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Last updated by author(s): Feb 23, 2025

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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ | 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | 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	BD FACSDiva (v9.0, BD Biosciences); CytExpert (v2.6, beckman coulter); LAS X (v4.7.0, Leica); inForm (v2.4.8, Akoya Biosciences)
Data analysis	ITK-SNAP (v 3.8.0); Image J (v2.1.0, NIH); Imaris (v9.01, Bitplane, Zurich); LipidSearch (v4.2, Thermo Fisher); Flowjo (v10.4, BD Biosciences); cellranger (v6.0.0, 10X genomics); Seurat package (v4.3.0); R (v 4.0.4); Harmony package (v0.1.1); GSEA (v4.1.0); Metascape (http://metascape.org); Monocle package (v2.26.0); Monocle3 package (v1.3.1); FateID package (v0.2.2); InterCellDB package (v0.9.2); AUCCell package (v1.10.0); Enrichr (https://maayanlab.cloud/Enrichr/); pySCENIC package (v0.11.2); GraphPad Prism (v8.2.1)

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 lipidomics data generated in this study have been deposited in the MetaboLights database⁸³ under accession code MTBLS12240 [<http://www.ebi.ac.uk/metabolights/MTBLS12240>]. The RNA-seq data generated in this study have been deposited in the GEO database under accession code GSE290191 [<https://>

www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE290191]. The scRNA-seq data generated in this study have been deposited in the GEO database under accession code GSE290194 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE290194]. The scRNA-seq data used in this study from our previously published work are available in the GEO database under accession code GSE171169 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE171169]. The flow cytometry gating strategies in this study are included in Supplementary data 6. All data generated or analyzed in this study, including differentially expressed genes and enriched functional terms, are available within the article and its supplementary information files. Source data are provided with this paper.

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	nA

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	The sample size was estimated based on prior experiments conducted in the laboratory and previously published data, as detailed in the main text, figure legends, and figures. We employed sample sizes that were sufficient to detect statistically significant effects.
Data exclusions	Low-quality cells and doublets were excluded in the scRNA-seq analysis based on the following criteria: (i) cells with a count of expressed genes below 200 or exceeding 6000, and (ii) cells with more than 10% of mitochondrial genes.
Replication	All experimental findings in this research were replicated across multiple biological samples. The number of replications for each experiment is detailed in the figure legends.
Randomization	Animals were randomly assigned to either the sham or stroke groups and received treatments determined by a lottery drawing method.
Blinding	The establishment of the MCAO model, behavioral tests, and statistical analyses were conducted by different researchers, ensuring blinding to treatment throughout the experiments. The recorded images were loaded into Image J (NIH) and manually quantified by two observers who were blinded to the grouping. All the data were quantified by 2 independent observers blinded to grouping.

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

For immunofluorescent: Goat anti-CD31 (R&D, AF3628, 1:200), Rabbit anti-ZO1 (Abcam, ab221547, 1:200), Rabbit anti-Pdgfra (Abcam, ab203491, 1:250), Goat anti-IBA1 (Abcam, ab5076, 1:250), Mouse anti-APC (Sigma-Aldrich, OP80, 1:100), Rat anti-BrdU (Abcam, ab6326, 1:100), Mouse anti-SMI32 (BioLegend, 801701, 1:250), Rat anti-MBP (Abcam, ab7349, 1:250), Rabbit anti-Plin2 (Abcam, ab108323, 1:100), Rabbit anti-GPNMB (Abcam, ab188222, 1:200), Rabbit anti-CD63 (Abcam, ab217345, 1:200) and Rabbit anti-MAP2 (Proteintech, 17490-1-AP, 1:500), Donkey anti-Goat Alexa Fluor 488 (Invitrogen, 1:500, A-11055), Donkey anti-Rabbit Alexa Fluor 488 (Invitrogen, 1:500, A-21206), Donkey anti-Mouse Alexa Fluor 488 (Invitrogen, 1:500, A-21202), Donkey anti-Rat Alexa Fluor 488 (Invitrogen, 1:500, A-21208), Goat anti-Rabbit Alexa Fluor 555 (Invitrogen, 1:500, A-21428), Donkey anti-Rabbit Alexa Fluor 594 (Invitrogen, 1:500, A-21207), Donkey anti-Goat Alexa Fluor 594 (Invitrogen, 1:500, A-11058), Donkey anti-Rat Alexa Fluor 594 (Invitrogen, 1:500, A-21209), Goat anti-Mouse Alexa Fluor 594 (Invitrogen, 1:500, A-11005), Donkey anti-Goat Alexa Fluor 647 (Invitrogen, 1:500, A-21447), Donkey anti-Rabbit Alexa Fluor 647 (Invitrogen, 1:500, A-31573).

For mIHC: Rabbit anti-CD4 (Abcam, ab183685, 1:200), Rabbit anti-Tmem119 (Abcam, ab209064, 1:200), Rabbit anti-Ly6G (Abcam, ab238132, 1:200) and Rabbit anti-NKP46 (Abcam, ab233558, 1:200).

For flow cytometry: Rat anti-CD45-Pacific blue (Biolegend, 103126), Mouse anti-CD45.1-APC-CY7 (Biolegend, 110715), Rat anti-CD163-APC (Biolegend, 155305), Rat anti-CD206-FITC (Biolegend, 141703), Rat anti-CD11b-PE-CY7 (Biolegend, 101216), Rat anti-Ly6G-PerCP-Cy5.5 (Biolegend, 127616), Rat anti-Gr-1-APC (Biolegend, 108412), Rat anti-CD19-PE (Biolegend, 115508), Mouse anti-NK1.1-BV605 (Biolegend, 108740), Hamster anti-CD3e-FITC (BD, 553061), Rat anti-CD4-APC-CY7 (BD, 552051), Rat anti-CD8a-APC (BD, 553035), Rat anti-CD63-FITC (Biolegend, 143919), Rat anti-CD63-PE (Biolegend, 143903), Rat anti-GPNMB-eFluor660 (Thermo Fisher Scientific, 50-5708-82) and Rat anti-CD115-BV605 (Biolegend, 135517), Rat anti-CD45-PE-Cy5 antibody (Thermo Fisher Scientific, 15-0451-81).

Validation

No new antibodies were generated as part of this study. All antibodies used in the study were from commercial sources and were validated by the vendors and previous studies performed by our laboratory or by others. Specific validation information for each antibody is available on the vendor's website.

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

Young (8-12 weeks old, n = 375) male C57BL/6 mice obtained from SLAC Laboratory Company (Shanghai, China) were used for in vivo experiments. Young (6-8 weeks old, n = 13) male CD45.1 C57BL/6 mice (Cat. NO. NM-KI-210226) were procured from Shanghai Model Organisms Center, Inc (Shanghai, China). Aged (20-22 months old, n = 14) male C57BL/6 mice were obtained from Beijing Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). CCR2-GFP (8-12 weeks old, n = 60) and CCR2-KO mice (8-12 weeks old, n=40), established on a C57BL/6 background, were procured from the Jackson Laboratory (Strain #: 027619, RRID: IMSR_JAX:027619).

Wild animals

This study did not involve wild animals.

Reporting on sex

All mice used in this study were male to reduce variability within this study.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

The ethical approval for all animal experimental procedures (Approval No. 2020-655) was granted by the Institutional Ethics Committee of the Second Affiliated Hospital, Zhejiang University School of Medicine. The procedures adhered to the guidelines set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

NA

Novel plant genotypes

NA

Authentication

NA

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

To obtain a single-cell suspension of intracerebral immune cells, mice were euthanized, and transcardial perfusion was performed using 25 ml of pre-cooled PBS. Left hemispheres were collected after brain dissection. Brain tissues were dissociated into homogenates using the Neural Tissue Dissociation Kit (T) with a gentleMACSTM Octo Dissociator with Heaters (Miltenyi Biotec), following the manufacturer's instructions. The resulting suspension was passed through a 70µm cell strainer, and the resulting cells were resuspended in 10 ml of 30% Percoll. Subsequently, 3 ml of 70% Percoll was slowly injected into the bottom of the suspension using a fine needle, while 1 ml of PBS was layered on top of the suspension. Centrifugation (800g, 30 minutes, 18°C) was performed using a density gradient of 30%/70% Percoll to separate myelin sheaths and debris. Cells at the interface, mainly consisting of immune cells, were collected, washed, and resuspended in pre-cooled PBS. For blood cells preparation, mice were deeply anesthetized, and 0.8 ml of peripheral blood was collected from the right atrium. The final 2 ml blood suspension (0.8 ml blood + 1.2 ml PBS) was layered onto 1 ml of Ficoll-Paque (GE, 17–1440-02). Cells at the interface were collected after density gradient centrifugation (800g, 20 minutes, 18°C) and then resuspended with pre-cooled PBS.

The single-cell suspension obtained as described above was incubated with antibodies to surface antigens in the dark for 30 minutes on ice at 4°C. For intracellular antigens, the suspension was washed with PBS twice after incubation with surface antigen antibodies. The cells were then fixed and permeabilized using Fixation/Permeabilization Diluent & Concentrate (eBioscience) according to the manufacturer's instructions, followed by incubation with corresponding intracellular antigen antibodies in the dark for 30 minutes on ice at 4°C. Fluorochrome compensation was established with single-stained UltraComp eBeads (Thermo Fisher Invitrogen).

Instrument

Flow cytometry was performed using the BD LSRFortessa flow cytometer (BD biosciences) or Beckman CytoFELX IX flow cytometer (Beckman).

Software

Flowjo (v10.4, BD Biosciences)

Cell population abundance

Sort purity was routinely over 95% on post-sort checks.

Gating strategy

The gating strategy is provided in the Supplementary Data 6.

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