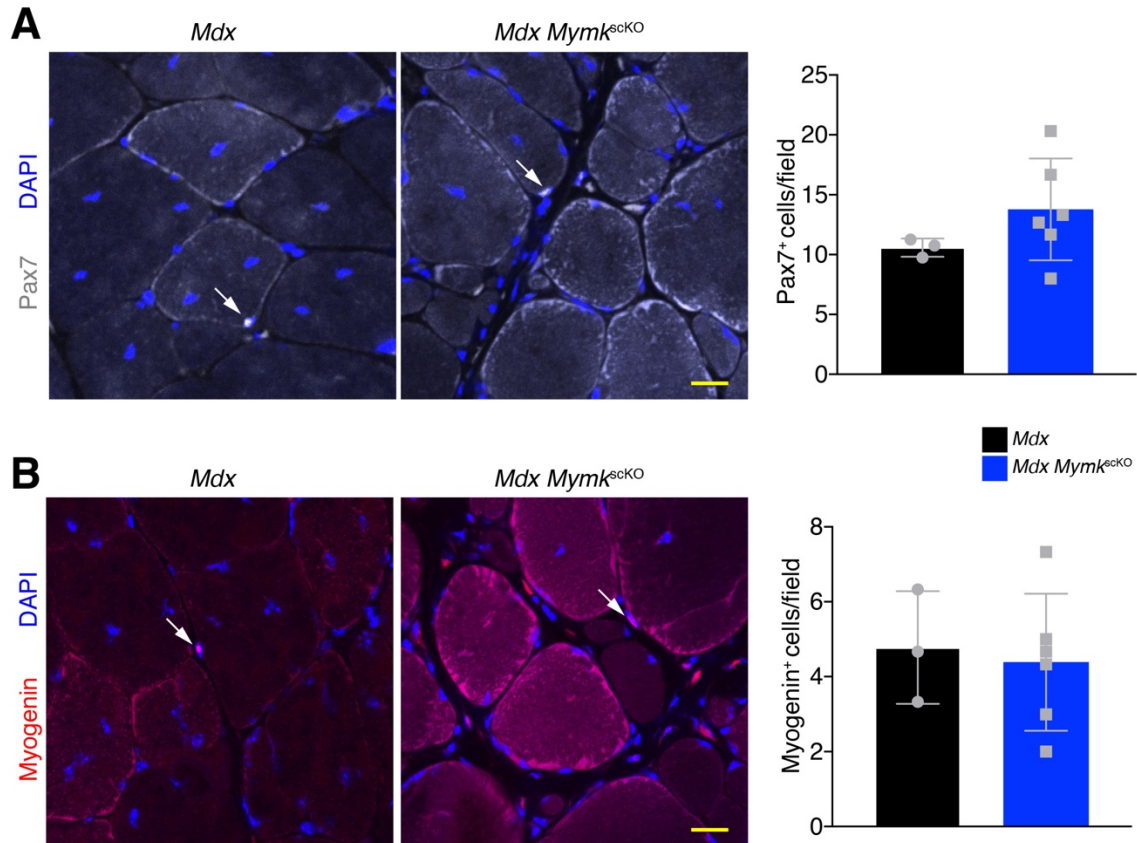
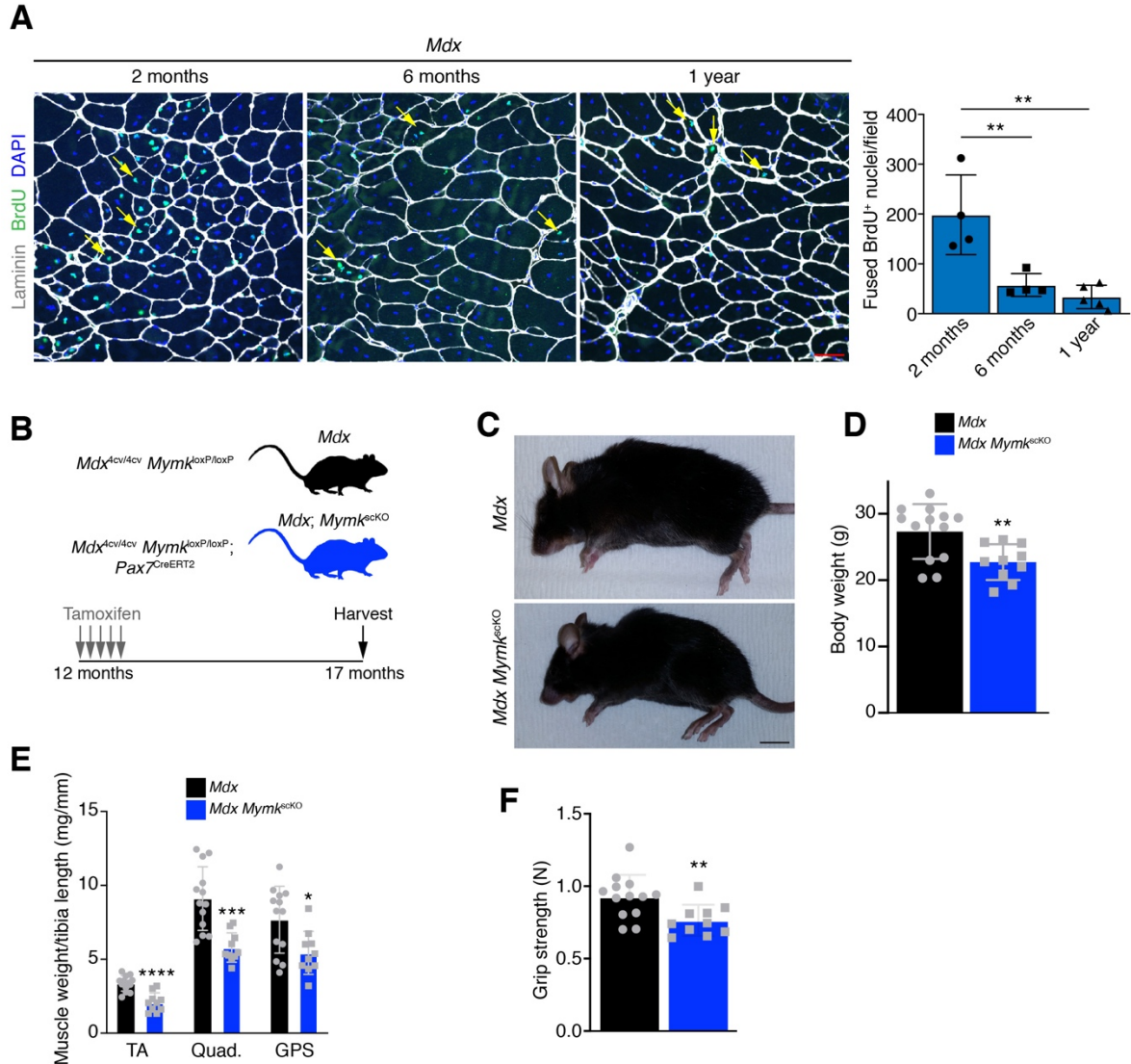


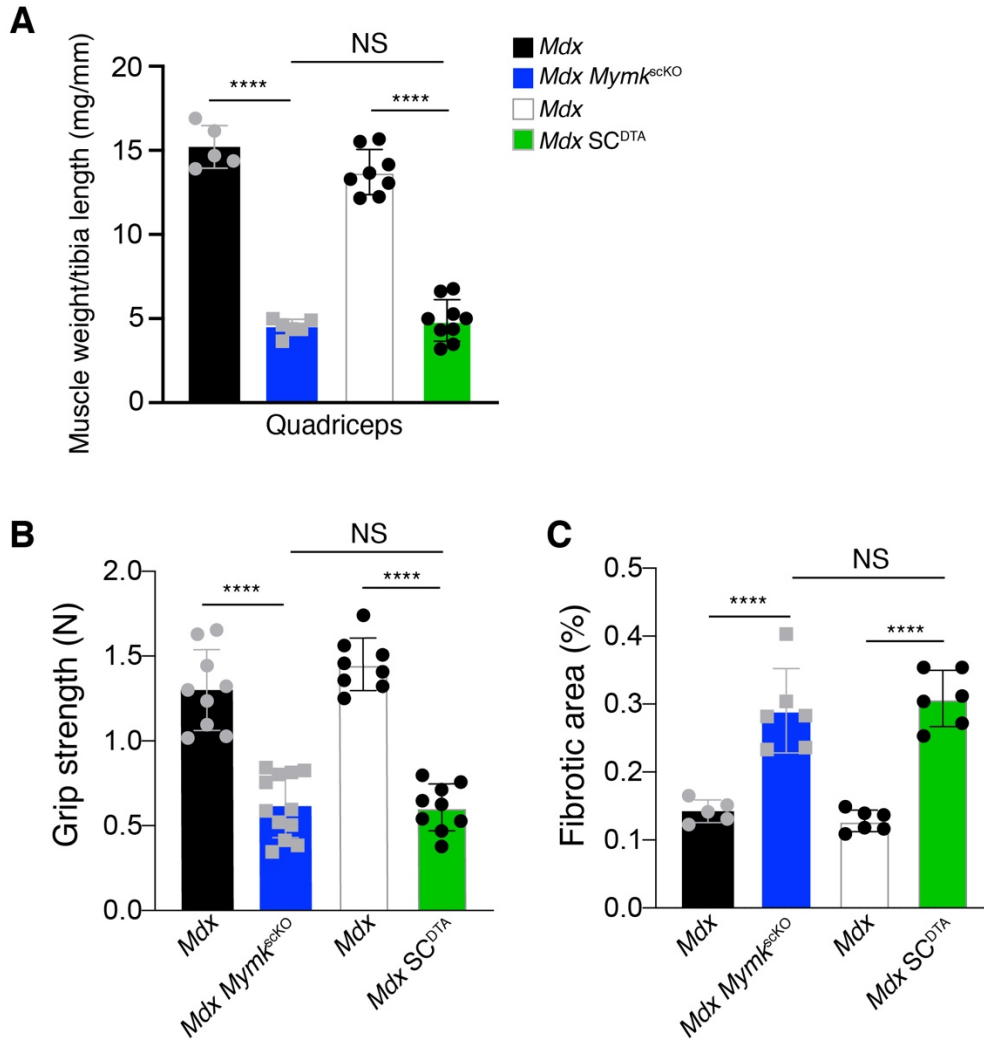
Supplemental Figure 1. Myomaker expression in myofibers of dystrophic muscle. X-gal stains of cross-sections of tibialis anterior (TA) and diaphragm muscle from 6-8 month old *mdx Myomaker^{LacZ/+}* mice showing expression of LacZ in dystrophic myofibers, both in concentrated clusters of myofibers as well as scattered in a punctate pattern (inset). Scale bars: 50 μ m.



Supplemental Figure 2. The satellite cell population is maintained in *mdx Mymk^{scKO}* mice. (A) Pax7 immunofluorescence shows persistence of SCs following deletion of Myomaker. Quantification of Pax7⁺/DAPI⁺ cells shows comparable tissue concentration to controls. (B) Myogenin immunofluorescence reveals similar levels of activated myoblasts in *mdx Mymk^{scKO}* mice and controls. Data are represented as mean ± SD. Scale bars: 20 μm. *n* = 3-6.

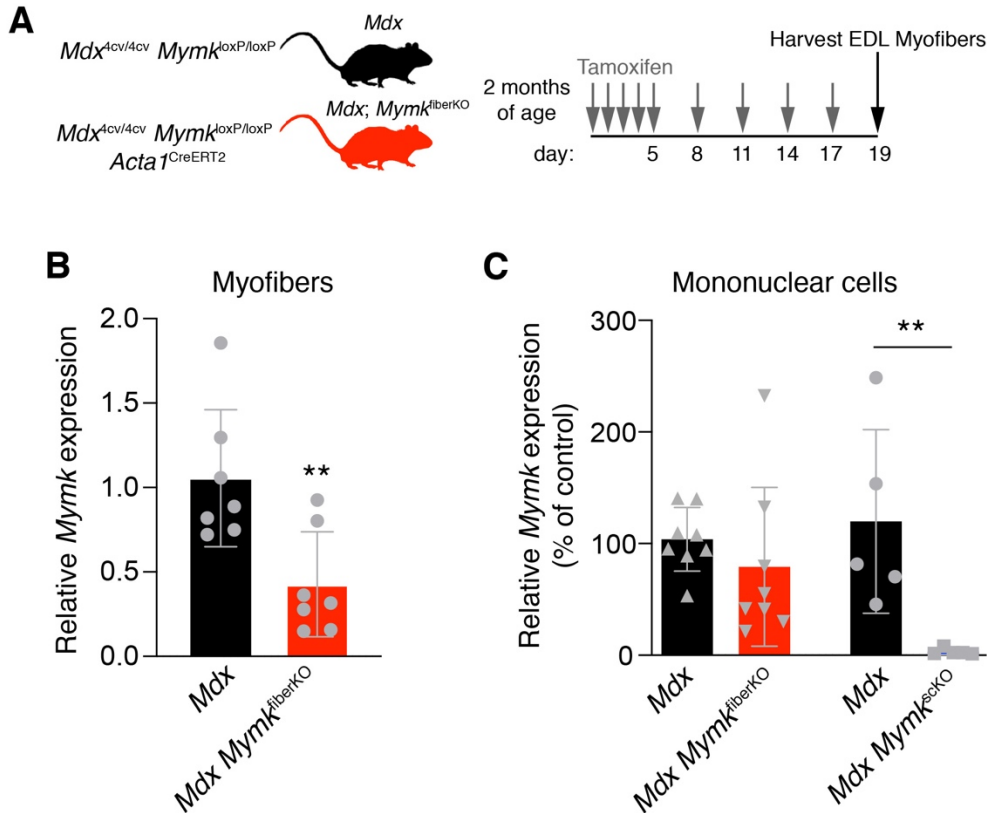


Supplemental Figure 3. Satellite cell fusion is required for regeneration in older *mdx* mice. (A) *Mdx* mice at various ages (2 months, 6 months, and 1 year) were injected with BrdU daily for 14 days prior to sacrifice in order to assess fusion rates. Quantification indicates a steep drop in fusion levels between 2 and 6 months ($n = 4$). (B) Schematic for ablation of Myomaker in SCs of 12 month old *mdx* mice. (C) Gross appearance of *mdx Mymk^{scKO}* mice showed smaller size, frail body habitus, and kyphosis of the spine. (D) Body weights of *mdx Mymk^{scKO}* mice were significantly reduced by 5 months following initial Myomaker deletion. (E) Individual dry muscle weights of tibialis anterior (TA), quadriceps, and gastrocnemius/plantaris/soleus (GPS) show loss of muscle mass. (F) Grip strength was measured on a forelimb meter and showed impaired function in dystrophic mice with Myomaker-deficient SCs. Statistical analyses and data presentation: (A) one-way ANOVA with Tukey post-hoc comparison, $**p < 0.01$. (D-F) unpaired t-test; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$. Data are represented as mean \pm SD. Scale bars: (A) 50 μ m; (C) 1 cm. $n = 10-13$ except where noted.

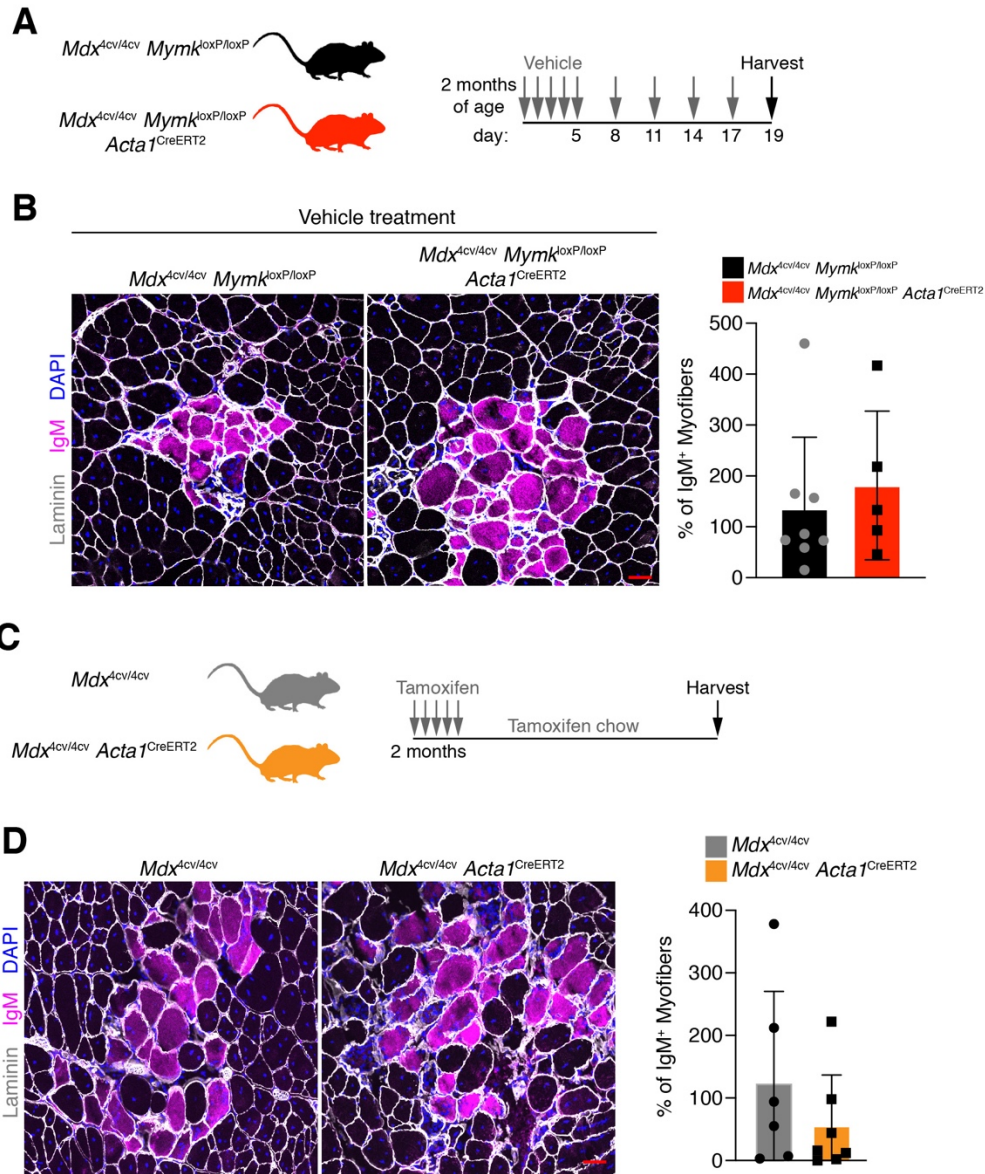


Supplemental Figure 4. Equivalence of *mdx Myrk^{scKO}* and *mdx SC^{DTA}* phenotypes.

(A) Juxtaposition of dry quadriceps weights from *mdx Myrk^{scKO}* and *mdx SC^{DTA}* mice and respective controls ($n = 5-11$). (B) Comparison of forelimb grip strength measurements from both models ($n = 8-11$). (C) Comparison of quantification of fibrotic area from Picosirius Red staining of tibialis anterior (TA) cross-sections ($n = 5-6$). Statistical analyses and data presentation: (A), (B), (C) one-way ANOVA with Tukey post-hoc comparison, **** $p < 0.0001$. Data are represented as mean \pm SD.



Supplemental Figure 5. *Mymk* transcription is downregulated in isolated muscle fibers of *mdx Mymk*^{fiberKO} mice. (A) Schematic showing tamoxifen administration of mice prior to sacrifice and myofiber isolation from the extensor digitorum longus muscle. (B) qPCR for *Mymk* mRNA from isolated muscle fibers ($n = 7$). (C) qPCR for *Mymk* in isolated mononuclear cells shows persistent expression in *mdx Mymk*^{fiberKO} mice but efficient deletion in *mdx Mymk*^{scKO} mice. Expression is presented relative to control for each model ($n = 5-8$). Statistical analyses and data presentation: (B) unpaired t-test, ** $p < 0.01$. (C) Mann-Whitney test, ** $p < 0.01$. Data are represented as mean \pm SD.



Supplemental Figure 6. No reduction in muscle damage is observed in vehicle-treated *mdx Mymk*^{fiberKO} mice or due to activation of *Acta1*^{CreER} alone. (A) Schematic of treatment with vehicle-only injections. (B) No reduction in IgM⁺ myofibers was seen in cross-sections of vehicle-treated *mdx Mymk*^{fiberKO} quadriceps (rectus femoris) muscle. (C) Schematic of experiment to test the effect of tamoxifen-induced *Acta1*^{CreERT2} activation in *mdx* mice in the absence of floxed Myomaker alleles. (D) *Acta1*^{CreERT2} activation alone does not reduce IgM⁺ myofibers in dystrophic rectus femoris muscle with quantification. Data are represented as mean ± SD. Scale bars: (B), (D) 50 μm.