nature portfolio

Corresponding author(s): Howard L. Weiner

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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	1.RNA-seq. Samples were processed for cDNA generation and Illumina Nextera XT library construction. Sequencing data was generated using 2x38 bp paired end sequencing on the NextSeq500. 2.Flow cytometry. acquisition was performed on FACSSymphony A5 (BD Biosciences) using DIVA software. 3.Immunofluorescence. Images were acquired using a Leica DMi8 widefield microscope and Zeiss LSM710 Confocal. 4.RT-qPCR. Data were collected using a Vii 7 real-time PCR system. 5.Cell sorting. Cells were sorted using FACSAria II. 6.Behavioral data acquisition. Noldus EthoVision XT software v.17.0.
Data analysis	1.RNA-seq. RNA-seq reads was assessed using FastQC quality control tool. Reads were concatenated then trimmed using Trimmomatic. The RNA reads were aligned to the mouse mm10 reference genome using HISAT2. HISAT2-generated SAM files were sorted and converted into BAM files using Samtools. The sorted reads were assembled into transcripts using StringTie104. Next, StringTie-generated transcript lengths and abundance estimates were converted into count matrices using Tximport. Differential gene expression analysis was performed with false discovery rate (FDR)-adjusted P values using DESeq2. Data visualization was performed in R (version 4.0.3). 2.Flow cytometry. Data were analyzed with FlowJo software version 10. 3.Immunofluorescence. Images were processed using Fiji. 4.RT-qPCR. Quantitative PCR data were analyzed by the delta-delta Ct method. 5. Statistical analysis.Statistical analysis was performed using GraphPad Prism 9 software.

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 Smartseq2 RNA-sequencing data that support the findings of this study have been deposited into Gene Expression Omnibus (GEO) under SuperSeries GSE276761.

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	In our study, 3-12 mice were used per group and every experiment was reproduced at least twice, which is commonly used for in vivo studies to alleviate unnecessary animal suffering. No sample calculation size was performed but was chosen in accordance with previous studies in the field: -Roth, T. L. et al. Transcranial amelioration of inflammation and cell death after brain injury. -Nature 505, 223-228 (2014), Kramer, T. J. et al. Depletion of regulatory T cells increases T cell brain infiltration, reactive astrogliosis, and interferon-gamma gene expression in acute experimental traumatic brain injury. J Neuroinflammation 16, 163 (2019) Brain and blood samples are pools of 5 mice, and sham-Iso brain samples are pools of 20 mice. Due to the low number of FoxP3$^+$ cells recruited to the brain, and ethical considerations, we limited the study to two biological replicates, following practices from previous studies in the field for blood and treg sequencing: -Shi, L. et al. Treg cell-derived osteopontin promotes microglia-mediated white matter repair after ischemic stroke. Immunity 54, 1527-1542 e1528 (2021
Data exclusions	Outliers were excluded as determined by the following: Any value that is 1.5 x IQR greater than the third quartile is designated as an outlier and any value that is 1.5 x IQR less than the first quartile is also designated as an outlier.
Replication	All experiments were performed at least twice to assure rigor and reproductibility, and all attempts at replication were successful.
Randomization	Mice were randomly assigned to the experimental groups. For in-vitro experiments, cells from the randomly assigned mice were sorted with each mouse and each well in the culture was from a different biological replicate.
Blinding	For all experiments the investigator was blinded to the different groups. Experimenter was also blinded to the different well conditions in the in-vitro experiement.

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

in-vivo/vitro antibodies:
hamster IgG CD3-specific antibody (clone 145-2C11.BioXCell), hamster IgG control antibody (BioXCell), monoclonal anti-IL-10R blocking antibody (clone 1B1.3A, BioXCell), IL-10 neutralizing antibody (JES5-2A5; BioXCell).

Flow Cytometry:
Zombie Aqua Fixable Viability Kit (Biolegend, #423102, 1:1000) or Zombie UV (Biolegend, #423108, 1:1000) was used to exclude dead cells. The staining antibodies used PE/Cyanine7 anti-mouse CD11b (M1/70, Biolegend, 1:300), APC/Cyanine7 anti-mouse TCR-beta (H57-597, Biolegend, 1:100), BUV661 anti-mouse CD45 (30-F11, BD Biosciences, 1:200), PE anti-mouse CD4 (GK1.5, Biolegend, 1:100), BUV805 anti-mouse CD8 (53-6.7, BD Biosciences 1:100), FITC anti-mouse FoxP3 (FJK-16s, eBioscience, 1:100), BV421 anti-mouse LAP (TW7-16B4, Biolegend, 1:100), PE/Dazzle 594 anti-mouse IL-10 (JES5-16E3, Biolegend, 1:100), BUV395 anti-mouse IL-17a (TC11-18H10, BD Biosciences, 1:100), BV785 anti-mouse IFN-g (XMG1.2, Biolegend, 1:100), BV605 anti-mouse Ly6G (1A8, Biolegend, 1:300), BV711 anti-mouse NK-1.1 (PK136, Biolegend, 1:100), AF700 anti-mouse Ly6C (HK1.4, Biolegend, 1:200), BV711 anti-mouse Ly6C (HK1.4, Biolegend, 1:200), and APC anti-mouse 4D4 (1:1000) provided by Dr. Butovsky.

For Cell Sorting:
PE/Cyanine7 anti-mouse CD11b (M1/70, Biolegend, 1:100), APC/Cyanine7 anti-mouse CD45 (30-F11, Biolegend, 1:100), FITC anti-mouse Ly6C (AL-21, BD Bioscience, 1:200) and APC anti-mouse 4D4 (marking resident microglia; 1:1000. APC/Cyanine7 anti-mouse CD45 (30-F11, Biolegend, 1:100), APC anti-mouse CD4 antibody (GK1.5, Biolegend, 1:100), and 7-AAD (BD Bioscience.

Tissue Staining:
Anti Iba1 (rabbit,1:1000, Wako), 647 goat anti-rabbit IgG (1:1000, Abcam, ab150075) haematoxylin and eosin (HE; Abcam, ab245880), and TUNEL (TUNEL Assay Kit - BrdU-Red, Abcam, ab66110),DAPI mounting media (Vector Laboratories, UX-93952-24). Alexa Flour 647 anti-mouse CD3 (17A2, Biolegend, 1:50).

Validation

Antibody dilutions were used as recommended by the manufactures. All antibodies employed in this study are well characterized and broadly used by the research community. Validation of all primary antibodies for the species and applications can be found at the website of the company, for example, Biolegend, Abcam, and BD Biosciences where the antibodies were purchased. Microglia staining 4D4 antibody was developed in our laboratory and has been extensively used. Krasemann, S. et al. The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. Immunity 47, 566-581 e569 (2017). Butovsky, O. et al. Modulating inflammatory monocytes with a unique microRNA gene signature ameliorates murine ALS. J Clin Invest 122, 3063-3087 (2012).

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

Studies were performed using 8-12-week-old C57BL6J mice (000664, Jackson Laboratories), B6.CD45.1 mice (002014, Jackson Laboratory), FoxP3-GFP mice (023800, Jackson Laboratory), FoxP3-DTR mice (016958, Jackson Laboratory), IL10-KO mice (002251Jackson Laboratory), Tmem119-CreETR2 mice (031820, Jackson Laboratory), IL-10raFlx mice (028146, Jackson Laboratory), and 10BiT.FoxP3GFP (kindly provided by Dr. Vijay Kuchroo). All mice were housed under specific pathogen free conditions, with food and water ad libitum. All animals were housed in temperature (20 ºC) - and humidity (60 %)-controlled rooms, maintained on a 12-h/12-h light/dark cycle (lights on at 7:00 AM). Mice were euthanized by CO_2 inhalation. The Institutional Animal Care and Use

	Committee (IACUC) at Harvard Medical School and Brigham and Women's Hospital has all experimental procedures involving animals.
Wild animals	No wild animals were used in the study.
Reporting on sex	Both males and females were used.
Field-collected samples	No field-collected samples were used in the study.
Ethics oversight	All experiments were reviewed and overseen by the institutional animal use and care committee at Brigham and Women's Hospital in accordance with NIH guidelines for the humane treatment of animals. IACUC Protocol number: 2018N000028.

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

Flow cytometry microglial sorting

For microglial cell sorting, mice were anesthetized with CO2 until respiration rate slowed and then transcardially perfused with 50mL hanks balanced salt solution (HBSS) containing heparin (1:1000). Following perfusion, the ipsilateral hemisphere homogenised using a dounce glass tissue homogeniser. Cells were separated through Percoll (GE Healthcare Life Sciences) 30% gradient centrifugation. Cells were isolated from the Percoll layer and stained on ice for 30 min with combinations of PE/Cyanine7 anti-mouse CD11b (M1/70, Biolegend, 1:100), APC/Cyanine7 anti-mouse CD45 (30-F11, Biolegend, 1:100), FITC anti-mouse Ly6C (AL-21, BD Bioscience, 1:200) and APC anti-mouse 4D4 (marking resident microglia; 1:1000) in blocking buffer containing 0.2% bovine serum albumin (BSA, Sigma-Aldrich) in HBSS. Cell sorting was performed using FACSAriaIII cell sorter (Becton Dickson). Microglial cells were identified as CD45+CD11b+ Ly6C-4D4+ and Dead cells were also excluded based on 7-AAD (BD Bioscience) staining. Cells were sorted directly in 1.5mL Eppendorf tubes and stored at -80°C. Phagocytic positive microglia was sorted as 4D4+ Alexa405+.

Flow cytometry Intracellular Staining

Intracellular cytokine staining and cell isolation was done as previously described. The meninges were carefully removed from the skull and the ipsilateral brain was further isolated. The enzyme dissociation mix used for the ipsilateral brain hemisphere and meninges was Collagenase P (0.5 mg/mL, Sigma cat. 11213865001) and DNAse-1 (250 U/mL, Worthingon cat. LK003172) diluted in RPMI-1640 with 10 mM HEPES. The samples were then finely minced and incubated in a room temp shaker for 1 hour. Following the enzyme dissociation, the cells were separated using Percoll (GE Healthcare Life Sciences) as described above. Cells isolated from the brain and meninges were only incubated for 2 hours instead of the 4 hours for cLN cells. Acquisition was performed on a Symphony (BD Biosciences) by using DIVA software (BD Biosciences) and data were analyzed with FlowJo software versions 9.9 or 10.1 (TreeStar Inc.). Intracellular staining antibodies used Zombie Aqua Fixable Viability Kit (Biolegend, #423102, 1:1000) or Zombie UV (Biolegend, #423108, 1:1000) was used to exclude dead cells. The staining antibodies used PE/Cyanine7 anti-mouse CD11b (M1/70, Biolegend, 1:300), APC/Cyanine7 anti-mouse TCR-beta (H57-597, Biolegend, 1:100), BUV661 anti-mouse CD45 (30-F11, BD Biosciences, 1:200), PE anti-mouse CD4 (GK1.5, Biolegend, 1:100), BUV805 anti-mouse CD8 (53-6.7, BD Biosciences 1:100), FITC anti-mouse FoxP3 (FJK-16s, eBioscience, 1:100), BV421 anti-mouse LAP (TW7-16B4, Biolegend, 1:100), PE/Dazzle 594 anti-mouse IL-10 (JES5-16E3, Biolegend, 1:100), BUV395 anti-mouse IL-17a (TC11-18H10, BD Biosciences, 1:100), BV785 anti-mouse IFN-g (XMG1.2, Biolegend, 1:100), BV605

anti-mouse Ly6G (1A8, Biolegend, 1:300), BV711 anti-mouse NK-1.1 (PK136, Biolegend, 1:100), AF700 anti-mouse Ly6C (HK1.4, Biolegend, 1:200), BV711 anti-mouse Ly6C (HK1.4, Biolegend, 1:200), and APC anti-mouse 4D4 (1:1000) provided by Dr. Butovsky.

Flow cytometry FoxP3(GFP) Tregs Sorting from the brain and blood

The ipsilateral brain hemisphere was processed with the same enzyme dissociation kit described above and then processed as the microglia cell sorting above. The sample were purified and enriched using CD4 cell isolation microbead kit (Militenyi Biotech, #130-104-454) on a magnetic MACS separator prior to sorting. Cell sorting was performed using FACSAriaIII cell sorter (Becton Dickson). APC/Cyanine7 anti-mouse CD45 (30-F11, Biolegend, 1:100), APC anti-mouse CD4 antibody (GK1.5, Biolegend, 1:100), and 7-AAD (BD Bioscience) was used to identify the live CD4+ population and the FITC channel was used to identify the FoxP3+ population. Each RNA sequencing sample is a pool of 5 ipsilateral hemispheres and 20 sham brain hemispheres. The blood samples were collected in heparin coated tubes and then transferred to a 15 mL tubes where ACK lysis buffer was used to lyse the erythrocytes. The sample was then strained through a 70 um filter and stained as decribed above. Each RNA sequencing sample is a pool of 5 mice.

Instrument

Flow cytometry acquisition was performed on FACSSymphony A5 (BD Biosciences) using DIVA software.
Cell were sorted using FACSAria III (Becton Dickson)

Software

Flow cytometry data were analyzed with FlowJo software version 10.

Cell population abundance

Gating Ly6C negative and CD11b positive yielded a cell population which was >95% positive for 4D4, a microglia-specific marker, which gates were drawn for and positive cells taken for analysis.

Gating strategy

Microglial cells were identified as CD45+CD11b+ Ly6C-4D4+ and Dead cells were also excluded based on 7-AAD (BD Bioscience) staining. Phagocytic positive microglia was sorted as 4D4+ Alexa405+

For FoxP3 Tregs were selected based on Dead cells were also excluded based on 7-AAD and CD4+ cells were gated then GFP+ cells were sorted as FoxP3 Tregs.

For Intracellular staining Dead cells were excluded with zombie aqua and CD45+ cells were gated, then CD11b+ was excluded followed by gating on TCR-b+ cells, Followed by gating both CD4+ and CD8+ T-cells. Different subtypes of CD4+ T cells were gated on CD4 and were chosen to be positive for that population based on an FMO for that marker.

Detailed gating strategies and FMOs are found in extended Data Fig 4,5,6,7,8, and 9.

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

Magnetic resonance imaging

Experimental design

Design type

One week post-TBI mice went through MRI imaging to measure the lesion volume of the injury.

Design specifications

Imaging was done using 7.0T Bruker BioSpect®USR. In brief, mice were gently handled and placed in isoflurane anesthesia chamber. Then mice were placed inside the imaging apparatus with their nose in front of tubes releasing 2% of isoflurane. Electrocardiogram (ECG) leads were placed on the animal's paws and a pneumatic pillow sensor will be placed under the abdomen for continuous ECG and respiratory rate monitoring of the anesthetized animal. These waveforms were closely monitored throughout MRI scanning by the MRI operator. The animal was placed on an MRI compatible bed, which will be placed inside the magnet for imaging. The imaging sessions last between 15-60 minutes. Mice were then returned to their cages and were monitored continuously after being returned to their cages prior to returning to a fully alert status. The. Following parameters were obtained to generate the T2 sequence images: Slice Thickness: 0.5mm, Repetition Time:3000ms, Echo Time: 50ms, Number of Averages: 3, Spacing Between Slices:0.5mm, Echo Train Length: 8, Acquisition Matrix: 200x200, Flip Angle:90, Field of View: 20mm. Serial Images were viewed and analyzed using the 3D Slicer platform

Behavioral performance measures

The MRI was used to quantify lesion volume by obtaining T2 sequences, and behavioral performance measures were not required to be obtained for that purpose.

Acquisition

Imaging type(s)	Structural
Field strength	7T
Sequence & imaging parameters	The following parameters were obtained to generate the T2 sequence images: Slice Thickness: 0.5mm, Repetition Time:3000ms, Echo Time: 50ms, Number of Averages: 3, Spacing Between Slices:0.5mm, Echo Train Length: 8, Acquisition Matrix: 200x200, Flip Angle:90, Field of View: 20mm.
Area of acquisition	The whole brain was used.

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	3D Slicer platform
Normalization	DICOM files were opened and serial images of the whole brain was obtained to analyze the TBI lesion volume
Normalization template	No data was normalized
Noise and artifact removal	The animal was well-sedated where noise and artifact removal were not required.
Volume censoring	The animal was well-sedated and positioned in which volume censoring was not needed.

Statistical modeling & inference

Model type and settings	A statistical model was not used and only lesion volumes were directly calculated from the T2 sequences.
Effect(s) tested	No statistical effects were tested and only lesion volumes were directly calculated from the T2 sequences.

Specify type of analysis: ☐ Whole brain ☒ ROI-based ☐ Both

Anatomical location(s)	The area of lesion was measured and analyzed using the Slicer 3D platform
Statistic type for inference (See Eklund et al. 2016)	Statistical inference was not used since lesion volumes were directly calculated from the T2 sequences.
Correction	Correction was not required since lesion volumes were directly calculated from the T2 sequences.

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis