Supplementary Materials and Methods

Cell lines. 10T1/2 (C3H/10T1/2, Clone 8) and C2C12 cells were obtained from ATCC and cultured at 37°C in 5% CO_2 in high glucose DMEM supplemented with 10% FBS. Cell lines were authenticated via STR repeat assay performed by the Integrated Genomics Operations Core Facility (MSKCC), and verified to be mycoplasma-free by MycoAlert Mycoplasma Detection Kit (Lonza).

Western Blotting. Protein extracts were prepared by using 1× RIPA buffer including protease inhibitors (Thermo # 1860932) and phosphatase inhibitors (Thermo # 78428). Equal amount of total protein was separated on NuPAGE Bis-Tris gels (Life Technologies), transferred to nitrocellulose membrane and incubated with indicated primary antibodies. After secondary antibody incubation and enhanced chemoluminescence solution incubation, blots were scanned using a Biorad ChemiDoc Touch Imaging System. Antibodies used are described in the accompanying reagents table.

GC-MS analysis of 2-hydroxyglutarate. 10T1/2 cells were plated at 750e3 cell/10cm^2 plate the day before harvesting. For harvesting, cells were rinsed with PBS once and incubated with 80% methanol containing 20 μM deuterated 2HG as an internal standard (D-hydroxyglutaric-2,3,3,4,4-d5) pre-chilled to -80°C for 30 minutes at -80°C. Three replicates per condition were harvested. Supernatant was sonicated three times for 30 seconds each in a Diagenode Bioruptor 300 and centrifuged at 20e3g for 20 minutes at 4°C. The supernatant was dried by spin vacuum, dissolved in 40 mg/mL methoxyamine in pyridine and derivatized with MSTFA with 1% TMCS

for 30 minutes at 30°C. One microliter of trimethylsilyl-derivatized organic acids was analyzed using an Agilent 7890A GC equipped with an HP-5MS capillary column and connected to an Agilent 5975 C mass selective detector operating in splitless mode with electron impact ionization. Relative quantification of 2HG was performed from extracted ion chromatograms for 2HG (m/z: 349) normalized to the D5-2HG internal standard (m/z: 354) and corrected by protein concentration per sample.

Molecular cloning and virus production. MyoD (26808) and MyoD-ER (26809) vectors were obtained from Addgene and subcloned into pCDH lentiviral vector (Clontech) or pMSCV-Puro-IRES-GFP (Addgene 21654) with addition of an N-terminal HA tag. Previously characterized human IDH2-WT and IDH2-R172 cDNAs (1) were subcloned into a modified version of pTRE-Tight (Clontech) (2). Myogenin was cloned from a 72 hour differentiated C2C12 cDNA library into pMSCV-Puro-GFP. Guide RNAs against murine EHMT1 and Mettl3 were cloned into pLentiCRISPRv2 vector. Rosa26 targeting guides (GAAGATGGGCGGGAGTCTTC) were used as a control. Lentiviral particles were produced in 293T cells by using psPAX2 and pMD.2 packaging plasmids (Addgene). Retroviral particles were produced in 293T cells by using pCG-gag-pol and pCMV-VSV-G packaging plasmids (Addgene). Cells were passed through a 0.45 μm nylon filter and transduced with viral supernatant in the presence of 8 μg/mL of polybrene overnight and subjected to puromycin (2.5 μg/mL) or hygromycin (250 μg/mL) antibiotic selection, or GFP sorting. For a list of used oligonucleotide sequences see accompanying reagents table.

Cell Culture and Differentiation. Inducible expression of IDH2-WT and IDH2-R172K vectors was achieved with doxycycline at a dose of 1 μg/ml. Differentiation of MyoD or MyoD-ER expressing cells was carried out in DMEM containing 2% horse serum in the presence of 1 μM 4-OH-tamoxifen in the case of the MyoD-ER construct. Cells were subsequently harvested or fixed for further analysis 48 hours after 4-OH-tamoxifen addition. For the 5-Azacytidine experiments, cells were plated at a density of 5e3 cells per well of a 6 well plate and 3 μM 5-Azacytidine added the following day. Cells were washed with regular growth media 24 hours later and harvested after 14 days.

Immunofluorescence and Immunocytochemistry. Cells were grown on cover slips at densities equivalent to those used for western blotting. 48 hours after differentiation induction, the cells were fixed using 100% methanol or 3.7% formaldehyde in PBS. Cells were permeabilized in 0.1% Triton-X in PBS and blocked with 0.2% Cold Water Fish Gelatin (Sigma) and 0.5% BSA. Primary antibodies were then added at a concentration of 1:100, washed, and secondary antibodies added at a concentration of 1:1000 in the presence of 4% normal goat serum in PBS. Cover slips were mounted on slides with Prolong Antifade and DAPI and imaged using a Zeiss Axio Observer Upright scope with an X-cite 120 LED lamp and a Hamamatsu C4742 camera. For immunocytochemistry, cells were grown on 6 well plates, fixed with 100% methanol, permeabilized, blocked and hybridized with primary and secondary antibodies as above and HRP signal detected using VECTASTAIN ABC Kit (Vector Laboratories) per the manufacturer's instructions.

Bisulfite Sequencing. 10T1/2 cells were grown on 6 well plates and DNA extracted one day after plating at 50% confluence with DNA lysis buffer (10 mM Tris pH 8.0, 100 mM, 10 mM EDTA pH 8.0, 10% SDS) containing Proteinase K and RNAse, precipitated with isopropanol and again with 70% ethanol. Bisulfite treatment was performed with Zymo Research EZ DNA Methylation Kit. PCR primers were designed using MethPrimer and amplified products subcloned into TOPO-TA vectors (Thermo Scientific) and colonies submitted for Sanger sequencing. Analysis of reads was done using BISMA. For a list of used oligonucleotide sequences see accompanying reagents table.

In situ EdU Incorporation and co-Immunofluorescence. EdU was added to proliferating or differentiating 10T1/2 cells grown on cover slips at a final concentration of 10 μM for 10 hours. Detection of EdU incorporation and concurrent immunofluorescence was carried out using the Click-iT EdU Imaging Kit (Invitrogen). Briefly, following incubation, cells were fixed in 3.7% formaldehyde in PBS for 15 minutes at room temperature. Cells were washed in PBS with 3% BSA and permeabilized with 0.5% Triton-X in PBS with 3% BSA. Click-iT reaction was then carried out with an Alexa-Fluor-488 dye. Cells were then incubated in primary and secondary antibodies as per immunofluorescence and DNA co-stained with Hoechst 33342. Coverslips were mounted on slides and imaged as per immunofluorescence.

Chromatin Immunoprecipitation. ChIP was carried out on 20e6 differentiated cells. Cells were crosslinked with 1% formaldehyde in PBS for 15 minutes and cross-linking quenched with addition of glycine to 125 mM and incubation for 5 minutes. Cells were then harvested, centrifuged at 500g for 5 minutes and washed with PBS. Pellets were stored in -80°C for up to a

month. For MyoG ChIP, lysis was performed with sequential lysis in Farnham Buffer (5 mM PIPES pH 8.0, 85 mM KCl, 0.5% NP40) and Lysis Buffer 2 (1% NP40, 0.5% sodium deoxycholate, 0.1% SDS in PBS) and samples sonicated in 1mL Covaris AFA Fiber milliTUBE using a Covaris E220 at 140 Watts, Intensity 3 and 200 bursts per second. Samples were centrifuged to remove debris after addition of Triton-X to 1% and protein quantified. 500 μg of protein were subsequently used for immunoprecipitation with primary antibody preincubated ProteinG Dynabeads and incubated for 16 hours at 4°C. Beads were sequentially washed with Wash Buffer 1 (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 2mM EDTA, 0.1% SDS, 1% Triton X-100), Wash Buffer 2 (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 2 mM EDTA, 0.1% SDS, 1% Triton X-100), Wash Buffer 3 (10 mM Tris-HCl pH 8.0, 250 mM LiCl, 1% NP40, 1% sodium deoxycholate, 1 mM EDTA) and TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA) and chromatin complexes eluted in 0.1 M NaHCO3, 1% SDS for 30 minutes at 65°C. After incubation in RNAse A at 0.2 mg/ml at 37°C for 2 hours and Proteinase K at 0.2 mg/ml at 55°C for 30 minutes, DNA was purified with phenol/chloroform/isoamyl alcohol, washed with EtOH, air dried and resuspended in TE. Quantitative PCR was done using indicated primers and SYBR green (ThermoFisher) per manufacturer's instructions on a Applied Biosystems QuantStudio 7 Flex. For a list of used oligonucleotide sequences see accompanying DNA oligos table. For H3K9me2 and H3K9me3 ChIP, cells were crosslinked and harvested as above. After washing in ice cold PBS, cells were lysed with SDS Buffer (100mM NaCl; 50mM Tris-HCl pH 8.1; 5mM EDTA; 0.2% NaN_3; 0.5% SDS) at room temperature and pelleted at max speed at room temperature. The pellet was then resuspended in ChIP Buffer (2 volumes of SDS buffer and 1 volume of Triton Buffer; 100mM Tris-HCl pH 8.6, 100mM NaCl, 5mM EDTA, 0.2% NaN_3, 5.0% Triton X-100) and samples sonicated in Covaris 1ml milliTUBEs using a Covaris E220 at

140 Watts, Intensity 3 and 200 bursts per second. 5µl were removed for input and appropriate antibody was added after volume adjustment. Lysate-antibody mix was incubated rotating overnight at 4°C and Protein A/G dynabead 1:1 mixture was added the next morning and incubated rotating at 4°C for 2 hours. Beads were subsequently washed sequentially in mixed micelle (150mM NaCl, 20mM Tris-HCl pH 8.1, 5mM EDTA, 5.2 w/v sucrose, 0.2% NaN_3, 1% Triton X-100, 0.2% SDS), B500 (0.1% deoxycholic acid, 1mM EDTA, 50mM HEPES pH 7.5, 1% Triton X-100, 0.2% NaN_3), LiCl (0.5% deoxycholic acid, 1mM EDTA, 250mM LiCl, 0.5% NP-40, 10mM Tris-HCl pH 8.0, 0.2% NaN_3) and TBS buffer (20mM Tris-HCl pH 7.4, 150mM NaCl), and elution carried out in elution buffer (1% SDS, 100mM $NaHCO_3$) at room temperature. Decrosslinking was carried out at 65°C overnight and DNA purified using 1.5X AmpureXP beads. QC was performed on D1000 Agilent 2200 TapeStation and ChIP-seq library preparation performed with NEB ChIPseq Library Preparation Kit using manufacturer's instructions. Samples were pooled for multiplexing and sequenced using paired-end sequencing technology on Illumina NextSeq 500 platform (MOLM-13).

Omni-ATAC-seq. Assay for Transposase Accessible Chromatin and sequencing was performed as previously described and modified (3, 4). Briefly, cells were harvested, centrifuged at 500g and resuspended in 50 µL of ATAC-Resuspension buffer (10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$) containing 0.1% NP40, 0.1% Tween-20 and 0.01% Digitonin. Lysate was incubated for 3 minutes on ice and lysis buffer diluted with cold ATAC-RSB containing 0.1% Tween-20. Nuclei were then precipitated at 500g for 10 min at 4°C, supernatant carefully aspirated and pellet resuspended in 50 µl of Transposition mix (10 mM Tris-HCL pH 7.6, 5 mM $MgCl_2$, Dimethyl 10% Formamide, 100 nM transposase, 0.01% digitonin, 0.1% Tween-20) and

incubated at 37°C for 30 minutes. DNA was then purified using the Zymo DNA Clean and Concentrator-5 Kit (Zymogen). Pre-amplification and barcoding were carried out with the NEBNext® High-Fidelity 2X PCR Master Mix (NEB) and Nextera PCR primers. Additional number of qPCR cycles were determined using 1/10 of the pre-amplified cycle with qPCR and final amplified libraries were purified with Zymo DNA Clean and Concentrator-5 Kit. Library quantification was performed with the KAPA Library Quantification kit quality control performed with Quant-iT Picogreen dsDNA assay kit (Life Technologies/Invitrogen) with median fragment size determined using an Agilent 2200 TAPE-Station. Samples were pooled for multiplexing and sequenced using paired-end sequencing technology on Illumina NextSeq 500 platform (MOLM-13).

Epigenomic Analysis

Raw reads from ChIP-seq and ATAC-seq data were trimmed and filtered for quality and adapter content using version 0.4.5 of TrimGalore! (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/), with a quality setting of 15, and running version 1.15 of cutadapt and version 0.11.5 of FastQC. Reads were aligned to mm9 with version 2.3.4.1 of bowtie2 (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml) and were deduplicated using MarkDuplicates in version 2.16.0 of Picard Tools. To ascertain regions of chromatin accessibility, peaks in read density were called with version 2.1.1.20160309 of MACS2 (https://github.com/taoliu/MACS), with a p-value setting of 0.001. The BEDTools suite (http://bedtools.readthedocs.io) was used to create read density profiles, with a read extension of the average library size for ChIP data and 0 for ATAC data. Within called peaks and normalization-defining regions, reads were tallied with version 1.6.1 of featureCounts

(http://subread.sourceforge.net/). Read density was normalized according to counts in windows of 4Kb around transcription start sites of genes known to be widely expressed, constructed as intersections of results from human and mouse transcriptomic studies (5-7). To normalize counts and analyze called peaks within accessibility data we used DESeq2 (https://bioconductor.org/packages/release/bioc/html/DESeq2.html), and version 1.0 of bwtool (https://github.com/CRG-Barcelona/bwtool) was used to extract and aggregate accessibility signal from a variety of regions. ChIP-seq signal was normalized to sequencing depth, and composite plots were based on average signal sampled in 25 bp windows with 'flanking' defined by the surrounding 3 kb. Box and violin plots were based on the total normalized read count in the entire window, without sub-sampling. During many phases of analysis after initial pipeline processing, we used simpleCache (https://github.com/databio/simpleCache) to conveniently store and reload data in intermediate analysis stages and forms. Version 2.27.1 of bedtools (https://github.com/arq5x/bedtools2/releases/tag/v2.27.1) and version 2.4.35 of bedops (https://github.com/bedops/bedops/releases/tag/v2.4.35) were used for manipulations of genomic regions in several specific sets of public data from ChIP-seq of DNA-binding proteins, with bedtools also used to generate a collection of randomized regions as a control baseline.

We were interested in how differences in chromatin accessibility changes following differentiation induction may be associated with the differentiation impairment observed with a particular genetic mutation to IDH2. Reasoning that such differences would likely behave nonuniformly across the genome, we compared regions flanking CTCF binding and those flanking MyoD or myogenin binding. Using the aggregate program from the bwtool software, we measured accessibility signal for each of the four (genotype + treatment) conditions, averaged

across all regions associated with CTCF, MyoD, or myogenin binding. Then for each genotype we measured accessibility difference following tamoxifen treatment by taking—at each distance from the metaregion's center—the difference between the treated and untreated subgroups within the genotype. The *difference-of-differences* given by subtracting this value for the mutant from the corresponding value for the wildtype is what is tracked for each DNA-binding protein in Figure 5C.

To quantify how the behavior of these measurements varies among these different genomic region sets, we partitioned the observations within a 5Kb window around each binding protein's metaregion center into 500bp subintervals. Within each subinterval, we performed a Wilcoxon signed-rank test comparing a particular protein's regions, and those of a "random shuffle" control generated with the shuffle program in the bedtools suite. For each DNA-binding protein, in all 500bp subregions the distributional center is significantly different from that of the pool of randomly selected regions, and using the confidence interval option in the wilcox.test function in R, we obtain intervals for the difference from random shuffle that do not contain zero.

Furthermore, moving inward from the distal flanks, in all subintervals the estimate of the difference between a protein's associated regions set and the randomized regions set holds relatively steady until the most peak-center-proximal bin is reached. That is, in all bins beyond 500bp of the metaregion center, difference-of-differences remains relatively stable. More important, each of the three myogenesis-related region sets attains maximal estimate for difference from random shuffle in the bin most proximal to metaregion center, and the difference between the estimate in that most proximal bin and the next-greatest estimate for a given

protein's set of subintervals represents the greatest percentage difference between any such pair of bins. CTCF dynamics were clearly a bit different; to hone in on those, we obtained finer resolution within the most proximal subinterval by further partitioning it in nonoverlapping 100bp intervals. From that closer perspective it's apparent that to observe a drop in the difference-of-differences measure, close proximity to a CTCF binding event is required. Again moving inward from the most distal flanking regions, until the most proximal (+/- 100bp) bins, estimates hold steady or even increase; in the most proximal bins, however, the estimate is negative (about -0.15).

To examine—without aggregation, and independently of any notion of a metaregion or distance from its center—the regional difference in accessibility dynamics upon differentiation induction response between the genotypes, we looked at the distribution of accessibility change within the CTCF-, MyoD, and MyoG-associated regions themselves. For regions associated with CTCF binding, there is clearly little difference between the genotypes, and a Kolmogorov-Smirnov test yielding a p-value of 0.3309 supports that visual observation. Conversely, for the regions associated with myogenin binding seven days after differentiation induction, a KS test yielding p-value less than 2.2e-16 affirms the visual observation from the empirical CDF plot that those regions are more responsive, in terms of accessibility increase, in the wildtypes than in the mutants.

References

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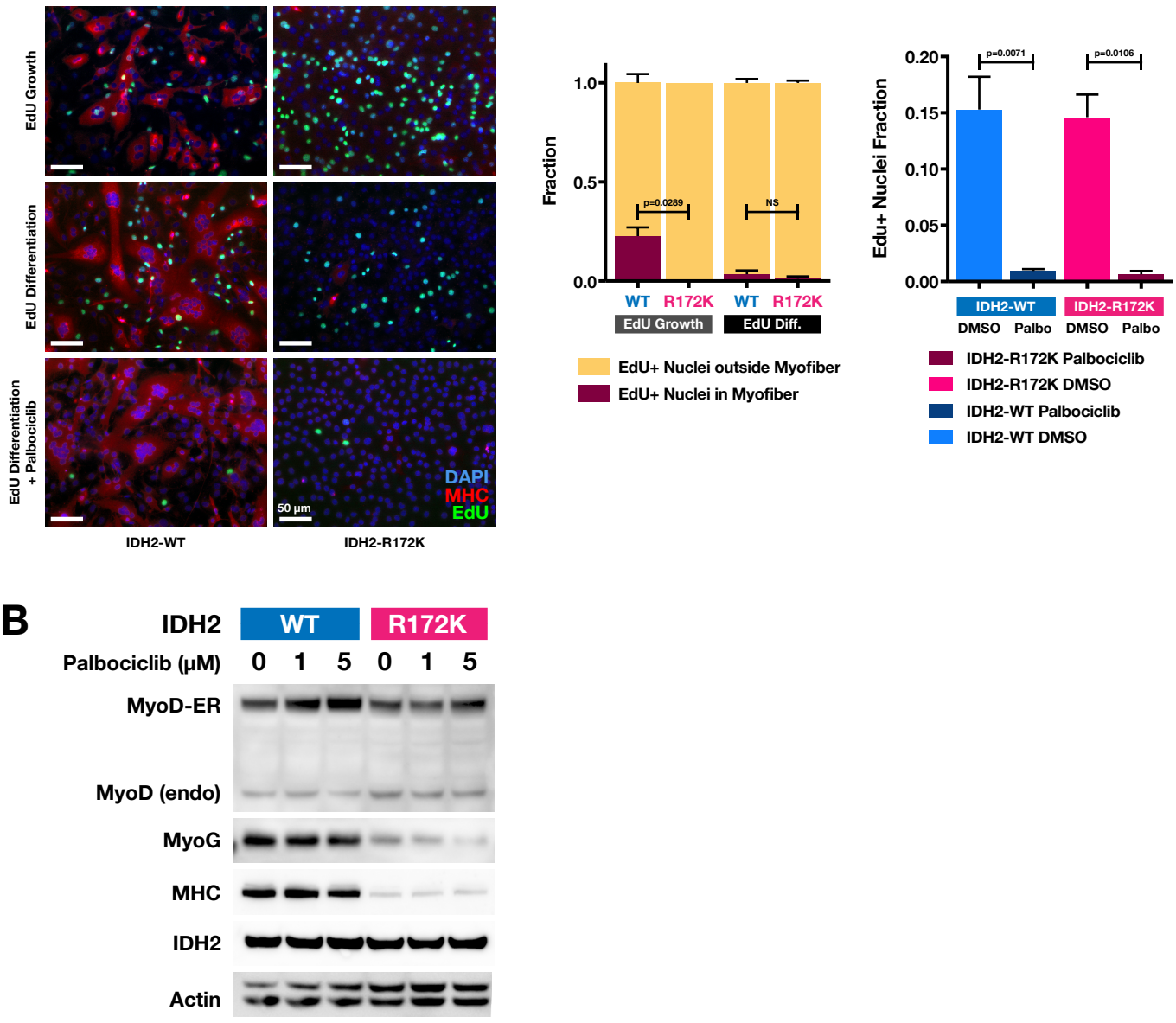
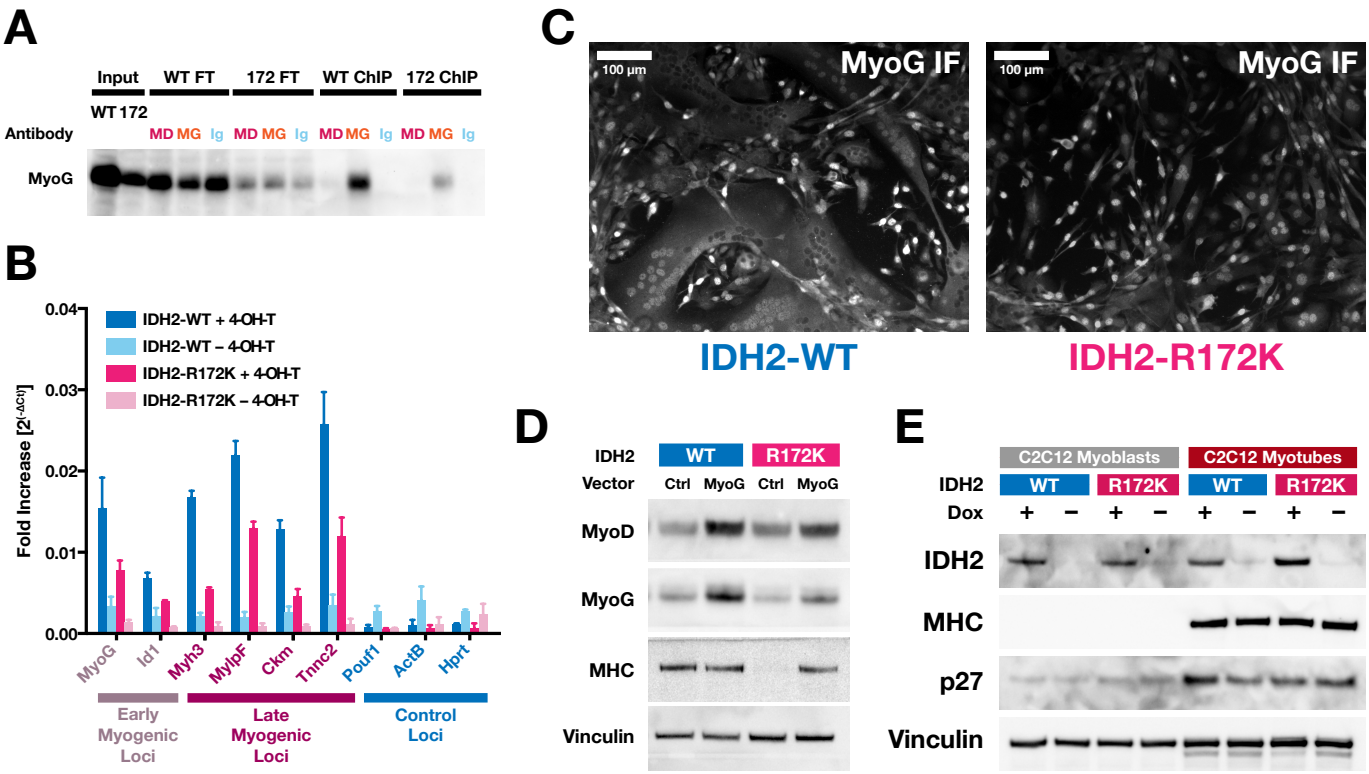
Figure S1



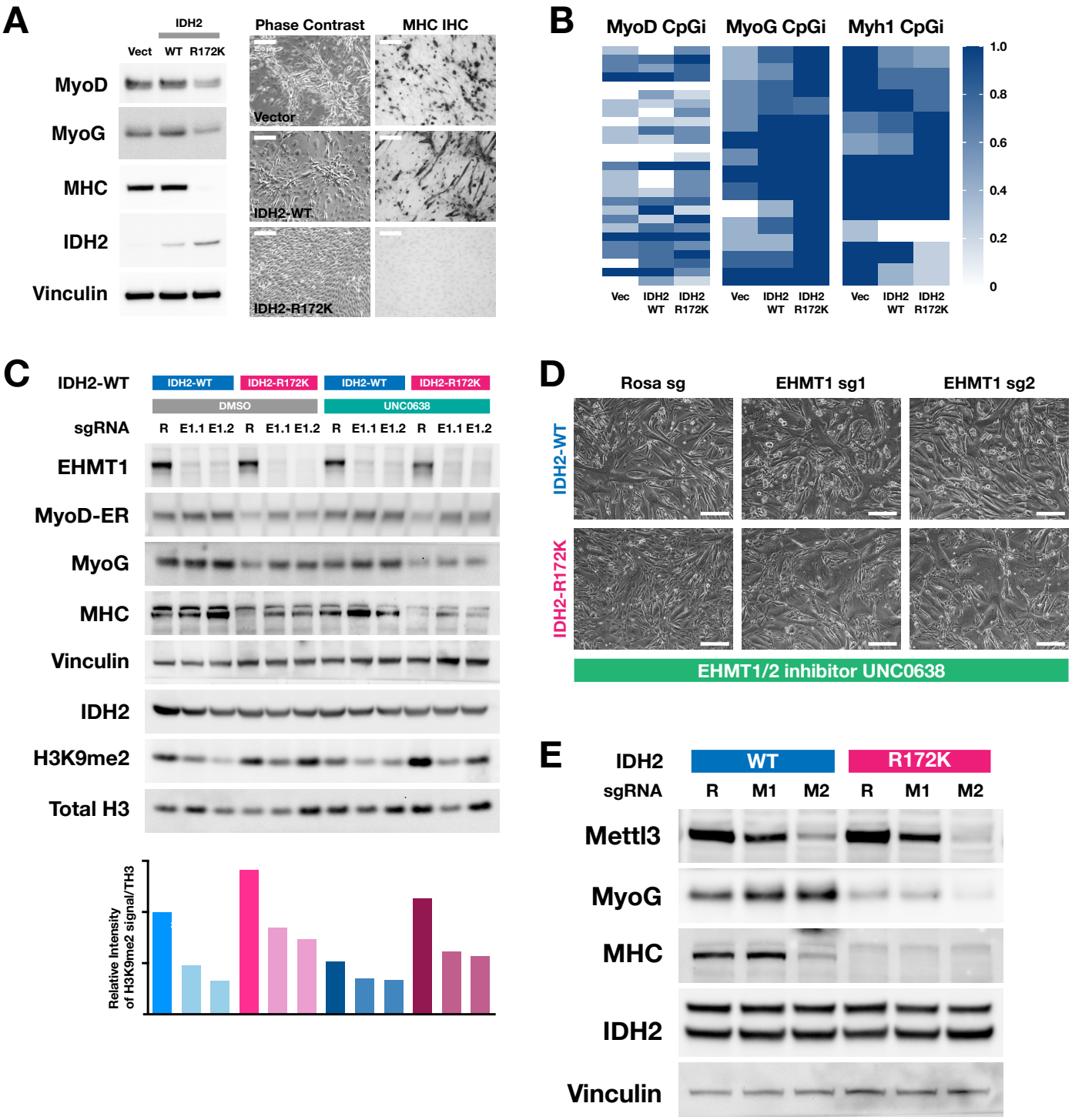
Supplementary Figure 1. The IDH2-R172K-mediated differentiation block is not a result of impaired cell cycle exit. A) 10T1/2 MyoD-ER cells were transduced with IDH2-WT or IDH2-R172K and treated with 4-OH-Tamoxifen for 48 hours to induce differentiation. EdU was incorporated at 10 µM during growth (top panels) or during differentiation (middle and bottom panels) together with 4-OH-Tamoxifen to label dividing cells. Bottom panels were also treated with the CDK4/6 inhibitor palbociclib (1 µM). DAPI in blue, MHC in red and EdU in green. Quantification of fraction of EdU positive nuclei inside and outside myofibers and effects of palbociclib on EdU incorporation is shown on the right. Data is mean ± SD of 3 representative images with at least 50 nuclei. P values were calculated using unpaired Student's t test. B) Western blot of 10T1/2 MyoD-ER cells expressing IDH2-WT or IDH2-R172K treated with indicated doses of palbociclib and induced to differentiate with 4-OH-Tamoxifen. Blotted proteins indicated on the left.

Figure S2



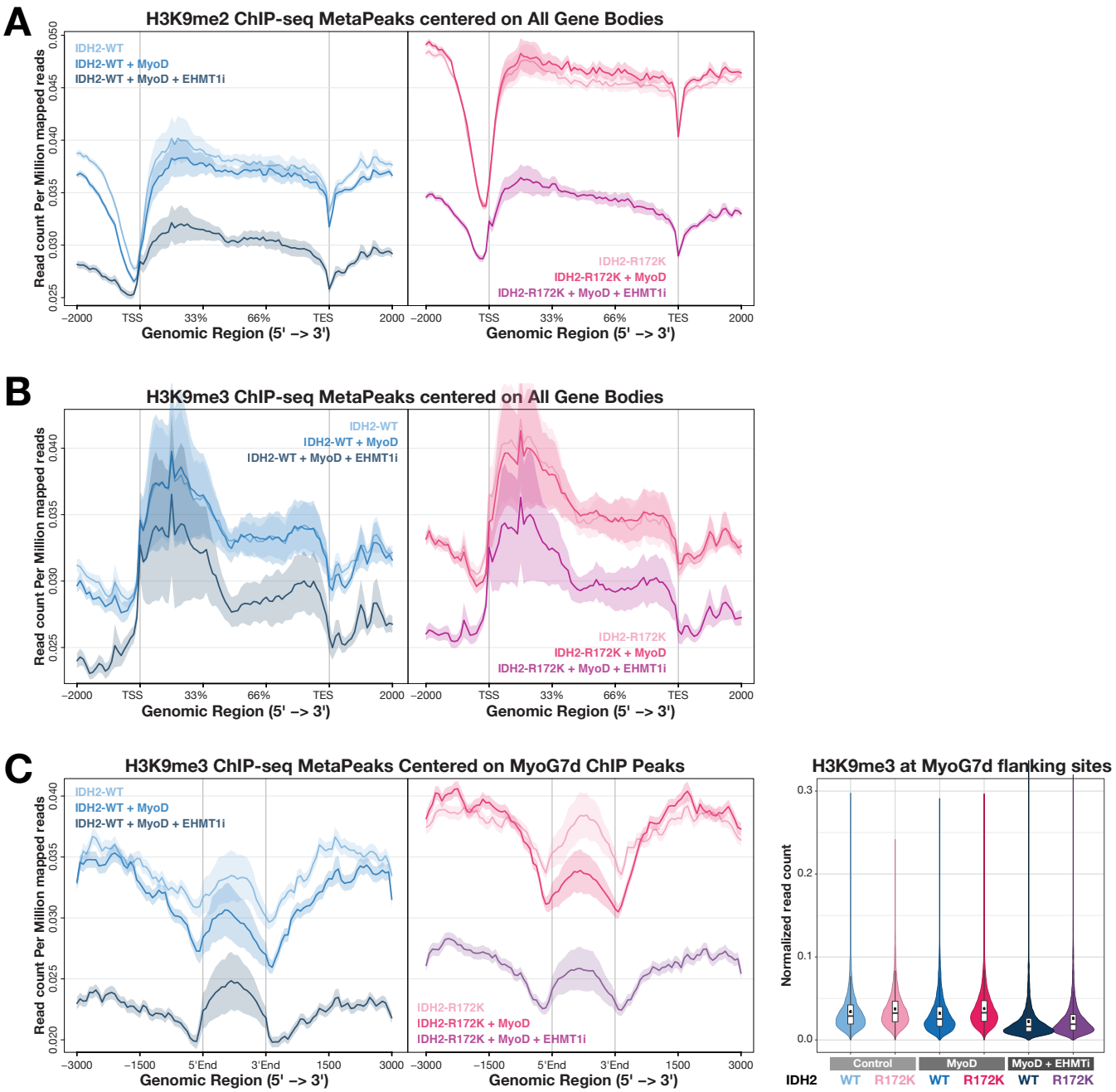
Supplementary Figure 2. IDH2-R172K and 2HG do not act by imparting a non-permissive metabolic environment for differentiation. A) ChIP-western blot of sonicated chromatin preparations from 10T1/2 MyoD-ER cells treated with 4-OH-T for 24 hours. Immunoprecipitation was carried out with MyoD (MD), Myogenin (MG) or Isotype control IgG antibodies. FT: flow-through. B) ChIP-qPCR for endogenous Myogenin in 10T1/2 MyoD-ER cells expressing IDH2-WT or IDH2-R172K in the presence or absence of 4-OH-T to activate MyoD. Early and late myogenic loci are indicated. Pouf1 (Oct4), ActB and Hprt serve as negative controls. All values are normalized to input and shown as mean ± SD of 3 technical replicates. C) Immunofluorescence for Myogenin in 10T1/2 MyoD-ER cells transduced with IDH2-WT or IDH2-R172 vectors and treated with 4-OH-T for 48 hours. D) Western blot of 10T1/2 MyoD-ER cells expressing IDH2-WT or IDH2-R172K, and transduced with a Myogenin-expressing or control vector. All four conditions were treated with 4-OH-T to trigger myogenic differentiation. E) Western blot of C2C12 before and 72 hours after differentiation in 1% serum containing media. Cells were transduced with doxycycline inducible IDH2-WT or IDH2-R172K vectors and probed with indicated antibodies.

Figure S3



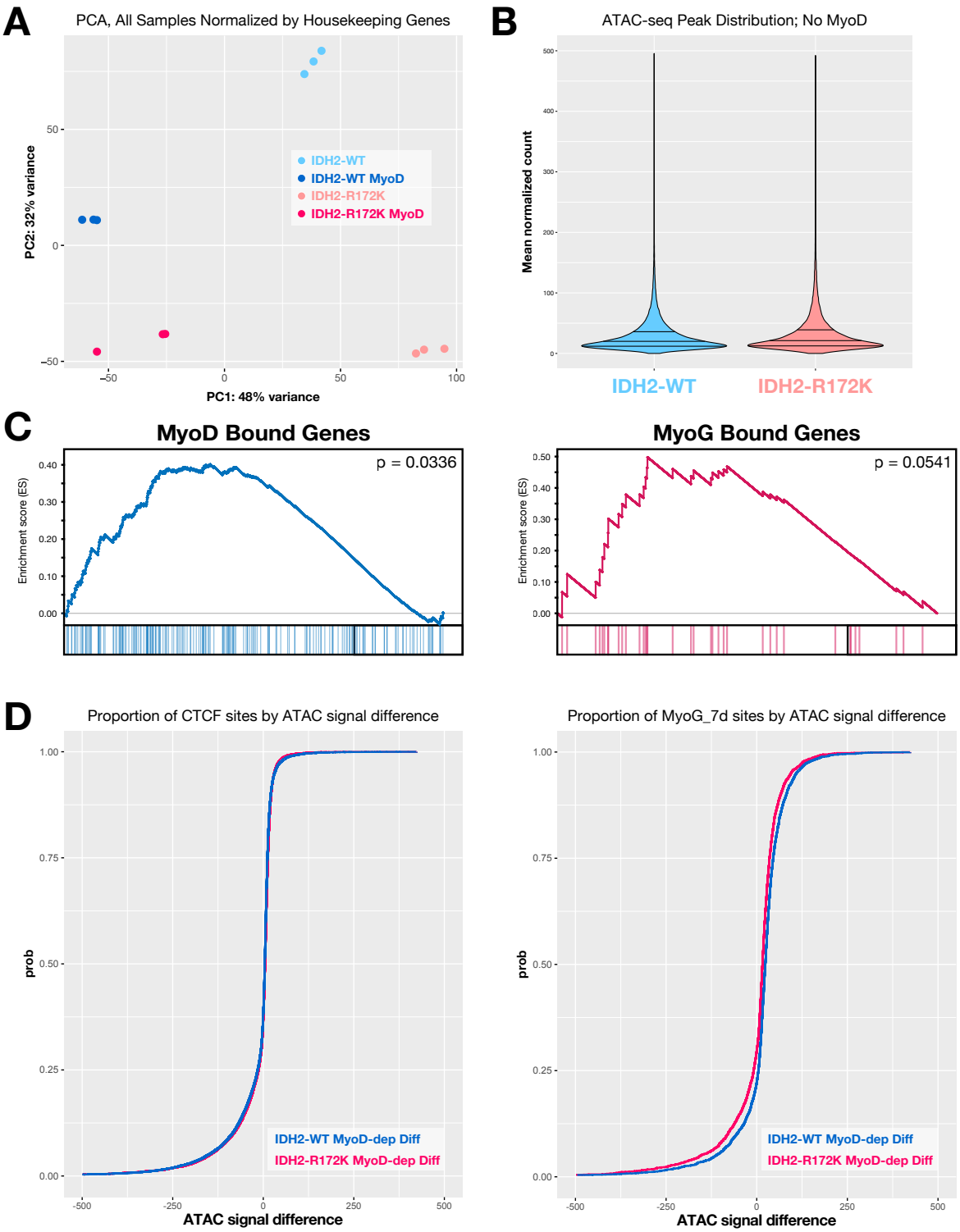
Supplementary Figure 3. The IDH2-R172K differentiation block is not a result of 5mC hypermethylation and is mediated by H3K9 hypermethylation. A) Western blot (left) and phase contrast and MHC immunoperoxidase staining (right) of 10T1/2 cells transduced with control, IDH2-WT and IDH2-R172K expressing vectors treated with a 24 hour pulse of 5-azacytidine. Blotted antibodies indicated on the left. B) Bisulfite sequencing analysis of indicated loci (CpGi, CpG island) of 10T1/2 cells as in A) not treated with 5-azacytidine. Intensity scale corresponds to degree of CpG methylation. C) and D) Western blot and phase contrast images of 10T1/2 MyoD-ER cells expressing IDH2-WT or IDH2-R172K and CRISPR Cas9/guide vectors targeting control Rosa26 (R) or EHMT1 using 2 different guides (E1.1 and E1.2) and grown in DMSO or the EHMT1 inhibitor UNC0638. Blotted proteins indicated on left in C). Quantification of H3K9me2 normalized to total H3 is shown below the western blot. E) Western blot of 10T1/2 MyoD-ER cells expressing IDH2-WT or IDH2-R172K and CRISPR Cas9/guide vectors targeting control Rosa26 (R) or Mettl3 using 2 different guides (M1 and M2). Blotted proteins indicated on left.

Figure S4



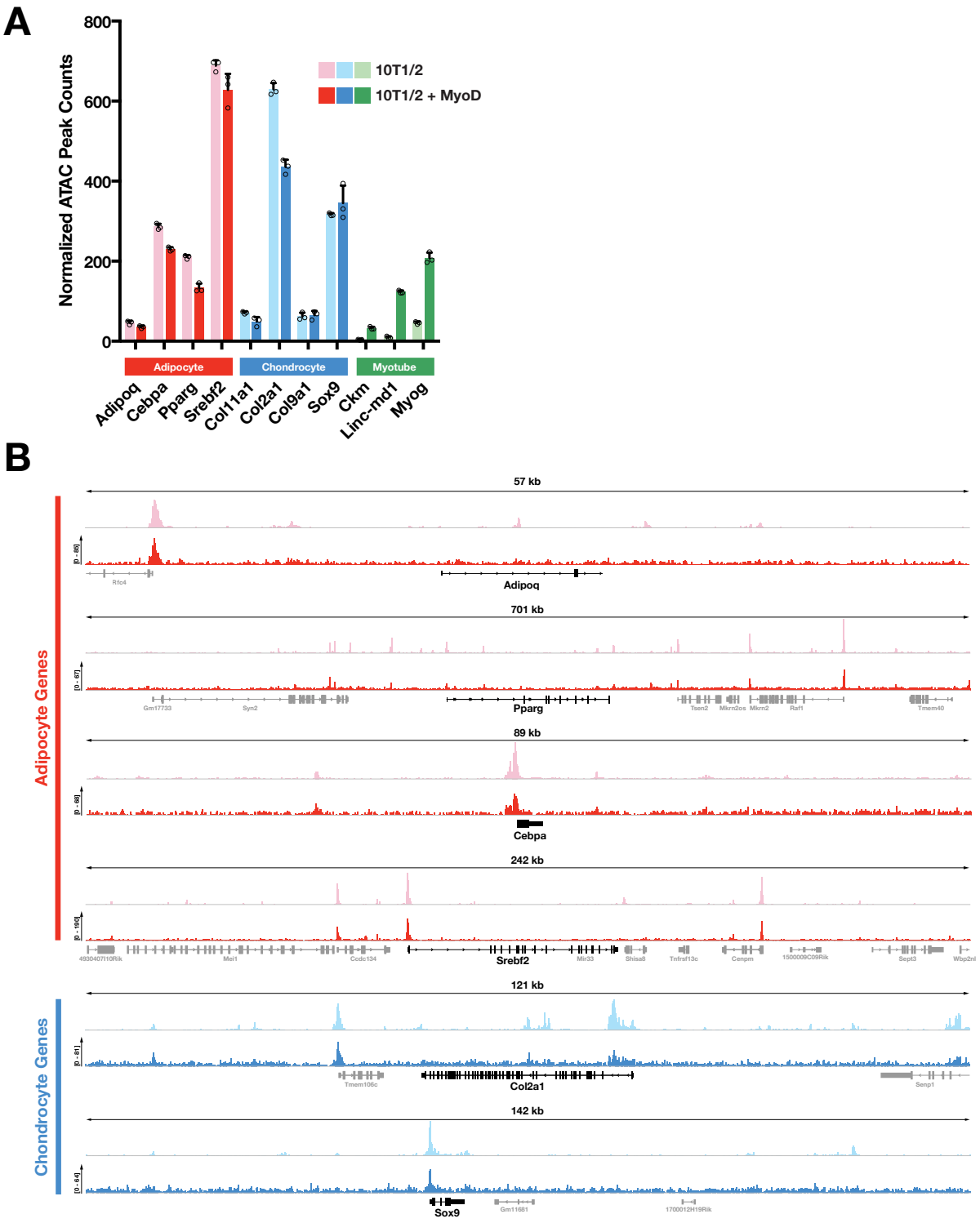
Supplementary Figure 4. Oncogenic IDH mutations do not impair global H3K9me2 demethylation. A) H3K9me2 Metapeaks for indicated samples centered around transcription start sites (TSS) and transcription end sites (TES). B) H3K9me3 Metapeaks (mean read count per million mapped reads) for indicated samples centered around TSS and TES. C) H3K9me3 Metapeaks (Left) and integrated normalized read count around flanking areas (Right) surrounding Myogenin binding sites in 7 day myotubes. Vertical grey bars delineate the MyoG 7 day summit region.

Figure S5



Supplementary Figure 5. IDH2-R172K mutation does not result in globally impaired chromatin accessibility. A) Principal component analysis plots of biological triplicates for the 4 indicated conditions. B) Violin plots of ATAC-seq peak distribution for IDH2-WT and IDH2-R172K samples without MyoD activation; 68377 peaks, estimated median shift of -1.910 (mutant more accessible), p-value for the 2-sample Wilcoxon signed rank test < 2.2e-16. C) Gene-set-enrichment-analysis plots for gene-sets containing MyoD (left) or MyoG (right) binding motifs. Black line indicates position right of which genes are negatively correlated. D) Cumulative distribution function plots of ATAC-seq signal difference between MyoD-inactive and MyoD-active treated samples surrounding CTCF (left) and MyoG day 7 (right) ChIP peaks for IDH2-WT and IDH2-R172K conditions, Kolmogorv-Smirnov test yields a p-value of 0.3309 for CTCF and < 2.2e-16 for MyoD day 7.

Figure S6



Supplementary Figure 6. 10T1/2 cells show ATAC-seq profiles consistent with multipotent mesenchymal progenitors . A) Quantification of largest ATAC-seq peaks in the vicinity of indicated lineage specific genes for adipocytes, chondrocytes and myotubes, respectively. B) Representative ATAC-seq genome tracks before (light color) and after (dark color) MyoD activation for 10T1/2 cells. Scale on left indicates normalized read count range.

Table S1: Reagents

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Myosin 4 Monoclonal Antibody (MF20), eBioscience™	Thermo Scientific	Cat#: 14-6503-80; RRID: AB_2572893
MyoD Antibody (M-318)	Santa Cruz	Cat#: sc-760; RRID:AB_2148870
Purified Mouse Anti-MyoD Clone 5.8A	BD	Cat#: 554130; RRID:AB_395255
Myogenin Antibody (F5D)	Thermo Scientific	Cat#: MA5-11486; RRID: AB_10977211
Anti-Myogenin antibody [EPR4789]	Abcam	Cat#: ab124800; RRID:AB_10971849
Myogenin Antibody (M-225)	Santa Cruz	Cat#: sc-576; RRID:AB_2148908
Normal rabbit IgG	Santa Cruz	Cat#: sc-2027; RRID:AB_737197
Anti-IDH2 Antibody	Abcam	Cat#: ab55271; RRID:AB_943793
Human/Mouse GLP/EHMT1 Antibody	R&D Systems	Cat#: PP-B0422-00; RRID:AB_1964250
Mouse IgG1 Isotype Control	Thermo Scientific	Cat#: MA5-14453; RRID: AB_10943239
Histone H3 (96C10) Mouse mAb	Cell Signaling Technology	Cat#: 3638S; RRID:AB_1642229
METTL3/MT-A70 Antibody, A301-568A	Bethyl Laboratories Inc	Cat#: A301-568A; RRID:AB_1040003
anti-p27Kip antibody	Cell Signaling Technology	Cat#: 2552; RRID:AB_2077837
Mouse monoclonal α-Vinculin	Sigma-Aldrich	Cat. #V9131; RRID: AB_477629
Alexa Fluor® 488 Goat anti-Mouse IgG	Life Technologies	Cat#: A-11001; RRID: AB_2534069
Alexa Fluor® 647 Goat Anti-Mouse IgG	Life Technologies	Cat#: A-21236; RRID: AB_2535805
Histone H3K9me3 antibody (pAb)	Active Motif	Cat#: 39765; RRID:AB_2687870
Di-Methyl-Histone H3 (Lys9) Antibody	Cell Signaling Technology	Cat#: 9753; RRID:AB_659849
Monoclonal Anti-ß-Actin antibody produced in mouse	Sigma	Cat#: A5441; RRID:AB_476744
Anti-Histone H3 (tri methyl K9) antibody	Abcam	Cat#: ab8898 RRID: AB_306848
Anti-Histone H3 (di methyl K9) antibody	Abcam	Cat#: ab1220 RRID: AB_449854
Anti-Mouse IgG, HRP-linked whole Ab (from sheep) Secondary Antibody	GE Healthcare Life Sciences	Cat#: NA931; RRID:AB_772210
Anti-Rabbit IgG, HRP-linked species-specific whole antibody (from donkey) Secondary Antibody	GE Healthcare Life Sciences	Cat#: NA934 RRID:AB_772206
Bacterial and Virus Strains		
NEB® 5-alpha competent cells	NEB®	Cat#: C2987
Stbl3™ competent cells	ThermoFisher	Cat#: C737303

Biological Samples		
N/A		
Chemicals, Peptides, and Recombinant Proteins		
MSTFA + 1% TMCS Silylation Reagent	ThermoFisher	Cat#: TS-48915
UNC0638 hydrate	Sigma-Aldrich	Cat#: U4885
Ezh2 Inhibitor III, GSK126	Millipore	Cat#: 5.00580.0001
5-Azacytidine	Sigma-Aldrich	Cat#: A2385; Cas#: 320-67-2
2'-Deoxycytidine	Sigma-Aldrich	Cat#: D3897; Cas#: 951-77-9
4-Hydroxytamoxifen	Sigma-Aldrich	Cat#: H6278; Cas#: 68392-35-8
GSK2879552	Chemietek	Cat#: CT-GSK287 Cas#: 1401966-69-5, 1401966-63-9
GSK343	Sigma-Aldrich	Cat#: SML0766 Cas#: 1346704-33-3
D-2-hydroxyglutarate	Sigma-Aldrich	Cat#: H8378 Cas#: 103404-90-6
Doxycycline hyclate	Sigma-Aldrich	Cat#: D9891 Cas#: 24390-14-5
Palbociclib	Sigma-Aldrich	Cat#: PZ0199 Cas#: 827022-33-3
Critical Commercial Assays		
VECTASTAIN ABC Kit (Standard*)	VECTOR LABORATORIES	Cat#: PK-4000
DAB Peroxidase (HRP) Substrate Kit	VECTOR LABORATORIES	Cat#: SK-4100
EZ DNA Methylation™ Kit	Zymo Research	Cat#: D5001
TOPO® TA Cloning® Kit for Sequencing	Thermo Scientific	Cat#: K457501
Click-iT® EdU Alexa Fluor® 488 Imaging Kit	Thermo Scientific	Cat#: C10337
Nextera DNA Library Prep Kit (24 samples)	Illumina, INC	Cat#: FC-121-1030
NEBNext® Q5® Hot Start HiFi PCR Master Mix	NEB	Cat#: M0543
Deposited Data		
See Accompanying High-throughput sequencing metadata file		
Experimental Models: Cell Lines		
C2C12	ATCC	Cat#: CRL-1772™; RRID:CVCL_0188
C3H/10T1/2, Clone 8	ATCC	Cat#: CCL-226™; RRID:CVCL_0190
Experimental Models: Organisms/Strains		
N/A		
Oligonucleotides		
See Supplementary Methods Table DNA Oligos		

Recombinant DNA		
pLv-CMV-MyoD-ER	Addgene	Cat#: 26809
pLv-CMV-MyoD	Addgene	Cat#: 26808
pLPC-IDH2-WT	(1)	
pLPC-IDH2-R172K	(1)	
pSIN-TREtight-HA-UbiC-rtTA3-IRES-Hygro	(2)	
pCDH-CMV-mcs-EF1-alpha-Neomycin	Clontech	Cat#: CD514B-1
pLentiCRISPR v2	Addgene (3)	Cat#: 52961
pMSCV PIG (Puro IRES GFP empty vector)	Addgene	Cat#: 21654
Software and Algorithms		
GraphPad Prism 7	GraphPad Software	www.graphpad.com
GuideScan	(4)	http://www.guidescan.com
BISMA	(5)	http://services.ibc.uni-stuttgart.de/BDPC/BISMA/index.php
MethPrimer	(6)	http://www.urogene.org/methprimer/
Other		
Dynabeads® Protein G for Immunoprecipitation	Thermo Scientific	10004D
DMEM culture medium	Media Preparation Facility (MSKCC)	N/A

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Table S2: DNA Oligos

CRISPR Oligos	
Rosa26 F	CACCgGAAGATGGGCGGGAGTCTTC
Rosa26 R	AAACGAAGACTCCCGCCCATCTTCc
sgEHMT1.1 F	CACCgGCTGCAGCTTTATCGGACAC
sgEHMT1.1 R	AAACGTGTCCGATAAAGCTGCAGCc
sgEHMT1.2 F	CACCgAGGTGCCCAGTGGGATATCC
sgEHMT1.2 R	AAACGGATATCCCACTGGGCACCTc
sgMetll3.1 F	CACCgTGGAAAGAGTCGATCAGCAC
sgMetll3.1 R	AAACGTGCTGATCGACTCTTTCCAc
sgMettl3.2 F	CACCgCAGTTGGGCTGCACATTGTG
sgMettl3.2 R	AAACCACAATGTGCAGCCCAACTGc
Bisulfite Sequencing PCR	
MyoD distal CpG F	GGGAGTAGGTAGATAGGTAAGGAGAGT
MyoD distal CpG R	ATAAACCAACCCCAACTAAAAAAAA
MyoG distal CpG F	GAGTATAGTTATAGTTTGGTTTATTTATTT
MyoG distal CpG R	CATACTAATAATAAATTCCTCCCC
Myh1 distal CpG F	GGTAGGTTTTTTTATTGGGTTGTAAT
Myh1 distal CpG R	ATCCCCAACTCTAAATATTTTTACTCAC
Myogenin ChIP qPCR	
Myogenin F	GAATCACATGTAATCCACTGGA
Myogenin R	ACACCAACTGCTGGGTGCCA
Id1 F	AGGGTAGAATTGGGTGCTGTAG
Id1 R	AGGTCGACTACACTGGGAGAGA
Myh3 4 F	CAGTGGCTTTTCCCCCAAA
Myh3 4 R	GATTCACGCCCCATCCTACTT
Mylpf F	CCAAGGGCAGCTGCTAAGTT
Mylpf R	GGCCCAGCCACCTGTTG
Ckm F	CCCGAGATGCCTGGTTATAATT
Ckm R	GCTCAGGCAGCAGGTGTTG
Tnnc2 F	CGGCCCTTGGCCTGAT
Tnnc2 R	CATTGCCCATGCCCAACTA
Pouf1 F	GTCCAGACGTCCCCAACCT
Pouf1 R	AAACTGAGGCGAGCGCTATC
ActB F	CCTCTGTCGTCTTTACGGGG
ActB R	GCAGTTTCGCCTTTGCAGAT
Hprt F	AGCATTAGGGGCAAGCAACT
Hprt R	CTGAGTATGGCCTGGAACCC