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Supplementary Materials for

Prevention of Muscle Aging by Myofiber-Associated Satellite Cell Transplantation

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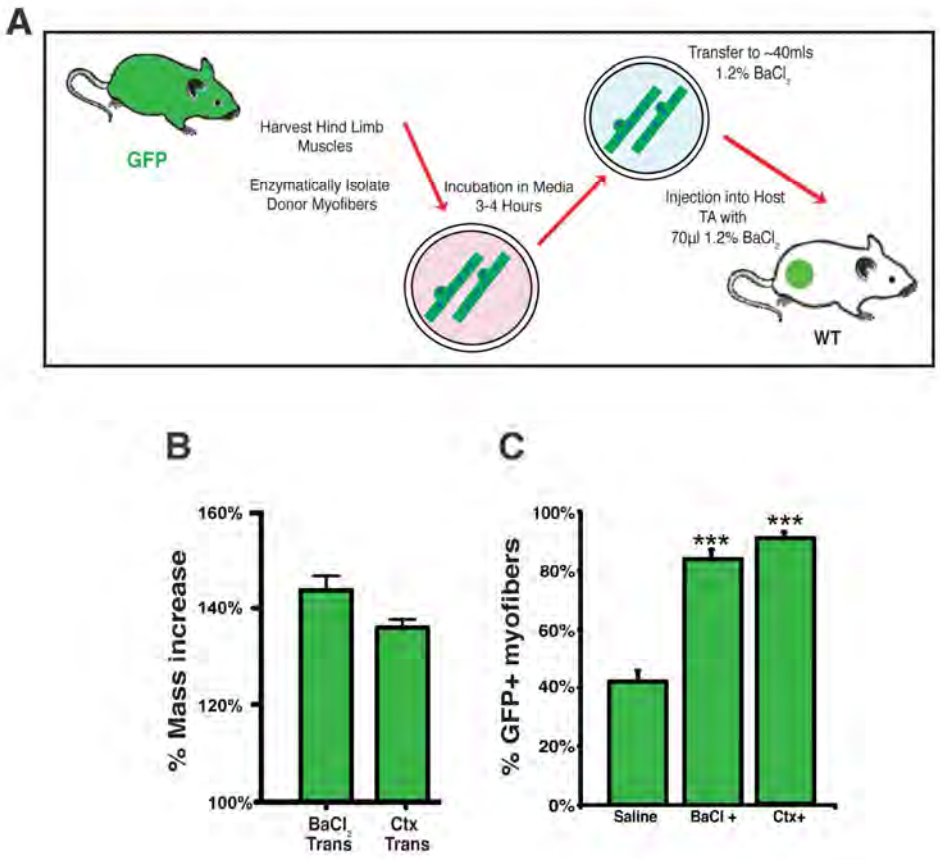

Fig. S1. Engraftment of donor cells is independent of injury agent.

A schematic illustrating myofiber transplantation. GFP+ myofibers isolated as previously described (1) from a donor *ßActGFP* mouse are placed in tissue culture for 3-4 h, then transferred to 1.2% $BaCl_2$ or cardiotoxin with a 27 ½ g needle. The cultured myofibers are then re-isolated in 70 µl of either $BaCl_2$ or cardiotoxin (where indicated), and injected into the TA muscle of a B6D2F1 host mouse. (**A**). Mass increase is independent of injury agent as 1.2% $BaCl_2$ and cardiotoxin (CTx) yield similar gains in mass (**B**) as well as donor engraftment quantified by the percent of GFP+ myofibers. GFP+ myofibers are scored after background subtraction of autofluorescence from a contralateral TA muscle section (**C**). *n*=12, for 1.2% $BaCl_2$ transplants and *n*=4 for Cardiotoxin transplants, Saline transplants and 1.2% $BaCl_2$ only. Number of myofibers transplanted is 5 (**B** and **C**). For all, *** indicates p value of <0.001.

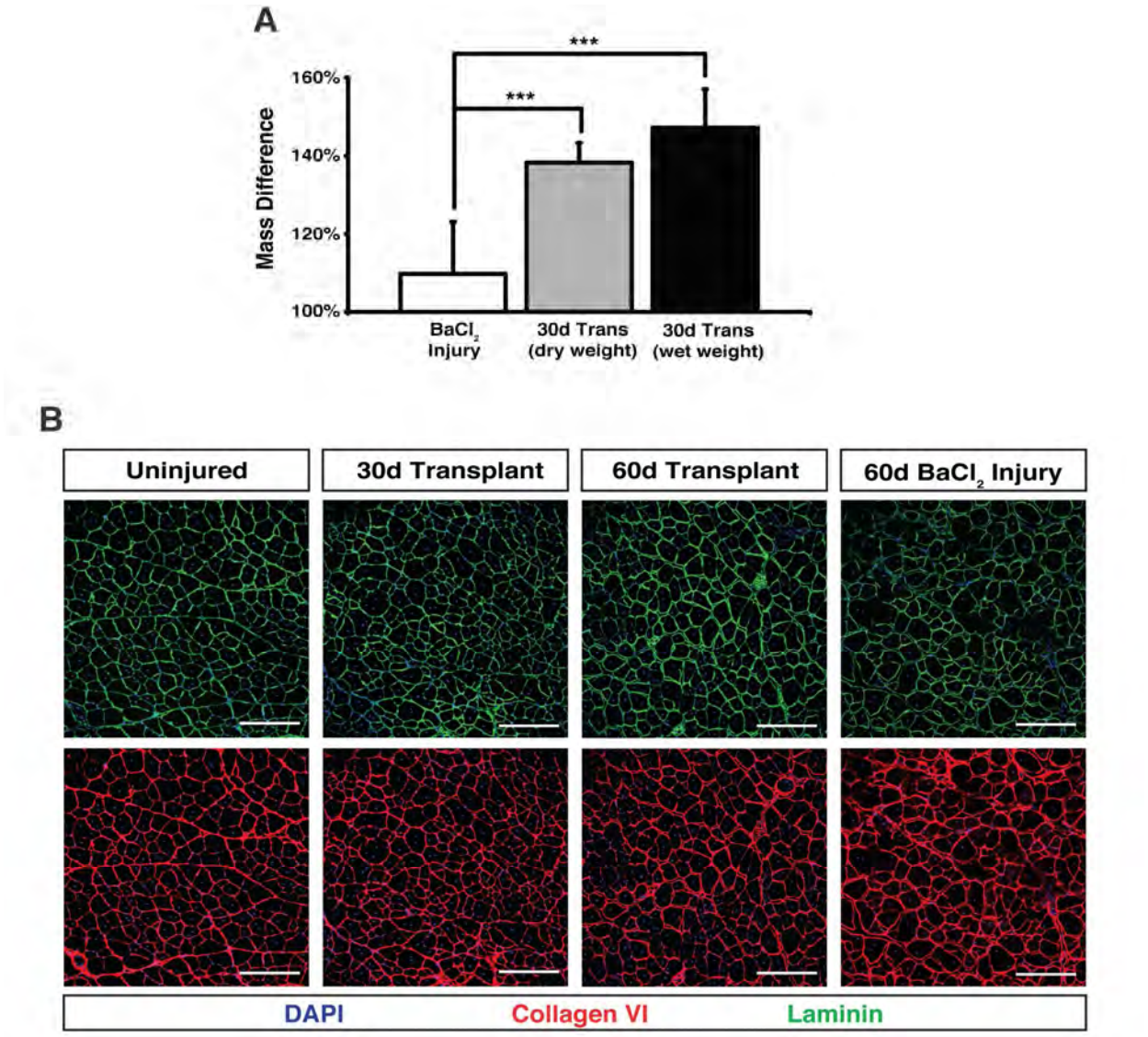

Fig. S2. Fibrosis does not contribute to increased muscle mass after transplantation.

Increased muscle mass following transplant is not due to increased fibrosis where transplanted TA muscles retain increased muscle mass when measured either wet or following desiccation (**A**). Collagen VI deposition is similar in TA muscle cross-sections of uninjured, 30d and 60d after transplant, and 60 d after $BaCl_2$ injury. Mice, n=4-10 (**A**) and n=3 for (**B**). Number of myofibers transplanted is 5 for all. For all, *** indicates p value of <0.001. Scale bars are 150μm (**B**).

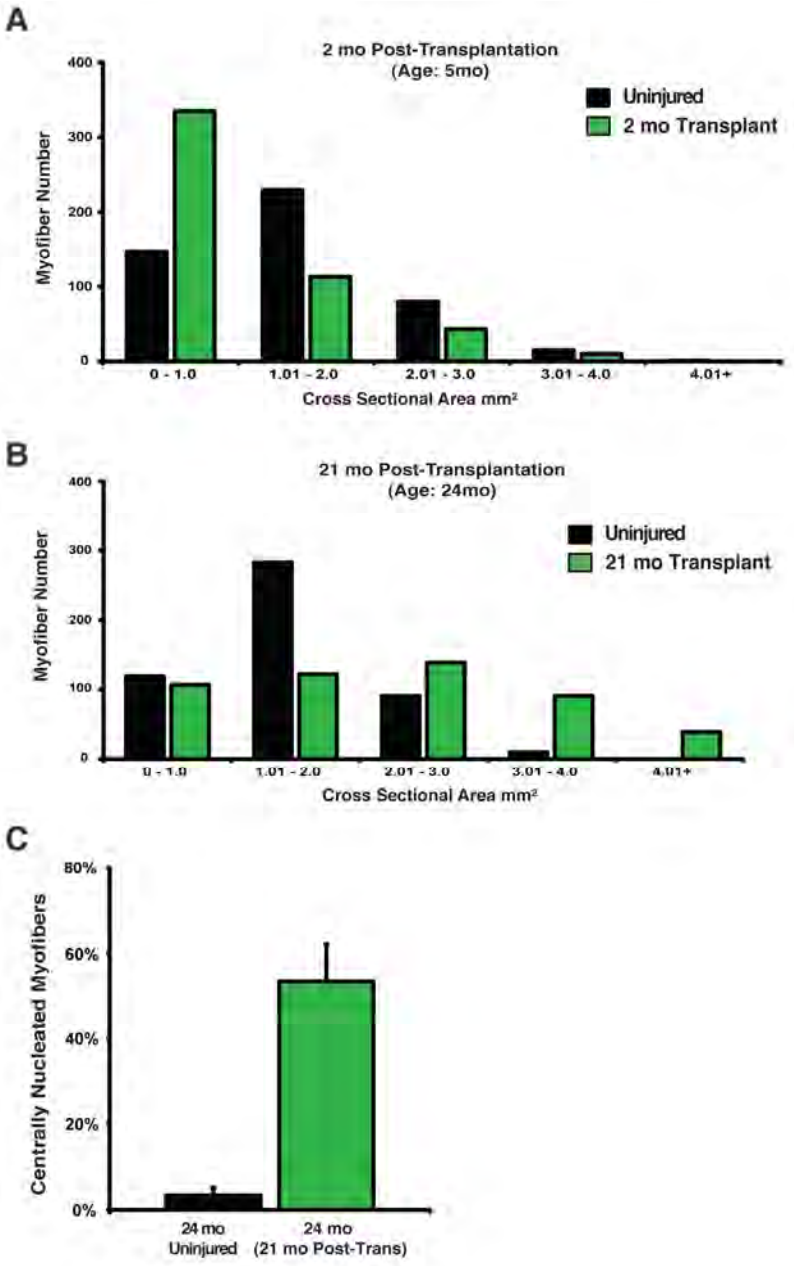

Fig. S3. Progressive hypertrophy in transplanted TA muscles.

The total, and GFP+ myofiber numbers were quantified by GFP fluorescence determined as described in the Supplemental Fig. 1 legend at 2 mo (**A**) and 21 mo (**B**) after transplant. Morphometric analysis was performed as described in Methods. The percent of centrally located myonuclei was scored for the engrafted and contralateral control TA muscles 21 mo post-transplant in 24 month-old mice (**C**). *n*=500 for **C**. Number of myofibers transplanted is 5 (**A**-**C**).

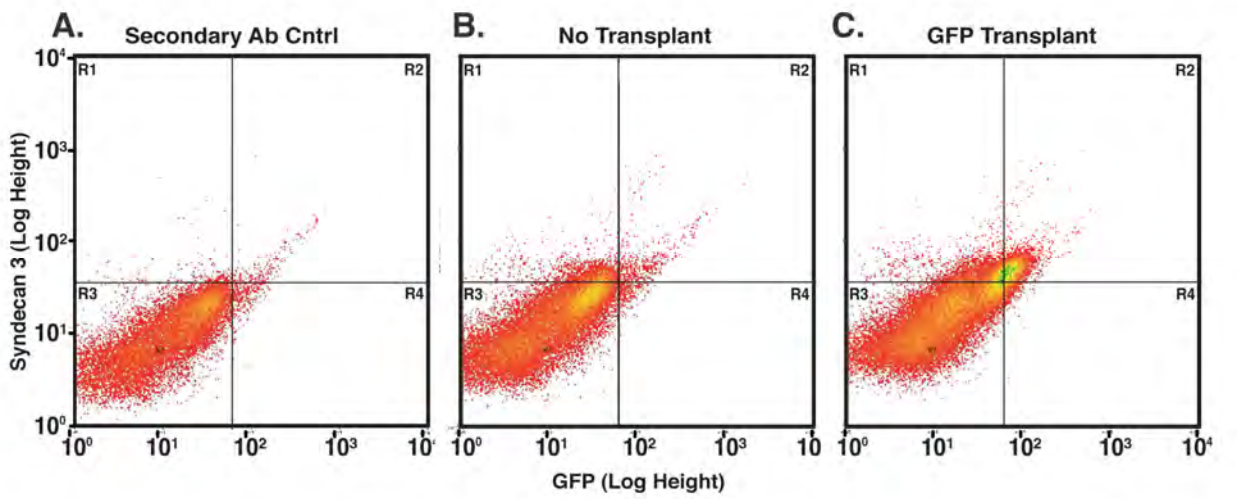

Fig. S4. Quantification of satellite cells in transplanted TA muscles by FACS.

The increase in GFP+, Syndecan-3+ mononuclear cells in transplanted as compared to uninjured, contralateral TA muscles was quantified using a Beckman Coulter Mo-Flo XDP flow cytometer (**A-C**). Similar numbers of events were plotted for each histogram. When plotting for Syndecan 3+ fluorescence vs. GFP+ fluorescence. Syndecan 3+ cells (**C, GFP Transplant; R1, R2**) increase 2.5-fold compared to the non-transplanted, uninjured contralateral muscle (**B, No Transplant; R1, R2**). GFP+ donor cells (**A, GFP Transplant; R2**) comprise ~60% of the total Syndecan-3+ cells in the transplanted host tissue. Ten *ßActGFP* myofibers were transplanted into the host TA muscle (**A-C**).

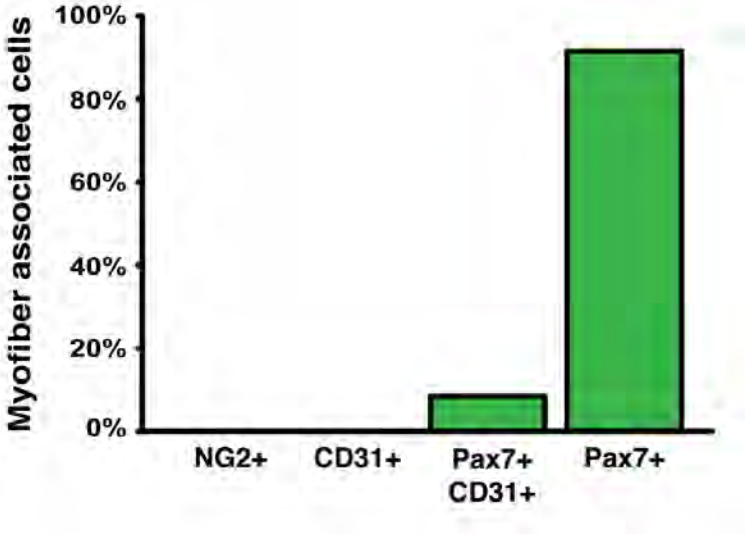

Fig. S5. Transplanted myofibers have only Pax7$^+$ cells.

All myofiber-associated cells on fibers selected for transplant were Pax7$^+$. Heterogeneity in cells was observed with 10% of cells expressing CD31 in addition to Pax7, however, no cells expressing either CD31+ or NG2+ were detected. *n*=20 myofibers possessing an average of 3 ± 2 Pax7$^+$ cells each. A subset of satellite cells are CD-31-positive (2).

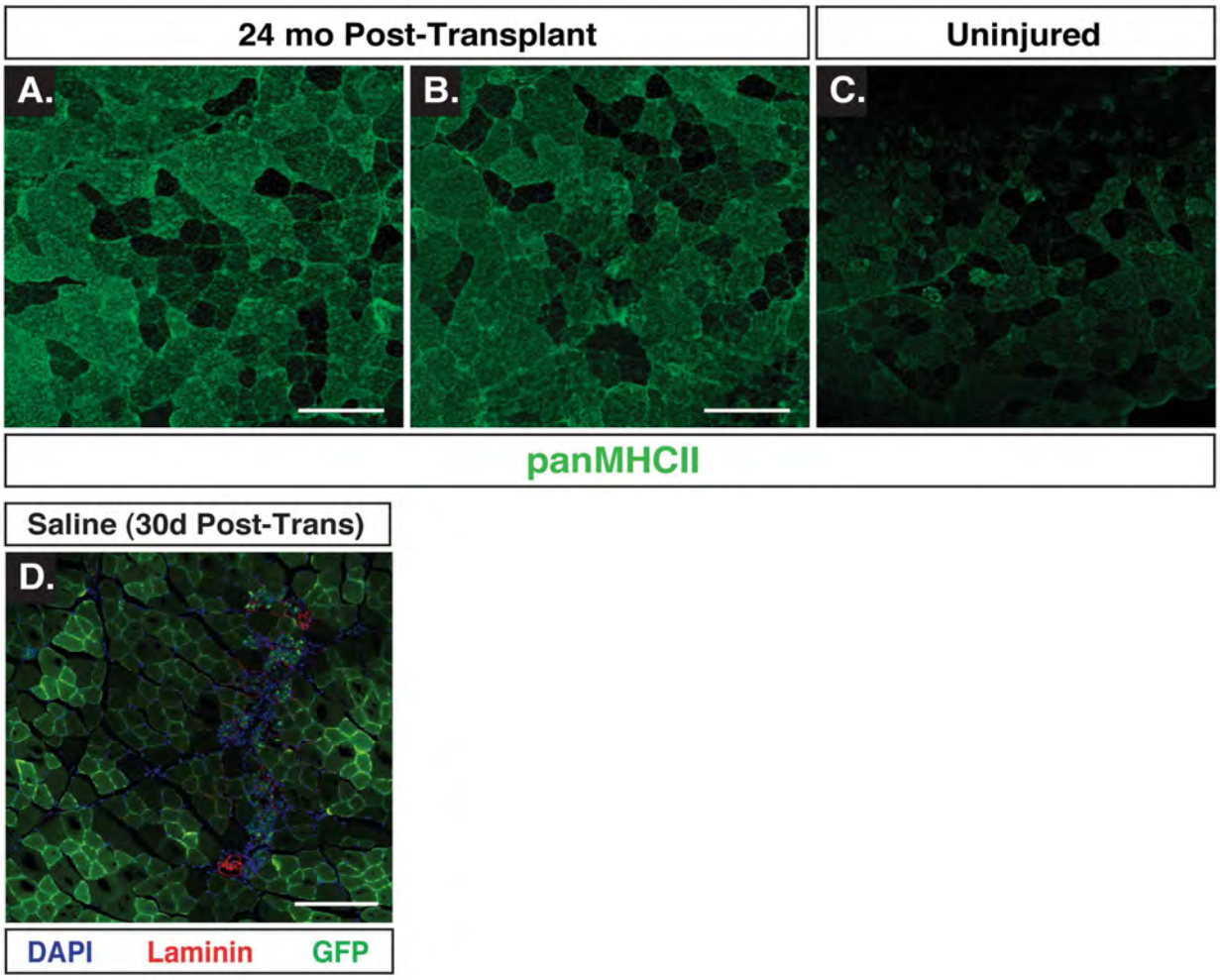

Fig. S6. Retention of MHC type II fibers 24 months after transplantation.

MHC type II myofibers (green) are retained in transplanted muscle, 21 mo post-injection (**A** and **B**) compared to uninjured, contralateral control muscle in 24 mo-old mice (**C**). Transplantation of GFP+ myofibers in the absence of injury yields minimal, localized engraftment within the host TA muscle (**D**). Scale bars are 75 µm (**A-C**) and 150 µm (**D**). *n*=4 for **A-D**. Number of myofibers transplanted is 5 (**A-D**).

Secondary Antibody Control

Gate	Count	% Histogram
Total	16660	100.00
R1	204	1.22
R2	184	1.10
R3	15970	95.86
R4	302	1.81

No Transplant (Uninjured, Contralateral TA)

Gate	Count	% Histogram
Total	25403	100.00
R1	2080	8.19
R2	479	1.89
R3	22602	88.97
R4	242	0.95

GFP Transplant

Gate	Count	% Histogram
Total	33636	100.00
R1	3949	11.74
R2	4406	13.10
R3	24587	73.10
R4	694	0.69

Table S1. FACS data for transplanted TA muscles.

Raw values for gated histograms of FACS analyzed primary cells depicted in Supplemental Figure 3.

Peak Force Measurements

Test Subject	Uninjured, No Transplant Contralateral	Transplant and Injury
Mouse #1	**1109 kN**	**2166 kN**
Mouse #2	**1266 kN**	**2138 kN**
Mouse #3	**1106 kN**	**2147 kN**
Mouse #4	**934 kN**	**1831 kN**
Mouse #5	**1117 kN**	**1896 kN**

Table S2. TA muscle peak force data at 24 months after transplantation.

Peak force measurement of transplanted TA muscles 24 mo after transplantation and concurrent injury.

Specific Force Measurements

Test Subject	Uninjured, No Transplant Contralateral	Transplant and Injury
Mouse #1	**258 kN/m^2**	**302 kN/m^2**
Mouse #2	**213 kN/m^2**	**269 kN/m^2**
Mouse #3	**289 kN/m^2**	**282 kN/m^2**
Mouse #4	**269 kN/m^2**	**305 kN/m^2**
Mouse #5	**233 kN/m^2**	**296 kN/m^2**

Table S3. TA muscle–specific force data at 24 months after transplantation.

Specific force measurement of transplanted TA muscles 24 mo after transplantation and concurrent injury.

Contraction Induced Injury Measurements

	Mouse #1	Mouse #2	Mouse #3	Mouse #4	Mouse #5	Mouse #1	Mouse #2	Mouse #3	Mouse #4	Mouse #5
Strain (% Optimal Length)	Uninjured, No Transplant Contralateral	Uninjured, No Transplant Contralateral	Uninjured, No Transplant Contralateral	Uninjured, No Transplant Contralateral	Uninjured, No Transplant Contralateral	Transplant and Injury	Transplant and Injury	Transplant and Injury	Transplant and Injury	Transplant and Injury
0	100	100	100	100	100	100	100	100	100	N/A
5	107	100	98	94	96	100	101	99	101	N/A
10	104	94	94	88	94	98	104	101	105	N/A
15	101	91	91	80	85	101	102	98	101	N/A
20	96	96	99	78	80	99	96	95	99	N/A
25	92	84	100	71	77	97	N/A	91	97	N/A
30	91	78	96	58	67	92	N/A	86	93	N/A
35	88	74	89	58	60	86	N/A	81	87	N/A
40	75	65	82	46	52	78	N/A	75	79	N/A
45	69	60	75	34	43	71	N/A	71	73	N/A

Table S4. TA muscle contraction–induced injury data at 24 months after transplantation.

Transplanted TA muscles 24 mo post-injection, and uninjured contralateral TA muscles were successively lengthened to assess contraction-induced injury. Values listed as N/A in mice #2 and #5 were the result of strong contractions in transplanted muscle that repeatedly removed the surgical ties to the TA muscle preventing data acquisition.

Supplementary References

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