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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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	None Used
Data analysis	XCalibur Qual Browser, XCalibur Quan Browser, Image J, Trim Galore!, Bowtie2, SAMtools

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/reviewers upon request. 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

NGS data generated in the present study are available from the BioProject database using accession number PRJNA479953.
Raw data is available for Fig. 1D (Fig. S9).
There are no restrictions on data availability.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	A naive, preliminary power calculation was performed at the outset, using speculative effect sizes we hoped to observe. The effects observed were much larger than anticipated, so the number of animals used in the study was scaled back, in accordance with the three R's principle (replacement, reduction, refinement), enshrined in UK animal research laws.
Data exclusions	No samples or data were excluded.
Replication	All experiments were subject to both biological and technical replicates to ensure reproducibility. The data described in the manuscript was collected from a number of independent experiments taking place over a ~3 year period.
Randomization	Randomization was not necessary for this study. Male animals were assembled into cohorts based on similar age and heteroplasmy. Homogeneity of the control and treatment cohorts (at the pre-treatment point) was the aim.
Blinding	Animals were given unique identifying numbers and the administration of substances was blinded. Samples for RNA, qPCR, amplicon resequencing and LC-MS analyses were blinded.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	Model cell lines and animals are available to academic and industrial scientists through the Max Planck Institute for Biology of Ageing. Requests for m.5024C>T specific mtZFNs should be directed to the corresponding authors.

Antibodies

Antibodies used	rabbit anti-TOM20 (Santa Cruz Biotechnology, sc-11415, 1:200), Alexa Fluor 647 anti-rabbit (Abcam, ab150079, 1:1000), mouse anti-FLAG (Sigma, F1804, 1:1000), Alexa Fluor 594 anti-mouse (Life Technologies, R37121, 1:1000), rat anti-HA (Roche, 11867431001, 1:200), Alexa Fluor 488 anti-rat (Life Technologies, A11006)
Validation	All antibodies have been verified using a variety of species-specific (human, mouse) cellular models. Verification of specific detection has been determined through a mixture of microscopy, subcellular fractionation and protein expression/purification studies.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Max Planck Institute for Biology of Ageing, Cologne.
Authentication	The m.5024C>T cells are unique, and were extensively characterized prior to publication (Kauppila et al., 2016, Cell Rep.)
Mycoplasma contamination	All cells were tested, and found to be negative, for mycoplasma.
Commonly misidentified lines (See ICLAC register)	None.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Mouse, tRNA-Ala C57/Bl6j, 2-8 months of age.
Wild animals	N/A
Field-collected samples	N/A