nature portfolio

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Last updated by author(s): Jun 20, 2024

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

The following softwares were used to collect the data in this study:
- Olympus V200 ASW
- CYTEK SpectroFlo
- Leica Application Suite v4.3.0.24308
- BioTek Gen5 3.11
- Illumina NextSeq Control Software
- Thermo Scientific Orbitrap Fusion Lumos Control Software
- Cellranger_1.1.0

Data analysis

The following softwares were used to analyze the data in this study:
- Fiji image processing software v2.1.0/1.53c
- Prism v9.4.0
-SnapGene v4.3.6
- FlowJo v10.7.1
- R v3.5.0
-10X genomics Cellranger software pipeline
- Seurat v3
- STAR v2.5.1a
- PEAKS Studio 10.6
- Custom code used for single-cell-seq analysis is available upon reasonable request.

- DropletUtils_1.10.3
- Custom code used for single-cell RNA-seq analysis is available from the corresponding authors upon request.

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

Adventitious Proteins database (www.thegpm.org/crap/)
Human Protein Atlas Database (https://www.proteinatlas.org)
Raw data of all single-cell sequencing are available on the Gene Expression Omnibus (GEO) under accession numbers GSE216391 and GSE189812.
Raw data of Mass Spec peptidome has been uploaded on MassIVE under accession numbers MSV000091130.

Human research participants

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

Reporting on sex and gender	Data from both male and female are involved. No preference of gender for the study.
Population characteristics	Cerebrospinal fluid (CSF) samples without obvious blood contamination are involved in the study. Five patients with complete/incomplete spinal cord injury were involved in this study. Patients involved in this study were with either thoracic or cervical spinal cord injury. Both male and female were involved in this study. Patients involved in this study were at their age of 20s to 70s.
Recruitment	Patients in Barnes-Jewish Hospital with complete/ incomplete spinal cord injury were involved in this study. These samples were obtained with informed consent for research use and were approved by the review board of Washington University in St. Louis.
Ethics oversight	The CSF samples used in the scRNA-seq experiments were obtained with informed consent for research use and were approved by the review board of Washington University in St. Louis under IRB# 202012050.

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 standard power calculations (with $\alpha = 0.05$ and power of 0.8) performed for similar experiments that were previously published (Drieu et al., Nature 2022). In general, statistical methods were not used to re-calculate or predetermine sample sizes.
Data exclusions	For BMS scoring (locomotion test) of spinal cord injury mice, if obvious infection of mice was observed, data from that individual mice will be removed. If the BMS score of SCI mice reaches 2 within the first two days after surgery, it indicates surgical failure. Data from such mice will be excluded from the analysis.
Replication	Number of reliable reproductions of each experimental finding is stated in each Figure legend. For single cell sequencing, mice (number mentioned individually in manuscript) were pooled in each group and sequence together. Other experiments were replicated at least once.
Randomization	Statistical analysis and reproducibility subsection: Mice were allocated randomly to cages with no more than 5 mice per cage after been shipped to the vivarium. Animals from different cages, but within the same experimental group, were selected to assure randomization. Mice from the same cage were received different treatments (for example, if there are four experimental groups in one experiment, will keep 4 mice per cage with each of them in different group). Treatment was given in a blind manner and could be identified by a corresponding ear tag.

For in vitro studies, cells from the same animal were harvested and subjects were randomly assigned into each experimental group. Treatments and controls were plated adjacent to one another.

Blinding

For single cell sequencing, at least one investigator responsible for sample collection was not blinded to ensure there was no cross-contamination between groups. Analyzer analyzed the data in blind.
For optic nerve injury, treatments were given in a blind manner and analyzer analyzed data in blind. For spinal cord injury and BMS scoring, treatments were given in a blind manner and BMS scoring was performed every other day by two independent investigators, both of whom were blinded.
No blinding was performed in the in vitro cell culture study to ensure there was no cross-contamination between 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-CD45 (103124, BioLegend), anti-MHC-II (107650, BioLegend), anti-CD3 (50-0032-82, eBioscience), anti-Iba1 (ab5076, Abcam), anti-Laminin (ab11575, Abcam), anti-GFAP (Z0334, Agilent Technologies), anti-Brn3a (411003, Synaptic system)), anti-NeuN (ab190195, Abcam) and FluoroMeylin Red (F34652, Fisher Scientific) were used for immunostaining.
Anti-CD3e (553057, BD Biosciences) and anti-CD28 (553294, BD Biosciences) were used for in vitro T cell activation.
Flow cytometry antibodies of CD45-FITC (11-0451-85), CD90.2-PE-Cy7 (25-0902-81), CD4-APC-eFluor780 (47-0041-82), CD8-APC-eFluor780 (47-0081-82), CD3-eFluor660 (50-0032-82), CD69-APC-eFluor780 (47-0691-82), CD8a-Alexa Fluor 532 (58-0081-80), CD45-BV750 (746947), TCRβ-BUV805 (748405), B220-APC (50-0452-82), TCRγ-FITC (11-5711-82), CD8a-Alexa Fluor 700 (557959), T-bet-PE (12-5825-82), T-bet-PE-Cy7 (25-5825-82), FoxP3-PE-eFluor610 (61-5773-82) FoxP3-APC (17-5773-80) and RORγt-BV650 (BDB564722) were purchased from eBioscience/ Thermo Fisher. Flow cytometry antibodies of CD11b-PE-Cy7 (552850), CD45-APC (559864), CD4-PE (553049), B220-PE (553090), Vβ5-PE (553190), CD4-PE-Cy7 (552775), CD11c-PE-Cy7 (558079) and CD4-BUV395 (563790) were purchased from BD Biosciences. Flow cytometry antibodies of TCRβ-PerCP/Cy5.5 (109228), CD45-BV510 (103137), CD45-PE (103106), CD11b-PerCP/Cy5.5 (101228), CD3-Alexa Fluor 594 (100240), TCRβ-BV421 (109230), TCRβ-FITC (109206), MHCII-BV650 (107641), Thy1.2-BV510 (105335), Thy1.1-PE (202524), Vβ11-FITC (125905), CD62L-BV785 (104440), TCRβ-BV510 (109233), CD69-PE (104508), CD44-PerCP/Cy5.5 (103032), Gata3-BV421 (653813), Gata3-APC (653806), IFNγ-BV711 (505836), Zombie Aqua (423102) and Zombie NIR Fixable Viability Kit (423105) were purchased from BioLegend. Antibodies for testing human CSF cells, HLA-DR (307638), TCRβ-BV785 (306741), CD8-APC-Cy7 (344713), CD4-APC-Fire 810 (344661), CD4-BV711 (300558), CD45-Alexa Fluor647 (304020) were purchased from BioLegend and CD11b-BUV563 (741242), CD235A-BUV395 (563810), CD5-BV421 (562646) were purchased from BD Biosciences.

Validation

Each antibody was validated for the species (mouse, human) and application (immunohistochemistry, Flow cytometry and fluorescence activated cell sorting, cell culture) by the correspondent manufacturer. All of antibody were tested using samples from control tissue (for example, spleen and blood).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Phoenix-ECO retroviral packaging cells (CRL-3214) were obtained from the American Type Culture Collection (ATCC).
The 58 α-β- hybridoma was generated by Dr. Kenneth Murphy and kindly provided by Dr. Dan Littman (New York University, United State).
Primary murine T cells: Spleen and lymph nodes from adult C57BL/6J mice were harvested, and primary CD4+ T cells were isolated using the EasySep mouse CD4+ T cell isolation kit (19852, STEMCELL Technologies). Both male and female were used depend on experimental design.
Primary murine neuron: The brain of E15-E19 embryo of C57BL/6J mice were used for primary neuron culturing. Both male and female were used.

Authentication

Primary murine CD4 T cells were identified by flow cytometry.
Identity of other cell lines by checking cellular morphological features but have not been authenticated by the short tandem repeat (STR) profiling.

Mycoplasma contamination	Morphological features of cells were frequently checked but mycoplasma contamination was not checked by PCR.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines are used in this study.

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	Wild type C57BL/6J mice (JAX: 000664, 7-10 weeks old) were purchased from the Jackson Laboratory and were kept in-house for at least 1 week before the start of an experiment. OT-II TCR transgenic mice (JAX: 004194), and 2D2 TCR transgenic mice (JAX: 006912), Ifng-/- mice (JAX: 002287), Thy1.1 mice (JAX: 000406), Rosa26-Cas9 mice (JAX: 026179) were purchased from the Jackson Laboratory and bred in-house. Both male and female were used in this study. All mice were housed and bred in a temperature-controlled (22 ℃) and humidity-controlled (33–39%) environment under a 12 h–12 h light–dark cycle and were provided with food and water ad libitum. No more than five mice were housed together per cage. Mice at their age between 2 months-4 months old were used.
Wild animals	No wild animal was involved in this study.
Reporting on sex	Only use female mice for spinal cord injury since spinal cord injury potentially induces urinary tract infection of male mice. Both male and female are involved in other experiments.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All experiments were approved by the Institutional Animal Care and Use Committee of the Washington University in St. Louis.

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	Mice were injected with a lethal dose of Euthasol (10% vol/vol) and transcardially perfused with PBS containing 0.025% heparin. Lymph nodes and spleen were directly collected using forceps. For spinal cord isolation, the spine was cut on both sides and the spinal cord was flushed out using a PBS filled syringe with a 18-gauge needle. The muscles were then removed and spinal cord dura was peeled from the vertebral. Optic nerves from the eye to the optic chiasm were collected for the following research. Lymph nodes and spinal cord dura were directly digested at 37 °C for 30 minutes in prewarmed digestion buffer (RPMI-1640 medium supplemented with 2% FBS, 1 mg mL-1 Collagenase VIII, and 0.5 mg mL-1 of DNase I). Spinal cord was minced using a surgical blade and digested in digestion buffer at 37 °C for 30 minutes, triturated with a 1 mL pipette, and digested for another 15 minutes. For the spleen, digestion buffer was injected into the tissue parenchyma and incubated at 37 °C for 5 minutes. Then spleen was then minced with a surgical blade and digested in digestion buffer at 37 °C for 20 minutes. After digestion, enzymes were neutralized with RPMI with 10% FBS and tissue samples were mechanically homogenized and filtered through 70 μm cell strainer. Cells were then centrifuged at 450 g for 4 minutes and the supernatant was removed. For lymph nodes, the cell pellet was directly resuspended with RPMI containing 10% FBS and kept on ice until use. For the spleen, red blood cells were lysed with ammonium-chloridepotassium (ACK) lysis buffer for 1 minute at room temperature and neutralized with the same volume of RPMI containing 10% of FBS. Cells were then centrifuged and cell pellets were resuspended with RPMI containing 10% FBS and kept on ice until use. For spinal cord and spinal cord dura, myelin was removed by resuspending cells with 30% percoll or 10-15% BSA in PBS and centrifuged at 800 g for 10 minutes with a slow brake. After centrifugation, the upper myelin-containing layer and supernatant were removed, and the cell pellet was resuspended with RPMI containing 10 % FBS. Single-cell suspensions were washed with PBS. The Zombie Aqua or Zombie NIR fixable Viability kit or DAPI was used to determine cell viability. Cell viability dyes were diluted in PBS and incubated with cells for 10 minutes at room temperature. After viability staining, Fc receptors were blocked for 5 min with the anti-CD16/CD32 antibody cocktail and cells were incubated with primary antibody cocktails for 10 minutes at room temperature for surface staining. Antibodies were all diluted in FACS buffer (2% BSA, 1 mM EDTA, 20 mM HEPES in PBS). After staining, samples were filtered with 70 μm cell strainer and ready for sample loading.
Instrument	Aurora spectral flow cytometer (Cytek) BD Biosciences FACSAria II

Software	CYTEK SpectroFlo for data collection. FlowJo v10.7.1 for data analyze.
Cell population abundance	Control cells or control tissue were always used for gating. For cell line study, post-sorted cells were cultured and purity were re-checked a few days after. If the purity of positive cell population is below 90%, cells would be performed the second round of sorting. For sorting cell from digested tissue for sequencing, a small proportion of sorted cells were re-loaded to Flow Cytometer to check the purify. Other contaminations were filtered out or specifically labeled based on quality of mRNA abundance and transcription characteristics after sequencing (described in Method).
Gating strategy	For all experiment, scatters (FSC-H>0.5M, SSC-H>0.5M), single cell and live/dead (DAPI- or zombie dye-) were performed to define starting cell population. For sorting TCR expressing hybridoma, target cell population were gated as CD3+ CD4+. For checking hybridoma activation, activated cell population were gated as CD11c- CD3+ CD4+ GFP+. For checking immune cell infiltration, cells were gated as CD45+ CD11b+/- B220+/- CD3+/- TCRb+ TCRrd+ CD4+/- CD8+/-. For sorting T cells from spinal cord or meninges for TCR sequencing, target cell population were gated as CD45+ Thy1.2+ CD4 + or CD8+. For sorting T cells from spinal cord for FACSseq, target cell population were gated as CD45+ CD11b- TCRβ+ CD4+ CD8- GFP+. For checking retrovirus based T cell proliferation, target cells were gated as CD3+ CD4+ GFP+. For checking mRNA based T cell proliferation, target cells were gated as CD11c- CD3+ CD4+. For checking 2D2/ OT-II T cell infiltration of optic nerve, target cells were gated as CD45+ CD3+ CD4+ Vb11+ or CD45+ CD11b- CD3+ TCRb+ CD4+ Vb11+ or Vb5+. For checking the proportion of injected T cells, target cell population were gated as CD45+ CD11b- B220- TCRβ+ Thy1.2+ Thy1.1-. For checking the phenotype of injected T cells, target cell population were gated as CD45+ CD11b- B220- TCRβ+ Thy1.2+ Thy1.1- CD4+ CD8-.

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