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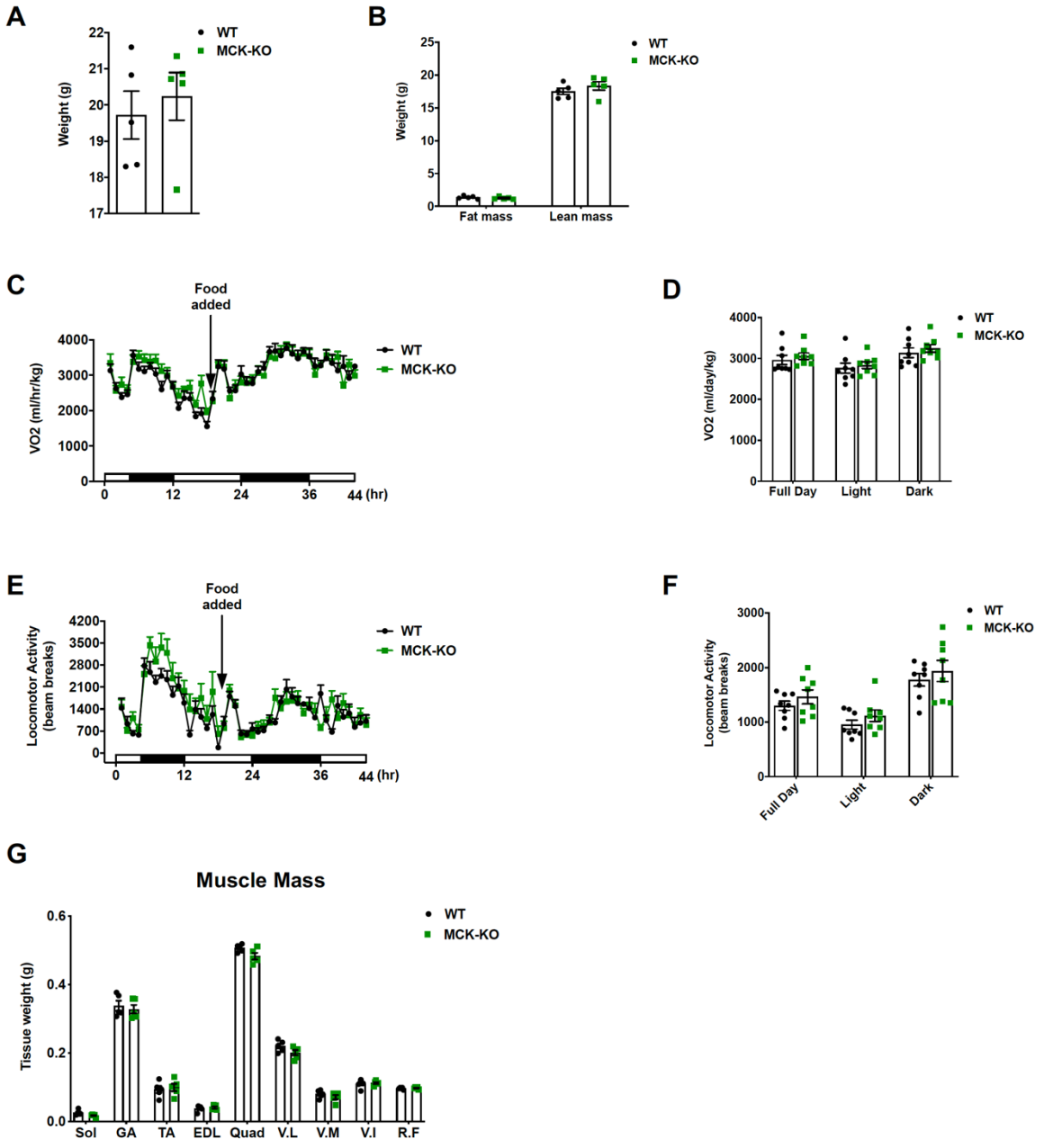
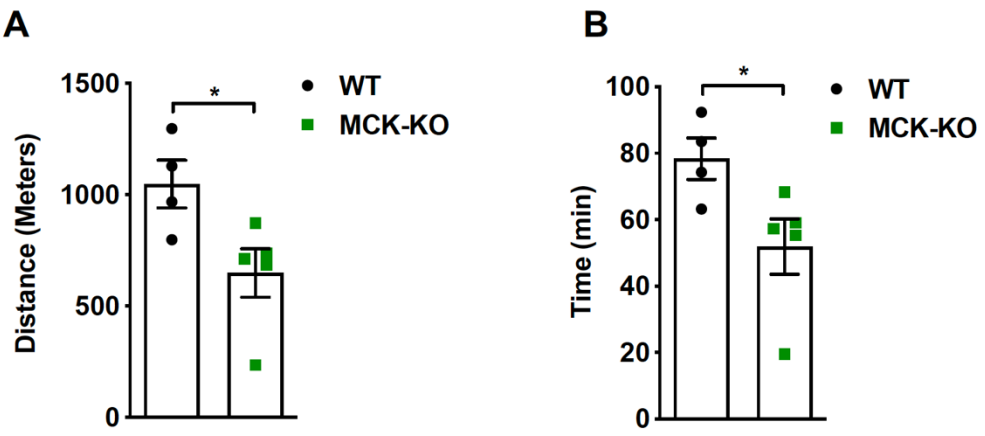
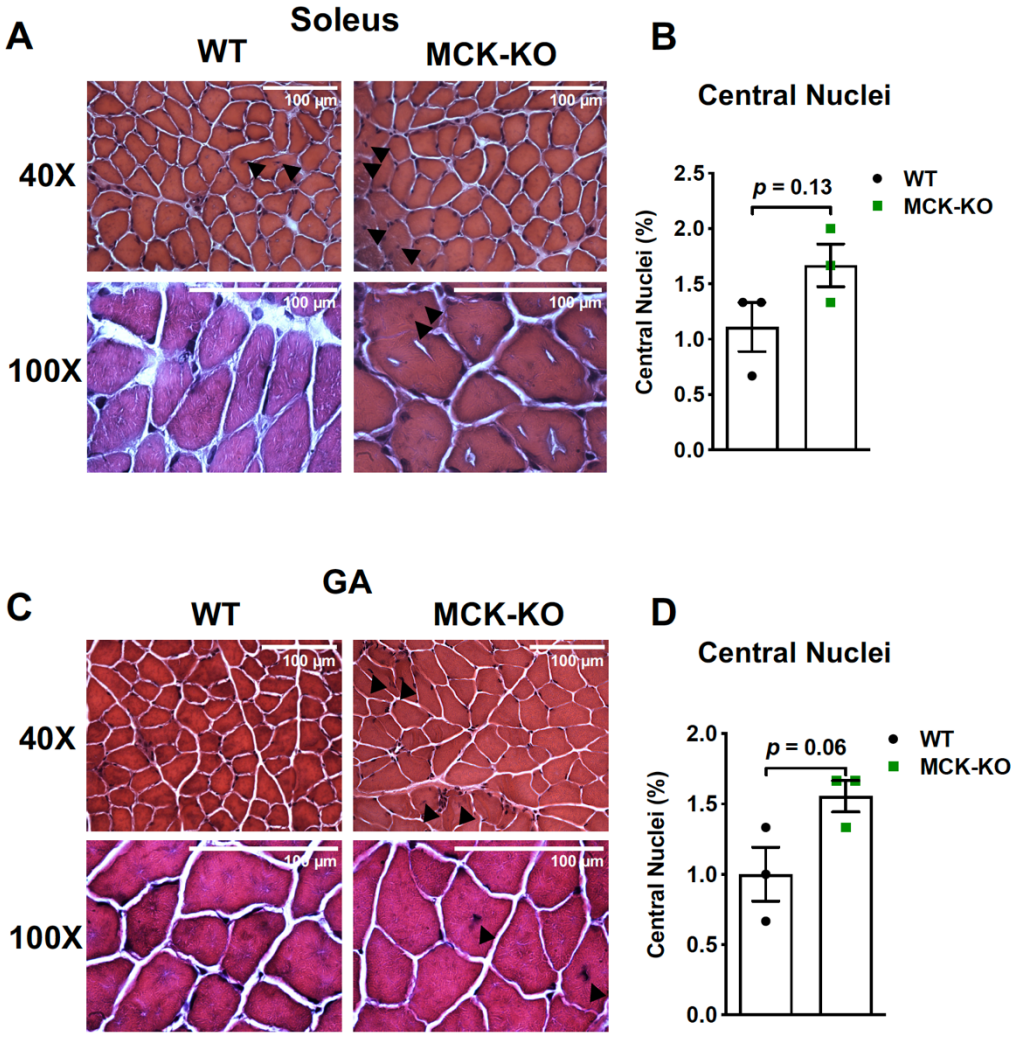


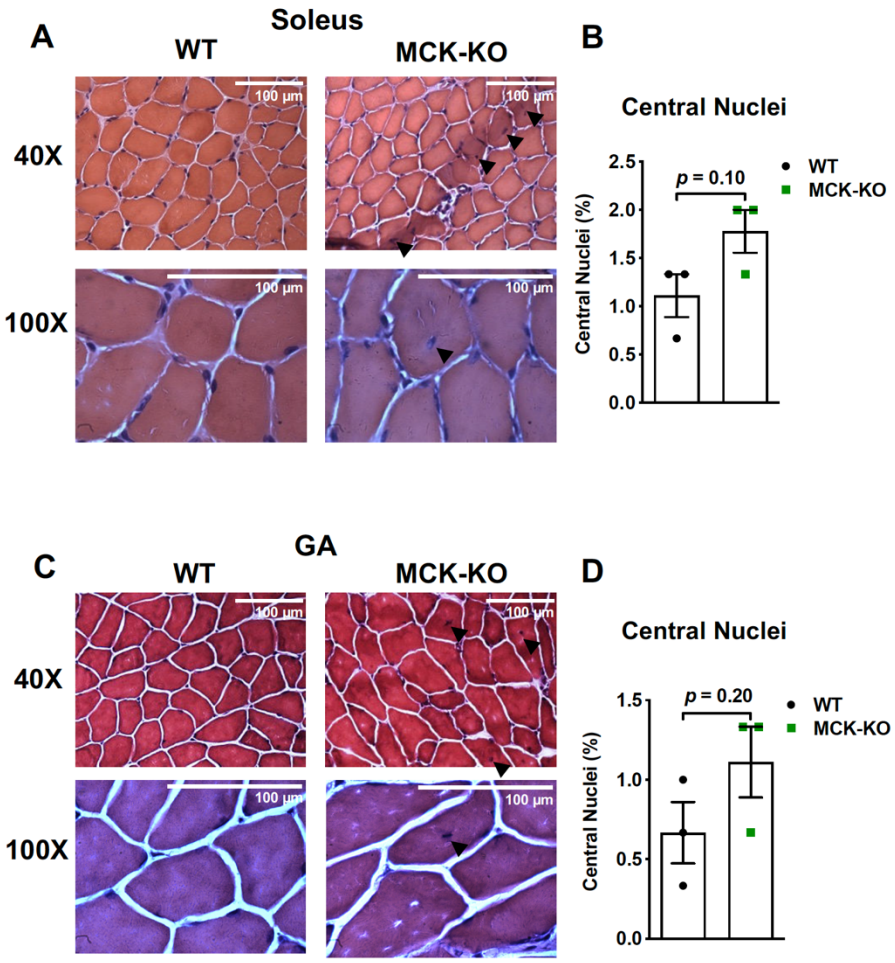
Appendix Figure S1. MCK-*Dnmt3a* KO mice do not show obvious change in energy homeostasis and muscle mass on chow diet. Body weight (**A**) and body composition (**B**) of MCK-KO and WT mice on chow (n = 5 , means ± SEM, * $p < 0.05$, two-tailed student's t-test). Shown is the whole-body oxygen consumption rate (VO2, **C**) and averaged VO2 (**D**) of MCK-KO and WT on chow during fasting (first 20 hrs) and fed (food added for next 25 hrs) conditions (n = 8, means ± SEM, * $p < 0.05$, two-tailed student's t-test and two-way ANOVA followed by Bonferroni post-hoc testing). (**E, F**) The whole-body locomotor activity of chow-fed MCK- KO and WT mice during fasting (first 20 hrs) and fed (food added for next 25 hrs) conditions (n = 8 mice, means ± SEM, * $p < 0.05$, two-tailed student's t-test and two-way ANOVA followed by Bonferroni post-hoc testing). (**G**) Tissue weight of different skeletal muscle types from chow-fed WT and MCK-KO mice. (n = 5, means ± SEM, * $p < 0.05$, two-tailed student's t-test and Bonferroni post-hoc testing).



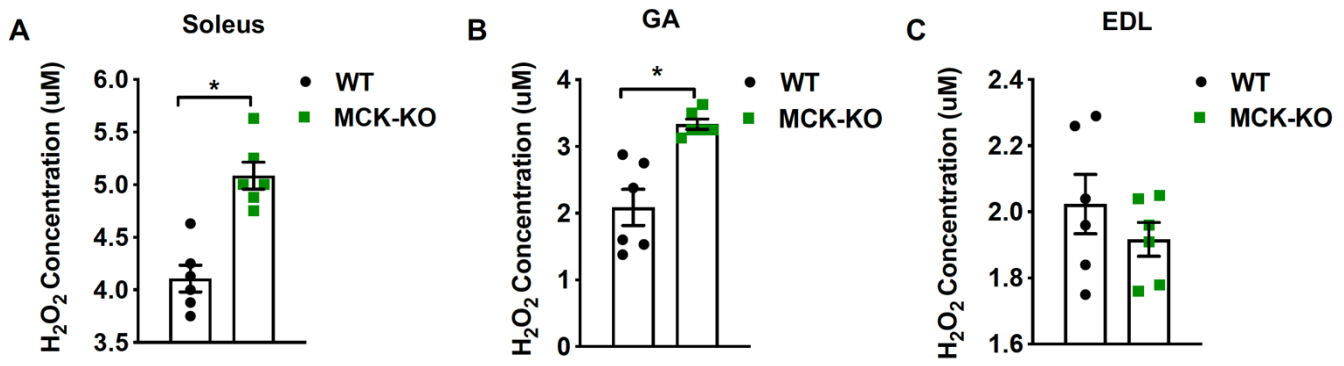
Appendix Figure S2. MCK-*Dnmt3a* KO mice display a reduced tolerance to endurance exercise at 5 weeks of age. (**A, B**) Exercise capacity of MCK-KO and WT mice from the low intensity regiment was conducted. (n = 4 WT and n = 5 MCK-KO, mean ±SEM, * $p < 0.05$, two-tailed student's t-test).



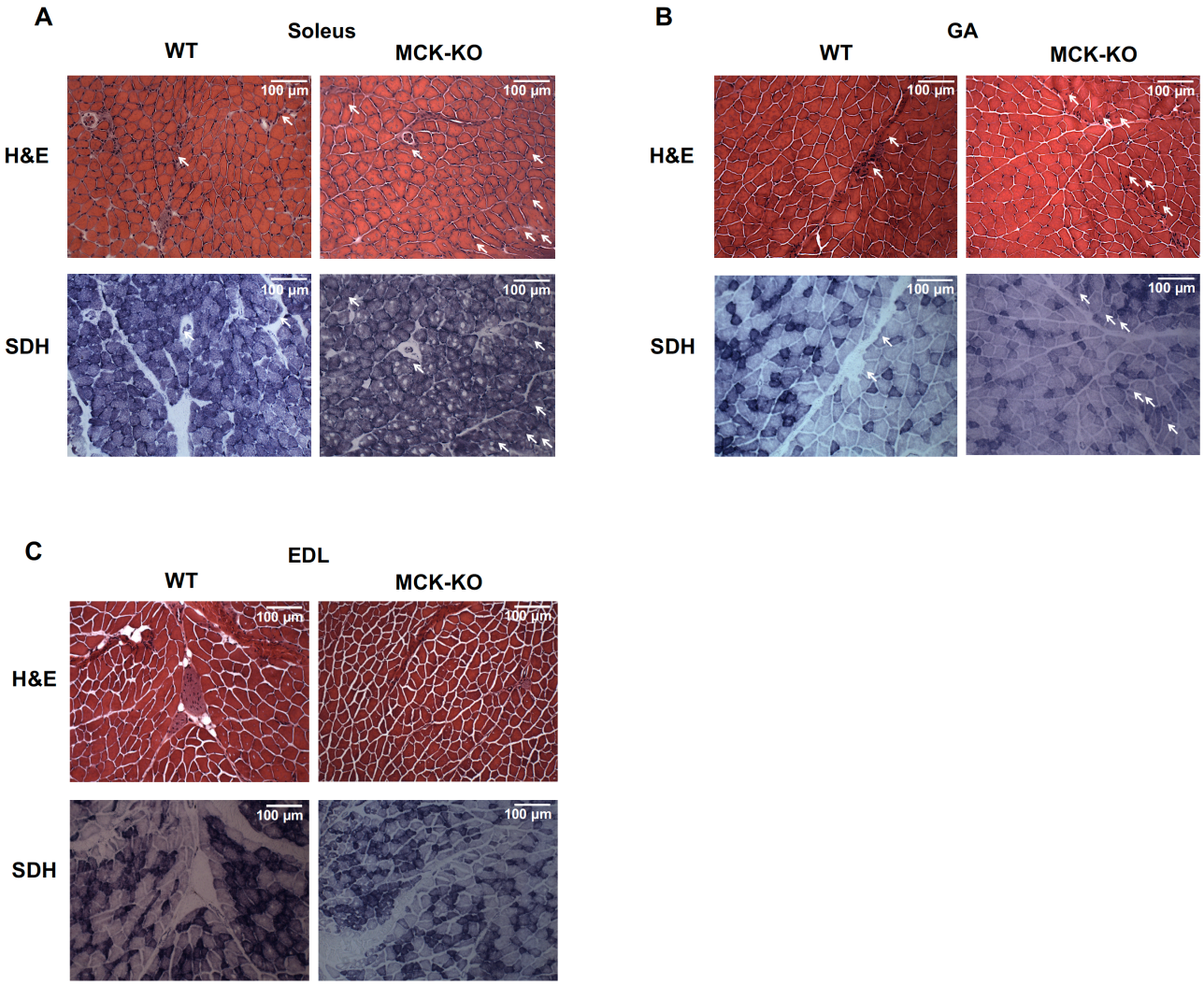
Appendix Figure S3. MCK-*Dnmt3a* KO mice display a trend towards increased muscle damage following exercise in 5 weeks old mice. (**A**) The H&E staining of WT and MCK-KO soleus muscle after a bout of low intensity treadmill running (top 40X, bottom 100X magnifications). Black arrow indicates centralized nuclei. (**B**) The percentage of myofibers with centralized nuclei was determined by manual counting 300 myofibers in 20X magnification. (n = 3, means ± SEM, two-tailed student's t-test). (**C, D**) The analogous set of data is shown with WT and MCK-KO GA muscles. (n = 3, means ± SEM, two-tailed student's t-test).



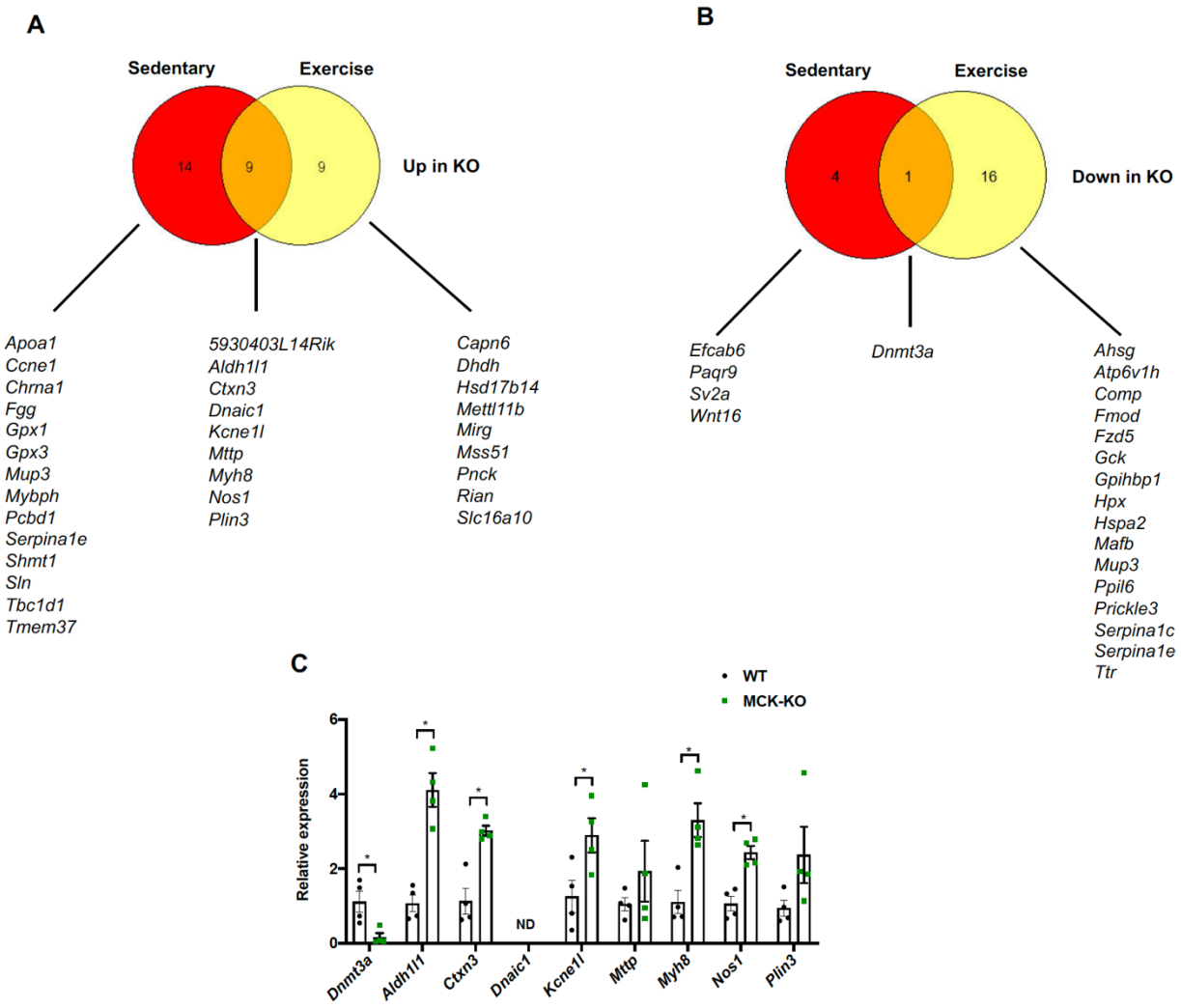
Appendix Figure S4. Muscle-specific *Dnmt3a* KO mice display an increased trend towards muscle at sedentary. (**A**) The H&E staining of WT and MCK-KO soleus muscle at sedentary (top 40X, bottom 100X magnifications). Black arrow indicates centralized nuclei. (**B**) The percentage of myofibers with centralized nuclei was determined by manual counting 300 myofibers in 20X magnification. (n = 3, means ± SEM, two-tailed student's t-test). (**C, D**) The analogous set of data is shown with WT and MCK-KO GA muscles. (n = 3, means ± SEM, two-tailed student's t-test).



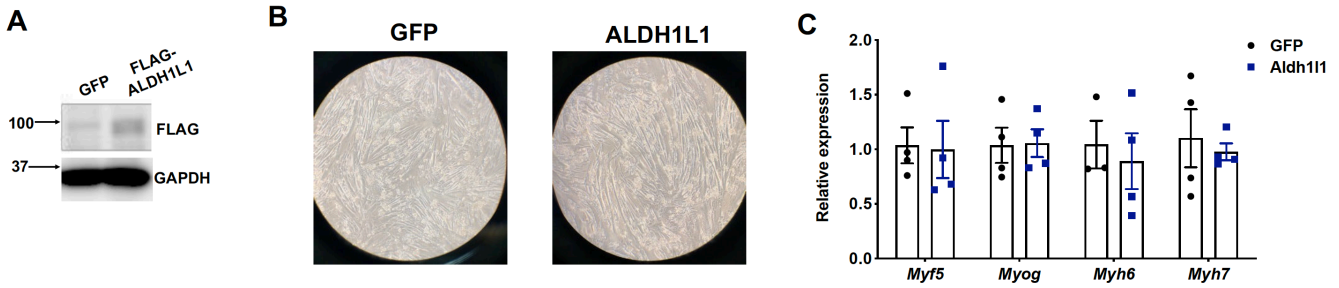
Appendix Figure S5. *Dnmt3a*-KO soleus and GA muscles display increased H2O2 levels following exercise in 5wks old mice. (**A-C**) Hydrogen peroxide (H_2O_2) levels were measured in WT and MCK-KO at rest and after a bout of exercise in soleus (**A**), GA (**B**) and EDL (**C**). (n = 6 per group means ± SEM, * $p < 0.05$, two-tailed student's t-test).



Appendix Figure S6. Successive staining of H&E and SDH from WT and MCK-KO muscles following exercise (**A-C**) H&E and SDH staining was performed using the successive sections of slide in WT and MCK-KO after a bout of low-intensity exercise for 50 min in soleus (**A**) GA (**B**) and EDL (**C**) (20X magnifications). Arrows indicates muscle fibers that have centralized nuclei in H&E slide and the same location of muscle fibers were depicted in the successive SDH slides.



Appendix Figure S7. Differentially regulated genes in Dnmt3a KO soleus. (A, B) Venn Diagram shows the number and the list of upregulated and downregulated genes in MCK-KO soleus from sedentary and exercise conditions. (**C**) The mRNA expression of Dnmt3a target genes were measured in single myofibers extracted from WT and MCK-KO soleus muscles at sedentary. (n = 4 per group means ± SEM, * $p < 0.05$, two-tailed student's t-test).



Appendix Figure S8. Overexpression of *Aldh1l1* does not affect differentiation status of myotubes. (**A**) L6 cells were transduced with lentiviral expression plasmids for Flag-ALDH1L1 and GFP. Flag western was performed to confirm the overexpression level of ALDH1L1. (**B**) Shown is the microscopic shot of L6 cells that overexpress Flag-ALDH1L1 and GFP from (**A**). (**C**) The mRNA expression of myogenesis markers were measured by qPCR in cells from (**A**). (n = 4, means ± SEM, *p<0.05, determined by two-tailed student's t-test).

Appendix Table S1. Oligonucleotide sequences used in this manuscript

Hairpin	(m/r) shDnmt3a	CGCTCCGCTGAAGGAATATTT
gRNA	(r) gAldh1l1	CGAGGTGGTGGGTGTGTTCA
Q-PCR	(m) Cyclophilin_f	GGTGGAGAGCACCAAGACAGA
Q-PCR	(m) Cyclophilin_r	GCCGGAAGTCGACAATGATG
Q-PCR	(r) Cyclophilin_f	CCAAACACAAATGGTTCCCAGT
Q-PCR	(r) Cyclophilin_r	ATTCCTGGACCCAAAACGCT
Q-PCR	(m) Dnmt3a_f	GTGGAGCCTGAAGCAGCTG
Q-PCR	(m) Dnmt3a_r	CTGGCACATGCCTCCAATGAA
Q-PCR	(m) Dnmt3b_f	CCATGGTGGTGTCCTGGAAA
Q-PCR	(m) Dnmt3b_r	CAGGACTGCTGGAGAAGGTCT
Q-PCR	(m) Ppargc1a_f	AGCCGTGACCACTGACAACGAG
Q-PCR	(m) Ppargc1a_r	GCTGCATGGTTCTGAGTGCTAAG
Q-PCR	(m) Aldh1l1_f	GGTGACCCTGTTTTCCCTACT
Q-PCR	(m) Aldh1l1_r	GGGATCTGCTTTCCCATCCT
Q-PCR	(r) Myf5_f	TGAGGGAGCAGGTAGAGAAC
Q-PCR	(r) Myf5_r	CTGTTCTTTCGGGACCAGAC
Q-PCR	(r) Myh6_f	CGAGACGGTGGTGGGGCTGT
Q-PCR	(r) Myh6_r	CCTTTCCCACTGTCACCGGTATC
Q-PCR	(r) Myh7_f	GATGTTTTTGTGCCTGATGA
Q-PCR	(r) Myh7_r	CAGTCACCGTCTTGCCATTCT
Q-PCR	(r) Myog_f	CTACAGGCCTTGCTCAGCTC
Q-PCR	(r) Myog_r	TGGAGTTGCATTCACTGG
Q-PCR	(m) MHC1_f	GCCAACTATGCTGGAGCTGATGCCC
Q-PCR	(m) MHC1_r	GGTGCGTGGAGCGCAAGTTTGTCATAAG
Q-PCR	(m) MHCIIA_f	GGCACAAACTGCTGAAGCAGAGGC
Q-PCR	(m) MHCIIA_r	GGTGCTCCTGAGGTTGGTCATCAGC
Q-PCR	(m) MHCIIX_f	GGCAGCAGCAGCTGCGGAAGCAGAGTCTGG
Q-PCR	(m) MCHIIX_r	GAGTGCTCCTCAGATTGGTCATTAGC
Q-PCR	(m) MHCIIB_f	GAGCTACTGGATGCCAGTGAGCGC
Q-PCR	(m) MHCIIB_r	CTGGACGATGTCTTCCATCTCTCC
Q-PCR	(m) Ctxn3_f	GGGCATCCTCATTGTCAGGT
Q-PCR	(m) Ctxn3_r	TCAGCCCATGTTGAGGTTGG
Q-PCR	(m) Dnaic1_f	CGAACTTTTCAGCCACAGCC
Q-PCR	(m) Dnaic1_r	CCGTGTCCCACTGCAAAAAG
Q-PCR	(m) Mttp_f	GGAAGGCAGAGCTTCATGGT
Q-PCR	(m) Mttp_r	GGCTTCAGCCTTGTCCATCT
Q-PCR	(m) Plin3_f	GAGCGGGGTGGACACAGTGC
Q-PCR	(m) Plin3_r	CAAGGGATGTGGCGAGGCGG
Q-PCR	(m) Myh8_f	CAGGAGCAGGAATGATGCTCTGAG
Q-PCR	(m) Myh8_r	AGTTCCTCAAACTTTCAGCAGCCAA
Q-PCR	(m) Kcne1l_f	GGTCGTCCCTGACCCTTTC
Q-PCR	(m) Kcne1l_r	CGGCTAGGCAGGCATAGAA
Q-PCR	(m) Nos1_f	CTCGGGCATACCCTCACTTC
Q-PCR	(m) Nos1_r	ATGTTGACGTCATCCCCCAC
MeDIP/ChIP	(m) Aldh1l1-P1_f	TAAGGAGTCTCAGCGGTGGT

MeDIP/ChIP	(m) Aldh1l1-P1_r	TGGGCAGAGTCATTGTCCTA
MeDIP/ChIP	(m) Aldh1l1-P2_f	GGCATGCTAGGCAATGAACT
MeDIP/ChIP	(m) Aldh1l1-P2_r	CTCAGGTCATCCGTCCATTT
MeDIP/ChIP	(m) Aldh1l1-P3_f	GGTGAAGGAAATGACCCAAA
MeDIP/ChIP	(m) Aldh1l1-P3_r	ATGAGTGAGGAGGCAAGGAG
MeDIP/ChIP	(m) Aldh1l1-P4_f	CTGCTTCCTGCCTCCTTG
MeDIP/ChIP	(m) Aldh1l1-P4_r	CTAGCATGCCCGGAACCTA
MeDIP/ChIP	(m) Aldh1l1-P5_f	AAATGGACGGATGACCTGAG
MeDIP/ChIP	(m) Aldh1l1-P5_r	CCTCCAGGCAGAGAAGAGG

(m); mouse
(r); rat

Appendix Supplementary Methods

Metabolic cages
Indirect calorimetry and food intake, as well as locomotor activity, were measured using the Comprehensive Lab Animal Monitoring System (CLAMS) (Columbus Instruments). The calorimetry system is an open-circuit system that determines O_2 consumption, CO_2 production, and RER. Data were collected after 3 hr of adaptation in acclimated singly housed mice.

Plasmids
Hairpins against *Dnmt3a* were purchased from Sigma. Lentiviral overexpression vectors for DNMT3A were subcloned into pCDH EcoRI/NotI sites. Single guide RNAs targeting *Aldh1l1* were cloned into lentiCRISPR v2 vectors. Briefly, lentiCRISPRv2 plasmid was digested and dephosphorylated with BsmBI. The oligos targeting *Aldh1l1* were phosphorylated and annealed followed by ligation into the digested lentiCRISPRv2 plasmid. Hairpin and gRNA sequences are shown in **Appendix Table S1**.

Myofiber isolation
Dissected muscles were digested with 4 mg/ml Collagenase A (Roche, #10103578001) in DMEM supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin at 37°C under 5% CO2. After 2–3 hrs, single myofibers were obtained by careful trituration using a pipette tip coated in horse serum.

RNA extraction and quantitative PCR
Total RNA was extracted from tissues using TRIzol reagent according to the manufacturer's instructions. cDNA was reverse transcribed from 1 μg of RNA using the cDNA High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative PCR (qPCR) was performed with SYBR green qPCR master mix (AccuPower 2X, Bioneer) using a CFX96 Touch (Bio Rad) or QuantStudio 5 (Applied Biosystems). The relative amount of mRNA normalized to cyclophilin B was calculated using the delta–delta method. Primer sequences are listed in **Appendix Table S1**.

Western blot analysis and antibodies.
Whole-cell protein lysates were prepared according to the manufacturer's protocol using RIPA lysis buffer and protease inhibitor cocktail. Proteins were size fractionated by SDS-PAGE and then transferred to polyvinylidene difluoride membranes. After blocking with 5% nonfat dried milk in TBS-Tween (0.25%), the membranes were incubated with the appropriate primary antibodies [DNMT3A (D23G1) Cell Signaling Technology Cat#3598 and ALDH1L1 Antibody (YY8): Cat#sc-100497 Santa Cruz Biotechnology. The loading control included GAPDH (14C10) Cell Signaling Technology Cat#2118 and Histone H3 (D1H2) Cell Signaling Technology Cat#4499]. The loading control included anti-GAPDH (#2118) and anti-Histone H3 (#14269). Immunoblots were quantified by ImageJ.

Histological analysis of skeletal muscle fibers
Dissected muscles were immediately embedded in T.F.M. compound (Tissue-Tek) and snap frozen using a Stand-Alone Gentle Jane (Instrumedics Inc.). We then prepared 10 μm sections from the muscle mid-belly using a Leica cryostat. Hematoxylin and eosin (H&E) staining was performed following fixation in ice-cold zinc formalin (Anatech Ltd. #175) for 60 min. Image analysis was performed using ImageJ software. For MHC staining, 10 μm transverse sections from the midportion of various muscles were obtained. Slides were air dried for 10 mins followed by washing with phosphate buffered saline (PBS) + 0.05% Triton X for 10 min and then for 5 min. Sections were blocked in PBS + 0.05% Triton X (PBST) containing 10% goat serum for 1 h at room temperature and then incubated overnight at 4°C with a mixture of three primary mouse monoclonal antibodies (MYH7 (BA-F8, IgG2b), MYH2 (SC-71, IgG1), and MYH4 (BF-F3, IgM), obtained from DSHB at the University of Iowa) in PBS + 0.05% Triton X (PBST) containing 10% goat serum. Next day, slides were washed with PBS + 0.05% Triton X for 10 min and then with new PBST for 5 min. After washes in PBST, sections were incubated for one hour with a mixture of three goat anti-mouse

secondary antibodies against IgG2b (Alexa 350), IgG1 (Alexa 488), and IgM (Alexa 555) in 10% GS/PBST, followed by washes with PBS + 0.05% Triton X for 10 min and then with new PBST for 5 min. Sections were then mounted with Fluromount-G, SouthernBiotech and sealed with nail polish. For SDH staining, ten-micrometer sections from the Gastrocnemius, Soleus, EDL muscles were cut onto glass slides and air-dried. Slides were incubated in the incubation medium (20 mM phosphate buffer, 0.027% sodium succinate (2378; Sigma) and 10 mg nitroblue tetrazolium (6639; Sigma) for 60 minutes at 37 ºC. The sections were dehydrated and then briefly rinsed in 30, 60 and 90% acetone in ddH2O in ascending and descending order, rinsed in H2O, air-dried and cover-slipped using Mounting medium, Permount, Fisher Scientific).

Measurement of plasma lactate

Mice were either kept at resting or allowed to run for 50 min on the low-intensity regime. Following this, blood was collected from the tail tip for WT and MCK-*Dnmt3a* KO mice from both groups. Blood plasma was separated by centrifugation for 15 min at 4,000 rpm. Lactate levels in the plasma were measured using a Lactate Colorimetric Assay Kit (Abcam).