Supplementary Information for:

Innate immunity to yeast prions:

Btn2p and Cur1p curing of the [URE3] prion is prevented by 60S ribosomal protein deficiency or ubiquitin/proteasome system overactivity

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Table S1. Co-localization of Btn2-RFP and Ure2N-GFP during prion curing.

Synthetic media	strain	Single Ure2N-GFP dot per cell	Two Ure2N-GFP dots per cell	Three Ure2N-GFP dot per cell	Colocalization Btn2-RFP with Ure2N-GFP dot	Btn2-RFP dot not at Ure2N-GFP dot
Raf	WT	83	19	1	-	-
Gal Raf	WT	144	10	-	22	7
Gal Raf	hsp42Δ	103	1	-	1	8
Gal Raf	ubr2Δ	72	11	1	8	12
Gal Raf	rpl4aΔ	113	14	1	20	5
Gal Raf	rpl21bΔ	60	6	-	9	3

Microscopy of strains carrying [URE3] and expressing overnight Btn2-RFP from galactose promoter (pYES52-BTN2-RFP (KRYNDUSHKIN *et al.* 2008)) and Ure2N-GFP from URE2 promoter (pVTG12 (EDSKES *et al.* 1999)). Growth of wild type in raffinose media (without galactose) was done without adenine, galactose induction was done in the presence of adenine. Note that not all cells had dots of both Ure2p and Btn2p, in part because some cells had already been cured of [URE3].

Table S2. Proteins levels altered in mutants affecting [URE3] curing measured by SILAC. See file SILACsummary.xlsx.

Table S3. GO process grouping of genes over or under expressed by ≥ 1.5-fold. The Gene Ontology Slim Term Mapper at the Saccharomyce genome database was used to sort over-espressed or underexpressed genes into functional groups (https://www.yeastgenome.org/goSlimMapper).

Mutant	GO process # (%)	Mutant # (%)	
hsp42Δ ↓ 161 genes	Ion transport 826 (12.8%)	27 (16.8%)	AFT1, AGP1, ATG19, BRL1, COS8, DIP5, ENA1, ENA2, FCY2, FET3, FTR1, FUI1, FUS3, GAP1, HIP1, HXT2, LTV1, OPT1, PHO87, PIR1, PTR2, RCF2, SDA1, STE6, TRE1, TRE2, YFH1
	Mitotic cell cycle 378 (5.8%)	23 (14.3%)	AFT1, ALK1, BCK2, BUD4, CDC27, CDC34, CHK1, CHS2, CIN8, CLB2, FAR1, FKH1, HSL1, IBD2, MCD1, MRC1, RAD51, RAD9, RME1, SDA1, SKG6, SRL3, YKL089W
	Transmembrane transport 460 (7.2%)	22 (13.7%)	AGP1, BAP3, DIP5, ENA1, ENA2, FCY2, FET3, FRT1, FTR1, FUI1, GAP1, GNP1, HIP1, HXT2, LYP1, OPT1, PHO87, PTR2, RCF2, STE6, TAT1, YNL095C
	Response to chemical 534 (8.3%)	22 (13.7%)	ATG19, CCW12, CUZ1, FAR1, FET3, FRT1, FUS3, GZF3, HLJ1, HSP42, LTV1, MET4, MGA2, MSN4, OPI1, PTR3, SAN1, STE2, STE6, TMC1, UBC6, YHB1
	regulation of cell cycle 299 (4.7%)	18 (11.2%)	BCK2, CDC27, CHK1, CIN8, CLB2, FAR1, FIR1, FKH1, FUS3, HSL1, IBD2, MRC1, RAD9, RME1, SDA1, SKG6, SPP1, YPL014W
	transcription by RNA polymerase II 556 (8/6%)	16 (8.6%)	AFT1, BAS1, DOT6, EAF7, FKH1, GZF3, INO2, MET4, MGA2, MSN4, OPI1, RAD9, RME1, SPT10, TOD6, YPR022C
	rRNA processing 359 (5.6%)	15 (9.3%)	BMT2, BUD21, CGR1, HCA4, MPP6, NOG1, NOP16, NOP4, NSA2, PNO1, PXR1, REX4, RPF1, SAS10, SLX9
	cellular response to DNA damage stimulus 226 (3.5%)	13 (8.1%)	ALK1, BDF2, CHK1, EAF7, MCD1, MKT1, MRC1, PIF1, RAD5, RAD51, RAD59, RAD9, RDH54
	ribosomal large subunit biogenesis 122 (1.9%)	9 (5.6%)	ALB1, NOG1, NOP16, NOP4, NSA2, REX4, RLP24, RPF1, SDA1
	ribosomal subunit export from nucleus 53 (0.8%)	7 (4.4%)	BUD20, LTV1, NOG1, NOG2, RPF1, SDA1, SLX9
	protein modification by small protein conjugation or removal 222 (3.5%)	6 (3.7%)	CDC27, CDC34, RAD5, SAN1, UBC6, YBR062C
hsp42Δ ↑ 59 genes	cellular amino acid metabolic process 220 (3.4%)	11 (18.6%)	ARG3, ARG4, ARG5,6, ARG7, ARG8, ARO10, ARO9, CPA1, GDH3, LYS1, YBR145W
	carbohydrate metabolic process 253 (3.9%)	10 (17%)	GCY1, GLG1, IGD1, INO1, NQM1, PGM2, SCW4, SOL4, UTH1, YNR034W
	response to chemical 534 (8.3%)	9 (15.2%)	TT1, GCY1, GRX2, HSP12, HSP31, NQM1, ROX1, STF2, YHI9
	generation of precursor metabolites and energy 129 (1.9%)	8 (12.6%)	GLG1, GRX2, IGD1, NQM1, PGM2, SOL4, YBR145W, YNR034W

*rpl4a*Δ↓ 102 genes	CHO metabolic process 253 (3.9%)	20 (19.6%)	DSF1, GCY1, GIP2, GLC3, GLG1, GPD1, GPH1, GPM2, GPP2, IGD1, INO1, NQM1, PGM2, SCW4, SOL4, TDH1, UTH1, YBR053C, YMR196W, YNR034W
	generation of precursor metabolites and energy 129 (1.9%)	15 (14.7%)	GIP2, GLC3, GLG1, GPH1, GPM2, GRX1, GRX2, IGD1, NQM1, PGM2, RGI1, SOL4, TDH1, YBR145W, YNR034W
	cellular amino acid metabolic process 220 (3.4%)	14 (13.7%)	ARG3, ARG4, ARG5,6, ARG7, ARG8, ARO10, ARO9, CPA1, GDH3, LYS1, ORT1, UTR4, YBR145W, YLR126C
	response to chemical 534 (8.3%)	13 (12.7%)	CTT1, GCY1, GPD1, GRX1, GRX2, HSP12, HSP31, HSP42, NQM1, ROX1, STF2, YHI9, YNL134C
	Ion transport 826 (12.8%)	10 (9.8%)	APE1, ATG1, ATG8, DUR3, HXT7, PBI2, SSU1, TRX2, UGA4, YRO2
	nucleobase-containing small molecule metabolic process 220 (3.4%)	9 (8.8%)	ADE2, CPA1, DAL1, GPM2, PGM2, PNC1, TDH1, TRX2, YNL200C
	response to oxidative stress 129 (2%)	9 (8.8%)	CTT1, GCY1, GRX1, GRX2, HMX1, HSP12, HSP31, HSP42, NQM1
*rpl4a*Δ↑ 59 genes	cellular amino acid metabolic process 220 (3.4%)	11 (18.6%)	ARG3, ARG4, ARG5,6, ARG7, ARG8, ARO10, ARO9, CPA1, GDH3, LYS1, YBR145W
	CHO metabolic process 253 (3.9%)	10 (17%)	GCY1, GLG1, IGD1, INO1, NQM1, PGM2, SCW4, SOL4, UTH1, YNR034W
	response to chemical 534 (8.3%)	9 (15.3%)	CTT1, GCY1, GRX2, HSP12, HSP31, NQM1, ROX1, STF2, YHI9
	generation of precursor metabolites and energy 129 (1.9%)	8 (13.6%)	GLG1, GRX2, IGD1, NQM1, PGM2, SOL4, YBR145W, YNR034W
*rpl21b*Δ↓ 80 genes	Mitotic cell cycle 378 (5.8%)	16 (20%)	ALK1, ATC1, BCK2, CDC4, CHS2, CIN8, CLB2, HSL1, MCD1, MET30, NDD1, RAD51, REI1, SKG6, SRL3, SWI5
	Transmembrane transport 460 (7.2%)	15 (18.8%)	AGP1, DIP5, ENA1, FCY2, FRT1, FUI1, GAP1, GNP1, HIP1, HXT2, OPT1, PHO87, PTR2, STE6, YJR054W
	Ion transport 826 (12.8%)	14 17.5%)	AGP1, DIP5, ENA1, FCY2, FUI1, GAP1, HIP1, HXT2, OPT1, PHO87, PTR2, STE6, TRE1, YJR054W
	response to chemical 534 (8.3%)	13 (16.3%)	CCW12, CUZ1, FRT1, GZF3, MET30, MET4, MSN4, SAN1, STE2, STE6, TMC1, UBC6, YHB1
	transcription by RNA polymerase II 556 (8/6%)	10 (12.5%)	DOT6, GZF3, MET4, MSN1, MSN4, MSS11, NDD1, STP2, SWI5, TOD6
	cellular amino acid metabolic process 220 (3.4%)	8 (10%)	ALT2, CAR1, CAR2, MET10, MET16, MET30, MET4, SPE1
	cellular response to DNA damage stimulus 226 (3.5%)	7 (8.8%)	ALK1, BDF2, MCD1, MKT1, PIF1, RAD51, SML1
	proteolysis involved in cellular protein catabolic process 265 (4.1%)	7 (8.8%)	CDC4, CUZ1, MET30, SAN1, TRE1, UBC6, YBR062C

*rpl21b*Δ↑ 84 genes	cellular amino acid metabolic process 220 (3.4%)	11 (13%)	ARG3, ARG5,6, ARG7, ARG8, ARO10, ARO9, CPA1, GCN4, GDH3, LYS1, YBR145W
	Ion transport 826 (12.8%)	11 (13%)	AFT1, APE1, ATX2, ENA2, HXT7, PIR1, ROG3, TOM7, UGA4, YOS1, YRO2
	response to chemical 534 (8.3%)	11 (13%)	CTT1, GCN4, GCY1, GRX2, HSP12, HSP31, MTL1, PDR15, RDR1, ROG3, YHI9
	Transmembrane transport 460 (7.2%)	9 (10.7%)	ATX2, ENA2, GGC1, HXT7, PDR15, TOM7, UGA4, YPQ2, YRO2
	mitochondrion organization 286 (4.4%)	9 (10.7%)	ATP23, CBS1, COX14, GGC1, MDM30, RRG9, SED1, TOM7, UTH1
	response to oxidative stress 129 (2%)	7 (8.3%)	CTT1, GCY1, GRX2, HMX1, HSP12, HSP31, MTL1
*rps30b*Δ↓ 67 genes	Mitotic cell cycle 378 (5.8%)	16 (24%)	ALK1, ASE1, BCK2, CHK1, CHS2, CIN8, GAC1, HSL1, IQG1, MCD1, MIH1, NDD1, RAD51, RME1, SDA1, SRL3
	Transmembrane transport 460 (7.2%)	12 (18%)	AQR1, FCY2, FRT1, FUI1, GAP1, GNP1, HIP1, HXT2, MUP1, POR2, SMF1, STE6
	Ion transport 826 (12.8%)	12 (18%)	AQR1, FCY2, FUI1, GAP1, HIP1, HXT2, MUP1, PIR1, POR2, SDA1, SMF1, STE6
	response to chemical 534 (8.3%)	11 (16%)	AQR1, CCW12, ECM38, FRT1, GZF3, MSN4, STE2, STE6, TMC1, UBC6, YHB1
	regulation of cell cycle 299 (4.7%)	10 (15%)	BCK2, CHK1, CIN8, FIR1, GAC1, HSL1, IQG1, MIH1, RME1, SDA1
	cellular response to DNA damage stimulus 226 (3.5%)	9 (13%)	ALK1, BDF2, CHK1, MCD1, MKT1, PIF1, RAD51, RDH54, SML1
*rps30b*Δ↑ 60 genes	cellular amino acid metabolic process 220 (3.4%)	12 (20%)	ADH2, ARG3, ARG4, ARG5,6, ARG7, ARG8, ARO10, ARO9, CPA1, GCN4, LYS1, YBR145W
	response to chemical 534 (8.3%)	10 (17%)	CTT1, GCN4, GCY1, GRX2, HSP12, HSP31, ROX1, STF2, TSA2, YHI9
	monocarboxylic acid metabolic process 163 (2.5%)	7 (12%)	BIO2, BIO3, BIO4, CAT2, GOR1, HSP31, YAT2
	response to oxidative stress 129 (2%)	7 (12%)	CTT1, GCY1, GRX2, HMX1, HSP12, HSP31, TSA2

Proteins over- or under-expressed $\geq$ 1.5-fold in mutant compared to w.t. as judged by SILAC were analyzed using the Genome Ontology Slim Mapper at the Saccharomyces Genome Database site https://www.yeastgenome.org/goSlimMapper. A total of 6437 genes have been categorized for GO processes. The number of proteins elevated (↑) or depressed (↓) more than the threshold is indicated. Only GO groups which were popular with genes affected in our mutants are shown.

Table S4. Test for Tma10p abrogation of [URE3] curing by overproduced Btn2p.

	0 gen	6.6 gen	13 gen
Plasmids	Fraction of red colonies		
-	0.0	0.0	0.0
pGAL-BTN2	0.0	.23	.27
pGAL-TMA10	0.008	.01	.02
pGAL-BTN2, pGAL-TMA10	0.008	.31	.37

Strain 6010 ([URE3]) was transformed with pGAL-BTN2 (pBEE1) or pGAL-TMA10 (p1678) or both. Cells were pregrown in raffinose, selecting for presence of the plasmid(s), then shifted to galactose/raffinose by 1:100 dilution. After growth for two days, cultures were diluted 1:100 into fresh medium. Samples were plated on ½ YPD and red/white colonies were counted (250-750 colonies in each case).

Fig. S1. Overproduction of Btn2p and Cur1p.

Strain BY241 (WT) was transformed with pBEE34 (*CUP1-BTN2*) or pBEE42 (*CUP1-CUR1*) and grown with or without added 0.25 mM Cu^{2+}. Extracts were compared by western blotting with the same strain without any plasmid and the *btn2*Δ *cur1*Δ mutant in the same background.

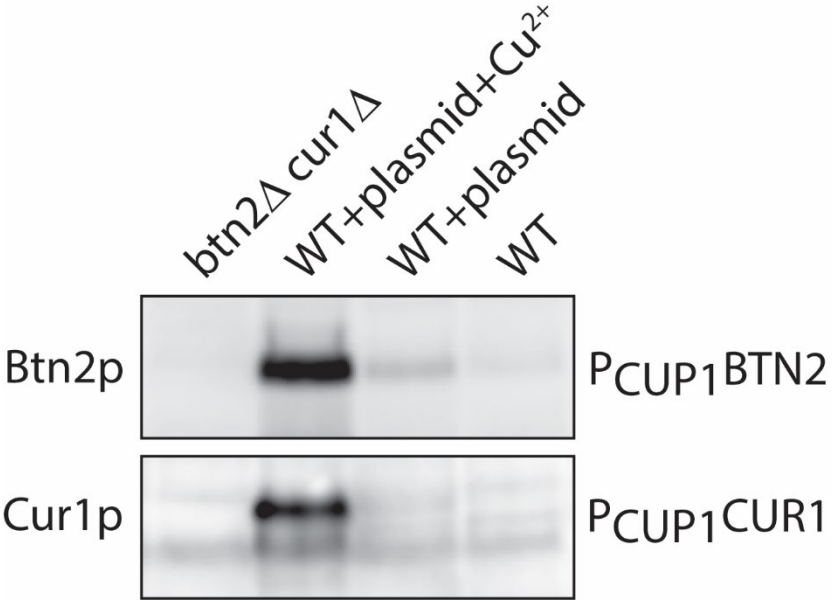


Figure S2. Cur1-overproduction curing of [URE3] in wild type and mutants.
Deletion mutants for genes identified in the HERMES screen for Btn2-overproduction incurable mutants were transformed with pBEE42 (*CEN TRP1 CUP1promoter-CUR1*), then grown with 0.25 mM $CuSO_4$ in synthetic complete medium from initial OD_{600} = 0.001 for 12 generations, then plated for single colonies on ½ YPD. Red colonies have lost [URE3]. The stability of [URE3] in the wild type and mutant strains was tested before introduction of the CUR1/BTN2 overexpression plasmids and loss of [URE3] was <1% in all cases.

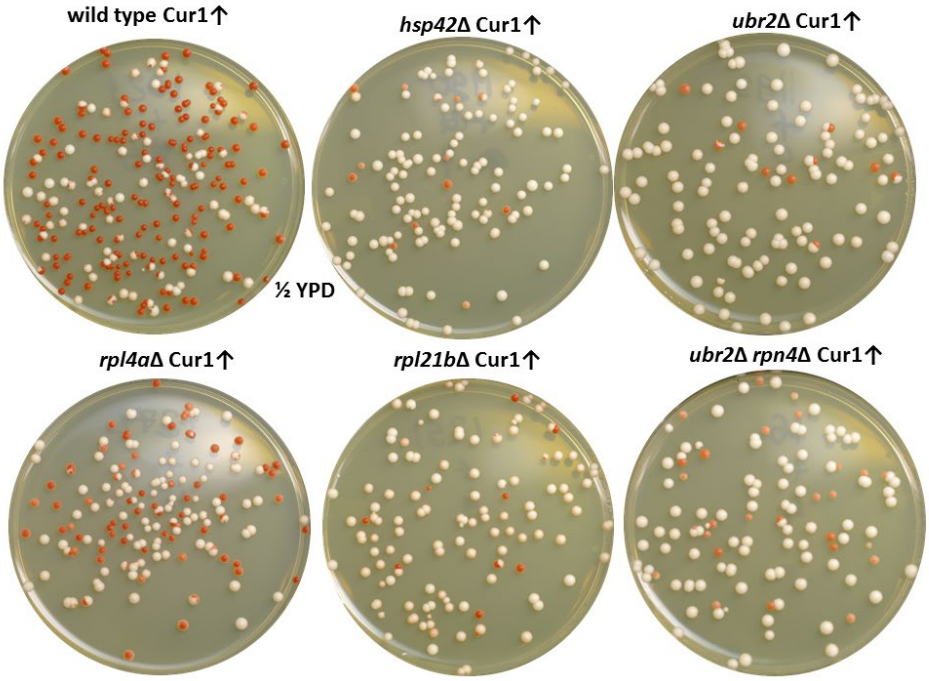
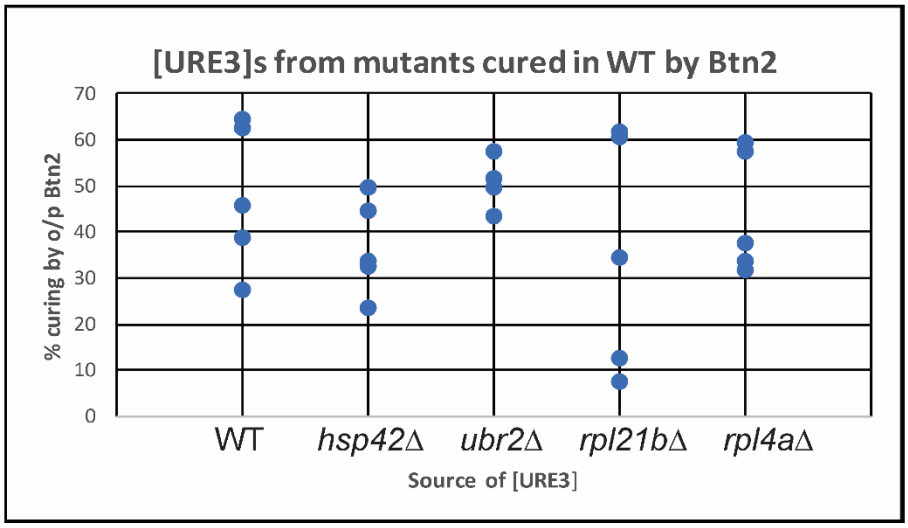


Fig. S3. Incurability in mutants is not due to an incurable [URE3] prion. The data used for Table 5 is shown as individual data points. Using the Mann-Whitney U test, the curability of [URE3] from none of the mutants is significantly different from that from the wild type, even at the $p < 0.1$ level.



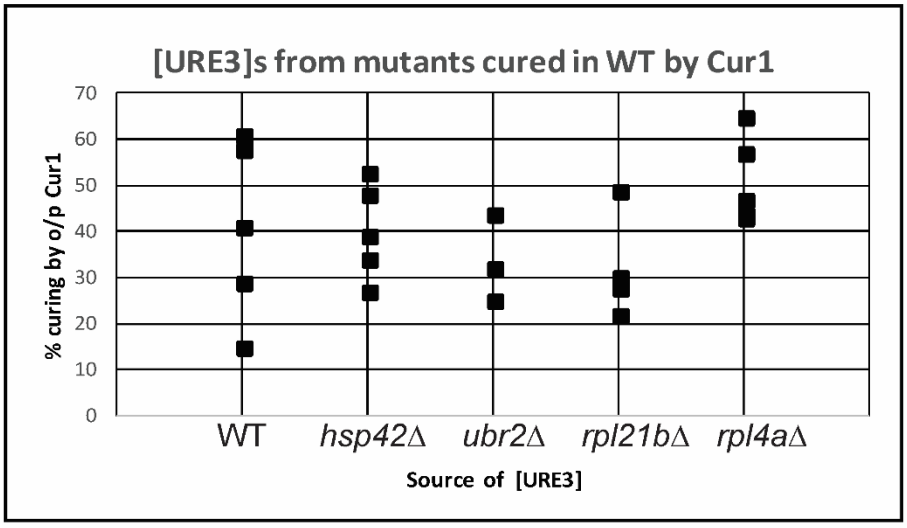


Fig. S4. Levels of Btn2p and Cur1p overproduction in incurable mutants. This blot is similar to that shown in Fig. 3.

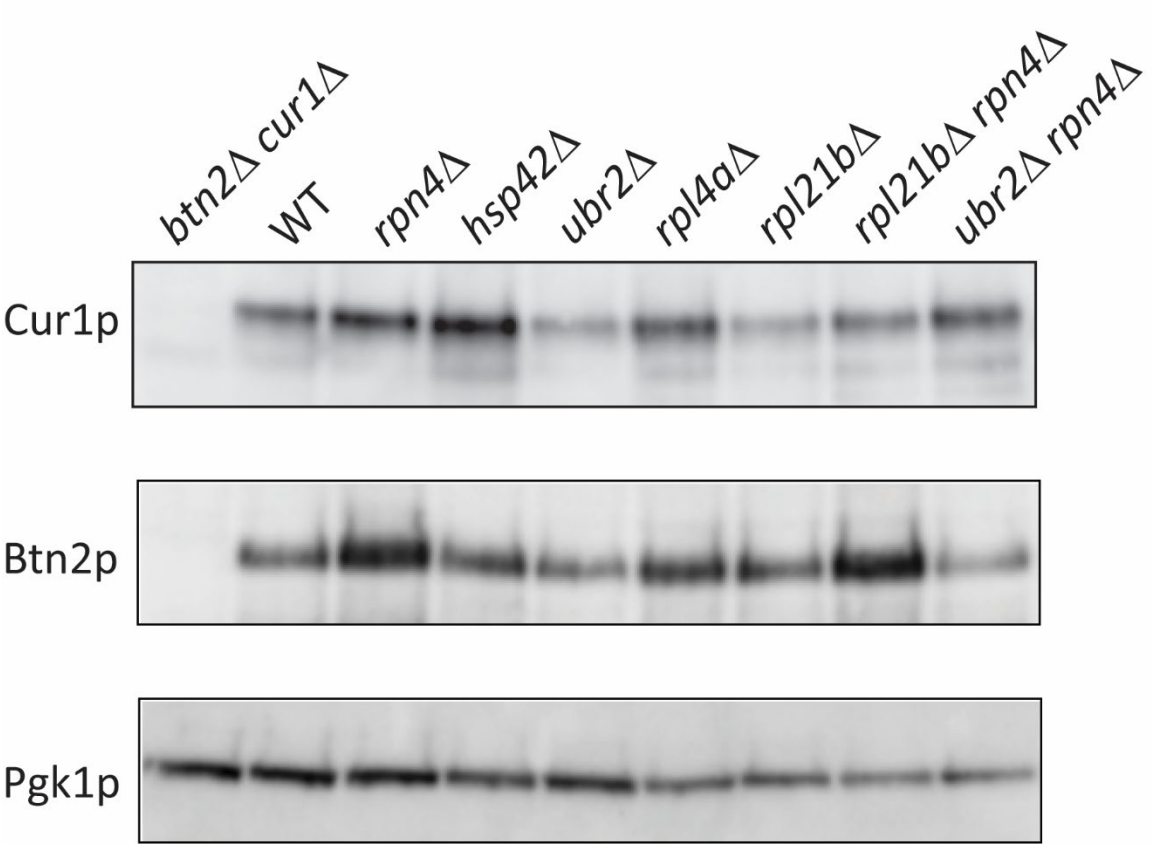


Fig. S5. Hsp104 prion-curing activity is not important in Btn2 or Cur1 overproduction curing of [URE3]. The data of Table 6 is plotted as individual data points. Although robust curing is seen by overproduction of either Btn2 or Cur1 in the *hsp104T160M* mutant whose prion-curing activity is completely eliminated (HUNG and MASISON 2006), the efficiency is slightly reduced ($p<0.01$).

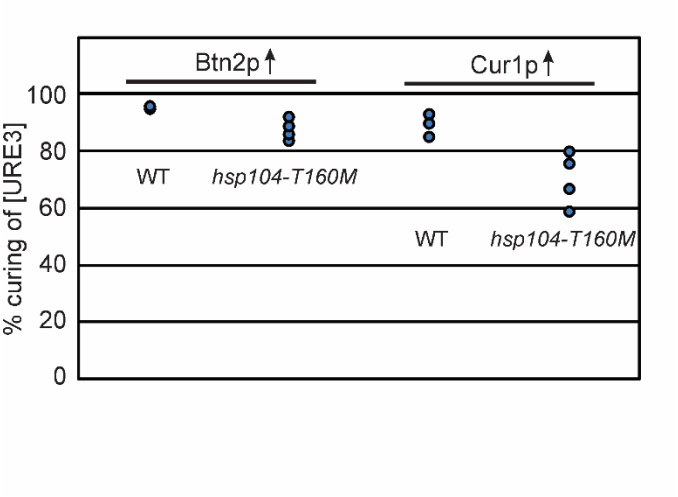
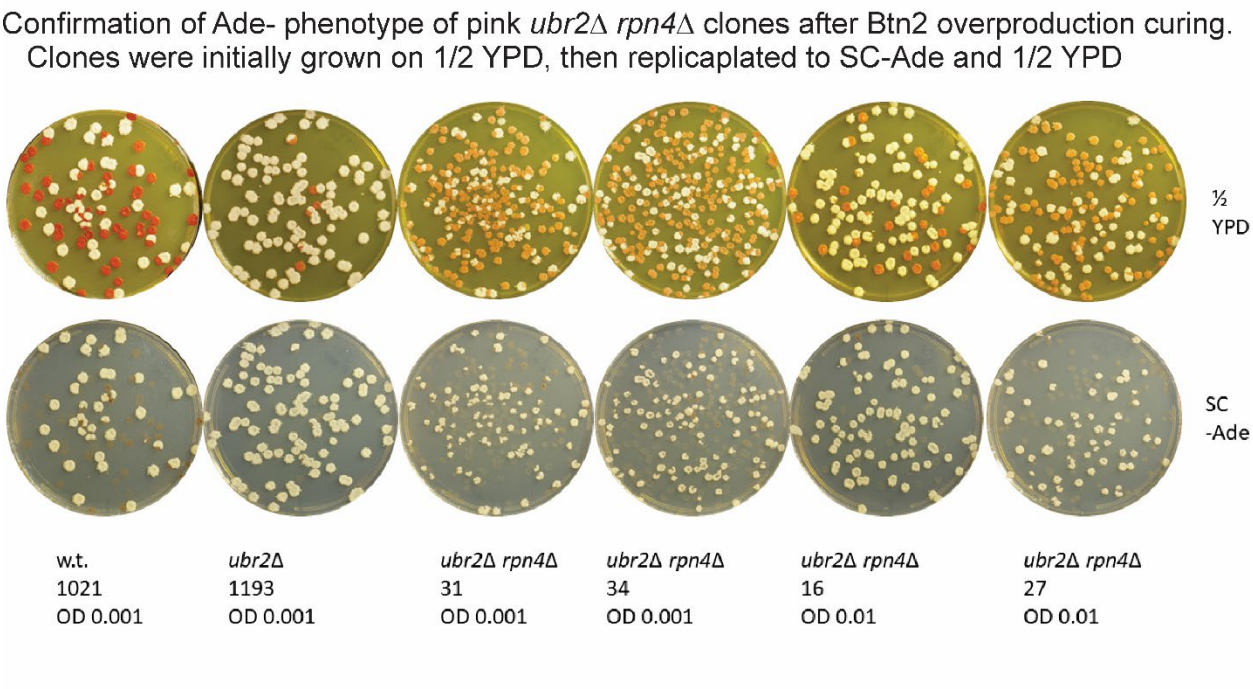
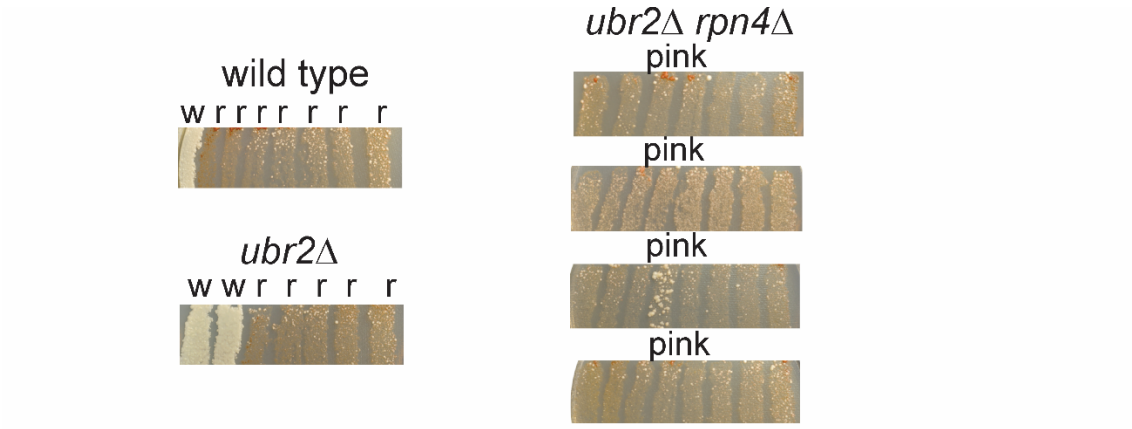


Fig. S6. Overproduction of Rpl4a does not cure [URE3].
Rpl4a was overproduced from pBEE64 in wild type strain BY241 [URE3-1] by growth on galactose for 6 generations, and cells were plated on ½ YPD to detect [ure-o] (red) cured colonies.

Fig. S7. Confirmation that *ubr2Δ rpn4Δ* pink clones are [ure-o].

Below strain genotype is the isolate number or strain number with the OD_{550} of the initial inoculum in galactose medium for the Btn2p overproduction.





After Btn2 overproduction curing of [URE3] from w.t., *ubr2*Δ, and *ubr2 rpn4* strains, red, pink and white colonies were mated with a wild type [ure-o] strain, diploids selected, and then replicaplated to SC-Ade medium. The failure to grow of most diploids shows that the Ade- red/pink phenotype was not due to any recessive chromosomal mutation (including *ubr2* or *rpn4*), but rather to the loss of [URE3]. 'w', 'r' or 'pink' refers to the color of uncured w.t.or *ubr2* colonies, cured w.t. or *ubr2* colonies or cured *ubr2 rpn4* colonies, respectively.