

Article Metadata	
Title	Silencing mitochondrial gene expression in living cells
Publication URL	<a href="https://doi.org/10.1126/science.adr3498">https://doi.org/10.1126/science.adr3498</a>
Data Availability Statement	Data and code availability <ul style="list-style-type: none"> <li>• This paper does not report original code.</li> <li>• Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.</li> </ul>
AAAS survey (please complete)	<a href="https://airtable.com/appEyo44c4qrc3VQE/shrOyR475L66qpU3v">https://airtable.com/appEyo44c4qrc3VQE/shrOyR475L66qpU3v</a>

Materials:				
Newly created materials	Materials Availability Statement	Article Subsection	DataSeer Notes	Author Response
The manuscript includes a dedicated "materials availability statement" providing transparent disclosure about availability of newly created materials including details on how materials can be accessed and describing any restrictions on access.	Materials availability Plasmids, cell lines, and other resources are available upon request to the lead contact.		Statement included in article text.	

Antibodies						
No Action Required		Optional				
Material is not required, but is recommended to be registered for an identifier to be included in the article text.						
Name	Associated sentence from text	Article Subsection	Source	Identifier	DataSeer Notes	Author Response
anti-TOM20	Following a washing step with PBS, the primary antibody solu	Immunofluorescence staining,	Abcam		Listed in article text	
secondary anti-rabbit antibody from goat	After three washing steps with PBS, the secondary antibody s	Immunofluorescence staining,	AffiniPure		Listed in article text	

DNA and RNA Sequences						
No Action Required	Done - Shared/Cited					
Material is sufficiently shared/cited						
Name	Associated sentence from text	Article Subsection	Source	Identifier	DataSeer Notes	Author Response
ND1 1-17	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		AGGTTGGCCATGGGTA	Sequence shared in article text	
ND2 12-29	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		TAGATGACGGGTGGG	Sequence shared in article text	
ND3 1-25	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		TTATTA <del>AA</del> ATTAAGGCG	Sequence shared in article text	
ND4L 6-30	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		TAGTATAATATTTATGTA	Sequence shared in article text	
ND4 [-15]-10	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		GTTTTAGCATTGGAGTA	Sequence shared in article text	
ND5 3-25	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		TGGTTATAGTAGTGTGC	Sequence shared in article text	
ND6 4-28	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		CACTCAACAGAAACAA	Sequence shared in article text	
CYTB 6-24	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		GTTAGTTTTGCGTATTG	Sequence shared in article text	
COX1 1-19	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		GTCAACGGTCGGCGAA	Sequence shared in article text	
COX1 181-199	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		AGATTATTACAAATGCA	Sequence shared in article text	
COX2 1-23	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		CCTACTTGCGCTGCATC	Sequence shared in article text	
COX3 2-19	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		CATGTGATTGGTGGGT	Sequence shared in article text	
ATP8 6-28	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		GCCATACGGTAGATTT	Sequence shared in article text	
ATP6 [-10]-15	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		CAGATTTTCGTTCAATT	Sequence shared in article text	
mCOX1 1-24	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		GAATAATCAACGATTAA	Sequence shared in article text	
xCOX1 1-22	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		ATAATCAACGAGTAATT	Sequence shared in article text	
COX1 19-42	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		GTCTTTGTGGTTTGTAG	Sequence shared in article text	
COX1 40-64	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		ATAATAGGTATAGTGT	Sequence shared in article text	
COX1 65-82	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		CTCCAGCTCATGCGCC	Sequence shared in article text	
COX1 85-104	The morpholinos used in this study were: ND1 1-17 , AGGTT	Synthesis of Jac1-and peptide		AGGCTTAGAGCTGTGC	Sequence shared in article text	

No Action Required	Optional					
Material is not required, but is recommended to be registered for an identifier to be included in the article text.						
Name	Associated sentence from text	Article Subsection	Source	Identifier	DataSeer Notes	Author Response
pCox4 1-25	pCox4 1-25 (Saccharomyces cerevisiae Cox4 presequence),	Synthesis of Jac1-and peptide			Listed in article text	
siRNA targeting ZNF703	siRNAs targeting ZNF703, TMEM186, and LINC00493 were	siRNA-mediated protein knock	Horizon Discovery (UK)		Listed in article text	
siRNA targeting TMEM186	siRNAs targeting ZNF703, TMEM186, and LINC00493 were	siRNA-mediated protein knock	Horizon Discovery (UK)		Listed in article text	
siRNA targeting LINC00493	siRNAs targeting ZNF703, TMEM186, and LINC00493 were	siRNA-mediated protein knock	Horizon Discovery (UK)		Listed in article text	
pcDNA3.1	The open reading frame (ORF) of TMEM186 (NM_015421.4)	Transient expression of FLAG			Listed in article text	
pCox4-COX1 1-19 chimera (COX1 1-19 c	Mitochondria-enriched fractions prepared from cells treated w	Quantitative mass spectromet			Listed in article text	
pCox4-ND 12-29 (ND2 12-29 or ND2 KD)	Mitochondria-enriched fractions prepared from cells treated w	Quantitative mass spectromet			Listed in article text	
pCox4-CYTB 6- 24 (CYTB 6-24 or CYTB	Mitochondria-enriched fractions prepared from cells treated w	Quantitative mass spectromet			Listed in article text	
pCox4 (Control)	Mitochondria-enriched fractions prepared from cells treated w	Quantitative mass spectromet			Listed in article text	

#### Cell Materials

No Action Required	Done - Shared/Cited					
Material is sufficiently shared/cited						
Name	Associated sentence from text	Article Subsection	Source	Identifier	DataSeer Notes	Author Response
AML12 (alpha mouse liver 12) cells	AML12 (alpha mouse liver 12) cells (CRL-2254 <sup>TM</sup> , ATCC) w	Mammalian cell culture	ATCC	CRL-2254	Listed in article text	

No Action Required	Optional					
Material is not required, but is recommended to be registered for an identifier to be included in the article text.						
Name	Associated sentence from text	Article Subsection	Source	Identifier	DataSeer Notes	Author Response
HEK293-Flp-In T-Rex	HEK293-Flp-In TM T-Rex TM (HEK293T), HEK293T-derived,	Mammalian cell culture			Listed in article text	
HeLa	HEK293-Flp-In TM T-Rex TM (HEK293T), HEK293T-derived,	Mammalian cell culture			Listed in article text	
Human iPS cell-derived cardiomyocytes	Human iPS cell-derived cardiomyocytes were cultured in RP	Mammalian cell culture			Listed in article text	
HEK293T mL45FLAG cells	HEK293T mL45FLAG cells (19) were treated with 0.6 mg/mL	Mammalian cell culture			Listed in article text	

#### Experimental Animals

*output type not detected in text*

#### Plants and Microbes

*output type not detected in text*

#### Human Research Participants

*output type not detected in text*

#### Design:

##### Study protocol

*output type not detected in text*

##### Laboratory protocol

*output type not detected in text*

##### Ethics

output type not detected in text

Analysis:

Data availability						
No Action Required		Done - Shared/Cited				
Research outputs are appropriately shared/cited						
Datatype	Associated sentence from text	Article Subsection	URL	Accession/DOI/PID	DataSeer Notes	Author Response
<a href="#">Genetic Data: High-Throughput Nucleotide Sequencing</a>	The NovaSeq X Plus sequencing platform (Illumina, USA) was used to perform 50 bp paired-end sequencing on the samples with 9 G raw data per sample.	RNA sequencing and analysis	<a href="https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE292101">https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE292101</a>	GSE292101672	Accession "GSE292101" is currently private and is scheduled to be released on Mar 15, 2026.	
<a href="#">Mass Spectrometry: Liquid Chromatography Mass Spectrometry</a>	Following colloidal Coomassie Blue staining, gel lanes were cut into five pieces each and processed for liquid chromatography-mass spectrometry (LC-MS) analysis as described before (46).	Quantitative mass spectrometry analysis	<a href="https://www.ebi.ac.uk/pride/archive/projects/PXD061846">https://www.ebi.ac.uk/pride/archive/projects/PXD061846</a>	PXD061846	Properly shared on PRIDE	
<a href="#">Mass Spectrometry: Liquid Chromatography Mass Spectrometry</a>	Following colloidal Coomassie Blue staining, gel lanes were cut into five pieces each and processed for liquid chromatography-mass spectrometry (LC-MS) analysis as described before (46).	Quantitative mass spectrometry analysis	<a href="https://www.ebi.ac.uk/pride/archive/projects/PXD061876">https://www.ebi.ac.uk/pride/archive/projects/PXD061876</a>	PXD061876	Properly shared on PRIDE	
<a href="#">Mass Spectrometry: Liquid Chromatography Mass Spectrometry</a>	Following colloidal Coomassie Blue staining, gel lanes were cut into five pieces each and processed for liquid chromatography-mass spectrometry (LC-MS) analysis as described before (46).	Quantitative mass spectrometry analysis	<a href="https://www.ebi.ac.uk/pride/archive/projects/PXD061877">https://www.ebi.ac.uk/pride/archive/projects/PXD061877</a>	PXD061877	Properly shared on PRIDE	

No Action Required	Optional					
Quality control and/or representative media are recommended but not required to be uploaded to a repository						
Datatype	Associated sentence from text	Article Subsection	URL	Accession/DOI/PID	DataSeer Notes	Author Response
<a href="#">Tabular Data: Assay</a>	The protein concentration was determined by the Bradford assay.	In vivo [ 35 S] methionine labe				
<a href="#">Image: Radiography</a>	Equivalent protein amounts were separated on Tris-Tricine 10-18% g	In vivo [ 35 S] methionine labe				
<a href="#">Image: Electrophoresis</a>	Finally, mitochondria were sedimented and the samples analyzed by	Downregulation of mtDNA-enc				
<a href="#">Image: Electrophoresis</a>	Equivalent amounts of material were analyzed by SDS-PAGE and we	Native immunoprecipitation of				
<a href="#">Tabular Data</a>	RNA quality control was assessed in a Fragment Analyzer.	RNA sequencing and analysis				
<a href="#">Image: Microscopy</a>	Images were acquired using a spinning disk microscope (Molecular I	Immunofluorescence staining,				
<a href="#">Tabular Data</a>	Cells were counted using the DAPI staining and the Stardist algorith	Immunofluorescence staining,				
<a href="#">Tabular Data</a>	Basal and maximal respiration were measured upon the addition of 3	Real-time respirometry				
<a href="#">Flow Cytometry</a>	BD-Canto flow cytometer (Becton Dickinson) was used to record 10,	Membrane potential measurer				
<a href="#">Tabular Data: Assay</a>	Mitochondrial respiratory chain complex activities were measured us	Measurement of mitochondria				
<a href="#">Tabular Data</a>	Autoradiographic and western blot signal intensities were quantified	Quantification and statistical a				

Code availability						
No Action Required	Done - Shared/Cited					
Research outputs are appropriately shared/cited						
Name or Type of Code	Associated sentence from text	Article Subsection	URL	DOI	DataSeer Notes	Author Response
R	The downstream analysis was performed in RStudio (R version 4.3.0) using packages from the Bioconductor repository (40, 41) and the Tidyverse suite.	RNA sequencing and analysis	<a href="https://doi.org/10.5281/zenodo.15260712">https://doi.org/10.5281/zenodo.15260712</a>		Properly shared on Zenodo	
Python	Data analyses for CYTB KD and ND2 KD as well as FLAG IP experiments were carried out using the autoprot package in Python).	RNA sequencing and analysis	<a href="https://doi.org/10.5281/zenodo.15241939">https://doi.org/10.5281/zenodo.15241939</a>		Properly shared on Zenodo	

Links or citations for software						
No Action Required	Optional					
Software objects are recommended but not required to be uploaded to a repository						
Name or Type of Software	Associated sentence from text	Article Subsection	URL	RRID	DataSeer Notes	Author Response

nSolver	The acquired data were analyzed with nSolver software (nan	Mitochondrial RNA detection b				
RStudio	The downstream analysis was performed in RStudio (R versi	RNA sequencing and analysis				
Bioconductor	The downstream analysis was performed in RStudio (R versi	RNA sequencing and analysis				
Tidyverse	The downstream analysis was performed in RStudio (R versi	RNA sequencing and analysis				
DESeq2	Differential gene expression analysis was conducted using D	RNA sequencing and analysis				
limma	Batch correction was applied using limma (version 3.56.2) (4)	RNA sequencing and analysis				
clusterProfiler	For Gene Ontology (GO) term enrichment analysis, the cluste	RNA sequencing and analysis				
org.Hs.eg.db	For Gene Ontology (GO) term enrichment analysis, the cluste	RNA sequencing and analysis				
UpSetR	UpSet plots were generated using UpSetR (version 1.4.0) co	RNA sequencing and analysis				
vsn	UpSet plots were generated using UpSetR (version 1.4.0) co	RNA sequencing and analysis				
ggplot2	Graphical representations were generated through the ggplot	RNA sequencing and analysis				
MaxQuant	The software MaxQuant/Andromeda (version 2.0.2.0 for COX	Quantitative mass spectromet				
UniProt	The software MaxQuant/Andromeda (version 2.0.2.0 for COX	Quantitative mass spectromet				
Perseus	The minimum peptide number required for LFQ was set to 1.	Quantitative mass spectromet				
impseq	The remaining missing values were imputed using sequential	Quantitative mass spectromet				
RankProd	Protein abundance ratios and p-values were calculated using	Quantitative mass spectromet				
Python	Data analyses for CYTB KD and ND2 KD as well as FLAG IP	Quantitative mass spectromet				
autoprot	Data analyses for CYTB KD and ND2 KD as well as FLAG IP	Quantitative mass spectromet				
ImageQuantTL	Autoradiographic and western blot signal intensities were qua	Quantification and statistical a				
ImageJ	Autoradiographic and western blot signal intensities were qua	Quantification and statistical a				
GraphPad Prism	Data were obtained from three or more biological replicates (	Quantification and statistical a				

To Be Completed by Authors

Design:

Experimental study design (statistics details)

For in vivo studies: State whether and how the following have been done	In vivo Study Information	Article Subsection	Author Response
Sample size determination			
Randomisation			
Blinding			
Inclusion/exclusion criteria			

Sample definition and in-laboratory replication

State number of times the experiment was replicated in laboratory.	Sample Information	Article Subsection	Author Response
Define whether data describe technical or biological replicates.			

Dual Use Research of Concern (DURC)

If study is subject to dual use research of concern regulations, state the authority granting approval and reference number for the regulatory approval.	Authority and Reference Number	Article Subsection	Author Response

Analysis:

Attrition	Article Subsection	Author Response
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Describe whether exclusion criteria were preestablished. Report if sample or data points were omitted from analysis. If yes report if this was due to attrition or intentional exclusion and provide justification.		
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Statistics			
Describe statistical tests used and justify choice of tests.			
Statistical Test	Justification	Article Subsection	Author Response

Reporting			
MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.			
Adherence to community standards	Guideline/Checklist	Article Subsection	Author Response
State if relevant guidelines (e.g., ICMJE, MIBBI, ARRIVE) have been followed, and whether a checklist (e.g., CONSORT, PRISMA, ARRIVE) is provided with the manuscript.			