Supplementary Materials and Methods

Satellite cell isolation by FACS and culture. TA and GA muscles dissected from mice were combined and subjected to mononuclear cell isolation using collagenase II combined with dispase digestion. Briefly, muscles were pooled and digested with 0.2% Collagenase II (Worthington Biochemical) in DMEM for 30 min at 37°C, followed by trituration with 20% FBS/DMEM. After washing, mixtures were further digested with 0.2% dispase (Gibco) and 0.00125% collagenase II in DMEM for 30 min at 37°C, with FBS later added to final concentration of 20%. After trituration by pipetting, cells were collected by filtering through 100 μm followed by 40 μm cell strainers (BD Falcon). Mononuclear cells were stained with Vcam-biotin (Clone 429; 1:50), PE-CD11b (Clone M1/70; 1:200), PE-Sca1 (Clone D7; 1:200), PE-CD45 (Clone 30-F11; 1:200) and PE-CD31 (Clone MEC 13.3; 1:200) on ice for 15 min (BD Biosciences). After washing, cells were incubated with streptavidin-APC-Cy7 (BD Biosciences; 1:100) on ice for 10 min to amplify the Vcam signal. Cell sorting was carefully optimized and conducted by facility managers with appropriate controls using FACSVantage sorter (BD Biosciences), MoFlo sorter (Beckman Coulter), or Astrios sorter (Beckman Coulter). Cells negative for PE and positive for APC-Cy7 (PE-/APC-Cy7+) were sorted as a SC population (Fig 1C and Supplementary Fig S1A). PE+/APC-Cy7- population was considered non-SCs. Most of the double positive population (PE+/APC-Cy7+) contained dead cells or muscle debris. An equivalent number of sorted SCs from control and KO muscle were seeded on gelatin-coated plates. Cells were cultured in DMEM supplemented with serum, GlutaMAX and penicillin/streptomycin. Different kinds of serum were supplied for experiments including 10% horse serum (HS), 2% HS (differentiation medium), and 20% fetal bovine serum containing 10 ng/ml of bFGF (growth medium).

Histology. TA muscles were dissected, frozen by OCT solution (Tissue-Tek) in methylbutane

chilled in liquid nitrogen, and cryosectioned with a thickness of 10 μm. Histological analysis was performed by haematoxylin and eosin (H&E) staining using a standard histological protocol. For Oil Red O staining, frozen sections were fixed with 4% paraformaldehyde (PFA) for 1 h at room temperature. After washing with water followed by 60% isopropanol, sections were incubated for 30 min with Oil Red O working solution freshly prepared and filtered. After rinsing with 60% isopropanol, slides were stained with haematoxylin for 0.5 min to stain nuclei, washed with water and mounted in 10% glycerol. Images for histology were captured using EVOS XL core microscope (AMG).

Immunofluorescence microscopy. For SC staining in muscle using Pax7 or MyoD antibodies, after fixing sections in 4% PFA for 10 min at room temperature, antigen retrieval was performed by placing slides in 10 mM citrate buffer (pH 6.5) including 0.05% Tween-20 at 92°C for 20 minutes. Endogenous peroxidases were blocked with 3% H_2O_2 in PBS. Sections were incubated in 1% blocking reagent (included in TSA kit; T-20935, Invitrogen) for 1 h at room temperature, followed by mouse monoclonal antibody for Pax7 (DSHB; Concentrate 1:50) or MyoD (Dako; Clone 5.8A; 1:100) in 1% blocking solution overnight at 4°C. After washing with PBS, sections were incubated with Biotin-SP-AffiniPure Goat Anti-Mouse IgG (Jackson ImmunoResearch; #115-065-205; 1:1000) in 1% blocking solution for 1.5 h at room temperature and subsequently with HRP-conjugated streptavidin (TSA kit; 1:200) in 1% blocking solution for 1 h at room temperature. Sections were then incubated with Alexa Fluor 594 tyramide solution in amplification buffer for 15 min (TSA kit; 1:200). To co-stain myofibers, samples were incubated with laminin antibody (Abcam; ab11575; 1:200) in 1% blocking solution overnight at 4°C, followed by Alexa Fluor 488 goat anti-rabbit IgG. For co-staining of Pax7 and UCP1 in muscle, sections were incubated with streptavidin, Alexa Fluor 546 conjugate (Molecular probe; S-11225; 1:200) to stain Pax7 after

incubating with Biotin-SP-AffiniPure Goat Anti-Mouse IgG. UCP1 antibody (Calbiochem; #662045; 1:200) was used at room temperature for 1 h, followed by HRP-conjugated anti-rabbit IgG (TSA kit; T-20922; 1:100) for 30 min and Alexa Fluor 488 tyramide (1:200) for 10 min. For staining of regenerating fibers using eMyHC antibody (DSHB; F1.652; Supernatant 1:10), sections were fixed in cold acetone at -20°C for 10 min. After washing sections with 0.2% Triton X-100 in PBS (PBT) at room temperature, sections were incubated with 1% blocking solution and further processed using TSA kit, similarly to Pax7 staining. For co-staining of BrdU and active caspase-3, SCs cultured in growth medium were treated with 10 μM of BrdU (BD Biosciences) for 3-4 hours before fixation. Cells were fixed in 4% PFA solution for 10 min and then permeabilized with 1% PBT for 10 min at room temperature. DNA was denatured by incubating with 2N HCl/1% PBT for 30 min at 37°C. After washing with PBS, cells were blocked with 5% normal goat serum (NGS) for 1 h at room temperature. Cells were incubated with caspase-3 antibody that detects only active forms (Cell signaling; #9661; 1:100) and monoclonal BrdU antibody conjugated with Alexa Fluor 488 (Invitrogen; clone MoBU-1; 1:50-100) overnight at 4°C, followed by Alexa Fluor 594 goat anti-rabbit IgG for 1 h at room temperature. For staining of myosin heavy chain, myotubes were fixed in 4% PFA solution for 10 min and permeabilized with 0.2% PBT for 10 min at room temperature. Cells were blocked with 5% NGS for 1 h at room temperature and incubated with MF20 antibody (DSHB; Concentrate; 1:50-100) overnight at 4°C, followed by Alexa Fluor 488 or 594 goat anti-mouse IgG for 1 h at room temperature. Images for immunofluorescence were captured using EVOS FL microscope (AMG) except for Fig 4F (Leica SP5 confocal microscope).

RNA analysis. RNA extraction and qRT-PCR were performed as described previously with slight modification [1]. TA muscles were homogenized in Trizol reagent (Invitrogen) by

bullet blender homogenizer (Next Advance) according to manufacturer's instructions. For RNA extraction of SCs, glycogen was added into Trizol reagent (100 μg/ml) to reduce RNA loss. cDNA synthesis for gene expression was carried out with oligo dT primer and an Improm II reverse transcription system (Promega) according to manufacturer's instructions. Real time PCR was performed using SsoAdvanced SYBR green supermix (Bio-rad) on the CFX384 real-time PCR detection system (Bio-Rad). Values were normalized to GAPDH. Primers were synthesized according to previous reports including MyoD [2], Myf5 [3], MyoG [1], Lix1[4], Mest [4], PlagL1 [4], Cipar1 [4], Pax3 [5], ID2 [3], ID3 [3], Prdm16 [6], UCP1 [7], and GAPDH [1]. Other primer sequences were as follows: HDAC4, 5'-AGCGACACCATATGGAATGAG-3' and 5'-TTGTACCCTCCAAGAGGTGTG-3'; HDAC5, 5'-GCCATCACAGCTAAACTCCTG-3' and 5'-TGCTCTGGATCTCGATGACTT-3'; Pax7, 5'-CATGAACCCTGTCAGCAATG-3' and 5'-GTGGACAGGCTCACGTTTTT-3'; Igfbp2, 5'-CCCCCTGGAACATCTCTACTC-3' and 5'-GGTATTGGGGTTCACACACC-3'. For miRNA analysis, total RNAs extracted by Trizol reagent were used as templates of cDNA synthesis. cDNA synthesis was performed using miScript II RT kit (Qiagen; #218160) according to manufacturer's instruction. Real-time PCR was carried out using miScript SYBR Green PCR kit (Qiagen; #218073). miScript primer assays (Qiagen) were used for miR-1a (#MS00011004), miR-133a (#MS00032305), and miR-206 (#MS00001869). Values were normalized to RNU6 (#MS00033740).

Western blot analysis. Equivalent numbers of SCs isolated by FACS sorting were plated and cultured for 2 days. Cells were solubilized in 2x laemmli sample buffer (Bio-rad; #161-0737) containing 5% β-mercaptoethanol and lysates were heat-denatured for 5min. Lysates were resolved on 4-15% SDS-PAGE, transferred to nitrocellulose membranes, and incubated with antibodies against HDAC4 [1], Pax7 [5], MyoG (Abcam; ab124800), or GAPDH (Cell

Signaling; #2118). Blots were detected using Supersignal West Pico Chemiluminescence (Pierce; #34080).

siRNA transfection. Equivalent numbers of SCs isolated by FACS sorting were reverse transfected with siRNAs using Lipofectamine RNAiMAX (Invitrogen). For KDs of Lix or Mest, Silencer Select siRNA system (Ambion) was used at final concentration of 10 nM for negative control #1 (#4390843), negative control #2 (#4390846), Lix1 #1 (#s83623), Lix1 #2 (#s83621), Mest #1 (#s69811) and Mest #2 (#s201693). For HDAC4 KD, stealth RNAi system (Invitrogen) was used at final concentration of 20 nM for negative control medium GC or HDAC4 siRNA (#MSS209678). The following siRNA sequences were used: Lix1 #1, 5'-CGGCUUUGGUCUUCAAAGAtt-3'; Lix1 #2, 5'-ACUACAUGCUGGAGUCAAAtt-3'; Mest #1, 5'-CGAUUAUGGAGAUAUCGUUtt-3'; Mest #2, 5'-GGUCAUCGACAGUCUUUUAtt-3'; HDAC4, 5'-GAGCAGCAGAGGAUCCACCAGUUAA-3'.

Chromatin immunoprecipitation (ChIP) assay. For ChIP assay, C2C12 myoblasts were reverse transfected with control or HDAC4 siRNA for 36 h. Chip analysis using anti-histone H4 (acetyl K8) antibody (Abcam; ab15823) was performed as described previously [8]. Primer sequences were as follows: Actin, 5'-GAGTATCCCCAGCTGTCTGC-3' and 5'-GCAAAGCAGTGACCTTTGGC-3'; r-sarcoglycan, 5'-CAGTTGTCACAGTGGACA-3' and 5'-GGCATGAATTAACATGGAC-3'; Pax7, 5'-CGCACGCTGGAGACGAAT-3' and 5'-CCCAGCTTCTGGAAGGG-3'; Lix1, 5'-CAGGAGCGCTAGTTTTCCCA-3' and 5'-GGCAGTGCACTCTGACAGTA-3'.

Supplementary References

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