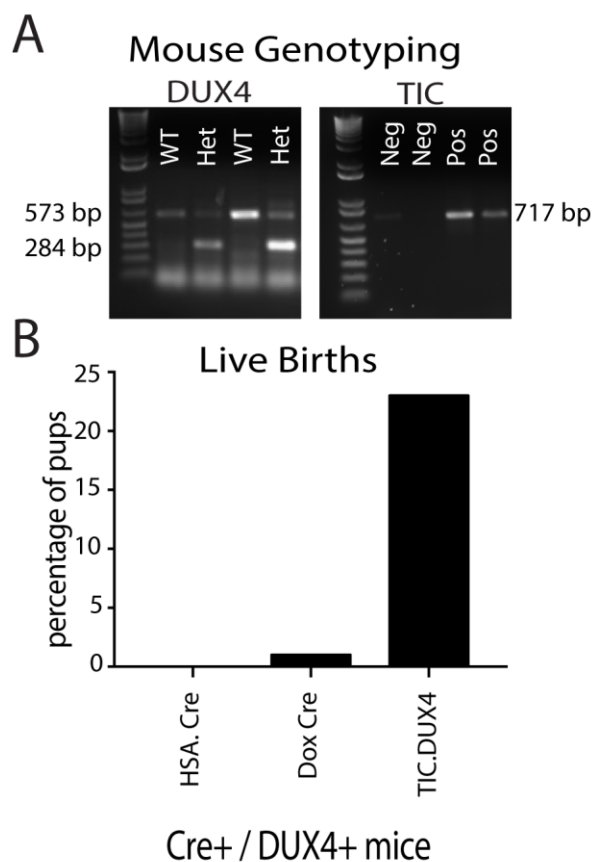
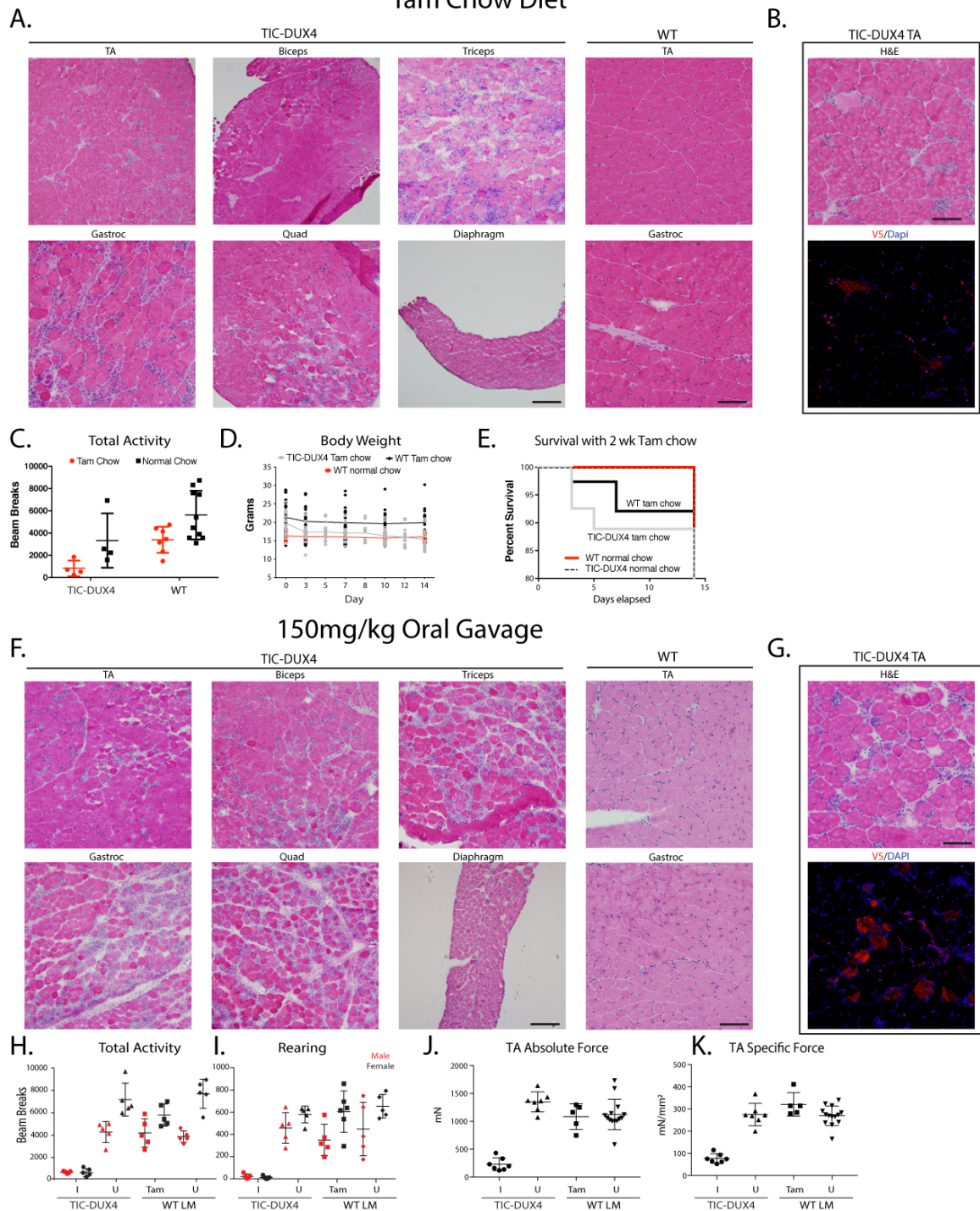




Supplemental Figure 1. Mouse genotyping and birth ratios

A. Genotyping of TIC-DUX4 mice. Image shows PCR amplified mouse genomic DNA electrophoresed on a 1% agarose gel stained with ethidium bromide. The 600 bp band indicated WT ROSA26 locus. The 300 bp band indicated presence of *DUX4* knock-in gene. **B.** 3 different Cre-expressing strains were crossed with ROSA26-DUX4 mice. In this experiment, constitutive HSA-Cre+/DUX4+ pup yield was 0/60 pups. Doxycycline (Dox)-inducible Cre+/DUX4+ pup yield was 2/148 pups. These 2 pups died on day P0. TIC.DUX4 pup yield was 28/119 pups (23.5%) which was close to the expected 25% ratio for heterozygous crosses.

Tam Chow Diet



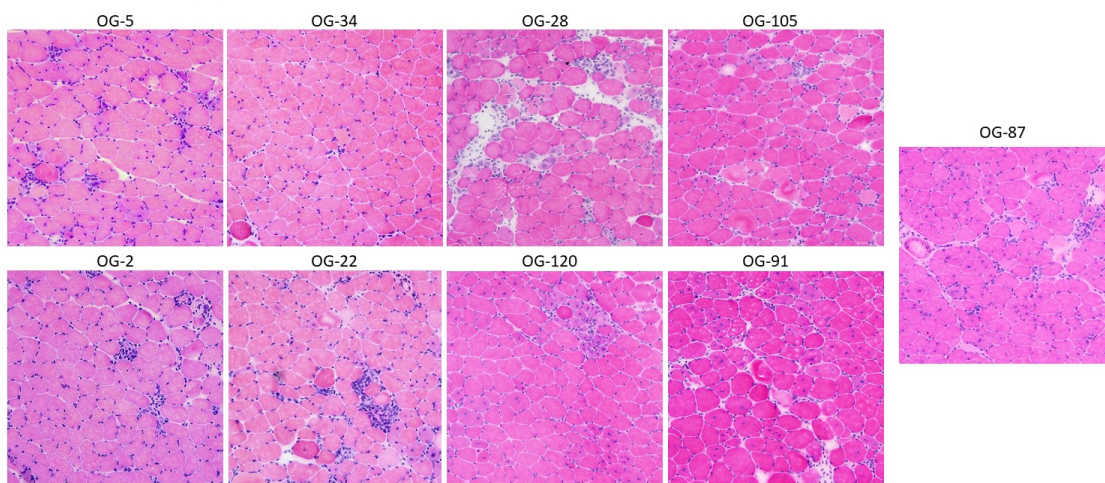
Supplemental Figure 2. High-dose Tamoxifen-treated TIC-DUX4 mouse characterization.

A. Histology of indicated muscles from 8-12 week old TIC-DUX4 animals fed Tamoxifen-laced chow diet for 2 weeks revealed widespread damage. H&E-stained 10X images, scale bar = 200 microns; 20X H&E C57/BL6 controls; scale bar = 100 microns. Images are representative of N = 4 male and 4 female mice.

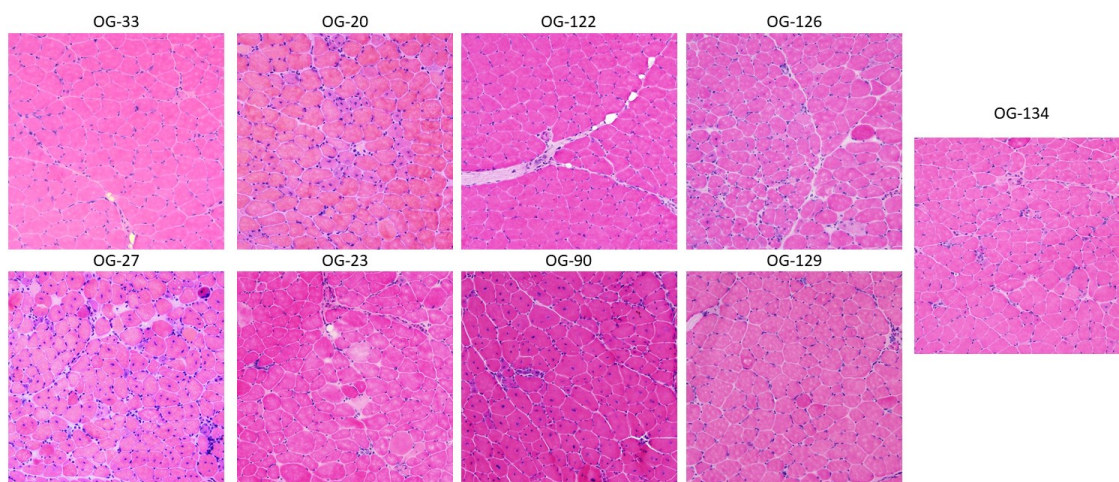
B. TIC-DUX4 Tamoxifen-induced TA muscle shows damage **(top)** co-localized with DUX4 protein expression by immunofluorescence (V5 staining, red) **(bottom)**. 20X images scale bar = 100 microns. **C-E.** TIC-DUX4 and WT animals fed Tamoxifen-laced chow showed reduced activity (C), body weight (D) and mortality (E), where ~7-10% of animals died within 2 weeks. N = 4 TIC-DUX4 mice on Tam chow; N=4 TIC-DUX4 mice on normal chow; N = 7 WT mice on Tam chow; N = 10 WT mice on normal chow. For initiating the body weight and survival studies, N = 23 induced TIC-DUX4 mice; N=30 Tam-treated WT; N=6 WT normal chow. **F.** Upper and lower limb skeletal muscles and diaphragm histology of 8-12 week old TIC-DUX4 induced with 2 doses of 150mg/kg Tam. H&E 10X images scale bar = 200 microns; 20X H&E C57/BL6 controls image scale bar = 100 microns; Images are representative of N= 4 male and 4 female mice. **G.** TIC-DUX4 Tamoxifen-induced TA muscle shows damage **(top)** co-localized with DUX4 protein expression by immunofluorescence (V5 staining, red) **(bottom)**. 20X images scale bar = 100 microns. **H.** High-dose Tam-induced TIC-DUX4 mice had significant activity impairments for both sexes compared to all controls (2-way ANOVA with Tukey's multiple comparisons test; $p < 0.0001$). N

= 5 male and 5 female mice for all groups. **I.** Tamoxifen-treated TIC-DUX4 mice had significantly reduced mean rearing behavior compared to all control groups. (2-way ANOVA with Tukey's multiple comparisons test; $p < 0.05$). N = 5 male and 5 female Tamoxifen-induced TIC-DUX4 mice; 5 male and 5 female uninduced TIC-DUX4; 5 male and 6 female Tam-treated; and 5 untreated WT littermates. **J-** **K.** High dose (150 mg/kg, 2 doses, 2 days apart) Tamoxifen-treated TIC-DUX4 mice showed significantly reduced TA muscle absolute and specific force (2-way ANOVA with Tukey's multiple comparisons test; $p < 0.0001$ for both measurements). N = 7 Tamoxifen-induced TIC-DUX4 legs; 7 uninduced TIC-DUX4 legs; 5 Tamoxifen-treated WT littermate legs, 12 untreated WT littermate legs. Data shown are force means $\pm$ SD.

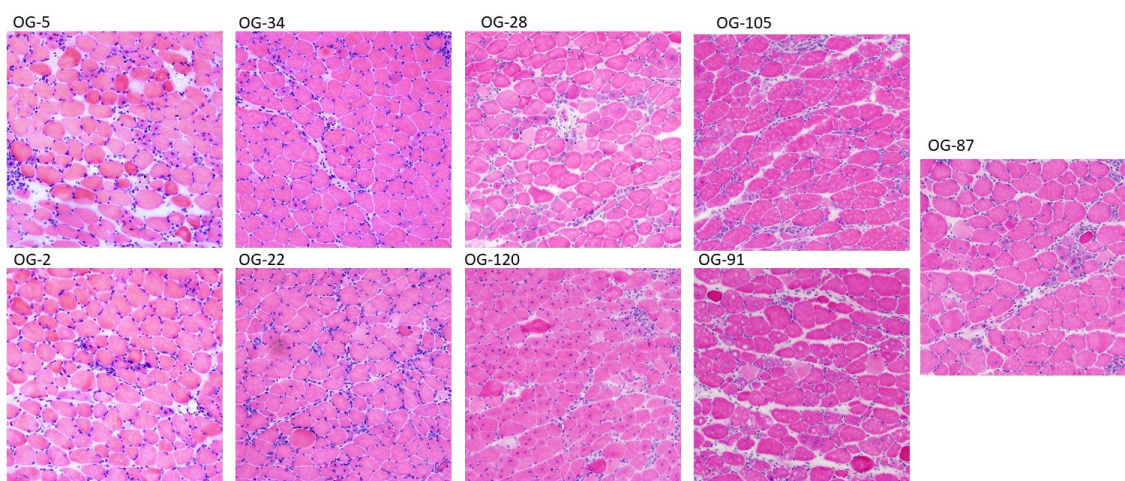
Female TA TIC-DUX4 5mg/kg



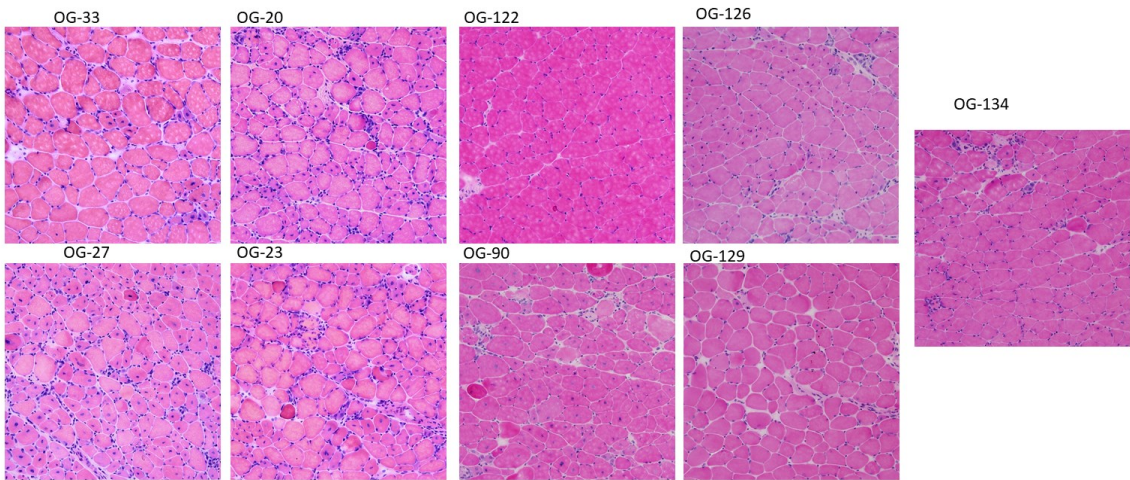
Male TA TIC-DUX4 5mg/kg



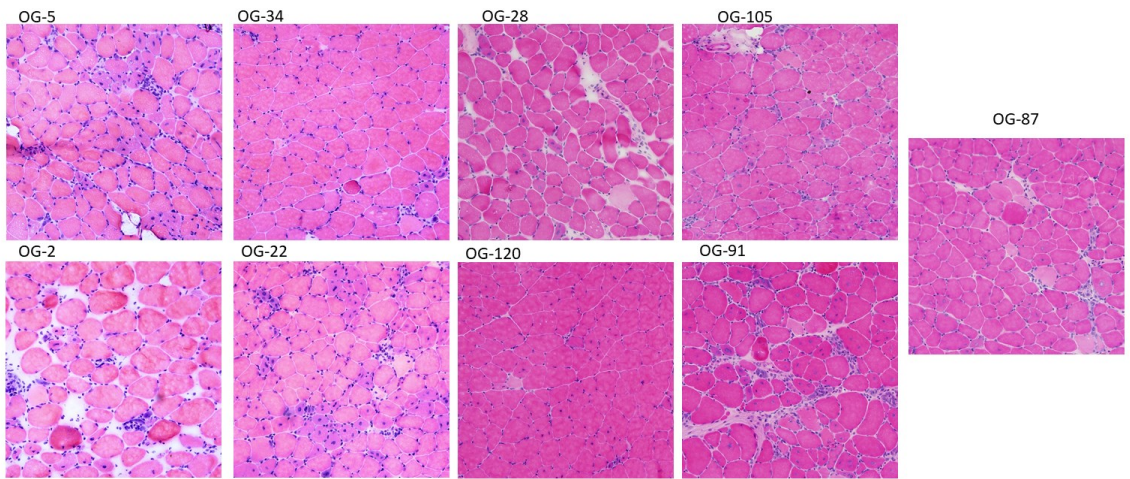
Female Gas TIC-DUX4 5mg/kg



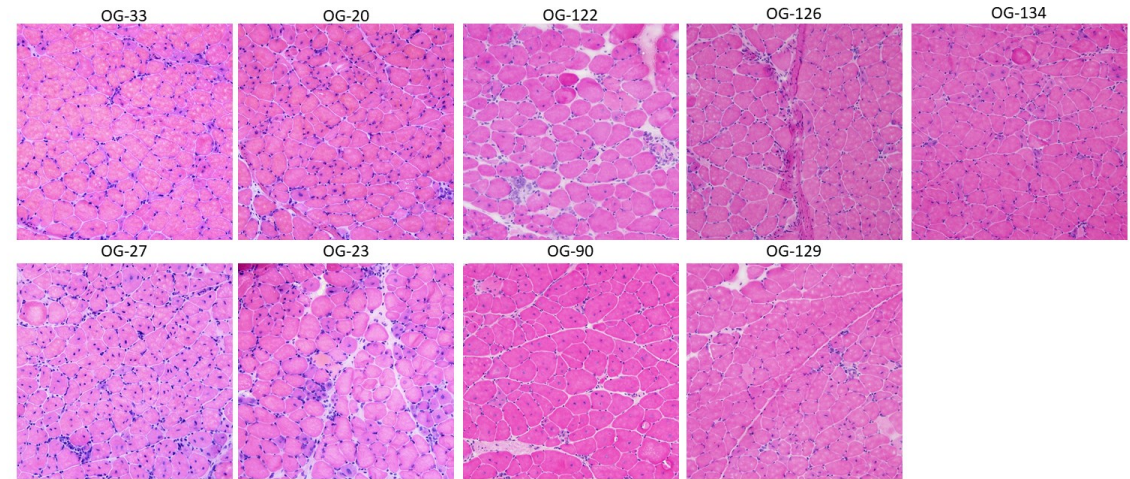
Male Gas TIC-DUX4 5mg/kg



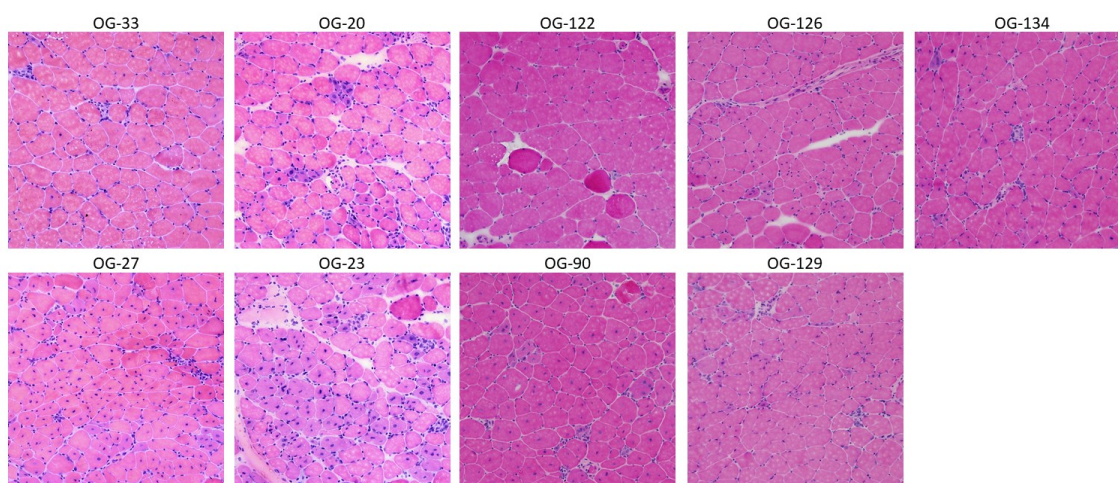
Female Quad TIC-DUX4 5mg/kg



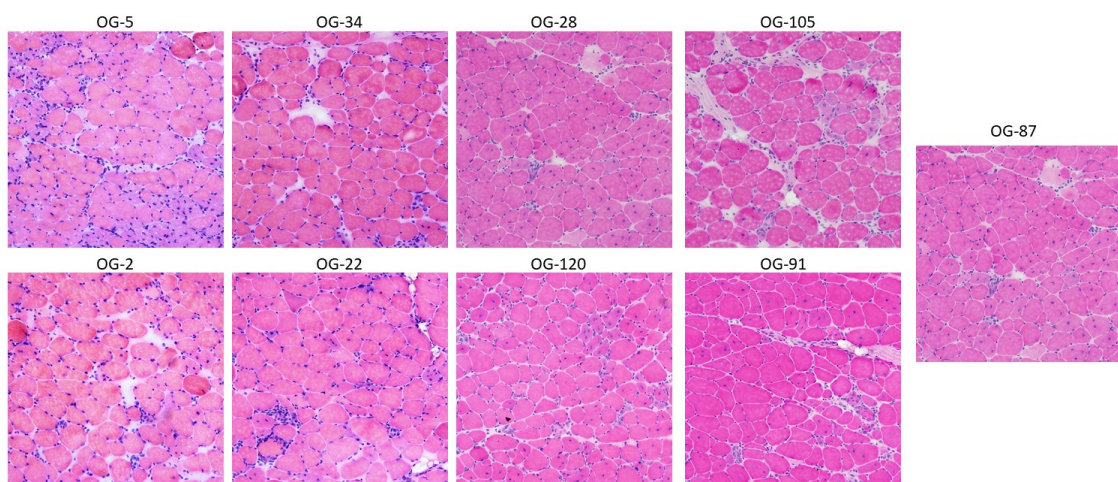
Male Quad TIC-DUX4 5mg/kg



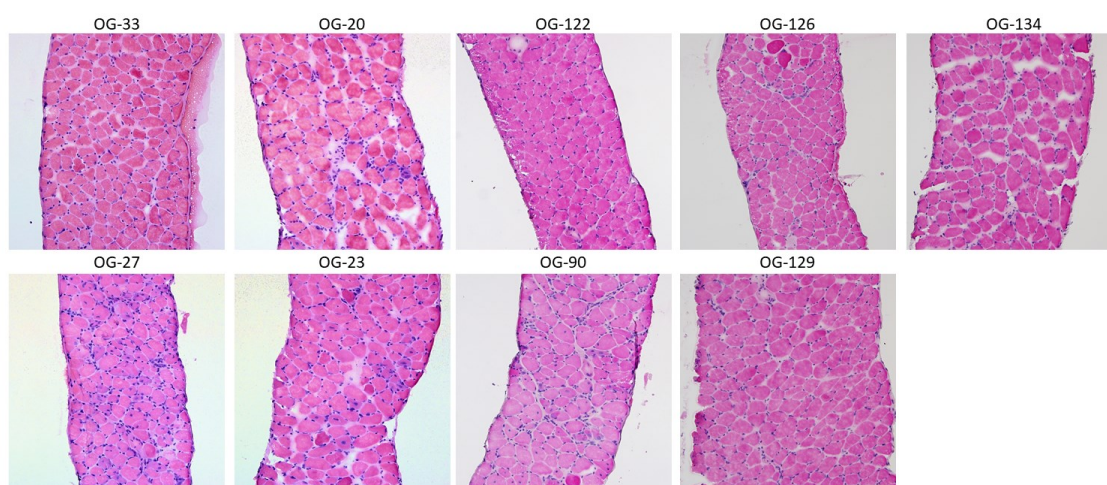
Male Triceps TIC-DUX4 5mg/kg



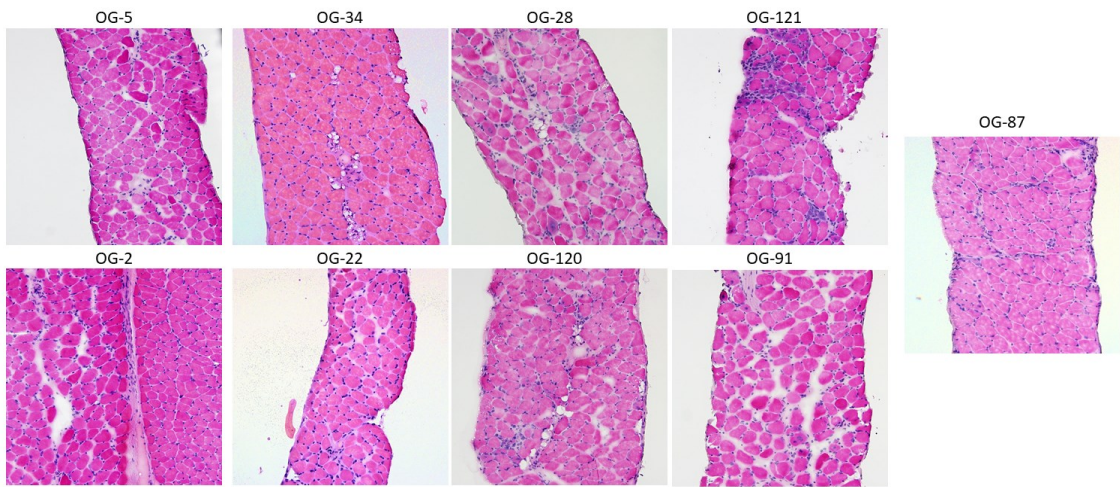
Female Triceps TIC-DUX4 5mg/kg



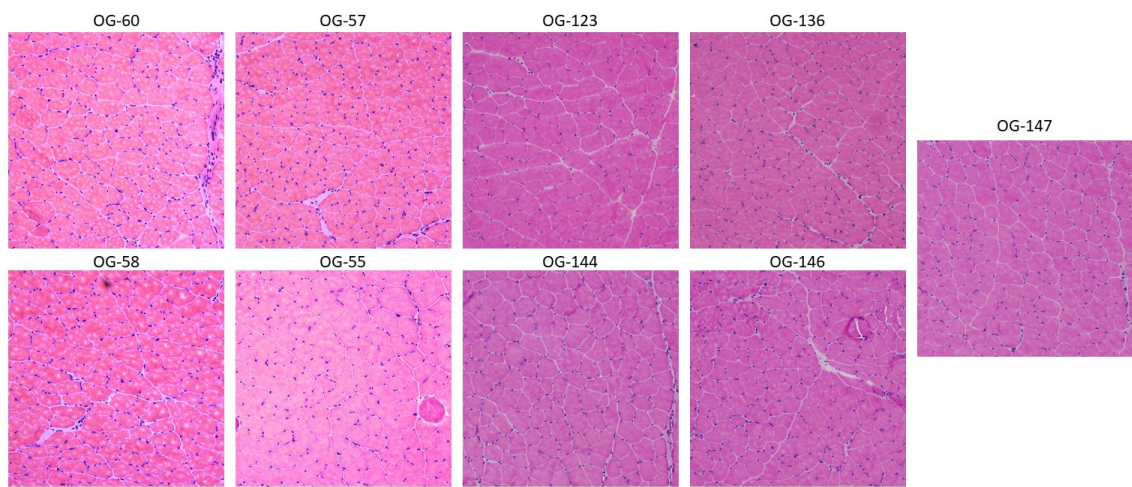
Male Diaphragm TIC-DUX4 5mg/kg



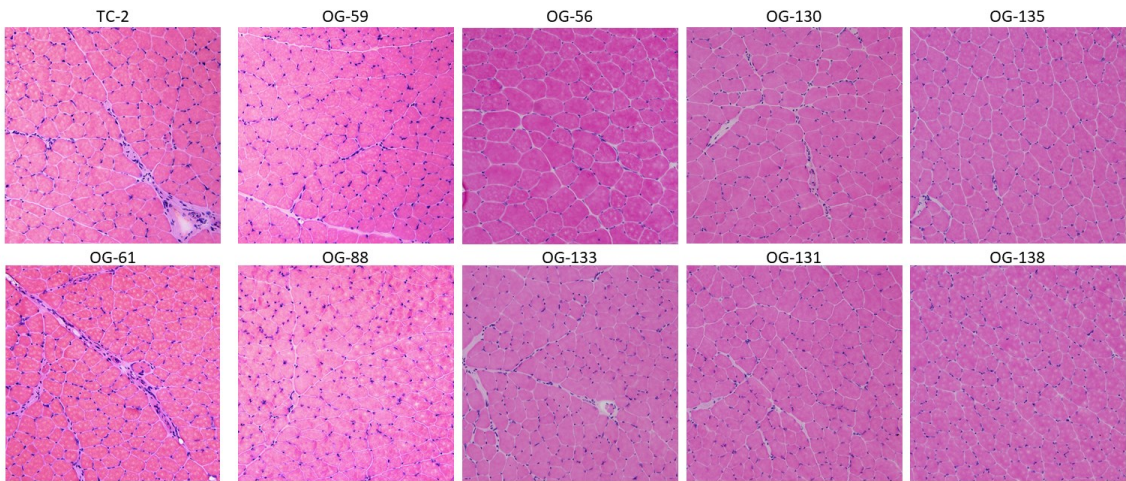
Female Diaphragm TIC-DUX4 5mg/kg



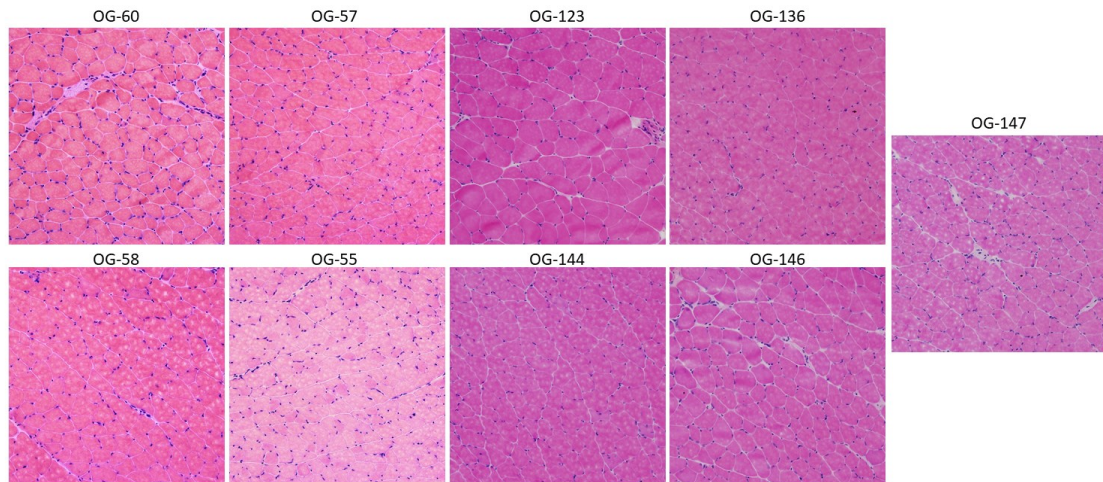
Male TA TIC-DUX4 0mg/kg



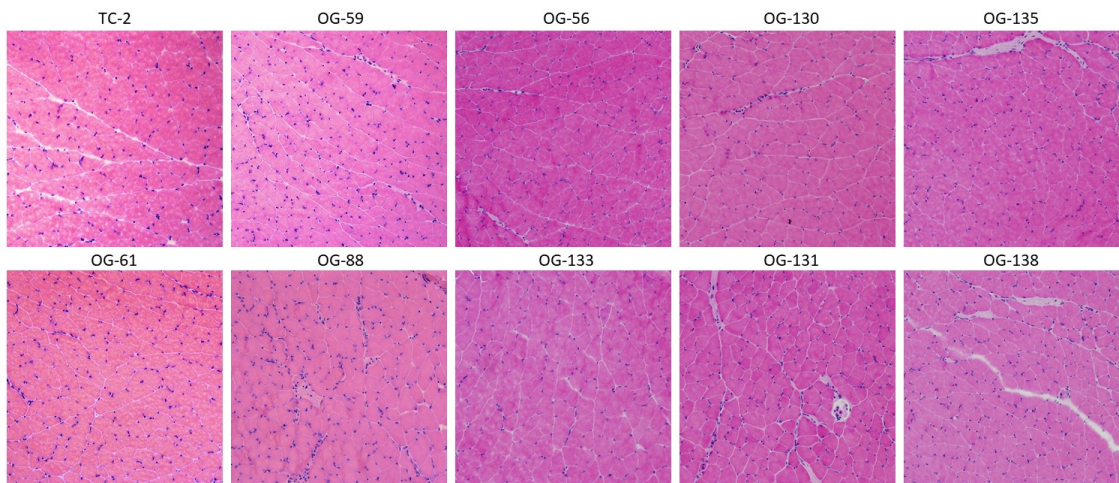
Female TA TIC-DUX4 0mg/kg



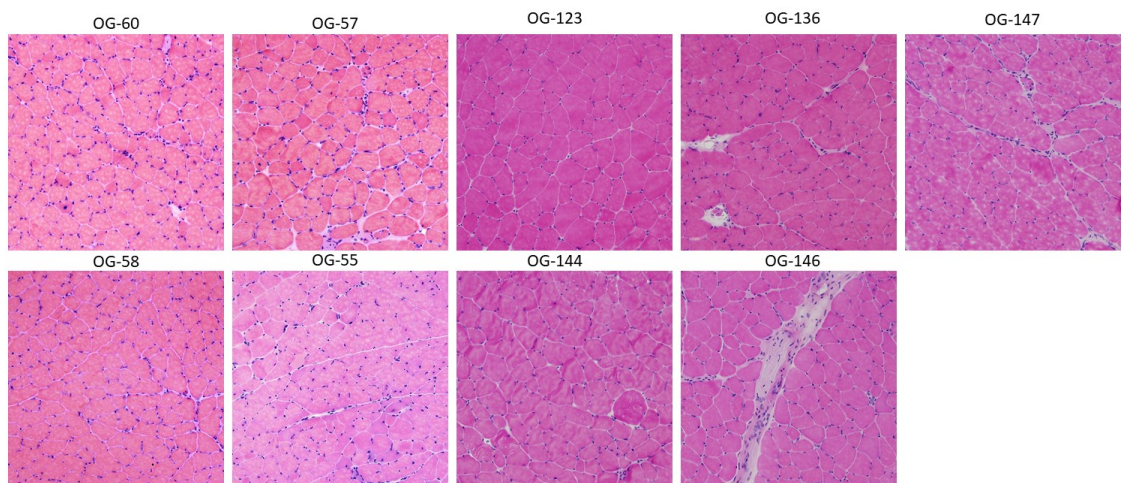
Male Gas TIC-DUX4 0mg/kg



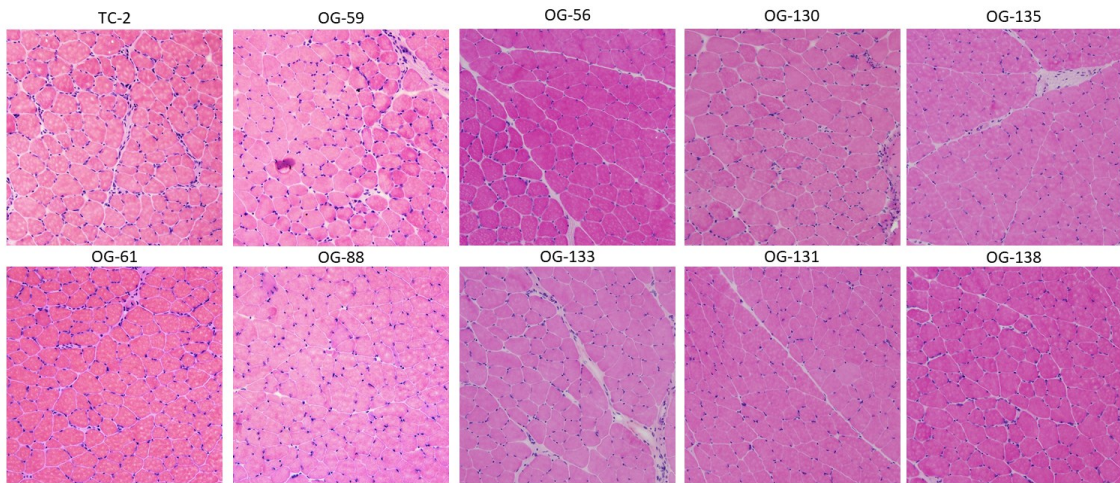
Female Gas TIC-DUX4 0mg/kg



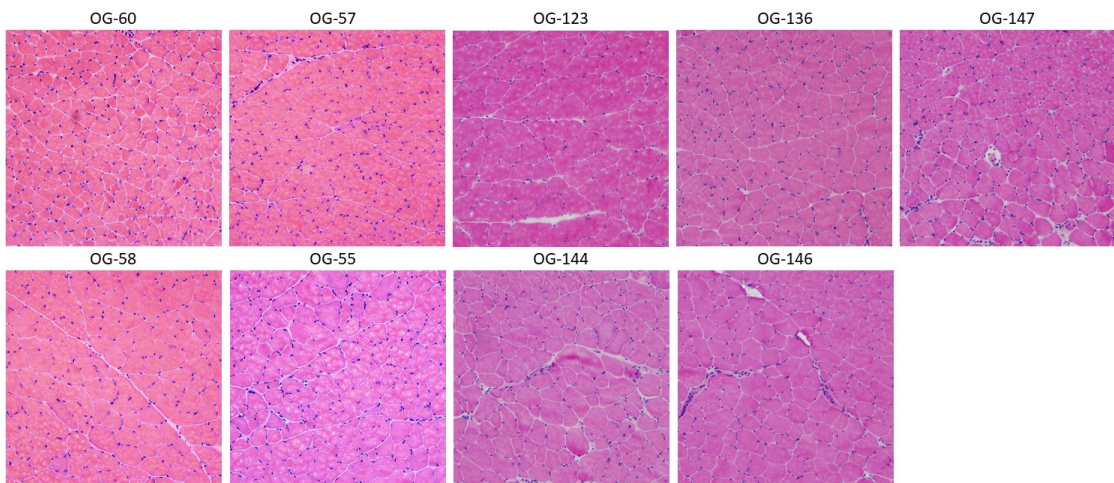
Male Quad TIC-DUX4 0mg/kg



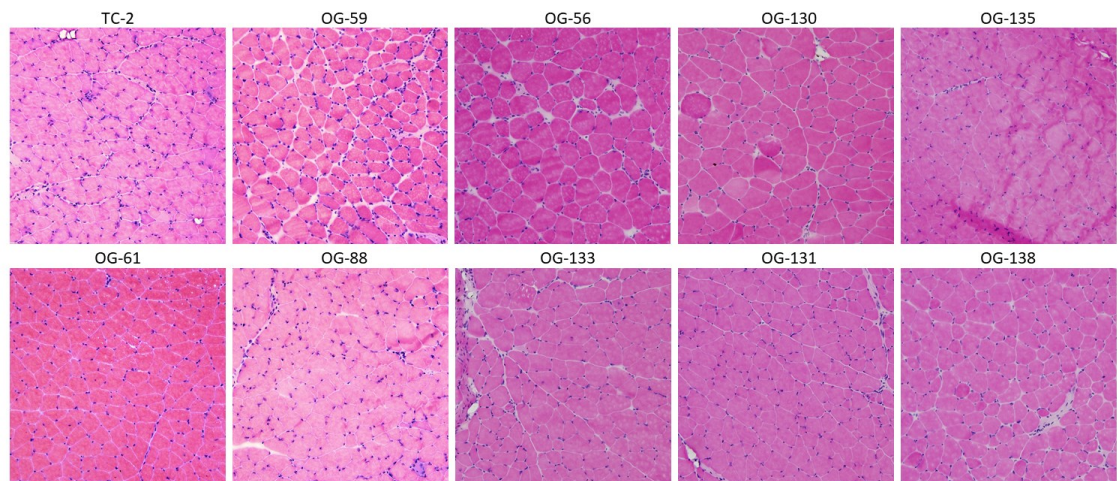
Female Quad TIC-DUX4 0mg/kg



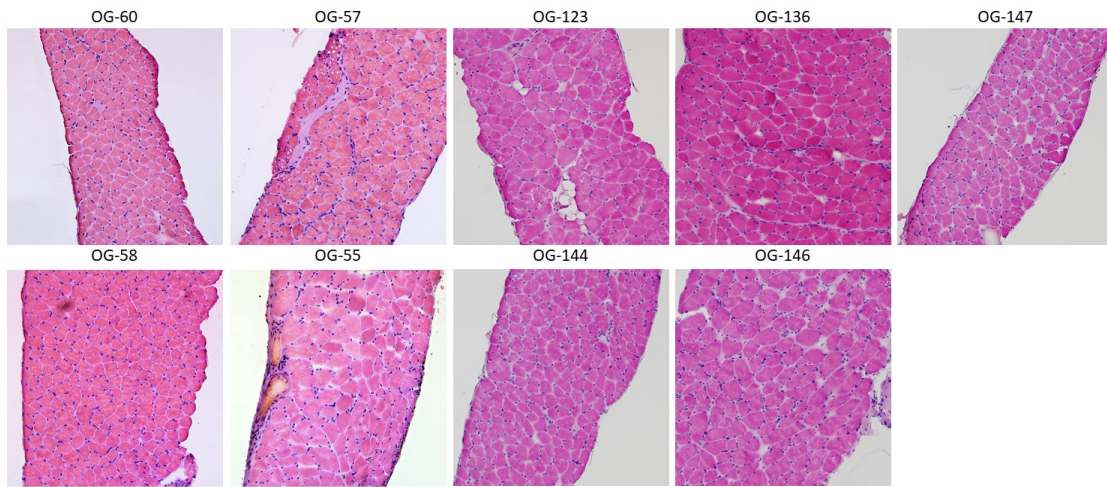
Male Triceps TIC-DUX4 0mg/kg



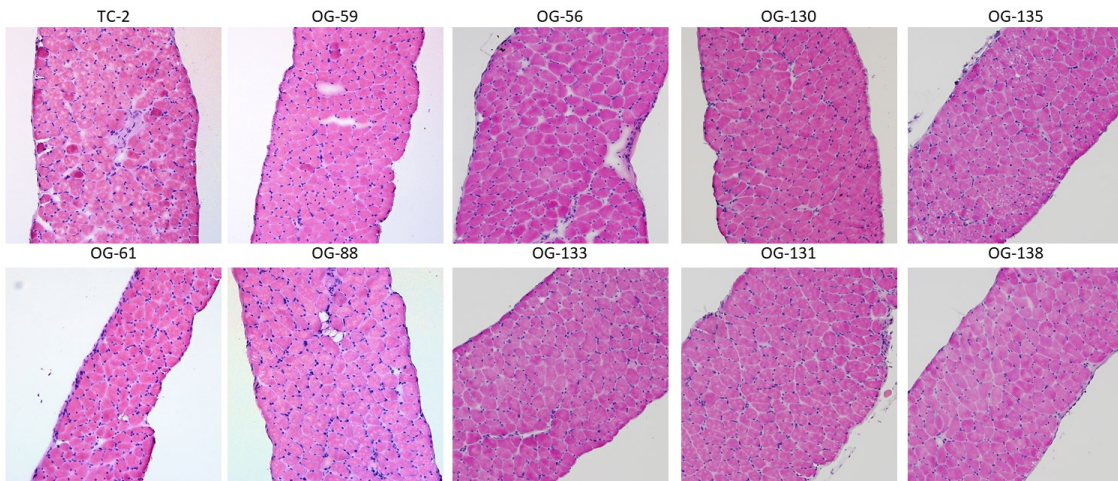
Female Triceps TIC-DUX4 0mg/kg



Male DiaphragmTIC-DUX4 0mg/kg

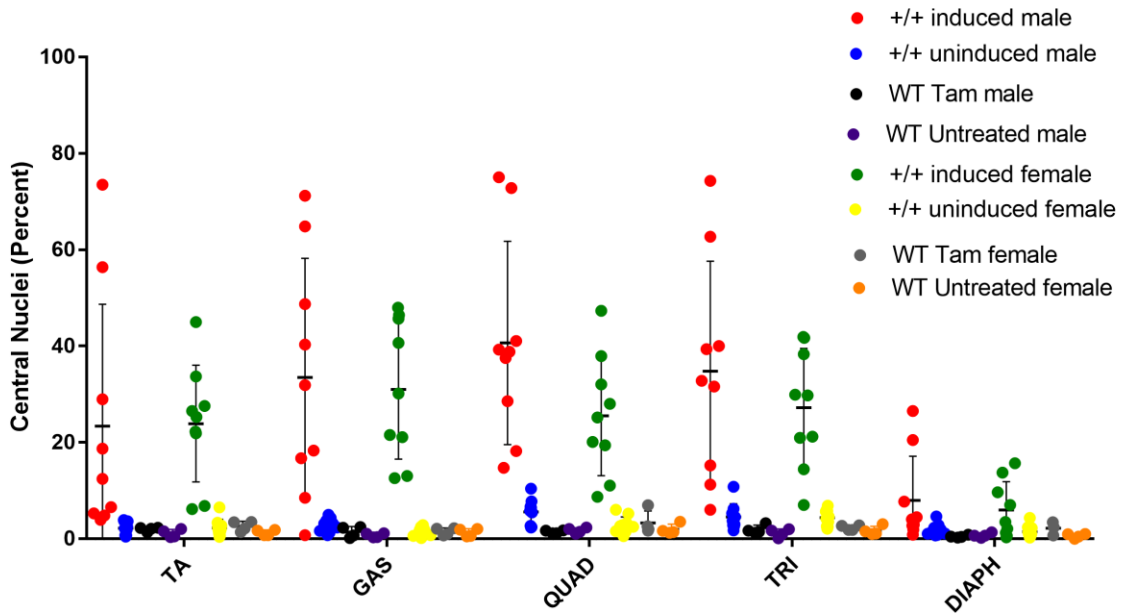


Female DiaphragmTIC-DUX4 0mg/kg



Supplemental Figure 3. Representative Histology for central nuclei and fiber diameter quantifications. Panels show representative images from all TIC-DUX4 mice used in histological quantifications. Images were taken 4 weeks after induction with 5 mg/kg Tamoxifen, 3 times per week. Histopathology, including degenerating myofibers, regenerated myofibers containing central nuclei, and immune infiltration, was evident in the 20X images throughout induced TIC-DUX4 muscles. Scale bars = 100 microns at 20X. Animals were 8-

immune infiltration, which are evident in the adjacent 20X images. Widespread damage was evident throughout induced TIC-DUX4 muscles. Increased percentage of myofibers with central nuclei and a shift toward smaller bore myofibers in induced TIC-DUX4 muscles support that muscles underwent degeneration and subsequent regeneration during this time course. Tamoxifen-induced TIC-DUX4 mice (TIC-DUX4 I) were significantly different from all controls (2-way ANOVA with Tukey's multiple comparison test; $p < 0.01$ for gas and $p < 0.05$ for quad). No significant sex differences were measured in induced TIC-DUX4 animals. Mean central nuclei percentages for gas muscle of induced TIC-DUX4 males: 33.5%; uninduced TIC-DUX4 mice, 2.8%; WT, Tam-treated 1.4%; WT untreated 0.7%. For female gas muscles: induced TIC-DUX4 muscles, 31.0%; uninduced TIC-DUX4, 1.4%; WT, Tam-treated, 1.5%; WT untreated 1.3%. For quad, mean central nucleic percentages in induced TIC-DUX4 males: 40.7%; uninduced TIC-DUX4, 5.6%; WT, Tam-treated 1.5%; WT untreated, 1.7%. For induced TIC-DUX4 female quad: 25.5%; uninduced TIC-DUX4, 2.9%; WT, Tam-treated 3.3%; WT untreated 2.0%. Scale bars = 500 microns in low power images; 100 microns at 20X. Animals were 8-12 weeks of age during this experiment. For male mice: N=9 induced (I) and uninduced (U) TIC-DUX4 mice; 4 Tamoxifen-treated WT littermates; 4 Untreated (U) WT littermates. For females: N=9 induced and 10 uninduced TIC-DUX4 mice; 4 Tamoxifen-treated WT littermates; 4 untreated (U) WT littermates.



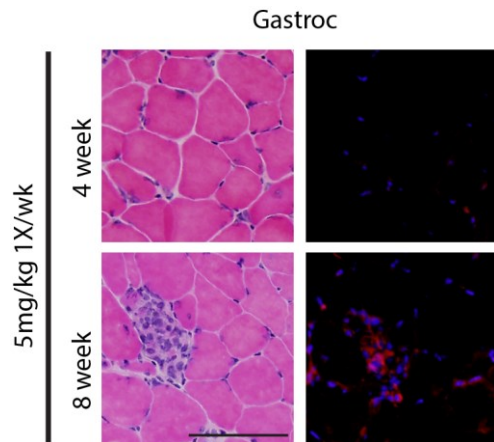
Supplemental Figure 5. Central Nuclei Percentages

Combined graph with central nuclei percentages reported in Figure 2 and Supplemental Figure 4. Mean values reported $\pm$ SD in all muscles and treatment groups. Induced TIC-DUX4 mice have increased average central nuclei percentages compared to Tam-treated and untreated WT controls.

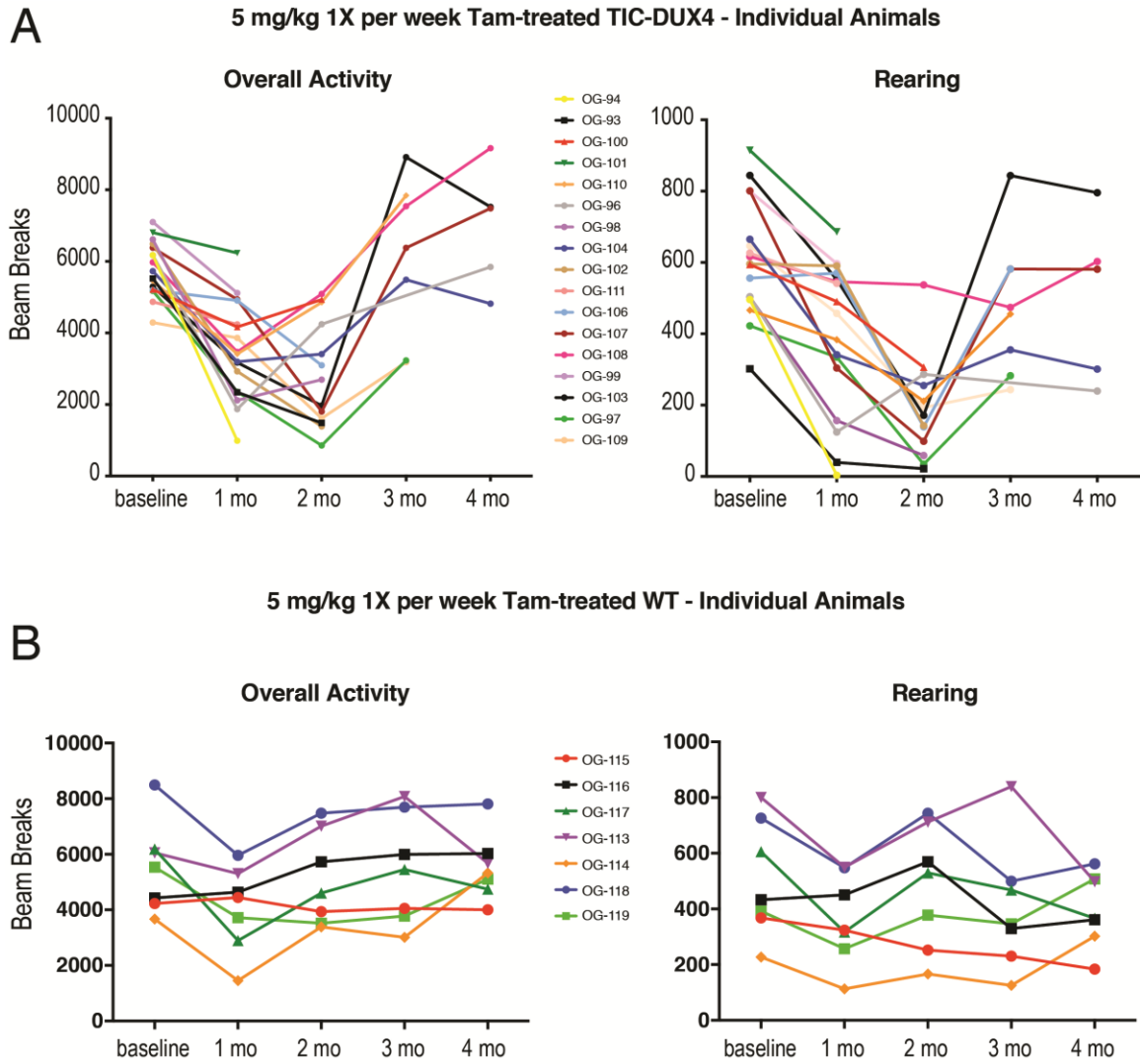
For male mice: N=9 induced (I) and uninduced (U) TIC-DUX4 mice; 4

Tamoxifen-treated WT littermates; 4 Untreated (U) WT littermates. For females:

N=9 induced and 10 uninduced TIC-DUX4 mice; 4 Tamoxifen-treated WT littermates; 4 untreated (U) WT littermates.



Supplemental Figure 6. TIC-DUX4 demonstrate DUX4 expression when induced with Tam (5mg/kg-1X/wk). Rare central nuclei and DUX4 positive nuclei occur at 1 month post induction. Focal lesions colocalize with more frequent DUX4 positive nuclei at 2 months post-induction. H&E 20X images scale bar = 100 microns. Representative of N = 4 induced TIC-DUX4 mice; 4 untreated WT mice.



Supplemental Figure 7. Supplemental figure to Figure 5 in the main text. Here we show total activity and rearing behavior in (A) TIC-DUX4 and (B) WT animals treated with low dose Tamoxifen (5 mg/kg, 1X per week) for 4 months. Tam-treated TIC-DUX4 mice declined in both measures between 1-2 months post-induction, while WT animals performed relatively constantly. Individual animals are indicated by an OG nomenclature, where OG = oral gavage. For TIC-DUX4 mice, at baseline and 1 mo timepoints, N = 17; at 2 mos, N=13; at 3 mos, N=7; at

A Tibialis Anterior Gastrocnemius

B 12-16 weeks

C 6 months

D 1 year

E 1.5 years

F 1.5 years

G 1.5 years

H 1.5 years

I 1.5 years

J 1.5 years

K 1.5 years

L 1.5 years

M 1.5 years

N 1.5 years

O 1.5 years

P 1.5 years

Q 1.5 years

R 1.5 years

S 1.5 years

T 1.5 years

U 1.5 years

V 1.5 years

W 1.5 years

X 1.5 years

Y 1.5 years

Z 1.5 years

AA 1.5 years

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HV 1.5 years

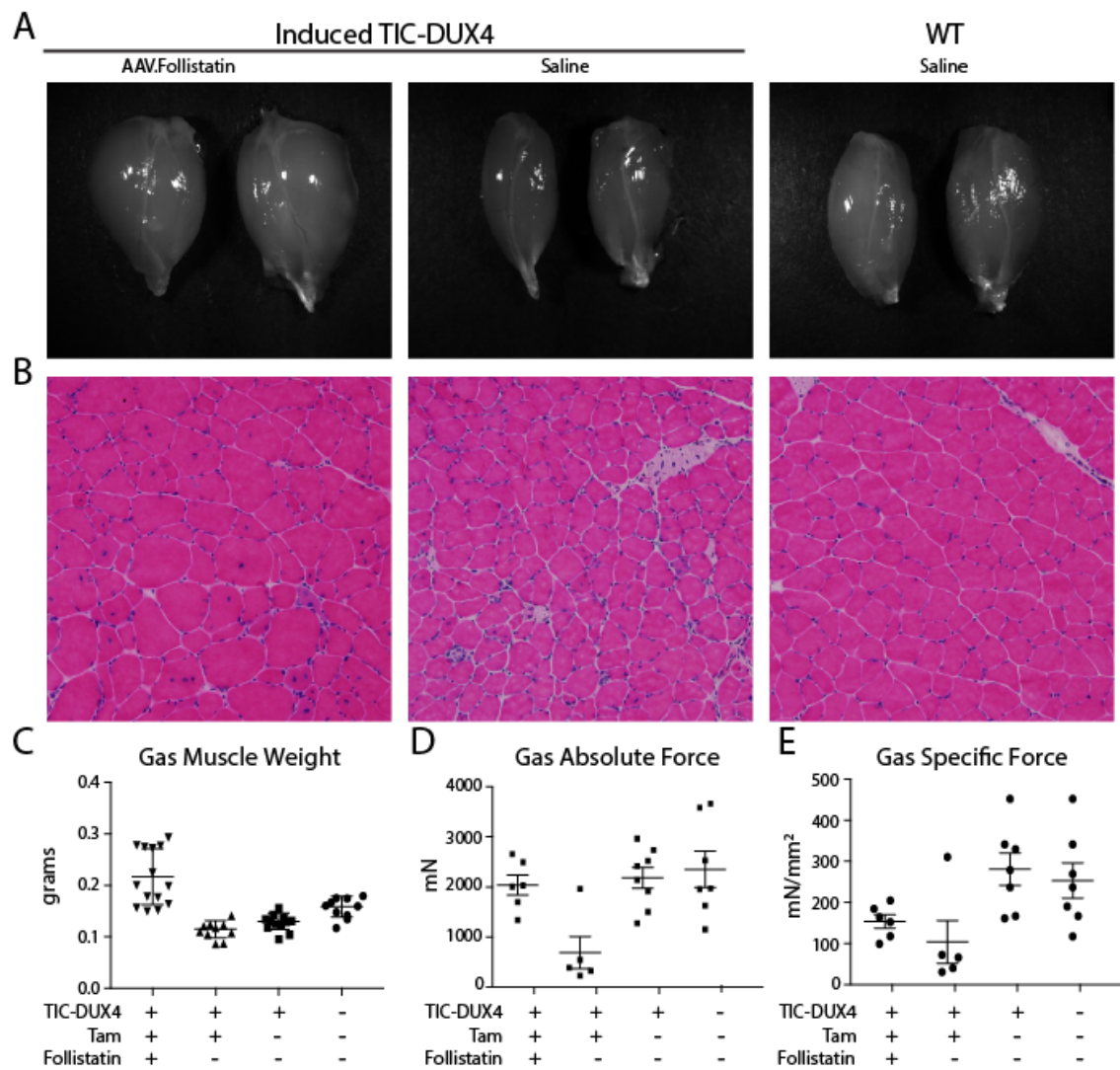
Supplemental Figure 8. Phenotypes in 1.5 yr-old uninduced TIC-DUX4 mice.

A-E. Animals were left untreated for up to 1.5 years old. Muscle cryosections from mice sacrificed at indicated ages were stained with H&E and immunofluorescence for DUX4 (V5 epitope, red) or nuclei (DAPI, blue). **A.** Shows the histology seen in TA and gastroc muscles at 12-16 weeks of age. Muscles appeared histologically normal with no DUX4 expression evident..

Representative images from N = 9 male and 10 female TIC-DUX4 mice and 4 male and 4 female untreated WT mice. **B.** Shows the histology seen in TA and gastroc muscles at 6 months of age with few or no degenerating fibers but some DUX4 positive nuclei. Representative of N = 4 male and 4 female TIC-DUX4 mice. **C.** Shows examples of uninduced TIC-DUX4 TA and gastroc muscles at 1 year of age displaying evidence of histopathology (mild inflammation, central nuclei, reduced eosin staining) colocalizing with DUX4 protein labeling.

Representative of N = 3 animals. **D.** Un-induced 1.5 year old uninduced TIC-DUX4 stain positive for sporadic DUX4 protein and have increased amounts of central nuclei. Image is representative of N = 4 uninduced TIC-DUX4 mice. **E.** Untreated HSA-mER-CRE-mER littermates do not stain positive for DUX4 protein and have normal histology. N = 4 littermates. H&E 20X images scale bar = 100 microns 20X scale bar = 100 microns. **F-G.** Old, uninduced TIC-DUX4 mice show no significant changes in total activity or rearing behavior compared to age-matched WT littermates. Difference in average activity or rearing levels (unpaired T-test, not significant). N=4 uninduced TIC-DUX4 mice, 4 untreated

WT mice. **H.** Absolute force (**Top**) was significantly reduced in TA muscles of 1.5 yr-old uninduced TIC-DUX4 mice (1056 mN) compared to age-matched WT littermates (1471 mN); 1-way ANOVA with Tukey's multiple comparisons test; $p < 0.01$. Uninduced TIC-DUX4 animals produced equivalent levels of force as compared to younger (16 wks) WT animals (16 wk WT muscles, absolute force 1090 mN). Specific force (**bottom**) comparison in TA muscles. No significant differences were found in specific force when comparing 1.5 year old TIC-DUX4 mice to 1.5 year old WT or 16 week old WT mice (1-way ANOVA with Tukey's multiple comparisons test). N = 6 uninduced TIC-DUX4 legs; 7 (1.5 year old) WT legs; 6 untreated 16 week old WT littermate legs. All graphs report force means $\pm$ SD. **I.** TA muscle weight compared in uninduced TIC-DUX4 and WT animals. Uninduced TIC-DUX4 TAs (32.7 mg) were significantly smaller than 1.5 year old WT mice (48.2 mg); unpaired t-test, $P < 0.01$. N=6 uninduced TIC-DUX4 legs; 7 (1.5 year old) WT legs; 6 untreated 16 week old WT littermate legs **J.** Old TIC-DUX4 mouse body weights were not significantly different from 1.5 year old WT mice (not significant; unpaired t-test). N = 6 uninduced TIC-DUX4 mice, 7 (1.5 year old) WT mice; 6 untreated 16 week old WT littermates. **K.** *Wfdc3* RT-qPCR in induced TIC-DUX4 gastroc muscles demonstrates that this *DUX4* target gene was significantly upregulated (~479-fold) in uninduced 1.5-year-old TIC-DUX4 mice (unpaired t-test, $p < 0.001$). Data shown are relative expression based on $\Delta\Delta C_q$ using *Rpl13* as a normalizer. For context, *Wfdc3* Cqs were >30 for WT controls but 21-24 for uninduced TIC-DUX4 mice. N=3 mice per group. All graphs report means $\pm$ SD.



Supplemental Figure 9. AAV.Follistatin increases gastroc muscle mass and absolute force in the presence of DUX4 expression.

A. 6-7 week old TIC-DUX4 and control mice were intramuscularly injected with 1×10^{11} particles of AAV1.Follistatin. 1-week later, mice were treated with 5 mg/kg Tamoxifen dosed 1X/week for 8 weeks. AAV1.Follistatin-injected gastroc muscles from Tamoxifen-treated TIC-DUX4 were overtly larger than saline-injected TIC-DUX4 controls and WT muscles. Image magnification 4X. **B.** H&E-stained cryosections show evidence of histopathology (myofibers with central nuclei) in Tamoxifen-treated TIC-DUX4 gastroc muscles expressing follistatin (left) and saline injected TIC-DUX4 controls. Tamoxifen-treated WT controls injected with saline appeared normal. scale bar = 100 μ m. **C.** Follistatin treatment significantly increased gas muscle weight compared to untreated controls (1-way ANOVA with Tukey's multiple comparisons test; $p < 0.001$). Mean gas muscle weights: Follistatin-treated, induced TIC-DUX4 mice, 213.9 mg; saline-treated induced TIC-DUX4 mice, 112 mg; uninduced TIC-DUX4 mice, 127.3 mg; age-matched WT, 155.7 mg. N=14 AAV.Follistatin-treated, Tamoxifen-induced TIC-DUX4 muscles; 10 saline-treated, Tamoxifen-induced TIC-DUX4 muscles; 12 uninduced TIC-DUX4 muscles; 9 untreated C57/BL6 controls. **D.** Gas absolute force was significantly increased in induced TIC-DUX4 mice treated with AAV1.Follistatin compared to induced, saline-treated TIC-DUX4 controls (1-way ANOVA with Tukey's multiple comparisons test; $p < 0.05$). Mean gas absolute force: Follistatin-treated, induced TIC-DUX4 mice, 2039 mN; saline-treated induced TIC-DUX4 mice, 693.6 mN; uninduced TIC-DUX4 mice, 2281 mN; age-

matched WT, 2356 mN. **E.** Specific force was determined by normalizing absolute force to muscle cross-sectional area. Follistatin treatment in induced TIC-DUX4 mice did not produce significant differences in specific force compared to all groups (1-way ANOVA with Tukey's multiple comparisons test), suggesting an intermediate phenotype. N=6 AAV.Follistatin-treated, Tamoxifen-induced TIC-DUX4 muscles; 5 saline-treated, Tamoxifen-induced TIC-DUX4 muscles; 7 uninduced TIC-DUX4 muscles; 8 untreated WT controls.

		Animal	TA	Gas	Quad	Tri	Diaphragm
Male	TIC-DUX4 5mg/kg 3X/week 4 week	N	28.97	40.33	41.05	32.80	2.98
		Q	4.86	48.74	39.26	40.00	7.76
		P	56.38	71.24	72.83	62.69	26.57
		O	5.32	18.34	18.21	6.05	0.85
		AS	73.53	64.87	75.07	74.29	20.54
		AV	3.96	0.78	38.84	15.23	1.92
		AX	18.74	31.89	37.56	39.37	4.50
		AY	6.57	8.52	14.75	11.28	4.06
		AZ	12.46	16.76	28.57	31.63	2.74
	TIC-DUX4 Uninduced	I	0.73	1.28	2.36	3.01	0.80
		W	2.54	4.99	10.43	6.24	2.30
		J	0.72	3.20	2.45	2.84	1.50
		H	3.64	3.16	7.76	3.76	1.08
		BA	0.47	0.76	2.70	1.77	1.12
		BC	3.91	2.34	6.50	5.32	4.65
		BG	2.17	4.37	5.48	10.82	0.75
		BI	1.92	1.66	5.85	2.63	1.99
		BJ	3.64	3.80	6.68	4.53	2.87
	WT 5mg/kg 3x/week 4 week	X	1.37	0.19	1.16	1.74	0.25
		AA	2.30	2.48	1.16	1.45	0.86
		AD	2.26	2.29	1.76	3.27	0.43
		AE	2.13	0.72	1.73	1.25	0.49
	WT uninduced	AH	2.07	0.38	2.05	2.02	0.23
		AJ	1.58	1.16	1.09	0.14	0.65
		AK	0.52	1.05	1.46	0.97	0.69
		AL	0.35	0.32	2.38	1.74	1.37
			Highest percentage in group				
			Lowest percentage in group				

Supplemental Table 1. Male Central Nuclei Percentages

Male central nuclei percentage averages across groups. Red text indicated highest central nuclei percentage per muscle and treatment group. Green text indicated lowest central nuclei percentage per muscle and treatment group

		Animal	TA	Gas	Quad	Tri	Diaphragm
Female	TIC-DUX4 5mg/kg 3X/week 4 week	L	22.24	21.59	28.02	29.91	1.28
		M	6.85	13.08	20.12	14.45	2.11
		U	21.92	21.14	25.18	21.21	0.62
		S	6.19	12.59	8.74	7.04	0.31
		AP	26.53	30.16	19.42	20.98	15.70
		AQ	25.28	45.67	37.92	29.77	9.70
		AU	33.73	40.66	32.06	41.70	7.02
		AT	45.02	46.42	47.35	41.90	3.53
		AR	27.59	47.97	11.08	38.34	13.78
	TIC-DUX4 Uninduced	Y	1.67	0.55	1.60	6.89	4.38
		V	3.37	1.18	3.25	2.61	0.29
		Z	0.65	0.62	1.46	3.57	0.96
		K	0.44	0.75	0.57	2.14	1.36
		AW	6.52	0.20	2.72	5.69	0.46
		BB	1.81	2.00	6.03	4.62	2.43
		BD	2.77	2.52	2.86	6.11	0.69
		BE	2.10	1.92	5.27	4.21	2.28
		BF	1.17	2.88	2.65	5.78	1.01
		BH	2.48	1.01	2.50	2.51	0.75
	WT 5mg/kg 3x/week 4 week	AB	3.45	2.20	2.00	2.69	0.73
		AC	2.16	2.13	7.00	1.85	2.44
		AG	1.40	1.04	2.60	1.86	1.90
		AF	3.53	0.70	1.72	2.81	3.47
	WT uninduced	T	1.87	0.62	1.41	1.57	0.00
		AI	0.78	1.91	1.27	1.12	0.98
		AM	1.69	2.06	3.58	3.05	0.45
		AN	0.78	0.50	1.68	1.04	1.04
			Highest percentage in group				
			Lowerest percentage in group				

Supplemental Table 2. Female Central Nuclei Percentages

Female central nuclei percentage averages across groups. Red text indicated highest central nuclei percentage per muscle and treatment group. Green text indicated lowest central nuclei percentage per muscle and treatment group.