nature portfolio

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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
☐	☒ 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	Fluorescent image acquisition: Zeiss ZEN software (version 2.6, Carl Zeiss) qRT-PCR: BioRad CFX manager version 3.1 (Bio-Rad Laboratories) Target number, size, and intensity: ImageJ (version 1.53c)
Data analysis	Excel 2021 and GraphPad Prism 9 were used for general data analysis. Images were analyzed using ImageJ 1.53c. Additional details are provided in the materials and methods section.

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 generated or analyzed during this study are included in the figures and text with representative images accompanying quantified results where applicable unless otherwise noted. Further information is available from the corresponding author upon reasonable request.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

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 pre-determine sample sizes. Sample sizes used in this study are similar to those reported in previous publications (Ulgherait et al., 2014, Cell Reports; Rana et al., 2017, Nat Commun; Aparicio et al., 2019, Cell Reports; Schmid et al., 2022 Nat Aging).
Data exclusions	No data were excluded from the analyses.
Replication	All data presented were from independent biological replicates or independent experiments, and all attempts at replication were successful. For lifespan assays, there were no attempts to replicate negative results, i.e. shortened lifespans upon dsGFP overexpression. When lifespan extensions were observed, results were confirmed in at least one independent experiment.
Randomization	Experimental and control flies were developed and maintained under the same conditions and were allocated to treatments/group randomly. Steps were taken to avoid batch effects.
Blinding	Blinding was not always possible during experimental setup given that investigators needed to carefully document the genotypes of flies when generating crosses or to track assigned groups being maintained on RU vs. vehicle throughout lifespans. Blinding was performed when possible, specifically when conducting microscopy for TMRE, ATG8a-tandem, and mitoQC. All experiments were conducted under the same conditions, and control and experimental samples were treated equally and in parallel to exclude bias. Additionally, all images were taken in the same location and depth in each tissue type.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

mouse anti-ATP5a (15H4C4, abcam)
mouse anti-actin (JLA20, DSHB)
mouse anti-FK2 (BML-PW8810-0500, ENZO)
rabbit anti-atg8a (home made, Rana et al., 2017)
mouse anti-dsDNA (ab27156, abcam)

anti-rabbit or anti-mouse AlexaFluor-488 (A-11001 or A-11008, Thermo Fisher Scientific)
anti-rabbit or anti-mouse AlexaFluor-568 (A-11031 or A-11036, Thermo Fisher Scientific)
phalloidin AlexaFlour-568 (A12380, Thermo Fisher Scientific)

Validation

All antibodies were used in accordance to the manufacturer guidelines.

mouse anti-ATP5a (15H4C4, abcam) was validated by the manufacturer and in the following publications:
Bawa S et al.,Elife (2020) 9:e52358.
Chen PL et al., Nat Commun (2020) 11(1):2592.
Aparicio R et al., Cell Rep (2019) 28(4):1029-1040.e5.

mouse actin (JLA20, DSHB) was validated by the manufacturer and the following publications:
Ordonez D et al. Neuron (2018) 97(1):108-124.
Bardai F et al. PLoS Biol (2018) 16(12):e2006265.

mouse anti-FK2 (BML-PW8810-0500, ENZO) was validated by the manufacturer and the following publications:
Tamai K et al., Am J Pathol (2008) 173(6):1806-17.
Aparicio R et al., Cell Rep (2019) 28(4):1029-1040.e5.
Schmid E et al., Nat Aging (2022) 2(6):494-507.

rabbit anti-atg8a (home made, Rana et al., 2017) was validated by the following publications:
Rana A et al., Nat Commun (2017) 8(1):448.
Aparicio R et al., Cell Rep (2019) 28(4):1029-1040.e5.
Schmid E et al., Nat Aging (2022) 2(6):494-507.

mouse anti-dsDNA (ab27156, abcam) was validated by the manufacturer and the following publications:
Hu Q et al., Cell Rep (2020) 30:1235-1245.e4.
Aparicio R et al., Cell Rep (2019) 28(4):1029-1040.e5.
Schmid E et al., Nat Aging (2022) 2(6):494-507.

anti-rabbit or anti-mouse AlexaFluor-488 (A-11001 or A-11008, Thermo Fisher Scientific) were validated by the manufacturer and the following publications:
Yun HY et al., PLoS Biol (2019) 17(7):e3000367.
Foggetti A et al., Cell Rep (2019) 27(13):3725-3732.e5.

anti-rabbit or anti-mouse AlexaFluor-568 (A-11031 or A-11036, Thermo Fisher Scientific) were validated by the manufacturer and the following publications:
Schips TG et al., Nat Commun (2019) 10(1):76.
Namiki S et al., Elife (2018) 7:e34272.
Sapmaz A et al., Nat Commun (2019) 10(1):1454.
Yip SH et al., Cell Rep (2019) 26(7):1787-1799.e5.

phalloidin AlexaFlour-568 (A12380, Thermo Fisher Scientific) was validated by the manufacturer and the following publications:
Helfand BT et al., J Cell Biol (2002) 157:795-806.
Meary F et al., J Biol Chem (2007) 282:14226-14237.
Ordonez D et al. Neuron (2018) 97(1):108-124.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Drosophila melanogaster. The strain, sex, and age are described in detail in the materials and methods section, and also in relevant figure legends.
Wild animals	Our study did not involve any wild animals
Reporting on sex	As a standard approach, mated female flies were used in experiments unless otherwise indicated. Findings, such as lifespans, were validated in male flies.
Field-collected samples	Our study did not involve any field-collected samples
Ethics oversight	No ethical approval was required for this study.

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