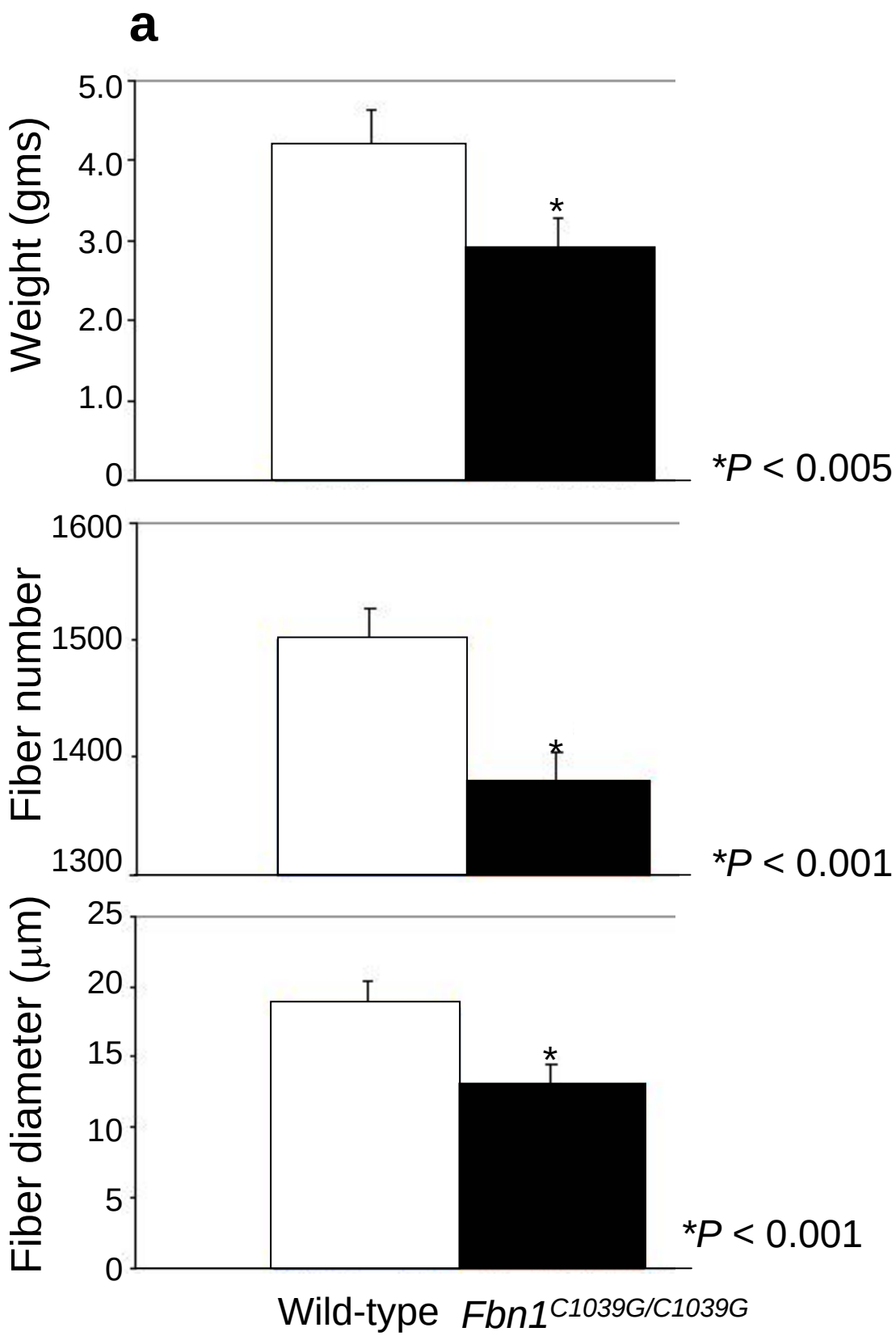
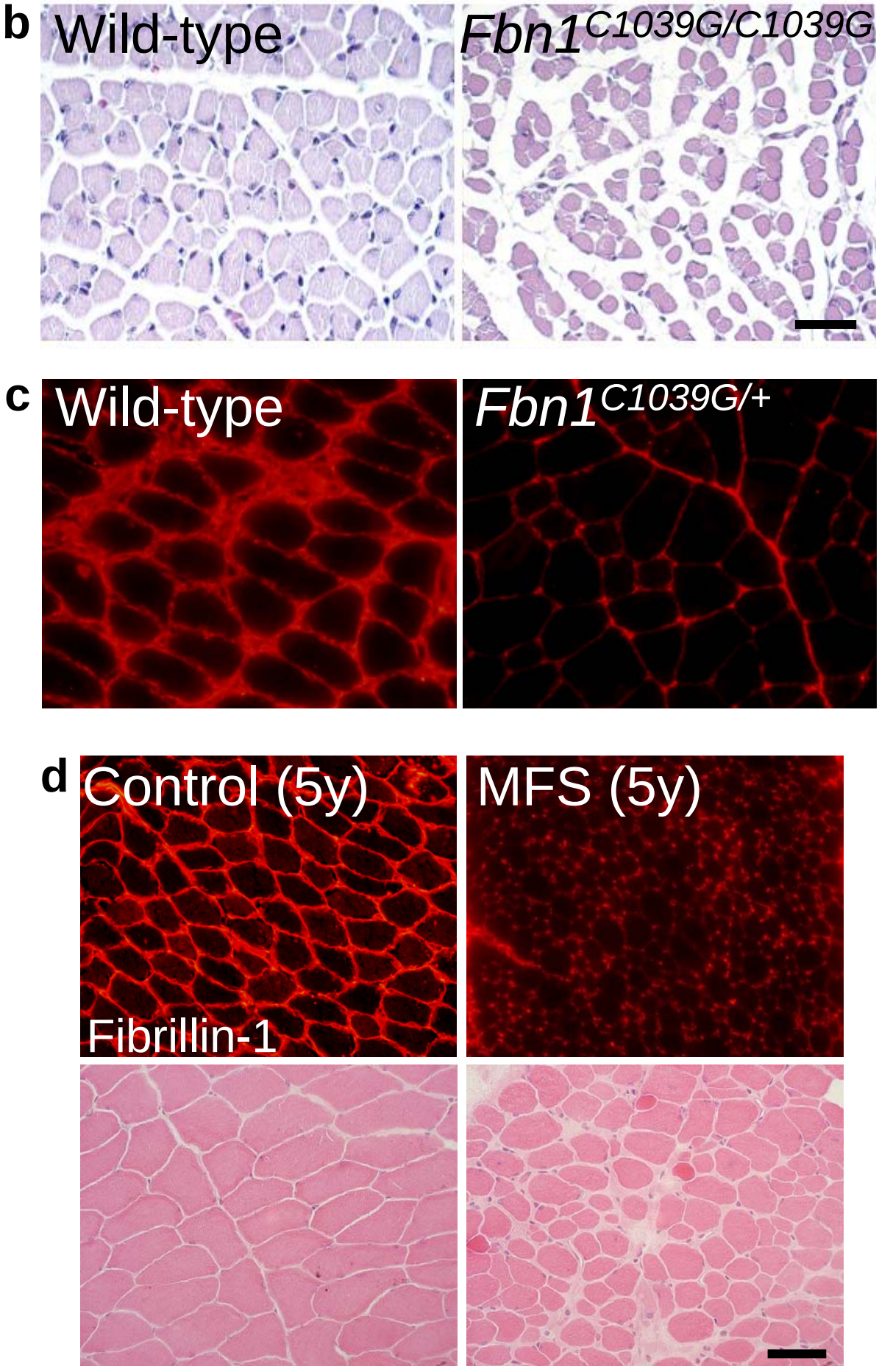
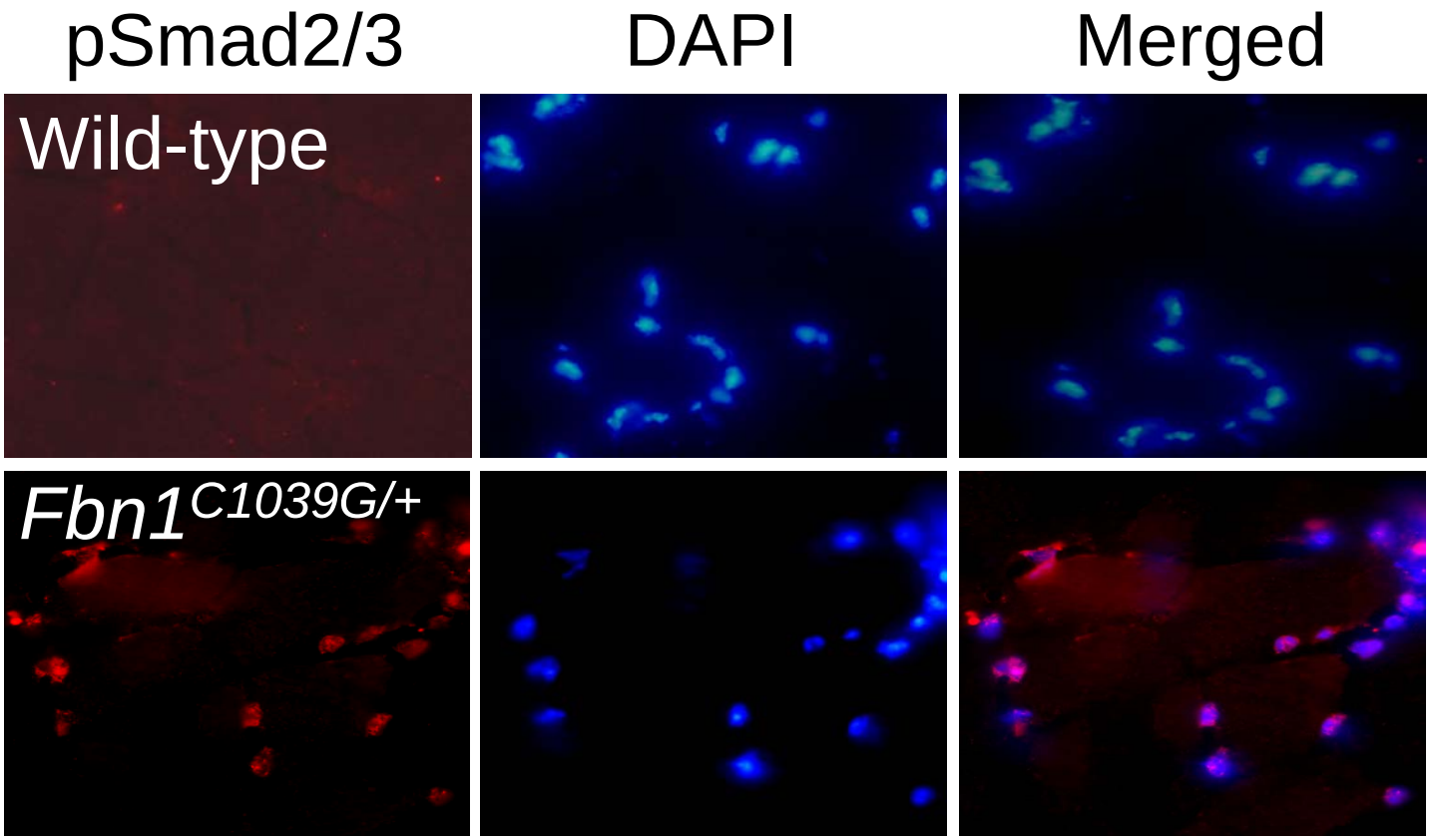
Supplementary Figure 1




b
Wild-type
Fbn1C1039G/C1039G
c
Wild-type
Fbn1C1039G/+
d
Control (5y)
MFS (5y)
Fibrillin-1

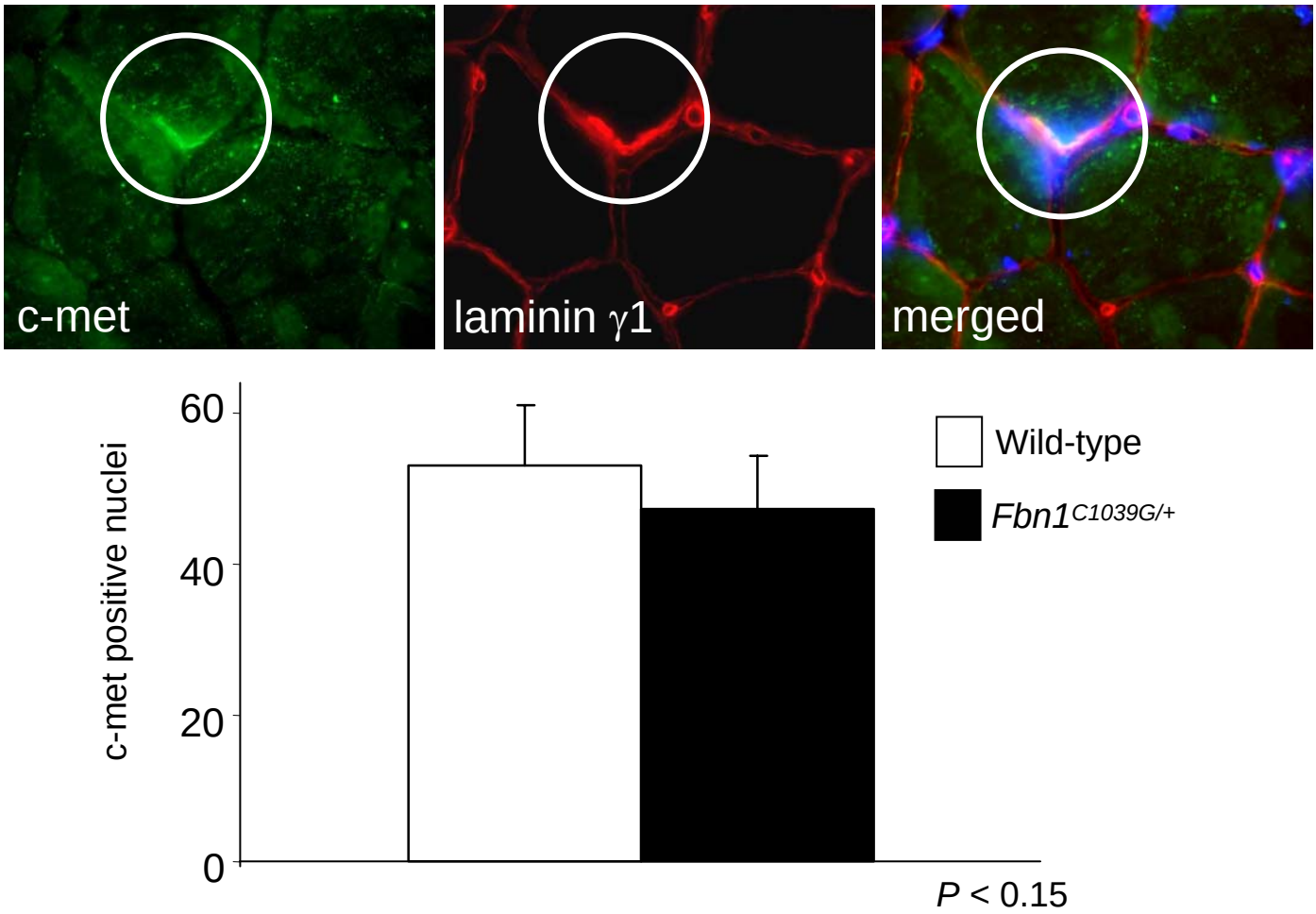
e



Supplementary Figure 1 Evidence for myopathy in fibrillin-1 deficient mouse and human skeletal muscles. (**a**) Total body weight is significantly reduced in *Fbn1*$^{C1039G/C1039G}$ mice. Mean muscle fiber number and mean fiber diameter are expressed ± SEM, demonstrating a decrease in both fiber number and size in *Fbn1*$^{C1039G/C1039G}$ mice. (**b**) Severe muscle hypoplasia and hypotrophy in *Fbn1*$^{C1039G/C1039G}$ mice. Hematoxylin and eosin staining reveals wide variation in skeletal muscle fiber size of *Fbn1*$^{C1039G/C1039G}$ mice at ten days with smaller fibers and increased interstitial tissue between fibers, when compared to age matched wild-type littermates (M. quadriceps). Scale bar, 40 mm. (**c**) Immunohistochemical staining for fibrillin-1 reveals decreased endomysial and perimysial expression in *Fbn1*$^{C1039G/+}$ mice, as compared to wild-type littermates. (**d**) Decreased endomysial expression of fibrillin-1 in a five year-old patient with Marfan syndrome (upper panels; MFS; M. lattissimus dorsi). Note the reduction and significant variation in fiber size and endomysial thickening as compared to a five year-old boy without any evidence of a skeletal muscle disorder (lower panels). Scale bar, 100 mm.
(**e**) Nuclear enrichment of pSmad2/3 in *Fbn1*$^{C1039G/+}$ mice as evidenced by merged staining with the nuclear marker DAPI.

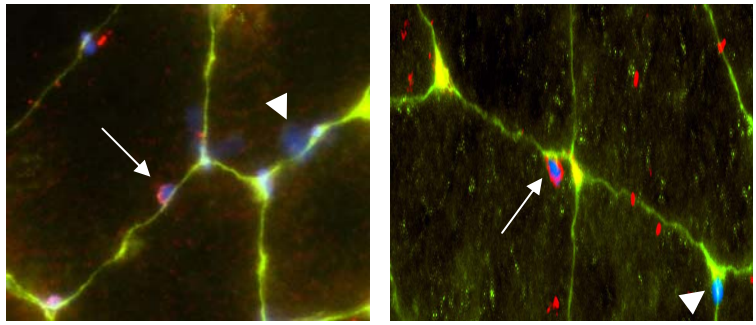
Supplementary Figure 2

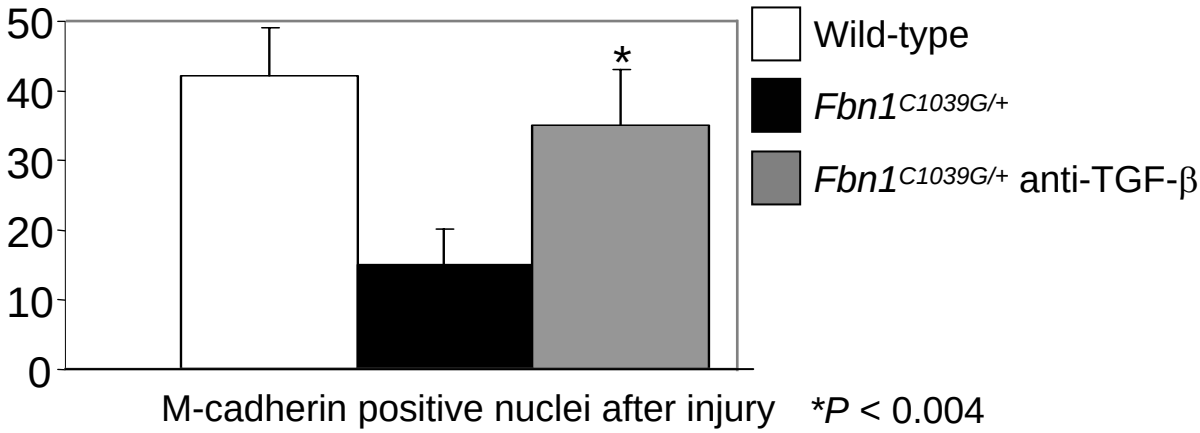
a



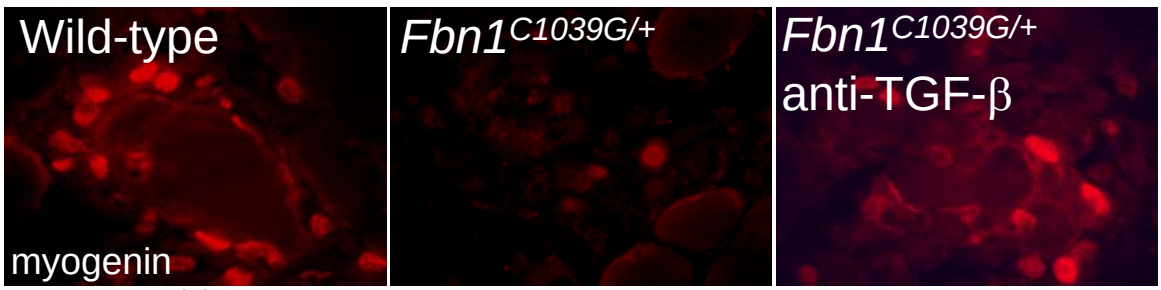
Dystrophin/M-cadherin/DAPI

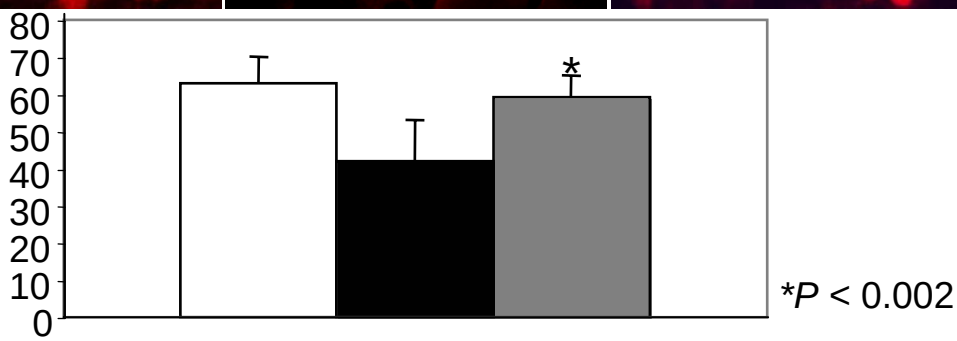
b





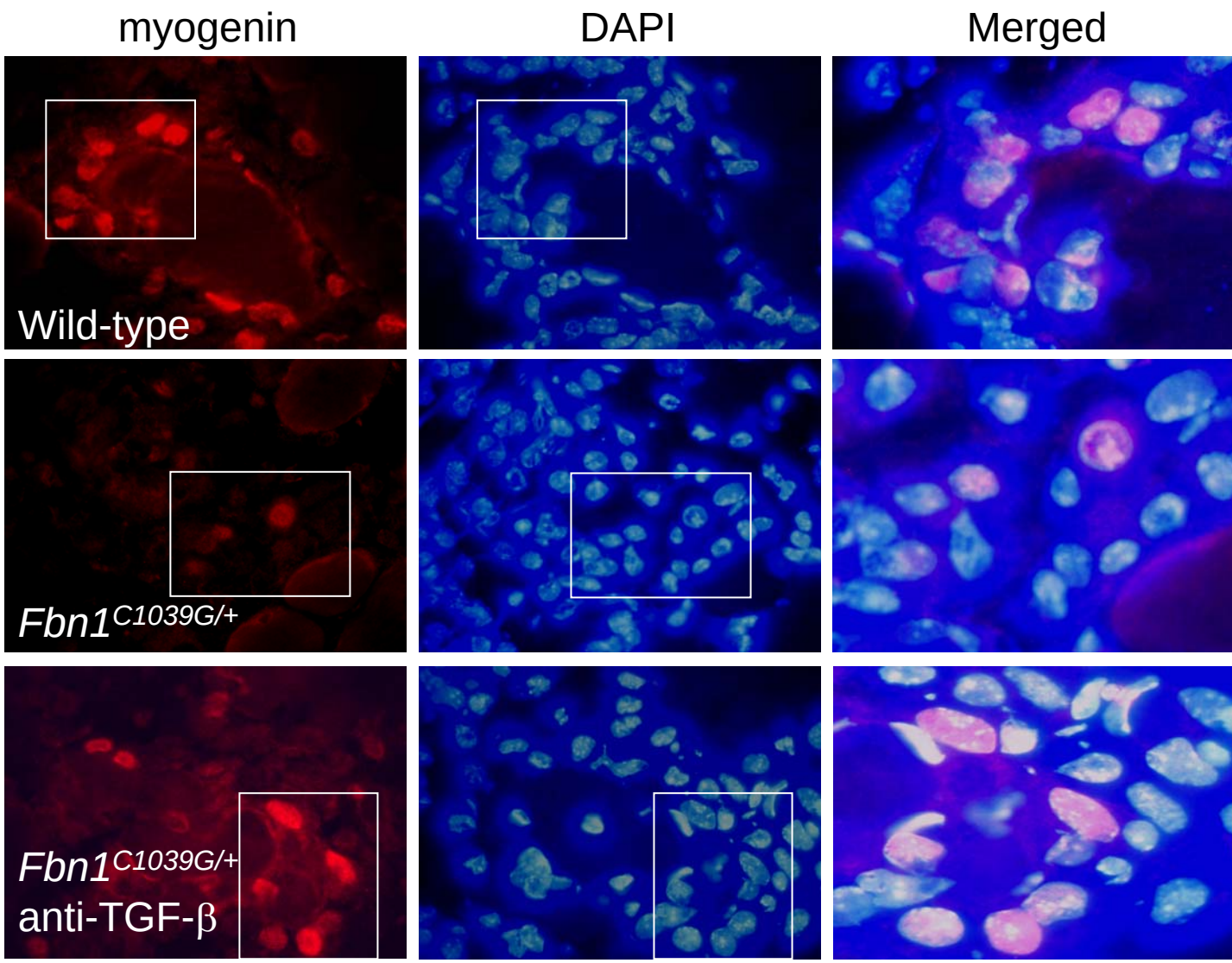
M-cadherin positive nuclei after injury *P < 0.004





*P < 0.002

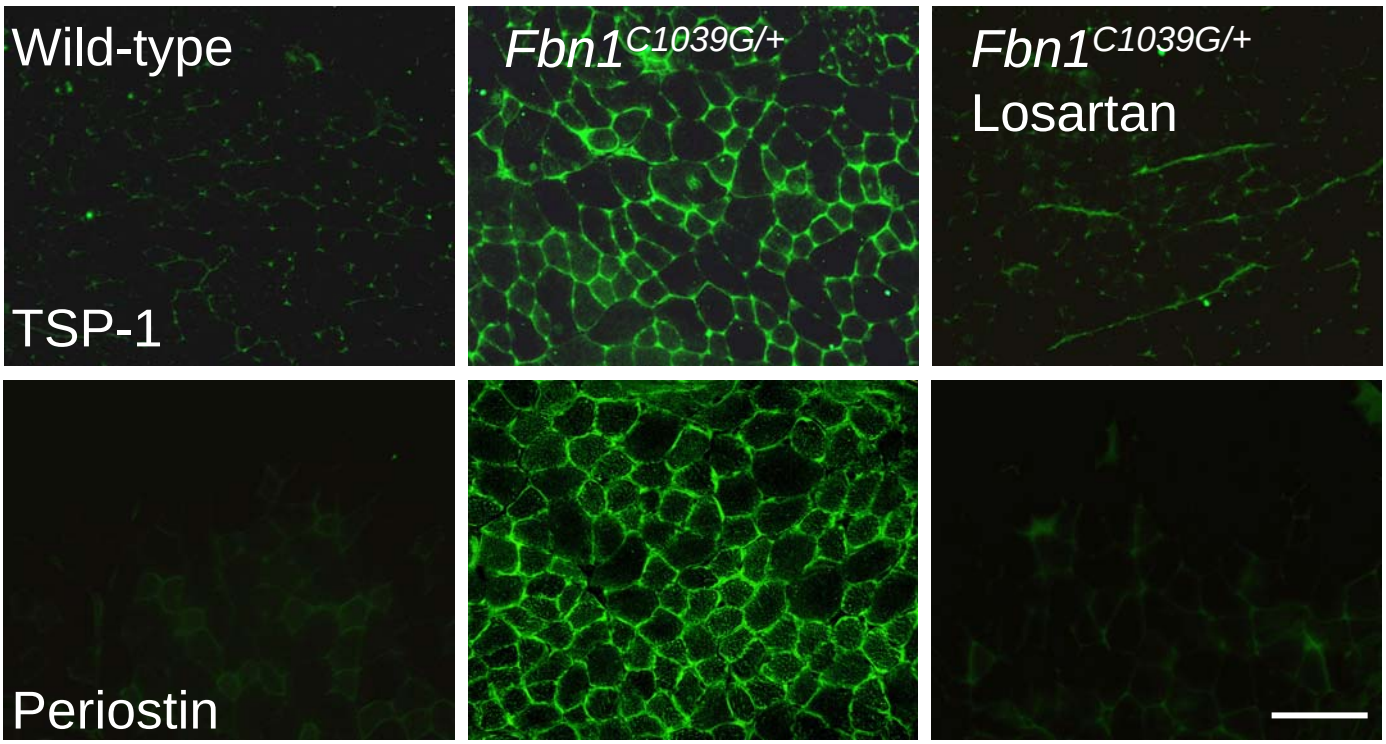
Myogenin positive nuclei after injury

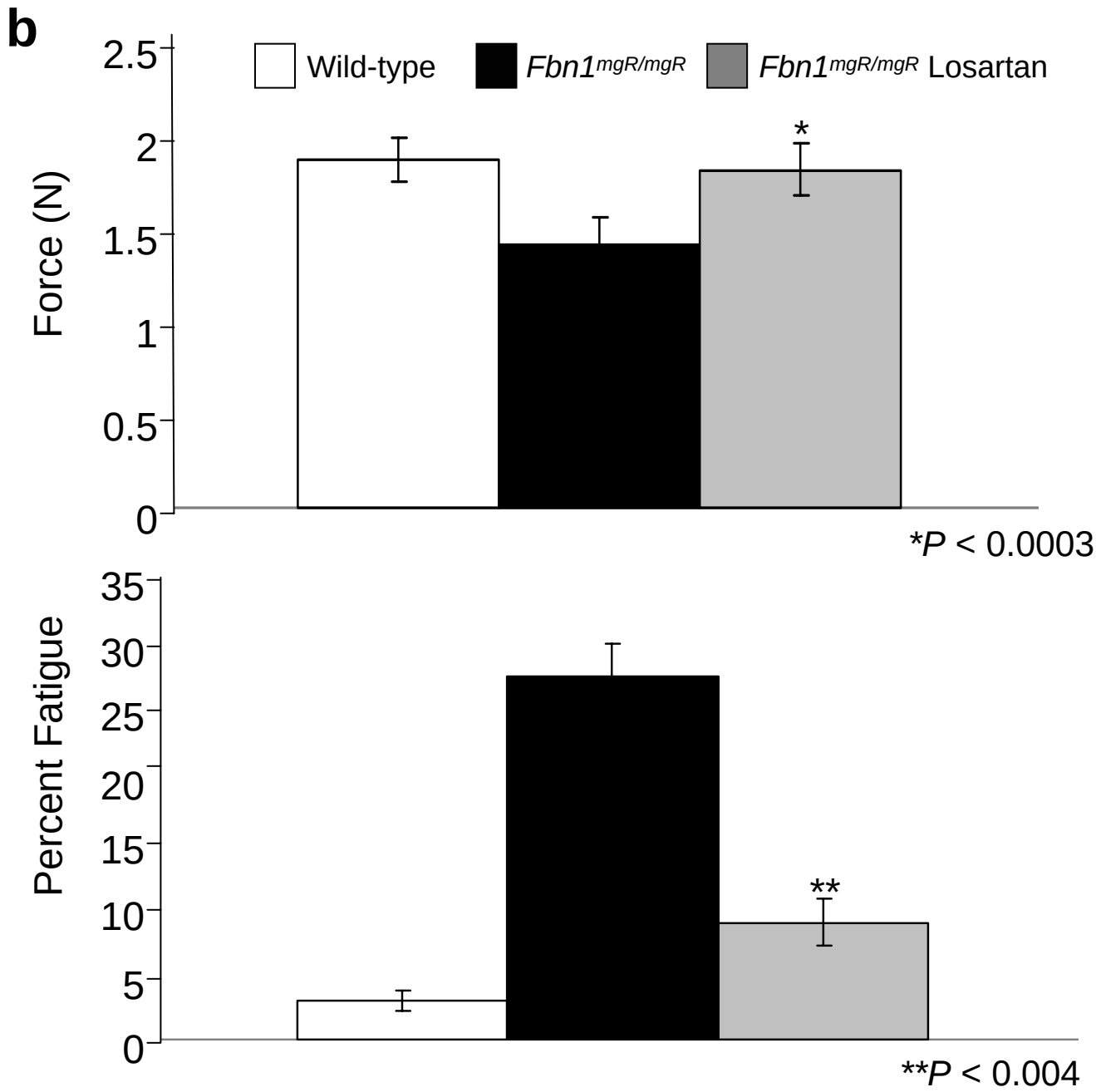


Supplementary Figure 2 Morphometric analyses of satellite cells in *Fbn1*$^{C1039G/+}$ mice. (**a**) There is no difference in the number of c-met positive muscle fibers, a marker for quiescent satellite cells, in wild-type and *Fbn1*$^{C1039G/+}$ mice. (**b**) Perturbed satellite cell function in *Fbn1*$^{C1039G/+}$ mice. At 48 and 72 hours after cardiotoxin injection, *Fbn1*$^{C1039G/+}$ mice exhibit significantly less M-cadherin- and myogenin-positive nuclei, as compared to wild-type and TGF-β neutralizing antibody treated *Fbn1*$^{C1039G/+}$ mice. Merged image shows a rim of M-cadherin (red, arrows) around DAPI-stained nuclei (blue), identifying satellite cells; dystrophin staining demarcates the sarcolemma (green), arrowheads show non-satellite cell nuclei. There is a significant decrease in M-cadherin (upper graph) and myogenin (lower graph) positive cells in *Fbn1*$^{C1039G/+}$ mice when compared to wild-type mice, which is rescued after administration of neutralizing antibody to TGF-β. (**c**) Myogenin positive cells co-localize with the nuclear marker DAPI.

Supplementary Figure 3

a

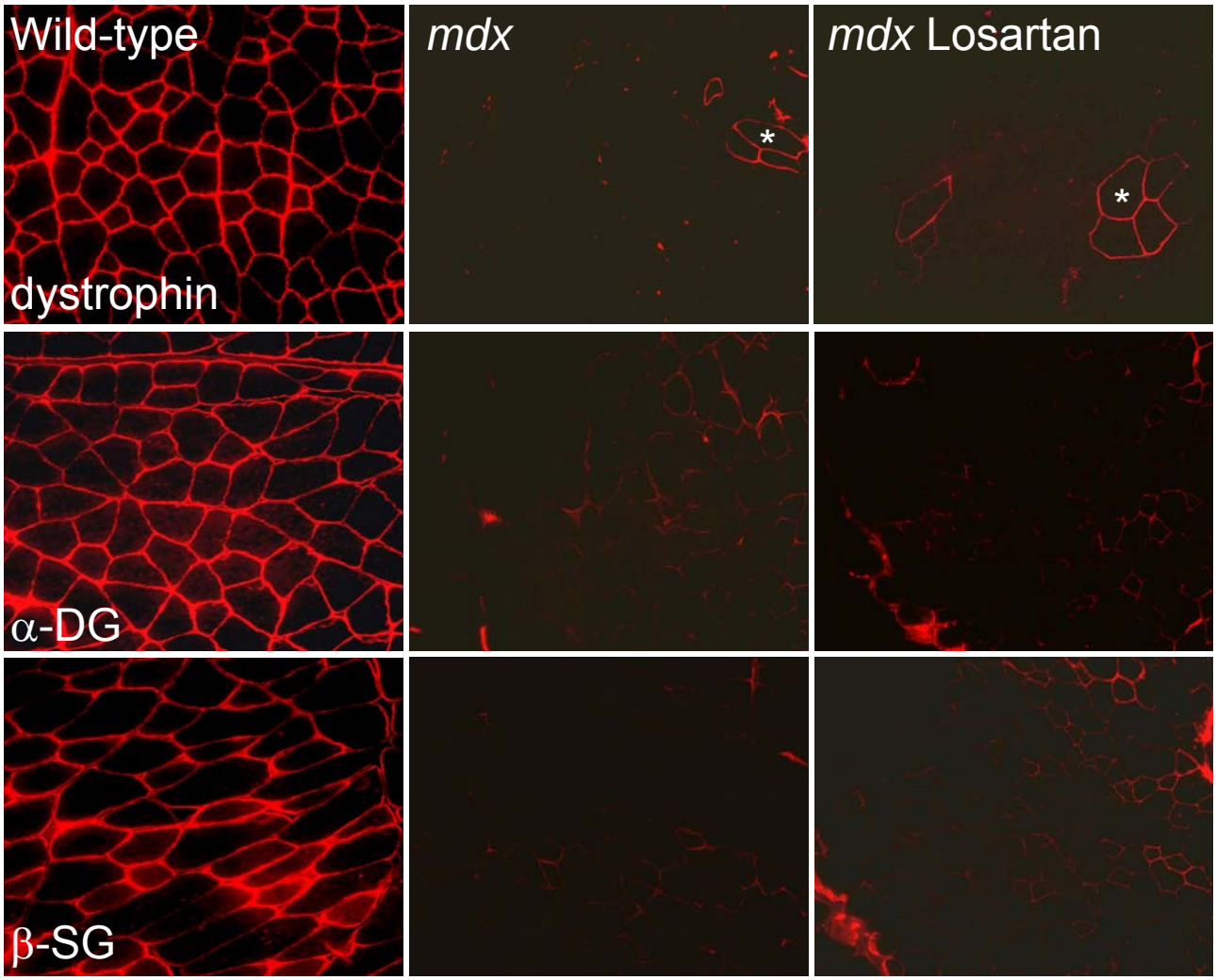


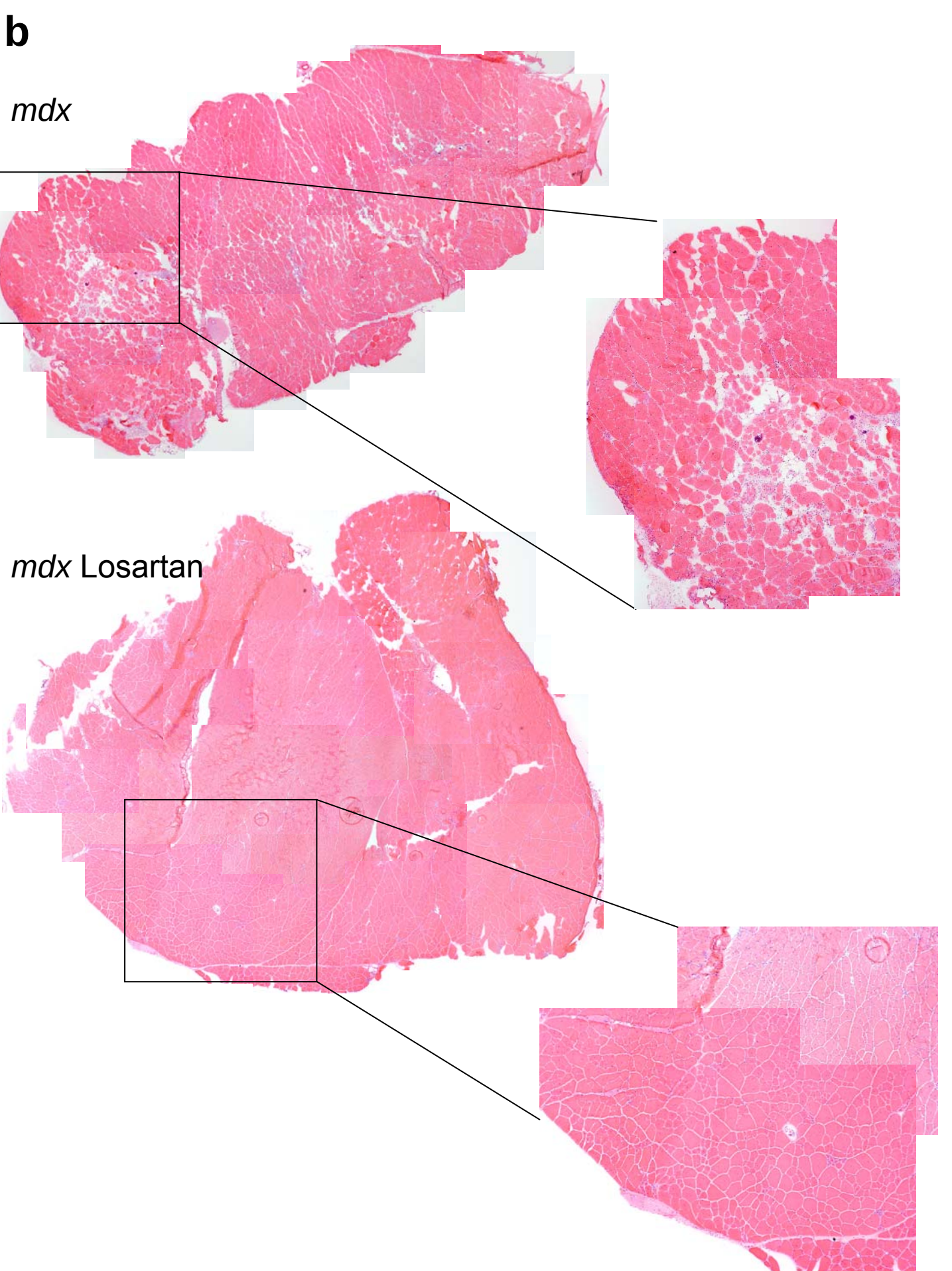


Supplementary Figure 3 Losartan treatment decreases thrombospondin-1 and periostin expression and improves *in vivo* muscle function of fibrillin-1 deficient mice.
(a) Decreased expression of thrombospondin-1 (TSP-1) and periostin in skeletal muscle of losartan-treated *Fbn1*$^{C1039G/+}$ mice . Scale bar 80 μm. (**b**) Functional benefit of losartan treatment on grip strength. Long-term losartan treatment rescues functional deficits in six month old mice deficient for fibrillin-1 (*Fbn1*$^{mgR/mgR}$). Data represent forelimb grip strength in Newton (N) and fatigue expressed as percentage. At six months of age, *Fbn1*$^{mgR/mgR}$ mice demonstrated a significant decrease in muscle strength when compared to age-matched wild-type mice (1.39 ± 0.16 N vs. 1.93 ± 0.12 N, respectively). In addition, *Fbn1*$^{mgR/mgR}$ mice demonstrate excessive muscle fatigue in response to repetitive challenge (28 ± 2.5% vs. 3 ± 0.8%, respectively). After five months of losartan treatment, *Fbn1*$^{mgR/mgR}$ mice showed improved forelimb grip strength (1.87 ± 0.15 N) and reduced fatigue (9 ± 1.8%) when compared to untreated *Fbn1*$^{mgR/mgR}$ littermates.

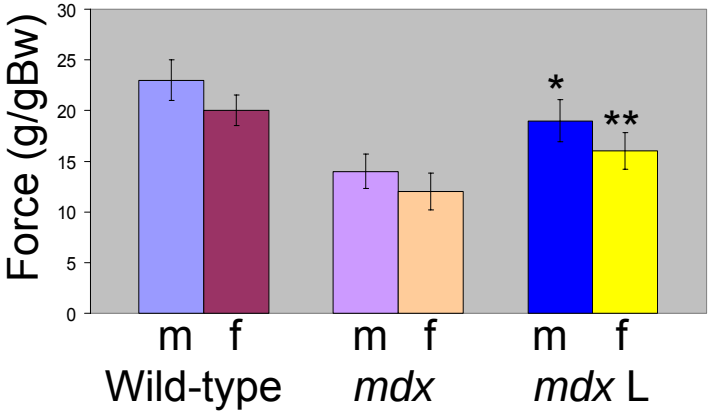
Supplementary Figure 4

a

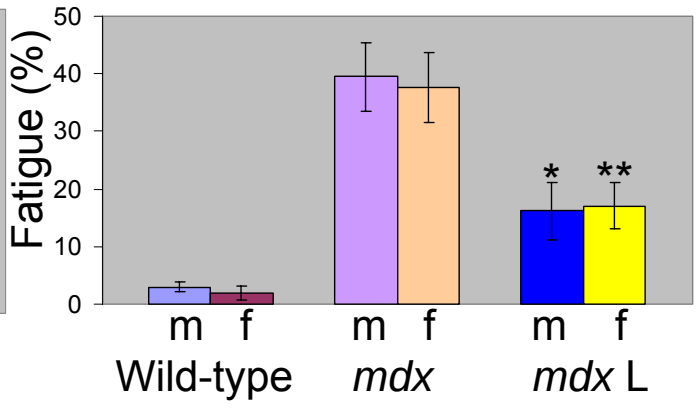



b
mdx
mdx Losartan

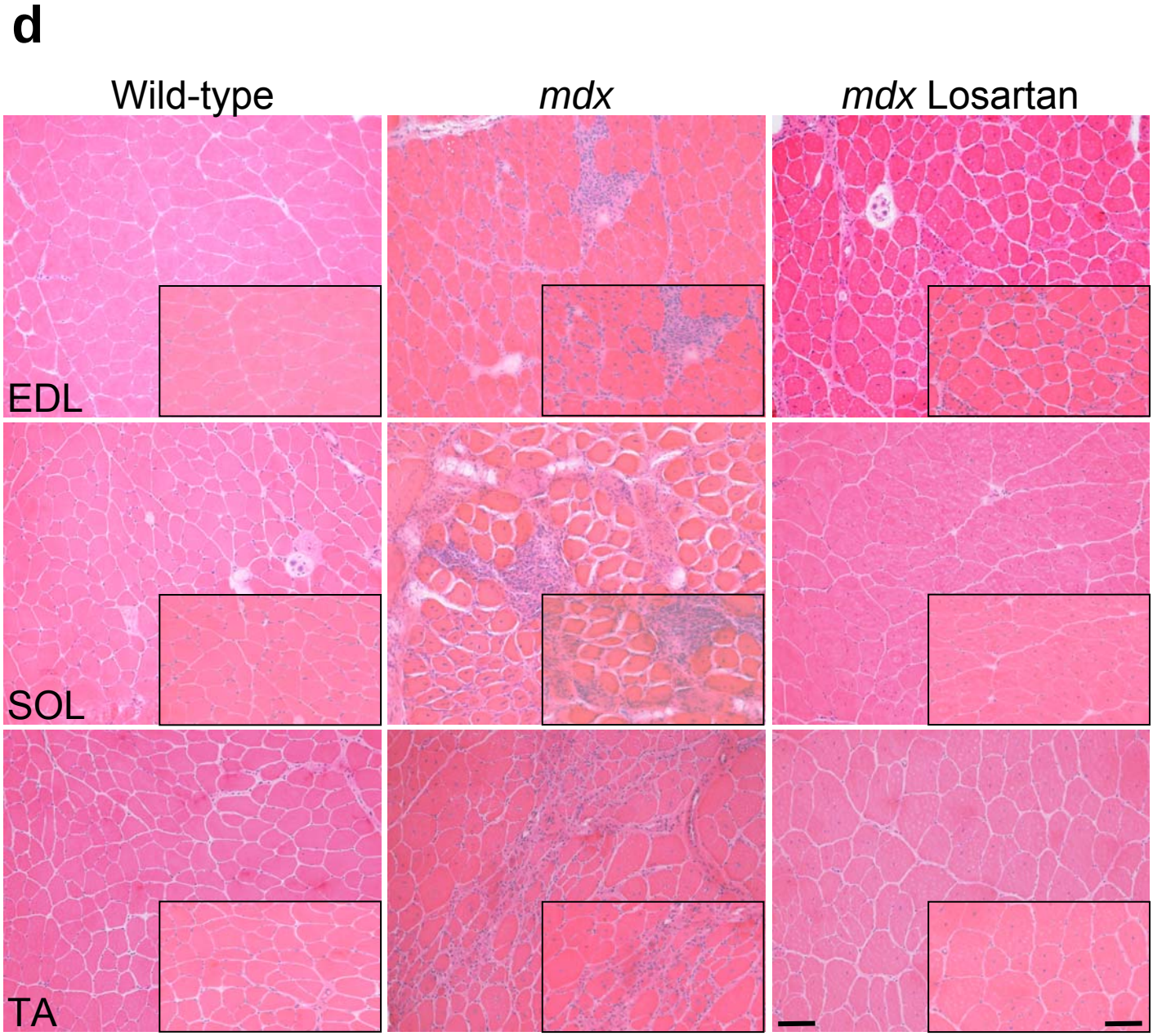
c



* $P < 0.005$ ** $P < 0.007$



* $P < 0.008$ ** $P < 0.005$


d
Wild-type
mdx
mdx Losartan
EDL
SOL
TA

Supplementary Figure 4 Losartan treatment does not alter expression of the dystrophin-glycoprotein complex but improves histologic and *in vivo* functional properties of skeletal muscles of *mdx* mice. (**a**) Dystrophin is absent in treated and untreated *mdx* mice (*denotes revertant fibers). In addition, sarcolemmal expression of α-dystroglycan and β-sarcoglycan are reduced in both groups of animal groups when compared to wild-type mice. (**b**) Representative montages of H&E images of whole gastrocnemius muscles from untreated *mdx* mice (upper panel) and losartan-treated mice (lower panel) demonstrating improved architecture and a significant decrease in fibrosis in losartan-treated *mdx* mice. (**c**) *In vivo* functional analysis of losartan-treated *mdx*mice. After six months, *mdx* mice treated with losartan (*mdx*L) demonstrate improved hind limb grip strength (measured as peak force in g/g bodyweight), m = male, f = female, n = 6 mice each group, left side, upper panel) when compared to untreated *mdx* mice (19 ± 2.1 g force/g bodyweight [male]/16 ± 1.8 g force/g bodyweight [female] versus 14 ± 1.5 g force/g bodyweight[male]/12 ± 2.2 g force/g bodyweight [female], respectively). In addition, losartan-treated mice showed significantly less muscle fatigue in response to repetitive challenge (16.2 ± 5%[male]/17.1 ± 4%[female] versus 39.5 ± 6%[male]/37.6 ± 6%[female]; right side, upper panel). (**d**) Corresponding hematoxylin-and-eosin stained representative sections of extensor digitorum longus (EDL), soleus (SOL) and tibialis anterior (TA) muscles of nine month-old wild-type, *mdx* and losartan-treated *mdx* mice demonstrating significantly improved architecture in skeletal muscle of losartan-treated *mdx* mice. Scale bars, 60 μm (low magnification) and 40 μm (high magnification).

Supplementary Table 1. Physiologic and morphometric analyses of explanted EDL muscles

	Wild-type	*mdx*	*mdx* Losartan
Body mass (g)	38.55 ± 0.04	34.9 ± 0.12	38.75 ± 0.42†
EDL weight (mg)	26.50 ± 0.21	19.83 ± 2.66	25.48 ± 1.57†
CSA (mm^2)	1.55 ± 0.02	1.19 ± 0.17	1.51 ± 0.11†
Absolute tetanic force (g)	39.34 ± 2.02	24.05 ± 3.14	36.84 ± 2.96†
Specific tetanic force (g/mm^2)	25.4 ± 1.12	20.11 ± 0.93	25.3 ± 0.92†
Twitch force (g)	5.41 ± 0.33	4.31 ± 0.71	5.81 ± 0.33†
Fibers per muscle	1,971.25 ± 51.32	1,358.25 ± 91.95	1,806.46 ± 73.06†
Minimal Feret's diameter*	197.01 ± 17.11	387.94 ± 24.46	235.44 ± 11.35†
Single fiber area, μm^2	1,597.34 ± 38.46	1,346.56 ± 482.97	1,439.77 ± 164.54

Values are presented as means ± s.e.m.; force values represent measurements at 150 Hz frequency. CSA, cross sectional area; *represents the variance coefficient for the minimal Feret's diameter. †Significantly different from untreated *mdx* mice at $P < 0.05$, using one-way ANOVA testing.

Supplementary Methods

In vivo muscle performance

After six months of losartan treatment, forelimb and hindlimb grip strength was assessed using an automated grip strength meter (Columbus Instruments, Columbus, OH). Grip strength testing in mice has previously been used as a method to monitor *in vivo* muscle function data in models of muscular dystrophy, Down syndrome and other motor neuron diseases[1,2,3,4]. The experiments for forelimb and hindlimb grip strength testing were conducted at different days allowing for a minimum recovery period of ten days between tests. Total peak force (in g/g bodyweight) was determined by an electronic strain gauge as previously described[5]. Five measurements within two minutes were taken from each animal. The three highest measurements for each animal were averaged to give the strength score. The degree of fatigue was calculated by comparing the first two pulls to the last two pulls with the decrement between pulls 1 + 2 and pulls 4 + 5 providing a measure of fatigue. Similarly, functional measurements were performed in 6 month-old fibrillin-1 deficient mice (*Fbn1*$^{mgR/mgR}$)[6] after five months of treatment.

In vitro muscle performance

Muscle performance of the EDL was assessed *in-vitro* using methods described previously[7,8]. In brief, single EDL muscles were surgically excised with ligatures at each tendon (5-0 silk suture) and mounted in an *in vitro* bath between a fixed post and force transducer (Aurora 300B-LR) operated in isometric mode. The muscle was maintained in physiological saline solution (PSS; pH 7.6) containing (in mM) 119 NaCl, 5 KCl, 1 $MgSO_4$, 5 $NaHCO_3$, 1.25 $CaCl_2$, 1 KH_2PO_4, 10 HEPES, 10 dextrose, and maintained at

30°C under aeration with 95% O_2 5% CO_2 throughout the experiment. Resting tension, muscle length and stimulation current were iteratively adjusted for each muscle to obtain optimal twitch force. Single twitches were elicited every 30 seconds during a five min. equilibration with electrical pulses (0.5 msec) via platinum electrodes running parallel to the muscle. Optimal resting tension was determined and isometric tension was evaluated by 250 msec. trains of pulses delivered at 1, 10, 20, 40, 60, 80, 100 and 150 Hz. After the experimental protocol, the muscle rested for five min. at which time muscle length was determined with a digital micrometer, muscle was trimmed proximal to the suture connections, blotted and weighed. The cross-sectional area for each muscle was determined by dividing the mass of the muscle (g) by the product of its length (L_o, mm) and the density of muscle (1.06 g/cm^3)[9] and was expressed as square millimeters. Muscle output was then expressed as isometric tension (g/mm^2) determined by dividing the tension (g) by the muscle cross-sectional area. Statistical comparisons between groups were performed using ANOVA procedures (SigmaStat v3.1) for tension (force frequency).

References

1. Connolly A.M., Keeling R.M., Mehta S., Pestronk A. & Sanes, J. R. Three mouse models of muscular dystrophy: the natural history of strength and fatigue in dystrophin-, dystrophin/utrophin-, and laminin alpha2-deficient mice. Neuromuscul. Disord. **11**, 703–712 (2001).
2. Hantai D. et al. Beneficial effects of insulin-like growth factor-I on wobbler mouse motoneuron disease. J. Neurol. Sci. **129**, 122–126 (1995).
3. Costa A.C., Walsh K. & Davisson, M.T. Motor dysfunction in a mouse model for Down syndrome. Physiol. Behav. **68**, 211–220. (1999).
4. Payne ET et al. Nutritional therapy improves function and complements corticosteroid intervention in mdx mice. Muscle Nerve. **33**, 66–77 (2006).
5. Wagner, K. R., McPherron, A. C., Winik, N. & Lee, S. J. Loss of myostatin attenuates severity of muscular dystrophy in mdx mice. Ann. Neurol. **52**, 832–836 (2002).
6. Pereira, L. et al. Targetting of the gene encoding fibrillin-1 recapitulates the vascular aspect of Marfan syndrome. Nat. Genet. **17**, 218–222 (1997).
7. Williams J.H., Ward C.W., Spangenburg, E. E. & Nelson, R. M. Functional aspects of skeletal muscle contractile apparatus and sarcoplasmic reticulum after fatigue. J. Appl. Physiol. **85**, 619–626. (1998).
8. Williams J.H., Ward C.W. & Klug G.A. Fatigue-induced alterations in Ca2+ and caffeine sensitivities of skinned muscle fibers. J. Appl. Physiol. **75**, 586-593 (1993).
9. Mendez J. & Keys A. Density and composition of mammalian muscle. Metabolism **9**, 184-188 (1960).