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Corresponding author(s): Marius Wernig

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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	For Droplet-based single nuclei RNA sequencing: Main cohort: Illumina sequencing of the libraries was performed by Novogene (https://en.novogene.com/) on an Illumina NovaSeq S4 (Illumina). Base calling, demultiplexing, and generation of FastQ files were conducted by Novogene. Control cohort: Illumina sequencing of the libraries was performed on an Illumina NextSeq 550 (Illumina). Base calling, demultiplexing, and generation of FastQ files for the control cohort data was conducted using the mkfastq command of the Cell Ranger software (v.6.1.2). The Cell Ranger (v.6.1.2) analysis pipelines were utilized to align reads to the mm10 reference genome as well as GFP, and count barcodes/UMIs. For the control cohort, cell demultiplexing by assigning hashtag antibody oligo tags to cell-associated barcodes was performed with the Cell Ranger multi pipeline. For, the human spinal cord dataset from Trobisch et al., the Cell Ranger (v.6.1.2) analysis pipelines were utilized to align reads to the GRCh38 reference genome. Flow cytometry: FACSDiva software (BD Biosciences) and Sony MA900 Multi-Application Cell Sorter software Microscopy: ZEN blue (Zeiss, version 3.7.97.04000), Zen black (Zeiss, version 14.0.28.201), Leica Application Suite X (LAS X, Leica, versions 3.7.4.23463 and 3.5.7.23225)
Data analysis	R version 4.1.2 and 4.2.0 RStudio 2022.02.2+485 and 2022.02.3+492

R packages: seurat 4.2.0, fgsea 1.24.0, ggplot2 3.4.0, ggpubr 0.5.0, UpSetR 1.4.0, VennDiagram 1.7.3, ggrepel 0.9.2, shiny 0.61.1, sctransform 0.3.5, MAST 1.20.0, VISION 3.0.0, dplyr 1.10.0, tidyr 1.2.1, biomaRt 2.50.3

Fiji ImageJ 1.53q

FlowJo 10.8.1

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 murine sequencing datasets generated and analyzed in the current study are available in the Gene Expression Omnibus repository under accession numbers GSE217529 and GSE242512.

The human spinal cord dataset from Trobisch et al. was accessed as 6 fastq files (3 control and 3 MS patients) via Sequence Read Archive (SRA; accession number PRJNA726991).

Human brain NucSeq datasets were obtained as expression matrices from the UCSC Cell Browser (https://cells.ucsc.edu)73 and were originally generated by Absinta et al. (3 control and 5 MS patients [Cell Browser dataset ID: chronic-ms]), Jäkel et al. (5 control and 4 MS patients [Cell Browser dataset ID: oligodendrocyte-ms]), and Schirmer et al. (9 control and 12 MS patients [Cell Browser dataset ID: ms]).

Reference genomes (refdata-gex-mm10-2020-A and refdata-gex-GRCh38-2020-A) were accessed via www.10xgenomics.com.

Human research participants

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

Reporting on sex and gender	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	No specific sample size calculation was performed due to the pilot character of the study exploring the only scarcely studied chronic stage of EAE and unknown effect sizes of hematopoietic cell transplantations in this model. However, previous publications on EAE in general were considered for choosing the sample sizes applied in this study.
Data exclusions	Sequencing data: Exclusion of nuclei that were deemed of low quality (debris) or suspected doublets. Specifically, for the murine dataset, Seurat objects were generated including features expressed by at least a minimum of 3 cells and including cells with expression of at least 50 features. Outliers with a ratio of mitochondrial relative to endogenous RNAs of >5%, homotypic doublets (>6,000 features in main and >4,000 features in control cohort) and debris (<400 features in main and <500 features in control cohort) were removed. After each integration, clustering, and subclustering step, clusters / subclusters were manually inspected for gene expression profiles and cell type-specific marker genes, and suspected doublets and debris were removed for downstream analysis. A small subcluster with Schwann cell signature was

removed in the T cell subcluster analysis.

EAE model: EAE animals without clinical presentation (clinical score = 0) were excluded from the experiments before the initiation of any therapeutic measures. Animals with mortality before day 50 were excluded from the analysis. One animal of the EAE-BMT-PLX group was sacrificed on day 76 due to non-EAE related reasons (with clinical score of 1.5) and was excluded from the analysis after this timepoint. Only animals surviving until the defined endpoints of the study were used for histological analysis.

For the human brain dataset of Schirmer et al., nuclei with a maximum number of 10,000 features were used and duplicated gene symbols were kept as distinct genes. No further adaptions were made to the other two human brain datasets before integration. For the human spinal cord dataset (generated by Trobisch et al.), Seurat objects were created per library, including features expressed by at least a minimum of 3 cells and including cells with expression of at least 50 features. Outliers with a ratio of mitochondrial relative to endogenous RNAs of >5% were removed. Thresholds for features were based on inspection of the separate objects and were as follows: SRR14408223: >150 & <1500; SRR14408224, SRR14408225, SRR14408231, SRR14408232: >300 & <3000; SRR14408226: >200 & <2000. After clustering and subclustering, the integrated human datasets were inspected and suspected doublet/debris cluster were removed as outlined above. Similarly, non-immune cell cluster were removed for the immune cell subcluster analysis.

Replication

Multiple biological replicates were used in this study, and clinical data was based on two independent cohorts. Specifically, the following total numbers of animals were used per experimental group:
Healthy controls: n = 8
CFA controls: n = 4
EAE (without intervention): n = 9
EAE with PLX: n = 4
EAE with BMT: n = 8
EAE with BMT+PLX: n = 9
All of these indicted EAE replicates demonstrated successful induction of the EAE phenotype as assessed with the EAE score. CFA control animals developed no EAE neurological phenotype but skin lesions. As detailed in the manuscript, we observed variability within the experimental groups. Therefore, we refer to the trends and significant effects between groups described in the manuscript for a detailed assessment of replication success.

Four independent, previously reported human datasets were analyzed.

Randomization

Animals were randomly assigned to experimental groups. Samples for downstream analysis were generated from these randomized animals.

Blinding

Investigators were blinded for clinical scoring (data collection), microscopy (data collection), and quantification of histological data (data analysis). No blinding was deemed feasible for the computational analysis of sequencing data due to the exploratory character of this analysis.

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

Primary antibodies for immunofluorescence staining:
C4 abcam ab11863 rat 1:100
CD206 R&D systems AF2535 goat 1:40
GFAP Invitrogen 13-0300 rat 1:500
GFP abcam ab13970 chicken 1:1000
Iba1 FUJIFILM Wako 019-19741 rabbit 1:1000
Iba1 abcam ab5076 goat 1:500
IQGAP1 abcam ab133490 rabbit 1:50 or 1:100
Olig2 Millipore Sigma AB9610 rabbit 1:1000 (polyclonal)
Spp1 Santa Cruz Biotechnology sc-21742 mouse 1:40
Stat1 Cell Signaling 14994 rabbit 1:200
Galectin3 Biolegend 125401 rat 1:100
NLRC5 abcam ab105411 rabbit 1:200

For flow cytometry:

NeuN Abcam ab190565 AlexaFluor647 1:200
Olig2 Sigma MABN50A4 AlexaFluor488 1:100 (clone 211F1.1)
CD45 Biolegend 103112 APC 1:200
CD11b Invitrogen 12-0112-82 PE 1:200

For cell hashing:
Anti-Nuclear Pore Complex Protein Hashtag antibodies (TotalSeq™-A0451, -A0452, -A0453, and -A0455, Biolegend, 682205, 682207, 682209, and 682213)

Validation

C4 abcam ab11863
https://www.abcam.com/products/primary-antibodies/c4-antibody-16d2-ab11863.html
Suitable for: IHC-Fr; Reacts with: Mouse

CD206 R&D systems AF2535
https://www.rndsystems.com/products/mouse-mmr-cd206-antibody_af2535
Species Reactivity Mouse
Immunohistochemistry: MMR/CD206 was detected in perfusion fixed frozen sections of mouse lung using Goat Anti-Mouse MMR/CD206 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2535) at 25 µg/mL overnight at 4 °C.

GFAP Invitrogen 13-0300
https://www.thermofisher.com/antibody/product/GFAP-Antibody-clone-2-2B10-Monoclonal/13-0300
Applications Immunohistochemistry (IHC)
Tested Dilution 10-50 µg/mL
Species Reactivity Bovine, Human, Mouse, Rat
Published species Ferret, Guinea pig, Human, Mouse, Pig, Rat

GFP abcam ab13970
https://www.abcam.com/products/primary-antibodies/gfp-antibody-ab13970.html
Suitable for: WB, ICC/IF
Reacts with: Species independent

Iba1 FUJIFILM Wako 019-19741
https://labchem-wako.fujifilm.com/us/product/detail/W01W0101-1974.html
Reactivity : mouse, rat
Applications Immunohistochemistry (Frozen Section) 1:500 - 1,000

Iba1 abcam ab5076
https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-022-02651-3#Sec2
PMID: 36463233 DOI: 10.1186/s12974-022-02651-3

IQGAP1 abcam ab133490
https://www.abcam.com/products/primary-antibodies/iqgap1-antibody-epr5220-ab133490.html
Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF
Knockout validated
Reacts with: Mouse, Human

Olig2 Millipore Sigma AB9610 rabbit 1:1000 (polyclonal)
https://www.emdmillipore.com/US/en/product/Anti-Olig-2-Antibody,MM_NF-AB9610
Species Reactivity Note: Human, rat and mouse. Other species have not been tested.
Application: Anti-Olig-2 Antibody is an antibody against Oligodendrocyte transcription factor 2 for use in IC, IH, IH(P), IP and WB.

Spp1 Santa Cruz Biotechnology sc-21742
https://www.scbt.com/p/opn-antibody-akm2a1
Applications: WB, IP, IF, IHC(P)
Species Reactivity/Detection: mouse, rat, human

Stat1 Cell Signaling 14994
https://www.cellsignal.com/products/primary-antibodies/stat1-d1k9y-rabbit-mab/14994
Specificity / Sensitivity: Stat1 (D1K9Y) Rabbit mAb recognizes endogenous levels of total Stat1 protein. This antibody also cross-reacts with an unidentified protein of 150 kDa.
Species Reactivity: Human, Mouse, Rat, Monkey
Application Immunofluorescence (Immunocytochemistry) Dilution 1:200 - 1:800
See also PMID: 29018057

Galectin3 Biolegend 125401
https://www.biolegend.com/en-ie/products/purified-anti-mouse-human-mac-2-galectin-3-antibody-4935
Verified Reactivity: Mouse, Human
Application: ICC, WB, IP - Verified

NLRC5 abcam ab105411
https://www.abcam.com/products/primary-antibodies/nlrc5-antibody-ab105411.html
Reacts with: Mouse
Application for Immunofluorescence see reference:
https://onlinelibrary.wiley.com/doi/10.1111/jnc.14876
PMID: 31549732 DOI: 10.1111/jnc.14876

NeuN Abcam ab190565 AlexaFluor647
https://www.abcam.com/products/primary-antibodies/alexa-fluor-647-neun-antibody-epr12763-neuronal-marker-ab190565.html
Suitable for: IHC-P, ICC/IF, IHC-Fr
Reacts with: Mouse, Rat, Human

Olig2 Sigma MABN50A4 AlexaFluor488
https://www.emdmillipore.com/US/en/product/Anti-Olig2-Antibody-clone-211F1.1-Alexa-Fluor488-Conjugate-MABN50A4,MM_NF-MABN50A4
Species Reactivity Note: Demonstrated to react with mouse and rat.
IC staining of undifferentiated rat OPCs using a 1:100 dilution of Anti Olig2 (MABN50) and counterstained with DAPI. Staining of this transcription factor is shown primarily nuclear.

CD45 Biolegend 103112 APC
https://punchout.biolegend.com/en-us/products/apc-anti-mouse-cd45-antibody-97
Verified Reactivity Mouse
Application FC - Quality tested

CD11b Invitrogen 12-0112-82 PE
https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/12-0112-82
Applications Flow Cytometry (Flow) Tested Dilution 0.125 µg/test
Species Reactivity Mouse
Published species Dog, Fish, Human, Mouse

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	Female C57BL/6J mice were used for the EAE model (JAX, Strain 000664). The C57BL/6-Tg(UBC-GFP)30Scha/J strain (JAX, Strain 004353) was crossed with wild-type C57BL/6J mice to generate hemizygote UBC-GFP animals. Female hemizygote UBC-GFP mice of 10-12 weeks were used as donor animals for BMT. EAE was induced in female C57BL/6J mice at the age of 11 weeks.
Wild animals	The study did not involve wild animals
Reporting on sex	Only female animals were used according to the instructions of the EAE induction kit (Hooke Laboratories)
Field-collected samples	The study did not involve field-collected samples
Ethics oversight	All animal procedures were approved by the administrative panel on laboratory animal care at Stanford University (APLAC21565).

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

Tissue collection for the transcriptomic analysis was performed 3 months after EAE induction or in an age-matched healthy control animal. Animals were anesthetized (100 mg/kg of ketamin and 10 mg/ kg of xylazine, i.p.) and transcardially perfused. Spinal cords were harvested by hydraulic expulsion and snap-frozen by submerging for 60 seconds in liquid nitrogen-cooled isopentane. Frozen spinal cords were stored at -80°C until further processing. Single-nuclei preparation was performed as previously described with the following modifications.63 The thoracolumbar section of spinal cords were first mechanically dissociated with an ice-cold razor blade in a petri dish with 1ml EZ Prep lysis buffer (Sigma, NUC-101) on ice. Transfer of tissue suspension into a 2ml glass dounce tissue grinder tube (Sigma, D8938). Petri dish was rinsed off with another 1ml EZ buffer, which was added to the grinder tube. Suspension was homogenized by hand 25 times with pestle A followed by 25 times with pestle B while incorporating a 180-degree twist. Tissue homogenate was transferred to a fresh 15ml tube on ice. The grinder tube was rinsed with 2 ml fresh lysis buffer, which was added to the 15ml tube for a total volume of 4 ml. Samples were incubated on ice for 5 minutes. Nuclei were centrifuged at 500 x g for 5 minutes at 4°C, supernatant was removed, and the pellet resuspended with 4 ml EZ lysis buffer and incubated on ice for 5 minutes. Centrifugation at 500 x g for 5 minutes at 4°C was repeated. After removing the supernatant, the pellet was resuspended with 4 ml chilled PBS and filtered through a 35-um cell strainer into a 5 ml round bottom FACS tube (Corning, 352235). Following centrifugation at 300

x g for 10 minutes at 4°C with break 3, supernatant was gently poured out leaving behind the nuclei pellet. The pellet was resuspended in 100µl FC block buffer (BD Biosciences, 553141, 1:50 in FACS buffer) and 1ul recombinant RNase inhibitor (Takara, 2313B) incubated on ice for 5min. FACS buffer consisted of PBS (Gibco, 10010049) containing 1% BSA (Thermo Fisher, BP9700100)). To 50µl of the cell suspension, we added 150µl FACS buffer with 2µl Olig2-Alexa Fluor488 (Sigma, MABN50A4), 1ul NeuN-AlexaFluor647 (Abcam, ab190565), and 1.5ul RNase inhibitor. Anti-Nuclear Pore Complex Protein Hashtag antibodies (1 µl) were additionally added to the samples of the control cohort (TotalSeq™-A0451, -A0452, -A0453, and -A0455, Biolegend, 682205, 682207, 682209, and 682213). After 30min incubation covered from light and shaking on ice, 3ml FACS buffer were added. Following centrifugation at 300 x g for 10 minutes at 4°C with break 3, supernatant was gently poured out. The pellet was resuspended in 700µl FACS buffer with 3.5ul recombinant RNase inhibitor and Hoechst dye (Thermo Fisher, H3570, 1:2000) and incubated 4min on ice covered from light. Single nuclei were sorted on a MA900 Multi-Application Cell Sorter (Sony Biotechnology) gating for NeuN negative nuclei.

Peripheral blood was collected from the facial vein and treated with 0.5M EDTA. Erythrocytes were removed by 10 minutes of incubation in 2ml ice-cold RBC lysis buffer.62 Cells were collected via centrifugation for 10min at 300g. Cells were washed with PBS and then stained for 15min at 4 °C in Flow buffer (PBS with 5% cosmic calf serum) containing the following antibodies: CD11b-PE (Invitrogen, 12-0112-82), CD45-APC (Biolegend, 103112). Stained cells were washed in PBS and resuspended in Flow buffer with DAPI (Sigma, D9542).

Instrument

FACS AriaII for peripheral blood analysis
MA900 Multi-Application Cell Sorter (Sony Biotechnology) for nuclei sorting

Software

Data collection: FACSDiva software (BD Biosciences) and Sony MA900 Multi-Application Cell Sorter software
Analysis: FlowJo 10.8.1

Cell population abundance

Nuclei were counted using a hemacytometer (Sigma, Z359629-1EA) and assessed for concentration and quality.

Gating strategy

Peripheral blood analysis: single cell gates (SSC-A/FSC-A -> FSC-H/FSC-A) -> live cell gate (DAPI negative) -> APC-CD45+ PE-CD11b+ gate -> GFP+ gate
Nuclei sorting: single nuclei (BSC-A/FSC-A -> Hoechst+) -> AlexaFluor647-NeuN- FITC-Olig2+

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