Increased expression of tryptophan and tyrosine tRNAs elevates stop codon readthrough of reporter systems in human cell lines.

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Running title: human tRNAs regulate readthrough

SUPPLEMENTAL INFORMATION

EXPERIMENTAL PROCEDURES

Construction of plasmids

List of all plasmids, PCR primers and Genestrings used throughout this study can be found in Tables S1, S2 and S3, respectively.

PBB166 (=SW148 lacking *Kpn*I site) was created by altering SW148 plasmid by digestion with *Kpn*I, trimming by T4 DNA polymerase and subsequent self-ligation.

PBB168 was created by inserting the undigested PCR product obtained with primers PB159 and PB160 using vector Addgene53188 as template into *Ssp*I digested PBB166.

PBB170 was created by inserting the *Sal*I-*Not*I digested fragment from SW174 into *Sal*I-*Not*I digested PBB168.

PBB171 was created by inserting the *Sal*I-*Not*I digested fragment from SW149 into *Sal*I-*Not*I digested PBB168.

PBB171 was created by inserting the *Sal*I-*Not*I digested fragment from SW150 into *Sal*I-*Not*I digested PBB168.

PBB180 was created by inserting the *Apa*I/*Kpn*I fragment from annealed primers PB162 and PB167 into *Apa*I/*Kpn*I digested PBB168.

PBB185 was created by inserting the *Sal*I-*Not*I digested fragment from PBB170 into *Sal*I-*Not*I digested PBB180.

PBB220 was created by inserting the *Sal*I-*Not*I digested fragment from PBB171 into *Sal*I-*Not*I digested PBB180.

PBB184 was created by inserting the *Apa*I/*Kpn*I fragment from annealed primers PB162 and PB167 into *Apa*I/*Kpn*I digested PBB172.

PBB181 was created by inserting the *Sal*I-*Not*I digested fragment from PBB168 into *Sal*I-*Not*I digested PBB187.

PBB187 was created by inserting the *Apa*I/*Kpn*I fragment from annealed primers PB164 and PB169 into *Apa*I/*Kpn*I digested PBB172.

PBB182 was created by inserting the *Apa*I/*Kpn*I fragment from annealed primers PB166 and PB171 into *Apa*I/*Kpn*I digested PBB168.

PBB189 was created by inserting the *Sal*I-*Not*I digested fragment from PBB172 into *Sal*I-*Not*I digested PBB182.

PBB204 was created by inserting the *Sal*I-*Not*I digested fragment from PBB168 into *Sal*I-*Not*I digested PBB198.

PBB206 was created by inserting the *Sal*I-*Not*I digested fragment from PBB170 into *Sal*I-*Not*I digested PBB198.

PBB207 was created by inserting the *Sal*I-*Not*I digested fragment from PBB171 into *Sal*I-*Not*I digested PBB198.

PBB198 was created by inserting the *Apa*I/*Kpn*I fragment from annealed primers PB177 and PB178 into *Apa*I/*Kpn*I digested PBB172.

PBB288 was created by inserting the *Pme*I digested fragment from Genestring SG7 into *Pme*I digested PBB207.

PBB292 was created by inserting the *Pme*I digested fragment from Genestring SG8 into *Pme*I digested PBB180.

PBB214 was created by inserting the *Sal*I-*Not*I digested fragment from PBB168 into *Sal*I-*Not*I digested PBB196.

PBB196 was created by inserting the *Apa*I/*Kpn*I fragment from annealed primers PB175 and PB176 into *Apa*I/*Kpn*I digested PBB172.

PBB313 was created by inserting undigested fragment from Genestring SG16 into *Ssp*I digested PBB166.

PBB314 was created by inserting undigested fragment from Genestring SG17 into *Ssp*I digested PBB166.

PBB315 was created by inserting undigested fragment from Genestring SG18 into *Ssp*I digested PBB166.

PBB316 was created by inserting undigested fragment from Genestring SG19 into *Ssp*I digested PBB166.

PBB317 was created by inserting undigested fragment from Genestring SG20 into *Ssp*I digested PBB166.

PBB318 was created by inserting undigested fragment from Genestring SG21 into *Ssp*I digested PBB166.

PBB319 was created by inserting undigested fragment from Genestring SG22 into *Ssp*I digested PBB166.

PBB320 was created by inserting undigested fragment from Genestring SG23 into *Ssp*I digested PBB166.

PBB336 was created by inserting *Pme*I digested fragment from Genestring SG24 into *Ssp*I digested PBB166.

PBB337 was created by inserting *Pme*I digested fragment from Genestring SG25 into *Ssp*I digested PBB166.

PBB338 was created by inserting *Pme*I digested fragment from Genestring SG26 into *Ssp*I digested PBB166.

PBB293 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG7 into *Sal*I/*Bam*HI digested GL3051.

PBB293 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG7 into *Sal*I/*Bam*HI digested GL3051.

PBB306 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG9 into *Sal*I/*Bam*HI digested GL3051.

PBB307 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG10 into *Sal*I/*Bam*HI digested GL3051.

PBB308 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG11 into *Sal*I/*Bam*HI digested GL3051.

PBB309 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG12 into *Sal*I/*Bam*HI digested GL3051.

PBB310 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG13 into *Sal*I/*Bam*HI digested GL3051.

PBB311 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG14 into *Sal*I/*Bam*HI digested GL3051.

PBB295 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG7 into *Sal*I/*Bam*HI digested GL3052.

PBB294 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG8 into *Sal*I/*Bam*HI digested GL3051.

PBB296 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG8 into *Sal*I/*Bam*HI digested GL3052.

PBB371 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG7 into *Sal*I/*Bam*HI digested GL3101.

PBB373 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG7 into *Sal*I/*Bam*HI digested GL3102.

PBB372 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG8 into *Sal*I/*Bam*HI digested GL3101.

PBB374 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG8 into *Sal*I/*Bam*HI digested GL3102.

PBB297 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG7 into *Sal*I/*Bam*HI digested GL3095.

PBB299 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG7 into *Sal*I/*Bam*HI digested GL3096.

PBB298 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG8 into *Sal*I/*Bam*HI digested GL3095.

PBB300 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG8 into *Sal*I/*Bam*HI digested GL3096.

HR4 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG7 into *Sal*I/*Bam*HI digested GL3107.

HR12 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG7 into *Sal*I/*Bam*HI digested GL3108.

HR5 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG8 into *Sal*I/*Bam*HI digested GL3107.

HR13 was created by inserting *Sal*I/*Bam*HI digested fragment from Genestring SG8 into *Sal*I/*Bam*HI digested GL3108.

PBB271 was created by inserting *Sal*I/*Sac*I digested fragment from Genestring SG1 into *Sal*I/*Sac*I digested PBB168.

PBB272 was created by inserting *Sal*I/*Sac*I digested fragment from Genestring SG2 into *Sal*I/*Sac*I digested PBB168.

PBB273 was created by inserting *Sal*I/*Sac*I digested fragment from Genestring SG3 into *Sal*I/*Sac*I digested PBB168.

PBB274 was created by inserting *Sal*I/*Sac*I digested fragment from Genestring SG4 into *Sal*I/*Sac*I digested PBB168.

PBB275 was created by inserting *Sal*I/*Sac*I digested fragment from Genestring SG1 into *Sal*I/*Sac*I digested PBB207.

PBB276 was created by inserting *Sal*I/*Sac*I digested fragment from Genestring SG2 into *Sal*I/*Sac*I digested PBB207.

PBB277 was created by inserting *Sal*I/*Sac*I digested fragment from Genestring SG3 into *Sal*I/*Sac*I digested PBB207.

PBB278 was created by inserting *Sal*I/*Sac*I digested fragment from Genestring SG4 into *Sal*I/*Sac*I digested PBB207.

PBB219 was created by inserting the *Sal*I-*Not*I digested fragment from PBB177 into *Sal*I-*Not*I digested PBB172.

PBB234 was created by inserting the *Sal*I-*Not*I digested PCR product obtained with primers PB43 and PB187 using PBB219 as template into *Sal*I-*Not*I digested PBB219.

PBB223 was created by inserting the *Sal*I-*Not*I digested fragment from PBB177 into *Sal*I-*Not*I digested PBB207.

PBB235 was created by inserting the *Sal*I-*Not*I digested fragment from PBB234 into *Sal*I-*Not*I digested PBB207.

PBB301 was created by inserting *Pme*I digested fragment from Genestring SG7 into *Sma*I digested PBB321 (p53BS-luci).

PBB407 was created by Site-directed mutagenesis of pCMV-p53-WT (PBB395) using primers PB212 and PB213.

PBB400 was created by Site-directed mutagenesis of pCMV-p53-WT (PBB395) using primers PB216 and PB217.

PBB301 was created by inserting two *Pme*I digested fragments from Genestring SG7 into *Sma*I digested PBB321 (p53BS-luci).

PBB332 was created by inserting *Pme*I digested fragment from Genestring SG8 into *Sma*I digested PBB321 (p53BS-luci).

PBB380 was created by inserting *Sal*I/*Bam*HI digested fragment from PBB375 into *Sal*I/*Bam*HI digested GL3101.

PBB382 was created by inserting *Sal*I/*Bam*HI digested fragment from PBB375 into *Sal*I/*Bam*HI digested GL3102.

REFERENCES

1. Grentzmann, G., Ingram, J.A., Kelly, P.J., Gesteland, R.F. and Atkins, J.F. (1998) A dual-luciferase reporter system for studying recoding signals. *RNA*, **4**, 479-486.
2. Loughran, G., Howard, M.T., Firth, A.E. and Atkins, J.F. (2017) Avoidance of reporter assay distortions from fused dual reporters. *RNA*, **23**, 1285-1289.
3. Floquet, C., Deforges, J., Rousset, J.P. and Bidou, L. (2011) Rescue of non-sense mutated p53 tumor suppressor gene by aminoglycosides. *Nucleic Acids Res*, **39**, 3350-3362.
4. Chan, P.P. and Lowe, T.M. (2016) GtRNAdb 2.0: an expanded database of transfer RNA genes identified in complete and draft genomes. *Nucleic Acids Res*, **44**, D184-189.
5. Chan, P.P. and Lowe, T.M. (2009) GtRNAdb: a database of transfer RNA genes detected in genomic sequence. *Nucleic Acids Res*, **37**, D93-97.
6. Firth, A.E., Wills, N.M., Gesteland, R.F. and Atkins, J.F. (2011) Stimulation of stop codon readthrough: frequent presence of an extended 3' RNA structural element. *Nucleic Acids Res*, **39**, 6679-6691.
7. Napthine, S., Yek, C., Powell, M.L., Brown, T.D. and Brierley, I. (2012) Characterization of the stop codon readthrough signal of Colorado tick fever virus segment 9 RNA. *RNA*, **18**, 241-252.

SUPPLEMENTAL TABLES

Table S1. Plasmids used in this study.

Plasmid	Description	Source of reference
SW148	p2luci_UAG-TMV	(1)
SW147	p2luci_UAA-TMV	(1)
SW149	p2luci_UGA-TMV	(1)
SW150	p2luci_CAG-TMV	(1)
PBB166	SW148noKpnI	This study
PBB168	p2luci-UAG-TMV-U6	This study
PBB170	p2luci-UAA-TMV-U6	This study
PBB171	p2luci-UGA-TMV-U6	This study
PBB172	p2luci-CAG-TMV-U6	This study
PBB180	p2luci-UAG-TMV-U6-tY01	This study
PBB185	p2luci-UAA-TMV-U6-tY01	This study
PBB220	p2luci-UGA-TMV-U6-tY01	This study
PBB184	p2luci-CAG-TMV-U6-tY01	This study
PBB181	p2luci-UAG-TMV-U6-tQ01	This study
PBB187	p2luci-CAG-TMV-U6-tQ01	This study
PBB182	p2luci-UAG-TMV-U6-tQ01s	This study
PBB189	p2luci-CAG-TMV-U6-tQ01s	This study
PBB204	p2luci-UAG-TMV-U6-tW01	This study
PBB206	p2luci-UAA-TMV-U6-tW01	This study
PBB207	p2luci-UGA-TMV-U6-tW01	This study
PBB198	p2luci-CAG-TMV-U6-tW01	This study
PBB288	p2luci-UGA-TMV-U6-tW01-U6-tW01	This study
PBB292	p2luci-UAG-TMV-U6-tY01-U6-tY01	This study
PBB214	p2luci-UAG-TMV-U6-tY02	This study
PBB196	p2luci-CAG-TMV-U6-tY02	This study
PBB313	p2luci_UAG-TMV_tY03	This study
PBB314	p2luci_UAG-TMV_tY04	This study
PBB315	p2luci_UAG-TMV_tY05	This study
PBB336	p2luci_UAG-TMV_tY05b	This study
PBB337	p2luci_UAG-TMV_tY05c	This study
PBB338	p2luci_UAG-TMV_tY05d	This study
PBB316	p2luci_UAG-TMV_tY06	This study
PBB317	p2luci_UAG-TMV_tY07	This study
PBB318	p2luci_UAG-TMV_tY08	This study
PBB319	p2luci_UAG-TMV_tY09	This study
PBB320	p2luci_UAG-TMV_tY10	This study
GL3051	pSGDluc_UGA-AQP4	(2)
GL3052	pSGDluc_UGG-AQP4	(2)

PBB293	pSGDluc_UGA-AQP4-U6-tW01	This study
PBB306	pSGDluc_ UGA-AQP4-U6-tW02	This study
PBB307	pSGDluc_ UGA-AQP4-U6-tW03	This study
PBB308	pSGDluc_ UGA-AQP4-U6-tW04	This study
PBB309	pSGDluc_ UGA-AQP4-U6-tW05	This study
PBB310	pSGDluc_ UGA-AQP4-U6-tW06	This study
PBB311	pSGDluc_ UGA-AQP4-U6-tW07	This study
PBB295	pSGDluc_UGG-AQP4-U6-tW01	This study
PBB294	pSGDluc_UGA-AQP4-U6-tY01	This study
PBB296	pSGDluc_UGG-AQP4-U6-tY01	This study
GL3101	pSGDluc_UGA-MDH1	(2)
GL3102	pSGDluc_UGG-MDH1	(2)
PBB371	pSGDluc_UGA-MDH1-U6-tW01	This study
PBB373	pSGDluc_UGG-MDH1-U6-tW01	This study
PBB372	pSGDluc_UGA-MDH1-U6-tY01	This study
PBB374	pSGDluc_UGG-MDH1-U6-tY01	This study
GL3095	pSGDluc_UGA-ACP2-2	(2)
GL3096	pSGDluc_UGG- ACP2-2	(2)
PBB297	pSGDluc_UGA-ACP2-2-U6-tW01	This study
PBB299	pSGDluc_UGG- ACP2-2-U6-tW01	This study
PBB298	pSGDluc_UGA-ACP2-2-U6-tY01	This study
PBB300	pSGDluc_UGG- ACP2-2-U6-tY01	This study
GL3107	pSGDluc_UGA-C	(2)
GL3108	pSGDluc_UGG-C	(2)
HR4	pSGDluc_UGA-C-U6-tW01	This study
HR12	pSGDluc_UGG-C-U6-tW01	This study
HR5	pSGDluc_UGA-C-U6-tY01	This study
HR13	pSGDluc_UGG-C-U6-tY01	This study
PBB271	p2luci-Sindbis_UGG-U6	This study
PBB272	p2luci-Sindbis_UGA-U6	This study
PBB273	p2luci-CTFV_UGG-U6	This study
PBB274	p2luci-CTFV_UGA-U6	This study
PBB275	p2luci-Sindbis_UGG-U6-tW	This study
PBB276	p2luci-Sindbis_UGA-U6-tW	This study
PBB277	p2luci-CTFV_UGG-U6-tW	This study
PBB278	p2luci-CTFV_UGA-U6-tW	This study
PBB219	p2luci-UGA-CTAG-U6	This study
PBB234	p2luci-CAG-CTAG-U6	This study
PBB223	p2luci-UGA-CTAG-U6-tW01	This study
PBB235	p2luci-CAG-CTAG-U6-tW01	This study
PBB301	p53BS-luci_U6-tW01-SmaI-a	This study
PBB321	p53BS-luci	(3)

PBB394	pCMV-LacZ	(3)
PBB395	pCMV-p53-WT	(3)
PBB406	pCMV-p53-W53UAG	(3)
PBB407	pCMV-p53-W53UGA	This study
PBB400	pCMV-p53-W146UGA	This study
PBB330	p53BS-luci_2x_U6-tW01-SmaI-a	This study
PBB332	p53BS-luci-U6-tY01	This study
PBB375	tet-U6-tW01	Invitrogen
PBB380	pSGDluc_UGA-MDH1-tetU6-tW01	This study
PBB382	pSGDluc_UGG-MDH1-tetU6-tW01	This study

Table S2. Primers used in this study.

Primer name	Primer sequence (5´to 3´)
PB159	ATGGCCAAAGGTCGGGCAGGAAGAG
PB160	AGGTACCACGCGTGGGCCCCGGTGTTTCGTCCTTTCCAC
PB162	AATAAGGGCCCCCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTGTAGATCCTTAGGtCGCTGGTTCGATTCCGGCTCGAAGGAGGTACCAATAA
PB167	TTATTGGTACCTCCTTCGAGCCGGAATCGAACCAGCGACCTAAGGATCTACAGTCCTCCGCTCTACCAGCTGAGCTATCGAAGGGGGCCCTTATT
PB164	AATAAGGGCCCGGTTCCATGGTGTAATGGTTAGCACTCTGGACTCTGAATCCAGCGATCCGAGTTCAAATCTCGGTGGAACCTGGTACCAATAA
PB169	TTATTGGTACCAGGTTCCACCGAGATTTGAACTCGGATCGCTGGATTCAGAGTCCAGAGTGCTAACCATTACACCATGGAACCGGGCCCTTATT
PB166	AATAAGGGCCCGGTTCCATGGTGTAATGGTTAGCACTCTGGACTCTAAATCCAGCGATCCGAGTTCAAATCTCGGTGGAACCTGGTACCAATAA
PB171	TTATTGGTACCAGGTTCCACCGAGATTTGAACTCGGATCGCTGGATTTAGAGTCCAGAGTGCTAACCATTACACCATGGAACCGGGCCCTTATT
PB177	AATAAGGGCCCGACCTCGTGGCGCAACGGTAGCGCGTCTGACTCCAGATCAGAAGGCTGCGTGTTCGAATCACGTCGGGGTCAGGTACCAATAA
PB178	TTATTGGTACCTGACCCCGACGTGATTCGAACACGCAGCCTTCTGATCTGGAGTCAGACGCGCTACCGTTGCGCCACGAGGTCGGGCCCTTATT
PB175	AATAAGGGCCCCCTTCAATAGTTCAGCTGGTAGAGCAGAGGACTATAGGTCCTTAGGTTGCTGGTTCGATTCCAGCTTGAAGGAGGTACCAATAA
PB176	TTATTGGTACCTCCTTCAAGCTGGAATCGAACCAGCAACCTAAGGACCTATAGTCCTCTGCTCTACCAGCTGAACTATTGAAGGGGGCCCTTATT
PB43	CTCGAAGCGGCCGCTCTAGAATTACAC
PB187	CAAATGTCGACGTGCGATCAGCTAGTCGGATCCTTCAACTTCCCTGAGCTCG
PB212	CGATATTGAACAATGATTCACTGAAGACCCA
PB213	TGGGTCTTCAGTGAATCATTGTTCAATATCG
PB216	CCCTGTGCAGCTGTGAGTTGATTCCACACCC
PB217	GGGTGTGGAATCAACTCACAGCTGCACAGGG
WARS_qPCR-f	GCTCATTGTTCGGTTTGGA

WARS_qPCR-r	GAAGTGGTGTGGTCTTTGG
YARS_qPCR-f	CTGAGGTGGTAAAGCAGGT
YARS_qPCR-r	AATACTCTTCATCCAAAGCCTG
eRF1_qPCR-f	GGTCTGGTTGTATACTGTGG
eRF1_qPCR-r	TGTAAGAGCCTCTGTATGGA
18S_qPCR-f	CCGCAGCTAGGAATAATG
18S_qPCR-r	CCGGTCCAAGAATTTCAC
ALAS_qPCR-f	CCACTGGAAGAGCTGTGTGATGTG
ALAS_qPCR-r	GCGATGTACCCTCCAACACAACC
SPIKE_qPCR-f	CGAAATGAGAGCCAAGTGG
SPIKE_qPCR-r	ATGCAATTTAGATCCATTATGAGG

Table S3. Genestrings (GeneArt™ Strings™ DNA Fragments by Invitrogen) used in this study.

Name	DNA sequence (5´to 3´)
SG1	GTTCTCAAAAATGAACAAATGTCGACGTACTGGCTAACC GGGGTAGGTGGGTACATATTTTCGACGGACACAGGCC CTGGGCACTTGCAAAAGAAGTCCGTTCTGCAGAACCAG CTTACAGAACCGACCTTGGAGCGCAATGTCCTGGAAAG AATTCATGCCCCGGTGCTCGACACGTCGAAAGAGGAAC AACTCAAACTCAGGTACCAGATGATGCCCACCGAAGCC AACAAAAGTAGGTACGGATCCTTCAACTTCCCTGAGCT CGAAGACGCCAAAAACATAAAG
SG2	GTTCTCAAAAATGAACAAATGTCGACGTACTGACTAACC GGGGTAGGTGGGTACATATTTTCGACGGACACAGGCC CTGGGCACTTGCAAAAGAAGTCCGTTCTGCAGAACCAG CTTACAGAACCGACCTTGGAGCGCAATGTCCTGGAAAG AATTCATGCCCCGGTGCTCGACACGTCGAAAGAGGAAC AACTCAAACTCAGGTACCAGATGATGCCCACCGAAGCC AACAAAAGTAGGTACGGATCCTTCAACTTCCCTGAGCT CGAAGACGCCAAAAACATAAAG
SG3	GTTCTCAAAAATGAACAAATGTCGACGTGTTGGCGGTG TTGGCCTGGTGAGCTGTACCTCATTAACATCGAGAGTG CAGGCTTTCGTGAAGGAGCTTCAGCGGCTGCGGCTTAC CAGGCGGGCTTTTTGCGTGGATCCTTCAACTTCCCTGA GCTCGAAGACGCCAAAAACATAAAG
SG4	GTTCTCAAAAATGAACAAATGTCGACGTGTTGACGGTGT TGGCCTGGTGAGCTGTACCTCATTAACATCGAGAGTGC AGGCTTTCGTGAAGGAGCTTCAGCGGCTGCGGCTTACC AGGCGGGCTTTTTGCGTGGATCCTTCAACTTCCCTGAG CTCGAAGACGCCAAAAACATAAAG
SG7	CTGATAAATGCTTCAATAATAGTTTAAACGGATCCTGGC CAAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATT CCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAG ATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAG TACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGT AGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCA TATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGC TTTATATATCTTGTGGAAAGGACGAAACACCGGGGCCC GACCTCGTGGCGCAACGGTAGCGCGTCTGACTCCAGA TCAGAAGGCTGCGTGTTCGAATCACGTCGGGGTCAGGT ACCTATTGAAAAATTTTTTGGAACCCGGGGTCGACGTTT AAACTATTGAAAAAGGAAGAGTATG
SG8	CTGATAAATGCTTCAATAATAGTTTAAACGGATCCTGGC CAAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATT

	CCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAG ATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAG TACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGT AGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCA TATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGC TTTATATATCTTGTGGAAAGGACGAAACACCGGGGCCC CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACTGTAGA TCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGAGGT ACCTATTGAAAAATTTTTTGGAACCCGGGGTCGACGTTT AAACTATTGAAAAAGGAAGAGTATG
SG9	CTGATAAATGCTTCAATAATAGTTTAAACGGATCCTGGC CAAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATT CCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAG ATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAG TACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGT AGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCA TATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGC TTTATATATCTTGTGGAAAGGACGAAACACCGGGGCCC GACCTCGTGGCGCAACGGTAGCGCGTCTGACTCCAGA TCAGAAGGTTGCGTGTTCAAATCACGTCGGGGTCAGGT ACCTATTGAAAAATTTTTTGGAACCCGGGGTCGACGTTT AAACTATTGAAAAAGGAAGAGTATG
SG10	CTGATAAATGCTTCAATAATAGTTTAAACGGATCCTGGC CAAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATT CCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAG ATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAG TACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGT AGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCA TATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGC TTTATATATCTTGTGGAAAGGACGAAACACCGGGGCCC GACCTCGTGGCGCAATGGTAGCGCGTCTGACTCCAGAT CAGAAGGTTGCGTGTTCAAGTCACGTCGGGGTCAGGTA CCTATTGAAAAATTTTTTGGAACCCGGGGTCGACGTTTA AACTATTGAAAAAGGAAGAGTATG
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SG25	CTGATAAATGCTTCAATAATAGTTTAAACGGATCCTGGC CAAAGGTCGGGCAGGAAGAGGGCCTATTTCCCATGATT CCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAG ATAATTGGAATTAATTTGACTGTAAACACAAAGATATTAG TACAAAATACGTGACGTAGAAAGTAATAATTTCTTGGGT AGTTTGCAGTTTTAAAATTATGTTTTAAAATGGACTATCA TATGCTTACCGTAACTTGAAAGTATTTCGATTTCTTGGC TTTATATATCTTGTGGAAAGGACGAAACACCGGGGCCC CCTTCGATAGCTCAGTTGGTAGAGCGGAGGACTGTAGA TCCTTAGGTCGCTGGTTCGAATCCGGCTCGGAGGAGGT ACCTATTGAAAAATTTTTTGGAACCCGGGGTCGACGTTT AAACTATTGAAAAAGGAAGAGTATG
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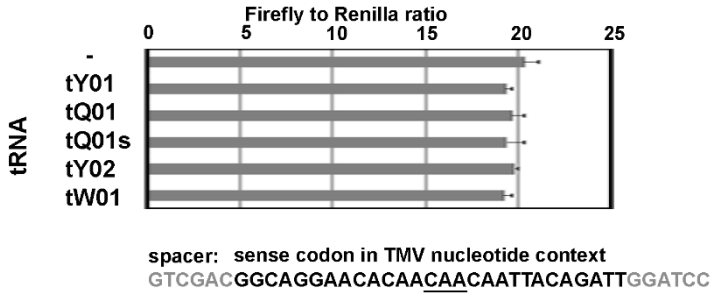
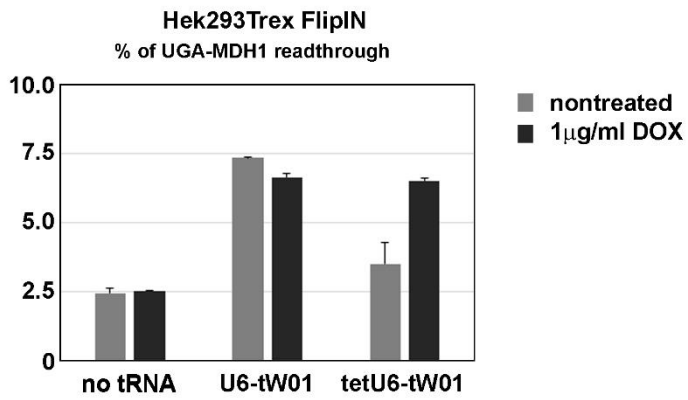
SUPPLEMENTARY FIGURES AND FIGURE LEGENDS



Supplementary Figure 1. Overexpression of human tRNAs do not affect Firefly to Renilla ratio for a reporter bearing the sense codon (CAA). The HEK293T cells were transfected with sense codon control reporters containing the U6-tRNAbox with or without indicated tRNAs and processed for luciferase activity measurements as described in Materials and Methods.

A



B

```
                                PmeI      BamHI     MscI
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    gactatttac gaagttatta tcaaatttgc ctaggaccgg tttccagccc gtccttctcc cggataaagg gtactaagga agtataaacg tatatgctat

101 caaggctgtt agagagataa ttggaattaa tttgactgta aacacaaaga tattagtaca aaatacgtga cgtagaaagt aataatttct tgggtagttt
    gttccgacaa tctctctatt aaccttaatt aaactgacat ttgtgtttct ataatcatgt tttatgcact gcatctttca ttattaaaga acccatcaaa

                                          NdeI
201 gcagttttaa aattatgttt taaaatggac tatcatatgc ttaccgtaac ttgaaagtcc ctatcagtga tagagattat atatctccct atcagtgata
    cgtcaaaatt ttaatacaaa attttacctg atagtatacg aatggcattg aactttcagg gatagtcact atctctaata tatagaggga tagtcactat
                                               PSE                 O2            TATA          O2

          ApaI                                                                                      KpnI
301 gagaccgggg cccgacctcg tggcgcaacg gtagcgcgtc tgactccaga tcagaaggct gcgtgttcga atcacgtcgg ggtcaggtac ctattgaaaa
    ctctggcccc gggctggagc accgcgttgc catcgcgcag actgagggtct agtcttccga cgcacaagct tagtgcagcc ccagtccatg gataactttt
        +1                                          tW01

                        SalI      PmeI
401 atttttttgga acccgggggtc gacgtttaaa ctattgaaaa aggaagagta tg
    taaaaaacct tgggccccag ctgcaaattt gataactttt tccttctcat ac
```
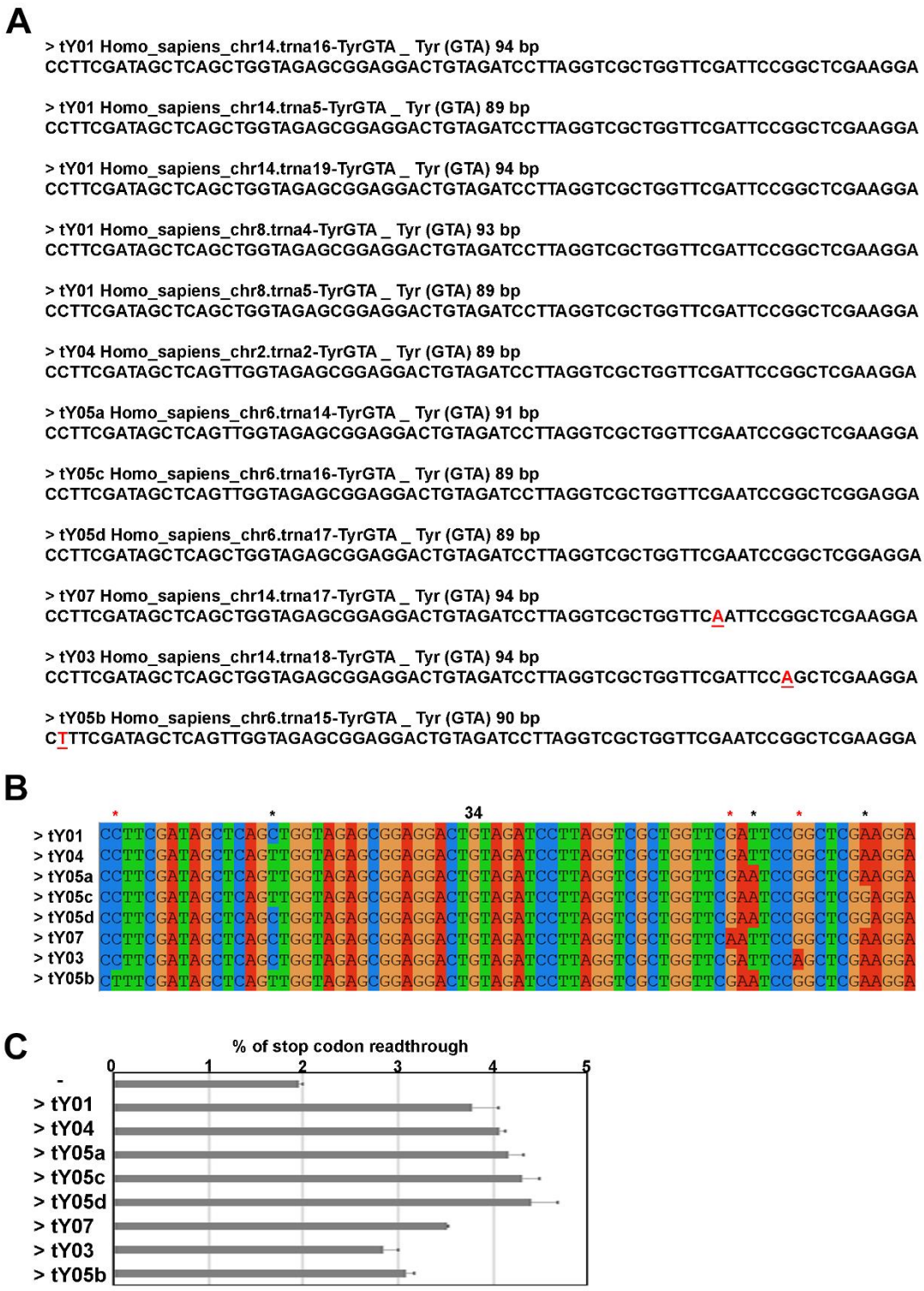
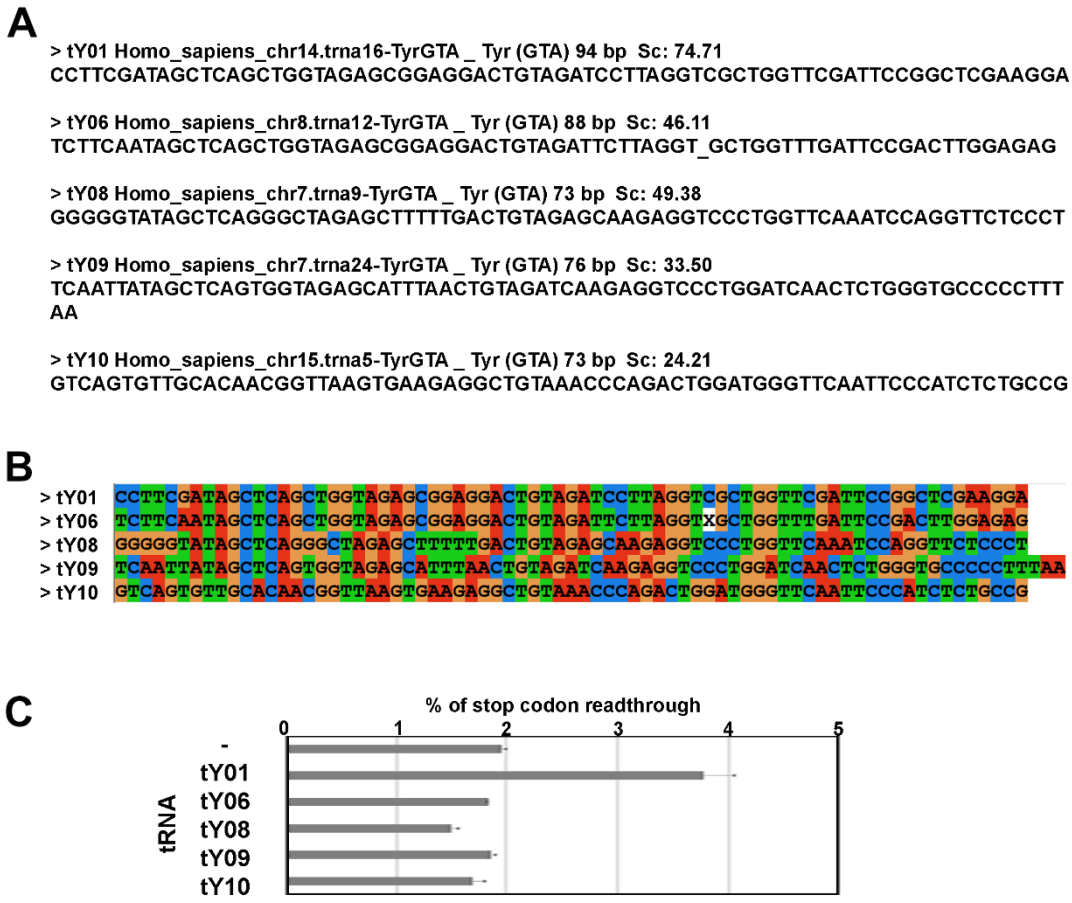

Supplementary Figure 2. Doxycycline inducible TetU6-tW01 expression in HEK293Trex cells increases SC-RT of the UGA-MDH1 reporter. (**A**) Readthrough measurements using UGA-MDH1 reporter with no tRNA control (no tRNA) and tryptophan tRNA under either the U6 promotor (U6-tW01) or the doxycycline inducible U6 promotor (tetU6-tW01) in the Hek293Trex FlipIN cells. The cells were transfected with stop codon constructs (GL3101, PBB371, PBB380) or their respective sense codon controls (GL3102, PBB373, PBB382) and treated with or without doxycycline 16h prior the readthrough measurement. (**B**) The sequence of tetU6-tW01 cassette including the U6 promotor features as in Figure 1 with insertions of two O2 repressor binding sequences and tW01 tryptophan tRNA. The PSE, TATAbox, +1 position, tRNA and O2 sequences are indicated in red.



Supplementary Figure 3. The single nucleotide polymorphism in human "high tRNA score" tyrosine iso-decoders influences the efficiency of readthrough. A) Sequences of all available tyrosine tRNAs retrieved from the GtRNAdb (4,5) (deprived of their computationally predicted introns) that were experimentally shown to increase the UAG readthrough. (**B**) The ClustalX2 alignment of the tRNA sequences from panel A. Single nucleotide polymorphisms are marked by an asterisk on the top of sequences; red boxes combined with red asterisks indicate a unique nucleotide

polymorphism. (**C**) The UAG–TMV context readthrough measurements with overexpression of the indicated Tyr tRNA iso-decoders under control of U6 in PBB168 (for UAG) or its sense control PBB172.



Supplementary Figure 4. Bioinformatically predicted "low tRNA score" tyrosine tRNA pseudogenes do not increase UAG readthrough. (**A**) tY01 and four other tyrosine tRNA sequences retrieved from the GtRNAdb (4,5) (deprived of their computationally predicted introns) that were experimentally shown not to increase the UAG readthrough. (**B**) The ClustalX2 alignment of the tRNA sequences from panel A and a high tRNA score tY01. (**C**) The UAG–TMV context readthrough measurements with overexpression of tY01 and the indicated Tyr tRNA pseudogenes under control of U6 in PBB168 (for UAG) or its sense control PBB172.

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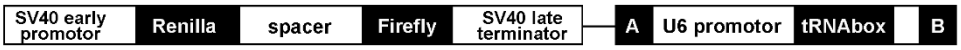
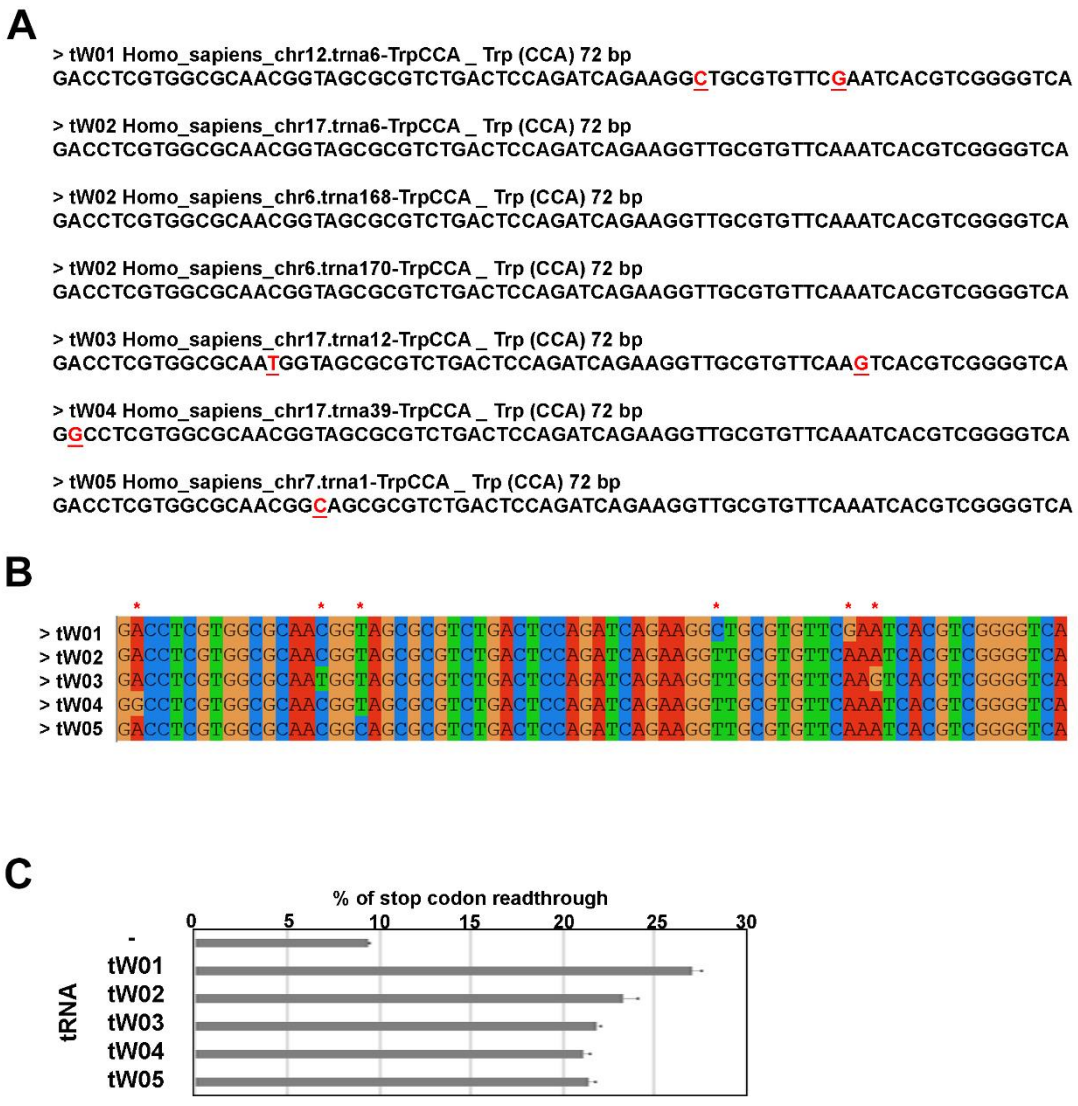


B

	tRNA	>gene name sequence	% of UAG readthrough
	-	none	1.96 ± 0.03
	tY01	>Homo_sapiens_chr14.trna16-TyrGTA CCTTCGATAGCTCAGCTGGTAGAGCGGAGGACT**GTA** GATCCTTAGGTCGCTGGTTCGATTCCGGCTCGAAGGA	3.61 ± 0.11
	tY02	>Homo_sapiens_chr2.trna14-TyrATA CCTTCAATAGTTCAGCTGGTAGAGCAGAGGACT**ATA** GGTCCTTAGGTTGCTGGTTCGATTCCAGCTTGAAGGA	1.91 ± 0.05

Supplementary Figure 5. The singleton human tyrosine tRNA with the ATA anticodon does not increase UAG readthrough (**A**) Schematic of the readthrough reporter containing the U6-tRNAbox cassette. For details please refer to the main text. (**B**) Readthrough measurements using the UAG reporter with or without the indicated human tRNAs in the HEK293T cells. The schematics of base-pairing between individual tRNAs and the UAG stop codon is shown to the right of the name of each construct, followed by the gene identity with the tRNA sequence and the relative percentage of the UAG readthrough. The tRNA sequences were individually placed under the U6 promoter of the PBB168 reporter or its sense control PBB172 reporter (see Figure 1).



Supplementary Figure 6. The single nucleotide polymorphism in human "high tRNA score" tryptophan iso-decoders does not impact the readthrough efficiency. A) Sequences of all available tryptophan tRNAs retrieved from the GtRNAdb (4,5). (**B**) The ClustalX2 alignment of the tRNA sequences from panel A. Single nucleotide polymorphisms (all are unique) are marked by a red asterisk on the top of sequences. (**C**) The UGA–AQP4 context readthrough measurements with overexpression of the indicated Trp tRNA iso-decoders under control of U6 in GL3051 (for UGA) or its sense control GL3052..

A

> tW01 Homo_sapiens_chr12.trna6-TrpCCA _ Trp (CCA) 72 bp Sc: 71.65
GACCTCGTGGCGCAACGGTAGCGCGTCTGACTCCAGATCAGAAGGcTGCGTGTTCGAATCACGTCGGGGTCA

> tW06 Homo_sapiens_chr9.trna3-TrpCCA _ Trp (CCA) 99 bp Sc: 23.38
GGCAGAGgagGGTGCAGTTGGCAGCCTGTCCAAGTCCAGcacggttggagcacaggatttagaATGGGATGGtCCTGG
GTTCAAACCCCAGCTGCGCCC

> tW07 Homo_sapiens_chr11.trna19-TrpCCA _ Trp (CCA) 74 bp Sc: 21.17
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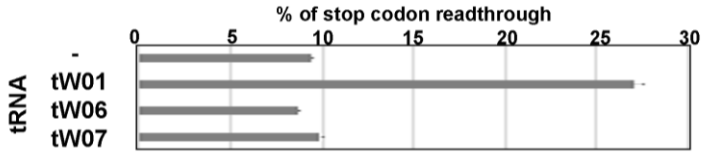
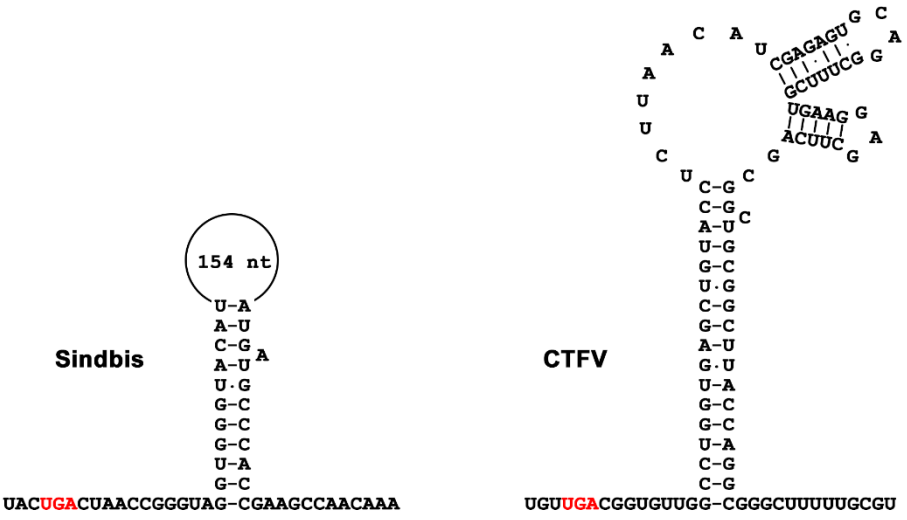
B



Supplementary Figure 7. Bioinformatically predicted "low tRNA score" tryptophan tRNA pseudogenes do not increase UAG readthrough. (**A**) tW01 and two other tryptophan tRNA sequences retrieved from the GtRNAdb (4,5) that were experimentally shown not to increase the UGA readthrough. (**B**) The UGA-AQP4 context readthrough measurements with overexpression of tW01 and the indicated Trp tRNA pseudogenes under control of U6 in GL3051 (for UGA) or its sense control GL3052.



Supplementary Figure 8. Schematics of the structural features of the Sindbis virus (SINV) (6) and Colorado thick fever virus (CTFV) (7) increasing the UGA readthrough. For details see the main text and Figure 5A.