

## 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

- |                                     |                                     |                                                                                                                                                                                                                                                            |
|-------------------------------------|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | A description of all covariates tested                                                                                                                                                                                                                     |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                        |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | 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

Data analysis

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 datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

## 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](https://www.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 statistical methods were used to pre-determine sample sizes. Wherever statistical tests were applied, we used at least n=4 to ensure non-parametric testing (except for Fig1 i, where no statistical tests were applied due to n=3). |
| Data exclusions | No data were excluded                                                                                                                                                                                                                   |
| Replication     | All experiments were successfully repeated at least twice.                                                                                                                                                                              |
| Randomization   | There was no allocation of test subjects in this study and thus, randomization was not applicable                                                                                                                                       |
| Blinding        | The genotype of the mice was blinded to the investigator performing scoring of the EAE mice. The genotype of the mice was unknown to the technician performing cell culture, stimulations, RNA-isolation, and qPCR.                     |

## 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                                           |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology          |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern           |

### Methods

|                                     |                                                    |
|-------------------------------------|----------------------------------------------------|
| n/a                                 | Involved in the study                              |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging    |

## Antibodies

|                 |                                                                                                                                                                                                                                                                                                                                     |
|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antibodies used | B220 (RA3-6B2, rat IgG, Biolegend; 1:200 dilution)<br>CD3 (17A2, rat IgG, Biolegend; 1:200 dilution)<br>CD4 (RM4-5, rat IgG, Biolegend; 1:200 dilution)<br>CD8 (53-6.7, rat IgG, Biolegend; 1:100 dilution)<br>CD11b (M1/70, rat IgG, Biolegend; 1:100 dilution),<br>CD11c (N418, Armenian hamster IgG, Biolegend; 1:100 dilution), |
|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

CD25 (PC61, rat IgG, Biolegend; 1:200 dilution),  
 CD45 (I3/2.3, rat IgG, Biolegend; 1:100 dilution),  
 CD83 (Michel19, rat IgG, Biolegend; 1:50 dilution),  
 CD86 (GL-1, rat IgG, Biolegend; 1:200 dilution),  
 CX3CR1 (SA011F11, mouse IgG, Biolegend; 1:200 dilution),  
 FoxP3 (FJK-16s, rat IgG, Thermo Fisher Scientific; 1:100 dilution),  
 IFN- $\gamma$  (XMG1.2, rat IgG, Biolegend; 1:200 dilution),  
 IL-17A (TC11-18H10.1, rat IgG, Biolegend; 1:300 dilution),  
 I-A/I-E (M5/114.15.2, rat IgG, Biolegend; 1:200 dilution),  
 Ly6C (HK1.4, rat IgG, Biolegend; 1:400 dilution),  
 Ly6G (1A8, rat IgG, Biolegend; 1:100 dilution),  
 P2RY12 (S16007D, rat IgG, Biolegend; 1:100 dilution)

## Validation

The validation of all primary commercial antibodies for the species and application was warranted by the vendors. Validation statements can be found on the manufacturers' website:

B220: <https://www.biolegend.com/en-us/products/fitc-anti-mouse-human-cd45r-b220-antibody-445>  
 CD3: <https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd3-antibody-7326>  
 CD4: <https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-cd4-antibody-1937>  
 CD8: <https://www.biolegend.com/en-us/products/percp-anti-mouse-cd8a-antibody-4256>  
 CD11b: <https://www.biolegend.com/en-us/products/percp-anti-mouse-human-cd11b-antibody-4315>  
 CD11c: <https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd11c-antibody-7149>  
 CD25: <https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd25-antibody-422>  
 CD45: <https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd45-antibody-9796>  
 CD83: was validated in the indicated dilution on CD83-deficient dendritic cells in our laboratory  
 CD86: <https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-cd86-antibody-6554>  
 CX3CR1: <https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-cx3cr1-antibody-11909>  
 FoxP3: <https://www.thermofisher.com/antibody/product/FOXP3-Antibody-clone-FJK-16s-Monoclonal/606-5773-82>  
 IFN- $\gamma$ : <https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-ifn-gamma-antibody-5865>  
 IL-17A: <https://www.biolegend.com/en-us/products/alexa-fluor-647-anti-mouse-il-17a-antibody-3536>  
 I-A/I-E: <https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-mouse-i-a-i-e-antibody-7997>  
 Ly6C: <https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-ly-6c-antibody-6758>  
 Ly6G: <https://www.biolegend.com/en-us/products/apc-anti-mouse-ly-6g-antibody-6115>  
 P2RY12: <https://www.biolegend.com/en-us/products/apc-anti-p2ry12-antibody-15402>

## 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

We used B6.129P2(C)-Cx3cr1tm2.1(cre/ERT2)Jung/J mice (purchased from Jackson) which were bred together with Cd83tm1.1Lnjt, which were established in our laboratory. In addition, STOCK Tg(Cx3cr1-cre)MW126Gsat/Mmucd mice bred to Cd83tm1.1Lnjt were used.

## Wild animals

The study did not involve wild animals.

## Reporting on sex

For the EAE model, we used only female mice since the model works better in female mice. For all other experiments both sexes were used, also with respect to adopting the 3R-principle (reduction of animals).

## Field-collected samples

The study did not involve field-collected samples.

## Ethics oversight

The present study was performed in accordance with the European Community Standards on the Care and Use of Laboratory Animals and were approved by the local ethics committee (Reference number 55.2.2-2532-2-1193).

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        | Microglia and other immune cells within the CNS were isolated by a one-step density centrifugation protocol to remove myelin debris. Cells were stained in PBS + 1 mM EDTA and the respective antibody mixes.                                                                                                                                                                                                                                                                                                                                                                                                         |
| Instrument                | BD FACS Canto II                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Software                  | The FACS Canto was running the BD FACS-Diva Software.<br>Data were evaluated using FlowJo V10                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Cell population abundance | In healthy mice, we acquired 20,000 cells in the CD45+ gate for further analysis. In EAE mice, 50,000 cells were acquired in the CD45+ gate.                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Gating strategy           | For all experiments, we first excluded doublets via FSA-A vs FSC-H. Then, we gated on living cells via FSC-A vs SSC-A, followed by gating on CD45 positive cells to select immune cells. Further populations were gated:<br>-Microglia: CD45low, CD11b positive, CX3CR1 positive<br>-Monocytes: CD45positive, CD11b positive, Ly6C positive, Ly6G negative<br>-Neutrophil granulocytes:CD45positive, CD11b positive, Ly6C positive, Ly6G positive<br>-Lymphocytes: CD45positive, CD11b negative:<br>-B cells: B220 positive<br>-CD4+ T cells: CD3 positive, CD4 positive<br>-CD8+ T cells: CD3 positive, CD8 positive |

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