Supporting Information

Yu et al. 10.1073/pnas.1321811111

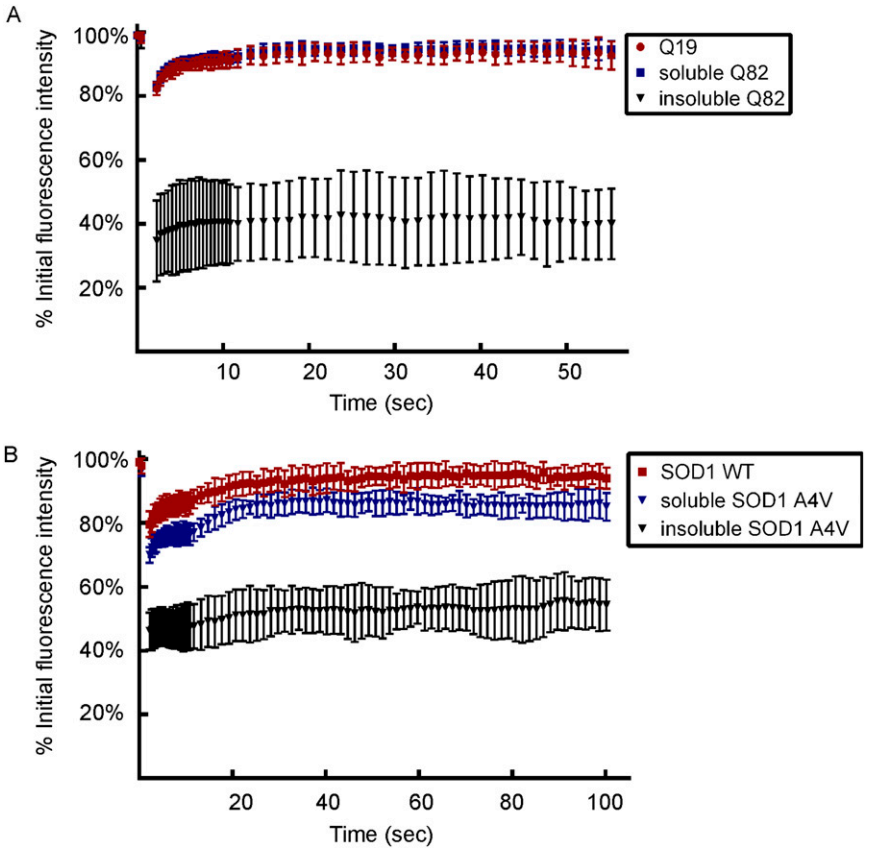

Fig. S1. Fluorescence recovery after photobleaching (FRAP) analysis of polyglutamine (polyQ) and superoxide dismutase-1 (SOD1) proteins. (*A*) FRAP analysis of Q19-GFP (red circles) and Q82-GFP when it appears diffuse (blue squares, soluble) or when it occurs in bright punctae (black triangles, insoluble). Error bars indicate SEs, where $n = 4$–12 cells per sample. (*B*) As in *A*, but of WT SOD1-GFP (red squares) and mutant SOD1 (A4V)-GFP when it appears diffuse (blue triangles) or in punctae (black triangles).

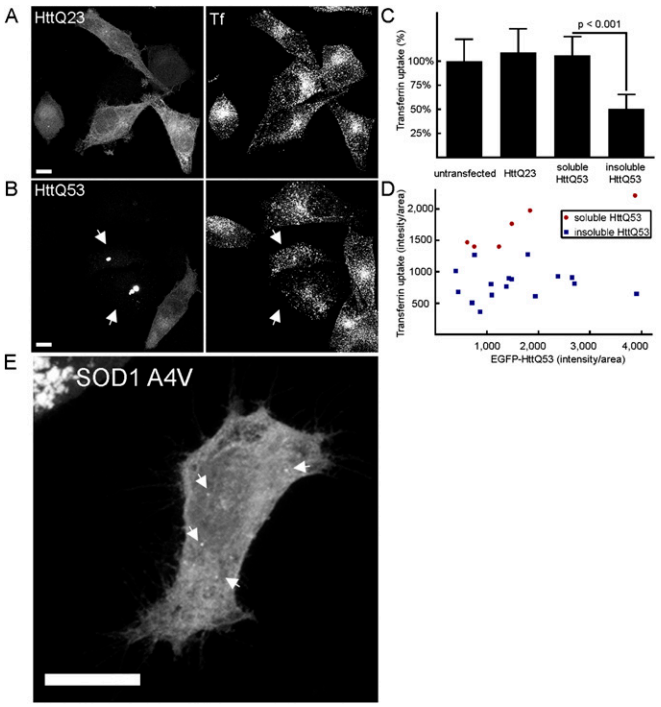

Fig. S2. Different types of protein aggregates inhibit clathrin-mediated endocytosis (CME). Maximal projections of confocal z-series of internalized Alexa555-transferrin (Tf, *Right*) in human PC-3 cells transiently expressing the soluble [*A*, huntingtin (Htt) Q23-EGFP] or aggregation-prone (*B*, Htt Q53-EGFP) form of huntingtin exon 1 fragment fused to EGFP (*Left*) are shown. Arrows in *B* point to cells containing aggregates. (*C*) Quantification of internalized Alexa555-transferrin in untransfected, HttQ23-EGFP–expressing, or Htt Q53-EGFP–expressing cells. Maximal projections of z-series representing the entire cell volume were quantified for sum fluorescence intensities of Alexa555-transferrin (n = 50–200 per sample). Cells containing soluble or aggregated Htt Q53-EGFP were visually subdivided into respective categories. Shown are the mean values, with error bars representing SDs. *P* values were determined using a Student *t* test. (*D*) Effect of relative Htt Q53-EGFP expression levels, as measured by mean fluorescence intensities of EGFP, on transferrin internalization was quantified. (*E*) Enlarged image of the cell labeled with an asterisk in Fig. 1*F* expressing SOD1 (A4V)-GFP, which has aggregated into small foci. Arrows in *E* point to aggregates. (Scale bars, 10 μm.)

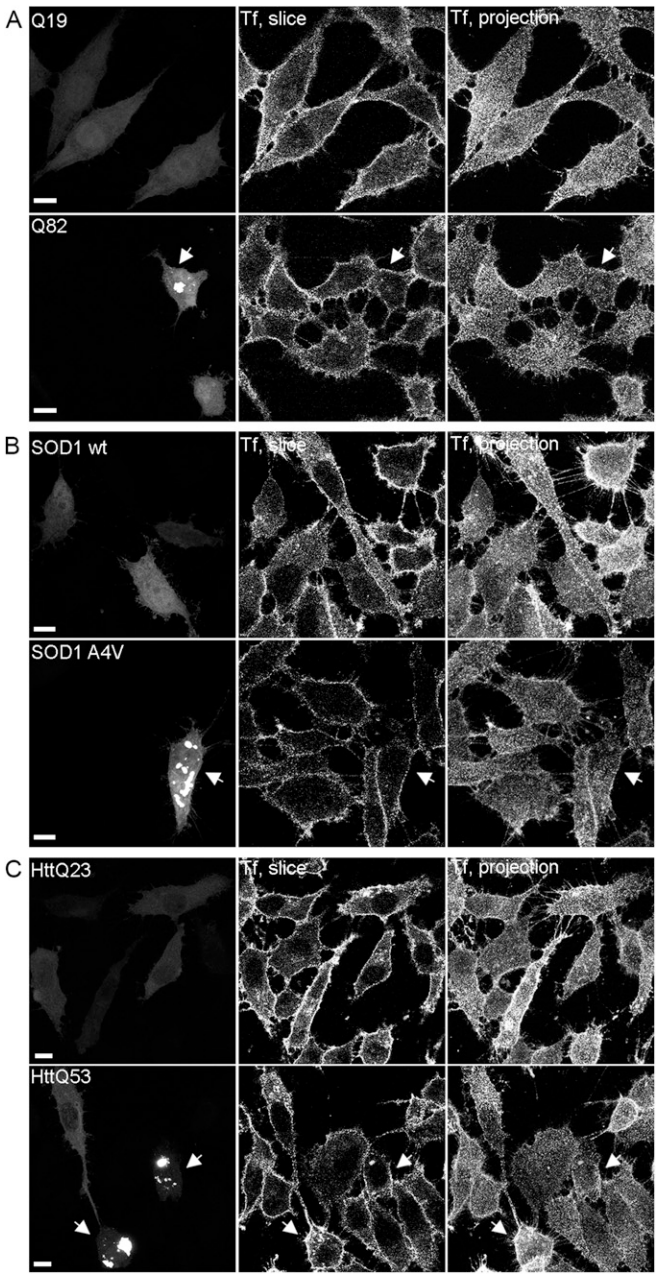

Fig. S3. Inhibition of CME is not caused by the redistribution of transferrin receptor in cells with protein aggregates. Distribution of endogenous transferrin receptor at the cell surface was assayed by fluorescence confocal microscopy in cells transiently expressing various aggregation-prone proteins fused to GFP. Images shown are maximal projections of a z-series of fluorescence confocal slices through the entire cell volume (*Left* and *Right*) or a single confocal slice through the middle of the cells (*Center*). (*A*) Binding of Alexa555-transferrin at 4 °C (*Center* and *Right*) to human PC-3 cells transiently expressing soluble or aggregation-prone Q19-EGFP or Q82-EGFP, respectively. Arrows indicate a cell containing aggregates. Note the aggregate-containing cell has similar fluorescence levels of transferrin at the cell surface compared with untransfected or soluble Q82-expressing cells (slice, *Center*). (Scale bar, 10 μm.) (*B*) As in *A*, but of PC-3 cells expressing WT (wt) or aggregation-prone mutant (A4V) SOD1-AcGFP, respectively. (*C*) As in *A*, PC-3 cells express the soluble (HttQ23-EGFP) or aggregation-prone (Htt Q53-EGFP) form of huntingtin exon 1 fragment fused to EGFP.

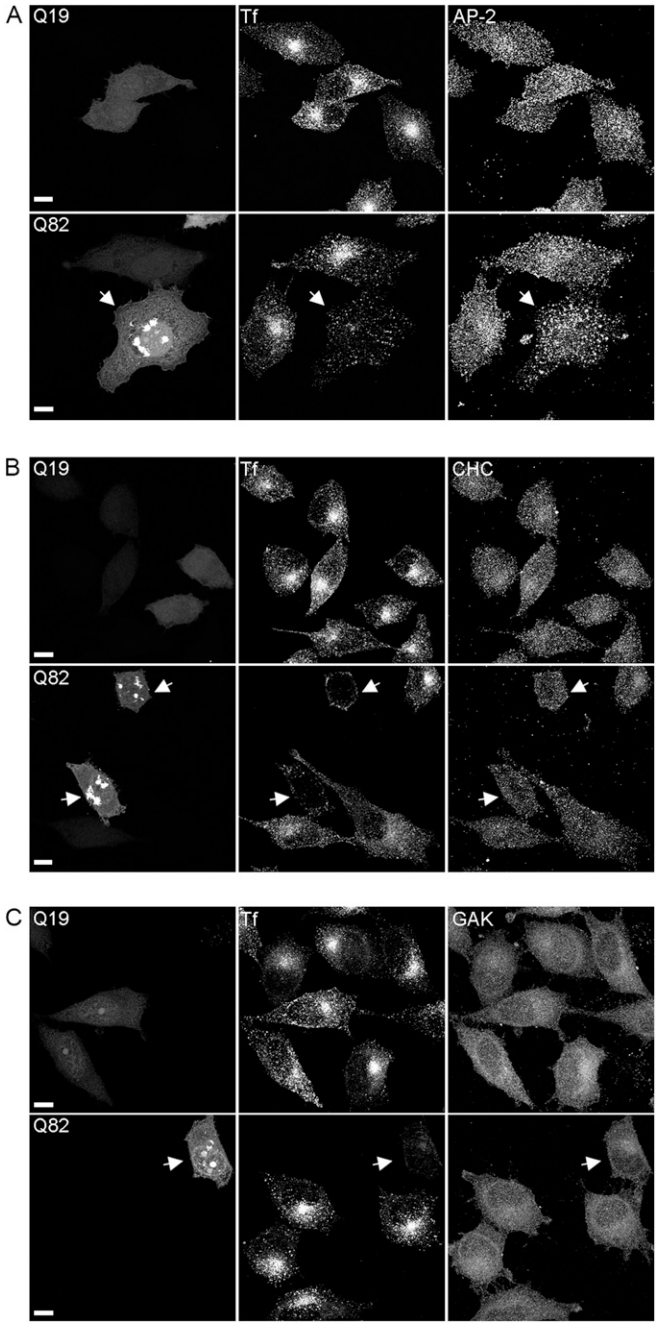

Fig. S4. Inhibition of CME does not relate to aggregate recruitment of other endocytosis factors. (*A*) PC-3 cells transiently expressing Q82-EGFP (*Left*) were assayed for internalized Alexa633-transferrin (*Center*) and immunostained for endogenous mu2, a subunit of the clathrin adaptor-related protein complex 2 (AP-2, *Right*) using specific antibodies. Arrows point to an aggregate-containing cell. (*B*) As in *A*, but immunostained for endogenous clathrin heavy chain (CHC) localization. (*C*) As in *A*, but assayed for endogenous auxilin 2 [G-activated kinase (GAK)] localization. Note the lack of protein recruitment of AP-2, CHC, and auxilin by the protein aggregates, although the transferrin internalization is reduced. (Scale bars, 10 μm.)

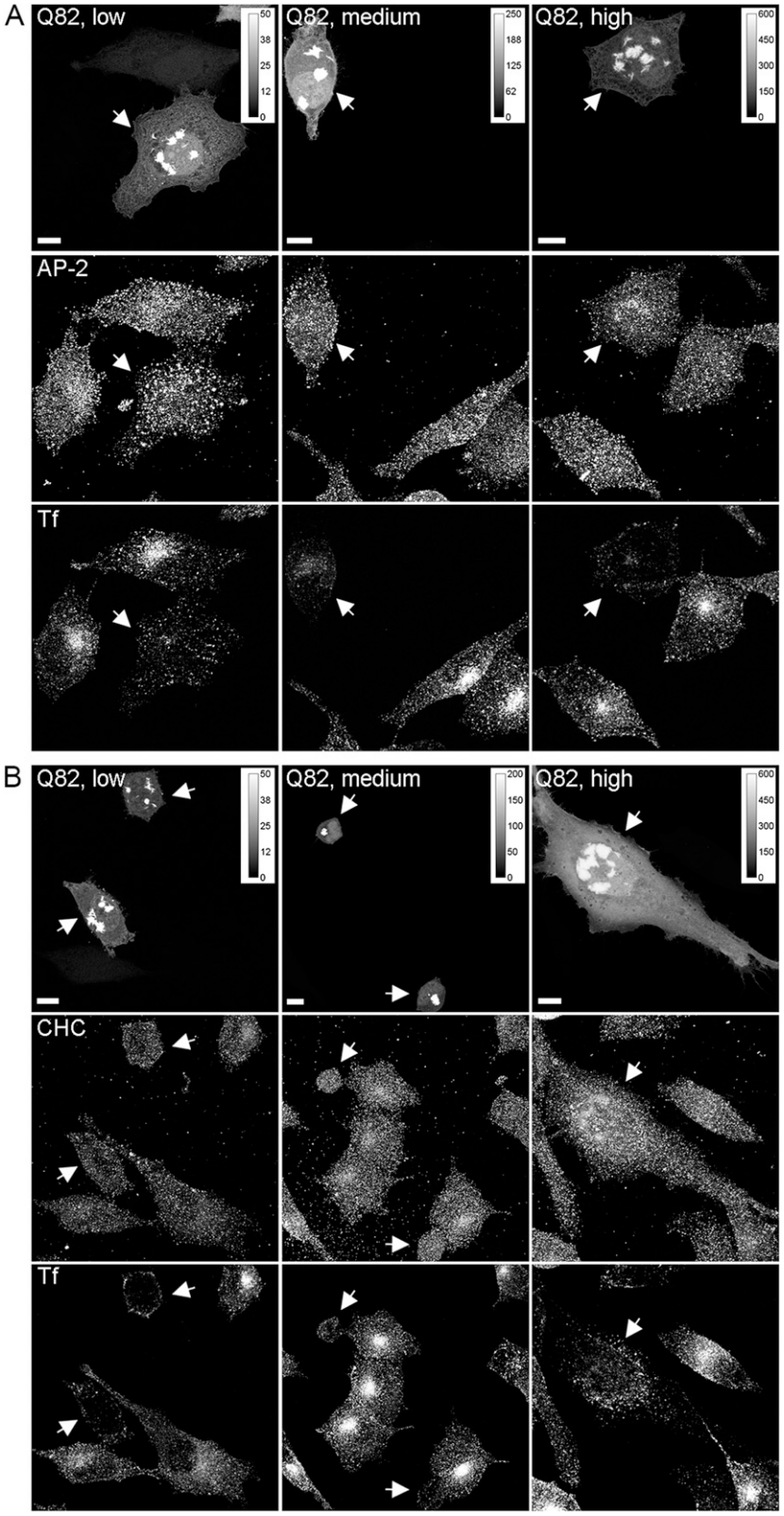

Fig. S5. Recruitment of clathrin coat components to aggregates occurs only in cells expressing Q82 at the highest levels. PC-3 cells transiently expressing Q82-EGFP (*Top*) were assayed for internalized Alexa633-transferrin (*Bottom*) and immunostained for endogenous mu2 (AP-2; *A*, *Middle*) or CHC (*B*, *Middle*). Shown are cells expressing Q82 at three different levels (*Left*, *Center*, and *Right*). Calibration bars (*Upper Right*) depict the range of raw integrated fluorescence intensity values of the Q82-GFP image. Arrows point to aggregate-containing cells. (Scale bars, 10 μm.)

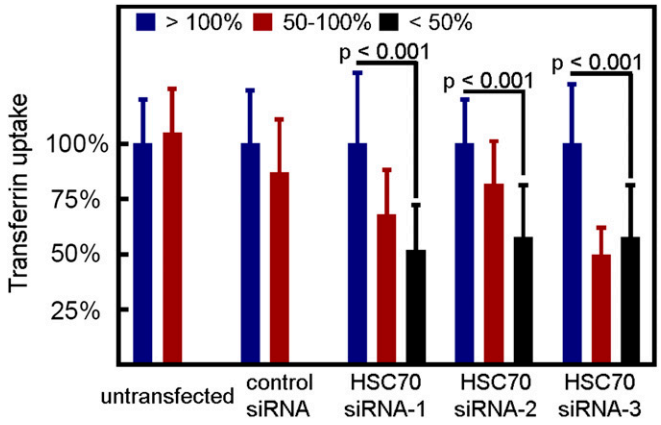

Fig. S6. RNAi depletion of heat shock cognate protein 70 (HSC70) inhibits CME in a concentration-dependent manner. Quantification of internalized transferrin levels in Neuro2a cells that were untransfected or treated with control or three independent siRNA oligonucleotides targeting different regions of HSC70. The untransfected, control siRNA, and HSC70 siRNA-1 samples are those shown in Fig. 2 *E* and *F*. Analyses were done as in Fig. 2*G*.

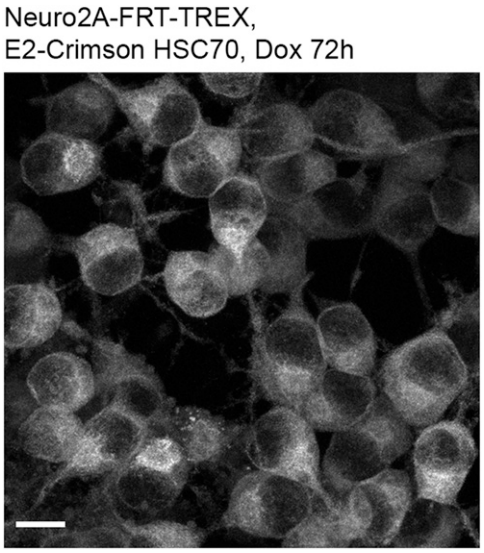

Fig. S7. E2-Crimson HSC70 is homogeneously expressed after doxycycline induction. A confocal image of Crimson fluorescence of Neuro2a E2-Crimson HSC70 cells after 72 h of doxycycline treatment is shown. (Scale bar, 10 μm.)