ONLINE METHODS

Plasmids.

The GST-HttQ51-exon1 fusion with a TEV protease cleavage site between GST and Htt-exon1, and an S-tag C-terminal to Htt-exon1[48] were previously described. We constructed the MBP-HttQ44-exon1 construct[49] as previously

described. Site-directed mutagenesis or inverse PCR using the Quik-Change kit (Stratagene, La Jolla, CA) generated all amino acid substitutions. Additional cloning details can be obtained upon request from the authors.

Antibodies.

Anti-TCPα (also known as TRiC subunit 1 or CCT1) antibodies (CTA-191) were from Stressgen (Ann Arbor, MI) and anti-HA antibodies from Sigma-Aldrich (St. Louis, MO). Anti-Htt antibodies were previously described[49].

Peptides.

Genscript (Piscataway, NJ) synthesized all peptides with amidated C-termini. Peptides used for crosslinking studies had an amino-terminal biotin and cysteine introduced at position 2 (A2C). Peptides used in aggregation assays were synthesized with free amino-termini. All peptides were dissolved to a final concentration of 1 mM in peptide buffer [50 mM Tris/Cl pH 7.5]. Mass spectrometry verified synthesis of full length peptide.

Crosslinking of $N17^{Htt}$ peptides to chaperones and Htt-exon1 truncations.

$N17^{Htt}$ peptides supplemented with 0.1 mM TCEP were reacted with benzophenone-4-iodacetamine (BPIA) (Molecular Probes) by addition to 0.3 mM for 90 min at room temperature. Unreacted BPIA was quenched by addition of 1 mM DTT for another 30 min. 2.5 μM BPIA-labeled peptide was incubated with 1 μM TRiC in buffer X [25 mM HEPES pH 7.5, 100 mM NaCl, 10% (v/v) glycerol], 5

μM BPIA-labeled peptide was incubated with equimolar concentrations of apical domains in 25 mM HEPES pH 7.5, 300 mM NaCl, 10% glycerol, and 12.5 μM BPIA-labeled peptide was incubated with 5 μM GST-HttQ51-exon1 truncations in 50 mM Tris/Cl pH 8.0, 0.5 mM EDTA, 1 mM DTT for 30 min at room temperature. Photolysis was carried out for 10 min under filtered UV light as previously described [22]. Biotinylated peptide crosslinks were probed with Streptavidin-HRP conjugate (Zymed, San Francisco, CA).

Crosslinking of cysteine-engineered MBP-Htt-exon1 to TRiC.

Cysteine-engineered MBP-HttQ44-exon1 in buffer X supplemented with 0.1 mM TCEP, were reacted with BPIA by addition to 0.125 mM for 90 min at room temperature. Unreacted BPIA was quenched by addition of 1 mM DTT for another 30 min. BPIA-modified MBP-HttQ44-exon1 constructs with cysteines at unique positions were incubated with 0.7 μM TRiC in buffer X for 30 min at room temperature. Photolysis was carried out as described for $N17^{Htt}$ peptide.

***In vitro* GST-Qn aggregation assay.**

3 μM GST-HttQ51-exon1 and assorted mutational variants were cleaved with TEV protease as described in TEV protocol (Invitrogen) in the presence of various chaperones, peptides, or negative control ovalbumin (Sigma). All samples had equivalent amounts of chaperone or peptide buffer to normalize any effect of buffering conditions on TEV cleavage and Htt-exon1 aggregation. In addition, the chaperones and peptides did not affect the efficiency of TEV

protease mediated cleavage (data not shown). At various time points, reactions were stopped by the addition of an equivalent volume of 4% SDS and 100 mM DTT, and boiled for 5 min as described. Reactions were then filtered through 0.22 µm cellulose acetate (Schleicher&Schuell, Dassel, Germany), washed twice with 0.1% SDS, and blocked in 3% BSA (in TBS) for 10 min. Htt-exon1 aggregates were probed with either S-protein-HRP (EMD Biosciences, San Diego, CA) or biotinylated S-protein (EMD Biosciences) and IRDye 800CW-labeled streptavidin followed by Li-Cor Odyssey analysis (Li-Cor Biosciences, Lincoln, NE).

Mammalian Cell culture.

HeLa S3 cells were maintained in DMEM/F12 (Gibco, Carlsbad, CA), supplemented with 10% fetal calf serum (FCS) and L-glutamine. Confluent cells were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) with Qn-GFP (n = 25, 103) according to manufacturer's protocol. Cells were analyzed 24 hrs later by microscopy. Transient transfection resulted in 60-70% transfection (as judged by GFP expression).

Protein Purification.

GST-HttQ51-exon1 truncations were purified from BL21 cells with pROSETTA as previously described[33]. TRiC was purified from bovine tissue as previously described[50,51]. TRiC subunit apical domains (Apicalx; x = subunit 1 or 3) were purified from BL21^{STAR} cells with pROSETTA by Co-TALON affinity

resin[22]. MBP-HttQ44-exon1 cysteine-engineered fusions were purified as previously described[49]. Purity of all proteins was determined by SDS-PAGE and coomassie staining to be >95%.

GST-Htt interaction with TRiC in complete lysate.

GST-WT and GST-NA (40 µg) were attached to 20 µl 50:50 Glutathione Sepharose 4 Fast Flow (GE Healthcare) equilibrated in 1X PBS for 1 hour in 4ºC. After 3 washes with 200 µl 1X PBS, 200 µl yeast lysate were added and incubated further for 1 hour in 4ºC. After 4 washes with 200 µl 1X TBST, the TRiC-huntingtin exon1 complex was eluted using 25 mM Glutathione in 1X PBS and ran on a 12% SDS-PAGE gel and analyzed by immunoblot against TRiC and Huntingtin exon1 essentially as described[19]. All membranes were scanned on Li-Cor Odyssey described above.