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Supplemental Information

Mutant *IDH1* Promotes Glioma Formation *In Vivo*

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Supplemental Experimental Procedures

Genotyping

Genotyping for the TVA transgene was carried out in 25 μL total volume containing 2.5 μL 10X PCR buffer (Life Technologies), 10 μL DMSO, 125 μL 50 mmol/l $MgCl_2$, 0.625 mmol/l of each nucleotide, 1 unit of Taq polymerase (Life Technologies), 6.25 μg/ml of primers TVA-386 sense and TVA-786 antisense to detect a 400-bp fragment. Samples were amplified for 30 cycles (94 °C for 30 seconds, 56 °C for 30 seconds, and 72 °C for 45 seconds) (Shin et al., 2017). PCR to detect a 180-bp *Cdkn2a*$^{lox/lox}$ allele and a 140-bp wild-type *Cdkn2a* allele was carried out with specific primers. Cycling conditions are 94 ˚C for 1 min followed by 30 cycles of 94 ˚C for 30 s, annealing for 30 s at 55 ˚C, elongation for 30 s and a final extension at 72 ˚C for 7 minutes (Shin et al., 2017). Genotyping for *Pten*^{lox}, and wild-type alleles was performed to detect 1000-bp *Pten*^{lox} allele and an 811-bp *Pten* wild type allele using CFP1, R1NEOCP and PTR14 primers. Cycling conditions are 95 ˚C for 3 min followed by 28 cycles of 95 ˚C for 1 min, annealing for 1 min at 60 ˚C and elongation at 72 ˚C for 1 min and a final extension at 72 ˚C for 6 min (Shin et al., 2017). PCR to detect a 2500-bp *Atrx*^{lox} allele and a 2000-bp wild-type *Atrx* allele was carried out using AccuStart II PCR SuperMix (Quantabio) per the manufacturer's specifications. Cycling conditions were as follows: 94 ˚C for 2 min followed by 34 cycles of 94 ˚C for 15 s, annealing for 30 s at 55 ˚C and elongation at 72 ˚C for 3 minutes.

Viral constructs and propagation

The avian retroviral vectors used in this study are replication-competent Avian Leukosis Virus (ALV) with Bryan polymerase subgroup A (designated RCASBP(A) and abbreviated RCAS). RCAS-*X* was a gift from Harold Varmus. RCAS-*Cre* has been described previously (Robinson et al., 2010). RCAS-*PDGFA* was cloned using LR recombination from the Gateway PLUS shuttle X03795.1 (Genecopoeia). *IDH1* (ENST00000345146.6) was PCR amplified from a cDNA library generated from normal human astrocytes (ScienCell Research Laboratories). The R132H mutation and C-terminal HA epitope tag were introduced using PCR (primer sequences are available upon request). The PCR products were cloned into the pCR8/GW/Topo vector (Thermo Fisher Scientific (TFS)), bidirectionally verified using Sanger sequencing, and Gateway cloned into the RCAS destination vector using LR Clonase II per the manufacturer's specifications (TFS). Viral infection was initiated by calcium phosphate transfection of proviral DNA into DF-1 avian fibroblasts. DF-1 cells were maintained in DMEM with 10% FBS, 100 IU/ml gentamicin, and 100 units/ml penicillin/streptomycin at 37 °C. Viral spread was monitored by expression of the p27 viral capsid protein as determined by western blot (Shin et al., 2015).

Viral delivery in vivo

Infected DF-1 cells from a confluent 10-cm culture dish were pelleted and re-suspended in 50 µl HBSS. Newborn pups were injected 2 mm ventral to Bregma with 5 µl of cell suspension using a gas-tight Hamilton syringe. In order to directly compare all of the different groups of mice in our study, three viruses were delivered to each mouse to maintain equal amounts of virus for each cohort. In groups with fewer than three delivered genes, an empty vector (RCAS-X) was co-injected to effectively dilute viral ratios to those equal to what would be expected for injections with three RCAS viruses.

Histological analysis

Brain tissue from injected mice was isolated, fixed in neutral buffered formalin (NBF), and paraffin embedded. 5 µm sections from tissue blocks were adhered to glass slides and stained by H&E or left unstained for further analysis.

Immunohistochemistry (IHC)

Tissue sections were deparaffinized as previously described (Vanbrocklin et al., 2012). IHC was performed with the following antibodies: HA (1/250), OLIG2 (1/300), ATRX (1/250), GFAP (1/200) PTEN (1/150), p19ARF (1/250), Ki67 (1/100), Endomucin (1/300) and $IDH1^{R132H}$ (1/30), 5hmc (1/2500), and PDGFA (1/250). Specific details regarding the staining procedures are available upon request. Slides were imaged on a Zeiss Axio microscope equipped with a 5 megapixel camera.

Development of primary astrocyte cell lines

N*::TVA*;$Cdkn2a^{lox/lox}$ astrocytes have been described (Robinson et al., 2010). N*::TVA*;$Atrx^{lox/lox}$; N*::TVA*;$Cdkn2a^{lox/lox}$;$Atrx^{lox/lox}$; and N*::TVA*;$Cdkn2a^{lox/lox}$;$Atrx^{lox/lox}$;$Pten^{lox/lox}$ primary astrocytes were established following dissection of newborn mouse brain tissue by physical disruption into single cells using scalpels and 0.25% trypsin. Cell cultures were maintained in DMEM (Invitrogen) containing 10% FBS, 100 IU/ml gentamicin, and 100 units/ml penicillin/streptomycin.

Viral infections in vitro

Astrocytes from newborn mice were seeded in six-well plates at a density of 5×10^4 cells/well and were maintained in DMEM with 10% FBS, 100 IU/ml gentamicin, and 100 units/ml penicillin/streptomycin at 37 °C. Prior to infection, the media was removed and replaced with 1 ml of filtered virus-containing media with 8 mg/ml polybrene

(Sigma) and incubated overnight at 37 °C. Infected astrocyte cultures were maintained as described above except the culture media was supplemented with 2 mM glutamine.

Immunoblotting

Whole cell lysates were collected using SDS-lysis buffer (200 mM Tris/HCl pH 6.8), 2% SDS, 0.1 M DTT, 10% Glycerol, 0.1% bromophenol Blue) or RIPA buffer with the addition of protease inhibitor cocktail (Bio-Rad Laboratories). Proteins were separated on 8-16% Tris-glycine polyacrylamide gels or 6% polyacrylamide gels (Invitrogen) for proteins above 200 KDa and transferred to nitrocellulose or PVDF membranes. Blots were probed with the indicated primary and secondary antibodies, incubated with Amersham ECL Western Blotting Detection Reagents (GE Healthcare), and exposed to film. Primary antibodies used were: Rabbit anti-IDH1 (1:1000); Mouse anti-IDH^{R132H} (1:1000) Mouse anti-GAPDH (1:1000); Rabbit anti-HA (1:1000); Rabbit polyclonal PDGFA (1:1000); Rabbit polyclonal ATRX (1:1000) Rabbit anti-PTEN (1:1000); p19ARF (1:1000). HRP-conjugated secondary antibodies used were: anti-Mouse IgG (1:1000) and anti-Rabbit IgG (1:1000). The source of the antibodies used is indicated in the Resource Table.

Synthesis of TFMB–(R)-2HG

To a solution of (*R*) oxotetrahydrofuran-2-carboxylic acid (650 mg, 5.0 mmol) in acetonitrile (15 ml) was added *i*-Pr2NEt (1.05 ml, 6.0 mmol, 1.2 equiv) and 3-(trifluoromethyl) benzyl bromide (0.92 ml, 6.0 mmol, 1.2 equiv). The mixture was heated to reflux for 10 minutes and allowed to stir at RT overnight. The mixture was concentrated under reduced pressure and the resulting residue taken up in ethyl acetate (50 ml). The organics were washed with 10% HCl (50 ml), 10% sat $NaHCO_3$ (50 ml) and brine (50 ml) and were dried over Na_2SO_4. Concentration gave a light yellow oil which was purified on silica gel, eluting with 1:1 hexanes:ethyl acetate to give the title compounds [(*R*)-enantiomer: 1.28 g, 89%] as a clear oil which later solidified upon standing in the freezer. Physical characteristics are detailed in (Losman et al., 2013).

Proliferation assay

Cell proliferation was assessed using an MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (Life technologies) assay. Briefly, 1×10^3 cells were seeded into each well of a 96 well plate. MTT, 20 µl (final concentration 1 mg/ml), was added to the cells at 24, 48, and 72 h post culture and incubated for 4 h at 37 °C. For proliferation assays using 2-HG, the media was supplemented with 3 mM TFMB–(R)-2HG. Excess MTT reagent

was removed and the formazan crystals were dissolved in 100 µl of DMSO and quantified via absorbance at 570 nm.

Soft agar colony formation assay

To assess anchorage-independent growth, 1.5×10^5 cells were suspended in 0.35% Difco Noble Agar (Becton Dickinson) in DMEM with 10% FBS and layered onto 0.65% pre-solidified Difco Noble Agar in DMEM with 10% FBS. Culture media with or without 3mM TFMB–(R)-2HG was replenished every 3 days for 3 weeks.

2-HG Analysis

Two 10 cm plates of N*::TVA;Cdkn2a*$^{lox/lox}$*;Atrx*$^{lox/lox}$*;Pten*$^{lox/lox}$ primary astrocytes infected with viruses containing the genes indicated were pelleted and snap frozen at -80 °C. For mass spectrometry analysis of 2-HG, cells were suspended in 90% methanol containing d_4-succinate. Absolute concentrations of R-2-HG in samples were determined from a calibration curve. Samples were extracted in 1 ml 90% ice cold MeOH, then spiked with the internal standard (10 ng of d3-2-HG), sonicated for 2 min and then incubated at -20 °C for 1 hr. Samples were then centrifuged at 4 °C for 10 min at 15,000 G. The supernatants were dried overnight in a speedVac. Samples were derivatized by the addition of 160 µl N -(p-toluenesulfonyl-L-phenylalanyl chloride (TSPC)) [1.25 mM in anhydrous acetonitrile (ACN)] and 2 µl of pyridine followed by 10 min incubation at room temperature, desiccated, resuspended in 100 µl ACN/H_2O (1:1) and transferred to a glass LC/MS vial for analysis. All experiments were performed using an Agilent 1290 Infinity UHPLC system and an Agilent 6490 triple quadrupole (QqQ) mass spectrometer interfaced with an Electron spray ionization (ESI) source. Separations were achieved using a RP column (Phenomenex Luna 5 µm C18 (2) 250 × 2.1 mm) operated at room temperature, while the autosampler was set at 4 °C. Injection volume was 3 µl. The chromatography gradient is isocratic for 18 min consisting of 38% mobile phase A (0.1% FA in water) and 62% mobile phase B (1:1 ACN:MeOH), then brought to 99% mobile phase B at 19 min and held for 6 min, and then returned to initial conditions and equilibrated for 8 min using a flow rate of 0.5 ml/min. The Agilent 6490 triple quadrupole mass was operated in the negative ion mode. The mass transitions (precursor → product ions) were 448.1 → 317.9 and 448.1 → 154.8 for TSPC-2HG, and 451.1 → 317.9 and 451.1 → 154.8 for internal standard d3-TSPC-2HG. The MRM acquisition parameters used were as follows: 4000 V capillary voltage, 500 V nozzle voltage, 12 l/min sheath gas flow at a temperature of 350 °C (ultra-high purity nitrogen), 11 l/min drying gas flow at a temperature of 150 °C, 35 psi nebulizer gas flow, 380 V default fragmentor voltage, 4 V or 1 V transition-specific cell accelerator potential, 9 - 41 V transitions-specific collision energy (CE),

and unit resolution. The MRM parameters were optimized to achieve maximum sensitivity. Results from LC-MS experiments were collected using Agilent Mass Hunter Workstation B.07.01 and analyzed using the software packages Mass Hunter Quant B.07.00 and Microsoft Excel.

NADPH assay

IDH1 activity was measured using an Isocitrate Dehydrogenase Activity Colorimetric Assay Kit (BioVision) per the manufacturer's protocol. Briefly, 1×10^6 cells were washed in ice cold PBS and homogenized in 200 µl IDH assay buffer. Five µg of protein extracted from each sample was transferred to a 96-well plate and mixed with the assay reaction mix in 1:1 ratio in triplicate. The plate was incubated at 37 °C and OD 450 was recorded every 5 min for 2 h using the Synergy HT Multi-Detection Microplate Reader (Bio-Tek). NADPH values were calculated from standard curves.

Immuno-Fluorescent In Situ Hybridization (Immuno-FISH)

To detect the ALT phenotype in tumor tissue, we performed Immuno-FISH. Briefly, 5 µm sections were de-paraffinized and subjected to heat induced antigen retrieval. Slides were washed with PBS, incubated in 0.005% pepsin solution for 5 min at 37 °C, followed by 2 washes in PBS and dehydration in ice cold ethanol. 200 nM of Alexa Fluor 488 conjugated C rich telomere probe (PNA Bio) prepared in 20 µl hybridization buffer (Roche Diagnostics) was applied to the slides and incubated for 10 min at 85 °C followed by 2 h incubation in the dark to allow hybridization. Post hybridization, the slides were incubated with PML antibody for 1 h followed by incubation with Alexa Flour 594 conjugated donkey anti-mouse secondary antibody. Cell nuclei were counterstained with Hoechst 33342 (Life Technologies) and mounted with ProLong Gold Antifade reagent (Life Technologies). Slides were imaged using a 100X oil immersion objective on a Zeiss Axioskop 2 MOT Plus fluorescence microscope.

Bioinformatics and Genomics

For RNAseq analysis, mouse tumors were microdissected from 10 µm FFPE sections and extracted using the Ambion Recoverall Kit (Ambion). Samples were prepped with TruSeq Stranded Total RNA with Ribo-Zero Gold and then sequenced on HiSeq 50 Cycle Single-Read Sequencing v4 (Illumina). Reads were aligned to the mouse genome (mm10) and differential gene expression was determined with the open source USeq/DESeq2 package. Genes were selected using two thresholds, an FDR of <10% and absolute log2 ratio > 1. GBM classifier genes were accessed from the TCGA data portal and were converted to mouse orthologues genes using Biomart (Verhaak et al., 2010). GSEA (v2. 2.0) was used to analyze the enrichment of signature gene sets from different GBM subtypes as

described (Lu et al., 2016). For Methylation array, Bisulfite sequencing library was prepared using an Agilent SureSelect XT Mouse Methyl-Seq capture kit following manufacturer's recommendations. The library was sequenced on an Illumina HiSeq 2500 multiplexed with 4 samples per lane. Reads were de-multiplexed and converted to Fastq using Illumina's pipeline. Reads were aligned to the mouse genome (build mm10, UCSC) using Novocraft Novoalign (www.novocraft.com, version 3.7.1) in bisulfite mode using options -b 2 -t 360 -h 120 -r random. Duplicate alignments were removed using Picard MarkDuplicates (http://broadinstitute.github.io/picard/index.html, version 2.9.0). Bisulfite analysis was performed with the USeq application (https://github.com/HuntsmanCancerInstitute/USeq/tree/master/Source, version 9.0.8). Cytosine methylation observations were converted to point data using USeq NovoalignBisulfiteParser, increasing minimum base quality to 20 and minimum map quality to 13. Point data was filtered for CpG contexts using USeq ParsePointDataContexts. For regional differential methylation analysis, USeq DefinedRegionBisSeq was run with a minimum base read coverage of 10. There were 220,894 target regions tested, representing intervals with a minimum mean base coverage of 10 reads across all samples and a minimum length of 100-bp. They were identified using Macs2 bdgpeakcall with a bedgraph representation of average read depth across all samples. Differentially methylated regions were filtered using criteria of absolute pseudomedian log2 ratio difference >= 1.0 and a -10log10 transformed Benjamini & Hochberg corrected FDR>30. To generate the regional methylation score, USeq ScoreMethylatedRegions was run with a minimum read coverage of 10 over the filtered targets; targets without a score across all samples were removed. Heat maps and figures were generated in R using the collected region sum fraction methylation score. Differentially methylated targets were annotated with the R package Chipseeker.

Cell viability assays

High-throughput *in vitro* cell viability assays were performed at the University of Utah Drug Discovery Core facility. Briefly, the activity levels of TMZ and olaparib as single agents in mouse astrocytes was determined by high-throughput CellTiter-Glo cell viability assay (Promega). Cells (1×10^3) were plated in each well of 384–well plates using the Tecan EVO100/MCA384 liquid handling station in a sterile bio-hood and incubated overnight. Drugs were added to the wells 24 hours after cell plating using the Tecan D300e Digital Dispenser, which allows for picoliter to microliter non-contact dispensing of small molecules directly into the assay plate without the need for serial dilutions. Each drug concentration was applied to 4 replicate wells with four additional control wells receiving vehicle only. After 72 hours of drug incubation, CellTiter-Glo reagent was added to each well at 1:1 ratio (v/v) with

the media using the BioTek Microplate Dispenser and incubated for 30 min at room temperature. Luminescence was measured on the Synergy 4 Microplate Reader. Percent viability was calculated and used to determine IC50 values.

Resource Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-IDH1^{R132H}	HistoBioTec	Cat#DIA-H09
Mouse anti-PTEN (6H2.1)	Dako	Cat#M3627
Mouse anti-PML	Millipore	Cat#MAB3738
Mouse anti-GAPDH	Millipore	Cat#MAB374
Mouse anti-5hmC	Active Motif	Cat#39769
Rabbit anti-HA	Cell Signaling	Cat# 3724
Rabbit anti-Olig 2	Millipore	Cat#Ab9610
Rabbit anti-ATRX (H-300)	Santa Cruz	Cat#SC-15408
Rabbit anti-CDKN2A/p19ARF	Abcam	Cat#ab80
Rabbit anti-IDH1 (D2H1)	Cell Signaling	Cat#8137
Rabbit anti-PTEN (D4.3)	Cell Signaling	Cat#9188
Rabbit anti-PDGFA (N30)	Santa Cruz	Cat#SC-128
Rabbit anti-p19ARF	Thermo Fisher	Cat#PA1-30670
Rat anti-GFAP mAb (2.2B10)	Thermo Fisher	Cat#130300
Rat anti-Endomucin (V.7C7)	Santa Cruz	Cat#65495
Rat anti-Ki67	Dako	Cat#M7249
Anti-mouse HRP conjugated	Cell Signaling	Cat#7076s
Anti-rabbit HRP conjugated	Cell Signaling	Cat#7074
Alexa flour 594 conjugated donkey anti-mouse	Thermo Fisher	Cat#R37115
Bacterial and Virus Strains		
Escherichia coli: recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F´ proAB lacIq ZΔM15 Tn10 (Tetr)	Agilent Technologies	XL1-Blue Cat# 200249

Escherichia coli: F- mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara- leu)7697 galU galK rpsL (StrR)	Thermo Fisher Scientific	One Shot™ TOP10 Cat# C404010
Escherichia coli: F- mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 araΔ139 Δ(ara-leu)7697 galU galK rpsL (StrR)	Thermo Fisher Scientific	One Shot™ *ccd*B survival™ 2 T1^{R} Cat# A10460
Escherichia coli: F- φ80(lacZ)ΔM15 ΔlacX74 hsdR(rK-mK+) ΔrecA1398 endA1 tonA	Thermo Fisher Scientific	One Shot™ Mach1™ T1 Cat# C862003
RCAS: replication-competent avian leukosis virus long terminal repeat with splice acceptor Bryan polymerase subgroup A	(Robinson et al., 2010)	NA
Biological Samples		
Mouse brain tissue	This paper	
Mouse tail biopsies	This paper	
Chemicals, Peptides, and Recombinant Proteins		
Pepsin	Sigma	Cat#P6887-1G
Hoechst 33342	Life Technologies	Cat#H3570
Prolong Gold anti-fade	Life Technologies	Cat#P10144
Temozolomide	Selleck Chemical	Cat#S1237
Olaparib	Selleck Chemical	Cat#S1060
TFMB-(*R*)-2HG	(Losman et al., 2013)	
Critical Commercial Assays		
RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	Thermo Fisher	Cat#AM1975
Ribo Zero Gold	Illumina	Cat#RS-122-2202
Isocitrate dehydrogenase kit	BioVision	Cat#K756-100
SureSelectXT Mouse Methyl-seq Reagent Kit	Agilent	Cat#931052
Qiagen DNEasy Kit	Qiagen	Cat#69504
CellTiter-Glo® Luminescent Cell Viability Assay	Promega	Cat#G7572

Deposited Data		
RNA seq processed data	This paper	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE107616
Methylation array processed data	This paper	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE107616
Experimental Models: Cell Lines		
N::*TVA*;*Cdkn2a*$^{lox/lox}$	(Robinson et al., 2010)	N/A
N::*TVA*;*Cdkn2a*$^{lox/lox}$;*Atrx*$^{lox/lox}$;*Pten*$^{lox/lox}$	This paper	N/A
N::*TVA*;*Cdkn2a*$^{lox/lox}$;*Atrx*$^{lox/lox}$	This paper	N/A
IDH1^{R132H} mouse tumor-derived cells	This paper	N/A
IDH1^{wt} mouse tumor-derived cells	This paper	N/A
DF-1	ATCC	Cat#CRL-12203
Experimental Models: Organisms/Strains		
N::*TVA*;*Cdkn2a*$^{lox/lox}$;*Atrx*$^{lox/lox}$;*Pten*$^{lox/lox}$	This paper	N/A
N::*TVA*;*Cdkn2a*$^{lox/lox}$;*Atrx*$^{lox/lox}$	This paper	N/A
N::*TVA*;*Cdkn2a*$^{lox/lox}$;*Pten*$^{lox/lox}$	(Shin et al., 2017)	N/A
N::*TVA*;*Atrx*$^{lox/lox}$;*Pten*$^{lox/lox}$	This paper	N/A
N::*TVA*;*Cdkn2a*$^{lox/lox}$	(Robinson et al., 2010)	N/A
N::*TVA*;*Pten*$^{lox/lox}$	(Shin et al., 2017)	N/A
N::*TVA*;*Atrx*$^{lox/lox}$	This paper	N/A
Oligonucleotides		
Primers for *TVA* 386 5′-AGCTGGTGAGATGGGACTGAAC-3′ 786 5′- CGAACATTCAAAGCCTCCAG-3′	IDT	N/A
Cdkn2a Fwd 5′-TTGTTGGCCCAGGATGCCGACATC-3′ Rev 5′-CCAAGTGTGCAAACCCAGGCTCC-3′	IDT	N/A

Primers for *Atrx* PPS1.15: 5'GGTTTTAGATGAAAATGAAGAG-3′ Mxnp30: 5'CACCATCTTCTTGCCATCTCTGTAG-3′ Neo801: 5' GGG CGC CCG GTT CTT TT-3′	IDT	N/A
Primers for *Pten* CPF1: 5'CTTCGGAGCATGTCTGGCAATGC-3′ R1NEOCP: 5'CTGCACGAGACTAGTGAGACG TGC-3′ PTR14: 5'AAGGAAGAGGGTGGGGATAC-3′	IDT	N/A
C-rich telomere probe: CCCTAACCCTAACCCTAA	PNA Bio	Cat#F1004
Recombinant DNA		
pCR8 GW TOPO HA IDH1	This paper	N/A
pCR8 GW TOPO HA IDH1 R132H	This paper	N/A
pCR8 GW TOPO PDGFA	This paper	N/A
RCAS HA IDH1	This paper	N/A
RCAS HA IDH1 R132H	This paper	N/A
RCAS HA PDGFA	This paper	N/A
RCAS X	Harold Varmus	
Software and Algorithms		
GraphPad Prism 7		*https://www.graphpad.com/scientific-software/prism/*
R statistical software Version 3.3.2		https://www.r-project.org/

Useq software version 9.0.8	(Nix et al., 2010)	https://github.com/HuntsmanCancerInstitute/USeq/tree/master/Source
Novocraft Novoalign version 3.7.1		www.novocraft.com
Image J	(Almeida et al., 2012)	http://imagejs.github.com
Mass Hunter Quant B.07.00	Agilent technologies	
Other		

Contact for Reagent and Resource Sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Sheri L. Holmen (sheri.holmen@hci.utah.edu).

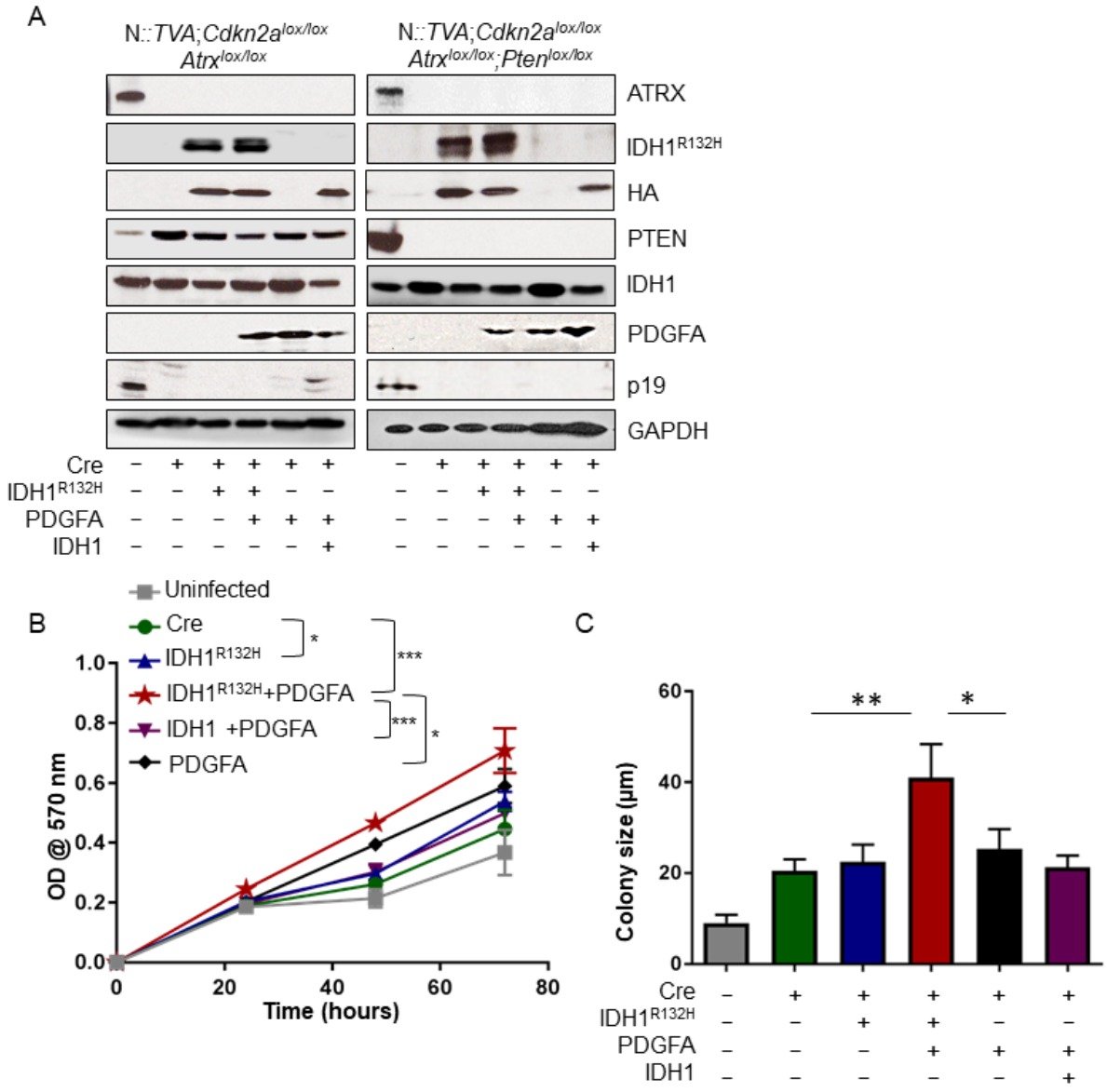

Figure S1. IDH1^{R132H} promotes proliferation and anchorage independent growth of immortal astrocytes.

Related to Figure 1. (A) Western blot analysis of ATRX, IDH1^{R132H}, HA, PTEN, IDH1, PDGFA, p19, and GAPDH in N*::TVA;Cdkn2a*$^{lox/lox}$*;Atrx*$^{lox/lox}$ (left panel) and N*::TVA;Cdkn2a*$^{lox/lox}$*;Atrx*$^{lox/lox}$*;Pten*$^{lox/lox}$ mouse astrocytes (right panel) expressing the genes indicated. (B) Analysis of cell proliferation was determined by MTT assay of astrocytes derived from N*::TVA;Cdkn2a*$^{lox/lox}$*;Atrx*$^{lox/lox}$ mice expressing the genes indicated. (C) Comparison of colony size from the soft agar colony formation assay shown in Figure 1B. Data are expressed as the mean ± S.E.M from three replicates. Statistical significance is shown by the following key: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

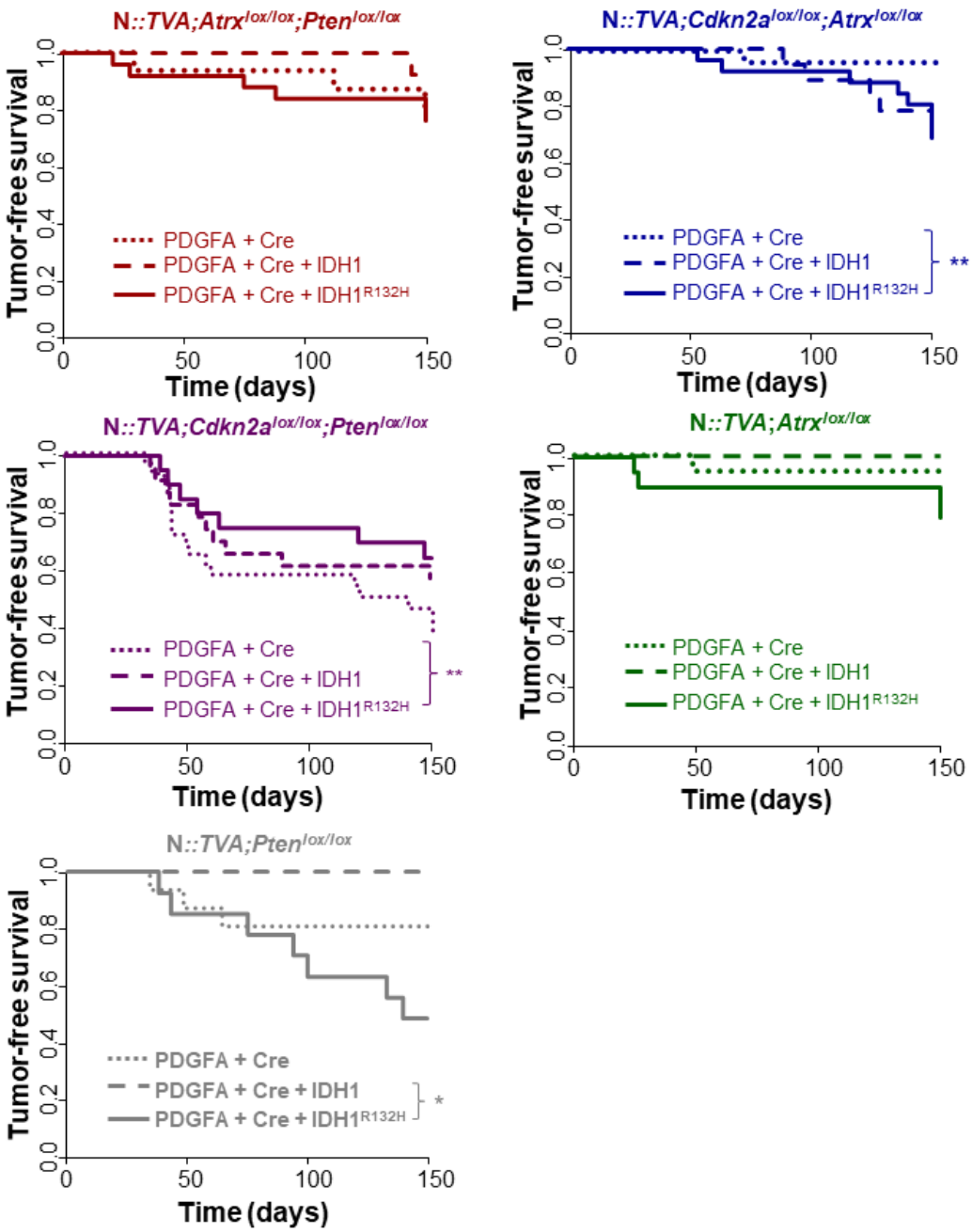

Figure S2. Loss of *Cdkn2a*, *Atrx* and *Pten* cooperate with IDH^{R132H} and PDGFA to promote glioma formation. Related to Figure 2, Table S1, and Table S2. Kaplan-Meier tumor-free survival analysis of the indicated mouse strains (denoted by color) infected with viruses containing PDGFA and Cre (round dotted line), PDGFA, Cre, and wild type IDH1 (dashed line), or PDGFA, Cre, and IDH1^{R132H} (solid line). The number of mice in each cohort is shown in Table S1. Statistical significance is shown by the following key: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. *ns* = not significant.

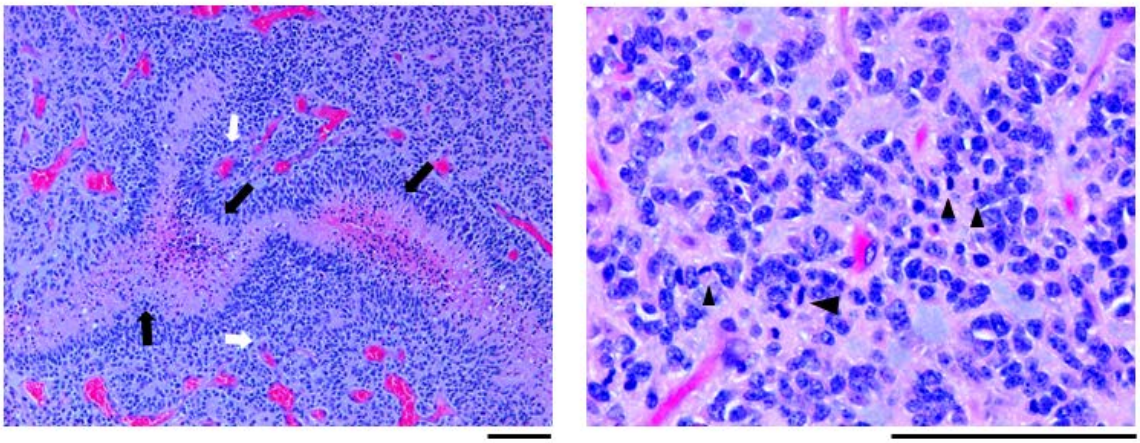

Figure S3. Histological examination of gliomas from N:*:TVA;Cdkn2a*$^{lox/lox}$*;Atrx*$^{lox/lox}$*;Pten*$^{lox/lox}$ mice injected with viruses containing PDGFA, Cre, and IDH1^{R132H}. Related to Figures 3 and S4. Representative H&E images showing features of GBM including pseudopalisading necrosis (black arrows), vascular proliferation (white arrows), and mitotic figures (black arrowheads). Scale bars represent 100 μm.

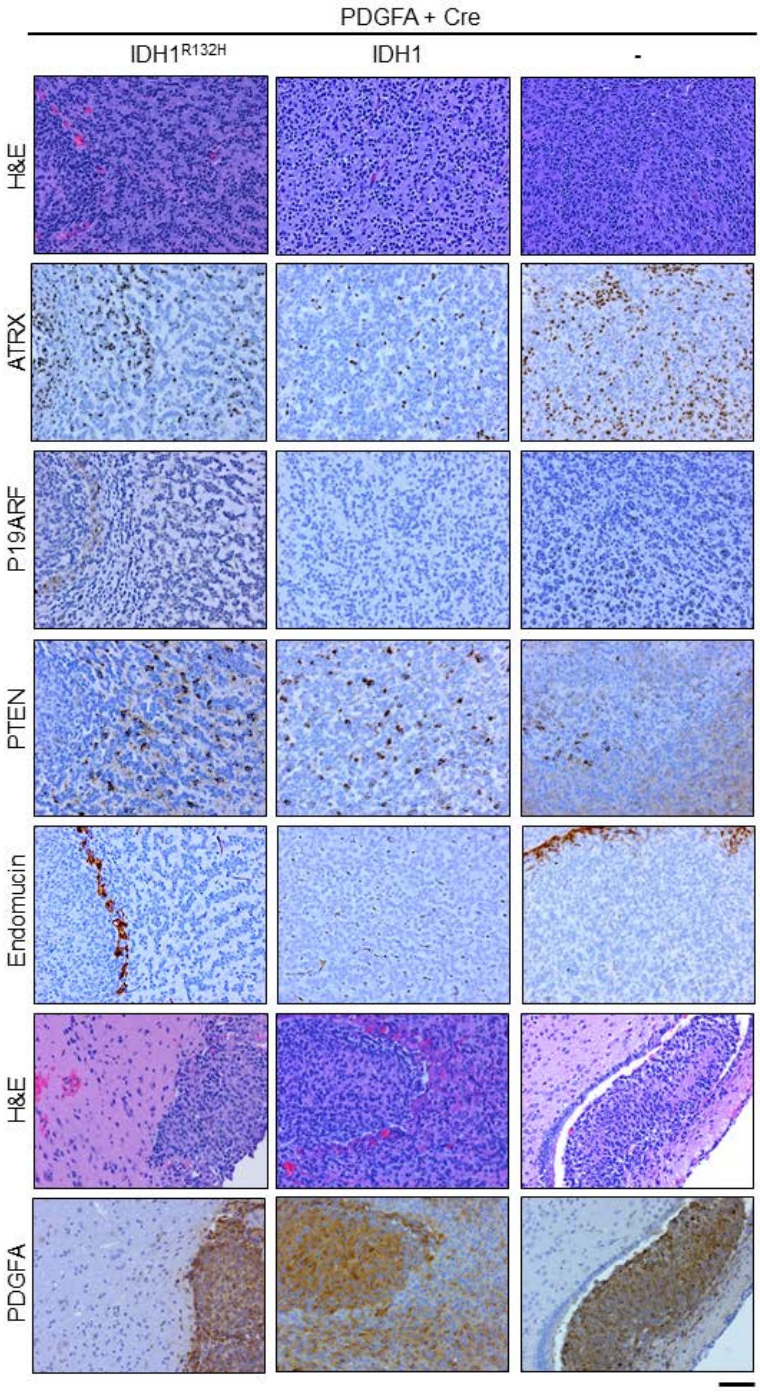

Figure S4. Immunohistochemical staining of gliomas from N::*TVA;Cdkn2a*$^{lox/lox}$*;Atrx*$^{lox/lox}$*;Pten*$^{lox/lox}$ injected mice. Related to Figures 3 and S3. Representative IHC sections of ATRX, P19ARF, PTEN, endomucin, and PDGFA in tumors from each strain of mice injected with viruses containing PDGFA and Cre alone (right column) or in combination with IDH1^{R132H} (left column) or wt IDH1 (middle column) as indicated. Corresponding H&E images are included for reference. Scale bar represents 100 μm.

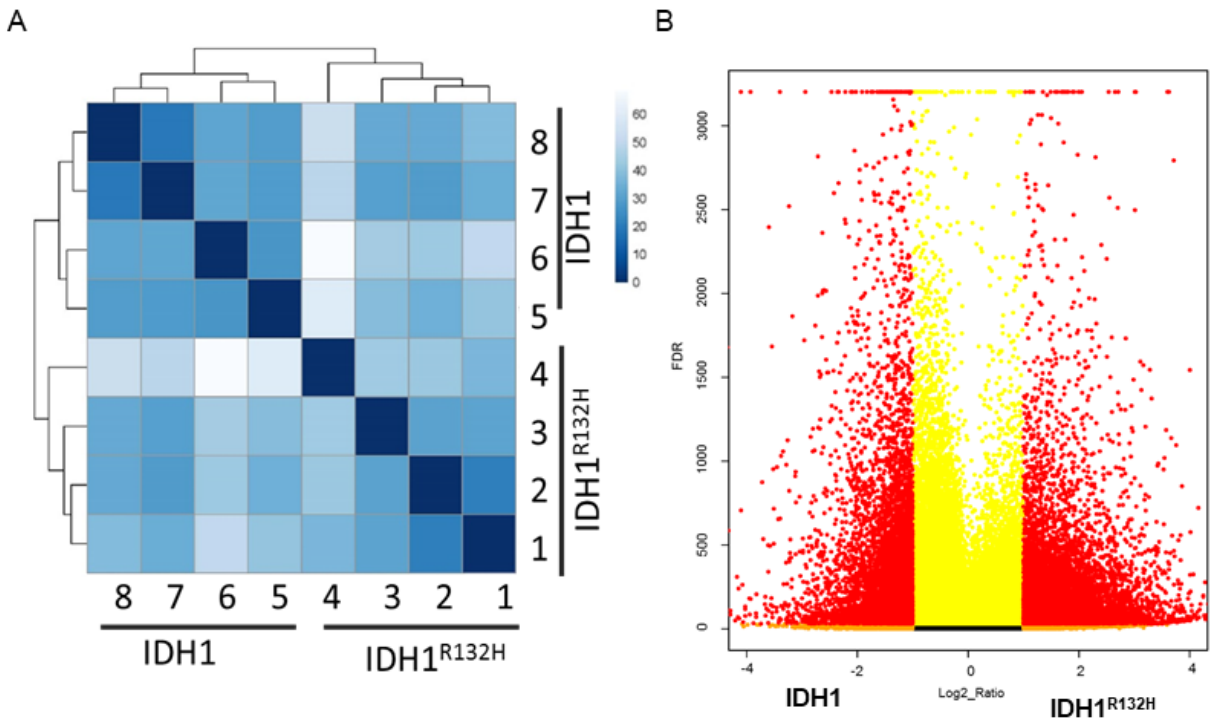

Figure S5. IDH1^{R132H} alters the methylation landscape in mouse gliomas. Related to Figure 6. (A) Heat map showing the relationship between all samples, where values represent a Euclidean distance calculated in R (B) Volcano plot showing differences in DNA methylation between IDH^{1R132H} and IDH1 samples. Each point represents a tested CpG region; points in red pass both significance and fold-change methylation.

Table S1. Tumor incidence by genotype and injection, Related to Figure 2, Figure S2, and Table S2.

Genotype	PDGFA + Cre + IDH^{R132H}	PDGFA + Cre + IDH1	PDGFA + Cre	Cre + IDH^{R132H}
N*::TVA;Atrx*$^{lox/lox}$	4/19(21%)	0/15 (0%)	1/18(5%)	0/4(0%)
N*::TVA;Cdkn2a*$^{lox/lox}$*;Atrx*$^{lox/lox}$	8/25(32%)	4/18(22%)	1/24(4%)	0/28(0%)
N*::TVA;Atrx*$^{lox/lox}$*;Pten*$^{lox/lox}$	6/26(23%)	1/13 (8%)	3/15(20%)	ND
N*::TVA;Cdkn2a*$^{lox/lox}$*;Atrx*$^{lox/lox}$*;Pten*$^{lox/lox}$	14/16(88%)	3/15(20%)	3/15(20%)	0/9(0%)
N*::TVA;Cdkn2a*$^{lox/lox}$*;Pten*$^{lox/lox}$	7/20 (35%)	10/24(42%)	15/29(59%)	0/10(0%)
N*::TVA;Pten*$^{lox/lox}$	7/13 (54%)	0/8(0%)	3/15(20%)	ND

ND: not done

Table S2. Tumor incidence and tumor-free survival by genotype for IDH1^{R132H} and IDH1 cohorts, Related to Figure 2, Figure S2, and Table S1.

Mouse Strain	Gene(s) delivered	Incidence	Median tumor-free survival (days)
N*::TVA;Atrx*$^{lox/lox}$	PDGFA + Cre + IDH1^{R132H}	4/19(21%)	150 ± 9.0
N*::TVA;Cdkn2a*$^{lox/lox}$*;Atrx*$^{lox/lox}$	PDGFA + Cre + IDH1^{R132H}	8/25(32%)	150 ± 5.2
N*::TVA;Atrx*$^{lox/lox}$*;Pten*$^{lox/lox}$	PDGFA + Cre + IDH1^{R132H}	6/26(23%)	150 ± 7.4
N*::TVA;Cdkn2a*$^{lox/lox}$*;Atrx*$^{lox/lox}$*;Pten*$^{lox/lox}$	PDGFA + Cre + IDH1^{R132H}	14/16(88%)	43.5 ± 11
N*::TVA;Cdkn2a*$^{lox/lox}$*;Pten*$^{lox/lox}$	PDGFA + Cre + IDH1^{R132H}	7/20(35%)	150 ± 10
N*::TVA;Pten*$^{lox/lox}$	PDGFA + Cre + IDH1^{R132H}	7/13(54%)	150 ± 12
N*::TVA;Atrx*$^{lox/lox}$	PDGFA + Cre + IDH1	0/15(0%)	150 ± 0.0
N*::TVA;Cdkn2a*$^{lox/lox}$*;Atrx*$^{lox/lox}$	PDGFA + Cre + IDH1	4/18(22%)	150 ± 4.5
N*::TVA;Atrx*$^{lox/lox}$*;Pten*$^{lox/lox}$	PDGFA + Cre + IDH1	1/13(8%)	150 ± 0.5
N*::TVA;Cdkn2a*$^{lox/lox}$*;Atrx*$^{lox/lox}$*;Pten*$^{lox/lox}$	PDGFA + Cre + IDH1	3/15(20%)	150 ± 8.0
N*::TVA;Cdkn2a*$^{lox/lox}$*;Pten*$^{lox/lox}$	PDGFA + Cre + IDH1	10/24(42%)	150 ± 10
N*::TVA;Pten*$^{lox/lox}$	PDGFA + Cre + IDH1	0/8(0%)	150 ± 0.0

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