Supplemental Data

Hsp70 and Hsp40 functionally interact with soluble mutant huntingtin oligomers in a classic ATP-dependent reaction cycle

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Figure S1 SEC elution profiles of marker proteins and htt fragments.
(A) The superdex200 10/30 provides steep selectivity curves with excellent resolution of marker proteins in the molecular weight range 13,200–545,000 kDa. (B) Uncleaved GST-HD53Q elutes as an apparent dimer ~130kDa that reacts with MW1, but with the A11 antibody. (C) 3 h after removal of GST, HD53Q resolves as several peaks by SEC, only one of which (~545 kDa) reacts with A11. (D) Coomassie blue stained SDS-PAGE of uncleaved GST-HD20Q and GST-HD53Q. This gel shows that GST-htt fusions proteins migrate at abnormally high apparent molecular masses by SDS-PAGE [GST-HD20Q migrates at ~50kDa (predicted MW: 38.7kDa) and GST-HD53Q migrates at ~62 kDa (predicted MW: 43kDa).

Figure S2 AFM analysis of mutant htt aggregation. Mutant htt oligomers are formed predominately at 3 h and fibrils at 5 h, with formation of globular oligomers in fraction 16 that are recognized by A11. Incubations with a protein concentration of 6 μM were imaged by AFM at different time-points after addition of protease. AFM images demonstrate the aggregation state of HD53Q before SEC analysis (i.e. prior to centrifugation) and in fraction 16 after SEC analysis. Histograms represent the number of aggregates versus height (nm) at 3 h before SEC analysis and in fraction 16 after SEC analysis. These histograms demonstrate that fraction 16 contains a subset of aggregates formed by mutant htt, as the height distribution of aggregates observed after SEC is much tighter.

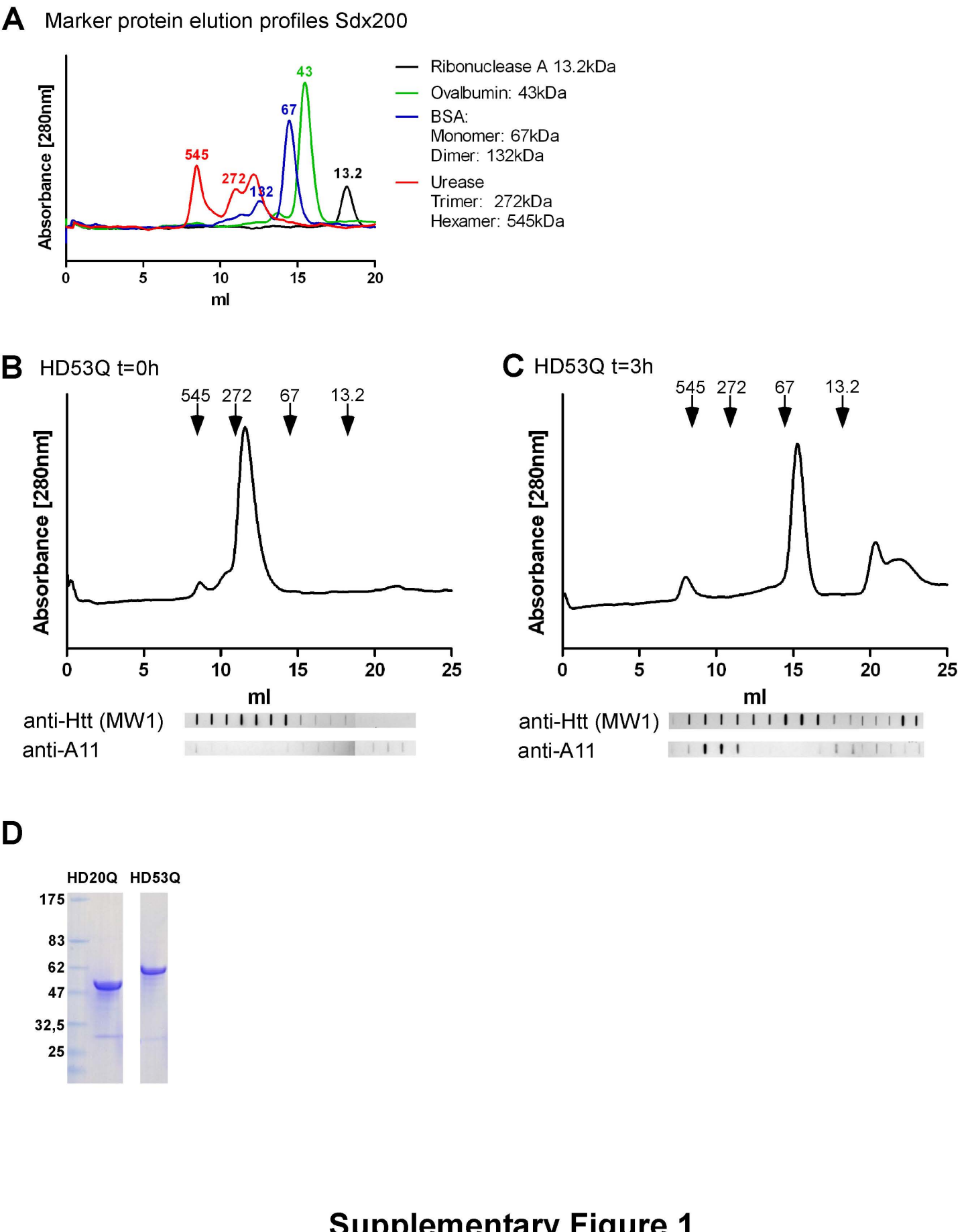
Figure S3 The formation of A11-reactive mutant htt oligomers is strictly dependent on polyQ-length. SEC of soluble supernatants from aggregation reactions of HD20Q, HD35Q, HD46Q, and HD53Q (6 μM) for 0, 1, 3, and 5 h. Fractions were analyzed as described in Fig. 2. Black square box indicates aggregates that reacted with antibody A11. Arrows indicate molecular mass markers (kDa).

Figure S4 Anti-oligomer A11 antibody does not react with active, full-length Hsp70 nor displays chaperone-like activity. (A) Dot-blot assays of HD53Q (2 μg) from a 5 h aggregation (6 μM), full-length Hsc70/Hsp70 purified from bovine liver, the recombinant substrate binding domain (SBD) of Hsp70, and Hsp40 (DNAJB1). Protein blots were analyzed with anti-htt (myc), anti-Hsp70, or anti-oligomer antibody A11. A11 reacted with the substrate binding domain of Hsp70, but not with full-length Hsp70. (B) Elution profiles of purified full-length Hsc70/Hsp70 and Hsp70 substrate binding domain loaded on a Superdex 75 column showing that the substrate binding domain of Hsp70 forms multimers. (C) Hsp70/Hsp40 refolds luciferase but A11 does not. Denatured 100 nM luciferase refolded by Hsp70/Hsp40 (5 μM each) measured at different times. Anti-oligomer antibody A11 (0.1 μg/μl) was added as indicated.

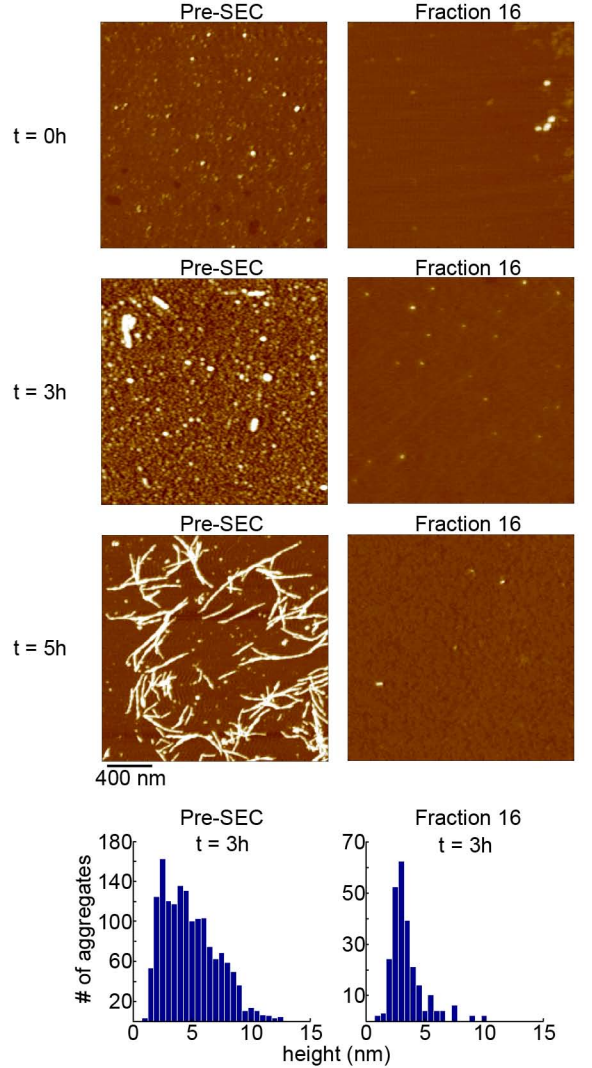
Figure S5 Hsp70 inhibits the aggregation of mutant htt in the presence of Hsp40 (DNAJB1) and ATP. (A) Western blot analysis with mutant htt antibody (MW8) of insoluble HD53Q aggregates (2 μg taken from a 6 μM reaction) after 0, 3, 5, 10 and 24 h of incubation. Molecular chaperones were added as indicated. (B) Supernatants from a 3 h HD53Q aggregation reaction in the absence or presence of Hsp70 and Hsp40 with or without ATP were loaded onto Superdex 200. Fractions 15, 16, and 17 of all runs were loaded in one gel and analyzed by SDS-PAGE and western blot with an Hsp70 antibody. Hsp70 associates with A11-reactive oligomers only in the presence of Hsp40 and ATP. (C) Analysis of intrinsic refolding activity of denatured luciferase (no chaperones in the buffer) in the presence or absence of mutant htt oligomers (6 μM). Intrinsic refolding was normalized relative to Hsp70/Hsp40-dependent

refolding activity at the same time points. Values are mean ± SEM of three independent experiments. ns = not significant (Student's *t*-test). (D) Analysis of native luciferase activity in the absence or presence of mutant htt oligomers (2.5 or 10 uM) or control protein (BSA).

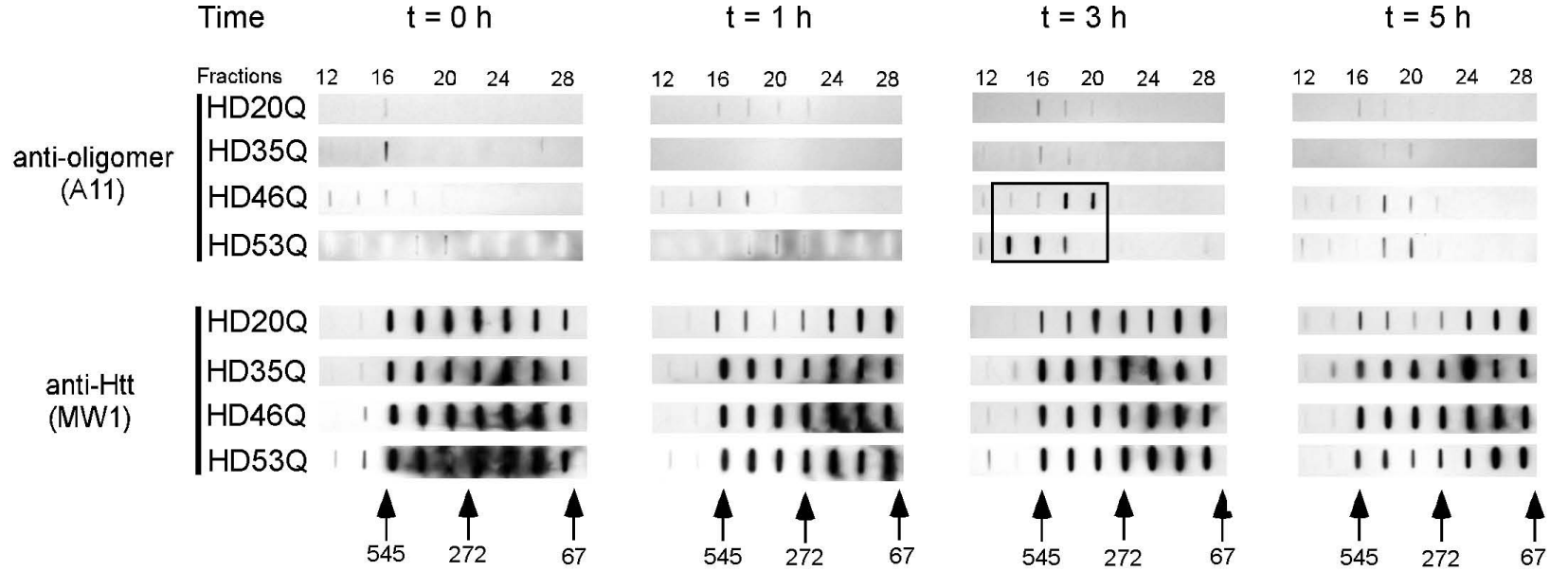
Figure S6 HD103Q-eGFP forms SDS-insoluble aggregates in PC12 cells after 24 h. (A) Western blot analysis with GFP antibody shows that insoluble aggregates (that remain in the wells) began to form at 24 h. (B) SEC fractions probed with anti-htt (GFP) and A11 antibodies to compare mutant htt oligomer detection in cell lysates. The soluble supernatant of lysate (60 μg) from HD103Q-expressing PC12 cells (36 h) was fractionated by SEC on a Superdex 200 column. Fractions were analyzed by slot-blot with GFP or A11 antibodies. Film was over-exposed to illustrate that GFP-positive fractions partially overlap with A11 positive fractions.



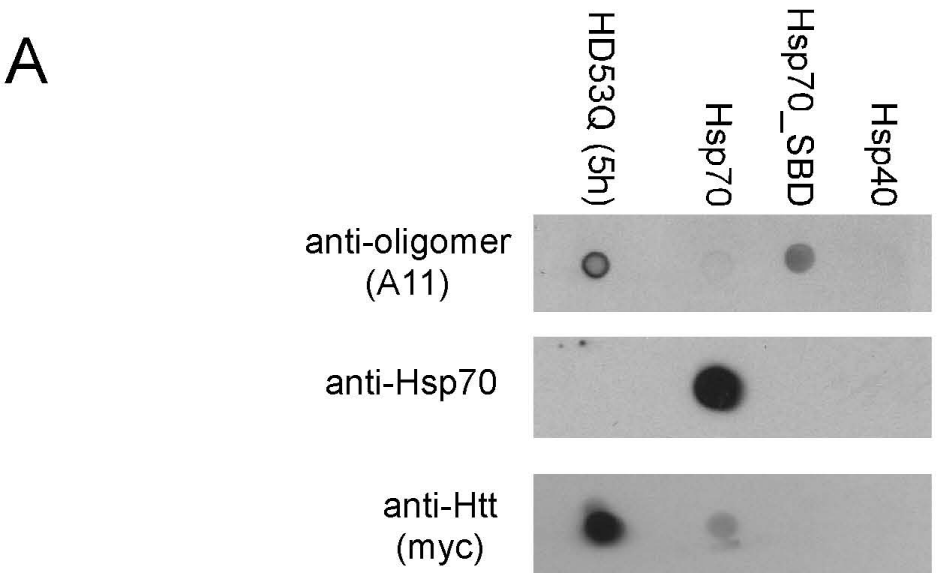
Supplementary Figure 1

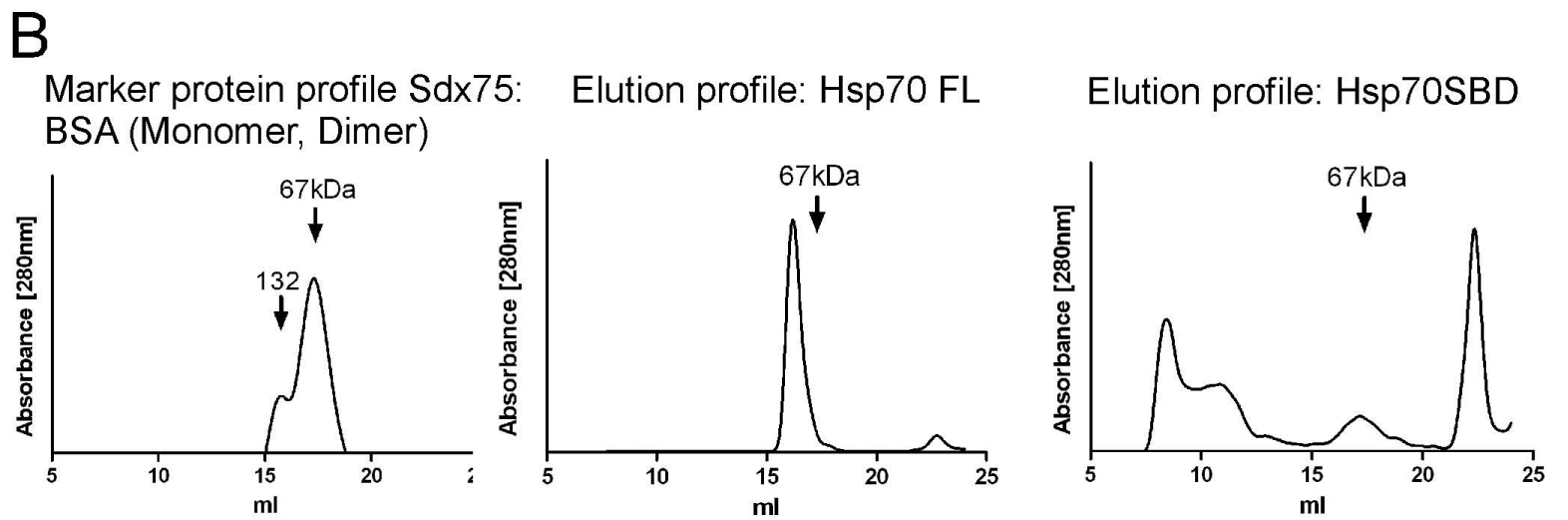


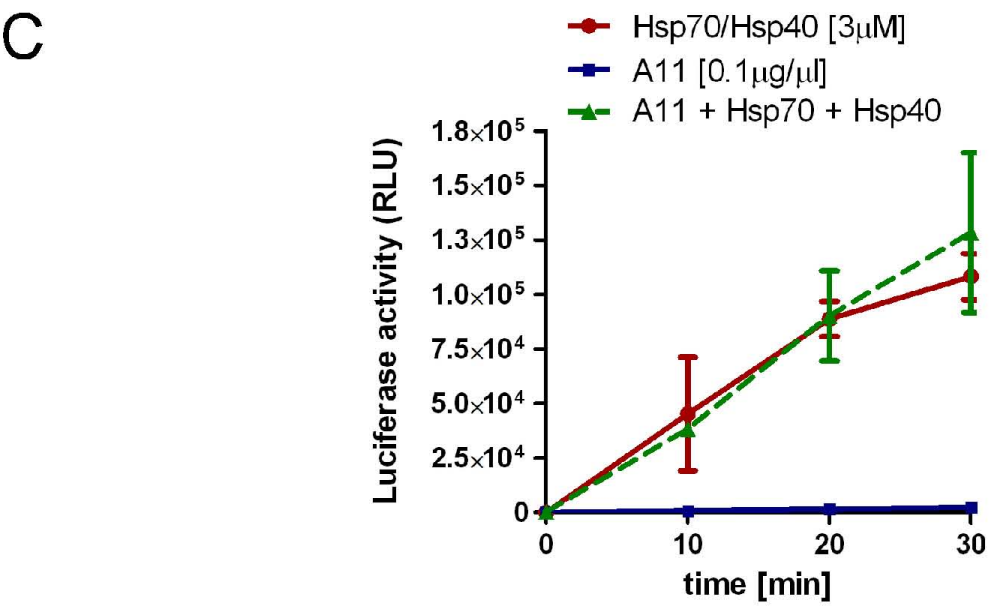
Supplementary Figure 2



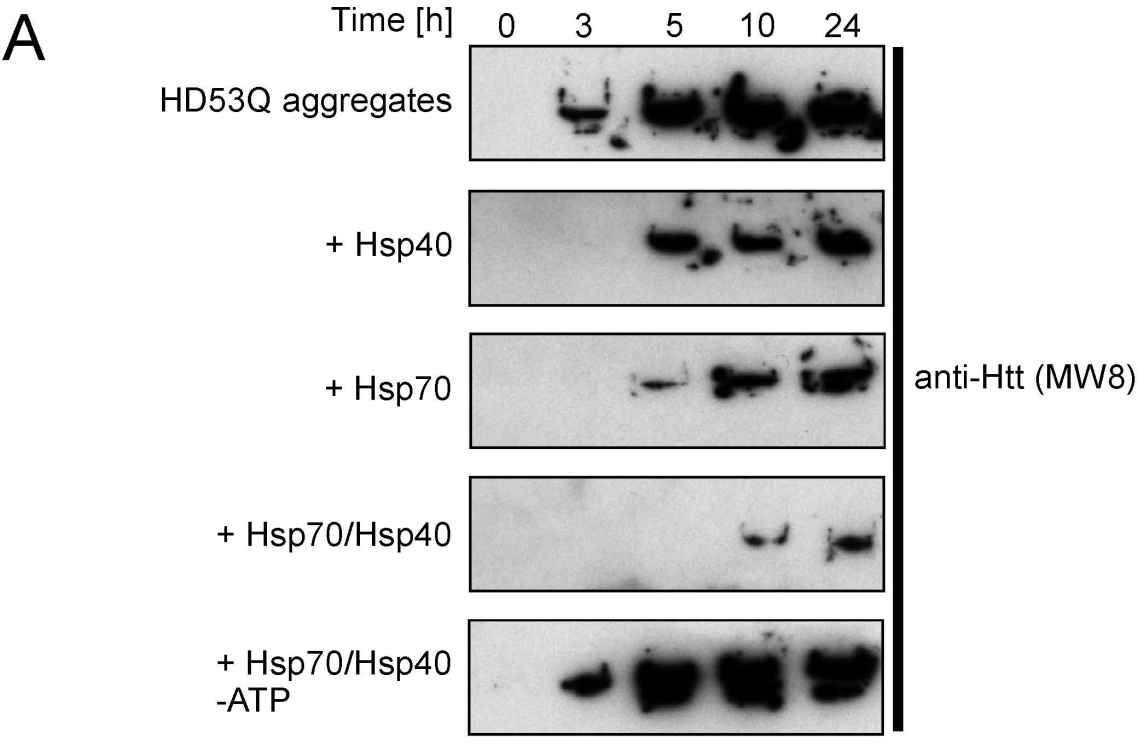
Supplementary Figure 3

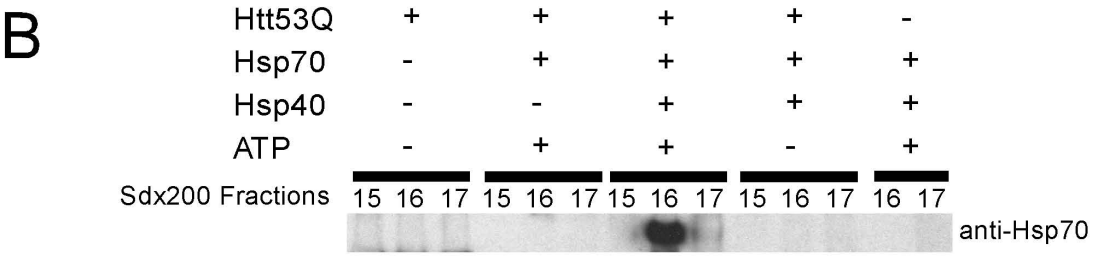


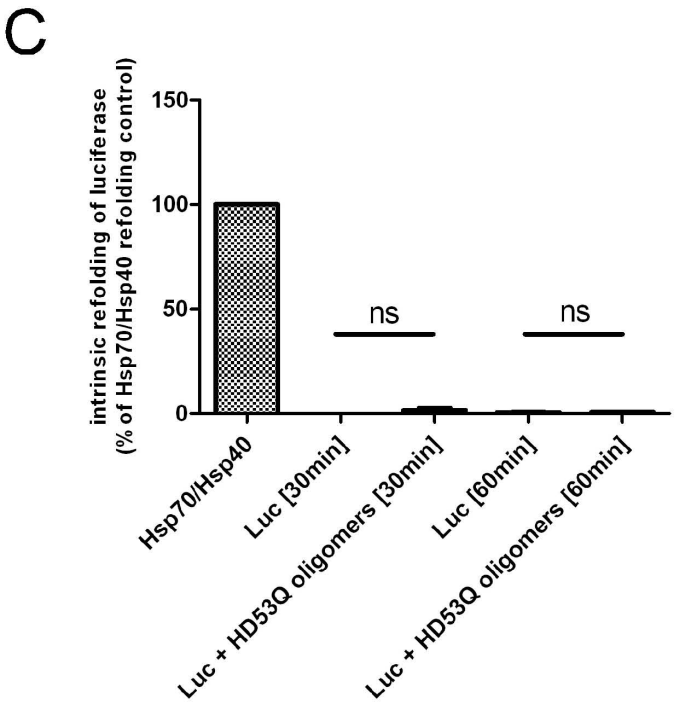


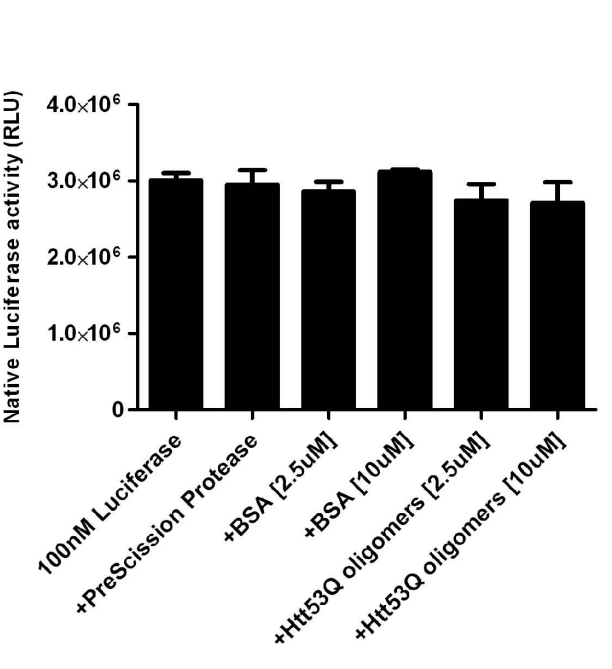


Supplementary Figure 4

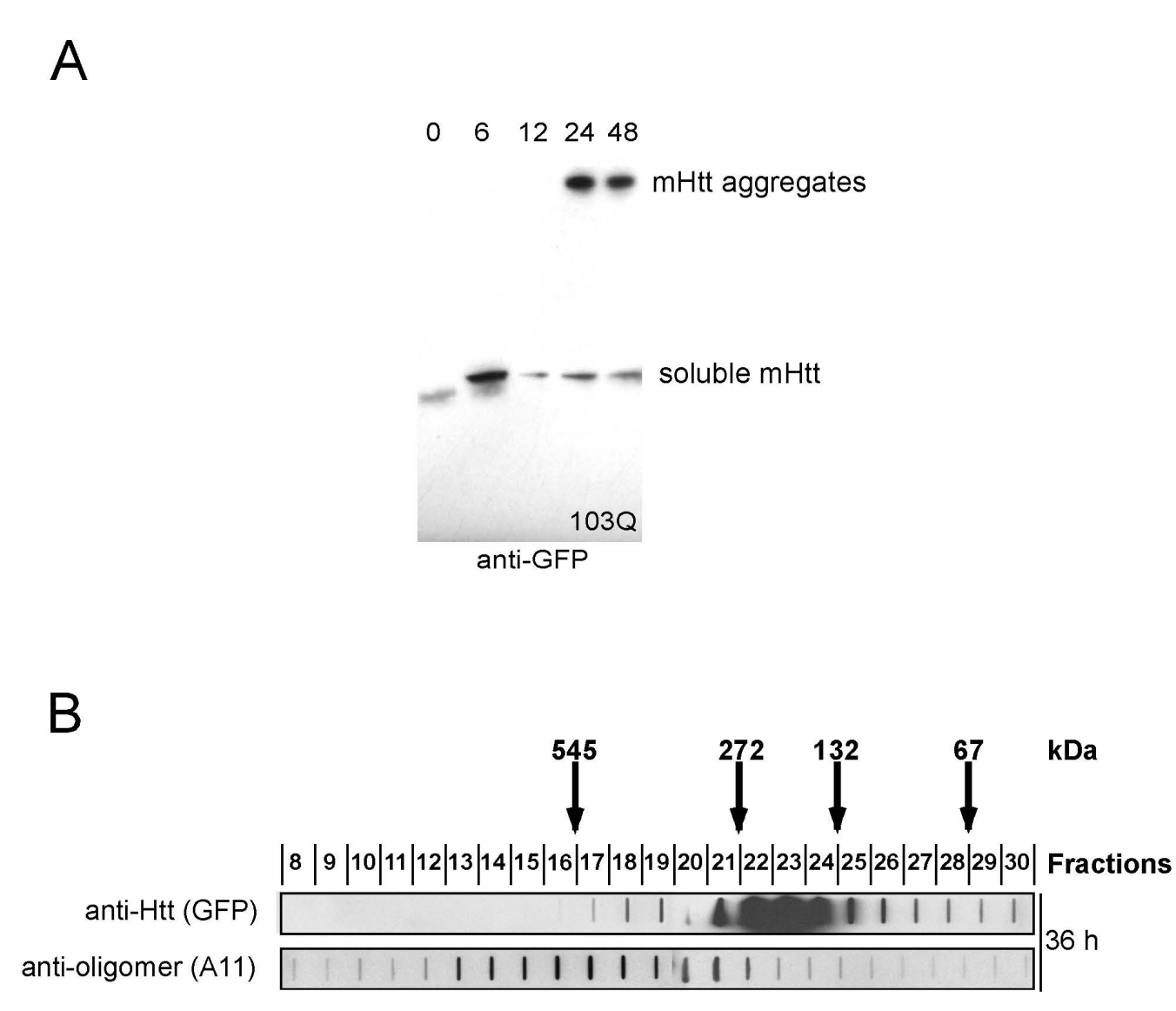








Supplementary Figure 5



Supplementary Figure 6