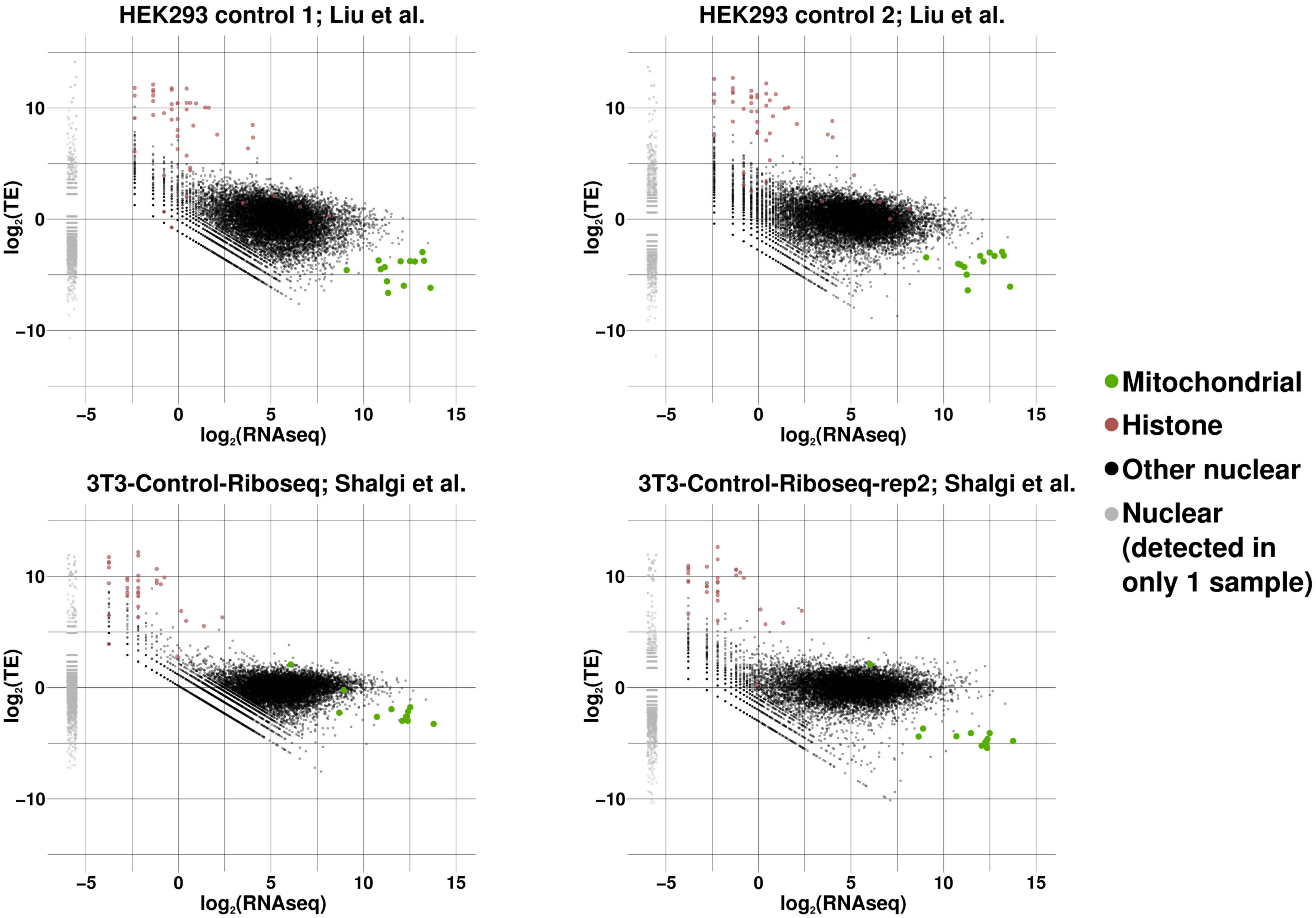
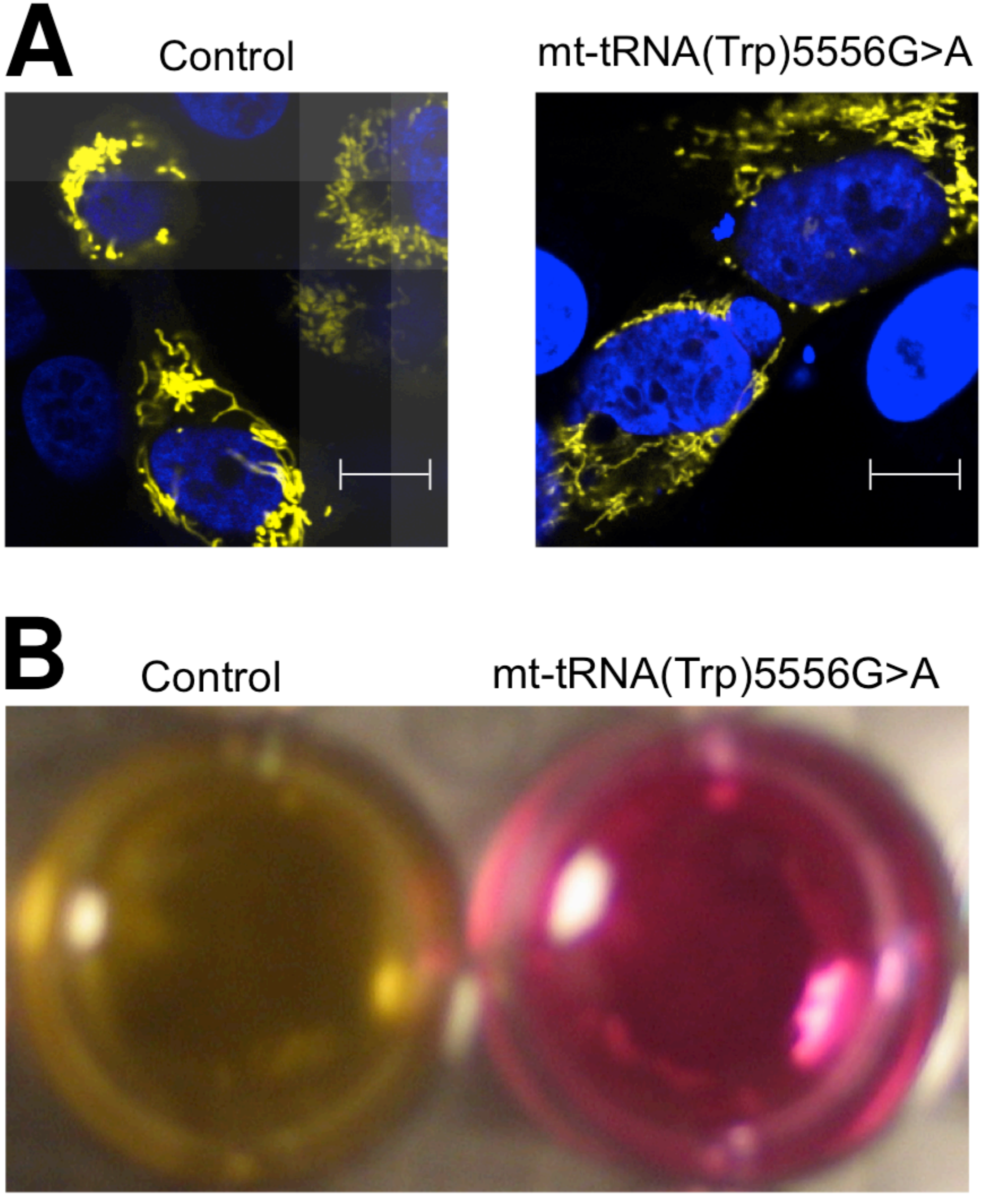
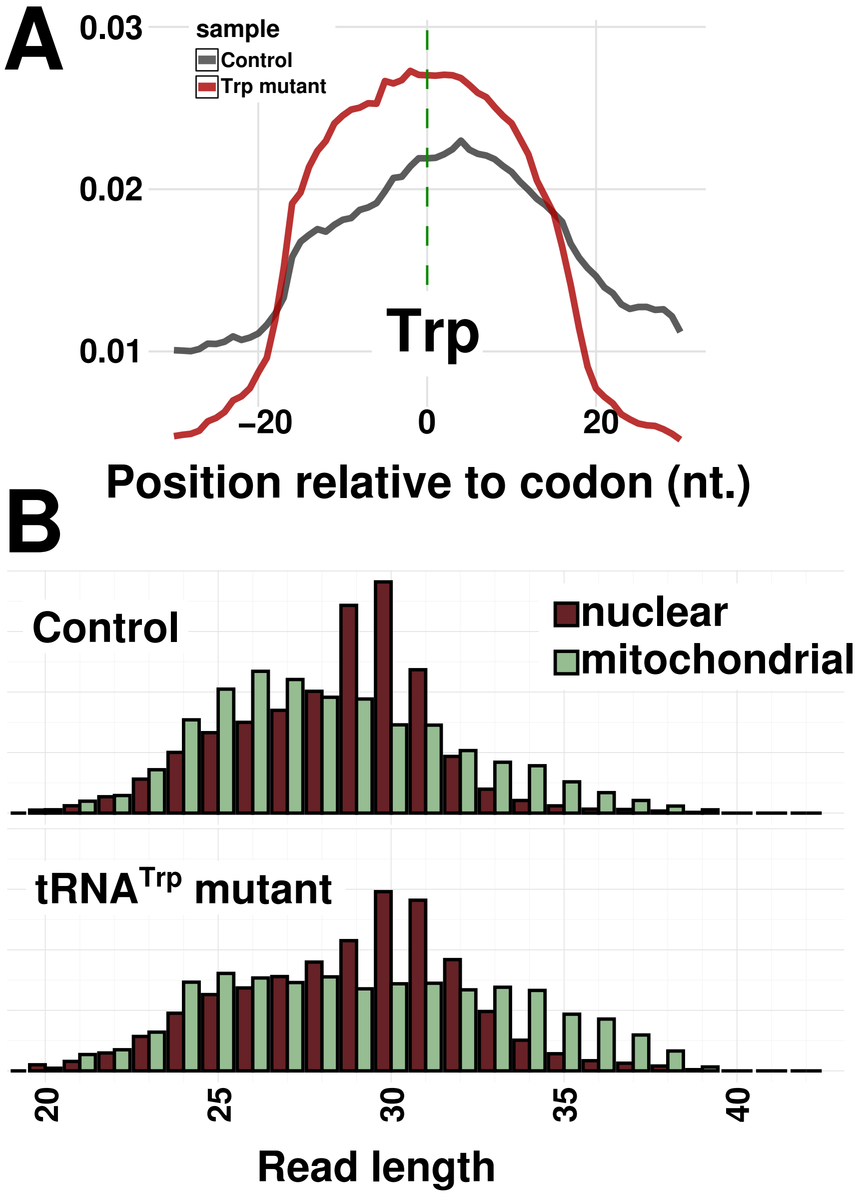


Supplementary Figure S1: Translational efficiency (TE) versus abundance (measured by RNAseq) in two recently published datasets from Liu et al.[10] and Shalgi et al.[11].

Since the data from Liu et al. was supplied without matching RNAseq of the samples, we used RNA sequencing data from matching cell line and control condition from Xiao et al.[20]. GEO sample identifiers of the used data are given in Supplementary Table S1. For the data from Liu et al. the same pipeline was used as the data described in the article. For the data from Shalgi et al. an adapted version of the protocol was used using the Mus Musculus NCBIM37 reference sequence for alignment and Ensembl v67 annotations for analysis.

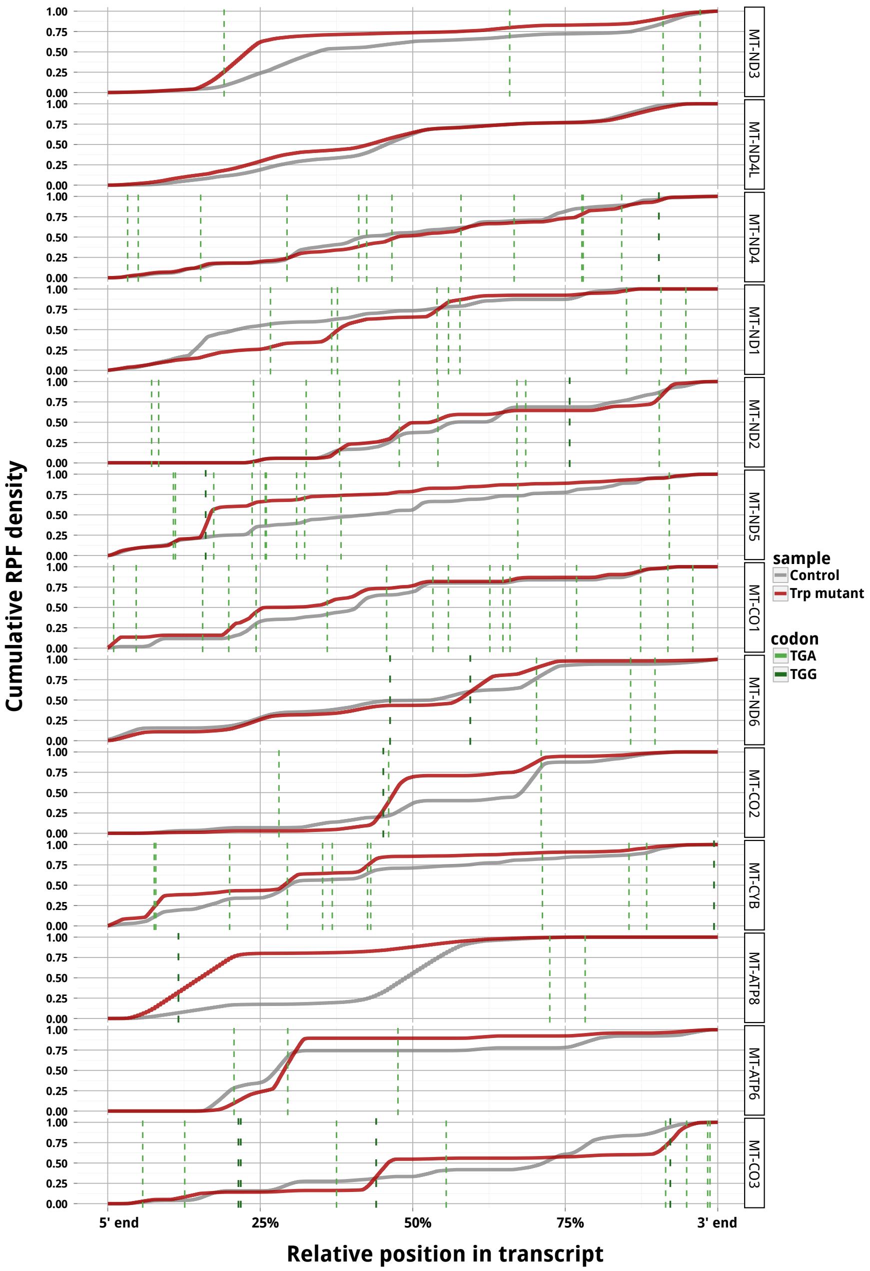


Supplementary Figure S2: Impact of tRNA(Trp)$^{5556G>A}$ on phenotype.
A. Immunofluorescence microscopy of Cybrids with wt and tRNA(Trp)$^{5556G>A}$ mutated mitochondria transfected with a mitochondria-targeted YFP vector. The scales measure 6.45 and 7.81 micrometer respectively. B. Image of wells containing control and tRNA(Trp)$^{5556G>A}$ mutant cells.



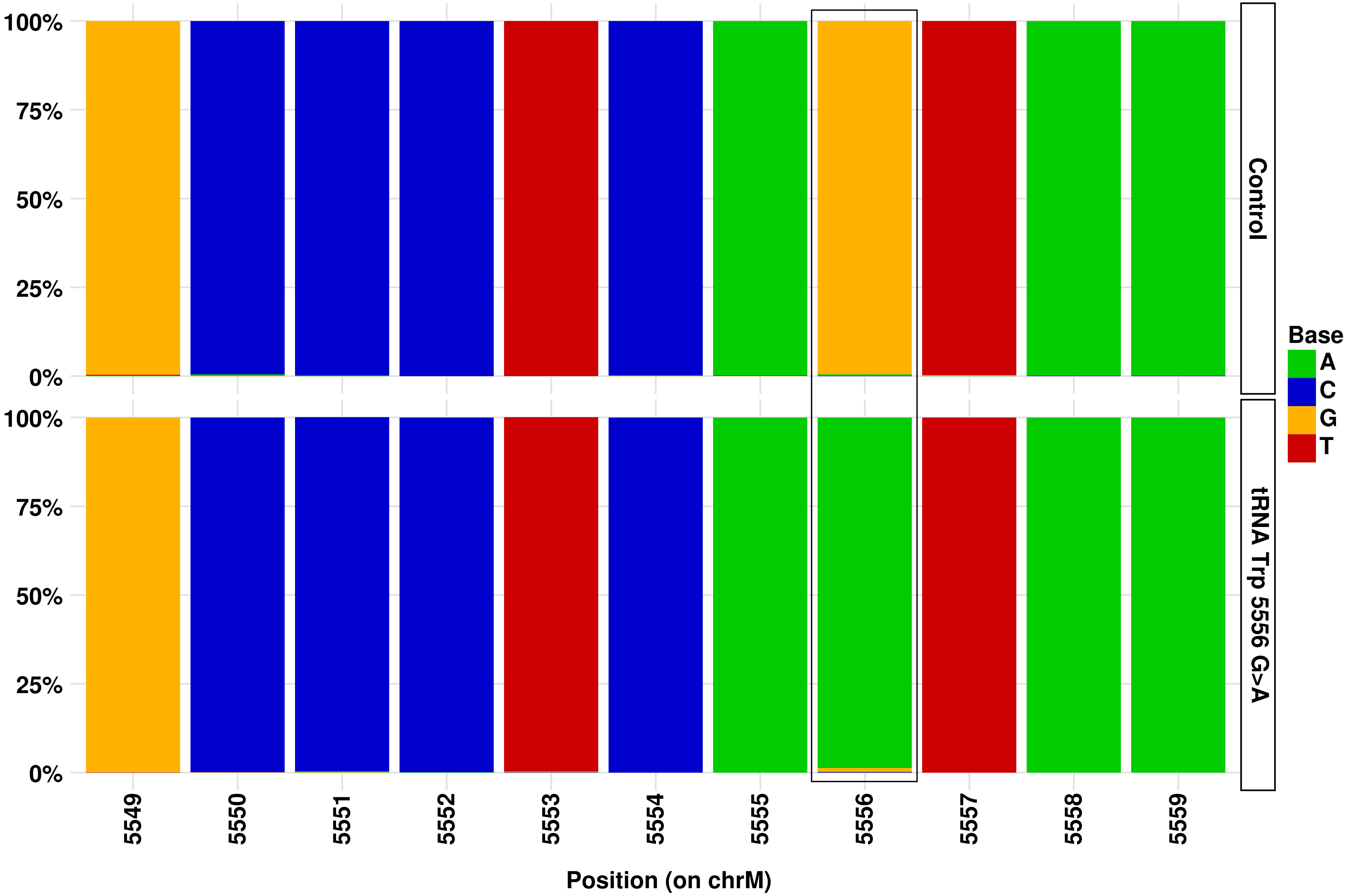
Supplementary Figure S3

Analysis of RP-sequencing data on an independent biological replicate pair of control and tRNA(Trp)$^{5556G>A}$ mutant cybrids. A. Normalized RPF density plot at tryptophan codon regions in the control and tRNA (Trp)$^{5556G>A}$ mutant sample, analogous to Fig. 3E. B. RPF length analysis analogous to Fig. 4D.



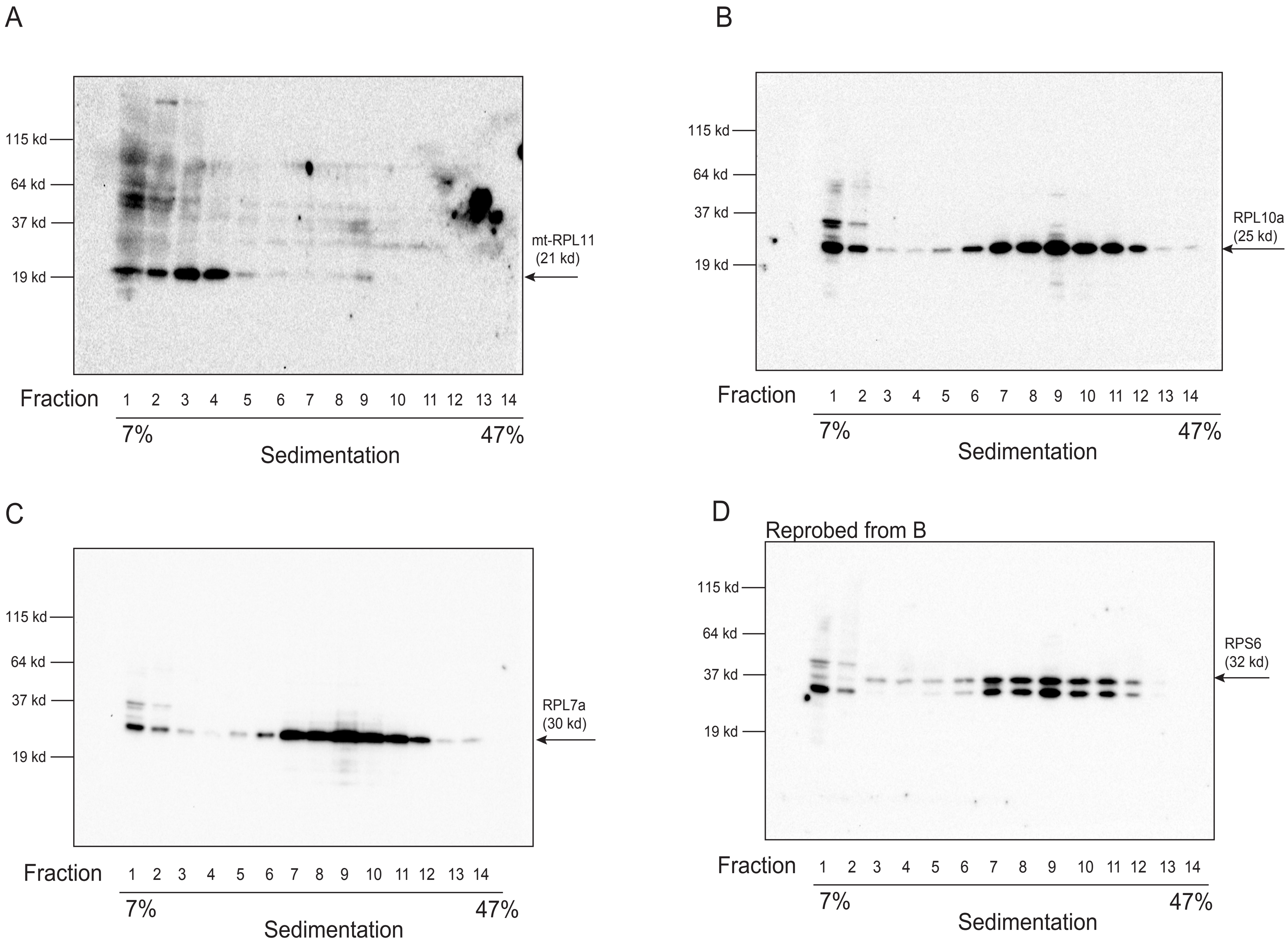
Supplementary Figure S4

Cumulative density plots for all 13 mitochondrial protein-coding genes in the wild-type (gray line) and tRNA(Trp)$^{5556G>A}$ mutant cybrid (red line) samples. Dashed lines indicate the positions of TGA (light green) and TGG (dark green) codons.



Supplementary Figure S5

Genotyping of the control and mutant cells based on reads covering the mitochondrial tRNA-Trp gene mutation.Y-axis shows the fraction of occurrence of each nucleotide for the positions 5549-5559.



Supplementary Figure S6
Full western blots from figure 1B.

Table S1 -- used datasets

	sample	SRR ID	GSM ID
Liu et al.	HEK293_C	SRR619082	(NA)
	HEK293_C	SRR619083	(NA)
Xiao et al.	Control-KD	SRR029285	GSM417716
Shalgi et al.	3T3-Control	SRR649749	GSM794848
	3T3-Control	SRR649752	GSM794854
	3T3-Control	SRR649755	GSM794857

Legend

Ribosome profiling sample

RNA sequencing sample

Supplementary Table S1: Overview of recently published datasets used to create Supplementary Fig. S1.

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