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# Nuclear and membrane expression of the angiogenesis regulator delta-like ligand 4 (DLL4) in normal and malignant human tissues.

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# Corrigendum

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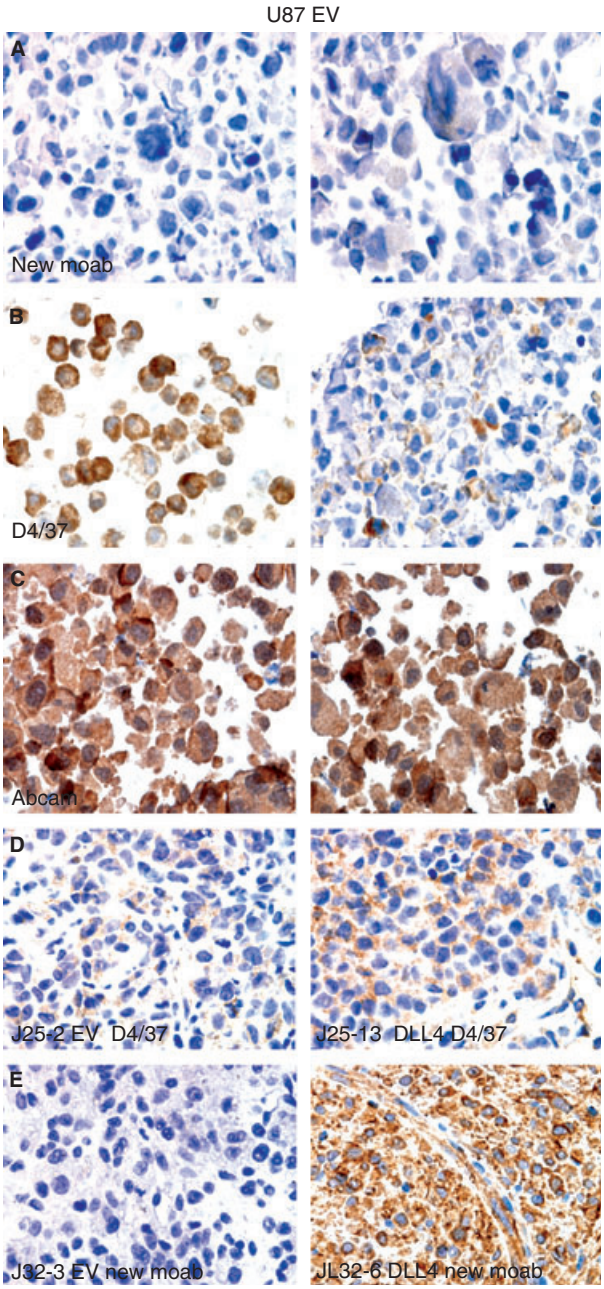
Martinez JC, Müller MM, Turley H, Steers G, Choteau L, Li J-L, Sainson R, Harris AL, Francesco Pezzella F, Gatter KC. Nuclear and membrane expression of the angiogenesis regulator Delta Like Ligand 4(DLL4) in normal and malignant human tissues. *Histopathology* 2009; **54**; 598–606.

Sir,

A paper from our group on the normal tissue distribution and tumour expression of a notch ligand, delta-like 4 was recently published in *Histopathology*.[1] We reported widespread expression in many tissues and a high proportion of tumours stained strongly positive. However, although in the paper we tested the antibodies against transfected cell lines, we have now done an experiment that we should have done earlier i.e. testing the same cell lines grown as xenografts, then formalin fixed and paraffin embedded [see figure below]. These results now show a significant background staining of the empty vector cell lines, and much less difference between control and transfected cells. So the dynamic range of the antibody is less than we found on frozen cell and tissues, and we can no longer be confident in these results. Furthermore, new antibodies are now available and we are also getting more results with *in situ* hybridization.

We therefore wish to withdraw our paper and re-submit our study in due course, as we will have completed a more extensive and detailed study including the use of new antibodies and more *in situ* hybridization experiments.

The human glioblastoma cell line U87 was analysed for expression of the notch ligand delta-like 4(Dll4). After 35 rounds of rt-PCR, no Dll4 transcript was detectable in these cells. They were transfected with the full-length human Dll4 construct and expressed high levels shown by western blotting and FACS analysis.




Three antibodies were tested: 1. D4/3, the subject of the paper. 2. A commercially antibody available from Abcam. 3. A new monoclonal antibody. Figure A shows two representative fields of the U87 cells in *in vitro* culture stained with the monoclonal antibody. Figure B shows two representative fields of the empty vector cell line stained with D4/3, showing significant background which is variable but can be high. Figure C shows a high background level with Abcam antibodies used in this study and were used in the confirmatory part of the work. Figure D shows xenografts of the U87 cell line, stained after formalin fixation the same way as the samples used in the paper. They show that the empty vector xenograft on the left, D, has rather similar staining to the Dll4 transfected cell lines on the right. There is a small difference in staining intensity. Figure E, in contrast, shows the empty vector xenograft has no staining with the new antibody and the transfected cell line shows striking upregulation of Dll4 in keeping with western blotting and FACS analysis for cell lines. Thus, the formalin fixation of sections has markedly reduced the specificity of the D4/3 antibody and it would seem that results with it are unreliable on sections handled in this way.

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1. Martinez JC, Müller MM, Turley H *et al.* Nuclear and membrane expression of the angiogenesis regulator Delta Like Ligand 4(DLL4) in normal and malignant human tissues. *Histopathology* 2009; **54;** 598–606.