

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	Some patient data included in Fig 1. was collected from www.deciphergenomics.org (v11.27), details of the included cases are provided in Supplementary Data 1. Public epigenomics data included in Fig 2. was collected from https://genome.ucsc.edu (ENCODE trackhub - DNase-seq/Histone ChIP-seq (by target)/TF ChIP-seq (by target); Comparative Genomic – UCSC 100 Vertebrates) and VISTA enhancer data was collected from https://vista-enhancer.lbl.gov . Public data supporting the identification of candidate cis-regulatory elements was collected from the Annotated Regulatory Index at meuleman.org and ENCODE SCREEN v3 (https://screen.wenglab.org). Single-cell RNA-seq data of the developing human brain (Braun et al.) was accessed through GitHub (https://github.com/linnarsson-lab/developing-human-brain). The single-cell RNA-seq data from the human neural organoid cell atlas HNOCA was sourced from CELLxGENE (https://cellxgene.cziscience.com/collections/de379e5f-52d0-498c-9801-0f850823c847).
Data analysis	CNV-seq data analysis made use of bowtie2, WisecondorX (v1.2.9) and Vivar (10,371/journal.pone.0113800). UMI-4C data was analyzed using the UMI-4C R package (https://github.com/tanaylab/umi4cpackage). Hi-C data was analyzed using the Juicer pipeline (v1.6) with BWA-MEM mapping (v0.7.17) and further processed using cooltools (v0.7.0) and FAN-C (github.com/vaquerizaslab/fanc). CUT&RUN analysis was performed using nf-core/cutandrun (v3.2.2) with Bowtie2 (v2.4.4) mapping. Quantitative RT-qPCR data was analyzed using qBase + software (Biogazelle, Ghent, Belgium). Packages installed to analyse and visualize qPCR and FC data: ggplot2 (v4.0.2), stringr (v1.6.0) tidyverse (v2.0.0), ggprism(v1.0.7), dplyr (v1.2.0), lme4 (v1.1.38), ggpubr (v0.6.2), ggokabeito (v0.1.0), emmeans (v2.0.1), broom.mixed (v0.2.9.7), modelsummary (v2.6.0)

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 datasets generated in this study are available through the Gene Expression Omnibus (GEO) repository under accession numbers GSE292880 and GSE292881. The Hi-C data is not publicly available as they contain patient-derived genomic information. The data generated in this study are provided in the Source Data/Source Data Supplementary file.

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	Sex -or gender based analyses were not included in the current study.
Reporting on race, ethnicity, or other socially relevant groupings	This study did not consider analyses related to race, ethnicity or other social groupings.
Population characteristics	In this study we describe the genetic diagnosis of a 7-year-old East-African boy and a 2-year-old girl. A full description of the clinical characteristics, as well as genotypic information is included in the manuscript.
Recruitment	The patients were identified by clinicians at UZ Gent (Belgium) and University Pediatric Hospital Sofia (Bulgaria). Informed consent was obtained from the patient prior to data collection, ensuring they fully understood the purpose of the study and the potential for their data to be made publicly available. All data were anonymized to protect patient confidentiality and comply with relevant data protection regulations.
Ethics oversight	This study was conducted in accordance with ethical guidelines and received approval from the Ethics Committee (EC) of UZ Gent under approval number ONZ-2022-0100 and ECD 2019/1430.

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](https://www.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	We did not use statistical models to predetermine sample size. When possible, experiments were performed with a minimum of three independent biological replicates. In cases where biological replicates were not feasible, for example due to limited availability of patient-derived material, technical replicates were included. These technical replicates were generated from independent culture or differentiation setups to ensure reproducibility.
Data exclusions	No data was excluded.
Replication	All experiments included in this study were experimentally replicated. The exact number of biological replicates for each assay is indicated in the manuscript.
Randomization	Randomization was not applicable to this study as no samples had to be allocated to specific treatment groups.
Blinding	Data collection was not blinded.

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

The following commercially-available antibodies were used. Catalog, clone, and lot numbers are provided whenever publicly available.

IgG (#3900, Cell Signalling Technologies, Danvers, MA, USA, 1:100 dilution, CUT&RUN);
H3K27ac (#MA5-24671, ThermoFisher, 1:100 dilution, CUT&RUN);
H3K4me1 (Ab8895, Abcam, 1:100 dilution, CUT&RUN);
H3K4me3 (Ab8580, Abcam, 1:100 dilution, CUT&RUN);
Live/Dead (L24957, Invitrogen, &/250 dilution, Flow Cytometry);
FOXG1 (ab196868, Abcam, 1:60 dilution, Flow Cytometry);
PAX6 (561664, BD Biosciences, 1:20 dilution, Flow Cytometry)
Donkey anti-Rabbit IgG (A21207, Invitrogen, 1:1000 dilution, Flow Cytometry)

Validation

Validation statements for all antibodies listed above can be found through the following links to the manufacturer's website.

From Cell Signalling Technologies (Danvers, MA, USA)

https://www.cellsignal.com/products/primary-antibodies/rabbit-da1e-mab-igg-xp-isotype-control/3900?srsltid=AfmBOooXY5I_pAP62gzUPQU_uur10Dn6PddrEXj1bel6PWchb8TrMzp8

From ThermoFisher Scientific (Waltham, MA, USA):

<https://www.thermofisher.com/antibody/product/H3K27ac-Antibody-clone-RM172-Recombinant-Monoclonal/MA5-24671>
<https://www.thermofisher.com/order/catalog/product/L34957>
<https://www.thermofisher.com/antibody/product/A-21207.html?CID=AFLCA-A-21207>

From Abcam (Cambridge, MA, USA):

https://www.abcam.com/en-us/products/primary-antibodies/histone-h3-mono-methyl-k4-antibody-chip-grade-ab8895?srsltid=AfmBOooY24O-pyKg_rfHlnmuajZbvmlGGC1Dh4N2JeR_6IslolCfoXi8
https://www.abcam.com/en-us/products/primary-antibodies/histone-h3-tri-methyl-k4-antibody-chip-grade-ab8580?srsltid=AfmBOOr4c5s0TZHTkVN613B4_khsgSmLdvQoovWx10xg-DCV1VJEUrD_
<https://www.abcam.com/en-us/products/primary-antibodies/foxg1-antibody-epr18987-ab196868>

From BD Biosciences (San Jose, CA, USA):

https://www.bdbiosciences.com/en-gb/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/alexa-fluor-488-mouse-anti-human-pax-6.561664?tab=product_details

Eukaryotic cell lines

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

Cell line source(s)

Human iPS cells were obtained from Axol Bioscience (lot #30270222072019, female, 75 years-of-age, dermal fibroblasts reprogrammed using Sendai virus methodology) and Stem Cell Technologies (SCTi003-A, Female, derived from healthy female donor peripheral blood mononuclear cells (PBMCs)). Case lymphoblastoid cell lines (LCLs) were derived in-house from patient blood samples. The following LCL lines were used in this study:
- control LCL lines: ND02050 (male), ND09729 (female), ND11929 (female), ND23969 (male), ND23993 (female), ND24284 (male), GM07544 (female), GM23712 (female); GM23712 (male)
- case LCL lines: del8 (male), bca4 (male), bca17 (female)
- parental control LCL lines: male and female

Authentication

Cell lines were authenticated using shallow whole genome sequencing.

Mycoplasma contamination

Cell lines were regularly tested for mycoplasma contamination and tested negative.

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

No commonly misidentified lines were 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	Danio rerio, age: up to three days post-fertilization Mus musculus, strain: FVB, age: up to e11.5, dark light cycle: 12 hrs on, 12 hrs off (light on 6am-6pm), temperature: 69-75F, humidity: 30-70%.
Wild animals	The study did not involve wild animals
Reporting on sex	No sex-based analyses were performed in this study.
Field-collected samples	The study did not involve field-collected samples.
Ethics oversight	All mouse experiments were reviewed and approved by the Lawrence Berkeley National Laboratory Animal Welfare and Research Committee under protocol numbers 290003 and 290008. All zebrafish experiments were performed prior to 5 days post-fertilization in accordance with applicable regulations and did not require ethical approval.

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

Plants

Seed stocks	<i>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.</i>
Novel plant genotypes	<i>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.</i>
Authentication	<i>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, mosaicism, off-target gene editing) were examined.</i>

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☐ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Day-14 fbNPCs were harvested by rinsing with D-PBS and incubating with 1 mL Accutase per well for 5.5 min. Detached cells were diluted in DMEM (2 mL per 1 mL Accutase), centrifuged (300 × g, 5 min), and resuspended in 1–2 mL NMM supplemented with Y-27632 (10 µg/mL) for counting and allocation. For staining, 5 × 10 ⁵ cells per sample were washed with PBS (490 × g, 6 min, RT). Dead cells were labelled using LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (0.2 µL dye in 48.9 µL PBS per sample, 405 nm excitation; Invitrogen L34957) for 20 min at RT in the dark, followed by two PBS washes. Cells were fixed and permeabilized using the FoxP3/Transcription Factor Staining Buffer Set (eBioscience 00-5523-00). A 1:4 dilution of fixation/permeabilization buffer (500 µL per sample) was applied for 30 min at RT, followed by one wash in 1× Permeabilization Buffer (PB) and two additional PBS washes. Samples were stored in PBS at 4°C until intracellular staining. Primary antibodies (Sup Table S12) were applied for 20 min at RT in 50 µL PB containing rabbit anti-FOXG1 (1:60, Abcam ab196868) and/or Alexa Fluor 488 mouse anti-human PAX6 (1:20, BD Biosciences 561664). After two washes in PB, secondary staining was performed for 20 min at RT in the dark with Alexa Fluor 594 donkey anti-rabbit IgG (1:1000 in 150 µL PB, Invitrogen A21207). Cells were washed once and resuspended in 150 µL PBS for acquisition.
Instrument	BD LSR Fortessa X-20 5-laser cytometer

Software	Flow data were processed in FlowJo (v10.6) and analysed in R (v4.5.1) using generalized additive mixed models as detailed in the Statistical Analysis section.
Cell population abundance	Cell population abundance was not used as a primary metric within this study. However, gate-based population percentages are included within Supplementary Figure 21.
Gating strategy	A starting gate was used to select appropriate cell size (X: FSC-A, Y: SSC-A). A second gate was used to discriminate doublets from the analysis (X: FSC-W, Y: FSC-H). Finally, the last gate was used to remove dead cells from the analysis (X: LiveDead Fixable Aqua, Y: FCS-A). Details on gate-based population percentages are included within Supplementary Figure 21.
☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.	