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Reporting Summary

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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	The original MR images were transferred to a workstation (GE Advantage Workstation 4.9). The raw ASL image data were imported to a GE workstation (ADW4.7) to generate CBF maps. Single-cell suspensions were loaded onto 10x Chromium to capture single cells. The subsequent cDNA amplification and library construction steps were performed according to standard protocols. Libraries were sequenced on an Illumina NovaSeq 6000 sequencing system (paired-end multiplexing run, 150 bp). The images of immunohistochemistry were detected using an Olympus BX41 microscope. The images of Immunofluorescence were taken using a Leica fluorescence microscope (DN4000B, Leica Microsystems, Germany).
Data analysis	Quantitative analysis of immunohistochemistry and Blotting band were calculated using the IHC toolbox plugin (IHC Profiler) of Image J Software (Vesion1.48, National Institutes of Health, Image J system, Bethesda, MD, USA). The sequencing results were demultiplexed and converted to FASTQ format using Illumina bcl2fastq software. Sample demultiplexing, barcode processing and single-cell 3' gene counting were performed by using the Cell Ranger pipeline (https://support.10xgenomics.com/single-cell-geneexpression/software/pipelines/latest/what-is-cell-ranger, version 3.1.0). The Cell Ranger output was loaded into Seurat (version 3.1.1) for dimensional reduction, clustering, and analysis of the scRNA-seq data. When

applying quality control, we set the criteria as min gene > 500 and proportion of mitochondria < 25% following doublet filtering via the R package 'DoubletFinder'(v2.0.3).

The cells were normalized to the total unique molecular identifier read count as instructed in the manufacturer's manual (http://satijalab.org/seurat/).

We used the Find Neighbors and Find Clusters functions in the Seurat package for cell clustering analysis and displayed the 2D map via tSNE/Umap.

The expression of selected genes was plotted with the Seurat function FeaturePlot and VlnPlot.

Heatmaps were generated via the heatmap function in Seurat.

An advanced single-cell volcano plot was generated via the OmicStudio tools at https://www.omicstudio.cn/tool.

Single-cell trajectory analysis was performed via the OmicStudio tools at https://www.omicstudio.cn/analysis/tenXMonocle.

CellPhoneDB Python package was using to conduct Cell-Cell interaction and Cellular communication analysis.

The GO analysis was performed based on the Database of Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/). The KEGG pathway analysis was conducted the same way as the GO analysis.

Statistical analyses were performed on GraphPad Prism Software (v 8.0.1, GraphPad Software, San Diego, CA, USA) and SPSS statistics software (v21.0, Chicago, USA).

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 raw data of scRNA-seq generated in this study have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA009021) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human. All other data included in this study are provided in Supplementary data and Source data. 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	The gender of participants in this study has been disclosed in Supplementary Table 1. Sex was determined by self-reporting, and it was not considered for study design. This is because that this study focuses on spatial heterogeneity in tumors and PBZ areas. The study did not involve an analysis of prognostic risk factors for clinical outcomes, and patient clinical characteristics (such as gender, age, course of disease, etc.) were not included in the study design. In addition, the relative few individuals in each group is another reason.
Reporting on race, ethnicity, or other socially relevant groupings	All human participants were of Han Chinese origin.
Population characteristics	Demographics of the subjects are summarized in Supplementary table1.
Recruitment	1.The detailed inclusion criteria(should be met at the same time): (1). The patients who received gross total resection (GTR). (2). Preoperative MRI showed significant T2 FLAIR abnormality area surrounding the contrast-enhancing/CE area. (3). Senior surgeon believe that partial resection of non-CE area beyond the CE tumor borders is beneficial but also harmless to the prognosis of patients. (4).The sampling locations of HBI and LBI, as determined by preoperative ASL-CBF imaging, were located in the non-CE area planned to be excised before surgery. (5)Individuals gave written informed consent for the sample donation. 2.The exclusion criteria: (1). Patients who receive only biopsy or partial resection of their tumor will not undergo additional PBZ removal programs. (2). If the PBZ sampling area delineated before surgery is considered dangerous/no beneficial for the patients (such as sampling lesions of PBZ are found in the eloquent area), the patients will not be enrolled in the study and the sampling of PBZ will not be performed.

There was no selection bias, as patients were not grouped in this study.

Ethics oversight

Ethical approval for the study was obtained from the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University (Ethics no. KY2021/172) and the Ethics Committee of Zhejiang Provincial People Hospital (Ethics no. 2021JS043). Ethical principles set out by the Declaration of Helsinki were strictly followed, and written consent was obtained from each patient in this study. The written consent was obtained from each patient in this study.

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 sizes are indicated in each figure and figure legend. No power analysis or statistical method were used to calculate the sample size, they were determinated based on our previous experimental designs and results. Data from all experiments were analyzed and p-values from statistical tests used to assess statistical significant and appropriateness of sample sizes.
Data exclusions	This study did not exclude any data from the analysis
Replication	All the data in this study are representative of at least three experiments, unless otherwise statements. Experiments were performed independently.
Randomization	This is not relevant to this study. In all the experiments carried out in this study, samples were assigned to experimental groups according to the specific anatomical regions from which they derived. The focus of the study was on the differences between these specific regions, so random allocation was not applicable.
Blinding	The investigators were blinded to group allocation during data collection and analysis

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 source and dilution of all antibodies are also summarized in Supplementary table 5.

Antibodies used for IHC:
CD31; Abcam; ab9498; 1/500
VEGFA; Wanleibio; WL00009b; 1/200
EGFR; Wanleibio; WL0682a; 1/200
HIF-1α; proteintech; 20960-1-AP; 1/500
MMP-9; proteintech; 10375-2-AP; 1/500
Ki-67; 27309-1-AP;proteintech; 1/2000
Goat Anti-Rabbit IgG H&L (HRP); Abcam; ab6721; 1/500
Goat Anti-Mouse IgG H&L (HRP); Abcam; ab6789 1/500

Antibodies used for Western blot
VEGFA; Wanleibio; WL00009b; 1/1000
EGFR; Wanleibio; WL0682a; 1/1000
HIF-1α; proteintech; 20960-1-AP; 1/2000
MMP-9; proteintech; 10375-2-AP; 1/1000
β-actin; Beyotime; AF0003; 1/1000
Goat Anti-Rabbit IgG H&L (HRP); Abcam; ab6721; 1/5000
Goat Anti-Mouse IgG H&L (HRP); Abcam; ab6789 1/3000

Antibodies used for IF
Nestin; Abcam; ab18102; 1/400
CD31; proteintech; 11265-1-AP; 1/200
CD86; CST; 91882S; 1/200
Arginase-1; CST; 93668T; 1/200
CD66b; Affinity; DF10151; 1/100
MPO; proteintech; 22225-1-AP; 1/300
OLIG2; Abcam; ab109186; 1/800
SOX2; Abcam; ab92494; 1/100
CD68; proteintech; 25747-1-AP; 1/1000
CD3; proteintech; 17617-1-AP; 1/1000
SOX9; Servicebio; GB14171-50; 1/200
GFAP; Servicebio; GB11096-100; 1/1000
TMEM119; Abcam; ab306583; 1/1000
iF440-Tyramide ; Servicebio ; G1250 ; 1/500
iF488-Tyramide ; Servicebio ; G1231 ; 1/500
iF555-Tyramide ; Servicebio ; G1233 ; 1/500
iF594-Tyramide ; Servicebio ; G1242 ; 1/500
iF647-Tyramide ; Servicebio ; G1232 ; 1/500

Validation

All primary antibodies were validated for the species (mouse) and application (immunohistochemistry, immunofluorescence, western blot) by the correspondent manufacturers or previous publications from Citeab (https://www.citeab.com); All antibodies were individually tested and titrated on relevant positive and negative biological controls prior to use.

Anti-CD31 ; Abcam; ab9498
Vendor information:https://www.abcam.cn/cd31-antibody-jc70a-ab9498.html

Anti-VEGFA ; Wanleibio ; WL00009b
Vendor information:https://www.wanleibio.cn/index/ProList?keywords=WL00009b

Anti-EGFR; Wanleibio; WL0682a
Vendor information:https://www.wanleibio.cn/index/ProList?keywords=WL0682a

Anti-HIF-1α ; proteintech ; 20960-1-AP
Vendor information:https://www.ptgcn.com/products/HIF1A-Antibody-20960-1-AP.htm

Anti-MMP-9 ; proteintech ; 10375-2-AP
Vendor information:https://www.ptgcn.com/products/MMP9-Antibody-10375-2-AP.htm

Anti-Ki-67 ; proteintech; 27309-1-AP
Vendor information:https://www.ptgcn.com/products/KI67-Antibody-27309-1-AP.htm

Goat Anti-Rabbit IgG H&L (HRP) Rabbit Goat ;Abcam; ab6721
Vendor information:https://www.abcam.cn/goat-rabbit-igg-hl-hrp-ab6721.html

Goat Anti-Mouse IgG H&L (HRP) Mouse Goat; Abcam ;ab6789
Vendor information:https://www.abcam.cn/goat-mouse-igg-hl-hrp-ab6789.html

Anti-β-actin Human Mouse; Beyotime ;AF0003
Vendor information:https://www.beyotime.com/product/AF0003.htm

Anti-Nestin ; Abcam; ab18102
Vendor information:https://www.abcam.cn/nestin-antibody-2c13a11-ab18102.html

CD86 (E2G8P) Rabbit mAb; CST; 91882S
Vendor information: https://www.cellsignal.cn/products/primary-antibodies/cd86-e2g8p-rabbit-mab/91882

Arginase-1 ;CST;93668T
Vendor information: https://www.cellsignal.cn/products/primary-antibodies/arginase-1-d4e3m-xp-rabbit-mab/93668

AAnti-CD66b; Affinity ;DF10151
Vendor information:http://www.affbiotech.cn/goods-10993-DF10151-CEACAM8_Antibody.html

MPO Polyclonal antibody; proteintech; 22225-1-AP
Vendor information: https://www.ptgcn.com/Products/MPO-Antibody-22225-1-AP.htm

OLIG2; Abcam; ab109186
Vendor information: https://www.abcam.cn/products/primary-antibodies/olig2-antibody-epr2673-ab109186.html

SOX2;Abcam; ab92494
Vendor information:https://www.abcam.cn/products/primary-antibodies/sox2-antibody-epr3131-ab92494.html

Anti-CD68 ; proteintech; 25747-1-AP
Vendor information:https://www.ptgcn.com/products/CD68-Antibody-25747-1-AP.htm

Anti-CD3 ; proteintech; 17617-1-AP
Vendor information:https://www.ptgcn.com/products/CD3E-Antibody-17617-1-AP.htm

SOX9;Servicebio; GB14171-50
Vendor information: https://www.servicebio.cn/goodsdetail?id=22108

GFAP; Servicebio; GB11096-100
Vendor information: https://www.servicebio.cn/goodsdetail?id=1376

TMEM119; Abcam; ab306583
Vendor information: https://www.abcam.cn/products/primary-antibodies/tmem119-antibody-epr25865-89-ab306583.html

iF440-Tyramide ; Servicebio ; G1250
Vendor information: https://www.servicebio.cn/goodsdetail?id=21652

iF488-Tyramide ; Servicebio ; G1231
Vendor information: https://www.servicebio.cn/goodsdetail?id=21962

iF555-Tyramide ; Servicebio ; G1233
Vendor information: https://www.servicebio.cn/goodsdetail?id=21964

iF594-Tyramide ; Servicebio ; G1242
Vendor information: https://www.servicebio.cn/goodsdetail?id=21965

iF647-Tyramide ; Servicebio ; G1232
Vendor information: https://www.servicebio.cn/goodsdetail?id=21963

Plants

Seed stocks: N/A

Novel plant genotypes: N/A

Authentication: N/A

Magnetic resonance imaging

Experimental design

Design type: resting state; block design.

Design specifications: Four blocks in each subject.

Behavioral performance measures: correct button press; range.

Acquisition

Imaging type(s)	structural, contrast-enhanced imaging , pseudo-continuous ASL(pCASL), diffusion-weighted imaging (DWI) ,diffusion tensor imaging (DTI)
Field strength	3.0 Tesla scanner (SIGNA Pioneer, GE, USA)
Sequence & imaging parameters	The sequences included T1-weighted imaging (axial spin–echo (SE) sequences, repetition time (TR)= 2,242.2ms; echo time (TE) = 29.5ms; field of view (FOV): 240*240 mm2; matrix:512*512; slice thickness: 5 mm without slice gap), T2-weighted imaging (axial turbo SE sequences: TR= 4,256.5ms; TE= 90.1ms; FOV: 240*240 mm2; matrix:512*512; slice thickness: 5 mm without slice gap), contrast-enhanced imaging (TR = 4,852ms; TE = 10.7ms; FOV = 240*240 mm2; matrix:128*128; slice thickness= 4 mm; 18 slices without slice gap), pseudo-continuous ASL(pCASL) (TR=4891ms; TE=10.6ms; post-labeling delay time=2025ms; FOV=240*240mm2; Flip angle =111°; slice thickness=4.0mm; NEX=3), diffusion-weighted imaging (DWI) (single-shot SE echo-planar sequence: TR = 3,467ms; TE =77.1ms; flip angle = 90; FOV: 240*240 mm2; matrix:256*256; slice thickness =6-7.5 mm, diffusion sensitizing gradients were applied sequentially in the X, Y, and Z directions with b values of 0 and 1000 mm2/s.), and diffusion tensor imaging (DTI) (single-shot echo-planar sequence: TR = 8,000 ms; TE =96.6 ms; flip angle = 90; FOV: 260*260mm2; matrix: 256*256; slice thickness =4-4.4 mm; b values (0, 1000 mm2/s) scanned in 24 directions).
Area of acquisition	Brain

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	Details of data processing and analysis are provided in the "Methods" section. The original MR images were transferred to a workstation (GE Advantage Workstation 4.7). Function Tool software was used to manually correct and denoise the images, and finally, images demonstrating cerebral blood flow (CBF) images were obtained.
Normalization	Details of normalization are provided in the "Method" section. The raw ASL image data were imported to a GE workstation (ADW4.7) to generate CBF maps. This software processes pCASL data in a standardized one-click mode.
Normalization template	Details of normalization template are provided in the "Methods" section The CBF maps were subsequently preprocessing using statistical parametric mapping (SPM, http://www.fil.ion.ucl.ac.uk/spm/software/spm12) implemented in MATLAB, which included registration, partial volume correction, normalization, and smoothing.
Noise and artifact removal	Function Tool software was used to manually correct and denoise the images, and finally, images demonstrating cerebral blood flow (CBF) images were obtained. Smoothing was performed with an isotropic Gaussian kernel filter of 6 mm full width at half maximum (FWHM).
Volume censoring	The original structural and multi-parametric MR images were also used to visualize the PBZ reconstructions in three-dimensions (3-D) on a medical imaging support platform (Tuomeng Technology, http://www.hljtmkj.com). The three regions PBZ(included Tumor), Tumor and HBI were extracted separately and the longest diameter and volume were calculated automatically in the support platform.

Statistical modeling & inference

Model type and settings	Not applicable.
Effect(s) tested	Not applicable.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☒ Both

Anatomical location(s)	1. The parenchymal part of the tumor (contrast-enhancing lesion) 2. The GI area (usually higher CBF/lower ADC area) and 3. The OPI area (the area usually lower CBF/higher ADC and far from the GI). Of note, in this study, the PBZ (consisting of GI and OPI) was defined as the tissue surrounding the contrast-enhancing lesion at a distance of < 1 cm and was considered the interface of the tumor and brain.
Statistic type for inference (See Eklund et al. 2016)	Not applicable.
Correction	Not applicable.

Models & analysis

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