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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	Image Studio Lite (Ver. 5.2), CLC Genomics Software WorkBench 2020, MACS2 (2.2.7), Deeptools(v3.4.3), Bowtie2(v2.3.5.1), Cell Quest Software (Becton Dickinson Immunocytometry), FlowJo(v10.6.2)
Data analysis	iDEP.94 DESeq2 Statistical packages in R, PRISM Graphpad 7.0, PGSEA(3.0) packages in R

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

H3K36me3 ChIP-Rx-Seq Data GEO accession# GSE160006
RNA-sequencing files deposited to GEO accession# GSE160088
Publicly available datasets used:
Reference sequence = Homo sapiens (hg19) sequence
Gene track = Homo_sapiens_ensembl_v91_o_Genes
mRNA track = Homo_sapiens_ensembl_v91_o_mRNA

Source data for all figures has been provided as source data files.

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	Minimum of 5-10 mice were used per experimental arm. Male and female mice were used. See methods and figure legends for specifics. there was no a priori sample size selection. A post power calculation was performed which ensured proper power, based on previous studies on average for brain tumor models 5-10 mice are sufficient. See Koncar RF, Dey BR, Stanton AJ, Agrawal N, Wassell ML, McCarl LH, Locke AL, Sanders L, Morozova-Vaske O, Myers MI, Hamilton RL, Carcaboso AM, Kohanbash G, Hu B, Amankulor NM, Felker J, Kambhampati M, Nazarian J, Becher OJ, James CD, Hashizume R, Broniscer A, Pollack IF, Agnihotri S. Identification of Novel RAS Signaling Therapeutic Vulnerabilities in Diffuse Intrinsic Pontine Gliomas. Cancer Res. 2019 Aug 15;79(16):4026-4041. doi: 10.1158/0008-5472.CAN-18-3521. Epub 2019 Jun 14. PMID: 31201162.
Data exclusions	No data was excluded
Replication	All experiments were done in biological replicates unless specifically stated otherwise
Randomization	in vivo studies: mice were injected with tumor and randomized to control KD or low methionine diet.
Blinding	Animal technician was blinded to which diet was control or low methionine, animal technician was also blinded to doxycycline water or sucrose water. Decoding was performed by the PI.

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	Antibodies and Concentrations for Western Blotting: Acetyl-Histone H3 (Lys27) (1:1000, Cell Signaling, cat# 8173, AHCY (1:1000, Thermo Fisher, cat# PA5-60038), Betaine--Homocysteine S-Methyltransferase (BHMT) (1:1000, Invitrogen (Thermo Fisher), cat# PA5-18772), Di-methyl Histone H3 (K27) (1:1000, Cell Signaling, cat# 9728T), HA-Tag (1:4000, Cell Signaling, cat# 3724), Histone H3 (1:1000, Cell Signaling, cat# 14269), Methionine Adenosyltransferase 2A (MAT2A) (1:4000, Novus, cat# NB110-94158SS), Methionine Adenosyltransferase 2B (MAT2B) (1:1000, Invitrogen (Thermo Fisher), cat# 703221), Methylthioadenosine Phosphorylase (MTAP) (1:500, Cell Signaling, cat#4158), Symmetric Di-Methyl Arginine Motif (1:1000, Cell Signaling, cat# 13222), Tri-Methyl-Histone H3 (Lys27) (1:1000, Cell Signaling, cat# 9733), Tri-Methyl-Histone H3 (Lys36) (1:1000, Thermo Fisher, cat# PA5-17109), Tri-Methyl-Histone H3 (Lys4) (1:1000, Cell Signaling, cat# 9751), V5-Tag (D3H8Q) (1:1000, Cell Signaling, cat# 13202), β-Actin (1:1000, Cell Signaling, cat# 3700), Adenosylmethionine Decarboxylase 1 (AMD1) (1:1000, ProteinTech, cat#11052-1-AP), 5-Methyltetrahydrofolate-Homocysteine Methyltransferase (MTR) (1:1000, Cell Signaling, cat#68796S), Methyltransferase 16, N6-Methyladenosine (METTL16) (1:1000, Cell Signaling, cat#17676), LAMIN A (1:1000, Cell Signaling, cat#86846s), IRDye® 680RD Goat anti-Mouse IgG Secondary Antibody (1:15,000, Fisher Scientific, cat# 925-68070) or IRDye® 800CW Donkey anti-Rabbit IgG Secondary Antibody (1:30,000, Fisher Scientific, cat# NC0964679) Antibodies and concentrations for IHC were as follows: V5-Tag (1:100, Cell Signaling, cat# 13202), HA-Tag (1:200, Cell Signaling, cat# 3724), Myc-Tag (1:100, Cell Signaling, cat# 2276), Tri-Methyl-Histone H3 (Lys27) (1:100, Cell Signaling, cat# 9733), Histone H3 K27M

(1:100,RM192, ThermoFisher Scientific Cat# MA5-27916), Ki-67 (1:500, Cell Signaling, cat#9027T), Oligodendrocyte Transcription Factor 2 (OLIG2) (1:100, ThermoFisher Scientific Cat#PA5-23456).

Validation

For manufacturer validation please look up catalog number for each antibody listed above. In house validation of MAT2A and AMD1 occurred through shRNA KD of targets.

Cell Signaling Technologies Statement: "To ensure product performance, we validate all of our antibodies, in-house, in multiple research applications."

Thermo Fisher:
AHCY "Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. A-431 cells were transfected with, control siRNA, AdoHcyase 2 siRNA probe #1, and a decrease in signal intensity was observed in Western Blot application using Anti-AdoHcyase 2 Polyclonal Antibody (Product # PA5-60038) used at a dilution of 0.4 µg/ml. Anti-GAPDH was used as a loading control. Knockdown validation info.

BHMT: "BHMT Antibody (PA5-18772) in WB Western Blot staining of Rat Liver lysate using Product # PA5-18772 at a concentration of 0.03 µg/mL, the primary antibody incubation was 1 hour and the detection method was chemiluminescence. (see product sheet for image).

MAT2B: "Antibody specificity was demonstrated by siRNA-mediated knockdown of target protein. Hep G2 cells were transfected with MAT2B siRNA and decrease in signal intensity was observed in western blot application using Anti-MAT2B Monoclonal Antibody (Product # 703221). Knockdown validation info."

H3K36me3:"Antibody specificity for modified targets can be established using peptide arrays by quantifying detection of the target protein along with closely related proteins. Peptide array of Histone H3K36me3 using Anti-Tri-Methyl-Histone H3 (Lys36) Antibody: An array of the specific peptide and other relevant peptides when tested using Anti-TriMethyl-Histone H3 (Lys36) Polyclonal Antibody (Product # PA5-17109), showed that the Histone H3K36me3 modification was specifically recognized by the antibody.
Peptide array validation info."

H3K27M:"Immunohistochemistry analysis of Histone H3 (K27M mutant) in brain tumor tissue. Sample was incubated with Histone H3 (K27M mutant) monoclonal antibody (Product # MA5-27916)."

OLIG2:"Antibody specificity was demonstrated by detection of differential basal expression of the target across tissues tested owing to their inherent genetic constitution. Higher expression of OLIG2 was observed in Mouse Pup Brain and Mouse Brain in comparison to Mouse Liver, a negative model for OLIG2 expression, using AntiOLIG2 Polyclonal Antibody (Product # PA5-23456) in Western Blot. Relative expression validation info."

MAT2A: Validated in house with over expression and shRNA knockdown of MAT2A
AMD1: Validated in house with over expression and shRNA knockdown of AMD1

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

DIPG cell lines (all with the H3K27M mutation) SU-DIPG-IV (DIPG04), SU-DIPG-13p (DIPG13p), BT-245, HSJ-19 through MTA, Michelle Monje. SF8628 cells purchased commercially, NSC through MTA Baoli Hu (Hu B, Wang Q, Wang YA, Hua S, Sauvé CG, Ong D, Lan ZD, Chang Q, Ho YW, Monasterio MM, Lu X, Zhong Y, Zhang J, Deng P, Tan Z, Wang G, Liao WT, Corley LJ, Yan H, Zhang J, You Y, Liu N, Cai L, Finocchiaro G, Phillips JJ, Berger MS, Spring DJ, Hu J, Sulman EP, Fuller GN, Chin L, Verhaak RGW, DePinho RA. Epigenetic Activation of WNT5A Drives Glioblastoma Stem Cell Differentiation and Invasive Growth. Cell. 2016 Nov 17;167(5):1281-1295.e18. doi: 10.1016/j.cell.2016.10.039. PMID: 27863244; PMCID: PMC5320931.). NHA was purchased commercially, Adult GBM lines U87 A172, SJG2, LN229 and HEK293T were purchased through ATCC, KNS42 were purchased through Cellbank.

Authentication

All cell lines were confirmed by STR profiling

Mycoplasma contamination

Cell lines tested negative for mycoplasma via PCR

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used

Animals and other organisms

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

Laboratory animals

mus musculus C57BL/6J mice were used to generate syngenic model, intracranial injections in <4week old C57BL/6 mice (both male and female). Sex and number is defined in figure legend by experiment. mus musculus NOD-scid IL2Rgamman^null injected 4-6 weeks (3 male, 2 female/condition). Mice are kept at 73-74F with 30% humidity and dark/light cycle of 14hrs with light, 10hrs in darkness.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve field collected samples

Ethics oversight	All animal procedures were carried out ethically according to protocols approved by Institutional Animal Care and Use Committee (IACUC) through the University of Pittsburgh

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://urldefense.proofpoint.com/v2/url?u=https-3A__www.ncbi.nlm.nih.gov_geo_query_acc.cgi-3Facc-3DGSE160006&d=DwIBAg&c=ZQs-KZ8oxEw0p81sqgiaRA&r=XA-VBrU-dm7JOQER7T84PJmVsjELWqHBwFpYX2hkWaA&m=vJwGNnbYf8UxrKz21G7TLsmAv-lqmSSemhbaerEg21k&s=Nx34rKzYljYZN1zUVHxW59Dms7_RtgeB6UxplucoFAY&e=
Files in database submission	DIPG4_noDOX, DIPG4_DOX, DIPG4_DOX input, DIPG13_noDOX, DIPG13_DOX, DIPG13 input
Genome browser session (e.g. UCSC)	No longer applicable

Methodology

Replicates	two separate models with comparable mutations as biological duplicates. These data were also validated using an orthogonal approach CUT&RUN to validate ChIP-seq findings
Sequencing depth	DIPG13 : Single-end Total number of trimmed reads and overall alignment rate : DIPG13 Dox : 38,676,666 (75bp, overall alignment rate 95%,~85% uniquely mapping reads) DIPG13 noDox: 51,435,136 (75bp, overall alignment rate 97.07%,~85% uniquely mapping reads) DIPG4: paired-end DIPG4 Dox: 59,972,372 (75bp, overall alignment rate 90.19%) DIPG4 noDox: 51,226,395 (75bp, overall alignment rate 93.09%)
Antibodies	H3K36me3 antibody (Cat# 91265 Active Motif)
Peak calling parameters	To visualize H3K36me3 signals over genes, the spike-in normalized read densities were plotted for 14904 genes passing the criteria of gene length >2kb and a gap distance of 4kb from the neighboring gene. To call broad H3K36me3 peaks, epic238 (implementation of SICER)39 was used with bin size of 400, gap size of 3 and FDR of 0.001. Peaks were annotated to genomic regions using R package ChipSeeker.40 To determine fold change difference in H3K36me3 signal between experimental conditions, multiBigwigSummary (deeptools, v3.4.3) was used to compute scores across Refseq genes.
Data quality	We used FDR cutoff of 0.01 since we used epic2 peak caller to call the broad peaks. Number of peaks: DIPG13 Dox : 13989 DIPG13 noDOX : 17767 DIPG4 Dox: 43211 DIPG4 noDOX: 45246
Software	Bowtie2(v2.3.5.1, --local --no-unal --no-mixed --no-discordant --phred33 -I 10 -X 700). The resulting alignments were sorted and marked for duplicates using picard tools (MarkDuplicates, v2.21.1) (http://broadinstitute.github.io/picard/), respectively, The normalized bam files were used to generate bigwig files using deeptools (v3.4.3). To call broad H3K36me3 peaks, epic238 (implementation of SICER)39 was used with bin size of 400, gap size of 3 and FDR of 0.001. Peaks were annotated to genomic regions using R package ChipSeeker.40 To determine fold change difference in H3K36me3 signal between experimental conditions, multiBigwigSummary (deeptools, v3.4.3)

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: Cells were grown in vitro harvested using accutase, washed with F12 Wash media, stained with Annexin PI for 45minutes and then processed for FLOW

Instrument: BD LSR II

Software: The flow cytometry results were analyzed using FlowJo™ v10.8 Software (BD Life Sciences)

Cell population abundance: Of the 30,000 events measured for each replicate, >60% of events were then used for Cell cycle analysis after gating.

Gating strategy: SSC-A and FSC-A parameters were used to define regions of bulk cells and exclude cell debris or clumps of cells. The initial gating included>70% of events. SSC-W and SSC-H then excluded remaining debris and broadly included >95% of events.

☒ 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: N/A

Acquisition

Imaging type(s): Structural

Field strength: 7-Tesla Field Strength, equipped with an actively shielded gradient system B-GA12S2 gradient with 440 mT/m gradient strength and slew rate 3440 T/m/s as well as a quadrature radio-frequency volume coil with an inner-diameter of 35 mm.

Sequence & imaging parameters: Multi-planar T2-weighted anatomical imaging covering the whole brain volume was acquired with Rapid Imaging with Refocused Echoes (RARE) pulse sequence with the following parameters: field of view (FOV) = 2 cm, matrix = 256 X 256, slice thickness = 0.6 mm, in-plane resolution = 78 µm X 78 µm, RARE factor = 8, effective echo time (TE) = 48 msec, repetition time (TR) = 1800 msec, flip angle (FA) = 1800.

Area of acquisition: Whole 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