FIGURE S1

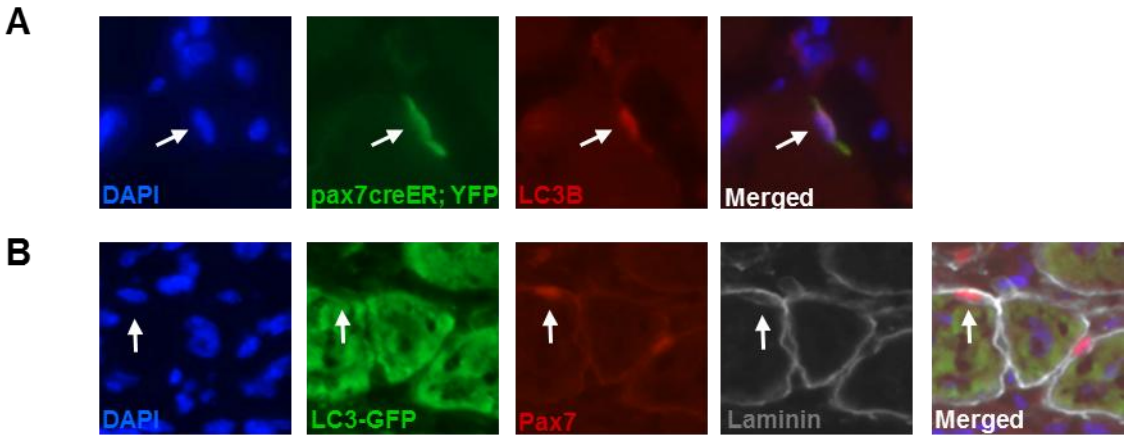

Figure S1. LC3B is expressed in SCs during early activation but not during self-renewal.

(**A**) Expression of LC3B in SCs during early activation. TAs of tamoxifen-treated Pax7$^{CreER/+}$; ROSA$^{eYFP/+}$ mice were injured and harvested on 1.5 days after injury. Cryosections were immunostained with anti-GFP, anti-LC3B, and anti-Pax7 antibodies.

(**B**) Expression of LC3B in self-renewing SCs. TAs of LC3-GFP mice were injured and harvested on 5 days after injury. Cryosections were immunostained with anti-GFP, anti-Pax7, and anti-Laminin antibodies to show that self-renewed SCs, Pax7^{+ve} cells under the basal lamina, are not expressing LC3B.

FIGURE S2

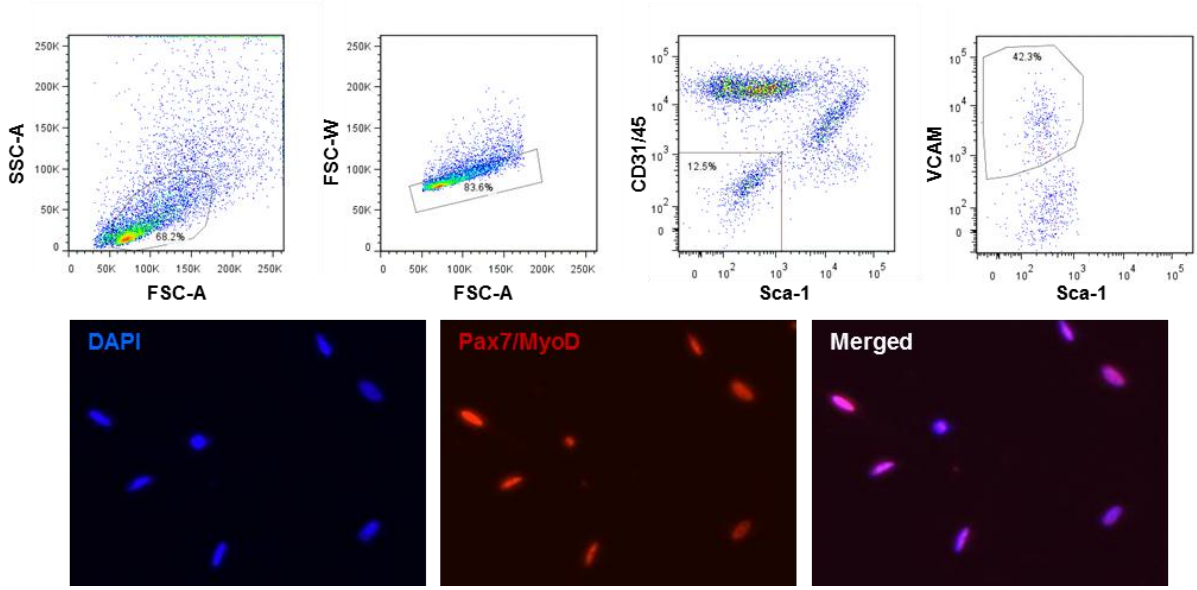

Figure S2. FACS scheme for purified population of SCs.

After muscles are digested and triturated into mononuclear cells, they are stained and subsequently sorted by negative selection for CD31, CD45, and Sca-1 and positive selection for VCAM. The resulting population of SCs is at least 98% pure by antibody staining for Pax7 and MyoD.

FIGURE S3

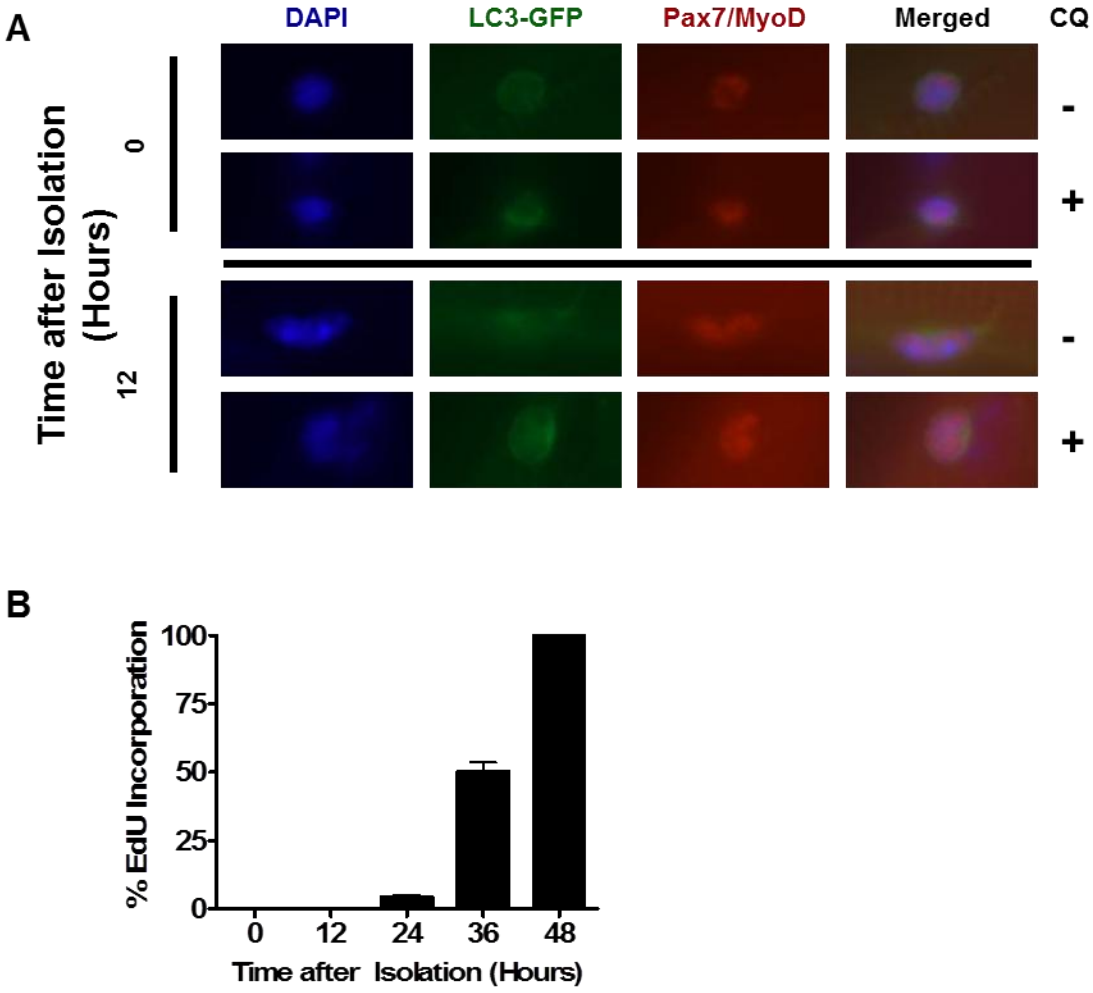

Figure S3. Timing of induction of autophagy and cell cycle entry of fiber-associated SCs.

(**A**) Immunostaining of single fibers from LC3-GFP mice cultured in the presence of EdU. Fibers were treated with CQ for 2 hours at every 12 hour time point prior to fixing.

(**B**) Kinetics of *ex vivo* activation of fiber-associated SCs. Single fibers from the EDL muscles were cultured with EdU for 48 hours, and fiber-associated SCs were assessed for EdU incorporation.

FIGURE S4

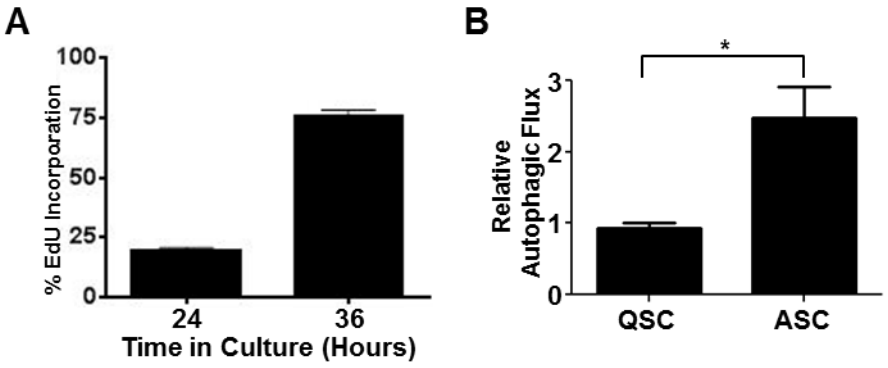

Figure S4. Timing of cell cycle entry and induction of autophagy in FACS-sorted SCs *in vitro*.

(**A**) Kinetics of EdU incorporation in *in vitro*-activated SCs. FACS-sorted QSCs were cultured with EdU for 24 hours and 36 hours and assessed for EdU incorporation. (*, $p<0.5$)

(**B**) Quantification of relative autophagic flux for QSCs and ASCs. The intensities of bands for LC3B-II in at least 3 independent western blots, of which a representative image is shown in Fig. 3C, were first normalized to the levels of GAPDH, and then the ratios of the intensities of +CQ to –CQ conditions were calculated for each population. (*, $p<0.05$)

FIGURE S5

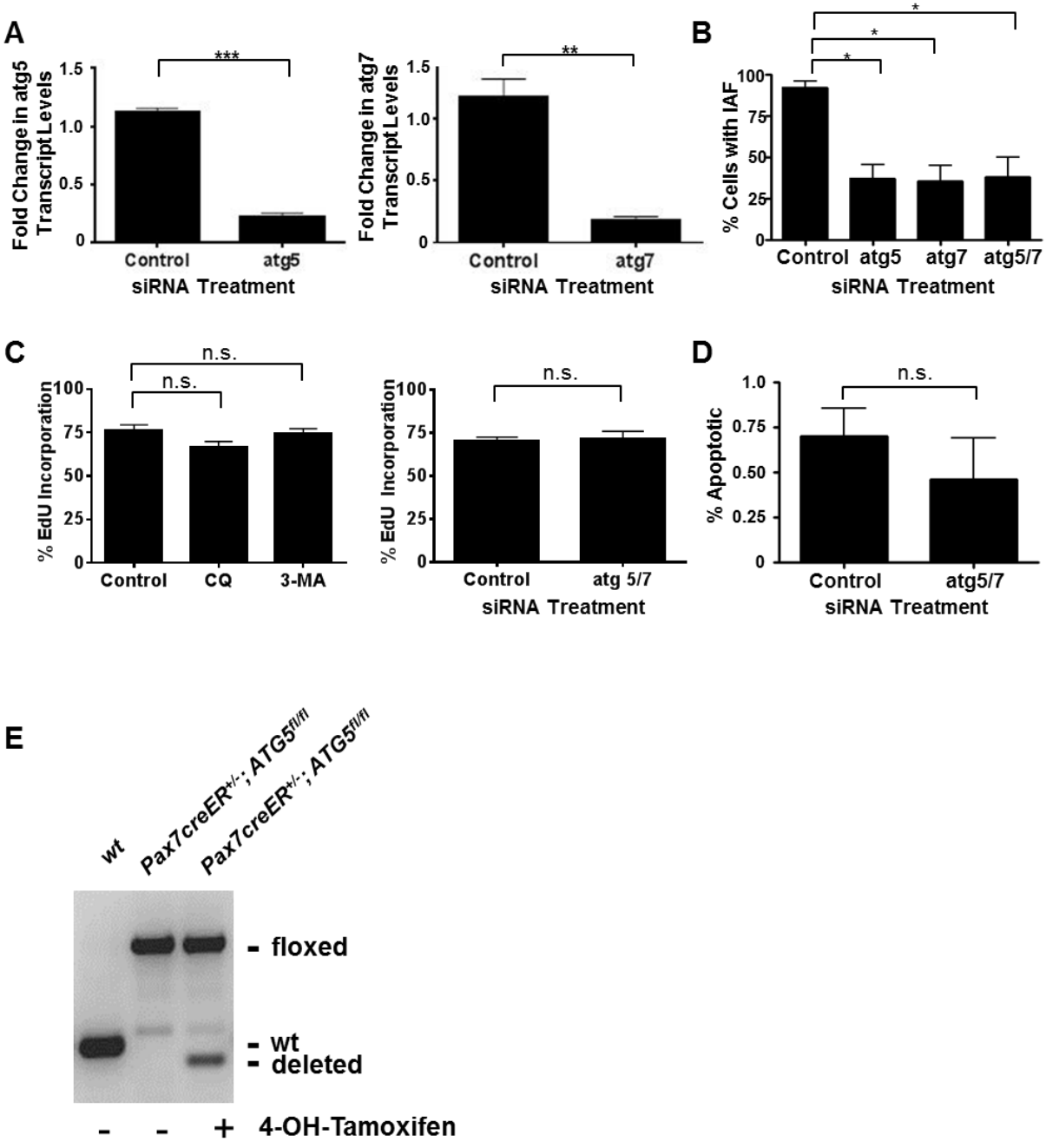
A
Fold Change in atg5 Transcript Levels
1.5
1.0
0.5
0
***
Control
atg5
siRNA Treatment
Fold Change in atg7 Transcript Levels
1.5
1.0
0.5
0
**
Control
atg7
siRNA Treatment
B
% Cells with IAF
100
75
50
25
0
*
*
*
Control
atg5
atg7
atg5/7
siRNA Treatment
C
% EdU Incorporation
100
75
50
25
0
n.s.
n.s.
Control
CQ
3-MA
% EdU Incorporation
100
75
50
25
0
n.s.
Control
atg 5/7
siRNA Treatment
D
% Apoptotic
1.0
0.75
0.50
0.25
0
n.s.
Control
atg5/7
siRNA Treatment
E
wt
Pax7creER+/-; ATG5fl/fl
Pax7creER+/-; ATG5fl/fl
- floxed
- wt
- deleted
- - + 4-OH-Tamoxifen

Figure S5. Knockdown of *atg5* and *atg7* leads to a reduction of transcript levels, decrease in autophagic flux, and delay in activation.

(**A**) Reduction in *atg5* and *atg7* transcripts. FACS-sorted QSCs from *wt* mice were transfected with siRNAs against *atg5* (left panel) and *atg7* (right panel) and cultured for 24 hours. RNA was then extracted and subjected to qPCR with TaqMan probes. (**, $p<0.005$; ***, $p<0.001$)

(**B**) Block in autophagy resulting in decreased autophagic flux during SC activation. QSCs were sorted from LC3-GFP mice and transfected with siRNAs against *atg5* and *atg7*. The cells were cultured for 24 hours, treated with CQ for 2 hours, and assessed for GFP-LC3 punctae. Cells with greater than 3 punctae were considered to have induced autophagic flux (IAF). Control cultures were transfected with a negative control siRNA. (*, $p<0.05$)

(**C**) Pharmacological or siRNA inhibition of autophagy does not lead to cell cycle arrest. (left panel) QSCs were sorted from *wt* mice, plated for culturing in the presence of EdU, and treated with CQ, 3-MA, or vehicle (control) the following day for 6-8 hours. The inhibitors were washed out and cultured with EdU until 36 hours after plating. (right panel) QSCs were also transfected with siRNAs against *atg5* and *atg7* after plating and cultured for 36 hours in the presence of EdU. EdU incorporation was then assessed. Control cultures were transfected with a negative control siRNA. (n.s., not significant)

(**D**) *atg5/7* siRNA transfection does not cause apoptosis in SCs. FACS-sorted QSCs from *wt* mice were transfected with siRNAs against *atg5* and *atg7* and cultured for 24 hours. The cells were then fixed and stained with an anti-activated Caspase 3 antibody. (n.s., not significant)

(**E**) Acute tamoxifen treatment leading to recombination of floxed *atg5* allele. 4-hyrdoxytamoxifen was added to all media in the procedure for the dissection and digestion of muscles for sorting from $Pax7^{CreER/+}$; $ATG5^{fl/fl}$ or *wt* animals. QSCs were FACS-sorted, cultured in media with 4-hydroxytamoxifen for 24 hours, and harvested for PCR. PCR primers and conditions were performed according to Hara et al (2006).

FIGURE S6

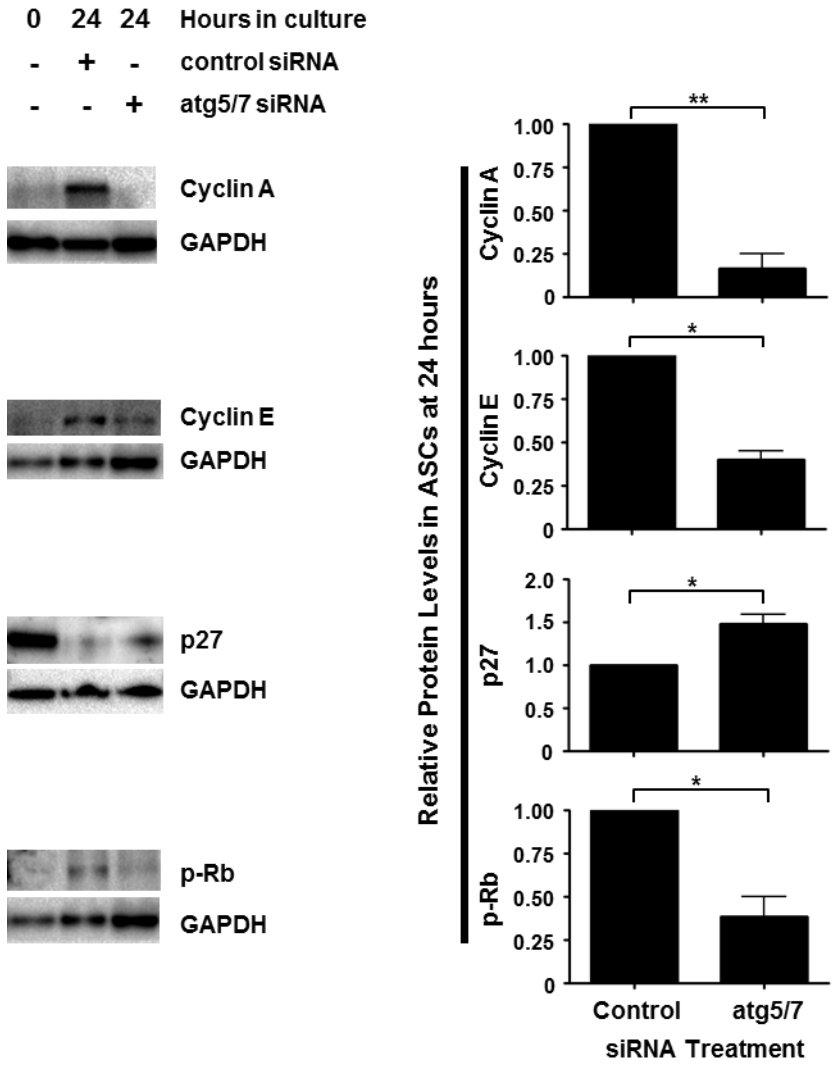

Figure S6. Assessment of G1/S cell-cycle markers upon *atg5/7* siRNA transfection.

FACS-sorted QSCs from *wt* mice were transfected with control or *atg5* and *atg7* siRNAs and cultured for 24 hours. (left panel) Western analyses for Cyclin A, Cyclin E, p27, and phospho-Rb (p-Rb) were performed on protein lysates from untransfected QSCs and from control- and *atg5/7* siRNA-transfected SCs cultured for 24 hours. (right panel) Relative protein levels were calculated from 3 independent experiments by normalizing bands for GADPH. (*, $p<0.05$; **, $p<0.01$)

FIGURE S7

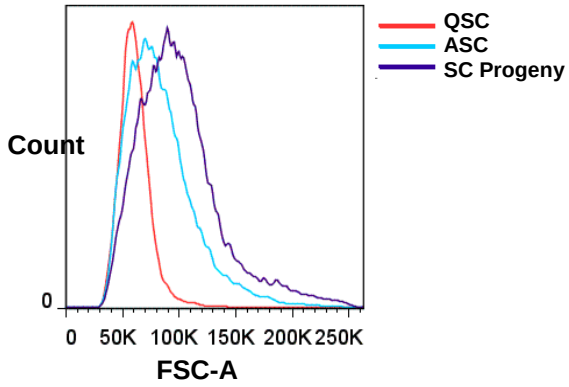

Figure S7. Quantification of QSC and ASC cell volumes.

QSCs, ASCs 1.5 days after injury, and SC progeny 2.5 days after injury were evaluated by FACS for their forward scatter (FSC) properties as a reflection of cell size. Quantitative analyses of replicate studies are presented in Fig. 4D.

FIGURE S8

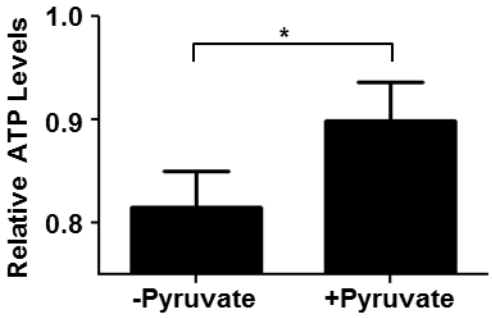

Figure S8. Increase in ATP levels in *atg5/7* siRNA-transfected SCs after sodium pyruvate treatment.

FACS-sorted QSCs from *wt* mice were transfected with *atg5/7* siRNAs and cultured in the presence or absence of sodium pyruvate for 24 hours. Cells were harvested, assayed for ATP content, and the ATP contents were normalized to those for control-transfected QSCs. (*, $p<0.01$)

FIGURE S9

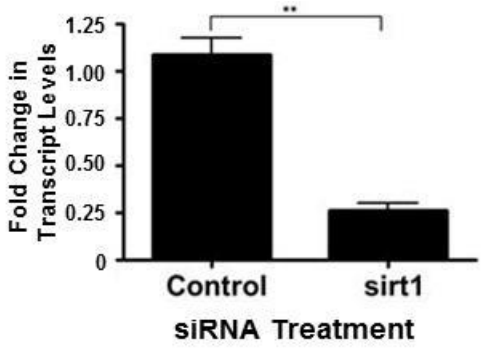

Figure S9. siRNA knockdown of *sirt1*.

FACS-sorted QSCs from *wt* mice were transfected with *sirt1* or negative control siRNAs and cultured for 24 hours. RNA was then extracted and subjected to qPCR with TaqMan probes. Relative transcript levels were determined by normalization to untransfected SCs. (**, $p<0.005$)

FIGURE S10

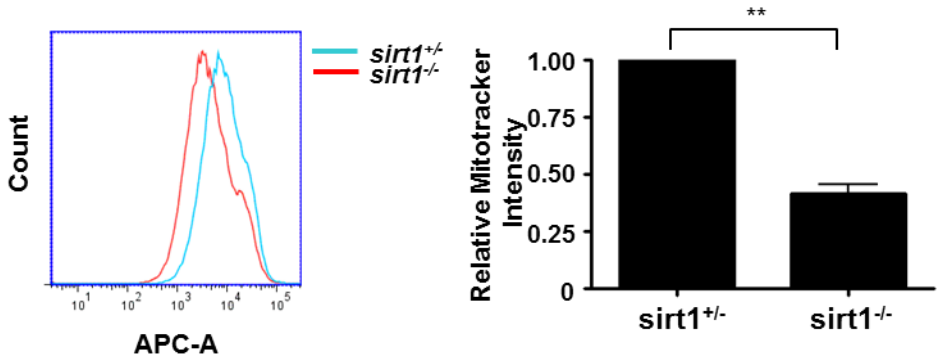

Figure S10. Mitochondrial activity in *sirt1*$^{-/-}$ and *sirt1*$^{+/-}$ QSCs.

Mitochondrial activity in *sirt1*$^{-/-}$ and *sirt1*$^{+/-}$ QSCs. (left panel) Mononuclear cells obtained from muscles of tamoxifen-treated Pax7$^{creER/+}$;sirt1$^{fl/+}$; ROSA$^{eYFP/+}$ and Pax7$^{creER/+}$;sirt1$^{fl/fl}$; ROSA$^{eYFP/+}$ were treated with MitoTracker Deep Red FM, sorted with the YFP marker, and analyzed by FACS. (right panel) Relative MitoTracker signal intensities were calculated from 3 independent experiments. (**, $p<0.01$)