

**Supporting Information File: DEPDC5 muscle specific inducible knockout**

Skeletal Muscle Specific Knockout of DEPDC5 Increases mTORC1 Signaling,

Muscle Cell Hypertrophy, and Mitochondrial Respiratory Capacity

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Running Title: DEPDC5 muscle specific inducible knockout

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**Supporting Information File**

<b>Table of Contents:</b>	<b>Page</b>
<b>Table S1 Muscle Fiber CSA and Fiber Typing.</b>	<b>S2</b>
<b>Table S2 Contractile and Muscle Properties.</b>	<b>S3</b>
<b>Figure S1 Genotyping the Depdc5 Knockout.</b>	<b>S4</b>
<b>Figure S2 Endurance Not Improved in Knockout Mice.</b>	<b>S5</b>
<b>Figure S3 Overall Motor Function Not Improved in Knockout Mice.</b>	<b>S6</b>
<b>Figure S4 Forelimb Grip Strength Not Improved in Knockout Mice.</b>	<b>S7</b>
<b>Figure S5 Activity Rate Not Improved in Knockout Mice.</b>	<b>S8</b>
<b>Figure S6 Mitochondrial Respiration in TA muscle.</b>	<b>S9</b>
<b>Figure S7 Succinate Dehydrogenase Enzymatic Activity Correlates with Cell Size.</b>	<b>S10</b>

**Supporting Information File: DEPDC5 muscle specific inducible knockout**

	Sex:	Male				Female			
Data Type	MHC Type	WT (n=5)	KO (n=6)	%dif	P	WT (n=5)	KO (n=8)	%dif	P
SOL CSA $\mu\text{m}^2$	MHC 1	<i>1322.4 ± 43.5</i>	<i>1458.5 ± 52.6</i>	13.2	0.034	1252.2 ± 48.2	1168.0 ± 59.1	-6.7	0.341
	MHC 2a	<i>1132.7 ± 42.9</i>	<i>1320.5 ± 71.8</i>	19.2	0.036	1069.9 ± 80.9	998.2 ± 50.6	6.7	0.443
	MHC 2x	<i>1304.8 ± 56.9</i>	<i>1588.2 ± 63.8</i>	21.7	0.010	1196.9 ± 150.6	1055.1 ± 51.2	-11.8	0.310
	MHC 2b	1418.3 ± 197.6	1391.6 ± 8.8	-1.9	0.933	N/A	2068.3a	N/A	N/A
	combined	1227.6 ± 34.5	<i>1424.0 ± 61.4</i>	16.0	0.027	1161.1 ± 63.2	1089.1 ± 54.8	-6.2	0.418
SOL Fiber Type Distribution %	MHC 1	<i>37.0 ± 2.0</i>	34.0 ± 1.1	-8.2	0.206	45.2 ± 2.4	45.4 ± 2.1	0.6	0.937
	MHC 2a	51.1 ± 1.6	54.2 ± 1.2	6.1	0.153	48.2 ± 2.4	45.6 ± 2.0	-5.5	0.420
	MHC 2x	10.1 ± 0.76	9.9 ± 1.00	-2.4	0.859	6.6 ± 1.3	8.1 ± 1.2	23.8	0.396
	MHC 2b	1.66 ± 0.72	1.85 ± 1.33	11.4	0.908	0.0	0.82 ± 0.77	N/A	0.452
	Sex:	Male				Female			
Data Type	MHC Type	WT (n=8)	KO (n=5)	%dif	P	WT (n=5)	KO (n=8)	%dif	P
TA CSA $\mu\text{m}^2$	MHC 1	501.8 ± 88.5	424.7 a	N/A	N/A	N/A		N/A	N/A
	MHC 2a	<i>865.4 ± 64.1</i>	<i>1012.3 ± 42.8</i>	17.0	0.089	841.0 ± 89.1	757.6 ± 64.4	-9.9	0.454
	MHC 2x	1557.6 ± 128.5	1546.4 ± 49.8	-0.7	0.634	1435.1 ± 163.4	1152.5 ± 91.1	-19.7	0.128
	MHC 2b	2614.4 ± 144.6	2689.7 ± 47.7	2.9	0.937	2451.8 ± 185.3	2082.6 ± 181.1	-15.1	0.204
	combined	1812.7 ± 125.9	2081.6 ± 127.5	14.8	0.164	1868.8 ± 133.5	1633.0 ± 143.2	-12.6	0.287
TA Fiber Type Distribution %	MHC 1	1.0 ± 0.34	0.2 ± 0.2	-79.3	0.051	0.0	0.1 ± 0.06	N/A	0.453
	MHC 2a	17.3 ± 4.9	5.9 ± 2.4	-65.8	0.064	16.0 ± 6.0	10.4 ± 3.2	-34.6	0.391
	MHC 2x	43.4 ± 3.4	43.8 ± 5.7	1.0	0.952	31.3 ± 2.8	31.8 ± 4.2	1.7	0.927
	MHC 2b	38.3 ± 6.4	50.1 ± 8.0	30.9	0.279	52.8 ± 2.8	57.7 ± 9.3	9.3	0.625

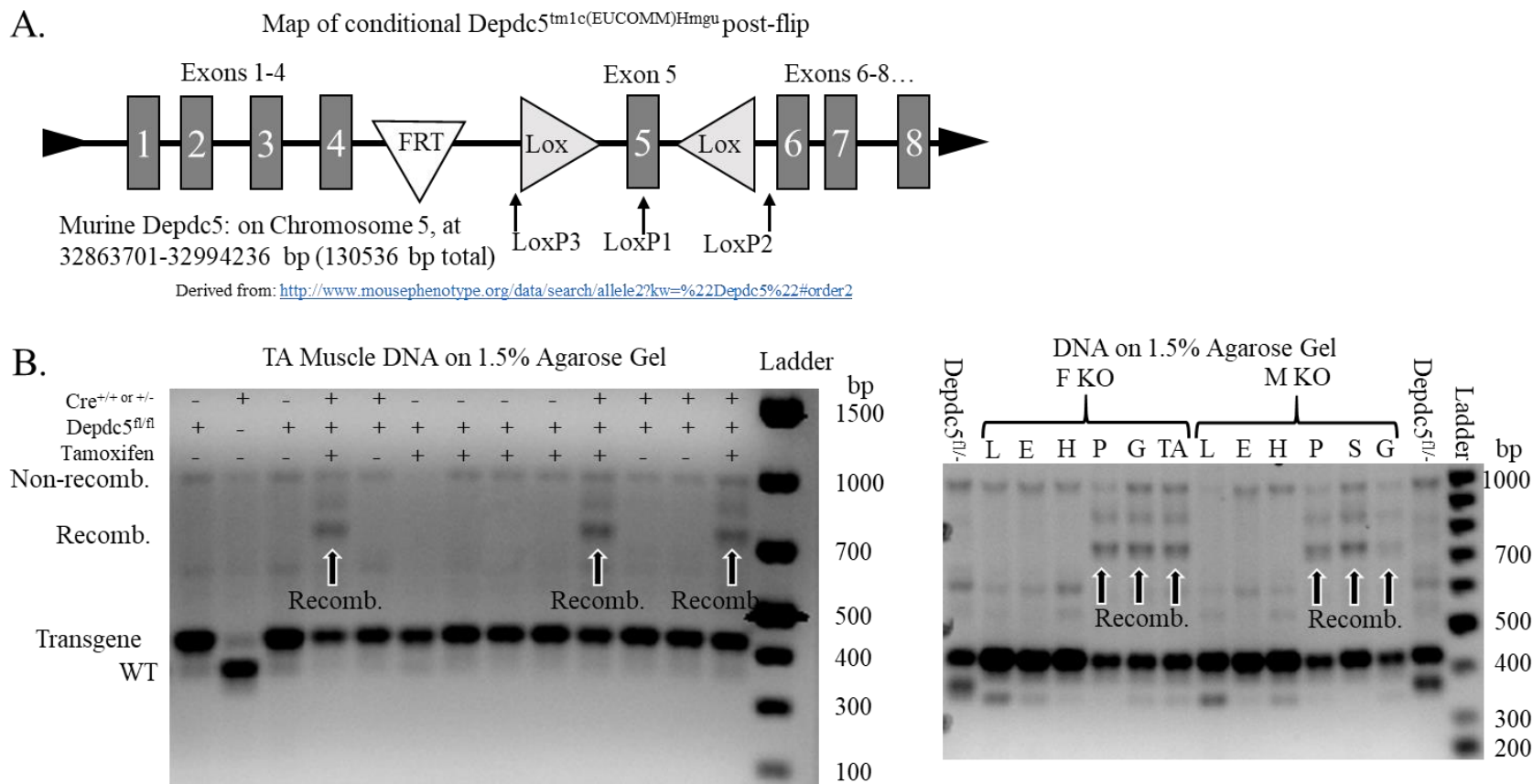
**Table SI1. Muscle Fiber CSA and Fiber Typing.** MHC = myosin heavy chain, SOL = soleus, TA = tibialis anterior, CSA = cross sectional area, WT = non-tamoxifen treated control mice, KO = tamoxifen treated experimental mice, %dif = percentage difference between WT and KO, P = P-value from 2-tailed Student's t-test, N/A = not applicable (due to division by zero, no data points in group, or other error). Data is expressed as mean of group ± the standard error. Numbers in italics indicates a trend (P<0.010) or significant difference (P<.050). <sup>a</sup> Only one mouse in the group expressed this MHC.

Supporting Information File: DEPDC5 muscle specific inducible knockout

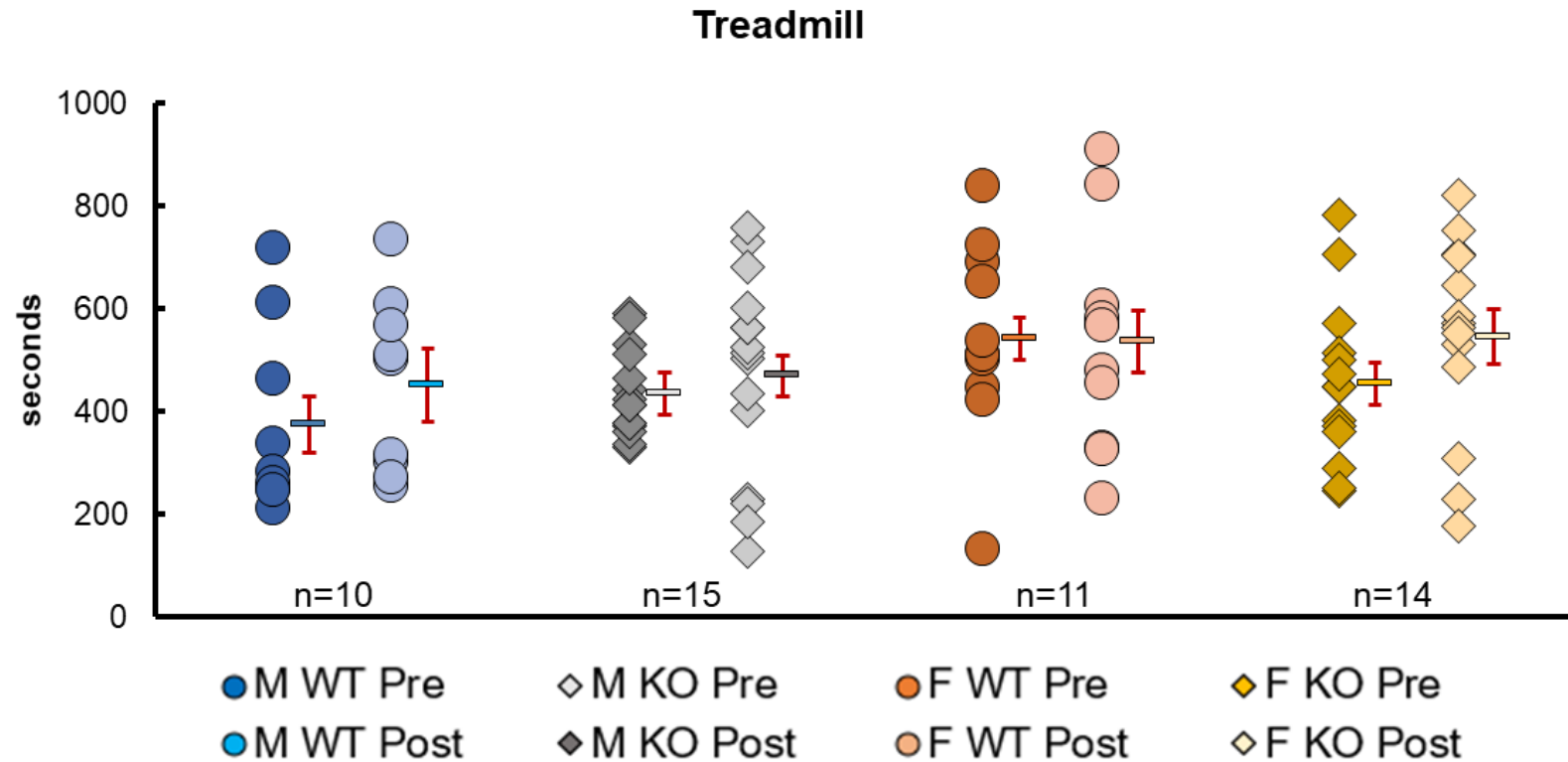
		Sex:	Male				Female			
Muscle	Data Type	Unit	WT (n=7)	KO (n=11)	%dif	P	WT (n=9)	KO (n=13)	%dif	P
EDL	P <sub>t</sub>	mN	78.6 ± 6.2	69.9 ± 4.5	-11.1	0.211	63.3 ± 4.5	61.8 ± 4.4	-2.4	0.825
	P <sub>0</sub>	mN	384.7 ± 19.2	378.1 ± 23.4	-1.7	0.211	309.7 ± 17.0	297.3 ± 10.1	-4.0	0.511
	P <sub>0</sub> Hz.	Hz	150.0 ± 9.3	152.7 ± 2.7	1.8	0.720	143.0 ± 4.4	156.9 ± 3.6	9.7	0.028
	Sp. F.	mN/mm <sup>2</sup>	41.7 ± 2.5	42.1 ± 2.5	1.0	0.902	41.1 ± 2.3	42.6 ± 2.0	3.6	0.628
	PCSA	mm <sup>2</sup>	9.60 ± 0.31	9.29 ± 0.46	-3.2	0.629	7.55 ± 0.19	7.04 ± 0.16	-6.8	0.055
	L <sub>0</sub>	mm	12.1 ± 0.3	12.3 ± 0.3	1.7	0.615	11.7 ± 0.2	12.2 ± 0.2	4.3	0.169
		Sex:	Male				Female			
Muscle	Data Type	Unit	WT (n=10)	KO (n=14)	%dif	P	WT (n=11)	KO (n=14)	%dif	P
SOL	P <sub>t</sub>	mN	49.4 ± 4.2	42.7 ± 3.4	-13.6	0.221	40.8 ± 4.2	39.1 ± 1.6	-4.2	0.695
	P <sub>0</sub>	mN	294.8 ± 16.4	277.3 ± 13.1	-5.9	0.409	234.1 ± 19.0	232.4 ± 9.7	-0.7	0.931
	P <sub>0</sub> Hz.	Hz	112.0 ± 3.3	111.4 ± 4.9	-0.5	0.930	120.9 ± 6.2	117.9 ± 5.3	-2.5	0.710
	Sp. F.	mN/mm <sup>2</sup>	27.2 ± 1.3	26.6 ± 1.4	-2.2	0.764	27.3 ± 2.8	26.1 ± 1.3	-4.4	0.697
	PCSA	mm <sup>2</sup>	10.95 ± 0.66	10.53 ± 0.34	-3.8	0.541	8.98 ± 0.51	9.05 ± 0.31	0.8	0.987
	L <sub>0</sub>	mm	11.8 ± 0.4	12.5 ± 0.2	5.9	0.059	10.8 ± 0.4	10.9 ± 0.2	0.9	0.948
		Sex:	Male				Female			
Muscle	Data Type	Unit	WT (n=10)	KO (n=15)	%dif	P	WT (n=11)	KO (n=14)	%dif	P
GAST	Mass	mg	155.7 ± 3.34	169.57 ± 5.3	8.9	0.061	118.5 ± 11.7	117.6 ± 9.4	-0.8	0.951
PLANT	Mass	mg	20.7 ± 1.0	22.4 ± 0.9	8.2	0.234	16.3 ± 0.8	16.1 ± 0.5	-1.2	0.788
TA	Mass	mg	53.00 ± 1.8	50.0 ± 1.6	-5.7	0.234	42.5 ± 1.2	39.2 ± 0.9	-7.8	0.040
SOL	Mass	mg	13.5 ± 1.1	14.1 ± 0.5	4.4	0.615	10.3 ± 0.7	10.4 ± 0.4	1.0	0.871
EDL	Mass	mg	12.1 ± 0.28	12.9 ± 0.4	6.6	0.108	9.9 ± 0.5	9.1 ± 0.2	-8.1	0.123
Bodymass	%change	%	1.50 ± 1.49	3.74 ± 1.02	149.3	0.211	6.3 ± 1.7	2.7 ± 1.4	-57.1	0.102

**Table SI2: Contractile and Muscle Properties.** EDL = extensor digitorum longus; SOL = soleus; WT = wild type; KO = knockout; P<sub>t</sub> = peak twitch force; P<sub>0</sub> = peak isometric tetanic force; P<sub>0</sub> Hz = frequency of stimulation in Hertz at P<sub>0</sub>; SP. F. = specific force; PCSA = physiological cross-sectional area, determined by formula; L<sub>0</sub> = muscle length at P; %dif = percent difference between WT and KO; P = P-value from independent t-test; data presented as mean of group ± standard error; italics = P<0.100.

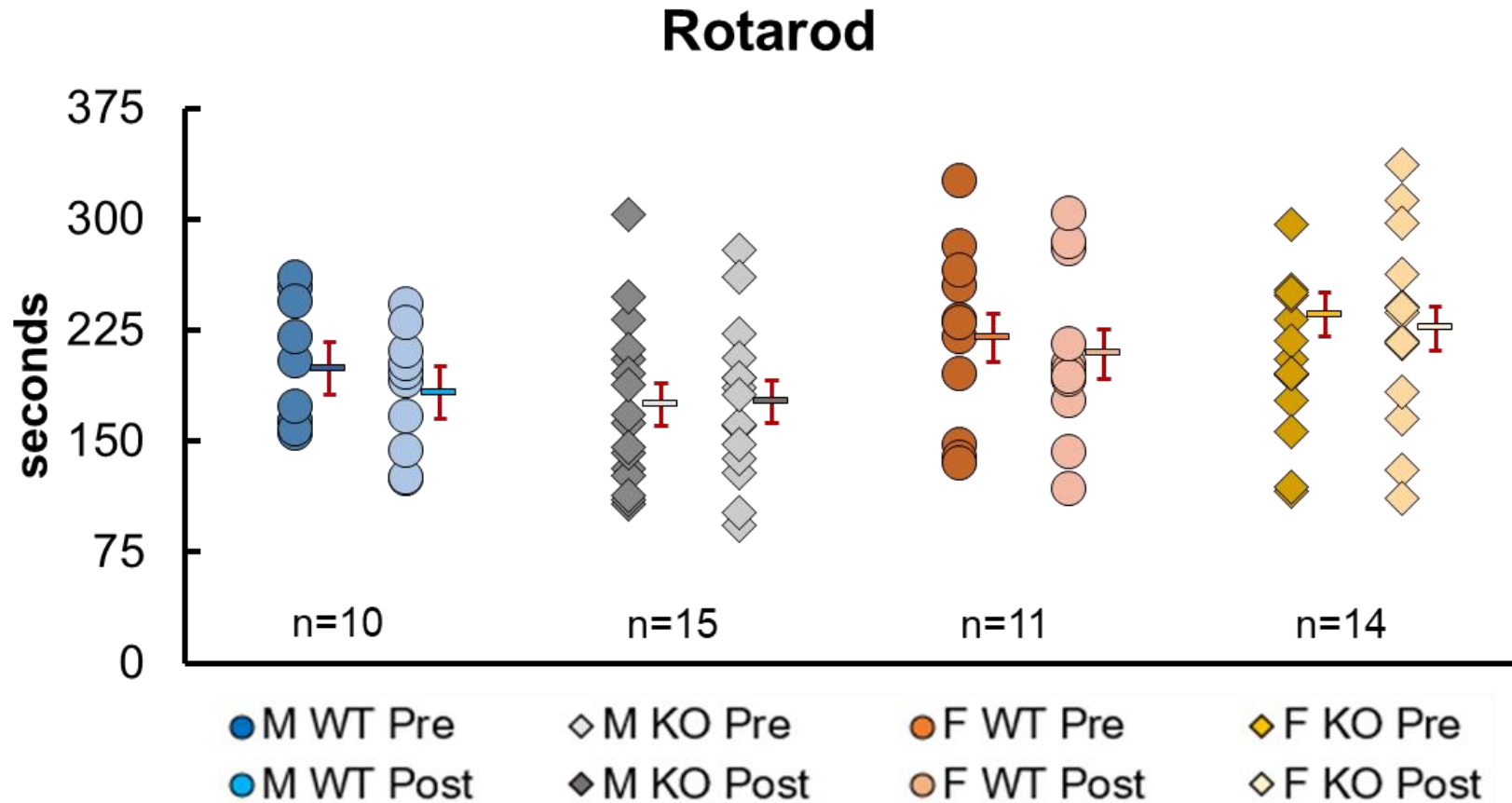
## Supporting Information File: DEPDC5 muscle specific inducible knockout



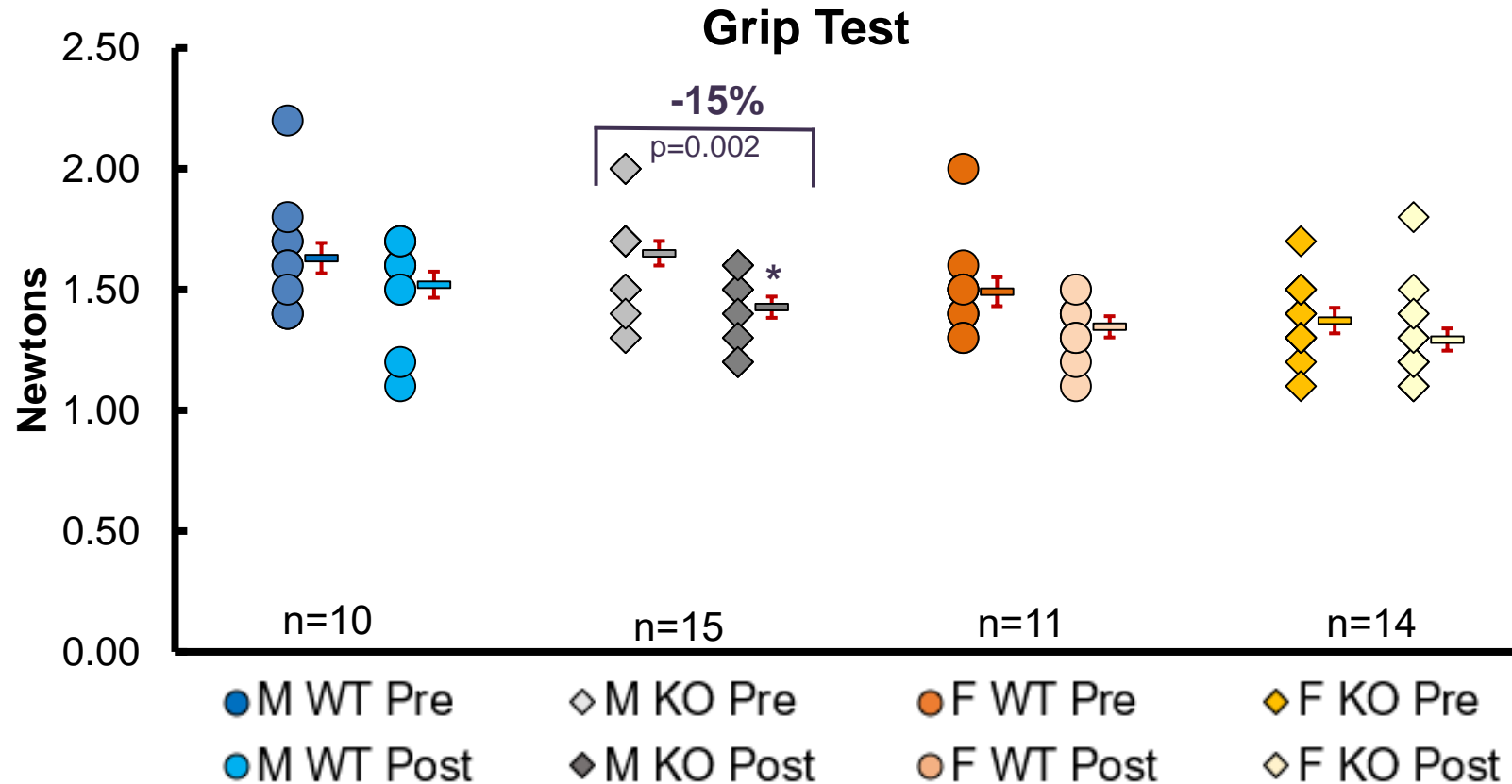
**Figure S11: A. Map of lox sites on the  $Depc5$  gene and primer locations.** The lox sites are on either side of exon 5. Exon 5 is expressed in all isoforms, thus transgenic ablation of exon 5 using cre-recombinase represses  $Depdc5$  gene expression. LoxP1, LoxP2 and LoxP3 on the figure show the location of primers. **B. Amplicons produced from PCR with LoxP1, P2 and P3.** The PCR product of LoxP1 and LoxP2 has amplicons at 431 bp if the lox site is present and at 368 bp if the allele is wild type. The PCR product of LoxP3 and LoxP2 has amplicons at 1349 bp for the floxed allele, 1069 bp if the allele is wild type, and at 749 bp in a recombinant allele (exon 5 not expressed). The amplicon at 1349 is not replicated when all 3 primers are used in one PCR Reaction. See Figure 1 for amplicons formed with only LoxP2 and LoxP3. Bp = base pairs; - or + signs = presence of allele in Cre<sup>+/+</sup> or +/- (homo- or heterozygous  $Tg^{(ACTA1-cre/Esr1^*)2Kesr}$  and  $Depdc5^{fl/fl}$  (homozygous  $Depdc5^{tm1c(EUCOMM)Hmgu}$ ) rows and whether or not tamoxifen was given;  $Depdc5^{fl/-}$  (heterozygous for  $Depdc5^{tm1c(EUCOMM)Hmgu}$ ), recomb. = recombination product, F = female, M = male, L = liver, E = skin (from ear punch), H = heart, P = plantaris, G = gastrocnemius, TA = tibialis anterior, S = soleus.



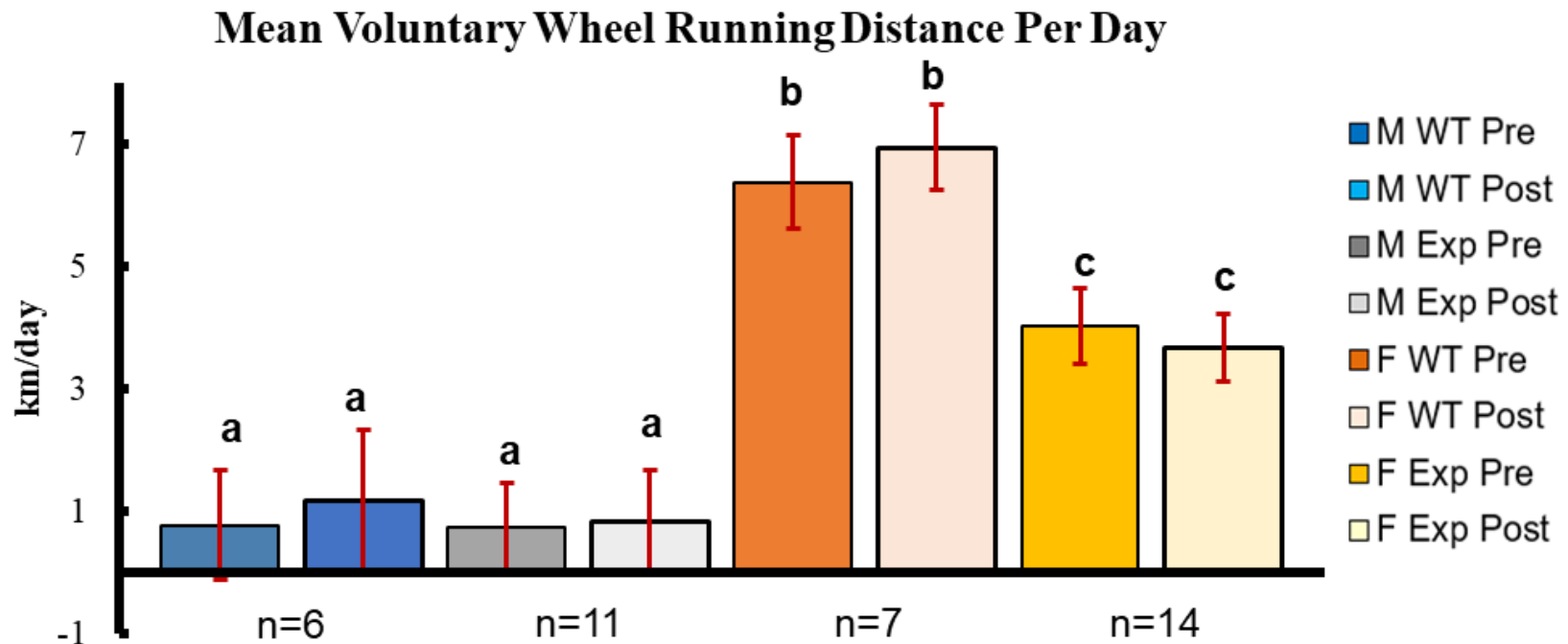
**Figure S12. Endurance Not Improved in Knockout Mice.** Function did not change significantly within any group from pre- to post- testing. WT = wild type mice, KO = transgenic knock-out mice, each symbol (circle for WT and diamond for KO) represents an individual mouse. To the right of each group the rectangle= the mean  $\pm$  standard error. M = male, F = female. Pre = before tamoxifen induced recombination, Post = 6 weeks after tamoxifen. A 2x2x2 ANCOVA (2 treatment groups, WT and KO; 2 sexes; 2 timepoints, before and 6-weeks after tamoxifen treatment in the EXP group, and 6 weeks after the pre-testing for the WT group) adjusted for body mass was used to determine differences with the least significant difference posthoc test (paired t-test) set at significance of  $P < 0.05$ .



**Figure SI3. Overall Motor Function Not Improved in Knockout Mice.** Function did not change significantly within any group from pre- to post-testing. WT = wild type mice, KO = transgenic knock-out mice, each symbol represents an individual mouse. To the right of each group the rectangle = the mean  $\pm$  standard error. M = male, F = female. Pre = before tamoxifen-induced recombination, Post = 6 weeks after tamoxifen-induced recombination. A 2x2x2 ANCOVA (2 treatment groups, WT and KO; 2 sexes; 2 timepoints, before and 6-weeks after tamoxifen treatment in the EXP group, and 6 weeks after the pre-testing for the WT group) adjusted for body mass was used to determine differences with the least significant difference posthoc test (paired t-test) set at significance of  $P < 0.05$ .

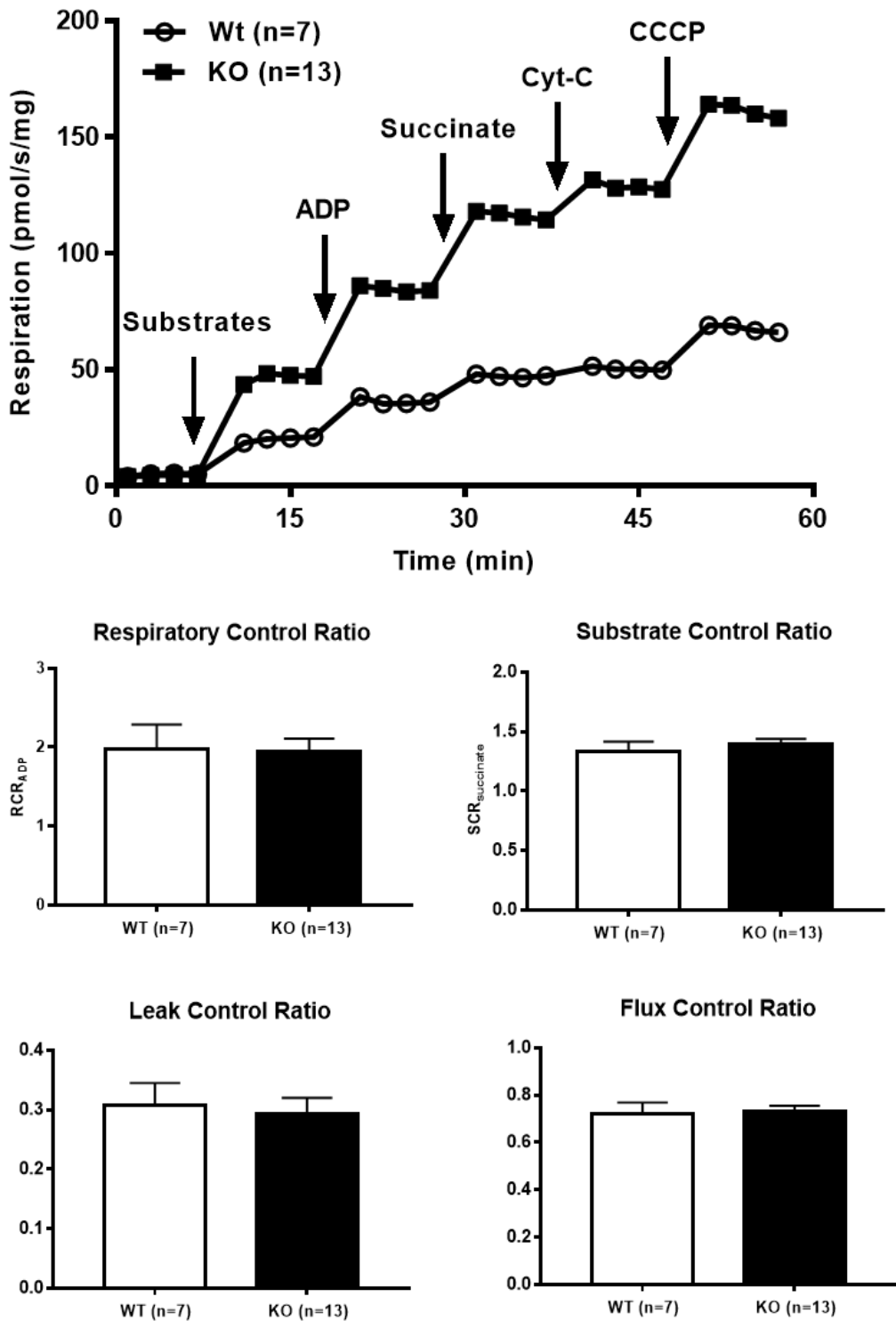


**Figure SI4. Forelimb Grip Strength Not Improved in Knockout Mice.** Other than the male KO who lost function, grip strength did not change significantly within any group from pre- to post- testing. WT = wild type mice, KO = transgenic knock-out mice, each symbol represents an individual mouse. To the right of each group the rectangle = the mean  $\pm$  standard error. M = male, F = female. Pre = before recombination, Post = 6 weeks after recombination. A 2x2x2 ANCOVA (2 treatment groups, WT and KO; 2 sexes; 2 timepoints, before and 6-weeks after tamoxifen treatment in the EXP group, and 6 weeks after the pre-testing for the WT group) adjusted for body mass was used to determine differences with the least significant difference posthoc test (paired t-test) set at significance of  $P < 0.05$ .



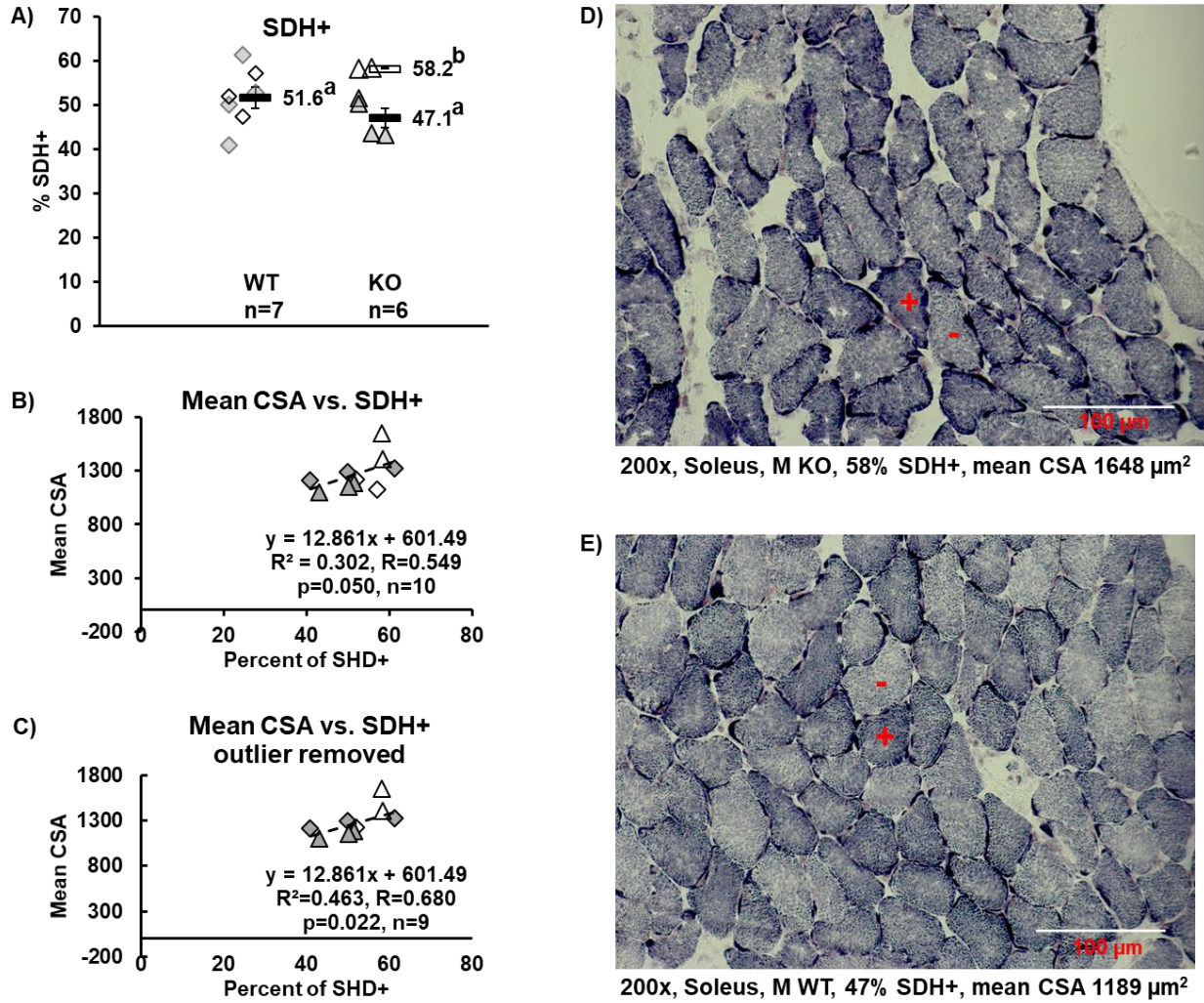
**Figure S15. Activity Rate Not Improved in Knockout Mice.** Function did not change significantly within any group from pre- to post-testing. WT = wild type mice, KO = transgenic knock-out mice. The error bar is  $\pm$  standard error. M = male, F = female. Pre = before tamoxifen-induced recombination, Post = 6 weeks after tamoxifen-induced recombination. A 2x2x2 ANCOVA (2 treatment groups, WT and KO; 2 sexes; 2 timepoints, before and 6-weeks after tamoxifen treatment in the EXP group, and 6 weeks after the pre-testing for the WT group) adjusted for body mass was used to determine differences with the least significant difference posthoc test (paired t-test) set at significance of  $P < 0.05$ . Different letter indicates significant difference.





**Figure SI6 Mitochondrial Respiration in TA muscle. A. Respiration.** Respiration in stages I-IV is higher in KO per unit of muscle. **B. Ratios.** The ratios demonstrate no difference in mitochondrial efficiency. KO = knockout mice; WT = wild type control

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**Figure SI7 SOL SDH Enzymatic Activity.** Cells were counted as either positive (dark stain) or negative (light stain). Degree of staining indicates higher SDH activity. **A)** M KO cells had significantly more ( $P < 0.05$ ) positive cells than the combined WT (M + F) or the F KO. Different letter indicates significance. **B)** There is a moderate positive correlation of cell size with SDH positive percentage. **C)** When the one outlier ( $> 2$  sd above mean for SDH+) is removed, the association becomes moderately strong. **D)** and **E)** are representative images. Each symbol represents the aggregate percentage of positive cells in one mouse. SD = standard deviation. SDH = succinate dehydrogenase, + = highly active cells, - = less active cells, SOL = soleus, Mean CSA is in microns squared, M = male, F = female, WT = wild type, KO = knockout, equations are simple linear regressions.