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INVESTIGATION OF CCN1 ROLE(S) IN MANTLE CELL LYMPHOMA (MCL)

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University of Plymouth

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INVESTIGATION OF CCN1 ROLE(S) IN MANTLE CELL LYMPHOMA (MCL)

By

Afak R S Zaidi

A thesis submitted to the University of Plymouth
In fulfilment for the degree of

Doctor of Philosophy

School of Biomedical Sciences
Faculty of Medicine and Dentistry

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Investigation of CCN1 role(s) in Mantle Cell Lymphoma (MCL)

Afak Rasheed Salman Zaidi

Abstract:

Mantle Cell Lymphoma (MCL) is a comparatively rare non-Hodgkin's lymphoma which is characterised by the overexpression of cyclin D1. Many patients present with or progress to advanced stage disease within three years. Disease progression involves down regulation of cyclin D1. Moreover, MCL is broadly considered as an incurable disease with median survival of patients being 3-4 years. CCN1, a matricellular protein involved in stem cell signalling within the haematopoietic microenvironment is highly expressed in early stage MCL cells and down-regulated in advanced stage disease.

We have used the human MCL cell lines REC1, G519, JVM2 as a model for disease aggression. We have investigated the role(s) of CCN1, cyclin D1 and cyclin dependent kinase inhibitors in MCL progression. CCN1 dysregulation is identified in MCL progression whereby the magnitude of CCN1 expression in human MCL cells is REC1 > G519 > JVM2 cells by RQ-PCR, depicting a decrease in CCN1 expression with disease progression. Further investigation of CCN1 protein expression by western blotting showed that whilst expression of full-length CCN1 (42kDa) barely altered through the cell lines, expression of the truncated form (20kDa) was high in REC1 cells (OD:1.0) reduced in G519 cells (OD:0.5) and barely detected in JVM2 cells depicting decreased with disease progression.

We have then demonstrated that cyclin D1 and cyclin dependent kinase inhibitors (p21^{CIP1} and p27^{KIP1}) are also involved in disease progression using the above MCL cell line model. Cyclin D1 was highly expressed in REC1 cells (OD: 1.0), reduced to one fifth in G519 cells (OD: 0.2) and not detected by western blotting in JVM2 cells. p27^{KIP1} followed a similar profile of expression as cyclin D1. Conversely, p21^{CIP1} was absent in

the REC1 cells and showed increasing expression in G519 and JVM2 cells. Subcellular localization detected p21^{CIP1}/ p27^{KIP1} primarily within the cytoplasm and absent from the nucleus, consistent with altered roles in treatment resistance. Mitochondrial detection of p21 in the JVM2 cell line supports an additional anti-apoptotic role.

CCN1 likely plays a key role in B cell development; dysregulation of CCN1 may support MCL progression with p21^{CIP1} and p27^{KIP1} forming molecular signatures associated with progressive disease.

REC1 cells and JVM2 cells were genetically modified using lentivirus to identify the potential pathways associated with CCN1. CCN1 knockdown was performed in REC1 cells (REC1 KD) and CCN1 overexpression in JVM2 cells (CCN1 OE). Proteomics analysis of JVM2 OE and REC1 KD revealed interesting results showing regulation of 44 proteins. 19 proteins regulated by CCN1 that simultaneously downregulated in the CCN1 KD model and up-regulated in CCN1 OE model. 25 proteins modulated by CCN1 that simultaneously up-regulated in CCN1 KD model and down-regulated in CCN1 OE model.

Our results suggest novel roles for CCN1. Whilst CCN1 roles are substantial in solid tumour research, CCN1 role(s) within the haematopoietic compartment are less well defined or investigated. CCN1 may have potential role(s) as a novel pro-inflammation regulator by modulating macrophage migration inhibitory factor (MIF) and within regulation of haematopoiesis via pre-B cell colony enhancing factor (PBEF1). CCN1 was shown to modulate calcium ion signalling by targeting intracellular calcium receptor protein Calmodulin 3 (CALM 3). CCN1 altered Apolipoprotein M (ApoM) and Talin 1 (TLN1) expression and could potentiate new targets to supplement treatment for MCL. However, these novel pathways would need further investigation to identify the role(s) of

CCN1 in B cell development and within the bone marrow microenvironment where regulation of haematopoiesis ensues.

DEDICATION

I dedicated this thesis to my beloved family, my dearest husband Sabri, my lovely daughters Zahraa, Zainab and Horaa for all their tolerance, support, patience, and encouragement, I am so lucky to have you....

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- Hands-On Proteomics and Data Analysis Workshop, arranged by University of Plymouth, 14th – 17th July 2014, Davy 209, Main Campus University of Plymouth.
- Welcome to the command line: a gentle practical introduction to UNIX and serious bioinformatics" session, arranged by Dr Robert Belshaw (University of Plymouth), 28th April 2015, Babbage 111, Main Campus University of Plymouth.
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Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

Work submitted for this research degree at the Plymouth University has not formed part of any other degree either at Plymouth University or at another establishment.

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Signed

Date

Abbreviations:

ACN	Acetonitrile
ACTRII	Activin receptor II
ALK1-7	Activity receptor-like kinase 1-7
AML	Acute myeloid leukaemia
ANK	Ankyrin repeats
ATM	Ataxia telangiectasia mutated
BCR	B cell receptor
BMP	Bone morphogenetic protein
BMPRII	Bone morphogenetic protein receptor II
BTK	Bruton's Tyrosine Kinase
CCI-779	Cell cycle inhibitor
CCND1	Cyclin D1
CDK4 or 6	Cyclin-dependent kinase 4 (CDK4) or cyclin-dependent kinase 6
CDKI	Cyclin dependent kinase inhibitor
CLL	Chronic lymphocytic leukaemia
CMV	Cytomegalovirus
Co-Smad	Common mediator Smad
CRC	Colorectal cancer
CT	Cysteine knot carboxyl terminal
CTGF	Connective tissue growth factor
CYR61	Cysteine-rich protein 61
DCIS	High-grade ductal carcinoma in situ
DKK	Dickkopf
DSL	Delta-Serrate-Lagz
DOR	Duration of response
ES	Embryonic stem cells
FDA	Food and Drug Administration
FKBP	FK506-binding protein

FOXO3a	Forkhead box O3
Fzd	Frizzled family
ECD	Extracellular domain
ECOG	Eastern Cooperative Oncology Group
EFS	Event free survival
EGF	Epidermal growth factors
ES	Embryonic stem cell
EU	European Union
G1&G2	Gap 1&2 phases
GDFs	Growth and differentiation factors
HCC	Hepatocellular carcinoma
hESCs	Human embryonic stem cells
HGSOC	High-grade serous ovarian cancer
HSPGs	Heparan sulfate proteoglycans
ICD	Intracellular domain
IGFBP	Insulin-like growth factor binding protein domain
IgH	Immunoglobulin heavy chain
IL-6	Interleukin-6
IMiD	Immunomodulatory drug
I-Smad	Inhibitory Smad
LNR	Lin-notch repeats
LPR5/6	Lipoprotein-receptor-related protein5/6
MAPK	Smad-independent mitogen activated protein kinase
MCL	Mantle cell lymphoma
MCL-ICs	MCL-initiating cells
MEK/ERK	Mitogen-activated protein kinase/extracellular-signal-regulated kinase
MIF	Mullerian inhibitory factor
MIPI	International Prognostic Index
MM	Multiple myeloma

MMPs	Matrix metalloproteinases
M phase	Mitosis phase
mTOR	Mammalian target of rapamycin
MWT	Molecular weight
NF _k B	Nuclear factor _k B
NHL	Non Hodgkin's Lymphoma
NK	Nature killer
NLS	Nuclear Localisation Signal
NLS	Nuclear localization sequence
NOV	Nephroblastoma overexpressed gene
NSCLC	Non-small-cell lung cancer
OAFs	Osteoclast activating factors
OBI	Osteoblast inhibitors
ORR	Overall response rate
OS	Overall survival
OSC	Oesophageal squamous carcinoma
OSCC	Oral squamous cell carcinoma
PCNA	Proliferating cell nuclear antigen
PEST	Prolin-, glutamate-, serine- and threonine-rich
PB	Peripheral Blood
PI	Proteasome inhibitor
PI3K	Phosphatidylinositol 3-kinase
pRb	Retinoblastoma protein
p21 ^{CIP1}	(Cdk Interacting Protein 1)
p27 ^{KIP1}	(Kinase Inhibitory Protein 1)
p57 ^{KIP2}	(Kinase Inhibitory Protein 2)
RAM	RBP-J-associated molecular
RCC	Renal cell carcinoma
SP	Signal peptide
S phase	DNA synthesis phase

ROR2/RYK	Receptor tyrosine kinase-like orphan receptors/receptor tyrosine kinase
R-Smad	Regulated Smad
TAD	Transactivation domain
TCF/LEF	T cell factor/lymphoid enhance binding factor
TGF- β	Transforming growth factor β
TNBC	Triple negative breast carcinoma
TSP-1	Thrombospondin type 1 domain
UPS	Ubiquitin proteasome system
VEGF	Vascular endothelial growth factor
vWC	von Willebrand type C repeat
WISPs	Wnt-induced secreted proteins
ZOL	Zoledronic acid

Chapter 1

Mantle Cell Lymphoma (MCL)

Chapter 1

1.1 Mantle Cell Lymphoma (MCL)

Mantle cell lymphoma (MCL) is a distinct subset of B-cell Non Hodgkin's Lymphoma (NHL) characterised by overexpression of cyclin D1 as a result of t(11;14) chromosomal translocation (Perez-Galan, Dreyling & Wiestner, 2011). This translocation juxtaposes the cyclin D1 (CCND1) gene on chromosome 11q13 with the immunoglobulin heavy chain (IgH) gene on 14q32 which leads to cyclin D1 overexpression and the dysregulation of the cell cycle (O'Connor, 2007; Zucca & Bertoni, 2013). Disease progression often involves down regulation of cyclin D1 (Peng, Chou & Hsu, 1998). In 1992, MCL was considered as a distinct entity (Banks *et al.*, 1992) and in 1994, accepted by the Revised European American Classification for Lymphoma (REAL) (Wang & Ma, 2014). MCL accounts for 3-10% of all NHL (Marco & Lorenzo, 2012). Median age of onset for MCL is 60, with a bias for males to females in the order of 4:1 and it is often diagnosed at advanced stage (Ahrens *et al.*, 2013). Patmore *et al.*, (2016) have found in the study of MCL management and outcome in the United Kingdom population that in the period of 2004-15 patients diagnosed with MCL were 327 with a median diagnostic age of 74 years (range 39-96) and 66% patients were male. In the United States (U.S.), (Wang & Ma, 2014) have found that racial differences have seen among patients with MCL and these patients were caucasian, male, and elderly. Furthermore, the incidence of MCL had increased between 1992-2007 (Howlader *et al.*, 2012; Zhou *et al.*, 2008). MCL is considered an aggressive and incurable B-cell malignancy with poor prognosis with median survival being 3-4 years from diagnosis (Fisher *et al.*, 2006; Habermann *et al.*, 2009). Many studies have indicated that the pathogenesis of MCL depends on epigenomic and genomic lesions which result in pathway dysregulation including cell cycle progression, protein homeostasis, DNA damage response, cell proliferation and apoptosis (Parekh, Weniger & Wiestner, 2011). In MCL, the genomic mutations involved in B-cell

development and maintenance, contribute to disrupt these gene pathways and epigenetic regulatory frameworks (Ahmed *et al.*, 2016). Epigenetic changes in DNA methylation and histone modifications in MCL can lead to either aberrant expression of oncogenes or inhibition of tumour suppressor genes (Leshchenko *et al.*, 2010; Sharma, Kelly & Jones, 2010). Hutter *et al.* (2006) have found that the genetic mutation and a rare promoter of methylation of p16 (9% MCL cases) were associated with enhanced cell proliferation and can be considered as additional molecular mutations that are involved in MCL cell cycle dysregulation. Mutations in the cell cycle regulator p53, correlated with high levels of proliferation and shortened survival associated with the aggressive variants of MCL (Hernandez *et al.*, 1996). Many gene alterations involved in the PI3K/AKT, WNT and TGF beta signalling pathways, which are also aberrantly expressed in MCL correlated with pathogenesis of this disease and may be useful as therapeutic targets (Rizzatti *et al.*, 2005). World Health Organization classification of haematopoietic and lymphoid tumours indicated that the diagnosis of MCL depends on “different clinicopathological manifestations and molecular pathogenetic pathways”. Classical MCL which consists of IGHV-unmutated or minimally mutated B cells expressing SOX11 involves lymph nodes and other extranodal sites. Additional molecular / cytogenetic aberrations can lead to a more aggressive blastoid or pleomorphic MCL. Other MCL develop from IGHV-mutated SOX11⁻ B cells which leads to leukemic non-nodal MCL, usually involving the Peripheral Blood (PB), bone marrow, and often spleen. These are often indolent unless additional molecular /cytogenetic aberrations have occurred (Swerdlow *et al.*, 2016) (Figure 1.1).

Classical MCL is derived from a mutated naïve B cell (Cyclin D1 translocation or those that are CCND1 negative frequently have aberrations in cyclin D2 or D3) (Schieber, Gordon & Karmali, 2018). Whilst various reports show conflicting evidence of cyclin D1 status by RNA or protein levels, SOX11 positivity or negativity is a much more reliable marker and is independent of Cyclin D1 status (Campo & Rule, 2015; Schieber, Gordon

& Karmali, 2018). Transient transition of cells through the mantle zone with SOX11+ causes sustained PAX5 expression which represses BLIMP-1 required for plasma cell differentiation, hence these tumours are unable to differentiate further (Nutt *et al.*, 2015; Swerdlow *et al.*, 2016) (Figure 1.1 B & D). In contrast, cells that are SOX11 negative traverse the germinal centre where somatic mutation of VDJ genes occur to enable repertoires of high affinity antibody producing cells to develop (Heesters, Myers & Carroll, 2014; Swerdlow *et al.*, 2016) (Figure 1.1 B & C). These cells then become hypermutated and give rise to leukaemic non-nodal MCL with plasmablast like cells (Figure 1.1 B).

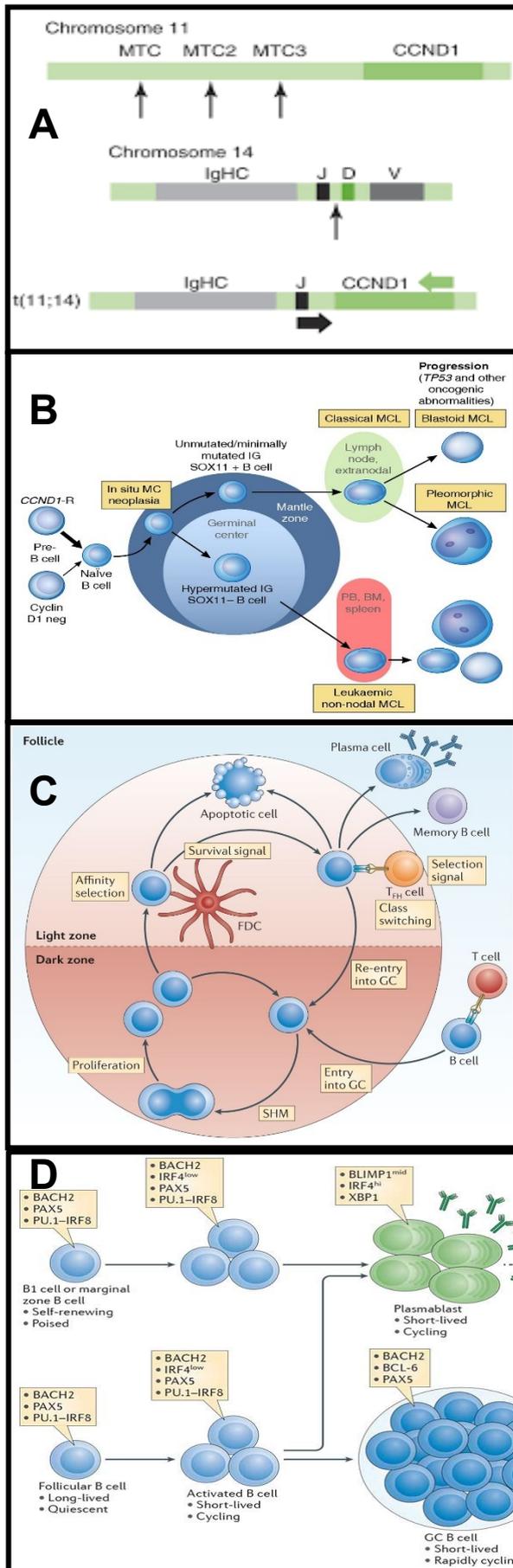


Figure 1.1 Origins of Mantle Cell Lymphoma.

(A) MCL is characterised by the t(11;14) (q13;q32) gene translocation which arises from Cyclin D1 (CCND1) on chromosome 11 being translocated and juxtaposed to the Immunoglobulin Heavy Chain (IgHC) between the J and D components for VDJ rearrangement required for antibody diversity.

(B) Classical MCL arises from naïve B cells with SOX11+ plus unmutated / minimally mutated IGHV which are transiently within the mantle zone of the lymph node, accrual of additional mutations give rise to more aggressive blastoid or pleomorphic phenotypes. Leukaemic non-nodal MCL arises from cells that are SOX11 negative traversing the germinal centre where somatic mutation of Ig occurs for antibody diversity giving rise to hypermutated IGHV presenting in the Peripheral Blood (PB), Bone Marrow (BM) and spleen (Swerdlow *et al.*, 2016).

(C) The germinal centre of the lymph node: B cells present antigen to T helper cells and receive co-stimulatory signals, selected cells enter the dark zone of the GC and undergo somatic hypermutation (SHM) by upregulating components of the SHM machinery, including activation-induced deaminase (AID). After one cycle (or possibly more cycles) of proliferation and SHM, the B cells migrate to the light zone; the mutated BCRs that are the product of SHM are now exposed to antigens that are incorporated into immune complexes on the follicular dendritic cells (FDCs). If the affinity of the BCR is very low, the B cell will not receive survival signals and will undergo apoptosis. The remaining B cells need to compete for limited T cell help, which favours the higher affinity B cells and forces the others to undergo apoptosis. Surviving B cells can re-enter the dark zone for further affinity maturation and SHM, they can exit the GC as plasma cells or they can exit as memory B cells. (Heesters, Myers & Carroll, 2014)

(D) SOX11 + cells in the marginal zone of the lymph node induce sustained PAX5 expression. PAX 5 inhibits BLIMP1 which is required for maturation of plasmablasts to plasma cells. In contrast, Follicular B cells require PAX5 and PU.1 for maturation of Memory B cells- (Nutt *et al.*, 2015)

Cyclin D1 status in MCL has conflicting reports depending on whether mRNA or protein levels were evaluated and disparity between RNA and protein evaluations exist (Amin *et al.*, 2003; Weinstein *et al.*, 2012). This may in part be explained by compartmentalisation of the protein in either nuclear or cytoplasmic fractions in MCL (Body *et al.*, 2017). Cytoplasmic Cyclin D1 expression results in increased invasive capacity and aggressive behaviour; increased engraftment into the bone marrow, spleen and brain in immunodeficient mice. Or perhaps, disparity in reports occur by lack of reporting on Cyclin D1a and b isoforms (Marzec *et al.*, 2006). Subsequently, SOX11 has been identified as a much better clinical marker for classical and blastoid MCL (Campo & Rule, 2015; Schieber, Gordon & Karmali, 2018).

IGHV mutation status can therefore be useful clinically; the presence of IGHV mutation shows transition through the germinal centre with usual somatic hypermutation occurring corresponding to a more favourable prognosis in MCL. However, MCL is derived from a naïve B cell whereas Chronic Lymphocytic Leukaemia (CLL) is derived from an antigen experienced mature B cell (Figure 1.2). IGHV status is a prognostic indicator in CLL whereby unmutated IGHV dictates a poor clinical course with reduced response to chemotherapy and progression free survival rates (Rozovski, Keating & Estrov, 2018). Mutated IGHV correlates with good responses to chemotherapy and good overall survival rates. The rationale for this has been elusive over many years. The current theory in CLL proposes that in high proliferating tumours DNA breaks are repaired by efficient high fidelity homology directed DNA repair mechanisms resulting in low or no IGHV mutation, whilst slowly dividing tumours are repaired by inefficient and low fidelity non-homologous end joining mechanisms resulting in IGHV mutation (Rozovski, Keating & Estrov, 2018) and better response to chemotherapy. This is consistent with Patten *et al.*, 2012, who identified that activation induced deaminase (AID) which is required for

'class switching' and IGHV mutation in B cells, showed a better overall survival rate for AID+ patients versus AID- patients (Fig 1.1 C).

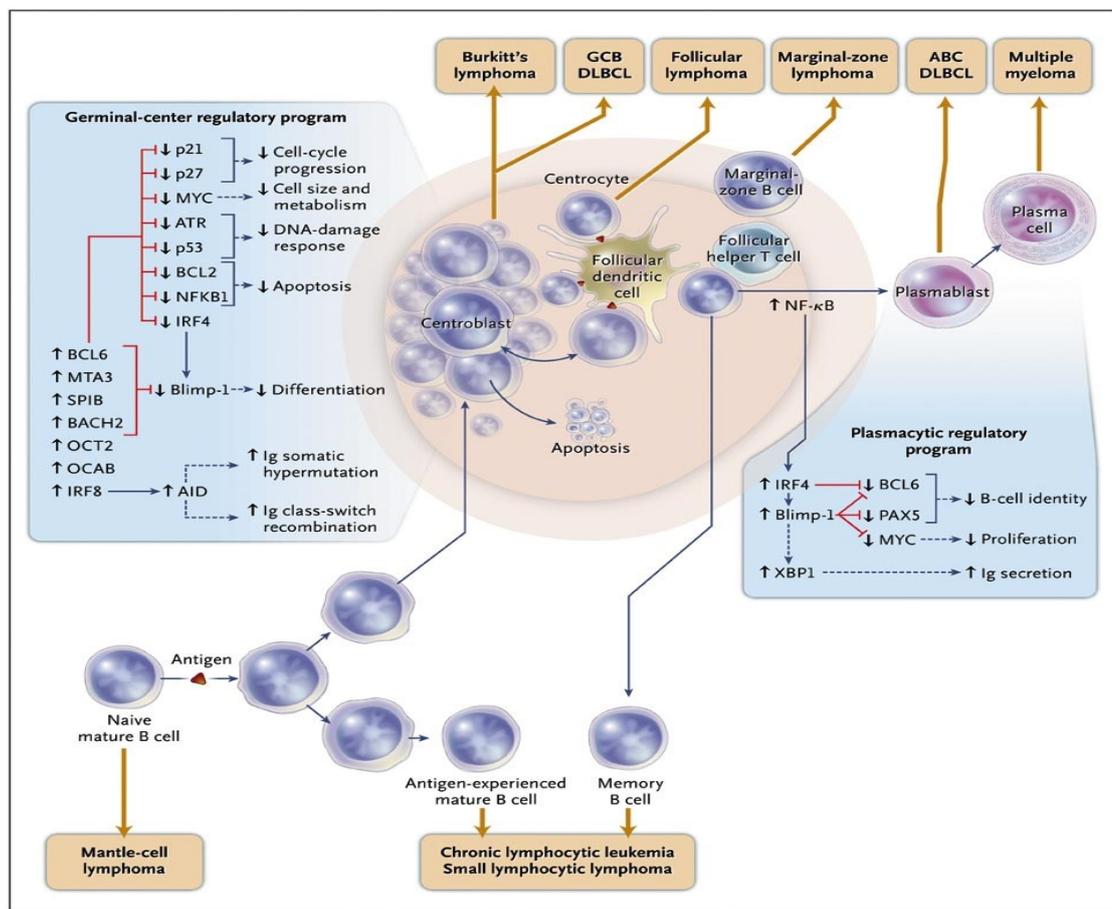


Figure 1.2 Origins of B cell disorders. Mantle cell lymphoma is derived from aberrations in naïve B cells either transiently transitioning across the mantle zone of the lymph node forming classical MCL or through the germinal centre forming non-nodal MCL. Chronic Lymphocytic Leukaemia is derived aberrations in antigen experienced mature B cells within the germinal centres of the lymph node required for production of high affinity antibodies by clonal selection and memory B cells. Frequently, de-regulated programming occurs within B cell malignancies for cell cycle regulation, DNA damage response mechanisms, differentiation, somatic mutation and class switching programmes required for antibody responses. Image taken from http://www.ufrgs.br/imunovet/molecular_immunology/pathohomotissueblood_WBCneoplasm.html

MCL is one of the most challenging of all NHL to treat, according to the poor overall survival post-treatment with conventional chemotherapy (Romaguera *et al.*, 2010). Many studies have suggested that the microenvironment plays an important role in the pathogenesis of several B-cell malignancies and indicated that tumour host interactions in MCL may be more important than was previously thought. The microenvironmental factors are potentially responsible for determination of disease localization or progression of malignant cell expansion or both (Kurtova *et al.*, 2009). MCL can involve bone marrow, secondary lymphoid organs and gastrointestinal tissue at advanced stages. The cross talk between MCL cells and their microenvironment can contribute to disease progression by enhancing tumour survival, growth and drug resistance (Burger & Ford, 2011; Kurtova *et al.*, 2009). Several aberrant signalling pathways are involved in growth of MCL cell including AKT, phosphatidylinositol 3-kinase (PI3K), mammalian target of rapamycin (mTOR), nuclear factor kappaB (NF κ B), WNT, and Notch (Kridel *et al.*, 2012; Noel, Friedberg & Barr, 2012; Rizzatti *et al.*, 2005). Consequently, novel approaches are urgently needed to effectively treat MCL patients by targeting specifically the MCL associated signalling pathways.

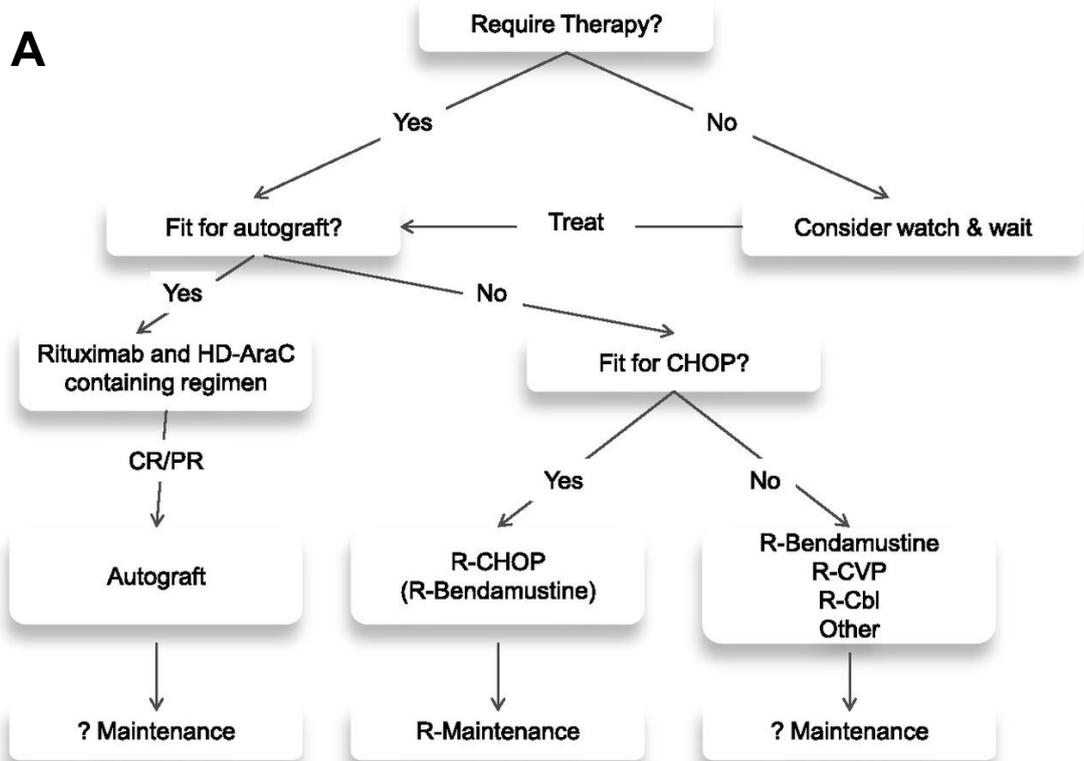
1.2 Stages of Mantle Cell Lymphoma (MCL)

MCL is a tumour derived from immature peripheral B lymphocytes, classified by stage of disease according to distinct morphologic, immunologic and genetic phenotype (Leitch *et al.*, 2003; Pervez, Haroon & Awan, 2015). MCL is characterised by classic (common variant) associated with early stage and blastoid and pleomorphic (aggressive and rare variants) associated with advanced stage of disease (Pervez, Haroon & Awan, 2015). The ‘classic’ variant has relatively favourable characteristics, with the ‘blastoid’ variant being aggressive with high proliferative capacity. The ‘pleomorphic’ variant mimicks diffuse

large B cell lymphoma (DLBCL) and is characteristically cyclin D1 negative with poor survival rates of under 1 year (Chuang *et al.*, 2017). The classic variant of MCL which carries t(11;14) chromosomal translocation leading to cyclin D1 overexpression have longer-survival than patients that are cyclin D1 negative (Yatabe *et al.*, 2000). Patients with cyclin D1 negative present aggressive behaviour (blastoid and pleomorphic), poor prognosis and short-survival with cyclin D2 translocation leading to cyclin D2 overexpression (Bernard *et al.*, 2001; Seto, 2013; Tiemann *et al.*, 2005). Recently, SOX11, a transcription factor, which is overexpressed in MCL cyclin D1 negative phenotype is considered as a biomarker (Narurkar, Alkayem & Liu, 2016). In 2009, (Mozos *et al.*) found overexpression of protein and mRNA of SOX11 in cyclin D1 negative cases as well as cases of cyclin D1 positive. All most all stages of MCL (except rare cases of blastoid and pleomorphic variants) present with CD20⁺, CD5⁺, cyclin D1⁺ and Bcl⁺ however, secondary genetic alterations are found in progressive MCL (aggressive blastoid and pleomorphic variants) (Kwatra *et al.*, 2016). For example, mutation in ATM, TP53, c-myc and BCL6 genes have been associated with MCL progression (Beà *et al.*, 1999; Hao *et al.*, 2002; Jares, Colomer & Campo, 2012; Royo *et al.*, 2011; Setoodeh *et al.*, 2013). In a rare case, CD5⁻ with CCND1/IGH and myc alterations which were identified in patient with blastoid MCL associated with resistance to conventional treatment, poor prognosis and poor-survival (1 month after diagnosis) (Seok *et al.*, 2012). Each stage of MCL has specific molecular characteristics associated with resistance to treatment, however subclassification of MCL is essential to infer prognostic indicators and potential beneficial treatment strands (Zhou *et al.*, 2015).

1.3 Treatment of Mantle Cell Lymphoma (MCL)

MCL is considered an aggressive disease and has one of the worst outcomes among B-cell lymphomas due to the dysregulation of the DNA damage response pathways accompanied with abnormal cell survival mechanisms suppressing apoptosis (Campo & Rule, 2015; Moros *et al.*, 2014). MCL is characterised by clinical course where variants are subdivided into indolent form (classic morphology) and aggressive (blastoid or pleomorphic appearance) (Kridel *et al.*, 2012). The MCL International Prognostic Index (MIPI) stratifies MCL patients into low, intermediate and high risk groups depending on age, Eastern Cooperative Oncology Group (ECOG) performance status, white blood cell counts and lactate dehydrogenase level (Hoster *et al.*, 2008). The heterogeneous biology and aggressive behaviour of MCL present a challenge for designing standard therapies (Smith, 2011). Moreover, patients with MCL who initially responded to first-line immunochemotherapy, but have shown chemoresistance in relapses are associated with poor outcome (Goy *et al.*, 2015). Similarly, short-term remissions are predominant with conventional chemotherapy and median survival is usually 3-6 years (Vose, 2015). High dose chemotherapy followed by autologous stem-cell transplantation shows improved outcomes with event free survival (EFS) >60% at 6 years however, the potential for cure resides within allogeneic bone marrow transplants, but this procedure is only feasible for younger patients (Avivi & Goy, 2015; Campo & Rule, 2015; Ghilmini & Zucca, 2009) (Figure 1.3). Recently, four novel agents have been approved for patients with MCL: Temsirolimus (mTOR inhibitor), Lenalidomide (anti-angiogenesis), Ibrutinib (Bruton's Tyrosine Kinase inhibitor), and Bortezomib (NF κ B inhibitor) (Avivi & Goy, 2015; Campo & Rule, 2015). The mechanism of action of each are reviewed in the subsequent sections.



B

Drug	Mechanism of Action	ORR/CR	Duration of Response	Toxicities
Bortezomib[29]	Proteasome inhibitor	ORR, 33% CR, 8%	9.2 mo	13% peripheral neuropathy
Lenalidomide[31]	Immunomodulator	ORR, 35% CR, 12%	Median PFS, 9 mo	46% grade 3/4 neutropenia, 30% grade 3/4 thrombocytopenia
Temsirolimus[30]	mTOR inhibitor	ORR, 41% CR, 4%	MTP; 6 mo	54% grade 3/4 hematologic toxicities
Ibrutinib[34]	BTK inhibitor	ORR, 68% CR, 21%	Median, 18 mo	16% grade 3/4 hematologic toxicities, 44% grade 1/2 diarrhea
Idelalisib[36]	PI3K inhibitor	ORR, 40% CR, 5%	Median, 3 mo	18% grade 3/4 diarrhea, 20% grade 3/4 AST/ALT elevation
Venetoclax[37]	BCL-2 inhibitor	ORR, 85% CR, 21%	Median PFS, 14 mo	16% grade 3/4 anemia, 12% grade 3/4 neutropenia

Figure 1.3 Mantle cell lymphoma first line treatment strands and clinical trials for refractory disease. (A) Schematic of first line treatment schedules for MCL including autograft and variations of CHOP (HyperCVAD alternating with high-dose methotrexate/cytarabine alternating with high-dose methotrexate/cytarabine regimen), R-Rituximab anti-CD20 monoclonal antibody (Campo & Rule, 2015). **(B)** Clinical trials for novel targeting agents used in MCL. ORR-Overall Response Rate, CR- Complete Response, PFS-Progression Free Survival, MTP- Months to Progression, AST – alanine amino transferase, ALT – aspartate amino transferase (Chen, Sanchez & Rosen, 2016).

1.3.1 Temsirolimus

Cell cycle inhibitor-779 (CCI-779) currently known as temsirolimus is derived from rapamycin and is a selective inhibitor of mammalian target of rapamycin (mTOR) signalling protein which is dysregulated in MCL (Galimberti & Petrini, 2010; Müller *et al.*, 2013). In the United States, temsirolimus has been approved to treat advanced renal cell carcinoma (RCC), in the European Union (EU) it has been approved to treat RCC and relapsed or refractory MCL.

mTOR is a downstream mediator in the phosphoinositide-3-kinase-protein kinase B (PI3K/Akt) pathway which is activated by growth factor receptor signalling, activation of PI3K/AKT pathways, and other cell stimuli. mTOR which structurally and functionally contains 2 distinct complexes mTORC1 and mTORC2 plays a central role in cell growth and proliferation, regulation of apoptosis, and angiogenesis by regulating synthesis of several proteins including cyclin D1, c-myc and HIF α (Kim, Cook & Chen, 2017; Le Tourneau *et al.*, 2008). In cancer, hyper activation of mTOR leads to stimulate the translation of mRNA encoding for cell cycle regulatory proteins (Morita *et al.*, 2015; Rini, 2008). Temsirolimus prevents the activation of mTOR through binding with FK506-binding protein (FKBP) and the protein-drug complex suppresses the kinase activity of mTORC1 (Figure 1.4). However, the mTORC2 isoform can be still activated even in the presence of temsirolimus (Le Tourneau *et al.*, 2008). In 2015, (Campo & Rule) have reviewed four drugs (Ibrutinib, Bortezomib, Lenalidomide, and Temsirolimus) which have been recently approved for MCL. The overall response rate (ORR) was 68%, 33%, 28%, and 22% and the duration of response (DOR) was 17.5, 9.2, 16.6, 7.1 months. Temsirolimus was the least active of these drugs, showing limited effect when used as a single agent.

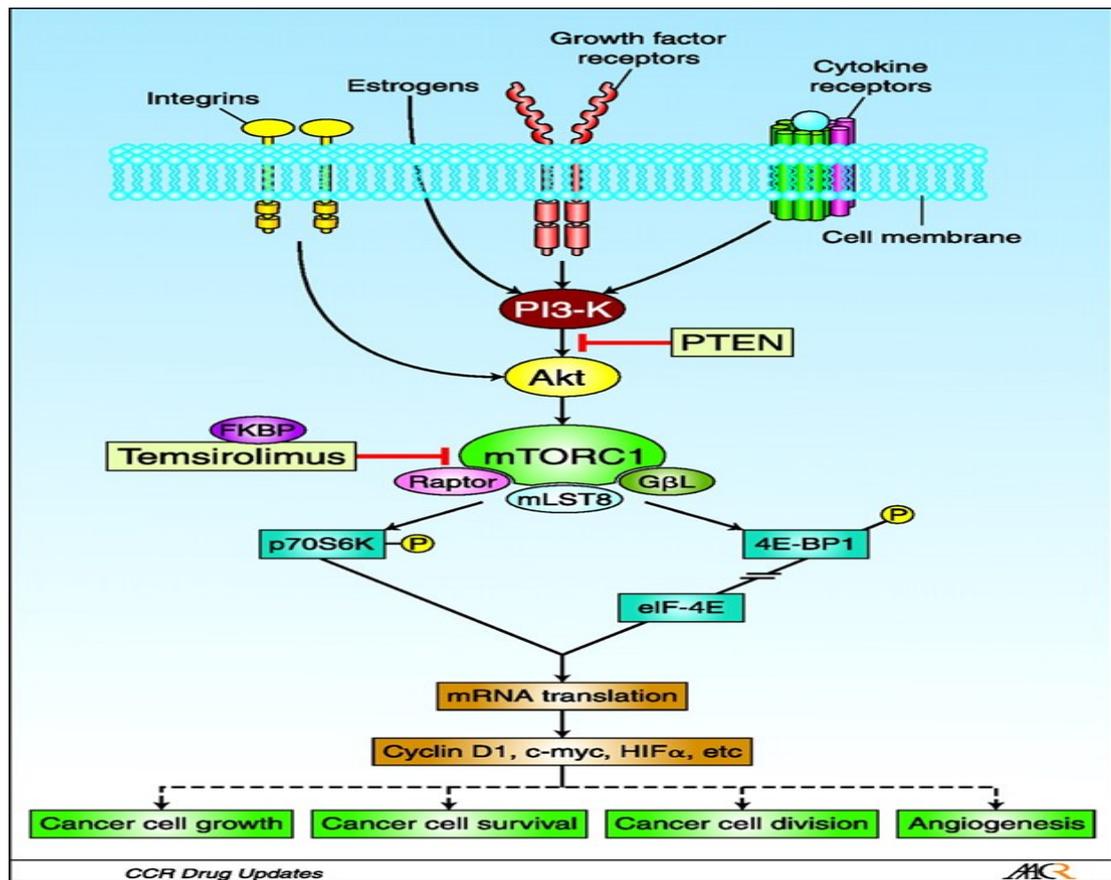


Figure 1.4 Mechanism of action of Temsirolimus and mTOR signalling: Temsirolimus inhibits mTOR signalling pathway by targeting mTOR Complex1 (mTORC1) that consists of regulatory-associated protein of mTOR (raptor), mLST8 and GβL. Activated mTORC1 which finds in cytoplasm phosphorylates p70S6K leading to induce cell growth and angiogenesis through promote translation and production of hypoxia-inducible factor α (HIF α). Furthermore, mTORC1 phosphorylates 4E-binding protein-1 (4E-BP1) leading to regulation of cell growth and apoptosis by regulating synthesis of several proteins including cyclin D1, c-myc. Temsirolimus prevents the activation of mTOR through binding with FK506-binding protein (FKBP) and protein-drug complex suppresses the kinase activity of mTORC1. Image from (Rini, 2008).

1.3.2 Bortezomib

Bortezomib, a reversible proteasome inhibitor (PI) was approved to treat patients with MCL by Food and Drug Administration (FDA, 2006) (Kane *et al.*, 2007). It is a boronic acid derivative which binds and suppresses the 26S proteasome that regulates critical cellular functions for example, inhibition of nuclear factor- κ B (NF κ B), inhibition of angiogenesis, apoptosis and regulation of cell cycle proteins (Figure 1.5) (Crawford &

Irvine, 2013). The 26S proteasome which composes of a core particle 20S and 2 regulatory particles 19S is a proteolysis part of ubiquitin proteasome system (UPS) (Wehmer & Sakata, 2016). In normal cells, the UPS regulates intracellular protein homeostasis (protein synthesis and degradation) leading to degradation of damaged, oxidized, or misfolded proteins. UPS plays a pivotal role in regulation of cell cycle control, transcription, DNA damage repair, antigen presentation and apoptosis (Streich Jr & Lima, 2014). In MCL, many studies have shown that the bortezomib has an anti-tumour impact with responses between 30%-50% of patients (Fisher *et al.*, 2006; Leshchenko *et al.*, 2015). It induced cell cycle halt and apoptosis through inhibition of NF κ B activation which is aberrantly active in MCL (Pham *et al.*, 2003). Furthermore, preclinical researches have shown that the anti-tumour effect of bortezomib has conducted through down regulation of anti-apoptotic proteins, up-regulation of pro-apoptotic proteins, and down regulation of proteins that implicated in DNA damage response (Mujtaba & Dou, 2011). However, Noxa, a pro-apoptotic BCL-2 family member, is hypomethylated is regulated in response to Bortezomib inducing drug resistance (Leshchenko *et al.*, 2015). Subsequently, increasing resistance to bortezomib has been shown in the large proportion of patients with MCL impeding its therapeutic activity (Zhao *et al.*, 2015).

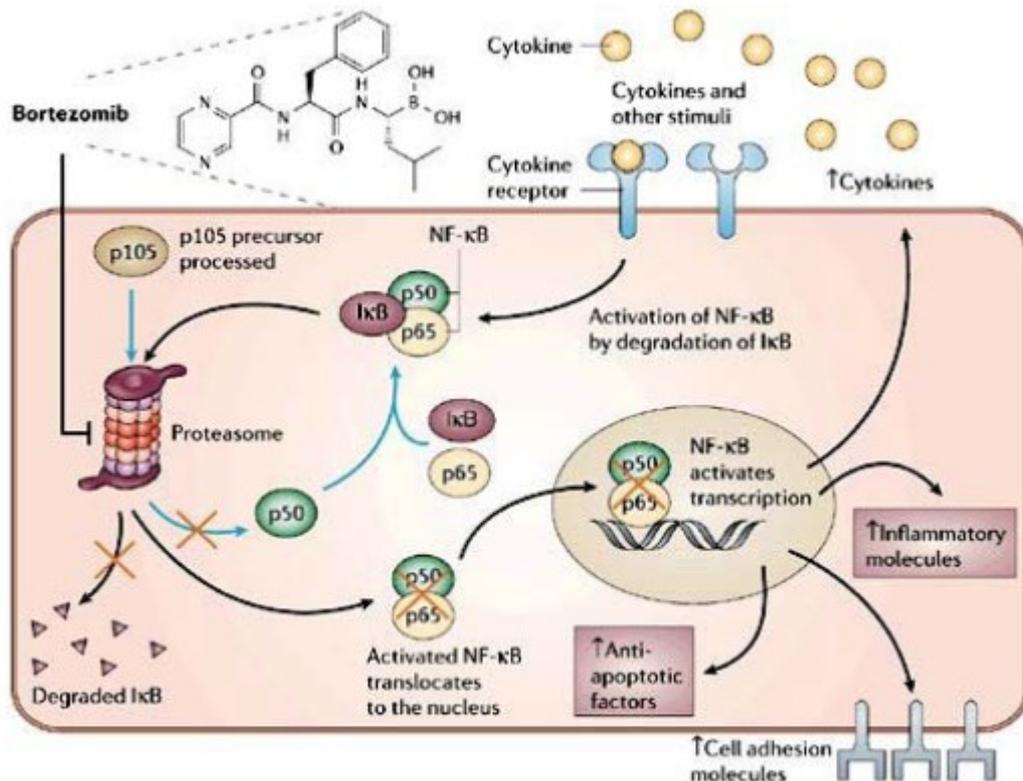


Figure 1.5 Mechanism of action of Bortezomib. Bortezomib inhibits NF- κ B nuclear localization that enables the transcription of target genes for cell proliferation and survival. Activation of NF- κ B translocated into the nucleus occurs by degradation of I κ B kinase in the cytoplasm. The 26S proteasome is responsible for degradation of damaged, oxidized, or misfolded proteins. Suppression the 26S proteasome by bortezomib inhibits I κ B degradation leading to inactivate NF- κ B which in turn prevents transcription of genes necessary for tumour cell proliferation and survival. Image from (Sánchez-Serrano, 2006)

1.3.3 Ibrutinib

In 2013, Ibrutinib was approved for treatment of relapsed MCL by FDA. Ibrutinib targets Bruton's Tyrosine Kinase (BTK) which is an intracellular signalling molecule downstream of the B cell receptor (BCR) (Figure 1.6) (Pan *et al.*, 2007; Roskoski Jr, 2016). Stimulation of the BCR is essential for B cell function including cell proliferation, apoptosis, differentiation, cell migration, and cell survival (Callard & Hodgkin, 2007; Del Nagro *et al.*, 2005; Richards *et al.*, 2008). Constitutive activation of signalling molecules downstream of the B-cell receptor, contributes to tumour proliferation and survival of

malignant B cells in several types of NHL including MCL (Maffei *et al.*, 2015; Tucker & Rule, 2015). BTK is essential mediator in the BCR signalling pathway by activating cell proliferation and survival (Byrd *et al.*, 2013). Ibrutinib inhibits phosphorylation of BTK leading to irreversible inactivation of B-cell proliferation and survival (Tucker & Rule, 2015). Furthermore, preclinical models have shown that the inactivation of downstream BTK pathways by ibrutinib leads to enhance tumour cell apoptosis and inhibits tumour cell proliferation (Akinleye *et al.*, 2013; Raedler, 2015). *In vivo*, Herman *et al.* (2014) have found that ibrutinib prevents activation of BCR and NF κ B signalling pathways resulting in inhibition of chronic lymphocytic leukaemia (CLL) cells proliferation. However, ibrutinib has antitumor activity in several types of NHL and resistance to ibrutinib has been observed in patients with CLL (Woyach *et al.*, 2014; Young & Staudt, 2014). Recently, Cheah *et al.* (2015) found that the MCL patients treated with Ibrutinib have a limited increased survival of around 8 months and a poor outcome for salvage therapy after treatment with ibrutinib. Resistance to ibrutinib predominantly implicates mutation of a cysteine residue C481S, within the ibrutinib binding site, causing reversible inhibition rather than irreversible inhibition of BCR signalling (Tucker & Rule, 2015).

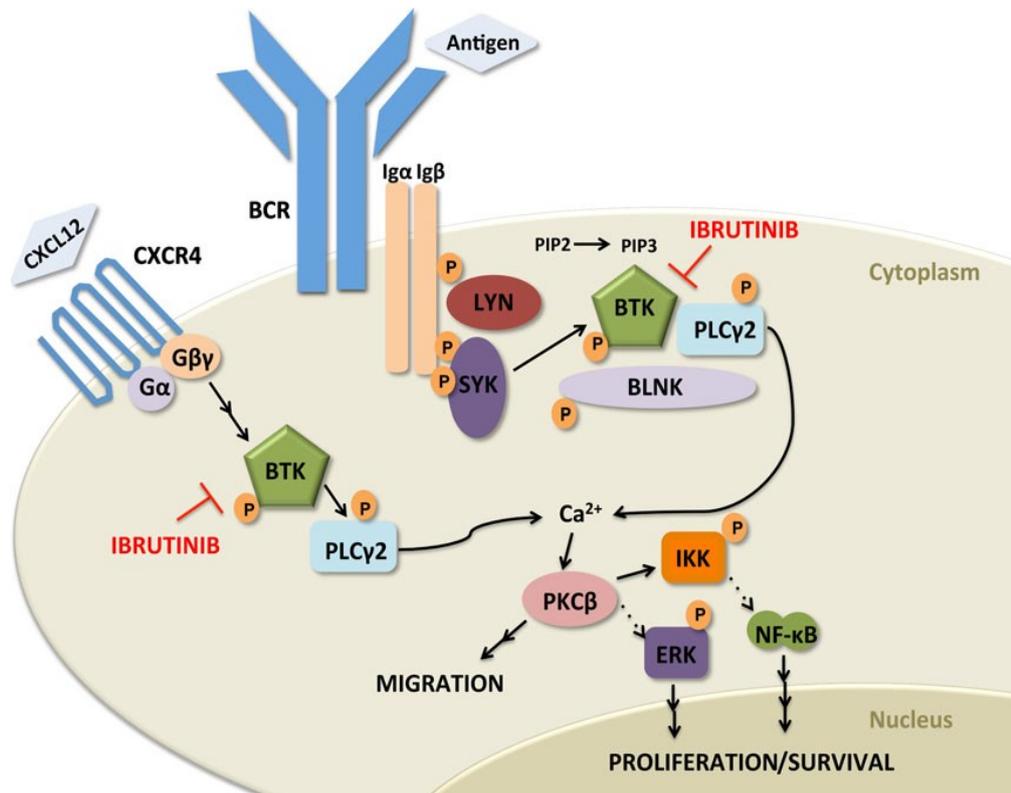


Figure 1.6 The mechanism of Ibrutinib blocking BTK signalling in hairy cell leukaemia. After stimulation of BCR receptors, LYN and SYK are phosphorylated leading to activate downstream BTK signalling which is phosphorylated leading to calcium release and induction of cell proliferation and survival. Ibrutinib inhibits BTK signalling reducing proliferation and survival. In conjunction with chemokine receptor CXCR4, Ibrutinib inhibits cell migration. Image from (Sivina *et al.*, 2012).

1.3.4 Lenalidomide

Lenalidomide is an immunomodulatory drug (IMiD) which has been used therapeutically (Dawar & Hernandez-Ilizaliturri, 2012). The mechanism of action of lenalidomide is not completely understood, but includes both immunomodulatory and non-immunomodulatory effects (Figure 1.7) (Habermann *et al.*, 2009). Lenalidomide has been found to increase tumour cell apoptosis by inhibiting the production of pro-inflammatory cytokines (TNF- α , IL-1, IL-6, and IL-12) and enhancing expression of the anti-inflammatory cytokine (IL-10) (Kotla *et al.*, 2009; Reddy *et al.*, 2008). Lenalidomide also inhibits CDK2 activity which led to induce G₀-G₁ cell cycle arrest (Qian *et al.*, 2011). Lenalidomide has also appeared to play a strong immunomodulating role through

activation of natural killer (NK) cells and improving the formation of so-called “immune synapses” between MCL cells and NK cells (Gaidarova *et al.*, 2009). Moreover, treatment of patients with refractory/relapsed MCL with lenalidomide after failing response to ibrutinib was affective with 27% overall response rate and 13% complete response (Wang *et al.*, 2016a). However, advanced stage MCL usually involves resistance to lenalidomide. Lenalidomide showed synergy when used in combination with dexamethasone by decreasing proliferation in cells taken from relapsed and refractory MCL patients and also within mouse models (Qian *et al.*, 2011).

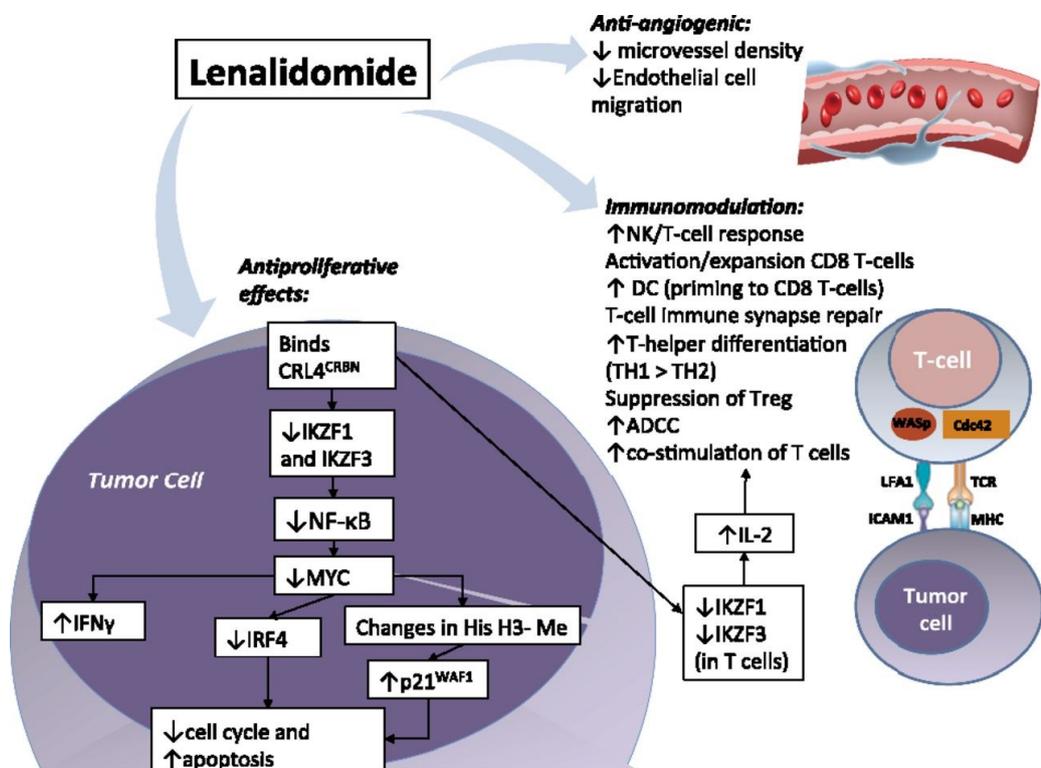


Figure 1.7 Mechanism of action of lenalidomide. Lenalidomide has immunomodulatory and non-immunomodulatory effects (anti-proliferative and anti-angiogenic) in lymphoid malignancies. Lenalidomide is considered as an anti-angiogenic, in part by decreasing microvessel density and inhibiting tumour growth and metastasis through depletion of monocytes/macrophages accompanied with lymphangiogenesis. Lenalidomide has anti-proliferative capacity through inhibition of cell cycle by ubiquitinating and degradation of the lymphoid transcription factors IKZF1 (Ikaros) and IKZF3 (Aiolos) leading to a decrease in NF-κB, decreased MYC and IRF4 (interferon regulatory factor 4) levels whilst increasing p21. Decreased IRF4 levels increase interferon production leading to enhanced apoptosis. The immunomodulatory effects of lenalidomide in lymphoid malignancies involve improvement of anti-tumour activity of T-cell and NK-cells. Image from (Kritharis *et al.*, 2015).

1.4 Combination treatment

MCL presents clinically and biologically a heterogeneous disease with combination of an indolent and aggressive characterisations (Maddocks & Blum, 2015). Whilst recent therapies are an improvement in the treatment of MCL patients who are mostly >60 yrs, the development of therapy-resistance is inevitable and considered a huge problem (Ahrens *et al.*, 2013). Patients with MCL initially respond to first line therapy but relapse, disease progression is frequent and considered a challenge to treat (Campo & Rule, 2015; Hitz *et al.*, 2013). (Zhao *et al.*) (2015) suggested that combination therapy could improve the outcome of patients; using a combination of arsenic trioxide with low dose bortezomib was beneficial in MCL cell lines and primary cells to avoid the toxicity associated using higher doses of bortezomib alone. Rituximab, an anti-CD20 monoclonal antibody, was used as a therapeutic agent to treat MCL alone or in combination with other therapies (Foran *et al.*, 2000; Wang *et al.*, 2016b). In clinical trial, combination of rituximab with ibrutinib was effective to treat patients with relapse or refractory MCL (Wang *et al.*, 2016b). Similarly, rituximab used in combination with lenalidomide was active against relapsed or refractory MCL with a median progression-free survival of 11.1 months and median overall survival of 24.3 months (Wang *et al.*, 2012). The combination of rituximab with lenalidomide was proposed as an initial therapy for MCL, improving the quality of life *in vivo* (Ruan *et al.*, 2015). Studies using rituximab plus lenalidomide inhibited cell growth and enhanced apoptosis in MCL cell lines and in primary MCL cells (Zhang *et al.*, 2009). The combination of rituximab with chemotherapy improved response rates and progression-free survival better than using chemotherapy alone in indolent MCL patients (Schulz *et al.*, 2007). However, irrespective of all of these promising regimens currently in use, MCL is still an incurable disease where patients succumb to progressive disease.

1.5 CCN1 protein

1.5.1 CCN family of matricellular proteins

Tumour development and progression of cancer is dependent on the interaction between the tumour cells and their microenvironment (Chong *et al.*, 2012). During this interaction the tumour cells and adjacent stromal cells abnormally secrete matricellular proteins which regulate cell-cell and cell-matrix interactions (Chong *et al.*, 2012). CCN1, a matricellular protein is involved in stem cell signalling within the haematopoietic microenvironment (Wells *et al.*, 2015). CCN1, also known as CYR61, belongs to CCN family of proteins named using acronym of the first three founding members CYR61 (cysteine-rich protein 61), CTGF (connective tissue growth factor) and NOV (nephroblastoma overexpressed gene). To date, another three members of the CCN protein family have been discovered; WISP-1 (CCN4), WISP-2 (CCN5) and WISP-3 (CCN6) (Wnt- induced secreted proteins) (Brigstock, 2003). All CCN proteins share about 40%-60% amino acid homology and comprise an N-terminal signal peptide followed by the four discrete domains with 38 conserved cysteine residues (Planque & Perbal, 2003). The discrete domains comprise an insulin-like growth factor binding protein domain (IGFBP), von Willebrand type C repeat (VWC), thrombospondin type 1 domain (TSP-1) and cysteine knot carboxyl terminal (CT) (McCallum & Irvine, 2009). Apart from CCN5, which is deficient of the C-terminal module, the other CCN proteins include the 4 structural modules (Figure 1.8) (Holbourn, Acharya & Perbal, 2008). The 4 structural domains of CCN1 may illustrate the diverse functions of the protein (McCallum & Irvine, 2009).

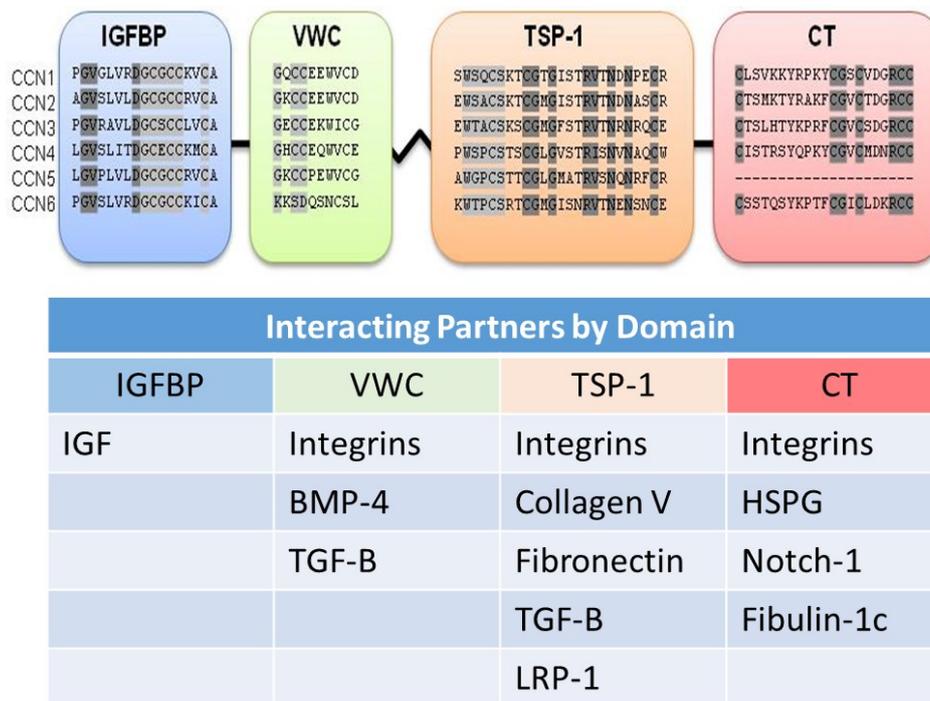


Figure 1.8 Structure of the CCN family protein. All CCN proteins share about 60% amino acid homology and comprise an N-terminal signal peptide followed by the four discrete domains with 38 conserved cysteine residues which shown in dark grey and sequences required for protein function in light grey. The discrete domains comprise an insulin-like growth factor binding protein domain (IGFBP), von Willebrand type C repeat (VWC), thrombospondin type 1 domain (TSP-1) and cysteine knot carboxyl terminal (CT). Apart from CCN5, which is deficient of the C-terminal module, CCN1, CCN2, CCN3, CCN4 and CCN6 contain the four structural domains. Image from (McCallum & Irvine, 2009). IGF- Insulin- like Growth Factor, BMP-4 Bone Morphogenic Protein 4, TGF-B Transforming Growth Factor Beta, LRP-1 low density lipoprotein receptor related 1, HSPG Heparan sulphate proteoglycans. Adapted from (Malik, Liszewska, & Jaworski, 2015)

1.5.2 CCN1 protein

CCN1 was originally considered as being a classic growth factor but later studies demonstrated that CCN1 induces expression of other growth factors for example, fibroblast growth factor and platelet-derived growth factor (Kireeva *et al.*, 1996). Results accumulated over the past decade have indicated that CCN1 is involved in a diverse array of cellular processes including regulation of cell migration, cell adhesion, proliferation, differentiation, apoptosis and angiogenesis through direct binding to cell surface receptors such as integrins and heparan sulfate proteoglycans (HSPGs) (Jandova *et al.*,

2012; Kireeva *et al.*, 1996) (Figure 1.8). CCN1 is a ligand for integrins (via the IGFBP domain) and acts through direct binding to integrins and HSPGs in order to enhance specific functions (Leu *et al.*, 2004). Increasing evidence has shown that CCN1 promotes cell adhesion by binding to integrin $\alpha 6\beta 1$ -HSPG co-receptors in fibroblasts (Todorovic *et al.*, 2005), $\alpha M\beta 2$ in murine macrophages (Bai, Chen & Lau, 2010), $\alpha D\beta 2$ in macrophage foam cells (Yakubenko, Yadav & Ugarova, 2006), $\alpha b\beta 3$ in activated platelets (Jedsadayamata *et al.*, 1999). CCN1 enhances cell migration in fibroblasts, smooth muscle cells, endothelial cells and certain cancer cells for example, CCN1 mediates tumour cell migration via integrin $\alpha v\beta 5$ in oesophageal squamous carcinoma (OSC) cell lines (Jandova *et al.*, 2012; Leu *et al.*, 2004). CCN1 has also specific non-canonical binding sites for a diverse set of integrins and these sites within CCN1 can function independently of one another (Chen *et al.*, 2004; Leu *et al.*, 2004). Some studies have shown that CCN1 mutants which disrupt its $\alpha 6\beta 1$ -HSPG abolish $\alpha 6\beta 1$ -HSPG-dependent activities while, $\alpha v\beta 3$ -mediated functions are not affected (Leu *et al.*, 2004). Furthermore, when CCN1 interaction with $\alpha v\beta 3$ is disrupted, this abolished $\alpha v\beta 3$ activity without affecting $\alpha 6\beta 1$ -mediated activities (Chen *et al.*, 2004). On the other hand, the cooperative interaction between CCN1 and TNF α is dependent on its interaction with both $\alpha v\beta 5$ and $\alpha 6\beta 1$ (Chen *et al.*, 2007). Thus, all these results suggest that CCN1 can independently bind specific integrins on the same cell and generate multiple signalling pathways (Lau, 2011). In addition to integrins and HSPG, CCN1 may interact with other growth factors such as bone morphogenetic proteins (BMP), transforming growth factor β (TGF- β) and vascular endothelial growth factor (VEGF) which need more thorough investigation (Lau, 2011).

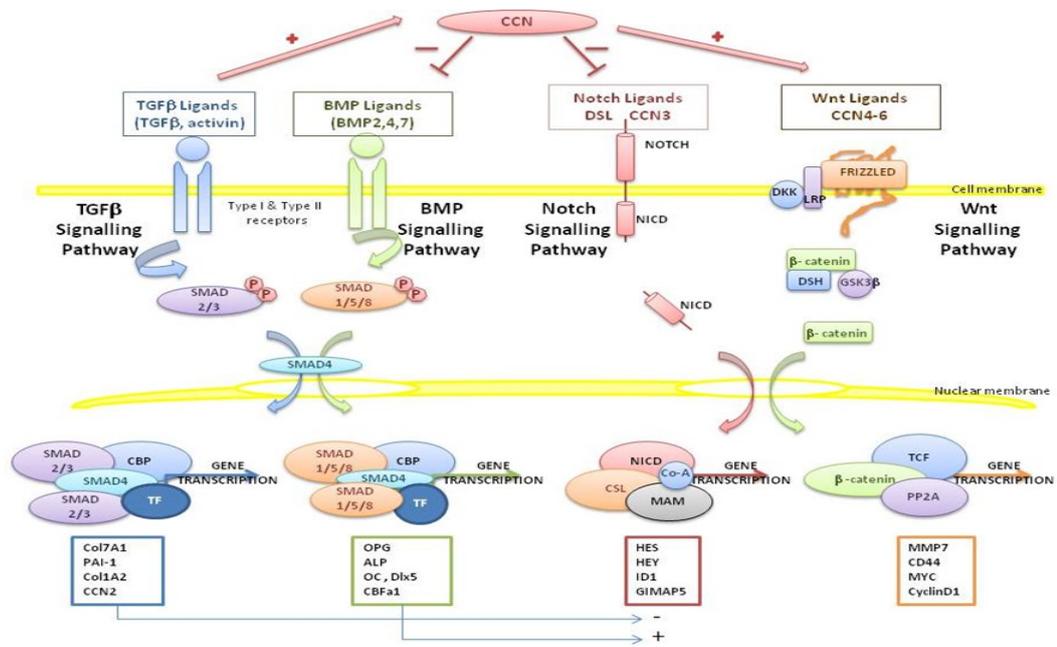
1.5.3 Roles of CCN1 proteins in cancer

Recently, Lau has described the role of CCN1 in cancer as “a double-edged sword” (Lau, 2011). CCN1 expression was altered in various cancers, depending on the cancer type, CCN1 may induce or suppress tumour growth (Feng, Wang & Ren, 2008; Holloway *et al.*, 2005). Consistent with these results, CCN1 plays unique roles in different cancers; it is a tumour promoter in cancers of the breast (Menendez *et al.*, 2005), prostate (Sun *et al.*, 2008), pancreatic (Maity *et al.*, 2014), gastric carcinogenesis (Cheng *et al.*, 2014), ovarian carcinoma (Gery *et al.*, 2005), colorectal (Jeong *et al.*, 2014), myeloma (Roodman, 2014) and acute myeloid leukaemia (AML) (Niu *et al.*, 2014) due to either overexpression of CCN1 or expression of truncated protein. Recent results have shown that full-length CCN proteins can play an anti-proliferative role, whilst CCN1 truncated proteins may induce tumour proliferation (Planque & Perbal, 2003). For example, CCN1 is cleaved by plasmin and releases a truncated protein of CCN1 (28kDa) which may support endothelial cell migration in breast cancer (Pendurthi *et al.*, 2005). This study also indicated that the truncated isoform has wider biological functions owing to the CCN1 partition into the soluble phase (28kDa) diffusing freely within tissue and may act as an antagonist towards the full-length form in the insoluble matrix (42kDa). In 2013, (Choi *et al.*) found CCN1 in the vitreous fluid secretome of proliferative diabetic retinopathy patients, these truncated proteins ranged from 11-23kDa rather than full-length protein 42kDa. Furthermore, a truncated protein of CCN1 which includes complete or partial length forms of the two-modules from IGFBP-VWC is generated by proteolytic cleavage by MMPs (Choi *et al.*, 2013). Additional to proteolytic activity generating various CCN1 truncated proteins in disease, a truncated isoform of CCN1 can also arise due to alternative mRNA splicing (Perbal, 2009). The gene structure of the CCN family of proteins comprises of five exons and four introns, however loss of one exon results in the production of a truncated CCN isoform, for example deletion of exon 4 leads to CCN1

lacking the TSP1 domain (Leng *et al.*, 2002; Zuo *et al.*, 2010). Paradoxically, CCN1 is a tumour suppressor in melanoma (Dobroff *et al.*, 2009), non-small cell lung cancer (Tong *et al.*, 2001) and endometrial adenocarcinoma (Chien *et al.*, 2004). The role of CCN1 in mantle cell lymphoma has not previously been investigated.

Increasing evidence suggests that CCN1 plays important roles in tumour development, including migration, survival, proliferation and metastasis (Sun *et al.*, 2008). In AML, CCN1 induces tumour survival through activation of the Ras/Raf/Mitogen-activated protein kinase/ERK kinase (MEK)/extracellular-signal-regulated kinase (ERK) (MEK/ERK pathway) by up regulating c-Myc and Bcl-xL (Bcl-2, Bcl-xL, and Mcl-1 are important inhibitors of apoptosis) and by down regulating Bax (apoptosis promoter) (Niu *et al.*, 2014). Similarly, CCN1 confers resistance to chemotherapeutic agent-induced apoptosis in human breast cancer and suppresses apoptosis by activating integrins and the NF- κ B/XIAP signalling pathway (Lin *et al.*, 2004). CCN1 has been found in sites of bone remodelling, involvement in enhancing osteoblast differentiation, whilst suppressing osteoclast formation (Crockett *et al.*, 2007). Furthermore, CCN1 has been implicated in Wnt3A-induced osteoblast differentiation of mesenchymal stem cells (Si *et al.*, 2006). In a preclinical model of myeloma, mesenchymal stromal cells in the multiple myeloma (MM) microenvironment produced CCN1 that has opposing function to myeloma cells which secrete and stimulate osteoclast activating factors (OAFs) and decrease osteoblast inhibitors (OBIs) (Roodman, 2014). Thus, high expression of CCN1 in the MM microenvironment can decrease MM tumour cell growth and osteoclast activity and promote osteoblast differentiation (Roodman, 2014) (Figure 1.9). However, CCN1 may enhance myeloma cell viability through supporting survival of the INA-6 myeloma cell line lacking interleukin-6 (IL-6) (Dotterweich *et al.*, 2014). Zoledronic acid (ZOL) an aminobisphosphonate and TGF- β inhibitor known to disrupt CCN1 signalling (Espinoza *et al.*, 2011), has a direct anti-tumour activity in skeletal complications secondary to bone

metastases including lymphoma, breast cancer, hormone-refractory prostate cancer, lung cancer and renal cell carcinoma (Lipton, Seaman & Zheng, 2004; Saad *et al.*, 2004; Westin *et al.*, 2010). In newly diagnosed lymphoma patients, treatment with ZOL blocks the bone mineral density loss that is commonly seen in lymphoma patients and causative to patient demise (Westin *et al.*, 2010). In triple negative breast carcinoma (TNBC) which has a high tendency for metastasis to bone, most patients receive ZOL as a companion to chemotherapy to reduce loss of bone density and reduce tumour spread (Espinoza *et al.*, 2011). Similarly, treatment of prostate cancer cells with ZOL decreased CCN1 expression which led to inhibition of proliferation (Marra *et al.*, 2009). Moreover, ZOL is considered a new therapeutic approach for cancer by targeting CCN1 overexpression on tumour cells through FOXO3a (forkhead box O3), suppressing bone resorption and blocking osteoclast activity. However, the mechanism of ZOL is not completely clear (Espinoza *et al.*, 2011).



CCN network regulation of Myelopoiesis, Lymphopoiesis: B & T-cell development (Notch, Hes, Hey), Osteogenesis / Chondrogenesis (ALP, OC), Adhesion / motility (MMPs, Collagens, Integrins, CD44).

Figure 1.9 CCN Stem cell signalling network. CCN1 is involved in regulating stem cell signalling cascades including TGFβ, BMP, Notch and Wnt-Beta catenin that are important in haematopoiesis including the B and T cell development lineages. Adapted from (McCallum & Irvine, 2009).

1.6 Cell cycle signalling

1.6.1 Stem cell signalling pathways

Stem cells are undifferentiated cells with the ability to self-renew and develop into other cell types during embryonic life or growth (Mishra, Derynck & Mishra, 2005). Embryonic stem cells (ES) and adult stem cells (also known somatic stem cells) are two kinds of stem cells. Human embryonic stem cells (hESCs) originated from the inner cell masses of a blastocyst. hESCs are considered pluripotent due to have the capacity to differentiate into cells from the three germ layers ectoderm, mesoderm and endoderm (Guo & Chen, 2015). Many studies have shown that the multiple signalling networks regulate the development and differentiation of ES and adult somatic stem cells into functional haematopoietic, neuronal, mesenchymal, and epithelial lineages (Mishra, Derynck & Mishra, 2005; Zhang & Crumpacker, 2015). The signalling pathways include

those of transforming growth factor beta (TGF- β), bone morphogenetic protein (BMP), Notch, and Wnt- β catenin. Aberrant expression of one of these signalling components leads to disruption within the complex co-ordination of signalling, resulting in carcinogenesis including that of the haematological malignancies (Harrison *et al.*, 2010; Rizzatti *et al.*, 2005; Watabe & Miyazono, 2009). Recently, many studies have focused on targeting of stem cell signalling pathways to provide more effective therapeutic strategies for different cancers.

1.6.1.a TGF- β family signalling

The Transforming growth factor β (TGF- β) signalling pathway and its ligands act as potent regulators of cellular processes such as proliferation, differentiation, migration and survival (Marino, Risbridger & Gold, 2015). It has essential roles in stem cell regulation by maintaining undifferentiated cells and initiating of differentiation (Mishra, Derynck & Mishra, 2005). The TGF- β superfamily includes TGF- β s, bone morphogenetic proteins (BMPs), growth and differentiation factors (GDFs), Mullerian inhibitory factor (MIF), nodal, activins, and inhibins (Krstic, Maslovaric & F Santibanez, 2014; Santibañez, Quintanilla & Bernabeu, 2011). TGF- β mediates downstream intracellular signalling by the Smad family of proteins which functionally subdivided into three groups: the receptor regulated Smads (R-Smad), which contain Smad 1, 2, 3, 5, 8; the common mediator Smad (Co-Smad), Smad4; and the inhibitory Smads (I-Smad), Smad6 and 7 (Heldin, Miyazono & Ten Dijke, 1997). TGF- β signalling is initiated by the three types of TGF β receptor (TGF β R); TGF β I, II and III. To date, seven TGF β RI, five TGF β RII and two TGF β RIII are known. TGF β RI include activin receptor-like kinase 1-7 (ALK1-7). TGF β RIIs contain the TGF β RII, bone morphogenetic protein receptor II (BMPRII), activin receptor II (ACTRII), while betaglycan and endoglin belong to the TGF β RIIIs and predominantly

acting as co-receptors to promote activin signalling (Bierie & Moses, 2006; Lewis *et al.*, 2000). The mechanism of TGF- β signalling starts by binding of ligands to two (type II) receptors and two (type I) receptors leading to form a stable receptor complex which consist of two receptors of each type. Binding of TGF- β ligands to TGF β RII leads to phosphorylation of the TGF β RI. Consequently, the activated TGF β RI phosphorylated Smad 2, 3 and bind with Smad 4 to make complex that accumulation in the nucleus to regulate the expression of target genes (Figure 1.10) (Derynck & Zhang, 2003). TGF- β signalling is essential in regulating tumorigenesis in human cancer; in early stage disease its acts as a tumour inhibitor through proliferation suppression but in advanced disease, it is often a tumour promoter through regulation of migration and invasion (Dai *et al.*, 2012; Derynck, Akhurst & Balmain, 2001; Roberts & Wakefield, 2003). Recently, it has seen that ALK5 suppression prevents TGF- β -induced CCN1 expression in human dermal fibroblasts (Thompson, Murphy-Marshman & Leask, 2014). More importantly, CCN1 is a transcriptional target of TGF- β and may potentiate an autocrine regulatory mechanism in tumorigenesis (Bartholin *et al.*, 2007). Further investigations of the relationship between CCN1 and TGF- β protein are required.

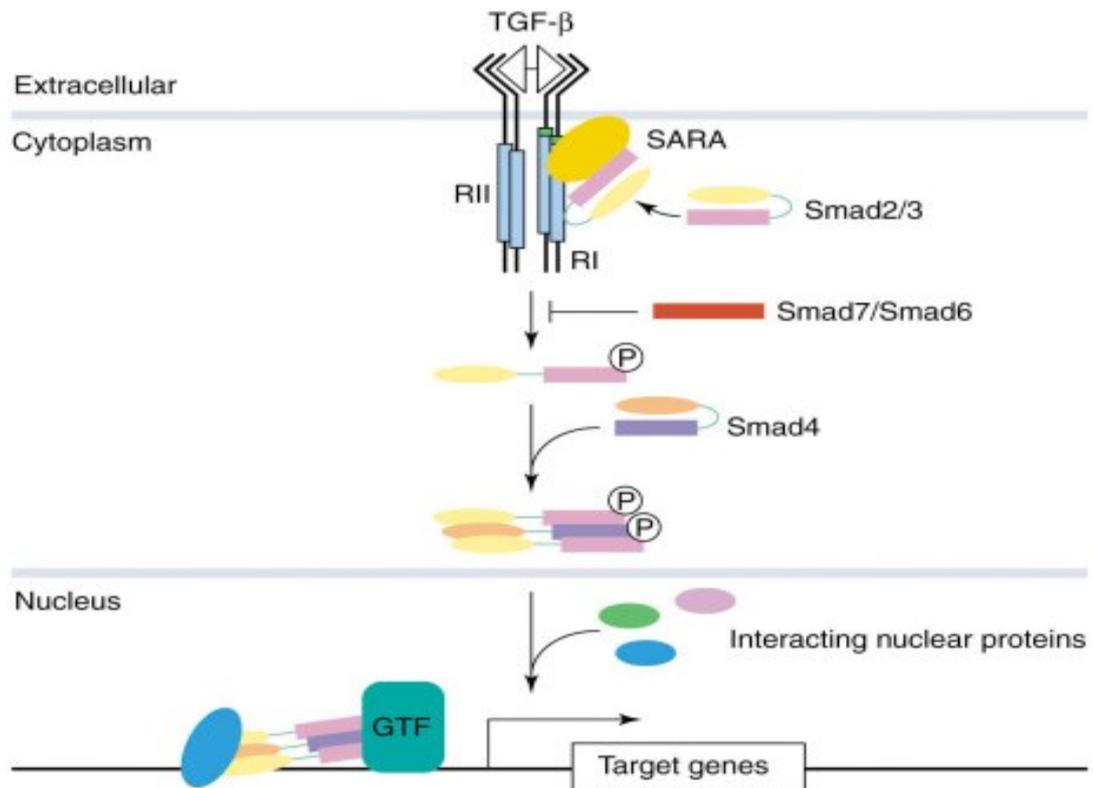


Figure 1.10 Schematic diagram illustrates the TGF β signalling pathway. Binding of TGF- β ligands to TGF β RII at the cell surface leads to phosphorylation of the TGF β RI by TGF β RII. Consequently, the activated TGF β RI phosphorylates Smad 2, 3 and binding with Smad 4 to make a complex which accumulates in the nucleus interacts with general transcription factors (GTF) and mediates transcriptional activation of genes. The inhibitory Smads (I-Smad), Smad6 and 7 inhibit R-Smad that is activated by TGF β RI in the cytoplasm. Image taken from (Akhurst & Derynck, 2001).

1.6.1.b BMP signalling

Bone Morphogenetic Proteins (BMPs) are secreted extracellular signalling molecules belonging to the TGF- β superfamily (Sartori & Sandri, 2015). BMPs are initially identified by their ability to stimulate endochondral bone formation (Chen *et al.*, 2014). Recently, more than 20 subgroups of BMPs have identified in the human body in a wide range of biological functions such as embryogenesis, skeletal formation, haematopoiesis and neurogenesis (Yang *et al.*, 2014). BMPs act an unique role in the formation of bone tissue through stimulating differentiation of bone marrow mesenchymal stem cells into osteoblastic lineage and then promoting the proliferation of osteoblasts and chondrocytes

(Yang *et al.*, 2014). Furthermore, they play important roles in regulation of variance of cellular responses including cell proliferation, differentiation, adhesion, migration and apoptosis (David, Feige & Bailly, 2009; Feng & Derynck, 2005; Wagner *et al.*, 2010). BMP pathways are activated by either a Smad-dependent pathway (canonical) or Smad-independent mitogen activated protein kinase (MAPK) pathway depending on signal transduction (Sánchez-Duffhues *et al.*, 2015; Yang *et al.*, 2014). In canonical Smad-dependent signalling, BMPs ligands bind BMPRI and BMPRII resulting in the activation of BMPRII which subsequently phosphorylates and activates of BMPRI (Miyazono, Kamiya & Morikawa, 2010). The activated BMPRI in the cytoplasm binds to R-Smad 1, 5, and Smad 8/9 that mediate BMPs signalling (Peng *et al.*, 2016). This complex leads to phosphorylate R-Smad 1, 5, 8/9, which in turn, complexes with co-Smad 4 enabling transduction into the nucleus where the complex collaborates with other transcription factors and accessory components to regulate the transcription of the target genes (Miyazono, Maeda & Imamura, 2005). While the non-canonical Smad-independent MAPK pathway, after binding of BMPs with either BMPRI or BMPRII, this complex activates BMPs-MAPKs signalling pathway which in turn migrate into the nucleus via JNK-1 and 2/3, ERK1/2, NF-kB and p38 signalling pathways to regulate the expression of target genes (Figure 1.11) (Yang *et al.*, 2014). On the other hand, the aberrant expression of BMP members have been involved in the progression of various cancers including breast cancer (Davies *et al.*, 2008), prostate carcinoma (Si, Feng & Yang, 2010), colon cancer (Kim *et al.*, 2015), lung squamous cell carcinoma and adenocarcinoma (Deng *et al.*, 2015). For example, the high expression of BMP2, BMP4 and BMP7 has found in gastric cancer associated with poor prognosis (Aoki *et al.*, 2011; Katoh & Terada, 1996; Park *et al.*, 2008). Clinical trials show various attempts to treat cancers by targeting the BMP signalling pathways (Peng *et al.*, 2016). However, the role of BMPs in MCL remains unknown.

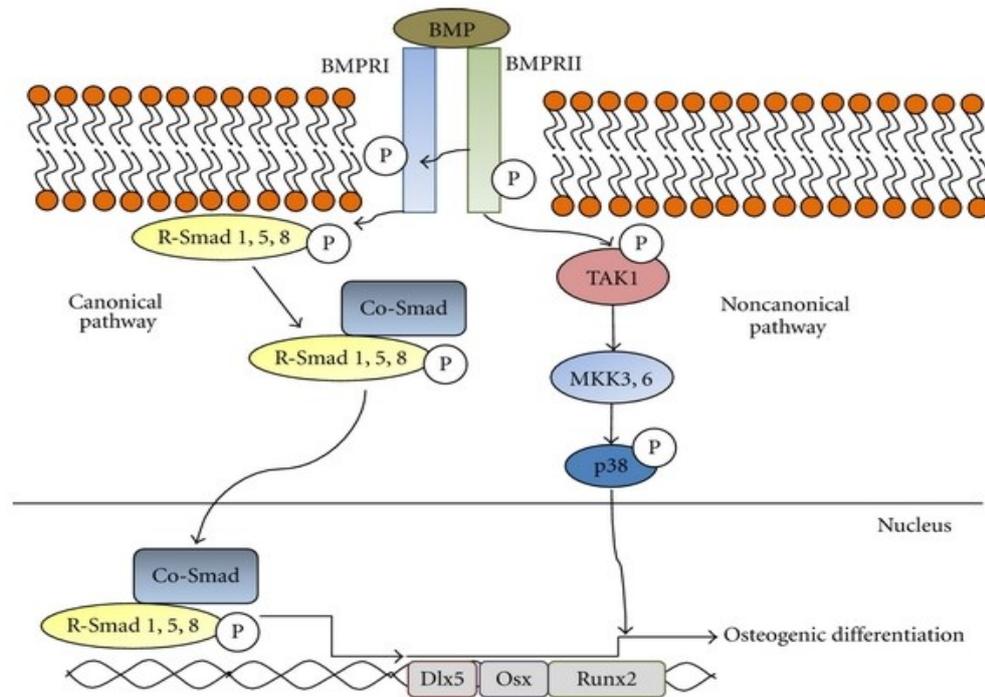


Figure 1.11 Schematic diagram overview of canonical Smad-dependent and Smad-independent BMP signalling. In canonical Smad-dependent signalling, BMPs ligands bind BMPRI and BMPRII resulting in the phosphorylation and activation of BMPRI that binds and activates R-Smad 1, 5, and Smad 8 which in turn complex with co-Smad 4 and transduce into the nucleus and mediate transcriptional activation of osteogenic genes Dlx5, Osterix and Runx2. While in the Smad-independent pathway, after binding of BMP ligand with BMP receptor, this complex phosphorylates TGF- β activation kinase (TAK1) leading to activation of the BMPs-MAPKs signalling pathway which in turn migrate into the nucleus via p38 MAP signalling to regulate the expression of Runx2. Image taken from (Chenard *et al.*, 2012).

1.6.1.c Notch signalling

Notch signalling is implicated in different cellular functions including cell fate specification, differentiation, proliferation and apoptosis (De Falco *et al.*, 2015). The notch family comprises of four notch receptors (notch 1-4) which consist of extracellular and intracellular domains. The extracellular domain (ECD) contains 29-36 epidermal growth factor (EGF) like repeats and three Lin-notch repeats (LNR). The intracellular domain (ICD) contains the RBP-J-associated molecule (RAM) domain, six ankyrin repeats (ANK), nuclear localization sequences (NLS), a transactivation domain (TAD)

and a proline-, glutamate-, serine- and threonine-rich sequence (PEST). The (TAD) is necessary to activate transcription while (PEST) is responsible to regulate notch degradation. Notch3 and notch4 are considered smaller proteins due to having fewer EGF repeats and absence of TAD. On the other hand, notch ligands consists of five canonical ligands belonging to Delta (Delta like 1, 3, and 4) and Jagged (Jag 1 and 2) which also having multiple EGF-like repeats and cysteine rich sequences known as the Delta-Serrate-Lag2 (DSL) motif (D'souza, Miyamoto & Weinmaster, 2008). Recently, many groups have shown that the notch activation can be performed by non-canonical ligands such as CCN3 (Suresh *et al.*, 2013) or the Wnt pathway (Andersen *et al.*, 2012) which signal independent of CSL transcription factors.

Notch signalling starts by ligands binding to the extracellular domain of notch receptors (NECD), this interaction allows series proteolytic cleavages of the receptors which termed S1, S2, S3, and S4. S1 is mediated by furin proteases (in the Golgi apparatus) and S2 is mediated by ADAM metalloproteases (anchored to the plasma membrane) (Brou *et al.*, 2000; Suresh & Irvine, 2015). Moreover, S3/S4 cleavage is mediated by the presenilin-dependent γ -secretase (De Strooper *et al.*, 1999; Okochi *et al.*, 2002). These proteolytic cleavages result in liberation and translocation of the intracellular domain of the notch receptor (NICD) to the nucleus, that in turn, binds to the DNA transcription factors and activates subsequent expression of Notch target genes (Figure 1.12) (Kovall, 2008). Recently, increasing evidence has demonstrated that abnormal notch signalling is implicated in tumour progression (Yuan *et al.*, 2015) including MCL. In 2012, (Kridel *et al.*) demonstrated that the 12% of cases and 20% of MCL cell lines had a notch mutation in the PEST domain. They also demonstrated that suppression of proliferation and promotion of apoptosis can be achieved by inhibiting the notch signalling pathway. Similarly, in colorectal cancer (CRC), reduced cell viability and cell cycle arrest has achieved by down-regulation of Jagged 1 which led to reduced expression of cyclin D1,

cyclin E, and c-Myc (Dai *et al.*, 2014). Furthermore, notch1 and notch2 signalling is involved in the resistance to apoptosis in chronic lymphocytic leukemia (CLL) (Rosati *et al.*, 2013; Rosati *et al.*, 2009). In cancer, it has been clear that the notch signalling blocks apoptosis by cell cycle and survival pathways such as the inhibition of p53 (Beverly, Felsher & Capobianco, 2005). Notch enhances the activation of the pro-survival PI3K/AKT pathway (Palomero, Dominguez & Ferrando, 2008) and notch suppresses the pro-apoptotic proteins Bax, Bim, and Noxa (Konishi *et al.*, 2010; Rizzo *et al.*, 2008) and increases the expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL (Konishi *et al.*, 2010). Overall, dysregulation of notch signalling or notch mutations have been involved in many cancers including MCL. Targeting this pathway may be beneficial as a novel therapeutic strategy but would need more investigation.

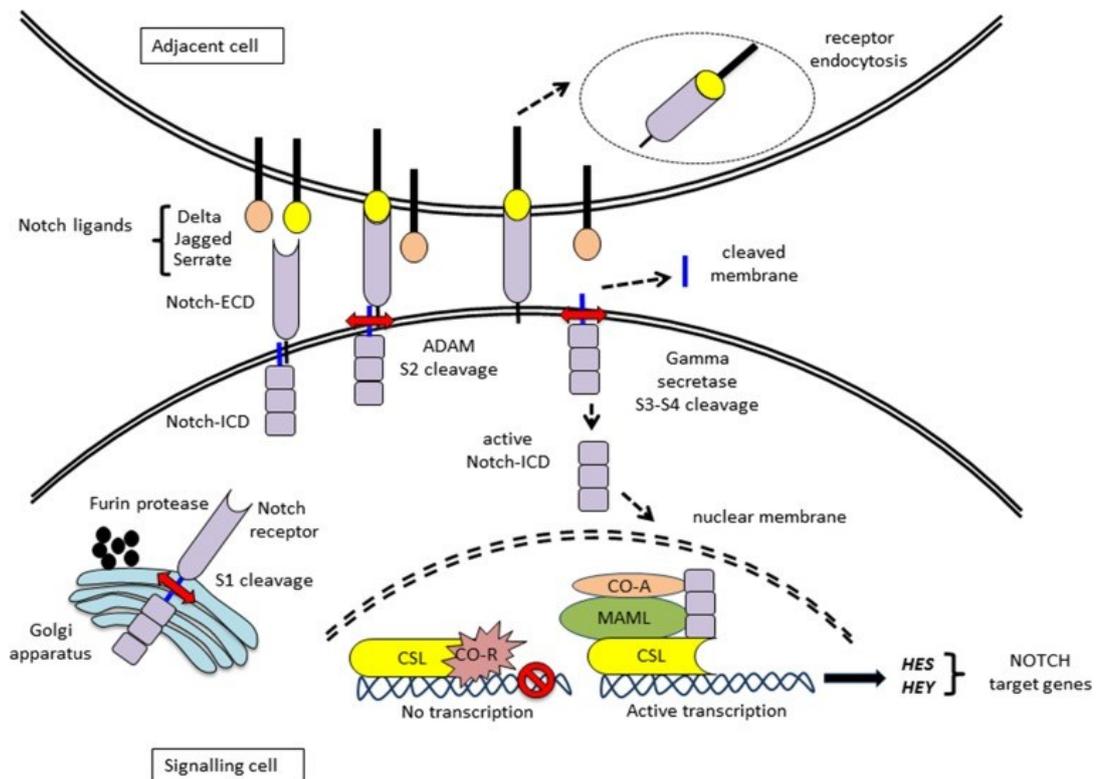


Figure 1.12 Schematic overview of the notch signalling pathway. The Notch signalling pathway starts in the Golgi apparatus when the notch receptor starts proteolytic cleavage 1 (S1) mediated by Furin proteases before the receptor can be released to the membrane. Notch ligands, Delta, Jagged and Serrate produced by neighbouring cells bind with notch-ECD leading to stimulate the S2 cleavage by ADAM metalloproteases. Once activated S3/S4 cleavage is mediated by the presenilin-dependent γ -secretase resulting in liberation and translocation of the notch intracellular domain (NICD) into the nucleus, binding to CSL protein resulting in the activation of complex with MAML and subsequent expression of Notch target genes including HES and HEY. Image taken from (Suresh & Irvine, 2015).

1.6.1.d Wnt- β catenin signalling

Wnt proteins are secreted glycoproteins that act as growth factors by regulating different cellular functions including cell proliferation, differentiation, migration and cell division (Willert *et al.*, 2003). In mammals, Wnts comprise a large family of cysteine rich secreted protein ligands that play essential roles in developmental and physiological processes (Kato, 2002; Logan & Nusse, 2004). The Wnt signalling mechanisms are subdivided into canonical pathway (cell fate determination) or via the non-canonical pathway

(control of cell movement and tissue polarity) (Sharma *et al.*, 2015). For the canonical pathway, Wnt ligands bind with seven pass transmembrane receptors of the Frizzled (Fzd) family (Vinson, Conover & Adler, 1989) and with single pass transmembrane co-receptors lipoprotein-receptor-related protein5/6 (LPR5/6) leading to activation of the β -catenin signalling cascade (Pinson *et al.*, 2000). In the lack of Wnt signalling, ubiquitination and degradation of β -catenin is conducted through phosphorylation of β -catenin interaction with GSK-3 β and axin-1 (Aberle *et al.*, 1997). Activation of the Wnt pathway blocks β -catenin phosphorylation-induced degradation, and accumulation of β -catenin in the nucleus, where it forms active transcription complexes with the T cell factor/lymphoid enhancer binding factor (TCF/LEF) family of DNA-binding transcription factors (Behrens *et al.*, 1996; Polakis, 2012). In the non-canonical pathway, Wnt ligands bind and complex with the receptors of FZD family and receptor tyrosine kinase-like orphan receptor 2/receptor tyrosine kinase (ROR2/RYK) (Oishi *et al.*, 2003). Additionally, Dickkopf (DKK) is considered another class of proteins family that interacts with Wnt receptor complex (Sharma *et al.*, 2015) and WISP-1/CCN4, WISP-2/CCN5, WISP-3/CCN6 are Wnt ligands that activate β -catenin signalling (Stephens *et al.*, 2015). Activation of canonical and non-canonical Wnt signalling pathways lead to expression of Wnt induced target genes (Figure 1.13). For example, Wnt proteins have essential roles in different types of stem cells including neural, mammary and embryonic stem cells (Nusse *et al.*, 2008). Recently, emerging evidence has shown that Wnt signalling has an important role in cancer stem cells (Holland *et al.*, 2013; Malanchi *et al.*, 2008). Cancer stem cells and haematopoietic stem cells have Wnt and Notch signalling pathways, which are essential for their growth and self-renewal (Holland *et al.*, 2013; Reya *et al.*, 2001). Dysregulation of Wnt signalling enhances tumourigenesis (Grady & Markowitz, 2002; Salahshor & Woodgett, 2005). Recently, many studies have shown that dysregulation of Wnt signalling pathway leads to lymphomagenesis of MCL

through maintenance and survival of MCL-initiating cells (MCL-ICs) (Kimura *et al.*, 2013; Mathur *et al.*, 2015). Similarly, Wnt increases chemotherapy resistance of high-grade serous ovarian cancer (HGSOC) by activation of Wnt/ β -catenin signalling pathway (Nagaraj *et al.*, 2015). Furthermore, inhibition of this pathway in ovarian CICs leads to eliminate cancer initiating cells (CIC) and sensitize cells to platinum-based therapies. Also, this study referred to using iCG-001, an inhibitor of Wnt/ β -catenin signalling pathway, “sensitized cells to cisplatin and decreased stem-cell frequency in platinum resistant cells” (Nagaraj *et al.*, 2015) .

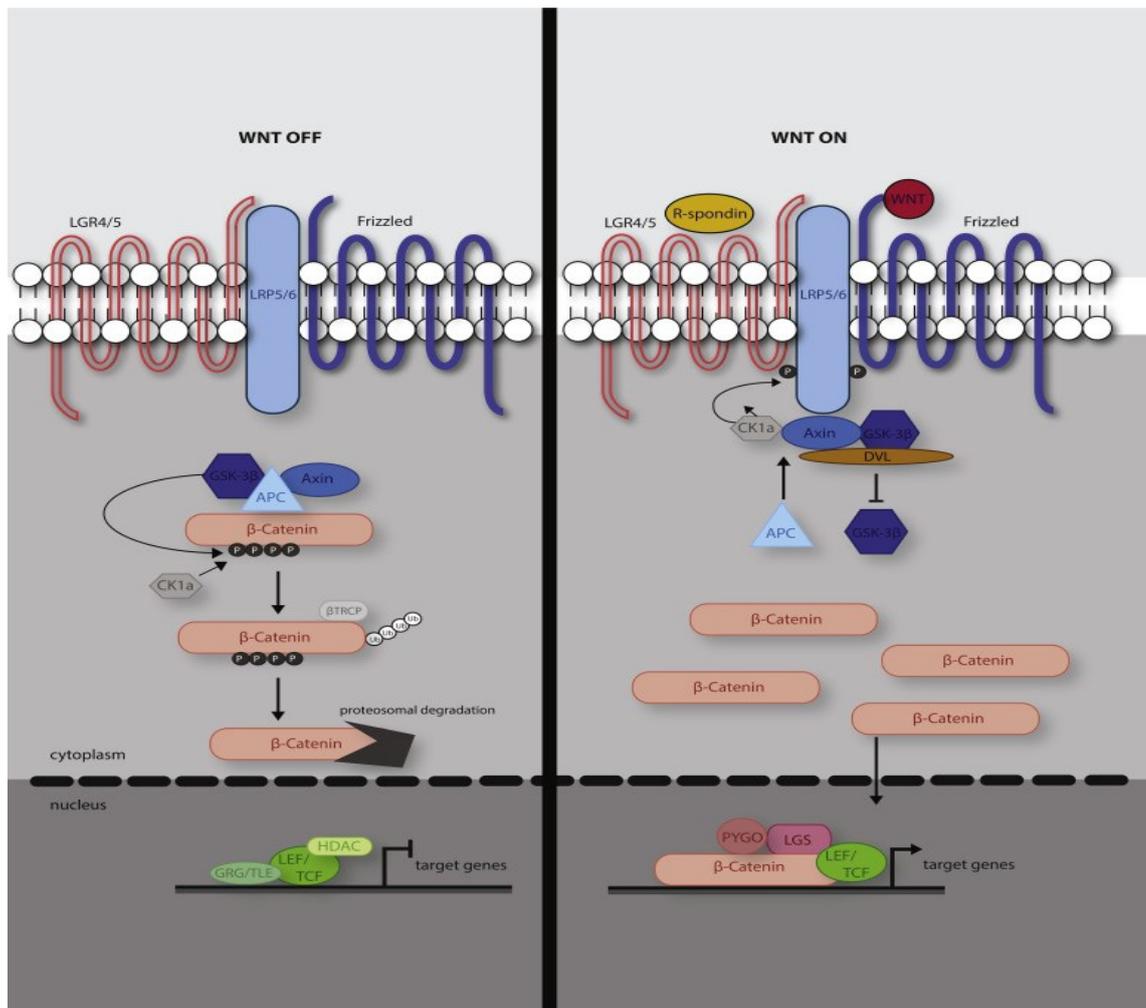


Figure 1.13 Schematic overview of canonical WNT signalling pathway. To activate transduction signalling for the canonical pathway, Wnt ligands bind with Frizzled (Fzd) receptor and with single pass transmembrane co-receptors lipoprotein-receptor-related protein5/6 (LPR5/6) leading to activate β -catenin signalling cascade (Right). In the lack of Wnt signalling, ubiquitination and degradation of β -catenin is conducted through phosphorylation of β -catenin and interaction with GSK-3 β and axin-1 (Left). Activation of the Wnt pathway blocks β -catenin phosphorylation-induced degradation, and accumulation of β -catenin in the nucleus, where it forms active transcription complexes with the TCF/LEF family of DNA-binding transcription factors. Image taken from (Staal, Chhatta & Mikkers, 2016).

1.6.2 Cell cycle

The cell cycle of eukaryotic cells comprises of four sequential phases; two gap phases, G_1 and G_2 , DNA synthesis phase (S phase) which comprising the replication of DNA in the nucleus and mitosis phase (M phase) which comprising the division of nucleus and cytoplasm (Zetterberg, Larsson & Wiman, 1995). The transition from one phase to the

next occurs in a precise consecutive pattern. It is controlled by cooperation of cyclins and cyclin dependent kinases (CDK's) which are found at G₁ and G₂ checkpoints (Bonelli *et al.*, 2014). Cell cycle progression is halted by cyclin dependent kinase inhibitors (CDKI's) in response to DNA damage, low levels of oxygen or nutrients Figure 1.14 (Zhang & Yan, 2012).

Several diseases and also tumorigenesis are associated with dysregulation of cell cycle checkpoints. In cancer, the cell cycle is dysregulated leading to loss differentiation and abnormal cell growth. Recently, more than 90% of cancers have been identified by oncogenic alteration of CDKs, and CDKIs which are frequently linked to the G₁ phase (Bonelli *et al.*, 2014). The molecular mechanisms which may be associated are gene overexpression, chromosomal translocation, point mutations, insertions and deletions, missense and frame shift mutation, splicing, or methylation. Moreover, the dysregulation of cell cycle associated with tumour relapse and treatment resistance is conferred by increasing proliferation. Recently, attention has focused on the restoration of cell cycle by therapeutic agents that act by modulating molecular targets in the cell (Bonelli *et al.*, 2014).

1.6.2.a Cyclin D1 and cell cycle progression

Overexpression of cyclin D1 (CCND1) as a result of t(11;14) chromosomal translocation is the hallmark feature of MCL (Cassaday *et al.*, 2015). Cyclin D1 plays a central role in the cell cycle regulation by binding to either cyclin-dependent kinase 4 (CDK4) or CDK6. The CCND1-CDK4 or CCND1-CDK6 complex phosphorylates the retinoblastoma protein (pRb) which leads to degradation of CCND1 suppressor effect on cell cycle progression. This process leads to release of the E2F family of transcription factors and then S-phase entry (Cassaday *et al.*, 2015). E2F transcription factor regulates genes that

encode for DNA replication and cell cycle control (Figure 1.14) (Nevins, 2001). In G₁ phase, there are some hormones, growth factors, and cytokines that regulate cell cycle. For example, CALMODULIN (CaM) which is an intracellular Ca²⁺ receptor protein regulates cell cycle by nuclear translocation of CDK4 and cyclin D1, activation of CDK4 and phosphorylation of pRb (Berchtold & Villalobo, 2014; Taulés *et al.*, 1999).

In MCL, CCND1 overexpression leads to hyperphosphorylation of pRb and accumulation of E2F which facilitates the G₁/S transition and uncontrolled cell proliferation (Cassaday *et al.*, 2015). CCND1 is upregulated in almost all MCL patients, however cyclin D1 alone is insufficient to promote MCL. Additional oncogenic aberrations have been implicated in the generation of MCL, including, c-Myc overexpression, lack of the ataxia telangiectasia mutated (ATM) gene or p53 dysregulation (Müller *et al.*, 2013). Furthermore, many secondary genetic events are involved in MCL lymphomagenesis and include inactivation of the DNA damage response pathways, activation of cell-survival pathways and suppression of apoptosis. Additional oncogenic aberrations are likely to contribute to the development of MCL involving cell proliferation, survival, and interactions with the microenvironment (Jares, Colomer & Campo, 2012; Müller *et al.*, 2013). Recently, stem cell signalling mutations have been identified in MCL that may contribute to the development or pathology of this disease. For example, Notch1 mutations are found in 12% of MCL and are associated with poor survival; suppression of the Notch pathway in MCL decreased cell proliferation and increased apoptosis (Kridel *et al.*, 2012). Similarly, the deregulation of the Wnt canonical pathway was found in MCL by inactivation of phospho-GSK3B which suggests that the Wnt pathway may also contribute to the pathogenesis of MCL (Gelebart *et al.*, 2008). However, these observations need further investigation toward developing novel, effective therapeutic agents for MCL.

In addition to overexpression of cyclin D1 by chromosomal translocation, additional mechanisms that lead to increased expression of cyclin D1 have also been observed in MCL (Jares, Colomer & Campo, 2012). These mechanisms include aberrant expression of PI3-K/AKT/mTOR signalling pathway which is implicated in tumour progression and responsible in part, to increased cyclin D1 expression in MCL (Rini, 2008). Moreover, cyclin D1 overexpression correlates with poor prognosis and mirrors the fact that prognosis of patients with MCL is the poorest of all B-cell lymphoma patients (Ghielmini & Zucca, 2009).

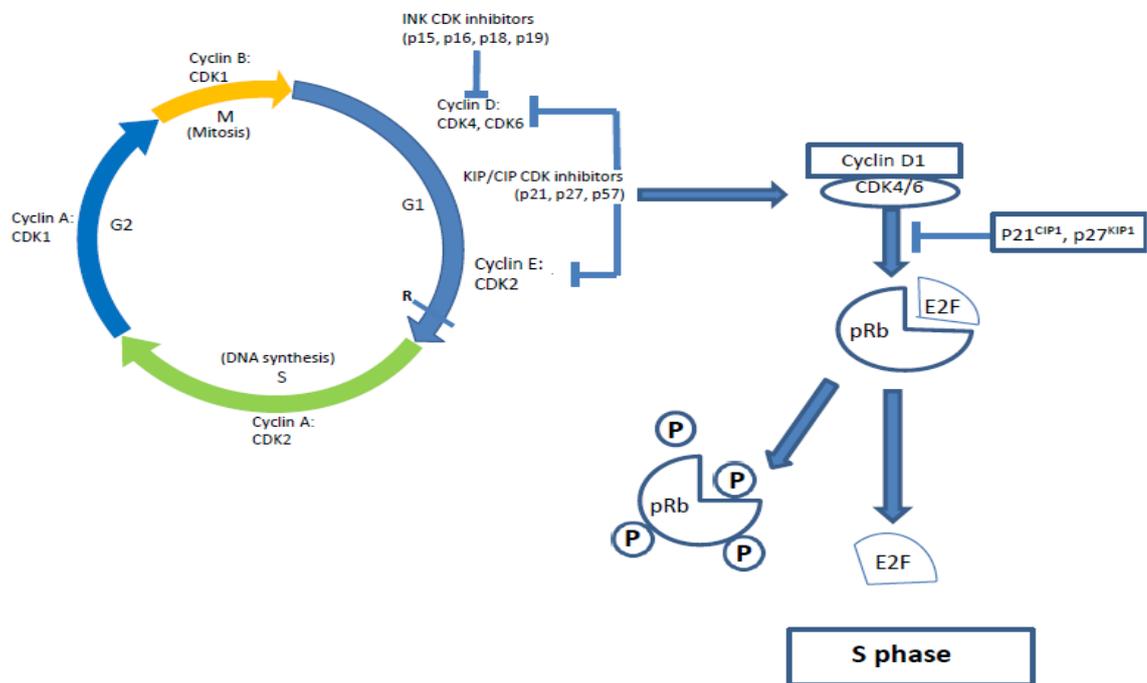


Figure 1.14 Cell cycle. Cyclin D1 is involved in promotion and progression of G₁ phase. p21 and p27 are cyclin-dependent kinase inhibitors (CDKI's) responsible for blocking G₁ phase progression. The Restriction checkpoint (R) is located at the end of the G₁ phase is responsible for assessing the integrity of the DNA. Updated from (Dehay & Kennedy, 2007) and (Zhang & Yan, 2012).

1.6.2.b P21

P21^{CIP1} (p21) belongs to the CIP/KIP family of proteins that regulate cell cycle progression. The CIP/KIP family comprises three members; p21^{CIP1} (Cdk Interacting

Protein 1), p27^{KIP1} (Kinase Inhibitory Protein 1) and p57^{KIP2} (Kinase Inhibitory Protein 2) (Bretones, Delgado & León, 2015). They bind and inhibit most cyclin-CDK complexes. The CIP/KIP family of proteins, particularly p21, plays an essential role in cell cycle control, halting the transition from G₁ phase to S phase (Pérez-Sayáns *et al.*, 2013) (Figure 1.14).

There are two different pathways to regulate p21, through (i) a p53-dependent pathway in response to DNA damage which activates p53 leading to upregulation of p21 and repression of cell growth in G₁ phase with potential DNA repair or stimulation of programmed cell death or (ii) a p53-independent pathway, in which cellular growth factors regulate p21 expression (Brennan *et al.*, 2002; Ciccarelli *et al.*, 2005). P21 binds to CCND-CDK4 or -CDK6 complex and inhibits the kinase activity of CDKs in response to many stimuli leading to regulation G₁/S progression, at a specific stage named the restriction point. This process leads to repression of phosphorylation of pRb protein which in turn prevents the expression of E2F transcription factor and blocks G₁/S transition (Figure 1.14) (Zhang & Yan, 2012). In addition to negative regulation of the cell cycle, p21 regulates gene transcription; for example, p21 suppresses E2F transcription factor through a pathway independent of CDKs or Rb (Perkins, 2002). P21 is involved in controlling cellular growth by suppressing of E2F1 transcription factor via *Wnt4* expression through Notch 1 activation (Devgan *et al.*, 2005). However, p21 stimulates NF κ B-mediated transcription by activation of transcriptional cofactors/deacetylases p300 and CBP (Perkins, 2002). Moreover, p21 is up-regulated in response to DNA damage which correlates with cell growth arrest and senescence (Chang *et al.*, 2000). Many studies have identified additional roles for p21, in cancer it is a tumour suppressor through cell cycle arrest and blocking DNA synthesis by binding to proliferating cell nuclear antigen (PCNA) (Abbas & Dutta, 2009 ; Ando *et al.*, 2001 ; Waga *et al.*, 1994). P21 can be observed as an oncogenic factor, where various functions

including enhanced proliferation and delocalisation of p21 from the nucleus are involved in promoting carcinogenesis and tumour development (Gartel, 2009; Roninson, 2002). Furthermore, the expression of p21 varies in different cancers, it is down regulated in small-cell lung (Komiya *et al.*, 1997), colorectal (Zirbes *et al.*, 2000), cervical (Lu *et al.*, 1998a), and head and neck cancers (Kapranos *et al.*, 2000) that associated with tumour progression. In contrast, it is upregulated in prostate (Baretton *et al.*, 1999), ovarian (Ferrandina *et al.*, 2000), breast, oesophageal squamous cell carcinomas, and in brain tumours (Roninson, 2002). For example, in patients with breast cancer, high expression of p21 is associated with disease progression, lymph node metastasis and short survival (Wei *et al.*, 2015).

The function of p21 in different cell types depends on localization, either in the nucleus or the cytoplasm. However p21 regulates cell proliferation and differentiation by localizing in the nucleus (in all normal cells except monocytes). In various cancers and in monocytes, it inhibits proteins essential for apoptosis and therefore enhances tumourigenesis by localisation to the cytoplasm (de Renty, DePamphilis & Ullah, 2014). Recently, it has been evident that p21 expression is entirely nuclear in some cancers which are associated with high proliferation rates, for example, oral squamous cell carcinoma (OSCC) (Nemes, Nemes & Márton, 2005; Queiroz *et al.*, 2010). Expression of p21^{CIP1} has not yet been investigated fully in MCL.

1.6.2.c P27

As mentioned above, p27^{KIP1} (p27) is one of the CIP/KIP family of cyclin dependent kinase inhibitors (CDKIs). Initially p27 and other members of CIP/KIP family have been identified as cell cycle inhibitors leading to growth inhibition. In G₁, p27 binds to CCNE-CDK2 complex and inhibits the catalytic activity of CDK2 resulting in cell cycle arrest

at the restriction point in response to DNA damage or anti-mitogenic signals (Hirama & Koeffler, 1995). As a result, this prevents the phosphorylation of pRb which leads to block in the transcription of genes required for G₁/S progression (Figure 1.14) (Toyoshima & Hunter, 1994). Beyond the G₁ restriction point in cell cycle, cell cycle progression can continue independent of mitogenic signals (Coats *et al.*, 1996). Interestingly, p27^{KIP1} and p21^{CIP1} have important roles in promotion of the assembly of CCND-CDK4/6 complexes (LaBaer *et al.*, 1997). This interaction leads to sequestration of p27 in CCND-CDK4 complex which blocks inhibition of the CCNE-CDK2 complex (Perez-Roger *et al.*, 1999). Furthermore, binding of p27^{KIP1} with CCND-CDK4 complex suppresses the kinase activity of CDK4 (Ray *et al.*, 2009). The regulation of p27^{KIP1} has been found primarily governed by transcriptional and translational mechanisms (Hengst & Reed, 1996). Many transcriptional factors such as FKHR-L1, AFX, FOXO, SPI, E2F1 and BRCA1 have been observed in promotion of its transcription (Andrés *et al.*, 2001; Dijkers *et al.*, 2000; Medema *et al.*, 2000; Stahl *et al.*, 2002; Wang *et al.*, 2008; Williamson, Dadmanesh & Koeffler, 2002). Simultaneously, c-myc, Id3 and Ap-1 have been observed to inhibit the transcription of p27^{KIP1} (Chassot *et al.*, 2007; Garrett-Engele *et al.*, 2007b; Khattar & Kumar, 2010; Yang *et al.*, 2001). Moreover, p27^{KIP1} degradation has emerged to control p27 expression (Pagano *et al.*, 1995). For example, degradation of p27^{KIP1} by CCNE-CDK2 complex which mediated phosphorylation of p27^{KIP1} in Thr187 makes it a target to the ubiquitin-proteasome pathway by the ubiquitin ligase F-box protein SKp2 (Lu & Hunter, 2010). However, there is no evidence to link involvement of activated CCND-CDK4 complex in the degradation of p27^{KIP1} (Sheaff *et al.*, 1997; Sherr & Roberts, 1999).

In cancer, loss of cell growth regulation and enhanced proliferation are often involved in aberrant expression of the cell cycle regulators which control the transition from G₁ to S phase of the cell cycle. P27^{KIP1} which is one of these cell cycle regulators, in cancers, is aberrantly expressed by transcriptional and post-transcriptional mechanisms, genetic

alterations of p27^{KIP1} are rare (Chu, Hengst & Slingerland, 2008; Garrett-Engele *et al.*, 2007b). Similarly, the expression of p27^{KIP1} is reduced in several types of cancer that are associated with poor prognosis including breast, prostate, lung, and colon cancer (He *et al.*, 2012; Hershko, 2008; Shapira *et al.*, 2005; Tian *et al.*, 2013; Timmerbeul *et al.*, 2006). However, p27^{KIP1} is overexpressed in hepatocellular carcinoma (HCC) which is associated with longer disease free survival (Qin & Ng, 2001). In MCL, Quintanilla-Martinez *et al.* (2003) suggested that overexpression of cyclin D1 contributed to a buffer change in p27^{KIP1} levels leading to inhibition of cellular growth. Furthermore, the role of p27^{KIP1} in the pathogenesis of MCL remains controversial.

In addition of cell cycle regulation, there is some evidence that p27^{KIP1} has important roles in apoptosis, transcriptional activation, and migration depending on its localization. In the nucleus, it has an essential role to inhibit cell growth and is considered as a tumour suppressor. However, phosphorylation of specific sites of p27^{KIP1} lead to its export into the cytoplasm where it can act as a tumour promotor (Besson, Dowdy & Roberts, 2008). Recently, many studies have shown that the cytoplasmic p27^{KIP1} is involved in many cancers including melanoma, ovarian carcinoma, renal cell carcinoma, osteosarcoma, acute myelogenous leukemia and breast cancer leading to cell migration, high tumour grade, poor prognosis, survival and metastasis (Chen *et al.*, 2011; Denicourt *et al.*, 2007; Duncan *et al.*, 2010; Liang *et al.*, 2002; Min *et al.*, 2004; Rosen *et al.*, 2005). Moreover, cytoplasmic p27^{KIP1} has been contributed to treatment resistance by suppressing apoptosis in Her2+ breast cancer cells. However, the mechanism of p27^{KIP1} in oncogenesis is not fully understood (Zhao *et al.*, 2014).

1.6.2.d p57

P57^{KIP2} together with p21 and p27 are members of CIP/KIP family of cyclin dependent kinase inhibitor proteins that regulate cell cycle progression by binding and inhibiting cyclin-CDK complexes and halting the transition from G₁ phase to S phase (Figure 1.14) (Vidal & Koff, 2000). p57 is considered a tumour suppressor protein. However, in different types of human cancers, p57 has been inactivated resulting in implication in promoting cancer cell growth instead of cancer cell suppression (Vlachos *et al.*, 2007). Moreover, down regulation of p57^{KIP2} in different tumours involved in tumorigenesis (Hatada *et al.*, 1996; Kobatake *et al.*, 2004; Li *et al.*, 2003; Nakai *et al.*, 2002; Pateras *et al.*, 2006; Shin *et al.*, 2000; Sui *et al.*, 2002). For example, in Wilm's tumours, down regulation of p57 involved in increasing invasiveness and metastasis suggesting a role of p57 in tumorigenesis (Orlow *et al.*, 1996). Importantly, no somatic mutations in p57 gene have been seen in these tumours (Li *et al.*, 2003; Nakai *et al.*, 2002; Shin *et al.*, 2000; Sui *et al.*, 2002). p57^{KIP2} has an anti-proliferation function in the nucleus (Lee, Reynisdottir & Massague, 1995), however p57^{KIP2} may accumulate in the cytoplasm playing a role in the regulation of the cytoskeletal dynamics (Yokoo *et al.*, 2003). In 2007, (Vlachos *et al.*,) have seen that p57^{KIP2} translocated to mitochondria and activated the intrinsic apoptotic pathway. Furthermore, p57 play a role in maintain haematopoietic stem cells (HSCs) quiescence by retaining Cyclin D into the cytoplasm (Matsumoto *et al.*, 2011; Zou *et al.*, 2011). However, functions of p57 need more investigations to detect its role in cancer development (Joaquin *et al.*, 2012).

1.7 Aims and objectives this study

1.7.1 Aims:

This study aims to:

- (1) Establish CCN1 roles in cell cycle regulation of progressive MCL.
- (2) Establish stable overexpression and knockdown cell line models using the REC1 and JVM2 cell lines which depict CCN1 driven disease progression:
- (3) Identify signalling pathways associated with CCN1 driven disease progression using proteomic technology and IPA / other software.

1.7.2 Objectives:

CCN1 expression is dysregulated in progressive Mantle Cell Lymphoma (MCL). This study will characterise the role of CCN1 in cell cycle regulation and develop stable CCN1 overexpression and knockdown cell line models in human MCL cells through viral infection. The stable cell lines will then be utilised to conduct proteomic investigation of the pathways affected; IPA / other software will be used to delineate the data sets generated and aid identification of novel small molecule inhibitors that may be useful clinically. We will screen a small number of compounds for efficacy on the human MCL cell lines and select those that may be useful for further detailed investigation.

1.7.3 Hypothesis:

Mantle cell lymphoma (MCL) is a distinct subset of B-cell non Hodgkin lymphoma (NHL) characterised by overexpression of cyclin D1 as a result of t(11;14) chromosomal translocation. Disease progression often involves down regulation of cyclin D1. We hypothesize that the CCN1 may play an active role in regulating MCL cells by altering B cell development via the stem cell signalling pathways.

Chapter 2

Materials and methods

2. Materials and methods

2.1 Cell culture

All cell lines used in this study were cultured *in vitro* in biological safety cabinet class II (Holten LaminAir, UK) under sterile conditions. The cells were maintained at 37 °C in 95% humidified atmosphere and 5% CO₂.

2.1.1 Human Mantle cell lymphoma (MCL) cell lines

2.1.1.a MCL cell lines

Human Mantle Cell Lymphoma cell lines REC1, G519 and JVM2 were purchased from the Deutsche Sammlung von Mikroorganismen and Zellkulturen (DSMZ; Germany). REC1, was established from a man 61 years old, human B cell lymphoma progressing to transformed mantle cell lymphoma blastoid variant (Raynaud *et al.*, 1993; Rimokh *et al.*, 1994) and is considered as early stage MCL. GRANTA-519 (G519), human B cell lymphoma, established from a Caucasian woman 58 years old derived from the peripheral blood at relapse of a high grade B-NHL (leukemic transformation of MCL, stage IV) with previous history of cervical cancer (Amin *et al.*, 2003; Drexler & MacLeod, 2002; Jadayel *et al.*, 1997; Rudolph *et al.*, 2004) is considered as an aggressive stage of MCL. JVM2, human chronic B cell leukaemia, established from a woman 63 years old with B-prolymphocytic leukaemia (B-PLL) derived from the peripheral blood (Melo *et al.*, 1988; Melo *et al.*, 1986) is considered as an advanced stage of MCL. All cell lines were cultured in Roswell Park Memorial Institute (RPMI 1640; Gibco, UK) supplemented with 10% Fetal Bovine Serum (FBS; Gibco, UK), and were incubated at 37 °C in a humidified atmosphere of 5% CO₂.

2.1.1.b REC1<G519<JVM2 model of MCL progression

In this study, we used three MCL cell lines which are REC1, G519 and JVM2 as a model for disease progression. The 2016 revision of World Health Organisation classification of lymphoid neoplasms describes the variants of MCL in addition to the characteristic Cyclin D1 rearrangement (i) 2 types of clinically indolent forms with either Immunoglobulin heavy chain variable region gene (IGHV) unmutated or minimally mutated and usually with expression of SOX11 typically involving lymph nodes or extranodal sites, (ii) acquisition of additional molecular or cytogenetic abnormalities leading to an aggressive blastoid or pleomorphic phenotype and (iii) leukaemic non-nodal MCL develops from IGHV mutated and SOX11 negative B cells with usual bone marrow and / or spleen involvement where abnormalities in TP53 contributes to enhance aggressive nature (Swerdlow *et al.*, 2016). REC1 cells display unmutated IGHV with expression of SOX11 (Beà *et al.*, 2013) consistent with the classic indolent forms of MCL. We used REC1 as early stage of MCL because it behaves like primary MCL in many studies in terms of sensitivity to conventional therapy in contrast to G519 and JVM2 cell lines which behave like aggressive stages (Nordgren, Hegde & Joshi, 2012; Rauert-Wunderlich *et al.*, 2016). However, data which are available for the cell line REC1 are limited (Drexler & MacLeod, 2002). G519 cells display blastoid phenotype with SOX11 positivity and amplification of BCL2 gene leading to Bcl2 overexpression enhancing cell survival associated with the aggressive blastoid variant forms (Queirós *et al.*, 2016; Rudolph *et al.*, 2004). G519 was established from peripheral blood of a patient with high grade of MCL in leukaemic transformation (Drexler & MacLeod, 2002) and we observed this as an intermediate stage in comparison to indolent and aggressive disease. In a comparative study between G519 and primary tumour of MCL, they found this cell line genetically similar to primary MCL in terms of expressing genes that upregulated in primary MCL when compared to normal B cells (Ek, Ortega &

Borrebaeck, 2005). G519 cell line carries t(11;14) chromosomal translocation, overexpressed cyclin D1 but it is un-similar to classic MCL (that is CD5⁺ and CD23⁻) in terms of having CD5⁻ and CD23⁺ (Amin *et al.*, 2003). In mouse models, Klanova *et al.* (2014) have found that the overall survival of immunodeficient NSG mice xenografted with REC1 and G519 separately was 54 ± 3 days and 22 ± 1 days respectively. The authors reported that G519 is more aggressive from REC1 cells and the other cell lines that were used in this study which are Jeko, Mino and Hbl-2 according to overall short survival time.

JVM2 cells are IGHV unmutated and SOX11 negative, expressing low levels of cyclinD1 with increased expression of cyclin D2, BCL2 positive (Tucker *et al.*, 2006) and is considered a blastoid variant (Camps *et al.*, 2006) consistent with the aggressive blastoid variant, SOX11 negativity overlaps with the aggressive leukaemic non-nodal MCL forms. In 2005, (Tiemann *et al.*) have found that additional to classic, small cell, pleomorphic and blastic subtypes of MCL there is cytological variants of MCL classified pleomorphic subgroups with mixtures of cells classical with pleomorphic cells. In 2006, (Tucker *et al.*) reported that JVM2 cell line harbouring both classic and variant characterisations of MCL. All the three cell lines carry t(11;14)(q13;32) chromosomal translocation and overexpressed cyclin D1 (Salaverria *et al.*, 2006) except JVM2 that expressed cyclin D2 instead (Tucker *et al.*, 2006). In addition, the cell lines show increasing resistance to lenalidomide in the order of REC1<G519<JVM2 (Zhang *et al.*, 2008) with G519 and JVM2 cells also showing increased resistance to Ibrutinib (Balsas *et al.*, 2017). REC1 cells are observed as early stage/ indolent MCL showing sensitivity to conventional therapy in contrast to G519 and JVM2 cell lines which behave consistently with aggressive stages (Nordgren, Hegde & Joshi, 2012; Rauert-Wunderlich *et al.*, 2016).

2.1.2 HEK293 cell line

The human embryonic kidney 293 (HEK 293) cells were kindly provided by Professor David Parkinson (Biomedical Research-Institute of Translational & Stratified Medicine, Faculty of Medicine and Dentistry, Plymouth University, UK) and used in transfection experiments. Cells were maintained at 37 °C in 5% CO₂ in Dulbeccos Modified Eagle Medium [+] 4.5g/L DMEM (1x) (DMEM; Gibco, UK) containing 10% FBS (Gibco, UK).

2.2 Maintenance, cryopreservation and reconstitution

REC1, G519 and JVM2 have been maintained in RPMI 1640 supplemented with 10% FBS and they were passaged twice weekly to maintain log phase. Experiments were conducted within 10 passages from cell recovery and cells were seeded at 2×10^5 cells per ml for experimental procedures. Cells were counted using a haemocytometer and cell viability assessed using the trypan blue dye exclusion assay as described at (4.3). HEK293 cells were maintained in DMEM supplemented with 10% FBS and were split three times a week. Adherent HEK293 cells were grown up to 80-90% confluence and then the medium was discarded, cells were passaged using 1-3 ml of Trypsin-EDTA (0.25%) with Phenol Red (Gibco, UK), incubated for 2-3 minutes in the cell culture incubator. DMEM supplemented with 10% FBS was added to stop trypsin and cells were centrifuged at 1300 rpm for 5 minutes, the supernatant was discarded and the pellet was re-suspended in culture medium and aliquoted 1:4 in 75 cm² tissue culture flasks. Cell morphology and cell density were checked using inverted microscopy (Olympus CK30, Japan). The colour and turbidity of the medium were also monitored.

For long-term storage, cells were prepared for cryopreservation; MCL cells were centrifuged at 1600 rpm for 5 minutes and the cell pellet was re-suspended in 90% FBS

and 10% dimethyl sulphoxide (DMSO) (Sigma-Aldrich, Germany) at a density $4-5 \times 10^6$ cells/vial. Vials were placed in cryopreservation stacks and stored at -80°C until required. Similarly, HEK293 cells were centrifuged at 1300 rpm for 5 minutes re-suspended in 45% DMEM, 45% FBS and 10% DMSO at a density $4-5 \times 10^6$ cells/vial. Vials were placed in cryopreservation racks and stored at -80°C until required. To reconstitute cells, vials were removed from -80°C and thawed at 37°C . Cells were transferred to a 15 ml tube with 10 ml supplemented medium. The cells centrifuged at 1600 rpm for 5 minutes for MCL cell lines and at 1300 rpm for 5 minutes for HEK293 cells and the cell pellet was re-suspended in 5 ml culture medium and placed in a 25 cm^2 tissue culture flask. The cells incubated as described above.

2.3 Cell viability assay

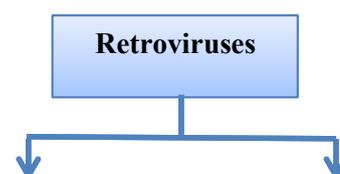
Cell viability was assessed by trypan blue (Sigma-Aldrich, UK) using a haemocytometer. The trypan blue dye exclusion assay was performed at 1:2 dilution and the percentage of unstained cells (live cells) counted and used to seed cells for each experiment. The stained cells (dead cells) were excluded by viewing the cells using inverted microscope at 400X magnification.

2.4 Lentiviral system

2.4.1 Characteristics of lentiviral vectors

Lentivirus-based vectors have been used as a robust tool for gene delivery. Lentiviruses are a complex subclass of retroviruses derived from human immunodeficiency virus 1 (HIV-1). In contrast to simple subclasses of retroviruses which are onco (γ -) retroviruses such as murine leukaemia viruses (MLV), they have the ability to stably integrate with

dividing and non-dividing genome. A comparative study between viral transduction vectors (using lentiviral vectors, adenovirus and adeno-associated vectors) and non-viral transduction vectors and ability to transduce rat mesenchymal stem cells showed that the lentivirus was an effective transduction tool with 95% of transgene incorporated and “low levels of cell toxicity” (McMahon *et al.*, 2006). Table 2.1 shows the summarised characteristics of lentiviral vectors compared with some other viral transgene systems.



Characteristics	Adeno-associated vectors	Adenoviral vectors	Lentiviruses vectors	γ -retroviral vectors
Transgene capacity	4.8kb ¹	38kb ⁵	8kb ⁹	8kb ¹¹
Infect cells	Dividing and non-dividing cells ²	Dividing and non-dividing cells ⁶	Dividing and non-dividing cells ⁹	Dividing cells ¹¹
Gene expression	Long-term gene expression ³	Too short term gene expression ⁷	Long-term stable gene expression ¹⁰	Long-term gene expression ¹²
Side effects (In vivo)	It has not associated with disease ⁴	It has caused death of some patients ⁸	No side effects ¹¹	It has developed leukaemia by integrating with LMO2 proto-oncogene promoter that leads to aberrantly transcription and expression of LMO2 ¹³

Table 2.1: Characteristics of lentiviral vectors and comparison with some other viral transgenes system. Summary of transgene capacity and advantages / disadvantages for a number of vector delivery systems. Information collated from ¹ (Grieger & Samulski, 2005), ² (Daya & Berns, 2008), ³ (Haberman, McCown & Samulski, 1998), ⁴ (Lai, Lai & Rakoczy, 2002), ⁵ (Vorburger & Hunt, 2002), ⁶ (Kafri *et al.*, 1998), ⁷ (Crystal, 2014), ⁸ (Reid, Warren & Kirn, 2002), ⁹ (Zufferey *et al.*, 1998), ¹⁰ (Mao *et al.*, 2015), ¹¹ (Nayerossadat, Maedeh & Ali, 2012), ¹² (Vargas *et al.*, 2016), ¹³ (Hacein-Bey-Abina *et al.*, 2003; Zhang *et al.*, 2008b).

Since lentiviral transduction will provide long-term expression and with no side effects, we have chosen to use the lentivirus system for CCN1/ CYR61 gene (1.146kb) in MCL cell lines. Furthermore, lymphoma cells are difficult cells to modify gene expression (Anastasov *et al.*, 2009). In 1998, (Huang *et al.*) have found that the primary T-helper

cells were resistant to transfection by conventional methods. Lentiviral vectors can deliver up to 10 kilobases (kb) of transgenes and leading to a naturally long-term expression of the transgene (Mátrai, Chuah & VandenDriessche, 2010) which makes the lentiviral system an attractive technique to use in lymphoma (Lois *et al.*, 2002).

2.4.2 The Lentivirus Expression System

Applied Biological Materials Inc (abm®) plasmids used in this study to overexpress and knockdown CCN1/CYR61 protein. pLenti and piLenti lentivirus expression system utilize 3rd generation system with replication-incompetent according to limited relation to the wild type (HIV-1 virus) through some safety characteristics that make using lentivirus expression system is safe. All lentiviral expression systems provided from Abm Inc. include the following safety features:

- ❖ Self-inactivation (SIN) recombinant according to deleting enhancer in the U3 region of the 3' Long Terminal Repeat (LTR) that making all lentivirus expression systems provided by abm Inc. replication-incompetent after transduction and integration with DNA of the targeted cells.
- ❖ By using Rous Sarcoma Virus (RSV) which is promoter upstream of 5' LTR Tet-independent to produce of viral RNA.
- ❖ The replication of virus is prevented (replication-incompetent) by deleting all accessory genes of wild type virus (HIV-1) and using necessary genes of Gag, Pol, and Rev for packaging, replication and transduction. The expression of these genes come from different (separate) plasmids that are losing packaged viral genome.

Lentivirus system that provided from abm Inc. is stable system to gene delivery, easy to grow in DH5 α competent cells and using “convergent promoters to avoid hairpin loop structure design” which helps to insert of 27-29 bp oligos. All pLenti and piLenti lentiviruses that used in this study depended on green fluorescence protein (GFP) expression as indicator to insert CCN1/CYR61 genes as well as including puromycin resistance gene in mammalian cells and kanamycin resistance in bacteria. For pLenti-GIII-CMV-hCYR61-GFP-2A-Puro, cytomegalo virus CMV promotor was used to express CCN/CYR61 overexpression. SV40 promoter was used to express GFP and kanamycin/puromycin resistance. For piLenti-siRNA-GFP, U6 and H1 promoters were used to direct CCN/CYR61 knockdown system. CMV promoter was used to express GFP and kanamycin/puromycin resistance (figure 2.1) (Lenti-siRNA-Expression-05-2014 (2).pdf).

A CYR61 Lentiviral Vector (Human) (CMV) (pLenti-GIII-CMV-GFP-2A-Puro) Lentiviral Vector

Cat. No.	Quantity	Accession Number
LV110412	1.0 µg DNA	BC016952
Gene Insert	CYR61	
Product Application	- Direct non-viral plasmid transfection for immediate expression - Package into Lentiviral particles for high efficiency transduction and stably integrated expression	
Species	Human	
Accession Number	BC016952	
Vector	pLenti-GIII-CMV-GFP-2A-Puro	
Vector Map		
Sequencing Primers	CMV sequencing primer 5'---CGC AAA TGG GCG GTA GGC GTG---3' SV40 reverse sequencing primer 5'---TAG TCA GCC ATG GGG CGG AGA ---3'	
Antibiotics	Bacterial: Kanamycin. Mammalian: Puromycin.	
Vector Size	8829bp	
Insert Size	1146bp	
Insert Notes	For any lentiviral vector, the maximum insert size is 5.0kb, above which will exceed the lentiviral virus packaging limit. This will result in minimal or no packaging of viral particles. For complex lentiviral vectors, such as dual promoter constructs or constructs with reporters and/or other features, maximum inset size can be as small as 3.0kb. For insert sizes over 5.0kb, removal of selection markers and selection marker promoters will help to accommodate these larger inserts.	
Tags	GFP	
Selection Marker	Bacterial: Kanamycin. Mammalian: Puromycin	
Format	Lentiviral Plasmid	

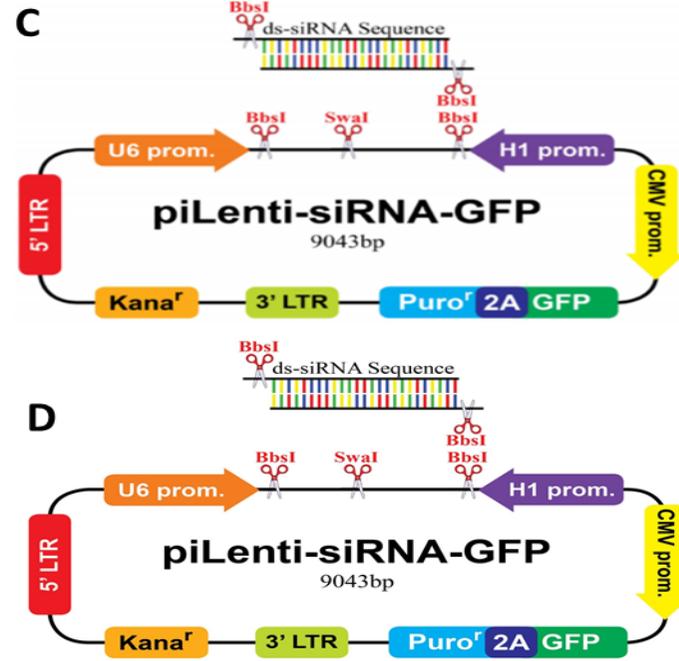
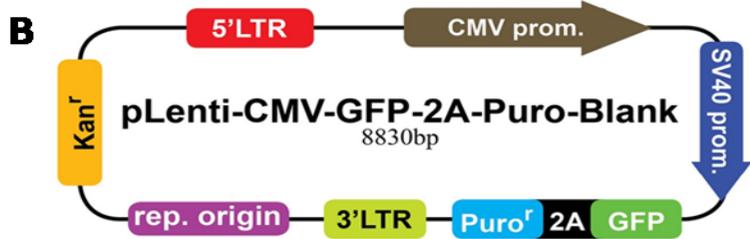


Figure 2.1: Maps of lentiviral vectors. A, CYR61 lentiviral vector used for overexpression of CCN1 protein. B, blank lentiviral control used for positive control of overexpression model. C, CYR61 siRNA lentiviral vector used for knockdown of CCN1 protein. D, scrambled lentiviral vector used for positive control of knockdown model.

Using lentivirus system in gene modification contains two parts which are lentivirus packaging and lentivirus infection. Lentivirus packaging includes recombination plasmids in packaging cells and lentivirus infection involves transcription viral RNA to double stranded DNA in targeted cells and integration with DNA cells leading to translation of targeted protein (Figure 2.2) (Durand & Cimarelli, 2011).

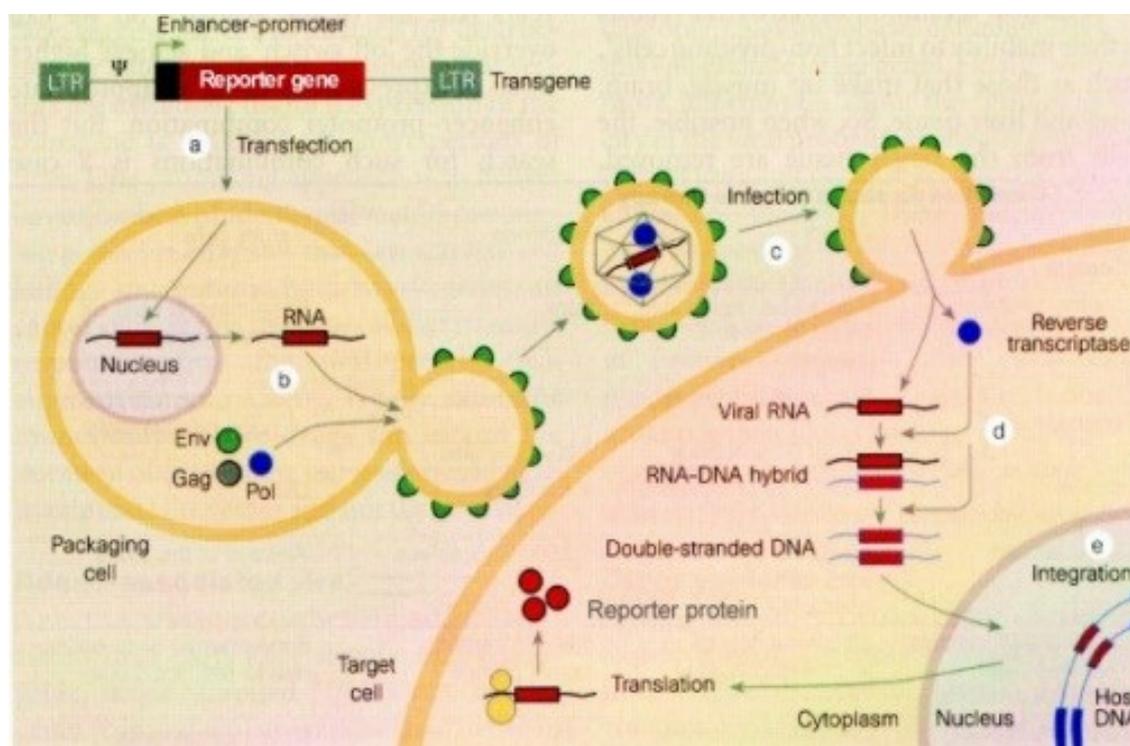


Figure 2.2: Schematic overviews lentivirus packaging using packaging cell line and infection of targeted cells. a, transfection of transfer vector which is part of HIV provirus carrying gene of interest, the packaging plasmid coding for *gag-pol* and the envelope glycoprotein coding for the *env*. The three plasmids transfected into packaging cells. b, the viral RNA, envelope, reverse transcriptase integrase enzymes package in lentivirus in supernatant that uses to infect targeted cells. c, infection lentivirus of targeted cells which started with engagement of specific targeted cellular-lentivirus ENV receptor triggering the fusion between plasma cells and viral membrane leading to release of viral RNA and enzymes into the cells. d, viral RNA reverse transcribed to double stranded DNA (dsDNA) utilizing viral transcriptase. e, integration of dsDNA with DNA of targeted cells in the nucleus leading to modify gene of interest. Image taken from <http://biology.kenyon.edu/slonc/gene-web/Lentiviral/Lentivi2.html>.

2.5 Bacterial techniques

2.5.1 Bacterial broth, agar and reagents

2.5.1.a Luria Bertani broth (LB)

20g of LB broth (Sigma-Aldrich, USA) was dissolved in 1 litre of distilled water and autoclaved at 121 °C for 20 minutes with pressure 151bs/in². Antibiotics ampicillin (Sigma-Aldrich, Belgium) or kanamycin (Sigma-Aldrich, UK)) was added to LB broth to a final concentration of 50µg/ml as required.

2.5.1.b Antibiotic-Luria Bertani agar

According to manufacturer's protocol 35g of LB agar (Sigma-Aldrich, UK), was dissolved in 1 litre of distilled water and autoclaved as described at (2.5.1.a). LB agar was cooled at room temperature (approximately 50-45 °C), appropriate antibiotic was added to a final concentration of 50 µg/ml. The mixture of LB agar and antibiotic was mixed, plated in a 94mm petri dish and stored at 4 °C until required.

2.5.1.c 1mg/ml of PEI reagent

To prepare 1 mg/ml polyethyleneimine (PEI), 0.01 µl of PEI (Sigma-Aldrich, UK) was dissolved in 8 ml molecular grade distilled water and mixed in a 37°C water bath. The pH was adjusted to 8.0 by adding HCl (4M) and topped up by distilled water to 10 ml final volume. The solution was filter sterilised using 0.2 µm pore filter (Fisher Scientific, UK) and aliquoted in 1 ml vials and stored at -20 C° until required.

2.5.2 Bacterial transformation

Escherichia coli (*E. coli*) DH5 α was purchased from Invitrogen and was used as the recipient for the plasmids. Vials of competent *E. coli* (50 μ l/vial) were thawed on ice. 100 ng of DNA was added to one vial of competent cells and mixed by gentle shaking. Cells were incubated on ice for 30 minutes, heat shocked at 42 °C for 30 seconds and recovered on ice for 5 minutes. 250 μ l of pre-warmed LB broth was added to the vial and incubated at 30 °C for 2 hours with shaking. 4 LB agar plates were used to sub clone each plasmid, 1 control plate without antibiotic was used to culture control *E. coli*. Another plate with appropriate antibiotic cultured with *E. coli* and 2 plates with relevant antibiotic as prepared at (2.5.1.b) cultured with 30 μ l and 270 μ l (respectively) using the mixture of bacteria and plasmid. All plates were incubated overnight at 30 °C with agitation at 230 rpm. LB agar plate was supplemented with 50 μ g/ml ampicillin or kanamycin (2.5.1.b) for VSV-G, p Δ 8.9 and lentiviral vectors respectively were used (Figure 2.3).

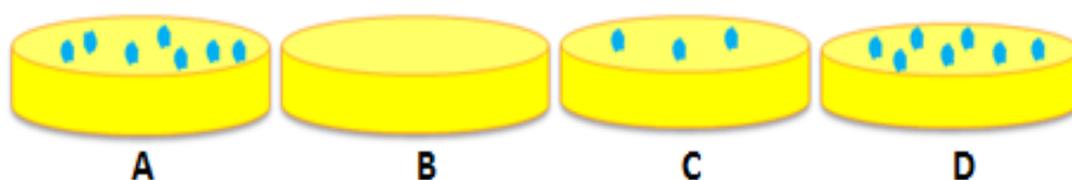


Figure 2.3 Sub-cloning a plasmid with *E. coli* DH5 α . A LB agar without antibiotic cultured with DH5 α without plasmid. B LB agar with antibiotic cultured with DH5 α without plasmid. C LB agar with antibiotic cultured with 30 μ l of DH5 α and plasmid. D LB agar with antibiotic cultured 270 μ l DH5 α and plasmid.

2.5.3 Preparation of Long-term glycerol stocks

For long-term storage, a single colony of bacteria was transferred from LB agar (2.5.2) into 5 ml LB broth supplemented with appropriate antibiotic and incubated overnight at 30 °C with agitation at 230 rpm. 800 μ l of overnight culture has added to 800 μ l of sterile

glycerol (Fisher Scientific, UK) and mixed by pipetting up and down many times. Glycerol stocks were stored at -80 C° until required.

2.5.4 Purification of plasmids (DNA)

The plasmid was isolated by using MiniPrep Genelute HP plasmid (Sigma-Aldrich, UK) following the supplier instructions. A single colony was transferred from LB agar (2.5.2) or using glycerol stock (2.5.3) (by scraping) into 5 ml LB broth supplemented with appropriate antibiotic and incubated (VWR, West Sussex, UK) overnight as described at (2.5.2). The culture was then centrifuged at 3,500 rpm for 15 minutes at room temperature and the supernatant was discarded. The bacterial pellet was re-suspended by adding 200 µl of resuspension solution containing RNase and gently pipetting up and down. To lyse the bacteria, 200 µl of alkaline lysis buffer was added and mixed by inversion 6-8 times. To precipitate the cell debris, 350 µl of neutralization/Binding buffer was added and mixed by inversion 4-6 times before centrifugation at 10,000 rpm (12,000 x g) for 10 minutes at room temperature. The clear solution is now absent of cell debris containing proteins, lipid and chromosomal DNA. The clear supernatant was transferred to the DNA binding column and centrifuged at 10,000 rpm for 1 minute at room temperature. The supernatant was discarded and the column was washed twice with wash solution 1 and 2. To remove excess ethanol from wash solution 2, the column was centrifuged for 1 minute at 10,000 rpm. 100 µl of Elution buffer was added to the column and centrifuged at 10,000 rpm for 1 minute to elute DNA. DNA quantity and purity was measured using a nanodrop spectrophotometer using A260/A280 ratios (Thermo-Fisher scientific, Waltham, MA, USA). Good quality DNA was regarded as 1.7-1.9 using A260/A280 ratio. The three sequences of CYR61 siRNA (i005683a, i005683b and i005683c) targeting three different sequences in CCN1 (Table 2.2) and pLenti-GIII-CMV-hCYR61-GFP-2A-Puro

(LV110412) (appendix 1) were purchased from Applied Biological Materials Inc (abm®) and were used for knockdown and overexpression of the target gene CCN1 respectively. Scrambled siRNA EGFP (LV015-G) and pLenti-III-CMV-GFP-2A-Puro-Blank Control Lentiviral Vector (LV590) were purchased from abm® and used for positive controls of knockdown and overexpression models respectively. VSV-G and pΔ8.9 plasmids were kindly provided by Dr Dan Rocca (University of Bristol).

System / Use	P-lenti vector	Sequence
CCN1 Knockdown Vectors and Control	Scrambled siRNA EGFP	GGGTGAACTCACGTCAGAA
	CYR61-288 siRNA/shRNA/RNAi Lentivector (Human) (Target a)	GGGCAGACCCTGTGAATATAACTCCAGA
	CYR61-582 siRNA/shRNA/RNAi Lentivector (Human) (Target b)	AAACAATGAATTGATTGCAGTTGGAA
	CYR61-676 siRNA/shRNA/RNAi Lentivector (Human) (Target c)	GGCCAGAAATGTATTGTTC
CCN1 Overexpression Vector and Control	pLenti-III-CMV-GFP-2A-Puro-Blank Control (LV590)	Empty Vector
	pLenti-III-CMV-hCYR61-GFP-2A-Puro (LV110412)	Vector containing full length human CCN1 sequence

Table 2.2: Composition of lentiviral vectors used in CCN1 overexpression and knockdown experiments.

2.5.5 Lentiviral production

The pLenti lentiviral vector was used to produce virus through transfection of HEK293 cells using 20 µg lentiviral vector, 10 µg VSV-G vector (envelope glycoprotein), 15 µg pΔ8.9 vector (packaging constructs) and 100 µl PEI (1 mg/ml) (2.5.1.c). In the present study, we used a three-plasmid expression system to generate lentivirus carrying CCN1/CYR61 gene. Two helper plasmids which are packaging and envelope plasmids and the transfer plasmid. The packaging plasmid was pΔ8.9 coding for *gag-pol* and the

envelope glycoprotein was VSV-G glycoprotein of the vesicular stomatitis virus coding for the *env*. The *gag* gene encodes for structural proteins which are matrix, capsid and nucleocapsid. *pol* gene encodes for the enzymes reverse transcriptase, integrase and protease that involved in reverse-transcription, integration, maturation and replication (Liechtenstein, Perez-Janices & Escors, 2013). VSV-G glycoprotein has broad tropism making the lentivirus a good transducer in a broad range of cell types. All the three plasmids were mixed in the following order (media, DNA and PEI) using 3 ml DMEM free serum. They were then incubated at 37 °C for 30 minutes to allow complex formation and then applied to the HEK293 packaging cells. Medium was changed to DMEM supplemented with 10% FBS 4-6 hours post transfection. After 48 hours, transfection was checked for fluorescence using a Lumascope. Supernatant containing lentivirus was collected and stored at 4 °C. Cells were maintained in fresh DMEM with 10% FBS. A second harvest of lentivirus was performed at 72 hours after the initial harvest. Supernatant containing lentivirus was spun down for 5 minutes at 1500 rpm to remove any cells or cell debris. The supernatant was then centrifuged for 2.5 hours at 48000 x g. Supernatant was discarded and the pellet was re-suspended in RPMI free serum and was then aliquoted and snap frozen in liquid nitrogen and stored at -80 °C until required.

2.5.6 Kill Curve

This experiment was performed to determine the optimal antibiotic concentration that enabled selection of transduced clones for the stable overexpression and knockdown models. JVM2 and REC1 cells were seeded separately at 0.3×10^5 , 1.5×10^4 , 7.5×10^3 , 3.5×10^3 , 2×10^3 and 1×10^3 in a 200 μ l RPMI media supplemented with 10% FBS using 96-well plates in duplicate. After 2 days incubation, various concentrations of puromycin (10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.5 and 0.1 μ g/ml) were added. For each cell number, a negative

control in duplicate was included that did not have addition of puromycin. Cell viability was checked every two days as described in 2.3, the medium was changed and fresh puromycin was added.

2.5.7 JVM2 preparation for single cell sorting by using Cell Trace Cell Proliferation Kit (CFSE)

Single cell sorting was performed for the JVM2 overexpression model to select clones with similar levels of CCN1 expression. JVM2 parent cells were harvested and centrifuged at 1600 rpm for 5 minutes. The supernatant discarded and the pellet re-suspended to 5×10^5 cells/ml and 1×10^6 cells/ml in PBS. Cell Trace CFSE Cell Proliferation Kit, for flow cytometry (ThermoFisher, USA) which contains 2 components (component A and B) was used. According to the manufacturer's protocol 18 μ l of component B (DMSO) was added to component A (5 mM STOCK CellTrace reagent) to make 5 μ M working concentration. 1 μ l of CellTrace reagent working concentration was added to each 1 ml of cell suspension and cells were incubated for 20 minutes at 37 °C with protection from light. RPMI supplemented with 10% FBS in a volume five times the original staining volume was added to the cells and incubated for 5 minutes. The cells were centrifuged to discard the supernatant, the pellet was re-suspended in culture medium and the cells were incubated for 10 minutes and applied to flow cytometry (Beckman Coulter FACS ARIA). To prepare a culture plate for cells post sorting, 200 μ l of culture medium was added into each well of a fresh 96-well plate. Single cells were sorted to each well of the 96-well plate and the plate was incubated at 37°C in a humidified atmosphere of 5% CO₂ to enable clones to grow.

2.5.8 Establishment of Stable CCN1/CYR61 expression models using MCL cell lines

2.5.8.1 CYR61 (CCN1) overexpression in JVM2 cells

JVM2 cells were seeded at 2 different cell densities 1.5×10^5 and 2.5×10^5 in 2 ml pure RPMI 1640 (Gibco, UK) using 3x 6-well plates. Two-wells of first plate were infected by 30 μ l of CYR61 lentivirus and two-wells of second plate by 30 μ l of empty lentivirus (blank) for positive control with two-wells of third plate as a negative control in the presence of polybrene (Merck Millipore,UK)(4 μ g/ml). Cells were incubated for 24 hours and then collected/spun down at 1600 rpm for 5 minutes at room temperature and re-suspended using 3 ml of fresh RPMI 1640 supplemented with 10% FBS. All three plates were incubated at 37 °C in 5% CO₂. After detection of green fluorescence protein (GFP) production, cells were sorted using flow cytometry (Beckman Coulter) to collect only GFP-positive cells which have CCN1 /CYR61 overexpression.

2.5.8.2 CYR61 knockdown model in REC1 cells

To generate a stable CYR61 knockdown model using REC1 cells, three different CYR61-siRNA/shRNA/RNAi Lentiviruses targeting three different sequences (a-c) were used (Table 2.2). After production of piLenti lentiviruses carrying siRNA sequences (section 2.5.5), REC1 cells were seeded at cell densities of 5.0×10^5 and 7.5×10^5 in 3ml of pure RPMI 1640 medium in 25 cm² tissue culture flask. The cells were infected by CYR61-R288, CYR61-582, CYR61-676 and scrambled lentiviruses in the presence of polybrene (4 μ g/ml) in duplicate (Table 2.2). Two flasks were left without infection to serve as a negative control and all the flasks incubated overnight. Cells were collected, spun down to discard the supernatant and the pellet re-suspended in 5 ml of RPMI 1640 supplemented with 10% FBS in 25 cm² tissue culture flasks. After detection of green

fluorescence protein (GFP) production, cells were selected by adding puromycin (0.1-3 $\mu\text{g/ml}$) to obtain CCN1 knockdown clones and 0.5 $\mu\text{g/ml}$ was used to maintain the cells.

2.5.8.3 CCN1 overexpression and knockdown models maintenance and long-term storage

Stable overexpression JVM2^{+CYR61} and knockdown REC1^{-CYR61} models were cultured in RPMI 1640 (Gibco, UK) supplemented with 10% Fetal Bovine Serum (Gibco, UK) incubated at 37 °C in 5% CO₂. Cells were passaged between 1 to 5 weeks to collect protein samples and were used to check gene expression changes associated with CCN1 models and correct modification before proceeding to proteomics.

For long-term storage, the cells were centrifuged at 1600 rpm for 5 minutes at room temperature. The cell pellet was re-suspended in 90% FBS and 10% DMSO and placed in a cryopreservation tube using a density 4-5x10⁶ cells/vial and stored at -80 °C until required.

2.5.8.4 Confirmation of CCN1 overexpression and knockdown

pLenti and piLenti lentiviral vectors contain green fluorescence protein (GFP) gene within the plasmid therefore enabling visualisation as an indicator of transfection efficacy using cell fluorescence using Lumascope. Within each weekly passage of the modified cell lines, 1 X 10⁶ cells were retained and used to extract mRNA for RQ-PCR (2.7.2) and 3-5 X 10⁶ cells were retained and used to extract protein lysate for western blotting (2.6.5.2) and proteomics (2.8).

2.6 Molecular biology techniques

2.6.1 RIPA buffer preparation

Radioimmune Precipitation Assay buffer (RIPA) was prepared by: Tris 50 mM, NaCl 150 mM, Triton X-100 1%, Na-Deoxycholate 0.5%, SDS 0.1%. To prepare 50 ml of RIPA buffer, 0.302 g Tris Base, 0.438 g NaCl, 0.05 g SDS, 0.25 g NaDeoxycholate, 0.5 ml TritonX 100 dissolved in ddH₂O in a 50 ml tube using a water bath at 37°C. The pH was adjusted to 7.8 and topped up to 50 ml by adding ddH₂O and stored at 4°C until required.

2.6.2 Total Protein Extraction and Subcellular Fractions

Total cell lysates for human MCL cell lines (REC1, G519 and JVM2) were prepared in RIPA buffer (2.6.1) supplemented with CompleteTM protease inhibitor (Roche, UK) which used according to manufacturer instructions and stored at -20 °C until required. Cells at density (1x10⁶ cells/ml) were collected and centrifuged at 1600 rpm for 5 minutes at RT. The supernatant was discarded and the pellet was re-suspended in 500-1000 µl of RIPA buffer with CompleteTM protease inhibitor. The suspension was incubated on ice for 10 minutes and sonicated by using a probe sonicator for 10 seconds at 4 °C and then centrifuged at 1400 rpm for 10 minutes at 4 °C. The supernatant was stored at -20 °C until required.

Nuclear, mitochondrial and cytoplasmic proteins from cell lines were extracted using Cell Fraction Kit-Standard (Abcam, UK) according to manufacturer instructions with some modifications. 1X buffer A was prepared using 5 ml 2X buffer A, 4900 µl dH₂O and 100

μl PI (Halt Protease Inhibitor Single-Use Cocktail EDTA-Free, ThermoScientific, UK). Cells were collected, counted using trypan blue dye exclusion, centrifuged and the cell pellet (1×10^6 cells/ml) was re-suspended with 500 μl of 1X buffer A. Buffer B was prepared by diluting Detergent I 1000-fold in 1X buffer A to extract cytoplasmic protein. 500 μl of buffer B was added to the cell suspensions and the cells were incubated for 6-7 minutes on a rotator at room temperature. The cells were then centrifuged twice at 5000 x g and 10,000 x g for 1 minute at 4 °C each time. Supernatants were collected in a fresh tube and labelled cytoplasmic fraction. Buffer C was prepared by diluting Detergent II 25-fold in 1X buffer A. The pellet was then re-suspended by pipetting with 500 μl of 1X buffer A and then buffer C was added. The cells were incubated for 8-9 minutes at room temperature on a rotator. After incubation, the cells were centrifuged twice at 5000 x g and 10,000 x g for 1 minute at 4 °C each time. Supernatants were collected in fresh tubes and labelled mitochondrial fraction. The pellet was then re-suspended in 1 ml of 1X buffer A, sonicated by using a probe sonicator for 2 minutes at 4 °C, centrifuged at 10,000 x g for 4 minutes at 4 °C. The supernatant was collected in a fresh tube and labelled nuclear fraction. All fractions were stored at -80 °C until required.

2.6.3 Protein quantification

The protein in each sample was quantified using micro BCA Assay Kit (Thermo Scientific, USA) following the supplier instructions. Micro BCA TM Protein Assay Kit contains 3 buffer components MA, MB and MC. Micro BCA working reagent (WR) was prepared by mixing 25:24:1 parts of reagent MA, MB and MC respectively. 10 μl of each sample or standard (2.6.4) was pipetted into replicates in each well of Nunc 96-well microplate (Thermo Scientific, UK) and then 90 μl of WR was added. The plate was mixed by a plate shaker for 30 seconds and incubated at 37 °C for 2 hours. The absorbance

was measured at 562 nm on a plate reader. A standard curve (BSA standards) was used to detect the protein concentration of known standards and calculate / estimate protein concentration of experimental samples.

2.6.4 Bovine Serum Albumin (BSA) standard

Bovine serum albumin stock solution (Sigma-Aldrich, USA) (2 mg/ml) was prepared in deionised water ddH₂O. A dilution series was prepared to have BSA standards ranging from 2mg/ml to 0.1 mg/ml as described at Table 2.3 below.

Table 2.3 BSA standards

Standard	Stock	ddH ₂ O	Final volume
2 mg/ml	500 µl	0 µl	500 µl
1.5 mg/ml	375 µl	125 µl	500 µl
1 mg/ml	250 µl	250 µl	500 µl
0.75 mg/ml	187.5 µl	312.5 µl	500 µl
0.5 mg/ml	125 µl	375 µl	500 µl
0.25 mg/ml	62.5 µl	437.5 µl	500 µl
0.1 mg/ml	25 µl	475 µl	500 µl
0 mg/ml	0 µl	500 µl	500 µl

2.6.5 Immunoblotting detection

2.6.5.1 Immunoblotting solutions

2.6.5.1.a MOPS SDS Running Buffer(1X)

To prepare running buffer (1X), 25 ml of MOPS (20X) (ThermoFisher Scientific, USA) placed in 500 ml cylinder and topped up to 500 ml by adding ddH₂O.

2.6.5.1.b NuPAGE® Transfer buffer (1X)

The transfer buffer was prepared by mixing 50 ml of transfer buffer NUPAGE® (20X) (ThermoFisher Scientific, USA), 50 ml of methanol (VWR, chemical, France) and 450 ml of ddH₂O and stored at 4 °C until required.

2.6.5.1.c 2.5% Milk Blocking buffer

2.5 g of skimmed milk was dissolved in 10 ml of phosphate buffer saline (PBS) (1X) (Fisher chemical, UK) and topped up to 100 ml by using distilled water.

2.6.5.1.d Washing buffer (0.05% PBS-T)

1 tablet of phosphate buffer saline (100XPBS) (Fisher chemical, UK) was dissolved in 100 ml of distilled water to prepare 1XPBS final concentration. 50 ml of 1X PBS was mixed with 500 µl of Tween® 20 (FisherScientific, UK) and topped up to 500 ml by distilled water and stored at room temperature until required.

2.6.5.1.e Membrane Stripping solution

Guanidine HCL 28.659 g (Fisher BioReagents, UK) was dissolved in 20 mM Tris-HCl pH 7.5 (Sigma-Aldrich, UK) which was prepared by weight 12.11 g of Tris Base (Molecular Weight 121.14) in 40 ml of distilled water, pH was adjusted and stored at 4 °C until required. Triton X-100 0.2% and β-Mercaptoethanol 0.1 M were added and topped up to 50 ml by adding distilled water.

2.6.5.2 SDS-PAGE and western blotting

Protein samples (10 µg) were placed in 1.5 ml sterilised Eppendorf tubes with one part of sample reducing sample buffer (5X SDS-PAGE) (ThermoFisher Scientific, USA) and denatured at 95 °C for 5 minutes. The protein samples were loaded on a 10% NuPAGE® Bis-Tris Gel (Invitrogen, USA) using the XCell SureLock™ Mini-cell system (Invitrogen, UK) and MOPS running buffer (1X) (2.6.5.1.a). Gels were run at 200V for 50 minutes at room temperature. Proteins were transferred onto a polyvinylidene difluoride PVDF membrane (Millipore, UK) by using transfer buffer (2.6.5.1.b) and current applied at 30V, 300 mA for 1 hour at room temperature. Membranes were then blocked using 2.5% skimmed milk (2.6.5.1.c) for 30 minutes and were then incubated overnight at 4 °C with antibody raised to detect the various human proteins. Specific antibodies used are as follows: rabbit anti-p21^{CIP1} (1:1000, Cell Signalling Technology) (2947S), rabbit anti-p27^{KIP1} (1:1000, New England Biolabs) (3688s), rabbit cyclin D1 (1:1000, Cell Signalling Technology) (2922s), rabbit anti-CCN1 (1:2000, Abcam) (ab24448, Lot # GR26258-7), rabbit anti-GAPDH (1:2500, Abcam) (ab9485), mouse anti-COX IV (1:1000, Abcam) (ab33985), rabbit anti Histone H3 (1:1000, New England Biolabs) (9717s) and mouse anti-beta actin (1:5000, proteingroup) (60008-1-Ig). Membranes were washed with 0.05% PBS-T (2.6.5.1.d) three times for 5 minutes each time to remove excess unbound primary antibody. Membranes were then incubated with an appropriate secondary antibody conjugated to horseradish peroxidase (HRP) (anti-rabbit-HRP (Cell Signalling Technology) or anti-mouse-HRP (Abcam) for 30 minutes at room temperature. All the incubations of membranes were performed using a shaker platform. Membranes were washed again with 0.05% PBS-T 3 times for 5 mins as described above. Bands were

detected using chemiluminescence (Thermo Scientific SuperSignal®West Dura Extended Duration Substrate, USA) and viewed using the Image Quant LAS4000 software.

2.6.5.3 Stripping and Re-probing membrane

This procedure was performed inside the fume cabinet. To re-probe membranes with loading control antibody, 25 ml of stripping solution buffer (2.6.5.1.e) was added to the membrane and incubated for 5 minutes at room temperature. Solution was discarded and another 25 ml stripping solution was used with incubation for 5 minutes at room temperature. The membrane was washed by 0.05% PBS-T (2.6.5.1.d) four times for 5 minutes each. Blocking buffer 2.5% skimmed milk (5.2.1.c) was added to the membrane for 30 minutes at room temperature with shaking. The membrane was then incubated with primary antibody overnight at 4 °C and procedures completed as detailed in (2.6.5.2).

2.7 RNA extraction and RQ-PCR

2.7.1 RNA extraction and cDNA synthesis

Total RNA was extracted using Trizol® reagent (Fisher Scientific, UK). Trizol® (1ml per 1×10^6 cells) was pipetted up and down several times until the samples were homogenised. 200 µl of chloroform was added per initial 1ml of Trizol®, shaken vigorously for 15 seconds and incubated at room temperature for 2-3 minutes. Samples were centrifuged at 13,000 rpm for 15 minutes at 4 °C. The upper layer was decanted to new tubes and 500 µl isopropanol added (per initial 1ml of Trizol®) and incubated at room temperature for 10 minutes. Samples were centrifuged at 13,000 rpm for 15 minutes at 4 °C. Supernatant was discarded and the white RNA pellet washed three times with 70%

ethanol. RNase free water was used to re-suspend the pellet. Samples were stored at -80 °C until required. RNA quantity and purity was measured using a nanodrop spectrophotometer using A260/A280 ratios (Thermo-Fisher scientific, Waltham, MA, USA). cDNA was synthesised using 2 µg of total RNA in 20 µl volume using the High Capacity RNA to cDNA kit (Applied Biosystems, UK). According to the manufacturer's instructions, the samples were heated at 37 °C for 60 minutes to perform reverse transcription and reaction stopped by heating at 95°C for 5 minutes to denature the enzyme. cDNA samples were stored at -20 °C until required. Negative template controls consisting of RT reaction without cDNA were run for all experiments.

2.7.2 RQ-PCR

Quantitative real-time PCR was performed by using Step One Plus and Software v2.3 for analyses (Applied Biosystems, USA). Reactions had a final volume of 12.5 µl containing 1µl cDNA (100ng), 0.625 µl primer primer probe set, 6.25 µl 2x Master Mix (Prime time Master Mix, Integrated DNA technologies). The PCR reaction conditions were 95 °C for 3 minutes, followed by 40 cycles comprising denaturation at 95 °C for 5 seconds and annealing and extension at 60 °C for 30 seconds. Predesigned assay reagents with FAM/TAMRA fluorescence were used for CCN1 (PT.58.827217), GAPDH (PT.39a.22214836) (Integrated DNA Technologies), p21 (00355782-M1), p27 (01597588-M1) and cyclin D1 (00765553-M1) (Applied Biosystems). GAPDH used as an endogenous control, gene expression levels were reported using the $\Delta\Delta CT$ method with experimental samples run in triplicate. Independent replicates of N=3 experiments were used for reporting.

2.8 Proteomics

2.8.1 Sample preparation for Mass Spectrometry

Cell pellets were lysed with RIPA buffer as described at (2.6.2). Protein estimation was performed by BCA Assay as described at (2.6.3). A total of 50ug of protein was digested using the Filter Aided Sample Preparation (FASP) procedure as described previously (Bouyer *et al.*, 2016). In day 1, 200 µl of Buffer A (8 M Urea in 0.1 M Tris-HCl pH 8.5 which made fresh) was added to Amicon Ultra-0.5 centrifugal filter device (Millipore, UK) and centrifuged at 14000 rpm for 5 minutes at room temperature to check the column. The samples were added to the column, topped up to 400 µl by buffer A and centrifuged for 5 minutes. 2 µl of DTT (final concentration 10mM) was added to the peptide concentrate and topped up to 200 µl with buffer A. The solution was incubated at room temperature for 30 minutes and centrifuged for 5 minutes at 14000 rpm and then the flow through was discarded. 20 µl of 50 mM chloroacetamide (CAA) (prepared in buffer A final concentration 500 mM) was added and topped up to 200 µl with buffer A. The column was incubated at room temperature in the dark for 20 minutes, centrifuged for 5 minutes at 14000 rpm and the flow through was discarded. 400 µl of buffer B (8M of urea in 0.1M Tris-HCl pH 8) was added, centrifuged for 5 minutes at 14000 rpm and the flow through was discarded. 400 µl of buffer ammonium bicarbonate (ABC) (50 mM prepared in water, the final concentration was 500 mM in ddH₂O and stored at -20 °C) was added, centrifuged for 10 minutes at 13300 rpm and the flow through was discarded. 5 µl of trypsin was added and incubated overnight at room temperature.

On day 2, collection tubes were changed and the column was centrifuged for 15 minutes at 14000 rpm with the flow through containing the proteins after digestion by trypsin. 50 µl of 500 mM sodium chloride was added to wash the column and centrifuged for 10-15 minutes at 14000 rpm. For acidity, 5% of trifluoroacetic acid (TFA) 100 µl was added to

each sample and 2 μ l taken from each sample on to pH paper to check the acidity which was 2/3. The samples were stored at -80 °C until required.

In stage-tip assembly; a high performance extraction disk (C18) was placed in a petri dish. A piece of the disk was picked using the picking tool (a cut off p200 tip and pushed down with Hamilton syringe plunger). The disk was transferred via pipet tip to a 2ml Eppendorf tube with a hole pierced in middle of the cap. The home-made stage-tip was pushed into the tube by pushing through lid.

For purification, 100 μ l of methanol was added and centrifuged for 10-15 minutes at 5000 rpm. Then 100 μ l of buffer C (80% of ACN 20% H₂O (0.5% acetic acid) was added and centrifuged until all the volume of fluid has passed the filter. 100 μ l of buffer D (aqueous phase 0.5% acetic acid prepared in water) was added and centrifuged for 10-15 minutes for 5000 rpm. 40 μ l of protein was added into the column and centrifuged until all the fluid has passed through the filter. Collection tubes were changed, 25 μ l of buffer C was added and centrifuged for 10-15 minutes at 5000 rpm. 400 μ l of buffer D was added, then transferred into glass vials for LC-MS analysis and stored at -20 °C until required.

2.8.2 Proteome analysis:

Peptides were separated on a Dionex Ultimate 3000 RSLC nano flow system (Dionex, Camberley, UK). 3 μ l of sample was loaded in 0.1% trifluoroacetic acid (TFA) and acetonitrile (2% acetonitrile in 0.1% TFA) onto an Acclaim Pep Map, 3 μ m C18 nano trap column, at a flow rate of 5 μ l/min, bypassing the analytical column. Elution of bound peptides was performed with the trap column in line with an Acclaim PepMap C18 nano column, 100 Å (Analytical Column) with a linear gradient of 96% buffer A and 4% buffer B to 60% buffer A and 40% buffer B, (Buffer A: 0.5% Acetic Acid, Buffer B: 80% acetonitrile in 0.5% acetic acid) at a constant flow rate of 300nl/min over 120 minutes.

The sample was ionized in positive ion mode using a Proxeon nano spray ESI source (Thermo Fisher Hemel UK) and analyzed in an Orbitrap Velos Pro FTMS (Thermo Finnigan, Bremen, Germany). MS spectra of intact peptides (m/z 350-1600) with an automated gain control accumulation target value of 1000000 ions were acquired with a resolution of 30000. The ten most intense ions were sequentially isolated and fragmented in the C trap by Higher Energy Dissociation (HCD) at a resolution of 7500. All the singly charged and unassigned charge state ions were excluded from sequencing. Typical mass spectrometric conditions were: spray voltage, 2.3 kV; no sheath and auxiliary gas flow; heated capillary temperature, 275°C; normalized HCD collision energy 45 for MS2 in C trap. The ion selection threshold was 50000 counts for MS2. An activation $q = 0.25$ and activation time of 20 ms were used.

2.8.3 Data Analysis by Max Quant

Peptides and proteins were identified by Andromeda via automated database searching of all tandem mass spectra against a curated target/decoy database (forward and reversed version of the Human protein sequence database (<http://www.uniprot.org/>, UniProt. Release May 2017) containing all Human protein entries from Swiss-Prot and TrEMBL. Spectra were initially searched with a mass tolerance of 6 ppm in MS mode and 0.5 Da in MS/MS mode and strict trypsin specificity and allowing up to 2 missed cleavage sites. Cysteine carbamidomethylation was searched as a fixed modification, whereas N-acetyl protein, deamidated NQ, oxidized methionine were searched as variable modification. The resulting Andromeda peak list-output files were automatically loaded into inbuilt MaxQuant software modules for further processing, label free quantitation (LFQ) and a maximum false discovery rate of 1% was fixed for the result output files (Cox *et al.*, 2014). Recent progress in the field has led to development of robust software tools for

analysis of label free proteomics datasets. Furthermore, label free offers a higher dynamic range and simplicity of analysing large datasets (Cox *et al.*, 2014). We performed proteomics analysis of samples in three biological replicates. Data analysis was performed on log 2 normalised LFQ intensities and ANOVA with permutation based FDR (>0.05) was used to determine significantly altered proteins. Gene Ontology (GO) Enrichment analysis was performed using DAVID webtool (Huang da, Sherman & Lempicki, 2009a; Huang da, Sherman, Lempicki, 2009b). Enrichment of GO terms was considered statistically significant, corrected for multiple testing by the Benjamini-Hochberg method with adjusted p-values < 0.05 .

We performed ANOVA to look for significantly altered proteins across multiple conditions. However, individual t tests or post hoc analysis could be performed before carrying out further validation studies in future.

2.9 Statistics

Statistical analysis was performed on data from at least 3 independent experiments. For RQ-PCR samples were run in triplicate and then independent sample replicates performed in triplicate for publication. Students t-test was performed to identify significance between samples where $p < 0.05$ was deemed significant.

Chapter 3

Role(s) of CCN1 (CYR61) protein and cell cycle regulators in MCL progression

Chapter 3

3. CCN1 (CYR61) protein and cell cycle regulators in MCL progression

3.1 Introduction

Mantle cell lymphoma (MCL) is a rare subset of B-NHL combines the poor-risk features of both indolent lymphoma with its incurability and aggressive lymphoma with its ability to proliferate and disseminate rapidly (Deng, Lee & O'Connor, 2012). It is characterised by cyclin D1 overexpression as a result of chromosomal translocation of t(11;14) (Bertoni, Zucca & Cotter, 2004). However, cyclin D1-Negative expression has been identified in blastoid MCL (Zeng *et al.*, 2012). Furthermore, lack of cyclin D1 expression has been observed in a rare case of MCL but with cyclin D2 overexpression. In 2013, (Igawa *et al.*) have found that cyclin D2 overexpressed in 98% of de novo CD5-positive DLBCLs (50/51) and in 28% of CD5-negative DLBCLs (14/51) and suggested that “this insight may be useful for overcoming the inferior survival of this aggressive lymphoma”. Cyclin D2 expression protein could plays a role in pathogenesis of MCL instead of cyclin D1 but the mechanism is not clear (Fu *et al.*, 2005). On the other hand, MCL lack cyclin D1 expression is considered difficulty to identify this type of NHL (Mozos *et al.*, 2009). However, SOX11 has been identified as a reliable biomarker for MCL with cyclin D1- patients which prognosis aggressive variants (Narurkar, Alkayem & Liu, 2016). Moreover, patients with cyclin D1-/SOX11+ MCL presented advanced stage of disease and poor outcome (5 year OS, 48%) (Salaverria *et al.*, 2013). Additionally, SOX11+ expression has seen in patients with MCL and not expressed in other low-grade B-cell NHL which making SOX11 transcriptional factor a valuable tool to distinguish cyclin D1 negative MCL from other type of lymphoma (Carvajal-Cuenca *et al.*, 2012; Dictor *et al.*,

2009: Ek *et al.*, 2008: Fernandez *et al.*, 2010: Hsiao *et al.*, 2012: Mozos *et al.*, 2009: Zeng *et al.*, 2012). While cyclin D2 expression was identified in small B-cell lymphoma additionally to patients with MCL which indicating that this type of cyclin is not useful to identify patients with cyclin D1 negative MCL (Metcalf *et al.*, 2010: Mozos *et al.*, 2009).

Increasing evidence refers to association of deregulated cyclin D1 expression with secondary genetic alterations involved in MCL progression (Sander *et al.*, 2016). For example, mutations of CDKN2A and TP53 associated with high proliferation rate of lymphoid neoplasms are common in blastoid and aggressive MCL but not in indolent MCL (Fernández *et al.*, 2010; Royo *et al.*, 2011). In 2007, (Salaverria *et al.*) reported that overexpression and down regulation of cyclin D1 in progressive MCL “share the same secondary genetic alterations”.

In MCL, as a result of dysregulation of cyclin D1, the cell cycle and DNA damage responses are disrupted (Fernández *et al.*, 2005). p21 and p27 which are inhibitors of the cell cycle progression through binding to CDK-cyclin D complex have observed dysregulated in MCL (Muñoz-Alonso *et al.*, 2005; Pinyol *et al.*, 1997). p27 expression has correlated with high rate of survival in typical MCL (Kremer *et al.*, 2001) Furthermore, p27 down expression associated with low rate of survival of patients with aggressive MCL variant (Chiarle *et al.*, 2000). It was suggested that the lack of p27 expression results from enhanced proteasome degradation of p27 protein in aggressive MCL when they have seen that normal mRNA of p27 was expressed (Chiarle *et al.*, 2000). Conversely, Izban *et al.* (2000) have reported that there was no difference in p21 expression at early and advanced stages of MCL.

The goal of therapy in patients with MCL, is to prolong survival while minimizing both disease-related and treatment-related side effects (Martin, Ghione & Dreyling, 2017).

Increasing evidence focuses on the extrinsic signalling from surrounding cells in microenvironment of MCL to overcome treatment resistance that is correlated with disease progression (Chiron *et al.*, 2017; Papin, Le Gouill & Chiron, 2018). Treatment resistance in MCL is produced from the surrounding cells in the microenvironment, specifically stromal cells have been identified as responsible or partially responsible for recurrence/relapse in MCL (Pham *et al.*, 2014). CCN1 (CYR61) which belongs to CCN family of proteins is involved in tumour microenvironment and has a role in potentiating chemoresistance (Long *et al.*, 2015). CCN1 is involved in many stem cell signalling pathways and active within the bone marrow microenvironment where haematopoiesis ensues; CCN1 can activate the Wnt- β catenin-TCF4 signalling pathway in glioma cells (Xie *et al.*, 2004a) and induces Wnt3A osteoblast differentiation of mesenchymal stem cells (Si *et al.*, 2006). CCN1 in some cancers plays important roles in enhancing apoptosis, suppressing tumour growth, such as non-small-cell lung cancer (NSCLC) cell lines through activating the β -catenin-c-myc-p53-p21 signalling pathway (Tong *et al.*, 2004). Moreover, CCN1 enhances pancreatic cancer cell motility *in vitro* and cell tumorigenic growth *in vivo* by regulating sonic-Hedgehog through integrin-Notch-signalling pathway (Haque *et al.*, 2012). In this chapter, we have investigated the expression of CCN1 and cell cycle regulators Cyclin D1, p21 and p27 in progressive MCL.

3.2 Results

3.2.1 CCN1 expression is inversely correlated with MCL aggressiveness

In order to assess the potential role(s) of CCN1 in MCL, we have investigated CCN1 expression using RQ-PCR and Western blotting. REC1, G519, JVM2 human MCL cell

lines were used as a model for MCL disease progression in the order from indolent to aggressive disease REC1<G519<JVM2 respectively.

RQ-PCR for CCN1 expression showed that JVM2 cells had the lowest CCN1 expression with a CT value 32.14573288. JVM2 expression was then set to a fold change of 1.0 to enable calculation of fold change for REC1 and G519 cells using the $\delta\delta$ CT method. CCN1 expression was high in REC1 cells and sequentially decreased in progressive G519 and JVM2 cells. The fold change of CCN1 in MCL cell lines REC1, G519 and JVM2 was 23.5, 7.0 and 1.0 respectively (Figure 3.1).

Investigation of CCN1 total protein expression using western blot analysis showed CCN1 protein expression at the following approximate molecular weights; 42kDa consistent with expression of full length CCN1, 28-30kDa and 18-20kDa consistent with expression of truncated isoforms. Expression of full-length CCN1 barely altered through the cell lines however, expression of the truncated isoform (18-20kDa) was high in REC1 cells (OD:1.0) reduced in G519 cells (OD:0.5) and barely detected in JVM2 cells (Figure 3.2A and B). Reports from a previous study (Choi *et al.*, 2013) suggests the 28-30kDa moiety could comprise the SP, IGFBP, VWC and TSP-1 domains whilst the 18-20 kDa moiety could be either the SP, IGFBP, VWC fragment or TSP-1 and CT fragment, or potentially a mix of both (Figure 3.3).

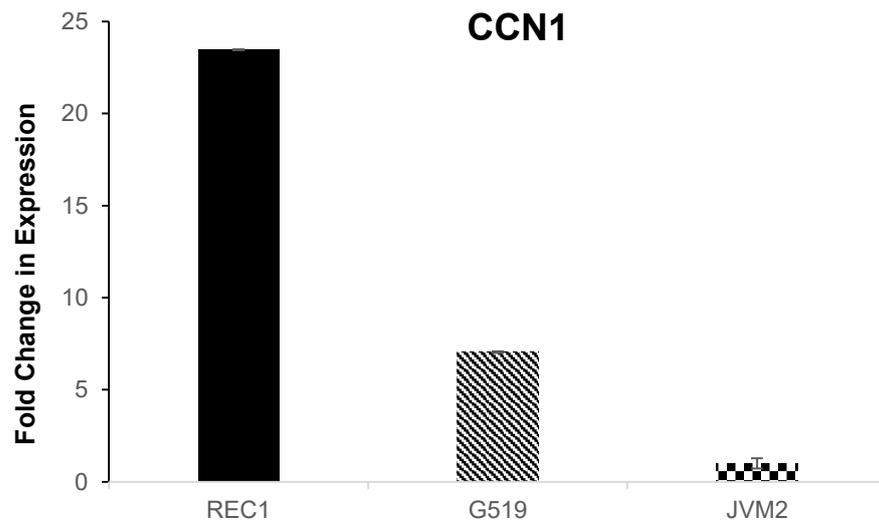
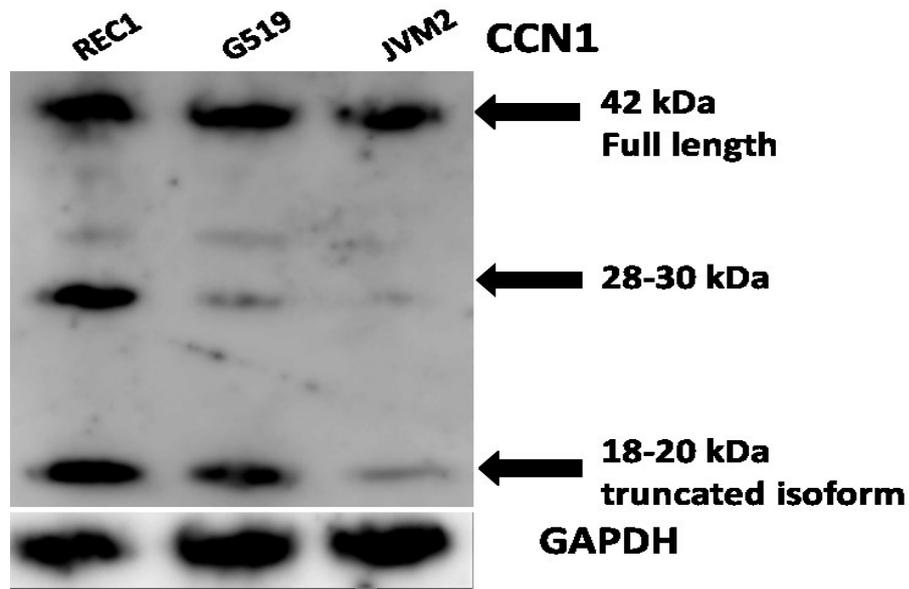


Figure 3.1: RQ-PCR screen of MCL cell lines for CCN1 expression shows that CCN1 expression is upregulated in the low-aggression phenotype (REC1) and down regulated in aggressive disease (G519 and JVM2). The fold change of CCN1 expression in MCL cell lines was 23.5 in REC1, 7.0 in G519 and 1.0 in JVM2. GAPDH is the housekeeping gene, data generated were normalised against GAPDH control (n=3 independent samples).

A



B

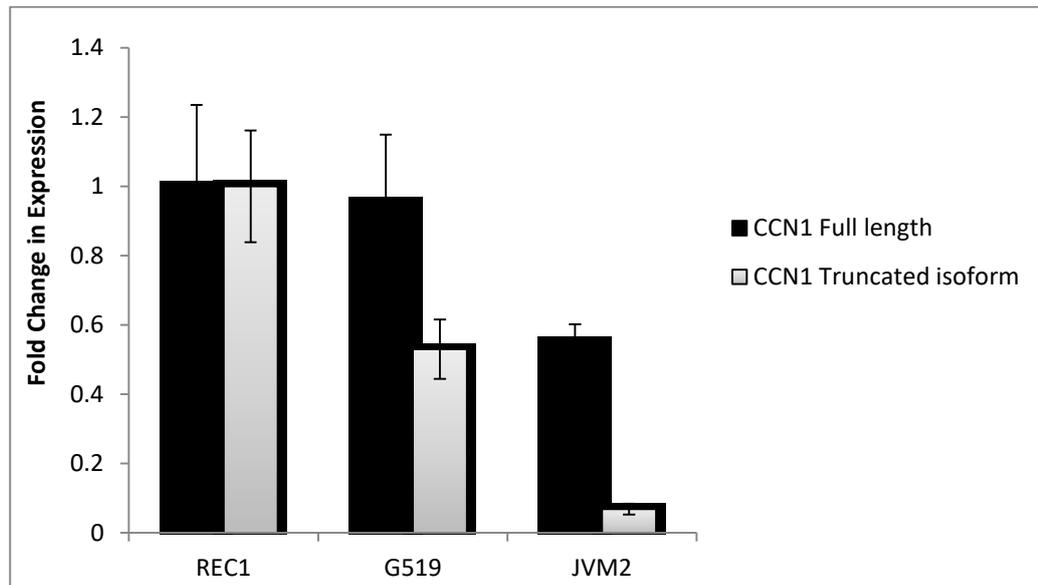


Figure 3.2: Western blot analysis of CCN1 expression for the MCL cell lines, REC1, G519 and JVM2 (A) Western blot showed full length CCN1 protein expression with 42 kDa barely altered in MCL progression. However, expression of truncated isoforms 28-30kDa and 18-20kDa were high in early stage REC1 and decreased in advanced stages G519 and JVM2. (B) Optical densitometry for CCN1 band pattern by Western Blotting expression shown expression of full-length CCN1 barely altered through the cell lines however, expression of the truncated isoform (18-20kDa) was high in REC1 cells (OD:1.0) reduced in G519 cells (OD:0.5) and barely detected in JVM2 cells. GAPDH was used as a loading control, data generated were normalised against GAPDH control (n=3 independent samples).

CCN1 domain structure

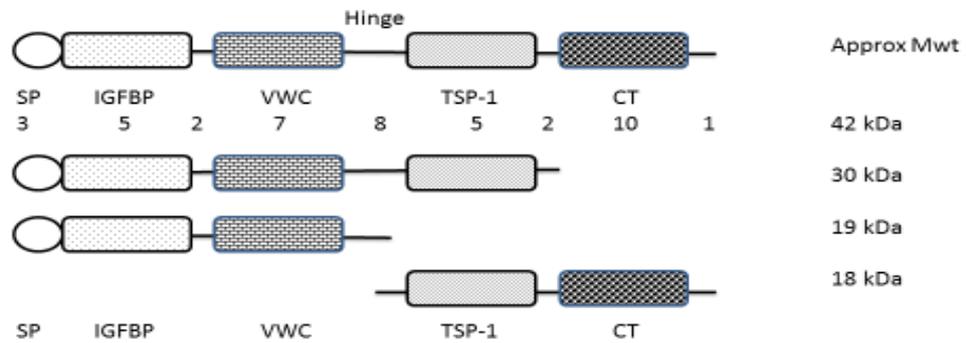


Figure 3.3: CCN1 domain structure of full length and potential truncated CCN1 proteins. The molecular weight (Mwt) of full length of CCN1 protein is 42 kDa that involves 5 kDa of an insulin-like growth factor binding protein domain (IGFBP), 7 kDa of von Willebrand type C repeat (VWC), 5 kDa of thrombospondin type 1 domain (TSP-1) and 10 kDa of cysteine knot carboxyl terminal (CT). Furthermore, 8 kDa of Hinge that contacts the N-terminus and C-terminus and 3 kDa of signal peptide (SP). The potential truncated CCN1 isoforms include C-terminus (IGFBP and VWC) with 19 kDa, N-terminus (TSP-1 and CT) with 18 kDa and C-terminus plus TSP-1 with 30 kDa. Adapted from (Choi *et al.*, 2013).

3.2.2 Cyclin D1 is downregulated in progressive MCL

We have investigated cyclin D1 expression using RQ-PCR and Western blotting using total protein extracts from the three MCL cell lines; REC1, G519 and JVM2. RQ-PCR shows cyclin D1 expression is high in REC1 cells and decreased in G519 and JVM2 cells consistent with deregulation of cyclin D1 in aggressive disease. Fold changes in cyclin D1 expression were 10.1, 4.6 and 1.0 for REC1, G519 and JVM2 respectively (Figure 3.4).

Cyclin D1 total protein expression mirrored that of the gene expression where cyclin D1 was highly expressed in the REC1 cells (OD: 1.0), reduced to one fifth in the G519 cells (OD: 0.2) and not detected by western blotting in the JVM2 cell line (Figure 3.5A and B).

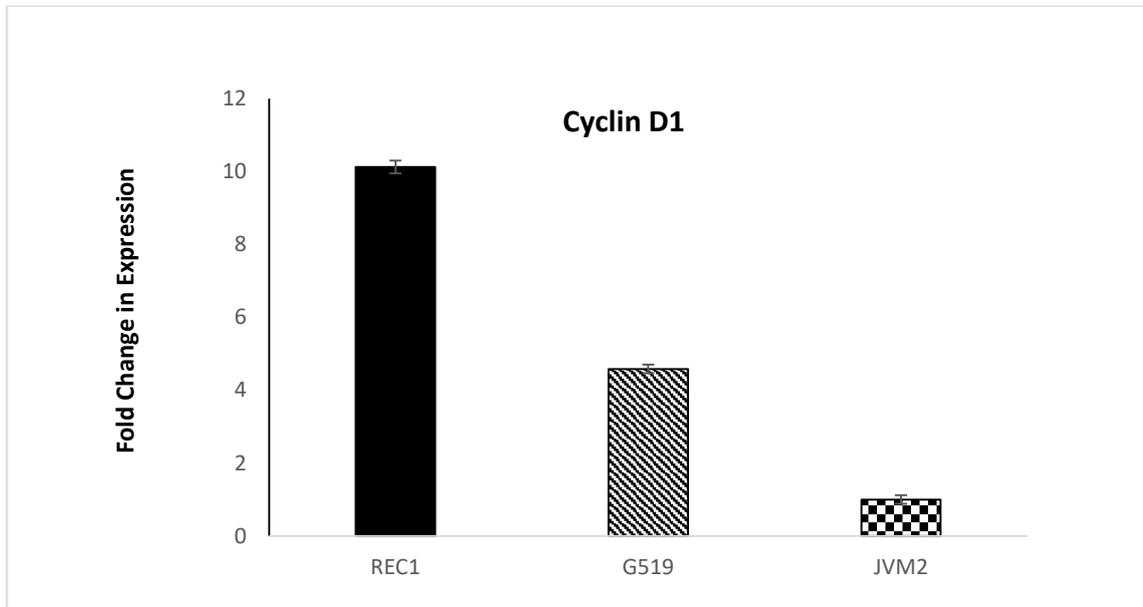
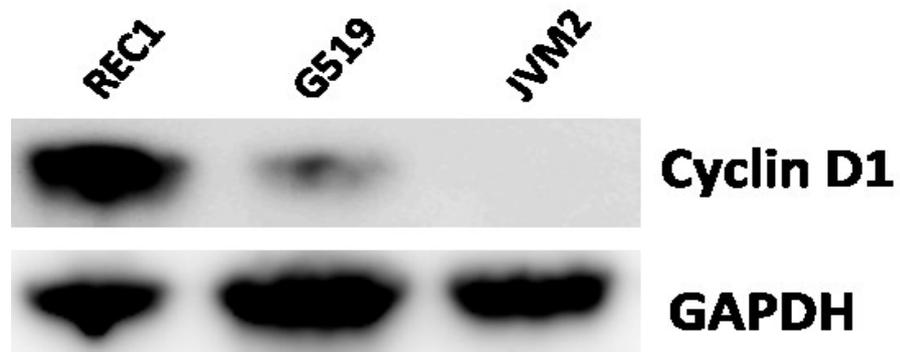


Figure 3.4: RQ-PCR screen of Cyclin D1 expression in MCL progression shown fold change in cyclin D1 expression in early stage of MCL (REC1) and down regulation in advanced stage of disease (G519 and JVM2). Fold changes in cyclin D1 expression were 10.1, 4.6 and 1.0 for REC1, G519 and JVM2 respectively. GAPDH was the housekeeping gene and data generated were normalised against GAPDH control (n=3 independent samples).

A



B

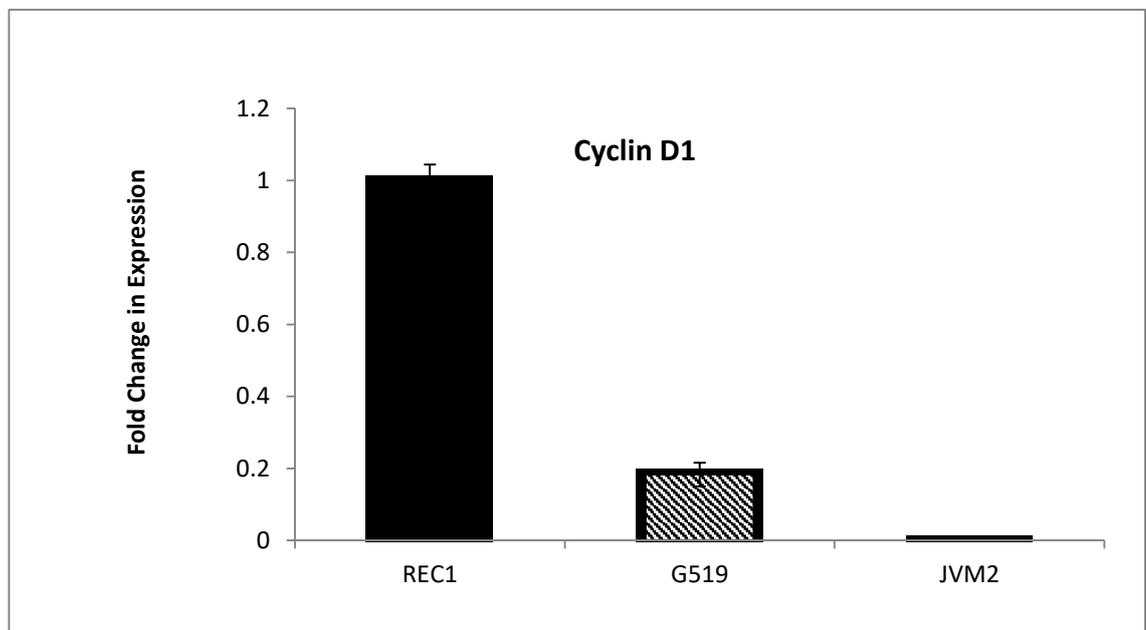


Figure 3.5: Western blotting for expression of total protein for cyclin D1 in MCL cell lines. (A) Western blot depicts high expression of cyclin D1 in REC1, decreases in G519 and barely detected in JVM2. **(B)** Optical densitometry for western blotting presents the expression of cyclin D1 total protein was highly expressed in the Rec1 cells (OD: 1.0), reduced to one fifth in the G519 cells (OD: 0.2) and not detected by western blotting in the JVM2 cell line. Optical densitometry was performed on banding and data generated normalised against GAPDH loading control (n=3 independent replicates).

3.2.3 High expression of p21^{CIP1} and down regulation of p27^{KIP1} in aggressive MCL

Cyclin dependent kinase inhibitors, p21^{CIP1} and p27^{KIP1}, were also investigated for an involvement in disease progression.

RQ-PCR shows increasing expression of p21 with disease progression (Figure 3.6) whilst expression levels of p27 are decreased with disease progression in G519 and JVM2 cells (Figures 3.8). Total cell lysates showed that p21^{CIP1} was not detected in REC1 cells but had increasing expression in G519 (OD: 0.6) and JVM2 cells (OD: 1.0) (Figure 3.7A and B). Whilst RQ-PCR and western blotting show that p27^{KIP1} expression was high in the REC1 and decreased with disease progression in G519 and JVM2 cells (Figures 3.8 and 3.9A). Total cell lysates showed that p27^{KIP1} was high expressed in REC1 (OD: 1.5) and decreased in G519 (OD: 1.0) and not detected in JVM2 cells (Figure 3.9B).

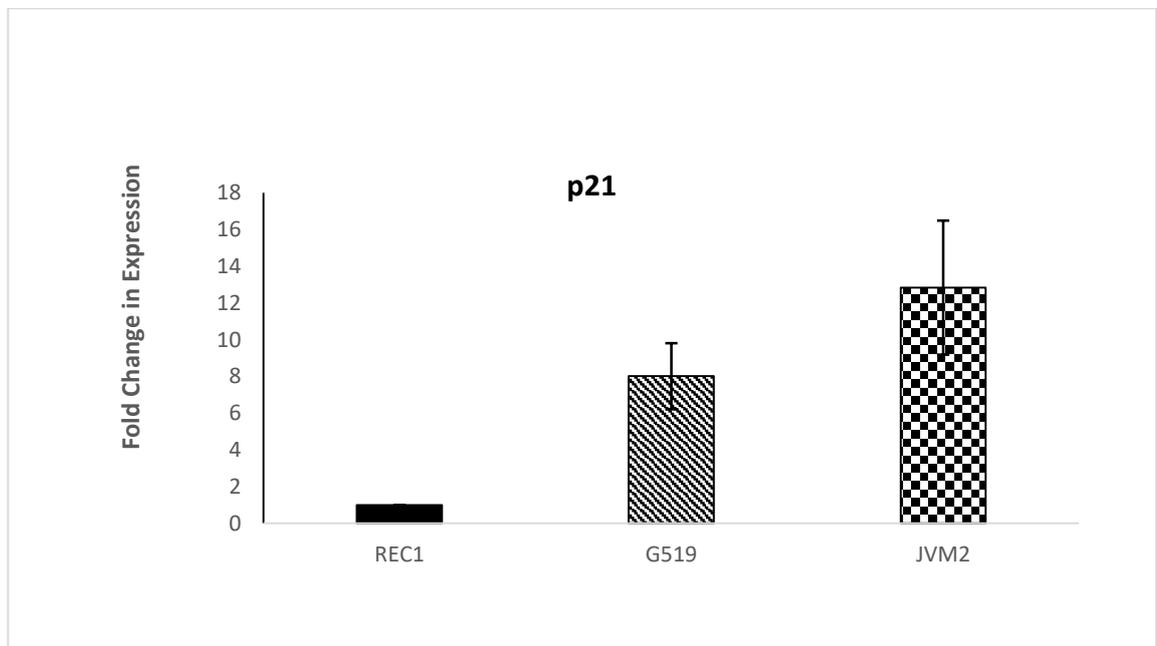
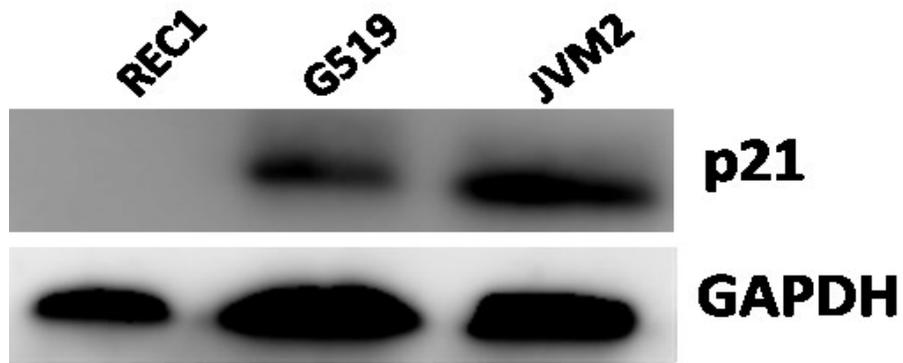


Figure 3.6: RQ-PCR screen p21 expression in MCL cell lines shown disease progression involved upregulation of p21 expression in the high-aggressive MCL and down regulation in low-aggressive disease. Fold change of p21 expression was 12.8 in JVM2 and reduced to 8.0 in G519 and 1.0 in REC1 mirrors association of p21 expression with progression disease. GAPDH was used the housekeeping gene and data generated were normalised against GAPDH control (n=3 independent samples).

A



B

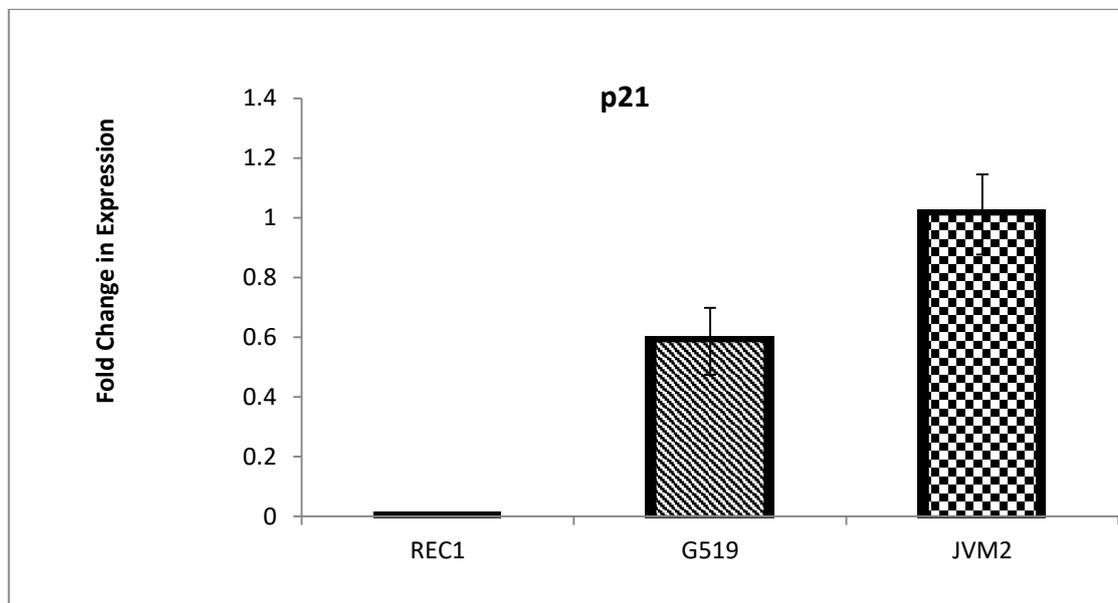


Figure 3.7: Western blotting for expression of total protein for p21 in MCL cell lines. (A) Western blot shows high expression of p21 in JVM2, decreases in G519 and not detected in REC1. **(B)** Optical densitometry for western blotting presents the expression of p21 total protein was highly expressed in the JVM2 cells (OD: 0.6), reduced in the G519 cells (OD: 1.0) and not detected by western blotting in the REC1 cell line. Optical densitometry was performed on banding and data generated normalised against GAPDH loading control (n=3 independent replicates).

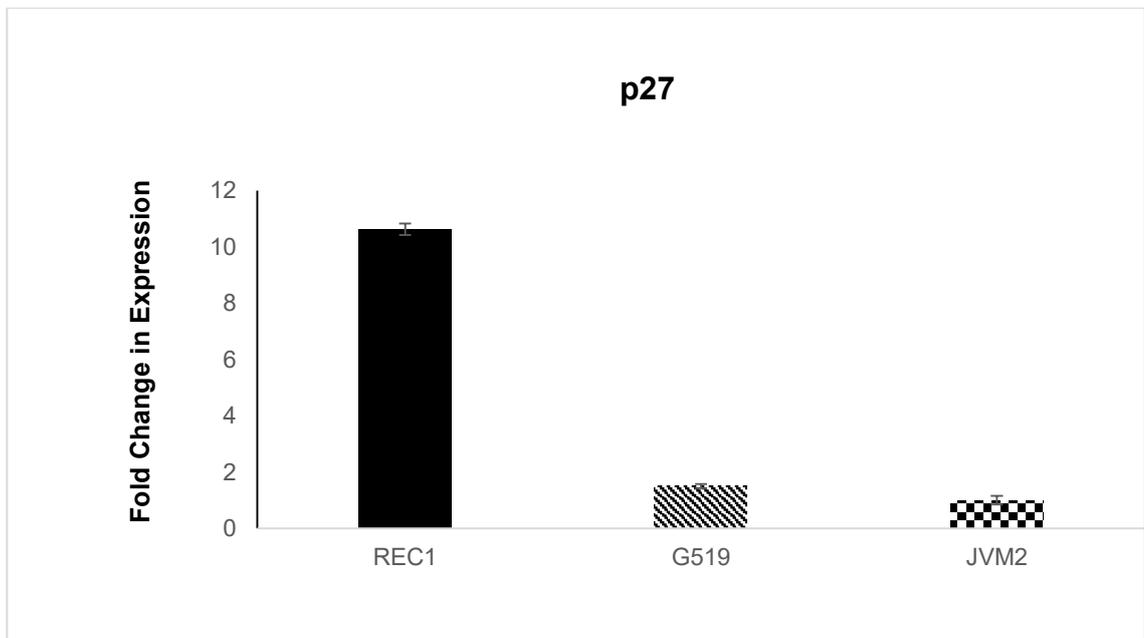
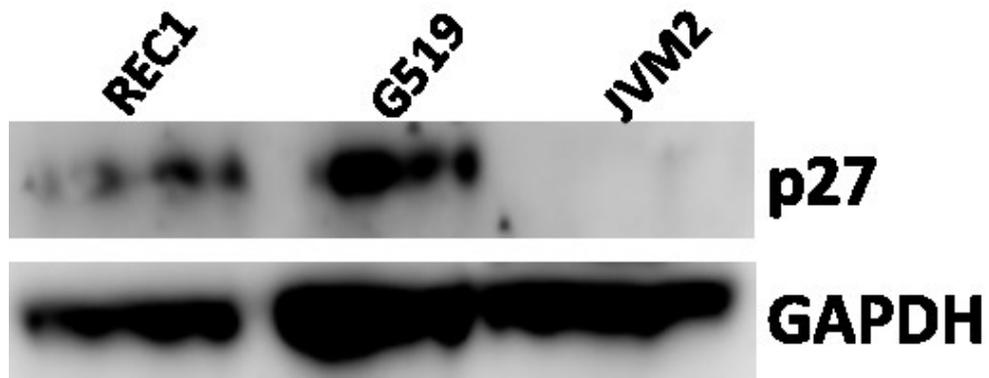


Figure 3.8: RQ-PCR screen p27 expression in MCL cell lines shown p27^{KIP1} expression was high in the REC1 and decreased with disease progression in G519 and JVM2 cells. Fold change of p27 was 10.6 in the REC1 decreased to 1.5 in G519 and 1.0 in JVM2 cells. GAPDH was used the housekeeping gene, data generated were normalised against GAPDH control (n=3 independent samples).

A



B

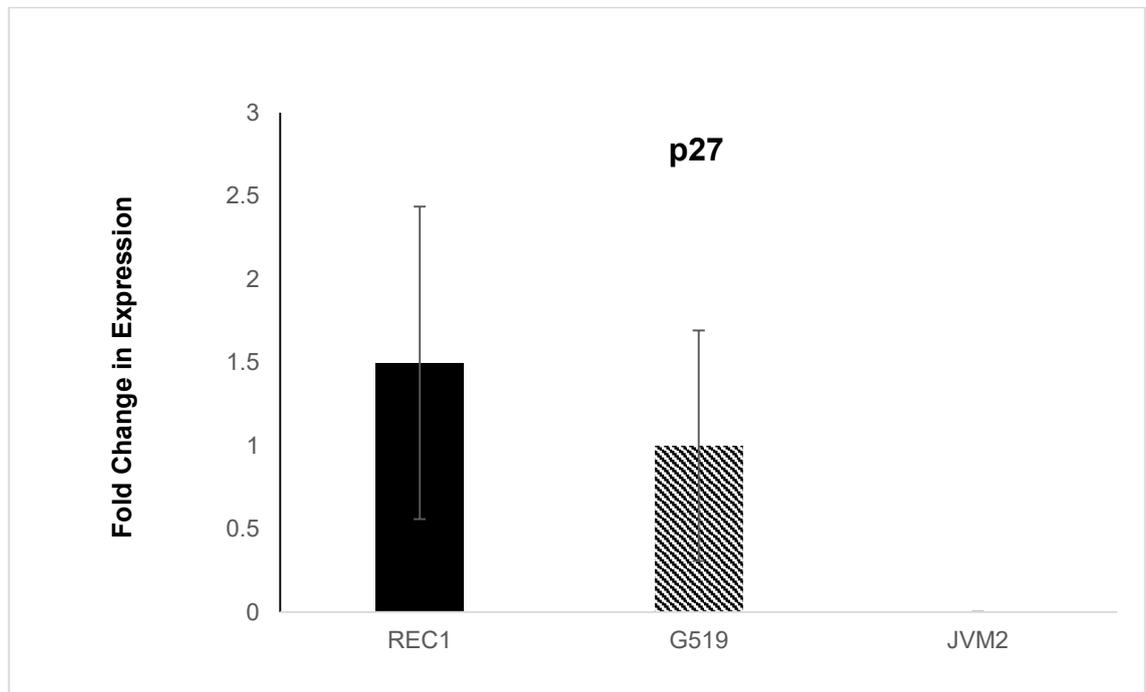
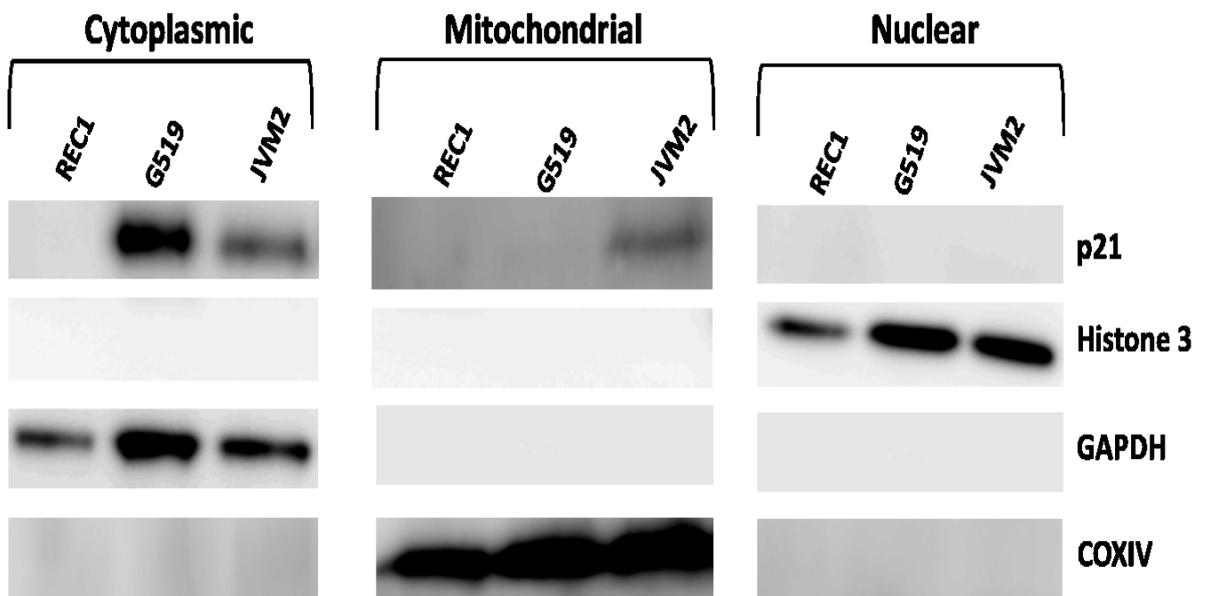


Figure 3.9: Western blot of total protein of p27 expression in MCL cell lines REC1, G519 and JVM2. (A) Western blot shown that p27 expression total protein decreased with disease progression and increased with early stage of disease. (B) Total cell lysates showed that p27^{KIP1} was high expressed in REC1 (OD: 1.5) and decreased in G519 (OD: 1.0) and not detected in JVM2 cells. Optical densitometry was performed on banding and data generated normalised against GAPDH loading control (n=3 independent replicates).

3.2.4 Altered subcellular localization of p21^{CIP1} and p27^{KIP1} portray resistance in progressive MCL

To investigate the subcellular localization of p21^{CIP1} and p27^{KIP1} in MCL, we extracted cytoplasmic, mitochondrial and nuclear protein from cells and performed western blotting. Expression of p21^{CIP1} was not detected in any fraction for early stage REC1 cells. For the progressive G519 and JVM2 cells, p21^{CIP1} was primarily expressed in the cytoplasm and was not detected in the nucleus. Expression of p21^{CIP1} was detected in the mitochondrial fraction for JVM2 cells (Figure 3.10 A). Blotting for GAPDH (cytoplasmic), COX IV (Mitochondrial) and Histone 3 (Nuclear) markers were used as controls for each fraction. For p27^{KIP1}, expression was only detected in the cytoplasmic fraction and not in mitochondrial or nuclear fractions (figure 3.10 B). GAPDH (cytoplasmic), COX IV (Mitochondrial) and Histone 3 (Nuclear) markers were used as loading controls and to ensure clean fractions were obtained for each, without cross contamination during the fractionation process.

A



B

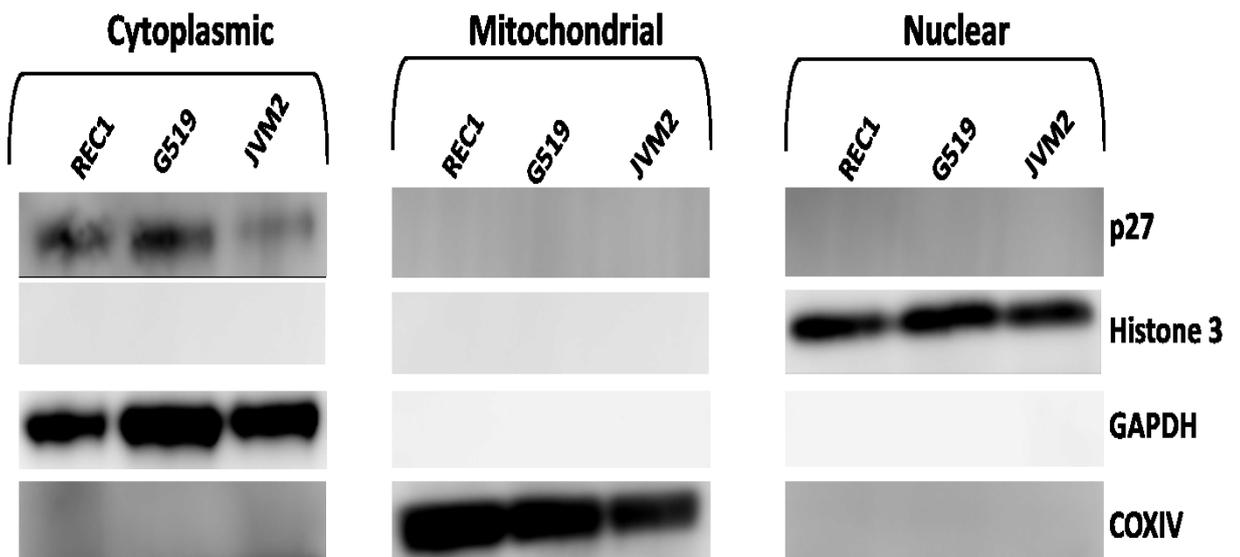


Figure 3.10: Subcellular localisation of p21 and p27 in MCL. (A) Western blot for p21 cellular content for cytoplasmic, mitochondrial and nuclear fractions for REC1, G519 and JVM2 cells. P21 was not detected in nuclear of all MCL cell lines. It is disappeared in early stage disease REC1, detected in the cytoplasm of advanced stage G519 and JVM2 and in mitochondria of JVM2. (B) Western blot for p27 cellular content for cytoplasmic, mitochondrial and nuclear fractions for REC1, G519 and JVM2 cells. P27 expression was detected in cytoplasm of MCL cell lines with decreased in expression with disease progression. It is disappeared in nucleus and mitochondria of all stages of MCL cell lines. GAPDH, Histone 3 and COXIV were used as a loading control and to ensure clean fractions free from cross contamination during the fractionation process were obtained (n=3 independent samples).

3.3 Discussion

MCL is considered an aggressive disease and has one of the worst outcomes among B-cell lymphomas due to the dysregulation of the DNA damage response pathways accompanied with abnormal cell survival mechanisms suppressing apoptosis (Campo & Rule, 2015; Moros *et al.*, 2014). MCL characterised by overexpression of cyclin D1 as a result of t(11;14) chromosomal translocation that leads to dysregulation of the cell cycle (O'Connor, 2007; Zucca & Bertoni, 2013). Furthermore, MCL is characterised by clinical course where variants are subdivided into indolent form (classic morphology) and aggressive (blastoid or pleomorphic appearance) (Kridel *et al.*, 2012). The heterogeneous biology and aggressive behaviour of MCL present a challenge for designing standard therapies (Smith, 2011) and therefore require further investigation to identify more effective treatment strategies.

In this study, we have investigated the role(s) of CCN1 in MCL and investigated cell cycle regulation using expression of cyclin D1, p21^{CIP1} and p27^{KIP1} in the a cell line model representing disease progression in MCL where the magnitude of aggressive behaviour is in the order REC1<G519<JVM2. CCN1 dysregulation was identified in MCL progression where CCN1 was highly expressed in the REC1 cells and reduced in the aggressive G519 and JVM2 cells. CCN1 expression decreases with disease progression. We found that the lower expression of CCN1 in aggressive MCL cell lines (G519 and JVM2) is additional risk factor for disease progression. However, many studies have indicated that the high expression of CCN1 is implicated in disease progression, tumorigenesis and invasion of hepatocellular carcinoma (HCC) (Li *et al.*, 2012), breast cancer (O'Kelly *et al.*, 2008), prostatic carcinoma (D'Antonio *et al.*, 2010), gliomas (Xie *et al.*, 2004b) and gastric cancer (Lin *et al.*, 2007).

Further investigation demonstrated that whilst full-length CCN1 remains relatively constant within the cell lines, the 18-20 kDa truncated form is decreased within aggressive G519 and JVM2 cells. Previous reports identify that this truncated form could potentially be a fragment consisting of the SP-IGFBP-VWC domains or the TSP-1-CT domains (Choi *et al.*, 2013), the latter would infer that CCN1 transcriptional control is lost within aggressive MCL. In 2006, (Planque *et al.*) have found that full length of CCN3 inhibited cell growth while truncated isoform induced “morphological transformation of chicken embryo fibroblast” and suggested a role in oncogenic activities. Moreover, truncated isoform of CCN3 translocated to the nucleus of cancer cell lines which is supporting of CT-CCN3 being involved in transcriptional regulation. Increasing evidence indicates the Nuclear Localisation Signal (NLS), a short peptide motif mediates nuclear localisation of proteins, located at the CT module in the CCN3 (lysine-rich PTDKKGKKCLRTRTKKSLKA) which is responsible for CT-translocated in the nucleus (Planque, 2006). Perbal (1999) found nuclear localisation of truncated isoform of NOV protein (31/32kDa) in the nucleus of 143 and HeLa cells. Furthermore, it is suggested the truncated isoform may be N-terminus which probably has a role in the gene expression of target cells because it is used antibodies against the C-terminus of NOV protein (KKGKKCLRTRTKKS). CCN1 (CYR61) and CCN3 (NOV) are members in the CCN cell growth regulators family sharing 40%-60% amino acid homology suggesting that the truncated isoform of CCN1 may be targeted to the nucleus and playing a role in the gene regulation (Perbal, 1999; Planque & Perbal, 2003). Furthermore, the CCN3 NLS region is highly conserved in CCN1 KKGKKCSKTKKS (Planque, 2006) which suggests the CT-CCN1 truncated isoform may translocate to the nucleus of MCL cell lines. This is consistent with CCN1 found in the nucleus of bladder smooth muscle cells (Chen & Du, 2007; Tamura *et al.*, 2001).

Full length CCN proteins can play an anti-proliferative role, while truncated isoforms may induce tumour proliferation (Planque & Perbal, 2003). The truncated isoform has wider biological functions owing to the CCN1 partition into the soluble phase (28kDa) diffusing freely within tissue and may act as an antagonist towards the full-length form in the insoluble matrix (42kDa) (Pendurthi *et al.*, 2005). For example, CCN1 is cleaved by plasmin and released a truncated isoform of CCN1 (28kDa) which may support endothelial cell migration in breast carcinoma cells (Pendurthi *et al.*, 2005). In 2013, (Choi *et al.*), found the CCN1 truncated isoform (11-23kDa) expressed in diabetic retinopathy patients instead of the full-length protein 42kDa. It is also postulated that the truncated form of CCN1 can also arise due to alternative mRNA splicing (Perbal, 2009).

In this study, Cyclin D1 was also deregulated in MCL and our findings are consistent with previous reports of cyclin D1 down regulation and disease progression (Peng, Chou & Hsu, 1998). Interestingly, Saglam *et al.* (2014) have demonstrated that the up regulation of cyclin D1 and p53 are activated by the CCN1 pathway in high-grade ductal carcinoma in situ (DCIS). Consistent with these findings, we showed a positive association between CCN1 and cyclin D1 expression in all MCL cell lines. In other solid tumours, where cyclin D1 is overexpressed; breast, liver, lung, and brain cancer (Gillett *et al.*, 1996; Hall & Peters, 1996; Molenaar *et al.*, 2008) requires consistent signalling from the extracellular matrix and growth factors (Assoian & Klein, 2008).

MCL progression involving down regulation of cyclin D1 and the up regulation of p21^{CIP1} may contribute to treatment resistance (Abukhdeir & Park, 2008). We have shown that p21^{CIP1} levels increase with disease progression. This is consistent with other studies that report overexpression of p21^{CIP1} was correlated with tumour progression; in breast (Ceccarelli *et al.*, 2001) and ovarian carcinoma (Ferrandina *et al.*, 2000) and in brain tumours (Jung *et al.*, 1995).

We have shown that p27^{KIP1} levels decrease with disease progression also consistent with findings from Izban *et al.* (2000), where p27^{KIP1} was overexpressed in early stage disease (typical MCL) and down regulated in aggressive stage (blastoid variants) where it was associated with a high proliferation rate of blastoid MCL. Conversely, in 1998 (Quintanilla-Martinez *et al.*) showed expression of p27^{KIP1} was inversely associated with the proliferation rate of MCL cells; undetected in typical MCL cells (classic disease) associated with low proliferation rate but was overexpressed in the blastic variant of MCL cells (aggressive disease) with higher proliferation rate.

P27^{KIP1} and p21^{CIP1} have important roles in promotion of assembly of CCND-CDK4/6 complexes (LaBaer *et al.*, 1997). This interaction leads to sequestration of p27^{KIP1} in CCND-CDK4 complex which blocks inhibition of the CCNE-CDK2 complex (Perez - Roger *et al.*, 1999). Furthermore, investigation of p21^{CIP1} expression in MCL progression showed down regulation at early stage disease and overexpression at advanced stage that mirrors its roles in MCL progression.

More importantly, in cancer, the tumour suppressor function of p21^{CIP1} and p27^{KIP1} depends on their nuclear localization (Jeannot *et al.*, 2015; Romanov, Pospelov & Pospelova, 2012). Many studies have found that phosphorylation of specific sites of p27^{KIP1} and p21^{CIP1} lead to their export into the cytoplasm where they can act as a tumour promoters and induce drug resistance (Ohkoshi, Yano & Matsuda, 2015; Zhao *et al.*, 2014). Furthermore, calmodulin plays important role in p21 accumulation in the nucleus but not p27 by binding to p21 and prevents phosphorylation of p21 at Ser153 (Rodríguez-Vilarrupla *et al.*, 2005; Taulés *et al.*, 1999). In this study, p21^{CIP1} and p27^{KIP1} were found in the cytoplasmic fractions and absent in nuclear fractions of all three cell lines (REC1, G519 and JVM2). This suggests in MCL, p21^{CIP1} and p27^{KIP1} have lost their tumour suppressor roles and acquired tumour promoter roles by localisation to the cytoplasm.

Whilst mutation of p21^{CIP1} has not been investigated here, mutation of the p21^{CIP1} gene frequently occurs in cancer cells leading to inactivation of p21^{CIP1} with loss of function to block the cell cycle, even when overexpressed (Lu *et al.*, 1998b; Lukas *et al.*, 1997). While regulation of p27^{KIP1} is different from other cell cycle inhibitors, p27^{KIP1} gene mutation is rare (Garrett-Engele *et al.*, 2007a).

CCN1 likely plays key role(s) in haematopoiesis and in B cell development through modulation of stem cell signalling pathways, TGF β , BMP, Notch, Wnt- β catenin (McCallum & Irvine, 2009; Wells *et al.*, 2015). CCN1 roles within haematological malignancy show CCN1 promotes survival and inhibits apoptosis in AML (Niu *et al.*, 2014) and overexpression of CCN1 in multiple myeloma (MM) postponed tumour growth and suppressed bone destruction (Johnson *et al.*, 2014). Recently, in 2018, (Niu *et al.*) have found the “genetic polymorphisms in the CYR61 gene may be considered potential AML risk factors in the Han Chinese population”. Additionally, CCN1 expression associated with osteosarcoma progression by promoting cell migration and metastasis through Raf-1/MEK/ERK/Elk-1/TWIST-1 signaling pathway (Hou *et al.*, 2014). CCN1 involved in the pathogenesis of acute lymphoblastic leukemia (ALL) by overexpression in the plasma and bone marrow samples from ALL patients compared with samples from normal control. Resulting in promoting tumour survival through the AKT/NF- κ B pathway by up-regulating Bcl-2 (Zhu *et al.*, 2016). CCN1 also decreases bone resorption by inducing osteoblastogenesis and suppressing osteoclastogenesis (Roodman, 2014). CCN1 may interact with other growth factors such as bone morphogenetic proteins (BMP), transforming growth factor β (TGF- β) and vascular endothelial growth factor (VEGF) which need more investigation (Lau, 2011). Recently, it has been seen that ALK5 suppression prevents TGF- β -induced CCN1 expression in human dermal fibroblasts (Thompson, Murphy-Marshman & Leask, 2014). More importantly, CCN1 is a

transcriptional target of TGF- β and may potentiate an autocrine regulatory mechanism in tumorigenesis (Bartholin *et al.*, 2007).

In conclusion, CCN1 appears to have a role in cell cycle regulation in progressive MCL. However, low expression of the truncated CCN1 protein in progressive MCL together with p21 / p27 changes in expression and subcellular localisation of p21 and p27 may contribute to aggressive disease and treatment resistance.

Chapter 4

CCN1 stable overexpression and knockdown models in MCL cell lines

Chapter 4

4. CCN1 overexpression and knockdown models in MCL cell lines

CCN1 expression is high in REC1 cells and reduced in aggressive JVM2 cells (Figure 3.1). In order to assess the role of CCN1 in disease progression, we used genetic modification of the cells lines as follows; knockdown CCN1 sequence in REC1 cells and CCN1 overexpression in JVM2 cells by lentivirus.

Western blot analysis of CCN1 expression for the MCL cell lines, REC1, G519 and JVM2 showed full length CCN1 protein expression with 42 kDa barely altered in MCL progression. However, expression of truncated isoforms 28-30kDa and 18-20kDa were high in early stage REC1 and decreased in advanced stages G519 and JVM2 as described at chapter 3 (Figure 3.2).

4.1 Introduction

Lentivirus expression has been chosen to transduce notoriously difficult to genetically modify haematopoietic cell lines, as this system has a higher efficacy of transduction with less toxicity.

In first attempt, JVM2 cells were seeded in 6 flasks 2 of them were infected by pLenti-hCYR61 lentivirus, 2 were infected by pLenti-Empty vector or Blank Control and 2 of flasks were used as negative control as described at (2.5.8.1). After successfully forming fluorescence, puromycin was added gradually on concentrations (0.1, 0.25, 0.5, 1, 2, 4, 8, 10 and 20) $\mu\text{g/ml}$. After 2 months adding puromycin to select transduced cells we noticed the negative control cells still viable and increased in number, so we stopped adding puromycin and cells were left to grow. After 1 month all the cells were dead, even

transduced cells were died. This problem occurred in second and third attempt with parent JVM2 (wild type) cells resistant to puromycin, therefore requiring further optimisation.

4.2 Optimisation of lentivirus delivery system

To identify the optimal antibiotic concentration that enabled selection of transduced clones for the stable overexpression and knockdown models, kill curves for REC1 and JVM2 cells were performed as described at (2.5.6). For REC1, cells were resistant to puromycin on concentration 0.1-2 $\mu\text{g/ml}$ and sensitive to 6-10 $\mu\text{g/ml}$ in the first dose of adding puromycin. Cells were sensitive to 4-5 $\mu\text{g/ml}$ concentration of puromycin after 25 days adding antibiotic. 3 $\mu\text{g/ml}$ of puromycin concentration was the minimum antibiotic concentration that killed the cells in all cell densities that used in this study (Figure 4.1).

For JVM2 cells, the cells were sensitive to 8, 9 and 10 $\mu\text{g/ml}$ of puromycin in the first dose and then resistance appeared in all concentrations after 25 days adding puromycin (Figure 4.2).

Since JVM2 cell line is resistant to antibiotic selection (puromycin) we have chosen single cell sorting to select transduced cells. This experiment was performed with parent JVM2 cells (without lentivirus infection) using CFSE kit as described at (2.5.7) to optimise the cell density and flow cytometry setting. The parameters for flow cytometry were Forward-scattered light (FSC), Side-scattered light (SSC) and Fluorescein isothiocyanate (FITC) were 155, 500 and 220 nm respectively (Figure 4. 3 A and B).

Figure 4.1: Kill curve of REC1 cells: Seeding density for REC1 cells was titred against puromycin concentration to ensure CCN1 knock down clones were obtained. Cell viability after addition of puromycin at 2 days (blue) and after 25 days (red) compared to cell seeding density (grey).

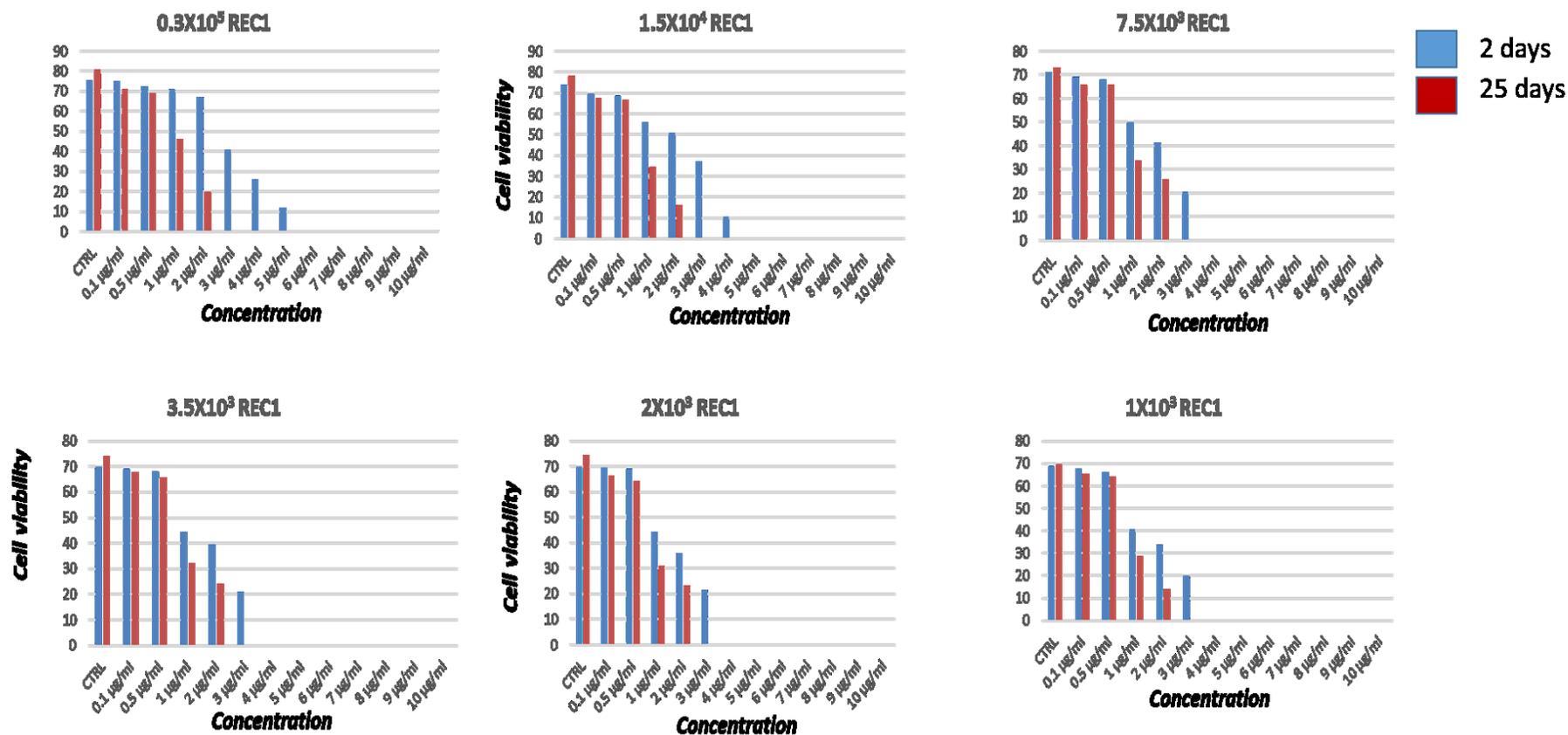
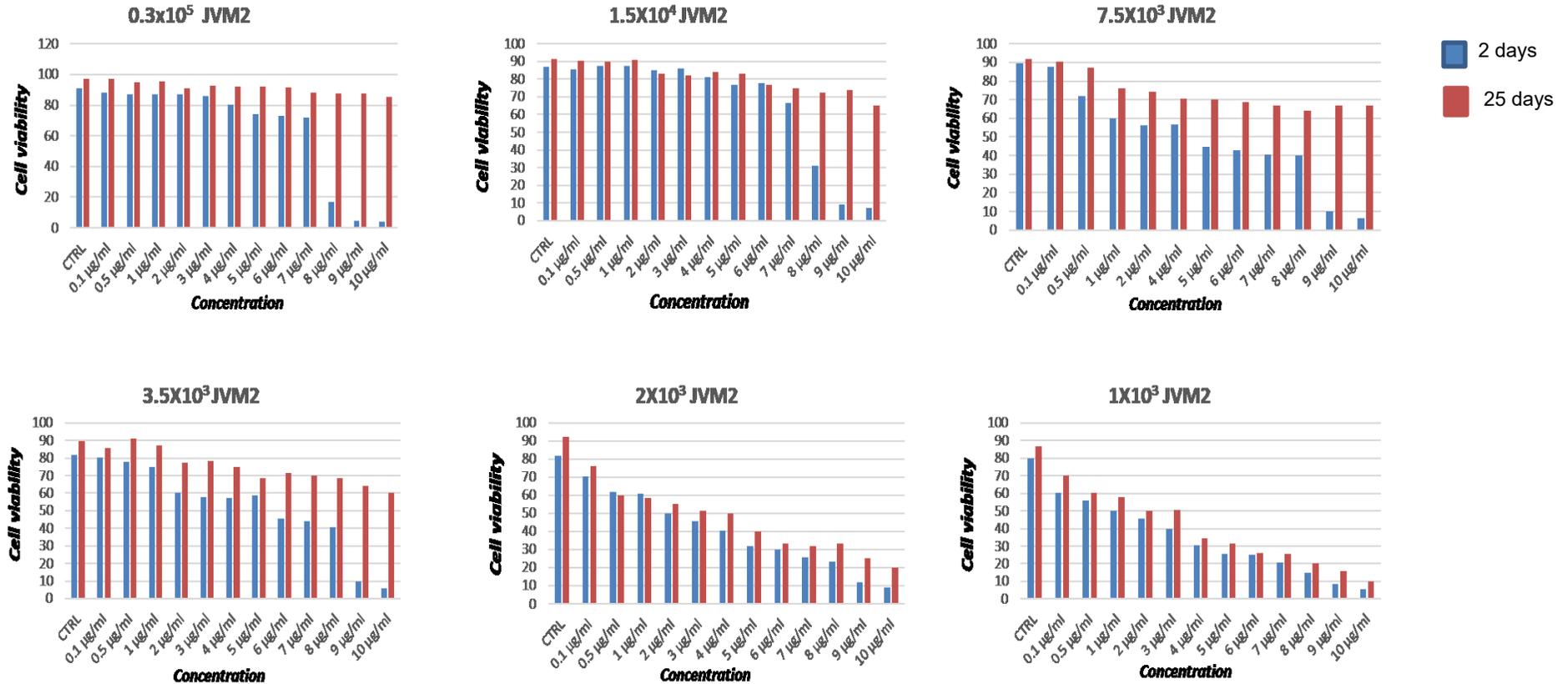


Figure 4.2 Kill curve of JVM2 cells: Seeding density for JVM2 cells was titred against puromycin concentration to ensure CCN1 overexpression clones were obtained. Cell viability after addition of puromycin at 2 days (blue) and after 25 days (red) compared to cell seeding density (grey).



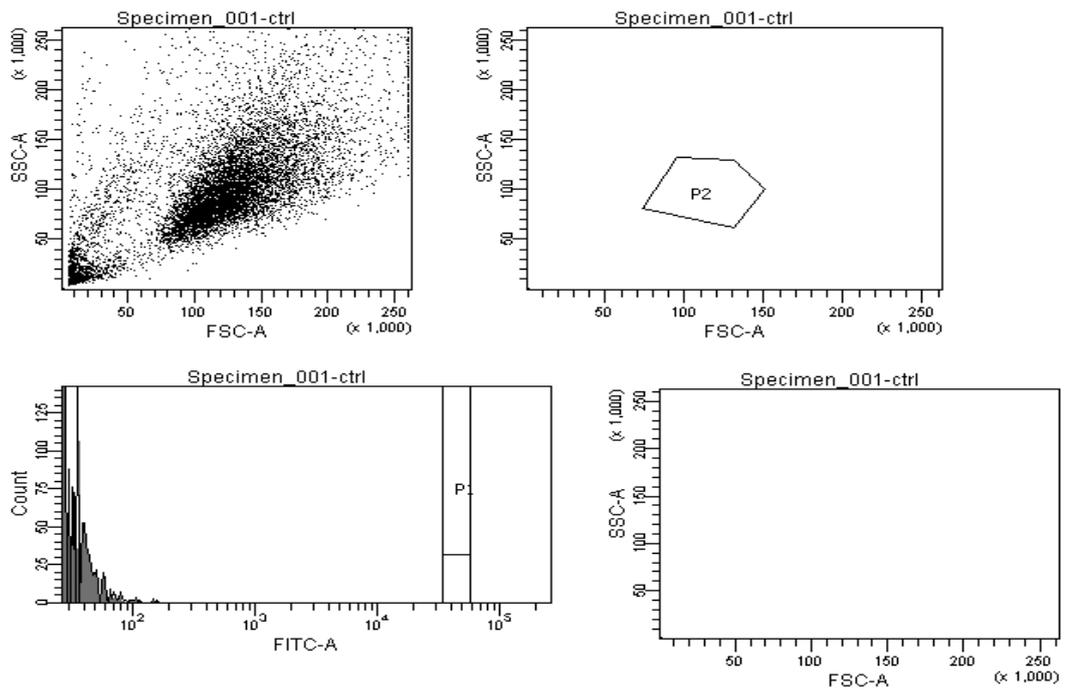
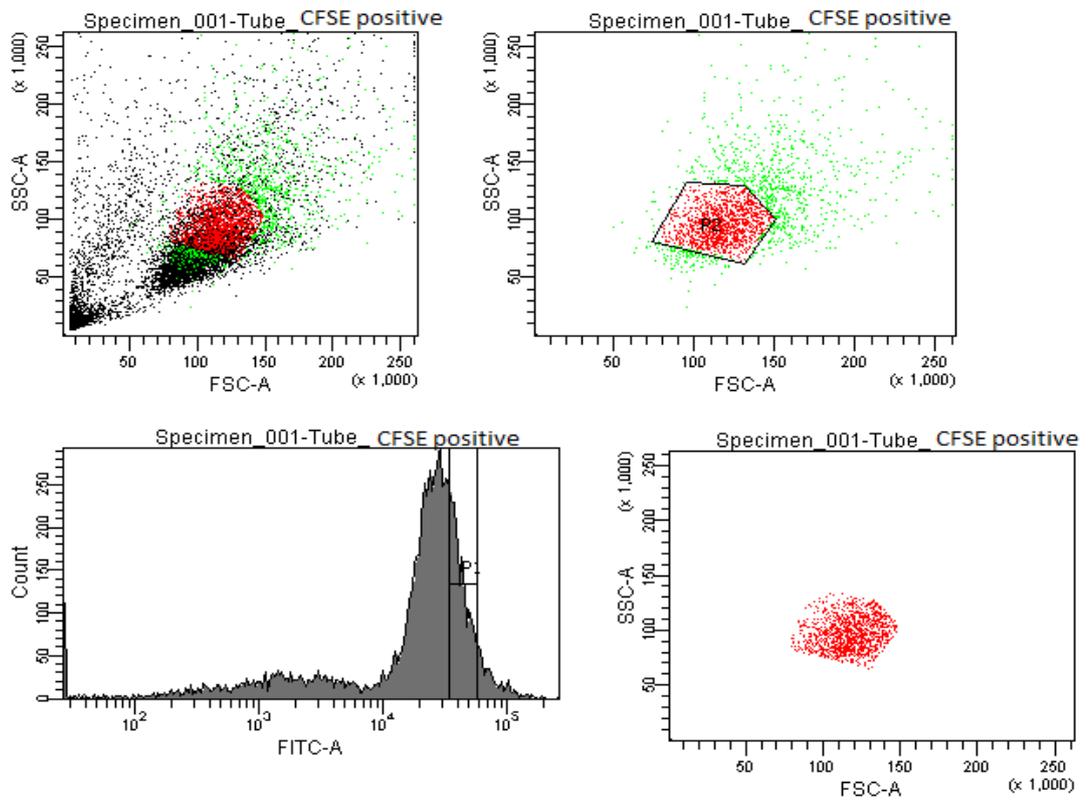
A**B**

Figure 4.3: Flow cytometry of single cell sorting of JVM2 cells. (A) Wild type of JVM2 cells display negative fluorescence of JVM2 cells. **(B)** JVM2 cells stained using CFSE kit display high fluorescence of cells that sorted.

4.2.1 Efficient lentivirus production

Lentivirus production was performed as described in (2.5.5). High green fluorescence indicated that high efficient lentivirus production because GFP is a reporter marker to package of lentiviral vector provided from abm® (Figure 4.4). In this study, lentiviral plasmid and two helper plasmids were used to generate lentivirus carrying CCN1/CYR61 gene. A three-plasmid expression system including two helper plasmids which are packaging and envelope plasmids and the transfer plasmid was used. The packaging plasmid was p Δ 8.9 coding for *gag-pol* and the envelope glycoprotein was VSV-G glycoprotein of the vesicular stomatitis virus coding for the *env*. The *gag* gene encodes for structural proteins which are matrix, capsid and nucleocapsid that used to package the virus. *pol* gene encodes for the enzymes reverse transcriptase, integrase and protease that involved in reverse-transcription, integration, maturation and replication (Liechtenstein, Perez-Janices & Escors, 2013).

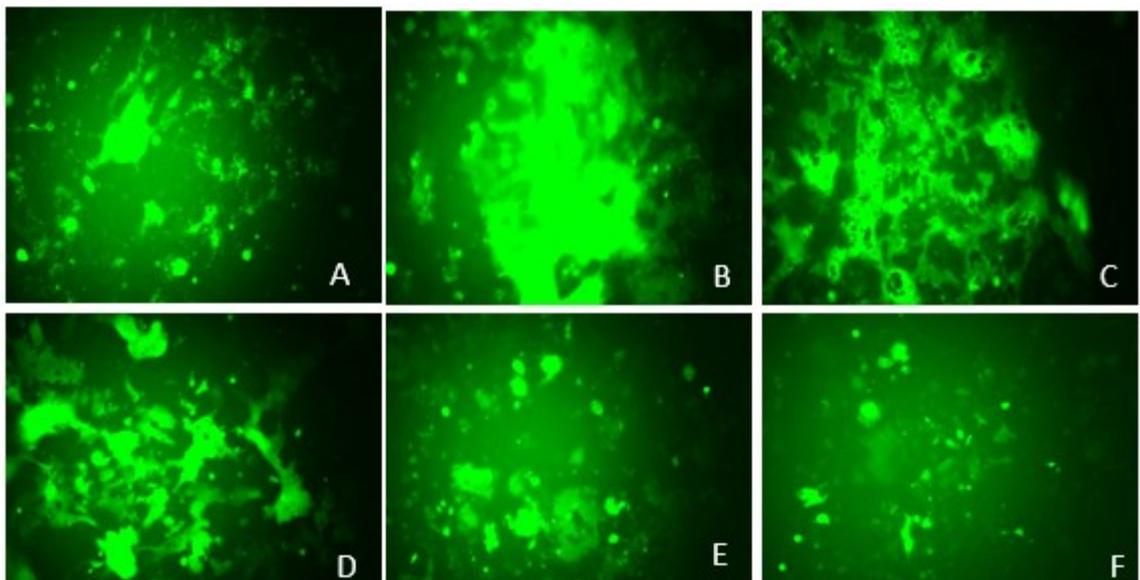


Figure 4.4: GFP expression after lentiviral production in HEK293 cells. A and E are scrambled and blank for CYR61 overexpression and knockdown respectively B, C and D are CYR61 siRNA lentiviruses. F is CYR61-overexpression lentivirus.

4.2.2 Effective transduction of MCL cell lines

JVM2 cells were seeded at 2 different cell densities 1.5×10^5 /well and 2.5×10^5 /well as described at (2.5.8.1) to generate CYR61/CCN1 overexpression (OE). High Green Fluorescence Protein (GFP) expression in both of cell densities reflected effective lentiviral transduction of JVM2 cells (figure 4,5 i and v). GFP is a reporter marker of insertion gene in to the DNA of targeted cells. After transduction of lentivirus carrying CYR61/CCN1 gene in MCL cells, the virus uncovered and released virus RNA and enzymes necessary for reverse transcription and integration into cells. In the cytoplasm of JVM2 cells, RNA transcribed to dsDNA which in turn was transported to nucleus integrated with DNA leading to translated CCN1 protein and gene modification.

GFP expression is highly expressed in picture B and C which are JVM2 cells infected by pLenti-III-CMV-GFP-2A-Puro-Blank Control as empty (blank) vector and pLenti-GIII-CMV-hCYR61-GFP-2A-Puro as vector containing full length human CCN1 sequence respectively (Figure 4.5 i and v). In comparison with wild type JVM2 cells which are GFP expression negative (figure 4.5 i and v picture A). However, during lentiviral transduction, not all cells will become transduced and there may be varying levels of gene transduction observed within the cell population displayed by a heterogenous GFP signal (figure 4.5 i and v picture B and C). Single cell sorting was used to identify GFP positive clones and enable growth of a more homogenous population of cells with the gene transduced to similar levels within the population (Figure 4.5 i and v picture D and E).

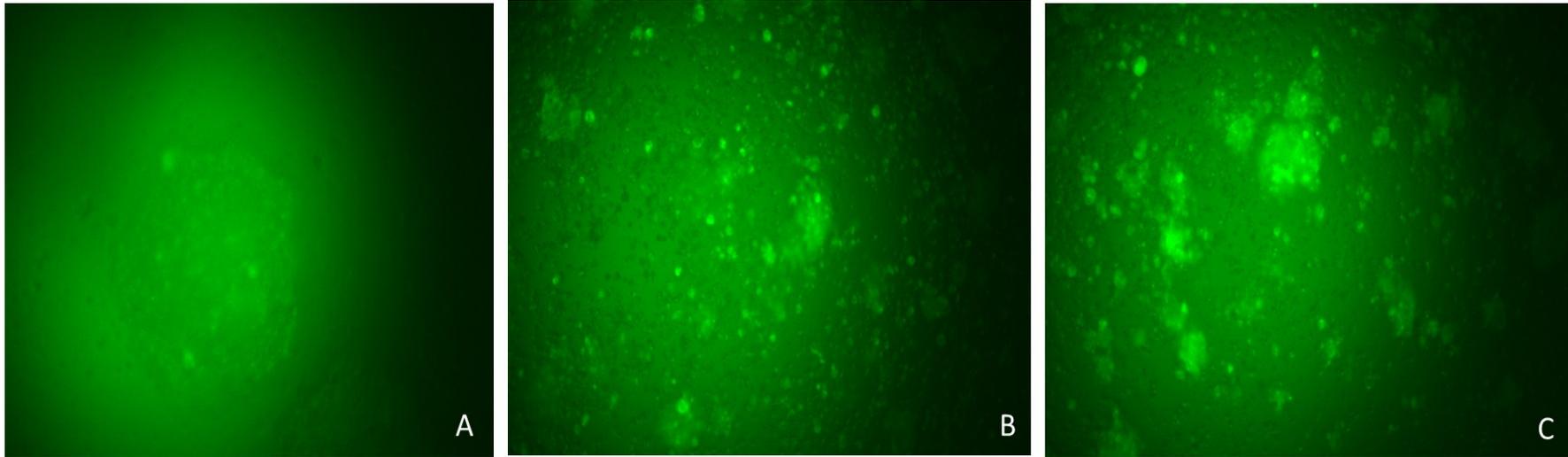
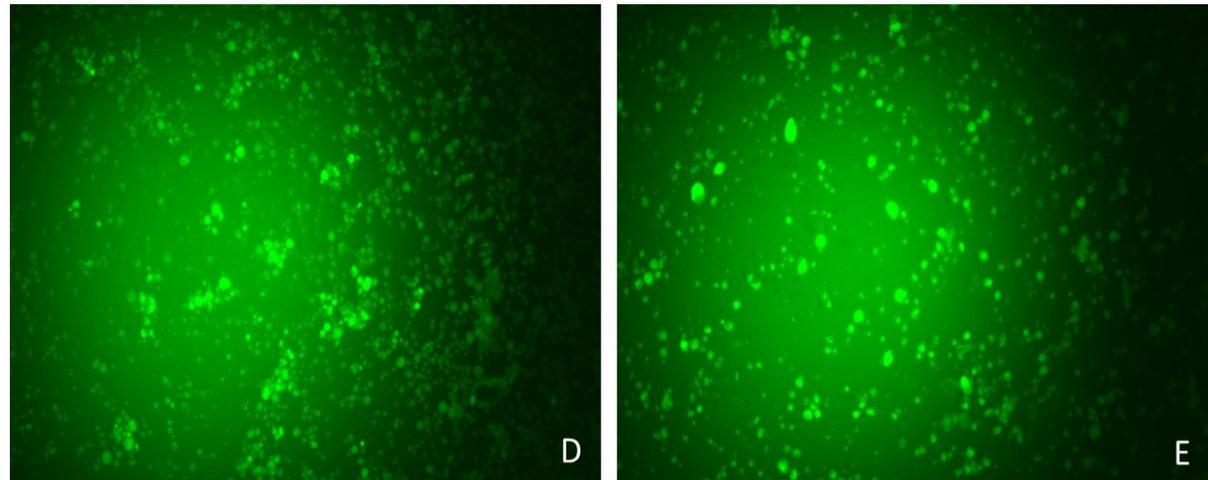
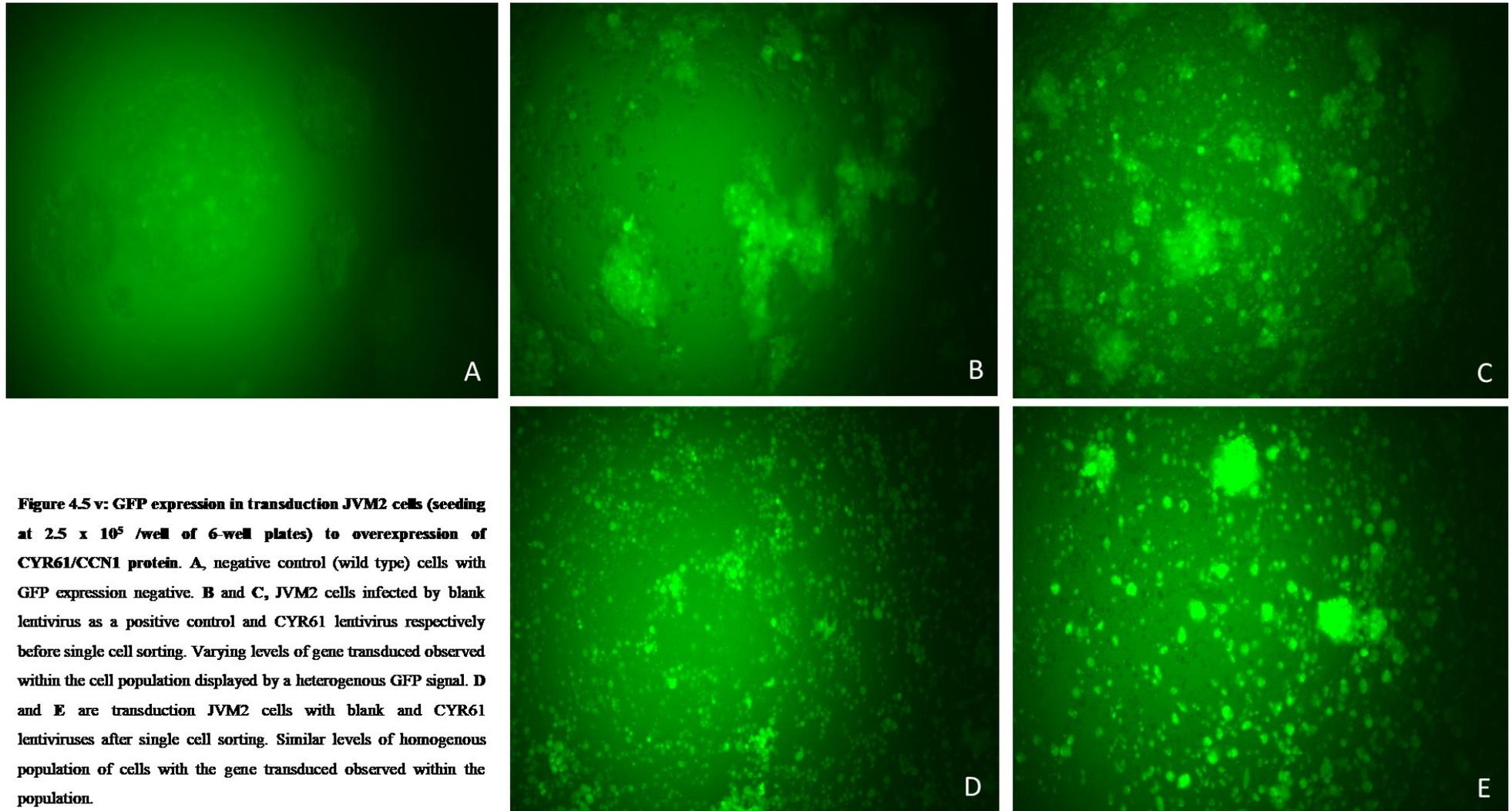


Figure 4.5 i: GFP expression in transduction JVM2 cells (seeding at 1.5×10^5 /well of 6-well plates) to overexpression of CYR61/CCN1 protein. A, negative control (wild type) cells with GFP expression negative. B and C, JVM2 cells infected by blank lentivirus as a positive control and CYR61 lentivirus respectively before single cell sorting. Varying levels of gene transduced observed within the cell population displayed by a heterogenous GFP signal. D and E are transduction JVM2 cells with blank and CYR61 lentiviruses after single cell sorting. Similar levels of homogenous population of cells with the gene transduced observed within the population.





To generate a stable CYR61/CCN1 knock down in REC1, three different CYR61-siRNA/shRNA/RNAi Lentiviruses targeting three different sequences (a-c) were used (Table 2.2). REC1 cells were seeded at cell densities of 5.0×10^5 /25 cm² tissue culture flask and 7.5×10^5 /25 cm² tissue culture flask as described at (2.5.8.2). High green fluorescence protein expression in both of cell densities referring to effective transduction of REC1. GFP is a reporter marker of insertion gene in to DNA of targeted cells. After detection of green fluorescence protein (GFP) production, cells were selected by adding puromycin (0.1-3 µg/ml) to obtain CCN1 knockdown clones and 0.5 µg/ml was used to maintain the cells.

GFP expression is highly expressed in picture B, C, D, and E in both of cell densities (Figure 4.6 i and v) which are infected by scrambled siRNA EGFP, CYR61-288 siRNA/shRNA/RNAi Lentivector (Human), CYR61-582 siRNA/shRNA/RNAi Lentivector (Human) and CYR61-676 siRNA/shRNA/RNAi Lentivector (Human) respectively. Comparing with wild type of REC1 which is negative to GFP expression (figure 4.6.i and v picture A).

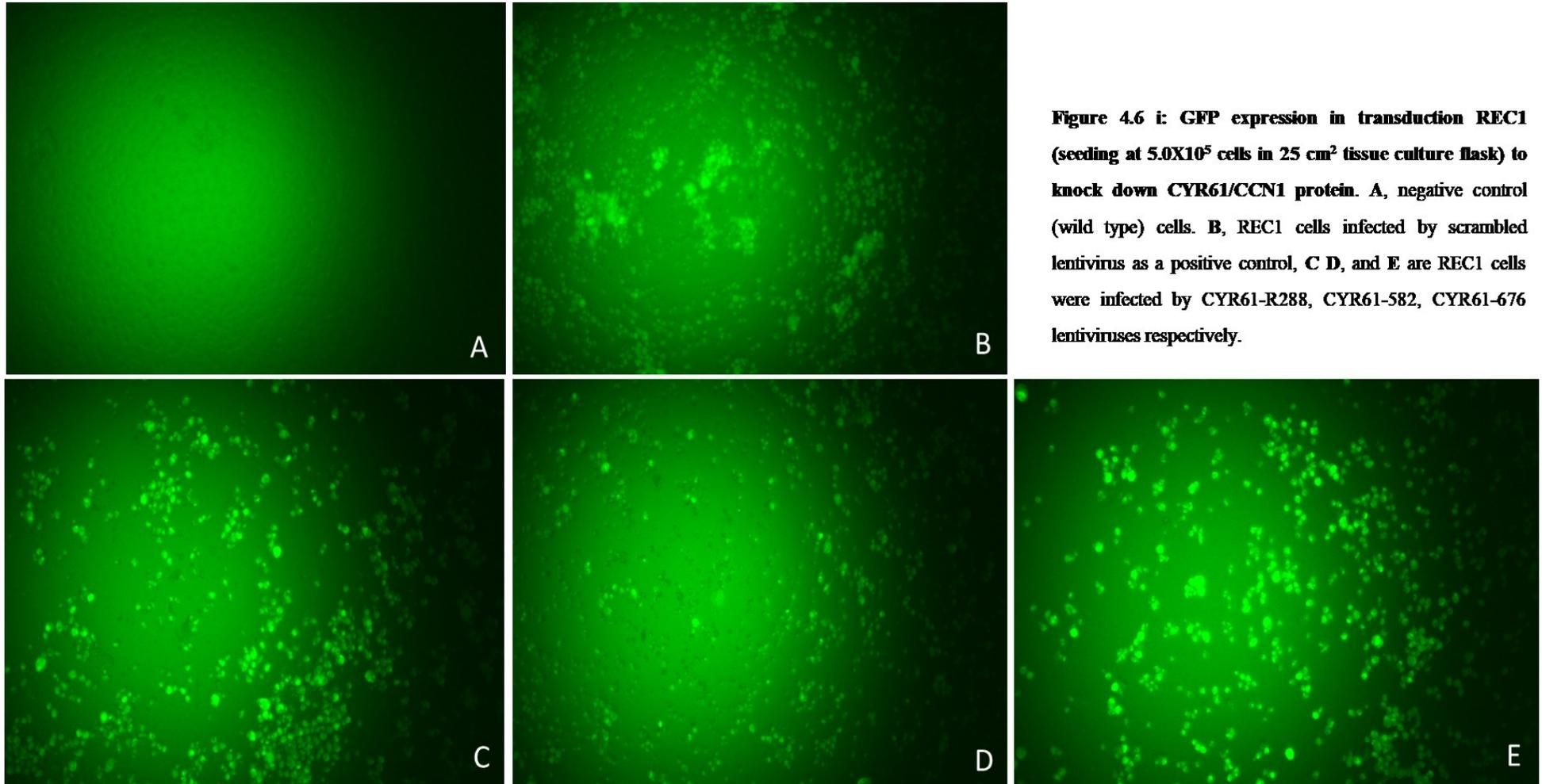


Figure 4.6 i: GFP expression in transduction REC1 (seeding at 5.0×10^5 cells in 25 cm^2 tissue culture flask) to knock down CYR61/CCN1 protein. A, negative control (wild type) cells. B, REC1 cells infected by scrambled lentivirus as a positive control, C D, and E are REC1 cells were infected by CYR61-R288, CYR61-582, CYR61-676 lentiviruses respectively.

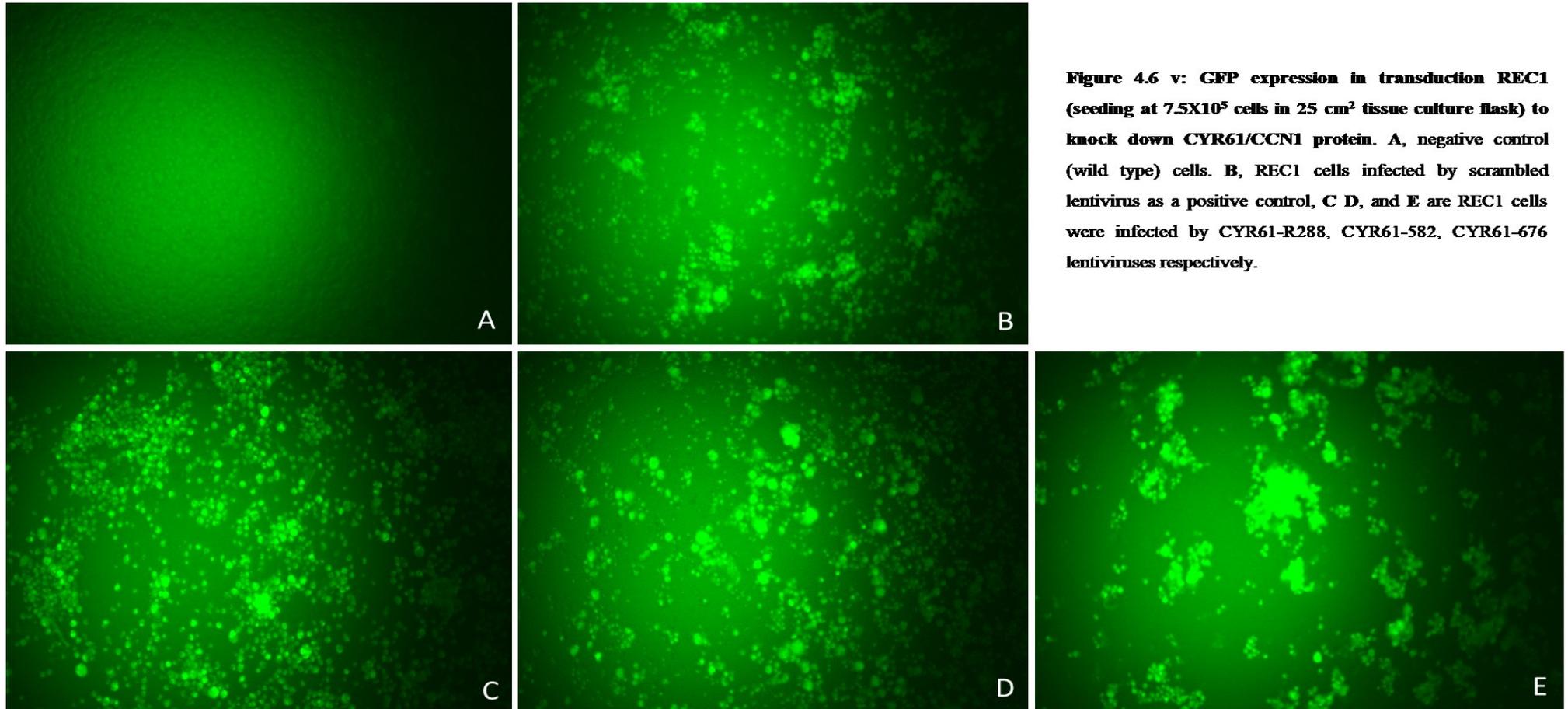
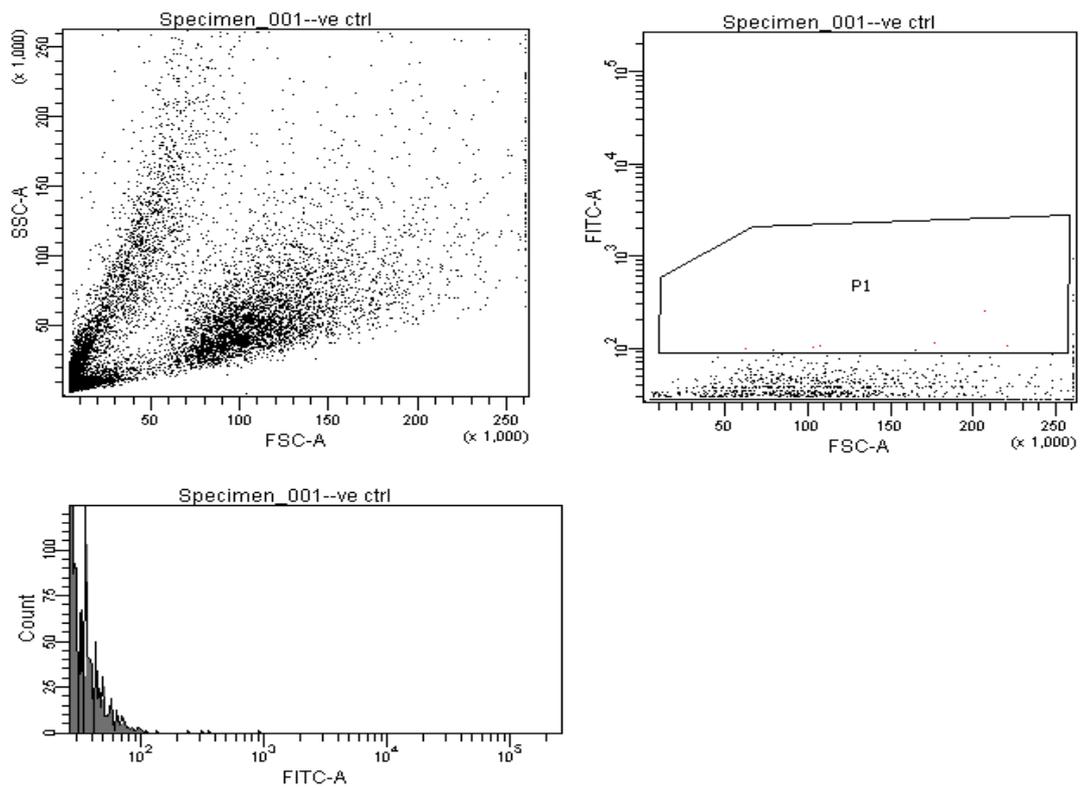


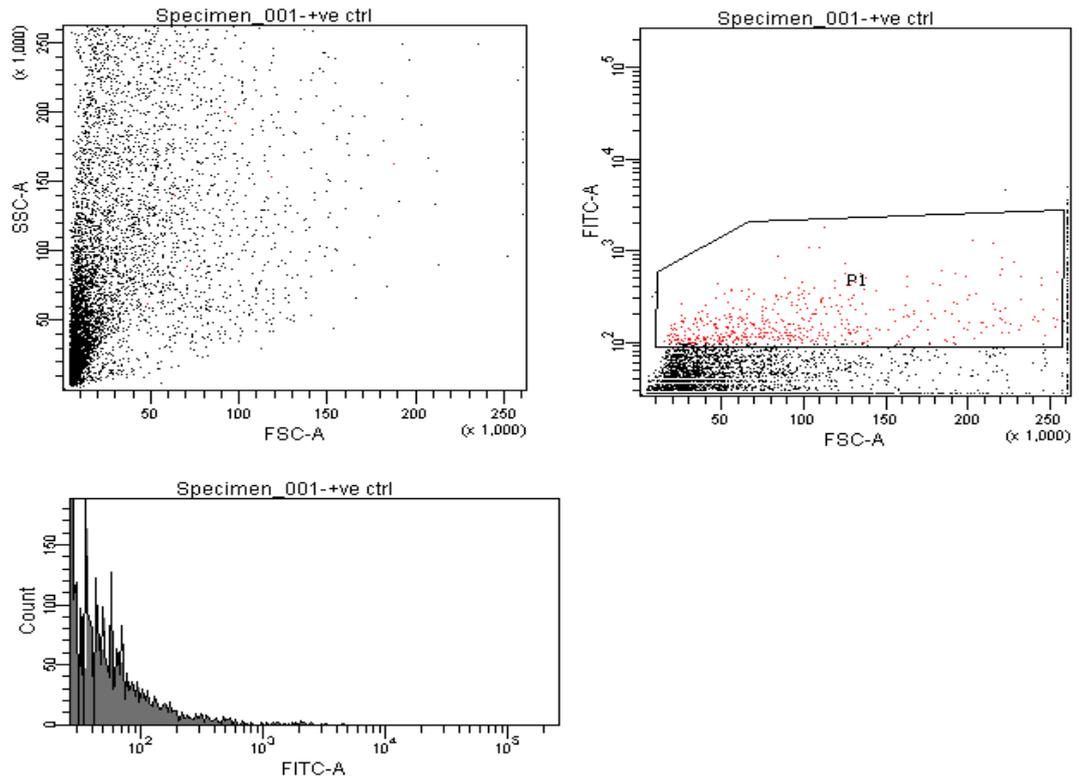
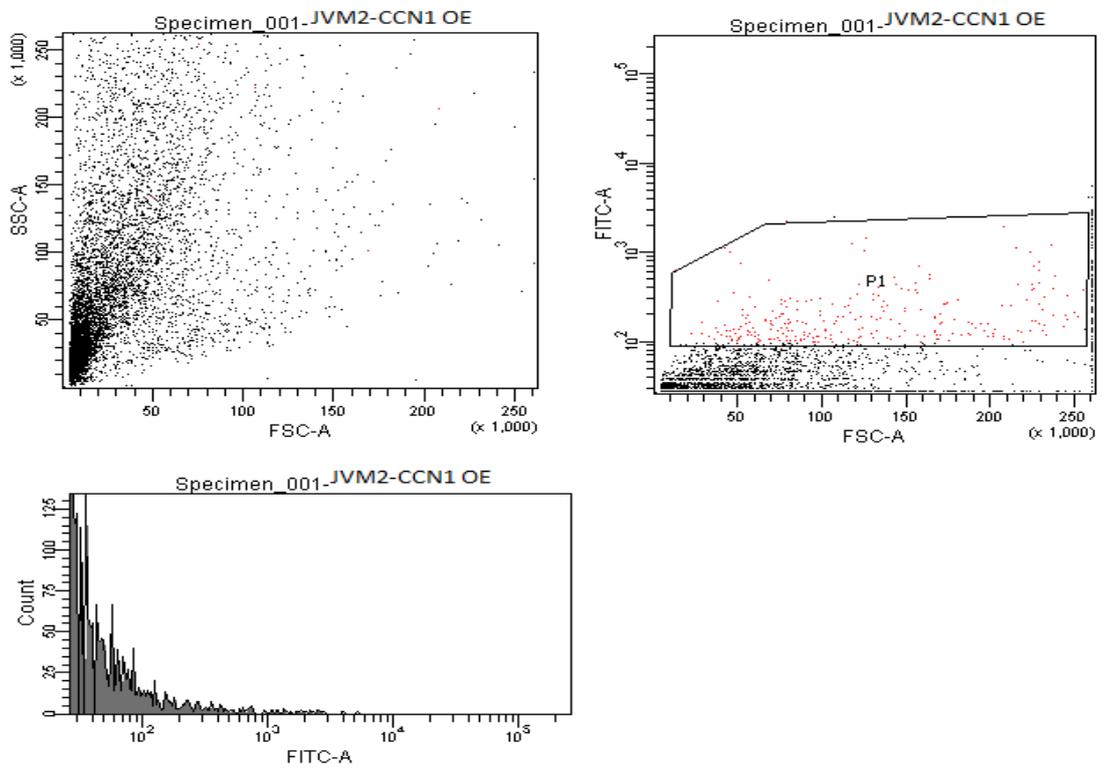
Figure 4.6 v: GFP expression in transduction REC1 (seeding at 7.5×10^5 cells in 25 cm^2 tissue culture flask) to knock down CYR61/CCN1 protein. A, negative control (wild type) cells. B, REC1 cells infected by scrambled lentivirus as a positive control, C D, and E are REC1 cells were infected by CYR61-R288, CYR61-582, CYR61-676 lentiviruses respectively.

Single cell sorting for selecting transduced JVM2 with CCN1/CYR61 gene that express fluorescence was performed (Figure 4.7).

Figure 4.7: Flow cytometry of sorting of JVM2-CCN1/CYR61 cells. (A) Negative control of JVM2 cells (wild type). (B) JVM2-Empty cells, P1 (red) to sort cells with empty gene. (C) JVM2-CCN1 OE cells, P1 (red) to sort cells with CCN1/CYR61 gene.

A



B**C**

4.2.3 Characterisation of CCN1 OE and KD cell line models

Western blots of samples taken forward for Proteomics

Western blotting as described at (2.6.5.2) was performed to confirm CYR61/CCN1 protein expression in OE and KD models of three sequential passages in JVM2 and REC1 cells respectively. High green fluorescence protein (GFP) expression was detected in both cell densities of OE and KD models but western blotting shows variations in bands in these models compared with positive controls (blank for OE and scrambled for KD). For CYR61/CCN1 OE model, western blotting has showing full length (42 kDa) and truncated forms (28-30 and 18-20 kDa) of CCN1 protein were overexpressed which means CYR61/CCN1 gene successfully inserted at cell densities 1.5×10^5 and 2.5×10^5 compared with positive control (blank vector). Protein was extracted from transduced CCN1-JVM2 cells with cell density 2.5×10^5 where CCN1 was highly expressed compared with empty vector or blank (positive control) and used for proteomics analysis in Chapter 5. Samples selected for proteomics were Blank b, CCN1 OEb1 passage 1, CCN1 OE b2 passage 2 and CCN1 OEb1 passage 3 (Figure 4.8).

For CYR61/CCN1 KD model, full length (42 kDa) and truncated forms 28-30 and 18-20 kDa of CCN1 protein were down regulated in REC1 cells at 7.5×10^5 cell density compared with positive control (scrambled). However, CCN1 protein (42kDa) at cell density 5.0×10^5 cells was still up-regulated compared with scrambled sequence. Protein was extracted from transduced siRNA/CCN1-REC1 from cell density 7.5×10^5 where CCN1 was down regulated compare with scrambled sequence (positive control) and used for proteomics analysis in chapter 5. Samples selected for proteomics were from passage 1; scrambled b, CCN1 KD R288b, CCN1 KD Y582b, CCN1 KD G676b; passage 2 scrambled b, CCN1 KD G676b, passage 3; scrambled b and CCN1 KD G676b (Figure 4.9).

Figure 4.8: Western blot of transduced JVM2 cells with CYR61/CCN1 protein of three sequential passages. Blank a, CCN1 OE a1 and 2 are protein extracted from transduced JVM2 with pLenti-III-CMV-GFP-2A-Puro-Blank Control and pLenti-GIII-CMV-hCYR61-GFP-2A-Puro lentivirus respectively at cell densities 1.5×10^5 . Blank b, CCN1 OE b1 and 2 are protein extracted from transduced JVM2 with pLenti-III-CMV-GFP-2A-Puro-Blank Control and pLenti-GIII-CMV-hCYR61-GFP-2A-Puro lentivirus respectively at cell density 2.5×10^5 . Full length (42 kDa) and truncated forms (28-30 and 18-20 kDa) of CCN1 protein were overexpressed at cell densities 1.5×10^5 and 2.5×10^5 compared with positive control (blank vector). However, in passages 1, 2 and 3 Blank b, CCN1 OE b1 and CCN1 OE b2 which are cell density 2.5×10^5 CCN1 full length and truncated forms were strongly overexpressed compare with Blank a, CCN1 OE a1 and 2 which are cell densities 1.5×10^5 . GAPDH was used as loading control

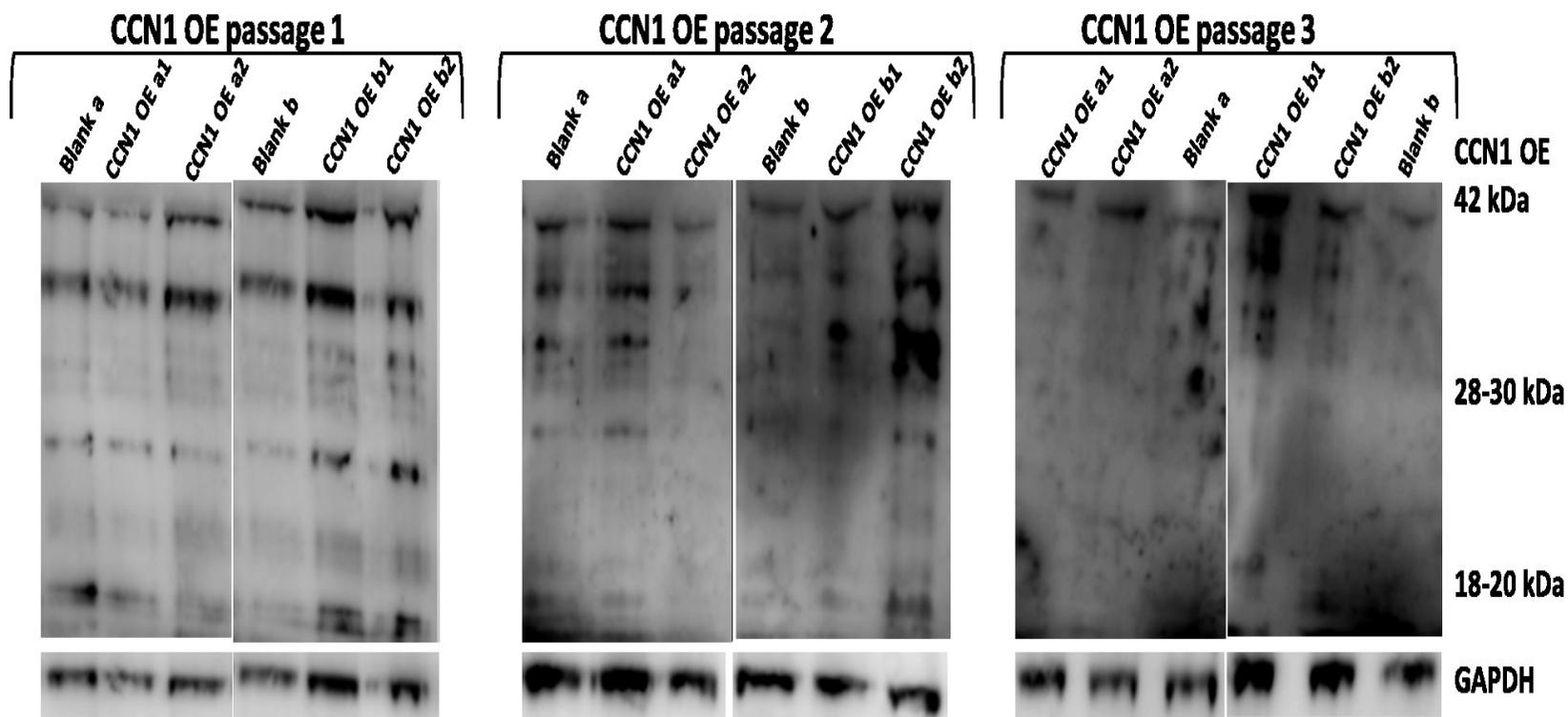
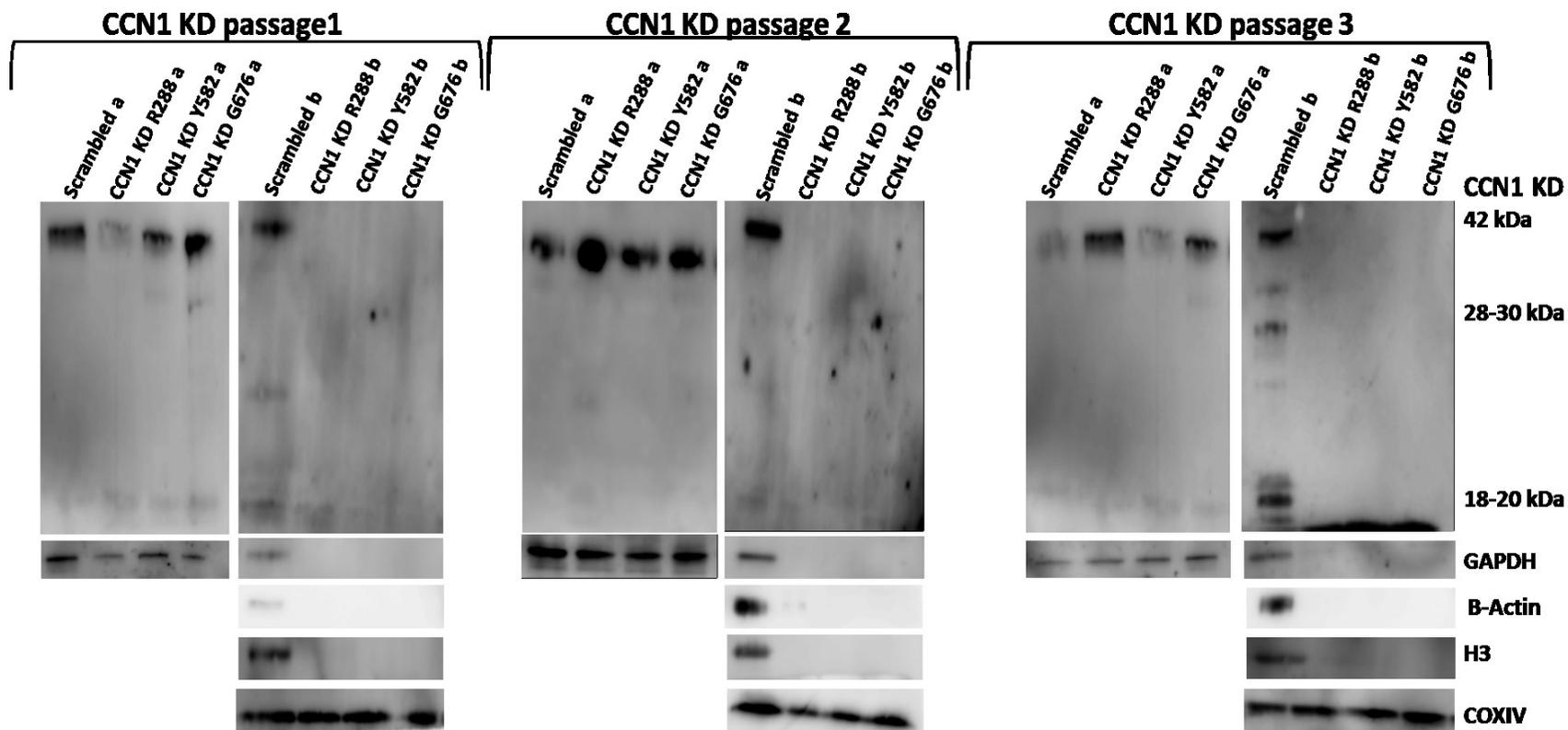


Figure 4.9: Western blot of transduced REC1 cells with CYR61/CCN1 protein of three sequential passages. scrambled a, CCN1 KD R288 a, CCN1 KD Y582 a and CCN1 G676 a are protein extracted from transduced REC1 with scrambled siRNA, CYR61-288, CYR61-582 and CYR61-676- si RNA lentivirus respectively at cell densities 5.0×10^5 . scrambled b, CCN1 KD R288 b, CCN1 KD Y582 b and CCN1 G676 b are protein extracted from transduced REC1 with scrambled siRNA, CYR61-288, CYR61-582 and CYR61-676- si RNA lentivirus respectively at cell densities 7.5×10^5 . Full length (42 kDa) and truncated forms 28-30 and 18-20 kDa of CCN1 protein were down regulated in REC1 cells at 7.5×10^5 cell density compared with scrambled. However, CCN1 protein (42kDa) at cell density 5.0×10^5 cells was still up-regulated compared with scrambled in three passages. GAPDH, B-Actin, H3 and COXIV were used as loading control.



4.3 Conclusion

Lentiviral vectors have been used as an effective transfer gene tool and optimisation of effective lentiviral transduction conditions are important for effective gene modification to occur experimentally (Denning *et al.*, 2013). Developing stable OE or KD cell lines require the optimal cell density and antibiotic concentration for selection to be evaluated (Delrue *et al.*, 2018). Kill curves were performed for wild type JVM2 and REC1 cells to optimise the optimal puromycin concentration and cell densities for effective CCN1 OE and CCN1 KD cells lines to be gained. The optimal puromycin concentration for REC1 was 3 µg/ml which was the minimum puromycin concentration that optimally killed the cells in all cell densities used in this study. Cells were sensitive to 4-5 µg/ml and resistant to 0.1-2 µg/ml concentration of puromycin after 25 days adding antibiotic (figure 4.1). In 2011, Jones *et al.*, have used 2 µg/ml puromycin to select transduced REC1 lentiviral shRNA knockdown of RRM2 to generate stable cell lines. However, 0.5 µg/ml puromycin concentration was used to select REC1 infected with lentiviral shRNA/pLOK.1 targeting rictor in mantle cell lymphoma (Liao *et al.*, 2019).

CCN1 OE in advanced stage JVM2 cells was particularly challenging because of the antibiotic resistance of JVM2 wild type cells which was observed in the kill curve experiments. In this study, we found that the JVM2 cells were resistant to all the recommended puromycin concentrations which are 0.1-10 µg/ml puromycin (Figure 4.2), so we used single cell sorting by flow cytometry to select CCN1 OE transduced cells (figure 4.3 A and B). Flow cytometry is rapid and reliable tool to select gene transferred cells (Nunez *et al.*, 2001; Wang *et al.*, 2015).

After optimisation of antibiotic concentration and cell densities of JVM2 and REC1 cells we have used lentivirus system for CCN1 OE and CCN1 KD respectively (figure 4.8 and 4.9). Lentiviral transduction has been found to be an attractive technique to deliver up to

10 kilobases of transgenes, naturally long-term expression with no side effects in lymphoma cells (Lois *et al.*, 2002; Mátrai, Chuah & VandenDriessche, 2010). In particular, lymphoma cells are difficult cells to modify gene expression (Anastasov *et al.*, 2009). In 1998, (Huang *et al.*) found that the primary T-helper cells were resistant to transfection by conventional methods. Protein was then extracted from CCN1 OE and CCN1 KD cell lines and western blotting performed to confirm gene insertion / deletion in the MCL cell lines. Protein was then extracted from three independent passages of the cell lines and used for proteomics analysis in chapter 5 (Figure 4.8 and 4.9).

Chapter 5

CCN1 dependent pathways in MCL

Chapter 5

5.1 Proteomics Analyses

We have genetically modified MCL cell lines in order to assess the role of CCN1 in MCL. We have used CCN1 knockdown in REC1 cells (parental: CCN1 high) and CCN1 overexpression in JVM2 cells (parental CCN1 low) as a model for investigating CCN1 potential roles and function(s). Proteomics was completed at the new Derriford Research Facility in conjunction with Dr Vikram Sharma.

Investigation of proteins in the knockdown model (REC1 KD) quantified changes in 559 proteins of which 112 proteins were downregulated to a value of less than 0.5. DAVID analyses was conducted to identify interactions within the network and Bonferoni was used to perform multiple testing correction and establish significance for $p < 0.05$. (Appendix 2). Similarly, 105 proteins were upregulated in this sample set greater than 2.0 fold, DAVID analyses was completed using Bonferoni to identify significance for pathways where $p < 0.05$ (Appendix 3).

Unfortunately the overexpression system did not yield many results with only 25 pathways associated using DAVID and will need further investigation (Appendix 4).

Proteomics has emerged as a promising tool to contribute to the prevention and treatment of cancer as it unravels protein modifications and networks enabling identification of biomarkers and treatment targets (Koomen *et al.*, 2008). Proteomics is the study of proteome and beyond genomics to identify protein-protein, protein-nucleic acid interactions, and post-translational modifications that affect protein function (Cho, 2007; Wu, Hu & Kavanagh, 2002). Many studies have found that Mass spectrometry (MS)-based proteomics is a useful technology to identify cancer biomarkers for diagnostic as well as therapeutic purposes (Elliott *et al.*, 2009; Lou *et al.*, 2007; Schirle, Bantscheff &

Kuster, 2012; Zhang, Chen & Huang, 2012; Zhu, Zhang & Humphreys, 2011). MS is an accurate measurement of mass (m) of a molecule to its charge (z) (m/z) ratio after ionizing to a gas phase (Savaryn, Toby & Kelleher, 2016). Protein ionization is performed by using two techniques which are electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) enabling proteins to be identified by a peptide mass ‘finger print’ (Huang *et al.*, 2017). In this study, we used ESI method to ionize protein samples of MCL cell lines of CYR61/CCN1 genetically modified models; CCN1 OE in JVM2 cells and CCN1 KD in REC1 cells. The principle of this technique described at Figure 5.1.

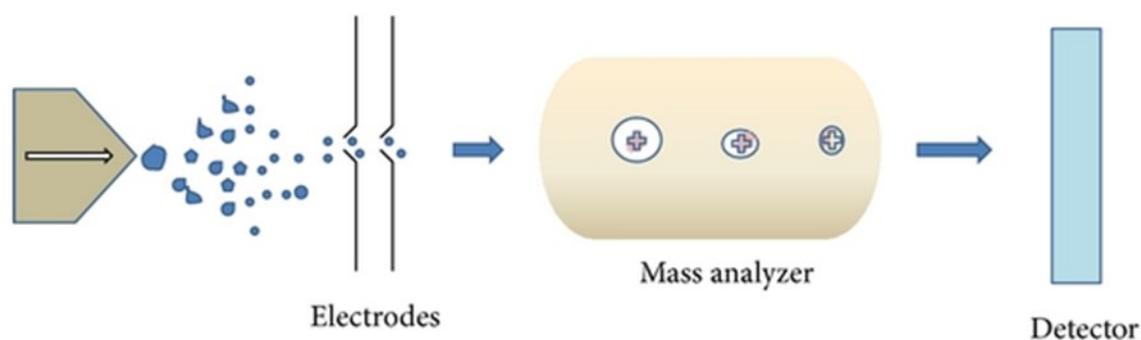


Figure 5.1 Overview of ESI-MS; sample comes out from the needle and generated charged droplets which in turn applying heat to desolvate, nebuliser gas and produce ions. Mass analyser separates the ions depending on m/z ratio and then the detector registers the ion resolved. Image from (Paul *et al.*, 2013).

Over last decade, mantle cell lymphoma prognosis has improved because of developing new treatment strategies but still MCL is more than a chronic disease with recurrent relapses and high morbidity (Sharma & Sweetenham, 2018). For young and fit patients with early stage MCL, there are many treatment options such as chemoimmunotherapy and stem cell transplantation are available. However, for elderly patients there is an urgent need for effective treatment (Sharma & Sweetenham, 2018). Investigation of CCN1 dependent pathways in MCL may lead to novel linkages within signalling pathways that

are specific to MCL or B cells and more importantly, provide novel targeting agents for MCL with higher selectivity and efficacy to target the disease effectively.

CCN1, a matricellular protein is involved in stem cell signalling within the haematopoietic microenvironment and involving in many cellular functions including cell adhesion, proliferation, migration, differentiation and survival (Franzen *et al.*, 2009; Wells *et al.*, 2015). Dysregulation of matricellular proteins have seen involving in the pathogenesis of disease and cancer generation and detection of the molecular mechanisms contributing to reveal new therapeutic regimes (Roberts, Kaur & Isenberg, 2017). CCN1/CYR61 implicates in the adhesion of prostatic carcinoma cells by binding to integrins and HSPGs and KD of CCN1/CYR61 expression in prostate cancer cells (PC-3 and DU-145) suppressed cells growth without causing apoptosis which reflects a role for CCN1 in promoting cell proliferation (Franzen *et al.*, 2009). Also, KD of CCN1 protein expression in gastric cells inhibited their migration and invasion indicating a role for CCN1 in metastasis (Wei *et al.*, 2016). Furthermore, expression of CCN1 associated inversely with the aggressiveness of NSCLC and addition of CCN1 in these cells which prompted cancer cell senescence provided a novel therapeutic strategy for lung cancer (Jim Leu *et al.*, 2013). Thus, utilising gene modification strategies for CCN1 expression in MCL could provide new therapeutic strategies for effective treatment (Chen *et al.*, 2017; Lau, 2011; Wei *et al.*, 2016).

5.2 Results

In this study, we used MS-based proteomics technology to identify protein changes in the MCL CCN1 OE and KD cell line models. Unfortunately, this technique could not detect CYR61/CCN1 protein which was genetically modified in REC1 and JVM2 cells but was detected by western blot (Figure 4.9). Specialists at the DRF proteomics laboratory have

suggested this is due to other proteins in the samples being of higher abundance than CCN1 itself. This consistent with studies that found the detection of low abundant proteins need depletion methodologies to deplete the high abundant proteins and enrichment approach to enhance the detection of low abundant protein (Millioni *et al.*, 2011; Qian *et al.*, 2008; Thulasiraman *et al.*, 2005). Protein concentrations should be at range 3-4 of magnitude to enable proteomics technology to analyse them, protein amplification cannot be performed like DNA and requires more complex processing (Cho, 2007). In the past decade, proteomics emerged as robust tool to detect biomarkers for cancer but has been hampered with the problem of difficult detection of low abundance of proteins (Srinivas *et al.*, 2001). Low-abundance proteins are often be hindered by the highly abundant ones, which mask them, requiring an increase in sensitivity to enhance information capture (Chandramouli & Qian, 2009; Srinivas *et al.*, 2001). For example in plasma, studies have found that highly abundant proteins in a sample mask low abundant proteins but depletion of high concentration proteins such as albumin in human blood plasma led to loss of low concentration protein and some cytokines which in turn confounded proteomic analysis (Granger *et al.*, 2005). Therefore the process of accessing data for low abundant protein remains complicated.

We have therefore investigated proteins that could be already associated with CCN1 from published literature to ensure CCN1 pathways were being affected in our genetically modified models. Many studies have found that integrins are receptors for the CCN1 protein to mediate diverse functions in cancer (Chai *et al.*, 2012; Espinoza *et al.*, 2014; Jandova *et al.*, 2012). In humans, there are 18 α subunit, 8 β subunit genomes and 24 different α - β combinations that have discovered at protein expression (Humphries, Byron & Humphries, 2006).

Using a STRING protein-protein search for Cyr61 (CCN1) (<https://string-db.org/>) for CCN1 UniProt ID O00622 showed various known interactions for CCN1. The String

database is useful to detect known ‘shells’ of protein interactions that have been determined theoretically, experimentally or thought to be possible by data mining. Interaction of CYR61/CCN1 protein with integrins ITGB2 (integrin subunit β 2), ITGB3 (integrin subunit β 3), ITGB5 (integrin subunit β 5), ITGAM (integrin subunit α M), ITGA2B (integrin subunit α 2b) and ITGAV (integrin subunit α V) are known by experimental determination (Figure 5.2). Interaction of integrins with ligands drives normal cellular and pathologies functions such as cell adhesion, metastasis, tumour cell proliferation, migration and tumour cell survival (Gahmberg *et al.*, 2009; Ganguly *et al.*, 2013). In breast cancer, CCN1 drives pro-metastasis pathway leading to disease progression through binding to α β 3 integrin in a paracrine manner and supporting VEGF secretion, an angiogenesis factor, in an autocrine manner (Espinoza *et al.*, 2014). Furthermore, CCN1- α β 3 signalling pathway is driving breast cancer angiogenesis through acting like an angiogenic promotor of endothelial cells (Brigstock, 2002; Espinoza *et al.*, 2014; Leu, Lam & Lau, 2002). Increasing evidence has shown that integrin α β 5 mediated CCN1 promoting tumour cell migration (Jandova *et al.*, 2012) and enhancing resistance to apoptosis in breast cancer (Lin *et al.*, 2004). In fact, integrin subunit β 5 is a receptor of CCN1 (Chen, Mo & Lau, 2001; Grzeszkiewicz *et al.*, 2001) and integrin subunit β 2 is considered a receptor of ICAM1, ICAM2 and ICAM3 (Long, 2011; Yun *et al.*, 2014).

Also, in Figure 5.2, STRING protein-protein interaction program shown that an interaction between CYR61/CCN1 protein and MAPK and APC. CCN1 is a ligand for integrins and acts through direct binding to integrins to enhance specific functions downstream signalling pathway including the PI3K and MAPK (Holbourn, Acharya & Perbal, 2008; Lin *et al.*, 2012; Sun *et al.*, 2015; Zhang *et al.*, 2009). In 2017, (Sun *et al.*) have found CCN1 protein as a novel pro-inflammatory regulator through binding to

integrin $\alpha6\beta1$ and activation of p38/MAPK signalling leading to enhance IL-1 β expression.

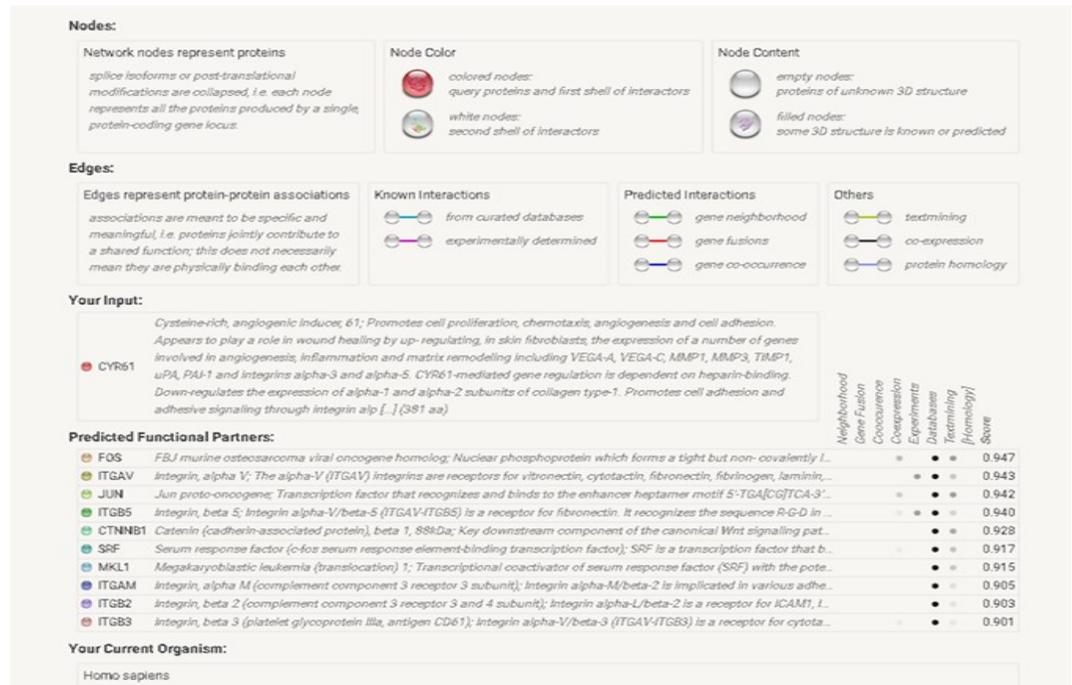
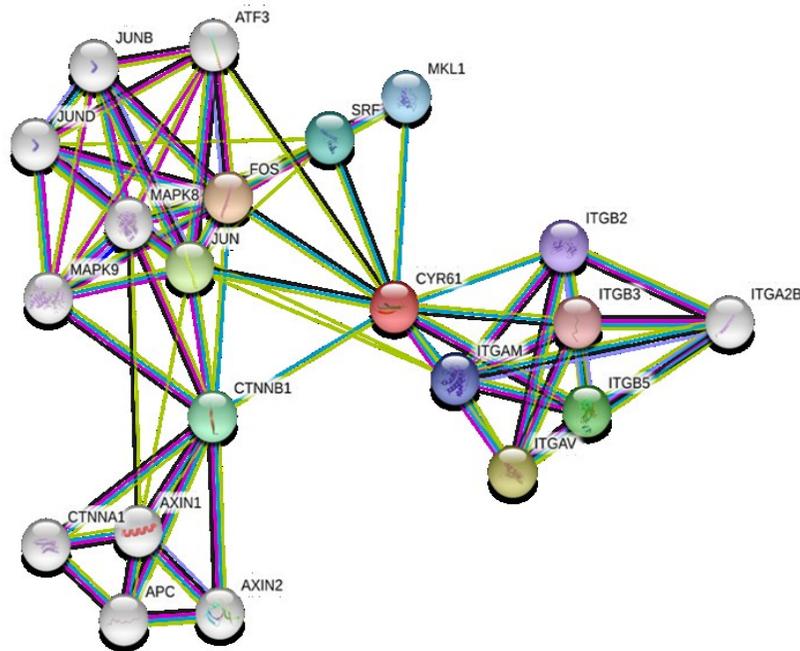


Figure 5.2 CCN1 (CYR61) associated proteins. STRING search identified a number of known CCN1 associated proteins including ITGB2, ITGA2B, ITGB3, ITGB5, ITGAV, ITGAM (Known experimentally determined interactions shown in Pink / Turquoise). Other components that may be involved in the CCN1 (CYR61) pathway are APC, AXIN2, AXIN1, CTNNA1, CTNNB1, JUN, JUND, JUNB, MAPK9, MAPK8, FOS, SRF, MKL1 and ATF3 (as determined by databases).

Furthermore, STRING network shown CYR61/CCN1 regulates integrins (ITGB5, ITGA5, ITGAV, ITGA2B, ITGA7, ITGA6, ITGA8 and ITGAP) and TLN1 (Figure 5.3). Integrin/ECM adhesion occurs through binding of TLN1 to the cytoplasmic part of β subunit of integrin (Anthis *et al.*, 2010) that suggest TLN1 is essential player in integrins activation to promote interaction between the cell and ECM leading to regulation of cell adhesion, proliferation, survival and cancer progression (Alam *et al.*, 2007; Das *et al.*, 2014; Fornaro, Manes & Languino, 2001).

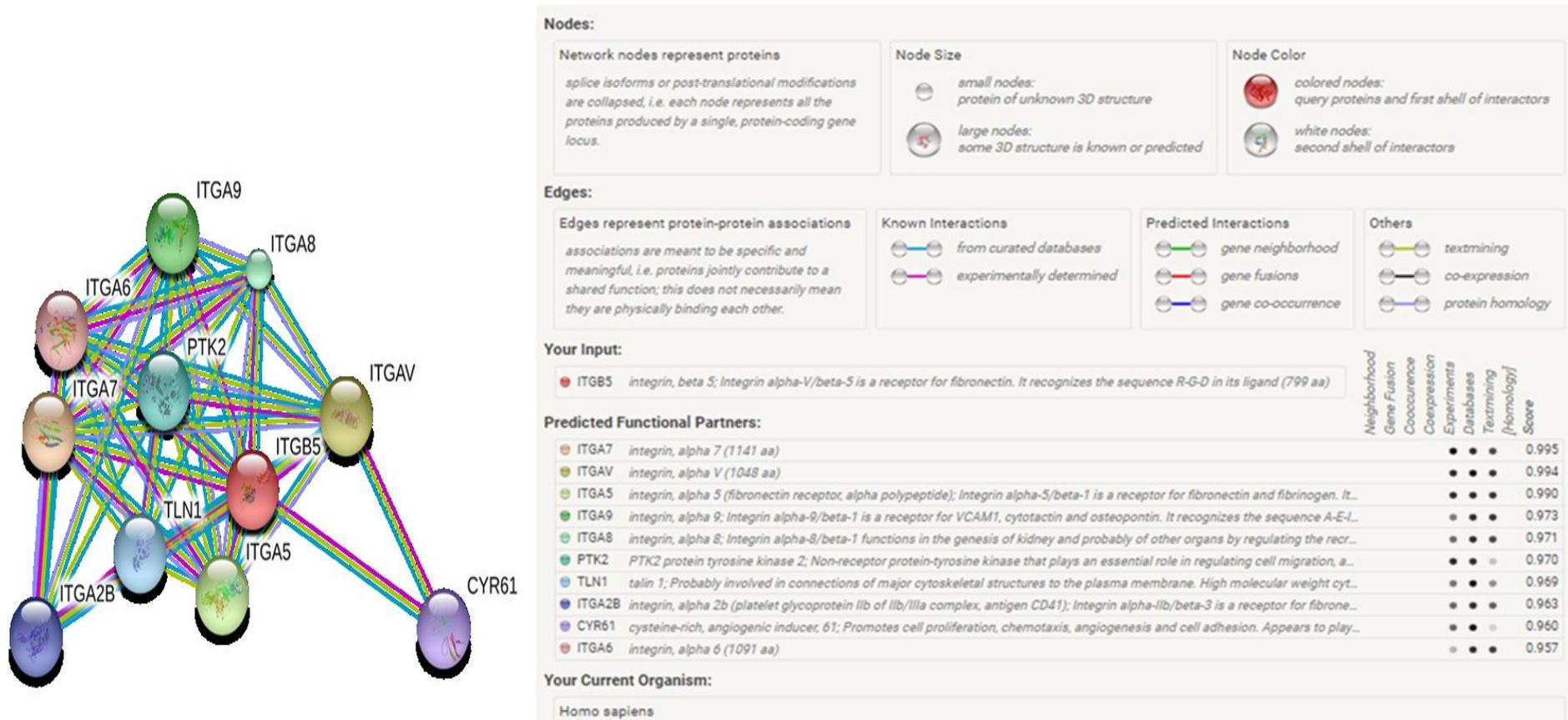
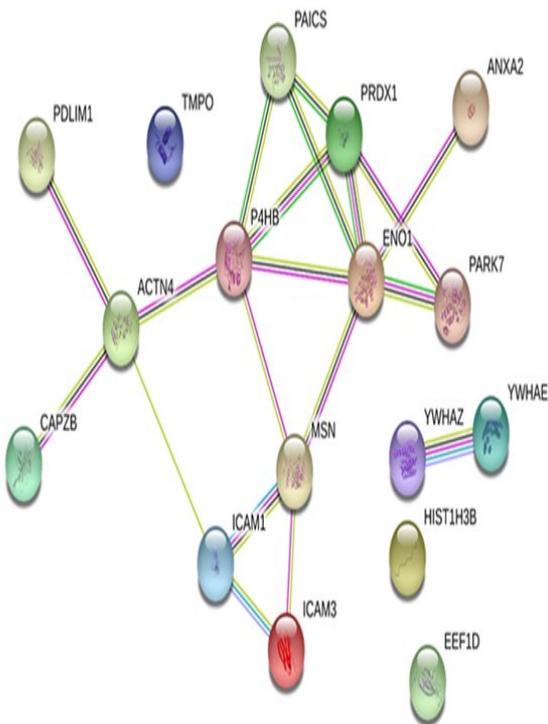


Figure 5.3: CYR61/CCN1 regulates integrins (ITGB5, ITGA5, ITGAV, ITGA2B, ITGA7, ITGA6, ITGA8, ITGAP and Talin1 (TLN1)). TLN1 was upregulated in KD model of CCN1 in REC1 and down regulated in OE model of CCN1 in JVM2.

5.2.1 REC1 CCN1 Knockdown model

112 proteins were down regulated and 107 proteins were up-regulated in REC1 KD model (Green 676 (G676) sequence compared to scrambled control). 418 pathways were down regulated (Appendix 2) and 252 pathways were up-regulated in REC1 CCN1 model (Appendix 3) using DAVID. By applying STRING protein-protein interaction program, intercellular adhesion molecules 1, and 3 (ICAM 1 and ICAM 3) were involved in three different adhesion pathways with other groups of proteins and were down regulated in REC1 KD model. These include cell adhesion molecule binding, cell-cell adhesion and cell adhesion. Gene Ontology (GO) analysis of CYR61/CCN1 KD and OE models was completed using DAVID functional analysis Cell adhesion molecule binding (GOTERM_BP_FAT GO:0050839) contains 17 proteins including MSN, ENO1, PARK7, P4HB, ACTN4, CAPZB, PDLIM1, PAICS, PRDX1, ANXA2, YWHAZ, YWHAE, HIST1H3B and EEF1D. P value was 1.17E-08, Bonferroni and Benjamini values were 0.0000 for both and was down regulated in REC1 KD (Figure 5.4).

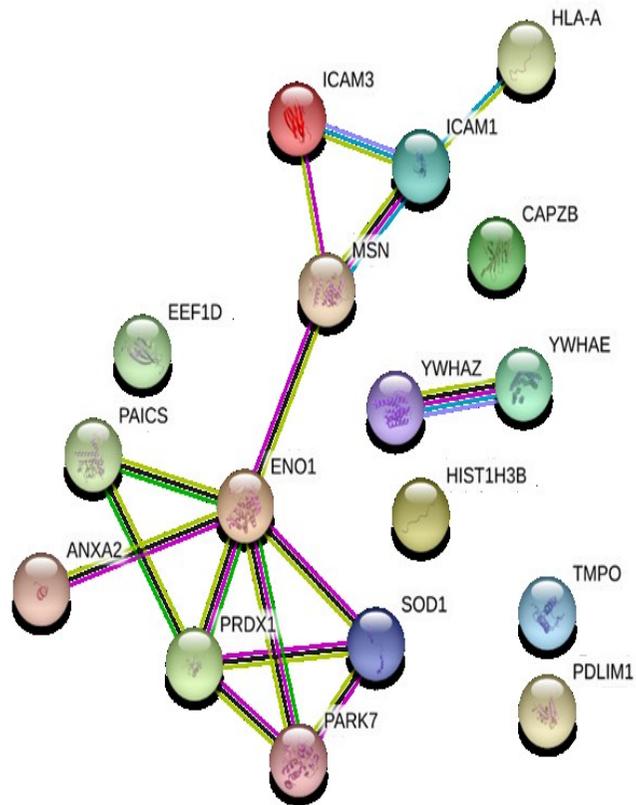


Your input:

ICAM3	Intercellular adhesion molecule 3; ICAM proteins are ligands for the leukocyte adhesion protein LFA-1 (integrin α 5 β 1/CD11a). ICAM3 is also a ligand for integrin α 5 β 1/CD11a (547 aa)
ENO1	Enolase 1, α form; Multifunctional enzyme that, as well as its role in glycolysis, plays a part in various processes such as growth control, hypoxia tolerance and allergic responses. May also function in the intravascular and pericellular fibrinolytic system due to its ability to serve as a receptor and activator of plasminogen on the cell surface of several cell-types such as leukocytes and neurons. Stimulates immunoglobulin production (434 aa)
HIST1H3B	Histone cluster 1, H2b (136 aa)
ACTN4	Actinin α 4; F-actin cross-linking protein which is thought to anchor actin to a variety of intracellular structures. This is a bundling protein (Probable). Probably involved in vesicular trafficking via its association with the GART complex. The GART complex is necessary for efficient transferrin receptor recycling but not for EGFR degradation (PubMed-15772161). Involved in tight junction assembly in epithelial cells probably through interaction with MICALL2. Links MICALL2 to the actin cytoskeleton and recruits it to the tight junctions (By similarity). May also function as a (aa [...]) (911 aa)
PRDX1	Peroxisiredoxin 1; Involved in redox regulation of the cell. Reduces peroxides with reducing equivalents provided through the thioredoxin system but not from glutaredoxin. May play an important role in eliminating peroxides generated during metabolism. Might participate in the signaling cascades of growth factors and tumor necrosis factor- α by regulating the intracellular concentrations of H ₂ O ₂ . Reduces an intramolecular disulfide bond in GOPD5 that gates the ability to GOPD5 to drive postmitotic motor neuron differentiation (By similarity) (199 aa)
CAPZB	Capping protein (actin filament) muscle Z-line β 2; F-actin-capping proteins bind in a Ca ²⁺ -independent manner to the fast growing ends of actin filaments (barbed end) thereby blocking the exchange of subunits at these ends. Unlike other capping proteins (such as gelsolin and severin), these proteins do not sever actin filaments. Plays a role in the regulation of cell morphology and cytoskeletal organization (277 aa)
YWHAE	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide; Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner (255 aa)
ICAM1	Intercellular adhesion molecule 1; ICAM proteins are ligands for the leukocyte adhesion protein LFA-1 (integrin α 5 β 1/CD11a). During leukocyte (trans-endothelial migration) ICAM1 engagement promotes the assembly of endothelial apical cups through ARHGAP26/SGEF and RHO G activation (522 aa)
TMPO	Thymopoietin; May be involved in the structural organization of the nucleus and in the post-mitotic nuclear assembly. Plays an important role, together with LMNA, in the nuclear anchorage of RB1 (694 aa)
YWHAZ	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide; Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner (245 aa)
P4HB	Prolin 4-hydroxylase, beta polypeptide; This multifunctional protein catalyzes the formation, breakage and rearrangement of disulfide bonds. At the cell surface, seems to act as a reductase that cleaves disulfide bonds of proteins attached to the cell. May therefore cause structural modifications of isoform proteins. Inside the cell, seems to form/rearrange disulfide bonds of nascent proteins. At high concentrations, functions as a chaperone that inhibits aggregation of misfolded proteins. At low concentrations, facilitates aggregation (anti-chaperone activity). May be involved with [...] (508 aa)
PARK7	Parkinson protein 7; Protein deglycase that repairs methylglyoxal- and glyoxal-glycated amino acids and proteins, and releases repaired proteins and lactate or glycolate, respectively. Deglycates cysteines, arginines and lysines residues in proteins, and thus reactivates these proteins by reversing glycation by glyoxals. Acts on early glycation intermediates (hemithioacetals and aminocarbinols), preventing the formation of advanced glycation endproducts (AGE) (PubMed-25416785). Plays an important role in cell protection against oxidative stress and cell death acting as oxidative stress [...] (189 aa)
ANXA2	Annexin A2; Calcium-regulated membrane-binding protein whose affinity for calcium is greatly enhanced by anionic phospholipids. It binds two calcium ions with high affinity. May be involved in heat-stress response. Inhibits PCSK9-enhanced LDLR degradation, probably reduces PCSK9 protein levels via a translational mechanism but also competes with LDLR for binding with PCSK9 (PubMed-18799458, PubMed-24808179, PubMed-22848640) (357 aa)
MSN	Moesin; Probably involved in connections of major cytoskeletal structures to the plasma membrane. May inhibit herpes simplex virus 1 infection at an early stage (577 aa)
PDLIM1	POZ and LIM domain 1; Cytoskeletal protein that may act as an adaptor that brings other proteins (like kinases) to the cytoskeleton (229 aa)
PAICS	Phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase (422 aa)
EEF1D	Eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein); Isoform 1 - EF1-beta and EF1-delta stimulate the exchange of GDP bound to EF1-alpha to GTP, regenerating EF1-alpha for another round of transfer of aminoacyl-tRNAs to the ribosome (647 aa)

Figure 5.4: Cell adhesion molecule binding. STRING protein-protein interaction network shows ICAM 1 and ICAM 3 involved in cell adhesion molecule interacting with different proteins including MSN, ENO1, PARK7, P4HB, ACTN4, CAPZB, PDLIM1, PAICS, PRDX1, ANXA2, YWHAZ, YWHAE, HIST1H3B and EEF1D.

Cell-cell adhesion proteins (GOTERM_BP_FAT GO:0098609) which involved 17 proteins ICAM 1, ICAM 3, MSN, ENO1, PRDX1, PARK7, DOD1, HLA-A, PAICS, EEF1D, ANXA2, CAPZB, YWHAE, YWHAZ, HIST1H3B, TMPO and PDIM1, were down expressed in KD model. The P value was 5.93E-04, Bonferroni and Benjamini were 0.7740 and 0.0207 respectively (Figure 5.5).

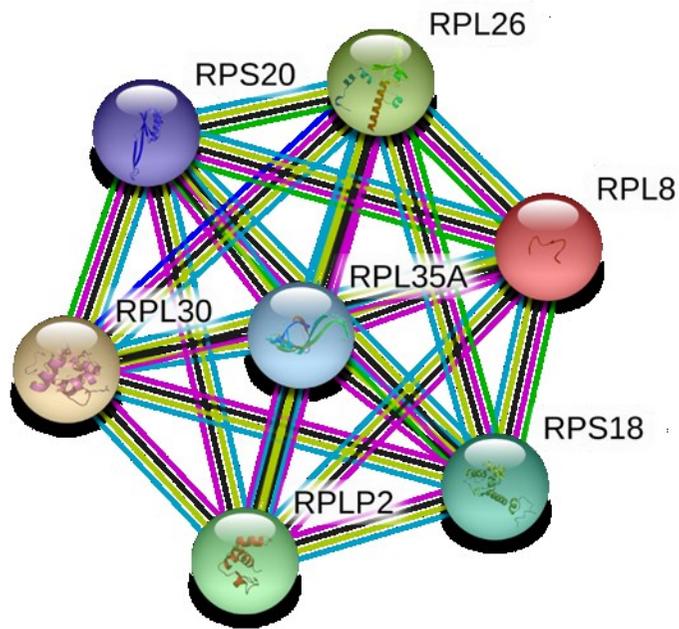


Your Input:	
ICAM3	Intercellular adhesion molecule 3, ICAM proteins are ligands for the leukocyte adhesion protein LFA-1 (integrin alpha L/beta 2). ICAM3 is also a ligand for integrin alpha G/beta 2 (347 aa)
ENO1	Enolase 1, [alpha]; Multifunctional enzyme that, as well as its role in glycolysis, plays a part in various processes such as growth control, hypoxia tolerance and allergic responses. May also function in the intracellular and pericellular fibrolytic systems due to its ability to serve as a receptor and activator of plasminogen on the cell surface of several cell types such as leukocytes and neurons. Stimulates monocyte/macrophage production (434 aa)
HIST1H3B	Histone cluster 1, H3b (736 aa)
PRDX1	Peroxiredoxin 1, involved in redox regulation of the cell. Reduces peroxides with reducing equivalents provided through the thioredoxin system but not from glutaredoxins. May play an important role in alleviating peroxides generated during metabolism. Might participate in the signaling cascades of growth factors and tumor necrosis factor alpha by regulating the intracellular concentrations of PCD/OC2. Reduces an intramolecular disulfide bond in GEP/OS that gives the ability to GEP/OS to drive postmitotic motor neuron differentiation (by accident) (199 aa)
CAPZB	Capping protein (actin filament) muscle Z-line, beta; F-actin-capping proteins bind as a Ca(2+)-independent dimer to the fast growing ends of actin filaments (barbed end) thereby blocking the anchorage of subunits at these ends. Unlike other capping proteins (such as gelsolin and severin), these proteins do not sever actin filaments. Plays a role in the regulation of cell morphology and cytoskeletal organization (277 aa)
YWHAZ	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide; Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner (238 aa)
ICAM1	Intercellular adhesion molecule 1, ICAM proteins are ligands for the leukocyte adhesion protein LFA-1 (integrin alpha L/beta 2). During leukocyte trans-endothelial migration, ICAM1 engagement promotes the assembly of endothelial apical cups through AIB1/SEF2b/SDF1 and H400 activation (302 aa)
TMPO	Thymopontin; May be involved in the structural organization of the nucleus and in the post mitotic nuclear assembly. Plays an important role, together with LMNA, in the nuclear anchorage of RBB1 (894 aa)
SOD1	Superoxide dismutase 1, soluble; Destroys radicals which are normally produced within the cells and which are toxic to biological systems (154 aa)
YWHAZ	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide; Adapter protein implicated in the regulation of a large spectrum of both general and specialized signaling pathways. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner (248 aa)
PARK7	Parkin protein 7; Protein disulfase that repairs methylglyoxal- and glyoxal glyoxylated amino acids and proteins, and releases repaired proteins and lactate or glycolate, respectively. Depletes cysteine, arginine and lysine residues in proteins, and thus reactivates these proteins by reversing glycation by glyoxals. Acts an early glycation intermediate (methylglyoxal and amino-carbinols), preventing the formation of advanced glycation endproducts (AGE) (PubMed 25416785). Plays an important role in cell protection against oxidative stress and cell death acting an oxidative stress [...] (189 aa)
ANXA2	Annexin A2; Calcium-regulated membrane-binding protein whose affinity for calcium is greatly enhanced by anionic phospholipids. It binds two calcium ions with high affinity. May be involved in heat stress responses. Inhibits PCSK9-enhanced LDLR degradation, probably reduces PCSK9 protein levels via a translational mechanism but also competes with LDLR for binding with PCSK9 (PubMed 18799438, PubMed 2488179, PubMed 23848840) (357 aa)
MSN	Microtubule; Probably involved in connections of major cytoskeletal structures to the plasma membrane. May inhibit herpes simplex virus 1 infection at an early stage (377 aa)
PDLIM1	PDZ and LIM domain 1; Cytoskeletal protein that may act as an adaptor that brings other proteins (like kinases) to the cytoskeleton (379 aa)
HLA-A	Major histocompatibility complex, class I, A; Involved in the presentation of foreign antigens to the immune system (365 aa)
PAICS	Phosphoenolpyruvate carboxylase, phosphoenolpyruvate carboxylase acetyl-coenzyme A synthetase (432 aa)
EEF1D	Eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein); Isoform 1-EP-1 delta and EP-1 delta stimulate the exchange of GDP bound to EF-1 alpha to GTP, regenerating EF-1 alpha for another round of transfer of aminoacyl-tRNAs to the ribosome (847 aa)

Figure 5.5: Cell-cell adhesion. STRING protein-protein net shown down regulation of ICAM 1 and ICAM 3 which involved in cell-cell adhesion with 15 proteins containing MSN, ENO1, PRDX1, PARK7, DOD1, HLA-A, PAICS, EEF1D, ANXA2, CAPZB, YWHAZ, YWHAZ, HIST1H3B, TMPO and PDIM1.

Cell adhesion proteins (GOTERM_BP_FAT GO:0007155) which contain 21 proteins, ICAM 1, ICAM 3, HLA-A, MSN, ACTN4, CAPZB, PDLIM1, ARHGDIA, ENO1, PRDX1, SOD1, NME1-NME2, PAICS, PARK7, ANXAZ, ENTPD1, YWHAE, YWHAZ, HIST1H3B, EEF1D and TMPO down regulated in CCN1 KD model of MCL disease. The P value was 8.35E-04, Bonferroni and Benjamini were 0.8769 and 0.0241 respectively (Figure 5.6).

In this study, proteomics analysis data by using DAVID bioinformatics resources shows that CYR61/CCN1 KD in REC1 cells identified ribosome biogenesis associated proteins, some of which were down-regulated and some that were increased expression. Ribosome biogenesis (GOTERM_BP_FAT GO:0042254) was down regulated in KD G676 compare with scrambled. Ribosome biogenesis involved 7 proteins RPL26, RPL8, RPS18, RPLP2, RPL30, RPS20 and RPL35A was in core of this interaction. The P value was 0.011, Bonferroni and Benjamini were 1.0000 and 0.1323 respectively (Figure 5.7).



Nodes:

Network nodes represent proteins
splice isoforms or post-translational modifications are collapsed, i.e. each node represents all the proteins produced by a single, protein-coding gene locus.

Node Color

- colored nodes: query proteins and first shell of interactors
- white nodes: second shell of interactors

Node Content

- empty nodes: proteins of unknown 3D structure
- filled nodes: some 3D structure is known or predicted

Edges:

Edges represent protein-protein associations
associations are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding each other.

Known Interactions

- from curated databases
- experimentally determined

Predicted Interactions

- gene neighborhood
- gene fusions
- gene co-occurrence

Others

- textmining
- co-expression
- protein homology

Your Input:

- RPL8 Ribosomal protein L8 (257 aa)
- RPL30 Ribosomal protein L30 (115 aa)
- RPL26 Ribosomal protein L26 (145 aa)
- RPLP2 Ribosomal protein, large, P2; Plays an important role in the elongation step of protein synthesis (115 aa)
- RPS18 Ribosomal protein S18; Located at the top of the head of the 40S subunit, it contacts several helices of the 18S rRNA (152 aa)
- RPL35A Ribosomal protein L35a; Required for the proliferation and viability of hematopoietic cells. Plays a role in 60S ribosomal subunit formation. The protein was found to bind to both initiator and elongator tRNAs and consequently was assigned to the P site or P and A site (110 aa)
- RPS20 Ribosomal protein S20 (142 aa)

Your Current Organism:

Homo sapiens
NCBI taxonomy id: 9606
Other names: H. sapiens, Homo, Homo sapiens, human, man

Figure 5.7: STRING protein-protein interaction shows ribosome biogenesis which is down regulated in CYR61/CCN1 KD model in REC1 involved RPL35A in core of this interaction that interacted with 6 proteins RPL26, RPL8, RPS18, RPLP2, RPL30 and RPS20.

Proteomics analysis of KD model of CYR61/CCN1 in REC1 cells had also shown 107 proteins that were up-regulated and involved in different pathways. 252 pathways were up-regulated in REC1 CCN1 model using DAVID (Appendix 3). Ribosome biogenesis proteins were overexpressed may be as response to KD of CYR61/CCN1 in this model. Because CCN1 involves in various signalling to perform many cellular function, REC1 cells struggled to resist genetic modification in this dynamic protein. Ribosome biogenesis (GOTERM_BP_FAT GO:0042254) contains 8 proteins, DDX21, RPS23, RPL14, RPL9, EIF4A3, RPL32, RPS4X and RPL4. The P value was 8.34E-04, Bonferroni and Benjamini were 0.8279 and 0.0280 respectively (Figure 5.8).

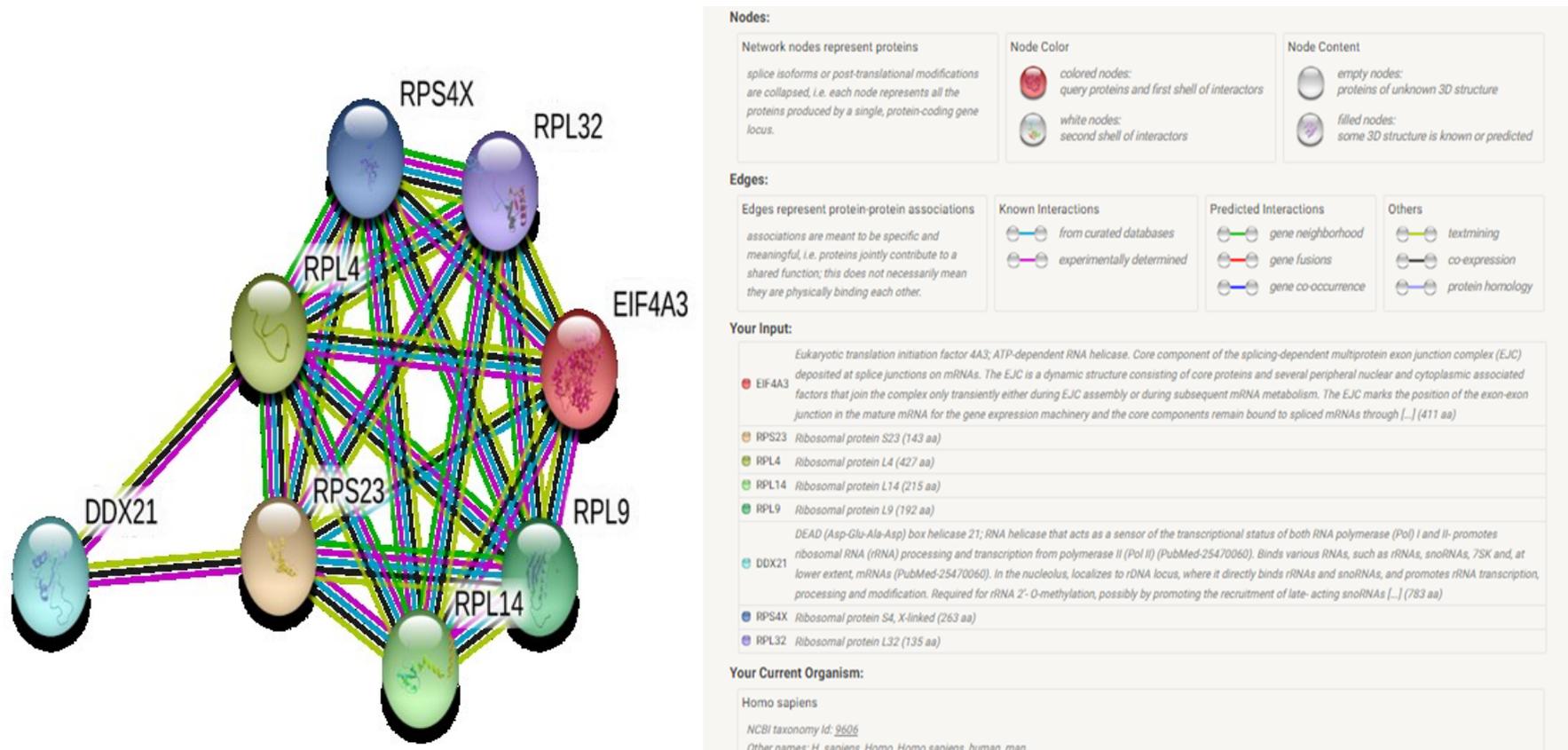
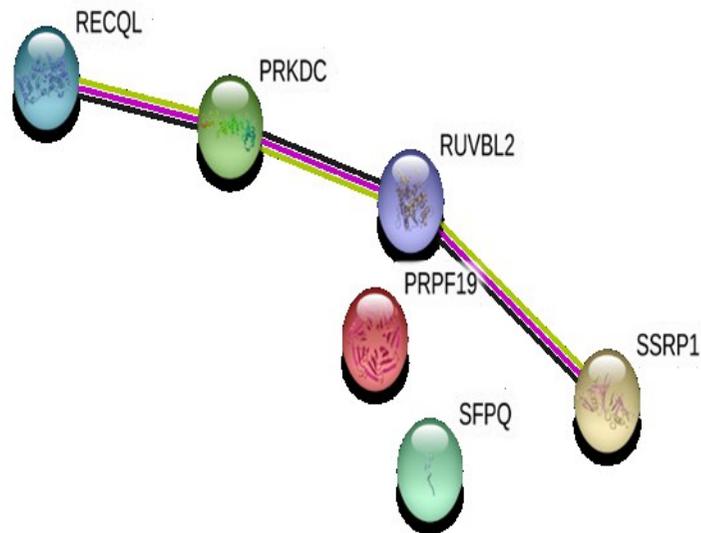


Figure 5.8: Ribosome biogenesis which were upregulated in KD model of CCN1 in REC1 contains 8 proteins, DDX21, RPS23, RPL14, RPL9, EIF4A3, RPL32, RPS4X and RPL4

DNA repair proteins (GOTERM_BP_FAT GO:0006281) were also identified and were potentially upregulated in CCN1 KD model but didn't reach significance. This data set contained 6 proteins, RECQL, RPKDC, RUVBL2, SSRP1, SFPQ and PRPF19. The P value was 0.095, Bonferroni and Benjamini were 1.0000 and 0.7547 respectively (Figure 5.9).



Nodes:

Network nodes represent proteins
splice isoforms or post-translational modifications are collapsed, i.e. each node represents all the proteins produced by a single, protein-coding gene locus.

Node Color

- colored nodes: query proteins and first shell of interactors
- white nodes: second shell of interactors

Node Content

- empty nodes: proteins of unknown 3D structure
- filled nodes: some 3D structure is known or predicted

Edges:

edges represent protein-protein associations
associations are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding each other.

Known Interactions

- from curated databases
- experimentally determined

Predicted Interactions

- gene neighborhood
- gene fusions
- gene co-occurrence

Others

- textmining
- co-expression
- protein homology

Your Input:

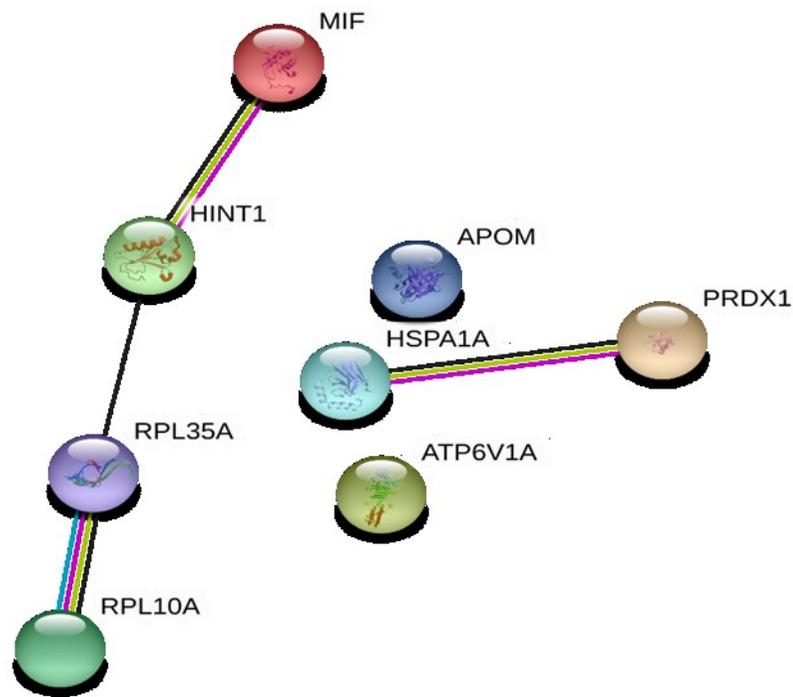
- PRPF19** (red sphere): PRPF19/PSO4 pre-mRNA processing factor 19 homolog (S. cerevisiae); Ubiquitin-protein ligase which is a core component of several complexes mainly involved pre-mRNA splicing and DNA repair. Core component of the PRP19C/Prp19 complex/NTC/Nineteen complex which is part of the spliceosome and participates in its assembly, its remodeling and is required for its activity. During assembly of the spliceosome, mediates Lys-63-linked polyubiquitination of the U4 spliceosomal protein PRPF3. Ubiquitination of PRPF3 allows its recognition by the US component PRPF8 and stabilizes the U4/US/U6 tri-s [...] (504 aa)
- SSRP1** (yellow sphere): Structure specific recognition protein 1; Component of the FACT complex, a general chromatin factor that acts to reorganize nucleosomes. The FACT complex is involved in multiple processes that require DNA as a template such as mRNA elongation, DNA replication and DNA repair. During transcription elongation the FACT complex acts as a histone chaperone that both destabilizes and restores nucleosomal structure. It facilitates the passage of RNA polymerase II and transcription by promoting the dissociation of one histone H2A-H2B dimer from the nucleosome, then subsequently promotes the re [...] (709 aa)
- PRKDC** (green sphere): Protein kinase, DNA-activated, catalytic polypeptide; Serine/threonine-protein kinase that acts as a molecular sensor for DNA damage. Involved in DNA non-homologous end joining (NHEJ) required for double-strand break (DSB) repair and V(D)J recombination. Must be bound to DNA to express its catalytic properties. Promotes processing of hairpin DNA structures in V(D)J recombination by activation of the hairpin endonuclease artemis (DCLRE1C). The assembly of the DNA-PK complex at DNA ends is also required for the NHEJ ligation step. Required to protect and align broken ends of DNA. May als [...] (4127 aa)
- SFPQ** (green sphere): Splicing factor proline/glutamine-rich; DNA- and RNA binding protein, involved in several nuclear processes. Essential pre-mRNA splicing factor required early in spliceosome formation and for splicing catalytic step II, probably as a heteromer with NONO. Binds to pre-mRNA in spliceosome C complex, and specifically binds to intronic polypyrimidine tracts. Involved in regulation of signal-induced alternative splicing. During splicing of PTPRD/CD45, a phosphorylated form is sequestered by THRAP3 from the pre-mRNA in resting T-cells; T-cell activation and subsequent reduced phosphorylation [...] (707 aa)
- RECQL** (blue sphere): RecQ protein-like (DNA helicase Q1-like); DNA helicase that may play a role in the repair of DNA that is damaged by ultraviolet light or other mutagens. Exhibits a magnesium-dependent ATP-dependent DNA-helicase activity that unwinds single- and double-stranded DNA in a 3'-5' direction (649 aa)
- RUVBL2** (purple sphere): RuvB-like 2 (E. coli); Involved in the endoplasmic reticulum (ER)-associated degradation (ERAD) pathway where it negatively regulates expression of ER stress response genes (463 aa)

Your Current Organism:
Homo sapiens

Figure 5.9: DNA repair proteins in CCN1 KD model and contained 6 proteins, RECQL, RPKDC, RUVBL2, SSRP1, SFPQ and PRPF19.

5.2.2 JVM2 CCN1 overexpression model

Unfortunately the overexpression system did not yield many results with only 25 pathways associated using DAVID and will need further investigation. JVM2 CCN1 OE model did not have up-regulated associated pathways, but showed 25 down regulated pathways using DAVID analyses (Appendix 3). Extracellular region part (GOTERM_CC_FAT GO:0044421) was down regulated in CCN1 OE model and contained 8 proteins. Macrophage migration inhibitory factor (MIF) was down regulated in CCN1 OE model and involved with 7 proteins which are HINT1, RPL35A, RPL10A, ATP6V1A, HSPA1A, APOM, and PRDX1. The P value was 0.005227832, Bonferroni and Benjamini were 0.345945849 and 0.191263856 respectively (Figure 5.10).



Nodes:

Network nodes represent proteins
splice isoforms or post-translational modifications are collapsed, i.e. each node represents all the proteins produced by a single, protein-coding gene locus.

Node Color

- colored nodes: query proteins and first shell of interactors
- white nodes: second shell of interactors

Node Content

- empty nodes: proteins of unknown 3D structure
- filled nodes: some 3D structure is known or predicted

Edges:

Edges represent protein-protein associations
associations are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding each other.

Known Interactions

- from curated databases
- experimentally determined

Predicted Interactions

- gene neighborhood
- gene fusions
- gene co-occurrence

Others

- textmining
- co-expression
- protein homology

Your Input:

- MIF** Macrophage migration inhibitory factor (glycosylation-inhibiting factor); Pro-inflammatory cytokine. Involved in the innate immune response to bacterial pathogens. The expression of MIF at sites of inflammation suggests a role as mediator in regulating the function of macrophages in host defense. Counteracts the anti-inflammatory activity of glucocorticoids. Has phenylpyruvate tautomerase and dopachrome tautomerase activity (in vitro), but the physiological substrate is not known. It is not clear whether the tautomerase activity has any physiological relevance, and whether it is impor [...] (115 aa)
- PRDX1** Peroxiredoxin 1; Involved in redox regulation of the cell. Reduces peroxides with reducing equivalents provided through the thioredoxin system but not from glutaredoxin. May play an important role in eliminating peroxides generated during metabolism. Might participate in the signaling cascades of growth factors and tumor necrosis factor-alpha by regulating the intracellular concentrations of H(2)O(2). Reduces an intramolecular disulfide bond in GDPD5 that gates the ability to GDPD5 to drive postmitotic motor neuron differentiation (By similarity) (199 aa)
- ATP6V1A** ATPase, H(+)-transporting, lysosomal 70kDa, V1 subunit A; Catalytic subunit of the peripheral V1 complex of vacuolar ATPase. V-ATPase vacuolar ATPase is responsible for acidifying a variety of intracellular compartments in eukaryotic cells (617 aa)
- HINT1** Histidine triad nucleotide binding protein 1; Hydrolyzes purine nucleotide phosphoramidates with a single phosphate group, including adenosine 5'monophosphoramidate (AMP-NH(2)), adenosine 5'monophosphorophosphidate (AMP-morpholidate) and guanosine 5'monophosphorophosphidate (GMP-morpholidate). Hydrolyzes lysyl-AMP (AMP-N-epsilon-(N-alpha-acetyl lysine methyl ester)) generated by lysine tRNA ligase, as well as Met-AMP, His-AMP and Asp-AMP; lysyl-GMP (GMP-N-epsilon-(N-alpha-acetyl lysine methyl ester)) and AMP-N-alanine methyl ester. Can also convert adenosine 5'-O-phosphorothioate and guan [...] (126 aa)
- RPL10A** Ribosomal protein L10a (217 aa)
- HSPA1A** Heat shock 70kDa protein 1A; In cooperation with other chaperones, Hsp70s stabilize preexistent proteins against aggregation and mediate the folding of newly translated polypeptides in the cytosol as well as within organelles. These chaperones participate in all these processes through their ability to recognize nonnative conformations of other proteins. They bind extended peptide segments with a net hydrophobic character exposed by polypeptides during translation and membrane translocation, or following stress-induced damage. In case of rotavirus A infection, serves as a post-attachme [...] (641 aa)
- APOM** Apolipoprotein M; Probably involved in lipid transport. Can bind sphingosine-1-phosphate, myristic acid, palmitic acid and stearic acid, retinol, all-trans-retinoic acid and 9-cis-retinoic acid (188 aa)
- RPL35A** Ribosomal protein L35a; Required for the proliferation and viability of hematopoietic cells. Plays a role in 60S ribosomal subunit formation. The protein was found to bind to both initiator and elongator tRNAs and consequently was assigned to the P site or P and A site (110 aa)

Your Current Organism:
Homo sapiens

Figure 5.10: Extracellular region part. Macrophage migration inhibitory factor (MIF) was down regulated in CCN1 OE mode and involved with 7 proteins which are HINT1, RPL35A, RPL10A, ATP6V1A, HSPA1A, APOM, and PRDX1.

5.2.3 Protein expression simultaneously modulated in OE and KD models

To identify proteins that were regulated in both the overexpression and knockdown models, we set parameters of fold change to less than 0.7 and over 1.5 fold. Rationale for choosing 0.7 and 1.5 were to ensure that we could identify more subtle changes in protein expression that may be associated with transcriptional components rather than only the larger changes in protein expression usually associated with cytoskeletal / cytoplasmic proteins using a 0.5/2 fold cut off. Simultaneously, 19 proteins were down-regulated in KD model and up-regulated in OE model (Table 5.1) and 25 proteins up-regulated in KD model and down-regulated in OE model (Table 5.2).

Some novel and interesting proteins have been identified in response to modulation of CCN1 expression in our MCL cell line models. In table 5.1, Pre-B cell enhancing colony factor 1 (Uniprot Accession: A0A024R718) was down regulated in CYR61/CCN1 KD in REC1 model (fold change: 0.489262) and up-regulated in CYR61/CCN1 OE in JVM2 model (fold change: 1.688939). ANOVA q-value was 0.002763. CALM 3 protein, calcium ion binding, (Uniprot Accession: Q9BRL5) was down expressed in CYR61/CCN1 KD model (fold change: 0.091035) and overexpressed in CYR61/CCN1 OE model (fold change: 2.086001). ANOVA q-value was 0.009052.

In table 5.2, 3 proteins were selected; apolipoprotein M, macrophage migration inhibitory factor and Talin-1. Apolipoprotein M (Uniprot Accession: I2D5J2) was overexpressed in REC1/CYR61/CCN1 KD model (fold change: 7.107762) and down expressed in JVM2/CYR61/CCN1 OE model (fold change: 0.251444). ANOVA q-value was 0.001623. Macrophage migration inhibitory factor (Uniprot Accession: I4AY87) was up-regulated in REC1/CYR61/CCN1 KD model (fold change: 3.795844) and down expressed in JVM2/CYR61/CCN1 OE model (fold change: 0.364793). ANOVA q-value was 0.389875.

Talin-1 (Uniprot Accession: Q9Y490) was up-regulated in REC1/CYR61/CCN1 KD model (fold change: 1.581185) and down expressed in JVM2/CYR61/CCN1 OE model (fold change: 0.650002) ANOVA q-value was 0.0066.

Table 5.1 Proteins simultaneously downregulated in KD model and up-regulated in OE model

(CCN1 KD676/SCRAMBLED less than 0.7 and OE/ EMPTY VECTOR greater than 1.5 fold)

Uniprot Accession	Fold Change KD 676/Scrm	Fold Change OE/Empty	ANOVA Significant	ANOVA q-value	Protein details
Q567R0	0.632598	1.634836	+	0.007845	UQCRH protein , ubiquinol-cytochrome-c reductase activity, Ubiquinol-Cytochrome C Reductase Hinge Protein, related pathways are Metabolism and Respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins.
B2RE46	0.659792	1.508077	+	0.004197	cDNA, FLJ96923, highly similar to Homo sapiens ribophorin II (RPN2), mRNA; protein N-linked glycosylation
H0YFI1	0.545737	1.50759	+	0.018239	Regulator complex protein LAMTOR1, REGULATION of MAPK and TOR , Late Endosomal/Lysosomal Adaptor, MAPK And MTOR Activator 1, As part of the Regulator complex it is involved in amino acid sensing and activation of mTORC1, a signaling complex promoting cell growth in response to growth factors, energy levels, and amino acids. Activated by amino acids through a mechanism involving the lysosomal V-ATPase, the Regulator functions as a guanine nucleotide exchange factor activating the small GTPases Rag. Activated Regulator and Rag GTPases function as a scaffold recruiting mTORC1 to lysosomes where it is in turn activated. LAMTOR1 is directly responsible for anchoring the Regulator complex to membranes. Also required for late endosomes/lysosomes biogenesis it may regulate both the recycling of receptors through endosomes and the MAPK signaling pathway through recruitment of some of its components to late endosomes. May be involved in cholesterol homeostasis regulating LDL uptake and cholesterol release from late endosomes/lysosomes. May also play a role in RHOA activation.
B4DDF7	0.589661	1.596185	+	0.003659	cDNA FLJ53296, highly similar to Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform , protein phosphatase regulator activity
H6VRG2	0.407092	3.52698	+	0.031124	Keratin 1, KRT1 , structural molecule activity, The type II cytokeratins consist of basic or neutral proteins which are arranged in pairs of heterotypic keratin chains coexpressed during differentiation of simple and stratified epithelial tissues
V9HWC6	0.46461	1.559968	+	0.002677	Peptidyl-prolyl cis-trans isomerase, peptidyl-prolyl cis-trans isomerase activity, protein folding.
H9ZYJ2	0.502747	1.674899	+	0.00169	Thioredoxin, TXN , peptide disulfide oxidoreductase activity, cell redox homeostasis, The protein encoded by this gene acts as a homodimer and is involved in many redox reactions. The encoded protein is active in the reversible S-nitrosylation of cysteines in

					certain proteins, which is part of the response to intracellular nitric oxide. This protein is found in the cytoplasm. Two transcript variants encoding different isoforms have been found for this gene, Diseases associated with TXN include Adult T-Cell Leukemia and Myocarditis. Among its related pathways are Gene Expression and Protein repair; Nitrosylates the active site Cys of CASP3 in response to nitric oxide (NO), and thereby inhibits caspase-3 activity.
A0A024R718	0.489262	1.688939	+	0.002763	Pre-B-cell colony enhancing factor 1, isoform CRA_a, PBEF1, NAMPT , https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4648259/
B4E2Z3	0.375945	1.802968	+	0.01138	cDNA FLJ54090, highly similar to 4F2 cell-surface antigen heavy chain, catalytic activity , (ie Similar to SL3A2)
A8KAQ5	0.425042	1.701171	+	0.002331	cDNA FLJ77404, highly similar to Homo sapiens small nuclear ribonucleoprotein 70kDa polypeptide (RNP antigen) (SNRP70) , transcript variant 1, mRNA. U1 snRNA binding
A0A024R3W7	0.375636	1.94946	+	0.003863	Eukaryotic translation elongation factor 1 beta 2, isoform CRA_a, EEF1B2 , translation elongation factor activity
F4ZW64	0.38961	1.583634	+	0.003639	NF90a, double-stranded RNA binding , This gene encodes a double-stranded RNA (dsRNA) binding protein that complexes with other proteins, dsRNAs, small noncoding RNAs, and mRNAs to regulate gene expression and stabilize mRNAs. This protein (NF90, ILF3) forms a heterodimer with a 45 kDa transcription factor (NF45, ILF2) required for T-cell expression of interleukin 2. This complex has been shown to affect the redistribution of nuclear mRNA to the cytoplasm. Knockdown of NF45 or NF90 protein retards cell growth, possibly by inhibition of mRNA stabilization. In contrast, an isoform (NF110) of this gene that is predominantly restricted to the nucleus has only minor effects on cell growth when its levels are reduced. Alternative splicing results in multiple transcript variants encoding distinct isoforms.[provided by RefSeq, Dec 2014]
A0A024QZ42	0.219351	1.73697	+	0.001867	HCG1985580, isoform CRA_c, PDCD6, calcium ion binding, Programmed cell death protein 6
V9HW98	0.25155	1.672154	+	0.003377	Epididymis luminal protein 2, HEL2, E3 ubiquitin-protein ligase HEL2 , Probable ubiquitin-protein ligase involved in the degradation-related ubiquitination of histones. Contributes to the post-translational regulation of histone protein levels by polyubiquitination of excess histones for subsequent degradation
Q496I0	0.186965	1.797549		0.102643	COX7A2 protein, COX7A2 , This protein is one of the nuclear-coded polypeptide chains of cytochrome c oxidase, the terminal oxidase in mitochondrial electron transport., Diseases associated with COX7A2 include Barrett's Adenocarcinoma. Among its related pathways are Respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins. and Cardiac muscle contraction
A0A024RCA7	0.111717	1.637925	+	0.001714	Ribosomal protein, large, P2, isoform CRA_a, RPLP2.

Q9BRL5	0.091035	2.086001	+	0.009052	CALM3 protein, calcium ion binding, CALMODULIN 3 , This gene encodes a member of a family of proteins that binds calcium and functions as a enzymatic co-factor. Activity of this protein is important in the regulation of the cell cycle and cytokinesis
J3KTF8	0.074175	1.556524	+	0.001739	Rho GDP-dissociation inhibitor 1, ARHGDI1 , Rho GDP Dissociation Inhibitor Alpha, This gene encodes a protein that plays a key role in the regulation of signaling through Rho GTPases. The encoded protein inhibits the disassociation of Rho family members from GDP (guanine diphosphate), thereby maintaining these factors in an inactive state. Activity of this protein is important in a variety of cellular processes, and expression of this gene may be altered in tumors.
Q76LA1	0.065526	1.662608	+	0.008658	CSTB protein, Cystatin B , The cystatin superfamily encompasses proteins that contain multiple cystatin-like sequences. Some of the members are active cysteine protease inhibitors, while others have lost or perhaps never acquired this inhibitory activity. There are three inhibitory families in the superfamily, including the type 1 cystatins (stefins), type 2 cystatins and kininogens. This gene encodes a stefin that functions as an intracellular thiol protease inhibitor. The protein is able to form a dimer stabilized by noncovalent forces, inhibiting papain and cathepsins l, h and b. The protein is thought to play a role in protecting against the proteases leaking from lysosomes

Table 5.2 Proteins simultaneously up-regulated in KD model and down-regulated in OE model

(CCN1 KD676/SCRAMBLED greater than 1.5 and OE/ EMPTY VECTOR less than 0.7 fold)

Uniprot Accession	Fold Change KD 676/Scrm	Fold Change OE/Empty	ANOVA Significance	ANOVA q-value	Protein details
A8K5I0	20.57224	0.455629	+	0.00168	Epididymis secretory protein Li 103, HSPA1A, HSPA1B, Proteosome P45 like ATPase
I2D5J2	7.107762	0.251444	+	0.001623	Apolipoprotein M, links with Rheumatoid Arthritis
H7BXH6	5.258516	0.542395	+	0.009607	Chitinase, CHIA, hydrolase, lysozyme activity
Q8TE92	3.96974	0.249509	+	0.001945	cDNA FLJ23782 fis, clone HEP20947
B4E266	3.70062	0.663747		0.390382	cDNA FLJ58466, highly similar to Leucyl-tRNA synthetase, cytoplasmic
A0A024RAM0	3.962813	0.639955		0.690141	Transportin 1, isoform CRA_a, for intracellular transport
A8K5I7	3.931517	0.69856		0.082126	Ribosomal protein S23, isoform CRA_a
I4AY87	3.795844	0.364793		0.389875	Macrophage migration inhibitory factor, links with Rheumatoid Arthritis and T cell development / disorders
M0QXS5	3.154703	0.691388		0.145811	Heterogeneous nuclear ribonucleoprotein L
P53621	2.693598	0.666542	+	0.040847	Coatomer subunit alpha, intracellular transport, ER Golgi vesicle mediated transport
P48047	3.030063	0.654179		0.270884	ATP synthase subunit O, mitochondrial, drug binding, ATP synthase activity
B4DDZ5	2.675871	0.674476		0.419119	DNA FLJ53969, highly similar to Trifunctional enzyme subunit alpha, mitochondrial, fatty acid beta-oxidation
V9HW24	2.673619	0.306314		0.168792	Epididymis secretory protein Li 73, HEL-S-73,
Q9NTK5	2.662313	0.688177		0.08092	, OLA1, ATPase requires Mg, binds cadherin
A0A140VK70	2.513442	0.684032	+	0.014443	Testis secretory sperm-binding protein Li 197a, ATP binding, cytoplasmic ribonucleoprotein granule

B8ZZI4	2.117774	0.5837	+	0.004156	Coiled-coil domain-containing protein 150, CCDC150 , Transcription factor / promoter / enhancer. Links with having transcription factor bindings sites for FOXO3b FOXO3a FOXO3 Pax-5 Ik-2 RORalpha2 TBP POU2F1c POU2F1b POU2F1a and upstream PKNOX1, SMAD1, FOXA2, TAF9B, CREB1, PKNOX1, CLOCK, JUN, CEBPB. Links to NOX4 potential oxygen sensor in response to HIF1a and also RAI1 retinoic acid induced gene regulated by CLOCK.
Q08ES8	1.765457	0.310873		0.777221	Cell growth-inhibiting protein 34 , Ribonucleoprotein, Ribosomal protein
Q5TFA5	1.643095	0.635322		0.218866	GINM1 , glycoprotein integral membrane 1 , single pass type I membrane protein https://www.genecards.org/cgi-bin/carddisp.pl?gene=GINM1
D3YTB1	2.076259	0.661053	+	0.026776	60S ribosomal protein L32
C9JXZ7	1.655766	0.585244		0.259281	RFC4, Replication factor 4 , plasma protein, predicted intracellular protein
Q4ZG57	1.642209	0.666008	+	0.00156	DNA helicase, MCM6 , https://www.sciencedirect.com/science/article/pii/S2211124717301778?via%3Dihub
Q9Y490	1.581185	0.650002	+	0.0066	Talin-1, TLN1 , involved in connections of major cytoskeletal structures to the plasma membrane. High molecular weight cytoskeletal protein concentrated at regions of cell-substratum contact and, in lymphocytes, at cell-cell contacts, Talin-1 functions to mediate cell-cell adhesion via the linkage of integrins to the actin cytoskeleton and in the activation of integrins, required for lymphocyte homing and development https://www.ncbi.nlm.nih.gov/pubmed/20923969
P30084	1.56129	0.574221		0.213159	ECHS1 Straight-chain enoyl-CoA thioesters from C4 up to at least C16 are processed, although with decreasing catalytic rate. Belongs to the enoyl-CoA hydratase/isomerase family. Amino Acid Metabolism - lysine degradation; Amino Acid Metabolism - tryptophan; Amino Acid Metabolism - valine, leucine and isoleucine degradation; Carbohydrate Metabolism - butanoate; Carbohydrate Metabolism - propanoate; EC 4.2.1.17; Lipid Metabolism - fatty acid; Lipid Metabolism - fatty acid elongation in mitochondria; Lyase; Mitochondrial; Other Amino Acids Metabolism - beta-alanine; Secondary Metabolites Metabolism - limonene and pinene degradation
V9HWF5	1.543778	0.694702		0.218976	Peptidyl-prolyl cis-trans isomerase, protein folding activity
A0A024RDH8	1.517251	0.664698	+	0.021775	Ribosomal protein L34, isoform CRA_a , RPL34, required for translation

5.3 Discussion

Despite the advances in understanding of the molecular pathogenesis of MCL, treatment remains inadequate (Weisenburger *et al.*, 2000). Proteomics is a robust tool to examine protein changes between samples. In this study, proteomics showed that in the CYR61/CCN1 KD in REC1 cells 112 proteins were down regulated. Intercellular adhesion molecules 1, and 3 (ICAM 1 and ICAM 3, which are ligands for integrins) were down regulated in KD model and are associated with adhesion pathways.

ICAM 1 belongs to the immunoglobulin superfamily mediating cell-cell adhesion (Lawson & Wolf, 2009; Zimmerman & Blanco, 2008) and was expressed by bladder high grade tumour cell line and played a role in cancer migration (Roche *et al.*, 2003). Many studies have found that CCN family proteins such as NOV and CCN6 involved in enhancing migration and metastasis of different types of cancer through up-regulation of ICAM 1 expression protein (Chen *et al.*, 2012; Fong *et al.*, 2012; Liu *et al.*, 2015). Furthermore, *in vivo*, CCN1 knock down mice showed less lung injury compared with wild type, CCN1 promoted ICAM 1 expression and mediated endothelial cell adhesion (Ringo *et al.*, 2011). CCN1 increased adhesion and interaction of thymic epithelial cells as well as thymocytes through binding to ICAM 1 and integrin $\alpha 6$ (Emre *et al.*, 2013). These findings support our result that CCN1 KD in REC1 cells downregulated ICAM 1, which suggests a role in cancer migration cells and may be involved metastasis. ICAM 3, is an adhesion molecule and has been found playing an important role in enhancing adhesion of T lymphocytes to endothelial cells and extracellular matrix proteins supporting immune activation (Cid *et al.*, 1994). However, high expression of ICAM 3 is related to increase treatment resistance in cancer cells and enhanced migration and invasion of human non-small cell lung cancer cells (Park *et al.*, 2010). Many studies suggested that down regulation of ICAM 1 and ICAM 3 can be anticancer therapy in different types of cancer (Di *et al.*, 2016; Park *et al.*, 2010). ICAM 1 is a ligand for the receptors CD11a (ITGAL) CD18

(ITGB2) and CD11b (ITGAM) and ICAM 3 is a ligand for integrin alpha D (ITGAD), Integrin beta 2 / CD18 (ITGB2), integrin alpha L (ITGAL). ITGAM and ITGB2 are known to be regulated by CCN1 (Figure 5.2). Whilst most studies to date have been completed in solid tumour investigations, our results suggest that CCN1 regulates the ligands ICAM 1 and ICAM3 in MCL and this regulatory network may be specific to MCL or potentially within B cell signalling. Further investigation is required to investigate the role of CCN1 modulating ICAM1 and ICAM3 expression.

The human ribosome which is essential for protein synthesis by translation of messenger RNA (mRNA) to protein comprises of 4 ribosomes (rRNAs) and 80 different ribosomal proteins (RPs) (Mao-De & Jing, 2007). Eukaryotic ribosome consists of 2 subunits which are large L (60S) and small S (40S) are responsible for different types of rRNAs and RPs (Chakraborty, Uechi & Kenmochi, 2011) as well as accessory factors named proteins associated with ribosome (PAR) for instance initiation factors (IFs) and elongation factors (EFs) (Mao-De & Jing, 2007). Ribosome biogenesis is the process of ribosome production and up-regulated in cancer in response to extracellular or intracellular stimuli stimulates ribosomal stress leading to an accumulation of free RPs (Montanaro, Treré & Derenzini, 2012; Zhang & Lu, 2009; Zhou *et al.*, 2012). In 2015, this was reviewed by (Zhou *et al.*) who identified potential reasons for ribosomal stress are “chemical agents or radiation that inevitably perturb rRNA production or mediates RP degradation, lack of nutrients, including serum or glucose starvation, hypoxia and gene deregulation e.g. malfunction of genes required for ribosome biogenesis resulting from genetic alterations or experimental manipulation” (Zhou *et al.*, 2015). In addition to protein synthesis, RPs have functions beyond the ribosome named extra ribosomal functions which are DNA replication, transcription, DNA repair, RNA splicing, regulation of cell growth and apoptosis and development of cellular transformation (Mao-De & Jing, 2007). Increasing evidences of dysregulation of RPs in cancer shown that individual RPs can play a tumour

promotor or tumour suppressor in tissue or disease specific (Zhou *et al.*, 2015). For example, the expression of numerous RPs was highly expressed at mRNA or protein level in various human cancers (Artero-Castro *et al.*, 2011; Kondoh *et al.*, 1992; Kondoh *et al.*, 2001; Shuda *et al.*, 2000). Additionally, in nude mice, overexpression of RPS3A enhanced transformation of NIH-3T3 cells and promoted tumour growth (Naora *et al.*, 1998). The expression level of RPL9 at mRNA was high in small-cell lung carcinoma, decreased in Adenocarcinoma, Large cell carcinoma and low expression in squamous cell lung carcinoma where it is associated with playing an important role in tumour development and cell proliferation (Dlamini & Mphahlele, 2006). Knockdown of RPL9 expression has found to inhibit colorectal cancer cell growth through decrease activation of Id-1/NF- κ B signalling in nude mice experiment (Baik *et al.*, 2016). On the other hand, knockdown of RPS4X decreased tumour cell growth but increased tumour cell resistance-treatment in ovarian cancer and breast cancer cell lines (Garand *et al.*, 2011; Tsofack *et al.*, 2013). Our result shown that 8 ribosomal proteins DDX21, RPS23, RPL14, RPL9, EIF4A3, RPL32, RPS4X and RPL4 involved in ribosome biogenesis were up-regulated in CYR61/CCN1 KD model (Figure 5.8). This result needs more investigation to reveal if these RPs overexpressed in response to gene modification or may have specific functions in regulation of treatment resistance, regulation of cell proliferation and apoptosis.

However, some RPs are considered tumour suppressors through activation of tumour inhibitors or inactivation of oncoproteins (de Las Heras-Rubio *et al.*, 2014; Zhou *et al.*, 2015). For example, many studies have found that different RPs such as RPL26, RPS20, RPS14 and RPS27A have involved in suppression of cancer cell proliferation through regulation of the MDM2/MDMX-p53 signalling (Daftuar *et al.*, 2013; Sun *et al.*, 2011; Zhang *et al.*, 2010; Zhou *et al.*, 2013). STRING protein-protein interaction network shows ribosome biogenesis which is down regulated in CYR61/CCN1 KD model in REC1 involved RPL35A in core of this interaction that interacted with 6 proteins RPL26, RPL8, RPS18, RPLP2, RPL30 and RPS20

(Figure 5.7). Down regulation of RPL35A in CCN1 KD may suggest a role in increased apoptosis and regulation of cell growth. Furthermore, RPL35A was down regulated in the JVM2 CCN1 OE model in extracellular region part pathway (Figure 5.10) that could be a promising target in enhancing apoptosis and regulation of proliferation of MCL at early and advance stages of disease. RPL35A-deficient hematopoietic cell lines shown decreased proliferation and enhanced apoptosis 45%-55% compared with control (Farrar *et al.*, 2008). Furthermore, low expression of RPL35A is associated with reduced cellular proliferation, enhanced apoptosis and defective ribosome biogenesis in Diamond Blackfan Anaemia (Caywood *et al.*, 2009).

In this study, RECQL, RPKDC, RUVBL2, SSRP1, SFPQ and PRPF19 were up-regulated in CCN1 KD model in a DNA repair pathway (Figure 5.9). RECQL, an ATP-dependent DNA helicase enzyme, plays crucial roles in DNA repair, recombination, replication and transcription by interaction with a unique set of protein partners (Croteau *et al.*, 2014). In 2018, (Xu *et al.*) have found that down regulation of RECQL protein associated with poor survival in breast cancer patients more than those with overexpression. Moreover, RECQL-deficient cancer cells were sensitive to treatment with DNA-toxic drug that reflects its role in DNA repair pathway for genomic integrity (Sharma & Brosh Jr, 2007; Sharma *et al.*, 2007; Wu & Brosh, 2010). This is consistent with our result that shows RECQL protein expression up-regulated in REC1-CCN1 KD model in response to the DNA repair process.

Pre-B-cell colony enhancing factor 1, (PBEF1 also known NAMPT) which is pro-inflammatory cytokine expresses by various cells lymphoid and non-lymphoid cells and contains 2 forms intracellular and extracellular (Rongvaux *et al.*, 2002; Sun, Lei & Zhang, 2013). Intracellular acts as cytokine-like molecule (PBEF) and extracellular has as enzymatic function in nicotinamide phosphoribosyl transferase (NAMPT) and both function in immune modulation, cell metabolism and inflammation (R Moschen, R Gerner & Tilg, 2010; Roberts

et al., 2013; Sun, Lei & Zhang, 2013). Several studies have demonstrated that overexpression of pre-B-cell colony enhancing factor involved in different tumour malignancies including breast, prostate, colorectal, ovarian, well-differentiated thyroid, endometrial carcinomas, myeloma, melanoma, and in malignant lymphomas (Folgueira *et al.*, 2005; Hufton *et al.*, 1999; Maldi *et al.*, 2013; Olesen, Hastrup & Sehested, 2011; Patel *et al.*, 2010; Shackelford *et al.*, 2013; Shackelford *et al.*, 2010; van Beijnum *et al.*, 2002; Venkateshaiah *et al.*, 2013; Wang *et al.*, 2011). In studying 53 samples of malignant lymphoma including diffuse large B-cell lymphoma, follicular B-cell lymphoma, Hodgkin's lymphoma and peripheral T-cell lymphoma, the NAMPT was overexpressed in the aggressive lymphoma malignancy more than indolent follicular lymphoma. Depletion of NAMPT by NAMPT inhibitor, APO866 which is in clinical phase II trials in lymphomas, revealed that follicular lymphoma was a promising target for inhibition of NAMPT while aggressive lymphoma particularly Hodgkin's lymphoma needed a combination treatment of NAMPT inhibitor with nicotinic acid (Olesen, Hastrup & Sehested, 2011). Reduced expression of pre-B-cell colony enhancing factor 1 in the REC1 CCN1 KD model could indicate a promising target for CCN1 positivity in early stage MCL.

CALMODULIN (CaM) is an intracellular Ca^{2+} receptor protein encoded by 3 genes CALM 1, CALM 2, and CALM 3 and regulates cell proliferation, cell cycle and apoptosis (Berchtold & Villalobo, 2014; Boczek *et al.*, 2016; Chen *et al.*, 2013; Monteith, Davis & Roberts-Thomson, 2012). CaM has been seen to regulate various intracellular enzymes including phosphodiesterases, adenylyl cyclases, ion channels, protein kinases, and protein phosphatases (Chin & Means, 2000). In cell cycle regulation, calcium/calmodulin ($\text{Ca}^{+2}/\text{CaM}$) complex regulates the phosphorylation of retinoblastoma protein at early G1 stage of cell cycle. Inhibition of CaM by antagonists enhanced arrest of the cell cycle at the G1 stage through inhibition of cdk4 and cdk2 resulting in hypophosphorylation of retinoblastoma protein (Taulés *et al.*, 1998). Many studies have demonstrated that overexpression of CaM in different tumour

cells and in cancer progression resulting in tumour metastasis (Berchtold & Villalobo, 2014; Karp *et al.*, 2007; Mishra, Siddique & Saleem, 2012). CaM has been reported to be involved in the initiation and progression of ductal cancers through activation of PI3K α /Akt and Raf/MEK/ERK pathways (Nussinov *et al.*, 2015). Blocking of calmodulin/PI3K α binding in a K-Ras4B/calmodulin/PI3K α model is considered a promising treatment strategy (Nussinov *et al.*, 2015). Furthermore, depletion of CaM by N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) prevented the metastasis development of primary lewis lung carcinoma in a mouse model (Ito, Wang & Shimura, 1991). Our result demonstrated that CaM (CALM 3) was down regulated in CCN1 KD model and up-regulated in CCN1 OE model (Table 5.1). Interestingly, a study revealed for the first time that CCN3 has a role in calcium ion signalling when it found CCN3 interacted with S100A4 (which is a calcium binding protein) in glioblastoma and neuroblastoma cells leading to S100A4 being considered as a partner of CCN3 (Li *et al.*, 2002). This interaction may associated with carcinogenesis and in normal tissues and addition of CCN2 and CCN3 enhanced intracellular calcium concentrations involved in regulation of the calcium flux which is associated with cell growth control and motility. These results indicated a role for CCN3 and all CCN family protein members in calcium ion signalling (Li *et al.*, 2002). This is consistent with our results that show knock down of CCN1 in REC1 results in depletion of intracellular Ca²⁺ receptor protein (CALMODULIN) and overexpression of CCN1 in JVM2 results in upregulation of CALMODULIN.

Additionally, CCN family proteins bind to other receptors such as the lipoprotein receptor-related proteins (LRPs) to mediate biological functions (Segarini *et al.*, 2001). For example, CCN2 which is a member of CCN family proteins modulated cell adhesion and Wnt signalling in different cells by binding to LRP1 and LRP6 respectively (Gao & Brigstock, 2003; Mercurio *et al.*, 2004). Apolipoprotein M (ApoM), which is a novel lipoprotein plasma protein of the

apolipoprotein family normally expressed in liver and kidney and is expressed in the human colorectal adenocarcinoma cell line (Caco-2 cells) (Calayir *et al.*, 2008; Luo *et al.*, 2010). In 2010, (Luo *et al.*) have found that the expression level of mRNA ApoM was substantially higher in the patients having colorectal cancer with lymph node metastasis than the patients without lymph node metastasis. Furthermore, they have reported that the expression level of mRNA ApoM was significantly increased in the patients with advanced stage of Dukes (stages 3 and 4) than the patients with early stage of Dukes (stage 1 and 2) and suggested that ApoM expression level could play a role in cancer progression (Luo *et al.*, 2010). Recently, another study has shown that the expression of ApoM protein level was increased in the non-small cell lung cancers tissues compare with adjacent tissue which suggested a role in tumour cell proliferation and invasion through activation of ERK1/2 and PI3K/AKT pathways (Zhu *et al.*, 2018). In the present study, overexpression of apolipoprotein M was detected in REC1 CYR61/CCN1 KD model and down regulation in JVM2 CYR61/CCN1 OE model. HIF1 alpha binds to the ApoM and drives ApoM expression (Huang *et al.*, 2015). This suggests ApoM levels may be modulated in a complex network with CCN1 in response to oxygen sensing in B cells and the Bone marrow microenvironment.

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine secreted by T lymphocytes, epithelial cells, endothelial cells and macrophages and participates in many cellular functions including carcinogenesis (Conroy, Mawhinney & Donnelly, 2010; Richard, Kindt & Saussez, 2015). Many studies have demonstrated that MIF overexpression in solid tumours such as lung, colorectal, breast, cervical and prostate cancers playing a critical role in cancer progression through enhancing tumour cell proliferation and invasion (Guo *et al.*, 2015; He *et al.*, 2009; Meyer-Siegler *et al.*, 2006; Rendon *et al.*, 2007; Richard *et al.*, 2014). Up-regulation of MIF was correlated with enhanced tumour invasion and metastasis to the liver, spleen, lymph nodes and intestine in pancreatic cancer cells (*in vivo* and *in vitro*) (Funamizu *et*

al., 2013). Inhibition of MIF activation by ISO-1(an inhibitor of MIF) attenuated tumour cell migration through activation of JNK pathway in adenoid cystic carcinoma (ACC) cells (Liu *et al.*, 2013). There is growing evidence that indicates knock down of MIF expression could be a therapeutic target in cancer treatment by enhancing apoptosis and decreasing tumour proliferation (Conroy, Mawhinney & Donnelly, 2010; Huang *et al.*, 2014). Moreover, inhibition of the ATPase activity of heat shock protein 90 (HSP90) in different cancer cells resulted in the down regulation of MIF (Schulz & Moll, 2014) which makes targeting HSP90 a novel strategy for the inhibition of MIF in cancer (Kindt *et al.*, 2016). Kindt *et al.* (2013) have revealed a role for MIF in head and neck squamous cell carcinoma (HNSCC) treatment when they have seen that the MIF-knock down cells grew slowly and were more sensitive to anti-cancer treatment than control cells. In this study, MIF was down regulated in the JVM2 CCN1 OE model (Figure 5.9) and up-regulated in the CCN1 KD model. This suggests that MIF has an inverse relationship with CCN1 expression in MCL.

Talin-1 (TLN1), is a cytoskeleton protein involved in cell migration and plays an important role in tumour formation and tumour migration through regulation of cell-cell adhesion by binding to different receptors such as integrins and actin (Desiniotis & Kyprianou, 2011; Sakamoto *et al.*, 2010). Overexpression of talin-1 has been reported in prostate cancer with enhanced tumour migration, invasion and mediates bone and lymph node metastasis (Jin *et al.*, 2015; Xu *et al.*, 2016; Zhang *et al.*, 2015). Moreover, high expression of talin-1 correlated with short-survival patients particularly in advanced stage of disease in nasopharyngeal carcinoma (NPC) patients (Xu *et al.*, 2015). Recently, talin-1 was found to regulate genes-associated hepatocellular carcinoma (HCC) progression; this study reported that knock down of talin-1 dysregulated 3099 genes (1924 genes up-regulated and 2175 down regulated (CYR61 which is a growth factor-inducible gene was down regulated) (Chen *et al.*, 2017)). Gene ontology (GO) profiled that TLN1 enhanced transcription of genes associated with cell-adhesion, ion

transport and cell growth which suggested a promising therapeutic targets (Chen *et al.*, 2017).

Whilst the ligands ICAM 1 and 3 are downregulated in the CCN1 KD model, the cell is possibly trying to compensate by up-regulation of the TLN1.

In present study, overexpression of ApoM, MIF and TLN1 in the CCN1 KD model and down regulation in CCN1 in the OE model may identify novel pathways / approaches for more effectively targeting MCL.

Chapter 6

General conclusion and future work

Chapter 6

6.1 General Conclusion and future work

CCN1 expression has not been investigated in MCL to our knowledge to date. CCN1 full length protein and truncated proteins were identified in a human cell line model for MCL progression; REC1, G519 and JVM2 where REC1 cells are consistent with early stage disease, G519 and JVM2 representative of aggressive disease. The magnitude of CCN1 expression by RQ-PCR in the cell lines is REC1>G519>JVM2 cells, depicting a decrease in CCN1 expression with disease progression. This is consistent with findings for Cyclin D1 levels associated with early stage disease and down-regulation in aggressive MCL (Zeng *et al.*, 2012). CCN1 mirrors Cyclin D1 expression in MCL. Investigation of CCN1 protein expression by Western blotting showed that whilst expression of full-length CCN1 was barely altered across the cell lines, expression of a truncated form (20kDa) was decreased in aggressive G519 and JVM2 cells. For future work, further investigations of the role(s) of the 20kDa protein in MCL would help elucidate the significance of this truncated form.

We investigated the expression of cell cycle regulators p21 and p27 in the cell line model. Whilst p21 expression was barely detectable in REC1 cells, increased expression was detected in G519 and JVM2 cells by RQ-PCR and Western blotting. Conversely, p27 was detected in REC1 cells and reduced in expression in G519 and JVM2 cells by RQ-PCR and Western Blotting. The subcellular localisation of both proteins have not been reported to date. This study detected expression of p21 and p27 in cytoplasm of the three MCL cell lines; absence from the nuclear compartment mirrors the loss of roles in cell cycle inhibition and further acquisition of tumour promoting roles via suppression of apoptosis, contributing to enhanced tumour proliferation and treatment resistance (Besson, Dowdy & Roberts, 2008; Gartel, 2009; Min *et al.*, 2004; Roninson, 2002). Mitochondrial

detection of p21 in the JVM2 cell line supports an additional anti-apoptotic role (Suzuki A, 1999).

Many studies have indicated that activation or upregulation of cell cycle regulators, p21, p27 and cyclin D1 are induced through CCN1 signalling (Sawai *et al.*, 2007; Tong *et al.*, 2004), whilst the functional effect or output appears to be cell type specific. In 2014, (Saglam *et al.*) have found that induction of cyclin D1 expression in grade ductal carcinoma in situ (DCIS) can occur through CCN1 signalling leading to cell cycle progression. CCN1 protein promoted cell cycle arrest by increased cell senescence at G0/G1 phase through activation notch-1-p21 pathway and reduced proliferation of human trophoblast cells (Kipkeew *et al.*, 2016). Similarly, CCN1 signalling induced accumulation of p53 and p21 driving cell senescence leading to suppression of lung cancer cell proliferation (Jim Leu *et al.*, 2013). A future work of investigation of cytoplasmic subcellular localisation of p21 and p27 proteins in REC1, G519 and JVM2 cells and mitochondrial subcellular of p21 protein in JVM2 cells signalling pathways would be interesting to study their roles in apoptosis and treatment resistant.

Stable overexpression and knockdown cell lines modules were completed in JVM2 (JVM2 OE) and REC1 (REC1 KD) cells respectively. Proteomics analysis of JVM2 OE and REC1 KD revealed interesting results showing regulation of 44 proteins (Table 5.1 and Table 5.2). Table 5.1 shows proteins simultaneously downregulated in the CCN1 KD model and up-regulated in CCN1 OE model and comprised of 19 proteins regulated by CCN1. Table 5.2 shows proteins simultaneously up-regulated in CCN1 KD model and down-regulated in CCN1 OE model and comprised of 25 proteins modulated by CCN1.

Our results suggest novel roles for CCN1. Whilst CCN1 roles are substantial in solid tumour research, CCN1 role(s) within the haematopoietic compartment are less well defined or investigated. CCN1 may have potential role(s) as a novel pro-inflammation

regulator by modulating macrophage migration inhibitory factor (MIF) and within regulation of haematopoiesis via pre-B cell colony enhancing factor (PBEF1). Liu *et al.* (2008) have suggested a role for CCN1 (CYR61) in contributing in MIF-mediated pro-inflammation, angiogenesis, cell proliferation and tumourgenesis when they have seen that the MIF knockdown cells led to downregulation of CCN1 expression in both mRNA and protein levels which inhibit CCN1 dependent signalling pathway (Figure 6.1).

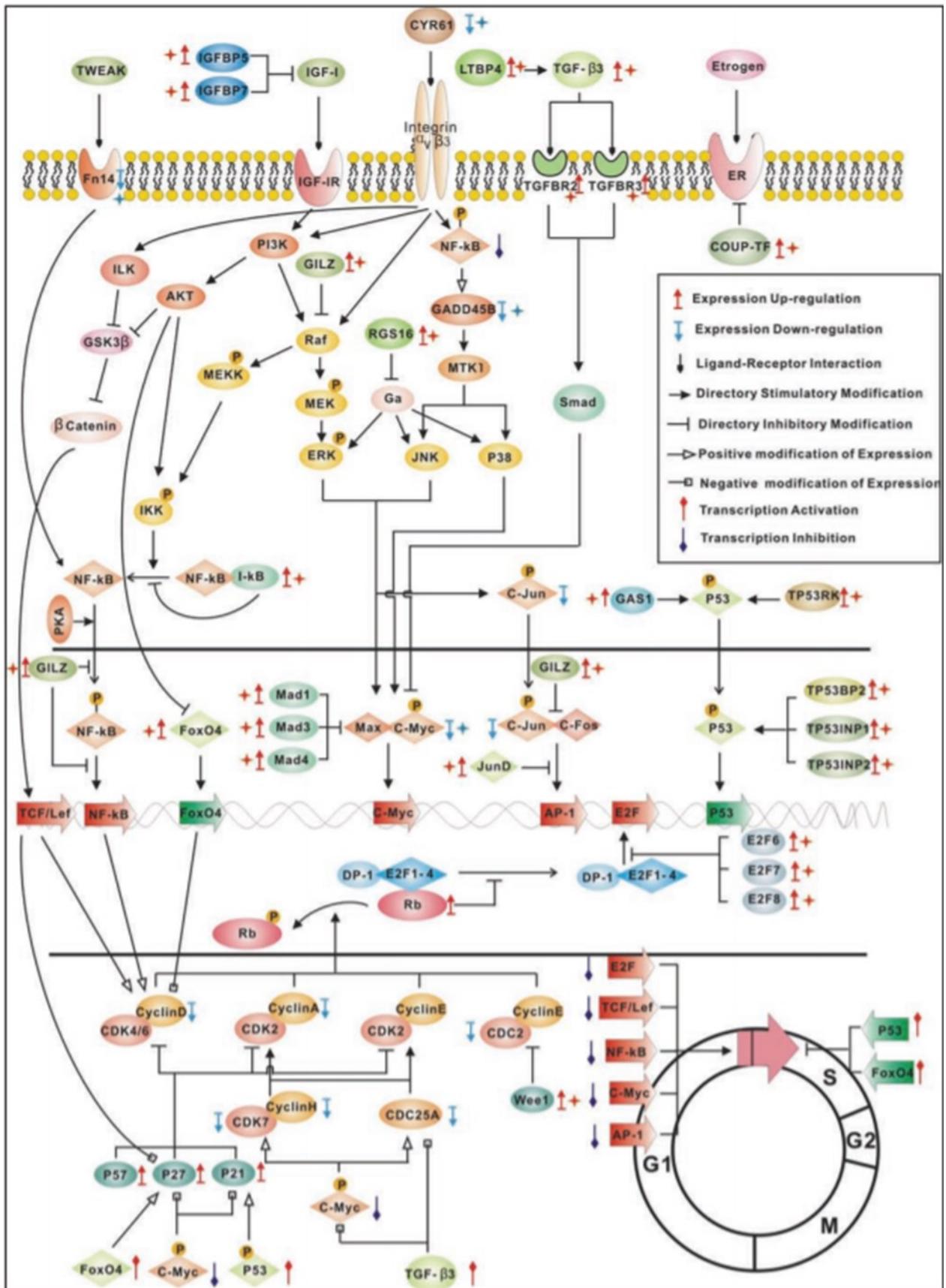


Figure 6.1: Schematic diagram overview of MIF knockdown led to down regulation of CCN1 (CYR61) and inhibition of CCN1-dependent signalling pathway. Image taken from (Liu *et al.*, 2008).

CCN1 was shown to modulate calcium ion signalling by targeting intracellular calcium receptor protein Calmodulin 3 (CALM 3). CCN1 altered Apolipoprotein M (ApoM) and Talin 1 (TLN1) expression and could potentiate new targets to supplement treatment for MCL. However, these novel pathways would need further investigations to identify the role(s) of CCN1 in B cell development and within the bone marrow microenvironment where regulation of haematopoiesis ensues.

REFERENCES

- Abbas, T. & Dutta, A. (2009) 'p21 in cancer: intricate networks and multiple activities'. *Nature Reviews Cancer*, 9 (6), pp. 400-414.
- Aberle, H., Bauer, A., Stappert, J., Kispert, A. & Kemler, R. (1997) ' β -catenin is a target for the ubiquitin–proteasome pathway'. *The EMBO journal*, 16 (13), pp. 3797-3804.
- Abukhdeir, A. M. & Park, B. H. (2008) 'P21 and p27: roles in carcinogenesis and drug resistance'. *Expert reviews in molecular medicine*, 10 pp. e19.
- Ahmed, M., Zhang, L., Nomie, K., Lam, L. & Wang, M. (2016) 'Gene mutations and actionable genetic lesions in mantle cell lymphoma'. *Oncotarget*, 7 (36), pp. 58638.
- Ahrens, A. K., Chaturvedi, N. K., Shukla, A., Nordgren, T. M., Hegde, G. V., Vose, J. M. & Joshi, S. S. (2013) 'Polo-like kinase 1: A novel target for the treatment of therapy-resistant mantle cell lymphoma'. *Lymphoma*, Volume 2013, Article ID 782903, 10 pages <http://dx.doi.org/10.1155/2013/782903>.
- Akhurst, R. J. & Derynck, R. (2001) 'TGF- β signaling in cancer—a double-edged sword'. *Trends in Cell Biology*, 11 (11), pp. S44-S51.
- Akinleye, A., Chen, Y., Mukhi, N., Song, Y. & Liu, D. (2013) 'Ibrutinib and novel BTK inhibitors in clinical development'. *Journal of Hematology & Oncology*, 6 (1), pp. 59.
- Alam, N., Goel, H. L., Zarif, M. J., Butterfield, J. E., Perkins, H. M., Sansoucy, B. G., Sawyer, T. K. & Languino, L. R. (2007) 'The integrin—growth factor receptor duet'. *Journal of Cellular Physiology*, 213 (3), pp. 649-653.
- Amin, H. M., McDonnell, T. J., Medeiros, L. J., Rassidakis, G. Z., Leventaki, V., O'Connor, S. L., Keating, M. J. & Lai, R. (2003) 'Characterization of 4 mantle cell lymphoma cell lines: establishment of an in vitro study model'. *Archives of Pathology and Laboratory Medicine*, 127 (4), pp. 424-431.
- Anastasov, N., Klier, M., Koch, I., Angermeier, D., Höfler, H., Fend, F. & Quintanilla-Martinez, L. (2009) 'Efficient shRNA delivery into B and T lymphoma cells using lentiviral vector-mediated transfer'. *Journal of Hematopathology*, 2 (1), pp. 9-19.
- Andersen, P., Uosaki, H., Shenje, L. T. & Kwon, C. (2012) 'Non-canonical Notch signaling: emerging role and mechanism'. *Trends in Cell Biology*, 22 (5), pp. 257-265.
- Ando, T., Kawabe, T., Ohara, H., Ducommun, B., Itoh, M. & Okamoto, T. (2001) 'Involvement of the interaction between p21 and proliferating cell nuclear antigen for the maintenance of G2/M arrest after DNA damage'. *The Journal of Biological Chemistry*, 16 (46), pp. 42971-7.
- Andrés, V., Ureña, J., Poch, E., Chen, D. & Goukassian, D. (2001) 'Role of Sp1 in the induction of p27 gene expression in vascular smooth muscle cells in vitro and after

- balloon angioplasty'. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 21 (3), pp. 342-347.
- Anthis, N. J., Wegener, K. L., Critchley, D. R. & Campbell, I. D. (2010) 'Structural diversity in integrin/talin interactions'. *Structure*, 18 (12), pp. 1654-1666.
- Aoki, M., Ishigami, S., Uenosono, Y., Arigami, T., Uchikado, Y., Kita, Y., Kurahara, H., Matsumoto, M., Ueno, S. & Natsugoe, S. (2011) 'Expression of BMP-7 in human gastric cancer and its clinical significance'. *British Journal of Cancer*, 104 (4), pp. 714.
- Artero-Castro, A., Castellvi, J., García, A., Hernández, J., y Cajal, S. R. & LLeonart, M. E. (2011) 'Expression of the ribosomal proteins Rplp0, Rplp1, and Rplp2 in gynecologic tumors'. *Human Pathology*, 42 (2), pp. 194-203.
- Assoian, R. K. & Klein, E. A. (2008) 'Growth control by intracellular tension and extracellular stiffness'. *Trends in Cell Biology*, 18 (7), pp. 347-352.
- Avivi, I. & Goy, A. (2015) 'Refining the Mantle Cell Lymphoma Paradigm: Impact of Novel Therapies on Current Practice'. *Clinical Cancer Research*, 21(17), pp. 3853-61
- Bai, T., Chen, C.-C. & Lau, L. F. (2010) 'Matricellular protein CCN1 activates a proinflammatory genetic program in murine macrophages'. *The Journal of Immunology*, 184 (6), pp. 3223-3232.
- Baik, I. H., Jo, G.-H., Seo, D., Ko, M. J., Cho, C. H., Lee, M. G. & Lee, Y.-H. (2016) 'Knockdown of RPL9 expression inhibits colorectal carcinoma growth via the inactivation of Id-1/NF- κ B signaling axis'. *International Journal of Oncology*, 49 (5), pp. 1953-1962.
- Balsas, P., Esteve-Arenys, A., Roldán, J., Jiménez, L., Rodríguez, V., Valero, J., G., Chamorro-Jorganes, A., de la Bellacasa, R., P., Teixidó, J., Matas-Céspedes, A., Moros, A., Martínez, A., Campo, E., Sáez-Borderías, A., Borrell, J., I., Pérez-Galán, P., Colomer, D. & Roué, G. (2017) 'Activity of the novel BCR kinase inhibitor IQS019 in preclinical models of B-cell non-Hodgkin lymphoma'. *Journal of Hematology & Oncology*, 10 (1), pp. 80.
- Banks, P. M., Chan, J., Cleary, M. L., Delsol, G., De Wolf-Peeters, C., Gatter, K., Grogan, T. M., Harris, N. L., Isaacson, P. G. & Jaffe, E. S. (1992) 'Mantle cell lymphoma. A proposal for unification of morphologic, immunologic, and molecular data'. *The American Journal of Surgical Pathology*, 16(7), pp. 637-40.
- Baretton, G., Klenk, U., Diebold, J., Schmeller, N. & Löhrs, U. (1999) 'Proliferation-and apoptosis-associated factors in advanced prostatic carcinomas before and after androgen deprivation therapy: prognostic significance of p21/WAF1/CIP1 expression'. *British Journal of Cancer*, 80 (3-4), pp. 546-555.
- Bartholin, L., Wessner, L. L., Chirgwin, J. M. & Guise, T. A. (2007) 'The human Cyr61 gene is a transcriptional target of transforming growth factor beta in cancer cells'. *Cancer Letters*, 246 (1), pp. 230-236.
- Beà, S. I., Ribas, M., Hernández, J. M., Bosch, F., Pinyol, M., Hernández, L., García, J. L., Flores, T., González, M. & López-Guillermo, A. (1999) 'Increased number of chromosomal imbalances and high-level DNA amplifications in mantle cell lymphoma are associated with blastoid variants'. *Blood*, 93 (12), pp. 4365-4374.

Beà, S., Valdés-Mas, R., Navarro, A., Salaverria, I., Martín-Garcia, D., Jares, P., Giné, E., Pinyol, M., Royo, C., Nadeu, F., Conde, L., Juan, M., Clot, G., Vizán, P., Croce, L., D., Puente, D., A., López-Guerra, M., Moros, A., Roue, G., Aymerich, M., Villamor, N., Colomo, L., Martínez, A., Valera, A., Martín-Subero, J., I., Amador, V., Hernández, L., Rozman, M., Enjuanes, A., Forcada, P., Muntañola, A., Hartmann, E., M., Calasanz, M., J., Rosenwald, A., Ott, G., Hernández-Rivas, J., M., Klapper, W., Siebert, R., Wiestner, A., Wilson, W., H., Colomer, D., López-Guillermo, A., López-Otin, C., Puente, X., S. & Campo, E. (2013) 'Landscape of somatic mutations and clonal evolution in mantle cell lymphoma'. *Proceedings of the National Academy of Sciences of the United States*, 110 (45), pp. 18250–18255.

Behrens, J., von Kries, J. P., Kühl, M., Bruhn, L., Wedlich, D., Grosschedl, R. & Birchmeier, W. (1996) 'Functional interaction of β -catenin with the transcription factor LEF-1'. *Nature*, 382 (6592), pp. 638.

Bernard, M., Gressin, R., Lefrere, F., Drenou, B., Branger, B., Caulet-Maugendre, S., Tass, P., Brousse, N., Valensi, F. & Milpied, N. (2001) 'Blastic variant of mantle cell lymphoma: a rare but highly aggressive subtype'. *Leukemia*, 15 (11), pp. 1785.

Berchtold, M. W. & Villalobo, A. (2014) 'The many faces of calmodulin in cell proliferation, programmed cell death, autophagy, and cancer'. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1843 (2), pp. 398-435.

Bertoni, F., Zucca, E. & Cotter, F. E. (2004) 'Molecular basis of mantle cell lymphoma'. *British Journal of Haematology*, 124 (2), pp. 130-140.

Besson, A., Dowdy, S. F. & Roberts, J. M. (2008) 'CDK inhibitors: cell cycle regulators and beyond'. *Developmental Cell*, 14 (2), pp. 159-169.

Beverly, L. J., Felsher, D. W. & Capobianco, A. J. (2005) 'Suppression of p53 by Notch in lymphomagenesis: implications for initiation and regression'. *Cancer Research*, 65 (16), pp. 7159-7168.

Bierie, B. & Moses, H. L. (2006) 'Tumour microenvironment: TGF β : the molecular Jekyll and Hyde of cancer'. *Nature Reviews Cancer*, 6 (7), pp. nrc1926.

Boczek, N. J., Gomez-Hurtado, N., Ye, D., Calvert, M. L., Tester, D. J., Kryshtal, D. O., Hwang, H. S., Johnson, C. N., Chazin, W. J. & Loporcaro, C. G. (2016) 'Spectrum and Prevalence of CALM1-, CALM2-, and CALM3-Encoded Calmodulin Variants in Long QT Syndrome and Functional Characterization of a Novel Long QT Syndrome–Associated Calmodulin Missense Variant, E141G'. *Circulation: Genomic and Precision Medicine*, 9 (2), pp. 136-146.

Body, S., Esteve-Arenys, A., Miloudi, H., Recasens-Zorzo, C., Tchakarska, G., Moros, A., Bustany, S., Vidal-Crespo, A., Rodriguez, V., Lavigne, R., Com, E., Casanova, I., Mangues, R., Weigert, O., Sanjuan-Pla, A., Menéndez, P., Marcq, B., Picquenot, J. M., Pérez-Galán, P., Jardin, F., Roué, G. & Sola, B. (2017) 'Cytoplasmic cyclin D1 controls the migration and invasiveness of mantle lymphoma cells'. *Scientific Reports*, 7(1) pp. 13946

Bonelli, P., Tuccillo, F. M., Borrelli, A., Schiattarella, A. & Buonaguro, F. M. (2014) 'CDK/CCN and CDKI alterations for cancer prognosis and therapeutic predictivity'. *BioMed research international*, 2014

Bouyer, G., Reininger, L., Ramdani, G., Phillips, L. D., Sharma, V., Egée, S., Langsley, G. & Lasonder, E. (2016) 'Plasmodium falciparum infection induces dynamic changes in the erythrocyte phospho-proteome'. *Blood Cells, Molecules, and Diseases*, 58 pp. 35-44.

Brennan, P., Palacios-Callender, M., Umar, T., Tant, S. & Langdon, J. (2002) 'Expression of type 2 nitric oxide synthase and p21 in oral squamous cell carcinoma'. *International Journal of Oral and Maxillofacial Surgery*, 31 (2), pp. 200-205.

Bretones, G., Delgado, M. D. & León, J. (2015) 'Myc and cell cycle control'. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, 1849 (5), pp. 506-516.

Brigstock, D. (2003) 'The CCN family: a new stimulus package'. *Journal of Endocrinology*, 178 (2), pp. 169-175.

Brigstock, D. R. (2002) 'Regulation of angiogenesis and endothelial cell function by connective tissue growth factor (CTGF) and cysteine-rich 61 (CYR61)'. *Angiogenesis*, 5 (3), pp. 153-165.

Brou, C., Logeat, F., Gupta, N., Bessia, C., LeBail, O., Doedens, J. R., Cumano, A., Roux, P., Black, R. A. & Israël, A. (2000) 'A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE'. *Molecular Cell*, 5 (2), pp. 207-216.

Burger, J. A. & Ford, R. J. (2011) 'The microenvironment in mantle cell lymphoma: Cellular and molecular pathways and emerging targeted therapies'. *Seminars in Cancer Biology*, 21 (5), pp. 308-312.

Byrd, J. C., Furman, R. R., Coutre, S. E., Flinn, I. W., Burger, J. A., Blum, K. A., Grant, B., Sharman, J. P., Coleman, M. & Wierda, W. G. (2013) 'Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia'. *New England Journal of Medicine*, 369 (1), pp. 32-42.

Calayir, E., Becker, T. M., Kratzer, A., Ebner, B., Panzenbock, U., Stefujl, J. & Kostner, G. M. (2008) 'LXR-agonists regulate ApoM expression differentially in liver and intestine'. *Current Pharmaceutical Biotechnology*, 9 (6), pp. 516-521.

Callard, R. & Hodgkin, P. (2007) 'Modeling T-and B-cell growth and differentiation'. *Immunological Reviews*, 216 (1), pp. 119-129.

Campo, E. & Rule, S. (2015) 'Mantle cell lymphoma: evolving management strategies'. *Blood*, 125 (1), pp. 48-55.

Camps, J., Salaverria, I., Garcia, M. J., Prat, E., Beà, S., Pole, J. C., Hernández, L., Del Rey, J., Cigudosa, J. C. & Bernués, M. (2006) 'Genomic imbalances and patterns of karyotypic variability in mantle-cell lymphoma cell lines'. *Leukemia Research*, 30 (8), pp. 923-934.

Carvajal-Cuenca, A., Sua, L. F., Silva, N. M., Pittaluga, S., Royo, C., Song, J. Y., Sargent, R. L., Espinet, B., Climent, F., Jacobs, S. A., Delabie, J., Naresh, K. N., Bagg, A.,

Brousset, P., Warnke, R. A., Serrano, S., Harris, N. L., Swerdlow, S. H., Jaffe, E. S. & Campo, E. (2012) 'In situ mantle cell lymphoma: clinical implications of an incidental finding with indolent clinical behavior'. *Haematologica*, 97(2), pp. 270–278.

Cassaday, R. D., Goy, A., Advani, S., Chawla, P., Nachankar, R., Gandhi, M. & Gopal, A. K. (2015) 'A Phase II, Single-Arm, Open-Label, Multicenter Study to Evaluate the Efficacy and Safety of P276-00, a Cyclin-Dependent Kinase Inhibitor, in Patients With Relapsed or Refractory Mantle Cell Lymphoma'. *Clinical Lymphoma Myeloma and Leukemia*,

Caywood, E., Farrar, J. E., Lipton, J. M. & Arceci, R. J. (2009) 'Differential Down-Regulation of RPL35a in Human Bone Marrow Progenitors Demonstrates a p53 Independent Mechanism Mediating the Diamond Blackfan Anemia Phenotype'. [in Am Soc Hematology. (Accessed: Caywood, E., Farrar, J. E., Lipton, J. M. & Arceci, R. J.

Ceccarelli, C., Santini, D., Chieco, P., Lanciotti, C., Taffurelli, M., Paladini, G. & Marrano, D. (2001) 'Quantitative p21WAF-1/p53 immunohistochemical analysis defines groups of primary invasive breast carcinomas with different prognostic indicators'. *International Journal of Cancer*, 95 (2), pp. 128-134.

Chai, J., Modak, C., Ouyang, Y., Wu, S.-Y. & Jamal, M. M. (2012) 'CCN1 Induces '. ISRN gastroenterology, 2012

Chakraborty, A., Uechi, T. & Kenmochi, N. (2011) 'Guarding the 'translation apparatus': defective ribosome biogenesis and the p53 signaling pathway'. *Wiley Interdisciplinary Reviews: RNA*, 2 (4), pp. 507-522.

Chandramouli, K. & Qian, P.-Y. (2009) 'Proteomics: challenges, techniques and possibilities to overcome biological sample complexity'. *Human genomics and proteomics: HGP*, 2009

Chang, B.-D., Watanabe, K., Broude, E. V., Fang, J., Poole, J. C., Kalinichenko, T. V. & Roninson, I. B. (2000) 'Effects of p21Waf1/Cip1/Sdi1 on cellular gene expression: implications for carcinogenesis, senescence, and age-related diseases'. *Proceedings of the National Academy of Sciences*, 97 (8), pp. 4291-4296.

Chassot, A., Turchi, L., Virolle, T., Fitsialos, G., Batoz, M., Deckert, M., Dulic, V., Meneguzzi, G., Buscà, R. & Ponzio, G. (2007) 'Id3 is a novel regulator of p27kip1 mRNA in early G1 phase and is required for cell-cycle progression'. *Oncogene*, 26 (39), pp. 5772-5783.

Cheah, C., Chihara, D., Romaguera, J., Fowler, N., Seymour, J., Hagemester, F., Champlin, R. & Wang, M. (2015) 'Patients with mantle cell lymphoma failing ibrutinib are unlikely to respond to salvage chemotherapy and have poor outcomes'. *Annals of Oncology*, pp. 1175-1179.

Chen, C.-C., Mo, F.-E. & Lau, L. F. (2001) 'The angiogenic factor Cyr61 activates a genetic program for wound healing in human skin fibroblasts'. *Journal of Biological Chemistry*, 276 (50), pp. 47329-37.

- Chen, C. C., Young, J. L., Monzon, R. I., Chen, N., Todorović, V. & Lau, L. F. (2007) 'Cytotoxicity of TNF α is regulated by integrin-mediated matrix signaling'. *The EMBO journal*, 26 (5), pp. 1257-1267.
- Chen, C. Y., Fuh, L. J., Huang, C. C., Hsu, C. J., Su, C. M., Liu, S. C., Lin, Y. M. & Tang, C. H. (2017) 'Enhancement of CCL2 expression and monocyte migration by CCN1 in osteoblasts through inhibiting miR-518a-5p: implication of rheumatoid arthritis therapy'. *Scientific Reports*, 24 (1), pp. 421.
- Chen, G., Cheng, Y., Zhang, Z., Martinka, M. & Li, G. (2011) 'Prognostic significance of cytoplasmic p27 expression in human melanoma'. *Cancer Epidemiology Biomarkers & Prevention*, 20 (10), pp. 2212-2221.
- Chen, J.-C., Yang, S.-T., Lin, C.-Y., Hsu, C.-J., Tsai, C.-H., Su, J.-L. & Tang, C.-H. (2014) 'BMP-7 enhances cell migration and α v β 3 integrin expression via a c-Src-dependent pathway in human chondrosarcoma cells'. *PloS One*, 9 (11), pp. e112636.
- Chen, N., Leu, S.-J., Todorović, V., Lam, S. C.-T. & Lau, L. F. (2004) 'Identification of a novel integrin α v β 3 binding site in CCN1 (CYR61) critical for pro-angiogenic activities in vascular endothelial cells'. *Journal of Biological Chemistry*, 279 (42), pp. 44166-44176.
- Chen, P.-C., Lin, T.-H., Cheng, H.-C. & Tang, C.-H. (2012) 'CCN3 increases cell motility and ICAM-1 expression in prostate cancer cells'. *Carcinogenesis*, 33 (4), pp. 937-945.
- Chen, P., Zheng, X., Zhou, Y., Xu, Y., Zhu, L. & Qian, Y. (2017) 'Talin-1 interaction network promotes hepatocellular carcinoma progression'. *Oncotarget*, 8 (8), pp. 13003.
- Chen, R., Sanchez, J. & Rosen, S.T. (2016) 'Clinical Management Updates in Mantle Cell Lymphoma'. *Oncology (Williston Park)*, 30(4) pp. 353-60.
- Chen, Y. & Du, X. Y. (2007) 'Functional properties and intracellular signaling of CCN1/Cyr61'. *Journal of Cellular Biochemistry*, 100 (6), pp. 1337-1345.
- Chen, Y.-F., Chen, Y.-T., Chiu, W.-T. & Shen, M.-R. (2013) 'Remodeling of calcium signaling in tumor progression'. *Journal of Biomedical Science*, 20 (1), pp. 23.
- Chenard, K. E., Teven, C. M., He, T.-C. & Reid, R. R. (2012) 'Bone morphogenetic proteins in craniofacial surgery: current techniques, clinical experiences, and the future of personalized stem cell therapy'. *BioMed research international*, 2012:601549.
- Cheng, T.-Y., Wu, M.-S., Hua, K.-T., Kuo, M.-L. & Lin, M.-T. (2014) 'Cyr61/CTGF/Nov family proteins in gastric carcinogenesis'. *World Journal of Gastroenterology: WJG*, 20 (7), pp. 1694.
- Chiarle, R., Budel, L. M., Skolnik, J., Frizzera, G., Chilosi, M., Corato, A., Pizzolo, G., Magidson, J., Montagnoli, A. & Pagano, M. (2000) 'Increased proteasome degradation of cyclin-dependent kinase inhibitor p27 is associated with a decreased overall survival in mantle cell lymphoma'. *Blood*, 95 (2), pp. 619-626.

Chien, W., Kumagai, T., Miller, C. W., Desmond, J. C., Frank, J. M., Said, J. W. & Koeffler, H. P. (2004) 'Cyr61 suppresses growth of human endometrial cancer cells'. *Journal of Biological Chemistry*, 279 (51), pp. 53087-53096.

Chin, D. & Means, A. R. (2000) 'Calmodulin: a prototypical calcium sensor'. *Trends in Cell Biology*, 10 (8), pp. 322-328.

Chiron, D., Papin, A., Bellanger, C., Amiot, M., Le Gouill, S. & Pellat-Deceunynck, C. (2017) 'Novel targeted strategies to overcome microenvironment-dependent resistance in mantle cell lymphoma'. *Hematological Oncology*, 35 (S2), pp. 258-258.

Cho, W. C. (2007) 'Proteomics technologies and challenges'. *Genomics, proteomics & bioinformatics*, 5 (2), pp. 77-85.

Choi, J., Lin, A., Shrier, E., Lau, L. F., Grant, M. B. & Chaqour, B. (2013) 'Degradome products of the matricellular protein CCN1 as modulators of pathological angiogenesis in the retina'. *Journal of Biological Chemistry*, 288 (32), pp. 23075-23089.

Chong, H. C., Tan, C. K., Huang, R.-L. & Tan, N. S. (2012) 'Matricellular proteins: a sticky affair with cancers'. *Journal of Oncology*, 2012

Chu, I. M., Hengst, L. & Slingerland, J. M. (2008) 'The Cdk inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy'. *Nature Reviews Cancer*, 8 (4), pp. 253-267.

Chuang, W. y., Chang, H., Chang, G. j., Wang, T. H., Chang, Y. s., Wang, T. h., Yeh, C. J., Ueng, S. H., Chien, H. P. & Chang, C. y. (2017) 'Pleomorphic mantle cell lymphoma morphologically mimicking diffuse large B cell lymphoma: common cyclin D1 negativity and a simple immunohistochemical algorithm to avoid the diagnostic pitfall'. *Histopathology*, 70 (6), pp. 986-999.

Ciccarelli, C., Marampon, F., Scoglio, A., Mauro, A., Giacinti, C., De Cesaris, P. & Zani, B. M. (2005) 'p21WAF1 expression induced by MEK/ERK pathway activation or inhibition correlates with growth arrest, myogenic differentiation and onco-phenotype reversal in rhabdomyosarcoma cells'. *Molecular cancer*, 4 (1), pp. 41.

Cid, M. C., Esparza, J., Juan, M., Miralles, A. i., Ordi, J., Vilella, R., Urbano-Márquez, A., Gayà, A., Vives, J. & Yagüe, J. (1994) 'Signaling through CD50 (ICAM-3) stimulates T lymphocyte binding to human umbilical vein endothelial cells and extracellular matrix proteins via an increase in β 1 and β 2 integrin function'. *European Journal of Immunology*, 24 (6), pp. 1377-1382.

Coats, S., Flanagan, W. M., Nourse, J. & Roberts, J. M. (1996) 'Requirement of p27Kip1 for restriction point control of the fibroblast cell cycle'. *Science*, 272 (5263), pp. 877.

Conroy, H., Mawhinney, L. & Donnelly, S. (2010) 'Inflammation and cancer: macrophage migration inhibitory factor (MIF)—the potential missing link'. *QJM: An International Journal of Medicine*, 103 (11), pp. 831-836.

Cox, J., Hein, M.Y., Lubner, C.A., Paron, I., Nagaraj, N. & Mann, M. (2014) 'Accurate proteome-wide label-free quantification by delayed normalization and maximal peptide ratio extraction, termed MaxLFQ'. *Molecular & Cellular Proteomics*, 13 pp. 2513-2526.

Crawford, L. J. & Irvine, A. E. (2013) 'Targeting the ubiquitin proteasome system in haematological malignancies'. *Blood Reviews*, 27 (6), pp. 297-304.

Crockett, J. C., Schütze, N., Tosh, D., Jatzke, S., Duthie, A., Jakob, F. & Rogers, M. J. (2007) 'The matricellular protein CYR61 inhibits osteoclastogenesis by a mechanism independent of $\alpha\beta3$ and $\alpha\beta5$ '. *Endocrinology*, 148 (12), pp. 5761-5768.

Croteau, D. L., Popuri, V., Opresko, P. L. & Bohr, V. A. (2014) 'Human RecQ helicases in DNA repair, recombination, and replication'. *Annual Review of Biochemistry*, 83 pp. 519-552.

Crystal, R. G. (2014) 'Adenovirus: the first effective in vivo gene delivery vector'. *Human Gene Therapy*, 25 (1), pp. 3-11.

Daftuar, L., Zhu, Y., Jacq, X. & Prives, C. (2013) 'Ribosomal proteins RPL37, RPS15 and RPS20 regulate the Mdm2-p53-MdmX network'. *PLoS One*, 8 (7), pp. e68667.

D'Antonio, K. B., Toubaji, A., Albadine, R., Mondul, A. M., Platz, E. A., Netto, G. J. & Getzenberg, R. H. (2010) 'Extracellular matrix associated protein CYR61 is linked to prostate cancer development'. *The Journal of urology*, 183 (4), pp. 1604-1610.

Das, M., Ithychanda, S. S., Qin, J. & Plow, E. F. (2014) 'Mechanisms of talin-dependent integrin signaling and crosstalk'. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1838 (2), pp. 579-588.

D'souza, B., Miyamoto, A. & Weinmaster, G. (2008) 'The many facets of Notch ligands'. *Oncogene*, 27 (38), pp. 5148.

Dai, M., Al-Odaini, A., Arakelian, A., Rabbani, S., Ali, S. & Lebrun, J. (2012) 'A novel function for p21Cip1 and the transcriptional regulator P/CAF as critical regulators of TGF β mediated breast cancer cell migration and invasion'. *Breast Cancer Research*, 14 (5), pp. R127.

Dai, Y., Wilson, G., Huang, B., Peng, M., Teng, G., Zhang, D., Zhang, R., Ebert, M., Chen, J. & Wong, B. (2014) 'Silencing of Jagged1 inhibits cell growth and invasion in colorectal cancer'. *Cell Death & Disease*, 5 (4), pp. e1170.

David, L., Feige, J.-J. & Bailly, S. (2009) 'Emerging role of bone morphogenetic proteins in angiogenesis'. *Cytokine and Growth Factor Reviews*, 20 (3), pp. 203-212.

Davies, S. R., Watkins, G., Douglas-Jones, A., Mansel, R. E. & Jiang, W. G. (2008) 'Bone morphogenetic proteins 1 to 7 in human breast cancer, expression pattern and clinical/prognostic relevance'. *Journal of Experimental Therapeutics and Oncology*, 7 (4),

Dawar, R. & Hernandez-Ilizaliturri, F. (2012) 'The emerging role of lenalidomide in the management of mantle cell lymphoma (MCL)'. *Best Practice & Research Clinical Haematology*, 25 (2), pp. 185-190.

Daya, S. & Berns, K. I. (2008) 'Gene therapy using adeno-associated virus vectors'. *Clinical Microbiology Reviews*, 21 (4), pp. 583-593.

De Falco, F., Sabatini, R., Del Papa, B., Falzetti, F., Di Ianni, M., Sportoletti, P., Baldoni, S., Screpanti, I., Marconi, P. & Rosati, E. (2015) 'Notch signaling sustains the expression of Mcl-1 and the activity of eIF4E to promote cell survival in CLL'. *Oncotarget*, 6 (18), pp. 16559.

de Las Heras-Rubio, A., Perucho, L., Paciucci, R., Vilardell, J. & Lleonart, M. E. (2014) 'Ribosomal proteins as novel players in tumorigenesis'. *Cancer and Metastasis Reviews*, 33 (1), pp. 115-141.

de Renty, C., DePamphilis, M. L. & Ullah, Z. (2014) 'Cytoplasmic localization of p21 protects trophoblast giant cells from DNA damage induced apoptosis'. *PLoS One*, 9, (5), pp. e97434.

De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfe, M. S. & Ray, W. J. (1999) 'A presenilin-1-dependent γ -secretase-like protease mediates release of Notch intracellular domain'. *Nature*, 398 (6727), pp. 518.

Dehay, C. & Kennedy, H. (2007) 'Cell-cycle control and cortical development'. *Nature Reviews Neuroscience*, 8 (6), pp. 438-450.

Del Nagro, C. J., Otero, D. C., Anzelon, A. N., Omori, S. A., Kolla, R. V. & Rickert, R. C. (2005) 'CD 19 function in central and peripheral B-cell development'. *Immunologic Research*, 31 (2), pp. 119-131.

Delrue, I., Pan, Q., Baczmanska, A. K., Callens, B. W. & Verdoodt, L. L. M. (2018) 'Determination of the Selection Capacity of Antibiotics for Gene Selection'. *Biotechnology journal*, 13(8), pp. e1700747.

Deng, C., Lee, S. & O'Connor, O. A. (2012) 'New strategies in the treatment of mantle cell lymphoma'. *Clinical Cancer Research*, 18 (13), pp. 3499-3508.

Deng, T., Lin, D., Zhang, M., Zhao, Q., Li, W., Zhong, B., Deng, Y. & Fu, X. (2015) 'Differential expression of bone morphogenetic protein 5 in human lung squamous cell carcinoma and adenocarcinoma'. *Acta Biochim Biophys Sin*, 47 (7), pp. 557-563.

Denicourt, C., Saenz, C. C., Datnow, B., Cui, X.-S. & Dowdy, S. F. (2007) 'Relocalized p27Kip1 tumor suppressor functions as a cytoplasmic metastatic oncogene in melanoma'. *Cancer Research*, 67 (19), pp. 9238-9243.

Denning, W., Das, S., Guo, S., Xu, J., Kappes, J. C. & Hel, Z. (2013) 'Optimization of the transductional efficiency of lentiviral vectors: effect of sera and polycations'. *Molecular Biotechnology*, 53(3), pp. 308-14.

Derynck, R., Akhurst, R. J. & Balmain, A. (2001) 'TGF- β signaling in tumor suppression and cancer progression'. *Nature Genetics*, 29 (2), pp. 117.

Derynck, R. & Zhang, Y. E. (2003) 'Smad-dependent and Smad-independent pathways in TGF- β family signalling'. *Nature*, 425 (6958), pp. 577.

Desiniotis, A. & Kyprianou, N. (2011) 'Significance of talin in cancer progression and metastasis', *International Review of Cell and Molecular Biology*. Elsevier, pp. 117-147.

Devgan, V., Mammucari, C., Millar, S. E., Brisken, C. & Dotto, G. P. (2005) 'p21WAF1/Cip1 is a negative transcriptional regulator of Wnt4 expression downstream of Notch1 activation'. *Genes and Development*, 19 (12), pp. 1485-1495.

Dictor, M., Ek, S., Sundberg, M., Warenholt, J., György, C., Sernbo, S., Gustavsson, E., Abu-Alsoud, W., Wadström, T. & Borrebaeck, C. (2009) 'Strong lymphoid nuclear expression of SOX11 transcription factor defines lymphoblastic neoplasms, mantle cell lymphoma and Burkitt's lymphoma'. *Haematologica*, 94(11), pp.1563–1568.

Di, D., Chen, L., Wang, L., Sun, P., Liu, Y., Xu, Z. & Ju, J. (2016) 'Downregulation of human intercellular adhesion molecule-1 attenuates the metastatic ability in human breast cancer cell lines'. *Oncology Reports*, 35 (3), pp. 1541-1548.

Dijkers, P. F., Medema, R. H., Pals, C., Banerji, L., Thomas, N. S. B., Lam, E. W.-F., Burgering, B. M., Raaijmakers, J. A., Lammers, J.-W. J. & Koenderman, L. (2000) 'Forkhead transcription factor FKHR-L1 modulates cytokine-dependent transcriptional regulation of p27KIP1'. *Molecular and Cellular Biology*, 20 (24), pp. 9138-9148.

Dlamini, Z. & Mphahlele, L. (2006) 'Molecular evaluation of ribosomal protein L9 gene (RPL9) in lung cancer'. [in AACR. (Accessed:Dlamini, Z. & Mphahlele, L.

Dobroff, A. S., Wang, H., Melnikova, V. O., Villares, G. J., Zigler, M., Huang, L. & Bar-Eli, M. (2009) 'Silencing cAMP-response element-binding protein (CREB) identifies CYR61 as a tumor suppressor gene in melanoma'. *Journal of Biological Chemistry*, 284 (38), pp. 26194-26206.

Dotterweich, J., Ebert, R., Kraus, S., Tower, R. J., Jakob, F. & Schütze, N. (2014) 'Mesenchymal stem cell contact promotes CCN1 splicing and transcription in myeloma cells'. *Cell Communication and Signaling*, 12 (1), pp. 36.

Drexler, H. G. & MacLeod, R. A. (2002) 'Malignant hematopoietic cell lines: in vitro models for the study of mantle cell lymphoma'. *Leukemia Research*, 26 (9), pp. 781-787.

Duncan, T. J., Al-Attar, A., Rolland, P., Harper, S., Spendlove, I. & Durrant, L. G. (2010) 'Cytoplasmic p27 expression is an independent prognostic factor in ovarian cancer'. *International Journal of Gynecologic Pathology*, 29 (1), pp. 8-18.

Durand, S. & Cimarelli, A. (2011) 'The inside out of lentiviral vectors'. *Viruses*, 3 (2), pp. 132-159.

Ek, S., Dictor, M., Jerkeman, M., Jirstrom, K. & Borrebaeck, C. A. (2008) 'Nuclear expression of the non B-cell lineage Sox11 transcription factor identifies mantle cell lymphoma'. *Blood*, 111(2), pp. 800–805.

Ek, S., Ortega, E. & Borrebaeck, C. A. (2005) 'Transcriptional profiling and assessment of cell lines as in vitro models for mantle cell lymphoma'. *Leukemia Research*, 29 (2), pp. 205-213.

Elliott, M. H., Smith, D. S., Parker, C. E. & Borchers, C. (2009) 'Current trends in quantitative proteomics'. *Journal of Mass Spectrometry*, 44 (12), pp. 1637-1660.

Emre, Y., Irla, M., Dunand-Sauthier, I., Ballet, R., Meguenani, M., Jemelin, S., Vesin, C., Reith, W. & Imhof, B. A. (2013) 'Thymic epithelial cell expansion through matricellular protein CYR61 boosts progenitor homing and T-cell output'. *Nature communications*, 4 pp. 2842.

Espinoza, I., Liu, H., Busby, R. & Lupu, R. (2011) 'CCN1, a candidate target for zoledronic acid treatment in breast cancer'. *Molecular Cancer Therapeutics*, 10 (5), pp. 732-741.

Espinoza, I., Menendez, J. A., Kvp, C. M. & Lupu, R. (2014) 'CCN1 promotes vascular endothelial growth factor secretion through $\alpha v \beta 3$ integrin receptors in breast cancer'. *Journal of cell communication and signaling*, 8 (1), pp. 23-27.

Farrar, J. E., Nater, M., Caywood, E., McDevitt, M. A., Kowalski, J., Takemoto, C. M., Talbot, C. C., Meltzer, P., Esposito, D. & Beggs, A. H. (2008) 'Abnormalities of the large ribosomal subunit protein, Rpl35a, in Diamond-Blackfan anemia'. *Blood*, 112 (5), pp. 1582-1592.

Feng, P., Wang, B. & Ren, E. C. (2008) 'Cyr61/CCN1 is a tumor suppressor in human hepatocellular carcinoma and involved in DNA damage response'. *The international journal of biochemistry & cell biology*, 40 (1), pp. 98-109.

Feng, X.-H. & Derynck, R. (2005) 'Specificity and versatility in TGF- β signaling through Smads'. *Annual Review of Cell and Developmental Biology*, 21 pp. 659-693.

Fernández, V., Hartmann, E., Ott, G., Campo, E. & Rosenwald, A. (2005) 'Pathogenesis of mantle-cell lymphoma: all oncogenic roads lead to dysregulation of cell cycle and DNA damage response pathways'. *Journal of Clinical Oncology*, 23 (26), pp. 6364-6369.

Fernández, V., Salamero, O., Espinet, B., Solé, F., Royo, C., Navarro, A., Camacho, F., Beà, S., Hartmann, E. & Amador, V. (2010) 'Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma'. *Cancer Research*, 70 (4), pp. 1408-1418.

Ferrandina, G., Stoler, A., Fagotti, A., Fanfani, F., Sacco, R., De Pasqua, A., Mancuso, S. & Scambia, G. (2000) 'p21WAF1/CIP1 protein expression in primary ovarian cancer'. *International Journal of Oncology*, 17 (6), pp. 1231-1236.

Fisher, R. I., Bernstein, S. H., Kahl, B. S., Djulbegovic, B., Robertson, M. J., De Vos, S., Epner, E., Krishnan, A., Leonard, J. P. & Lonial, S. (2006) 'Multicenter phase II study of

- bortezomib in patients with relapsed or refractory mantle cell lymphoma'. *Journal of Clinical Oncology*, 24 (30), pp. 4867-4874.
- Folgueira, M. A. A. K., Carraro, D. M., Brentani, H., da Costa Patrão, D. F., Barbosa, E. M., Netto, M. M., Caldeira, J. R. F., Katayama, M. L. H., Soares, F. A. & Oliveira, C. T. (2005) 'Gene expression profile associated with response to doxorubicin-based therapy in breast cancer'. *Clinical Cancer Research*, 11 (20), pp. 7434-7443.
- Fornaro, M., Manes, T. & Languino, L. R. (2001) 'Integrins and prostate cancer metastases'. *Cancer and Metastasis Reviews*, 20 (3-4), pp. 321-331.
- Fong, Y. C., Lin, C. Y., Su, Y. C., Chen, W. C., Tsai, F. J., Tsai, C. H., Huang, C. Y. & Tang, C. H. (2012) 'CCN6 enhances ICAM-1 expression and cell motility in human chondrosarcoma cells'. *Journal of Cellular Physiology*, 227 (1), pp. 223-232.
- Foran, J. M., Cunningham, D., Coiffier, B., Solal-Celigny, P., Reyes, F., Ghielmini, M., Johnson, P. W., Gisselbrecht, C., Bradburn, M. & Matthews, J. (2000) 'Treatment of mantle-cell lymphoma with Rituximab (chimeric monoclonal anti-CD20 antibody): analysis of factors associated with response'. *Annals of Oncology*, 11 (suppl_1), pp. S117-S121.
- Franzen, C. A., Chen, C. C., Todorović, V., Juric, V., Monzon, R. I. & Lau, L. F. (2009) 'Matrix protein CCN1 is critical for prostate carcinoma cell proliferation and TRAIL-induced apoptosis'. *Molecular Cancer Research*, 7(7), pp. 1045-55.
- Fu, K., Weisenburger, D. D., Greiner, T. C., Dave, S., Wright, G., Rosenwald, A., Chiorazzi, M., Iqbal, J., Gesk, S., Siebert, R., De Jong, D., Jaffe, E. S., Wilson, W. H., Delabie, J., Ott, G., Dave, B. J., Sanger, W. G., Smith, L. M., Rimsza, L., Braziel, R. M., Müller-Hermelink, H. K., Campo, E., Gascoyne, R. D., Staudt, L. M. & Chan, W. C. (2005) 'Cyclin D1-negative mantle cell lymphoma: a clinicopathologic study based on gene expression profiling'. *Blood*, 106(13), pp. 4315-21.
- Funamizu, N., Hu, C., Lacy, C., Schetter, A., Zhang, G., He, P., Gaedcke, J., Ghadimi, M. B., Ried, T. & Yfantis, H. G. (2013) 'Macrophage migration inhibitory factor induces epithelial to mesenchymal transition, enhances tumor aggressiveness and predicts clinical outcome in resected pancreatic ductal adenocarcinoma'. *International Journal of Cancer*, 132 (4), pp. 785-794.
- Gahmberg, C. G., Fagerholm, S. C., Nurmi, S. M., Chavakis, T., Marchesan, S. & Grönholm, M. (2009) 'Regulation of integrin activity and signalling'. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1790 (6), pp. 431-444.
- Gaidarova, S., Corral, L., Glezer, E., Schafer, P. & Lopez-Girona, A. (2009) 'Treatment of MCL Cells with Combined Rituximab and Lenalidomide Enhances NK-Mediated Synapse Formation and Cell Killing'. *Blood*, 114 (22), pp. 673-673.
- Galimberti, S. & Petrini, M. (2010) 'Temsirolimus in the treatment of relapsed and/or refractory mantle cell lymphoma'. *Cancer Management and Research*, 2 pp. 181.
- Ganguly, K. K., Pal, S., Moulik, S. & Chatterjee, A. (2013) 'Integrins and metastasis'. *Cell adhesion & migration*, 7 (3), pp. 251-261.
- Gao, R. & Brigstock, D. R. (2003) 'Low density lipoprotein receptor-related protein (LRP) is a heparin-dependent adhesion receptor for connective tissue growth factor (CTGF) in rat activated hepatic stellate cells'. *Hepatology Research*, 27 (3), pp. 214-220.

Garand, C., Guay, D., Sereduk, C., Chow, D., Tsofack, S. P., Langlois, M., Perreault, E., Yin, H. H. & Lebel, M. (2011) 'An integrative approach to identify YB-1-interacting proteins required for cisplatin resistance in MCF7 and MDA-MB-231 breast cancer cells'. *Cancer Science*, 102 (7), pp. 1410-1417.

Garrett-Engele, C. M., Tasch, M. A., Hwang, H. C., Fero, M. L., Perlmutter, R. M., Clurman, B. E. & Roberts, J. M. (2007a) 'A mechanism misregulating p27 in tumors discovered in a functional genomic screen'. *PLoS genetics*, 3 (12), pp. e219.

Garrett-Engele, C. M., Tasch, M. A., Hwang, H. C., Fero, M. L., Perlmutter, R. M., Clurman, B. E. & Roberts, J. M. (2007b) 'A mechanism misregulating p27 in tumors discovered in a functional genomic screen'. *PLoS Genet*, 3 (12), pp. e219.

Gartel, A. L. (2009) 'p21WAF1/CIP1 and cancer: A shifting paradigm?'. *Biofactors*, 35 (2), pp. 161-164.

Gelebart, P., Anand, M., Armanious, H., Peters, A. C., Bard, J. D., Amin, H. M. & Lai, R. (2008) 'Constitutive activation of the Wnt canonical pathway in mantle cell lymphoma'. *Blood*, 112 (13), pp. 5171-5179.

Gery, S., Xie, D., Yin, D., Gabra, H., Miller, C., Wang, H., Scott, D., William, S. Y., Popoviciu, M. L. & Said, J. W. (2005) 'Ovarian carcinomas: CCN genes are aberrantly expressed and CCN1 promotes proliferation of these cells'. *Clinical Cancer Research*, 11 (20), pp. 7243-7254.

Ghielmini, M. & Zucca, E. (2009) 'How I treat mantle cell lymphoma'. *Blood*, 114 (8), pp. 1469-1476.

Gillett, C., Smith, P., Gregory, W., Richards, M., Millis, R., Peters, G. & Barnes, D. (1996) 'Cyclin D1 and prognosis in human breast cancer'. *International Journal of Cancer*, 69 (2), pp. 92-99.

Goy, A., Kalayoglu Basisik, S., Drach, J., Ramchandren, R., Robertson, M. J., Avivi, I., Rowe, J. M., Herbrecht, R., Van Hoof, A. & Zhang, L. (2015) 'Longer-term follow-up and outcome by tumour cell proliferation rate (Ki-67) in patients with relapsed/refractory mantle cell lymphoma treated with lenalidomide on MCL-001 (EMERGE) pivotal trial'. *British journal of haematology*, 170 (4), pp. 496-503.

Grady, W. M. & Markowitz, S. D. (2002) 'Genetic and epigenetic alterations in colon cancer'. *Annual review of genomics and human genetics*, 3 (1), pp. 101-128.

Granger, J., Siddiqui, J., Copeland, S. & Remick, D. (2005) 'Albumin depletion of human plasma also removes low abundance proteins including the cytokines'. *Proteomics*, 5 (18), pp. 4713-4718.

Grieger, J. C. & Samulski, R. J. (2005) 'Packaging capacity of adeno-associated virus serotypes: impact of larger genomes on infectivity and postentry steps'. *Journal of Virology*, 79 (15), pp. 9933-9944.

Grzeszkiewicz, T. M., Kirschling, D. J., Chen, N. & Lau, L. F. (2001) 'CYR61 stimulates human skin fibroblast migration through integrin $\alpha\beta 5$ and enhances mitogenesis through integrin $\alpha\beta 3$, independent of its carboxyl-terminal domain'. *Journal of Biological Chemistry*, 276 (24), pp. 21943-21950.

Guo, P., Wang, J., Liu, J., Xia, M., Li, W. & He, M. (2015) 'Macrophage immigration inhibitory factor promotes cell proliferation and inhibits apoptosis of cervical adenocarcinoma'. *Tumor Biology*, 36 (7), pp. 5095-5102.

Guo, X.-L. & Chen, J.-S. (2015) 'Research on induced pluripotent stem cells and the application in ocular tissues'. *International journal of ophthalmology*, 8 (4), pp. 818.

Haberman, R., McCown, T. & Samulski, R. (1998) 'Inducible long-term gene expression in brain with adeno-associated virus gene transfer'. *Gene Therapy*, 5 (12), pp. 1604.

Habermann, T. M., Lossos, I. S., Justice, G., Vose, J. M., Wiernik, P. H., McBride, K., Wride, K., Ervin-haynes, A., Takeshita, K., Pietronigro, D., Zeldis, J. B. & Tuscano, J. M. (2009) 'Lenalidomide oral monotherapy produces a high response rate in patients with relapsed or refractory mantle cell lymphoma'. *British Journal of Haematology*, 145 (3), pp. 344-349.

Hacein-Bey-Abina, S., Von Kalle, C., Schmidt, M., McCormack, M., Wulffraat, N., Leboulch, P. a., Lim, A., Osborne, C., Pawliuk, R. & Morillon, E. (2003) 'LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1'. *Science*, 302 (5644), pp. 415-419.

Hall, M. & Peters, G. (1996) 'Genetic alterations of cyclins, cyclin-dependent kinases, and Cdk inhibitors in human cancer'. *Advances in cancer research*, 68 pp. 67-108.

Hao, S., Sanger, W., Onciu, M., Lai, R., Schlette, E. J. & Medeiros, L. J. (2002) 'Mantle cell lymphoma with 8q24 chromosomal abnormalities: a report of 5 cases with blastoid features'. *Modern Pathology*, 15 (12), pp. 1266.

Haque, I., De, A., Majumder, M., Mehta, S., McGregor, D., Banerjee, S. K., Van Veldhuizen, P. & Banerjee, S. (2012) 'The matricellular protein CCN1/Cyr61 is a critical regulator of Sonic Hedgehog in pancreatic carcinogenesis'. *Journal of Biological Chemistry*, 287 (46), pp. 38569-38579.

Harrison, H., Farnie, G., Howell, S. J., Rock, R. E., Stylianou, S., Brennan, K. R., Bundred, N. J. & Clarke, R. B. (2010) 'Regulation of breast cancer stem cell activity by signaling through the Notch4 receptor'. *Cancer Research*, 70 (2), pp. 709-718.

Hatada, I., Inazawa, J., Abe, T., Nakayama, M., Kaneko, Y., Jinno, Y., Niikawa, N., Ohashi, H., Fukushima, Y., Iida, K., Yutani, C., Takahashi, S., Chiba, Y., Ohishi, S. & Mukai, T. (1996) 'Genomic imprinting of human p57KIP2 and its reduced expression in Wilms' tumors'. *Human Molecular Genetics*, 5, pp. 783-788.

He, W., Wang, X., Chen, L. & Guan, X. (2012) 'A crosstalk imbalance between p27kip1 and its interacting molecules enhances breast carcinogenesis'. *Cancer Biotherapy and Radiopharmaceuticals*, 27 (7), pp. 399-402.

He, X.-X., Chen, K., Yang, J., Li, X.-Y., Gan, H.-Y., Liu, C.-Y., Coleman, T. R. & Al-Abed, Y. (2009) 'Macrophage migration inhibitory factor promotes colorectal cancer'. *Molecular Medicine*, 15 (1-2), pp. 1.

Heesters, B. A., Myers, R. C. & Carroll, M. C. (2014) 'Follicular dendritic cells: dynamic antigen libraries'. *Nature Reviews Immunology*, 14, pp. 495–504

Heldin, C.-H., Miyazono, K. & Ten Dijke, P. (1997) 'TGF- β signalling from cell membrane to nucleus through SMAD proteins'. *Nature*, 390 (6659), pp. 465.

Hengst, L. & Reed, S. I. (1996) 'Translational control of p27Kip1 accumulation during the cell cycle'. *Science*, 271 (5257), pp. 1861.

Herman, S. E., Mustafa, R. Z., Gyamfi, J. A., Pittaluga, S., Chang, S., Chang, B., Farooqui, M. & Wiestner, A. (2014) 'Ibrutinib inhibits BCR and NF- κ B signaling and reduces tumor proliferation in tissue-resident cells of patients with CLL'. *Blood*, 123 (21), pp. 3286-3295.

Hernandez, L., Fest, T., Cazorla, M., Teruya-Feldstein, J., Bosch, F., Peinado, M. A., Piris, M., Montserrat, E., Cardesa, A. & Jaffe, E. (1996) 'p53 gene mutations and protein overexpression are associated with aggressive variants of mantle cell lymphomas'. *Blood*, 87 (8), pp. 3351-3359.

Hershko, D. D. (2008) 'Oncogenic properties and prognostic implications of the ubiquitin ligase Skp2 in cancer'. *Cancer*, 112 (7), pp. 1415-1424.

Hirama, T. & Koeffler, H. P. (1995) 'Role of the cyclin-dependent kinase inhibitors in the development of cancer'. *Blood*, 86 (3), pp. 841-854.

Hitz, F., Bargetzi, M., Cogliatti, S., Lohri, A., Taverna, C., Renner, C. & Mey, U. (2013) 'Diagnosis and treatment of mantle cell lymphoma'. *Swiss Medical Weekly*, 143 pp. w13868.

Holbourn, K. P., Acharya, K. R. & Perbal, B. (2008) 'The CCN family of proteins: structure–function relationships'. *Trends in Biochemical Sciences*, 33 (10), pp. 461-473.

Holland, J. D., Klaus, A., Garratt, A. N. & Birchmeier, W. (2013) 'Wnt signaling in stem and cancer stem cells'. *Current Opinion in Cell Biology*, 25 (2), pp. 254-264.

Holloway, S. E., Beck, A. W., Girard, L., Jaber, M. R., Barnett, C. C., Brekken, R. A. & Fleming, J. B. (2005) 'Increased expression of Cyr61 (CCN1) identified in peritoneal metastases from human pancreatic cancer'. *Journal of the American College of Surgeons*, 200 (3), pp. 371-377.

Hoster, E., Dreyling, M., Klapper, W., Gisselbrecht, C., van Hoof, A., Kluin-Nelemans, H. C., Pfreundschuh, M., Reiser, M., Metzner, B. & Einsele, H. (2008) 'A new prognostic index (MIPI) for patients with advanced-stage mantle cell lymphoma'. *Blood*, 111 (2), pp. 558-565.

Hou, CH., Lin, F. L., Hou, S. M. & Liu, J. F. (2014) 'Cyr61 promotes epithelial-mesenchymal transition and tumor metastasis of osteosarcoma by Raf-1/MEK/ERK/Elk-1/TWIST-1 signaling pathway'. *Molecular Cancer* 2014 13 pp 236.

Howlader, N., Noone, A. M., Krapcho, M., Neyman, N., Aminou, R., Waldron, W., Altekruse, S. F., Kosary, C. L., Ruhl, J., Tatalovich, Z., Cho, H., Mariotto, A., Eisner, M. P., Lewis, D. R., Chen, H. S., Feuer, E. J. & Cronin, K. A. (2012) 'SEER Cancer Statistics Review 1975–2009'. *Bethesda, MD: National Cancer Institute*, pp. 2012.

Hsiao, S. C., Cortada, I. R., Colomo, L., Ye, H., Liu, H., Kuo, S. Y., Lin, S. H., Chang, S. T., Kuo, T. U., Campo, E. & Chuang, S. S. (2012) 'SOX11 is useful in differentiating cyclin D1-positive diffuse large B-cell lymphoma from mantle cell lymphoma'. *Histopathology*, 61(4), pp. 685–693.

Huang da, W., Sherman, B.T. & Lempicki, R.A. (2009a) 'Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources'. *Nature Protocols*, 4(1), pp. 44-57.

Huang da, W., Sherman, B.T. & Lempicki, R.A. (2009b). 'Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists'. *Nucleic Acids Research*, 37(1), pp. 1-13.

Huang, H., Pannetier, C., Hu-Li, J. & Paul, W. E. (1998) 'Transient transfection of primary T helper cells by particle-mediated gene transfer'. *Journal of Immunological Methods*, 215 (1-2), pp. 173-177.

Huang, L. Z., Gao, J. L., Pu, C., Zhang, P. H., Wang, L. Z., Feng, G. & Zhang, Y. (2015) 'Apolipoprotein M: Research progress, regulation and metabolic functions'. *Molecular Medicine Reports*, 12 (2), pp. 1617-1624.

Huang, R., Chen, Z., He, L., He, N., Xi, Z., Li, Z., Deng, Y. & Zeng, X. (2017) 'Mass spectrometry-assisted gel-based proteomics in cancer biomarker discovery: approaches and application'. *Theranostics*, 7 (14), pp. 3559.

Huang, X.-h., Jian, W.-h., Wu, Z.-f., Zhao, J., Wang, H., Li, W. & Xia, J.-t. (2014) 'Small interfering RNA (siRNA)-mediated knockdown of macrophage migration inhibitory factor (MIF) suppressed cyclin D1 expression and hepatocellular carcinoma cell proliferation'. *Oncotarget*, 5 (14), pp. 5570.

Hufton, S. E., Moerkerk, P. T., Brandwijk, R., de Bruïne, A. P., Arends, J.-W. & Hoogenboom, H. R. (1999) 'A profile of differentially expressed genes in primary colorectal cancer using suppression subtractive hybridization'. *FEBS Letters*, 463 (1-2), pp. 77-82.

Humphries, J. D., Byron, A. & Humphries, M. J. (2006) 'Integrin ligands at a glance'. *Journal of Cell Science*, 119 (19), pp. 3901-3903.

Hutter, G., Scheubner, M., Zimmermann, Y., Kalla, J., Katzenberger, T., Hübler, K., Roth, S., Hiddemann, W., Ott, G. & Dreyling, M. (2006) 'Differential effect of epigenetic alterations and genomic deletions of CDK inhibitors [p16 (INK4a), p15 (INK4b), p14

- (ARF)] in mantle cell lymphoma'. *Genes, Chromosomes and Cancer*, 45 (2), pp. 203-210.
- Igawa, T., Sato, Y., Takata, K., Iwaki, N., Tanaka, T., Asano, N., Maeda, Y., Orita, Y., Nakamura, N., Nakamura, S. & Yoshino, T. (2013) 'De novo CD5-positive diffuse large B-cell lymphomas show high specificity for cyclin D2 expression'. *Diagnostic Pathology*, 15 (8), pp. 81
- Ito, H., Wang, J. & Shimura, K. (1991) 'Inhibition of lung metastasis by a calmodulin antagonist, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7), in mice bearing Lewis lung carcinoma'. *Anticancer Research*, 11 (1), pp. 249-252.
- Izban, K. F., Alkan, S., Singleton, T. P. & Hsi, E. D. (2000) 'Multiparameter immunohistochemical analysis of the cell cycle proteins cyclin D1, Ki-67, p21WAF1, p27KIP1, and p53 in mantle cell lymphoma'. *Archives of Pathology and Laboratory Medicine*, 124 (10), pp. 1457-1462.
- Jadayel, D., Lukas, J., Nacheva, E., Bartkova, J., Stranks, G., De Schouwer, P., Lens, D., Bartek, J., Dyer, M. & Kruger, A. (1997) 'Potential role for concurrent abnormalities of the cyclin D1, p16 CDKN2 and p15 CDKN2B genes in certain B cell non-Hodgkin's lymphomas. Functional studies in a cell line (Granta 519)'. *Leukemia*, 11 (1), pp. 64.
- Jandova, J., Beyer, T. E., Meuillet, E. J. & Watts, G. S. (2012) 'The matrix protein CCN1/CYR61 is required for α V β 5-mediated cancer cell migration'. *Cell Biochemistry and Function*, 30 (8), pp. 687-695.
- Jares, P., Colomer, D. & Campo, E. (2012) 'Molecular pathogenesis of mantle cell lymphoma'. *The Journal of clinical investigation*, 122 (10), pp. 3416.
- Jeannot, P., Callot, C., Baer, R., Duquesnes, N., Guerra, C., Guillermet-Guibert, J., Bachs, O. & Besson, A. (2015) 'Loss of p27Kip1 promotes metaplasia in the pancreas via the regulation of Sox9 expression'. *Oncotarget*, 6 (34), pp. 35880.
- Jedsadayanmata, A., Chen, C.-C., Kireeva, M. L., Lau, L. F. & Lam, S. C.-T. (1999) 'Activation-dependent adhesion of human platelets to Cyr61 and Fisp12/mouse connective tissue growth factor is mediated through integrin α IIb β 3'. *Journal of Biological Chemistry*, 274 (34), pp. 24321-24327.
- Jeong, D., Heo, S., Ahn, T. S., Lee, S., Park, S., Kim, H., Park, D., Bae, S. B., Lee, S. S. & Lee, M. S. (2014) 'Cyr61 expression is associated with prognosis in patients with colorectal cancer'. *BMC Cancer*, 14 (1), pp. 164.
- Jim Leu, S. J., Sung, J. S., Chen, M. Y., Chen, C. W., Cheng, J. Y., Wang, T. Y. & Wang, J. J. (2013) 'The matricellular protein CCN1 suppresses lung cancer cell growth by inducing senescence via the p53/p21 pathway'. *Journal of Cellular Biochemistry*, 14(9), pp. 2082-93.
- Jin, J.-K., Tien, P.-C., Cheng, C.-J., Song, J. H., Huang, C., Lin, S.-H. & Gallick, G. E. (2015) 'Talin1 phosphorylation activates β 1 integrins: a novel mechanism to promote prostate cancer bone metastasis'. *Oncogene*, 34 (14), pp. 1811.

Joaquin, M., Gubern, A., González-Nuñez, D., Josué Ruiz, E., Ferreiro, I., de Nadal, E., Nebreda, A. R. & Posas, F. (2012) 'The p57 CDKi integrates stress signals into cell-cycle progression to promote cell survival upon stress'. *EMBO Journal*, 31 (13), pp. 2952-64.

Johnson, S. K., Stewart, J. P., Bam, R., Qu, P., Barlogie, B., van Rhee, F., Shaughnessy Jr, J. D., Epstein, J. & Yaccoby, S. (2014) 'CYR61/CCN1 overexpression in the myeloma microenvironment is associated with superior survival and reduced bone disease'. *Blood*, 124 (13), pp. 2051-2060.

Jones, R. J., Baladandayuthapani, V., Neelapu, S., Fayad, L. E., Romaguera, J. E., Wang, M., Sharma, R., Yang, D. & Orlowski, R. Z. (2011) 'HDM-2 inhibition suppresses expression of ribonucleotide reductase subunit M2, and synergistically enhances gemcitabine-induced cytotoxicity in mantle cell lymphoma'. *Blood*, 118(15), pp. 4140-9.

Jung, J.-M., Bruner, J. M., Ruan, S., Langford, L. A., Kyritsis, A. P., Kobayashi, T., Levin, V. A. & Zhang, W. (1995) 'Increased levels of p21WAF1/Cip1 in human brain tumors'. *Oncogene*, 11 (10), pp. 2021-2028.

Kafri, T., Morgan, D., Krahl, T., Sarvetnick, N., Sherman, L. & Verma, I. (1998) 'Cellular immune response to adenoviral vector infected cells does not require de novo viral gene expression: implications for gene therapy'. *Proceedings of the National Academy of Sciences*, 95 (19), pp. 11377-11382.

Kane, R. C., Dagher, R., Farrell, A., Ko, C.-W., Sridhara, R., Justice, R. & Pazdur, R. (2007) 'Bortezomib for the treatment of mantle cell lymphoma'. *Clinical Cancer Research*, 13 (18), pp. 5291-5294.

Kapranos, N., Stathopoulos, G., Manolopoulos, L., Kokka, E., Papadimitriou, C., Bibas, A., Yiotakis, J. & Adamopoulos, G. (2000) 'p53, p21 and p27 protein expression in head and neck cancer and their prognostic value'. *Anticancer research*, 21 (1B), pp. 521-528.

Karp, C., Shukla, M., Buckley, D. & Buckley, A. (2007) 'HRPAP20: a novel calmodulin-binding protein that increases breast cancer cell invasion'. *Oncogene*, 26 (12), pp. 1780.

Katoh, M. (2002) 'WNT and FGF gene clusters'. *International Journal of Oncology*, 21 (6), pp. 1269-1273.

Katoh, M. & Terada, M. (1996) 'Overexpression of bone morphogenic protein (BMP)-4 mRNA in gastric cancer cell lines of poorly differentiated type'. *Journal of Gastroenterology*, 31 (1), pp. 137-139.

Khattar, E. & Kumar, V. (2010) 'Mitogenic regulation of p27Kip1 gene is mediated by AP-1 transcription factors'. *Journal of Biological Chemistry*, 285 (7), pp. 4554-4561.

Kim, B. R., Oh, S. C., Lee, D.-H., Kim, J. L., Lee, S. Y., Kang, M. H., Lee, S. I., Kang, S., Joung, S. Y. & Min, B. W. (2015) 'BMP-2 induces motility and invasiveness by promoting colon cancer stemness through STAT3 activation'. *Tumor Biology*, 36 (12), pp. 9475-9486.

- Kim, L. C., Cook, R. S. & Chen, J. (2017) 'mTORC1 and mTORC2 in cancer and the tumor microenvironment'. *Oncogene*, 36 (16), pp. 2191.
- Kimura, Y., Arakawa, F., Kiyasu, J., Miyoshi, H., Yoshida, M., Ichikawa, A., Niino, D., Sugita, Y., Okamura, T. & Yasuda, K. (2013) 'The Wnt signaling pathway and mitotic regulators in the initiation and evolution of mantle cell lymphoma: Gene expression analysis'. *International Journal of Oncology*, 43 (2), pp. 457-468.
- Kindt, N., Journe, F., Laurent, G. & Saussez, S. (2016) 'Involvement of macrophage migration inhibitory factor in cancer and novel therapeutic targets'. *Oncology Letters*, 12 (4), pp. 2247-2253.
- Kindt, N., Preillon, J., Kaltner, H., Gabius, H.-J., Chevalier, D., Rodriguez, A., Johnson, B. D., Megalizzi, V., Decaestecker, C. & Laurent, G. (2013) 'Macrophage migration inhibitory factor in head and neck squamous cell carcinoma: clinical and experimental studies'. *Journal of Cancer Research and Clinical Oncology*, 139 (5), pp. 727-737.
- Kipkeew, F., Kirsch, M., Klein, D., Wuelling, M., Winterhager, E. & Gellhaus, A. (2016) 'CCN1 (CYR61) and CCN3 (NOV) signaling drives human trophoblast cells into senescence and stimulates migration properties'. *Cell Adhesion & Migration*, 10 (1-2), pp. 163-178.
- Kireeva, M. L., Mo, F.-E., Yang, G. P. & Lau, L. F. (1996) 'Cyr61, a product of a growth factor-inducible immediate-early gene, promotes cell proliferation, migration, and adhesion'. *Molecular and Cellular Biology*, 16 (4), pp. 1326-1334.
- Klanova, M., Soukup, T., Jaksa, R., Molinsky, J., Lateckova, L., Maswabi, B. C., Prukova, D., Brezinova, J., Michalova, K. & Vockova, P. (2014) 'Mouse models of mantle cell lymphoma, complex changes in gene expression and phenotype of engrafted MCL cells: implications for preclinical research'. *Laboratory Investigation*, 94 (7), pp. 806.
- Kobatake, T., Yano, M., Toyooka, S., Tsukuda, K., Dote, H., Kikuchi, T., Toyota, M., Ouchida, M., Aoe, M., Date, H., Pass, H. I., Doihara, H. & Shimizu, N. (2004) 'Aberrant methylation of p57KIP2 gene in lung and breast cancers and malignant mesotheliomas'. *Oncology Reports*, 12, pp. 1087-1092.
- Komiya, T., Hosono, Y., Hirashima, T., Masuda, N., Yasumitsu, T., Nakagawa, K., Kikui, M., Ohno, A., Fukuoka, M. & Kawase, I. (1997) 'p21 expression as a predictor for favorable prognosis in squamous cell carcinoma of the lung'. *Clinical Cancer Research*, 3 (10), pp. 1831-1835.
- Kondoh, N., Schweinfest, C. W., Henderson, K. W. & Papas, T. S. (1992) 'Differential expression of S19 ribosomal protein, laminin-binding protein, and human lymphocyte antigen class I messenger RNAs associated with colon carcinoma progression and differentiation'. *Cancer Research*, 52 (4), pp. 791-796.
- Kondoh, N., Shuda, M., Tanaka, K., Wakatsuki, T., Hada, A. & Yamamoto, M. (2001) 'Enhanced expression of S8, L12, L23a, L27 and L30 ribosomal protein mRNAs in human hepatocellular carcinoma'. *Anticancer Research*, 21 (4A), pp. 2429-2433.
- Konishi, J., Yi, F., Chen, X., Vo, H., Carbone, D. P. & Dang, T. P. (2010) 'Notch3 cooperates with the EGFR pathway to modulate apoptosis through the induction of bim'. *Oncogene*, 29 (4), pp. 589.

- Koomen, J. M., Haura, E. B., Bepler, G., Sutphen, R., Remily-Wood, E. R., Benson, K., Hussein, M., Hazlehurst, L. A., Yeatman, T. J. & Hildreth, L. T. (2008) 'Proteomic contributions to personalized cancer care'. *Molecular & Cellular Proteomics*, 7 (10), pp. 1780-1794.
- Kotla, V., Goel, S., Nischal, S., Heuck, C., Vivek, K., Das, B. & Verma, A. (2009) 'Mechanism of action of lenalidomide in hematological malignancies'. *Journal of hematology & oncology*, 2 pp. 36.
- Kovall, R. (2008) 'More complicated than it looks: assembly of Notch pathway transcription complexes'. *Oncogene*, 27 (38), pp. 5099.
- Kremer, M., Dirnhofer, S., Nickl, A., Hoefler, H., Quintanilla-Martínez, L. & Fend, F. (2001) 'p27 Kip1 immunostaining for the differential diagnosis of small B-cell neoplasms in trephine bone marrow biopsies'. *Modern Pathology*, 14 (10), pp. 1022.
- Kridel, R., Meissner, B., Rogic, S., Boyle, M., Telenius, A., Woolcock, B., Gunawardana, J., Jenkins, C., Cochrane, C. & Ben-Neriah, S. (2012) 'Whole transcriptome sequencing reveals recurrent NOTCH1 mutations in mantle cell lymphoma'. *Blood*, 119 (9), pp. 1963-1971.
- Kritharis, A., Coyle, M., Sharma, J. & Evens, A. M. (2015) 'Lenalidomide in non-Hodgkin lymphoma: biological perspectives and therapeutic opportunities'. *Blood*, 125 (16), pp. 2471-2476.
- Krstic, J., Maslovaric, I. & Santibanez, J. (2014) 'Novel patents and cancer therapies for transforming growth factor-beta and urokinase type plasminogen activator: potential use of their interplay in tumorigenesis'. *Recent Patents on Anti-Cancer Drug Discovery*, 9 (3), pp. 354-371.
- Kurtova, A., Tamayo, A., Ford, R. & Burger, J. (2009) 'Mantle cell lymphoma cells express high levels of CXCR4, CXCR5, and VLA-4 (CD49d): importance for interactions with the stromal microenvironment and specific targeting'. *Blood*, 113 (19), pp. 4604-4613.
- Kwatra, K. S., Paul, P. A., Dhaliwal, D., Calton, N. & John, J. M. (2016) 'Mantle Cell Lymphoma and Variants: A Clinicopathological and Immunohistochemical Study'. *International Journal Of Scientific Study*, 3 (12), pp. 162-168.
- LaBaer, J., Garrett, M. D., Stevenson, L. F., Slingerland, J. M., Sandhu, C., Chou, H. S., Fattaey, A. & Harlow, E. (1997) 'New functional activities for the p21 family of CDK inhibitors'. *Genes and Development*, 11 (7), pp. 847-862.
- Lai, C. M., Lai, Y. K. & Rakoczy, P. E. (2002) 'Adenovirus and adeno-associated virus vectors'. *DNA and Cell Biology*, 21 (12), pp. 895-913.

- Lau, L. F. (2011) 'CCN1/CYR61: the very model of a modern matricellular protein'. *Cellular and Molecular Life Sciences*, 68 (19), pp. 3149.
- Lawson, C. & Wolf, S. (2009) 'ICAM-1 signaling in endothelial cells'. *Pharmacological Reports*, 61 (1), pp. 22-32.
- Lee, M. H., Reynisdottir, I. & Massague, J. (1995) 'Cloning of p57KIP2, a cyclin-dependent kinase inhibitor with unique domain structure and tissue distribution'. *Genes & Development*, 9, pp. 639–49.
- Le Tourneau, C., Faivre, S., Serova, M. & Raymond, E. (2008) 'mTORC1 inhibitors: is temsirolimus in renal cancer telling us how they really work&quest'. *British journal of cancer*, 99 (8), pp. 1197-1203.
- Leitch, H., Gascoyne, R., Chhanabhai, M., Voss, N., Klasa, R. & Connors, J. (2003) 'Limited-stage mantle-cell lymphoma'. *Annals of Oncology*, 14 (10), pp. 1555-1561.
- Leng, E., Malcolm, T., Tai, G., Estable, M. & Sadowski, I. (2002) 'Organization and expression of theCyr61 gene in normal human fibroblasts'. *Journal of Biomedical Science*, 9 (1), pp. 59-67.
- Leshchenko, V. V., Kuo, P.-Y., Jiang, Z., Weniger, M. A., Overbey, J., Dunleavy, K., Wilson, W. H., Wiestner, A. & Parekh, S. (2015) 'Harnessing Noxa demethylation to overcome Bortezomib resistance in mantle cell lymphoma'. *Oncotarget*, 6 (29), pp. 27332.
- Leshchenko, V. V., Kuo, P.-Y., Shaknovich, R., Yang, D. T., Gellen, T., Petrich, A., Yu, Y., Remache, Y., Weniger, M. A. & Rafiq, S. (2010) 'Genomewide DNA methylation analysis reveals novel targets for drug development in mantle cell lymphoma'. *Blood*, 116 (7), pp. 1025-1034.
- Leu, S.-J., Chen, N., Chen, C.-C., Todorović, V., Bai, T., Juric, V., Liu, Y., Yan, G., Lam, S. C.-T. & Lau, L. F. (2004) 'Targeted mutagenesis of the angiogenic protein CCN1 (CYR61). Selective inactivation of integrin $\alpha 6\beta 1$ -heparan sulfate proteoglycan coreceptor-mediated cellular functions'. *Journal of Biological Chemistry*, 279 (42), pp. 44177-44187.
- Leu, S.-J., Lam, S. C.-T. & Lau, L. F. (2002) 'Proangiogenic activities of CYR61 (CCN1) mediated through integrins $\alpha v\beta 3$ and $\alpha 6\beta 1$ in human umbilical vein endothelial cells'. *Journal of Biological Chemistry*,
- Lewis, K. A., Gray, P. C., Blount, A. L., MacConell, L. A., Wiater, E., Bilezikjian, L. M. & Vale, W. (2000) 'Betaglycan binds inhibin and can mediate functional antagonism of activin signalling'. *Nature*, 404 (6776), pp. 411.
- Li, C., Martinez, V., He, B., Lombet, A. & Perbal, B. (2002) 'A role for CCN3 (NOV) in calcium signalling'. *Molecular Pathology*, 55 (4), pp. 250.
- Li, J. Q., Wu, F., Usuki, H., Kubo, A., Masaki, T., Fujita, J., Bandoh, S., Saoo, K., Takeuchi, H., Kuriyama, S., Ishida, T. & Imaida, K. (2003) 'Loss of p57KIP2 is associated with colorectal carcinogenesis'. *International Journal of Oncology*, 23, pp. 1537–1543.

Li, Z.-Q., Ding, W., Sun, S.-J., Li, J., Pan, J., Zhao, C., Wu, W.-R. & Si, W.-K. (2012) 'Cyr61/CCN1 Is Regulated by Wnt/ β -Catenin Signaling and Plays an Important Role in the Progression of Hepatocellular Carcinoma (Cyr61 Is Regulated by Wnt and Plays a Role in HCC)'. *PLoS ONE*, 7 (4), pp. e35754.

Liang, J., Zubovitz, J., Petrocelli, T., Kotchetkov, R., Connor, M. K., Han, K., Lee, J.-H., Ciarallo, S., Catzavelos, C. & Beniston, R. (2002) 'PKB/Akt phosphorylates p27, impairs nuclear import of p27 and opposes p27-mediated G1 arrest'. *Nature Medicine*, 8 (10), pp. 1153-1160.

Liao, W., Jordaan, G., Benavides-Serrato, A., Holmes, B., Gera, J. & Sharma, S. (2019) 'Targeting the mTORC2 signaling complex in B cell malignancies'. doi: <https://doi.org/10.1101/564500>.

Liechtenstein, T., Perez-Janices, N. & Escors, D. (2013) 'Lentiviral vectors for cancer immunotherapy and clinical applications'. *Cancers*, 5 (3), pp. 815-837.

Lin, J., Zhou, Z., Huo, R., Xiao, L., Ouyang, G., Wang, L., Sun, Y., Shen, B., Li, D. & Li, N. (2012) 'Cyr61 induces IL-6 production by fibroblast-like synoviocytes promoting Th17 differentiation in rheumatoid arthritis'. *The Journal of Immunology*, pp. 1103201.

Lin, M.-T., Chang, C.-C., Chen, S.-T., Chang, H.-L., Su, J.-L., Chau, Y.-P. & Kuo, M.-L. (2004) 'Cyr61 expression confers resistance to apoptosis in breast cancer MCF-7 cells by a mechanism of NF- κ B-dependent XIAP up-regulation'. *Journal of Biological Chemistry*, 279 (23), pp. 24015-24023.

Lin, M.-T., Chang, C.-C., Chen, S.-T., Chang, H.-L., Su, J.-L., Chau, Y.-P. & Kuo, M.-L. (2004) 'Cyr61 expression confers resistance to apoptosis in breast cancer MCF-7 cells by a mechanism of NF- κ B-dependent XIAP up-regulation'. *Journal of Biological Chemistry*, 279 (23), pp. 24015-24023.

Lin, M.-T., Chang, C.-C., Lin, B.-R., Yang, H.-Y., Chu, C.-Y., Wu, M.-H. & Kuo, M.-L. (2007) 'Elevated expression of Cyr61 enhances peritoneal dissemination of gastric cancer cells through integrin α 2 β 1'. *Journal of Biological Chemistry*, 282 (47), pp. 34594-34604.

Lipton, A., Seaman, J. & Zheng, M. (2004) 'Long-term efficacy and safety of zoledronic acid in patients with bone metastases from renal cell carcinoma', *Bone*. ELSEVIER SCIENCE INC 360 PARK AVE SOUTH, NEW YORK, NY 10010-1710 USA, pp. S62-S63.

Liu, H., Chen, G., Zhang, W., Zhu, J.-Y., Lin, Z.-Q., Gong, Z.-C., Wang, F.-Q., Jia, J., Sun, Z.-J. & Zhao, Y.-F. (2013) 'Overexpression of macrophage migration inhibitory factor in adenoid cystic carcinoma: correlation with enhanced metastatic potential'. *Journal of Cancer Research and Clinical Oncology*, 139 (2), pp. 287-295.

Liu, L., Ji, C., Chen, J., Li, Y., Fu, X., Xie, Y., Gu, S. & Mao, Y. (2008) 'A global genomic view of MIF knockdown-mediated cell cycle arrest'. *Cell Cycle*, 7 (11), pp. 1678-92.

Liu, S., Han, L., Wang, X., Liu, Z., Ding, S., Lu, J., Bi, D., Mei, Y. & Niu, Z. (2015) 'Nephroblastoma overexpressed gene (NOV) enhances RCC cell motility through upregulation of ICAM-1 and COX-2 expression via Akt pathway'. *International Journal of Clinical and Experimental Pathology*, 8 (2), pp. 1302.

Logan, C. Y. & Nusse, R. (2004) 'The Wnt signaling pathway in development and disease'. *Annual Review of Cell and Developmental Biology*, 20 pp. 781-810.

Lois, C., Hong, E. J., Pease, S., Brown, E. J. & Baltimore, D. (2002) 'Germline transmission and tissue-specific expression of transgenes delivered by lentiviral vectors'. *Science*, 295 (5556), pp. 868-872.

Long, E. O. (2011) 'ICAM-1: getting a grip on leukocyte adhesion'. *The Journal of Immunology*, 186 (9), pp. 5021-5023.

Long, X., Yu, Y., Perlaky, L., Man, T. K. & Redell, M. S. (2015) 'Stromal CYR61 confers resistance to mitoxantrone via spleen tyrosine kinase activation in human acute myeloid leukaemia'. *British Journal of Haematology*, 170 (5), pp. 704-718.

Lou, X., Xiao, T., Zhao, K., Wang, H., Zheng, H., Lin, D., Lu, Y., Gao, Y., Cheng, S. & Liu, S. (2007) 'Cathepsin D is secreted from M-BE cells: its potential role as a biomarker of lung cancer'. *Journal of Proteome Research*, 6 (3), pp. 1083-1092.

Luo, G., Zhang, X., Mu, Q., Chen, L., Zheng, L., Wei, J., Berggren-Söderlund, M., Nilsson-Ehle, P. & Xu, N. (2010) 'Expression and localization of apolipoprotein M in human colorectal tissues'. *Lipids in Health and Disease*, 9 (1), pp. 102.

Lu, X., Toki, T., Konishi, I., Nikaido, T. & Fujii, S. (1998a) 'Expression of p21WAF1/CIP1 in adenocarcinoma of the uterine cervix'. *Cancer*, 82 (12), pp. 2409-2417.

Lu, Y., Yamagishi, N., Yagi, T. & Takebe, H. (1998b) 'Mutated p21 (WAF1/CIP1/SDI1) lacking CDK-inhibitory activity fails to prevent apoptosis in human colorectal carcinoma cells'. *Oncogene*, 16 (6), pp. 705-712.

Lu, Z. & Hunter, T. (2010) 'Ubiquitylation and proteasomal degradation of the p21Cip1, p27Kip1 and p57Kip2 CDK inhibitors'. *Cell cycle*, 9 (12), pp. 2342-2352.

Lukas, J., Groshen, S., Saffari, B., Niu, N., Reles, A., Wen, W.-H., Felix, J., Jones, L. A., Hall, F. L. & Press, M. F. (1997) 'WAF1/Cip1 gene polymorphism and expression in carcinomas of the breast, ovary, and endometrium'. *The American journal of pathology*, 150 (1), pp. 167.

Maddocks, K. & Blum, K. A. (2015) 'Treatment strategies in mantle cell lymphoma', *Non-Hodgkin Lymphoma*. Springer, pp. 251-270.

Maffei, R., Fiorcari, S., Martinelli, S., Potenza, L., Luppi, M. & Marasca, R. (2015) 'Targeting neoplastic B cells and harnessing microenvironment: the double face of ibrutinib and idelalisib'. *Journal of Hematology & Oncology*, 8 (1), pp. 60.

Maity, G., Mehta, S., Haque, I., Dhar, K., Sarkar, S., Banerjee, S. K. & Banerjee, S. (2014) 'Pancreatic tumor cell secreted CCN1/Cyr61 promotes endothelial cell migration and aberrant neovascularization'. *Scientific Reports*, 4

- Malanchi, I., Peinado, H., Kassen, D., Hussenet, T., Metzger, D., Chambon, P., Huber, M., Hohl, D., Cano, A. & Birchmeier, W. (2008) 'Cutaneous cancer stem cell maintenance is dependent on β -catenin signalling'. *Nature*, 452 (7187), pp. 650.
- Maldi, E., Travelli, C., Caldarelli, A., Agazzone, N., Cintura, S., Galli, U., Scatolini, M., Ostano, P., Miglino, B. & Chiorino, G. (2013) 'Nicotinamide phosphoribosyltransferase (NAMPT) is over-expressed in melanoma lesions'. *Pigment cell & melanoma research*, 26 (1), pp. 144-146.
- Malik, A. R., Liszewska, E. & Jaworski, J. (2015) 'Matricellular proteins of the Cyr61/CTGF/NOV (CCN) family and the nervous system'. *Front Cell Neurosci*, 24(9), pp. 237
- Mao-De, L. & Jing, X. (2007) 'Ribosomal proteins and colorectal cancer'. *Current genomics*, 8 (1), pp. 43-49.
- Mao, Y., Yan, R., Li, A., Zhang, Y., Li, J., Du, H., Chen, B., Wei, W., Zhang, Y. & Summers, C. (2015) 'Lentiviral vectors mediate long-term and high efficiency transgene expression in HEK 293T cells'. *International Journal of Medical Sciences*, 12 (5), pp. 407.
- Marco, G. & Lorenzo, F. (2012) 'Therapeutic Activity of Lenalidomide in Mantle Cell Lymphoma and Indolent Non-Hodgkin's Lymphomas'. *Advances in Hematology*, 2012
- Marino, F. E., Risbridger, G. & Gold, E. (2015) 'Re-evaluating the role of activin- β C in cancer biology'. *Cytokine and Growth Factor Reviews*, 26 (4), pp. 463-470.
- Marra, M., Santini, D., Meo, G., Vincenzi, B., Zappavigna, S., Baldi, A., Rosolowski, M., Tonini, G., Loeffler, M. & Lupu, R. (2009) 'Cyr61 downmodulation potentiates the anticancer effects of zoledronic acid in androgen-independent prostate cancer cells'. *International Journal of Cancer*, 125 (9), pp. 2004-2013.
- Martin, P., Ghione, P. & Dreyling, M. (2017) 'Mantle cell lymphoma—Current standards of care and future directions'. *Cancer Treatment Reviews*, 58 pp. 51-60.
- Marzec, M., Kasprzycka, M., Lai, R., Gladden, A. B., Wlodarski, P., Tomczak, E., Nowell, P., Deprimo, S. E., Sadis, S., Eck, S., Schuster, S. J., Diehl, J. A. & Wasik, M. A. (2006) 'Mantle cell lymphoma cells express predominantly cyclin D1a isoform and are highly sensitive to selective inhibition of CDK4 kinase activity'. *Blood*, 108(5) pp. 1744-50.
- Mathur, R., Sehgal, L., Braun, F. K., Berkova, Z., Romaguerra, J., Wang, M., Rodriguez, M. A., Fayad, L., Neelapu, S. S. & Samaniego, F. (2015) 'Targeting Wnt pathway in mantle cell lymphoma-initiating cells'. *Journal of Hematology & Oncology*, 8 (1), pp. 63.
- Mátrai, J., Chuah, M. K. & VandenDriessche, T. (2010) 'Recent advances in lentiviral vector development and applications'. *Molecular Therapy*, 18 (3), pp. 477-490.
- Matsumoto, A., Takeishi, S., Kanie, T., Susaki, E., Onoyama, I., Tateishi, Y., Nakayama, K. & Nakayama, K. I. (2011) 'p57 is required for quiescence and maintenance of adult hematopoietic stem cells'. *Cell Stem Cell*, 9, pp. 262–271.

- McCallum, L. & Irvine, A. (2009) 'CCN3—a key regulator of the hematopoietic compartment'. *Blood Reviews*, 23 (2), pp. 79-85.
- McMahon, J., Conroy, S., Lyons, M., Greiser, U., O'shea, C., Strappe, P., Howard, L., Murphy, M., Barry, F. & O'brien, T. (2006) 'Gene transfer into rat mesenchymal stem cells: a comparative study of viral and nonviral vectors'. *Stem cells and development*, 15 (1), pp. 87-96.
- Medema, R. H., Kops, G. J., Bos, J. L. & Burgering, B. M. (2000) 'AFX-like Forkhead transcription factors mediate cell-cycle regulation by Ras and PKB through p27kip1'. *Nature*, 404 (6779), pp. 782-787.
- Melo, J., Foroni, L., Brito-Babapulle, V., Luzzatto, L. & Catovsky, D. (1988) 'The establishment of cell lines from chronic B cell leukaemias: evidence of leukaemic origin by karyotypic abnormalities and Ig gene rearrangement'. *Clinical and Experimental Immunology*, 73 (1), pp. 23.
- Melo, J. V., Brito-Babapulle, V., Foroni, L., Robinson, D. S., Luzzatto, L. & Catovsky, D. (1986) 'Two new cell lines from B-prolymphocytic leukaemia: Characterization by morphology, immunological markers, karyotype and Ig gene rearrangement'. *International Journal of Cancer*, 38 (4), pp. 531-538.
- Menendez, J. A., Vellon, L., Mehmi, I., Teng, P. K., Griggs, D. W. & Lupu, R. (2005) 'A novel CYR61-triggered 'CYR61- α v β 3 integrin loop' regulates breast cancer cell survival and chemosensitivity through activation of ERK1/ERK2 MAPK signaling pathway'. *Oncogene*, 24 (5), pp. 761-779.
- Mercurio, S., Latinkic, B., Itasaki, N., Krumlauf, R. & Smith, J. (2004) 'Connective-tissue growth factor modulates WNT signalling and interacts with the WNT receptor complex'. *Development*, 131 (9), pp. 2137-2147.
- Metcalf, R. A., Zhao, S., Anderson, M. W., Lu, Z. S., Galperin, I., Marinelli, R. J., Cherry, A. M., Lossos, I. S. & Natkunam, Y. (2010) 'Characterization of D-cyclin proteins in hematolymphoid neoplasms: lack of specificity of cyclin-D2 and D3 expression in lymphoma subtypes'. *Modern Pathology*, 23(3), pp. 420-33.
- Meyer-Siegler, K. L., Iczkowski, K. A., Leng, L., Bucala, R. & Vera, P. L. (2006) 'Inhibition of macrophage migration inhibitory factor or its receptor (CD74) attenuates growth and invasion of DU-145 prostate cancer cells'. *The Journal of Immunology*, 177 (12), pp. 8730-8739.
- Millioni, R., Tolin, S., Puricelli, L., Sbrignadello, S., Fadini, G. P., Tessari, P. & Arrighoni, G. (2011) 'High abundance proteins depletion vs low abundance proteins enrichment: comparison of methods to reduce the plasma proteome complexity'. *PloS One*, 6 (5), pp. e19603.
- Mishra, S. K., Siddique, H. R. & Saleem, M. (2012) 'S100A4 calcium-binding protein is key player in tumor progression and metastasis: preclinical and clinical evidence'. *Cancer and Metastasis Reviews*, 31 (1-2), pp. 163-172.
- Montanaro, L., Treré, D. & Derenzini, M. (2012) 'Changes in ribosome biogenesis may induce cancer by down-regulating the cell tumor suppressor potential'. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, 1825 (1), pp. 101-110.

Monteith, G. R., Davis, F. M. & Roberts-Thomson, S. J. (2012) 'Calcium channels and pumps in cancer: changes and consequences'. *Journal of Biological Chemistry*, 287 (38), pp. 31666-31673.

Min, Y. H., Cheong, J.-W., Kim, J. Y., Eom, J. I., Lee, S. T., Hahn, J. S., Ko, Y. W. & Lee, M. H. (2004) 'Cytoplasmic mislocalization of p27Kip1 protein is associated with constitutive phosphorylation of Akt or protein kinase B and poor prognosis in acute myelogenous leukemia'. *Cancer Research*, 64 (15), pp. 5225-5231.

Mishra, L., Derynck, R. & Mishra, B. (2005) 'Transforming growth factor- β signaling in stem cells and cancer'. *Science*, 310 (5745), pp. 68-71.

Miyazono, K., Kamiya, Y. & Morikawa, M. (2010) 'Bone morphogenetic protein receptors and signal transduction'. *The journal of biochemistry*, 147 (1), pp. 35-51.

Miyazono, K., Maeda, S. & Imamura, T. (2005) 'BMP receptor signaling: transcriptional targets, regulation of signals, and signaling cross-talk'. *Cytokine and Growth Factor Reviews*, 16 (3), pp. 251-263.

Molenaar, J. J., Ebus, M. E., Koster, J., van Sluis, P., van Noesel, C. J., Versteeg, R. & Caron, H. N. (2008) 'Cyclin D1 and CDK4 activity contribute to the undifferentiated phenotype in neuroblastoma'. *Cancer Research*, 68 (8), pp. 2599-2609.

Morita, M., Gravel, S.-P., Hulea, L., Larsson, O., Pollak, M., St-Pierre, J. & Topisirovic, I. (2015) 'mTOR coordinates protein synthesis, mitochondrial activity and proliferation'. *Cell cycle*, 14 (4), pp. 473-480.

Moros, A., Bustany, S., Cahu, J., Saborit-Villarroya, I., Martínez, A., Colomer, D., Sola, B. & Roué, G. (2014) 'Antitumoral activity of lenalidomide in in vitro and in vivo models of mantle cell lymphoma involves the destabilization of cyclin D1/p27KIP1 complexes'. *Clinical Cancer Research*, 20 (2), pp. 393-403.

Mozos, A., Royo, C., Hartmann, E., De Jong, D., Baró, C., Valera, A., Fu, K., Weisenburger, D. D., Delabie, J. & Chuang, S.-S. (2009) 'SOX11 expression is highly specific for mantle cell lymphoma and identifies the cyclin D1-negative subtype'. *Haematologica*, 94 (11), pp. 1555-1562.

Mujtaba, T. & Dou, Q. P. (2011) 'Advances in the understanding of mechanisms and therapeutic use of bortezomib'. *Discovery Medicine*, 12 (67), pp. 471.

Müller, A., Zang, C., Chumduri, C., Dörken, B., Daniel, P. T. & Scholz, C. W. (2013) 'Concurrent inhibition of PI3K and mTORC1/mTORC2 overcomes resistance to rapamycin induced apoptosis by down-regulation of Mcl-1 in mantle cell lymphoma'. *International Journal of Cancer*, 133 (8), pp. 1813-1824.

Muñoz-Alonso, M. J., Acosta, J. C., Richard, C., Delgado, M. D., Sedivy, J. & León, J. (2005) 'p21Cip1 and p27Kip1 induce distinct cell cycle effects and differentiation

programs in myeloid leukemia cells'. *Journal of Biological Chemistry*, 280 (18), pp. 18120-18129.

Nagaraj, A. B., Joseph, P., Kovalenko, O., Singh, S., Armstrong, A., Redline, R., Resnick, K., Zanotti, K., Waggoner, S. & DiFeo, A. (2015) 'Critical role of Wnt/ β -catenin signaling in driving epithelial ovarian cancer platinum resistance'. *Oncotarget*, 6 (27), pp. 23720.

Nakai, S., Masaki, T., Shiratori, Y., Ohgi, T., Morishita, A., Kurokohchi, K., Watanabe, S. & Kuriyama, S. (2002) 'Expression of p57(KIP2) in hepatocellular carcinoma: relationship between tumor differentiation and patient survival'. *International Journal of Oncology*, 20, pp. 769–775.

Naora, H., Takai, I., Adachi, M. & Naora, H. (1998) 'Altered cellular responses by varying expression of a ribosomal protein gene: sequential coordination of enhancement and suppression of ribosomal protein S3a gene expression induces apoptosis'. *The Journal of cell biology*, 141 (3), pp. 741-753.

Narurkar, R., Alkayem, M. & Liu, D. (2016) 'SOX11 is a biomarker for cyclin D1-negative mantle cell lymphoma'. *Biomarker research*, 4 (1), pp. 6.

Nayerossadat, N., Maedeh, T. & Ali, P. A. (2012) 'Viral and nonviral delivery systems for gene delivery'. *Advanced biomedical research*, 1: 27.

Nemes, J. A., Nemes, Z. & Márton, I. J. (2005) 'p21WAF1/CIP1 expression is a marker of poor prognosis in oral squamous cell carcinoma'. *Journal of Oral Pathology and Medicine*, 34 (5), pp. 274-279.

Nevins, J. R. (2001) 'The Rb/E2F pathway and cancer'. *Human Molecular Genetics*, 10 (7), pp. 699-703.

Niu, C.-C., Zhao, C., Yang, Z., Zhang, X.-L., Pan, J. & Si, W.-K. (2014) 'Inhibiting CCN1 blocks AML cell growth by disrupting the MEK/ERK pathway'. *Cancer Cell International*, 14 (1), pp. 74.

Niu, C. C., Wan, Y. F., Yang, C., Li, T. & Liao, P. ' Polymorphisms of the CYR61 gene in patients with acute myeloid leukemia in a Han Chinese population'. *Medicine* 97(34) pp e11963.

Noel, M. S., Friedberg, J. W. & Barr, P. M. (2012) 'Novel agents in mantle cell lymphoma'. *Best practice & research Clinical haematology*, 25 (2), pp. 191-200.

Nordgren, T. M., Hegde, G. V. & Joshi, S. S. (2012) 'Ritonavir exhibits limited efficacy as a single agent in treating aggressive mantle cell lymphoma'. *Journal of Cancer Science and Therapy*, 4 (4), pp. 61-68.

Nunez, R., Ackermann, M., Saeki, Y., Chiocca, A. & Fraefel, C. (2001) 'Flow cytometric assessment of transduction efficiency and cytotoxicity of herpes simplex virus type 1-based amplicon vectors'. *Cytometry Journal*, 44(2), pp. 93-9.

Nusse, R., Fuerer, C., Ching, W., Harnish, K., Logan, C., Zeng, A., Ten Berge, D. & Kalani, Y. (2008) 'Wnt signaling and stem cell control', *Cold Spring Harbor Symposia on Quantitative Biology*. Cold Spring Harbor Laboratory Press, pp. 59-66.

Nussinov, R., Muratcioglu, S., Tsai, C.-J., Jang, H., Gursoy, A. & Keskin, O. (2015) 'The key role of calmodulin in KRAS-driven adenocarcinomas'. *Molecular Cancer Research*, pp. molcanres. 0165.2015.

Nutt, S. L., Hodgkin, P. D., Tarlinton, D. M. & Corcoran, L. M. (2015) 'The generation of antibody-secreting plasma cells'. *Nature Reviews Immunology*, 15 (3), pp. 160-71.

O'Kelly, J., Chung, A., Lemp, N., Chumakova, K., Yin, D., Wang, H.-J., Said, J., Gui, D., Miller, C. W. & Karlan, B. Y. (2008) 'Functional domains of CCN1 (Cyr61) regulate breast cancer progression'. *International journal of oncology*, 33 (1), pp. 59-67.

O'Connor, O. A. (2007) 'Mantle cell lymphoma: identifying novel molecular targets in growth and survival pathways'. *ASH Education Program Book*, 2007 (1), pp. 270-276.

Ohkoshi, S., Yano, M. & Matsuda, Y. (2015) 'Oncogenic role of p21 in hepatocarcinogenesis suggests a new treatment strategy'. *World Journal of Gastroenterology*, 21 (42), pp. 12150.

Oishi, I., Suzuki, H., Onishi, N., Takada, R., Kani, S., Ohkawara, B., Koshida, I., Suzuki, K., Yamada, G. & Schwabe, G. C. (2003) 'The receptor tyrosine kinase Ror2 is involved in non-canonical Wnt5a/JNK signalling pathway'. *Genes to Cells*, 8 (7), pp. 645-654.

Okochi, M., Steiner, H., Fukumori, A., Tanii, H., Tomita, T., Tanaka, T., Iwatsubo, T., Kudo, T., Takeda, M. & Haass, C. (2002) 'Presenilins mediate a dual intramembranous γ -secretase cleavage of Notch-1'. *The EMBO journal*, 21 (20), pp. 5408-5416.

Olesen, U. H., Hastrup, N. & Sehested, M. (2011) 'Expression patterns of nicotinamide phosphoribosyltransferase and nicotinic acid phosphoribosyltransferase in human malignant lymphomas'. *APMIS*, 119 (4-5), pp. 296-303.

Orlow, I., Iavarone, A., Crider-Miller, S. J., Bonilla, F., Latres, E., Lee, M. H., Gerald, W. L., Massagué, J., Weissman, B. E. & Cordon-Cardó, C. (1996) 'Cyclin-dependent kinase inhibitor p57KIP2 in soft tissue sarcomas and Wilms'tumors'. *Cancer Research*, 56 (6), pp. 1219-21.

Pagano, M., Tam, S. W., Theodoras, A. M., Beer-Romero, P., Del Sal, G., Chau, V., Yew, P. R., Draetta, G. F. & Rolfe, M. (1995) 'Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27'. *Science*, 269 (5224), pp. 682-685.

Palomero, T., Dominguez, M. & Ferrando, A. A. (2008) 'The role of the PTEN/AKT Pathway in NOTCH1-induced leukemia'. *Cell cycle*, 7 (8), pp. 965-970.

Pan, Z., Scheerens, H., Li, S. J., Schultz, B. E., Sprengeler, P. A., Burrill, L. C., Mendonca, R. V., Sweeney, M. D., Scott, K. C. & Grothaus, P. G. (2007) 'Discovery of selective irreversible inhibitors for Bruton's tyrosine kinase'. *ChemMedChem*, 2 (1), pp. 58-61.

Papin, A., Le Gouill, S. & Chiron, D. (2018) 'Rationale for targeting tumor cells in their microenvironment for mantle cell lymphoma treatment'. *Leukemia and Lymphoma*, 59 (5), pp. 1064-1072.

Parekh, S., Weniger, M. A. & Wiestner, A. (2011) 'New molecular targets in mantle cell lymphoma', *Seminars in Cancer Biology*. Elsevier, pp. 335-346.

Park, J. K., Park, S. H., So, K., Bae, I. H., Yoo, Y. D. & Um, H.-D. (2010) 'ICAM-3 enhances the migratory and invasive potential of human non-small cell lung cancer cells by inducing MMP-2 and MMP-9 via Akt and CREB'. *International Journal of Oncology*, 36 (1), pp. 181-192.

Park, Y., Kim, J. W., Kim, D. S., Kim, E. B., Park, S. J., Park, J. Y., Choi, W. S., Song, J. G., Seo, H. Y. & Oh, S. C. (2008) 'The bone morphogenesis protein-2 (BMP-2) is associated with progression to metastatic disease in gastric cancer'. *Cancer research and treatment: official journal of Korean Cancer Association*, 40 (3), pp. 127.

Patel, S. T., Mistry, T., Brown, J. E., Digby, J. E., Adya, R., Desai, K. M. & Randeva, H. S. (2010) 'A novel role for the adipokine visfatin/pre-B cell colony-enhancing factor 1 in prostate carcinogenesis'. *Peptides*, 31 (1), pp. 51-57.

Pateras, I. S., Apostolopoulou, K., Koutsami, M., Evangelou, K., Tsantoulis, P., Liloglou, T., Nikolaidis, G., Sigala, F., Kittas, C., Field, J. K., Kotsinas, A. & Gorgoulis, V. G. (2006) 'Downregulation of the KIP family members p27(KIP1) and p57(KIP2) by SKP2 and the role of methylation in p57(KIP2) inactivation in nonsmall cell lung cancer'. *International Journal of Cancer*, 119, pp. 2546–2556.

Patmore, R., Smith, A., Appleton, S., Howell, D., Johnson, R., J., Burton, C., H. & Roman, E. (2016) 'Mantle Cell Lymphoma Management and Outcome in the U.K's Population-Based Haematological Malignancy Research Network'. *Blood*, 128 (22), pp.1112.

Patten, P. E., Chu, C. C., Albesiano, E., Damle, R. N., Yan, X. J., Kim, D., Zhang, L., Magli, A. R., Barrientos, J., Kolitz, J. E., Allen, S. L., Rai, K. R., Roa, S., Mongini, P. K., MacCarthy, T., Scharff, M. D. & Chiorazzi, N. (2012). 'IGHV-unmutated and IGHV-mutated chronic lymphocytic leukemia cells produce activation-induced deaminase protein with a full range of biologic functions'. *Blood*, 120(24) pp. 4802-11

Paul, D., Kumar, A., Gajbhiye, A., Santra, M. K. & Srikanth, R. (2013) 'Mass spectrometry-based proteomics in molecular diagnostics: discovery of cancer biomarkers using tissue culture'. *BioMed research international*, 2013

Pendurthi, U. R., Tran, T. T., Post, M. & Rao, L. V. M. (2005) 'Proteolysis of CCN1 by plasmin: functional implications'. *Cancer Research*, 65 (21), pp. 9705-9711.

Peng, J., Yoshioka, Y., Mandai, M., Matsumura, N., Baba, T., Yamaguchi, K., Hamanishi, J., Kharma, B., Murakami, R. & Abiko, K. (2016) 'The BMP signaling pathway leads to enhanced proliferation in serous ovarian cancer—a potential therapeutic target'. *Molecular Carcinogenesis*, 55 (4), pp. 335-345.

Peng, S.-Y., Chou, S.-P. & Hsu, H.-C. (1998) 'Association of downregulation of cyclin D1 and of overexpression of cyclin E with p53 mutation, high tumor grade and poor prognosis in hepatocellular carcinoma'. *Journal of Hepatology*, 29 (2), pp. 281-289.

Perbal, B. (1999) 'Nuclear localisation of NOVH protein: a potential role for NOV in the regulation of gene expression'. *Molecular Pathology*, 52 (2), pp. 84.

Perbal, B. (2009) 'Alternative splicing of CCN mRNAs.... it has been upon us'. *Journal of cell communication and signaling*, 3 (2), pp. 153-157.

Perez-Galan, P., Dreyling, M. & Wiestner, A. (2011) 'Mantle cell lymphoma: biology, pathogenesis, and the molecular basis of treatment in the genomic era'. *Blood*, 117, pp. 26-38.

Pérez-Sayáns, M., Suárez-Peñaranda, J. M., Gayoso-Diz, P., Barros-Angueira, F., Gándara-Rey, J. M. & García-García, A. (2013) 'The role of p21Waf1/CIP1 as a Cip/Kip type cell-cycle regulator in oral squamous cell carcinoma (Review)'. *Medicina oral, patología oral y cirugía bucal*, 18 (2), pp. e219.

Perez-Roger, I., Kim, S. H., Griffiths, B., Sewing, A. & Land, H. (1999) 'Cyclins D1 and D2 mediate Myc-induced proliferation via sequestration of p27Kip1 and p21Cip1'. *The EMBO journal*, 18 (19), pp. 5310-5320.

Perkins, N. D. (2002) 'Not just a CDK inhibitor: regulation of transcription by p21WAF1/CIP1/SDI1'. *Cell cycle*, 1 (1), pp. 35-37.

Pervez, S., Haroon, S. & Awan, D. (2015) 'Ki-67 labeling indices in 'classic' versus 'blastoid' mantle cell lymphomas--proposed cutoff values for routine diagnostic workup'. *Asian Pacific Journal of Cancer Prevention*, 16 pp. 6591-6594.

Pham, L. V., Tamayo, A. T., Pogue, E., Lu, G., Challagundla, P., Jorgensen, J. L., Zhang, L., Wang, M. & Ford, R. J. (2014) 'The Tumor Microenvironment in Mantle Cell Lymphoma (MCL): Novel Targets to Overcome Chemo-Resistance in MCL'. *Hematology*, 124 (21), pp. 494.

Pham, L. V., Tamayo, A. T., Yoshimura, L. C., Lo, P. & Ford, R. J. (2003) 'Inhibition of constitutive NF- κ B activation in mantle cell lymphoma B cells leads to induction of cell cycle arrest and apoptosis'. *The Journal of Immunology*, 171 (1), pp. 88-95.

Pinson, K. I., Brennan, J., Monkley, S., Avery, B. J. & Skarnes, W. C. (2000) 'An LDL-receptor-related protein mediates Wnt signalling in mice'. *Nature*, 407 (6803), pp. 535.

Pinyol, M., Hernandez, L., Cazorla, M., Balbín, M., Jares, P., Fernandez, P. L., Montserrat, E., Cardesa, A., Lopez-Otín, C. & Campo, E. a. (1997) 'Deletions and loss of expression of p16INK4a and p21Waf1 genes are associated with aggressive variants of mantle cell lymphomas'. *Blood*, 89 (1), pp. 272-280.

Planque, N. (2006) 'Nuclear trafficking of secreted factors and cell-surface receptors: new pathways to regulate cell proliferation and differentiation, and involvement in cancers'. *Cell Communication and Signaling*, 4 (1), pp. 7.

Planque, N., Long Li, C., Saule, S., Bleau, A. M. & Perbal, B. (2006) 'Nuclear addressing provides a clue for the transforming activity of amino-truncated CCN3 proteins'. *Journal of Cellular Biochemistry*, 99 (1), pp. 105-116.

Planque, N. & Perbal, B. (2003) 'A structural approach to the role of CCN (CYR61/CTGF/NOV) proteins in tumourigenesis'. *Cancer Cell International*, 3 (1), pp. 15.

Polakis, P. (2012) 'Wnt signaling in cancer'. *Cold Spring Harbor Perspectives in Biology*, 4 (5), pp. a008052.

Qian, W.-J., Kaleta, D. T., Petritis, B. O., Jiang, H., Liu, T., Zhang, X., Mottaz, H. M., Varnum, S. M., Camp, D. G. & Huang, L. (2008) 'Enhanced detection of low abundance human plasma proteins using a tandem IgY12-SuperMix immunoaffinity separation strategy'. *Molecular & Cellular Proteomics*, 7 (10), pp. 1963-1973.

Qian, Z., Zhang, L., Cai, Z., Sun, L., Wang, H., Yi, Q. & Wang, M. (2011) 'Lenalidomide synergizes with dexamethasone to induce growth arrest and apoptosis of mantle cell lymphoma cells in vitro and in vivo'. *Leukemia Research*, 35 (3), pp. 380-386.

Qin, L.-F. & Ng, I. O.-I. (2001) 'Expression of p27 KIP1 and p21 WAF1/CIP1 in primary hepatocellular carcinoma: Clinicopathologic correlation and survival analysis'. *Human Pathology*, 32 (8), pp. 778-785.

Queirós, A., C., Beekman, R., Vilarrasa-Blasi, R., Duran-Ferrer, M., Clot, G., Merkel, A., Raineri, E., Russiñol, N., Castellano, G., Beà, S., Navarro, A., Kulis, M., Verdaguer-Dot, N., Jares, P., Enjuanes, A., Calasanz, M., J., Bergmann, A., Vater, I., Salaverría, I., van de Werken, H., J., G., Wilson, W., H., Datta, A., Flicek, P., Royo, R., Martens, J., Giné, E., Lopez-Guillermo, A., Stunnenberg, H., G., Klapper, W., Pott, C., Heath, S., Gut, I., G., Siebert, R., Campo, E. & Martín-Subero, J. I. (2016) 'Decoding the DNA Methylome of Mantle Cell Lymphoma in the Light of the Entire B Cell Lineage'. *Cancer Cell*, 30 (5), pp. 806-821.

Queiroz, A. B., Focchi, G., Dobo, C., Gomes, T. S., Ribeiro, D. A. & Oshima, C. T. (2010) 'Expression of P27, P21WAF/Cip1, and P16INK4a in normal oral epithelium, oral squamous papilloma, and oral squamous cell carcinoma'. *Anticancer Research*, 30 (7), pp. 2799-2803.

Quintanilla-Martinez, L., Davies-Hill, T., Fend, F., Calzada-Wack, J., Sorbara, L., Campo, E., Jaffe, E. S. & Raffeld, M. (2003) 'Sequestration of p27^{Kip1} protein by cyclin D1 in typical and blastic variants of mantle cell lymphoma (MCL): implications for pathogenesis'. *BLOOD-NEW YORK*, 101 (8), pp. 3181-3187.

Quintanilla-Martinez, L., Thieblemont, C., Fend, F., Kumar, S., Pinyol, M., Campo, E., Jaffe, E. S. & Raffeld, M. (1998) 'Mantle cell lymphomas lack expression of p27 Kip1, a cyclin-dependent kinase inhibitor'. *The American journal of pathology*, 153 (1), pp. 175-182.

Raedler, L. A. (2015) 'Imbruvica (Ibrutinib), First-in-Class Bruton's Tyrosine Kinase Inhibitor, Receives Expanded Indications for Patients with Relapsed Chronic Lymphocytic Leukemia'. *American health & drug benefits*, 8 (Spec Feature), pp. 66.

Rauert-Wunderlich, H., Rudelius, M., Ott, G. & Rosenwald, A. (2016) 'Targeting protein kinase C in mantle cell lymphoma'. *British Journal of Haematology*, 173 (3), pp. 394-403.

Ray, A., James, M. K., Larochele, S., Fisher, R. P. & Blain, S. W. (2009) 'p27Kip1 inhibits cyclin D-cyclin-dependent kinase 4 by two independent modes'. *Molecular and Cellular Biology*, 29 (4), pp. 986-999.

Raynaud, S. D., Bekri, S., Leroux, D., Grosgeorge, J., Klein, B., Bastard, C., Gaudray, P. & Simon, M. P. (1993) 'Expanded range of 11q13 breakpoints with differing patterns of cyclin D1 expression in B-cell malignancies'. *Genes, Chromosomes and Cancer*, 8 (2), pp. 80-87.

Reddy, N., Hernandez-Ilizaliturri, F., Deeb, G., Roth, M., Vaughn, M., Knight, J., Wallace, P. & Czuczman, M. S. (2008) 'Immunomodulatory drugs stimulate natural killer-cell function, alter cytokine production by dendritic cells, and inhibit angiogenesis enhancing the anti-tumour activity of rituximab in vivo'. *Br. J. Haematol.*, 140 (1), pp. 36-45.

Reid, T., Warren, R. & Kirn, D. (2002) 'Intravascular adenoviral agents in cancer patients: lessons from clinical trials'. *Cancer Gene Therapy*, 9 (12), pp. 979.

Rendon, B. E., Roger, T., Teneng, I., Zhao, M., Al-Abed, Y., Calandra, T. & Mitchell, R. A. (2007) 'Regulation of human lung adenocarcinoma cell migration and invasion by macrophage migration inhibitory factor'. *Journal of Biological Chemistry*, 282 (41), pp. 29910-29918.

Reya, T., Morrison, S. J., Clarke, M. F. & Weissman, I. L. (2001) 'Stem cells, cancer, and cancer stem cells'. *Nature*, 414 (6859), pp. 105.

Richard, V., Kindt, N., Decaestecker, C., Gabius, H. J., Laurent, G., Noël, J.-C. & Saussez, S. (2014) 'Involvement of macrophage migration inhibitory factor and its receptor (CD74) in human breast cancer'. *Oncology Reports*, 32 (2), pp. 523-529.

Richard, V., Kindt, N. & Saussez, S. (2015) 'Macrophage migration inhibitory factor involvement in breast cancer'. *International Journal of Oncology*, 47 (5), pp. 1627-1633.

Richards, S., Watanabe, C., Santos, L., Craxton, A. & Clark, E. A. (2008) 'Regulation of B-cell entry into the cell cycle'. *Immunological Reviews*, 224 (1), pp. 183-200.

Rimokh, R., Berger, F., Delsol, G., Dignonet, I., Rouault, J. P., Tigaud, J. D., Gadoux, M., Coiffier, B., Bryon, P. A. & Magaud, J. P. (1994) 'Detection of the chromosomal translocation t (11; 14) by polymerase chain reaction in mantle cell lymphomas'. *Blood*, 83 (7), pp. 1871-1875.

Ringo, K., Norman, R., Bijli, K. M., Rahman, A. & Young, J. L. (2011) 'Extracellular Matrix Protein CCN1 (Cyr61) Modulates Neutrophil Recruitment To The Lung', A61. LUNG

ENDOTHELIUM FUNCTIONS: THE BARRIER AND BEYOND. *American Thoracic Society*, pp. A1967-A1967.

Rini, B. I. (2008) 'Temsirolimus, an inhibitor of mammalian target of rapamycin'. *Clinical Cancer Research*, 14 (5), pp. 1286-1290.

Rizzatti, E. G., Falcão, R. P., Panepucci, R. A., Proto-Siqueira, R., Anselmo-Lima, W. T., Okamoto, O. K. & Zago, M. A. (2005) 'Gene expression profiling of mantle cell lymphoma cells reveals aberrant expression of genes from the PI3K-AKT, WNT and TGF β signalling pathways'. *British Journal of Haematology*, 130 (4), pp. 516-526.

Rizzo, P., Miao, H., D'Souza, G., Osipo, C., Yun, J., Zhao, H., Mascarenhas, J., Wyatt, D., Antico, G. & Hao, L. (2008) 'Cross-talk between notch and the estrogen receptor in breast cancer suggests novel therapeutic approaches'. *Cancer Research*, 68 (13), pp. 5226-5235.

R Moschen, A., R Gerner, R. & Tilg, H. (2010) 'Pre-B cell colony enhancing factor/NAMPT/visfatin in inflammation and obesity-related disorders'. *Current Pharmaceutical Design*, 16 (17), pp. 1913-1920.

Roberts, A. B. & Wakefield, L. M. (2003) 'The two faces of transforming growth factor β in carcinogenesis'. *Proceedings of the National Academy of Sciences*, 100 (15), pp. 8621-8623.

Roberts, D. D., Kaur, S. & Isenberg, J. S. (2017) 'Regulation of Cellular Redox Signaling by Extracellular Matrix Proteins in Vascular Biology, Immunology, and Cancer'. *Antioxidants & Redox Signaling*, 27(12), pp. 874-911.

Roberts, K. J., Cross, A., Vasieva, O., Moots, R. J. & Edwards, S. W. (2013) 'Inhibition of pre-B cell colony-enhancing factor (PBEF/NAMPT/visfatin) decreases the ability of human neutrophils to generate reactive oxidants but does not impair bacterial killing'. *Journal of Leukocyte Biology*, 94 (3), pp. 481-492.

Roche, Y., Pasquier, D., Rambeaud, J.-J., Seigneurin, D. & Duperray, A. (2003) 'Fibrinogen mediates bladder cancer cell migration in an ICAM-1-dependent pathway'. *Thrombosis and Haemostasis*, 90 (06), pp. 1089-1097.

Rodríguez-Vilarrupla, A., Jaumot, M., Abella, N., Canela, N., Brun, S., Díaz, C., Estanyol, J. M., Bachs, O. & Agell, N. (2005) 'Binding of calmodulin to the carboxy-terminal region of p21 induces nuclear accumulation via inhibition of protein kinase C-mediated phosphorylation of Ser153'. *Molecular and Cellular Biology*, 25(16), pp. 7364-74.

Romaguera, J. E., Fayad, L. E., Feng, L., Hartig, K., Weaver, P., Rodriguez, M. A., Hagemester, F. B., Pro, B., McLaughlin, P., Younes, A., Samaniego, F., Goy, A., Cabanillas, F., Kantarjian, H., Kwak, L. & Wang, M. (2010) 'Ten-year follow-up after intense chemoimmunotherapy with Rituximab-HyperCVAD alternating with Rituximab-high dose methotrexate/cytarabine (R-MA) and without stem cell transplantation in patients with untreated aggressive mantle cell lymphoma'. *British Journal of Haematology*, 150 (2), pp. 200-208.

Romanov, V., Pospelov, V. & Pospelova, T. (2012) 'Cyclin-dependent kinase inhibitor p21Waf1: Contemporary view on its role in senescence and oncogenesis'. *Biochemistry (Moscow)*, 77 (6), pp. 575-584.

Rongvaux, A., Shea, R. J., Mulks, M. H., Gigot, D., Urbain, J., Leo, O. & Andris, F. (2002) 'Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamide phosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis'. *European Journal of Immunology*, 32 (11), pp. 3225-3234.

Roninson, I. B. (2002) 'Oncogenic functions of tumour suppressor p21 Waf1/Cip1/Sdi1: association with cell senescence and tumour-promoting activities of stromal fibroblasts'. *Cancer Letters*, 179 (1), pp. 1-14.

Roodman, G. D. (2014) 'CCN1: a sticky issue in myeloma'. *Blood*, 124 (13), pp. 2006-2008.

Rosati, E., Sabatini, R., De Falco, F., Del Papa, B., Falzetti, F., Di Ianni, M., Cavalli, L., Fettucciari, K., Bartoli, A. & Screpanti, I. (2013) ' γ -Secretase inhibitor I induces apoptosis in chronic lymphocytic leukemia cells by proteasome inhibition, endoplasmic reticulum stress increase and notch down-regulation'. *International Journal of Cancer*, 132 (8), pp. 1940-1953.

Rosati, E., Sabatini, R., Rampino, G., Tabilio, A., Di Ianni, M., Fettucciari, K., Bartoli, A., Coaccioli, S., Screpanti, I. & Marconi, P. (2009) 'Constitutively activated Notch signaling is involved in survival and apoptosis resistance of B-CLL cells'. *Blood*, 113 (4), pp. 856-865.

Rosen, D. G., Yang, G., Cai, K. Q., Bast, R. C., Gershenson, D. M., Silva, E. G. & Liu, J. (2005) 'Subcellular localization of p27kip1 expression predicts poor prognosis in human ovarian cancer'. *Clinical Cancer Research*, 11 (2), pp. 632-637.

Roskoski Jr, R. (2016) 'Ibrutinib inhibition of Bruton protein-tyrosine kinase (BTK) in the treatment of B cell neoplasms'. *Pharmacological Research*, 113 pp. 395-408.

Royo, C., Salaverria, I., Hartmann, E. M., Rosenwald, A., Campo, E. & Beà, S. (2011) 'The complex landscape of genetic alterations in mantle cell lymphoma', *Seminars in Cancer Biology*. Elsevier, pp. 322-334.

Rozovski, U., Keating, M. J. & Estrov, Z. (2018) 'Why Is the Immunoglobulin Heavy Chain Gene Mutation Status a Prognostic Indicator in Chronic Lymphocytic Leukemia? '. *Acta Haematol*, 140(1), pp. 51-54

Ruan, J., Martin, P., Shah, B., Schuster, S. J., Smith, S. M., Furman, R. R., Christos, P., Rodriguez, A., Svoboda, J. & Lewis, J. (2015) 'Lenalidomide plus rituximab as initial treatment for mantle-cell lymphoma'. *New England Journal of Medicine*, 373 (19), pp. 1835-1844.

Rudolph, C., Steinemann, D., Von Neuhoff, N., Gadzicki, D., Ripperger, T., Drexler, H., Mrasek, K., Liehr, T., Claussen, U. & Emura, M. (2004) 'Molecular cytogenetic

characterization of the mantle cell lymphoma cell line GRANTA-519'. *Cancer Genetics and Cytogenetics*, 153 (2), pp. 144-150.

Saad, F., Gleason, D. M., Murray, R., Tchekmedyan, S., Venner, P., Lacombe, L., Chin, J. L., Vinholes, J. J., Goas, J. A. & Zheng, M. (2004) 'Long-term efficacy of zoledronic acid for the prevention of skeletal complications in patients with metastatic hormone-refractory prostate cancer'. *Journal of the National Cancer Institute*, 96 (11), pp. 879-882.

Saglam, O., Dai, F., Husain, S., Zhan, Y., Toruner, G. & Haines, G. K. (2014) 'Matricellular protein CCN1 (CYR61) expression is associated with high-grade ductal carcinoma in situ'. *Human pathology*, 45 (6), pp. 1269-1275.

Sakamoto, S., McCann, R. O., Dhir, R. & Kyprianou, N. (2010) 'Talin1 promotes tumor invasion and metastasis via focal adhesion signaling and anoikis resistance'. *Cancer Research*, pp. 0008-5472. CAN-0009-2833.

Salahshor, S. & Woodgett, J. (2005) 'The links between axin and carcinogenesis'. *Journal of Clinical Pathology*, 58 (3), pp. 225-236.

Salaverria, I., Perez-Galan, P., Colomer, D. & Campo, E. (2006) 'Mantle cell lymphoma: from pathology and molecular pathogenesis to new therapeutic perspectives'. *Haematologica*, 91 (1), pp. 11-16.

Salaverria, I., Royo, C., Carvajal-Cuenca, A., Clot, G., Navarro, A., Valera, A., Song, J. Y., Woroniecka, R., Rymkiewicz, G., Klapper, W., Hartmann, E. M., Sujobert, P., Wlodarska, I., Ferry, J. A., Gaulard, P., Ott, G., Rosenwald, A., Lopez-Guillermo, A., Quintanilla-Martinez, L., Harris, N. L., Jaffe, E. S., Siebert, R., Campo, E. & Beà, S. (2013) 'CCND2 rearrangements are the most frequent genetic events in cyclin D1(-) mantle cell lymphoma'. *Blood*, 121(8), pp. 1394-402.

Salaverria, I., Zettl, A., Beà, S., Moreno, V., Valls, J., Hartmann, E., Ott, G., Wright, G., Lopez-Guillermo, A. & Chan, W. C. (2007) 'Specific secondary genetic alterations in mantle cell lymphoma provide prognostic information independent of the gene expression-based proliferation signature'. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*, 25 (10), pp. 1216.

Sánchez-Duffhues, G., Hiepen, C., Knaus, P. & ten Dijke, P. (2015) 'Bone morphogenetic protein signaling in bone homeostasis'. *Bone*, 80 pp. 43-59.

Sánchez-Serrano, I. (2006) 'Success in translational research: lessons from the development of bortezomib'. *Nature Reviews Drug Discovery*, 5 (2), pp. 107.

Sander, B., Quintanilla-Martinez, L., Ott, G., Xerri, L., Kuzu, I., Chan, J. K., Swerdlow, S. H. & Campo, E. (2016) 'Mantle cell lymphoma—a spectrum from indolent to aggressive disease'. *Virchows Archiv*, 468 (3), pp. 245-257.

Santibañez, J. F., Quintanilla, M. & Bernabeu, C. (2011) 'TGF- β /TGF- β receptor system and its role in physiological and pathological conditions'. *Clinical Science*, 121 (6), pp. 233-251.

- Sartori, R. & Sandri, M. (2015) 'BMPs and the muscle–bone connection'. *Bone*, 80 pp. 37-42.
- Savaryn, J. P., Toby, T. K. & Kelleher, N. L. (2016) 'A researcher's guide to mass spectrometry-based proteomics'. *Proteomics*, 16 (18), pp. 2435-2443.
- Sawai, K., Mukoyama, M., Mori, K., Kasahara, M., Koshikawa, M., Yokoi, H., Yoshioka, T., Ogawa, Y., Sugawara, A. & Nishiyama, H. (2007) 'Expression of CCN1 (CYR61) in developing, normal, and diseased human kidney'. *American Journal of Physiology-Renal Physiology*, 293 (4), pp. F1363-F1372.
- Schieber, M., Gordon, L. I. & Karmali, R. (2018) 'Current overview and treatment of mantle cell lymphoma'. *F1000Res*, 25, pp. 1136.
- Schirle, M., Bantscheff, M. & Kuster, B. (2012) 'Mass spectrometry-based proteomics in preclinical drug discovery'. *Chemistry and Biology*, 19 (1), pp. 72-84.
- Schulz, H., Bohlius, J. F., Trelle, S., Skoetz, N., Reiser, M., Kober, T., Schwarzer, G., Herold, M., Dreyling, M. & Hallek, M. (2007) 'Immunochemotherapy with rituximab and overall survival in patients with indolent or mantle cell lymphoma: a systematic review and meta-analysis'. *Journal of the National Cancer Institute*, 99 (9), pp. 706-714.
- Schulz, R. & Moll, U. M. (2014) 'Targeting the heat shock protein 90: a rational way to inhibit macrophage migration inhibitory factor function in cancer'. *Current Opinion in Oncology*, 26 (1), pp. 108-113.
- Segarini, P. R., Nesbitt, J. E., Li, D., Hays, L. G., Yates, J. R. & Carmichael, D. F. (2001) 'The low density lipoprotein receptor-related protein/ α 2-macroglobulin receptor is a receptor for connective tissue growth factor'. *Journal of Biological Chemistry*, 276 (44), pp. 40659-40667.
- Seok, Y., Kim, J., Choi, J. R., Kim, Y. R., Park, S.-J., Kim, S. J., Song, J. & Lee, K.-A. (2012) 'CD5-negative blastoid variant mantle cell lymphoma with complex CCND1/IGH and MYC aberrations'. *Annals of Laboratory Medicine*, 32 (1), pp. 95-98.
- Seto, M. (2013) 'Cyclin D1-negative mantle cell lymphoma'. *Blood*, 121 (8), pp. 1249-1250.
- Setoodeh, R., Schwartz, S., Papenhausen, P., Zhang, L., Sagatys, E. M., Moscinski, L. C. & Shao, H. (2013) 'Double-hit mantle cell lymphoma with MYC gene rearrangement or amplification: a report of four cases and review of the literature'. *International Journal of Clinical and Experimental Pathology*, 6 (2), pp. 155.
- Shackelford, R., Hirsh, S., Henry, K., Abdel-Mageed, A., Kandil, E. & Coppola, D. (2013) 'Nicotinamide phosphoribosyltransferase and SIRT3 expression are increased in well-differentiated thyroid carcinomas'. *Anticancer Research*, 33 (8), pp. 3047-3052.
- Shackelford, R. E., Bui, M. M., Coppola, D. & Hakam, A. (2010) 'Over-expression of nicotinamide phosphoribosyltransferase in ovarian cancers'. *International Journal of Clinical and Experimental Pathology*, 3 (5), pp. 522.
- Shapira, M. a., Ben-Izhak, O., Linn, S., Futerman, B., Minkov, I. & Hershko, D. D. (2005) 'The prognostic impact of the ubiquitin ligase subunits Skp2 and Cks1 in colorectal carcinoma'. *Cancer*, 103 (7), pp. 1336-1346.

Sharma, G., Sharma, A. R., Seo, E.-M. & Nam, J.-S. (2015) 'Genetic polymorphism in extracellular regulators of Wnt signaling pathway'. *BioMed research international*, 2015

Sharma, S. & Brosh Jr, R. M. (2007) 'Human RECQ1 is a DNA damage responsive protein required for genotoxic stress resistance and suppression of sister chromatid exchanges'. *PloS One*, 2 (12), pp. e1297.

Sharma, S., Kelly, T. K. & Jones, P. A. (2010) 'Epigenetics in cancer'. *Carcinogenesis*, 31 (1), pp. 27-36.

Sharma, S., Stumpo, D. J., Balajee, A. S., Bock, C. B., Lansdorp, P. M., Brosh, R. M. & Blackshear, P. J. (2007) 'RECQL, a member of the RecQ family of DNA helicases, suppresses chromosomal instability'. *Molecular and Cellular Biology*, 27 (5), pp. 1784-1794.

Sharma, S. & Sweetenham, J. W. (2018) 'Mantle Cell Lymphoma: Are New Therapies Changing the Standard of Care?'. *Oncology - European Medical Journal*, 6(1), pp. 109-119.

Sheaff, R. J., Groudine, M., Gordon, M., Roberts, J. M. & Clurman, B. E. (1997) 'Cyclin E-CDK2 is a regulator of p27Kip1'. *Genes and Development*, 11 (11), pp. 1464-1478.

Sherr, C. J. & Roberts, J. M. (1999) 'CDK inhibitors: positive and negative regulators of G1-phase progression'. *Genes and Development*, 13 (12), pp. 1501-1512.

Shin, J. Y., Kim, H. S., Lee, K. S., Kim, J., Park, J. B., Won, M. H., Chae, S. W., Choi, Y. H., Choi, K. C., Park, Y. E. & Lee, J. Y. (2000) 'Mutation and expression of the p27KIP1 and p57KIP2 genes in human gastric cancer'. *Experimental & Molecular Medicine*, 32, pp. 79–83.

Shuda, M., Kondoh, N., Tanaka, K., Ryo, A., Wakatsuki, T., Hada, A., Goseki, N., Igari, T., Hatsuse, K. & Aihara, T. (2000) 'Enhanced expression of translation factor mRNAs in hepatocellular carcinoma'. *Anticancer Research*, 20 (4), pp. 2489-2494.

Si, W., Kang, Q., Luu, H. H., Park, J. K., Luo, Q., Song, W.-X., Jiang, W., Luo, X., Li, X. & Yin, H. (2006) 'CCN1/Cyr61 is regulated by the canonical Wnt signal and plays an important role in Wnt3A-induced osteoblast differentiation of mesenchymal stem cells'. *Molecular and Cellular Biology*, 26 (8), pp. 2955-2964.

Si, X.-h., Feng, Z.-j. & Yang, L.-j. (2010) 'Bone morphogenetic proteins and prostate carcinoma'. *Chinese Journal of Cancer Biotherapy*, 5 pp. 028.

Sivina, M., Kreitman, R. J., Arons, E., Buggy, J. J., Ravandi, F. & Burger, J. A. (2012) 'Bruton's tyrosine kinase (BTK) inhibitor ibrutinib (PCI-32765) blocks hairy cell leukemia (HCL) survival, proliferation, and BCR signaling: A new therapeutic approach for HCL', *ASH Annual Meeting Abstracts*. pp. 1802.

Smith, M. R. (2011) 'Should there be a standard therapy for mantle cell lymphoma?'. *Future Oncology*, 7 (2), pp. 227-237.

Srinivas, P. R., Srivastava, S., Hanash, S. & Wright, G. L. (2001) 'Proteomics in early detection of cancer'. *Clinical Chemistry*, 47 (10), pp. 1901-1911.

Staal, F. J., Chhatta, A. & Mikkers, H. (2016) 'Caught in a Wnt storm: Complexities of Wnt signaling in hematopoiesis'. *Experimental Hematology*, 44 (6), pp. 451-457.

Stahl, M., Dijkers, P. F., Kops, G. J., Lens, S. M., Coffey, P. J., Burgering, B. M. & Medema, R. H. (2002) 'The forkhead transcription factor FoxO regulates transcription of p27Kip1 and Bim in response to IL-2'. *The Journal of Immunology*, 168 (10), pp. 5024-5031.

Stephens, S., Palmer, J., Konstantinova, I., Pearce, A., Jarai, G. & Day, E. (2015) 'A functional analysis of Wnt inducible signalling pathway protein- 1 (WISP-1/CCN4)'. *Journal of cell communication and signaling*, 9 (1), pp. 63-72.

Streich Jr, F. C. & Lima, C. D. (2014) 'Structural and functional insights to ubiquitin-like protein conjugation'. *Annual review of biophysics*, 43 pp. 357.

Sui, L., Dong, Y., Ohno, M., Watanabe, Y., Sugimoto, K. & Tokuda, M. (2002) 'Expression of p57kip2 and its clinical relevance in epithelial ovarian tumors'. *Anticancer Research*, 22, pp. 3191-3196.

Sun, X.-X., DeVine, T., Challagundla, K. B. & Dai, M.-S. (2011) 'Interplay between ribosomal protein S27a and MDM2 in p53 activation in response to ribosomal stress'. *Journal of Biological Chemistry*, pp. jbc. M111. 223651.

Sun, Y., Zhang, J., Zhai, T., Li, H., Li, H., Huo, R., Shen, B., Wang, B., Chen, X. & Li, N. (2017) 'CCN1 promotes IL-1 β production in keratinocytes by activating p38 MAPK signaling in psoriasis'. *Scientific Reports*, 7 pp. 43310.

Sun, Y., Zhang, J., Zhou, Z., Wu, P., Huo, R., Wang, B., Shen, Z., Li, H., Zhai, T. & Shen, B. (2015) 'CCN1, a pro-inflammatory factor, aggravates psoriasis skin lesions by promoting keratinocyte activation'. *Journal of Investigative Dermatology*, 135 (11), pp. 2666-2675.

Sun, Z., Lei, H. & Zhang, Z. (2013) 'Pre-B cell colony enhancing factor (PBEF), a cytokine with multiple physiological functions'. *Cytokine and Growth Factor Reviews*, 24 (5), pp. 433-442.

Sun, Z., Wang, Y., Cai, Z., Chen, P., Tong, X. & Xie, D. (2008) 'Involvement of Cyr61 in growth, migration, and metastasis of prostate cancer cells'. *British Journal of Cancer*, 99 (10), pp. 1656-1667.

Suresh, S. & Irvine, A. E. (2015) 'The NOTCH signaling pathway in normal and malignant blood cell production'. *Journal of cell communication and signaling*, 9 (1), pp. 5-13.

Suresh, S., McCallum, L., Crawford, L. J., Lu, W. H., Sharpe, D. J. & Irvine, A. E. (2013) 'The matricellular protein CCN3 regulates NOTCH1 signalling in chronic myeloid leukaemia'. *The Journal of pathology*, 231 (3), pp. 378-387.

Suzuki, A., Tsutomi, Y., Yamamoto, N., Shibutani, T. & Akahane, K. (1999) 'Mitochondrial regulation of cell death: mitochondria are essential for procaspase 3-p21 complex formation to resist Fas-mediated cell death'. *Molecular and Cellular Biology*, 19 (5), pp. 3842-7.

Swerdlow, S. H., Campo, E., Pileri, S. A., Harris, N. L., Stein, H., Siebert, R., Advani, R., Ghielmini, M., Salles, G. A., Zelenetz, A. D. & Jaffe, E. S. (2016) 'The 2016 revision of the World Health Organization classification of lymphoid neoplasms'. *Blood*, 127(20), pp. 2375-90

Tamura, I., Rosenbloom, J., Macarak, E. & Chaqour, B. (2001) 'Regulation of Cyr61 gene expression by mechanical stretch through multiple signaling pathways'. *American Journal of Physiology-Cell Physiology*, 281 (5), pp. C1524-C1532.

Taulés, M., Rius, E., Talaya, D., López-Girona, A., Bachs, O. & Agell, N. (1998) 'Calmodulin is essential for cyclin-dependent kinase 4 (Cdk4) activity and nuclear accumulation of cyclin D1-Cdk4 during G1'. *Journal of Biological Chemistry*, 273 (50), pp. 33279-33286.

Taulés, M., Rodríguez-Vilarrupla, A., Rius, E., Estanyol, J. M., Casanovas, O., Sacks, D. B., Pérez-Payá, E., Bachs, O. & Agell, N. (1999) 'Calmodulin binds to p21(Cip1) and is involved in the regulation of its nuclear localization'. *Journal of Biological Chemistry*, 274(35), pp. 24445-8.

Thompson, K., Murphy-Marshman, H. & Leask, A. (2014) 'ALK5 inhibition blocks TGF β -induced CCN1 expression in human foreskin fibroblasts'. *Journal of cell communication and signaling*, 8 (1), pp. 59-63.

Thulasiraman, V., Lin, S., Gheorghiu, L., Lathrop, J., Lomas, L., Hammond, D. & Boschetti, E. (2005) 'Reduction of the concentration difference of proteins in biological liquids using a library of combinatorial ligands'. *Electrophoresis*, 26 (18), pp. 3561-3571.

Tian, Y.-F., Chen, T.-J., Lin, C.-Y., Chen, L.-T., Lin, L.-C., Hsing, C.-H., Lee, S.-W., Sheu, M.-J., Lee, H.-H. & Shiue, Y.-L. (2013) 'SKP2 overexpression is associated with a poor prognosis of rectal cancer treated with chemoradiotherapy and represents a therapeutic target with high potential'. *Tumor Biology*, 34 (2), pp. 1107-1117.

Tiemann, M., Schrader, C., Klapper, W., Dreyling, M. H., Campo, E., Norton, A., Berger, F., Kluin, P., Ott, G. & Pileri, S. (2005) 'Histopathology, cell proliferation indices and clinical outcome in 304 patients with mantle cell lymphoma (MCL): a clinicopathological study from the European MCL Network'. *British Journal of Haematology*, 131 (1), pp. 29-38.

Timmerbeul, I., Garrett-Engele, C. M., Kossatz, U., Chen, X., Firpo, E., Grünwald, V., Kamino, K., Wilkens, L., Lehmann, U. & Buer, J. (2006) 'Testing the importance of p27 degradation by the SCFskp2 pathway in murine models of lung and colon cancer'. *Proceedings of the National Academy of Sciences*, 103 (38), pp. 14009-14014.

Todorovic, V., Chen, C.-C., Hay, N. & Lau, L. F. (2005) 'The matrix protein CCN1 (CYR61) induces apoptosis in fibroblasts'. *The Journal of cell biology*, 171 (3), pp. 559-568.

Tong, X., O'Kelly, J., Xie, D., Mori, A., Lemp, N., McKenna, R., Miller, C. W. & Koeffler, H. P. (2004) 'Cyr61 suppresses the growth of non-small-cell lung cancer cells via the β -catenin-c-myc-p53 pathway'. *Oncogene*, 23 (28), pp. 4847-4855.

Tong, X., Xie, D., O'Kelly, J., Miller, C. W., Muller-Tidow, C. & Koeffler, H. P. (2001) 'Cyr61, a member of CCN family, is a tumor suppressor in non-small cell lung cancer'. *Journal of Biological Chemistry*, 276 (50), pp. 47709-47714.

Toyoshima, H. & Hunter, T. (1994) 'p27, a novel inhibitor of G1 cyclin-Cdk protein kinase activity, is related to p21'. *Cell*, 78 (1), pp. 67-74.

Tsofack, S. P., Meunier, L., Sanchez, L., Madore, J., Provencher, D., Mes-Masson, A.-M. & Lebel, M. (2013) 'Low expression of the X-linked ribosomal protein S4 in human serous epithelial ovarian cancer is associated with a poor prognosis'. *BMC Cancer*, 13 (1), pp. 303.

Tucker, C. A., Bebb, G., Klasa, R. J., Chhanabhai, M., Lestou, V., Horsman, D. E., Gascoyne, R. D., Wiestner, A., Masin, D. & Bally, M. (2006) 'Four human t (11; 14)(q13; q32)-containing cell lines having classic and variant features of mantle cell lymphoma'. *Leukemia Research*, 30 (4), pp. 449-457.

Tucker, D. L. & Rule, S. A. (2015) 'A critical appraisal of ibrutinib in the treatment of mantle cell lymphoma and chronic lymphocytic leukemia'. *Therapeutics and Clinical Risk Management*, 11 pp. 979.

van Beijnum, J. R., Moerkerk, P. T., Gerbers, A. J., de Bruijne, A. P., Arends, J. W., Hoogenboom, H. R. & Hufton, S. E. (2002) 'Target validation for genomics using peptide-specific phage antibodies: A study of five gene products overexpressed in colorectal cancer'. *International Journal of Cancer*, 101 (2), pp. 118-127.

Vargas, J. E., Chicaybam, L., Stein, R. T., Tanuri, A., Delgado-Cañedo, A. & Bonamino, M. H. (2016) 'Retroviral vectors and transposons for stable gene therapy: advances, current challenges and perspectives'. *Journal of Translational Medicine*, 14 (1), pp. 288.

Venkateshaiah, S. U., Khan, S., Ling, W., Bam, R., Li, X., van Rhee, F., Usmani, S., Barlogie, B., Epstein, J. & Yaccoby, S. (2013) 'NAMPT/PBEF1 enzymatic activity is indispensable for myeloma cell growth and osteoclast activity'. *Experimental Hematology*, 41 (6), pp. 547-557. e542.

Vidal, A. & Koff, A. (2000) 'Cell-cycle inhibitors: three families united by a common cause'. *Gene*, 247, pp. 1-15.

Vinson, C. R., Conover, S. & Adler, P. N. (1989) 'A Drosophila tissue polarity locus encodes a protein containing seven potential transmembrane domains'. *Nature*, 338 (6212), pp. 263.

Vlachos, P., Nyman, U., Hajji, N. & Joseph, B. (2007) 'The cell cycle inhibitor p57(Kip2) promotes cell death via the mitochondrial apoptotic pathway'. *Cell Death & Differentiation - Nature*, 14(8), pp. 1497-507.

Vorburger, S. A. & Hunt, K. K. (2002) 'Adenoviral gene therapy'. *The oncologist*, 7 (1), pp. 46-59.

Vose, J. M. (2015) 'Mantle cell lymphoma: 2015 update on diagnosis, risk-stratification, and clinical management'. *American Journal of Hematology*, 90 (8), pp. 739-745.

Waga, S., Hannon, G. J., Beach, D. & Stillman, B. (1994) 'The p21 inhibitor of cyclin-dependent kinases controls DNA replication by interaction with PCNA'. *Nature*, 369 (6481), pp. 574-578.

Wagner, D. O., Sieber, C., Bhushan, R., Börgermann, J. H., Graf, D. & Knaus, P. (2010) 'BMPs: from bone to body morphogenetic proteins'. *Science Signaling*, 3 (107), pp. mr1.

Wang, B., Hasan, M., Alvarado, E., Yuan, H., Wu, H. & Chen, W. (2011) 'NAMPT overexpression in prostate cancer and its contribution to tumor cell survival and stress response'. *Oncogene*, 30 (8), pp. 907.

Wang, M., Fayad, L., Wagner-Bartak, N., Zhang, L., Hagemeister, F., Neelapu, S. S., Samaniego, F., McLaughlin, P., Fanale, M. & Younes, A. (2012) 'Lenalidomide in combination with rituximab for patients with relapsed or refractory mantle-cell lymphoma: a phase 1/2 clinical trial'. *The Lancet Oncology*, 13 (7), pp. 716-723.

Wang, M., Martin, P., Phillips, T., Goy, A., Lossos, I. S., Rule, S. A., Hamadani, M., Ghosh, N., Reeder, C. B. & Barnett, E. (2016a) 'Effectiveness of lenalidomide in patients with mantle cell lymphoma who relapsed/progressed after or were refractory/intolerant to ibrutinib: the MCL-004 study'. *Journal of Hematology & Oncology*, 10 (1), pp. 171.

Wang, M. L., Lee, H., Chuang, H., Wagner-Bartak, N., Hagemeister, F., Westin, J., Fayad, L., Samaniego, F., Turturro, F. & Oki, Y. (2016b) 'Ibrutinib in combination with rituximab in relapsed or refractory mantle cell lymphoma: a single-centre, open-label, phase 2 trial'. *The Lancet Oncology*, 17 (1), pp. 48-56.

Wang, X. L., Yu, L., Ding, Y., Guo, X. R., Yuan, Y. H. & Li, D. S. (2015) 'Gene Manipulation of Human Embryonic Stem Cells by In Vitro-Synthesized mRNA for Gene Therapy'. *Current Gene Therapy*, 15(4), pp. 428-35.

Wang, X. Q., Lui, E. L. H., Cai, Q., Ching, W. Y. P., Liu, K. S. Y., Poon, R. T. P. & Fan, S. T. (2008) 'p27Kip1 promotes migration of metastatic hepatocellular carcinoma cells'. *Tumor Biology*, 29 (4), pp. 217-223.

Wang, Y. & Ma, S. (2014) 'Racial differences in mantle cell lymphoma in the United States'. *BioMed Central Cancer*, 15(14), pp. 764.

Watabe, T. & Miyazono, K. (2009) 'Roles of TGF- β family signaling in stem cell renewal and differentiation'. *Cell Research*, 19 (1), pp. 103.

Wehmer, M. & Sakata, E. (2016) 'Recent advances in the structural biology of the 26S proteasome'. *The international journal of biochemistry & cell biology*, 79 pp. 437-442.

Wei, C.-Y., Tan, Q.-X., Zhu, X., Qin, Q.-H., Zhu, F.-B., Mo, Q.-G. & Yang, W.-P. (2015) 'Expression of CDKN1A/p21 and TGFBR2 in breast cancer and their prognostic significance'. *International Journal of Clinical and Experimental Pathology*, 8 (11), pp. 14619.

Wei, J., Yu, G., Shao, G., Sun, A., Chen, M., Yang, W. & Lin, Q. (2016) 'CYR61 (CCN1) is a metastatic biomarker of gastric cardia adenocarcinoma'. *Oncotarget*, 7(21), pp. 31067-78.

Weinstein, S., Emmanuel, R., Jacobi, A. M., Abraham, A., Behlke, M. A., Sprague, A. G., Novobrantseva, T. I., Nagler, A. & Peer, D. (2012) 'RNA inhibition highlights cyclin D1 as a potential therapeutic target for mantle cell lymphoma'. *PLoS One*, 7(8), pp. e43343.

Weisenburger, D. D., Vose, J. M., Greiner, T. C., Lynch, J. C., Chan, W. C., Bierman, P. J., Dave, B. J., Sanger, W. G. & Armitage, J. O. (2000) 'Mantle cell lymphoma. A clinicopathologic study of 68 cases from the Nebraska Lymphoma Study Group'. *American Journal of Hematology*, 64 (3), pp. 190-196.

Wells, J., Howlett, M., Cheung, L. & Kees, U. R. (2015) 'The role of CCN family genes in haematological malignancies'. *Journal of cell communication and signaling*, 9 (3), pp. 267-278.

Westin, J. R., Thompson, M. A., Cataldo, V. D., Toth, B. B., Sanjorjo, P., Bourgeois, S., Jimenez, C., Murphy, W. A., Fanale, M. & Fayad, L. (2010) 'Bone Loss in Lymphoma Patients Prior to Receiving Front-line Therapy'. *Clinical Lymphoma, Myeloma and Leukemia*, 10 (3), pp. E32.

Willert, K., Brown, J. D., Danenberg, E., Duncan, A. W., Weissman, I. L., Reya, T., Yates III, J. R. & Nusse, R. (2003) 'Wnt proteins are lipid-modified and can act as stem cell growth factors'. *Nature*, 423 (6938), pp. 448.

Williamson, E. A., Dadmanesh, F. & Koeffler, H. P. (2002) 'BRCA1 transactivates the cyclin-dependent kinase inhibitor p27 (Kip1)'. *Oncogene*, 21 (20), pp. 3199-3206.

Woyach, J. A., Furman, R. R., Liu, T.-M., Ozer, H. G., Zapatka, M., Ruppert, A. S., Xue, L., Li, D. H.-H., Steggerda, S. M. & Versele, M. (2014) 'Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib'. *New England Journal of Medicine*, 370 (24), pp. 2286-2294.

Wu, W., Hu, W. & Kavanagh, J. (2002) 'Proteomics in cancer research'. *International Journal of Gynecological Cancer*, 12 (5), pp. 409-423.

Wu, Y. & Brosh, R. M. (2010) 'Distinct roles of RECQ1 in the maintenance of genomic stability'. *DNA repair*, 9 (3), pp. 315-324.

Xie, D., Yin, D., Tong, X., O'Kelly, J., Mori, A., Miller, C., Black, K., Gui, D., Said, J. W. & Koeffler, H. P. (2004a) 'Cyr61 is overexpressed in gliomas and involved in integrin-linked kinase-mediated Akt and β -catenin-TCF/Lef signaling pathways'. *Cancer research*, 64 (6), pp. 1987-1996.

Xie, D., Yin, D., Wang, H.-J., Liu, G.-T., Elashoff, R., Black, K. & Koeffler, H. P. (2004b) 'Levels of expression of CYR61 and CTGF are prognostic for tumor progression and survival of individuals with gliomas'. *Clinical Cancer Research*, 10 (6), pp. 2072-2081.

Xu, H., Xu, Y., Ouyang, T., Li, J., Wang, T., Fan, Z., Fan, T., Lin, B. & Xie, Y. (2018) 'Low expression of RECQL is associated with poor prognosis in Chinese breast cancer patients'. *BMC Cancer*, 18 (1), pp. 662.

Xu, N., Chen, H.-J., Chen, S.-H., Xue, X.-Y., Chen, H., Zheng, Q.-S., Wei, Y., Li, X.-D., Huang, J.-B. & Cai, H. (2016) 'Upregulation of Talin-1 expression associates with advanced pathological features and predicts lymph node metastases and biochemical recurrence of prostate cancer'. *Medicine*, 95 (29),

Xu, Y.-F., Ren, X.-Y., Li, Y.-Q., He, Q.-M., Tang, X.-R., Sun, Y., Shao, J.-Y., Jia, W.-H., Kang, T.-B. & Zeng, M.-S. (2015) 'High expression of Talin-1 is associated with poor prognosis in patients with nasopharyngeal carcinoma'. *BMC Cancer*, 15 (1), pp. 332.

Yakubenko, V. P., Yadav, S. P. & Ugarova, T. P. (2006) 'Integrin α D β 2, an adhesion receptor up-regulated on macrophage foam cells, exhibits multiligand-binding properties'. *Blood*, 107 (4), pp. 1643-1650.

Yang, J., Shi, P., Tu, M., Wang, Y., Liu, M., Fan, F. & Du, M. (2014) 'Bone morphogenetic proteins: relationship between molecular structure and their osteogenic activity'. *Food Science and Human Wellness*, 3 (3-4), pp. 127-135.

Yang, W., Shen, J., Wu, M., Arsur, M., FitzGerald, M., Suldan, Z., Kim, D. W., Hofmann, C. S., Pianetti, S. & Romieu-Mourez, R. (2001) 'Repression of transcription of the p 27 Kip 1 cyclin-dependent kinase inhibitor gene by c-Myc'. *Oncogene*, 20 (14), pp. 1688-1702.

Yatabe, Y., Suzuki, R., Tobinai, K., Matsuno, Y., Ichinohasama, R., Okamoto, M., Yamaguchi, M., Tamaru, J.-i., Uike, N. & Hashimoto, Y. (2000) 'Significance of cyclin D1 overexpression for the diagnosis of mantle cell lymphoma: a clinicopathologic comparison of cyclin D1-positive MCL and cyclin D1-negative MCL-like B-cell lymphoma'. *Blood*, 95 (7), pp. 2253-2261.

Yokoo, T., Toyoshima, H., Miura, M., Wang, Y., Iida, K. T., Suzuki, H., Sone, H., Shimano, H., Gotoda, T., Nishimori, S., Tanaka, K. & Yamada, N. (2003) 'p57Kip2 regulates actin dynamics by binding and translocating LIM-kinase 1 to the nucleus'. *Journal of Biological Chemistry*, 278, pp. 52919-23.

Young, R. M. & Staudt, L. M. (2014) 'Ibrutinib treatment of CLL: the cancer fights back'. *Cancer Cell*, 26 (1), pp. 11-13.

Yuan, X., Wu, H., Xu, H., Xiong, H., Chu, Q., Yu, S., Wu, G. S. & Wu, K. (2015) 'Notch signaling: an emerging therapeutic target for cancer treatment'. *Cancer Letters*, 369 (1), pp. 20-27.

Yun, J., Duan, Q., Wang, L., Lv, W., Gong, Z., Yang, F. & Song, Y. (2014) 'Differential expression of leukocyte β 2 integrin signal transduction-associated genes in patients with symptomatic pulmonary embolism'. *Molecular Medicine Reports*, 9 (1), pp. 285-292.

Zeng, W., Fu, K., Quintanilla-Fend, L., Lim, M., Ondrejka, S. & Hsi, E. D. (2012) 'Cyclin D1-negative blastoid mantle cell lymphoma identified by SOX11 expression'. *The American journal of surgical pathology*, 36 (2), pp. 214-219.

Zetterberg, A., Larsson, O. & Wiman, K. G. (1995) 'What is the restriction point?'. *Current Opinion in Cell Biology*, 7 (6), pp. 835-842.

Zhang, H., Chen, S. & Huang, L. (2012) 'Proteomics-based Identification of Proapoptotic Caspase Adapter Protein as A Novel Serum Marker of Non-small Cell Lung Cancer'. *Chinese Journal of Lung Cancer*, 15 (5),

Zhang, J. & Crumpacker, C. (2015) 'Hematopoietic stem and immune cells in chronic HIV infection'. *Stem cells international*, 2015: 148064.

Zhang, L., Qian, Z., Cai, Z., Sun, L., Wang, H., Bartlett, J. B., Yi, Q. & Wang, M. (2009) 'Synergistic antitumor effects of lenalidomide and rituximab on mantle cell lymphoma in vitro and in vivo'. *American Journal of Hematology*, 84 (9), pp. 553-559.

Zhang, L., Schafer, P., Muller, G., Stirling, D. & Bartlett, B. (2008a) 'The ratio of cyclin D1/p21kip baseline gene expression and SPARC gene expression can be potential predictors of non-Hodgkin's lymphoma (NHL) patient response to lenalidomide therapy'. *Journal of Clinical Oncology*, 26 (15_suppl), pp. 22150-22150.

Zhang, Q., Wu, J., Cao, Q., Xiao, L., Wang, L., He, D., Ouyang, G., Lin, J., Shen, B. & Shi, Y. (2009) 'A critical role of Cyr61 in interleukin-17-dependent proliferation of fibroblast-like synoviocytes in rheumatoid arthritis'. *Arthritis & Rheumatism: Official Journal of the American College of Rheumatology*, 60 (12), pp. 3602-3612.

Zhang, W., Mao, Y.-q., Wang, H., Yin, W.-j., Zhu, S.-x. & Wang, W.-c. (2015) 'MiR-124 suppresses cell motility and adhesion by targeting talin 1 in prostate cancer cells'. *Cancer Cell International*, 15 (1), pp. 49.

Zhang, X.-B., Beard, B. C., Trobridge, G. D., Wood, B. L., Sale, G. E., Sud, R., Humphries, R. K. & Kiem, H.-P. (2008b) 'High incidence of leukemia in large animals after stem cell gene therapy with a HOXB4-expressing retroviral vector'. *The Journal of clinical investigation*, 118 (4), pp. 1502-1510.

Zhang, Y. & Lu, H. (2009) 'Signaling to p53: ribosomal proteins find their way'. *Cancer Cell*, 16 (5), pp. 369-377.

Zhang, Y., Wang, J., Yuan, Y., Zhang, W., Guan, W., Wu, Z., Jin, C., Chen, H., Zhang, L. & Yang, X. (2010) 'Negative regulation of HDM2 to attenuate p53 degradation by ribosomal protein L26'. *Nucleic Acids Research*, 38 (19), pp. 6544-6554.

Zhang, Y. & Yan, B. (2012) 'Cell cycle regulation by carboxylated multiwalled carbon nanotubes through p53-independent induction of p21 under the control of the BMP signaling pathway'. *Chemical research in toxicology*, 25 (6), pp. 1212-1221.

Zhao, H., Faltermeier, C. M., Mendelsohn, L., Porter, P. L., Clurman, B. E. & Roberts, J. M. (2014) 'Mislocalization of p27 to the cytoplasm of breast cancer cells confers resistance to anti-HER2 targeted therapy'. *Oncotarget*, 5 (24), pp. 12704.

Zhao, L. L., Liu, Y. F., Peng, L. J., Fei, A. M., Cui, W., Miao, S. C., Hermine, O., Gressin, R., Khochbin, S. & Chen, S. J. (2015) 'Arsenic trioxide rewires mantle cell lymphoma response to bortezomib'. *Cancer medicine*, 4 (11), pp. 1754-1766.

Zhou, D. M., Chen, G., Zheng, X. W., Zhu, W. F. & Chen, B. Z. (2015) 'Clinicopathologic features of 112 cases with mantle cell lymphoma'. *Cancer Biology & Medicine*, 12:46-52.

- Zhou, X., Hao, Q., Liao, J.-m., Zhang, Q. & Lu, H. (2013) 'Ribosomal protein S14 unties the MDM2-p53 loop upon ribosomal stress'. *Oncogene*, 32 (3), pp. 388.
- Zhou, X., Liao, J.-M., Liao, W.-J. & Lu, H. (2012) 'Scission of the p53-MDM2 loop by ribosomal proteins'. *Genes & Cancer*, 3 (3-4), pp. 298-310.
- Zhou, X., Liao, W.-J., Liao, J.-M., Liao, P. & Lu, H. (2015) 'Ribosomal proteins: functions beyond the ribosome'. *Journal of Molecular Cell Biology*, 7 (2), pp. 92-104.
- Zhou, Y., Wang, H., Fang, W., Romaguer, J. E, Zhang, Y., Delasalle, K. B., Kwak, L., Yi, Q., Du, X. L. & Wang, M. (2008) 'Incidence trends of mantle cell lymphoma in the United States between 1992 and 2004'. *Cancer*, 113 (4), pp. 791-798.
- Zhu, M., Zhang, H. & Humphreys, W. G. (2011) 'Drug metabolite profiling and identification by high resolution mass spectrometry'. *Journal of Biological Chemistry*, pp. jbc. R110. 200055.
- Zhu, X., Song, Y., Wu, C., Pan, C., Lu, P., Wang, M., Zheng, P., Huo, R., Zhang, C., Li, W., Lin, Y., Cao, Y. & Li, N. (2016) 'Cyr61 participates in the pathogenesis of acute lymphoblastic leukemia by enhancing cellular survival via the AKT/NF- κ B signaling pathway'. *Scientific Reports*, 6: 34018.
- Zhu, Y., Luo, G., Jiang, B., Yu, M., Feng, Y., Wang, M., Xu, N. & Zhang, X. (2018) 'Apolipoprotein M promotes proliferation and invasion in non-small cell lung cancers via upregulating S1PR1 and activating the ERK1/2 and PI3K/AKT signaling pathways'. *Biochemical and Biophysical Research Communications*, 501 (2), pp. 520-526.
- Zimmerman, T. & Blanco, F. J. (2008) 'Inhibitors targeting the LFA-1/ICAM-1 cell-adhesion interaction: design and mechanism of action'. *Current Pharmaceutical Design*, 14 (22), pp. 2128-2139.
- Zirbes, T. K., Baldus, S. E., Moenig, S. P., Nolden, S., Kunze, D., Shafizadeh, S. T., Schneider, P. M., Thiele, J., Hoelscher, A. H. & Dienes, H. P. (2000) 'Prognostic impact of p21/waf1/cip1 in colorectal cancer'. *International Journal of Cancer*, 89 (1), pp. 14-18.
- Zou, P., Yoshihara, H., Hosokawa, K., Tai, I., Shinmyozu, K., Tsukahara, F., Maru, Y., Nakayama, K., Nakayama, K. I. & Suda, T. (2011) 'p57(Kip2) and p27(Kip1) cooperate to maintain hematopoietic stem cell quiescence through interactions with Hsc70'. *Cell Stem Cell*, 9, pp. 247-261.
- Zucca, E. & Bertoni, F. (2013) 'Toward new treatments for mantle-cell lymphoma?'. *N Engl J Med*, 369 (6), pp. 571-572.
- Zufferey, R., Dull, T., Mandel, R. J., Bukovsky, A., Quiroz, D., Naldini, L. & Trono, D. (1998) 'Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery'. *Journal of Virology*, 72 (12), pp. 9873-9880.
- Zuo, G.-W., Kohls, C. D., He, B.-C., Chen, L., Zhang, W., Shi, Q., Zhang, B.-Q., Kang, Q., Luo, J. & Luo, X. (2010) 'The CCN proteins: important signaling mediators in stem cell differentiation and tumorigenesis'. *Histology and Histopathology*, 25 (6), pp. 795.

Appendices

Appendix 1: Full length of DNA CCN1/CYR61 from gene bank, yellow colour is Y582, green colour is G676 and red colour is R288. Taken from https://www.ncbi.nlm.nih.gov/nuccore/NC_000001.11?report=genbank&from=85580761&to=85583967

ORIGIN

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1 agaccgagcgag cgagagcgcc cccgagcagc gcccgcgccc tccgcgcctt ctccgcccggg
61 acctcgagcgc aaagacgccc gcccgcgccc cagccctcgc ctccctgccc accgggcccga
121 ccgcgcccgc accccgaccc cgctgcgcac ggcctgtccg ctgcacacca gcttgttggc
181 gtcttcgctcg ccgcgctcgc cccgggctac tcctgcgcgc cacaatgagc tcccgcacgc
241 ccagggcgct cgcttagtc gtcacccttc tccacttgac caggetggtg agttggactc
301 tccttttgcc acctattccc cgtccgctct ccagcccctt cccctggtcc cagattgccc
361 acggcaggaa aagttaaaaa gttcgcgacg gtttgcgggt agccgtttct ttaagcactc
421 tccccctccc cccgaagacg tgcgaggacc tcttgggtggg agcagccttc cgagggtggcc
481 gggctggacg agatcagagg ctccccgctc atagggtcgg agacccccgt cctcactgc
541 ggcagccgcg gcgcccctcc tgcgcaccgc gccgagtctc acgcgatatc tctccccctt
601 ccaggcgctc tccacctgcc ccgctgcctg ccaactgccc ctggaggcgc ccaagtgcgc
661 gccgggagtc gggctggtcc gggacgctg cggtgctgt aaggctcgcg ccaagcagct
721 caacgaggac tgcagcaaaa cgcagccctg cgaccacacc aaggggctgg aatgcaactt
781 cggcgccagc tccaccgctc tgaaggggat ctgcagaggt aagatgcttg tggtttggcc
841 cttttaaaaa aaatactagt ccccatagtc cagaagtta gtaattctga gaccatgtat
901 ggtgatgctt tgttgttggg agcttggcag aaaggcaccat gatttcagca gtctggaatg
961 caattcagtg tgtctgggcc caacgaaagc gcatacggaa aagtgattcc tgactaactt
1021 attgttctgg tctggagtct ccggggaatt gtagggaact ttaccataaa ttgaatttaa
1081 cagcagaaaa tatgtatgag tttcagcgcg tggtttggaa tcttaacttt atcccccttct
1141 acctttctct tttggtgatc tttgcagctc agtcagaggg cagaccctgt gaatataact
1201 ccagatctca ccaaacggg gaaagtttcc agcccaactg taaacatcag tgcacatgta
1261 ttgatggcgc cgtgggctgc attcctctgt gtcccccaaga actatctctc cccaacttgg
1321 gctgtcccaa cctcggctg gtcaaagtta ccgggcagtg ctgcgaggag tgggtctgtg
1381 acgaggatag tatcaaggac cccatggagg accaggacgg cctccttggc aaggagctgg
1441 gattcgatgc ctccgaggtg gagttgacga gaaacaatga attgattgca gttggaa aag
1501 gcagctcact gaagcggctc cctggtaagt ggagactgag cacttcagac actgtactga
1561 gatgcatttc tggctctaaat cttttagaaa atgagtgctt gagcctgttt gtgtcggat
1621 gcctctgaga agtcttcct cttatatgct tctagttttt ggaatggagc ctcgcacact
1681 atacaacctt ttacaaaggcc agaaatgtat tgttdaaaca acttcatggt cccagtgtc
1741 aaagacctgt ggaactggta tctccacacg agttaccaat gacaaccctg agtgccgct
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1801 tgtgaaagaa acccggatth gtgaggtgcy gccttgthga cagccagtht acagcagcct
1861 gaaagthaagt tccttcaggg acgtgtagac thttgcctgg caggtgggtg ggatgtgaa
1921 atctthtttga agaaagagaa atatcacccc taactthtct tctctcttht ctcttacaga
1981 agggcaagaa atgcagcaag accaagaaat cccccgaacc agtcaggtth acttacgctg
2041 gatgthttgag thtgaagaaa taccggccca agtactgcyg thctgcygth gacggccgat
2101 gctgcacgcc ccagctgacc aggactgthga agatgcygth ccygthcygaa gatggggaga
2161 cattthtcaa gaacgtcatg atgatccagth cctgcaaatg caactacaa thcccgcath
2221 ccaatgaagc agcythttccc thctacaggc thttcaatga cattcacaaa thtagggact
2281 aaatgctacc thgggtthtcca gggcacacct agacaaacaa gggagaagag thtcagaa
2341 agaatcathg agaaaatggg cgggggtgth gtgggtgath ggactcath thagaaaggaa
2401 gcctthgctca thctthgagga gcattaagth atthtcaa thccaaggt thtggthcyg
2461 atggacacta atgcagccac gattggagaa tactthgct catagthatt gagcacath
2521 tactgctthca thttggagct thtggagtht atgactthtct gthttctgth thtaaatth
2581 thgctaaagca ththttctct aggctthttt cthtttggg thctacagth gtaaaagaga
2641 taataagath agthggacag thtaagctt thattcgtcc thtgacaaaa gtaaatggga
2701 gggcattcca thcctthctg aagggggaca thccatgagth gthtthgaga ggcagctath
2761 thgactctaa actgcaaaac gaaatcagth gthtttaagac thaatgthtt atthtcaaa
2821 atgtagctth thggggaggga ggggaaatgth aactgthgaa thattthgaa atgattthaa
2881 thttatathc agthgaaaaga thttattht ggaatthacc atthtaataa gaaatthtth
2941 cctaathatct gagthgathc cattcgtath thttagagth gthccaaagth cattaggaac
3001 aacctagctc acgthactca thattcaaac aggactthatt gggatacagc agthgaaatth
3061 gctathtaaaa thagataath atthgctthtth tactthcagth agagaaaagth cthtgcath
3121 aaagthaath thaaaaaca thgaththgaa acgacathth atgaaagca ataaagathc
3181 thgaagctaaa thtthgathh aagaaaa

//

Appendix 2: Down regulated proteins in REC1 CYR61/CCN1 KD model.

Category	Term	GO TERM	Count	%	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
GOTERM_BP_FAT	GO:0070972	protein localization to endoplasmic reticulum	9	9.474	5.31E-07	29082, 6181, 2923, 6132, 6224, 6222, 6154, 6165, 6156	93	128	16650	12.5882	0.0013	0.0013	0.0009
GOTERM_BP_FAT	GO:0002474	antigen processing and presentation of peptide antigen via MHC class I	8	8.421	7.46E-07	5687, 5688, 2923, 5243, 5721, 5685, 3105, 5684	93	92	16650	15.5680	0.0019	0.0009	0.0013
GOTERM_BP_FAT	GO:0090150	establishment of protein localization to membrane	13	13.684	8.41E-07	6181, 302, 6132, 6154, 6165, 6156, 29082, 6224, 6222, 7534, 7532, 7531, 26520	93	368	16650	6.3245	0.0021	0.0007	0.0015
GOTERM_BP_FAT	GO:0045047	protein targeting to ER	8	8.421	1.72E-06	29082, 6181, 6132, 6224, 6222, 6154, 6165, 6156	93	104	16650	13.7717	0.0043	0.0011	0.0030
GOTERM_BP_FAT	GO:0072599	establishment of protein localization to endoplasmic reticulum	8	8.421	2.21E-06	29082, 6181, 6132, 6224, 6222, 6154, 6165, 6156	93	108	16650	13.2616	0.0055	0.0011	0.0039
GOTERM_BP_FAT	GO:0033238	regulation of cellular amine metabolic process	7	7.368	3.31E-06	11315, 5687, 5688, 3251, 5721, 5685, 5684	93	74	16650	16.9355	0.0083	0.0014	0.0058
GOTERM_BP_FAT	GO:0009117	nucleotide metabolic process	16	16.842	3.50E-06	3251, 654364, 1329, 4190, 4710, 11315, 2079, 7167, 51727, 10606, 10135, 808, 7086, 2023, 1345, 3945	93	667	16650	4.2946	0.0087	0.0013	0.0062
GOTERM_BP_FAT	GO:0006753	nucleoside phosphate metabolic process	16	16.842	4.05E-06	3251, 654364, 1329, 4190, 4710, 11315, 2079, 7167, 51727, 10606, 10135, 808, 7086, 2023, 1345, 3945	93	675	16650	4.2437	0.0101	0.0013	0.0071

GOTERM_BP_FAT	GO:0045454	cell redox homeostasis	7	7.368	4.17E-06	10130, 9601, 2923, 10935, 9352, 5034, 5052	93	77	16650	16.2757	0.0104	0.0012	0.0074
GOTERM_BP_FAT	GO:0016032	viral process	19	20.000	4.70E-06	6181, 6132, 6154, 293, 6165, 5034, 5685, 5684, 8665, 3383, 6156, 29082, 2224, 5688, 6224, 6222, 2023, 7531, 3105	93	966	16650	3.5213	0.0117	0.0012	0.0083
GOTERM_BP_FAT	GO:0044764	multi-organism cellular process	19	20.000	5.20E-06	6181, 6132, 6154, 293, 6165, 5034, 5685, 5684, 8665, 3383, 6156, 29082, 2224, 5688, 6224, 6222, 2023, 7531, 3105	93	973	16650	3.4960	0.0129	0.0012	0.0092
GOTERM_BP_FAT	GO:0006605	protein targeting	16	16.842	6.39E-06	6181, 302, 6132, 2923, 6154, 293, 6165, 5052, 6156, 29082, 6224, 6222, 7534, 7532, 7531, 26520	93	701	16650	4.0863	0.0159	0.0013	0.0113
GOTERM_BP_FAT	GO:0044403	symbiosis, encompassing mutualism through parasitism	19	20.000	7.38E-06	6181, 6132, 6154, 293, 6165, 5034, 5685, 5684, 8665, 3383, 6156, 29082, 2224, 5688, 6224, 6222, 2023, 7531, 3105	93	998	16650	3.4084	0.0183	0.0014	0.0130
GOTERM_BP_FAT	GO:0044419	interspecies interaction between organisms	19	20.000	7.38E-06	6181, 6132, 6154, 293, 6165, 5034, 5685, 5684, 8665, 3383, 6156, 29082, 2224, 5688, 6224, 6222, 2023, 7531, 3105	93	998	16650	3.4084	0.0183	0.0014	0.0130
GOTERM_BP_FAT	GO:0006886	intracellular protein transport	19	20.000	9.95E-06	6181, 302, 6132, 2923, 10961, 6154, 293, 6165, 5052, 6156, 29082, 11315, 10016, 6224, 6222, 7534, 7532, 7531, 26520	93	1020	16650	3.3349	0.0246	0.0018	0.0176
GOTERM_BP_FAT	GO:0055086	nucleobase-containing small molecule metabolic process	16	16.842	1.01E-05	3251, 654364, 1329, 4190, 4710, 11315, 2079, 7167, 51727, 10606, 10135, 808, 7086, 2023, 1345, 3945	93	728	16650	3.9348	0.0249	0.0017	0.0177
GOTERM_BP_FAT	GO:0006612	protein targeting to membrane	9	9.474	1.02E-05	29082, 6181, 302, 6132, 6224, 6222, 6154, 6165, 6156	93	190	16650	8.4805	0.0252	0.0016	0.0180
GOTERM_BP_FAT	GO:1901566	organonitrogen compound	22	23.158	1.48E-05	1936, 6181, 3251, 654364, 1933, 6132, 1329, 6154, 293, 6165, 8665,	93	1375	16650	2.8645	0.0363	0.0022	0.0260

		biosynthetic process				6156, 11315, 2079, 6224, 51727, 10606, 6222, 10135, 808, 5859, 6576							
GOTERM_BP_FAT	GO:0006614	SRP-dependent cotranslational protein targeting to membrane	7	7.368	1.50E-05	6181, 6132, 6224, 6222, 6154, 6165, 6156	93	96	16650	13.0544	0.0369	0.0021	0.0265
GOTERM_BP_FAT	GO:0072657	protein localization to membrane	13	13.684	1.55E-05	6181, 302, 6132, 6154, 6165, 6156, 29082, 6224, 6222, 7534, 7532, 7531, 26520	93	489	16650	4.7595	0.0381	0.0020	0.0273
GOTERM_BP_FAT	GO:0072594	establishment of protein localization to organelle	15	15.789	1.64E-05	6181, 6132, 2923, 6154, 293, 6165, 5052, 6156, 29082, 6224, 6222, 7534, 7532, 7531, 26520	93	666	16650	4.0323	0.0404	0.0021	0.0290
GOTERM_BP_FAT	GO:0006613	cotranslational protein targeting to membrane	7	7.368	2.24E-05	6181, 6132, 6224, 6222, 6154, 6165, 6156	93	103	16650	12.1672	0.0547	0.0027	0.0396
GOTERM_BP_FAT	GO:0002223	stimulatory C-type lectin receptor signaling pathway	7	7.368	2.50E-05	5687, 5688, 7334, 3385, 5721, 5685, 5684	93	105	16650	11.9355	0.0609	0.0029	0.0442
GOTERM_BP_FAT	GO:0002479	antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent	6	6.316	2.52E-05	5687, 5688, 5721, 5685, 3105, 5684	93	63	16650	17.0507	0.0612	0.0027	0.0445
GOTERM_BP_FAT	GO:1902582	single-organism intracellular transport	15	15.789	2.64E-05	6181, 6647, 302, 6132, 1329, 2923, 6154, 6165, 5052, 6156, 29082, 6224, 6222, 26520, 81	93	695	16650	3.8640	0.0640	0.0028	0.0466
GOTERM_BP_FAT	GO:0002220	innate immune response activating cell surface receptor signaling pathway	7	7.368	2.79E-05	5687, 5688, 7334, 3385, 5721, 5685, 5684	93	107	16650	11.7124	0.0675	0.0028	0.0492

GOTERM_BP_FAT	GO:0033365	protein localization to organelle	17	17.895	3.14E-05	6181, 6132, 2923, 6154, 293, 6165, 5052, 6156, 29082, 11315, 4478, 6224, 6222, 7534, 7532, 7531, 26520	93	902	16650	3.3742	0.0757	0.0030	0.0554
GOTERM_BP_FAT	GO:0042590	antigen processing and presentation of exogenous peptide antigen via MHC class I	6	6.316	3.16E-05	5687, 5688, 5721, 5685, 3105, 5684	93	66	16650	16.2757	0.0762	0.0029	0.0558
GOTERM_BP_FAT	GO:0019882	antigen processing and presentation	9	9.474	3.67E-05	5687, 5688, 2923, 5243, 5721, 5685, 3105, 5684, 3383	93	227	16650	7.0982	0.0880	0.0033	0.0648
GOTERM_BP_FAT	GO:0044106	cellular amine metabolic process	7	7.368	4.19E-05	11315, 5687, 5688, 3251, 5721, 5685, 5684	93	115	16650	10.8976	0.0997	0.0036	0.0739
GOTERM_BP_FAT	GO:0046907	intracellular transport	23	24.211	4.25E-05	6181, 6647, 302, 2923, 6132, 1329, 10961, 6154, 293, 6165, 5052, 6156, 29082, 11315, 4478, 10016, 6224, 6222, 7534, 7532, 81, 7531, 26520	93	1594	16650	2.5833	0.1011	0.0035	0.0750
GOTERM_BP_FAT	GO:0051438	regulation of ubiquitin-protein transferase activity	7	7.368	5.07E-05	11315, 5687, 5688, 7334, 5721, 5685, 5684	93	119	16650	10.5313	0.1195	0.0041	0.0895
GOTERM_BP_FAT	GO:0015031	protein transport	25	26.316	5.20E-05	6181, 9601, 6165, 29082, 10016, 4678, 81, 302, 2923, 6132, 10961, 293, 6154, 5052, 6156, 11315, 4082, 6224, 2665, 6222, 7534, 7532, 7531, 26520, 5878	93	1860	16650	2.4063	0.1222	0.0041	0.0917
GOTERM_BP_FAT	GO:0048002	antigen processing and presentation of peptide antigen	8	8.421	5.99E-05	5687, 5688, 2923, 5243, 5721, 5685, 3105, 5684	93	179	16650	8.0014	0.1396	0.0045	0.1057
GOTERM_BP_FAT	GO:0000184	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	7	7.368	6.11E-05	6181, 6132, 6224, 6222, 6154, 6165, 6156	93	123	16650	10.1888	0.1420	0.0045	0.1077

GOTERM_BP_FAT	GO:0006979	response to oxidative stress	11	11.579	6.30E-05	11315, 654364, 9124, 6647, 10935, 3848, 9352, 5034, 5052, 5660, 4710	93	394	16650	4.9984	0.1461	0.0045	0.1111
GOTERM_BP_FAT	GO:0009308	amine metabolic process	7	7.368	6.39E-05	11315, 5687, 5688, 3251, 5721, 5685, 5684	93	124	16650	10.1067	0.1480	0.0044	0.1127
GOTERM_BP_FAT	GO:0051444	negative regulation of ubiquitin-protein transferase activity	6	6.316	8.00E-05	11315, 5687, 5688, 5721, 5685, 5684	93	80	16650	13.4274	0.1819	0.0054	0.1412
GOTERM_BP_FAT	GO:0006413	translational initiation	8	8.421	8.44E-05	6181, 6132, 6224, 6222, 6154, 6165, 8665, 6156	93	189	16650	7.5781	0.1908	0.0056	0.1488
GOTERM_BP_FAT	GO:0019080	viral gene expression	8	8.421	9.31E-05	6181, 6132, 6224, 6222, 6154, 6165, 8665, 6156	93	192	16650	7.4597	0.2083	0.0060	0.1642
GOTERM_BP_FAT	GO:1902580	single-organism cellular localization	18	18.947	9.89E-05	6181, 6647, 302, 6132, 1329, 2923, 6154, 6165, 5052, 6156, 29082, 6224, 6222, 7534, 7532, 7531, 26520, 81	93	1102	16650	2.9243	0.2197	0.0062	0.1744
GOTERM_BP_FAT	GO:0044248	cellular catabolic process	22	23.158	1.56E-04	6181, 3251, 302, 6132, 6154, 6165, 5721, 5052, 5685, 5684, 6156, 5687, 11315, 4478, 5688, 7167, 6224, 7334, 10935, 6222, 808, 1632	93	1616	16650	2.4373	0.3244	0.0095	0.2755
GOTERM_BP_FAT	GO:0044033	multi-organism metabolic process	8	8.421	1.83E-04	6181, 6132, 6224, 6222, 6154, 6165, 8665, 6156	93	214	16650	6.6928	0.3673	0.0108	0.3216
GOTERM_BP_FAT	GO:0045184	establishment of protein localization	25	26.316	1.92E-04	6181, 9601, 6165, 29082, 10016, 4678, 81, 302, 2923, 6132, 10961, 293, 6154, 5052, 6156, 11315, 4082, 6224, 2665, 6222, 7534, 7532, 7531, 26520, 5878	93	2021	16650	2.2146	0.3822	0.0111	0.3383
GOTERM_BP_FAT	GO:0019058	viral life cycle	11	11.579	2.05E-04	29082, 6181, 6132, 6224, 6222, 6154, 6165, 5034, 8665, 3383, 6156	93	455	16650	4.3283	0.4020	0.0116	0.3611
GOTERM_BP_FAT	GO:0006412	translation	13	13.684	2.08E-04	1936, 6181, 1933, 6132, 6224, 6222, 6154, 293, 6165, 5859, 6576, 8665, 6156	93	641	16650	3.6309	0.4058	0.0115	0.3656

GOTERM_BP_FAT	GO:0002181	cytoplasmic translation	5	5.263	2.08E-04	6181, 6132, 6154, 6165, 8665	93	53	16650	16.8898	0.4062	0.0113	0.3661
GOTERM_BP_FAT	GO:0019693	ribose phosphate metabolic process	12	12.632	2.17E-04	11315, 3251, 654364, 1329, 7167, 10606, 51727, 808, 7086, 2023, 1345, 4710	93	549	16650	3.9133	0.4204	0.0115	0.3830
GOTERM_BP_FAT	GO:0006521	regulation of cellular amino acid metabolic process	5	5.263	2.23E-04	5687, 5688, 5721, 5685, 5684	93	54	16650	16.5771	0.4291	0.0116	0.3937
GOTERM_BP_FAT	GO:0009161	ribonucleoside monophosphate metabolic process	9	9.474	2.37E-04	11315, 3251, 1329, 7167, 10606, 51727, 2023, 1345, 4710	93	297	16650	5.4252	0.4485	0.0121	0.4179
GOTERM_BP_FAT	GO:0051443	positive regulation of ubiquitin-protein transferase activity	6	6.316	2.64E-04	5687, 5688, 7334, 5721, 5685, 5684	93	103	16650	10.4291	0.4840	0.0131	0.4645
GOTERM_BP_FAT	GO:0034599	cellular response to oxidative stress	8	8.421	2.69E-04	11315, 654364, 6647, 10935, 9352, 5034, 5052, 5660	93	228	16650	6.2818	0.4906	0.0131	0.4734
GOTERM_BP_FAT	GO:0044802	single-organism membrane organization	15	15.789	2.73E-04	6181, 6647, 302, 6132, 5243, 6154, 6165, 6156, 29082, 6224, 6222, 7534, 7532, 7531, 26520	93	866	16650	3.1010	0.4961	0.0131	0.4811
GOTERM_BP_FAT	GO:0002478	antigen processing and presentation of exogenous peptide antigen	7	7.368	2.77E-04	5687, 5688, 5243, 5721, 5685, 3105, 5684	93	162	16650	7.7360	0.5003	0.0130	0.4869
GOTERM_BP_FAT	GO:0044265	cellular macromolecule catabolic process	16	16.842	2.79E-04	6181, 302, 6132, 6154, 6165, 5721, 5685, 5684, 6156, 11315, 5687, 4478, 5688, 6224, 7334, 6222	93	976	16650	2.9350	0.5035	0.0129	0.4915
GOTERM_BP_FAT	GO:0043043	peptide biosynthetic process	13	13.684	2.99E-04	1936, 6181, 1933, 6132, 6224, 6222, 6154, 293, 6165, 5859, 6576, 8665, 6156	93	667	16650	3.4894	0.5271	0.0135	0.5256

GOTERM_BP_FAT	GO:0055114	oxidation-reduction process	16	16.842	3.01E-04	6647, 1329, 2923, 9352, 4190, 5052, 4710, 11315, 7167, 10935, 2665, 808, 2023, 1632, 1345, 3945	93	983	16650	2.9141	0.5304	0.0134	0.5305
GOTERM_BP_FAT	GO:0038061	NIK/NF-kappaB signaling	6	6.316	3.15E-04	5687, 5688, 81, 5721, 5685, 5684	93	107	16650	10.0392	0.5460	0.0138	0.5542
GOTERM_BP_FAT	GO:0019674	NAD metabolic process	5	5.263	3.15E-04	7167, 10135, 4190, 2023, 3945	93	59	16650	15.1722	0.5461	0.0135	0.5543
GOTERM_BP_FAT	GO:0009123	nucleoside monophosphate metabolic process	9	9.474	3.24E-04	11315, 3251, 1329, 7167, 10606, 51727, 2023, 1345, 4710	93	311	16650	5.1810	0.5558	0.0137	0.5694
GOTERM_BP_FAT	GO:0019637	organophosphate metabolic process	17	17.895	3.45E-04	3251, 654364, 1329, 4190, 4710, 2224, 11315, 2079, 7167, 51727, 10606, 10135, 808, 7086, 2023, 1345, 3945	93	1108	16650	2.7469	0.5786	0.0143	0.6063
GOTERM_BP_FAT	GO:0019884	antigen processing and presentation of exogenous antigen	7	7.368	3.47E-04	5687, 5688, 5243, 5721, 5685, 3105, 5684	93	169	16650	7.4155	0.5812	0.0142	0.6106
GOTERM_BP_FAT	GO:0006796	phosphate-containing compound metabolic process	32	33.684	3.66E-04	5660, 5685, 5684, 3383, 2224, 7167, 51727, 10135, 7086, 1345, 3945, 3251, 654364, 6647, 302, 1329, 10961, 4190, 476, 5721, 5052, 4710, 5687, 11315, 5688, 2079, 10935, 10606, 808, 2023, 7532, 7531	93	3083	16650	1.8583	0.6008	0.0147	0.6440
GOTERM_BP_FAT	GO:0006793	phosphorus metabolic process	32	33.684	3.79E-04	5660, 5685, 5684, 3383, 2224, 7167, 51727, 10135, 7086, 1345, 3945, 3251, 654364, 6647, 302, 1329, 10961, 4190, 476, 5721, 5052, 4710, 5687, 11315, 5688, 2079, 10935, 10606, 808, 2023, 7532, 7531	93	3089	16650	1.8547	0.6137	0.0150	0.6670
GOTERM_BP_FAT	GO:0051649	establishment of localization in cell	24	25.263	4.45E-04	6181, 6647, 302, 2923, 6132, 1329, 10961, 6154, 293, 6165, 5052, 6156, 29082, 11315, 4478, 10016, 6224,	93	2004	16650	2.1441	0.6727	0.0173	0.7827

						6222, 7534, 808, 7532, 81, 7531, 26520							
GOTERM_BP_FAT	GO:0009116	nucleoside metabolic process	10	10.526	4.60E-04	11315, 3251, 2079, 654364, 1329, 7167, 51727, 2023, 1345, 4710	93	413	16650	4.3349	0.6845	0.0176	0.8085
GOTERM_BP_FAT	GO:0038095	Fc-epsilon receptor signaling pathway	7	7.368	4.71E-04	5687, 5688, 7334, 808, 5721, 5685, 5684	93	179	16650	7.0013	0.6934	0.0178	0.8283
GOTERM_BP_FAT	GO:1903362	regulation of cellular protein catabolic process	8	8.421	4.80E-04	11315, 5687, 4478, 5688, 302, 5721, 5685, 5684	93	251	16650	5.7062	0.6999	0.0178	0.8433
GOTERM_BP_FAT	GO:0019083	viral transcription	7	7.368	5.00E-04	6181, 6132, 6224, 6222, 6154, 6165, 6156	93	181	16650	6.9239	0.7145	0.0183	0.8783
GOTERM_BP_FAT	GO:0006518	peptide metabolic process	14	14.737	5.42E-04	1936, 6181, 1933, 6647, 6132, 6154, 293, 6165, 8665, 6156, 6224, 6222, 5859, 6576	93	818	16650	3.0641	0.7430	0.0195	0.9515
GOTERM_BP_FAT	GO:0008104	protein localization	27	28.421	5.74E-04	6181, 9601, 51596, 6165, 29082, 10016, 4678, 81, 302, 2923, 6132, 10961, 6154, 293, 5052, 6156, 11315, 4478, 4082, 6224, 2665, 6222, 7534, 7532, 7531, 26520, 5878	93	2448	16650	1.9746	0.7631	0.0204	1.0084
GOTERM_BP_FAT	GO:0098609	cell-cell adhesion	17	17.895	5.93E-04	7112, 1936, 832, 6647, 302, 3385, 5052, 3383, 11315, 4478, 9124, 8350, 10606, 7534, 2023, 7531, 3105	93	1164	16650	2.6147	0.7740	0.0207	1.0412
GOTERM_BP_FAT	GO:0046496	nicotinamide nucleotide metabolic process	6	6.316	5.97E-04	7167, 10135, 4190, 7086, 2023, 3945	93	123	16650	8.7333	0.7764	0.0206	1.0486
GOTERM_BP_FAT	GO:0019362	pyridine nucleotide metabolic process	6	6.316	5.97E-04	7167, 10135, 4190, 7086, 2023, 3945	93	123	16650	8.7333	0.7764	0.0206	1.0486
GOTERM_BP_FAT	GO:0080134	regulation of response to stress	18	18.947	6.05E-04	6647, 302, 3848, 10961, 3385, 293, 5721, 5052, 5660, 5034, 5685, 5684, 5687, 11315, 5688, 7334, 7531, 3105	93	1285	16650	2.5078	0.7811	0.0206	1.0631

GOTERM_BP_FAT	GO:0051436	negative regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	5	5.263	6.39E-04	5687, 5688, 5721, 5685, 5684	93	71	16650	12.6079	0.7989	0.0214	1.1221
GOTERM_BP_FAT	GO:1901657	glycosyl compound metabolic process	10	10.526	6.67E-04	11315, 3251, 2079, 654364, 1329, 7167, 51727, 2023, 1345, 4710	93	435	16650	4.1157	0.8126	0.0221	1.1714
GOTERM_BP_FAT	GO:0051439	regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	5	5.263	6.74E-04	5687, 5688, 5721, 5685, 5684	93	72	16650	12.4328	0.8157	0.0220	1.1828
GOTERM_BP_FAT	GO:1904667	negative regulation of ubiquitin protein ligase activity	5	5.263	7.10E-04	5687, 5688, 5721, 5685, 5684	93	73	16650	12.2625	0.8316	0.0229	1.2458
GOTERM_BP_FAT	GO:0043604	amide biosynthetic process	13	13.684	7.19E-04	1936, 6181, 1933, 6132, 6224, 6222, 6154, 293, 6165, 5859, 6576, 8665, 6156	93	736	16650	3.1623	0.8354	0.0229	1.2617
GOTERM_BP_FAT	GO:0009967	positive regulation of signal transduction	19	20.000	7.25E-04	1936, 6647, 2923, 10961, 5721, 5660, 5685, 5684, 3383, 5687, 11315, 5688, 2079, 7334, 7534, 808, 7532, 7531, 81	93	1428	16650	2.3821	0.8376	0.0227	1.2709
GOTERM_BP_FAT	GO:0009259	ribonucleotide metabolic process	11	11.579	7.33E-04	11315, 3251, 654364, 1329, 7167, 10606, 51727, 808, 2023, 1345, 4710	93	535	16650	3.6810	0.8409	0.0227	1.2851
GOTERM_BP_FAT	GO:0031397	negative regulation of protein ubiquitination	6	6.316	7.41E-04	11315, 5687, 5688, 5721, 5685, 5684	93	129	16650	8.3271	0.8443	0.0227	1.2999
GOTERM_BP_FAT	GO:0023056	positive regulation of signaling	20	21.053	7.84E-04	1936, 6647, 302, 2923, 10961, 5721, 5660, 5685, 5684, 3383, 5687, 11315,	93	1563	16650	2.2909	0.8602	0.0237	1.3750

						5688, 2079, 7334, 7534, 808, 7532, 7531, 81							
GOTERM_BP_FAT	GO:0072524	pyridine-containing compound metabolic process	6	6.316	7.94E-04	7167, 10135, 4190, 7086, 2023, 3945	93	131	16650	8.2000	0.8638	0.0237	1.3928
GOTERM_BP_FAT	GO:0051437	positive regulation of ubiquitin-protein ligase activity involved in regulation of mitotic cell cycle transition	5	5.263	8.27E-04	5687, 5688, 5721, 5685, 5684	93	76	16650	11.7784	0.8743	0.0244	1.4487
GOTERM_BP_FAT	GO:0009057	macromolecule catabolic process	17	17.895	8.31E-04	6181, 302, 6132, 6154, 6165, 5721, 5685, 5684, 6156, 11315, 5687, 4478, 5688, 6224, 7334, 6222, 808	93	1201	16650	2.5342	0.8757	0.0242	1.4564
GOTERM_BP_FAT	GO:0007155	cell adhesion	21	22.105	8.35E-04	7112, 1936, 832, 654364, 6647, 302, 3385, 953, 5052, 3383, 11315, 4478, 9124, 8350, 10606, 396, 7534, 2023, 81, 7531, 3105	93	1699	16650	2.2129	0.8769	0.0241	1.4630
GOTERM_BP_FAT	GO:0022610	biological adhesion	21	22.105	8.73E-04	7112, 1936, 832, 654364, 6647, 302, 3385, 953, 5052, 3383, 11315, 4478, 9124, 8350, 10606, 396, 7534, 2023, 81, 7531, 3105	93	1705	16650	2.2051	0.8880	0.0248	1.5286
GOTERM_BP_FAT	GO:0001738	morphogenesis of a polarized epithelium	6	6.316	9.10E-04	5687, 4478, 5688, 5721, 5685, 5684	93	135	16650	7.9570	0.8980	0.0256	1.5933
GOTERM_BP_FAT	GO:0031145	anaphase-promoting complex-dependent catabolic process	5	5.263	9.56E-04	5687, 5688, 5721, 5685, 5684	93	79	16650	11.3312	0.9091	0.0266	1.6735
GOTERM_BP_FAT	GO:0033036	macromolecule localization	29	30.526	9.61E-04	6181, 9601, 51596, 6165, 5660, 29082, 10016, 4678, 81, 302, 2923, 6132, 5243, 10961, 6154, 293, 5052,	93	2817	16650	1.8431	0.9104	0.0264	1.6830

						6156, 11315, 4478, 4082, 6224, 6222, 2665, 7534, 7532, 7531, 26520, 5878								
GOTERM_BP_FAT	GO:0000956	nuclear-transcribed mRNA catabolic process	7	7.368	9.83E-04	6181, 6132, 6224, 6222, 6154, 6165, 6156	93	206	16650	6.0836	0.9151	0.0267	1.7208	
GOTERM_BP_FAT	GO:0046364	monosaccharide biosynthetic process	5	5.263	0.001001978	7167, 4190, 7086, 2023, 6576	93	80	16650	11.1895	0.9191	0.0270	1.7536	
GOTERM_BP_FAT	GO:0009167	purine ribonucleoside monophosphate metabolic process	8	8.421	0.001013814	11315, 3251, 1329, 7167, 10606, 2023, 1345, 4710	93	285	16650	5.0255	0.9214	0.0270	1.7741	
GOTERM_BP_FAT	GO:0009126	purine nucleoside monophosphate metabolic process	8	8.421	0.001034643	11315, 3251, 1329, 7167, 10606, 2023, 1345, 4710	93	286	16650	5.0079	0.9254	0.0272	1.8102	
GOTERM_BP_FAT	GO:0006733	oxidoreduction coenzyme metabolic process	6	6.316	0.001036949	7167, 10135, 4190, 7086, 2023, 3945	93	139	16650	7.7280	0.9259	0.0270	1.8142	
GOTERM_BP_FAT	GO:0034613	cellular protein localization	20	21.053	0.001039676	6181, 302, 6132, 2923, 10961, 6154, 293, 6165, 5052, 6156, 29082, 11315, 4478, 10016, 6224, 6222, 7534, 7532, 7531, 26520	93	1600	16650	2.2379	0.9264	0.0268	1.8190	
GOTERM_BP_FAT	GO:1903321	negative regulation of protein modification by small protein conjugation or removal	6	6.316	0.001070723	11315, 5687, 5688, 5721, 5685, 5684	93	140	16650	7.6728	0.9319	0.0273	1.8728	
GOTERM_BP_FAT	GO:0043603	cellular amide metabolic process	15	15.789	0.001071749	1936, 6181, 1933, 6647, 6132, 6154, 293, 6165, 8665, 6156, 6224, 6222, 10135, 5859, 6576	93	994	16650	2.7017	0.9321	0.0271	1.8746	

GOTERM_BP_FAT	GO:1904668	positive regulation of ubiquitin protein ligase activity	5	5.263	0.001098825	5687, 5688, 5721, 5685, 5684	93	82	16650	10.9166	0.9365	0.0275	1.9215
GOTERM_BP_FAT	GO:0070727	cellular macromolecule localization	20	21.053	0.001145052	6181, 302, 6132, 2923, 10961, 6154, 293, 6165, 5052, 6156, 29082, 11315, 4478, 10016, 6224, 6222, 7534, 7532, 7531, 26520	93	1613	16650	2.2199	0.9435	0.0283	2.0016
GOTERM_BP_FAT	GO:2001233	regulation of apoptotic signaling pathway	9	9.474	0.001199795	11315, 6647, 2923, 10961, 7534, 7532, 7531, 5034, 3383	93	380	16650	4.2402	0.9508	0.0294	2.0963
GOTERM_BP_FAT	GO:0090407	organophosphate biosynthetic process	11	11.579	0.001216942	2224, 3251, 2079, 654364, 1329, 7167, 10606, 51727, 10135, 808, 7086	93	572	16650	3.4429	0.9528	0.0295	2.1260
GOTERM_BP_FAT	GO:0043488	regulation of mRNA stability	6	6.316	0.001251986	5687, 5688, 7534, 5721, 5685, 5684	93	145	16650	7.4082	0.9568	0.0300	2.1866
GOTERM_BP_FAT	GO:0098869	cellular oxidant detoxification	5	5.263	0.001256145	11315, 6647, 10935, 9352, 5052	93	85	16650	10.5313	0.9573	0.0299	2.1937
GOTERM_BP_FAT	GO:0009141	nucleoside triphosphate metabolic process	8	8.421	0.001286771	11315, 654364, 1329, 7167, 51727, 2023, 1345, 4710	93	297	16650	4.8224	0.9604	0.0303	2.2467
GOTERM_BP_FAT	GO:0009119	ribonucleoside metabolic process	9	9.474	0.001303855	11315, 3251, 654364, 1329, 7167, 51727, 2023, 1345, 4710	93	385	16650	4.1852	0.9621	0.0304	2.2762
GOTERM_BP_FAT	GO:1904666	regulation of ubiquitin protein ligase activity	5	5.263	0.00131191	5687, 5688, 5721, 5685, 5684	93	86	16650	10.4089	0.9628	0.0303	2.2901
GOTERM_BP_FAT	GO:0048584	positive regulation of response to stimulus	23	24.211	0.001319694	1936, 6647, 2923, 3848, 10961, 293, 3385, 5721, 5660, 5685, 5684, 3383, 5687, 11315, 5688, 2079, 7334, 7534, 808, 7532, 81, 7531, 3105	93	2030	16650	2.0284	0.9636	0.0302	2.3035
GOTERM_BP_FAT	GO:1990748	cellular detoxification	5	5.263	0.001369383	11315, 6647, 10935, 9352, 5052	93	87	16650	10.2892	0.9678	0.0310	2.3893

GOTERM_BP_FAT	GO:0006402	mRNA catabolic process	7	7.368	0.001410314	6181, 6132, 6224, 6222, 6154, 6165, 6156	93	221	16650	5.6707	0.9710	0.0317	2.4598
GOTERM_BP_FAT	GO:0061024	membrane organization	15	15.789	0.001463054	6181, 6647, 302, 6132, 5243, 6154, 6165, 6156, 29082, 6224, 6222, 7534, 7532, 7531, 26520	93	1027	16650	2.6149	0.9746	0.0325	2.5507
GOTERM_BP_FAT	GO:0043487	regulation of RNA stability	6	6.316	0.001542674	5687, 5688, 7534, 5721, 5685, 5684	93	152	16650	7.0671	0.9792	0.0340	2.6877
GOTERM_BP_FAT	GO:0015992	proton transport	6	6.316	0.001587931	11315, 1329, 476, 1347, 81, 1345	93	153	16650	7.0209	0.9814	0.0347	2.7656
GOTERM_BP_FAT	GO:0098754	detoxification	5	5.263	0.001616917	11315, 6647, 10935, 9352, 5052	93	91	16650	9.8369	0.9827	0.0350	2.8154
GOTERM_BP_FAT	GO:2000060	positive regulation of protein ubiquitination involved in ubiquitin-dependent protein catabolic process	5	5.263	0.001616917	5687, 5688, 5721, 5685, 5684	93	91	16650	9.8369	0.9827	0.0350	2.8154
GOTERM_BP_FAT	GO:0035567	non-canonical Wnt signaling pathway	6	6.316	0.001681367	5687, 5688, 808, 5721, 5685, 5684	93	155	16650	6.9303	0.9853	0.0360	2.9260
GOTERM_BP_FAT	GO:0006818	hydrogen transport	6	6.316	0.001681367	11315, 1329, 476, 1347, 81, 1345	93	155	16650	6.9303	0.9853	0.0360	2.9260
GOTERM_BP_FAT	GO:0009165	nucleotide biosynthetic process	8	8.421	0.001736839	3251, 2079, 654364, 1329, 10606, 51727, 10135, 808	93	313	16650	4.5759	0.9872	0.0369	3.0212
GOTERM_BP_FAT	GO:0006732	coenzyme metabolic process	8	8.421	0.001800761	7167, 10135, 4190, 4144, 7086, 2023, 3945, 6576	93	315	16650	4.5469	0.9891	0.0379	3.1307
GOTERM_BP_FAT	GO:1901293	nucleoside phosphate biosynthetic process	8	8.421	0.001833401	3251, 2079, 654364, 1329, 10606, 51727, 10135, 808	93	316	16650	4.5325	0.9900	0.0383	3.1866

GOTERM_BP_FAT	GO:0010647	positive regulation of cell communication	19	20.000	0.001917745	1936, 6647, 2923, 10961, 5721, 5660, 5685, 5684, 3383, 5687, 11315, 5688, 2079, 7334, 7534, 808, 7532, 7531, 81	93	1555	16650	2.1875	0.9919	0.0396	3.3308
GOTERM_BP_FAT	GO:0019725	cellular homeostasis	13	13.684	0.002071558	10130, 654364, 6647, 9601, 2923, 10935, 9352, 808, 476, 5034, 5052, 1207, 3383	93	833	16650	2.7940	0.9945	0.0424	3.5934
GOTERM_BP_FAT	GO:0002758	innate immune response-activating signal transduction	7	7.368	0.002277192	5687, 5688, 7334, 3385, 5721, 5685, 5684	93	243	16650	5.1573	0.9967	0.0462	3.9434
GOTERM_BP_FAT	GO:0038093	Fc receptor signaling pathway	7	7.368	0.002277192	5687, 5688, 7334, 808, 5721, 5685, 5684	93	243	16650	5.1573	0.9967	0.0462	3.9434
GOTERM_BP_FAT	GO:0009150	purine ribonucleotide metabolic process	10	10.526	0.002301811	11315, 3251, 654364, 1329, 7167, 10606, 808, 2023, 1345, 4710	93	520	16650	3.4429	0.9969	0.0463	3.9852
GOTERM_BP_FAT	GO:0050852	T cell receptor signaling pathway	6	6.316	0.002329105	5687, 5688, 7334, 5721, 5685, 5684	93	167	16650	6.4323	0.9971	0.0464	4.0316
GOTERM_BP_FAT	GO:2000058	regulation of protein ubiquitination involved in ubiquitin-dependent protein catabolic process	5	5.263	0.00236885	5687, 5688, 5721, 5685, 5684	93	101	16650	8.8630	0.9974	0.0468	4.0990
GOTERM_BP_FAT	GO:0006401	RNA catabolic process	7	7.368	0.002571544	6181, 6132, 6224, 6222, 6154, 6165, 6156	93	249	16650	5.0330	0.9984	0.0504	4.4423
GOTERM_BP_FAT	GO:0002218	activation of innate immune response	7	7.368	0.00262326	5687, 5688, 7334, 3385, 5721, 5685, 5684	93	250	16650	5.0129	0.9986	0.0509	4.5297
GOTERM_BP_FAT	GO:0031396	regulation of protein ubiquitination	7	7.368	0.002783109	11315, 5687, 5688, 7334, 5721, 5685, 5684	93	253	16650	4.9535	0.9991	0.0536	4.7993

GOTERM_BP_FAT	GO:0060071	Wnt signaling pathway, planar cell polarity pathway	5	5.263	0.002919943	5687, 5688, 5721, 5685, 5684	93	107	16650	8.3660	0.9993	0.0557	5.0296
GOTERM_BP_FAT	GO:2001235	positive regulation of apoptotic signaling pathway	6	6.316	0.002994205	11315, 6647, 2923, 7534, 7532, 7531	93	177	16650	6.0689	0.9995	0.0566	5.1543
GOTERM_BP_FAT	GO:0090175	regulation of establishment of planar polarity	5	5.263	0.003225864	5687, 5688, 5721, 5685, 5684	93	110	16650	8.1378	0.9997	0.0604	5.5425
GOTERM_BP_FAT	GO:0006163	purine nucleotide metabolic process	10	10.526	0.0032267	11315, 3251, 654364, 1329, 7167, 10606, 808, 2023, 1345, 4710	93	547	16650	3.2730	0.9997	0.0600	5.5439
GOTERM_BP_FAT	GO:0031398	positive regulation of protein ubiquitination	6	6.316	0.003373815	5687, 5688, 7334, 5721, 5685, 5684	93	182	16650	5.9022	0.9998	0.0622	5.7896
GOTERM_BP_FAT	GO:0002682	regulation of immune system process	17	17.895	0.003421312	654364, 6647, 5243, 3848, 3385, 293, 5721, 5685, 5684, 3383, 5687, 4478, 5688, 7334, 8294, 808, 3105	93	1378	16650	2.2087	0.9998	0.0626	5.8688
GOTERM_BP_FAT	GO:0010941	regulation of cell death	18	18.947	0.003451738	1936, 654364, 6647, 2923, 10961, 5034, 5660, 3336, 3383, 29082, 11315, 10016, 10935, 396, 7534, 7532, 81, 7531	93	1509	16650	2.1356	0.9998	0.0627	5.9195
GOTERM_BP_FAT	GO:0046128	purine ribonucleoside metabolic process	8	8.421	0.003563413	11315, 3251, 654364, 1329, 7167, 2023, 1345, 4710	93	356	16650	4.0232	0.9999	0.0642	6.1054
GOTERM_BP_FAT	GO:0023051	regulation of signaling	29	30.526	0.00359071	1936, 5660, 5685, 5684, 3383, 10016, 7334, 81, 832, 6647, 302, 2923, 10961, 293, 476, 5721, 5052, 5034, 5687, 11315, 5688, 4082, 2079, 2665, 396, 7534, 808, 7532, 7531	93	3070	16650	1.6912	0.9999	0.0642	6.1508
GOTERM_BP_FAT	GO:0051348	negative regulation of	8	8.421	0.003618618	11315, 5687, 5688, 10935, 7532, 5721, 5685, 5684	93	357	16650	4.0119	0.9999	0.0642	6.1972

		transferase activity											
GOTERM_BP_FAT	GO:0009205	purine ribonucleoside triphosphate metabolic process	7	7.368	0.003627631	11315, 654364, 1329, 7167, 2023, 1345, 4710	93	267	16650	4.6937	0.9999	0.0639	6.2121
GOTERM_BP_FAT	GO:1903364	positive regulation of cellular protein catabolic process	6	6.316	0.003701561	5687, 4478, 5688, 5721, 5685, 5684	93	186	16650	5.7752	0.9999	0.0647	6.3349
GOTERM_BP_FAT	GO:0042278	purine nucleoside metabolic process	8	8.421	0.003730985	11315, 3251, 654364, 1329, 7167, 2023, 1345, 4710	93	359	16650	3.9896	0.9999	0.0648	6.3837
GOTERM_BP_FAT	GO:0006364	rRNA processing	7	7.368	0.00376229	6181, 6132, 6224, 6222, 6154, 6165, 6156	93	269	16650	4.6588	0.9999	0.0648	6.4356
GOTERM_BP_FAT	GO:0045088	regulation of innate immune response	8	8.421	0.003788157	5687, 5688, 7334, 3385, 5721, 5685, 3105, 5684	93	360	16650	3.9785	0.9999	0.0648	6.4784
GOTERM_BP_FAT	GO:0009199	ribonucleoside triphosphate metabolic process	7	7.368	0.004042723	11315, 654364, 1329, 7167, 2023, 1345, 4710	93	273	16650	4.5906	1.0000	0.0686	6.8993
GOTERM_BP_FAT	GO:0031347	regulation of defense response	11	11.579	0.004097381	11315, 5687, 5688, 7334, 3848, 293, 3385, 5721, 5685, 3105, 5684	93	676	16650	2.9132	1.0000	0.0690	6.9894
GOTERM_BP_FAT	GO:0009144	purine nucleoside triphosphate metabolic process	7	7.368	0.004115192	11315, 654364, 1329, 7167, 2023, 1345, 4710	93	274	16650	4.5738	1.0000	0.0688	7.0188
GOTERM_BP_FAT	GO:1903320	regulation of protein modification by small protein conjugation or removal	7	7.368	0.004263015	11315, 5687, 5688, 7334, 5721, 5685, 5684	93	276	16650	4.5407	1.0000	0.0708	7.2620
GOTERM_BP_FAT	GO:0016072	rRNA metabolic process	7	7.368	0.004263015	6181, 6132, 6224, 6222, 6154, 6165, 6156	93	276	16650	4.5407	1.0000	0.0708	7.2620

GOTERM_BP_FAT	GO:0042176	regulation of protein catabolic process	8	8.421	0.004397314	11315, 5687, 4478, 5688, 302, 5721, 5685, 5684	93	370	16650	3.8710	1.0000	0.0724	7.4825
GOTERM_BP_FAT	GO:0010035	response to inorganic substance	9	9.474	0.004401799	11315, 10016, 6647, 10935, 51596, 808, 5052, 5660, 3383	93	469	16650	3.4356	1.0000	0.0720	7.4899
GOTERM_BP_FAT	GO:0072521	purine-containing compound metabolic process	10	10.526	0.004474043	11315, 3251, 654364, 1329, 7167, 10606, 808, 2023, 1345, 4710	93	575	16650	3.1136	1.0000	0.0727	7.6083
GOTERM_BP_FAT	GO:0052548	regulation of endopeptidase activity	8	8.421	0.004527575	11315, 10016, 1476, 10935, 7531, 5721, 3336, 5684	93	372	16650	3.8502	1.0000	0.0731	7.6959
GOTERM_BP_FAT	GO:0090263	positive regulation of canonical Wnt signaling pathway	5	5.263	0.00453398	5687, 5688, 5721, 5685, 5684	93	121	16650	7.3980	1.0000	0.0727	7.7064
GOTERM_BP_FAT	GO:0032270	positive regulation of cellular protein metabolic process	17	17.895	0.004587283	6647, 302, 10961, 5721, 5660, 5685, 3336, 5684, 3383, 11315, 5687, 4478, 5688, 10016, 7334, 808, 5859	93	1420	16650	2.1433	1.0000	0.0731	7.7935
GOTERM_BP_FAT	GO:0001736	establishment of planar polarity	5	5.263	0.004668171	5687, 5688, 5721, 5685, 5684	93	122	16650	7.3374	1.0000	0.0738	7.9257
GOTERM_BP_FAT	GO:0007164	establishment of tissue polarity	5	5.263	0.004668171	5687, 5688, 5721, 5685, 5684	93	122	16650	7.3374	1.0000	0.0738	7.9257
GOTERM_BP_FAT	GO:1903322	positive regulation of protein modification by small protein conjugation or removal	6	6.316	0.004821753	5687, 5688, 7334, 5721, 5685, 5684	93	198	16650	5.4252	1.0000	0.0757	8.1761
GOTERM_BP_FAT	GO:0031329	regulation of cellular catabolic process	8	8.421	0.004866021	11315, 5687, 4478, 5688, 302, 5721, 5685, 5684	93	377	16650	3.7991	1.0000	0.0759	8.2481

GOTERM_BP_FAT	GO:0097190	apoptotic signaling pathway	10	10.526	0.004892065	11315, 10016, 6647, 2923, 10961, 7534, 7532, 7531, 5034, 3383	93	583	16650	3.0709	1.0000	0.0758	8.2905
GOTERM_BP_FAT	GO:0006091	generation of precursor metabolites and energy	8	8.421	0.005006625	11315, 1329, 7167, 808, 4190, 2023, 1345, 4710	93	379	16650	3.7790	1.0000	0.0770	8.4767
GOTERM_BP_FAT	GO:0022613	ribonucleoprotein complex biogenesis	9	9.474	0.005051978	6181, 6132, 6224, 6222, 6154, 6165, 1207, 8665, 6156	93	480	16650	3.3569	1.0000	0.0772	8.5502
GOTERM_BP_FAT	GO:0034655	nucleobase-containing compound catabolic process	8	8.421	0.005078067	6181, 3251, 6132, 6224, 6222, 6154, 6165, 6156	93	380	16650	3.7691	1.0000	0.0772	8.5926
GOTERM_BP_FAT	GO:0034614	cellular response to reactive oxygen species	5	5.263	0.005086743	11315, 6647, 10935, 5052, 5660	93	125	16650	7.1613	1.0000	0.0768	8.6066
GOTERM_BP_FAT	GO:0044093	positive regulation of molecular function	20	21.053	0.005115516	6647, 302, 10961, 5721, 5660, 5685, 3336, 5684, 3383, 11315, 5687, 5688, 10016, 7334, 10935, 2665, 396, 808, 81, 6742	93	1840	16650	1.9460	1.0000	0.0768	8.6533
GOTERM_BP_FAT	GO:0051186	cofactor metabolic process	8	8.421	0.005446882	7167, 10135, 4190, 4144, 7086, 2023, 3945, 6576	93	385	16650	3.7202	1.0000	0.0811	9.1887
GOTERM_BP_FAT	GO:0045089	positive regulation of innate immune response	7	7.368	0.005591102	5687, 5688, 7334, 3385, 5721, 5685, 5684	93	292	16650	4.2919	1.0000	0.0827	9.4208
GOTERM_BP_FAT	GO:0030162	regulation of proteolysis	11	11.579	0.005650545	11315, 5687, 5688, 10016, 1476, 10935, 7531, 5721, 5685, 3336, 5684	93	708	16650	2.7816	1.0000	0.0830	9.5163
GOTERM_BP_FAT	GO:0010646	regulation of cell communication	28	29.474	0.005741003	1936, 5660, 5685, 5684, 3383, 10016, 7334, 81, 832, 6647, 302, 2923, 10961, 293, 5721, 5052, 5034, 11315, 5687, 5688, 4082, 2079, 2665, 396, 7534, 808, 7532, 7531	93	3020	16650	1.6599	1.0000	0.0838	9.6615

GOTERM_BP_FAT	GO:0009966	regulation of signal transduction	26	27.368	0.005938727	1936, 5660, 5685, 5684, 3383, 10016, 7334, 81, 832, 6647, 302, 2923, 10961, 5721, 5052, 5034, 11315, 5687, 5688, 2079, 2665, 396, 7534, 808, 7532, 7531	93	2728	16650	1.7063	1.0000	0.0861	9.9780
GOTERM_BP_FAT	GO:0030048	actin filament-based movement	5	5.263	0.006159717	4637, 7171, 476, 81, 7170	93	132	16650	6.7815	1.0000	0.0886	10.3305
GOTERM_BP_FAT	GO:0000302	response to reactive oxygen species	6	6.316	0.006164486	11315, 6647, 10935, 5034, 5052, 5660	93	210	16650	5.1152	1.0000	0.0882	10.3381
GOTERM_BP_FAT	GO:0045087	innate immune response	12	12.632	0.006288685	5687, 5688, 8339, 7334, 3848, 3385, 5721, 5052, 5685, 3105, 5684, 3383	93	836	16650	2.5698	1.0000	0.0894	10.5356
GOTERM_BP_FAT	GO:0052547	regulation of peptidase activity	8	8.421	0.006328939	11315, 10016, 1476, 10935, 7531, 5721, 3336, 5684	93	396	16650	3.6168	1.0000	0.0894	10.5996
GOTERM_BP_FAT	GO:0002768	immune response-regulating cell surface receptor signaling pathway	8	8.421	0.006587051	5687, 5688, 7334, 808, 3385, 5721, 5685, 5684	93	399	16650	3.5896	1.0000	0.0924	11.0085
GOTERM_BP_FAT	GO:0031349	positive regulation of defense response	8	8.421	0.00685295	5687, 5688, 7334, 293, 3385, 5721, 5685, 5684	93	402	16650	3.5628	1.0000	0.0954	11.4279
GOTERM_BP_FAT	GO:0033209	tumor necrosis factor-mediated signaling pathway	5	5.263	0.007373761	5687, 5688, 5721, 5685, 5684	93	139	16650	6.4400	1.0000	0.1017	12.2440
GOTERM_BP_FAT	GO:0044772	mitotic cell cycle phase transition	9	9.474	0.007535185	5687, 5688, 5243, 808, 7532, 7531, 5721, 5685, 5684	93	514	16650	3.1348	1.0000	0.1033	12.4955
GOTERM_BP_FAT	GO:0046700	heterocycle catabolic process	8	8.421	0.007698774	6181, 3251, 6132, 6224, 6222, 6154, 6165, 6156	93	411	16650	3.4848	1.0000	0.1048	12.7497
GOTERM_BP_FAT	GO:0032268	regulation of cellular protein metabolic process	23	24.211	0.007897456	6647, 1476, 302, 10961, 5721, 5052, 5660, 5685, 3336, 5684, 8665, 3383, 5687, 11315, 5688, 4478, 10016, 7334, 10935, 808, 7532, 5859, 7531	93	2346	16650	1.7552	1.0000	0.1068	13.0575

GOTERM_BP_FAT	GO:0051247	positive regulation of protein metabolic process	17	17.895	0.008103349	6647, 302, 10961, 5721, 5660, 5685, 3336, 5684, 3383, 11315, 5687, 4478, 5688, 10016, 7334, 808, 5859	93	1508	16650	2.0183	1.0000	0.1089	13.3754
GOTERM_BP_FAT	GO:0044270	cellular nitrogen compound catabolic process	8	8.421	0.008408394	6181, 3251, 6132, 6224, 6222, 6154, 6165, 6156	93	418	16650	3.4265	1.0000	0.1122	13.8443
GOTERM_BP_FAT	GO:0050851	antigen receptor-mediated signaling pathway	6	6.316	0.008642865	5687, 5688, 7334, 5721, 5685, 5684	93	228	16650	4.7114	1.0000	0.1145	14.2031
GOTERM_BP_FAT	GO:0042180	cellular ketone metabolic process	6	6.316	0.008798073	11315, 5687, 5688, 5721, 5685, 5684	93	229	16650	4.6908	1.0000	0.1159	14.4399
GOTERM_BP_FAT	GO:0006457	protein folding	6	6.316	0.008798073	10130, 9601, 2923, 10961, 9352, 3336	93	229	16650	4.6908	1.0000	0.1159	14.4399
GOTERM_BP_FAT	GO:0051347	positive regulation of transferase activity	10	10.526	0.008820658	11315, 5687, 5688, 6647, 7334, 10961, 808, 5721, 5685, 5684	93	640	16650	2.7974	1.0000	0.1155	14.4742
GOTERM_BP_FAT	GO:0019439	aromatic compound catabolic process	8	8.421	0.008944084	6181, 3251, 6132, 6224, 6222, 6154, 6165, 6156	93	423	16650	3.3860	1.0000	0.1164	14.6620
GOTERM_BP_FAT	GO:1903050	regulation of proteolysis involved in cellular protein catabolic process	6	6.316	0.008955203	11315, 5687, 5688, 5721, 5685, 5684	93	230	16650	4.6704	1.0000	0.1160	14.6789
GOTERM_BP_FAT	GO:0016310	phosphorylation	22	23.158	0.009079375	654364, 6647, 302, 1329, 10961, 5721, 5052, 5660, 5685, 5684, 4710, 3383, 5687, 11315, 5688, 7167, 51727, 10935, 808, 2023, 7532, 1345	93	2229	16650	1.7670	1.0000	0.1169	14.8674
GOTERM_BP_FAT	GO:0010033	response to organic substance	26	27.368	0.009149939	10130, 1933, 5660, 3336, 5685, 5684, 3383, 10016, 10135, 4678, 3105, 3251, 654364, 6647, 302, 6132, 476, 5721, 11315, 5687, 4478, 5688, 10935, 396, 808, 7532	93	2822	16650	1.6495	1.0000	0.1172	14.9743

GOTERM_BP_FAT	GO:0006334	nucleosome assembly	5	5.263	0.009804026	8339, 8350, 4673, 8294, 4678	93	151	16650	5.9282	1.0000	0.1244	15.9594
GOTERM_BP_FAT	GO:0051641	cellular localization	24	25.263	0.00992952	6181, 6647, 302, 2923, 6132, 1329, 10961, 6154, 293, 6165, 5052, 6156, 29082, 11315, 4478, 10016, 6224, 6222, 7534, 808, 7532, 81, 7531, 26520	93	2541	16650	1.6910	1.0000	0.1253	16.1471
GOTERM_BP_FAT	GO:0030177	positive regulation of Wnt signaling pathway	5	5.263	0.010483725	5687, 5688, 5721, 5685, 5684	93	154	16650	5.8127	1.0000	0.1312	16.9716
GOTERM_BP_FAT	GO:0042254	ribosome biogenesis	7	7.368	0.010639501	6181, 6132, 6224, 6222, 6154, 6165, 6156	93	335	16650	3.7410	1.0000	0.1323	17.2020
GOTERM_BP_FAT	GO:0044770	cell cycle phase transition	9	9.474	0.01073764	5687, 5688, 5243, 808, 7532, 7531, 5721, 5685, 5684	93	547	16650	2.9457	1.0000	0.1328	17.3468
GOTERM_BP_FAT	GO:0043085	positive regulation of catalytic activity	17	17.895	0.010980235	6647, 10961, 5721, 5660, 5685, 3336, 5684, 3383, 11315, 5687, 5688, 10016, 7334, 2665, 396, 808, 6742	93	1559	16650	1.9522	1.0000	0.1350	17.7038
GOTERM_BP_FAT	GO:0046034	ATP metabolic process	6	6.316	0.011177965	11315, 1329, 7167, 2023, 1345, 4710	93	243	16650	4.4205	1.0000	0.1366	17.9936
GOTERM_BP_FAT	GO:0071356	cellular response to tumor necrosis factor	6	6.316	0.011363187	5687, 5688, 5721, 5685, 5684, 3383	93	244	16650	4.4024	1.0000	0.1380	18.2643
GOTERM_BP_FAT	GO:0010565	regulation of cellular ketone metabolic process	5	5.263	0.011436496	5687, 5688, 5721, 5685, 5684	93	158	16650	5.6656	1.0000	0.1382	18.3712
GOTERM_BP_FAT	GO:1901361	organic cyclic compound catabolic process	8	8.421	0.011603843	6181, 3251, 6132, 6224, 6222, 6154, 6165, 6156	93	445	16650	3.2186	1.0000	0.1394	18.6147
GOTERM_BP_FAT	GO:0050776	regulation of immune response	12	12.632	0.011685485	5687, 5688, 7334, 3848, 808, 293, 3385, 5721, 5685, 3105, 5684, 3383	93	912	16650	2.3557	1.0000	0.1396	18.7333
GOTERM_BP_FAT	GO:0016071	mRNA metabolic process	10	10.526	0.011888562	6181, 3189, 6132, 6224, 6222, 6154, 6165, 1207, 6156, 51691	93	672	16650	2.6642	1.0000	0.1412	19.0274

GOTERM_BP_FAT	GO:000209	protein polyubiquitination	6	6.316	0.012921023	5687, 5688, 7334, 5721, 5685, 5684	93	252	16650	4.2627	1.0000	0.1519	20.5076
GOTERM_BP_FAT	GO:0090090	negative regulation of canonical Wnt signaling pathway	5	5.263	0.013234772	5687, 5688, 5721, 5685, 5684	93	165	16650	5.4252	1.0000	0.1546	20.9523
GOTERM_BP_FAT	GO:0044085	cellular component biogenesis	26	27.368	0.013573988	6181, 8339, 6165, 3383, 29082, 4678, 81, 3251, 654364, 832, 302, 6132, 4673, 6154, 8665, 4710, 6156, 11315, 4478, 8350, 6224, 6222, 8294, 7534, 4144, 1207	93	2914	16650	1.5974	1.0000	0.1575	21.4305
GOTERM_BP_FAT	GO:0043065	positive regulation of apoptotic process	9	9.474	0.013767865	11315, 10016, 6647, 2923, 7534, 7532, 7531, 3336, 3383	93	572	16650	2.8169	1.0000	0.1588	21.7026
GOTERM_BP_FAT	GO:0031497	chromatin assembly	5	5.263	0.013779796	8339, 8350, 4673, 8294, 4678	93	167	16650	5.3602	1.0000	0.1583	21.7193
GOTERM_BP_FAT	GO:0045732	positive regulation of protein catabolic process	6	6.316	0.013964905	5687, 4478, 5688, 5721, 5685, 5684	93	257	16650	4.1797	1.0000	0.1595	21.9782
GOTERM_BP_FAT	GO:0009260	ribonucleotide biosynthetic process	6	6.316	0.014180305	3251, 654364, 1329, 10606, 51727, 808	93	258	16650	4.1635	1.0000	0.1610	22.2784
GOTERM_BP_FAT	GO:0045862	positive regulation of proteolysis	7	7.368	0.014380113	5687, 5688, 10016, 5721, 5685, 3336, 5684	93	358	16650	3.5006	1.0000	0.1624	22.5560
GOTERM_BP_FAT	GO:0043068	positive regulation of programmed cell death	9	9.474	0.014442365	11315, 10016, 6647, 2923, 7534, 7532, 7531, 3336, 3383	93	577	16650	2.7925	1.0000	0.1623	22.6422
GOTERM_BP_FAT	GO:0031400	negative regulation of protein modification process	9	9.474	0.01458012	11315, 5687, 5688, 808, 7532, 7531, 5721, 5685, 5684	93	578	16650	2.7877	1.0000	0.1630	22.8328

GOTERM_BP_FAT	GO:0046390	ribose phosphate biosynthetic process	6	6.316	0.014839931	3251, 654364, 1329, 10606, 51727, 808	93	261	16650	4.1157	1.0000	0.1650	23.1911
GOTERM_BP_FAT	GO:1903052	positive regulation of proteolysis involved in cellular protein catabolic process	5	5.263	0.014912274	5687, 5688, 5721, 5685, 5684	93	171	16650	5.2349	1.0000	0.1650	23.2906
GOTERM_BP_FAT	GO:0034612	response to tumor necrosis factor	6	6.316	0.015290964	5687, 5688, 5721, 5685, 5684, 3383	93	263	16650	4.0844	1.0000	0.1681	23.8093
GOTERM_BP_FAT	GO:0010608	posttranscriptional regulation of gene expression	8	8.421	0.015602898	5687, 5688, 7534, 5721, 5859, 5685, 5684, 8665	93	472	16650	3.0344	1.0000	0.1705	24.2341
GOTERM_BP_FAT	GO:0045333	cellular respiration	5	5.263	0.015799204	11315, 1329, 4190, 1345, 4710	93	174	16650	5.1446	1.0000	0.1717	24.5003
GOTERM_BP_FAT	GO:0051338	regulation of transferase activity	12	12.632	0.016020593	11315, 5687, 5688, 6647, 7334, 10961, 10935, 808, 7532, 5721, 5685, 5684	93	955	16650	2.2496	1.0000	0.1732	24.7995
GOTERM_BP_FAT	GO:0002429	immune response-activating cell surface receptor signaling pathway	7	7.368	0.016066863	5687, 5688, 7334, 3385, 5721, 5685, 5684	93	367	16650	3.4148	1.0000	0.1729	24.8618
GOTERM_BP_FAT	GO:0050790	regulation of catalytic activity	22	23.158	0.016556598	6647, 1476, 302, 10961, 5721, 5660, 5685, 3336, 5684, 3383, 5687, 11315, 5688, 10016, 7334, 10935, 2665, 396, 808, 7532, 7531, 6742	93	2357	16650	1.6711	1.0000	0.1770	25.5191
GOTERM_BP_FAT	GO:0051246	regulation of protein metabolic process	23	24.211	0.01695505	6647, 1476, 302, 10961, 5721, 5052, 5660, 5685, 3336, 5684, 8665, 3383, 5687, 11315, 5688, 4478, 10016, 7334, 10935, 808, 7532, 5859, 7531	93	2512	16650	1.6392	1.0000	0.1801	26.0498
GOTERM_BP_FAT	GO:0034728	nucleosome organization	5	5.263	0.017032567	8339, 8350, 4673, 8294, 4678	93	178	16650	5.0290	1.0000	0.1801	26.1527
GOTERM_BP_FAT	GO:0015980	energy derivation by oxidation of	6	6.316	0.01718697	11315, 1329, 808, 4190, 1345, 4710	93	271	16650	3.9638	1.0000	0.1808	26.3571

		organic compounds											
GOTERM_BP_FAT	GO:0043086	negative regulation of catalytic activity	11	11.579	0.017288684	11315, 5687, 5688, 1476, 302, 10935, 7532, 7531, 5721, 5685, 5684	93	839	16650	2.3473	1.0000	0.1810	26.4915
GOTERM_BP_FAT	GO:0031331	positive regulation of cellular catabolic process	6	6.316	0.017434456	5687, 4478, 5688, 5721, 5685, 5684	93	272	16650	3.9492	1.0000	0.1817	26.6837
GOTERM_BP_FAT	GO:0000165	MAPK cascade	11	11.579	0.017549109	5687, 5688, 6647, 10961, 808, 5721, 5052, 5660, 5685, 5684, 3383	93	841	16650	2.3417	1.0000	0.1820	26.8345
GOTERM_BP_FAT	GO:0044283	small molecule biosynthetic process	8	8.421	0.018391588	2224, 11315, 6647, 7167, 4190, 7086, 2023, 6576	93	488	16650	2.9350	1.0000	0.1892	27.9339
GOTERM_BP_FAT	GO:0006839	mitochondrial transport	6	6.316	0.018707452	1329, 7534, 293, 7532, 26520, 7531	93	277	16650	3.8780	1.0000	0.1913	28.3420
GOTERM_BP_FAT	GO:0010942	positive regulation of cell death	9	9.474	0.019007367	11315, 10016, 6647, 2923, 7534, 7532, 7531, 3336, 3383	93	607	16650	2.6545	1.0000	0.1933	28.7275
GOTERM_BP_FAT	GO:1901135	carbohydrate derivative metabolic process	14	14.737	0.01930743	3251, 654364, 1329, 5660, 4710, 11315, 2079, 7167, 10606, 51727, 808, 7086, 2023, 1345	93	1247	16650	2.0100	1.0000	0.1953	29.1113
GOTERM_BP_FAT	GO:0072655	establishment of protein localization to mitochondrion	5	5.263	0.020374809	7534, 293, 7532, 26520, 7531	93	188	16650	4.7615	1.0000	0.2042	30.4606
GOTERM_BP_FAT	GO:0032269	negative regulation of cellular protein metabolic process	12	12.632	0.020641929	11315, 5687, 5688, 1476, 302, 10935, 808, 7532, 7531, 5721, 5685, 5684	93	992	16650	2.1657	1.0000	0.2058	30.7945
GOTERM_BP_FAT	GO:0042981	regulation of apoptotic process	15	15.789	0.021186504	654364, 6647, 2923, 10961, 5034, 3336, 3383, 11315, 10016, 10935, 7534, 396, 7532, 7531, 81	93	1400	16650	1.9182	1.0000	0.2099	31.4705

GOTERM_BP_FAT	GO:0006333	chromatin assembly or disassembly	5	5.263	0.021450825	8339, 8350, 4673, 8294, 4678	93	191	16650	4.6867	1.0000	0.2114	31.7963
GOTERM_BP_FAT	GO:0070585	protein localization to mitochondrion	5	5.263	0.021450825	7534, 293, 7532, 26520, 7531	93	191	16650	4.6867	1.0000	0.2114	31.7963
GOTERM_BP_FAT	GO:0009894	regulation of catabolic process	8	8.421	0.022563486	11315, 5687, 4478, 5688, 302, 5721, 5685, 5684	93	509	16650	2.8139	1.0000	0.2203	33.1520
GOTERM_BP_FAT	GO:0043067	regulation of programmed cell death	15	15.789	0.022746632	654364, 6647, 2923, 10961, 5034, 3336, 3383, 11315, 10016, 10935, 7534, 396, 7532, 7531, 81	93	1413	16650	1.9006	1.0000	0.2211	33.3727
GOTERM_BP_FAT	GO:0023014	signal transduction by protein phosphorylation	11	11.579	0.022752415	5687, 5688, 6647, 10961, 808, 5721, 5052, 5660, 5685, 5684, 3383	93	877	16650	2.2456	1.0000	0.2203	33.3797
GOTERM_BP_FAT	GO:0031401	positive regulation of protein modification process	13	13.684	0.023213773	5687, 11315, 5688, 6647, 302, 7334, 10961, 808, 5721, 5660, 5685, 5684, 3383	93	1143	16650	2.0362	1.0000	0.2234	33.9325
GOTERM_BP_FAT	GO:0016051	carbohydrate biosynthetic process	5	5.263	0.023320608	7167, 4190, 7086, 2023, 6576	93	196	16650	4.5671	1.0000	0.2235	34.0599
GOTERM_BP_FAT	GO:0065003	macromolecular complex assembly	17	17.895	0.02446403	3251, 832, 654364, 302, 8339, 4673, 4710, 8665, 3383, 29082, 11315, 4478, 8350, 8294, 4678, 4144, 1207	93	1710	16650	1.7799	1.0000	0.2323	35.4091
GOTERM_BP_FAT	GO:0043281	regulation of cysteine-type endopeptidase activity involved in apoptotic process	5	5.263	0.024885848	11315, 10016, 10935, 7531, 3336	93	200	16650	4.4758	1.0000	0.2349	35.9002

GOTERM_BP_FAT	GO:0030178	negative regulation of Wnt signaling pathway	5	5.263	0.025286866	5687, 5688, 5721, 5685, 5684	93	201	16650	4.4535	1.0000	0.2374	36.3638
GOTERM_BP_FAT	GO:0009636	response to toxic substance	5	5.263	0.025691782	11315, 6647, 10935, 9352, 5052	93	202	16650	4.4315	1.0000	0.2399	36.8287
GOTERM_BP_FAT	GO:0034470	ncRNA processing	7	7.368	0.02630744	6181, 6132, 6224, 6222, 6154, 6165, 6156	93	411	16650	3.0492	1.0000	0.2440	37.5295
GOTERM_BP_FAT	GO:0006323	DNA packaging	5	5.263	0.026929988	8339, 8350, 4673, 8294, 4678	93	205	16650	4.3666	1.0000	0.2482	38.2306
GOTERM_BP_FAT	GO:0006461	protein complex assembly	15	15.789	0.026957351	3251, 832, 654364, 302, 8339, 4673, 4710, 3383, 29082, 11315, 4478, 8350, 8294, 4678, 4144	93	1445	16650	1.8585	1.0000	0.2475	38.2612
GOTERM_BP_FAT	GO:0070271	protein complex biogenesis	15	15.789	0.027097733	3251, 832, 654364, 302, 8339, 4673, 4710, 3383, 29082, 11315, 4478, 8350, 8294, 4678, 4144	93	1446	16650	1.8572	1.0000	0.2478	38.4182
GOTERM_BP_FAT	GO:0002764	immune response-regulating signaling pathway	8	8.421	0.027597257	5687, 5688, 7334, 808, 3385, 5721, 5685, 5684	93	531	16650	2.6973	1.0000	0.2509	38.9738
GOTERM_BP_FAT	GO:0007005	mitochondrion organization	9	9.474	0.027897372	11315, 10935, 7534, 293, 7532, 26520, 7531, 6742, 4710	93	653	16650	2.4675	1.0000	0.2524	39.3054
GOTERM_BP_FAT	GO:0010038	response to metal ion	6	6.316	0.027977156	11315, 10016, 6647, 51596, 808, 3383	93	308	16650	3.4876	1.0000	0.2521	39.3932
GOTERM_BP_FAT	GO:0070887	cellular response to chemical stimulus	23	24.211	0.029390706	654364, 10130, 6647, 6132, 9352, 476, 5721, 5034, 5052, 5660, 5685, 5684, 3383, 5687, 11315, 5688, 4478, 10016, 10935, 396, 10135, 7532, 3105	93	2648	16650	1.5550	1.0000	0.2622	40.9299
GOTERM_BP_FAT	GO:0051248	negative regulation of protein metabolic process	12	12.632	0.029608854	11315, 5687, 5688, 1476, 302, 10935, 808, 7532, 7531, 5721, 5685, 5684	93	1049	16650	2.0480	1.0000	0.2630	41.1638
GOTERM_BP_FAT	GO:0022603	regulation of anatomical	12	12.632	0.029967652	5687, 4478, 5688, 10016, 832, 3848, 396, 81, 5721, 5685, 5684, 3383	93	1051	16650	2.0441	1.0000	0.2649	41.5465

		structure morphogenesis											
GOTERM_BP_FAT	GO:1901700	response to oxygen-containing compound	15	15.789	0.030021775	654364, 6647, 1933, 476, 5052, 5660, 5034, 3383, 11315, 4478, 10935, 10135, 808, 4678, 7532	93	1466	16650	1.8318	1.0000	0.2644	41.6040
GOTERM_BP_FAT	GO:1902589	single-organism organelle organization	16	16.842	0.030510155	832, 6647, 302, 3383, 29082, 11315, 83473, 7534, 396, 7171, 7532, 7531, 26520, 81, 6742, 7170	93	1612	16650	1.7770	1.0000	0.2672	42.1207
GOTERM_BP_FAT	GO:0051336	regulation of hydrolase activity	14	14.737	0.031915947	11315, 10016, 6647, 1476, 10935, 2665, 396, 808, 5721, 7531, 6742, 3336, 5684, 3383	93	1337	16650	1.8747	1.0000	0.2768	43.5841
GOTERM_BP_FAT	GO:0002253	activation of immune response	8	8.421	0.032522411	5687, 5688, 7334, 3848, 3385, 5721, 5685, 5684	93	550	16650	2.6041	1.0000	0.2804	44.2045
GOTERM_BP_FAT	GO:0033554	cellular response to stress	17	17.895	0.032866322	10130, 654364, 6647, 9601, 2923, 10961, 9352, 5034, 5052, 5660, 3383, 11315, 10016, 7334, 10935, 8294, 7531	93	1773	16650	1.7166	1.0000	0.2820	44.5535
GOTERM_BP_FAT	GO:0072593	reactive oxygen species metabolic process	5	5.263	0.034618071	11315, 6647, 10935, 5052, 3383	93	222	16650	4.0323	1.0000	0.2938	46.2994
GOTERM_BP_FAT	GO:0042787	protein ubiquitination involved in ubiquitin-dependent protein catabolic process	5	5.263	0.034618071	5687, 5688, 5721, 5685, 5684	93	222	16650	4.0323	1.0000	0.2938	46.2994
GOTERM_BP_FAT	GO:0050778	positive regulation of immune response	9	9.474	0.035316596	5687, 5688, 7334, 3848, 3385, 5721, 5685, 3105, 5684	93	684	16650	2.3557	1.0000	0.2979	46.9810
GOTERM_BP_FAT	GO:2000116	regulation of cysteine-type endopeptidase activity	5	5.263	0.035598253	11315, 10016, 10935, 7531, 3336	93	224	16650	3.9963	1.0000	0.2989	47.2535

GOTERM_BP_FAT	GO:0042592	homeostatic process	16	16.842	0.035641704	10130, 654364, 6647, 9601, 2923, 3848, 9352, 476, 5034, 5052, 94081, 3383, 10935, 8294, 808, 1207	93	1645	16650	1.7413	1.0000	0.2982	47.2954
GOTERM_BP_FAT	GO:0006936	muscle contraction	6	6.316	0.036468057	6647, 4637, 808, 7171, 476, 7170	93	331	16650	3.2453	1.0000	0.3031	48.0868
GOTERM_BP_FAT	GO:0044257	cellular protein catabolic process	9	9.474	0.036628028	11315, 5687, 4478, 5688, 302, 7334, 5721, 5685, 5684	93	689	16650	2.3386	1.0000	0.3033	48.2386
GOTERM_BP_FAT	GO:0009896	positive regulation of catabolic process	6	6.316	0.03727382	5687, 4478, 5688, 5721, 5685, 5684	93	333	16650	3.2258	1.0000	0.3068	48.8476
GOTERM_BP_FAT	GO:0034660	ncRNA metabolic process	8	8.421	0.038312893	6181, 6132, 6224, 6222, 6154, 6165, 5859, 6156	93	570	16650	2.5127	1.0000	0.3130	49.8132
GOTERM_BP_FAT	GO:0008219	cell death	18	18.947	0.038623652	654364, 6647, 2923, 10961, 293, 5034, 5660, 3336, 3383, 29082, 11315, 10016, 10935, 396, 7534, 7532, 7531, 81	93	1958	16650	1.6459	1.0000	0.3141	50.0986
GOTERM_BP_FAT	GO:0031399	regulation of protein modification process	16	16.842	0.038870462	5687, 11315, 5688, 6647, 302, 7334, 10961, 808, 7532, 5721, 7531, 5660, 5052, 5685, 5684, 3383	93	1664	16650	1.7215	1.0000	0.3148	50.3242
GOTERM_BP_FAT	GO:0002684	positive regulation of immune system process	11	11.579	0.038906381	5687, 5688, 5243, 7334, 3848, 3385, 5721, 5685, 3105, 5684, 3383	93	960	16650	2.0514	1.0000	0.3141	50.3569
GOTERM_BP_FAT	GO:0044092	negative regulation of molecular function	12	12.632	0.039770833	11315, 5687, 5688, 1476, 302, 10935, 808, 7532, 7531, 5721, 5685, 5684	93	1100	16650	1.9531	1.0000	0.3189	51.1391
GOTERM_BP_FAT	GO:0051091	positive regulation of sequence-specific DNA binding transcription factor activity	5	5.263	0.040207683	11315, 5687, 7334, 10935, 3383	93	233	16650	3.8419	1.0000	0.3209	51.5299

GOTERM_BP_FAT	GO:0007596	blood coagulation	6	6.316	0.041035431	832, 302, 8350, 3848, 7534, 953	93	342	16650	3.1409	1.0000	0.3254	52.2623
GOTERM_BP_FAT	GO:0065004	protein-DNA complex assembly	5	5.263	0.041276209	8339, 8350, 4673, 8294, 4678	93	235	16650	3.8092	1.0000	0.3260	52.4734
GOTERM_BP_FAT	GO:0080135	regulation of cellular response to stress	8	8.421	0.041771254	11315, 6647, 7334, 10961, 7531, 5034, 5052, 5660	93	581	16650	2.4652	1.0000	0.3282	52.9046
GOTERM_BP_FAT	GO:0060828	regulation of canonical Wnt signaling pathway	5	5.263	0.044578327	5687, 5688, 5721, 5685, 5684	93	241	16650	3.7144	1.0000	0.3453	55.2808
GOTERM_BP_FAT	GO:0006955	immune response	15	15.789	0.045102661	8339, 3848, 931, 3385, 293, 5721, 5052, 5685, 5684, 3383, 5687, 5688, 7334, 808, 3105	93	1551	16650	1.7315	1.0000	0.3476	55.7119
GOTERM_BP_FAT	GO:0005996	monosaccharide metabolic process	5	5.263	0.045711204	7167, 4190, 7086, 2023, 6576	93	243	16650	3.6838	1.0000	0.3504	56.2074
GOTERM_BP_FAT	GO:0009152	purine ribonucleotide biosynthetic process	5	5.263	0.046860155	3251, 654364, 1329, 10606, 808	93	245	16650	3.6537	1.0000	0.3565	57.1286
GOTERM_BP_FAT	GO:0051259	protein oligomerization	7	7.368	0.047005405	29082, 3251, 654364, 302, 8350, 8294, 4144	93	473	16650	2.6495	1.0000	0.3564	57.2437
GOTERM_BP_FAT	GO:0010638	positive regulation of organelle organization	8	8.421	0.048214351	11315, 4478, 302, 7334, 7534, 7532, 7531, 3383	93	600	16650	2.3871	1.0000	0.3628	58.1909
GOTERM_BP_FAT	GO:0050817	coagulation	6	6.316	0.0482759	832, 302, 8350, 3848, 7534, 953	93	358	16650	3.0005	1.0000	0.3621	58.2386
GOTERM_BP_FAT	GO:0007599	hemostasis	6	6.316	0.0482759	832, 302, 8350, 3848, 7534, 953	93	358	16650	3.0005	1.0000	0.3621	58.2386
GOTERM_BP_FAT	GO:0034622	cellular macromolecular complex assembly	11	11.579	0.050119056	29082, 832, 8339, 8350, 4673, 8294, 4678, 1207, 8665, 4710, 3383	93	1004	16650	1.9615	1.0000	0.3722	59.6431
GOTERM_BP_FAT	GO:0006164	purine nucleotide biosynthetic process	5	5.263	0.051007881	3251, 654364, 1329, 10606, 808	93	252	16650	3.5522	1.0000	0.3764	60.3043

GOTERM_BP_FAT	GO:2000027	regulation of organ morphogenesis	5	5.263	0.051007881	5687, 5688, 5721, 5685, 5684	93	252	16650	3.5522	1.0000	0.3764	60.3043
GOTERM_BP_FAT	GO:0071310	cellular response to organic substance	19	20.000	0.052212466	10130, 654364, 6647, 6132, 476, 5721, 5660, 5685, 5684, 3383, 5687, 11315, 4478, 5688, 10016, 10135, 396, 7532, 3105	93	2186	16650	1.5561	1.0000	0.3825	61.1842
GOTERM_BP_FAT	GO:0015672	monovalent inorganic cation transport	7	7.368	0.052348837	11315, 1329, 476, 1347, 7531, 81, 1345	93	486	16650	2.5787	1.0000	0.3822	61.2826
GOTERM_BP_FAT	GO:0035556	intracellular signal transduction	22	23.158	0.053124928	1936, 832, 6647, 10961, 5721, 5034, 5052, 5660, 5685, 5684, 3383, 5687, 11315, 5688, 10016, 7334, 2665, 396, 808, 7531, 81, 5878	93	2657	16650	1.4824	1.0000	0.3857	61.8384
GOTERM_BP_FAT	GO:0043161	proteasome-mediated ubiquitin-dependent protein catabolic process	6	6.316	0.056751806	11315, 5687, 5688, 5721, 5685, 5684	93	375	16650	2.8645	1.0000	0.4053	64.3374
GOTERM_BP_FAT	GO:1903047	mitotic cell cycle process	10	10.526	0.057115731	5687, 29082, 5688, 5243, 808, 7532, 7531, 5721, 5685, 5684	93	890	16650	2.0116	1.0000	0.4062	64.5794
GOTERM_BP_FAT	GO:0071824	protein-DNA complex subunit organization	5	5.263	0.057272898	8339, 8350, 4673, 8294, 4678	93	262	16650	3.4166	1.0000	0.4060	64.6835
GOTERM_BP_FAT	GO:0002757	immune response-activating signal transduction	7	7.368	0.057600108	5687, 5688, 7334, 3385, 5721, 5685, 5684	93	498	16650	2.5165	1.0000	0.4067	64.8992
GOTERM_BP_FAT	GO:0072522	purine-containing compound biosynthetic process	5	5.263	0.057921267	3251, 654364, 1329, 10606, 808	93	263	16650	3.4037	1.0000	0.4074	65.1097

GOTERM_BP_FAT	GO:0060548	negative regulation of cell death	10	10.526	0.059431665	11315, 29082, 654364, 6647, 10935, 396, 7534, 7531, 5660, 3383	93	897	16650	1.9959	1.0000	0.4146	66.0838
GOTERM_BP_FAT	GO:0055085	transmembrane transport	13	13.684	0.059759516	1329, 5243, 293, 476, 94081, 11315, 808, 7531, 26520, 81, 1347, 1345, 6576	93	1322	16650	1.7605	1.0000	0.4153	66.2918
GOTERM_BP_FAT	GO:0034976	response to endoplasmic reticulum stress	5	5.263	0.061894458	11315, 10130, 9601, 2923, 5034	93	269	16650	3.3277	1.0000	0.4256	67.6173
GOTERM_BP_FAT	GO:0065009	regulation of molecular function	23	24.211	0.062078531	6647, 1476, 302, 10961, 5721, 5660, 5685, 3336, 5684, 3383, 5687, 11315, 5688, 10016, 7334, 10935, 2665, 396, 808, 7532, 7531, 81, 6742	93	2863	16650	1.4383	1.0000	0.4255	67.7292
GOTERM_BP_FAT	GO:0006396	RNA processing	10	10.526	0.063893096	6181, 3189, 6132, 6224, 6222, 6154, 6165, 1207, 6156, 51691	93	910	16650	1.9674	1.0000	0.4339	68.8134
GOTERM_BP_FAT	GO:1901701	cellular response to oxygen-containing compound	10	10.526	0.064598087	11315, 4478, 654364, 6647, 10935, 10135, 7532, 5052, 5660, 3383	93	912	16650	1.9631	1.0000	0.4365	69.2253
GOTERM_BP_FAT	GO:0060429	epithelium development	11	11.579	0.067309492	5687, 4478, 5688, 654364, 3189, 6647, 5721, 5660, 5685, 5684, 3383	93	1060	16650	1.8579	1.0000	0.4492	70.7622
GOTERM_BP_FAT	GO:0060070	canonical Wnt signaling pathway	5	5.263	0.072443197	5687, 5688, 5721, 5685, 5684	93	284	16650	3.1520	1.0000	0.4735	73.4757
GOTERM_BP_FAT	GO:0071103	DNA conformation change	5	5.263	0.072443197	8339, 8350, 4673, 8294, 4678	93	284	16650	3.1520	1.0000	0.4735	73.4757
GOTERM_BP_FAT	GO:0010498	proteasomal protein catabolic process	6	6.316	0.072474493	11315, 5687, 5688, 5721, 5685, 5684	93	403	16650	2.6655	1.0000	0.4725	73.4915
GOTERM_BP_FAT	GO:0048585	negative regulation of	13	13.684	0.073660851	5687, 11315, 5688, 10016, 302, 3848, 808, 5721, 5660, 5685, 3105, 5684, 3383	93	1369	16650	1.7001	1.0000	0.4771	74.0835

		response to stimulus											
GOTERM_BP_FAT	GO:0003012	muscle system process	6	6.316	0.073680998	6647, 4637, 808, 7171, 476, 7170	93	405	16650	2.6523	1.0000	0.4760	74.0935
GOTERM_BP_FAT	GO:0006952	defense response	14	14.737	0.073831264	8339, 3848, 3385, 293, 5721, 5052, 5685, 5684, 3383, 11315, 5687, 5688, 7334, 3105	93	1518	16650	1.6512	1.0000	0.4756	74.1675
GOTERM_BP_FAT	GO:0071822	protein complex subunit organization	15	15.789	0.075500329	3251, 832, 654364, 302, 8339, 4673, 4710, 3383, 29082, 11315, 4478, 8350, 8294, 4678, 4144	93	1674	16650	1.6042	1.0000	0.4824	74.9769
GOTERM_BP_FAT	GO:1901615	organic hydroxy compound metabolic process	6	6.316	0.076745527	2224, 11315, 3251, 6647, 7167, 808	93	410	16650	2.6200	1.0000	0.4870	75.5650
GOTERM_BP_FAT	GO:0030029	actin filament-based process	8	8.421	0.078218289	832, 4637, 396, 7171, 476, 81, 7170, 3383	93	672	16650	2.1313	1.0000	0.4927	76.2438
GOTERM_BP_FAT	GO:0030163	protein catabolic process	9	9.474	0.07934631	11315, 5687, 4478, 5688, 302, 7334, 5721, 5685, 5684	93	811	16650	1.9868	1.0000	0.4967	76.7517
GOTERM_BP_FAT	GO:0012501	programmed cell death	16	16.842	0.082980733	654364, 6647, 2923, 10961, 293, 5034, 3336, 3383, 11315, 10016, 10935, 396, 7534, 7532, 7531, 81	93	1852	16650	1.5467	1.0000	0.5118	78.3192
GOTERM_BP_FAT	GO:0032880	regulation of protein localization	10	10.526	0.083856467	11315, 4478, 4082, 10961, 396, 7534, 293, 7532, 7531, 5052	93	962	16650	1.8610	1.0000	0.5145	78.6817
GOTERM_BP_FAT	GO:0010467	gene expression	37	38.947	0.084339108	1936, 6181, 1933, 6165, 5685, 5684, 3383, 51691, 9124, 7334, 3012, 10135, 7112, 654364, 6132, 10961, 6154, 293, 5721, 8665, 6156, 5687, 11315, 5688, 4478, 3189, 3182, 8350, 6224, 10935, 6222, 7534, 8294, 2023, 5859, 1207, 6576	93	5306	16650	1.2484	1.0000	0.5154	78.8790
GOTERM_BP_FAT	GO:0019221	cytokine-mediated signaling pathway	7	7.368	0.086185876	5687, 5688, 5721, 5685, 3105, 5684, 3383	93	554	16650	2.2621	1.0000	0.5223	79.6183

GOTERM_BP_FAT	GO:000278	mitotic cell cycle	10	10.526	0.086380059	5687, 29082, 5688, 5243, 808, 7532, 7531, 5721, 5685, 5684	93	968	16650	1.8495	1.0000	0.5219	79.6946
GOTERM_BP_FAT	GO:1902531	regulation of intracellular signal transduction	15	15.789	0.08777809	1936, 832, 6647, 10961, 5052, 5660, 5034, 3383, 11315, 10016, 7334, 2665, 396, 808, 81	93	1714	16650	1.5668	1.0000	0.5267	80.2360
GOTERM_BP_FAT	GO:0006812	cation transport	10	10.526	0.089382026	11315, 1329, 808, 476, 1347, 7531, 81, 1345, 94081, 3383	93	975	16650	1.8362	1.0000	0.5323	80.8403
GOTERM_BP_FAT	GO:1901990	regulation of mitotic cell cycle phase transition	5	5.263	0.094414626	5687, 5688, 5721, 5685, 5684	93	312	16650	2.8691	1.0000	0.5517	82.6254
GOTERM_BP_FAT	GO:0030111	regulation of Wnt signaling pathway	5	5.263	0.09778665	5687, 5688, 5721, 5685, 5684	93	316	16650	2.8328	1.0000	0.5639	83.7324
GOTERM_BP_FAT	GO:0010604	positive regulation of macromolecule metabolic process	22	23.158	0.098263848	654364, 6647, 302, 10961, 5721, 5660, 5685, 3336, 5684, 3383, 5687, 11315, 5688, 4478, 10016, 3182, 7334, 8350, 8294, 10135, 808, 5859	93	2856	16650	1.3791	1.0000	0.5646	83.8836
GOTERM_BP_FAT	GO:0006915	apoptotic process	15	15.789	0.098828425	654364, 6647, 2923, 10961, 293, 5034, 3336, 3383, 11315, 10016, 10935, 7534, 396, 7532, 7531	93	1747	16650	1.5372	1.0000	0.5656	84.0608
GOTERM_BP_FAT	GO:0006511	ubiquitin-dependent protein catabolic process	7	7.368	0.099857191	11315, 5687, 5688, 7334, 5721, 5685, 5684	93	577	16650	2.1720	1.0000	0.5684	84.3788
GOTERM_CC_FAT	GO:0070062	extracellular exosome	71	74.737	5.45E-31	10130, 1476, 552900, 3385, 6165, 5685, 3336, 5684, 3383, 7167, 3012, 51727, 10135, 1347, 81, 3105, 3251, 832, 654364, 6647, 1329, 302, 10961, 931, 9352, 4190, 6154, 476, 5034, 6156, 5687, 5688, 4082, 6224, 8350, 6222, 2665, 8294, 7171, 2023, 5878, 6742, 7170, 6181, 8339, 3848, 51596, 5660, 29082, 10016, 4637, 7334, 7086, 3945, 2923, 5243, 953, 5721, 5052, 4710, 11315, 4478, 10606,	95	2811	14527	3.8623	0.0000	0.0000	0.0000

						10935, 396, 7534, 808, 7532, 7531, 1632, 6576							
GOTERM_CC_FAT	GO:1903561	extracellular vesicle	71	74.737	7.53E-31	10130, 1476, 552900, 3385, 6165, 5685, 3336, 5684, 3383, 7167, 3012, 51727, 10135, 1347, 81, 3105, 3251, 832, 654364, 6647, 1329, 302, 10961, 931, 9352, 4190, 6154, 476, 5034, 6156, 5687, 5688, 4082, 6224, 8350, 6222, 2665, 8294, 7171, 2023, 5878, 6742, 7170, 6181, 8339, 3848, 51596, 5660, 29082, 10016, 4637, 7334, 7086, 3945, 2923, 5243, 953, 5721, 5052, 4710, 11315, 4478, 10606, 10935, 396, 7534, 808, 7532, 7531, 1632, 6576	95	2825	14527	3.8432	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:0043230	extracellular organelle	71	74.737	7.70E-31	10130, 1476, 552900, 3385, 6165, 5685, 3336, 5684, 3383, 7167, 3012, 51727, 10135, 1347, 81, 3105, 3251, 832, 654364, 6647, 1329, 302, 10961, 931, 9352, 4190, 6154, 476, 5034, 6156, 5687, 5688, 4082, 6224, 8350, 6222, 2665, 8294, 7171, 2023, 5878, 6742, 7170, 6181, 8339, 3848, 51596, 5660, 29082, 10016, 4637, 7334, 7086, 3945, 2923, 5243, 953, 5721, 5052, 4710, 11315, 4478, 10606, 10935, 396, 7534, 808, 7532, 7531, 1632, 6576	95	2826	14527	3.8418	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:0031988	membrane-bounded vesicle	73	76.842	5.66E-26	10130, 9601, 1476, 552900, 3385, 6165, 5685, 3336, 5684, 3383, 7167, 3012, 51727, 10135, 81, 1347, 3105, 3251, 832, 654364, 6647, 1329, 302, 10961, 931, 9352, 4190, 6154, 476, 5034, 6156, 5687, 5688, 4082, 6224, 8350, 6222, 2665, 8294, 7171, 2023, 5878, 6742, 7170, 6181, 8339, 3848, 51596, 5660, 29082, 10016, 4637, 7334, 7086, 3945, 2923, 5243, 4673, 953, 5721, 5052, 4710, 11315, 4478,	95	3611	14527	3.0913	0.0000	0.0000	0.0000

						10606, 10935, 396, 7534, 808, 7532, 7531, 1632, 6576							
GOTERM_CC_FAT	GO:0044421	extracellular region part	71	74.737	4.23E-22	10130, 1476, 552900, 3385, 6165, 5685, 3336, 5684, 3383, 7167, 3012, 51727, 10135, 1347, 81, 3105, 3251, 832, 654364, 6647, 1329, 302, 10961, 931, 9352, 4190, 6154, 476, 5034, 6156, 5687, 5688, 4082, 6224, 8350, 6222, 2665, 8294, 7171, 2023, 5878, 6742, 7170, 6181, 8339, 3848, 51596, 5660, 29082, 10016, 4637, 7334, 7086, 3945, 2923, 5243, 953, 5721, 5052, 4710, 11315, 4478, 10606, 10935, 396, 7534, 808, 7532, 7531, 1632, 6576	95	3878	14527	2.7996	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:0005576	extracellular region	71	74.737	1.80E-17	10130, 1476, 552900, 3385, 6165, 5685, 3336, 5684, 3383, 7167, 3012, 51727, 10135, 1347, 81, 3105, 3251, 832, 654364, 6647, 1329, 302, 10961, 931, 9352, 4190, 6154, 476, 5034, 6156, 5687, 5688, 4082, 6224, 8350, 6222, 2665, 8294, 7171, 2023, 5878, 6742, 7170, 6181, 8339, 3848, 51596, 5660, 29082, 10016, 4637, 7334, 7086, 3945, 2923, 5243, 953, 5721, 5052, 4710, 11315, 4478, 10606, 10935, 396, 7534, 808, 7532, 7531, 1632, 6576	95	4623	14527	2.3485	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:0005912	adherens junction	25	26.316	8.33E-12	1936, 6181, 3383, 9124, 81, 7112, 832, 302, 2923, 6132, 5052, 5034, 6156, 11315, 4478, 4082, 8350, 10606, 2665, 6222, 7534, 7171, 2023, 7532, 7531	95	694	14527	5.5085	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:0070161	anchoring junction	25	26.316	1.39E-11	1936, 6181, 3383, 9124, 81, 7112, 832, 302, 2923, 6132, 5052, 5034, 6156, 11315, 4478, 4082, 8350, 10606, 2665, 6222, 7534, 7171, 2023, 7532, 7531	95	711	14527	5.3768	0.0000	0.0000	0.0000

GOTERM_CC_FAT	GO:0042470	melanosome	12	12.632	7.67E-11	10130, 9601, 302, 2923, 10961, 4673, 7534, 476, 7531, 5034, 5878, 5052	95	106	14527	17.3112	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:0048770	pigment granule	12	12.632	7.67E-11	10130, 9601, 302, 2923, 10961, 4673, 7534, 476, 7531, 5034, 5878, 5052	95	106	14527	17.3112	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:0043209	myelin sheath	14	14.737	8.63E-11	654364, 6647, 302, 10963, 2923, 4190, 476, 5052, 4478, 10935, 2665, 7086, 7532, 3945	95	174	14527	12.3036	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:0005925	focal adhesion	16	16.842	2.89E-08	6181, 6132, 2923, 5034, 3383, 6156, 4478, 4082, 9124, 2665, 6222, 7534, 7171, 7532, 7531, 81	95	391	14527	6.2574	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:0005924	cell-substrate adherens junction	16	16.842	3.20E-08	6181, 6132, 2923, 5034, 3383, 6156, 4478, 4082, 9124, 2665, 6222, 7534, 7171, 7532, 7531, 81	95	394	14527	6.2098	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:0030055	cell-substrate junction	16	16.842	3.78E-08	6181, 6132, 2923, 5034, 3383, 6156, 4478, 4082, 9124, 2665, 6222, 7534, 7171, 7532, 7531, 81	95	399	14527	6.1320	0.0000	0.0000	0.0001
GOTERM_CC_FAT	GO:0005829	cytosol	47	49.474	5.48E-08	1936, 6181, 1933, 6165, 5685, 5684, 2224, 29082, 7167, 7334, 4637, 51727, 10135, 7086, 3945, 3251, 654364, 832, 6647, 302, 6132, 6154, 4190, 5721, 5052, 8665, 6156, 5687, 11315, 56896, 5688, 6224, 10606, 10935, 6222, 2665, 7534, 396, 7171, 4144, 808, 2023, 7532, 7531, 5859, 1207, 7170	95	3399	14527	2.1145	0.0000	0.0000	0.0001
GOTERM_CC_FAT	GO:0030054	cell junction	27	28.421	3.84E-07	1936, 6181, 3383, 9124, 10135, 81, 7112, 832, 302, 2923, 6132, 476, 5052, 5034, 6156, 11315, 4478, 4082, 8350, 10606, 2665, 6222, 7534, 7171, 2023, 7532, 7531	95	1379	14527	2.9940	0.0001	0.0000	0.0005
GOTERM_CC_FAT	GO:0005913	cell-cell adherens junction	12	12.632	9.58E-06	11315, 1936, 7112, 832, 9124, 302, 8350, 10606, 7534, 2023, 7531, 5052	95	332	14527	5.5271	0.0031	0.0002	0.0129
GOTERM_CC_FAT	GO:0005739	mitochondrion	26	27.368	5.55E-05	6165, 5660, 3336, 2224, 1347, 3945, 1345, 654364, 6647, 1329, 9352,	95	1700	14527	2.3387	0.0176	0.0010	0.0749

						4190, 293, 5052, 94081, 4710, 11315, 10935, 7534, 7532, 1632, 7531, 5859, 26520, 6742, 6576							
GOTERM_CC_FAT	GO:0000502	proteasome complex	6	6.316	6.67E-05	5687, 5688, 9352, 5721, 5685, 5684	95	66	14527	13.9014	0.0211	0.0012	0.0899
GOTERM_CC_FAT	GO:0044445	cytosolic part	9	9.474	1.57E-04	6181, 6132, 6224, 10935, 6222, 6154, 2023, 6165, 6156	95	239	14527	5.7583	0.0491	0.0026	0.2121
GOTERM_CC_FAT	GO:0022626	cytosolic ribosome	7	7.368	1.58E-04	6181, 6132, 6224, 6222, 6154, 6165, 6156	95	125	14527	8.5633	0.0493	0.0025	0.2129
GOTERM_CC_FAT	GO:0005911	cell-cell junction	14	14.737	2.49E-04	11315, 1936, 7112, 832, 9124, 302, 8350, 10606, 7534, 2023, 476, 81, 7531, 5052	95	644	14527	3.3243	0.0767	0.0038	0.3360
GOTERM_CC_FAT	GO:0031012	extracellular matrix	12	12.632	6.13E-04	6647, 302, 4637, 6224, 3848, 6222, 8294, 293, 6165, 5034, 5052, 6156	95	530	14527	3.4622	0.1783	0.0089	0.8245
GOTERM_CC_FAT	GO:0022625	cytosolic large ribosomal subunit	5	5.263	9.76E-04	6181, 6132, 6154, 6165, 6156	95	68	14527	11.2438	0.2683	0.0135	1.3086
GOTERM_CC_FAT	GO:0030017	Sarcomere	7	7.368	0.001375839	5687, 832, 808, 7171, 2023, 81, 7170	95	188	14527	5.6937	0.3563	0.0182	1.8407
GOTERM_CC_FAT	GO:0016023	cytoplasmic, membrane-bounded vesicle	18	18.947	0.001405836	10130, 832, 6647, 9601, 302, 2923, 10961, 4673, 476, 5034, 5052, 29082, 7534, 7532, 81, 7531, 5878, 3105	95	1183	14527	2.3267	0.3625	0.0178	1.8805
GOTERM_CC_FAT	GO:0044391	ribosomal subunit	7	7.368	0.00141365	6181, 6132, 6224, 6222, 6154, 6165, 6156	95	189	14527	5.6635	0.3641	0.0173	1.8909
GOTERM_CC_FAT	GO:0030529	intracellular ribonucleoprotein complex	14	14.737	0.001601561	6181, 6132, 6154, 6165, 5685, 8665, 6156, 51691, 5687, 3189, 3182, 6224, 6222, 81	95	786	14527	2.7237	0.4012	0.0188	2.1397
GOTERM_CC_FAT	GO:1990904	ribonucleoprotein complex	14	14.737	0.001619842	6181, 6132, 6154, 6165, 5685, 8665, 6156, 51691, 5687, 3189, 3182, 6224, 6222, 81	95	787	14527	2.7202	0.4047	0.0184	2.1639
GOTERM_CC_FAT	GO:0044429	mitochondrial part	16	16.842	0.001652483	654364, 6647, 1329, 293, 3336, 94081, 4710, 11315, 10935, 1347, 5859, 26520, 1632, 6742, 1345, 6576	95	990	14527	2.4714	0.4109	0.0181	2.2071

GOTERM_CC_FAT	GO:0044449	contractile fiber part	7	7.368	0.002133423	5687, 832, 808, 7171, 2023, 81, 7170	95	205	14527	5.2215	0.4951	0.0225	2.8409
GOTERM_CC_FAT	GO:0030016	Myofibril	7	7.368	0.00252352	5687, 832, 808, 7171, 2023, 81, 7170	95	212	14527	5.0491	0.5545	0.0257	3.3522
GOTERM_CC_FAT	GO:0019866	organelle inner membrane	11	11.579	0.003181315	11315, 7112, 1329, 293, 1347, 1632, 26520, 1345, 94081, 6576, 4710	95	557	14527	3.0199	0.6393	0.0314	4.2087
GOTERM_CC_FAT	GO:0043292	contractile fiber	7	7.368	0.003314209	5687, 832, 808, 7171, 2023, 81, 7170	95	224	14527	4.7786	0.6543	0.0317	4.3809
GOTERM_CC_FAT	GO:0005743	mitochondrial inner membrane	10	10.526	0.004962268	11315, 1329, 293, 1347, 1632, 26520, 1345, 94081, 6576, 4710	95	499	14527	3.0644	0.7965	0.0457	6.4926
GOTERM_CC_FAT	GO:0005840	Ribosome	7	7.368	0.005975961	6181, 6132, 6224, 6222, 6154, 6165, 6156	95	253	14527	4.2309	0.8531	0.0533	7.7700
GOTERM_CC_FAT	GO:0015934	large ribosomal subunit	5	5.263	0.007196283	6181, 6132, 6154, 6165, 6156	95	118	14527	6.4795	0.9009	0.0622	9.2862
GOTERM_CC_FAT	GO:0005740	mitochondrial envelope	12	12.632	0.007547774	11315, 654364, 6647, 1329, 293, 1347, 1632, 26520, 1345, 94081, 6576, 4710	95	732	14527	2.5068	0.9115	0.0634	9.7187
GOTERM_CC_FAT	GO:0044455	mitochondrial membrane part	6	6.316	0.01000643	11315, 1329, 293, 1347, 26520, 4710	95	202	14527	4.5421	0.9600	0.0812	12.6906
GOTERM_CC_FAT	GO:0098800	inner mitochondrial membrane protein complex	5	5.263	0.010859403	11315, 1329, 293, 26520, 4710	95	133	14527	5.7487	0.9696	0.0857	13.7002
GOTERM_CC_FAT	GO:0031966	mitochondrial membrane	11	11.579	0.013748384	11315, 654364, 1329, 293, 1347, 1632, 26520, 1345, 94081, 6576, 4710	95	691	14527	2.4343	0.9881	0.1048	17.0402
GOTERM_CC_FAT	GO:0005615	extracellular space	17	17.895	0.022374487	6647, 1476, 302, 8339, 3848, 931, 4190, 5052, 5660, 3383, 4478, 7167, 7534, 10135, 396, 2023, 81	95	1444	14527	1.8003	0.9993	0.1619	26.3143
GOTERM_CC_FAT	GO:0098798	mitochondrial protein complex	5	5.263	0.023557666	11315, 1329, 293, 26520, 4710	95	168	14527	4.5511	0.9995	0.1661	27.5086

GOTERM_CC_FAT	GO:0031967	organelle envelope	14	14.737	0.033439041	7112, 654364, 6647, 1329, 293, 94081, 4710, 11315, 10016, 26520, 1632, 1347, 1345, 6576	95	1149	14527	1.8632	1.0000	0.2236	36.8059
GOTERM_CC_FAT	GO:0031975	Envelope	14	14.737	0.03446355	7112, 654364, 6647, 1329, 293, 94081, 4710, 11315, 10016, 26520, 1632, 1347, 1345, 6576	95	1154	14527	1.8551	1.0000	0.2251	37.7038
GOTERM_CC_FAT	GO:0005788	endoplasmic reticulum lumen	5	5.263	0.042063147	10130, 9601, 2923, 10961, 5034	95	202	14527	3.7850	1.0000	0.2633	44.0048
GOTERM_CC_FAT	GO:0099568	cytoplasmic region	6	6.316	0.044590952	4082, 832, 6647, 302, 7171, 81	95	299	14527	3.0685	1.0000	0.2719	45.9662
GOTERM_CC_FAT	GO:0005759	mitochondrial matrix	7	7.368	0.057437985	11315, 6647, 10935, 1632, 5859, 6742, 3336	95	425	14527	2.5186	1.0000	0.3315	54.9882
GOTERM_CC_FAT	GO:0005938	cell cortex	5	5.263	0.076285371	4082, 832, 302, 7171, 81	95	247	14527	3.0955	1.0000	0.4108	65.7271
GOTERM_CC_FAT	GO:0000785	Chromatin	7	7.368	0.085446603	11315, 7112, 3012, 8339, 8350, 8294, 4678	95	472	14527	2.2678	1.0000	0.4420	70.0403
GOTERM_MF_FAT	GO:0003723	RNA binding	39	41.053	2.74E-14	1936, 6181, 1933, 1476, 9601, 6165, 3336, 51691, 2224, 7334, 81, 3105, 302, 10963, 2923, 6132, 4673, 6154, 5034, 5052, 8665, 6156, 5687, 11315, 4478, 2079, 3189, 3182, 6224, 6222, 2665, 7534, 8294, 7532, 2023, 7531, 5859, 6742, 1207	93	1656	15478	3.9195	0.0000	0.0000	0.0000
GOTERM_MF_FAT	GO:0044822	poly(A) RNA binding	32	33.684	6.87E-13	1476, 9601, 6165, 3336, 51691, 2224, 7334, 81, 3105, 302, 2923, 6132, 10963, 4673, 6154, 5034, 5052, 6156, 11315, 2079, 3189, 3182, 6224, 6222, 2665, 7534, 8294, 7532, 2023, 7531, 6742, 1207	93	1196	15478	4.4530	0.0000	0.0000	0.0000
GOTERM_MF_FAT	GO:0050839	cell adhesion molecule binding	17	17.895	1.17E-08	7112, 1936, 832, 302, 3385, 5034, 5052, 3383, 11315, 4478, 9124, 8350, 10606, 7534, 2023, 7531, 81	93	461	15478	6.1373	0.0000	0.0000	0.0000

GOTERM_MF_FAT	GO:0016860	intramolecular oxidoreductase activity	7	7.368	7.68E-07	10130, 9601, 2923, 7167, 10961, 1632, 5034	93	54	15478	21.5743	0.0003	0.0001	0.0011
GOTERM_MF_FAT	GO:0098641	cadherin binding involved in cell-cell adhesion	12	12.632	1.16E-06	11315, 1936, 7112, 832, 9124, 302, 8350, 10606, 7534, 2023, 7531, 5052	93	290	15478	6.8868	0.0005	0.0001	0.0016
GOTERM_MF_FAT	GO:0098632	protein binding involved in cell-cell adhesion	12	12.632	1.66E-06	11315, 1936, 7112, 832, 9124, 302, 8350, 10606, 7534, 2023, 7531, 5052	93	301	15478	6.6351	0.0007	0.0001	0.0024
GOTERM_MF_FAT	GO:0098631	protein binding involved in cell adhesion	12	12.632	1.95E-06	11315, 1936, 7112, 832, 9124, 302, 8350, 10606, 7534, 2023, 7531, 5052	93	306	15478	6.5267	0.0009	0.0001	0.0028
GOTERM_MF_FAT	GO:0045296	cadherin binding	12	12.632	2.01E-06	11315, 1936, 7112, 832, 9124, 302, 8350, 10606, 7534, 2023, 7531, 5052	93	307	15478	6.5054	0.0009	0.0001	0.0028
GOTERM_MF_FAT	GO:0016864	intramolecular oxidoreductase activity, transposing S-S bonds	5	5.263	7.88E-06	10130, 9601, 2923, 10961, 5034	93	22	15478	37.8250	0.0034	0.0004	0.0111
GOTERM_MF_FAT	GO:0003756	protein disulfide isomerase activity	5	5.263	7.88E-06	10130, 9601, 2923, 10961, 5034	93	22	15478	37.8250	0.0034	0.0004	0.0111
GOTERM_MF_FAT	GO:0003676	nucleic acid binding	44	46.316	2.26E-05	1936, 6181, 1933, 9601, 1476, 8339, 6165, 3336, 51691, 2224, 3012, 7334, 81, 3105, 7112, 654364, 302, 10963, 6132, 2923, 4673, 6154, 5034, 5052, 8665, 6156, 5687, 11315, 4478, 2079, 3189, 3182, 8350, 6224, 6222, 2665, 7534, 8294, 7532, 2023, 7531, 5859, 6742, 1207	93	4097	15478	1.7874	0.0099	0.0010	0.0320
GOTERM_MF_FAT	GO:0003735	structural constituent of ribosome	9	9.474	5.22E-05	6181, 6132, 6224, 6222, 6154, 293, 6165, 6576, 6156	93	222	15478	6.7472	0.0226	0.0021	0.0738

GOTERM_MF_FAT	GO:1901363	heterocyclic compound binding	55	57.895	7.05E-05	1936, 1933, 9601, 1476, 6165, 3336, 3012, 51727, 81, 3105, 3251, 654364, 10963, 302, 4190, 6154, 476, 5034, 6156, 5687, 3189, 3182, 8350, 6224, 6222, 2665, 8294, 2023, 5878, 6742, 6181, 8339, 51691, 2224, 7334, 3945, 7112, 6132, 2923, 5243, 4673, 953, 5052, 8665, 11315, 4478, 2079, 83473, 10606, 7534, 4144, 7532, 5859, 7531, 1207	93	5971	15478	1.5330	0.0304	0.0026	0.0996
GOTERM_MF_FAT	GO:0097159	organic cyclic compound binding	55	57.895	1.07E-04	1936, 1933, 9601, 1476, 6165, 3336, 3012, 51727, 81, 3105, 3251, 654364, 10963, 302, 4190, 6154, 476, 5034, 6156, 5687, 3189, 3182, 8350, 6224, 6222, 2665, 8294, 2023, 5878, 6742, 6181, 8339, 51691, 2224, 7334, 3945, 7112, 6132, 2923, 5243, 4673, 953, 5052, 8665, 11315, 4478, 2079, 83473, 10606, 7534, 4144, 7532, 5859, 7531, 1207	93	6052	15478	1.5125	0.0459	0.0036	0.1516
GOTERM_MF_FAT	GO:0051087	chaperone binding	6	6.316	1.19E-04	6647, 10963, 10961, 476, 26520, 3336	93	81	15478	12.3282	0.0509	0.0037	0.1685
GOTERM_MF_FAT	GO:0016853	isomerase activity	7	7.368	4.48E-04	10130, 9601, 2923, 7167, 10961, 1632, 5034	93	165	15478	7.0607	0.1784	0.0130	0.6319
GOTERM_MF_FAT	GO:0005198	structural molecule activity	14	14.737	7.01E-04	6181, 6132, 3848, 6154, 293, 6165, 6156, 4478, 6224, 4637, 6222, 7171, 6576, 7170	93	782	15478	2.9796	0.2646	0.0190	0.9866
GOTERM_MF_FAT	GO:0046983	protein dimerization activity	17	17.895	0.001242935	3251, 6647, 8339, 10961, 5034, 29082, 11315, 10016, 3012, 8350, 8294, 10135, 7086, 7531, 26520, 81, 1207	93	1160	15478	2.4391	0.4200	0.0315	1.7423
GOTERM_MF_FAT	GO:0016209	antioxidant activity	5	5.263	0.001371502	11315, 6647, 10935, 9352, 5052	93	81	15478	10.2735	0.4518	0.0328	1.9209

GOTERM_MF_FAT	GO:0032403	protein complex binding	13	13.684	0.001887506	832, 4082, 8350, 931, 7171, 3385, 4678, 81, 7531, 5034, 7170, 3105, 3383	93	766	15478	2.8245	0.5629	0.0426	2.6346
GOTERM_MF_FAT	GO:0042802	identical protein binding	18	18.947	0.002457616	3251, 6647, 10961, 5721, 5052, 29082, 11315, 5688, 10016, 10606, 10935, 7534, 10135, 4144, 7086, 81, 26520, 3945	93	1358	15478	2.2060	0.6596	0.0525	3.4177
GOTERM_MF_FAT	GO:0019899	enzyme binding	21	22.105	0.003727522	6647, 1476, 302, 51596, 476, 5034, 29082, 11315, 4478, 4082, 7167, 7334, 10935, 2665, 6222, 7534, 808, 2023, 7532, 7531, 3945	93	1789	15478	1.9536	0.8052	0.0749	5.1408
GOTERM_MF_FAT	GO:0051015	actin filament binding	5	5.263	0.007921699	4082, 832, 7171, 81, 7170	93	132	15478	6.3042	0.9693	0.1464	10.6310
GOTERM_MF_FAT	GO:0019900	kinase binding	10	10.526	0.008810583	11315, 4478, 4082, 10935, 6222, 7534, 808, 476, 7532, 3945	93	595	15478	2.7971	0.9793	0.1551	11.7560
GOTERM_MF_FAT	GO:0008092	cytoskeletal protein binding	11	11.579	0.02842315	56896, 4478, 4082, 832, 302, 83473, 808, 7171, 476, 81, 7170	93	846	15478	2.1640	1.0000	0.4092	33.4688
GOTERM_MF_FAT	GO:0042803	protein homodimerization activity	10	10.526	0.029314969	11315, 29082, 3251, 10016, 6647, 10961, 10135, 7086, 26520, 81	93	730	15478	2.2799	1.0000	0.4062	34.3267
GOTERM_MF_FAT	GO:0019901	protein kinase binding	8	8.421	0.038649177	4478, 4082, 10935, 6222, 7534, 808, 476, 7532	93	531	15478	2.5074	1.0000	0.4852	42.7093
GOTERM_MF_FAT	GO:0044877	macromolecular complex binding	14	14.737	0.044779149	832, 4082, 8350, 931, 7171, 3385, 4678, 81, 7531, 5034, 6742, 3105, 7170, 3383	93	1306	15478	1.7841	1.0000	0.5244	47.6612
GOTERM_MF_FAT	GO:0046982	protein heterodimerization activity	7	7.368	0.058561408	3012, 8339, 8350, 8294, 7531, 5034, 1207	93	465	15478	2.5054	1.0000	0.6109	57.3790
GOTERM_MF_FAT	GO:0003779	actin binding	6	6.316	0.087674464	4478, 4082, 832, 7171, 81, 7170	93	397	15478	2.5153	1.0000	0.7499	72.6580
KEGG_PATHWAY	hsa04260:Cardiac muscle contraction		6	6.316	0.001045987	1329, 7171, 476, 1347, 1345, 7170	73	75	6910	7.5726	0.1272	0.1272	1.2113

KEGG_PATHWAY	hsa03050:Proteasome	5	5.263	0.001073877	5687, 5688, 5721, 5685, 5684	73	44	6910	10.7565	0.1304	0.0675	1.2435
KEGG_PATHWAY	hsa03010:Ribosome	7	7.368	0.002804538	6181, 6132, 6224, 6222, 6154, 6165, 6156	73	136	6910	4.8721	0.3059	0.1146	3.2177
KEGG_PATHWAY	hsa01130:Biosynthesis of antibiotics	8	8.421	0.006297631	2224, 654364, 7167, 10606, 4190, 7086, 2023, 3945	73	212	6910	3.5720	0.5601	0.1856	7.0930
KEGG_PATHWAY	hsa05012:Parkinson's disease	6	6.316	0.015884239	11315, 1329, 293, 1347, 1345, 4710	73	142	6910	3.9996	0.8753	0.3405	17.0112
KEGG_PATHWAY	hsa05203:Viral carcinogenesis	7	7.368	0.019509958	8339, 7534, 8294, 7532, 7531, 81, 3105	73	205	6910	3.2322	0.9228	0.3475	20.5028
KEGG_PATHWAY	hsa05169:Epstein-Barr virus infection	6	6.316	0.047436263	7534, 953, 7532, 7531, 3105, 3383	73	190	6910	2.9892	0.9982	0.5945	43.2180
KEGG_PATHWAY	hsa05016:Huntington's disease	6	6.316	0.049232298	6647, 1329, 293, 1347, 1345, 4710	73	192	6910	2.9580	0.9986	0.5597	44.4523
KEGG_PATHWAY	hsa05322:Systemic lupus erythematosus	5	5.263	0.050598681	3012, 8339, 8350, 8294, 81	73	134	6910	3.5320	0.9988	0.5276	45.3749
KEGG_PATHWAY	hsa05010:Alzheimer's disease	5	5.263	0.097452179	1329, 808, 1347, 1345, 4710	73	168	6910	2.8172	1.0000	0.7363	69.7010
KEGG_PATHWAY	hsa04141:Protein processing in endoplasmic reticulum	5	5.263	0.099063949	10130, 9601, 2923, 10961, 5034	73	169	6910	2.8005	1.0000	0.7085	70.3251

Appendix 3: Up-regulated proteins in REC1 CYR61/CCN1 KD model.

Category	Term	GO TERM	Count	%	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
GOTERM_BP_FAT	GO:0016071	mRNA metabolic process	23	29.114	1.43E-13	23020, 4670, 26528, 6637, 6635, 6124, 6133, 3303, 6161, 10236, 1660, 6421, 9045, 6228, 27339, 11338, 22913, 9775, 10949, 1655, 3191, 6050, 6191	77	672	16650	7.4009	0.0000	0.0000	0.0000

GOTERM_BP_FAT	GO:0006396	RNA processing	25	31.646	9.30E-13	6637, 6635, 6124, 6161, 10521, 3028, 6421, 9045, 27339, 9775, 10949, 3191, 23020, 26528, 4670, 6133, 3303, 10236, 1660, 6228, 11338, 22913, 1655, 9188, 6191	77	910	16650	5.9405	0.0000	0.0000	0.0000
GOTERM_BP_FAT	GO:0000398	mRNA splicing, via spliceosome	15	18.987	6.78E-11	23020, 4670, 6637, 26528, 6635, 10236, 1660, 6421, 11338, 27339, 22913, 9775, 10949, 1655, 3191	77	299	16650	10.8478	0.0000	0.0000	0.0000
GOTERM_BP_FAT	GO:0000377	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	15	18.987	6.78E-11	23020, 4670, 6637, 26528, 6635, 10236, 1660, 6421, 11338, 27339, 22913, 9775, 10949, 1655, 3191	77	299	16650	10.8478	0.0000	0.0000	0.0000
GOTERM_BP_FAT	GO:0000375	RNA splicing, via transesterification reactions	15	18.987	8.08E-11	23020, 4670, 6637, 26528, 6635, 10236, 1660, 6421, 11338, 27339, 22913, 9775, 10949, 1655, 3191	77	303	16650	10.7046	0.0000	0.0000	0.0000
GOTERM_BP_FAT	GO:0008380	RNA splicing	16	20.253	2.95E-10	23020, 4670, 6637, 26528, 6635, 3303, 10236, 1660, 6421, 11338, 27339, 22913, 9775, 10949, 1655, 3191	77	400	16650	8.6494	0.0000	0.0000	0.0000
GOTERM_BP_FAT	GO:0006397	mRNA processing	16	20.253	2.78E-09	23020, 4670, 6637, 26528, 6635, 3303, 10236, 1660, 6421, 11338, 27339, 22913, 9775, 10949, 1655, 3191	77	471	16650	7.3455	0.0000	0.0000	0.0000
GOTERM_BP_FAT	GO:0044085	cellular component biogenesis	33	41.772	4.39E-07	55920, 6637, 6635, 6124, 9380, 10856, 6161, 3842, 9361, 38, 9045, 27339, 9775, 6472, 4905, 23020, 6133, 3303, 1731, 55937, 5701, 5341, 3615, 4282, 6228, 81876, 10938, 1213, 70, 6191, 9188, 83706, 2058	77	2914	16650	2.4488	0.0009	0.0001	0.0008

GOTERM_BP_FAT	GO:190156 6	organonitrogen compound biosynthetic process	22	27.84 8	5.39E-07	8565, 6124, 6133, 10236, 2618, 7284, 6161, 51520, 51649, 3615, 38, 790, 9045, 6228, 10128, 9775, 1213, 539, 4677, 6472, 6191, 2058	77	1375	1665 0	3.4597	0.0011	0.0001	0.0009
GOTERM_BP_FAT	GO:004360 4	amide biosynthetic process	16	20.25 3	9.57E-07	8565, 6124, 6133, 10236, 6161, 7284, 51649, 51520, 790, 9045, 6228, 10128, 9775, 4677, 6191, 2058	77	736	1665 0	4.7007	0.0020	0.0002	0.0017
GOTERM_BP_FAT	GO:000641 2	translation	15	18.98 7	1.03E-06	8565, 6124, 6133, 6161, 7284, 10236, 51649, 51520, 9045, 6228, 10128, 9775, 4677, 6191, 2058	77	641	1665 0	5.0601	0.0022	0.0002	0.0018
GOTERM_BP_FAT	GO:004304 3	peptide biosynthetic process	15	18.98 7	1.65E-06	8565, 6124, 6133, 6161, 7284, 10236, 51649, 51520, 9045, 6228, 10128, 9775, 4677, 6191, 2058	77	667	1665 0	4.8628	0.0035	0.0003	0.0029
GOTERM_BP_FAT	GO:002261 3	ribonucleoprotein complex biogenesis	13	16.45 6	1.67E-06	23020, 6637, 6635, 6133, 6124, 6161, 10856, 9045, 6228, 27339, 9775, 9188, 6191	77	480	1665 0	5.8563	0.0035	0.0003	0.0029
GOTERM_BP_FAT	GO:000640 1	RNA catabolic process	10	12.65 8	1.93E-06	9045, 6228, 10128, 9775, 6124, 6133, 10236, 6161, 6050, 6191	77	249	1665 0	8.6841	0.0041	0.0003	0.0033
GOTERM_BP_FAT	GO:190331 1	regulation of mRNA metabolic process	8	10.12 7	2.45E-06	27339, 11338, 26528, 9775, 3303, 10236, 1655, 3191	77	133	1665 0	13.0065	0.0051	0.0004	0.0042
GOTERM_BP_FAT	GO:004690 7	intracellular transport	22	27.84 8	5.82E-06	6637, 1434, 6635, 6124, 7846, 6133, 10960, 6161, 1314, 3842, 9045, 6228, 11338, 10128, 81876, 9775, 10938, 1213, 539, 6191, 4905, 10452	77	1594	1665 0	2.9844	0.0122	0.0008	0.0101
GOTERM_BP_FAT	GO:000640 2	mRNA catabolic process	9	11.39 2	7.37E-06	9045, 6228, 9775, 6124, 6133, 10236, 6161, 6050, 6191	77	221	1665 0	8.8059	0.0154	0.0010	0.0128
GOTERM_BP_FAT	GO:003312 0	positive regulation of RNA splicing	5	6.329	7.55E-06	27339, 11338, 26528, 9775, 3303	77	28	1665 0	38.6132	0.0158	0.0009	0.0131

GOTERM_BP_FAT	GO:0050684	regulation of mRNA processing	7	8.861	7.88E-06	27339, 11338, 26528, 9775, 3303, 1655, 3191	77	104	16650	14.5542	0.0165	0.0009	0.0136
GOTERM_BP_FAT	GO:0043603	cellular amide metabolic process	17	21.519	8.41E-06	8565, 6124, 6133, 10236, 6161, 7284, 51649, 51520, 790, 9045, 6228, 10128, 9775, 4677, 6472, 6191, 2058	77	994	16650	3.6982	0.0176	0.0009	0.0146
GOTERM_BP_FAT	GO:0034660	ncRNA metabolic process	13	16.456	9.69E-06	8565, 6133, 6124, 6161, 51520, 3028, 9045, 6228, 9775, 4677, 9188, 6191, 2058	77	570	16650	4.9316	0.0202	0.0010	0.0168
GOTERM_BP_FAT	GO:0043484	regulation of RNA splicing	7	8.861	1.03E-05	27339, 11338, 26528, 9775, 3303, 1655, 3191	77	109	16650	13.8866	0.0215	0.0010	0.0179
GOTERM_BP_FAT	GO:0051641	cellular localization	28	35.443	1.06E-05	55920, 6637, 1434, 6635, 6124, 7846, 6133, 10856, 10960, 6161, 1731, 1314, 1660, 3842, 9045, 6228, 27339, 11338, 10128, 81876, 9775, 10938, 4627, 1213, 539, 6191, 4905, 10452	77	2541	16650	2.3827	0.0220	0.0010	0.0183
GOTERM_BP_FAT	GO:0048024	regulation of mRNA splicing, via spliceosome	6	7.595	1.67E-05	27339, 11338, 26528, 9775, 1655, 3191	77	70	16650	18.5343	0.0345	0.0015	0.0288
GOTERM_BP_FAT	GO:0006518	peptide metabolic process	15	18.987	1.71E-05	8565, 6124, 6133, 6161, 7284, 10236, 51649, 51520, 9045, 6228, 10128, 9775, 4677, 6191, 2058	77	818	16650	3.9652	0.0355	0.0015	0.0296
GOTERM_BP_FAT	GO:0051649	establishment of localization in cell	24	30.380	1.82E-05	6637, 1434, 6635, 6124, 7846, 6133, 10960, 6161, 1731, 1314, 3842, 9045, 6228, 11338, 10128, 81876, 9775, 10938, 4627, 1213, 539, 6191, 4905, 10452	77	2004	16650	2.5896	0.0377	0.0015	0.0315
GOTERM_BP_FAT	GO:0000184	nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	7	8.861	2.06E-05	9045, 6228, 9775, 6124, 6133, 6161, 6191	77	123	16650	12.3060	0.0425	0.0017	0.0356

GOTERM_BP_FAT	GO:0006082	organic acid metabolic process	15	18.987	5.34E-05	8565, 9380, 47, 2618, 51520, 3028, 38, 790, 4282, 9775, 51144, 4677, 6472, 10728, 2058	77	907	16650	3.5761	0.1065	0.0042	0.0923
GOTERM_BP_FAT	GO:0010608	posttranscriptional regulation of gene expression	11	13.924	5.45E-05	10128, 9775, 3303, 10949, 10236, 51520, 6191, 3842, 1660, 5701, 2058	77	472	16650	5.0393	0.1085	0.0041	0.0942
GOTERM_BP_FAT	GO:0034655	nucleobase-containing compound catabolic process	10	12.658	5.65E-05	9045, 6228, 10128, 9775, 6124, 6133, 10236, 6161, 6050, 6191	77	380	16650	5.6904	0.1123	0.0041	0.0977
GOTERM_BP_FAT	GO:0006614	SRP-dependent cotranslational protein targeting to membrane	6	7.595	7.67E-05	9045, 6228, 6124, 6133, 6161, 6191	77	96	16650	13.5146	0.1493	0.0054	0.1326
GOTERM_BP_FAT	GO:1903313	positive regulation of mRNA metabolic process	5	6.329	8.51E-05	27339, 26528, 9775, 3303, 10236	77	51	16650	21.1994	0.1643	0.0058	0.1472
GOTERM_BP_FAT	GO:0046700	heterocycle catabolic process	10	12.658	1.03E-04	9045, 6228, 10128, 9775, 6124, 6133, 10236, 6161, 6050, 6191	77	411	16650	5.2612	0.1951	0.0068	0.1779
GOTERM_BP_FAT	GO:0006613	cotranslational protein targeting to membrane	6	7.595	1.07E-04	9045, 6228, 6124, 6133, 6161, 6191	77	103	16650	12.5961	0.2022	0.0068	0.1852
GOTERM_BP_FAT	GO:0045047	protein targeting to ER	6	7.595	1.12E-04	9045, 6228, 6124, 6133, 6161, 6191	77	104	16650	12.4750	0.2106	0.0069	0.1939
GOTERM_BP_FAT	GO:0044270	cellular nitrogen compound catabolic process	10	12.658	1.17E-04	9045, 6228, 10128, 9775, 6124, 6133, 10236, 6161, 6050, 6191	77	418	16650	5.1731	0.2186	0.0070	0.2022
GOTERM_BP_FAT	GO:0019439	aromatic compound catabolic process	10	12.658	1.28E-04	9045, 6228, 10128, 9775, 6124, 6133, 10236, 6161, 6050, 6191	77	423	16650	5.1119	0.2365	0.0075	0.2212
GOTERM_BP_FAT	GO:0072599	establishment of protein localization	6	7.595	1.34E-04	9045, 6228, 6124, 6133, 6161, 6191	77	108	16650	12.0130	0.2462	0.0076	0.2316

		to endoplasmic reticulum											
GOTERM_BP_FAT	GO:0065003	macromolecular complex assembly	20	25.316	1.86E-04	23020, 6637, 6635, 9380, 10856, 55937, 9361, 3842, 5701, 5341, 3615, 38, 4282, 27339, 81876, 10938, 6472, 83706, 4905, 2058	77	1710	16650	2.5290	0.3238	0.0102	0.3206
GOTERM_BP_FAT	GO:1901361	organic cyclic compound catabolic process	10	12.658	1.87E-04	9045, 6228, 10128, 9775, 6124, 6133, 10236, 6161, 6050, 6191	77	445	16650	4.8592	0.3261	0.0101	0.3234
GOTERM_BP_FAT	GO:0033036	macromolecule localization	27	34.177	1.91E-04	55920, 1434, 6124, 6133, 10856, 6161, 10960, 55937, 1314, 1660, 3842, 5341, 4282, 9045, 6228, 27339, 11338, 10128, 81876, 9775, 27159, 10938, 4627, 1213, 6191, 4905, 10452	77	2817	16650	2.0725	0.3319	0.0100	0.3304
GOTERM_BP_FAT	GO:0045184	establishment of protein localization	22	27.848	2.00E-04	55920, 1434, 6124, 6133, 10856, 6161, 10960, 1314, 3842, 5341, 4282, 9045, 6228, 11338, 81876, 27159, 10938, 4627, 1213, 6191, 4905, 10452	77	2021	16650	2.3539	0.3438	0.0102	0.3452
GOTERM_BP_FAT	GO:0033365	protein localization to organelle	14	17.722	2.02E-04	55920, 1434, 6124, 6133, 6161, 10856, 3842, 1660, 9045, 6228, 11338, 10938, 6191, 10452	77	902	16650	3.3562	0.3473	0.0101	0.3495
GOTERM_BP_FAT	GO:0006364	rRNA processing	8	10.127	2.25E-04	9045, 6228, 9775, 6124, 6133, 6161, 9188, 6191	77	269	16650	6.4307	0.3772	0.0110	0.3878
GOTERM_BP_FAT	GO:0016072	rRNA metabolic process	8	10.127	2.63E-04	9045, 6228, 9775, 6124, 6133, 6161, 9188, 6191	77	276	16650	6.2676	0.4252	0.0125	0.4533
GOTERM_BP_FAT	GO:0070972	protein localization to endoplasmic reticulum	6	7.595	2.96E-04	9045, 6228, 6124, 6133, 6161, 6191	77	128	16650	10.1360	0.4647	0.0138	0.5115
GOTERM_BP_FAT	GO:0009057	macromolecule catabolic process	16	20.253	2.97E-04	6124, 6133, 3303, 6161, 10236, 9361, 5701, 9045, 6228, 10128,	77	1201	16650	2.8807	0.4655	0.0135	0.5127

						27339, 9775, 27159, 6050, 6191, 4905							
GOTERM_BP_FAT	GO:0010467	gene expression	40	50.633	3.27E-04	6637, 6635, 6124, 6161, 10856, 3842, 10521, 3028, 6421, 9045, 27339, 9775, 10949, 3191, 4677, 6749, 23020, 4670, 26528, 8565, 6133, 3303, 10236, 7284, 51520, 51649, 1660, 5701, 4282, 6228, 10128, 11338, 22913, 4627, 1655, 70, 6191, 9188, 5591, 2058	77	5306	16650	1.6301	0.4976	0.0145	0.5633
GOTERM_BP_FAT	GO:0019752	carboxylic acid metabolic process	13	16.456	3.40E-04	4282, 790, 8565, 47, 9380, 2618, 51144, 4677, 51520, 6472, 10728, 2058, 38	77	826	16650	3.4032	0.5120	0.0148	0.5870
GOTERM_BP_FAT	GO:0000956	nuclear-transcribed mRNA catabolic process	7	8.861	3.55E-04	9045, 6228, 9775, 6124, 6133, 6161, 6191	77	206	16650	7.3477	0.5268	0.0152	0.6121
GOTERM_BP_FAT	GO:0043436	oxoacid metabolic process	13	16.456	3.59E-04	4282, 790, 8565, 47, 9380, 2618, 51144, 4677, 51520, 6472, 10728, 2058, 38	77	831	16650	3.3827	0.5313	0.0150	0.6199
GOTERM_BP_FAT	GO:0008104	protein localization	24	30.380	4.05E-04	55920, 1434, 6124, 6133, 10856, 6161, 10960, 1314, 1660, 3842, 5341, 4282, 9045, 6228, 27339, 11338, 81876, 27159, 10938, 4627, 1213, 6191, 4905, 10452	77	2448	16650	2.1199	0.5741	0.0166	0.6980
GOTERM_BP_FAT	GO:0022607	cellular component assembly	25	31.646	4.18E-04	55920, 23020, 6637, 6635, 3303, 9380, 10856, 1731, 55937, 9361, 3842, 5701, 5341, 3615, 38, 4282, 27339, 81876, 10938, 1213, 70, 6472, 83706, 4905, 2058	77	2618	16650	2.0649	0.5854	0.0168	0.7200
GOTERM_BP_FAT	GO:0044265	cellular macromolecule catabolic process	14	17.722	4.34E-04	6124, 6133, 3303, 6161, 10236, 9361, 5701, 9045, 6228, 10128, 27339, 9775, 6050, 6191	77	976	16650	3.1017	0.5995	0.0171	0.7482

GOTERM_BP_FAT	GO:0010033	response to organic substance	26	32.911	5.11E-04	10856, 6161, 10521, 9361, 38, 9775, 10949, 6472, 4670, 3303, 2618, 7284, 55937, 5701, 3615, 4282, 790, 10938, 1655, 10148, 70, 6191, 9188, 5591, 10728, 2058	77	2822	16650	1.9922	0.6598	0.0198	0.8808
GOTERM_BP_FAT	GO:0043488	regulation of mRNA stability	6	7.595	5.26E-04	3303, 10949, 10236, 3842, 1660, 5701	77	145	16650	8.9476	0.6699	0.0199	0.9054
GOTERM_BP_FAT	GO:0015031	protein transport	20	25.316	5.44E-04	1434, 6124, 6133, 6161, 10960, 1314, 3842, 5341, 9045, 4282, 6228, 11338, 81876, 27159, 10938, 4627, 1213, 6191, 4905, 10452	77	1860	16650	2.3251	0.6821	0.0203	0.9360
GOTERM_BP_FAT	GO:0034470	ncRNA processing	9	11.392	5.60E-04	9045, 6228, 9775, 6124, 6133, 6161, 9188, 6191, 3028	77	411	16650	4.7350	0.6933	0.0205	0.9651
GOTERM_BP_FAT	GO:0043487	regulation of RNA stability	6	7.595	6.51E-04	3303, 10949, 10236, 3842, 1660, 5701	77	152	16650	8.5355	0.7467	0.0234	1.1205
GOTERM_BP_FAT	GO:0006886	intracellular protein transport	14	17.722	6.58E-04	1434, 6124, 6133, 6161, 1314, 3842, 9045, 6228, 11338, 10938, 1213, 6191, 4905, 10452	77	1020	16650	2.9679	0.7505	0.0233	1.1329
GOTERM_BP_FAT	GO:0034613	cellular protein localization	18	22.785	7.32E-04	55920, 1434, 6124, 6133, 10856, 6161, 1314, 3842, 1660, 9045, 6228, 11338, 27339, 10938, 1213, 6191, 4905, 10452	77	1600	16650	2.4326	0.7865	0.0254	1.2593
GOTERM_BP_FAT	GO:0070727	cellular macromolecule localization	18	22.785	8.03E-04	55920, 1434, 6124, 6133, 10856, 6161, 1314, 3842, 1660, 9045, 6228, 11338, 27339, 10938, 1213, 6191, 4905, 10452	77	1613	16650	2.4130	0.8159	0.0274	1.3792
GOTERM_BP_FAT	GO:0042254	ribosome biogenesis	8	10.127	8.34E-04	9045, 6228, 9775, 6124, 6133, 6161, 9188, 6191	77	335	16650	5.1638	0.8279	0.0280	1.4337
GOTERM_BP_FAT	GO:0072594	establishment of protein localization to organelle	11	13.924	8.63E-04	9045, 6228, 11338, 1434, 6124, 6133, 10856, 6161, 6191, 3842, 10452	77	666	16650	3.5714	0.8381	0.0285	1.4829

GOTERM_BP_FAT	GO:0006520	cellular amino acid metabolic process	7	8.861	9.81E-04	790, 8565, 2618, 4677, 51520, 6472, 2058	77	250	16650	6.0545	0.8737	0.0318	1.6833
GOTERM_BP_FAT	GO:0019058	viral life cycle	9	11.392	0.001084581	9045, 6228, 81876, 6124, 6133, 3303, 1655, 6161, 6191	77	455	16650	4.2772	0.8985	0.0346	1.8597
GOTERM_BP_FAT	GO:1902582	single-organism intracellular transport	11	13.924	0.001192514	9045, 6228, 10128, 1434, 6124, 6133, 6161, 539, 6191, 3842, 10452	77	695	16650	3.4224	0.9192	0.0374	2.0430
GOTERM_BP_FAT	GO:0016070	RNA metabolic process	35	44.304	0.00123791	6637, 6635, 6124, 10856, 6161, 10521, 3028, 6421, 9045, 27339, 9775, 10949, 3191, 4677, 6749, 23020, 4670, 26528, 8565, 6133, 3303, 10236, 51520, 1660, 5701, 6228, 10128, 11338, 22913, 1655, 6050, 6191, 9188, 5591, 2058	77	4648	16650	1.6283	0.9265	0.0382	2.1200
GOTERM_BP_FAT	GO:1902580	single-organism cellular localization	14	17.722	0.001342295	1434, 6133, 6124, 6161, 3842, 9045, 6228, 10128, 4627, 1213, 539, 6191, 4905, 10452	77	1102	16650	2.7471	0.9411	0.0408	2.2968
GOTERM_BP_FAT	GO:0019083	viral transcription	6	7.595	0.001422734	9045, 6228, 6124, 6133, 6161, 6191	77	181	16650	7.1680	0.9503	0.0426	2.4328
GOTERM_BP_FAT	GO:0044283	small molecule biosynthetic process	9	11.392	0.001691317	4282, 790, 47, 51144, 6472, 50814, 10728, 5341, 38	77	488	16650	3.9879	0.9718	0.0497	2.8858
GOTERM_BP_FAT	GO:0006413	translational initiation	6	7.595	0.001721203	9045, 6228, 6124, 6133, 6161, 6191	77	189	16650	6.8646	0.9735	0.0499	2.9361
GOTERM_BP_FAT	GO:0006612	protein targeting to membrane	6	7.595	0.001761508	9045, 6228, 6124, 6133, 6161, 6191	77	190	16650	6.8284	0.9757	0.0503	3.0038
GOTERM_BP_FAT	GO:0019080	viral gene expression	6	7.595	0.001844191	9045, 6228, 6124, 6133, 6161, 6191	77	192	16650	6.7573	0.9796	0.0519	3.1427
GOTERM_BP_FAT	GO:0043933	macromolecular complex subunit organization	22	27.848	0.002697816	23020, 6637, 6635, 9380, 10856, 55937, 51649, 9361, 3842, 5701, 5341, 6421, 3615, 38, 4282,	77	2461	16650	1.9330	0.9966	0.0741	4.5657

						27339, 81876, 10938, 6472, 83706, 4905, 2058							
GOTERM_BP_FAT	GO:0044033	multi-organism metabolic process	6	7.595	0.002951972	9045, 6228, 6124, 6133, 6161, 6191	77	214	16650	6.0626	0.9980	0.0797	4.9855
GOTERM_BP_FAT	GO:0016032	viral process	12	15.190	0.004248983	9045, 6228, 81876, 6124, 6133, 3303, 1655, 6161, 9188, 6191, 3842, 5701	77	966	16650	2.6861	0.9999	0.1114	7.1011
GOTERM_BP_FAT	GO:0044764	multi-organism cellular process	12	15.190	0.004485701	9045, 6228, 81876, 6124, 6133, 3303, 1655, 6161, 9188, 6191, 3842, 5701	77	973	16650	2.6668	0.9999	0.1158	7.4824
GOTERM_BP_FAT	GO:0006605	protein targeting	10	12.658	0.004527472	9045, 6228, 11338, 1434, 6124, 6133, 6161, 6191, 3842, 10452	77	701	16650	3.0846	0.9999	0.1154	7.5496
GOTERM_BP_FAT	GO:0044419	interspecies interaction between organisms	12	15.190	0.005418773	9045, 6228, 81876, 6124, 6133, 3303, 1655, 6161, 9188, 6191, 3842, 5701	77	998	16650	2.6000	1.0000	0.1350	8.9711
GOTERM_BP_FAT	GO:0044403	symbiosis, encompassing mutualism through parasitism	12	15.190	0.005418773	9045, 6228, 81876, 6124, 6133, 3303, 1655, 6161, 9188, 6191, 3842, 5701	77	998	16650	2.6000	1.0000	0.1350	8.9711
GOTERM_BP_FAT	GO:0044248	cellular catabolic process	16	20.253	0.005783249	6124, 6133, 3303, 6161, 10236, 9361, 3028, 5701, 38, 9045, 6228, 10128, 27339, 9775, 6050, 6191	77	1616	16650	2.1409	1.0000	0.1417	9.5464
GOTERM_BP_FAT	GO:0090150	establishment of protein localization to membrane	7	8.861	0.006673482	9045, 6228, 6124, 6133, 6161, 6191, 4905	77	368	16650	4.1131	1.0000	0.1599	10.9373
GOTERM_BP_FAT	GO:0072657	protein localization to membrane	8	10.127	0.00689237	9045, 6228, 6124, 6133, 1213, 6161, 6191, 4905	77	489	16650	3.5376	1.0000	0.1629	11.2762
GOTERM_BP_FAT	GO:0046394	carboxylic acid biosynthetic process	6	7.595	0.006920858	4282, 790, 47, 51144, 6472, 10728	77	262	16650	4.9519	1.0000	0.1617	11.3203

GOTERM_BP_FAT	GO:0051260	protein homooligomerization	6	7.595	0.007480299	4282, 10938, 6472, 9361, 38, 3615	77	267	16650	4.8592	1.0000	0.1717	12.1805
GOTERM_BP_FAT	GO:0016053	organic acid biosynthetic process	6	7.595	0.008820994	4282, 790, 47, 51144, 6472, 10728	77	278	16650	4.6669	1.0000	0.1973	14.2101
GOTERM_BP_FAT	GO:0006399	tRNA metabolic process	5	6.329	0.010169291	8565, 4677, 51520, 3028, 2058	77	185	16650	5.8442	1.0000	0.2216	16.2067
GOTERM_BP_FAT	GO:0009161	ribonucleoside monophosphate metabolic process	6	7.595	0.011514393	790, 3303, 2618, 539, 29789, 3615	77	297	16650	4.3684	1.0000	0.2447	18.1548
GOTERM_BP_FAT	GO:0001649	osteoblast differentiation	5	6.329	0.012154079	23020, 1213, 9188, 1660, 5701	77	195	16650	5.5445	1.0000	0.2539	19.0662
GOTERM_BP_FAT	GO:1901605	alpha-amino acid metabolic process	5	6.329	0.012154079	790, 9775, 2618, 6472, 38	77	195	16650	5.5445	1.0000	0.2539	19.0662
GOTERM_BP_FAT	GO:0006461	protein complex assembly	14	17.722	0.013190405	9380, 3842, 9361, 5701, 5341, 3615, 38, 4282, 81876, 10938, 6472, 83706, 4905, 2058	77	1445	16650	2.0950	1.0000	0.2698	20.5225
GOTERM_BP_FAT	GO:0070271	protein complex biogenesis	14	17.722	0.013261335	9380, 3842, 9361, 5701, 5341, 3615, 38, 4282, 81876, 10938, 6472, 83706, 4905, 2058	77	1446	16650	2.0935	1.0000	0.2685	20.6213
GOTERM_BP_FAT	GO:0009123	nucleoside monophosphate metabolic process	6	7.595	0.013826135	790, 3303, 2618, 539, 29789, 3615	77	311	16650	4.1717	1.0000	0.2757	21.4036
GOTERM_BP_FAT	GO:0022618	ribonucleoprotein complex assembly	5	6.329	0.016068534	23020, 27339, 6637, 6635, 10856	77	212	16650	5.0999	1.0000	0.3101	24.4385
GOTERM_BP_FAT	GO:0061024	membrane organization	11	13.924	0.017963155	9045, 6228, 81876, 6124, 6133, 4627, 3303, 1213, 6161, 6191, 4905	77	1027	16650	2.3160	1.0000	0.3369	26.9164
GOTERM_BP_FAT	GO:0071822	protein complex subunit organization	15	18.987	0.018254071	9380, 51649, 3842, 9361, 5701, 5341, 3615, 38, 4282, 81876, 10938, 6472, 83706, 4905, 2058	77	1674	16650	1.9376	1.0000	0.3384	27.2900

GOTERM_BP_FAT	GO:0071826	ribonucleoprotein complex subunit organization	5	6.329	0.018979959	23020, 27339, 6637, 6635, 10856	77	223	16650	4.8483	1.0000	0.3464	28.2144
GOTERM_BP_FAT	GO:0006417	regulation of translation	6	7.595	0.020242606	10128, 9775, 10236, 51520, 6191, 2058	77	343	16650	3.7825	1.0000	0.3618	29.7960
GOTERM_BP_FAT	GO:0051259	protein oligomerization	7	8.861	0.020909363	4282, 10938, 9380, 6472, 9361, 38, 3615	77	473	16650	3.2001	1.0000	0.3682	30.6179
GOTERM_BP_FAT	GO:0001503	ossification	6	7.595	0.024081293	23020, 1213, 1655, 9188, 1660, 5701	77	359	16650	3.6139	1.0000	0.4080	34.4053
GOTERM_BP_FAT	GO:0034248	regulation of cellular amide metabolic process	6	7.595	0.02808273	10128, 9775, 10236, 51520, 6191, 2058	77	374	16650	3.4690	1.0000	0.4548	38.9055
GOTERM_BP_FAT	GO:0007005	mitochondrion organization	8	10.127	0.02927297	10128, 11338, 3303, 7284, 51649, 9361, 3028, 10452	77	653	16650	2.6491	1.0000	0.4654	40.1870
GOTERM_BP_FAT	GO:0009260	ribonucleotide biosynthetic process	5	6.329	0.030329283	790, 2618, 539, 38, 3615	77	258	16650	4.1906	1.0000	0.4742	41.3029
GOTERM_BP_FAT	GO:0009119	ribonucleoside metabolic process	6	7.595	0.031271943	790, 3303, 539, 29789, 38, 3615	77	385	16650	3.3699	1.0000	0.4814	42.2823
GOTERM_BP_FAT	GO:0046390	ribose phosphate biosynthetic process	5	6.329	0.031454096	790, 2618, 539, 38, 3615	77	261	16650	4.1424	1.0000	0.4801	42.4697
GOTERM_BP_FAT	GO:0001819	positive regulation of cytokine production	6	7.595	0.031572734	4282, 27159, 3303, 10148, 1660, 5591	77	386	16650	3.3611	1.0000	0.4781	42.5915
GOTERM_BP_FAT	GO:0009259	ribonucleotide metabolic process	7	8.861	0.035293854	790, 3303, 2618, 539, 29789, 38, 3615	77	535	16650	2.8292	1.0000	0.5139	46.2902
GOTERM_BP_FAT	GO:0009199	ribonucleoside triphosphate metabolic process	5	6.329	0.036198083	790, 3303, 539, 29789, 3615	77	273	16650	3.9603	1.0000	0.5196	47.1545
GOTERM_BP_FAT	GO:0098609	cell-cell adhesion	11	13.924	0.038297861	9045, 55379, 10938, 4627, 3303, 10148, 83706, 5591, 10598, 29789, 5341	77	1164	16650	2.0434	1.0000	0.5367	49.1111

GOTERM_BP_FAT	GO:0097193	intrinsic apoptotic signaling pathway	5	6.329	0.038717595	4282, 3303, 1655, 5591, 6421	77	279	16650	3.8752	1.0000	0.5373	49.4940
GOTERM_BP_FAT	GO:0019693	ribose phosphate metabolic process	7	8.861	0.03925364	790, 3303, 2618, 539, 29789, 38, 3615	77	549	16650	2.7571	1.0000	0.5390	49.9790
GOTERM_BP_FAT	GO:0009116	nucleoside metabolic process	6	7.595	0.040391198	790, 3303, 539, 29789, 38, 3615	77	413	16650	3.1414	1.0000	0.5462	50.9937
GOTERM_BP_FAT	GO:0009167	purine ribonucleoside monophosphate metabolic process	5	6.329	0.04133579	3303, 2618, 539, 29789, 3615	77	285	16650	3.7936	1.0000	0.5514	51.8215
GOTERM_BP_FAT	GO:0010628	positive regulation of gene expression	14	17.722	0.041749113	26528, 3303, 10856, 10521, 5701, 11338, 27339, 9775, 4627, 1655, 70, 9188, 6191, 5591	77	1692	16650	1.7892	1.0000	0.5519	52.1796
GOTERM_BP_FAT	GO:0009126	purine nucleoside monophosphate metabolic process	5	6.329	0.041781759	3303, 2618, 539, 29789, 3615	77	286	16650	3.7803	1.0000	0.5490	52.2078
GOTERM_BP_FAT	GO:0044802	single-organism membrane organization	9	11.392	0.04336906	9045, 6228, 6124, 6133, 3303, 1213, 6161, 6191, 4905	77	866	16650	2.2472	1.0000	0.5595	53.5590
GOTERM_BP_FAT	GO:0009141	nucleoside triphosphate metabolic process	5	6.329	0.046868536	790, 3303, 539, 29789, 3615	77	297	16650	3.6403	1.0000	0.5852	56.4118
GOTERM_BP_FAT	GO:1901657	glycosyl compound metabolic process	6	7.595	0.048588449	790, 3303, 539, 29789, 38, 3615	77	435	16650	2.9825	1.0000	0.5955	57.7526
GOTERM_BP_FAT	GO:0009893	positive regulation of metabolic process	21	26.582	0.053577659	26528, 47, 3303, 10856, 10236, 10521, 5701, 5341, 4282, 27339, 11338, 81876, 9775, 4627, 1655, 10148, 70, 9188, 6191, 5591, 4905	77	3052	16650	1.4878	1.0000	0.6292	61.4256
GOTERM_BP_FAT	GO:0014070	response to organic cyclic compound	9	11.392	0.053966483	790, 9775, 1655, 10856, 6161, 9188, 10521, 10728, 38	77	906	16650	2.1480	1.0000	0.6288	61.6988

GOTERM_BP_FAT	GO:0010564	regulation of cell cycle process	7	8.861	0.054220847	55920, 4282, 27339, 1213, 5591, 5701, 6421	77	595	16650	2.5439	1.0000	0.6275	61.8766
GOTERM_BP_FAT	GO:0048511	rhythmic process	5	6.329	0.054339068	10236, 1655, 1660, 5591, 6421	77	312	16650	3.4653	1.0000	0.6252	61.9589
GOTERM_BP_FAT	GO:0009165	nucleotide biosynthetic process	5	6.329	0.054858925	790, 2618, 539, 38, 3615	77	313	16650	3.4542	1.0000	0.6258	62.3191
GOTERM_BP_FAT	GO:0006732	coenzyme metabolic process	5	6.329	0.055906789	47, 2618, 51144, 6472, 38	77	315	16650	3.4323	1.0000	0.6299	63.0353
GOTERM_BP_FAT	GO:1901293	nucleoside phosphate biosynthetic process	5	6.329	0.056434792	790, 2618, 539, 38, 3615	77	316	16650	3.4214	1.0000	0.6305	63.3913
GOTERM_BP_FAT	GO:0006913	nucleocytoplasmic transport	6	7.595	0.061254778	11338, 6637, 1434, 6635, 9775, 3842	77	465	16650	2.7901	1.0000	0.6586	66.4950
GOTERM_BP_FAT	GO:0051169	nuclear transport	6	7.595	0.064923397	11338, 6637, 1434, 6635, 9775, 3842	77	473	16650	2.7429	1.0000	0.6776	68.6894
GOTERM_BP_FAT	GO:0071310	cellular response to organic substance	16	20.253	0.067445687	4670, 3303, 10856, 6161, 10521, 5701, 3615, 790, 4282, 9775, 10938, 1655, 10148, 5591, 10728, 2058	77	2186	16650	1.5827	1.0000	0.6891	70.1187
GOTERM_BP_FAT	GO:0034654	nucleobase-containing compound biosynthetic process	27	34.177	0.073781534	6637, 6635, 6124, 10856, 6161, 10521, 6421, 38, 9045, 9775, 539, 6749, 6133, 3303, 2618, 5701, 3615, 790, 6228, 11338, 10128, 22913, 1655, 9188, 6191, 5591, 10728	77	4357	16650	1.3400	1.0000	0.7198	73.4428
GOTERM_BP_FAT	GO:0006974	cellular response to DNA damage stimulus	8	10.127	0.074884164	4282, 27339, 1655, 10856, 5965, 6749, 5591, 6421	77	807	16650	2.1436	1.0000	0.7225	73.9844
GOTERM_BP_FAT	GO:0006629	lipid metabolic process	11	13.924	0.079722598	4282, 27339, 5049, 47, 51144, 55937, 50814, 3028, 10728, 5341, 38	77	1330	16650	1.7884	1.0000	0.7427	76.2404

GOTERM_BP_FAT	GO:0046128	purine ribonucleoside metabolic process	5	6.329	0.079745536	3303, 539, 29789, 38, 3615	77	356	16650	3.0370	1.0000	0.7401	76.2507
GOTERM_BP_FAT	GO:0050878	regulation of body fluid levels	6	7.595	0.079764419	790, 10938, 4627, 1314, 83706, 5341	77	503	16650	2.5793	1.0000	0.7375	76.2591
GOTERM_BP_FAT	GO:0034645	cellular macromolecule biosynthetic process	30	37.975	0.081403221	6637, 6635, 6124, 10856, 6161, 9361, 10521, 6421, 9045, 9775, 4677, 6749, 8565, 6133, 3303, 10236, 7284, 51520, 51649, 5701, 6228, 10128, 11338, 22913, 1655, 6191, 9188, 5591, 10728, 2058	77	5012	16650	1.2943	1.0000	0.7423	76.9800
GOTERM_BP_FAT	GO:0042278	purine nucleoside metabolic process	5	6.329	0.081662598	3303, 539, 29789, 38, 3615	77	359	16650	3.0116	1.0000	0.7408	77.0922
GOTERM_BP_FAT	GO:0071396	cellular response to lipid	6	7.595	0.082935987	4282, 1655, 10856, 6161, 10521, 10728	77	509	16650	2.5489	1.0000	0.7438	77.6355
GOTERM_BP_FAT	GO:0007155	cell adhesion	13	16.456	0.083147384	55920, 9045, 55379, 10938, 4627, 3303, 51144, 10148, 83706, 10598, 5591, 29789, 5341	77	1699	16650	1.6545	1.0000	0.7422	77.7245
GOTERM_BP_FAT	GO:0018130	heterocycle biosynthetic process	27	34.177	0.083502269	6637, 6635, 6124, 10856, 6161, 10521, 6421, 38, 9045, 9775, 539, 6749, 6133, 3303, 2618, 5701, 3615, 790, 6228, 11338, 10128, 22913, 1655, 9188, 6191, 5591, 10728	77	4411	16650	1.3236	1.0000	0.7412	77.8732
GOTERM_BP_FAT	GO:0009117	nucleotide metabolic process	7	8.861	0.08384236	790, 3303, 2618, 539, 29789, 38, 3615	77	667	16650	2.2693	1.0000	0.7401	78.0148
GOTERM_BP_FAT	GO:0022610	biological adhesion	13	16.456	0.084864456	55920, 9045, 55379, 10938, 4627, 3303, 51144, 10148, 83706, 10598, 5591, 29789, 5341	77	1705	16650	1.6487	1.0000	0.7420	78.4352
GOTERM_BP_FAT	GO:0019438	aromatic compound biosynthetic process	27	34.177	0.085971574	6637, 6635, 6124, 10856, 6161, 10521, 6421, 38, 9045, 9775, 539, 6749, 6133, 3303, 2618, 5701, 3615, 790, 6228, 11338,	77	4424	16650	1.3197	1.0000	0.7442	78.8821

						10128, 22913, 1655, 9188, 6191, 5591, 10728							
GOTERM_BP_FAT	GO:0006631	fatty acid metabolic process	5	6.329	0.086224643	4282, 47, 51144, 10728, 38	77	366	16650	2.9540	1.0000	0.7428	78.9830
GOTERM_BP_FAT	GO:0031325	positive regulation of cellular metabolic process	19	24.051	0.086337667	26528, 47, 3303, 10856, 10236, 10521, 5701, 5341, 4282, 11338, 27339, 81876, 9775, 4627, 1655, 10148, 6191, 5591, 4905	77	2839	16650	1.4471	1.0000	0.7407	79.0280
GOTERM_BP_FAT	GO:0070887	cellular response to chemical stimulus	18	22.785	0.086686605	4670, 3303, 10856, 6161, 10521, 9361, 5701, 6421, 3615, 790, 4282, 9775, 10938, 1655, 10148, 5591, 10728, 2058	77	2648	16650	1.4699	1.0000	0.7397	79.1661
GOTERM_BP_FAT	GO:0006753	nucleoside phosphate metabolic process	7	8.861	0.087602326	790, 3303, 2618, 539, 29789, 38, 3615	77	675	16650	2.2424	1.0000	0.7411	79.5245
GOTERM_BP_FAT	GO:0009150	purine ribonucleotide metabolic process	6	7.595	0.088924066	3303, 2618, 539, 29789, 38, 3615	77	520	16650	2.4950	1.0000	0.7442	80.0316
GOTERM_BP_FAT	GO:0071495	cellular response to endogenous stimulus	10	12.658	0.089783172	790, 4670, 9775, 10938, 3303, 1655, 6161, 10521, 5591, 10728	77	1185	16650	1.8248	1.0000	0.7453	80.3548
GOTERM_BP_FAT	GO:0010604	positive regulation of macromolecule metabolic process	19	24.051	0.090309212	26528, 3303, 10856, 10236, 10521, 5701, 4282, 11338, 27339, 81876, 9775, 4627, 1655, 10148, 70, 9188, 6191, 5591, 4905	77	2856	16650	1.4385	1.0000	0.7450	80.5503
GOTERM_BP_FAT	GO:0009719	response to endogenous stimulus	12	15.190	0.092284269	790, 4670, 9775, 10938, 3303, 1655, 6161, 10521, 9361, 5591, 10728, 38	77	1548	16650	1.6762	1.0000	0.7505	81.2680
GOTERM_BP_FAT	GO:0009725	response to hormone	8	10.127	0.094350228	790, 1655, 6161, 10521, 9361, 5591, 10728, 38	77	854	16650	2.0256	1.0000	0.7562	81.9920
GOTERM_BP_FAT	GO:0006281	DNA repair	6	7.595	0.094560581	27339, 10856, 5965, 6749, 5591, 6421	77	530	16650	2.4479	1.0000	0.7547	82.0642

GOTERM_BP_FAT	GO:0048545	response to steroid hormone	5	6.329	0.096411958	790, 1655, 6161, 10521, 10728	77	381	16650	2.8377	1.0000	0.7594	82.6882
GOTERM_BP_FAT	GO:0033993	response to lipid	8	10.127	0.097024157	4282, 790, 10949, 1655, 10856, 6161, 10521, 10728	77	860	16650	2.0115	1.0000	0.7594	82.8900
GOTERM_BP_FAT	GO:0051186	cofactor metabolic process	5	6.329	0.099221399	47, 2618, 51144, 6472, 38	77	385	16650	2.8082	1.0000	0.7652	83.5961
GOTERM_CC_FAT	GO:0030529	intracellular ribonucleoprotein complex	25	31.646	7.25E-13	6637, 6635, 7846, 6124, 6161, 9045, 27339, 9775, 10949, 3191, 23020, 4670, 6133, 10236, 51649, 1660, 5701, 6228, 10128, 11338, 22913, 1655, 6191, 10728, 2058	77	786	14527	6.0007	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:1990904	ribonucleoprotein complex	25	31.646	7.45E-13	6637, 6635, 7846, 6124, 6161, 9045, 27339, 9775, 10949, 3191, 23020, 4670, 6133, 10236, 51649, 1660, 5701, 6228, 10128, 11338, 22913, 1655, 6191, 10728, 2058	77	787	14527	5.9931	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:0070062	extracellular exosome	38	48.101	7.34E-09	1434, 6635, 6124, 7846, 9380, 10856, 1314, 3842, 38, 9045, 539, 3191, 4677, 6472, 4905, 4670, 47, 10960, 2618, 7284, 55937, 3615, 4282, 790, 81876, 10938, 5049, 4627, 1213, 1655, 70, 6050, 6191, 83706, 10598, 29789, 10728, 10452	77	2811	14527	2.5504	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:1903561	extracellular vesicle	38	48.101	8.44E-09	1434, 6635, 6124, 7846, 9380, 10856, 1314, 3842, 38, 9045, 539, 3191, 4677, 6472, 4905, 4670, 47, 10960, 2618, 7284, 55937, 3615, 4282, 790, 81876, 10938, 5049, 4627, 1213, 1655, 70, 6050, 6191, 83706, 10598, 29789, 10728, 10452	77	2825	14527	2.5378	0.0000	0.0000	0.0000

GOTERM_CC_FAT	GO:0043230	extracellular organelle	38	48.101	8.53E-09	1434, 6635, 6124, 7846, 9380, 10856, 1314, 3842, 38, 9045, 539, 3191, 4677, 6472, 4905, 4670, 47, 10960, 2618, 7284, 55937, 3615, 4282, 790, 81876, 10938, 5049, 4627, 1213, 1655, 70, 6050, 6191, 83706, 10598, 29789, 10728, 10452	77	2826	14527	2.5369	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:0071013	catalytic step 2 spliceosome	9	11.392	2.52E-08	23020, 4670, 27339, 6637, 22913, 6635, 9775, 10236, 1655	77	92	14527	18.4561	0.0000	0.0000	0.0000
GOTERM_CC_FAT	GO:0044421	extracellular region part	43	54.430	1.23E-07	1434, 6635, 6124, 7846, 9380, 10856, 1314, 3842, 38, 9045, 539, 3191, 4677, 6472, 4905, 4670, 8565, 3303, 47, 10960, 2618, 7284, 55937, 3615, 4282, 790, 81876, 27159, 10938, 5049, 4627, 1213, 1655, 51144, 10148, 70, 6050, 6191, 83706, 10598, 29789, 10728, 10452	77	3878	14527	2.0919	0.0000	0.0000	0.0002
GOTERM_CC_FAT	GO:0005681	spliceosomal complex	10	12.658	3.75E-07	23020, 4670, 27339, 11338, 6637, 22913, 6635, 9775, 10236, 1655	77	179	14527	10.5398	0.0001	0.0000	0.0005
GOTERM_CC_FAT	GO:0042645	mitochondrial nucleoid	6	7.595	3.56E-06	55379, 10128, 7284, 55210, 6472, 9361	77	45	14527	25.1550	0.0013	0.0001	0.0049
GOTERM_CC_FAT	GO:0009295	Nucleoid	6	7.595	4.43E-06	55379, 10128, 7284, 55210, 6472, 9361	77	47	14527	24.0846	0.0016	0.0002	0.0061
GOTERM_CC_FAT	GO:0031988	membrane-bounded vesicle	38	48.101	5.85E-06	1434, 6635, 6124, 7846, 9380, 10856, 1314, 3842, 38, 9045, 539, 3191, 4677, 6472, 4905, 4670, 47, 10960, 2618, 7284, 55937, 3615, 4282, 790, 81876, 10938, 5049, 4627, 1213, 1655, 70, 6050, 6191, 83706, 10598, 29789, 10728, 10452	77	3611	14527	1.9854	0.0021	0.0002	0.0080

GOTERM_CC_FAT	GO:0005576	extracellular region	44	55.696	6.93E-06	1434, 6635, 6124, 7846, 9380, 10856, 1314, 3842, 38, 9045, 539, 3191, 4677, 6472, 4905, 4670, 8565, 3303, 47, 10960, 2618, 7284, 55937, 5341, 3615, 4282, 790, 81876, 27159, 10938, 5049, 4627, 1213, 1655, 51144, 10148, 70, 6050, 6191, 83706, 10598, 29789, 10728, 10452	77	4623	14527	1.7956	0.0025	0.0002	0.0095
GOTERM_CC_FAT	GO:0005829	Cytosol	36	45.570	1.17E-05	55920, 6637, 1434, 6635, 6124, 7846, 9380, 6161, 1314, 3842, 55114, 9045, 9775, 4677, 4905, 8565, 6133, 3303, 47, 2618, 51520, 1660, 5701, 5341, 3615, 790, 6228, 5049, 4627, 1213, 70, 6191, 10598, 5591, 10728, 2058	77	3399	14527	1.9982	0.0042	0.0003	0.0160
GOTERM_CC_FAT	GO:0005654	nucleoplasm	33	41.772	1.71E-05	6637, 1434, 6635, 10856, 9361, 10521, 6421, 27339, 9775, 10949, 3191, 5965, 6749, 23020, 4670, 26528, 3303, 47, 10236, 1660, 5701, 4282, 790, 6228, 11338, 10128, 1655, 6050, 6191, 9188, 5591, 10728, 10452	77	2996	14527	2.0781	0.0061	0.0004	0.0234
GOTERM_CC_FAT	GO:0022626	cytosolic ribosome	6	7.595	4.94E-04	9045, 6228, 6124, 6133, 6161, 6191	77	125	14527	9.0558	0.1633	0.0118	0.6761
GOTERM_CC_FAT	GO:0005730	nucleolus	14	17.722	6.02E-04	55920, 4670, 6124, 6133, 10236, 1660, 10521, 6228, 5049, 1655, 6749, 9188, 5591, 29789	77	882	14527	2.9946	0.1954	0.0135	0.8243
GOTERM_CC_FAT	GO:0005912	adherens junction	12	15.190	9.20E-04	9045, 55379, 6124, 10938, 6133, 4627, 3303, 1213, 70, 6191, 10598, 29789	77	694	14527	3.2622	0.2826	0.0193	1.2560
GOTERM_CC_FAT	GO:0070161	anchoring junction	12	15.190	0.001119626	9045, 55379, 6124, 10938, 6133, 4627, 3303, 1213, 70, 6191, 10598, 29789	77	711	14527	3.1842	0.3326	0.0222	1.5274

GOTERM_CC_FAT	GO:0035770	ribonucleoprotein granule	6	7.595	0.001417712	6637, 7846, 3191, 6191, 1660, 5701	77	158	14527	7.1644	0.4008	0.0266	1.9304
GOTERM_CC_FAT	GO:0044445	cytosolic part	7	8.861	0.001558271	9045, 6228, 6124, 6133, 6161, 6191, 5701	77	239	14527	5.5257	0.4305	0.0278	2.1199
GOTERM_CC_FAT	GO:0031012	extracellular matrix	10	12.658	0.001733336	4670, 6133, 4627, 1213, 1655, 539, 51144, 6191, 5591, 6421	77	530	14527	3.5597	0.4654	0.0294	2.3554
GOTERM_CC_FAT	GO:0005840	ribosome	7	8.861	0.002077599	9045, 6228, 6124, 6133, 6161, 51649, 6191	77	253	14527	5.2199	0.5280	0.0336	2.8171
GOTERM_CC_FAT	GO:0043209	myelin sheath	6	7.595	0.00216554	4282, 10938, 7846, 1213, 7284, 4905	77	174	14527	6.5056	0.5428	0.0335	2.9347
GOTERM_CC_FAT	GO:0044391	ribosomal subunit	6	7.595	0.003096517	9045, 6228, 6124, 6133, 6161, 6191	77	189	14527	5.9893	0.6736	0.0456	4.1716
GOTERM_CC_FAT	GO:0034399	nuclear periphery	5	6.329	0.003926015	790, 4670, 10856, 3842, 6421	77	123	14527	7.6692	0.7583	0.0552	5.2614
GOTERM_CC_FAT	GO:0005759	mitochondrial matrix	8	10.127	0.006732247	55379, 10128, 7284, 55210, 6472, 9361, 3028, 38	77	425	14527	3.5513	0.9127	0.0895	8.8635
GOTERM_CC_FAT	GO:0036464	cytoplasmic ribonucleoprotein granule	5	6.329	0.007351141	6637, 7846, 6191, 1660, 5701	77	147	14527	6.4171	0.9303	0.0939	9.6406
GOTERM_CC_FAT	GO:0005913	cell-cell adherens junction	7	8.861	0.00778836	9045, 55379, 10938, 4627, 3303, 10598, 29789	77	332	14527	3.9778	0.9405	0.0959	10.1859
GOTERM_CC_FAT	GO:0005925	focal adhesion	7	8.861	0.016452293	6124, 6133, 4627, 3303, 1213, 70, 6191	77	391	14527	3.3776	0.9975	0.1866	20.3820
GOTERM_CC_FAT	GO:0005924	cell-substrate adherens junction	7	8.861	0.017020467	6124, 6133, 4627, 3303, 1213, 70, 6191	77	394	14527	3.3519	0.9980	0.1866	21.0116
GOTERM_CC_FAT	GO:0030055	cell-substrate junction	7	8.861	0.017997412	6124, 6133, 4627, 3303, 1213, 70, 6191	77	399	14527	3.3099	0.9986	0.1906	22.0834
GOTERM_CC_FAT	GO:0030054	cell junction	14	17.722	0.025801752	9045, 55379, 6124, 10938, 6133, 4627, 3303, 1213, 70, 6191, 83706, 10598, 29789, 55114	77	1379	14527	1.9154	0.9999	0.2554	30.1739

GOTERM_CC_FAT	GO:0044429	mitochondrial part	11	13.924	0.032943627	55379, 10128, 7284, 55210, 539, 51649, 6472, 9361, 3028, 10452, 38	77	990	14527	2.0962	1.0000	0.3068	36.8880
GOTERM_CC_FAT	GO:0031967	organelle envelope	12	15.190	0.036019207	55379, 10128, 1434, 7284, 55210, 539, 51649, 6472, 3842, 10452, 489, 38	77	1149	14527	1.9704	1.0000	0.3226	39.5906
GOTERM_CC_FAT	GO:0031975	Envelope	12	15.190	0.037004262	55379, 10128, 1434, 7284, 55210, 539, 51649, 6472, 3842, 10452, 489, 38	77	1154	14527	1.9618	1.0000	0.3222	40.4333
GOTERM_CC_FAT	GO:0005911	cell-cell junction	8	10.127	0.051050855	9045, 55379, 10938, 4627, 3303, 10598, 29789, 55114	77	644	14527	2.3436	1.0000	0.4087	51.3229
GOTERM_CC_FAT	GO:0005694	chromosome	10	12.658	0.055107003	55920, 10128, 27339, 1213, 10856, 5965, 6749, 5591, 10728, 6421	77	937	14527	2.0135	1.0000	0.4248	54.1051
GOTERM_CC_FAT	GO:0005739	mitochondrion	15	18.987	0.056106888	3303, 7284, 55210, 51649, 9361, 3028, 38, 10128, 55379, 81876, 1213, 539, 4677, 6472, 10452	77	1700	14527	1.6647	1.0000	0.4222	54.7679
GOTERM_CC_FAT	GO:0015630	microtubule cytoskeleton	11	13.924	0.059920941	55920, 10128, 27339, 7846, 4627, 3303, 1213, 1731, 6472, 1660, 29789	77	1100	14527	1.8866	1.0000	0.4356	57.2155
GOTERM_CC_FAT	GO:0019866	organelle inner membrane	7	8.861	0.071098926	10128, 7284, 55210, 539, 51649, 6472, 38	77	557	14527	2.3710	1.0000	0.4860	63.6997
GOTERM_CC_FAT	GO:0005819	Spindle	5	6.329	0.07424049	55920, 27339, 4627, 1213, 1731	77	303	14527	3.1132	1.0000	0.4930	65.3506
GOTERM_CC_FAT	GO:0005635	nuclear envelope	6	7.595	0.079180926	55379, 10128, 1434, 51649, 3842, 489	77	438	14527	2.5844	1.0000	0.5079	67.8067
GOTERM_MF_FAT	GO:0003723	RNA binding	37	46.835	7.04E-16	55920, 6637, 6635, 6124, 6161, 3842, 9361, 10521, 3028, 6421, 9045, 55379, 9775, 10949, 3191, 6749, 23020, 4670, 26528, 8565, 6133, 10236, 7284, 51649, 1660, 3615, 6228, 10128, 11338,	77	1656	15478	4.4912	0.0000	0.0000	0.0000

						22913, 4627, 1213, 1655, 6191, 9188, 5591, 2058							
GOTERM_MF_FAT	GO:0044822	poly(A) RNA binding	32	40.506	1.54E-15	55920, 6637, 6124, 6161, 10521, 3842, 3028, 6421, 9045, 55379, 9775, 10949, 3191, 6749, 23020, 4670, 26528, 8565, 10236, 7284, 51649, 1660, 6228, 11338, 10128, 22913, 4627, 1213, 1655, 6191, 9188, 5591	77	1196	15478	5.3783	0.0000	0.0000	0.0000
GOTERM_MF_FAT	GO:0000166	nucleotide binding	40	50.633	3.06E-13	7846, 9380, 55210, 10856, 9361, 10521, 6421, 489, 9775, 10949, 3191, 4677, 5965, 4905, 23020, 4670, 26528, 8565, 3303, 47, 1731, 10236, 2618, 7284, 51520, 1660, 5701, 3615, 790, 11338, 22913, 81876, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	2389	15478	3.3656	0.0000	0.0000	0.0000
GOTERM_MF_FAT	GO:1901265	nucleoside phosphate binding	40	50.633	3.10E-13	7846, 9380, 55210, 10856, 9361, 10521, 6421, 489, 9775, 10949, 3191, 4677, 5965, 4905, 23020, 4670, 26528, 8565, 3303, 47, 1731, 10236, 2618, 7284, 51520, 1660, 5701, 3615, 790, 11338, 22913, 81876, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	2390	15478	3.3642	0.0000	0.0000	0.0000
GOTERM_MF_FAT	GO:0036094	small molecule binding	41	51.899	5.98E-13	7846, 9380, 55210, 10856, 9361, 10521, 6421, 489, 9775, 10949, 3191, 4677, 5965, 4905, 23020, 4670, 26528, 8565, 3303, 47, 10960, 1731, 10236, 2618, 7284, 51520, 1660, 5701, 3615, 790, 11338, 22913, 81876, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	2571	15478	3.2056	0.0000	0.0000	0.0000

GOTERM_MF_FAT	GO:0016887	ATPase activity	17	21.519	3.04E-10	23020, 3303, 10856, 9361, 1660, 10521, 5701, 489, 9775, 4627, 1655, 539, 5965, 70, 9188, 4905, 29789	77	439	15478	7.7841	0.0000	0.0000	0.0000
GOTERM_MF_FAT	GO:0042623	ATPase activity, coupled	15	18.987	5.42E-10	23020, 3303, 10856, 9361, 1660, 10521, 5701, 489, 9775, 4627, 1655, 539, 5965, 9188, 4905	77	326	15478	9.2491	0.0000	0.0000	0.0000
GOTERM_MF_FAT	GO:1901363	heterocyclic compound binding	56	70.886	2.20E-09	7846, 6124, 9380, 6161, 10856, 489, 6421, 9045, 55379, 3191, 23020, 26528, 3303, 7284, 2618, 10236, 51520, 1660, 5701, 3615, 11338, 1655, 70, 6191, 5591, 29789, 2058, 55920, 6637, 6635, 55210, 3842, 9361, 10521, 3028, 9775, 10949, 5965, 4677, 6749, 4905, 4670, 8565, 6133, 47, 1731, 51649, 790, 6228, 10128, 22913, 81876, 10938, 4627, 1213, 9188	77	5971	15478	1.8852	0.0000	0.0000	0.0000
GOTERM_MF_FAT	GO:0035639	purine ribonucleoside triphosphate binding	30	37.975	3.68E-09	7846, 55210, 10856, 9361, 10521, 489, 9775, 5965, 4677, 4905, 23020, 8565, 3303, 47, 2618, 7284, 1731, 51520, 1660, 5701, 790, 81876, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	1841	15478	3.2756	0.0000	0.0000	0.0000
GOTERM_MF_FAT	GO:0097159	organic cyclic compound binding	56	70.886	3.89E-09	7846, 6124, 9380, 6161, 10856, 489, 6421, 9045, 55379, 3191, 23020, 26528, 3303, 7284, 2618, 10236, 51520, 1660, 5701, 3615, 11338, 1655, 70, 6191, 5591, 29789, 2058, 55920, 6637, 6635, 55210, 3842, 9361, 10521, 3028, 9775, 10949, 5965, 4677, 6749, 4905, 4670, 8565, 6133, 47, 1731, 51649, 790, 6228, 10128, 22913, 81876, 10938, 4627, 1213, 9188	77	6052	15478	1.8600	0.0000	0.0000	0.0000

GOTERM_MF_FAT	GO:0032550	purine ribonucleoside binding	30	37.975	4.12E-09	7846, 55210, 10856, 9361, 10521, 489, 9775, 5965, 4677, 4905, 23020, 8565, 3303, 47, 2618, 7284, 1731, 51520, 1660, 5701, 790, 81876, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	1850	15478	3.2597	0.0000	0.0000	0.0000
GOTERM_MF_FAT	GO:0032549	ribonucleoside binding	30	37.975	4.28E-09	7846, 55210, 10856, 9361, 10521, 489, 9775, 5965, 4677, 4905, 23020, 8565, 3303, 47, 2618, 7284, 1731, 51520, 1660, 5701, 790, 81876, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	1853	15478	3.2544	0.0000	0.0000	0.0000
GOTERM_MF_FAT	GO:0001883	purine nucleoside binding	30	37.975	4.28E-09	7846, 55210, 10856, 9361, 10521, 489, 9775, 5965, 4677, 4905, 23020, 8565, 3303, 47, 2618, 7284, 1731, 51520, 1660, 5701, 790, 81876, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	1853	15478	3.2544	0.0000	0.0000	0.0000
GOTERM_MF_FAT	GO:0097367	carbohydrate derivative binding	33	41.772	4.42E-09	7846, 55210, 10856, 9361, 10521, 489, 9775, 5965, 4677, 4905, 23020, 8565, 3303, 47, 10960, 2618, 7284, 1731, 51520, 1660, 5701, 790, 81876, 27159, 10938, 4627, 1655, 51144, 70, 9188, 5591, 29789, 2058	77	2241	15478	2.9600	0.0000	0.0000	0.0000
GOTERM_MF_FAT	GO:0001882	nucleoside binding	30	37.975	4.67E-09	7846, 55210, 10856, 9361, 10521, 489, 9775, 5965, 4677, 4905, 23020, 8565, 3303, 47, 2618, 7284, 1731, 51520, 1660, 5701, 790, 81876, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	1860	15478	3.2421	0.0000	0.0000	0.0000

GOTERM_MF_FA T	GO:003255 5	purine ribonucleotide binding	30	37.97 5	6.27E-09	7846, 55210, 10856, 9361, 10521, 489, 9775, 5965, 4677, 4905, 23020, 8565, 3303, 47, 2618, 7284, 1731, 51520, 1660, 5701, 790, 81876, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	1884	1547 8	3.2008	0.0000	0.0000	0.0000
GOTERM_MF_FA T	GO:001707 6	purine nucleotide binding	30	37.97 5	7.34E-09	7846, 55210, 10856, 9361, 10521, 489, 9775, 5965, 4677, 4905, 23020, 8565, 3303, 47, 2618, 7284, 1731, 51520, 1660, 5701, 790, 81876, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	1897	1547 8	3.1789	0.0000	0.0000	0.0000
GOTERM_MF_FA T	GO:003255 3	ribonucleotide binding	30	37.97 5	7.61E-09	7846, 55210, 10856, 9361, 10521, 489, 9775, 5965, 4677, 4905, 23020, 8565, 3303, 47, 2618, 7284, 1731, 51520, 1660, 5701, 790, 81876, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	1900	1547 8	3.1739	0.0000	0.0000	0.0000
GOTERM_MF_FA T	GO:000552 4	ATP binding	26	32.91 1	2.09E-08	10856, 55210, 9361, 10521, 489, 9775, 5965, 4677, 4905, 23020, 8565, 47, 3303, 2618, 51520, 1660, 5701, 790, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	1495	1547 8	3.4959	0.0000	0.0000	0.0000
GOTERM_MF_FA T	GO:001711 1	nucleoside- triphosphatase activity	19	24.05 1	2.81E-08	23020, 7846, 3303, 10856, 7284, 10521, 1660, 9361, 5701, 489, 9775, 4627, 1655, 539, 5965, 70, 9188, 4905, 29789	77	779	1547 8	4.9028	0.0000	0.0000	0.0000
GOTERM_MF_FA T	GO:003255 9	adenyl ribonucleotide binding	26	32.91 1	3.35E-08	10856, 55210, 9361, 10521, 489, 9775, 5965, 4677, 4905, 23020, 8565, 47, 3303, 2618, 51520, 1660, 5701, 790, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	1531	1547 8	3.4137	0.0000	0.0000	0.0000

GOTERM_MF_FAT	GO:0030554	adenyl nucleotide binding	26	32.911	3.87E-08	10856, 55210, 9361, 10521, 489, 9775, 5965, 4677, 4905, 23020, 8565, 47, 3303, 2618, 51520, 1660, 5701, 790, 10938, 4627, 1655, 70, 9188, 5591, 29789, 2058	77	1542	15478	3.3893	0.0000	0.0000	0.0001
GOTERM_MF_FAT	GO:0016462	pyrophosphatase activity	19	24.051	6.62E-08	23020, 7846, 3303, 10856, 7284, 10521, 1660, 9361, 5701, 489, 9775, 4627, 1655, 539, 5965, 70, 9188, 4905, 29789	77	824	15478	4.6350	0.0000	0.0000	0.0001
GOTERM_MF_FAT	GO:0016818	hydrolase activity, acting on acid anhydrides, in phosphorus-containing anhydrides	19	24.051	6.86E-08	23020, 7846, 3303, 10856, 7284, 10521, 1660, 9361, 5701, 489, 9775, 4627, 1655, 539, 5965, 70, 9188, 4905, 29789	77	826	15478	4.6238	0.0000	0.0000	0.0001
GOTERM_MF_FAT	GO:0016817	hydrolase activity, acting on acid anhydrides	19	24.051	7.12E-08	23020, 7846, 3303, 10856, 7284, 10521, 1660, 9361, 5701, 489, 9775, 4627, 1655, 539, 5965, 70, 9188, 4905, 29789	77	828	15478	4.6126	0.0000	0.0000	0.0001
GOTERM_MF_FAT	GO:0008026	ATP-dependent helicase activity	8	10.127	8.04E-07	23020, 9775, 1655, 10856, 5965, 9188, 10521, 1660	77	105	15478	15.3153	0.0003	0.0000	0.0011
GOTERM_MF_FAT	GO:0070035	purine NTP-dependent helicase activity	8	10.127	8.04E-07	23020, 9775, 1655, 10856, 5965, 9188, 10521, 1660	77	105	15478	15.3153	0.0003	0.0000	0.0011
GOTERM_MF_FAT	GO:0003676	nucleic acid binding	40	50.633	3.30E-06	55920, 6637, 6635, 6124, 10856, 6161, 3842, 9361, 10521, 3028, 6421, 9045, 55379, 9775, 10949, 3191, 4677, 5965, 6749, 23020, 4670, 26528, 8565, 6133, 10236, 7284, 51649, 1660, 3615, 6228, 10128, 11338, 22913, 4627, 1213, 1655, 6191, 9188, 5591, 2058	77	4097	15478	1.9625	0.0012	0.0000	0.0045

GOTERM_MF_FAT	GO:0004386	helicase activity	8	10.127	1.28E-05	23020, 9775, 1655, 10856, 5965, 9188, 10521, 1660	77	159	15478	10.1139	0.0046	0.0002	0.0175
GOTERM_MF_FAT	GO:0004004	ATP-dependent RNA helicase activity	6	7.595	1.77E-05	23020, 9775, 1655, 9188, 10521, 1660	77	66	15478	18.2739	0.0064	0.0002	0.0243
GOTERM_MF_FAT	GO:0008186	RNA-dependent ATPase activity	6	7.595	1.90E-05	23020, 9775, 1655, 9188, 10521, 1660	77	67	15478	18.0012	0.0069	0.0002	0.0262
GOTERM_MF_FAT	GO:0003724	RNA helicase activity	6	7.595	2.05E-05	23020, 9775, 1655, 9188, 10521, 1660	77	68	15478	17.7364	0.0074	0.0002	0.0281
GOTERM_MF_FAT	GO:0003735	structural constituent of ribosome	7	8.861	7.71E-04	9045, 6228, 6124, 6133, 6161, 51649, 6191	77	222	15478	6.3382	0.2442	0.0090	1.0550
GOTERM_MF_FAT	GO:0019899	enzyme binding	19	24.051	0.002164193	55920, 1434, 3303, 3842, 9361, 5341, 6421, 38, 790, 10128, 11338, 27159, 10938, 1213, 10949, 1655, 5591, 4905, 2058	77	1789	15478	2.1349	0.5445	0.0243	2.9352
GOTERM_MF_FAT	GO:0016616	oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	5	6.329	0.002617371	9380, 51144, 50814, 3028, 3615	77	117	15478	8.5903	0.6138	0.0284	3.5397
GOTERM_MF_FAT	GO:0098641	cadherin binding involved in cell-cell adhesion	7	8.861	0.002996115	9045, 55379, 10938, 4627, 3303, 10598, 29789	77	290	15478	4.8520	0.6635	0.0315	4.0422
GOTERM_MF_FAT	GO:0098632	protein binding involved in cell-cell adhesion	7	8.861	0.003597792	9045, 55379, 10938, 4627, 3303, 10598, 29789	77	301	15478	4.6747	0.7297	0.0367	4.8354
GOTERM_MF_FAT	GO:0098631	protein binding involved in cell adhesion	7	8.861	0.003899236	9045, 55379, 10938, 4627, 3303, 10598, 29789	77	306	15478	4.5983	0.7578	0.0386	5.2305
GOTERM_MF_FAT	GO:0016874	ligase activity	8	10.127	0.003899682	790, 27339, 8565, 2618, 4677, 51520, 2058, 38	77	409	15478	3.9318	0.7579	0.0376	5.2311

GOTERM_MF_FAT	GO:0045296	cadherin binding	7	8.861	0.003961711	9045, 55379, 10938, 4627, 3303, 10598, 29789	77	307	15478	4.5834	0.7633	0.0372	5.3122
GOTERM_MF_FAT	GO:0016614	oxidoreductase activity, acting on CH-OH group of donors	5	6.329	0.004606485	9380, 51144, 50814, 3028, 3615	77	137	15478	7.3362	0.8129	0.0421	6.1516
GOTERM_MF_FAT	GO:0050839	cell adhesion molecule binding	8	10.127	0.007409724	9045, 55379, 10938, 4627, 3303, 83706, 10598, 29789	77	461	15478	3.4883	0.9328	0.0653	9.7214
GOTERM_MF_FAT	GO:0005198	structural molecule activity	10	12.658	0.014119737	9045, 6228, 6124, 6133, 7846, 1213, 6161, 51649, 1314, 6191	77	782	15478	2.5705	0.9943	0.1183	17.7612
GOTERM_MF_FAT	GO:0051020	GTPase binding	6	7.595	0.019534061	55920, 1434, 10938, 3842, 4905, 2058	77	316	15478	3.8167	0.9992	0.1568	23.7589
GOTERM_MF_FAT	GO:0019904	protein domain specific binding	8	10.127	0.030853769	55920, 4670, 11338, 7846, 4627, 3303, 4905, 55114	77	614	15478	2.6191	1.0000	0.2325	35.0109
GOTERM_MF_FAT	GO:0005525	GTP binding	6	7.595	0.040389456	81876, 10938, 7846, 7284, 1731, 29789	77	384	15478	3.1408	1.0000	0.2883	43.2730
GOTERM_MF_FAT	GO:0017016	Ras GTPase binding	5	6.329	0.043274775	55920, 1434, 10938, 3842, 4905	77	269	15478	3.7363	1.0000	0.3001	45.5740
GOTERM_MF_FAT	GO:0032561	guanyl ribonucleotide binding	6	7.595	0.048820586	81876, 10938, 7846, 7284, 1731, 29789	77	405	15478	2.9780	1.0000	0.3263	49.7555
GOTERM_MF_FAT	GO:0019001	guanyl nucleotide binding	6	7.595	0.049246287	81876, 10938, 7846, 7284, 1731, 29789	77	406	15478	2.9706	1.0000	0.3230	50.0638
GOTERM_MF_FAT	GO:0044877	macromolecular complex binding	12	15.190	0.053345628	10128, 6637, 9775, 4627, 10856, 51144, 6749, 6472, 83706, 4905, 29789, 6421	77	1306	15478	1.8470	1.0000	0.3394	52.9445
GOTERM_MF_FAT	GO:0031267	small GTPase binding	5	6.329	0.054845358	55920, 1434, 10938, 3842, 4905	77	291	15478	3.4538	1.0000	0.3416	53.9593
KEGG_PATHWAY	hsa03040:Spliceosome		9	11.392	3.01E-06	23020, 4670, 27339, 11338, 6637, 6635, 9775, 3303, 1655	49	133	6910	9.5427	0.0002	0.0002	0.0032

KEGG_PATHWAY	hsa03010:Ribosome	6	7.595	0.0023817	9045, 6228, 6124, 6133, 6161, 6191	49	136	6910	6.2215	0.1658	0.0866	2.4780
KEGG_PATHWAY	hsa01130:Biosynthesis of antibiotics	6	7.595	0.015209883	47, 2618, 6472, 50814, 3028, 38	49	212	6910	3.9911	0.6880	0.3218	14.8949
KEGG_PATHWAY	hsa01100:Metabolic pathways	14	17.722	0.071424399	9380, 47, 2618, 3028, 3615, 38, 790, 5049, 539, 51144, 6472, 50814, 10728, 2058	49	1228	6910	1.6077	0.9964	0.7554	54.1498

Appendix 4: Down regulated proteins in JVM2 CYR61/CCN1 OE model.

Category	Term	GO TERM	Count	%	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
GOTERM_BP_FAT	GO:190170	response to oxygen-containing compound	5	45.45454545	0.008150698	4282, 523, 55937, 4736, 5052	11	1466	16650	5.162470544	0.998782504	0.998782504	11.8414847
GOTERM_BP_FAT	GO:0044248	cellular catabolic process	5	45.45454545	0.011499516	3094, 3303, 4736, 6165, 5052	11	1616	16650	4.683280828	0.999923959	0.991279862	16.31552214
GOTERM_BP_FAT	GO:0042592	homeostatic process	5	45.45454545	0.012238948	4282, 523, 55937, 5052, 94081	11	1645	16650	4.600718431	0.999958832	0.965470893	17.274359
GOTERM_BP_FAT	GO:0033036	macromolecule localization	6	54.54545455	0.016439709	4282, 523, 55937, 4736, 6165, 5052	11	2817	16650	3.223932617	0.99999875	0.966564926	22.52948703
GOTERM_BP_FAT	GO:0015031	protein transport	5	45.45454545	0.018730641	4282, 523, 4736, 6165, 5052	11	1860	16650	4.068914956	0.999999815	0.954993942	25.26220698
GOTERM_BP_FAT	GO:0006796	phosphate-containing compound metabolic process	6	54.54545455	0.023925461	4282, 2079, 523, 3094, 3303, 5052	11	3083	16650	2.945773007	0.999999998	0.963467907	31.12846442
GOTERM_BP_FAT	GO:0006793	phosphorus metabolic process	6	54.54545455	0.02411757	4282, 2079, 523, 3094, 3303, 5052	11	3089	16650	2.940051208	0.999999998	0.942721358	31.33691687
GOTERM_BP_FAT	GO:0045184	establishment of protein localization	5	45.45454545	0.024842135	4282, 523, 4736, 6165, 5052	11	2021	16650	3.744770816	0.999999999	0.924110779	32.117823

GOTERM_BP_FA T	GO:000810 4	protein localization	5	45.4545454 5	0.04680027 8	4282, 523, 4736, 6165, 5052	11	244 8	16650	3.09157754	1	0.987311139	52.1991366
GOTERM_BP_FA T	GO:000996 6	regulation of signal transduction	5	45.4545454 5	0.06604963	4282, 2079, 3094, 3303, 5052	11	272 8	16650	2.77426019 7	1	0.996314033	65.0866445
GOTERM_BP_FA T	GO:001003 3	response to organic substance	5	45.4545454 5	0.07340082 1	4282, 523, 3303, 55937, 4736	11	282 2	16650	2.68185039 6	1	0.996596369	69.0870028 6
GOTERM_BP_FA T	GO:004408 5	cellular component biogenesis	5	45.4545454 5	0.08102963 7	4282, 3303, 55937, 4736, 6165	11	291 4	16650	2.59717975 9	1	0.996893513	72.7824214 6
GOTERM_BP_FA T	GO:001064 6	regulation of cell communication	5	45.4545454 5	0.09034968	4282, 2079, 3094, 3303, 5052	11	302 0	16650	2.50602047	1	0.997453589	76.7365568 5
GOTERM_BP_FA T	GO:002305 1	regulation of signaling	5	45.4545454 5	0.09494181 6	4282, 2079, 3094, 3303, 5052	11	307 0	16650	2.46520580 4	1	0.997099448	78.4808291 6
GOTERM_CC_FA T	GO:000573 9	mitochondrion	6	54.5454545 5	0.00330940 2	8402, 523, 3303, 6165, 5052, 94081	11	170 0	14527	4.66106951 9	0.235479265	0.235479265	3.47111249 8
GOTERM_CC_FA T	GO:004442 1	extracellular region part	8	72.7272727 3	0.00522783 2	4282, 523, 3094, 3303, 55937, 4736, 6165, 5052	11	387 8	14527	2.72436588 7	0.345945849	0.191263856	5.43292869 7
GOTERM_CC_FA T	GO:007006 2	extracellular exosome	7	63.6363636 4	0.00534770 8	4282, 523, 3094, 55937, 4736, 6165, 5052	11	281 1	14527	3.28867113	0.352299415	0.13478356	5.55430841 8
GOTERM_CC_FA T	GO:190356 1	extracellular vesicle	7	63.6363636 4	0.0054882	4282, 523, 3094, 55937, 4736, 6165, 5052	11	282 5	14527	3.27237329	0.359668034	0.105456847	5.69638174 1
GOTERM_CC_FA T	GO:004323 0	extracellular organelle	7	63.6363636 4	0.00549834 3	4282, 523, 3094, 55937, 4736, 6165, 5052	11	282 6	14527	3.27121533 8	0.360196824	0.085446158	5.70663177 7
GOTERM_CC_FA T	GO:000557 6	extracellular region	8	72.7272727 3	0.01497885	4282, 523, 3094, 3303, 55937, 4736, 6165, 5052	11	462 3	14527	2.28533223 2	0.705496804	0.184329037	14.8573529 8
GOTERM_CC_FA T	GO:003198 8	membrane- bounded vesicle	7	63.6363636 4	0.01914115 4	4282, 523, 3094, 55937, 4736, 6165, 5052	11	361 1	14527	2.56008156 9	0.791009011	0.200395249	18.6143436
GOTERM_MF_FA T	GO:004482 2	poly(A) RNA binding	5	45.4545454 5	0.00325896 3	8402, 2079, 4736, 6165, 5052	10	119 6	15478	6.47073578 6	0.278504044	0.278504044	3.55829294 8
GOTERM_MF_FA T	GO:000372 3	RNA binding	5	45.4545454 5	0.01056989 3	8402, 2079, 4736, 6165, 5052	10	165 6	15478	4.67330917 9	0.654449021	0.412164156	11.1253588 5
GOTERM_MF_FA T	GO:190136 3	heterocyclic compound binding	8	72.7272727 3	0.02014108 3	8402, 2079, 523, 3094, 3303, 4736, 6165, 5052	10	597 1	15478	2.07375649	0.869276124	0.492481779	20.2150151 8
GOTERM_MF_FA T	GO:009715 9	organic cyclic compound binding	8	72.7272727 3	0.02183307	8402, 2079, 523, 3094, 3303, 4736, 6165, 5052	10	605 2	15478	2.04600132 2	0.890024074	0.424130064	21.7309076

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