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SUPPLEMENTARY MATERIAL FOR:

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

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1 **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 |

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3 ¹NA, not applicable

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

7 ³ND, not determined

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

12 ND, not determined

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1 **Table S2. Yeast strains employed in this work.**

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| Strain | Genotype | Source |
|--------|--|-----------------------|
| H2995 | <i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG)</i> | (Valasek et al. 2004) |
| HD2738 | <i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> | This study |
| HD2754 | <i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> pDH412 [lc ^a <i>LEU2 RPS3</i> ⁺ in pRS315] | This study |
| HD2755 | <i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> pRS315 [lc <i>LEU2</i> vector] | This study |
| HD2765 | <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] | This study |
| HD2764 | <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] | This study |
| HD2767 | <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] | This study |
| HD2766 | <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] | This study |
| HD2769 | <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] | This study |
| HD2768 | <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] | This study |
| HD3120 | <i>MATa ura3-52 trp1Δ-63 leu2-3,112 his4-301(ACG) P_{GAL}-RPS3::kanMX</i> pDH13-37 [lc <i>LEU2 rps3K108D</i> in pRS315] | This study |
| HD2772 | <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] | This study |
| HD2779 | <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] | This study |
| HD2836 | <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] p4281 [sc ^a <i>TRP1 SUI5</i> in YCplac22] | 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 |

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

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1 **Table S3. Plasmids employed in this work.**

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| 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) |

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

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

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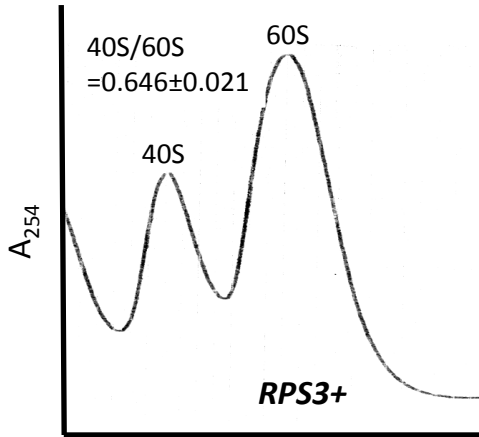
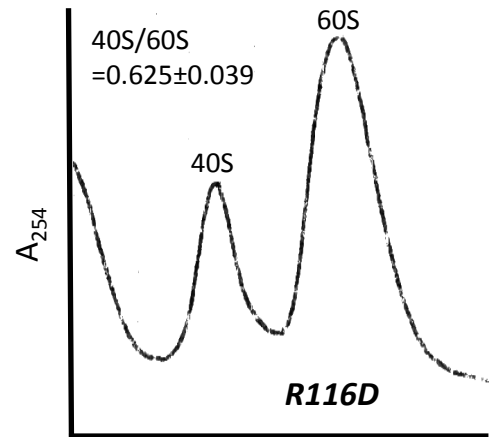
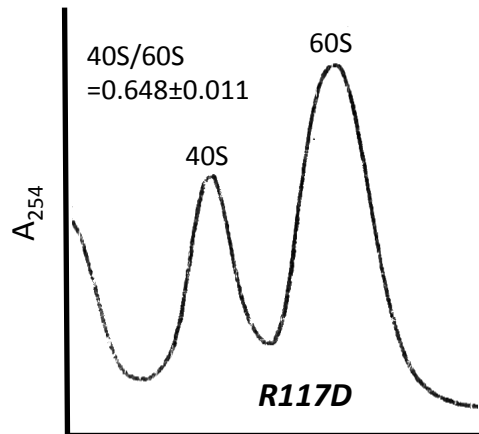
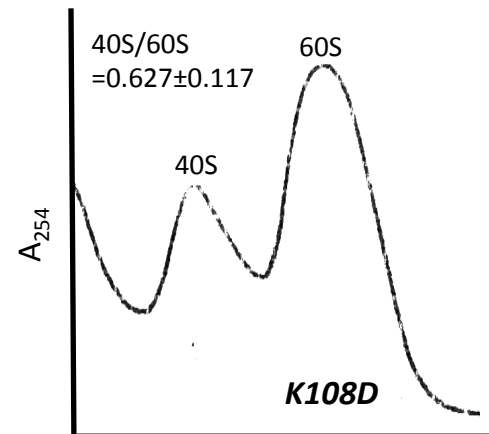
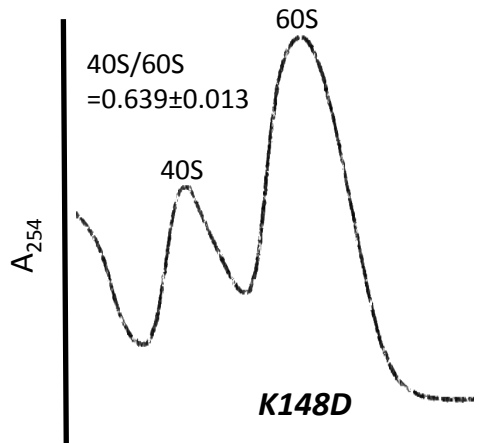
| Primer | Sequence (5' to 3') | Mutation |
|--------------|---|--------------|
| RPS3-K148D | CTAAGAGCTGCCAGAGCT <u>GAT</u> GCTATGAAATTTGCTGAC | <i>K148D</i> |
| RPS3-K148D-r | GTCAGCAAATTTTCATAGC <u>ATC</u> AGCTCTGGCAGCTCTTAG | <i>K148D</i> |
| RPS3-K148A | CTAAGAGCTGCCAGAGCT <u>GCT</u> GCTATGAAATTTGCTGAC | <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>CT</u> GCTGCTTACGGTGTCGTCAG | <i>R117A</i> |
| RPS3-R117A-r | CTGACGACACCGTAAGCAGC <u>AGCT</u> CTGATAGCCAAACC | <i>R117A</i> |
| RPS3-R117D | GGTTTGGCTATCAGAG <u>AT</u> GCTGCTTACGGTGTCGTCAG | <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> |

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1 **Figure S1. *RPS3* alleles do not significantly alter 40S:60S subunit ratios.**
2 *rps3Δ::kanMX* deletion strains harboring plasmid-borne WT *RPS3*⁺ (HD3240) or *rps3* alleles *R116D*
3 (HD3241), *R117D* (HD3242), *K108D* (HD3243), *K148D* (HD3244) or *R146D* (HD3277) were grown in
4 YPD medium to A₆₀₀ ~1, and WCEs prepared in the absence of Mg⁺² were resolved by velocity
5 sedimentation through 5 to 30% sucrose gradients and scanned at 254 nm. Mean (± S.E.M.) 40S/60S
6 subunit ratios calculated from three biological replicates are indicated. Asterisks indicate significant
7 differences between mutant and WT as judged by a two-tailed, unpaired Student's t-test (*, P<0.05; **,
8 P<0.01).

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