Supporting Information:
Visualizing tRNA-dependent mistranslation in human cells

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Supporting Methods

Production of T7 RNA polymerase. T7 RNA polymerase (6xHis-tagged T7 RNAP) was expressed from pT7-911 vector in 1L *E. coli* culture at 37^{0}C in LB + 100 μg/mL ampicillin. Cultures were induced with 1 mM Isopropyl β-D-1-thiogalactopyranoside at OD_{600} = 0.6 and incubated for 3 hours at 20°C before harvesting cells by centrifugation. Recombinant T7 RNAP was purified by Ni-NTA affinity chromatography. The column was washed with 20 column volumes wash buffer (10 mM imidazole, 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 5% glycerol, 5 mM β-mercaptoethanol) and eluted with 10 column volumes elution buffer (500 mM imidazole, 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 5% glycerol, 5 mM β-mercaptoethanol). Elution fractions were analyzed by coomassie-stained SDS-PAGE and fractions containing T7 RNA polymerase at the correct molecular weight were pooled for dialysis. Pooled elution fractions were dialyzed overnight at 4°C in storage buffer (20 mM HEPES-KOH pH 8.0, 1 mM EDTA, 100 mM NaCl, 3 mM dithiothreitol, 50% glycerol) and stored at -20°C.

In vitro tRNA transcription and radiolabeling. Transcription reactions were performed as previously[1] in a reaction containing 200 ng/μl DNA template in the presence 40 mM HEPES-KOH pH 8.0, 22 mM $MgCl_2$, 5 mM dithiothreitol, 1 mM spermidine, 4 mM of NTPs (ATP, GTP, CTP, and UTP), and 30 nM T7 RNAP. Samples were run on 12% urea (7M) polyacrylamide gels at 40 W. Correct-sized bands were visualized with a UV transilluminator and excised. Gel plugs were vortexed and dissolved in 10 ml of 3 M sodium acetate (pH 5.2) at 4°C for 2 hours or overnight. The solubilized tRNAs solution was then filter sterilized (2 μm). Filtrates from three reactions (2 × 2-hour, 1 × overnight incubation in 3 M sodium acetate pH 5.2) were pooled together and precipitated with an equal volume of 100% isopropanol at -80°C overnight. Samples were centrifuged at 10,000 × g for one hour, supernatant was decanted, and the pellet was washed with 70% ethanol. Centrifugation was then repeated, supernatant was removed and pellets were air-dried before re-suspension in sterile milli-Q water.

Folding and radiolabeling of tRNA. tRNAs were denatured in a 95°C water bath for 5 min then cooled gradually to room temperature. At 65°C, $MgCl_2$ was added to a final concentration of 10

mM to assist with folding. 50 µl reactions were prepared with 1 µM tRNA, 100 mM Tris-HCl pH 8.0, 10 mM dithiothreitol, 40 mM $MgCl_2$, 10 µM NaPPi, 200 nCi of α-^{32}[P]-ATP (PerkinElmer) and 5 µl of CCA adding enzyme (produced as previously[1]). The reaction was incubated at 37°C for 1 hour. Radio-labelled tRNAs were purified using Biospin30 column (Bio-Rad).

Supporting References

1. O'Donoghue P, Sheppard K, Nureki O, Soll D. Rational design of an evolutionary precursor of glutaminyl-tRNA synthetase. Proc Natl Acad Sci USA 2011; 108:20485-90.

Supporting Tables

Table S1. Sequences of tRNA construct oligos

	Sequence (5' to 3')
$tRNA^{Ala}$ Watson strand	CTAGATAATACGACTCACTATAGGGGGTATAGCTCAGT GGTAGAGCGCGTGCTTAGCATGCACGAGGTCCTGGGTT CGATCCCCAGTACCTCCACCATGCATG
$tRNA^{Ala}$ Crick strand	GATCCATGCATGGTGGAGGTACTGGGGATCGAACCCAG GACCTCGTGCATGCTAAGCACGCGCTCTACCACTGAGC TATACCCCCTATAGTGAGTCGTATTAT
$tRNA^{Pro}$ Watson strand	CTAGATAATACGACTCACTATAGGCTCGTTGGTCTAGTG GTATGATTCTCGCTTTGGGTGCGAGAGGTCCCGGGTTC AAATCCCGGACGAGCCCCCATGCATG
$tRNA^{Pro}$ Crick strand	GATCCATGCATGGGGGCTCGTCCGGGATTTGAACCCGG GACCTCTCGCACCCAAAGCGAGAATCATACCACTAGA CCAACGAGCCTATAGTGAGTCGTATTAT
$tRNA^{Pro}$ mutant Watson strand	CTAGATAATACGACTCACTATAGGCTCGTTGGTCTAGTG GTATGATTCTCGCTTTGGGTGCGAGAGGTCCCGGGTTC AAATCCCGGACGATCCCCCATGCATG
$tRNA^{Pro}$ mutant Crick strand	GATCCATGCATGGGGGATCGTCCGGGATTTGAACCCGG GACCTCTCGCACCCAAAGCGAGAATCATACCACTAGA CCAACGAGCCTATAGTGAGTCGTATTAT

Table S2. Sequences of primers for template PCR

	Sequence (5' to 3')
Upstrean T7 promoter forward	GTTGGGAAGGGCGATCGGTG
$tRNA^{Ala}$ forward	CTAGATAATACGACTCACTATAGGGGG
$tRNA^{Ala}$ reverse	TGGTGGAGGTACTGGGGAT
$tRNA^{Pro}$ forward	CTAGATAATACGACTCACTATAGGC
$tRNA^{Pro}$ reverse	TGGGGGCTCGTCCGGGA
$tRNA^{Pro}$ mutant forward	TAATACGACTCACTATAGGGTCG
$tRNA^{Pro}$ mutant reverse	TGGGGGATCGTCCGGGA

Supporting Figures

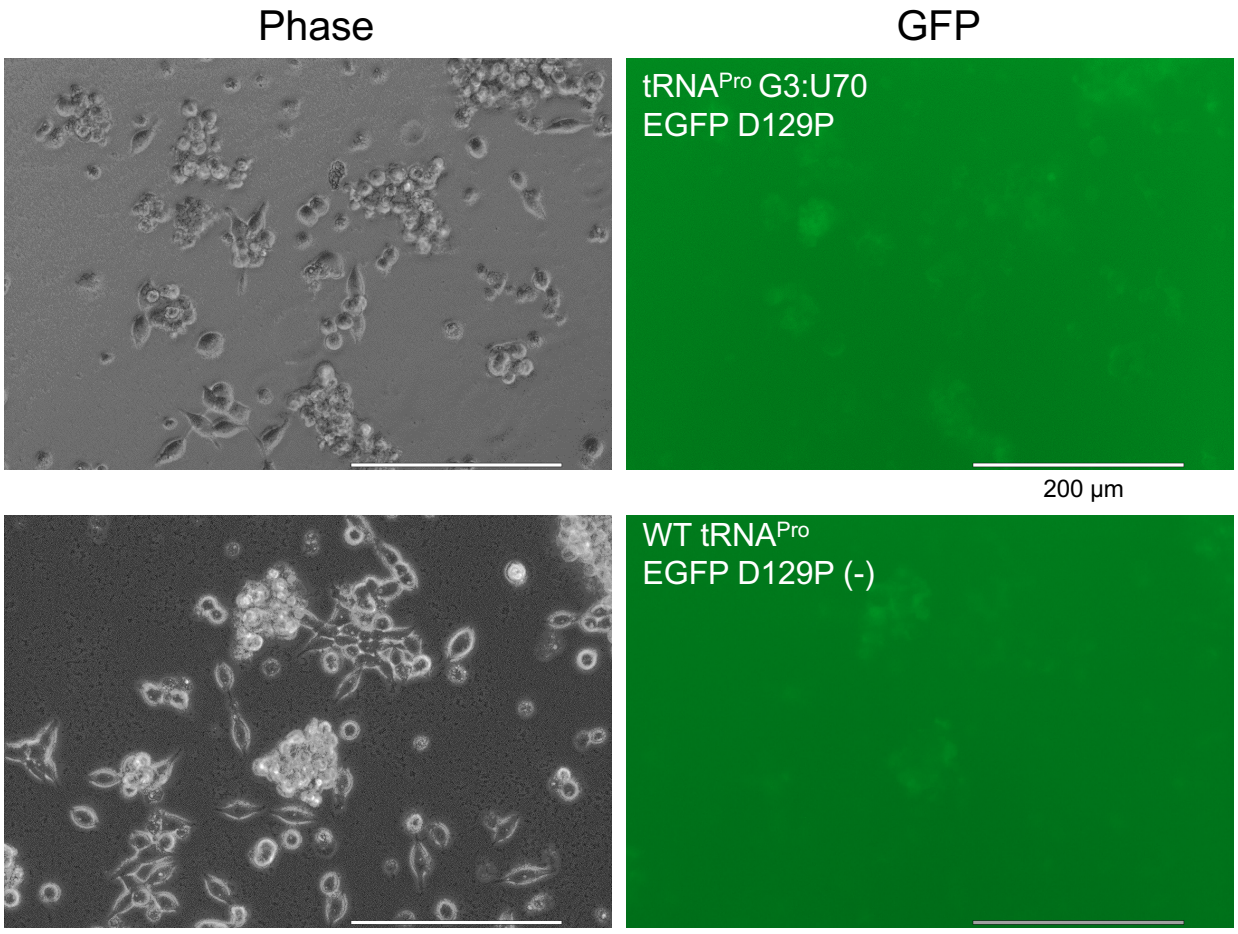


Figure S1. Overexpression of tRNA^{Pro} G3:U70 had no obvious effect on cell morphology compared to wildtype tRNA^{Pro}. Images captured by light (phase) or fluorescence (GFP; ex/em = 470/525 nm) microscopy on day three low serum, low glucose media (see main text, Fig 3). Scale bars depict 200 µm distance.

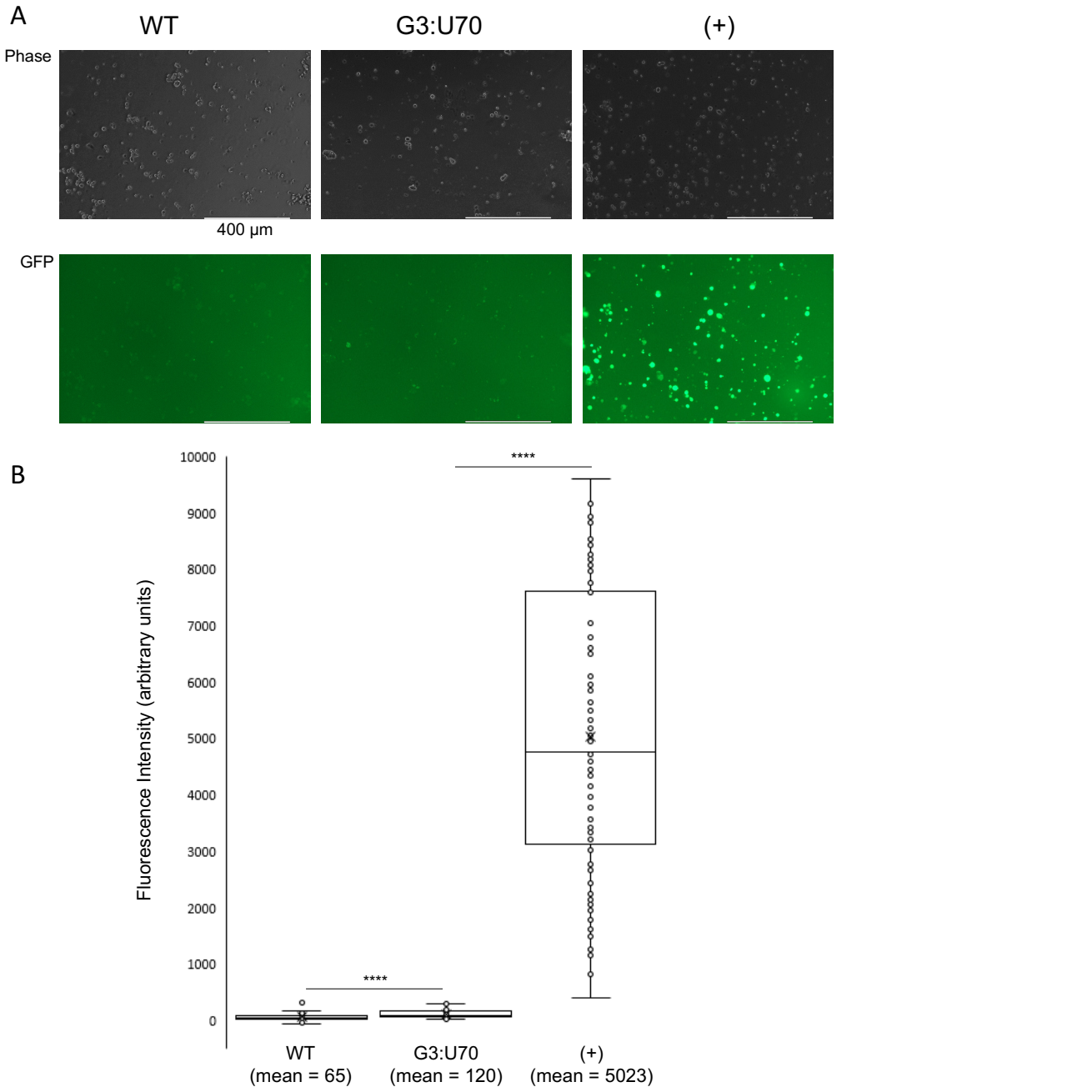


Figure S2. Visualizing mistranslation after selection in Geneticin. HEK 293T cell cultures were transfected with plasmid harboring $tRNA^{Pro}$ and EGFP D129P (WT); $tRNA^{Pro}$ G3:U70 and EGFP D129P (G3:U70); or $tRNA^{Pro}$ and EGFP D129A (+). All plasmids have a pCDNA 3.1 (+) backbone containing the bacterial NeoR gene. Selection cultures were maintained in Geneticin (G418) containing media for 25 days prior to quantification. (A) representative images captured by light (phase) or fluorescence (GFP; ex/em = 470/525) microscopy at 4 × magnification. Scale bars represent 400 µm distance. (B) Box and whisker plots of EGFP foci intensity. Horizontal demarcations depict quartiles with median centered. Dots represent the general distribution of 100 foci measured in each plot. Stars indicate statistically significant differences according to single-factor ANOVA (**** = $p < 0.0001$). Means are noted below each blot.

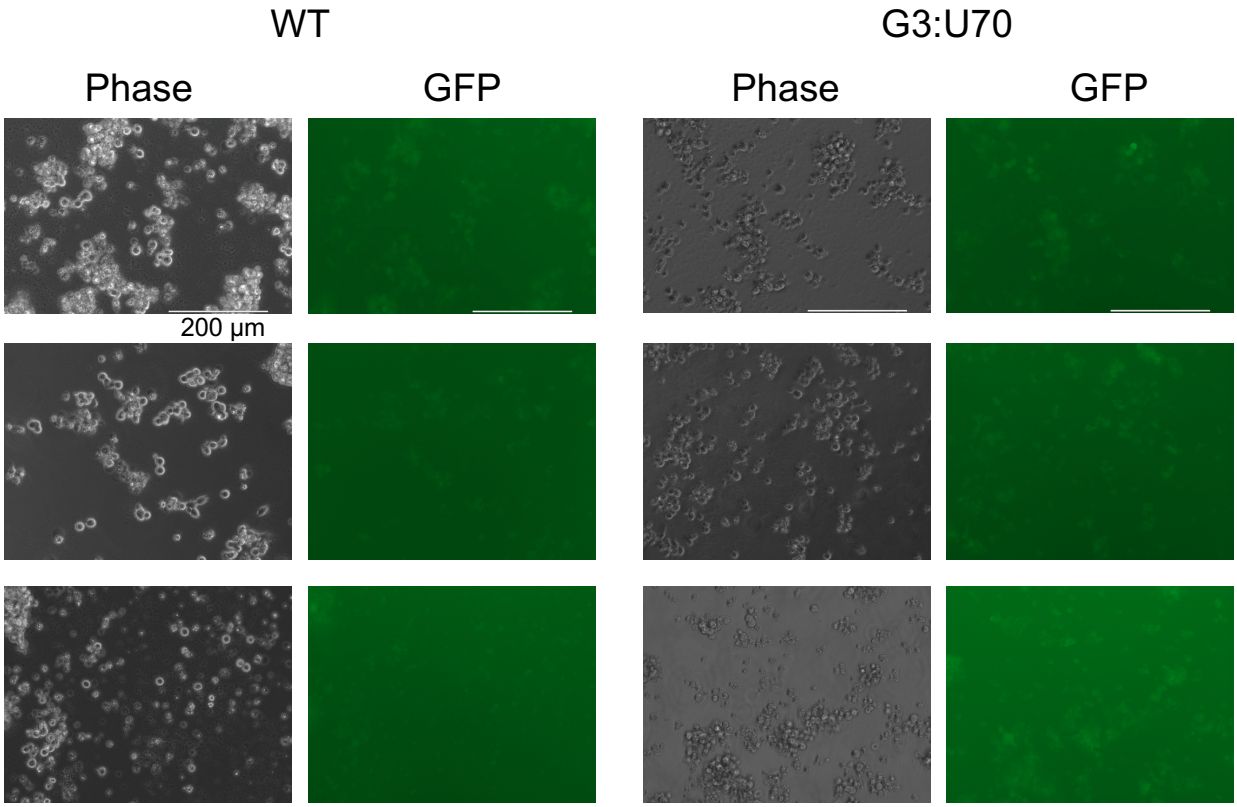
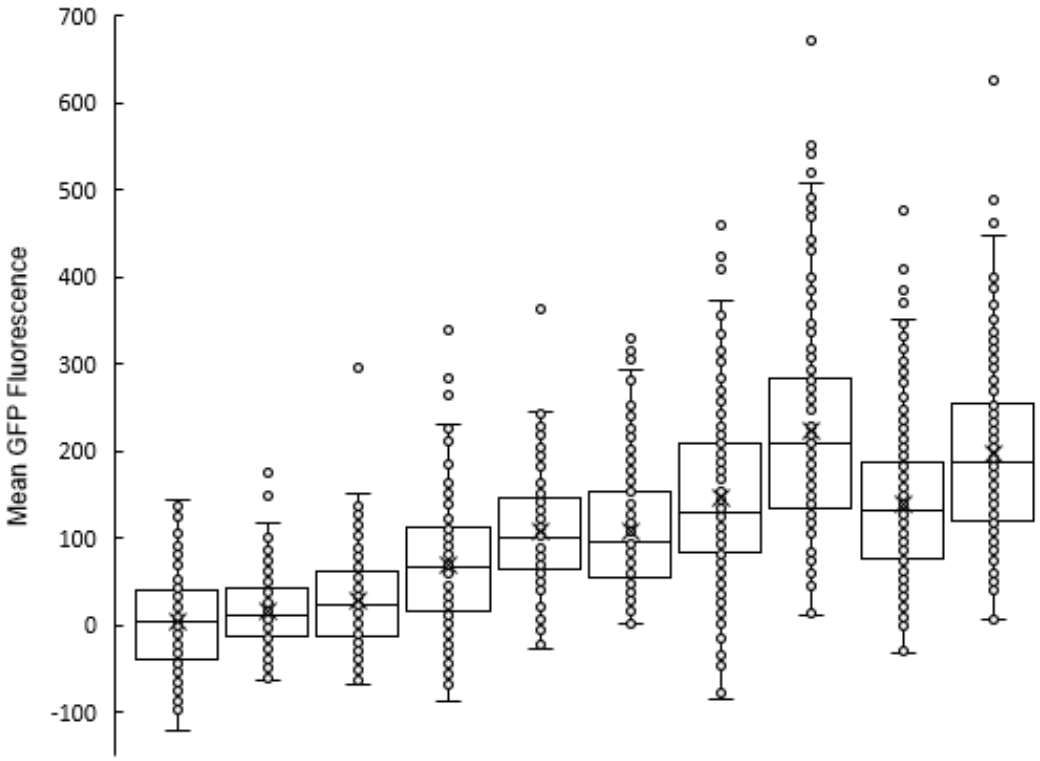


Figure S3. Additional images from low serum low glucose growth, experiment day 5. HEK 293T cell cultures were transfected with plasmid harboring $tRNA^{Pro}$ and EGFP D129P (WT); or $tRNA^{Pro}$ G3:U70 and EGFP D129P (G3:U70). Images captured by light (phase) or fluorescence (GFP; ex/em = 470/525) microscopy at 20 × magnification. Scale bars represent 200 µm distance. See main text (Fig. 3 and methods) for experimental details.



tRNA	WT	G3:U70	WT	G3:U70	WT	G3:U70	WT	G3:U70	WT	G3:U70
Day	1		2		3		4		5	
Media	High		Low		Low		Low		Low	
Estimated mistranslation	0.7%, p = 0.0268		1%, $p = 7x10^{-07}$		0%, p = 0.9984		2%, $p = 3x10^{-08}$		1.4%, $p = 2x10^{-10}$	

Figure S4. Mistranslation under glucose and serum starvation. Box and whisker plots showing data from figure 3 (main text) with EGFP D129A data set removed and vertical axis zoomed in to more clearly show differences caused by mistranslating tRNA in comparison to the wild type tRNA. HEK 293T cell cultures were transfected with plasmid harboring $tRNA^{Pro}$ and EGFP D129P (WT); or $tRNA^{Pro}$ G3:U70 and EGFP D129P (G3:U70). See main text (Fig. 3 and methods) for experimental details.