

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

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

Data collection	MaxLab Live (version 19.2.19, 22.2.6, 22.2.8, 25.1.6.1), Maxwell Biosystems
Data analysis	MATLAB (MathWorks), Python, R; SpikeInterface 0.13.0 in Python (https://github.com/SpikeInterface); custom MATLAB (version 2018b), Python (version 3.6 and 3.11) and R (version 4.2.1) code for MEA analysis as described in methods is here: https://github.com/braingeneers/Protosequences .

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](#)

The data supporting the findings of this study are available within the article and its supplementary information. Raw and curated electrophysiology recordings can

be found here <https://dandiarchive.org/dandiset/001603>. scRNA-seq data have been deposited and are publicly available in the NCBI Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo>) under accession GSE290330.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	Sample size for organoid and primary culture recordings were determined by the data available in Sharf et al. and Alam El Din et al. Murine organoids were set by the number of available organoids. Acute murine slice recordings were set by the number of available pups.
Data exclusions	Due to intrinsic neurophysiological variability occurring during brain organoid development, organoids exhibited a range in the number of active spiking units. Organoids with less than 20 units were not considered for recordings in the murine, Sharf et al. and Alam El Din et al. data. In order to compare the same number of burst events across all samples the first x minutes of the recordings were used so each recording had a similar number of burst events.
Replication	All experiments were performed with sufficient biological replicates across biological models. Additionally, experimental approaches are documented with sufficient detail for precise replication.
Randomization	Single-unit spike times were randomized to preserve both each neuron's mean firing rate and the population-averaged firing rate distribution. The resulting spike matrix contained an equal number of spikes per unit (same average firing rate) and per frame (same population rate). For murine cortical brain slice experiments, neonatal pups of both sexes were selected at random, and slices from the somatosensory cortex were chosen randomly prior to acute electrophysiology. Murine cortical organoids were also selected randomly for electrophysiological recordings.
Blinding	Human and murine brain organoids and murine cortical slices were grown or prepared in separate laboratories, so sample collection could not be blinded. Data analyses were performed blind to the conditions of the experiments.

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 used for murine organoids: rabbit anti-Map2 (Proteintech # 17490-1-AP, 1:2000); mouse anti-Pax6 (BD Biosciences # 561462, 1:100); rabbit anti-Nkx2.1 (Abcam # ab76013, 1:400); rat anti-Ctip2 (Abcam # ab18465, 1:250); rabbit anti-Brn2 (ThermoFisher # PA530124, 1:400); anti-Gaba (Thermo Fisher Scientific # PA5-32241, 1:375). Secondary antibodies were of the Alexa series (Thermo Fisher Scientific), used at a concentration of 1:750. Nuclear counterstain was performed using 1.0 µg/ml Hoechst 33342 (ThermoFisher # H1399)

Primary antibodies used for human organoids: mouse anti-Gephyrin (Synaptic Systems #147 011, Monoclonal Mouse IgM Clone mAb7a ,1:1000); chicken anti-MAP2 (Invitrogen #PA1-10005,1:5000), rabbit anti-GFAP (Dako #Z0334, 1:400); mouse anti-O4 (R&D #MAB1326, Monoclonal Mouse IgM Clone # O4, 1:200)

Validation

Antibodies used for murine cortical organoids:

MAP2 (Proteintech 17490-1-AP): Microtubule-associated protein 2 (MAP2) is a tubulin binding protein regulating the spacing and stability of microtubules and contributing to elongation of dendrites. MAP2 has multiple isoforms that arise from alternative splicing (PMID: 3121794, 7854050, and 10383434). They are classified into two groups - MAP2A and MAP2B, which are known as high molecular weight (HMW) isoforms, run as ~280 kDa species, while low molecular weight (LMW) isoforms MAP2C and MAP2D are around ~70 kDa. MAP2 proteins are heavily phosphorylated, which contributes to a large discrepancy between their predicted and observed molecular weight in SDS-PAGE (220 vs 280 kDa for HMW forms). In neurons, MAP2 proteins are found in the cell body and dendrites, where they associate with microtubules, while they can also be present in the nuclei of testicular cells.

Tested Reactivity: human, mouse, rat

Host / Isotype: Rabbit / IgG

Class: Polyclonal

Calculated Molecular Weight: 200 kDa

Observed Molecular Weight: 280 kDa, 70-85 kDa

Purification Method: Antigen affinity purification

Pax-6 (BD Biosciences 561462): Pax-6 is a member of the paired box (pax) gene family whose protein products are transcription factors involved in development. Pax family members share a highly conserved DNA binding domain that contains six alpha helices (paired domain) and a homeo box domain. Pax-6 has important roles in the development of the eye, nose, central nervous system, and pancreas. Tissue culture supernatant is purified by either protein A/G or affinity purification methods. Both methods yield antibody in solution that is free of most other soluble proteins, lipids, etc. This format provides pure antibody that is suitable for a number of downstream applications including: secondary labeling for flow cytometry or microscopy, ELISA, Western blot, etc.

Reactivity: Human (QC Testing)

Isotype: Mouse BALB/c IgG2a, κ

Clone: O18-1330

Immunogen: Human Pax-6 aa 406-422 Peptide

Molecular Weight: 46-48 kDa

Nkx2.1 (abcam ab76013): Nkx2.1 is a transcription factor with a molecular mass of approximately 38 kDa. It plays a significant role in regulating gene expression. This target is mainly expressed in tissues such as the thyroid lung and the brain.

Host species: Rabbit

Clonality: Monoclonal

Clone number: EP1584Y

Isotype: IgG

Reacts with: Mouse, Rat, Human

Purification technique: Affinity purification Protein A

Observed Molecular Weight: 40 kDa

CTIP2 (Abcam ab18465): CTIP2, also known as BCL11B, is a transcription factor crucial in neuro research for its role in neural development and differentiation. It is particularly important for the development of medium spiny neurons (MSNs) in the striatum, which are essential for motor control and are affected in conditions like Huntington's disease. CTIP2 regulates gene expression involved in neuronal differentiation, axon guidance and synaptic connectivity, making it vital for understanding the cellular architecture and function of the nervous system.

Isotype: IgG2a

Host species: Rat

Clonality: Monoclonal

Clone number: 25B6

Purification technique: Multi-step chromatography

Specificity: Detects 2 bands representing Ctip2 at about 120kD. Ctip2 is highly expressed in brain and in malignant T-cell lines derived

from patients with adult T-cell leukemia/lymphoma
 Predicted band size: 96 kDa
 Observed band size: ~100 kDa

Brn2 (ThermoFisher PA530124): This gene encodes a member of the POU-III class of neural transcription factors. The encoded protein is involved in neuronal differentiation and enhances the activation of corticotropin-releasing hormone regulated genes. Overexpression of this protein is associated with an increase in the proliferation of melanoma cells.

Species Reactivity: Chimpanzee, Human, Mouse, Non-human primate, Rat

Host/Isotype: Rabbit / IgG

Class: Polyclonal

Immunogen: Recombinant protein encompassing a sequence within the C-terminus region of human Brn2.

Purification: Antigen affinity chromatography

Gaba (Thermo Fisher Scientific PA5-32241): This chemical acts as a neurotransmitter in the central nervous system. It reduces neuronal excitation and regulates muscle tone.

Species Reactivity: Chemical, Human, Mouse

Host/Isotype: Rabbit / IgG

Class: Polyclonal

Immunogen: GABA.

Purification: Antigen affinity chromatography

Antibodies used for human brain organoids:

Gephyrin (Synaptic Systems 147 011): Gephyrin is a scaffolding protein associated with inhibitory neurotransmitter receptors

Clone: mAb7a

Subtype: IgG1 (κ light chain)

Immunogen: Nativ Protein corresponding to AA 1 to 768 from rat Gephyrin

Epitop: AA 264 to 276 from rat Gephyrin

Reactivity: human, rat, mouse, pig, goldfish, zebrafish, chicken

Specificity: Specific for the brain specific 93 kDa splice variant phosphorylated at Ser-270. validated PubMed: 9812897

MAP2 (Invitrogen PA1-10005): MAP2 (Microtubule Associated Protein 2) exists in two high molecular weight forms (MAP2a & MAP2b) and a low molecular weight form. The expression of MAP2 is developmentally regulated and its multiple forms arise by alternative splicing of a single gene. MAP2 is involved in microtubule assembly, which is an essential step in neurogenesis. The products of similar genes in rat and mouse are neuron-specific cytoskeletal proteins that are enriched in dendrites, implicating a role in determining and stabilizing dendritic shape during neuron development.

Species Reactivity: Human, Mouse, Rat

Published species: Human, Mouse, Pig, Rat

Host/Isotype: Chicken / IgY

Class: Polyclonal

Immunogen: Mix of recombinant human constructs of projection domain sequences, amino acids 235-1588.

Western blot was performed using Anti-MAP2 Polyclonal Antibody (Product # PA1-10005) and a 160 kDa band corresponding to MAP2 was observed across Mouse Brain and Rat Brain and not in Mouse Heart and Mouse Skeletal Muscle.

Observed Molecular Weight: ~160 kDa

GFAP (Dako Z0334): GFAP (Glial Fibrillary Acidic Protein) is a key marker in neuro research, particularly for its role in identifying astrocyte activity. It is predominantly expressed in astrocytes, the most abundant glial cells in the brain and spinal cord.

Clone: polyclonal

Immunogen: GFAP isolated from cow spinal cord

Species: Rabbit Anit-

Specificity: The antibody has been solid-phase absorbed with human and cow serum proteins. In crossed immunoelectrophoresis using 50 µL antibody per cm² gel area, no reaction with 2 µL human plasma and 2 µL cow serum is observed. The antibody shows one distinct precipitate (GFAP) with cow brain extract. Staining: Coomassie Brilliant Blue. In indirect ELISA, the antibody shows no reaction with human plasma and cow serum. GFAP shows 90-95% homology between species, and as demonstrated by immunohistochemistry, the antibody reacts strongly with human GFAP.

O4 (R&D MAB1326): Oligodendrocyte Marker O4. Oligodendrocytes are myelinating cells in the central nervous system (CNS) and form the myelin sheath of axons to support rapid nerve conduction. Oligodendrocyte Marker O4 is an antigen on the surface of oligodendrocyte progenitors (1, 2). It has been commonly used as the earliest recognized marker specific for the oligodendroglial lineage

Species Reactivity: Human/Mouse/Rat/Chicken

Specificity: Detects human, mouse, rat, and chicken Oligodendrocyte Marker O4.

Source Monoclonal: Mouse IgM Clone # O4

Purification: IgM-specific Affinity-purified from hybridoma culture supernatant

Immunogen: Bovine brain corpus callosum white matter

Eukaryotic cell lines

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

Cell line source(s)

Three mouse embryonic stem cell lines were used: (1) C57BL/6 genetic background (BRUCE-4 ES Cells; Millipore Sigma); (2) E14TG2a (ATCC deposited by T. Doetschman), and (3) KH2 (Jaenisch; <https://doi.org/10.1002/gene.20180>).
 Two human iPSC lines used: (1) F12442.4 (Karch; <https://doi.org/10.1186/s13195-018-0400-0>); (2) NIBSC8 (National Institute for Biological Standards and Control, NIBSC, UK).

Authentication

Mouse ESCs were characterized using standard methods, and validated using qPCR and immunohistochemistry to assess pluripotency and correct cell type patterning. Additional single cell and bulk RNA sequencing was performed to authenticate pluripotency and correct cell type patterning of our organoids. Human iPSC line 1 was analyzed for pluripotency markers and chromosomal abnormalities by G-band karyotyping and were confirmed to possess the appropriate genotype by Sanger sequencing. Human iPSC line 2 was analyzed for genetic stability every 10 passages using the qPCR-based hPSC Genetic Analysis kit (StemCell Technologies™) and authenticated by Short Tandem Repeat profiling.

Mycoplasma contamination

Mycoplasma testing was routinely performed on our ESCs, iPSCs and organoids and all tested negative

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

No commonly misidentified cell lines were used.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

mouse C57BL/6JrJ from Janvier Labs (P12-14; both sexes)

Wild animals

n/a

Reporting on sex

Neonatal cortical slices were used from both sexes. We do not consider sex as a factor in our analyses, but results apply to all animals studied.

Field-collected samples

No field collected samples were used in the study

Ethics oversight

All experiments involving murine neonatal acute slice recordings were approved by the Basel-Stadt veterinary office according to Swiss federal laws on animal welfare.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a