HuR and GRSF1 modulate the nuclear export and mitochondrial localization of IncRNA $RMRP$

Ji Heon Noh$^{1,4}$, Kyoung Mi Kim$^{1,4}$, Kotb Abdelmohsen$^1$, Je-Hyun Yoon$^1$, Amaresh C. Panda$^1$, Rachel Munk$^1$, Jiyoung Kim$^1$, Jessica Curtis$^2$, Christopher A. Moad$^3$, Christina M. Wohler$^3$, Fred E. Indig$^3$, Wilson de Paula$^1$, Dawood B. Dudekula$^1$, Supriyo De$^1$, Yulan Piao$^1$, Xiaoling Yang$^1$, Jennifer L. Martindale$^1$, Rafael de Cabo$^2$, and Myriam Gorospe$^1$*

SUPPLEMENTAL DATA:

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Use of CRISPR/Cas9 system to prevent GRSF1 expression in HEK293 cells

The genome editing CRISPR/Cas9 system was used in HEK293 cells to generate stable knockout cells. Three single guide RNAs (sgRNAs) were designed according to a CRISPR design tool provided at http://crispr.mit.edu, and the guide sequence oligos were cloned into a plasmid containing Cas9 and the sgRNA scaffold (pSpCas9(BB)-2A-Puro (PX459) V2.0) (Addgene, #62988) following a ‘scarless’ cloning strategy described previously (Ran et al. 2013). HEK293 cells were transfected with each CRISPR plasmid using lipofectamine 2000 and maintained in culture for 3 days. After 2 days of puromycin (3 μg/mL) selection, cells were seeded at low density and maintained in culture media with puromycin for isolating single clones (2-3 weeks).

Western blot analysis

Protein lysates, prepared in RIPA buffer (50 mM Tris-HCl [pH 7.2], 150 mM NaCl, 1% NP40, 0.1% SDS, 0.5% DOC, 1 mM PMSF, 25 mM MgCl₂, supplemented with a phosphatase inhibitor cocktail) or modified extraction buffer (MEB) (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM MgCl₂, 1% NP-40, protease inhibitor cocktail, 1 mM PMSF) were separated by electrophoresis in SDS-containing polyacrylamide gels (SDS-PAGE), and transferred onto nitrocellulose membranes (Invitrogen iBlot Stack). Primary antibodies recognizing HuR, CRM1, Lamin B, HSP90, p53, TOMM40, PNPase, GAPDH, β-actin, GST, and GFP were from Santa Cruz Biotechnology. Primary antibodies recognizing ACO2, AUH, Twinkle, IMMT, ATP5A, PCB, OGDH, and TIMM23 were from Abcam. Anti-GRSF1 and anti-Flag were from Sigma, and anti-phospho-AMPKα (Thr172) was from Cell Signaling Technology. HRP-conjugated secondary antibodies were from GE Healthcare.

RIP analysis

For immunoprecipitation (IP) of endogenous RNP complexes (RIP analysis) from whole-cell extracts (Lee et al. 2010), cells were lysed in 20 mM Tris-HCl [pH 7.5], 100 mM KCl, 5 mM MgCl₂ and 0.5% NP-40 for 10 min on ice and centrifuged at 10,000 × g for 15 min at 4°C. The supernatants were incubated with protein A-Sepharose beads (GE Healthcare) coated with antibodies that recognized HuR or with control IgG (Santa Cruz Biotechnology) for 1.5 h at 4°C. After the beads were washed with NT2 buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM MgCl₂ and 0.05% NP-40), the complexes were incubated with 20 units of RNase-free DNase I (15 min at 37°C) and further incubated with 0.1% SDS/0.5 mg/ml Proteinase K (15 min at 55 °C) to remove DNA and proteins, respectively. The RNAs isolated from the IP materials was further assessed by RT-qPCR analysis. To detect mitochondrial interactions of RBPs (GRSF1 and PNPase) with
RNA, isolated mitochondria were lysed in MEB, and IP was performed following the procedure as previously described (Jourdain et al. 2013) with minor modifications.

**Biotin pulldown analysis**

To transcribe biotinylated transcripts, PCR fragments were prepared using forward primers that contained the T7 RNA polymerase promoter sequence, as described (Abdelmohsen et al. 2009); primers are listed in the supplemental Table S1. After purification of the PCR products, biotinylated transcripts were synthesized using MEGAscript™ T7 kit (Life Technologies). Whole-cell lysates (500 μg per sample) were incubated with 1 μg of purified biotinylated transcripts for 1 h at room temperature, followed by isolation of RNP complexes using Streptavidin-coupled Dynabeads (Invitrogen). The proteins present in the pulldown material were detected by Western blot analysis. Biotinylated RMRP was synthesized by T7 RNA polymerase using linearized pcDNA3-RMRP as a template. For affinity pulldown of endogenous human RMRP, biotin-labeled DNA oligomers complementary to human RMRP (0.5-1 μg) were incubated with HEK293 cell lysates (a 15-cm dish was used per reaction) for 2 h and the complexes were isolated with Streptavidin-coupled agarose beads. Antisense and sense oligomer sequences are listed (Table S1). Antisense oligomers ASO-1 and ASO-2 are shown in Fig. S2A.

**Subcellular fractionation**

Cytoplasmic and nuclear fractions were prepared using NE-PER extraction reagents (PIERCE) according to the manufacturer’s instructions. The purity of each fraction was assessed by immunoblotting using antibodies that recognized exclusively nuclear (Lamin B) or cytoplasmic (HSP90) proteins or by RT-qPCR analysis to amplify nuclear RNA (NEAT1). To obtain the nuclear fractions with minimal cytoplasmic contamination, buffer daunry (0.5% NP-40, 140 mM NaCl, 1.5 mM MgCl₂, 10 mM Tris-HCl, pH 8.4) including 10 mM EDTA was used as described previously (Weil et al. 2000). Crude mitochondria were isolated from cultured HEK293 cells. Briefly, cells were scraped, centrifuged, and washed once with ice-cold PBS. After subsequent centrifugation, the cell pellets were resuspended in a hypotonic buffer (20 mM HEPES [pH 7.3], 1.5 mM MgCl₂, 10 mM KCl), vortexed at low speed for 5 sec, and incubated on ice for 2 min. CHAPS buffer (20 mM HEPES [pH 7.3], 8% (w/v) CHAPS) was added and vortexed vigorously 5 times while incubating on ice for 5 min. Hypertonic buffer (20 mM HEPES [pH 7.3], 420 mM mannitol, 140 mM sucrose, 2 mM EGTA) was then added with equal volume of hypotonic buffer, and gently mixed by inverting the tubes several times. After centrifugation at 2,000 × g, 4°C for 10 min, supernatants were collected and centrifuged again at 2,000 × g for 10 min to remove nuclei. From the supernatant, mitochondria were pelleted down at 12,000 × g for 15 min. The crude mitochondria were washed with an isotonic buffer (20 mM HEPES [pH 7.3], 210 mM mannitol, 70 mM sucrose) and stored on ice until used.
For sub-mitochondrial fractionation, crude mitochondria were first incubated with hypotonic buffer on ice for 30 min, whereupon the same volume of hypertonic buffer was added to stop the swelling. After centrifugation, samples were treated with RNase A (2 mg/mL) (Life technologies) to remove nuclear and cytosolic RNAs, as previously described (Mercer et al. 2011). RNase A activity was neutralized by addition of proteinase K (PK; 50 μg/mL). RNaseOUT (Invitrogen) was used to inhibit RNase A activity before RNA isolation. The mitoplast pellet was obtained by centrifugation at 12,000 × g and treated with RNase A again as described above. Purified mitochondria or matrix were incubated in mitochondria solubilizing buffer at 65 ºC for 5 min. RNA was isolated using TRIzol reagent and treated with RNase-free DNase I (Roche) for 1 h at 37 ºC. RNA was then extracted by acidic phenol (Ambion).

**RNA import assay**

*In vitro* RNA import assay was performed as previously described (Wang et al. 2010; Wang et al. 2015) with minor modifications. Mitochondria were isolated from mouse liver tissue by using percoll (GE Healthcare) gradients. Briefly, the liver was excised and washed in PBS to remove blood, placed in 20-30 mL of homogenization buffer (20 mM HEPES [pH 7.3], 210 mM mannitol, 70 mM sucrose) supplemented with 2 mM EGTA, and chopped into pieces. The chopped liver was homogenized using a Potter-Elvehjem homogenizer with the Teflon pestle rotating at ~1,600 rpm at 4 ºC. The homogenate was transferred into a fresh tube and centrifuged at 2,000 × g for 5 min; that supernatant was transferred into another tube and centrifuged at 13,000 × g for 15 min. The pellet was resuspended in homogenization buffer and centrifuged again at 2,000 × g for 5 min; the supernatant was transferred to a fresh tube and centrifuged at 13,000 × g for 15 min to get a crude mitochondrial pellet. Three layers of Percoll gradients (40, 23, and 15% from bottom to top) were prepared; the crude mitochondria were resuspended in 15% Percoll and laid on the top of Percoll gradients, and centrifuged at 30,700 × g for 5 min. The fraction with the intact mitochondria was removed carefully, washed in excess homogenization buffer, and centrifuged at 13,000 × g several times to remove the remaining Percoll. Crude mitochondria from HEK293 cells (2 × 10⁹ cells per sample) were isolated as described previously (Clayton and Shadel, 2014) and purified by Percoll gradient as explained above.

The purified mitochondria (100-200 μg) were incubated with *in vitro*-transcribed RNA (labeled with α-³²P-CTP) in 200 μL of import assay buffer (0.2 M mannitol, 75 mM sucrose, 25 mM KCl, 5 mM MgCl₂, 5 mM ATP, 2 mM DTT, 15 mM succinate, 10 mM HEPES [pH 7.4]) at 30°C for 10 min. After import, mitochondria preparations were incubated with RNase A (50 μg/mL) at 30°C for 20 min. The mixture was transferred to a new tube and incubated at 30°C for an additional 10 min. Mitochondria were pelleted down at 12,000 × g for 5 min at RT and incubated at 65°C for 5 min in 100 μL of mitochondria solubilizing buffer (1% SDS, 100 mM NaCl, 10 mM Tris-HCl, pH 7.4) which was preheated at 95°C with 25 μg/mL of
proteinase K. RNA was isolated from the solubilized mitochondria using TRIzol. The imported RNA was separated by 6% urea-PAGE (Invitrogen) and visualized using a PhosphorImager.

Biotinylation and purification of 4-SU labeled RNA
Nascent RNA was metabolically labeled by addition of 4-thiouridine (4-SU, Sigma, 100 μM final concentration) to the culture medium for 12 h. Total RNA was extracted using TRIzol, and used in biotinylation reactions as previously described with minor modifications (Rabani et al. 2011). 4-SU-labeled RNA (15 μg) was then biotinylated in a labeling reaction that included 30 μL of Biotin-HPDP (Pierce, Cat #21341) dissolved in dimethylformamide (DMF) at a concentration of 1 mg/mL and 20 μL of 10× biotinylation buffer (100 mM Tris-HCl, pH 7.4, 10 mM EDTA) at room temperature for 1.5 h with rotation. RNA was purified using chloroform/isoamylalcohol (24:1) extraction. RNA was precipitated at 20,000 × g for 20 min with a 1/10 volume of 5 M NaCl and an equal volume of isopropanol. Pellets were washed once with an equal volume of 75% ethanol and centrifuged again at 20,000 × g for 10 min. The RNA pellet was resuspended in 100 μL of RNase-free water with 0.1 mM EDTA. The labeled RNA was heated at 65°C for 5 min, chilled on ice quickly, and incubated with 100 μL of streptavidin beads (Miltenyi Biotec) with rotation for 15 min. Beads were applied to the equilibrated μMacs columns (Miltenyi Biotec) and washed with 65°C or room temperature washing buffer (100 mM Tris-HCl, pH 7.5, 10 mM EDTA, 1 M NaCl, and 0.1% Tween20) three times each. Biotinylated RNA was then eluted directly into 700 μL Buffer RLT (Qiagen) using 100 μL of freshly prepared 100 mM dithiothreitol (DTT) twice with a 3 min interval. RNA was recovered using RNeasy MinElute spin columns (Qiagen) following the manufacturer’s instructions and studied by RT-qPCR analysis.

Confocal microscopy
Cells were imaged with an instant structured illumination microscope (iSIM) built in the lab of Dr. Hari Shroff, NIBIB (York et al. 2013). Z-sections (100 nm) were obtained with an Olympus Plan Apo 60x/NA 1.45 TIRFM objective and deconvolved with iSIM deconvolution software. Images were opened with Image J (FUJI64) and brightness/contrast was adjusted with the auto B/C function. Several sections (average 0.6 μm) were merged into a maximum intensity projection, which were colored and saved as .tif files, or binary masks were created after background reduction for quantitation in Image J. Statistics were performed on six sections of each sample group, with Student’s unpaired t test used for determining significance.

Mitotracker staining and immunofluorescence
Cells were grown in MatTek dishes with phenol-free DMEM (Invitrogen). For Mitotracker staining, 100-200 nM of Mitotracker Green FM or CMXROS-Red (Invitrogen) were added to the dishes and cells were
incubated at 37°C for 20 min. Cells were imaged in a heated stage supplied with humidified air supplemented with 5% CO\textsubscript{2}, mounted on a Zeiss LSM 710 confocal microscope under a 40×/NA 1.3 objective. For indirect immunofluorescence, HEK293 cells on MatTek dishes were processed as described previously (Partridge et al. 2003). Primary antibodies used were anti-Complex IV mAb (1D6E1A8, Invitrogen), rabbit anti-VDAC1 (Abcam, ab135585) and anti-Mitofilin (Abcam, ab110329), all used at 1:100 dilution. Secondary antibodies were goat anti-mouse IgG or goat anti-rabbit IgG, highly cross-adsorbed, conjugated to Alexa Fluor 488 or 568 (Invitrogen).

**RNA secondary structure**

The secondary structures of human RMRP (Pluk et al. 1999) and predicted in silico structures of deletion mutant RMRPs were drawn by using VARNA software (Darty et al. 2009).

**Transmission Electron Microscopy**

Samples were fixed in 2.5% glutaraldehyde, 3 mM MgCl\textsubscript{2}, in 0.1 M sodium cacodylate buffer [pH 7.2] overnight at 4 °C. After rinsing with the buffer, samples were postfixed in 1% osmium tetroxide in buffer (1 h) on ice in the dark followed by rinsing with 0.1 M sodium cacodylate buffer. Samples were left at 4 °C for 16 h in buffer, rinsed with 0.1 M maleate buffer, and stained with 2% uranyl acetate (0.22-µm filtered) for 1 h in 0.1 M maleate, dehydrated in a graded series of ethanol, propylene oxide and embedded in Eponate 12 (Ted Pella) resin. Samples were polymerized at 60 °C overnight. Thin sections, 60- to 90-nm thick, were cut with a diamond knife on the Reichert-Jung Ultracut E ultramicrotome and picked up with naked 200 mesh copper grids. Grids were stained with 2% uranyl acetate (aq.) followed by lead citrate, and observed with a Philips CM120 at 80 kV. Images were captured with an AMT XR80 high-resolution (16-bit) 8 M pixel camera.

**Detection of free 5’ ends of the newly synthesized mtDNA (O\textsubscript{H})**

Mitochondrial DNA was prepared by using a mtDNA isolation kit (Abcam, ab65321), and ligation-mediated PCR (LMPCR) was performed as described (Kang et al. 1997) with some modifications. Briefly, a unidirectional linker was prepared by annealing 6 nmol of LMPR1 (5’-GCGGTGACCCGGAGATCTGTATTC-3’) and 2 nmol of LMPR2 (5’-GAATACAGATC-3’) in 40 µL of annealing buffer (10 mM Tris-HCl [pH 7.5], 50 mM NaCl, 1 mM EDTA) by heating at 95°C in a water-filled heat block for 5 min followed by cooling to RT for 40-60 min. The annealed linker was stored on ice. Primer 1 (5’-ACATCACGATGGAGATCT-3’) was extended in 20 µL of the first-strand synthesis reaction mixture consisting of 2 µL of 10× Vent pol buffer, 2 µL of 0.25 mM dNTP, 6 µL of 0.1 pmol/µL of primer 1, 1 µL of Vent pol (NEB, M0254), and 0.4 µg of DNA. DNA was then denatured at 95 °C for 5 min,
and the primer was annealed at 55 °C for 30 min, after which polymerization was performed at 75 °C for 10 min. For ligation of the linker, 50 µL of ice-cold ligation mixture (45 mM Tris-HCl [pH7.5], 8 mM MgCl₂, 20 mM DTT, 5 mg/mL BSA, 1 mM ATP, 2 µL of double-stranded linker (100 pmol), 3 Weiss of T4 DNA ligase) including the synthesized DNA above was prepared, and the ligation reaction was performed at 16 °C for 20 h. DNA was precipitated by addition of 1/10 vol of 3M NaOAc and 2.5 vol of EtOH, and incubation at -20°C. After washing with 75% EtOH once, the DNA pellet was solubilized in 60 µL of water, and then 40 µL of PCR amplification mix [10 µL of 10× Vent polymerase buffer, 10 µL of LMPR1 (1 pmol/µL stock), 10 µL of ligation PCR primer 2 (5’-GAGCTCTCCATGCATTTGGT-3’) (2 pmol/µL stock), 4 µL of 5 mM dNTP mix, 1.5 µL of Vent polymerase (NEB), 4.5 µL of water] was added to the DNA solution. DNA was denatured at 95 °C for 5 min, and then the reaction continued for 25 PCR cycles of 95 °C for 1 min, 60 °C for 2 min, and 75 °C for 3 min plus an extra 5 sec for each cycle. Final extension was allowed to proceed at 75 °C for 10 min.

The sequencing library was prepared using Illumina TruSeq ChIP Sample Prep according to the manufacturer’s protocol (Illumina, San Diego, CA) with slight modifications. In short, the ends of the fragments were repaired and As (adenosines) were added to the 3’ end. Adapters were ligated to the DNA fragments and a 18-cycle PCR amplification reaction was performed after size selection (100-400 bases) on a 4.5% agarose gel followed by cluster generation and paired-end (PE) sequencing for 2×10⁶ bases with Illumina HiSeq 2500 sequencer (Rapid-Run mode). The resulting BCL files were converted to FASTQ files for analysis.

For analysis, linkers LMPR1 and LMPR2 as well as Illumina adapters were carefully removed using the cutadapt program; low-quality bases were removed using a Q20 cut-off. After cleaning, sequences were aligned using Bowtie2 using the high sensitivity option against the hg19 mitochondrial sequence. The aligned locations and strand information were exported into MS Excel file. The number of reads from both + and - strands at each nucleotide position (the first nucleotide following the linker sequence) was normalized by the total number of reads counted from each sequencing reaction. For analysis of the replication start sites, the region spanning 145 to 225 nucleotide position covering the most upstream site among the 5’ ends previously reported [nucleotide position 250 (Kang et al. 1997)] was selected.
SUPPLEMENTAL REFERENCES

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Additional information on the mitochondrial transcriptome. (A) Mitochondrial transcripts encoded by nuclear DNA (total 555 transcripts) identified previously (Mercer et al. 2011). Protein-coding, non-coding, and pseudogene RNAs are grouped; FPKM, fragments per kilobase of transcript per million mapped reads. (B) Transcripts (476 total) enriched in mitoplasts purified from WI-38 human diploid fibroblasts were identified by RNA-Seq analysis (GSE73458).

Figure S2. Predicted RMRP secondary structure and RMRP-interacting RBPs. (A) Secondary structure of RMRP drawn by using the VARNA software. The cleavage site for RNA processing in mitochondria (processing region), the complementary sequences targeted by antisense oligomers (ASO-1 and ASO-2), and the predicted sequence pair forming an internal stem (internal stem) are indicated. (B) Partial list of RNA-binding proteins (RBPs) interacting with RMRP identified by biotin pulldown and mass spectrometry; cut-off for inclusion was set at 15 peptides per protein minimum. (C) Venn diagram representing the intersections among three groups of proteins: MitoCarta (mitochondria-localized proteins), RBP DB (RNA-binding proteins in database), and RMRP targets (RMRP-interacting proteins). (D) RIP analysis of the interaction of HuR with a known target transcript, VHL mRNA (Abdelmohsen et al. 2009), in HeLa cells.

Figure S3. Further characterization of HuR binding to RMRP. (A) The levels of a nuclear transcript, the lncRNA NEAT1, were measured to monitor the specificity of HuR silencing upon nuclear export of RMRP. (B) Forty-eight hours after transfecting HuR or Ctrl siRNAs, HEK293 cells were either left intact (whole-cell lysate, WCL) or were fractionated into cytosolic (Cytosol) and mitochondrial (Mitoch.) components (Materials and Methods). The levels of HuR, GRSF1, and p53 were assessed by Western blot analysis. (C) Regions of HuR interaction with RMRP, as determined by HuR PAR-CLIP analysis (Kishore et al. 2011). (D) In silico-predicted structures of the RMRP fragments shown in Fig. 3D.

Figure S4. Further characterization of GRSF1 binding to RMRP. (A) Western blot analysis of outer mitochondria membrane-resident protein TOMM40 and matrix-resident proteins Aconitase 2 (Aco2) and GRSF1 in mitoplasts that were either left untreated or treated with Proteinase K (PK) and RNase A. After osmotic shock, the supernatant (S) and mitoplast pellet (P) were prepared for analysis (left). Relative levels of four mitochondria DNA-encoded RNAs (mtRNAs) and two negative controls (nuclear RNAs NEAT1 and MALAT1) in matrix samples prepared from HEK293 cells (right). (B) A construct expressing chimeric protein YFP-MS2 (Materials and Methods), bearing a strong nuclear localization signal (NLS), was
overexpressed in HEK293 cells to monitor the extent of nuclear contamination in the matrix preparation. GFP and the mitochondrial matrix protein Twinkle were detected by Western blot analysis. (C) The levels of mt-RNR1, normalized to GAPDH mRNA (left), and the levels of mt-ATP6 and mt-CYB mRNAs normalized to noncoding RNA mt-RNR1 (right), were compared between control and GRSF1-silenced HEK293 cells. (D-F) HEK293 cells were transfected with control siRNA, GRSF1 siRNA or IMMT (mitofilin) siRNA; 48 h later, mitochondria were isolated and fractionated into the mitoplast pellet and the supernatant as described above. GRSF1, IMMT, and PNPase were assessed by Western blot analysis (D). The levels of 18S rRNA and 5S rRNA were assessed by RT-qPCR analysis (E), and mt-CYB and mt-RNR1 RNAs were detected by RT-qPCR analysis in IMS and matrix (F). (G) PCR templates for in vitro transcription of the full-length RMRP, which also includes the specific nucleotide stretch from the vector backbone (RMRP FL-Vec, lane 4) and a fragment which has no GRSF1 binding motif (RMRP f2/f3-Vec, lane 6) were amplified (left). After in vitro transcription, biotin-labeled RNAs were subjected to electrophoresis through 6% urea-polyacrylamide gels to assess the quality of the RNA (right). (H) Full-length, radiolabeled RMRP was imported into mitochondria isolated from mouse liver. After incubation, imported RNA was isolated, subjected to 6% UREA PAGE, and detected by PhosphoImager. (I) Schematic depiction of a full-length RMRP and three fragments synthesized in vitro (f1, f2, and f3 as shown in Fig. 3D, top). GRSF1 interaction with the different segments of RMRP was assessed by Western blot analysis in each of the biotin pulldown reactions (bottom). Input, 10, 5 μg of lysates; Beads, pulldown reactions without biotinylated RNA.

Figure S5. Submitochondrial localization of GRSF1. (A) Basal OCR in HeLa cells after silencing (left) and after overexpressing (right) GRSF1 compared with the respective control populations. (B) Genomic DNA was extracted from control and GRSF1-silenced HEK293 cells, and qPCR analysis was used to determine the relative number of mitochondrial DNA by estimating the cycle numbers of mitochondrial genes ND1 and ND2, and the nuclear β-globin gene, HBB. (C) Seventy-two h after silencing RMRP using AS-LNA, mitochondria were isolated and the levels of mitochondria-encoded mt-ATP6, mt-CO1, and mt-CYB mRNAs were quantified by RT-qPCR analysis. (D) Representative bioanalyzer electropherograms for amplified DNA fragments for size selection (100-400 bases). (E) RMRP sequences from four different species were aligned by using LocARNA algorithm (Will et al. 2012) (http://rna.informatik.uni-freiburg.de/LocARNA/Input.jsp) to illustrate the partial conservation in RMRP among these species. The blue square indicates the reported GRSF1-interaction motif A(G)₄A.
Figure S6. Integrated model of the subcellular transport and localization of RMRP by RBPs HuR and GRSF1. HuR is involved in exporting RMRP from the nucleus to the cytosol via the CRM1 export factor. Once RMRP is internalized into the mitochondria matrix, GRSF1 binds RMRP, retains it in the matrix, facilitates RMRP promotion of mitochondrial respiration, and enhances RMRP-mediated generation of primers for mtDNA replication. See text for details.
### Nuclear DNA-encoded ncRNAs

- **Antisense:** 8
- **LncRNA:** 9
- **rRNA:** 75
- **Sense-intronic:** 1
- **snRNA:** 21

**Total:** 555 transcripts

(Cut-off = FPKM > 1.0) (Mercer et al., 2011)

| Transcript ID | Chr | Str. | Start | End | Transcript Name | Transcript Biotype | HG19 Description |
|---------------|-----|------|-------|-----|-----------------|-------------------|-------------------|
| ENST00000416718 | chr1 | +    | 569755 | 570302 | RP5-857K21.11-001 | pseudogene | |
| ENST00000387059 | chr1 | +    | 28975111 | 28975245 | RNU11-201 | snRNA | RNA, U11 small nuclear |
| ENST00000386451 | chr1 | -    | 228763894 | 228764013 | RNU55-201 | rRNA | RNA, 55 ribosomal 9 |
| ENST000003864991 | chr10 | -    | 327994 | 328065 | RNU9P298-201 | RNA | RNA, 55 ribosomal pseudogene 298 |
| ENST00000602946 | chr12 | -    | 6649599 | 6647536 | RPS-94D5.9-001 | antisense | |
| ENST000003655568 | chr12 | -    | 120729565 | 120729706 | RNU4-A2-201 | snRNA | RNA, U4 small nuclear 2 |
| ENST00000355637 | chr14 | -    | 5033559 | 5035359 | RN75L2-001 | antisense | RNA, 7SL cytoplasmic 1 |
| ENST00000578231 | chr14 | -    | 50320345 | 50320362 | RN75L3-201 | misc_RNA | RNA, 7SL, cytoplasmic 3 |
| ENST00000480232 | chr14 | -    | 50325270 | 50325657 | RN75L2-201 | misc_RNA | RNA, 7SL, cytoplasmic 2 |
| ENST000003813825 | chr19 | +    | 49468557 | 49470139 | FTL-001 | protein_coding | ferritin, light polypeptide |
| ENST00000582110 | chr21 | +    | 35677429 | 35677943 | AP000318.1-201 | miRNA | |
| ENST00000584058 | chr3 | +    | 15780021 | 15780315 | RN75L4-201 | misc_RNA | RNA, 7SL, cytoplasmic 4, pseudogene |
| ENST00000365124 | chr3 | +    | 150905885 | 150906010 | RNU55P145-201 | rRNA | RNA, 55 ribosomal pseudogene 145 |
| ENST00000368445 | chr3 | +    | 134502277 | 134502397 | RNU55P141-201 | rRNA | RNA, 55 ribosomal pseudogene 141 |
| ENST00000364932 | chr3 | +    | 179876573 | 179876765 | RNU55P149-201 | rRNA | RNA, 55 ribosomal pseudogene 149 |
| ENST00000465508 | chr9 | +    | 79186730 | 79186787 | AL161626.1-201 | miRNA | |
| ENST00000602291 | chr9 | +    | 35657747 | 35658025 | RP7-859F3.10-001 | lincRNA | RMRP |
| ENST00000387347 | chrM | +    | 1670 | 3229 | MT-RNR2-201 | Mt_tRNA | mitochondrially encoded 16S RNA |
| ENST000003631390 | chrM | +    | 3306 | 4262 | MT-ND1-201 | protein_coding | mitochondrially encoded NADH dehydrogenase 1 |
| ENST000003812789 | chrM | +    | 14746 | 15887 | MT-CYB-201 | protein_coding | mitochondrially encoded cytochrome b |
| ENST00000387405 | chrM | -    | 5760 | 5826 | MT-TC-201 | Mt_tRNA | mitochondrially encoded tRNA cysteine |
| ENST00000361681 | chrM | -    | 11418 | 14673 | MT-ND6-201 | protein_coding | mitochondrially encoded NADH dehydrogenase 6 |
| ENST00000419932 | chrX | +    | 108297360 | 108297792 | RNU285-001 | pseudogene | RNA, 285 ribosomal 5 |
| ENST00000518596 | chrY | +    | 10037763 | 10037915 | RNU85-85F6-201 | RNA | RNA, 5.85 ribosomal pseudogene 8 |
### Figure S2

#### A

![ASO-2 processing region with PNPase](image)

#### B

| # | RBPs identified in the upper band (# peptides >=15) | Gene Symbol | Accession Number | # Peptides |
|---|--------------------------------------------------|-------------|-----------------|-----------|
| 1 | heterogeneous nuclear ribonucleoprotein H        | HNRPH1      | gi|5031753       | 68        |
| 2 | heterogeneous nuclear ribonucleoprotein F        | HNRPF       | gi|4835760      | 64        |
| 3 | far upstream element-binding protein 3           | FUBP3       | gi|15065892     | 46        |
| 4 | heterogeneous nuclear ribonucleoprotein H2       | HNRPH2      | gi|74090687     | 42        |
| 5 | G-rich sequence factor 1 isoform 1               | GRSF1       | gi|14919321     | 32        |
| 6 | tumor susceptibility gene 101 protein           | TSG101      | gi|5454140      | 28        |
| 7 | plasminogen activator inhibitor 1 RNA-binding protein isoform 1 | SERBP1 | gi|55340879 | 24 |
| 8 | tubulin beta chain                               | TUBB        | gi|29765785     | 24        |
| 9 | TNF receptor-associated factor 2                 | TRAP2       | gi|22027612     | 23        |
| 10 | RNA-splicing ligase RtcB homolog                 | C22orf2B    | gi|7057015      | 22        |
| 11 | RNA-binding motif protein, X chromosome isoform 1 | RBMX       | gi|56896405     | 19        |
| 12 | heterogeneous nuclear ribonucleoprotein M isoform a | HNRPM      | gi|14141152(+1) | 18        |
| 13 | insulin-like growth factor 2 mRNA-binding protein 1 isoform 1 | IGF2BP1 | gi|62372072 | 17 |

#### C

![AKAP1 (A kinase (PRKA) anchor protein 1), GRSF1 (G-rich RNA sequence binding factor 1), PNPT1 (polyribonucleotide nucleotidyltransferase 1)](image)

#### D

| # | RBPs identified in the lower band (# peptides >=15) | Gene Symbol | Accession Number | # Peptides |
|---|--------------------------------------------------|-------------|-----------------|-----------|
| 1 | heterogeneous nuclear ribonucleoproteins A2/B1 isoform B1 | HNRNA2B1 | gi|14043072     | 124       |
| 2 | heterogeneous nuclear ribonucleoprotein A1 isoform b | HNRNA1      | gi|14043070(+)   | 100       |
| 3 | heterogeneous nuclear ribonucleoprotein H3 isoform a | HNRPH3      | gi|14114167(+)   | 62        |
| 4 | serine protease HTRA2, mitochondrial isoform 1 preprotein | HTRA2 | gi|7091477      | 40        |
| 5 | eukaryotic translation initiation factor 2 subunit 1 | EIF2S1     | gi|4785566      | 40        |
| 6 | eukaryotic translation initiation factor 3 subunit I | EIF3I      | gi|4503513      | 38        |
| 7 | RNA-binding protein Musashi homolog 2 isoform a | MUS2        | gi|20373175(+)  | 28        |
| 8 | caspin ion I isoform alpha isoform 2 | CASKIA1     | gi|89303572      | 27        |
| 9 | eukaryotic translation initiation factor 3 subunit M | EIF3M      | gi|23397429     | 28        |
| 10 | heterogeneous nuclear ribonucleoprotein A0 | HNRNA0      | gi|6803036      | 24        |
| 11 | transcriptional activator protein P53-beta | PURB       | gi|15147219      | 23        |
| 12 | heterogeneous nuclear ribonucleoprotein A3 | HNRNA3      | gi|34740329      | 22        |
| 13 | RNA 2-O-methyltransferase ribozyme | FSL        | gi|2056468      | 21        |
| 14 | RNA-binding protein 4 isoform 1 | RBM4       | gi|93277122      | 21        |
| 15 | heterogeneous nuclear ribonucleoproteins C1/C2 isoform a | HNRNC1      | gi|11716997(+)  | 19        |
| 16 | heterogeneous nuclear ribonucleoprotein A/B isoform a | HNRNA1      | gi|55666619(+1) | 19        |
| 17 | mitochondrial import inner membrane translocase subunit TIM50 | TIMM50 | gi|48520559 | 19 |
| 18 | ELAV-like protein 1 | ELAVL1 | gi|38201714 | 18 |
| 19 | leucine-rich repeat-containing protein 56 | LRR59 | gi|40525624 | 16 |
| 20 | eukaryotic translation initiation factor 3 subunit J | EIF3J | gi|30281438 | 16 |
| 21 | replication factor C subunit 4 | RFC4 | gi|459491 | 16 |
| 22 | RNA/np complex 1 interacting phosphatase | DUSP11 | gi|205627447 | 16 |
| 23 | heterogeneous nuclear ribonucleoprotein F | HNRNPF | gi|4820760 | 15 |
| 24 | replication factor C subunit 6 isoform 4 | RFC6 | gi|32194786(+1) | 15 |
| 25 | serine-threonine kinase receptor-associated protein | STRAP | gi|148727341 | 16 |
Supernatant
Mitoplast
PNPase
GRSF1
IMMT
siRNA:

C

D

E

F

H

I

A

B

PCR products on Agarose gel

1 μl of RNA
6% UREA PAGE
Recruitment to replication sites?

Oxygen consumption

OX-PHOS Complexes

RMRP

GRSF1

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Noh_FigS6

HuR

RMRP

HuR

CRM1

MATRIX

CYTOPLASM

NUCLEUS

MITOCHONDRIA