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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	scRNA-Seq data was produced with the 10X Chromium Controller. Bulk RNA-seq data was produced with the Illumina NovaSeq 6000 platform. CyTOF data was acquired using the Helio3 CyTOF Mass Cytometer (Fluidigm). Flow cytometry data were collected using BD FACSDiva Software (v8.0.1).
Data analysis	Softwares: Trimmomatic (v0.39), FastQC (v0.11.9), fastp (v0.22.0), HISAT2 (v2.1.0), StringTie (v2.1.3b), Change-O (v1.3.0), Cell Ranger (v3.0.2), R (v4.3.0), Python (v3.11.0), FlowJo (v10.8.1), Cytoscape (v3.10.1), R packages: AUCell (v1.22.0), biomaRt (v2.56.1), clusterProfiler (v4.0.5), ComplexHeatmap (v2.16.0), flowCore (v2.14.0), CATALYST (v1.26.0), edgeR (v3.42.4), ggplot2 (v3.5.1), ggpubr (v0.6.0), ggrepel (v0.9.5), monocle (v2.6.1), org.Hs.eg.db (v3.17.0), pheatmap (v1.0.12), randomForest (v4.7-1.1), rstatix (v0.7.2), scales (v1.3.0), scater (v1.30.1), SCENIC (v1.3.1), Seurat (v5.0.1), SingleCellExperiment (v1.24.0), Stats (v4.3.0), tidyverse (v2.0.0), vegan (v2.6-4), VennDiagram (v1.7.3), tibble (v3.2.1), RColorBrewer (1.1-3), STARTRAC (v0.1.0), DescTools (v0.99.54), immunarch (v1.0.0), SCOPer (v1.3.0), cytofWorkflow (V1.14.0). Python package: Scrublet (v0.2.3), scvi-tools (v1.1.0), torch (v2.0.1), umap-learn (v0.5.6) Other software: GraphPad Prism (v8.4.3), Cytoscape (v3.10.1).

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

The raw single-cell RNA sequencing data reported in this study are available from Genome Sequence Archive for human with accession number: HRA009014 (https://bigd.big.ac.cn/gsa-human/browse/HRA009014). The processed gene expression of single-cell RNA sequencing data (Seurat object RDS file), the expression profiles of bulk-RNA sequencing data, and CyTOF single-cell protein data were uploaded into the SYNAPSE database (https://www.synapse.org), with accession numbers: syn61592786, syn61853526 and syn61765870, respectively. The machine learning-based immune age prediction framework has been made publicly available on the online website (https://pu-lab.sjtu.edu.cn/shiny/siage-model/). The source code of the model is available at GitHub (https://github.com/shanzha9/siAgeM-odel.git). data are for academic purposes only.
The external validation datasets can be accessed under ID: GSE158055, GSE168732, GSE162025, GSE135779, and HRA000150.
The CellTalkDB database can be accessed at http://tcm.zju.edu.cn/celltalkdb/. The CKTTD database can be accessed at https://www.ckttdb.org/. The Molecular Signatures Database can be accessed at https://www.gsea-msigdb.org/gsea/msigdb.

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	We deliberately balanced the sex ratio of participants during enrollment. Detailed participant information is provided in Supplementary Table 1.
Reporting on race, ethnicity, or other socially relevant groupings	All the healthy donors in this study are of East Asian ancestry.
Population characteristics	Characteristics of all participants are detailed in Supplementary Table 1.
Recruitment	After providing written informed consent in accordance with the criteria set by the Declaration of Helsinki (2013), 220 healthy volunteers aged 0 to ≥90 years were recruited from Shanghai Pudong Cohort (NCT05206643). All participants met the strict healthy criteria originally developed for enrollment in the Baltimore Longitudinal Study of Aging and in accordance with the WHO classification. Participants declared no specific disease issues. Participants with any of the following conditions were excluded: (1) cardio-cerebrovascular diseases (e.g., hypertension, coronary artery disease, congestive heart failure, and stroke); (2) neurodegenerative disorders or cognitive impairment; (3) endocrine and metabolic diseases (e.g., diabetes mellitus, dyslipidemia, and hypo- or hyperthyroidism); (4) hematological disorders; (5) renal or hepatic dysfunction; (6) infectious diseases (e.g., human immunodeficiency virus infection, human papillomavirus infection, hepatovirus infection, COVID-19 infection, and cytomegalovirus infection); (7) autoimmune diseases and motor dysfunctions; (8) cancer; (9) smoking; and (10) steroid usage and vaccination within three months. The potential for self-selection bias in recruiting healthy participants is that individuals who volunteer for research studies may have different health-seeking behaviors or socioeconomic statuses compared to the general population. To mitigate this, we ensured diverse recruitment channels to reach a broader demographic and included thorough participant screening to align with population health characteristics. This may impact the generalizability of our findings in reflecting the immune status of less health-aware or less accessible populations.
Ethics oversight	All human samples were obtained with written informed consent in accordance with protocols approved by the Institutional Review Board at Shanghai Jiao Tong University. All donors were enrolled with approval by the Ethics Committee of Ren Ji Hospital (KY2019-136), School of Medicine, Shanghai Jiao Tong University.

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	A total of 220 healthy volunteers aged 0 to ≥ 90 years were recruited from Shanghai Pudong Cohort (NCT05206643), including 61 for scRNA and scTCR/scBCR sequencing, 70 for independent CyTOF-based single-cell protein validation experiments, 34 for independent bulk RNA-seq

validation experiments, and 55 for flow cytometry validation experiments.
This study is observational, and the sample size depends on the number of eligible volunteers we were able to recruit. The conclusions of this study have undergone proper statistical testing and reflect the actual values observed in the sample.

Data exclusions

No samples were excluded from scRNA-seq, bulk RNA-seq, CyTOF and flow cytometry datasets.
scRNA-seq cell exclusions: doublets (doublet rate = 0.075, doubletScore <0.25), cells expressing <400 genes, cells with >2500 detected genes, cells with mitochondrial gene count percentage >10%. Cells with two TCR α- and two TCR β-chains, or more than two TCR α- and/or TCR β-chains, were assumed to be doublets and discarded.
Single cells expressing two sets of well-studied canonical markers of major cell types were labeled as doublets and excluded from the following analyses.

Replication

For scRNA-seq, CyTOF, and bulk-RNA seq cohort, data was analyzed at a cohort level using the relevant samples/time points to derive statistically meaningful conclusions.
For experiments, immunofluorescence staining, flow cytometric analyses and animal study were replicated independently at least three times.

Randomization

This was an observation cohort with only one experimental group, hence randomization was not relevant to scRNA-seq, CyTOF, and bulk-RNA seq cohort in this study.
Stimulation experiments for both PBMCs and B cells, cells from each donor were present in both basic and stimulated conditions.
Mice of matching age and sex were randomly distributed for each group.

Blinding

This study is observational, and we did not intervene with the volunteers; therefore, a double-blind experiment was not conducted

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

Specificity; label; clone; manufacturer; identifier
anti-human CD3; 115In; UCHT1; Bioxcell; BE0231
anti-human CD56; 141Pr; NCAM16.2; BD; 559043
anti-human TCR γ/δ; 142Nd; 5A6.E9; PLT; 100P001A
anti-human CCR6; 143Nd; G034E3; Biolegend; 353402
anti-human CD14; 144Nd; M5E2; Biolegend; 301862
anti-human IgD; 145Nd; IA6-2; Biolegend; 348202
anti-human CD38; 146Nd; HIT2; Biolegend; 303502
anti-human CXCR3; 147Sm; G025H7; Biolegend; 353750
anti-human CD1c; 148Nd; L161; Biolegend; 331502
anti-human CD62L; 149Sm; DREG-56; Biolegend; 304854
anti-human ICOS; 150Nd; C398.4A; Biolegend; 313502
anti-human GNLY; 151Eu; RB1; BD; 558254
anti-human CD45RA; 152Sm; HI100; Biolegend; 304102
anti-human CCR4; 153Eu; L291H4; Biolegend; 359402
anti-human HLA-DR; 154Sm; L243; Biolegend; 307648
anti-human IL-3Rα; 155Gd; 6H6; Biolegend; 306002
anti-human ZNF683; 156Gd; Sanquin-Hobit/1; BD; 624382
anti-human IgG; 157Gd; M1310G05; Biolegend; 410701
anti-human CCR7; 158Gd; G043H7; Biolegend; 353256
anti-human CD11c; 159Tb; Bu15; Biolegend; 337202
anti-human Helios; 160Gd; 22F6; Biolegend; 137202
anti-human CTLA-4; 161Dy; BN13; Bioxcell; BE0190
anti-human CD27; 162Dy; O323; Biolegend; 302802
anti-human Syndecan-1; 163Dy; DL-101; Biolegend; 352302
anti-human RORC; 164Dy; 600214; RD; MAB6109
anti-human CD161; 165Ho; HP-3G10; Biolegend; 339902
anti-human CD69; 166Er; FN50; Biolegend; 310902
anti-human GZMB; 167Er; QA16A02; Biolegend; 372202

anti-human IgM; 168Er; MHM-88; Biolegend; 314502
anti-human CD16; 169Tm; 3G8; Biolegend; 302057
anti-human Tim-3; 170Er; F38-2E2; Biolegend; 345010
anti-human PD-1; 171Yb; EH12.2H7; Biolegend; 329926
anti-human CD19; 172Yb; HIB19; Biolegend; 302268
anti-human IL-2Rα; 173Yb; 24212; RD; MAB1020
anti-human IL-7Rα; 174Yb; A019D5; Biolegend; 351302
anti-human TCR Vα7.2; 175Lu; 3C10; Biolegend; 351702
anti-human ITGAE; 176Yb; B-Ly7; eB; 14-1038-82
anti-human CD4; 197Au; RPA-T4; Biolegend; 301074
anti-human CD8a; 198Pt; RPA-T8; Biolegend; 301074
anti-human CD11b; 209Bi; M1/70; Bioxcell; BE0007
anti-human CD45; 89Y; HI30; Biolegend; 304002
anti-human IgM; APC; G20-127(RUO); BD; 551062
anti-human CD19; BB700; SJ25C1(RUO); BD; 566396
anti-human GNLY; -; EPR22110-101; Abcam; ab241333
anti-human IgM; -; SA-DA4; Thermo Fisher Scientific; 14-9998-82
anti-human J Chain; -; OTI3B3; Thermo Fisher Scientific; MA5-25840
anti-human IgA ; -; B-12; Santa Cruz; SC-166334

Validation

All antibodies are commercially available and validation statements can be found on the manufacturers' websites using the catalogue number. Antibodies were titrated to achieve optimal separation between negative and positive populations.
Anti-human CD3 antibody (https://bioxcell.com/invivomab-anti-human-cd3-be0231). The UCHT1 (Leu-4)(T3) monoclonal antibody reacts with human CD3ε tested by flow cytometry.
Anti-human CD56 antibody (https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/purified-mouse-anti-human-cd56.559043). Anti-CD56 monoclonal antibody reacts with immunoaffinity-enriched adult human brain NCAM and has been tested by flow cytometry.
Anti-human TCR gamma/delta antibody (https://www.thermofisher.cn/cn/zh/antibody/product/TCR-gamma-delta-Antibody-clone-5A6-E9-Monoclonal/TCR1061). Anti-TCR gamma/delta monoclonal antibody reacts with Human TCR gamma/delta. This antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human CCR6 antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-cd196-ccr6-antibody-7512). Anti-CCR6 monoclonal antibody reacts with Human, Cynomolgus, Rhesus CCR6. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human CD14 antibody (https://www.biolegend.com/en-gb/products/ultra-leaf-purified-anti-human-cd14-antibody-9582). Anti-CD14 monoclonal antibody reacts with Human, Cynomolgus, Rhesus CD14. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human IgD antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-igd-antibody-6527). Anti-IgD monoclonal antibody reacts with Human IgD. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human CD38 antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-cd38-antibody-748). Anti-CD38 monoclonal antibody reacts with Human CD38. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human CXCR3 antibody (https://www.biolegend.com/en-gb/products/ultra-leaf-purified-anti-human-cd183-cxcr3-antibody-17829). Anti-CXCR3 monoclonal antibody reacts with Human, Cynomolgus, Rhesus CXCR3. Each lot of this antibody is quality control tested by flow cytometric analysis.
Anti-human CD62L antibody (https://www.biolegend.com/en-gb/products/ultra-leaf-purified-anti-human-cd62l-antibody-19308). Anti-CD62L monoclonal antibody reacts with Human CD62L. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human/mouse/rat CD278 (ICOS) antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-mouse-rat-cd278-icos-antibody-2477). Anti-ICOS monoclonal antibody reacts with Human, Mouse, Rat ICOS. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-Human Granulysin (GNLY) antibody (https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/alexa-fluor-488-mouse-anti-human-granulysin.558254). Anti-GNLY monoclonal antibody reacts with Human GNLY. Each lot of this antibody is quality control tested by flow cytometric analysis.
Anti-human CD45RA antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-cd45ra-antibody-689). Anti-CD45RA monoclonal antibody reacts with Human CD45RA. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human CD194 (CCR4) antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-cd194-ccr4-antibody-8744). Anti-CCR4 monoclonal antibody reacts with Human CCR4. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human HLA-DR antibody (https://www.biolegend.com/en-gb/products/ultra-leaf-purified-anti-human-hla-dr-antibody-8092). Anti-HLA-DR monoclonal antibody reacts with Human, Cynomolgus, Rhesus HLA-DR. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by CyTOF.
Anti-human CD123 (IL-3Rα) antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-cd123-antibody-578). Anti-IL-3Rα monoclonal antibody reacts with Human IL-3Rα. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by CyTOF.
Anti-human IgG Fc antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-igg-fc-11779). Anti-IgG monoclonal antibody reacts with Human IgG. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by CyTOF.
Anti-human CD197 (CCR7) antibody (https://www.biolegend.com/en-gb/products/ultra-leaf-purified-anti-human-cd197-ccr7-antibody-18257). Anti-CCR7 monoclonal antibody reacts with Human CCR7. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human CD11c antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-cd11c-antibody-5392). Anti-CD11c monoclonal antibody reacts with Human CD11c. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by CyTOF.
Anti- mouse/human Helios antibody (https://www.biolegend.com/en-gb/products/purified-anti-mouse-human-helios-

antibody-7174). Anti-Helios monoclonal antibody reacts with Mouse, Human Helios. Each lot of this antibody is quality control tested by intracellular flow cytometry, and verified by IP.
Anti-human CD152 (CTLA-4) antibody (https://www.bioxcell.com.cn/in-vivo-antibodies/invivomab-anti-human-ctla-4-cd152-be0190.html). Anti-CTLA-4 monoclonal antibody reacts with Human CTLA-4. Each lot of this antibody is reported by flow cytometric analysis.
Anti-human CD27 antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-cd27-antibody-812). Anti-CD27 monoclonal antibody reacts with Human, Cynomolgus, Rhesus CD27. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by CyTOF.
Anti-human CD138 (Syndecan-1) antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-cd138-syndecan-1-antibody-7312). Anti-Syndecan-1 monoclonal antibody reacts with Human Syndecan-1. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by IHC-P.
Anti-human /mouse ROR gamma /RORC/NR1F3 antibody (https://www.rndsystems.com/cn/products/human-mouse-rorgamma-rorc-nr1f3-antibody-600214_mab6109). Anti-NR1F3 monoclonal antibody reacts with Human, Mouse NR1F3. Each lot of this antibody is quality control tested by western blot.
Anti-human CD161 antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-cd161-antibody-5672). Anti-CD161 monoclonal antibody reacts with Human, Cynomolgus, Rhesus CD161. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by CyTOF.
Anti-human CD69 antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-cd69-antibody-1670). Anti-CD69 monoclonal antibody reacts with Human CD69. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by CyTOF.
Anti- human/mouse GZMB recombinant antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-mouse-granzyme-b-recombinant-antibody-14428). Anti-GZMB recombinant antibody reacts with Human, Mouse GZMB. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human IgM antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-igm-antibody-2877). Anti-IgM monoclonal antibody reacts with Human IgM. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human CD16 antibody (https://www.biolegend.com/en-gb/products/ultra-leaf-purified-anti-human-cd16-antibody-8089). Anti-CD16 monoclonal antibody reacts with Human, Cynomolgus, Rhesus CD16. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by CyTOF.
Anti-human CD366 (Tim-3) antibody (https://www.biolegend.com/en-gb/products/ultra-leaf-purified-anti-human-cd366-tim-3-antibody-7748). Anti-Tim-3 monoclonal antibody reacts with Human Tim-3. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human CD279 (PD-1) antibody (https://www.biolegend.com/en-gb/products/ultra-leaf-purified-anti-human-cd279-pd-1-antibody-7747). Anti-PD-1 monoclonal antibody reacts with Human PD-1. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human CD19 antibody (https://www.biolegend.com/en-gb/products/ultra-leaf-purified-anti-human-cd19-antibody-18785). Anti-CD19 monoclonal antibody reacts with Human CD19. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by CyTOF.
Anti-human CD25 (IL-2Rα) antibody (https://www.rndsystems.com/cn/products/human-cd25-il-2r-alpha-antibody-24212_mab1020). Anti-IL-2Rα monoclonal antibody reacts with Human IL-2Rα. Each lot of this antibody is quality control tested by western blot, flow cytometric analysis, blockade of receptor-ligand Interaction and CyTOF.
Anti-human CD127 (IL-7Rα) antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-cd127-il-7ralpha-antibody-7093). Anti-IL-7Rα monoclonal antibody reacts with Human IL-7Rα. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human TCR Vα7.2 antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-tcr-valpha7-2-antibody-7122). Anti- TCR Vα7.2 monoclonal antibody reacts with Human TCR Vα7.2. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.
Anti-human CD103 (ITGAE) antibody (https://www.thermofisher.cn/cn/zh/antibody/product/CD103-Integrin-alpha-E-Antibody-clone-B-Ly7-Monoclonal/14-1038-82). Anti-ITGAE monoclonal antibody reacts with Human ITGAE. Each lot of this antibody is quality control tested by flow cytometric analysis, and verified by flow cytometric analysis and IHC.
Anti-human CD4 antibody (https://www.biolegend.com/en-gb/products/ultra-leaf-purified-anti-human-cd4-antibody-17876). Anti-CD4 monoclonal antibody reacts with Human CD4. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by CyTOF.
Anti-human CD8a antibody (https://www.biolegend.com/en-gb/products/ultra-leaf-purified-anti-human-cd8a-antibody-18046). Anti-CD8a monoclonal antibody reacts with Human CD8a. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by CyTOF.
Anti- mouse/human CD11b antibody (https://www.biolegend.com/en-gb/products/purified-anti-mouse-human-cd11b-antibody-351). Anti- CD11b monoclonal antibody reacts with Mouse, Human CD11b. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by CyTOF and IHC-F.
Anti-human CD45 antibody (https://www.biolegend.com/en-gb/products/purified-anti-human-cd45-antibody-710). Anti-CD45 monoclonal antibody reacts with Human CD45. Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis, and verified by CyTOF and IHC-P.
Anti-human IgM antibody (https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-mouse-anti-human-igm.551062). Anti-IgM monoclonal antibody reacts with Human IgM. This antibody is quality control tested by Flow cytometry.
Anti-human CD19 antibody (https://www.bdbiosciences.com/zh-cn/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bb700-mouse-anti-human-cd19.566396). Anti-CD19 monoclonal antibody reacts with Human CD19. This antibody is quality control tested by Flow cytometry.
Anti-human Granulysin (GNLY) antibody (https://www.abcam.cn/products/primary-antibodies/gnlygranulysin-antibody-epr22110-101-ab241333.html). Anti-GNLY monoclonal antibody reacts with Human GNLY. This antibody is tested by IHC-P, WB, ICC/IF.
Anti-human IgM antibody (https://www.thermofisher.cn/cn/zh/antibody/product/IgM-Antibody-clone-SA-DA4-Monoclonal/14-9998-82). Anti-IgM monoclonal antibody reacts with Human IgM. This antibody is tested by flow cytometric analysis.
Anti-human J Chain antibody (https://www.thermofisher.cn/cn/zh/antibody/product/J-Chain-Antibody-clone-OTI3B3-Monoclonal/MA5-25840). Anti-J Chain monoclonal antibody reacts with Human J Chain. This antibody is tested by WB and IHC.

Anti-human IgA antibody (https://www.scbt.com/zh/p/iga-antibody-b-12). Anti-IgA monoclonal antibody raised against amino acids 1-300 mapping at the N-terminus of IgA of human origin. This antibody is tested by IHC-P, WB, IF, FCM.

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	To generate mice with overexpression of GNLY, cDNA for human GNLY was cloned and used to construct the transgene vector CAG-loxP-STOP-loxP-GNLY-WPRE-pA (Shanghai Model Organisms Center, Inc). The transgene vector was knocked in at the ROSA26 locus using CRISPR/Cas9 methods. To induce B-cell specific GNLY expression, CAG-loxP-STOP-loxP-GNLY-WPRE-pA TG mice were bred with CD19-CAG-Cre transgenic mice; littermate CD19-Cre mice were used as controls. Both male and female mice used in this study were on a C57BL/6 background and between 6-8 weeks of age. All mice were kept at 24°C ± 2°C, humidity of 40% ± 5%, under a constant 12-hour light/dark cycle with free access to diet and water.
Wild animals	This study did not involve wild animals.
Reporting on sex	Male and female.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The protocols for randomized animal experiments were approved by the Institute's Animal Ethics Committee of Shanghai Jiao Tong University, School of Medicine, Renji Hospital and adhered to the NIH Guidelines for the Care and Use of Laboratory Animals.

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	N/A
Study protocol	No clinical trial was performed - prospective observational cohort study.
Data collection	1. Epidemiological survey: Design the Public Health and Chronic Disease Prevention and Control Questionnaire in accordance with the principles and requirements of the cohort study. On the premise of informed notification, data are collected through face-to-face interviews between the studied cohort and investigators who signed the informed consent and received uniform training. Quality control staff will review the questionnaire for quality control. The main content includes demographic sociology, lifestyle, physical exercise, daily eating habits, usage of dietary supplements, personal and family medical history, psychological assessment, and female fertility history. 2. Physical examination: the examination is performed by the clinical professional physician in the health service center. The examination content includes height, weight, blood pressure, hearing, vision, internal surgery, body fat composition (optional), chest radiography, electrocardiogram, B-ultrasound, carotid ultrasound (optional), and other imaging examinations. 3. Clinical biochemical test: fasting blood collection should be performed by all individuals upon their enrollment. The blood samples collected are tested for biochemistry, liver and kidney function, blood lipid analysis, CMV/EBV/HBV DNAs and antibodies, hsCRP, and electrolyte analysis in accordance with clinical testing requirements. 4. Biological specimen collection: collect 10 ml blood samples using a health checkup, then send them to Renji Sample Bank for separation within 2 hours, and store in -80°C ultra-low temperature refrigerator. The blood samples are for multi-omics and serological studies.
Outcomes	N/A

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	PBMCs isolation: Blood samples were collected from the peripheral vein of each donor and processed within 2 hours of collection. UCB was obtained from placental cords. PBMCs were separated using Vacutainer CPT tubes (362761, BD, San Diego, CA, USA)

following the manufacturer's protocols. The viability of the PBMCs in each sample was examined by Trypan Blue staining and confirmed to be >90%. PBMCs were immediately used for scRNA-seq library construction and sequencing, or they were cryopreserved at −80°C in a medium containing 10% dimethylsulfoxide and 90% fetal bovine serum for CyTOF experiments.

B cells isolation:
Human PBMCs were separated from whole blood samples using Vacutainer CPT tubes (362761, BD) following the manufacturer's protocols. B cells were isolated from PBMCs using the Human Pan B Cell Isolation Kit (130-101-638, Miltenyi Biotec, Auburn, CA, USA) following the manufacturer's instructions. The purity of B cells was confirmed by flow cytometry (> 95% purity). Human B cells were cultured and expanded in ImmunoCult™ Human B Cell Expansion Kit (100-0645, STEMCELL Technologies, Vancouver, Canada) for further use.

Instrument

BD LSRFortessa X-20

Software

Flow cytometry data were collected using BD FACSDiva Software (v8.0.1). Flow cytometry data were analysed using FlowJo (v10.8.1; BD Biosciences).

Cell population abundance

The amounts of cells in flow tube tested by flow cytometry are 5*10^5/tube. The viability of the PBMCs in each sample was examined by Trypan Blue staining and confirmed to be >90%. The purity of B cells was confirmed by flow cytometry (> 95% purity).

Gating strategy

Representative gating strategy for select populations was provided in the Supplementary Fig.2. Additionally, single cells were gated for live/dead in all presented figures.

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