

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

Data analysis

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

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](https://www.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	Sample size was determined based on similar studies in this field.
Data exclusions	No data exclusions carried out.
Replication	The reproducibility of all experimental findings in this study was confirmed.
Randomization	Randomization of the data was not required.
Blinding	Blinding was not required.

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	Involvement in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	Detailed information about the antibodies used in this study are provided in Supplementary Data 3.
Validation	<p>Anti-LONP1 Methods section in this manuscript</p> <p>Anti-CLPP abcam CAT# ab124822 https://www.abcam.com/clpp-antibody-epr7133-ab124822.html</p> <p>Anti-AFG3L2 Proteintech CAT# 14631-1-AP https://www.ptglab.com/Products/AFG3L2-Antibody-14631-1-AP.htm</p> <p>Anti-mtHSP70 CST CAT# 35935 https://www.cellsignal.com/products/primary-antibodies/grp75-d13h4-xp-rabbit-mab/3593</p> <p>Anti-mtHSP60 SANTA CRUZ CAT# sc-59567 https://www.scbt.com/p/hsp-60-antibody-1k1?productCanUrl=hsp-60-antibody-1k1&_requestid=2382054</p> <p>Anti-TID1 abcam CAT# ab181024 https://www.abcam.com/tid1-antibody-epr12414-ab181024.html?#description_protocols</p> <p>Anti-TRAP1 abcam CAT# ab109323 https://www.abcam.com/trap1-antibody-epr5381-ab109323.html</p> <p>Anti-CPN10 abcam CAT# ab108600 https://www.abcam.com/epf-antibody-epr4476-ab108600.html</p> <p>Anti-CLPX abcam CAT# ab168338 https://www.abcam.com/clpx-antibody-ep8772-ab168338.html</p> <p>Anti-PMPCA SANTA CRUZ CAT# sc-390718 https://www.scbt.com/p/pmpca-antibody-f-4?productCanUrl=pmpca-antibody-f-4&_requestid=2382531</p> <p>Anti-PMPCB Proteintech CAT# 16064-1-AP https://www.ptglab.com/Products/PMPCB-Antibody-16064-1-AP.htm</p> <p>Anti-TOMM40 Proteintech CAT# 18409-1-AP</p>

<https://www.ptglab.com/Products/TOMM40-Antibody-18409-1-AP.htm>
 Anti-TIMM22 Proteintech CAT# 14927-1-AP
<https://www.ptglab.co.jp/products/TIMM22-Antibody-14927-1-AP.htm>
 Anti-TIMM29 (C19orf52) Proteintech CAT# 25652-1-AP
<https://www.ptglab.co.jp/products/C19orf52-Antibody-25652-1-AP.htm>
 Anti-AGK GeneTex CAT# GTX107413
<https://www.genetex.com/Product/Detail/Acylglycerol-kinase-antibody/GTX107413>
 Anti-TIMM44 abcam CAT# ab194829
<https://www.abcam.com/tim44-antibody-epr16821-ab194829.html>
 Anti-TIMM23 Proteintech CAT# 11123-1-AP
<https://www.ptglab.com/Products/TIMM23-Antibody-11123-1-AP.htm>
 Living Colors Av. Peptide Antibody (AcGFP) TAKARA CAT# 632377
<https://www.takarabio.com/assets/documents/Certificate%20of%20Analysis/632377-PA923065.pdf>
 Anti-DsRED TAKARA CAT# 632496
<https://www.takarabio.com/products/antibodies-and-elisa/fluorescent-protein-antibodies/red-fluorescent-protein-antibodies/catalog=632496>
 anti-DDDDK (FLAG) MBL CAT# PM020
<https://ruo.mbl.co.jp/bio/e/dtl/A/?pcd=PM020>
 Anti-VDAC Proteintech CAT# 63345-1-Ig
<https://www.ptglab.com/products/VDAC1-Porin-Antibody-66345-1-Ig.htm>
 Anti-Tuba MBL CAT# PM054
<https://ruo.mbl.co.jp/bio/e/dtl/A/?pcd=PM054>
 Anti-MRPS15 abcam CAT# ab137070
<https://www.abcam.com/mrps15-antibody-epr9361-ab137070.html>
 Anti-MRPS26 abcam CAT# ab181863
<https://www.abcam.com/mrps26-antibody-epr14396-ab181863.html>
 Anti-MRPS35 abcam CAT# ab182160
<https://www.abcam.com/mrps35-antibody-epr117312-ab182160.html>
 Anti-MRPL4 abcam CAT# ab180165
<https://www.abcam.com/mrpl4-antibody-epr13151-ab180165.html>
 Anti-MRPL16 abcam CAT# ab181834
<https://www.abcam.com/mrpl16-antibody-epr14351-ab181834.html>
 Anti-MRPL30 abcam CAT# ab179819
<https://www.abcam.com/mrpl30-antibody-epr12502-ab179819.html>
 Anti-POLRMT SANTA CRUZ CAT# sc-365082
<https://www.scbt.com/p/mtrpol-antibody-b-1?requestFrom=search>
 Anti-TFB2M ABGENT CAT# AP10145b
<http://www.abcepta.com/products/AP10145b-TFB2M-Antibody-C-term>
 Anti-MRPPP3 abcam CAT# ab185942
<https://www.abcam.com/mrpp3-antibody-epr14321-30-ab185942.html>
 Anti-POLGA CST CAT# 13609S
<https://www.cellsignal.jp/products/primary-antibodies/dna-polymerase-g-d1y6r-rabbit-mab/13609>
 Anti-mtSSB home made
 Takamatsu, C., Umeda, S., Ohsato, T., Ohno, T., Abe, Y., Fukuoh, A., Shinagawa, H., Hamasaki, N., and Kang, D. (2002). Regulation of mitochondrial D-loops by transcription factor A and single-stranded DNA-binding protein. EMBO Rep 3, 451-456. Anti-TFAM home made
 Takamatsu, C., Umeda, S., Ohsato, T., Ohno, T., Abe, Y., Fukuoh, A., Shinagawa, H., Hamasaki, N., and Kang, D. (2002). Regulation of mitochondrial D-loops by transcription factor A and single-stranded DNA-binding protein. EMBO Rep 3, 451-456. Anti-SDHA abcam CAT# ab14715
<https://www.abcam.com/sdha-antibody-2e3gc12fb2ae2-ab14715.html>
 Anti-SDHB SANTA CRUZ CAT# sc-25851
https://www.scbt.com/p/sdhb-antibody-fl-280?productCanUrl=sdhb-antibody-fl-280&_requestid=2387382
 Anti-COX1 abcam CAT# ab14705
<https://www.abcam.com/mtco1-antibody-1d6e1a8-ab14705.html>
 Anti-COX2 abcam CAT# ab79393
<https://www.abcam.com/mtco2-antibody-epr3314-ab79393.html>
 Anti-COX4 MBL CAT# PM063
<https://ruo.mbl.co.jp/bio/e/dtl/A/?pcd=PM063>
 Anti-ATPa abcam CAT# ab14748
<https://www.abcam.com/atp5a-antibody-15h4c4-mitochondrial-marker-ab14748.html>
 Anti-Cytrate synthase CST CAT# 14309S
https://www.cellsignal.jp/products/primary-antibodies/citrate-synthase-d7v8b-rabbit-mab/14309?site-search-type=Products&N=4294956287&Ntt=14309s&fromPage=plp&_requestid=2961995
 Anti-Fumarase CST CAT# 4567S
https://www.cellsignal.jp/products/primary-antibodies/fumarase-d9c5-rabbit-mab/4567?site-search-type=Products&N=4294956287&Ntt=4567s&fromPage=plp&_requestid=2962032
 Anti-MDH2 CST CAT# 11908S
https://www.cellsignal.jp/products/primary-antibodies/mdh2-d8q5s-rabbit-mab/11908?site-search-type=Products&N=4294956287&Ntt=11908s&fromPage=plp&_requestid=2962060
 Anti-ACO2 abcam CAT# ab110312
<https://www.abcam.com/hsp60-antibody-1d11bd8-mitochondrial-marker-ab110312.html>
 Anti-DLAT abcam CAT# ab110332
<https://www.abcam.com/pyruvate-dehydrogenase-e2-antibody-15d3g9c11-ab110332.html>
 Anti-PDHE1A abcam CAT# ab168379
<https://www.abcam.com/pyruvate-dehydrogenase-e1-alpha-subunit-antibody-epr11098-ab168379.html>
 Anti-PDHE1B abcam CAT# ab155996

<https://www.abcam.com/pdhh-antibody-epr11097b-ab155996.html>
 Anti-CPS1 abcam CAT# ab45956
<https://www.abcam.com/cps1-antibody-ab45956.html>
 Anti-HADHA abcam CAT# ab54477
<https://www.abcam.com/hadha-antibody-ab54477.html>
 Anti-SOD2 CST CAT# 13194S
https://www.cellsignal.jp/products/primary-antibodies/sod2-d9v9c-rabbit-mab/13194?site-search-type=Products&N=4294956287&Ntt=13194s&fromPage=plp&_requestid=2962217
 Anti-DDDK -tag mAB Magnetic Beads MBL CAT# M185-11
<https://ruo.mbl.co.jp/bio/e/dtl/A/?pcd=M185-11>
 Anti-rabbit IgG, HRP-Linked GE CAT# NA934-1ML
<https://www.sigmaaldrich.com/catalog/product/sigma/gena9341ml?lang=ja®ion=JP>
 Anti-mouse IgG, HRP-Linked GE CAT# NA931-1ML
<https://www.sigmaaldrich.com/catalog/product/sigma/gena9311ml?lang=ja®ion=JP>
 TidyBlot™ Western Blot Detection Reagent:HRP Biorad CAT# STAR209P
<https://www.bio-rad-antibodies.com/reagent/tidyblot-ac>

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HeLa and HEK cells. The stable cell lines used in the study were derived from the corresponding parental cells.
Authentication	HeLa and HEK cells were purchased from ATCC and Thermo Fisher Scientific, respectively. https://www.atcc.org/products/all/CCL-2.aspx https://www.thermofisher.com/order/catalog/product/R78007#/R78007
Mycoplasma contamination	All cell lines tested were negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.