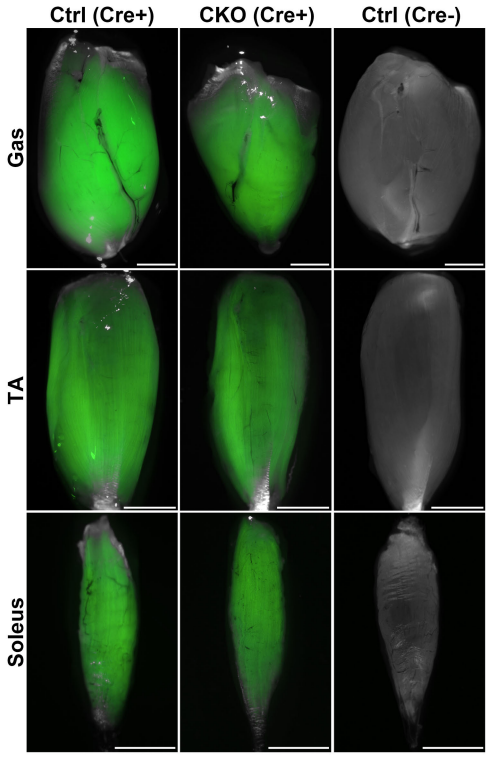
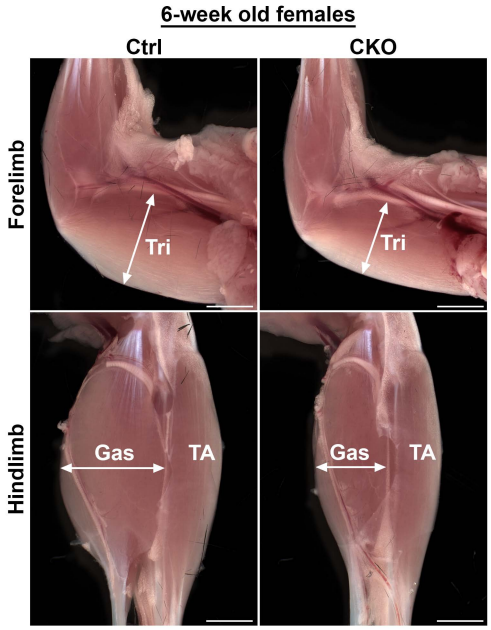
Suppl. Table S1. Good correlation between gene expression data obtained by RNA-seq and real-time PCR.

Gene	RNA-seq	Real-Time PCR
Regeneration		
Pax3	NS	NS
Pax7	↑ 1.76	↑ 1.58
Myod1	NS	NS
Myog	↑ 2.54	↑ 2.44
Myh3	↑ 11.07	↑ 12.88
Myh8	NS	NS
CNM-Associated		
Bin1	NS	NS
Dnm2	NS	NS
Mtm1	NS	NS
Mtmr14	NS	NS
Ptpla	↓ 0.82	↓ 0.39
**Ryr1*	NS	↑ 1.63
MyHC Isoforms		
Myh1	↑ 2.14	↑ 2.94
Myh2	↑ 2.39	↑ 3.21
Myh4	↑ 1.74	↑ 2.08
Myh7	↑ 13.64	↑ 14.91
Smyd Family		
Smyd2	↓ 0.39	↓ 0.35
Smyd3	NS	NS
Smyd4	NS	NS
Smyd5	NS	NS

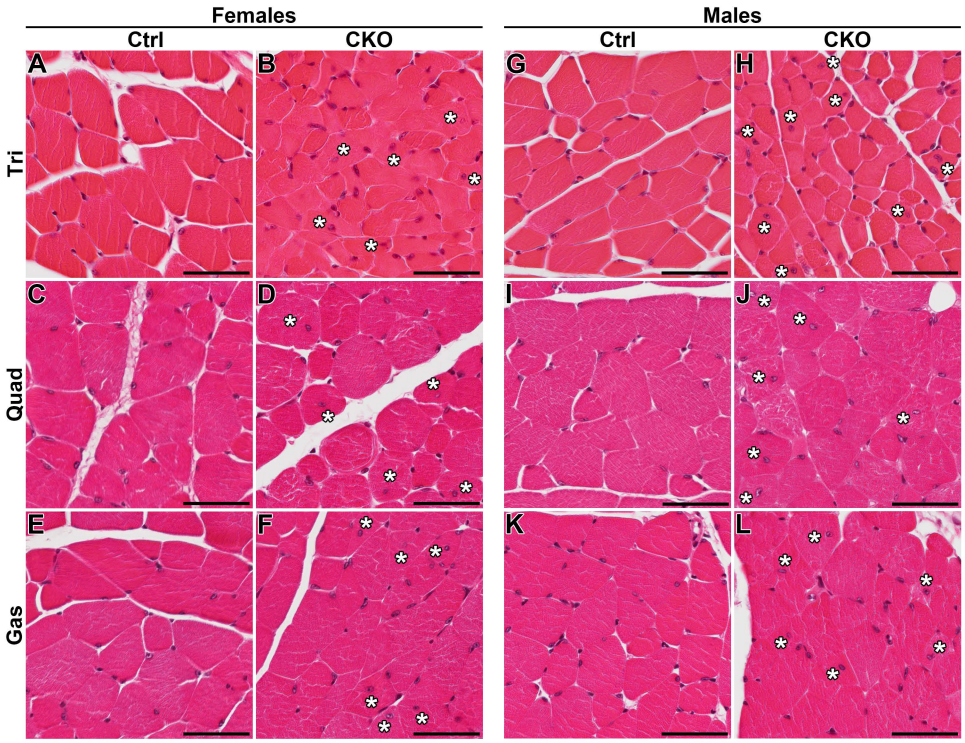
Right column is a summary of all real-time PCR gene expression assays reported in this manuscript. Center column contains the RNA-seq results for those same genes. Tissues were tibialis anterior. Values reported are fold change (*Smyd1* CKO/Control). Up and down arrows indicate upregulation and downregulation, respectively. Values reported if $P < 0.05$. *Only for *Ryr1* did RNA-seq and real-time PCR data not correlate. NS, difference was not significant.



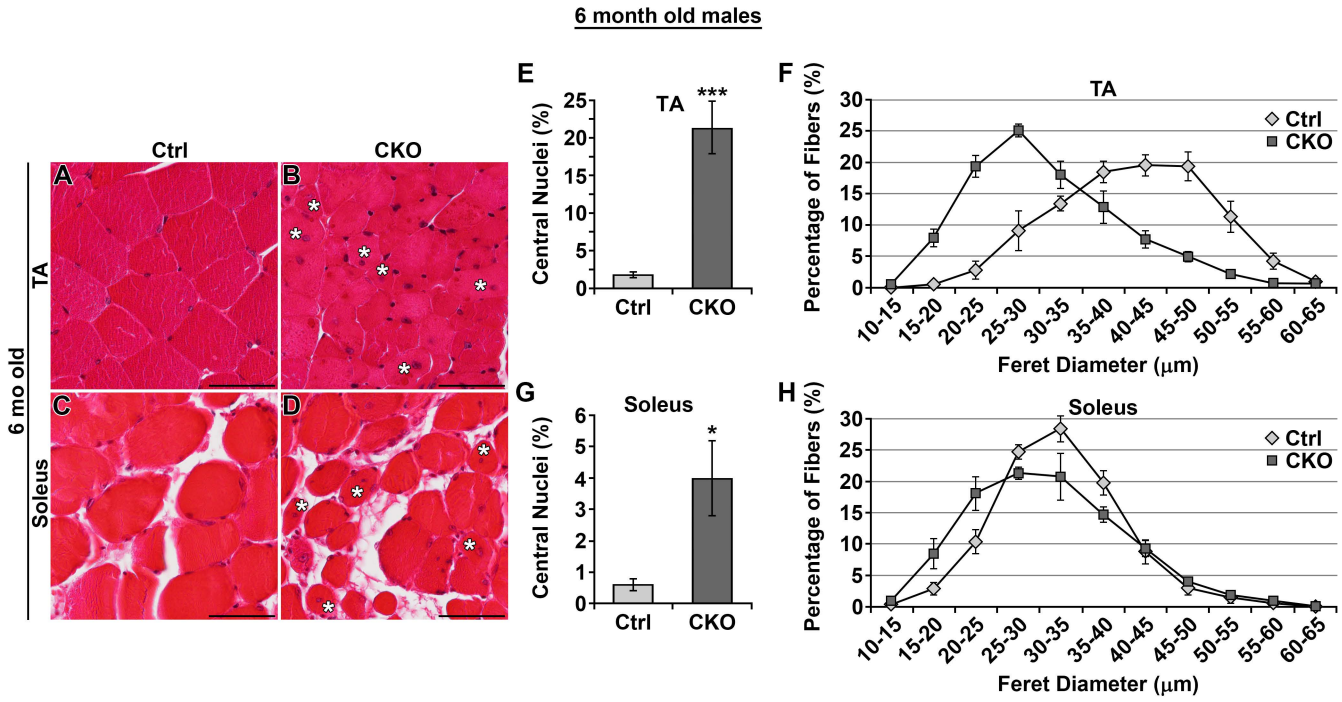
Suppl. Fig. S1. *Myf6*^{cre} induces recombination of *loxP* sites in skeletal muscles. Our genetic approach included the *Rosa26*^{YFP} allele (*Myf6*$^{cre/+}$*; Smyd1*$^{+/-}$ x *Smyd1*$^{flox/flox}$*; Rosa26*$^{YFP/YFP}$), which allowed us to monitor cells derived from *Myf6*^{cre}-expressing cells. We confirmed that *Myf6*^{cre} induces recombination of *loxP* sites in all muscles examined by imaging YFP fluorescence under a stereofluorescence microscope. Muscles from *Myf6*$^{+/+}$*; Smyd1*$^{flox/-}$*; Rosa26*$^{YFP/+}$ animals [Ctrl (Cre-)], which should not express YFP, were used as a negative control (rightmost column). Scale bars are 2 mm. Ctrl, control; CKO, conditional knockout; Gas, gastrocnemius; TA, tibialis anterior.



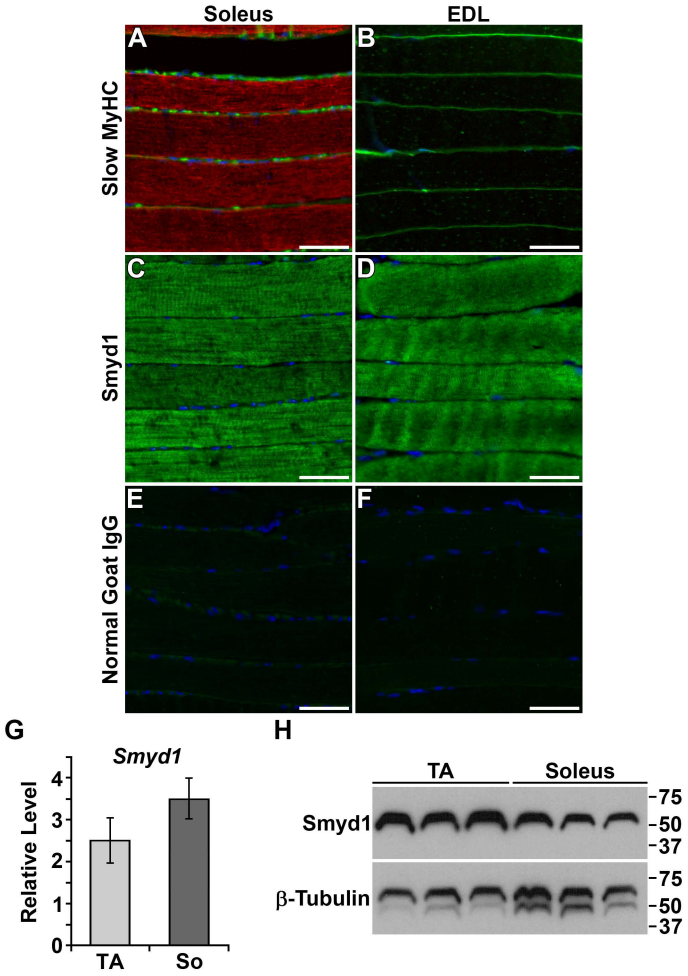
Suppl. Fig. S2. Reduced muscle mass in *Smyd1* CKO females. Stereoimages of the muscles of the forelimbs and hindlimbs of female control and *Smyd1* CKO mice at 6 weeks of age. Scale bars are 2 mm. Ctrl, control; CKO, conditional knockout; Gas, gastrocnemius; TA, tibialis anterior; Tri, triceps.



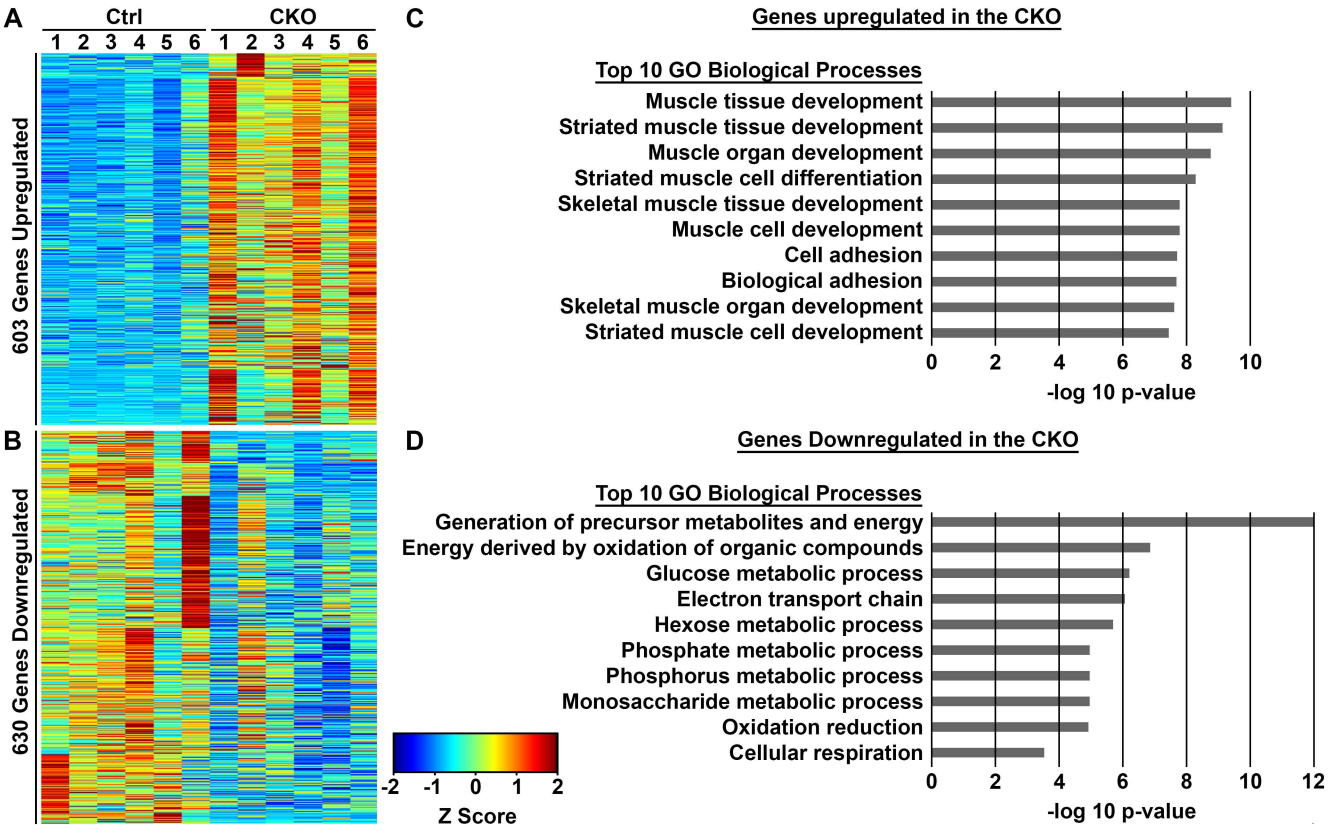
Suppl. Fig. S3. High prevalence of fibers with central nuclei in triceps, quadriceps and gastrocnemius. Images of H&E-stained muscle sections from 6-week old female (A-F) and male (G-L) mice. Very few fibers with central nuclei were observed in the muscles of control mice (A, C, E, G, I and K). Central nuclei were prevalent in male and female *Smyd1* CKO triceps (B and H), quadriceps (D and J) and gastrocnemius (F and L). White asterisks (*) indicate fibers with central nuclei. Scale bars are 50 μm. Ctrl, control; CKO, conditional knockout; Gas, gastrocnemius; Tri, triceps; Quad, quadriceps.



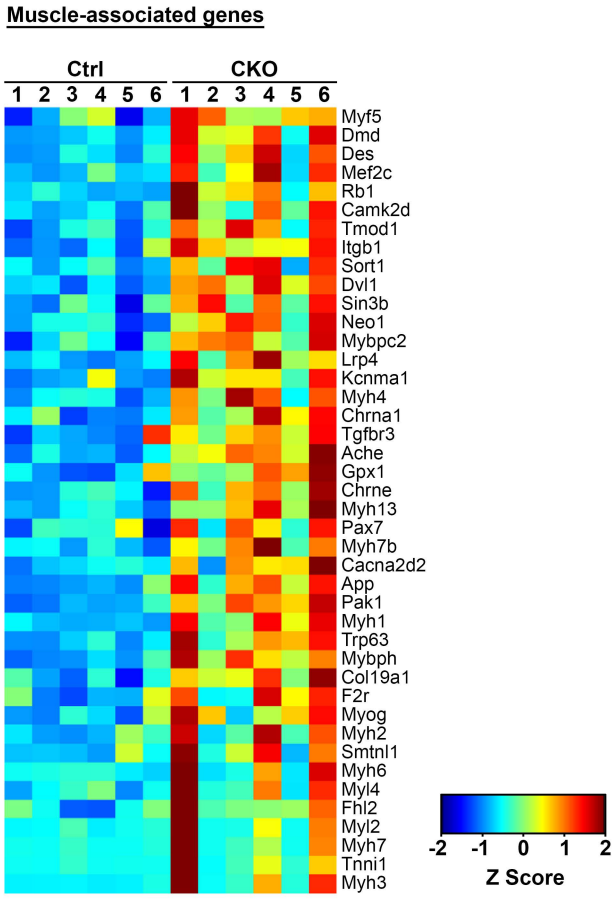
Suppl. Fig. S4. Slight disease progression by 6 months of age. (A-D) Images of H&E-stained muscle sections from 6-month old mice. White asterisks (*) indicate central nuclei. Central nuclei were prevalent in *Smyd1* CKO muscles. (E) Quantification of the percentage of myofibers in the tibialis anterior with central nuclei ($n \geq 3$ animals/group). (F) Distribution of myofiber minimum Feret diameter in the tibialis anterior. *Smyd1* CKO tibialis anterior had increased numbers of hypotrophic fibers ($P = 0.0018$, $n \geq 3$ animals/group). (G) Quantification of the percentage of myofibers in the soleus with central nuclei ($n \geq 6$ animals/group). (H) Distribution of myofiber minimum Feret diameter in the soleus. Mean myofiber minimum Feret diameter was not significantly different for the soleus ($n \geq 3$ animals/group). *P < 0.05, ***P < 0.001, Student's *t* test. Error bars show SEM. Scale bars are 50 μm. Ctrl, control; CKO, conditional knockout; TA, tibialis anterior.



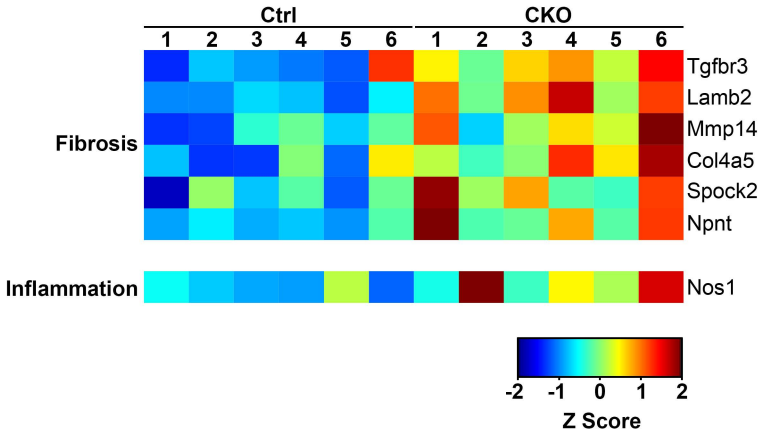
Suppl. Fig. S5. *Smyd1* is expressed in all fiber types. (A-F) Immunolocalization of slow MyHC (red) (A, B) and Smyd1 (green) (C, D) in the EDL and soleus. Normal goat IgG was used as a negative control (E, F). For A and B, sarcolemmas are labeled with WGA-488 (green). Smyd1 protein was detectable in all fibers. Scale bars are 50 μm. (G) Real-time PCR assay. The *Smyd1* gene is expressed to equivalent levels in the tibialis anterior and soleus (*n* = 6 animals/group). (H) Western blot to compare SMYD1 protein levels in tibialis anterior and soleus. Each lane contains protein extract from an individual animal. SMYD1 protein levels appeared to be slightly lower in the soleus. Anti-β-tubulin was used as a loading control. Molecular weight (kDa) is indicated to the right of each blot.



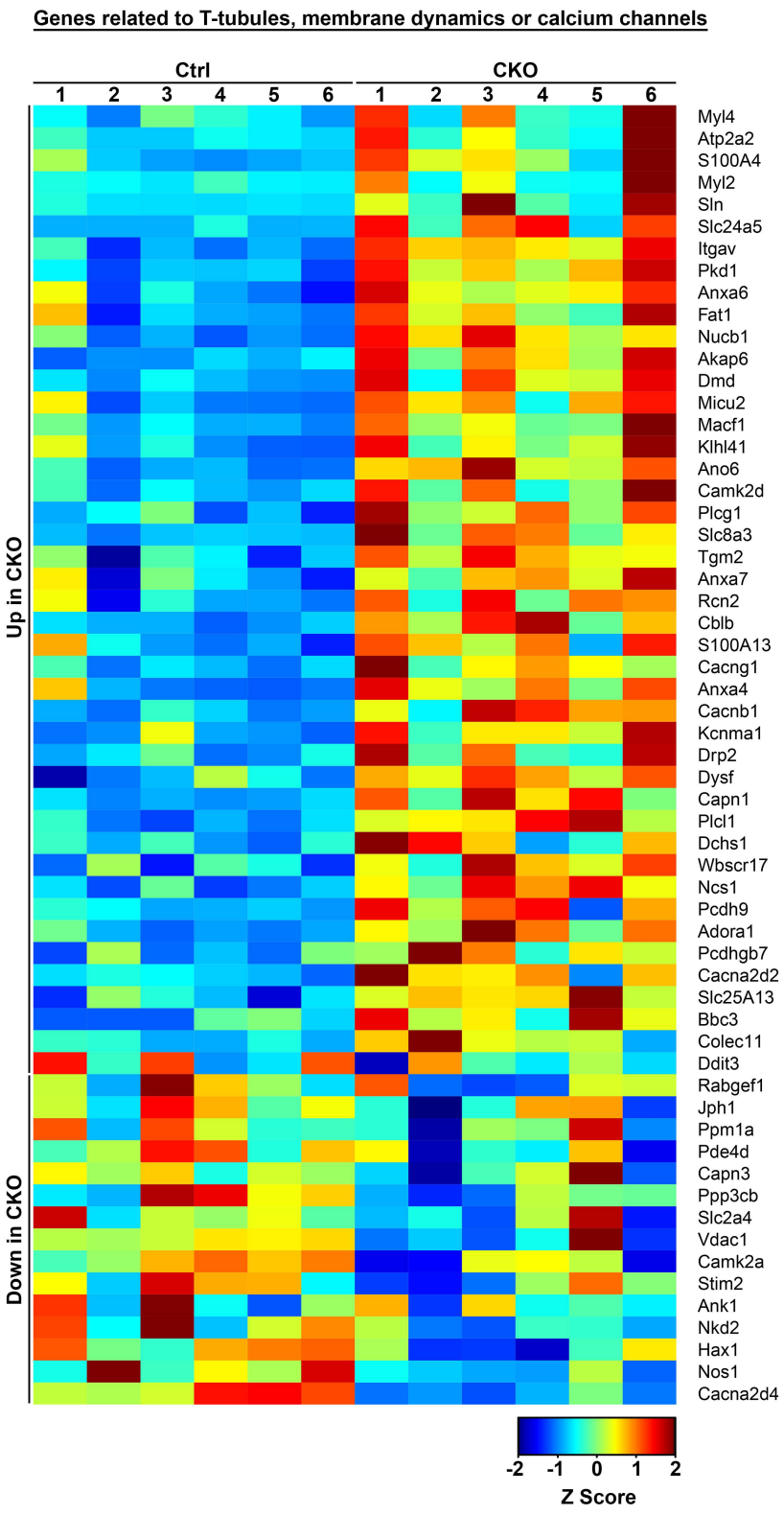
Suppl. Fig. S6. Genes involved in muscle development are upregulated in *Smyd1* CKO muscle. Results of RNA-seq analysis. (A and B) Heat maps of all genes upregulated (A) or downregulated (B) in the *Smyd1* CKO (cutoff was $P < 0.05$). (C and D) Top 10 biological processes gene ontology terms associated with those genes upregulated (C) or downregulated (D) in the *Smyd1* CKO tibialis anterior. Tissues (tibialis anterior) were collected from 6-week old mice. $n = 6$ animals/group. Ctrl, control; CKO, conditional knockout; GO, gene ontology.



Suppl. Fig. S7. Heat map of muscle-associated genes upregulated in the *Smyd1* CKO. Each column contains RNA-seq results from a single animal. Tissues (tibialis anterior) were collected from 6-week old mice (n = 6/group). Ctrl, control; CKO, conditional knockout.



Suppl. Fig. S8. Very few genes associated with fibrosis or inflammation showed altered expression in the *Smyd1* CKO. Each column contains RNA-seq results from a single animal. Tissues (tibialis anterior) were collected from 6-week old mice (*n* = 6/group). Ctrl, control; CKO, conditional knockout.



Suppl. Fig. S9. Altered expression of genes related to T-tubules, membrane dynamics or calcium channels in the *Smyd1* CKO. Each column contains RNA-seq results from a single animal. Tissues (tibialis anterior) were collected from 6-week old mice (n = 6/group). Ctrl, control; CKO, conditional knockout.