Supplemental Figure S1. Expression patterns of Lkb1 during muscle development and satellite cells activation. (A) $Lkb1^{+}$ cells in cross sections of embryos from E10.5 to E17.5. Scale bars: 100 μm. (B-D) Relative mRNA levels *Lkb1* and *Myod* in anterior somite (B), posterior somite (C) and hindlimb (D). (E, F) Immunostaining (E) and realtime PCR (F) results showing expression and relative mRNA levels of Lkb1 in quiescent and activated satellite cells. Scale bars: 30 μm.

Supplemental Figure S2. Strategy and specificity of *MyoD* ^{Cre} mediated deletion of Lkb1 in myogenic lineage cells. $Lkb1^{flox/flox}$ targeting strategy. Exons 3-6 encode the kinase domain and the deletion of Exon 3-6 results in frame shift and a premature stop coden in Exon7. (B, C) Relative levels of Lkb1 mRNA (B) and protein (C) in different muscle tissues and myoblasts. (D, E) Relative levels of Lkb1 mRNA (D) and protein (E) in several non-muscle tissues. (F) Body weight of the WT and MyoD-$Lkb1^{flox/+}$ mice at birth and 10-wk old. n = 3-5. (G) Organ mass and relative weight of organs normalized to body weight of WT and MyoD-Lkb1 mice at 10-wk-old. n=5.

Supplemental Figure S3. Myogenic genes expression in WT and MyoD-Lkb1 embryos at E10.5. (A) $Pax7^{+}$ cells in somites of WT and MyoD-Lkb1 embryos at E10.5. (B) MyoD and MyoG expressing cells in somites of WT and MyoD-Lkb1 embryos at E10.5. (C) $MF20^{+}$ cells in somites of WT and MyoD-Lkb1 embryos at E10.5. Scale bars: 100 μm.

Supplemental Figure S4. MyoD-Lkb1 muscles exhibit features of muscle pathology. (A) Evans blue (EB) dye incorporation in resting TA muscles at 10-week-old. (B) CD11b (red), dystrophin

(green) and DAPI staining (blue) reveals increased inflammatory macrophasges in the MyoD-Lkb1 Gas muscle at 10-week-old. (C) Masson's Trichrome staining reveals increased fibrotic tissue (blue) in MyoD-Lkb1 Gas muscle at 10- week-old. (D, E) Relative expression levels of mitochondrial biogenesis genes in TA and Sol muscles at 10-wk-old. n=5. (F, G) Immunostaining of myosin heavy chain type I (green) and IIA (red) and IIA (blue) of Sol muscle cross sections from 10-wk-old WT (F) and MyoD-Lkb1 (G) mice. Note that the MyoD-Lkb1 muscle is magnified 3 times relative to the WT muscle. Scale bars: 100 μm. (H) Abundance of type I and IIA myofibers in Sol muscles of 10-wk-old mice. n = 3 (WT), 4 (MyoD-Lkb1). (I) Myofiber type distribution of TA muscles labeled with antibodies specific for Type I (green), IIA (red) and IIB (blue) myosin heavy chain at 10-wk-old. Note that Type IIA fibers are mainly restricted to the right half (dotted line indicates the center) of the TA muscle in the MyoD-Lkb1 muscle. Scale bars: 100 μm. Error bars represent SEM, * $P< 0.05$, ** $P< 0.01$.

Supplemental Figure S5. Lkb1 deletion inhibits muscle regeneration. (A-C) Abundance of $Pax7^{+}$ (A), $Ki67^{+}$ (FB) and $MyoG^{+}$ (C) cells in regenerating muscles of WT and $Pax7^{CreER}$ -Lkb1 mice at day 7 after CTX injection. Pax7 labels satellite cells and myoblasts, Ki67 labels proliferating cells, MyoG labels differentiated myoblasts. Error bars represent SEM, n = 3. ** $P < 0.01$.

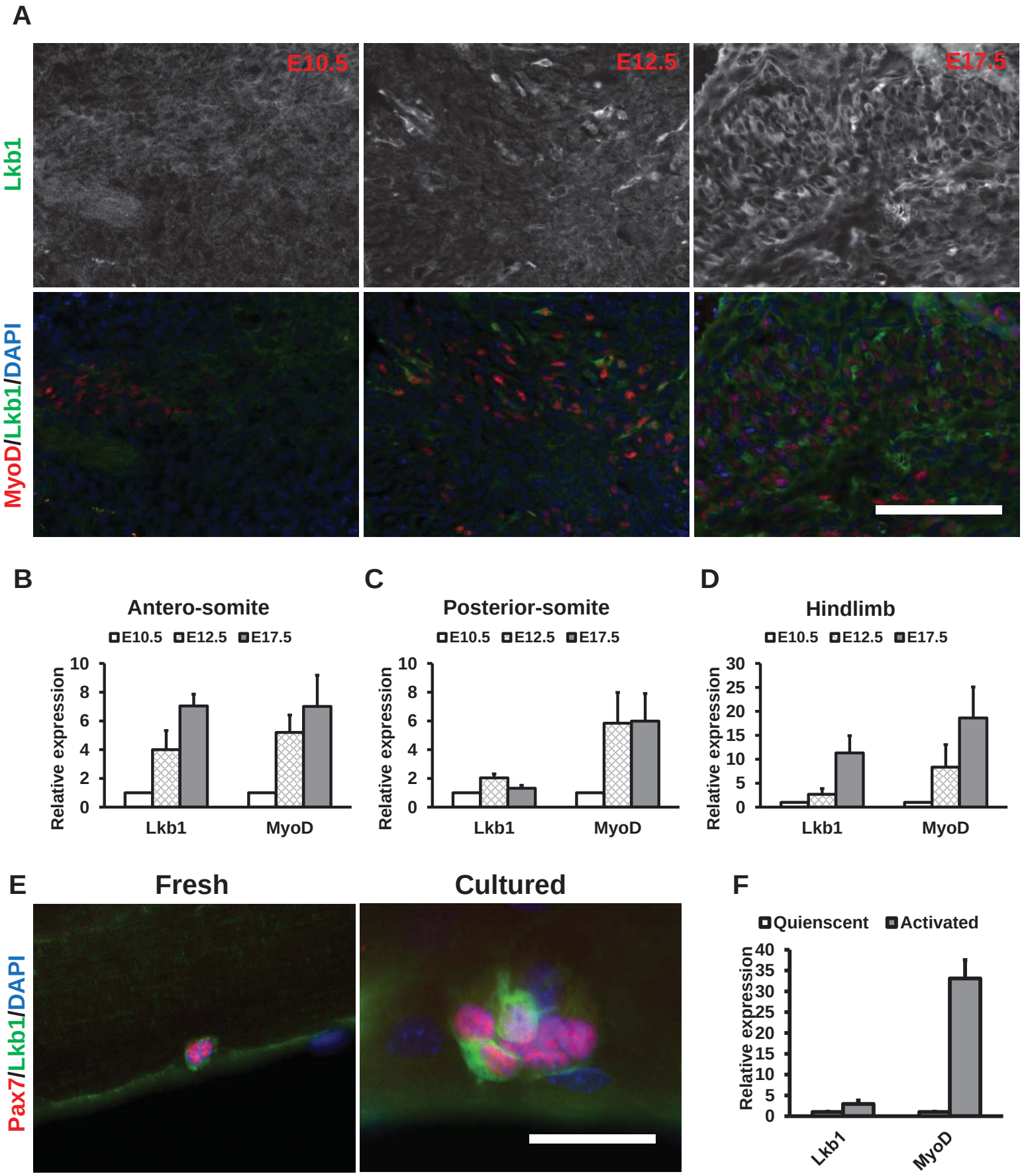
Supplemental Figure S6. Expression of myogenic related genes in $Pax7^{CreER}$-Lkb1 myoblast. (A, B) The mRNA (A) and protein (B) levels of myogenic related genes in $Pax7^{CreER}$-Lkb1 myoblast after TMX treatment. Error bars represent SEM, n=3. * means $P< 0.05$, ** $P< 0.01$.

Supplemental Figure S7. Lkb1 regulates myoblast proliferation through mTOR pathway. (A, B) Relative expression of the myogenic and proliferation marker genes in $mTOR^{flox/flox}$ myoblast treated with adenovirus-GFP and adenovirus-Cre (A), and (B) $Pax7^{CreER}$-$mTOR^{flox/flox}$ myoblast treated with 4-OH-TMX or vehicle control. (C) Proliferation marker Ki67 expression in WT and MyoD-Lkb1 myoblasts with or without Rapa treatment. (D) Colony assay of WT and MyoD-Lkb1 myoblasts treated with Rapa or vehicle control. (E) Growth curve of WT and MyoD-Lkb1 myoblasts treated with Rapa or vehicle control. Error bars represent SEM, n=5. * $P< 0.05$, ** $P< 0.01$. Scale bars: 100 μm.

Supplemental Figure S8. Lkb1 regulates myogenin and myogenic differentiation independent of the AMPK-mTOR pathway. (A, B) Activation of AMPK by AICAR failed to rescue the inhibitory effect of Lkb1 mutation on MyoG and eMHC expression at both protein (A) and mRNA (B) levels. (C) Inhibition or deletion of mTOR reduced the mRNA levels of MyoG in WT myoblasts. (D) Inhibition of mTOR with Rapa further reduced $MyoG^{+}$ cells and failed to rescue MyoG expression in MyoD-Lkb1 myoblasts. (E) A model summarizing the role Lkb1 in inhibiting proliferation but promoting differentiation of muscle stem cells. Error bars represent SEM, n = 5. * $P< 0.05$, ** $P< 0.01$. Scale bar = 100 μm.

Supplementary Movie 1. Severe compromised mobility of MyoD-Lkb1 mice at 12-week-old.

Supplemental Figure S1



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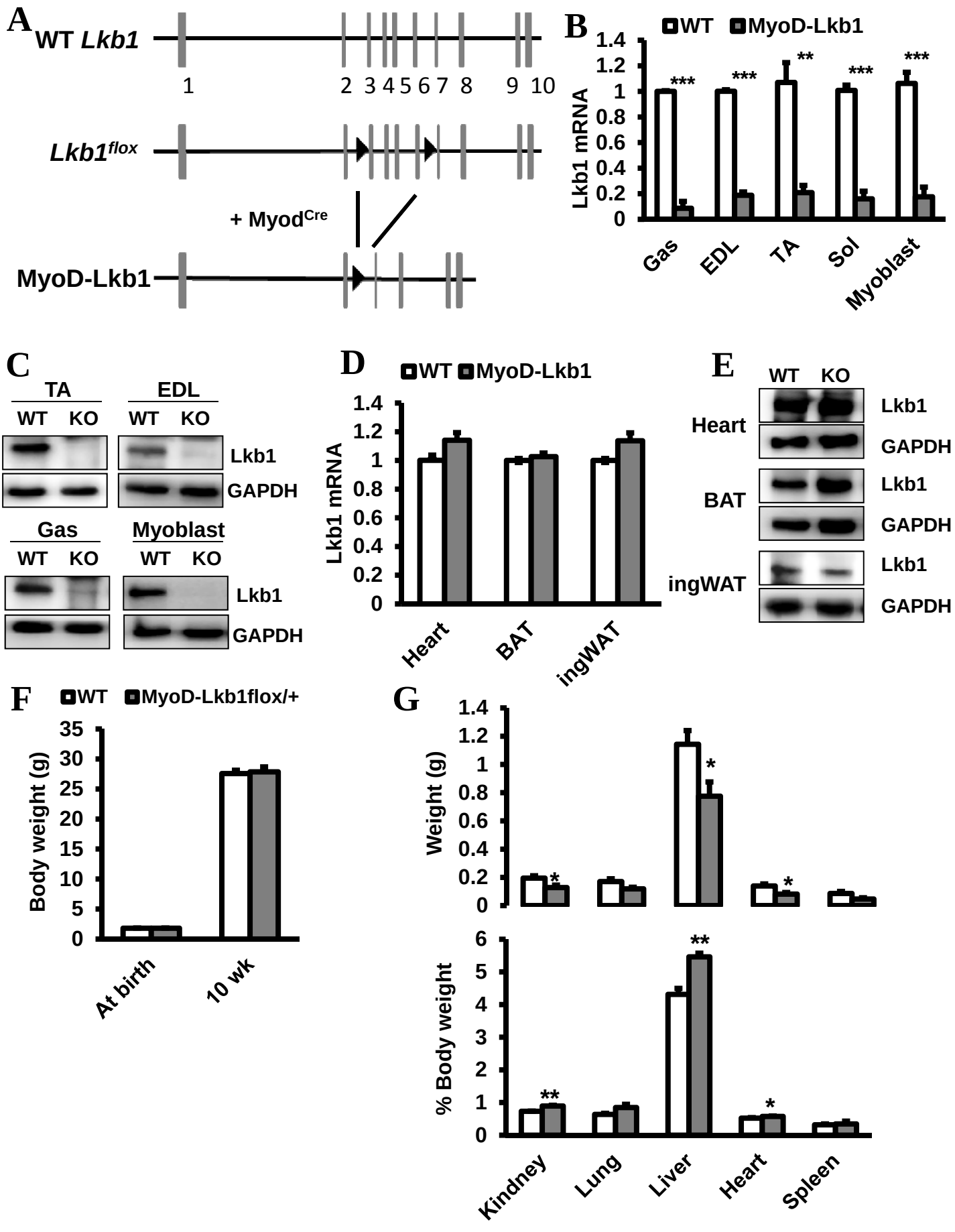

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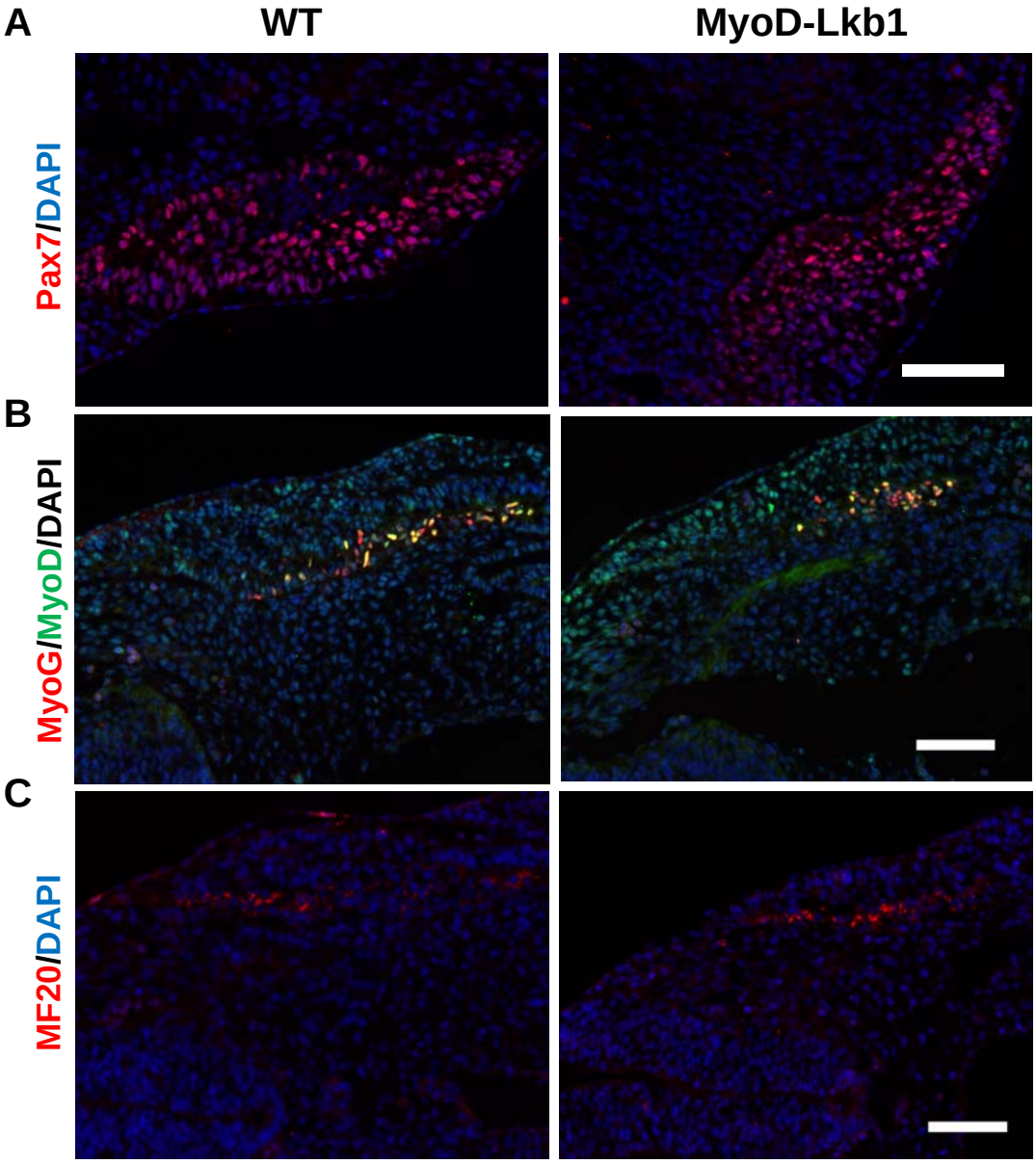

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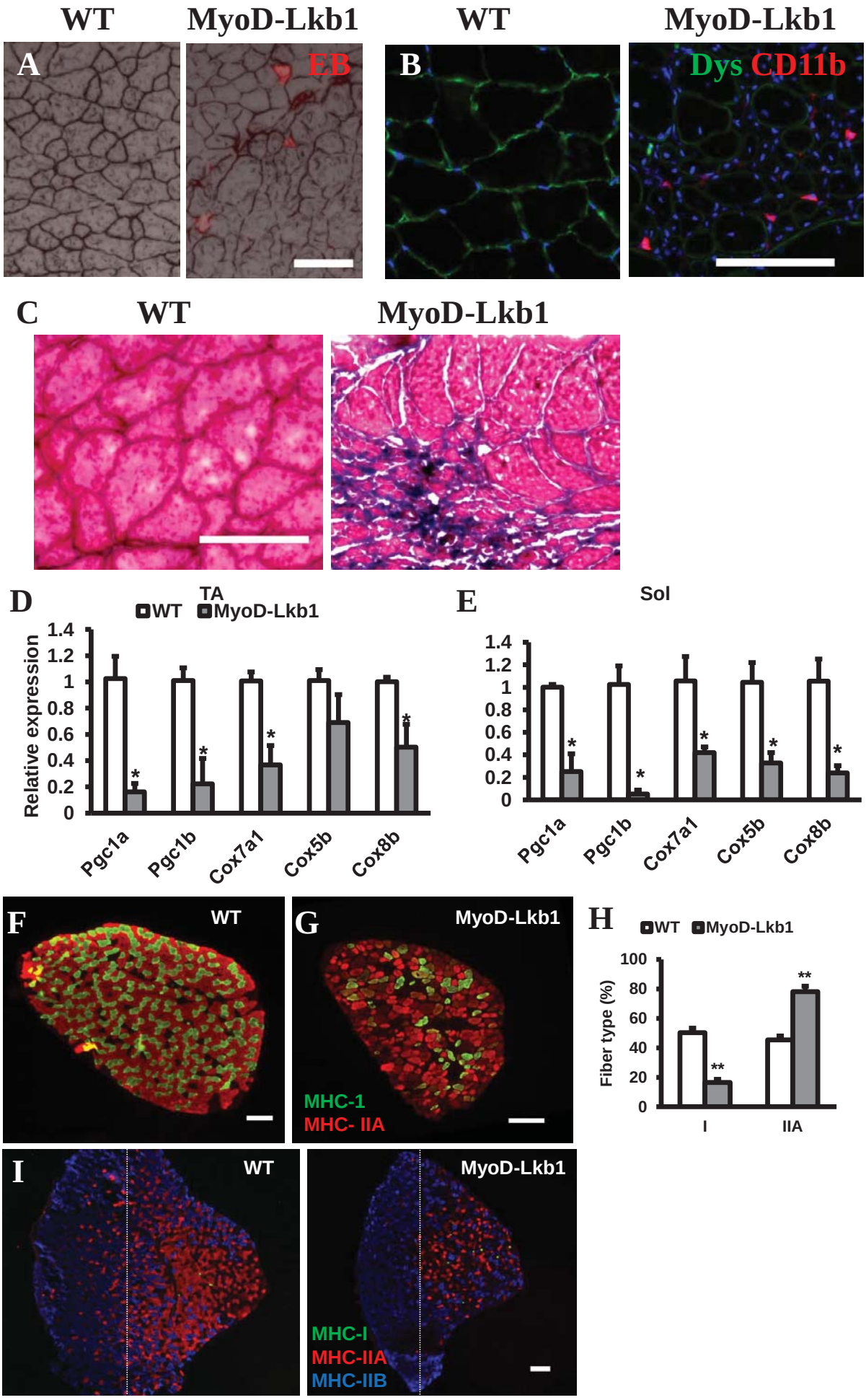

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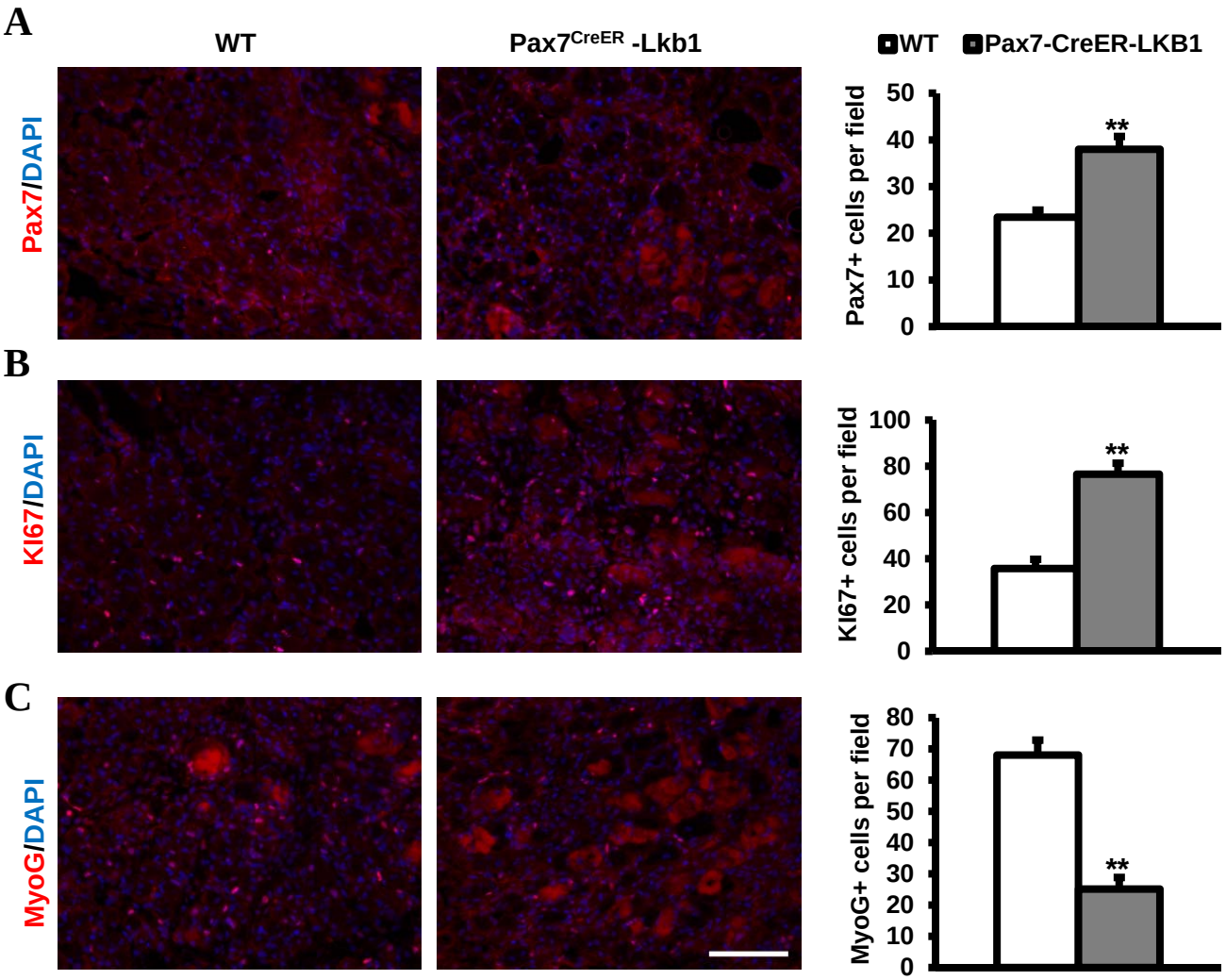

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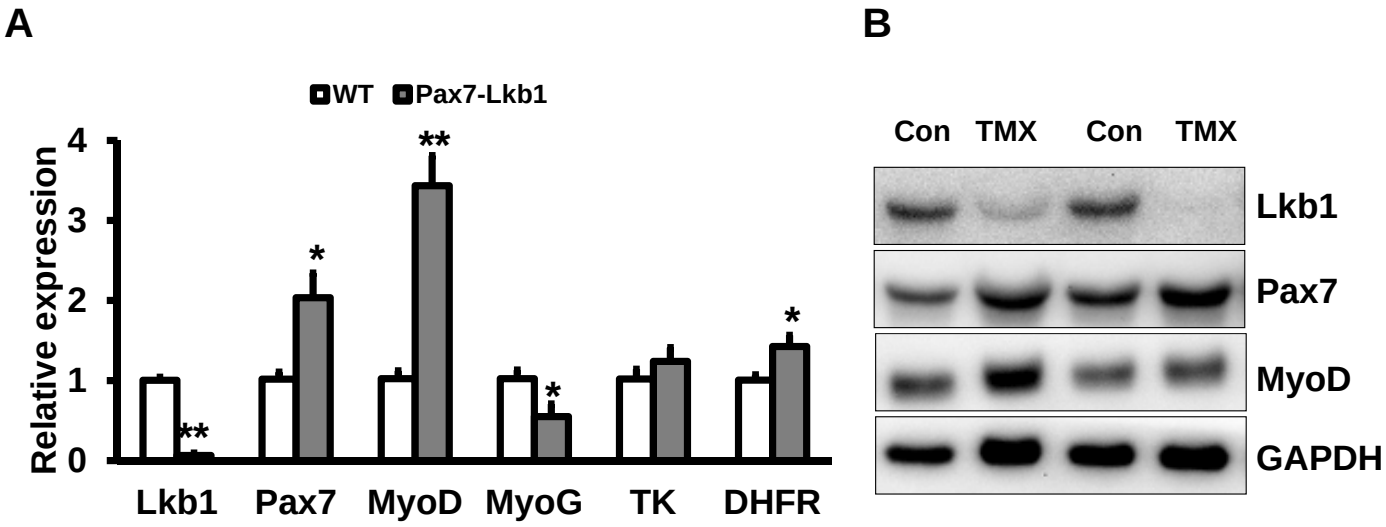

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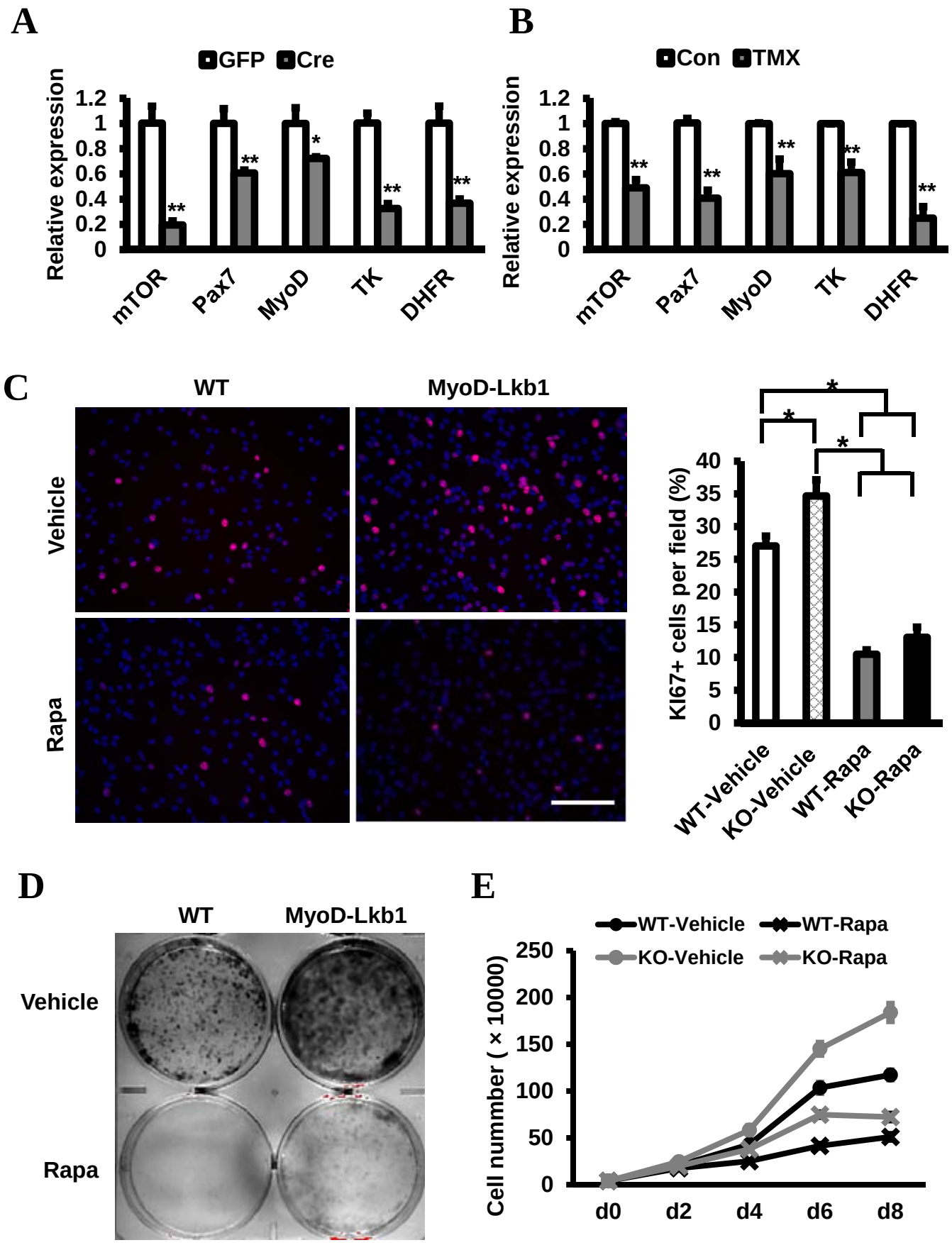
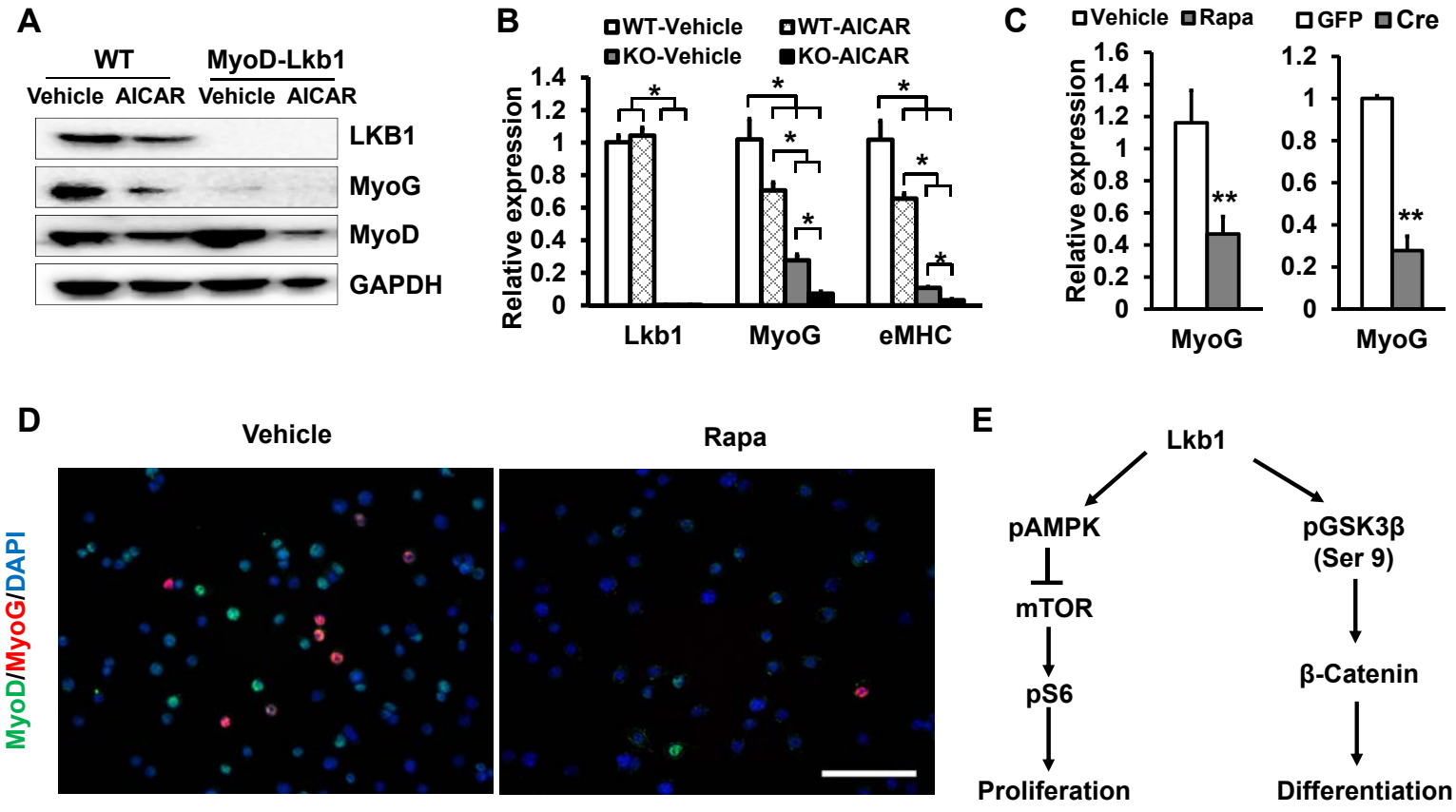


Figure S8



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