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	Cytek SpectroFlo software (Version 2.0.2), BD FACSChorus (v2)
Data analysis	FlowJo v10.10, FlowSOM v2.10.0, umap-learn v0.5.7, Cell Ranger v3.1.0, BarCounter v1.0.0, Scanpy v1.9.6, ArchR v1.0.2, NMFproj v1.0.1, rstatix v0.7.2, lme4 v1.1-35.3, DESeq2 v1.42.0, MACS3 v3.0.1, MAGIC v3.0.0, scyan v1.6.2, fgsea v1.28.0, Single Sample Enrichment Analysis(https://github.com/aifimmunology/IHA-Figure/blob/master/Extended_Figure5_1/03_DEGs_and_EnrichmentAnalysis/03_SLEA.ipynb) , CellPhoneDB v5.0.1,Celltypist v1.6.1-v1.6.2, Multicelltypist (https://github.com/aifimmunology/multicelltypist), CLR transformation(https://github.com/aifimmunology/IHA-Figure/blob/master/helper_function/helper_function_IHA.r), milopy v.0.1.0, ggplot 2 v3.4.0 and v3.5.1, matrixStats v1.4.1, BSgenome.Hsapiens.UCSC.hg38 v1.4.5, GenomicRanges v1.54.1, dplyr v1.1.4, SummarizedExperiment v1.32.0. coin v1.4.3, stats v4.3.2. Slingshot v2.10.0 All original code has been deposited at GitHub and Zenodo: https://github.com/aifimmunology/aifi-healthy-pbmc-reference/ (https://zenodo.org/records/13352146) for generation of the Immune Health Atlas, https://github.com/aifimmunology/sound-life-scrna-analysis/ (https://zenodo.org/records/13352142) for labeling and assembly of the Sound Life Cohort data, and https://github.com/aifimmunology/IHA-Figure (https://zenodo.org/records/17114439) for downstream analysis and figure generation. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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

Processed single-cell RNA-seq data have been deposited in the GEO database. (Sound Life cohort: GSE271896 and Follow-up cohort: GSE275067). Raw single-cell RNA-seq fastq files have been deposited in dbGaP (phs003841.v1.p1). All processed data, data objects, and clinical metadata derived from de-identified human subjects is available at the Human Immune System Explorer (HISE) website (doi:10.57785/e9e1-wh09 for AIFI Immune cell Atlas; doi:10.57785/bn17-3h16 for Dynamics of Immune Health & Age). This paper also analyzes existing, publicly available datasets (TEAseq dataset: GEO (GSE214546), dbGaP (phs003400.v1); Aging Influenza memory B cell dataset: GEO (GSE167823); Terekhova's aging dataset: Synapse (syn49637038); preRA dataset: https://apps.allenimmunology.org/aifi/insights/ra-progression/downloads/scrna/, dbGaP (phs003944.v1.p1, doi:10.57785/c0pq-s567); Onek1K dataset: https://cellxgene.cziscience.com/collections/dde06e0f-ab3b-46be-96a2-a8082383c4a1; Tissue aging dataset: https://cellxgene.cziscience.com/collections/cc431242-35ea-41e1-a100-41e0dec2665b).

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	Both males and females were recruited into this study, aiming for equal distribution of the sexes. We considered sex in the study design, determining biological sex based on self-report. Sex was included as a confounding factor in the DEG analysis.
Reporting on race, ethnicity, or other socially relevant groupings	We collected race and ethnicity information based on the self-report, but we didn't conduct any analysis or control them as confounding factor in our study.
Population characteristics	For the sound life cohort, young healthy adults (25-35 yr olds) and older healthy adults (55-65 yr olds) were recruited for the study. For the follow-up cohort, healthy adults (40-89+yr olds) were recruited for the study. Relevant covariates include age, sex and CMV status and are available in Supplemental Table 1.
Recruitment	For sound life cohort: Healthy 25-35 year old and 55-65 year old adult subjects were recruited from the greater Seattle area as part of the Sound Life project at Benaroya Research Institute (BRI). Patients were excluded from enrollment if they had a history of chronic disease, autoimmune disease, severe allergy, or chronic infection. Subjects enrolled by BRI were compensated for their time, effort and incidental expenses related to the research visits with $50 per research visit that involved a blood draw. For follow up cohort, adults (40-89+ yr old) were recruited in and around Palo Alto, CA. Volunteers aged 40+ were recruited via ads and flyers. Eligible participants completed a phone screening, then scheduled a visit after fasting for at least 8 hours. Exclusion criteria included recent infections, cancer, and immune-modulating medications. Participants were screened and enrolled based on age-sex balance. The assessments involved a detailed questionnaire, blood draws, and physical activity evaluation. Participants 65 and older underwent additional assessments. For both cohorts, access to specific blood draw sites could lead to selection bias based on geographical location and local demographics.
Ethics oversight	All studies were approved by Institutional Review Boards at Benaroya Research Institute (sound life cohort), Stanford University (follow up cohort), and/or Allen Institute (all sample usage). All adult participants gave informed consent prior to participation in these studies.

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	For the sound life cohort, we selected a sample size of 96 donors based on inclusion/exclusion criteria of healthy across all time points in the longitudinal study as well as sample availability. For the follow-up cohort, sample size was selected based on sample availability when balancing for age and sex across racial/ethnic groups present in the cohort.
Data exclusions	For the sound life cohort, we excluded three donors from our deep immune-profiling studies due to pre-malignacy in B cells identified during longitudinal monitoring over the course of the study, as the focus of this study was the non-diseased, healthy immune system

Replication	All experiments have been biologically replicated in at least three donors and results were successfully reproduced. All sequencing was performed using a common PBMC batch control for technical replication confirmation and normalization.
Randomization	For our deep immune profiling studies, all samples were analyzed, no randomization was required. Samples were randomized across experimental batches with common batch controls for higher data quality purposes. For flu and functional studies, donor time point samples were grouped together. No other randomization was performed.
Blinding	Experiments and analyses were not performed blinded as the same investigator(s) oversaw the sample processing, data generation and data analyses.

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

Antibody information including titers are included in Supplemental Table 4.

TotalSeq-A0251 anti-human Hashtag 1 AntibodyBioLegendCat # 394601, RRID: AB_2750015
TotalSeq-A0252 anti-human Hashtag 2 AntibodyBioLegendCat # 394603, RRID: AB_2750016
TotalSeq-A0253 anti-human Hashtag 3 AntibodyBioLegendCat # 394605, RRID: AB_2750017
TotalSeq-A0254 anti-human Hashtag 4 AntibodyBioLegendCat # 394607, RRID: AB_2750018
TotalSeq-A0255 anti-human Hashtag 5 AntibodyBioLegendCat # 394609, RRID: AB_2750019
TotalSeq-A0256 anti-human Hashtag 6 AntibodyBioLegendCat # 394611, RRID: AB_2750020
TotalSeq-A0257 anti-human Hashtag 7 AntibodyBioLegendCat # 394613, RRID: AB_2750021
TotalSeq-A0258 anti-human Hashtag 8 AntibodyBioLegendCat # 394615, RRID: AB_2750022
TotalSeq-A0259 anti-human Hashtag 9 AntibodyBioLegendCat # 394617, RRID: AB_2750023
TotalSeq-A0260 anti-human Hashtag 10 AntibodyBioLegendCat # 394619, RRID: AB_2750024
TotalSeq-A0262 anti-human Hashtag 12 AntibodyBioLegendCat # 394623, RRID: AB_2750025
TotalSeq-A0263 anti-human Hashtag 13 AntibodyBioLegendCat # 394625, RRID: AB_2750026
TotalSeq-A0264 anti-human Hashtag 14 AntibodyBioLegendCat # 394627, RRID: AB_2750027
TotalSeq-A0265 anti-human Hashtag 15 AntibodyBioLegendCat # 394629, RRID: AB_2750028
Mouse anti-human CD3/BUV395 (UCHT1)BD BiosciencesCat # 563546, RRID: AB_2744387
Mouse anti-human CD45/BUV496 (HI30)BD BiosciencesCat # 750179, RRID: AB_2868405
Mouse anti-human CD56/BUV563 (NCAM16.2)BD BiosciencesCat # 612928, RRID: AB_2870213
Mouse anti-human CD19/BUV615 (HIB19)BD BiosciencesCat # 751273, RRID: AB_2875287
Mouse anti-human CD27/BUV661 (L128)BD BiosciencesCat # 750167, RRID: AB_2874372
Mouse anti-human CD8/BUV737 (RPA-T8)BD BiosciencesCat # 749367, RRID: AB_2868408
Mouse anti-human CD39/BUV805 (Tu66)BD BiosciencesCat # 748472, RRID: AB_2872887
Mouse anti-human CD103/BV421 (Ber-ACT8)BioLegendCat # 350214, RRID: AB_2563514
Mouse anti-human abTCR/BV480 (IP26)BD BiosciencesCat # 746544, RRID: AB_2743835
Mouse anti-human CD223/BV605 (11C3C64)BioLegendCat # 369324, RRID: AB_2721541
Mouse anti-human CD95/BV650 (DX2)BioLegendCat # 305642, RRID: AB_2632622
Mouse anti-human CD127/BV711 (A019D5)BioLegendCat # 351328, RRID: AB_2562908
Mouse anti-human CD278/BV750 (DX29)BD BiosciencesCat # 746858, RRID: AB_2871662
Mouse anti-human CD45RA/BV786 (HI100)BioLegendCat # 304140, RRID: AB_2563816
Mouse anti-human CD185/BB515 (RF8B2)BD BiosciencesCat # 564624, RRID: AB_2738871
Mouse anti-human CD14/BB660 (MϕP9)BD BiosciencesCat # 624295 (custom), RRID: N/A
Mouse anti-human CD4/BB700 (SK3)BD BiosciencesCat # 566392, RRID: AB_2744421
Mouse anti-human HLA-DR/BB790 (G46-6)BD BiosciencesCat # 624296 (custom), RRID: N/A
Mouse anti-human CD279/PE (EH12.2H7)BioLegendCat # 329906, RRID: AB_940483
Mouse anti-human TIGIT/PE-Dazzle594 (A15153G)BioLegendCat # 372716, RRID: AB_2632931
Mouse anti-human CD38/PE-Cy5 (HIT2)BD BiosciencesCat # 555461, RRID: AB_395854
Mouse anti-human CD197/PE-Cy7 (G043H7)BioLegendCat # 353226, RRID: AB_11126145
Mouse anti-human CD69/APC (FN50)BioLegendCat # 310910, RRID: AB_314845
Mouse anti-human CD25/APC-R700 (2A3)BD BiosciencesCat # 565106, RRID: AB_2744339

Mouse anti-human KLRG1/APC-Fire750 (SA231A2)BioLegendCat # 367718, RRID: AB_2687392
Fixable Viability Stain 510BD BiosciencesCat # 564406, RRID: AB_2869572
Mouse anti-human CD3/PE-CF594 (UCHT1)BD BiosciencesCat # 562280, RRID: AB_11153674
Mouse anti-human CD7/BV650 (M-T701)BD BiosciencesCat # 740565, RRID: AB_2740266
Mouse anti-human CD14/BUV395 (M5E2)BD BiosciencesCat # 569102, RRID: AB_3099635
Mouse anti-human CD15/BUV395 (W6D3)BD BiosciencesCat # 740318, RRID: AB_2740054
Mouse anti-human CD16/FITC (3G8)BioLegendCat # 302006, RRID: AB_314205
Mouse anti-human CD19/BUV395 (HIB19)BD BiosciencesCat # 740287, RRID: AB_2740026
Mouse anti-human CD45/BUV496 (HI30)BD BiosciencesCat # 750179, RRID: AB_2868405
Mouse anti-human CD56/BV421 (NCAM16.2)BD BiosciencesCat # 562751, RRID: AB_2732054
Mouse anti-human CD57/APC (QA17A04)BioLegendCat # 393306, RRID: AB_2734460
Mouse anti-human NKG2A/APC-Fire750 (S19004C)BioLegendCat # 375116, RRID: AB_2888866
Mouse anti-human NKG2C/PE (S19005E)BioLegendCat # 375004, RRID: AB_2888871
Mouse anti-human Ki67/BV786 (B56)BD BiosciencesCat # 563756, RRID: AB_2732007
Mouse anti-human GranzymeB/PerCP-Cy5.5 (QA16A02)BioLegendCat # 372212, RRID: AB_2728379
Mouse anti-human GranzymeK/PE-Cy7 (GM26E7)BioLegendCat # 370516, RRID: AB_2715970
Mouse anti-human CD10/BV750 (HI10a)BD BiosciencesCat# 747517, RRID: AB_2872180
Mouse anti-human CD11c/AF700 (Bu15)BioLegendCat# 337220 (also 337219), RRID: AB_2561503
Mouse anti-human CD16/PE-CY7 (3G8)BioLegendCat# 302015 (also 302016), RRID: AB_314215
Mouse anti-human CD183/BV785 (G025H7)BioLegendCat# 353737 (also 353738), RRID: AB_2565923
Mouse anti-human CD19/BV650 (HIB19)BioLegendCat# 302238 (also 302237), RRID: AB_2562097
Mouse anti-human CD20/APC-Cy7 (2H7)BioLegendCat# 302314 (also 302313), RRID: AB_314262
Mouse anti-human CD21/BUV661 (1048)BD BiosciencesCat# 750187, RRID: AB_2874389
Mouse anti-human CD23/APC (EBVCS-5)BioLegendCat# 338514 (also 338513), RRID: AB_1501112
Mouse anti-human CD24/BV605 (ML5)BioLegendCat# 311124 (also 311123), RRID: AB_2562288
Mouse anti-human CD27/BV421 (L128)BD BiosciencesCat# 742731, RRID: AB_2741005
Mouse anti-human CD32/FITC (FUN-2)BioLegendCat# 303204, RRID: AB_314336
Mouse anti-human CD38/PE-Dazzle594 (HIT2)BioLegendCat# 303538 (also 303537), RRID: AB_2564105
Mouse anti-human CD45RB/PE (MEM-55)BioLegendCat# 310204, RRID: AB_314807
Mouse anti-human CD69/BUV395 (FN50)BD BiosciencesCat# 564364, RRID: AB_2738770
Mouse anti-human CD3/PE-Cy7 (UCHT1)BD BiosciencesCat# 563423, RRID: AB_2738196
Mouse anti-human CD7/PE-Cy7 (CD7-6B7)BioLegendCat# 343114 (also 343113), RRID: AB_2563941
Mouse anti-human CD14/PE-Cy7 (M5E2)BD BiosciencesCat# 557742, RRID: AB_396848
Mouse anti-human CD33/PE-Cy7 (P67.6)BioLegendCat# 366618 (also 366617), RRID: AB_2566420
Mouse anti-human CD61/PE-Cy7 (VI-PL2)BioLegendCat# 336416 (also 336415), RRID: AB_2566692
Mouse anti-human CD235a/PE-Cy7 (HIR2)BioLegendCat# 306619 (also 306620), RRID: AB_2716163
Mouse anti-human CD73/BUV805 (AD2)BD BiosciencesCat# 748584, RRID: AB_2872991
Mouse anti-human CD95/BV711 (DX2)BioLegendCat# 305644 RRID: AB_2632623
Mouse anti-human HLA-DR/BUV737 (G46-6)BD BiosciencesCat# 748339, RRID: AB_2872758
Mouse anti-human IgA/PerCP-Vio700 (IS11-8E10)MiltenyiCat# 130-113-478, RRID: AB_2733052
Mouse anti-human IgD/PE-Cy5 (IA6-2)BioLegendCat# 348249 (also 348250), RRID: AB_2876661
Mouse anti-human IgG/BUV496 (G18-145)BD BiosciencesCat# 741172, RRID: AB_2870743
Mouse anti-human IgM/BUV563 (UCH-B1)BD BiosciencesCat# 748929, RRID: AB_2873332
Purified Mouse IgGBioradCat#PMP01X, RRID: AB_482871
Mouse anti-human CD3/BUV395 UCHT1 BD Bioscience Cat#563546, RRID: AB_2744387
Mouse anti-human CD45RA/BUV496 HI100 BD Bioscience Cat#750258, RRID: AB_2874456
Mouse anti-human CD27/BUV805 L128 BD Bioscience Cat#569167, RRID: AB_3099637
Rat-anti-human IL-4/BV421 MP4-25D2 BD Bioscience Cat#564110, RRID:AB_2738599
Mouse anti-human CD185(CXCR5)/BV711 J252D4 Biolegend Cat#356934, RRID: AB_2629526
Mouse anti-human CD8/SparkBlue 550 SK1 Biolegend Cat#344760, RRID: AB_2819983
Mouse anti-human CD4/RB613 SK3 Biolegend Cat#571073, RRID: AB_3683575
Mouse anti-human CD40L/RB780 TRAP1 BD Bioscience Cat#755457, RRID:AB_3683576
Mouse anti-human CCR7/APC-Fire 750 G043H7 Biolegend Cat#353246, RRID:AB_2750147
Mouse anti-human IFNg/AlexaFluor 647 B27 BD Bioscience Cat#557729, RRID:AB_396837

Validation

All antibodies were purchased from established vendors with strict quality control assurances and validation statements can be found on the manufacturers' websites using the catalogue number or in the Antibody Registry database (https://antibodyregistry.org).

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.

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	All blood samples were collected, processed to PBMCs using a Ficoll-based approach and frozen in FBS with 10% DMSO within 4 hours of blood draw. Cryopreserved PBMCs were rapidly thawed, washed, and counted to assess viability and recovery. For T cell stimulations, T cells were then enriched from PBMC using StemCell EasySep Human T Cell Isolation Kit
Instrument	Cytek Aurora (5 laser)
Software	FlowJo v10.10, BD FACSChorus (v2), Cytek SpectroFlo software (Version 2.0.2)
Cell population abundance	To address the constant sum issue in compositional data, we applied a centered log ratio (CLR) transformation to frequency data. CLR was based on total T cells (Tfh data, cytokine stims) or 9 B cell types plus 1 non-B cell type (B cell data). For B cell isotype analysis, CLR was performed per cell type across isotypes.
Gating strategy	B cell analyses were done by unsupervised clustering. Adaptive NK cells were defined at single/CD3-CD14-CD15-CD19-CD56+ +CD16-CD57+NKG2C+ cells. Memory T cell subsets were defined using CD45RA, CCR7 and CD27 expression. Gating strategies are provided in Supplementary Information.

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