

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	Zen Black Version 14.0.23.201 MetaSystems VSViewer software version: v 2.1.112 SlideBook - Intelligent Imaging Innovations, Inc. (3i) Leica Application Suite X MAPS software
Data analysis	ImageJ2 Version 2.3.0/1.53f ImageJ2 Version 2.16.0/1.54p for the TUNEL analysis ImageJ2 Version 2.3.0/1.53f for the Nfh/MBP analysis Dragonfly 3D world software (Gaussian filter for denoising and CLAHE filter for contrast improvement) Illumina BCL Convert 4.1.5 pipeline for single cell sequencing scDbfFinder Cell Counter plugin in FIJI NIS Elements, version 5.42.07 Metamorph software (Molecular Devices): v 7.10.5.476 CellRanger DecontX Scater Seurat scDbfFinder

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](#)

All data supporting the findings of this study are available within the paper and its Supplementary Information. RNA-seq data that support the findings in this study have been deposited in the Gene Expression Omnibus (GEO) under accession number GSE303406. Previously published RNA-seq data referenced in this study and compared to our dataset were downloaded from <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE279183>, GEO GSE279183.

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	Sex of donors acknowledged in the methods but not incorporated into analyses.
Reporting on race, ethnicity, or other socially relevant groupings	No race or ethnicity grouping noted.
Population characteristics	Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."
Recruitment	Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.
Ethics oversight	Identify the organization(s) that approved the study protocol.

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	No sample-size calculation was performed but, to the best of our capacity, we sampled across fields of view, across organoids, across independent differentiations and across independent iPSC lines to account for variability to a level that matches, if not exceeds, what is commonly seen in the literature.
Data exclusions	No data were excluded.
Replication	Data were replicated in a second iPSC line to verify the reproducibility of findings.
Randomization	Each well of a 6 well plate (containing 16 organoids) was randomised to LPC or Ethanol and then organoids were further randomised for individual analyses.
Blinding	Investigators were blinded to experimental group during data accrual.

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

Biolegend Cat Code: #368507 Clone: 2D1 Lot: B286627 5 ug/mL
 Biolegend Cat Code: #982604 Clone: ICRF44 Lot: B336395 10 ug/mL
 Biolegend Cat Code: #392113 Clone: S16001E Lot: B351221 20 ug/mL
 Biolegend Cat Code: #341607 Clone: 2A9-1 Lot: B338961 5 ug/mL
 BioLegend Cat Code: #822601 clone: Poly28226 Lot: B384954 1:10000
 Abcam Cat Code: AB7349 Lot 1015269-14 1:1000
 Abcam Cat Code: Ab155279 Lot: 1014930-22 1:300
 Abcam Cat Code: Ab218400 Clone: FTL/1386 Lot: GR3361785-4 1:1000
 Abcam Cat Code: Ab783 Clone: PG-M1 Lot: GR3371622-2 1:200
 Abcam Cat code: #ab66110 1:20
 Abcam Cat code: #ab185966 Clone: EPR14335-78 1:200
 Abcam Cat Code: #ab104225 1:500
 Wako Cat Code: 019-19741 Lot: WTF4691 1:300
 Atlas Cat Code: AMAB91072 Clone: CL2887 Lot: MAB04122 1:2000
 Dako Cat Code: M0775 Clone: CR3/43 Lot: 20034792 1:1000
 Synaptic Systems Cat Code: #234-004 Lot: 2-26 1:1000
 Merck Cat Code: #MABN69 Clone: K65/35 Lot: 3823691 1:300
 Merck Cat Code: #AB144P Lot number: 3854469 1:250
 Swant Cat Code: #PV27 1:500
 Thermo Cat Code: #36-4500 Lot: AA406895 1:75
 Cell Signaling Technologies Cat Code: #5241 Clone: D13C6 1:200

Validation

Catalog #: AF2864 Verified reactivity in humans as per https://www.rndsystems.com/products/human-sox10-antibody_af2864
 Catalog # ab185966 Verified in human, mouse, rat as per <https://www.abcam.com/en-us/products/primary-antibodies/sox9-antibody-epr14335-78-ab185966?srsltid=AfmBOorza-sGq4kKNGOmriPl-q1Tn-eJ5Bm78mkWG6EUAtUbxrY6KleQ#application=ihc-p>
 #MABN69: Verified reactivity in Rat as per <https://www.sigmaaldrich.com/US/en/product/mm/mabn69#product-documentation>
 Catalog #AB144P Verified reactivity with Av, Ch, Gp, H, M, Mk, R, Op as per <https://www.sigmaaldrich.com/US/en/product/mm/ab144p#product-documentation>
 Catalog #PV27 Verified reactivity in human, mouse, rat, zebrafish, domestic sheep, rhesus macaque as per <https://www.labome.com/product/SWant/235.html>
 Catalog #822601 Verified reactivity in Human, Rat, Mouse, Cat, Chicken as per <https://www.biolegend.com/en-gb/products/purified-anti-neurofilament-h-nf-h-antibody-11474>
 Catalog #36-4500 Verified reactivity in Human, Mouse as per <https://www.thermofisher.com/antibody/product/Claudin-11-Antibody-Polyclonal/36-4500>
 Catalog #ab66110 Verified reactivity in mammals as per https://www.abcam.com/en-us/products/assay-kits/tunel-assay-kit-brdu-red-ab66110?srsltid=AfmBOop7rsmUqkhRe6zHCl_p56yqZhlJkst7LjhqjDCd0EOqm9PnhW0#tab=datasheet
 Catalog #ab104225 Verified in humans, Mouse, rat as per https://www.abcam.com/en-us/products/primary-antibodies/neun-antibody-neuronal-marker-ab104225?srsltid=AfmBOop4hm-SXfGvD_TS-SGRJCWjJfkzoE4OVxEhAJiwGtLiFk7ytu#
 Catalog #5241 Verified in humans as per <https://www.cellsignal.com/products/primary-antibodies/pdgf-receptor-a-d13c6-xp-rabbit-mab/5241?srsltid=AfmBOor4BY6PWOj54v7iPucEhuXsrbtob41Vrhxm2Bl9xGgnkGmtr0qp>
 Catalog #: AF2864 Verified reactivity in humans as per https://www.rndsystems.com/products/human-sox10-antibody_af2864
 Catalog # ab185966 Verified in human, mouse, rat as per <https://www.abcam.com/en-us/products/primary-antibodies/sox9-antibody-epr14335-78-ab185966?srsltid=AfmBOorza-sGq4kKNGOmriPl-q1Tn-eJ5Bm78mkWG6EUAtUbxrY6KleQ#application=ihc-p>
 Catalog #MABN69: Verified reactivity in Rat as per <https://www.sigmaaldrich.com/US/en/product/mm/mabn69#product-documentation>
 Catalog #AB144P Verified reactivity with Av, Ch, Gp, H, M, Mk, R, Op as per <https://www.sigmaaldrich.com/US/en/product/mm/ab144p#product-documentation>
 Catalog #PV27 Verified reactivity in human, mouse, rat, zebrafish, domestic sheep, rhesus macaque as per <https://www.labome.com/product/SWant/235.html>
 Catalog #822601 Verified reactivity in Human, Rat, Mouse, Cat, Chicken as per <https://www.biolegend.com/en-gb/products/purified-anti-neurofilament-h-nf-h-antibody-11474>
 Catalog #36-4500 Verified reactivity in Human, Mouse as per <https://www.thermofisher.com/antibody/product/Claudin-11-Antibody-Polyclonal/36-4500>

Eukaryotic cell lines

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

Cell line source(s)	18a iPSC (female) was provided by a collaborator and was reprogrammed from fibroblasts. C005 (female) was reprogrammed from fibroblasts within our laboratory under full ethics approval. PB001 iPSC (male) was provided by a collaborator and was reprogrammed from PBMCs.
Authentication	all iPSC lines have been authenticated and published previously 18a: DOI:10.1038/nbt.1783 PB001: DOI:10.1016/j.scr.2018.101380 C005: DOI: 10.1038/s41593-025-02118-7
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	<i>Name any commonly misidentified cell lines used in the study and provide a rationale for their use.</i>

Plants

Seed stocks	<i>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.</i>
Novel plant genotypes	<i>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.</i>
Authentication	<i>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, mosaicism, off-target gene editing) were examined.</i>

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	CBA array: Sample preparation was performed according to the manufacturer's instructions. Briefly, the supernatant from iPSC-derived microglial-like cells was incubated with IL-6, IL-10, TNF- α , and IL-13 capture beads and then with the PE detection reagent. Protein standards and compensation beads were also prepared as per the manufacturer's instructions. Microglial phenotyping: iPSC-derived macrophage-like cells were thawed, washed and resuspended in FACS buffer and incubated with antibody. Organoids were dissociated using papain as per kit protocol (Worthington) and washed in FACS buffer prior to incubation in primary antibody.
Instrument	Beckman Coulter CytoFLEX S Becton Dickinson FACS Aria III
Software	FlowJo 10.10.0
Cell population abundance	The abundance of our tdT+ cell population in organoids was 1.3%
Gating strategy	CBA array: Step 1: To eliminate debris, gated on SSC-H Vs FSC-H Step 2: To identify bead populations, gated by FL7-H vs FL19-H. Step 3: Software automatically detected MFI on FL12-H Microglial phenotyping: Step 1: To eliminate debris, SSC-A Vs FSC-A Step 2: FSC-W Vs FSC-H for doublet discrimination Step 3: SSC-W Vs SSC-H to ensure single cells

Step 4: Cell isolations without TdTomato+ cells +/- antibodies to ensure no autofluorescence and reduce the risk of false positives

Step 5: Quadrant gates were drawn with tdTomato only positive cells as our cell population of interest

Step 6: Percentages of markers of interest were calculated by considering the percentage of double positive cells to the total tdTomato cells.

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