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Supporting Information

A Specific Mini-Intrabody Mediates Lysosome Degradation of Mutant Huntingtin

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Supplementary Materials

A specific mini-intrabody mediates lysosomal degradation of mutant huntingtin

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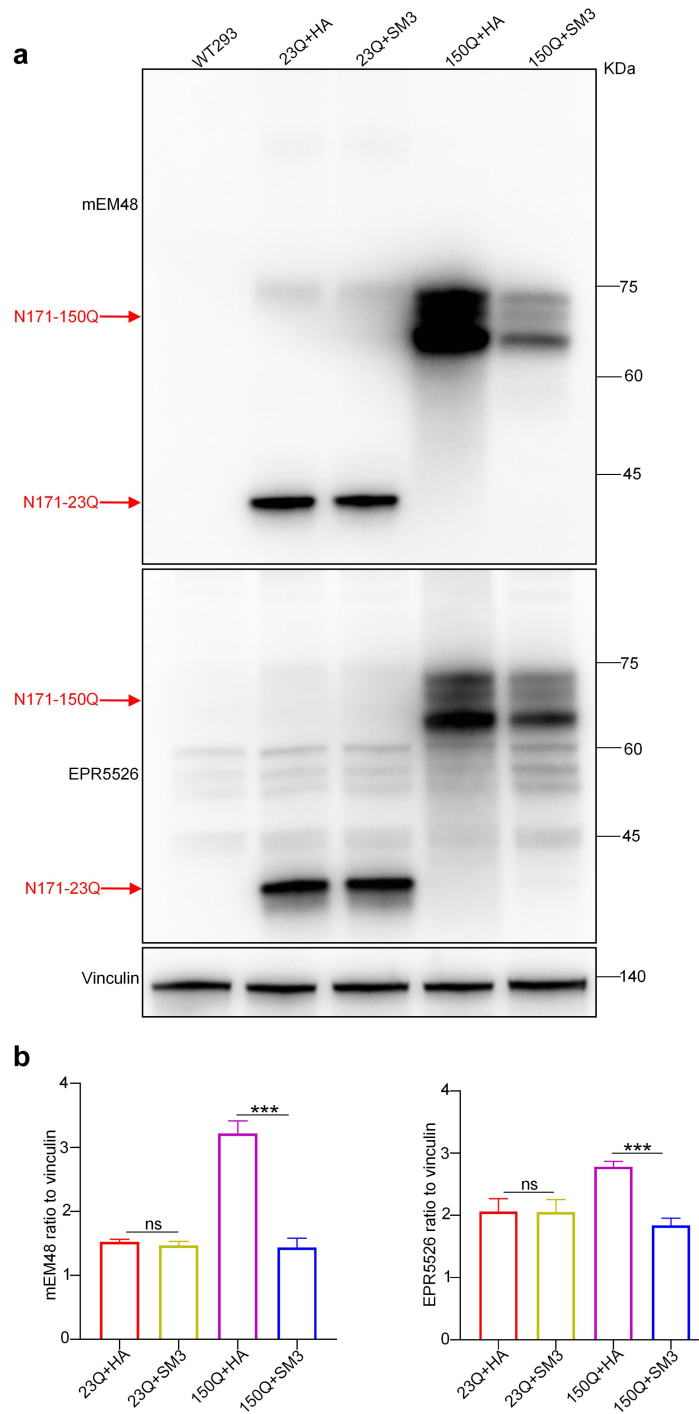[†]These authors contributed equally to this work

Content

Figure S1. Comparison of N-terminal HTT-23Q and -150Q binding to SM3. a) Western blotting analysis of the transfected 293T cells and HTT N171-23Q or -150Q with two anti-HTT antibodies. **b)** Quantification of the ratios of HTT recognized by HTT antibodies (mEM48 and EPR5526) to vinculin on the western blots. The data were obtained from four independent experiments (n = 4). Data were analyzed by Student's t test and presented as mean ± SEM. ***P=0.0003 (mEM48); ***P=0.0006 (EPR5526).

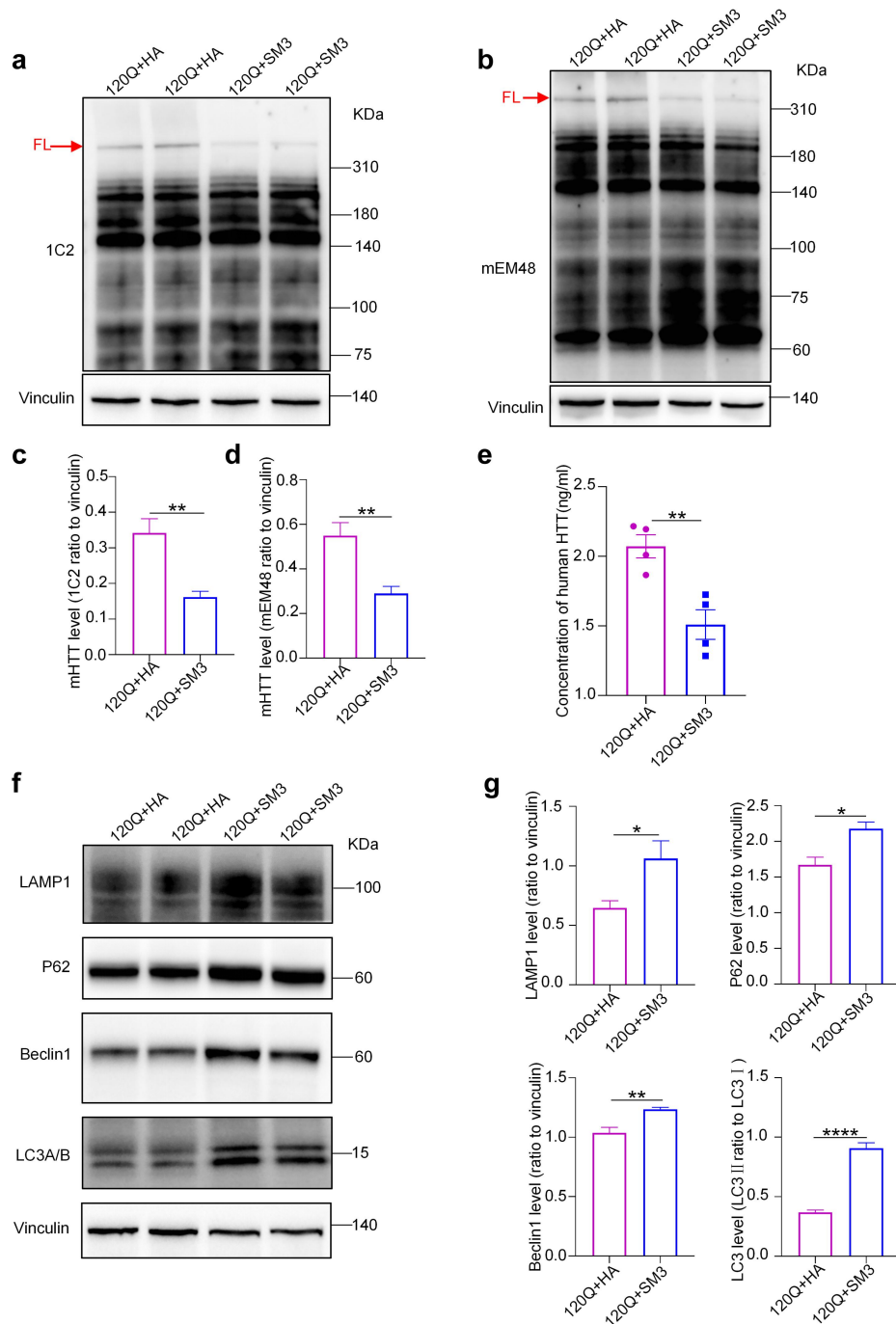**Figure S2. Analysis of the effect of SM3 on the full-length HTT-120Q transfected 293 cells. a, b)** Western blotting analysis of the full-length HTT-120Q stably expressed 293T cells, which were transfected with SM3 or vector expressing HA only. Full-length mHTT (arrow pointed) were detected with 1C2 or mEM48 antibodies. **c, d)** Quantitation of the ratios of mHTT detected by 1C2 or mEM48 to vinculin on the western blots. The data were obtained from four independent experiments (n = 4). Data are analyzed by two-tailed Student's t test and presented as mean ± SEM; **P = 0.0058 (1C2); **P = 0.0081(mEM48). **e)** Quantification of human HTT using ELISA. n = 4 per group. Data were analyzed by two-tailed Student's t test and presented as mean ± SEM. **P=0.0058. **f)** Western blot analysis of HTT-120Q 293 cells transfected with SM3 or the vector expressing HA only. The

blots were probed with LAMP1, P62, Beclin 1 and LC3 antibodies. Vinculin served as a loading control. **g)** Quantitation of the ratios of LAMP1, P62 and Beclin 1 to vinculin on the western blots, and quantitation of the ratios of LC3II to LC3I (LC3A/B) on the western blots. The data were obtained from four independent experiments (n = 4). Data are analyzed by two-tailed Student's t test and presented as mean ± SEM. LAMP1: *P = 0.0304. P62: *P = 0.0124. Beclin 1: **P = 0.007. LC3: ****P<0.0001.
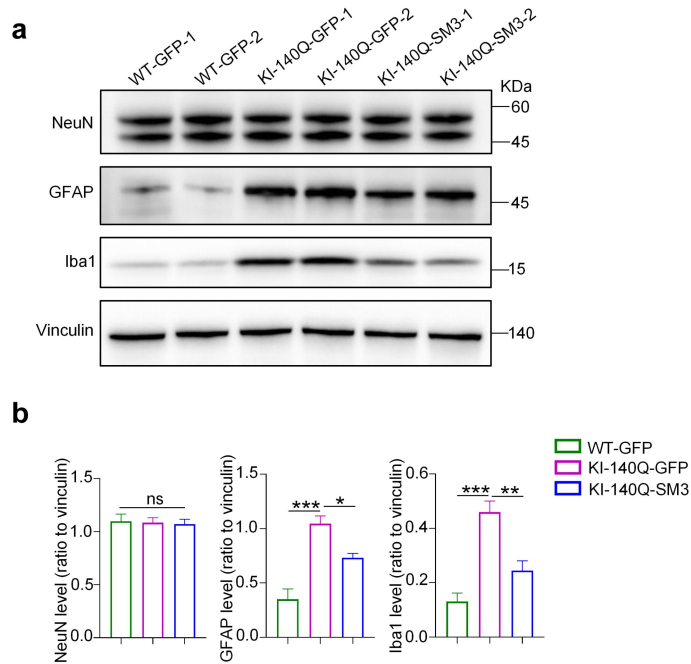

Figure S3. Analysis of the HD KI-140Q mouse brain injected with SM3. a) Western blotting analysis of the expression of neuronal (NeuN) and glial (GFAP, Iba1) proteins in the striatum of HD KI-140Q and wild type (WT) mice injected with GFP (control) or SM3 at 7 months of age. **b)** Quantification of the ratios of NeuN, GFAP, and Iba1 to vinculin on the western blots. The data were obtained from four independent experiments (n = 4). Data were analyzed by one-way ANOVA with Dunnett's multiple comparisons test and are presented as mean ± SEM. GFAP: *P =0.0249; ***P = 0.0002. Iba1: **P = 0.0044; ***P = 0.0003.
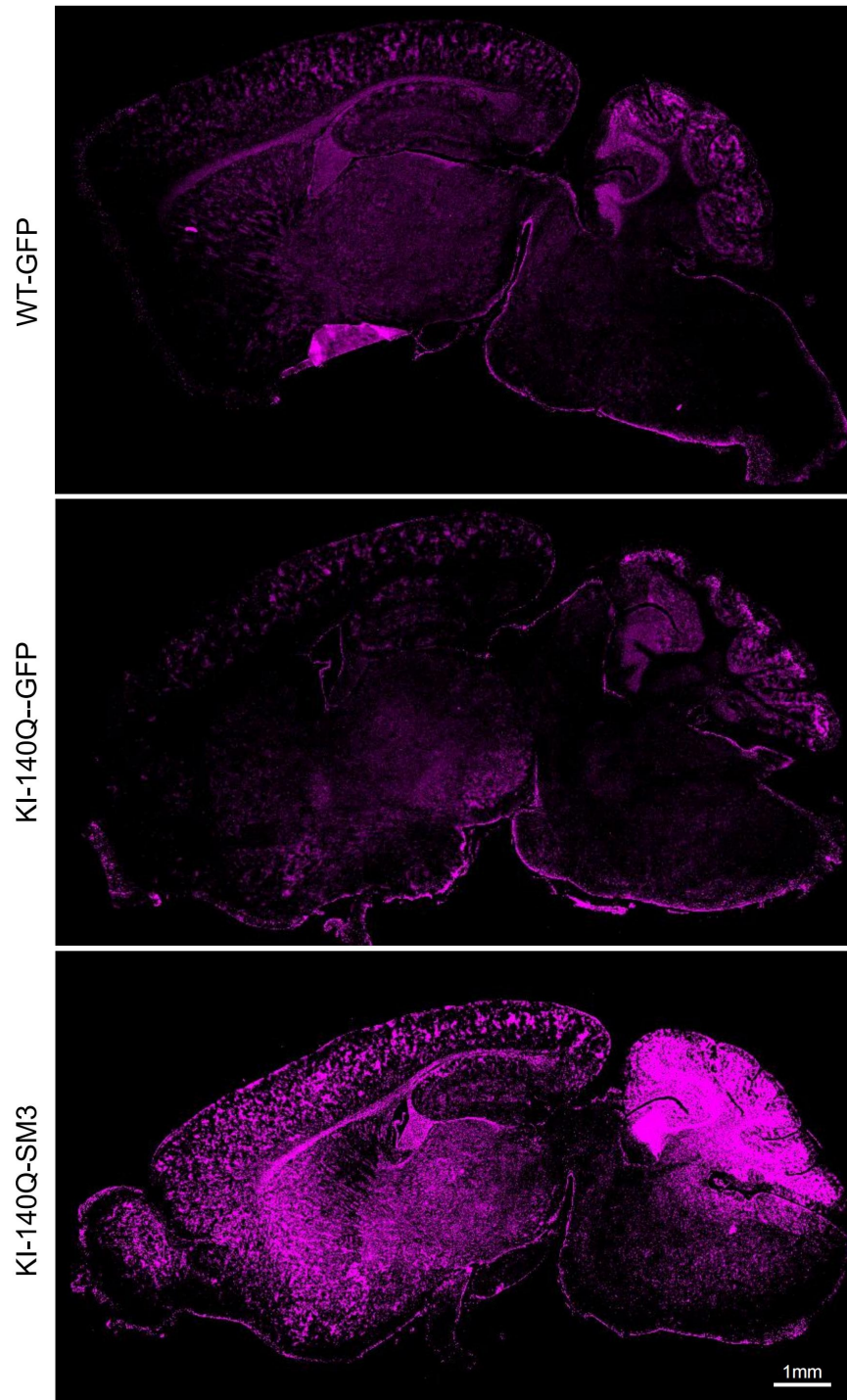
Figure S4. Sagittal images of HA antibody immunofluorescent staining of SM3 in the HD KI-140Q mouse brain.
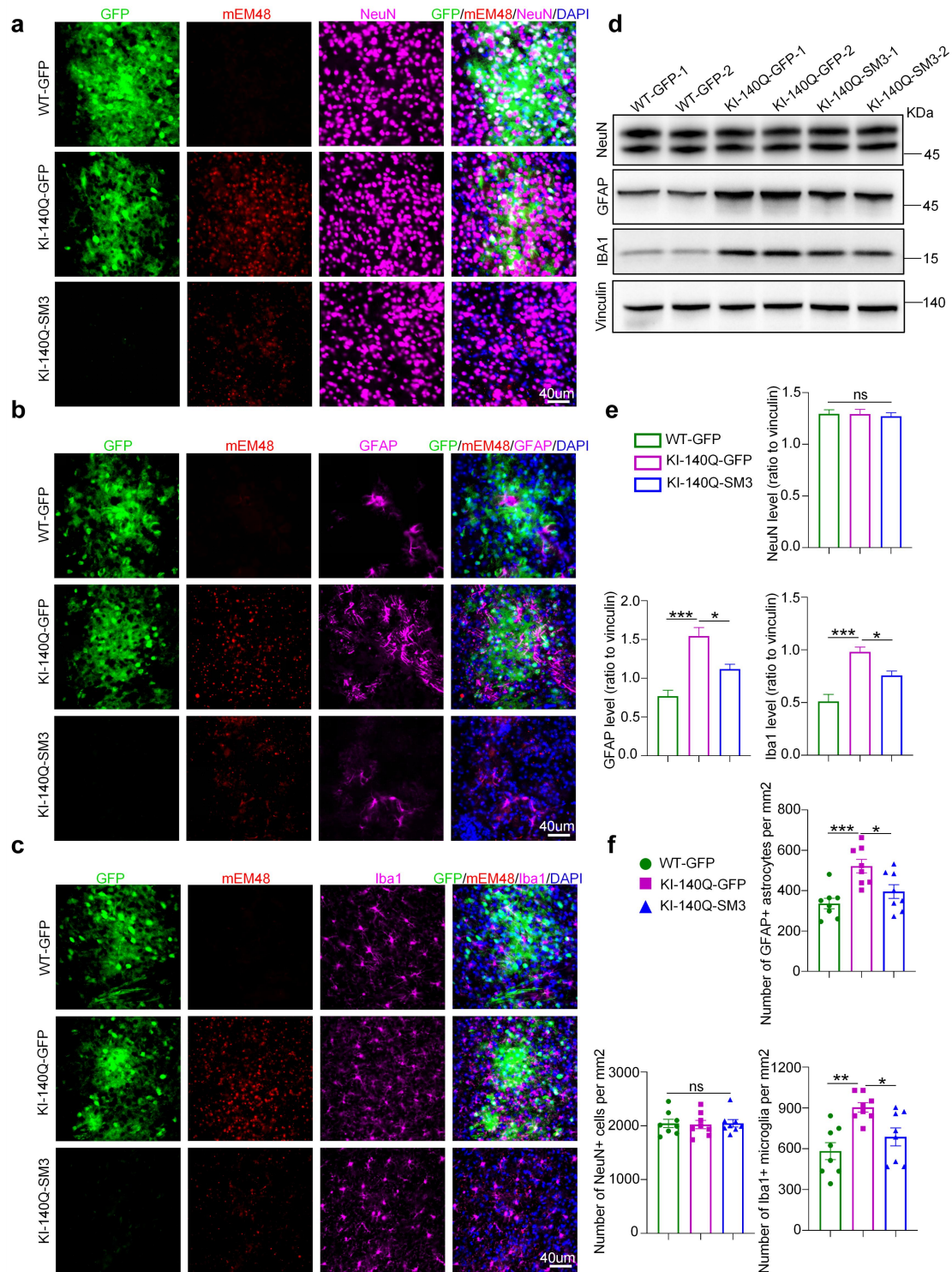
Figure S5. Analysis of HD KI-140Q mouse brain after intravenous injection of SM3. Immunostaining and western blotting of HD KI-140Q mouse striatum one month after intravenous injection of SM3. **a-c)** Representative immunofluorescent fluorescent images of the striatum from GFP or SM3 injected HD KI-140Q mice. Antibodies for NeuN, GFAP and Iba1 were used. All the sections were stained with mEM48 and anti-GFP as well as DAPI. GFP injection was used as control. Note that the numbers of NeuN-positive neurons did not change in HD KI-140Q mice in all treatments. SM3 injection significantly reduced gliosis

shown by GFAP or Iba1staining. Scale bars: 40 μm. **d)** Western blotting analysis of neuronal (NeuN) and glial (GFAP, Iba1) proteins in the HD KI-140Q or WT mice injected with GFP or SM3. **e)** Quantification of the ratios of NeuN, GFAP, and Iba1 to vinculin on the western blots. The data were obtained from four independent experiments (n = 4). Data were analyzed by one-way ANOVA with Dunnett's multiple comparisons test and presented as mean ± SEM. GFAP: ***P= 0.0002; *P= 0.0107. Iba1: ***P= 0.0003; *P= 0.0257. **f)** Quantification of the NeuN, GFAP, Iba1 immunostaining. Data are analyzed by one-way ANOVA with Dunnett's multiple comparisons test and presented as mean ± SEM. n= 8 mice per group; GFAP: *P = 0.0183 (WT-GFP verses HD KI-140Q+SM3); ***P = 0.0008 (WT-GFP verses HD KI-140Q). Iba1: *P = 0.0228 (WT-GFP verses HD KI-140Q+SM3); **P = 0.0011 (WT-GFP verses HD KI-140Q).
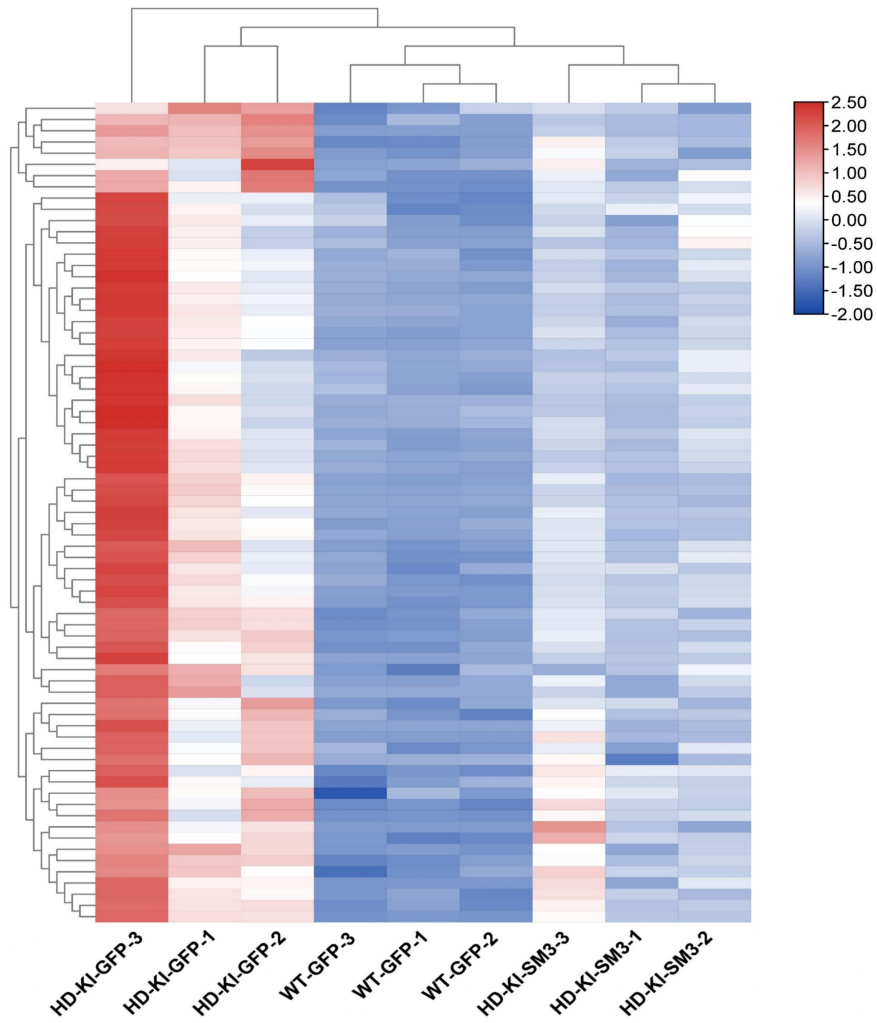
Figure S6. RNA-seq analysis of HD-KI mice after SM3 treatment. Total RNAs from WT and HD KI-140Q treated with GFP or SM3 were examined. The striatum from three animals of each group were examined. The mice at 6 months of age were stereotaxically injected with AAV and then their striatal tissues were isolated for RNA-seq analysis one month after injection. The heat map of the RNA-seq analysis reveals the alterations in the transcripts related to immune and inflammatory responses in HD KI mouse brain.
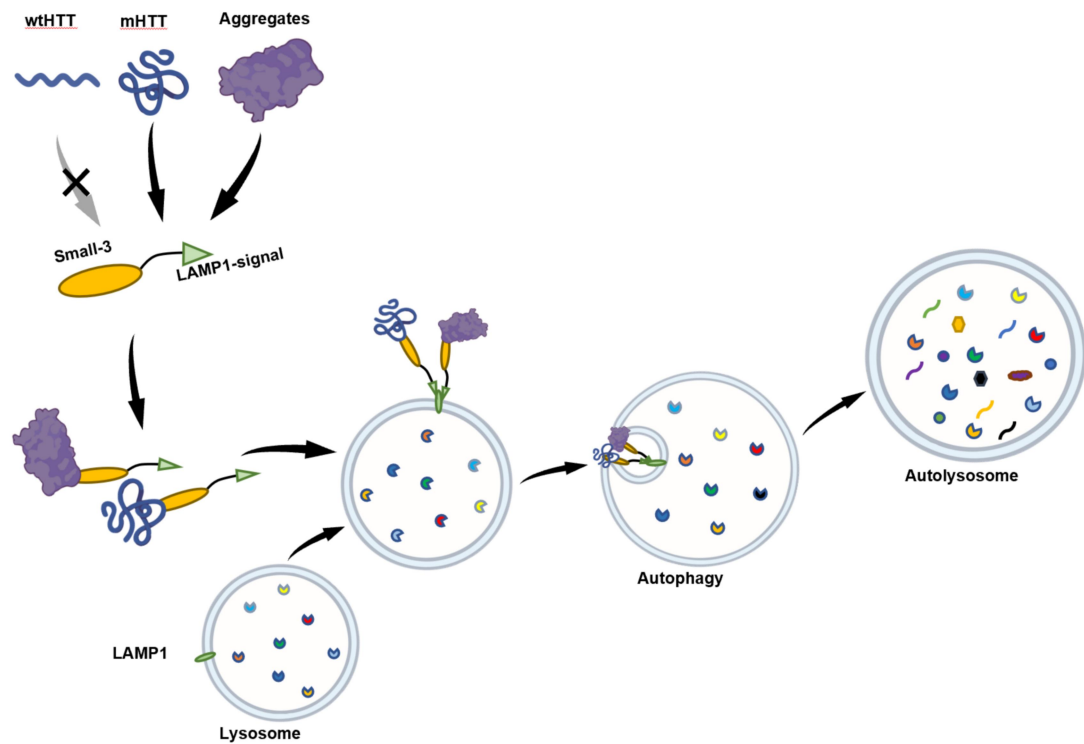
Figure S7. A proposed model of how SM3 induces mHTT degradation. A small peptide derived from HTT intrabody and linked with the lysosomal targeting signal can selectively bind mHTT and bring it to the lysosome for degradation.