Supporting Information

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SI Text

Elimination of Toxic PolyQ Amyloid by Gpg1. Propagation of polyQ nonprion amyloids was also examined by using a yeast-based assay for polyQ toxicity developed by Sherman and colleaguess (1). Because it is known that toxicity of polyQ is seen only in the presence of [*PSI*$^+$] or [*PIN*$^+$] prion (1, 2), over-expression of Gpg1 is likely to hamper polyQ toxicity in [*PIN*$^+$] cells. We expressed a GFP-tagged polypeptide derived from mutant (103Q) form of huntingtin under the galactose-inducible *GAL1* promoter in [*PIN*$^+$] cells (NPK562). As expected, expression of 103Q on galactose plates was toxic to yeast, whereas the same cells grew normally on glucose plates (Fig. S1*A*). When Gpg1 was over-expressed from pGPG1, 103Q-induced toxicity was completely blocked (see Fig. S1*A*). Under these conditions, 103Q-GFP formed aggregates with strong fluorescence foci in the absence of pGPG1, whereas 103Q-GFP appeared diffusely distributed in the cytoplasm in the presence of pGPG1 (Fig. S1*B*). These findings confirm that Gpg1 hampers toxic polyQ aggregates in [*PIN*$^+$] cells, as expected.

Methods. ***Strains and manipulations.*** Elimination of [*PSI*$^+$], [*URE3*], or [*PIN*$^+$] prion by Gpg1 was examined by transforming cells with plasmid pGPG1 expressing Gpg1 from the strong constitutive *GPD* promoter: the relevant prion phenotype of transformants was monitored by colony color based on the expression of the *ade1–14* allele or the *DAL5-ADE2* fusion, and by formation of Rnq1-GFP fluorescent foci and SDS-stable Rnq1 polymers, respectively, as described previously (3). To monitor colony color based on the *ade1–14* nonsense suppression or transcriptional (de)repression of *ADE2* expression, yeast cells were grown on YPD plates for 4 days at 30 °C. The null *gpg1*Δ strain was constructed by transformation of NPK265 strain with a PCR product amplified from the *gpg1*::*KanMX* DNA (American Type Culture Collection strain no. 14488) by using primers ≈100-bp upstream and downstream of the *GPG1* coding sequence (5′-CACTCAATTTCAACGAGG-3′ and 5′-CCCATTTGCCAC-CAGCG-3′). Transformation was performed as described previously (3).

Plasmids. The original plasmid pN5 and its *Sau*3AI-BamHI deletion derivative pN5–1 (Fig. 1*A*) are derivatives of pRS423 (*HIS3* marker). The 378-bp coding sequence of *GPG1* was amplified by PCR using primers 5′-TTTGGATCCAATGTTT-TATCTAAGTGAC-3′ and 5′-TTTCTCGAGCTAGTGATA-GAACCTTCTC-3′, and cloned into expression plasmids pRS413GPDp (*HIS3* marker) and pRS414GPDp (*TRP1* marker), giving rise to Gpg1-expression plasmid pGPG1 marked with *HIS3* or *TRP1*. The Rnq1-GFP expression plasmid, pRS414CUP1p-Rnq1-GFP, and the polyQ-toxicity assay plasmid are described previously (1, 3). Hsp104-expression plasmid pHSP104 was constructed by cloning PCR-amplified *HSP104* coding sequence (primers: 5′-GGGGGATCCATATGAAC-GACCAAACGCAATT-3′ and 5′-GGGGCTCGAGTTATAT-TACTGATTCTTGTTCGAA-3′) into expression plasmids pRS423GPDp (*HIS3* marker) and pRS425GPDp (*LEU2* marker). The YFP reporter fusion to Sup35NM domain, pRS413CUP1p-NM-YFP, was described previously (3). The CFP reporter fusion to Gpg1, pRS424CUP1p-Gpg1-CFP, was constructed as described previously except that a peptide linker, GGGSGGGSG, was inserted at the junction (3).

1. Meriin AB, *et al.* (2002) Huntington toxicity in yeast model depends on polyglutamine aggregation mediated by a prion-like protein Rnq1. *J Cell Biol* 157:997–1004.
2. Gokhale KC, Newnam GP, Sherman MY, Chernoff YO (2005) Modulation of prion-dependent polyglutamine aggregation and toxicity by chaperone proteins in the yeast model. *J Biol Chem* 280:22809–22818.
3. Kurahashi H, Ishiwata M, Shibata S, Nakamura Y (2008) A regulatory role of the Rnq1 nonprion domain for prion propagation and polyglutamine aggregates. *Mol Cell Biol* 28:3313–3323.
4. Bailleul PA, Newnam GP, Steenbergen JN, Chernoff YO (1999) Genetic study of interactions between the cytoskeletal assembly protein SIa1 and prion-forming domain of the release factor Sup35 (eRF3) in *Saccharomyces cerevisiae*. *Genetics* 153:81–94.
5. Brachmann A, Baxa U, Wickner RB (2005) Prion generation *in vitro*: Amyloid of Ure2p is infectious. *EMBO J* 24:3082–3092.
6. Kurahashi H, Nakamura Y (2007) Channel mutations in Hsp104 hexamer distinctively affect thermotolerance and prion-specific propagation. *Mol Microbiol* 63:1669–1683.
7. Brachmann CB, *et al.* (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: A useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14:115–132.

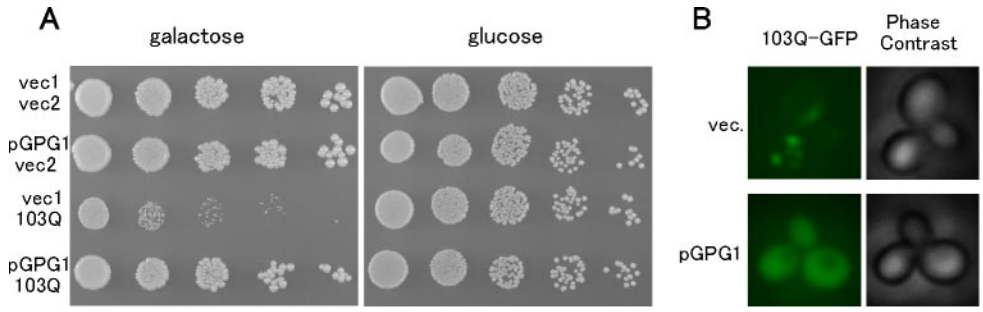

Fig. S1. Effect of Gpg1 over-expression on polyQ aggregation and toxicity. (*A*) 103Q-induced toxicity and the blockage of this toxicity by Gpg1 in [*PIN*$^+$] strains. Mid-log phase cultures of [*PIN*$^+$] cells (NPK562) carrying the indicated sets of plasmids were serially diluted 5-fold and spotted on SC (glucose) (*Right*) and SGal (galactose) (*Left*) plates and then grown for 3 days (*Right*) or 6 days (*Left*), respectively. Plasmids: vec1, empty vector pRS413; pGPG1, pRS413GPDp derivative expressing Gpg1; vec2, empty vector pYES2; 103Q, pYES2 derivative expressing 103Q from the *GAL1* promoter. (*B*) 103Q-GFP aggregates monitored by fluorescent microscopy. The [*PIN*$^+$] strain (NPK562) carrying the 103Q-GFP plasmid was transformed with the empty plasmid pRS413 (vec.) and with pGPG1. The resulting double transformants were grown in SC broth and mid-log phase cultures were transferred to SGal liquid medium for 6 h. Phase contrast (*Right*) and fluorescent (*Left*) images of 103Q-GFP are shown.

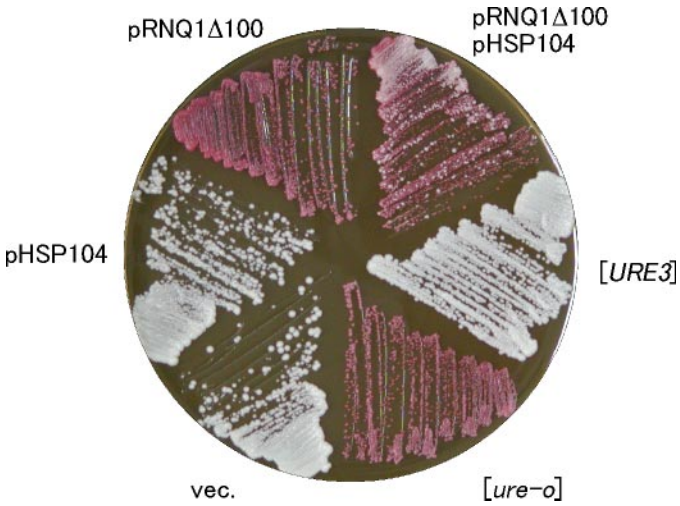

Fig. S2. Compensatory effect of over-expressing Hsp104 on Rnq1Δ100-induced prion elimination. NPK435 ([*URE3*] [*PIN*$^+$]) strain was transformed with pHSP104 and/or pRNQ1Δ100 (pRS415 derivative carrying *rnq1Δ100*; constructed in the present study), and transformants were selected on SC-his-leu after 3 days and regrown on YPD for 4 days to monitor colony color as shown in Fig. 3*C*.

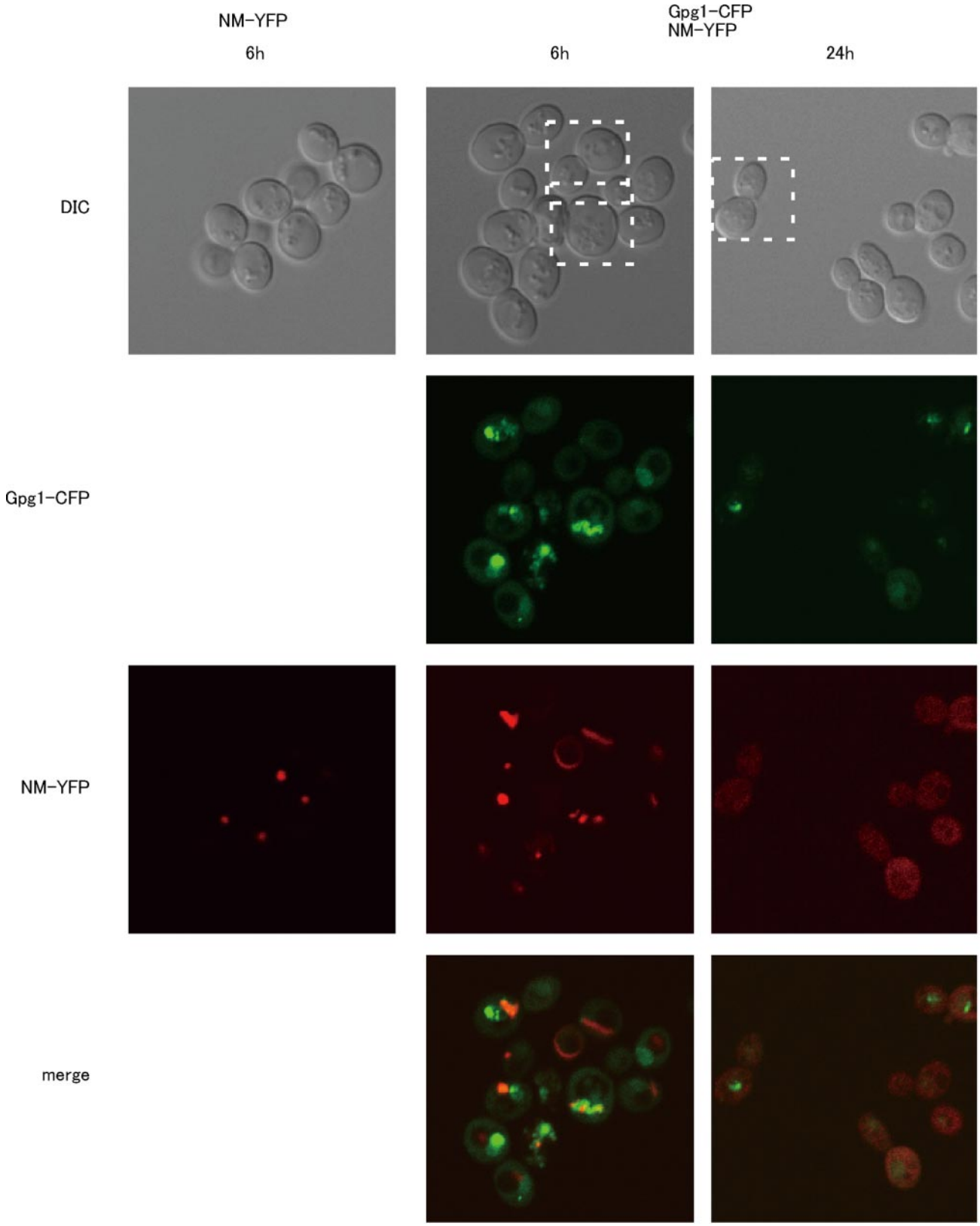

Fig. S3. Wider field image of confocal fluorescence microscopy of Sup35NM-YFP and Gpg1-CFP expressed in [*PSI*$^{+}$] strain (NPK265). Experimental procedures and condition are the same as in Fig. 4*C*, except that an NM-YFP single expression image is shown (*Left*). Boxed areas are shown in Fig. 4*C*.

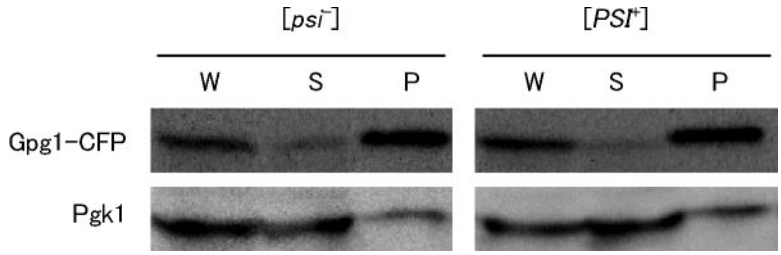

Fig. S4. Pelletable aggregation of Gpg1-CFP. Gpg1-CFP was expressed in NPK265 ([*PSI*$^{+}$]) and NPK564 ([*psi*$^{-}$]) cells as shown in Fig. 4*C*, and cell lysates were fractionated by centrifugation and analyzed by SDS/PAGE and Western blotting, as shown in Fig. 4*A*.

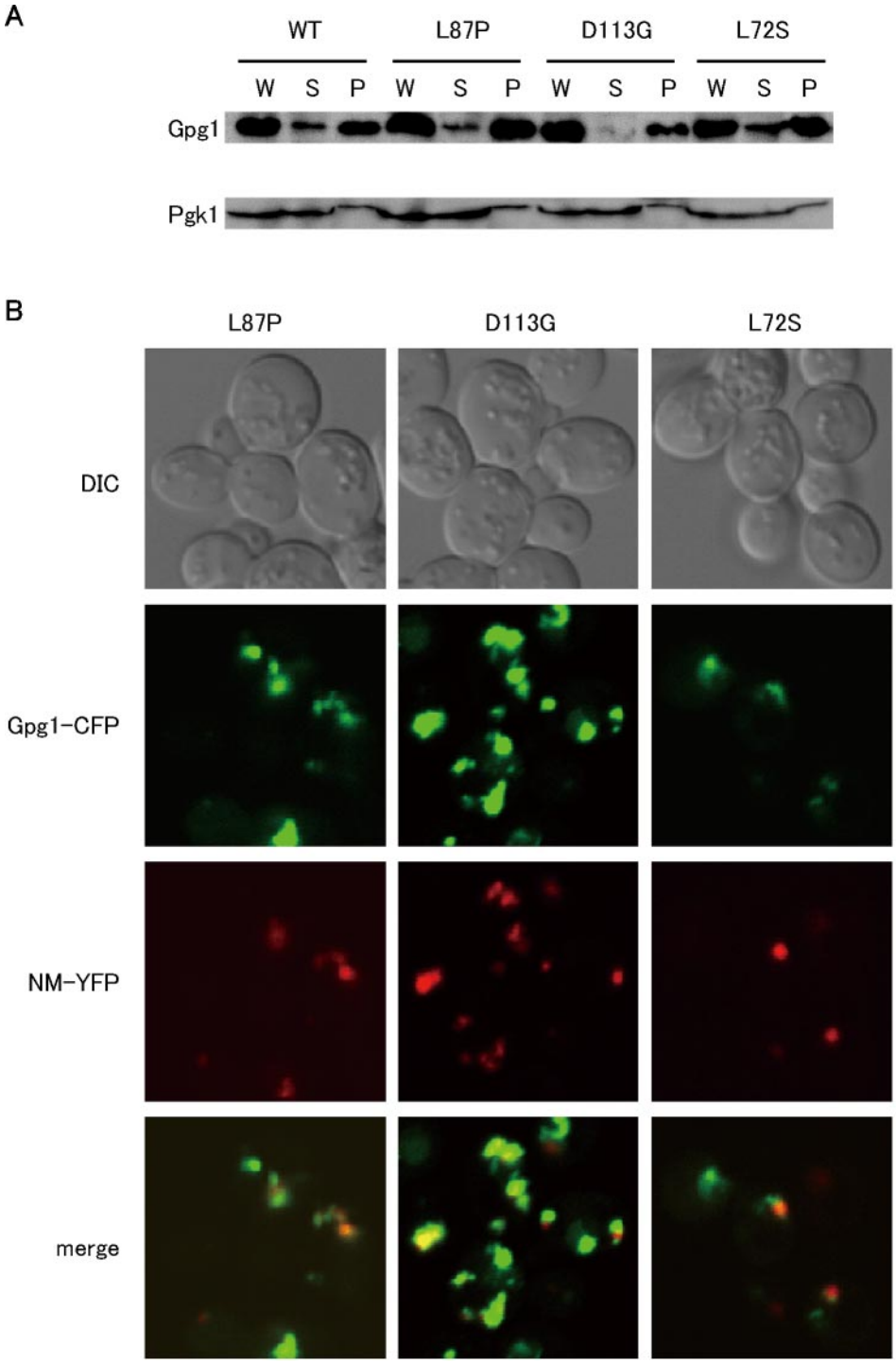

Fig. S5. Biochemical and morphological study of *gpg1* mutant aggregates. (*A*) Wild-type and mutant (L72S, L87P, and D113G) Gpg1s were expressed from pGPG1 plasmids carrying wild-type and *gpg1* mutations in NPK564 ([*psi*$^{-}$]), as shown in Fig. 5*C*, and each protein aggregation was monitored by centrifugation assay, as shown in Fig. 4*A*. (*B*) Confocal fluorescence microscopy of Sup35NM-YFP and mutant Gpg1-CFP coexpressed for 6 h in [*PSI*$^{+}$] strain (NPK265). Experimental procedures and condition are the same as in Fig. 4*C*.

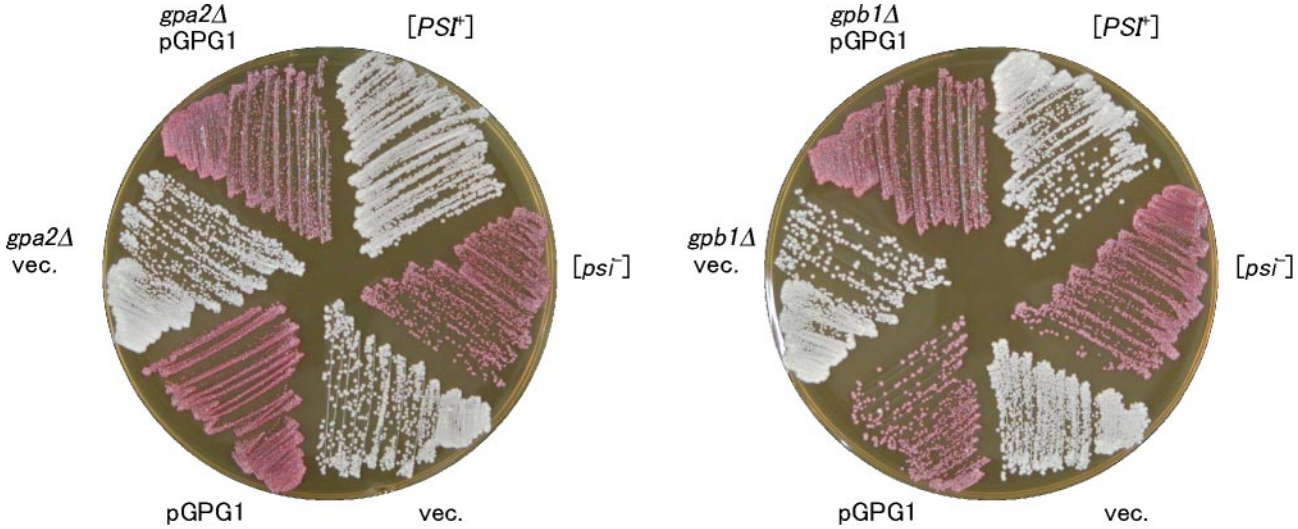

Fig. S6. Prion elimination by Gpg1 over-expression in G-protein α and β subunit deletion strains. NPK265 ([*PSI*$^{+}$] *ade1–14*) strain carrying *gpa2*Δ (*Left*) or *gpb1*Δ (*Right*) chromosomal allele (nullified by *KanMX*; present study) was transformed with an empty vector (vec.) or pGPG1. Transformants were selected on SC-his after 3 days and regrown on YPD for 4 days to monitor colony color. [*PSI*$^{+}$] and [*psi*$^{-}$] control cells are also shown.

Table S1. Summary of defects of *gpg1* mutants in prion elimination

gpg1 alleles	Prion elimination activity		
	[*PSI*$^+$]	[*PIN*$^+$]	[*URE3*]
Wild type	+	+	+
L34H	−*	−	−*
L34P	±	−	−*
V38D	−*	−	−*
L44P	−	−	−*
D46Y	±	−	±
L65P	−*	−	−*
L69S	−*	−	−*
L72S	±	−	−*
L87P	−	−	−*
K90E	−	−	−*
R111G	±	−	−*
D113G	−*	−	−*
R121W	−*	−	−*
R122S	±	−	−*

The data in Fig. 5 and analogous not-shown data are summarized together. Symbols representing the activity: +, strong elimination; ±, weak (or partial) elimination; −*, subtle (or nearly no) elimination; −, no elimination.

Table S2. Strains

Laboratory name	Plasmotype	Genotype	Source or origin, ref.
NPK50	strong [*PSI*$^+$], [*pin*$^-$]	*MATa ade1–14 leu2–3,112 ura3–52 his3Δ200 trp1–289*	74-D694
NPK197	weak [*PSI*$^+$], [*pin*$^-$]	*MATa ade1–14 leu2Δ0 ura3–197 his3Δ200 trp1–289*	Present study
NPK200	[*psi*$^-$], [*PIN*$^+$]	*MATa ade1–14 leu2–3,112 ura3–52 his3Δ200 trp1–289*	OT60 (4)
NPK265	strong [*PSI*$^+$], [*PIN*$^+$]	*MATa ade1–14 leu2Δ0 ura3–197 his3Δ200 trp1–289*	Present study
NPK302	[*URE3*], [*pin*$^-$]	*MATa PD-ADE2 his3 leu2 trp1 kar1 PD-CAN1*	BY242 [*URE3*] (5)
NPK304	[*URE3*], [*PIN*$^+$]	*MATα PD-ADE2 leu2 trp1 ura3 kar1 PD-CAN1*	BY319 [*URE3*] (5)
NPK339	[*psi*$^-$], [*pin*$^-$]	*MATa ade1–14 leu2Δ0 ura3–197 his3Δ200 trp1–289 hsp104::LEU2*	(6)
NPK435	[*URE3*], [*PIN*$^+$]	*MATa PD-ADE2 his3 leu2 trp1 kar1 PD-CAN1*	(3)
NPK562	[*psi*$^-$], [*PIN*$^+$]	*MATα leu2Δ0 ura3Δ0 his3Δ200 trp1Δ63 lys2Δ0 met15Δ0*	BY4727 (7)
NPK563	strong [*PSI*$^+$], [*PIN*$^+$]	*MATa ade1–14 leu2Δ0 ura3–197 his3Δ200 trp1–289 gpg1::KanMX*	Present study
NPK564	[*psi*$^-$], [*pin*$^-$]	Same as NPK265: Prions were cured by GuHCl treatment	Present study