

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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ | 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | 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

FlowJo software version 10.9 was used to generate population frequencies and representative flow cytometry plots. For R-based analyses, package versions are detailed in the renv lockfile that is deposited in GitHub.

Data analysis

All original code has been deposited on GitHub [<https://github.com/betts-lab/hpap-tissue-citeseq>] and in Zenodo under the digital object identifier 10.5281/zenodo.15027610 [<https://zenodo.org/records/15027610>]. Exceptions are the code used to generate HLA-GRS [<https://github.com/USF-HII/hla-prs-toolkit>] version 0.22a, and code for ADT and HTO preprocessing [<https://github.com/betts-lab/scc-proc>]. All code are publicly available as of the date of publication.

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](#)

All de-identified human flow cytometry and clinical data have been deposited in Zenodo under the digital object identifier 10.5281/zenodo.15001886 [<https://zenodo.org/records/15001886>].

zenodo.org/records/15001886], and on PANC-DB (<https://hpap.pmacs.upenn.edu/>). Raw sequencing -based data are also deposited in Genbank under accession number GSE221787 [<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE221787>]. All data are publicly available as of the date of publication. Source Data are provided with this paper. All data are included in the Supplementary Information or available from the authors, as are unique reagents used in this Article. The raw numbers for charts and graphs are available in the Source Data file whenever possible.

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

Sex was considered to balance the number of male and female donors between disease groups. Sex was identified by the local organ procurement organization or via University of Florida/nPOD. Sex-based analyses were not performed in this study due to a balance of male and female donors within each disease group.

Reporting on race, ethnicity, or other socially relevant groupings

Race was considered in order to balance the distributions of race between disease groups in order to eliminate race as a covariate.

Population characteristics

All population characteristics were balanced across disease groups. There were no identified co-variables between disease groups except a slight, statistically insignificant, covariation of disease status and cold ischemia time. This was accounted for in statistical analyses (please see the "Statistical Analyses" section of the manuscript).

Recruitment

Whole organ samples are obtained via the local organ procurement organization or via University of Florida/nPOD.

Ethics oversight

All procedures were in accordance with federal guidelines for organ donation and the University of Florida or University of Pennsylvania Institutional Review Board, or the Institutional Review Board of local organ procurement programs.

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](https://www.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 size was determined by sample availability and balancing donor characteristics between disease groups. For the flow cytometry dataset, sample size was determined by including all donors in the study that were freshly-acquired via flow cytometry (never frozen). For the CITEseq dataset, sample size was determined by balancing available donor samples with matching donor characteristics between disease groups. Additionally, the depth of sampling (ie number of cells) per tissue sample required for the diversity of immune cells limited the total number of tissue samples put through the CITEseq pipeline.

Data exclusions

Only freshly-acquired flow cytometry data was included in the study. The freezing and thawing process is known to introduce changes in surface levels of activation markers acquired via flow cytometry. The exclusion of frozen/thawed samples from the flow cytometry dataset was determined before analysis of the dataset begun.

Replication

Reproducibility was verified by two methods. First, a relatively high number of donors were selected to account for the inherent variability of human-based studies. Second, many of the phenotypes highlighted in this manuscript were identified by two independent analysis methodologies, flow cytometry and CITEseq, thus increasing the robustness findings.

Randomization

Donors were allocated into disease groups by measuring several factors used to detect Type 1 Diabetes (T1D) or β cell auto-antibody positive T1D negative (AAb+) conditions. T1D diagnosis was required for donors to be placed into the T1D group. Donors were placed into the AAb+ group after confirmation of at least one AAb in serum and no clinical T1D diagnosis. Control donors (AAb- with no diabetes) were acquired to match the group characteristics of the AAb+ and T1D groups.

Blinding

Blinding was not done in this study. Blinding could not be performed due to the structure of nPOD and HPAP. The investigators in this study were often directly involved in tissue processing and distribution of said tissues to other labs. Certain assays could only be performed on certain disease groups by certain labs in the network, and the investigators in the study knew the donors' categories in order to distribute tissues and execute the assays for certain disease groups.

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

Mouse Anti-CD15 Antibody, FITC, 1:50 dilution, Clone HI98, BD Biosciences Cat# 555401;
 Mouse Anti-CD4, BB790, 1:2500 dilution, Clone SK3, BD Biosciences Custom Conjugation;
 Rat Anti-CXCR5 Antibody, Alexa Fluor 647, 1:250 dilution, Clone RF8B2, BD Biosciences Cat# 558113;
 Mouse Anti-CD45 Antibody, Alexa Fluor 700, 1:400 dilution, Clone HI30, BD Biosciences Cat# 560566;
 Mouse Anti-CCR7 Antibody, APC-Cy7, 1:67 dilution, Clone G043H7, BioLegend Cat# 353212;
 Mouse Anti-CD11c Antibody, BUV395, 1:400 dilution, Clone B-ly6, BD Biosciences Cat# 563787;
 Mouse Anti-CD8 Antibody, BUV496, 1:200 dilution, Clone RPA-T8, BD Biosciences Cat# 612942;
 Mouse Anti-CD45RA Antibody, BUV563, 1:500 dilution, Clone HI100, BD Biosciences Cat# 612927;
 Mouse Anti-CD38 Antibody, BUV661, 1:400 dilution, Clone HIT2, BD Biosciences Cat# 612969;
 Mouse Anti-CD25 Antibody, BUV737, 1:200 dilution, Clone 2A3, BD Biosciences Cat# 612807;
 Mouse Anti-CD3 Antibody, BUV805, 1:125 dilution, Clone UCHT1, BD Biosciences Cat# 612895;
 Mouse Anti-PD1 Antibody, BV421, 1:83 dilution, Clone RH12.2H7, BioLegend Cat# 565935;
 Mouse Anti-CD14 Antibody, BV480, 1:1000 dilution, Clone MφP9, BD Biosciences Cat# 566141;
 Mouse Anti-CD56 Antibody, BV570, 1:200 dilution, Clone HCD56, BioLegend Cat# 318330;
 Mouse Anti-HLA-DR Antibody, BV605, 1:400 dilution, Clone G46-6, BD Biosciences Cat# 562845;
 Mouse Anti-CD27 Antibody, BV650, 1:400 dilution, Clone O323, BioLegend Cat# 302828;
 Mouse Anti-CD16 Antibody, BV711, 1:400 dilution, Clone 3G8, BioLegend Cat# 302044;
 Mouse Anti-CD19 Antibody, BV785, 1:125 dilution, Clone HIB19, BioLegend Cat# 302240;
 Mouse Anti-CD123 Antibody, PE, 1:400 dilution, Clone 9F5, BD Biosciences Cat# 555644;
 Mouse Anti-CD127 Antibody, PE/CF594, 1:200 dilution, Clone HIL-7R-M21, BD Biosciences Cat# 562397;
 Mouse Anti-CD69 Antibody, PE/Cy5, 1:500 dilution, Clone FN50, BioLegend Cat# 310908;
 Mouse Anti-CD34 Antibody, PE/Cy5.5, 1:100 dilution, Clone 581, Invitrogen Cat# CD34-581-18;
 Mouse Anti-CD21 Antibody, PE/Cy7, 1:2000 dilution, Clone Bu32, BioLegend Cat# 354912;
 Human TruStain FcX (Fc Receptor Blocking Solution), 1:100 dilution, BioLegend Cat# 422302;
 TotalSeq-A Human Universal Cocktail, V1.0, recommended dilution, BioLegend Cat# 399907;
 TotalSeq-A0251 anti-human Hashtag 1 Antibody, 1:37.5 dilution, BioLegend Cat# 394601;
 TotalSeq-A0252 anti-human Hashtag 2 Antibody, 1:37.5 dilution, BioLegend Cat# 394603;
 TotalSeq-A0253 anti-human Hashtag 3 Antibody, 1:37.5 dilution, BioLegend Cat# 394605;
 TotalSeq-A0254 anti-human Hashtag 4 Antibody, 1:37.5 dilution, BioLegend Cat# 394607;
 TotalSeq-A0256 anti-human Hashtag 6 Antibody, 1:37.5 dilution, BioLegend Cat# 394611;
 TotalSeq-A0257 anti-human Hashtag 7 Antibody, 1:37.5 dilution, BioLegend Cat# 394613;
 TotalSeq-A0258 anti-human Hashtag 8 Antibody, 1:37.5 dilution, BioLegend Cat# 394615;
 TotalSeq-A0259 anti-human Hashtag 9 Antibody, 1:37.5 dilution, BioLegend Cat# 394617;

Validation

All validation information is available on the webpages from the antibody vendors.

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

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

To isolate pancreatic and mesenteric LN lymphocytes, LNs obtained from brain-dead organ donors were submerged in 5 mL R10 media (RPMI + 10% FBS + 1% penicillin/streptomycin + 2mM L-glutamine) supplemented with 10U/mL DNase I (Roche), cleaned of visceral fat, and cut into small pieces (~2mm x ~2mm). Tissue fragments were placed into a sterile 70 µm mesh (Miltenyi Biotech) and pushed through the mesh with the blunt end of a sterile 5 mL syringe plunger. Residual tissues and cells in the mesh were washed with 45 mL of R10 + DNase, centrifuged at 500 xg for 7 minutes, resuspended in 10 mL of R10 + DNase, and counted. To isolate splenocytes, approximately 30 g of spleen tissue was placed in R10 supplemented with 10U/mL DNase I and 3 mg/ml collagenase D (Sigma), the capsule was removed, and remaining tissue was cut into 4mm x 4mm pieces. The tissue was then mechanically dissociated using a GentleMACS (Miltenyi Biotech) and incubated at 37 C for 15 min with gentle inversion. After digestion, the cell suspension was filtered through a 100 µm strainer, and erythrocytes were lysed by ACK buffer (Corning). Post-ACK, cells were resuspended in R10 media and filtered through a sterile 70 µm mesh. Splenic mononuclear cells were isolated by density gradient centrifugation using Ficoll-Paque. Samples were immediately placed into a flow cytometry protocol after isolation.

Instrument

All flow cytometry data was collected on a BD FACSymphony A5 cytometer (BD Biosciences)

Software

BD FACSDiva software version 9.3 was used to acquire flow cytometry data. FlowJo software version 10.9 was used to generate population frequencies and representative flow cytometry plots.

Cell population abundance

N/A

Gating strategy

Preliminary gating was performed to enrich single immune cells. The first gate was Time X SSC, in order to eliminate events at the beginning (or end) of an acquisition that could occur due to unstable event rate or the sample running out. After the Time gate, the second gate was FSC-A X FSC-H along the 1:1 axis of the two parameters in order to isolate single cells. After the Single Cell gate, the third gate was selecting Live-Dead stain negative and CD45+ in events in order to gate live immune cells.

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