Corresponding author(s): Tony Wyss-Coray (twc@stanford.edu)
Benoit Lehallier (lehallib@stanford.edu)

Last updated by author(s): 10/22/2019

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.

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	No software was used for data collection
Data analysis	R version 3.6.1 with packages: DEswan (0.0.0.9001), car (3.0-3), topGO (2.36), clusterProfiler(3.12.0) , org.Hs.eg.db (3.8.2) , NHANES (2.1.0), ade4 (1.7-13), SuperExactTest (1.0.7), qgraph (1.6.3). Details and references can be found within text in the relevant Methods sections.

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. 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

We created a searchable web interface to mine the human INTERVAL and LonGenity datasets (https://twc-stanford.shinyapps.io/aging_plasma_proteome/)
The Human independent cohorts and mouse protein data are available in Supplementary Tables 16 and 17. The INTERVAL data is available through the European Genome-Phenome Archive (https://ega-archive.org/studies/EGAS00001002555).

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	Methods, "Human cohorts characteristics" and "Validation of the aging signature in mice" subsections. Human cohorts. This is a discovery study, not focusing on specific effect-size. Power calculation are not applicable for our study but we are well-powered (4000+ subjects) and we identified hundreds of highly significant changes after adjustment for multiple comparisons. Sample sizes of the Human cohorts are listed in Extended Data 1) Mouse cohorts. No statistical methods were used to predetermine sample size. Sample size was determined based on the number of animals used in prior experiments conducted in the Wyss-Coray lab (Villeda et al., Nature 2011; Villeda et al., Nature Medicine 2014, Yousef et al., Nature Medicine 2019). Again, we are well powered (110 mice) and we identified hundreds of highly significant changes. Sample sizes of the mouse cohorts are listed in Supplementary Table 9.
Data exclusions	Methods, "Human cohorts characteristics" and "Validation of the aging signature in mice" subsections. For the 4 independent cohorts and the mouse data. "Data for 1305 SOMAmer probes were obtained and no sample or probe data were excluded" For the Interval cohort, we used the dataset publicly available without excluding samples / proteins For the LonGenity cohort. "Sixty-eight subjects without clinical and functional data were excluded from the analysis." By excluding these samples, the same subjects (and not different subsets) were used in the whole paper, making the interpretation of the results more straightforward. For the Addneuromed data, no exclusion criteria were pre-established but "we filtered out 4 addneuromed samples based on visual inspection of the results of a Principal Component Analysis (PCA)".
Replication	All attempts at replication were successful (See Extended Data 2, 3a, 4).
Randomization	Human cohorts. Within each subcohort, the age was balanced and age distribution between cohorts overlapped to assess cohort and batch effect. Sex was balanced. Mouse cohorts. Number of male mice per group was balanced. Female mice were available only at 3m, 12, 18 and 21 months.
Blinding	Somalogic measured proteins levels without any information about the samples.

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
☐	☒ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data

Methods

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

Animals and other organisms

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

Laboratory animals	Methods, "Validation of the aging signature in mice" subsection. A total of 110 male and virgin female C57BL/6JN mice were used. Mouse groups are summarized in ST9. In the aging cohort, 6 1 months old (mo), 10 3mo, 6 6mo, 6 9mo, 10 12mo, 6 15mo, 10 18mo, 10 21 mo, 5 24 mo, 6 27mo and 6 30mo were used. In the parabiosis cohort, 11 4mo and 18 19mo were used.
Wild animals	This study did not involve wild animals

Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	Methods, "Validation of the aging signature in mice" subsection. All animal care and procedures were carried out in accordance with institutional guidelines approved by the VA Palo Alto Committee on Animal Research

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Participants were healthy blood donors from the Interval, LonGenity, VAseattle, GEHA, PRIN06 and PRIN09 cohorts. For Interval, age ranged from 18 to 76 years with a median age of 45 (1st Quartile=31, 3rd Quartile=55). For LonGenity, age ranged from 61 to 95 years with a median age of 74 (1st Quartile=69, 3rd Quartile=80). For the 4 independent cohorts (combined). Age ranged from 21 to 107 years with a median of 70 years (1st Quartile=58, 3rd Quartile=89).
Recruitment	Cohorts characteristics are summarized in Extended data 1 Participants in the INTERVAL randomized controlled trial were recruited with the active collaboration of the National Health Service Blood and Transplant England (www.nhsbt.nhs.uk), which has supported field work and other elements of the trial. As described by Sun et al. (Nature, 558, pages73–79, 2018), 50,000 participants were enrolled in the randomized trial of varying blood donation intervals. People with a history of major diseases (e.g. myocardial infarction, stroke, cancer, HIV, and hepatitis B or C) or with recent illness or infection were excluded . For proteomics measurements, subjects were randomly selected. LonGenity is an ongoing longitudinal study initiated in 2008, designed to identify biological factors that contribute to healthy aging. The LonGenity study enrolls older adults of Ashkenazi Jewish descent, age 65-94 years at baseline. Approximately 50% of the cohort consists of offspring of parents with exceptional longevity (OPEL), defined by having at least one parent that survived to 95 years of age. Subjects were randomly selected within each cohort. Samples from the 4 independent cohort were obtained from cohorts in US (VAseattle) and Europe (GEHA, PRIN06 and PRIN09). GEHA cohort (Franceschi et al. Ann N Y Acad Sci 2007) and VAseattle samples (Britschgi, M., et al. Mol Cell Proteomics 2011) used were described previously. Subjects from the PRIN06 and PRIN09 cohorts were enrolled by multiple Italian study centers. Subjects were randomly selected within each cohort.
Ethics oversight	All participants from the Interval cohort gave informed consent before joining the study and the National Research Ethics Service approved this study (11/EE/0538) The LonGenity study was approved by the Institutional Review Board (IRB) at the Albert Einstein College of Medicine. The IRB has determined that our research using VAseattle, GEHA, PRIN06 and PRIN09 cohorts does not meet the definition of human subject research per STANFORD's HRPP policy because 1) we are not obtaining or receiving private individually identifiable information 2) data or specimens were not collected specifically for this study 3) no direct intervention or interaction. For these reasons, this part of the study did not require approval from the IRB

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	ISRCTN24760606
Study protocol	Study protocol is described by Moore et al (Trials. 17;15:363, 2014, https://www.ncbi.nlm.nih.gov/pubmed/25230735)
Data collection	According to Moore et al (Trials. 17;15:363, 2014), INTERVAL is a randomised trial of whole blood donors enrolled from all 25 static centres of NHS Blood and Transplant. Recruitment of about 50,000 male and female donors started in June 2012 and was completed in June 2014.
Outcomes	According to Moore et al (Trials. 17;15:363, 2014), the primary outcome is the number of blood donations made. Multiple secondary outcome were investigated. The most important are (i) donor quality of life (assessed using the Short Form Health Survey) and (ii) the number of 'deferrals' due to low haemoglobin (and other factors), iron status, cognitive function, physical activity, and donor attitudes. Several papers published the results of this clinical trial 2016 results in: https://www.ncbi.nlm.nih.gov/pubmed/27645285 2017 results in: https://www.ncbi.nlm.nih.gov/pubmed/28941948 2019 extension study results in: https://www.ncbi.nlm.nih.gov/pubmed/31383583