Supplement to:

Proteolysis suppresses spontaneous prion generation in yeast

by

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Supplimental Fig. S1

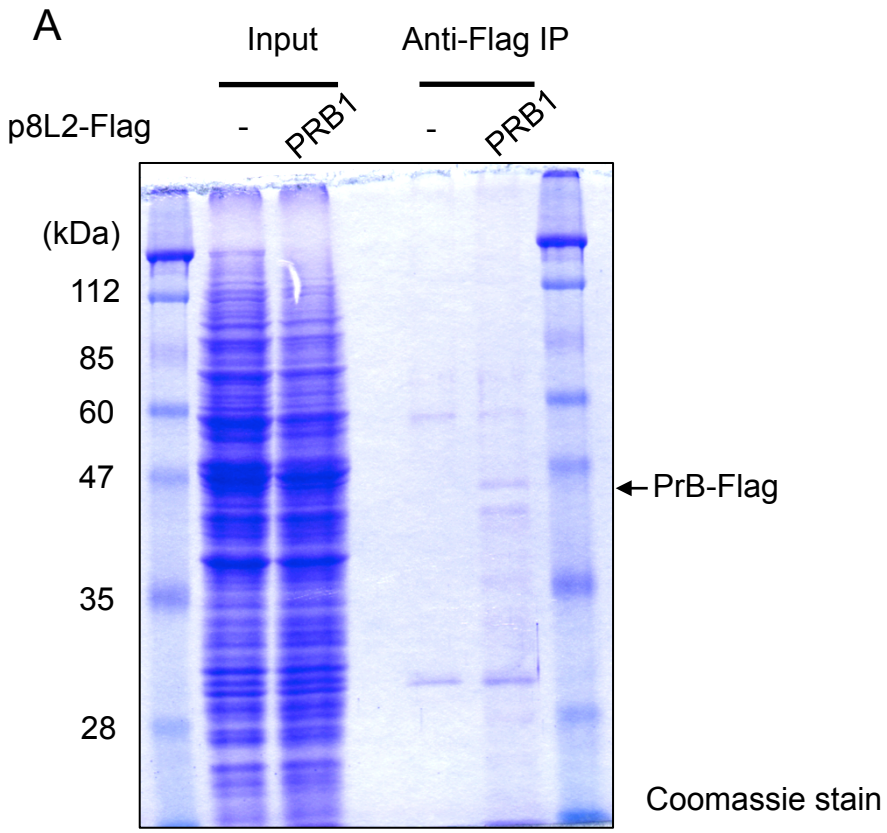

FIGURE S1 **The anti-Flag immunopurified PrB.** **(*A*)** PrB was purified from the cell extract isolated from *pep4Δ* strain carrying a PRB1-Flag single copy plasmid. Input and IP fractions were separated by SDS-PAGE followed by Coomassie brilliant blue staining.

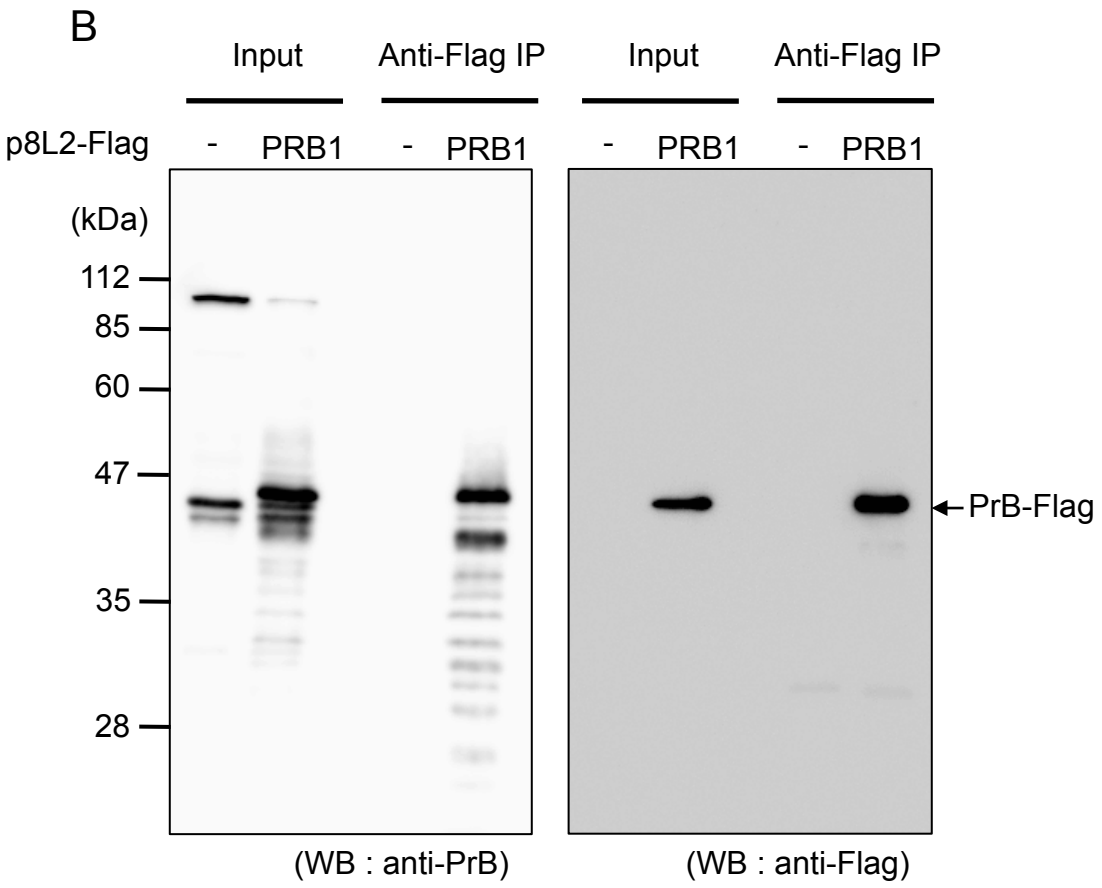

FIGURE S1 **The anti-Flag immunopurified PrB.**
(*B*) Input and IP fractions in (A) were analyzed by western blotting using anti-PrB or anti-Flag.

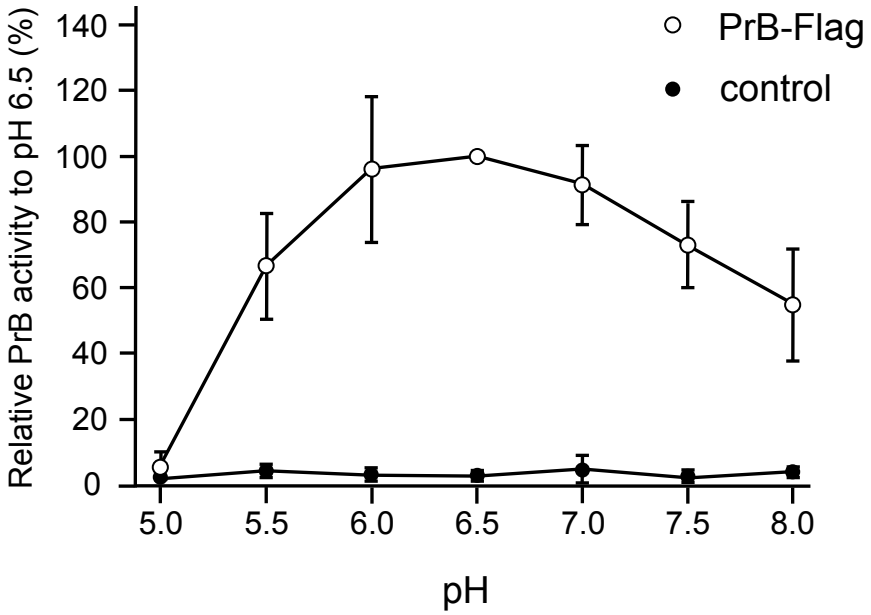

FIGURE S2 **The optimal pH for the purified PrB was around pH 6.5.** The activity of purified PrB between pH 5.0 and 8.0 was measured by azocoll assay. Relative PrB activity is expressed as a percentage of the maximum activity at pH 6.5 (n=3; bars, s.d.).

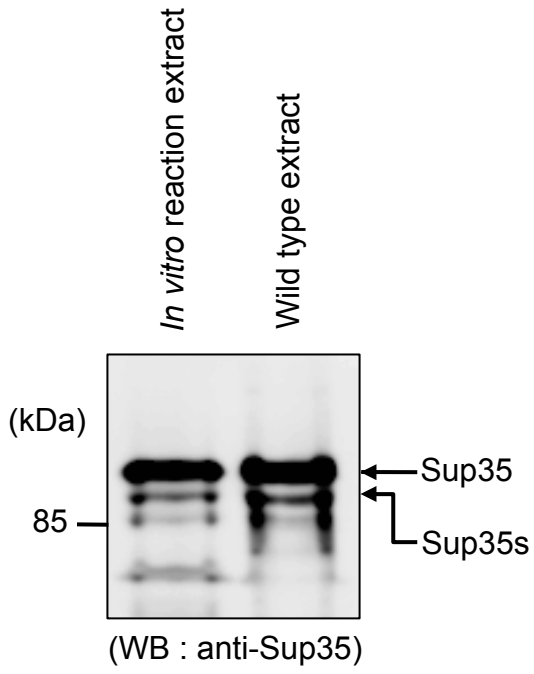

FIGURE S3 **The molecular weight of the cleaved Sup35 *in vitro* corresponds to Sup35s *in vivo*.** After *in vitro* Sup35 cleavage reaction at 30°C for 15 min (as in Fig. 3B), an aliquot of the reaction mixture was analyzed by western blotting using anti-Sup35 to compare the *in vitro* cleaved Sup35 with Sup35s in the lysate of wild type strain.

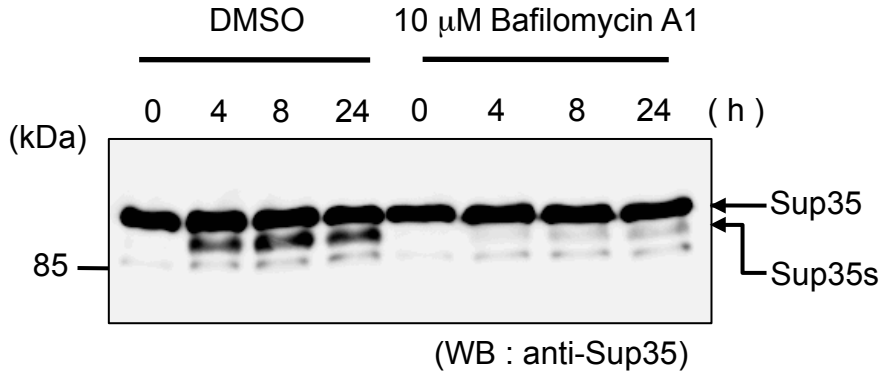

FIGURE S4 **Bafilomycin A1 inhibited the glucose-induced appearance of Sup35s.** Glucose-starved GT17 [*psi*$^{-}$ *pin*$^{-}$] cells were cultured in YPDA containing either 10 mM bafilomycin A1 or its vehicle control. Cells were harvested at the specified time points and analyzed by western blotting using anti-Sup35.

Yeast strains constructed in this study

Strain	Genotypic background	Genotype
GT17	74-D694	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*pin*$^-$]
OT60	74-D694	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*PIN*$^+$]
OT55	74-D694	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*PSI*$^+$,*PIN*$^+$] weak
OT56	74-D694	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*PSI*$^+$,*PIN*$^+$] strong
BY4741		*MAT*a *his3Δ1 leu2Δ0 lysΔ0 ura3Δ0*
yKO1	BY4741	*MAT*a *his3Δ1 leu2Δ0 lysΔ0 ura3Δ0 nma111*Δ*::kanMX4*
yKO2	BY4741	*MAT*a *his3Δ1 leu2Δ0 lysΔ0 ura3Δ0 imp2*Δ*::kanMX4*
yKO4	BY4741	*MAT*a *his3Δ1 leu2Δ0 lysΔ0 ura3Δ0 ste13*Δ*::kanMX4*
yKO5	BY4741	*MAT*a *his3Δ1 leu2Δ0 lysΔ0 ura3Δ0 prb1*Δ*::kanMX4*
yKO8	BY4741	*MAT*a *his3Δ1 leu2Δ0 lysΔ0 ura3Δ0 pcp1*Δ*::kanMX4*
yKO14	BY4741	*MAT*a *his3Δ1 leu2Δ0 lysΔ0 ura3Δ0 pep4*Δ*::kanMX4*
yKO15	BY4741	*MAT*a *his3Δ1 leu2Δ0 lysΔ0 ura3Δ0 prc1*Δ*::kanMX4*
yAO53	OT60	*MAT*a *ade1-14 his3 leu2 trp1 ura3 sup35*Δ*::CgHIS3* YCplac22-SUP35-HA (WT)
yAO66	OT60	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*PIN*$^+$] *prb1*Δ*::CgHIS3*
yAO90	OT60	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*PIN*$^+$] YCplac22
yAO91	OT60	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*PIN*$^+$] YCplac22-Myc-Ub-SUP35 (1M)
yAO92	OT60	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*PIN*$^+$] YCplac22-Myc-Ub-SUP35 (39A)
yAO121	OT60	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*PIN*$^+$] *pep4*Δ*::CgHIS3*
yAO109	OT60	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*PIN*$^+$] p8L2-Flag
yAO110	OT60	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*PIN*$^+$] p8L2-PRB1
yAO117	OT55	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*PSI*$^+$,*PIN*$^+$] weak p8L2-Flag
yAO118	OT55	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*PSI*$^+$,*PIN*$^+$] weak p8L2-PRB1
yAO119	OT56	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*PSI*$^+$,*PIN*$^+$] strong p8L2-Flag
yAO120	OT56	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*PSI*$^+$,*PIN*$^+$] strong p8L2-PRB1
yAO149	GT17	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*pin*$^-$] YCplac22-SUP35-Flag
yAO150	GT17	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*pin*$^-$] YCplac22-Flag-SUP35
yAO179	OT60	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*PIN*$^+$] *prc1*Δ*::CgHIS3*
yAO226	OT60	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*PIN*$^+$] *pep4*Δ*::CgHIS3* p8L2-Flag
yAO227	OT60	*MAT*a *ade1-14 his3 leu2 trp1 ura3* [*psi*$^-$,*PIN*$^+$] *pep4*Δ*::CgHIS3* p8L2-PRB1-Flag

Table S1. **Yeast strains used in this study.**

Oligonucleotide primers used in this study

Primer	Sequence 5' – 3'
AO0009	TCT TCA TCG ACT TGC TCG GAA TAA CAT CTA TAT CTG CCC ACT AGC AAC ATC GAG GTC GAC GGT ATC GAT
AO0010	CGG TAT TAT TGT GTT TGC ATT TAC TTA TGT TTG CAA GAA ATT TAC TCG GCC CGC TCT AGA ACT AGT GGA T
AO0015	TCC TCG GAT TCA AAC CAA GGC AAC AAT CAG
AO0016	TCC CAT TGT TGC TAG TGG GCA GAT ATA GAT
AO0018	ATG GCA GGA TCC CAG AAG CTG ATC TCA GAG
AO0019	GAA GGG GGA TCC ACC ACC ACG AAG TCT CAA
AO0020	ACC ACC ACG AAG TCT CAA CAC AAG ATG AAG
AO0022	GCC CAA CCT GCA GGT GGG TAC TAC CAA AAT
AO0025	ATG TCG GAT TCA AAC CAA GGC AAC AAT CAG
AO0036	GGA GTT CTT CCC ATA CAA ACT TAA GAG TCC AAT TAG CTT CAT CGC TCG AGG TCG ACG GTA TCG AT
AO0037	GAT AGT GAA GAG GGA CTC CGA CTT GTA ACC TCG AGA CGC CTA AGG CCG CTC TAG AAC TAG TGG AT
AO0051	TAA CAA GGA TCC ATG AAG TTA GAA AAT ACT CTA
AO0052	CTA AAT GGA TCC TTA AAT AAT ATT CAA TTT ATC
AO0069	GTG ACC TAG TAT TTA ATC CAA ATA AAA TTC AAA CAA AAA CCA AAA CTA ACT CGA GGT CGA CGG TAT CGA T
AO0070	CTC TCT AGA TGG CAG AAA AGG ATA GGG CGG AGA AGT AAG AAA AGT TTA GCC CGC TCT AGA ACT AGT GGA T
AO0073	AAG TTA GAA AAT ACT CTA TTT ACA CTC GGT GCC CTA
AO0074	CAT GGT GGC AAT TTA GTG TGT GTA TTT GTG TTT GCG
AO0080	GAC GAC GAT AAG TAA ATT TCT TGC AAA CAT AAG TAA ATG C
AO0081	ATC CTT GTA ATC CTC GGC AAT TTT AAC AAT TTT ACC AAT TGC
AO0082	GAC GAC GAT AAG TCG GAT TCA AAC CAA GGC AAC AAT CAG C
AO0083	ATC CTT GTA ATC CAT TGT TGC TAG TGG GCA GAT ATA GAT G
AO0086	CAC GGA TCC GCC ACC ATG GAT TAC
AO0087	AGC GGA TCC AAT CAA TGA ATC GAA AAT G
AO0119	GACGACGATAAGTAAGGATCCGTGAATTTACTTTAAATCTTGC
AO0120	ATCCTTGTAATCAATAATATTCAATTTATCAAGAATATCTCTC
NH0206	ATG GAT TAC AAG GAT GAC GAC GAT AAG CAA AAG ATC ACC ACT GCT TCC
NH0207	GGT GGC AAT TTA GTG TGT GTA TTT G
NH0209	CCA AGA TCT GTG AAT TTA CTT AAA ATC
NH0210	AGC CTG CAG AAT CAA TGA ATC GAA AAT G
FN01	CCA GAC TAC GCT TAA ATT TCT TGC AAA CAT AAG TAA ATG
FN02	GAC GTC GTA TGG GTA CTC GGC AAT TTT AAC AAT TTT ACC

Table S2. **Oligonucleotide primers used in this study.**

		Ade+	Cured	Cured rate (%)
Fig. 5B	Early (growth phase)	26	20	77
	Late (growth phase)	32	20	63
Fig. 5D,5F,5H	Wild type	55	38	69
	pep4Δ	62	50	81
	prb1Δ	42	36	86
	prc1Δ	68	42	62
Fig. 5J	vector	48	37	71
	PRB1	58	37	68
Fig. 6C	vector	76	46	61
	1M	66	60	91
	39A	67	45	67

Table S3. **Guanidine curability of all strains used in this study.** Guanidine curability of all strains is shown.

		Mitotic stability (%)
[*psi*$^-$]	vector	70 ± 5
	PRB1	75 ± 4
[*PSI*$^+$] ^{weak}	vector	79 ± 3
	PRB1	74 ± 8
[*PSI*$^+$] ^{strong}	vector	60 ± 1
	PRB1	79 ± 13

Table S4. **Mitotic stability of plasmids used in Fig. 7D.** Mitotic stability of strains used in Fig. 7D is shown. Average results of 3 repeats are shown, with standard deviations.