Supplemental Information

The human RNA-binding protein RBFA promotes the maturation of the mitochondrial ribosome.

Agata Rozanska, Ricarda Richter-Dennerlein, Joanna Rorbach, Fei Gao, Richard J. Lewis, Robert N. Lightowlers and Zofia M. Chrzanowska-Lightowlers

Supplemental Methods

Synthetic siRNA used for depletion studies (starting nucleotide)
#1 si-ICT1-ORF A (220) sense CUA GAU CGC UUG ACA AUA U dTdT
#2 si-ICT1-ORF B (419) sense GCC GCU AUC AGU UCC GGA A dTdT
#3 si-ICT1-ORF C (179) sense GGG UCC CGA AUG GUG CAA A dTdT
#4 si-Rbfa-ORF A (481) sense GGA GCU GUA UGA CCU UAA C dTdT
#5 si-Rbfa-ORF B (691) sense GGG AAA UGC AGC UCU AGC U dTdT
#6 si-Rbfa-ORF C (262) sense GAA CUG GCU CAA GAA AUU U dTdT
#7 si-Rbfa-ORF D (862) sense GGC GCU CAA CAA GCA GAU U dTdT
#8 si-Rbfa-ORF E (668) sense CCG AUA GUG UUU GUU CAA G dTdT
#9 si-Rbfa-UTR F (1242) sense GGC AGU UGA UGG AGU UAA A dTdT
#10 si-control siRNA negative control duplex OR-0030-NEG05
siRNAs were stored as 20 µM or 100 µM stocks in RNase free water at -20°C. All siRNAs were custom synthesised by Eurogentec.

Northern analysis
Northern blots were performed as described in (Chrzanowska-Lightowlers et al., 1994). Briefly, RNA was extracted from HEK293T cells with TRIzol (Invitrogen), 3 to 4 µg/ sample electrophoresed through 1.2% (w/v) agarose under denaturing conditions and transferred to GenescreenPlus membrane (NEN duPont) following manufacturer's protocol. Probes were generated using random hexamers on PCR-generated templates corresponding to internal regions of the relevant genes. Detection of signal was performed with a Storm PhosphorImager and analysed with Image-Quant software (Molecular Dynamics, GE Healthcare).

Analysis of apoptosis
The proportion of apoptotic cells was analysed using the APODIRECT kit (BD Biosciences) following the manufacturer's protocol as described in (Dennerlein et al., 2010).

FACS analysis of mitochondrial mass (NAO), reactive oxygen species (MitoSox) and mitochondrial membrane potential (JC1) after mtRbfA depletion
HEK293T cells were treated with si-NT or si-RBFA for 6 days, harvested, resuspended in PBS and treated with the dyes NAO (10 µM), MitoSox Red (5 µM) or JC1 (2 µM), respectively for 15 min at 37°C 5% CO_2. Samples were then washed in PBS and measurements taken at the individual wavelengths using BD FACS CANTO II machine. Results from a minimum of 4 experiments were analysed using BD FACSDIVATM software.

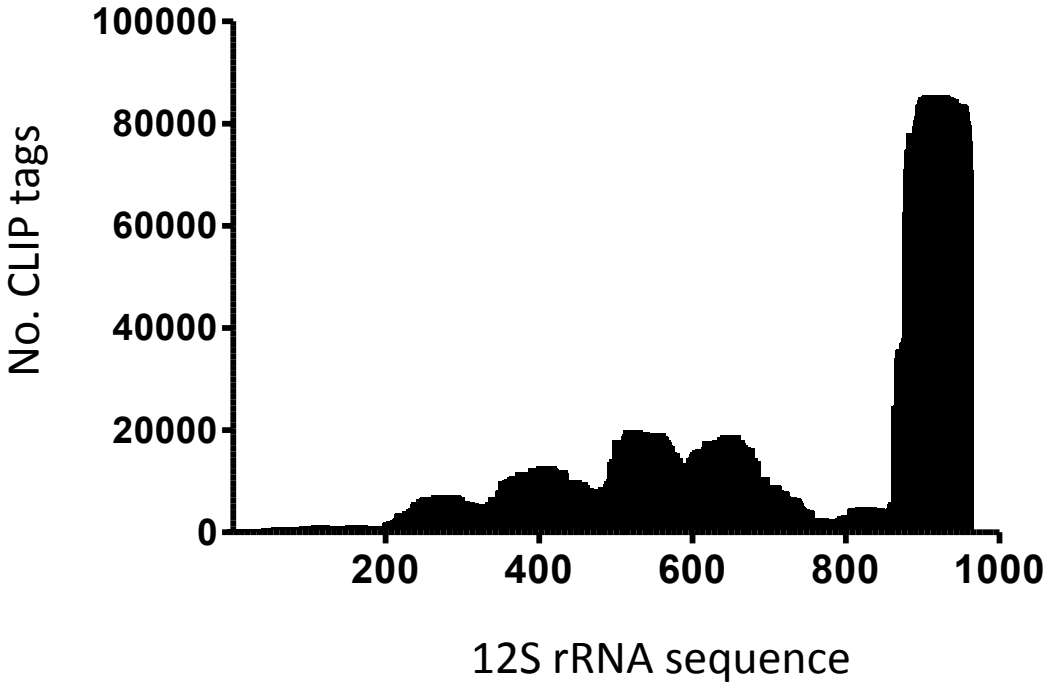

Figure S1. *Location of RBFA-bound CLIP tags within 12S rRNA.*
Following immunoprecipitaton and extraction as described in experimental procedures, cDNA libraries were generated from all RNA CLIP tags and sequenced. All tags identified within the 12S rRNA are shown against the corresponding nucleotides. The 5' nucleotide of 12S rRNA corresponds to position 648 of the revised mtDNA sequence (Andrews RM et al. 1999) and the 3' terminus (nt953) at position 1601.

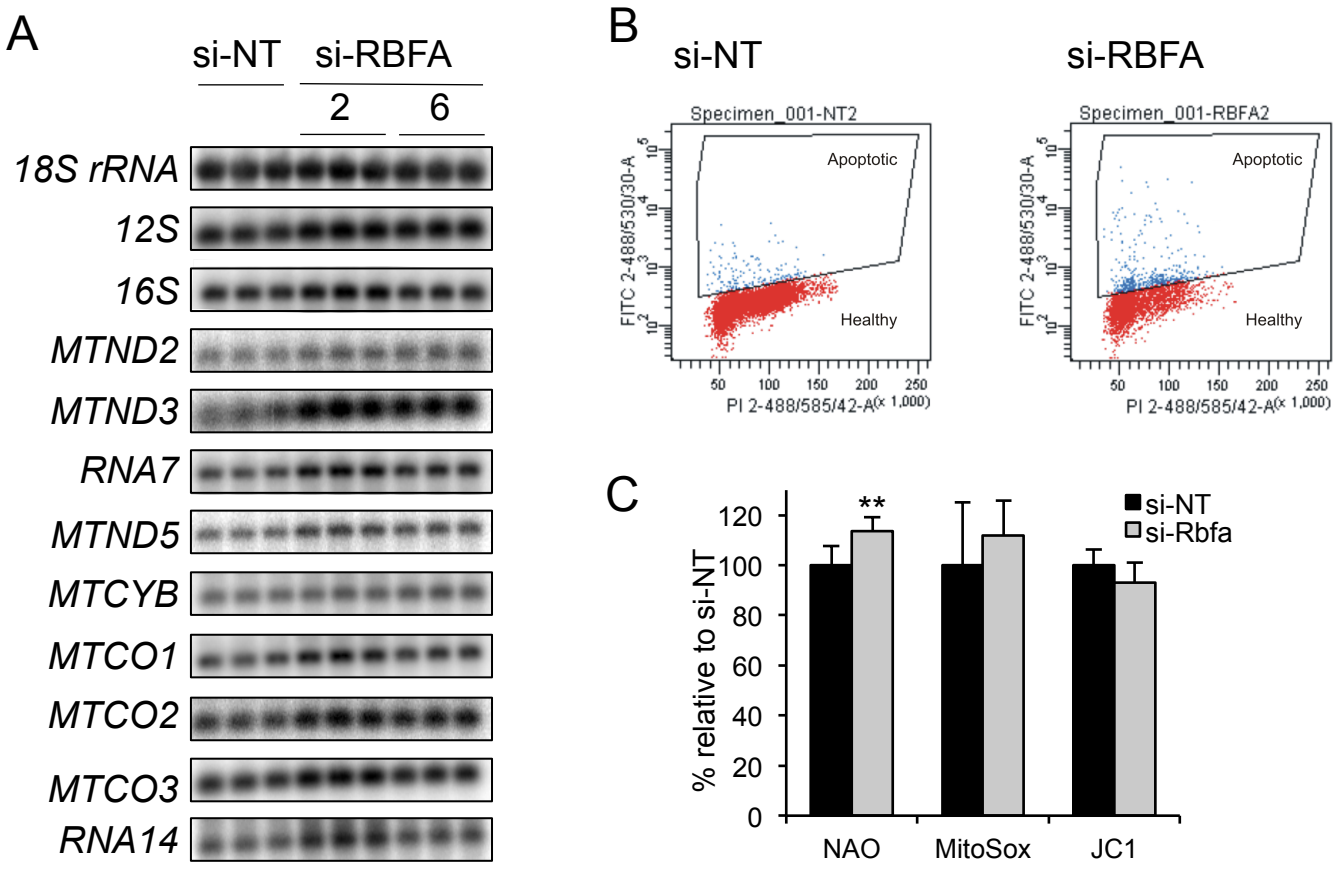

Figure S2. *Short-term depletion of RBFA causes little disruption to mitochondrial homeostasis*.
A. Northern blot analysis of mitochondrial RNA steady state level was performed after 3 days RbfA depletion. RNA (3 µg) was separated through 1.2% denaturing agarose, transferred to GenescreenPlus membrane and probed for the mt-RNA species indicated. The cytosolic 18S rRNA was used as a loading control. The blot is representative of 6 experimental repeats. **B**. HEK293T cells treated for 6 days with si-NT or si-RBFA were assessed for apoptosis. Cells were then measured for DNA fragmentation using APO-DIRECT kit to estimate the proportion of apoptotic cells. FITC-dUTP (measured at 530 nm, y axis) indicated the apoptotic cells and PI (measured at 585 nm, x axis) stained the DNA of the whole population. The results of the flow cytometry (using ~ 5,000 cells) were analysed using BD FACSDivaTM software. The images are representative examples for primary FACS data for si-NT and si-RBFA treated samples. Healthy cells are labelled in red and the apoptotic in blue. The depletion of RBFA resulted in 7.4% (+ 3.3%) apoptotic cells, compared to si-NT with 1.7% (+ 0.5%) (n = 3, p = 0.0167*). **C**. FACS analysis of mitochondrial mass, reactive oxygen species and mitochondrial membrane potential was performed on HEK293T cells after 6 days siRNA treatment with si-NT (black bars) or si-RBFA (grey bars). Cells were analysed for changes in mitochondrial mass (NAO), reactive species (MitoSox Red) or membrane potential (JC1). Measurements were taken at the relevant wavelengths (BD FACS CANTOII machine) and the results analysed using BD FACSDIVATM software. NAO: n = 7, p = 0.0028**; MitoSox: n = 4, p = 0.4433; JC1: n = 4, p =0.2180.

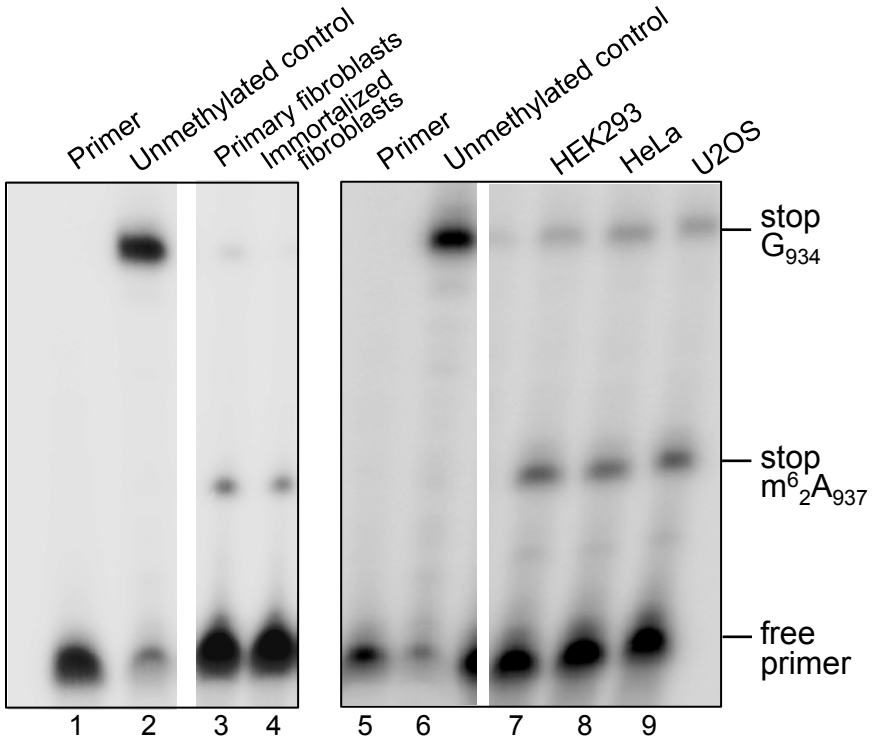

Figure S3. *Dimethylation levels differ modestly between cell types.*
Modification status of helix 45 was determined in different cell lines by primer extension. Dimethylation at position A_{937} (stop $m^6{}_2A$) arrests extension. Lack of modification permits readthrough (stop G_{934}) past the 2 consecutive A_{936}, A_{937} sites, as shown in the unmethylated template control (lanes 2 and 6). Analysis of fibroblasts showed a modest difference between the percentage of dimethylation, with primary cultures displaying 86% (lane 3) compared to 93% (lane 4) in immortalized lines. These levels were similar to the modification levels in HeLa (lane 8, 73%), U2OS (lane 9, 79%) and HEK293 cells (85%, lane 7).