nature portfolio

Corresponding author(s): Paola Arlotta

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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	Imaging software Zen Blue (ZEN2 .6 - blue edition, Zeiss; Nikon Spinning disk) was used . Imaging Software Zen Blue(ZEN2.6 and ZEN blue 3.1 - blue edition, Zeiss Confocal LSM 90 0 inverted) was used. ImageJ2 (2. 14.0/ 1. 54f, 2. 16.0/ 1. 54p) was used to process images for quantification Nuclei segmentation and quantification ZEN blue 3.1 Image Analysis with add-on Zen Intellesis software packages (no version number). Electron microscopy (EM) images were obtained using a FEI Magellan Scanning Electron Microscope (Thermo Fisher Scientific) equipped with custom image acquisition software (WaferMapper).
Data analysis	Cell Ranger version 7.2.0 was used to process raw scRNA-seq data into count matrices. Cell Bender v0.3.0 was used to remove systematic biases and background noises by taking Cell Ranger's raw count matrices as inputs. The command-line tool demuxlet vl.0, souporcell v2.5, and demuxafy v2.0.l were used to identify the genotype/cell line of individual cells in scRNA-seq datasets. Downstream scRNA-seq analysis was performed in R version 4.2.2, using packages Seurat v. 4.3.0/5.1.0, harmony version 1.2.0, DIALOGUE 1.0, lme4 v. 1.1.33, emmeans version 1.8.5, stats v. 4.2.2, robCompositions version 2.3.1, edgeR v. 3.40.2, DESeq2 version 1.38.3 and clusterProfiler version 4.6.2. Scripts written by the authors for data analysis is available at https://github.com/tfaits/Arlotta_Lab_LongTermOrganoids. WGBS reads were aligned to the reference genome using BSMAP (v2.90). Methylation rates were called using mcall from MOABS package (vl.3.9.6). DMRs were called using metilene (v0.2-8). DMRs were annotated to nearest genes using GREAT (v4.0.4). DNA methylation age was performed with MethylClock package (vl.10.0).

Automatic synaptic detection in EM images was performed using VAST Lite and a deep neural network (U-Net architecture; Ronneberger et al., 2015) implemented
via the mEMbrain software package in MATLAB (Pavarino et al., 2023).
ImageJ2 (2.16.0/1.54p) was used to process the images and quantify the number of cells that were SATB2 and C-FOS positive.
Electrical signals were recorded using the Accura 3D CMOS-HD-MEA system by 3Brain (BioCAM DupleX system)
Spike detection was carried out by Kilosort4, followed by analysis of the spike train using custom MATLAB scripts (version 24.1, R2024b, The MathWorks, Inc.).

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

Code Availability
ode used during data analysis is available at GitHub (https://github.com/tfaits/Arlotta_Lab_LongTermOrganoids), with the exception of custom code used for MEA analysis, which is available upon
request.

Data Availability
Novel raw scRNA-seq data that support the findings of this study are available through dbGaP, accession number <pending>, along with processed data from one organoid from the CW50037 cell line. Raw data from four organoids from the Mito210 cell line cannot be publicly shared due to limitations of donor consent. Novel processed scRNA-seq data that support the findings of this study are available at the Broad Single Cell Portal (SCP) (singlecell.broadinstitute.org, study ID SCP3697), and via the Gene Expression Omnibus (GEO) (ncbi.nlm.nih.gov/geo, with accession number GSE333707), with the exception of processed data from one organoid from the CW50037 cell line, which is included in the dbGaP submission due to limitations of donor consent, and four organoids from the Mito210 cell line, which cannot be publicly shared due to limitations of donor consent. Processed WGBS data that support the findings of this study are available at GEO, with accession number GSE333708. Raw WGBS data is available upon request to the authors. Also see Supplementary Table 1.

Previously published data that support the findings of this study are available as follows: Processed data from Anton Bolanos et al., 2024 (DOI: 10.1038/s41586-024-07578-8) at the Broad SCP with study ID SCP2609, and raw data at Synapse.org with accession number syn52132869. Processed data from Uzquiano et al., 2022 (DOI: 10.1016/j.cell.2022.09.010) are all at the Broad SCP with study ID SCP1756, and raw data at Synapse.org with accession number syn26346373. Processed data from Paulsen et al., 2022 (DOI: 10.1038/s41586-021-04358-6) are available at the Broad SCP with study ID SCP1129, and raw data at Synapse.org with accession number syn26346373. Processed data from Velasco et al., 2019 (DOI: 10.1038/s41586-019-1289-x) are available at the Broad SCP with study ID SCP282, and raw data can be accessed from GEO at accession GSE129519. Processed data from Trevino et al., 2021 (DOI: 10.1016/j.cell.2021.07.039) from GEO at accession GSE162170. Processed data from Velmeshev et al., 2023 (DOI: 10.1126/science.adf0834) from the UCSC Cell Browser at cells.ucsc.edu/?ds=pre-postnatal-cortex+all+rna. Processed data from Wang et al., 2025 (DOI: 10.1038/s41586-024-08351-7) from the UCSC Cell Browser at cell.ucsf.edu/snMultiome. Postnatal DLPFC methylation data from Price et al., 2019 (DOI: 10.1186/s13059-019-1805-1) from Synapse.org with accession syn5842535. (Also see Supplementary Table 1.)

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	In this study, 6 lines were used in total, 5 male lines (4 hiPSC, and 1 ESC) and 1 female hiPSC line: Psychiatric control Mito210 male iPS cell line The GM08330 male iPS cell line. The HI male hES cell line (also known as WA0I). The IIa male iPS cell line The PGPI male iPS cell line The CW50037 iPS cell line All features regarding the human pluripotent stem cell lines are summarized in Methods. No active enrollment has been performed for this study.
Reporting on race, ethnicity, or other socially relevant groupings	No active enrollment has been performed for this study.
Population characteristics	HI (male, embryonic stem cell [ESC]), Mito210 (male,induced pluripotent stem cell [iPSC], PGPI (male, iPSC, control),GM08330 (hereafter 'GM'; male, IIa (male, iPSC, control), and CW50037 (female, iPSC control).
Recruitment	No active recruitment has been performed for this study.
Ethics oversight	All experiments involving human cells were approved by the Harvard University IRB and ESCRO committees.

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 size was not predetermined using specific methods; instead, at least three individual organoids were utilized for each experiment when possible, guided by the analysis from previous published work (https://www.nature.com/articles/s41586-021-04358-6#Sec1, https://www.nature.com/articles/s41586-019-1289-x, https://www.cell.com/ce11/pdf/S0092-8674(22)01168-0.pdf). This prior research demonstrated the reproducibility of organoids and confirmed that using three organoids adequately captured the variation.
Data exclusions	Individual cells were excluded from single-cell analyses if they expressed fewer than 200 genes, had fewer than 500 or more than 20,000 UM ls, or if mitochondrial RNA comprised greater than 15% of UM ls. Cells were also removed when they were determined to be ambiguous droplets or heterogenous doublets by demuxlet, and doublets or unassigned droplets by souporcell. These filtering thresholds were established prior to data collection and processing. scRNA-seq profiles from organoids without matched genetic backgrounds between CDM4 and APM culture conditions at 6, 9, 12, 18 months were excluded for data representation and downstream analysis.
Replication	scRNA-seq (data source indicated unless de nova generated in this study): CDM4-treated organoids: 15 days: Anton-Bolanos et al. (2024): MD (Multi-Donor)-Chimeroids (n = 1) 23 days: Uzquiano et al. (2022): PGPl (n = 3), Mito210 (n = 3) 1 month: Paulsen et al. (2022): Mito210 (n = 6) 1.5 months: Uzquiano et al. (2022): PGPl (n = 3), Mito210 (n = 3) 2 months: Uzquiano et al. (2022): PGPl (n = 3), Mito210 (n = 3) 3 months: Anton-Bolanos et al. (2024): SD (Single-Donor)-Chimeroids (n = 25 replicates) Uzquiano et al. (2022): PGPl (n = 6) 4 months: Uzquiano et al. (2022): PGPl (n = 3), Mito210 (n = 3) 5 months: Uzquiano et al. (2022): PGPl (n = 3), Mito210 (n = 3) 6 months: MD (Multi-Donor)-Chimeroids (n = 2) Velasco et al. (2019): PGPl (n = 5) Paulsen et al. (2022): Mito210 (n = 3) Hl (n = 2) 9 months: GM (n = 2), Hl (n = 2), PG Pl (n = 2), lla (n = 4) 12 months: Hl (n = 2), PGPl (n = 2) 18 months: lla (n = 2), PGPl (n = 2), Hl (n = 2) 24 months: lla (n = 2), PGPl (n = 2) 36 months: lla (n = 2) 42 months: lla (n = 2) 60 months: lla (n = 1), PGPl (n = 1) APM-treated organoids: 4 months: Hl (n = 1), lla (n = 1) 6 months: Hl (n = 4), PGPl (n= 3), Mito210 (n = 2) 9 months: PGPl (n = 2), lla (n = 1) 12 months: Hl (n = 2), PGPl (n = 1) 18 months: Hl (n = 2) Monochronic Chimeroids (10-15 organoids pooled for each replicate): 9 months: lla/H1/CW (n=1), GM (n=1) 11 months: GM (n=l pool from 15-20 organoids) Heterochronic Chimeroid (25 organoids pooled): 9 months: GM/PGPl (n=1) Reproducibility: detail information can be found in Methods under "Cluster annotation and data integration". Replication: All results obtained and reported in this study were reproducible across the replicates examined. The number of replicates per experiment has been described in the figures, legends, tables, main text or supplementary materials.
Randomization	Organoids used for analysis or treatment were chosen from each batch to be representative of the morphology seen in that differentiation. For every experiment organoids were collected without a preconceived selection strategy or priority.
Blinding	The investigators did not use blinding in the study. However, our analytical pipeline for each experiment followed uniform criteria applied to all samples, allowing us to analyze our data in an unbiased manner. All bioinformatics analyses were applied uniformly to all samples without considering genotype adjustments.

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 cFOS {Cell signaling 312545,Monoclonal [clone: E2I7R], Lot:3, 1/100)
Chicken GFAP {MilliporeSigma AB5541,Polyclonal, Lot: 3843668, 1/500)
Rabbit Cryab {Abeam AB13496,Monoclonal [clone: 1B6.1-3G4] Lot: 1013789-11, 1/100)
Chicken MAP2 {Abeam AB5392,Polyclonal, Lot: 1012833-1, 1/5,000)
Mouse SATB2 {Abeam AB51502, Monoclonal [clone: SABTA4B10] Lot: GR273053-7, 1/50)
Goat SOX2 {RD Systems AF2018,Polyclonal, Lot: KOY0622061, 1/50)
Rabbit TBRI {Abeam AB31940,Polyclonal, Lot: 1015782-1, 1/500)
Goat Sox9 {R&D Biotechne AF3075,Polyclonal, Lot: WIL0522101, 1/1000)
Mouse NEFH { Invitrogen 13-1300,Monoclonal [clone: RMdO-20], Lot:XC348907, 1/100)
Alexa Fluor" 647 Anti-SATB2 antibody { Abcam (ab196536), Monoclonal [clone: EPNCIR130A], Lot:1033844-9, 1/200)
Rat MBP {MilliporeSigma MAB386, Monoclonal [clone: 12], Lot:3510774, 1/100)
Alexa Fluor" 488 anti-RBFOX3 (NeuN) Antibody (RRID: AB_2927957, Clone W18230A; Biolegend, 1/200)
Rat MAbl {Hypoxyprobe Inc, Monoclonal, HPl-100, lot: 1.3.25, 1/50)
Secondary antibodies:
All secondary antibodies were diluted 1/1,000
Alexa Fluor donkey anti-rabbit 647, 546 (Life Technologies A31573, A10040)
Alexa Fluor donkey anti-mouse 546 (Life Technologies A10036)
Alexa Fluor Goat anti-mouse 647 (ThermoFisher Scientific A21241)
Alexa Fluor donkey anti-goat 488 (Life Technologies A21121)
Alexa Fluor donkey anti-rat 546 (Life Technologies A21208)
Alexa Fluor donkey anti-chicken 488, 647 {Jackson lmmunoResearch Laboratories 703-545-155, Millipore AP194SA6)
Alexa Fluor goat anti-mouse 647 (ThermoFisher Scientific, A21241)
Alexa Fluor anti-rat, 488 {Jackson lmmunoResearch Laboratories, 712-545-153 ,1/1000)

Validation

According to the manufacturer's website:

The chicken MAP2 polyclonal antibody (Abeam, AB5392) is reactive to human, and validated for use in ICC/IF, IHC-P and WB and has been cited in 681 publications.It's performance in IHC and Immunofluorescence in human, mouse and rat samples is trusted by the scientific community. https://www.abcam.com/en-us/products/primary-antibodies/map2-antibody-neuronal-marker-ab5392

The mouse SATB2 monoclonal antibody (Abeam, AB51502) is reactive to human and has been cited in 269 publications. The specificity SATB2 antibody (ab51502) has been confirmed by testing in knockout sample https://www.abcam.com/en-us/products/primary-antibodies/satb1-satb2-antibody-satba4b10-c-terminal-ab51502

The goat SOX2 polyclonal antibody {Isotype: IgG, Manufacturer: R&D Systems, AF2018) is reactive to humans and has been cited in 186 publications. Has been validated by for use in Immunohistochemistry, Western Blot, Immunocytochemistry, Simple Western, Chromatin Immunoprecipitation (ChIP). https://www.rndsystems.com/products/human-mouse-rat-sox2-antibody_af2018

The rabbit TBR1 polyclonal antibody {isotype: IgG, Manufacturer: Abcam AB31940) is reactive to humans and has been cited in 425 publications.Has been validated for use in IHC-P, WB. https://www.abcam.com/en-us/products/primary-antibodies/tbr1-antibody-ab31940

The Goat Sox9 polyclonal antibody {Isotype: IgG, Manufacturer: R&D Biotechne AF3075) is reactive to humans, has been cited in 326 publications, and has been validated for use in Western Blot, Immunocytochemistry, Simple Western. https://www.bio-techne.com/p/antibodies/human-sox9-antibody_af3075#product-specifications

The rabbit cFOS monoclonal antibody (Cell signaling 312545) is reactive to human and has been cited in 6 publications. The antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits and can be used for Western Blotting, Simple Western, Immunoprecipitation. Immunohistochemistry (Paraffin), Immunofluorescence (Frozen), Immunofluorescence

(Immunocytochemistry), and Chromatin IP. https://www.cellsignal.com/products/primary-antibodies/c-fos-e2i7r-rabbit-monoclonal-antibody/31254

The chicken GFAP polyclonal antibody (MilliporeSigma AB5541) is reactive to human, and has been cited in 333 publications. It is suitable for Immunohistochemistry(paraffin) , Immunocytochemistry, Western blot, and Immunohistochemistry. https://www.sigmaaldrich.com/US/en/product/mm/ab5541#product-documentation
The mouse NEFH monoclonal antibody {clone: RMdO-20, Manufacturer: Invitrogen 13-1300) is reactive to humans and has been cited in 15 publications. It is suitable for Western Blot, Immunohistochemistry, Immunohistochemistry (IHC (P)), Immunohistochemistry (IHC (F)), Immunocytochemistry (ICC/IF) 1 ELISA (ELISA) and Immunoprecipitation (IP).https://www.thermofisher.com/antibody/product/NEFH-Antibodyclone-RMdO-20-Monoclonal/13-1300.

The conjugated mouse SATB2 monoclonal antibody {Clone: EPNCIR130A, Manufacturer: Abcam, ab196536) is reactive to human and has been cited in 2 publications. Can be used for ICC/IF, Flow Cyt (Intra), and IHC-P. https://www.abcam.com/en-us/products/primary-antibodies/alexa-fluor-647-satb2-antibody-epncir130a-ab196536

The rat MBP monoclonal antibody {Clone: 12, MilliporeSigma, MAB386) is reactive to human and has been cited in 314 publications. It is suitable for ELISA, immunocytochemistry, immunohistochemistry, radioimmunoassay, and western blot. https://www.sigmaaldrich.com/US/en/product/mm/mab386

The rabbit Cryab monoclonal antibody {Clone: 1B6.1-3G4, Manufacturer: Abcam, AB13496) is reactive to human and has been cited in 56 publications. Validated for use in Western Blot (WB), Flow Cytometry (Flow Cyt), Immunohistochemistry (IHC-P) in Cow, Human samples.https://www.abcam.com/en-us/products/primary-antibodies/alpha-b-crystallin-antibody-1b61-3g4-ab13496

The conjugated rat NeuN monoclonal antibody {Clone: W18230A, Manufacturer: Biolegend, 608456) is reactive to mouse and human, and has been cited in 2 publications. It has been applied for IHC-F and IHCP. https://www.biolegend.com/en-ie/products/alexa-fluor-488-anti-rbfox3-neun-antibody-25085

The Rat monoclonal antibody {Hypoxyprobe Inc, HPI-100), has been cited in Uzquiano et al., 2022(DOI:10.1016/j.cell.2022.09.010). http://www.hypoxyprobe.com/hp1-100kit.html

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	The psychiatric control Mito210 male iPS cell line was provided by B. Cohen (Mclean Hospital); the PG Pl male iPS cell line was provided by G. Church (Harvard University); the GM08330 male iPS cell line was provided by M. Talkowski (MGH) and was originally derived from fibroblasts obtained from the Coriell Institute for Medical Research. The Hl male hESC line (also known as WA0l) was purchased from WiCell; and the lla male iPS cell line was obtained from the Harvard Stem Cell Institute. The female CW50037 iPSC line was from the California Institute for Regenerative Medicine (CIRM) iPSC collection.
Authentication	The PGPl line was authenticated using STR analysis completed by TRIPath (2018). The 11a cell line was authenticated by karyotyping as described in (Quadrato et al., 2017a). The Hl and GM08330 was authenticated by STR analysis (performed by WiCell in 2021). As previously described, the GM08330 parental line has an interstitial duplication in the long (q) arm of chromosome 20 (Paulsen et al., Nature, 2022). The Mito210 lines were authenticated by genotyping analysis (Fluidigm FPVS chip) performed by the Broad Institute Genomics Platform (2017). The CW50037 iPSC line was authenticated with SNP Genotyping (lllumina Global Screening Array (GSA) from lllumina; processed at the Genomics Platform, Broad Institute) for cell line identification and detection of chromosomal abnormalities
Mycoplasma contamination	All human PSC cultures were negative for mycoplasma (assayed with MycoAlert PLUS Mycoplasma Detection Kit, Lonza).
Commonly misidentified lines (See ICLAC register)	No misidentified lines were used in this study.

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	To develop the Mouse-endogenous Chimeroid system we used wild-type CD-1 and C57BL/6 mice from Charles River Laboratories. We used embryos of both sexes collected at embryonic stages (E) Ell.5. Mice were maintained under standard housing conditions in a temperature- and humidity-controlled facility on a 12 h light/12 h dark cycle (7AM-7PM) with ad libitum access to food and water. Ambient temperature was maintained at 20–24°C and relative humidity at 30–70%.
Wild animals	The study did not involve wild animals.
Reporting on sex	Findings do not apply to one sex. Male and Female embryos were used for analysis. In Mouse-endogenous chimeroids experiments, the individual sex of each embryo was not determined prior to the experiment.
Field-collected samples	The study did not involve field-collected animals
Ethics oversight	All animals were handled according to protocols approved by the Institutional Animal Care and Use Committee (IACUC-1106) of Harvard University.

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.*