nature portfolio

Corresponding author(s): Cameron McAlpine

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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	Spectro Flow, Keyence Image aquisition, Seahorse XF Cell Mito Stress Test and Glycolysis Stress Test Report Generator (Agilent), Chromium platform (10x Genomics) , QuBit (ThermoFisher) and TapeStation (Agilent),
Data analysis	FlowJo v10.8.1, ImageJ/QuPath, Keyence BZ-X, GraphPad, CellRanger Multi v8.0.0 (10x Genomics), CellChat (version 2.1.0), Neuroscore, FIJI software and JACoP plugin, Design and Analysis 2 software (Applied Boiosystems),

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

scRNAseq data is deposited to NCBI-GEO record number: GSE317642. UK Biobanks and All of Us data are available to investigators by application. All other necessary data are contained within the manuscript. Requests for material can be made to the corresponding author.

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	Study was performed on men and women
Reporting on race, ethnicity, or other socially relevant groupings	Population is reported in Extended data Fig 3 and as previously described: Schuermans, A. et al. Clonal haematopoiesis of indeterminate potential predicts incident cardiac arrhythmias. Eur Heart J 45, (2024) and Khurshid, S., Al-Alusi, M. A., Churchill, T. W., Guseh, J. S. & Ellinor, P. T. Accelerometer-Derived 'weekend Warrior' Physical Activity and Incident Cardiovascular Disease. JAMA 330, (2023).
Population characteristics	82,834 individuals from the UK Biobank and 8,404 individuals from the All of Us dataset. Population is reported in Extended data Fig 3 and as previously described: Schuermans, A. et al. Clonal haematopoiesis of indeterminate potential predicts incident cardiac arrhythmias. Eur Heart J 45, (2024) and Khurshid, S., Al-Alusi, M. A., Churchill, T. W., Guseh, J. S. & Ellinor, P. T. Accelerometer-Derived 'weekend Warrior' Physical Activity and Incident Cardiovascular Disease. JAMA 330, (2023). The UK Biobank is a prospective study of UK adults aged 40-69 years at the time of recruitment between 2006-2010, of whom 454,787 underwent whole exome sequencing of blood DNA (median sequencing depth ~40x)46. A subset of 103,695 UK Biobank participants completed an accelerometer sub-study in which participants wore an Axivity AX3 wrist accelerometer for one week47. MVPA was quantified using a validated machine learning algorithm47–49. The All of Us Program is an ongoing U.S. cohort including 245,394 participants with available whole genome sequencing of blood DNA (median sequencing depth ≥30x)50. A subset of 15,538 All of Us participants submitted data from a Fitbit wrist-worn accelerometer, with MVPA duration quantified using the Fitbit algorithm49. After excluding individuals with prevalent hematologic malignancy and missing covariate data, we analyzed 82,834 individuals from the UK Biobank and 8,404 individuals from All of Us with available accelerometer and sequencing data.
Recruitment	N/A
Ethics oversight	N/A exempt

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	Power calculations were performed to determine sample size. Additionally, based on prior experience with mouse models and variation, sample number were determined.
Data exclusions	No data was excluded
Replication	All experiments were repeated at least 3 times. All replications were successful.
Randomization	In every experiment, mice were randomized to intervention within their genotype, sex, and experimental group.
Blinding	Investigators were blinded to group allocation during sample collection and analysis.

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

Single-cell suspensions were stained with antibody cocktail in MACS buffer (0.5% BSA and 2 mM EDTA in PBS) for 30 minutes at 4 °C, protected from light. To differentiate between live and dead cells, the cell suspensions were then stained with Live/Dead Blue (Thermo Fisher) at a dilution of 1:1.000 in PBS at 4 °C for 30 min. The following monoclonal antibodies were used for flow cytometry analyses at a dilution of 1:700 unless otherwise indicated: anti-CD45 (BioLegend, 30-F11), anti-CD45.1 (BioLegend, A20), anti-CD45.2 (BioLegend or Invitrogen, both 104), anti-CD11b (BioLegend, M1/70), anti-Ly-6G (BioLegend, 1A8), anti-Ly-6C (BioLegend, HK1.4), anti-f4/80 (BioLegend, BM8), anti-CD64 (BioLegend, X54-5/7.1), anti-CD68 (BioLegend FA-11), anti-CD11c (BioLegend, N418), anti-CD90.2 (Invitrogen, 53-2.1 and BioLegend, 30-H12), anti-CD4 (BioLegend RM4-5), anti-CD8a (BD Horizon, 53-6.7), anti-B220 (BioLegend, RA3-6B2), anti-CD49b (BioLegend, DX5), anti-Ter119 (BioLegend, TER-119), anti-CD11c (BioLegend, N418), anti-CD19 (BioLegend, 1D3/CD19), anti-CD127 (BioLegend, S18006K), anti-cKit (BioLegend, 2B8), anti-Sca-1 (BioLegend, E13-161.7), anti-CD135 (BioLegend, A2F10), anti-CD48 (BioLegend, HM48-1), anti-CD150 (BioLegend, TC15-12F12.2), anti-CD34 (eBioscience, RAM34), anti-CD16/32 (BioLegend, 93), anti-CD115 (BioLegend, AFS98; 16A8), anti-ki67 (Invitrogen, SolA15); anti-IL1b, anti-IL1R1 (Invitrogen, MA5-40958 or MA5-40959), Anti-MPO (1:100, Bio-Techne, AF3667), Anti-Histone H3 (1:100, abcam, ab5103). For intracellular staining, extracellular antibody staining was performed first for 30 min at 4 °C, followed by fixation with BD Cytofix for 20 min at 4 °C, and permeabilization for 30 min with BD Perm/Wash Buffer (BD Biosciences). Intracellular cytokine staining was performed at a dilution of 1:200 in BD Perm/Wash Buffer for 45 min at room temperature, protected from light. CountBright™ Absolute Counting Beads (Invitrogen Reference #C36950) were used to determine absolute cell counts. Data were collected on an Cytek Aurora cytometer with Spectroflow software and analyzed using FlowJo. FACS sorting: anti-CD45 (BioLegend, 30-F11), anti-CD45.1 (BioLegend, A20), anti-CD45.2 (BioLegend or Invitrogen, 104), and anti-CD11b (BioLegend, M1/70), all at a dilution of 1:700. Histology: antibodies: Anti-Clec4e (1:75, MBL, D292-3), Anti-Mac-2 (1:1000, Cedarlane, CL8942AP), Anti-IL-1β (1:500, Invitrogen, P420B), Anti-NLRP3 (1:500, Invitrogen, SC06-23), Anti-AIM2 (1:500, Abcam, ab119791), Anti-Iba1 (1:100, Synaptic Systems, 234308), Anti-CD64 (1:200, Invitrogen, MA5-29704), Anti-GSDMD (1:75, Inivtrogen, PA5115330), anti-c-Fos Rabbit IgG (1:5000 Synaptic Systems, 226 008). After washing three times for 5 minutes each in 0.1% PBS-Tween, sections were incubated with secondary antibodies for 2 hours at room temperature in the dark, all at a 1:300 dilution: Donkey anti-rabbit AlexaFluor 647 (Thermo Scientific, A-31573), Goat anti-rat AlexaFluor 647 (Thermo Fisher, A-21247), Goat anti-rat AlexaFluor 594 (Thermo Fisher, A-11007), Goat anti-rabbit AlexaFluor 594 (Thermo Fisher, A-11012), Donkey anti-guinea pig FITC (MilliporeSigma), and anti-CD45.2-Biotin (1:100, Biolegend, 109804), Anti-CD68 (1:200, Bio-Rad, MCA1957) and Anti-ADRB2 (1:250, Bioss, BS-0947R). Slides were then washed 3 times in 0.1% PBS-Tween and incubated with DAPI (1:1000 in PBS, Invitrogen, PI62247) for 10 minutes at RT in the dark, followed by another 3 washes in 0.1% PBS-Tween. In vivo blocking antibodies included: IgG2a anti-Clec4e antibody (clone 6G5, InvivoGen, mabg-mmcl-2) at a dosage of 10 µg per injection; anti-Ly6G IgG2a monoclonal antibody (clone 1A8, Absolute Antibody, Ab00295–2.0) at 200 µg per injection; anti-IL-1β antibody (clone B122, Bio X Cell, BE0246) at a dosage of 200 µg per injection.

Validation

These antibodies are all used in flow cytometry and have been validated by the corresponding companies. The validation materials can be found on the companies' website.

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

Wild-type C57BL6J (WT; Strain#000664), CD45.1 (JAXBoy (PtprcK302E); C57BL/6J-Ptprcem6Lutzy/J; Strain#033076) and Ldlr-/- (B6.129S7-Ldlrtm1Her/J; Strain#002077) mice were purchased from the Jackson Laboratory and bred in-house. Mx1-Cre (B6.Cg-Tg(Mx1-cre)1Cgn/J; Strain#003556), Jak2+/flox (B6N.129S6(SJL)-Jak2tm1.1Ble/AmlyJ; Strain#031658), p53flox (B6.129P2-Trp53tm1Brn/J; Strain#008462), Tet2flox (B6;129S-Tet2tm1.1Iaai/J; Strain#017573), and Dnmt3afl-R878HB6(B6(Cg)-Dnmt3atm1Trow/J) mice were purchased from the Jackson Laboratory and bred in-house. Jak2+/flox, Tet2flox, p53flox and Dnmt3a +/flox mice were crossed to Mx1-Cre to generate Jak2V617F/wtMx1-Cre or Tet2-/-Mx1-Cre, p53-/-Mx1-Cre mice, or Dnmt3aR878H/wtMx1-Cre. Controls were flox-negative, Cre-positive, sex-matched littermates. The PAC1flox mice (Adcyap1r1loxP/loxP) were generated with a conditional knockout allele through the NIH-funded knockout mouse project (KOMP) as previously described58. Female mice were used. All animal protocols were approved by the Animal Review Committee at the Icahn School of Medicine at Mount Sinai (Protocol numbers: IPROTO202300000060, IPROTO202400000080, IPROTO202400000058, and IPROTO20190055) and followed relevant ethical regulations. All experimental procedures were conducted in accordance with guidelines set by the Institutional Animal Care and Use Committee at the Icahn School of Medicine at Mount Sinai. All mice used were cohoused, female, aged 8-12 weeks and on a C57BL/6J background. Mice were provided ad libitum access to food and water in a light- and temperature-controlled vivarium in a standard 12 h light/dark cycle (light Zeitgeber (ZT) 0–12 and dark ZT 12–24) at 23 °C and 65% humidity. Animals were randomly assigned to experimental groups. Investigators were blinded to group allocation during outcome assessment and data analysis. All mice were group housed in the same room, and cage location did not differ between experimental groups. To control for circadian effects, all mice, including controls, were euthanized at the same time of day (Zeitgeber time 2, ZT2).

	All experiments were performed in at least two or three independent biological cohorts unless otherwise stated, with similar results observed.
Wild animals	No wild animals were used in this study
Reporting on sex	Experiments were performed in female mice.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	All animal protocols were approved by the Animal Review Committee at the Icahn School of Medicine at Mount Sinai (Protocol numbers: IPROTO202300000060, IPROTO202400000080, IPROTO202400000058, and IPROTO20190055) and followed relevant ethical regulations. All experimental procedures were conducted in accordance with guidelines set by the Institutional Animal Care and Use Committee at the Icahn School of Medicine at Mount Sinai.

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

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	At sacrifice, mice were anesthetized using 3% isoflurane and peripheral venous blood was drawn through retro-orbital bleeding. Red blood cells were eliminated using RBC lysis buffer (BioLegend, Catalog #420302). Bone marrow cells were obtained by flushing the bone with PBS from a 25-gauge needle, then mechanically dissociated by passing through a 19-gauge needle to generate a single-cell suspension. Remaining red blood cells were subsequently lysed with RBC lysis buffer. After performing cardiac puncture and perfusing with 10-20 mL PBS, the aorta was carefully dissected and placed in cold PBS. The tissue was then digested in a solution containing 450 U/ml collagenase I, 125 U/ml collagenase XI, 60 U/ml DNase I, and 60 U/ml hyaluronidase (Sigma) in PBS for 45 minutes at 37°C with gentle agitation to generate a single-cell suspension. After digestion, the suspension was passed through a 100-µm strainer, washed with PBS, and processed for further analysis.Sequential cheek bleeds were performed every 2-4 weeks as indicated to monitor expansion in competitive BM chimeras. Mice were anesthetized with 3% isoflurane prior to blood collection. A 25-gauge needle was used to obtain a 50 µL sample from the cheek vein. Samples were immediately processed for flow cytometry to evaluate cellular expansion based on CD45.1 and CD45.2 expression.
Instrument	Data were acquired on the Cytek Aurora and analyzed with FlowJo software (Tree Star, Ashland, OR).
Software	Spectroflo and FlowJo
Cell population abundance	Cell population abundance information is provided within each figure
Gating strategy	Detailed gating strategies are provided throughout

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