nature portfolio

Corresponding author(s): Angeliki Louvi, Murat Gunel, Kaya Bilguvar

Last updated by author(s): 3 October 2024

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	NovaSeq 6000 (Illumina) and HiSeq 2500 (Illumina), Zeiss Zen
Data analysis	Single-cell RNA seq and mass spec R pipeline is deposited at github.com/danliangunc/PIDD1-lissencephaly. Software used in this study: GATK 4.1 (https://www.broadinstitute.org/gatk); PLINK version 1.9 (http://pngu.mgh.harvard.edu/~purcell/plink); CADD version 1.6/ANNOVAR (http://annovar.openbioinformatics.org); Burrows-Wheeler Aligner tool (v0.7.15), Picard's MarkDuplicates, (v2.18.29), KING (v2.2.7), Picard (v2.25.6), SnpEff (v5.1), Ensembl Variant Effect Predictor (v107) , CellRanger (v6.1.2), R v4.3.0, Seurat version v4.3.0. Mass spec: Progenesis QI software (Nonlinear Dynamics, version 4.2), Bioconductor v3.18. GraphPad Prism version 10.3.1 was used for statistical analyses.

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

Summary statistics for WES data analyses are provided in Supplementary Tables 1 and 3. Individual-level phenotype data of patients with PIDD1 mutations are provided in Supplementary Table 2. Sequencing data of patients with PIDD1 mutations are publicly restricted due to consent forms not allowing broad sharing of raw genomic data. Single-cell RNA-Seq files have been deposited in Sequence Read Archive (SRA) at Accession PRJNA1168541. Mass spectrometry data have been deposited in Proteomics Identification Database (PRIDE) at Accession PXD056364. Alphafold (https://alphafold.ebi.ac.uk/) was used to predict PIDD1 protein structure. Human Brain Transcriptome (HBT) (https://hbatlas.org/pages/hbtd) was used to display PIDD1 mRNA expression across brain regions and developmental time points. Pathway enrichment analysis was performed using the MSigDB_2020 gene set from GSEA (https://www.gsea-msigdb.org/gsea/index.jsp), OMIM dataset (www.omim.org), and Reactome 2022 pathway database (https://reactome.org/).

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Sex or gender had no impact on data; we generated an iPSC line from a female patient.
Population characteristics	The patient line was generated from a female recruited at age 11, and harbors a homozygous R331X PIDD1 mutation, diagnosed with diffuse pachygyria. The MDLS line was generated from a female sampled at age 1 per Coriell (https://www.coriell.org/0/sections/Search/Sample_Detail.aspx?Ref=GM26025&Product=CC) and harbors a heterozygous 46,XX,del(17)(p13.1p13.3)[25].arr[hg19] 17p13.3p13.2(513-4,242,178) genotype (https://www.coriell.org/0/sections/Search/Sample_Detail.aspx?Ref=GM26025&Product=CC), and was diagnosed with lissencephaly. The outside-family control iPSC lines were derived by the Yale Stem Cell Core from a healthy female subject at age 26. The patient, MDLS, and control iPSC lines were treated with and without NV-5138 (mTORC1 activator) for a subset of experiments reported in the study. The participants did not receive any treatment.
Recruitment	Participants were diagnosed with lissencephaly confirmed by imaging studies and were referred by treating physicians. No additional recruitment criteria were applied.
Ethics oversight	The study was approved by the Yale Human Investigations Committee (HIC protocol 9406007680). Institutional Review Board approvals for genetic and imaging studies, including written consent forms from all study subjects, were obtained by the referring physicians at the participating institutions.

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	No sample size calculation was performed. Previous studies have shown that in a well-established organoid protocol, batches and not individual organoids are the greatest source of variability (Camp et al. 2015, Kanton et al., 2019). A consistent phenotype was demonstrated when observed in a minimum of two independent batches. All organoid immunostaining experiments were performed with at least n=6 cortical regions, n=2 organoids, and n=2 independent batches. For proteomic analyses, n=3 biological replicates (each replicate with 3 organoids) were used per genotype. For scRNA-seq analyses, at least n=2 organoids/replicates were used for each genotype. For Figure 3:C, n=4; P, n=4; KI, n=2, PR, n=2. For Ext. Figure 8: n=2; C+NV, n=2; P, n=2, P+NV, n=2, MDLS, n=2, MDLS+NV, n=2. For western blot analyses, n=4 independent batches for p-S6/S6, n=3 independent batches for pAKT/AKT, 3 organoids harvested per batch per genotype
Data exclusions	Single-cells in scRNA-seq were excluded based on established quality control criteria prior to data collection; no data samples were excluded.
Replication	All quantifications were performed on a minimum of two independent organoid batches, with a minimum of 6 cortical regions per genotype. All attempts at replication were successful. For the full list of exact number of cortical regions analyzed, please see supplementary table 4.

Randomization	Organoids were transferred to culture dishes in a random order and by mixing organoids of the same genotype within the same batch.
Blinding	The investigators were not blinded during data collection and analysis, as most of the tissue collection, processing, and analyses were performed by one researcher.

Behavioural & social sciences study design

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

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

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

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*

Blinding

Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions

Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).

Location

State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).

Access & import/export

Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).

Disturbance

Describe any disturbance caused by the study and how it was minimized.

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

Methods

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

Antibodies

Antibodies used

Primary antibodies (immunostaining)
mouse anti-MAP2 (1:500, MAB3418, Millipore)
mouse anti-SOX2 (1:500, sc-365823; Santa Cruz)
rat anti-SOX2 (1:500, 14-9811-82, Invitrogen)
rat anti-CTIP2 (1:500, ab18465; Abcam)
rabbit anti-SATB2 (1:500, ab34735, Abcam)
rabbit anti-TBR1 (1:500, ab31940, Abcam)
rabbit anti-HOPX (1:2500, HPA030180, Sigma)
mouse anti-HOPX (1:250, sc-398703, Santa Cruz)
rabbit anti-cleaved caspase 3 (1:1000, 9661, Cell Signaling Technologies)
mouse anti-PH3 (1:500, 9706, Cell Signaling Technologies)
mouse anti-Ki67 (1:500, 556003, BD Biosciences)
rabbit anti-EOMES (TBR2) (1:500, HPA028896, Sigma)
sheep anti-EOMES (TBR2) (1:200, AF6166, R&D)
rabbit anti-Phospho-S6 Ribosomal Protein (PS6) (1:200, 4858, Cell Signaling Technologies)

Secondary antibodies (immunostaining)
Alexa Flour 488 anti-sheep (1:1000, A11015, Thermofisher)
Alexa Fluor 488 ant-rat (1:1000, A21208, Thermofisher)
Alexa Fluor 568 anti-rabbit (1:1000, A10042, Thermofisher)
Alexa Fluor 647 anti-mouse (1:1000, A31571, Thermofisher)

Primary antibodies (western blotting)
mouse anti-PIDD1 (Anto-1) (1:500, ALX-804-837-C100, Enzo)
rabbit anti-PS6 (1:1000, 4858, Cell Signaling Technologies)
rabbit anti-S6 Ribosomal protein (5G10) (1:1000, 2217, Cell Signaling Technologies)
rabbit anti-Phospho-AKT (Ser473) (1:1000, 4060, Cell Signaling Technologies)
rabbit anti-Akt (pan) (C67E7), (1:1000, 4691, Cell Signaling Technologies)
mouse anti-beta-actin (1:5000, A2228, Sigma)

Secondary antibodies (western blotting)
anti-rabbit IgG, HRP (1:2000, 7074, Cell Signaling Technologies)
anti-mouse IgG, HRP (1:2000-5000, 7076, Cell Signaling Technologies)

Validation

Antibodies were chosen if reported to work on human tissue and in the method used (i.e., immunohistochemistry, western blot). Each antibody was validated based on previously published data listed on the manufacturer's website and antibody validation statement.
mouse anti-MAP2 (1:500, MAB3418, Millipore) is reactive to human and cited in 100 publications.
mouse anti-SOX2 (1:500, sc-365823; Santa Cruz) is reactive to human and cited in 300 publications.
rat anti-SOX2 (1:500, 14-9811-82, Invitrogen) is reactive to human and cited in 67 publications.
rat anti-CTIP2 (1:500, ab18465; Abcam) is reactive to human and cited in 841 publications.
rabbit anti-SATB2 (1:500, ab34735, Abcam is reactive to human and cited in 90 publications.
rabbit anti-TBR1 (1:500, ab31940, Abcam is reactive to human and cited in 476 publications.
rabbit anti-HOPX (1:2500, HPA030180, Sigma) is reactive to human and cited in 31 publications.
mouse anti-HOPX (1:250, sc-398703, Santa-Cruz) is reactive to human and cited in 82 publications.
rabbit anti-cleaved caspase 3 (1:1000, 9661, Cell Signaling Technologies) is reactive to human and cited in 10478 publications.
mouse anti-PIDD1 (Anto-1) (1:500, ALX-804-837-C100, Enzo) is reactive to human and cited in 4 publications.
rabbit anti-PS6 (1:1000, 4858, Cell Signaling Technologies) is reactive to human and cited in 1590 publications.
rabbit anti-S6 Ribosomal protein (5G10) (1:1000, 2217, Cell Signaling Technologies) is reactive to human and cited in 2378 publications.
rabbit anti-Phospho-AKT (Ser473) (1:1000, 4060, Cell Signaling Technologies) is reactive to human and cited in 7877 publications.
rabbit anti-Akt (pan) (C67E7), (1:1000, 4691, Cell Signaling Technologies) is reactive to human and cited in 5474 publications.
mouse anti-beta-actin (1:5000, A2228, Sigma) is reactive to human and cited in 2979 publications.
mouse anti-PH3 (1:500, 9706, Cell Signaling Technologies) is reactive to human and cited in 473 publications.
mouse anti-Ki67 (1:500, 556003, BD Biosciences) is reactive to human and cited in 299 publications.
rabbit anti-EOMES (TBR2) (1:500, HPA028896, Sigma) is reactive to human and cited in 4 publications.
sheep anti-EOMES (TBR2) (1:200, AF6166, R&D) is reactive to human and cited in 25 publications.
rabbit anti-Phospho-S6 Ribosomal Protein (PS6) (1:200, 4858, Cell Signaling Technologies) is reactive to human and cited in 1590 publications.

Manufacturer company validation statements/protocols:
Abcam: https://www.abcam.com/primary-antibodies/how-we-validate-our-antibodies
BD Biosciences: https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/quality-and-reproducibility
Cell Signaling Technologies: https://www.cellsignal.com/about-us/cst-antibody-validation-principles
Enzo: https://www.enzolifesciences.com/ALX-804-837/pidd-monoclonal-antibody-anto-1
Milipore: https://www.emdmillipore.com/US/en/life-science-research/antibodies-assays/antibodies-overview/Antibody-Development-and-Validation/cFOb.qB.8McAAAFOb64qQvSS,nav
Santa Cruz: https://www.scbt.com/resources/protocols/immunofluorescence-cell-staining
Sigma: https://www.sigmaaldrich.com/US/en/products/protein-biology/antibodies/prestige-antibodies
Thermofisher: https://www.thermofisher.com/us/en/home/life-science/antibodies/invitrogen-antibody-validation.html
Sigma (mouse beta actin): https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/protein-biology/elisa/antibody-standard-validation

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

MDLS iPSCs (Coriell Repository, GM26025); Patient iPSCs (derived in-house from patient hair follicle keratinocytes), and Control iPSCs (derived in-house at the Yale Stem Cell Core). The Knock-In (KI) line was derived via CRISPR-Cas9 gene editing in-house from the Control line. The Patient Rescue (PR) line was derived via CRISPR-Cas9 gene editing in-house from the Patient line.

Authentication

MDLS iPSCs were obtained from Coriell (catalog # GM26025) and authenticated using Agilent 60k Standard microarray comparative genomic hybridization and karyotyping performed by Cell LIne Genetics (Ext. Data Fig .3). Control iPSC line was obtained from the Yale School of Medicine Stem Cell Core and authenticated through karyotyping, teratoma assay, and Sanger sequencing confirming no R331X mutation (Ext. Data Fig. 3). The Patient line was generated using the CytoTune 2.0 Sendai virus kit and authenticated by karyotyping, pluripotency marker staining, and Sanger sequencing confirming the homozygous R331X mutation (Ext. Data Fig 3). The Patient Rescue and Knock-In lines were authenticated by karyotyping and Sanger sequencing confirming the intended edit (WT/WT and R331X/R331X) respectively (Ext. Data Fig. 3).

Mycoplasma contamination

iPSC lines were tested and found to be negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified lines was used in the study.

Palaeontology and Archaeology

Specimen provenance

Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.

Specimen deposition	*Indicate where the specimens have been deposited to permit free access by other researchers.*
Dating methods	*If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

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

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	*For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.*
Wild animals	*Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*
Reporting on sex	*Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.*
Field-collected samples	*For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*
Ethics oversight	*Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.*

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	*Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.*
Study protocol	*Note where the full trial protocol can be accessed OR if not available, explain why.*
Data collection	*Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*
Outcomes	*Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.*

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes	
☐	☐	Public health
☐	☐	National security
☐	☐	Crops and/or livestock
☐	☐	Ecosystems
☐	☐	Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes	
☐	☐	Demonstrate how to render a vaccine ineffective
☐	☐	Confer resistance to therapeutically useful antibiotics or antiviral agents
☐	☐	Enhance the virulence of a pathogen or render a nonpathogen virulent
☐	☐	Increase transmissibility of a pathogen
☐	☐	Alter the host range of a pathogen
☐	☐	Enable evasion of diagnostic/detection modalities
☐	☐	Enable the weaponization of a biological agent or toxin
☐	☐	Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

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	*Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.*
Instrument	*Identify the instrument used for data collection, specifying make and model number.*

Software

Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.

Cell population abundance

Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy

Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Lissencephaly diagnosis was confirmed by at least one imaging study (Magnetic Resonance Imaging) in all index cases and family members. All available imaging for study participants who underwent whole-exome sequencing were obtained and reviewed by a minimum of two clinicians (attending neuroradiologists).

Design specifications

Available brain MRI scans were reviewed for lissencephaly/pachygyria.

Behavioral performance measures

N/A

Acquisition

Imaging type(s)

Structural

Field strength

1.5T - 3T

Sequence & imaging parameters

Clinical imaging protocols varied depending on institution, but were within the "standard" for structural MRI brain scans. Sequences evaluated included T1-weighted, T2-weighted, and T2-FLAIR.

Area of acquisition

Entire brain

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software

Processing was performed by the clinical institution

Normalization

Processing was performed by the clinical institution

Normalization template

Processing was performed by the clinical institution

Noise and artifact removal

Processing was performed by the clinical institution

Volume censoring

Processing was performed by the clinical institution

Statistical modeling & inference

Model type and settings

No statistical modeling was performed.

Effect(s) tested

N/A

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)

N/A

Correction

N/A

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation,

Functional and/or effective connectivity	*mutual information).*
Graph analysis	*Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).*
Multivariate modeling and predictive analysis	*Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.*