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	BD FACSDiva Software Version 8.0.1, Hamamatsu Photonics NDP.view2 version 2.9
Data analysis	Commercial: FlowJo version 10.9.0, GraphPad Prism version 9.4.0, NDP.view2 version 2.9. Open-source: R version 4.2.2, OpenCyto (opencyto.org), SEARchways (http://github.com/BIGslu/SEARchways). Published manuscripts for the above open-source software are included in the References of this study.

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

Source data are provided with this paper. The accession number for the bulk RNA-seq data reported in this paper is found at GEO accession number GSE253755 [https://www.ncbi.xyz/geo/query/acc.cgi?acc=GSE253755]. Any additional requests should be directed to the corresponding author.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	No statistical methods were used to pre-determine samples sizes but our sample sizes are similar to those reported in previous publications: Olsen et al. J Immunol (2018); Vom Steeg et al. Vaccine (2019). In general, 2-5 mice were used per group and then experiments repeated 2-3 times and results pooled.
Data exclusions	Data analyzed included outliers unless explained by pre-established documented technical error.
Replication	Number of biological and technical replicates are specified in figure legends, and all replicates where stated were successful. RNAseq data in Figure 3 was not replicated due to high cost of RNAseq experiment. In Figure S9d one group was not repeated since it was a proof-of-function readout. In Figure 7, one group was not replicated due to unexpected post-surgical mortality and is identified as such as n=5 mice in the legend.
Randomization	For each biological replicate, the order of treatments were randomized for each group. For each experiment replicated, all samples were acquired in one batch (acquired on the same day with same flow cytometer parameters).
Blinding	Individuals who acquired samples were not blinded to treatment of groups. Data collection and analysis were also not performed blind to the conditions of the experiments, with the exception of immunohistochemistry quantification, which was conducted by a blinded reviewer. When blinding was not conducted, it was because of number of personnel limitations.

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

All information is provided in the Supplementary Table 2 . Also find the information included below:

Marker ; Fluorochrome; Clone; Source Catalog; Titer (in 50 µL MM)
Live/Dead Zombie yellow Biolegend 1:250
CD3E BUV395 145-2C11 BD Bioscience 563565 1 µL
B220 BV711 RA3-6B2 Biolegend 103255 0.25µL
CD4 BB700 GK1.5 BD Bioscience 745922 0.5µL
CD8a APC-H7 53-6.7 BD Bioscience 560182 0.5µL
CD44 BUV737 IM7 BD Bioscience 612799 0.5µL
CD62L PE-CY7 MEL-14 BD Bioscience 560516 0.5µL
CD69 BV786 H1-2F3 BD Bioscience 564683 1µL
CXCR3 BV650 CXCR3-173 BD Bioscience 740630 1µL
PD1 PE/DAZZLE594 29F.1A12 Biolegend 135228 0.5µL
LAG3 BB515 C9B7W BD Bioscience 564672 0.5µL
TCF1 BV421 S33-966 BD Bioscience 566692 1µL
CD107a PE 1D4B BD Bioscience 558661 0.5µl (in 200µL MM)
IFNG BV421 XMG1.2 Biolegend 505830 1µL
TNF FITC MP6-XT22 BD Bioscience 554418 1µL
Granzyme B AF647 GB11 BD Bioscience 560212 0.5µL
CSP-tetramer APC NIH tetramer facility 1µL

IHC antibodies
anti-CD8a (Rat monoclonal IgG2a eBioscience Cat no.14-0808-02; 1:1000)
anti-CD11c (Rabbit monoclonal, Cell Signaling Cat no. 97585; 1:500)

Validation

All flow antibodies used in this study come from commercial manufacturers as specified in Supplementary Table 2 or included in methods section. Antibodies were used in accordance with manufacturer recommendations. All antibodies used were validated for appropriate species reactivity in mice and for use in flow cytometry applications. Specified dilutions, clones, and catalog numbers are indicated. Antibody staining procedures are specified in the Method section of the paper.

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

Animal studies were performed according to the regulations of the institutional animal care and use committee. Approval was obtained from the University of Washington (UW) Institutional Animal Care and Use Committee (IACUC) under protocol 4317-01. The UW IACUC adheres to the NIH Office of Laboratory Animal Welfare standards (OLAW welfare assurance # D16-00292). Male and female BALB/cj and C57BL/6 mice (4-6 wk old) from Jackson Laboratories were used. Mice experience a 14-hour light and 10-hour dark cycle in a 72F and 30-70% humidity room. Primary euthanasia was conducted with CO2 inhalation, followed by a secondary dissection method.
Adult Anopheles stephensi mosquitoes strain STE2 were used.

Wild animals

No wild animals were used in this study.

Reporting on sex

Consideration of biological sex is at the forefront of this manuscript and findings should be considered applicable to males and females. For each experiment, the sex of the individual was included on the figure. When a single sex was excluded from an experiment, it was to specifically assess the impact of testicular or ovarian hormones on males and females, respectively.

Field-collected samples

This study did not involve field collected samples.

Ethics oversight	Approval was obtained from the University of Washington (UW) Institutional Animal Care and Use Committee (IACUC) under protocol 4317-01. The UW IACUC adheres to the NIH Office of Laboratory Animal Welfare standards (OLAW welfare assurance # D16-00292).

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

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	Unperfused livers were collected into RPMI media containing, 5% fetal bovine serum (FBS), and supplemented with glutamine (RPMI mixture). Each liver was gently mashed through a 100-µm cell strainer and washed with RPMI mixture. Cell suspensions were centrifuged at 80 x g for 1 min at 22C with no brake. Isolated supernatants were then centrifuged at 450 x g for 10 min to pellet cells. Cells were resuspended in 10 mL of 35% Percoll (GE Healthcare) in HBSS (Life Technologies) supplemented with 100 U heparin (Millipore Sigma) before centrifugation at 830 x g at 22C with no brake. The cell pellet was incubated in 3 mL RBC lysis solution (150 mM NH4Cl, 10 mM KHCO3; 0.1 mM EDTA. Na2·2H2O) for 3 min before being washed with 10 mL of RPMI mixture and spun at 500 x g at 4C for 8 min. Final pellets were resuspended in 200 µL of RPMI mixture and moved to 96-well plates for antibody staining. Splenic lymphocytes were isolated by teasing tissue through a 100-µm cell strainer, washed with RPMI mixture and centrifuged at 460 x g at 4C for 5 min. The cell pellet was incubated in 3 mL RBC lysis solution for 3 min before being washed with 10 mL of RPMI mixture and spun at 460 x g at 4C for 5 min. Cells were resuspended in 1 mL of RPMI mixture and counted. Cells were diluted, and one million splenocytes per sample were used for antibody staining.
Instrument	BD LSR Fortessa
Software	FlowJo version 10.9.0 software (TreeStar) / BD FACSDiva Software Version 8.0.1
Cell population abundance	Lymphocytes were isolated from tissue and viability was assessed under a microscope prior to staining. Further inclusion of a live/dead dye identified dead cells. CD8+ T cells represented between 15-20% of total CD3+ cells. The proportion of CSP-tetramer positive cells was established by flow cytometry and shown in representative figures in manuscript.
Gating strategy	Cell populations were from isolated lymphocytes, gated based on FSC-A and SSC-A and doublet exclusion. Cell death was evaluated with a liver/dead dye. Further gating on lineage markers (i.e. B220, CD3, CD4, CD8, etc.), functional markers (TNF, IFNg, and, CD107a), and transcription factors (TCF1), was specified for each respective panel and outlined within the Supplemental Figures 3 and 4.

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