Supporting Information

Primers

Target	Forward primer (5'-3')	Reverse primer (5'-3')
Jun	CCTTCTACGACGATGCCCTC	GGTTCAAGGTCATGCTCTGTTT
Junb	TTTGTGTATTTAACAGGGAGGG	AAGTGCGTGTTTCTTCTCCA
Fos	CGGGTTTCAACGCCGACTA	TTGGCACTAGAGACGGACAGA
Fosb	TTTTCCCGGAGACTACGACTC	GTGATTGCGGTGACCGTTG
Myc	TCAGTGGTCTTTCCCTACCC	CTTCTTGCTCTTCTTCAGAGTCG
Egr1	TCGGCTCCTTTCCTCACTCA	CTCATAGGGTTGTTCGCTCGG
Apaf1	AGTGGCAAGGACACAGATGG	GGCTTCCGCAGCTAACACA
Cdkn1a	AACATCTCAGGGCCGAAA	TGCGCTTGGAGTGATAGAAA
Mdm2	TGTCTGTGTCTACCGAGGGTG	TCCAACGGACTTTAACAACTTCA
Pmaip1	GCAGAGCTACCACCTGAGTTC	CTTTTGCGACTTCCCAGGCA
Hmox1	AAGCCGAGAATGCTGAGTTCA	GCCGTGTAGATATGGTACAAGGA
Srxn1	CCCAGGGTGGCGACTACTA	GTGGACCTCACGAGCTTGG
Gapdh	TCCTTGGAGGCCATGTAG	CCCACTCTTCCACCTTCG
Paxbp1	GCTTAAAGACAGGTTGGACTC	AAGTTCATTAATCAGTGGCACC
Aco2	ATCGAGCGGGGAAAGACATAC	TGATGGTACAGCCACCTTAGG
Fh1	GAATGGCAAGCCAAAATTCCTT	CGTTCTGTAGCACCTCCAATCTT
Cs	GGACAATTTTCCAACCAATCTGC	TCGGTTCATTCCCTCTGCATA
Pkm	CGCCTGGACATTGACTCTG	GAAATTCAGCCGAGCCACATT
Ywhaz	CAGAAGACGGAAGGTGCTGAGA	CTTTCTGGTTGCGAAGCATTGGG

Antibodies

Antibodies	Source	Identifier
Mouse monoclonal anti-Pax7	Hybridoma Bank (DSHB)	RRID:AB_528428
Rabbit polyclonal anti-Myod1	Santa Cruz	Cat#SC-760
Mouse monoclonal anti-Myod1	Dako	Cat#M3512
Rabbit polyclonal anti-Paxbp1	This paper	N/A
Mouse monoclonal anti-Gapdh	Invitrogen	Cat#AM4300
Mouse monoclonal anti-Tubulin	Sigma-Aldrich	Cat#T4026
Mouse monoclonal anti-eMHC	Hybridoma Bank (DSHB)	RRID:AB_528358
Mouse monoclonal anti-Plin1	Vala sciences	Cat#4854
Rabbit monoclonal anti-pS6 (Ser235/236)	Cell signaling Technology	Cat#4858s
Rabbit polyclonal anti-total S6	Cell signaling Technology	Cat#2217s
Mouse monoclonal anti-Cdkn1a	Santa Cruz	Cat#sc-6246
Rabbit polyclonal anti-Tomm20	Santa Cruz	Cat#sc-11415
Rabbit monoclonal anti-p4ebp1 (Thr37/46)	Cell signaling Technology	Cat#2855
Rabbit polyclonal anti-total 4ebp1	Cell signaling Technology	Cat#9452s
Mouse monoclonal anti-Idh2	Santa Cruz	Cat#sc-374476
Mouse monoclonal anti-Ndufs1	Santa Cruz	Cat#sc-271510
Goat polyclonal anti-GFP	Abcam	Cat#Ab6658
Rat monoclonal Alexa Fluor® 647 anti-mouse CD45 Antibody	biolegend	Cat#103124
Rat monoclonal Alexa Fluor® 647 anti-mouse CD31 Antibody	biolegend	Cat#102516
Rat monoclonal Pacific Blue™ anti-mouse Ly-6A/E (Sca-1) Antibody	biolegend	Cat#108120
Rat monoclonal Biotin anti-mouse CD106 Antibody	biolegend	Cat#105704
Rabbit monoclonal anti-Mki67	Abcam	Cat#Ab16667
Rabbit polyclonal anti-laminin	Sigma-Aldrich	Cat#L9393
Rabbit polyclonal anti-Sestrin2	proteintech	Cat#10795-1-AP
Rabbit polyclonal anti-Ddit4	proteintech	Cat#10638-1-AP
Mouse monoclonal anti-Myog conjugated with Alexa Fluor® 594	Santa Cruz	Cat#sc-12732

Materials and Methods

Animals

Paxbp1$^{flox/flox}$ mice were generated by the Model Animal Research Center in Nanjing University using the targeting vector of *Paxbp1* (*Paxbp1*$^{tm44128(L1L2\_gt0)}$) purchased from the International Mouse Phenotyping Consortium (IMPC, http://www.mousephenotype.org) with exon8 of *Paxbp1* flanked by loxP sites. The *LacZ-Neo(R)* cassette flanked by FRT sites between exons 7 and 8 was removed by crossing with FLP-expressing mice. The resulting *Paxbp1*$^{flox/flox}$ mice were viable, fertile and normal in body weight and size. *Pax7*$^{CreERT2(Gaka)}$ (Stock No: 017763) and *R26R-EYFP* (Stock No: 006148) mice were from Jackson Laboratory (Bar harbor, ME, USA). To delete *Paxbp1* in adult MuSC, tamoxifen (TMX) (75 μg/g of body weight) was intraperitoneally injected for 7 doses, with five doses in the first week and 2 doses in the second week. All the experiments were performed in accordance with protocols approved by the Animal ethics committee at HKUST.

Muscle injury by Cardiotoxin (CTX) injection

Adult mice were anesthetized by intraperitoneal injection of Avertin (0.5 mg/g body weight). For TA muscle injury, 30 μl of 10 μM CTX prepared in 0.9% NaCl was injected to induce muscle damage. For lower hindlimb muscle injury, 50 μl of 10 μM CTX was injected into 8-10 spots in the lower hindlimb muscle. Muscles were then collected at various time points after injury for histological or FACS analysis.

Single myofiber isolation

Extensor digitorum longus (EDL) muscles were carefully dissected and subjected to digestion by 800 U/ml Collagenase II (#LS004177; Worthington Biochemical Corp, NJ, USA) for 75 min at 37°C. Digested EDL muscles were then gently dispersed via trituration with a glass pipet. Single myofibers were then collected and cultured in Ham's F10 medium with 10% horse serum.

Isolation of adult MuSC by FACS

Hindlimb muscles were dissected, minced and subjected to Collagenase II (800 U/ml) digestion in washing medium (Ham's F10 with 10% horse serum) at 37°C for 90 min. The digested muscles were then triturated, washed, and subjected to further digestion by 80 U/ml of collagenase II and 1 U/ml of Dispase (#17105041, ThermoFisher) for 30 min. The suspension

was further triturated by passing through a 20-G needle for 15 times and filtered by a 40 μm cell strainer (BD Biosciences). The single-cell suspension was then incubated with the following fluorophore-conjugated antibodies from Biolegend (CA, USA) (all antibodies were used at a dilution of 1:75): Alexa 647 anti-CD31 (#102516), Alexa 647 anti-CD45 (#103124), Pacific Blue anti-Sca-1 (#108120), Biotin anti-Vcam1 (#105704) and PE/Cy7 streptavidin (#405206). MuSC were sorted by FACSAria III or BD Influx (BD Bioscience, CA, USA) with the gating: $CD31^-/CD45^-/Sca\text{-}1^-/Vcam1^+$. Alternatively, YFP^+ MuSC were sorted using the autofluorescence of YFP.

Histology and Histochemistry

TA muscles were dehydrated in 20% sucrose and embedded in O.C.T. Embedded muscles were sectioned to 8 μm slices using a Thermo Fisher Cryostat. For hematoxylin and eosin (H&E) staining, TA muscle sections were fixed with 4% paraformaldehyde (PFA) for 5 min. After rinsed with ddH_2O, sections were immersed in hematoxylin staining solution for 30 min. Sections were then differentiated in 1% HCl and neutralized in ammonia solution. This was followed by eosin staining for 3 min and subsequent sequential dehydration in EtOH and xylene. For Oil Red O staining, Oil Red O stock solution was prepared by dissolving 0.35 g of Oil Red O (#O0625, Sigma) in 100 mL isopropanol and filtered through a 0.22 μm filter. Sections were fixed in 10% formalin for 10 min, washed with ddH_2O twice and with 60% isopropanol for 5 min. After dried at room temperature, Oil Red O working solution (60% Oil Red O stock solution in ddH_2O) was applied for 10 min. Sections were then immediately rinsed by ddH_2O and stained with hematoxylin for 5 min.

OCR and ECAR measurement

The oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured by a Seahorse XFp analyzer (Agilent, CA, USA) using the "Cell Mito Stress" test kit. Briefly, 5×10^4 of FISC were seeded into each well of a miniplate and cultured in growth medium (F10 with 10% horse serum). Prior to assays, cells were first incubated with XF DMEM (pH 7.4) medium (supplemented with 1 mM of pyruvate, 1 mM of glutamine, and 1 g/L of glucose) in a CO_2 free incubator at 37°C for 60 min. The "Cell Mito Stress" assays were performed following manufacturer's instructions.

ATP concentration measurement

ATP concentration was determined using the ATP determination kit (A22066, Invitrogen). Briefly, 8 x 10^4 of FISC were seeded in 24-well culture dish for various time. Cells were lysed with the lysis buffer (50 mM Tris, pH 7.4, 10% glycerol, 1% Triton, 150 mM NaCl) after harvest. 5 µl of cell lysate was then mixed with 95 µl of the reaction buffer and subjected to luminescence measurement. ATP concentration was then calculated according to the standard curve following manufacturer's instructions.

RNA-seq and data processing

10^5 of YFP^+ ASC were isolated by FACS from control and *Paxbp1*-iKO mice 24 h after CTX-induced injury. Total RNA was extracted with the NucleoSpin RNA XS kit (MACHEREY-NAGEL) following manufacturer's instructions. cDNA was then synthesized and amplified according to the Smart-Seq2 protocol (66). Purified cDNA was then fragmented using a Covaris ultrasonicator followed by library construction using the Ovation® RNA-Seq System V2 (NuGEN Technologies, CA, USA). The paired-end library was sequenced on an Illumina Nextseq 500 machine. Raw sequencing reads were mapped to the mouse reference genome (GRCm38/mm10) using STAR aligner (67). Raw counts table was then generated with featureCount (v1.6.4) (68), followed by differential gene expression analysis with DEseq2 R package (69). Differentially expressed genes having absolute log2 fold change >1 and padj <0.05 were considered significant. Raw data were uploaded to GEO (accession number: GSE141881). For GSEA analysis (37), DEseq2 normalized count table was used as input file using default parameters. Two gene set databases, Hallmark and C5 (Gene ontology) (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp), were used for differential pathway enrichment.

Immunostaining

Cells or myofibers were fixed in either 4% PFA or ice-cold methanol for 5 min, washed with PBS for 3 times, permeabilized in PBS with 0.5% Triton (0.5% PBST) for 15 min and subsequently blocked in 4% IgG-free BSA for 1 h. After removal of the blocking solution and washing, samples were incubated with primary antibodies at 4°C overnight followed by washing and incubation with a secondary antibody for another 1 h. After washing with PBS, slides were mounted using Mowiol mounting medium. Immunostaining of muscle sections was performed similarly as described above, except that 0.3% of PBST was used for washing. For Pax7 and eMHC staining on muscle sections, antigen retrieval was carried out by boiling

sections in 0.01 M of citric acid (pH6.0) at 90°C twice (5 min/each time) prior to blocking. For Myog staining, TA muscles were first prefixed in 0.5% PFA after dissection. After cryosectioning, muscle sections were then fixed with 4% PFA for 5 min and blocked with (1:50 dilution) an anti-mouse Fab in 0.3% PBST for 2 h followed by incubation with (1:50 dilution) an Alexa Fluor® 594 conjugated Myog antibody overnight. For EU and EdU labeling, 1 mM of EU and 10 μM of EdU were added in culture media during labeling. Cells were then fixed and the click chemistry detection was performed according to manufacturer's instructions. 100 ng/ml of 4',6-diamidino-2-pheny-lindole (DAPI) was used to counterstain nuclei.

Cell cycle analysis and measurement of ROS levels by flow cytometry

For cell cycle analysis, cells were first incubated with 10 μg/ml of Hoechst 33342 at 37°C for 45 min followed by staining with 0.5 μg/ml of Pyronin Y for 15 min. For ROS detection, FISC were suspended in DMEM plus 10% fetal bovine serum. CellROX Deep Red (Thermo Fisher) was added at a final concentration of 5 μM and stained at 37°C for 30 min. Cells stained with Pyronin Y or CellROX Deep Red were then analyzed on a BD Influx™ cell sorter.

Western blot

Cells were washed with ice cold PBS and lysed in lysis buffer (50 mM Tris, pH7.4, 1% Triton, 150 mM NaCl, 10% glycerol, supplemented with protease and phosphatase inhibitors). Samples were then centrifuged at 13,000 rpm for 10 min and soluble whole cell lysates were collected. 20~50 μg of cell lysates were heat-denatured in SDS sample buffer and proteins in the lysates were separated by 8%-12% SDS-PAGE followed by protein transfer to PVDF membrane. The membrane was first blocked in 10% milk for 1 h, then incubated with a primary antibody at 4°C overnight. After washing, the membrane was incubated with a secondary antibody for 1 h and subjected to chemiluminescence detection.

Super-resolution Microscopy

This was adapted from the protocol by Wu et al (Mol Cell, 2019. 73: 971-84). Briefly, FISC were seeded on poly-D-Lysine-coated coverslips. Before imaging, fresh imaging buffer (PBS with 10% D-glucose, 5.6 mg/mL glucose oxidase, 0.4 mg/mL Catalase and 143 mM β-mercaptoethanol) was added to the chamber to replace the original buffer. Super-resolution images were taken by a custom-built dSTORM microscope with a channel for samples labeled with Alexa 647, a 100x oil TIRF lens and an electron-multiplying charge-coupled device

camera (Andor, IXon-Ultra). For each sample, 50 frames of TIRF images were captured first (exposure: 30 ms/frame) followed by 30 s of bleaching, then 4000 frames of super-resolution images were taken (exposure: 30 ms/frame). The laser intensity was kept at 4 kW/cm^2 during 30 s of bleaching and the super-resolution imaging. The raw images were processed by Rohdea (Nanobioimaging Ltd., Hong Kong) and reconstructed by ImageJ.

Time-lapse imaging and apoptosis detection

FISC were seeded in a 24-well or 96-well plate and cultured in growth media (F10 with 10% horse serum). Time-lapse imaging was then performed by a Nikon Ti-E-PFS microscope or Zeiss Cell Discoverer 7 automated microscope for up to 72 h. Images were taken every 5 -10 min and videos were generated using FIJI or Zen blue software. For apoptosis detection, FISC were cultured in growth medium in the presence of 4 μM of CellEvent™ Caspase-3/7 Green Detection Reagent (Thermo Fisher) for up to 72 h. Live-cell imaging was recorded with a Zeiss cell discoverer 7 automated microscope.

RNA extraction and RT-PCR

Total RNA was extracted with TRIzol reagent (Thermo Fisher), followed by cDNA synthesis using ImProm-II reverse transcription system (Promega). Real time PCR was performed on a Roche LightCycler 480 machine using SYBR green master mix (Roche) or TB Green Premix Ex Taq II (Takara). The results were quantified using $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All the error bars represent standard deviation (s.d.). The experimental data were analyzed with GraphPad Prism 7/8. Unpaired, two-tailed Student's *t*-test was used for comparison between two groups. Multiple *t*-tests between control and iKO were corrected using Holm-Sidak's method. To compare multiple groups of data with control, one way ANOVA was applied with subsequent Holm-Sidak's multiple comparisons tests. For mTOR inhibitor treatment and RT-qPCR after H_2O_2 treatment, two-way ANOVA followed by Holm-Sidak's multiple comparison tests were conducted. $P<0.05$ was considered statistically significant.

Supplemental Figures S1-S6

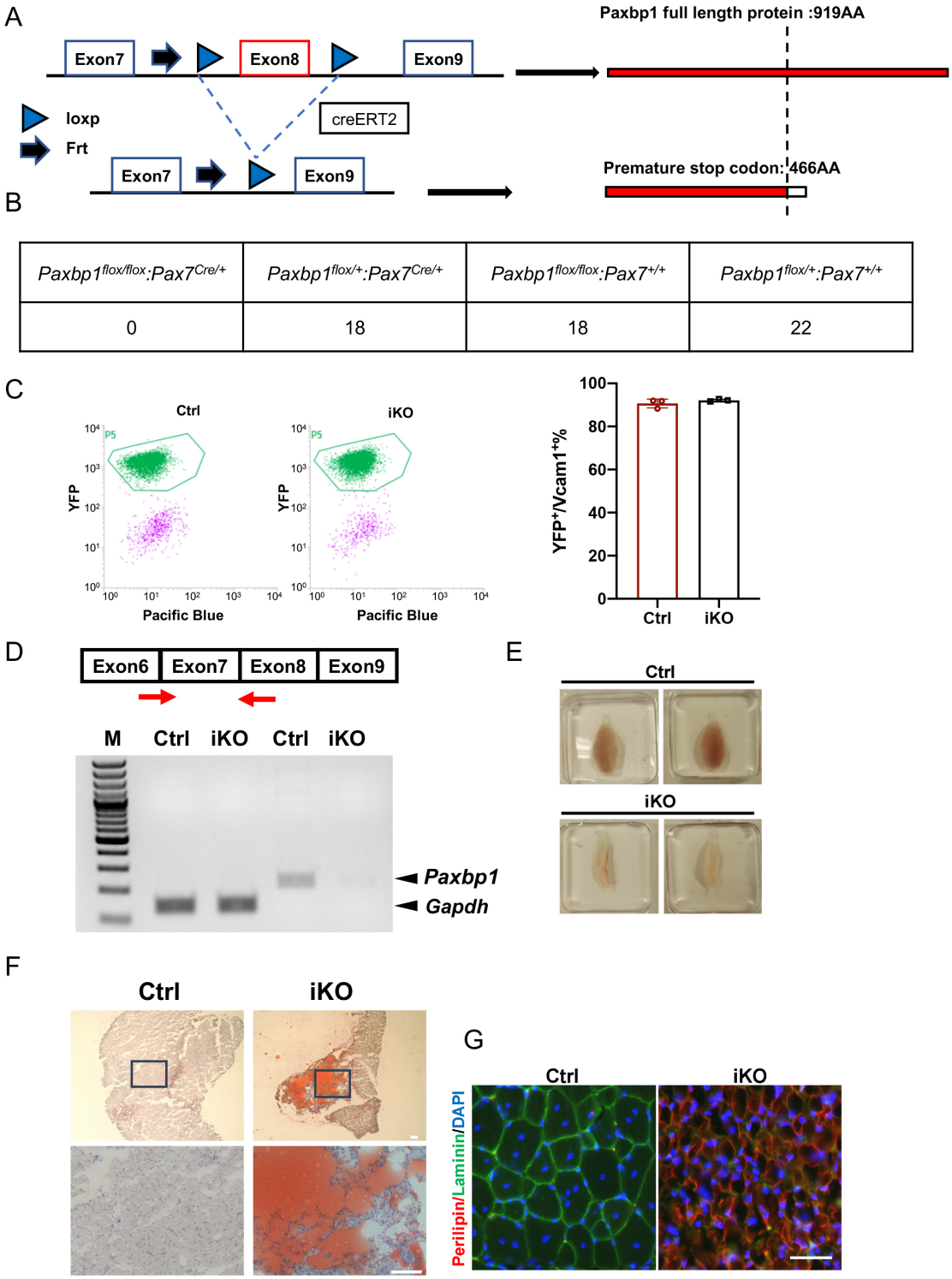

Fig. S1. Generation of *Paxbp1*-iKO mice. (*A*) Schematic showing a floxed *Paxbp1* allele and the predicted protein product after Cre-mediated recombination. (*B*) Enumeration of viable pups of different genotypes after crossing $Pax7^{Cre/+}$; $Paxbp1^{flox/+}$ mice with $Paxbp1^{flox/flox}$ mice. (*C*) Representative FACS plots (left) to determine the efficiency of tamoxifen-induced recombination in FISC. MuSC were first selected as the $CD31^{-}/CD45^{-}/Sca1^{-}/Vcam1^{+}$

population and then checked for YFP expression. The percentage of YFP^+ MuSC was quantified (right) (n = 3 mice/group). (*D*) Top: a diagram showing relevant exons of *Paxbp1* mRNA and locations of the PCR primers (red) for genotyping. Bottom: representative genotyping results to monitor *Paxbp1* deletion at the mRNA level using FISC from Ctrl and iKO mice. *Gapdh*: loading control. (*E*) Representative images of TA muscles at 30 dpi. (*F* and *G*) TA muscle cross sections at 30 dpi from control and iKO mice were subjected to either Oil red O staining (*F*) or immunostaining for Perilipin-1 (red) and laminin (green) (*G*). Data were presented as mean ± SD. Scale bars: 100 μm in (*F*) and 50 μm in (*G*).

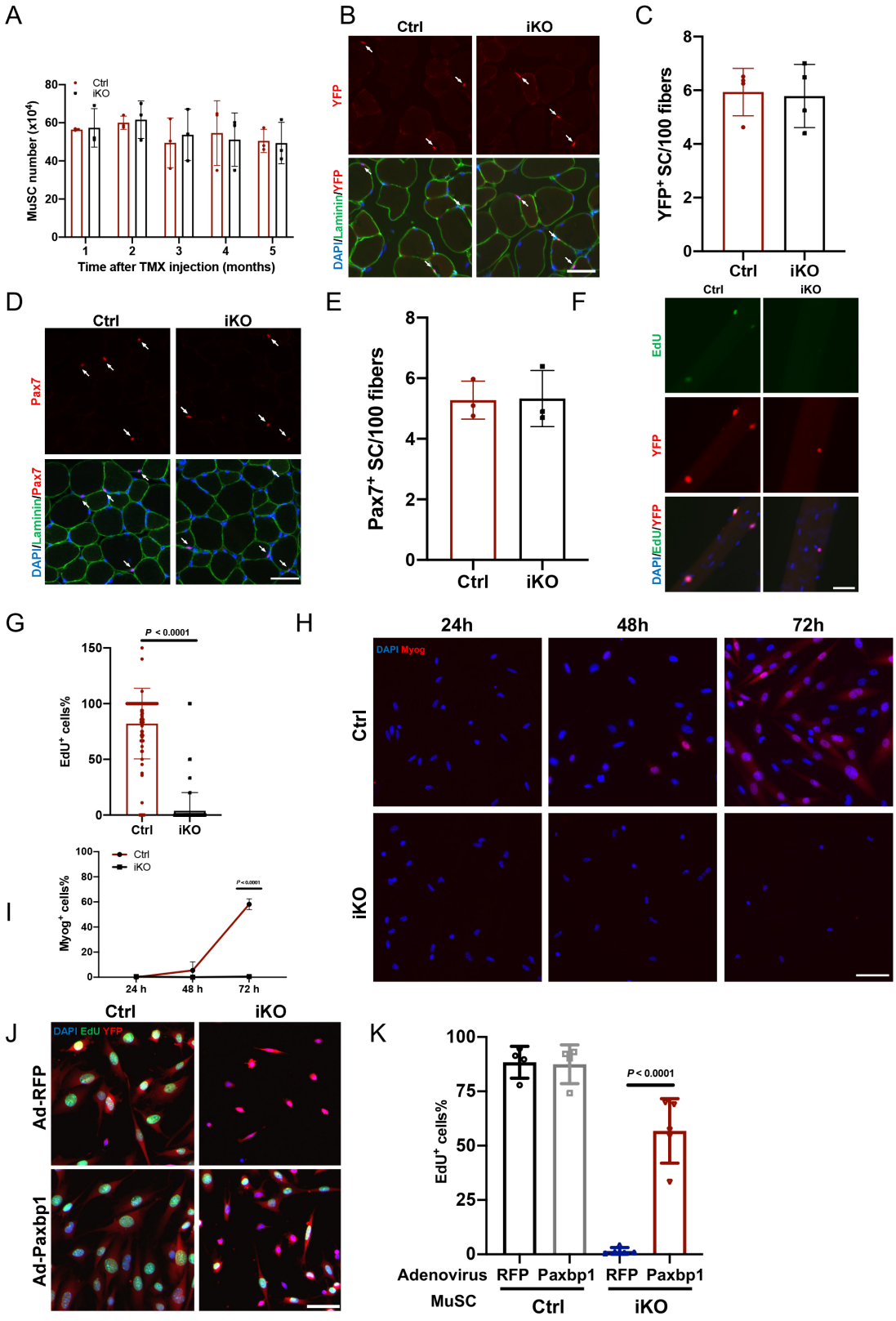

Fig. S2. Paxbp1 was indispensable for cell cycle re-entry upon activation of MuSC without affecting their quiescence maintenance. (*A*) FACS-mediated measurement of the number of YFP$^+$ MuSC from hindlimb muscles of Ctrl and *Paxbp1*-iKO mice at various time points as indicated after the last tamoxifen (TMX) injection (n = 3 mice/group for each time point). (*B*)

TA muscle sections from uninjured Ctrl and *Paxbp1*-iKO mice were subjected to immunostaining for YFP and laminin. (*C*) Quantification of the number of YFP^+ MuSC/100 myofibers in (*B*) (n = 4 mice/group). (*D*) TA muscle sections from uninjured Ctrl and *Paxbp1*-iKO mice were subjected to immunostaining for Pax7 and laminin. (*E*) Quantification of the number of $Pax7^+$ MuSC/100 myofibers in (*D*) (n = 3 mice/group). In (*B* and *D*), white arrows indicated YFP^+ (*B*) and $Pax7^+$ (*D*) MuSC respectively. Nuclei were counter stained with DAPI. (*F*) Single myofibers were isolated and cultured with EdU for 48 hours. Fibers were then fixed and stained for EdU and YFP. (*G*) Quantification of the percentage of EdU^+ MuSC over YFP^+ MuSC in (*F*). ~90 fibers from 3 mice/group were used for quantification. (*H*) FISC from Ctrl and *Paxbp1*-iKO mice were cultured for 24, 48 and 72 hours followed by immunostaining for Myogenin. (*I*) Quantification of the percentage of $Myog^+$ cells in (*H*) (n=3 mice per group). (*J*) FISC from Ctrl and *Paxbp1*-iKO mice were infected with RFP- or Paxbp1-expressing adenovirus and cultured for 3 days with 10 μM of EdU. Cells were then fixed and stained for EdU and YFP. (*K*) Quantification of the percentage of EdU^+ cells over total YFP^+ SC in (*J*) (n = 4 for Ctrl SC and n = 5 for *Paxbp1*-null SC). Data were presented as mean ± SD. Scale bars: 50 μm.

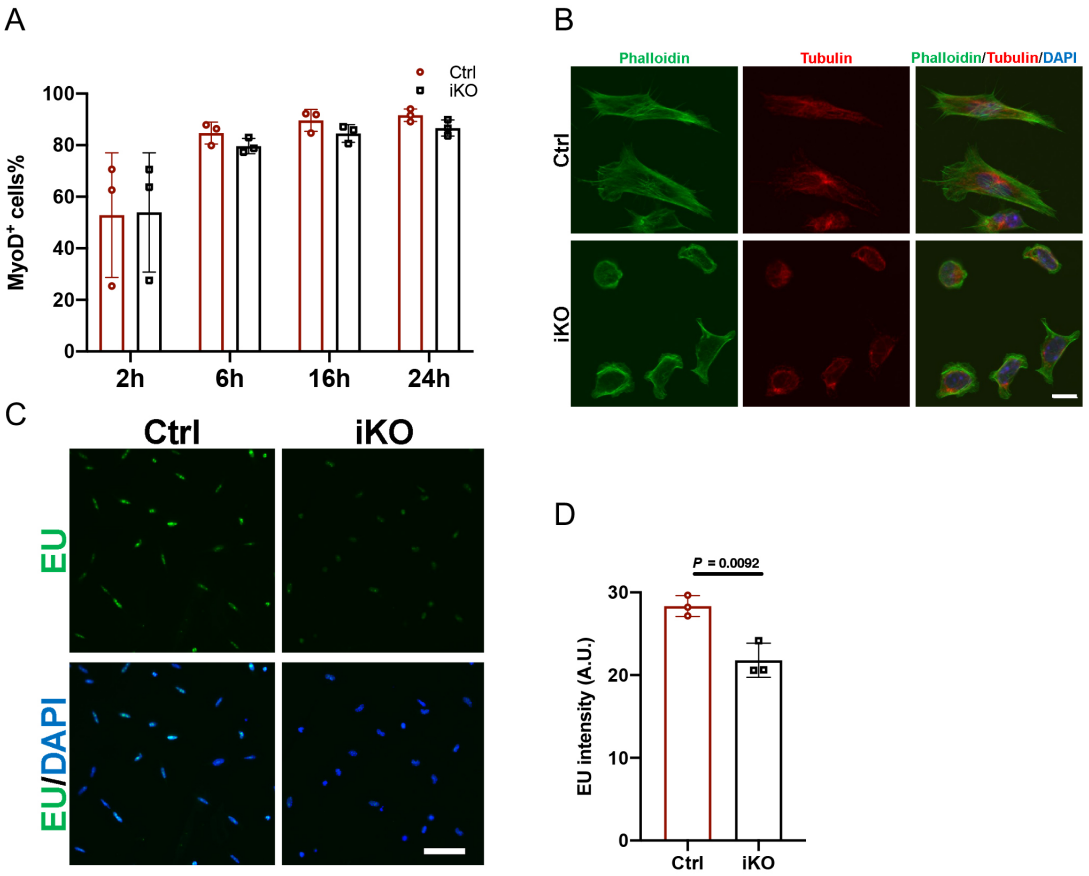

Fig. S3. Loss of *Paxbp1* impaired actin cytoskeleton and RNA synthesis upon activation of MuSC without affecting MyoD expression. (*A-C*) FISC from Ctrl and *Paxbp1*-iKO mice were cultured for various times as indicated. In (*A*), cells were subjected to immunostaining for MyoD and the percentage of MyoD$^+$ ASC was calculated and presented (n = 3 mice/group). In (*B*), cells were subjected to staining for phalloidin and Tubulin. In (*C*), cells were cultured for 24 h and pulse labeled by 1 mM EU for 1 h before fixation followed by EU staining. A representative image of EU staining was shown. (*D*) Quantification of the relative EU intensity in (*C*) (n = 3 mice/group). Data were presented as mean ± SD. Scale bars: 50 μm in (*C*) and 10 μm in (*B*).

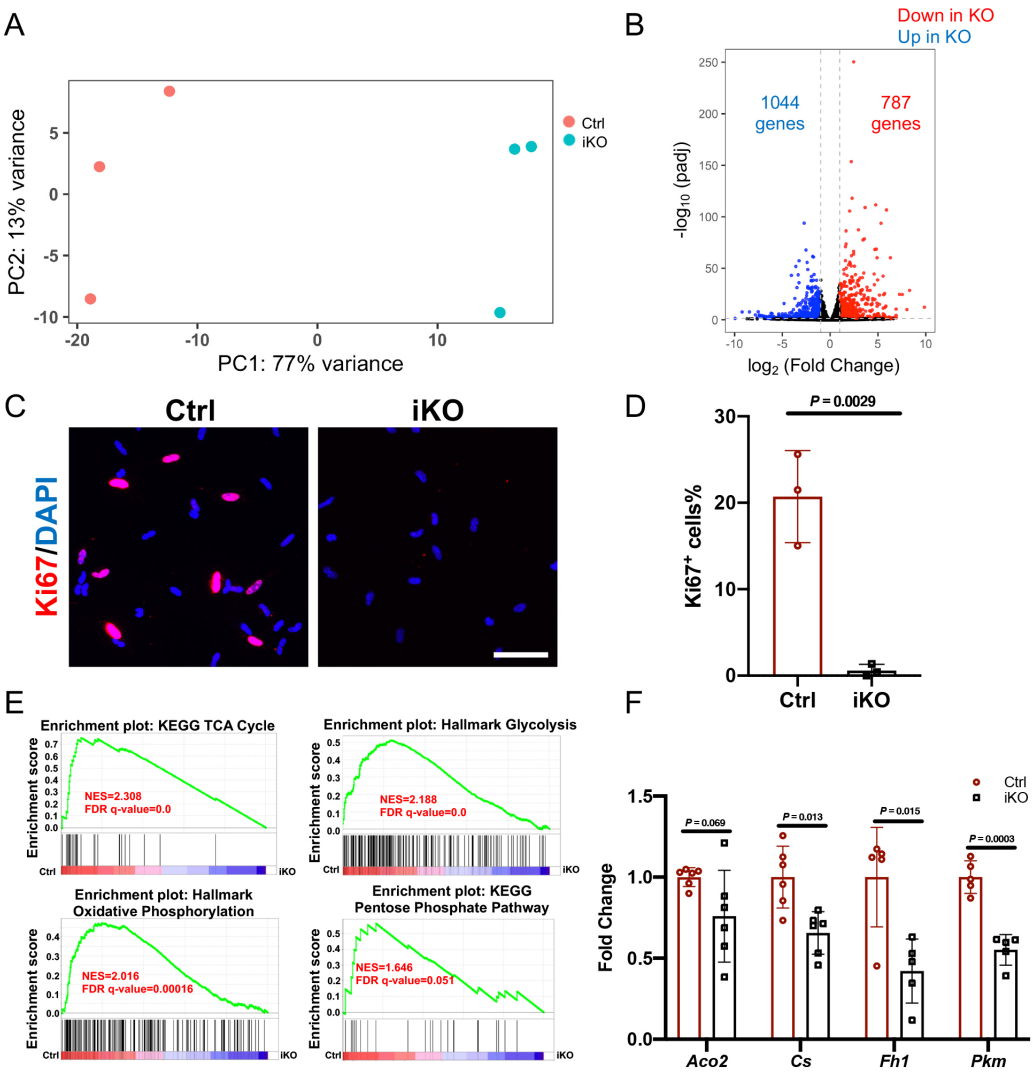

Fig. S4. RNA-seq revealed dysregulated metabolic genes in *Paxbp1*-null ASC. (*A*) PCA plot of our RNA-seq data derived from freshly-isolated ASC 24 h after CTX-induced muscle injury from both control (Ctrl) and *Paxbp1*-iKO mice. (*B*) Volcano plot of RNA-seq results. Genes with absolute fold change > 2 and padj < 0.05 were highlighted as significantly changed genes. (*C*) FISC from Ctrl and *Paxbp1*-iKO mice were cultured for 24 h before fixation followed by immunostaining for Ki67. (*D*) Quantification of the percentage of $Ki67^{+}$ ASC over total ASC in (*C*) (n = 3 mice/group). (*E*) GSEA plots showing multiple anabolic pathways were downregulated in *Paxbp1*-null ASC. (*F*) Measurement of relative mRNA levels for selected metabolic genes by RT-qPCR using ASC after 24 h in culture (n = 5~6 mice/group). Data were presented as mean ± SD. Scale bar: 50 μm.

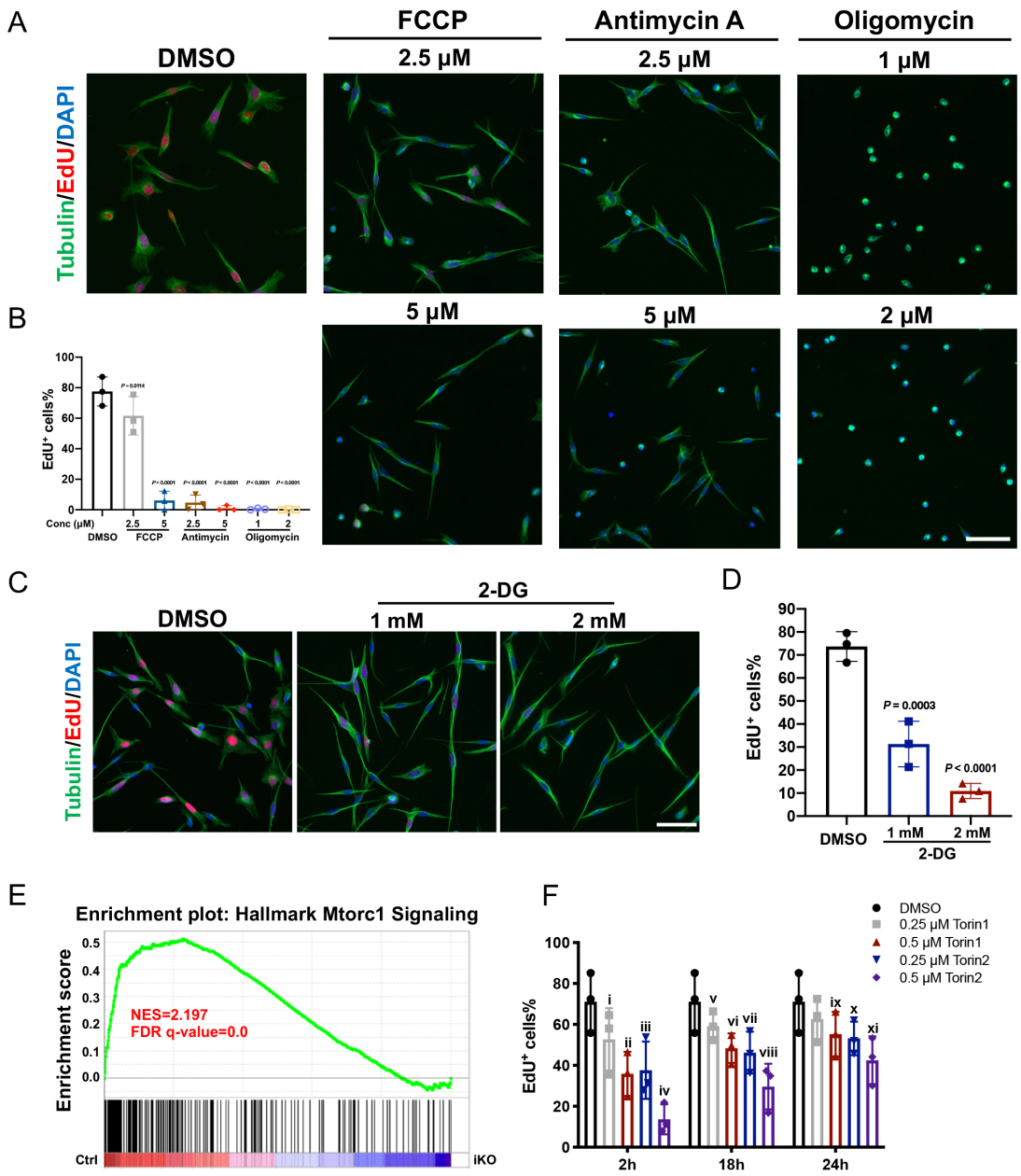

Fig. S5. The mTORC1 signaling and anabolic metabolism were essential for QSC to re-enter the cell cycle. (*A-D*) Representative images of FISC from wildtype adult mice that were cultured in the presence of various ETC inhibitors (*A*) or 2-DG (*C*) for 40 h before fixation followed by staining for EdU and Tubulin. In (*B* and *D*), quantification of the percentage of EdU^+ ASC over total ASC in (*A*) and (*C*) (n = 3 independent experiments). (*E*) The GSEA plot showing defective mTORC1 signaling in *Paxbp1*-null ASC. (*F*) FISC from wildtype adult mice were cultured for 40 h in the absence or presence of Torin1 or Torin2 that were added at the indicated time points after plating followed by staining for EdU. The percentage of EdU^+ ASC over total ASC was shown (n = 3 independent experiments). Adjusted *P* values: i, 0.000764; ii, <0.000001; iii, <0.000001; iv, <0.000001; v, 0.020104; vi, 0.000154; vii, 0.000073; viii, <0.000001; ix, 0.005812; x, 0.002928; xi, 0.000014. Data were presented as mean ± SD. Scale bars: 50 μm.

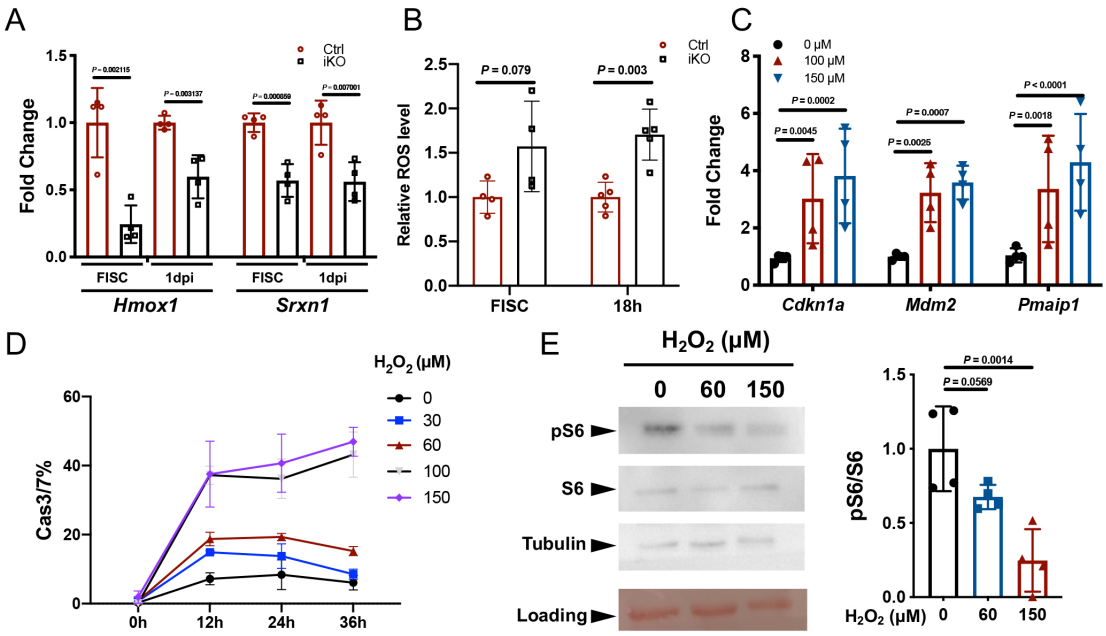

Fig. S6. Paxbp1-dependent redox regulation influenced mTORC1 and p53 signaling in ASC. (*A*) Measurement of mRNA levels of two antioxidant genes in FISC and ASC from Ctrl and *Paxbp1*-iKO mice at 1 dpi by RT-qPCR (n = 4 mice/group). *Hmox1*: heme oxygenase 1; *Srxn1*: sulfiredoxin 1. Data were presented as fold change with the relative expression of a gene from *Paxbp1*-iKO mice over that from Ctrl mice. (*B*) Measurement of relative ROS levels in FISC and ASC that were cultured for 18 h using CellROX Deepred (n ≥ 4 mice/group for each time point). (*C-E*) FISC were cultured in the absence or presence of different doses of H_2O_2 for various times (24 h in (*C*) and (*E*) and 36 h in (*D*)). In (*C*), the relative mRNA levels of selected p53 target genes were determined by RT-qPCR. The fold changes were quantified using relative levels of genes with H_2O_2 over that without H_2O_2 (n = 4 independent experiments). In (*D*), the cells were cultured in the presence of the CellEvent™ Caspase-3/7 Green Detection Reagent for 36 h and observed under a Zeiss cell discoverer 7 automated microscope. The percentage of apoptotic cells was calculated from cells in duplicate wells. In (*E*), the protein levels of S6 and pS6 were determined by Western blot. Quantification of the ratio of pS6 over total S6 by densitometry from 4 independent experiments was shown on the right. Data were presented as mean ± SD.

Movies S1-S6

Movies S1 and S2. Freshly-isolated muscle satellite cells (FISC) from control (S1) or *Paxbp1*-iKO (S2) mice were seeded in 24-well culture plate and cultured for 12 h. Cells were then observed by time-lapse microscopy for 48 h. Images were taken every 5 min.

Movies S3 and S4. FISC from *Paxbp1*-iKO were infected with adenovirus expressing RFP (S3) or Paxbp1 (S4) after plating. Viral medium was removed 24 h post infection and cultured for another 12 h before live-cell imaging. Videos were then recorded for 48 h. Images were taken every 5 min.

Movies S5 and S6. FISC from control (S5) and *Paxbp1*-iKO mice (S6) were seeded in 96 - well plate together with 4 μM of CellEvent™ Caspase-3/7 Green Detection Reagent (Thermo Fisher) and observed under a Zeiss cell discoverer 7 automated microscope for 72 hours. Images were taken every 10 min.