Supporting Information

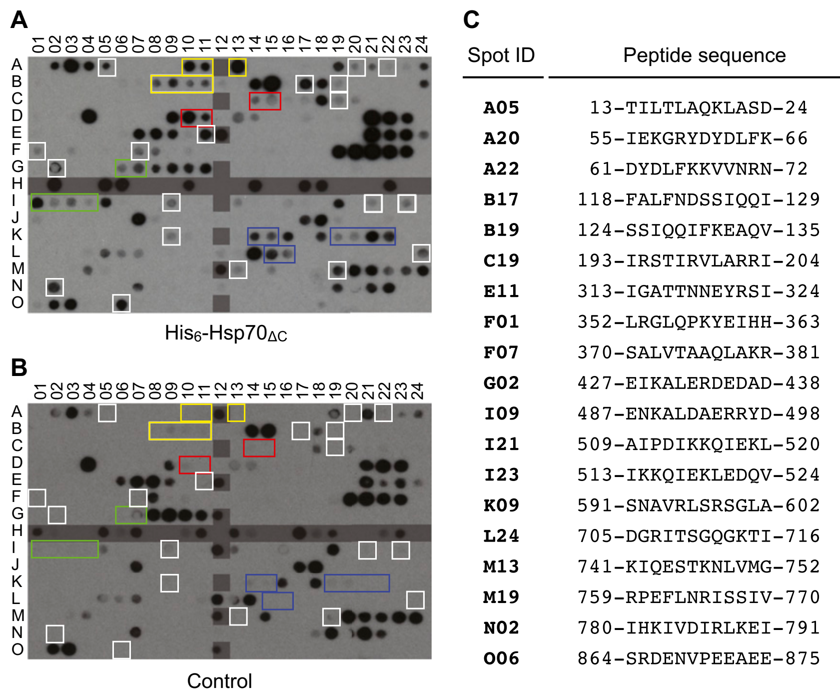
Lee et al. 10.1073/pnas.1217988110

SI Text

Thermotolerance Assay. *Saccharomyces cerevisiae* heat shock protein (Hsp) 104 variants and *Escherichia coli* ClpB were cloned into pYS104 (kind gift from Yury Chernoff, Georgia Institute of Technology, Atlanta), which featured Hsp104 under the control of the *Hsp104* promoter, by swapping the *Strep*-tag–containing HindIII fragments or replacing the entire Hsp104 sequence with *E. coli* ClpB. Plasmids were transformed into *S. cerevisiae* OT46 (Δ*hsp104*) (1). Yeast cells were grown to midlog phase at 25 °C in yeast extract-peptone-dextrose (YPD) medium and divided into two sets. One set was treated with heat shock (basal thermotolerance). The other set was incubated at 37 °C for 30 min to induce heat-shock protein synthesis before heat shock (induced thermotolerance). Cells were heat-shocked at 50 °C for 20 min and immediately chilled on ice. Cells were diluted with sterile water and spotted on YPD plates. Viability was scored after 2 d of incubation at 30 °C.

Preparation of $Hsp104_{PI}$ and $Hsp104_{PR}$ Variants. Two Hsp104 *Strep*-tag variants were made in which the *Strep*-tag is inserted after Ser740 ($Hsp104_{PI}$) or replaced 13 residues of the D2-domain (ΔSer736–Asn748; $Hsp104_{PR}$). $Hsp104_{PI}$ and $Hsp104_{PR}$ constructs were generated by cassette mutagenesis and expressed and purified as described for other Hsp104 *Strep*-tag variants.

1. Derkatch IL, Bradley ME, Zhou P, Chernoff YO, Liebman SW (1997) Genetic and environmental factors affecting the de novo appearance of the [*PSI*+] prion in *Saccharomyces cerevisiae*. *Genetics* 147(2):507–519.



Spot ID	Peptide sequence
A05	13-TILTLAQKLASD-24
A20	55-IEKGRYDYDLFK-66
A22	61-DYDLFKKVVNRN-72
B17	118-FALFNDSSIQQI-129
B19	124-SSIQQIFKEAQV-135
C19	193-IRSTIRVLARRI-204
E11	313-IGATTNNEYRSI-324
F01	352-LRGLQPKYEIHH-363
F07	370-SALVTAAQLAKR-381
G02	427-EIKALERDEDAD-438
I09	487-ENKALDAERRYD-498
I21	509-AIPDIKKQIEKL-520
I23	513-IKKQIEKLEDQV-524
K09	591-SNAVRLSRSGLA-602
L24	705-DGRITSGQGKTI-716
M13	741-KIQESTKNLVMG-752
M19	759-RPEFLNRISSIV-770
N02	780-IHKIVDIRLKEI-791
O06	864-SRDENVPEEAEE-875

Fig. S1. Hsp70-binding peptides identified by peptide array technology. (*A*) Hsp104 peptide array probed with His_6-$Hsp70_{\Delta C}$ and (*B*) with $Hsp70_{\Delta C}$ (without His_6-tag), which functions as a negative control. Overlapping peptides containing at least two consecutive spots are boxed using the same color scheme shown in Fig. 1 *B* and *C*. The remaining 19 peptides, which are present as single spots on the membrane, are boxed in white. Peptides that function as either positive or negative internal binding controls, including His_6- and poly-alanine–containing peptides, are highlighted in gray. (*C*) Sequences of the 19 $Hsp70_{\Delta C}$-binding peptides boxed in white in *A*.

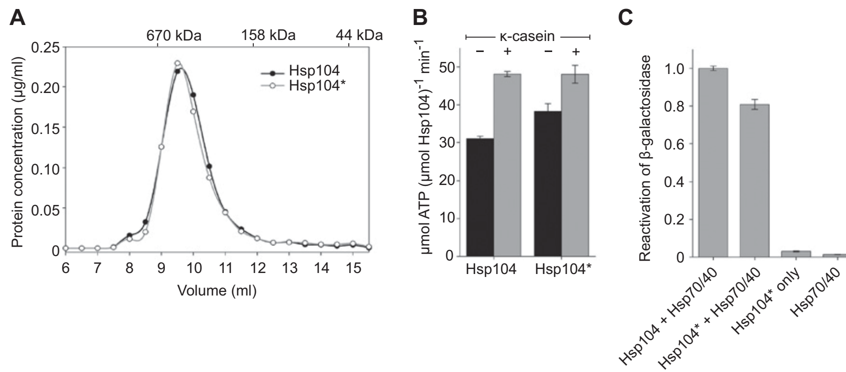


Fig. S2. The cysteine-less Hsp104 variant (Hsp104*) is functional. (*A*) $Hsp104_{C209S/C399S/C643S/C876L}$ (Hsp104*) forms a hexamer that is indistinguishable from wild-type Hsp104. Hexamers were analyzed on a Superdex 200 10/30 column in 25 mM Hepes (pH 7.5), 150 mM KOAc, 10 mM $Mg(OAc)_2$, and 1 mM DTT. (*B*) Basal and casein-stimulated ATPase activities of Hsp104* and wild-type Hsp104. (*C*) Coupled chaperone assay using heat-aggregated β-gal. Recovered enzymatic activities are expressed relative to the wild-type bichaperone system. Averages of three independent measurement ± SD are shown.

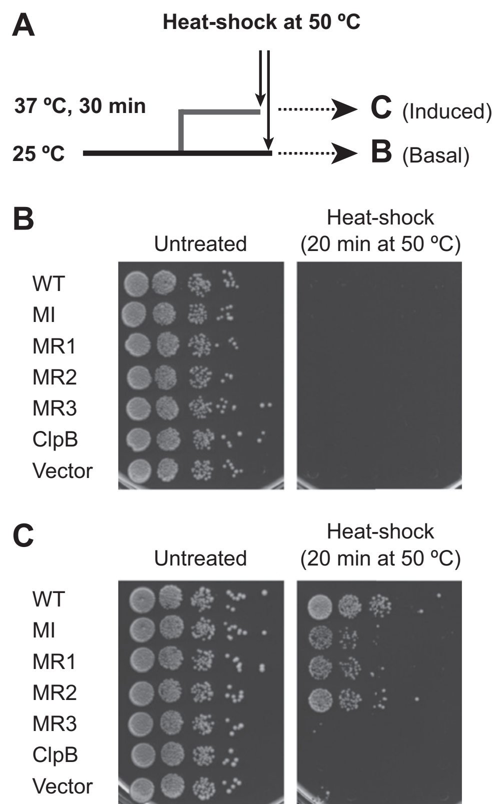


Fig. S3. Functional analysis of engineered Hsp104 *Strep*-tag variants in vivo. (*A*) Schematic outline of the thermotolerance assay. (*B*) Basal and (*C*) induced thermotolerance of Δ*hsp104* yeast expressing the indicated wild-type or mutant protein. Shown are 10-fold serial dilutions.

Fig. S4. Hsp104$_{MI}$ is activated by *Strep*-Tactin and recovers functional protein in the absence of Hsp70/Hsp40. (*A*) Time-course analyses of recovered β-gal activity shown in Fig. 4*A* and (*B*) of α-glucosidase shown in Fig. 4*C*. Recovered enzymatic activity was measured every 60 min (β-gal) or 30 min (α-glucosidase) and is shown with the initial substrate activity set at 100%. *Strep*-Tactin (tetramer; 4 × 15 kDa) and BSA (monomer; 67 kDa) were added in *n*-fold excess of Hsp104 (WT) or Hsp104$_{MI}$ (MI) monomer. Averages of three independent measurements ± SD are shown.

Fig. S5. Only *Strep*-tag–containing M-domain variants are activated by *Strep*-Tactin. (*A*) Hsp104 hexamer model depicting the location of the *Strep*-tag insertions. In Hsp104$_{PI}$ and Hsp104$_{PR}$, the *Strep*-tag was inserted into the so-called ClpP-binding motif (1), which confers the ability of Hsp104 to cooperate with the bacterial ClpP peptidase (2). The location of the *Strep*-tag insertions is indicated by the red (M-domain) and blue spheres (ClpP-binding motif). (*B*) Protein disaggregation assay using Hsp104 wild-type and Hsp104 *Strep*-tag variants in conjunction with Hsp70/Hsp40 or a fourfold molar excess of *Strep*-Tactin. Recovered β-gal activities are expressed relative to the wild-type bichaperone system. Averages of three independent measurement ± SD are shown.

1. Lee S, Sielaff B, Lee J, Tsai FTF (2010) CryoEM structure of Hsp104 and its mechanistic implication for protein disaggregation. *Proc Natl Acad Sci USA* 107(18):8135–8140.
2. Sielaff B, Tsai FTF (2010) The M-domain controls Hsp104 protein remodeling activity in an Hsp70/Hsp40-dependent manner. *J Mol Biol* 402(1):30–37.

Fig. S6. Hsp70 activates the Hsp104 protein disaggregating activity in a protein concentration-dependent manner. (*A* and *B*) Hsp70 (monomer) was added in *n*-fold excess over Hsp104 monomer. (*A*) Time-course analyses with the initial substrate activity set at 100% of protein disaggregation assay with β-gal shown in Fig. 5*A*. Recovered enzymatic activity was measured every 60 min. Averages of three independent measurements ± SD are shown. (*B*) Real-time recovery of EGFP fluorescence over 120 min. A representative curve from three independent measurements is shown.