

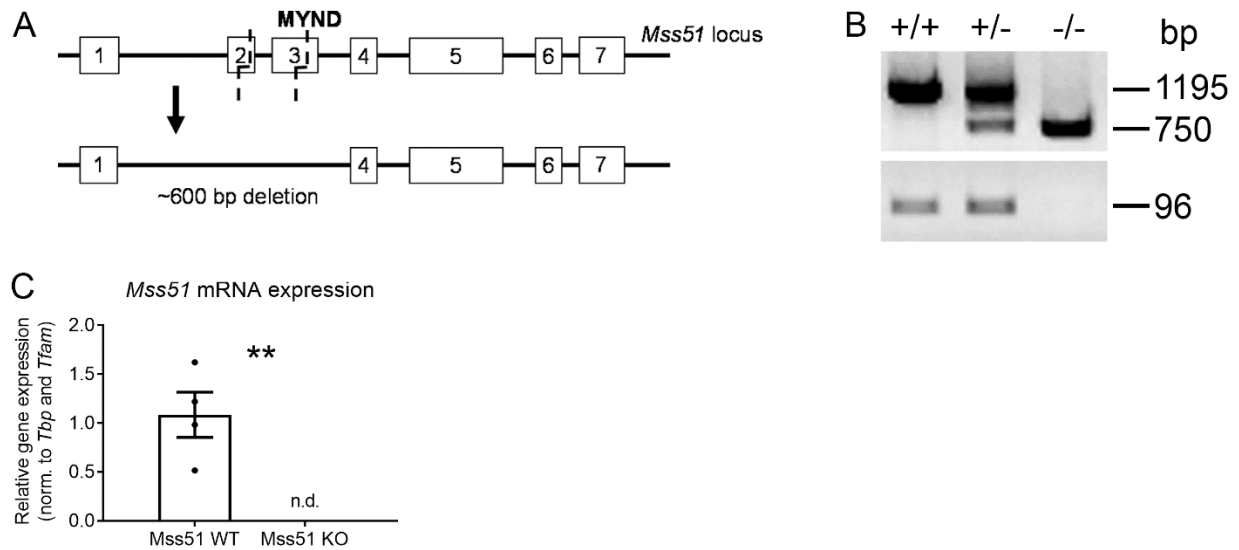
Supplementary Tables and Figures:

Gene	Accession number	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')	Amplicon size (bp)
<i>Tbp</i>	NM_013684.3	AGTGCCCAGCATCACTATTT	GGTCCATGATTCTCCCTTTCTT	126
<i>Tfam</i>	NM_009360.4	ATTCCGAAGTGTTTTCCAGCA	TCTGAAAGTTTTGCATCTGGGT	122
<i>B2m</i>	NM_009735.3	GGTCTTTCTATATCCTGGCTCAC	ACATGTCTCGATCCCAGTAGA	120
<i>Mss51</i>	NM_029104.1	GTCCCAGTTGATCCTCCAAA	CGAAATCCAAAGCTTGATACTGG	96

Supplemental Table 1. Primer sequences for the quantitative RT-PCR of specified mouse genes.

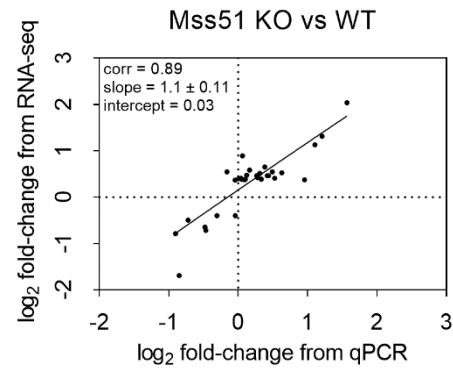
Genes	RNA-seq	qPCR
<i>Acaa2</i>	+0.52	+0.31
<i>Acadl</i>	+0.41	+0.52
<i>Acsl1</i>	+0.42	+0.28
<i>Acss1</i>	+0.46	+0.42
<i>Acta2</i>	-0.40	-0.31
<i>Adcy2</i>	+0.65	+0.38
<i>Atp5g1</i>	+0.54	+0.49
<i>Atp5g3</i>	+0.40	+0.01
<i>Bdh1</i>	+2.04	+1.57
<i>Cox6b1</i>	+0.39	+0.10
<i>Cox7a1</i>	+0.46	+0.27
<i>Cox7a2</i>	+0.39	+0.33
<i>Eci1</i>	+0.39	+0.05
<i>Egr1</i>	-1.68	-0.85
<i>Fads6</i>	+0.58	+0.16
<i>Got2</i>	+0.38	+0.10
<i>Hadh</i>	+0.38	+0.96
<i>Hadhb</i>	+0.47	+0.44
<i>Idh2</i>	+0.89	+0.06
<i>Ldhd</i>	+1.32	+1.21
<i>Mdh1</i>	+0.54	-0.16
<i>Myl2</i>	+1.13	+1.11
<i>Myl3</i>	+0.53	+0.63
<i>Ndufb10</i>	+0.37	-0.04
<i>Ndufb3</i>	+0.37	+0.34
<i>Ndufb7</i>	+0.38	+0.09
<i>Oxct1</i>	+0.48	+0.12
<i>Pck1</i>	-0.79	-0.90
<i>Ppp1r3c</i>	-0.70	-0.47
<i>Prkar2b</i>	-0.49	-0.72
<i>Scd1</i>	-0.65	-0.48
<i>Scd2</i>	-0.39	-0.04
<i>Tomm5</i>	+0.42	+0.04

Supplemental Table 2. Comparison of log2(fold change) values detected by RNA-seq versus qPCR. Gene expression from quadriceps of Mss51 WT versus Mss51 KO muscle.



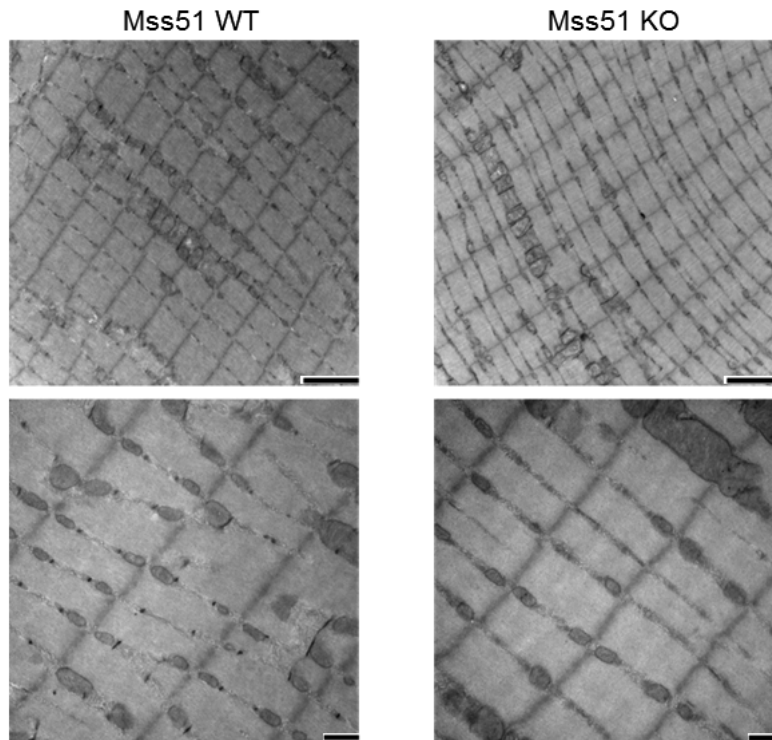
Supplemental Figure 1. *Mss51* KO mouse model design.

(**A**) Schematic of mouse design for knocking out *Mss51* gene. The dashed lined represent the cut sites for the guide RNAs for introduction of indels through non-homologous end joining (NHEJ). Note that the cut sites encompass part of the highly conserved zinc finger mynd domain (MYND). (**B**) Genotyping of WT (+/+), heterologous (+/-), and knockout (-/-) mice. The top panel represents the PCR amplification of whole *Mss51* locus in the stated mouse sample while the bottom panel shows the PCR amplification of just the sections that were deleted (exons 2 and 3) in the knockout mice. (**C**) *Mss51* gene expression analysis done in quadriceps muscle of 6-month-old mice on standard chow diet. Normalized to *Tbp* and *Tfam*. Data are expressed as mean $\pm$ SEM ($n = 4-6$ per group, all females) $**P < 0.01$ by Student's *t* test. n.d. = not detected.



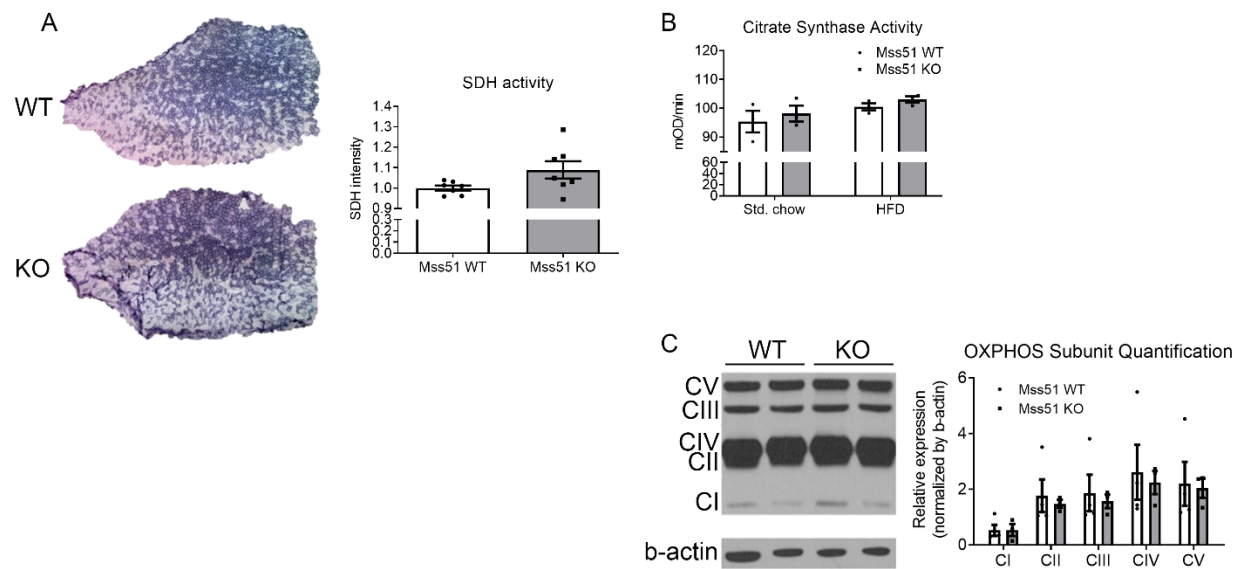
Supplemental Figure 2. Correlation between RNA-seq and qPCR.

Correlation between log₂ changes from RNA-seq and from qPCR for Mss51 WT vs KO mice. Each dot represents a differentially expressed gene. For qPCR, expression levels were normalized to *Tbp* and *Tfam*.



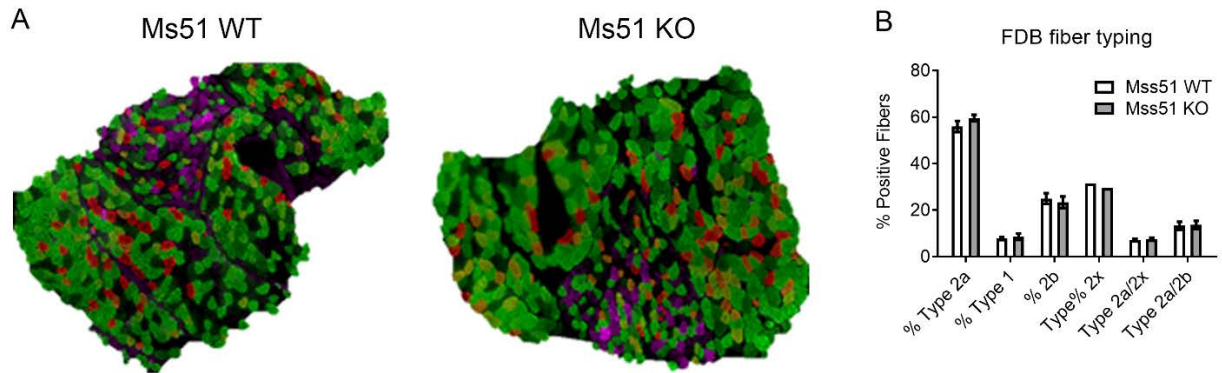
Supplemental Figure 3. Electron microscopy of EDL muscle.

Top panel: Scale bar = 2 µm; Magnification = 12,000x. Bottom panel: Scale bar = 500 nm; Magnification = 30,000x.



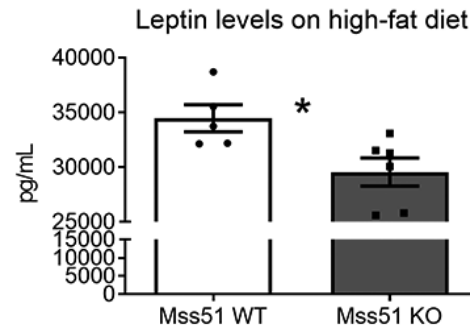
Supplemental Figure 4. Mitochondrial capacity in Mss51 WT and Mss51 KO mice.

(A) Succinate dehydrogenase (SDH) staining of TA muscle of Mss51 WT and KO mice and SDH activity quantitation (n = 7 per group) on standard chow diet. (B) Citrate synthase assay in muscle of Mss51 WT and KO mice (n = 3 per group) on standard chow and HFD. (C) Representative western blot and quantitation of unchanged total OXPHOS proteins in quadriceps muscle of Mss51 WT and KO mice (n = 4 per group). Data are expressed as mean $\pm$ SEM.



Supplemental Figure 5. Fiber typing of Flexor digitorum brevis (FDB) muscle in Mss51 WT and KO mice.

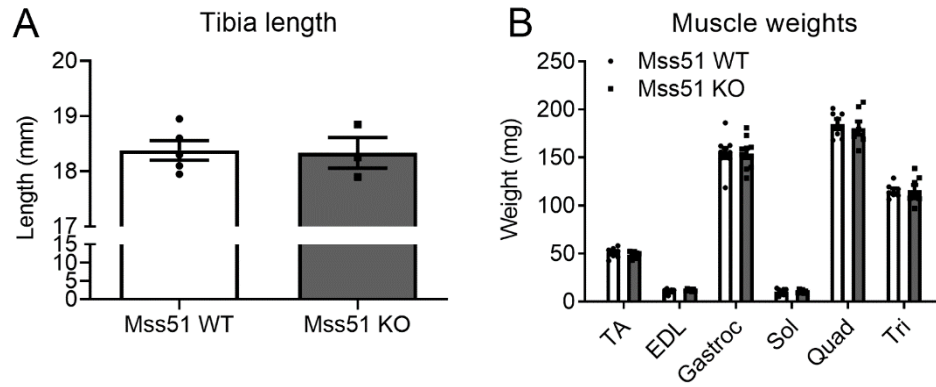
(A) Representative images of immunostaining in which type 1 fibers are magenta, type 2a are green, type 2b are red, and type 2x are black. (B) Percent positive fibers of each fiber type in FDB muscle from Mss51 WT and KO mice (n = 6-7 per group). Data are expressed as mean $\pm$ SEM (n = 3-4 per group, all females).



Supplemental Figure 6. Serum leptin levels.

Results from 2 mo animals fed HFD for 13 weeks. Data are expressed as mean $\pm$ SEM (n = 5-6 per group, all males)

* $P < 0.05$ by Student's t test.



Supplemental Figure 7. Muscle mass and tibia length in HFD-fed Mss51 WT and KO mice.

(A) Tibia length (n = 3-5, males) and (B) muscle weights (n = 7-10, males) of HFD-fed Mss51 WT and KO mice. Data are expressed as mean ± SEM.