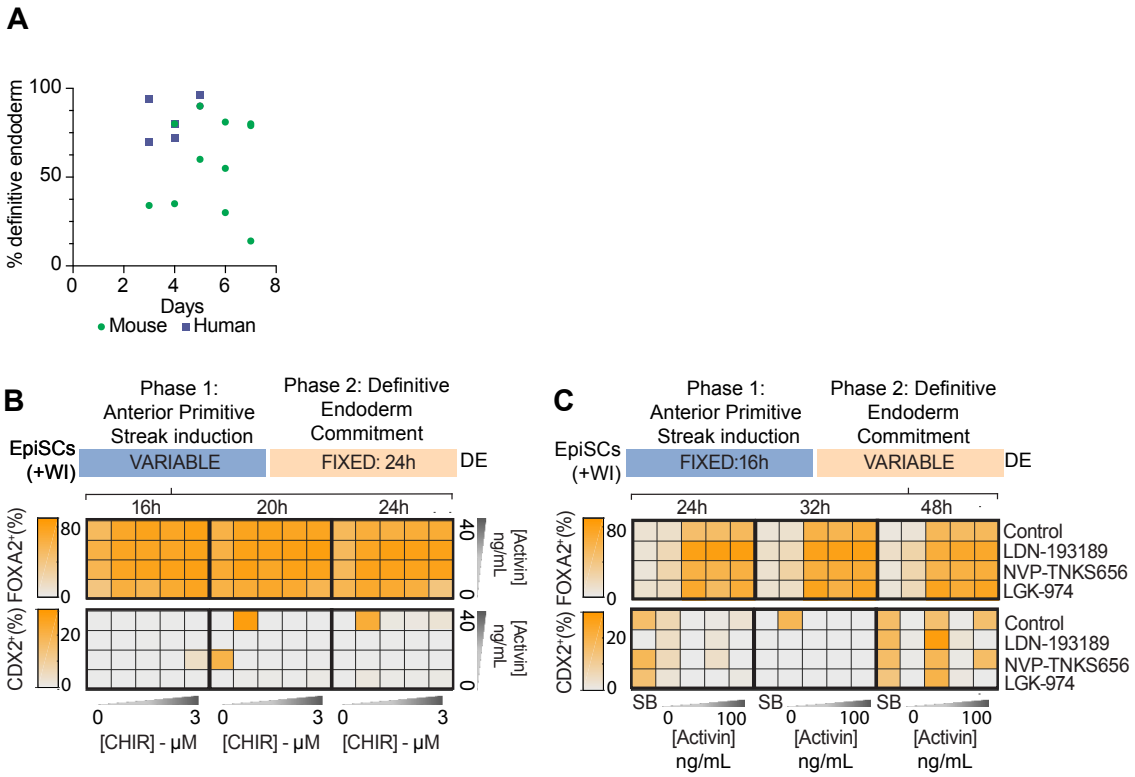


Fig. S1. Systematic optimization of conditions for EpiSC-DE differentiation. (**A**) Overview of state-of-the-art differentiation protocols for human (purple) and mouse (green) PSCs. Further details of each study are provided in **Supplementary Table 1**). (**B-C**) Results of DE protocol optimization experiments. For each condition, immunofluorescence staining was performed following 40 h of differentiation (end of Stage 2). Immunofluorescence images for the indicated antibodies were quantified using CellProfiler. Markers of DE (FOXA2) and mesoderm (CDX2) are shown. (**B**) Summary of results from anterior primitive streak (Stage 1) optimization experiments (n=2 technical replicates). After the indicated conditions for Stage 1, all conditions were exposed to the same Stage 2 conditions, and then quantified. (**C**) Summary of results for optimization of DE commitment (Stage 2) conditions (n=2 technical replicates). For Stage 1, the optimal conditions identified in (**B**) were used, and then cells were switched into the indicated conditions for Stage 2.

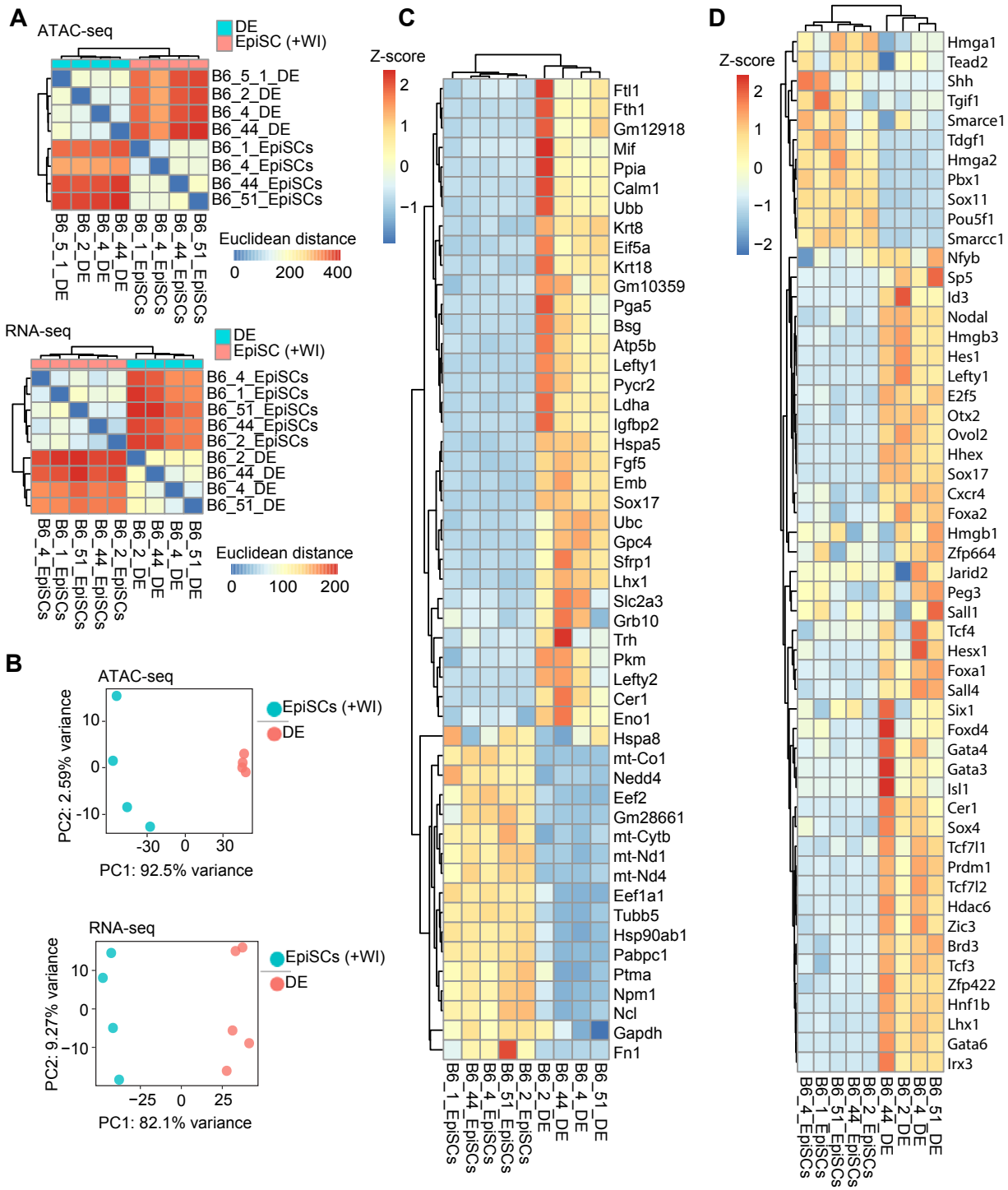


Fig. S2. Extended characterization of EpiSC-derived definitive endoderm. (**A**) Euclidean distance between samples based on ATAC-seq (n=4 per condition, left) and RNA-seq (n=5 for EpiSCs and n=4 for MACs-sorted DE samples, right). (**B**) PCA plot of bulk chromatin accessibility (left) and gene expression (right) of the same C57Bl/6J EpiSC and DE samples as (**A**). (**C**) Unbiased clustered heatmap of the top variable genes as detected by RNA-seq. (**D**) Expression of genes previously identified as DE markers in single cell gene expression studies of hPSC-derived DE and DE isolated from E7.0 embryos (Genga et al., 2019; Nowotschin et al., 2019).

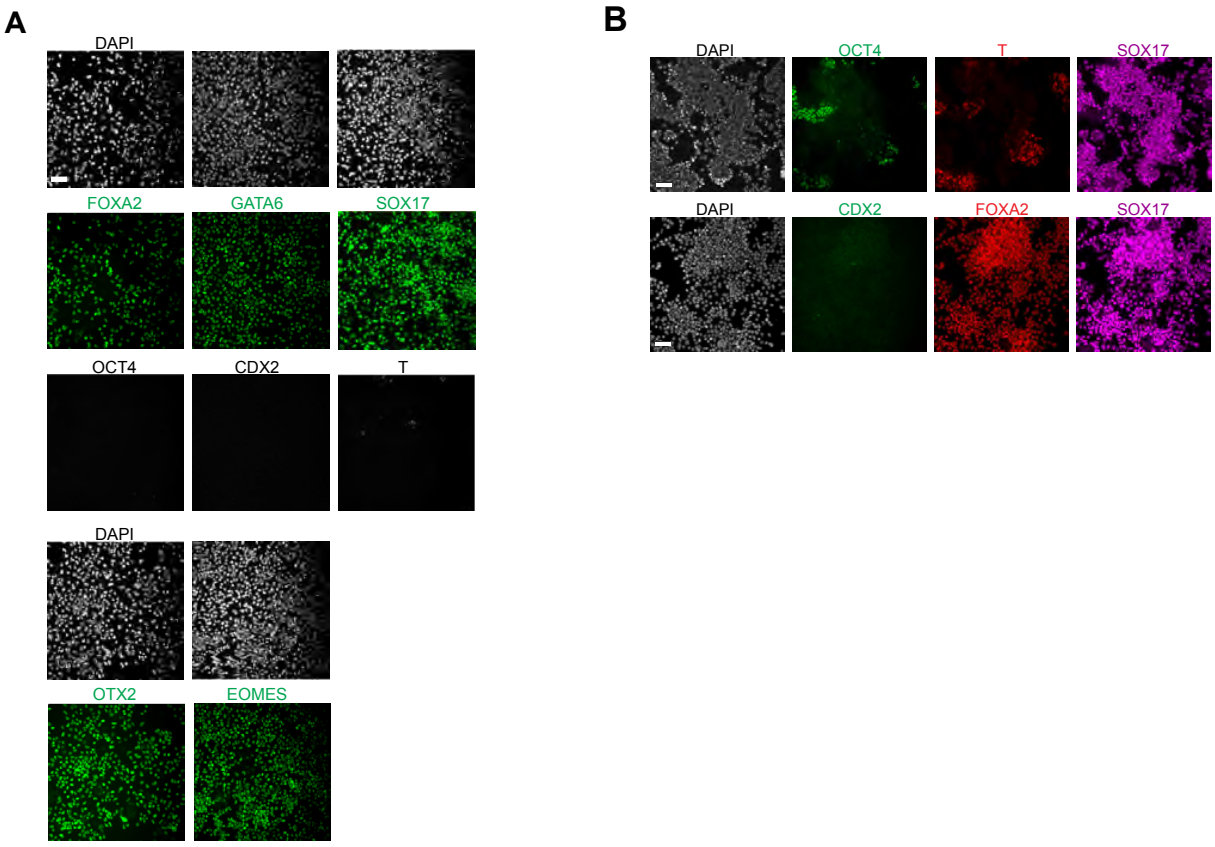


Fig. S3. Immunostainings of EpiSC-derived definitive endoderm. (**A**) Representative immunofluorescence images from DE differentiations for a panel of DE markers (FOXA2, GATA6, SOX17, OTX2, EOMES), pluripotency markers (OCT4), and primitive streak/mesoderm markers (T, CDX2). (**B**) Immunofluorescence images from feeder-free derived DE for SOX17, FOXA2, T, OCT4 and CDX2). All images represent the average results after 4 or more experiments, with at least 3 biological replicates each. Scale bar = 50 μm.

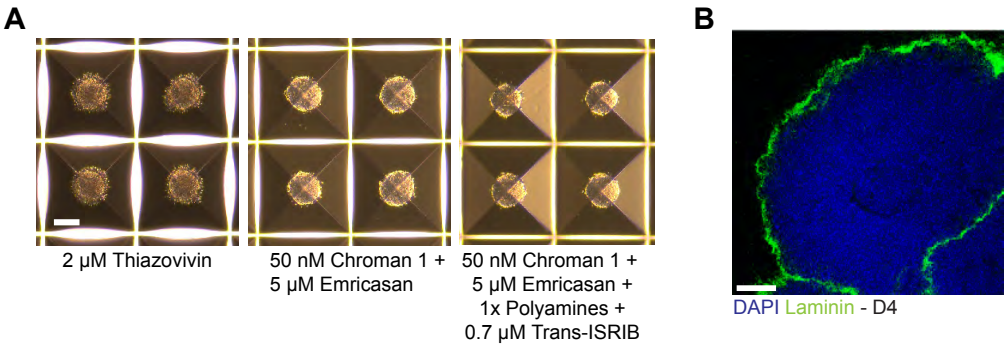


Fig. S4. Optimization of conditions for forebrain organoid generation from mouse EpiSCs. (**A**) Brightfield images of d1 organoids in Aggrewell wells. EBs were formed in the presence of Thiazovivin or chroman 1 + emricasan (CE), or the recently published CEPT cocktail (chroman 1, emricasan, polyamine mix, trans-ISRIB) (Chen et al., 2021). Scale bar = 100 µm. (**B**) Confocal immunofluorescence image of a day 4 EB/organoid. Scale bar = 25 µm.

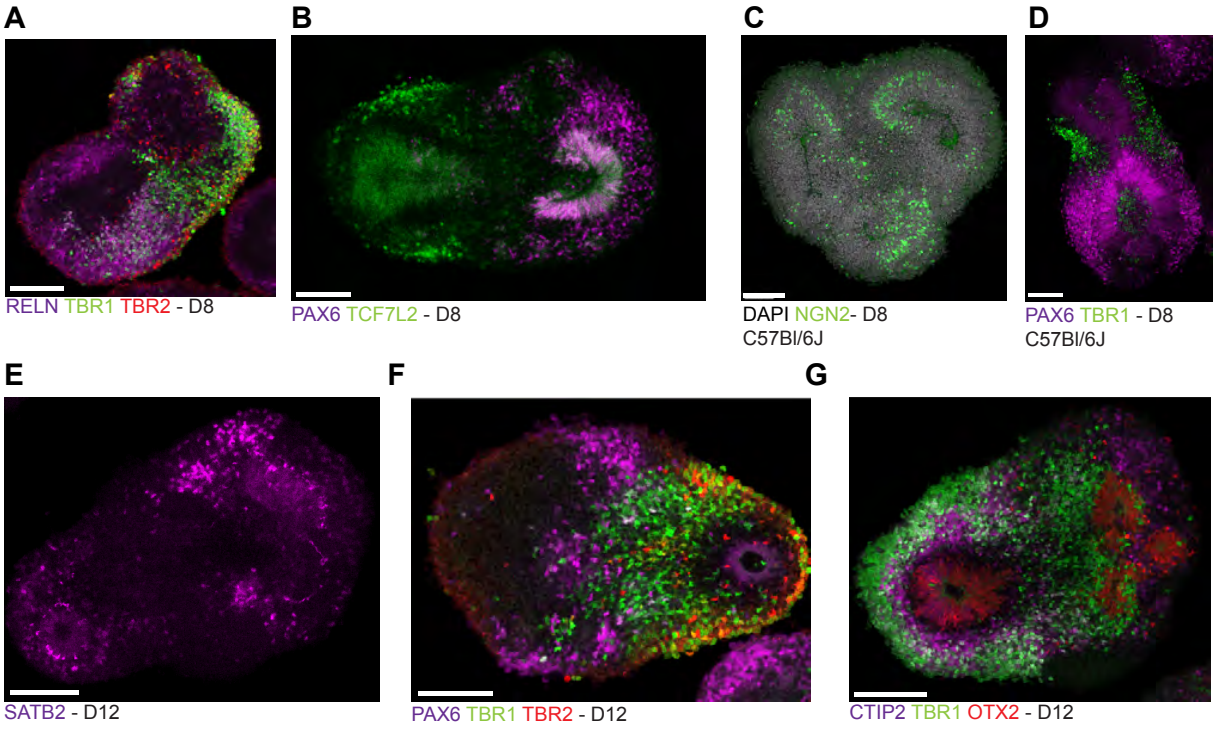


Fig. S5. EpiSC-derived brain organoids generate prethalamic and cortical-like neuronal populations. (**A,B**) Confocal immunofluorescence images of d8 organoids stained with antibodies against classical cortical markers (TBR1, TBR2, RELN and PAX6), which are also found in other regions of the brain such as the hippocampus, the cortical hem and the prethalamus, and thalamic markers (TCF7L2). Scale bar = 100 μm. (**C-D**) Confocal immunofluorescence of d8 organoids from C57Bl/6J background. Scale bar = 100 μm. (**E**) Confocal immunofluorescence image of a d12 organoid stained using antibodies against the classical upper-layer cortical marker SATB2. Scale bar = 100 μm. (**F-G**) Confocal immunofluorescence images of d12 organoids. Scale bar = 100 μm.

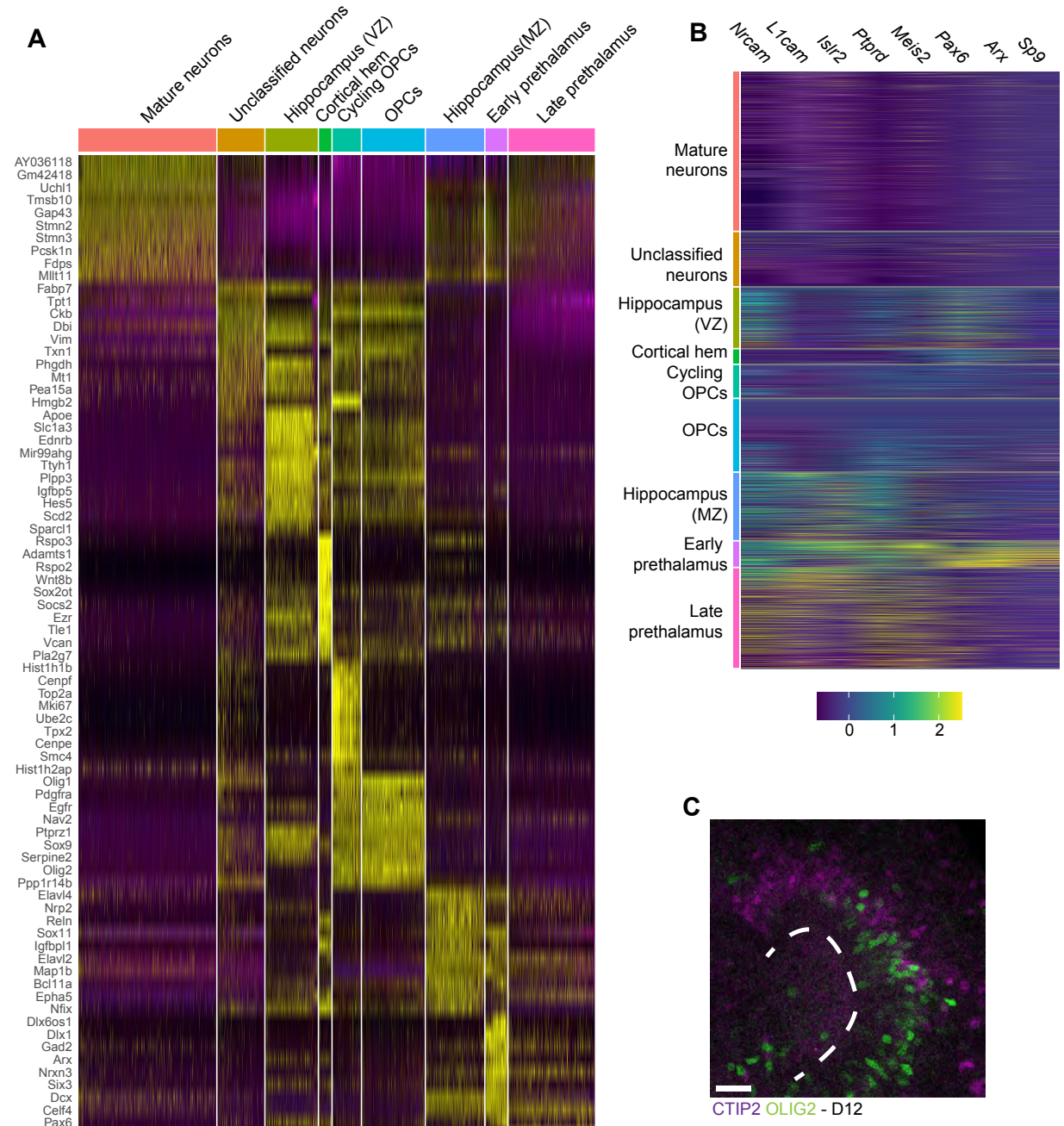


Fig. S6. scRNA-seq analyses on day 12 organoids reveals three main cell types. (**A**) Heatmap of the top 10 genes that define each cluster. Abbreviations: VZ = ventricular zone, MZ = mantle zone. (**B**) Expression profile, across clusters, of several prethalamic (*Sp9, Arx, Pax6, Meis2, Ptprd, Islr2*) and axonal (*L1cam, Nrcam*) markers. (**C**) Confocal immunofluorescence image of a day 12 organoid. Scale bar = 30 µm.

Table S1. Summary of previous mESC, mEpiLC, and hPSC directed differentiation protocols (related to Figure 2). References used to generate the plot in Fig. 2A (Borowiak et al., 2009; Chen et al., 2013; D'Amour et al., 2005; Diekmann et al., 2019; Green et al., 2011; Kinoshita et al., 2020; Korostylev et al., 2017; Li et al., 2011; Loh et al., 2014; Mfopou et al., 2014; Morrison et al., 2016; Morrison et al., 2008; Mou et al., 2012; Mulas et al., 2017; Ortmann et al., 2020; Sherwood et al., 2011; Shi et al., 2017).

PMID	Organism	Days	Efficiency	Journal	Published year
26675138	Mouse	7	80	The Embo journal	2016
33271069	Mouse	3	FS cells: 34%, EpiSCs 4%	Cell Stem Cell	2021
28669603	Mouse	5-6	60%	Stem Cell Reports	2017
24239964	Mouse	6	54.92	Stem cell research	2014
19341624	Mouse	6	81	Cell stem cell	2009
23293299	Mouse	7	79	Development	2013
28702321	Mouse	4	35	Molecular metabolism	2017
32795399	Mouse	6	30	Cell stem cell	2020
18940732	Mouse	7	14	Cell stem cell	2008
21400570	Mouse	4	80	Cellular Biochemistry	2011
22482504	Mouse	5	>90%	Cell Stem Cell	2012
21854845	Mouse	5	>90%	Mechanisms of Development	2011
24412311	Human	3	94	Cell stem cell	2014
28196600	Human	4	72	Cell stem cell	2017
16258519	Human	4	80	Nature biotechnology	2005
30700818	Human	3	70	nature scientific reports	2019
21358635	Human	5	96	Nature biotechnology	2011

Table S2. List of antibodies used.

Antibody	Concentration	Manufacturer	Cat.No.
Alexa Fluor 488 Anti-Human SOX17 conjugated antibody	5uL/10^6 cells	R&D Systems, Inc.	614013
Alexa Fluor 647 Anti-mouse CD184 conjugated antibody	0.5ug/10^6 cells	BioLegend	146504
Anti-CDX2	1/250	Abcam	Ab157524
Anti-CTIP2	1/200	Abcam	Ab18465
Anti-FOXA2	1/500	Abcam	Ab108422
Anti-GATA4	1/200	Santa Cruz	sc-25310
Anti-GATA6	1/500	Cell signaling technology	5851S
Anti-GFAP	1/1000	Invitrogen	13-0300
Anti-Human SOX17	1/200	R&D Systems	AF1924
Anti-Human/Mouse BRACHYURY antibody	1/200	R&D systems	AF2085
Anti-LAMININ	1/1000	Sigma	L9393
Anti-NESTIN	1/500	Aves	NES
Anti-NEUROGENIN-2	1/200	R&D Systems	MAB3314-SP
Anti-OCT4	1/500	Invitrogen	701756
Anti-OLIG2	1/500	Abcam	Ab109186
Anti-OTX1+OTX2	1/500	Abcam	Ab21990
Anti-OTX2	1/500	R&D Systems	AF1979
Anti-PAX6	1/200	BD Biosciences	561462
Anti-PDX1	1/100	Abcam	Ab47308
Anti-PH3	1/500	Cell Signaling	9706S
Anti-REELIN	1/200	Millipore	MAB5366
Anti-SATB2	1/200	Abcam	Ab51502
Anti-SOX1	1/200	R&D Systems	AF3369
Anti-SOX2	1/500	Sigma	AB5603
Anti-TBR1	1/500	Abcam	Ab31940
Anti-TBR2 (for brain organoids)	1/500	Millipore	AB15894

Anti-TBR2/ EOMES (for 2D staining)	1/500	Abcam	Ab23345
Anti-TCF7L2	1/200	Cell Signaling	2569
Anti-TUJ1	1/1000	BioLegend	801213
Anti-ZO-1	1/500	Thermo	617300
Live/dead zombie UV staining	1uL/10^6 cells	Invitrogen	L34962
DAPI	300nM (ICC) 1uM (organoid)	Invitrogen	D1306
Donkey anti Rabbit IgG Secondary Antibody, Alexa Fluor 488	1/500	Invitrogen	A-21206
Donkey anti-Goat highly cross-adsorbed secondary antibody, Alexa Fluor Plus 488	1/500	Invitrogen	A-32814
Donkey anti Rat IgG Highly Cross Adsorbed Secondary Antibody, Alex Fluor 488	1/500	Invitrogen	A-21208
Donkey anti Mouse IgG Highly Cross Adsorbed Secondary Antibody, Alexa Fluor 488	1/500	Invitrogen	A-21202
Goat anti-Chicken IgY Secondary Antibody, Alexa Fluor 555	1/500	Invitrogen	A-21437
Donkey anti Mouse IgG Secondary Antibody, Alexa Fluor 555	1/500	Invitrogen	A-31570
Donkey anti Rabbit IgG Secondary Antibody, Alexa Fluor 555	1/500	Invitrogen	A-31572
Donkey anti Goat IgG Cross Adsorbed Secondary Antibody Alexa Fluor 555	1/500	Invitrogen	A-21432
Chicken anti-Rat IgG Cross Adsorbed Secondary Antibody, Alexa Fluor 647	1/500	Invitrogen	A-21472

Chicken ant Goat IgG Secondary Antibody, Alexa Fluor 647	1/500	Invitrogen	A-21469
Donkey anti Mouse IgG Secondary Antibody, Alexa Fluor 647	1/500	Invitrogen	A-31571
Donkey anti Rabbit IgG Highly Cross Adsorbed Secondary Antibody, Alexa Fluor 647	1/500	Invitrogen	A-31573

Table S3. List of reagents used.

Reagent	Manufacturer	Reference #
2-mercaptoethanol	Gibco	21985023
Accutase	Gibco	A1110501
Activin A	Peprotech	120-14P
Aggrewell plate	STEMCELL	34415
Anti-Rat IgG Microbeads	Miltenyi Biotec	130-048-502
Apo-transferrin	InVitra	777TRF029
B27 supplement	ThermoScientific	17504044
B27 supplement (without vitamin A)	Gibco	12587010
BDNF	PeproTech	450-02
BenchMark Fetal bovine serum	Gemini	100-106
bFGF	Gibco	12587010
BMP-7	PeproTech	120-03P
Bovine Serum Albumin	Sigma	A2153
Cell recovery solution	Corning	354253
Chemically Defined Lipid Concentrate	Gibco	11905031
CHIR99201	Tocris Bioscience	252917-06-9
Chroman 1	MedChem Express	HY-15392
Chromium Next GEM Chip G Single Cell Kit, 48 rxns	10X genomics	1000120
Chromium Next GEM Single Cell 3' Kit v3.1, 16 rxns	10X genomics	1000268
CytoTune emGFP Sendai fluorescence reporter	ThermoScientific	A16519
Collagenase Type IV	Gibco	17104019
DAPI	Thermo Scientific	D1306
DeepClear	Celexplorer	DC-201
Digitonin	Sigma	D-141
DMEM with glutamax	Gibco	10564029
DMEM/F12	Gibco	11320082
Dual Index Kit TT Set A 96 rxns	10x genomics	1000215
eBioscience Fixation/Permeabilization Concentrate	Invitrogen	00-5123

eBioscience Fixation/Permeabilization Dilent	Invitrogen	00-5223-56
eBioscience Permeabiliation buffer (10X)	Invitrogen	00-8333
EDTA (0.5 M), pH 8.0, RNase-free	Invitrogen	AM9261
Emricasan	Selleckchem	S7775
ESGRO LIF	Sigma	ESG1107
F12 with GlutaMAX	Gibco	31765035
Fetal bovine serum	Gemini	100-106
Fgf-8b protein, CF	R&D Systems	423-F8-025/CF
Fibronectin	Sigma	FC010
Fixation/permeabilization concentrate	Invitrogen	00-5223-43
Fixation/permeabilization diluent	Invitrogen	00-5223
Flowmi cell strainer	Bel-Art	136800040
GDNF	PeproTech	450-10
Glutamax	Gibco	35050061
Heat stable recombinant human bFGF	Gibco	PHG0360
Hyclone fetal bovine serum	Cytiva	SH3007003
IMDM	Gibco	12440053
Insulin	Sigma	91077C
KAPA Hyper Prep Kit	Kapa Biosystems	KK8504
KingFisher Flex Magnetic Particle Processor	Thermo Scientific	5400630
Knockout serum replacement	Gibco	10828028
Laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane	Sigma	L2020
LDN-193189	Stemgent	04-0074
LGK-974	Selleck Chemicals	S7143
LY294002	Selleckchem	S1105
MACS multistand	Miltenyi Biotech	130-042-303
MagMAX *mir*Vana Total RNA Isolation Kit	Thermo Scientific	A27828
Matrigel	Corning	354230
Monothioglycerol	Sigma	M6145
MS column	Miltenyi Biotech	130-122-727
N2 supplement	Gibco	17502048

NEBNext® High-Fidelity 2X PCR Master Mix	NEB	M0541L
Neurobasal Media	Gibco	21103049
Non-adherent 6 well plate	Corning	3471
Non-essential amino acids	Gibco	11140050
NP-40 detergent	Sigma	74385
NVP-TNKS656	Selleck Chemicals	S7238
OctoMACS separator	Miltenyi Biotech	130-042-109
PD0325901	Tocris	4192
PD173074	STEMCELL Technologies	72164
Penicllin/streptomycin (100X)	Gibco	15140122
Permeabilization buffer	Invitrogen	00-8333
PiK-90	Sigma Aldrich	528117
Polyvinyl Alcohol	Sigma	341584
Putrescine	Sigma	P5780
rhLaminin-521	Gibco	A29249
SB-431542	Tocris	1614
SMART-Seq v4 Ultra Low Input RNA Kit	Clonetech	63488
Sodium pyruvate	Gibco	11360070
Spermidine	Sigma	S2626
Spermine	Sigma	S4264
16% Formaldehyde (w/v), Methanol-free	Thermo Scientific	28908
Thiazovivin	Sigma	SML1045
Tn5 enzyme and tagmentation buffer	Illumina	20034198
Trans-isrib	Tocris	5284
Tri Reagent	Sigma	T9424
TruSeq Stranded mRNA LT Kit	Illumina	RS-122-2102
TrypLE	Thermo Scientific	12605028
Triton X-100	Sigma-Aldrich	9002-93-1
Tween-20	Boston Bioproducts	P-934

Name	B6.129_17	B6.129_9	B6.129_4	DBA/2J_55	C57Bl/6J_44	C57Bl/6J_51	C57Bl/6J_1	C57Bl/6J_4	C57Bl/6J_2	PWK/PhJ_AC705	PWK/PhJ_AC698	PWK/PhJ_AC695	PWK/PhJ_AC699
Background	B6129SF1/J	B6129SF1/J	B6129SF1/J	DBA/2J	C57Bl/6J	C57Bl/6J	C57Bl/6J	C57Bl/6J	C57Bl/6J	PWK/PhJ	PWK/PhJ	PWK/PhJ	PWK/PhJ
Received from	Matthias Stadtfeld, PhD - Weill Cornell			Christopher L. Baker, Ph.D. - The Jackson Laboratory			inhouse, through IVF			Laura Reinholdt, Ph.D. - The Jackson Laboratory			
Culture conditions (mESCs)	On gelatin-coated dishes with irradiated mouse embryonic fibroblast feeder cells												
Media composition (mESCs)	Serum/LIF media: DMEM with glutamax, 1% nonessential amino acids, 1% sodium pyruvate, 1% penicillin-streptomycin, 0.1% 2-mercaptoethanol, 10% fetal bovine serum and 1% ESGRO LIF.			Serum/LIF media: DMEM with glutamax, 1% nonessential amino acids, 1% sodium pyruvate, 1% penicillin-streptomycin, 0.1% 2-mercaptoethanol, 10% fetal bovine serum, 1% ESGRO LIF, 3 uM CHIR99201 and 1 uM PD0325901.									
Culture conditions (mEpiSCs)	On gelatin-coated dishes with irradiated mouse embryonic fibroblast feeder cells												
Media composition (mEpiSCs)	N2B27 media: 50% DMEM-F12, 50% Neurobasal, 0.5% N2 supplement, 1% B27 supplement without vitamin A, 2 mM glutamax, 1% penicillin-streptomycin, 0.1% 2-mercaptoethanol, 20 ng/ml activin A, 12.5 ng/ml heat stable bFGF and 175 nM NVP-TNKS656												
Passage at EpiLC-to-EpiSC conversion	P5-10	P5-10	P5-10	P10	P5	P14	P6	P6	P11	P14	P10	P10	P8
Passage at experimental time	P5-10	P5-10	P5-10	P17	P13	P20	P12	P12	P18	P21	P17	P17	P15
Mycoplasma results	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Karyotyped by LC-WGS		Yes - Normal			Yes - Normal	Yes - Normal					Yes - Normal		Yes - Normal

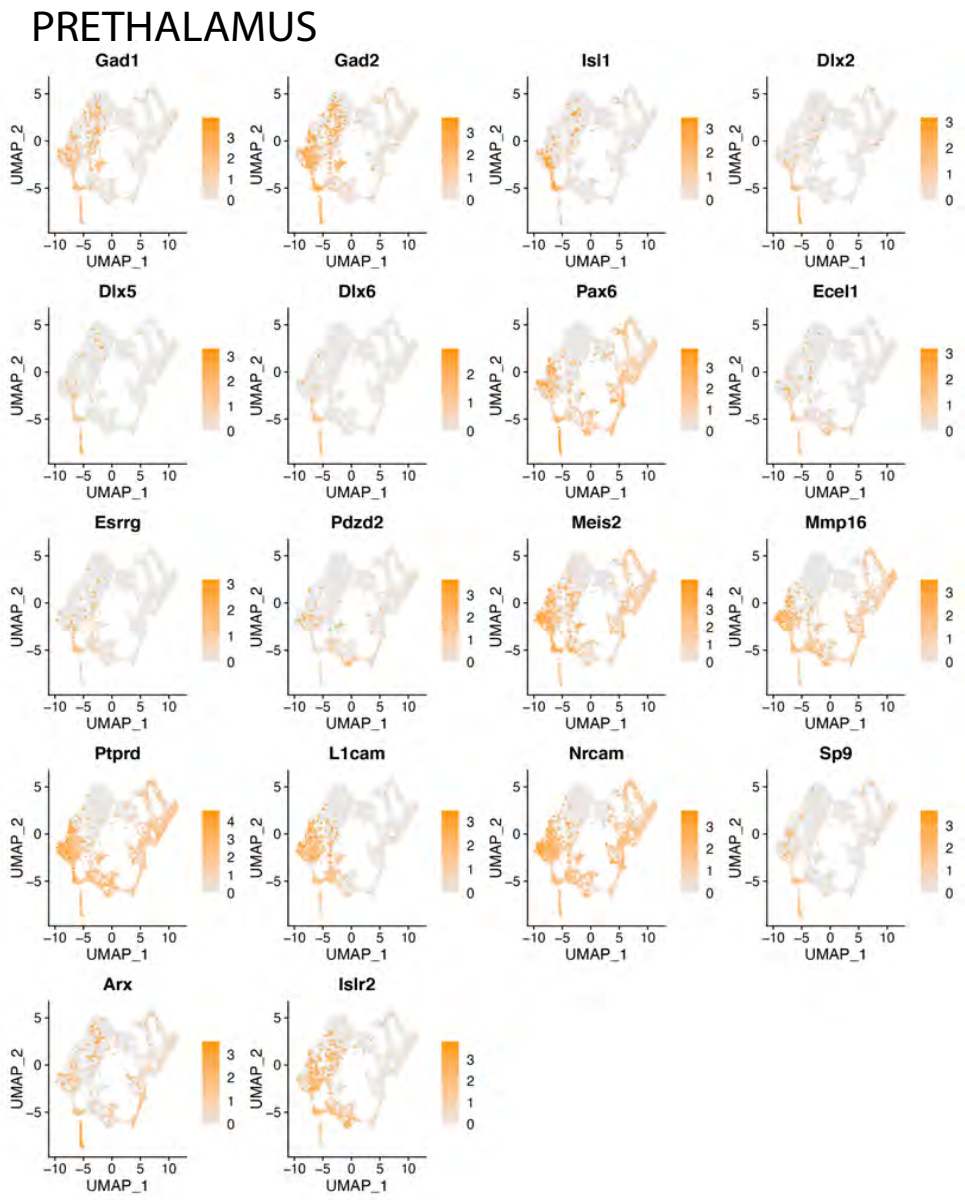
Table S4. Mouse embryonic stem cell lines

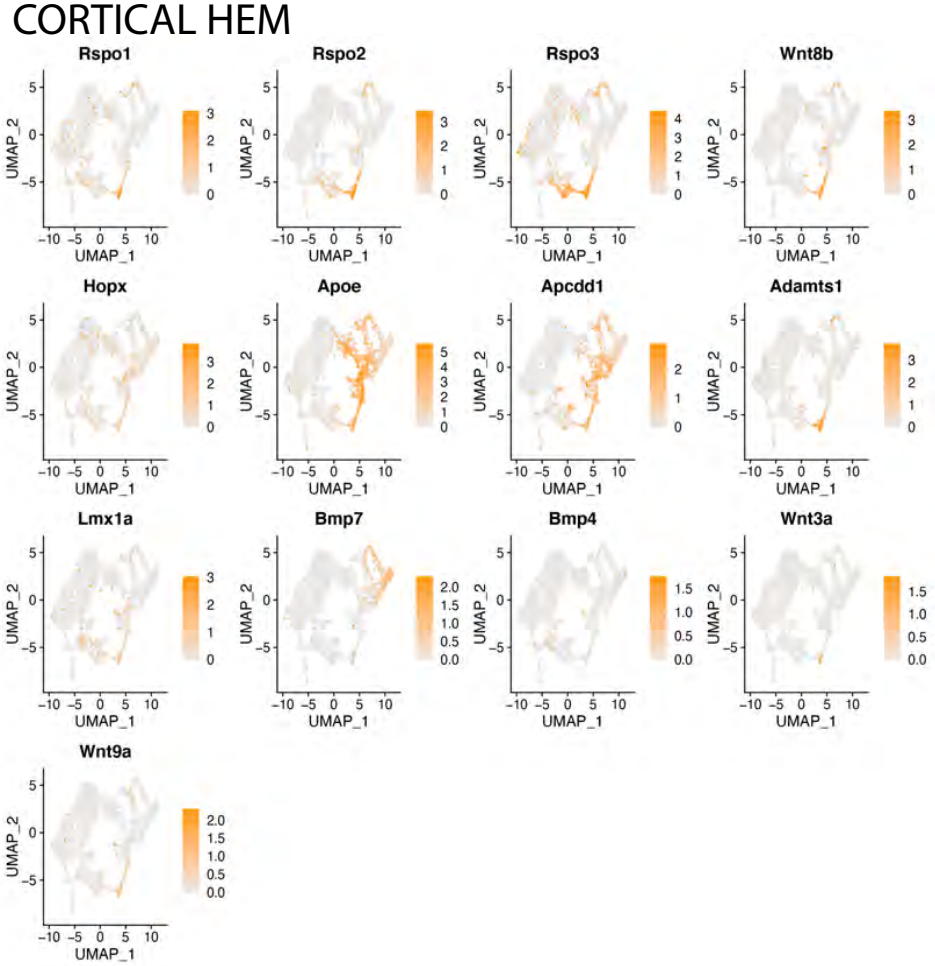
Development: doi:10.1242/dev.200561: Supplementary information

Table S5. EpiSC lines used in brain organoid experiments.

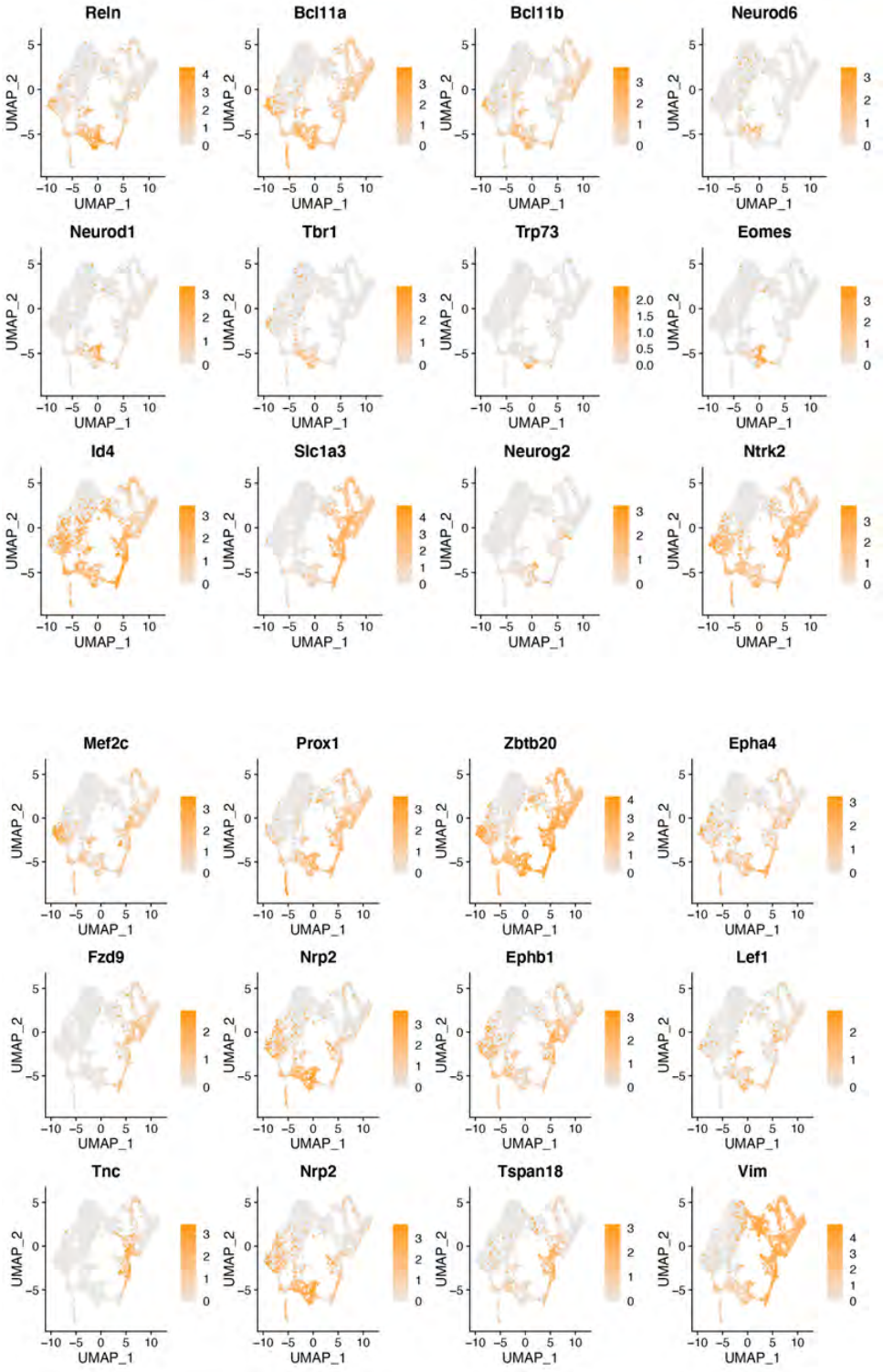
Genetic background	Biological replicates	Technical replicates
B6129SF1/J	3	>10
C57Bl/6J	1	2
C57Bl/6J X CAST/EiJ	1	2

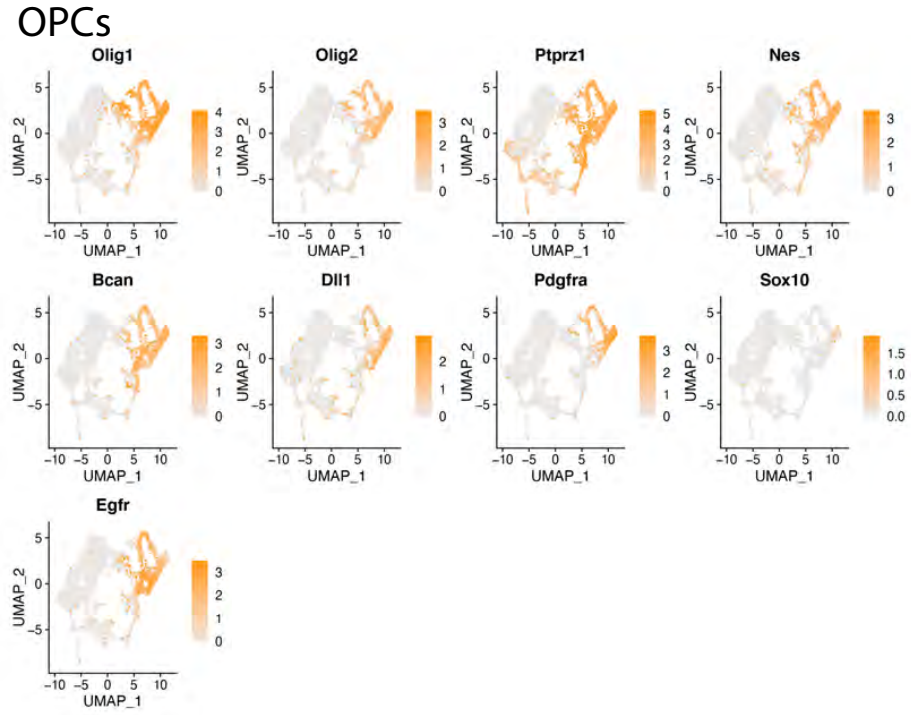
Fig. S7. Complete UMAPs, related to figure 6.

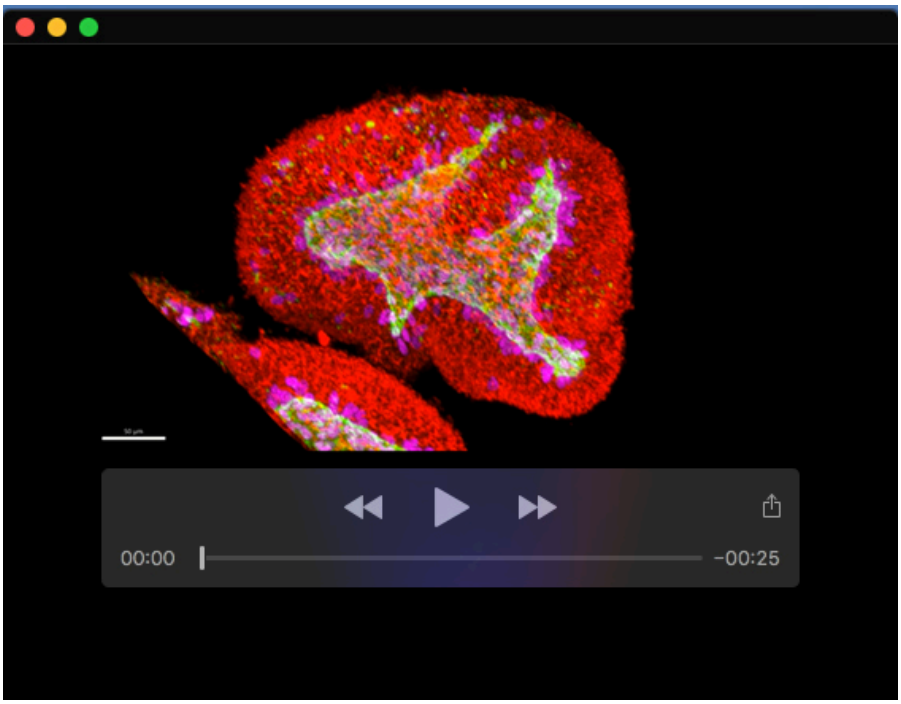




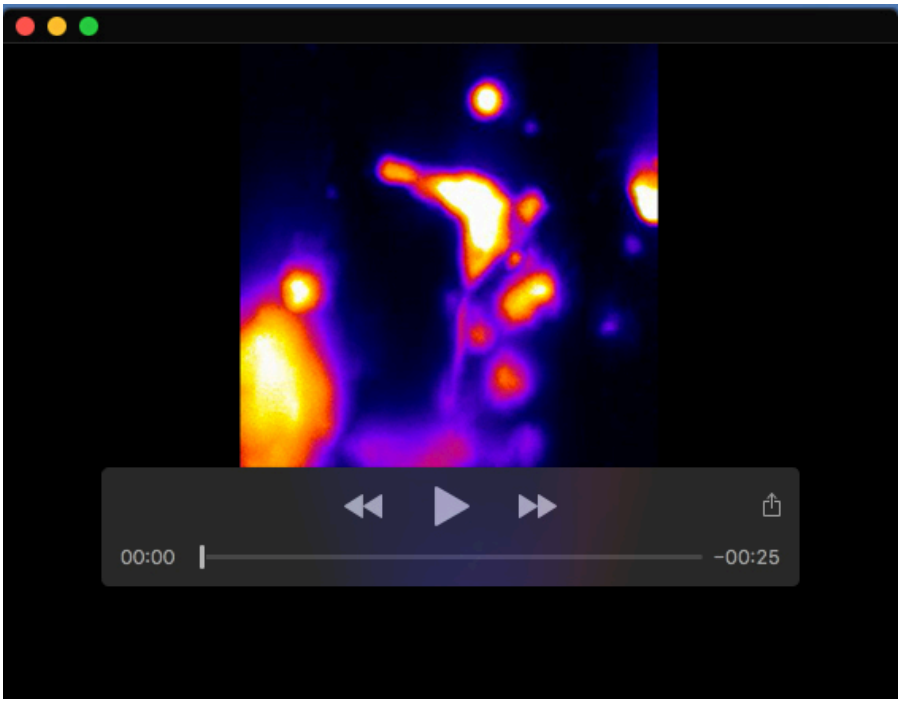
HIPPOCAMPUS







Movie 1. Day 4 forebrain organoid stained with antibodies against ZO-1 (green), phospho-histone H3 (purple) and Nestin (red).



Movie 2. Live imaging of radial glial-like cells in a d3 organoid. emGFP Sendai virus was added to the EB formation media during the EB formation period to sparsely label developing neuroepithelial progenitor cells. The organoid was then embedded in Matrigel and imaged with a light sheet microscope from day 3 to day 4. The apical membrane is at the bottom of the video and the basal lamina on top.

Supplementary Materials and Methods

Protocol: Mouse ESC to EpiSC conversion and EpiSC culture

Notes before starting:

- Before use, all media should be warmed to 37 °C using a water or bead bath.
- All steps should be performed in a sterile BSC using rigorous aseptic technique.

Thawing ESCs

1. Remove cryovial of ESCs from liquid nitrogen storage and thaw rapidly with warmed media.
2. Once thawed, transfer into 15 mL falcon containing 5 mL Serum/Lif media (Table S7) to dilute the DMSO in the freezing media.
3. Centrifuge at 200 g for 3 minutes.
4. Aspirate media and resuspend cell pellet in appropriate volume of Serum/Lif media (Table S6).
5. Remove pre-plated feeders from the incubator and aspirate MEF media.
6. Add single cell suspension of ESCs to feeders and gently shake the plate left-to-right and up-and-down 3 times each to ensure cells are evenly distributed throughout.

Maintaining ESCs

Important notes

- Change media daily and passage cells upon 70% confluence at a 1:6 ratio.
- Feeders plated on gelatin should be prepared at least 8 hours in advance.

Passaging ESCs

1. Aspirate media and wash cells twice with PBS without calcium and magnesium ($PBS^{-/-}$).
2. Add warmed Accutase to wells for 2-3 minutes to dissociate the cells from the plate.
    a. *To speed up the dissociation process, place the plate with Accutase at 37 ℃ for 1-2 minutes.*
3. Gently tap the sides of the tissue culture plate to enhance cell dissociation.
4. Once all cells are lifted, collect Accutase and cells in a 15 mL centrifuge tube and dilute with equal amounts of $PBS^{-/-}$.
5. Centrifuge at 200 g for 3 minutes.
6. Aspirate supernatant and resuspend cell pellet in Serum/Lif media.
7. Remove MEF media from feeders and add the appropriate amount of ESC suspension to the plate for a 1:6 dilution.
8. If passaging long-term as ESCs, use collagenase to lift cells and accutase to bring to a single cell suspension to avoid accumulation of feeders. See "Passaging as EpiSCs" below.

ESC to EpiLC Conversion

Prepare plate

1. Coat the desired tissue culture plate with fibronectin diluted to 16.7 uL/mL in $PBS^{-/-}$. Rest plate at room temperature for ≥30 minutes.
2. Wash the coated plate twice with $PBS^{-/-}$.
3. Add additional $PBS^{-/-}$ to the plate until ready to add the cells to prevent desiccation. The plate can be stored with $PBS^{-/-}$ at 37 degrees for up to 4 hours.

Create single cell suspension and plate

1. Confirm ESC quality using a phase contrast microscope.
2. Aspirate ESC media from plate.
3. Use a serological pipette to wash each well twice with $PBS^{-/-}$.
4. Remove $PBS^{-/-}$ from the plate and apply pre-warmed Collagenase IV to each well to preferentially dissociate EpiSC colonies without feeder contamination.
    a. *Optional: place the plate with collagenase back in the incubator to increase the speed of dissociation.*
    b. *Gently tap the sides of the tissue culture plate to encourage colony lifting.*
    c. *Do not leave collagenase on wells for over 25-30 minutes as this will cause feeder cells to dissociate.*
5. Once the ESC colonies are lifted, gently collect colonies using serological pipette and transfer to a 15 mL centrifuge tube.
    a. *Optional: wash each well with HBSS++ to collect any remaining colonies and combine with the collagenase in the 15 mL tube.*
6. Centrifuge cells at 125 g for 4 minutes to pellet the ESC colonies and aspirate the supernatant.
7. Using a serological pipette, resuspend the pellet with 1 mL Accutase to break colonies into a single cell suspension. Leave the cell suspension under the hood for 2-4 minutes to ensure single cell suspension.
    a. *Optional: remove one drop of the suspension (~10 uL) and place on microscope slide. Observe with the microscope for residual colonies.*
8. Once the colonies are completely dissociated, add 5-10 mL $PBS^{-/-}$ to dilute the Accutase.
9. Centrifuge the solution at 200 g for 3 minutes to pellet single cells.
10. Aspirate supernatant and resuspend the pellet in EpiLC media (Table S7).
11. Count cells and plate at a density of 17,500 cells/cm^2.
12. Change the media 24 hours after plating. Cells should be cultured in EpiLC media for 48 hours before being converted to EpiSCs.

Conversion and Maintenance of EpiSCs

Important notes

- Change media daily and passage cells at 70% confluence, generally every other day
- Feeders plated on gelatin should be at least 8 hours before.

Converting EpiLCs to EpiSCs

1. After 48 hours of culture in EpiLC conditions, wash cells twice with $PBS^{-/-}$.
2. Add warmed Accutase to wells and collect in 15ml falcon tube once cells are lifted. Break the cells into small clumps of 5-10 cells (approx. 2-4 mins). Add equal volume of $PBS^{-/-}$ to dilute Accutase.
3. Centrifuge at 200 g for 3 minutes to pellet cells.
4. Resuspend in pre-warmed EpiSC media (Table S7).
5. Wash feeders twice with $PBS^{-/-}$. Aspirate the final PBS wash and plate 50,000-100,000 EpiLCs/cm^2 onto the feeders.
    a. *Note: Roughly, 1 well of a 12-well plate of EpiLCs can be seeded to 1 well of a 6-well plate for EpiSC culture.*

Passaging EpiSCs

1. Aspirate EpiSC media.
2. Use a serological pipette to wash each well twice with $PBS^{-/-}$.
3. Add pre-warmed Collagenase IV to each well to preferentially dissociate EpiSC colonies without feeder contamination.
    a. *Optional: place the plate with collagenase in the incubator to increase the speed of dissociation.*
    b. *Gently tap the sides of the tissue culture plate to encourage colony lifting.*
    c. *Do not leave collagenase on wells for over 25-30 minutes as this will cause feeder cells to dissociate.*
4. Once EpiSC colonies are lifted, gently collect colonies using serological pipette and transfer to a 15 mL centrifuge tube.
    a. *Optional: wash each well with HBSS++ to collect any remaining colonies and combine with collagenase in the 15 mL tube.*
5. Centrifuge cells at 125 g for 4 minutes to pellet EpiSC colonies and aspirate supernatant.
6. Using a serological pipette, resuspend the pellet with 1 mL Accutase to break colonies into clumps of 3-8 cells. Leave the cell suspension in hood for 1-2 minutes to ensure dissociation.
    a. *Optional: remove one drop of the suspension (~10uL) and place it on microscope slide. Observe colony size under the microscope.*
7. Once cells are dissociated, add 5-10 mL $PBS^{-/-}$ to dilute Accutase.
8. Centrifuge the solution at 200 g for 3 minutes to pellet cells.
9. Gently resuspend in EpiSC media to avoid additional dissociation.
10. Wash pre-plated feeders twice with $PBS^{-/-}$ to remove all MEF media and any traces of serum.
11. Plate EpiSCs on washed feeders with appropriate volume of media (Table S6) and place in incubator.
    a. *Optional: Check the size of the cell clumps after plating to ensure small colonies of 3-8 cells.*

12. EpiSCs grow very rapidly and therefore require daily media changes and are generally passaged every 48 hours.

Freezing EpiSCs

1. Aspirate media from each well of the plate and wash two times with $PBS^{-/-}$.
2. Add appropriate amount of accutase to each well (Table S6) and place in the incubator until the colonies are lifted.
3. Collect the mixture with a serological pipette and transfer to a 15 mL centrifuge tube.
4. Dilute Accutase with $PBS^{-/-}$.
5. Centrifuge cell solution at 200 g for 3 minutes.
6. Aspirate the supernatant.
7. Gently resuspend in EpiSC media with 10% DMSO, add to a cryotube and freeze.

Thawing EpiSCs

1. Remove EpiSCs from liquid nitrogen tank and thaw rapidly with warmed media.
2. Once thawed, add to a 15 mL falcon with 4 mL of N2B27 to dilute the DMSO.
3. Centrifuge at 200 g for 3 minutes to pellet cells.
4. Aspirate supernatant and gently resuspend the cells with EpiSC media.
5. Wash overnight feeders 2x with $PBS^{-/-}$ and plate cells directly onto feeders.

Frequent questions and concerns

Minimizing EpiSC differentiation (Figure S8B)

- Once there are many spontaneously differentiating colonies within the culture, it can be difficult to bring the line back to pluripotency. Prevention by culturing carefully is the best method to avoid spontaneous differentiation.
- Passage frequently, preferentially every other day, with no longer than 3 days on the same plate of feeders.
- Use collagenase to passage, as this will avoid propagation of differentiated cells.

Protocol: Directed differentiation of mouse EpiSCs into definitive endoderm

Notes before starting:

- Before use, all media should be warmed to 37 °C using a water or bead bath.
- All steps should be performed in a sterile BSC using rigorous aseptic technique.

Preparing the laminin coated cell culture dish

1. Dilute laminin-521 in PBS with calcium and magnesium to a final concentration of 10 ug/mL. Optionally, laminin from EHS murine sarcoma basement membrane (Sigma, L2020) can be used as a replacement at 20 ug/mL.

2. Once combined, use a serological pipette to dispense the diluted laminin into each well of a tissue culture plate (see Table S8 for volume recommendations). Shake plate to ensure that the entire surface is covered.
3. Place the laminin-coated tissue culture plate(s) in cell culture incubator at 37°C for 2 hours or O/N at 4°C.

Preparing and plating EpiSCs

1. Confirm EpiSC quality using a phase contrast microscope (Figure S8A).
2. Aspirate EpiSC media from plate.
3. Use a serological pipette to wash each well twice with $PBS^{-/-}$.
4. Remove the $PBS^{-/-}$ from the plate and apply pre-warmed Collagenase IV to each well to preferentially dissociate EpiSC colonies without feeder contamination.
    a. *Optional: place the plate with collagenase back in the incubator to increase the speed of dissociation.*
    b. *Gently tap the sides of the tissue culture plate to encourage colony lifting.*
    c. *Do not leave collagenase on wells for over 25-30 minutes as this will cause feeder cells to dissociate as well and should be avoided.*
5. Once the EpiSC colonies are lifted, gently collect colonies using serological pipette and transfer to a 15 ml centrifuge tube.
    a. *Optional: wash each gently with HBSS++ to collect any remaining colonies and combine with collagenase in the 15 mL tube.*
6. Centrifuge cells at 125 g for 4 minutes to pellet EpiSC colonies and aspirate the supernatant.
7. Using a serological pipette, resuspend the pellet with 1 mL Accutase to break colonies into a single cell suspension. Leave the cell suspension in hood for 2-4 minutes to ensure single cell suspension
    a. *Optional: remove one drop of the suspension (~10 uL) and place on microscope slide. Observe under the microscope for residual colonies.*
8. Once cells are completely dissociated, add 5-10 mL $PBS^{-/-}$ to dilute Accutase.
9. Centrifuge the solution at 200 g for 3 minutes to pellet single cells.
10. Aspirate the supernatant, resuspend in plating media (Table S9) and count the number of cells.
11. Calculate the appropriate volume of media and number of cells to seed the desired plate, then prepare master solution with 110,000 cells/cm^2 (Table S8).
12. Remove the laminin coated plate from incubator and aspirate laminin solution. Do not allow plate to dry—the cell suspension must be added immediately.
13. Homogenize the cell solution and immediately add the cell suspension to the plate.
14. Place the plate in the incubator for 5-6 hours. During this time cells will attach to the plate.

Changing media

1. After 5-6 hours, gently remove the plate from the incubator and check under microscope to confirm that most cells have attached. At this stage, the cells can be easily washed off if media is added too vigorously.
2. Slowly aspirate media off all wells at a 45-degree angle to the BSC surface. Gently add $PBS^{-/-}$ to wash off any residual media.
    a. *IMPORTANT: Use one hand to tilt the tissue culture plate towards you at a 45° angle and maintain this angle while gently removing and dispensing media into the side of the well (slow ejection speed on the pipettor). This will ensure minimal loss of cells.*
3. Using same technique, add appropriate amount of Differentiation Media 1 (Table S9).
4. Place the tissue culture dish in the incubator and allow to sit for 16 more hours.
5. After 16 hours, aspirate Differentiation Media 1.
6. While resting serological pipette tip on bottom wall of well, gently add $PBS^{-/-}$ to dilute any residual media.
7. Using same technique as $PBS^{-/-}$ application, add appropriate amount of Differentiation Media 2 (Table S9).
8. Return the tissue culture dish to the incubator for 24 hours.
9. After 24 hours in Differentiation Media 2, the cells should have reached the definitive endoderm stage. Examine all wells to confirm appropriate density and morphology.

Fixing and immunostaining

1. To fix cells, wash each well two times with $PBS^{-/-}$.
2. Add 4% PFA to each well and allow to rest at room temperature for 15 minutes.
3. After 15 minutes, aspirate the PFA and wash twice with $PBS^{-/-}$.
4. Add $PBS^{-/-}$ to each well and refrigerate at 4 °C until staining.

Frequent questions and concerns

Low cell density after plating

- Ensure the laminin was diluted and left for specified amount of time (2 hours). Do not rinse culture plates after laminin coating.
- Some cell lines have weak attachment. If this is a recurring issue for specific lines, increase the starting density when plating.

Cell loss during media changes

- Use one hand to tilt the tissue culture plate towards you at a 45° angle and maintain this angle while gently removing and dispensing media into the side of the well (slow ejection speed on the pipettor). This will ensure minimal loss of cells.
- Increase the size of the wells you are using. Cell loss is more common on smaller sized wells.
- Wait a longer period of time (6-8 hours) before changing plating media.

Low efficiency of differentiation

- It is important to begin with high quality EpiSCs with minimal spontaneous differentiation. At the beginning of the directed differentiation protocol, EpiSCs colonies should number ~50-200 and the plate should be no more than 70-80% confluent.
- This protocol was optimized on EpiSCs passaged no more than 18 passages as EpiSCs and it is unknown how high passage lines may respond to the differentiation protocol. In general, it is best to begin with low-passage ESCs to convert to EpiSCs and avoid prolonged culture.

Timing

- The timing of each media change was optimized to produce the highest Sox17 and FoxA2 expression at 40 hours of culture. Changing the timing of Step 1 and Step 2 can lead to reduced efficiency.

Protocol: Generation of forebrain organoids from mouse EpiSCs

Notes before starting:

- Before use, all media should be warmed to 37 °C using a water or bead bath.
- All steps should be performed in a sterile BSC using rigorous aseptic technique.

Formation of EBs

1. Confirm EpiSC quality using a phase contrast microscope (Figure S8A).
2. Aspirate EpiSC media from plate.
3. Use a serological pipette to wash each well twice with $PBS^{-/-}$.
4. Remove the $PBS^{-/-}$ from the plate and apply pre-warmed Collagenase IV to each well to preferentially dissociate EpiSC colonies without feeder contamination.

- *Optional: place the plate with collagenase back in the incubator to increase the speed of dissociation.*
- *Gently tap the sides of the tissue culture plate to encourage colony lifting.*
- *Do not leave collagenase on wells for over 25-30 minutes as this will cause feeder cells to dissociate as well and should be avoided.*

5. Once the EpiSC colonies are lifted, gently collect colonies using serological pipette and transfer to a 15 mL centrifuge tube.

- *Optional: wash each gently with HBSS++ to collect any remaining colonies and combine with collagenase in the 15 mL tube.*

6. Centrifuge cells at 125 g for 4 minutes to pellet EpiSC colonies and aspirate the supernatant.
7. Using a serological pipette, resuspend the pellet with 1 mL Accutase to break colonies into a single cell suspension. Leave the cell suspension in the hood for 2-4 minutes to ensure single cell suspension.

- *Optional: remove one drop of the suspension (~10 uL) and place on microscope slide. Observe under the microscope for residual colonies.*

8. Once cells are completely dissociated, add 5-10 mL $PBS^{-/-}$ to dilute Accutase.
9. Centrifuge the solution at 200 g for 3 minutes to pellet single cells.
10. Aspirate the supernatant, resuspend in EB formation media (Table S10) and count cells.
11. Calculate the appropriate volume of media and number of cells to seed the desired number of wells, then prepare master solution with 1,200,000 cells/well of Aggrewell, with each well containing a total of 2 mL media.
12. Using a serological pipette, disperse cell solution into well(s) of Aggrewell.
13. Using a P1000 pipette, gently pipet up and down to equally distribute the cells within the well(s).
14. Centrifuge the Aggrewell plate at 100 g for 3 minutes. Confirm cells are evenly distributed using microscope.
15. Place Aggrewell in incubator for 24 hours to form EBs. Avoid moving plate during the EB formation phase.

Collection and embedding of organoids

Important note:

- Matrigel should be thawed overnight on ice at 4°C (or for at least 3-4h)

1. Following 24 hours in EB formation media, Aggrewell should contain uniform, well-formed developing organoids. Using a P1000 pipette, gently remove as much media as possible from the Aggrewell without disturbing organoids.
2. Using a P1000, add 1 mL of Neural Induction media (Table S10) to Aggrewell and, using a wide orifice pipette tip, gently resuspend the developing organoids.
3. Transfer organoids to a 6 cm Petri dish containing 5 mL Neural Induction media and swirl briefly to dilute EB formation media. Transfer organoids to a 10 cm Petri dish containing 10 mL Neural induction media.
   a. *Note: washing with $PBS^{-/-}$ should be avoided as this may cause organoids to adhere to the plate.*
4. Determine the total number of organoids desired and how many wells of a low-adherence 6 well plate are needed.
   a. *Note: Each well of the plate should contain 20-50 organoids.*
5. Then, carefully gather 20-50 organoids in 67 uL of Neural Induction media and mix with 100 uL of Matrigel. Carefully dispense the mix of Matrigel, media, and organoids in the center of a well, being sure to avoid the walls of the well (Derived from Qian et al., 2018).
6. Repeat for desired number of wells then place the plate in the incubator for 30 minutes to solidify the Matrigel.
7. Following the 30-minute incubation, carefully remove the plate from the incubator and gently add 3 mL of Neural Induction media to each well. Return to incubator for 24 hours.
   a. *Note: when adding the media, Matrigel domes may float off plate. This does not affect organoid quality.*

Maintenance and patterning of organoids

1. Following 24 hours in Neural Induction media, gently remove as much media as possible from the well without disturbing Matrigel domes.
2. Add 3 mL of Neuroepithelial Expansion media (Table S10) to each well. Place plate in incubator for 48 hours.
3. After 48 hours in Neuroepithelial Expansion media, gently remove media and wash twice with $PBS^{-/-}$.
    a. *Note: Wash and media change should be performed under a dissection microscope to avoid organoid loss.*
4. After removing second $PBS^{-/-}$ wash, add 2mL Corning Cell Recovery Solution to each well of the 6 well plate. Gently swirl plate and place in incubator for 30 minutes to remove Matrigel from organoids.
    a. *Optional: To improve recovery, gently swirl plate every 5-10 min while in incubator.*
5. Following Matrigel removal, gently add 2 mL of Neuronal media (Table S10) to each well of the 6 well plate to dilute solution.
6. Using a wide orifice pipette tip, transfer the organoids to a freshly prepared low adherence 6 well plate containing 2 mL Neuronal media per well.
7. Using a wide orifice pipette tip, transfer the organoids to an additional freshly prepared low adherence 6 well plate containing 2 mL Neuronal media per well to ensure maximum dilution of Corning Cell Recovery solution.
8. Finally, using a wide orifice pipette tip, transfer organoids to a 10 cm Petri dish containing 12.5mL of Neuronal media and place on shaker.
    a. *Note: speed of shake will depend on the specific shaker being used. For Celltron benchtop shaker, 65 rpm was sufficient in preventing organoid merging while minimizing impact.*
9. Change media using fresh Neuronal media every other day until collection day.

Protocol: Immunostaining of mouse EpiSC-derived forebrain organoids (method derived from Dekkers et al., 2019)

Fixation and immunostaining of organoids

1. Coat a 15 mL falcon tube with 1% BSA to avoid organoid attachment and place on ice.
2. While on ice, gently add organoids to the pre-coated falcon tube and wash three times with 4°C $PBS^{-/-}$.
    a. *Note: Organoids at day 4 or earlier should be centrifuged at 70 g for 3 minutes at 4 ℃ for each wash step. Organoids later than day 4 will naturally settle to the bottom of the tube due to their size.*
3. To fix organoids, add 4% PFA to 15 mL falcon tube until organoids are completely submerged. Place on ice for 45 minutes and gently resuspend halfway through incubation to ensure proper fixation.
4. Following fixation, wash three times with $PBS^{-/-}$ containing 0.1% Triton-X (PBST) to remove any remaining PFA.

5. To permeabilize organoids, add PBS$^{-/-}$ containing 0.5% Triton-X until organoids are completely submerged and incubate for 15 minutes at 4°C.
6. Following 15-minute incubation, remove permeabilization buffer and block organoids for 15 minutes in organoid washing buffer (OWB) containing 0.2% Triton-X, 0.02% SDS and 0.2% BSA in PBS$^{-/-}$. Calculate appropriate volume of OWB by determining number of staining conditions (200 uL OWB per staining condition).
7. Equally distribute the organoids in a low-adhesion 24-well plate with a total of 200 uL OWB and desired number of organoids in each well. Each well will represent a single staining condition.
8. Following 15-minute blocking, directly add primary antibodies of interest prediluted in 200 uL OWB to each well for a total volume of 400 uL per well.
9. Place plate on shaker and incubate in primary antibodies overnight at 4°C.
10. Following overnight incubation, add 800 uL of OWB directly to each well and place on shaker at room temperature for 5 minutes.
11. Next, remove 1 mL of solution, replace with a fresh 1mL of OWB, and place on shaker for two hours at room temperature to wash organoids.
12. Repeat this wash step two additional times, for a total of 6 hours of washing.
13. After 6 hours of washing, remove 1 mL of OWB from each well and directly add 200 uL of prediluted secondary antibodies and DAPI (final concentration of 1 µM) in OWB, for a total volume of 400 uL per well.
14. Place on shaker and incubate overnight at 4°C.
15. Following overnight secondary antibody incubation, remove from 4°C, and add 800 uL of OWB with 1 µM DAPI per well. Place on shaker at room temperature and allow to shake for 5 minutes.
16. Next, remove 1 mL of OWB from each well and replace with a fresh 1 mL of OWB with 1 µM DAPI. Place on shaker at room temperature for 2 hours to wash organoids.
17. Repeat this wash step twice more, for a total of 6 hours of washing.

Preparation of slide for imaging

1. Using a standard hole puncher, punch a hole (0.5cm Ø) into a sticky silicone pad (20x20x1 mm).
2. Place sticky pad on imaging slide. (Figure S9)

Mounting of organoids for imaging

1. Using a wide orifice pipette tip, Transfer the organoids to a 1.5 mL Eppendorf.
2. Remove as much supernatant as possible without disturbing the organoids
    a. *Note: If the organoids at day 4 or less, centrifuge at 70 g for 3 minutes to pellet.*
3. Using a wide orifice pipette tip, add 35 uL of DeepClear solution, gently resuspend organoids, and transfer to prepared slide. Add a coverslip and image.

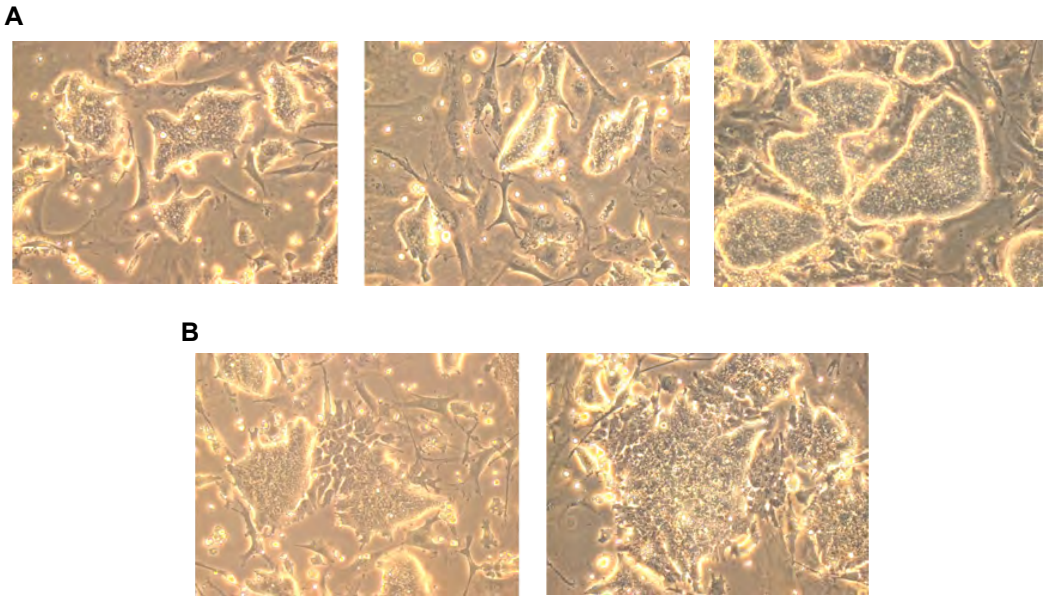


Fig. S8. EpiSCs in culture. (A) Standard culture of EpiSCs. (B) Spontaneous differentiation in EpiSC culture.

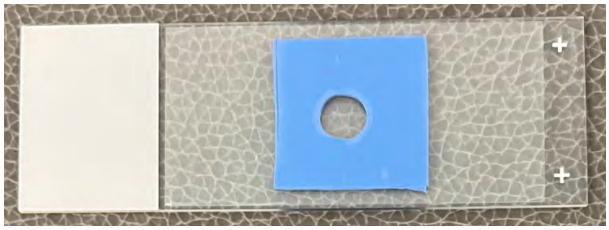


Fig. S9. Slide for organoid imaging.

Table S6. Plate sizes and suggested volumes for stem cell culture

Plate size	Surface area (cm^2)	Volume of collagenase, $PBS^{-/-}$ and Accutase (mL)	Media Volume (mL)
96 well	0.32	0.1	0.2
48 well	1.1	0.3	0.6
24 well	1.9	0.4	0.8
12 well	3.5	0.5	2
6 well	9.6	1	5
10cm dish	56.7	5	15

Table S7. Media composition for stem cell culture

MEF	ESC	EpiLC	EpiSC
MEF media	S/L media	N2B27	N2B27
	3uM CHIR99021*	12.5 ng/ml Heat stable recombinant human bFGF	12.5 ng/ml Heat stable recombinant human bFGF
	1uM PD0325901*	20 ng/ml Activin A	20 ng/ml Activin A
		1% KOSR	175 nM NVP-TNKS656

*Optional components to create 2i/SL culture conditions

Table S8. Plate sizes and suggested volumes for DE differentiation

Plate size	Surface area (cm^2)	Volume of laminin, collagenase, PBS (ml)	Media Volume (ml)	Number of cells per well
96 well	0.32	0.1	0.2	35,000
48 well	1.1	0.3	0.6	120,000
24 well	1.9	0.4	0.8	208,000
12 well	3.5	0.5	2	382,000
6 well	9.6	1	5	1,050,000
10cm dish	56.7	5	15	6,200,000

Table S9. DE differentiation media composition

Plating Media	Differentiation media 1	Differentiation media 2
CDM + 0.7 µg/ml insulin	CDM + 0.7 µg/ml insulin	CDM + 0.7 µg/ml insulin
12.5 ng/ml FGF	3 µM CHIR99021	100 ng/ml Activin A
20 ng/ml Activin	40 ng/ml Activin A	100 nM LDN-193189 (BMP inhibitor)
175 nM NVP-TNKS656		
1% Knockout Serum Replacement		
2 µM Thiazovivin		

Table S10. Media composition for neural organoids

EB Formation media	Neural induction media	Neuroepithelial expansion media	Neuronal media
N2B27 (B27 without vitamin A)	N2B27 (B27 without vitamin A)	N2B27 (B27 without vitamin A)	N2B27 (B27 with vitamin A)
50 nM chroman-1	100 nM LDN	100ng/ml Fgf8b	20ng/mL BDNF
5 µM emricasan	10 µM SB431542		20ng/mL GDNF
100 nM LDN	100 nM PD173074		
10 µM SB431542	4 nM LGK974		
100 nM PD173074			
4 nM LGK974			

MEF MEDIA: [**For 500ml**] Sterile filter and store at 4˚C

Solution (stock concentration)	Volume	Final concentration
DMEM w/ glutamine (1x)	445 mL	2 mM (L-glutamine)
Penicillin-streptomycin (100x)	5 mL	100 ug/mL
FCS (Hyclone)	50 mL	10%

SERUM/LIF MEDIA: [**For 500ml**] Sterile filter and store at 4˚C

Solution (stock concentration)	Volume	Final concentration
DMEM w/ glutamine (1x)	430ml	2mM (L-glutamine)
NEAA (100x)	5ml	0.1mM
Sodium pyruvate (100x)	5ml	1mM
Penicillin-streptomycin (100x)	5ml	100ug/ml
2-mercaptoethanol (1000x)	500ul	0.1mM
FCS (Hyclone)	50ml	10%
ESGRO LIF (100x)	5ml	1000U/ml

N2B27 MEDIA: [**For 500ml**] Sterile filter and store at 4˚C

Solution (stock concentration)	Volume	Final concentration
DMEM- F12	241ml	
Neurobasal medium	241ml	
N2 supplement	2.5ml	1:200
B27 supplement	5ml	1:100
GlutaMax	1.875ml	2mM
Penicillin-streptomycin (100x)	5ml	100ug/ml
2-mercaptoethanol (1000x)	500ul	0.1mM

CHEMICALLY DEFINED MEDIA (CDM): [**For 500ml**] Sterile filter and store at 4°C

Solution (stock concentration)	Volume	Final Concentration
IMDM (1x)	243 ml	50%
F12 with GlutaMAX (1x)	243 ml	50%
Chemically Defined Lipid Concentrate (100x)	5 ml	1x
Monothioglycerol (11.5 M)	19.3 µL	450 µM
Polyvinyl Alcohol (100 mg/mL)	5 ml	1 mg/mL
Apo transferrin (10 mg/mL)	750 µL	15 ug/mL
Glutamax (100x)	2.5 ml	100x
Insulin (10 mg/mL)	35 µL	0.7 µg/ml

Preparation of stock solutions

Activin A: Peprotech (120-14P)

- Reconstitute lyophilized protein to 100ug/ml in sterile H_2O with 0.1% BSA (5,000X solution)
- Aliquot and store at -80 C for up to 3 months.

Heat-stable bFGF2: Gibco (PHG0360)

- Reconstitute FGF to 1 g/L in sterile diH2O.
- Add sterile PBS + 0.1% BSA to dilute solution to 25 ug/ml (2,000X stock for a working concentration of 12.5 ng/mL).
- Store at -20 for up to 12 months.

NVP-TNKS656: Selleck chemicals (S7238)

- Dilute powder with DMSO to make a 35 mM solution (for 10 mg, add 577 µL DMSO)
- Dilute 35 mM stock further to 350 uM
- Aliquot 350uM stock (2,000X) and store at -80 C for up to 2 years.

Collagenase Type IV: Gibco (17104109)

- Add 10 ml of HBSS++ directly to the vial of collagenase.
- Vortex to complete dissolution.
- Determine final volume of HBSS++ required to bring collagenase solution to 5 u/µL (10X) & vortex.

- Filter to sterilize stock solution with a low protein binding filtration unit (PES Membrane, 0.2 µm).
- Aliquot and store at -20C for up to 24 months.
- Add HBSS++ to bring concentration to 5X before use.

Thiazovivin: Sigma (SML1045)

- Resuspend in DMSO to make 2mM stock (for 5 mg, 8.030 mL DMSO for a 1,000X solution).
- Aliquot and store at -20C for up to 6 months.

CHIR99021: Tocris Bioscience (252917-06-9)

- Resuspend in DMSO to make 5mM stock (for 10mg, 4.2979 mL DMSO for a 5,000X working solution).
- Aliquot and store at -20C.

Apo-transferrin: InVitra (777TRF029)

- Resuspend 1g in 100 mL diH2O, mix until complete homogenization and filter.
- This will give a 666 X stock solution. Aliquot and store at -80C.

Lif: Sigma (ESG1107)

- Each vial contains 10^7 units/mL. Dilute it 100 times in sterile tissue culture media to make a 100x solution, which is stored at 4C until use.

Insulin: Sigma (91077C)

- Take 250 mg, add 25 mL diH2O, leading to a cloudy solution.
- Add HCl until a pH=3.0 is achieved.
- Filter and store at 4C.

LDN-193189: Stemgent (04-0074)

- Dilute the powder (2 mg) with 22.75 mL DMSO to make a 200 µM solution (2,000X).
- Aliquot and store at -20C for up to 6 months.

PD0325901: Tocris (4192)

- Add 830 µL of DMSO to 10mg powder to make a 25 mM stock solution.
- Aliquot and store at -20C.

Polyvinyl alcohol (PVA): Sigma (341584)

- Within a clean, autoclaved bottle (around 2 L capacity), add 20 g of PVA and 200 mL diH2O (100 mg/mL stock, 100X).
- Autoclave (wet settings) with the cap slightly opened for 1-2h until the powder fully dissolves.
- Store at 4C and use it within the next 3 months.

Chroman 1: MedChem Express (HY-15392)

- Dilute the powder (5 mg) with 11.4548 mL DMSO to make a 1 mM solution (20,000X).
- Aliquot and store at -80C for up to 6 months.

Emricasan: Selleckchem (S7775)

- Dilute the powder (25 mg) with 877 µL of DMSO to make a 50mM solution (10,000X).
- Aliquot and store at -80C for up to 2 years.

SB-431542: Tocris (1614)

- Dilute the powder (10 mg) with 2.6 mL 100% ethanol to make a 200 µM solution (1,000X).
- Aliquot and store at -20C for up to 1 month.

PD-173074: STEMCELL technologies (72164)

- Dilute the powder (10 mg) with 19.09 mL of DMSO to make a 1 mM solution (10,000X).
- Aliquot and store at -80C for up to 2 years.

LGK-974: Selleck Chemicals (S7143)

- Dilute the powder (1 mg) with 63.06 mL DMSO, warmed at 50C in a water bath if needed to fully dissolve, to make a 40 µM solution (10.000X) .
- Aliquot and store at -80C for up to 2 years.

FGF8b: R&D systems (423-F8-025/CF)

- Dilute the powder (25 µg) in 250 uL PBS-/- to make a 1,000X solution, store at 4C for up to 3 months.

BDNF: Peprotech (450-02)

- Resuspend the powder (50 µg) in 2.5 mL PBS-/- with 0.1% BSA to make a 1.000X solution.
- Aliquot and store at -20C for up to a year.

GDNF: Peprotech (450-10)

- Resuspend the powder (50 µg) in 2.5 mL PBS-/- with 0.1% BSA to make a 1.000X solution.
- Aliquot and store at -20C for up to a year.

REFERENCES

Dekkers, J.F., Alieva, M., Wellens, L.M., Ariese, H.C.R., Jamieson, P.R., Vonk, A.M., Amatngalim, G.D., Hu, H., Oost, K.C., Snippert, H.J.G., et al. (2019). High-resolution 3D imaging of fixed and cleared organoids. Nat Protoc 14, 1756-1771.

Qian, X., Jacob, F., Song, M.M., Nguyen, H.N., Song, H., and Ming, G.L. (2018). Generation of human brain region-specific organoids using a miniaturized spinning bioreactor. Nat Protoc 13, 565-580.