

Supplementary Materials

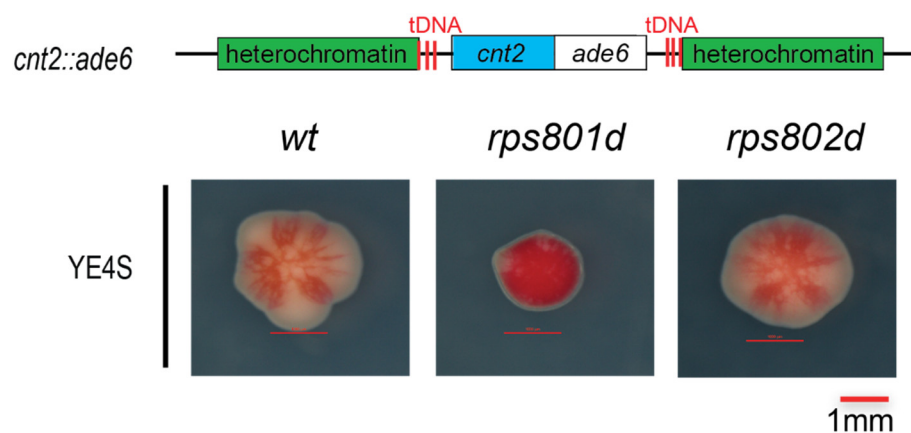


Figure S1. *rps8* paralog deletions show differential colony morphology of CEN-PEV. Cells derived from the red colony among wild type, *rps801d* and *rps802d* were planted at the density of one cell/cm² by microscopic manipulation on YE+4S plates, incubated at 25°C for eight days. The representative colony for each strain were shown. Scale bar is 1mm.

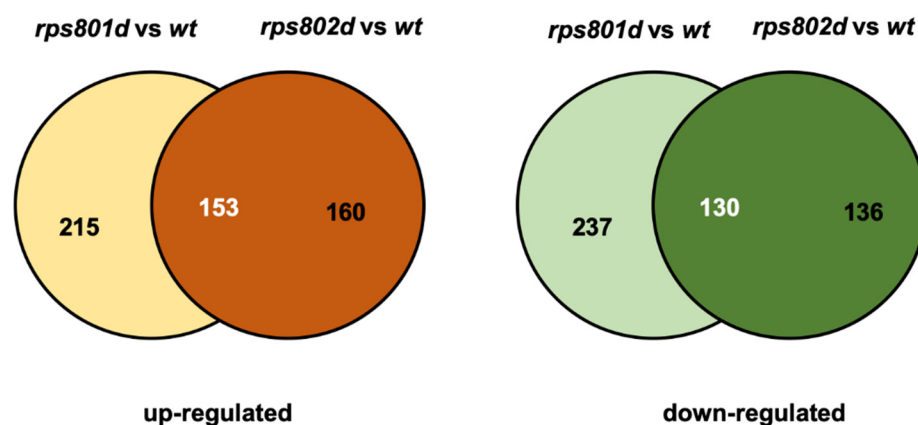


Figure S2. Venn diagram showing the overlap between *rps8* paralog deletions vs wild type. Proteins whose abundance was significantly increased are shown in the left; decreased are shown in the right.

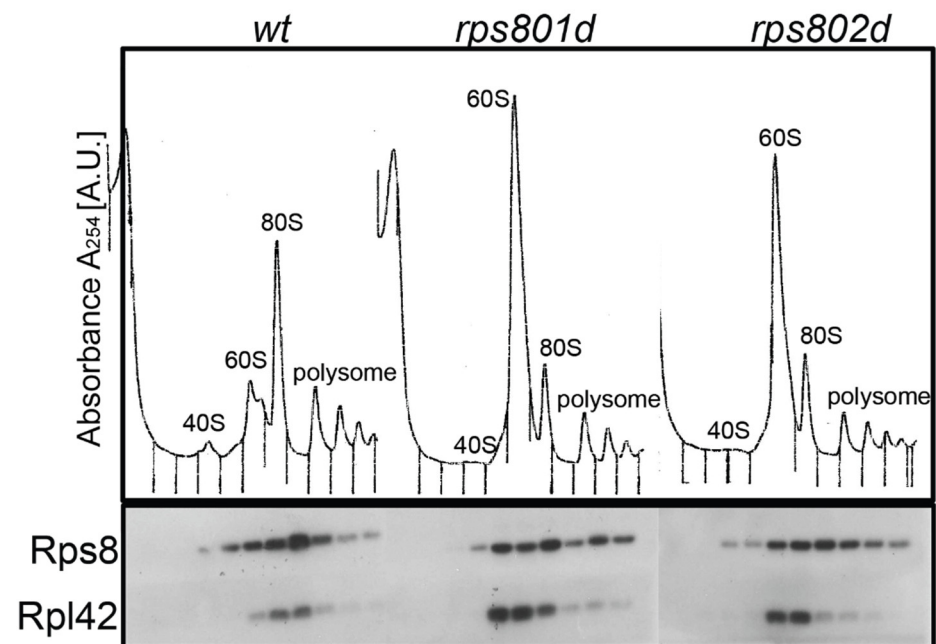


Figure S3. Rpl42/eL42 protein is present in accumulated 60S subunit in *rps8* paralog deletions. Polysome analysis by sucrose-gradient-based centrifugation to assess relative abundance of 40S, 60S, 80S ribosomes and polysomes. And Rpl42/eL42 protein level is detected in each fraction among *wt*, *rps801d* and *rps802d* cells by western blotting.

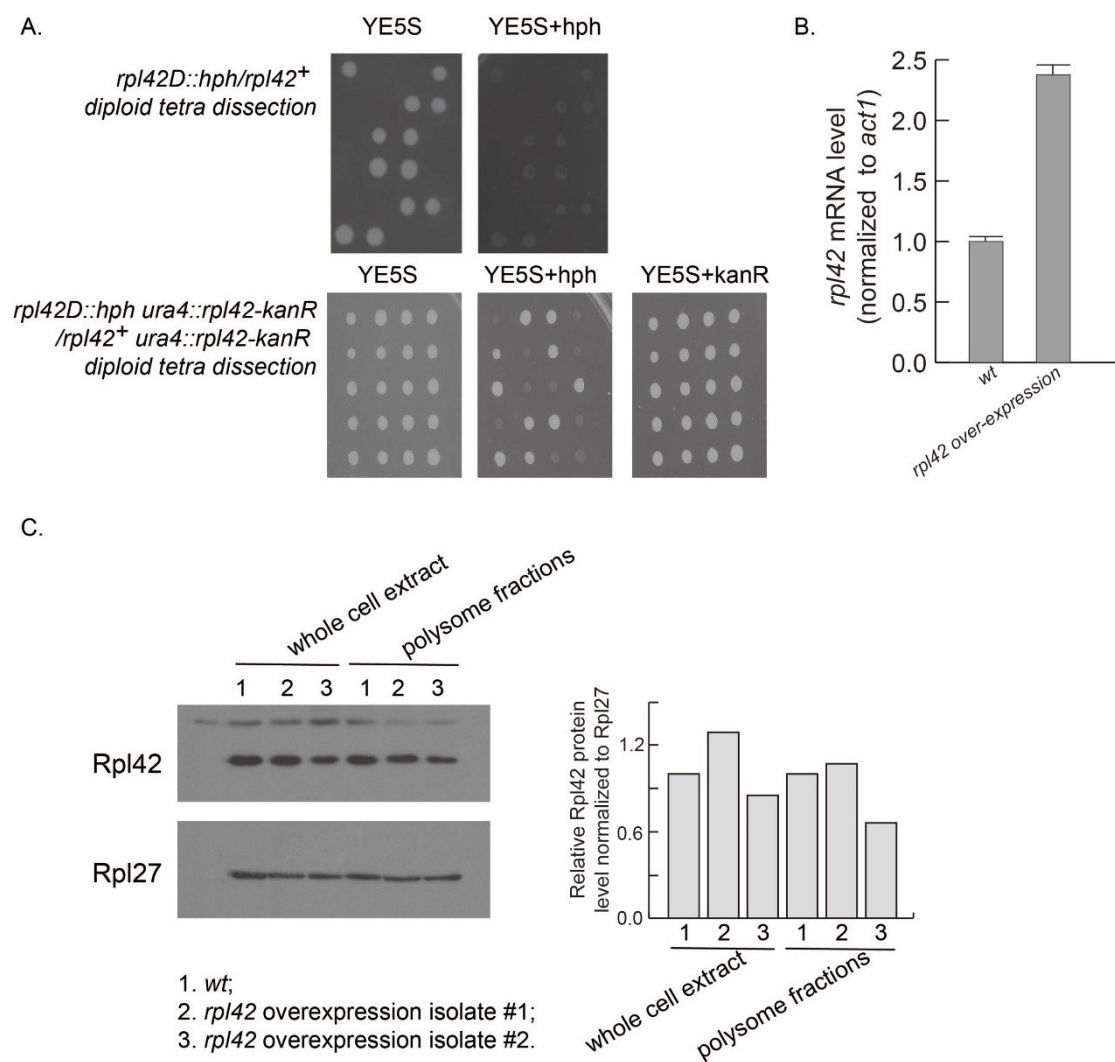


Figure S4. Quantification of *rpl42* mRNA and protein levels when *rpl42* is overexpressed in haploid wild type cells. *rpl42* is an essential gene in haploid wild type cells. B. Quantification of *rpl42* mRNA level by qRT-PCR. C. Quantification of Rpl42/eL42 protein level in whole cell extract and polysome fractions.

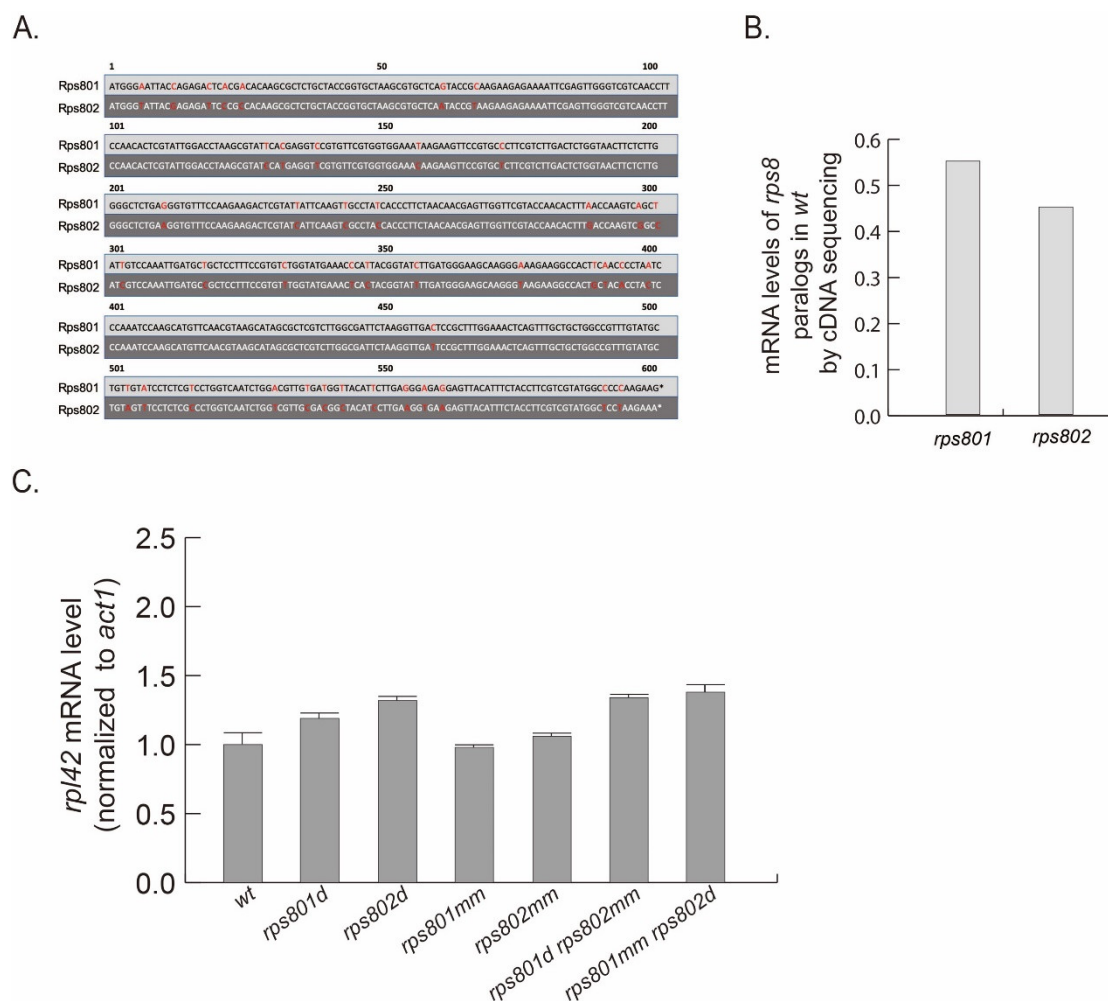


Figure S5. Quantification of Rps8/eS8 paralogs mRNA expression level in wild type cells and quantification of *rpl42* mRNA level in wild type and *rps8* mutants. Comparison of Rps8/eS8 paralogs DNA sequence. B. Quantification of *rps8* paralogs mRNA expression level in wild type cells by cDNA sequencing. C. Quantification of *rpl42* mRNA expression level in wild type and *rps8* mutants by qRT-PCR.

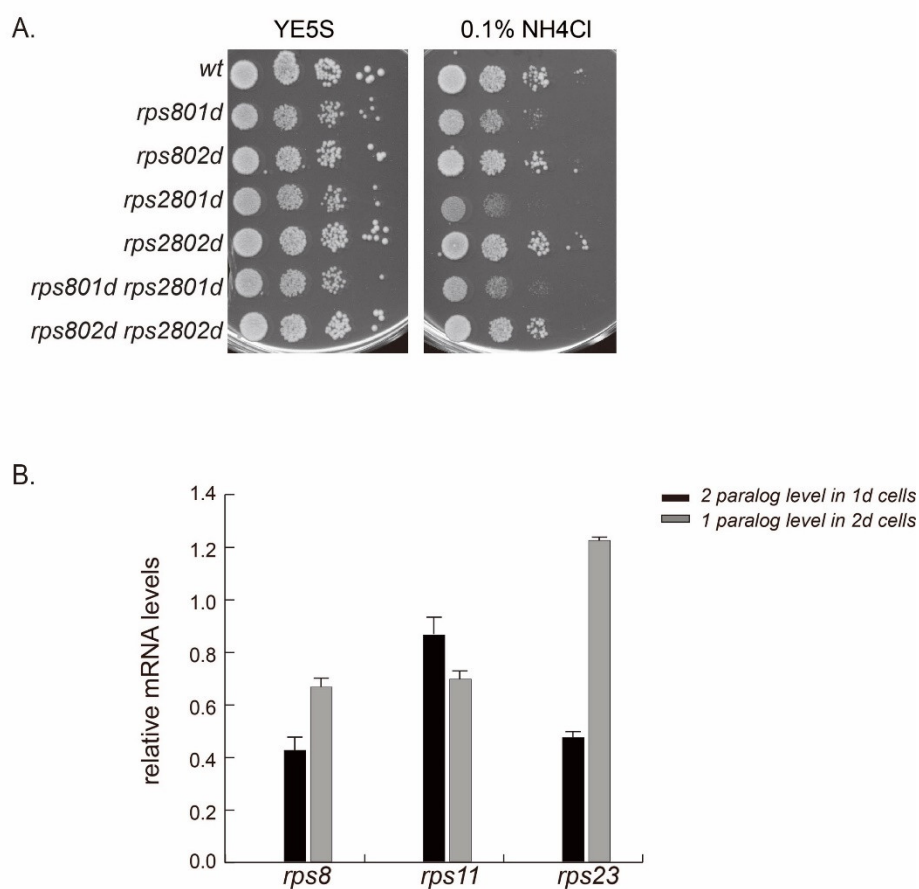


Figure S6. Epistasis analysis between *rps8* paralogs and *rps28* paralogs by spotting assay(A) and the impact of deletion one RP paralog gene on the RP total expression level by qRT-PCR (B).

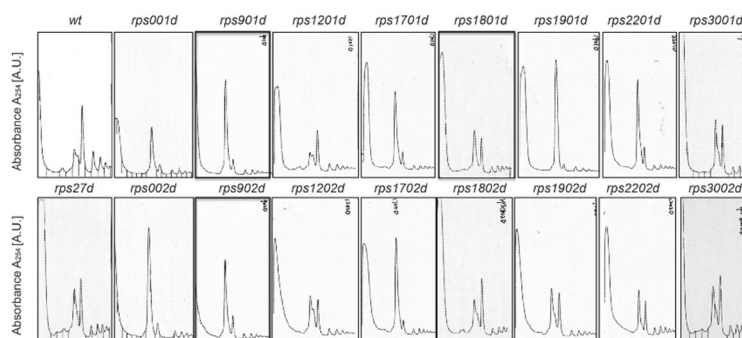


Figure S7. 60S subunits accumulation was observed in other *rps* deletion mutants.

Table S1. Ribosome paralog deletion mutants show multiple paralog-specific phenotypes.

Ribosome proteins	Ribosome paralogs	<i>cnt2::ade6</i> PEV	High NH ₄ Cl sensitiv- ity	Cycloheximide sensi- tivity
Rps0/uS2	<i>Rps001</i>	WT-like	Resistant	Sensitive
	<i>Rps002</i>	Reduced	Sensitive	Sensitive
Rps1/eS1	<i>Rps101</i>	WT-like	NA	NA

	<i>Rps102</i>	NA	NA	NA
	<i>Rps401</i>	NA	NA	NA
Rps4/eS4	<i>Rps402</i>	WT-like	NA	NA
	<i>Rps403</i>	WT-like	NA	NA
Rps5/uS7	<i>Rps5</i>	NA	NA	NA
	<i>Rps502</i>	WT-like	NA	NA
Rps6/eS6	<i>Rps601</i>	NA	NA	NA
	<i>Rps602</i>	NA	NA	NA
Rps8/eS8	<i>Rps801</i>	Reduced	Sensitive	Sensitive
	<i>Rps802</i>	WT-like	Resistant	WT-like
Rps9/uS4	<i>Rps901</i>	Reduced	Resistant	Sensitive
	<i>Rps902</i>	Reduced	Resistant	WT-like
Rps10/eS10	<i>Rps1001</i>	NA	NA	NA
	<i>Rps1002</i>	NA	NA	NA
Rps11/uS17	<i>Rps1101</i>	Reduced	Resistant	WT-like
	<i>Rps1102</i>	WT-like	Sensitive	Sensitive
Rps12/eS12	<i>Rps1201</i>	WT-like	Resistant	WT-like
	<i>Rps1202</i>	WT-like	Resistant	WT-like
Rps14/uS11	<i>Rps1401</i>	NA	NA	NA
	<i>Rps1402</i>	WT-like	NA	NA
Rps15/uS19	<i>Rps1501</i>	NA	NA	NA
	<i>Rps1502</i>	Reduced	NA	NA
Rps17/eS17	<i>Rps1701</i>	Reduced	Resistant	Sensitive
	<i>Rps1702</i>	Reduced	Resistant	Sensitive
Rps18/uS13	<i>Rps1801</i>	WT-like	Resistant	NA
	<i>Rps1802</i>	WT-like	Resistant	NA
Rps19/eS19	<i>Rps1901</i>	NA	NA	NA
	<i>Rps1902</i>	WT-like	WT-like	NA
Rps22/uS8	<i>Rps2201</i>	WT-like	Resistant	Sensitive
	<i>Rps2202</i>	Reduced	Resistant	WT-like
Rps23/uS12	<i>Rps23</i>	Reduced	Sensitive	Sensitive
	<i>Rps2302</i>	WT-like	Resistant	WT-like
Rps24/eS24	<i>Rps2401</i>	WT-like	NA	NA
	<i>Rps2402</i>	NA	NA	NA
Rps25/eS25	<i>Rps2501</i>	NA	NA	NA
	<i>Rps2502</i>	NA	NA	NA
Rps26/eS26	<i>Rps2601</i>	NA	NA	NA
	<i>Rps2602</i>	NA	NA	NA
Rps28/eS28	<i>Rps2801</i>	Reduced	Sensitive	Sensitive
	<i>Rps2802</i>	Bi-stable	Resistant	WT-like
Rps30/eS30	<i>Rps3001</i>	Reduced	WT-like	WT-like
	<i>Rps3002</i>	Reduced	WT-like	WT-like
Rpl5/uL18	<i>Rpl501</i>	WT-like	Resistant	Sensitive
	<i>Rpl502</i>	WT-like	WT-like	WT-like
Rpl8/eL8	<i>Rpl801</i>	NA	NA	NA
	<i>Rpl802</i>	NA	NA	NA
	<i>Rpl803</i>	Enhanced	NA	NA
Rpl9/uL6	<i>Rpl901</i>	Reduced	Resistant	NA
	<i>Rpl902</i>	Reduced	WT-like	NA
Rpl10/uL16	<i>Rpl1001</i>	NA	NA	NA

	<i>Rpl1002</i>	WT-like	NA	NA
Rpl11/uL5	<i>Rpl1101</i>	All white	Sensitive	NA
	<i>Rpl1102</i>	WT-like	Sensitive	NA
Rpl12/uL11	<i>Rpl1201</i>	WT-like	WT-like	Sensitive
	<i>Rpl1202</i>	WT-like	WT-like	Sensitive
Rpl15/eL15	<i>Rpl15</i>	NA	Resistant	NA
	<i>Rpl1502</i>	WT-like	Resistant	NA
	<i>Rpl1601</i>	NA	NA	NA
Rpl16/uL13	<i>Rpl1602</i>	NA	NA	NA
	<i>Rpl1603</i>	WT-like	NA	NA
	<i>Rpl1701</i>	NA	WT-like	NA
Rpl17/uL22	<i>Rpl1702</i>	WT-like	Resistant	NA
	<i>Rpl1801</i>	NA	NA	NA
Rpl18/eL18	<i>Rpl1802</i>	NA	NA	NA
	<i>Rpl1901</i>	WT-like	WT-like	NA
Rpl19/eL19	<i>Rpl1902</i>	WT-like	Resistant	NA
	<i>Rpl2001</i>	WT-like	Sensitive	NA
Rpl20/eL20	<i>Rpl2002</i>	All white	Resistant	NA
	<i>Rpl2101</i>	All white	WT-like	NA
Rpl21/eL21	<i>Rpl2102</i>	NA	WT-like	NA
	<i>Rpl24</i>	WT-like	WT-like	WT-like
Rpl24/eL24	<i>Rpl2402</i>	WT-like	WT-like	WT-like
Rpl27/eL27	<i>Rpl2701</i>	NA	NA	NA
	<i>Rpl2702</i>	Reduced	NA	NA
	<i>Rpl2801</i>	Enhanced	WT-like	WT-like
Rpl28/uL15	<i>Rpl2802</i>	Reduced	Resistant	Sensitive
	<i>Rpl3001</i>	NA	NA	NA
Rpl30/eL30	<i>Rpl3002</i>	WT-like	NA	NA
	<i>Rpl3201</i>	NA	Resistant	NA
Rpl32/eL32	<i>Rpl3202</i>	NA	WT-like	NA
Rpl36/eL36	<i>Rpl3601</i>	Reduced	Resistant	Sensitive
	<i>Rpl3602</i>	Reduced	Resistant	Sensitive
Rpl37/eL37	<i>Rpl3702</i>	WT-like	NA	NA
	<i>Rpl3703</i>	NA	NA	NA
Rpl38/eL38	<i>Rpl3801</i>	WT-like	Resistant	NA
	<i>Rpl3802</i>	NA	WT-like	NA
Rpl41/eL41	<i>Rpl4101</i>	NA	NA	NA
	<i>Rpl4102</i>	NA	NA	NA
Rpl43/eL43	<i>Rpl4301</i>	Reduced	Resistant	Sensitive
	<i>Rpl4302</i>	Enhanced	WT-like	WT-like

Note: CEN-PEV phenotypes of the RP paralog deletions were examined by visual inspection of the colony color sectoring morphology. NA: not available; Reduced: colonies showed significant reduction in frequency of color switching; Enhanced: colonies showed significant enhancement in frequency of color switching; Bi-stable: the colonies showed both reduced and enhanced PEV phenotype. The growth rates of the RP paralog deletions on YE media supplied with excess NH₄Cl or different concentrations of cycloheximide were assessed with spotting assay analysis. Resistant: the mutant cells grow better than wild type cells; Sensitive: the wild type cells grow better than the mutants.

Table S2. Summary of specific information in different Rps8/eS8 constructs.

Genotype	Gene dosage of Rps8	Protein identity of Rps8	Promoter of Rps8
<i>wt</i>	2	Rps801 and Rps802	P1 and P2

<i>rps801d</i>	1	Rps802	P2
<i>rps802d</i>	1	Rps801	P1
<i>rps801mm</i> (S130A N133T)	2	Rps802	P1 and P2
<i>rps802mm</i> (A130S T133N)	2	Rps801	P1 and P2
<i>rps801mm</i> (S130A N133T)	1	Rps802	P1
<i>rps802mm</i> (A130S T133N)	1	Rps801	P2

Table S3. Strain list.

Name	Genotype	Source
446	<i>h- ade6-210 leu1-32 ura4-D18</i>	Lab stock
447	<i>h+ ade6-216 leu1-32 ura4-D18</i>	Lab stock
LW258	<i>rps802d::kanR</i>	This study
LW261	<i>rps802d::hph</i>	This study
LW262	<i>rps801d ::kanR</i>	This study
LW284	<i>rps801d ::hph</i>	This study
LW314	<i>rps802d ::hph rpl31-GFP ::kanR</i>	This study
LW316	<i>rps801d ::hph rpl31-GFP ::kanR</i>	This study
LW318	<i>h- rpl31-GFP ::kanR</i>	This study
LW539	<i>rpl42+/- ::hph ura4-rpl42 ::kanR diploid #1</i>	This study
LW540	<i>rpl42+/- ::hph ura4-rpl42 ::kanR diploid #2</i>	This study
LW553	<i>rpl42+/- ::hph diploid</i>	This study
LW515	<i>rps801mm(S130A N133T)::hph</i>	This study
LW454	<i>rps802mm(A130S T133N)::hph</i>	This study
LW471	<i>rps802mm(A130S T133N)::hph rps801d::kanR</i>	This study
LW537	<i>rps801mm(S130A N133T)::hph rps802d::kanR</i>	This study
LW701	<i>rps801d ::kanR rpl3201-GFP ::kanR</i>	This study
LW703	<i>rps802d ::kanR rpl3201-GFP ::kanR</i>	This study
ZJ-L3201G	<i>rpl3201-GFP ::kanR</i>	This study
ZJ-25-8	<i>rpl42(G51S F58S) ::kanR</i>	This study
ZJ-25-12	<i>rps801d ::hph rpl42(G51S F58S) ::kanR</i>	This study
ZJ-25-6	<i>rps802d ::hph rpl42(G51S F58S) ::kanR</i>	This study
ZJ-25-7	<i>rpl42(K100M A103T) ::kanR</i>	This study
ZJ-25-15	<i>rps801d ::hph rpl42(K100M A103T) ::kanR</i>	This study
ZJ-25-4	<i>rps802d ::hph rpl42(K100M A103T) ::kanR</i>	This study
ZJ-DM-5	<i>rps801d ::hph rps2801d ::kanR</i>	This study
ZJ-DM-2	<i>rps802d ::hph rps2802d ::kanR</i>	This study
LW688	<i>rps1101d ::kanR rpl42(K100M A103T) ::kanR</i>	This study
LW689	<i>rps23d ::kanR rpl42(K100M A103T) ::kanR</i>	This study
LW691	<i>rps2302d ::kanR rpl42(K100M A103T) ::kanR</i>	This study
LW693	<i>rps1102d ::kanR rpl42(K100M A103T) ::kanR</i>	This study
LW695	<i>rps2801d ::kanR rpl42(K100M A103T) ::kanR</i>	This study
LW697	<i>rps2802d ::kanR rpl42(K100M A103T) ::kanR</i>	This study
B08-C06	<i>rps001d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B27-A11	<i>rps002d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B22-A03	<i>rps101d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B03-H10	<i>rps402d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B34-G02	<i>rps403d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B06-G02	<i>rps502d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B30-F11	<i>rps801d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock

B25-A06	<i>rps802d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B36-G11	<i>rps901d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B29-H01	<i>rps902d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B27-C12	<i>rps1101d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B23-A04	<i>rps1102d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B09-B08	<i>rps1201d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B26-E10	<i>rps1202d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B07-B07	<i>rps1402d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B31-B08	<i>rps1502d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B24-G04	<i>rps1701d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B04-G03	<i>rps1702d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B24-D02	<i>rps1801d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
J01-G12	<i>rps1802d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
LW-S1901D	<i>rps1901d ::kanR leu1-32 ura4-D18 ade6 ?</i>	This study
B33-E07	<i>rps1902d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
ZJ-S2201D	<i>rps2201d ::kanR leu1-32 ura4-D18 ade6 ?</i>	This study
B31-F02	<i>rps2202d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B28-B01	<i>rps23d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B11-G04	<i>rps2302d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B09-A08	<i>rps2401d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B01-C11	<i>rps2801d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B29-H08	<i>rps2802d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B05-G05	<i>rps3001d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
ZJ-S3002D	<i>rps3002d ::kanR leu1-32 ura4-D18 ade6 ?</i>	This study
B06-F05	<i>rpl501d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B21-B12	<i>rpl502d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B30-C03	<i>rpl803d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B26-E03	<i>rpl901d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B17-D11	<i>rpl902d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B27-A08	<i>rpl1002d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B22-F07	<i>rpl1101d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B32-B02	<i>rpl1102d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B39-C06	<i>rpl1201d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B20-B01	<i>rpl1202d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B18-H10	<i>rpl15d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B26-B12	<i>rpl1502d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B02-A10	<i>rpl1603d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B36-F08	<i>rpl1701d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B28-B04	<i>rpl1702d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B39-C10	<i>rpl1901d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B23-B02	<i>rpl1902d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B36-G12	<i>rpl2001d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B24-C11	<i>rpl2002d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B28-B12	<i>rpl2101d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B30-H07	<i>rpl2102d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B02-E02	<i>rpl24d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B30-H08	<i>rpl2402d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B03-E03	<i>rpl2702d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B29-E08	<i>rpl2801d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
ZJ-L2802D	<i>rpl2802d ::kanR leu1-32 ura4-D18 ade6 ?</i>	This study
B04-H09	<i>rpl3002d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock

B03-B10	<i>rpl3201d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B27-D12	<i>rpl3202d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B21-H11	<i>rpl3601d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B38-A02	<i>rpl3602d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B08-D11	<i>rpl3702d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B01-D06	<i>rpl3801d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
LW-L3802D	<i>rpl3802d ::kanR leu1-32 ura4-D18 ade6 ?</i>	This study
B08-A07	<i>rpl4301d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock
B30-C10	<i>rpl4302d ::kanR cnt2 -ade6 ::nat leu1-32 ura4-D18 ade6D ::hph</i>	Lab stock