Supplemental Information

Supplemental Figures

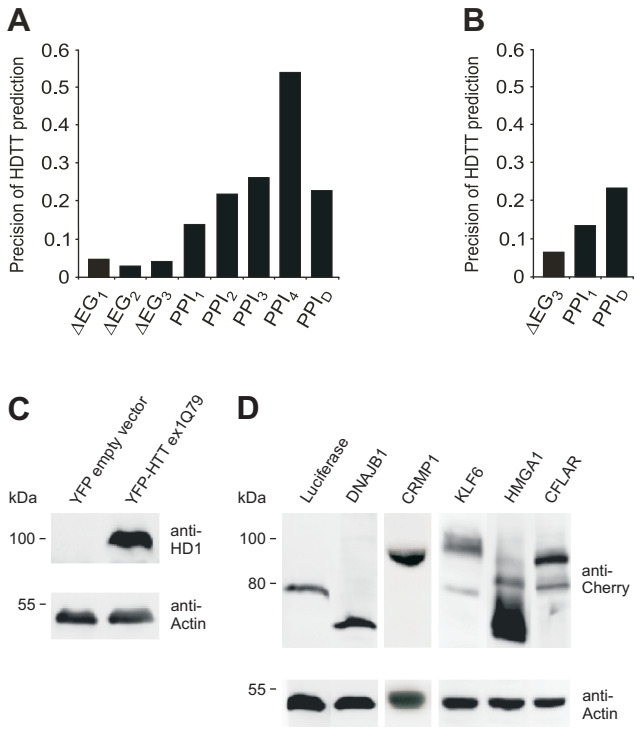


Figure S1

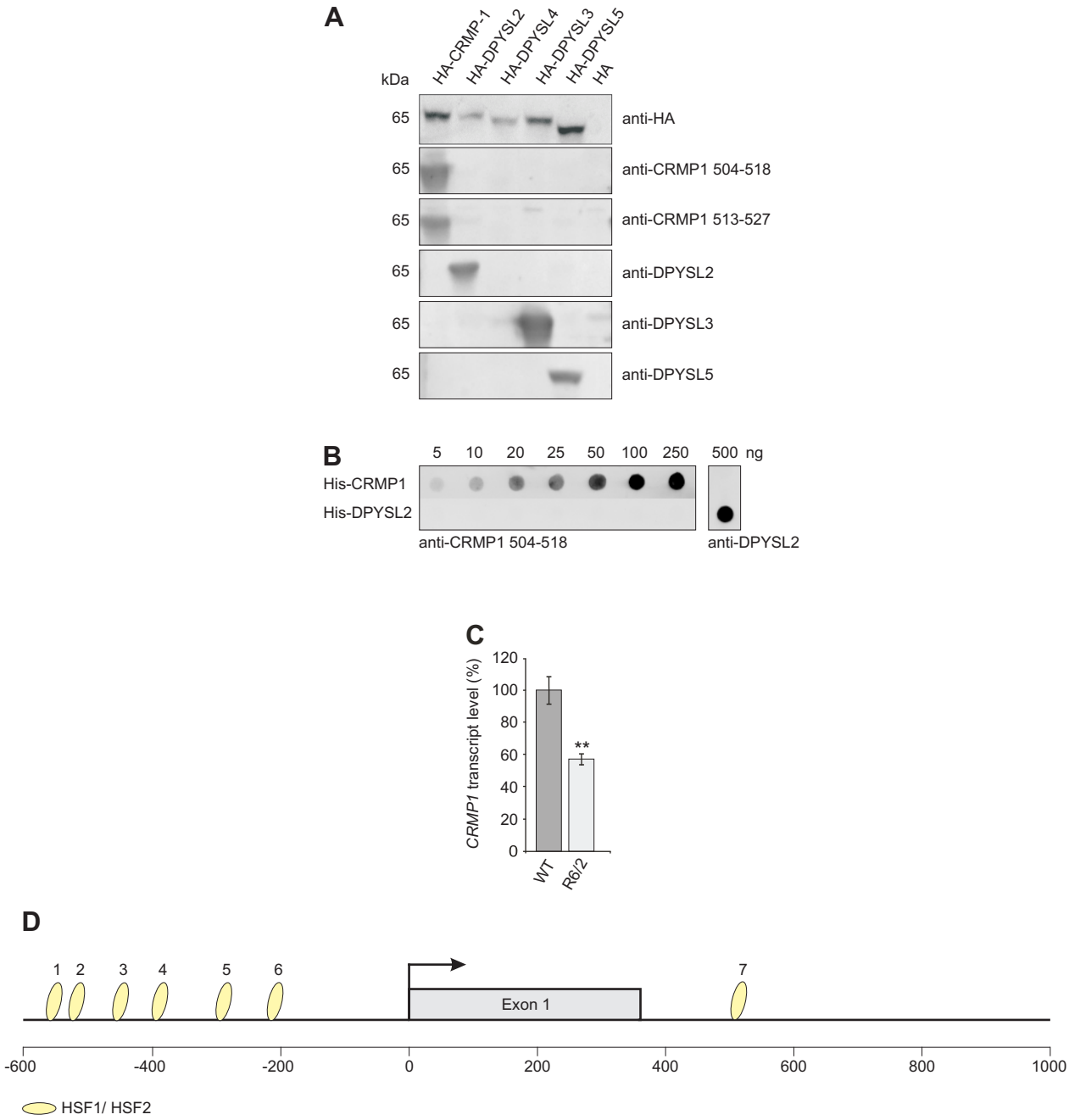

A
HA-CRMP-1
HA-DPYSL2
HA-DPYSL4
HA-DPYSL3
HA-DPYSL5
HA
kDa
65
anti-HA
anti-CRMP1 504-518
anti-CRMP1 513-527
anti-DPYSL2
anti-DPYSL3
anti-DPYSL5
B
5 10 20 25 50 100 250 500 ng
His-CRMP1
His-DPYSL2
anti-CRMP1 504-518
anti-DPYSL2
C
CRMP1 transcript level (%)
120
100
80
60
40
20
0
**
WT
R6/2
D
1 2 3 4 5 6 7
Exon 1
-600 -400 -200 0 200 400 600 800 1000
HSF1/ HSF2

Figure S2

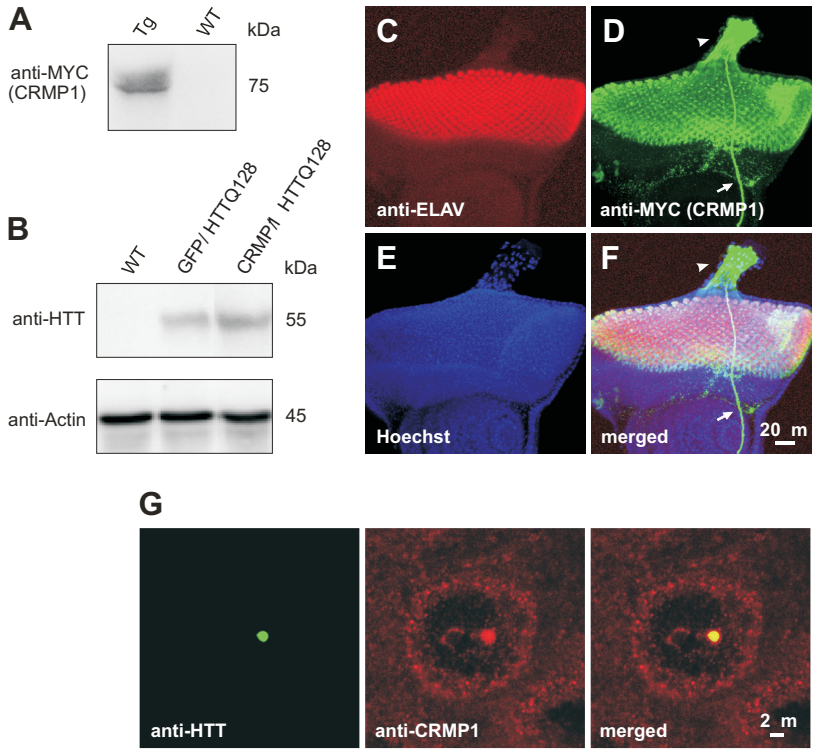

A
Tg
WT
kDa
anti-MYC
(CRMP1)
75
B
WT
GFP/HTTQ128
CRMP1/HTTQ128
kDa
anti-HTT
55
anti-Actin
45
C
anti-ELAV
D
anti-MYC (CRMP1)
E
Hoechst
F
merged
20 m
G
anti-HTT
anti-CRMP1
merged
2 m

Figure S3

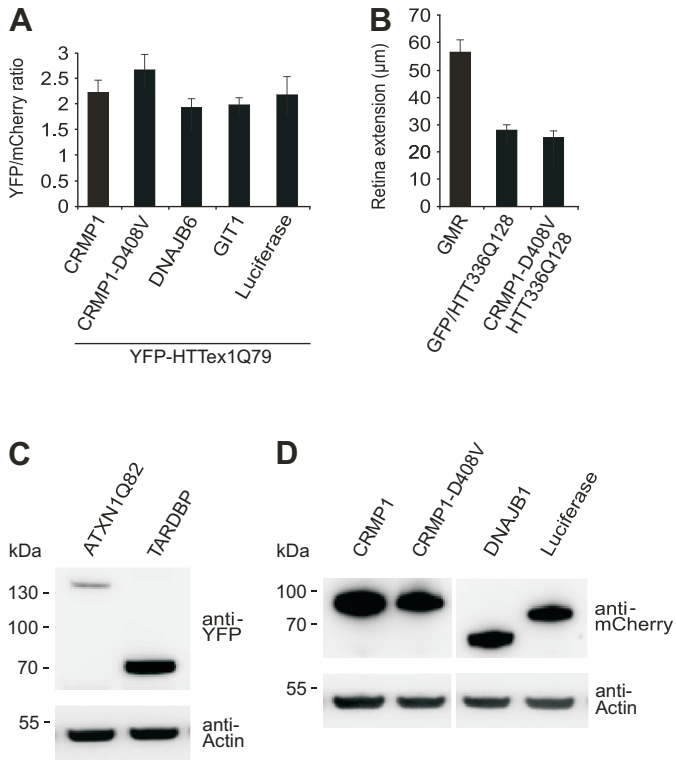
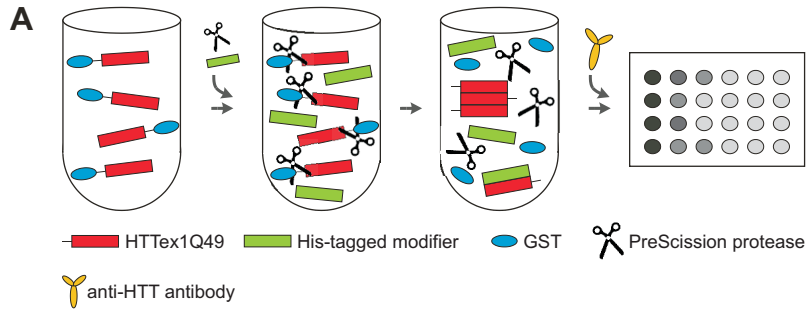
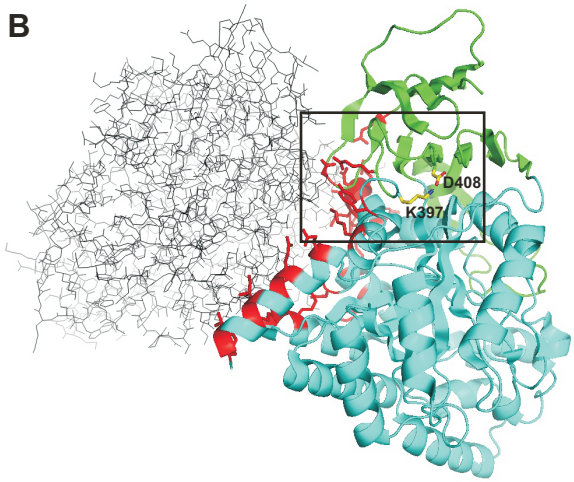
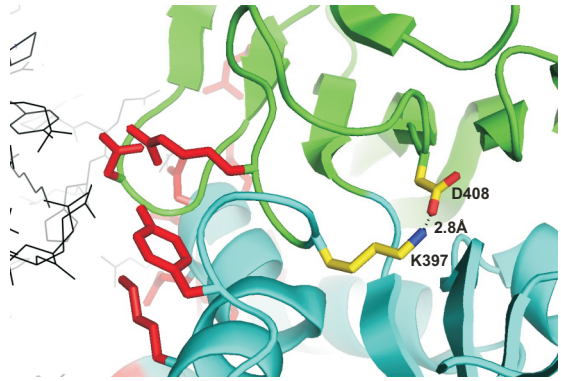
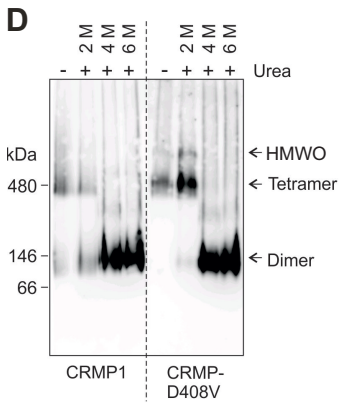

A
YFP/mCherry ratio
3
2.5
2
1.5
1
0.5
0
CRMP1
CRMP1-D408V
DNAJB6
GIT1
Luciferase
YFP-HTTex1Q79
B
Retina extension (µm)
70
60
50
40
30
20
10
0
GMR
GFP/HTT336Q128
CRMP1-D408V HTT336Q128
C
kDa
ATXN1Q82
TARDBP
130
100
70
55
anti-YFP
anti-Actin
D
kDa
CRMP1
CRMP1-D408V
DNAJB1
Luciferase
100
70
55
anti-mCherry
anti-Actin

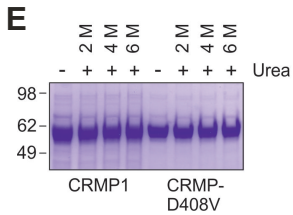
Figure S4


A
HTTex1Q49
His-tagged modifier
GST
PreScission protease
anti-HTT antibody


B
D408
K397


C
D408
2.8Å
K397


D
2 M
4 M
6 M
Urea
kDa
480
146
66
HMWO
Tetramer
Dimer
CRMP1
CRMP-D408V


E
2 M
4 M
6 M
Urea
98
62
49
CRMP1
CRMP-D408V

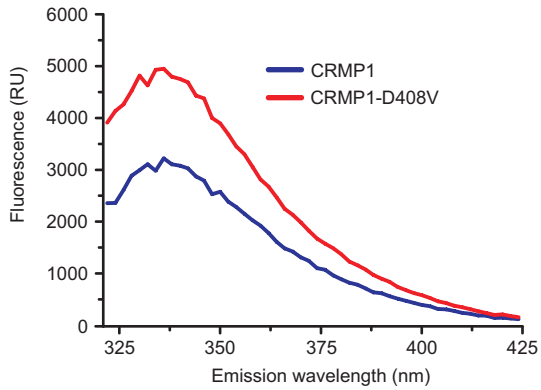

F
Fluorescence (RU)
6000
5000
4000
3000
2000
1000
0
325
350
375
400
425
Emission wavelength (nm)
CRMP1
CRMP1-D408V

Figure S5

Supplemental Figure Legends

Figure S1. Benchmarking of the protein interaction filtering strategy and comparison with available literature information; expression controls in cell model systems. (*A*) The precision of prediction of known HDTTs was determined for different PPI data sets (PPI_{1-4} and PPI_D) as well as for data sets with differentially expressed genes (ΔEG_{1-3}). Step-by-step data filtering reduces the size of PPI data sets but increases the relative number of HDTTs in PPI networks. (*B*) Optimization of PPI_D by optimizing the threshold to define ΔEG_3 does not increase the precision of prediction of known HDTTs in the PPI_D network compared to (*A*). (*C*) SDS-PAGE and immunoblotting confirms the expression of YFP-tagged HTTex1Q79 in transfected SH-EP cells using the HTT specific HD1 antibody. (*D*) Expression control of the mCherry tagged proteins luciferase, CRMP1, DNAJB1, KLF6, HMGA1 and CFLAR, which were co-expressed with the aggregation prone HTTex1Q79 protein using an anti-mCherry antibody. All proteins were expressed in SH-EP cells with their expected sizes.

Figure S2. Characterization of anti-CRMP1 antibodies and potential HSF1 and HSF2 binding sites around the transcriptional start site in the transcript encoding CRMP1. (*A, B*) Investigating the specificity of polyclonal anti-CRMP1 antibodies. (*A*) Protein extracts with different HA-tagged CRMP proteins were prepared from transfected COS7 cells and analyzed by SDS-PAGE and immunoblotting. The anti-CRMP1 antibodies 504-518 and 513-527 specifically recognize CRMP1. (*B*) Dot blot assay. Different amounts of natively purified His7-CRMP1 protein were spotted onto a nitrocellulose membrane. The anti-CRMP1 antibody 504-518 recognizes 5-10 ng of the purified antigen. No cross-reactivity with the related protein His7-DPYSL2 protein was observed. (*C*) *Crmp1* transcript levels were significantly decreased in brains of 12-week-old R6/2 mice compared to brains of wild type littermates as measured by Taqman qPCR. Taqman qPCR values were normalized to the

geometric mean of three housekeeping genes: Atp5b, Eif4a2 and Rpl13a. Error bars are SEM using Student's t-test (n=8). **p<0.01. (*D*) 600 bp upstream and 1000 bp downstream of the CRMP1 transcriptional start site (including exon 1) were analyzed for potential evolutionary conserved heat shock factor (HSF) binding sites using the ConTra v2 software. Several potential HSF binding sites were found upstream of the open reading frame, indicating that CRMP1 is a heat shock inducible protein. Parameters used for the identification of potential HSF binding sites are described in Table S11.

Figure S3. Detection of the recombinant proteins CRMP1 and HTT336Q128 in transgenic flies and co-localization of CRMP1 with HTT aggregates in HD transgenic mice. (*A, B*) The recombinant proteins MYC-CRMP1 and HTT336Q128 were produced in HD transgenic (Tg) flies and detected by SDS-PAGE and immunoblotting. Protein lysates of whole larvae were analyzed. (*A*) The recombinant protein MYC-CRMP1 (75 kDa) was detected in transgenic HD flies but not in wild-type flies. An anti-MYC antibody was used for detection. (*B*) Analysis of protein extracts by SDS-PAGE and immunoblotting revealed that aggregation-prone HTT336Q128 protein with a size of 55 kDa is produced in HD transgenic flies but not in wild-type (WT) flies. The endogenous protein actin was used as a loading control. (*C-F*) CRMP1 is expressed in differentiating neuronal photoreceptor cells and localized to neuronal projections. Confocal micrographs of a triple-labelled third instar eye/antennal imaginal discs of w^{1118}*;GMR-GAL4/+;UAS-Hsap\CRMP1[1-1-10M]/+* *Drosophila* larvae. (*C*) The anti-Elav antibody 7E8A10 labels neuronal cell bodies in the eye imaginal disc. (*D* and *F*) Human CRMP1 (green) is specifically expressed in the eye imaginal disc and co-localizes (*F*) with the neuronal ELAV protein (*C*) in the differentiating photoreceptors. CRMP1, which is recognized by the anti-MYC antibody, is highly enriched in the Bolwig's nerve (arrow) originating from 12 larval photoreceptor cells (Bolwig's organ) and projecting to the eye/antennal disc via the optic stalk (arrow head) into the optic lobe anlage. The optic stark is the second structure where CRMP1 is enriched. The structures,

stained by the antibodies, mainly represent axonal projections of developing photoreceptor cells of the third instar eye imaginal disc. These results indicate that CRMP1 is actively transported into neuronal projections, supporting previous observations that the protein is critical for axonal outgrowth and guidance. (*E*) Cell nuclei were detected by Hoechst 33258 staining. (*G*) Immunofluorescence analysis of brain slices prepared from 12-week old R6/2 HD transgenic mice. Endogenous CRMP1 co-localizes with neuronal intranuclear inclusions with insoluble HTTQ150 aggregates in brains of R6/2 HD transgenic mice. HTTQ150 and CRMP1 were detected with the antibodies EM48 and anti-CRMP1 (504-518), respectively.

Figure S4. Expression of recombinant proteins in mammalian cells and quantification of retinal degeneration in *Drosophila*. (*A*) YFP-HTTex1Q79 and mCherry tagged proteins are co-expressed at similar levels in SH-EP cells. Ratios were determined by comparison of YFP and mCherry fluorescence. YFP/mCherry ratios range from 1.9 to 2.7. **(***B*) Quantification of retinal degeneration in control (*GMR*) and HD transgenic flies. n ≥ 6 flies per genotype. **(***C*) Analysis of protein extracts obtained from transfected SH-EP cells by SDS-PAGE and immunoblotting confirms the expression of the aggregation-prone proteins YFP-ATXN1Q82 and YFP-TARDBP with the size of ~140 and ~75 kDa, respectively. (*D*) Expression control of the mCherry-tagged proteins CRMP1, CRMP1-D408V, DNAJB6 and luciferase, which were co-expressed with the aggregation prone proteins in (*C*). All proteins were expressed in SH-EP cells with their expected sizes.

Figure S5. A schematic display of a cell-free HTTex1Q49 aggregation assay and investigations of CRMP1 oligomers with computational and biochemical methods. (*A*) Schematic representation of a spontaneous HTTex1Q49 aggregation assay. Soluble GST-HTTex1Q49 fusion protein was incubated with PreScission protease (PP) and either His-CRMP1 or His-CRMP1-D408V. Incubation of GST-HTTex1Q49 with PP initiates spontaneous HTTex1Q49 aggregation by removal of the GST tag. The spontaneous formation

of insoluble HTTex1Q49 aggregates was quantified over time by filter retardation assay using the anti-HTT CAG53b antibody. (*B*) CRMP1 homodimer with one subunit represented with wireframe and the other with ribbon (using the structure of murine CRMP1, PMID:14685275). Both subunits adopt the same structure. We focus on the subunit that is in front. CRMP1 contains two structural domains named upper lobe (green) and lower lobe (blue). D408 in CRMP1 possibly establishes a salt bridge with K397 across these lobes, contributing to the stabilization of the structure. The amino acids in the dimerization interphase region are represented by their side chains (red sticks). A higher magnification of the framed area in (B) is shown in (*C*). (*D*) CRMP1-D408V is more stable under increasing urea conditions compared to the wild-type CRMP1. Equal amounts of CRMP1 and CRMP-D408V were incubated with different concentrations of urea and analyzed by blue native PAGE and immunoblotting using an anti-CRMP1 specific antibody. (*E*) The urea treated proteins CRMP1 and CRMP-D408V in (*D*) were also analyzed under denaturing conditions using SDS-PAGE and Serva Blue staining. (*F*) Fluorescence emission wavelengths scan of CRMP1 and CRMP1-D408V. The proteins show a difference in intrinsic protein fluorescence, indicating the formation of different oligomeric structures. 1 mg/ml solutions of CRMP1 and CRMP1-D408V were excited at 295 nm; the emission was recorded from 320 to 425 nm. The data were collected using a M200 plate reader (Tecan, Switzerland).

Supplemental Materials and Methods

Cell culture conditions

COS1 cells: Cells were grown in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA, USA) supplemented with 5% fetal calf serum (FCS), penicillin (5 U/ml) and streptomycin (5 µg/ml).

PC12 cells: Cells were maintained in complete medium with high glucose Dulbecco´s modified Eagle´s medium (DMEM with 10% HS, 5% FCS, 1% penicillin/streptomycin, 200 µg/ml G418, 2 mM glutamine and Zeocin) at 37°C and 5% CO_2. Expression of HTTQ103-EGFP was induced by incubating the cells in media with 5 µM of Ponasterone A (Invitrogen).

SH-SY5Y and SH-EP cells: *Cells* were grown at 37°C and 5% CO_2 in Dulbecco's modified Eagle medium (DMEM) supplemented with 4.5 g/l D-glucose, 10% fetal calf serum, penicillin (100 µg/ml) and streptomycin (100 µg/ml).

HEK293 cells: Cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 4.5 g/l D-glucose, 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 µg/ml), 4 mM L-Alanyl-L-Glutamine (GlutaMAX^{TM}) and 1 mM sodium pyruvate at 37°C and 5% CO_2.

Tissue preparation for SDS-PAGE and immunoblotting

Cell cultures: Total protein lysates from cultured cells were generated with lysis buffer [50mM HEPES pH7.4 with 150 mM NaCl, 2 mM $MgCl_2$, 1 mM EDTA, 0.5% NP40, 25 U/ml Benzonase, and a protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland)]. Protein concentrations were determined using a Bio-Rad protein assay. Protein solutions were adjusted to equal amounts of total protein.

Drosophila: Eye imaginal discs of third instar wandering larvae were prepared and rapidly frozen by immersion in liquid nitrogen. The eye imaginal discs were homogenized in NP-40 buffer (1% NP-40, 25 U/ml Benzonase and protease inhibitors). Total protein concentrations were determined using a Bio-Rad protein assay and lysates were adjusted to a concentration of 10 μg/μl total protein. Equal amounts of total protein extracts were analyzed by SDS-PAGE and Western blotting.

Mice: Whole brains or striatal tissues were homogenized in lysis buffer containing 50 mM Tris-HCl (pH 7.4), 100 mM NaCl, 5 mM $MgCl_2$, 1% NP-40, 10% glycerol, 1 mM EGTA, 25 U/ml Benzonase, and a protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland). Total protein concentrations were determined using a Bio-Rad protein assay; lysates were adjusted to 12 μg/μl of total protein. Equal amounts of protein extracts were analyzed.

SDS-PAGE and immunoblotting

Purified proteins or total cell lysates were denatured, and separated by electrophoresis on 4-12% NuPAGE gels (Invitrogen). Then, proteins were transferred onto Protran BA 83 nitrocellulose membranes (Whatman, Maidstone, Kent, United Kingdom). Membranes were blocked with 5% non-fat dry milk-phosphate-buffered saline (PBS) containing 0.05% Tween-20 and probed with an appropriate primary antibody overnight at 4° C. Secondary antibodies included alkaline phosphatase-conjugated anti-mouse or anti-rabbit IgGs. Finally, membranes were incubated with ChemiGlow (ProteinSimple) and the chemiluminescence signals were detected using a LAS-3000 imaging system (Fujifilm, Tokyo, Japan). For quantification of signals the AIDA software was used.

Detection of insoluble HTT aggregates in HD transgenic mice

For detection of neuronal intranuclear inclusions with insoluble HTT aggregates in HD mice, 12-week-old R6/2 transgenic mice (Mangiarini et al. 1996) were anaesthetized and perfused through the left cardiac ventricle with 4% paraformaldehyde (PFA). Brains were removed, post-fixed in 4% PFA for 4 hours and subsequently cryoprotected in 30% sucrose. Serial sections of 30 μm were cut using a vibratome (Leica Microsystems GmbH, Wetzlar, Germany) and free-floating sections were collected in PBS for staining. Sections were permeabilized with 0.3% Triton X-100 and blocked in PBS supplemented with 2% bovine serum albumin (BSA) and 10% normal donkey serum (NDS). Sections were incubated overnight at 4° C with primary antibodies against HTT (EM48, 1:200) and CRMP1 (anti-CRMP1, 1:400). Alexa 549- or Alexa 488-conjugated antibodies were used as secondary antibodies (1:500, Invitrogen, Carlsbad, CA, USA). A Leica DM 2500 confocal microscope was used to detect fluorescence.

Used *Drosophila* stocks and genotypes

Fly lines for expression of GFP (1522) and CRMP (*CRMP*^{EY04380}, *19693*) were obtained from the Bloomington *Drosophila* Stock Center (Indiana University, Bloomington, IN, USA). *y*^{1}*w*^{118}*; GMRGAL4/CyO; UAS-Hsap\HTT336Q128[M64]* and *Elav-GAL4; UAS-Hsap\HTT336Q128[F27B]* flies were provided by Juan Botas (Fred Hutchinson Cancer Research Center, Seattle, Washington). Transgenic CRMP1 flies were generated by BestGene Inc (Chino Hills, CA, USA). For all experiments, flies were kept at 25°C on standard medium.

The following genotypes were produced und used for different analyses:

Analysis of retina degeneration: GMR-GAL4/+;UAS-Hsap\HTT336Q128/UAS-Hsap\CRMP1 [1-1-10M], GMR-GAL4/+;UAS-Hsap\HTT336Q128/UAS-Hsap\CRMP^{EY04380},

GMR-GAL4/+;UAS-Hsap\HTT336Q128/UAS-Hsap\CRMP-D408V, *GMR-GAL4/+;UAS-Hsap\HTT336Q128/UAS-GFP[1522]*, *GMR-GAL4/+;UAS-Hsap\CRMP1 [1-1-10M]/+ and GMR-GAL4/+* .

Analysis of polyQ-mediated HTT *aggregation:* y^1w^{118}*;GMR-GAL4/+;UAS-Hsap\HTT336Q128[M64]/CRMP1[1-1-10M]* (n=11 larvae), y^1w^{118}*;GMR-GAL4/+;UAS-Hsap\HTT336Q128[M64]/ CRMP*^{EY04380} (n=14 larvae) and y^1w^{118}*;GMR-GAL4/+;UAS-Hsap\HTT336Q128/UAS-GFP[1522]* control larvae (n=9).

Analysis of motor activity: Wild-type flies and HD transgenic flies co-expressing HTT336Q128 and CRMP1 (*Elav-GAL4;UAS-Hsap\HTT336Q128[F27B]/UAS-Hsap\CRMP1[1-1-10M])* or HTT336Q128 and GFP (*Elav-GAL4;UAS-Hsap\HTT336Q128[F27B]/UAS-GFP[1522])* were analyzed.

Supplemental Reference

Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, Lawton M, Trottier Y, Lehrach H, Davies SW et al. 1996. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell* **87**(3): 493-506.