Specht et al., http://www.jcb.org/cgi/content/full/jcb.201106037/DC1

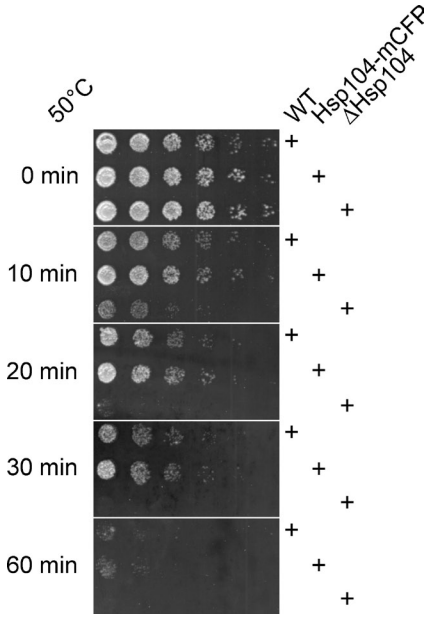

Figure S1. ***S. cerevisiae* Hsp104-mCFP provides WT-like thermotolerance.** *S. cerevisiae* WT, Hsp104-mCFP–expressing, and hsp104Δ cells were grown at 30°C and shifted to 37°C for 60 min. Subsequently, the cells were incubated at 50°C for the indicated time periods and spotted in a serial dilution onto YPD plates. Images were acquired after 2 d of growth at 30°C.

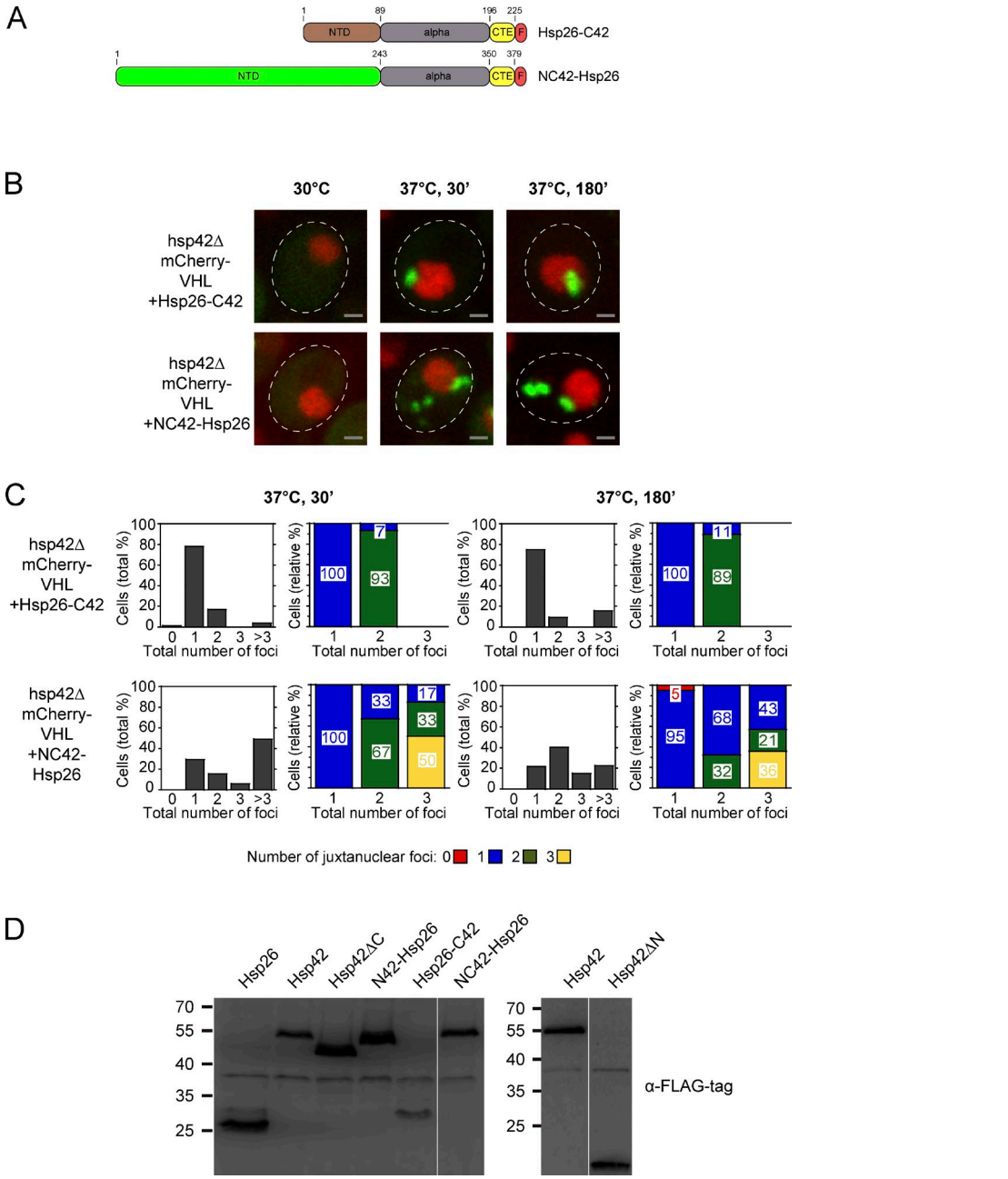

Figure S2. **The NTD of Hsp42 mediates sorting of aggregated proteins to peripheral inclusions.** (A) Domain organization of Hsp26-C42 and NC42-Hsp26. Both constructs consist of an NTD, a conserved α-crystallin domain (alpha), and a CTE and were C-terminally fused to a FLAG tag (F). Domain boundaries are indicated by residue numbers. Both constructs were under control of the native *HSP42* promoter and integrated at the Hsp42 locus in hsp42Δ cells. (B) *S. cerevisiae* hsp42Δ cells expressing mCherry-VHL and the indicated sHSP constructs were grown at 30°C and shifted to 37°C (+MG132). mCherry-VHL localization (green) was determined at the indicated time points. Nuclei were visualized by coexpressing HTB1-cerulean (red). The dashed lines indicate the border of respective yeast cells. Bars, 1 µm. (C) Number (shaded bars) and localization (colored bars) of mCherry-VHL inclusions in hsp42Δ cells expressing the indicated sHSP construct after incubation at 37°C for 30 and 180 min. The proteasome inhibitor MG132 was added before the temperature shift. The color code deciphers the foci localization. The total number of foci per cell is depicted in all diagrams on the x axis. Quantifications are based on the analysis of 50–100 cells and are representatives of two experimental repeats. (D) The levels of the Hsp42 domain deletion and Hsp26–Hsp42 domain swap constructs are similar. *S. cerevisiae* hsp42Δ cells were grown to midlog phase at 30°C, and the level of the indicated sHSP construct was determined by Western blotting using FLAG tag–specific antibodies. Molecular mass is indicated in kilodaltons.

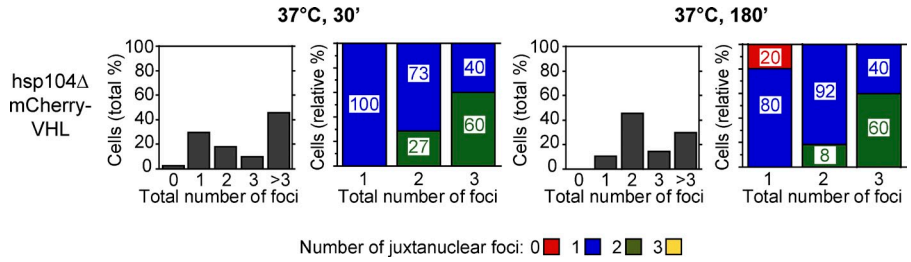

Figure S3. **Formation of mCherry-VHL foci in hsp104Δ cells.** Number (shaded bars) and localization (colored bars) of mCherry-VHL foci in hsp104Δ cells after incubation at 37°C for 30 and 180 min. The total number of foci per cell (juxtanuclear and peripheral) is depicted in all diagrams on the x axis. Quantifications are based on the analysis of 60 cells and are representatives of an experimental repeat.

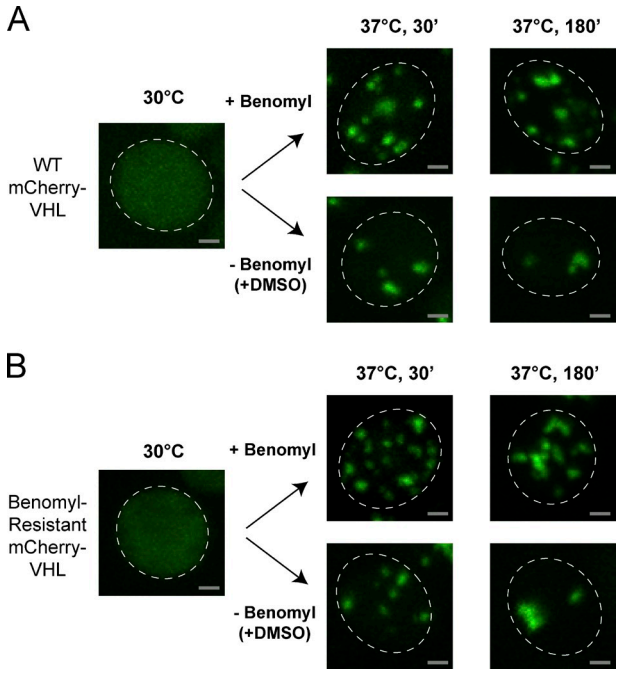

Figure S4. **Microtubule-independent effects of benomyl prevent aggregate sorting of misfolded mCherry-VHL into JUNQ and IPOD-like compartments.** (A and B) mCherry-VHL (green) localization was analyzed after stress application in the presence of the microtubule-depolymerizing drug benomyl in WT cells (A) and a yeast strain containing a mutation in tubulin-2, which renders the microtubule cytoskeleton resistant to benomyl (B). After 30 and 180 min of incubation at 37°C (+MG132) in the presence of benomyl, both WT and benomyl-resistant cells contained multiple dispersed mCherry-VHL foci and did not exhibit JUNQ and IPOD-like compartments. Instead of benomyl, control cells were treated with the same amount of DMSO. The dashed lines indicate the border of respective yeast cells. Bars, 1 μm.

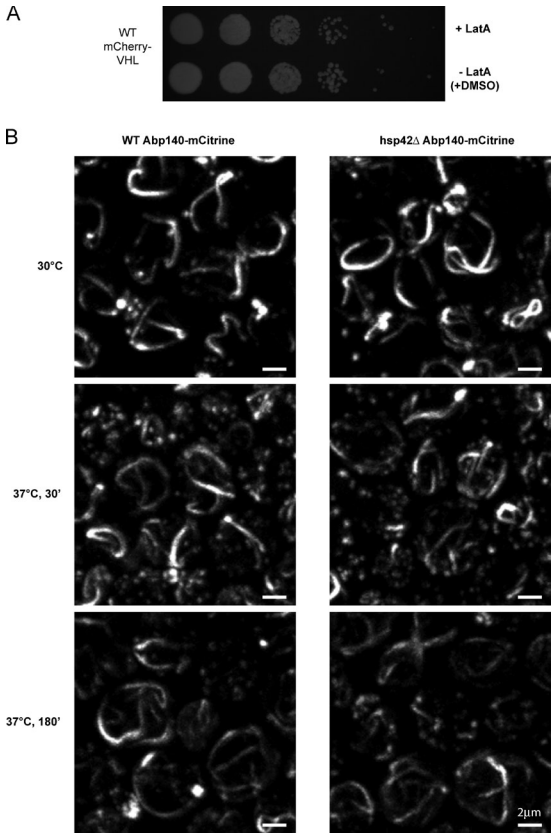

Figure S5. **LatA treatment does not reduce cell viability, and the actin cytoskeleton is not altered in hsp42Δ cells at both physiological and folding stress conditions.** (A) WT cells were incubated for 180 min at 37°C in the presence of the actin-depolymerizing drug LatA and proteasome inhibitor MG132. Instead of LatA, control cells were treated with same amount of DMSO. Subsequently, the cells were washed and spotted in a serial dilution onto an agar plate. The image was acquired after 2 d of growth at 30°C. (B) *S. cerevisiae* WT and hsp42Δ cells were incubated at 30°C (top) and shifted to 37°C (+MG132) for 30 min (middle) and 180 min (bottom). The actin cytoskeleton was visualized via a genomic C-terminal fusion of mCitrine to the actin-binding protein 140 (Abp140).

Table S1. **Plasmids used in this study**

Plasmid	Description	Source
pBS10	pFA6a-link-cerulean-hphMX4	NCRR Yeast Resource Center, Rizzo et al., 2004
pESC-mCherry-VHL	GAL1 promoter; mCherry-VHL; Amp^R; URA3; 2μ	Kaganovich et al., 2008, J. Frydman[a]
pFA6-kanMX4	kanMX4 (G418/Geneticin)	Wach et al., 1994
pGA25	natMX4 (CloNat)	Goldstein and McCusker, 1999
pKT211	pFA6a-link-yEmCFP-SpHIS5	Sheff and Thorn, 2004
pKT212	pFA6a-link-yEmCitrine-SpHIS5	Sheff and Thorn, 2004
pRS303	pBluescript based; Amp^R; HIS3; YIP	Sikorski and Hieter, 1989
pRS303-ACT-yEmCitrine-luciferase	pRS303; ACT1 promoter; yEmCitrine-luciferase	This study
pRS303-ADH-HTB-cerulean	pRS305; ADH1 promoter; HTB1-cerulean	This study
pRS303-PHsp42	pRS303; HSP42 including promoter and terminator (500 bp up- and downstream of HSP42)	This study
pRS305	pBluescript based; Amp^R; LEU2; YIP	Sikorski and Hieter, 1989
pRS305-ADH-HTB-cerulean	pRS305; ADH1 promoter; HTB1-cerulean	This study
pRS305-ADH-HTB-mCherry	pRS305; ADH1 promoter; HTB1-mCherry	This study
pRS305-GAL-RNQ1-YFP	pRS305; Gal1, 10 promoter; RNQ1-YFP	Laboratory collection
p425-GAL1-RNQ1-mCherry	Gal1 promoter; RNQ1-mCherry; Amp^R; LEU2; 2μ	Laboratory collection
pRS306	pBluescript based; Amp^R; URA3; YIP	Sikorski and Hieter, 1989
pRS306-ACT-yEmCitrine-luciferase	pRS306; ACT1 promoter; yEmCitrine-luciferase	This study
pSM006	pRS303; HSP42 promoter and terminator (500 bp up- and downstream of HSP42); Hsp42	This study
pSM012	pRS303; HSP42 promoter and terminator (500 bp up- and downstream of HSP42); N42-Hsp26	This study
pSM013	pRS303; HSP42 promoter and terminator (500 bp up- and downstream of HSP42); Hsp42-FLAG	This study
pSM014	pRS303; HSP42 promoter and terminator (500 bp up- and downstream of HSP42); Hsp42ΔN	This study
pSM015	pRS303; HSP42 promoter and terminator (500 bp up- and downstream of HSP42); Hsp42ΔC	This study
pSM016	pRS303; HSP42 promoter and terminator (500 bp up- and downstream of HSP42); N26-Hsp42	This study
pSM017	pRS303; HSP42 promoter and terminator (500 bp up- and downstream of HSP42); Hsp26-C42	This study
pSM018	pRS303; HSP42 promoter and terminator (500 bp up- and downstream of HSP42); NC42-Hsp26	This study
pSM019	pRS303; HSP42 promoter and terminator (500 bp up- and downstream of HSP42); Hsp26-FLAG	This study

Features of the vector are given in the Description column. 2μ, 2-μm origin of replication; NCRR, National Center for Research Resources; YIP, yeast integration vector.
[a]Stanford University, Stanford, CA.

Table S2. ***S. cerevisiae* strains used in this study**

Name	Genotype	Source
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	EUROSCARF
hsp26Δ	*BY4741 hsp42Δ::kanMX4*	EUROSCARF
hsp42Δ	*BY4741 hsp42Δ::kanMX4*	EUROSCARF
hsp104Δ	*BY4741 hsp104Δ::kanMX4*	EUROSCARF
pdr5Δ	*BY4741 pdr5Δ::natMX4*	This study
hsp26Δ pdr5Δ	*BY4741 hsp26Δ::kanMX4 pdr5Δ::natMX4*	This study
hsp42Δ pdr5Δ	*BY4741 hsp42Δ::kanMX4 pdr5Δ::natMX4*	This study
hsp104Δ pdr5Δ	*BY4741 hsp104Δ::kanMX4 pdr5Δ::natMX4*	This study
KAY0173 (LatA resistant)	*ura3-52 his3Δ200 leu2-3,112 ade4 can1-1 tub2-201 Act1-117*	Ayscough et al., 1997, K.R. Ayscough[a]
KAY0173 (LatA resistant) hsp42Δ	*KAY0173 hsp42Δ::kanMX4*	This study
KAY0159 (benomyl resistant)	*ura3-52 his3Δ200 leu2-3,112 cry1 tub2-201 Act1::His3*	Ayscough et al., 1997, K.R. Ayscough
WT (HSP104-mCFP)	BY4741 *HSP104-yEmCFP-spHIS5*	This study
hsp26Δ (HSP104-mCFP)	BY4741 *hsp26Δ::kanMX4 HSP104-yEmCFP-spHIS5*	This study
hsp42Δ (HSP104-mCFP)	BY4741 *hsp42Δ::kanMX4 HSP104-yEmCFP-spHIS5*	This study
WT pdr5Δ (HSP104-mCFP)	BY4741 *pdr5Δ::natMX4 HSP104-yEmCFP-spHIS5*	This study
hsp26Δ pdr5Δ (HSP104-mCFP)	BY4741 *hsp26Δ::kanMX4 pdr5Δ::natMX4 HSP104-yEmCFP-spHIS5*	This study
hsp42Δ pdr5Δ (HSP104-mCFP)	BY4741 *hsp42Δ::kanMX4 pdr5Δ::natMX4 HSP104-yEmCFP-spHIS5*	This study
WT (ABP140-yEmCitrine)	*BY4741 Abp140-yEmCitrine-URA3*	This study
WT pdr5Δ (ABP140-yEmCitrine)	*BY4741 pdr5Δ::natMX4 Abp140-yEmCitrine-URA3*	This study
hsp42Δ pdr5Δ (ABP140-yEmCitrine)	*BY4741 hsp42Δ::kanMX4 pdr5Δ::natMX4 Abp140-yEmCitrine-URA3*	This study

EUROSCARF, European Saccharomyces Cerevisiae Archive for Functional Analysis.

[a]University of Sheffield, Sheffield, England, UK.

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