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Corresponding author(s): Gonçalo Castelo-Branco

Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Fast-Q alignment done using STAR aligner. For flow cytometry analysis a Gallios Flow Cytometer (Beckman Coulter) was used and data analysis was perfomed using a Kaluza 1.5a Flow Cytometry Analysis Software (Beckman Coulter).
Data analysis	R statistical computing platform (Microsoft R Open 3.4.2), https://github.com/Castelo-Branco-lab/GeneFocus (custom code and functions, including necessary packages.), we also provide a full R session info file containing all packages and their versions used. Furthermore, essential packages used include destiny 2.6.1, e1071 R package (https://CRAN.R-project.org/package=e1071, spdep 0.6-15, facto extra 1.0.5 , MAST R package v1.4.1, clusterProfiler package v3.6.0, MetaNeighbor v1, Cytoscape v3.5.1, Imaris version 9.1.1, Fiji version 1.0.

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

No restrictions apply on data availability. All figures have associated raw data, these are: Fig 1-4 and Supplementary Fig 1-9, accession codes are provided.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed to pre-determine sample sizes. Rationale for sample size choice is provided for each of the experiments described below. For single cell RNA-seq, sample size was n=4 mice per condition, control or EAE. These 8 mice were sufficient to obtain the number of cells necessary to perform a confident data analysis and comparisons between controls and EAE mice. For Immunohistochemistry and RNAscope ISH performed in mouse tissue, the sample size was n=3 per condition, control or EAE (at the exception of RNAscope/IHC in Supplementary Fig. 6a, b,c). This sample size was found sufficient for validations of findings from single-cell RNA-seq because it is to demonstrate mostly presence of absence in EAE and control, respectively, of a certain protein and RNA. For Immunohistochemistry of human samples the sample size was 2. This sample size is sufficient to prove the occurrence of our candidate genes in oligodendrocyte lineage cells in Multiple Sclerosis patients. For qPCR analysis in tissue collected from control and EAE mice, sample size was n=4 and n=5, respectively, this sample size is often reported in previous publications with qPCRs analysis. For primary cultures experiments the sample size was as it follows: qPCR: n=3, this sample size was found sufficient to the study since the expected differences between controls and treatments were robust. Immunocytochemistry for IFN-G and Dexamethasone treatment: n=3 RNAscope ISH in OPCs and OLs: n=3 Immunocytochemistry for co-culture of OPCs and EAE-derived CD45+ cells: n=3 The 3 techniques above, qPCR, RNAscope ISH and ICC demonstrate mostly presence or absence of a given protein and RNA upon treatment. As such, we found n=3 sufficient for each technique. Immunocytochemistry for fluorescent microspheres experiments: n=3 pHrodo-labeled myelin experiment: n=3 These phagocytosis experiments demonstrate the capacity for phagocytosis, as such, n=3 for each experiment was sufficient. Furthermore, we also use 2 different methods to prove the same result. Co-culture studies of OPCs with CD4+ T cells: n=7. This sample size was sufficient to allow us to take conclusions on CD4+ T cell survival/ proliferation and cytokine production.
Data exclusions	For single cell RNA-Seq data, we excluded data points through our quality control pipeline, as indicated in the methods section in the paper. In short, data was excluded based on thresholding of cells expressing less than 220 000 counts, less than 2 500 genes, a count to mitochondrial count ratio of more than 2, and a count to ERCC spike in ratio of more than 35. Additionally we also removed cells that were clustered in clusters with less than 3 cells, these cells seemed to be doublets based on mixed expression profiles. These cutoffs were chosen based on data distribution curves, and are custom for the data itself. As of yet, there are no standard cutoffs for any cell-types, nor possible due to differences in aim, scope, cell types, cell sizes and cell numbers. Our cutoffs were relatively strict to ensure good quality cells.
Replication	Verification of the experimental findings derived from single cell RNA-seq is performed through validation of RNA-seq data with protein, RNA ISH staining experiments and qPCRs. All replications were successful.
Randomization	For single-cell RNA-seq, we distributed females and males with similar ages equally in Controls and EAE. We also used two different transgenic mice: - 4 Pdgfra-HistoneH2B-GFP, distributed in 2 controls and 2 EAE - 4 PdgfraCre-RCE, distributed in 2 controls and 2 EAE.
Blinding	All analysis/counting involving immunohistochemistry/immunocytochemistry and RNAscope ISH were performed blindly.

Reporting for specific materials, systems and methods

Materials & experimental systems		Methods	
n/a	Involved in the study	n/a	Involved in the study
☒	☐ Unique biological materials	☒	☐ ChIP-seq
☐	☒ Antibodies	☐	☒ Flow cytometry
☒	☐ Eukaryotic cell lines	☒	☐ MRI-based neuroimaging
☒	☐ Palaeontology		
☐	☒ Animals and other organisms		
☐	☒ Human research participants		

Antibodies

Antibodies used

For Immunocytochemistry and Immunohistochemistry the antibodies description is the following:
- anti-GFP from Abcam Cat. number ab150169 and Lot number GR191620-1; dilution 1:1000
- anti-IBA1 from Wako Cat. number 019-19741 and Lot number STH7964; dilution 1:400
- anti-NG2 from MerckMillipore Cat. number AB5320 and Lot 2768460; dilution 1:200
- anti-CNP from Abcam Cat. number ab6319, clone number 11-5B and Lot number GR265011-1; dilution 1:200
- anti-MHCII or anti-I-A/I-E (clone 2G9) from BD Bioscience Cat. number 553622 and Lot number 12304; dilution 1:600
- anti-OLIG2 from R&D, Cat. number AF2418; dilution 1:200
- anti-SOX10 from R&D Cat. number AF2864 and Lot number VRY0616121; dilution 1:100
- anti-PLIN4 from Sigma Cat. number ABS526 and Lot number 2909935; dilution 1:200
- anti-OLIG1 (for human IHC) from Abcam, Cat. number ab68105 and Lot number GR236765-4; dilution 1:100
- anti-OLIG2 (for human IHC) from Atlas Antibodies, Cat. number HPA003254 and Lot number CC81836; dilution 1:200
- anti-MHCII (for human IHC) from Dako, Cat. number M0775 , clone CR3/43 and Lot number 20047190; dilution 1:100

For Flow Cytometry the antibodies description is the following:
- anti-CD4 PE-Dazzle from BioLegend Cat.number 100566, clone RM4-5 and Lot number B233575; dilution 1: 1:500
- anti-Ki67 V450 from BD biosciences cat. number 561281, clone B56 and Lot number 5302586; dilution 1:1:300
- anti-TCR Vbeta11 BV510 from BD biosciences Cat. number 743677, clone RR3-15 and Lot number 8043531; dilution 1:500
- anti-TNF PE-Cy7 from eBioscience Cat. number 25-7423-82, clone TN3-19.12 and Lot number E07682-1634; dilution 1:300
- anti-IFNg APC from BD biosciences Cat. number 554413, clone XMG1.2 and Lot number 4226904; dilution 1:300

Validation

All antibodies used in this study have been tested by the company and have been cited by other authors and references are available on the webpage of the provider company. In addition, regarding antibodies used in IHC and ICC, we have further evaluated the specificity of the antibodies in our tissue by analyzing the presence of the antibody signal in regions where the protein should be expressed and its absence in regions where the protein shouldn't be expressed (for instance MHC-II cannot be expressed in controls OL and indeed it was not, the same for control spinal cord tissue where rare cells MHC-II positive were found). We have further evaluated the location /morphology of the signal within the cell, for transcription factors such as Olig2 and Sox10 where we expect a nuclear signal, the antibody signal was indeed nuclear, whether for proteins like PDGFRA, CNP, IBA-1, PLIN4, OLIG1 and NG2 the signal was cytoplasmic, as expected, with NG2, PDGFRA, MHC-II and IBA-1 presenting antibody signal in ramified processes, as expected.
For flow cytometry analysis all antibodies used are broadly used in the field. Plenty references of these antibodies from BD Bioscience, Biolegend and eBioscience can be found at the company webpage. Additionally, in our hands these antibodies were tested in a broad set of samples and proved specific, as an example, anti-TNF antibody signal was only found in immune cells after specific activation.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Mouse lines used in this study included C57BL/6NJ wild type (WT) mice, Pdgfra-Cre-LoxP-GFP, Sox10Cre-LoxP-GFP, Pdgfra-H2B-GFP knock-in mice and 2D2 transgenic mice. Pdgfra-Cre-LoxP-GFP mice are a strain of mice obtained originally by crossing mice with Cre recombinase under the control of a Pdgfra genomic DNA fragment (with a C57BL/6NJ genetic background) (The Jackson Laboratories, CA, USA) with reporter mice RCE:loxP-GFP (with CD1 background) (Gord Fishell, NYU Neuroscience Institute) to label the complete OL lineage. Sox10-Cre-LoxP-GFP mice are a strain of mice obtained originally by crossing mice with Cre recombinase under the control of the Sox10 promotor (The Jackson Laboratories, CA, USA) (with a C57BL/6 genetic background) with reporter mice RCE:loxP-GFP (with CD1 background) to label the complete OL lineage. Pdgfra-H2B-GFP, with a C57BL/6NJ background, presents an H2B-eGFP fusion gene expressed under the promoter of the OPC marker, Pdgfra. Mice homozygous for the knock-in targeted mutation have an embryonic lethal phenotype, with half of the embryos failing to survive past embryonic day 12.5 and the remainder failing to survive beyond embryonic day 15.5 (https://www.jax.org/strain/007669). Animals were used in adult stage, between 10-12 weeks old and both genders were included in CFA control mice and EAE. More specifically, for the Single cell RNA-seq analysis: 4 CFA controls comprised 2 females Pdgfra-H2B-GFP, 1 male Pdgfra-Cre-LoxP-GFP and 1 female Pdgfra-Cre-LoxP-GFP; EAE comprised 2 females Pdgfra-H2B-GFP and 2 males Pdgfra-Cre-LoxP-GFP. For the

	primary cultures, the P7 pup litters were pooled and the genders were mixed. For the 2D2 T-cells used in co-culture experiments both genders were used with ages between 8-16 weeks old.
Wild animals	The study did not involve wild animals
Field-collected samples	The study did not involve samples collected from the field.

Human research participants

Policy information about studies involving human research participants

Population characteristics	The human tissue used in this study comprised one female and one male , with ages of 38 and 50 years old both with secondary progressive multiple Sclerosis. No patient had specific disease treatment.
Recruitment	All participants gave prospective pre-mortem written consent for their brains to be banked and used for research.

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	Naïve and memory T cells were isolate from the spleens of C57Bl/6 2D2 transgenic mice. Splenocytes were isolated by tissue disruption through a 100um mesh and erythrocytes were lysed with ACK buffer. Splenocytes were depleted from non-T cells using a naive CD4+ T Cell Isolation Kit (130-104-453; Miltenyi), and the flow through was further sorted into CD44 low (naïve) and CD44 high (memory) cells using anti-CD44 beads from the same kit.
Instrument	Gallios (Beckman Coulter)
Software	Kaluza for Gallios for sample acquisition; Kaluza analysis software for data analysis
Cell population abundance	The naïve T cell fraction was 81% CD4+ and 19% CD8+ and contained no detectable CD44hi (memory cells); the memory fraction was 94% CD4+ with a minor fraction of CD8+ T cells, but still contained 50% naïve T cell. Since naïve T cells are unresponsive in our experimental set up, their presence does not affect the results presented. Purity was determined by staining flow through and column-bound fractions with antibodies against CD3, CD4, Vb11, CD62L and CD44.
Gating strategy	Cells were identified first on FSC/SSC plots; dead cells were subsequently gated away using a LIVE/DEAD Fixable Near-IR Dead Cell Stain (Invitrogen); live cells were plotted against CD4 and Vb11 for the determination of double positive cells; these were in turn analyzed for the expression of Ki67, IFNg or TNF.

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