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	Excel v2108 was used for data collection and GraphPad v10.0.2 was used for data collection and generation of graphs. Biorender.com (latest update Jan 13,2025) was used to generate schematic figures. Fluorescent microscopy images were taken on a Keyence BZ-X800 (2018 Keyence Corporation).
Data analysis	Fluorescent microscopy images were processed (stitched and full focus) using Keyence BZ-X800 Analyzer v1.1.1.8. Fiji 2.9.0 was used for subsequent image processing and analysis. GraphPad v10.0.2 was used for statistical analyses of experimental data. Publicly available code was implemented in Python for the UMAP (https://github.com/lmcinnes/umap) and HDBSCAN (https://github.com/scikit-learn-contrib/hdbscan) analyses. Figures in these analyses were generated using Matplotlib (https://matplotlib.org/) and Seaborn (https://seaborn.pydata.org/). Transcriptomic data was processed in python 3.10 using scanpy-1.10.1, anndata-0.10.7, numpy-1.26.4, scipy-1.11.4, pandas-2.2.2, statsmodels-0.14.2 software packages. Differential gene expression significance was calculated using the Wilcoxon rank-sum test, as provided by the SciPy software package. All code used to reanalyze publicly available datasets and generate associated figures will be posted at: https://github.com/Tawfik-Lab/ Donovan_Senescence_2024 and https://doi.org/10.5281/zenodo.14902120.

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

Data that support the findings of this study will be available on Dryad at the following location https://doi.org/10.5061/dryad.fbg79cp5v within 6 months of publication.

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	Human lumbar L4 DRG tissues were used in this study taken from de-identified post-mortem organ donors. Three female (32yo, 33yo, 65yo) and one male (65yo) donor was collected from in this study.
Reporting on race, ethnicity, or other socially relevant groupings	Human lumbar L4 DRG tissues were obtained from one white female donor (age 33), one Hispanic or Latino female donor (age 32), one male donor whose ethnicity was not reported (age 65), and one white female donor (age 65).
Population characteristics	Two donors were categorized as 'young' (32 & 33yo), and two donors were catagorized as 'aged' (65yo) in this study.
Recruitment	Donors were post-mortem. Human lumbar L4 DRG tissues were obtained from young female donors (age 32&33yo), and two aged donors one male, one female (both age 65). All patients died from stroke or head trauma.
Ethics oversight	Use of human post-mortem DRG received Stanford University Institutional Review Board for human subjects exemption.

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	Cohort sizes were determined based on historical data from our laboratory using a power analysis to provide >80% power to discover 25% differences with p<0.05 between groups to require a minimum of 4 animals per group for all behavioral outcomes. Power analysis was used to determine sample sizes for molecular analyses (RNAscope).
Data exclusions	No data were excluded from analyses.
Replication	Each experiment was replicated in separate cohorts of mice. Experiments using RNAscope detection of p21, p16, IL6, and Trpv1 were replicated in separate experiments and with several different secondary dyes. All data and trends were consistent in each replicated experiment. No experiment failed to replicate the results presented in this study.
Randomization	For all experiments presented, mice were allocated into experimental groups in a random manner, with each cage of mice representing an experimental block.
Blinding	Experimenters were blinded to group allocation during data collection for behavior analyses. Experimenters were blinded to age/sex/ timepoint for RNAscope quantification cell counts. In all electrophysiology experiments, the identity of each neuron was unknown to the electrophysiologist during experimentation.

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

Primary antibodies used: Rabbit anti-ATF3 (Novus Bio, Cat#NBP1-85816) and Rabbit anti-Cleaved-caspase-3 (Cell Signaling Technology, Cat#9661). Secondary antibodies used included: AlexaFluor secondary antibodies (Donkey anti-rabbit-A488, LifeTechnologies, Cat# A21206 and Donkey anti-rabbit Alexa-555, LifeTechnologies, Cat#A31572).

Validation

For ATF3 primary antibody validation, Novus Bio confirmed reactivity in human and mouse tissues among other species and validated applications such as IHC used in this study. Further validation by both Genetic and Biological strategies is noted on supplier website. Details include: 1) Genetic Strategy Validation- Expression of the target protein is compared before and after knockout or knockdown using CRISPR/CAS9 or siRNA/shRNA. If protein expression following knockout or knockdown is substantially reduced, then antibody specificity is ensured. and 2) Biological Strategies Validation- These strategies use defined biological or chemical modulation of protein expression to demonstrate antibody specificity to the target protein. The data is compared across multiple cell lines including positive and negative expressing cells, and multiple species.

For Cleaved caspase-3 antibody validation, Cell Signaling Technology confirmed: Cleaved Caspase-3 (Asp175) Antibody detects endogenous levels of the large fragment (17/19 kDa) of activated caspase-3 resulting from cleavage adjacent to Asp175. This antibody does not recognize full length caspase-3 or other cleaved caspases. This antibody detects non-specific caspase substrates by western blot. Non-specific labeling may be observed by immunofluorescence in specific sub-types of healthy cells in fixed-frozen tissues (e.g. pancreatic alpha-cells). Nuclear background may be observed in rat and monkey samples.

Primary antibody controls (no-primary conditions) were used throughout to validate immuno-positive signal.

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

All animal procedures were approved by the Stanford University Administrative Panel on Laboratory Animal Care and Institutional Animal Care and Use Committee (IACUC; 34760) in accordance with American Veterinary Medical Association guidelines and the International Association for the Study of Pain. C57BL/6J male and female mice at 11-16 weeks old and C57BL/6JN male and female mice at 20-24 months old from NIA aged rodent colony (C57BL/6JN). All mice were housed 2-5 per cage maintained on a 12-hour light/dark cycle in a temperature-controlled environment (Temp:68-74F; Humidity:30-70%) with ad libitum access to food and water.

Wild animals

The study did not involve wild animals.

Reporting on sex

Male and female mice were used throughout the study as described.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

Stanford University Administrative Panel on Laboratory Animal Care and Institutional Animal Care and Use Committee (IACUC; 34760)

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.