nature portfolio

Corresponding author(s): Aron E. Lukacher

Last updated by author(s): May 1, 2026

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	Relevant code for the MERFISH data was made available previously on GitHub in the stratton-lab/alexander-2025-cxcr repository, with a DOI available via Zenodo at zenodo.21179919 (European Organization For Nuclear Research and OpenAIRE 2013). Code for the single-cell RNA-seq data analysis has been deposited via Zenodo at https://doi.org/10.5281/zenodo.21243082. Code for analyzing the Luminex data has been deposited via Zenodo at DOI: 10.5281/zenodo.21076315.
Data analysis	FlowJo (v. 10.1, BD Biosciences), GraphPad Prism (v. 10.3.1, Dotmatics), Seurat R package v. 5.3.0, R v. 4.4.3, ggplot2 R package (RRID:SCR_014601), dplyr (RRID:SCR_016708) tidyr (RRID:SCR_017102), ProcartaPlex software (Thermo Fisher), ropls (v.1.44.0)

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

The MERFISH data generated in this study have been deposited in the Gene Expression Omnibus (GEO) database under accession code GSE296357. The single-cell sequencing RNA-seq data have been deposited in the GEO database under accession code GSE337457 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE337457]. Non-sequencing data generated in this study are provided in the source data file.

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	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

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 based on our extensive experience using polyomavirus-infected mice, where individual groups of 3-5 mice in 2-3 biological replicates, are sufficient for statistical analyses.
Data exclusions	No data was excluded
Replication	2-3 biological replicates for each experiment. All replicates were successful.
Randomization	Male and female mice were randomly assigned to each experimental group.
Blinding	For the Luminex assay study, our collaborators were unaware of the study sample sources until their analyses were completed. For flow cytometry and qPCR studies, the authors were not deliberately blinded to the study group assignments but used appropriate negative and positive controls for each experimental group. Blinding was not relevant to flow cytometry and qPCR studies, as analyses of these samples are based on software algorithms and are performed in the same manner (e.g., the same gating strategy is used for all brain samples in one experiment).

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
[ ]	[x] Antibodies
[x]	[ ] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used	Refer to Supplementary Table 1.
Validation	All primary antibodies used were either validated by the manufacturer and/or validated in lab at different concentrations in mice.

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	Strains are listed in Supplementary Table 1. Both males and females were used at 2 to 4 months old. Mice were bred and housed in pathogen-free facilities, with virus-infected experimental mice housed separately from non-infected breeding mice. Mice are under a 12-hour light/dark cycle, and rooms are kept at 22°C and at an ambient humidity range of 30 to 70 %.
Wild animals	*Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Reporting on sex	Both male and female mice were used.
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	Penn State College of Medicine Institutional Animal Care and Use committee (Protocol PRAMS201447619)

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:

[x] The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

[x] 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).

[x] All plots are contour plots with outliers or pseudocolor plots.

[x] A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	For lymphocyte isolation from brain tissue, brains were minced, digested with collagenase type I (Worthington) and deoxyribonuclease I (DNAse) (Worthington) for 20 min at 37°C, then passed through a 70 µm filter. Lymphocytes were then separated via a 44%/66% Percoll (Cytiva) step gradient, collected from the interface, and resuspended in wash media [DMEM with 2% fetal bovine serum (FBS)]. For lymphocyte isolation from spleens, spleens were passed through a 70 µm filter, and red blood cells (RBC) were lysed with ammonium-chloride-potassium (ACK) buffer for 5 min at room temperature (RT). Single cells were then resuspended in wash media. For glial cell isolation, brains were dissected, minced, and digested with collagenase type I (Worthington) for 20 min at 37°C. Homogenate was passed through a 100 µm filter, centrifuged on a 37% Percoll gradient, then single cells were resuspended in wash media. For blood samples, blood was collected from mice through submandibular bleeds into tubes containing an equal volume of heparin. RBCs were lysed with two 5-min ACK treatments, washed with wash media, and resuspended in 500 µl of wash media.
Instrument	23-color BD FACS Symphony flow cytometer (BD Biosciences)
Software	FlowJo (BD Biosciences)
Cell population abundance	Purity of the sorted cell populations was greater than 95%.
Gating strategy	Representative gating strategies for lymphocytes and glial cells are included as supplementary figures. Lymphocytes were gated via FSC and SSC. From the lymphocyte gate, single cells were gated. Live cells were then gated via negative expression for fixable viability dye, then gated for CD8 and CD4. CD8+ cells were gated for positive expression of tetramer and CD44. Glial cells were gated via CD45 and CD11b expression.

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