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Supplemental Information

CHD4 ensures stem cell lineage fidelity during skeletal muscle regeneration

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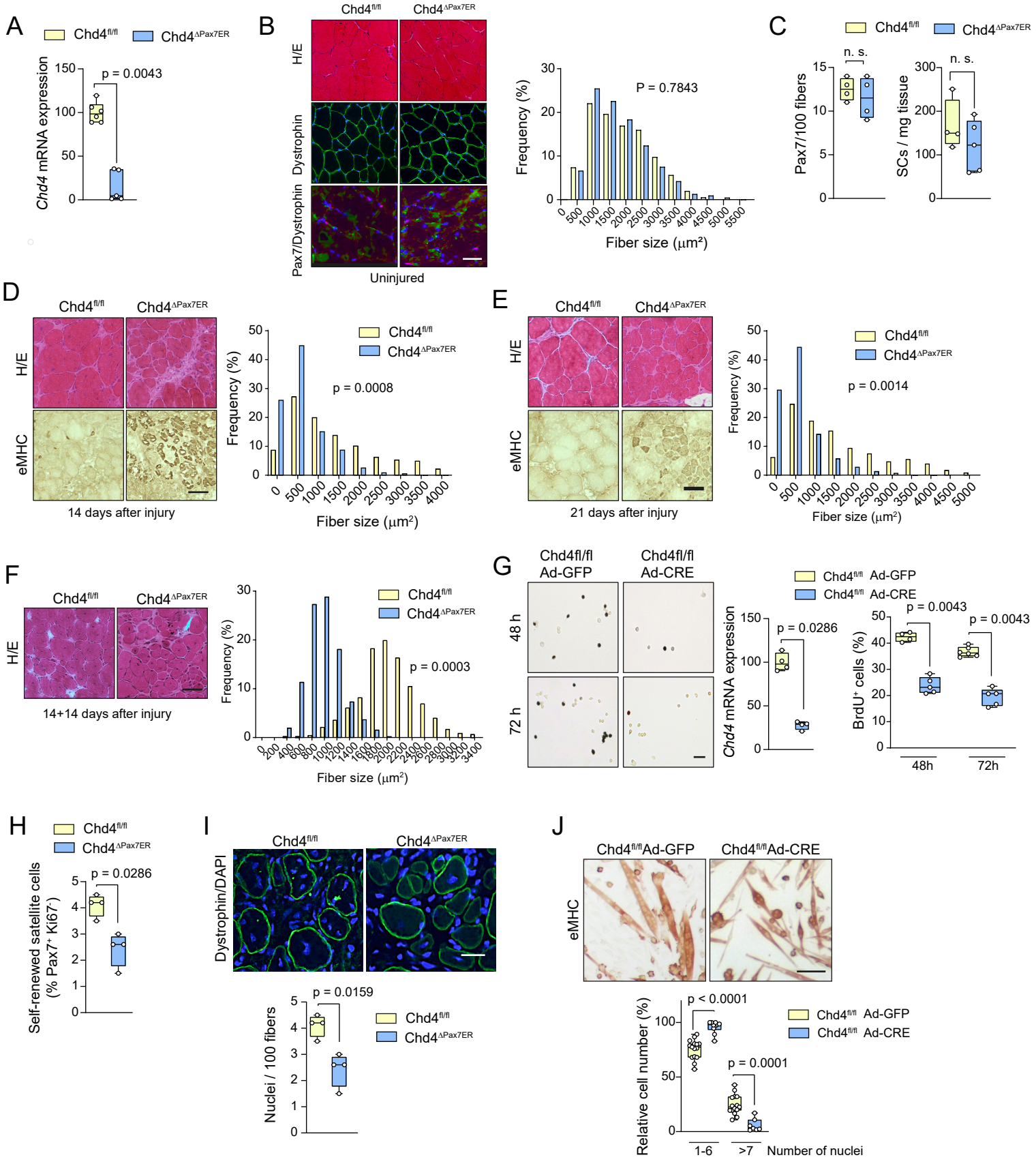
A
Chd4fl/fl
Chd4ΔPax7ER
Chd4 mRNA expression
p = 0.0043
B
H/E
Dystrophin
Pax7/Dystrophin
Uninjured
P = 0.7843
Frequency (%)
Fiber size (μm²)
C
n. s.
Pax7/100 fibers
SCs / mg tissue
D
14 days after injury
p = 0.0008
E
21 days after injury
p = 0.0014
F
14+14 days after injury
p = 0.0003
G
Chd4fl/fl Ad-GFP
Chd4fl/fl Ad-CRE
48 h
72 h
p = 0.0286
p = 0.0043
BrdU+ cells (%)
H
Self-renewed satellite cells (% Pax7+ Ki67-)
I
Dystrophin/DAPI
p = 0.0159
Nuclei / 100 fibers
J
eMHC
p < 0.0001
p = 0.0001
Relative cell number (%)
1-6
>7
Number of nuclei

Figure S1

Figure S1, Related to Figure 1. (A) *Chd4* mRNA expression in FACS-isolated satellite cells from *Chd4* $^{fl/fl}$ or *Chd4* $^{\Delta Pax7ER}$ mice at 30 days after tamoxifen treatment. Data were normalized to the housekeeping gene *Rpl7* (n = 6 or 4 mice, respectively). **(B)** Representative pictures and fiber size distribution of uninjured muscles from tamoxifen-treated *Chd4* $^{fl/fl}$ or *Chd4* $^{\Delta Pax7ER}$ mice (n = 4 mice/group). Scale bar, 50 μm. **(C)** Satellite cell numbers in muscles of *Chd4* $^{fl/fl}$ and *Chd4* $^{\Delta Pax7ER}$ mice at 14 days (left, n = 4 mice/group) and 90 days (right, n = 4 or 5 mice, respectively) after tamoxifen treatment. **(D)** Representative pictures and fiber size distribution of muscles from tamoxifen-treated *Chd4* $^{fl/fl}$ or *Chd4* $^{\Delta Pax7ER}$ mice at 14 days post-injury (n = 3 mice/ group). Scale bar, 50 μm. **(E)** Representative pictures and fiber size distribution of muscles from tamoxifen-treated *Chd4* $^{fl/fl}$and *Chd4* $^{\Delta Pax7ER}$ mice at 21 days post-injury (n = 3 mice/group). Scale bar, 50 μm. **(F)** Representative pictures and fiber size distribution of muscles from tamoxifen-treated *Chd4* $^{fl/fl}$ or *Chd4* $^{\Delta Pax7ER}$ mice at 14 days post-injury (n = 5 or 4 mice, respectively). Scale bar, 50 μm. **(G)** *Chd4* mRNA relative expression and proliferation rate in satellite cells from *Chd4* $^{fl/fl}$ satellite cells transduced with Ad-CRE or Ad-GFP (control) and examined after 48 h (n = 4 or 5 independent experiments, respectively) or 72 h (n = 6 or 5 independent experiments, respectively). Representative pictures of BrdU-stained cells are shown. Scale bar, 50 μm. **(H)** Proportion of quiescent ($Pax7^{+}/Ki67^{-}$) satellite cells in muscles from tamoxifen-treated *Chd4* $^{fl/fl}$ or *Chd4* $^{\Delta Pax7ER}$ mice at 21 dayspost-injury (n = 4 mice/group).
(I) Representative pictures of nuclei and dystrophin staining of muscles from tamoxifen-treated *Chd4* $^{fl/fl}$ or *Chd4* $^{\Delta Pax7ER}$ mice at 7 days post-injury. Quantification of nuclei number inside the myofibers is shown (n = 4 or 5 mice, respectively). Scale bar, 50 μm. **(J)** Representative pictures of cultured myotubes derived from satellite cells of *Chd4* $^{fl/fl}$ mice transduced with Ad-CRE or Ad-GFP (control) and cultured in DM for 48 h. The proportion of myotubes containing the indicated nuclei number is shown. Data were from two independent experiments. Scale bar, 50 μ m.

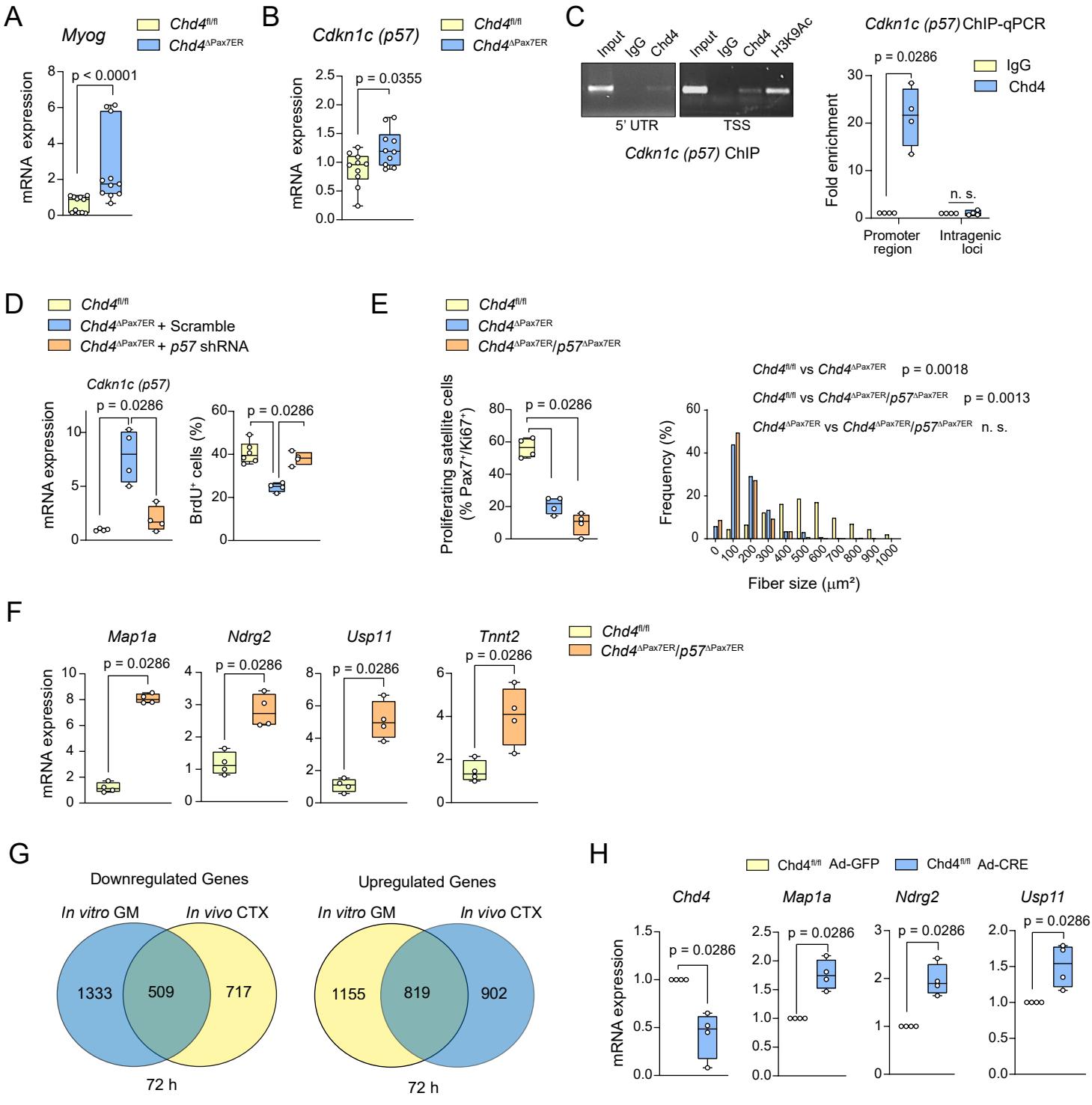
A
Myog
Chd4fl/fl
Chd4ΔPax7ER
mRNA expression
p < 0.0001
B
Cdkn1c (p57)
p = 0.0355
C
Input
IgG
Chd4
H3K9Ac
5' UTR
TSS
Cdkn1c (p57) ChIP
Cdkn1c (p57) ChIP-qPCR
p = 0.0286
n. s.
Fold enrichment
Promoter region
Intragenic loci
D
Chd4ΔPax7ER + Scramble
Chd4ΔPax7ER + p57 shRNA
BrdU+ cells (%)
E
Chd4ΔPax7ER/p57ΔPax7ER
Proliferating satellite cells (% Pax7+/Ki67+)
Chd4fl/fl vs Chd4ΔPax7ER p = 0.0018
Chd4fl/fl vs Chd4ΔPax7ER/p57ΔPax7ER p = 0.0013
Chd4ΔPax7ER vs Chd4ΔPax7ER/p57ΔPax7ER n. s.
Frequency (%)
Fiber size (μm²)
F
Map1a
Ndrg2
Usp11
Tnnt2
G
Downregulated Genes
Upregulated Genes
In vitro GM
In vivo CTX
1333
509
717
1155
819
902
72 h
H
Chd4fl/fl Ad-GFP
Chd4fl/fl Ad-CRE
Chd4
Map1a
Ndrg2
Usp11

Figure S2

Figure S2, Related to Figure 2. (A, B) mRNA expression in FACS-purified satellite cells at 3 days after muscle injury from tamoxifen-treatment of *Chd4* $^{fl/fl}$ or *Chd4*$^{\Delta Pax7ER}$ mice. Data were normalized to the housekeeping gene *Rpl7*, with the *Chd4* $^{fl/fl}$ set to 1 **(**n = 11 mice/group for *Myog*, and n= 10 mice/group for *Cdkn1c*). **(C)** ChIP-PCR analysis of the *Cdkn1c (p57)* promoter region showing binding of the Chd4 protein. Qualitative PCR gel pictures (left) and qPCR analysis (right) are shown. n = 3 samples/group from two biological replicates. **(D)** shRNA-mediated knockdown of *p57* (left) with a lentiviral vector (shRNA p57) in *Chd4*$^{\Delta Pax7ER}$ satellite cells (n= 3 independent experiments); percentage of proliferating satellite cells at 72 h after *p57* silencing as compared to control sRNA-scramble cells, is shown (right) (n = 6, 3, and 3 independent experiments, respectively). **(E)** Quantification of proliferating cells and frequency distribution of cross-sectional area (μm^2) of regenerating fibres from tamoxifen-treated *Chd4* $^{fl/fl}$, *Chd4*$^{\Delta Pax7ER}$ and *Chd4*$^{\Delta Pax7ER}$*/p57*$^{\Delta Pax7ER}$ double-mutant mice at 3 or 7 days post-injury (n= 3 mice/group). **(F)** Relative mRNA expression of the indicated genes in SCs from *Chd4*$^{fl/f}$ or *Chd4*$^{\Delta Pax7ER}$*/p57*$^{\Delta Pax7ER}$ mice at 3 days post-injury (n = 4 mice/group). Data were normalized to the housekeeping gene *Rpl7* with the *Chd4*$^{fl/f}$ set to 1. **(G)** Venn diagram showing the common significantly ($p < 0.01$) upregulated or downregulated genes in the RNA-seq of Ad-CRE or Ad-GFP-treated *Chd4* $^{fl/fl}$ SCs (n = 4 independent experiments), and the RNA-seq of SCs obtained from tamoxifen-treated *Chd4* $^{fl/fl}$ or *Chd4*$^{\Delta Pax7ER}$ mice isolated at 3 days post-injury of muscle (n= 4 mice/group). Data analyzed using DESeq2. **(H)** Relative mRNA expression of the indicated genes in *Chd4*$^{fl/fl}$ SCs transduced with Ad-CRE or Ad-GFP for 48 h. Data were normalized to housekeeping gene *Rpl7* with the Ad-GFP set to 1 (n=4 independent experiments).

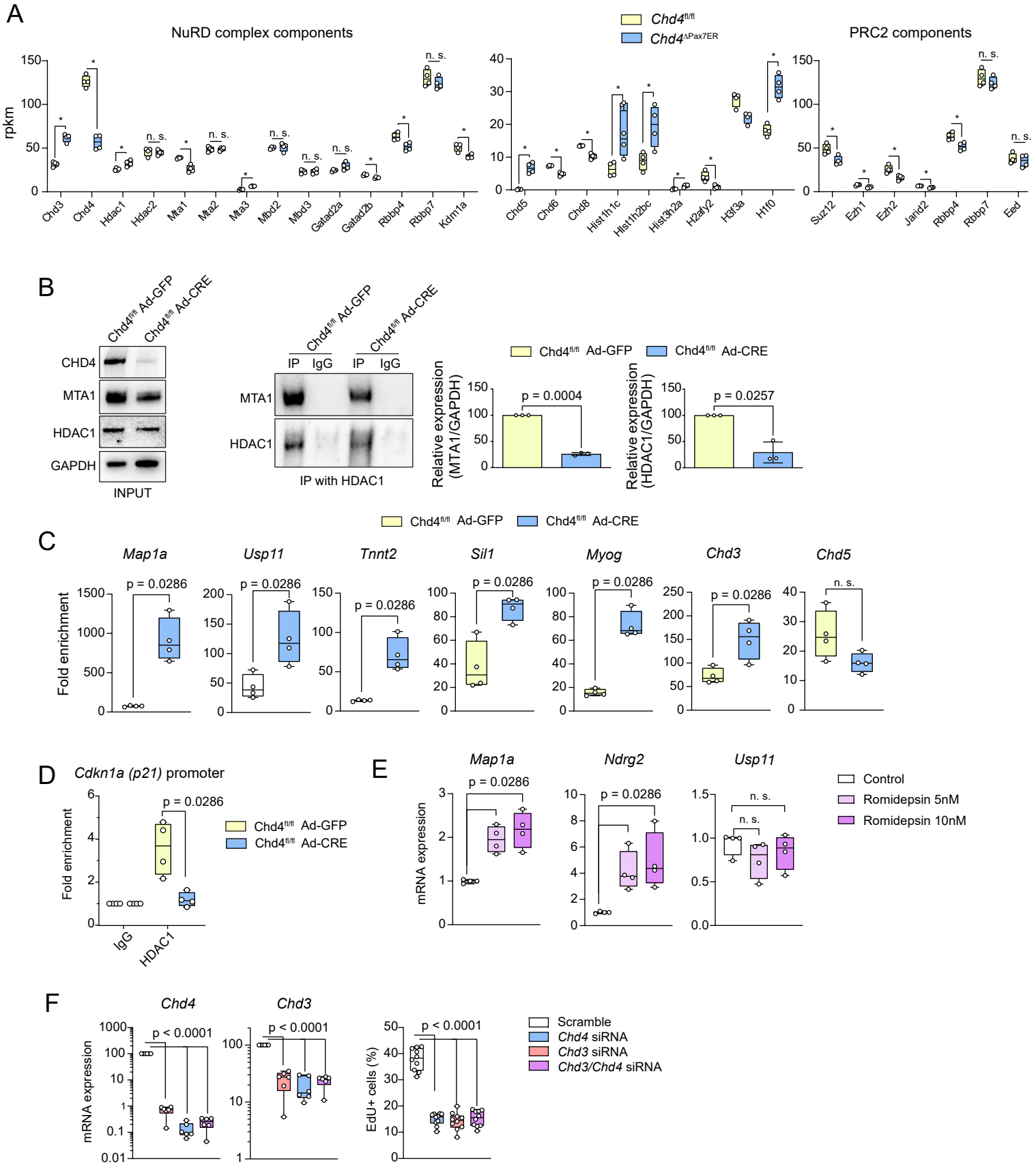
A
NuRD complex components
PRC2 components
Chd4fl/fl
Chd4ΔPax7ER
rpkm
Chd3
Chd4
Hdac1
Hdac2
Mta1
Mta2
Mta3
Mbd2
Mbd3
Gatad2a
Gatad2b
Rbbp4
Rbbp7
Kdm1a
Chd5
Chd6
Chd8
Hist1h1c
Hist1h2bc
Hist3h2a
H2afy2
H3f3a
H1f0
Suz12
Ezh1
Ezh2
Jarid2
Rbbp4
Rbbp7
Eed
n. s.
B
Chd4fl/fl Ad-GFP
Chd4fl/fl Ad-CRE
CHD4
MTA1
HDAC1
GAPDH
INPUT
IP
IgG
IP with HDAC1
Relative expression (MTA1/GAPDH)
Relative expression (HDAC1/GAPDH)
p = 0.0004
p = 0.0257
C
Map1a
Usp11
Tnnt2
Sil1
Myog
Chd3
Chd5
Fold enrichment
p = 0.0286
n. s.
D
Cdkn1a (p21) promoter
IgG
HDAC1
E
Ndrg2
mRNA expression
Control
Romidepsin 5nM
Romidepsin 10nM
F
Chd4
Chd3
p < 0.0001
mRNA expression
EdU+ cells (%)
Scramble
Chd4 siRNA
Chd3 siRNA
Chd3/Chd4 siRNA

Figure S3

Figure S3, Related to Figure 3. (A) Reads per kilobase per million mapped reads (RPKM) of the indicated NuRD complex components, members of the chromodomain helicase DNA-binding (CHD) family, histone variants, and components of the PRC2 complex obtained in the RNA-seq of SCs from tamoxifen-treated *Chd4* $^{fl/fl}$ or *Chd4*$^{\Delta Pax7ER}$ mice, isolated three days post-injury to muscle (n = 4 mice/group). Data were analyzed using DESeq2. (**B**) Western blots of co-immunoprecipitation (IP) experiments showing interactions between MTA1 and HDAC1 in *Chd4*$^{fl/fl}$ SCs transduced with Ad-CRE or Ad-GFP for 48 h. Left, input blots, showing also CHD4 and GAPDH as a control; right, blots of MTA1 and HDAC1 from IP, performed using HDAC1 and IgG antibodies. Quantification of three different experiments is shown. (**C**) ChIP-qPCR of H4 pan-acetyl protein from *Chd4*$^{fl/fl}$ SCs transduced with Ad-CRE or Ad-GFP for 48 h at the indicated genes loci. Data were normalized to IgG (set to 1) (n = 4 independent experiments). (**D**) ChIP-qPCR of HDAC1 protein from *Chd4*$^{fl/fl}$ SCs transduced with Ad-CRE or Ad-GFP for 48 h at the *p21* loci. Data were normalized to IgG (set to 1) (n = 4 independent experiments). (**E**) Effects of HDAC1 inhibition with romidepsin (5 or 10 nM concentrations) in WT SCs on the expression of the indicated genes. Data were normalized to the housekeeping gene *Rpl7*, with the control cells set to 1 (n = 4 independent experiments). (**F**) siRNA-mediated knockdown of *Chd4*, *Chd3*, or both genes using siRNA pools in WT SCs (n = 6 independent experiments). Left, efficiency of the knockdown on *Chd4* and *Chd*3 gene expression; right, percentage of proliferating SCs at 72 h after silencing as compared to control cells (treated with siRNA-scramble).

Supplemental Experimental Procedures

Mice

For mouse experiments, no specific blinding method was used but mice in each sample group were selected randomly.

Isolation of satellite cells by fluorescence-activated cell sorting (FACS)

Dissection of fore and hind limb muscles was done with a scalpel and retrieved in cold DMEM (Dulbecco's Modified Eagle Medium, Gibco). Muscle tissues were finely minced and digested with Liberase 5 mg/mL (Roche/Sigma Aldrich), 0.03% Dispase II (Sigma), DMEM, 1% penicillin/streptomycin, BSA 0.2% (Sigma), 1 M $CaCl_2$, and 1 M $MgCl_2$ at 37ºC. Samples were then centrifuged 10 min at 50*g* at 4ºC. Supernatant was filtered through 100-µm and then a 70-µm cell strainers filters and then centrifuged at 1700 rpm for 15 min. Pellets were then resuspended in Lysis Buffer 1× (BD) and incubated for 10 min. Samples were resuspended in cold DMEM, filtered through 40-µm cell strainer filters, and centrifuged again for 15 min at 1700 rpm. Cells were resuspended in PBS, and 2.5% of goat serum (FACS buffer). Samples were incubated 30 min at 4ºC with the following antibodies: anti-CD45 PE-Cy7 (Biolegend), anti-Sca1 PE-Cy7 (Biolegend), anti-α7-integrin PE (Ablab.ca), and anti-CD34 Alexa-647 (BDPharmingen). Samples were centrifuged at 1700 rpm for 15 min and finally resuspended in FACS buffer with 1 µg/mL DAPI. After antibody labelling, $SCA1^{-}/CD45^{-}/\alpha7\text{-integrin}^{+}/CD34^{+}$ cells were sorted by fluorescence-activated cell sorting (FACS) in the BD Influx cell sorter, and then cultured as described.

Satellite cell culture

Freshly isolated satellite cells from *Pax7*$^{CreERT2/+}$*/Chd4*$^{fl/fl}$ or *Chd4*$^{fl/fl}$ mice were cultured on collagen-coated dishes with HAM's F10 medium (Gibco), 20% Fetal Bovine Serum (FBS), 1:10000 Fibroblast Growth Factor (FGF) and 1% penicillin/streptomycin and kept in an incubator (37ºC, 5% CO_2). *In vitro* genetic ablation of *Chd4* was induced by Adenovirus-Cre 1:2000 transduction during 48 h. Adenovirus-GFP 1:2000 transduction was used as control.

Cell and tissue immunohistochemistry

Tissue samples from tibialis anterior muscles were embedded in optimal cutting temperature (OCT) and frozen in liquid nitrogen-cooled isopentane. Sections (10-µm-thick) were collected and either stained with hematoxylin and eosin (H&E) or immunostained. Freshly isolated or cultured satellite cells were fixed with 4% formaldehyde. Samples were rinsed with PBS, permeabilized with 0.5% Triton X-100 in PBS for 10 min at room temperature, rinsed again with PBS, and then incubated in blocking solution (10% goat serum, 5% BSA) before applying the indicated primary antibodies. For Pax7, Ki67, dystrophin and myogenin immunostainings, samples were incubated for 20 min in 4% PFA, washed with PBS and incubated for 6 min in cold methanol. Sections were then washed with PBS, followed by an antigen retrieval procedure (incubation twice for 5 min at 100 ºC with citric acid 0.1 M pH 6). Afterwards, slides were washed with PBS again and incubated for 2 h in blocking solution (5% BSA IgG-Free with 10% goat serum), followed by a wash with PBS and incubation during 30 min with M.O.M blocking solution (Vector Laboratories). Samples incubated overnight at 4ºC with a primary antibody against Pax7 (sc-81648 Santa Cruz, diluted 1/50 in blocking solution), Ki67 (ab15580, Abcam, diluted 1/100 in blocking solution), dystrophin (Sigma D8168, 1/400), or MyoG (sc-576, Santa Cruz, diluted 1/50). After PBS washing, samples were incubated with the appropriated secondary antibody (Rhodamine Red-X (RRX) AffiniPure Goat Anti-Mouse IgG1, no. 115-295-205; Alexa Fluor 488 AffiniPure Goat AntiRabbit IgG, no. 111-545-144; both from Jackson ImmunoResearch; both were diluted 1/400 in PBS and 1% BSA for use) and rinsed with PBS; nuclei were stained with DAPI (1 µg/mL in PBS) before mounting with Fluoromount (Sigma). For embryonic myosin heavy chain (eMHC) immunostaining, samples were incubated for 30 min with 0.3% H_2O_2, washed with PBS, and incubated 1 h with M.O.M blocking solution (Vector Laboratories). Samples were then incubated with primary anti-eMHC antibody (F1652,

Developmental Studies Hybridoma Bank, undiluted), followed by three PBS washes and incubation with secondary antibody (M.O.M. biotinylated anti-mouse IgG, Vector Laboratories, 1/250 in PBS). Samples were washed with PBS and then incubated with M.O.M ABC solution for 5 min. Slides were then briefly incubated with 3,3'-diaminobenzidine (DAB), and the reaction was stopped by adding H_2O. Slides were dehydrated with ethanol, cleared in xylol, and mounted in di(n-butyl)phthalate in xylene solution (DPX, Sigma-Aldrich). Images were taken using the Zeiss Cell Observer HS widefield microscope with a Zen Blue software, and the image analysis was performed using Fiji image analysis software.

BrdU and EdU incorporation assays

Cultured satellite cells were incubated with BrdU (1.5 µg/mL; Sigma) for 1 h and fixed with formaldehyde 3.7%. Samples were then washed twice with PBS and incubated during 45 min with 1 M HCl at 45ºC, and washed and further incubated for 10 min with 0.1 M borate buffer (7.8 g sodium tetraborate, 4.96 g boric acid, 4L H_2O) at room temperature. Blocking solution (5% donkey serum, 0.5% BSA, 0.25% Triton X-100) was added for 1 h at room temperature. The primary antibody rat anti-BrdU (Ab6326 Abcam, diluted 1/500 in blocking solution) was added and incubated overnight at 4ºC. Samples were then rinsed 3 times with PBS-Tween 0.025% and then incubated with the secondary antibody biotin-Donkey anti-rat (712-066-150 Jackson ImmunoResearch, diluted 1/250 in PBS-Tween 0.025%) for 1 h at room temperature. Samples were washed 3 times with PBS-Tween and then incubated for 30 min with the VECTASTAIN Elite ABC-HRP solution (Vector Labs) at room temperature. Cells were then washed 3 times in PBS-Tween and finally incubated briefly with DAB solution (10 ml PBS, 60 µl DAB 50 µg/ml, 2 µl H_2O_2 30%). The reaction was stopped using distilled water. Samples were stored in PBS and 0.025% sodium azide. EdU incorporation assay was performed with Click-iT™ EdU Cell Proliferation Kit for Imaging (Invitrogen) following manufacturer's instructions. Images were taken using the Zeiss Cell Observer HS widefield microscope with Zen Blue software and the image analysis was performed using Fiji image processing software.

Clonogenic assay

FACS-purified satellite cells were cultured as described in collagen-coated 96 well plates. Total number of cells and colonies were manually counted progressively throughout 4 days.

Time-Lapse Live microscopy

FACS-purified satellite cells were plated in collagen coated µ-Slide Angiogenesis (Ibidi) plates with 1:1 DMEM:HAMs F10 medium containing 20% serum FBS and ITS (Insulin, Transferrin and Selenium; GIBCO) and recorded during 72 h using the Zeiss Cell Observer HS widefield microscope with Zen Blue software. The number of cell divisions and the time for every division was analyzed manually using Fiji image processing software.

RNA isolation

For qPCR and in vitro-based RNA-Seq, total RNA was isolated from cultured satellite cells with the RNeasy Micro kit (QIAGEN) with on-column DNA digestion. For the RNA-Seq experiment in freshly isolated cells, total RNA was extracted with the Arcturus PicoPure RNA Isolation Kit (Thermo Fisher) together with on-column DNA digestion. Both RNA isolation kits were used according to the manufacturer's instructions. RNA concentration was measured with NanoDrop 2000/2000c (Thermo Fisher).

cDNA and qPCR

Reverse transcription of RNA into cDNA was performed by random-primed reverse transcription using the SuperScript II reverse transcriptase (Invitrogen). cDNA quantification was obtained by real-time qPCR (LightCycler 480, Roche) using PowerUp SYBR Green Universal (Thermo Fisher) according to the manufacturer's instructions. Primers used for qPCR are depicted in Supplemental table 1. *Rpl7* transcript levels were used as a reference for the normalization of each target within each sample.

Protein Mass Spectrometry

Whole-cell lysates were run on SDS-PAGE gels and stained with colloidal protein staining solution (Invitrogen). The gels were subjected to digestion with trypsin. Released peptides were measured using LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific) equipped with a nanoelectrospray source (Proxeon). Raw data from (Sreenivasan et al., 2020) was analyzed using the MaxQuant software package 1.6.1.0.

RNA sequencing

RNA isolation, cDNA, and qPCR are indicated in Supplemental information. RNA integrity and library preparation were checked with a BioAnalyzer (RNA Pico Chip, Agilent). For the in vitro-based RNA-Seq, low input library preparation and single-end sequencing was performed in the Centre de Regulació Genomica (CRG) genomics unit using the HiSeqTM Sequencing Kit and the TruSeq v3 Cluster (Illumina). For the RNA-seq experiment in freshly isolated cells standard Directional RNA-seq library preparation and single-end sequencing (Illumina HiSeq 2500) was performed in the Genomic Unit of Centro Nacional de Investigaciones Cardiovasculares (CNIC). For both datasets RNA-Seq raw reads were assessed for quality, adaptor content and duplication rates with FastQC 0.11.8, trimmed by Cutadapt 1.18 and terminally aligned to the Ensemble mouse genome version mm10 (GRCm38) by HISAT2 2.1.0. Differentially expressed genes were identified using DESeq2 3.6. Only genes with a minimal p-value of 0.01 were classified as significantly differentially expressed. Gene ontology (GO) analysis was performed using Gene Set Enrichment Analysis (GSEA) using MSigDB 6.2 database. Functional annotation clustering was done using DAVID 6.8.

ATAC Sequencing

Library preparation was done on FACS-purified satellite cells following the Omni-ATAC protocol (http://www.nature.com/articles/nmeth.4396). Time of incubation with transposase was set to 40 min after extensive setup with *Chd4* $^{fl/fl}$ satellite cells. Primers used for amplification and sequencing are stated in **Supplemental table 2**. Library quality was assessed by Agilent BioAnalyzer (DNA High Sensitivity Chip). Paired-end sequencing (Illumina HiSeq 2500) was performed in the Genomic Unit of the CRG. Raw reads were assessed for quality, adaptor content and duplication rates with FastQC 0.11.8, trimmed by Cutadapt 1.18 and terminally aligned to the Ensemble mouse genome version mm10 (GRCm38) by Picard 2.18.15. Regions of open chromatin were identified by MACS2 2.1.2 while HOMER was used for peak annotation. Differentially peaks near the Transcriptional Start Site (TSS ± 500 bp) were identified using DESeq2 3.6 ($p < 0.01$). Gene ontology analysis was performed using Gene Set Enrichment Analysis (GSEA) using MSigDB 6.2 database.

ChIP and RT–qPCR

Chromatin immunoprecipitation (ChIP) experiments were performed as previously described (Gomez-del Arco et al., 2016)(Sreenivasan *et al.*, 2020) Chromatin Shearing Kit with SDS (Covaris) was used for chromatin shearing. Briefly, cells were fixed using 1% formaldehyde for 10 minutes and the reaction was quenched with 0.125M glycine for 5 minutes at room temperature. The chromatin were sheared to approximately 300-500bp using Bioruptor (Diagenode). Sheared chromatin was subjected to immunoprecipitation with indicated antibodies. Corresponding mouse or rabbit IgG antibody served as a negative control. Chromatin DNA was verified via quantitative real-time PCR. Primers used in this study are listed in Supplemental Table 1.

Co-immunoprecipitation and western blot analysis

SCs were harvested from *Chd4*$^{fl/fl}$ mice. Genetic ablation of Chd4 was induced *in vitro* by adenoviral transduction with a recombinant adenovirus harboring the Cre recombinase (AdCRE). Control cells were infected with green fluorescent protein (GFP)-expressing virus (AdGFP). Co-immunoprecipitation and western blot analysis was performed as previously described (Ianni et al., 2021), 72 h post-transduction. In this study, anti-HDAC1 antibody (Cell Signaling Technology; 5356) was used for immunoprecipitation and anti-HDAC1 (Cell Signaling Technology; 34589), anti-MTA1

(Cell Signaling Technology; 5647) and anti-CHD4 (Cell Signaling Technology; 12011) antibodies for western blot analysis.

Quantification of Western blot bands and statistical analysis

Intensity of bands was quantified using Image Lab™ software (version 6.0.1; Bio-Rad laboratories) and normalized to loading control (GAPDH). The normalized signal of the control sample for each experiment was set to 100. The abundance of target protein is displayed as fold-change relative to controls (relative expression). Statistical significance was assessed by paired Student's t-test using GraphPad Prism 5.0 Software. *$p<0.05$; ***$p<0.001$.

Data availability

RNA–seq and ATAC-seq data have been deposited in the Gene Expression Omnibus (GEO) under accession code GSE179683. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

Table S1. ChIP and qPCR primers and sequences

Gene	**Forward primer (5′ – 3′)**	**Reverse primer (5′ – 3′)**
Rpl7	GAAGCTCATCTATGAGAAGGC	AAGACGAAGGAGCTGCAGAAC
Chd4	AAGGCCATTGAACGACTGCT	CTCCTCTTCCTCCCCCATCT
p57	CGAGGAGCAGGACGAGAATC	GAAGAAGTCGTTCGCATTGGC
Ndrg2	CAAGGCATGGGCTACATGGC	GGAGAGTCCCAGACTCGCTA
Map1a	GGGGAGAACCTTCAGGTGAC	GACCAGGACGTTCAGTTGCT
Usp11	ATGCGTGATGGACACTATAC	GGCGCTGATAGAACAAGACA
Chd5	TGGACCCTGACTACTGGGAG	CATCCTGCCACTCCTGGTC
Tnnt2	AAGATGCTGAAGAAGGTCCAGTAGA	CACTCTCTCTCCATCGGGG
ChIP qPCR primers		
MyoG	TTATCTGGCTGGGAAGTGGC	GGCAAGGAAGGACAGAGACC
Tnnt2	GACAGTTGGTGGTGGGAGAG	AGCAGGGAGGTAGACCAGAG
Tbx21	CATACAGGAGGCAGCAACAA	TTTCTCTCCCCCAGGAAGTT
Chd5	GGCCCATTGGAGGGGTTATT	ATTTCTCGGGTACCCTCCGT
Chd3	GGGGGTAGAGAGAAGTCGAAAA	GCACCACCCCCAAAAATGTTC
Sil1	TAATAGCCACGCGCCTTCA	CCACCTCCCCTACCTTCGG
Map1a	TCACTTTACCTCCGATCCCAC	CTCAGCATGGCTCAGTTCCA
Ndrg2	TCTTGAGTCATAGCATAGTCATTCT	ACTATTCCTGCTGGAGAACAGC
Usp11	TCCTTTGTGAGGCCCTAAACAC	TCTGCAACCAATACCAGGGG
Cdkn1b	CTAGGTTTCGCGGGCAAAG	AACTAGCCAAACGGCCGGA
p57 promoter	GGTCGAATATGGCCTGAC	CTGAGGAACAATACTGTA
p57 Intragenic	TAACGGCCAGAGAGAACT	TTCTCAATACATTGCACA

Table S2. Primers used for the ATAC-seq, Related to Figure 3

Primer	**Sequence (5′ – 3′)**
Ad1_noMX	AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGCGTCAGATGTG
Ad2.7	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGT
Ad2.8	CAAGCAGAAGACGGCATACGAGATCCTCTCTGGTCTCGTGGGCTCGGAGATGT
Ad2.9	CAAGCAGAAGACGGCATACGAGATAGCGTAGCGTCTCGTGGGCTCGGAGATGT
Ad2.10	CAAGCAGAAGACGGCATACGAGATCAGCCTCGGTCTCGTGGGCTCGGAGATGT
Ad2.11	CAAGCAGAAGACGGCATACGAGATTGCCTCTTGTCTCGTGGGCTCGGAGATGT
Ad2.12	CAAGCAGAAGACGGCATACGAGATTCCTCTACGTCTCGTGGGCTCGGAGATGT
Ad2.13	CAAGCAGAAGACGGCATACGAGATATCACGACGTCTCGTGGGCTCGGAGATGT
Ad2.14	CAAGCAGAAGACGGCATACGAGATACAGTGGTGTCTCGTGGGCTCGGAGATGT

Ad1_noMX is the common primer for all samples, while the other primers are specific for each of the eight total samples sequenced and act as a barcode.

Supplemental references

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