Expanded View Figures

Figure EV1. Controls for Htt fibrilization and the kinetics, point mutations in HSP-1 and HSP-110, and analysis of ATPase rates of HSP-70s and HSP-110.

A TEM image of untagged HttExon1Q_{48} 24 h post-PreSP treatment. Scale bar: 200 nm.

B Sedimentation analysis of the kinetics of HttExon1Q_{48}-CyPet fibrilization. Depicted are the ratios of the supernatant (soluble HttExon1Q_{48}-CyPet) and the pellet (insoluble, aggregated HttExon1Q_{48}-CyPet) at the indicated time points. The graph shows the average of two independent analyses.

C Analysis of the GST-cleavage reaction of GST-HttExon1Q_{48} via SDS–PAGE in the presence (bottom) and absence (top) of HSP-1, DNJ-13, and HSP-110. The time points are indicated on top, and the migration of the full-length and cleaved GST-HttExon1Q_{48} protein is indicated on the right.

D SDS–PAGE and Coomassie staining of all purified proteins used in this study.

E CD analysis of HSP-1 (red) and the point mutants HSP-1_D10S (blue) and HSP-1_K71E (green) and the blank control (black).

F CD analysis of HSP-110 (red) and the point mutants HSP-110_D7S (blue) and HSP-110_N578Y/E581A (green) and the blank control (black).

G Basal ATPase rates of HSP-1 and HSP-110 and its point mutants. The ATPase rate is indicated on the *y*-axis in pmol ATP/μM Hsp/min ($N = 2$).

H ATPase rates of the Hsp70s: HSP-1 (white), F44E5.4 (light gray), C12C8.1 (dark gray), and F11F1.1 (black) alone and in the presence of the DNJ proteins, DNJ-12, DNJ-13, DNJ-19, and DNJ-24 ($N = 2$).

I Sedimentation analysis of disaggregation of HttExon1Q_{48} fibrils by HSP-1, HSP-110, and DNJ-13 in the presence or absence of ATP after 12 h. Depicted are the ratios of the supernatant (soluble HttExon1Q_{48}) and the pellet (insoluble, aggregated HttExon1Q_{48}). The error bars represent the standard deviation of three independent experiments ($N = 3$).

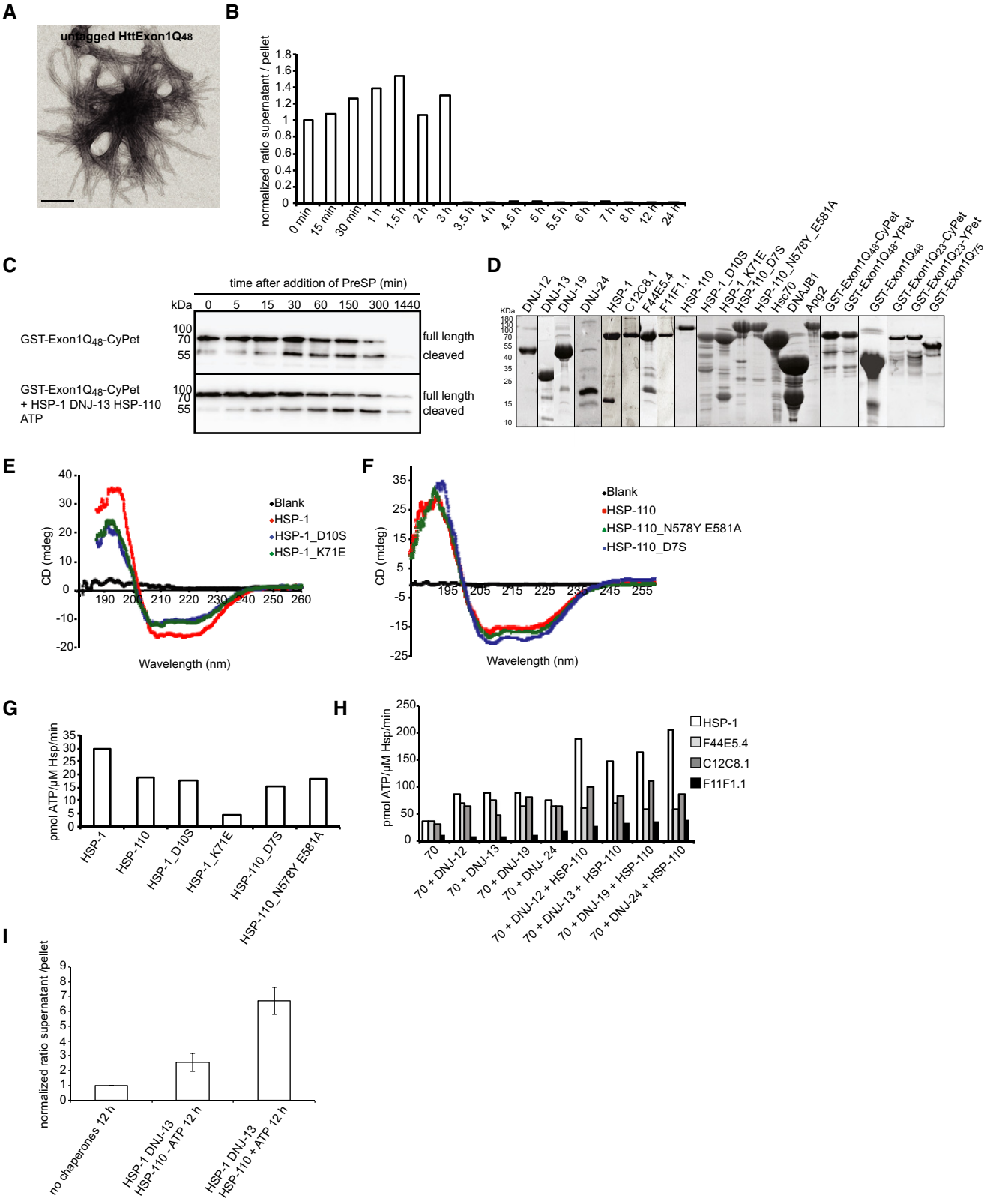
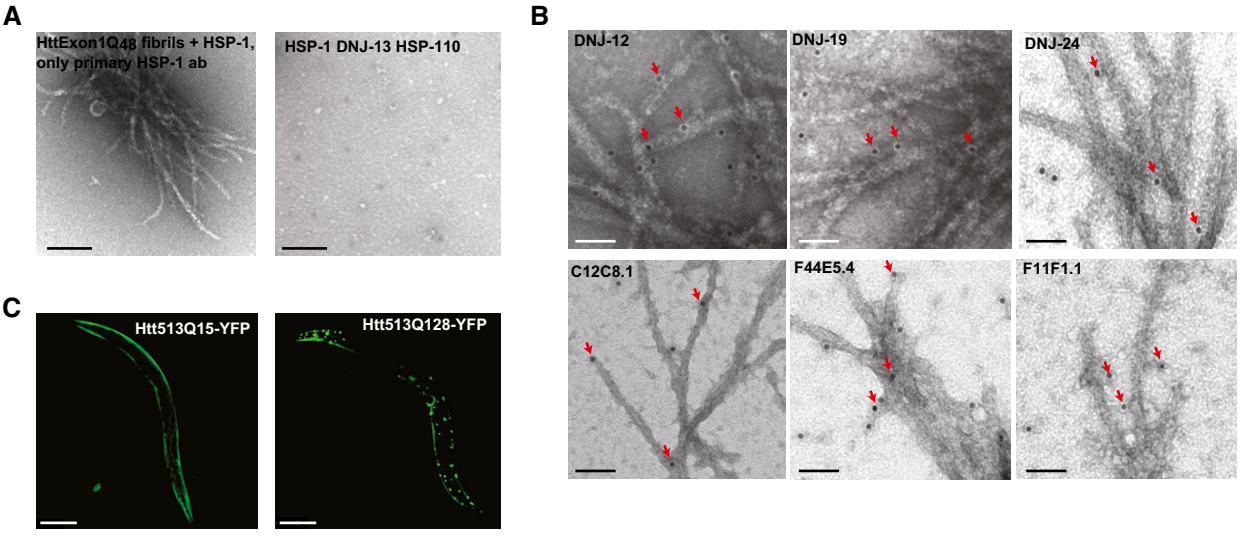


Figure EV1.



D

	Htt513 Q15	Htt513 Q128
HSP-1	231 +/- 85	249 +/- 98
C12C8.1	6 +/- 4	4 +/- 4
F44E5.4	2 +/- 2	3 +/- 3
HSP-110	651 + /- 157	740 +/- 338
DNJ-12	27 +/- 6	27 +/- 4
DNJ-13	47 +/- 16	51 +/- 11
DNJ-19	22 +/- 8	33 +/- 19

all in nmol / g total protein

	young adult (4 days old)	older animals (10 days old)
HSP-1	525 +/- 136	537 +/- 148
C12C8.1	69 +/- 17	18 +/- 6
F44E5.4	1 +/- 1	0
HSP-110	566 +/- 72	705 +/- 78
DNJ-12	45 +/- 13	66 +/- 40
DNJ-13	64 +/- 7	74 +/- 18
DNJ-19	54 +/- 35	48 +/- 33

all in nmol / g total protein

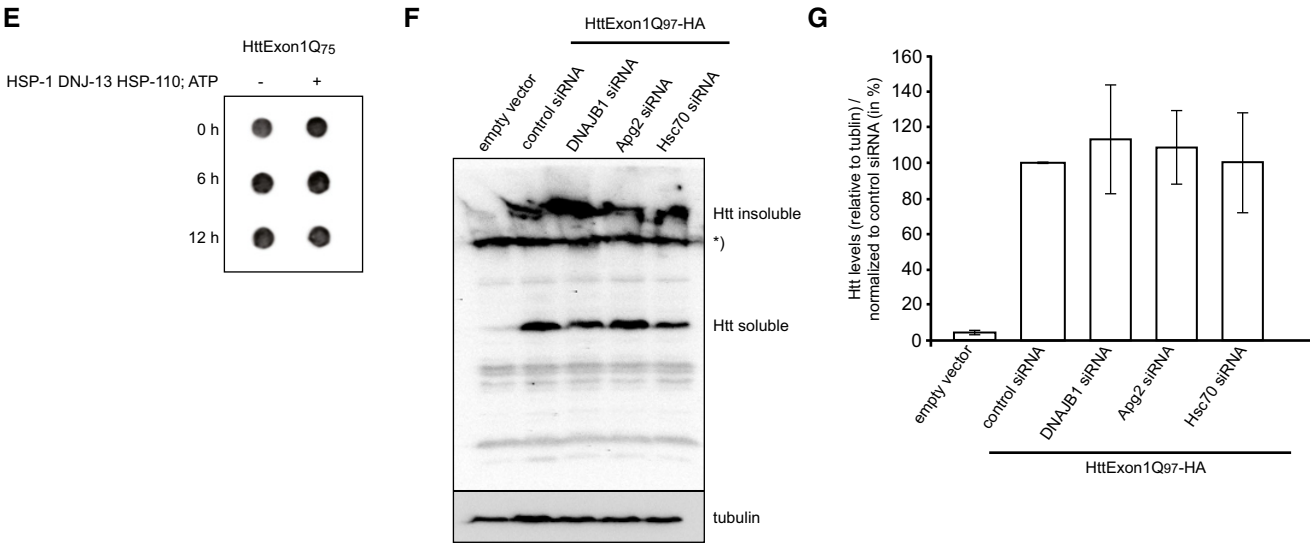


Figure EV2.

Figure EV2. Controls for *in vitro* and *in vivo* immunostaining *Caenorhabditis elegans* models of Htt513Q_{15}/Q_{128}-YFP and quantification of chaperones.

A On the left: TEM analysis of a control of HttExon1Q_{48} fibrils incubated with HSP-1 and only the primary antibody against HSP-1. On the right: TEM analysis of only the chaperones HSP-1, DNJ-13, and HSP-110. Scale bars: 200 nm.

B Immunostaining of chaperones with HttExon1Q_{48} fibrils for DNJ-12, DNJ-19, DNJ-24, C12C8.1, F44E5.4, and F11F1.1. Scale bars: 50 nm. The red arrows mark the positive immunogold labeling.

C Fluorescence images of nematode lines that were used in this study for the analysis of chaperone expression (Fig 5B and C): Htt513Q_{15}-YFP (HttQ_{15}) and Htt513Q_{128}-YFP (HttQ_{128}). Scale bars: 200 μm.

D Tables depict the quantification of the chaperone concentrations in Htt513Q_{15}-YFP vs. Htt513Q_{128}-YFP (top) and wild-type nematodes of 4-day-old (young adults) vs. 10-day-old animals (bottom). Protein levels are depicted as nmol/g total protein. Data show an average of three independent analyses. Error ranges represent the standard deviation.

E Filter retardation analysis of the disaggregation analysis of HttExon1Q_{75} aggregates by HSP-1, DNJ-13, and HSP-110 using an Htt antibody. The time course of the experiment is depicted on the left. No difference in the aggregated state of HttExon1Q_{75} can be observed in the chaperone-containing sample (right lane vs. left lane).

F Western blot analysis of the soluble and insoluble moiety of HttExon1Q_{97} of HEK293T cells upon siRNA-mediated depletion of DNAJB1, Apg2, and Hsc70. The migration of the insoluble and soluble HttExon1Q_{97} protein is indicated on the right. An asterisk indicates a non-specific signal that also appears in the non-transfected HEK393T cells (left lane).

G Quantification of the overall HttExon1Q_{97} levels (soluble + insoluble) of (F). The intensities were normalized to tubulin and represent an average of 3 independent experiments. Error bars represent the standard deviation.