

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

- | | | |
|-------------------------------------|-------------------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | 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> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | A description of all covariates tested |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | 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) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | 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

Zeiss Imaging Software (2.6) and Zeiss LSM700 and LSM880 was used for confocal image acquisition.
Beckman Coulter Moflo XDP cell sorter was used for flow cytometry.
Roche LightCycler 96 Real-Time PCR system was used for Real-Time PCR analysis.
LI-COR Odyssey® XF Imaging System was used for western blotting.

Data analysis

GraphPad Prism (v7.0e), Zeiss Imaging Software (2.6), FlowJo (v10.5.3), LightCycler 96 SW 1.1 Real-Time PCR Analysis, R (4.0), RStudio (1.4.1717), STAR (v2.7.6a), Reference Component Analysis (RCA) (2.0), pheatmap (version 1.0.12), DESeq2 (version 1.32.0), ggplot2 (version 3.3.5), 10xGenomics Cell Ranger (version 5.0.0), Seurat V3, org.Dr.eg.db (3.11.4), DAVID Bioinformatics Resources 5.8 (<https://david.ncifcrf.gov/home.jsp>), IMARIS (v9.3), Image J (v1.53e), BioRender (<https://biorender.com>), photoshop CS6, Adobe Illustrator CC 2019.

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 data that support the findings of this study, including experimental animals, antibodies, vector constructions, and chemicals, are available within the article, its

Supplementary information, or from the corresponding author upon reasonable request. Data from the bulk and single-cell RNA-seq analyses have been deposited in Sequence Read Archive (SRA) with the accession number PRJNA660936 (<https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA660936>) and PRJNA661142 (<https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA661142>) and NCBI Gene Expression Omnibus (GEO) with the accession number GSE183340 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE183340>).

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	It is generally considered that a sample size containing at least 3 independent replicates can provide adequate statistical power in biochemical analysis. We have described the exact sample size for each experiment in our manuscript.
Data exclusions	No data captured was excluded from the subsequent analyses.
Replication	The exact number of replication for all experiments was described in figure legends and our attempts at replication were successful.
Randomization	Samples and animals were allocated randomly.
Blinding	The experimenters were blinded to the animal genotype, grouping information and data 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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used

The following antibodies were used for immunoblotting or immunostaining: goat anti-GFP (Abcam, #ab6658, 1:400), mouse anti-DsRed (Santa Cruz, #sc-101526, 1:400), rabbit anti-DsRed (Takara Bio Clontech, 632496, 1:400), rabbit anti-Lcp1 (GTX124420, GeneTex, 1:400), mouse anti-zBax (This paper, 1:400), mouse anti-Bax (B-9) (Santa Cruz, #sc-7480, 1:200), rabbit anti-SOX2 (Abcam, #ab94959, 1:200), rabbit anti-CaMKII (pan) (Cell Signalling technology, #4436, 1:400), rabbit anti-phospho-CaMKII (Thr286) (Cell Signalling technology, #12716, 1:400), rabbit anti-CREB (Abcam, #ab32515, 1:400), rabbit anti-CREB (phospho S133) (Abcam, #ab32096, 1:400), rabbit anti-NICD1a (Abcam, #ab83232, 1:400), rabbit anti-DLL3 (Abcam, #ab103102, 1:200), rabbit β -catenin (Merck Millipore, #06-734, 1:400), Rat anti-F4/80 (Abcam, #ab6640, 1:100), goat anti-IBA1 (Abcam, #ab5076, 1:200), mouse anti-Hes1 (Abcam, #ab119776, 1:400), rabbit anti-activated Notch1 (Abcam, #ab8925, 1:400), rabbit anti-Rbpj (BOSTER, #A00767-1, 1:400), rabbit Anti- Active Caspase-3 (BD Biosciences, #559565, 1:400), mouse anti-PCNA (Sigma-Aldrich, P8825, 1:800), mouse anti-FLAG (Sigma-Aldrich, #F1804, 1:400), mouse anti- β tubulin (Thermo Fisher Scientific, #MA5-11732, 1:1000), FITC anti-mouse/human CD11b antibody (Elabscience, #E-AB-F1081C, 1:20), Alexa Fluor 700 anti-mouse CD45 antibody (BioLegend, #103128, 1:50), 488 Goat Anti-Rat IgG (Abcam, ab150165, 1:400), Alexa Fluor 488 Donkey Anti-Goat IgG (Thermo Fisher Scientific, #A-11055, 1:400), Alexa Fluor 555 Donkey Anti-Rabbit IgG (Thermo Fisher Scientific, #A-31572, 1:400), Alexa Fluor 555 Donkey Anti-Mouse IgG (Thermo Fisher Scientific, #A-31570, 1:400), Alexa Fluor 647 Donkey Anti-Mouse IgG (Thermo Fisher Scientific, #A-31571, 1:400), Goat anti-Mouse IgG (Thermo Fisher Scientific, #31430, 1:1000), and Goat anti-Rabbit IgG (Thermo Fisher Scientific, #31460, 1:1000)

Validation

All antibodies except for mouse anti-zBax were commercial in origin and validated by the company. Information regarding validation processes can be easily accessed on the company's web site using the product numbers listed above. Additionally, we validated antibodies: rabbit anti-NICD1a (Abcam, #ab83232, 1:400), rabbit anti-DLL3 (Abcam, #ab103102, 1:200), rabbit anti-CREB (phospho S133) (Abcam, #ab32096, 1:400), and rabbit anti-phospho-CaMKII (Thr286) (Cell Signaling Technology, #12716, 1:400), by western

blot. The validation results were presented in Supplementary fig. 2d, 4e, 5i, 7a, and 7h. The related description was included in the Methods section. The detailed information about a monoclonal antibody, mouse anti-zBax, against zebrafish Bax antigen has been provided in our Methods section. The appropriate dilution for all the antibodies was determined through preliminary experiments.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

Zebrafish embryos were kept at a constant temperature (28.5°C) on an incubator.

According to the purpose of each experiment, different transgenic and mutant zebrafish lines were used in this study:

Tg(*coro1a*:DsRed), Tg(*coro1a*:Kaede), Tg(*mfap4*:GFP), Tg(*apoeb*:GFP), Tg(*tp1bglob*:eGFP), Tg(*tp1bglob*:hmgbl1-mCherry), Tg(*hsp70*:DN-MAML-GFP), Tg(*HuC*:GCaMP6s), Tg(*HuC*:GFP), Tg(*HuC*:dlb), Tg(NBT:DenNTR), Tg(NBT:creb1a), Tg(NBT:bax), Tg(*hsp70*:bax), Tg(*coro1a*:bax), Tg(*coro1a*:NICD1a), Tg(*ubiq*:secAnnexinV-mVenus), and Tg(*coro1a*:DN-MAML-FLAG), *mibta52b* mutant, *baxacq55* mutant, *baxΔ1/Δ1* mutant, *baxΔ58/Δ58* mutant, and *dlb*^{-/-} mutant. AB and WIK strains were used as control and as a background line for experiments. 52 hpf, 55 hpf, 2 dpf, 2.5 dpf, 3 dpf, 4 dpf, and 6 dpf zebrafish were used for experiments.

Mice were bred at 23°C, with 40-60% relative humidity, 12hrs light cycle (8am-8pm).

According to the purpose of each experiment, WT C57BL/6J (The Jackson Laboratory, #000664), Bax⁺/tm1Sjk (The Jackson Laboratory, #002994), RBP-J(f/f) (The Jackson Laboratory Stock, #034200) and Cx3cr1-Cre (The Jackson Laboratory Stock, #025524) mice were used and both genders were used in the entire study. E12.5 and E14.5 mice embryos was used.

Wild animals

No wild animals involved in this study.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All animal experiments were approved by the Institutional Review Board of Southwest University (Chongqing, China).

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

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

Zebrafish microglia isolation: The green-kaede+ cells in 3 dpf siblings and Bax cq55 Tg(*coro1a*:Kaede) zebrafish brain were labeled, and whole brain were dissected for isolating labeled *coro1a*-kaede+ cells to do bulk RNA seq and RNA extraction by REPLI-g WTA Single Cell Kit (QIAGEN, #150063). The brain of 4 dpf WT Tg(*coro1a*:DsRed) zebrafish was dissected for DsRed+ microglia isolation to do single cell RNA seq.

Zebrafish neuron isolation: The whole brain of 3 dpf siblings and Bax cq55 Tg(*HuC*:GFP) zebrafish brain were dissected to isolate GFP+ neurons to do bulk RNA seq and RNA extraction by REPLI-g WTA Single Cell Kit (QIAGEN, #150063). The whole brain of 55 hpf WT Tg(*HuC*:GFP) zebrafish brain were dissected to isolate GFP+ neurons to perform transplantation assay.

To prepare single cell suspensions. Zebrafish were anesthetized with 0.02% tricaine. 200 brains were freshly isolated in ice-cold dulbecco's phosphate-buffered saline (dPBS) and incubated with papain (10U/mL) at 30°C for 20 min. After centrifuging with 800 rpm and a brief washing, the digested tissues were re-suspended with 5% FBS (dissolved in dPBS) and passed through 1.2 μm and 0.5 μm glass pipette 7 times, respectively. Then, the samples were filtered through a 40 μm Cell Strainer to gain single cell suspension for FACS sorting.

Mice microglia isolation: Whole E12.5 or E14.5 mouse embryonic brains, excluding olfactory bulbs, cerebella and spine cords, were dissected for microglia isolation. The brains were minced to ~1mm³ with scissors under a dissecting microscope. Brain tissues were digested by papain at 37°C for around 40 min, and then centrifuged with 800 rpm at 4°C for 5 min. Cell pellets were washed by dBS, resuspended in 5 mL 5% FBS, passed through 1.2 μm and 0.5 μm glass and pushed through a 40 μm cell strainer. Cell suspensions were stained with CD11b antibody for FACS sort to do primary microglia culture in vitro. Cell suspensions were stained with CD11b and CD45 antibody for FACS analysis and RNA extraction by REPLI-g WTA Single Cell Kit (QIAGEN, #150063).

Instrument

Beckman Coulter Moflo XDP cell sorter

Software

FlowJo Software (10.5.3) of Beckman Coulter Moflo XDP cell sorter was used for cell sorting.

Cell population abundance

About 3x10⁴ *coro1a*-DsRed+ cells in zebrafish larval brains were collected by Moflo XDP cell sorter with 99% of purity and prepared to conduct single-cell sequencing. About 500 *HuC*-GFP+ neurons and 100 photo-converted red *coro1a*-Kaede+ microglial cells in zebrafish larval brains were sorted by Beckman Coulter Moflo XDP cell sorter with 99% of purity, to isolate total RNA by REPLI-g WTA Single Cell Kit and establish the cDNA libraries by Anoroda corporation for Bulk RNA Sequencing. About 1x10⁶ *HuC*-GFP+ cells in zebrafish larval brains were collected by Beckman Coulter Moflo XDP cell sorter with 99% of

purity and prepared to perform zebrafish transplantation. About 6×10^4 CD11b⁺ primary microglia were isolated from E12.5 embryonic mice cerebral cortexes (largely immature microglia at embryonic stages) by Beckman Coulter Moflo XDP cell sorter with 95% of purity and prepared for culture in vitro. About 4×10^4 CD11bhiCD45lo E14.5 mouse brains microglia were sorted by Beckman Coulter Moflo XDP cell sorter with 99% of purity and prepared for FACS analysis. About 1000 CD11bhiCD45lo E14.5 mouse brains microglia were sorted by Beckman Coulter Moflo XDP cell sorter with 99% of purity and prepared for RNA extraction by REPLI-g WTA Single Cell Kit.

Gating strategy

Gating strategy for microglia isolated from 4 dpf WT Tg(*coro1a*:DsRed) transgenic zebrafish brain samples as presented in Supplementary Fig. 4f. Samples of mouse brains microglia were gated accordingly to specific gene markers (CD11bhiCD45lo), refer to Methods for details. Gating strategies for FACS sorted cells are shown in Supplementary fig. 8e and 10a.

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