

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

Data analysis

strawr (v0.0.1)
 plotgardener (v0.1.0)
 Trimmomatic (v0.32)
 Integrative Genomics Viewer (v2.11.1)
 GraphPad Prism (v9.3.1)

All analyses were performed in the R computing environment (versions 3.6 and 4.1).

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](#)

ChIPseq for human cell lines and scRNAseq data for normal murine samples have been deposited in the Gene Expression Omnibus (GEO) under accession number GEO: GSE188625. Bulk RNAseq, ChIPseq, scRNAseq, scATACseq, and scMultiome sequencing data for human tumors have been deposited in the European Genome-phenome Archive (EGA) under accession number EGA: EGAS00001005773. Accession numbers for published data used in this study are provided in Supplementary Table 1, Supplementary Table 2, and Supplementary Table 3.

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 measures were used to determine sample size. For human samples, sample size was determined by the availability of donor and patient-derived material. For the healthy mouse timecourse, sample size was determined based on developmental events in the pons and forebrain relevant to tumor types of focus. Post-hoc acquisition of the reference, we used two methods to confirm that this sample size was sufficient for the necessary analyses: 1) we performed downsampling experiments to confirm that cell type projections were robust to cell numbers; 2) we performed a cross-species analysis to confirm that cell type projections based on the mouse reference was consistent with a human reference. For mouse xenografts, experiments were performed with 3 to 6 replicates. For patient-derived cell lines, all conclusions were drawn from multiple cell lines from independent patients with the same mutations, with the exception of the rare H3.1K27M PFA, which is clearly indicated in the manuscript.
Data exclusions	All of the data acquired was utilized for analysis, and filtering of single-cell RNAseq/scATAC cells and clusters not retained for downstream analysis is specified in the Methods.
Replication	We replicated our findings on tumor cell-of-origin on several patient samples from independent source (institutions and cohorts) for each tumor type, as well as across data types (RNAseq, ChIPseq, scRNAseq, scATACseq). NKX6-1 expression was validated using immunohistochemistry (including on independent tumor samples). For ddPCR measurements, experiments were performed with 3 to 4 technical replicates. For mouse xenografts, survival was assessed on replicates.
Randomization	Samples were allocated to groups according to genotype and brain region. No randomization was required because the sequencing of nucleic acid libraries are not affected by sample randomization.
Blinding	Blinding was not applicable to this study as no effect of treatment or perturbations to the system were assessed.

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

Methods

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	<p>For ChIP, the following primary antibodies were used: For ChIP, the following primary antibodies were used: anti-H3K27me3 (1:40, Cell Signaling Tech, 9733), anti-H3K27me2 (1:50, Cell Signaling Tech, 9728), anti-SUZ12 (1:150, Cell Signaling Tech, 3737), anti-CTCF (1:400, Diagenode, C15410210), anti-H3K27ac (1:100, Diagenode, C15410196).</p> <p>For IHC, the following antibodies were used: - Phospho-Smad1 (Ser463/465)/ Smad5 (Ser463/465)/ Smad9 (Ser465/467) (D5B10) Rabbit mAb (1:100, Cell Signaling, #13820, lot3) - Recombinant Anti-NKX6.1 antibody (EPR20405) Rabbit (1:250, abcam, #221549, Lot GR3245957-4)</p> <p>For Western Blot, the following antibodies were used: - anti-rabbit IgG Horseradish Peroxidase linked whole antibody (1:1000, GE Healthcare) was applied for 1h in 3% BSA/skim milk in TBST - anti-H3K27M (1:200, Millipore ABE419) - anti-total H3 (1:2000, Abcam 1791)</p>
Validation	For ChIP, the specificity of antibodies was tested by dot blot on a histone modification peptide array. Primary antibodies were validated for human and mouse species by respective manufacturers, with the information available on manufacturers' websites. Primary antibodies for Western blot used have been used in the literature and also validated by manufacturers.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	All cell lines were derived from primary human tumors. Patient demographic information is listed in Supplementary Table 1. The original source of each line is as follows: SU-DIPGIV, SU-DIPGXXI, SU-DIPGXXXVI, SU-DIPGXIII (Michelle Monje) BT245, BT869 (Keith Ligon) HSJD-DIPG007 (Angel Carcaboso) HSJ-019 (Nada Jabado)
Authentication	Cell lines have been authenticated as unique by distinct RNA-seq and chromatin patterns. The identity of cell lines were checked by microsatellite typing (DNA fingerprinting). CRISPR/Cas9 edited clones were verified by Sanger sequencing and targeted resequencing by MiSeq to confirm specific genome editing event.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used in this study.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	<p>For the healthy mouse developmental timecourse, C57BL6 mice were used at the following time points: E10.5, E13.5, E16.5, E18.5. Both male and female mice were used. Mice were housed in compliance with the CCAC Guidelines and the OMAFRA Animals for Research Act. Mice were housed with a dark/light cycle of 12hrs each, in individually ventilated cages, supplied with 100% fresh air and received 10-15 air changes per hour. Room temperature was maintained at 20-22°C.</p> <p>For xenograft experiments, female NOD.Cg-Prkdcscid mice (4-6 weeks) (Jackson Laboratory, strain # 005557) were used. Mice were housed under a cycle of 14-hours light and 10-hours dark (14:10) as per CCAC guidelines-3.1.1. Room temperature was continuously monitored by a building automation system equipped with an alarm or notification system, and maintained in the range of 20-26 °C (CCAC-3.1.2). Room relative humidity was kept at between 40-60%, as stated in the CCAC guidelines on: laboratory animal facilities – characteristics, design and development (CCAC, 2003).</p>
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve animals collected from the field.

Ethics oversight

Animal protocols for this study were approved by the following:
 Animal Compliance Office, McGill University and Affiliated Hospitals Research Institutes
 Animal Care Committee of The Centre for Phenogenomics, Joseph and Wolf Lebovic Centre

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

Patients diagnosed with pediatric brain tumors (high-grade glioma or posterior fossa ependymoma) were recruited from McGill University Health Centre and the Hospital for Sick Children/The Arthur and Sonia Labatt Brain Tumour Research Centre Biobank. All relevant covariates and demographic information is included in Supplementary Table 1.

Recruitment

Patients diagnosed with pediatric brain tumors were recruited from the above centers. Informed consent was obtained from all research participants. Compensation was not provided.

Ethics oversight

Protocols for this study involving human samples were approved by the following:
 Research Ethics Board, McGill University and Affiliated Hospitals Research Institutes
 Research Ethics Board, Hospital for Sick Children

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.

ChIPseq for human cell lines and scRNAseq data for normal murine samples have been deposited in the Gene Expression Omnibus (GEO) under accession number GEO: GSE188625. Bulk RNAseq, ChIPseq, scRNAseq, scATACseq, and scMultiome sequencing data for human tumors have been deposited in the European Genome-phenome Archive (EGA) under accession number EGA: EGAS00001005773. Accession numbers for published data used in this study are provided in Supplementary Table 1, Supplementary Table 2, and Supplementary Table 3.

Files in database submission

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 BT245_KO_c5p8_H3K27ac_SE_blacklist_bs1_rpkmapq0_nodup_rx.bw
 BT245-ko-c2p25-H3-1K27M-p6-Rx_H3K27me2.bw
 BT245-ko-c2p25-H3-1WT-p6-Rx_H3K27me2.bw
 BT245-ko-c2p25-H3-1K27M-p6-Rx_H3K27me3-C.bw
 BT245-ko-c2p25-H3-1WT-p6-Rx_H3K27me3-C.bw
 BT416-C11-Rx_cells_ChIP1_H3K27me3_1.bw
 HSJ019_KO_c8p30_H3K27ac_PE_blacklist_bs1_rpkmapq0_nodup_rx.bw
 HSJ019_parental_C4_H3K27ac_SE_blacklist_bs1_rpkmapq0_nodup_rx.bw

H5J019-C7-Rx_cells_ChIP1_H3K27ac_1.bw
 H5J019_KO_c10p29_H3K27ac_PE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 H5J019_parental_p25_H3K27ac_PE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 H5J051-p32-Rx_XChIP_H3K27ac.bw
 H5J051-p32.H3K27me2.bw
 H5J051-p32-Rx_XChIP_H3K27me3-C.bw
 AB33_PFA2-Rx-XChIP_K27me2.bw
 AB29_PFA4-Rx-XChIP_K27me2.bw
 PFA4_hypox_p3.H3K27me3.bw
 AB30_PFA5-Rx-XChIP_K27me2.bw
 PFA9_hypox_p3.H3K27me3.bw
 P-1741_S-2756_1_Input.bw
 P-1741_S-2756_2_Input.bw
 P-1752_SX-2748_1_Input.bw
 P-2200_S-2200_1_Input.bw
 P-3147_S-3146_1_Input.bw
 P-1601_S-1601_1_Input.bw
 P-1601_S-1601_2_Input.bw
 P-1982_S-1982_1_Input.bw
 P-4111_S-4496_1_Input.bw
 P-1192_S-1193_2_Input.bw
 P-1192_S-1193_1_Input.bw
 P-1425_S-1425_2_Input.bw
 P-1425_S-1425_1_Input.bw
 P-1411_S-1411_2_Input.bw
 P-5425_S-6886_1_Input.bw
 P-5426_S-6887_1_Input.bw
 P-5428_S-6889_1_Input.bw
 P-5430_S-6891_1_Input.bw
 P-2077_S-2077_1_Input.bw
 ASH171_DIPG21-pX-Rxx_Input.bw
 DIPG21_NKO_c4p6_Input_PE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 DIPG21_KO_c7p6_Input_PE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 DIPG21_parental_p28_Input_SE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 DIPG36_NKO_c1S2_Input_PE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 DIPG36_KO_c2p4_Input_PE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 DIPG36_KO_c3S2p6_Input_SE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 DIPG36_parental_p20_Input_SE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 DIPGIV_KO_c1p5_Input_SE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 DIPGIV_NKO_c3p5_Input_SE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 DIPGIV_KO_c9p9_Input_PE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 DIPGIV_parental_p22_Input_SE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 ASH213_DIPGIV-ACVR1-ko-c129p7-Rx_Input.bw
 DIPGIV-nko-c3p5-Rx_Input.bw
 BT869p21-Rx_Input.bw
 DIPG007p149-Rx_Input.bw
 DIPGVI-C30-Rx_cells_ChIP1_Input_1.bw
 DIPGXIII-pool_cells_ChIP1_Input_1.bw
 DIPGXIII-ko-c5p32-H3-1K27M-p6-Rx_Input.bw
 DIPGXIII-ko-c5p32-H3-1WT-p6-Rx_Input.bw
 BT245-pool_cells_ChIP1_Input_1.bw
 BT245_KO_c5p8_Input_SE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 BT245-ko-c2p25-H3-1K27M-p6-Rx_Input.bw
 BT245-ko-c2p25-H3-1WT-p6-Rx_Input.bw
 BT416-C11-Rx_cells_ChIP1_Input_1.bw
 H5J019_KO_c8p30_Input_PE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 H5J019_parental_C4_Input_SE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 H5J019_parental_C7_Input_SE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 H5J019_KO_c10p29_Input_PE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 H5J019_parental_p25_Input_PE_blacklist_bs1_rpkms_MAPQ0_noDup_rx.bw
 H5J051-p32-Rx_XChIP_Input.bw
 PFA2-Rx_cells_ChIP1_Input.bw
 PFA4-Rx_cells_ChIP1_Input.bw
 PFA5-Rx_cells_ChIP1_Input.bw
 PFA9_hypox_p3.Input.bw
 P-1741_S-2756_1_Input.bam
 P-1741_S-2756_2_Input.bam
 P-1752_SX-2748_1_Input.bam
 P-2200_S-2200_1_Input.bam
 P-3147_S-3146_1_Input.bam
 P-1601_S-1601_1_Input.bam
 P-1601_S-1601_2_Input.bam
 P-1982_S-1982_1_Input.bam
 P-4111_S-4496_1_Input.bam
 P-1192_S-1193_2_Input.bam
 P-1192_S-1193_1_Input.bam

P-1425_S-1425__2__Input.bam
 P-1425_S-1425__1__Input.bam
 P-1411_S-1411__2__Input.bam
 P-5425_S-6886__1__Input.bam
 P-5426_S-6887__1__Input.bam
 P-5428_S-6889__1__Input.bam
 P-5430_S-6891__1__Input.bam
 P-2077_S-2077__1__Input.bam
 ASH171_DIPG21-pX-Rxx_Input.sorted.bam
 DIPG21_NKO_c4p6_Input_PE.bam
 DIPG21_KO_c7p6_Input_PE.bam
 DIPG21_parental_p28_Input_SE.bam
 DIPG36_NKO_c1S2_Input_PE.bam
 DIPG36_KO_c2p4_Input_PE.bam
 DIPG36_KO_c3S2p6_Input_SE.bam
 DIPG36_parental_p20_Input_SE.bam
 DIPGIV_KO_c1p5_Input_SE.bam
 DIPGIV_NKO_c3p5_Input_SE.bam
 DIPGIV_KO_c9p9_Input_PE.bam
 DIPGIVp22-Rx_cells_ChIP1_Input_1.sorted.dup.bam
 ASH213_DIPGIV-ACVR1-ko-c129p7-Rx_Input.sorted.bam
 DIPGIV-nko-c3p5-Rx_Input.sorted.bam
 BT869p21-Rx_cells_ChIP1_Input_1.sorted.dup.bam
 DIPG007p149-Rx_cells_ChIP1_Input_1.sorted.dup.bam
 DIPGVI-C30-Rx_cells_ChIP1_Input_1.sorted.dup.bam
 DIPGXIII-pool_cells_ChIP1_Input_1.sorted.dup.bam
 HI.4862.006.Index_4.DIPGXIII-ko-c5p32-H3-1K27M-p6-Rx_ChIP1_Input_1.bam
 HI.4862.006.Index_2.DIPGXIII-ko-c5p32-H3-1WT-p6-Rx_ChIP1_Input_1.bam
 BT245-pool_cells_ChIP1_Input_1.sorted.dup.bam
 BT245_KO_c5p8_Input_SE.bam
 HI.4862.006.Index_25.BT245-ko-c2p25-H3-1K27M-p6-Rx_ChIP1_Input_1.bam
 HI.4862.006.Index_23.BT245-ko-c2p25-H3-1WT-p6-Rx_ChIP1_Input_1.bam
 BT416-C11-Rx_cells_ChIP1_Input_1.sorted.dup.bam
 HSJ019_KO_c10p29_Input_PE.bam
 HSJ019_parental_C4_Input_SE.bam
 HSJ019-C7-Rx_cells_ChIP1_Input_1.sorted.dup.bam
 HSJ019_KO_c8p30_Input_PE.bam
 HSJ019_parental_p25_Input_PE.bam
 HSJ051-p32-Rx_XChIP_Input.sorted.dup.bam
 HI.4630.002.Index_10.PFA2-Rx_cells_ChIP1_Input_1.bam
 PFA4-Rx_cells_ChIP1_Input_1.sorted.dup.bam
 PFA5-Rx_cells_ChIP1_Input_1.sorted.dup.bam
 PFA9_hypox_p3.Input.sorted.dup.bam

Genome browser session
(e.g. [UCSC](#))

Not applicable - data visualization using IGV and BentoBox/plotgardener.

Methodology

Replicates	Biological replicates and/or independent samples were used throughout human tumor analyses, mouse xenografts, mass spectrometry, and cell line experiments.
Sequencing depth	ChIP libraries were sequenced using Illumina HiSeq 2000, 2500 or 4000 at 50bp single reads or Illumina NovaSeq 6000 at 50bp paired-end reads (one read used in the analysis for compatibility).
Antibodies	For ChIP, the following primary antibodies were used: anti-H3K27me3 (1:40, Cell Signaling Tech, 9733), anti-H3K27me2 (1:50, Cell Signaling Tech, 9728), anti-SUZ12 (1:150, Cell Signaling Tech, 3737), anti-CTCF (1:400, Diagenode, C15410210), anti-H3K27ac (1:100, Diagenode, C15410196). All ChIP primary antibodies were validated for human and mouse species by respective manufacturers, the information is available on manufacturers' websites.
Peak calling parameters	Not applicable - no peak calling was performed on ChIPseq data
Data quality	ChIP-sequencing experiments were assessed for their percent of mapped reads (to hg19 and dm6, when applicable) to ensure proper coverage. Reads with poor mapping quality were discarded from further analysis. Antibody pulldown efficacy was visually assessed by assessing tracks and comparing with spiked-in distribution of marks, when applicable.
Software	GenPipes (v3.1.2 and v3.1.0) bwa-mem (v0.7.12) Picard (v2.0.1) VisRSeq (v0.9.40) SeqMonk (v1.46) Homer (v4.9.1) Rsubread (v2.4.2) changepoint (v2.2.2)

