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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	The commercially available softwares used in this study to collect, stitch and visualize the data are: ImSpector (Version 5.295, LaVision BioTec GmbH), Vision4D (Version 2.12.6 x64, Arivis AG), Amira (Version 6.3.0, FEI Visualization Sciences Group), Imaris (Version 9.1, Bitplane AG), Zen 2 (Version 10.0.4.910, Carl Zeiss AG), Zen 2 (Version 2.0.0.0, Carl Zeiss AG).
Data analysis	The commercially available softwares used in this study to analyze the data are: Amira (Version 6.3.0, FEI Visualization Sciences Group), Imaris (Version 9.1, Bitplane AG), GraphPad Prism (Version 6, GraphPad Software Inc.). The already publicly or published codes/softwares used in this study to stitch and analyze the data are: Fiji (Version 1.51, https://fiji.sc/), TeraStitcher (Version 1.10, https://abria.github.io/TeraStitcher/), NeuroGPS-Tree (https://www.nature.com/articles/nmeth.3662), ClearMap (https://www.sciencedirect.com/science/article/pii/S0092867416305554). All the custom-made codes: the code to pre-process the data for NeuroGPS-Tree in Fiji, the custom-made macros for Fiji to remove region of interests in images, the custom-script to calculate the volume of specific brain regions for the cellular density are available upon request.

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

The data 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	Sample sizes were chosen based on prior experience with similar models. Sample sizes are specified in figure legends.
Data exclusions	Exclusion criteria were pre-established. Animals that resulted negative for the expression of fluorescent proteins by genotyping were excluded from the study. For MCAO experiments, exclusion criteria were as follows: insufficient MCA occlusion (a reduction in blood flow to 15% of the baseline value) and blood flow recovery >80% within 10 min of reperfusion.
Replication	The indication of how many times each experiment was repeated independently showing similar results is written in the corresponding figure legend. Overall, the protocols described in this study were replicated successfully more than 5 times in independent experiments and they were also reproduced at least by 3 different operators.
Randomization	The animals used in this study were selected for each experiment based on their genetic background (wt or fluorescent transgenes). Within each strain, animals were randomly selected.
Blinding	Littermates were housed in the same cage. The investigator was not blinded to group allocation for data analysis as this was obvious during the experiments (e.g. stroke vs sham or TBI vs unlesioned).

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

Antibodies

Antibodies used	Atto594 conjugated anti-RFP nanobooster (Chromotek, rba594-100), Atto647N conjugated anti-GFP nanobooster (Chromotek, gba647n-100), Atto488N conjugated anti-GFP nanobooster (Chromotek, gba488-100), Atto488 conjugated anti-Vimentin nanobooster (Vimentin-Label_Atto488, Chromotek, vba488-100), Alexa647 conjugated anti-GFP antibody (Invitrogen, A31852). All the nanoboosters were used in dilution 1:500 to label brain slices, 1:600 to label whole brains and 1:7000 to label whole bodies. The antibody was used in dilution 1:850 to label brain slices, 1:730 to label whole brains and 1:7000 to label whole bodies.

Validation	All the nanoboosters were coming from the same company which is the manufacturer: Chromotek https://www.chromotek.com/products/nano-boosters/. The manufacturer validated the nanoboosters and in its website (see link) they provide references, images of the validation from published papers and from validation experiments. We used nanobooster to label fluorescent proteins and vimentin expressed in mouse tissue, as indicated in example images from the manufacturer website. Before any deep tissue staining such as vDISCO whole body labeling, we further validated the compatibility of the nanoboosters with vDISCO in brain slices from transgenic animals as indicated in Method part of the manuscript. The Alexa647 conjugated anti-GFP antibody (GFP Polyclonal Antibody Alexa Fluor 647, Invitrogen, A31852) has been validated by the manufacturer: Invitrogen https://www.thermofisher.com/antibody/product/GFP-Antibody-Polyclonal/A-31852. In the manufacturer website (see link) they provide references, images of the validation from published papers and from validation experiments. We used this antibody to label GFP expressed in mouse tissue, and this application has already been shown by references indicated in the manufacturer website. We further validated the compatibility of this antibody with iDISCO+ protocol in Supplementary Figure 3.

Animals and other organisms

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

Laboratory animals	We used the following mixed gender animals in the study: 6 weeks-12 months old CX3CR1GFP/+ (B6.129P-Cx3cr1tm1Litt/J, Jackson Laboratory strain code: 005582), 6 weeks-6 months old Thy1-GFPM , 3-7 months old Thy1-YFPH, 4 weeks old Prox1-EGFP (Tg(Prox1-EGFP)KY221Gsat/Mmucd, MMRRC strain code: 031006-UCD), 6 months old VEGFR3-YFP, 2 months old CX3CR1GFP/+ x CCR2RFP/+ (B6.129(Cg)-Ccr2tm2.1Ifc/J, Jackson Laboratory strain code: 017586), 6 months old LysM-EGFP (Lyz2tm1.1Graf, MGI: 2654931), 3 months old CD68-EGFP (C57BL/6-Tg(CD68-EGFP)1Drg/j, Jackson Laboratory strain code: 026827), 3 weeks-4 months old C57BL/6J, 3-4 months old NMRI nu/nu mice.
Wild animals	This study does not involve wild animals
Field-collected samples	This study does not involve Field-collected samples