nature portfolio

Corresponding author(s): Jiamin Miao

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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 (*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	All relevant data supporting the findings of this study are available within the article and its supplementary information files. The single-cell RNA-seq dataset used for the analysis (GSE208766) is publicly available from the Gene Expression Omnibus (GEO) database. Further datasets generated or analyzed during the current study are available from the corresponding author upon reasonable request.
Data analysis	All relevant data supporting the findings of this study are available within the article and its supplementary information files. The single-cell RNA-seq dataset used for the analysis (GSE208766) is publicly available from the Gene Expression Omnibus (GEO) database. Further datasets generated or analyzed during the current study are available from the corresponding author upon reasonable 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 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 supports the findings of this study are available in the manuscript and supplementary materials.

The sequencing data generated in this study have been deposited in the National Center for Biotechnology Information (NCBI) under BioProject accession number PRJNA1325451. The raw sequencing reads are available in the Sequence Read Archive (SRA) under the following accession numbers:
Sham group: SRR35334752, SRR35334751, SRR35334750
CCI group: SRR35334749, SRR35334748, SRR35334747
CCI+TF/PS/TDP-43-IN-1 group: SRR35334746, SRR35334745, SRR35334744
All data are publicly accessible through the NCBI SRA database.

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	Not applicable – this study did not involve human participants.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable – this study did not involve human participants.
Population characteristics	Not applicable – this study did not involve human participants.
Recruitment	Not applicable – this study did not involve human participants.
Ethics oversight	Not applicable – this study did not involve human participants.

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 were determined based on previous studies and standard practices for behavioral and molecular assays in mouse neuropathic pain models. At least five mice per group were used, which was considered sufficient to detect statistically significant differences. No formal sample size calculation was performed.
Data exclusions	No data were excluded from the analyses unless animals died due to non-protocol-related causes. All exclusion criteria were pre-established.
Replication	All key experiments were independently replicated at least three times with similar results. Biological and technical replicates were included to confirm reproducibility.
Randomization	Mice were randomly assigned to experimental groups using a computer-generated randomization protocol. Randomization was applied during group allocation before surgery and drug administration.
Blinding	Investigators were blinded to group allocation during data collection and analysis, particularly for behavioral tests and immunofluorescence quantification. Blinding was maintained until the completion of data processing.

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	Primary antibodies: rabbit anti-C3 (ab97462, Abcam), rabbit anti-S100A10 (NBP1-89370, Bio-Techne), mouse anti-CD86 (NBP2-25208, Bio-Techne), rabbit anti-CD206 (NBP1-90020, Bio-Techne), rabbit anti-Iba1 (ab178846, Abcam), rabbit anti-GFAP (NB300-141, Bio-Techne), etc. Full list in manuscript.
Validation	Antibodies were validated for use in mouse tissues by the suppliers. Specificity confirmed by Western blot and immunofluorescence. Validation data are available on manufacturer websites and match prior published data.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Primary astrocytes and microglia were isolated from neonatal C57BL/6J mouse brains (sex not specified).
Authentication	Not applicable – primary cultures were used, not established cell lines.
Mycoplasma contamination	Cells were cultured under sterile conditions; no mycoplasma testing was performed.
Commonly misidentified lines (See ICLAC register)	Not applicable – no established or 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	Male C57BL/6J mice, 8 weeks old, obtained from Zhejiang University Laboratory Animal Center. Neonatal mice were used for primary cell isolation.
Wild animals	Not applicable – no wild animals were used in this study.
Reporting on sex	Only male mice were used for in vivo experiments. The sex of neonatal mice used for cell culture was not specified.
Field-collected samples	Not applicable – no field-collected samples were used.
Ethics oversight	All animal experiments were approved by the Ethics Committee of Sir Run Run Shaw Hospital, Zhejiang University School of Medicine. Protocols followed the guidelines of the International Association for the Study of Pain.

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

Plants

Seed stocks	Not applicable – this study did not involve any plant materials.
Novel plant genotypes	Not applicable – this study did not involve any plant materials.
Authentication	Not applicable – this study did not involve any plant materials.

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	Primary astrocytes and microglia were isolated from neonatal C57BL/6J mice, cultured for 14–21 days, treated with DiR-labeled liposomes, digested with trypsin, and resuspended in PBS before flow cytometry.
Instrument	BD FACSCanto II (BD Biosciences, USA) was used for data acquisition.
Software	FlowJo v10.0.7 (Tree Star, USA) was used for analysis.
Cell population abundance	The percentage of DiR-positive cells among total astrocytes or microglia was quantified. Purity was confirmed with GFAP (Ast) and CD68 (Mic) immunostaining.
Gating strategy	Gating was based on FSC/SSC for size/granularity, followed by DiR-positive gating to quantify liposome uptake. Negative controls (no DiR) and compensation controls were used.

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