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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	SpectroFlo Software (Cytek Biosciences); BD FACS Diva; Olympus FluoView; Olympus OlyVIA
Data analysis	FlowJo v10.1; Imaris v10.2.0 software; Graphpad Prism 10; ImageJ v2.14 (FIJI)

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

All data that support the findings of this study are presented in the manuscript, and are available from the corresponding author upon reasonable request. Data are located in controlled access data storage at Monash University.

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	Sample size is estimated from power calculations using DSS Researcher's Tookit. Animal numbers based on infarct size from a previous study (Shim et al Translational stroke research DOI: 10.1007/s12975-019-00738-3), 40% difference, α=0.05, β=0.2 to result N=/>6.
Data exclusions	The animal was excluded from analysis if cerebral infarct was not visible following stroke induction. This occurred <5% of total animals used.
Replication	All experiments were performed at least twice. Flow cytometry experiments were repeated 3 times (i.e. in three separate cohort of animals).
Randomization	Cage littermates were randomised to sham or stroke surgery evenly.
Blinding	Image analysis was performed in a blinded manner. Images were acquired and analysed by different investigators. Behaviour tests were performed in a blinded manner. Investigators analysing the animal behaviour did not have information of whether the mouse underwent sham or stroke surgery.

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	All information on antibodies used in this study is listed in the manuscript. Please see Methods section of the paper.

Validation	All antibodies against surface expressed proteins used in this study have been previously validated by the manufacturer as stated on their associated product webpages, and our own lab in previous experiments. Full information on antibodies used in this study is in the Methods section.

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	Adult male mice aged 7-12 weeks on C57Bl/6J background were used. Cx3cr1gfp/+ mice were generated in-house by crossing Cx3cr1gfp/gfp mice, with C57BL/6J wildtype mice. Neutrophil-reporter (CatchupIVM-red) mice, and CCR2-deficient mice (Ccr2rfp/rfp) were also used.
Wild animals	N/A
Reporting on sex	Only male mice were used in this study due to potential hormonal influence on neuroinflammation.
Field-collected samples	N/A
Ethics oversight	All procedures were performed in accordance with Australian law (Victorian Prevention of Cruelty to Animals Act 1986), and were approved by the Monash Medical Centre Animal Ethics Committee (MMCB/2021/32).

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	Brains were processed using an Adult Brain Dissociation Kit (#130-107-677, Miltenyi Biotech), modified from manufacturers instruction, using a "C tube" (#130-093-237, Miltenyi Biotech) with the 37C_ABDK_01 protocol of a GentleMACS Octo Disassociator with heaters. Upon dissociation, cells were isolated by centrifugation using a 30%/80% Percoll gradient (Bio-Strategy) at 1,850 xg for 20 min without brake, at room temperature. Cells were collected from Percoll and washed twice with ice-cold PBS-EDTA (2 mM), before incubation with fixable viability stain (FVS)780 (1:1,000, #565388 BD Biosciences) for 20 min on ice. Cells were washed, before blockade with PBS-fetal calf serum (FCS; 10%) with anti-CD16/32 (1:100, #553142 BD Biosciences) for 20 min on ice. Cells were then incubated with conjugated antibodies (key resources table) against cell surface proteins in PBS-EDTA (2 mM) for 30 min on ice. Cells were fixed and permeabilized using a CytoPerm Solution Kit (#555028, BD Biosciences) for 20 min on ice, before incubation with conjugated antibodies (key resources table) against intracellular proteins in permeabilization wash buffer for 30 min on ice.
Instrument	Aurora Spectral Flow Cytometer (Cytek Biosciences)
Software	SpectroFlo Software (Cytek Biosciences)
Cell population abundance	Microglia comprises around 15-20% of all cells in the brain, and for this reason we do not need to enrich this population to

Cell population abundance	examine their inflammatory profile. Notably, we did not enrich for any population examined.
Gating strategy	Flow plots signifying gating strategy do not have the axis labelled, as this was simply used to demonstrate how the cells were identified. However, all plots displaying MFI showed axis for quantification purposes.

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