
Supplementary information

Engineered tRNAs suppress nonsense mutations in cells and in vivo

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Engineered tRNAs suppress nonsense mutations in cells and in vivo

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Supplementary Figures

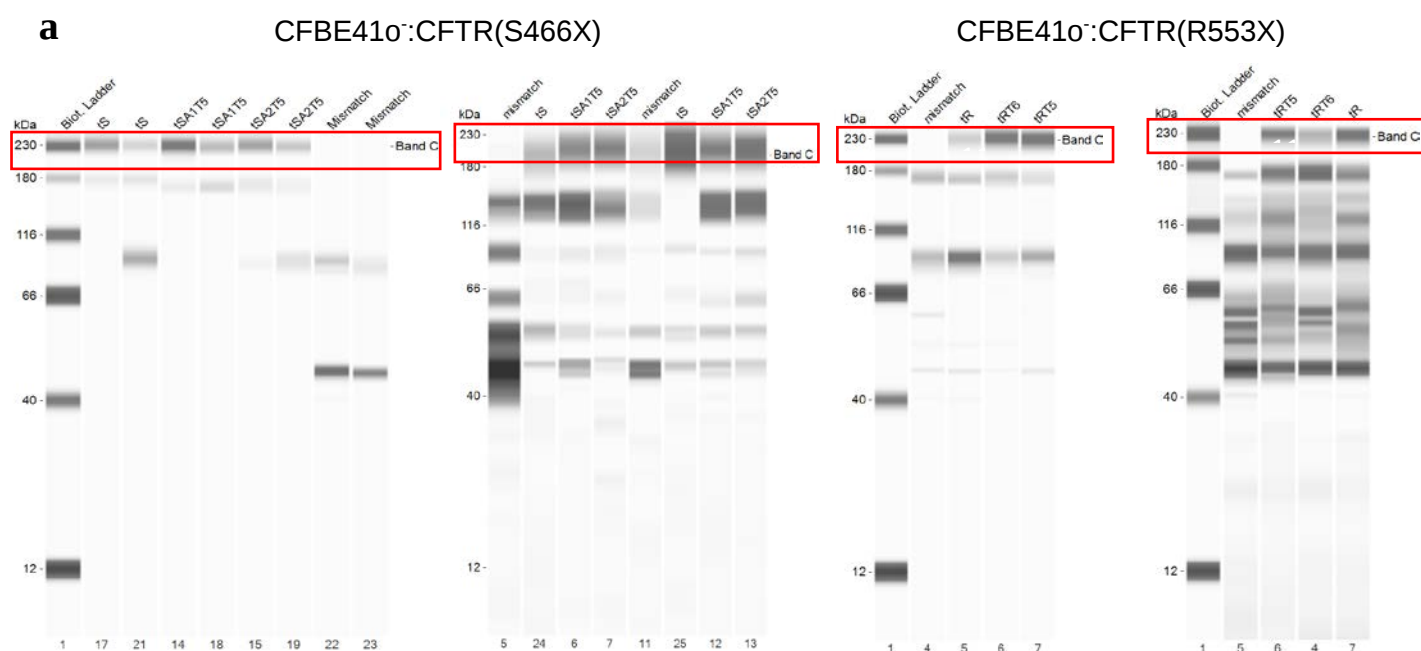
Supplementary Figure 1. Raw data of gel images from the capillary electrophoresis

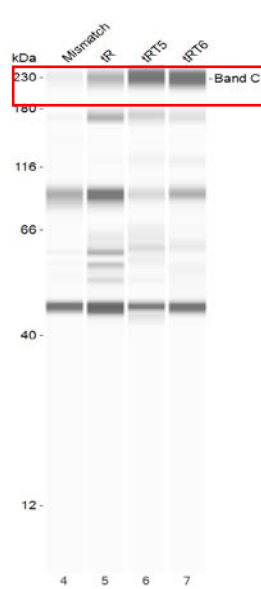
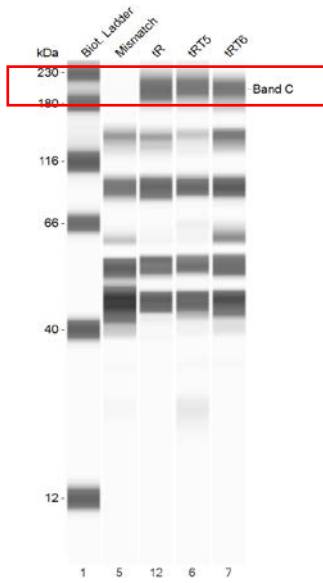
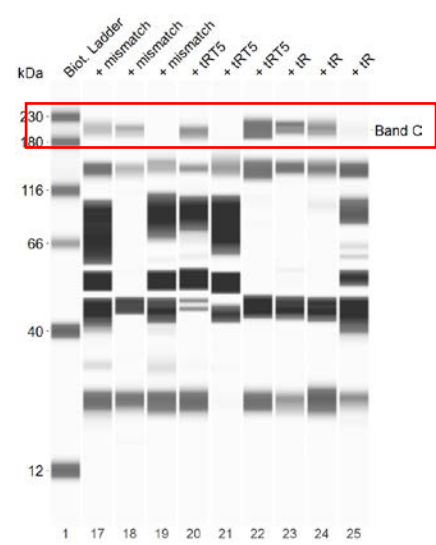
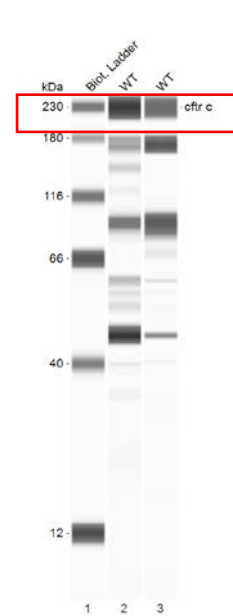
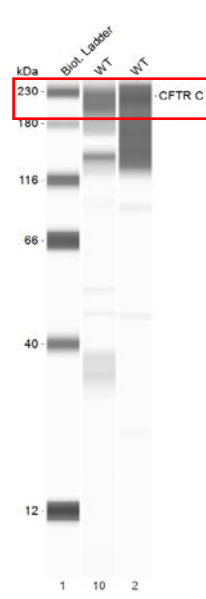
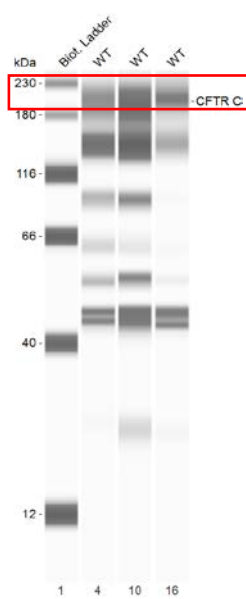
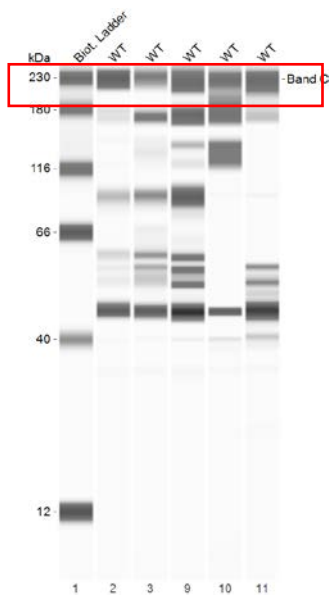
system (Jess, ProteinSimple). Designated areas (red boxes) correspond to CFTR band C and are used in the main figures for quantification of CFTR protein expression.

a-c, Gels used in Fig. 3a to determine the efficacy of tS and tR variants in restoring expression of full-length CFTR(S466X), CFTR(R553X) or CFTR(R1162X) in CFBE41o⁻ cells

d, Gels used in Fig. 4b to determine the efficacy of tR or tRT5 efficacy alone or by co-treatment with NMD inhibitor (5 μ M NMD14), or treatment with 10 μ M and 30 μ M PTC124 in 16HBEg^{R1162X/-} (X represents UGA) and 16HBE14o⁻ expressing wild-type *CFTR* cells.

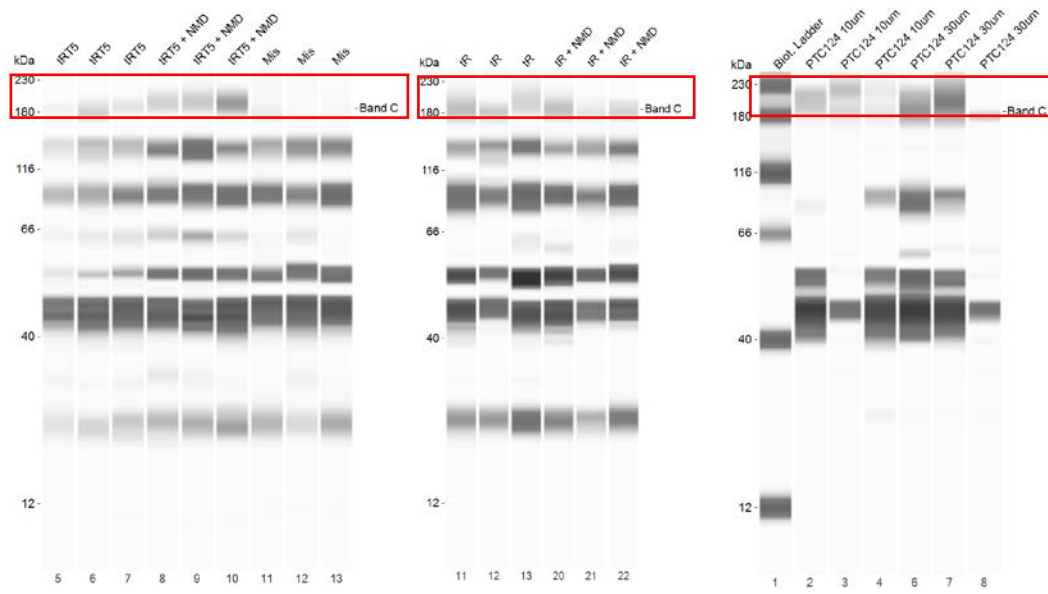
e, Gels used in Fig. 4c to determine the efficacy of tRT5 alone or by co-treatment with NMD inhibitor (5 μ M NMD14) in hNE^{R1162X/R1162X} and hNE expressing WT-CFTR.



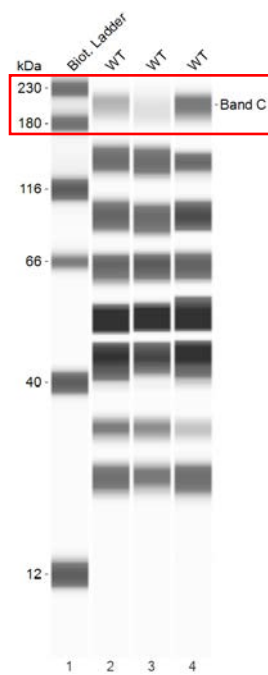
bCFBE410⁻: CFTR(R553X)CFBE410⁻: CFTR(R1162X)**c**CFBE410⁻: WT-CFTR

d

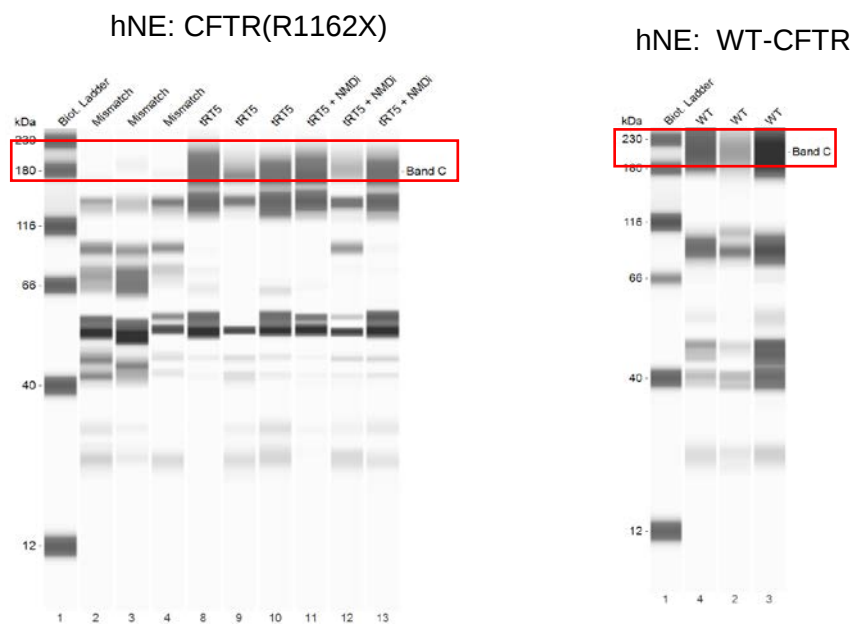
16HBEge: CFTR(R1162X)



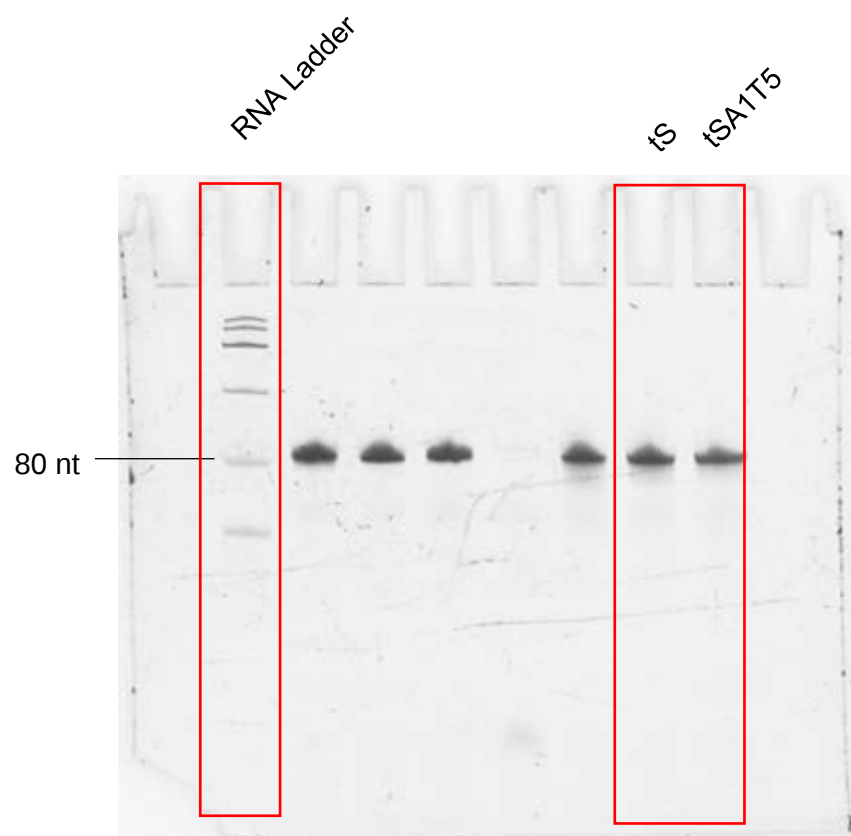
16HBE14o: WT-CFTR



e



Supplementary Figure 2. Raw gel image. Cropped area (red box) is shown in Extended Data Fig. 2a.



Supplementary Tables

Supplementary Table 1: Sequences of the tRNA variants. Substitutions in the anticodon are underlined, in the AC-stem and TΨC-stem highlighted in blue (according to Fig. 1a and Extended Data Fig. 1).

tRNA variant	tRNA ^{scan} -SE ID ^a	Sequence (5'-3')
tRNA ^{Ser} (AGA)	chr6.tRNA151	GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> GA <u>AAU</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tRNA ^{Ser} (UGA)	chr6.tRNA53	GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> GA <u>AAU</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tS		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> CA <u>AAU</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tSA1		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> CU <u>CAA</u> AA <u>U</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tSA2		GUAGUCGUGGCCGAGUGGUUAAGGCGC <u>GAAGG</u> CU <u>U</u> CA <u>AA</u> CC <u>U</u> GUUGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tST1		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> CA <u>AAU</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tST2		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> CA <u>AAU</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tST3		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> CA <u>AAU</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tST4		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> CA <u>AAU</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tST5		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> CA <u>AAU</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tST6		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> CA <u>AAU</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tSA1T4		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> CU <u>CAA</u> AA <u>U</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tSA1T5		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> CU <u>CAA</u> AA <u>U</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tSA1T6		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> CU <u>CAA</u> AA <u>U</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tSA2T5		GUAGUCGUGGCCGAGUGGUUAAGGCGC <u>GAAGG</u> CU <u>U</u> CA <u>AA</u> CC <u>U</u> GUUGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tRNA ^{Arg} (UCU)	chr9.tRNA5	GGCUCUGUGGCGCAUAGGGAUAGCGCAUUGGACU <u>U</u> CU <u>AAU</u> UCA <u>AA</u> GGUUUGGGUUCGAGUCCACCAAGAGUGGCCA
tR	chr11.tRNA2	GGCUCUGUGGCGCAUAGGGAUAGCGCAUUGGACU <u>U</u> CA <u>AAU</u> UCA <u>AA</u> GGUUUGGGUUCGAGUCCACCAAGAGUGGCCA
tRA2		GGCUCUGUGGCGCAUAGGGAUAGCGCA <u>GAAGG</u> CU <u>U</u> CA <u>AA</u> CC <u>U</u> GUAGGUUGGGUUCGAGUCCACCAAGAGUGGCCA
tRT5		GGCUCUGUGGCGCAUAGGGAUAGCGCAUUGGACU <u>U</u> CA <u>AAU</u> UCA <u>AA</u> GGUU <u>AC</u> GGGUUCGAGUCCCGU <u>U</u> CAGAGUGGCCA
tRT6		GGCUCUGUGGCGCAUAGGGAUAGCGCAUUGGACU <u>U</u> CA <u>AAU</u> UCA <u>AA</u> GGUU <u>GC</u> GGGUUCGAGUCCCGU <u>U</u> CAGAGUGGCCA
tRT7		GGCUCUGUGGCGCAUAGGGAUAGCGCAUUGGACU <u>U</u> CA <u>AAU</u> UCA <u>AA</u> GGUU <u>AC</u> GGGUUCGAGUCCCGU <u>U</u> CAGAGUGGCCA
tRT8		GGCUCUGUGGCGCAUAGGGAUAGCGCAUUGGACU <u>U</u> CA <u>AAU</u> UCA <u>AA</u> GGUU <u>GC</u> GGGUUCGAGUCCCGU <u>U</u> CAGAGUGGCCA
tRT9		GGCUCUGUGGCGCAUAGGGAUAGCGCAUUGGACU <u>U</u> CA <u>AAU</u> UCA <u>AA</u> GGUU <u>GC</u> GGGUUCGAGUCCCGU <u>U</u> CAGAGUGGCCA
tRA2T5		GGCUCUGUGGCGCAUAGGGAUAGCGCA <u>GAAGG</u> CU <u>U</u> CA <u>AA</u> CC <u>U</u> GUAGGUU <u>AC</u> GGGUUCGAGUCCCGU <u>U</u> CAGAGUGGCCA
tRNA ^{Gly} (UCC)	chr17.tRNA9	GCGUUGGUGGUUAUAGUGGUUAAGCAUAGCGCCU <u>U</u> CA <u>AA</u> AGCAGUUGACCCGGGUUCGAU <u>U</u> CCCGGCCAACGCACCA
tG1		GCGUUGGUGGUUAUAGUGGUUAAGCAUAGCGCCU <u>U</u> CA <u>AA</u> AGCAGUUGACCCGGGUUCGAU <u>U</u> CCCGGCCAACGCACCA
tG1T5		GCGUUGGUGGUUAUAGUGGUUAAGCAUAGCGCCU <u>U</u> CA <u>AA</u> AGCAGUUGACCCGGGUUCGAU <u>U</u> CCCGGCCAACGCACCA
tG1T6		GCGUUGGUGGUUAUAGUGGUUAAGCAUAGCGCCU <u>U</u> CA <u>AA</u> AGCAGUUGACCCGGGUUCGAU <u>U</u> CCCGGCCAACGCACCA
tRNA ^{Gly} (UCC)	chr19.tRNA2	GCGUUGGUGGUUAUAGUGGUUAAGCAUAGCGCCU <u>U</u> CA <u>AA</u> AGCAGUUGACCCGGGUUCGAU <u>U</u> CCCGGCCAACGCACCA
tG2		GCGUUGGUGGUUAUAGUGGUUAAGCAUAGCGCCU <u>U</u> CA <u>AA</u> AGCAGUUGACCCGGGUUCGAU <u>U</u> CCCGGCCAACGCACCA
tG2T5		GCGUUGGUGGUUAUAGUGGUUAAGCAUAGCGCCU <u>U</u> CA <u>AA</u> AGCAGUUGACCCGGGUUCGAU <u>U</u> CCCGGCCAACGCACCA
tG2T6		GCGUUGGUGGUUAUAGUGGUUAAGCAUAGCGCCU <u>U</u> CA <u>AA</u> AGCAGUUGACCCGGGUUCGAU <u>U</u> CCCGGCCAACGCACCA
mis-match		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> U <u>AAU</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
tRNAs		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> U <u>AA</u> AA <u>U</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> U <u>AA</u> AA <u>U</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA
		GUAGUCGUGGCCGAGUGGUUAAGGCGAUGGACU <u>U</u> U <u>AA</u> AA <u>U</u> CCA <u>U</u> UGGGUUUCCCGCGCAGGUUCGAAUCCUGCCGACUACGCCA

^atRNA^{scan} IDs are from the tRNA data base (GtRNAdb), <http://gtRNAdb.ucsc.edu/>

Supplementary Table 2: Summary of the sequence context of pathologic PTCs used in this study. Nucleotide and amino sequence of human PTCs are incorporated downstream of the start codon of *FLuc* (PTC-FLuc variants). The PTC is underlined and is flanked by seven codons each site.

Disease	Protein	Mutation	PTC	Nucleotide and amino acid sequence of PTC contexts
CLN2	TPP1	R208X	UGA	ACCCCCTCTGTGATCCGTAAGT <u>GAT</u> TACAACCTTG
				ACCTCACAAGAC
			UAG	ACCCCCTCTGTGATCCGTAAGT <u>AGT</u> TACAACCTTG
				ACCTCACAAGAC
			UAA	ACCCCCTCTGTGATCCGTAAGT <u>AA</u> TACAACCTTG
				ACCTCACAAGAC
CF	CFTR	S466X	UGA	-T--P--S--V--I--R--K--X--Y--N--L--T-- S--Q--D-
				TCCACTGGAGCAGGCAAGACTT <u>GACT</u> TCTAATGATGATTATGGGA
				TCCACTGGAGCAGGCAAGACTT <u>AGCT</u> TCTAATGATGATTATGGGA
				TCCACTGGAGCAGGCAAGACTTAACTTCTAATGATGATTATGGGA
				-S--T--G--A--G--K--T--X--L--L--M--M--I--M--G-

Supplementary Table 3. LUNAR1 and LUNAR2 nanoparticulate encapsulations.

LUNAR formulations	Particle size [nm]	Polydispersity index, PDI	% Encapsulation
LUNAR 1	83.45	0.133	96.4
LUNAR 2	75.61	0.168	99.4

Supplementary Table 4. Total number of transcripts detected in the ribosome profiling data sets.

Murine liver			
	Treated with tS Replicate #1	Treated with tS Replicate #2	Control
UAA	2791	2636	2504
UAG	2552	2424	2295
UGA	5747	5441	5109
Total	10900	10501	9908

Murine lungs			
	Treated with tSA1T5 Replicate #1	Treated with tSA1T5 Replicate #2	Control
UAA	3325	2580	3080
UAG	3012	2338	2778
UGA	6690	5259	6215
Total	13027	10117	12073