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Corresponding author(s): Kilian Schober

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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	No software was used for data collection.
Data analysis	Flow-cytometric data were analyzed with FlowJo v10.7.2. For visualization and some statistical analysis, GraphPadPrism (v10.2.0) was used. We performed analysis of scRNA seq data with CellRanger version 7.1.0 (10X Genomics), scanpy (v1.10.1), scirpy (v0.14.0), HashSolo, sklearn package (v1.5.0), Decoupler package (v1.8.0), visionpy (v0.2.0), R (v4.5.1), oligo (v1.72.0), AnnotationDbi (v1.70.0), dyplr (v1.1.4), Python (v3.13.5). All code is available on Github (https://github.com/SchoberLab/YF_scRNAseq).

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 data generated or analyzed during this study are included in this article, its supplementary information files or are publicly available on NCBI GEO with the accession number GSE298237. Additional raw data are available from the corresponding authors upon reasonable request.

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 reported for all donors as shown in Table S1. Gender identity was not assessed.
Reporting on race, ethnicity, or other socially relevant groupings	Donors were of European Caucasian ethnicity. Ethnicity was self-reported and used to describe the population's background. No race- or ethnicity-based subgroup analyses were performed, and ethnicity was not used as a proxy for any other variable.
Population characteristics	The relevant charactersitics of all donors can be found in Table S1.
Recruitment	Donors were recruited through local university hospitals and gave informed consent before participation. The study excluded individuals with chronic medication.
Ethics oversight	Human studies were conducted under the oversight and approval of the Ethics Committee of the Medical Faculty of the University Hospital of Erlangen, Friedrich-Alexander University Erlangen-Nürnberg, Germany and the Medical Faculty of LMU, Germany.

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 sizes were chosen on the basis of previously published studys. No power analysis was performed before experiments.
Data exclusions	In flow cyometry experiments, T-cell subsets with less than 10 cells were excluded for functional and metabolic quantifications. For quantifications in scRNA-sequencing, cells from Leiden cluster 7 and 8 wer excluded, as these are likely pre-apoptotic cells and clusters 11, 12, and 13 were mostly excluded as these were most likely KIR cells (cluster 11), MAIT cells (cluster 12) and JUN/FOS effector T cells (cluster 13), which were not relevant to the analysis.
Replication	All experiments were perfomed with multiple human donors in individual experiments. Mouse study was perfomed in two individual experiments with each 6 mice. Individual data points are shown.
Randomization	No randomization was performed.
Blinding	No blinding was performed since the purpose of the study was not a comparison between different donors, but to provide methodological proof-of-concept in multiple donors.

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

Human samples: anti-CD3-BUV496 (741206;1:100), anti-CD4-PE-CF595 (562316;1:200), anti-CD4-BV786 (740962, 1:400), anti-CD8-BUV395 (563795;1:200), anti-CD8-BUV496 (612942;1:200), anti-CD19-PE/CF594 (562294;1:200), anti-CD56-PE/CF595 (564963;1:200), anti-HLA-DR-BV421 (562805;1:400), anti-HLA-DR-APC (560744;1:200), anti-CD38-BV605 (562666;1:400), anti-CD38-BUV395 (563812;1:200), anti-CD95-BUV737 (612790;1:20), anti-CD95-BV421 (566258;1:25), anti-Ki-67-BV711 (563755;1:20), anti-CD69-PC7 (561928;1:100) anti-Bcl2-PE (556535;1:100) and anti-γH2AX-PE (562377;1:20) anti-HLA-A2-FITC (551285;1:100) from BD Biosciences; anti-CD8-APC (301049;1:200), anti-CD4-BV510 (300545;1:50), anti-CD62L-FITC (304804;1:200), anti-CD62L;-APC/Cy7 (304813;1:100), anti-CCR7-FITC (353215;1:100), anti-mouse TCR ß chain-APC/fire750 (109246;1:100) and anti-CD45RA-PerCP/Cy5.5 (304121;1:400) from BioLegend; anti-CD4-PE (12-0049-42;1:400), anti-CD8-eF450 (48-0086-42;1:200), anti-CD56-FITC (11-0566-42;1:200), anti-CD45-PerCP/Cy5.5 (45-0459-42;1:100) and anti-CD45-PE/Cy7 (25-9459-42;1:400) from eBioScience; anti-puromycin-AF647 (MABE343-AF647;1:200), anti-puromycin-AF488 (MABE343-AF488;1:200) from Sigma-Aldrich; anti-CD8-FITC (A07756;1:200) from Beckman Coulter; anti-CD45-PB (PB986, 1:50) DAKO; anti-CD137-PE (130-119-885;1:100) from Miltenyi; anti-cleavedCaspase3-PC7 (64772S;1:50) from Cell Signaling Technology. Viability staining: ZombieAqua or ZombieNIR Fixable Viability Kit (BioLegend;4231017/ 423102;1:500).

Murine samples (see below): anti-puromycin-AF647 (MABE343-AF647;1:200) from Sigma-Aldrich; anti-CD45.1-FITC (110706;1:100); anti-KLRG1-PE/Cy7 (138416;1:100); anti-CD27-mCherry (124228;1:100); anti-CD4-APC/Cy7 (100414;1:300); anti-CD8-BV785 (100750;1:200); anti-CD19-APC/Cy7 (115530;1:300) from BioLegend; anti-CD62L-BUV737 (612833;1:200) from BD Biosciences. Viability staining: Fixable Viability Dye eFluor-780 (Thermo Fischer, 65-0865-18;1:1000).

Validation

Antibody validations were performed by antibody suppliers per quality assurance literature provided by each supplier.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Jurkat TCR-KO cells and RD114 cells were provided by the laboratory of Dirk Busch, TUM, Munich.

Authentication

Cells were used within 2 months after thawing. No additional authentification was performed.

Mycoplasma contamination

All cell lines were routinely tested for mycoplasma contamination (test results were negative).

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell line was 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

8-week-old female C57BL/6 mice

Wild animals

This study did not involve wild animals.

Reporting on sex

Only female mice were used.

Field-collected samples

This study does not involve samples collected from field.

Ethics oversight

Experimental protocols were approved by the district government of upper Bavaria (Department 5 - Environment, Health and Consumer Protection)

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	DRKS00034356 and ISRCTN17974967
Study protocol	Details are described in the methods section.
Data collection	Donors were recruited from the vaccination clinic of the university hospital Erlangen (2022-2024) or the LMU Munich.
Outcomes	No outcomes were defined for this study.

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	Human PBMCs were isolated from citrated peripheral blood by density gradient centrifugation using a BioColl density medium with a density of 1.077 g/mL (BioSell, BS.L 6115). Cells were resuspended in heat-inactivated FCS + 10% DMSO and stored in liquid nitrogen. In murine blood samples lysis was performed with Ammonium chloride-Tris (90% (v/v) 0.17 M NH4Cl, 10% (v/v) 0.17 M Tris HCl, pH 7.2) directly after blood collection. PBMCs or murine cells were washed twice with 200 µl cold FACS-buffer. When required, pHLA-multimers were added at a volume of 25 µL per sample with 1–2 Mio. cells, and the samples were incubated on ice for 25 min. Subsequently, 25 µL of FACS-buffer with surface antibodies and the viability stain was directly added, followed by an additional 20 min of incubation on ice. For samples without pHLA-multimer staining, cells were resuspended in 25 µL of FACS-buffer with surface antibodies and viability stain and incubated on ice for 20 min. After staining with surface antibodies, cells were washed twice with cold FACS-buffer. If intracellular staining was required, cells were fixed/permeabilized using the BD Cytofix/Cytoperm Kit (BD Biosciences, 554714) for human samples and eBioscienceTM Foxp3/Transcription Factor Staining Buffer Set (Invitrogen by Thermo Fisher Scientific, 00-5523-00) for murine samples, in accordance with the manufacturer's instructions. For intracellular antibody staining, cells were incubated on ice for 60 min in 50 µL PermWash (1x) per 1 Mio. cells with the respective antibodies. Cells were then washed twice with cold PermWash (1x) and once with cold FACS buffer. Samples were then analyzed using an LSRFortessa Cell Analyzer (BD Biosciences) and FlowJo software version 10.7.2 (Tree Star Inc.). Unless indicated otherwise, we gated on single, living, CD19-CD56-CD4-CD3+CD8+ A2/NS4B+ or A2/NS4B- (unspecific).
Instrument	Flow cytometry samples were analyzed using an LSRFortessa Cell Analyzer (BD Biosciences). Human CD8+ T cells and OT-1 murine cells were sorted at a FACS Aria II cell sorter (BD Biosciences).
Software	Flow-cytometric data were analyzed with FlowJo v10.7.2. For visualization and some statistical analysis, GraphPadPrism (v10.2.0) was used.
Cell population abundance	For human epitope specific CD8+ T cells no purity sort but an enrichement sort was perfomed. Number of sorted cells varied between donors and timepoints. Cell numbers recovered in scRNAseq analysis are shown in Table S4.

Gating strategy

Lymphocyte gates were defined according to FSC and SSC area. Singlets were gated with FSC and FSC width. Living cells were gated based on viability dyes. Additional gating was performed for individual experiments, as described in figure legends.

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