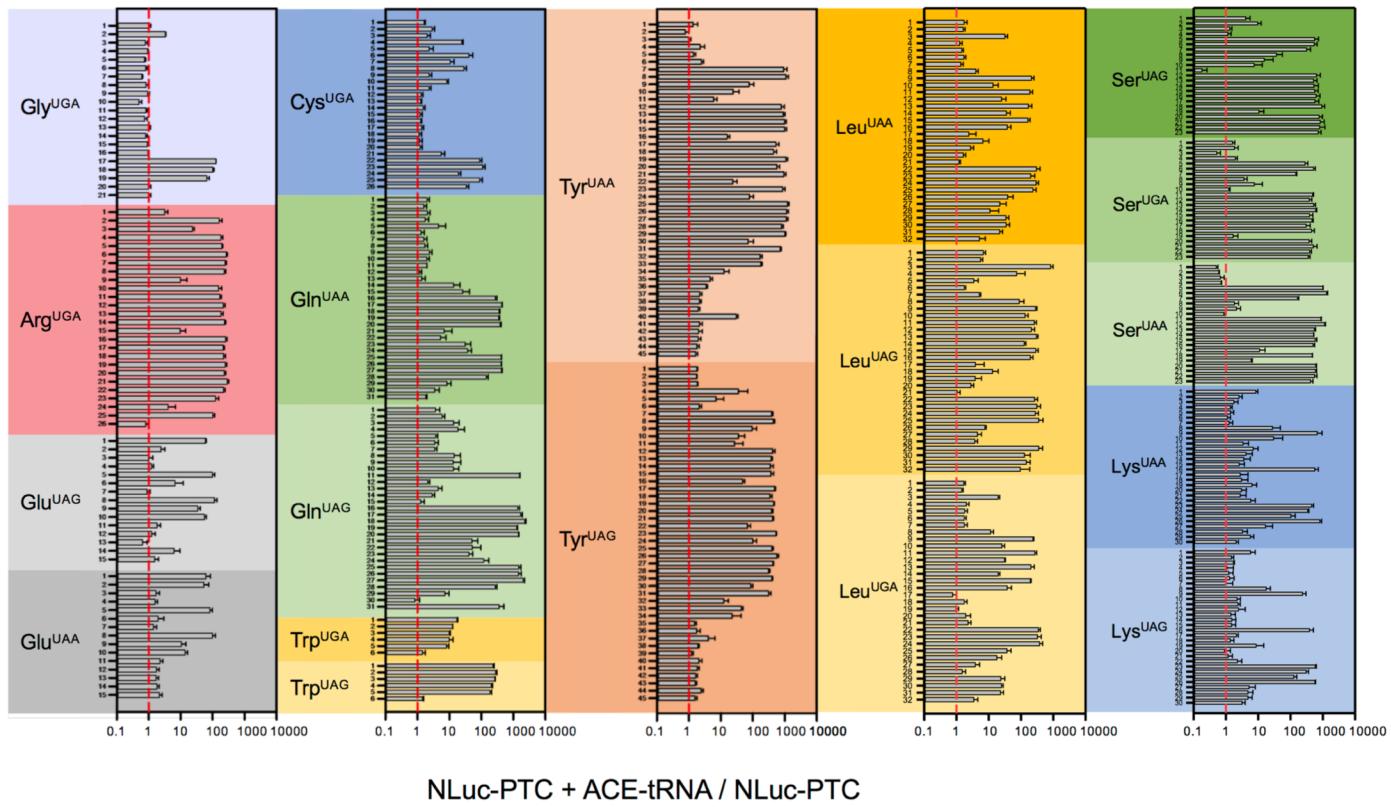


Engineered transfer RNAs for suppression of premature termination codons.

Lueck et al

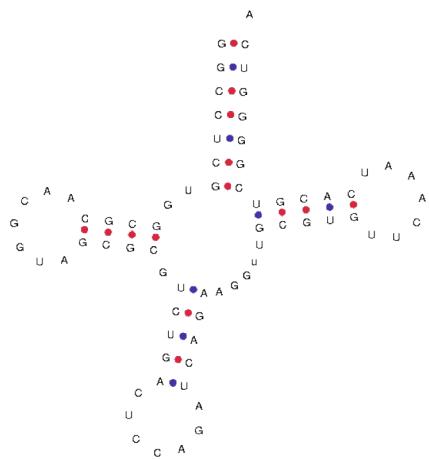
Amino Acid			Codons					
W	Trp	Tryptophan	TGG					
Y	Tyr	Tyrosine	TAC	TAT				
C	Cys	Cysteine	TGC	TGT				
E	Glu	Glutamic acid	GAA	GAG				
K	Lys	Lysine	AAA	AAG				
Q	Gln	Glutamine	CAA	CAG				
S	Ser	Serine	AGC	AGT	TCA	TCC	TCG	TCT
L	Leu	Leucine	TTA	TTG	CTA	CTC	CTG	CTT
R	Arg	Arginine	AGA	AGG	CGA	CGC	CGG	CGT
G	Gly	Glycine	GGA	GGC	GGG	GGT		
F	Phe	Phenylalanine	TTC	TTT				
D	Asp	Aspartic acid	GAC	GAT				
H	His	Histidine	CAC	CAT				
N	Asn	Asparagine	AAC	AAT				
M	Met	Methionine	ATG					
A	Ala	Alanine	GCA	GCC	GCG	GCT		
P	Pro	Proline	CCA	CCC	CCG	CCT		
T	Thr	Threonine	ACA	ACC	ACG	ACT		
V	Val	Valine	GTA	GTC	GTG	GTT		
I	Ile	Isoleucine	ATA	ATC	ATT			
X	STP	Stop codon	TAA	TAG	TGA			

Codon usage for common PTC. Red highlight indicates the most common codons and corresponding amino acid type which can be converted to stop codons by a single nucleotide substitution. Engineered tRNAs have been developed for each type.

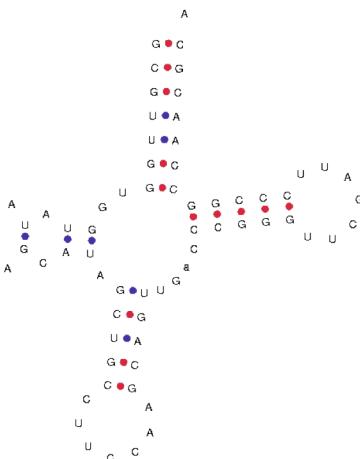


Number referenced ACE-tRNA activity plot.

Supplementary Fig.2A

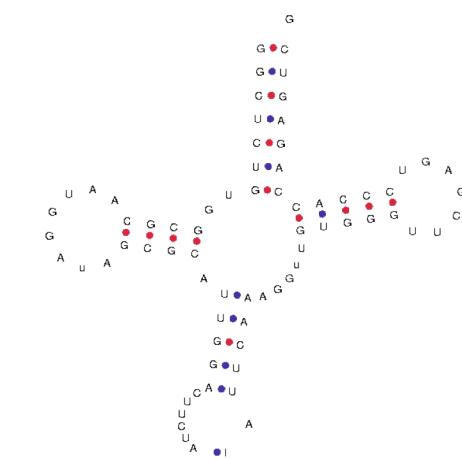


Trp-chr17.tRNA39
UGA and UAG



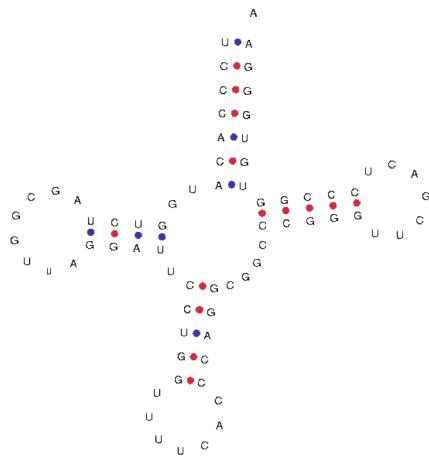
Gly-chr19.tRNA2

UGA

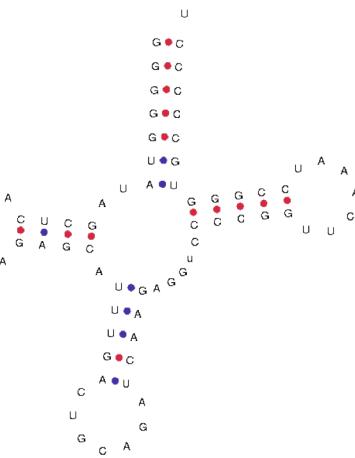


Arg-chr9.trna6

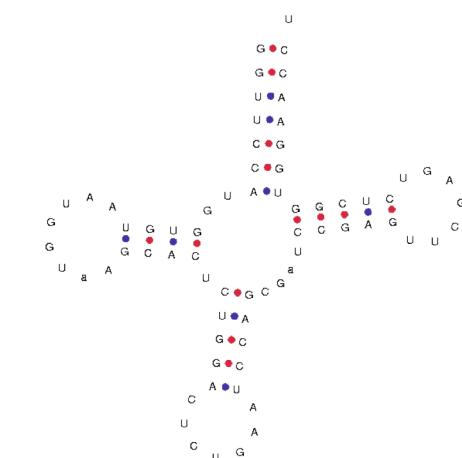
UGA



Glu-chr13.trna2 UAG and UAA

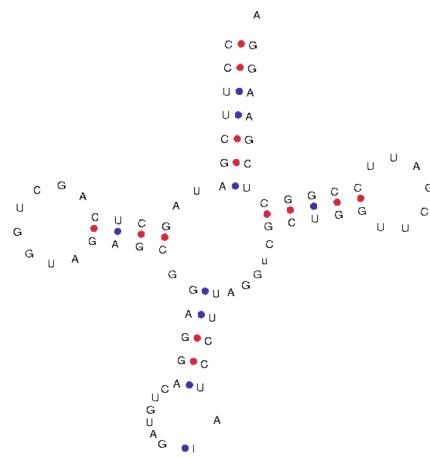


Cys-chr15.tRNA3 UGA

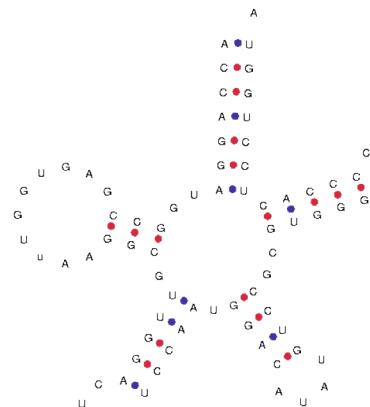


Gln-chr1.trna101

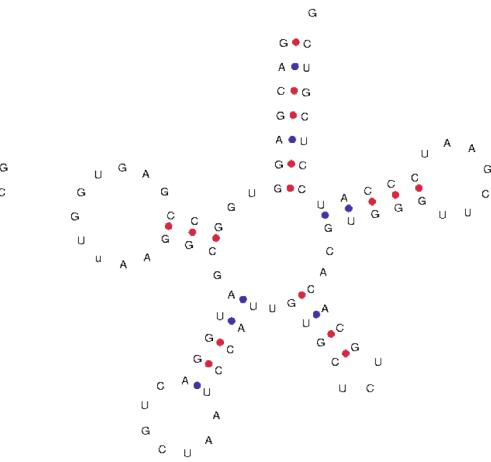
UAA not UAG



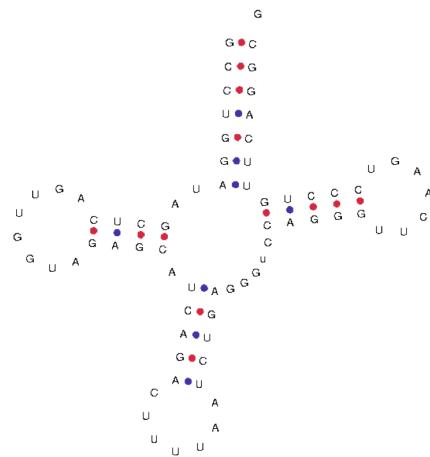
Tyr-chr14.trna17
UAA not UAG



Leu-chr6.trna81
UGA and UAA, not UAG

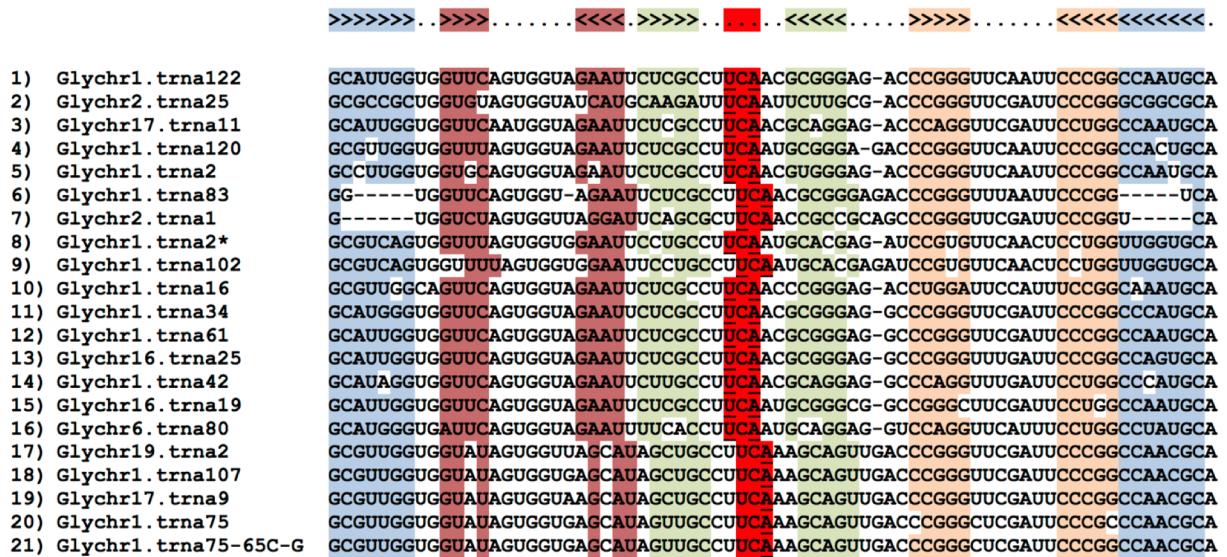
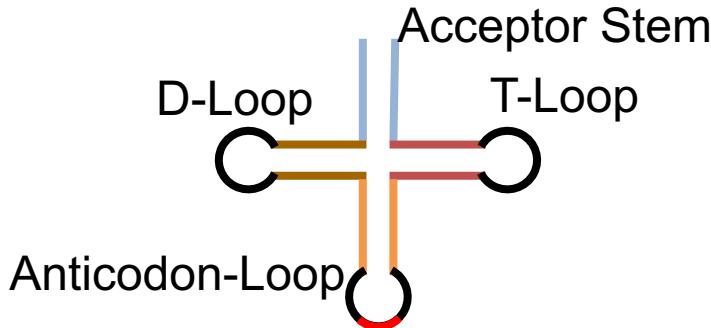


Ser-chr6.trna61

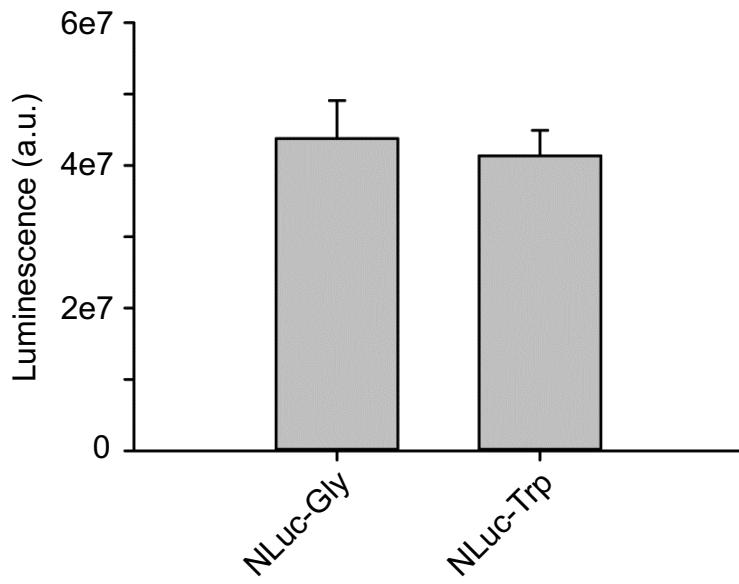


Lys-chr11.trna11
UAG not UAA

Predicted cloverleaf structures for ACE-tRNA examples used in this study. Anticodon type noted for versions that were functional for suppression. Cloverleaf predictions were based off the parent sequence (non-mutated) of each ACE-tRNA using tRNAscan-SE (<http://lowelab.ucsc.edu/tRNAscan-SE/>).



Alignment of Glycine tRNA sequences. 21 tRNA^{Gly} human sequences demonstrate high sequence homology amongst tRNA clades. Color coding indicated in tRNA image.

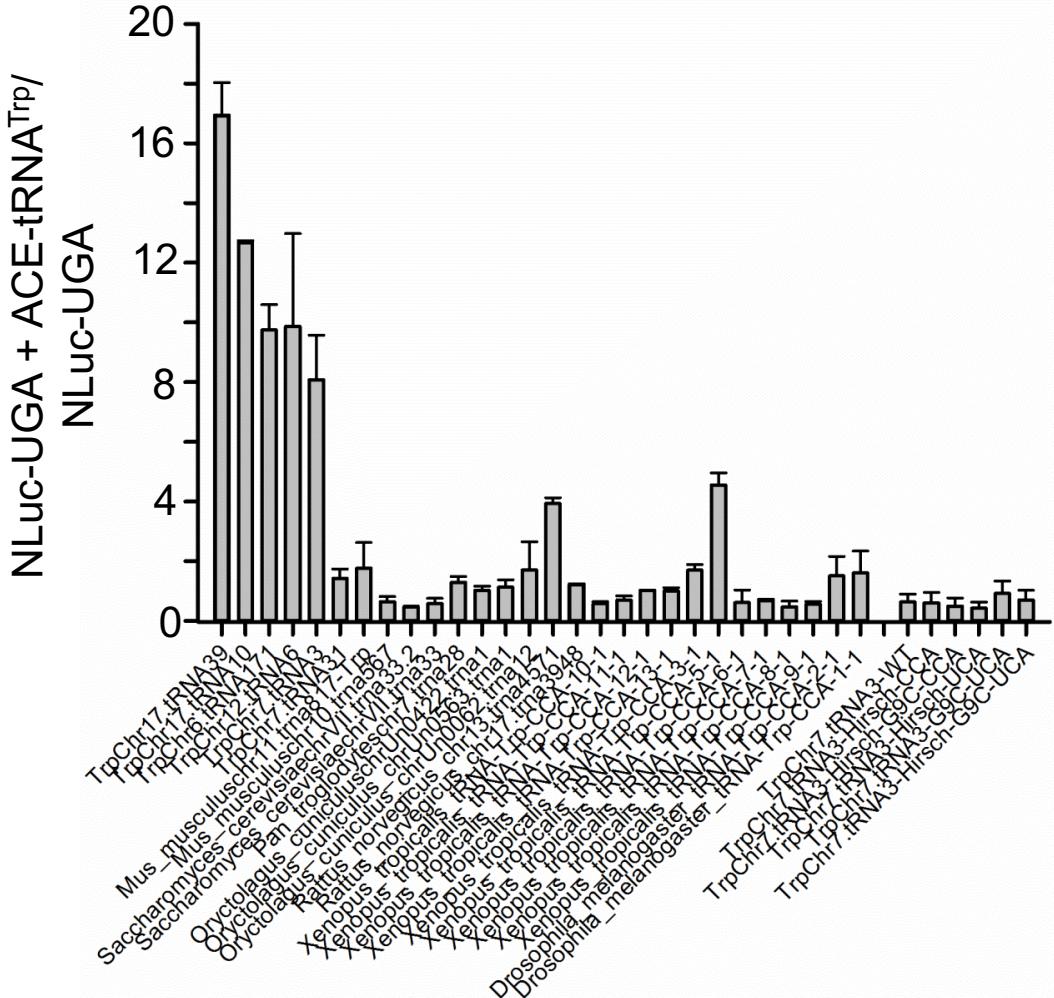


Side-chain identity at p.162 in Nanoluciferase does not effect activity. Total luminescence activity is indicated for each mutation at site.

a

TrpChr17.tRNA39	-GACCUCUGGGCGCACACGGCAGCGCUGACU <u>cacG</u>	-AUCAAGAAGGUUGCGUGUCAAUCAGCUGGGGUCA
TrpChr17.tRNA10	-GACCUCUGGGCGCACACGGCAGCGCUGACU <u>cacG</u>	-AUCAAGAAGGUUGCGUGUCAAUCAGCUGGGGUCA
TrpChr6.tRNA171	-GCCUCUGGCCAAC <u>GUAGCG</u> CUGUCACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAUCAGCAGGGGUCA
TrpChr12.tRNA6	-GACCUCUGGGCGCAC <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUGCGUGUCAAUCAGCUGGGGUCA
TrpChr7.tRNA3	-GCCUCUGGGCGCAC <u>GG</u> AGCGCUGACU <u>cacG</u>	-AUCAAGAAGGUUGCGUGUCAAUCAGCUGGGGUCA
TrpChr7.tRNA31	-GACCUCUGGGCGCAC <u>GG</u> AGCGCUGACU <u>cacG</u>	-AUCAAGAAGGUUGCGUGUCAAUCAGCUGGGGUCA
Mus_musculuschr11.tRNA817	-GACCUCUGGGCGCAA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUGCGUGUCAAUCAGCUGGGGUCA
Mus_musculuschr10.tRNA567	-GACCUCUGGGCGCAA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUGCGUGUCAAUCAGCUGGGGUCA
Saccharomyces_cerevisiaechrVII.tRNA33	-GA <u>ACGGGG</u> CGGCCAA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUGCGUGUCAAUCAGCUGGGGUCA
Saccharomyces_cerevisiaechrVII.tRNA33	-GA <u>ACGGGG</u> CGGCCAA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUGCGUGUCAAUCAGCUGGGGUCA
Pan_trichodyleschr7.tRNA28	-GGCCUCAU <u>G</u> GGCAAC <u>GUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>U</u> GUUCAAACACATGGGGUCA
Oryctolagus_cuniculuschrUn0422.tRNA1	-GACCUCUGGGCG <u>A</u> AA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Oryctolagus_cuniculus_chrUn0563.tRNA1	-GACCUCUGGGCG <u>A</u> AA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Oryctolagus_cuniculus_chrUn0062.tRNA12	-GACCUCUGGGCG <u>A</u> AA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Rattus_norvegicus_chrl3.TRNA4571	-GACCUCUGGG <u>C</u> AA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Rattus_norvegicus_chrl7.tRNA3948	-GACCUCUGGG <u>C</u> AA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Xenopus_tropicalis.tRNA-trp-cca-10-1	-GACCUCUGGGCG <u>A</u> AC <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Xenopus_tropicalis.tRNA-trp-cca-11-1	-GACCUCUGGGCG <u>A</u> AC <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Xenopus_tropicalis.tRNA-trp-cca-12-1	-GACCUCUGGGCG <u>A</u> AC <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Xenopus_tropicalis.tRNA-trp-cca-13-1	-GACCUCUGGG <u>C</u> AA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Xenopus_tropicalis.tRNA-trp-cca-3-1	-GACCUCUGGG <u>C</u> AA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Xenopus_tropicalis.tRNA-trp-cca-5-1	-AG <u>GGGUA</u> T <u>A</u> GG <u>U</u> CA <u>UU</u> GG <u>A</u> CA <u>GG</u> CG <u>U</u> GU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Xenopus_tropicalis.tRNA-trp-cca-6-1	-GACCUCUGGG <u>C</u> AA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Xenopus_tropicalis.tRNA-trp-cca-7-1	-GACCUCUGGG <u>C</u> AA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Xenopus_tropicalis.tRNA-trp-cca-8-1	-AC <u>GGG</u> GU <u>A</u> GG <u>U</u> CA <u>UU</u> GG <u>A</u> CA <u>GG</u> CG <u>U</u> GU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
Xenopus_tropicalis.tRNA-trp-cca-9-1	-GACCUCUGGG <u>C</u> AA <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
D._melanogaster.tRNA-trp-cca-2-1	-GAC <u>U</u> GG <u>U</u> GG <u>C</u> CA <u>ACGGU</u> AG <u>CGG</u> GU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
D._melanogaster.tRNA-trp-cca-1-1	-GAC <u>U</u> GG <u>U</u> GG <u>C</u> CA <u>ACGGU</u> AG <u>CGG</u> GU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
TrpChr7.tRNA3-WT	-GCCUCUGGGCG <u>A</u> AC <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
TrpChr7.tRNA3-Hirsch-CCA	-GCCUCUGGGCG <u>A</u> AC <u>GGUAGCG</u> CUGACU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
TrpChr7.tRNA3-Hirsch-G9C-CCA	-GCCUCUGGG <u>C</u> CA <u>ACGGU</u> AG <u>CGG</u> GU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
TrpChr7.tRNA3-Hirsch-UCA	-GCCUCUGGG <u>C</u> CA <u>ACGGU</u> AG <u>CGG</u> GU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
TrpChr7.tRNA3-G9C-UCA	-GCCUCUGGG <u>C</u> CA <u>ACGGU</u> AG <u>CGG</u> GU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA
TrpChr7.tRNA3-Hirsch-G9C-UCA	-GCCUCUGGG <u>C</u> CA <u>ACGGU</u> AG <u>CGG</u> GU <u>cacG</u>	-AUCAAGAAGGUUG <u>GU</u> UCAAACAGCAGGGGUCA

b

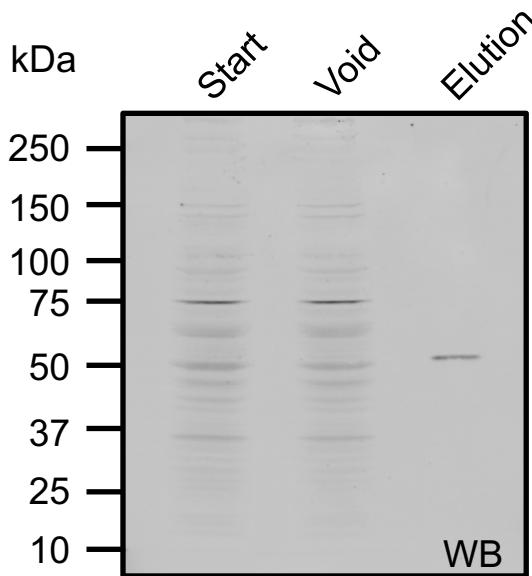
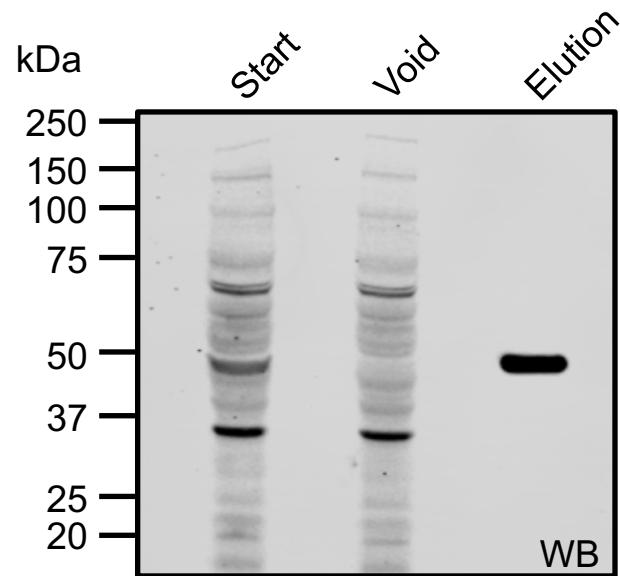


Analysis of ACE-tRNA^{Trp} sequences from multiple species and suppressor tRNA mutations.

Supplementary Fig. 5

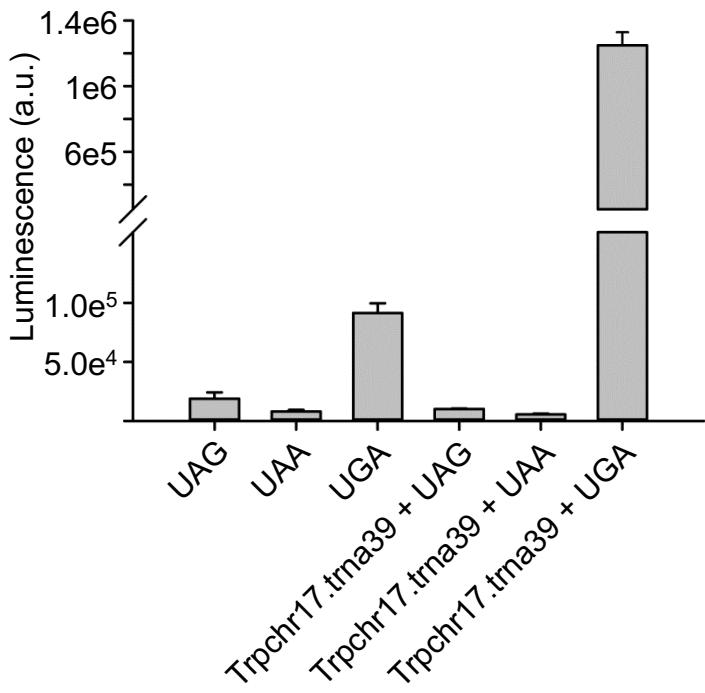
a

MSFNTIIDWNSCTAEQQRQLLMRPAISASESITRTVNDILDNV
KARGDEALREYSAKFDKTTVTALKVSAEEIAAASERLSDELKQ
AMAVAVK**NIEFHTAQKLPPVDVETQPGVRCQQVTR**PVASVGL
YIPGGSAPLFSTVMLATPASIAGCKKVVLCSPPPIADEILYA
AQLCGVQDVFNVGGAQAI^AALA^FGTE^SVPKVDKIFGPGNAFVT
EAKRQVSQRLDGAAIDMPAGPSEVLVIADSGATPDFVASDLLS
QAEHGPDSQVILLTPAADMARRVAEAVERQLAEPLPRAETARQ**A**
LNASRLIVTKDIAQCVEISNQYGPEHLIIQTRNAREELVDSITS
AGSVFLGDWSPESAGDYASGTNHVLPTYGYTATCSSLGLADFQ
KRMTVQELSKEGFSALASTIETLAAAERLTAHKNAVTLR**VNAL**
KEQA**HHHHHHHHHS GGSAWSHPQFEK**

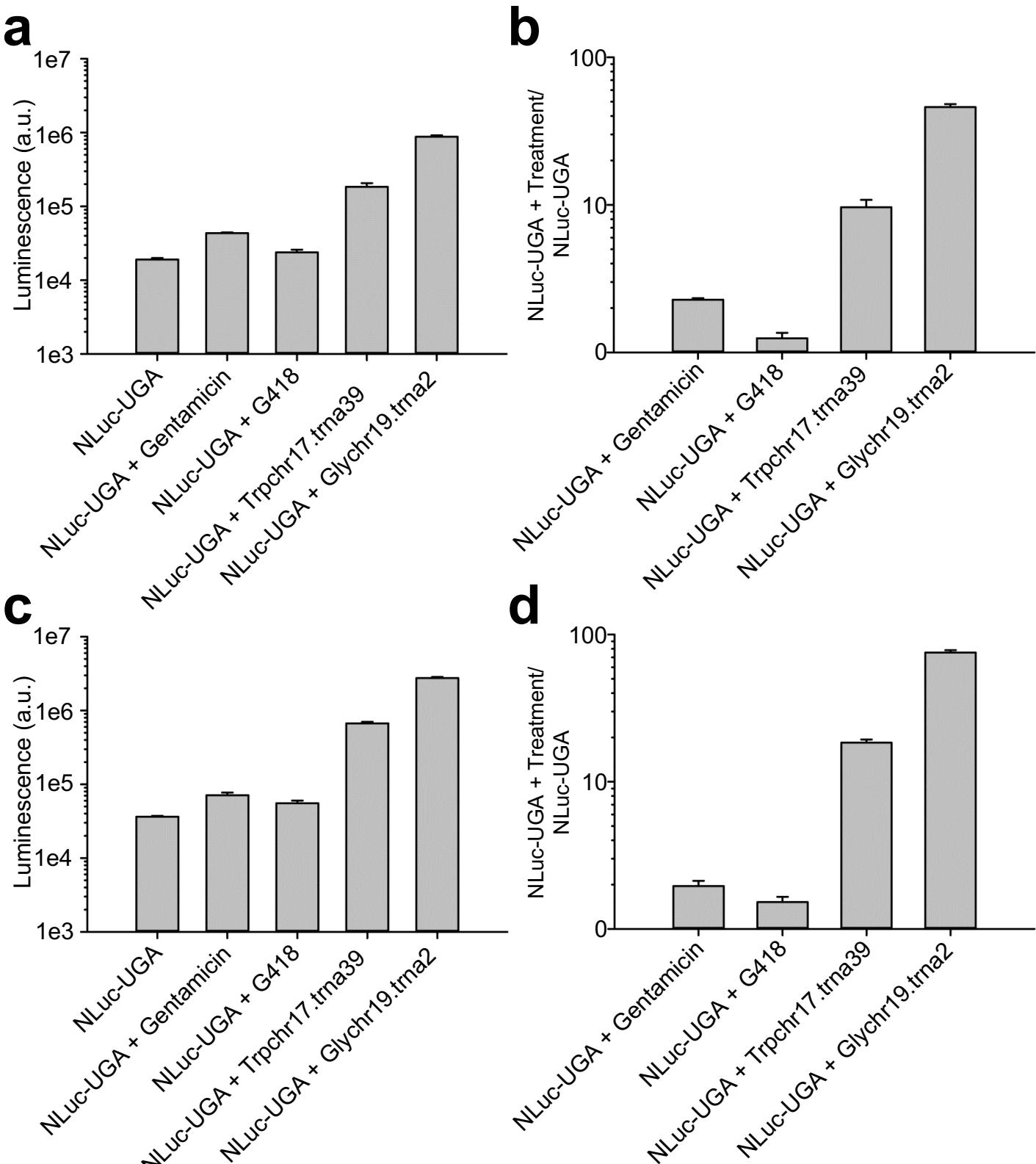
bHDH N94tga + ACE-tRNA_{Trp}**c**HDH N94tga + ACE-tRNA_{Gly}

Histidinol dehydrogenase (HDH) His(8)-streptactin expression construct allows for efficient one-step isolation of protein from HEK293 cells. a) Protein sequence of HDH expression construct.

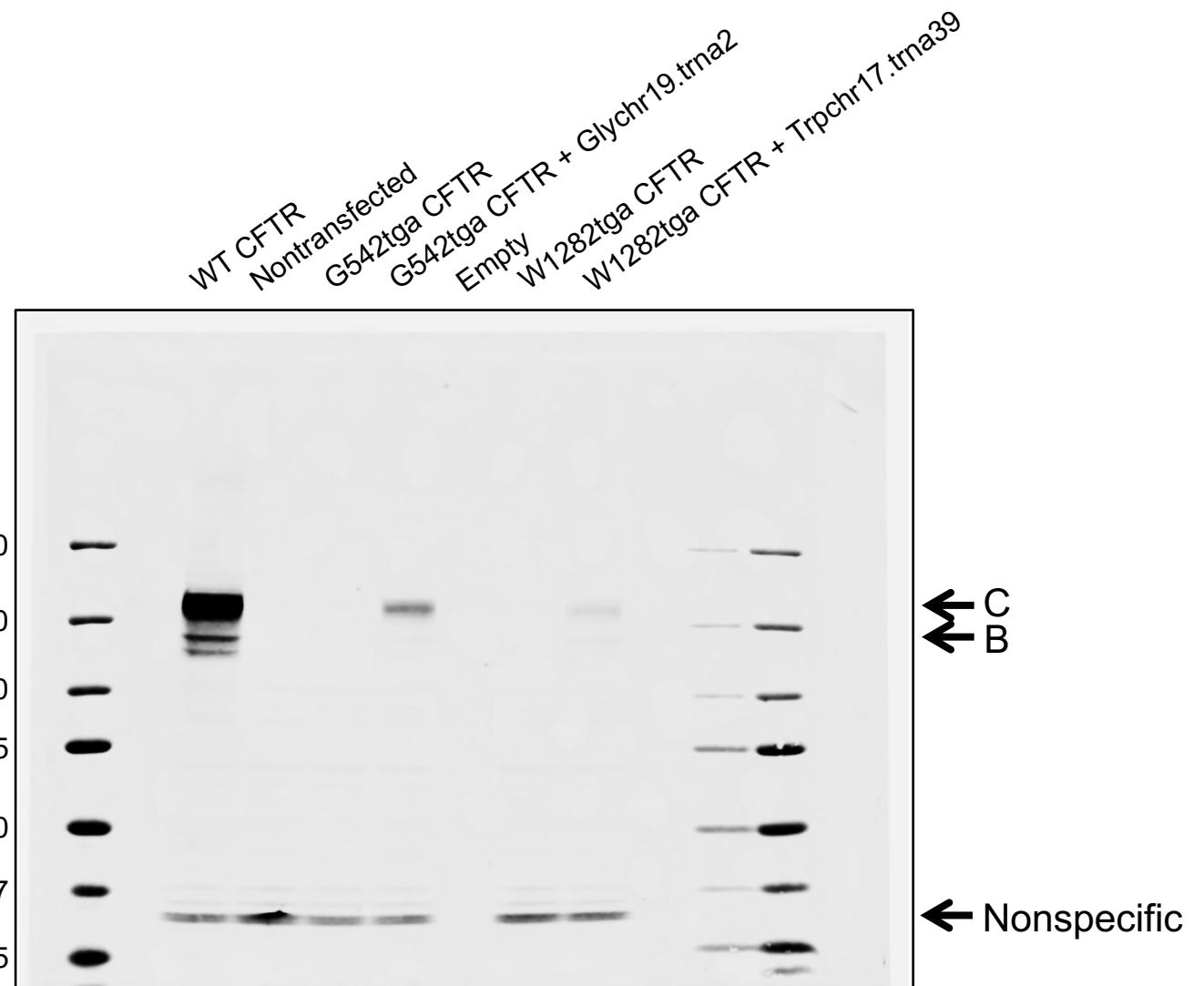
Underlined sequence indicates coverage by mass spectrometry. The red asparagine (a.a. position 94) is the residue mutated to a TGA PTC for determining ACE-tRNA fidelity. The dual affinity tag is indicated in green. Silver stain of HDH protein following PTC suppression with **b)** Trpchr17.tRNA39 and **c)** Glychr19.tRNA2 .



Stop codon specificity is maintained for ACE-tRNA^{Trp}. Suppression activity for tRNA Trp^{TGA} Trpchr17.tRNA39, the top performing Trp^{TGA} suppressor tRNA, Figure 2. This tRNA was co-expressed with the indicated pNano-STOP plasmid.

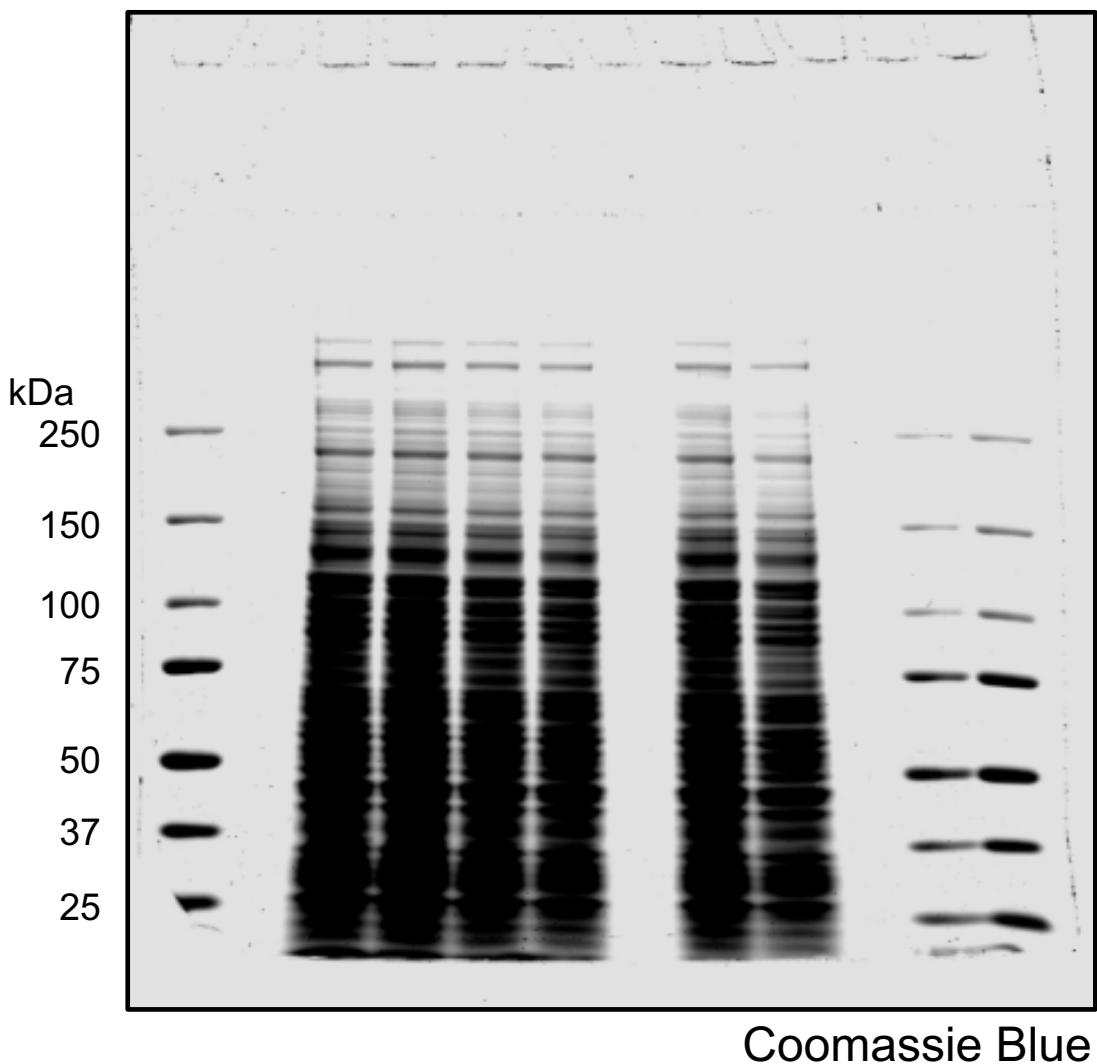


ACE-tRNAs are more efficient than aminoglycoside PTC suppression. **a)** Raw and **b)** normalized luminescence measured 24hrs following addition of gentamicin (40uM), G418 (150uM) and transfection with Trpchr17.tRNA39 and Glychr19.tRNA2 in HEK293 cells stably expressing PTC reporter Nluc-UGA. **c)** Raw and **d)** normalized luminescence measured 24hrs following addition of gentamicin (40uM), G418 (150uM) and co-transfection with Trpchr17.tRNA39 and Glychr19.tRNA2 in HEK293 cells.

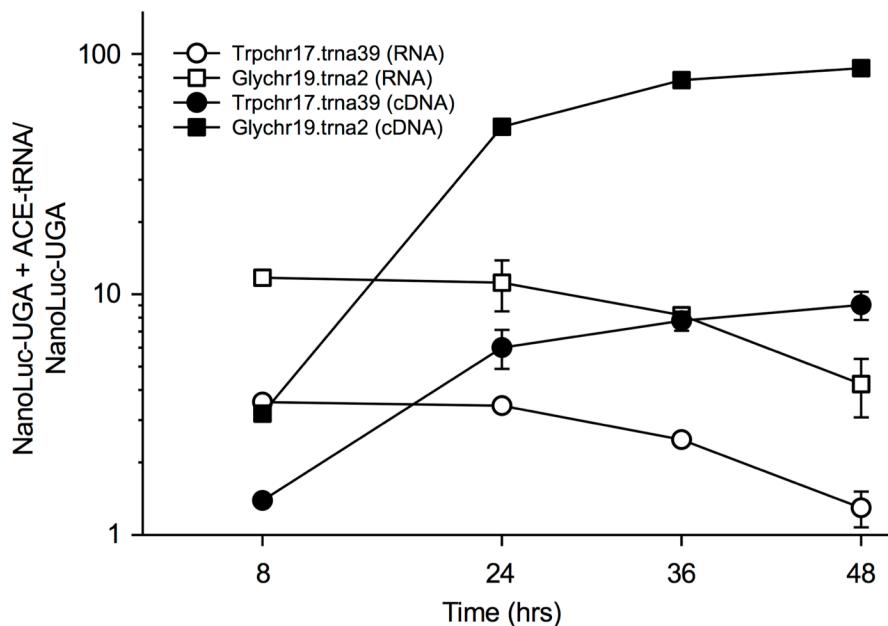


Full-length Western blot figure 5d.

WT CFTR Nontransfected G542tga CFTR G542tga CFTR + Glychr19.tma2
Empty W1282tga CFTR W1282tga CFTR + Trpchr17.tma39



Full-length Coomassie stain of replicate SDS-PAGE gel for figure 5d.



Comparison of time courses of ACE-tRNA activity following delivery as RNA or cDNA. ACE-tRNAs were delivered to HEK293 cells that stably express pNanoluc-UGA as described in the methods, however only 5 μ l of the reaction mix was added to the cells to reduce the effect of transfection reagents on cell viability. ACE-tRNA delivered as RNA (open symbols), was more rapid in rescuing expression of the PTC reporter than cDNA constructs (closed circles). However, ACE-tRNA activity continued to rise over the 48 hrs when expressed from cDNA and decreased as a RNA deliverable.

Supplementary Table 1. rRNA depletion oligos

Reference	Sequence (5' to 3')
NR_003285	TCCTCCGGGGCTACGCCTGTCTGAGCGTCGCT
NR_003286	GGGGGGATGCGTGCATTATCAGATCA TCCCCGCCCTTGCCCTCGGCC TTGGTGACTCTAGATAACCTCGGGCCGATCGC GAGCCGCCTGGATACCGCAGCTAGGAATAATGGA
NR_003287	TCGTGGGGGCCAAGTCCTCTGATCGAGGCC TCCGCCGAGGGCGCACCAACCGGCCGTCTGCC GAGCCTGGTTGGCCTCGGATAGCCGGTCCCCGC GGGGCCGGGCCACCCCTCCCACGGCGCG GCGCCCCGGCGGGTCGCGCCGTGGGCC CTCGCTTCTGGCGCCAAGCGCCCGC GCGCGCCGGCTGGACGAGGCGCCGCC

Supplementary Table 2: Oligo Sequences (5'-3')

	pNanoLuc plasmid for High-throughput cloning and screening mutagenesis primers.
pNanoLuc-For	GCCACCATGGTATTCACACTCGAAGATTCGTTG
pNanoLuc- TGA-Rev	TTACGCCAGAATGCGTCGCACAGCCGCCAGCCGGTCACTCATCC
pNanoLuc- TAA-Rev	TTACGCCAGAATGCGTCGCACAGCCGCCAGCCGGTCACTTATCC
pNanoLuc- TAG-Rev	TTACGCCAGAATGCGTCGCACAGCCGCCAGCCGGTACCTATCC
pNanoLuc- WT-Rev	TTACGCCAGAATGCGTCGCACAGCCGCCAGCCGGTCACTCCACC
	pNanoLuc plasmid for High-throughput cloning and screening Gibson assembly primers.
pNanoLuc-GAFor	ctggctagcgtaacttaagctt GCCACCATGGTATTCACAC
pNanoLuc- TGA-GARev	actgtgctggatatctgcagaattc TTACGCCAGAATGCGTTC
pNanoLuc- TAA-GARev	actgtgctggatatctgcagaattc CTACGCCAGAATGCGTTC
pNanoLuc- TAG-GARev	actgtgctggatatctgcagaattc TTACGCCAGAATGCGTTC
pNanoLuc- WT-GARev	actgtgctggatatctgcagaattc TTACGCCAGAATGCGTTC
	Primers for generation of 4 x ACE-tRNA expression Golden Gate puc57 plasmid
tRNA 1F	cacagacgaagactgttcataatacgactcactatagagcg
tRNA 1R	cacagacgaagactggattaaattaaccctcaactaaaggaaa
tRNA 2F	cacagacgaagacgtatacgcactcactatagagcg
tRNA 2R	cacagacgaagacgttgcattaaattaaccctcaactaaaggaaa
tRNA 3F	cacagacgaagacctacgcactcactatagagcgc
tRNA 3R	cacagacgaagacctaaccctcaactaaaggaaaaaa
tRNA 4F	cacagacgaagacacgttaatttaatacgactcactatagagcg
tRNA 4R	cacagacgaagacaccctgaatttaaccctcaactaaaggaaa
	Oligos for Golden Gate MCS in puc57 (EcoRI/HindIII)
GoldenGateMCS-For	aattcttcccgagacgTTCCAAGTCTTCatGAAGACTACAGGcgctccagga
GoldenGateMCS-Rev	agttccctggagacgcCTGTAGTCTTCatGAAGACTTGGAACgtctcggaag
	Primers for amplification SV40 polyA termination signal and Gibson Assembly
SV40 term-For	acagtggcgccgcgtcgagt TTGTTTATTGCAGCTTATAATG
SV40 term-Rev	tggccgattcataatgcagctg ATAAGATACATTGATGAGTTGG

Synthesized ccdB Golden Gate cDNA (digested plasmid with PciI and Gibson assembled cDNA)

```
tcttatcatgtctggatcgaCTAGAGGGCCTCCTAATACGACTCACTATAGAGCGCTCCGGTTTCTG  
TGCTGAACCTCAGGGACGCCACACACGTACACGTCTAGTCTCGGGCGCATTAAGGCACCCCAGGCT  
TTACACTTATGCTCCGGCTCGTATAATGTGTGGATTTGAGTTAGGATCCGGCGAGATTTCAGGAGC  
TAAGGAAGCTAAAATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCAATGGCATCGTAA  
GAACATTTGAGGCATTCAGTCAGTTGCTCAATGTACCTATAACCAGACCGTCAGCTGGATATTACGG  
CCTTTAAAGACCGTAAAGAAAAATAAGCACAAAGTTTATCCGGCTTATTACACATTCTGCCGCCT  
GATGAATGCTCATCCGAATTCGTATGGCAATGAAAGACGGTGAGCTGGTATGGATAGTGTTCAC  
CCTTGTACACCGTTCCATGAGCAAACGTAAACGTTCATCGCTCTGGAGTGAATACCACGACGATT  
TCCGGCAGTTCTACACATATTCGCAAGATGTGGCTTACGGTGAAACCTGGCTATTCCTAA  
AGGGTTATTGAGAATATGTTTCTCGTCAGCCAATCCCTGGGTGAGTTACCACTGGTAAAC  
GTGGCCAATATGGACAACCTCTCGCCCCCGTTTACCATGGCAAATATTATACGCAAGGCACAAGG  
TGCTGATGCCGCTGGCATTAGTTCATCATGCCGCTGTGATGGCTTACATCGGCAGAATGCTTAA  
TGAATTACAACAGTACTGCGATGAGTGGCAGGGGGGGCTAAAGATCTGGATCCGGTTACTAAAGCC  
AGATAACAGTATGCGTATTGCGCCTGATTGGCGGTATAAGAATATATACTGATATGTATACCGAA  
GTATGTCAAAAGAGGTGTGCTATGAAGCAGCGTATTACAGTGACAGTGACAGCAGCTATCAGTG  
CTCAAGGCATATATGATGTCAATATCTCGGTCTGGTAAGCACAAACATGCAGAATGAAGCCGTCGTCT  
GCGTGCACGCTGGAAAGCGGAAATCAGGAAGGGATGGCTGAGGTGCGCCGGTTATTGAAATGAAC  
GGCTTTGCTGACGAGAACAGGGACTGGTGAATGCAGTTAAGGTTACACCTATAAAAGAGAGAGC  
CGTTATCGTCTGTTGTGGATGTACAGAGTGATATTGACACGCCGGCAGGGATGGTATCCCC  
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AAGCTGGCGATGATGACCACCGATATGCCAGTGTGCCGGTCTCCGTTATGGGAAAGAAGTGGCTGAT  
CTCAGCCACCGCGAAAATGACATCAAAACGCCATTAACCTGATGTTCTGGGAAATAAATGTCAGGCT  
CCGTTATACACAGCCAGTCTGCAGGGAAgaagaccgGTCCTTTGCTTAGTGAGGGTTAATTCA  
GAGCAAAAGGC
```

The following mutants gibson assembled into the naturally occurring HindIII-EcoRI sites:

pNanoLuc-TGA

```
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HDH-TGA-HIS-STREP

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Genbank sequence for pNanoLuc-TGA-pcDNA3.1

pNanoLuc-TGA-pcDNA3.1

LOCUS NanLuc_pcDNA3.1_BbsI_le 5025 bp ds-DNA circular 8-
DEC-2018

DEFINITION .

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