Supplementary information

Figure S1. Characterization of muscular dystrophy in wild-type, *mdx*, microdystrophin$^{\Delta R4\text{-}R23}$/*mdx* and minidysGFP/*mdx* mice. A) Muscles were frozen in OCT in 2-methylbutane cooled in liquid nitrogen. For gross muscle structure, 10 µm thick frozen sections were stained with hematoxylin and eosin. Note the presence of skeletal muscle fiber necrosis, fibrosis and the infiltration of mononuclear cells in *mdx* muscles. Microdystrophin$^{\Delta R4\text{-}R23}$, minidysGFP transgenes were able to prevent these signs of dystrophy. B) The proportion of centrally nucleated myofibers was quantified in the diaphragm, gastrocnemius and quadriceps muscles from n = 4 microdystrophin$^{\Delta R4\text{-}R23}$/*mdx* transgenic mice. More than 500 fibers were counted per muscle and the proportion of centrally nucleated fibers was compared using a non-parametric Students t-test. C) Frozen sections were immunostained with developmental myosin (1:20; Novocastra) to examine regenerating myofibers. Note that developmental myosin was nearly undetectable in ΔR4-R23/*mdx* transgenic mice. $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to wild-type; $^{\#\#\#}P < 0.001$ compared to *mdx*. Scale bar = 100 µm.

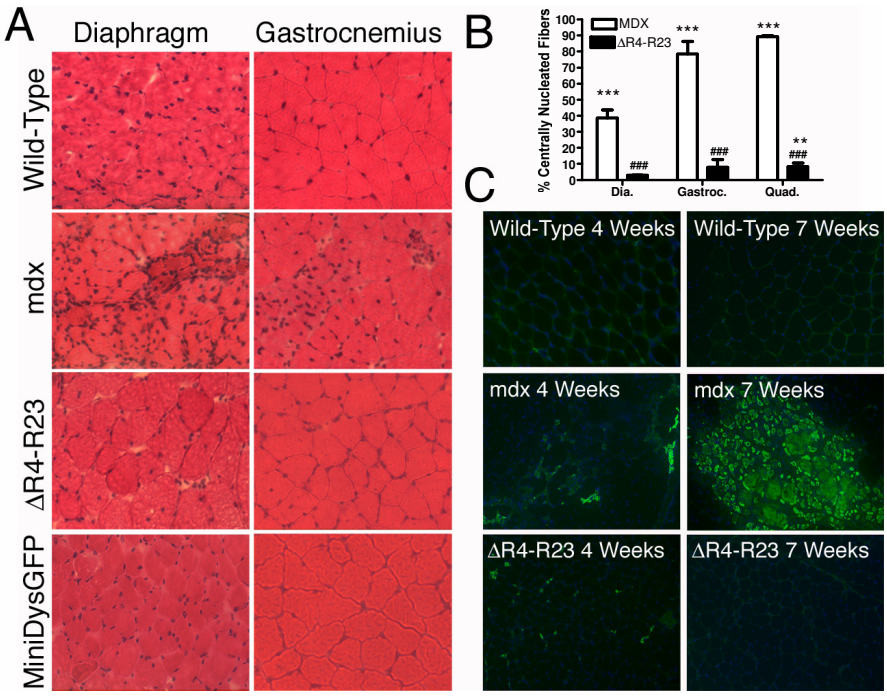
Figure S2. Longitudinal extent of ringed fibers. A) Shown is a montage of a single muscle fiber from microdystrophin$^{\Delta R4\text{-}R23}$/*mdx* mice. The inset shows the ringed myofibrils begin near the site of injury at the MTJ. B) The rings can also begin at the MTJ or C) the myomuscular junction.

Figure S3. Expression of microdystrophin$^{\Delta R4\text{-}R23}$ transgene in *mdx* mice did not cause fiber splitting or denervation in the gastrocnemius muscles. Gastrocnemius muscles

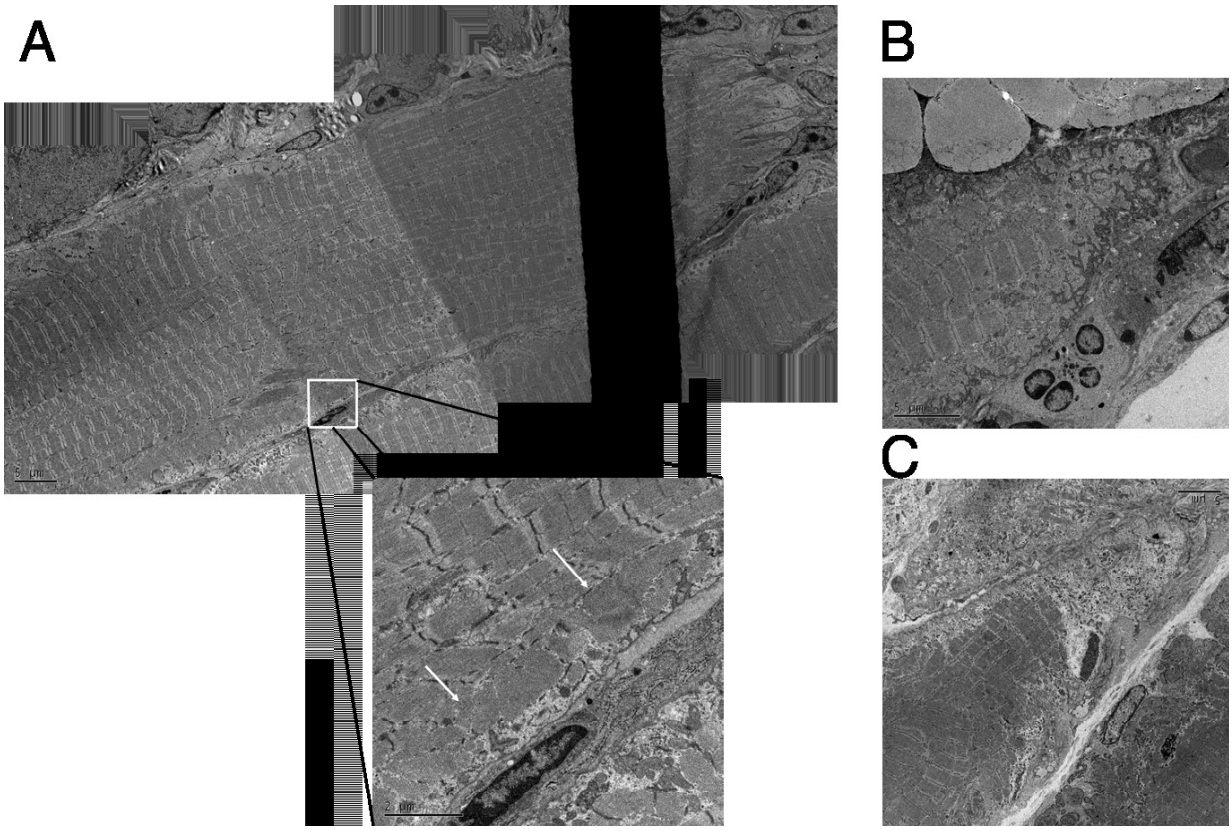
from *mdx* and ΔR4-R23/*mdx* mice were fixed for 2 hours in 2% paraformaldehyde. Individual muscle fibers were mechanically teased under a dissecting microscope and imaged using a Nikon eclipse E1000 fluorescent microscope (Nikon; NY). 80 teased gastrocnemius muscles fibers were analyzed from a single *mdx* mouse and over 350 muscle fibers from 5 microdystrophin$^{\Delta R4\text{-}R23}$/*mdx* mice. Shown are phase contrast images of teased wholemount muscle fibers from *mdx* (A) and microdystrophin$^{\Delta R4\text{-}R23}$/*mdx* mice (B). Example of a split fiber in *mdx* muscles identified using this method (A). No split fibers were found in microdystrophin$^{\Delta R4\text{-}R23}$/*mdx* transgenic mice. Bar = 50 μm C) All 38 axon terminals from 4 microdystrophin$^{\Delta R4\text{-}R23}$/*mdx* mice immunostained with synaptophysin (green; 1:100 dilution; Sigma) colocalized with acetylcholine receptors stained with α-bungarotoxin in the postsynaptic apparatus (red; 1:800 dilution; Molecular Probes) in transverse sections of gastrocnemius muscles. Bar = 20 μm.

Figure S4. A) Gastrocnemius muscles after intravenous administration of evans blue dye (EBD), before and after 33% stretch. EBD enters muscle fibers that have holes in the sarcolemma. B) Cross section of *mdx* muscle showing EBD without experimental stretch induced injury. Nuclei are shown in blue. C) Cross section of *mdx* muscle stretched 33% beyond its optimal length. Note that many *mdx* muscle fibers have EBD (in red) showing contraction-induced injury tears the sarcolemma. E) Representative section of minidysGFP/*mdx* muscle that has not been stretched. Note the lack of EBD. F) Cross

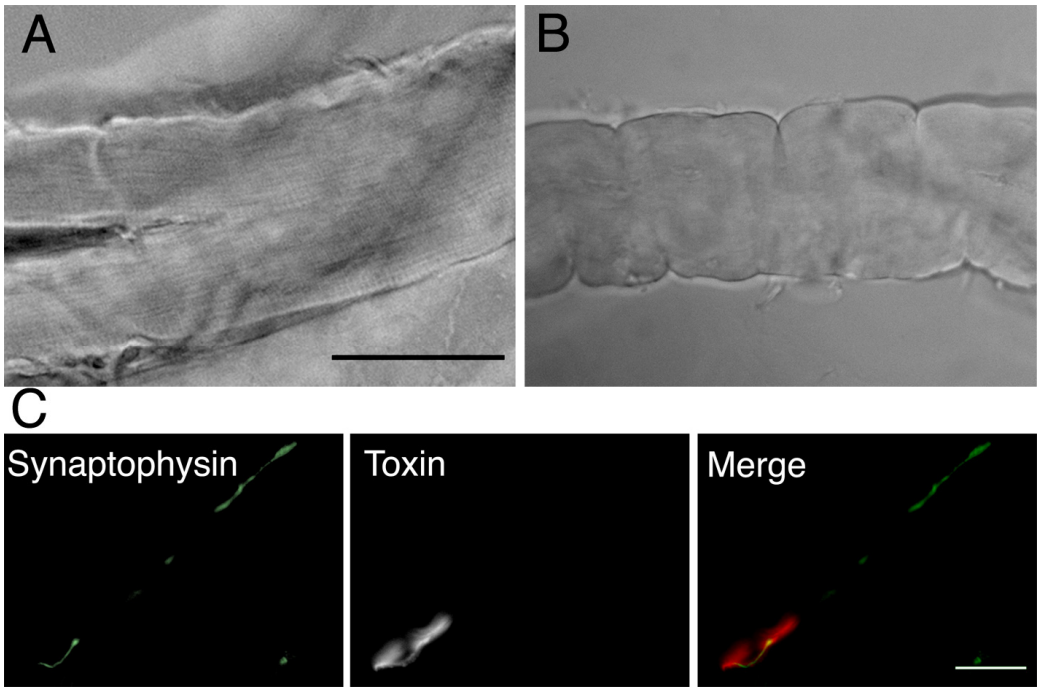
section of minidysGFP/*mdx* muscle stretched 33% beyond its optimal length. Note the presence of EBD showing the sarcolemma was injured during strain. Scale Bar = 50 μm.



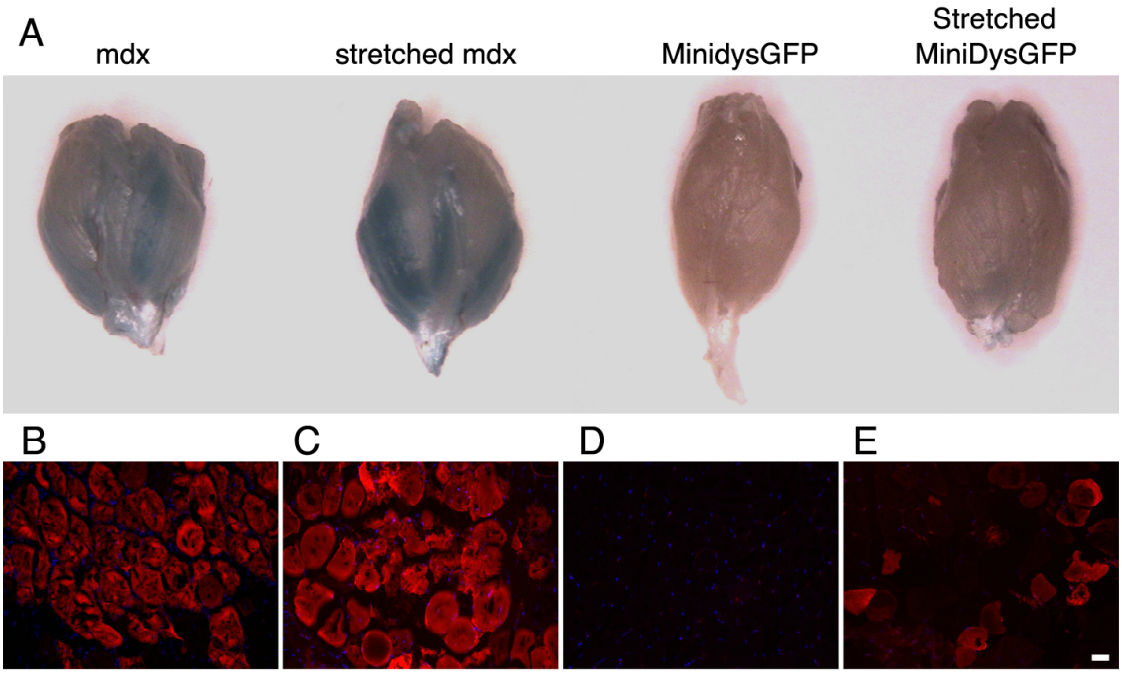
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4.

Table 1. Number of EBD positive fibers in the gastrocnemius muscles before and after 33% strain.

	Unstrained Gastroc.	Strained Gastroc.
Wild-type	0	84 +/- 30**
mdx	417 +/- 25	683 +/- 124*
Microdystrophin$^{\Delta R4\text{-}R23}$	1 +/- 1	3 +/- 2
MinidysGFP	1 +/- 1	99 +/- 33**

Significant increase compared to unstrained muscles *$P < 0.05$; ** $P < 0.01$. Students t-test.