

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 (<i>n</i>) 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> 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 <i>d</i> , Pearson's <i>r</i>), 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	Fluorescence images were acquired using a SLIDEVIEW VS200 slide scanner (Olympus, Tokyo, Japan) and a STELLARIS 8 FALCON confocal microscope (Leica, Wetzlar, Germany). Near-infrared fluorescence imaging was performed using an IVIS Spectrum system (PerkinElmer, Waltham, MA, USA). Magnetic resonance imaging (MRI) was performed on a 9.4T MRI system (Bruker, Billerica, MA, USA). Raw RNA sequencing data were generated using the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA).
Data analysis	GraphPad Prism (version 9.5.1; GraphPad Software, La Jolla, CA, USA) was used for statistical analysis and data visualization. ImageJ (version 1.54g; NIH, Bethesda, MD, USA) and Imaris (version 11.0; Oxford Instruments, Abingdon, UK) were used to analyze immunofluorescence images. Living Image software (version 4.4; PerkinElmer, Waltham, MA, USA) was used to analyze IVIS images. ImageJ (version 1.54g; NIH, Bethesda, MD, USA) was used to analyze MRI images. The CT images were processed and analyzed using MATLAB (version R2024b; MathWorks, Natick, MA, USA). Space Ranger (version 3.1.2; 10x Genomics, Pleasanton, CA, USA), Seurat R package (version 4.3.0; New York Genome Center, New York, NY, USA), R (version 4.0.3; R Foundation for Statistical Computing, Vienna, Austria), RCTD (version 1.1.0; Broad Institute, Cambridge, MA, USA) were used for spatial transcriptomics data analysis. Schematics were created with BioRender.com. or Blender (version 5.2; Blender Foundation, Amsterdam, Netherlands).

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 supporting the findings of this study are provided in the manuscript, supplementary information, and source data files. The raw sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1373366 [<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1373366>]. The remaining raw datasets have not been deposited in a public repository because of their large size, but are available upon sending requests to the corresponding author at chengbb@shanghaitech.edu.cn, specifying the datasets required and the intended use. Access is limited to academic researchers for non-commercial research. Responses will be provided within two weeks, and access instructions will be provided by email upon approval. Data access will remain available for 6 months from the date access is granted.

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	All analyses were performed using publicly available data from the ISLES22 dataset. No sex- or gender-based analyses were conducted.
Reporting on race, ethnicity, or other socially relevant groupings	None.
Population characteristics	None.
Recruitment	None.
Ethics oversight	None.

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 predetermine sample size. Our sample sizes were comparable to those reported in previously published studies: Nat. Biomed. Eng 9, 1531–1546 (2025). https://doi.org/10.1038/s41551-025-01390-z ; Nature 560, 185–191 (2018). https://doi.org/10.1038/s41586-018-0368-8 ; Nat Commun. 2021 Nov 26;12(1):6943. doi: 10.1038/s41467-021-27248-x.
Data exclusions	For the mouse model of transient focal cerebral ischemia, mice were excluded if they exhibited < 70% reduction in cerebral blood flow during vessel occlusion, or < 50% recovery of cerebral blood flow relative to baseline after reperfusion. This exclusion criterion was applied to ensure the consistency and reliability of the ischemic model.
Replication	All experiments included a minimum of three independent biological replicates per experimental condition, and all key findings were consistently reproduced across replicates to ensure result reproducibility.
Randomization	All animals and samples were randomly allocated to experimental and control groups using a computer-generated random number sequence to reduce potential allocation bias.
Blinding	Investigators performing data collection and outcome assessment were blinded to group allocation for the full duration of the study.

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	The following antibodies were used for immunofluorescence staining in this study: anti-CD90 (1:200, abcam, #ab225), anti-CD34 (1:200, abcam, #ab81289), anti-CD45 (1:200, abcam, #ab10558), anti-lba1 (1:1000, FUJIFILM WAKO, #019-19741), anti-CD31 (1:200, R&D, #AF3628), anti-Nestin (1:100, Millipore, #MAB353), anti-Ki67 (1:200, abcam, #ab15580), donkey anti-rabbit-Alexa Fluor 555 (1:500, Beyotime, #A0453), donkey anti-goat-Alexa Fluor 488 (1:500, abcam, #ab150129), or goat anti-mouse-Alexa Fluor 488 (1:500, Beyotime, #A0428).
Validation	All primary antibodies used in this study were validated by the manufacturer and/or had citations in published articles. CD90: https://www.abcam.com/en-us/products/primary-antibodies/cd90-thy1-antibody-mrc-ox-7-hematopoietic-stem-cell-marker-ab225 ; CD34: https://www.abcam.com/en-us/products/primary-antibodies/cd34-antibody-ep373y-ab81289 ; CD45: https://www.abcam.com/en-us/products/primary-antibodies/cd45-antibody-ab10558 ; lba1: https://labchem-wako.fujifilm.com/europe/product/detail/W01W0101-1974.html ; CD31: https://www.rndsystems.com/products/human-mouse-rat-cd31-pecam-1-antibody_af3628?utm_source=distributor&utm_medium=referral&utm_campaign=product&utm_term=primaryantibodies ; Nestin: https://www.sigmaaldrich.cn/CN/zh/product/mm/mab353 ; Ki67: https://www.abcam.com/en-us/products/primary-antibodies/ki67-antibody-ab15580 ; donkey anti-rabbit-Alexa Fluor 555: https://www.beyotime.com/product/A0453.htm ; donkey anti-goat-Alexa Fluor 488: https://www.abcam.cn/products/secondary-antibodies/donkey-goat-igg-h-l-alex-fluor-488-ab150129 ; goat anti-mouse-Alexa Fluor 488: https://www.beyotime.com/product/A0428.htm .

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	BV2 mouse microglial cell line, originally established from primary microglia of female C57BL/6 mice, was provided by the laboratory of Dr. Guo-Yuan Yang (School of Biomedical Engineering, Shanghai Jiao Tong University, Shanghai, China). Male Sprague-Dawley rats (4–6 weeks old; 120–220 g) obtained from Jihui (Shanghai, China) were used to isolate primary bone marrow-derived MSCs. All animal procedures were approved by the the Animal Care and Use Committee of ShanghaiTech University. No human-derived cells were used in this study.
Authentication	BV2 cells were authenticated by the providing laboratory (Dr. Guo-Yuan Yang, Shanghai Jiao Tong University) via morphological assessment and marker expression, consistent with established phenotypic characteristics of BV2 cells. Primary rat bone marrow-derived MSCs were validated via immunostaining for canonical MSC markers (CD90 ⁺ , CD34 ⁺ , CD45 ⁺).
Mycoplasma contamination	BV2 cells were obtained from Dr. Guo-Yuan Yang's laboratory (Shanghai Jiao Tong University) with negative mycoplasma documentation. Primary rat MSCs showed no contamination signs via routine morphological and viability monitoring.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

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 C57BL/6J mice (8–10 weeks old; 21–28 g) obtained from Jihui (Shanghai, China) were used in all animal experiments. Male Sprague-Dawley rats (4–6 weeks old; 120–220 g) obtained from Jihui (Shanghai, China) were used to isolate primary bone marrow-derived MSCs.
Wild animals	No wild animals were used in this study.
Reporting on sex	Only male mice were included in this initial proof-of-concept study to minimize variability arising from sex-dependent differences in stroke pathophysiology, BBB integrity, and ultrasound-mediated BBB opening. No sex-based comparative analysis was performed, and all findings are restricted to male mice.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal experiments were approved by the Animal Care and Use Committee of ShanghaiTech University.

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

Plants

Seed stocks	none.
Novel plant genotypes	none.
Authentication	none.

Magnetic resonance imaging

Experimental design

Design type	Structural imaging only. No task-based, resting-state, event-related or block design paradigms were used.
Design specifications	Not applicable, as no task-based or event-related experimental paradigm was employed in this study.
Behavioral performance measures	Not applicable. MRI scans were performed on anesthetized mice following Middle Cerebral Artery Occlusion (MCAO) surgery, and no behavioral tasks were administered during scanning.

Acquisition

Imaging type(s)	Structural (T2-weighted).
Field strength	9.4 Tesla.
Sequence & imaging parameters	Imaging parameters at 3 d were as follows: repetition time (TR)/echo time (TE) = 2005/35 ms, flip angle = 90°, number of excitations = 8, in-plane resolution = 0.1 × 0.1 mm, slice thickness = 0.3 mm, inter-slice gap = 0.3 mm, and receiver bandwidth = 81967.2 Hz. Imaging parameters at 28 and 42 d were as follows: TR/TE = 3011.71/26 ms, field of view (FOV) = 18 × 18 mm, acquisition matrix = 256 × 256, total number of slices = 45, slice thickness = 0.3 mm, two mean values, and Rapid Acquisition with Relaxation Enhancement factor = 8.
Area of acquisition	Whole-brain MRI scans were performed in this study.
Diffusion MRI	☐ Used ☒ Not used

Preprocessing

Preprocessing software	All T2-weighted MRI images were preprocessed using ImageJ (NIH, Bethesda, MD, USA).
Normalization	None.
Normalization template	None.
Noise and artifact removal	None.
Volume censoring	None.

Statistical modeling & inference

Model type and settings	Mass univariate analysis using t-tests or Mann–Whitney U tests.
Effect(s) tested	Intergroup differences in cerebral atrophy volume and lateral ventricular volume were compared between paired subgroups: MCAO+EVs vs MCAO+HaMBO+EVs, MCAO+MSCs vs MCAO+HaMBO+MSCs, MCAO vs MCAO+HaMBO.
Specify type of analysis:	☐ Whole brain ☒ ROI-based ☐ Both
Anatomical location(s)	Manual ROI segmentation guided by anatomical landmarks.
Statistic type for inference	ROI-level volumetric analysis was performed, with no voxel-wise or cluster-wise inference conducted.
(See Eklund et al. 2016)	
Correction	No multiple comparison correction was performed.

Models & analysis

n/a	Involvement in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis