nature portfolio

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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	LAS-X (Leica) software was used for acquiring the microscopy data. Image Studio (V5.2) was used to acquire western blotting data. QuantStudio Real-Time PCR software (V1.7.1) was used to collect qPCR data. RHX software (V3.1.0) was used for acquiring the extracellular recording data. Infinity Capture software (Teledyne Lumenera) and Clampex (v10.7) was used for patch clamp recording.
Data analysis	The GraphPad Prism (V10.0.0) was used for statistical analyses. Fiji (ImageJ, V2.14.0) was used for image quantification and processing. MATLAB(R2022a and R2023a) was used to analyze calcium imaging and extracellular recording data. The codes for scaled correlation analysis (SCA) were described in Miura et al., bioRxiv (2024) and have been deposited at Zenodo (DOI: 10.5281/zenodo.13921410). ImarisViewer (V10.0.1) was used to process immunostaining images. CellRanger (7.0.1, 7.1.0,7.2.0, 10x Genomics), R (4.2.2), and Seurat (V4.3.0, R package) were used for single-cell RNA-seq analysis. Clampfit (v10.7) was used to analyze patch-clamp recording data.

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

Source data were provided with this manuscript. Single cell RNA-seq data is available in GEO under accession number GSE224766. The following datasets were used in this study: GRCh38/Ensembl 98 reference for Cell Ranger (https://cf.10xgenomics.com/supp/cell-exp/refdata-gex-GRCh38-2020-A.tar.gz); single cell RNA-seq data of hCO by Khan et al. (GSE145122); single cell RNA-seq data of hDiO by Kim et al. (GSE224766); human fetal spinal cord single cell RNA-seq data by Rayon et al. (GSE171892); human fetal brain single cell RNA-seq data by Braun et al. (https://github.com/linnarsson-lab/developing-human-brain).

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 sizes were estimated empirically, based on previous studies (Birey et al., Nature 2017; Marton et al., Nature Neuroscience; Pasca et al, Nature Medicine 2019; Yoon et al., Nature Methods 2019; Miura et al., Nature Biotechnology 2020; Birey et al., Cell Stem Cell 2022; Meng et al., 2023, Chen et al, Nature 2024).
Data exclusions	Organoids or assembloids displaying no activity were not included
Replication	Data shown in representative experiments were repeated with similar results in multiple independent experiments. Sample sizes for other experiments were indicated in Figure legends.
Randomization	Organoids and assembloids were randomly picked from cell culture plates for each assay.
Blinding	Blinding of experimental conditions was used for calcium imaging analysis, projection imaging analysis of assembloids. Blinding was not used or was not relevant for other experiments due to constraints in sample processing.

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

Anti-GAPDH (mouse, GeneTex, GTX627408, 1:5000 dilution), lot#41323 Anti-FOXG1 (rabbit, Takara, M227, 1:400 dilution), lot#AM62662A
Anti-TCF7L2 (rabbit, Cell Signaling Technology, 2569S, 1:200 dilution), lot#4Ref07/2019
Anti-PHOX2A (rabbit, Abcam, ab155084, 1:200 dilution), lot#GR117345-3
Anti-BRN3A (mouse, Millipore, MAB1585, 1:200 dilution), lot#3728208
Anti-mCherry (goat, Biorbyt, orb153320, 1:1000 dilution), lot#VS2124
Anti-NK1R (rabbit, Sigma, S8305, 1:200 dilution), lot#UD280591
Anti-SCN9A (rabbit, 1:1,000, Alomone Labs, ASC-008), lot#3728208
Anti-VGLUT2 (mouse, 1:200, Millipore, MAB5504)
Anti-NF-H (mouse, 1:200, Millipore, NE1023)
Anti-PAX2 (goat, 1:200, R&D Systems, AF3364), lot# XOT0823051

Goat Anti-Mouse IgG Polyclonal Antibody (IRDye 680RD, 1:10,000, LI-COR Biosciences, 926-68070)
Goat Anti-Rabbit IgG Polyclonal Antibody (IRDye 800CW, 1:10,000, LI-COR Biosciences, 926-32211)
Alexa Fluor 647 AffiniPure donkey anti-rabbit IgG (H&L) (1:1,000, Jackson ImmnunoResearch, 711-605-152)
Alexa Fluor 568 donkey anti-goat IgG (H&L) (1:1,000, Thermo Fisher Scientific, A11057)
Alexa Fluor 568 donkey anti-rabbit IgG (H&L) (1:200, Thermo Fisher Scientific, A10042)
Alexa Fluor 647 donkey anti-mouse IgG (H&L) (1:200, Thermo Fisher Scientific, A31571)
Alexa Fluor 568 donkey anti-mouse IgG (H&L) (1:1,000, Thermo Fisher Scientific, A10037)

Validation

Anti-GAPDH: This antibody has been used in at least several hundreds of studies according to the manufacture's website.
Anti-FOXG1: This antibody has been used by several studies, including Iwata et al., Science, 2020; Ideno et al., iScience, 2022.
Anti-TCF7L2: This antibody has been used in more than 150 studies according to the manufacture's website.
Anti-PHOX2A: This antibody has been used by Roome et al., Cell Reports, 2020.
Anti-BRN3A: This antibody has been used in more than 50 studies according to the manufacture's website.
Anti-mCherry: This antibody was used in our previous study (Andersen et al., Cell, 2020).
Anti-NK1R: This antibody has been used in more than 60 studies according to the manufacture's website.
Anti-SCN9A: This antibody has been used in more than 100 studies according to the manufacture's website.
Anti-VGLUT2: This antibody was used in our previous study (Kim et al., Neuron 2024).
Anti-NF-H: We have used this antibody in our previous study (Andersen et al., Cell, 2020). This antibody has been validated and used in 76 studies according to the manufacture's website.
Anti-PAX2: This antibody has been used by Roome et al., Cell Reports, 2020.

Goat Anti-Mouse IgG Polyclonal Antibody: We have used this antibody in our previous study (Birey et.al., Cell Stem Cell, 2022). Based on the manufacture's technical note, this antibody has been validated and used in multiple studies.
Goat Anti-Rabbit IgG Polyclonal Antibody: We have used this antibody in our previous study (Birey et.al., Cell Stem Cell, 2022). Based on the manufacture's technical note, this antibody has been validated and used in multiple studies.
Alexa Fluor 647 AffiniPure donkey anti-rabbit IgG (H&L): This antibody has been used in 659 studies according to the manufacture's website.
Alexa Fluor 568 donkey anti-goat IgG (H&L) : This antibody has been used in 855 studies according to the manufacture's website.
Alexa Fluor 568 donkey anti-rabbit IgG (H&L): This antibody has been used in 1538 studies according to the manufacture's website.
Alexa Fluor 647 donkey anti-mouse IgG (H&L): This antibody has been used in 1813 studies according to the manufacture's website.
Alexa Fluor 568 donkey anti-mouse IgG (H&L): This antibody has been used in 898 studies according to the manufacture's website.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Four hiPS cell lines (1205-4, 1208-2, 8119-1, and 0410-1) were reprogrammed at Stanford University from fibroblasts harvested with IRB approval and following written consent. These hiPS cell lines were reported our previous publication (Khan et al., Nature Medicine, 2020)
KOLF2.1J was derived at Jackson Laboratory and the line was obtained under an inter-institutional MTA.

Authentication

The hiPS cell lines (1205-4, 1208-2, 8119-1, 0410-1, and KOLF2.1J) were authenticated by SNP arrays.

Mycoplasma contamination

All cell lines were regularly tested and maintained Mycroplasma free.

Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines 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	E13.5 CD1 embryos were used in this study without knowing the sex of the embryos.
Wild animals	No wild animals were used in the study.
Reporting on sex	Mouse embryos were used in the experiment without knowing the sex of the embryos.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	Animal work was performed under protocols approved by the Stanford University's Administrative Panel on Laboratory Animal Care (APLAC).

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.*