**Supplemental Material**

**Tables**

**Table S1.** Primers used in this study

|  |  |  |
| --- | --- | --- |
| **Primer Number** | **Sequence** | **Description** |
| UK9789 | ACCTTGAATTCCGTACGCTGCAGGTCGAC | *natNT2* |
| UK9790 | ACCTTGAATTCATCGATGAATTCGAGCTCG | *natNT2* |
| UG5953 | TCTAAGCTTCGGACGATTGCCAACCGCCGAA | *SUP17* |
| VB2609 | TCTAAGCTTCGCGGAAATTAGCACGGCCTC | *SUP17* |

**Table S2.** Number of peptides identified in mass spectrometry-based analysis of mistranslation rates.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Strain | Mistranslation Type | Replicate | # peptides containing WT residue | # peptides with mistranslated residue | % mistranslatedpeptides |
| CY8611  | Pro→Ala | 1 | 9664 | 23 | 0.24 |
| (Control) |  | 2 | 8866 | 19 | 0.21 |
|  |  | 3 | 9438 | 27 | 0.29 |
|  |  | 4 | 7747 | 24 | 0.31 |
|  |  | 5 | 7653 | 15 | 0.20 |
| CY8612 | Pro→Ala | 1 | 9540 | 312 | 3.27 |
| (tRNAProG3:U70) |  | 2 | 9790 | 341 | 3.48 |
|  |  | 3 | 9070 | 287 | 3.16 |
|  |  | 4 | 6879 | 200 | 2.91 |
|  |  | 5 | 6729 | 185 | 2.75 |
| CY8614 | Pro→Ala | 1 | 7787 | 15 | 0.19 |
| (tRNASerUCU, G26A) |  | 2 | 7626 | 18 | 0.24 |
|  |  | 3 | 7687 | 20 | 0.26 |
|  |  | 4 | 7290 | 9 | 0.12 |
|  |  | 5 | 6496 | 10 | 0.15 |
| CY8611  | Arg→Ser | 1 | 7184 | 12 | 0.17 |
| (Control) |  | 2 | 5991 | 5 | 0.08 |
|  |  | 3 | 6686 | 15 | 0.22 |
|  |  | 4 | 5084 | 8 | 0.16 |
|  |  | 5 | 4930 | 9 | 0.18 |
| CY8612 | Arg→Ser | 1 | 7185 | 13 | 0.18 |
| (tRNAProG3:U70) |  | 2 | 7471 | 8 | 0.11 |
|  |  | 3 | 6699 | 6 | 0.09 |
|  |  | 4 | 4135 | 6 | 0.14 |
|  |  | 5 | 4007 | 8 | 0.20 |
| CY8614 | Arg→Ser | 1 | 5142 | 158 | 3.07 |
| (tRNASerUCU, G26A) |  | 2 | 4894 | 149 | 3.04 |
|  |  | 3 | 5005 | 157 | 3.14 |
|  |  | 4 | 5053 | 127 | 2.51 |
|  |  | 5 | 4009 | 129 | 3.22 |

**Table S3.** Percent of arginine and proline content in the RNA processing and transport genes identified to have negative genetic interactions with tRNASerUCU, G26A or tRNAProG3:U70.

|  |  |  |
| --- | --- | --- |
| **Protein** | **Arginine****Content** | **Proline****Content** |
| Sen1 | 4.93 | 4.84 |
| Sad1 | 3.79 | 4.24 |
| Sof1 | 7.77 | 2.86 |
| Rna1 | 2.46 | 2.70 |
| Crm1 | 3.51 | 3.23 |
| Fip1 | 3.36 | 8.26 |
| Nop2 | 5.34 | 4.37 |
| Cus1 | 5.73 | 5.05 |
| Rpr1 | 5.76 | 2.52 |
| Rpr4 | 5.57 | 3.06 |
| Prp3 | 7.46 | 4.26 |
| Prp18 | 4.78 | 4.78 |
| Prp4 | 5.59 | 2.37 |
| Srm1 | 3.94 | 4.77 |
| Pwp1Snm1 | 3.133.54 | 3.473.54 |
| *Whole Genome* | 4.42 | 4.39 |

**Figures**

 GCGGCCGC ATCGCGAAAA TTCATGTCAT GTCCACATTA ACATCATTGC AGAGCAACAA TTCATTTTCA TAGAGAAATT TGCTACTATC ACCCACTAGT ACTACCATTG GTACCTACTA CTTTGAATTG TACTACCGCT GGGCGTTATT AGGTGTGAAA CCACGAAAAG TTCACCATAA CTTCGAATAA AGTCGCGGAA AAAAGTAAAC AAGCTTATAT GAATTC**ATG**C TTTCTG AAAACACGAC TATTCTGATG GCTAACGGTG AAATTAAAGA CATCGCAAAC GTCACGGCTA ACTCTTACGT TATGTGCGCA GATGGCTCCG CTGCCCGCGT CATAAATGTC ACACAGGGCT ATCAGAAAAT CTATAATATA CAGCAAAAAA CCAAACACAG AGCTTTTGAA GGTGAACCTG GTAGGTTAGA GCGGCCGC

**Figure S1.** Synthetic *HO* DNA. Underlined sequences are engineered restriction sites used for cloning. Bold sequence is the start codon of the *HO* gene.

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**Figure S2.** Mass spectrometry analysis of the cellular proteome was performed on a wild-type control strain (CY8611) or strains expressing the mistranslating tRNA tRNAProG3:U70 (CY8612) or tRNASerUCU, ­G26A (CY8614). Mistranslation of alanine at proline codons (**A**) or serine at arginine codons (**B**) at their respective codons.