Supporting Information

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SI Methods

Muscle Bath Measurements. Mice were deeply anesthetized (2 mg xylazine, 20 mg ketamine per 100 g of body mass, intraperitoneally), fast-twitch extensor digitorum longus (EDL) and slow-twitch soleus muscles were excised, and nonabsorbable silk surgical suture was tied to the proximal and distal myotendinous junctions. Muscles were incubated at 30 °C in an oxygenated (95% O_2–5% CO_2) physiological salt solution (pH 7.6 containing in mM: 120.5 NaCI, 4.8 KCI, 1.2 $MgSO_4$, 20.4 $NaHCO_3$, 1.6 $CaCI_2$, 1.2 NaH_2PO_4, 10.0 dextrose, 1.0 pyruvate), as previously described (1, 2). To obtain the contractile properties, muscles were fixed between a clamp and arm of a dual-mode servomotor system (300B; Aurora Scientific) at a resting tension (L_o) of 1.0 g. Muscle activation was provided by computer-generated electrical stimulation delivered through closely flanking platinum electrodes. EDL and soleus muscles were maintained at L_o by a stepper motor. The servomotor arm, stepper motor, and electrical stimulation were controlled by Dynamic Muscle Control software (DMC Version 4.1.6, Aurora Scientific) to obtain the position and force output data from the activated muscles (1).

The protocol to obtain contractile properties consisted of five steps: (*i*) a pretwitch and tetanus, followed by a 10-min rest at L_o; (*ii*) a stress frequency protocol, followed by a 5-min rest at L_o; (*iii*) a force-velocity protocol, followed by a 5-min rest at L_o; (*iv*) a fatigue protocol; and (*v*) a fatigue recovery protocol. In the first step, muscles were subjected to three isometric twitches and tetani (150 Hz) spaced 1-min apart. In the second step, the muscle was subjected to stimulation at frequencies of 1, 30, 50, 80, 100, and 150 Hz, with each stimulation lasting 1 s and separated by 1 min. For the third step, maximal force output was determined from the 150-Hz tetanus determined at the end of the second step, and then corresponding shortening velocities were determined after-loaded isotonic contractions of 5%, 10%, 25%, 50%, 75%, and 90% of this maximal force, using a tetanic afterload protocol (3). The force–velocity relationship was established by plotting the mean isotonic loads (percentage of maximum force in grams) against the peak shortening velocities (mm/s) and fitting with the Hill equation (GraphPad Prism, GraphPad Software, Inc.), as previously described (4). The maximum shortening velocity for each muscle (i.e., V_{max}) was determined by solving the fitted Hill equation at zero load (3). In the fourth step, muscles were fatigued by stimulating at 60 Hz (EDL) or 100 Hz (soleus) for 800 ms, once every 5 s, for a total of 300 s, as previously described (5). Finally, in the fifth step, fatigue recovery was determined by collecting muscle force at 60 Hz (EDL) or 100 Hz (soleus) at 5, 10, 15, and 30 min postfatigue. At the conclusion of the protocols, muscle length was determined to the nearest 0.1 mm with a micrometer, muscle mass determined to the nearest 0.1 mg using an A-200D electronic analytical balance (Denver Instruments), and then the muscle was snap-frozen in liquid nitrogen for subsequent analysis.

Microarray Analysis. Pooled triplicate samples of total RNA were analyzed on an Illumina MouseWG-6 v2.0 BeadChip. Relative fold-change was then calculated by comparison of signal intensities. Fold-changes greater or less than twofold were subject to gene ontology analysis (Ingenuity Systems) with a statistical threshold of $P = 0.01$.

ChIP Assay. Snap-frozen tibialis anterior (TA) muscle from WT and Sox6 conditional knockout (cKO) mice was minced on ice and fixed with 4% PFA at room temperature for 15 min. Samples were quenched, washed, and dounce-homogenized in lysis buffer (EZ ChIP kit; Millipore). Samples were sonicated using a bioruptor (Diagenode) and further prepared using EZ-ChIP kit (Millipore) according to the manufacturer's instructions. Antibody directed against the N terminus of Sox6 (6) was kindly provided by Veronique Lefebvre (The Cleveland Clinic). C2C12 myoblasts were transfected with N-terminal tagged FLAG-Sox6. C2C12 ChIP was performed using EZ-ChIP kit and anti-FLAG antibody (Sigma).

Western Blotting. Protein was isolated from muscle by dounce homogenization in RIPA buffer containing protease inhibitor mixture (Roche). Protein concentrations were determined by BCA Protein assay (Thermo Scientific) and samples were resolved by SDS/PAGE electrophoresis. Tnni1 and myoglobin were detected by anti-Tnni1 primary antibody (sc-8119) at a concentration of 1:1,000, and α-myoglobin primary antibody (Dako) at a concentration of 1:5,000. Appropriate HRP-conjugated secondary antibodies (Biorad) at a concentration of 1:5,000 and chemiluminescent reagent (Santa Cruz) were used for detection.

Gene-Expression Analysis. Whole skeletal muscle was flash-frozen in liquid nitrogen, and total RNA was isolated following tissue homogenization in TRIzol (Invitrogen) according to the manufacturer's instructions. cDNA was generated by reverse-transcription using random hexamer primers (Invitrogen). Gene expression was measured by quantitative real-time PCR either with purchased gene-specific Taqman probes (ABI) or primers designed for SYBR green qPCR.

ChIP Primers.

myl3-F GAGGCCTCTGTCTCCCTCTC
myl3-R CTGCTCAAGGGGCTATTTTG
myl2-F CTCCGCCTCACCTACAACTG
myl2-R ACCTCTGGAGAGTTCGAGGA
myh7-F TAAAAGCATTTCCCCCGACT
myh7-R CCTGGAACTCAGACCCTGAA
myh1-F GGGCTCAGTCTCAGTCATGC
myh1-R CAGCAGCCTCGGTGGTAT
ATP2a2-F CTAGATTGTGAAGTGCCATTGAA
ATP2a2-R GACTGGGTTTCTGTCCCTTC

Gel-Shift Oligos.

myh7-F GCTAGGAAACAATTGGAAGTGGTCGTCATTGTTGTGGCA
myh7-R TGCCACAACAATGACGACCACTTCCAATTGTTTCCTAGC
myl3-F CCATTCCTGGGCTTGAACAATGCCCTCTCCAGGA
myl3-R TCCTGGAGAGGGCATTGTTCAAGCCCAGGAATGG
myl2-F GAGAGGTATTTATTGTTCCACAGCAGGGGCAG
myl2-R CTGCCCCTGCTGTGGAACAATAAATACCTCTC
myh1-F TTAAAATAGGAAACAATGTTCAGGTGTTATT
myh1-R AATAACACCTGAACATTGTTTCCTATTTTAA
ATP2a2-F AATGTTGGCAAAACAATGTCTGTTACAATA
ATP2a2-R TATTGTAACAGACATTGTTTTGCCAACATT
myh7-MUT-F GCTAGGAGAGACGTGGAAGTGGTCGTCCGTCTCGTGGCA
myh7-MUT-R TGCCACGAGACGGACGACCACTTCCACGTCTCTCCTAGC

myh1-MUT-F TTAAAATAGGAGAGACGGTTCAGGTG-TTATT
myh1-MUT-R AATAACACCTGAACCGTCTCTCCTATT-TTAA
myl2-MUT-F GAGAGGTATTTCGTCTCCCACAGCAGG-GGCAG
myl2-MUT-R CTGCCCCTGCTGTGGGAGACGAAATAC-CTCTC
myl3-MUT-F CCATTCCTGGGCTTGGAGACGGCCCTC-TCCAGGA
myl3-MUT-R TCCTGGAGAGGGCCGTCTCCAAGCCCA-GGAATGG
ATP2a2-MUT-F AATGTTGGCAAGAGACGGTCTGTTA-CAATA
ATP2a2-MUT-R TATTGTAACAGACCGTCTCTTGCCA-ACATT

Sybr Green Primers.

sv2b-SYBR-F TTCCCAGTTTATCAGCCAGG
sv2b-SYBR-R TAGTGAGCTTTGCTCTGCCA
cryba4-SYBR-F GCCCCTCTCCAGCACATATT
cryba4-SYBR-R TCACAGCTGAGTGTCCCAGT
chrna1-SYBR-F ACAATCTCACGGTGGTCCTC
chrna1-SYBR-R CTGTTCTCCTGCTGCTAGGC
Psat1-SYBR-F CATTTGGGATTTGATGGAGC
Psat1-SYBR-R ACTACAAAGTGCAGGCTGGG
crybb1-SYBR-F GGAAGGCAGATTGCTCAAAG
crybb1-SYBR-R GATCGTCTTCGAGCAGGAAA
Vlrd6-SYBR-F GGCGCCTTCAGGATCATA
Vlrd6-SYBR-R TCATGGCTCAGCACTCATCT
Lrba-SYBR-F GCCTCAAGTCATCTTCCCAG
Lrba-SYBR-R GAGACCATGTGACCGCAAC
Sln-SYBR-F CACACCAAGGCTTGTCTTCA
Sln-SYBR-R CCCTCAGACTACATTAGGCCC
Pdrg1-SYBR-F AAAACCTTTCAGCTCCGGTT
Pdrg1-SYBR-R ATGCCTCACCCTAAGACGAA
Mod1-SYBR-F ATGCCATATTTGGAAGACGC
Mod1-SYBR-R TCACTTTGGATGTGGGAACA
Psdm8-SYBR-F TGTGTGGAACTCAGCCACTC
Psdm8-SYBR-R TACATGGCCCAACTCAAATG
Rpl29-SYBR-F TCTTGTTGTGCTTCTTGGCA
Rpl29-SYBR-R GAACCACACCACACACAACC
Lrrc2-SYBR-F AGGTGATCCCCACTGACAAC
Lrrc2-SYBR-R CTGAGTGACCTGCCACAAGA
Smox-SYBR-F CGCCACTGGATTCACAACTT
Smox-SYBR-R TGCAGGAGGAAGCCCAGT
kcnc4-SYBR-F CTACCCGATGAATCTCCGTC
kcnc4-SYBR-R GAGGATCCCTACTCATCCCG
opn1mw-SYBR-F GTCTTGGAGGTGCTGGAAAG
opn1mw-SYBR-R GGCCTCCCTACCATCCTACT
LOC225594-SYBR-F ATGTGAAGAGCCTGGAGCAC
LOC225594-SYBR-R CAGTGAAGCTGGACTCCAAA
dysfip1-SYBR-F CTTGGACCACATTCGACAAG
dysfip1-SYBR-R ATGTCAGCCCCGTATTTGAC
LOC383308-SYBR-F CACGAAACACACCACACACA
LOC383308-SYBR-R TTCAGGGCTTTCTTGTGCTT
zymnd17-SYBR-F GTCATGGAATGGCTTCTGGT
zymnd17-SYBR-R GCCCAAAGCATGGTCATAGA
KLK26-F1-sybr CCCCACAAATGTGTTCCTTT
KLK26-R1-sybr GTTCCTGATCCTGTTCCCAG
KLK22-F1-sybr GACTCCCCCGCATAGGTACT
KLK22-R1-sybr CCTGTTCCTAACCCTGTCCC
LRRN1-F1-sybr CACAGGTCCTTGTGGGAGTT
LRRN1-R1-sybr TCCTCATCCTCCGGCTAGT
DUPD1-F1-sybr GCCGGACTACTACCGAGACA
DUPD1-R1-sybr GCGGTTCTTAGCCACTTGTT
RSPO3-F1-sybr ATCTGAACACGTTGCACAGC
RSPO3-R1-sybr CTTTATGGAATACATTGGCAGC

1. Wolff AV, et al. (2006) Passive mechanical properties of maturing extensor digitorum longus are not affected by lack of dystrophin. *Muscle Nerve* 34:304–312.
2. Grange RW, Gainer TG, Marschner KM, Talmadge RJ, Stull JT (2002) Fast-twitch skeletal muscles of dystrophic mouse pups are resistant to injury from acute mechanical stress. *Am J Physiol* 283:C1090–C1101.
3. Brooks SV, Faulkner JA (1988) Contractile properties of skeletal muscles from young, adult and aged mice. *J Physiol* 404:71–82.
4. Grange RW, Cory CR, Vandenboom R, Houston ME (1995) Myosin phosphorylation augments force-displacement and force-velocity relationships of mouse fast muscle. *Am J Physiol* 269:C713–C724.
5. Call JA, et al. (2008) Endurance capacity in maturing mdx mice is markedly enhanced by combined voluntary wheel running and green tea extract. *J Appl Physiol* 105:923–932.
6. Han Y, Lefebvre V (2008) L-Sox5 and Sox6 drive expression of the aggrecan gene in cartilage by securing binding of Sox9 to a far-upstream enhancer. *Mol Cell Biol* 28: 4999–5013.

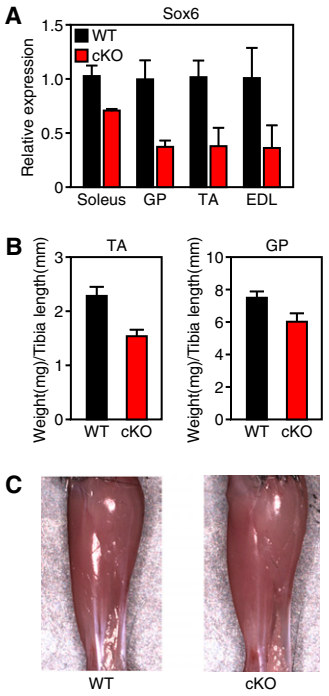

Fig. S1. (*A*) Measurement of Sox6 expression in 8-wk-old WT and Sox6 cKO mice by real-time qPCR. (*B*) Sox6 cKO TA and gastrocnemius/plantaris (GP) muscle from 8-wk-old mice demonstrate a reduction in muscle mass. (*C*) Grossly, WT and Sox6 cKO hindlimb musculature appear identical at P10.

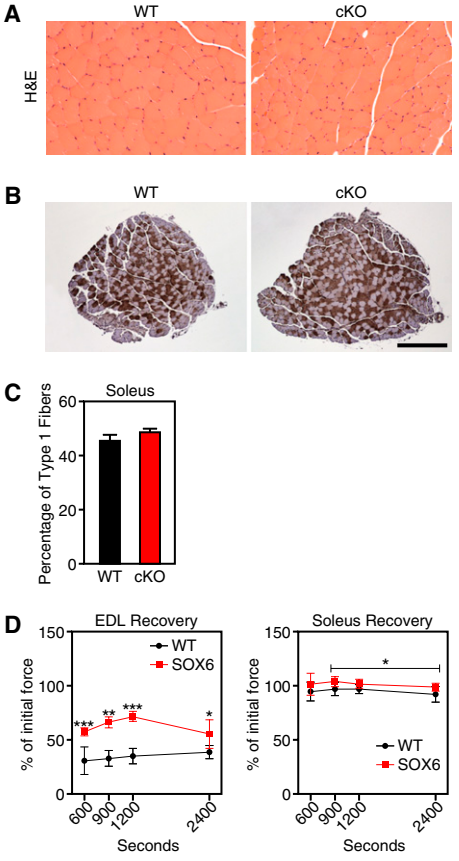

Fig. S2. (*A*) H&E staining of WT and Sox6 cKO TA muscle reveals healthy-looking muscle, with noticeably smaller myofibers in the Sox6 cKO TA at 8 wk of age. (*B*) Immunohistochemistry against Type I myosin in Sox6 cKO soleus displays normal fiber type patterning at P10. Slow fibers are stained brown. (Scale bar, 400 μm.) (*C*) Quantification of Type I fibers in the soleus at P10 confirms preservation of WT fiber-type patterning at P10. (*D*) Sox6 cKO EDL and soleus generate a greater percentage of initial force than WT control muscle following fatigue, indicating enhanced recovery. Datapoints are a continuation of the plot from Fig. 2*C*. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

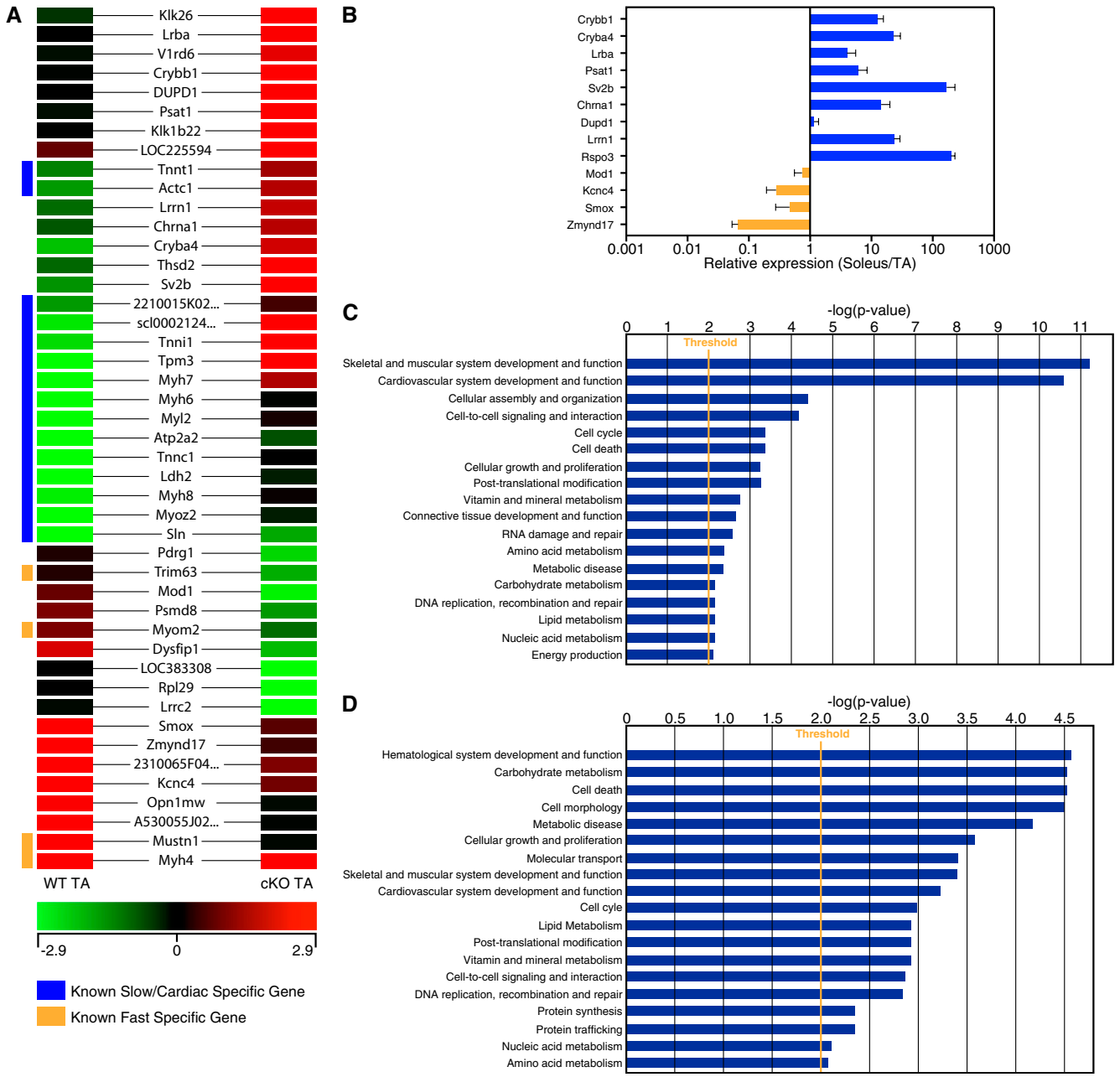

Fig. S3. (*A*) Heat-map analysis of genes regulated >fivefold in Sox6 cKO TA by microarray. Transcripts enriched in fast or slow myofibers are noted. Of 49 regulated genes, 26 have no known fiber-type specificity or function in skeletal muscle. (*B*) Thirteen transcripts from the microarray heatmap (Fig. 4*A*) display novel fiber-type enrichment in WT mice by comparison of expression in slow soleus muscle relative to WT fast TA muscle by qPCR. (*C*) Gene ontology analysis of all genes up-regulated by twofold in Sox6 cKO TA muscle reveals significant enrichment of genes involved in muscle function and metabolism. (*D*) Gene ontology analysis of all genes down-regulated by twofold in Sox6 cKO TA muscle indicates enrichment of genes critical for muscle structure/function and myofiber metabolism.

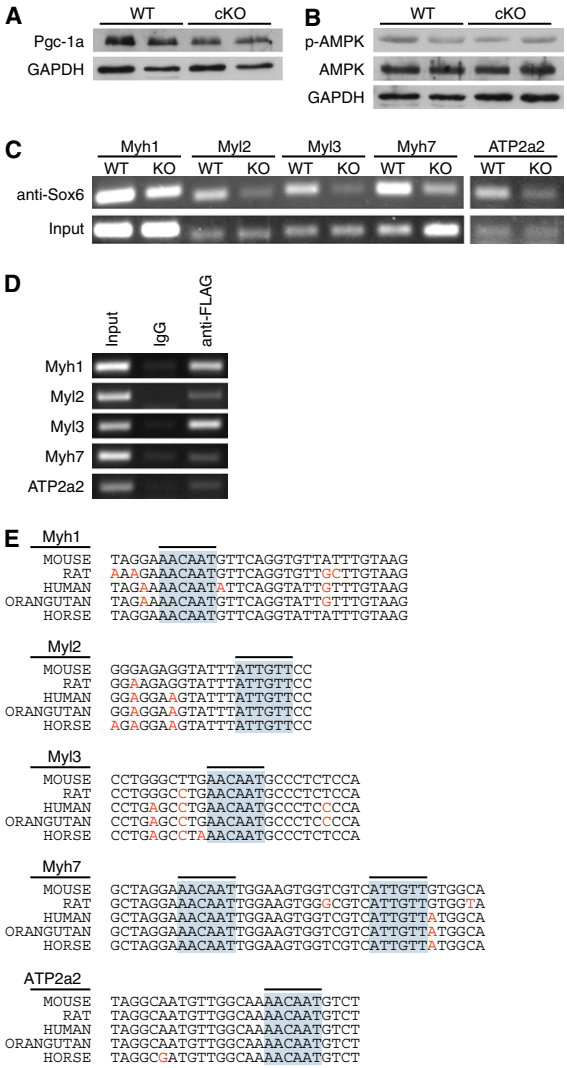

Fig. S4. (*A*) PGC-1α expression is not altered in adult Sox6 cKO TA muscle as detected by Western blot. (*B*) Phosphorylated and total AMP-kinase levels are not changed in adult Sox6 cKO TA muscle compared with WT by Western blot. (*C*) Semiquantitative PCR analysis of anti-Sox6 ChIP chromatin from WT and cKO TA samples at loci containing conserved Sox6 consensus binding sequences. (*D*) Semiquantitative PCR analysis of ChIP chromatin immunoprecipitated with anti-FLAG antibody and IgG negative control from FLAG-Sox6 transfected C2C12 cells. (*E*) The Sox6 binding motifs upstream of five target loci are strongly conserved among mammals.