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Reporting Summary

Nature Research 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 Research 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	Hi-Seq4000 ImageJ MultiClamp 700A amplifier (Molecular Devices) Axon Digidata 1550B digitizer (Molecular Devices) Clampex 11.0 software (Molecular Devices) SP8 confocal microscope (Leica) LI-COR Odyssey CLx imaging system (LI-COR)
Data analysis	The code used for this manuscript can be found at: https://github.com/dhglab/human_cortical_organoid_maturation Bioinformatic tools used picard tools v2.5.0, STAR v2.5.2b, rsem v1.3.0, GATK v3.3, HISAT 2.1.0, R 3.5.1 and 3.6.0 R packages used: cqn 1.28.0, fgsea 1.8.0, sva 3.30.0, edgeR 3.24.0, limma 3.38.2,WGCNA 1.68, EWCE 0.99.2, clusterProfiler 3.12.0 , ggplot2 v3.3.2, betareg 3.1-3 Western blots were quantified using Image Studio Lite (LI-COR).

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Gene expression data has been deposited in the Gene Expression Omnibus (GEO) under accession numbers GSE150122 and GSE150123. The data in this study are available on request from the corresponding author.

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	Six hiPSC lines were used in this paper (Supplementary Table 1 and 2). A total of 62 samples for RNA sequencing (from 4 individuals, 5 hiPSC lines) and a total of 50 samples for DNA methylation (from 5 individuals, 6 hiPSC lines) at 13 time periods were collected. For WB, 3 hiPSC were used, for electrophysiological experiments 2 hiPSC lines were used and for immunocytochemistry 4 hiPSC lines were used. No statistical methods were used to pre-determine sample sizes, but our sample sizes per time point are similar to those reported in previous publications (Pasca et al., Nature Methods, 2015; Sloan et al., Neuron 2017; Trevino et al., Science, 2020)
Data exclusions	The predetermined exclusion criteria were: For RNA-seq, samples were excluded if 3 standard deviations away from the mean standardized sample network connectivity. For electrophysiology experiments, cells which deviated by more than 15% in their access resistance during the course of the recording were not used in the analysis.
Replication	hiPSC lines were differentiated from 6 hiPSC lines derived from 5 individuals to assess reliability of the methods and maintained long term (previously described in Yoon et al., Nature Methods, 2019).
Randomization	hCS of similar diameter were randomly selected for experiments. Neurons for patching were randomly selected using a fluorescent reporter.
Blinding	This study did not include case-control comparison. For western blotting the investigators were not blinded to the differentiation status of the hCS samples. Patch clamping was performed blindly to the stage of differentiation of hCS.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

anti-BRN2 (Mouse, 1:500, Millipore, MABD51, clone 8C4.2, Lot#2375594)
anti-CTIP2 (Rat, 1:300, Abcam, ab18465, Lot#GR322373-7)
anti-FXR1 (B-2, Mouse, 1:50; Santa Cruz, sc-374148, Lot#B2018)
anti-GFAP (Rabbit, 1:1,000, Dako, Z0334, Lot#20073982)
anti-GFAP (Rat, 1:1,000, Thermo Fisher Scientific, Clone 2.2B10, 13-0300)
anti-HDAC2 (C-8, Mouse, 1:50, Santa Cruz, sc-9959, Lot#G1320),
anti-MAP2 (Guinea pig, 1:5,000, Synaptic Systems, 188004, Lot#2-26)
anti-SOX9 (Goat, 1:500, R&D Systems, AF3075)
anti-NR2A(GRIN2A) (Rabbit, 1:1000, Cell Signaling #4205)
anti-NR2B(GRIN2b) (Rabbit, 1:1000, Cell Signaling #4207)
anti-β-actin (Mouse, 1:50,000, Sigma, A5316)
anti-Synapsin1 (Rabbit, 1:1000, Cell Signaling, 5297S)
anti-Mouse IgG Polyclonal Antibody Goat, IRDye 680RD, 926-68070)
anti-Rabbit IgG Polyclonal Antibody (Goat, IRDye 800CW, 926-32211)

Validation

All antibodies were commercially available.
We have previously used and/or validated some of these antibodies (Pasca et al., Nature Methods 2015; Sloan et al., Neuron 2017; Trevino et al., Science 2020).
The anti-BRN2 antibody was validated in human neural cells (Trevino et al., Science 2020).
The anti-CTIP2 has been referenced in 401 publications according to the manufacturer's website, and validated in human neural cells (Pasca et al., Nature Methods 2015; Trevino et al., Science 2020).
The anti-FXR1 antibody has been referenced in 2 publications according to the manufacturer's website.
The anti-GFAP (Rabbit) has been used in 8 studies according to the manufacturer's website, and has been validated in human neural cells (Pasca et al., Nature Methods 2015; Sloan et al., Neuron 2017; Trevino et al., Science 2020).
The anti-GFAP (Rat) has been used in 130 publications according to the manufacturer's website and has been validated in human neural cells (Trevino et al., Science 2020).
The anti-HDAC2 has been used in 64 publications according to the manufacturer's website.
The anti-MAP2 has also been used in 71 studies for ICC and 13 studies for IHC according to the manufacturer's website, and has been validated in human neural cells (Pasca et al., Nature Methods 2015; Trevino et al., Science 2020).
The anti-SOX9 has been used in 36 studies according to the manufacturer's website, and has been validated in human neural cells (Trevino et al., Science 2020).
The rabbit anti-NMDAR-2A (Cell Signaling, #4205) was used in 38 publications according manufacturer's website.
The mouse anti-NMDAR-2B (Cell Signaling, #4207) was used in 31 publication according manufacturer's website.
The mouse anti-β-actin (Sigma, A5316) has been used in 1,670 publications according manufacturer's website, and tested for western blot analysis in human cells (Gabriel-Salazar et al., 2018).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Five hiPSC lines from were derived at Stanford University with IRB approval and following written consent; one line (H20961) was obtained from the Gilad lab at University of Chicago under an MTA.
Inactivated mouse fibroblasts EmbryoMax PMEF were purchased from EMD Millipore.

Authentication

All hiPSC lines were assessed for pluripotency, like previously described in Pasca et al, Nature Medicine 2011 or Yazawa et al., Nature 2011.
hiPSC lines were assessed for genomic integrity by SNP microarray "GSAMD–24v2–0" (with 759,993 probes).

Mycoplasma contamination

All cell lines and P-MEF cells were tested for Mycoplasma contamination and tested negative

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used