

Supplementary Materials

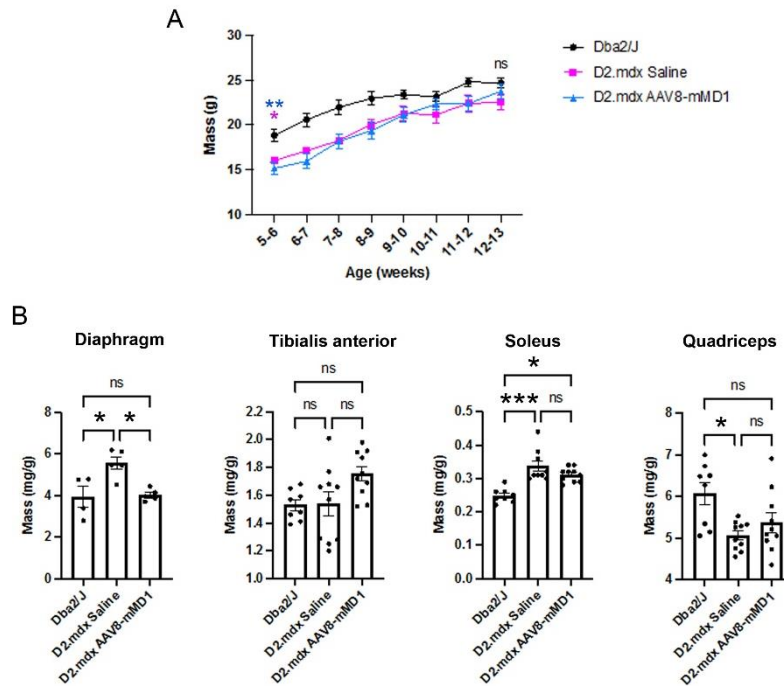


Figure S1. Effect of AAV8-MD1 on body and muscle mass of *D2.mdx* mice. Male *Dbal2/J* control mice and *D2.mdx* treatment groups were weighed weekly starting from 6 weeks of age. Mice at 6 weeks of age were subsequently treated with a single IV injection of either saline or AAV8-MD1 at 4×10^{12} vg/mouse. Muscles were weighed when harvested at the end of the experiment. (A) The weekly mean body mass per group shows that while the body mass at the beginning of the experiment was significantly different between *Dbal2/J* and *D2.mdx* mice, at the end of the treatment (13 weeks of age) all mice had a similar weight. Data shown as means \pm S.E.M., $n = 4-5$ mice. Statistical analysis was performed by a two-way ANOVA followed by a Tukey's multiple comparisons test, * $p < 0.05$ (*Dbal2/J* vs saline treated *D2.mdx*), ** $p < 0.01$ (*Dbal2/J* vs AAV8-MD1 treated *D2.mdx*). (B) At 14 weeks of age, mice were placed under terminal anesthesia and the diaphragm, tibialis anterior, soleus, and quadriceps muscles were harvested and muscle mass was normalized to corresponding body mass (mg/g). No substantial change in muscle mass was detected for skeletal muscles except for the diaphragm muscle. Data shown as means \pm S.E.M., $n = 4-5$ mice. Statistical analysis was performed using a one-way ANOVA followed by a Tukey's multiple comparisons test, $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), ns: no significant difference was observed.

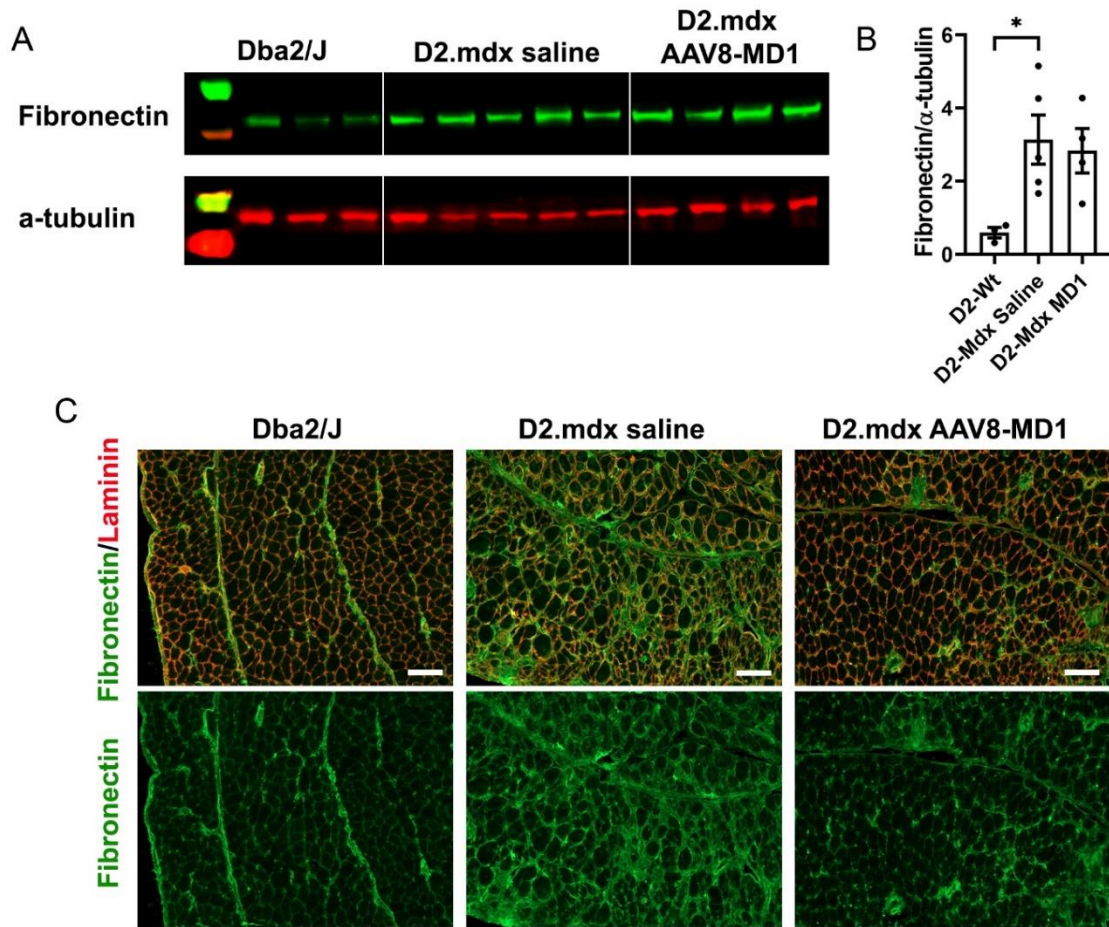


Figure S2. Fibronectin expression shows a minimal reduction in treated mice. Male *Dbal2/J* control and *D2.mdx* mice were treated with a single IV injection of either saline or AAV8-MD1 at 4×10^{12} vg/mouse at 6 weeks of age. (A) Western blot analysis displaying fibronectin protein expression shows an increase in protein expression in *D2.mdx* mice compared to *Dbal2/J* mice but no reduction after AAV treatment. (B) subsequent quantification of the level of fibronectin, normalised to α -tubulin, $n = 4-5$. (C) Representative images of fibronectin deposition in mouse diaphragms for each treatment group. Laminin staining is in red. Scale bar: 100 μ m). Statistical analysis was performed by a one-way ANOVA followed by a Tukey's multiple comparisons test, $p < 0.05$ (*).

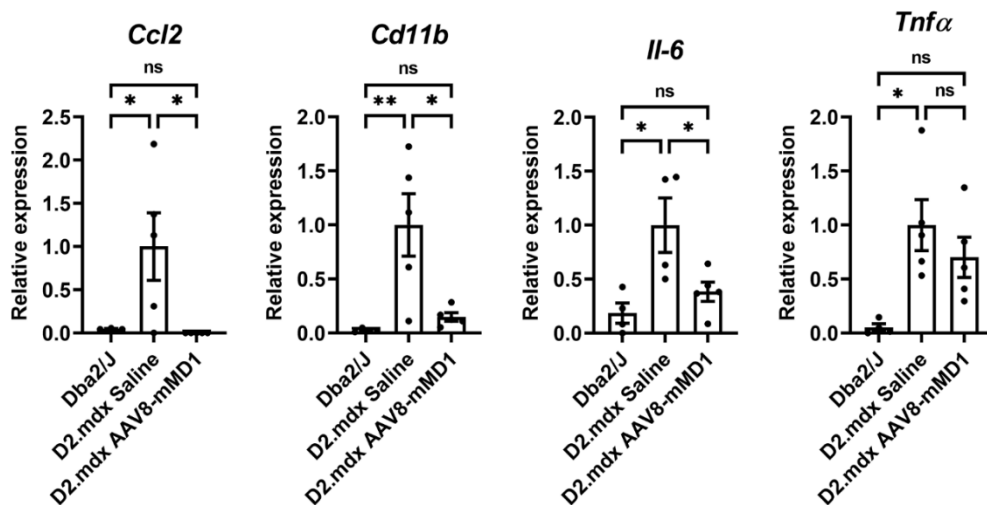


Figure S3. qPCR for inflammatory markers. qPCRs performed for Ccl2, Cd11b, IL-6 and Tnf- α show a general substantial reduction of the first 3 markers after treatment, while Tnf- α expression remained unaltered. Statistical analysis was performed by a one-way ANOVA followed by a Tukey's multiple comparisons test, $p < 0.05$ (*), $p < 0.01$ (**), ns: not significant.

Table S1. qPCR primer sequences.

| Target genes (mouse) | Primer sequence 5'-3' | PCR product size (bp) |
|---|--|-----------------------|
| Alpha actin 2 (<i>Acta2</i>) | Forward primer: 5'-GAGCTACGAACTGCCTGAC-3' Reverse primer: 5'-CTGTTATAGGTGGTTTCGTGGA-3' | 129 |
| Fibronectin 1 (<i>Fn1</i>) | Forward primer: 5'-GAGCTATCCATTTACCTTCAGA-3' Reverse primer: 5'-TTGTTTCGTAGACACTGGAGAC-3' | 96 |
| Periostin (<i>Postn</i>) | Forward primer: 5'-CCTGTAAGAACTGGTATCAAGGT-3' Reverse primer: 5'-CCTTTCATCCCTTCCATTCTCA-3' | 97 |
| Tissue inhibitor metalloproteinase 1 (<i>Timp1</i>) | Forward primer: 5'-AGACAGCCTTCTGCAACTC-3' Reverse primer: 5'-CAGCCTTGAATCCTTTTAGCATC-3' | 129 |
| Tissue inhibitor metalloproteinase 2 (<i>Timp2</i>) | Forward primer: 5'-GACCTGACAAAGACATCGAGT-3' Reverse primer: 5'-GCCATCTCCTTCTGCCTTT-3' | 119 |
| Ribosomal protein lateral stalk subunit P0 (<i>Rplp0</i>) | Forward primer: 5'-TTATAACCCTGAAGTGCTCGA-3' Reverse primer: 5'-CGCTTGTACCCATTGATGATG-3' | 147 |
| Matrix metalloproteinases 9 (<i>Mmp9</i>) | Forward primer: 5'-GACATAGACGGCATCCAGTATC-3' Reverse primer: 5'-GTGGGAGGTATAGTGGGACA-3' | 125 |
| Periostin exon 17 (<i>Postn-17</i>) | Forward primer: 5'-ATAACCAAAGTCGTGGAACCAA-3' Reverse primer: 5'-CTTCCGTTTTGATAATAGGCTGAA-3' | 70 |
| Lysyl oxidase (<i>Lox</i>) | Forward primer: 5'-ACGCTGTGACATTCGCTACA-3' Reverse primer: 5'-TGTCCAAACACCAGGTACGG-3' | 115 |
| Collagen, type I, alpha 1 (<i>Col1a1</i>) | Forward Primer: 5'-GAAACTTTGCTTCCCAGATGTC-3' Reverse Primer: 5'-AGACCACGAGGACCAGAA-3' | 94 |
| Serine (or cysteine) peptidase inhibitor, clade E, member 1 (<i>Serpine1</i>) | Forward Primer: 5'-AGAGGTAAAGGAAGAGGGTCCA-3' Reverse Primer: 5'-TTTTCCATTGTCCATCGGCG-3' | 128 |
| Connective tissue growth factor (<i>Ctgf</i>) | Forward Primer: 5'-GTGCACTGCCAAAGATGGTG-3' Reverse Primer: 5'-CTTTGGAAGGACTCACCGCT-3' | 72 |
| Transforming Growth factor β 1 (<i>Tgf-β1</i>) | Forward primer: 5'-CTGCTGACCCCCACTGATAC-3' Reverse primer: 5'-GCCCTGTATTCCGTCTCCTT-3' | 93 |
| Glyceraldehyde 3-phosphate dehydrogenase (<i>Gapdh</i>) | Forward primer: 5'-GTGGAGTCATACTGGAACATGTAG-3' Reverse primer: 5'-AATGGTGAAGGTCGGTGTG-3' | 150 |
| Tumour Necrosis Factor alpha (<i>Tnfα</i>) | Forward primer: 5'-AGCCGATGGGTTGTACCTTG-3' Reverse primer: 5'-ATAGCAAATCGGCTGACGGT-3' | 99 |
| Interleukin 6 (<i>Il-6</i>) | Forward primer: 5'-CCCCAATTTCCAATGCTCTCC-3' Reverse primer: 5'-CGCACTAGGTTTGCCGAGTA-3' | 141 |
| Chemokine (C-C motif) ligand 2 (<i>Ccl2</i>) | Forward primer: 5'-CCACAACCACCTCAAGCACT-3' Reverse primer: 5'-AGGCATCACAGTCCGAGTCA-3' | 75 |
| Integrin alpha M (<i>Cd11b</i>) | Forward primer: 5'-AGTGCTGGGAGACGTGAATG-3' Reverse primer: 5'-GCACTGAGGCTGGCTATTGA-3' | 120 |