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	For flow cytometry, data were collected on FACSDiva software (BD Biosciences). Bulk-RNAseq was performed on NovaSeq (Illumina). TCRb sequencing was performed by Adaptive Biotechnologies.
Data analysis	For flow cytometry, data were analyzed with FlowJo software (Version 10.8.1; BD Biosciences and FlowJo LLC). Data analysis and statistical comparisons were done using GraphPad Prism Version 9.3.1 and R version 4.0.1. RNA-Seq data: Sequencing quality control was performed with FastQC v0.11.9 and MultiQC version v1.12. RNA-seq reads were trimmed using Ilumina's DRAGEN FASTQ toolkit version 1.0.0. The STAR (v2.7.1 with default parameters) aligner was used to map the transcriptomes of the human and mouse bulk RNAseq data to GENCODE GRCh38.p13 and GENCODE GRCm39 respectively. Differential expression analysis using DESeq2 v1.3457 or Seurat v4.0.6 (FindAllMarkers and FindMarkers function) . We used the support vector machine (SVM) classifier from Scikit-learn (v1.1)26 machine learning library to classify data points. Packages such as Numpy (v1.23.2), Matplotlib (v3.5.2), Pandas (v 1.4.3), and Seaborn (v0.11.2) were used along with Scikit-learn to aid preprocessing. Gene Set Enrichment Analysis (v4.2.3) with default parameters. GGplot2 v3.3.5 and ComplexHeatmaps v2.12.1 were used to make bar plots and heatmaps, Feature plots and UMAPs were generated using Seurat's FeaturePlot and DimPlot functions. Dot plots were generated using the package Ggpubr v0.4.0. TCRb data were analyzed with ImmunoSEQ analyzer and CDR3 data was analyzed using GLIPH v.2. Further downstream analysis was done in R using packages dplyr v1.0.9 and stats v4.1.1.

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

Mouse and human bulk RNA-seq have been uploaded to the NCBI GEO and accessible under accession no. GSE217010. Human scRNA-seq data can be accessed at NCBI GEO with accession no. GSE190570. Human TCR sequencing data was generated and processed by Adaptive Biotechnologies. Details of productive TCR sequences, accessed through their immunoSEQ Analyzer portal, are provided in Supplementary Table 14. Source data files for main and extended data figures are provided. All other data supporting the findings are available in the paper or from corresponding authors upon request.

Human research participants

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

Reporting on sex and gender	Both male and female samples were used with no bias. Sequencing experiments using human samples were sex balanced. Informations are provided in Supplementary Table 13.
Population characteristics	Population characteristics of donors used in bulk RNAseq and TCRseq are shown in the Supplementary Table 13.
Recruitment	Healthy volunteers were recruited by the Clinical core at the La Jolla Institute for Immunology (LJI). All participants received financial compensation according to guidelines approved by LJI's Institutional Review Board. Written informed consents were obtained from all enrolled participants. Donors self-reported ethnicity and race details, and tested negative for hepatitis B, hepatitis C and HIV. None of the donors had any ongoing infection. They had no known conditions of cancer, diabetes, heart or kidney or liver disease. Donors were neither pregnant nor nursing. De-identified blood or PBMC samples for the study were made available by LJI's Clinical core.
Ethics oversight	The Institutional Review Board (IRB) of the La Jolla Institute for Immunology (IRB protocol: IB248 and VD-057) approved the 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	All sample sizes are indicated in figure legends. Sample sizes were based on previous experience (Ref 3-4, 6-7) providing enough statistic robustness, reproducibility and are based on resource availability. For flow cytometry, a minimum of 3 donors was used up to 16 to confirm the findings. For human Bulk-RNAseq, 7 donors were used to provide enough power for any statistical analyses.
Data exclusions	No data were excluded from the analysis.
Replication	Findings have been replicated from 2 to 5 independent experiments to confirm reproducibility. Biological replicates are indicated in legend.
Randomization	For mice data, no randomization was performed as no experimental groups were performed. For human TCRb and bulk-RNAseq, both men and women and age-matched samples were used to decrease sex-biased effect.
Blinding	No blinding was performed as there were no experimental 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

Methods

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

Antibodies

Antibodies used

anti-hCD8a-APC-Cy7; clone RPA-T8; Cat. No. 301016; BioLegend; d; dilution 1:40
anti-hCD19-APC-Cy7; clone 6D5; Cat. No. 115530; BioLegend; dilution 1:40
anti-hCD14-APC-Cy7; clone HCD14; Cat. No. 325620; BioLegend; dilution 1:40
anti-hCD3-PE-Cy7; clone SK7; Cat. No. 557851; BD Biosciences; dilution 1:40
anti-hCD3 (unconjugated); clone OKT3; Cat. No. 566685; BD Biosciences; dilution 1:200
anti-hCD4-PerCp-Cy5.5; clone RPA-T4; Cat. No. 560650; BD Biosciences; dilution 1:80
anti-hCD56-BV785; clone 5.1H11; Cat. No. 362550; BioLegend; dilution 1:20
anti-hCD16-BV570; clone 3G8; Cat. No. 302036; BioLegend; dilution 1:40
anti-hCD25-AF647; clone M-A251; Cat. No. 356128; BioLegend; dilution 1:40
anti-hCD127-PE; clone HIL-7R-M21; Cat. No. 557938; BD Biosciences; dilution 1:50
anti-hCD45RA-BV605; clone HI100; Cat. No. 304133; BioLegend; dilution 1:40
anti-hCCR7-PE; clone REA108; Cat. No. 130-120-603; Miltenyi; dilution 1:50
anti-hFOXP3-FITC; clone 206D; Cat. No. 320106; BioLegend; dilution 1:20
anti-hGranzymeB-AF647; clone GB11; Cat. No. 515405; BioLegend; dilution 1:50
anti-hPerforin-AF488; clone B-D48; Cat. No. 353319; BioLegend; dilution 1:50
anti-hTNF-BV650; clone MAb11; Cat. No. 502937; BioLegend; dilution 1:50
anti-hCD3-AF700; clone OKT3; Cat. No. 317340; BioLegend; dilution 1:40
anti-hCD4-BV711; clone SK3; Cat. No. 344647; BioLegend; dilution 1:40
anti-hCD8a-PE-Cy7; clone SK1; Cat. No. 344750; BioLegend; dilution 1:40
anti-hCD14-PE-Cy7; clone HCD14; Cat. No. 325617; BioLegend; dilution 1:40
anti-hCD19-PE-Cy7; clone HIB19; Cat. No. 302215; BioLegend; dilution 1:40
anti-hCD16-BV785; clone 3G8; Cat. No. 302046; BioLegend; dilution 1:40
anti-hCD56-PE; clone MEM-188; Cat. No. 304606; BioLegend; dilution 1:40
anti-hCXCR2-PE/Dazzle 594; clone 5E8/CXCR2; Cat. No. 320721; BioLegend; dilution 1:12.5
anti-hCCL3-APC; clone 11A3; Cat. No. 551533; BD Biosciences; dilution 1:3.5
anti-hCCL4-APC-H7; clone D21-1351; Cat. No. 561280; BD Biosciences; dilution 1:20
anti-hCCL5-BV421; clone 2D5; Cat. No. 564754; BD Biosciences; dilution 1:20
anti-hCD19-APC-Cy7; clone HIB19; Cat. No. 302218; Biolegend; dilution 1:100
anti-hCD3-AF700; clone UCHT1; Cat. No. 300424; Biolegend; dilution 1:100
anti-hCD4-Pacific blue; clone RPA-T4; Cat. No. 300521; Biolegend; dilution 1:100
anti-hCD56-PE-Cy5; clone 5.1H11; Cat. No. 362516; Biolegend; dilution 1:60
anti-hCD127-AF647; clone eBioDR5; Cat. No. 51-1278-42; Invitrogen; dilution 1:60
anti-hCD25-PE-Cy7; clone M-A251; Cat. No. 560920; BD Biosciences; dilution 1:60
anti-hTCRab-PerCp-Cy5.5; clone IP26; Cat. No. 306724; Biolegend; dilution 1:100
anti-hCCR7-FITC; clone REA108; Cat. No. 130-117-700; Miltenyi; dilution 1:60
anti-hCD27-BV711; clone M-T271; Cat. No. 356430; Biolegend; dilution 1:60
anti-hCD56-Pe-Cy7; clone 5.1H11; Cat. No. 362509; Biolegend; dilution 1:60
anti-hPD-1-BV785; clone EH12.2H7; Cat. No. 329929; Biolegend; dilution 1:60
anti-hGITR-PE; clone eBioAITR; Cat. No. 12-5875-41; Invitrogen; dilution 1:60
anti-hTIGIT-AF488; clone MBSA43; Cat. No. 53-9500-41; Invitrogen; dilution 1:60
anti-hLAG3-BV650; clone 11C3C65; Cat. No. 369315; Biolegend; dilution 1:60
anti-hPerforin-PE; clone B-D48; Cat. No. 353303; Biolegend; dilution 1:50
anti-hFASLG-PE-Cy7; clone NOK-1; Cat. No. 306417; Biolegend; dilution 1:50
anti-hCD107a-BV785; clone H4A3; Cat. No. 328643; Biolegend; dilution 1:100
anti-hCD40L-PE; clone 24-31; Cat. No. 12-1548-42; Invitrogen; dilution 1:50
anti-hIFNg-PE-Cy7; clone 4S.B3; Cat. No. 502528; BioLegend; dilution 1:50
anti-hFOXP3-PE; clone 206D; Cat. No. 320107; Biolegend; dilution 1:40
anti-hCCR5-BV785; clone J418F1; Cat. No. 359131; Biolegend; dilution 1:60
anti-hCXCR3-BV650; clone G025H7; Cat. No. 353730; Biolegend; dilution 1:60
anti-hCXCR4-APC; clone 12G5; Cat. No. 306509; Biolegend; dilution 1:60
anti-hCX3CR1-BV785; clone 2A9-1; Cat. No. 341627; Biolegend; dilution 1:60
anti-mTCRb-AF700; clone H57-597; Cat. No. 109224; BioLegend; dilution 1:100
anti-mCD4-PerCp-Cy5.5; clone GK1.5; Cat. No. 100434; BioLegend; dilution 1:100
anti-mCD25-APC; clone PC61; Cat. No. 557192; BD Biosciences; dilution 1:75
anti-mCD45-APC-Cy7; clone 30-F11; Cat. No. 103116; Biolegend; dilution 1:100
anti-mTCRb-Pacific blue; clone H57-597; Cat. No. 109226; Biolegend; dilution 1:100

anti-mCD4-BV785; clone GK1.5; Cat. No. 100453; Biolegend; dilution 1:100
anti-mKI67-AF647; clone 16A8; Cat. No. 652408; Biolegend; dilution 1:50
anti-mFOXP3-AF488; clone MF-14; Cat. No. 126406; BioLegend; dilution 1:50
anti-mBrDU-PE-Cy7; clone 3D4; Cat. No. 364118; BioLegend; dilution 1:50

Dilutions of each antibody is provided here. These are based on technical datasheets from the vendor as well as experiences from preliminary experiments.

Validation

All above antibodies are commercially available and have been validated for immunofluorescent staining with flow cytometry by the vendor. Data sheets are available at vendors' websites provided below.
hCD8a-APC-Cy7-https://www.biolegend.com/en-us/cell-separation/apc-cyanine7-anti-human-cd8a-antibody-832?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=APC/Cyanine7%20anti-human%20CD8a%20Antibody.pdf&v=20230114043032
hCD19-APC-Cy7-https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-cd19-antibody-3903?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=APC/Cyanine7%20anti-mouse%20CD19%20Antibody.pdf&v=20230628033023
hCD14-APC-Cy7-https://www.biolegend.com/en-us/products/apc-cyanine7-anti-human-cd14-antibody-3959?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=APC/Cyanine7%20anti-human%20CD14%20Antibody.pdf&v=20230114013553
hCD3-PE-Cy7-https://www.bdbiosciences.com/content/bdb/paths/generate-tds-document.us.557851.pdf
hCD3-unconjugated-https://www.bdbiosciences.com/content/bdb/paths/generate-tds-document.us.566685.pdf
hCD4-PerCp-Cy5.5-https://www.bdbiosciences.com/content/bdb/paths/generate-tds-document.us.560650.pdf
hCD56-BV785-https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-human-cd56-ncam-antibody-12129?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Brilliant%20Violet%20785%E2%84%A2%20anti-human%20CD56%20(NCAM)%20Antibody.pdf&v=20230114013553
hCD16-BV570-https://www.biolegend.com/en-us/products/brilliant-violet-570-anti-human-cd16-antibody-7466?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Brilliant%20Violet%20570%E2%84%A2%20anti-human%20CD16%20Antibody.pdf&v=20230630093043
hCD25-AF647-https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-human-cd25-antibody-9867?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Alexa%20Fluor%C2%AE%20647%20anti-human%20CD25%20Antibody.pdf&v=20230114013553
hCD127-PE-https://www.bdbiosciences.com/content/bdb/paths/generate-tds-document.us.557938.pdf
hCD45RA-BV605-https://www.biolegend.com/en-us/search-results/brilliant-violet-605-anti-human-cd45ra-antibody-7661?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Brilliant%20Violet%20605%E2%84%A2%20anti-human%20CD45RA%20Antibody.pdf&v=20230701123045
hCCR7-PE-https://www.miltenyibiotec.com/US-en/products/cd197-ccr7-antibody-anti-human-reafinity-rea108.html#conjugate=pe:size=100-tests-in-200-ul
hFOXP3-FITC-https://www.biolegend.com/en-us/products/fitc-anti-human-foxp3-antibody-2946?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=FITC%20anti-human%20FOXP3%20Antibody.pdf&v=20230628033023
hGranzymeB-AF647-https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-human-mouse-granzyme-b-antibody-6067?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Alexa%20Fluor%C2%AE%20647%20anti-human/mouse%20Granzyme%20B%20Antibody.pdf&v=20230701123045
hPerforin-AF488-https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-human-perforin-antibody-16596?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Alexa%20Fluor%C2%AE%20488%20anti-human%20Perforin%20Antibody.pdf&v=20230114013553
hTNF-BV650-https://www.biolegend.com/en-us/products/brilliant-violet-650-anti-human-tnf-alpha-antibody-7680?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Brilliant%20Violet%20650%E2%84%A2%20anti-human%20TNF-%CE%B1%20Antibody.pdf&v=20230114013553
hCD3-AF700-https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-human-cd3-antibody-9625?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Alexa%20Fluor%C2%AE%20700%20anti-human%20CD3%20Antibody.pdf&v=20230630093043
hCD4-BV711-https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-human-cd4-antibody-7942?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Brilliant%20Violet%20711%E2%84%A2%20anti-human%20CD4%20Antibody.pdf&v=20230114043032
hCD8a-PE-Cy7-https://www.biolegend.com/en-us/products/pe-cyanine7-anti-human-cd8-antibody-6390?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=PE/Cyanine7%20anti-human%20CD8%20Antibody.pdf&v=20230316073101
hCD14-PE-Cy7-https://www.biolegend.com/en-us/products/pe-cyanine7-anti-human-cd14-antibody-3958?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=PE/Cyanine7%20anti-human%20CD14%20Antibody.pdf&v=20230628033023
hCD19-PE-Cy7-https://www.biolegend.com/en-us/products/pe-cyanine7-anti-human-cd19-antibody-1911?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=PE/Cyanine7%20anti-human%20CD19%20Antibody.pdf&v=20230701123045
hCD16-BV785-https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-human-cd16-antibody-7966?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Brilliant%20Violet%20785%E2%84%A2%20anti-human%20CD16%20Antibody.pdf&v=20230630102944
hCD56-PE-https://www.biolegend.com/en-us/products/pe-anti-human-cd56-ncam-antibody-1605?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=PE%20anti-human%20CD56%20(NCAM)%20Antibody.pdf&v=20230606063319
hCXCR2-PE/Dazzle 594-https://www.biolegend.com/en-us/search-results/pe-dazzle-594-anti-human-cd182-cxcr2-antibody-16314?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=PE/Dazzle%E2%84%A2%20594%20anti-human%20CD182%20(CXCR2)%20Antibody.pdf&v=20230114013553
hCCL3-APC-https://www.bdbiosciences.com/content/bdb/paths/generate-tds-document.us.551533.pdf
hCCL4-APC-H7-https://www.bdbiosciences.com/content/bdb/paths/generate-tds-document.us.561280.pdf
hCCL5-BV421-https://www.bdbiosciences.com/content/bdb/paths/generate-tds-document.us.564754.pdf

hCD19-APC-Cy7-https://www.biolegend.com/en-us/products/apc-cyanine7-anti-human-cd19-antibody-1910?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=APC/Cyanine7%20anti-human%20CD19%20Antibody.pdf&v=20230628033023
hCD3-AF700-https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-human-cd3-antibody-3394?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Alexa%20Fluor%C2%AE%20700%20anti-human%20CD3%20Antibody.pdf&v=20230114013553
hCD4-Pacific blue-https://www.biolegend.com/en-us/search-results/pacific-blue-anti-human-cd4-antibody-2850?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Pacific%20Blue%E2%84%A2%20anti-human%20CD4%20Antibody.pdf&v=20230114013553
hCD56-PE-Cy5-https://www.biolegend.com/en-us/products/pe-cyanine5-anti-human-cd56-ncam-antibody-10213?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=PE/Cyanine5%20anti-human%20CD56%20(NCAM)%20Antibody.pdf&v=20230114013553
hCD127-AF647-https://www.thermofisher.com/antibody/product/CD127-Antibody-clone-eBioRDR5-Monoclonal/51-1278-42
hCD25-PE-Cy7-https://www.bdbiosciences.com/content/bdb/paths/generate-tds-document.us.560920.pdf
hTCRab-PerCp-Cy5.5-https://www.biolegend.com/en-us/search-results/percp-cyanine5-5-anti-human-tcr-alpha-beta-antibody-9232?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=PerCP/Cyanine5.5%20anti-human%20TCR%20%CE%B1/%CE%B2%20Antibody.pdf&v=20230606063319
hCCR7-FITC-https://static.miltenyibiotec.com/asset/150655405641/document_3oc1m6viqt6nf5fcs8cvv86p0p?content-disposition=inline
hCD27-BV711-https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-human-cd27-antibody-13982?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Brilliant%20Violet%20711%E2%84%A2%20anti-human%20CD27%20Antibody.pdf&v=20230524063105
hCD56-Pe-Cy7-https://www.biolegend.com/en-us/search-results/pe-cyanine7-anti-human-cd56-ncam-antibody-9959?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=PE/Cyanine7%20anti-human%20CD56%20(NCAM)%20Antibody.pdf&v=20230630093043
hPD-1-BV785-https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-human-cd279-pd-1-antibody-7980?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Brilliant%20Violet%20785%E2%84%A2%20anti-human%20CD279%20(PD-1)%20Antibody.pdf&v=20230524063105
hGITR-PE-https://www.thermofisher.com/antibody/product/CD357-AITR-GITR-Antibody-clone-eBioAITR-Monoclonal/12-5875-42
hTIGIT -AF488-https://www.thermofisher.com/antibody/product/TIGIT-Antibody-clone-MBSA43-Monoclonal/53-9500-42
hLAG3-BV650-https://www.biolegend.com/en-us/products/brilliant-violet-650-anti-human-cd223-lag-3-antibody-13941?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=Brilliant%20Violet%20650%E2%84%A2%20anti-human%20CD223%20(LAG-3)%20Antibody.pdf&v=20230114013553
hPerforin-PE-https://www.biolegend.com/en-us/products/pe-anti-human-perforin-antibody-7516?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=PE%20anti-human%20Perforin%20Antibody.pdf&v=20221115073101
hFASLG-PE-Cy7-https://www.biolegend.com/en-us/products/pecyanine7-anti-human-cd178-fas-l-antibody-18204?pdf=true&displayInline=true&leftRightMargin=15&topBottomMargin=15&filename=PE/Cyanine7%20anti-human%20CD178%20(Fas-L)%20Antibody.pdf&v=20220817064306
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Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	P815 cell line is derived from Mus musculus mastocytoma. Cell type origin is a mast cell. Available from ATCC (American Type Culture Collection).
Authentication	Cell line was not authenticated in-house. Relevant details are available at ATCC's website. https://www.atcc.org/products/tib-64?matchtype=&network=g&device=c&adposition=&keyword=&gad=1&gclid=Cj0KCQjwtamlBhD3ARIsAARoaEyv2hBoR31ET6NC3DdDOUNeAJ7ul4vkgkGVumQZ0Wf2FcHgc9F3PB0aAtQ4EALw_wcB
Mycoplasma contamination	Not detected (ATCC). No further tests were done in-house.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

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	For fate mapping of exTregs, Foxp3eGFP-Cre-ERT2 (Jackson; #016961) mice were crossed to B6.CgGt(ROSA)26Sortm14(CAGtdTomato)Hze/J (Jackson; #007914) and B6.129P2-Apoetm1Unc/J (Jackson; #002052) to obtain the lineage tracker Apoe-/- mice (Foxp3eGFP-Cre-ERT2;Cg-Gt(ROSA)26Sortm14(CAG-tdTomato)Hze/J;129P2-Apoetm1Unc/J, ROSA26-SORT-CAG/FoxP3-eGFP-ERT-Cre/Apoe-/-). Mice used were from 8 weeks to 28 weeks old. B6.129P2-Apoetm1Unc/J were used only for breeding purposes and not for any experiment in this study.
Wild animals	No wild animals were used.
Reporting on sex	Both males and females were used as exTregs/Tregs can be found in both sexes after tamoxifen injection.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All mouse experiments were approved by the La Jolla Institute for Immunology Animal Care and Use Committee (protocol no.AP00001019).

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	Human flow cytometry: fresh PBMC were isolated from blood using SepMate tubes and Ficoll gradient method. Mouse flow cytometry: cells from spleens and lymph nodes. Organs were crushed on 100um filters using PBS, cells were washed and RBC were discarded (if needed) using RBC lysis buffer. Single cells suspensions were then stained.
Instrument	BD FACSCanto, LSR-II, LSR Fortessa, FACSAria IIu, FACSARIA Fusion
Software	Flow cytometry data was acquired on cytometers using the FACSDiva software (BD Biosciences), and was analyzed with FlowJo software (V10.8.1; BD Biosciences and FlowJo LLC).

Cell population abundance	Frequencies of relevant populations are shown. Cell suspensions were sorted on a 70um nozzle at a high pressure putting a 0.32.0 mask.
Gating strategy	For human: After gating on morphology, singlets, live cells (DAPI- or Ghost Dye510) and CD8a-CD14-CD19-, Tregs were defined as CD3+CD4+CD25highCD127low, exTregs candidate as CD3+CD4+CD56+CD16+, Teff as CD3+CD4+CD25-, Tnaive as CD3+CD4+CD45RA+CCR7+, CD8 CTL as CD14-CD19-CD3+CD4-CD45RA- , NK cells as CD3-CD4-CD16+CD56+. For mouse: after gating on morphology, singlets, live cells (zombie yellow or Ghost Dye510), mouse Tregs were defined as TCRb+CD4+GFP+TdTomato+ and exTregs as TCRb+CD4+GFP-TdTomato+. All gates were set using either FMO or isotype controls antibodies. For BrDU, untreated "no BrDU" control was used.

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