

SUPPLEMENTARY MATERIAL FOR:

Rps3/uS3 promotes mRNA binding at the 40S ribosome entry channel and stabilizes preinitiation complexes at start codons

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Table S1. Summary of key phenotypes for Rps3 substitutions

Rps3 substitution	Relevant 48S PIC contacts ($\leq 5 \text{ \AA}$)	Cell Growth (Slg ⁻)	Suppression of <i>SUI5</i> His ⁺ phenotype	Suppression of <i>SUI5</i> Slg ⁻ phenotype at 37°C	<i>HIS4-lacZ</i> UUG:AUG expression in <i>SUI5</i> cells
none (WT)	NA ¹	++++	none (WT)	-	0.151±0.019
K108D	none	++++	strong	strong	0.039±0.006
R116A	mRNA	-/+	NA ²	NA ²	ND ³
R116D	mRNA	++++	moderate	strong	0.032±0.003
R117A	mRNA	++++	none	none	ND
R117D	mRNA	++++	strong	strong	0.026±0.004
K141A	latch	lethal	NA ²	NA ²	ND
K141D	latch	++	NA ²	NA ²	ND
R146A	latch/mRNA	lethal	NA ²	NA ²	ND
R146D	latch/mRNA	+++	slight	Moderate ⁴	0.036±0.006
K148A	mRNA	++++	none	none	ND
K148D	mRNA	++++	moderate	slight	0.026±0.004

¹NA, not applicable

²NA, as suppression of the effects of *SUI5* on growth in -His medium or in +His medium at 37°C could not be scored owing to lethality (*K141A* & *R146A*) or strongly impaired growth (*R116A*) conferred by the *rps3* mutation in the absence of *SUI5* (Fig. 2B).

³ND, not determined

⁴Although growth of the *R146D/SUI5* strain is only slightly better than that of the WT/*SUI5* control strain on +His medium at 37°C (cf. rows 1 and row 12 in Fig. 2B), taking into account the reduced growth of the *R146D/SUI5* strain relative to the WT/*SUI5* strain at 30°C (left panel) suggests a moderate versus slight degree of suppression.

ND, not determined

Table S2. Yeast strains employed in this work.

Strain	Genotype	Source
H2995	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)</i>	(Valasek et al. 2004)
HD2738	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i>	This study
HD2754	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX pDH412 [lc^a LEU2 RPS3⁺ in pRS315]</i>	This study
HD2755	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX pRS315 [lc LEU2 vector]</i>	This study
HD2765	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX pDH424 [lc LEU2 rps3-K148D in pRS315]</i>	This study
HD2764	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX pDH431 [lc LEU2 rps3-K148A in pRS315]</i>	This study
HD2767	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX pDH425 [lc LEU2 rps3-R116D in pRS315]</i>	This study
HD2766	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX pDH432 [lc LEU2 rps3-R116A in pRS315]</i>	This study
HD2769	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX pDH482 [lc LEU2 rps3-R117D in pRS315]</i>	This study
HD2768	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX pDH433 [lc LEU2 rps3-R117A in pRS315]</i>	This study
HD3120	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX pDH13-37 [lc LEU2 rps3K108D in pRS315]</i>	This study
HD2772	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX pDH429 [lc LEU2 rps3-R146D in pRS315]</i>	This study
HD2779	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX pDH430 [lc LEU2 rps3-K141D in pRS315]</i>	This study
HD2836	<i>MATα ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX pDH412 [lc LEU2 RPS3⁺ in pRS315] p4281 [sc^a TRP1 SUI5 in YCplac22]</i>	This study

Strain	Genotype	Source
HD2911	<i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> pDH412 [lc <i>LEU2 RPS3⁺</i> in pRS315] YCplac22 [sc <i>TRP1</i> vector]	This study
HD2846	<i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> pDH424 [lc <i>LEU2 rps3-K148D</i> in pRS315] p4281 [sc <i>TRP1 SUI5</i> in YCplac22]	This study
HD2868	<i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> pDH431 [lc <i>LEU2 rps3-K148A</i> in pRS315] p4281 [sc <i>TRP1 SUI5</i> in YCplac22]	This study
HD2848	<i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> pDH425 [lc <i>LEU2 rps3-R116D</i> in pRS315] p4281 [sc <i>TRP1 SUI5</i> in YCplac22]	This study
HD2861	<i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> pDH432 [lc <i>LEU2 rps3-R116A</i> in pRS315] p4281 [sc <i>TRP1 SUI5</i> in YCplac22]	This study
HD2850	<i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> pDH482 [lc <i>LEU2 rps3-R117D</i> in pRS315] p4281 [sc <i>TRP1 SUI5</i> in YCplac22]	This study
HD2849	<i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> pDH433 [lc <i>LEU2 rps3-R117A</i> in pRS315] p4281 [sc <i>TRP1 SUI5</i> in YCplac22]	This study
HD3145	<i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> pDH13-37 [lc <i>LEU2 rps3-K108D</i> in pRS315] p4281 [sc <i>TRP1 SUI5</i> in YCplac22]	This study
HD2841	<i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> pDH429 [lc <i>LEU2 rps3-R146D</i> in pRS315] p4281 [sc <i>TRP1 SUI5</i> in YCplac22]	This study
HD2860	<i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> pDH430 [lc <i>LEU2 rps3-K141D</i> in pRS315] p4281 [sc <i>TRP1 SUI5</i> in YCplac22]	This study
HD2973	<i>MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX</i> pDH459 [lc <i>URA3 RPS3⁺</i> in pRS316]	This study
HD3240	<i>MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX</i> pDH412 [lc <i>LEU2 RPS3⁺</i> in pRS315]	This study

Strain	Genotype	Source
HD3241	<i>MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX</i> pDH425 [lc <i>LEU2 rps3-R116D</i> in pRS315]	This study
HD3242	<i>MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX</i> pDH482 [lc <i>LEU2 rps3-R117D</i> in pRS315]	This study
HD3243	<i>MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX</i> pDH13-37 [lc <i>LEU2 rps3-K108D</i> in pRS315]	This study
HD3244	<i>MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX</i> pDH424 [lc <i>LEU2 rps3-K148D</i> in pRS315]	This study
HD3277	<i>MATa ura3Δ0 leu2Δ0 his3Δ0 met15Δ0 rps3Δ::kanMX</i> pDH429 [lc <i>LEU2 rps3-R146D</i> in pRS315]	This study

^alc, low copy number; sc, single copy

Table S3. Plasmids employed in this work.

Plasmid	Description	Source
pRS315	lc ^a <i>LEU2</i> vector	(Sikorski and Hieter 1989)
pRS316	lc <i>URA3</i> vector	(Sikorski and Hieter 1989)
p4281	sc <i>TRP1 TIF5-G31R</i> in YCplac22	(Valasek et al. 2004)
YCplac22	sc <i>TRP1</i> vector	(Gietz and Sugino 1988)
pDH412	lc <i>LEU2, RPS3</i> ⁺	This study
pDH459	lc <i>URA3, RPS3</i> ⁺	This study
pDH424	lc <i>LEU2, rps3-K148D</i>	This study
pDH431	lc <i>LEU2, rps3-K148A</i>	This study
pDH425	lc <i>LEU2, rps3-R116D</i>	This study
pDH432	lc <i>LEU2, rps3-R116A</i>	This study
pDH482	lc <i>LEU2, rps3-R117D</i>	This study
pDH433	lc <i>LEU2, rps3-R117A</i>	This study
pDH13-37	lc <i>LEU2, rps3-K108D</i>	This study
pDH429	lc <i>LEU2, rps3-R146D</i>	This study
pDH430	lc <i>LEU2, rps3-K141D</i>	This study
p3218	pFA6a-kanMX6-PGAL1	(Longtine et al. 1998)
p367	sc <i>URA3 HIS4(ATG)-lacZ</i>	(Donahue and Cigan 1988)

Plasmid	Description	Source
p391	sc <i>URA3 HIS4(TTG)-lacZ</i>	(Donahue and Cigan 1988)
pPMB24	sc <i>URA3 SUI1-lacZ</i>	(Martin-Marcos et al. 2011)
pPMB25	sc <i>URA3 SUI1-opt-lacZ</i>	(Martin-Marcos et al. 2011)

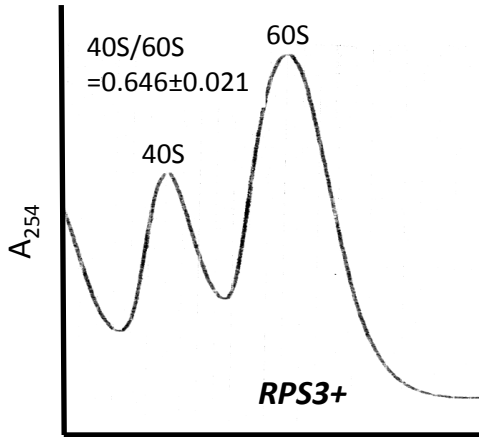
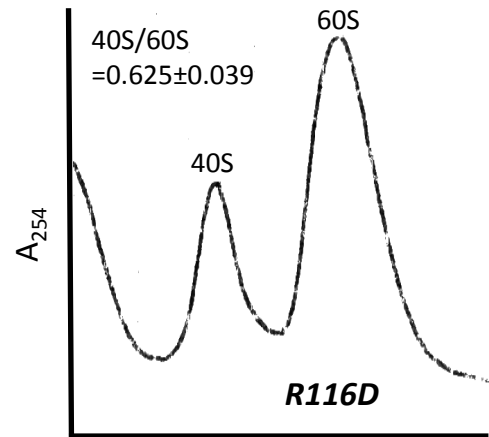
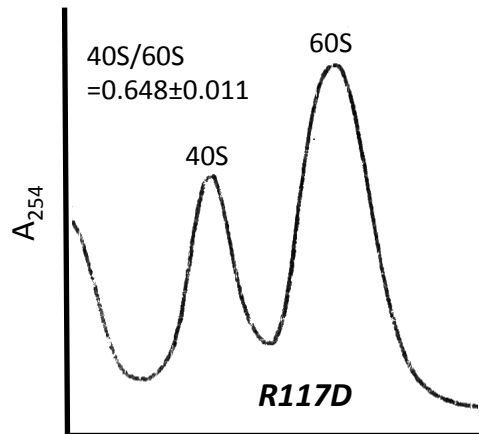
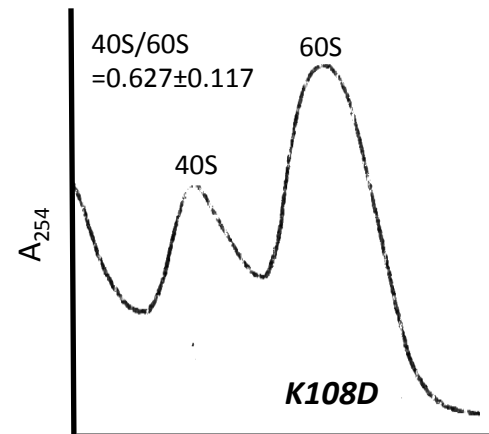
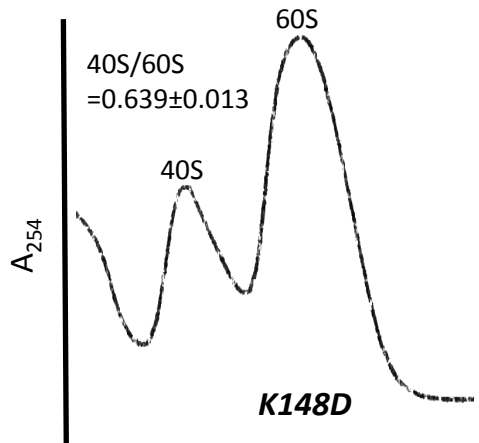
^alc, low copy number; sc, single copy.

Table S4. Primers used for mutagenesis (with mutated nucleotides underlined).

Primer	Sequence (5' to 3')	Mutation
RPS3-K148D	CTAAGAGCTGCCAGAGCT <u>GAT</u> GCTATGAAATTTGCTGAC	<i>K148D</i>
RPS3-K148D-r	GTCAGCAAATTTTCATAGC <u>ATC</u> AGCTCTGGCAGCTCTTAG	<i>K148D</i>
RPS3-K148A	CTAAGAGCTGCCAGAGCT <u>GCT</u> GCTATGAAATTTGCTGAC	<i>K148A</i>
RPS3-K148A-r	GTCAGCAAATTTTCATAGC <u>AGC</u> AGCTCTGGCAGCTCTTAG	<i>K148A</i>
RPS3-R116D	GAACGGTTTGGCTATC <u>GAT</u> AGAGCTGCTTACGGTGTCG	<i>R116D</i>
RPS3-R116D-r	CGACACCGTAAGCAGCTCT <u>ATC</u> GATAGCCAAACCGTTC	<i>R116D</i>
RPS3-R116A	GAACGGTTTGGCTATC <u>GCT</u> AGAGCTGCTTACGGTGTCG	<i>R116A</i>
RPS3-R116A-r	CGACACCGTAAGCAGCTCT <u>AGC</u> GATAGCCAAACCGTTC	<i>R116A</i>
RPS3-R117A	GGTTTGGCTATCAGAG <u>CTG</u> CTGCTTACGGTGTCGTCAG	<i>R117A</i>
RPS3-R117A-r	CTGACGACACCGTAAGCAGC <u>AGCT</u> CTGATAGCCAAACC	<i>R117A</i>
RPS3-R117D	GGTTTGGCTATCAGAG <u>ATG</u> CTGCTTACGGTGTCGTCAG	<i>R117D</i>
RPS3-R117D-r	CTGACGACACCGTAAGCAGC <u>ATCT</u> CTGATAGCCAAACC	<i>R117D</i>
RPS3-K108D	GCTGAATCTATGAAATTC <u>GAT</u> TTGTTGAACGGTTTGGC	<i>K108D</i>
RPS3-K108D-r	GCCAAACCGTTCAACAA <u>ATC</u> GAATTTTCATAGATTCAGC	<i>K108D</i>
RPS3-R146D	GGTAAACTAAGAGCTGCC <u>GAT</u> GCTAAGGCTATGAAATTT	<i>R146D</i>
RPS3-R146D-r	AAATTTTCATAGCCTTAGC <u>ATC</u> GGCAGCTCTTAGTTTACC	<i>R146D</i>
RPS3-K141D	GAAGTTGTTGTTTCCGGT <u>GAT</u> CTAAGAGCTGCCAGAGCT	<i>K141D</i>
RPS3-K141D-r	AGCTCTGGCAGCTCTTAG <u>ATC</u> ACCGGAAACAACAACCTTC	<i>K141D</i>

Figure S1. *RPS3* alleles do not significantly alter 40S:60S subunit ratios.

rps3Δ::kanMX deletion strains harboring plasmid-borne WT *RPS3*⁺ (HD3240) or *rps3* alleles *R116D* (HD3241), *R117D* (HD3242), *K108D* (HD3243), *K148D* (HD3244) or *R146D* (HD3277) were grown in YPD medium to A₆₀₀ ~1, and WCEs prepared in the absence of Mg⁺² were resolved by velocity sedimentation through 5 to 30% sucrose gradients and scanned at 254 nm. Mean (± S.E.M.) 40S/60S subunit ratios calculated from three biological replicates are indicated. Asterisks indicate significant differences between mutant and WT as judged by a two-tailed, unpaired Student's t-test (*, P<0.05; **, P<0.01).

A**B****C****D****E****F**