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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 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	The computational code used to perform machine learning based analyses on EVs are uploaded on https://github.com/ankitbhatia/bioimage_aging. The code for RNAseq analysis is available on https://github.com/sruthi-hub/Aging_EV/tree/main.
Data analysis	To generate PCAs the OriginLab plugin called "Principal Component Analysis for Spectroscopy" was used.

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

The raw data that support the experimental findings are included as supplementary information. The image files used for computational analysis are uploaded at https://github.com/ankitbhatia/bioimage_aging. RNAseq data has been deposited to NCBI GEO database and is publicly available. The GEO accession number is GSE176478.

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	Based on the preliminary investigations of peak muscle tetanic force in young and aged animals and after adjusting for unforeseen circumstances estimated at 20%, the final sample size for functional testing was 8-10 animals/group.
Data exclusions	Animals displaying evidence of external injuries and/or tumor growths were not included in the study. Statement for exclusion criteria has been listed in the supplementary methods section.
Replication	In vivo studies for the experimental groups were performed in two-three independent cohorts, wherever possible. In vitro studies were repeated in two-three independent runs whenever possible, to ensure reproducibility. To enhance transparency, data from different cohorts or experimental repeats have been displayed in different colors in the dot-plot graphs wherever possible. All attempts at replication were successful.
Randomization	Aged animals (C57/BL6, 21-23 months, NIA) were randomized into their experimental groups based on their overall muscle endurance scores as displayed by modified hanging-grid test. Statement for randomization has been made in main paper (Study design and steps taken to ensure rigor section) supplemental methods.
Blinding	All investigators were blinded for the experimental hypothesis and/or end-point analysis. Statement for blinding has been made in Study design and steps taken to ensure rigor section of the main manuscript.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	The antibodies used in the manuscript are described in Supplemental document under Immunofluorescence imaging. Briefly, the antibodies product information and dilutions are as follows: MyoD(SCBT, sc-760, 1:500), MyoD(sc-377460, 1:500),Desmin(Abcam, ab15200, 1:500), Klotho(R&D systems, MAB1819, Lot# KGN0315101, 1:400), Laminin(Abcam, ab11575, 1:500),Collagen I(Abcam, ab21286, 1:500),SDHA(Abcam, ab14715, 1:500),Pax-7 (Abcam, ab187339, 1:100),Pax-7(DSHB, 5 µg/mL)
Validation	MyoD, Pax7, SDHA, collagen I, Desmin and Laminin antibodies have been cited in several publications. Antibody against Klotho (R&D systems, MAB1819, Lot# KGN0315101) has been validated using a Klotho knockout muscle in a two independent publications from the laboratory (Sahu et al, Nat Comm, 2018; Iijima et al, BioRxiv, 2021).

Animals and other organisms

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

Laboratory animals	Aged male C57BL/6 mice used in these studies were obtained from NIA (21-23 months). The original breeders for Klotho strain mice were obtained from MMRRC, UC Davis. The mouse colony for the laboratory was obtained through further breeding by Dr. Fabrisia

Ambrosio's laboratory. KI homozygotes (3-6 months, KI+/+, B6; 129S5-Kltm1-Lex) and KI heterozygotes (3-6 months, KI+/-; B6; 129S5-Kltm1-Lex) were used obtain EVs. Aged male mice and KI knockout mice (4-6 weeks, KI-/-; B6; 129S5-Kltm1-Lex) were used to isolate muscle progenitor cells.

Wild animals | N/A

Field-collected samples | N/A

Ethics oversight | All animal experiments were performed with prior approval from the Institutional Animal Care and Use Committee of the University of Pittsburgh (IACUC protocol # 20087744, 20098045).

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