Supplementary Files



Figure S1. EDL decellularization and collagenase experiments. (A-B) Muscle length and PCSA were not different between wildtype and D2.*mdx* EDL muscles. (C) Muscle mass was significantly reduced following decellularization in EDL muscles. (D) Muscle mass was significantly decreased in collagenase digested EDL muscles. (E) Elastic stiffness was not significantly altered in decellularized EDL muscles. (F) Collagenase digested EDL muscles had

significantly lower elastic stiffness compared to raw muscles. (G) Elastic index was not significantly altered in decellularized EDL muscles. (H) Collagenase digestion significantly reduced the elasticity of EDL muscles. (I) There were no significant differences between the percent of raw elastic stress maintained in the decellularized EDL muscles across genotype or strain. (J) There were no significant differences between the percent of raw elastic stress maintained in the collagenase treated EDL muscles across genotype or strain. (K) There were no significant differences in total collagen content by genotype or treatment in EDL muscles. (L) There was a significant effect of treatment on the percentage of insoluble collagen in EDL muscles. (M) There were no significant effects of genotype or treatment on Sirius Red area fraction in EDL muscles. (N) There was significantly less loosely packed collagen in the D2.mdx EDL compared to wildtype. Shaded areas represent ±SEM. For all panels flat bars represent twoway ANOVA significant effects by treatment: p < 0.05, p < 0.01, p < 0.001, p < 0.001; and by genotype: **p < 0.01. Bracketed bars represent post-hoc Sidak test significant effects by treatment: p < 0.05, p < 0.01, p < 0.001, p < 0.001.



Figure S2. Fluorescent images of *D2.mdx* **raw, decellularized, and collagenase treated muscles.** Representative fluorescent images of raw, collagenase (coll-ase), and decellularized (decell) D2.*mdx* muscles. Scale bars are equal to 100µm.



Figure S3. Validation of collagenase effectiveness against a sham treatment in wildtype muscles. (A) Muscle mass was significantly reduced in sham and collagenase treated diaphragms compared to raw controls. Collagenase digested soleus muscles had significantly reduced mass compared to raw controls. (B) Collagenase digested diaphragm and solei muscles were significantly less stiff than raw controls, but sham treated muscles did not show a significant change in elastic stiffness. (C) Total collagen was not significantly altered by sham or collagenase treatment in diaphragm and soleus muscles. (D) Percentage of insoluble collagen was not significantly altered by collagenase or sham treatment in diaphragm and soleus muscles. Bracketed bars represent post-hoc Tukey multiple comparisons test significant differences: p<0.05, p=0.01, p=0.01, p=0.001, p=0.001.



Figure S4. Comparison between 1-hour and 2-hour collagenase treatments in *D2.mdx*

muscles. (A) Muscle mass was significantly decreased following 1-hour and 2-hour collagenase treatments in diaphragm muscles but not in soleus muscles. (B) Diaphragm and soleus muscles digested for 1 and 2 hours in collagenase had significantly reduced stiffness compared to raw muscles. However, there were no significant differences in stiffness between the 1-hour and 2-hour groups of either muscle. (C-D) Total collagen and percentage of insoluble collagen were not significantly altered following 1-hour or 2-hour collagenase treatment in diaphragm and soleus muscles. Flat bars represent one-way ANOVA significant main effects: $^{\#}p$ <0.01. Bracketed bars represent post-hoc Tukey multiple comparisons test significant differences: $^{\#}p$ <0.001.