

Supplemental Materials

Molecular Biology of the Cell

Wolfe et al.

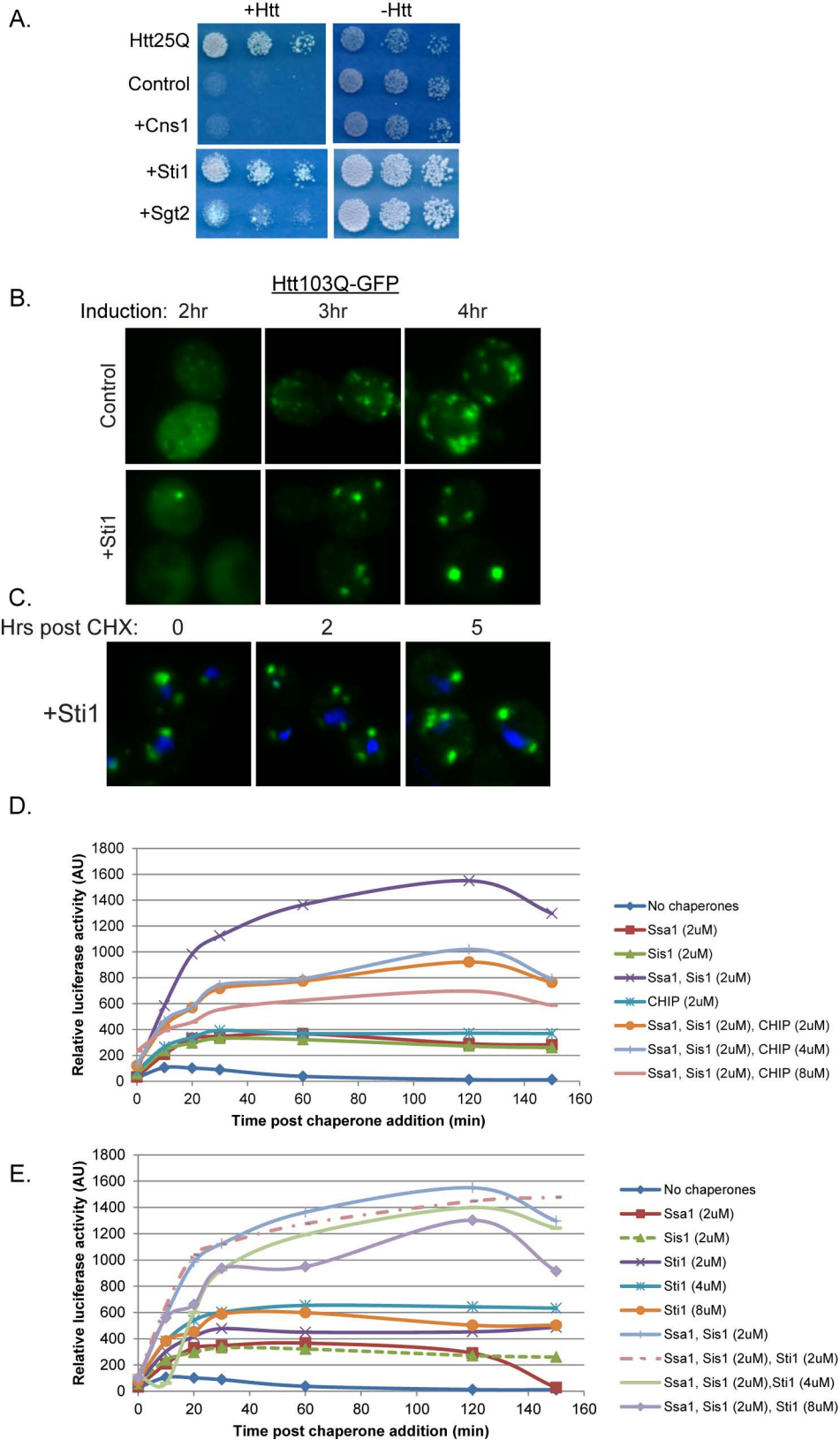
Supplemental Legends

Supplemental Figure 1. Sti1 specifically alters Htt103Q-GFP spatial and temporal foci formation. (A) Sgt2 does not suppress the Htt103Q growth defect as monitored by cell growth assay plated in fivefold dilutions. The toxicity assay was plated on synthetic media containing 500uM CuSO₄ to induce Sgt2 expression. (B) Impact of Sti1 upon Htt103Q-GFP foci formation over time. Live images were captured at the indicated times after galactose induction of Htt103Q-GFP. (C) Stability of Htt103Q-GFP foci during Sti1 overexpression as monitored by cycloheximide decay. After 4 h of Htt103Q-GFP induction, cells were treated with cycloheximide, then fixed and imaged at the indicated times. Nuclei were visualized using DAPI staining. (D and E) Influence of (D) CHIP or (E) Sti1 upon Ssa1 and Sis1 chaperone activity as measured by in vitro luciferase activity assay. Chaperones were added at the indicated concentrations to GndHCl treated luciferase and luminescence was measured as a readout for chaperone mediated refolding.

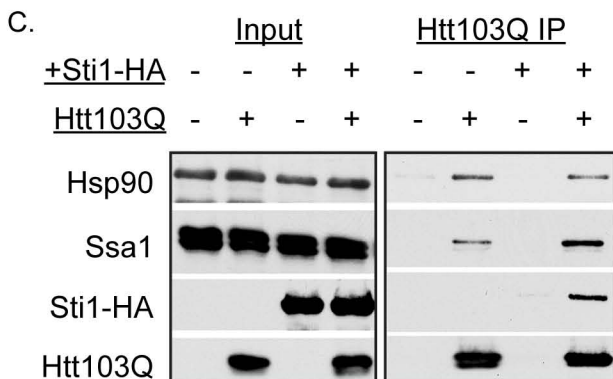
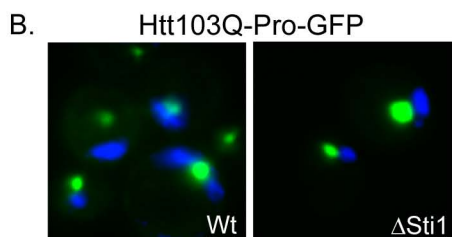
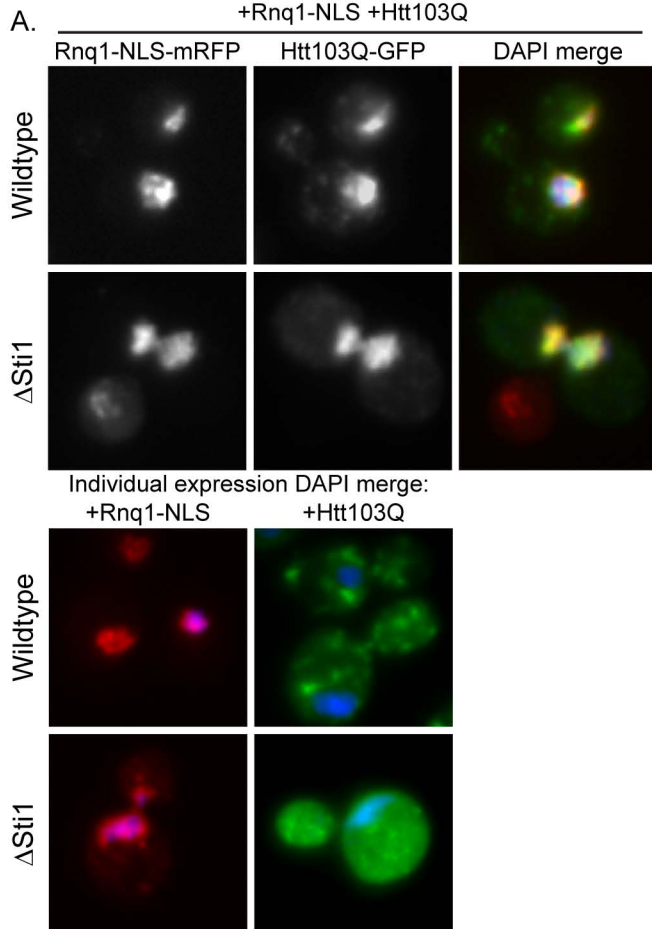
Supplemental Figure 2. Sti1 is not required for Rnq1-NLS relocalization of Htt103Q to the nucleus. (A) Lack of Sti1 dependence for Rnq1-mRFP-NLS to target Htt103Q-GFP to the nucleus. Rnq1 was tagged with the SV40 NLS signal and expressed at low levels from the CUP1 promoter as in other Rnq1-mRFP localization studies. Samples expressed either Rnq1-mRFP-NLS, Htt103Q-GFP, or both as indicated. (B) Impact of Sti1 deletion upon foci formation of Htt103Q-GFP containing a proline rich region. Nuclei were visualized using DAPI staining. (C) Sti1 immunoprecipitates with Hsp70 and Hsp90. Analysis of Sti1 and indicated chaperones in complex with Htt103Q in the presence and absence of Htt103Q. Immunoprecipitation was carried out as in Figure 6. Sti1 with a C terminal HA tag was overexpressed from a high copy plasmid under control of its endogenous promoter.

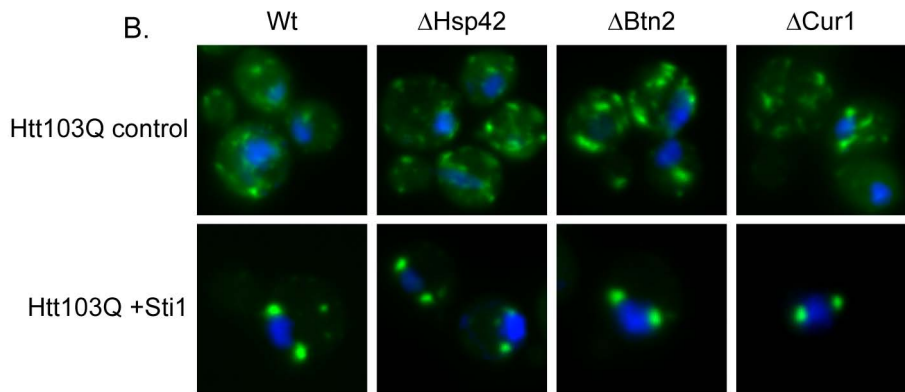
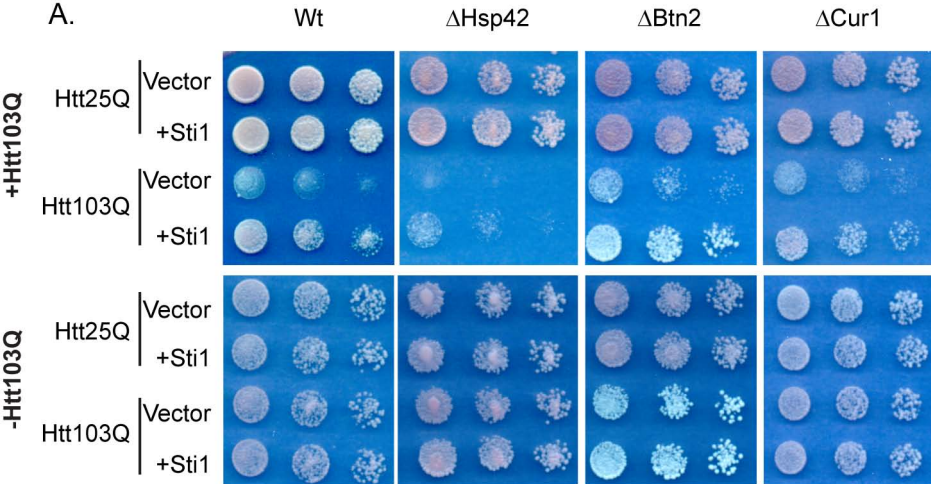
Supplemental Figure 3. Other cellular sorting factors are not necessary for Sti1's mechanism of action (A) Impact of deletion of indicated proteins upon Sti1's ability to suppress Htt103Q growth defect as monitored by cell growth assay plated in fivefold dilutions. (B) Impact of deletion of indicated proteins upon Sti1's ability to reorganize Htt103Q-GFP. Toxicity and fluorescence microscopy assays were carried out as in Figure 1.

Supplemental Figure 4. Sti1 attenuates Hsp104 shearing activity in the presence of GndHCl (A) Curing rate of [RNQ+] is increased in the presence of GndHCl when Sti1 is overexpressed as indicated by triton solubility assay followed by Western blot. Since [RNQ+] is triton insoluble, we measured the Rnq1 triton solubility from lysates with endogenous and overexpressed Sti1 at the indicated generations post GndHCl treatment. Increased Sti1 does not completely inactivate Hsp104 and cure the cells of [RNQ+] as indicated in the left panel without GndHCl treatment. (B) Quantitation of curing rate in (A) as measured by ratio of soluble to insoluble Rnq1. (C) Sti1 levels increase during heat shock. Protein levels of Sti1 were examined by Western blot analysis during a shift from 25°C to 37°C for 1hr compared to overexpression of Sti1 from a high copy plasmid.

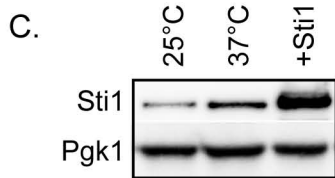
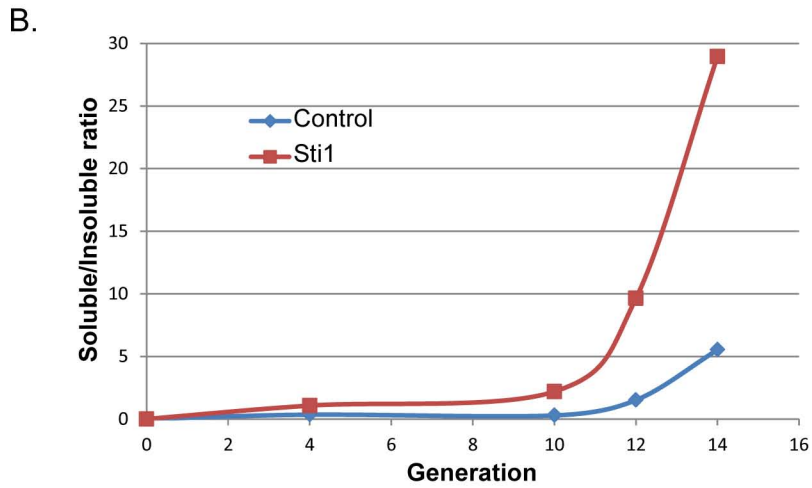
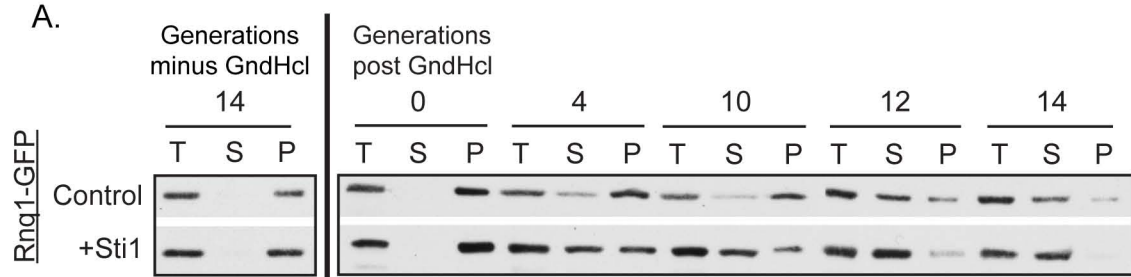


Supplemental Figure 1





Supplemental Figure 3



Supplemental Table 1. Yeast Strains

Strain	Genotype	Source
W303, MAT α	leu2-3,112; trp1-1; can1-100; ura3-1; ade2-1; his3-11,15	Open Biosystems
Htt25Q, MAT α	leu2-3,112; trp1-1; can1-100; ura3-1; ade2-1; his3-11,15; LEU2::Htt25Q	This study
Htt103Q, MAT α	leu2-3,112; trp1-1; can1-100; ura3-1; ade2-1; his3-11,15; LEU2::Htt103Q	This study
W303 $\Delta sti1$, MAT α	leu2-3,112; trp1-1; can1-100; ura3-1; ade2-1; his3-11,15; sti1 Δ ::KanMX4	This study
Htt25Q $\Delta sti1$, MAT α	leu2-3,112; trp1-1; can1-100; ura3-1; ade2-1; his3-11,15; sti1 Δ ::KanMX4; LEU2::Htt25Q	This study
Htt103Q $\Delta sti1$, MAT α	leu2-3,112; trp1-1; can1-100; ura3-1; ade2-1; his3-11,15; sti1 Δ ::KanMX4; LEU2::Htt103Q	This study
Htt25Q $\Delta hsp42$, MAT α	leu2-3,112; trp1-1; can1-100; ura3-1; ade2-1; his3-11,15; hsp42 Δ ::KanMX4; LEU2::Htt25Q	This study
Htt103Q $\Delta hsp42$, MAT α	leu2-3,112; trp1-1; can1-100; ura3-1; ade2-1; his3-11,15; hsp42 Δ ::KanMX4; LEU2::Htt103Q	This study
Htt25Q $\Delta btn2$, MAT α	leu2-3,112; trp1-1; can1-100; ura3-1; ade2-1; his3-11,15; btn2 Δ ::KanMX4; LEU2::Htt25Q	This study
Htt103Q $\Delta btn2$, MAT α	leu2-3,112; trp1-1; can1-100; ura3-1; ade2-1; his3-11,15; btn2 Δ ::KanMX4; LEU2::Htt103Q	This study
Htt25Q $\Delta cur1$, MAT α	leu2-3,112; trp1-1; can1-100; ura3-1; ade2-1; his3-11,15; cur1 Δ ::KanMX4; LEU2::Htt25Q	This study
Htt103Q $\Delta cur1$, MAT α	leu2-3,112; trp1-1; can1-100; ura3-1; ade2-1; his3-11,15; cur1 Δ ::KanMX4; LEU2::Htt103Q	This study
Sis1GFP	BY4741 MAT a; his3 Δ ; leu2 Δ ; met15 Δ ; ura3 Δ ; SIS1-GFP::HIS3	Invitrogen
Hsp42GFP	BY4741 MAT a; his3 Δ ; leu2 Δ ; met15 Δ ; ura3 Δ ; Hsp42-GFP::HIS3	Invitrogen
Spc42GFP	BY4741 MAT a; his3 Δ ; leu2 Δ ; met15 Δ ; ura3 Δ ; Spc42-GFP::HIS3	Invitrogen

Supplemental Table 2. Plasmids

Plasmid	Source
pRS305-GAL1-FLAG-Htt25Q-GFP	This study
pRS305-GAL1-FLAG-Htt103Q-GFP	This study
pRS426-CUP1-FLAG-Htt103Q-GFP	This study
pRS423-CUP1-Rnq1-mRFP	This study
pRS315-CUP1-Rnq1-mRFP	Douglas et al, 2009
pRS315-CUP1-Rnq1-GFP	Douglas et al, 2008
pRS316-CUP1-Rnq1-mRFP-NLS	Douglas et al, 2009
yEPlac-Sti1 (endogenous promoter)	Vitaly Kushnirov
yEPlac-Sti1 point mutations	This study
yEPlac-Sti1-mRFP	This study
pRS426-ACT1-mCitrine-Luciferase	This study
pRS316-CUP1-Sgt2	This study
Yep24-Cns1	Zuehlke et al, 2012
pRS414-GPD-Sis1	Douglas et al, 2008
Addgene plasmid 15583:	
pRS416-GAL1-Htt103Q-GFP	Susan Lindquist

Supplemental Table 3. Antibodies

Antibody	Host	Source	Catalogue number
GFP	Mouse	Roche	11814460001
Flag	Mouse	Sigma	F3165
RFP	Rabbit	Rockland	600-401-379
Sti1	Rabbit	This study	
Pgk1	Mouse	Molecular Probes	A-6457
Hsp104	Rabbit	Stressgen	SPA-1040
Ssa1	Rabbit	Lee et al, 2002	
Sis1	Rabbit	Lee et al, 2002	
Ydj1	Rabbit	Lee et al, 2002	
Hsp42	Rabbit	Johanes Buchner	
anti-mouse HRP	Goat	Thermo	31436
anti-rabbit HRP	Goat	Thermo	31461
