nature portfolio

Corresponding author(s): Ian Parish, Maria Nogueira de Menezes

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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	Flow cytometry data was collected using the flow cytometer listed in the methods section using FACSDiva Software (BD Biosciences).
Data analysis	Flow cytometry data was analyzed using FlowJo software (BD Biosciences v10.7.2), data calculations were conducted in Microsoft Excel (v16.90.2) and data was graphed and statiscally analysed using GraphPad Prism (GraphPad Software v10.3.1). For the bioinformatic analysis, the following software were used: 10x Cell Ranger ARC pipeline (version 2.0.0), R (version 4.4.2), CITE-seq-Count (version 1.4.5), Seurat (version 5.0.2), scDblFinder (v1.16.0), DoubletFinder (version 2.0.4), Signac (version 1.12.0), ArchR (version 1.0.2), MACS3, clustree package (version 0.5.0), ProjecTILs (version 3.0.3), RunChromVar (version 1.4.3), dittoSeq (version 1.8.1), ComplexHeatmap (version 2.14.0).

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 in this study are provided in the article itself, its supplementary information and in the Source Data file. The multi-ome (scRNAseq and scATACseq) sequencing data from Figure 6 and Supplementary Figure 4 have been deposited in GEO NCBI under the accession code GSE312328 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE312328].

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	This information has not been collected.
Reporting on race, ethnicity, or other socially relevant groupings	No human data.
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	Sample sizes were chosen in mouse experiments based on prior experience of the "n" required to achieve significance given the known experimental variation from our previous work (eg. Nussing et al JI 2020).
Data exclusions	No data was excluded.
Replication	All reported experiments were conducted at least two times, and all findings reported were successfully reproduced.
Randomization	In experiments where there were different treatment conditions (eg. adoptive transfer experiments), mice were randomly assigned to groups, and age and sex matched mice were used.
Blinding	While blinding was not always feasible due to cage labelling and animal housing requirements, where possible, downstream analysis of samples (ie. sample processing, flow cytometric analysis) was conducted in a manner blinded to treatment condition.

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

Zombie Aqua Fixable Viability Kit (BioLegend, Cat. no. 423102,1:1000), CD8-BUV395 (Clone 53-6.7, BD Biosciences, Cat. no. 563786, 1:200), CD45.1-Pacific Blue (Cat. no. 110722, 1:200), -FITC (Cat. no. 110706, 1:200) or -APC (Cat. no. 110714, 1:200) (Clone A20 - BioLegend), Thy1.2-PE (Clone 53-2.1, BD Biosciences, Cat. no. 553005, 1:1000), CD44-Pacific Blue (Cat. no. 103020, 1:200) or -APC (Cat. no. 103012, 1:200) (Clone IM7, BioLegend), CXCR5-PECy7 (clone 2G8, BD Biosciences, Cat. no. 560617, 1:25), PD-1-BV785 (clone 29F.1A12, BioLegend, Cat. no. 135225, 1:100), Anti-mouse rabbit TCF-1 mAb (clone C63D9, Cell Signaling Technology, Cat. no. 2203S, 1:400), secondary anti-rabbit IgG AlexaFluor594 (ThermoFisher Scientific, Cat. no. A-11012, 1:1000), Granzyme-B-APC (Clone GB11, Invitrogen, Cat. no. GRB05, 1:200) and TOX-PE (clone TXRX10, Invitrogen, Cat. no. 12-6502-82, 1:100), IFNg-PECy7 (clone XMG1.2, eBioscience, Cat. no. 25-7311-82, 1:2000) and TNFa-PE (clone MP6-XT22, BioLegend, Cat. no. 506306, 1:2000).

Validation

Representative staining is shown for a number of antibodies within Figures 1, 2, 3, 4, 5 and Supplementary Figures 1 and 3. Further validation data is provided on the manufacturer's website, which illustrates target staining relative to isotype control antibodies. Validation data can be accessed by searching for the listed catalog numbers on the Biolegend (https://www.biolegend.com/), eBioscience/Invitrogen/Thermofisher (https://www.thermofisher.com/), BD Biosciences (https://www.bdbiosciences.com/) or Cell Signaling Technology (https://www.cellsignal.com/) websites.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK293T tumor cell lines were obtained from the American Type Culture Collection.

Authentication

The cell line was not authenticated but was utilized within 10 passages of a master stock

Mycoplasma contamination

Cells were tested negative for mycoplasma contamination

Commonly misidentified lines
(See ICLAC register)

None of the cell lines are listed on the ICLAC database

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

CD45.2+ C57BL/6 (B6) mice were purchased from the Walter and Eliza Hall Institute Kew Animal Facility (Kew, VIC, Australia), whereas CD45.1+ P14 transgenic mice were bred in-house with constant backcrossing to the Kew Animal Facility C57BL/6 background. Both male and female mice were used at 6-14 weeks of age, and all mice were maintained in specific pathogen free conditions.

Housing was as follows;
Cage type - Allentown IVC
Bedding - irradiated corncob
Diet - Barastoc irradiated commercial rat and mouse pellets
Light cycle - 10 hour dark/14 hour light with a half hour sunrise and a half hour sunset period
Ambient temperature is set at 20oC +/- 1degree
Relative humidity is 40%

Wild animals

The study did not involve wild animals.

Reporting on sex

Both male and female mice were used in the study and sex was assigned according to mouse availability and donor mouse sex match. We do not see sex-dependent differences in exhausted T cell differentiation after chronic LCMV infection, although mice within each experiment were still sex matched across groups.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All animal work was in accordance with protocols approved by the Peter MacCallum Cancer Centre Animal Experimentation

Ethics oversight	Ethics Committees, and current guidelines from the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

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

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	Spleens were homogenised through a 70 µm strainer to obtain a single cell suspension prior to red blood cell lysis in 0.83% NH4Cl. For cell surface staining, cells were stained on ice in FACS buffer
Instrument	BD Fortessa X20
Software	Samples were collected using the FACSDiva Software and analyzed using FlowJo software (BD Biosciences v10.7.2).
Cell population abundance	In experiments where cells were sorted by FACS, purity was confirmed post-sort by sample re-analysis
Gating strategy	A broad FSC/SSC gate was used to eliminate small debris, followed by doublets exclusion using FSC-H vs FSC-A. Dead cells were then excluded using a fixable viability marker and CD8+ CD45.1+ cells were then gated for subsequent analysis.

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