Supplementary information

Supplementary Methods

Protein analysis

Yeast cultures were grown in 50 ml to OD_{600} between 0.4 to 0.7. Cells were harvested by centrifugation, were transferred to a 1.5 ml tube and resuspend in 400 µl of gradient buffer ((1), 20 mM HEPES pH7.5, 1 mM EGTA, 5 mM $MgCl_2$, 10 mM KCl, 10% glycerol, 1 × complete mini protease inhibitor cocktail from Roche, 3 mM phenylmethanesulfonyl fluoride and 100 µg ml^{-1} of cycloheximide). As described previously (1), cells were disrupted by agitation with an equal volume of glass beads for 5 min (30 sec beating, 30 sec cooling on ice). Cell debris was removed by spinning down for 2 min at 4000 *g,* and the supernatants (total fraction; T) were used for electrophoresis and Western blot analysis. Protein concentrations were determined by Bradford assay or absorbance at 280 nm.

For sucrose fractionation analysis, 200 µl of extracted proteins were layered on top of 200 µl of a 25% sucrose pad. Samples were centrifuged in 3.5 ml (13 × 51 mm) open-top thick wall polycarbonate tubes, using an SW55Ti rotor at 260,000 *g* for 80 min. Soluble fractions (S) were transferred and the pellets were resuspended in 100 µl of gradient buffer (pellet fractions; P). For western blot analysis (2), samples were resolved on a NuPAGE™ 4-12% Bis-Tris gel (Thermo Fisher Scientific) and transferred to a PVDF transfer membrane (Thermo Fisher Scientific) in transfer buffer (25 mM Tris, 380 mM glycine, 20% methanol) at 100 V for 40 min. Membranes were blocked for 1 h in Tris-buffered saline−Tween 20 (TBST; 10 mM Tris, pH 7.4, 100 mM NaCl, 0.05% [vol/vol] Tween 20)) plus 2.5% skim milk. The membranes were then probed with corresponding anti-bodies, as described below, in TBST plus 1% skim milk for 1 h at room temperature. The membranes were washed five times with TBST over the course of 30 min and probed with horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (Bio-Rad) at 1:1,000 dilution for 1 h. The membranes were then washed five times in TBST over the course of 30 min and evaluated for chemiluminescence using the Amersham ECL Western blotting detection reagents and analysis system (GE Healthcare) according to the manufacturer's protocol.

References:

1. F. Sherman, "Getting started with yeast" in Guide to yeast genetics and molecular biology, C. Guthrie, G. R. Fink, Eds. (Academic Press, San Diego, 1991), vol. 194, pp. 3 - 21.

2. M. Son *et al.*, The HEX1 gene of Fusarium graminearum is required for fungal asexual reproduction and pathogenesis and for efficient viral RNA accumulation of Fusarium graminearum virus 1. *J. Virol.* 87, 10356 - 10367 (2013).
3. M. Son, R. B. Wickner, Nonsense-mediated mRNA decay factors cure most [PSI+] prion variants. *Proc. Natl. Acad. Sci. USA* 115, E1184-E1193 pii: 201717495. doi: 201717410.201711073/pnas.1717495115 (2018).
4. A. Brachmann, U. Baxa, R. B. Wickner, Prion generation *in vitro*: amyloid of Ure2p is infectious. *Embo J* 24, 3082 - 3092 (2005).
5. R. B. Wickner, A. C. Kelly, E. E. Bezsonov, H. E. Edskes, Prion propagation is controlled by inositol polyphosphates. *Proc. Natl. Acad. Sci. USA* 114, E8402-E8410 (2017).
6. R. S. Sikorski, P. Hieter, A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. *Genetics* 122, 19 - 27 (1989).
7. T. Nakayashiki, C. P. Kurtzman, H. K. Edskes, R. B. Wickner, Yeast prions [URE3] and [*PSI*$^{+}$] are diseases. *Proc Natl Acad Sci U S A* 102, 10575-10580 (2005).
8. H. K. Edskes, R. B. Wickner, Conservation of a portion of the *S. cerevisiae* Ure2p prion domain that interacts with the full - length protein. *Proc. Natl. Acad. Sci. USA* 99 (Suppl. 4), 16384-16391 (2002).

Supplementary Figures

Fig. S1. [PSI+] prion variants isolated in *ssb1*/*2*Δ, *zuo1*Δ, or *ssz1*Δ are lost on cytoduction to an isogenic WT strain. Strains WT [PSI+] MS224, WT [psi−] MS109 strain, and Ura⁻ cytoductants from *ssb1*/*2*Δ [PSI+sbs], *zuo1*Δ [PSI+zos], or *ssz1*Δ [PSI+szs] into a WT recipient (top to bottom) were transformed with a 2 μ plasmid pH770 (Vector) or pM18 encoding Sup35NM-GFP controlled by the GAL1 promoter. Ura⁻ cytoductants were obtained by transfer of cytoplasm from the mutant [PSI+] donor strain into the WT recipient [psi-] ρ° strain MS173 (Table 7). After GAL induction for 16 h in 2% (wt/vol) raffinose, 2% (wt/vol) galactose minimal medium, Sup35NM-GFP aggregates were observed using fluorescence confocal microscopy.

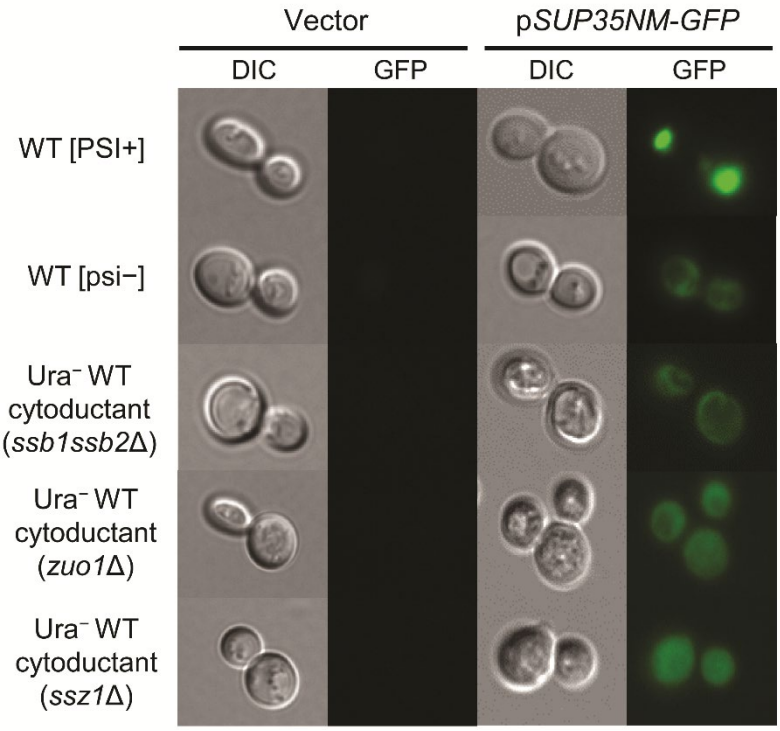


Fig. S2. The effects of each RAC deletion on the stability or fraction of its partner. (A) Zuo1p is destabilized in *ssz1*Δ [psi−] strain, but less so in *ssz1*Δ [PSI+] strain. Same amount of total cell extracts from each strain were separated by SDS/PAGE and further blotted with antibody specific to Zuo1p. (B) Ssz1p is completely destabilized in *zuo1*Δ [psi−] strain, but stably remained only in soluble fraction of *zuo1*Δ [PSI+]. Corresponding amounts of T, S, and P fraction were separated by SDS/PAGE and further analyzed by western blot, with the use of antibody specific to Ssz1p.

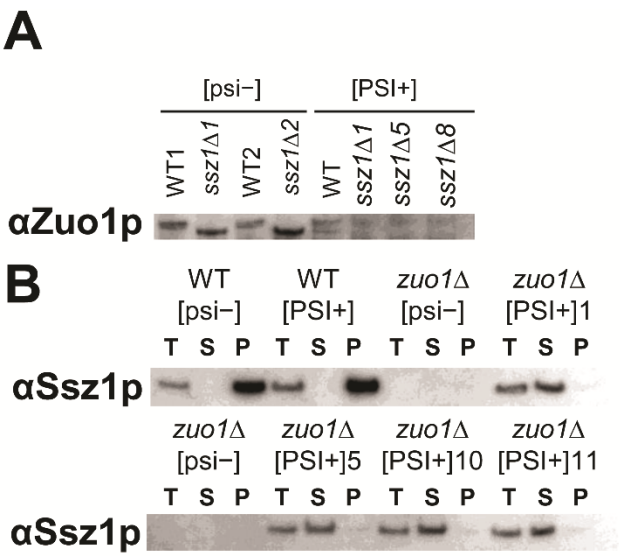
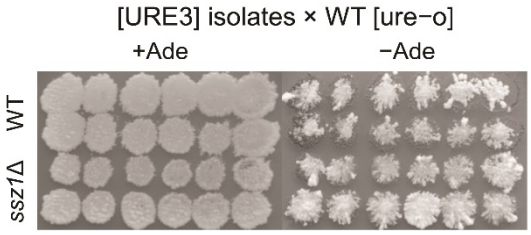


Fig. S3. Most [URE3] prion variants isolated in *ssz1*Δ strains are not cured by restoration of the WT allele of *SSZ1*. [URE3] isolates generated in WT BY241 or *ssz1::kanMX* deletion strains MS581 and MS582 were mated for 2 days on YPAD with isogenic WT MS574 and replica-plated to minimal media with and without adenine. The presence of *DAL5* promoted *ADE2* in all strains enables scoring [URE3]. All diploids formed with WT are generally Ade^+ as a result of maintenance of [URE3] (see also Table S3).



Supplementary Tables

Table S1. Strains used in this study

Strain	Genotype	Reference
BY4741/MS157	*MAT*a *ura3 leu2 his3 met15* [psi−][PIN+]	C. Brachmann
BY4742/MS317	*MAT*α *ura3 leu2 his3 lys2* [psi−][PIN+]	C. Brachmann
MS327	*MAT*a *ura3 leu2 his3 met15 ade1-14 kar1Δ15* [psi−][PIN+]	(3)
MS173	*MAT*α *ura3 leu2 his3 lys2 ade1-14 kar1Δ15* [psi−][PIN+]	(3)
MS515	*MAT*a *ura3 leu2 his3 met15 ade1-14 ssb1::kanMX ssb2::natMX* [psi−][PIN+]	This study
MS520	*MAT*α *ura3 leu2 his3 lys2 ade1-14 kar1Δ15 ssb1::kanMX ssb2::natMX* [psi−][PIN+]	This study
MS527	*MAT*a *ura3 leu2 his3 met15 ade1-14 kar1Δ15 zuo1::kanMX* [psi−][PIN+]	This study
MS528	*MAT*α *ura3 leu2 his3 lys2 ade1-14 kar1Δ15 zuo1::kanMX* [psi−][PIN+]	This study
MS510	*MAT*a *ura3 leu2 his3 met15 ade1-14 ssz1::kanMX* [psi−][PIN+]	This study
MS514	*MAT*α *ura3 leu2 his3 lys2 ade1-14 kar1Δ15 ssz1::kanMX* [psi−][PIN+]	This study
MS560	MS515 *zuo1::kanMX*	This study
MS224	*MAT*a *ura3 leu2 his3 met15 ade1-14 kar1Δ15* [PSI+s]	(3)
MS562	*MAT*α *ura3 leu2 his3 lys2 ade1-14 kar1Δ15* [psi−][PIN+] $^{WT\ (MS327)}$	This study
MS563	*MAT*α *ura3 leu2 his3 lys2 ade1-14 kar1Δ15* [psi−][PIN+] $^{ssb1/2\Delta\ (MS515)}$	This study
MS564	*MAT*α *ura3 leu2 his3 lys2 ade1-14 kar1Δ15* [psi−][PIN+] $^{zuo1\Delta\ (MS527)}$	This study
MS565	*MAT*α *ura3 leu2 his3 lys2 ade1-14 kar1Δ15* [psi−][PIN+] $^{ssz1\Delta\ (MS510)}$	This study
BY241/MS573	*MAT*a *ura3 leu2 trp1 kar1Δ15* P_{DAL5}*:ADE2* P_{DAL5}*:CAN1*	(4)
αBY241/MS574	*MAT*α *his3::TRP1 leu2 trp1 kar1Δ15* P_{DAL5}*:ADE2* P_{DAL5}*:CAN1*	H. K. Edskes
MS581, 582	*MAT*a *ura3 leu2 trp1 kar1Δ15* P_{DAL5}*:ADE2* P_{DAL5}*:CAN1 ssz1::kanMX*	This study
MS80	*MAT*α *ura3-52 leu2-3, 112 his3Δ200 trp1-89 ade1-14* [PSI+] strong	S. Liebman
MS81	*MAT*α *ura3-52 leu2-3, 112 his3Δ200 trp1-89 ade1-14* [PSI+] weak	S. Liebman

Table S2. Plasmids used in this study

Name	Description	Source
p1520	*CEN LEU2 URA3-14* P_{GAL1}*:SUP35NM*	(5)
pRS313/pM24	*CEN HIS3*	(6)
pM76	pRS313 P_{SSB1}*:SSB1*	This study
pH75	pRS313 P_{ZUO1}*:ZUO1*	This study
pM78	pRS313 P_{SSZ1}*:SSZ1*	This study
pRS423/pM84	2 µ *HIS3*	(6)
pM85	pRS423 P_{SSB1}*:SSB1*	This study
pM87	pRS423 P_{ZUO1}*:ZUO1*	This study
pM86	pRS423 P_{SSZ1}*:SSZ1*	This study
pH770/pM14	pRS423 P_{GAL1}	(3)
pM18	pRS423 P_{GAL1}*:SUP35NM-GFP*	(3)
pM60	*CEN LEU2* P_{ADH1}*:RNQ1-GFP*	(7)
pH382/pM88	*CEN LEU2* P_{GAL1}*:URE2N* (1-65)	H. Edskes (8)
pRS316/pM2	*CEN URA3*	(6)
pM89	pRS316 P_{SSZ1}*:SSZ1*	This study

Table S3. Confirmation for elimination of each [PSI+] variant in WT recipient.

		Cytoductants	
Donor	Recipient	Ura^+	Total
*ssb1/2*Δ [PSI+sb12s]1	WT ρ^o	1	8
*ssb1/2*Δ [PSI+sb12s]2		1	12
*ssb1/2*Δ [PSI+sb12s]3		3	9
*ssb1/2*Δ [PSI+sb12s]4		5	10
*ssb1/2*Δ [PSI+sb12s]5		0	6
*ssb1/2*Δ [PSI+sb12s]6		1	4
*ssb1/2*Δ [PSI+sb12s]7		0	12
*ssb1/2*Δ [PSI+sb12s]8		0	8
*ssb1/2*Δ [PSI+sb12s]9		0	9
*ssb1/2*Δ [PSI+sb12s]10		0	4
*zuo1*Δ [PSI+zo1s]1	WT ρ^o	2	13
*zuo1*Δ [PSI+zo1s]2		5	12
*zuo1*Δ [PSI+zo1s]3		3	8
*zuo1*Δ [PSI+zo1s]4		0	9
*zuo1*Δ [PSI+zo1s]5		0	10
*zuo1*Δ [PSI+zo1s]6		1	10
*zuo1*Δ [PSI+zo1s]7		0	7
*zuo1*Δ [PSI+zo1s]8		0	10
*zuo1*Δ [PSI+zo1s]9		1	10
*zuo1*Δ [PSI+zo1s]10		0	14
*ssz1*Δ [PSI+sz1s]1	WT ρ^o	2	8
*ssz1*Δ [PSI+sz1s]2		1	12
*ssz1*Δ [PSI+sz1s]3		0	6
*ssz1*Δ [PSI+sz1s]4		0	10
*ssz1*Δ [PSI+sz1s]5		3	10
*ssz1*Δ [PSI+sz1s]6		1	8
*ssz1*Δ [PSI+sz1s]7		1	6
*ssz1*Δ [PSI+sz1s]8		0	5
*ssz1*Δ [PSI+sz1s]9		2	10
*ssz1*Δ [PSI+sz1s]10		3	7

Ssb1/2p, Zuo1p, and Ssz1p-sensitive [PSI+] variants were transferred by cytoduction (cytoplasmic mixing) from [PSI+] isolates in each SSB-RACΔ strain into the WT strain. In the case of ssb-racΔ recipients, cytoductants were so few that analysis was impossible.

Table S4. The stability of [PSI+] variant is differentiated by stability in doubly heterozygous diploids with identical ribosome-associated chaperone composition.

	[PSI+] isolate	× *zuo1*Δ [psi−] (Ura+/total)	× *ssz1*Δ [psi−] (Ura+/total)	× *ssb1/2*Δ*zuo1*Δ [psi−] (Ura+/total)
*ssb1/2*Δ	sbs1	4/10	3/10	10/10
	sbs2	5/10	1/10	10/10
	sbs3	3/10	0/10	10/10
	sbs4	3/10	1/10	10/10
	sbs5	4/10	1/10	9/10
	sbs6	2/10	2/10	9/10
	sbs7	4/10	0/10	9/10
	sbs8	3/10	1/10	10/10
	sbs9	4/10	1/10	9/10
	sbs10	3/10	1/10	10/10
	Total	35/100	11/100	96/100
	[PSI+] isolate	× *ssb1/2*Δ (Ura+/total)	× *ssz1*Δ (Ura+/total)	× *ssb1/2*Δ*zuo1*Δ (Ura+/total)
*zuo1*Δ	zos1	0/10	9/10	10/10
	zos2	0/10	10/10	10/10
	zos3	1/10	10/10	9/10
	zos4	0/10	8/10	10/10
	zos5	1/10	8/10	9/10
	zos6	0/10	8/10	10/10
	zos7	2/10	9/10	10/10
	zos8	0/10	8/10	10/10
	zos9	0/10	9/10	10/10
	zos10	0/10	8/10	10/10
	Total	4/100	87/100	98/100
	[PSI+] isolate	× *ssb1/2*Δ (Ura+/total)	× *zuo1*Δ (Ura+/total)	× *ssb1/2*Δ*zuo1*Δ (Ura+/total)
*ssz1*Δ	szs1	0/10	10/10	9/10
	szs2	0/10	10/10	9/10
	szs3	0/10	8/10	10/10
	szs4	1/10	9/10	10/10
	szs5	0/10	9/10	10/10
	szs6	0/10	10/10	10/10
	szs7	0/10	9/10	9/10
	szs8	1/10	10/10	10/10
	szs9	0/10	10/10	9/10
	szs10	0/10	10/10	8/10
	Total	2/100	95/100	94/100

Ten of [PSI+sbs], [PSI+zos], or [PSI+szs] variants carrying strains were mated with isogenic *ssb1/2*Δ [psi−] MS520, *zuo1*Δ [psi−] MS528, *ssz1*Δ [psi−] MS514 and *ssb1/2*Δ*zuo1*Δ [psi−] MS560 strain and the doubly hetero-zygous diploid formed were subcloned on YPAD medium. Diploids were replica-plated to −Ura plate to test the stability of [PSI+].

Table S5. The effects of increased expression level of Ssb1p on each [PSI+] variant.

	CEN plasmid Transformants (Ura+/total)		2 μ plasmid Transformants (Ura+/total)	
[PSI+] isolates	pRS313	pRS313 -*SSB1*	pRS423	pRS423 -*SSB1*
sbs1	50/50	15/35	50/50	2/50
sbs2	48/50	11/50	50/50	2/50
sbs3	47/50	8/50	45/50	0/50
sbs4	49/50	14/32	48/50	0/12
sbs5	48/50	7/50	46/50	3/50
sbs6	48/50	6/50	46/50	0/50
sbs7	45/50	13/50	48/50	3/50
sbs8	49/50	14/50	47/50	4/50
sbs9	50/50	13/50	48/50	0/30
sbs10	48/50	5/50	48/50	0/50
Total % of Ura+	482/500 96.4%	106/467 22.7%	476/100 95.2%	14/442 3.2%
zos1	46/50	48/50	46/50	50/50
zos2	47/50	48/50	49/50	45/50
zos3	50/50	42/50	44/50	38/50
zos4	49/50	46/50	49/50	40/50
zos5	47/50	49/50	50/50	39/50
zos6	50/50	48/50	50/50	48/50
zos7	45/50	49/50	48/50	48/50
zos8	48/50	45/50	45/50	45/50
zos9	50/50	49/50	49/50	48/50
zos10	44/50	44/50	48/50	38/50
Total % of Ura+	476/500 95.2%	468/500 93.6%	477/500 95.4%	439/500 87.8%
szs1	49/50	50/50	48/50	32/50
szs2	50/50	49/50	50/50	30/50
szs3	48/50	49/50	47/50	22/50
szs4	46/50	48/50	48/50	24/50
szs5	50/50	50/50	46/50	24/50
szs6	49/50	50/50	48/50	27/50
szs7	50/50	49/50	49/50	12/50
szs8	48/50	48/50	48/50	18/50
szs9	49/50	49/50	48/50	17/50
szs10	50/50	48/50	49/50	32/50
Total % of Ura+	489/500 97.8%	490/500 98.0%	482/500 96.4%	262/500 52.4%

For each of *ssb1/2*Δ*, zuo1*Δ and *ssz1*Δ, ten [PSI+] isolates were transformed with pRS313 (pM24), pRS313-*SSB1* (pM76), 2 μ plasmids pRS423 (pM84) and pRS423-*SSB1* (pM85). In each case, *SSB1* is expressed under its native promoter. Transformants were selected in the presence of uracil and were replica-plated to a plate lacking uracil to test the stability of [PSI+].

Table S6. The effects of overproduced Ssb1p, Zuo1p, and Ssz1p on [PSI+szs] variants.

	*ssz1*Δ [PSI+szs] transformant (Ura$^+$/total transformants)			
Isolate no.	Vector	p*SSB1*	p*ZUO1*	p*SSZ1*
1	8/9	3/6	26/28	1/3
2	8/8	3/5	10/11	1/11
3	11/12	6/10	2/3	0/22
4	14/15	3/9	3/5	1/16
5	16/16	4/11	5/8	1/14
6	23/25	10/18	2/5	2/16
7	39/40	13/20	3/4	3/41
8	22/23	6/9	11/23	0/5
9	6/7	14/18	6/8	1/34
10	17/17	7/12	7/8	4/49
11	38/40	8/16	5/7	2/48
12	12/12	17/26	9/16	1/10
total	214/224 95.5%	94/160 58.5%	89/126 70.6%	17/274 6.2%

Each of ten [PSI+szs] isolates were transformed with the high copy 2 μ plasmids pRS423 (pM84), pRS423-*SSB1* (pM85), pRS423-*ZUO1* (pM87) or pRS423-*SSZ1*. Each gene is expressed under their native promoter. Transformants were selected in the presence of uracil and were replica-plated to a plate lacking uracil to test the stability of [PSI+].

Table S7. The stability of [URE3] isolated in a WT strain or *ssz1*Δ strain in their original host or in a diploid formed with a wild type strain.

Isolate no.	[URE3] isolates in (no. of Ade$^+$/total subclones)			
	WT	WT/$^+$	*ssz1*Δ	*ssz1*Δ/$^+$
1	36/50	10/15	38/50	11/15
2	34/50	10/15	34/50	12/15
3	37/50	9/15	35/50	12/15
4	38/50	11/15	36/50	14/15
6	38/50	12/15	40/50	12/15
7	36/50	11/15	41/50	13/15
8	38/50	12/15	42/50	12/15
9	41/50	12/15	48/50	14/15
10	38/50	11/15	41/50	14/15
11	36/50	11/15	49/50	15/15
12	37/50	12/15	48/50	15/15
total	409/550 74.4%	121/165 73.3%	489/600 81.5%	144/165 87.2%

Eleven [URE3] isolates in a WT strain (BY241) or BY241 *ssz1*Δ strains (MS581 or MS582) were either subcloned on YPAD medium or mated with WT strain αBY241 and the diploids were subcloned on selective plates with adenine. Haploid and diploid colonies were replica-plated to −Ade plates to test the stability of [URE3]. Each [URE3] prion variant in all cases showed similar stability.

Table S8. Restored normal level of Ssz1p does not affect the loss of [URE3] variants in an *ssz1*Δ strain.

	WT [URE3] transformant (Ade$^+$/total transformants)			*ssz1*Δ [URE3] transformant (Ade$^+$/total transformants)	
Isolate no.	Vector	p*SSZ1*		Vector	p*SSZ1*
1	28/40	24/40		27/40	28/40
2	26/40	27/40		29/40	31/40
3	27/40	30/40		31/40	26/40
4	31/40	29/40		30/40	27/40
6	25/40	27/40		24/40	29/40
7	30/40	31/40		31/40	28/40
8	32/40	28/40		29/40	29/40
9	31/40	29/40		39/40	40/40
10	30/40	34/40		39/40	39/40
11	29/40	26/40		40/40	39/40
12	15/20	31/40		40/40	39/40
Total % of Ura+	304/420 72.4%	316/440 71.8%		359/440 81.6%	355/440 80.6%

Eleven WT or *ssz1*Δ strains carrying [URE3] were transformed with the *CEN* plasmid pRS316 or with the same plasmid carrying *SSZ1* under its native promoter (pM89=p*SSZ1*). Transformants were selected in the presence of adenine and were replica-plated to a plate lacking adenine to test the loss of [URE3].