

Reporting Summary

Nature Portfolio 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 Portfolio 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 |
|-------------------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of all covariates tested |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> 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 Portfolio [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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

All data is available from the authors upon request.
There are no restrictions on data availability.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	<input type="text" value="The study did not involve human participants."/>
Reporting on race, ethnicity, or other socially relevant groupings	<input type="text" value="The study did not involve human participants."/>
Population characteristics	<input type="text" value="The study did not involve human participants."/>
Recruitment	<input type="text" value="The study did not involve human participants."/>
Ethics oversight	<input type="text" value="The study did not involve human participants."/>

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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	<input type="text" value="Sample sizes were not predetermined based on pre-specified effect size, but were selected based on commonly adopted standards in the field taking into account previous experience and literature to ensure statistically meaningful comparisons and adequate statistical power. The numbers of samples analyzed are provided in the figure legends for all experiments."/>
Data exclusions	<input type="text" value="No samples were excluded."/>
Replication	<input type="text" value="For all quantified experiments, the number of independent replications is indicated in the figure legends. All graphs clearly indicate biological, technical or other replicates (e.g., individual cells)."/>
Randomization	<input type="text" value="For all treatments, cultured cells of the required type were randomly allocated into control and treatment group without using a formalized randomization procedure."/>
Blinding	<input type="text" value="Investigators were generally not blinded as the experimental work flow required investigators to know the identity of the samples. However, data analysis occurred in an unbiased manner by applying analysis routines from image analysis programs in an identical manner to all images of the different experimental groups."/>

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
<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"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved 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

Primary antibodies
 MIC60 Proteintech Cat# 10179-1-AP; RRID:AB_2127193
 MIC27/APOOL Sigma Cat# HPA000612; RRID:AB_1078594
 YME1L1 Proteintech Cat# 11510-1-AP; RRID:AB_2217459
 LC3B Novus Biologicals Cat# NB600-1384; RRID:AB_669581
 p62 abcam Cat# ab56416; RRID:AB_945626
 OMA1 Proteintech Cat#17116-1-AP; RRID:AB_2299053
 LAMP1 (D2D11) Cell Signalling Cat#9091; RRID: AB_2687579)
 LAMP2 Santa Cruz Cat # sc-18822; RRID: AB_626858
 MRPL44 Proteintech Cat #16394-1-AP; RRID: AB_2146062
 MRPS27 Proteintech Cat #17280-1-AP; RRID: AB_2180510
 OPA1 BD Biosciences Cat #612606; RRID: AB_399888
 v-ATPase ATP6AP1 Santa Cruz Cat# sc-81886; RRID: AB_1119179
 v-ATPase ATP6AP2 Sigma Aldrich Cat# HPA003156; RRID: AB_1078245
 v-ATPase B1/2 Santa Cruz Cat # sc-55544; RRID: AB_831844
 V-ATPase A1 Santa Cruz Cat # sc-374475; RRID: AB_10987694
 SHMT2 ThermoFisher Scientific Cat # PA5-32228; RRID: AB_2549701
 HSP60 Abcam Cat # ab46798; RRID: AB_881444
 VDAC1/Porin Abcam Cat # ab14734; RRID: AB_443084
 CTH / CSE Proteintech Cat #12217-1-AP; RRID: AB_2087497
 TOM40 Santa Cruz Cat #sc-11414; RRID: AB_793274
 NDUFA9 Abcam Cat # ab14713; RRID: AB_301431
 MT-CO1 Abcam Cat # ab14607; RRID: AB_2084810
 MT-CO2 Abcam Cat # ab79393; RRID: AB_1603751
 SDHA Abcam Cat# ab14715; RRID: AB_301433
 ATP5B Abcam Cat# ab14748; RRID: AB_301447
 UQCRCF1 Abcam Cat #ab14746; RRID: RRID:AB_301445
 VINCULIN Abcam Cat# ab129002; RRID: AB_11144129
 PHGDH Proteintech Cat#14719-1-AP; RRID: AB_2283938
 TOM20 Santa Cruz Cat#sc-17764; RRID: AB_628381
 TOM20 Abcam Cat# ab186735; RRID: AB_2889972
 FLAG M2 Sigma Cat # F1804, RRID: AB_262044

Secondary antibodies
 HRP-linked anti-mouse Jackson ImmunoResearch Cat# 115-035-146; RRID: AB_2307392
 HRP-linked anti-rabbit Jackson ImmunoResearch Cat# 111-035-144; RRID: AB_2307391
 Alexa488 anti-mouse ThermoFisher Scientific Cat# A-11001; RRID: 2534069
 Alexa594 anti-rabbit ThermoFisher Scientific Cat# A-21207; RRID: AB_141637

Validation

Antibodies against ATP6AP1, MRPL44 were validated by gene knockout (this study) and FLAG by overexpression. Antibodies against OXPHOS complexes have been validated extensively for immunoblotting in denaturing and native PAGE analyses (Jackson et al., 2019). All other antibodies used in this study have been validated before and unanimously confirmed convergent mass spectrometry results in this study.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	HEK293(#CRL-1573) and U2OS (#HTB-96) cell lines were originally obtained from ATCC. Fibroblasts cell lines were obtained as indicated in respective publications with the respective gender indicated.
Authentication	Cell lines from ATCC are regularly authenticated by STR profiling.
Mycoplasma contamination	All cell lines were regularly tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	There were no commonly misidentified cell lines used in this study.

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a