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Supplementary information

Oncometabolites suppress DNA repair by disrupting local chromatin signalling

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Supplementary File 3

Methods.

Cell Culture. We assembled a collection of genetically engineered matched pair cell lines, and cell lines harboring endogenous IDH1, IDH2, or FH mutations. YUNK1 cells have been previously described[6] and were generated in culture from uninvolved cortical renal tissue from a patient undergoing a radical nephrectomy for kidney cancer. They were immortalized with a lentiviral vector containing SV40 Large T antigen (Addgene plasmid #22298). YUNK1 cells with shRNA suppression of SDHB or FH using the GIPZ lentiviral constructs (GE Dharmacon) have been previously described[6]. shCTRL, shSDHB, and shFH cells were maintained in DMEM+10% FBS+1% Pen/Strep + 2µg/mL Puromycin. shRNA suppression of SDHB and FH in the HEK293FT cell lines was achieved using TRIPZ lentiviral knockdowns and these cells are previously described[6]. After lentiviral infections with TRIPZ-shRNA or GIPZ lentiviruses, cells were maintained in DMEM+10%FBS+1% Pen/Strep + 2 µg/mL Puromycin. For dox inducible shRNAs, expression was induced with 2 µg/mL doxycycline. Doxycycline (DOX) induced knockdown was monitored by western blot as well as by RFP fluorescence from the internal and dox-inducible reporter on the TRIPZ construct. HEK293FT cells were cultured in DOX for 96h before initiating the indicated assays and maintained in DOX throughout the assays. U2OS DR-GFP cells have been previously described[14] and were maintained in DMEM+10% FBS+1% Pen/Strep. Hela cells (ATCC), and heterozygous IDH1 R132H/+ HeLa cells have been previously described[5] and were maintained un DMEM+10% FBS and 1% pen/strep. HCT116 cells and HCT116 IDH1 R132H/+ cells were obtained from Horizon Discovery (Waterbeach, UK). Immortalized astrocytes expressing WT or mutant IDH1 have been previously described[9,28]

and were a gift from T. Chan (Sloan Kettering). U87 IDH1 WT and IDH1 R132H/WT glioblastoma cells were obtained from the ATCC and maintained in DMEM/F12 media +10% FBS+1% Pen/Strep. SN1079 and RBE cells were obtained from the RIKEN cell bank and were maintained in RPMI media +10% FBS+1% Pen/Strep. SW1353 cells were obtained from ATCC and were maintained in DMEM/F12 media +10% FBS+1% Pen/Strep. UOK 262 cells were a gift from M. Linehan and have been previously described[29]; these cells were maintained in DMEM +10% FBS+1% Pen/Strep + 1% sodium pyruvate. NCCFH1 have been previously described[30] and were maintained in +10% FBS+1% Pen/Strep + 1% sodium pyruvate. UOK262 cells complemented with FH overexpression were generated by transfecting UOK 262 cells with pCMV-6-FH-GFP, selecting in G418 for 4 days, and then cloning cells by limiting dilution. Clones were generated in G418 media and then screened for FH expression by western blot for validation. After clonal isolation cells were no longer maintained in G418. All cell lines were regularly tested for mycoplasma contamination (MycoAlert, Lonza).

CRISPR/Cas9 mediated genetic knockouts. To generate a specific knockout of the mutant IDH1 allele in HT1080 (IDH1 R132C/WT) cells, three spCas9 guide RNAs overlapping the R132C region were designed to harbor a mutant-specific nucleotide variant (394 C>T) within the binding region. Guide RNA sequences are as follows with the R132C mutation binding site bolded, GAC**A**ACCUAUGAUGAUAG, GAC**A**ACCUAUGAUGAUAG, UAGGT**U**GUCAUGCUUAUG. The guide RNA sequences were cloned into the GeneArt CRISPR nuclease vector (Invitrogen) to express selected sgRNAs, spCas9, and an OFP reporter in a single construct. The expression constructs were transfected into HT1080 cells using Lipofectamine 3000. 200,000 cells were transfected in a 6-well format, using 2 μg of plasmid

DNA and 2 µL of Lipofectamine 3000. For each guide RNA, a portion of the transfected cells was collected 48 h later and assayed for cleavage efficiency by T7 endonuclease assay (EnGen Mutation Detection Kit, NEB). Efficient cleavage was detected in cells transfected with the expression vector for the guide RNA sequence UAGGU**U**GUCAUGCUUAUG, and these cells were then seeded for limiting dilution. Clones were screened in 96 well format for reduced 2HG production using the Pico probe D-2-Hydroxyglutarate (D2HG) Assay Kit (BioVision), screened by western blot analysis for the presence or absence of the mutant IDH1, and finally verified by Sanger sequencing to have lost the IDH1 R132C allele (but to have retained the WT allele). These cells were designated knockout/wild-type (KO/WT), and then verified again by LC/MS to no longer be producing 2HG. To generate KDM4A and KDM4B knockout YUNK1 cells, spCas9 guide RNAs for KDM4A and KDM4B, respectively, were designed using the CRISPRko feature on GPP sgRNA Designer (https://portals.broadinstitute.org/gpp/public/analysis-tools/sgrna-design). Guide RNA sequences were cloned into GeneArt CRISPR nuclease vector (Invitrogen) to express the selected sgRNA, spCas9, and an OFP reporter in a single construct. These constructs were transfected into YUNK1 cells, and cleavage efficiency was assayed by T7 endonuclease assay (EnGen Mutation Detection Kit, NEB). Clones were generated by limiting dilution, and those with KDM4A or KDM4B knockout were identified by western blot analysis. KDM4A KO clones were generated using the following guide RNA sequences Clone 1: UCUUCAAACUCACUAUAGCG, and Clone 2: AGCUCCUUGGGAUUCAAUGU. KDM4B knockout YUNK1 cells were generated using the gRNA sequence: CUUUGUCUCCCCGAUCUACG. Knockouts for KDM4A and KDM4B in the U87 cells were generated as above, using the following gRNA sequences, KDM4A: AGCUCCUUGGGAUUCAAUGU, and KDM4B: CUUUGUCUCCCCGAUCUACG.

Chemicals. Succinate (S9512, Sigma), dimethyl-fumarate (242926, Sigma) and dimethyl-alpha-ketoglutarate (349631, Sigma) were obtained from Sigma. (2R)-octyl-alpha-hydroxyglutarate (No.16366) was obtained from Cayman Chemical. AGI-5198(S7185) and AG-221 (S8205) were obtained from Selleckchem.

Oncometabolite and metabolite treatments for cell-based assays. For cell based DNA repair assays, (2R)-octyl-alpha-hydroxyglutarate, referred to as 2HG in figures, was added to a final working concentration of 500uM; dimethyl-fumarate, referred to as fumarate in figures, was added to a final working concentration of 30uM; and succinate was added at a final concentration of 2mM, unless otherwise indicated. All 2HG, fumarate, or succinate treatments were for 24 h before starting the indicated assay, unless otherwise indicated, and done in parallel with treatment with equal volume of vehicle control, DMSO. Dimethyl-alpha-ketoglutarate (αKG), also referred to as dimethyl-2-oxoglutarate, was added to cells at 1mM or at otherwise indicated concentrations for 5 days before initiating indicated assays. AGI-5198 and AG-221 treatments were carried out at 1uM and 5 uM final working concentrations, respectively, and cells were pretreated with these inhibitors for 5 days before starting the indicated assays, with fresh inhibitors added at the time of assay initiation.

Western blot analyses. Western blot analysis of protein levels was conducted by loading 30 μg of whole cell lysate onto a 4-20% polyacrylamide gel. Total protein was transferred to a nitrocellulose membrane. After blocking with 5% milk, membranes were incubated with primary antibodies. Antibodies used were as follows: mouse anti-ATM (phospho S1981) antibody

(Abcam, ab36810) 1:100 in 5% milk, rabbit anti-ATM (Millipore, Ab-3) 1:1000 in 5% milk, mouse anti-ß-Actin HRP (Proteintech, HRP-60008) 1:1000 in 5% milk, anti-Histone 3 Lysine 9 trimethyl (D4W1U, Cell Signaling Technology) 1:1000 in 5% BSA, rabbit polyclonal anti-Histone H3 (ab1791, Abcam) 1:5000 in 5% BSA, rabbit anti-HA-Tag (C29F4, Cell Signaling Technology) 1:2000 in 5% BSA, mouse anti-GAPDH HRP (HRP-60004, Proteintech), mouse anti-IDH1 R132H (H09, Dianova) 1:2000 in 5% milk, rabbit anti-IDH1 (D2H1, Cell Signaling Technology) 1:1000 in 5% BSA, rabbit monoclonal anti-Fumarase (D9C5, Cell Signaling Technologies) 1:1000 in 5% BSA, mouse monoclonal anti-SDHB (21A11AE7, Abcam) 1:1000 in 5% milk, mouse monoclonal anti-RAD51(14B4 Novus bio) 1:1000 in 5% milk, mouse anti-BRCA2 (ab1, Millipore) 1:1000 in 5% milk, rabbit anti-T60 (NBP2-24613, Novus Biologicals) 1:1000 in 5% BSA, rabbit anti-RPA32 (#52448, Cell Signaling Technology) 1:1000 in 5% BSA, Rabbit anti -MRE11 (H-300, Sant Cruz Biotechnology sc-22767) 1:1000 in 5% milk, rabbit anti-Phospho-(Ser/Thr) ATM/ATR Substrate Antibody (#2851,Cell Signaling Technology) 1:1000 in 5% BSA, rabbit anti-KDM4A (A300-861A, Bethyl) 1:1000 in 5% BSA, rabbit anti-KDM4A (C37E5, Cell Signaling Technology) 1:1000 in 5% BSA, anti-KDM4B (A301-478A, Bethyl) 1:1000 in 5% BSA, anti-KDM4B (D7E6, Cell Signaling Technology) 1:1000 in 5% BSA, rabbit anti-FLAG (SAB4301135, Sigma) 1:1000 in 5% BSA, rabbit anti-SUV39H1 (D11B6, Cell Signaling Technology) 1:1000 in 5% BSA, mouse anti-Ku80 80 (Clone 7, BD Biosciences) 1:1000 in 5% Milk, rabbit anti-Histone H2A.X (D17A3, Cell Signaling Technology) 1:1000 in 5% BSA, mouse anti-Poly-ADP-Ribose (PAR) (4335-mc-100, Trevigen), mouse anti-phospho-ATM S1981 (ab36810, Abcam) 1:1000 in 5% Milk, rabbit anti-phospho-RPA32 (E5A2F, Cell Signaling Technology) 1:1000 in 5% BSA, mouse anti-CHK1 (2G1D5, Cell Signaling Technology) 1:1000 in 5% BSA, rabbit anti-phospho-CHK1 (#2341, Cell Signaling Technology)

1:1000 in 5% BSA, rabbit anti-CHK2 (#2662, Cell Signaling Technologies) 1:1000 in 5% BSA, rabbit anti-phospho-CHK2 (C13C1, Cell Signaling Technologies) 1:1000 in 5% BSA, rabbit anti-DNA-PKcs (ab70250, Abcam) 1:1000 in 5% milk, rabbit anti phospho-DNA-PKcs (ab 18192, Abcam) 1:1000 in 5% milk, mouse anti-HIF1a (NB100-105, Novus) 1:1000 in 5% BSA, rabbit anti-H3K36me3 (D5A7, Cell Signaling Technologies) 1:1000 in 5% BSA, rabbit anti-H3K27me3 (C36B11, Cell Signaling Technologies) 1:1000 in 5% BSA, rabbit anti-H3K4me3 (C42D8, Cell Signaling Technologies) 1:1000 in 5% BSA. Where indicated, Histone H3, β-Actin, GAPDH and vinculin were used as sample prep loading controls. In Extended Data Figure 3m-u, membranes were stripped using a stripping buffer of 62.5mM Tris-HCl, 2% SDS, and 700 µL BME per 100ml, at 50 °C for 20 min. Then re-blocked with 5% milk for 1h at room temperature and re-probed with β-Actin HRP (Proteintech, HRP-60008) 1:1000 in 5% milk, overnight for a loading control.

Neutral comet assays. Neutral comet assays on cell line samples were performed as previously described[5,6] For doxycycline-inducible shRNA of FH and SDHB, the assays were performed 4 days after induction of shRNA with 2 µg/mL doxycycline. For plasmid transfection assays with the αKG-dependent dioxygenases, $1x10^5$ cells per well were seeded in a 12-well dish and allowed to attach overnight. The next day the cells were transfected with 1 µg of plasmid DNA using 1 µL of Lipofectamine 3000 and 1 µL of p3000 reagent. Plasmid DNA for the H3 mutants were transfected at a ratio of 0.2 µg of plasmid DNA using 0.5 µL of Lipofectamine 3000 and 0.5 µL of p3000 reagent 24 h later cells were collected on ice, suspended in PBS, and then adjusted to a concentration of 2000 cells per assay in low melt agarose. For assays following treatment with exogenous metabolites, these were added at the indicated time points before

performing the neutral comet assay. Data are presented as means of three biological replicates ± SEM. Replicates reflect independent cultures for *in vitro* assays.

LC-MS/MS analyses. The concentrations of 2HG, succinate, and fumarate in cell samples were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method[5,6], as described below. The calibration curve was prepared in distilled water, with the concentration range of 0.002 – 10 μM for succinate and 0.02 – 100 μM for fumarate. The intra- and inter-day precision and accuracy of the quality control samples met the generally accepted criterial for bioanalytical methods (i.e., <15%). *Sample preparation.* Cell homogenate was prepared by adding 500 μL water into cell pellets (~5 million cells per pellet) followed by sonication. Into tissue or cell homogenate (original or 20-fold diluted sample), the stable isotope labeled internal standards, D_6-succinate and 1,4-$^{13}C_2$, 2,3-D_2-fumarate, were added and followed by vortexed-mixing. Then, 60 μL was taken and protein precipitated by adding 240 μL of ice-cold methanol. The supernatant was transferred to a 2-mL Eppendorf tube; 0.1 mL of ice-cold 80% methanol was added to the precipitated pellet followed by vortex-mixing and centrifugation (at 10,000 rpm, 4°C for 10 min). The supernatants from two extractions were combined, and dried-down using a CentriVap® Refrigerated Centrifugal Concentrator (Kansas City, MO) at 10°C. The residue was reconstituted in 60 μL distilled water followed by vortex-mixing and centrifugation, and 5 μL of the supernatant was injected into the LC-MS/MS system. *LC-MS/MS analysis.* All LC-MS/MS analyses were performed on an AB SCIEX (Foster City, CA) QTRAP 6500 system, which consists of an enhanced high-performance hybrid triple quadrupole / linear ion trap mass spectrometer, interfaced with a SHIMADZU (Kyoto, Japan) Nexera ultra-high-performance liquid chromatography (UHPLC) system. Chromatographic separation was

achieved on a Synergi RP column (2.0 mm x 150 mm, 4 μm) under a gradient elution consisting of mobile phase A (0.03% formic acid in water) and mobile phase B (0.03% formic acid in acetonitrile), at a flow rate of 0.25 mL/min. Succinate, fumarate, and their respective internal standards were monitored under the negative electrospray ionization mode at the mass transitions of 117.0 > 73.0, 114.9 > 71.0, 121.0 > 76.9, and 119.0 > 74.0, respectively. Analyst® 1.6 software was used for system control and data acquisition and MultiQuant® 3.0 software was used for data processing and quantitation.

Tumor irradiation and tumor growth delay assay. Female athymic nu/nu mice (Hsd:Athymic Nude-Foxn1nu, Envigo) 8 weeks of age were used for all *in vivo* xenograft studies. The authors confirm that all animal experiments were performed in accordance with relevant guidelines and regulations. All studies were approved by the Yale University Institutional Animal Care and Use Committee. Mice were quarantined for at least 1 week before experimental manipulation. Tumors were formed by subcutaneous implantation of $1x10^6$ human U87 IDH1 WT cells or $11x10^6$ U87 IDH1 R132H/WT cells in 100μl of phosphate-buffered saline (PBS) in the flanks of athymic nu/nu mice. Mice were randomized into irradiated and mock irradiated groups (8 mice/group, based on a calculated power of 80% to detect a difference of 1.25 standard deviations between two groups at 5% significance level) once the average tumor volume reached 150 mm^3. The tumors were irradiated with a single dose of 8 Gy using a Seimen's Stabilipan. Mice were visually observed daily, and tumors were measured three times per week by calipers to determine tumor volume using the formula: Volume = L x W x W x 0.532. Mean (geometric) tumor volume (mm^3) was plotted over time to monitor tumor growth. P-values were determined by ANOVA. The maximal allowed tumor size approved by Yale IACUC was 1,000 mm^3, and in

none of the experiments were these limits exceeded. The experiments were not blinded to investigators.

DSB-ChIP assays. U2OS DR-GFP cells expressing the dual ligand responsive I-SceI fused to a degradation-domain and glucocorticoid receptor[15] were grown to 70% confluence in DMEM media + 10% FBS + 1% Pen/Strep. Shield-1 was added to a final working concentation of 1 μM from a 1000X stock, and triamcinolone was added to a final working concentration of 100 nM from a 500X stock. Cells were trypsinized and collected using 10 mL cell culture media per 15 cm dish, at indicated time points. Trypsin was quenched by collecting cells with media containing 1% formaldehyde. The cell suspension was incubated at room temp, in 1% formaldehyde for 15 min with gentle shaking. After 15 min, glycine was added to a final concentration of 150 mM to quench the formaldehyde fixation. Cells were pelleted at 1500 rpm for 5 min at 4 ºC. The cell pellets were then washed twice with cold PBS. To isolate the chromatin, the cell pellets were first resuspended in 1 mL cell lysis buffer (5 mM PIPES pH 8.0, 85 mM KCl, 0.5% Nonidet P-40) per 15 cm dish of cells and incubated for 10 minutes on ice. Next, nuclei were pelleted by centrifugation at 1000 rpm for 5 min at 4 ºC, and the supernatant was aspirated. The nuclei were lysed with nuclear lysis buffer (50 mM Tris-Cl pH 8.0, 10 mM EDTA, and 1% SDS) using 1 mL per 15 cm dish of cells, for 10 min on ice. Chromatin samples were sonicated using an Active Motif Q800R1 water bath sonicator for 6 cycles of 30 sec on and 30 sec off at 4 ºC. Successful DNA shearing was determined using gel electrophoresis. Chromatin was cleared by adding 200 μl of Protein A Agarose/Salmon Sperm DNA beads (Millipore 16-157) to 1 mL of chromatin extract and incubating at 4 ºC under constant agitation for 90 min. Samples were then centrifuged at max speed for 2 min and the supernatant was

collected to use as the chromatin for the immunoprecipitation. 30 µg of chromatin per replicate were used in each immunoprecipitation, with 10% kept as input. To immunoprecipitate the proteins of interest, 30 µg of chromatin were diluted to a total volume to 300 µl using dilution buffer (16.7 mM Tris-Cl, 167 mM NaCl, 1.2 MM EDTA, 0.01% SDS, and 1.1% Triton X-100). Antibodies were added at a dilution of 1:100 and the samples were incubated at 4 ºC overnight under constant agitation. The antibodies used were as follows: γH2Ax: rabbit anti-pHistone H2A.X, (S139) (20E3) (Cell Signaling Technologies #9718), SUV39H1: rabbit anti-SUV39H1 (D11B6) (Cell Signaling Technologies #8729), H3K9me3: rabbit anti-Tri-Methyl-Histone H3 (lys9) (D4W1U) (Cell Signaling Technologies #13969), H3K36me3: rabbit anti-H3K36me3 (D5A7) Cell Signaling Technologies), H3K27me3: rabbit anti-H3K27me3 (C36B11) (Cell Signaling Technologies), H3K4me3: rabbit anti-H3K4me3(C42D8) (Cell Signaling Technologies), MRE11: rabbit anti-MRE11 (H-300) (Sant Cruz Biotechnology sc-22767), Tip60: rabbit anti-Tip60 (Novus Biologicals NBP2-24613), ATM: rabbit anti-ATM(Ab-3) (Millipore 819-844), RPA: rabbit anti RPA32/RPA2 (X8X5P XP) (Cell Signaling Technologies #35869), BRCA1: mouse anti-BRCA1 (17F8) (GenTex GTX70111), Rad51: mouse anti-Rad51 (14B4) (Novus NB100-148), Total H3: rabbit anti-Histone H3 (D1H2) XP (Cell Signaling Technologies #4499). 50 µl of Protein A agarose/salmon sperm DNA beads (Millipore 16-157) were added per sample and incubated at 4 ºC for 90 min under constant agitation. Next, beads were pelleted at max speed for 2 min. The beads were washed successively 2 times each with the following buffers, high salt buffer (50mM HEPES pH 7.9, 500 mM NaCl, 1mM EDTA, 0.1% SDS, 1% Triton X-100 and 0.1% deoxycholate), low salt buffer (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20 mM Tris-HCl ph8.0 and 150 mM NaCl), and TE buffer (10 mM Tris pH 8.0 and 1mM EDTA). After the final wash, the beads were resuspended in elution buffer (50 mM

Tris-Cl pH 8.0, 10 mM EDTA and 1% SDS) and 5 µl of 5M NaCl and 2 µl RNAse A was added to each sample, then the samples were then incubated overnight at 65 ºC. The next morning, 2 µl of 20 mg/mL proteinase K was added to each sample and the samples were incubated at 55 ºC. The DNA was purified using the column based QIAquick PCR purification kit, and the 50 µl of eluted DNA was diluted 1:5 in TE buffer for analysis by Realtime PCR. Analysis of the precipitated DNA was done with the following primers 400 bp upstream of the I-SceI site, 5' TTATTGTGCTGTCTCATCATT 3' (forward) and 5'GTGCTGCATGCTTCTTCGGCA 3' (reverse). Realtime PCR was done using the Power SYBR green master mix and the Applied Biosystems Step One Plus Real Time PCR machine per manufacturer's instructions. ChIP PCR signals were calculated as percent input, equal to the quantity enriched/input x 100. Beads only controls were used as negative controls for all time points and antibodies used. To generate heatmaps, % input values for each data point were normalized to the uninduced break in the vehicle treated control cells. Statistical analysis for each ChIP assay curve for the indicated factor is by 2-way ANOVA comparing the vehicle control (DMSO) to each indicated metabolite treatment. These p-values appear on the line graphs of % inputs for the DSB-ChIP data.

PCR validation of the I-SceI-induced DNA DSB. Genomic DNA was isolated from U2OS cells 1 h after addition of Shield-1 and triamcinolone using the Wizard gDNA isolation kit (Promega). PCR across the DSB was run using the Power SYBR green 2X master mix (ThermoFischer). The primers spanning the break were: 5' GATCAGGCAGAGCAGGAAC 3' (forward) and 5' AATACCTACGGCAAGCTGAC 3' (reverse).

Assay for DNA end resection using qPCR restriction enzyme protection assay. DNA end resection at the site of the I-SceI-induced DSB was monitored using the same approach as previous studies[31,32] in which ssDNA produced by end resection causes protection from ApoI digestion as assessed by qPCR. qPCR primers were used that amplify a product across the ApoI cut site 442 bps upstream of the I-SceI site in the DR-GFP integrated reporter construct. Mock HincII digestions (do not affect qPCR products) and additional control primers designed to span an ApoI site in the ß-actin gene (at 1559 bps) were used to normalize for ApoI digestion efficiency. U2OS DR-GFP cells harboring the ligand-inducible I-SceI were grown in in DMEM + 10% FBS and 1% Pen/Strep in the presence of DMSO (vehicle control), 500 µM octyl-(R)-2HG, 2 mM succinate, or 30 µM dimethyl-fumarate. The I-SceI DNA DSB was induced as above with triamcinolone and Shield-1 and cells were collected 3 h after the addition of the ligands. Genomic DNA was extracted with a Phenol-chloroform extraction. Genomic DNA was digested with ApoI or HincII for 4 hrs 30 min at 37C followed by heat inactivation at 80C for 20 min. qPCR was carried out using the iTaq Universal SYBR Green Kit (Bio-Rad, 1725121) and a CFX96 real time PCR detection system (Bio-Rad). The primers used flanking the restriction enzyme sites upstream of the I-SceI cut site in DR-GFP were F: GCTAACCATGTTCATGCCTTCT and R: TAGTGGCGGCGGATCTGAAT. The β-Actin control primers were F: GGGCCTCAGGTGATAAATTCTG and R: CCTGAGTCCAAAGGCTGTTTG.

Laser micro-irradiation. Sterile grid cover slips (10816; ibidi inc.) were placed in 50mm glass-bottom dishes (P50GC-1.5-14-F; MatTek corp.) and coated overnight with 20 µg/mL Collagen A (A1048301; Thermo Fisher Scientific) in PBS at 4°C. 300,000 to 400,000 cells were seeded per

dish under normal growth conditions. After one day 10 µM BrdU (19-160; EMD Millipore) was added to the medium to sensitize the cells. Cells were incubated additional 48h prior to irradiation. Shortly before irradiation, cells were washed with warm PBS and growth medium was replaced with warm low absorption medium (31053; Thermo Fisher Scientific). Cells were mounted on a Leica TCS SP8 X microscope system (Leica Microsystems) with an incubator chamber at 37 °C with 5% CO_2. Cells were exposed to a 405 nm diode laser (95% with FRAP booster, 35 iterations, 150 µJ/pixel total power) and irradiation field of multiple 5-pixel wide stripes. After 35 iterations at a speed of 130 Hz bidirectional (pixel dwell time: 3.75 µs, frame rate 0.503/s, zoom 0.75, resolution 512x512), cells were and permeabilized with 3% formaldehyde, 0.2% Triton X-100 and 8% sucrose for 15 min at RT.

Immunofluorescence staining. The following antibodies and corresponding dilutions were used to stain laser micro-irradiated samples and foci assays, anti-Rad51 (1:200; PC130-100µl; EMD Millipore) , anti-BRCA1 (1:100; OP92; EMD Millipore), anti-ATM (1:200; 2873S; Cell Signaling), anti-phospho-ATM (S1981) (1:250; 4526S; Cell Signaling), anti-Tip60/Kat5 (1:250; NBP2-24613; NovusBio), anti-trimethyl H3K9 (1:200; 05-1250; EMD Millipore), anti-phospho-DNA-PKcs (1:300; E9J4G; Cell Signaling Technologies), anti-rabbit IgG Alexa Fluor Plus 488, anti-mouse IgG Alexa Fluor Plus 488, anti-rabbit IgG Alexa Fluor Plus 555, anti-mouse IgG Alexa Fluor Plus 555, anti-rabbit IgG Alexa Fluor Plus 647, anti-mouse IgG Alexa Fluor Plus 647, (1:500; A32731, A32723, A32732, A32727, A32733, A32728; Thermo Fisher Scientific). For foci assays, 25,0000 – 40,000 cells were seeded in Collagen A (20 µg/ml; A1048301; Thermo Fisher Scientific) pre-coated 8-well chamber slides (PEZGS08; EMD Millipore). After overnight incubation, cells were treated and fixed at the indicated time points. After one washing

step with PBS, cells were incubated with 3% PFA, 0.5% Triton X-100, 8% Sucrose in PBS for 20 min at RT followed by one washing step with PBS. Cells were then blocked overnight at 4°C with 5% FBS, 5% NGS, 0.2% Triton X-100 in PBS. Primary antibody staining was performed three hours at RT or overnight at 4°C for foci assays in blocking buffer, followed by three washing steps with PBS + 0.5% Triton X-100 and secondary antibodies staining for two hours at RT protected from light. After three washing steps with PBS + 0.5% Triton X-100. Cells were stained with DAPI/Hoechst33342 mix (each 5 µg/mL) in PBS + 0.5% Triton X-100 for 15min. Chambers were removed from slides and a cover slip was mounted on top using Dako Fluorescence Mounting (S3023; Dako NA Inc.). Images were analyzed with a Nikon Eclipse Ti fluorescence microscope with a Nikon Plan Fluor 40x/1.30 Oil DIC N2 objective or a Nikon Plan Apo λ 60x/1.40 Oil DIC N2 objective, a CSU-W1 confocal spinning disk unit, an iXon Ultra camera (Andor Technology), MLC 400B laser unit (Agilent Technologies) and NIS Elements 4.30 software (Nikon Corporation). Foci images were evaluated with the Focinator v2 software as previously described[33]. Images of the laser micro-irradiation assays were analyzed with our in-house-programmed software[34]. Data is plotted as % foci positive nuclei, whereas foci positive nuclei is defined as containing ≥ 10 foci of the indicated protein. For single time point post IR data, Tip60 and pATM foci analysis was conducted 1 h after 2 Gy IR and RAD51 and BRCA1 foci analysis was performed at 4h after IR after optimization of the foci assays over a time course.

Plasmid transfection and factor overexpression. 24 h prior to starting the indicated assays, cells were transfected with the following expression constructs for KDM4A (pCMV-HA-KDM4A Addgene 24180), KDM4A H188A (pCMV-HA-KDM4A H188A), KDM4B (pCMV-

HA-KDM4B, Addgene 24181), KDM4B H189A (pCMV-HA-KDM4B H189A), KDM4C (pCMV-HA-KDM4C Addgene 24214), KDM6A (pCMV-HA-KDM6A Addgene 24168), ALKBH2 (pCMV6-ALBH2, Origene RC226507), ALKBH3 (pCMV6-ALBH3, Origene RC212873), JMJD4 (pCMV6-JMJD4, Origene RC228358), pSLIK-IDH1-R132H (Addgene # 66802). Catalytically inactive mutants of KDM4A (H188A) and KDM4B (H189A), were generated using the Q5 Site-directed mutagenesis kit from New England Biolabs, per the manufacturer's protocol and verified by Sanger sequencing. The primers used were: CTTTGCTTGGGCCACTGAAGACATGG (forward) and GATGTCTTCCACATGCCAAAG (reverse) for KDM4A, and CTTCGCCTGGGCCACCGAGGAC (forward) and GTGGTCTTCCACATGCCG (reverse) for KDM4B. Plasmids to overexpress the above enzymes were transfected using Lipofectamine 3000, per the manufacturer's instructions, at a ratio of 1 µg of plasmid DNA: 1 µL of Lipofectamine 3000: 100,00 cells. Experiments to overexpress WT H3 and the indicated H3 mutants were performed using the pCVM6-H.3.A(H3AFA) vector (Origene RC209908) in which the respective site-directed mutations were introduced to alter the H3K4, H3K9, H3K27, and H3K36 lysine residues as indicated. Histone mutants were generated using the Q5 Site-directed mutagenesis kit from New England Biolabs, per the manufacturer's protocol and verified by Sanger sequencing. The primers for site-directed mutagenesis are as indicated below: The primers used for the H3K9 mutant expression constructs were as follows, F: ACTGCTCGGAGGTCTACTGG and R:TTGCTTAGTGCGAGCCATT to generate H3K9R; F:AACTGCTCGGCAGTCTACTGG and R: TGCTTAGTGCGAGCCATT to generate H3K9Q; F: AACTGCTCGGGGGTCTACTGGTG and R: TGCTTAGTGCGAGCCATT to generate H3K9G; and F: ACTGCTCGGATGTCTACTGGTG and R: TTGCTTAGTGCGAGCCAT to

generate H3K9M. The primers used to generate the H3K4 mutant expression constructs were, F: GCTCGCACTAGGCAAACTGCT and R: CATTCACGCCCTCTCTCC for H3K4R; F: GGCTCGCACTCAGCAAACTGC and R: ATTCACGCCCTCTCTCCG; for H3K4Q, F: GGCTCGCACTGGGCAAACTGCTC and R: ATTCACGCCCTCTCTCCG for H3K4G; and F: GCTCGCACTATGCAAACTGCTC and R: CATTCACGCCCTCTCTCC for H3K4M. The primers used to generate the H3K27 mutant expression constructs were, F: GCAGCCCGCAGAAGCGCTCCG and R: CTTAGTGGCCAACTGTTTGCGTGG for H3K27R; F: GGCAGCCCGCCAAAGCGCTCC and R: TTAGTGGCCAACTGTTTGCGTGG for H3K27Q; F: GGCAGCCCGCGGAAGCGCTCCG and R: TTAGTGGCCAACTGTTTGCGTG for H3K27G; and F: GCAGCCCGCATGAGCGCTCCGG and R: CTTAGTGGCCAACTGTTTGCGTG for H3K27M. The primers used to generate the H3K36 mutant expression constructs were, F: GGCGGCGTGAGAAAGCCCCAC and R: GGTGGCCGGAGCGCTTTT for H3K36R; F: CGGCGGCGTGCAAAAGCCCCA and R: GTGGCCGGAGCGCTTTTG for H3K36Q; F: CGGCGGCGTGGGAAAGCCCCAC and R: GTGGCCGGAGCGCTTTTG for H3K36G; and F: GGCGGCGTGATGAAGCCCCACC and R: GGTGGCCGGAGCGCTTTT for H3K36M.

siRNA knockdowns. All siRNA knockdowns for the indicated target genes were performed using the ON-TARGETplus smart from GE Dharmacon at a final concentration of 20 nM and were transfected with a 1:1000 dilution of equal parts Dharmafect 1 and Dharmafect 2 siRNA knockdowns were done with Dharmacon SMARTpool siRNAs. The RNA sequences were as follows KDM4A: GUAUGAUCUUCCAGACUUA, GCACGGACAUCAACCUUUC, GGGAUUCUAUCUCUUCUGA, GUGCGGAGUCUACCAAUUU, KDM4B:

CCGCAGGUCUCACCGGAAA, GCGCAGAAUCUACCAACUU,
CAAAUACGUGGCCUACAUA, CAUCAGCGGCUCUUUGUAU, H2AFX:
GGGACGAAGCACUUGGUAA, CGACUAGAACCUUAGGCAU,
GGAAAGAGCUGAGCCGCUU, GAACUGGAAUUCUGCAGCU, SUV39H1:
CUAAGAAGCGGGUCCGUAU, GGUGAAAUGGCGUGGAUAU,
UCGAGUACCUGUGCGAUUA, CAAAUCGUGUGGUACAGAA, TIP60 (KAT5):
CGUAAGAACAAGAGUUAUU, AUGAAUGGGUGACGCAUGA,
GGACAGCUCUGAUGGAAUA, GACCAAGUGUGACCUACGA, MRE11 :
GAUGAGAACUCUUGGUUUA, GAAAGGCUCUAUCGAAUGU,
GCUAAUGACUCUGAUGAUA, GAGUAUAGAUUUAGCAGAA, ATM:
GCAAAGCCCUAGUAACAUA, GGUGUGAUCUUCAGUAUAU,
GAGAGGAGACAGCUUGUUA, GAUGGGAGGCCUAGGAUUU, BRCA1,
CAACAUGCCCACAGAUCAA, CCAAAGCGAGCAAGAGAAU,
UGAUAAAGCUCCAGCAGGA, GAAGGAGCUUUCAUCAUUC, RPA32 (RPA2):
AACAUGAAGUUCUGCGGUA, UGGAACAGUGGAUUCGAAA,
GAGCAGGACCAGGGCGUUA, GGAAGUAGGUUUCAUCUAU, RAD51:
UAUCAUCGCCCAUGCAUCA, CUAAUCAGGUGGUAGCUCA,
GCAGUGAUGUCCUGGAUAA, CCAACGAUGUGAAGAAAUU. siCTRL is the ON-TARGETplus non-targeting Pool from GE Dharmacon. Log phase cells were transfected with siRNAs 72 hours before initiating the indicated downstream assay. siRNA suppression of target proteins was validated by western blot analysis. Quantification of band intensity for siRNA knockdown of repair factors in the DSB-ChIP assay was done with Image J64 software, with all

replicates on the same gel, which was re-probed for loading controls. For these experiments loading controls were re-probed on the same membrane.

ChIP-seq analysis and visualization. Raw ChIP-seq data corresponding to dox-induced IDH1 R132H mutant (MUT) and uninduced parental (PAR) astrocyte cell lines after 40 passages in culture, as well as corresponding input data, were accessed from publicly available SRA data (SRP082568) from experiments previously published in Turcan et al., 2018[28]. Raw sequencing data was aligned to the GRhg38 (hg38) genome build using Bowtie2 (v2.3.4.1). H3K9me3 peaks were called using HOMER (v4.10) using findPeaks with options -style histone -size 1000 -minDist 2500, filtered by P = 0.01. All peaks were called relative to input. Number of peaks per chromosome for called peak locations for two replicates in the MUT and PAR conditions were quantified.

ChIP of DNA repair factors to Cas9-induced DNA DSBs. H3K9me3 ChIP-seq peak data published in Turcan et al., 2018[28] were accessed via Gene Expression Omnibus (accession GSE85940) and visualized as tracks on the UCSC Genome Browser using hg19 assembly. Genomic locations of trimethylation peaks were identified that showed differential H3K9me3 status in dox-induced IDH1 R132H mutant (MUT) versus uninduced parental (PAR) astrocyte lines, or showed low in H3K9me3 in both MUT and PAR cell lines, or showed high in H3K9me3 in both the MUT and PAR samples. These loci are as follows: differential H39me3, Chr7: 30,830,457-30,831,521, hypermethylated H3K9me3 Chr7: 157,331,880-157,333,036, and hypomethylated Chr1: 82,408,624-82,409,455. The methylation status of these loci was verified by ChIP-PCR using the ChIP primers listed below. spCas9 guide RNA sequences were designed

to target these loci for induction of DSB. The following gRNA sequences were cloned into the MLM3636 human sgRNA expression vector: (1) differential H3K9me3 target gRNA: CUGCACCCAACAGGACAUUC, (2) hypermethylated target gRNA: UUCCCACAGCAUUCCUCCAU, and (3) hypomethylated target gRNA: ACUGCUAGGUCGAUAUCAGU. sgRNA expression vectors were transfected using Lipofectamine 3000. Ten million immortalized astrocytes either overexpressing WT IDH1 or IDH R132H in a 15 cm cell culture dish were transfected with 20 μg of DNA using 20 μL of Lipofectamine 3000. 24 hours later, 45 pmol of purified Cas9-NLS protein (PNA BIO, CP02) was electroporated into cells using a Lonza Nucleofector IIb, using the "astrocytes" program. DSB induction by Cas9 was confirmed by PCR across the DSB target loci at 6 h post nucleofection. For analysis of repair factor recruitment, cells were collected at 12 h post Cas9-nucleofection, and samples were prepared and analyzed using the DSB-ChIP assay as described above. H3K9me3 loci occupancy was determined by collecting samples at the indicated time-points after Cas9 electroporation. Loci occupancy of repair factors was quantified as %input using the primers below. ChIP primers for Cas9 induced breaks are (1) differential H3K9me3, F: GCAAAGGTGACGGCTTTTCA, R: TTCCAGGTGTGTGAGCTCTG; (2) hypermethylated, F: TCGGCTGGTTTCTCACTTCC, R: GAGCAAGCTATGAGGGTCCC; and (3) hypomethylated F: TGAAGCAACGTGGGAGACTG, R: TGGTTGGGGAAGGGAACATC.

NHEJ reporter assays. EJ5, EJ2 and the luciferase-based plasmid based NHEJ assays have been previously described[35]. GFP fluorescence to quantify end-joining efficiency in EJ2 and EJ5 was measured by flow cytometry 96 h after transfection of the cells with the p-I-SceI construct. Cells were treated with oncometabolites 24 h before I-SceI transfection and were maintained in

oncometabolites for the duration of the assay. The luciferase based NHEJ assay cells were performed as previously described[5,6,36]. Briefly, cells were treated with oncometabolites for 24 h before transfection of a HindIII digested Firefly-luciferase NHEJ reporter plasmid. Luciferase activity was quantified 24 h after transfection and was normalized to Renilla luciferase activity, based on transfection with a Renilla luciferase control plasmid to account for transfection efficiency differences. Data was normalized to WT or vehicle-treated controls. Ku80 knockdown served as a positive control for EJ5 assay and the luciferase based NHEJ assay. PARP inhibition served as a positive control from the EJ2 assay.

PARP inhibitor sensitivity assays. PARP inhibitor sensitivity assays were performed as previously described[5,6]. Briefly, cells were seeded in solid white 96 well plates at a density of 5,000 cells per well. 24 h later they were then transfected with the indicated histone 3 expression construct. 24 h after transfection the PARP-inhibitor, BMN-673, was added at the indicated concentrations. 96 h after the addition of the PARP-inhibitor cells were assayed for viability using the Cell Titer Glow Kit (Promega).

Cell growth analysis and cell cycle analysis. To measure cell growth rates, cells were seeded at indicated densities in either 10 cm dishes or 6 well plates. Cells were counted at the indicated time points to generate serial cell counts over time. To analyze cell cycle phase distribution, log phase cells were collected and stained with propidium iodide (PI) and then analyzed using flow cytometry. Data was analyzed using Flo-Jo software.

Data analysis for lower grade glioma cohort in TCGA database. Processed data was downloaded from cbioportal.com[37] and plotted using PRISM 7.

Statistical analyses. Comparisons between two groups were done with two tailed, unpaired t-test. For comparisons of curves over time, ANOVA was used. The number of replicates, df, and F values for ANOVAs are indicated in each figure legends.

Code Availability. There is no custom code in this study.

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