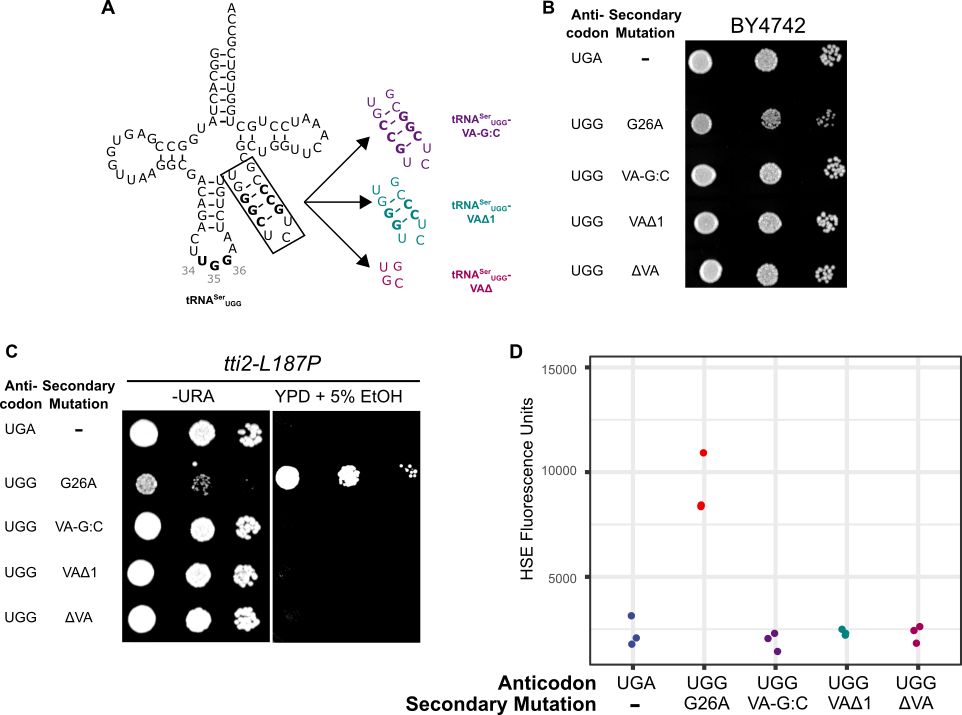
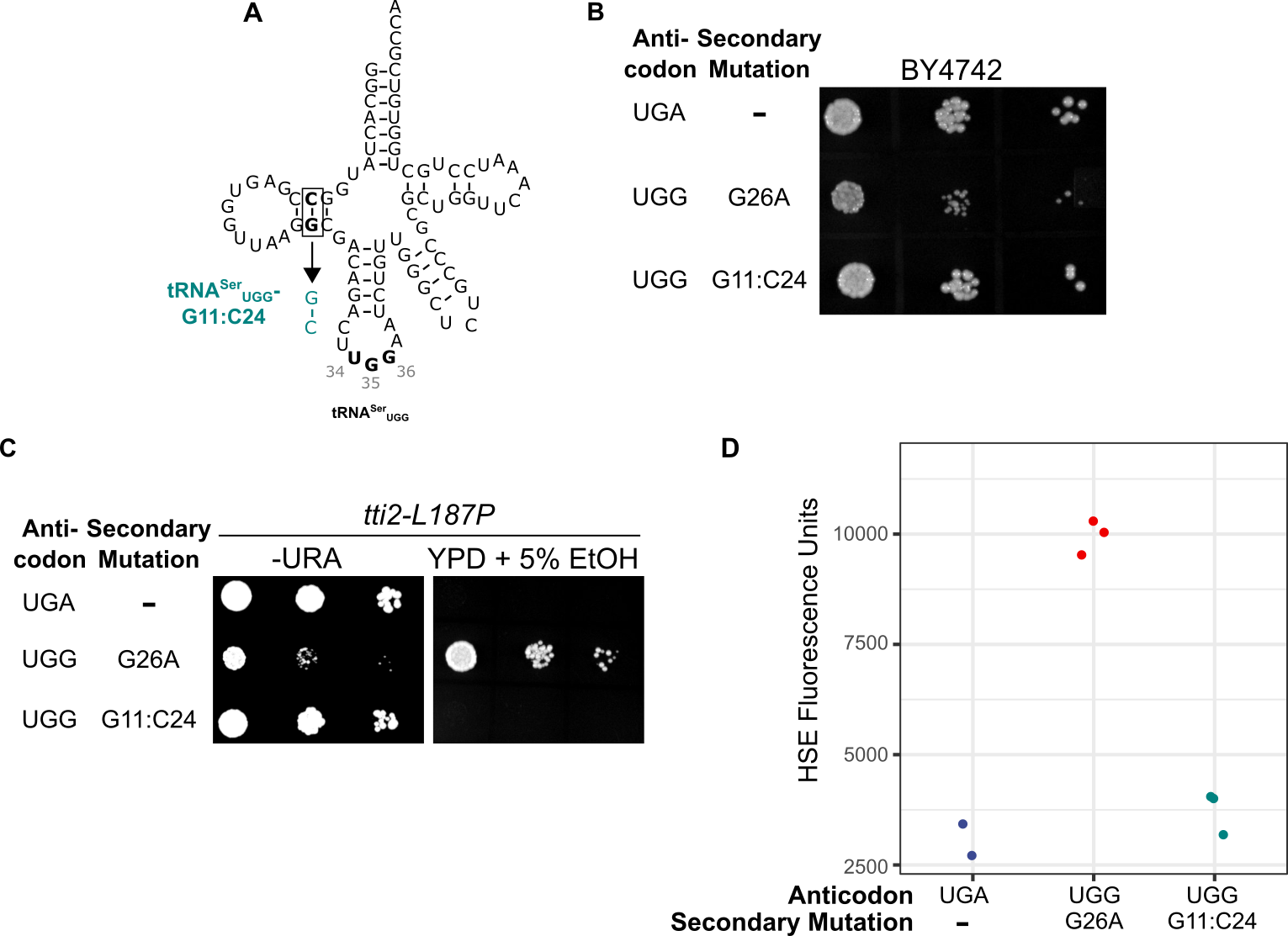
**Supplemental Material**

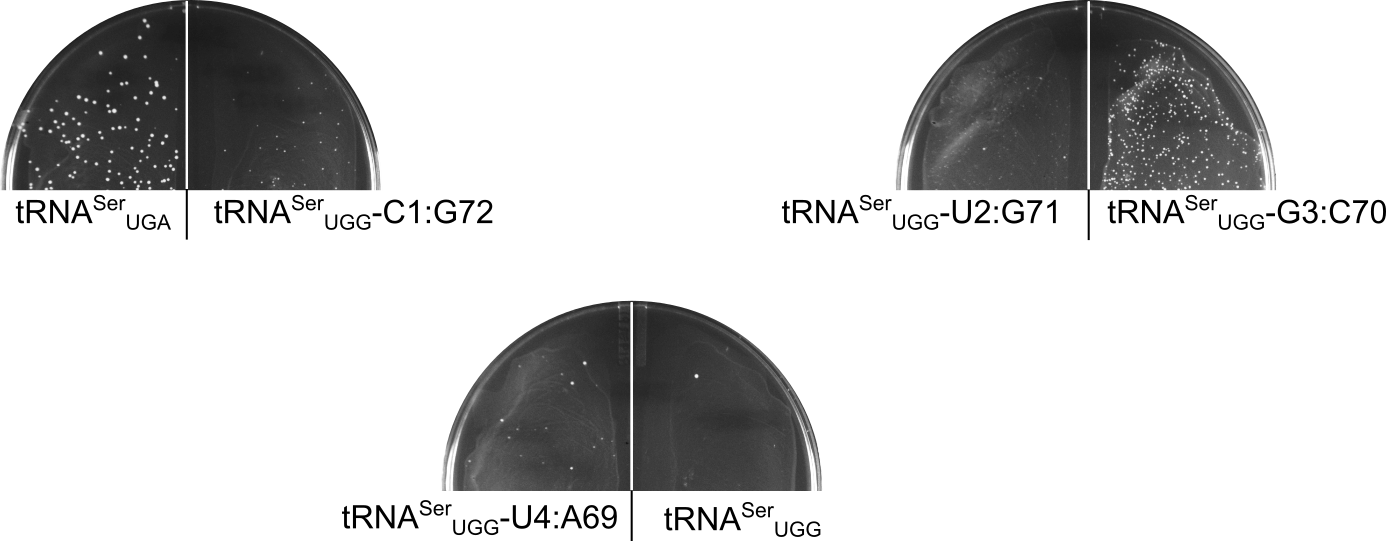
**Supplemental Figures**

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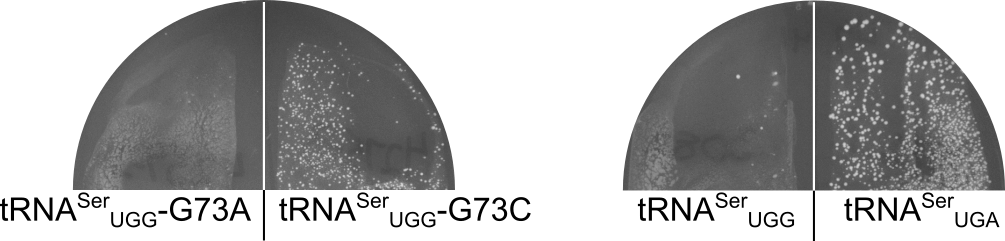
**Figure S1.** Targeted mutations to the variable arm of tRNASer do not mistranslate. (A) Structures of tRNASerUGG-VA-G:C, tRNASerUGG-VAΔ1 and tRNASerUGG-VAΔ. (B) Wild-type strain (BY4742) containing either a wild-type tRNASerUGA, tRNASerUGG-G26A or the tRNASerUGG alleles described in (A) were grown to saturation in media lacking uracil and spotted in 10-fold serial dilutions on the same media. (C) Yeast strains containing *tti2-L187P* (CY7020) andexpressing one of the tRNAs described in (B) were grown to saturation in media lacking uracil and spotted in 10-fold serial dilutions on media lacking uracil or YPD containing 5% ethanol. (D) Wild-type strains containing a variant tRNASerUGG described in (B) and a fluorescent heat shock reporter were grown in media lacking uracil and histidine. Cells were diluted 1:20 in the same media and grown for 6 hours. Cell densities were normalized and fluorescence measured. Each point represents one biological replicate.



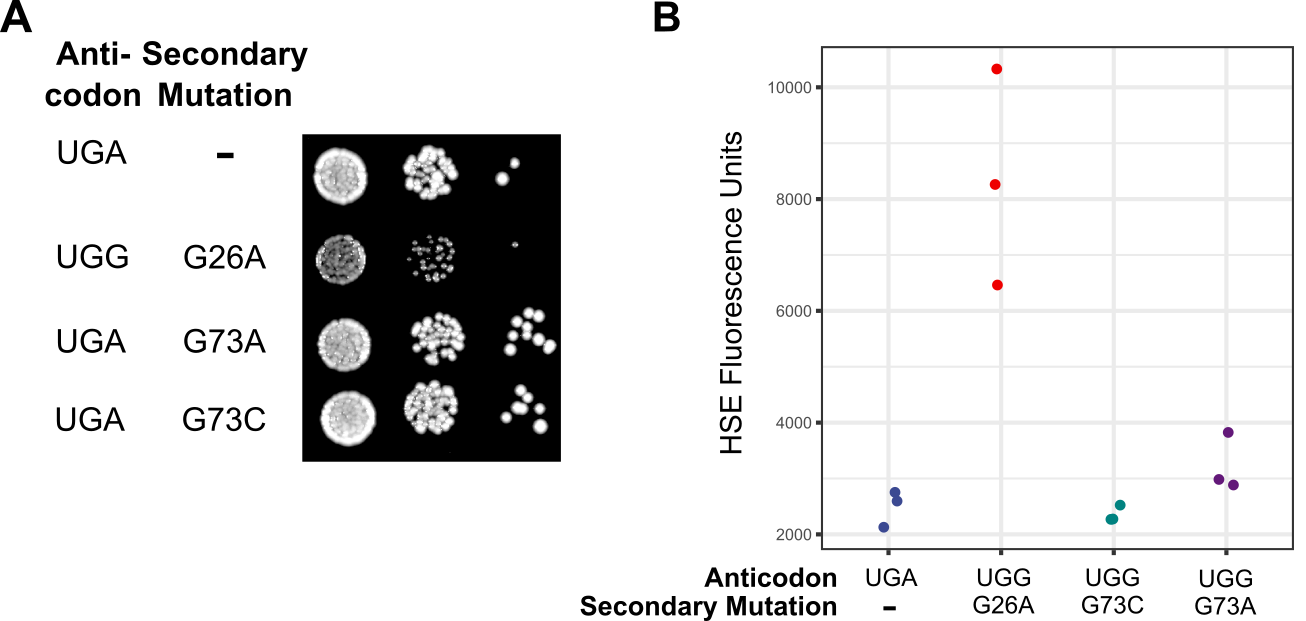
**Figure S2.** The C11:G24 base pair in the D-stem is required for tRNASer function. (A) Structure of tRNASerUGG-G11:C24. (B) Wild-type strain (BY4742) containing either wild-type tRNASerUGA, tRNASerUGG-G26A or tRNASerUGG-G11:C24 were grown to saturation in media lacking uracil and spotted in 10-fold serial dilutions on media lacking uracil. (C) Yeast strains containing *tti2-L187P* (CY7020) andexpressing one of the tRNAs described in (B) were grown to saturation in media lacking uracil and spotted in 10-fold serial dilutions on media lacking uracil or complete media containing 5% ethanol. (D) Wild-type strains containing a variant tRNASerUGG described in (B) and an HSE-GFP reporter were grown in media lacking uracil and histidine. Cells were diluted 1:20 in the same media and grown for 6 hours. Cell densities were normalized and fluorescence measured. Each point represents one biological replicate.



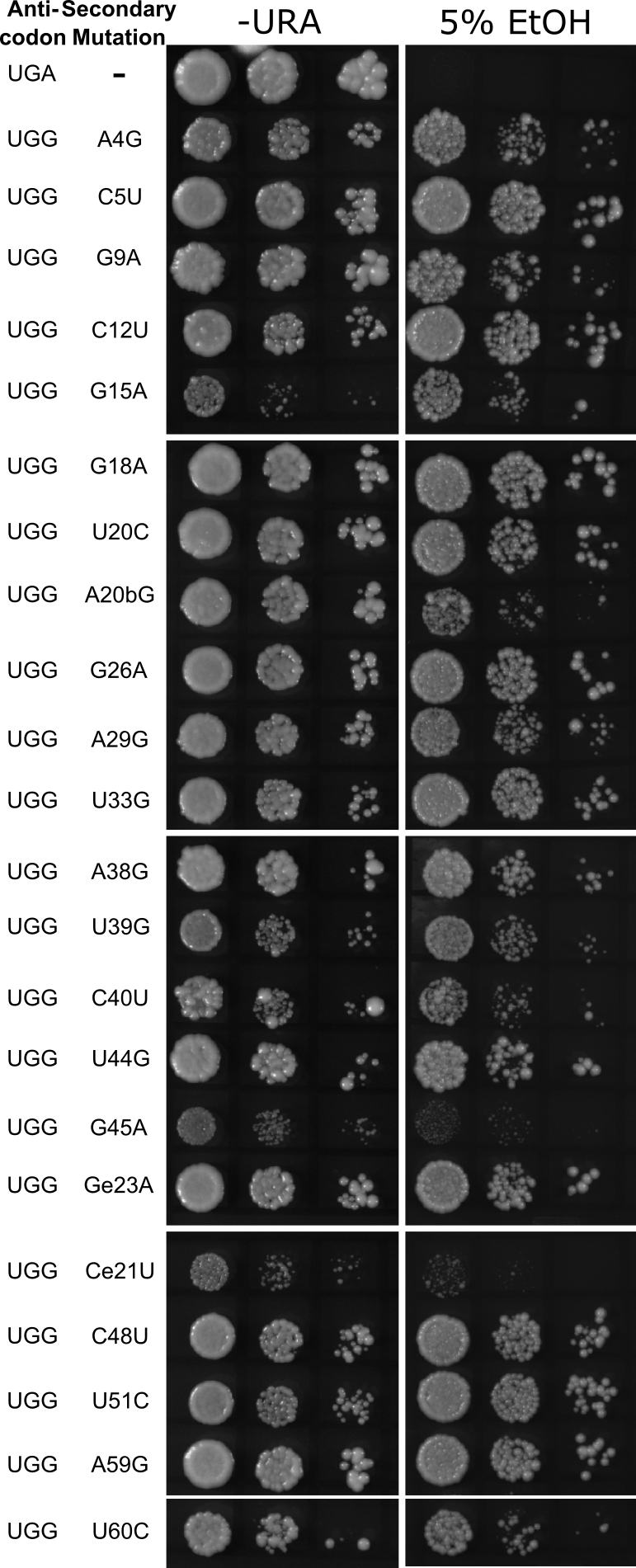
**Figure S3.** Transformation of 100 ng of *URA3*+ centromeric plasmids expressing tRNASerUGA,tRNASerUGG, or tRNASerUGG variants with mutations in the acceptor stem into a wild-type yeast strain (BY4742). Transformations were plated on media lacking uracil and grown for two days at 30°.



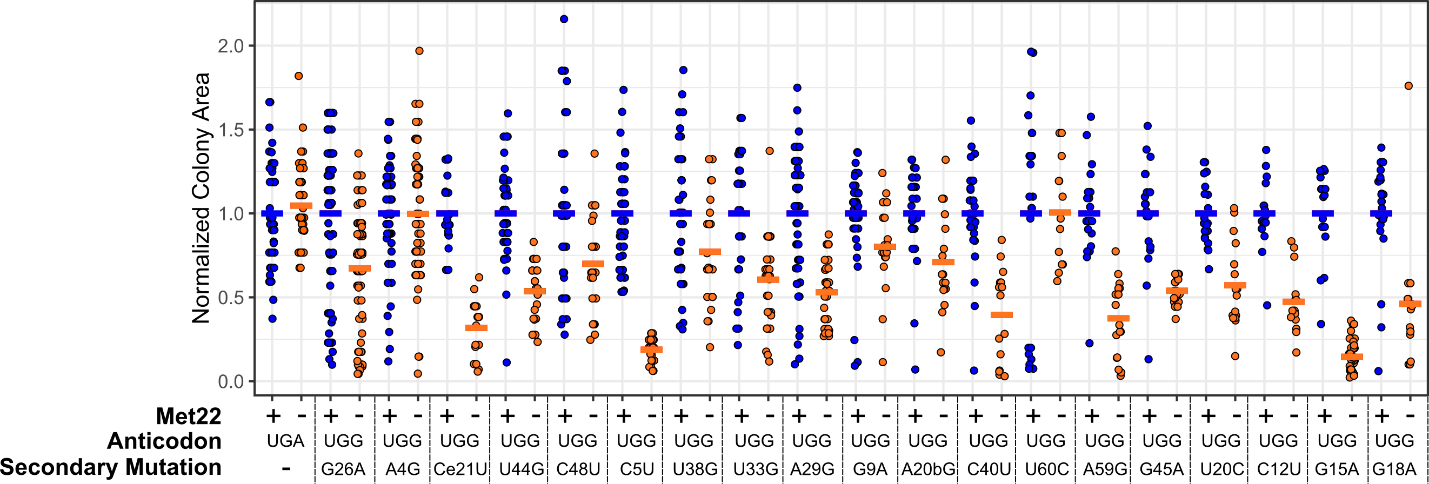
**Figure S4.** Transformation of 100 ng of *URA3*+ centromeric plasmids expressing tRNASerUGA, tRNASerUGG or variants of tRNASerUGG with mutations (G73A or G73C) at the discriminator base into a wild-type yeast strain (BY4742). Transformations were plated on media lacking uracil and grown for two days at 30°.



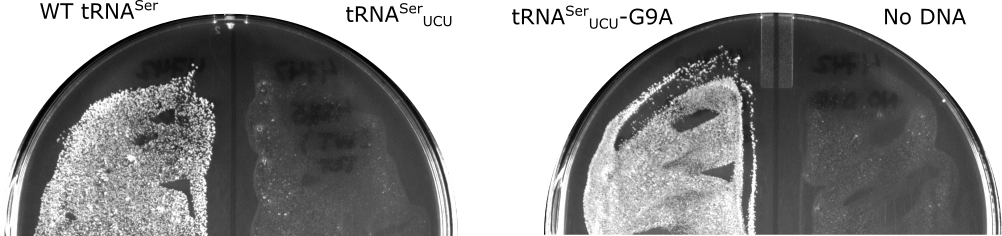
**Figure S5.** (A)Wild-type strain (BY4742) containing either wild-type tRNASerUGA, tRNASerUGG-G26A, tRNASerUGA-G73A or tRNASerUGA-G73C were grown to saturation in media lacking uracil and spotted in 10-fold serial dilutions on media lacking uracil. (B) Strains described in (A) containing an HSE-GFP reporter were grown in media lacking uracil and histidine. Cells were diluted 1:20 in the same media and grown for 6 hours. Cell densities were normalized and fluorescence measured. Each point represents one biological replicate.



**Figure S6.** *tti2-L187P* suppression by randomly selected tRNASerUGG variants. Strains containing *tti2-L187P* (CY7020) and tRNASerUGA or a randomly selected tRNASerUGG variants were grown to saturation in media lacking uracil and spotted in 10-fold serial dilutions on media lacking uracil or complete media containing 5% ethanol.

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**Figure S7.** Colony sizes for tRNASerUGG variants in *MET22* and *met22* strains. Strains were grown in media lack uracil, diluted 1:10,000, plated for single colonies on media lacking uracil and allowed to grow for two days at 30°. Colony area was measured using ImageJ.



**Figure S8.** Transformation of 100 ng of *HIS3*+ centromeric plasmids expressing tRNASerUGA,tRNASerUCU, or tRNASerUCU-G9A into a wild-type yeast strain (BY4742). Transformations were plated on media lacking histidine and grown for two days at 30°.

**Supplemental Tables**

**Table S1. Yeast strains used in this study**

|  |  |  |  |
| --- | --- | --- | --- |
| Strain | Genotype/Parent | Plasmid(s) | Reference |
| BY4741 | *MATa his3∆0 leu2∆0 met15∆0 ura3∆0* |  | Winzeler and Davis, 1997 |
| BY4742 | *MATα his3∆0 leu2∆0 lys2∆0 ura3∆0* |  | Winzeler and Davis, 1997 |
| CY6963 | *MATα his3Δ1 leu2Δ0 lys2Δ0 met15Δ0*  *ura3Δ0 tti2Δ-met5Δ-mTn10luk* | YCplac33-*DED1pr-TTI2* | Hoffman *et al.,* 2016 |
| CY6874 | CY6963 | YCplac111-*DED1pr*-*tti2Q276TAA* | Hoffman *et al.,* 2016 |
| CY7020 | CY6963 | YCplac111-*DED1pr-tti2L187P* | Hoffman *et al.,* 2016 |
| CY8150 | CY6963 | pRS303-*sup17(UCU)-G9A* and  YCplac111-*DED1pr*-*tti2L187R* | This work |
| CY8588 | *MATa his3∆0 leu2∆0 ura3∆0 met22::KanMX* |  | Tong *et al.* 2001 |
| CY8589 | *MATa his3∆0 leu2∆0 ura3∆0 MET22* |  | Tong *et al.* 2001 |

**Table S2. Oligonucleotides used in this study**

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence** | **Description** |
| VI1382 | aatctgttgcgcgctggttcaaatcctgc | *sup17(UGG)-VAΔ* |
| VI1383 | gaaccagcgcgcaacagattccaagtctgtcg | *sup17(UGG)-VAΔ* |
| UG5953 | TCTAAGCTTCGGACGATTGCCAACCGCCGAA | *SUP17* |
| UG5954 | CTGCAGAATTCCGCGGAAATTAGCACGGCC | *SUP17* |
| VJ2697 | atggGcgagtggttaagCcgacagactTGGA | *sup17(UGG)-G11:C24* |
| VJ2698 | gtcgGcttaaccactcgCccatagtGCCtaa | *sup17(UGG)-G11:C24* |
| VJ2766 | CTGTTGccgTCTcggCGCGCTGGttcaaatcctg | *sup17(UGG)-VA-G:C* |
| VJ2767 | GCGCCccgAGAcggCAACAGATTCCAagtctgt | *sup17(UGG)- VA-G:C* |
| WA5536 | caaatcctgctggtgtcAttatttttttatttttatttttt | *sup17(UGG)-G73A* |
| WA5537 | taaaaaaataaTgacaccagcaggatttgaaccagcg | *sup17(UGG)-G73A* |
| WA6571 | caaatcctgctggtgtcCttatttttttatttttatttttt | *sup17(UGG)-G73C* |
| WA6572 | taaaaaaataaGgacaccagcaggatttgaaccagcg | *sup17(UGG)-G73C* |
| VK4593 | AATCTGTTGGCTCTGCCGCGCTGGTTcaaatcc | *sup17(UGG)-VAΔ1* |
| VK4394 | GCGCGGCAGAGCCAACAgATTCCAAgtctgtc | *sup17(UGG)-VAΔ1* |
| VL5002 | AAATCCTGCTGGTCTCGTTATTTTTTTATTTTTAT | *sup17(UGG)-G70C* |
| VL5003 | AAATAACGAGACCAGCAGGATTTGAACCAGCG | *sup17(UGG)-G70C* |
| VF8661 | tgttttagggactatggccgagtggttaa | *sup17(UGG)-C3G* |
| VF8662 | cggccatagtccctaaaacaatgtaggtta | *sup17(UGG)-C3G* |
| WC8504 | CAACAGACTGAAAATCTGTTGGGCTCTGCC | *sup17(GAA)-G26A* |
| WC8505 | CCCAACAGATTTTCAGTCTGTTGCCTTAACC | *sup17(GAA)-G26A* |
| WF1163 | attgttttaGGCTCTATGGCCGAGTGGTT | *sup17(UGG)-A4T* |
| WF1164 | GGCCATAGAGCCTAAAACAATGTAGGTTATTT | *sup17(UGG)-A4T* |
| WF1165 | AATCCTGCTGGAGTCGTTATTTTTTTATTTTTATTT | *sup17(UGG)-T69A* |
| WF1166 | TAAAAAAATAACGACTCCAGCAGGATTTGAACCAG | *sup17(UGG)-T69A* |
| VJ2409 | CGACAGACTTCTAATCTGTTGGGCTCTGCC | *sup17(UCU)* |
| VJ2410 | CCCAACAGATTAGAAGTCTGTCGCCTTAACC | *sup17(UCU)* |
| VK4595 | GCGACAGACTttaAATCTGTTGGGctctgcc | *sup17(UUA)* |
| VK4596 | CCAACAGATTtaaAGTCTGTCGCCTTAACcac | *sup17(UUA)* |
| VF7765 | TGGTTAAGGCAACAGACTCTGAATCTGTTGGGC | *sup17(CUG)-G26A* |
| VF7766 | TTCAGAGTCTGTTGCCTTAACCACTCGGCCAT | *sup17(CUG)-G26A* |
| VL4943 | ACAGACTTctAATCTGTTGGGCTCTGCCC | *sup17(UCU)-G26A* |
| VL4945 | CCCAACAGATTagAAGTCTGTTGCCTTAACCACT | *sup17(UCU)-G26A* |
| 5693-1 | ataagaatgcggccgcaatgacggccgtaactgatatc | *TTI2* |
| 5693-2 | atacgagctctgcatttgtctgtgtctgtgt | *TTI2* |
| 6856-1 | ttctgctcagacagacgctattaaatcatacc | *tti2-L187R* |
| 6856-2 | tagcgtctgtctgagcagaacaccacgaagttta | *tti2-L187R* |

**Table S3.** Number of peptides identified in mass spectrometry based analysis of mistranslation rates.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| tRNA Variant | Replicate | # of wild-type peptides containing proline | | # peptides with Pro to Ser substitutions | % mistranslated  peptides |
| tRNASerUGA | 1 | 5985 | 7 | | 0.1 |
| tRNASerUGA | 2 | 5241 | 5 | | 0.1 |
| tRNASerUGA | 3 | 5238 | 5 | | 0.1 |
| tRNASerUGG-G9A | 1 | 5767 | 24 | | 0.4 |
| tRNASerUGG-G9A | 2 | 5363 | 33 | | 0.6 |
| tRNASerUGG-G9A | 3 | 5287 | 34 | | 0.6 |
| tRNASerUGG-G26A | 1 | 5516 | 285 | | 5.2 |
| tRNASerUGG-G26A | 2 | 5068 | 222 | | 4.4 |
| tRNASerUGG-G26A | 3 | 5225 | 234 | | 4.5 |
| tRNASerUGG-U44G | 1 | 5327 | 19 | | 0.4 |
| tRNASerUGG-U44G | 2 | 5376 | 12 | | 0.2 |
| tRNASerUGG-U44G | 3 | 4575 | 9 | | 0.2 |
| tRNASerUGG-U33G | 1 | 5085 | 147 | | 2.9 |
| tRNASerUGG-U33G | 2 | 5408 | 180 | | 3.3 |
| tRNASerUGG-U33G | 3 | 4483 | 125 | | 2.8 |
| tRNASerUGG-U39G | 1 | 5166 | 205 | | 4.0 |
| tRNASerUGG-U39G | 2 | 5134 | 200 | | 3.9 |
| tRNASerUGG-U39G | 3 | 5023 | 215 | | 4.3 |

**Table S4.** Eufind and Infernal tRNA score for tRNASer variants analyzed in this paper.

|  |  |  |
| --- | --- | --- |
| tRNA Variant | Eufind Score | ΔEufind Score |
| tRNASerUGA | -15.97 | 0 |
| VA-G:C | -15.97 | 0 |
| VAΔ1 | -15.97 | 0 |
| VAΔ | -14.6 | 1.37 |
| G11:C24 | -22.32 | -6.35 |
| G73C | -15.97 | 0 |
| G73A | -15.97 | 0 |
| G1:C70 | -15.97 | 0 |
| U2:G71 | -15.97 | 0 |
| C3:G70 | -15.97 | 0 |
| U4:A69 | -15.97 | 0 |
| A4G | -15.97 | 0 |
| C5T | -15.97 | 0 |
| G9A | -16.27 | -0.3 |
| C12T | -16.02 | -0.05 |
| G15A | -17.79 | -1.82 |
| G18A | -26.48 | -10.51 |
| T20C | -18.74 | -2.77 |
| A20bG | -19.43 | -3.46 |
| G26A | -15.97 | 0 |
| A29G | -15.97 | 0 |
| T33G | -15.97 | 0 |
| A38G | -15.97 | 0 |
| T39G | -15.97 | 0 |
| C40T | -15.97 | 0 |
| T44G | -15.97 | 0 |
| G45A | -15.97 | 0 |
| Ge23A | -15.97 | 0 |
| Ce21T | -15.97 | 0 |
| C48T | -15.97 | 0 |
| T51C | -15.97 | 0 |
| A59G | -17.32 | -1.35 |
| T60C | -17.01 | -1.04 |

**Table S5.** tRNASerUGG mistranslating tRNA variants investigated in this study. All have the UGG anticodon, except for the wild-type tRNASerUGA.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Mutation** | **Lethal** | **Suppresses *tti2-L187P*** | **Doubling Time (min)** | **Heat Shock relative to tRNASerUGAa** |
| tRNASerUGA | NA | NA | 65.5 | 1 |
| **Engineered Variants** | | | | |
| VA-G:Cb | No | No | n.d. | 1.0 |
| VAΔ1c | No | No | n.d. | 1.0 |
| VAΔd | No | No | n.d. | 0.8 |
| G11:C24 | No | No | n.d. | 1.2 |
| C1:G72 | No | Yes | n.d. | 3.6 |
| U2:G71 | Yes | NA | NA | NA |
| G3:C70 | No | Yes | n.d. | 1.8 |
| U4:A69 | Yes | NA | NA | NA |
| G73C | Yes | n.d. | n.d. | 2.8 |
| G73A | Yes | NA | NA | NA |
| **Genetically Selected Variants** | | | | |
| A4G | No | Yes | 77.9 | 1.7 |
| C5T | No | Yes | 75.5 | 1.9 |
| G9A | No | Yes | 68.3 | 1.2 |
| C12T | No | Yes | 105 | 2.8 |
| G15A | No | Yes | 107 | 2.9 |
| G17A | No | Yes | 102 | 2.5 |
| T19C | No | Yes | 85.3 | 3.4 |
| A20bG | No | Yes | 67.4 | 1.6 |
| G26A | No | Yes | 88.9 | 3.2 |
| A29G | No | Yes | 81.6 | 3.3 |
| T33G | No | Yes | 98.0 | 2.8 |
| A38G | No | Yes | 80.4 | 2.2 |
| T39G | No | Yes | 129 | 2.4 |
| C40T | No | Yes | 116 | 3.8 |
| T44G | No | Yes | 71.6 | 2.4 |
| G45A | No | Yes | 79.4 | 2.3 |
| Ge23A | No | Yes | 105 | 4.2 |
| Ce21T | No | Yes | 77.7 | 1.4 |
| C48T | No | Yes | 109 | 6.5 |
| T51C | No | Yes | 101 | 3.8 |
| A59C | No | Yes | 127 | 2.3 |
| A59G | No | Yes | 85.4 | 2.7 |
| T60C | No | Yes | 113 | 1.7 |

a as determined with *HSE-eGFP*

b switches the G:C base pairs in the variable arm to C:G

c deletes one G:C base pair from the variable arm

d deletes three G:C base pairs from the variable arm