

Human mtRF1 terminates COX1 translation and its ablation induces mitochondrial ribosome-associated quality control

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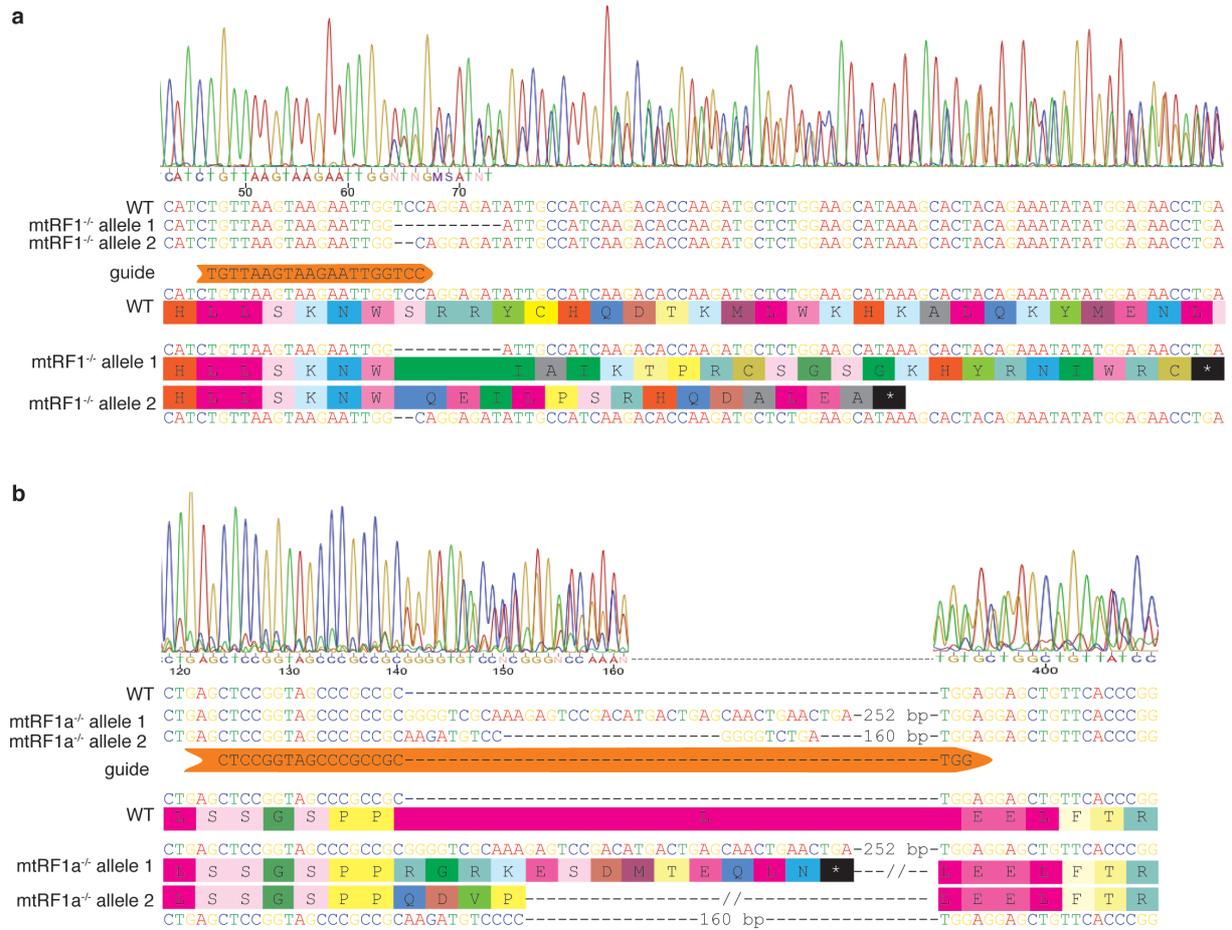
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Supplementary information

Supplementary Figures

	α5-helix →	PxT	GGQ
T.thermophilus RF1	...VEIRAG-- TGGEEAALF ARDLNFNMYLRF...ESGGHRVQRV PVT ---ETQGRHTSTA...IRIDVMRASGP GGQ GVNNTDS...		
E.coli RF1	...LEVRAG-- TGGDEAALF AGRLFRMYSRY...ESGGHRVQRV PAT ---ESQGRHTSAC...LRIDTFRSSG GGQ HVNTTDS...		
mtRF1a	...LEVTAG-- VGGQEAAMLF TSEIFDMYQQY...EGGVHRVQRV PKT ---EKQGRVHTSTM...LRIDTKRASG GGQ HVNTTDS...		
mtRF1	...LEVTAGRT TGGDICQQT REIFDMYQNY...EGGIHRVQRI PEVGLS SRMQRIHTGTM...LRIDTFRAKG GGQ HVNKTDS...		
ICT1 (mL62)	...LLPPPAR-----CPRRALHKQK...-----LTISYCRSSG GGQ NVNKVN...		
C12ORF65	...-MSTVG-----LFH---FPTPLTR...-----LEEQFVKGHG GGQ ATNKTSN...		

Supplementary Fig. S1. Sequence alignment of mitochondrial and bacterial release factors. Extended alignment as shown in Fig. 1a is provided with the decoding motifs labeled in green and blue and the GGQ motif in red.



Supplementary Fig. S2. Genomic sequences of *mtRF1*^{-/-} and *mtRF1a*^{-/-}. Genomic DNA was isolated from the respective knockout cell lines, respective target region was PCR amplified and products were analyzed using TOPO cloning and subsequent sequencing. Chromatograms and respective DNA sequences of allele 1 and 2 with corresponding amino acid sequences are provided.

a COX1 termination codons

Homo sapiens

5'...CCACCCUACCACACAUUCGAAGAACCCGUAUACAUAAAAUC**UAGA**CAAAAAAGGAAGGAAUCGAA...3'
... P P Y H T F E E P V Y M K S *

Pan troglodytes

5'...CCACCCUACCACACAUUCGAAGAACCCGUAUACAUAAAAUC**UAGA**CAAAAAAGGAAGGAAUCGAA...3'
... P P Y H T F E E P V Y M K S *

Mus musculus

5'...CCAUAUCACACAUUCGAGGAACCAACCUAUGUAAAAAGUAAAA**UAA**GAAAGGAAGGAAUCGAACCC...3'
... P Y H T F E E P T Y V K V K *

Rattus norvegicus

5'...CCCUACCACACAUUCGAAGAACCUUCCUAUGUAAAAAGUAAAA**UAA**GAAAGGAAGGAUUCGAACCC...3'
... P Y H T F E E P S Y V K V K *

Bos taurus

5'...CCAUAUCACACAUUUGAAGAACCCACCUAUGUUAACCUAAAA**UAA**GAAAGGAAGGAAUCGAACCC...3'
... P Y H T F E E P T Y V N L K *

Sus scrofa

5'...CCCUAUCACACAUUUGAAGAACCAACAUAUAUCAACCUAAAA**UAA**GCAUAAGAAAGGAAGGAAUC...3'
... P Y H T F E E P T Y I N L K *

b ND6 termination codons

Homo sapiens

5'...UUUGUUGGUGUAUAUAUUGUAAUUGAGAUUGCUCGGGGGAA**UAGG**UUAUGUGAUUAGGAGUAGGG...3'
... F V G V Y I V I E I A R G N *

Pan troglodytes

5'...UUUGUUGGUGUAUAUAUUGUAAUUGAGAUUGCUCGGGGGAA**UAGG**UUAUGUGAUUAGGAGUAAGG...3'
... F V G V Y I V I E I A R G N *

Mus musculus

5'...UUGUUUGCGGGUAAUUUUUAUUUAUUCGAGAUUACUCGAGAU**UAA**UUGAGUAUAAGAUAAUAAUU...3'
... L F A G I F I I I E I T R D *

Rattus norvegicus

5'...UUGUUUGCGGGGAUUUUUAUUUAUUGAAAUCACUCGGGAU**UAA**GUGUGUGUAAGAUAAAAAUU...3'
... L F A G I F I I I E I T R D *

Bos taurus

5'...UUAUUGGUGUUGUGGUUAUUUAUAGAAAUCUCGUGGAAA**UAA**AUAAGAUUAUGCUGAUUAGG...3'
... L I G V V V I M E I T R G N *

Sus scrofa

5'...CUUAUUGGAGUUGUUGUUAUUUAUAGAGAUUACUCGUGGUA**UAA**AUAAGUGUUAUGCUGAUUAUA...3'
... L I G V V V I M E I T R G N *

Supplementary Fig. S3. Comparison of COX1 (a) and ND6 (b) termination codons in different species. Only the 3' end for COX1 and ND6 mRNA including the UTRs are presented with the corresponding amino acid sequence. Termination codons are marked in bold.

Supplementary Table S1. Key reagents

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-uS15m	ProteinTech	Cat# 17006-1-AP
Rabbit polyclonal anti-uL23m		PRAB1716
Mouse monoclonal anti-FLAG	Sigma Prestige	Cat# F1804
Rabbit polyclonal anti-TIM23		PRAB1527
Rabbit polyclonal anti-TIM70		PRAB3280
Mouse monoclonal anti-Calnexin	ProteinTech	Cat#66903-1-Ig
Rabbit polyclonal anti-ND1		PRAB5021
Rabbit polyclonal anti-ND2	ProteinTech	Cat# 19704-1-AP
Rabbit polyclonal anti-NDUFB8		PRA3765
Mouse monoclonal anti-SDHA	Invitrogen	Cat#459200
Rabbit polyclonal anti-CYTB		PRAB5131
Rabbit polyclonal anti-RIESKE		PRAB1512
Rabbit polyclonal anti-COX1		PRAB5121
Mouse monoclonal anti-COX2	Abcam	Cat# ab110258
Rabbit polyclonal anti-COX4l		PRAB1522
Rabbit polyclonal anti-ATP5B		PRAB4826
Rabbit polyclonal anti-ATP6		PRAB5159
Rabbit polyclonal anti-C12orf62		PRAB 4845
Rabbit polyclonal anti-MITRAC12		PRAB3761
Rabbit polyclonal anti-MITRAC15		PRAB4814
Rabbit polyclonal anti-mtRF1		RRDAB5461
Rabbit polyclonal anti-mtRF1a	ProteinTech	Cat# 16694-1-AP
Rabbit polyclonal anti-mL62/ICT1	ProteinTech	Cat# 10403-1-AP
Rabbit polyclonal anti-C12ORF65	ProteinTech	Cat# 24646-1-AP
Rabbit polyclonal anti-MTRES1	Sigma	Cat# HPA049535
Chemicals		
L-[³⁵ S]methionine	Hartmann Analytic	Cat# SCM-01
Adenosine 5'-triphosphate, [γ - ³² P]	Hartmann-Analytic	Cat# SRP-501
Emetine dihydrochloride hydrate	Sigma-Aldrich	Cat# 219282
Lipofectamine 3000	Invitrogen	Cat# L3000-015
GeneJuice	Novagen	Cat# 70967-3
Alt-R® CRISPR-Cas9 tracrRNA, ATTO™ 550	Integrated DNA technologies	Cat# 1075927
Alt-R® S.p. Cas9 Nuclease V3	Integrated DNA technologies	Cat# 1081058
TRIzol® Reagent	Ambion	Cat# 15596018
Critical Commercial Assays		
Rapid DNA Ligation Kit	ThermoFisher Scientific	Cat# K1423
T4 Polynucleotide Kinase (T4 PNK)	ThermoFisher Scientific	Cat# EK0031
KOD Hot Start DNA Polymerase	Merck	Cat# 71086-3
Wizard® Plus SV Minipreps DNA Purification System	Promega	Cat# A1460
Wizard® SV Gel and PCR Clean-Up System	Promega	Cat# A9282
QuikChange Lightning Site-Directed Mutagenesis Kit	Agilent Technologies	Cat# 210519-5

TOPO TA Cloning® Kit	ThermoFisher Scientific	Cat# 45-0030
OneShot®TOP10 Chemically Competent Cells	ThermoFisher Scientific	Cat# C404004
Complex I Enzyme Activity Assay Kit (Colorimetric)	abcam	Cat# ab109721
Seahorse FluxPaks	Agilent Technologies	Cat# 102416-100
MitoSOX™ Red Mitochondrial Superoxide Indicator	ThermoFisher Scientific	Cat# M36008
Experimental Models: Cell Lines		
HEK293-Flp-In T-Rex	ThermoFisher Scientific	R78007
HEK293-Flp-In T-Rex- <i>mtRF1</i> ^{-/-}	This study	N/A
HEK293-Flp-In T-Rex- <i>mtRF1a</i> ^{-/-}	This study	N/A
HEK293-Flp-In T-Rex- <i>mtRF1</i> ^{FLAG-GGQ}	This study	N/A
HEK293-Flp-In T-Rex- <i>mtRF1</i> ^{FLAG-AAQ}	This study	N/A
HEK293-Flp-In T-Rex- <i>mtRF1a</i> ^{FLAG-GGQ}	This study	N/A
HEK293-Flp-In T-Rex- <i>mtRF1a</i> ^{FLAG-AAQ}	This study	N/A
Oligonucleotides		
Guide RNA: targeting the Exon 2 of <i>mtRF1</i> : 5'-TGTTAAGTAAGAATTGGTCC-3'	This study; IDT	N/A
Guide RNA: targeting the Exon 1 of <i>mtRF1a</i> : 5'-CTCCGGTAGCCCCGCCGCTGG-3'	This study; IDT	N/A
siRNA C12orf65 Oligo: GCAAAGGAAACCCUGGAAA	This study; Eurogentec	N/A
Primer: Generation of the FLAG-tagged version of <i>mtRF1</i> Forward: 5'- CTCTCCAAGCTTCCACCATGAATCGTCACCTGTGTGTTTGG C-3'	This study; Microsynth	N/A
Primer: Generation of the FLAG-tagged version of <i>mtRF1</i> Reverse: 5'- CTTTCTCTCGAGCTACTTATCGTCGTCATCCTTGTAATCTTT TGCTGATTTAAGGTGTTTCATCC-3'	This study; Microsynth	N/A
Primer: Generation of the FLAG-tagged mutant (GGQ→AAQ) version of <i>mtRF1</i> : Forward: 5'- GATACATTTTCGAGCCAAAGGAGCAGCAGCGCAGCATGTTA ATAAAAC-3'	This study; Microsynth	N/A
Primer: Generation of the FLAG-tagged mutant (GGQ→AAQ) version of <i>mtRF1</i> Reverse: 5'- CACTATCAGTTTTATTAACATGCTGCGCTGCTGCTCCTTTGG CTC-3'	This study; Microsynth	N/A
Primer: Generation of the FLAG-tagged version of <i>mtRF1a</i> Forward: 5'-CTCTCCAAGCTTCCACCATGCGGTCCCGGTTCTGTGGG- 3'	This study; Microsynth	N/A
Primer: Generation of the FLAG-tagged version of <i>mtRF1a</i> Reverse: 5'- CTTTCTGATATCCTACTTATCGTCGTCATCCTTGTAATCAAC TTTTTGGAATAATTTCTACTAAAGATTC-3'	This study; Microsynth	N/A

Primer: Generation of the FLAG-tagged mutant (GGQ→AAQ) version of mtRF1a Forward: 5'-GACACTAAGCGAGCCAGTGGAGCTGCGGCGCAGCATGTAAATAC-3'	This study; Microsynth	N/A
Primer: Generation of the FLAG-tagged mutant GGQ→AAQ) version of mtRF1a Reverse: 5'-CACTGTCCGTGGTATTTACATGCTGCGCCGACGCTCCACTG GCTC-3'	This study; Microsynth	N/A
Probe: targeting MTRNR1 (12S rRNA) 5'-TCGATTACAGAACAGGCTCCTCTAG-3'	¹	N/A
Probe for northern blot: targeting MTRNR2 (16S rRNA) 5'-GTTTGGCTAAGGTTGTCTGGTAGTA-3'	¹	N/A
Probe for northern blot: targeting MTCO1 5'-GTCAGTTGCCAAAGCCTCCGATTATG-3'	¹	N/A
Probe for northern blot: targeting MTCO2 5'-GACGTCCGGGAATTGCATCTGTTTT-3'	¹	N/A
Probe for northern blot: targeting MTCYTB 5'-CGTGTGAGGGTGGGACTGTCTACTG-3'	This study; Microsynth	N/A
Probe for northern blot: targeting 18S-rRNA 5'-TTACTTCCTCTAGATAGTCAAGTTCGACC-3'	²	N/A
Recombinant DNA		
pOG44 Flp-Recombinase Expression Vector	ThermoFisher Scientific	Cat# V600520
pcDNA5/FRT/TO	ThermoFisher Scientific	Cat# V6520-20
Software and Algorithms		
ImageJ	³	https://imagej.nih.gov/ij/

Supplementary Table S2. nCounter tube sequences for NanoString analyses (provided by IDT)

Mito_JCM_008.1 :591 T001	CTTCATCAGGGTTTGTGTAAGATGGCGGTATATAGGCTGAGCAAGAGGTGCCTCAAGACCTAAGCGACAGCGTGACCTTGTTTCA
Mito_JCM_009.1 :481 T002	CTTTCTTAATTGGTGGCTGCTTTTAGGCCTACTATGGGTGTAAATTTTTCATCCTCTTCTTTTCTGGTGTGAGAAGATGCTC
HSMT_ND1.1:46 1 T003	GTTCTTGTGTTGTGATAAGGGTGGAGAGGTTAAAGGAGCCACTTATTAGTCAACAATCTGCGGGTTAGCAGGAAGGTTAGGGAAC
HSMT_ND2.1:44 4 T004	CCTGCTATGATGGATAAGATTGAGAGAGTGAGGAGAAGGCTTACGTTTACGTGTTGAGATTATTGAGCTTCATCATGACCAGAAG
HSMT_COX1.1:8 73 T005	GCGGAGGTGAAATATGCTCGTGTGTCTACGTCTATTCCTACTGTAAATATCAAAGACGCCTATCTCCAGTTTGATCGGGAAACT
HSMT_COX2.1:2 16 T006	TCGTCTGTTATGTAAAGGATGCGTAGGGATGGGAGGGCGATGAGGACTAGCGAACCTAACTCCTCGCTACATTCCTATTGTTTTT
HSMT_ATP8.1:82 T007	TATTTTTATGGGCTTTGGTGAGGGAGGTAGGTGGTAGTTTGTGTTAATACC AATTTGGTTTTACTCCCCTCGATTATGCGGAGT
HSMT_ATP6.1:33 1 T008	GTGGGCTAGGGCATTITTAATCCTTAGAGCGAAAGCCTATAATCACTGTGCCTTTCGGGTTATATCTATCATTACTTGACACCCT
HSMT_COX3.1:5 39 T009	ATGTTGAGCCGTAGATGCCGTGCGAAATGGTGAAGGGAGACTCGAAGTACC AACAGCCACTTTTTTTCCAAATTTTGCAAGAGCC
HSMT_ND3.1:13 5 T010	AAGGTAATAGCTACTAAGAAGAATTTTATGGAGAAAGGGACGCGGGCGGG CACCGTGTGGACGGCAACTCAGAGATAACGCATAT
HSMT_ND4L.1:5 8 T011	TATTCCTTCTAGGCATAGTAGGGAGGATATGAGGTGTGAGCGATATACTAC CTGGAGTTTATGTATTGCCAACGAGTTTGCTTT
HSMT_ND4.1:96 7 T012	ACTGTGAGTGCGTTCGTAGTTTGAGTTTGCTAGGCAGAATAGTAATGAGGC AGATAAGGTTGTTATTGTGGAGGATGTTACTACA

HSMT_ND5.1:1147_T013	TGCGGTTTCGATGATGTGGTCTTTGGAGTAGAAACCTGTGAGGAAAGGTACTTCCTTCTGTGTTCCAGCTACAACTTAGAAAC
HSMT_ND6.1:191_T014	CCTCAGGATACTCCTCAATAGCCATCGCTGTAGTATATCCAAAGACAACCCATAAAATTGGTTTTGCCTTTCAGCAATTCAACTT
HSMT_CYTB.1:946_T015	GAGGTCTGCGGCTAGGAGTCAATAAAGTGATTGGCTTAGTGGGCGAAATAC TGGTCAAGACTTGCATGAGGACCCGCAAATTCCT
NR_003286.2:1640_T016	AGGGCAGGGACTTAATCAACGCAAGCTTATGACCCGCACCTACTGGGAATCTTTCGTTGGGACGCTTGAAGCGCAAGTAGAAAAC
NR_023379.1:8_T017	TGCTTAGCTTCCGAGATCAGACGAGATCGGGCGCGTTCAGGGCCAGCAGACCTGCAATATCAAAGTTATAAGCGCGT
Mito_JCM_008.1:591_ProbeB	CGAAAGCCATGACCTCCGATCACTCTACACCTTGACCTAACGTCTTTACGTGGTACTTGCGCTTACTTTGTAGC
Mito_JCM_009.1:481_ProbeB	CGAAAGCCATGACCTCCGATCACTCTTCAGTTATATGTTTGGGATTTTTTAGGTAGTGGGTGTTGAGCTTGAACG
HSMT_ND1.1:461_ProbeB	CGAAAGCCATGACCTCCGATCACTCGATAAATCATATTATGGCCAAGGGTCATGATGGCAGGAGTAATCAGAGGT
HSMT_ND2.1:444_ProbeB	CGAAAGCCATGACCTCCGATCACTCAGTATGCTAAGATTTTGCGTAGCTGGTTTTGGTTAATCCACCTCAACTG
HSMT_COX1.1:873_ProbeB	CGAAAGCCATGACCTCCGATCACTCCGAGTCAGCTAAATACTTTGACGCCGTGGGGATAGCGATGATTATGGTA
HSMT_COX2.1:216_ProbeB	CGAAAGCCATGACCTCCGATCACTCAGTACCATTGGTGGCCAATTGATTGATGGTAAGGGAGGGATCGTTGACC
HSMT_ATP8.1:82_ProbeB	CGAAAGCCATGACCTCCGATCACTCAGCGAACAGATTTTCGTTCAATTTGGTTCTCAGGGTTTGTATAAATTTTT
HSMT_ATP6.1:331_ProbeB	CGAAAGCCATGACCTCCGATCACTCATAAATACTAGTATGGGGATAAGGGGTGTAGGTGTGCCTTGTGGTAAGAA
HSMT_COX3.1:539_ProbeB	CGAAAGCCATGACCTCCGATCACTCAGTTGAGCCAATAATGACGTGAAGTC CGTGGAAGCCTGTGGCTACAAAAA
HSMT_ND3.1:135_ProbeB	CGAAAGCCATGACCTCCGATCACTCGGGCTCATGGTAGGGGTAAAAGGAGG GCAATTTCTAGATCAAATAATAAG
HSMT_ND4L.1:58_ProbeB	CGAAAGCCATGACCTCCGATCACTCGAGTGGGTGTTGAGGGTTATGAGAGTAGCTATAATGAACAGCGATAGTAT
HSMT_ND4.1:967_ProbeB	CGAAAGCCATGACCTCCGATCACTCGCTATTAGTGGGAGTAGAGTTTGAAGTCCTTGAGAGAGGATTATGATGCG
HSMT_ND5.1:1147_ProbeB	CGAAAGCCATGACCTCCGATCACTCGTAGCGATGAGAGTAATAGATAGGGCTCAGGCGTTTGTGTATGATATGTT
HSMT_ND6.1:191_ProbeB	CGAAAGCCATGACCTCCGATCACTCCGCTAACCCCACTAAAACACTACCAAGACCTCAACCCCTGACCCCATG
HSMT_CYTB.1:946_ProbeB	CGAAAGCCATGACCTCCGATCACTCATGGTAAAAGGGTAGCTTACTGGTTGTCCTCCGATTCAGGTTAGAATGAG
NR_003286.2:1640_ProbeB	CGAAAGCCATGACCTCCGATCACTCGCCTCACTAAACCATCCAATCGGTAGTAGCGACGGGCGGTGTGTACAA
NR_023379.1:8_ProbeB	CGAAAGCCATGACCTCCGATCACTCCGGTCTCCCATCCAAGTACTAACCAGGCCGACCC

References

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