

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	BD FACSDiva Software, version 8.0.2; Nextseq 2000 and NovaSeq platforms (Illumina); Leica Application Suite X (version 1.8.0); Vizgen software (version 233b).
Data analysis	R (version 4.3.2); CellRanger (version 7.2.0); DoubletFinder (version 2.0.4); Seurat (version 5.2.0); Harmony (version 0.1.162); BBMap (version 38.33; sourceforge.net/projects/bbmap/); Bowtie (version 5.2.1); UMI-tools (version 0.5.1); Vizgen software (version 233b); Cellpose (version 2.0); Solo (doi: 10.1016/j.cels.2020.05.010); MapMyCells (RRID:SCR_024672); ImageJ/Fiji (version 1.54g); Custom codes: https://github.com/guohua-git/p4-brain-lineage .

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](#)

scRNA-seq transcriptomic data and STICR barcode data are available at the Gene Expression Omnibus (GEO; <https://www.ncbi.nlm.nih.gov/geo/>) under accession number GSE341480. An interactive browser of single-cell data and MERFISH data can be found at the University of California, Santa Cruz (UCSC) cell browser <https://p4-brain-lineage.cells.ucsc.edu>. The MERFISH dataset is available at BIL (<https://api.brainimagelibrary.org/web/view?bildid=ace-let-lax>).

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](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	No statistical method was used to pre-determine sample sizes. Suitable sample size was estimated based on those reported in previous publications in the field [22,23].
Data exclusions	No data were excluded from the analyses, except for low-quality cells were removed for further analysis.
Replication	The number of times that each experiment was repeated independently with similar results were provided in the figure legends and supplementary table 1.
Randomization	For all animal experiments, mice were allocated randomly. For scRNA-seq and MERFISH experiments, no randomization was performed as they do not involve multiple study groups.
Blinding	Imaging data were analyzed by blinded researchers. Investigators were not blind to the genotypes during experiments, because no multiple study groups involved in this study.

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

Methods

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

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Primary antibodies in this study include: Rat anti-BrdU antibody (1:200, ab6326, Abcam), Rabbit anti-NEUROD1 antibody (1:200, ab109224, Abcam), Mouse anti-DLX2 antibody (1:50, sc-393879, Santa Cruz biotechnology), Rabbit anti-TBR1 antibody (1:500, ab183032, Abcam), and Mouse anti-SATB2 antibody (1:200, sc-81376, Santa Cruz biotechnology). Secondary antibodies in this study include: donkey anti mouse 647 (1:500, A31571, Thermo), donkey anti rabbit 594 (1:500, A11012, Thermo), and donkey anti rat 488(1:500, A21208, Thermo).
Validation	All the antibodies used in the study have been used and validated in the previous studies.

Eukaryotic cell lines

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

Cell line source(s)	Lenti-X HEK293T cells (Takara Bio, 632180)
Authentication	Cell lines were not authenticated.
Mycoplasma contamination	Cell lines were tested by PCR and no mycoplasma contamination was found.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines 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	C57BL/6 wild-type females were purchased from Charles River Laboratories(California, United States). All animals are maintained at the University of California, San Francisco. For timed pregnant mice (8-15 weeks), the plug date was designated as E0. Pregnant dams were kept in single cages and pups before P21 were kept with their mothers until collection. Mice were housed in the institutional animal facility under standard conditions, with a 12-h light/12-h dark cycle, an ambient temperature of 23±2 °C, and a relative humidity of 30–70%.
Wild animals	No wild animal was used.
Reporting on sex	Both of male and female mice were analyzed.
Field-collected samples	No field-collected sample was used.
Ethics oversight	All mouse experiments were performed in accordance with the protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, San Francisco.

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, mosaicism, 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	Brains were dissected in ice-cold pre-bubbled artificial cerebrospinal fluid (aCSF), and sectioned into coronal sections. Each brain region was manually dissected out from sliced brains based on the guidance of anatomy atlas from Allen institute. Collected tissue was then dissociated in the solution of papain (Worthington, LK003178) supplemented with 0.5 mg/ml DNase (Sigma Aldrich, #DN25-1G) diluted with HBSS (Gibco, 14025134) at 37C. The tissues were incubated at 37C for 30-60 min, with gentle trituration every 15 min until samples had no visible chunks. Papain was quenched by adding 1%BSA (Militeny) to the solution. Samples were spun down and resuspended in HBSS + 0.1% BSA and passed through the 35 um filter into a FACS tube (Falcon # 352235). To isolate and collect virally infected cells, flow cytometry was done using a BD FACSAria III Cell Sorter (BD FACSDiva Software, version 8.0.2) with a 100-µm nozzle. The cell suspensions were first gated on forward scatter, then within this population based on eGFP expression. eGFP-expressing cells were sorted into HBSS + 0.1% BSA for downstream processing on the 10x Genomics Chromium platform. Samples were kept on ice before and after FACS, and the chamber and collection tubes were maintained at 4C during sorting.
Instrument	BD Aria Cell Sorter
Software	BD FACSDiva Software, version 8.0.2; FlowJo, version 10.8.1
Cell population abundance	the live cells sorted out for sequencing are more the 90% of total cells.
Gating strategy	The cell suspension is first gated based on FSC and SSC to select cell out from debris, then the GFP positive population is considered as live cells to be sorted out.

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