

2019-10-30

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<http://hdl.handle.net/10026.1/15145>

10.1038/s41586-019-1722-1

Nature

Springer Science and Business Media LLC

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1 Allele-selective Lowering of Mutant HTT Protein by HTT-LC3 Linker Compounds

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31 **Summary**

32 Accumulation of mutant proteins is the major cause for many diseases
33 (proteopathies), and lowering the level of these proteins is highly desired for treatment.
34 We hypothesized that compounds interacting with both the autophagosome protein
35 LC3¹ and the disease-causing protein may target the latter for autophagic clearance.
36 We tested this hypothesis in the context of lowering mutant HTT protein (mHTT),
37 which contains an expanded polyglutamine (polyQ) tract and causes Huntington's
38 disease (HD), an incurable neurodegenerative disorder². Through
39 small-molecule-microarray based screening, we identified four compounds interacting
40 with both LC3 and mHTT, but not the wild-type HTT protein (wtHTT). Some of these
41 compounds targeted mHTT to autophagosomes, reduced mHTT levels in an
42 allele-selective manner, and rescued HD-relevant phenotypes in cells and *in vivo* in
43 the fly and mouse HD models. We further revealed that these compounds interacted
44 with the expanded polyQ stretch and could lower the level of mutant ATXN3, another
45 disease-causing protein with expanded polyQ³. Our study provides candidate
46 compounds for lowering mHTT and potentially other disease-causing proteins with
47 polyQ expansion, demonstrating the concept of lowering disease-causing proteins by
48 autophagosome-tethering compounds (ATTEC).

49 **Background**

50 An emerging approach for disease treatment is to lower the levels of
51 disease-causing proteins, especially those with unknown activities. Biological tools
52 such as RNAi or CRISPR may achieve this goal⁴⁻⁶, but their clinical delivery is
53 challenging. Enhancing proteasomal degradation of target proteins by “PROTAC” is a
54 promising emerging approach⁷, but proteasomes alone are inefficient in degrading
55 certain large proteins or aggregates⁸. Another independent protein degradation
56 pathway is macroautophagy (referred to as autophagy hereafter), which is a bulk
57 degradation system that engulfs proteins into autophagosomes for subsequent
58 lysosomal degradation⁹. Autophagy is present in all eukaryotic cells, and thus
59 harnessing the power of autophagy to degrade certain target proteins may open new
60 windows for drug discovery. Here we investigate this possibility in the context of
61 lowering mHTT, which contains an expanded polyglutamine (polyQ) stretch ($\geq 36Q$)
62 and causes HD, an incurable monogenetic neurodegenerative disorder².

63 mHTT could be degraded by autophagy, during which protein substrates are
64 engulfed into double-membrane autophagosomes associated with lipidated LC3¹. We
65 thus hypothesized that linker compounds interacting with both mHTT and LC3 may
66 tether them together to enhance the recruitment of mHTT into autophagosomes,
67 facilitating mHTT degradation. In addition, mHTT-LC3 linker compounds that do not
68 interact with wtHTT may promote allele-selective degradation of mHTT. Since no
69 mHTT/LC3-interacting compounds have been reported, we performed
70 small-molecule-microarray-based screening for desired compounds, and utilized

71 wtHTT for the counter-screen to identify allele-selective candidates.

72 **Results**

73 *Identification of mHTT-LC3 linker compounds*

74 We stamped 3375 compounds (Fig. 1a) in duplicates into a microarray on
75 isocyanate-functionalized glass slides *via* the nucleophile-isocyanate reaction, which
76 forms covalent bonds between the compounds and the glass slides^{10,11}. We then
77 purified the human LC3B protein¹ (Extended Data Fig. 1a-b & Supplementary Table 1),
78 a pathogenic mHTT exon1 fragment¹² with expanded polyQ (mHTT_{exon1-Q72}), and a
79 control wtHTT exon1 fragment (HTT_{exon1-Q25}) (Extended Data Fig. 1c-d) for the
80 screen. We fused a maltose-binding-protein (MBP) tag to both HTT exon1 proteins to
81 increase their solubility required for later experiments.

82 To identify LC3B- and mHTT- interacting compounds, we had these purified
83 proteins flow through the SMMs, and detected the compound-protein interaction using
84 an optical biosensor, the scanning oblique-incidence reflectivity difference (OI-RD)
85 microscope. OI-RD is a widely used interaction measurement technology¹³⁻¹⁵, whose
86 working principle has been validated and reported previously¹⁶⁻¹⁸. We then performed
87 experiments with HTT_{exon1-Q25} or buffer alone to exclude non-specific signals, and
88 identified two compounds that interact with both LC3B and mHTT_{exon1-Q72}, but not
89 HTT_{exon1-Q25}: 10O5 (GW5074,
90 3-3-[(3,5-Dibromo-4-hydroxyphenyl)methylidene]-5-iodo-1H-indol-2-one) and 8F20
91 (ispinesib,
92 N-(3-aminopropyl)-N-[(1R)-1-[7-chloro-4-oxo-3-(phenylmethyl)-2-quinazolinyl]-2-meth

93 ylpropyl]-4-methylbenzamide) (Fig. 1b, annotated based on their ID in the compound
94 library). We then measured the K_{on} and K_{off} of these interactions to confirm our
95 observation (Fig. 1c-d) and revealed ~ 100 nM K_d of the compounds' interaction with
96 LC3B or mHTT_{exon1-Q72}. As shown in Fig. 1e, these compounds also interacted
97 with the full-length mHTT (flHTT-Q73, Extended Data Fig. 1e), but not wtHTT
98 (HTT_{exon1-Q25} or flHTT-Q23, Fig. 1c&e) or irrelevant proteins (Extended Data Fig.
99 2a) including MBP-His8 (MBP), superfolder GFP (sfGFP), and Rpn10 (a proteasomal
100 ubiquitin-receptor) (Extended Data Fig. 1f). We then validated the interaction by an
101 orthogonal assay, microscale thermophoresis (MST), and obtained consistent results
102 (Extended Data Fig. 2b).

103 *mHTT-LC3 linker compounds reduced mHTT levels via autophagy in an*
104 *allele-selective manner*

105 We then tested if these potential mHTT-LC3 linker compounds decrease mHTT
106 levels *via* autophagy as predicted. Both hits decreased levels of mHTT in cultured
107 primary cortical neurons from a well-established HD knockin mouse model
108 (Hdh^{Q7/Q140})¹⁹ (Fig. 2a), while had little or no effect on levels of wtHTT in the
109 heterozygous HD neurons (Hdh^{Q7/Q140}) (Fig. 2a) and wild-type neurons (Hdh^{Q7/Q7}) (Fig.
110 2b), consistent with their lack of wtHTT interaction. We then looked for other
111 mHTT-LC3 linker compounds based on the common features of the two hit
112 compounds 10O5 and 8F20. The –OH group in 10O5 and the –NH₂ group in 8F20
113 were utilized in the nucleophile-isocyanate reaction for stamping of the SMMs, and
114 these groups were inaccessible to mHTT and LC3B for the compound-protein

115 interaction during the screening (Fig. 2c). Thus, while these two hit compounds have
116 different structures, their exposed chemical groups on the SMMs shared similarities
117 by containing an aryl ring connected with a lactam-based bicyclic structure with
118 halogen-substituted aryl group (Fig. 2c). We tested several compounds with similar
119 features and identified two additional mHTT-LC3 linker compounds (Fig. 2c, AN1
120 (3-5-Bromo-3-[(3-bromo-4,5-dihydroxyphenyl)methylidene]-1H-indol-2-one) and AN2
121 (5,7-Dihydroxy-4-phenylcoumarin), for analog1 and analog2, respectively) that
122 interact with both mHTT and LC3B, but not wtHTT or irrelevant control proteins
123 (Extended Data Fig. 2c-d). They also reduced the levels of mHTT in an
124 allele-selective manner in cultured HD mouse neurons (Fig. 2d). No cytotoxicity was
125 observed in cultured neurons treated with these compounds at the tested
126 concentration range (Extended Data Fig. 2e), confirming that the mHTT lowering was
127 not due to cell loss.

128 Most of these compounds showed an optimal dose (hook effect) in lowering
129 mHTT (Fig. 2a&d): a sufficient concentration is desired for tethering mHTT and LC3
130 together, but excessively high concentrations may cause the compound molecules to
131 interact with mHTT and LC3 separately, without tethering them. Similar concentration
132 dependent effects were observed in HD patient fibroblasts (Fig. 3c, *right* panel) and
133 have been reported for PROTAC²⁰. Consistent with the prediction that the mHTT
134 lowering is mediated by degradation *via* autophagy, the autophagy inhibitor NH₄Cl or
135 chloroquine blocked the mHTT-lowering effects (Extended Data Fig. 3a), confirming
136 that the compounds targeted mHTT for autophagic degradation. Meanwhile, the

137 compound-induced mHTT-lowering effects were only slightly enhanced by the mTOR
138 inhibitor rapamycin, an enhancer of autophagosome formation (Extended Data Fig.
139 3a, *right* panels; also see Fig. 3b).

140 The mHTT lowering could be detected by multiple mHTT antibodies, including
141 3B5H10, which detects a toxic species of the expanded polyQ stretch^{21,22} (Fig. 2d,
142 *right* panel), suggesting that the detected mHTT lowering was not due to affinity
143 changes to a specific antibody. In addition, we did not observe any obvious increase
144 of possible polyQ-containing mHTT fragments at lower molecular weights (Extended
145 Data Fig. 3b-c), suggesting that the mHTT lowering was not due to increased
146 site-specific cleavages of mHTT.

147 We further investigated the compounds' effects in HD patient cells by the
148 well-established HTRF (Homologous Time-Resolved Fluorescence) assay^{23,24}, which
149 is more quantitative than Western-blot, although inapplicable to mouse mHTT
150 proteins due to non-specific signals²⁵. We observed autophagy-dependent lowering of
151 mHTT by these compounds in HD patient fibroblasts and iPSC-derived neurons (Fig.
152 3a-b&Extended Data Fig. 3d), but not wtHTT in the wild-type or Parkinson's disease
153 (PD) patient fibroblasts (Fig. 3a). To further confirm the role of autophagic degradation,
154 we tested the compounds' effect with or without lowering of ATG5, a key autophagy
155 gene required for autophagosome formation²⁶. ATG5 knockdown in HD patient
156 fibroblasts (Q47) significantly decreased LC3-II and nullified the mHTT lowering
157 effects induced by the mHTT-LC3 linker compounds (Extended Data Fig. 3e). Similar
158 results were obtained in ATG5 knockout mouse embryonic fibroblasts²⁶ (MEFs,

159 Extended Data Fig. 3f), confirming that the compounds' effects were mediated by
160 autophagic degradation.

161 The two hit compounds 10O5 and 8F20 are known to inhibit c-Raf and KSP^{27,28},
162 respectively, whereas AN1 and AN2 had unknown activities on these targets. We thus
163 tested their potential influences on c-Raf and KSP. Based on the *in vitro* c-Raf kinase
164 assay, 10O5, the known c-Raf inhibitor, but not the other three compounds inhibited
165 c-Raf at the concentrations tested (Extended Data Fig. 4a). We then tested Mek/Erk
166 phosphorylation levels in the cultured neurons treated with these compounds at
167 optimal mHTT-lowering concentrations to evaluate Raf activity²⁹ and found no
168 significant effects of all tested compounds (Extended Data Fig. 4b, *left* panel). We
169 also tested phospho-BUBR1 levels to evaluate KSP activities³⁰, and observed no
170 significant effects either (Extended Data Fig. 4b, *right* panel). We made similar
171 observations in the HD patient fibroblasts (Q47) (Extended Data Fig. 4c). Thus, the
172 observed mHTT-lowering is probably irrelevant to c-Raf or KSP inhibition. To further
173 confirm this, we examined the effects of several known c-Raf or KSP inhibitors, and
174 found that they had no HTT-lowering effects (Fig. 3c, *left* panel). Two of these
175 inhibitors, PLX-4720 and BAY1217389, have structures somewhat similar to 10O5
176 and 8F20, respectively (Fig. 3c, *middle* panel). They showed no effects in lowering
177 mHTT in patient cells at sub-micromolar concentrations (Fig. 3c, *right* panel), probably
178 because they had very weak affinity to LC3 and mHTT, if any (Extended Data Fig. 2c,
179 *right panels*). In comparison, AN2 showed a dose-dependent mHTT lowering in the
180 same cells (Fig. 3c, *right* panel).

181 We then investigated the compounds' effects *in vivo*. Since the *Drosophila* LC3
182 homolog Atg8 has a predicted structure highly similar to LC3B (Extended Data Fig.
183 5a), we tested the compounds in HD transgenic flies expressing human full-length
184 mHTT. All of the discovered mHTT-LC3 linker compounds significantly reduced mHTT
185 levels (Extended Data Fig. 5b), validating the *in vivo* efficacy of these compounds.

186 We further investigated the compounds' effects *in vivo* in the HD knockin mouse
187 model (Hdh^{Q7/Q140})¹⁹ by intracerebroventricular (icv) injections. Three out of the four
188 linker compounds (10O5, AN1 or AN2, but not 8F20) led to significant lowering of
189 mHTT in cortices of HD mice (Extended Data Fig. 6a). We then performed
190 intraperitoneal (ip) injection of 10O5 and AN2 at 0.5 mg/kg in HD knockin mice. The
191 compounds crossed the blood-brain barrier and reached the brain at detectable
192 concentrations (Extended Data Fig. 5c, ~20 to 200 nM for 10O5 and ~20 to 40 nM for
193 AN2; no signal was detected in the DMSO injected control group) 0.5 ~ 6 hours after
194 injection. Consistent with this, we observed significant allele-selective lowering of
195 mHTT in mouse cortices and striata (Extended Data Fig. 6b-c). The observed
196 lowering was not due to changes in mHTT solubility, because no increase of mHTT
197 aggregates was observed in the cortical tissues of mice treated with these
198 compounds (Extended Data Fig. 6d).

199 *mHTT-LC3 linker compounds tethered mHTT to autophagosomes*

200 We then examined whether these compounds truly function as linkers between
201 mHTT and LC3 to target mHTT for autophagosome engulfment. Presence of 10O5 or
202 AN2, the two compounds that were effective *in vivo* by ip-injection, markedly

203 enhanced the mHTT-LC3 interaction in the *in vitro* pull-down experiments (Fig. 4a,
204 comparing lane 11 with 12&13; Fig. 4b, comparing lane 11 with 12 for both *upper* and
205 *lower* panels). No enhancement effect was observed for the wtHTT-LC3 interaction
206 (Fig. 4a, lane 8-10; Fig. 4b, lane 9-10 in both *upper* and *lower* panels). Consistent with
207 this, these compounds led to elevated engulfment of mHTT by autophagosomes both
208 in transiently transfected HeLa cells expressing exogenous GFP-LC3B and
209 HTTexon1-MBP-His fragments (Fig. 4c), and in mouse striatal cells (STHdh^{Q111/Q111})³¹
210 expressing endogenous LC3 and full-length mHTT proteins (Fig. 4d).

211 The data above confirmed that the compounds tethered mHTT and
212 LC3B/autophagosomes *in vitro* and in cells, although the detailed structural
213 information remains to be resolved.

214 *mHTT-LC3 linker compounds did not influence autophagy function and reduced*
215 *mHTT in a relatively specific manner*

216 The mHTT-lowering by the linker compounds was unlikely due to an
217 enhancement of autophagy functions, because the number and size of
218 autophagosomes remained unchanged (Extended Data Fig. 7a). We then further
219 investigated whether the compounds could influence autophagy by established
220 approaches in the literature³²⁻³⁴. Neither 10O5 nor AN2 influenced the
221 autophagosome-lysosome fusion or autophagy activity (Extended Data Fig. 7b-d).
222 Furthermore, we observed no changes of the LC3-II level in the cultured cortical
223 neurons treated with 10O5 or AN2 in the absence or presence of the lysosome
224 inhibitor bafilomycin-A1 (bafA1) (Extended Data Fig. 7e). The level of the known

225 autophagy-selective substrate protein SQSTM1/p62 was also unaffected *in vivo* and
226 in cultured neurons (Extended Data Fig. 7f & 8a). In addition, other wild-type polyQ
227 proteins (Atxn3 and Tbp) and control proteins (Nbr1, Ncoa4, Actin, Gapdh, Tubulin)
228 were not influenced (Extended Data Fig. 8a, all changes < 10%).

229 We then performed proteomics analysis to obtain a more complete scope of
230 proteins that may have been influenced. We observed significant lowering (~20%, $p <$
231 0.01) of HTT levels in cortices of mice ip-injected with 10O5 or AN2 (Extended Data
232 Fig. 8b, bar plots). Considering that the proteomics analysis was unable to distinguish
233 mHTT from wtHTT, the actual mHTT lowering was expected to be more. Meanwhile,
234 using the criteria of $p < 0.01$, we observed changes of only a small percentage of
235 proteins (Extended Data Fig. 8b, and see Supplementary Table 2 for details). No
236 autophagy-specific substrate proteins exhibited significant changes and there was no
237 enrichment of autophagy pathway-related proteins (Supplementary Table 2), further
238 confirming that autophagy was unaffected. Proteomics analysis in cultured neurons
239 revealed consistent results (Extended Data Fig. 8c, and see Supplementary Table 3
240 for details).

241 *mHTT-LC3 linker compounds lowered other proteins with expanded polyQ*

242 The discovered linker compounds interacted with and lowered mHTT but not
243 wtHTT (Fig. 1). The simplest explanation is that the compounds specifically interact
244 with the expanded polyQ tract, possibly by recognizing its emergent conformation that
245 is different from the short polyQ stretch^{21,35}. If so, the discovered linker compounds
246 may reduce other proteins with expanded polyQ. Consistent with this prediction, the

247 linker compounds 10O5, AN1 and AN2 reduced the levels of mutant but not wild-type
248 ATXN3 in SCA3 patient fibroblasts (Extended Data Fig. 9a) and exogenously
249 expressed 72Q-GFP, 46Q-GFP, 38Q-GFP but not 25Q-GFP proteins (containing just
250 the Met-polyQ-sfGFP sequences) in HEK293T cells (Extended Data Fig. 9b). These
251 data suggest that the compounds distinguished the expanded from the short polyQ
252 stretch at a threshold between 25Q and 38Q. To further confirm this, we tested the
253 compound-polyQ interactions (Extended Data Fig. 9c), and confirmed that 10O5, AN1
254 and AN2 interacted with polyQ-GFP with 38Q or longer polyQ, but not 25Q-GFP or
255 GFP alone (Extended Data Fig. 9d-e; Extended Data Fig. 2).

256 *mHTT-LC3 linker compounds rescued HD-relevant phenotypes*

257 We further investigated the therapeutic potential of the compounds in treating HD.
258 All the mHTT-LC3 linker compounds rescued mHTT toxicity in HD patient
259 iPSC-derived neurons (Fig. 5a-b). They also rescued HD-relevant behavioral deficits
260 and increased the lifespan of flies expressing human mHTT, while having no influence
261 on the flies expressing wtHTT (Fig. 5c).

262 Finally, we investigated the disease-relevant behavioral phenotypes in 10-months
263 old heterozygous HD knockin mouse ($Hdh^{Q7/Q140}$). Significant deficits of HD mice were
264 observed in several behavioral tests including rotarod, balance beam, and gripping
265 force tests, while the DMSO (110 $\mu\text{g}/\text{kg}$) injection alone did not have an effect
266 (Extended Data Fig. 9f-h). In comparison, ip injection of the 10O5 or AN2 significantly
267 improved HD-relevant behavioral deficits in these tests, without influencing the
268 wild-type mice (Fig. 5d-f), demonstrating a rescue of HD-relevant phenotypes. This is

269 a proof of principle study, and further investigations will be required to establish the
270 suitability for therapeutic application.

271 **Discussion**

272 In this study, we have identified mHTT-LC3 linker compounds that were able to
273 reduce mHTT levels at ~nM concentrations in HD cells and at 0.5 mg/kg by ip
274 injection *in vivo* (Extended Data Table 1). The compounds did not influence wtHTT,
275 which has essential functions especially during development and young adulthood³⁶.
276 These features of the compounds are highly desired for HD treatment or potentially
277 other polyQ diseases (Extended Data Fig. 9a-e), although preclinical studies of
278 longitudinal efficacy and safety are necessary for therapeutic development.

279 From a broader perspective, we demonstrate the concept of using small molecule
280 compounds to “glue” the target protein (mHTT) and LC3 for autophagic degradation
281 (Fig. 5g). We selected mHTT as our target protein, because wtHTT provides a perfect
282 control for screening. We had the fortune to discover hits that interact with both LC3B
283 and mHTT. If no such hits had been identified, linker compounds could still be
284 generated by conjugating an mHTT-interacting compound and an LC3-interacting
285 compound by the nucleophile-isocyanate reaction utilized for the stamping of SMMs.
286 To develop this concept, the critical next step is to resolve the core chemical
287 compartment that interacts with LC3 without influencing its function. Comprehensive
288 medicinal chemistry and structural studies are needed to resolve the compound-LC3
289 interaction interface, which may then be developed to a general degradation tool for
290 conjugation with other compounds interacting with specific targets of interest.

291 In summary, we have identified mHTT-LC3 linker compounds that are capable of
292 lowering mHTT *in vivo* in an allele-selective manner, and demonstrated the possibility
293 of utilizing autophagosome-tethering compounds (ATTEC) to lower target proteins,
294 providing new entry points for drug discovery.

295

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405

406 **Main figure legends**

407 **Figure 1. Identification of potential mHTT-LC3 linker compounds by SMM-based**
408 **screening and validation**

409 a) The OI-RD image of a small molecule microarray (SMM). Each compound was
410 printed in duplicates in adjacent vertical positions.

411 b) Magnified view of surface mass density changes after incubation with
412 HTTexon1-Q25-MBP, HTTexon1-Q72-MBP, and LC3B, respectively. Two hits (10O5
413 and 8F20) were highlighted by red rectangular boxes.

414 c-e) Association-dissociation curves of surface immobilized compounds 8F20 and
415 10O5 with HTTexon1-Q72-MBP (Q72), HTTexon1-Q25-MBP (Q25), LC3B, full-length
416 HTT-Q73 (Q73), or full-length HTT-Q23 (Q23) at the indicated concentrations of
417 purified proteins. For details see “Methods”.

418

419 **Figure 2. mHTT-LC3 linker compounds lowered mHTT but not wtHTT through**
420 **autophagy in cultured mouse neurons.**

421 a) Western-blot (HTT detected by the 2166 antibody) and quantifications of
422 compound-treated cultured cortical neurons from $Hdh^{Q7/Q140}$ HD knockin mice. The
423 statistical analysis was performed by two-way ANOVA tests. For 10O5, $F(1, 72) =$
424 $50.93, p < 0.0001$; for 8F20, $F(1, 40) = 8.903, p = 0.0048$.

425 b) Representative Western-blot (from 3 biological repeats) of cultured wild-type
426 cortical neurons treated with the indicated compounds.

427 c) 2D-structures of the hit compounds and the other effective linker compounds
428 identified. The red lines indicate the glass-chips used to stamp the hit compounds.
429 The dotted ovals indicate the possible chemical groups exposed for
430 protein-compound interactions in the screening.

431 d) *Left* and *middle* panels: similar to a), but treated with the compounds AN1 or AN2.
432 For AN1, $F(1, 70) = 32.96, p < 0.0001$; For AN2, $F(1, 69) = 23.03, p < 0.0001$. *Right*
433 panels: similar to a), but blotted with different HTT antibodies (10O5 and 8F20: 100nM;
434 AN2: 50 nM).

435 For all panels, n indicates the number of independently plated wells, and bars
436 represent mean and s.e.m.. Full-blot of cropped gels are shown in Extended Data
437 Fig. 3b or Supplementary Fig. 1.

438

439 **Figure 3. mHTT-LC3 linker compounds lowered mHTT in HD patient cells.**

440 a) HTT levels measured by HTRF (2B7/MW1 for mHTT, and 2B7/2166 for total HTT)
441 in the indicated HD, WT or PD patient primary fibroblasts treated with the indicated
442 compounds (100 nM). All signals were normalized to the averaged signals from the
443 DMSO control group. The statistical analysis was performed by one-way ANOVA with
444 post-hoc Dunnett's tests. “****”: $p < 0.0001$.

445 b) Similar to a), but in immortalized HD patient fibroblasts treated with or without the
446 autophagy inhibitors NH_4Cl /chloroquine/bafilomycin-A1, or the autophagy enhancer
447 rapamycin.

448 c) *Left* panel: similar to a), but in immortalized HD patient fibroblasts (Q47) treated
449 with indicated c-Raf or KSP inhibitors at 100 nM. *Middle* panel: 2D structure of

450 inhibitors. The dotted ovals indicate the parts of the compounds that share similarities
451 with the hit compounds. *Right* panel: dose-dependent curves of the indicated
452 compounds.

453 For all panels, n indicates the number of independently plated wells, and bars
454 represent mean and s.e.m..

455

456 **Figure 4. Linker compounds enhanced mHTT-LC3 interaction and tethered**
457 **mHTT to autophagosomes**

458 a-b) Representative results (from 3 biological repeats) of *in vitro* pull-down
459 experiments using purified HTT and LC3B proteins. For details see “Methods”.

460 c-d) Representative images (*scale* bar: 10 μ m) and quantifications of the
461 co-localization between HTT and autophagosomes. For details see “Methods”.

462 Bars present mean and s.e.m.. The n number indicates the number of cells. The
463 statistical analysis was performed by one-way ANOVA with post-hoc Dunnett’s tests.

464 “****”: $p < 0.0001$.

465

466 **Figure 5. Linker compounds rescued HD-relevant phenotypes in cells and *in***
467 ***vivo*.**

468 a) Representative immunostaining images (*scale* bar: 50 μ m) and quantifications of
469 the neuronal specific tubulin marker TUBB3 and DAPI staining showing neuronal
470 morphology of patient iPSC-derived striatal neurons (HD: Q47; WT: Q19) treated with
471 indicated compounds. For details see “Methods”.

472 b) Neuronal apoptosis measurement at different time points after BDNF removal using
473 a green fluorescent dye (NucView 488) detecting active caspase-3. For details see
474 “Methods”.

475 c) *Left*: Kaplan-Meier survival curves of transgenic *Drosophila* with the indicated
476 transgenes and compound treatments. *Right*: similar to the *left* panel, but plotting the
477 climbing performance as a function of age after eclosion. For details see “Methods”.

478 d-f) Mouse behavioral tests showing the improvement of HD-relevant phenotypes by
479 ip-injection of the indicated compounds at 0.5 mg/kg. For details see “Methods”.

480 g) A schematic model showing how mHTT-LC3 linker compounds may induce mHTT
481 degradation, illustrating the concept of lowering target proteins by
482 autophagosome-tethering compounds (ATTEC). The images representing HTT
483 proteins were published previously³⁷.

484

485

486 **Methods**

487 **Additional details of figure legends**

488 Figure 1. Identification of potential mHTT-LC3 linker compounds by SMM-based
489 screening and validation.

490 c-e) For all association-dissociation curves, vertical dash lines mark the starts of
491 association and dissociation phases of the binding event. The red dash curves are
492 global fits to a Langmuir reaction model with the fitting parameters listed at the bottom
493 of each plot. No binding signals were observed for HTT_{exon1}-Q25-MBP or full-length
494 HTT-Q23 proteins, and thus the parameters were not presented.

495 Figure 4. Linker compounds enhanced mHTT-LC3 interaction and tethered mHTT
496 to autophagosomes.

497 a-b) For MBP pull-down (a), purified HTT_{exon1}-MBP (10 µg) or MBP (10 µg) bound
498 MBP-resin were incubated with the purified LC3B protein (40 µg) and the indicated
499 compounds. The HTT_{exon1}-MBP or the MBP proteins were pulled down and the
500 eluates were tested for co-precipitated LC3B. 4% of the total eluate was loaded in
501 each lane, and the input : pull-down loading ratio was 100%. Both 10O5 and AN2
502 enhanced LC3B's interaction with HTT_{exon1}-Q72-MBP, but not HTT_{exon1}-Q25-MBP.
503 Note that the MBP blot signals were much weaker for the Q72 protein, possibly
504 because recognition of the MBP tag by the antibody was affected in the fusion protein.
505 Meanwhile, data interpretation was not influenced, because compound treatments did
506 not alter the MBP signals for the Q72 protein (last three lanes). The GST pull-down (b)
507 was performed similarly, except using full-length HTT-Q73 or full-length HTT-Q23
508 (both without fusion tags) and GST-LC3B proteins for the *in vitro* GST pull-down
509 experiments to precipitate GST-LC3B or GST alone with its binding proteins, and then
510 eluted for detection. Note that the pull-down is in the reverse direction of the pull-down
511 in (a). The input : pull-down loading ratio for the GST blot was 100%, whereas the
512 ratio for the HTT blot was 10% to avoid over-exposure of the input. Both 10O5 and
513 AN2 enhanced LC3B's interaction with the full-length HTT-Q73 but not the full-length
514 HTT-Q23 protein.

515 c-d) Representative confocal microscopy images (*scale* bar: 10 µm) and
516 quantifications of the co-localization between HTT_{exon1}-MBP-His (red, detected by
517 anti-His immunofluorescence) and LC3B-GFP (green, detected by GFP fluorescence
518 directly) in transiently transfected HeLa cells (c) or between endogenous mHTT and
519 LC3-II in the HD knockin mouse striatal cells (STHdh^{Q111/Q111}) (d). For over-expressed
520 proteins (c), the LC3B-GFP alone transfected or the HTT_{exon1}-MBP-His alone
521 transfected cells were imaged at both channels to ensure the specificity of the signals
522 (*upper* panels). The white arrows indicate representative co-localization puncta. Parts

523 of the images have been amplified to show co-localization puncta more clearly
524 (pointed by orange arrows). Since the puncta were obvious, co-localization was
525 analyzed by counting the red⁺green⁺ (yellow) and the total red⁺ puncta directly, and
526 then calculate their ratio for each cell. Blind analysis was performed for quantifications.
527 For endogenous proteins (d), mHTT was detected by the anti-HTT antibody 2166, and
528 the endogenous LC3-II was detected by an anti-LC3 antibody that has been reported
529 to specifically detect LC3-II³². Since the signals of endogenous proteins were more
530 dispersed, the co-localization analysis was performed blindly by measuring the
531 red⁺green⁺ (yellow) and the total red⁺ pixels by ImageJ, and then calculate their ratio
532 for each cell.

533 Figure 5. Linker compounds rescued HD-relevant phenotypes in cells and *in vivo*.

534 a) Loss of processes and shrinkage of neurons were observed in HD neurons after
535 BDNF removal. Bar plots: quantification of the TUBB3 signal covered area (TUBB3
536 area) normalized to the nuclei counts based on DAPI. The lower TUBB3 area *per cell*
537 reflects neuronal processes shrinkage and loss. Data were normalized to the average
538 of WT controls. The statistical analysis was performed by one-way ANOVA (F (5, 60) =
539 94.78) with post-hoc Dunnett's tests: "****": p<0.0001. The n number indicates the
540 number of independently plated wells.

541 b) The images were captured every 3 hours inside the incubator using Incucyte, and
542 the caspase-3 active cells were quantified by the fluorescent object count per field.
543 The statistical analysis was performed by two-way ANOVA (F (43, 516) = 12.85) with
544 post-hoc Dunnett's tests, comparing to the HD_DMSO group. "****": p<0.0001. The
545 numbers in brackets indicate the number of independently plated wells, with 4 fields
546 *per well* imaged and averaged for quantification. Three batches were tested and
547 showed consistent results.

548 c) *Left*: the *Drosophila* expressed full-length HTT proteins (Q128 or Q16) in the
549 nervous system driven by *elav-GAL4*. 75 flies were tested for each group. The
550 statistics was performed by Log-rank (Mantel-Cox) test, comparing compound treated
551 groups with DMSO controls in Q128 flies. ****: p < 0.0001. *Right*: similar as the *left*
552 panel, but plotting the climbing performance as a function of age after eclosion. The
553 statistics was performed by two-way ANOVA (F (4, 275) = 122.1) with post-hoc
554 Dunnett's tests, comparing the compound treated groups with the DMSO controls in
555 Q128 flies. Numbers in brackets indicate the number of vials (each containing 15 flies)
556 tested. ****: p < 0.0001.

557 d-f) The numbers in brackets indicated the number of mice tested. The statistical
558 analysis was performed by two-way ANOVA with post-hoc Dunnett's tests, and the p

559 values compared with the DMSO control were indicated (“****”: $p < 0.0001$). For HD
560 mice, $F(2, 195) = 4.963$ in rotarod tests, $F(2, 195) = 37.31$ in balance beams tests,
561 and $F(2, 156) = 7.068$ in gripping force tests. No significant difference was detected in
562 the wild-type mice injected with different compounds. The investigators were blinded
563 to the compounds and genotypes when performing the experiments. In all panels, the
564 error bars represent mean and s.e.m..

565 **Compound stamping on the microarray**

566 Small molecule microarrays (SMMs) containing 3,375 bioactive compounds were
567 used for high-throughput screening of target proteins. The compound library
568 containing 1,527 drugs approved by Food and Drug Administration (FDA) of United
569 States, 1,053 natural products from traditional Chinese medicine, and 795 known
570 inhibitors were stamped onto the SMMs. Each compound was dissolved in DMSO at
571 a concentration of 10 mM and printed in duplicates along vertical direction on
572 homemade phenyl-isocyanate functionalized glass slides with a contact microarray
573 printer (SmartArrayer 136, CapitalBio Corporation). Biotin-BSA at a concentration of
574 7,600 nM in 1× phosphate-buffered saline (PBS) and biotin-(PEG)₂-NH₂ at a
575 concentration of 5 mM in DMSO were printed as the inner and outer borders of SMMs,
576 respectively. The diameter of each spot was about 150 μm and spacing between two
577 adjacent spots was 250 μm. The printed SMMs were then dried at 45 °C for 24 h to
578 facilitate covalent bonding of nucleophilic groups of small molecules to isocyanate
579 groups of the functionalized slides. Afterwards, the SMMs were stored in a -20 °C
580 freezer.

581 **Expression and purification of recombinant proteins**

582 The human microtubule associated protein 1 light chain 3 beta (MAP1LC3B,
583 LC3B) gene (GenBank: NM_022818.4) was amplified by PCR and cloned into a
584 pGEX-6P1 (GE Healthcare) derived vector pGHT, which is a prokaryotic expression
585 vector reconstructed by adding a His8 tag and a TEV protease cleavage site before
586 the pGEX-6P1 multiple cloning site. After sequencing verification, the expression
587 plasmid pGHT-LC3B was introduced into *Escherichia coli* BL21 (DE3) pLysS, in which
588 the recombinant GST-LC3B protein was expressed by IPTG induction. When the
589 bacterial culture reached OD₆₀₀=0.8, its temperature was decreased to 18 °C, and 0.2
590 mM IPTG was added into the culture for an additional 20 h incubation. The cells were
591 then harvested by centrifugation (6,000g, 4 °C, 15 min) and the cell pellet was
592 suspended in 50 mM Tris-HCl buffer, pH 7.5, with 150 mM NaCl and 5% glycerol.
593 Cells were then disrupted by sonication, followed by centrifugation (20,000g, 4 °C, 60
594 min). The supernatants were then loaded onto a HisTrap HP column (GE Healthcare,

595 cat. no. 17524701), and eluted with 50 mM Tris-HCl buffer, pH 7.5, containing 150 mM
596 NaCl, 5% glycerol and 300 mM imidazole. The LC3B eluate was then mixed with TEV
597 protease (Sigma, cat. no. T4455; eluted protein: TEV protease = 100:1) and dialyzed
598 against the dialysate buffer (50 mM Tris-HCl buffer, pH 7.5, containing 100 mM NaCl)
599 in 4 °C overnight. After TEV protease treatment, the samples were then loaded onto a
600 HisTrap HP column again, the flow through fraction which mainly contains tag
601 removed recombinant LC3B. Afterwards, the proteins were concentrated and further
602 purified by Superose 6 Increase 10/300 GL (GE Healthcare) size exclusive
603 chromatography. Finally, the purified proteins were concentrated to approximately 10
604 mg/ml in 50 mM HEPES buffer with 100 mM NaCl for further analysis. The MBP-His8
605 and Rpn10 proteins were purified similarly.

606 The full-length HTT proteins, HTTExon1-MBP, polyQ-sfGFP and sfGFP proteins
607 were purified from mammalian cells. For full-length HTT proteins, the human *HTT*
608 gene (GenBank: NM_002111.8) with (CAG)₂₃ or (CAG)₇₃ (23Q or 73Q for proteins)
609 were *de novo* synthesized (by Genewiz Inc.), sequence validated and then cloned into
610 a modified pCAG vector with an N-terminal protein A tag. The plasmid was transfected
611 to human embryonic kidney E293 cells using polyethylenimine (PEI, from
612 Polysciences, cat. no. 23966). After culture at 37 °C for 48 to 60 h, cells were
613 collected and lysed at 4 °C for 1 h in lysis buffer containing 50 mM Tris-HCl, pH 8.0,
614 150 mM NaCl, 5% glycerol, 0.5% CHAPS, 3 mM DTT, 1% PMSF, 1 µg/ml pepstatin, 1
615 µg/ml leupeptin and 1 µg/ml aprotinin, 5 mM ATP and 5 mM MgCl₂. After
616 centrifugation at 15000 rpm for 40 min, the supernatants were then incubated with
617 IgG monoclonal antibody-agarose (Smart-lifesciences, cat. no. SA030010) for 2 h and
618 unbound proteins were extensively washed away. The HTT proteins were then
619 digested using TEV protease overnight to remove the protein A tag and eluted protein
620 was further purified by ion exchange and gel filtration chromatography using Mono Q
621 and Superose 6 (5/150 GL) columns from GE healthcare. The peak fractions were
622 pooled for further biochemical analysis. The HTTExon1 with 25Q or 72Q cDNA were
623 also *de novo* synthesized and cloned into a mammalian expression vector
624 pTT5SH8Q2 for large scale production in HEK293T cells. In order to improve the
625 production yield and increase the solubility, a C terminal MBP tag was added after the
626 HTTExon1 sequences to generate the pTT-HTTExon125Q-MBP and
627 pTT-HTTExon125Q-MBP plasmids. For protein production and purification, the
628 HEK293T cells were transfected by pTT-HTT25QExon1-MBP and
629 pTT-HTT72QExon1-MBP plasmids with linear PEI (PolySciences #24765), and then
630 collected after 48 h. The cells were then lysed by sonication in buffer containing 50
631 mM Tris-HCl, pH 7.5, 150 mM NaCl, 20 mM imidazole, 5% glycerol, protease inhibitor

632 cocktail (Sigma) and 50U/ml benzonase (Sigma). After centrifugation, the
633 supernatants were loaded onto HisTrap HP column (GE Healthcare), and eluted with
634 the buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 300 mM imidazole, 5%
635 glycerol and protease inhibitor cocktail. The MBP tag was not cleaved to avoid
636 precipitation. Afterwards, the proteins were concentrated and further purified by
637 Superose 6 Increase 10/300 GL (GE Healthcare) size exclusive chromatography.

638 **Verifications of the recombinant proteins by matrix assisted laser desorption** 639 **ionization-time of flight mass spectrometry (MALDI-TOF).**

640 The purified LC3B, HTT_{exon1Q25}-MBP, and HTT_{exon1Q72}-MBP proteins were
641 dialyzed into 5 mM NH₄Ac by Superose 6 Increase size exclusive chromatography for
642 linear mode MALDI-TOF analysis on a Bruker FLEX MALDI-TOF instrument. A total of
643 1500-2500 scans were averaged for each spectrum using an accelerating voltage of
644 25 kV. Sinapinic acid (SA, Bruker, cat. no. 820135) was used as the matrices for
645 protein and peptide analyses. SA was made into 20 mg/mL solutions in 70% ACN, 0.1%
646 TFA. For the acquisition of spectra from 10,000 to 100,000 amu, 2 μ L of sample was
647 mixed with 2 μ L of SA solution in an Eppendorf tube, and 2 μ L of the mixture was
648 loaded onto the MALDI plate. The calibration peptides for this range were BSA (M +
649 66431) (Sigma, cat. no. A1933). All spectra were obtained in positive linear mode.
650 The amount of full-length HTT proteins were limited, and thus not validated by
651 MALDI-TOF. Instead, they were further purified by ion exchange and gel filtration
652 chromatography, and validated by Coomassie-blue staining (Extended data Fig. 1e)
653 and Western-blot (Fig. 4b).

654 **Verifications of the recombinant LC3B by X ray diffraction crystallography**

655 Since the deletion of G120 (lipidation site) stabilizes LC3B protein, we utilized
656 LC3B Δ G120 protein to get high resolution diffraction data. Purified LC3B Δ G120
657 protein was concentrated in the following buffer: 20 mM HEPES pH7.5, 150 mM NaCl.
658 The LC3B Δ G120 crystal was grown in reservoir solutions consisting of 0.16 M
659 ammonium sulfate, 0.08 M sodium acetate pH 4.6, 20% (w/v) PEG4000, 20% (v/v)
660 glycerol and 0.01 M Taurine.

661 **Refinement**

662 The X-ray diffraction data were collected at 100 K in the beamline BL17U1 and
663 BL19U1, SSRF. The wavelength for data collection was 0.97892 Å. Diffraction images
664 were indexed and processed by HKL2000. The structure of LC3B Δ G120 (PDB ID
665 6J04, 1.90Å) was solved by molecular replacement with the Phaser 2.8 program from
666 the CCP4 crystallography package using the (PDB ID code 1UGM) as the search
667 model. The refinement was performed by Refmac 5.5 and Phenix 1.14. There are no

668 Ramachandran outliers to report. The related figure was drawn using PyMOL 2.2.

669 **Compound-protein interaction measurements by oblique-incidence reflectivity**
670 **difference (OI-RD)**

671 For high-throughput preliminary screening of target proteins, a SMM was
672 assembled into a fluidic cartridge and washed *in situ* with a flow of 1× PBS to remove
673 excess unbound small molecules. After washing, the SMM was scanned with a
674 label-free OI-RD scanning microscope to image small molecules immobilized on glass
675 slides. After it was blocked with 7,600 nM BSA in 1× PBS for 30 min, SMM was
676 incubated with the target protein for 2 h. HTTexon1-Q25-MBP at a concentration of
677 454 nM, HTTexon1-Q72-MBP at a concentration of 238 nM, and LC3B at a
678 concentration of 680 nM were screened on separate fresh SMMs. OI-RD images were
679 scanned for each operation, including washing, blocking, and incubation. The OI-RD
680 difference images (images after incubation – images before incubation) were utilized
681 for analysis, and vertical bright doublet spots indicated compounds that bind with
682 target proteins in both replicates. 8F20 and 10O5 were identified to bind to
683 HTTexon1-Q72-MBP and LC3B, but not HTTexon1-Q25-MBP. The binding was
684 further confirmed by the kinetics measurements (see below).

685 To measure binding kinetics of target proteins with compounds, we prepared new
686 SMMs consisting of 8F20, 10O5, and AN2. Six identical microarrays were printed on
687 one glass slide and each compound was printed in triplicates in a single microarray.
688 The printed small SMMs were assembled into a fluidic cartridge with each microarray
689 housed in a separate chamber. Before the binding reaction, the slide was washed *in*
690 *situ* with a flow of 1× PBS to remove excess unbound samples, followed by blocking
691 with 7,600 nM BSA in 1× PBS for 30 min. For binding kinetics measurement, 1 × PBS
692 was first flowed through a reaction chamber at a flow rate of 0.01 mL/min for 5 min to
693 acquire the baseline. 1 × PBS was then quickly replaced with the probe solution of the
694 target protein at a flow rate of 2 mL/min for 9 sec followed by a reduced flow rate at
695 0.01 mL/min to have the microarray incubated in the probe solution under the flow
696 condition for 35 min (association phase of the reaction). The probe solution was then
697 quickly replaced with 1 × PBS at a flow rate of 2 mL/min for 9 sec followed by a
698 reduced flow rate of 0.01 mL/min to allow dissociation of probe for 30 min
699 (dissociation phase of the reaction). By repeating the binding reactions of the target
700 protein at three different concentrations on separate fresh microarrays, binding curves
701 of compounds with the target protein at three concentrations were recorded with
702 scanning OI-RD microscope. Reaction kinetic rate constants were extracted by fitting
703 the binding curves globally using 1-to-1 Langmuir reaction mode.

704 **Compound-protein interaction measurements by microscale thermophoresis**

705 **(MST)**

706 The purified recombinant proteins were dialyzed into 1× PBS, and then labelled
707 according to the protocol of Protein labeling kit RED-NHS (Nanotemper, cat. no. L001).
708 All the tested stock compounds (10 mM) dissolved in DMSO were also diluted into the
709 same buffer for the final MST assay. The MST experiment was performed using
710 Monolith NT.115 instrument (NanoTemper Technologies). 500 nM of labelled proteins
711 were mixed with the indicated concentrations of candidate compounds in the reaction
712 buffer containing 20 mM HEPES, pH 7.4, 150 mM NaCl. The MST data were then
713 collected under 40% infrared laser power and 20% light-emitting diode power. The
714 data were analyzed by Nanotemper analysis software (1.5.41) and the K_d was
715 determined.

716 **cDNA plasmids for transfection in mammalian cells**

717 The pEX-GFP-hLC3WT plasmid was obtained from Addgene (#24987) to
718 express LC3B. The pTT-HTTExon1-Q72-MBP-His and pTT-HTTExon1-Q25-MBP-His
719 were generated by subcloning HTTExon1 cDNAs into the mammalian expression
720 vector pTT-MBP-His and then transiently transfected into HeLa cells to express
721 HTTExon1 proteins for the colocalization experiments. The polyQ-GFP sequences
722 (expressing Met-polyQ-sfGFP) were *de novo* synthesized and subcloned into the
723 pcDNA vector. All plasmids were sequence validated. For transient transfections, the
724 cells were plated at 50% confluence. After 24 h, the cDNAs were transfected with
725 Lipofectamine 2000 (ThermoFisher Scientific, cat. no. 11668019) using the forward
726 transfection protocol provided by the manufacturer.

727 **Cell culture**

728 For mouse primary cortical neuron cultures, cortices were isolated from P0 pups
729 following genotyping. Cortices were dissected into cold Ca^{2+} - and Mg^{2+} -free PBS
730 buffer. Chopped small pieces were digested in solution containing 2.5% trypsin
731 (Sigma, cat. no. P1005) and DNase I (0.1 mg/mL, Sigma, cat. no. D5025), for 20–30
732 min at 37 °C. Tissues were transferred to 10% FBS containing DMEM (ThermoFisher
733 Scientific, cat. no. 11965) to cease digestion. Neurons were then dissociated by
734 trituration with fire-polished glass pipettes, collected by spinning and plated onto
735 polylysine-coated dishes at 4×10^5 cell/35 mm dish. The growth medium was
736 composed of Neurobasal A medium (ThermoFisher Scientific, cat. no. 10888022) with
737 1× B-27 (ThermoFisher Scientific, cat. no. 17504044) and 1× N2 supplement
738 (ThermoFisher Scientific, cat. no. 17504048). Cytosine-arabinofuranoside (Sigma,
739 cat. no. C1768) was added at 6 μ M to inhibit glial growth.

740 Some of the primary patient fibroblasts were obtained from HD patients (Q47,
741 Q49, Q55) and healthy sibling (WT, Q19) controls in a Mongolian Huntington's

742 disease family. The HD Q68 fibroblast line was obtained from Coriell Cell Repositories
743 (Camden, NJ, USA). The PD line was obtained from an idiopathic Parkinson's
744 disease patient, and the SCA3 line was obtained from a SCA3 patient with the ATXN3
745 expansion mutation (Q74). The studies were approved by The Ethic Community of
746 Institutes of Biomedical Sciences at Fudan University (#28) for obtaining the HD and
747 wild-type patient fibroblasts, and by Huashan Hospital Institutional Review Board at
748 Fudan University (#174) for obtaining the PD and SCA3 patient fibroblasts. Verbal
749 and written consent was obtained from patients. The procedures were in compliance
750 with all relevant ethical regulations. The immortalized fibroblasts were generated by
751 infection of lentivirus expressing SV40T. For generation of iPSCs (iPSCs), the
752 primary fibroblasts were transduced with the retroviral STEMCCA polycistronic
753 reprogramming system (Millipore, cat. no. SCR548). The iPSCs were confirmed
754 positive for Tra-1-81, Tra-1-60, SSEA-4 and Nanog by immunofluorescence and
755 flow-cytometry. All four vector-encoded transgenes were found to be silenced and the
756 karyotype was normal. iPSCs were cultured in E8 medium (ThermoFisher Scientific,
757 cat. no. A1517001) on Matrigel (Corning, cat. no. 354277) surface. iPSCs were
758 differentiated to Pax6-expressing primitive neuroepithelia (NE) for 10-12 days in a
759 neural induction medium. Sonic hedgehog (SHH, 200 ng/ml) was added at days
760 10-25 to induce ventral progenitors. For neuronal differentiation, neural progenitor
761 clusters were dissociated and placed onto poly-ornithine/laminin-coated coverslips at
762 day 26 in Neurobasal medium (ThermoFisher Scientific, cat. no. 21103049), with 1×
763 B-27 (ThermoFisher Scientific, cat. no. 17504044), 1× N-2 (ThermoFisher Scientific,
764 cat. no. 17504048), brain derived neurotrophic factor (BDNF, 20 ng/ml, Protech, cat.
765 no. 450-02), glial-derived neurotrophic factor (GDNF, 10 ng/ml, Protech, cat. no.
766 450-10), insulin-like growth factor 1 (IGF1, 10 ng/ml, Protech, cat. no. 100-11) and
767 Vitamin C (Sigma cat. no. D-0260, 200 ng/ml). The mouse striatal cells (STHdh) were
768 obtained from Coriell Cell Repositories (Camden, NJ, USA). The HEK293T cells and
769 the HeLa cells were originally obtained from American Type Culture Collection
770 (ATCC). STHdh, HeLa and HEK293T cells were cultured in DMEM (ThermoFisher
771 Scientific, cat. no. 11965) with 10% (vol/vol) FBS (ThermoFisher Scientific, cat. no.
772 10082-147). Atg5 WT and KO MEFs were from N. Mizushima. All the mammalian cell
773 lines were maintained at 37 °C incubator with 5% CO₂, except STHdh cells, which
774 were maintained at 33 °C with 5% CO₂. The cells were tested every two months by a
775 TransDetect PCR Mycoplasma Detection Kit (Transgen Biotech, cat. no. FM311-01)
776 to ensure that they are mycoplasma free. The CellTiter-glo assay was performed to
777 measure cell viability with the indicated compound treatment (Extended Data Fig. 2e)
778 following the protocol provided in the kit (Promega, cat. no. G7570).

779 **HD *Drosophila* models**

780 The nervous system driver line *elav-GAL4 (c155)*, and the HTT-expressing lines
781 *UAS-fIHHTT-Q16* and the *UAS-fIHHTT-Q128* (expressing human full-length HTT with
782 16Q and 128Q, respectively, when crossed to the GAL4 line) lines were obtained from
783 the Bloomington *Drosophila* Stock Center at University of Indiana
784 (<http://flystocks.bio.indiana.edu/>), and maintained in a 25 °C incubator. Crosses were
785 set up between virgin female flies carrying *elav-GAL4* driver and the *UAS-fIHHTT-Q16*
786 or *UAS-fIHHTT-Q128* male flies to generate the desired genotypes.

787 **HD mouse models**

788 The generation and characterization of the Hdh140Q knock-in mice have been
789 previously described¹⁹. Mice were group-housed (up to 5 adult mice *per* cage) in
790 individually vented cages with a 12 h light/dark cycle. The mouse experiments were
791 carried out following the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments)
792 guidelines, and they were in compliance with all relevant ethical regulations. The
793 Animal Care and Use Committee of the School of Medicine at Fudan University
794 approved the protocol used in animal experiments (Approval #20140904 and
795 #20170223-005).

796 **Compound treatment in cells and animals**

797 The compounds utilized in this study were all commercially available, and quality
798 controlled by the vendors using NMR. 1005: GW5074 (DC Chemicals; cat.no.
799 DC8810); 8F20: ispinesib (Selleck; cat.no. S1452); AN1:
800 5-bromo-3-[(4-hydroxyphenyl)methylidene]-2,3-dihydro-1H-indol-2-one (Specs; cat.
801 no. AN-655/15003575); AN2: 5,7-Dihydroxy-4-phenylcoumarin (ChemDiv; cat.no.
802 D715-2435); GSK923295 (Selleck, cat.no. S7090), BAY1217389 (Selleck, cat.no.
803 S8215), PLX-4720 (Selleck, cat.no. S1152), Dabrafenib (Selleck, cat.no. S2807),
804 Sorafenib Tosylate (Selleck, cat.no. S1040), rapamycin (Sigma-Aldrich, cat. no.
805 R8781).

806 For compound treatment in the cells, the compounds were diluted in culture
807 medium to 10X concentrations and added to the plated cells: for primary cultured
808 neurons and iPSC-derived neurons, the compounds were added 5 days after plating;
809 for patient fibroblasts and other cell lines, the compounds were added 1 day after
810 plating. The cells were then collected 2 days later for measurement of HTT levels. For
811 detection of HTT-LC3 colocalization, the cells were fixed 4 h after compound
812 treatment. For caspase-3 activation detection, the cells were stressed (BDNF removal
813 for iPSC-derived neurons) 1 day after compound treatment, and tested at the
814 indicated time points.

815 For compound treatment in the *Drosophila*, flies were maintained in standard
816 maize food at 25 °C. For drug feeding, maize media was heated to 45 °C until liquid
817 and distributed into vials. Compounds were freshly prepared in DMSO and added to
818 the media. New adult flies were transferred to vials with 400 µL the control (DMSO) or
819 compound-containing food, which was changed every other day.

820 For compound treatment in mice using intracerebral ventricular (icv) injection, the
821 3-month-old mice were anesthetized using a small animal anesthesia machine
822 (MSS-3, MSS International, Keighley, UK) by Isoflurane (1.5% solution). We surgically
823 implanted each mouse with a guide cannula directed toward the lateral ventricle. The
824 coordinates for implantation were determined utilizing “The Mouse Brain in
825 Stereotaxic Coordinates” and the guide cannulas were placed at 0.6 mm posterior,
826 1.5 mm lateral (left), and 1.7 mm dorsal with respect to bregma. A cap with stylus was
827 then inserted into the guide cannula to seal its opening. Mice were then allowed to
828 recover from surgery for a week before being treated. For injection, we first inserted
829 an internal injector cannula so that it extended 0.5 mm beyond the tip of the guide
830 cannula to reach the lateral ventricle. We then injected the mice through the internal
831 injector cannula using a 25 µL syringe (Hamilton 1700 Series Microliter Syringes ,
832 Bonaduz, GR, CH) at a flow rate of 0.25 µL/min powered by a syringe pump (KDS
833 Legato 130, Holliston, MA, USA) to administer 2 µL of compounds-containing artificial
834 cerebrospinal fluid (ACSF: 1 mM glucose, 119 mM NaCl, 2.5 mM KCl, 1.3 mM MgSO₄,
835 2.5 mM CaCl₂, 26.2 mM NaHCO₃, 1 mM NaH₂PO₄) at a concentration of 25 µM
836 (containing 0.125% vol/vol DMSO). 2 µL ACSF containing equivalent amount of
837 DMSO (0.125% vol/vol) was used as the control. The injector cannula was left in
838 place for approximately 60 s to allow for diffusion before placing the caps with stylus
839 back in guide cannulas.

840 For compound treatment in mice using intraperitoneal (ip) injection, each mouse
841 was weighed. The compounds were diluted with 0.9% NaCl intravenous infusion
842 solution to 0.05 µg/µL (containing 0.011 µg/µL DMSO) and injected into each mouse
843 based on the weight of the mouse (500 µg/kg, containing 110 µg/kg DMSO). As
844 controls, equivalent amount of DMSO was diluted and injected in the same way.
845 Injection of 0.9% NaCl intravenous infusion solution alone was also tested and
846 showed no difference (Extended data Fig. 9f-h). One injection *per day* was performed
847 for two weeks before subsequent behavioral experiments or tissue extractions.

848 Note that in some of the experiments (Fig. 4-5 & Extended Data Fig. 6b-d), 8F20
849 and/or AN1 were not tested. 8F20 was not tested because it did not have an effect *in*
850 *vivo* by icv-injection (Extended Data Fig. 6a). AN1 was not tested because its

851 structure is highly similar as 10O5 while it had a weaker HTT-lowering effect by
852 icv-injection (Extended Data Fig. 6a).

853 **Protein extraction from cells and tissues**

854 For protein extraction from cells, the cell pellets were collected and lysed on ice
855 for 30 min in 1× PBS+1% Triton X-100+1× complete protease inhibitor (Sigma-Aldrich,
856 cat. no. 11697498001), sonicated for 10 sec, and spun at >20,000 g at 4 °C for 15 min.
857 The supernatants were then loaded and transferred onto nitrocellulose membranes
858 for Western-blots. For mouse brain tissues, the mouse striata and cortices were
859 dissected on ice and grinded by a tissue grinder for 5 min 60 Hz and lysed on ice for
860 60 min in brain lysis buffer (50 mM Tris, 250 mM NaCl, 5 mM EDTA, 1% Triton X-100
861 PH7.4) + 1× complete protease inhibitor (Roche, cat. no. 4693159001). The samples
862 were then sonicated for 10 cycles, 15 s on and 20 s off, and then collected for
863 Western-blots.

864 For protein extraction from the mouse brain, the brains were collected and the
865 cortices were acutely dissected on ice and homogenized with a tissue grinder for 5
866 min at 60 Hz and lysed on ice for 60 min in brain lysis buffer (50 mM Tris, 250 mM
867 NaCl, 5 mM EDTA, 1% (vol/vol) Triton X-100, 1× complete protease inhibitor (Roche,
868 cat. no. 4693159001), pH=7.4). The samples were then sonicated for 10 cycles, 15 s
869 on and 20 s off, and then collected for Western-blots, HTRF or dot blots.

870 For mHTT measurements in the HD *Drosophila* model, the fly heads were
871 collected at the age of 7 days and lysed on ice for 30 min in PBS + 1% (vol/vol) Triton
872 X-100 + 1× complete protease inhibitor (Roche, cat. no. 4693159001), sonicated for
873 10 cycles, 15 s on and 20 s off, and then collected for HTRF.

874 For all the samples, the protein concentrations were measured to correct the
875 loadings. Different protein concentrations or cell numbers per well were tested to
876 ensure that the signals were in the linear range. Background corrections were
877 performed by subtracting the background signals from blank samples.

878 **Western-blot and filter trap assays**

879 For Western-blots, the samples were loaded onto the SDS page gel (5-12%
880 depending on the molecular weight of the protein of interest). The proteins on the gel
881 were then transferred to the nitrocellulose membranes for blocking and antibody
882 detection. The signal was detected with ECL (Bio-Rad, cat. no. 1705061) after 1 h
883 incubation of the membrane with secondary antibody 1:10,000.

884 The filter trap assay was performed similarly as previously described²³, 2 µL (10
885 µg) aliquots of each sample were loaded onto nitrocellulose membranes stacked in
886 the Bio-Dot microfiltration apparatus (Bio-Rad). The membrane was blocked for 1 h
887 with 5% milk and incubated overnight with the antibody 4C9 at a concentration of 1.5

888 $\mu\text{g}/\mu\text{l}$ in 5% milk diluted in PBS + 0.1% Tween-20. The signal was detected with ECL
889 (Bio-Rad, cat. no. 1705061) after 1 h incubation of the membrane with secondary
890 antibody 1:10,000.

891 **Homogeneous Time Resolved Fluorescence (HTRF) assays**

892 For HTRF, the assays were similar as previously described²⁵. The cell or tissue
893 lysates were diluted with the original lysis buffer PBS + 1% (vol/vol) Triton X-100 + 1 \times
894 complete protease inhibitor (Roche), utilized for lysing the samples, and then detected
895 with indicated antibody pairs diluted in the HTRF assay buffer (50 mM NaH_2PO_4 , 400
896 mM NaF, 0.1% BSA, 0.05% (vol/vol) Tween-20, 1% (vol/vol) Triton X-100, pH 7.4).
897 The donor antibody concentration was 0.023 ng/ μL and the acceptor antibody
898 concentration was 1.4 ng/ μL , both in HTRF assay buffer. Different antibody pairs were
899 used for different experiments as indicated in the figure legends. For all the samples,
900 the signals were normalized to the total protein concentrations to ensure equal
901 loadings. Different protein concentrations were pre-tested to ensure that the signals
902 were in the linear range. Background corrections were performed by subtracting the
903 background signals from blank samples.

904 ***In vitro* c-Raf kinase assay**

905 *In vitro* c-Raf kinase assays were carried out with a c-Raf kinase assay kit (BPS
906 Bioscience, cat. no. 79570). The assays were performed in a 96-well plate according
907 to the manufacturing instruction. The samples and non-reactive negative controls
908 were tested in duplicate according to the instruction.

909 For details, 25 μL of the mixture containing 5x kinase assay buffer (6 μL), ATP (1 μL),
910 5x Raf substrate (10 μL) and water (8 μL) was added to a well. 5 μL of water solution
911 containing a test compound at a 10x desired concentration (DMSO was at 10% at the
912 water solution) was added to the 25 μL of mixture, and 20 μL of 1x kinase assay
913 buffer containing 2 ng/ μL c-Raf kinase was added to the mixture in a well to initiate the
914 kinase reaction (at this stage compounds were at 1x desired concentration, and
915 DMSO was at 1% concentration). For a non-reactive negative control, 20 μL of 1x
916 kinase assay buffer containing no c-Raf was added to the mixture instead. The plate
917 was incubated at 30 $^\circ\text{C}$ for 45 min. After the 45-min reaction, 50 μL of kinase
918 Kinase-Glo Max reagent (Promega, cat. no. V6071) was added to each well, and the
919 plate was incubated at room temperature for 15 min, at dark. The plate was read with
920 a microplate reader (BMG Labtech) for luminescence reading. The luminescence
921 reading value measures the levels of ATP remaining, which is inversely related to
922 kinase activity. The non-reactive negative control read value, indicating the level of
923 initial added ATP, subtracted the level of ATP remaining (the luminescence reading)
924 for the value of consumed ATP in the reaction that represents a kinase activity.

925 ***In vitro* pull-down assays**

926 We performed *in vitro* pull-down assays to test the compounds' influence on the
927 HTT-LC3 interactions. The purified HTT_{exon1} (with the indicated tags), full-length
928 proteins and the control proteins were incubated with amylose resin (New England
929 BioLabs, cat. no. E8021L) at 4 °C for 30min. Immobilized amylose resins were then
930 washed three times with HBS (20 mM HEPES pH7.5, 150 mM NaCl, 0.05%
931 Tween-20). The resulting amylose resins containing about 10 µg of MBP-fused
932 proteins were incubated with the indicated compounds (1 µM for 1005 and 100 nM for
933 AN2) or the DMSO control at the same volume in 300 µl of HBS at 4 °C for 1 h using
934 sample mixer. 40 µg of purified LC3B protein were then added and incubated at 4 °C
935 for another 2 h using sample mixer. The resin-bound proteins were eluted with 40 µl
936 maltose buffer (10 mM maltose, 20 mM HEPES, 150 mM NaCl, pH 7.5) and then
937 added with 20 µl SDS-PAGE sample loading buffer. Samples were then analyzed by
938 SDS-PAGE and Western-blot.

939 GST pulldown was performed as the same procedures described above, except
940 that GST-fused LC3B was immobilized onto magnetic conjugated GST mouse mAb
941 beads (Cell Signaling Technology, cat.no.11847S) and eluted with SDS-PAGE
942 protein loading buffer by vortex according to the instruction manual.

943 **Imaging-based autophagy assays**

944 Analysis of GFP-LC3 puncta for measuring autophagosomes: HeLa cells stably
945 expressing GFP-LC3 were generated by transfection of pEGFP C1-LC3, and positive
946 clones were selected by 500 µg/ml G418. The cells were then treated with vehicle
947 (DMSO, 0.1%), 1005, or AN2 for the indicated concentration, chloroquine (CQ, 20
948 µM) treatment was used as a control. After 24 hours, cells were fixed in 4%
949 paraformaldehyde (PFA) for 10 min. Images were acquired with confocal microscopy
950 (Leica SP8) by the observer blinded to the identity of the slides. The number and size
951 of GFP vesicles per cell was determined by Image J software. Images were
952 processed with the despeckle function to decrease the noise, and a threshold was set
953 to highlight puncta. Cells were selected by the freehand drawing tool. The
954 analyze-particle function was used for the sizes and numbers of GFP puncta.

955 The mRFP-GFP-LC3 assay: this assay allows us to monitor autophagosome
956 synthesis and maturation/fusion by labelling autophagosomes (green and red) and
957 autolysosomes (red), since the low lysosomal pH in autolysosomes quenches the
958 GFP signals³². HeLa cells stably expressing mRFP-GFP-LC3³² were treated with
959 vehicle (DMSO, 0.1%), 1005, or AN2 for the indicated concentration, bafilomycin-A1
960 (bafA1, 10 nM) treatment was used as a control. After 24 hours, cells were fixed in 4%
961 PFA for 10 min. Images were acquired with confocal microscopy (Leica SP8) by the

962 observer blinded to the identity of the slides. The green and red single channel
963 images were analyzed by Image J to quantify green and red puncta in the same way
964 as in the GFP-LC3 assay described above.

965 **Detection of long-lived proteins by click-chemistry**

966 As an indicator of autophagy activity, the degradation of long-lived proteins was
967 measured similarly as previously reported³³. Basically, the HeLa cells with 70~80%
968 confluency in a 6-well plate were washed with warm PBS and cultured in Met-free
969 DMEM (ThermoFisher Scientific, cat. no.21013) added with dialyzed FBS for 1 h to
970 deplete intracellular free Met reserves. The Met analog L-AHA (50 μ M) was then
971 added to label the proteins for 18 h. After labeling, the cells were washed with PBS
972 and cultured in regular culture medium containing 10x L-Met (2 mM) for 2 h to chase
973 out short-lived proteins. The cells were then treated with the compounds versus the
974 DMSO controls for 6 h before cell lysis and protein extraction. For the starvation
975 sample, the culture medium was replaced with EBSS (ThermoFisher Scientific, cat.
976 no. 24010043) for 6 h. The protein lysates were then used for the click reaction by the
977 Click-it reaction kit (Click Chemistry tools, cat. no. C1001) following manufacturer's
978 instructions, and the remaining L-AHA containing long-lived proteins were then
979 conjugated with biotin. These proteins were then analyzed by electrophoresis and
980 detected by the HRP-conjugated streptavidin (Beyotime, cat. no. A0303).

981 **Immunofluorescence and caspase-3 imaging**

982 For immunofluorescence of cultured cells, cells were fixed in 4% PFA for 10 min
983 after washing with 1 \times PBS for three times, and then washing and permeabilized in 0.5%
984 (vol/vol) TritonX-100 for 10 min. The cells were then blocked in blocking buffer (4%
985 BSA + 0.1% (vol/vol) Triton X-100 in 1 \times PBS) for 30 min and incubated overnight at
986 4 $^{\circ}$ C with primary antibodies, and then washed three times with blocking buffer and
987 incubated with secondary antibody at room temperature for 1 h. Coverslips were then
988 washed three times, stained with 0.5 mg/ml DAPI for 5 min at room temperature, and
989 then mounted in vectashield mounting medium (Vector, cat.no. H-1002). Images were
990 taken by Zeiss Axio Vert A1 confocal microscopes and analyzed blindly by ImageJ for
991 co-localization and TUBB3 quantifications. For co-localization experiments of
992 transfected HeLa cells (Fig. 4c), the GFP signals were used to detect GFP-LC3B, and
993 anti-His was used to detect HTT_{Exon1-MBP-His} proteins. Empty vector transfected
994 cells were imaged to ensure the specificity of the signals. The co-localization was
995 analyzed by calculating the ratio between overlapping puncta and the HTT (red)
996 puncta for each cell, and the puncta numbers were counted blindly. For co-localization
997 experiments of STHdh^{Q111/Q111} cells, the endogenous mHTT protein was stained with
998 the HTT antibody (Millipore, cat. no. MAB2166), and autophagosomes were stained

999 with the LC3B antibody (ThermoFisher Scientific, cat. no. 700712), which
1000 preferentially detects LC3-II³⁸. The co-localization was analyzed by ImageJ to
1001 calculate the ratio between overlapping pixels and the HTT (red) positive pixels,
1002 because the signals of the endogenous proteins were more dispersed and could not
1003 be counted accurately. For TUBB3, the total area of TUBB3 signals and the DAPI
1004 counts were analyzed by ImageJ. The former is then divided by the latter to calculate
1005 the averaged area of TUBB3 in each neuron as an index for neurodegeneration *in*
1006 *vitro*.

1007 For caspase-3 activity measurements of the iPSC-derived neurons, the NucView
1008 488 caspase-3 dye (Biotium, cat. no. 30029) was used for the caspase 3 activity
1009 detection as an indicator for apoptosis. The images were then taken every 3 h using
1010 the Incucyte technology (Essen Bioscience, IncuCyte FLR), which takes images of 4
1011 different fields in each well inside the cell culture incubator. The quantification was
1012 performed by the Incucyte 2011A software, which identified the green fluorescent
1013 puncta and quantified the fluorescent object count per field. The 4 fields *per* well were
1014 quantified and averaged, and 4 independent wells were used for statistical analysis.

1015 **Antibodies**

1016 Antibodies used for Western-blot, HTRF and/or
1017 immunofluorescence/immunohistochemistry are as follows: the HTT antibodies 2B7³⁹,
1018 ab1⁴⁰ and MW1⁴¹ have been described previously; commercially purchased
1019 antibodies include HTT antibody 2166 (Millipore, cat. no. MAB2166), anti-polyQ
1020 antibody 3B5H10 (Sigma, cat. no. P1874), anti-HTT antibody (D7F7)XP (Cell
1021 Signaling Technologies, cat. no. 5656s), anti- β -tubulin (Abcam, cat. no. ab6046),
1022 anti-TUBB3 (Biologends (previously Covance), cat. no. 801202), anti-ATXN3
1023 (Millipore, cat. no. MAB5360); anti-Gapdh (Proteintech, cat. no. 60004-1), anti-NBR1
1024 (ThermoFisher Scientific, cat. no. PA5-54660), anti- β -actin (Beyotime, cat. no.
1025 AA128); anti-TBP (Abcam, cat. no. ab818); anti-P62 (ThermoFisher Scientific, cat. no.
1026 PA5-27247); anti-spectrin (Millipore, cat. no. MAB1622); anti-Ncoa4 (Santa cruz,
1027 cat.no. sc-373739); anti-GST (ProteinTech, cat. no. HRP-66001); anti-GFP (Cell
1028 Signaling Technologies, cat. no. 2956); anti-MBP (ProteinTech, cat. no. 15089-1-AP);
1029 anti-His (Beyotime, cat. no. AH367); anti-BUBR1 (BD Transduction, cat.no, 612503);
1030 anti-phospho-p44/42 MAPK (ERK1/2) and anti-phospho-MEK1/2 in the
1031 Phospho-Erk1/2 Pathway Sampler Kit (Cell Signaling Technology, cat.no. 9911);
1032 anti-LC3B (ThermoFisher Scientific, cat.no. PA1-16930 (for Western-blot) & cat. no.
1033 700712 (for immunofluorescence)). All the antibodies used for immunofluorescence in
1034 this study have been validated by knock-down experiments. All the HTT, polyQ and
1035 ATXN3 antibodies used for HTRF and/or Western-blot have been validated by

1036 knock-down experiments and by comparing the signals from different genotypes in
1037 previous studies from us and others. All the other antibodies have been validated by
1038 previous literature or the vendor.

1039 **Compound detection *in vivo* in brain tissue from ip-injected mice**

1040 The experiments were performed by the SIM-Servier joint laboratory. The mice
1041 ip-injected with DMSO or the indicated compounds were anesthetized by chloral
1042 hydrate (200 μ L/kg of 10% stock) at indicated time points, and the heart blood was
1043 collected by vacuum blood collection tubes. The heart blood samples were further
1044 spun at 10,000 rpm for 5 min to generate the heart plasma. The mice were then
1045 perfused with 1X PBS to remove the blood. The mice were then sacrificed and the
1046 brain samples were dissected. 5 times of volume of methanol: acetonitrile (50: 50,
1047 vol/vol) were added to each sample, which was then homogenized. Following
1048 ultrasonic treatment for 15 min, the homogenates were centrifuged for 5 min, then 20
1049 μ L supernatant liquid was mixed with 20 μ L water for 30s before injection. Linear
1050 range of 10O5 was 10-30000 ng/mL, and the linear range of AN2 was 0.3-10000
1051 ng/mL. The LC-MS/MS analyses were performed on an Acquity ultra performance
1052 liquid chromatography (UPLC) system (Waters Corporation, Milford, MA, USA)
1053 coupled to a Xevo TQ-S mass spectrometer (Waters Corporation, Milford, MA, USA).
1054 Chromatographic separation was performed using an Acquity UPLC BEH C18 (1.7
1055 μ m 2.1 \times 50 mm) column supplied by Waters at a flow of 0.5 mL/min. Gradient elution
1056 was used with a mobile phase composed of solvent A (water containing 0.1% formic
1057 acid and 5 mM NH_4AC) and solvent B (acetonitrile: methanol (9:1, vol/vol) containing
1058 0.1% formic acid).

1059 **Proteomics analysis**

1060 Samples were analyzed on Orbitrap Fusion Lumos mass spectrometers (Thermo
1061 Fisher Scientific, Rockford, IL, USA) coupled with an Easy-nLC 1000 nanoflow LC
1062 system (Thermo Fisher Scientific). Dried peptide samples were re-dissolved in
1063 Solvent A (0.1% formic acid in water) and loaded to a trap column (100 μ m \times 2 cm;
1064 particle size, 3 μ m; pore size, 120 \AA ; SunChrom, USA) with a max pressure of 280 bar
1065 using Solvent A, then separated on a 150 μ m \times 15 cm silica microcolumn (particle size,
1066 1.9 μ m; pore size, 120 \AA ; SunChrom, USA) with a gradient of 5–35% mobile phase B
1067 (acetonitrile and 0.1% formic acid) at a flow rate of 600 nL/min for 75min. The FAIMS
1068 device was placed before the mass spectrometer. FAIMS separation was performed
1069 with the following settings: inner electrode temperature = 100 $^\circ\text{C}$, outer electrode
1070 temperature = 100 $^\circ\text{C}$, carrier gas flow= 4.6 L/min, Dispersion Voltage = -5000 V,
1071 entrance plate voltage = 250 V. The FAIMS carrier gas is N_2 only. The noted CVs were
1072 applied to the FAIMS electrodes. Each of the selected CVs was applied to sequential

1073 survey scans and MS/MS cycles (1s); the MS/MS CV was always paired with the
1074 appropriate CV from the corresponding survey scan. For detection with Fusion or
1075 Fusion Lumos mass spectrometry, a precursor scan was carried out in the Orbitrap by
1076 scanning m/z 300–1400 with a resolution of 120,000. The most intense ions selected
1077 under top-speed mode were isolated in Quadrupole with a 1.6m/z window and
1078 fragmented by higher energy collisional dissociation (HCD) with normalized collision
1079 energy of 30%, then measured in the linear ion trap using the rapid ion trap scan rate.
1080 Automatic gain control targets were 5×10^5 ions with a max injection time of 50 ms for
1081 full scans and 1×10^4 with 35 ms for MS/MS scans. Dynamic exclusion time was set at
1082 18 s. Data were acquired using the Xcalibur software (Thermo Scientific).

1083 Rawfiles were searched against the human National Center for Biotechnology
1084 Information (NCBI) Refseq protein database (updated on 04-07-2013, 32,015 entries)
1085 by Mascot 2.3 (Matrix Science) implemented on Proteome Discoverer 2.2 (Thermo
1086 Scientific). The mass tolerances were 20 ppm for precursor and 0.5 Da for product
1087 ions for Fusion Lumos. Up to two missed cleavages were allowed. The search engine
1088 set cysteine carbamidomethylation as a fixed modification and N-acetylation,
1089 oxidation of methionine as variable modifications. Precursor ion score charges were
1090 limited to +2, +3, and +4. The data were also searched against a decoy database so
1091 that protein identifications were accepted at a false discovery rate of 1%. Label-free
1092 protein quantifications were calculated using a label-free, intensity-based absolute
1093 quantification (iBAQ) approach.

1094 Proteins with at least 2 unique peptides with 1% FDR at the peptide level and
1095 Mascot ion score greater than 20 were selected for further analysis. The file used for
1096 protein inference and protein FDR calculation was derived from Mascot search results,
1097 and the peptide spectrum match (PSM) was filtered via Percolator and customized
1098 parameters, and then the proteins were assembled. The protein FDR was calculated
1099 depending on the ratio of NPD (the number of assembled proteins from decoy
1100 database searches) and NPT (the number of assembled proteins from target
1101 database searches). The FOT was used to represent the normalized abundance of a
1102 particular protein across samples. FOT was defined as a protein's iBAQ divided by the
1103 total iBAQ of all identified proteins within one sample. The FOT was multiplied by 10^5
1104 for the ease of presentation. Only the proteins detection in all compared samples
1105 were utilized for comparison.

1106 **Behavioral and lifespan experiments in HD *Drosophila* models**

1107 For behavioral experiments, we placed 15 age-matched virgin female flies in an
1108 empty vial and tapped them down. The percentage of flies that climbed past a
1109 7-cm-high line after 15 s was recorded. The mean of five observations is plotted for

1110 each vial on each day, and data from multiple vials containing different batches of flies
1111 were plotted and analyzed by two-way ANOVA tests. The flies were randomly placed
1112 into each tube. For lifespan measurements, we placed 75 age-matched virgin female
1113 flies in an empty plastic vial and recorded the survival situation for each vial on each
1114 day. For both behavioral and lifespan measurement experiments, the person who
1115 performed the experiments were blinded to the drugs fed until data analysis.

1116 **Behavioral experiments in HD mouse models**

1117 All the behavioral experiments were performed during the light phase and the
1118 experimenters were blinded to the compound treatment and the genotype of each
1119 mouse. Both males and females have been used. All the mice were kept in the
1120 behavioral test room in dim red light for 1 h before starting the experiments. For
1121 rotarod experiments, mice were pre-trained on 3 consecutive days on the rotarod
1122 rotating at 4 rpm for 2 min. Mice were then tested for five days at an accelerating
1123 speed ranging from 4 to 40 rpm within 2 min. Each performance was recorded as the
1124 time in seconds spent on the rotating rod until falling off or until the end of the task.
1125 Each test included three repetitions with an inter-trial interval of 60 min in order to
1126 reduce stress and fatigue, and the means from these three runs were analyzed for
1127 each mouse. The balance beam test was run using a 2 cm thick meter stick
1128 suspended from a platform on both sides by metal grips. The total length is 100 cm.
1129 There was a bright light at the starting point and a dark box with food at the endpoint.
1130 The total time for each mouse to walk through the beam was recorded. For gripping
1131 force measurements, mice were allowed to grip the metal grids of a grip meter
1132 (Ametek Chatillon) with their forelimbs, and they were gently pulled backwards by the
1133 tail until they could no longer hold the grids. The peak grip strength observed in 10
1134 trials was recorded.

1135 **Statistics**

1136 To ensure to reach a statistical power > 0.8, power analyses were performed for
1137 each assay based on estimated values by PASS 16
1138 (<https://www.ncss.com/software/pass/>) before experiments. Estimation was based on
1139 our previously published results on similar experiments and preliminary experiments.
1140 The effect size was also estimated by Cohen's d, two means divided by the standard
1141 deviation for the data. The power analysis suggested $n \geq 3$ for mHTT level
1142 measurements and $n \geq 5$ for behavioral experiments. In all the experiments we
1143 performed, we have used a larger n than these numbers in case the effect was
1144 smaller than preliminary results, and we also performed post-experiment power
1145 analyses to ensure that power ≥ 0.8 for all the significant differences. Statistical
1146 comparisons between two groups were conducted by the unpaired two-tailed t tests.

1147 Statistical comparisons among multiple groups were conducted by one-way ANOVA
1148 tests and post-hoc tests for the indicated comparisons (Dunnett's tests for comparison
1149 with a single control, and Bonferroni's tests for comparisons among different groups).
1150 Statistical comparisons for series of data collected at different time points were
1151 conducted by two-way ANOVA tests. The similarity of variances between groups to be
1152 compared was tested when performing statistics in GraphPad Prism 7 and Microsoft
1153 Excel 2016. Normality of data sets was assumed for ANOVA and t tests, and was
1154 tested by Shapiro-Wilk tests. When the data were significantly different from normal
1155 distribution, nonparametric tests were used for statistical analysis. All statistical tests
1156 were unpaired and two-tailed.

1157

1158 **Reference for Methods**

1159

1160 38 Hancock, M. K., Hermanson, S. B. & Dolman, N. J. A quantitative TR-FRET
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1172

1173 **Acknowledgements**

1174 The authors would like to thank Drs. Junmei Lu, Min Jiang, Linyun Liu and Qian Huang for
1175 their technical support of mouse behavioral experiments. Dr. Yanhui Xu for technical
1176 supports of the protein purification. Dr. Hexige Saiyin for the help with obtaining human
1177 patient fibroblasts. The authors would also like to thank the following funding supports:
1178 National Key Research and Development Program of China (2016YFC0905100), National
1179 Natural Science Foundation of China (8192500069, 81870990, 31961130379, 91649105,
1180 31470764, 91527305, and 61505032), Science and Technology Commission of Shanghai
1181 Municipality (18410722100), Natural Science Foundation of Shanghai (19ZR1405200),
1182 Shanghai Municipal Science and Technology Major Project (No.2018SHZDZX01) and
1183 ZJLab, and Hereditary Disease Foundation.

1184

1185 **Author Contribution**

1186 B Lu perceived the idea, initiated the project, designed experiments, analysed data and
1187 wrote the manuscript. Y Fei and C Zhu performed the OI-RD screening and K_{on}/K_{off}
1188 measurements with data analysis. Y Ding, Z Wang, J Li, C Gao performed protein
1189 purification, *in vitro* pull-down and structural biology experiments with data analysis. With
1190 the help from others for blinding, Z Li performed the HTT measurements in cells and in
1191 mouse models, the mouse phenotype experiments, the autophagy-related mechanistic
1192 experiments, the control protein measurements, and neurotoxicity measurements. C Wang
1193 replicated Z Li's HTT measurement and autophagy-related mechanistic experiments, and
1194 performed additional HTT measurement and phenotypic experiments in HD fly models,
1195 patient iPSC-derived neurons and MEFs, as well as all the measurements of other polyQ
1196 proteins and all the MST experiments. Y Dang provided the compound library for the
1197 screen. T Sha and C Ding performed proteomics experiments and analysis. S Luo and Y
1198 Yang performed the measurements of autophagy function. L Ma, Y Sun and J Wang
1199 provided and characterized the patient cell lines. X Sun did the initial subcloning of
1200 full-length HTT and found the explanation for the observed "hook effects". C Lu
1201 (biostatistician) performed biostatistical analysis. M Difiglia and Y Mei helped designing
1202 the experiments and interpreting the data.

1203

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1208 (yuding@fudan.edu.cn). B Lu, Y Fei, Y Ding and Y Dang has filed two patents together
1209 based on this study to the State Intellectual Property Office of China (201910180674.7
1210 and 201910180717.1).

1211

1212 **Data Availability Statement**

1213 The protein structure data has been uploaded to the PDB database with entry number
1214 6J04. Source data for all figure plots are provided with the paper. The full gel blots and the
1215 proteomics datasets have been provided in supplementary information. The data that
1216 support the findings of this study are available from the corresponding authors upon
1217 reasonable request.

1218

1219 **Extended data figure/table legends**

1220 **Extended Data Fig. 1. Protein purifications.**

1221 a) SDS-PAGE and linear mode MALDI-TOF mass spectrometry analysis of the
1222 expression and purification of recombinant LC3B protein. For SDS-PAGE, lane 1, the

1223 whole cell lysate before induction; lane 2, the whole cell lysate after induction; lane 3, the
 1224 supernatants of induced cells; lane 4, the flow through fraction of Ni-NTA chromatography;
 1225 lane 5, the eluates of Ni-NTA chromatography (GST-His8-LC3); lane 6, the eluates of
 1226 GST-His8 tag removed LC3B by TEV protease; lane 7, the eluates of size exclusive
 1227 chromatography; lane 8, molecular weight marker. The obtained mass spectrometry m/z
 1228 peak of recombinant LC3B is 14660.811, consistent with theoretical calculations
 1229 b) Structural alignment of purified recombinant LC3BΔG120 (PDB: 6J04, yellow) with
 1230 published LC3B structure (PDB: 1UGM, cyan) by Pymol.
 1231 c-d) SDS-PAGE and linear mode MALDI-TOF mass spectrometry analysis of the
 1232 HTTexon1 proteins. For HTTexon1-Q72-MBP SDS-PAGE, lane 1, the supernatants of
 1233 induced cells; lane 2, the insoluble fraction of induced cells; lane 3, the flow through
 1234 fraction of Ni-NTA chromatography; lane 4, the eluates of Ni-NTA chromatography; lane 5,
 1235 the eluates of size exclusive chromatography; lane 6, molecular weight marker. For
 1236 HTTexon1-Q25-MBP SDS-PAGE, lane 1, molecular weight marker; lane 2, the induced
 1237 cell lysate; lane 3, the supernatant fraction of induced cells; lane 4, the flow through
 1238 fraction of Ni-NTA chromatography; lane 5 and 6, the eluates of Ni-NTA chromatography;
 1239 lane 7 and 8, the eluates of size exclusive chromatography. The obtained mass
 1240 spectrometry m/z peak of HTTexon1-Q72-MBP and HTTexon1-Q25-MBP are 64225.946
 1241 and 58228.893, consistent with theoretical calculations.
 1242 e) *Left* and *middle* panels: size exclusive chromatography of the recombinant full-length
 1243 HTT-Q73 (flHTT-Q73) and HTT-Q23 (flHTT-Q23) proteins using Superose 6 5/150 GL.
 1244 The major peak fractions were then collected pooled together for the SDS-PAGE analysis
 1245 (*right* panel).
 1246 f) SDS-PAGE analysis of purified MBP-His8 (MBP), sfGFP (GFP), and Rpn10 proteins.

1247
 1248 **Extended Data Fig. 2. Negative controls for OI-RD measurements and validation of**
 1249 **the compounds' interaction with HTT and LC3 by MST.**

1250 a) Similar to Fig. 1c-e, but for negative control proteins MBP-His8 (MBP), superfolder GFP
 1251 (sfGFP), and ubiquitinated-Rpn10 (Ub-Rpn10). Association-dissociation curves of surface
 1252 immobilized compounds 8F20 and 10O5 with these proteins were measured by OI-RD,
 1253 and no compound-protein interactions were detected. For all association-dissociation
 1254 curves, vertical dash lines mark the starts of association and dissociation phases of the
 1255 binding event.
 1256 b) Binding of 10O5 and 8F20 to full-length HTT-Q73 (flHTT-Q73, black dots) or LC3B (red
 1257 dots) in standard treated capillaries measured by MST. The compound bound protein
 1258 fractions (bound / total) were calculated by the MST signals (F_{norm}) at each compound
 1259 concentrations, as well as the bound (F_{norm_bound} , set as 100%) and the unbound
 1260 ($F_{norm_unbound}$, set as 0%) MST signals: bound / total = $(F_{norm} - F_{norm_unbound}) / (F_{norm_bound} -$
 1261 $F_{norm_unbound}) \times 100\%$. The fitted curves and calculated K_d values by the Nanotemper
 1262 analysis software (1.5.41) for flHTT-Q73 and LC3B were indicated in each panel.
 1263 Consistent with the OI-RD measurements (Fig. 1e), no binding was observed for the
 1264 flHTT-Q23 protein (blue dots). The MST experiments were repeated > 3 times and
 1265 showed consistent results.
 1266 c) Similar to b), except testing the compounds indicated in the X-axis. MST measurements

1267 of the binding of indicated compounds to full-length HTT-Q73 (flHTT-Q73), full-length
1268 HTT-Q23 (flHTT-Q23) and LC3B in standard treated capillaries. The proteins tested were
1269 indicated in the legends.

1270 d) Similar to Fig. 1c-e, but plotting the association-dissociation curves of surface
1271 immobilized compound AN2 with full-length HTT-Q73 (Q73), or full-length HTT-Q23 (Q23),
1272 or LC3B, or the negative control proteins MBP-His8 (MBP), superfolder GFP (sfGFP), and
1273 Rpn10.

1274 For all association-dissociation curves, vertical dash lines mark the starts of association
1275 and dissociation phases of the binding event. The red dash lines are global fits to a
1276 Langmuir reaction model with the global fitting parameters listed at the bottom of each plot.
1277 No binding signals were observed for full-length HTT-Q23 proteins, and thus the
1278 parameters were not presented.

1279 e) Cell viability measurement of cultured HD neurons measured by the CellTiter-glo assay.
1280 No toxicity was observed within the concentration range presented in Fig. 2, although the
1281 compound 8F20 became toxic to the cells when the concentration reached 300 nM.

1282

1283 **Extended Data Fig. 3. mHTT lowering effects by mHTT-linker compounds could be**
1284 **detected by multiple antibodies and were dependent on autophagy.**

1285 a) Representative Western-blot (HTT detected by the 2166 antibody) and quantifications
1286 of compound-treated cultured cortical neurons from $Hdh^{Q7/Q140}$ HD knockin mice. The
1287 neurons were treated with the indicated compounds (100 nM for 10O5, 8F20 and AN1; 50
1288 nM for AN2) with or without the autophagy inhibitor NH_4Cl (*upper panels*) or chloroquine
1289 (*lower left*), or the autophagy activator rapamycin (*lower right*). Same amount of culture
1290 medium was added in the controls (*upper panels*). The statistical analysis was performed
1291 by one-way ANOVA with post-hoc Dunnett's tests, and the F/degree of freedom/post-hoc
1292 p values have been indicated in each bar plot.

1293 b) Western-blot using indicated HTT/polyQ antibodies for samples from cultured cortical
1294 neurons treated with the indicated compounds: 10O5 (100 nM), 8F20 (100 nM), AN2 (50
1295 nM). The HTT gel blot presented in Fig. 2d (*right panel*) were cropped from first four blots.
1296 The low molecular weight bands were run out in these blots so that the wtHTT and mHTT
1297 could be better separated. Note that the weak bands just above 250 kDa in the first two
1298 blots were leftover signals from the Spectrin blotting. The Spectrin signals were too strong
1299 to be stripped completely.

1300 c) Western-blot using the antibody MW1 or 3B5H10 that detects mHTT specifically. The
1301 relatively low molecular weight proteins were kept. No increase of potential
1302 polyQ-containing mHTT N-terminal fragments was observed.

1303 d) HD patient iPSC-derived striatal neurons (Q47) were treated with the indicated
1304 compounds (100 nM, with 0.1% DMSO) in presence of an additional 0.1% DMSO or 10
1305 mM NH_4Cl , and the mHTT levels were measured by HTRF using the 2B7/MW1 antibody
1306 pair. All signals were normalized to the averaged signals from the DMSO control group.
1307 The statistical analysis was performed by one-way ANOVA with post-hoc Dunnett's tests,
1308 and the F/degree of freedom/post-hoc p values have been indicated in each bar plot. "****":
1309 $p < 0.0001$. The post-hoc analysis was not performed if the ANOVA tests did not show
1310 significance ($p > 0.05$).

1311 e) The HD patient immortalized fibroblasts (Q47) were transfected with the non-targeting
1312 control siRNA (Neg siRNA) or the ATG5 siRNA (target sequence:
1313 GCCUGUAUGUACUGCUUUA; ATG5 mRNA was knocked down to $17.7 \pm 3.0\%$, $n=3$, as
1314 tested by qPCR), and then after 24 hours treated with the indicated compounds (100 nM)
1315 for another 48 hours. mHTT levels were then measured by HTRF using the 2B7/MW1
1316 antibody pair. All signals were normalized to the averaged signals from the DMSO control
1317 group. The statistical analysis was performed by one-way ANOVA with post-hoc Dunnett's
1318 tests, and the F/degree of freedom/post-hoc p values have been indicated in each bar plot.
1319 "****": $p < 0.0001$. The post-hoc analysis was not performed if the ANOVA tests did not
1320 show significance. The Western-blot of LC3 confirmed the partial inhibition of autophagy
1321 in the ATG5 knockdown cells.
1322 f) Similar to e), but in wild-type (WT) or Atg5 knockout (Atg5 KO) mouse embryonic
1323 fibroblast lines (MEF) transfected with full-length mHTT (flHTT-Q73). The Western-blot of
1324 LC3 confirmed the inhibition of autophagy in the Atg5 KO cells.
1325 For all panels, n indicates the number of independently plated wells, and bars represent
1326 mean and s.e.m.. Full-blots of cropped gels are shown in Supplementary Fig. 1.

1327

1328 **Extended Data Fig. 4. Potential influence of c-Raf and KSP pathways by treatment**
1329 **of the mHTT-LC3 linker compounds.**

1330 a) Representative results (from 3 biological repeats) of the *in vitro* c-Raf kinase assay (see
1331 "Methods") showing that only 10O5 inhibits c-Raf activity within the concentration range
1332 tested.

1333 b) Representative Western-blots and quantifications of phospho-MEK/ERK as an indicator
1334 of Raf activities (*left*) and phospho-Bub1 as an indicator of KSP inhibition (*right*) in
1335 cultured cortical neurons treated with indicated compounds (100 nM for 10O5, 8F20, AN1,
1336 and 50 nM for AN2) or the DMSO control.

1337 c) Similar to b), but in immortalized HD patient fibroblasts (Q47). Note that the
1338 phospho-Bub1 is essentially absent and too weak to quantify, indicating that KSP was not
1339 inhibited by any of the compounds at the concentration tested.

1340 For all panels, error bars represent mean and s.e.m.. For b, all data were corrected by the
1341 loading control (β -Tubulin) and then normalized to the averaged signal of the DMSO
1342 control group. The statistical analysis was performed by one-way ANOVA and the
1343 F/degree of freedom/p values have been indicated in each bar plot. The n number
1344 indicates the number of independently plated and treated wells.

1345

1346 **Extended Data Fig. 5. mHTT-LC3 linker compounds lowered mHTT in transgenic HD**
1347 **flies.**

1348 a) Overlay between LC3B and predicted Atg8 structure showing high structural similarities.

1349 b) Transgenic flies expressing full-length HTT-Q128 driven by *elav*-GAL4 were fed with
1350 indicated compounds at 10 μ M for 6 days, and their heads were then extracted for protein
1351 lysates. mHTT were then measured by HTRF using the 2B7/MW1 antibody pair.

1352 Each dot represents the HTRF signal from each individual sample extracted from 5 fly
1353 heads. All the data were normalized to the average of the DMSO-fed control samples. The
1354 statistical analysis was performed by one-way ANOVA and Dunnett's post hoc tests. F (4,

1355 31) = 15.67; “*****”: $p < 0.0001$.
1356 c) 10O5 (*upper panel*) and AN2 (*lower panel*) concentrations in heart plasma and brain
1357 tissues were measured by mass-spectrum at indicated time points for compound-injected
1358 mice (0.5 mg/kg). In the brain tissue, the 10O5 concentrations were ~20 to ~200 nM, and
1359 the AN2 concentrations were ~20 to ~40 nM, close to the effective doses that were
1360 capable of lowering mHTT in cultured neurons. Data were plotted by mean and s.e.m..
1361

1362 **Extended Data Fig. 6. mHTT-LC3 linker compounds lowered mHTT *in vivo* in the**
1363 **mouse brains.**

1364 a) Western-blot (4 mice (3 months old) for each group) and quantifications of mHTT and
1365 wtHTT in the cortices from Hdh^{Q7/Q140} knockin mice icv-injected with the indicated
1366 compounds (2 μ L at 25 μ M for each mouse) for 10 days at one-dose *per day*. HTT were
1367 detected by Western-blot using the 2166 antibody, and the statistical analysis was
1368 performed by one-way ANOVA and post-hoc Dunnett’s tests. The F/degree of
1369 freedom/post-hoc p values have been indicated under each bar plot.

1370 b) Similar to a), except that the compounds were delivered by ip-injection (0.5 mg/kg) to
1371 Hdh^{Q7/Q140} mice at 5 months-old for 14 days at one-dose *per day*.

1372 c) Similar to b), but from striata of ip-injected mice. The mice were injected at 10
1373 months-old for 14 days at one-dose *per day*.

1374 d) *Left panel*: representative dot-blot results (from two technical replicates) of the protein
1375 lysates in b) using the 4C9 antibody, which preferentially detects mHTT aggregates²³.
1376 *Middle panel*: quantification of the dot-blot based on the averaged signals from two
1377 technical replicates. *Right panel*: measurement of mHTT aggregates by the 4C9/4C9
1378 HTRF assay²³.

1379 For all panels, n indicates the number of mice tested, and bars represent mean and s.e.m..
1380 For quantification, two to three technical replicates were averaged for each mouse. The
1381 statistical analysis was performed by one-way ANOVA with post-hoc Dunnett’s tests, and
1382 the F/degree of freedom/post-hoc p values have been indicated in each bar plot.
1383

1384 **Extended Data Fig. 7. mHTT-LC3 linker compounds did not influence autophagy.**

1385 a) HeLa cells stably expressing GFP-LC3B were treated with 2 μ L vehicle (0.1% DMSO),
1386 10O5, or AN2 for the indicated concentration for 24 hours; chloroquine (CQ, 20 μ M)
1387 treatment was used as a control. After 24 hours, cells were fixed, and images were
1388 acquired with confocal microscopy. The number and size of GFP vesicles *per cell* was
1389 determined by Image J software (n number indicated on top of each plot). For each
1390 treatment, over 20,000 puncta were quantified (~100 puncta *per cell* in 226 cells). *Scale*
1391 *bar*: 10 μ m.

1392 b) Representative images and quantifications of the numbers of autophagosomes (green⁺
1393 puncta) and autolysosomes (red⁺;green⁻ puncta) in HeLa cells stably expressing
1394 mRFP-GFP-LC3B. *Scale bar*: 10 μ m. Autophagosome numbers or sizes were not
1395 influenced by 10O5 and AN2 at indicated concentrations after 24 hours treatment (or 4
1396 hours treatment, not shown). The autophagosome fusion was also unaffected as indicated
1397 by the autolysosome number. Note that the autophagosome/autolysosome numbers/sizes
1398 were based on image analysis of the puncta, some of which may reflect multiple vesicles.

1399 Green vesicles are considered to be autophagosomes (GFP+ puncta) and red vesicles
1400 are considered to be both autophagosomes and autolysosomes. The number of
1401 autolysosomes (RFP+ GFP- puncta) was achieved by subtracting the number of green
1402 vesicles from that of the red vesicles. >10,000 puncta from 194 cells were analyzed.
1403 c) Representative Western-blots and quantifications of HeLa cells stably expressing
1404 GFP-LC3B. The “free GFP” was generated by lysosomal cleavage, and thus the “free
1405 GFP”/“GFP-LC3B” ratio was used as an index for autophagy flux, which was unaffected
1406 by 10O5 or AN2, but decreased by the autophagy flux inhibitor chloroquine (CQ).
1407 d) Representative Western-blots and quantifications of the chase signal of long-lived
1408 proteins in HeLa cells as an indicator of autophagy flux (see “Methods”). Consistent with
1409 previous reports³³, starvation reduced the long-live protein chase signal, whereas
1410 rapamycin treatment had a milder effect. The mHTT-LC3 linker compounds 10O5 and
1411 AN2 showed no influence in this assay.
1412 e) Representative Western-blots and quantifications of LC3 in cultured cortical neurons
1413 treated with the indicated compounds. Normalized LC3-II/LC3-I was used as the indicator
1414 of autophagy. In the right panels, the 10O5 and AN2 concentrations were 100 nM and 50
1415 nM, respectively.
1416 f) The SQSTM1/p62 levels were tested by Western-blots for the cortical tissues from mice
1417 injected by the indicated compounds or the DMSO control. Bars indicate mean and s.e.m..
1418 For all panels, the n numbers indicated in each bar indicate the cell numbers (a-b), the
1419 number of independently plated wells (c-e) or mouse numbers (f). Error bars indicate
1420 mean and s.e.m.. The statistical analysis was performed by one-way ANOVA with
1421 post-hoc Dunnett’s tests for a-e, and by two-tailed unpaired t tests for f. Note that the
1422 post-hoc tests were not performed if the ANOVA tests failed to show significance. “****”:
1423 $p < 0.0001$ (post-hoc).
1424

1425 **Extended Data Fig. 8. Investigation on the specificity of mHTT lowering effects of**
1426 **mHTT-LC3 linker compounds.**

1427 a) Representative Western-blots and quantifications of cultured cortical neurons treated
1428 with the indicated compounds. None of the proteins tested had an obvious change (>
1429 10%).
1430 b) Volcano plots of the proteomics analysis of cortices from ip-injected HD mice (10 m, 4
1431 mice *per* group, injected for 14 days). The concentration injected was 0.5 mg/kg with 110
1432 μ g/kg DMSO, and equal amount of vehicle containing DMSO was injected as the controls.
1433 Only those proteins detected in both groups of samples used for comparisons were
1434 calculated and plotted. The dots plotted for HTT were pointed by red arrows. See
1435 Supplementary Table 2 for complete datasets. The bar plots indicate the total HTT levels
1436 normalized to the DMSO control. The actual mHTT reduction is anticipated to be more,
1437 because the compounds reduced mHTT allele-selectively.
1438 c) Similar to b), but in cultured cortical neurons (from p0 pups, 3 wells *per* group). See
1439 Supplementary Table 3 for complete datasets.
1440 In all panels, the error bars represent mean and s.e.m..
1441

1442 **Extended Data Fig. 9. mHTT-LC3 linker compounds lowered the mutant ATXN3**

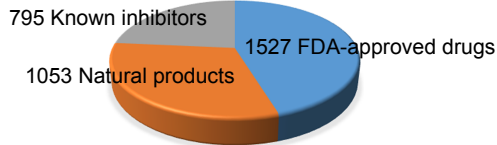
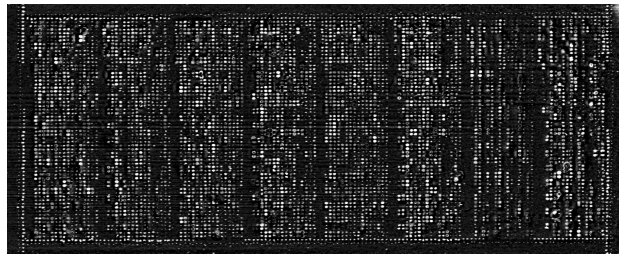
1443 **protein with polyQ expansion in an allele-selective manner.**
1444 a) Representative Western-blots and quantifications of ATXN3 levels in a SCA3 patient
1445 fibroblast line treated with the indicated compounds. The lowering of mutant (Q74) but not
1446 wild-type (Q27) ATXN3 was observed by treatment of linker compounds tested.
1447 b) Quantification of the GFP intensity as an indicator of polyQ-sfGFP (25Q-GFP, 38Q-GFP,
1448 46Q-GFP, and 72Q-GFP) protein levels in transfected HEK293T cells treated with the
1449 indicated compounds using Incucyte. The lowering of 72Q-GFP, 46Q-GFP and 38Q-GFP,
1450 but not 25Q-GFP was observed.
1451 For both a) and b), the compound concentrations were 100 nM for 10O5 and AN1, and 50
1452 nM for AN2. Bar plots present mean and s.e.m., and n numbers indicate independently
1453 plated wells.
1454 c) SDS-PAGE analysis of polyQ-sfGFP proteins (25Q, 38Q, 46Q, 53Q and 72Q) purified
1455 from HEK293T cells. The protein purification methods were similar to the ones for HTT
1456 proteins.
1457 d) Binding of 10O5, AN1 and AN2 to sfGFP (GFP) or different polyQ-sfGFP (25Q-GFP,
1458 38Q-GFP, 72Q-GFP) proteins in standard treated capillaries measured by MST,
1459 performed and analyzed similarly as in Extended Data Fig. 2b. All these compounds
1460 interact with 38Q-GFP and 72Q-GFP, but not 25Q-GFP or GFP.
1461 e) Association-dissociation curves of surface immobilized compounds 10O5, AN1 and
1462 AN2 with polyQ-sfGFP (72Q, 53Q, 46Q, 38Q and 25Q) proteins. For all
1463 association-dissociation curves, vertical dash lines mark the starts of association and
1464 dissociation phases of the binding event. The red dash curves are fits to a Langmuir
1465 reaction model with the fitting parameters listed at the bottom of each plot. No binding
1466 signals were observed for 25Q-sfGFP (25Q).
1467 f-h) Results of mouse behavioral test performed similarly as in Fig. 5d-f, except that the
1468 mice were injected with saline (0.9% NaCl) with DMSO (110 µg/kg) or without DMSO. The
1469 statistical analysis was performed by two-way ANOVA with post-hoc Bonferroni's tests,
1470 and the F/p values and degrees of freedom were indicated in the table underneath each
1471 plot.
1472 In all panels, the error bars represent mean and s.e.m..

1473
1474 **Extended Data Table 1. Summary of data on mHTT lowering or rescue of**
1475 **HD-relevant phenotypes**

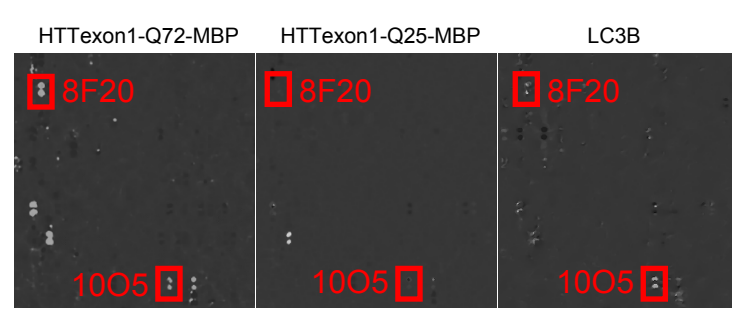
1476 A summary table showing the percentage lowering of the mHTT or HTT levels, as well as
1477 the percentage rescue of HD-relevant phenotypes (normalized to the difference between
1478 HD and WT) in different HD models assayed by different approaches under optimal
1479 conditions. The corresponding data figures have been indicated in the *middle* column. The
1480 percentage change/rescue were presented in the form of mean ± s.e.m..
1481

Figure 1

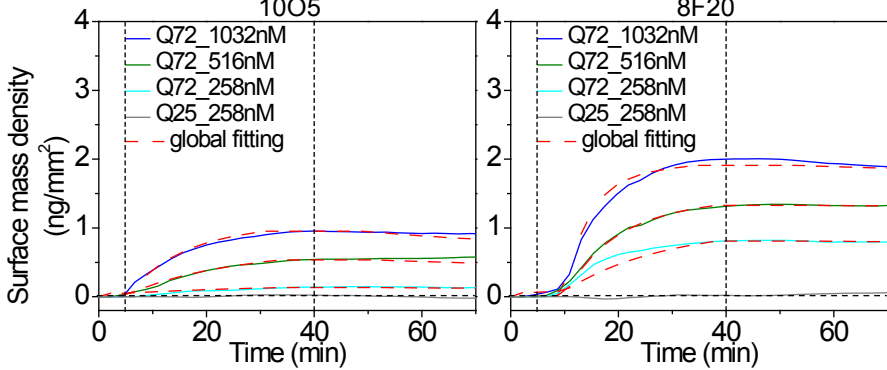
a Small molecule microarray (SMM)



b OI-RD screening signals

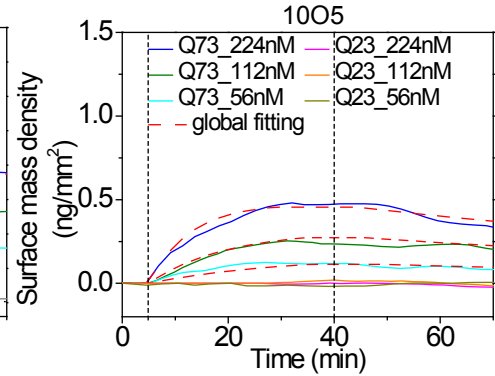


c K_{on}/K_{off} measurements for compound-HTTexon1 interactions



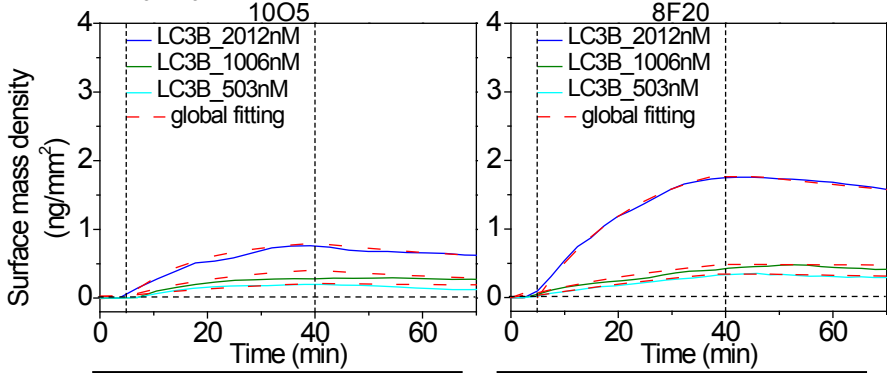
Compound	K_{on} (min·nM) ⁻¹	K_{off} (min) ⁻¹	K_d (nM)
Q72 (1005)	9.07×10^{-5}	1.43×10^{-2}	157.6
Q72 (8F20)	2.13×10^{-4}	6.23×10^{-3}	29.2

e K_{on}/K_{off} measurements for compound-HTT(full-length) interactions

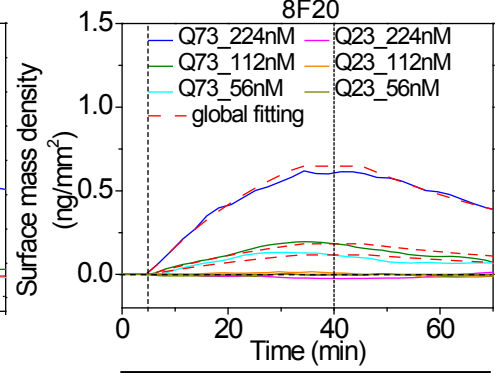


Compound	K_{on} (min·nM) ⁻¹	K_{off} (min) ⁻¹	K_d (nM)
Q73 (1005)	5.43×10^{-4}	7.97×10^{-3}	14.7

d K_{on}/K_{off} measurements for compound-LC3B interactions



Compound	K_{on} (min·nM) ⁻¹	K_{off} (min) ⁻¹	K_d (nM)
LC3B (1005)	3.72×10^{-5}	1.74×10^{-2}	467.7
LC3B (8F20)	2.46×10^{-5}	5.86×10^{-3}	238.2



Compound	K_{on} (min·nM) ⁻¹	K_{off} (min) ⁻¹	K_d (nM)
Q73 (8F20)	6.43×10^{-5}	2.07×10^{-2}	321.9

Figure 2

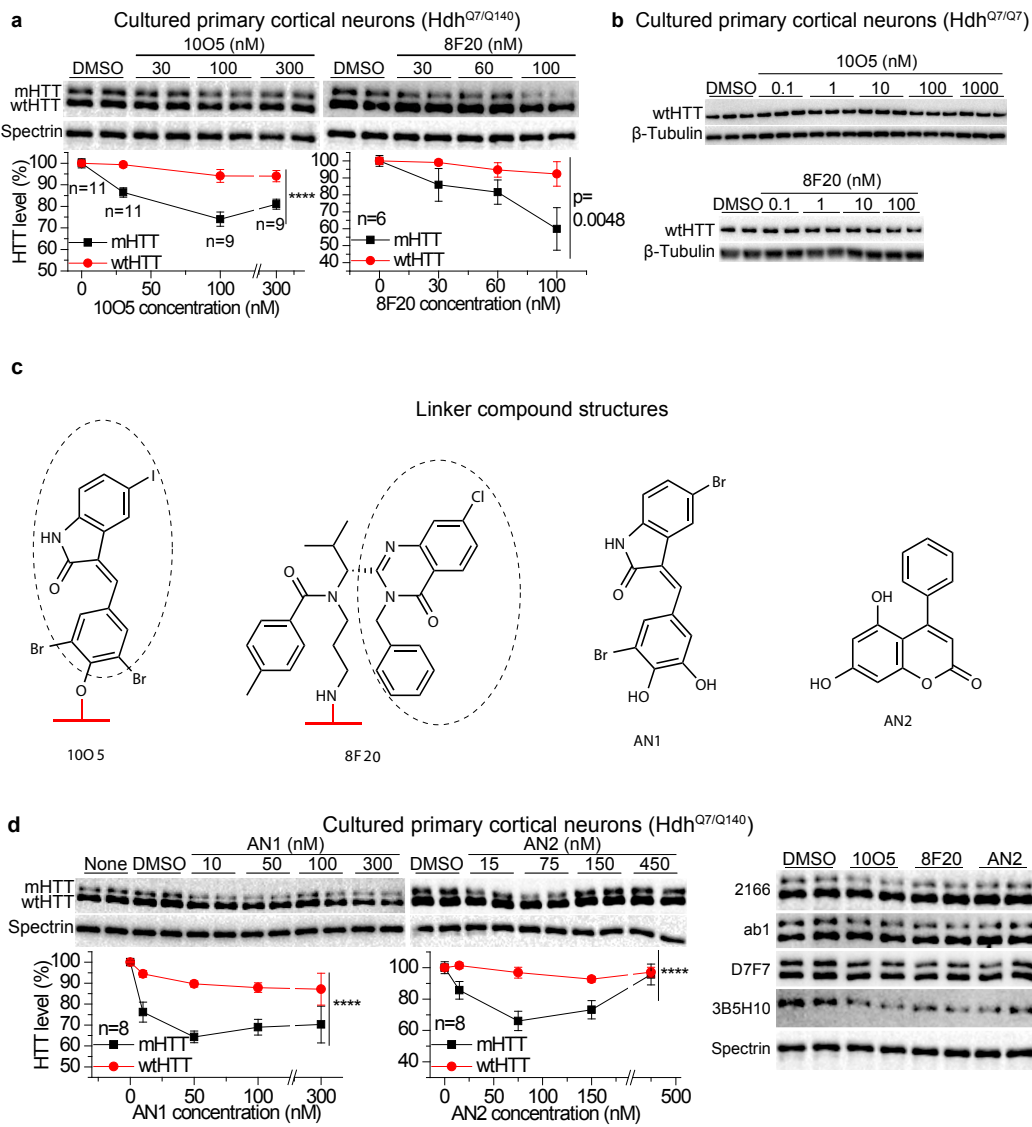


Figure 3

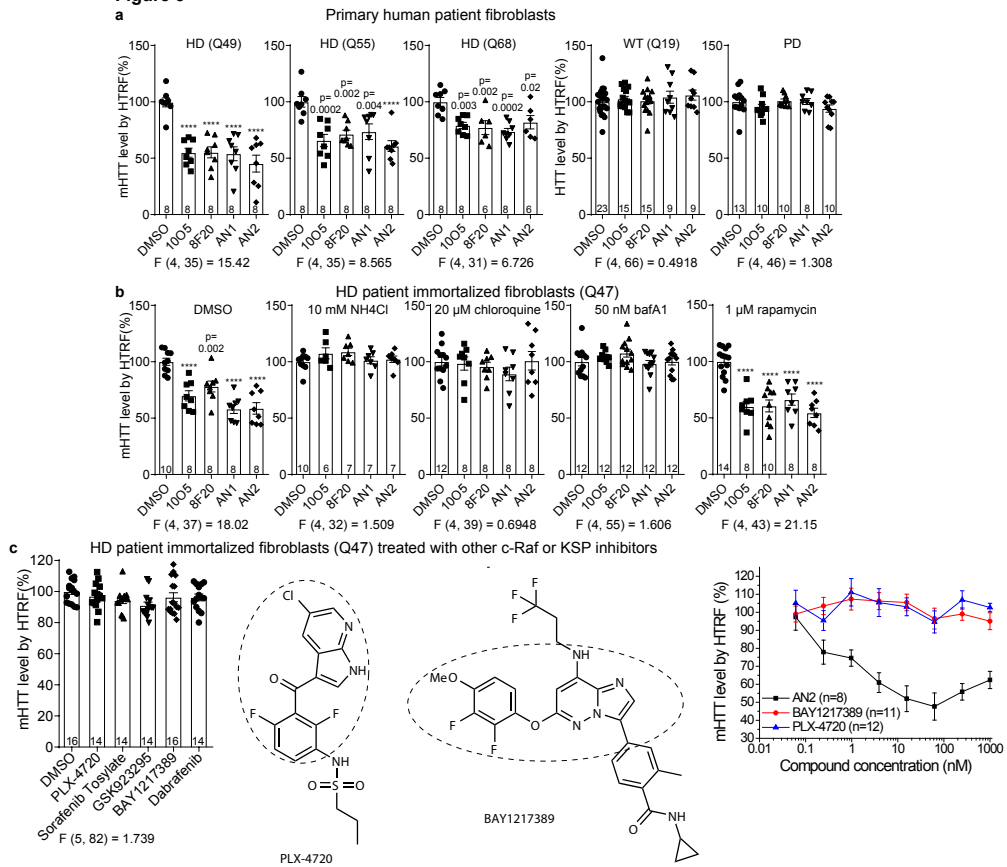


Figure 4

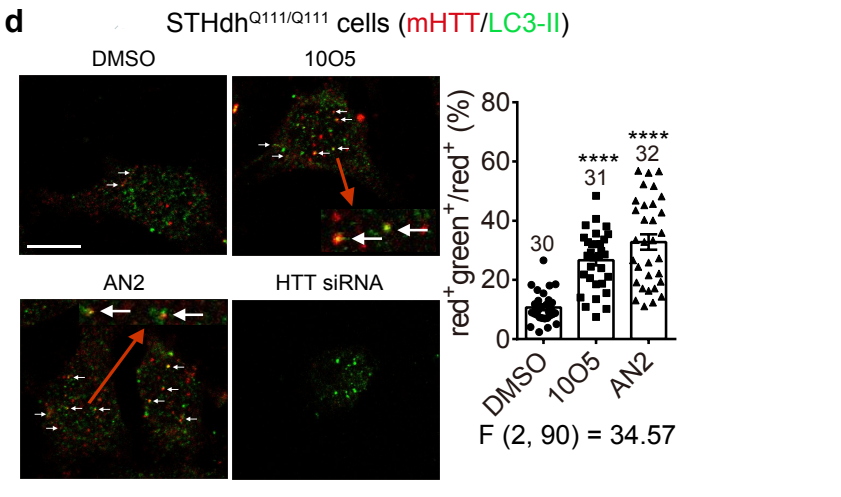
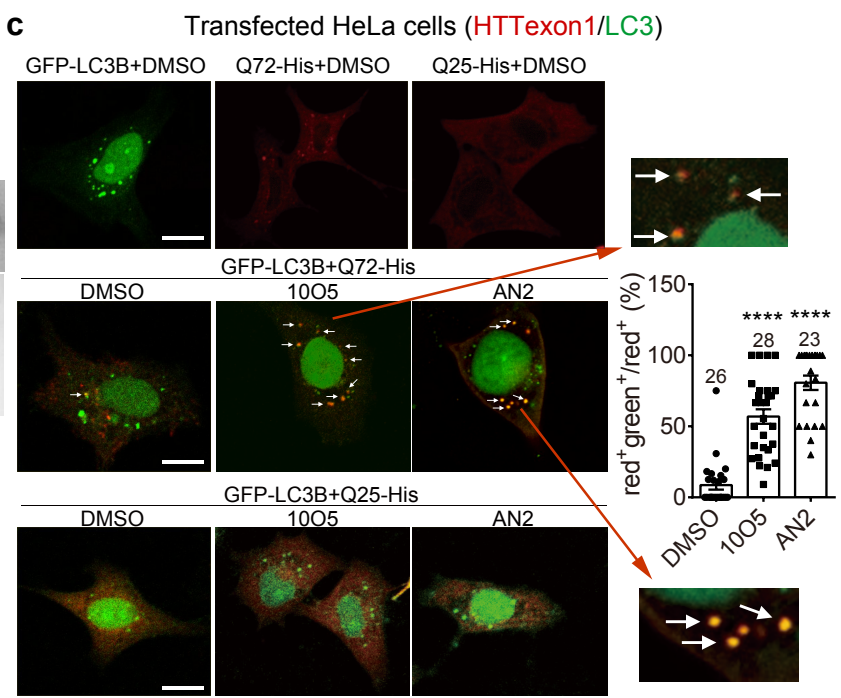
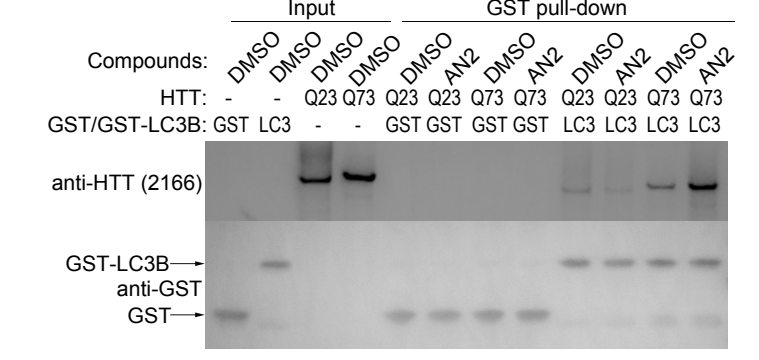
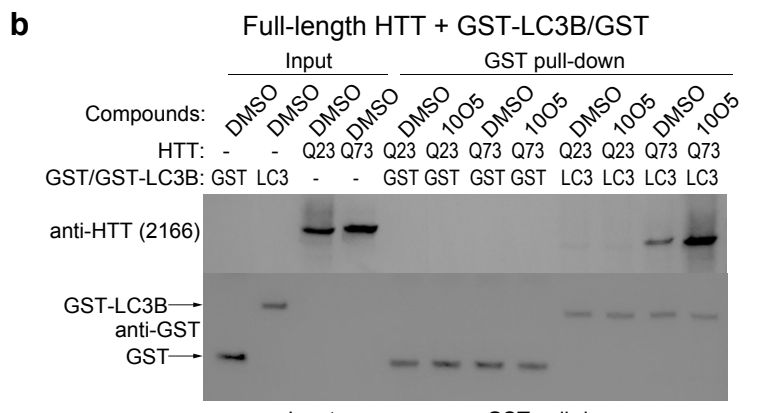
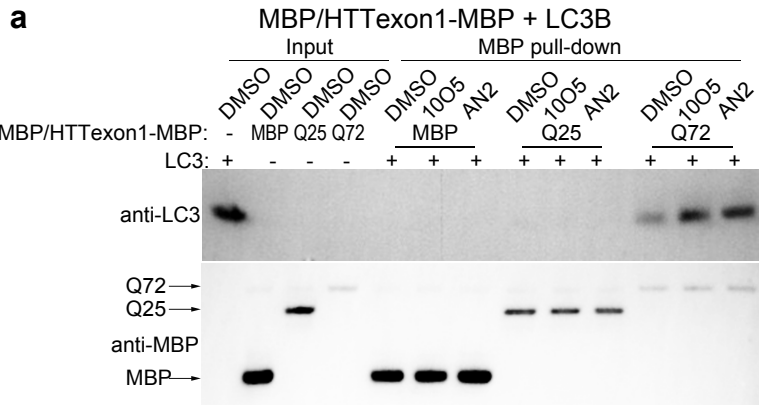
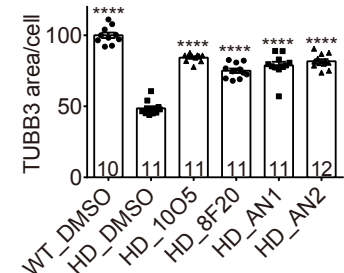
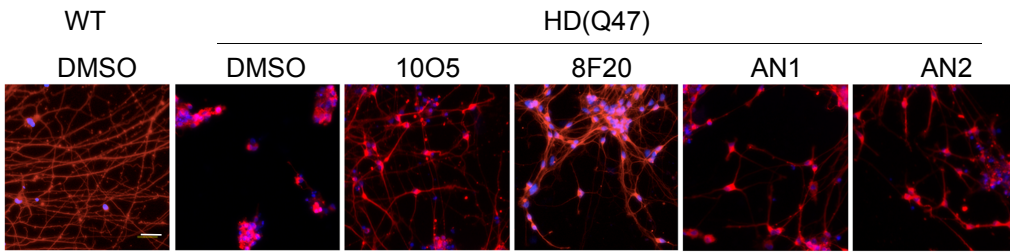


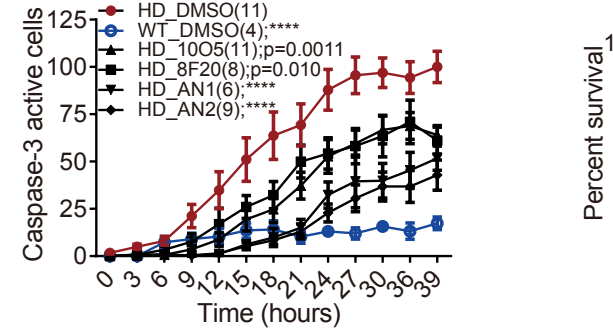
Figure 5

a

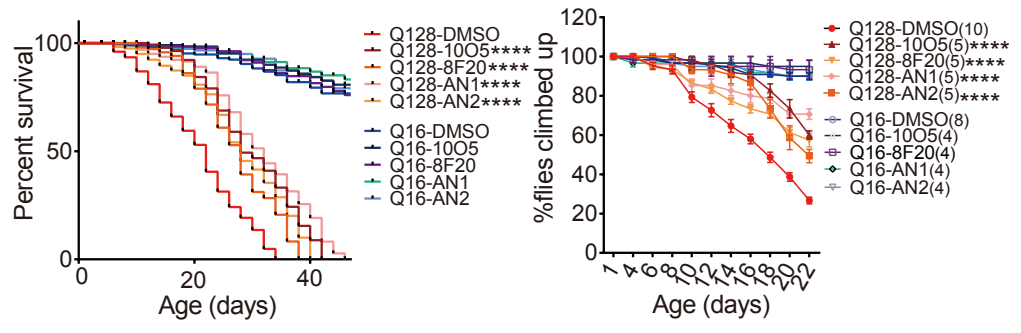
Human iPSC-derived neurons (TUBB3/DAPI; -BDNF)



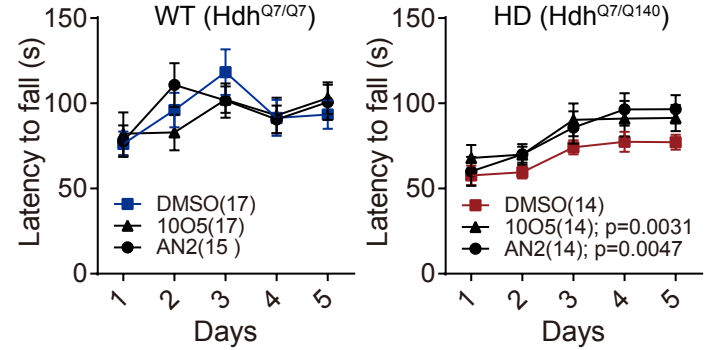
b Human iPSC-derived neurons (-BDNF at 0 h)



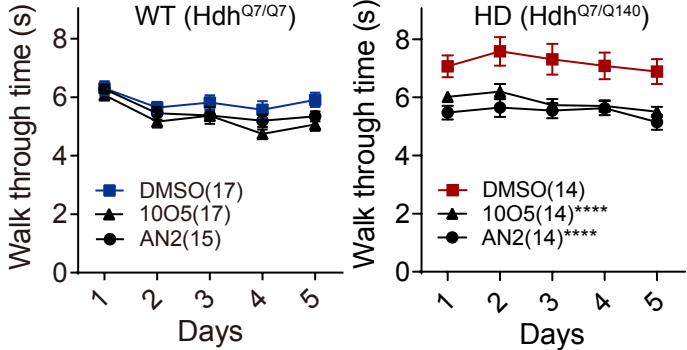
c *Drosophila* models expressing human full-length HTT



d Mouse rotarod tests



e Mouse balance-beam tests



f Mouse gripping force tests

