nature portfolio

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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	N/A
Data analysis	All RNA seq and SnRNA seq analysis was performed in R; The pipeline will be deposited on Github

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

Bulk and SnRNA-seq datasets generated in this study are in the process of being uploaded to GEO and will be available at acceptance.
This paper does not report original code.
Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Power analysis was performed to determine the sample size
Data exclusions	No data was excluded in the study.
Replication	All key findings were reproduced in three independent experiments (N=3) except bulk and SnRNA seq experiments where minimum of n=3 is used.
Randomization	Plate layout and acquisition order were randomized for the kinase screen and flow cytometry screen to control potential batch or positional effects.
Blinding	Blinding was not relevant in our study design due to objective and quantitative nature of the experimental assays and the use of automated analysis pipelines.

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	GFAP (Thermo Fisher Scientific, 13-0300) β-Tubulin (Thermo Fisher Scientific, MAB1637) IBA1 (FUJIFILM Wako, 019-19741) ICAM-1 (Abcam, Ab17123) VCAM1 (Abcam, Ab134047) ICOSL (Thermo Fisher Scientific, Ab2852462) BST2 (Abcam, Ab88523) β-actin (Cell

Signaling Technology, 8H10D10) HSP27 (R&D Systems, AF1580) pHSP27 (R&D Systems, AF2314) CD38-BV711 (BioLegend, 303527, clone HIT2) CD82-PE-Cy7 (BioLegend, 342109, clone ASL-24) CD275/ICOSL-PE (BioLegend, 329806, clone 9F.8A4) CD317-APC (BioLegend, 348410, clone RS38E) CD106-BV421 (BioLegend, 305815, clone STA) CD54-FITC (BioLegend, 353107, clone HA58) anti-ICAM1 (clone G-5; Santa Cruz Biotechnology, sc-8439) anti-C3 (Invitrogen, PA5-21349)

Validation

All antibodies used in this study were validated for immunocytochemistry and immunohistochemistry in human cells and tissue samples, as confirmed by the manufacturers. Specificity was supported by:
1- Morphologically and spatially appropriate staining in target cell types
2- Manufacturer validation and published studies using the same antibodies
3- Secondary -only controls were used to confirm specificity in selected experiments

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Human Primary Gibco Astrocytes (Gibco, K1884) Human Primary ScienCell Astrocytes (ScienCell, 1800), Human ReNcell VM (Millipore, SCC008), iAstrocytes 1.0 (Fujifilm, 01434), iMotor Neurons (Fujifilm, 01279), iGlutamatergic Neurons (Fujifilm, 01279), iMicroglia (Fujifilm, 01279)
Authentication	All cell lines used in the study were authenticated by short tandem repeat (STR) profiling by the respective vendor. Primary cells were maintained for a limited number of passages (as specified in the methods section) to minimize genetic drift.
Mycoplasma contamination	Routine mycoplasma contamination testing was performed . Only mycoplasma negative cultures were used for experiments.
Commonly misidentified lines (See ICLAC register)	No such lines were used in the study.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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	Human primary Gibco and ScienCell astrocytes were dissociated with Accutase (3–5 min, 37°C), washed, and resuspended in FACS buffer (PBS supplemented with 2% fetal bovine serum and 2 mM EDTA). Unstimulated cells were labeled with CellTrace™ Far Red (Thermo Fisher Scientific, C34572) following the manufacturer's instructions. Equal numbers of labeled unstimulated and unstimulated ITC-treated cells were mixed at a 1:1 ratio prior to antibody staining. Cells were incubated with PE-conjugated antibodies from the LEGENDScreen™ Human PE kit (BioLegend, 700011) for 20 min at 4°C, washed, and resuspended in FACS buffer before acquisition.
Instrument	BD LSRII or ZE5 flow cytometers
Software	FlowJo v10
Cell population abundance	Across all screening and validation experiments, the total number of events collected per well ranged from 10,000-20,000 single live cells.

Gating strategy	Initial gating excluded debris and doublets by forward scatter (FSC) and side scatter (SSC) parameters. Live cells were gated based on viability dye exclusion (if used) or scatter properties. CellTrace™ Far Red labeling enabled discrimination between unstimulated and stimulated populations. Single, live cells were gated before analysis of marker expression. Gates for positivity were set using fluorescence-minus-one (FMO) controls or unstained controls where applicable. MFI ratios between unstimulated and stimulated populations were used to assess differential marker expression

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