

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
<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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P 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 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	Mutagenic primer designed: NEBaseChanger. CRISPR gRNA design: CHOPCHOP v.3 software. Microscopy image acquisition: Zeiss Zen 3.1, Leica Application Suite X (LAS X), Volocity, MetaMorph
Data analysis	Variant calling, annotation and visualization: bcbio-nextgen (GATK 4.0.7.0), VEP, GEMINI, Vcfanno, Integrative Genomics Viewer (IGV). ddPCR copy number analysis: QuantaSoft v1.4 (Bio-Rad). Microscopy image analysis: HALO, CaseViewer 2.4, SlideViewer 2.5, Fiji (Coloc 2, TrackMate, Kappa plugins). 3D protein structure: PyMOL Molecular Graphics System 2.0. Protein stability analysis: FoldX. Flow cytometry data: FlowJo 10.8. Statistical analysis: GraphPad Prism 9.4.1, BioRender Graph (using R 4.2.2)

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 experimental data supporting the findings of this study are available in the main text or in the supplementary information files. Source data are provided with

this paper. MRI data generated in this study are not publicly available due to data sharing restrictions. Relevant genotype-phenotype data are provided within the article. Whole genome sequencing data cannot be publicly shared, as the study protocol permits publication of findings and results but does not allow deposition of raw data in public repositories. Access to whole genome sequencing data may be granted to qualified researchers upon request to the corresponding author for non-commercial research purposes only, subject to approval by the relevant ethics board. We aim to respond to requests within 20 business days.

The following Protein Data Bank (PDB) entries were used in this study: 6i2i [https://doi.org/10.2210/pdb6i2i/pdb], 6o2s [https://doi.org/10.2210/pdb6o2s/pdb], 4i4t [https://doi.org/10.2210/pdb4i4t/pdb], 5nqu [https://doi.org/10.2210/pdb5nqu/pdb], 5yl2 [https://doi.org/10.2210/pdb5yl2/pdb], 5ca1 [https://doi.org/10.2210/pdb5ca1/pdb].

The following NCBI Sequence Read Archive (SRA) experiment ID was accessed for this study: SRX858057 [https://www.ncbi.nlm.nih.gov/sra/?term=SRX858057].

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 (male) was known for the first patient and their family members; sex of the second patient was unknown. Sex and gender were not considered in the study design, as patients were identified based on clinical phenotype (first patient) and genetic variants in the autosomal gene of interest (second patient).
Reporting on race, ethnicity, or other socially relevant groupings	Data on race, ethnicity, or other socially relevant groupings were not collected for this study. These variables were not considered in the study design, as participants were identified based on clinical phenotype (first patient) and genetic variants in the gene of interest (second patient).
Population characteristics	The study included two patients: the first patient was assessed during early childhood and early adulthood, while the second patient was assessed only during early childhood.
Recruitment	Participants were recruited based on clinical phenotype (first patient) and genetic variant in the gene of interest (second patient). The second patient was identified due to a ClinVar submission, which led to a match and connection through the GeneMatcher platform. The second patient was recruited with prior knowledge of the functional context (Figure 10b) of the TUBB E108K variant, which may introduce assessor bias during MRI evaluation. This was mitigated, as the reported radiological features represent structural findings independently assessed by two qualified radiologists using standard clinical criteria.
Ethics oversight	This study was approved by the Research Ethics Board of The Hospital for Sick Children, Toronto, Ontario, Canada. Informed written consent to participate in research and to publish potentially identifying clinical information was obtained from the first patient and their family members. A separate study allowing the sharing of deidentified patient information with second patient's consent was approved by the Review Board of the Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.

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	For all experiments, sample sizes were determined based on literature precedents for microtubule and cilia studies. Sample size was not predetermined using statistical methods.
Data exclusions	No data or samples were excluded from the analyses.
Replication	Cell culture experiments were independently replicated at least three times, with exceptions described below. Mouse experiments were conducted using independent animals. Results were reproducible across independent experiments. The flow cytometry experiment shown in Supplementary Figure 9 was performed once. It tested an alternative hypothesis that impaired cell cycle exit might underlie the ciliation defect. As the data did not support this, the experiment was not repeated. The primary mechanism, impaired ciliogenesis, was identified and confirmed through independently replicated experiments. The overexpression screen shown in Supplementary Figure 11b was performed once. It assessed the impact on ciliation of TUBB variants with undetermined clinical significance in ClinVar. All variants except E108K showed no effect and were not pursued further. The E108K variant showed reduced ciliation and was tested in follow-up experiments. Its effect was confirmed in subsequent independently replicated experiments presented in Figure 10b. The experiment shown in Figure 3h was independently replicated twice.
Randomization	Experimental groups for cell culture and mouse experiments were defined by genotype. Where applicable, variables such as age and sex were

controlled by matching samples across groups. All experiments were conducted under consistent conditions to minimize batch effects.

Blinking

For cell culture and mouse experiments, analyses were performed blinded to sample identity. MRI evaluations were conducted by radiologists blinded to patient clinical information and, for the first patient, also to the genetic variant and any associated functional data.

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

For immunostaining experiments, the following primary antibodies were used: mouse monoclonal anti-alpha-tubulin (DM1A, Sigma-Aldrich T6199, 1:500 dilution. Figure 2), mouse monoclonal anti-aplha-tubulin (DM1A, Sigma-Aldrich T9026, 1:10000 dilution. Figure 3 and 8), chicken polyclonal anti-V5 tag (Abcam ab9113, 1:500 dilution), mouse monoclonal anti-tyrosinated tubulin (TUB-1A2, Sigma-Aldrich T9028, 1:500 dilution), mouse monoclonal anti-gamma-tubulin (GTU-88, Sigma-Aldrich T6557, 1:500 dilution for figure 3, 1:2000 dilution for figure 4, 5 and 9), rabbit polyclonal anti-ninein (Sigma-Aldrich ABN1720, 1:300 dilution), mouse monoclonal anti-ARL13B (NeuroMab 75-287, 1:300 dilution. Figure 6, S5 and S6), rabbit polyclonal anti-ARL13B (Proteintech 17711-1-AP, 1:300 dilution), mouse monoclonal anti-acetylated tubulin (6-11B-1, Sigma-Aldrich T7451, 1:500 dilution), rabbit polyclonal anti-CEP135 (Sigma-Aldrich SAB4503685, 1:500 dilution), chicken polyclonal anti-calbindin-D28k (ThermoFisher PA5-143561, 1:1000 dilution), rabbit monoclonal anti-IBA1 (EPR16588, Abcam ab178846, 1:1000 dilution), mouse monoclonal anti-VGLUT2 (N29/29, ThermoFisher MA5 27613, 1:1000 dilution), chicken polyclonal anti-GFAP (Abcam ab4674, 1:1000 dilution).

For immunoblotting, the following primary antibodies were used: mouse monoclonal anti-beta-tubulin (AA2, Sigma-Aldrich T8328, 1:500 dilution), mouse monoclonal anti-beta-actin (AC-15, Sigma-Aldrich A5441, 1:1000 dilution).

The following secondary antibodies were used: goat anti-rabbit IgG (H+L) Alexa Fluor™ 488 (ThermoFisher A-11008), goat anti-rabbit IgG (H+L) Alexa Fluor™ 555 (ThermoFisher A-21429), goat anti-mouse IgG (H+L) Alexa Fluor™ 488 (ThermoFisher A-11001), goat anti-mouse IgG (H+L) Alexa Fluor™ 555 (ThermoFisher A-21422), goat anti-chicken IgY (H+L) Alexa Fluor™ 488 (ThermoFisher A-11039), donkey anti-mouse IgG (H+L) Alexa Fluor™ Plus 647 (ThermoFisher A 32787), goat anti-mouse IgG (H+L) HRP-conjugated (Jackson ImmunoResearch 115-035-146).

Validation

All antibodies used in this study were previously validated for the specific application either by the companies they were purchased from (as stated on their websites) or by both the companies and cited references.

Eukaryotic cell lines

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

Cell line source(s)

Primary fibroblasts were derived from a skin biopsy of the patient (male) and an age-matched control subject (male) and obtained from The Centre for Applied Genomics (TCAG), Tissue Culture Facility – Biobanking Services at The Hospital for Sick Children, Toronto, Ontario, Canada. hTERT-RPE1 cells (ATCC CRL-4000; female origin) were obtained from ATCC. p53-null hTERT-RPE1 cells (female origin) were a gift from Daniel Durocher (Department of Molecular Genetics, University of Toronto).

Authentication

hTERT-RPE1 cells used in this study were authenticated by short tandem repeat analysis performed in our laboratory on the same cell stock. Primary fibroblasts were used below passage 10 to ensure integrity.

Mycoplasma contamination

Mycoplasma testing was performed periodically, with no contamination detected.

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

hTERT-RPE1 cells are not listed in the ICLAC register of commonly misidentified cell lines.

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

C57BL/6NCrI control mice and CD-1 (ICR) mice used for generating chimeric animals were either purchased from Charles River

Laboratories or sourced from an in-house colony at The Centre for Phenogenomics (Toronto, Ontario, Canada). Experiments were conducted on juvenile animals at postnatal day 17 (P17) and on adult animals aged between 19 and 21 weeks.

Wild animals

This study did not involve wild animals.

Reporting on sex

No sex-specific hypotheses were tested in this study. However, only male chimeric mice with the mutation of interest were obtained. Therefore, only male control animals were used to maintain consistency across experimental groups. This is reported in the Methods section of the manuscript.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight

All procedures involving animals were performed in compliance with the Animals for Research Act of Ontario and the Guidelines of the Canadian Council on Animal Care. The Animal Care Committee at The Centre for Phenogenomics (Toronto, Ontario, Canada) reviewed and approved all procedures.

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

This study did not involve a clinical trial.

Study protocol

This study did not involve a clinical trial. Therefore, no trial protocol was developed or registered.

Data collection

Clinical data referenced in this study consisted of qualitative diagnoses made by qualified medical professionals. No quantitative clinical measurements or assessments were collected as part of this study. Therefore, details on settings, locales, and recruitment periods do not apply.

Outcomes

This study did not involve predefined primary or secondary clinical outcome measures, nor were clinical outcomes directly assessed. Clinical diagnoses referenced were used solely as background information.

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, mosaicism, off-target gene editing) were examined.

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

For cycling samples, 1.5×10^5 cells/well were plated in complete medium in 12-well plates. For serum-starved samples, the same conditions as the ciliation assay were used: 3×10^5 cells/well were plated in complete medium in 12-well plates, and serum starved in Opti-MEM (Thermo Fisher Scientific) for 48 hours the following day, as described above. In preparation for flow cytometry, approximately 2×10^6 cells/sample were collected, resuspended in 50 μ l eBioscience Flow Cytometry Staining Buffer (Thermo Fisher Scientific 00-4222-26), and fixed with 1 ml of ice-cold 80% ethanol overnight at 4°C. Fixed cells were then collected, incubated with 500 μ l of 2 mg/ml RNase A (Qiagen 19101) in HBSS with calcium and magnesium (Thermo Fisher Scientific 14025-092) for 5 minutes at room temperature, and stained for 30 minutes at room temperature with 500 μ l of 0.1 mg/ml PI (Thermo Fisher Scientific P3566) in HBSS with 0.6% NP-40 (Sigma-Aldrich I8896). Cells were then filtered through a 40 μ m cell strainer (VWR CA21008-949).

Instrument	Flow cytometry was performed using a BD LSR II analyzer (BD Biosciences). Analysis of PI was performed using 561 nm (yellow-green) laser excitation and a 575/26 nm bandpass filter.
Software	FlowJo software version 10.8 (BD Biosciences).
Cell population abundance	The purity of the single nuclei population was estimated to be approximately 98%, based on flow cytometry gating shown in the gating strategy figure in Source Data file. Purity varied slightly between samples.
Gating strategy	Cells were first gated on FSC-A vs SSC-A to identify the main population. Doublets were excluded by sequential gating on FSC-H vs FSC-A and SSC-H vs SSC-A to select single cells. Finally, single nuclei were gated using propidium iodide (PI) signal (PI width vs PI area). Gates distinguishing positive and negative populations were set manually based on visual inspection of signal distribution, guided by unstained control samples.

☒ 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	Clinical / Research MRI with conventional sequences only (to assess structural abnormalities)
Design specifications	One MRI per patient
Behavioral performance measures	None

Acquisition

Imaging type(s)	Structural. Note: For the scan in Figure 10c, only the sequence names are available, as the scan was performed at an external institution and the acquisition parameters could not be retrieved.
Field strength	3T
Sequence & imaging parameters	Figure 1a and Supplementary Figure 1a: Sagittal 3DT1 (TR:5ms; TE:2.3ms; Slice thickness [ST]:1mm); Axial DWI (TR:5759ms; TE:86.3ms; ST:3mm); Axial FLAIR (TR:10000ms; TE:141ms; TI: 2850ms; ST: 3mm); Axial/Coronal T2 (TR:5712ms; TR: 80ms; ST: 3mm); Coronal T2 fat saturated orbits (TR:3679ms; TE: 81ms; ST: 2mm); FIESTA (TR: 6.5ms; TE: 3.2ms; ST: 0.5mm; matrix 528 x 528). Figure 10c: Sagittal T1 SPGR; T2 FLAIR CUBE; Axial T2 FRF FSE; T1 MT; 3D SWAN; DTI; Coronal T2 FRFSE; High-resolution coronal T2 FRFSE through the hippocampus.
Area of acquisition	Whole head
Diffusion MRI	☒ Used ☐ Not used
Parameters	Specify # of directions, b-values, whether single shell or multi-shell, and if cardiac gating was used.

Preprocessing

Preprocessing software	None
Normalization	None
Normalization template	None
Noise and artifact removal	None
Volume censoring	None

Statistical modeling & inference

Model type and settings	Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).
Effect(s) tested	Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.
Specify type of analysis:	☐ Whole brain ☐ ROI-based ☐ Both
Statistic type for inference	Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.
(See Eklund et al. 2016)	
Correction	Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

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