Supplemental Materials, Methods and References

Cell culture and differentiation. Myogenic differentiation potential was very similar in cells of the same genotypes derived from two different litters, thus we used WT, *Kdm5a*$^{-/-}$ and DKO MEFs from a single litter throughout the project. MEFs were maintained in DMEM (CellGro) containing 10% FBS (HyClone) growth medium (GM) in 10% CO_2 in a humidified incubator at 37^{o}C. MEFs were seeded on 16-well glass chamber slides (Lab-Tek #12565-110N) pre-coated with poly-D-lysine and fibronectin (Sigma) at 2.2 x 10^4 cells per well or on 12-well dishes pre-coated with fibronectin at 2.5 x 10^5 cells per well. The following day the cells were transduced with Adeno-MyoD (Vector BioLabs) (2.2 x 10^8 PFU/well in 12-well dish) or with concentrated Lenti-CMV-MyoD (Lenti-MyoD herein) and Lenti-CMV-MyoDER[T] (MyoDER[T] herein) (constructs deposited by Jeff Chamberlain, Addgene #26808 and #26809) overnight. For KDM5A/Mfn2 overexpression experiments, DKO MyoDER[T] MEFs were transduced with the pLenti-CMV-Hygro-3xFLAG-KDM5A viruses overnight. The medium was changed on GM and recovery after the virus lasted for 8 h, after which the transduction with the pLenti-CMV-Hygro-3xFLAG-Mfn2 viruses was performed again overnight. The medium was changed on GM again and after the 8-h recovery cells were induced in DM with OHT for upto 24 h, followed by additional induction in DM upto 72 h. C2C12 cells were transduced with lentiviruses overnight. Next morning, cells were washed in PBS twice and placed in GM for 2 h. Then cells were plated on coated chamber slides in DM and stained 48 h later.

As non-differentiated controls, we used empty Adeno (Vector BioLabs) and Lenti vectors, or DM lacking OHT, where appropriate. For experiments with MyoDER[T], the 0 h-time point fully confluent cells were grown in GM and 30 min before the harvest the medium was changed to DM, containing OHT if necessary. All cells were routinely tested for mycoplasma contamination.

To generate lentiviruses, the viral packaging cell line Lenti-X 293T (Clontech) was transfected with psPAX2 and pMD2.G packaging plasmids (Addgene) using Lipofectamine 2000 reagent (Life Technologies) on a p100 plate. Harvesting the virus was performed at 48 and 72 h after transfection and the collected media was pooled. The virus was concentrated using Lenti-X Concentrator (Clontech) and resuspended in 500 µl of serum-free DMEM to increase its concentration twentyfold.

For testing sensitivity of differentiation to mitochondrial inhibitors, cells were transduced with Adeno-MyoD (1 x 10^7 PFU/well) or concentrated Lenti-MyoD. 20 mM sodium azide and 10 nM rotenone treatments were performed after Adeno-MyoD transductions of WT MEFs. In particular, the DM containing adenoviruses was replaced with fresh DM with or without inhibitors. For cells with the BCL2 overexpression, the cells were first transduced with Adeno-BCL2 (1 x 10^7 PFU/well in chamber slide) (Vector BioLabs) 24 h prior to transduction with Adeno-MyoD. The rotenone, metformin and phenformin treatments were performed with Lenti-MyoD. Transduction was performed without the drugs. After the overnight incubation with Lenti-MyoD, cells were washed twice with PBS and increasing concentrations of metformin, phenformin or rotenone in GM were added to the cells. After 24 h, cells were plated in DM with the drugs. After 24 more hours, media was changed to DM without drugs for additional 48 h before the analysis took place.

Multiple compounds have been tested for rescue of differentiation in *Rb1*$^{-/-}$ MEFs. TSA was used at 50 nM and sodium butyrate was used at 5 mM, cells were treated for the first 24 h of induction of differentiation. Mdivi-1 was used at 30 µM, cells were treated in GM with Mdivi-1 for the first 12 h, then followed by DM with Mdivi-1 for the next 12 h. The control was 0.2% DMSO-treated cells. Treatment with inhibitors of glycolysis, dichloroacetate DCA (at 5 mM and 10 mM concentration), 2-deoxyglucose (2DG, at 0.25 mM and 1 mM concentration), 3-BrPA (30 µM and 100 µM), or with peroxisome proliferator-activated receptor agonists bezafibrate (in the

concentration range from 100 μM to 1 mM), GW7647 (0.1 μM and 10 μM), WY-14643 (10 μM for selective activation of PPARα and 200 μM for activation of both PPARα and PPARδ), and CP-775146 (10 nM and 100 nM) was performed in parallel with treatment of cells with 0.1% DMSO that was used as a solvent.

Purification of myotubes. Cells were induced for differentiation for 72 h as above. Then, the medium was removed from the cells and kept aside (conditioned medium). Cells were trypsinized and resuspended in GM. The plate was left to sit undisturbed for 1 minute to allow myotubes sank to the bottom of the tissue culture dish, after which ¾ of the media containing mostly undifferentiated MEFs was carefully removed by a pipette. New GM was added back to the well and the process was repeated two more times to generate a purified population of myotubes. Cells were allowed to recover and reattach to the dish for 3 h in GM, after which the GM was removed and replaced with the conditioned medium. Cells were then allowed to recover for 21 h. Thus, the full differentiation protocol took 96 h. RNA-seq data showed that the purification procedure was highly reproducible as Pearson correlation co-efficient was over 0.98 between the two replicates of WT or the two replicates of DKO purified myotubes (data not shown).

Cell culture of *Rb*-negative cancer cell lines. SAOS-2 cells were cultured in GM in 10% CO_2 at 37^0C. Cells were first transfected with pcDNA3-HA-CBFA1 (Thomas et al. 2001) using ExtremeGeneHD (Roche) overnight. In the morning, medium was changed, 4 h after cells were replated and then transduced (see below) later the same day. Y79 and NCI-H446 cells were cultured in 5% CO_2 at 37^0C, in the RPMI growth medium containing 20% FBS and 10% FBS, respectively. RB-355 cells were cultured in 5% CO_2 at 37^0C, in DMEM/20% FBS. For transduction of SAOS-2,

Y79, RB-355 and NCI-H446 cells, $1.5x10^6$ cells were seeded in a well of on a 6-well plate and 8 h later 100 µl lentivirus and 7.5 µg ml^{-1} polybrene was added for transduction overnight. Next morning cells were washed twice with PBS to remove viral particles and fed with 2 ml of respective growth media. In the evening, $5x10^4$ cells were plated per well in 12-well plate format in duplicates for cell counting at day 2 and 4. Cells were also plated for RNA and protein extraction in EBC lysis buffer on day 7. C33A cells were cultured in growth media in 5% CO_2 at 37^0C. $8x10^5$ cells were plated per well in a 6 well plate and transduced with 50 µl of lentivirus (10x stock) and 7.5 µg ml^{-1} polybrene overnight. RNA was extracted on day 3 and the protein lysates were prepared on day 4. Images of SAOS-2, Y79 and NCI-H446 cells were taken on day 3 after plating and of C33A on day 7 after plating at 20x magnification of the Nikon TMS-F microscope, and images were acquired using Olympus Camedia C-5050 ZOOM digital camera. For all cell lines, growth medium was renewed every second day and cells were split when necessary but not later then 24 h before the analyses. ~90% of cells expressed GFP at 48 h after transduction with lentiviruses.

Immunocytochemistry (ICC), cell cycle analysis, Oil Red O staining and immunoblotting. Cells were fixed for 15 min in 10% formalin followed by three washes with PBS. Then the cells were permeabilized and blocked with a solution of 0.5% Triton X-100 and 3% Bovine Serum Albumin (BSA) in PBS for 30 min at room temperature. The cells were then rinsed once with PBS. Primary antibodies were diluted in a solution of 1% BSA in PBS. The cells were incubated with the antibody solution for 2 h at room temperature followed by three fifteen-minute washes with PBS at room temperature. Secondary antibodies were diluted in a solution of 1% BSA in PBS. The cells were incubated with secondary antibody for 25 minutes followed by three fifteen-

minute washes with PBS. During the second wash, 4',6-diamidino-2-phenylindole (DAPI) was added to stain the nuclei.

ICC images were taken with a Zeiss LSM confocal microscope. Detection of MyHC was performed as described previously (Lin et al. 2011) with MY-32 mouse monoclonal antibody (Sigma) at a 1:300 dilution. MyHC quantitation was performed using a 40x objective. Detection of myogenin (Myog) was performed with anti-Myog mouse antibody (DSHB, Clone F5D) at a 1:50 dilution. Staining with MitoTracker Red CMXRos (Life Technologies) was performed at a 1:1,500 dilution using serum-free DMEM. After 45 min incubation, cells were washed, fixed and permeabilized as for the ICC. For analysis of cell cycle re-entry, cells were removed from DM after 72 h and put into DMEM and 20% FBS for 12 h to promote cell cycle reentry. After 12 h, 5-ethynyl-2'-deoxyuridine (EdU) (Invitrogen #C10340) was added at 10 μM concentration to the culture media and cells were incubated for an additional 12 h. Staining for EdU was done according to the manufacturer's protocol using reagents included in the EdU kit, and EdU-positive cells were counted from three-five microscope fields (20X power) per well. COX IV quantitation was performed using a 63x objective. Rabbit polyclonal antibodies were used for Histone 3 phosphorylated at serine 10, H3pS10 (Millipore, 06-570), at a 1:400 dilution, and COX IV (Abcam ab16056) at a 1:500 dilution. Cy3-labeled anti-mouse (Life Technologies) and Alexa Fluor 488 anti-rabbit (Jackson ImmunoResearch Lab) antibodies were used as the secondary antibodies at a 1:800 dilution and a 1:500 dilution, respectively. EdU was detected with Alexa Fluor 647 antibody. Apoptosis was detected with Cleaved Caspase 3 (Cell Signaling) and CaspaACE FITC-VAD-FMK *in situ* marker (Promega). Caspase quantitation was performed using a 63x objective. Oil Red O staining was performed as described previously (Benevolenskaya et al. 2005) and cell images were acquired using 20x objective of Leica DM IRB microscope and QCapture PRO software.

Protein extraction for immunoblotting was performed in EBC 450 mM lysis buffer (50 mM Tris-HCl, pH 8.0, 450 mM NaCl, 0.5% NP-40 with proteinase inhibitors). Immunoblotting was performed using our rabbit KDM5A anti-serum 2469 (1:5,000 dilution) and the following commercial antibodies: rabbit anti-PGC-1α (Santa Cruz sc-13067, 1:800 dilution), anti-COX IV (Abcam ab16056, 1:2,000 dilution), pACC Ser79 (Cell Signaling 3661), and total histone H3 (Abcam ab-1791), or mouse monoclonal anti-VDAC1/porin (Abcam ab14734, 1:1,000 dilution), anti-FLAG M2 (Sigma F1804), anti-MyHC MY-32 (1:800 dilution), anti-MFN2 (Abcam ab56889, 1:500 dilution), anti-vinculin ascites fluid (Sigma hVIN-1, 1:15,000 dilution) and anti-α-tubulin (Sigma T9026, 1:5,000 dilution). Blots were developed using ECL.

Electron Microscopy. MEFs containing MyoDER[T] lentiviruses were seeded on p100 fibronectin-coated dishes and induced in DM and OHT for 72 h (see above). Cells were fixed for 5 minutes at room temperature in 5 mL of EM fixative (2% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate buffer). Quantification of mitochondrial size was performed using NIH ImageJ 1.42q software. The manual trace tool was used to establish the area of each mitochondrion to be measured. The area was then determined with the measure tool. Measurements were taken for all mitochondria in the image and then averaged.

Metabolic Analysis. 10^4 of WT and DKO MEFs, and 1.5 x 10^4 of *Rb1*$^{-/-}$ and *Kdm5a*$^{-/-}$ MEFs were plated per well of a Seahorse XF^{e}96 cell culture microplate coated with Poly-D-lysine and fibronectin. The cells were transduced the night before with Lenti-MyoD for induced cells or Lenti-empty vector for the 0 h condition, and allowed to recover for 3 h before plating on microplates.

Plating on microplates was performed in GM, and 6 h afterwards the medium was changed on DM or GM, where appropriate. 1 h before the measurements, the medium was replaced on assay medium: for XF Cell Mito stress test, in XF base DMEM pH 7.4 with 2 mM glutamine, 10 mM glucose and 1 mM pyruvate; for XF glycolysis stress test, in XF base DMEM pH 7.4 with 2 mM glutamine; for glucose oxidation, in XF Base DMEM pH 7.4 with 2.5 mM glucose; and for fatty-acid oxidation (FAO), in KHB media (5 mM Hepes pH 7.4, 2.5 mM glucose and 0.5 mM L-carnitine hydrochloride). The cells were placed in 0% CO_2 in a humidified incubator at 37^{o}C. Treatment with metabolic perturbing drugs was performed with 1 μM oligomycin, 0.5 μM carbonylcyanide p-trifluoromethoxyphenylhydrazone (FCCP), and the mix of 0.5 μM antimycin and 50 nM rotenone. Glycolysis stress test was performed with 10 mM glucose, 0.5 μM oligomycin and 100 mM 2DG. Rate of oxygen consumption was measured on a XF^{e}96 Extracellular Flux Analyzer (Seahorse Bioscience). For substrate utilization experiments, 3 measurements (1-3) were taken before the substrate injection, three measurements (4-6) after the injection, followed by the injection of corresponding inhibitor and three more measurements (7-9). The compounds for substrate utilization experiments were used as below: 25 mM glucose, 100 mM 2-DG, 150 μM sodium palmitate conjugated to ultra fatty acid-free BSA, 5 mM sodium butyrate, and 40 μM (+)-etomoxir sodium salt hydrate. For metformin-treated cells, transduced MEFs were pre-treated with 2 mM metformin hydrochloride for 24 h and then induced with or without 2 mM metformin for 18 h. Data normalization was performed to the total protein content, for which cells were lysed in 62 mM Tris-HCl (pH 6.8), 1.5% SDS buffer immediately after the Seahorse experiment.

Chromatin immunoprecipitation (ChIP). *Rb1*$^{-/-}$ cells containing MyoDER[T] lentiviruses were plated on 6-well plates and transduced with corresponding lentiviruses. For induction of

differentiation, the cells were then plated from one well in a 6-well plate on a p100 plate. Cells were allowed to reach confluent state, and at the indicated times before the harvesting, media was changed to DM, supplemented with OHT. For 0 h time point, media was replaced with GM and 30 min before the harvesting, was changed to DM, supplemented with OHT. ChIP was performed as previously described (Beshiri et al. 2010). Cells were fixed in 1% formaldehyde for 10 min and sonicated 12 cycles at 60% amplitude (Branson sonicator). A mixture of monoclonal M2 F1804 and polyclonal F7425 from Sigma were used for FLAG ChIP, a mixture of the affinity purified antibody 1416 and anti-serum 2469 (4 µl and 0.5 µl, respectively) was used for Kdm5a ChIP; also we used rabbit anti-MyoD (Santa Cruz, sc-760), anti-PGC-1α (Santa Cruz, sc-13067) and anti-H3K4me3 (Millipore, 17-614) antibodies. Real-time PCR was performed using the SYBR Green PCR master mix and iCycler CFX96 system (Bio-Rad).

RT-qPCR. For induced MEFs or C2C12 cells, RNA was extracted using Trizol. MEFs transduced with Adeno-MyoD were differentiated in 24-well plates. C2C12 cells were seeded 8 x 10^6 cells per p100 plate and were induced to differentiate for 24, 48 and 96 h. For human cell lines, RNA was extracted using Zyppy RNA Mini kit. Absolute quantification was done using standard curves generated from known copy numbers of three different *Drosophila* RNA transcripts, converted to cDNA (LD22368, LD05461 and dE2F2). Five dilutions covering 3 orders of magnitude of each control cDNA were made and qPCR was performed in triplicate. The threshold cycle C[t] values from each cDNA control were averaged and used to generate the standard curve. Control RNAs were spiked into the unknown sample RNA and processed into cDNA with the sample RNA. Copy numbers were determined with the following calculation: copy # =10^((C[t]-36.056)/-3.3806). The value 36.056 is the y intercept of the standard curve and -3.3806 is the slope of the standard curve. C[t] values were derived for each gene of interest by qPCR. Absolute copy numbers were

represented as relative fold difference compared to the WT 0 h condition. For reverse transcriptase reaction 0.9 µg RNA was used along with $1.5x10^6$ RNA molecules of each spikes in 10 µl total reaction using Superscript Vilo Kit (Life Technologies). Real-time PCR was performed as for ChIP assays.

Constructs. The lentiviral constructs were used for protein overexpression and were designed as follows. The cDNA sequence was first subcloned into pENTR4-3xFLAG entry vector. The pENTR4-3xFLAG vector was generated from the pENTR4-FLAG vector (Addgene cat#17423) by replacement of the FLAG sequence on three repeats of FLAG sequence. Second, the entry vector with the insert was recombined with pLenti-CMV-Hygro-DEST destination vector (Addgene cat#17454) using Gateway LR II Clonase Enzyme Mix (Life Technologies). The pLenti-CMV-Hygro-3xFLAG was generated using the same technique and was used as an empty control in all experiments involving this backbone plasmid. The RB1 sequence was first PCR-amplified from pSG5L-HA-RB plasmid(Sellers et al. 1998) using forward 5'-CTTGCGGCCGCAGGGTACCCATACGATGTTCCAGATTAC – 3' and reverse 5' – CTTCTCGAGTCATTTCTCTTCCTTGTTTGAGGTATC – 3' primers. The PCR product was then digested with Not I and Xho I and cloned into pENTR4-3xFLAG, and then recombined to generate pLenti-CMV-Hygro-3xFLAG-HA-RB (pLenti-FLAG-RB). KDM5A sequences were generated by PCR from pcDNA3-HA-RBP2 and pcDNA3-HA-RBP2mut plasmids, respectively (Lopez-Bigas et al. 2008) using forward 5' – CTTGCGGCCGCAGCGGGCGTGGGGCCGGGGGGCTAC – 3' and reverse 5' – ATCTAGACCTAACTGGTCTCTTTAAGATCCTCCATTG – 3' primers. The frame shift version of KDM5A contained the KDM5A cDNA with a stop codon preventing KDM5A protein expression. The PCR reactions were performed using Elongase Enzyme Mix (Life

Technologies). The PCR product was then digested with Not I and Xba I and cloned into pENTR4-3xFLAG vector, and then recombined to generate pLenti-CMV-Hygro-3xFLAG-KDM5A or pLenti-PGK-FLAG-CMV-3xFLAG-Hygro-KDM5Amut. The KDM5A variant with point mutations at H483 and E485 in the iron-binding site of the JmjC domain was used as a catalytic domain mutant, KDM5mut. The occurrence of other amino acids at these positions is common in JmjC domain proteins that specifically lack demethylase activity, however, they still able to interact with the chromatin and to regulate transcription (Klose et al. 2006). Mfn2 was cloned from cDNA generated from WT MEFs using forward 5' - CTTGCGGCCGCATCCCTGCTCTTTTCTCGATGCAACTCC - 3' and reverse 5' - ATCTAGACTATCTGCTGGGCTGCAGGTACTGGTGTG - 3' primers. The PCR product was then digested with Not I and Xba I and recombined to generate pLenti-CMV-Hygro-3xFLAG-Mfn2. Pgc-1α was cloned from cDNA generated from WT MEFs using forward 5' - CTT GTCGACGCTTGGGACATGTGCAGCCAAGACTCTG - 3' and reverse 5' - CTTCTCGAGTTACCTGCGCAAGCTTCTCTGAGCTTCCTTC - 3' primers. The PCR product was then digested with Sal I and Xho I and recombined to generate pLenti-CMV-Hygro-3xFLAG-Pgc-1α. The following vectors were available for production of lentiviruses: pLVTHM-shKDM5A-GFP or the control pLVTHM-GFP (Beshiri et al. 2012), mouse TRC shRB (6 different clones) (GE Dharmacon RMM4534-EG19645) and GIPZ *Mfn2* shRNA (GE Healthcare, V3LMM_417856). The corresponding mouse *Kdm5a* shRNA construct, pLVTHM-shKdm5amouse-GFP, targeting the sequence 5' - GCTGTAAGAGAATATACAC - 3', was created by site-directed mutagenesis.

RNA-seq library preparation. The obtained RNA sample was first fragmented for 5 minutes at 94^{o}C using 10X fragmentation buffer from the NEBNext mRNA Sample Prep Master Mix Set 1 (NEB cat# E6110S). First strand cDNA synthesis was performed with random primers (NEB) and

Superscript II reverse transcriptase (Invitrogen). Second strand synthesis was done with 10X Second Strand Synthesis Reaction Buffer (NEB) and Second Strand Synthesis Enzyme Mix (NEB). Double stranded cDNA was purified with 1.8 volumes of AMPure XP Beads (Beckman Coulter) followed by end repair using NEBNext End Repair Enzyme Mix and Reaction Buffer (NEB). End-repaired DNA was cleaned up with 1.8 volumes of AMPure XP Beads. For mRNA, dA-Tailing was performed using 10X NEBNext dA-Tailing Reaction Buffer and Klenow Fragment (3'>>>5' exo$^-$). dA-Tailed DNA was cleaned up with 1.6 volumes of AMPure XP Beads followed by adapter ligation using PE adapters designed for Illumina sequencing. Adapter-ligated DNA was cleaned up and size selected for a range of 250-300 bp using 1 volume of AMPure XP Beads. cDNA was enriched by 15 cycles of PCR using Phusion DNA Polymerase (NEB) and PE primers designed for Illumina sequencing. Enriched DNA was cleaned up with 1 volume AMPure XP Beads. For mRNA, single-read sequencing for 36 bases was done on an Illumina Genome Analyzer II. For RiboMinus RNA, end-repaired DNA was dissolved in 17.5 µl of Tris buffer 10 mM, pH 8, and was integrated into the standard TruSeq Illuminal protocol at the step of "Adenylate 3' ends". Single-read sequencing for 40 bases was done on an Illumina HiSeq Analyzer.

Read quality assessment and filtering. Basic assessment of Illumina output reads (FastQ) quality including GC bias were checked by FastQC program (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/). Poor quality reads were eliminated before mapping based on default quality flag by Illumina pipeline in FastQ file.

Mapping of reads. Illumina sequenced RNA-seq data was mapped against reference genome/transcriptome (UCSC, mm9) using TopHat (version 1.2.0) (Trapnell et al. 2009) with

Ensembl 59 (Cunningham et al. 2014) gene/transcript model. Specifically, identified short reads were uniquely aligned allowing at best two mismatches to the reference genome (mm9). Sequence that matched more than one place with equally quality were discarded to avoid bias. The reads that were not mapped to the genome were utilized to map against the transcriptome (junctions mapping). Any residual mapping bias was checked using RSEQtools (Habegger et al. 2011). Consistency between two replicate data were checked by Pearson correlation co-efficient (PCC) by counting reads in 500-bp bins.

Differential expression (DE) analysis. BedTools (version 2.10) (Quinlan and Hall 2010) were applied to calculate read abundance (read count, rc) for each transcript associated with an Ensembl 59 gene. Since we used random hexamers in cDNA library generation, which could has introduced bias in the nucleotide composition at the beginning of the reads, we utilized read count reweighting scheme (Hansen et al. 2010) to calculate weighted counts (wc) using Genominator (version 1.2.4). For DE analysis, we applied R/Bioconductor (Gentleman et al. 2004) package DESeq (version 1.2.1) (Anders et al. 2013) that uses a model based on the negative binomial distribution. We normalized the data based on total mapped reads in combined analyzed samples, and using inherited functions, we checked fitting of the model density of residual variance ratios and also produced MA plot for overall visualization of differential expression pattern. Independently, DE analysis was also done using DEGseq MARS algorithm (Wang et al. 2010) (data not shown). Both DESeq and DEGseq methods resulted in a very high overlap of DE genes (86.4% DE genes identified by DESeq between DKO-myo and WT-myo were also identified as DE by DEGseq). Thus, the results from DESeq algorithm were used for further analysis.

Input gene lists for analyses. To allow comparison across all sequenced samples, the baseMean values (wcn) were obtained using normalization to the total reads in the matrix of count-data. For the purposes of this study, we call the baseMean values "counts" hereafter. The list of mapped genes (the total of 36,229 genes) was next filtered as follows: 1) genes on the X and Y chromosomes as well as genes encoded by the mitochondrial genome were removed (to the total of 33,602 genes), 2) noncoding RNAs were removed (to the total of 21,517 genes), and 3) genes with fewer than 20 counts were removed (to the total of 11,241 genes). The lists of differentially expressed genes were generated with the following thresholds:

"Increased in *Rb1*$^{-/-}$" with Log2 > 0.76 compared to the WT-myo. The adjusted *P* value for differential expression was set at ≤ 0.05.

"Decreased in *Rb1*$^{-/-}$" with Log2 < -0.76 compared to the WT-myo. The adjusted *P* value for differential expression was set at ≤ 0.05.

"In DKO-myo rescued all" - increased in DKO-myo with Log2 > 0.76 compared to the condition "Decreased in *Rb1*$^{-/-}$", i.e. these are "rescued" genes. The adjusted *P* value for differential expression was set at ≤ 0.05.

"In DKO-myo rescued Kdm5a targets" - increased in DKO-myo with Log2 > 0.38 compared to the genes decreased in *Rb1*$^{-/-}$. The adjusted *P* value for differential expression was set at ≤ 0.05.

"DE" (differentially expressed) - Kdm5a target genes (Kdm5a peak ≤1 kb from the TSS) changed in DKO-myo with Log2 > 1 or Log2 < -1, compared to the WT-myo. The adjusted *P* value for differential expression was set at ≤ 0.001.

"Non-DE" (non differentially expressed) - Kdm5a target genes (Kdm5a peak ≤1 kb from the TSS) with adjusted *P* value for differential expression ≥ 0.001 in DKO-myo compared to the WT-myo.

"Increase in H3K4me3" – Kdm5a target genes (Kdm5a peak ≤1 kb from the TSS) with increased H3K4me3 (KO vs. f/f) ≤1 kb from the TSS; increase in H3K4me3 was determined by SICER with FDR cut off 0.01.

Overlap analysis. Overlap of genomic position range data was done using BedTools (Quinlan and Hall 2010) and significance of overlap was analyzed by Hypergeometric test. However, venn-diagram generation and overlap of target genes analysis was performed using in-house R programming language script. Significance of overlap calculated based on χ^2 test. Peak overlap was defined as overlap by at least one bp.

Generation of H3K4me3 ChIP-seq data. Two separate *Kdm5a*$^{f/f}$ clones and two *Kdm5a*$^{-/-}$ clones were analyzed. In particular, samples were prepared cells of the two *Kdm5a*$^{f/f}$ clones or two *Kdm5a*$^{-/-}$ clones mixed in a 1:1 ratio. Reference DNA was the total genomic DNA sample. After adapter ligation, DNA was PCR-amplified for 18 cycles with Illumina primers and library fragments of ~320 bp were isolated from an agarose gel. The purified DNA was captured on an Illumina flow cell for cluster generation. Libraries were sequenced on the Genome Analyzer following the manufacturer's protocols.

Peak caller program SICER (version 1.1) (Zang et al. 2009) was used with the following parameters: redundancy threshold=1, window size=200, fragment size=150, effective genome fraction=0.75, gap size=200, and FDR=0.01. 33,824 and 27,797 significant islands of H4K4me3 were detected in *Kdm5a*$^{f/f}$ and *Kdm5a*$^{-/-}$, respectively. Differential enrichment of H3K4me3 in *Kdm5a*$^{-/-}$ versus *Kdm5a*$^{f/f}$ was determined using SICER program script "SICER-df.sh" with similar parameter settings.

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