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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

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	ZEN SP1.2 2008 v5.0.0.276 (Carl Zeiss GmbH, Jena), BD FACSDiva v8.0.2
Data analysis	Image J v1.47, FlowJo v10.1 (LLC), BD FACSDiva v8.0.2; GraphPad Prism v6.01; CASAVA v1.82; Partek Genomics Suite V6.6, DeSeq2,DAVID v6.8, REVIGO, R (v.3.5.0 2018-04-23), bcl2fastq2 v2.20, MotiQ, Code for CENA is available at: https://github.com/UlasThomas/CoCena2

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

Our gene expression data are deposited in the GEO database (GSE120701). The data that support the findings of this study are available from the corresponding author upon reasonable request.

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 statistical methods were used to pre-determine sample sizes but our sample sizes are similar to those reported in previous publications (e.g. references 26,35,36).
Data exclusions	Outliers were removed after identification by the GraphPad Prism ROUT method using standard settings.
Replication	All experiments were replicated and all attempts to replicate were successful.
Randomization	The available Cxcr4cKO and Cxcr4-control animals were allocated to stroke experiment based on genotype and irrespective of sex. There were no other selection criteria for the allocated animals. Other experiments did not require randomization.
Blinding	Monocyte counting in Cxcr4cKO and Cxcr4-control animals at day 1 after tMCAO and infarct size measurements were performed by a researcher blinded to the experimental group.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	Cxcr4-CreER animals are unique and available from the Stumm lab upon request. Cxcr4-GFP and Cxcr4-Loxp animals are commercially available. All antibodies are also commercially available.

Antibodies

Antibodies used	All antibodies are detailed with clone or product number, supplier, and dilution in Supplemetary Table 6
Validation	Anti-CXCR4 (2B11) was validated using Cxcr4 knockout mice. All other antibodies were published before and titrated prior to initial experiment. References are provided for all antibodies in Supplementary Table 6.

Animals and other organisms

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

Laboratory animals	We used C57Bl6 and CD1 mice at foetal and adult age. Mice were used regardless of sex. Foetal mice were assessed between E9.5 and E18.5. Postnatal mice were assessed between P0 and P45. Stroke experiments were performed in adult animals (between 3 and 6 months).
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve samples collected from the field.

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	FSC/SSC gates and dead cell stain (DAPI or Hoechst) were used to define single live cells. Doublets were removed based on FSC-A/FSC-W. In primary experiments all gates were defined based on FMO controls. In consecutive experiments, gates in fluorescence channels were drawn based on clear separation between negative and positive populations as shown in Supplementary Information.
Instrument	BD FACSAria Fusion or LSRFortessa.
Software	BD FACSDiva v8.0.2 and FlowJo v10.1 (LLC)
Cell population abundance	The overlap of GFP and tdTomato is reported for monocytes, microglia, leukocytes and hematopoietic cells. Animals lacking GFP or tdTomato were used to define the signal threshold of the fluorescent proteins. This is demonstrated in histograms in the manuscript.
Gating strategy	Gating strategies are shown in the manuscript. More detailed information will be provided upon request.

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