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	CyTOF2 (Fluidigm) equipped with a SuperSampler fluidics system (Victorian Airships) CyTOF software v.6.7 (Fludigm) Cytobank v7 (Beckman Coulter) MiSeq (Illumina) NovaSeq 6000 (Illumina) NextSeq 500 (Illumina)
Data analysis	openTSNE v0.3.11 Phenograph v1.5.2 Seaborn v0.9.1 pRESTO: v0.7.0 ImmuneDB: v0.29.9 VDJtools v1.2.1 factoextra v1.0.7 immunarch v0.6.7 GGally v2.1.2 network v1.17.1 kallisto v0.45.2 bustools v0.40.0 CellRanger pipeline v6.0.1 scanpy v1.9.0.

bioinfokit v2.0.8.
matplotlib_venn v0.11.6.
Prism GraphPad v8.4.3

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

High-throughput T cell receptor sequence data that support the findings of this study have been deposited in NCBI SRA with the accession code PRJNA861254. single-cell RNA sequencing with V(D)J data that support the findings of this study have been deposited in NCBI GEO with the accession code GSE206507.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Not applicable; no human research participants involved in this study.
Population characteristics	Not applicable; no human research participants involved in this study.
Recruitment	Not applicable; no human research participants involved in this study.
Ethics oversight	Not applicable; no human research participants involved in this study.

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	Sample size is variable depending upon the type of experiment due to the restricted availability some of the donor tissues. All the donors that fit a certain criteria was used. The donors have no previous history of heavy smoking, and they were free of COVID-19, cancer, active hepatitis B, C and HIV.
Data exclusions	No data were excluded from any of these analysis.
Replication	Figures show data from multiple individuals. Measures were taken to verify the reproducibility of the experimental findings by examining the same tissues from multiple donors of varying ages. While there were individual variation, consistent findings were demonstrated across individuals.
Randomization	All donors samples obtained were analyzed. No treatment groups were assigned in this study. Therefore, no randomization was required.
Blinding	The experiments did not require blinded analysis, as no treatment groups were assigned or evaluated in this study.

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

Methods

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

Antibodies

Antibodies used

CyTOF:
CD45 89 Y HI30 Fluidigm Cat No.: 3089003B
CD57 113 In HCD57 Biolegend Cat No.: 322302
CD28 141 Pr CD28.2 Biolegend Cat No.: 302902
CD19 142 Nd HIB19 Biolegend Cat No.: 302202
CD45RA 143 Nd HI100 Biolegend Cat No.: 304102
CD103 144 Nd Ber-Act8 Biolegend Cat No.: 350202
CD4 145 Nd RPA-T4 Biolegend Cat No.: 300502
CD8a 146 Nd RPA-T8 Biolegend Cat No.: 301002
Perforin 147 Sm dG9 Biolegend Cat No.: 308102
CD16 148 Nd 3G8 Biolegend Cat No.: 302014
CD127 149 Sm A019D5 Biolegend Cat No.: 351302
CD1c 150 Nd L161 Biolegend Cat No.: 331502
CD123 151 Eu 6H6 Biolegend Cat No.: 306002
CD66b 152 Sm G10F5 Biolegend Cat No.: 305102
TIGIT 153 Eu MBSA43 Fluidigm Cat No.: 3153019B
ICOS 154 Sm C398.4A Biolegend Cat No.: 313502
CD27 155 Gd O323 Biolegend Cat No.: 302802
CCR5 156 Gd NP-6G4 Fluidigm Cat No.: 3156015A
Tcf1 159 Tb 7F11A10 Biolegend Cat No.: 655202
CD14 160 Gd M5E2 Biolegend Cat No.: 301810
CD56 161 Dy B159 BD Biosciences Cat No.: 555513
gdTCR 162 Dy REA591 Miltenyi Cat No.: 130-122-291
CXCR5 163 Dy J252D4 Biolegend Cat No.: 356902
CD69 164 Dy FN50 Biolegend Cat No.: 310902
CRTH2 165 Ho REA598 Miltenyi Cat No.: 130-122-305
CD25 166 Er M-A251 Biolegend Cat No.: 356102
CCR7 167 Er G043H7 Biolegend Cat No.: 353222
CD3 168 Er UCHT1 Biolegend Cat No.: 300402
Tbet 169 Tm 4B10 Biolegend Cat No.: 644802
CD38 170 Er HB-7 Biolegend Cat No.: 356602
CD95 171 Yb DX2 Biolegend Cat No.: 305602
LAG3 172 Yb 11C3C65 Biolegend Cat No.: 369302
CXCR4 173 Yb 12G5 Fluidigm Cat No.: 3173001B
HLADR 174 Yb L243 Biolegend Cat No.: 307602
PD-1 175 Lu EH12.2H7 Fluidigm Cat No.: 329912
GranzymeB 176 Yb GB11 Invitrogen Cat No.: MA1-80734
CD11b 209 Bi ICRF44 Fluidigm Cat No.: 3209003B
FACS:
Anti-Human CD3 APC Biolegend SK7 Cat No.: 344812 (Dilution 1:50)
Anti-Human CD4 PE-Cy7 Tonbo RPA-T4 Cat No.: 60-0049 (Dilution 1:50)
Anti-Human CD45 Alexa Fluor 700 BioLegend HI30 Cat No.: 304023 (Dilution 1:50)
Anti-Human CD8 PE BioLegend SK1 Cat No.: 980902 (Dilution 1:50)

Validation

All antibodies were validated for specificity by the manufacturer.
For antibodies with catalog numbers 322302, 302902, 302202, 304102, 350202, 300502, 301002, 308102, 302014, 351302, 331502, 306002, 305102, 313502, 302802, 655202, 301810, 356902, 310902, 356102, 353222, 300402, 644802, 356602, 305602, 369302, 307602, 329912, 344812, 304023, 980902:
Per Biolegend, "each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is ≤ 0.5 µg per 106 cells in 100 µl volume or 100 µl whole blood."

For antibodies with catalog numbers 3089003B, 3153019B, 3173001B, 3209003B:
Per Fluidigm, "Each lot of conjugated antibody is quality control tested by CyTOF® analysis of stained cells using the appropriate positive and negative cell staining and/or activation controls."

For antibodies with catalog number 555513:
Per BD Biosciences, "All flow cytometry reagents are titrated on the relevant positive or negative cells. To save time and cell samples for researchers, test size reagents are bottled at an optimal concentration with the best signal-to-noise ratio on relevant models

during the product development. To ensure consistent performance from lot-to-lot, each reagent is bottled to match the previous lot MFI."

For antibodies with catalog number MA1-80734:
Per Invitrogen, "Part 1. Target specificity verification helps ensure the antibody will bind to the correct target; out antibodies are being tested using at least one of the following methods: knockout, knockdown, independent antibody verification, cell treatment, relative expression, neutralization, peptide array, SNAP-ChIP validation, immunoprecipitation/massspectrometry. Part 2. Functional application validation. These tests help ensure the antibody works in particular applications(s) of interest, which may include (but are not limited to: western blotting, immunofluorescence imaging, flow cytometry, ChIP, immunohistochemistry."

For antibodies with catalog number 60-0049:
Per Tonbo, "Tonbo flow cytometry reagents & antibodies are manufactured with the highest quality and precision and validated for consistent performance in multiparametric flow cytometry experiments. Tonbo offers a carefully selected portfolio of antibodies and fluorophores designed to provide researchers with a core resource for flow cytometry reagents that are used in the majority of staining protocols. Made in San Diego, CA, our manufacturing process includes optimization of fluorophore to protein ratio (F:P), meticulous quality control testing, and stringent final release criteria, resulting in consistently high-performing reagents for multicolor protocols."

For antibodies with catalog numbers 130-122-291, 130-122-305:
Per Miltenyi, "In order to compare the epitope specificity of an antibody, the clone being used is compared with other known clones recognizing the same antigen in a competition assay...Cells were incubated with an excess of purified unconjugated antibody followed by staining with fluorochrome-conjugated antibodies of other known clones against the same marker. Based on the fluorescence signal obtained, the clones were identified as recognizing completely overlapping (++), partially overlapping (+), or completely different epitopes (-) of the marker. Selected fluorochrome conjugated antibodies from Miltenyi Biotec were compared to commercially available hybridoma clones in flow cytometry analysis...Flow cytometric comparison of different clones. Human peripheral blood mononuclear cells (PBMCs) were stained with antibodies and with a suitable counterstaining. As a control, antibody staining was omitted and cells were measured in the same channels. Flow cytometry was performed with the MACSQuant® Analyzer. Cell debris, dead cells, and cell doublets were excluded from the analysis based on scatter signals and 4',6-diamidino-2-phenylindole (DAPI) fluorescence. No FcR Blocking Reagent was used. The recommended titers of respective antibodies from different suppliers were used...To provide an indication on how an antibody performs after fixation of cells, in-house data on staining results before and after fixation with 3.7% formaldehyde using Miltenyi Biotec antibodies are provided. Different experimental settings may lead to different results...Comparison of staining pattern on non-fixed and fixed cells. The performance of the antibody after fixation was tested by comparing the staining pattern on fresh (no fixation) versus fixed (post fixation) cells."