Expanded View Figures

Figure EV1. Muscle stem cell extraction and effects of AMPKα2 loss or tamoxifen injection on muscle stem cell fate *in vitro* and *in vivo*.
Related to Fig 1.

A Gating strategy to sort MuSCs from hindlimbs. MuSCs were CD45/CD31/Sca1$^-$ and CD34/α7int$^+$ cells. The dot plot on the right is a representative example of sorted MuSCs.

B Pax7Ki67MyoD labeling was performed on WT and AMPKα1$^{-/-}$ MuSCs at the time of the induction of differentiation (activation). Percentage of Pax7$^+$Ki67/MyoD$^-$, Pax7$^+$Ki67/MyoD$^+$, Pax7$^-$Ki67/MyoD$^+$ and Pax7$^-$Ki67/MyoD$^-$ cells were quantified.

C, D Pax7Ki67MyoD labeling was performed on WT and AMPKα2$^{-/-}$ MuSCs after 48 h of culture in differentiation conditions: (C) percentages of Pax7$^+$Ki67/MyoD$^-$, Pax7$^+$Ki67/MyoD$^+$, Pax7$^-$Ki67/MyoD$^+$ and Pax7$^-$Ki67/MyoD$^-$ cells were quantified and (D) Pax7 (green), Ki67/MyoD (red), nuclei (blue) labeling of MuSCs. White arrows show Pax7$^+$ quiescent cells.

E MuSCs (CD45/CD31/Sca1$^-$CD34/α7int$^+$) and non-myogenic cells (CD45/CD31/Sca1$^+$) were extracted from total hindlimbs at day 0 and from injured TA (28 days post-CTX) of Pax7-CreER$^{T2/+}$:AMPKα1$^{fl/fl}$ mice injected with PBS (Pax7-α1$^{+/+}$) or from Pax7-CreER$^{T2/+}$:AMPKα1$^{fl/fl}$ injected with tamoxifen (2 mg/mouse, Pax7-α1$^{-/-}$) during 4 days. DNA was extracted, and PCR was performed to verify the deletion of AMPKα1 at day 0 (i.e., 1 week after the first tamoxifen injection, see Fig 1F).

F–K Tibialis anterior muscles from Pax7-CreER$^{T2/+}$ mice daily injected with PBS or tamoxifen were removed before (day 0) or 28 days after CTX damage (see Fig 1F). Muscle cryosections were prepared for Pax7Ki67MyoD labeling or hematoxylin–eosin staining. (F) Hematoxylin–eosin staining, (G) Pax7 (green), Ki67/MyoD (red), nuclei (blue) labeling. White arrows show Pax7$^+$ quiescent cells. (H) Number of fibers per muscle, (I) number of nuclei per fiber, (J) number of Pax7$^+$Ki67/MyoD$^-$ cells per muscle and (K) ratio of muscle mass per body mass were calculated.

Data information: Data are means ± SEM from at least three *in vitro* independent experiments or three *in vivo* independent experiments. $^{**}P < 0.01$ versus WT; $^{\$}P < 0.05$, $^{\$\$\$}P < 0.001$ versus day 0. Student's *t*-test. Scale bars = 100 μm (D) or 50 μm (F, G).

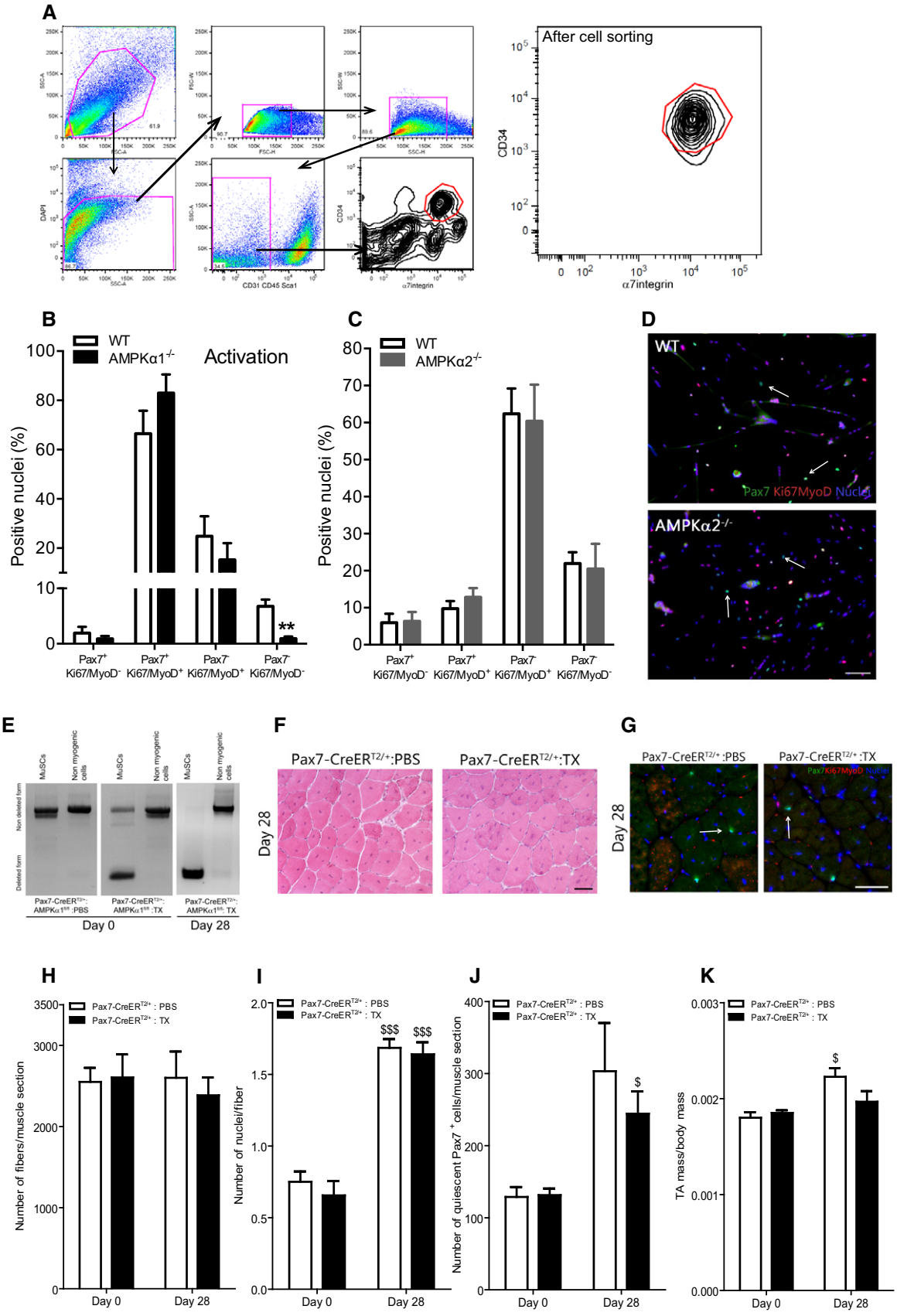
A
After cell sorting
CD34
α7integrin
B
WT
AMPKα1-/-
Activation
Positive nuclei (%)
Pax7+ Ki67/MyoD-
Pax7+ Ki67/MyoD+
Pax7- Ki67/MyoD+
Pax7- Ki67/MyoD-
**
C
WT
AMPKα2-/-
Positive nuclei (%)
D
WT
Pax7 Ki67MyoD Nuclei
AMPKα2-/-
E
MuSCs
Non myogenic cells
Non deleted form
Deleted form
Day 0
Day 28
F
Pax7-CreER T2/+:PBS
Pax7-CreER T2/+:TX
Day 28
G
Pax7-CreER T2/+:PBS
Pax7-CreER T2/+:TX
Day 28
H
Pax7-CreER T2/+ : PBS
Pax7-CreER T2/+ : TX
Number of fibers/muscle section
Day 0
Day 28
I
Number of nuclei/fiber
$$$
$$$
J
Number of quiescent Pax7 + cells/muscle section
$
K
TA mass/body mass
$

Figure EV1.

Figure EV2. Muscle stem cell microenvironment.
Related to Fig 2.

A Mass of gastrocnemius (GAS) muscles from Pax7-CreER$^{T2/+}$:AMPKα1$^{fl/fl}$ mice injected with PBS (Pax7-α1$^{+/+}$ mice) or tamoxifen (2 mg/mouse; Pax7-α1$^{-/-}$ mice) were analyzed 28 days after cardiotoxin (CTX) damage. Ratio of muscle mass per body mass was calculated.

B Populations of Ly-6C/G^{hi}F4/80^{low} and Ly-6C/G^{neg}F4/80^{hi} macrophages in regenerating tibialis anterior (TA) muscles of Pax7-α1$^{+/+}$ or Pax7-α1$^{-/-}$ mice were quantified in percentage of total F4/80^{+} cells.

C Representative dotplots of (B) at day 3 of regeneration.

D–H Tibialis anterior (TA) muscles from AMPKα1$^{fl/fl}$ (HSA-α1$^{+/+}$) and HSA-Cre$^{+/-}$:AMPKα1$^{fl/fl}$ (HSA-α1$^{-/-}$) mice were removed before (day 0) or 28 days after CTX damage. (D) Number of Pax7^{+} per 100 fibers, (E) cross-sectional area and (F) ratio of muscle mass per body mass were calculated. (G) Hematoxylin–eosin staining of day 28 regenerating TA muscles. (H) AMPKα1 expression in liver (Li), heart (He), soleus (So) muscle, GAS (Gc) muscle and TA muscle from AMPKα1$^{fl/fl}$ (HSA-α1$^{+/+}$) and HSA-Cre$^{+/-}$:AMPKα1$^{fl/fl}$ (HSA-α1$^{-/-}$) was verified by Western blot.

I–K MuSCs were extracted from total hindlimbs of Pax7-LKB1$^{+/+}$ and Pax7-LKB1$^{-/-}$ mice. Pax7Ki67MyoD labeling was performed after 48 h of culture in differentiation conditions. (I) Percentage of Pax7^{+}Ki67/MyoD^{-} (quiescent cells), Pax7^{+}Ki67/MyoD^{+} (activated cells), Pax7^{-}Ki67/MyoD^{+} (differentiating cells) and Pax7^{-}Ki67/MyoD^{-} cells (differentiated cells), and (J) fusion index were calculated. (K) Pax7 (green), Ki67/MyoD (red), nuclei (blue) labeling of MuSCs. White arrows show quiescent Pax7^{+} cells.

Data information: Data are means ± SEM from at least three animals. *$P < 0.05$, **$P < 0.01$ versus Pax7-α1$^{+/+}$ or HSA-α1$^{+/+}$, $^{SS}P < 0.01$, $^{SSS}P < 0.001$ versus day 0. Student's *t*-test. Scale bars = 50 μm (G) or 100 μm (K).

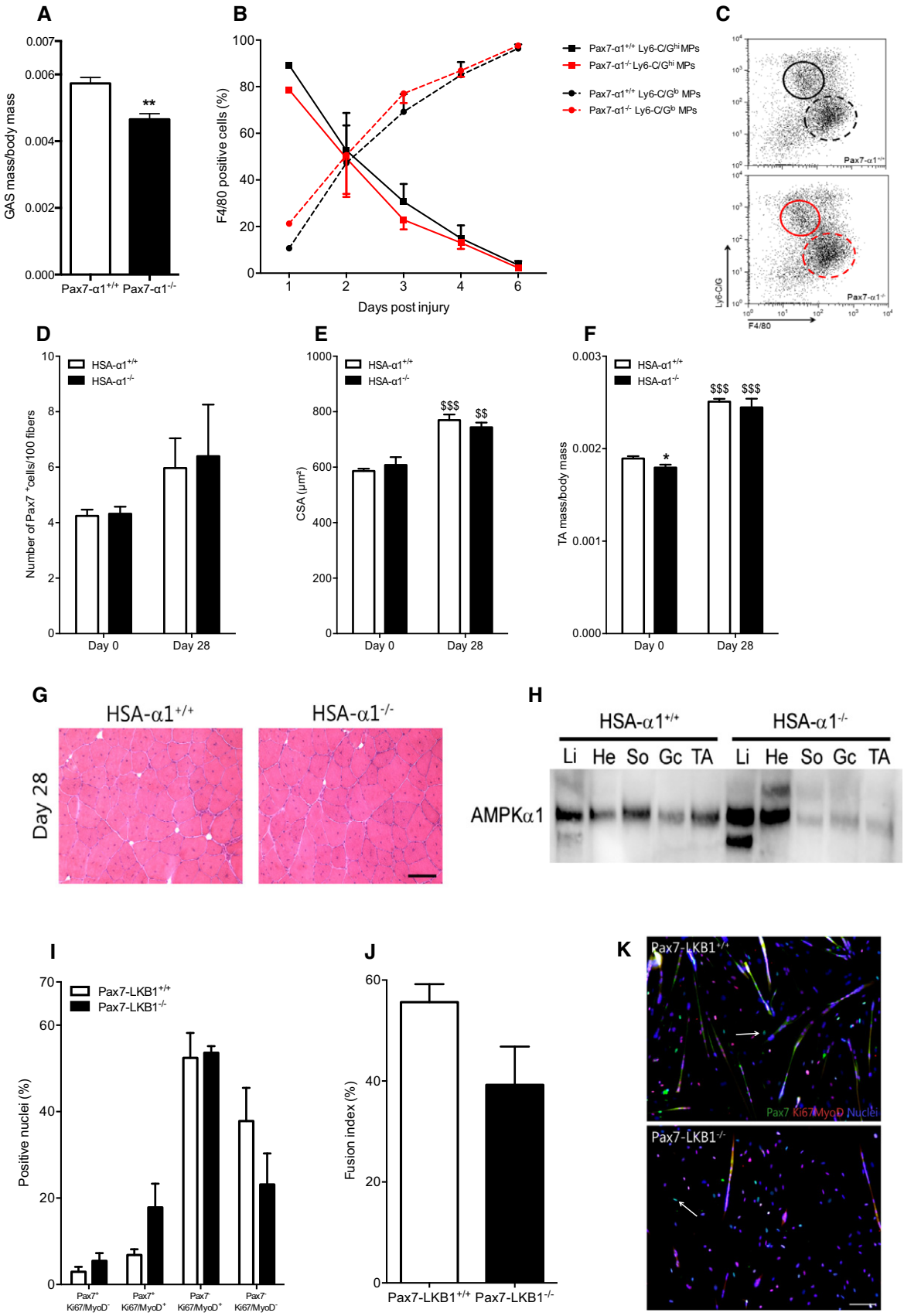
A
GAS mass/body mass
0.007
0.006
0.004
0.002
0.000
**
Pax7-α1+/+ Pax7-α1-/-
B
F4/80 positive cells (%)
100
80
60
40
20
0
1
2
3
4
6
Days post injury
Pax7-α1+/+ Ly6-C/Ghi MPs
Pax7-α1-/- Ly6-C/Ghi MPs
Pax7-α1+/+ Ly6-C/Gb MPs
Pax7-α1-/- Ly6-C/Gb MPs
C
Pax7-α1+/+
Pax7-α1-/-
Ly6-C/G
F4/80
D
Number of Pax7 +cells/100 fibers
HSA-α1+/+
HSA-α1-/-
Day 0
Day 28
E
CSA (µm²)
$$$
$$
F
TA mass/body mass
*
G
HSA-α1+/+
HSA-α1-/-
Day 28
H
HSA-α1+/+
HSA-α1-/-
Li He So Gc TA
AMPKα1
I
Positive nuclei (%)
Pax7-LKB1+/+
Pax7-LKB1-/-
Pax7+ Ki67/MyoD-
Pax7+ Ki67/MyoD+
Pax7- Ki67/MyoD+
Pax7- Ki67/MyoD-
J
Fusion index (%)
Pax7-LKB1+/+ Pax7-LKB1-/-
K
Pax7-LKB1+/+
Pax7 Ki67 MyoD Nuclei
Pax7-LKB1-/-

Figure EV2.

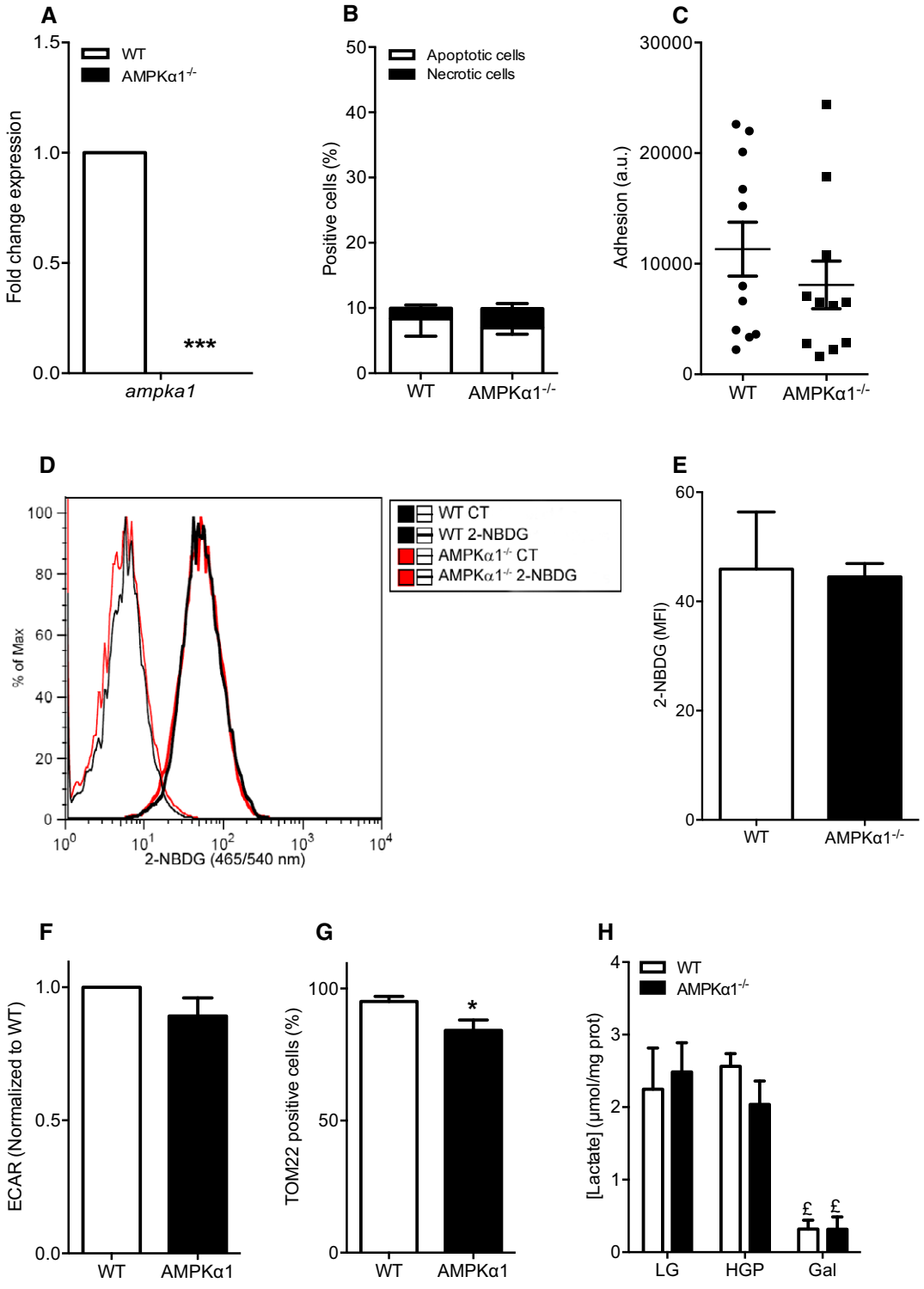

Figure EV3

Figure EV3. Metabolic characteristics of AMPKα1-depleted muscle precursor cells.
Related to Fig 3.

A Gene expression of *ampka1* in MPCs was quantified by qPCR.
B Apoptosis and necrosis of WT and AMPKα1$^{-/-}$ MPCs in proliferating conditions were analyzed by flow cytometry using annexin V/propidium iodide labeling.
C MPC adhesion was quantified 6 h after seeding.
D, E MPCs were cultured in proliferating conditions for 24 h and further incubated 3 h with 20 μM 2-NBDG: (D) representative histogram of 2-NBDG labeling and (E) median fluorescence intensity (MFI) of 2-NBDG labeling in MPCs.
F Extracellular acidification rate (ECAR) of WT and AMPKα1$^{-/-}$ MPCs was measured.
G Percentage of TOM22-positive MPCs was quantified. MPCs that express TOM22 below the level of detection for TOM22 antibody are negative for TOM22 in these conditions.
H MuSCs were cultured for 48 h in differentiation conditions under glycolytic [25 mM glucose + 1 mM pyruvate (HGP) or 5 mM glucose (LG)] or oxidative [10 mM galactose (Gal)] stimulation and lactate concentration were quantified in supernatants.

Data information: Results are means ± SEM from at least four *in vitro* experiments. *$P < 0.05$, ***$P < 0.01$ versus WT. $^{£}P < 0.05$ versus LG. Student's *t*-test.

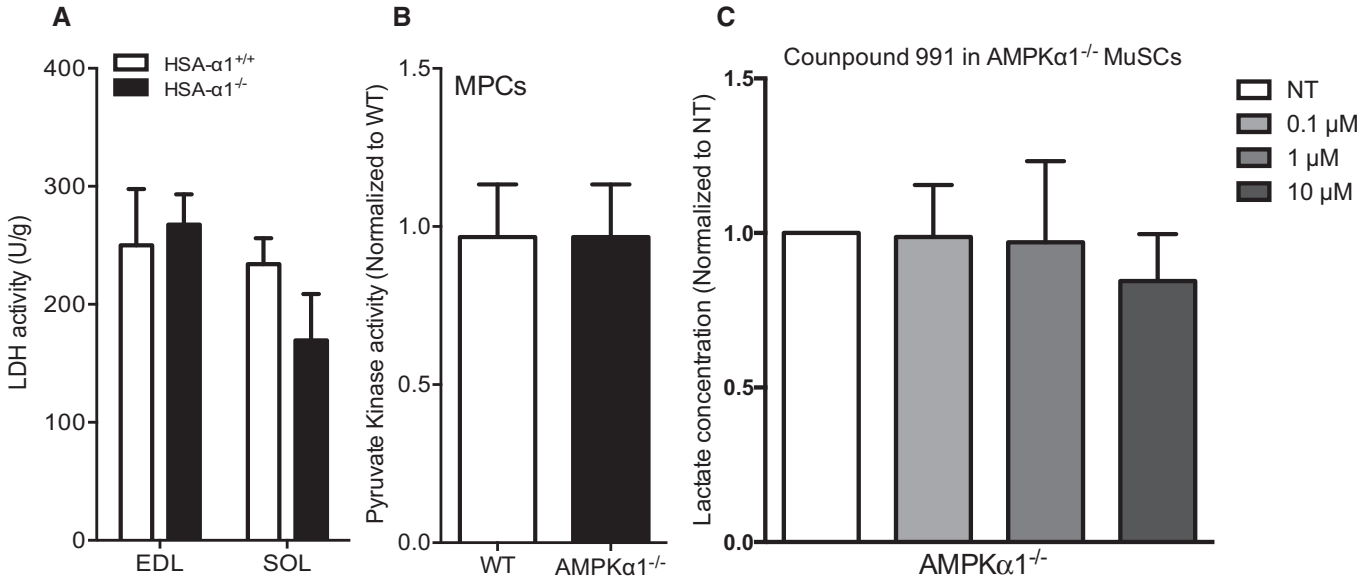

Figure EV4. LDH activity is regulated by AMPKα1.
Related to Fig 4.

A LDH activity from HSA-α1$^{+/+}$ and HSA-α1$^{-/-}$ EDL and soleus (SOL) muscle fibers was quantified.
B Pyruvate kinase activity was quantified in WT and AMPKα1$^{-/-}$ MPCs.
C Lactate concentration in the culture medium from AMPKα1$^{-/-}$ MuSCs cultured in differentiation conditions with 991 (0.1, 1 or 10 μM) during 48 h.

Data information: Data are means ± SEM from at least three *in vitro* and *in vivo* independent experiments.

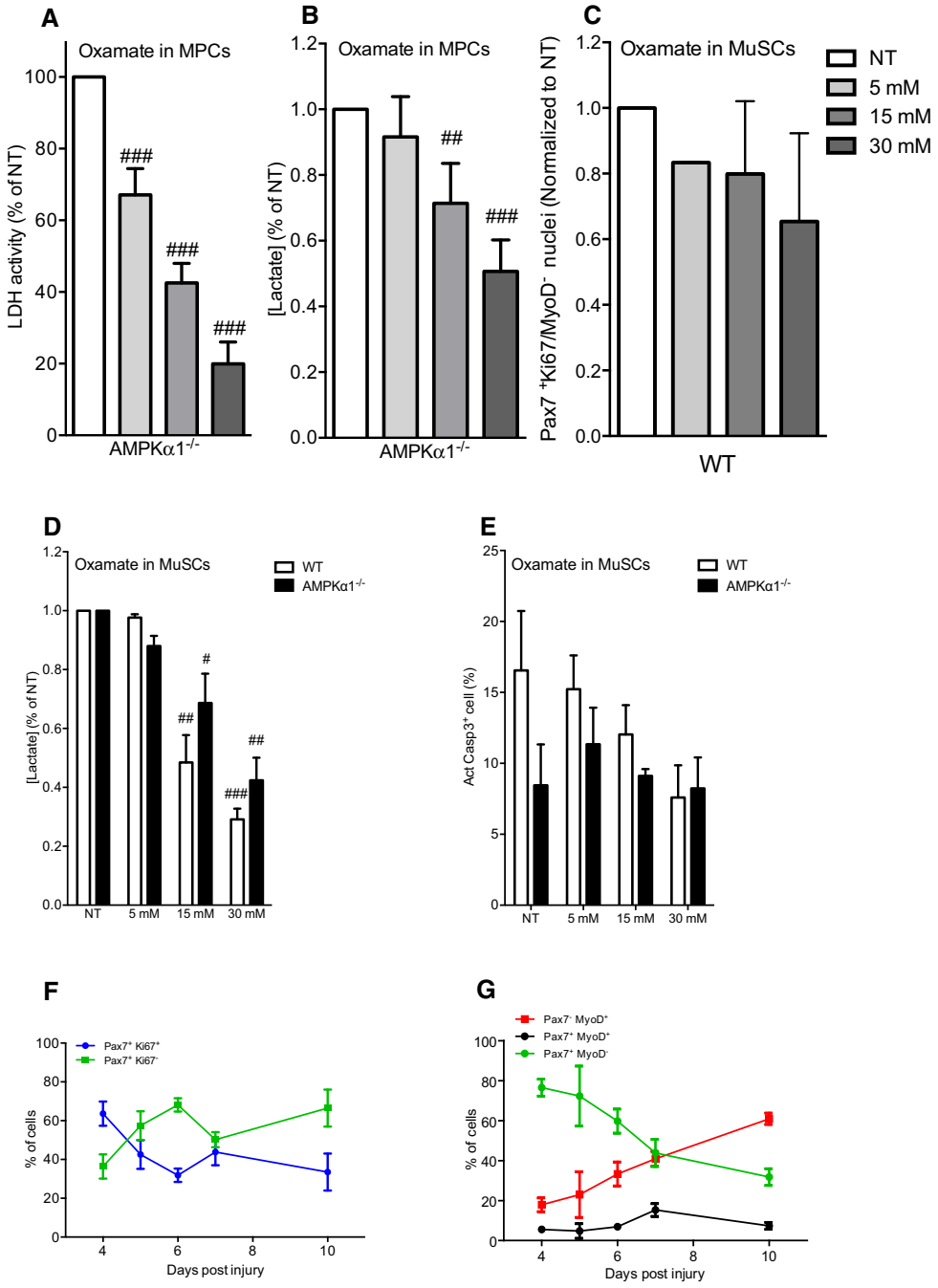

Figure EV5. Regulation of LDH activity *in vitro* and muscle stem cell fate *in vivo*.
Related to Fig 5.

A, B (A) LDH activity and (B) lactate concentration were quantified from AMPKα1$^{-/-}$ MPCs cultured in differentiation conditions with oxamate (0 (NT), 5, 15 and 30 mM) during 48 h.

C Percentage of Pax7^{+}Ki67/MyoD^{-} (quiescent cells) from WT MuSCs cultured in differentiation conditions with oxamate (0 (NT), 5, 15 and 30 mM) during 48 h was calculated.

D Lactate concentration in the culture medium of WT and AMPKα1$^{-/-}$ MuSCs cultured in differentiation conditions with oxamate (0 (NT), 5, 15 and 30 mM) during 48 h was quantified.

E Apoptosis was assessed by the number of positive cell for active Casp3 by immunofluorescence in presence of oxamate (0 (NT), 5, 15 and 30 mM) during 48 h.

F Pax7^{+}Ki67^{+} (proliferating) and Pax7^{+}Ki67^{-} (non-proliferating) MuSCs were quantified.

G Pax7^{+}Myo^{-} (undifferentiated), and Pax7^{+}MyoD^{+} (activated,) and Pax7^{-}MyoD^{+} (differentiated) MuSCs were quantified during skeletal muscle regeneration.

Data information: Data are means ± SEM from at least three *in vitro* and *in vivo* independent experiments (n = 2 for the oxamate 5 mM in MuSCs, Fig EV5C). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.01$ versus NT. Student's t-test.