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	EVOS FL Auto 2 Software Revision 2.0.2094.0, Illumina NovaSeq 6000 System, Keyence BZ-X800 epifluorescence microscope, Akoya Biosciences PhenoCycler system
Data analysis	R 4.1, ArchR 1.0.1, Seurat 4.1, Cell Ranger ARC 2.0.2, Matlab 2020b, Signac 1.8, SpatialGlue 1.0, QuPath 0.5.1, FigR 1.0, clusterProfiler 4.0.5, cell2location 0.1.3 , mgcv_1.9 , Python 3.9, cellchat 2.1, NICHES 1.0.0, Cellpose 3.1 Scripts for data analysis were written in R and python with code available at Github (https://github.com/di-0579/spatial_tri-omics) and archived at Zenodo (https://zenodo.org/records/17121652)

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 raw and processed data have been deposited in the Neuroscience Multi-omic Data Archive (NeMO; https://assets.nemoarchive.org/col-1w5kt2k), and the mouse subset is also deposited in the Gene Expression Omnibus with accession code GSE308623. These datasets are available as a web resource and can be browsed within the tissue spatial coordinates in our own data portal: https://spatial-omics.yale.edu/.CODEX data have been deposited at Zenodo (https://zenodo.org/records/17121652).

The resulting fastq files were aligned to the human reference genome (GRCh38) (https://hgdownload.soe.ucsc.edu/goldenPath/hg38/chromosomes/) or mouse reference genome (GRCm38) (https://hgdownload.soe.ucsc.edu/goldenPath/mm10/chromosomes/). Published data for integration and quality comparison are available online: Atlas of gene regulatory elements in adult mouse cerebrum (http://catlas.org/mousebrain/#!/downloads); atlas of the adolescent mouse brain (http://mousebrain.org/adolescent/downloads.html); developing mouse brain data (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE110823); mouse brain (https://assets.nemoarchive.org/dat-qwqfftg); CNS inflammation (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE130119); and atlas of adult mouse brain (https://assets.nemoarchive.org/dat-qg7n1b0); FANTOM5 ligand–receptor 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	Sex and gender were not used in any scenario as criteria for sample collection. All human samples are de-identified in this research.
Reporting on race, ethnicity, or other socially relevant groupings	No race, ethnicity, or other socially relevant groupings were performed in this study.
Population characteristics	We used de-identified tissue samples and no population characteristics other than age were used in this research.
Recruitment	De-identified tissue samples were collected with previous patient consent in strict observance of the legal and institutional ethical regulations. This was performed by the clinic and no recruitment criteria were used. This research don't take sex into consideration so the sex won't provide bias for this research.
Ethics oversight	Human Gamete, Embryo and Stem Cell Research Committee (Institutional Review Board) at the University of California, San Francisco; Institutional Review Board at the University of Maryland Brain & Tissue Bank

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 directly relevant. No sample size calculation was performed. Samples sizes were chosen primarily based on experiment length, sample availability, and sequencing costs.
Data exclusions	No data were excluded from the study.
Replication	All attempts at replication were successful. At least two replicates have been done for each time point for developing and LPC mouse brains. Human tissue was processed once per stage.
Randomization	Randomization was performed to assign samples to time-point groups.
Blinding	Experimental blinding was implemented during retro-AAV-eGFP image 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
☐	☒ 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

Anti-H3K27me3 antibody (clone number: C36B11), 9733, Cell Signaling Technology;
Secondary antibody (Guinea Pig anti-Rabbit IgG (Heavy & Light Chain) Antibody), ABIN101961, Antibodies-Online;
Anti-PDGFRα antibody (clone number: EPR22059-270), ab234965, Abcam;
Anti-Olig2 antibody (clone number: EPR2673), ab220796, abcam;
Anti-APC antibody (clone number: EP701Y), ab239828, abcam;
Anti-Myelin Basic Protein antibody (clone number: EPR21188), ab230378, abcam;
Anti-Iba1 antibody (clone number: EPR16588) - BSA and Azide free, ab220815, abcam;
Anti-GFAP antibody (clone number: EPR1034Y) - BSA and Azide free, ab218309, abcam;
Anti-Ctip2 antibody (clone number: EPR23120-25) - BSA and Azide free, ab269367, abcam;
Anti-CUX1+CUX2 antibody (clone number: EPR26509-154) - BSA and Azide free, ab309140, abcam;
Anti-TBR1 antibody (clone number: EPR8138(2)) - BSA and Azide free, ab239000, abcam;
Anti-NeuN antibody (clone number: EPR12763) - BSA and Azide free, ab209898, abcam;
Anti-Myelin oligodendrocyte glycoprotein antibody (clone number: EPR22629-310) - BSA and Azide free, ab255266, abcam;
Anti-SATB2 antibody (clone number: EPNCIR130A) - BSA and Azide free, ab212177, abcam;
TotalSeq™-A Mouse Universal Cocktail, V1.0, AB_2910353 (BioLegend Cat. No. 199901);
TotalSeq™-A Human Universal Cocktail, V1.0, AB_2888692 (BioLegend Cat. No. 399907);
CD31 antibody (clone number: MEC13.3)–Akoya Biosciences, Cat# 4250001.
CD4 antibody (clone number: RM4-5)–Akoya Biosciences, Cat# 4250016.
CD8a antibody (clone number: 53-6.7)– Akoya Biosciences, Cat# 4250017
CD19 antibody (clone number: 6D5)–Akoya Biosciences, Cat# 4250014.
CD45R/B220 antibody (clone number: RA3-6B2)–Akoya Biosciences, Cat# 4450006.
CD11c antibody (clone number: N418) – Akoya Biosciences, Cat# 4550108.
Ki67 antibody (clone number: B56)–Akoya Biosciences, Cat# 4250019
CD11b antibody (clone number: M1/70)–Akoya Biosciences, Cat# 4450015
LY6G antibody (clone number: 1A8)– Akoya Biosciences, Cat# 4550110.
CD3 antibody (clone number: 17A2) —Akoya Biosciences, Cat# 4550109.
CD169 antibody (clone number: 3D6.112) –Akoya Biosciences, Cat# 4550100.

Validation

All antibodies used have been validated by the manufacturer.
Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb #9733, Cell Signaling Technology, citation: Nat Cancer 3(9):1071-1087 (2022); Nat Commun 13(1):5883 (2022); Cancer Discov 12(7):1760-1781 (2022), et al. Please refer to manufacturer's description: https://www.cellsignal.com/products/primary-antibodies/tri-methyl-histone-h3-lys27-c36b11-rabbit-mab/9733
Guinea Pig anti-Rabbit IgG (Heavy & Light Chain) Antibody - Preadsorbed, Antibodies-Online, According to the manufacturer's description: ABIN101961 is tested via ELISA to ensure that the titer against the antigen (Rb IgG) is above a certain threshold. We also test to make sure the titer against potentially cross-reactive human IgG, goat IgG, and mouse IgG is below a certain threshold. In addition, we test ABIN101961 against anti-guinea pig Serum, rabbit IgG, and rabbit serum in an immunoelectrophoresis assay. Please refer to manufacturer's description: https://www.antibodies-online.com/antibody/101961/Guinea+Pig+anti-Rabbit+IgG+Heavy++Light+Chain+antibody+-+Preadsorbed/
Anti-PDGFR alpha antibody [EPR22059-270] - BSA and Azide free, abcam. Please refer to manufacturer's description: https://www.abcam.com/en-us/products/primary-antibodies/pdgfr-alpha-antibody-epr22059-270-bsa-and-azide-free-ab234965
Anti-Olig2 antibody [EPR2673] - BSA and Azide free, abcam. Please refer to manufacturer's description: https://www.abcam.com/en-us/products/primary-antibodies/olig2-antibody-epr2673-bsa-and-azide-free-ab220796
Anti-APC antibody [EP701Y] - BSA and Azide free, abcam. Please refer to manufacturer's description: https://www.abcam.com/en-us/products/primary-antibodies/apc-antibody-ep701y-bsa-and-azide-free-ab239828
Anti-Myelin Basic Protein antibody [EPR21188] - BSA and Azide free, abcam. Please refer to manufacturer's description: https://www.abcam.com/en-us/products/primary-antibodies/myelin-basic-protein-antibody-epr21188-bsa-and-azide-free-ab230378
Anti-Iba1 antibody [EPR16588] - BSA and Azide free, abcam. Please refer to manufacturer's description: https://www.abcam.com/en-us/products/primary-antibodies/iba1-antibody-epr16588-bsa-and-azide-free-ab220815
Anti-GFAP antibody [EPR1034Y] - BSA and Azide free, abcam. Please refer to manufacturer's description: https://www.abcam.com/

en-us/products/primary-antibodies/gfap-antibody-epr1034y-bsa-and-azide-free-ab218309
Anti-Ctip2 antibody [EPR23120-25] - BSA and Azide free, abcam. Please refer to manufacturer's description: https://www.abcam.com/en-us/products/primary-antibodies/ctip2-antibody-epr23120-25-bsa-and-azide-free-ab269367
Anti-CUX1+CUX2 antibody [EPR26509-154] - BSA and Azide free, abcam. Please refer to manufacturer's description: https://www.abcam.com/en-us/products/primary-antibodies/cux1cux2-antibody-epr26509-154-bsa-and-azide-free-ab309140
Anti-TBR1 antibody [EPR8138(2)] - BSA and Azide free, abcam. Please refer to manufacturer's description: https://www.abcam.com/en-us/products/primary-antibodies/tbr1-antibody-epr81382-bsa-and-azide-free-ab239000
Anti-NeuN antibody [EPR12763] - BSA and Azide free, abcam. Please refer to manufacturer's description: https://www.abcam.com/en-us/products/primary-antibodies/neun-antibody-epr12763-bsa-and-azide-free-ab209898
Anti-Myelin oligodendrocyte glycoprotein antibody [EPR22629-310] - BSA and Azide free, abcam. Please refer to manufacturer's description: https://www.abcam.com/en-us/products/primary-antibodies/myelin-oligodendrocyte-glycoprotein-antibody-epr22629-310-bsa-and-azide-free-ab255266
Anti-SATB2 antibody [EPNCIR130A] - BSA and Azide free, abcam. Please refer to manufacturer's description: https://www.abcam.com/en-us/products/primary-antibodies/satb2-antibody-epncir130a-bsa-and-azide-free-ab212177
For all the abcam antibodies, the manufacturer's description is as follow: We have tested this species and application combination and it works. It is covered by our product promise.
CD31 antibody – Anti-Mu CD31(AKYP0002)-BX002—Atto 550 for PhenoCycler (Formerly CD31-BX002 (MEC13.3)—Atto 550-RX002), Akoya Biosciences, Cat# 4250001. Please refer to manufacturer's description : https://my.akoyabio.com/ccrz__ProductDetails?sku=4250001
CD4 antibody – Anti-Mu CD4(AKYP0041)-BX026—Atto 550 for PhenoCycler (Formerly CD4-BX026 (RM4-5)—Atto 550-RX026), Akoya Biosciences, Cat# 4250016. Please refer to manufacturer's description : https://my.akoyabio.com/ccrz__ProductDetails?sku=4250016
CD8a antibody – Anti-Mu CD8a(AKYP0044)-BX029—Atto 550 for PhenoCycler (Formerly CD8a-BX029 (53-6.7)—Atto 550-RX029), Akoya Biosciences, Cat# 4250017. Please refer to manufacturer's description : https://my.akoyabio.com/ccrz__ProductDetails?sku=4250017
CD19 antibody – Anti-Mu CD19(AKYP0033)-BX020—Atto 550 for PhenoCycler (Formerly CD19-BX020 (6D5)—Atto 550-RX020), Akoya Biosciences, Cat# 4250014. Please refer to manufacturer's description : https://my.akoyabio.com/ccrz__ProductDetails?sku=4250014
CD45R/B220 antibody – Anti-Mu CD45R/B220(AKYP0014)-BX010—Alexa Fluor™ 750 for PhenoCycler (Formerly CD45R/B220-BX010 (Ra3-6B2)—Alexa FluorTM 750-RX010), Akoya Biosciences, Cat# 4450006. Please refer to manufacturer's description : https://my.akoyabio.com/ccrz__ProductDetails?sku=4450006
CD11c antibody – Anti-Mu CD11c(AKYP0045)-BX030—Alexa Fluor™ 647 for PhenoCycler (Formerly CD11c-BX030(N418)—Alexa Fluor™ 647-RX030) (Replaces 4350013 CD11c-BX030 (N418)—Cy5-RX030), Akoya Biosciences, Cat# 4550108. Please refer to manufacturer's description : https://my.akoyabio.com/ccrz__ProductDetails?sku=4550108
Ki67 antibody – Anti-Hu/Mu Ki67(AKYP0052)-BX047—Atto 550 for PhenoCycler (Formerly Ki67-BX047 (B56)—Atto 550-RX047), Akoya Biosciences, Cat# 4250019. Please refer to manufacturer's description : https://my.akoyabio.com/ccrz__ProductDetails?sku=4250019
CD11b antibody – Anti-Mu CD11b(AKYP0040)-BX025—Alexa Fluor™ 750 for PhenoCycler (Formerly CD11b-BX025 (M1/70)—Alexa FluorTM 750-RX025), Akoya Biosciences, Cat# 4450015. Please refer to manufacturer's description : https://my.akoyabio.com/ccrz__ProductDetails?sku=4450015
LY6G antibody – Anti-Mu Ly6g(AKYP0039)-BX024—Alexa Fluor™ 647 for PhenoCycler (Formerly Ly6g-BX024(1A8)—Alexa Fluor™ 647-RX024) (Replaces 4350015 Ly6g-BX024(1A8)—Cy5-RX024), Akoya Biosciences, Cat# 4550110. Please refer to manufacturer's description : https://my.akoyabio.com/ccrz__ProductDetails?sku=4550110
CD3 antibody – Anti-Mu CD3(AKYP0035)-BX021—Alexa Fluor™ 647 for PhenoCycler (Formerly CD3-BX021(17A2)—Alexa Fluor™ 647-RX021) (Replaces 4350014 CD3-BX021(17A2)—Cy5-RX021), Akoya Biosciences, Cat# 4550109. Please refer to manufacturer's description : https://my.akoyabio.com/ccrz__ProductDetails?sku=4550109
CD169 antibody – Anti-Mu CD169(AKYP0015)-BX015—Alexa Fluor™ 647 for PhenoCycler (Formerly CD169-BX015(3D6.112)—Alexa Fluor™ 647-RX015) (Replaces 4350005 CD169-BX015(3D6.112)—Cy5-RX015), Akoya Biosciences, Cat# 4550100. Please refer to manufacturer's description : https://my.akoyabio.com/ccrz__ProductDetails?sku=4550100

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	The present study followed some applicable aspects of the PREPARE planning guidelines checklist such as the formulation of the in vivo study, dialogue between scientists and the animal facility, and quality control of the in vivo components in the study. All animals were born, bred, and housed at Karolinska Institutet, Comparative Medicine Biomedicum animal facility (KMB). Mouse brain tissues (postnatal days P0, P2, P5, P7, P10, and P21) were obtained from a mouse line generated by crossing Sox10:Cre animals (The Jackson Laboratory mouse strain 025807) on a C57BL/6j genetic background with RCE:loxP (eGFP) animals (The Jackson Laboratory mouse strain 32037-JAX) on a C57BL/6xCD1 mixed genetic background. Females with a hemizygous Cre allele were mated with males lacking the Cre allele, while the reporter allele was kept in hemizygosity in both females and males. In the resulting Sox10:Cre-RCE:LoxP (eGFP) progeny the entire OL lineage was labelled with eGFP. None of the experimental animals in this study were subjected to previous procedures before enrollment in the study. All animals were free from mouse viral pathogens, ectoparasites endoparasites, and mouse bacteria pathogens. Mice were kept with the following light/dark cycle: dawn 6:00-7:00, daylight 7:00-18:00, dusk 18:00-19:00, night 19:00-6:00 and housing to a maximum number of 5 per cage in individually ventilated cages (IVC Sealsafe plus GM500, Tecniplast, Italy). General housing parameters such as relative humidity, temperature, and ventilation follow the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes treaty ETS 123, Strasbourg 18.03.1996/01.01.1991. Briefly, consistent relative air humidity of 55%±10, 22 °C and the air quality is controlled with the use of stand-alone air handling units supplemented with HEPA filtrated air. Monitoring of husbandry parameters is done using ScanClime® (Scanbur) units. Cages contained hardwood bedding (TAPVEI, Estonia), nesting material, shredded paper, gnawing sticks and card box shelter (Scanbur). The mice received a regular chow diet (CRM(P) SDS, United Kingdom and CRM(P), SAFE, Augy, France). Water was provided by using a water bottle, which was changed weekly. Cages were changed every other week. Cage changes were done in a laminar air-flow cabinet (NinoSafe MCCU mobile cage changing unit) provided with a HEPA H14 EN1822 filter (0.3 um particle size). Facility personnel wore dedicated scrubs, socks and shoes. Respiratory masks were used when working outside of the laminar air-flow cabinet. Both sexes were included in the study.

Wild animals	No wild animals were used in the study.
Reporting on sex	We don't take sex into consideration in this research. Animals of both sexes were used for experiments.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	All experimental procedures on animals were performed following the European directive 2010/63/EU, local Swedish directive L150/SJVFS/2019:9, Saknr L150, Karolinska Institutet complementary guidelines for procurement and use of laboratory animals, Dnr. 1937/03-640 and Karolinska Institutet Comparative Medicine veterinary guidelines and plans (version 2020/12/18). The procedures described were approved by the regional committee for ethical experiments on laboratory animals in Sweden (Stockholms Norra Djurförsöksetiska Nämnd, Lic. nr. 1995/2019 and 7029/2020).

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

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.*