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	*Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.*
Data analysis	The scRNA-seq data were analyzed using Parse software to identify cells by bar code, and to map transcripts to the mouse transcriptome. Data were then analyzed using our previously reported pipeline (Malawsky et al., 2021; Ocasio et al., 2019). All other statistical analysis were conducted using Prism9.0 (Graphpad software). Confocal images analysis was done in Zen software.

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 scRNA seq data reported in this manuscript are deposited at GEO submission (accession number-GSE233519). For the data used to investigate the relationship

of OLIG2 to survival in MB subtypes (Figure 1 AND Supplementary Figure 1), we analyzed transcriptomic datasets previously published by Cavalli et al in the R2 database (https://hgserver1.amc.nl/cgi-bin/r2/main.cgi, Cavalli et al., 2017).

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	Not relevant. For the purposes of this study we used organiod cultures from 3 medulloblastoma patient samples to show that CT-179 treatment induces cell death.
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	No patients were recruited as part of this study.
Ethics oversight	The use of patient-derived medulloblastoma samples for PDX and oragnoid models was reviewed by the uman Ethics Committee of the Queensland Institute of Medical Research (QIMR, Brisbane, QLD), Queensland Children's Hospital (QCH) and Queensland Children's Tumor Bank (QCTB) and approved as protocols P3420-A2102-601M and P2324-A1706-612M.

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 in vitro experiments n was always at least done three three times unless stated otherwise in the figure legends/methods. For PDX animal experiments number of animals per group was 10. This sample size of 10 had a 80% power to detect an effect size of 1.16 (as per Cohen's d (SESs)) assuming a 5% significance level in a one-sided t-test, which was used to detect an extension in survival.
Data exclusions	As described in the text, a small number of mice developed treatment-related toxicities and were removed from the study. One mouse in the CT-179 single treatment arm had skin irritation at the injection site and was censored on Day 28 and another animal showed swelling of the abdomen at the injection site and was censored on Day 35. In the Med-813 PDX studies, we noted toxicities in both the RT group and the CT-179 + RT group. Two animals from the RT group and three animals from the combination group were censored due to radiation-induced side effects. In the scRNA-seq analysis, cells failing to meet QC cutoffs were excluded from analysis, as described in the text.
Replication	All studies were performed in replicates as enumerated in the text.
Randomization	In all animal treatment studies, individual animals were randomly assigned to each treatment group.
Blinding	Blinding was not feasible in our animal studies. However, animal survival time was determined by a strict set of humane endpoints as determined by the QIMR Ethic Committee and the UNC IACUC.

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

Antibodies from Cell Signaling Technology included mouse monoclonal anti-β-actin (clone 8H10D10), cat# 3700; rabbit monoclonal anti- β-actin (clone 13E5), cat# 4970; mouse monoclonal anti_Bcl-2 (clone 124), cat# 15071; rabbit monoclonal anti-Bcl-xL (clone 54H6), cat# 2764; rabbit monoclonal anti-Mcl-1 (clone D2W9E), cat# 94296; rabbit monoclonal anti-cleaved caspase-3 (Asp175) (clone 5A1E), cat# 9664; rabbit polyclonal anti-caspase-3, cat# 9662; rabbit monoclonal anti-cleaved PARP (Asp214) (clone D64E10), cat# 5625; mouse monoclonal anti-Cdc2 (clone POH1), cat# 9116; rabbit polyclonal anti-phospho-Cdc2 (clone Thr161), cat# 9116; rabbit monoclonal anti-cyclin B1 (clone D5C10), cat# 12231; rabbit monoclonal anti-phospho-Rb (Ser807/811) (clone D20B12), cat# 8974; rabbit monoclonal anti_NeuN (clone D4G4O), cat# 24307; rabbit monoclonal anti-SOX10 (clone E2V9N), cat# 78330; rabbit monoclonal anti-α-tubulin (clone 11H10), cat# 2125. Antibodies from Sigma-Aldrich included mouse monoclonal anti-OLIG2 (clone 211F1.1), cat# MABN50. Antibodies from Millipore included rabbit polyclonal anti-OLIG2, cat# ab9610. Antibodies from Cell Marque included rabbit monoclonal anti-OLIG2 (clone EP112), cat# 387R-14. Antibodies from Biocare Medical included rabbit polyclonal anti-cleaved caspase-3, cat# CP229. Antibodies from Dako included rabbit polyclonal anti-CD3, cat# A0452; mouse monoclonal anti-CD31 (clone JC-70A), cat# M0823; mouse monoclonal anti-Ki67 (clone MIB-1), cat# M7240. Antibodies from Abcam included rabbit monoclonal anti-Iba1 (clone EPR16588), cat# ab178846; rabbit monoclonal anti-SOX2 (clone EPR3131), cat# 92494. Antibodies from Daka Cytomation included goat anti-rabbit horseradish peroxidase, cat# P0448; rabbit anti-mouse horseradish peroxidase, cat# P0260.

Validation

All antibodies employed in this study were validated by the commercial vendors/manufacturers. In addition, specificity was for determined based on size, absence/reduced intensity in KD cells/positive/negative controls and available prior validation/use of these antibodies in the literature.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Cell lines obtained from the American Type Culture Collection were Daoy (male) and D283 (male). Cell lines obtained from Dr. Tobias Schoep at the Telethon Kids Institute, WA were UW228 (female), MB002 (male), D425 (male), and PER547. Cell lines obtained from the Olson Laboratory were Med-2312 PDX (female), Neo-113 PDX (unspecified), Med-2112 PDX (male), Med-113 PDX (male) and Med-813 PDX (male). MB-R201 PDX is a medulloblastoma PDX model generated in our lab. MB-R203 is a primary medulloblastoma cell line generated from patient specimen in our lab.

Authentication

All cell lines are routinely authenticated by short tandem repeat (STR) profiling

Mycoplasma contamination

All cell lines are routinely tested for mycoplasma contamination and all tested negative.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

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

NOD.Cg-Rag1tm1Mom Il2rgtm1Wjl/SzJArc (NRG) animals were used for PDX experiments. hGFAP-Cre and SmoM2 mice were used for GEMM animals experiments.

Wild animals

No wild animals were used

Reporting on sex

Female 4-8week old NRG animals were used for PDX experiments. Male and female G-Smo mice were included with equal probability in all studies.

Field-collected samples

not applicable

Ethics oversight

Medulloblastoma-bearing mice were maintained at UNC in accordance with UNC IACUC protocols 19-098 and 21-011 and at QIMR under protocol 1572 and 2324.

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	Cell cycle analysis in MB cells Cell cycle analysis was performed as descried previously (Linsley et al., 2007). Cells were fixed with 70% ice-cold ethanol and subsequently stained with PI and analyzed using a Fortessa 5 Flow Cytometer (Becton Dickinson) and data was analyzed using FlowJo® software (Tree Star). Annexin V staining in MB cells At the indicated time points, cells were stained using FITC-conjugated Annexin V and Annexin V binding buffer (BD PharmingenTM) according to the manufacturer's instructions. Annexin V or propidium iodide (PI)-positive cells were analyzed using a Fortessa 5 Flow Cytometer (Becton Dickinson) and data was analyzed using FlowJo® software (Tree Star). Cell cycle analysis in G-Smo mice Tumors were dissociated using the Worthington Papain Dissociation System Kit, then fixed for 15 minutes on ice and washed with FACS Wash buffer (2% FBS in PBS). Fixed cells were stained with fluorophore markers for DNA (FX Cycle stain, Thermo Fisher Scientific), EdU (Click-it Edu Kit, Thermo Fisher Scientific), and phospho-RB content (phospho-RBSer807/811, Cell Signalling Technology) as previously described (Hwang et al., 2021). Stained cells were resuspended in sheath fluid and ran on LSR II flow cytometer for 50000 events at the UNC Flow Cytometry core, using appropriate compensation controls and analyzed using FlowJo® software (Tree Star). Cell cycle analysis in MB cells Cell cycle analysis was performed as descried previously (Linsley et al., 2007). Cells were fixed with 70% ice-cold ethanol and subsequently stained with PI and analyzed using a Fortessa 5 Flow Cytometer (Becton Dickinson) and data was analyzed using FlowJo® software (Tree Star).
Instrument	Cell viability and cell cycle analysis – Fortessa 5 Flow Cytometer (BD) Cell cycle analysis in G-Smo mice – LSR II Flow Cytometer
Software	FlowJo (Tree Star)
Cell population abundance	The abundance of cells in each phase of the cell cycle is reported in the relavant sections of the results.
Gating strategy	Gating strategies used are demonstrated in Supplementary Figure 3A and Supplementary Figure 6C.

☒ 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	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s) *Specify: functional, structural, diffusion, perfusion.*

Field strength *Specify in Tesla*

Sequence & imaging parameters *Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*

Area of acquisition *State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software *Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*

Normalization *If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*

Normalization template *Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*

Noise and artifact removal *Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*

Volume censoring *Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

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