Expanded View Figures

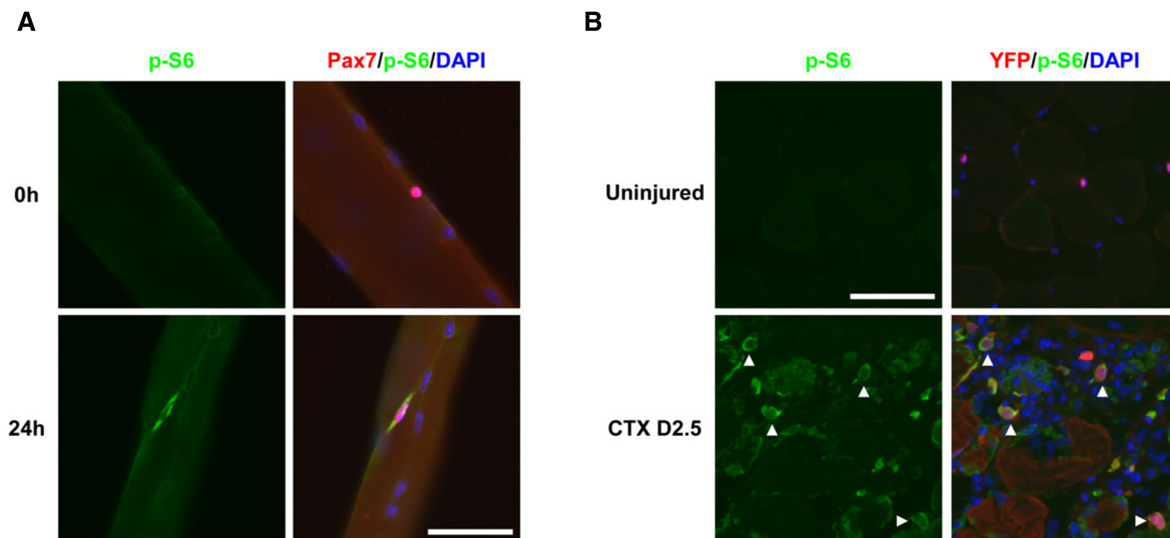

Figure EV1. The PI3K pathway was active in ASC but not QSC.

A Freshly isolated single myofibers from 2-month-old wild-type mice were fixed either immediately or 24 h after culturing.

B TA muscles from uninjured or cardiotoxin (CTX)-injured mice (2.5 days post-injury) were dissected, cryosectioned, and fixed. Myofibers from (A) and TA muscle sections from (B) were then subjected to immunostaining for p-S6 (green) together with Pax7 (A) and YFP (B) (red), respectively. The nuclei were counterstained with DAPI (blue). Arrowheads: YFP^+ MuSCs. Scale bars: 25 µm.

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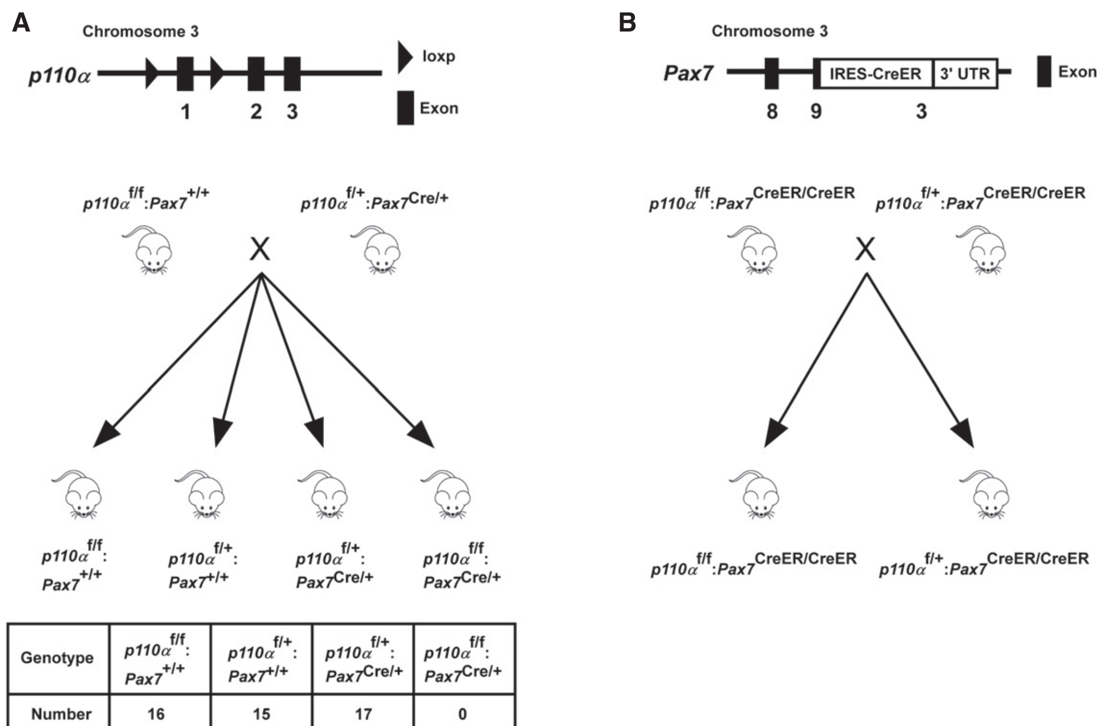

Figure EV2. Generation of MuSC-specific *p110α* knockout (KO) mice.

A Non-inducible *p110α* KO mice. Top: the schematic showing the floxed exon at the *p110α* locus. Middle: the mating strategy to generate the non-inducible $p110\alpha^{f/f}$:$Pax7^{Cre/+}$ mice. Bottom: the number of offspring of different genotypes obtained based on the mating strategy above.

B Tamoxifen-inducible *p110α* KO mice. Top: the schematic showing the location of CreER in $Pax7^{CreER(GaKa)}$ mice. Bottom: the mating strategy to generate the tamoxifen-inducible $p110\alpha^{f/f}$:$Pax7^{CreER/CreER}$ mice.

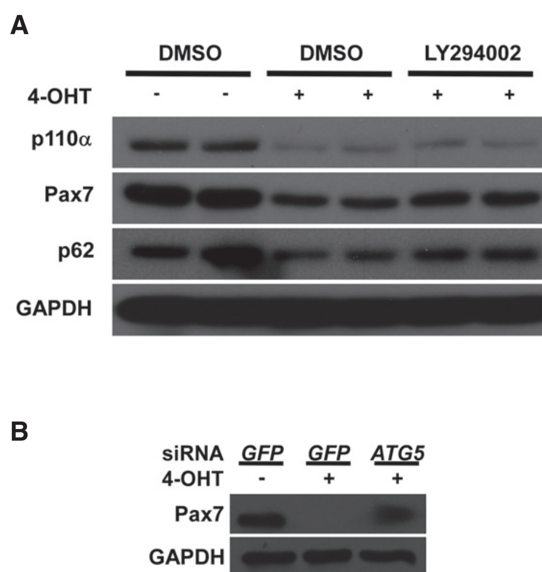

Figure EV3. Reduction in Pax7 protein in *p110α*-null MuSCs was mediated by autophagy.

A, B FACS-isolated MuSCs from iKO mice (without Tmx) were cultured for 2 days followed by 1 day of 4-OHT treatment. Then, the cells were either treated with DMSO or LY294002 (10 μM) in (A) or transfected with siRNAs in (B) as indicated and grown in the presence of 4-OHT for another day before harvest. Whole-cell lysates were subjected to Western blotting.

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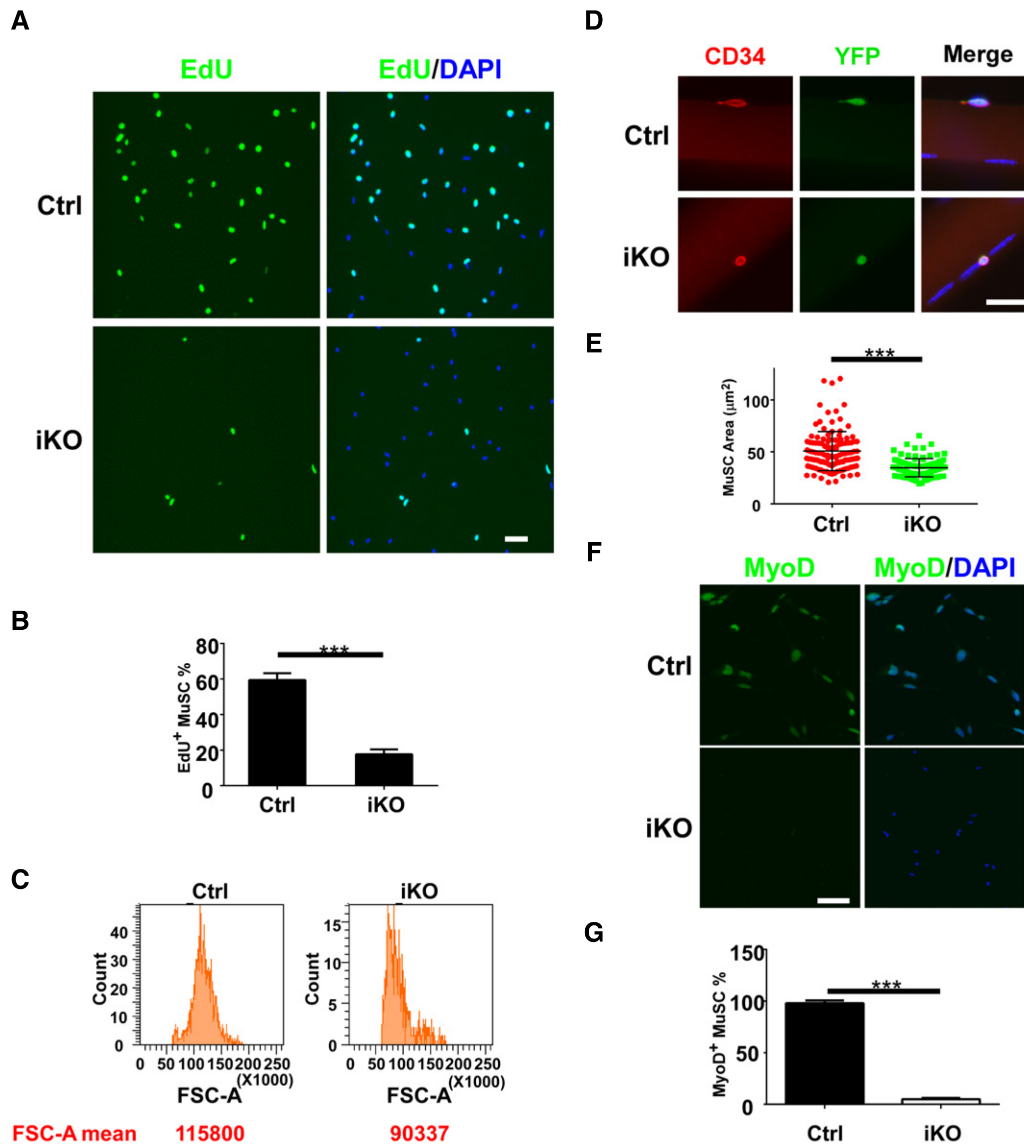

Figure EV4. ***p110α*-null MuSCs were arrested in the G_0 state.**

A FACS-isolated MuSCs from the tamoxifen (Tmx)-treated Ctrl and iKO mice were cultured for 36 h followed by a 4-h EdU pulse. Cells were fixed and stained for EdU (green) and DAPI (blue).

B Quantification of the EdU^+ MuSCs from (A) by counting ~600 MuSCs from triplicate wells.

C Histograms showing the mean FSC-A values of MuSCs freshly isolated from the Ctrl and iKO mice.

D Freshly isolated single myofibers ($n > 20$) from the Ctrl and iKO mice ($n = 3$ in each group) were immediately fixed and stained for CD34 (red), YFP (green), and DAPI (blue). A representative image is shown.

E Quantification of the areas of MuSCs from (D) based on CD34 staining. More than 100 MuSCs were calculated for each group of mice.

F FACS-isolated MuSCs from the Tmx-treated Ctrl and iKO mice were cultured for 36 h followed by staining for MyoD (green) and DAPI (blue).

G Quantification of the $MyoD^+$ ASCs from (F) by counting ~600 ASCs in triplicate wells. Scale bars: 25 μm. In (B, E, G), the data are presented as mean ± s.d. ***$P < 0.001$.

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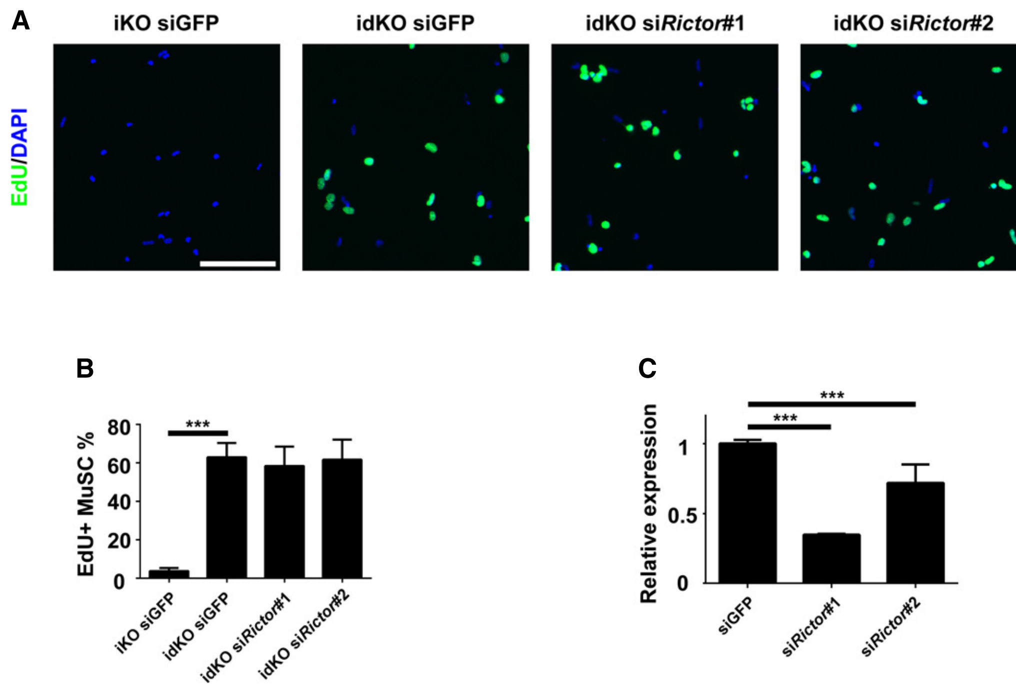

Figure EV5. mTORC2 was not required for the cell cycle re-entry of MuSCs from idKO mice.

A FACS-isolated MuSCs from iKO and idKO mice were transfected with siRNAs as indicated in the presence of EdU for 36 h followed by cell fixation and staining for EdU (green) and DAPI (blue).

B Quantification of the EdU^+ MuSCs in (A) by counting ~600 MuSCs in triplicate wells.

C Triplicate C2C12 cells were transfected with indicated siRNAs for 24 h. Total RNAs were extracted. The mRNA levels of *Rictor* were measured by RT–qPCR. Scale bar: 50 μm. In (B, C), the data are presented as mean ± s.d. ***$P < 0.001$.

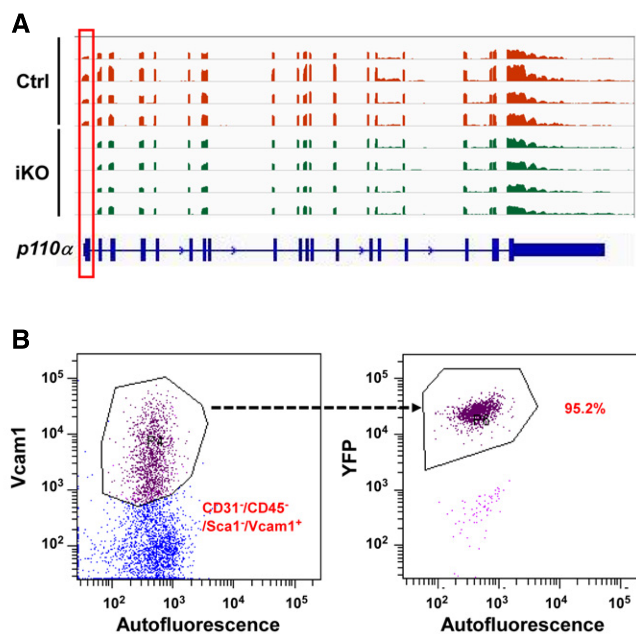

Figure EV6. Examination of the Cre-mediated gene deletion efficiency.

A The genome browser view showing the expression of *p110α* exons in MuSCs from the Ctrl and iKO mice based on our RNA-seq data. Note the absence of the transcripts from the 1st exon in MuSCs from four mutant iKO mice.

B The representative FACS plots showing the percentage of YFP^+ MuSCs among the $CD31^-$/$CD45^-$/$Sca1^-$/$Vcam1^+$ cell population, which indirectly revealed the Cre-mediated gene deletion efficiency at the *p110α* locus.

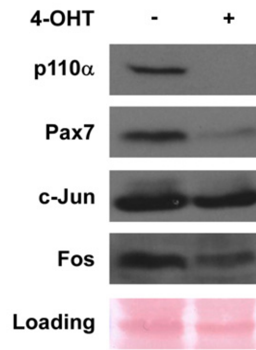

Figure EV7. Fos protein levels were reduced in cultured *p110α*-null primary myoblasts.

FACS-isolated MuSCs from iKO mice (without Tmx) were cultured for 2 days followed by 2 days of 4-OHT treatment before harvest. Whole-cell lysates were subjected to Western blotting.

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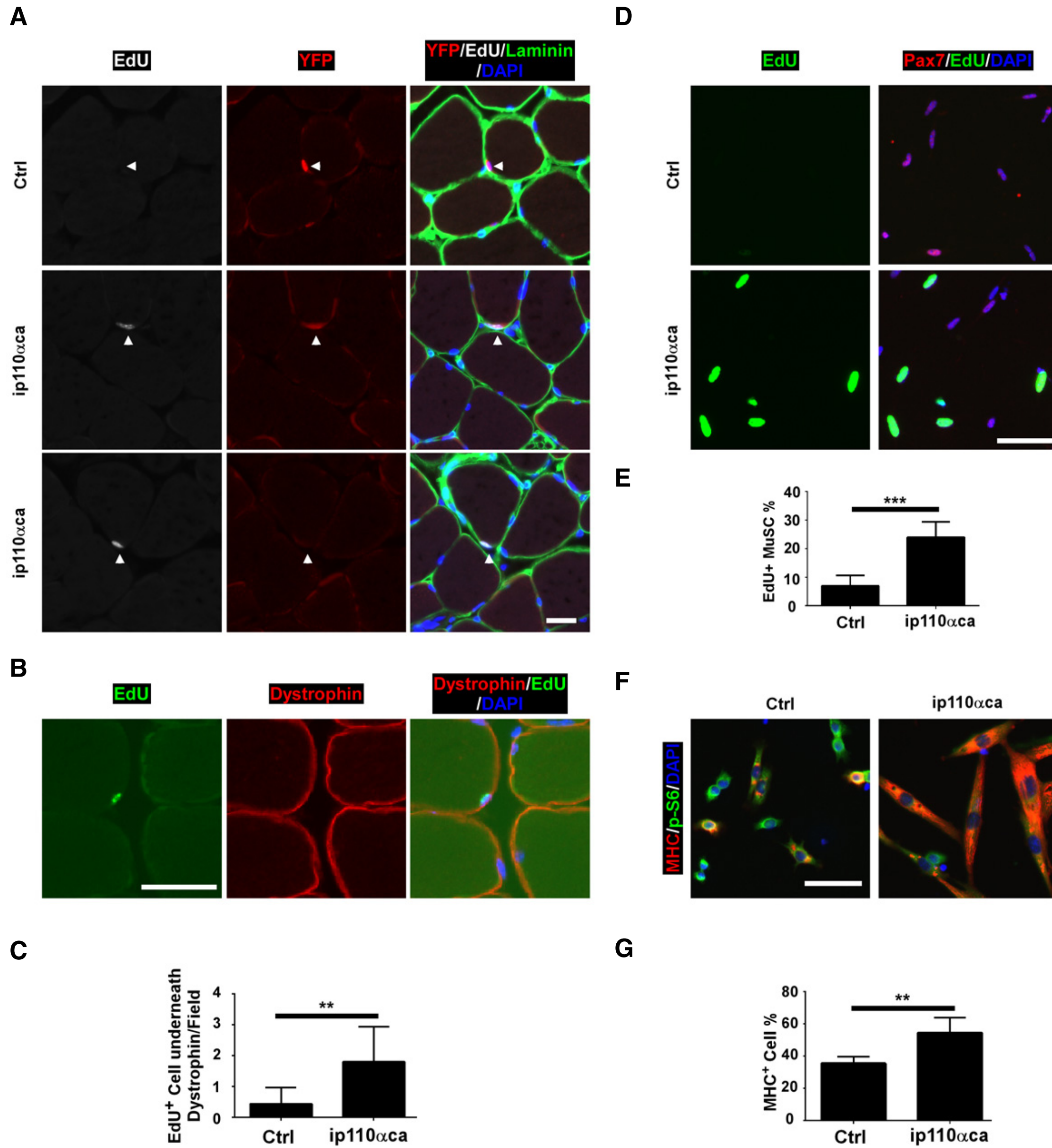

Figure EV8. MuSCs from ip110αca mice proliferated and differentiated faster.

A TA muscle sections from the Ctrl and ip110αca mice as described in Fig 7A were stained for EdU (white), YFP (red), laminin (green), and DAPI (blue). Arrowheads: YFP^+/EdU^-, YFP^-/EdU^+, or YFP^+/EdU^+ cells.

B TA muscle sections as described above were stained for EdU (green) and dystrophin (red).

C Quantification of the EdU^+ cells underneath the $dystrophin^+$ sarcolemma in (B). Three pairs of mice were examined including five TA muscle sections per mouse and three microscopic fields per section.

D FACS-isolated MuSCs from the Ctrl or ip110αca mice were cultured for 24 h followed by a 4-h EdU pulse.

E Quantification of the EdU^+ ASCs in (D) by counting ~600 ASCs in triplicate wells.

F FACS-isolated MuSCs from the Ctrl or ip110αca mice were cultured for 3 days followed by cell fixation and staining for MHC (red), p-S6 (green), and DAPI (blue).

G Quantification of the MHC^+ cells in (F) by counting ~600 nuclei in triplicate wells. Scale bars: 25 μm. In (C, E, G), the data are presented as mean ± s.d. $^{**}P < 0.01$; $^{***}P < 0.001$.

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