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Corresponding author(s): Richter-Dennerlein, Ricarda; Hillen, Hauke

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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☒	☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Serial EM 3.8 beta 8
Data analysis	RELION 3.0 beta-2, UCSF Chimera 1.13, UCSF ChimeraX v1.2, COOT 0.9.5, Warp v1.0.7, PHENIX 1.18, MaxQuant 1.6.0.1

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The electron density reconstructions and structure coordinates were deposited with the Electron Microscopy Database (EMDB) under accession codes EMD-12865, EMD-12872, EMD-12867, EMD-12868, EMD-12870, EMD-12869, EMD-12871and with the Protein Data Bank (PDB) under accession codes 7OF0, 7OF7, 7OF2, 7OF3, 7OF5, 7OF4, 7OF6.
The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Perez-Riverol et al., 2018) partner repository with the dataset identifier PXD023502. Project Name: GTPase driven maturation of the human mitoribosomal peptidyl transferase center; Project accession: PXD023502.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample size. All biochemical experiments were replicated two or more times.
Data exclusions	No data were excluded from the analyses.
Replication	All attempts at replication were successful.
Randomization	Samples were not allocated to groups.
Blinding	Investigators were not blinded during data acquisition and analysis because it is not a common procedure for the methods employed.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Primary polyclonal anti-rabbit antibodies used in this study (dilution is indicated in brackets): anti-NSUN4 [1:1000] (Lavdovskaia et al., 2020; ProteinTech; #16320-1-AP), anti-MTERF4 [1:1000] (Lavdovskaia et al., 2020; Sigma Prestige; #HPA027097; RRID:AB_10603879), anti-MALSU1 [1:1000] (Lavdovskaia et al., 2018; Proteintech; #22838-1-AP; RRID:AB_11182483), anti-GTPBP5 [1:1000] (Sigma Prestige; #HPA047379; RRID:AB_10965845), anti-GTPBP10 [1:1000] (Lavdovskaia et al., 2018; Novusbio; #NBP1-85055; RRID:AB_11037644), anti-bL32m [1:1000] (Lavdovskaia et al., 2018; gift from Prof. P. Rehling; PRAB4957), anti-uS14m [1:1000] (Proteintech; #16301-1-AP; RRID:AB_2878240). Primary monoclonal anti-mouse antibody used in this study (dilution is indicated in brackets): anti-uL3 [1:500] (Proteintech; #66130-1-IG; RRID: AB_2881529).
Validation	Antibodies have been validated by ProteinTech (anti-uL3) and in recent studies: PMID: 30085210 (anti-GTPBP10, anti-MALSU1, anti-bL32m), PMID: 33264405 (anti-uS14m, anti-NSUN4, anti-MTERF4, anti-GTPBP5) PMID: 32652011 (anti-MTERF4, anti-GTPBP5).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293-Flp-In T-Rex - wild type (ThermoFisher Scientific, #R78007), HEK293-Flp-In T-Rex - Gtpbp6-/- (Generated by Elena Lavdovskaia, Research group Richter-Dennerlein, University Medical Center Göttingen; Lavdovskaia et al., 2020)
Authentication	HEK293-Flp-In T-Rex - wild type cell line was not authenticated. CRISPR/Cas9 mediated knockout of GTPBP6 was confirmed by sequencing (Lavdovskaia et al., 2020).
Mycoplasma contamination	Cells were systematically confirmed to be negative for the presence of Mycoplasma by GATC Biotech.

Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.