Dulle et al., http://www.jcb.org/cgi/content/full/jcb.201307040/DC1

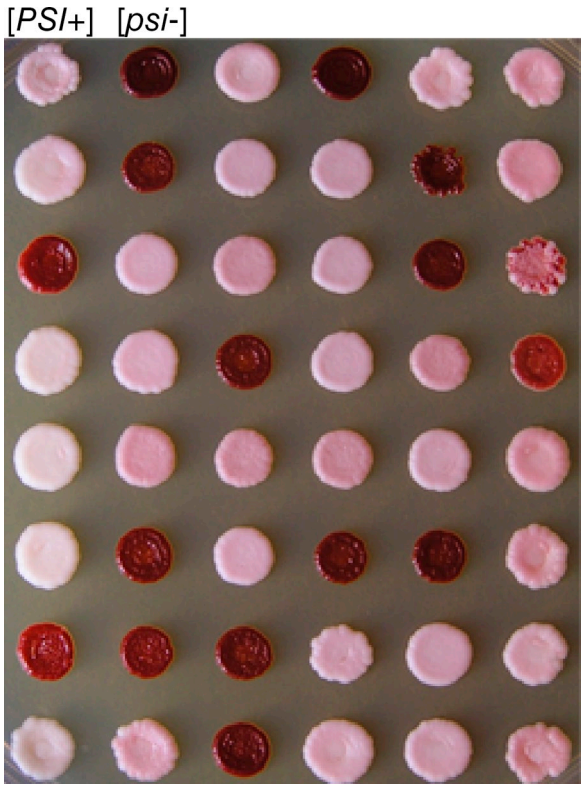

Figure S1. ***hsp104-R830S* haploids crossed to [*psi*−] haploids results in [*PSI+*] diploids.** The presence of phenotypically undetectable [*PSI+*] prion propagons was apparent when red *hsp104-R830S* haploids were mated to wild type red [*psi*−] haploids. Though the *hsp104-R830S* haploids appear [*psi*−] (red in color due to efficient translation termination of *ade1-14*), prion propagons are still present that can efficiently template soluble Sup35 in the presence of wild-type *HSP104* to produce mostly pink [*PSI+*] *HSP104:hsp104-R830S* heterogeneous diploids. Diploids from the cross were spotted onto YPD plates and [*PSI+*] and [*psi*−] controls are labeled in the top left corner of the plate.

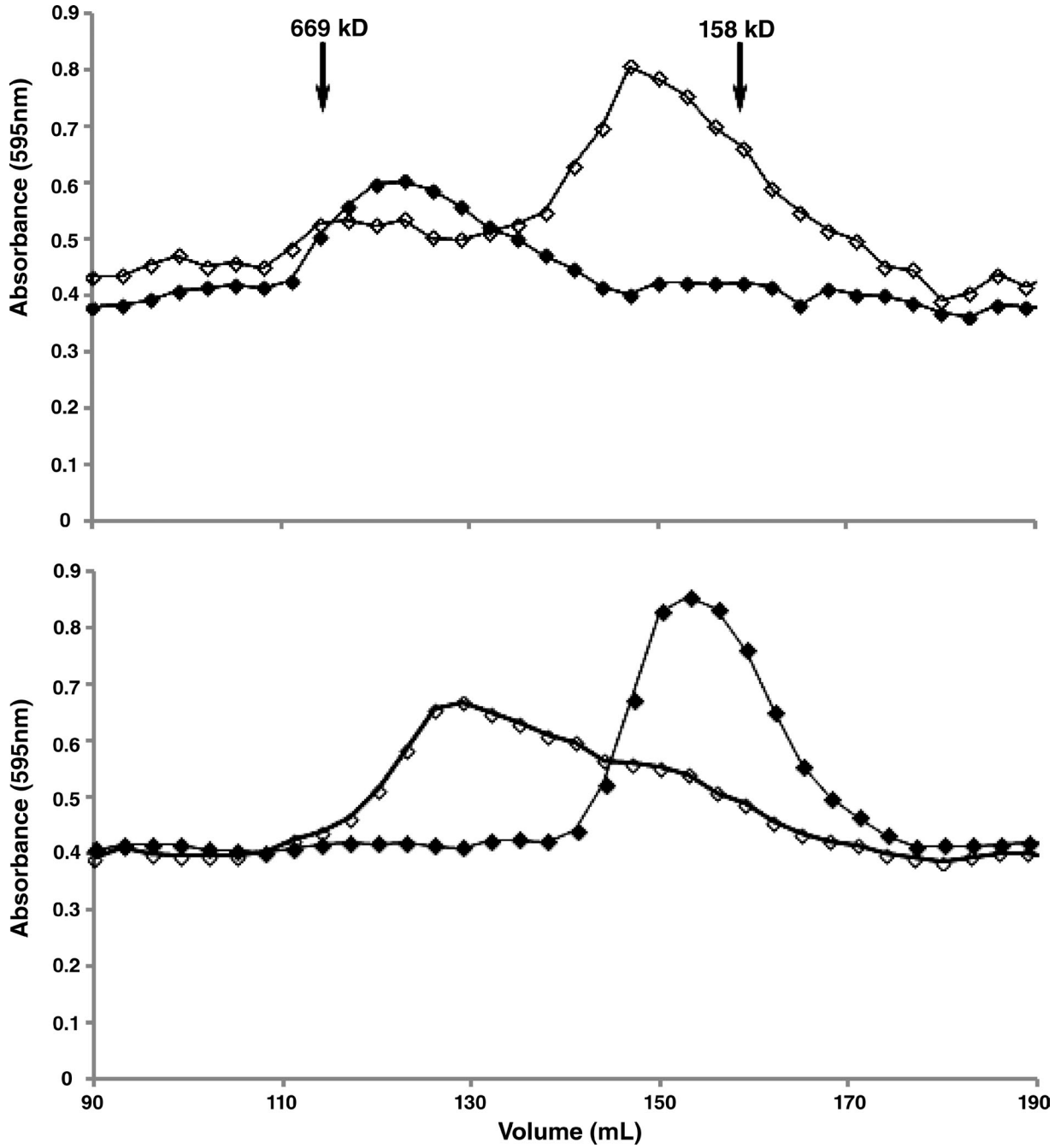

Figure S2. **Hsp104-R830S does not form efficient hexamers.** Recombinant Hsp104 (top) and Hsp104-R830S (bottom) were incubated either with ATP (◆) or without ATP (◇) and applied to an S-300 size-exclusion column. Fractions of the eluate were collected and Bradford analysis (absorbance at 595 nm) was performed to quantify the amount of Hsp104 protein in each fraction. Both recombinant Hsp104 and Hsp104-R830S without ATP migrate mainly as monomers or dimers. Incubation of wild-type Hsp104 with ATP causes hexamers to form, but Hsp104-R830S incubated with ATP is distributed across several fractions, suggesting an inability to efficiently hexamerize (or maintain stable hexamers) in response to ATP binding. Proteins of known molecular weights, thyroglobulin (669 kD) and aldolase (158 kD), were also applied to the column and their elution peaks are labeled for reference.

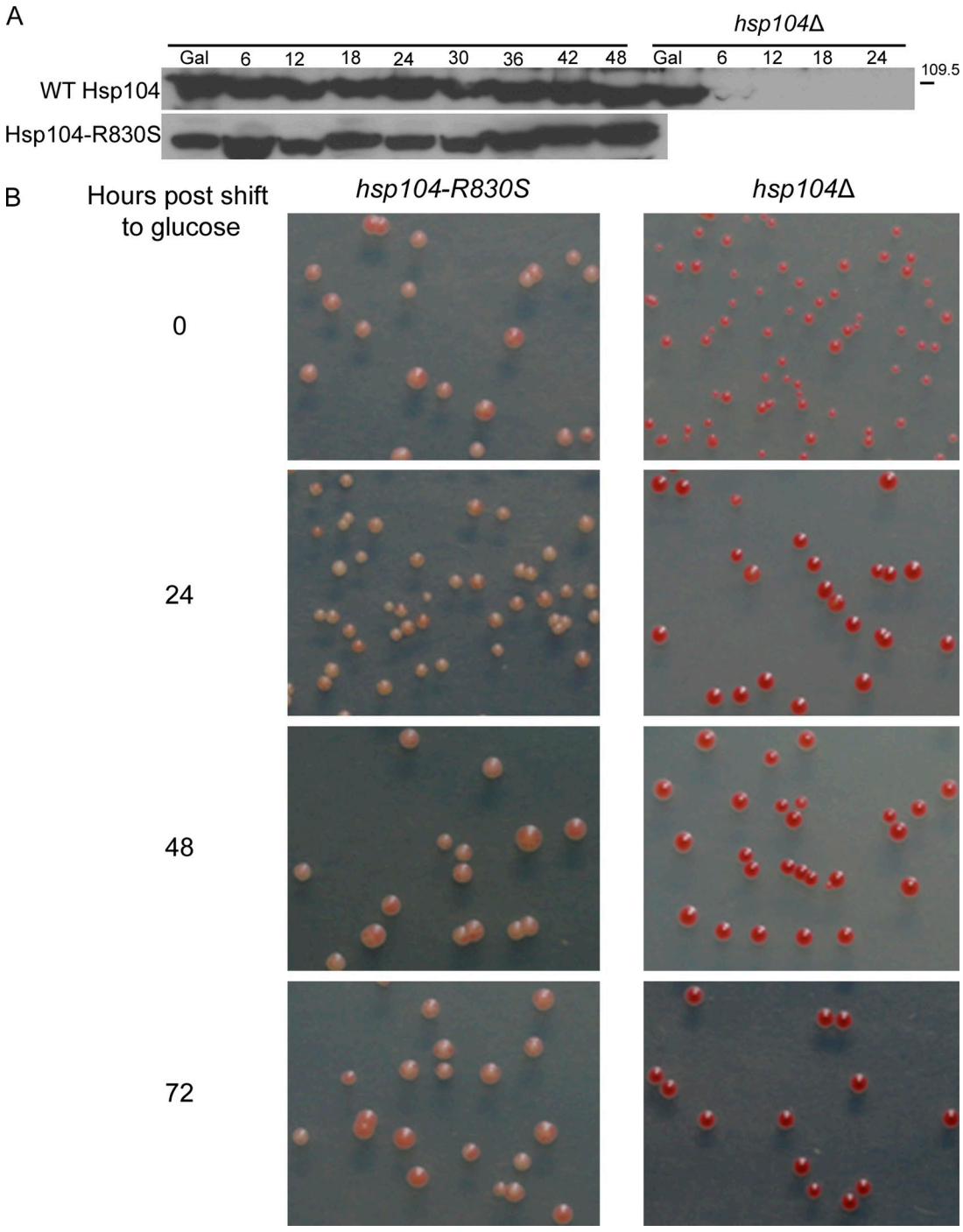

Figure S3. **Glucose represses expression of wild-type *HSP104* but [*PSI+*] propagons persist in the absence of SDS-resistant aggregates.** (A) To establish a system whereby we could determine the effect of *hsp104-R830S* on pre-existing [*PSI+*] aggregates, we transformed a plasmid expressing wild-type *HSP104* driven by the galactose promoter (pRS416-GAL-*HSP104*) into heterozygous diploids expressing either wild-type *HSP104* or *hsp104-R830S* and *hsp104*Δ on the chromosome. To determine if switching the cells expressing pRS416-GAL-*HSP104* to glucose efficiently repressed wild-type *HSP104*, we performed a Western blot to detect the amount of Hsp104 expressed while growing in galactose (plasmid-borne wild-type *HSP104* is expressed, Gal) and for various amounts of time after switching the cells to glucose (plasmid-borne wild-type *HSP104* is repressed, 6–48 h for wild-type and *hsp104-R830S* and 6–24 h for *hsp104*Δ). We compared wild-type *HSP104* and *hsp104-R830S* cells containing pRS416-GAL-*HSP104* to *hsp104*Δ cells containing pRS416-GAL-*HSP104*. After only 6 h in glucose, we could detect no Hsp104 in the *hsp104*Δ cells. This suggests that over the time course of our assay (Fig. 3 C), wild-type *HSP104* from the plasmid was efficiently repressed while growing in glucose. Total protein loading was assessed by membrane stain. (B) We investigated the effect of *hsp104-R830S* on pre-existing aggregates of Sup35 by both SDD-AGE (Fig. 3, C and E) and by nonsense suppression phenotype (using *ade1-14*) after repression of wild-type *HSP104*. Briefly, cells (*hsp104-R830S* or *hsp104*Δ) carrying galactose-inducible wild-type *HSP104* on a plasmid were grown in low galactose (0.25%) to maintain [*PSI+*]. Cells were then switched to glucose media to repress wild-type *HSP104*, grown for various times in liquid glucose media (hours after shift to glucose), and then plated on media plates lacking uracil and containing galactose (0.25%) to de-repress wild-type *HSP104*. The restoration of wild-type *HSP104* allowed for assessment of whether the cells contained any species capable of propagating [*PSI+*]. Throughout the time course tested, the *hsp104-R830S* cells were phenotypically [*PSI+*] on galactose plates while the *hsp104*Δ cells had truly lost the prion phenotype (red in color indicating efficient translation termination).