

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 [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
- ☒ ☐ An indication of 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 statistics 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
- ☒ ☐ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection

Publicly available ZEN Blue/Black 2012 and Volocity 4.1 software were used during imaging.

Data analysis

For the single cell data, we used the publicly available Cell Ranger 2.0.1 pipeline (10X Genomics), the Seurat R package v2.3.4, Scrublet python package v0.1, and the Monocle R package v2.99.1 for analysis. Custom code used in conjunction with these packages for data analysis is available on GitHub at <https://github.com/AmandaKedaigle/BrainOrganoidsReproducibility>.

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/reviewers upon request. 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

Single-cell RNA-seq data that support the findings of this study have been deposited at Gene Expression Omnibus, accession number GSE129519, and at the Single

Cell Portal (portals.broadinstitute.org/single_cell/study/reproducible-brain-organoids). The reference datasets used for comparison are available in the Gene Expression Omnibus at accession numbers GSE86153, GSE116470, and GSE103723, or in dbGaP at accession phs000989.v3, and phs000424.v8.p1.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	A total of 21 single organoids collected at 3 and 6 months were profiled by single-cell RNA-seq. Individual organoids were sequenced separately. The number of cells sampled were chosen to maximize the availability of the sequencing lane per organoid. A total of 82 single organoids collected at multiple time points were analyzed by immunohistochemistry (IHC). No methods were used to predetermine sample size.
Data exclusions	All 21 organoids that were sequenced are reported and the data has been made available. Droplets excluded by default Cell Ranger pipeline parameters, as well as droplets expressing less than 500 genes, were excluded from analysis. This is common practice to avoid empty droplets, and thresholds were determined before data analysis began. All other cells were included in the analysis. For the IHC data, we showed representative images of gene marker expression across multiple cell lines and also a summary table of all the stainings performed to assess protocol efficiency.
Replication	About 95% of the organoids profiled by single-cell RNA-seq, derived from 4 different cell lines, from 6 independent experimental batches (PGP1: 3 batches, HUES66, GM08330, and 11a: 1 batch each) efficiently generated dorsal forebrain cell types. About 90% of organoids analyzed by IHC, derived from 5 different lines, from 8 independent experimental batches (PGP1: 3 batches, HUES66: 2 batches, GM08330, 11a, and Mito 210: 1 batch each) expressed dorsal forebrain markers. Statistics and reproducibility of all the organoids analyzed by IHC is reported in Methods. Organoids derived from the H9 cell line didn't show the typical morphology during the early stages of neural differentiation and further optimization might be required for their culture.
Randomization	Samples were not randomized to different groups, as there was no such experimental groups in this study.
Blinding	Not relevant, as there were no such experimental groups in this study.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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

Unique biological materials

Policy information about [availability of materials](#)

Obtaining unique materials All unique materials used are readily available from the authors.

Antibodies

Antibodies used	Primary antibodies: Rabbit Cleaved Caspase 3 (Asp 175) (Cell Signaling Technologies 9661, 1/300) Rat CTIP2 [25B6] (Abcam AB18465, 1/100) Rabbit EMX1 (Atlas Antibodies HPA006421, Lot: E114426, 1/50)
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Rabbit FOXG1 [EPRI8987] (Abcam 196868, Lot: 196779-2, 1/100)
 Mouse GFAP (Sigma-Aldrich G3893, Lot: 038M4864V, 1/400)
 Rabbit HOPX (Sigma-Aldrich HPA030180, 1/2,500)
 Mouse Ki67 (BD Biosciences 550609, Lot: 4127608, 1/400)
 Chicken MAP2 (Abcam AB5392, 1/5,000)
 Rabbit PAX6 [19013] (Biolegend 901301, 1/400)
 Rabbit PSD95 (Thermo Fisher 51-6900, Lot: SL257842, 1/350)
 Rabbit S100B (Abcam AB41548, 1/2,000)
 Mouse SATB2 [SABTA4B10] (Abcam AB51502, 1/50)
 Goat SOX2 (RD Systems AF2018, 1/50)
 Rabbit TBR1 (Abcam AB31940, Lot: GR3182037-1, 1/500)
 Rabbit TBR2 (Millipore AB2283, Lot: 3012123, 1/2,000)
 Guinea Pig VGluT1 (Millipore AB5905, Lot: 3076459, 1/2,000)

Secondary antibodies:

Except for staining of synaptic markers PSD95 and VGluT1, when secondary antibodies were diluted 1/750, all secondary antibodies were diluted 1/1,200.

Alexa Fluor donkey anti-rabbit 647, 546 (Life Technologies A31573, A10040)
 Alexa Fluor donkey anti-mouse 546, 488 (Life Technologies A10036, A21202)
 Alexa Fluor donkey anti-goat 647 (Life Technologies A21447)
 Alexa Fluor donkey anti-rat 488 (Life Technologies A21208)
 Alexa Fluor donkey anti-chicken 488,647 (Jackson ImmunoResearch Laboratories 703-545-155, Millipore AP194SA6)
 Alexa Fluor donkey anti-guinea pig 647 (Jackson ImmunoResearch Laboratories 706-605-148)

Validation

According to the manufacturer's website:

The rabbit Cleaved Caspase 3 antibody (Cell Signaling Technologies, 9661) is reactive to human tissue, and has been cited in 3,488 publications.

The rat CTIP2 antibody (Abcam, AB 18465) has been cited in 295 publications, including Quadrato et al., 2017, from our lab, which used this antibody on human brain organoids.

The rabbit EMX1 antibody (Atlas Antibodies, HPA006421) has been cited in 8 publications, including Lancaster et al., 2013 and Quadrato et al., 2017, which used this antibody on human brain organoids.

The rabbit FOXG1 antibody (Abcam, 196868) is reactive to human.

The mouse GFAP antibody (Sigma-Aldrich, G3893) is reactive to human, and has been cited in 541 publications.

The rabbit HOPX antibody (Sigma-Aldrich, HPA030180) is reactive to human, and has been cited in 10 publications.

The mouse Ki67 antibody (BD Biosciences, 550609) is reactive to human, and has been cited in 14 publications.

The chicken MAP2 antibody (Abcam, AB5392) is reactive to human, and has been cited in 277 publications.

The rabbit PAX6 antibody (Biolegend, 901301) is reactive to human, and has been cited in 73 publications.

The rabbit PSD-95 antibody (Thermo Fisher, 51-6900) is reactive to human, and has been cited in 46 publications.

The rabbit S100b antibody (Abcam, 41548) has been cited in 32 publications, including Bedner et al., 2015, which used this antibody in human hippocampal tissue.

The mouse SATB2 antibody (Abcam, AB51502) is reactive to human and has been cited in 99 publications.

The goat SOX2 antibody (RD Systems, AF2018) is reactive to human and has been cited in 72 publications.

The rabbit TBR1 antibody (Abcam AB31940) is reactive to human and has been cited in 190 publications.

The rabbit TBR2 antibody (Millipore AB2283) is reactive to human and has been cited in 8 publications.

The guinea pig VGluT1 antibody (Millipore AB5905) has been cited in over 55 publications, including Pasca et al., 2015, which used this antibody in human brain organoids.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

The PGP1 (Personal Genome Project 1) hiPSC line was from the lab of George Church (Harvard University); the HUES66 hESC and the 11a hiPSC lines from the Harvard Stem Cell Institute; the GM08330 hiPSC line from the lab of Michael Talkowski (MGH Hospital); and the Mito 210 line from the Cohen lab (McLean Hospital).

Authentication

The PGP1 line was authenticated using STR analysis completed by TRIPath (2018), the HUES66 line was authenticated using STR analysis completed by GlobalStem Inc (2008). For authentication of the 11a cell line refer to Quadrato et al., Nature 2017. The GM08330 line was not authenticated. The Mito 210 line was authenticated by genotyping analysis (Fluidigm FPV5 chip) performed by the Broad Institute (2017).

Mycoplasma contamination

All cell lines tested negative for mycoplasma contamination.

Commonly misidentified lines (See [ICLAC](#) register)

No misidentified lines were used in the study.