Supplementary Methods

Peptides

The $K_2Q_{44}K_2$ and Aβ (1-40) peptides used in these experiments were synthesized with FITC, TMR, or biotin conjugated via the ε-amino group of the N-terminal lysine, by Fmoc chemistry at the Keck Biotechnology Resource Laboratory of Yale University. The α-amino group of Aβ (1-40) was conjugated to biotin.

Antibodies

Monoclonal rab9 antibody was a kind gift from Dr. Suzanne Pfeffer (Stanford University). Polyclonal cathepsin D antibody was purchased from Oncogene (San Diego, CA). Monoclonal GFP antibody was purchased from Roche (Mannheim, Germany). Ubiquitin polyclonal antibodies were from Chemicon International (Temecula, CA). Polyclonal Hsp70 antibody was from Assay Designs. Monoclonal proteasome S7 antibody was obtained from Biomol. Monoclonal γ-tubulin was purchased from Sigma. Monoclonal antibody to LAMP1 was obtained from the Iowa Hybridoma Bank.

Immunofluorescence Microscopy

COS7 cells were incubated for 60 min with 0.5 μM monomer equivalents of TMR-$K_2Q_{44}K_2$, alexa-546-Sup35-NM, or biotin-Aβ (1-40) aggregates at 4°C. Cells were washed and "chased" in complete growth medium at 37°C for 8 hours before fixation with 4% paraformaldehyde. Cells stained for γ-tubulin were fixed by methanol precipitation at –20°C for 10 minutes and did not undergo Triton X-100 permeabilization. Paraformaldehyde-fixed cells on poly-L-lysine-coated coverslips were permeabilized with 0.1% Triton X-100 in PBS for 3 minutes, washed, and blocked with 2% BSA in PBS for 30 minutes. Cells on coverslips were incubated with antibodies to ubiquitin, cathepsin D, hsp70, proteasome S7, γ-tubulin, or LAMP1 in 2% BSA for 1 hour. Antibodies to mouse or rabbit IgG conjugated to Alexa 488 or 594 were used as secondary antibodies. Biotin-Aβ (1-40) was visualized using Alexa-594 streptavidin. Nuclei were stained with 10 μg/ml bisbenzimide. Plasma membrane was stained using Oregon Green wheat germ agglutinin (Invitrogen) diluted 1:100 dilution in PBS on coverslips for 1 min before fixation.

Cell Culture

Human embryonic kidney (HEK)293, COS7, and Neuro-2A cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum at 37°C in 5% CO_2. Plasmid transfection was performed using either the calcium phosphate method or Lipofectamine 2000. Cells were exposed to aggregated peptides or proteins ~40 hours post transfection. Cells were lysed in 0.5% NP-40, 100 mM NaCl, 50 mM Tris-HCl pH 8.8, 5 mM $MgCl_2$, 1 mM EDTA with protease inhibitor cocktail tablets (Roche). Clonal cell lines of GFP-$httQ_{25}$, GFP-$httQ_{71}$, CFP-$httQ_{25}$ and chFP-$httQ_{25}$ were selected and maintained at 400 μg/mL of G418. Neuro-2A cell lines with inducible GFP-$httQ_{60}$, was a generous gift from Dr. Nobuyuki Nukina (RIKEN, Japan).

Legends to supplementary online figures

Figure S1. *In vitro* nucleation of Htt aggregation by K_2Q44K_2 aggregates. a. Schematic diagram of GST-S-tag Htt fusion protein. b. GST-S-tag Htt fusion protein was cleaved with TEV protease in the presence of K_2Q44K_2 aggregates as indicated and subjected to filter retardation assay.

Figure S2. a. Infection with K_2Q44K_2 aggregates induces focal redistribution of intracellular Htt in neuroblastoma cells. The fraction of cells with GFP-HttQ60 puncta was determined in neuro2A cells at different times following induction of Htt expression in the absence (solid circles) or presence (open circles) of K_2Q44K_2 aggregates. b. Filter retardation assay of cells in panel a.

Figure S3. Electron micrographs of aggregated biotin-Aβ (1-40) peptides and aggregated Sup35-NM protein. Aggregated proteins and peptides were freshly sonicated before experiments. Aggregated peptides were placed on copper grids and stained with uranyl acetate, visualized using transmission electron microscope. Scale bar = 0.2 μm in Aβ (1-40) and 0.1 μm in Sup35-NM.