Supplementary Material

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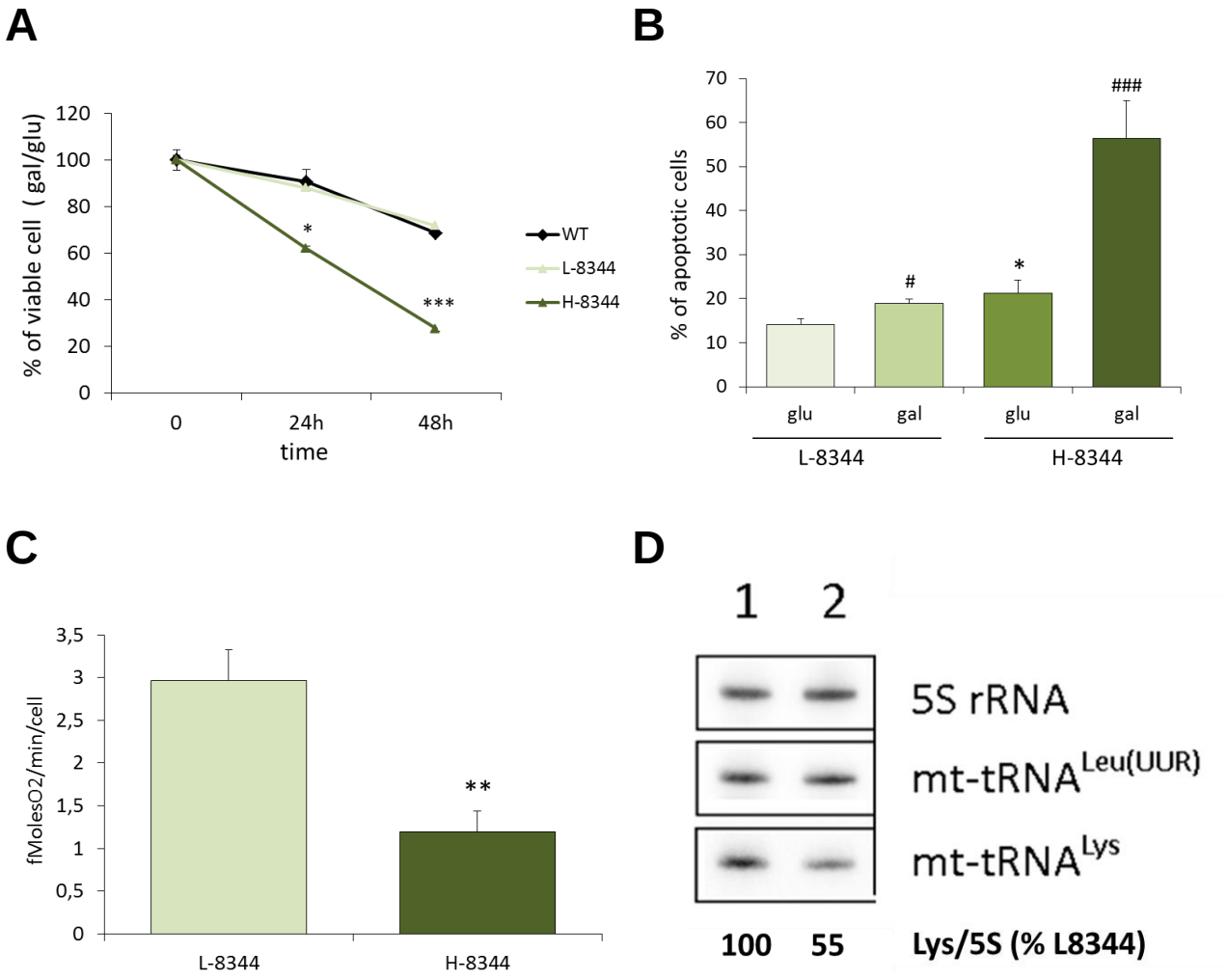


Supplementary Material Figure S1. Pathological phenotype of m.8344A>G mutant cybrids.

A. Viability of cells in galactose medium. Cells were maintained in glucose medium for 24 hours and then harvested and plated (30×10^4) in either glucose or galactose medium. The number of viable cells in galactose medium was evaluated after 24-48 hours and normalized for the number of viable cells in glucose at the same time point. WT=wild type, L-8344=low mutant, H-8344=high mutant.

B. Apoptotic cell death of mutant cybrids evaluated after 6 hours incubation in glucose or galactose medium.

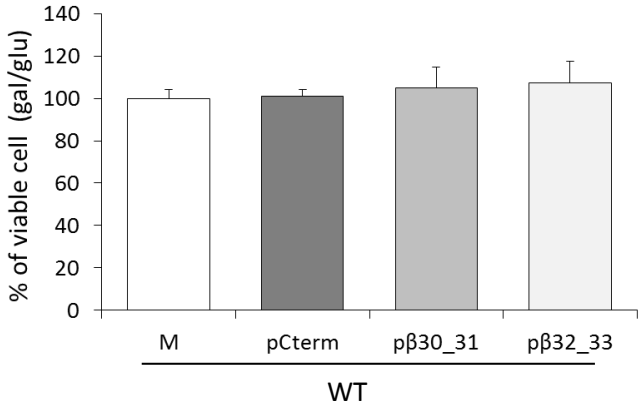
C. Rate of oxygen consumption measured in mutant cybrids maintained in glucose medium.

D. Steady state levels of mutant mt-tRNA^{Lys} in either L-8344 (1) or H-8344 (2) mutant cybrids.

Results are the mean ± SEM of triplicate experiments.

# p<0.05, ### p<0.001 for galactose vs glucose; * p<0.05, **p< 0.01, ***p< 0.001 for high mutant cybrids (H-8344) versus low mutant cybrids (L-8344).

A



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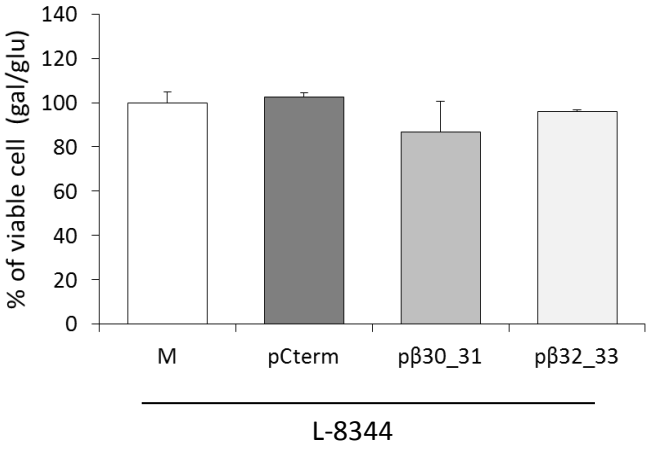
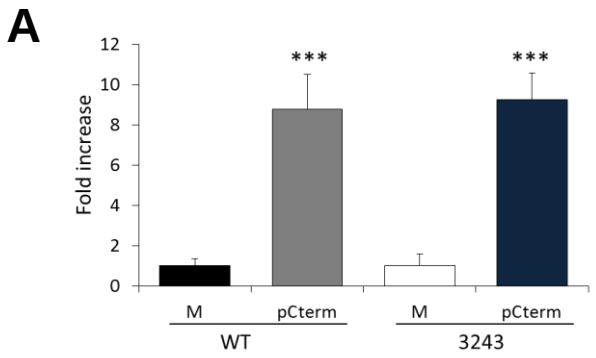
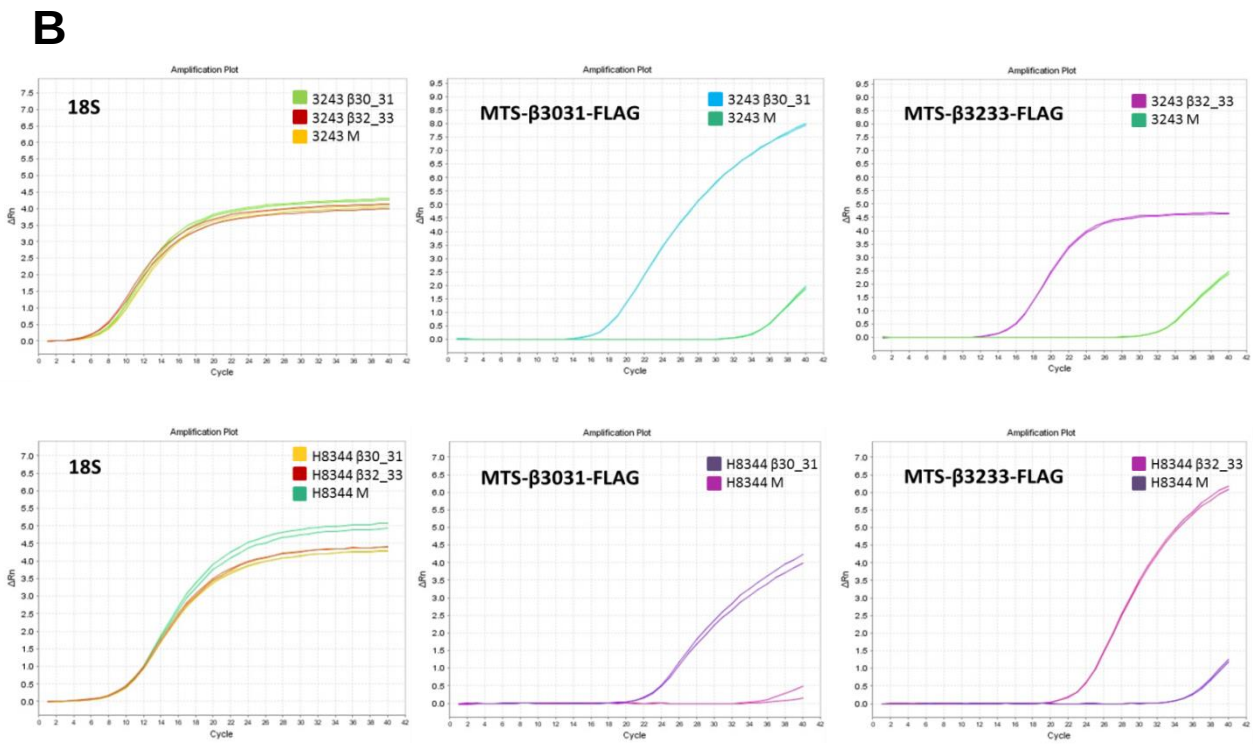


Supplementary Material Figure S2. Peptides overexpression had no effect on the viability of both WT and L-8344 cybrids.

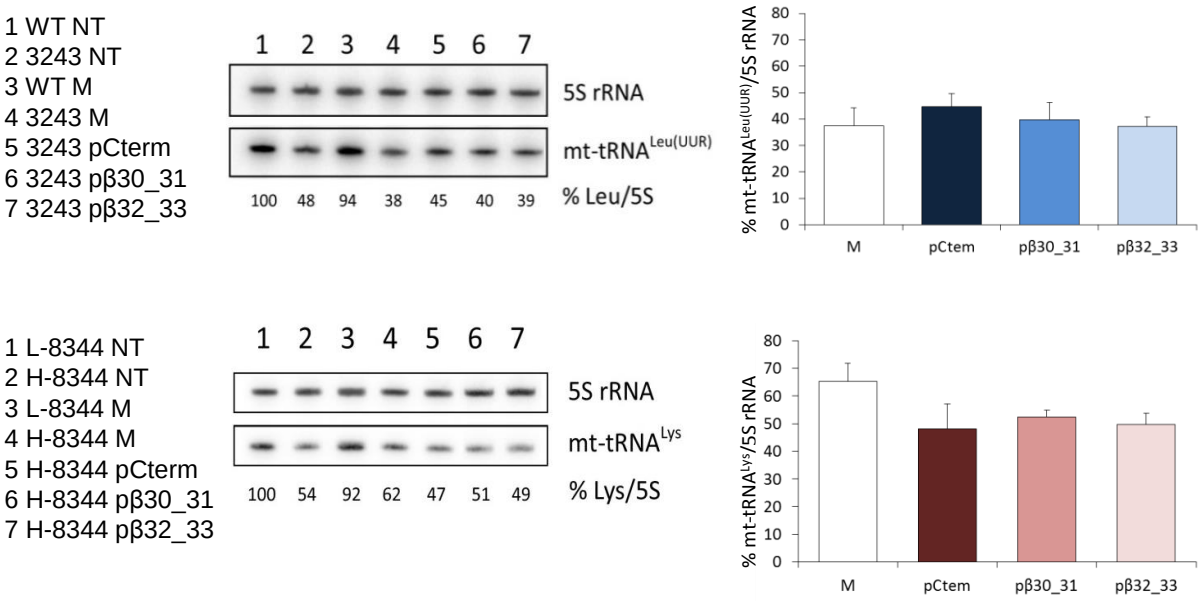
A-B. Viability of mock, Cterm, β30_31 and β32_33 transformants (M, pCterm, pβ30_31 and pβ32_33) evaluated after 24 hours incubation in galactose medium. The number of viable cells in galactose medium is normalized to the number of viable cells in glucose at the same time point. WT=wild type, L-8344=low mutant.
Results are the mean ± SEM of triplicate experiments.





Supplementary Material Figure S3. RNA over-expression analysis
A. Relative expression levels of Cterm in trasformant cybrids with respect to 18S gene. Gene expression levels are normalized to the gene expression level of the relative mock. Value represent the mean of all transfection experiments.
B. Relative expression levels of MTS-β30_31-FLAG and MTS-β32_33-FLAG in mock and transformant cybrids with respect to 18S gene. The amplification plot are representative of all real-time experiments.

A



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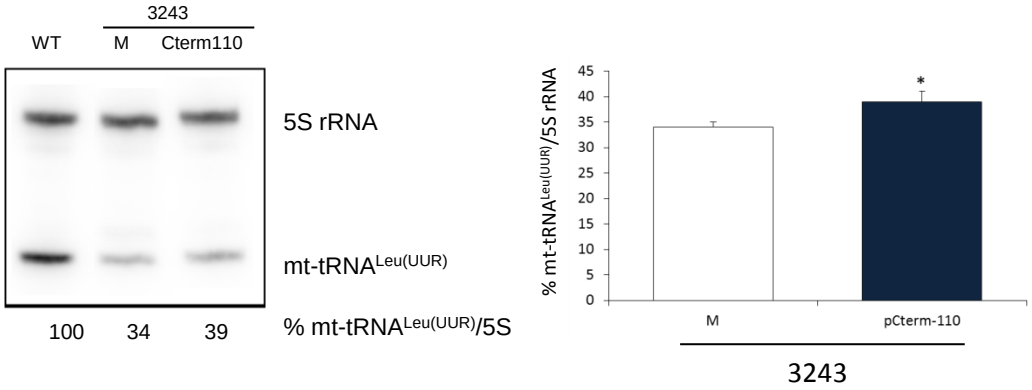


Supplementary Material Figure S4. Steady state levels of mt-tRNA$^{Leu(UUR)}$ and mt-tRNA^{Lys} in transfected cybrids.

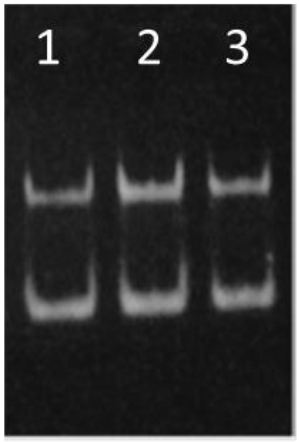
A. Mutant transformants cybrids (pCterm, pβ30_31 and pβ32_33) were maintained in glucose medium for 12 hours before RNA extraction. RNA (2µg) was electrophoresed through 13% denaturing polyacrylamide gel and hybridized with radiolabelled probes for 5S-rRNA, mt-tRNA$^{Leu(UUR)}$ and mt-tRNA^{Lys}. Transfection with either of the constructs does not result in a detectable increase in mutated tRNA steady state levels, as compared to the mock (M).

B. Evaluation of mt-tRNA$^{Leu(UUR)}$ steady-state levels in 3243 stable transfected cybrids. Left: representative northern blot; right: quantification of mt-tRNA$^{Leu(UUR)}$ levels normalized for 5S rRNA (results are the mean ± SEM of seven experiments).

* p<0.05 for transformants Cterm cybrids versus mock cybrids.

WT=wild type, L-8344=low mutant, H-8344=high mutant, NT=non-transfected

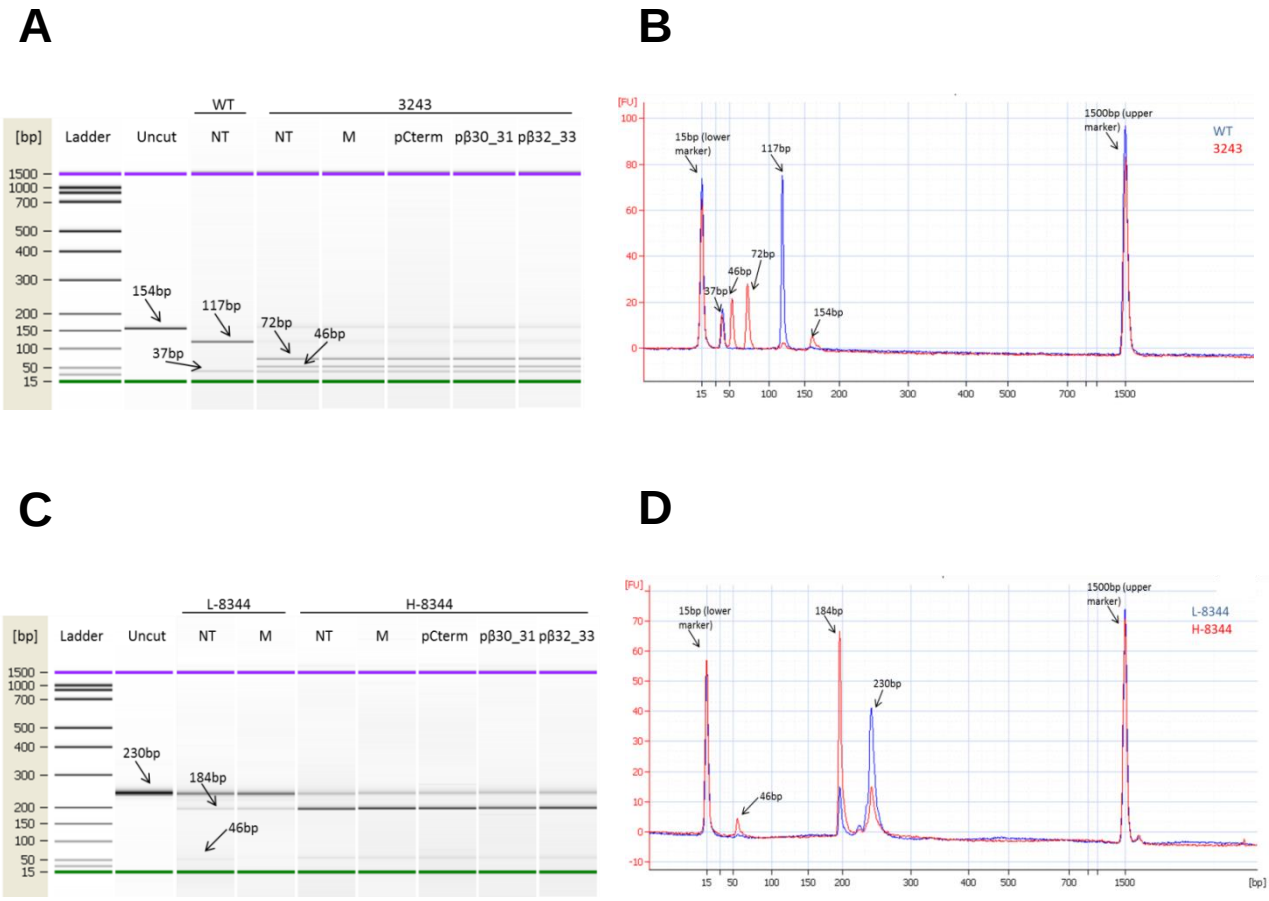
A



B

Sample	mt-tRNA$^{Leu(UUR)}$ m.3243A>G		
+ peptide	β30_31 10μM	-	β32_33 10μM
% dimerization	35%	40%	36%

Supplementary Material Figure S5. Dimerization of mt-tRNA$^{Leu(UUR)}$ m.3243A>G
A. Gel electrophoresis of 1 μM mt-tRNA$^{Leu(UUR)}$ m.3243A>G samples, incubated in 20 mM HEPES (pH 7.4), 150mM NaCl, 1mM MgCl2 and 0.1mM Spm for 15' in the presence of β30_31 or β32_33 peptides. The interaction with the peptides decreases mt-tRNA$^{Leu(UUR)}$ m.3243A>G dimerization (upper band), observed by Wittenhagen and Kelley (2002).
Lane 1: mt-tRNA$^{Leu(UUR)}$ m.3243A>G + 10 μM β30_31; lane 2: mt-tRNA$^{Leu(UUR)}$ m.3243A>G lane 3: mt-tRNA$^{Leu(UUR)}$ m.3243A>G + 10 μM β32_33; in each lane, 0.4 μg of tRNA were loaded.
B. Dimerization percentages in each lane of **A**, calculated using the Fiji suite.



Supplementary Material Figure S6. RFLP analysis of cybrid cells bearing m.3243A>G (A and B) or m.8344A>G (C and D) mutations.

Restriction fragments were separated using the Agilent 2100 bioanalyzer instrument with the 1000 Lab Chip kit.

A. LabChip gel image. 154-bp PCR products, amplified with mismatched forward and reverse primers (see methods), were cut with HaeIII enzyme.

B. Overlay of two different electropherograms (blue = wild type; red = 3243 mutant). The bands are separated and identified by the software as separate peaks.

C. LabChip gel image. 230-bp PCR products, amplified with mismatched forward and reverse primers (see methods), were cut with BglI enzyme.

D. Overlay of two different electropherograms (blue = low 8344 mutant; red = high 8344 mutant). The bands are separated and identified by the software as separate peaks.

NT= non transformant, WT= wild type, L-8344= low 8344 mutant, H-8344= high 8344 mutant, M=mock transformants, pCterm= Cterm transformants, pβ30_31= β30_31 transformants, pβ32_33 = β32_33 transformants.

WT contain a single restriction site, which cleaves the amplicons into two fragments of 117 and 37-bp. The m.3243A>G mutation introduces a second HaeIII recognition site that cuts the 117-bp fragment into two smaller fragments of 45 and 36-bp.

The m.8344A>G mutation introduces a BglI recognition site that cuts the 230-bp fragment into two smaller fragments of 184 and 46-bp.

Supplementary Material Table S1. Cybrid cell lines

Cell line	Mutation	Mutation load	Reference
HP27*	WT	nd	*Pello et al. 2008*
RN164*	m.3243A>G	~100%	*King et al. 1992*
HMP58	m.8344A>G	~34%	*Unpublished*
HMP87	m.8344A>G	~86%	*Unpublished*

* Generous gift from Dr Monica Montopoli and Valerio Carelli

Supplementary Material Table S2. pcDNA3.2 vectors for transient and stable transfections.

Plasmid name	Construct (nucleotide)
MTS-Cterm-FLAG	ATGTCCGTCCTGACGCCGCTGCTGCTGCGGGGCTT GACAGGCTCGGCCCGGCGGCTCCCAGTGCCGCGC GCCAAGGGTGGGAGCGGTGGGGAGGTTGTCCAGA TGGCAGTTCTGATCAACAATAAAGCTTGTGGCAAAA TTCCTGTGCCCCAACAAGTTGCCCGGGACCAGGAC AAAGTCCACGAATTTGTTCTTCAAAGCGAGCTGGGT GTCAGGCTTTTGCAAGGACGAAGCATCAAGAAGTC CTTCCTTTCCCCGAGAACTGCCCTCATCAACTTCCT GGTGCAAGATGGTGGGAGCGGTGGGGATTACAAG GATGACGACGATAAGTAG
MTS-β3031-FLAG	ATGTCCGTCCTGACGCCGCTGCTGCTGCGGGGCTT GACAGGCTCGGCCCGGCGGCTCCCAGTGCCGCGC GCCAAGGGTGGGAGCGGTGGGATGGCAGTTCTGA TCAACAATAAAGCTTGTGGCAAAATTCCTGTGGGTG GGAGCGGTGGGGATTACAAGGATGACGACGATAAG TAG
MTS-β3233-FLAG	ATGTCCGTCCTGACGCCGCTGCTGCTGCGGGGCTT GACAGGCTCGGCCCGGCGGCTCCCAGTGCCGCGC GCCAAGGGTGGGAGCGGTGGGAAGAAGTCCTTCC TTTCCCCGAGAACTGCCCTCATCAACTTCCTGGTGG GTGGGAGCGGTGGGGATTACAAGGATGACGACGAT AAGTAG

Black: start and stop codons
Red: Mitochondrial target sequence (MTS) from human COX8 gene
Green: linker
Purple: LeuRS fragment
Blue: FLAG sequence

Supplementary Material Table S3. Real-time PCR assays used to assess gene expression levels

Gene	Assay ID or primers and probe sequences
18S	Hs99999901_s1
MTS-Cterm-FLAG	For 5'-AAATTCCTGTGCCCCAACAA-3' Rev 5'-TGAAGAACAAATTCGTGGACTTTG-3' Probe 6FAM-TGCCCGGGACCAGG-MGB
MTS-β30_31-FLAG	For 5'-TTATCGTCGTCATCCTTGTAATCC-3' Rev 5'-TCAACAATAAAGCTTGTGGCAAA-3' Probe 6FAM -TCCCACCCACAGGAA- MGB
MTS-β32_33-FLAG	For 5'-TTATCGTCGTCATCCTTGTAATCC-3' Rev 5'-CCGAGAACTGCCCTCATCAA-3' Probe 6FAM -TCCCACCCACCAGGAA- MGB

Supplementary Material References

King MP, Koga Y, Davidson M, Schon EA (1992) Defects in mitochondrial protein synthesis and respiratory chain activity segregate with the tRNA(Leu(UUR)) mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes. Mol Cell Biol 12:480-490

Pello R, Martín MA, Carelli V, Nijtmans LG, Achilli A, Pala M, Torroni A, Gómez-Durán A, Ruiz-Pesini E, Martinuzzi A et al. (2008) Mitochondrial DNA background modulates the assembly kinetics of OXPHOS complexes in a cellular model of mitochondrial disease. Hum Mol Genet 17:4001-4011

Wittenhagen LM, Kelley SO (2002) Dimerization of a pathogenic human mitochondrial tRNA. Nat Struct Biol 9:586-90