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	Immunofluorescence images were acquired with confocal laser scanning microscope ZEISS LSM 900. Brightfield images were acquired with the Vectra Polaris system (v1.0, PerkinElmer) or Leica Aperio VERSA 200 system. Metagenome libraries were sequenced on the MGI DNBSEQ-T7 platform. Bulk RNA-seq libraries were sequenced on the MGI DNBSEQ-T7 platform. Metabolome were sequenced on the LC-MS (1290 Infinity LC, Agilent Technologies and AB Sciex TripleTOF 6600).
Data analysis	GraphPad Prism (version 8.0.1) kneadData (version 0.10.0) Bowtie2 (version 2.4.1) MetaPhlAn (version 3.0.13) HUMAnN3 (version 3.0.0) CAMERA (version 3.6) ProteoWizard MSConvert (version 3.0.6428) XCMS (version 3.14.1) vegan (version 2.6.8) shortBRED (version 0.9.3_dev_702e3ef) Scikit-Learn (version 0.24.1) MaAsLin2 (version 1.16.0) glmnet (version 4.1.8) QIIME 2 (version 2021.11.0) DADA2 (version 2021.11.0)

Trim Galore (version 0.6.7)
STAR (version 2.7.1a)
StringTie (version 2.1.7)
DESeq2 (version 1.42.1)
Clusterprofiler (version 3.1.0)
Megahit (version 1.2.9)
metaWRAP (version 1.3.2)
dRep (version 3.4.0)
checkM (version 1.2.1)
iTOL (version 7)
Prodigal (version 2.6.3)
BLASTx (version 2.13.0)

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

Raw metagenomic sequencing data generated in this study for the discovery cohort have been deposited in the National Genomics Data Center (NGDC) under accession number CRA028886 (BioProject PRJCA039092). The remaining discovery cohort multi-omics datasets were generated in our previous study and are publicly available, including RNA-seq (HRA003766), proteomics (PXD041432), and metabolomics (OMIX003055). Validation cohort datasets (BioProject PRJCA037542), which were generated in previous study, are available through the Aging Biobank database of the China National Center for Bioinformation (https://cbb.cncb.ac.cn/abb). The corresponding RNA-seq, metagenomic, proteomic, and metabolomic datasets are available under accession numbers HRA011466, CRA025612, OMIX016647, and OMIX010131, respectively.
For mouse multi-omics data, raw mouse RNA-seq data have been uploaded to the GSA database under accession number CRA029004, and the full mouse metabolomics dataset is deposited in the OMIX database under accession number OMIX011411.

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	Discovery cohort: 257 individuals (141 male, 116 female) Validation cohort: 1,483 individuals (618 male, 865 female)
Reporting on race, ethnicity, or other socially relevant groupings	Han Chinese individuals
Population characteristics	Discovery cohort: Age 20-87 years Validation cohort: Age 18-90 years
Recruitment	Across all participating cohorts, a standardized protocol was implemented. Volunteers were required to have abstained from antibiotic use for at least three months before sample collection and consumed a uniform, standardized diet on the day before sampling to minimize confounding variables.
Ethics oversight	All human experimental procedures were approved by the respective Research and Ethical Committees of the following institutions: Quzhou People's Hospital (LSYD-2020-12-001), Ningbo Hangzhou Bay Hospital (KY2019-101-CR-04), Xuanwu Hospital Capital Medical University (LYS-2024-162-001), The First Affiliated Hospital of Nanchang University (IIT-2024-394), Beijing Institute of Genomics (China National Center for Bioinformation), Chinese Academy of Sciences (2023H001, 2024H022, 2024H023, 2024H032), and Institute of Zoology, Chinese Academy of Sciences (DYLZ-2024-024, DYLZ-2024-025, DYLZ-2024-027). All participants provided written informed consent prior to their inclusion in the study. The collection of biological samples and data complied with the guidance of the Human Genetic Resource Administration, Ministry of Science and Technology of the People's Republic of China.

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 method was used to predetermine sample size, but similar sample sizes as previous studies were used in this study. The exact sample sizes (i.e.,n) are provided in the figure legends.
Data exclusions	In the analysis of metagenome data, we focused on bacterial and archaeal species/pathways/gene families with a mean relative abundance >0.01% that were present in at least 5% of participants, to deal with sparse microbial data in the downstream analysis.
Replication	The information has been presented in the figure legends.
Randomization	
Blinding	Data collection and analysis were performed in a blinded manner including behavioral recordings and histological markers.

Behavioural & social sciences study design

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

Study description	
Research sample	
Sampling strategy	
Data collection	
Timing	
Data exclusions	
Non-participation	
Randomization	

Ecological, evolutionary & environmental sciences study design

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

Study description	
Research sample	
Sampling strategy	
Data collection	
Timing and spatial scale	
Data exclusions	
Reproducibility	
Randomization	
Blinding	

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	
Location	
Access & import/export	
Disturbance	

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 Rabbit monoclonal anti-p21 antibody (Abcam, Cat# ab188224, RRID: AB_2734729) Rabbit monoclonal anti-S100A8 antibody (Abcam, Cat# ab180735; RRID: AB_2184122) Rabbit monoclonal anti-p21 Waf1/Cip1 (12D1) antibody (Cell Signaling Technology, Cat# 2947; RRID: AB_823586) Rabbit polyclonal anti-Ki67 antibody (Abcam, Cat# ab15580, RRID: AB_443209) Mouse monoclonal anti-Ki-67 antibody reagent (ZSGB-Bio, Cat# ZM-0166, RRID: AB_2890067) Rabbit polyclonal anti-Phospho-Histone H2A.X (Ser139) Antibody (Cell Signaling Technology, Cat# 2577, RRID: AB_2118010) Rabbit polyclonal anti-H3K9me3 antibody (Abcam, Cat# ab8898, RRID: AB_306848) Rabbit polyclonal anti-CD45 antibody (Abcam, Cat# ab10558, RRID: AB_442810) Rabbit polyclonal anti-Lamin B1 antibody (Abcam, Cat# ab16048, RRID: AB_443298) Secondary antibodies Goat-anti-rabbit secondary antibody kit (ZSGB-BIO, PV-9001) anti-Mouse IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor™ 488 (Invitrogen, Cat# A-21202; RRID:AB_141607) anti-Mouse IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor 568 (Invitrogen, Cat# A10037; RRID:AB_11180865) anti-Rabbit IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor™ 488 (Invitrogen, Cat# A21206; RRID:AB_2535792) anti-Rabbit IgG (H+L) highly cross-adsorbed secondary antibody, Alexa Fluor™ 568 (Invitrogen, Cat# A10042; RRID:AB_2534017) Mitochondrial Superoxide Assay Kit with MitoSO™ Red (Beyotime, Cat# S0061M)
Validation	All the antibodies used in this study have been tested by the manufacturer and have been cited by other authors and the references are available on the manufacturer's websites. We have further evaluated the specificity of the antibodies in our samples by analyzing the distribution of the antibody signals and the absence of the antibody signals in the regions where the target protein was not supposed to be expressed.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Human primary small intestinal mucosal epithelial cells (HUM-iCell-d007) from a 20-year-old male donor were purchased from iCell Bioscience Inc, Shanghai. Human mesenchymal stem cells were directed differentiation from hESCs directed differentiation as described in the previous study (DOI: 10.1093/procel/pwad027).
Authentication	The human primary small intestinal mucosal epithelial cells were authenticated by detecting the expression of pan-CK and CK18.
Mycoplasma contamination	All cell lines tested were negative for mycoplasma contamination.

Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Palaeontology and Archaeology

Specimen provenance	
Specimen deposition	
Dating methods	

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	

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

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	C57BL/6 mice (Mus musculus) were obtained from Zhejiang Vital River Experimental Animal Technology Co., LTD (certificate number: SCXK (Zhe) 2024-0001) and bred by the Experimental Animal Center of Quzhou People's Hospital. Standardized breeding conditions were maintained as previously described in detail. Specifically, the animals were housed in an artificial climate-controlled room set at a constant temperature of 25°C and subjected to a 12-hour light/dark cycle. Ad libitum access to food and drinking water was provided throughout the study. Prior to the experiment, rigorous selection criteria were applied to ensure that all selected animals were free from potential pathological factors that could interfere with the assessment of aging-related phenotypes.
Wild animals	This study did not involve wild animals.
Reporting on sex	Only male mice were analyzed to avoid the influence of gender.
Field-collected samples	No field-collected samples were used.
Ethics oversight	All mouse experimental procedures in this study were conducted in accordance with the guidelines set by the Animal Care and Use Institutional Committee of the Beijing Institute of Genomics, Chinese Academy of Sciences (BIG-2024A014) and Quzhou People's Hospital (202309-06).

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	
Study protocol	
Data collection	
Outcomes	

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☐	☐	Demonstrate how to render a vaccine ineffective
☐	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
☐	☐	Enable evasion of diagnostic/detection modalities
☐	☐	Enable the weaponization of a biological agent or toxin
☐	☐	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	
Files in database submission	
Genome browser session (e.g. UCSC)	

Methodology

Replicates	
Sequencing depth	
Antibodies	
Peak calling parameters	
Data quality	
Software	

Flow Cytometry

Plots

Confirm that:

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	N/A
Instrument	N/A
Software	N/A
Cell population abundance	N/A
Gating strategy	N/A

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	
Design specifications	
Behavioral performance measures	

Acquisition

Imaging type(s)	
Field strength	
Sequence & imaging parameters	
Area of acquisition	

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	
Normalization	
Normalization template	
Noise and artifact removal	
Volume censoring	

Statistical modeling & inference

Model type and settings	
Effect(s) tested	

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

(See Eklund et al. 2016)

Correction

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Graph analysis

Multivariate modeling and predictive analysis