Figure S1  Abundance of Hsp104/ClpB hybrid proteins. Lysates of cells of strain 1408 expressing the indicated wild type or hybrid chaperones were separated on SDS-PAGE gels, blotted and probed with antibodies to Hsp104 or ClpB as indicated. Load panel shows representative portion of the blotted membrane stained by amido-black. There are noticeable variations in ability to detect hybrid proteins due to re-assortment of epitopes. It is evident that the ClpB antibodies recognize a major epitope in the M region, but do not recognize the NTD or NBD1. The Hsp104 antibodies react well with the Hsp104 NTD and an epitope that spans the M-NBD2 region, but do not react with NBD1 and NBD2. Thus, the weaker signals for B44B and BB4B are due at least in part to the lower recognition of these proteins by the antibodies.
Figure S2  \([PSI^-]\) propagation in BKE cells is cured by guanidine. Cells were grown for six days at 23° without adenine (upper plates) or with limiting adenine (lower plates) and either lacking (left plates) or containing (right plates) 3 mM guanidine-hydrochloride. Guanidine impairment of ClpB and Hsp104 weakens \([PSI^-]\) and therefore reduces growth on medium without adenine (upper right). Prion weakening caused by guanidine is also seen as increased accumulation of pigment on limiting adenine (lower right). Bottom panels show magnification of the limiting adenine plates to show color difference more clearly.
Figure S3  Confirmation of [PSI+] propagation in BKEJ cells and in BK*E cells expressing J*, Y* and S*. Control cells in lanes 1, 2 and 3 express ClpB (B), Hsp104 (104) or BKE as indicated. Cells in lanes 4 and 5 express BKEJ and are [PSI+] (+) and [psi−] (−), respectively. Cells in lanes 6-9 express BK*E and the indicated J-protein (same strains as in Figure 4B). All strains were crossed with [psi−] strain 621. Growth of diploids on medium lacking adenine (lower panel) indicates presence of [PSI+] (e.g. samples 2, 3, 4, 9).
Figure S4 Thermotolerance of strain BY4741 hsp104Δ cells expressing Hsp104 (104) or empty vector (ev), or various combinations of E. coli chaperones ClpB (B), DnaK (K), DnaJ (J) and GrpE (E). Cells express indicated chaperones from genes regulated by the constitutively active glyceraldehyde-3-phosphate dehydrogenase promoter on single-copy plasmids. Cells grown at 30° on liquid medium selecting for plasmids to OD₆₀₀ = 0.25 were pretreated at 37° for 30 minutes and then exposed to 50° for 30 min. Five microliters of five-fold serial dilutions of cultures were grown three days at 30° on YPAD plates.