

The Role Of Skeletal Muscle Akt Signaling In The Regulation Of Muscle Growth And Function

Abstract

Skeletal muscle accounts for more than 40% of the body mass, providing means for locomotion at the expense of cellular energy. They rely on distinct metabolic pathways to meet this energetic demand. Insulin and insulin-like-growth factor 1 signaling control skeletal muscle growth and function and defects in these underlie a host of skeletal muscle diseases; however, the molecular mechanisms mediating these effects remain ill-defined. To understand the signaling mechanisms controlling insulin and IGF-1 regulation of skeletal muscle physiology, we generated mice lacking Akt, an essential downstream signaling molecule with skeletal muscle specific deletion of Akt2 (M-Akt2KO), the predominant Akt isoform in skeletal muscle, and Akt1 and Akt2 (M-AktDKO), to delete both the Akt isoforms in muscles. Surprisingly, despite a reduction of ~90% of total Akt following Akt2 deletion alone, M-Akt2KO had normal muscle mass and no apparent phenotype. In contrast, M-AktDKO mice have significant reduction in muscle mass resulting in lower absolute force generation and impaired *in vivo* performance accompanied by the loss of oxidative fibers. Mechanistically, the activation of mTORC1 and inhibition of FOXO1 are both required and sufficient for the regulation of muscle mass and exercise performance downstream of Akt.

Complete loss of Akt in muscle decreases myofibril size and alters muscle morphology

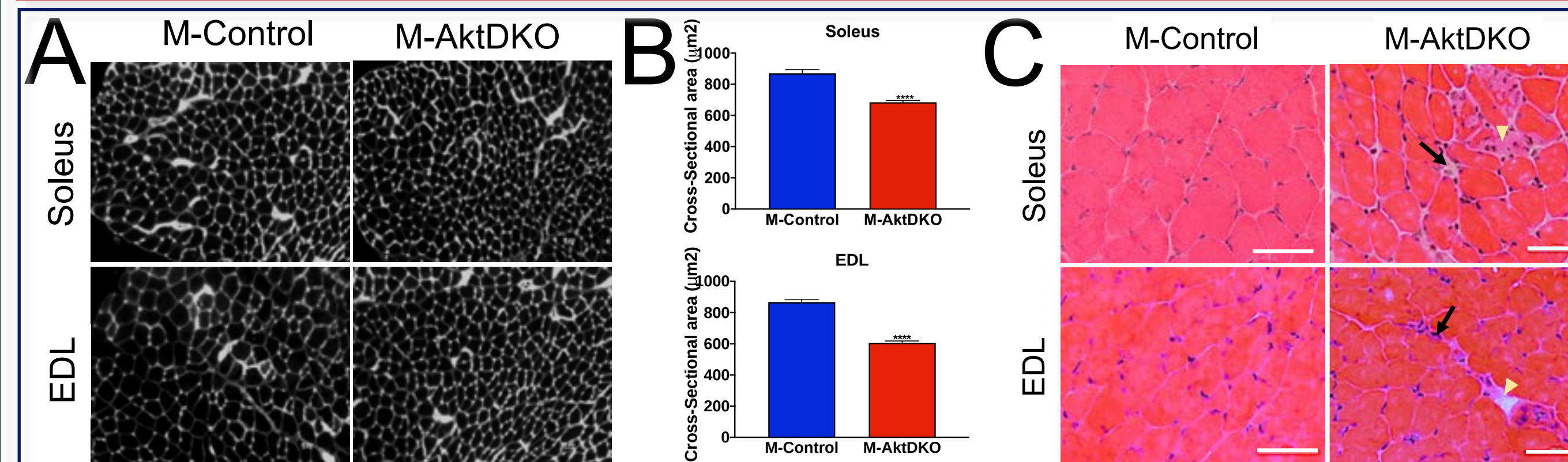


Figure 3: Complete deletion of Akt in muscle dramatically decreases muscle fiber size and alters muscle morphology. A) WGA staining of Soleus and the EDL muscle cross sections (scale bar 20 μm) B) Muscle fiber cross-sectional area and myofiber size distribution C) Hematoxylin and eosin (H&E) staining of muscle cross-sections from the EDL and soleus muscle M-AktDKO mice (scale bar 100 μm). ****p<0.0001

Inhibition of FOXO1 and activation of mTORC1 alone are not sufficient to rescue muscle mass

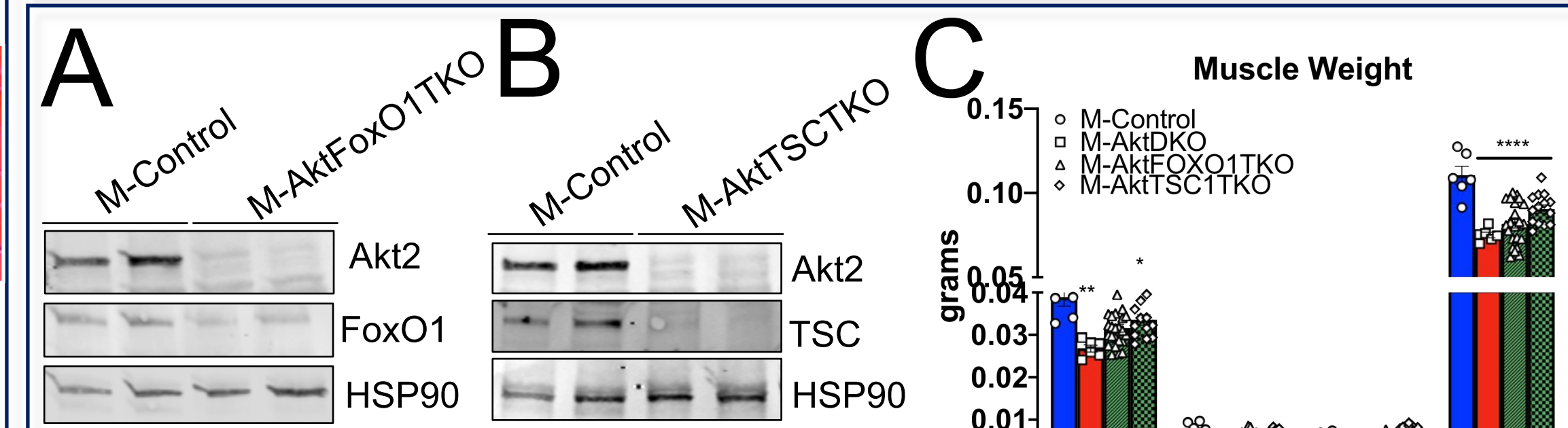


Figure 8: Inhibition of FOXO1 and activation of mTORC1 alone are not sufficient to rescue muscle mass in M-AktDKO mice. Western blot of gastrocnemius muscle in A) M-AktFoxO1TKO and B) M-AktTSC1TKO mice and C) muscle mass of the genotypes as compared to M-controls. *p<0.05 **p<0.01 ****p<0.0001

Role of Insulin/IGF-1 signaling in skeletal muscle physiology

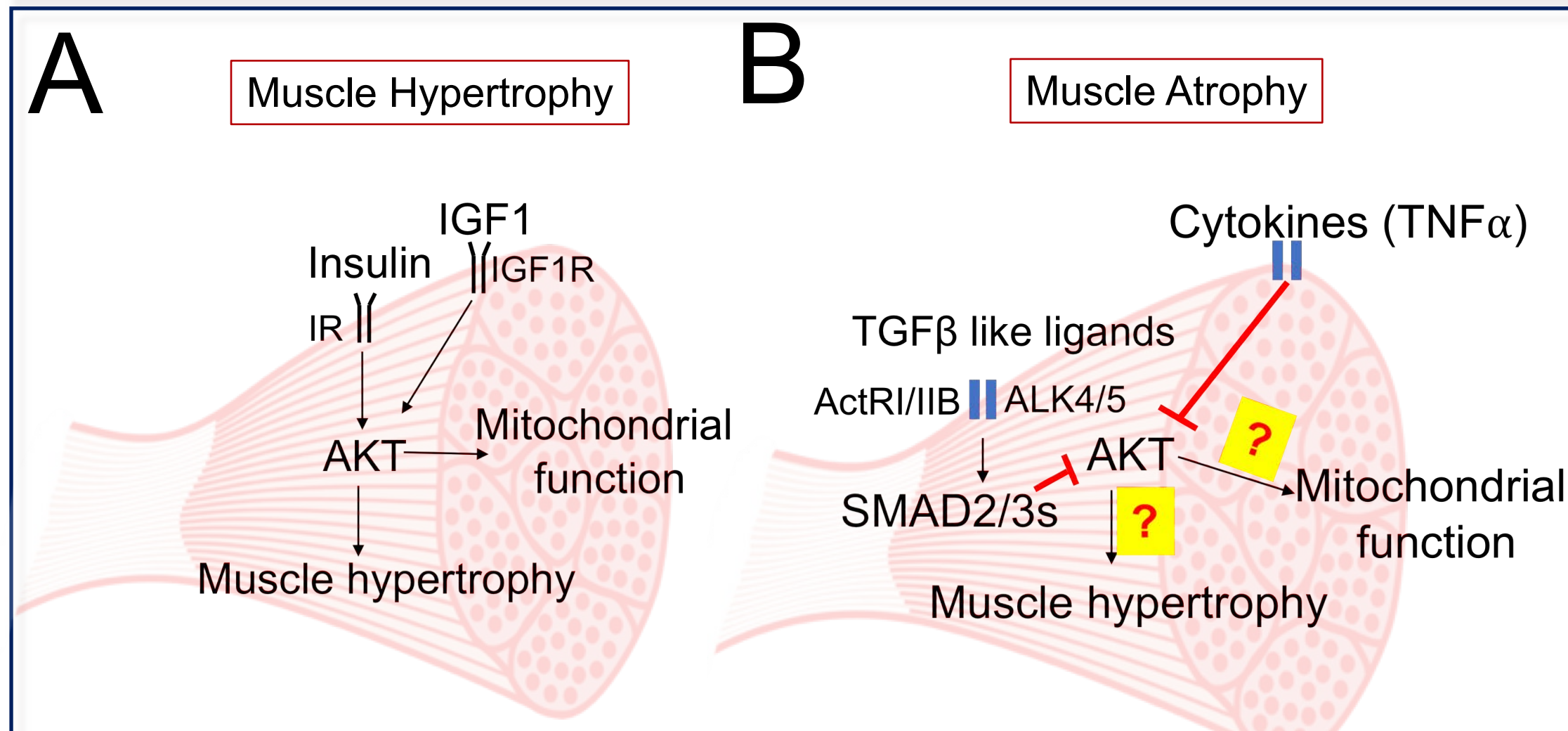


Figure 1: Insulin signaling in skeletal muscle. A) Insulin signals through muscle IR and IGF1-R to activate skeletal muscle Akt which stimulates muscle growth and function B) Predicted effects of loss of Akt on muscle growth and whole body performance

Akt is critical for the oxidative property of skeletal muscle

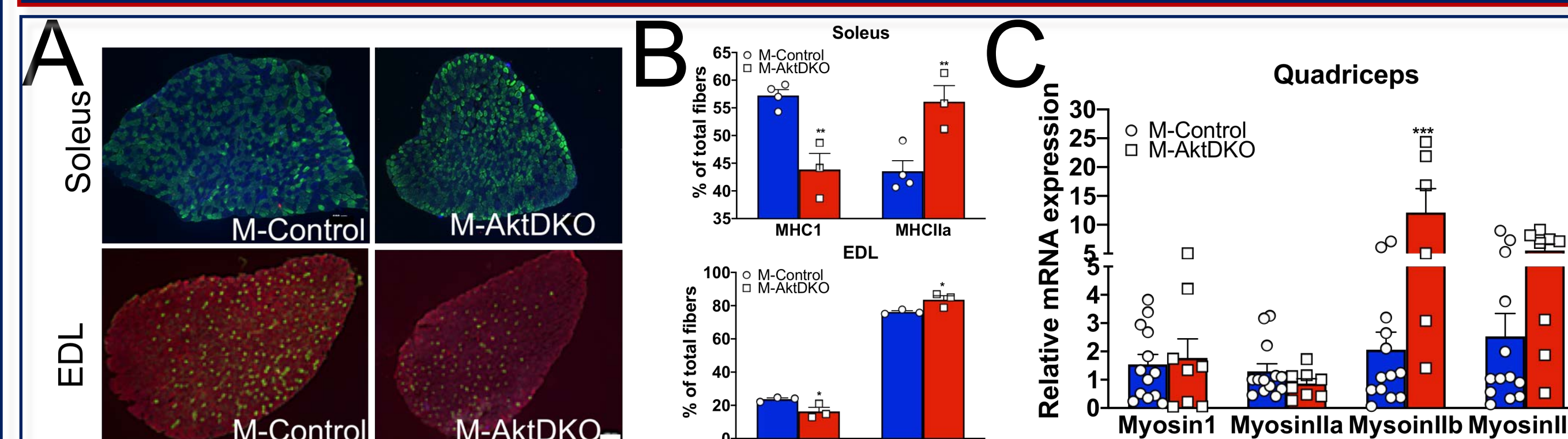


Figure 4: M-AktDKO muscles display a switch in muscle fiber type towards more glycolytic fibers. A) Immunostaining of Myosin I (blue), Myosin IIa (green) and myosin IIb (red) of deep portion of EDL and Soleus muscle from control and knockout mice (scale bar: 20 μm) B) Quantification of the Fiber type density C) Gene expression level of MHC protein in Gastrocnemius muscle of M-AktDKO mice as compared to the M-Control. *p<0.05 **p<0.01 ***p<0.001

Loss of Akt induces mitochondrial function

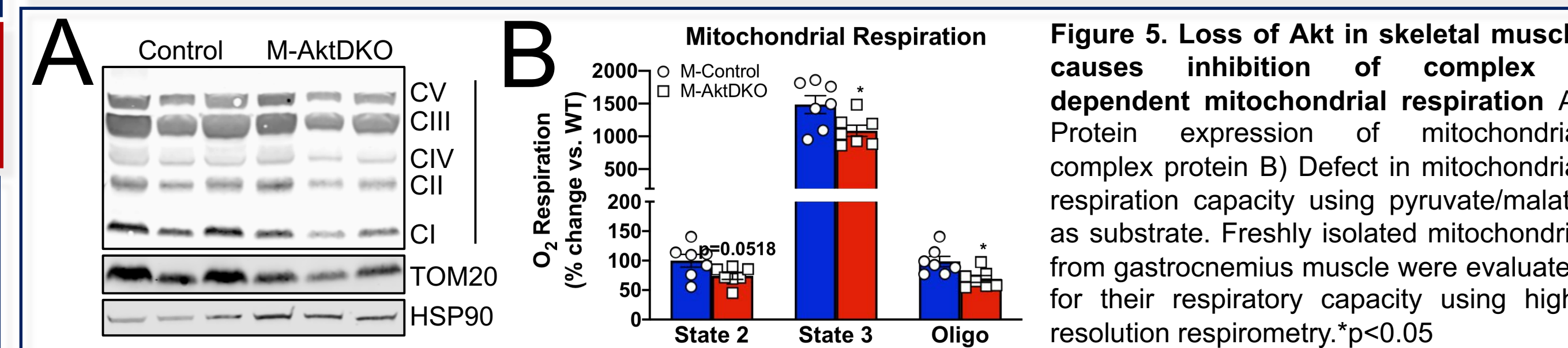


Figure 5: Loss of Akt in skeletal muscle causes inhibition of complex I dependent mitochondrial respiration A) Protein expression of mitochondrial complex protein B) Defect in mitochondrial respiration capacity using pyruvate/malate as substrate. Freshly isolated mitochondria from gastrocnemius muscle were evaluated for their respiratory capacity using high-resolution respirometry. *p<0.05

Akt deletion in skeletal muscle alters body performance

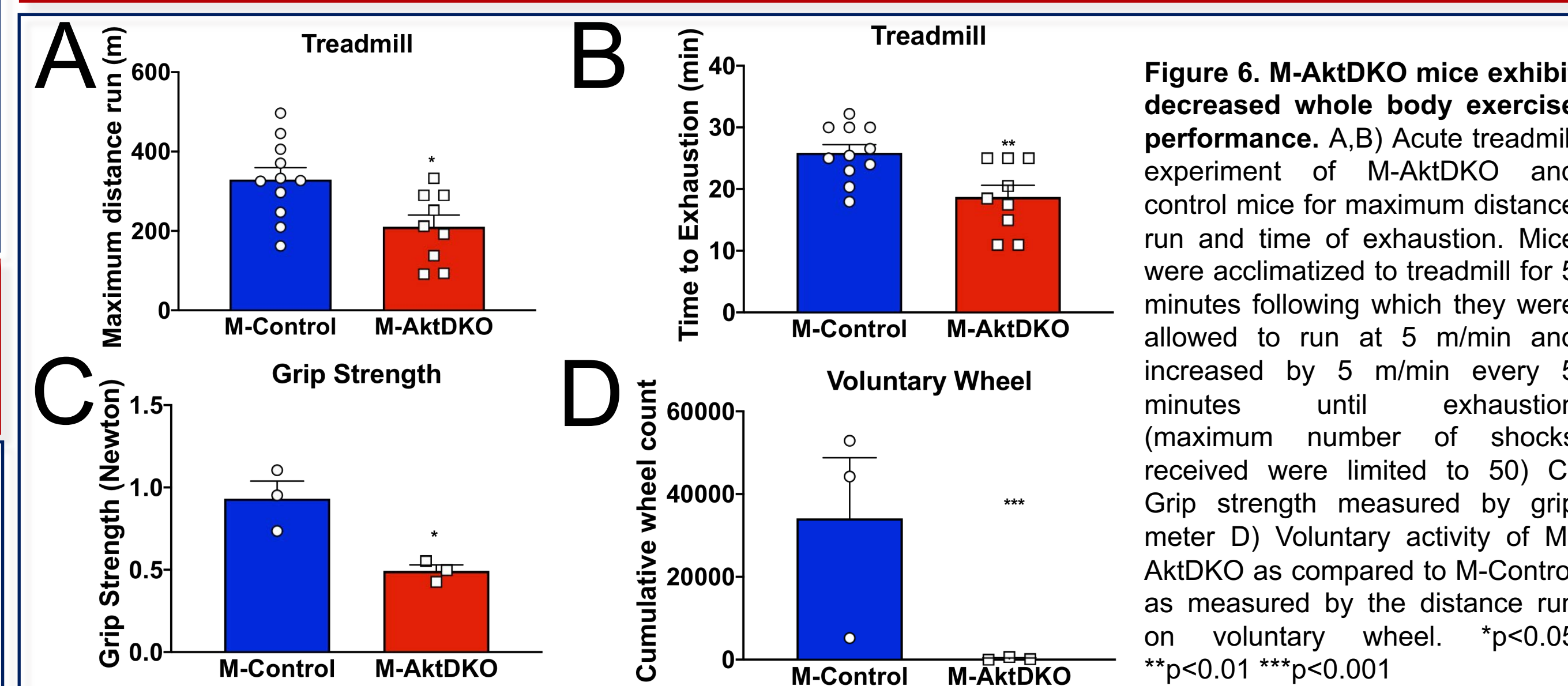


Figure 6: M-AktDKO mice exhibit decreased whole body exercise performance. A,B) Acute treadmill experiment of M-AktDKO and control mice for maximum distance run and time of exhaustion. Mice were acclimatized to treadmill for 5 minutes following which they were allowed to run at 5 m/min and increased by 5 m/min every 5 minutes until exhaustion (maximum number of shocks received were limited to 50) C) Grip strength measured by grip meter D) Voluntary activity of M-AktDKO as compared to M-Control as measured by the distance run on voluntary wheel. *p<0.05 **p<0.01 ****p<0.001

Loss of Akt in skeletal muscle reduces muscle strength

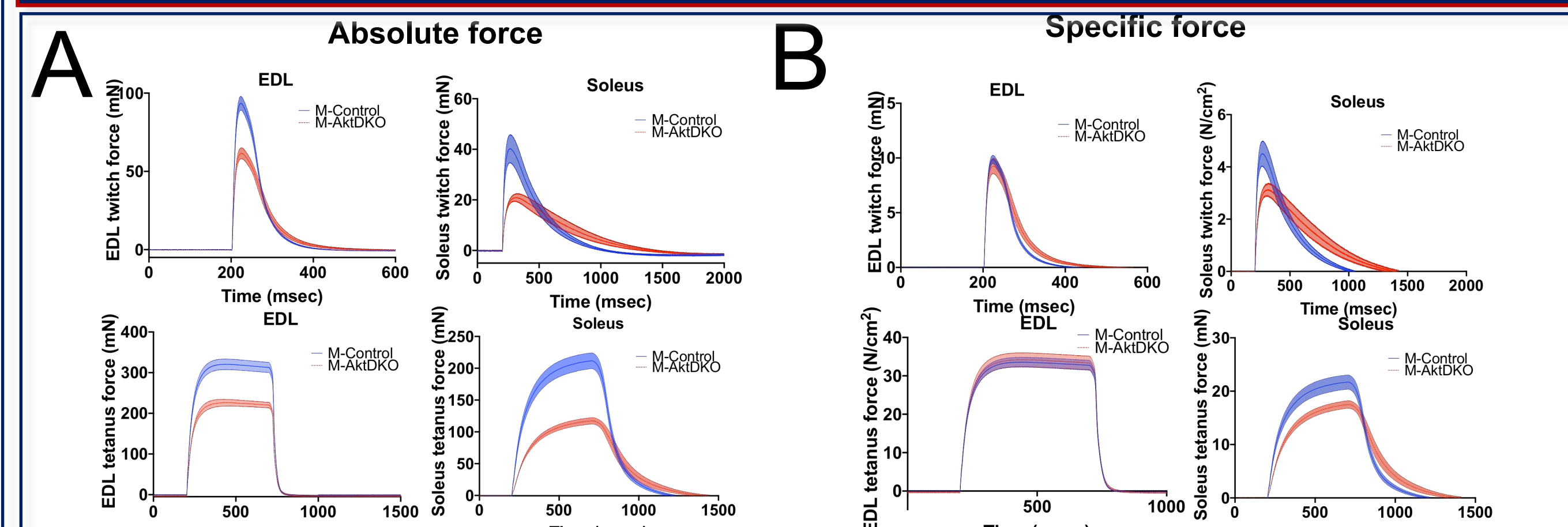


Figure 7: Loss of Akt in skeletal muscle causes significant reduction in muscle function. Contraction properties of the M-AktDKO muscle during *Ex vivo* physiology experiment A) Absolute force generation and B) Specific Twitch and Tetanic force.

Activation of mTORC1 and inhibition of Foxo1 are both required and sufficient to rescue muscle mass and myofiber size in the absence of Akt

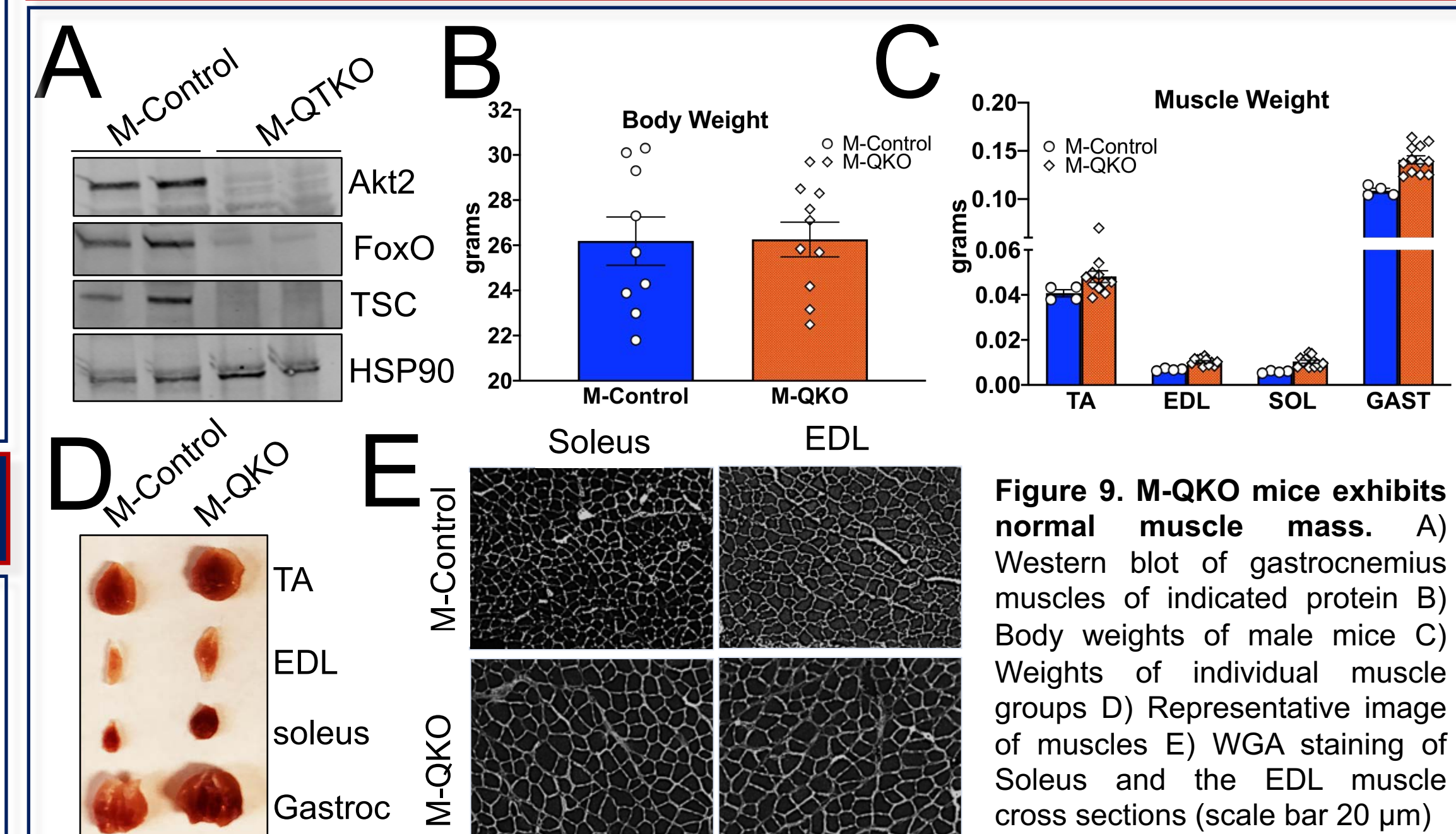


Figure 9: M-QKO mice exhibit normal muscle mass. A) Western blot of gastrocnemius muscles of indicated protein B) Body weights of male mice C) Weights of individual muscle groups D) Representative image of muscles E) WGA staining of Soleus and the EDL muscle cross sections (scale bar 20 μm)

Combined activation of mTORC1 and inhibition of Foxo1 are sufficient to rescue oxidative fibers and exercise performance in the absence of Akt

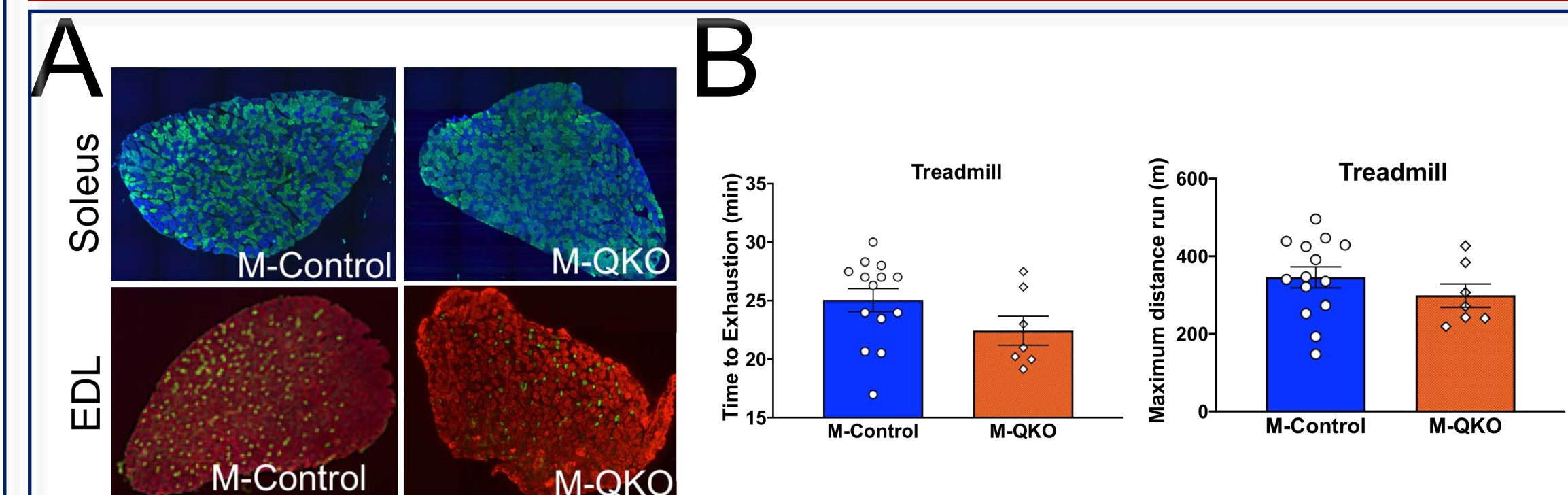


Figure 10: M-QKO muscles muscle fiber type and exercise performance comparable to M-Control. A) Immunostaining of Myosin I (blue), Myosin IIa (green) and myosin IIb (red) of deep portion of EDL and Soleus muscle from control and knockout mice (scale bar 20 μm) B) Acute treadmill experiment of M-QKO and M-Control mice for maximum distance run and time of exhaustion as described previously

Conclusions

- Complete loss of skeletal muscle Akt signaling leads to reduced muscle mass.
- Akt signaling is critical to maintain the oxidative properties of skeletal muscle *in vivo* and combined deletion of both Akt isoforms in skeletal muscle alters their morphology.
- Skeletal muscle Akt regulates exercise performance and muscle strength
- Activation of mTORC1 and inhibition of Foxo1 are both required and sufficient to rescue the muscle size and exercise performance in the absence of Akt *in vivo*

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Mouse model of Akt deficiency in skeletal muscle

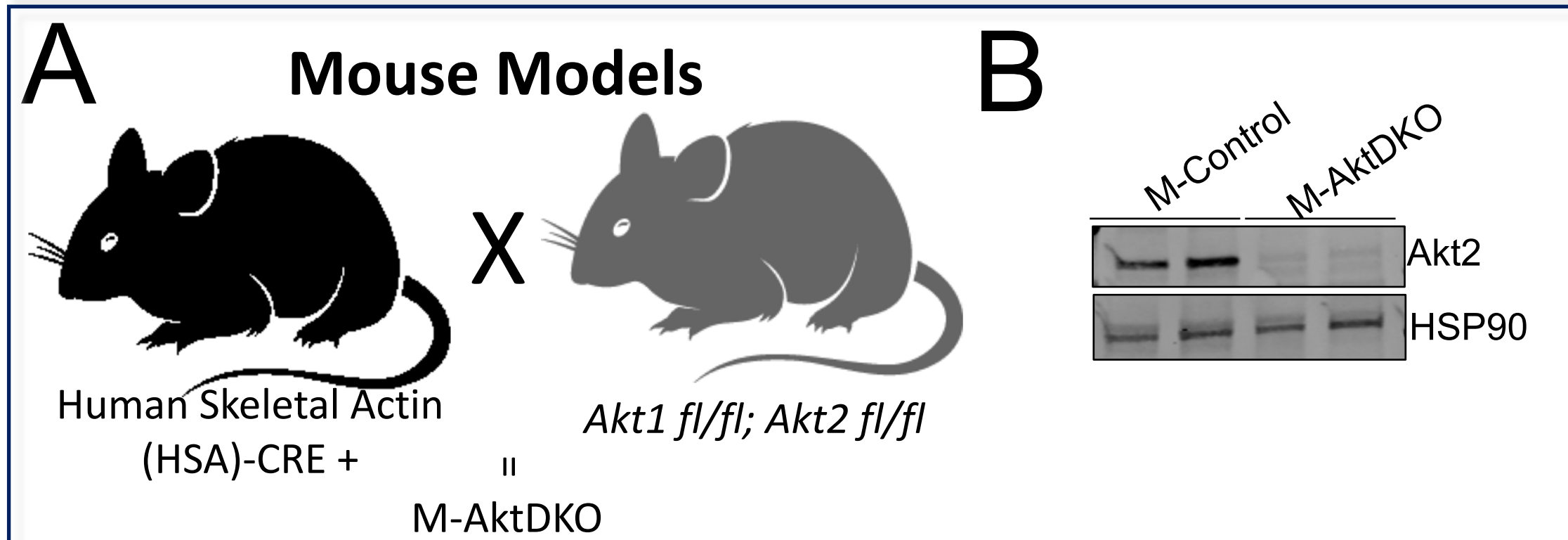


Figure 2: Mouse Model. A) Schematic of genetic mouse models used in this study B) Western Blot of Akt2 and total Akt in Soleus and Gastrocnemius (Gastroc) muscles from genetic mice

Loss of both Akt isoforms in skeletal Muscle reduces muscle mass

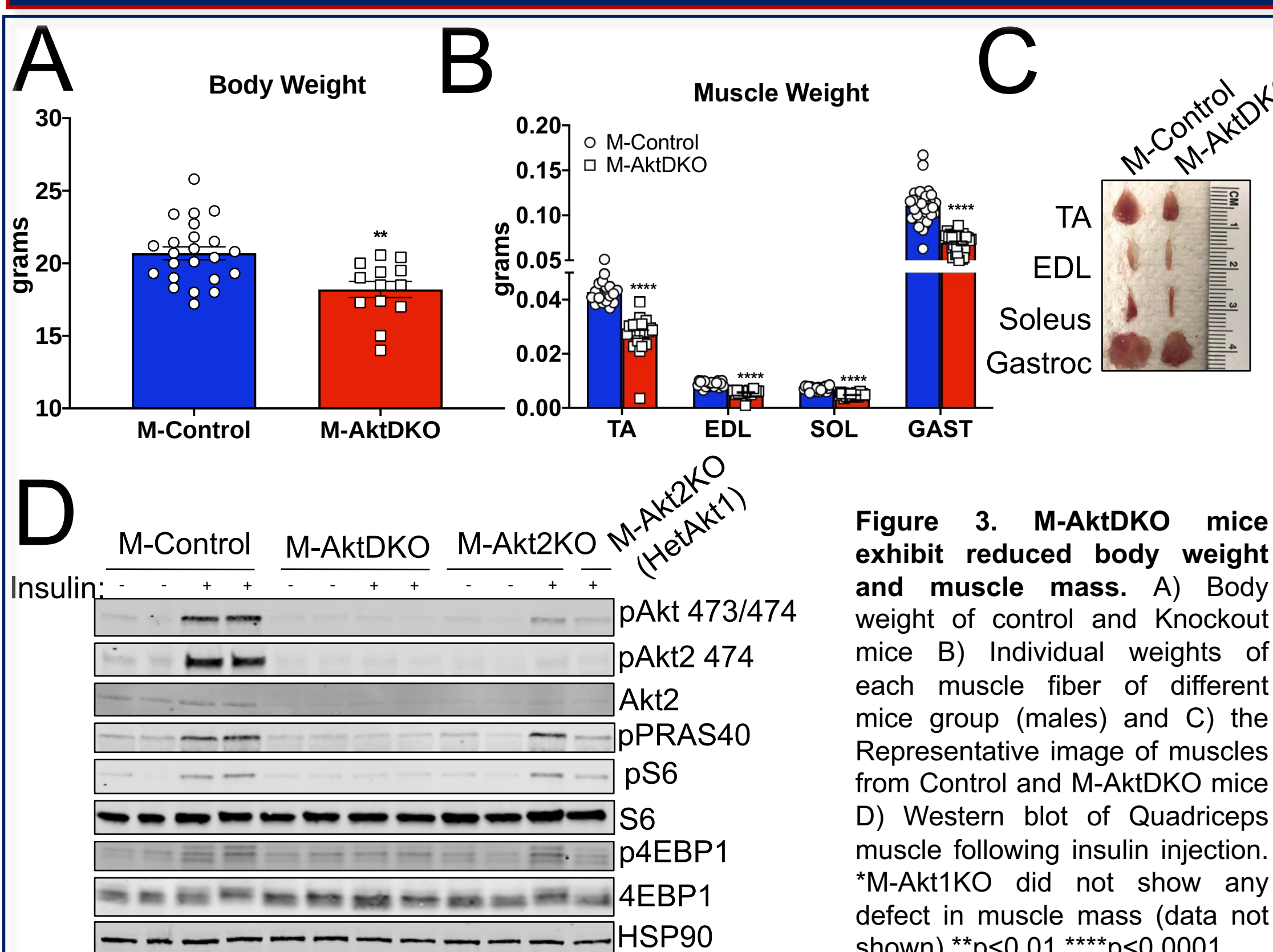


Figure 3: M-AktDKO mice exhibit reduced body weight and muscle mass. A) Body weight of control and Knockout mice B) Individual weights of each muscle fiber of different mice group (males) and C) the Representative image of muscles from Control and M-AktDKO mice D) Western blot of Quadriceps muscle following insulin injection. *M-Akt1KO did not show any defect in muscle mass (data not shown) **p<0.01 ****p<0.0001