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Supplemental Data

Chaperonin TRiC Promotes the Assembly of polyQ Expansion Proteins into Nontoxic Oligomers

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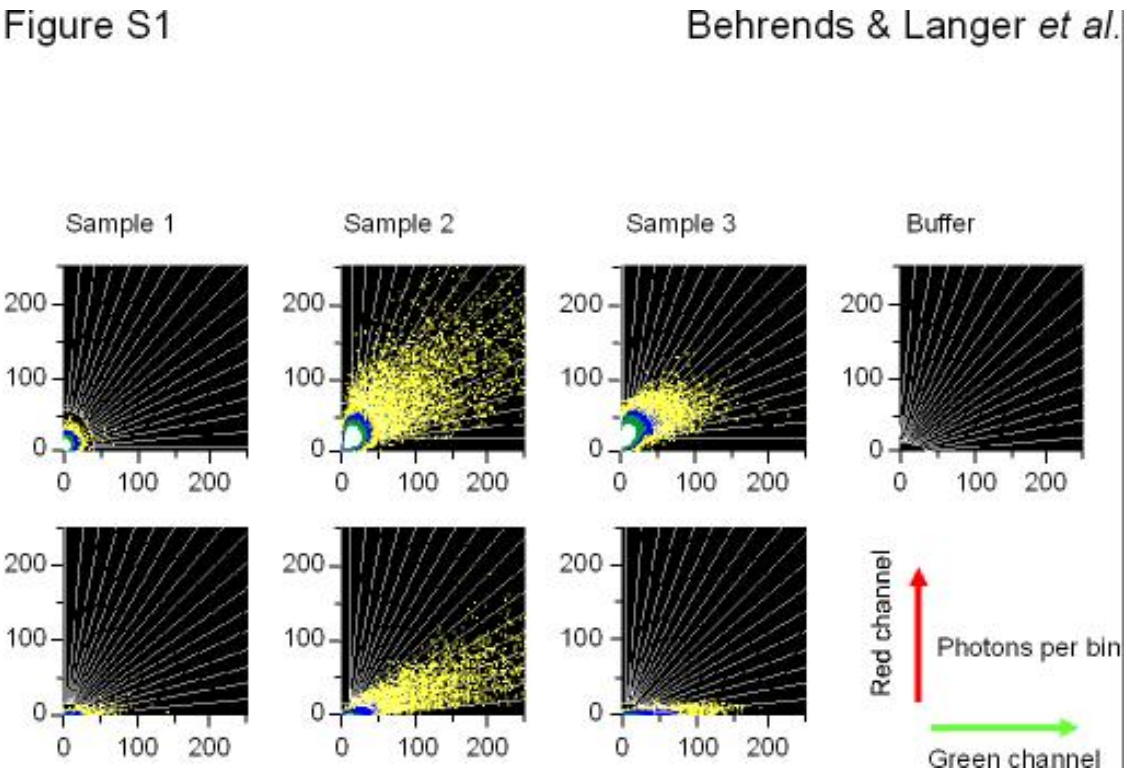

Figure S1. Analysis of Htt Aggregation by Single-Particle FRET

Aggregation of Htt53Q was performed as in Figure 1. In a mixture of Htt labeled with Alexa 488 and Htt labeled with Alexa 633 at a concentration of 25 nM each, virtually no aggregates were detectable upon incubation overnight (sample 1). When incubation was performed at a 20-fold higher concentration followed by 20-fold dilution immediately

before measurement (sample 2), pronounced formation of aggregates with high particle brightness in the green and red channels was seen (upper panels). Notably, these aggregates also exhibited a significant red fluorescence when excited only at 488 nm (lower panels), indicating efficient inter-molecular FRET within the aggregates. Fluorescence distribution intensity analysis of the red fluorescence thus allowed characterization of particle concentration and particle brightness (i.e. size) of these aggregates without any interference from non-aggregated monomers. When aggregation was performed at the same total concentration of Htt53Q but in the additional presence of a 20-fold excess of non-labeled monomers (sample 3), the resulting aggregates showed a lower particle brightness due to the reduced number of fluorophores per aggregate. Moreover, no FRET was detectable under these conditions (lower panels).

Figure S2 Behrends & Langer *et al.*

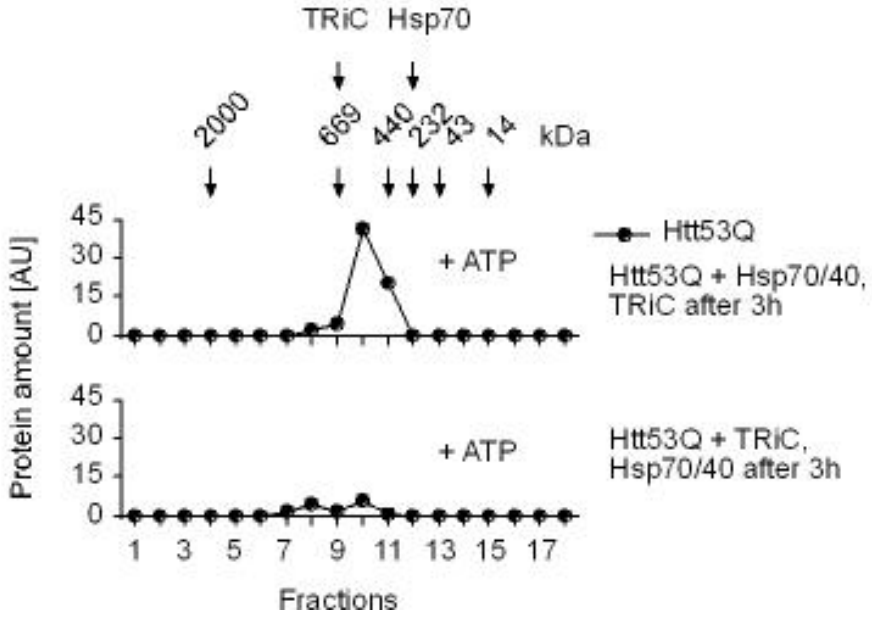

Figure S2. The Hsp70 System Stabilizes Htt53Q for Subsequent Interaction with TRiC

Aggregation reactions of Htt53Q were analyzed by size exclusion chromatography as in Figure 2A, except that Hsp70/Hsp40 and TRiC were added sequentially. Top panel: Htt53Q aggregation was initiated by GST-Htt53Q cleavage in the presence of Hsp70/Hsp40, followed by addition of TRiC after 3 hours and continuation of incubation for another 5 hours. Bottom panel: Aggregation was initiated in the presence of TRiC followed by addition of Hsp70/Hsp40 after 3 hours and continuation of incubation for another 5 hours. Fractions were analyzed by immunoblotting and quantified by densitometry. Amounts of Htt53Q are given in arbitrary units (AU). Peak positions of molecular weight markers and of TRiC and Hsp70 are indicated.

Figure S3 Behrends & Langer *et al.*

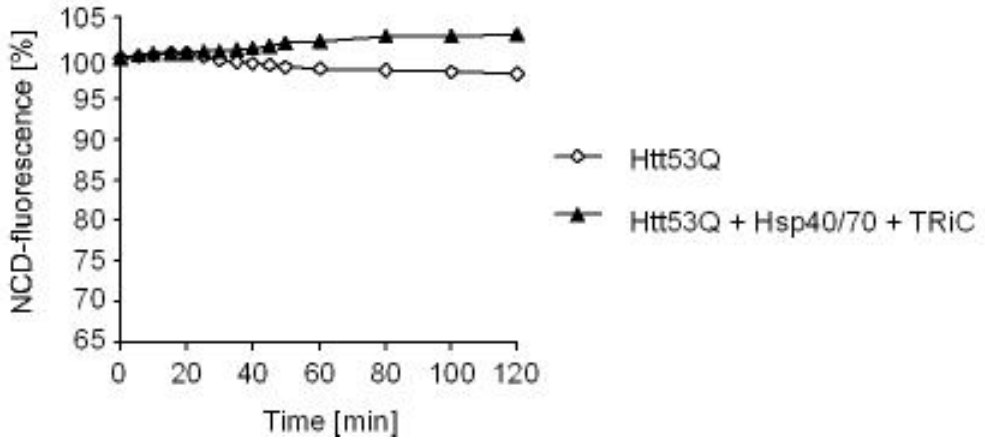

Figure S3. Conformational Analysis of Htt53Q by FRET

This figure presents controls for the FRET experiments described in Figure 2B. It is shown that the donor (NCD) fluorescence of NCD-labeled Htt53Q remains constant upon cleavage of GST-Htt53Q and is not significantly affected by the presence of Hsp70/Hsp40 and TRiC in the aggregation reaction. Thus, the decrease in donor fluorescence observed with NCD-IANBD double-labeled Htt53Q is due to FRET between donor and acceptor fluorophores and indicates a conformational compaction of the Htt53Q molecule. See Schaffar et al. (2004) for a detailed description of this experimental system.

Figure S4 Behrends & Langer *et al.*

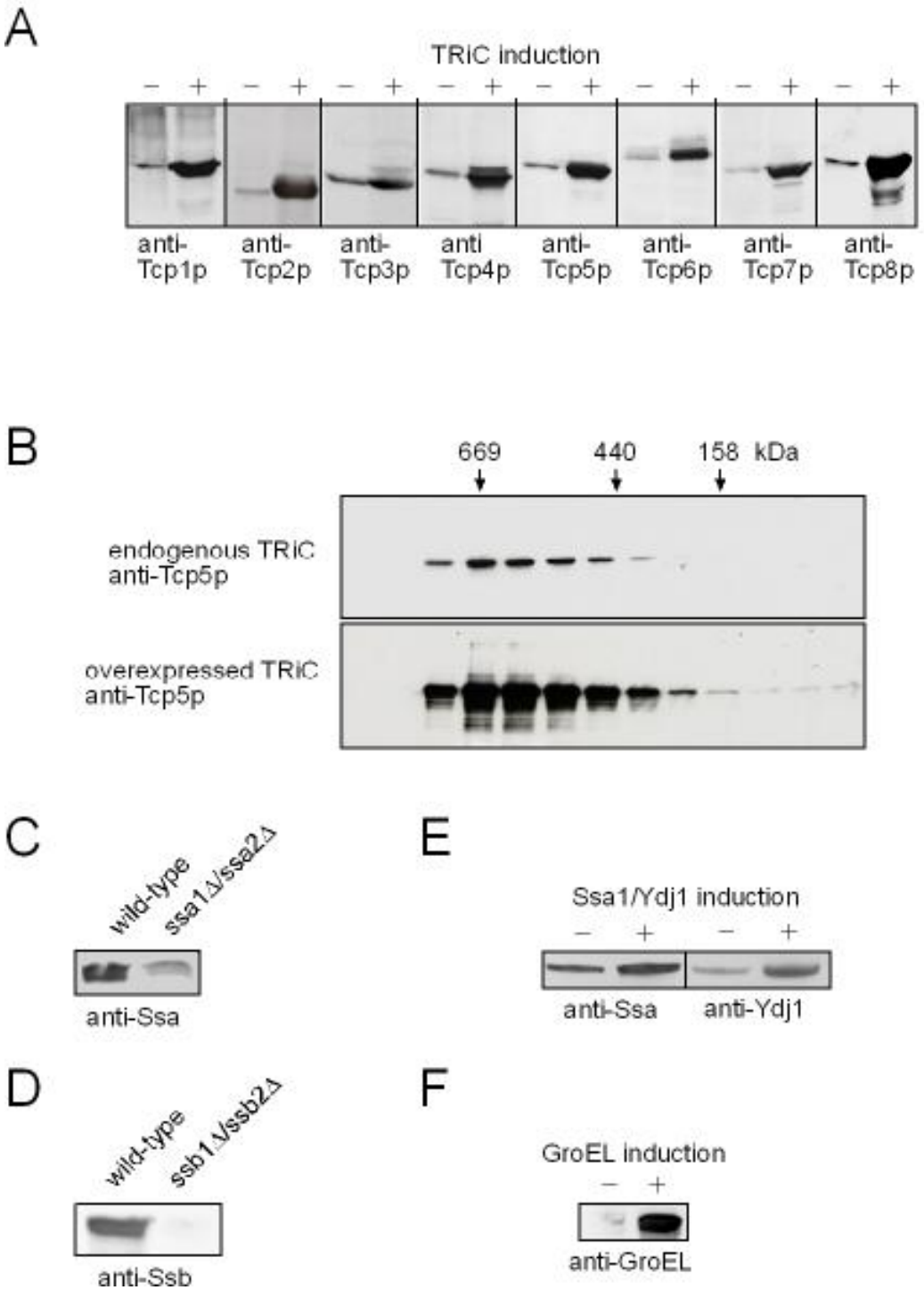

Figure S4. Overexpression and Deletion of Chaperones in *S. cerevisiae*

(A) Overexpression of TRiC. TRiC subunits (Tcp1p-Tcp8p) were simultaneously expressed in wild-type yeast from 4 plasmids under galactose regulated promoters (see Experimental Procedures). Cell lysate was analyzed by SDS-PAGE and immunoblotting with subunit-specific antibodies.

(B) Size exclusion chromatography of lysate from wild-type and from TRiC overexpressing cells. The distribution of endogenous and overexpressed TRiC was analyzed by immunoblotting with anti-Tcp5p antibody. The position of size markers is indicated. Note that, similar to purified bovine TRiC, yeast TRiC fractionates below its nominal mass under the conditions used.

(C) Reduced amount of Ssa proteins in *ssa1Δ/ssa2Δ* yeast compared to wild-type cells. Ssa protein was detected in cell extracts by Western blotting with anti-Ssa antibody.

(D) Absence of Ssb1p and Ssb2p in *ssb1Δ/ssb2Δ* strain compared to wild-type cells. Ssb1p and Ssb2p were detected by Western blotting with anti-Ssb antibody.

(E) Overexpression of Ssa1p and Ydj1p from galactose-inducible promoters detected by immunoblotting in soluble cell extracts with anti-Ssa and anti-Ydj1p antibodies, respectively.

(F) Expression of E. coli GroEL from a galactose-inducible promoter detected by immunoblotting in soluble cell extracts with anti-GroEL antibody.

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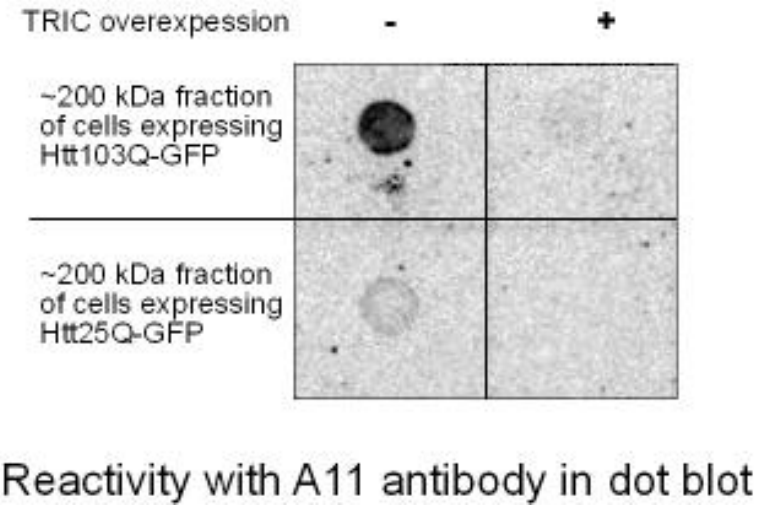

Figure S5. Reactivity with A11 Antibody in 200 kDa Fractions of Cell Extracts Depends on Expression of Htt103Q-GFP

~200 kDa Peak fractions obtained by size exclusion chromatography of extracts from cells expressing Htt103Q-GFP or Htt25Q-GFP with and without overexpression of TRiC were prepared as in Figure 5C and analyzed by dot blot assay for reactivity with A11 antibody.