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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	HiSeq (Illumina)
Data analysis	Our in-house R and python pipelines will be provided by the authors on request. The software used to analyze the data in this study includes: GATK (https://www.broadinstitute.org/gatk/); TrioDeNovo (http://genome.sph.umich.edu/wiki/Triodenovo); DenovolyzeR (http://denovolyzer.org); Plink (http://pngu.mgh.harvard.edu/~purcell/plink); MetaSVM/CADD13/ANNOVAR (http://annovar.openbioinformatics.org); Seurat v4 (PMID34062119); ggplot2_3.3.0; igraph_1.2.5; clusterprofile 4.2.2; Cibersort (https://cibersortx.stanford.edu/); DeSeq2 (v1.26.0); R versions 3.5.0 and 3.6.1; Python 2.7; EIGENSTRAT; DMLE+2.3. Mouse brain MRI data were analyzed using ITK-SNAP v3.8.0-beta and BioImage Suite Web. FACS data were analyzed by FlowJo 10.6.1. Immunoblots were quantified by Fiji (2.3.0/1.53f). Immunofluorescent images were analyzed using Fiji (2.1.0/1.53c) and QuPath-0.2.0-m8.

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

The databases used in this study included ExAC03 (http://exac.broadinstitute.org; gnomAD (https://gnomad.broadinstitute.org); BRAVO (https://bravo.sph.umich.edu/). Unprocessed scans of all immunoblots in the paper are included as Source Data Fig. 1. WES data have been deposited in the database of Genotypes and Phenotypes (dbGaP) under accession numbers phs000744. A Life Sciences Reporting Summary is available for this paper. Bulk RNAseq data of mESCs have been deposited at the GEO database under the accession number GSE189420.

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 statistical methods were used to predetermine sample size a priori, but they are similar to those published elsewhere (PMID22508726, PMID26057209, PMID28430184)
Data exclusions	No data were excluded from analyses.
Replication	All experiments described were replicated successfully at least three times in the laboratory or in at least two independent cohort of animals.
Randomization	Randomization was not relevant to this study as controls and mutant mouse lines did not receive different treatments, human studies were descriptive studies, and cell line experiments were generated using the same experimental conditions.
Blinding	All experiments were performed and analyzed in a blinded manner.

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
☐	☒ Human research participants
☒	☐ 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:
rabbit anti-MIK67 1/250 (Cell Signaling Technology, catalog number 9129S, https://www.cellsignal.com/products/primary-antibodies/ki-67-d3b5-rabbit-mab/9129)

rabbit anti-pH3 1/250 (Cell Signaling Technology, catalog number 3377S, https://www.cellsignal.com/products/primary-antibodies/phospho-histone-h3-ser10-d2c8-xp-rabbit-mab/3377)

chicken anti-DCX 1/500 (Abcam, ab153668, https://www.abcam.com/doublecortin-antibody-ab153668.html)

rabbit anti-cleaved caspase 3 Alexa Fluor 488 conjugate 1/500 (Cell Signaling Technology, catalogue number 9669S, https://www.cellsignal.com/products/antibody-conjugates/cleaved-caspase-3-asp175-antibody-alexa-fluor-488-conjugate/9669)

rat anti-BCL11B/CTIP2 1/500 (Abcam, catalogue number 18465, https://www.abcam.com/ctip2-antibody-25b6-ab18465.html)

rabbit anti-POU3F2/BRN2 1/500 (Cell Signaling Technology, catalogue number 12137S, https://www.cellsignal.com/products/primary-antibodies/brn2-pou3f2-d2c1l-rabbit-mab/12137)

rabbit anti-TBR1 Alexa Fluor 647 conjugate 1/500 (Cell Signaling Technology, catalog number 45664S, https://www.cellsignal.com/products/antibody-conjugates/tbr1-d6c6x-rabbit-mab-alexa-fluor-647-conjugate/45664)

mouse anti-acetylated tubulin antibody 1/500 (Sigma, catalog number T7451-200UL, https://www.sigmaaldrich.com/US/en/product/sigma/t7451)

rabbit anti-ARL13B 1/500 (Invitrogen, catalog number PA5-61840, https://www.thermofisher.com/antibody/product/ARL13B-Antibody-Polyclonal/PA5-61840)

rabbit anti-S100B 1/200 (Novus, catalog number NB200-538, https://www.novusbio.com/products/s100a-b-antibody_nb200-538)

mouse anti-FoxJ1 1/500 (Invitrogen, catalog number 14-9965-82, https://www.thermofisher.com/antibody/product/FOXJ1-Antibody-clone-2A5-Monoclonal/14-9965-82)

mouse anti-E-cadherin 1/500 (R and D system, catalog number MBA7481, https://www.rndsystems.com/products/mouse-e-cadherin-antibody-114420_mab7481)

rabbit anti-OTX2 1/200 (Abcam, catalog number 183951, https://www.abcam.com/otx2-antibody-epr20375-ab183951.html)

rabbit anti-TRIM71 1/500 (gift from Dr. Shinya Yamanaka, Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA, PMID24239284)

rabbit anti-pNKCC1 1/500 (Millipore, ABS 1004, https://www.emdmillipore.com/US/en/product/Anti-phospho-NKCC1-Antibody-Thr212-Thr217,MM_NF-ABS1004?ReferrerURL=https%3A%2F%2Fwww.google.com%2F)

rabbit anti-NKCC1 1/500 (Cell Signaling, catalog number 85403S, https://www.cellsignal.com/products/primary-antibodies/nkcc1-d2o8r-xp-rabbit-mab-rodent-specific-if-specific/85403)

rabbit anti-pSPAK 1/250 (Millipore, 07-2273, https://www.emdmillipore.com/US/en/product/Anti-phospho-SPAK-Antibody-Ser373-phospho-OSR1-Antibody-Ser325,MM_NF-07-2273?ReferrerURL=https%3A%2F%2Fwww.google.com%2F)

mouse anti-SPAK 1/200 (Santa Cruz, catalog number sc-517361, https://www.scbt.com/browse/spak-Antibodies/_/N-qvhajp)

mouse anti-FLAG (M2) 1/5000 (Sigma-Aldrich, F1804, https://www.sigmaaldrich.com/DE/de/product/sigma/f1804)

mouse anti-GAPDH (6C5) 1/3000 (Acris, ACR001P, https://m1.acris-antibodies.com/pdf/ACR001P.pdf)

rabbit anti-TRIM71 1/1000 (Abnova, PAB19293, https://www.biozol.de/en/product/abn-pab19293)

rabbit anti-SHCBP1 1/1000 (Proteintech, 12672-1-AP, https://www.thermofisher.com/antibody/product/SHCBP1-Antibody-Polyclonal/12672-1-AP)

rabbit anti-CDKN1A/P21 1/1000 (Cell Signaling Technology, #2947, https://www.cellsignal.com/products/primary-antibodies/p21-waf1-cip1-12d1-rabbit-mab/2947)

rabbit anti-EGR1 1/1000 (Cell Signaling Technology, #4154, https://www.cellsignal.com/products/primary-antibodies/egr1-44d5-rabbit-mab/4154)

rabbit anti-UPF1 1/1000 (Cell Signaling Technology, #9435, https://www.cellsignal.com/products/primary-antibodies/upf1-antibody/9435)

mouse anti-VINCULIN (hVIN-1) 1/5000 (Sigma-Aldrich, V9131, https://www.sigmaaldrich.com/DE/de/product/sigma/v9131)

mouse anti-TUBULIN (DM1A) 1/3000 (Sigma-Aldrich, T9026, https://www.sigmaaldrich.com/DE/de/product/sigma/t9026)

rabbit anti-Ubiquitin 1/1000 (Cell Signaling Technology, #3933, https://www.cellsignal.com/product/productDetail.jsp?productId=3933)

Secondary antibodies:

goat anti-Chicken IgY (H+L) Alexa Fluor 555 1/400 (Invitrogen, catalog number A-21437, https://www.thermofisher.com/antibody/product/Goat-anti-Chicken-IgY-H-L-Secondary-Antibody-Polyclonal/A-21437)

goat anti-Rat IgG (H+L) Antibody Alexa Fluor 555 1/400 (Invitrogen, catalog number A-21434, https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21434)

goat anti-Mouse IgG (H+L) Alexa Fluor 488 1/400 (Invitrogen, catalog number A-11001, https://www.thermofisher.com/antibody/

product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11001)

goat anti-Mouse IgG (H+L) Alexa Fluor 555 1/400 (Invitrogen, catalog number A-21422, https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21422)

goat anti-Mouse IgG (H+L) Alexa Fluor 647 1/400 (Invitrogen, catalog number A-21235, https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21235)

goat anti-Rabbit IgG (H+L) Alexa Fluor 488 1/400 (Invitrogen, catalog number A-11008, https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11008)

goat anti-Rabbit IgG (H+L) Alexa Fluor 555 1/400 (Invitrogen, catalog number A-21428, https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21428)

goat anti-Rabbit IgG (H+L) Alexa Fluor 647 1/400 (Invitrogen, catalog number A-21245, https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21245)

horse anti-mouse IgG HRP linked 1/10000 (Cell Signaling Technology, #7076S, https://www.cellsignal.com/products/secondary-antibodies/anti-mouse-igg-hrp-linked-antibody/7076)

goat anti-rabbit IgG HRP linked 1/10000 (Cell Signaling Technology, #7074S, https://www.cellsignal.com/products/secondary-antibodies/anti-rabbit-igg-hrp-linked-antibody/7074)

Validation

Information regarding antibody validation is provided as a link to the manufacturer's website or the PMID in case of the rabbit anti-TRIM71 antibody from Yamanaka lab (PMID24239284) as above.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	R595H mutant mESC lines were generated as described in the Methods. Trim71 knock-out mESC lines were obtained from PMID26057209. HEK293T cells were obtained from ATCC.
Authentication	mESC lines were authenticated by morphology, genotyping, and expression analysis of TRIM71. HEK293T cells were verified by morphology.
Mycoplasma contamination	All cell lines tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Mice were reared in group housing less than five mice per cage at 25 °C and 56% humidity in a 12-h light:12-h dark cycle and provided food and water ad libitum with veterinary care provided by Yale Animal Resource Center. Mice were maintained on the C57BL/6J background. Trim71R595H mutant mice were generated as described in Methods. Nestin-cre (Jackson Laboratory, Stock No: 003771) mice were mated with Trim71fl/fl mice (PMID26057209) to obtain Nestin-Trim71fl/fl mice in which Trim71 is conditionally deleted from embryonic NSCs. Emx1-Cre (Jackson Laboratory, Stock No: 005628) were mated with a novel Cre-dependent Trim71 allele (Fig. 3q) to obtain Emx1-Trim71fl/- in which Trim71 is conditionally deleted from embryonic NSCs. TetO-cre (No.006234) and R26rtTA*M2 (No. 006965) mice were purchased from Jackson Laboratories (Bar Harbor, ME). We crossed TetO-cre tg; Trim71fl/fl males/females with Trim71fl/fl;R26rtTA*M2/rtTA*M2 males/females to obtain Trim71 KO (TetO-cre tg; R26rtTA*M2/+; Trim71fl/fl) and control littermates (R26rtTA*M2/+;Trim71fl/fl). To induce Cre expression, doxycycline (dox) was delivered to time-mated females via ad libitum access to feed containing 2 g/kg dox. Mice of both sexes were used. Number of mice used in each experiment is included in Supplementary Table 13.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve field-collected samples.
Ethics oversight	Mouse care and experiments were performed in accordance with protocols approved by the Yale University Institutional Animal Care and Use Committee.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Available relevant population characteristics have been provided in Supplementary Table 1. All patients had undergone therapeutic CSF diversion (shunt placement and/or endoscopic third ventriculostomy).

Recruitment	All sequenced trios composed of 483 primary CH probands including 289 parent-offspring trios and 194 singletons (Supplementary Table 1). All probands had undergone surgery for therapeutic CSF diversion (shunt placement and/or endoscopic third ventriculostomy). Patients and participating family members provided buccal swab samples (Isohelix SK-2S DNA buccal swab kits), medical records, neuroimaging studies, operative reports, and CH phenotype data. The diversity of clinical and neuroimaging presentations of CH, ranging clinically from asymptomatic to severely disabled or obtunded, and radiologically from mild isolated ventriculomegaly to massively dilated ventricles with associated cortical malformations, prompted our use of a "real world", practical metric of neurosurgically-treated CH (i.e. via shunt or ETV), to bias our cohort towards clinically-relevant cases of ventriculomegaly (as identified by practicing neurosurgeons/neurologists around the world). This does, however, bias our cohort away from subclinical forms of ventriculomegaly. Despite this aforementioned bias, we believe our identification of many cases with primary symptomatology outside those clasically associated with elevated ICPs strengthens our assertion that current prediction of surgical efficacy is complex at best. Further, by focusing on clinically relevant hydrocephalus, we believe this may increase the likelihood of identifying rare variants. In the setting of trio analysis, however, this in no way decreases the validity of any finding.
Ethics oversight	The study protocol was approved by the Yale Human Investigation Committee. Written informed consent was obtained from all adult participants. Parent or legal guardian authorization was obtained in writing for sample collection of all minors in this study.

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

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	mESC were labelled with the Cell Proliferation Dye eFluor670 (ThermoFisher) according to manufacturer's instructions. In short, mESC were dissociated, counted and washed in PBS. 1.4 million cells were stained with the proliferation dye eFluo670 diluted to 5 µM in 1 ml of PBS by incubation for 10 min at 37°C. Staining reaction was stopped by addition of 5 ml FCS and incubation for 5 min on ice. Cells were pelleted, washed once with medium and cultured in N2B27 medium for neural differentiation. 400000 cells were cultured in 3 wells of a 12 well plate, one well for each time point. The remaining cells were used for flow-cytometry (FACSCanto II, BD) to measure the initial fluorescence intensity later used as time point day 0. Cells were dissociated at indicated time points and flourescence intensity was measured by flow-cytometry.
Instrument	FACSCanto II by Becton Dickinson
Software	FlowJo 10.6.1
Cell population abundance	Approximately 10000 sorted cells were analyzed.
Gating strategy	Initial cell populations were gated according to size and sideward scatter using FSC/SCC. eFluor670 positive cells gate was set between unstained and max fluorescence intensity.

☐ 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	Live mice at resting state were imaged as described in the Methods
Design specifications	N/A
Behavioral performance measures	N/A

Acquisition

Imaging type(s)	Structural
Field strength	11.7 Tesla
Sequence & imaging parameters	We use a RARE (Rapid Acquisition with Relaxation Enhancement) imaging sequence. In 31 minutes, 30 seconds, using a TR/TE of 4500/ll.25ms, 20 repetitions, and rare-factor 8, we obtain a coronal 0.07x0.30x0.07mm3 image of the whole brain (FOV 1.40x8.40xl2.0mm3). This sequence is repeated, and the data averaged after motion correction using customized modules within Bioimage Suite (BIS) Web (www.bioimagesuite.org) developed in-house and available free online. In post-processing, again using BIS, we used signal intensity thresholding to delineate the ventricles from surrounding brain tissue and computed ventricular volume.
Area of acquisition	Whole mouse brain

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	N/A
Normalization	N/A
Normalization template	N/A
Noise and artifact removal	N/A
Volume censoring	N/A

Statistical modeling & inference

Model type and settings	N/A
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