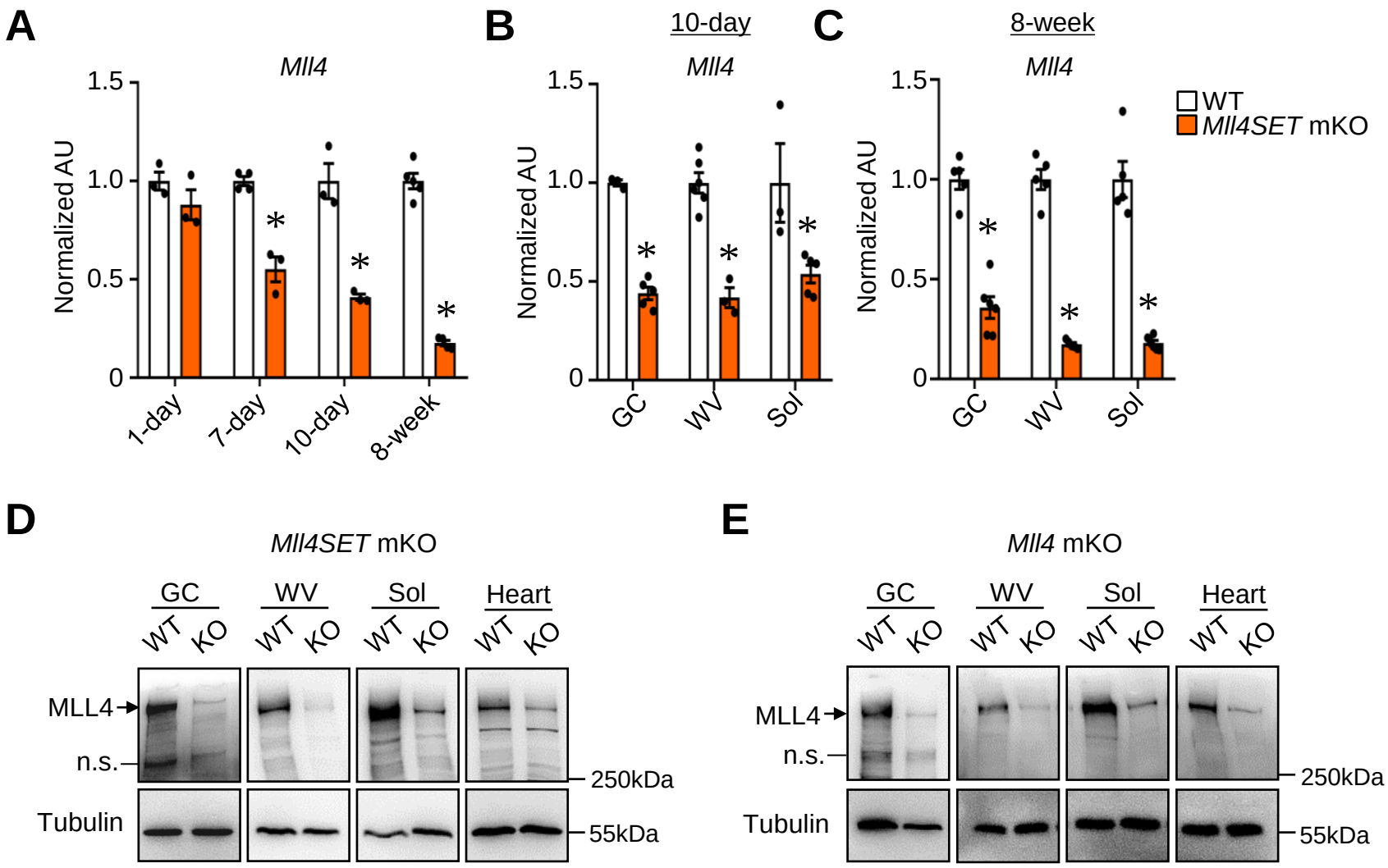
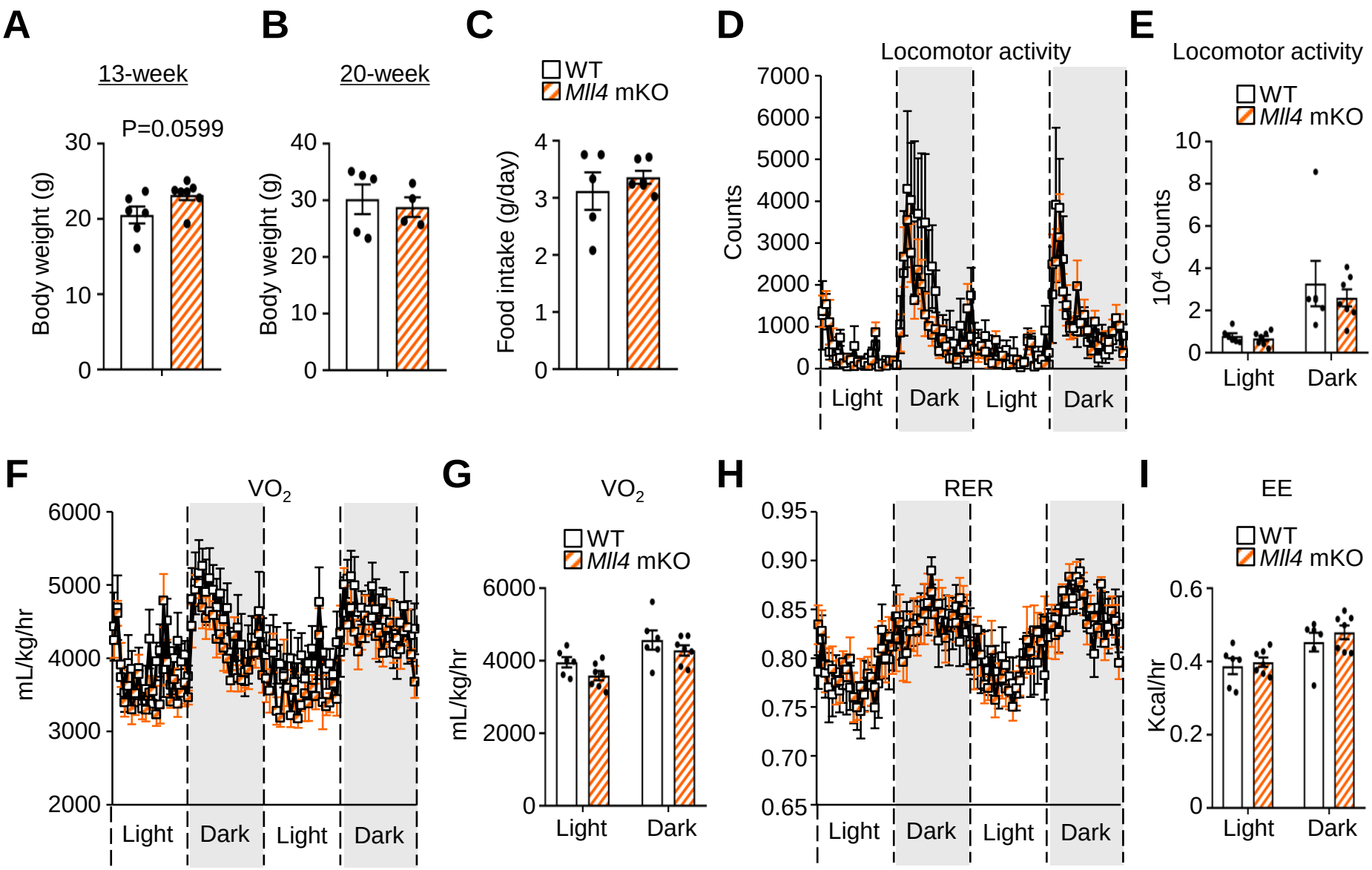
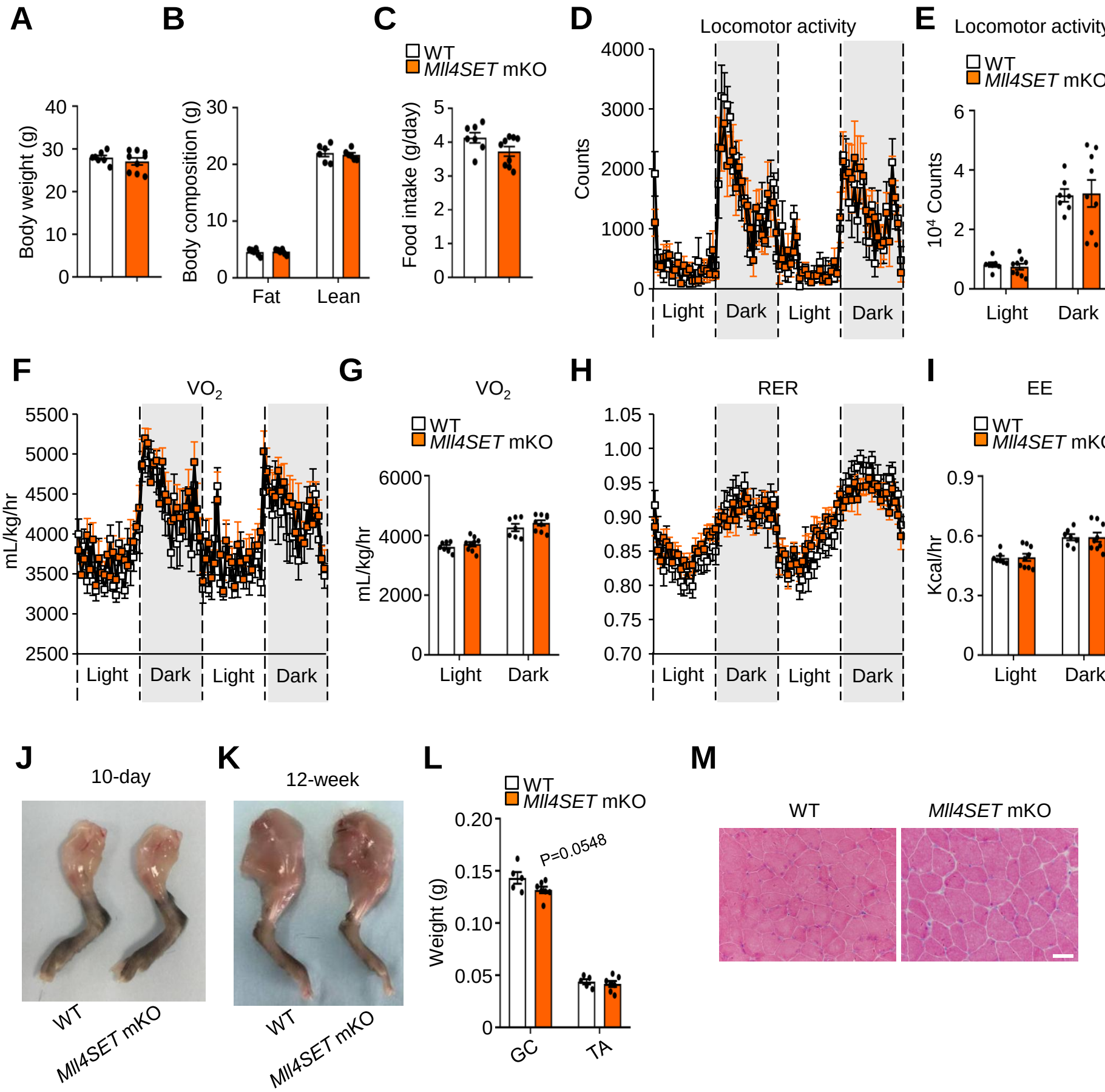


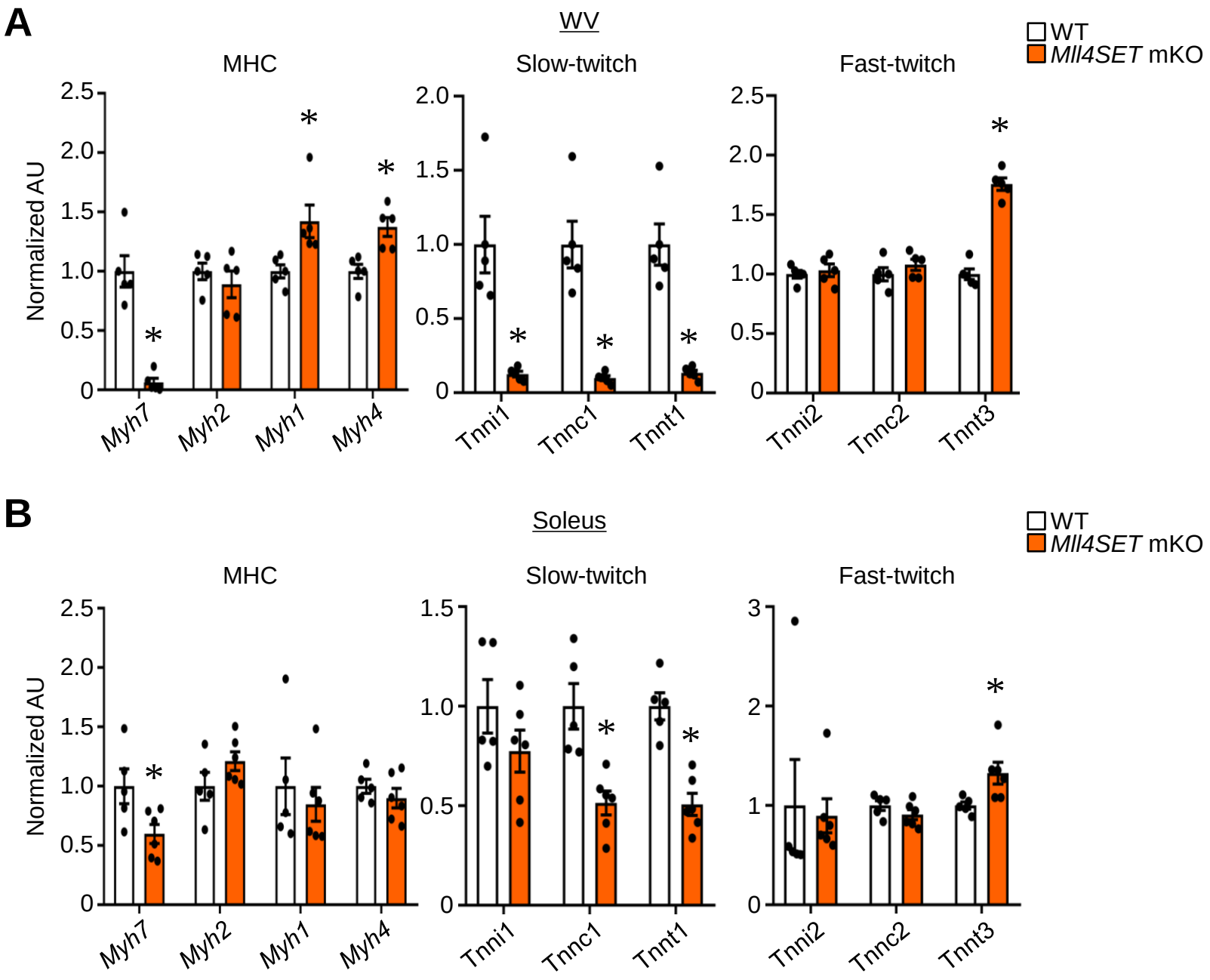
Supplemental Figure 1. Generation of two independent skeletal muscle-specific *Mll4*-knockout mouse lines. (**A**-**D**) Efficient postnatal deletion of *Mll4* in skeletal muscle by *Mck-Cre*. (**A**) qRT-PCR analysis of *Mll4* expression in skeletal muscle from indicated genotypes at the age of 1, 7, 10 days and at 8 weeks. n = 3-5 mice per group. (**B**, **C**) Bars represent mean ± SEM gene expression levels of *Mll4* using qRT-PCR from gastrocnemius (GC), white vastus lateralis (WV) and soleus muscles of WT and *Mll4SET* mKO mice (**B**) at the age of 10 days and (**C**) at 8 weeks. n = 3-6 mice per group. (**D**, **E**) Western blot analysis of MLL4 expression in skeletal muscle and heart from indicated genotypes. n.s., none-specific band, n = 3 mice per group. Values represent mean ± SEM, **P* < 0.05 vs. corresponding WT controls, *P*-value was determined using two-tailed unpaired Student's *t*-test.



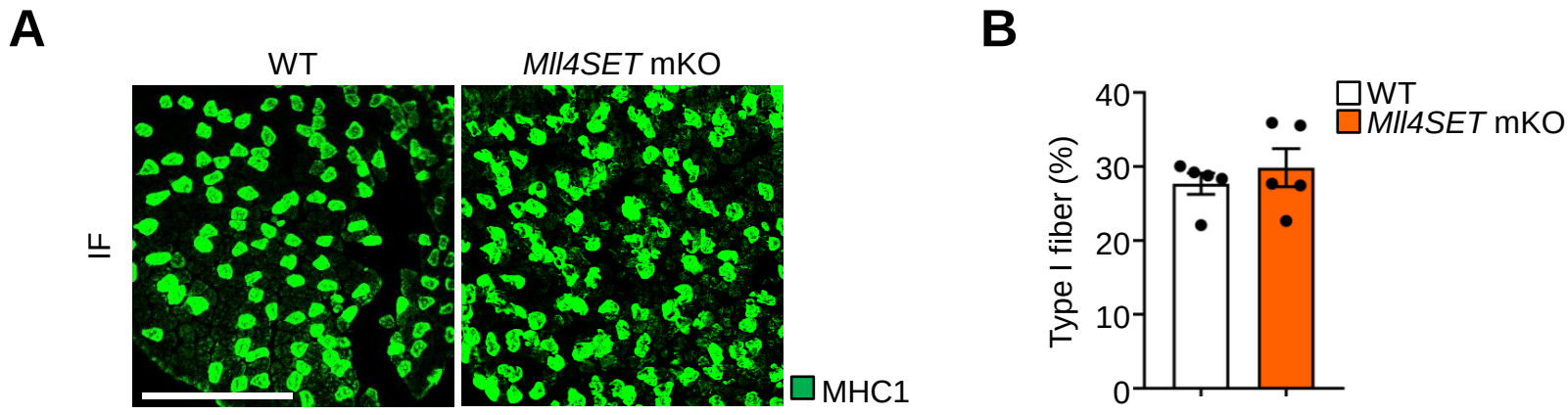
Supplemental Figure 2. Metabolic characterization of *Mll4* mKO mice fed on a normal chow diet. (**A**) Body weight of 13-week-old male WT or *Mll4* mKO mice. n = 6-7 mice per group. (**B**) Body weight in a second set of 20-week-old male WT or *Mll4* mKO mice. n = 4-5 mice per group. (**C**-**I**) Metabolic cage measurements of 13-week-old male WT and *Mll4* mKO mice. n = 5-7 mice per group. (**C**) Food consumption. (**D**, **E**) Locomotor activity over 12 hour light/dark cycle. (**F**, **G**) Oxygen consumption per hour during the light/dark cycle normalized to body weight. (**H**) Respiratory exchange rate (RER) during the light/dark cycle. (**I**) Energy expenditure per mouse. Data are represented as mean ± SEM. *P*-value was determined using two-tailed unpaired Student's *t*-test.



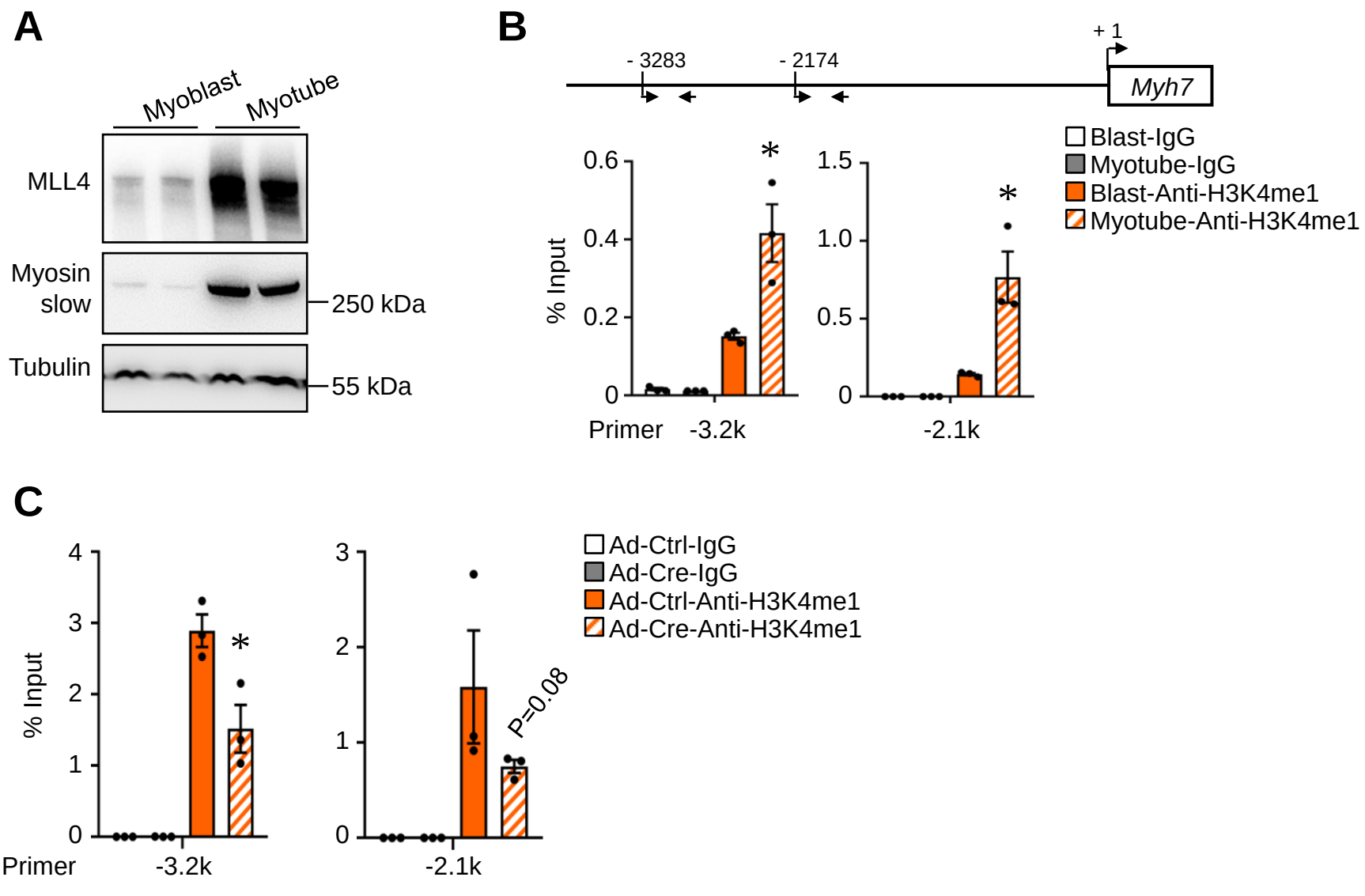
Supplemental Figure 3. Metabolic characterization of *Mll4SET* mKO mice fed on a normal chow diet. (**A**) Body weight of 9-week-old male WT or *Mll4SET* mKO mice. n = 7-9 mice per group. (**B**) Body composition measured to determine fat mass and lean tissue mass the age of 10 weeks. n = 6 mice per group. (**C**-**I**) Metabolic cage measurements of 9-week-old male WT and *Mll4SET* mKO mice. n = 7-9 mice per group. (**C**) Food consumption. (**D**, **E**) Locomotor activity over 12 hour light/dark cycle. (**F**, **G**) Oxygen consumption per hour during the light/dark cycle normalized to body weight. (**H**) Respiratory exchange rate (RER) during the light/dark cycle. (**I**) Energy expenditure per mouse. (**J**, **K**) Representative hindlimbs from WT and *Mll4SET* mKO mice (**J**) at the age of 10 days and (**K**) at 12 weeks. n = 5-7 mice per group. (**L**) Weight of gastrocnemius (GC) and tibialis anterior (TA) muscles from 12-week-old male WT and *Mll4SET* mKO mice. n = 5-7 mice per group. (**M**) H&E staining of the GC muscle from 8-week-old male WT and *Mll4SET* mKO mice. Representative images were shown. Scale bar: 50 μm. n = 3-5 mice per group. Data are represented as mean ± SEM. *P*-value was determined using two-tailed unpaired Student's *t*-test.



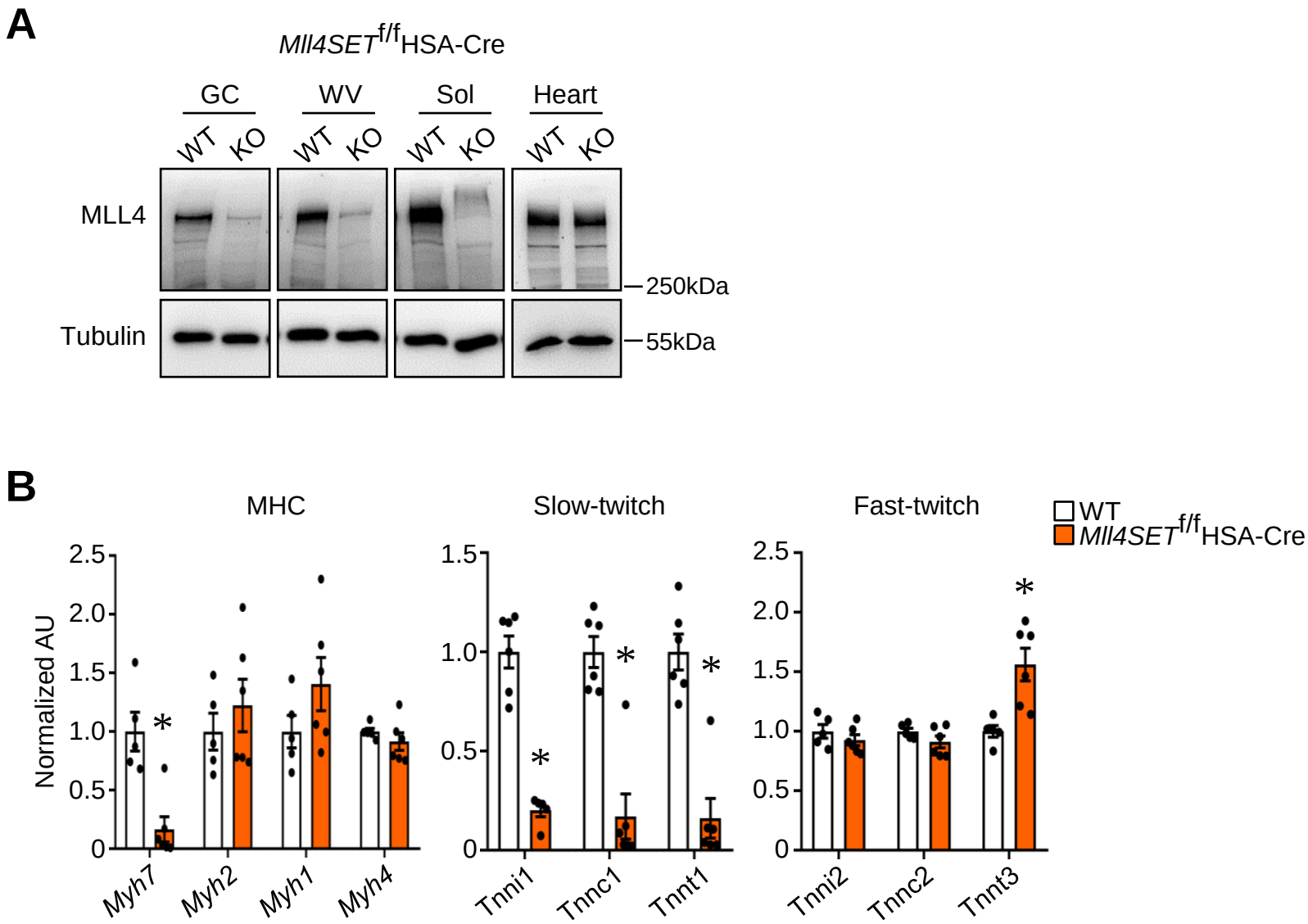
Supplemental Figure 4. MLL4 regulates slow-twitch muscle fiber type program. (**A**, **B**) Expression of myosin heavy chain (MHC) and representative slow/fast-twitch troponin genes (qRT-PCR) in WV (**A**) and soleus (**B**) muscle of *Mll4SET* mKO mice and WT controls. n = 5-6 mice per group. Values represent mean ± SEM, **P* < 0.05 vs. corresponding WT controls, *P*-value was determined using two-tailed unpaired Student's *t*-test.



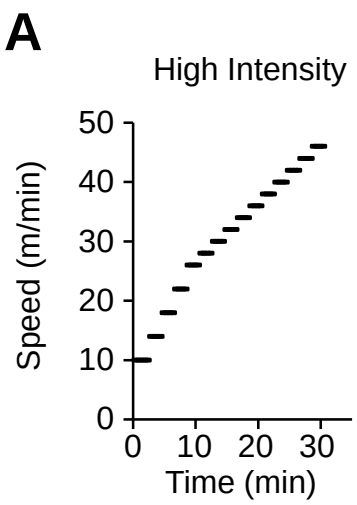
Supplemental Figure 5. Characterization of *Mll4SET* mKO mice at P10. (**A**) Cross-section of soleus muscle from P10 *Mll4SET* mKO mice stained for MHC1 by immunofluorescence (IF). MHC1 (green). Representative images were shown. Scale bar: 250 μm. (**B**) Quantification of IF data shown in (**A**) expressed as mean % total muscle fibers. n = 5 mice per group.

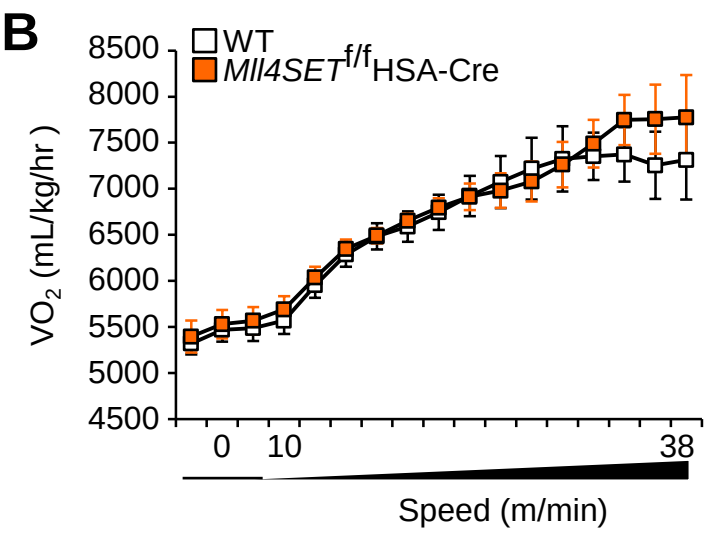


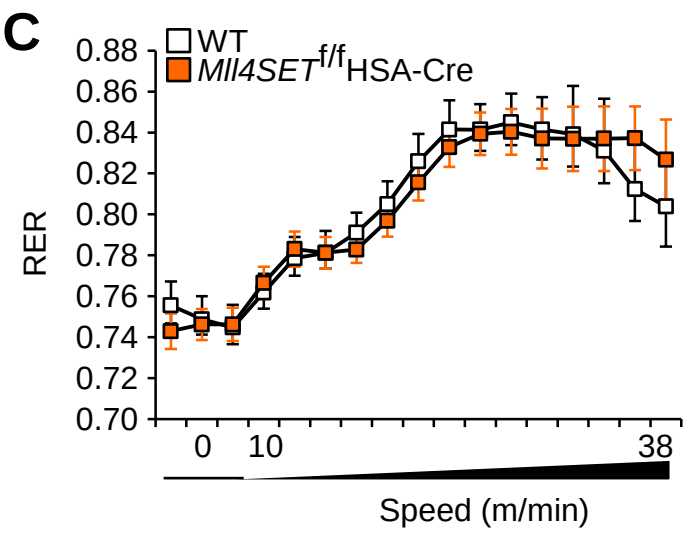
Supplemental Figure 6. MLL4-mediated H3K4me1 parallels type I muscle gene expression in primary muscle cells. (**A**) Representative Western blot analysis of MLL4 and slow myosin protein levels during differentiation of myoblasts into mature myotubes (n = 3). (**B**) Results of SYBR green-based quantification of H3K4me1 ChIP assays performed on WT primary myoblasts or myotubes (n = 3). The graphs show enrichment relative (%) to input, while the schematic shows PCR primer set location relative to the *Myh7* gene transcription start site (= +1). (**C**) Primary myoblasts isolated from *Mll4SET*$^{f/f}$ mice were infected with an adenovirus overexpressing Cre or control virus (Ctrl), followed by differentiation into myotubes. ChIP assays in myotubes using anti-H3K4me1 antibody (n = 3). Values represent mean ± SEM, **P* < 0.05 vs. corresponding controls. *P*-value was determined using one-way ANOVA coupled to a Fisher's least-significant difference (LSD) post-hoc test.

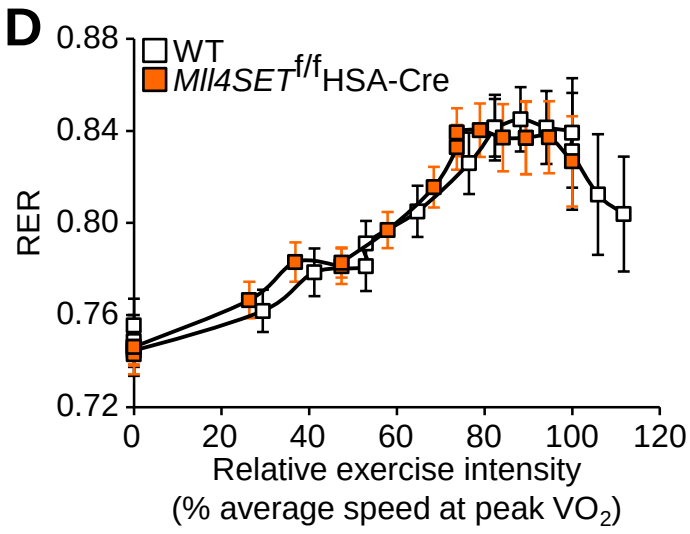


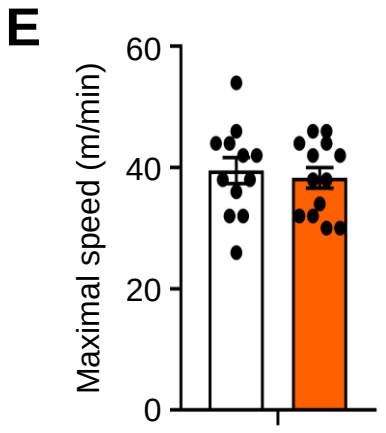
Supplemental Figure 7. Generation and characterization of a third skeletal muscle-specific *Mll4*-knockout mouse line. (**A**) Western blot analysis of MLL4 expression in skeletal muscle and heart from indicated genotypes. (**B**) Expression of myosin heavy chain (MHC) and representative slow/fast-twitch troponin genes (qRT-PCR) in GC muscle of *Mll4SET*$^{f/f}$*HSA-Cre* mice and WT controls. n = 5-6 mice per group. Values represent mean ± SEM, **P* < 0.05 vs. corresponding WT controls, *P*-value was determined using two-tailed unpaired Student's *t*-test.

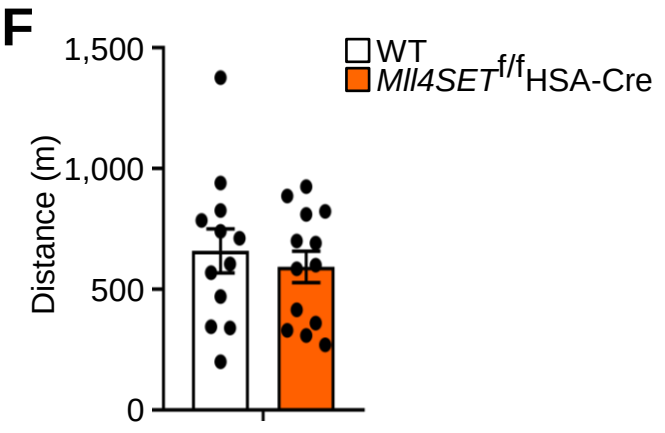


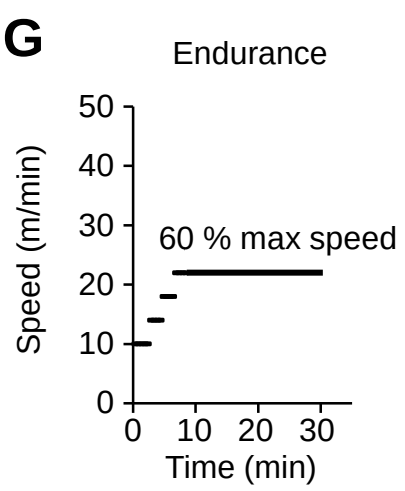


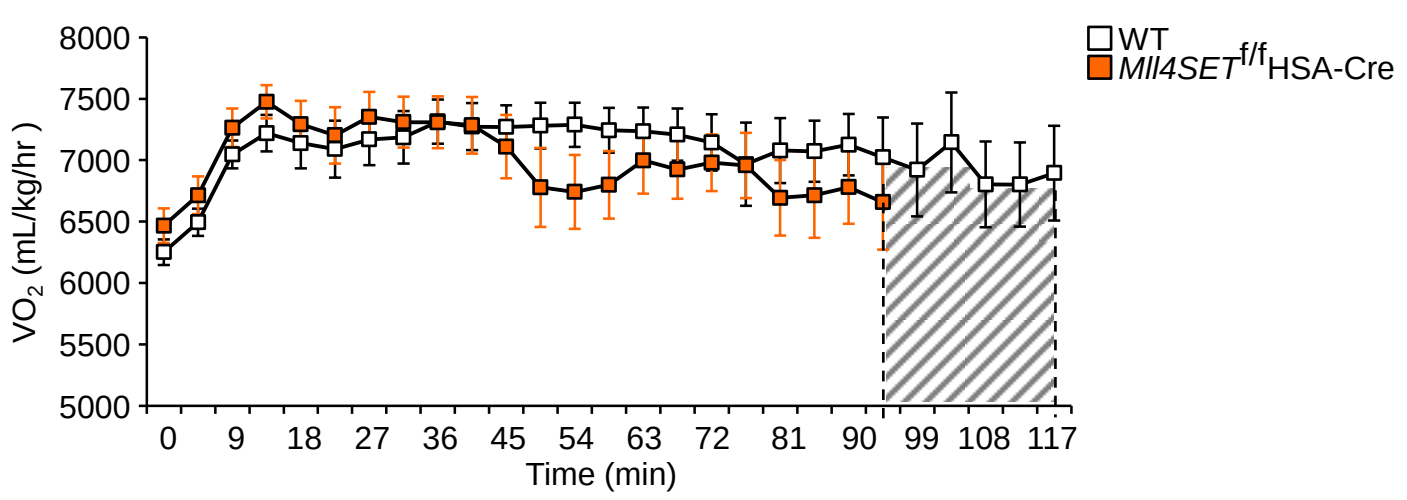


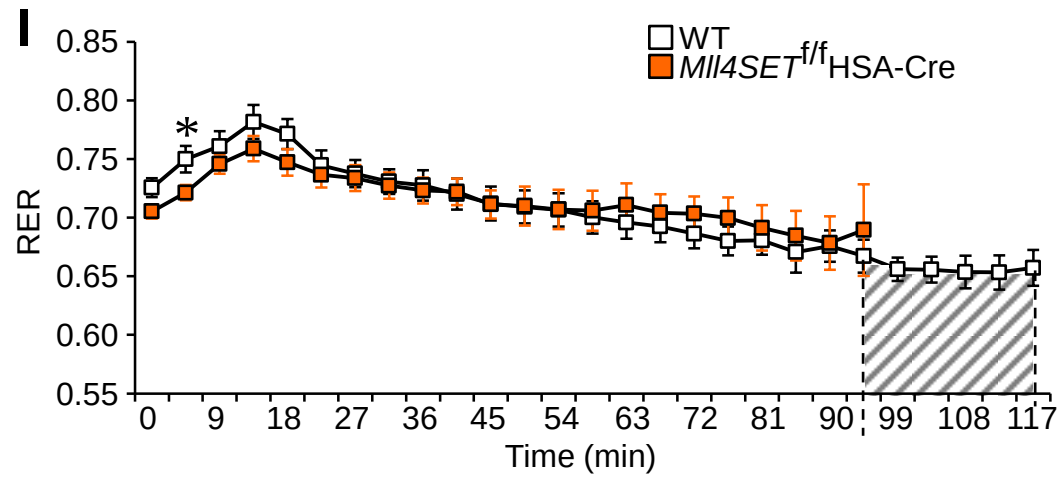


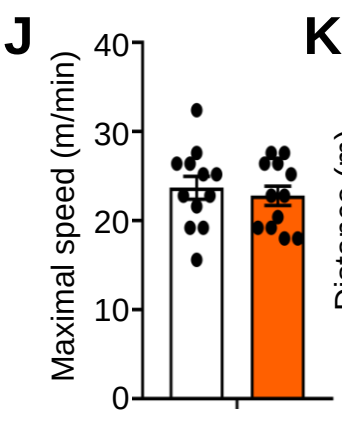


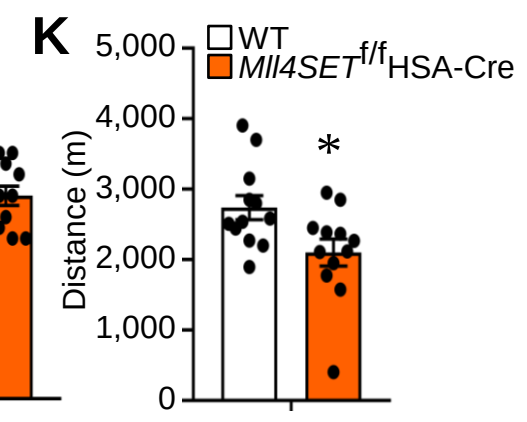




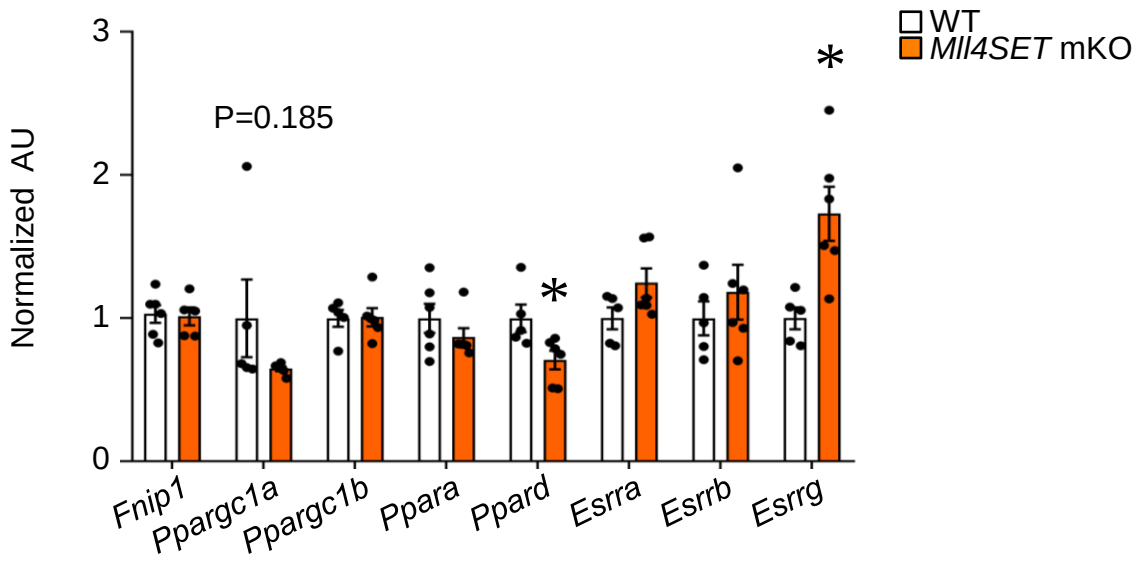








Supplemental Figure 8. *Mll4SET*$^{f/f}$*HSA-Cre* mice show reduced running endurance capacity. (**A**) Schematic depicts the increments of speed over time during maximal exercise capacity test (VO_{2max} test). (**B**) VO_2 (oxygen consumption) and (**C**) respiratory exchange ratio (RER) during the course of the high intensity exercise in 8 week-old male *Mll4SET*$^{f/f}$*HSA-Cre* mice and WT controls. n = 12-13 mice per group. (**D**) RER values were plotted against relative exercise intensity, as estimated by the percentage of mean speed at which peak VO_2 occurred for each genotype. n = 12-13 mice per group. (**E**) Maximal speed and (**F**) total distance reached at exhaustion. n = 12-13 mice per group. (**G**) Schematic depicts the increments of speed over time during low intensity endurance exercise. (**H**) VO_2 and (**I**) RER during endurance exercise challenge. n = 12 mice per group. The grey shaded area in the VO_2 and RER line graphs illustrate the difference in time to exhaustion in *Mll4SET*$^{f/f}$*HSA-Cre* mice (91 ± 12 min) compared to WT controls (118 ± 10 min**)**. (**J**) Maximal speed and (**K**) total distance reached at exhaustion. n = 12 mice per group. Values represent mean ± SEM, **P* < 0.05 vs. corresponding controls, *P*-value was determined using two-tailed unpaired Student's *t*-test.

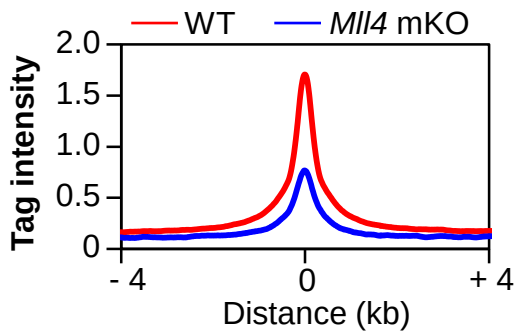


Supplemental Figure 9. Relative expression of genes involved in muscle fiber type in *Mll4SET* mKO mice. Expression of the indicated genes (qRT-PCR) in gastrocnemius muscles from *Mll4SET* mKO mice compared to WT controls. n = 5-6 mice per group. Values represent mean ± SEM, and shown as arbitrary units (AU) normalized (=1.0) to the value of WT controls. **P* < 0.05 vs. WT controls, *P*-value was determined using two-tailed unpaired Student's *t*-test.

A

Genotype	Antibody	Total reads
Input	------	17818116
WT	MLL4	15774289
Mll4 mKO	MLL4	12571708

B



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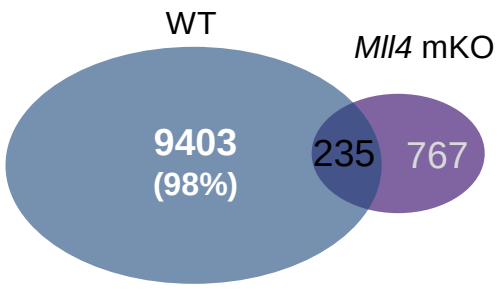

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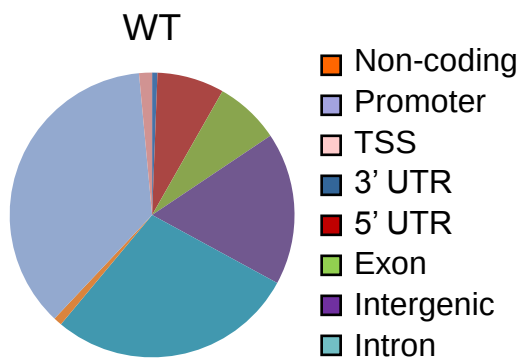

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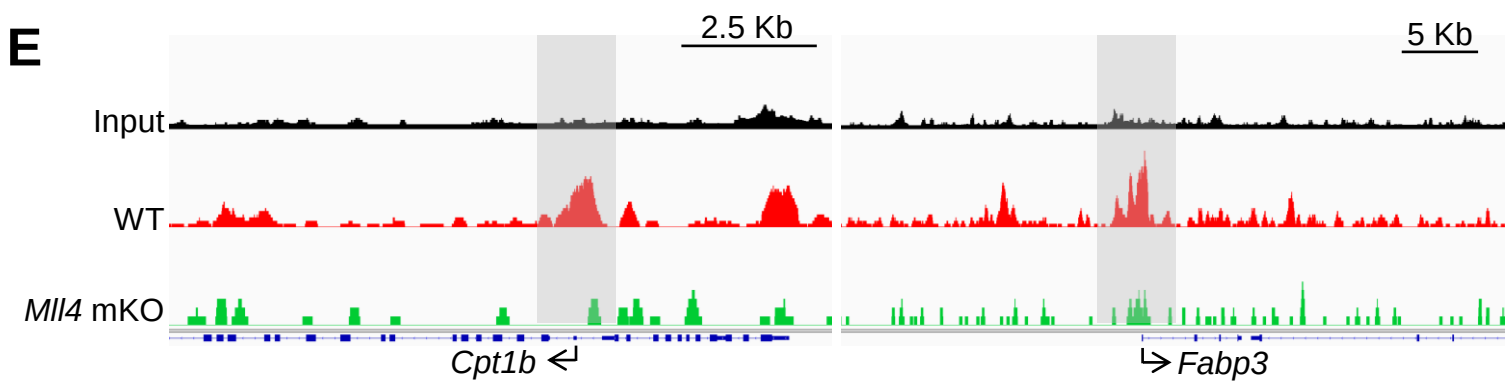

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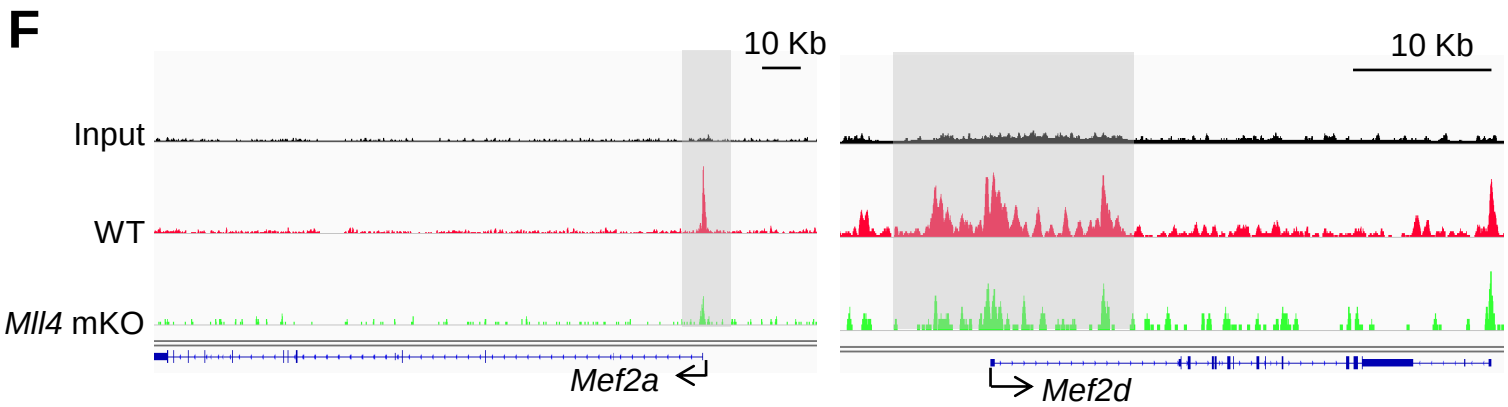
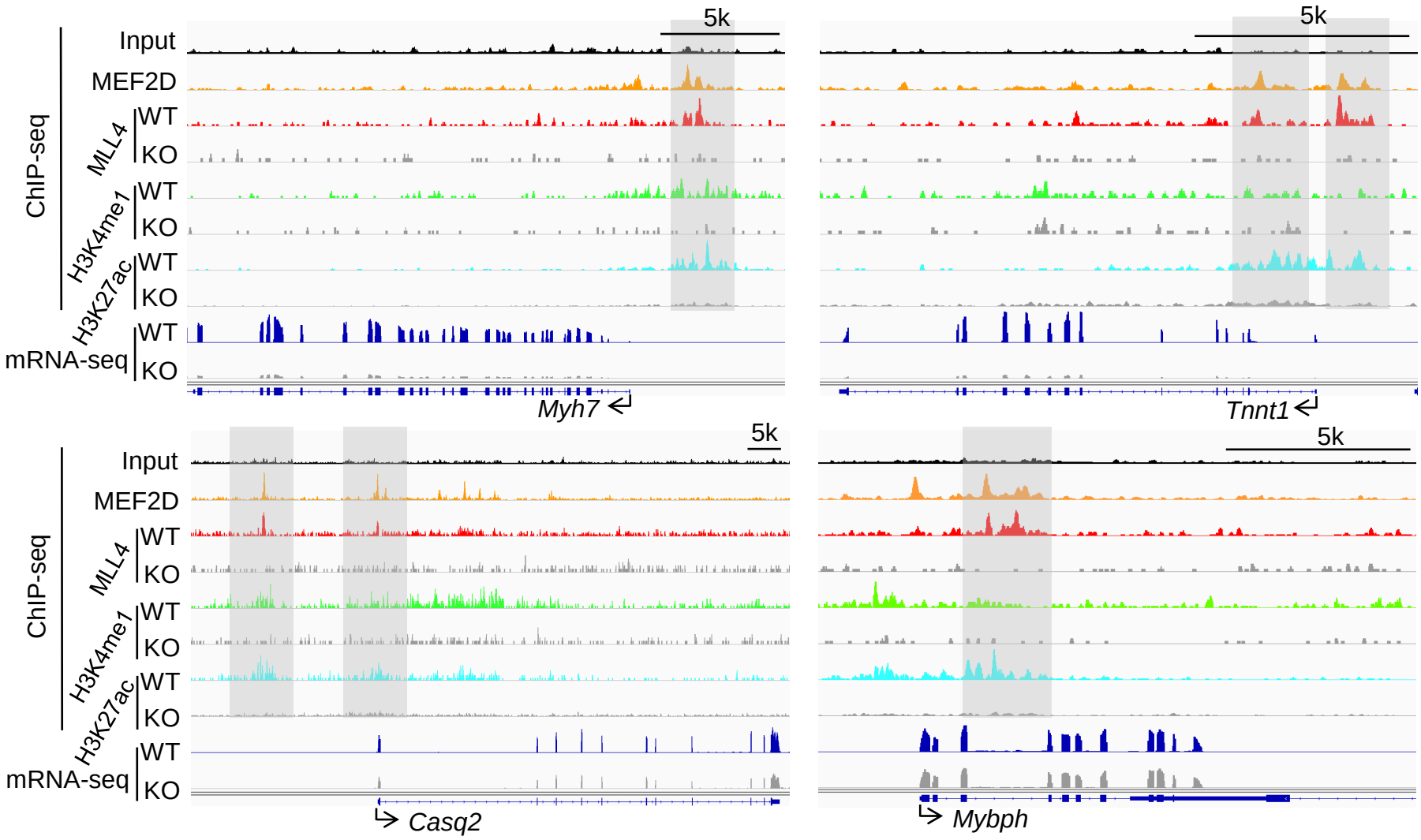
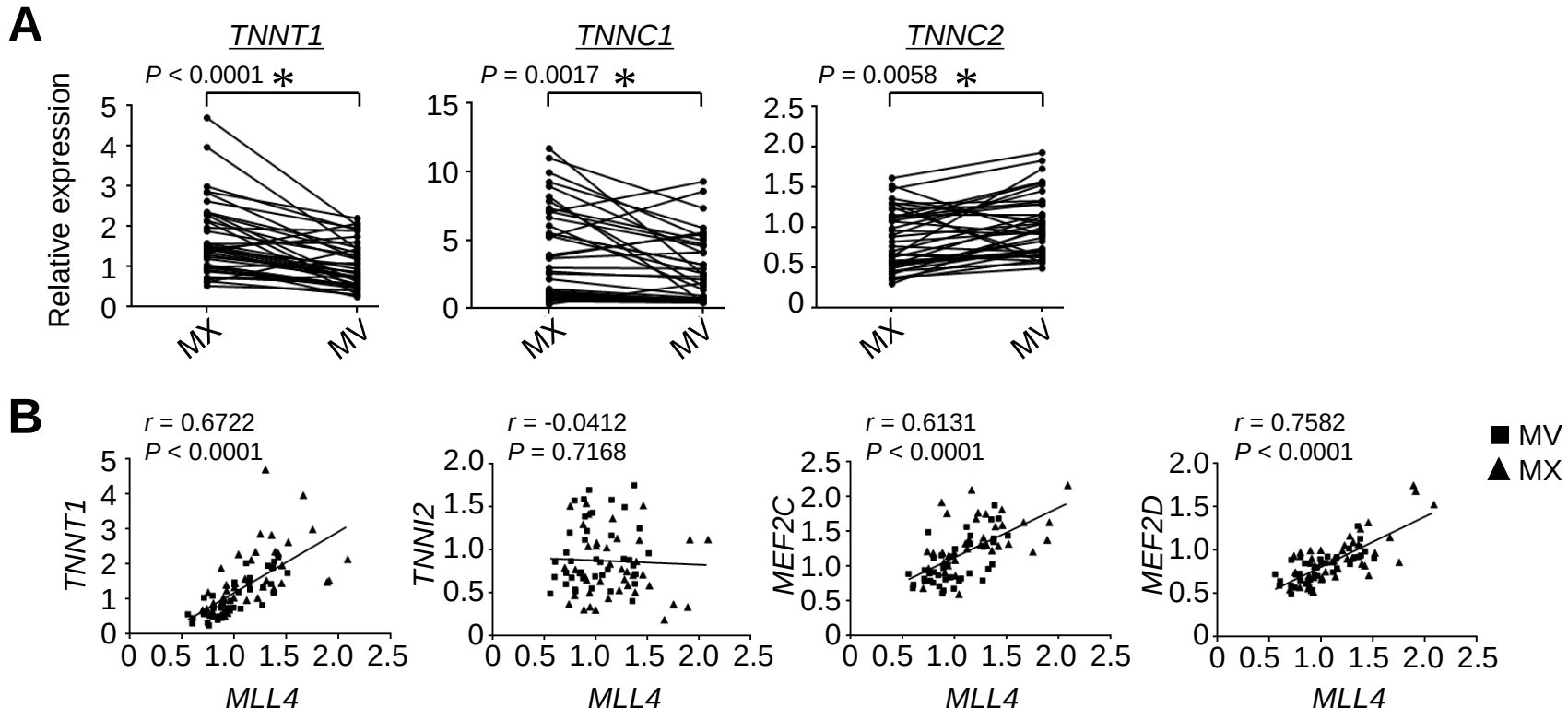


Supplemental Figure 10. Cistromic analysis of MLL4 in skeletal muscle. (**A**) Total ChIP-seq reads are shown in WT or *Mll4* mKO chromatin enriched with an anti-MLL4. (**B**) Signal intensity plot of MLL4 ChIP-Seq signal in WT or *Mll4* mKO chromatin are shown. The peak distribution of MLL4 binding in WT muscle is shown in red, whereas the peak in the *Mll4* mKO is shown in blue. (**C**) MLL4-binding peaks in WT muscle were compared with those of *Mll4* mKO. A total of 9403 high-confidence binding regions were identified. (**D**) Genomic distribution of MLL4-binding sites relative to mouse RefSeq genes. The promoter regions are defined as 1 kb upstream to 200 bp downstream of a RefGene transcription start sites (TSS). (**E**, **F**) ChIP-Seq binding profiles of MLL4 in WT or *Mll4* mKO muscle are shown. Input, genomic DNA from muscle; Gray box indicates the high confidence MLL4 binding regions.



Supplemental Figure 11. MLL4 co-localizes with MEF2 on enhancer of slow-myofiber genes. MLL4-dependent active enhancers on slow-myofiber gene loci are shown (data obtained from published data sets GSE50466 and GSE43223, as well as current data set GSE137368). (Top) ChIP-seq binding profiles for MLL4, MEF2D and histone modifications in WT or *Mll4* KO myocytes are shown. mRNA-seq data from WT and *Mll4SET* mKO muscle is shown at the bottom. Input, genomic DNA from myocytes; Gray box indicates the high confidence MLL4 binding regions.



Supplemental Figure 12. MLL4 regulatory circuit is associated with muscle fiber type remodeling in humans. Paraspinal muscle samples from both the convex ("MX") and concave ("MV") sides of the curvature from 40 patients with adolescent idiopathic scoliosis (AIS) were used for this analysis. (**A**) mRNA expression levels of muscle contractile genes were determined by qRT-PCR. Significant differences were analyzed using paired Student's *t* test. (**B**) Correlation between *MLL4* and *MEF2s* gene expression and that of muscle contractile genes. Pearson correlation analysis was used to determine the correlation.

Supplemental Table 1. Human Subject Characteristics		
	Mean (n = 40 female)	Range
Age (yr)	13.8 ± 0.3	10 - 18
Height (cm)	159.3 ± 0.8	146 - 171
Weight (kg)	45.3 ± 1.0	35 - 66
BMI (kg/m^2)	17.8 ± 0.3	13.7 - 23.7
Initial Major Cobb (°)	54.0 ± 2.4	40 - 115

Data represent the mean ± SEM.

Supplemental Table 2. RT-PCR primers		
Mouse Gene	***Forward***	***Reverse***
36b4	*5'-ATCCCTGACGCACCGCCGTGA*	*5'-TGCATCTGCTTGGAGCCCACGT*
Mll4	*5'-CAGTTGAGCTAGTCAAGTGATT*	*5'-TTCAATGTGGAGGGGAGTGACAG*
Myh7	*5'-GCCAACTATGCTGGAGCTGATGCCC*	*5'-GGTGCGTGGAGCGCAAGTTTGTCATAAG*
Tnni1	*5'-TGAAGCCAAATGCCTCCACAACAC*	*5'-ACACCTTGTGCTTAGAGCCCAGTA*
Tnnt1	*5'-TGGATCCACCAGCTGGAATCAGAA*	*5'-GCTGATGCGGTTGTAGAGCACATT*
Tnnc1	*5'-AGCTCATGAAGGACGGTGACAAGA*	*5'-AACCGTGCAAGACCAGCATCTACT*
Tnni2	*5'-AGCAGCAAGGAGCTGGAAGA*	*5'-ATGGCGTCGGCAGACATAC*
Tnnc2	*5'-CCATCATCGAGGAGGTGGAC*	*5'-CTTCCCCTTCGCATCCTCTT*
Tnnt3	*5'-AACTGGAGACTGACAAATTCGAGT*	*5'-GCTGTGCTTCTGGGTTTGGT*
Myl2	*5'-GGACACATTTGCTGCCCTA*	*5'-ATCGTGAGGAACACGGTGA*
Myl3	*5'-GGCTCTGGGTCAGAATCCTA*	*5'-CATCATCTTGGAATTGAGCTCTT*
Tpm3	*5'-GAGCTGGACAAGTATTCGGAAG*	*5'-AGGAGGCCACCTCAGCTT*
Myom3	*5'-AGAGGCGGGTAGGCTTTG*	*5'-CTCTCGGACCTTCTCCTCTG*
Atp2a2	*5'-TCGACCAGTCAATTCTTACAGG*	*5'-CAGGGACAGGGTCAGTATGC*
Casq2	*5'-CCGCACGATTGAGTTTGAC*	*5'-CACGATCTCCACTGGGTCTT*
Smtnl1	*5'-GGATTGGCTCCAGAGTCTACA*	*5'-CTCACTGGGCGAAGATTCA*
Myoz2	*5'-TTGTCCCATTTCAGTAATCGTG*	*5'-TGCATGGCGATATTGTGATT*
Ldhb	*5'-AGTCTCCCGTGCATCCTCAA*	*5'-AGGGTGTCCGCACTCTTCCT*
Ppp1r1a	*5'-CACCTGGGATCCCAGACACA*	*5'-GGGGTTGGATTCTGCAGACT*
Dgat2	*5'-GCTGGTGCCCTACTCCAAG*	*5'-CCAGCTTGGGGACAGTGA*
Fads6	*5'-GGTAGCTCTTGAGCATTTGAGG*	*5'-AGTAAAGGCCCAGGCAGATG*
Phyhd1	*5'-CTCTGCATGCCCATGACCC*	*5'-CTTCGCCGCCAAAGTGAG*
Myh2	*5'-GGCACAAACTGCTGAAGCAGAGGC*	*5'-GGTGCTCCTGAGGTTGGTCATCAGC*
Myh1	*5'-GGCAGCAGCAGCTGCGGAAGCAGAGTCTGG*	*5'-GAGTGCTCCTCAGATTGGTCATTAGC*
Myh4	*5'-GAGCTACTGGATGCCAGTGAGCGC*	*5'-CTGGACGATGTCTTCCATCTCTCC*
Cpt1b	*5'-GAGTGACTGGTGGGAAGAATATG*	*5'-GCTGCTTGCACATTTGTGTT*
Slc27a1	*5'-CGCTTTCTGCGTATCGTCTG*	*5'-GATGCACGGGATCGTGTCT*
Fabp3	*5'-ACCTGGAAGCTAGTGGACAG*	*5'-TGATGGTAGTAGGCTTGGTCAT*
Fnip1	*5'-TCCGTCAGTGCCTGGTATC*	*5'-ACAGCTTCTGCTATTGGTTCATC*
Ppargc1a	*5'-CGGAAATCATATCCAACCAG*	*5'-TGAGAACCGCTAGCAAGTTTG*
Ppargc1b	*5'-TCCAGAAGTCAGCGGCCT*	*5'-CTGAGCCCGCAGTGTGG*
Ppara	*5'-ACTACGGAGTTCACGCATGTG*	*5'-TTGTCGTACACCAGCTTCAGC*
Ppard	*5'-GTATGCGCATGGGACTCAC*	*5'-GTCTGAGCGCAGATGGACT*
Esrra	*5'-AGGAGTACGTCCTGCTG*	*5'-CCTCAGCATCTTCAATG*
Esrrb	*5'-ACGGCTGGATTCGGAGAAC*	*5'-TCCTGCTCAACCCCTAGTAGATTC*
Esrrg	*5'-TGACTTGGCTGACCGAG*	*5'-CCGAGGATCAGAATCTCC*
ChIP primers	***Forward***	***Reverse***
Myh7-2.1k	*5'-CATTGAGGGCCAACTTTGAG*	*5'-CAGGGGTCTTTGCTTTGGTT*
Myh7-3.2k	*5'-CTCCCTGAGACAGTTTTGCC*	*5'-TGTGCTAGGTCCCTCACCTG*

Human Gene	***Forward***	***Reverse***
MLL4	*5′-TGCCCATGAAGGTGAAAGA*	*5′-GTTTCTGTCAGCCACACACC*
MYH7	*5′-ACACCCTGACTAAGGCCAAA*	*5′-TCCAGGGATCCTTCCAGAT*
GAPDH	*5′-AGCCACATCGCTCAGACAC*	*5′-GCCCAATACGACCAAATCC*
TNNI1	*5′-GGGCCAACCTCAAGTCTGT*	*5′-AGACATGGCCTCCACGTT*
TNNC1	*5′-TGAGTTCCTGGTCATGATGG*	*5′-CGATGTAGCCATCAGCATTTT*
TNNI2	*5′-AGGACCTCAGGATGGGAGAT*	*5′-CGCTATCTGCAGCATCACAC*
MEF2A	*5′-TGATGCGGAATCATAAAATCG*	*5′-TGGAACTGTGACAGACATTGAA*
MEF2C	*5′-ACAAACTCAGACATCGTGGAGA*	*5′-TTGTTCAATGCGGAATCGT*
MEF2D	*5′-GCCCACTGCCTACAACACA*	*5′-CCCCCAGGTGAACTAAAGG*
TNNC2	*5′-GGTCACCAGCAACCATGAC*	*5′-TCATCTCTTCGCTGAGGTAGG*
TNNT1	*5′-CAGAGGATGATGCCAAGAAAA*	*5′-TTACCACGCTTCTGTTCTGC*
LDHB	*5′-GATGGATTTTGGGGGAACAT*	*5′-AACACCTGCCACATTCACAC*