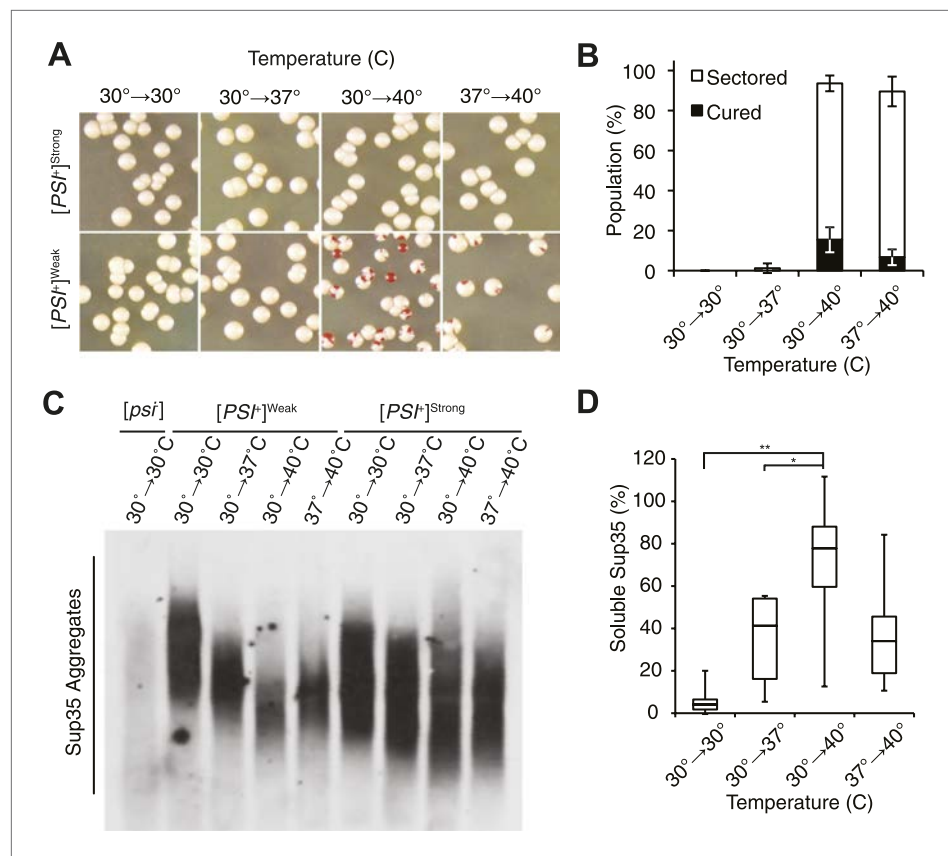


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## Figures and figure supplements

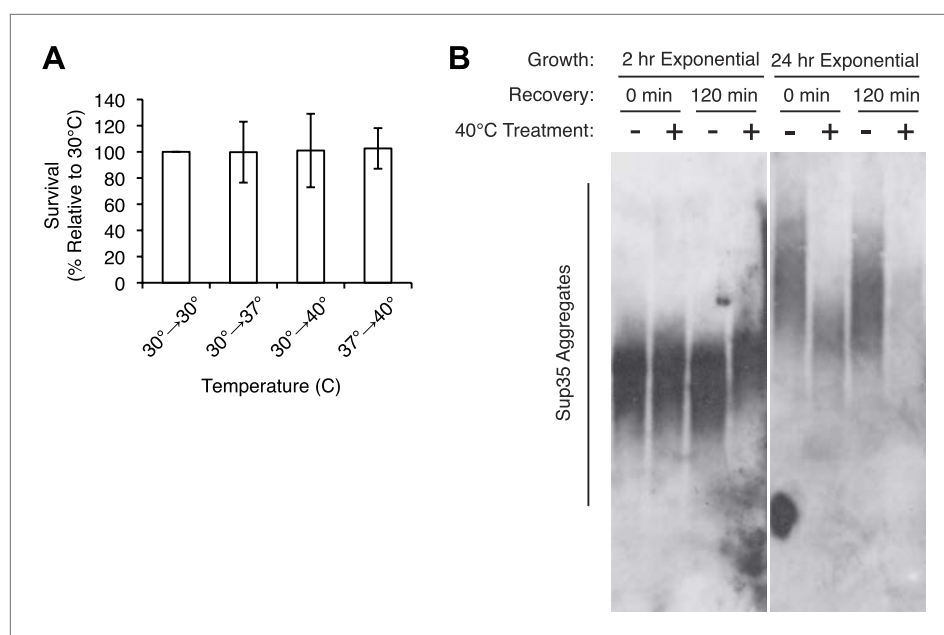
Spatial quality control bypasses cell-based limitations on proteostasis to promote prion curing

**Courtney L Klaips, et al.**



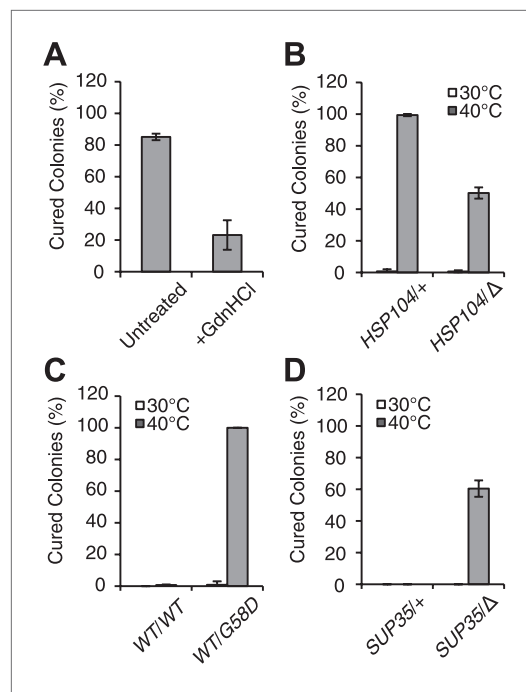
**Figure 1.** Thermal stress induces curing through resolution of Sup35 amyloid. **(A)**  $[PSI^{+}]^{Strong}$  (SLL2606) and  $[PSI^{+}]^{Weak}$  (SLL2600) cultures were incubated for 30 min at the indicated temperatures before plating on rich medium at 30°C to analyze curing by colony color phenotype, as described in the text. **(B)** Quantification of  $[PSI^{+}]^{Weak}$  (SLL2600) colony color phenotypes following treatment as described in **(A)**. Colonies were scored as completely  $[psi^{-}]$  (black), or sectored (partially  $[psi^{-}]$ , white). Data represent averages; error bars represent standard deviations;  $n = 3$ . **(C)** Semi-native lysates of  $[psi^{-}]$  (SLL2119),  $[PSI^{+}]^{Weak}$  (SLL2600), and  $[PSI^{+}]^{Strong}$  (SLL2606) cultures were analyzed by semi-denaturing detergent agarose gel electrophoresis (SDD-AGE) and immunoblotting for Sup35 after treatment as described in **(A)**. **(D)** Sup35 released from amyloid aggregates in a  $[PSI^{+}]^{Weak}$  strain (SLL2600) following treatment as described in **(A)** and recovery at 30°C in the presence of cycloheximide was determined by treating lysates with 2% SDS at 53°C, followed by SDS-PAGE and quantitative immunoblotting for Sup35. Lines represent medians; boxes represent upper and lower quartiles, and whiskers represent maximum and minimum;  $n = 5$ ; \* $p = 0.02$ , \*\* $p = 0.01$  by paired t-test.

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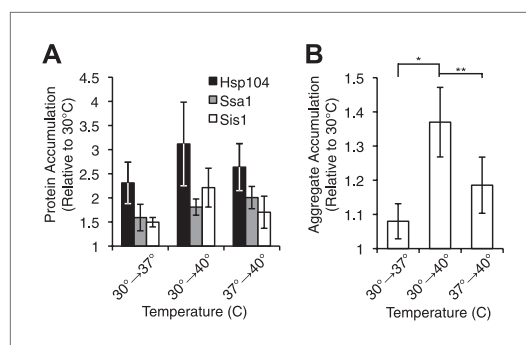
**Figure 1—figure supplement 1.** Characterization of thermal stress effects. **(A).** Exponentially growing [*PSI*<sup>+</sup>]<sup>Weak</sup> cultures (SLL2600) were incubated at 30°C, 37°C, 40°C, or 37°C before 40°C for 30 min and plated to YPD at 30°C to quantify colony forming units. Data represent means; error bars represent standard deviations;  $n \geq 3$ . **(B)** [*PSI*<sup>+</sup>]<sup>Weak</sup> cultures (SLL2600) grown exponentially for 2 hr after dilution from a saturated overnight culture (left) or for at least 24 hr (right) were incubated at 40°C for 30 min and allowed to recover for 2 hr at 30°C. Lysates isolated from these cultures were analyzed by SDD-AGE and immunoblotting for Sup35.

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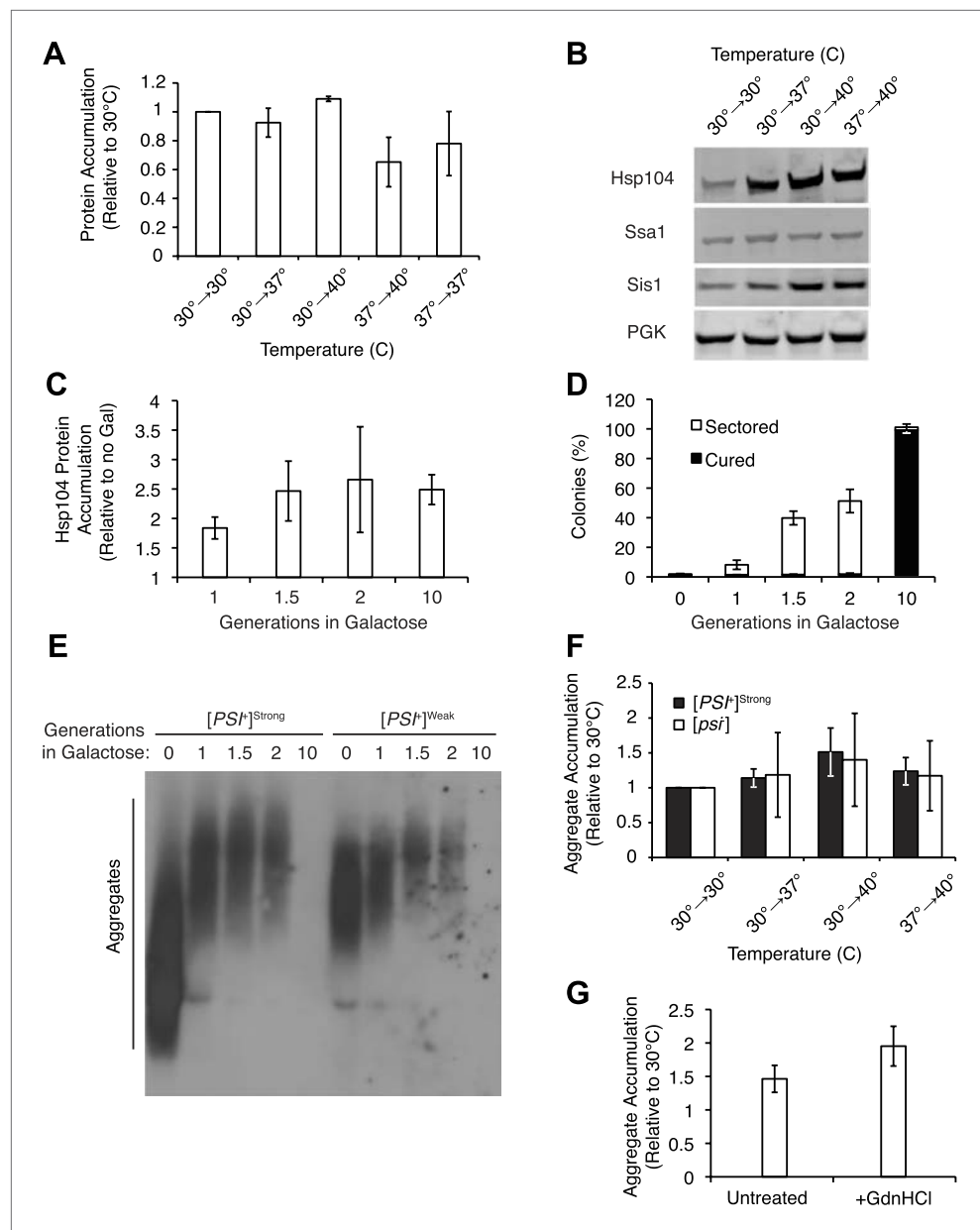
**Figure 2.** Curing is mediated by Hsp104 and depends upon propagation efficiency. **(A)** [*PSI*<sup>+</sup>]<sup>Weak</sup> cultures (SLL2600) were incubated at 40°C for 30 min in the absence (untreated) or presence of guanidine HCl (GdnHCl) and plated on YPD to quantify prion loss by colony color phenotype. Data represent means; error bars represent standard deviations; n = 3; p = 0.0004 by unpaired t-test. **(B)** A WT (*HSP104*<sup>+/+</sup>; SY945) and a heterozygous disruption (*HSP104*<sup>Δ/Δ</sup>; SY591) [*PSI*<sup>+</sup>]<sup>Weak</sup> diploid strain were incubated at 40°C for 90 min and plated on YPD to quantify prion loss by colony color phenotype. Data represent means; error bars represent standard deviations; n = 3; p < 0.0001 by unpaired t-test. **(C)** [*PSI*<sup>+</sup>]<sup>Strong</sup> strains expressing an extra copy of either WT (SY1646) or G58D (SY1648) Sup35 were incubated at 40°C for 90 min and plated on YPD to quantify prion loss by colony color phenotype. Data represent means; error bars represent standard deviations; n = 4; p < 0.0001 by unpaired t-test. **(D)** A WT (*SUP35*<sup>+/+</sup>; SLL3071) and a heterozygous disruption (*SUP35*<sup>Δ/Δ</sup>; SY957) diploid [*PSI*<sup>+</sup>]<sup>Strong</sup> strain were incubated at 40°C for 90 min and plated on YPD to quantify prion loss by colony color phenotype. Data represent means; error bars represent standard deviations; n = 3; p < 0.0001 by unpaired t-test.

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**Figure 3.** Heat-induced aggregate accumulation but not chaperone levels correlate with temperature. **(A)** A [*PSI<sup>+</sup>*]<sup>Weak</sup> strain (SLL2600) was incubated at 30°C, 37°C, 40°C, or 37°C before 40°C for 30 min, and lysates were prepared and analyzed by SDS-PAGE and quantitative immunoblotting for Hsp104 (black), Ssa1 (gray), and Sis1 (white). Data represent means; error bars represent standard deviations;  $n \geq 3$ . **(B)** Aggregates from lysates of a [*PSI<sup>+</sup>*]<sup>Weak</sup> strain (SLL2600) following treatment as described in **(A)** were prepared and analyzed by differential centrifugation and Bradford assay. Data represent means; error bars represent standard error;  $n = 6$ ; \* $p = 0.0014$ , \*\* $p = 0.0052$  by paired t-test.

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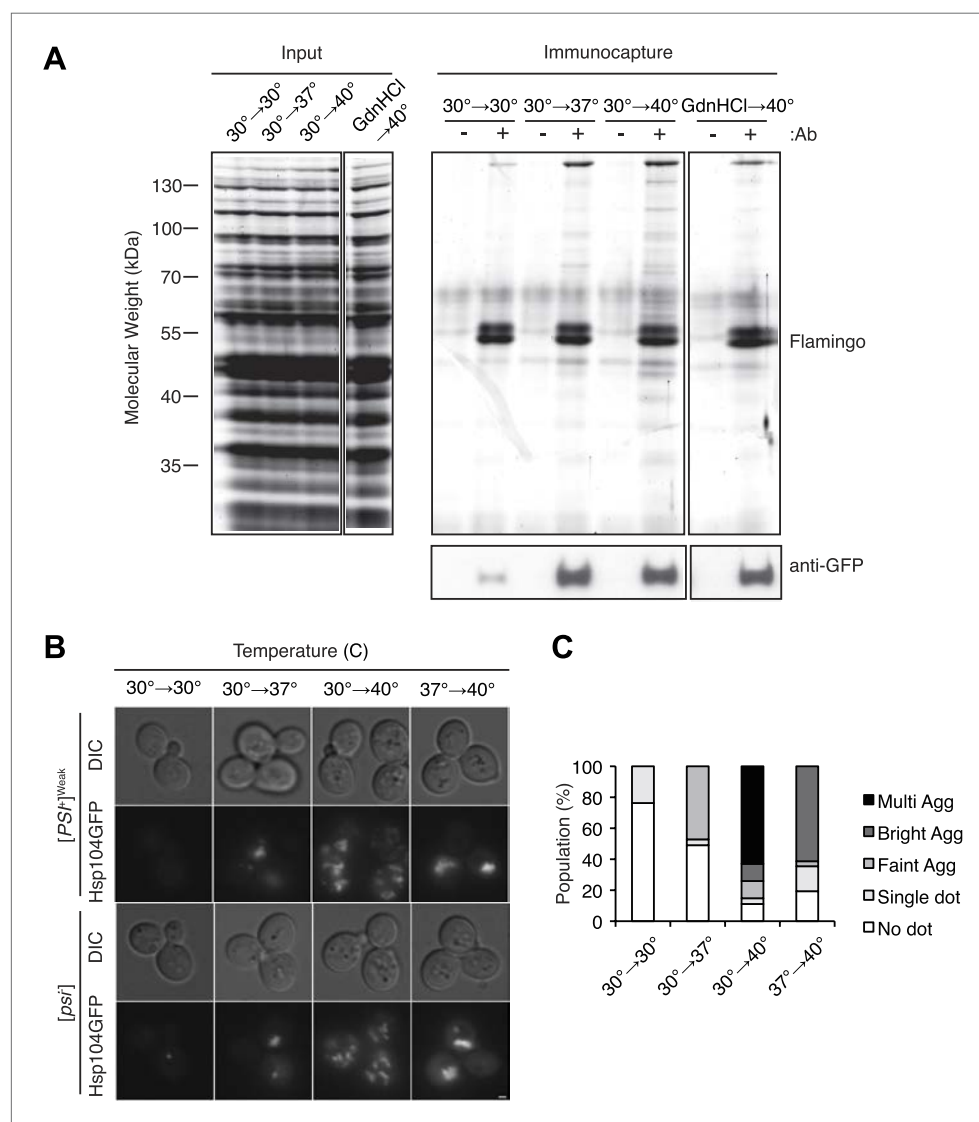
**Figure 3—figure supplement 1.** Effects of thermal stress and Hsp104 on protein accumulation. **(A)** Lysates were isolated from [PSI<sup>+</sup>]<sup>Weak</sup> strains (SLL2600) incubated at 30°C, 37°C, 40°C, or 37°C before 40°C for 30 min and analyzed by SDS-PAGE and quantitative immunoblotting for Sup35. Data represent means; error bars represent standard deviations; n = 4. **(B)** Lysates were isolated from [PSI<sup>+</sup>]<sup>Weak</sup> strains (SLL2600) incubated at 30°C, 37°C, 40°C, or 37°C before 40°C for 30 min and analyzed by SDS-PAGE and quantitative immunoblotting for Hsp104, Ssa1, Sis1 or phosphoglycerate kinase (PGK) as a loading control (representative blot; see **Figure 3A** for quantification). **(C)** Lysates were isolated from a [PSI<sup>+</sup>]<sup>Weak</sup> strain containing a galactose-inducible *HSP104* at the endogenous locus (SY1749) after galactose treatment and Hsp104 protein was quantified by SDS-PAGE and immunoblotting. Data represent means; error bars represent standard deviations; n = 3. **(D)** Galactose-inducible *HSP104* [PSI<sup>+</sup>]<sup>Weak</sup> strains (SY1749) were grown in the presence of galactose for various times and plated on YPD for analysis of [PSI<sup>+</sup>] phenotype. Data represent means; error bars represent standard deviation; n = 3. **(E)** Lysates isolated from galactose-inducible *HSP104* [PSI<sup>+</sup>]<sup>Strong</sup> (SY1748) or [PSI<sup>+</sup>]<sup>Weak</sup> (SY1749) cultures treated as described in **(D)** were analyzed by SDD-AGE and immunoblotting for Sup35. **(F)** Lysates were isolated from [PSI<sup>+</sup>]<sup>Strong</sup> (black) (SLL2606) and [psi<sup>-</sup>] (white) (SLL2119) strains that were treated as described in **(B)**, and heat-induced protein aggregates were quantified following differential centrifugation and Bradford assay. Data represent means; error bars represent standard errors; n ≥ 5. **(G)** Lysates were isolated from a [PSI<sup>+</sup>]<sup>Weak</sup> strain (SLL2600) that was incubated at 30°C or 40°C

Figure 3—figure supplement 1. Continued on next page

Figure 3—figure supplement 1. Continued

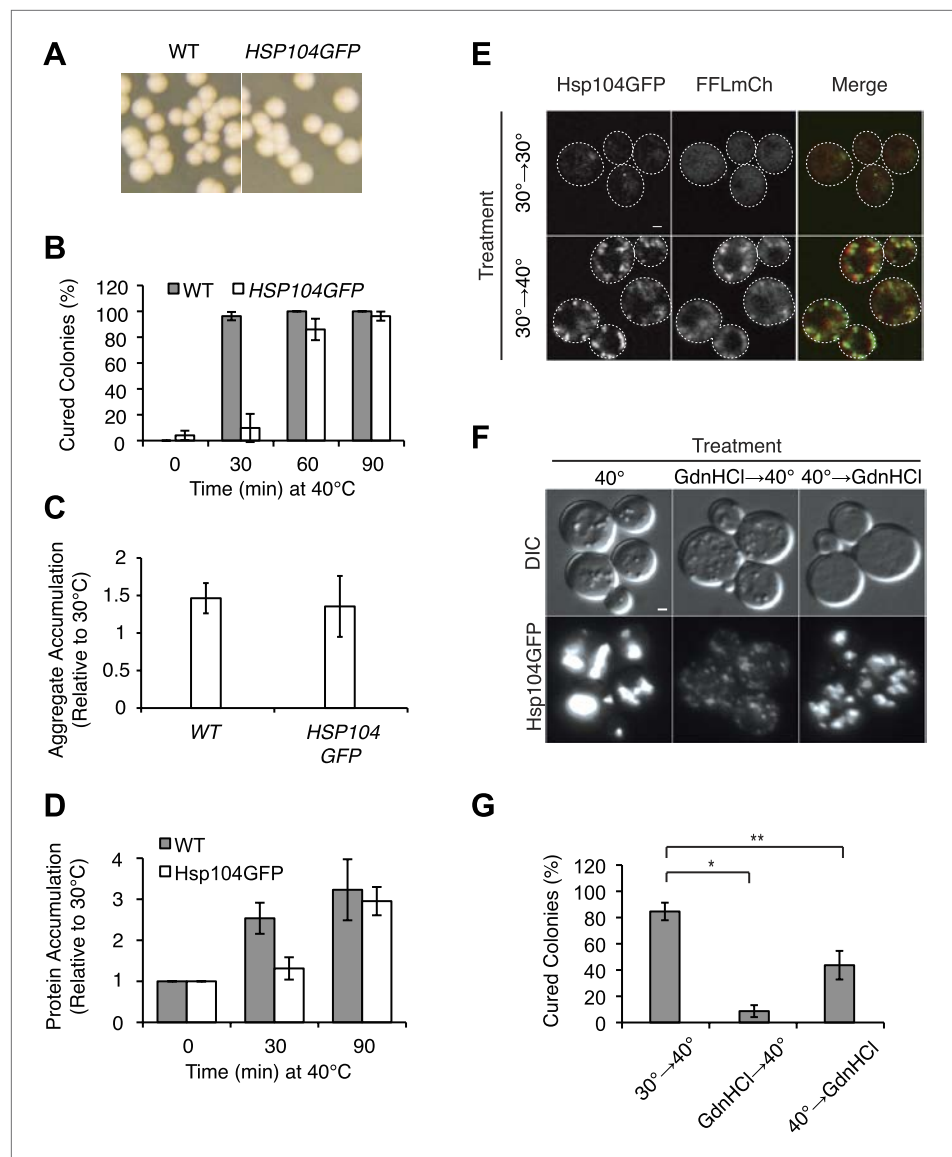
for 30 min in the absence (untreated) or presence of GdnHCl, and heat-induced protein aggregates were quantified following differential centrifugation and Bradford assay. Data represent means; error bars represent standard error;  $n = 3$ .

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**Figure 4.** Hsp104 engages heat-induced substrates upon thermal stress. **(A)** A [PSI<sup>+</sup>]<sup>Weak</sup> strain with a GFP-tagged endogenous Hsp104 (SY2126) was incubated at 30°C, 37°C, 40°C, or 40°C with GdnHCl for 30 min, and immunocapture in the presence (+) or absence (–) of anti-GFP antibodies (Ab) was performed on native lysates. Proteins were analyzed by SDS-PAGE and general protein staining (Flamingo, top), or immunoblotting for GFP (bottom). **(B)** [PSI<sup>+</sup>]<sup>Weak</sup> (SY2126) or [psi<sup>-</sup>] (SY2125) HSP104GFP strains were incubated at 30°C, 37°C, 40°C, or 37°C before 40°C for 90 min, and the pattern of Hsp104-GFP fluorescence was examined by microscopy. Scale bar = 1  $\mu$ m. **(C)** Quantification of Hsp104-GFP fluorescence pattern in [PSI<sup>+</sup>]<sup>Weak</sup> (SY2126) cells, treated as described in **(B)**: no localization (white); single dot (light gray); faint aggregate (medium gray); bright aggregate (dark gray); multiple bright aggregates (black);  $n > 25$ .

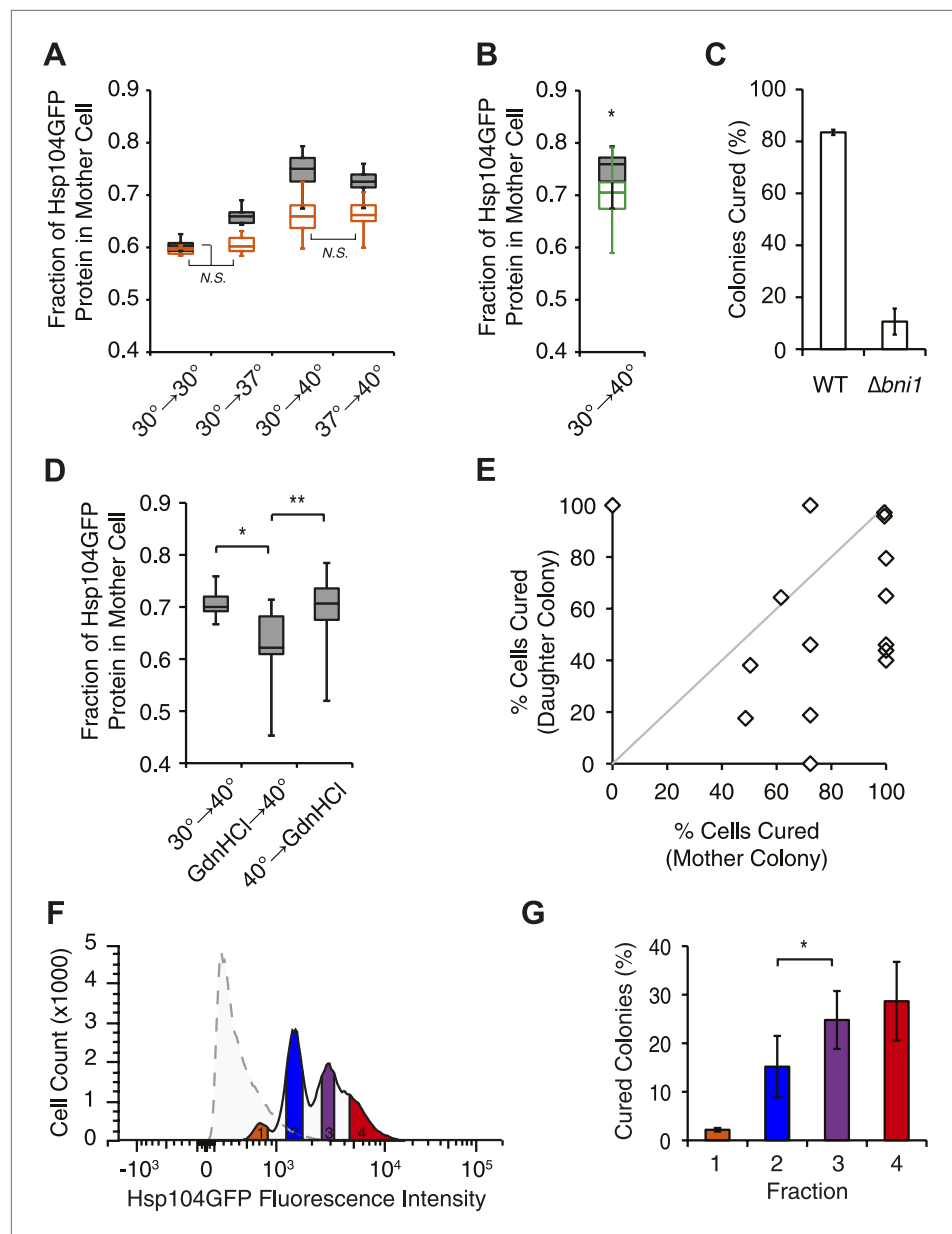
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**Figure 4—figure supplement 1.** Characterization of HSP104GFP strain. **(A)**  $[PSI^+]^{Weak}$  (SLL2600) and  $[PSI^+]^{Weak}$  HSP104GFP (SY2126) strains were grown at 30°C, plated on YPD, and incubated at 30°C for analysis of  $[PSI^+]$  colony color phenotype. **(B)**  $[PSI^+]^{Weak}$  (SLL2600, gray) and  $[PSI^+]^{Weak}$  HSP104GFP (SY2126, white) strains were incubated at 40°C for the indicated times and plated on YPD at 30°C for analysis of prion curing by colony color phenotype. Data represent means; error bars represent standard deviations;  $n = 3$ . **(C)** Lysates were isolated from WT (SLL2600) or HSP104GFP (SY2126)  $[PSI^+]^{Weak}$  strains that were incubated at 30°C or 40°C for 30 min, and heat-induced protein aggregates were quantified by differential centrifugation and Bradford assay. Data represent means; error bars represent standard error;  $n = 3$ . **(D)** Quantitative western blotting for Hsp104 was performed on lysates from  $[PSI^+]^{Weak}$  (SLL2600) and  $[PSI^+]^{Weak}$  HSP104GFP (SY2126) strains after incubation at 40°C for the indicated times. Data represent means; error bars represent standard deviations;  $n = 3$ . **(E)** Hsp104GFP and an mCherry-tagged firefly-luciferase (FFLmCh) reporter were visualized in a  $[PSI^+]^{Weak}$  strain (SY2802) by microscopy following incubation at 30°C or after a 30-min recovery from an incubation at 40°C for 90 min (30°C → 40°C). Scale bar = 1  $\mu$ m. **(F)** Hsp104GFP was visualized in a  $[PSI^+]^{Weak}$  strain (SY2126) by microscopy after a 90-min recovery from incubation at 40°C for 90 min in the absence (40°C) or presence of GdnHCl added before (GdnHCl → 40°C) or after (40°C → GdnHCl) heat treatment. Scale bar = 1  $\mu$ m. **(G)**  $[PSI^+]^{Weak}$  HSP104GFP cultures (SY2126) treated as described in **(F)** were plated on YPD and incubated at 30°C for analysis of  $[PSI^+]$  colony color phenotype. Data represent means; error bars represent standard deviations;  $n = 3$ ; \* $p = 0.0001$ , \*\* $p = 0.0089$ .

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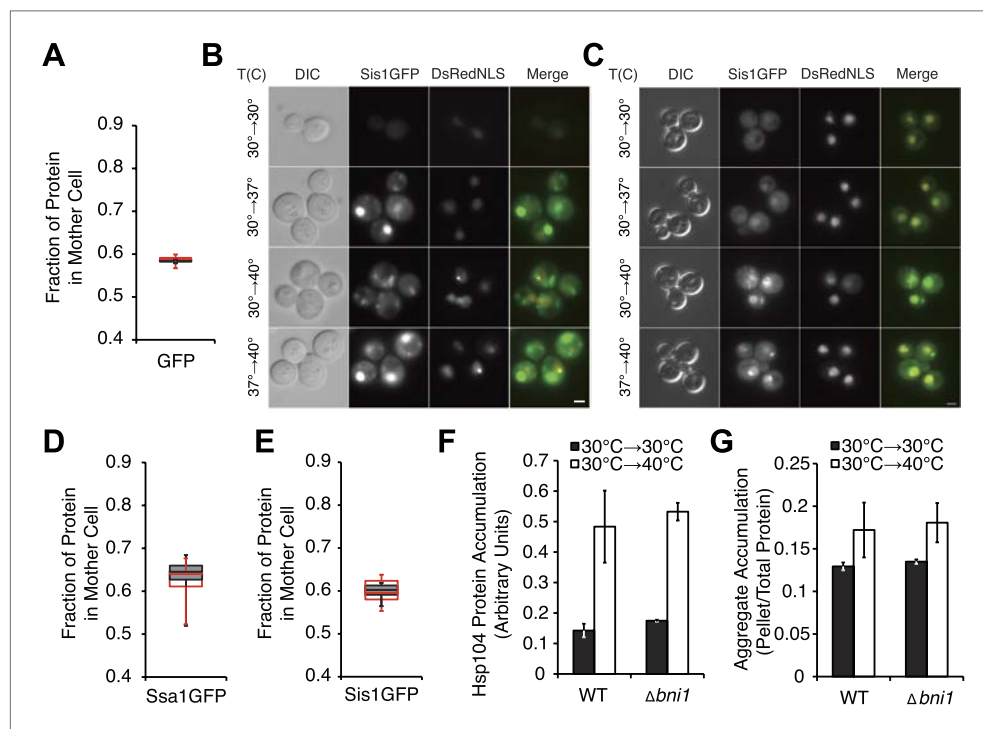




**Figure 5.** Curing results from the asymmetric localization of Hsp104 following thermal stress. **(A)** A  $[PSI^-]^{Weak}$  *HSP104GFP* culture (SY2126) was imaged over time in a microfluidics chamber at 30°C after a 30 min incubation at 30°C, 37°C, 40°C, or 37°C before 40°C. Fluorescence intensity in daughter and mother cells was quantified at the first cell division in cells that were budded (gray) or unbudded (orange) after thermal stress. Lines represent medians; boxes represent upper and lower quartiles, and whiskers represent maximum and minimum. All pairwise comparisons are significantly distinct, with a  $p < 0.015$ , except where indicated (N.S.), by unpaired t-test;  $n \geq 10$ . **(B)** A  $[PSI^-]^{Weak}$  *HSP104GFP* WT (SY2126, gray) or *BNI1* deletion strain ( $\Delta bni1$ ) (SY2486, green) was imaged over time in a microfluidics chamber at 30°C after a 30 min incubation at 40°C. Fluorescence intensity in daughter and mother cells was quantified at the first cell division. Lines represent medians; boxes represent upper and lower quartiles; and whiskers represent maximum and minimum;  $n \geq 14$ ;  $p = 0.0075$  by unpaired t-test. **(C)**  $[PSI^-]^{Weak}$  WT (SLL2600) or  $\Delta bni1$  strains (SY1888), treated as described in **(B)**, were plated on YPD to analyze curing by colony color phenotype. Data represent means; error bars represent standard deviations;  $n = 3$ ;  $p < 0.0001$  by unpaired t-test. **(D)** A  $[PSI^-]^{Weak}$  *HSP104GFP* strain (SY2126) was imaged over time in a microfluidics chamber at 30°C after a 30 min incubation at 40°C and with GdnHCl added before or after the 40°C incubation. Fluorescence intensity in daughter and mother cells was quantified at the first cell division. Lines represent medians; boxes represent upper and lower quartiles; and whiskers represent maximum and minimum;  $n > 11$ ; \* $p = 0.0003$ , \*\* $p = 0.0026$  by unpaired t-test. **(E)** Figure 5. Continued on next page

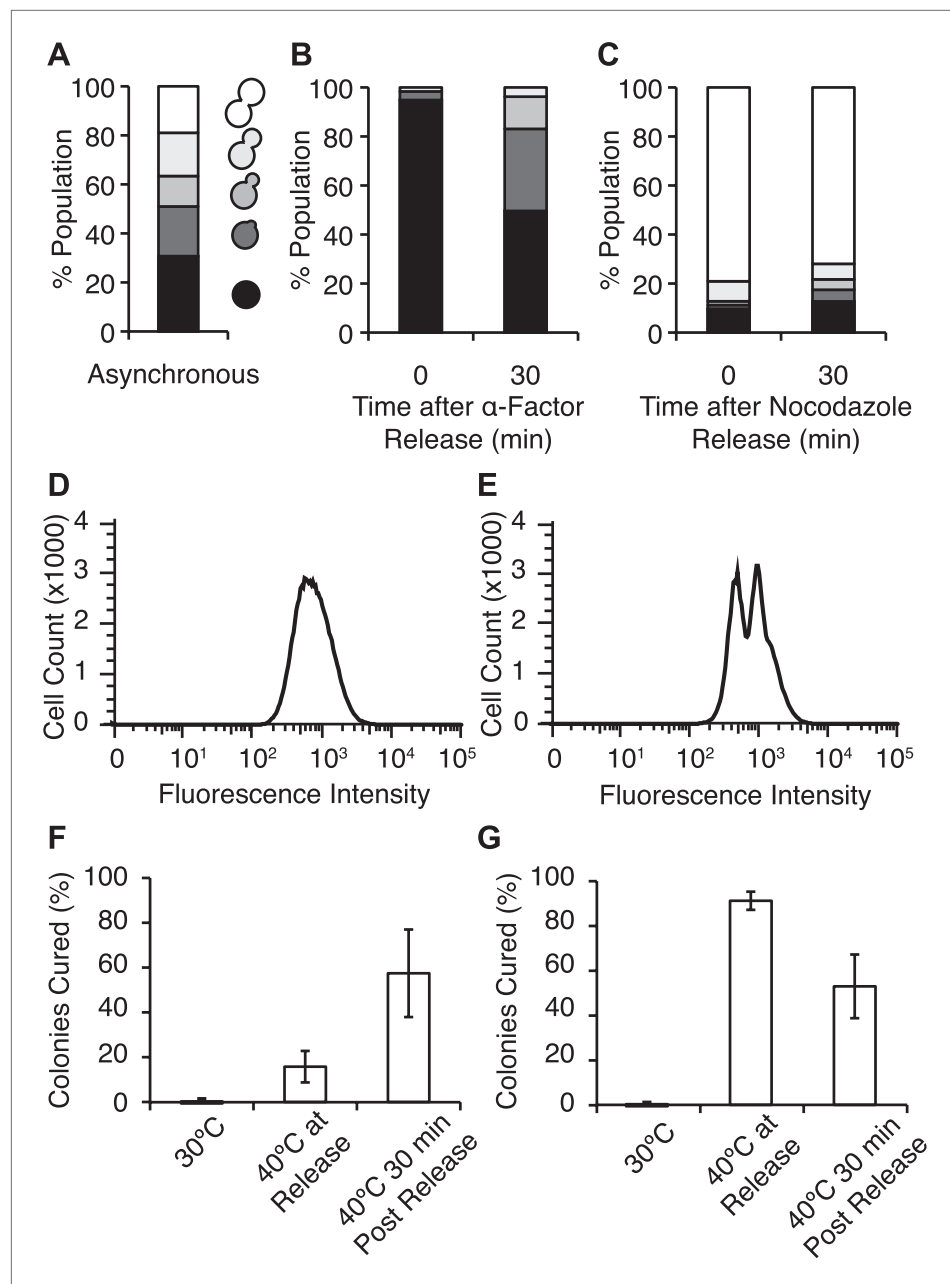
Figure 5. Continued

A [*PSI*<sup>+</sup>]<sup>Weak</sup> strain (SLL2600) was incubated at 40°C for 30 min and plated on rich medium. Mother and daughter pairs were separated by micromanipulation and allowed to form colonies, which were then dispersed to YPD for analysis of curing by colony color phenotype. *n* = 15. (F) A [*PSI*<sup>+</sup>]<sup>Weak</sup> *HSP104GFP* culture (SY2126) was incubated at 30°C (dotted) or at 40°C for 30 min and allowed to recover for 30 min at 30°C (solid) before analysis of GFP fluorescence intensity by flow cytometry. Based on these intensities, cells were sorted into four fractions (orange, blue, purple, red) by FACS. (G) Cells collected in (F) were plated on YPD to analyze curing by colony color phenotype. Data represent means; error bars represent standard deviations; *n* = 2; \**p* = 0.02 by paired *t*-test. DOI: 10.7554/eLife.04288.010



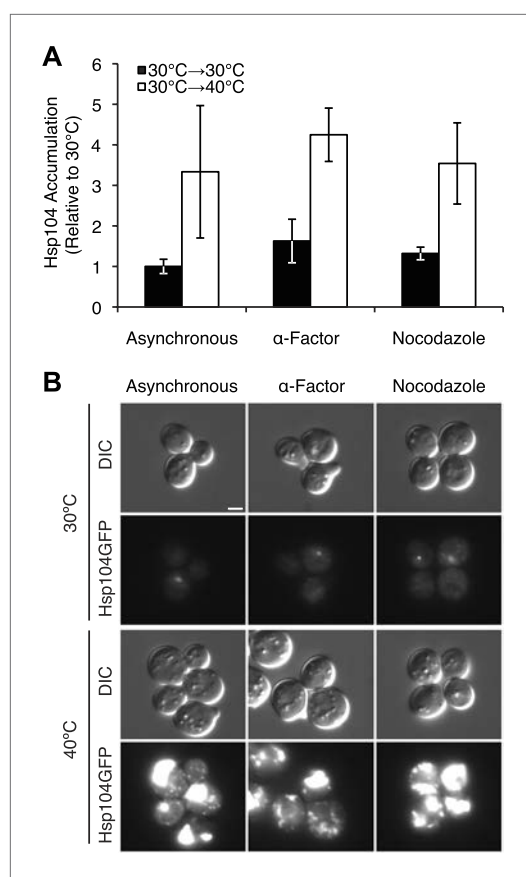
**Figure 5—figure supplement 1.** Characterization of chaperone asymmetric retention following thermal stress. (A) A [*psi*<sup>+</sup>] strain expressing heat-inducible untagged GFP (SY2091) was imaged over time in a microfluidics chamber at 30°C after 30 min incubation at 40°C (red) or 30°C (gray). Fluorescence intensity in daughter and mother cells was quantified at the first cell division in budded cells. Lines represent medians, boxes represent upper and lower quartiles, and whiskers represent maximum and minimum; *n* ≥ 11. (B) A [*PSI*<sup>+</sup>]<sup>Weak</sup> strain expressing a GFP-tagged endogenous Ssa1 and DsRedNLS (SY2659) was imaged after a 90 min incubation at 30°C, 37°C, 40°C, or 37°C before 40°C. Scale bar = 2 μm. (C) A [*PSI*<sup>+</sup>]<sup>Weak</sup> strain expressing a GFP-tagged endogenous Sis1 and DsRedNLS (SY2485) was imaged after a 90-min incubation at 30°C, 37°C, 40°C, or 37°C before 40°C. Scale bar = 2 μm. (D) A [*PSI*<sup>+</sup>]<sup>Weak</sup> *SSA1GFP* culture (SY2658) was imaged over time in a microfluidics chamber at 30°C after a 30 min incubation at 40°C (red) or 30°C (gray). Fluorescence intensity in daughter and mother cells was quantified at the first cell division in budded cells. Lines represent medians, boxes represent upper and lower quartiles, and whiskers represent maximum and minimum; *n* > 15. (E) A [*PSI*<sup>+</sup>]<sup>Weak</sup> *SIS1GFP* culture (SY2447) was imaged over time in a microfluidics chamber at 30°C after a 30 min incubation at 40°C (red) or 30°C (gray). Fluorescence intensity in daughter and mother cells was quantified at the first cell division in budded cells. Lines represent medians, boxes represent upper and lower quartiles, and whiskers represent maximum and minimum; *n* ≥ 7. (F) Quantitative immunoblotting for Hsp104 was performed on lysates from WT (SLL2600) or Δ*bni1* (SY1888) [*PSI*<sup>+</sup>]<sup>Weak</sup> cultures treated at 30°C (black) and 40°C (white) for 30 min following SDS-PAGE. Data represent means; error bars represent standard deviations; *n* = 3. (G) Lysates were isolated from WT (SLL2600) or Δ*bni1* (SY1888) [*PSI*<sup>+</sup>]<sup>Weak</sup> strains that were incubated at 30°C or 40°C for 30 min, and heat-induced protein aggregates were analyzed by differential centrifugation and Bradford assay. Data represent means; error bars represent standard error; *n* = 3.

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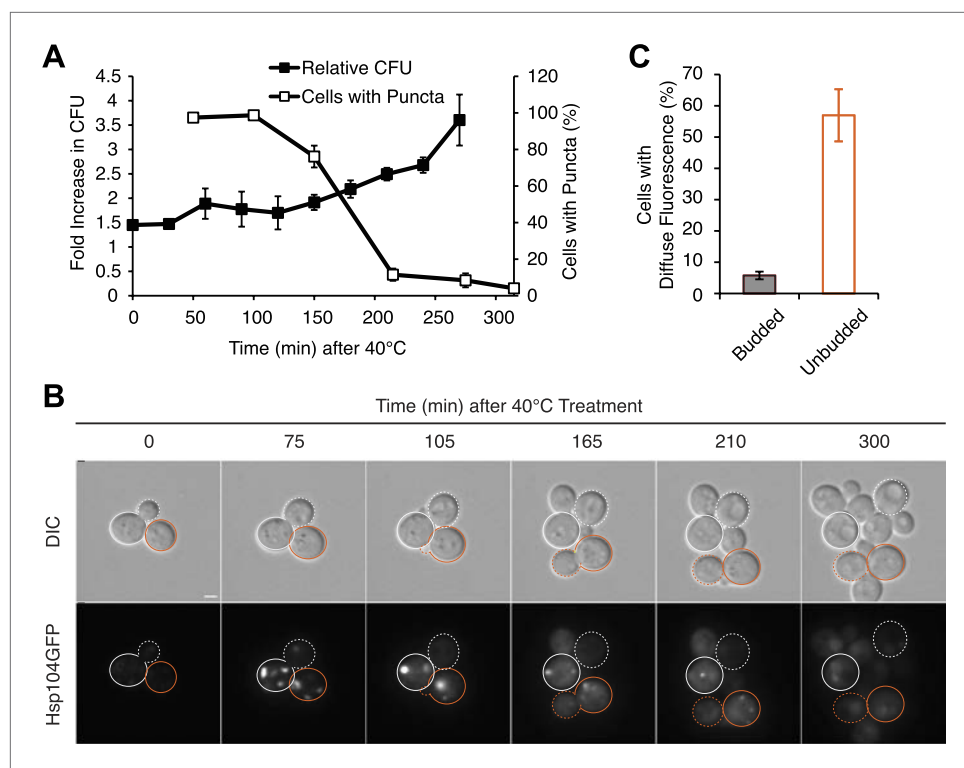
**Figure 6.** Efficient curing occurs in late cell-cycle staged cells following thermal stress. **(A)** Single cells from an asynchronous WT  $[PSI^+]^{Weak}$  culture (SLL2600) were scored for morphology following bright-field imaging by microscopy: unbudded (black), tiny bud (dark gray), small bud (gray), medium bud (light gray), large bud (white).  $n = 153$ . **(B)**  $\alpha$ -factor-arrested cultures were analyzed as in **(A)** over time after release.  $n \geq 250$ . **(C)** Nocodazole-arrested cultures were analyzed as in **(A)** over time after release.  $n \geq 175$ . **(D)** A  $[PSI^+]^{Weak}$  HSP104GFP strain (SY2126) released from  $\alpha$ -factor arrest was incubated at 40°C (solid black lines) for 30 min before analysis by flow cytometry. 100,000 cells were analyzed per sample. **(E)** A  $[PSI^+]^{Weak}$  HSP104GFP strain (SY2126) released from nocodazole arrest was incubated at 40°C (black lines) for 30 min before analysis by flow cytometry. 100,000 cells were analyzed per sample. **(F)**  $\alpha$ -factor-arrested cultures (SLL2600) were incubated at 40°C for 30 min immediately or 30 min after release, and curing was quantified by colony color phenotype after plating on YPD at 30°C. Data represent means; error bars represent standard deviations;  $n = 3$ ;  $p = 0.0255$  by unpaired t-test. **(G)** Nocodazole-arrested cultures (SLL2600) were incubated at 40°C for 30 min immediately or 30 min after release, and curing was quantified colony color phenotype after plating on YPD at 30°C. Data represent means; error bars represent standard deviations;  $n = 3$ ;  $p = 0.0263$  by unpaired t-test.

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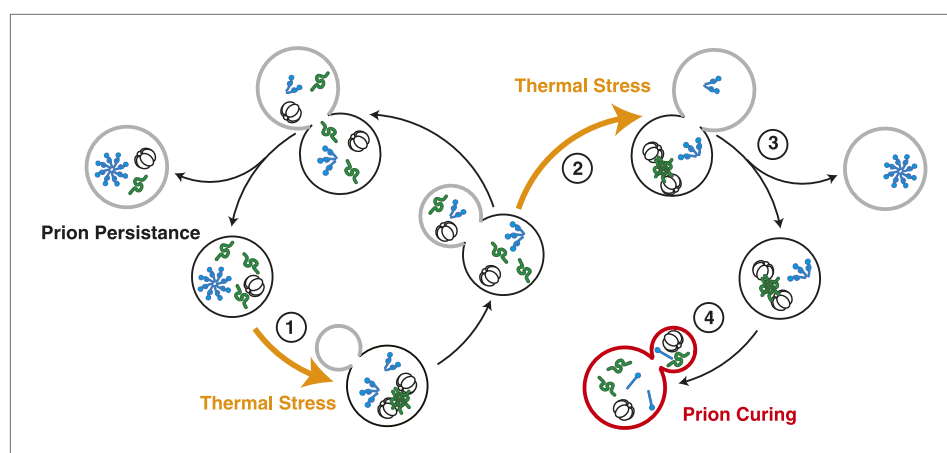
**Figure 6—figure supplement 1.** Characterization of chaperone accumulation and engagement in arrested cultures. **(A)** Lysates were isolated from asynchronous,  $\alpha$ -factor-arrested, or nocodazole-arrested  $[PSI^+]^{Weak}$  cultures (SLL2600) following incubation at 30°C (black) or 40°C (white) for 30 min, and the levels of Hsp104 were determined by quantitative immunoblotting following SDS-PAGE. Data represent means; error bars represent standard deviations;  $n = 3$ . **(B)** Asynchronous,  $\alpha$ -factor-arrested, and nocodazole-arrested  $[PSI^+]^{Weak}$  *HSP104GFP* (SY2126) cultures were treated for 90 min at 30°C or 40°C and imaged by microscopy. Scale bar = 2  $\mu$ m.

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**Figure 7.** Substrate–chaperone engagement must exceed time to cell division to induce curing. **(A)** The number of  $[PSI^{+}]^{Weak}$  *HSP104GFP* (SY2126) cells containing fluorescent foci was quantified in cultures recovering at 30°C over time following a 90 min incubation at 40°C (white). Colony forming units in these cultures were quantified by plating (black). Data represent means; error bars represent standard deviations;  $n = 3$ . **(B)**  $[PSI^{+}]^{Weak}$  *HSP104GFP* cells (SY2126) treated for 30 min at 40°C and imaged over time in a microfluidics chamber are shown. Cells that were budded at the time of thermal stress are outlined in white, while unbudded cells are outlined in orange. Solid lines mark mothers, and dotted lines mark daughters. Scale bar = 1  $\mu$ m. **(C)** A  $[PSI^{+}]^{Weak}$  *HSP104GFP* strain (SY2126) was imaged over time in a microfluidics at 30°C after a 30 min incubation at 40°C chamber. Budded or unbudded cells were scored at the first cell division for the presence or absence of fluorescent aggregates. Data represent means; error bars represent standard deviations;  $n = 3$ ;  $p = 0.0005$  by unpaired t-test.

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**Figure 8.** Model for Sup35 amyloid resolubilization and curing upon thermal stress. Upon thermal stress, cellular proteins (green) misfold and aggregate, leading to the induction and recruitment of Hsp104 (barrel). If thermal stress occurs in unbudded cells (1), these aggregates are resolved prior to cell division, allowing the partitioning of Hsp104 to both mother (black) and daughter (gray) cells (left). If thermal stress occurs in budded cells (2), heat-induced aggregates persist upon cell division (3), leading to the asymmetric retention of Hsp104 in mother cells. Both heat-induced aggregates (green) and Sup35 amyloid (blue corkscrews) are resolved in cells accumulating high levels of Hsp104, leading to curing (red, 4).

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