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Corresponding author(s): Tamra Werbowetski-Ogilvie

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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	No software was used
Data analysis	Trimmomatic 0.36, R (v.3.5.0), Seurat (R package) (v3.0.0), DESeq2 (R package) (v1.20.0), CellRanger (v3.0.2), GSEA (v3.0), Cell Seek (https://cellseek.stjude.org/ cerebellum/), Partek Genomics Suite software, Prism 8.0, Cytoscape v3.7.2, P, Kaluza 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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Novel ChIP-seq, RNA-seq and scRNA-seq data have all been deposited into GEO under the SuperSeries accession code GSE132558. Each dataset can be accessed separately through the following subseries: GSE132269 (ChIP-seq), GSE132506 (RNA-seq), and GSE137758 (scRNA-seq). All supportive data corresponding to the GEO files are displayed in Figures 1,3,5,6 and 7, as well as Supplementary Fig. 5-7. All other supporting data are available within the article and supplementary files. Additional publicly available datasets utilized include Ensembl GRCh38 (ChIP-seq Fig. 1) , GEO acession codes GSE92585 (Fig. 3d), GSE85217 (Fig. 1 and Supplementary Figure 1), and European Genome-phenome Archive accession code EGAS00001003170 (Fig. 2a-b).

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 power calculations were utilized to determine required sample size for in vitro and in vivo experiments. For in vivo studies, sample size was determined based on availability of animals and cost of the drug. For in vitro studies, all experiments were performed on a minimum of 3 independent biological replicates for each cell line with the majority of cell culture-based assays also being repeated with an additional 1-2 independent cell lines accordingly. For patient sample transcriptome, proteome, single cell RNA seq and IHC studies, sample size was based on the number of samples already present in publicly available datasets or made available to the lab through collaboration.
Data exclusions	For Figure 7d, two animals in the 50 mg/kg group were removed from the study as they died during oral gavage treatment due to tracheal injuries. Pre-established exclusion criteria included animals that die during oral gavage treatment due to tracheal injuries or develop any severe health issues unrelated to tumor growth that require euthanasia during the course of the experiment.
Replication	For vitro/molecular experiments (ie. tumorsphere assays, qPCR, ChIP-qPCR, bulk RNA sequencing), studies were carried out in 3 or greater independent biological replicates for each cell line with the majority also being replicated in 1-2 additional cell lines for reproducibility. All statistics were performed on independent biological replicates. Single cell RNA sequencing was performed on tumorspheres derived from 3 biologically independent Group 3 MB cell lines and data from individual cell lines was compared to results obtained from integrating the 3 datasets. Slides from multiple control and treatment group tumor sections were stained for IHC and representative images shown. In vivo studies for PAX3 and PAX6 overexpressing cells were performed twice, and in vivo PQR620 treatment studies were performed on 3 sets of NOD SCID mice.
Randomization	Following intracerebellar transplantation, NOD SCID mice were randomly distributed into groups for drug treatment in Figure 7. For all other experiments, cells/samples were randomly assigned into groups.
Blinding	Blinding was performed during MRI imaging and tissue preparation for IHC. For tumorsphere experiments, measurements were performed by 2 independent observers to minimize bias and ensure reproducibility. Remaining studies were not blinded but replicated as described above and/or repeated in 1-2 additional cell lines.

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
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

The following antibodies were used in this study (epitope, experimental procedure, company, catalog number, lot number, concentration/dilution):

H3K27me3, ChIP-sequencing, Millipore, 07-449, 2919706, 10ug
H3K4me3, ChIP-sequencing, Abcam, ab8580, N/A, 5 ug
OTX2, ChIP/ChIP-sequencing, Abcam, ab21990, N/A, 10 ug
OTX2, immunoblotting, Abcam, ab21990, N/A, 1/500
OTX2, immunoprecipitation, Proteintech, 13497-1-AP, N/A, 1/500
p-4E-BP1, immunoblotting, Cell Signaling Technology, 9451, N/A , 1/1000

p-4E-BP1, IHC, Cell Signaling Technology, 2855, N/A ,1/1000
Total 4E-BP1, immunoblotting, Cell Signaling Technology, 9644, N/A, 1/2000
Beta actin, immunoblotting, Sigma-Aldrich, A2228, N/A, 1/1000
p-AKT, immunoblotting, Cell Signaling Technology, 9611, N/A, 1/500
AKT, immunoblotting, Cell Signaling Technology, 9272, N/A, 1/500
Cleaved Caspase-3, Cell Signaling Technology, 9664, N/A, 1/500
Doublecortin, immunoblotting, Cell Signaling Technology, 4604, N/A, 1/500
EZH2, immunoblotting, Cell Signaling Technology, 5246, N/A, 1/1000
GAPDH, immunoblotting, Santa Cruz Biotechnology, sc-47724, 1/3000
PAX3, Immunoblotting, Abcam, ab180754, N/A, 1/500
PAX3, IHC, Developmental Studies Hybridoma Bank, AB528426, N/A, 1/400
PAX6, immunoblotting, ThermoFisher Scientific, 42-6600, N/A, 1/500
PAX6, IHC, ThermoFisher Scientific, 42-6600, N/A, 1/150
p-Raptor, immunoblotting, Cell Signaling Technology, 2083, N/A, 1/1000
Raptor, immunoblotting, Cell Signaling Technology, 2280, N/A, 1/1000
Rheb, immunoblotting, Cell Signaling Technology, 13879, N/A, 1/500
p-S6, immunoblotting, Cell Signaling Technology, 2211, N/A, 1/1000
p-S6, IHC, Cell Signaling Technology, 2211 N/A, 1/400
Total S6, immunoblotting, Cell Signaling Technology, 2317, N/A, 1/1000
SOX2, immunoblotting, Cell Signaling Technology, 3579, N/A, 1/500
STEM121, IHC , Clontech, Y40410, N/A, 1/100
SUZ12, immunoblotting, Cell Signaling Technology, 3737, N/A, 1/1000
TUJ1,immunoblotting and IHC, R&D Systems, MAB1195, HGQ0116081, 1/1000 and 1/250 for IHC
Ki67, IHC, Cell Signaling Technology, 9449, N/A, 1/800
Annexin V, Flow cytometry, BD Biosciences, 561012, N/A, as per manufacturer's guidelines
BrdU, flow cytometry, BD Biosciences, 552598, N/A, as per manufacturer's guidelines
Biotin-SP sheep anti-mouse IgG,IHC, Jackson ImmunoResearch, 515-065-003, N/A, 1/500
Biotin-SP goat anti-rabbit IgG, IHC, Jackson ImmunoResearch, 111-065-144, N/A, 1/500
Goat anti-mouse HRP, immunoblotting, Abcam, ab6789, N/A, 1/3000
Donkey anti-rabbit HRP, immunoblotting, Jackson Immunoresearch, 711-035-152, N/A, 1/5000

Validation

H3K27me3 (07-449) antibody underwent dot blot testing for trimethylated lysine 27 specificity and validated by IP.
H3K4me3 (ab8580) antibody is tested in peptide arrays where specific recognition to the H3K4 modification was observed. Manufacturer tested applications include ChIP and ChIP-seq.
OTX2 (ab21990) manufacturer tested applications include ChIP
OTX2 (13497-1-AP) antibody underwent KD/KO validation and was tested for IP with Y79 lysates by the manufacturers.
p-4E-BP1 (9451), Total 4E-BP1 (9644), p-AKT (9611), AKT (9272), Cleaved Caspase-3 (9664), Doublecortin (4604), EZH2 (5246), p-Raptor (2083) , Raptor (2280), Rheb (13879), p-S6 (2211), total S6 (2317), Sox2 (3579), Suz12 (3737), antibodies are validated according to Cell Signaling Technologies Hallmarks of Antibody Validation™ which for immunoblotting may include KD/KO validation, validation across several cell lines/tissues, etc.
p-4E-BP1 (2855), p-S6 (2211) and Ki-67 (9449) antibodies are validated according to Cell Signaling Technologies Hallmarks of Antibody Validation™ which for IHC may include staining with the use of blocking peptides, staining on human cancer tissue arrays, etc.
GAPDH (516214) has been tested in immunoblotting by the manufacturers.
PAX3 (ab180754) antibody has been tested in immunoblotting by the manufacturers.
PAX3 (AB528426) antibody has been used for IHC in a number of publications including: 1. Lang D, et al., "Pax3 functions at a nodal point in melanocyte stem cell differentiation". Nature 433.7028 (2005 Feb 24): 884-7; 2. Medic and Zinman, "PAX3 expression in normal skin melanocytes and melanocytic lesions (naevi and melanomas)", PloS one 5.4 (2010 Apr 22): e9977.
PAX6 (42-6600) antibody was validated by knockdown by the manufacturers.
Stem121 (Y40410) antibody has been widely used for detection of human cell engraftment: Kelly S, et al . "Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex". PNAS . (2004) 101: 11839-11844.; 2. Cummings BJ, et al . "Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice". PNAS. (2005) 102: 14069-14074.; 3. Tamaki SJ, et al . "Neuroprotection of host cells by human central nervous system stem cells in a mouse model of infantile neuronal ceroid lipofuscinosis". Cell Stem Cell. (2009) 5: 310-319.; 4. Kallur T, et al . "Human fetal cortical and striatal neural stem cells generate region-specific neurons in vitro and differentiate extensively to neurons after intrastriatal transplantation in neonatal rats" .J Neurosci Res. (2006) 84:1630-1644. ; 5.SalazarDL,etal. "Human neural stem cells differentiate and promote locomotor recovery in an early chronic spinal cord injury NOD-scid mouse model" .PLoS ONE. (2010) 5:e12272.
BIII tubulin (Tuj1) (MAB1195) has been tested for immunoblots in human and mouse neural tissue by the manufacturers.
Annexin V (561012) and BrdU (552598) were titrated by investigators to attain the appropriate concentrations.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	D283 was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA) HDMB03 was kindly provided by Dr. Till Milde (Milde T, et al., Journal of Neuro-oncology, 2012) MB3W1 was kindly provided by Dr. Matthias Wölfl (Dietl S, et al., BMC cancer, 2016)
Authentication	All cell lines have been authenticated by STR profiling (ATCC)
Mycoplasma contamination	Cell lines were not tested for Mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No cell lines utilized in our study are listed in the ICLAC register

Animals and other organisms

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

Laboratory animals	NOD-SCID male mice aged 7-9 weeks were utilized for all in orthotopic xenograft in vivo analysis.
Wild animals	Our study did not utilize wild animals
Field-collected samples	Our study did not involve field-collected samples
Ethics oversight	All in vivo procedures were approved by the University of Manitoba Animal Care Committee (AUP-17-008).

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	These data are based on the samples collected in 2 recently published studies: 1. Cavalli et al., Cancer Cell 2017 Jun 12;31(6):737-754.e6. doi: 10.1016/j.ccell.2017.05.005 2. Vladiou et al., Nature, 2019 Aug;572(7767):67-73. doi: 10.1038/s41586-019-1158-7. Epub 2019 May 1. See these studies for all details regarding patient populations.
Recruitment	All medulloblastoma samples were collected at diagnosis after obtaining informed consent from subjects as part of the Medulloblastoma Advanced Genomics International Consortium. Approval was obtained from institutional research ethics boards at all contributing institutions (Cavalli et al, 2017; Vladiou et al., Nature, 2019)
Ethics oversight	See 2 published studies above for all details.

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

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.*	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE132269
Files in database submission	GSE132269_diffrepsH3K27me3.hotspot.txt.gz GSE132269_diffrepsH3K27me3.txt.gz GSE132269_diffrepsH3K4me3.hotspot.txt.gz GSE132269_diffrepsH3K4me3.txt.gz

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

one replicate of each condition:

GSM3855384 H3K4me3OTX2high
GSM3855385 H3K4me3OTX2low
GSM3855386 H3K27me3OTX2high
GSM3855387 H3K27me3OTX2low
GSM3855388 OTX2low (input control)
GSM3855389 OTX2high (input control)

Sequencing depth

Single end 75bp reads:
H3K27me3OTX2high: 79587453 total, 55830223 uniquely mapped
H3K27me3OTX2low: 83779457 total, 55101562 uniquely mapped
H3K4me3OTX2high: 53158040 total, 38592295 uniquely mapped
H3K4me3OTX2low: 44076908 total, 29747824 uniquely mapped
OTX2high: 96361808 total, 72228795 uniquely mapped
OTX2low: 60765131 total, 44855374 uniquely mapped

Antibodies

H3K27me3, Millipore, catalog number 07-449, lot number 2919706
H3K4me3, Abcam, catalog number ab8580
OTX2, Abcam, catalog number ab21990

Peak calling parameters

#note genome name hg19 is used in both of these as a required argument, diffreps does not directly support hg38 so the hg19 argument was used for genome length estimation only.

diffReps.pl --treatment H3K27me3OTX2high_trimmed_sorted_dedup.bed --control H3K27me3OTX2low_trimmed_sorted_dedup.bed --btr OTX2high_trimmed_sorted_dedup.bed --bco OTX2low_trimmed_sorted_dedup.bed --mode block --gname=hg19 --meth gt --nproc 8 --report diffrepsH3K27me3

diffReps.pl --treatment H3K4me3OTX2high_trimmed_sorted_dedup.bed --control H3K4me3OTX2low_trimmed_sorted_dedup.bed --btr OTX2high_trimmed_sorted_dedup.bed --bco OTX2low_trimmed_sorted_dedup.bed --mode peak --meth gt --gname=hg19 --nproc 8 --report diffrepsH3K4me3

Specifics for Supplementary Dataset 1 are as follows:
• Counts of MACS2 peaks associated with –5kb to +2kb regions around the TSS and gene bodies for H3K4me3 and H3K27me3 respectively.
• Count of MACS2 OTX2 peaks associated with –5kb to +2kb regions around the TSS.
• The most significant diffReps peaks adjusted pvalue and fold change associated with –5kb to +2kb regions around the TSS and gene bodies for H3K4me3 and H3K27me3 respectively.
• FPKM and count values for reads in –5kb to +2kb regions around the TSS and gene bodies for H3K4me3 and H3K27me3 respectively.
• Indication of membership in neuronal and axon associated genes, association of genes with Gene Ontology terms GO:0003677 (DNA binding), GO:0003700 (DNA-binding transcription factor activity)

Data quality

FASTQC analysis was performed
Mapping statistics were determined

Software

ChIP-Sequencing reads were trimmed using Trimmomatic 0.36. The resulting trimmed sequences were mapped to GRCh38 using bowtie2 2.3.3.1. Histone differential peak analysis was performed using diffReps G-test comparisons were performed in "block" mode for H3K27me3 and in "peak" mode for H3K4me3, utilizing the OTX2 high and low input samples treatment group backgrounds.

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	BrdU incorporation was analysed using a BD Pharmingen BrdU Flow Kit (BrDU-APC) (BD Biosciences) according to manufacturer's instructions as previously described (Liang et al., Cancer Research, 2018) in PAX GOF tumorspheres grown for 72 hours in stem cell conditions. The Annexin V Apoptosis Detection Kit (Annexin V-APC) (BD Biosciences) was used to evaluate cell death as previously described (Liang et al., Cancer Research, 2018). In both cases, tumorsphere populations were dissociated using Accutase into single cell suspensions for further analyses.
Instrument	MoFlo XDP cell sorter (Beckman Coulter, Mississauga, ON, Canada)
Software	Kaluza software (Beckman Coulter)
Cell population abundance	Cell sorting was not performed in these studies, only analytical studies by flow cytometry.
Gating strategy	For BrDU and Annexin V staining, "dead/dying" cells were not gated out, as they were included in the analysis. For BrDU incorporation, gates were set based on the 7AAD only negative control to determine the G0/G1, S, and G2-M gate delineations. For Annexin V, gates were set based on 1. unstained controls. and 2. 7AAD only without Annexin V staining. Plots depicting gating strategy are included in Figure 4 of the manuscript.

☒ 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	Resting state
Design specifications	Not applicable
Behavioral performance measures	Not applicable

Acquisition

Imaging type(s)	Structural
Field strength	7 Tesla
Sequence & imaging parameters	Fast spin echo T1, FOV 30 x 30 mm, matrix 512x 256, TR= 1000ms, TE=11ms, FA=90, coronal slice, 0.3mm slice thickness Fast spin echo T2, FOV 30 x 30 mm, matrix 256x 245, TR= 5000ms, TE=45ms, FA=90, coronal slice, 0.3mm slice thickness
Area of acquisition	Whole brain

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	Not applicable
Normalization	Not applicable
Normalization template	Not applicable
Noise and artifact removal	Not applicable
Volume censoring	Not applicable

Statistical modeling & inference

Model type and settings	Not applicable
Effect(s) tested	Not applicable

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

Statistic type for inference (See Eklund et al. 2016)	Not applicable
Correction	Not applicable

Models & analysis

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