Winkler et al., http://www.jcb.org/cgi/content/full/jcb.201201074/DC1

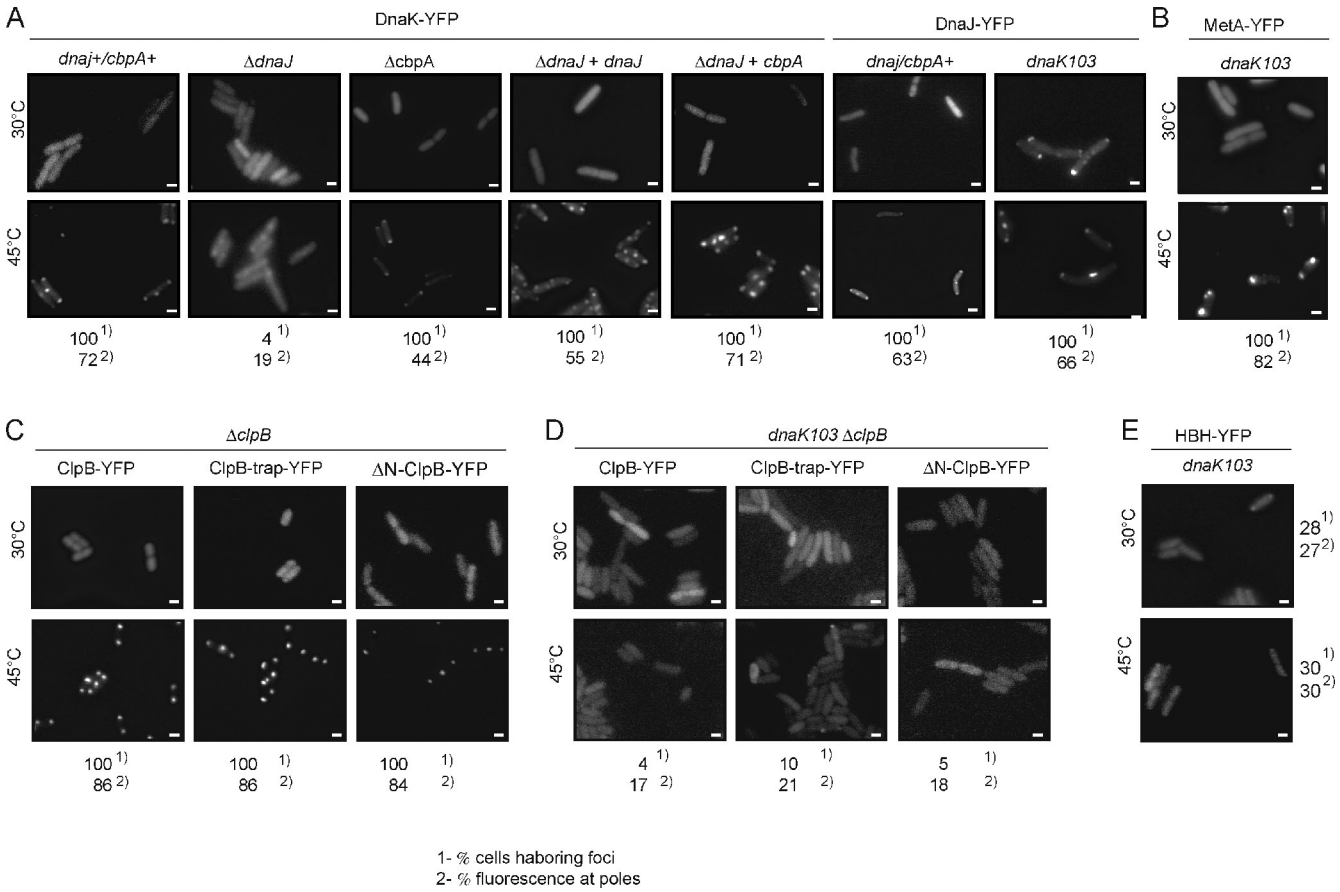

Figure S1. **Hierarchic association of disaggregating chaperones with protein aggregates in *E. coli* cells.** (A) J proteins target DnaK to protein aggregates. *E. coli* wild type (*dnaJ+/cbpA+*), *dnaK103*, *ΔdnaJ*, and *ΔcbpA* mutant cells expressing DnaK-YFP or DnaJ-YFP fusions were grown at 30°C to mid-log phase and shifted to 45°C for 20 min. The occurrence of stress-induced polar foci indicated binding to protein aggregates. Polar localization of DnaK-YFP in *ΔdnaJ* cells was restored by in trans expression of either *dnaJ* or *cbpA*. (B) Thermolabile proteins aggregated in *E. coli dnak103* cells. Formation of stress-induced protein aggregates in *E. coli dnaK103* cells. *E. coli dnaK103* cells expressing the thermolabile model protein MetA-YFP were grown to mid-exponential log phase at 30°C and shifted to 45°C for 20 min. (C) ClpB-trap and ΔN-ClpB bind to stress-induced protein aggregates in a DnaK-dependent manner. *E. coli* MC4100 *ΔclpB* cells expressing ClpB-YFP, ClpB-trap-YFP, or ΔN-ClpB-YFP were grown to mid-exponential log phase at 30°C and shifted to 45°C for 20 min. The occurrence of heat-induced foci indicates binding to protein aggregates. The fraction of cells harboring ClpB-YFP foci was determined (n = 200). The degree of aggregate binding was calculated by determining the fluorescence intensity of ClpB-YFP foci (n = 20). (D) DnaK-dependent interaction of ClpB-trap-YFP and ΔN-ClpB-YFP with protein aggregates. *E. coli dnaK103* cells expressing ClpB-YFP, ClpB-trap-YFP, or ΔN-ClpB-YFP were grown to mid-exponential log phase at 30°C and shifted to 45°C for 20 min. Quantification of stress-induced ClpB-YFP foci formation was performed as described in C. (E) The hybrid protein HBH-YFP requires DnaK for efficient binding to protein aggregates in *E. coli* cells. *E. coli dnaK103* expressing HBH-YFP were grown to mid-exponential log phase at 30°C and shifted to 45°C for 20 min. The fraction of cells harboring HBH-YFP foci was determined (n = 200). The degree of aggregate binding was calculated by determining the fluorescence intensity of HBH-YFP foci (n = 20). Bars, 1 µm.

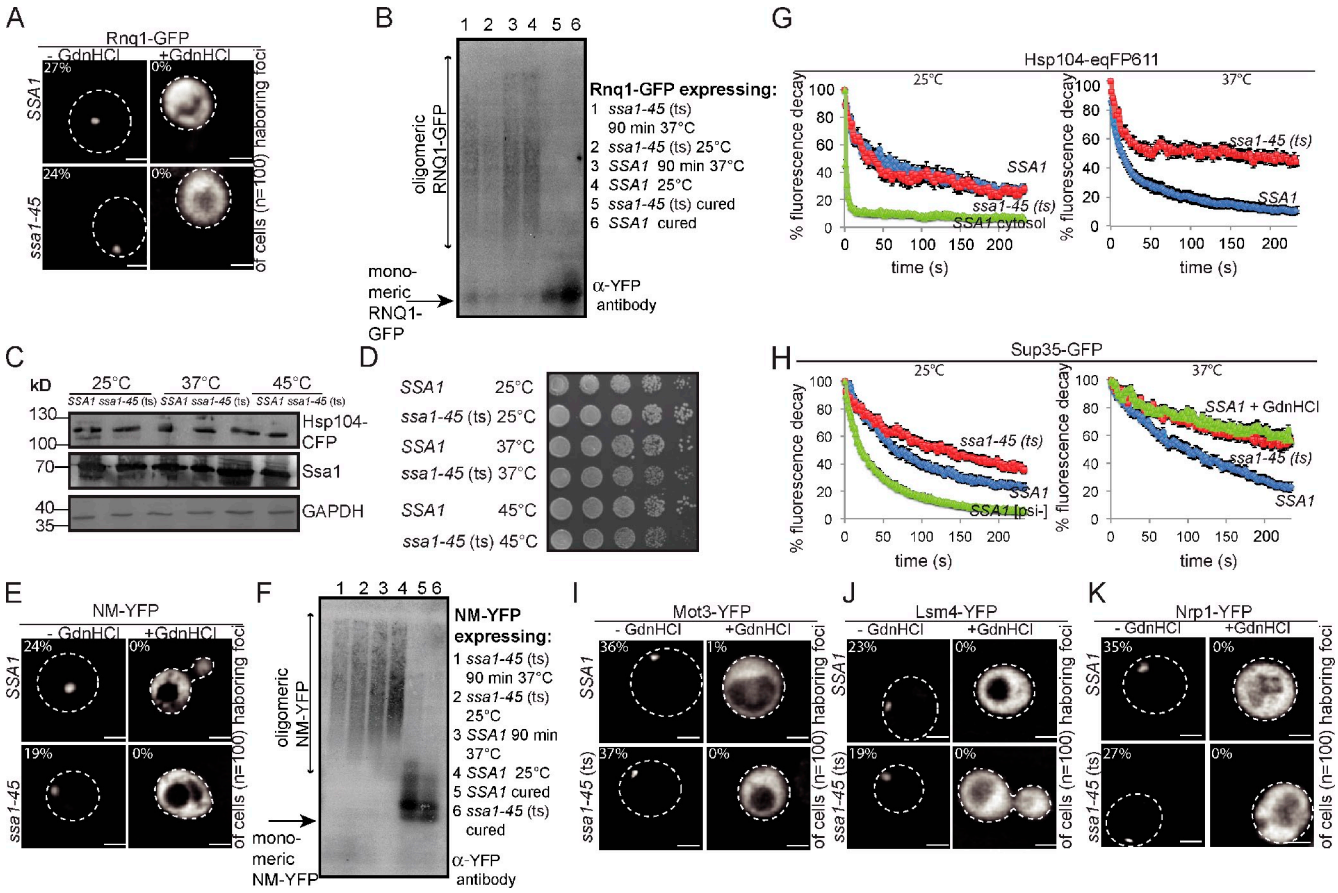

Figure S2. **Diffuse cytosolic localization of Rnq1-mcherry upon inhibition of Hsp104 by guanidinium hydrochloride (GdnHCl).** *S. cerevisiae SSA1* or *ssa1-45*(ts) cells expressing Rnq1-mCherry were passaged three times on SD plates with or without (±) 3 mM GdnHCl. Afterward, the cells were grown in SD media supplemented with 3 mM GdnHCl to mid-exponential growth phase at 25°C and were analyzed for their ability to form prion fibrils. The detection of diffuse cytosolic fluorescence indicates curing of the prion phenotype. Bars, 2 µm. (B) Rnq1p-GFP forms SDS-resistant aggregates in *S. cerevisiae SSA1* and *ssa1-45*(ts) cells. *S. cerevisiae SSA1* or *ssa1-45*(ts) cells expressing Rnq1-GFP were grown at 25°C or heat shocked to 37°C for 90 min. Total cell lysates were prepared, separated by SDD-AGE, and immunostained with YFP-specific antibodies. Furthermore, cell lysates of *SSA1* or *ssa1-45*(ts) cells, which were initially treated with 3 mM GdnHCl, expressing soluble Rnq1-GFP were included in the analysis as control. The positions of soluble, monomeric protein and SDS-resistant oligomeric forms are indicated. (C) Comparable expression levels of Ssa1/Ssa1-45ts and Hsp104-CFP in *SSA1* or *ssa1-45*(ts) cells. Yeast cells were grown at 25°C to mid-exponential growth phase. 12.5 µg/ml cycloheximide was added and cells were shifted for 90 min to 37°C before being heat shocked for 20 min at 45°C. Samples were collected at 25°C, 37°C, and 45°C and subjected to Western blot analysis using Ssa1- or Hsp104-specific antibodies. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) levels were determined by specific antibodies and served as a loading control. (D) Viability of *SSA1* or *ssa1-45*(ts) cells is not affected during the applied heat treatments. *SSA1* or *ssa1-45*(ts) cells expressing Hsp104-CFP were grown to exponential growth phase at 25°C. Cycloheximide was added and cells were incubated at 37°C for 90 min and then heat shocked for 20 min at 45°C. At the indicated time points, cells were washed and spotted in fivefold dilutions on YPD agar plates. Plates were incubated at 25°C for 2 d. (E) Diffuse cytosolic localization of NM-YFP upon inhibition of Hsp104 by guanidinium hydrochloride (GdnHCl). *S. cerevisiae SSA1* or *ssa1-45*(ts) cells expressing NM-YFP were passaged three times on SD plates with or without (±) 3 mM GdnHCl. Afterward, the cells were grown in SD media to mid-exponential growth phase at 25°C and were analyzed for their ability to form prion fibrils. The detection of diffuse cytosolic fluorescence indicates curing of the prion phenotype. Bars, 2 µm. (F) NM-YFP forms SDS-resistant aggregates in *S. cerevisiae SSA1* and *ssa1-45*(ts) cells. *S. cerevisiae SSA1* or *ssa1-45*(ts) cells expressing NM-YFP were grown at 25°C or heat shocked to 37°C for 90 min. Total cell lysates were prepared, separated by SDD-AGE, and immunostained with YFP-specific antibodies. Furthermore, cell lysates of *SSA1* or *ssa1-45*(ts) cells, which were initially treated with 3 mM GdnHCl, expressing soluble NM-YFP were included in the analysis as control. The positions of soluble, monomeric protein and SDS-resistant oligomeric forms are indicated. (G and H) FLIP measurements of Hsp104-eqFP611 (G) and Sup35-GFP (H) were performed in *SSA1* and *ssa1-45*(ts) cells at 25°C and 37°C. For comparison, the mobilities of diffuse Hsp104-eqFP611 located outside the foci (cytosol) or of diffuse Sup35-GFP in [*psi*$^-$] cells were determined. The mobility of Sup35-GFP was also monitored in *SSA1* cells at 37°C after the addition of GdnHCl, resulting in Hsp104 inactivation. Curves represent the mean of 15–25 cells and the corresponding standard errors. Because of the reduced photostability of eqFP611 compared with CFP, FLIP curves recorded for the different fluorophores (Fig. 4 B) cannot be directly compared (Wiedenmann et al., 2002). (I–K) Diffuse cytosolic localizations of the prion model proteins Mot3-YFP (J), Lsm4-YFP (K), and Nrp1-YFP (L) upon inhibition of Hsp104 by guanidinium hydrochloride (GdnHCl). *S. cerevisiae SSA1* or *ssa1-45*(ts) cells expressing the indicated fluorescent prion domains were passaged three times on SD plates ± 3 mM GdnHCl. Afterward, the cells were grown in SD media to mid-exponential growth phase at 25°C and were analyzed for their ability to form prion fibrils. The detection of diffuse cytosolic fluorescence indicates curing of the prion phenotype. The broken lines indicate the borders of respective yeast cells. Bar, 2 µm

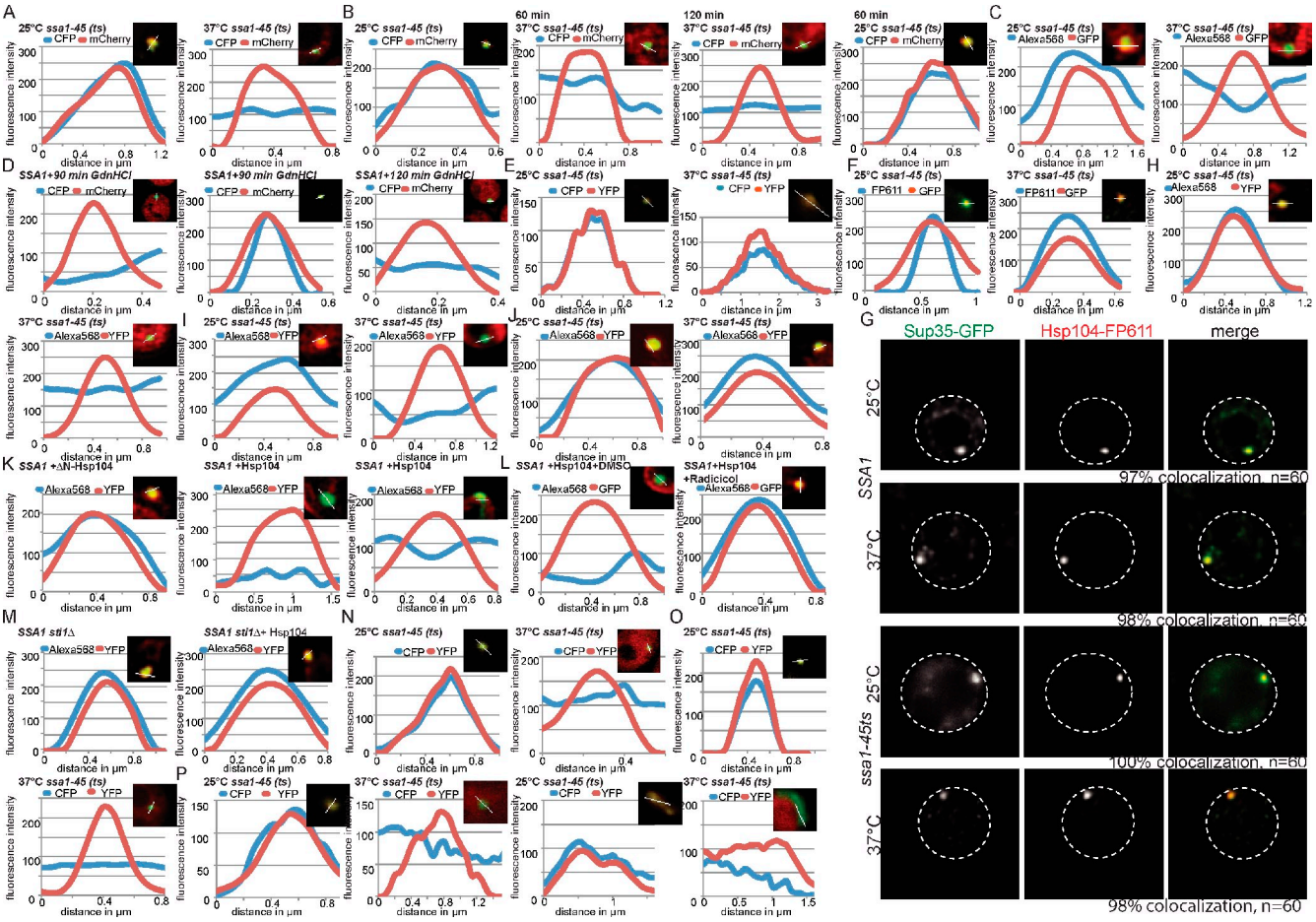

Figure S3. **Colocalizations were confirmed by line intensity plots of deconvoluted wide-field images corresponding to various figures.** (A–C) Fig. 3 A–C; (D) Fig. 5 A; (E) Fig. 6 A; (H) Fig. S4 A; (I) Fig. 7 A; (J) Fig. S5 A; (K) Fig. 7 B; (L–M) Fig. 8, A and B; (N–P) Fig. 9, A–C. Line intensity plots of *ssa1-45*(ts) cells are depicted (25°C and 37°C). Co-localizations observed in *SSA1* cells were also confirmed by line intensity plots but are not depicted. (F and G) Co-localization of Hsp104-eqFP611 and Sup35-GFP in *SSA1* and *ssa1-45*(ts) cells. Cells were grown at 25°C and shifted to 37°C. The frequency of Sup35-GFP and Hsp104-eqFP611 colocalization is given (n = 100). The broken lines indicate the borders of respective yeast cells. Sup35-GFP formed multiple foci in *SSA1* and *ssa1-45*(ts) cells. Labeling of the chromosomal copy Hsp104 with the red fluorescent protein eqFP611 caused the formation of fewer but more intense Sup35-GFP foci, which remained SDS-resistant and were converted to soluble and diffuse Sup35-GFP upon GdnHCl treatment (not depicted).

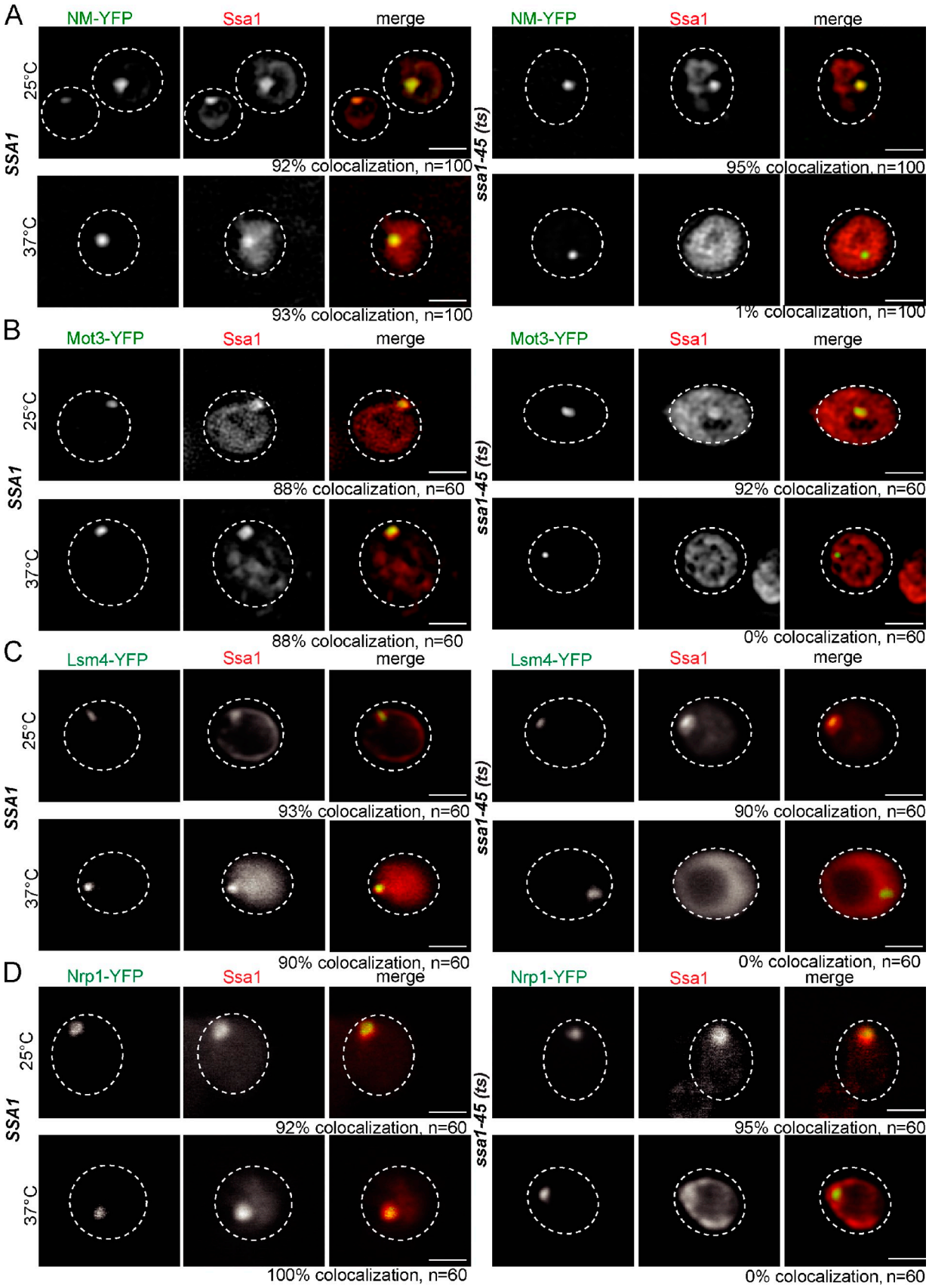

Figure S4. **Ssa1-45 is deficient in binding to prion fibrils at 37°C.** (A) NM-YFP; (B) Mot3-YFP; (C) Lsm4-YFP; (D) Nrp1-YFP. *S. cerevisiae SSA1* or *ssa1-45*(ts) cells expressing the indicated fluorescent prion domains and Hsp104-CFP were grown at 25°C to mid-log phase and shifted to 37°C for 90 min, resulting in inactivation of Ssa1-45. Cells were fixed, and association of Ssa1-45 with prion aggregates was monitored by immunofluorescence using Ssa-specific antibodies. The broken lines indicate the borders of respective yeast cells. Bars, 2 µm.

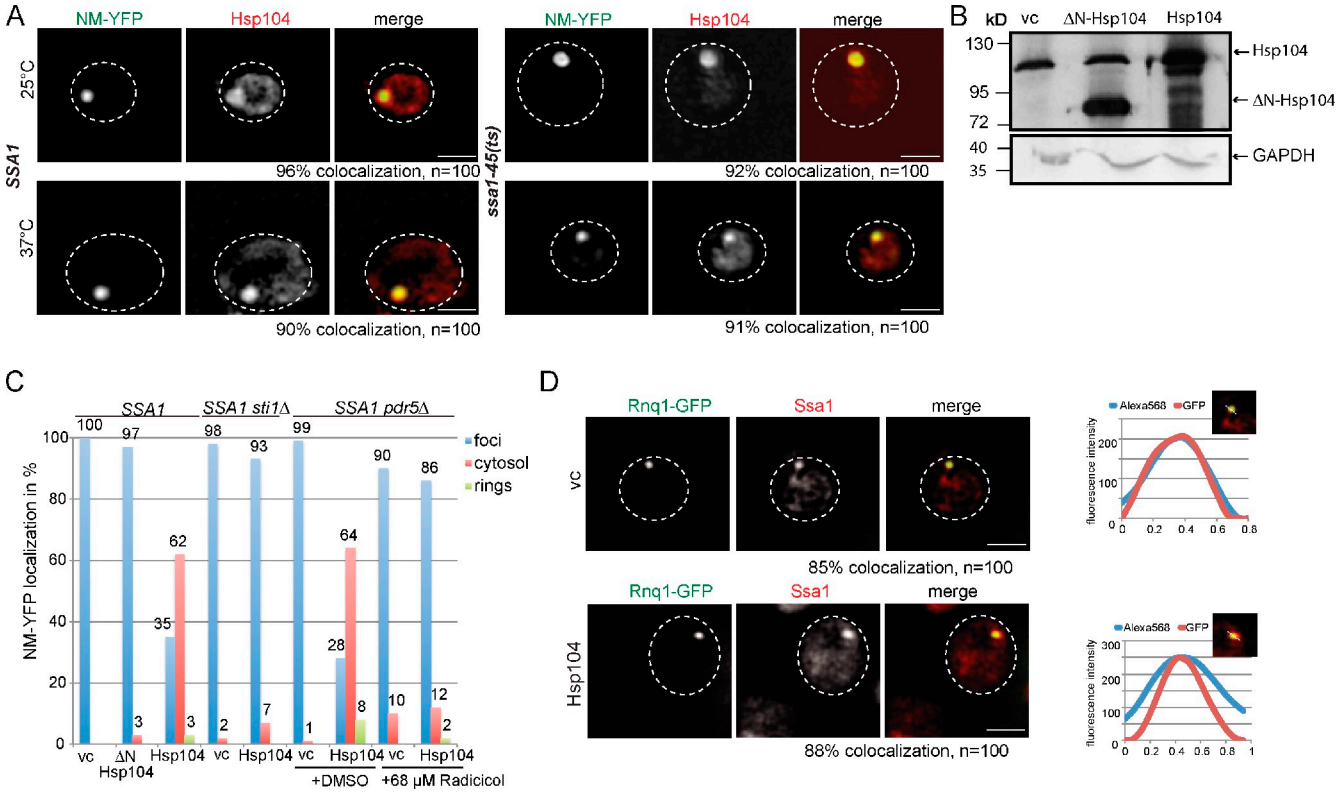

Figure S5. **Overproduction of full-length Hsp104 impairs NM-YFP prion propagation.** (A) Full-length Hsp104 associates with NM-YFP fibrils under permissive conditions. *SSA1* or *ssa1-45*(ts) cells expressing NM-YFP were grown to mid-exponential growth phase at 25°C and shifted to 37°C for 90 min. Cells were fixed and binding of Hsp104 to NM-YFP fibrils was monitored by immunofluorescence using Hsp104-specific antibodies. The broken lines indicate the borders of respective yeast cells. Bars, 2 μm. (B) Degree of Hsp104 and/or ΔN-Hsp104 overproduction upon induction of the copper-inducible promoter. Plasmid-encoded Hsp104 or ΔN-Hsp104 were expressed from a copper-inducible promoter in *S. cerevisiae SSA1* cells expressing NM-YFP. First, NM-YFP was induced for 2 h with galactose before copper was added to induce the expression of Hsp104 or ΔN-Hsp104 for 8 h. Total cell extracts were prepared and analyzed by Western blotting using Hsp104-specific antibodies. The degree of Hsp104 and ΔN-Hsp104 overproduction was verified from three to four independent experiments. Levels of GAPDH were determined by immunoblot analysis using GAPDH-specific antibodies and served as a loading control. (C) Accumulation of soluble NM-YFP and NM-YFP ribbons upon overproduction of full-length Hsp104. Plasmid-encoded Hsp104 or ΔN-Hsp104 were expressed from a copper-inducible promoter in *S. cerevisiae SSA1* cells, SSA1 *pdr5*Δ, and *SSA1 sti1*Δ expressing NM-YFP. First, NM-YFP was induced for 2 h with galactose before copper was added to induce the expression of the Hsp104 variants for 22 h. Formation of diffuse, dot- and ring-like NM-YFP fluorescence was quantified from 200 cells for SSA1 cells and from 100 cells for *SSA1 pdr5*Δ and *SSA1 sti1*Δ cells expressing NM-YFP. (D) Binding of Ssa1 to RNQ-GFP is not perturbed in the presence of high Hsp104 levels. Hsp104-overexpressing SSA1 cells were fixed, and Ssa1 colocalization with Rnq1-GFP was visualized by immunofluorescence using SSA1-specific antibodies. The frequency of colocalization (%) is given (n = 100). vc, empty vector control. The broken lines indicate the borders of respective yeast cells. Bars, 2 μm.

Table S1. ***E. coli* plasmids used in this study**

Plasmid	Resistance	Origin	Induction levels	Reference
placIq	Spectinomycin	p15A	Constitutive	Bukau laboratory
pDM1.1	Kanamycin	p15A	Constitutive	Bukau laboratory
pHSG *clpB-yfp*	Chloramphenicol	pSC101	200 μM IPTG	Bukau laboratory
pHSG *dnaK-yfp*	Chloramphenicol	pSC101	100 μM IPTG	Bukau laboratory
pDK66 *dnaJ-yfp*	Chloramphenicol	pSC101	10 μM IPTG	Sourjik laboratory
pHSG *hsp104-yfp*	Chloramphenicol	pSC101	100 μM IPTG	This study
pHSG *hbh-yfp*	Chloramphenicol	pSC101	100 μM IPTG	This study
pDK66 *metA-yfp*	Ampicillin	pBR322	50 μM IPTG	Bukau laboratory
pHSG-*clpB trap-yfp*	Chloramphenicol	pSC101	200 μM IPTG	This study
pHSG *clpB ΔN-yfp*	Chloramphenicol	pSC101	200 μM IPTG	This study
pUHE21 *dnaJ*	Ampicillin	pBR322	100 μM IPTG	Bukau laboratory
pUHE21 *cbpA*	Ampicillin	pBR322	100 μM IPTG	Bukau laboratory
pUHE21 *dnaK*	Ampicillin	pBR322	100 μM IPTG	Bukau laboratory
pUHE 21 *dnaKV436F*	Ampicillin	pBR322	100 μM IPTG	Bukau laboratory
pBAD *luciferase*	Ampicillin	pBR322	0.015% arabinose	Bukau laboratory

Table S2. ***E. coli* strains used in this study**

Strain	Genotype	Reference
MC4100	F-araD139 Δ(argF-lac)U169* rspL150 relA1 flbB5301 fruA25‡ deoC1 ptsF25 e14-	Bukau laboratory
BB7140	MC4100 *clpB::kan*	Bukau laboratory
BB6735	MC4100 *ΔclpB*	Bukau laboratory
	MC4100 *ΔclpB* pHSG *clpB-yfp* placIq	This study
	MC4100 *ΔclpB pHSG clpB trap-yfp* placIq	This study
	MC4100 *ΔclpB pHSG clpB ΔN-yfp* placIq	This study
BB6735	MC4100 *clpB::kan*	
	MC4100 *clpB::kan* pHSG *hsp104-yfp* placIq	This study
	MC4100 *clpB::kan* pHSG *clpB-yfp* placIq	This study
	MC4100 *clpB::kan* pHSG *hbh-yfp* placIq	This study
	MC4100 *clpB::kan* pHSG *dnaK-yfp* placIq	This study
	MC4100 *clpB::kan* pHSG *clpB-yfp* placIq pBAD *luciferase*	This study
	MC4100 *clpB::kan* pHSG *hbh-yfp* placIq pBAD *luciferase*	This study
	MC4100 *clpB::kan* pHSG placIq pBAD *luciferase*	This study
	MC4100 *clpB::kan* pHSG *hsp104-yfp* placIq pBAD *luciferase*	This study
BB1064	MC4100 *dnaJ::tet*	Bukau laboratory
	MC4100 *dnaJ::tet pHSG dnaK-yfp* pUHE21	This study
	MC4100 *dnaJ::tet pHSG dnaK-yfp* pUHE21 *dnaJ*	This study
	MC4100 *dnaJ::tet pHSG dnaK-yfp* pUHE21 *cbpA*	This study
	MC4100 *cbpA::kan*	Bukau laboratory
	MC4100 *cbpA::kan pHSG dnaK-yfp* pUHE21	This study
C600	F- tonA21 thi-1 thr-1 leuB6 lacY1 glnV44 rfbC1 fhuA1 λ-	Bukau laboratory
	C600 pHSG *clpB-yfp* placIq	This study
BB2393	C600 *dnaK103*	Bukau laboratory
	C600 *dnaK103* pDK66 *metA-yfp*	This study
	C600 *dnaK103* pHSG *clpB-yfp placIq* pUHE21	This study
	C600 *dnaK103* pHSG *clpB-yfp placIq* pUHE21 *dnaK*	This study
	C600 *dnaK103* pHSG *clpB-yfp placIq* pUHE21 *dnaKV436F*	This study

Table S3. **Yeast plasmids used in this study**

Plasmid	Marker	Description	Reference
pBS10	HygB	pFA6a-link-Cerulean-hphMx4	NCRR Yeast Resource Center
pYM51	HygB	eqFP611-hphMx4	EUROSCARF
p423 cup	His	Copper promoter, 2μ	This study
p423 cup *ΔN-hsp104*	His	Copper promoter, 2μ	This study
p423 cup *hsp104*	His	Copper promoter, 2μ	This study
p426 *nm-yfp*	Ura	Gal1 promoter, 2μ	S. Lindquist laboratory
p416 *nm-yfp*	Ura	Gal1 promoter, CEN	This study
p426 *mot3-yfp*	Ura	Gal1 promoter, 2μ	This study
p426 *lsm4-yfp*	Ura	Gal1 promoter, 2μ	This study
p426 *nrp1-yfp*	Ura	Gal1 promoter, 2μ	This study
p426 *rnq1-mcherry*	Ura	Gal1 promoter, 2μ	This study
p426 Gal *rnq1-gfp*	Ura	Gal1 promoter, 2μ	This study
pRS306 *citrine luciferase*	Ura	ACT1 promoter, integrative plasmid	Bukau laboratory
pSB20	Ura	Integrative plasmid encoding P_{sup35} Sup35-GFP	T. Serio laboratory

Table S4. **Yeast strains used in this study**

Strain	Genotype	Reference
YWO0625	Mat a *ura3-52 leu2-3 his3-11 lys2 trp1-D1 ssa2::Leu2 ssa3::TRP1 ssa4::Lys2*	D. Wolf laboratory
	hsp104::cfp hphMX4 p426 *nm-yfp*	This study
	hsp104::eqFP 611 hphMX4 pSB20 sup35-gfp	This study
	p426 *nm-yfp*	This study
	p426 *rnq1-gfp*	This study
	hsp104::cfp hphMX4 p426 *rnq1-mcherry*	This study
	hsp104::cfp hphMX4 p426 *mot3-yfp*	This study
	hsp104::cfp hphMX4 p426 *lsm4-yfp*	This study
	hsp104::cfp hphMX4 p426 *nrp1-yfp*	This study
	p416 *nm-yfp* p423 cup	This study
	p416 *nm-yfp* p423 cup *hsp104*	This study
	p416 *nm-yfp* p423 cup *ΔN-hsp104*	This study
	hsp104::ΔN-hsp104-cfp hphMX4 p426 *nm-yfp*	This study
	sti1Δ:: hphMX4 p416 *nm-yfp* p423 cup	This study
	sti1Δ:: hphMX4 p416 *nm-yfp* p423 cup *hsp104*	This study
	pdr5Δ hphMX4 p423 cup	This study
	pdr5Δ hphMX4 p423 *hsp104*	This study
	p426 Gal *rnq1-gfp, p423* cup	This study
	p426 Gal *rnq1-gfp, p423* cup *hsp104*	This study
	p426 Gal *rnq1-gfp, p423* cup *ΔN-hsp104*	This study
YWO0622	*Mat a ura3-52 leu2-3 his3-11 lys2 trp1-D1 ssa1-45 ssa2::Leu2 ssa3::TRP1 ssa4::Lys2*	D. Wolf laboratory
	hsp104::cfp hphMX4 p426 *nm-yfp*	This study
	hsp104::eq FP611 hphMX4 pSB20 sup35-gfp	This study
	p426 *nm-yfp*	This study
	p426 *rnq1-gfp*	This study
	hsp104::cfp hphMX4 p426 *rnq1-mcherry*	This study
	hsp104::cfp hphMX4 p426 *mot3-yfp*	This study
	hsp104::cfp hphMX4 p426 *lsm4-yfp*	This study
	hsp104::cfp hphMX4 p426 *nrp1-yfp*	This study
	hsp104::ΔN-hsp104-cfp hphMX4 p426 *nm-yfp*	This study

Reference

Wiedenmann, J., A. Schenk, C. Röcker, A. Girod, K.D. Spindler, and G.U. Nienhaus. 2002. A far-red fluorescent protein with fast maturation and reduced oligomerization tendency from Entacmaea quadricolor (Anthozoa, Actinaria). *Proc. Natl. Acad. Sci. USA*. 99:11646–11651. http://dx.doi.org/10.1073/pnas.182157199