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	Image analyses data were collected with Fiji- ImageJ, CellProfiler Image Analysis Software v.2,2 and Nikon's NIS-Elements AR v5.11.

Data analysis	qPCR data were analyzed by CFX Manager Software (Bio-Rad).
	Statistics were performed by GraphPad PRISM software v. 6.
	
	Here, we list all the packages employed with the respective documentation pages.
	- Pre-processing of raw scRNAseq data into count matrices:
	  - Cellranger 10x Genomics v2.1.1
	  - [Cellranger Documentation](https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger)
	
	- Downstream analysis of scRNAseq, including filtering, normalization, clustering, etc:
	  - Scanpy v1.7.1-v1.8.2 (Wolf et al. 2018)
	  - [Scanpy GitHub](https://github.com/theislab/scanpy)
	
	- Trajectory analysis:
	  - Velocyto v.0.17.17 (La Manno et al., 2018)
	  - [Velocyto GitHub](https://velocyto.org/velocyto.py/index.html)
	  - scVelo v.0.2.3 (Bergen et al., 2020)
	  - [scVelo GitHub](https://github.com/theislab/scvelo)
	  - Monocle3 v.0.1.0 (Cao et al., 2019)
	  - [Monocle3 Documentation](https://cole-trapnell-lab.github.io/monocle3/docs/trajectories/)

- Cell Scoring and Cell-Cell Communication analysis:
  - Scoring Cells (Della Chiara et al., 2021)
    - [Della Chiara GitHub](https://github.com/paganilab/DellaChiara_et_al_2021/tree/main/sc_RNAseq/scr)
  - CellChat v.1.1.1 (Jin et al., 2021)
    - [CellChat GitHub](https://github.com/sqjin/CellChat)
  - NeuronChat v.1.0.0 (Zhao et al., 2023)
    - [NeuronChat GitHub](https://github.com/Wei-BioMath/NeuronChat)

- Transcription Factor Activity Inference, and Gene Ontology/Gene Set Enrichment Analysis:
  - Decoupler v1.6.0 (Badia-i-Mompel et al., 2022)
    - [Decoupler GitHub](https://github.com/saezlab/decoupler-py)
  - TopGO v2.44.0 (Alexa et al., 2007)
    - [TopGO GitHub](https://github.com/ycl6/topGO-feat)
  - GSEApy v1.1.1 (Fang et al., 2022)
    - [GSEApy GitHub](https://github.com/zqfang/GSEApy)

- Comparisons with Human Fetal Datasets:
  - CellTypist v1.6.2 (Domínguez Conde et al., 2022)
    - [CellTypist GitHub](https://github.com/Teichlab/celltypist)
  - CellHint 0.1.1 (Xu et al., 2023)
    - [CellHint GitHub](https://github.com/Teichlab/cellhint)
  - VoxHunt v1.0.1 (Fleck et al., 2023)
    - [VoxHunt GitHub](https://github.com/quadbio/VoxHunt)

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

All scRNA-seq data have been deposited in the ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress/) under accession no E-MTAB-12924. All other data are present in the main paper or the supplement.

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	In this study 5 hESC RUES2 lines were used in total, which are all female lines. All features regarding the human pluripotent stem cell lines are summarized in Methods "Culture of human pluripotent stem cells". No active enrollment has been performed for this study.
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	The use of RUES2 human embryonic stem cell lines and derivatives in the laboratory has been approved by the ethical committee of the University of Milano

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	Replicates are indicated specifically in each Figure legend. In general, The sample size was not predetermined using any specific methods. However, prior research showed that organoids are reproducible and confirmed that using three organoids adequately captured the experiment's variability (https://www.nature.com/articles/s41586-021-04358-6, https://www.nature.com/articles/s41586-019-1289-x, https://www.cell.com/cell/pdf/S0092-8674(22)01168-0.pdf). Specifically, for the single cell RNA sequencing a total of 46 organoids were used. We sequenced 6 individual organoid (3 per genotype) at DIV 45 and 8 samples (4 pools of 10 organoids that are FACS sorted, with 2 cell lines per genotype) at DIV 120. Immunohistochemistry and brightfield images (such as Fig 1B-C, 2B and D, 2J-K, and 4A) derived from three independent experiments with similar results, collecting at least 12 individual organoids per time point. For SEM analysis, six individual organoids from independent experiments were analyzed. For qPCR pools of four organoids from two to five independent experiments were used.
Data exclusions	No organoids were excluded from scRNAseq analyses. In immunohistochemistry and qPCR quantifications only outlier values were excluded.
Replication	For all analyses were considered three or more individual organoids from batches of independent differentiation experiments. Details are indicated in the sample size and specifically in each Figure legend.
Randomization	Samples were not randomized because pathological HD lines were paired with their isogenic control from the same differentiation batches.
Blinding	Investigators were not blinded. All analyses were performed equally to all samples without adjustments for genotypes.

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

ARL13B (Rabbit, 1:500, Abcam, cat. n ab83879),
ASCL1 (mouse, 1:500; Becton Dickinson, cat. n. 556604),
BASSOON (Mouse, 1:500, Enzo Life Sciences, cat. n. ADI-VAM-PS003-F),
BLBP (Rabbit, 1:500; Millipore, cat. n. ABN14),
βIII-TUBULIN (rabbit, 1:1,000; BioLegend, cat. n. 802001),
CASPASE-3 (Rabbit, 1:400, Cell Signaling, cat. n. 9661),
CTIP2 (rat, 1:500; Abcam, cat. n. ab18465),
EBF1 (Mouse, 1:1000; Santa Cruz, cat. n. sc-137065),
GABA (Rabbit, 1:500; Sigma, cat. n. A2052),
GAD67 (Mouse, 1:1000, Millipore, cat. n. MAB5406),
GFP (chicken, 1:1000, Abcam, cat. n. ab13970),
GSX2 (rabbit, 1:200, GeneTex, cat. n. gtx129390),
HOMER1 (Rabbit, 1:300, Genetex, cat. n. GTX103278),
ISLT1/2 (mouse, 1:1,000; Hybridoma Bank, cat. n. 39.4D5),
KI67 (rabbit, 1:500; Abcam, cat. n. ab16667),
MAP2 (mouse, 1:500; Becton Dickinson, cat. n. 556320),

N-CADHERIN (mouse, 1:800; Becton Dickinson, cat. n. 610921),
NESTIN (mouse, 1:300; Millipore, cat. n. MAB5326),
NKX2.1/TTF1 (rabbit, 1:200; Abcam, cat. n. ab76013),
PALS1 (rabbit, 1:500; Protein tech, cat. n. 17710-1-AP),
PAX6 (rabbit, 1:300; Biolegend cat. n. 901302),
PERICENTRIN (Rabbit, 1:1000, Abcam, cat. n. ab4448),
PH3 (Mouse, 1:1000, Cell Signaling, cat. n. #9706),
RFP (Rabbit, 1:500, MBL, cat. n. PM005),
SATB2 (Mouse, 1:500, Abcam, cat. n. ab51502),
SOX2 (rabbit, 1:200; Millipore, cat. n. AB5603),
TBR1 (rabbit, 1:1,000; Abcam, cat. n. ab31940),
TBR2 (rabbit, 1:100; Abcam, ab23345),
VIMENTIN (mouse, 1:100; Becton Dickinson, cat. n. 550513),
p-VIMENTIN (Mouse, 1:1000, MBL, D076-35),
ZO-1 (Mouse, 1:100, Invitrogen, cat. n. 33-9100).
AlexaFluor Goat Anti-Rabbit 488 Life Technologies Cat#A11008;
AlexaFluor Goat Anti-Rabbit 514 Life Technologies Cat#A31558;
AlexaFluor Goat Anti-Rabbit 568 Life Technologies Cat#A11011;
AlexaFluor Goat Anti-Rabbit 647 Life Technologies Cat#A27040;
AlexaFluor Goat Anti-Mouse 488 Life Technologies Cat#A11029;
AlexaFluor Goat Anti-Mouse 568 Life Technologies Cat#A11004;
AlexaFluor Goat Anti-Mouse 647 Life Technologies Cat#A21235;
AlexaFluor Goat Anti-Rat 488 Life Technologies Cat#A11006;
AlexaFluor Goat Anti-Rat 568 Life Technologies Cat#A11077;
AlexaFluor Goat Anti-Rat 647 Life Technologies Cat#A21247;
AlexaFluor Goat Anti-Chicken 488 Life Technologies Cat#A11039;
Hoechst 33258 (5 µg/ml; Thermo Fisher Scientific)

Validation

The ARL13B (Rabbit, Abcam) has been cited in 12 publications, is now discontinued by Abcam,
The ASCL1 (mouse, Becton Dickinson) has been cited in 241 publications, including Conforti et al, Cell Rep Methods (2022), Bocchi et al, Science (2021) and Conforti et al, Neurobiol Dis (2020) from our lab and has been tested on human fetal brain tissue and mouse E15,
The BASSOON (mouse, Enzo Life Sciences) has been cited in 39 publications,
The BLBP (rabbit, Millipore) has been cited in 90 publications, including Magni et al Int J Mol Sci (2022) from our lab
The βIII-TUBULIN (rabbit, BioLegend) has been cited in 179 publications,
The CASPASE-3 (Rabbit, Cell Signaling) has been cited in 9679 publications, and is reactive to human tissue
The CTIP2 (rat, Abcam) has been cited in 1027 publications, including Quadrato et al, Nature (2017), which used this antibody on human brain organoids, Antón-Bolanos et al, Nature (2024), Shanshan et al, PNAS (2024), Onorati et al, Nat Neurosci (2014), Conforti et al, Neurobiol Dis (2020), Bocchi et al, Science (2021) Magni et al, Int J Mol Sci (2022), Conforti et al, Cell Rep Methods (2022), Schellino et al, Stem Cell Res Ther (2023) from our lab and has been tested on human fetal brain tissue and mouse E15,
The EBF1 (mouse, Santa Cruz), has been cited in 14 publications, including Onorati et al, Nat Neurosci (2014) and Bocchi et al, Science (2021) from our lab and has been tested on human fetal brain tissue,
The GABA (Rabbit, Sigma) has been cited in 790 publications, including Onorati et al, Nat Neurosci (2014), Magni et al, Int J Med Sci (2022) from our lab,
The GAD67 (Mouse, Millipore) has been cited in 940 publications, including Shanshan et al, PNAS (2024) and Conforti et al, Cell Rep Methods (2022), Magni et al, Int J Med Sci (2022) from our lab,
The GFP (chicken, Abcam) has been cited in 4269 publications,
The GSX2 (rabbit, GeneTex) has been cited in 3 publications, is now discontinued by Abcam, including Conforti et al, Neurobiol Dis (2020), Magni et al, Int J Med Sci (2022), Conforti et al, Cell Rep Methods (2022) from our lab
The HOMER1 (rabbit, Genetex) has been cited in 6 publications,
The ISLT1/2 (mouse, Hybridoma Bank) has been cited in 643 publications, including Onorati et al, Nat Neurosci (2014), Bocchi et al, Science (2021), Conforti et al Cell Rep Methods (2022) from our lab and has been tested on human fetal brain tissue,
The KI67 (rabbit, Abcam) has been cited in 3283 publications, including Conforti et al, Neurobiol Dis (2020), Bocchi et al, Science (2021), Magni et al, Int J Med Sci (2022), Conforti et al, Cell Rep Methods (2022) from our lab and has been tested on human fetal brain tissue,
The MAP2 (mouse, Becton Dickinson) has been cited in 31 publications, including Onorati et al, Nat Neusci (2014), Magni et al, Int J Med Sci (2022), Conforti et al, Cell Rep Methods (2022) from our lab and has been tested on human fetal brain tissue and mouse E15,
The N-CADHERIN (mouse, Becton Dickinson) has been cited in 226 publications, including Onorati et al, Nat Neusci (2014), Conforti et al, Neurobiol Dis (2020) from our lab,
The NESTIN (mouse, Millipore) has been cited in 510 publications,
The NKX2.1/TTF1 (rabbit, Abcam) has been cited in 216 publications, including Conforti et al, Neurobiol Dis (2020) from our lab,
The PALS1 (rabbit, Protein tech) has been cited in 25 publications, including Magni et al, Int J Med Sci (2022) from our lab
The PAX6 (rabbit, Biolegend) has been cited in 21 publications, and has been tested on human fetal brain tissue and mouse E15,
The PERICENTRIN (rabbit, Abcam) has been cited in 576 publications,
The PH3 (mouse, Cell Signaling) has been cited in 436 publications, including Onorati et al, Nat Neurosci (2014)
The RFP (rabbit, MBL) has been cited in 255 publications,
The SATB2 (mouse, Abcam) has been cited in 367 publications, including Antón-Bolanos et al, Nature (2024) and Onorati et al, Nat Neurosci (2014), Magni et al, Int J Med Sci (2022), Schellino et al, Stem Cell Res Ther (2023) from our lab,
The SOX2 (rabbit, Millipore) has been cited in 725 publications, including Onorati et al, Nat Neurosci (2014), Magni et al, Int J Med Sci (2022) from our lab,
The TBR1 (rabbit, Abcam), has been cited in 564 publications, including Antón-Bolanos et al, Nature (2024) and Onorati et al, Nat Neurosci (2014), Conforti et al, Neurobiol Dis (2020), Magni et al, Int J Med Sci (2022) from our lab,
The TBR2 (rabbit, Abcam), has been cited in 604 publications, including Antón-Bolanos et al, Nature (2024) and Onorati et al, Nat Neurosci (2014), Conforti et al, Neurobiol Dis (2020) from our lab,
The VIMENTIN (mouse, Becton Dickinson) has been cited in 239 publications,
The p-VIMENTIN (mouse, MBL) has been cited in 32 publications, including Magni et al, Int J Med Sci (2022) from our lab,

The ZO-1 (Mouse, Invitrogen) has been cited in 1129 publications, including Antón-Bolanos et al, Nature (2024) and has been tested on human fetal brain tissue and mouse E15.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	The hESC RUES2 lines were derived from Prof. Ali Brinvalou's Laboratory (Rockefeller University) and provided by the CHDI Foundation.
Authentication	The karyotype for each cell line was monitored every 3 months by Q-banding analyses (by the ISENET group). Routine immunocytochemistry analyses for pluripotent markers were performed.
Mycoplasma contamination	The hES Rues2 lines were regularly tested and maintained mycoplasma-free (Eurofins).
Commonly misidentified lines (See ICLAC register)	No misidentified lines were used in the study