nature cell biology

Figure S1
Tam et al.

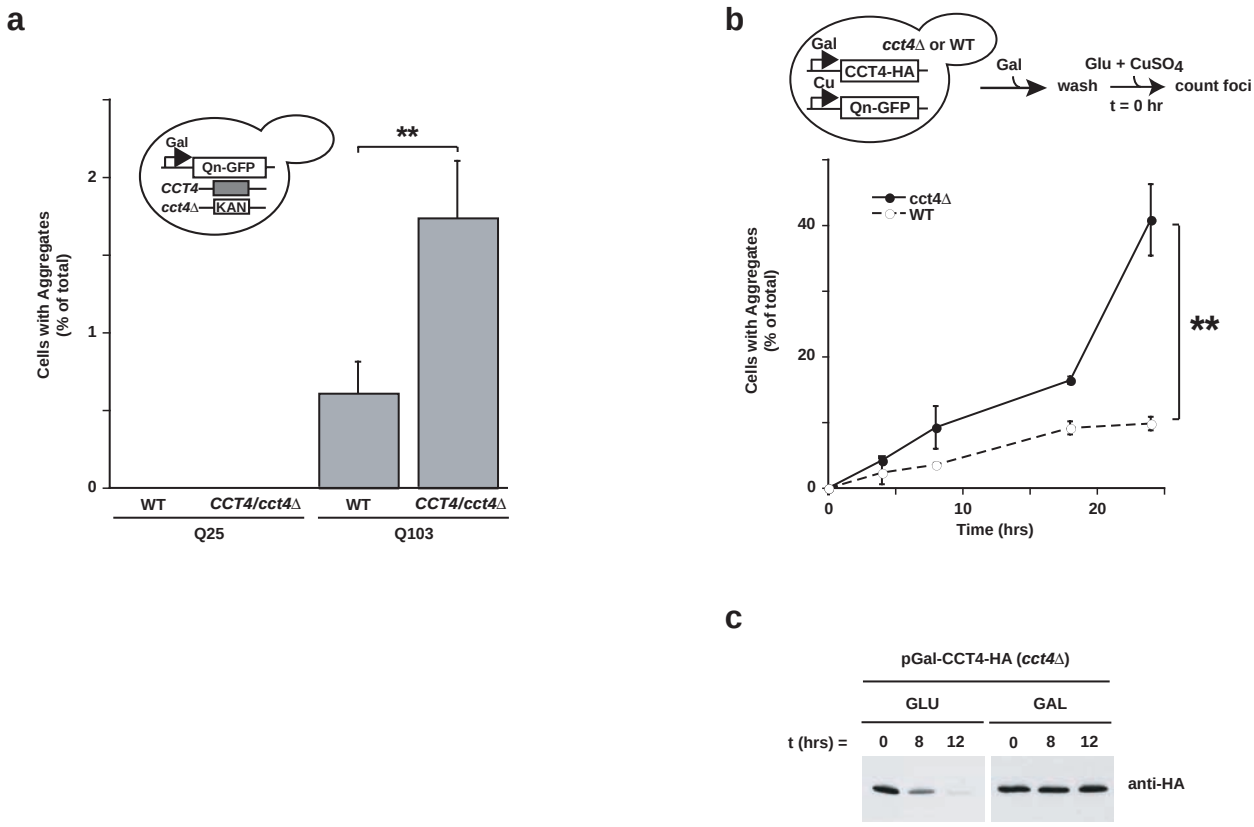

Figure S1 Reduced levels of TRiC increases polyglutamine-expanded huntingtin aggregation in budding yeast. **(a)** TRiC haploinsufficiency enhances aggregation: Qn-GFP (n = 25, 103) was expressed in wild-type (WT) or TRiC haploinsufficient mutant strain *(CCT4/cct4Δ)* yeast as described in methods. Yeast cells were scored for foci by visual inspection of GFP aggregates. Statistical analysis was performed using the one-sided, paired Student's *t*-test: Means + SE of five independent experiments of at least 200 cells each are shown. **(b)** TRiC depletion enhances aggregation: TRiC was depleted by glucose-mediated transcriptional repression of subunit CCT4 expressed in either *cct4Δ* or wild-type (WT) cells. Q103-GFP expression was induced at t = 0 hr with 200 μM $CuSO_4$ for 24 hrs concomitantly with CCT4 repression by growth at 30 ºC in liquid media containing 2% glucose. Yeast cells were scored for foci by visual inspection of GFP aggregates at the indicated times post-chase. Broken line, wild-type; Solid line, *cct4Δ* deletion. Statistical analysis was performed using the one-sided, paired Student's *t*-test: Means +/- SE of three independent experiments with at least 200 cells each are shown (**p < 0.01). **(c)** Depletion of CCT4-HA was assessed by anti-HA immunoblot at 0, 8 and 12 hrs post-chase, grown at 30 ºC in liquid media containing either 2% glucose (non-inducing) or 2% galactose (inducing).

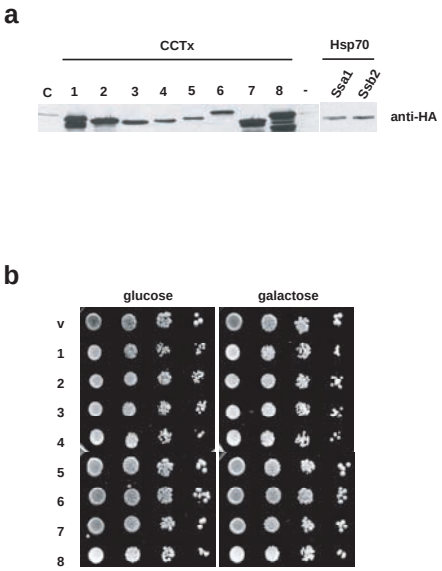

Figure S2 TRiC subunits are soluble upon overexpression and do not adversely affect cell viability. **(a)** HA-tagged TRiC subunits, CCT1 thru CCT8, under galactose control, or Ssa1/Ssb2 under copper control, were overexpressed for 24 hrs in wild-type (WT) yeast cells as described in methods. Lysates were prepared and equivalent total protein amounts were analyzed by anti-HA immunoblot. C: Endogenous CCT1-HA levels, from lysates containing a chromosomally tagged CCT1 subunit (see below). Yeast expressing HA-tagged TRiC subunits were lysed and clarified by centrifugation at 30,000xg. Supernatants were analyzed by immunoblot against the HA-tag. To assess the level of overexpression over endogenous levels, control cells carrying a chromosomally tagged version of CCT1-HA were analyzed in parallel (lane C, contains a ten-fold excess in total lysate protein compared to lanes 1-8). Lane (-): Uninduced control for CCT5-HA cells, no galactose addition. **(b)** Overexpression of individual TRiC subunits does not affect growth. Viability was assessed by 10-fold serial dilutions onto glucose (non-inducing) or galactose (inducing) plates.

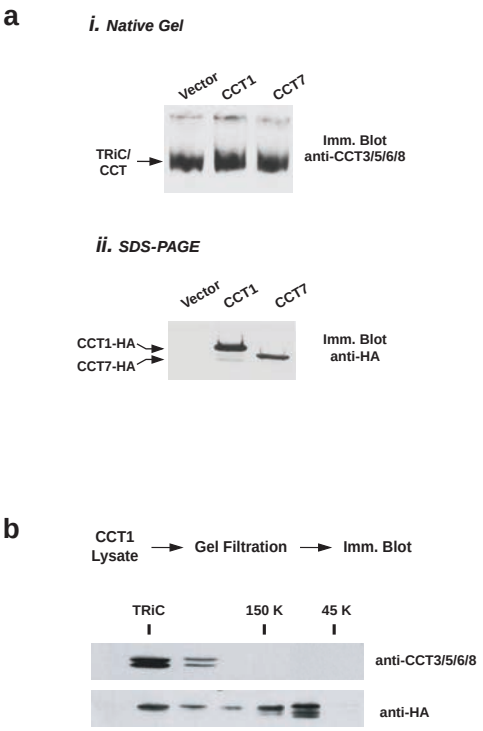

Figure S3 Biochemical analysis of overexpressed chaperonin subunits. **(a)** Overexpression of chaperonin subunits does not affect endogenous levels of the TRiC complex. HA-tagged TRiC subunits, CCT1 and CCT7, under galactose control, were overexpressed for 24 hrs in wild-type (WT) yeast cells as described in methods. ***(i)*** Native lysates were prepared and equivalent total protein amounts were analyzed by native gel. The TRiC complex, migrating with a characteristic mobility, was detected followed by immunoblot with antibodies against the endogenous TRiC subunits (anti-CCT3/5/6/8). A control lysate corresponding to the vector control was also analyzed. ***(ii)*** The same lysates were also analyzed by SDS-PAGE followed by anti-HA immunoblot to detect the overexpressed subunit. **(b)** Gel filtration analysis of lysates from cells overexpressing CCT1. Lysates from cells overexpressing CCT1-HA were fractionated by size exclusion chromatography on a Superose 12 column. Individual fractions were analyzed by SDS-PAGE followed by western blotting with antibodies against the endogenous TRiC complex (anti-CCT3/5/6/8), and the HA-tag. A fraction of the overexpressed CCT1 subunit is incorporated into the assembled TRiC complex while the remainder elutes as a small, probably monomeric species.

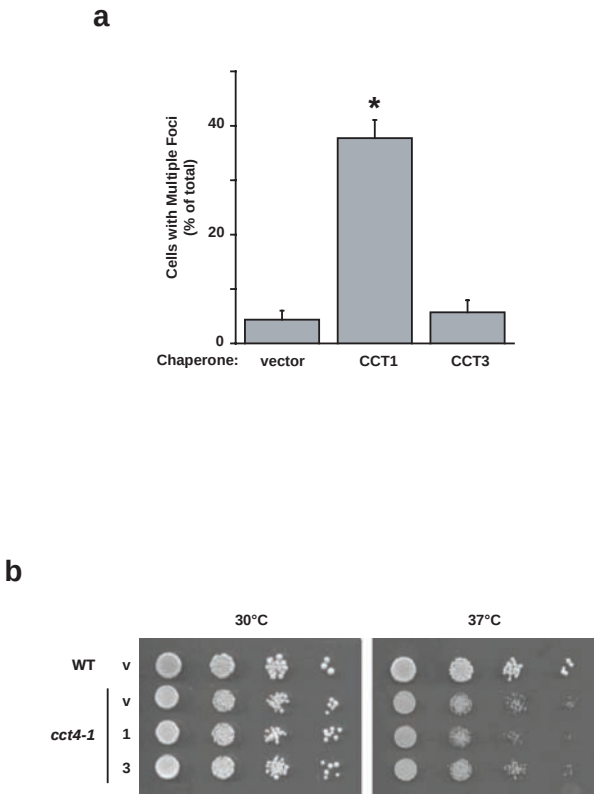

Figure S4 Specific TRiC/CCT subunits modulate polyglutamine-expanded huntingtin aggregate morphology, without TRiC functional complementation. **(a)** Q103-GFP and individual TRiC subunits (CCTx; x = 1, 3) were co-overexpressed in TRiC mutant (*cct4-1*) cells for 24 hrs as described in methods. Q103-GFP aggregate morphology was assessed by visual inspection of GFP aggregates. Statistical analysis was performed using the one-sided, paired Student's *t*-test: Means + SE of three independent experiments counting at least 200 cells each are shown (*$p < 0.05$). **(b)** Overexpression of individual TRiC subunits does not rescue TRiC mutant (*cct4-1*) temperature-sensitivity. Viability was assessed by 10-fold serial dilutions onto galactose (inducing) plates at permissive (30ºC) or non-permissive (37ºC) temperatures.

References and Notes

1. Camasses, A., Bogdanova, A., Shevchenko, A. & Zachariae, W. The CCT chaperonin promotes activation of the anaphase-promoting complex through the generation of functional Cdc20. *Mol Cell* **12**, 87-100 (2003).
2. Deutschbauer, A. M. et al. Mechanisms of haploinsufficiency revealed by genome-wide profiling in yeast. *Genetics* **169**, 1915-25 (2005).

Figure S5
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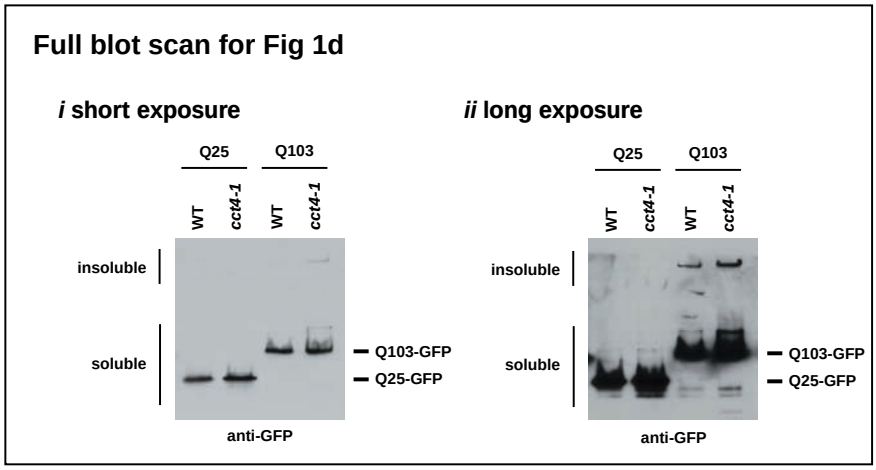

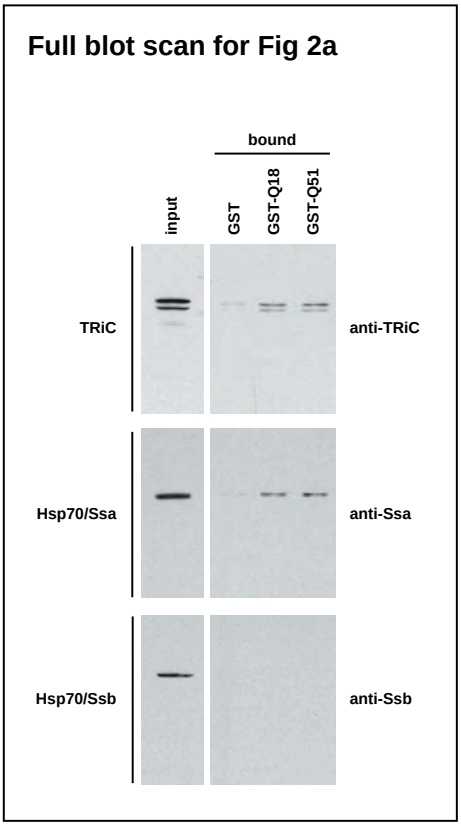

Figure S5 Expanded blots from selected figures.

NCB-F09632 - Supporting Online Material

SUPPORTING TABLE

Table S1 Genotypes of strains used in study.

Yeast Strain	Genotype	Source
W303	*ade2-1 trp1-1 can1-100 leu2-3, 112 his3-11, 15 ura3 CCT4*	[1]
cct4-1	*ade2-1 trp1-1 can1-100 leu2-3, 112 his3-11, 15 ura3 cct4-1*	[1]
RDY 49	*his3Δ1/ his3Δ1 leu2Δ0/ leu2Δ0 lys2Δ0 leu2Δ0 ura3Δ0/ ura3Δ0 CCT4/CCT4*	[2]
RDY 56	*his3Δ1/ his3Δ1 leu2Δ0/ leu2Δ0 lys2Δ0 leu2Δ0 ura3Δ0/ ura3Δ0 CCT4/cct4Δ::KAN*	[2]
CCT4 (WT)	*his3Δ1 leu2Δ0 lys2Δ0 leu2Δ0 ura3Δ0 kanMX4* CCT4-HA-pESC-URA	This study
CCT4(*cct4Δ*)	*his3Δ1 leu2Δ0 lys2Δ0 leu2Δ0 ura3Δ0 cct4Δ:kanMX4* CCT4-HA-pESC-URA	This study