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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	The OPUS spectroscopic software was used for data handling. All acquisitions for the flow-cytometry experiments were performed using CytExpert for CytoFLEX Acquisition and Analysis software.
Data analysis	All statistical analysis was performed using GraphPad Prism version 7.0 software. The ACD/NMR processor spectroscopic software was used for NMR data handling. Confocal images were analyzed using FIJI (ImageJ software). All analyses of the flow-cytometry experiments were performed using CytExpert for CytoFLEX Acquisition and Analysis software.

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 main data supporting the results in this study are available within the paper and its Supplementary Information. The raw and analysed datasets generated during the study are too large to be publicly shared, yet they are available for research purposes from the corresponding authors on reasonable request. RNA-sequencing data are available as Supplementary Information.

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	Sample sizes for the in vitro experiments were chosen on the basis of preliminary experiments and previous laboratory experience. Sample sizes for the in vivo experiments were chosen on the basis of preliminary experiments .
Data exclusions	No data were excluded.
Replication	The in vitro experiments were performed using a minimum of three biological replicates. The allogeneic kidney transplantation experiment was repeated 8 times, and other transplantation experiments were repeated five times. The experiments were reproducible.
Randomization	Randomization of these experiments was not relevant as the nature of the experiment (allograft and syngraft transplantations) dictated the group of mice used.
Blinding	Blinding for the experiments was not relevant, as their nature (allograft and syngraft transplantations) dictated the group of mice used. Data analysis was blinded to minimize bias; this was done by creating codes for each sample, with no identifiers.

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
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	Antibodies used in this study: PE-Cy5, mouse, anti-human CD54 (BD Biosciences, 555512, HA58, 5205518) PE-Cy5, mouse IgG1 isotype control, (BD Biosciences, 555750, MOPC-21, 5023610) FITC, anti-human CD45 (BD Biosciences, 555482, HI30, 8120939) FITC mouse IgG1 isotype control (BD Biosciences, 555748, MOPC-21, 8241736) APC, mouse, anti-human CD8 (BD Biosciences, 555369, RPA-T8, 12780) FITC, mouse, anti-human CD69 (BD Biosciences, 347823, L78, 70422) FITC, anti-human CD8 (STEMCELL Technologies, 60022FI1, RPA-T8, BX29950) APC, anti-human siglec-7, CD328 (Biolegend, 339205, 6-434, B203411) FITC, rat, anti-mouse IgG (Biolegend, 406001,Poly4060, B298540) PE, rat, anti-mouse CD3 (BD Biosceinces, 555275, 17A2, 8176542)
Validation	For the flow-cytometry experiments, antibodies were validated by comparison with any negative control sample and isotypes.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	EA.hy926 (human somatic cell hybrid endothelium, ATCC CRL-2922); HMEC-1 (human dermal microvscular endothelium, ATCC CRL-3243); HUVEC (human umbilical endothelial, ATCC PCS-100-013).

Authentication	None of the cell lines used were authenticated.
Mycoplasma contamination	The cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	None commonly misidentified cell lines were used in this study.

Animals and other organisms

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

Laboratory animals	Male and female C57BL/6 and Balb/c mice were obtained from Jackson Laboratories (Bar Harbor, Maine), bred and used for aortic allograft transplanation experimentations at 8-to-12 weeks of age. The donor and recipient mice were sex-matched. Male and female C3H mice were obtained from Jakcson Laboratories, bred and used for skin transplantation experiments at 8-to-12 weeks of age. The donor and recipient mice were sex-matched. Male C57BL/6 and Balb/c mice were obtained from Jackson Laboratories (Bar Harbor, Maine), bred and used for kidney transplantation experimentation at 8-to-12 weeks of age.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The animal experiments were performed in accordance with The Canadian Council on Animal Care (CCAC) guidelines, and were approved by the University of British Columbia Animal Care Committee (certificate number A16-0271) or the Simon Fraser University Animal Care Committee (certificate number 1235MB-08) or the Institutional Animal Care and Use Committee of Northwestern University (certificate number IS00002313).

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	Healthy adults, with no blood-related disorders and normal cell blood counts, 18 years or older, over 120 lb, and who had not donated blood in the last 2 months were selected for the study.
Recruitment	We used direct recruitment of donors through posted recruitment flyers (no database or contact information stored).The participants were randomly recruited and there was no direct relationship between the study team members and the participants. Care was taken to assure adequate representation of males and females, and no individual was excluded on the basis of race or gender. We excluded participants with low haemoglobin level, blood cells, and coagulation disorders, for example, anemia, hemophilia, thalassemia, sickle cell anemia, anemic diseases, as well as participants taking medication that can interfere with blood-cell functions, such as Aspirin and Ibuprofen. In addition, we excluded those who had donated blood in the last 2 months.
Ethics oversight	Procedures involving human subjects in the Centre for Blood Research at the University of British Columbia were approved by the Institutional Review Board (IRB) within the University of British Columbia (UBC Ethics approval no: H20-00084 and H18-02553).

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

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	To obtain PBMCs, EDTA anticoagulated blood from healthy donors was subjected to ficoll density gradient centrifugation Histopaque-1077 (Sigma Aldrich). PBMCs were cultured for 24 h in RPMI-1640 containing 10% FBS (v/v), 1000 U/mL recombinant human IL-2 (Cedarlane)11 and 1% P/S. Then the cells were incubated with the appropriate fluorescently-labelled antibody at room temperature prior to being subjected to flow cytometry.
Instrument	Flow cytometry profiles were acquired using a 3-laser CytoFLEX flow cytometer from Beckman Coulter Life Sciences (10,000 events).

Software	All experimental acquisition and analysis was performed by using CytExpert for CytoFLEX Acquisition and Analysis software.
Cell population abundance	A total of 10,000 events were collected for every sample.
Gating strategy	To obtain PBMCs, EDTA anticoagulated blood from healthy donors was subjected to ficoll density gradient centrifugation Histopaque-1077 (Sigma Aldrich). PBMCs were cultured for 24 h in RPMI-1640 containing 10% FBS (v/v), 1000 U/mL recombinant human IL-2 (Cedarlane)11 and 1% P/S. Prior to fluorescent labelling, cultured PBMCs were tested for absence of platelets by labelling an aliquot of cells with FITC-labelled anti-human CD45 (1:200 Beckman Coulter) and analyzed by flow cytometry (CytoFLEX, Beckman Coulter). Following PBMC isolation from whole blood, IL-2 activated PBMCs were selectively depleted for NK cells by immunomagnetic separation using an EasySep™ isolation kit (STEMCELL Technologies Inc) through positive selection for CD56+ cells. NK content in depleted and non-depleted populations was assessed through flow cytometry (Cytoflex, Beckman Coulter) by labeling lymphocyte populations with both a general Anti-Human CD45 (FITC conjugated, BD Biosciences, 1:40 dilution) and Anti Human Siglec-7 (APC conjugated, Biolegend, 1:20 dilution). CD8+ T-cells were depleted from IL-2 activated PBMCs through positive selection using Anti-Human CD8+ Dynabeads (Thermo Scientific). CD8+ T-cell content in depleted and non-depleted populations was assessed through flow cytometry (Cytoflex, Beckman Coulter) by labeling lymphocyte populations with Anti-Human CD8a (FITC conjugated, STEMCELL Technologies, 1:40 dilution).

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