Supporting Information

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Fig. S1. Analysis of mitochondrial ribosomal proteins and ribosome assembly in MRPL12-depleted cells. (A) Steady-state levels of indicated mitochondrial ribosomal proteins. (B) Western blot analysis of 10–30% sucrose gradient density centrifugation fractions. Antibodies used for immunoblotting of input and sucrose gradient fractions are indicated. Fraction numbers are shown, and fractions enriched for small (285) and large (395) ribosomal subunits, and assembled mitochondrial ribosomes (555) are underlined. In both boxes, extracts from MRPL12 knockdown (sh2, 3) and shGFP control HeLa cells were used for analysis.



Fig. 52. Analysis of mtDNA transcript levels and mitochondrial biogenesis in HA-POLRMT HeLa cells depleted of MRPL12 or MRPS10. (*A*) Immunoblotting of MRPL12 protein in cells expressing shMRPL12 (sh1-3) or shGFP (GFP). (*B*) Steady-state levels of indicated mitochondrial transcripts in control (GFP) and MRPL12 knockdown (sh2, 3) cells measured by quantitative RT-PCR. (*C*) Western blot analysis of MRPS10 protein (left box) and quantitative RT-PCR measurement of mtRNA steady-state levels in shGFP (GFP) control cells and cells depleted of MRPS10 (S10sh). (*D*) Quantitative PCR results for mtDNA copy number in shGFP (GFP) and shMRPL12 (sh1-sh3) cells. (*E*) Western blot analysis of indicated peptides in control (GFP) and MRPL12 knockdown (sh2, 3) cells. In all boxes, HeLa cells encoding HA-POLRMT were used for shRNA-mediated knockdown of mitochondrial ribosomal proteins. In (*B*, *C*, and *D*), results from three biological replicates are quantified and shown as mean + / – standard deviation.



Fig. S3. Investigation of mitochondrial transcript stability in vivo in MRPL12 knockdown cell lines. Shown are levels of ND6 and COII mitochondrial transcripts following 0, 4, 8, or 16 h of EtBr treatment. Results from three biological replicates are quantified as mean + / - standard deviation.

Antibody	Source/Reference
ΑΤΡβ	Mitosciences MS503
Coll	Mitosciences MS405
Complex III subunit core 2	Mitosciences MS304
Cox5B	Mitosciences MS410
HSP60	Santa Cruz Biotechnology 1052
HSP70 (GRP75)	Santa Cruz Biotechnology 1058
HA	Abcam 9110
h-mtTFB1	(1)
h-mtTFB2	(1)
h-mtTFA	Aviva Systems Biology ARP31400
MRPL10	Sigma HPA021234
MRPL12	Novus Biologicals H00006182-M01
MRPL37	Sigma HPA025826
MRPL45	Sigma HPA023337
MRPS10	Sigma HPA029134
MRPS18B	ProteinTech Group 16139-1-AP
MRPS27	ProteinTech Group 17280-1-AP
MRPS29	BD Transduction Laboratories 610662
ND6	Sigma-Aldrich AV50255
NDUFS3	Mitosciences MS110
POLRMT	(2)
SDHA	Mitosciences MS204
Tubulin	NeoMarkers MS 581

Table S1. List of antibodies used in the study

1 Cotney J, Wang Z, Shadel GS (2007). Relative abundance of the human mitochondrial transcription system and distinct roles for hmtTFB1 and h-mtTFB2 in mitochondrial biogenesis and gene expression. *Nucleic Acids Res* 35:4042–4054.

2 Seidel-Rogol BL, Shadel GS (2002). Modulation of mitochondrial transcription in response to mtDNA depletion and repletion in HeLa cells. Nucleic Acids Res 30:1929–1934.

Table S2. PCR primers used in the study

Gene	Forward oligo	Reverse oligo
ND1	5' CCCTAAAACCCGCCACATCT 3'	5' GAGCGATGGTGAGAGCTAAGGT 3'
ND6	5' CCCCGAGCAATCTCAATTACA 3'	5' TGATTATGGGCGTTGATTAGTAGTAGTT 3'
Coll	5' CCGACTACGGCGGACTAATC 3'	5' CGCCTGGTTCTAGGAATAATGG 3'
12S	5' CATCAAGCACGCAGCAATGCAG 3'	5' GTTAATCACTGCTGTTTCCCGTG 3'
16S	5' CCAAGCATAATATAGCAAGGAC 3'	5' CTTAGCTTTGGCTCTCCTTG 3'

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