

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	Olympus FV3000, FV1200 and FV1000 were used to collect immunofluorescence data including Atomic force and Lipid-lipid phase separation data too; RNA sequencing: MGISEQ-2000 platform (BGI Genomics, Shenzhen, China)
Data analysis	ImageJ (Java 8) for immunofluorescence and westernblot results;GraphPad Prism 10.1.2 for statistics; RNA sequencing analysis : the GO or KEGG pathway analysis were conducted using the ClusterProfiler and enrichplot Bioconductor R packages, following website guidelines.;

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](#)

RNA-sequencing data related to Figure 5 and Figure S4 had been submitted to the National Genomics Data Center (accession: PRJNA1242536; accession: PRJNA1371673). RNA-sequencing data related to Figure 6 and Extended Data Fig 6 from previous research used here are accessible via PRJCA01939312.

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](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	Sample sizes are described in the methods and/or figure legends. Sample sizes were determined on the basis of estimates from preliminary experiments and from similar studies. For the in vitro and in vivo experiments, we followed standards of good scientific practice. We used at least 3 biological replicates or more animals per group, to calculate means and standard deviations and to perform statistical analyses.
Data exclusions	No data were excluded.
Replication	All the experimental findings were reliably reproduced as validated by at least three independent experiments.
Randomization	All mice and cells used in the study were randomly divided into groups.
Blinding	Blinding was not performed. Experiments were reproduced by multiple individuals.

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

β -Actin antibody (Cat #RM200; 1:4000)
 MAP2 Antibody (Cat #4542s; WB: 1:1000; IF: 1:100)
 HSP70 antibody (Cat #MA3-008; 1:1000)
 recombinant heatshockprotein70 (r-HSP70) (Cat #ab113187)
 Anti-TNF alpha antibody [EPR20972] (Cat #ab215188; 1:1000)
 Caspase-3 Rabbit pAb (Cat #A2156; 1:1000)
 HSP70 Polyclonal antibody (Cat #A1507; 1:1000)
 Doublecortin Rabbit pAb (Cat # A17469; 1:2000)
 IL1 β Rabbit pAb (Cat #A1112; 1:200)
 AIF1/IBA1 Rabbit mAb (Cat #A19776; 1:200)
 Piezo1 (Extracellular Domain) Polyclonal Antibody (Cat #15939-1-AP, WB, 1:1000, IF: 1:200)
 Anti-GFAP Antibody (Cat #R1308-9; 1:200)
 IGG(H+L) Peroxidase AffiniPure Goat Anti-Rabbit; 1:500
 IGG(H+L) Peroxidase AffiniPure Goat Anti-Mouse IgG (H+L) Jackson
 M594 ALEXA FLUOR 594 DONKEY (A21203; 1:500)
 R488 ALEXA FLUOR 488 DONKEY (A21206; 1:500)
 IL-6 Rabbit Antibody (Cat # 12912; 1:1000)
 IL-4 Rabbit Antibody (Cat # A14660; 1:1000)
 IL-10 Rabbit antibody (Cat # A2171; 1:1000)
 Cleaved Caspase-3 Rabbit antibody (Cat # 9664; WB, 1:1000, IF: 1:200)
 R594 ALEXA FLUOR 594 DONKEY (A21207; 1:500)
 Purified anti-mouse CD16/32 Antibody (Cat # 101335; 1:100)
 APC/Cyanine7 anti-mouse/human CD11b (Cat # 101226; 1:100)
 PerCP/Cyanine5.5 anti-mouse CD45 (Cat # 103132; 1:100)
 FITC anti-mouse Ly-6G (Cat # 127606; 1:200)

Validation

β -Actin antibody (Cat #RM2001) Beijing Ray Antibody Biotech
<http://www.rayantibody.com/uploads/2014/07/101627017904.pdf>
 MAP2 Antibody (Cat #4542s) Cell Signaling Technology
<https://www.cellsignal.cn/products/primary-antibodies/map2-antibody/4542>
 HSP70 antibody (Cat #MA3-008) Invitrogen
<https://www.thermofisher.cn/cn/zh/antibody/product/HSP70-Antibody-clone-2A4-Monoclonal/MA3-008>
 recombinant heatshockprotein70 (r-HSP70) (Cat #ab113187) Abcam
<https://www.abcam.cn/products/proteins-peptides/recombinant-mouse-hsp70-protein-ab113187.html>
 Anti-TNF alpha antibody [EPR20972] (Cat #ab215188) Abcam
<https://www.abcam.cn/products/primary-antibodies/tnf-alpha-antibody-epr20972-ab215188.html>
 Caspase-3 Rabbit pAb (Cat #A2156) Abclonal
<https://abclonal.com.cn/catalog/A2156>
 HSP70 Polyclonal antibody (Cat #A1507) Abclonal
<https://abclonal.com.cn/catalog/A1507>
 Doublecortin Rabbit pAb (Cat # A17469) Abclonal
<https://abclonal.com.cn/catalog/A17469>
 IL1 β Rabbit pAb (Cat #A1112) Abclonal
<https://abclonal.com.cn/catalog/A1112>
 AIF1/IBA1 Rabbit mAb (Cat #A19776) Abclonal
<https://abclonal.com.cn/catalog/A19776>
 Piezo1 (Extracellular Domain) Polyclonal Antibody (Cat #15939-1-AP) proteintech
<https://www.ptgcn.com/products/FAM38A-Antibody-15939-1-AP.htm>
 Anti-GFAP Antibody (Cat #R1308-9) HUABIO
<https://huabio.cn/products/GFAP-antibody-R1308-9>
 IGG(H+L) Peroxidase AffiniPure Goat Anti-Rabbit Jackson (Cat #111-035-003)
<https://www.jacksonimmuno.com/catalog/products/111-035-003>
 IGG(H+L) Peroxidase AffiniPure Goat Anti-Mouse IgG (H+L) Jackson (Cat #115-035-003)
<https://www.jacksonimmuno.com/catalog/products/115-035-003>
 M594 ALEXA FLUOR 594 DONKEY (A21203) ThermoFisher
 Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed, Alexa Fluor™ 594 (A-21203)
 R488 ALEXA FLUOR 488 DONKEY (A21206) ThermoFisher

<https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21206>
 R594 ALEXA FLUOR 594 DONKEY(A21207) ThermoFisher
<https://www.thermofisher.cn/cn/zh/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21207>
 IL-6 Rabbit Antibody (Cat # 12912)
<https://www.cellsignal.cn/products/primary-antibodies/il-6-d5w4v-rabbit-monoclonal-antibody/12912>
 IL-4 Rabbit Antibody (Cat # A14660)
<https://abclonal.com.cn/catalog/A14660>
 IL-10 Rabbit antibody (Cat # A2171)
<https://abclonal.com.cn/catalog/A14660>
 Cleaved Caspase-3 Rabbit antibody (Cat # 9664)
<https://www.cellsignal.cn/products/primary-antibodies/cleaved-caspase-3-asp175-5a1e-rabbit-monoclonal-antibody/9664>
 Purified anti-mouse CD16/32 Antibody(Cat # 101335)
<https://www.fishersci.com/shop/products/pure-anti-mu-cd16-32-maxpar-r/502069563?searchHijack=true&searchTerm=pure-anti-mu-cd16-32-maxpar-r&searchType=Rapid&matchedCatNo=502069563>
 APC/Cyanine7 anti-mouse/human CD11b(Cat # 101226)
<https://www.fishersci.com/shop/products/apc-cy7-anti-mu-cd11b/NC9908054?searchHijack=true&searchTerm=apc-cy7-anti-mu-cd11b&searchType=Rapid&matchedCatNo=NC9908054>
 PerCP/Cyanine5.5 anti-mouse CD45(Cat # 103132)
<https://www.fishersci.com/shop/products/percp-cy5-5-anti-mouse-cd45-ab/NC0217834#?keyword=103132>
 FITC anti-mouse Ly-6G(Cat # 127606)
<https://www.fishersci.com/shop/products/fitc-anti-mu-ly-6g-2/50164390#?keyword=127606>

Eukaryotic cell lines

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

Cell line source(s)	HEK293(CRL-1573), HT22(CRL-3595), and MLO-Y4 (CRL-12424) cell lines were purchased from ATCC Cell Bank(https://www.atcc.org/), Primary neurons cells were isolated from postnatal (2-5 day old) mice.
Authentication	The cell lines used were originally authenticated by the ATCC, but were not re-authenticated after purchase.
Mycoplasma contamination	All cell lines were routinely tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

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	All animal experimental protocols were approved by the Animal Care and Use Committee of the Southern Medical University, Guangzhou, China (SMUL2020141). No author was a member of this committee. Animal care was conducted in accordance with the guidelines of the US National Institutes of Health and the Chinese National Institute of Health. Male C57BL/6J mice (12 weeks) were purchased from Guangdong Weitong Lihua Laboratory Animal Technology Co. Ltd. (Foshan, China). Male NOD/SCID mice (12 weeks) were purchased from Hunan SLaik Jingda Laboratory Animal Co. Ltd. (Changsha, China). Sost-Cre/ERT2 mice were purchased from Shanghai Model Organisms Center, Inc. (Shanghai, China). Piezo1-flox mice were from the UCDAVIS KOMP repository (strain ID: Piezo1 tm1a). Conditional KO mice were produced by interbreeding Piezo1fl/fl mice with Sost-Cre/ERT2 mice and Piezo1fl/fl mice littermates were used as controls for all experiments. Mice were utilized and housed in plastic cages under a controlled temperature of 22 ± 1°C and a 12-h light/dark cycle. The mice were provided with standard rodent chow and water ad libitum throughout the duration of the study. Only male mice were used to avoid estrogen fluctuation interference as previously reported. Male Bama miniature pigs (4 months) were obtained from Taizhou Taihe Biotechnology Co., Ltd. (Taixing City, China). All animal experimental procedures were reviewed and approved by the Laboratory Animal Ethics Committee of South China Agricultural University (2024H028). All experimental animals were purchased from a qualified supplier with a laboratory animal production license, and all experiments were performed in laboratories holding a valid laboratory animal use license. The animal care and experimental protocols strictly complied with the 3R principles and animal welfare requirements.
Wild animals	The study did not involve wild animals.
Reporting on sex	male mice was included in all analyses reported in this manuscript.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Animal studies were conducted in accordance with the guidelines set by the Ministry of Science and Technology of China, after approval by the Ethical Committee for Animal Research at Southern Medical University (Guangzhou, China).

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	The mice were perfused with cold Hank's balanced salt solution and brains were collected and digested with a Neural Tissue Dissociation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) using a gentleMACS™ Octo Dissociator with heaters (Miltenyi Biotec). Brain immune cells were enriched with Percoll® medium (GE Healthcare, Chicago, IL, USA), blocked with rat anti-mouse CD16/32 (BioLegend, San Diego, CA, USA), and then incubated with the following fluorescence-conjugated antibodies: peridinin chlorophyll protein-cyanine 5-conjugated antibody against CD45 (BioLegend), allophycocyanin- cyanine 7-conjugated antibody against CD11b (BioLegend), Alexa Fluor® 700-conjugated antibody against Ly6C (BioLegend), and fluorescein isothiocyanate-conjugated antibody against Ly6G (BioLegend). Stained cells were analyzed on BD LSRFortessa™ X-20 Cell Analyzer (BD Biosciences, Franklin Lakes, NJ, USA). The data were analyzed with FlowJo software (BD Biosciences).
Instrument	BD LSRFortessa™ X-20 Cell Analyzer (BD Biosciences, Franklin Lakes, NJ, USA)
Software	FlowJo software (BD Biosciences).
Cell population abundance	N/A
Gating strategy	N/A

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

Magnetic resonance imaging

Experimental design

Design type	Resting state
Design specifications	T2-weight imaging of porcine brain tissue 4 weeks after TBI
Behavioral performance measures	N/A

Acquisition

Imaging type(s)	Structural
Field strength	3T
Sequence & imaging parameters	Spin-echo images from 22 continuous slices in the axial plane were acquired using the following parameters: sequences: repetition time (TR) =2700 ms; echo time (TE) =100 ms; slice thickness=6 mm, FOV=250x250 mm ² ; MTX=200x180.
Area of acquisition	N/A
Diffusion MRI	☐ Used ☒ Not used

Preprocessing

Preprocessing software	N/A
Normalization	N/A
Normalization template	N/A
Noise and artifact removal	N/A
Volume censoring	N/A

Statistical modeling & inference

Model type and settings	N/A
Effect(s) tested	N/A
Specify type of analysis:	☐ Whole brain ☒ ROI-based ☐ Both
Anatomical location(s)	N/A
Statistic type for inference	N/A
(See Eklund et al. 2016)	
Correction	N/A

Models & analysis

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