**Supporting information**

## Supplementary methods

### Western Immunoblotting Methodology

Frozen diaphragm tissue was lysed with a polytron PT 1200 homogenizer and suspended in buffer containing: 50 mM Tris pH 7.5, 150 mM NaCl, 25 mM EDTA, 25 mM EGTA, 1% Triton X-100, protease inhibitor cocktail, calpain 1 inhibitor and phosphatase inhibitor. After 30 min of incubation on ice, homogenates were centrifuged at 15,800 x g for 30 min at 4°C and the supernatant was removed. Muscle lysates were kept on ice for 10 min and then centrifuged at 800 x g for 10 min at 4°C. Protein concentration in the supernatants was determined using the Bradford assay. A total of 20 μg protein per well was loaded into a 4 %-12 % gradient gels. Proteins were separated by SDS-PAGE and transferred to a nitrocellulose membrane and blocked for 30 min with 5% skim milk powder in PBS-Tween 20 at room temperature. Membranes were incubated with an anti-fibronectin antibody (Sigma-Aldrich) diluted 1: 1000 in blocking buffer for 1 h at room temperature. After rinsing with PBS-Tween 20, membranes were incubated for 1 h at room temperature with HRP-conjugated anti-rabbit secondary antibodies (Jackson Immunoresearch) at a dilution of 1:8000. Protein bands were developed using an ECL Plus detection kit (Amersham Pharmacia Biotech, UK) and visualized with an Alpha Innotech FluoChem SP Imaging System. Protein bands were quantified using IMAGE J software V1.44P. Sample protein concentrations were determined using BCA assay (Pierce) and equal

protein loadings were confirmed by measuring Ponceau S intensity on nitrocellulose membranes post-transfer. *Mdx* values were normalized to wild-type in each gel.

**Immunohistochemistry, Histology and the Degeneration / Regeneration Index Assay**

Diaphragm fibrosis was determined by immunolabeling 10-μm-thick frozen muscle sections with collagen I (Abcam) and fibronectin (Sigma-Aldrich) polyclonal antibodies. Sections were fixed with 2 % paraformaldehyde in PBS, blocked for 20 minutes (50 mM glycine, 0.25% BSA dissolved in PBS), then incubated for 1 hour at room temperature with primary antibodies. Sections were washed in blocking buffer and then incubated with an Alexa Fluor 488 donkey anti-rabbit secondary antibody for 1 hour at room temperature. Sections were again washed in blocking buffer, mounted in Prolong Gold antifade reagent and imaged by confocal microscopy on Leica TCS SP and Zeiss LSM5 microscopes at the W.M. Keck Center for Advanced Studies in Neural Signaling (University of Washington).

To determine the percentage of centrally nucleated muscle fibers for the regeneration / regeneration index assay, 10-μm-thick diaphragm cryosections were stained with hematoxylin and eosin using standard methods. The fraction of centrally nucleated muscle cells was determined from all muscle cells within an entire section. At least 5 sections spaced at 100 μm intervals were analyzed to maximize sampling efficiency. To determine the percentage of centrally nucleated muscle fibers for the regeneration / regeneration index assay, 10-μm-thick diaphragm cryosections were stained with hematoxylin and eosin using standard methods. The fraction of centrally nucleated muscle cells was determined from all muscle cells within an entire

section. At least 5 sections spaced at 100 μm intervals were analyzed to maximize sampling efficiency. Approximately 3000-5000 myofibers were investigated for centrally localized nuclei per mouse muscle. Muscles from at least 4 mice per group were analyzed.

**Quantitative Real Time Polymerase Chain Reaction Assays**

Mouse diaphragms were dissected and immediately frozen in liquid nitrogen for RNA isolation. Total RNA was prepared from diaphragms using Trizol (Invitrogen) and further purified with an RNeasy Kit (Qiagen). First strand cDNA was generated from 2 μg of RNA using the $RT^2$ First Strand kit (SABiosciences). cDNA was amplified on a fibrosis PCR Array (PAMM-120-SABiosciences) using an ABI 7000 detection system (Applied BioSystems). Analysis was performed separately for each test group. The resulting data were normalized to hypoxanthine-guanine phosphoribosyltransferase and analyzed using the Δ/Δ CT method using the manufacturer's online analysis software.

**References**

**(Note: reference numbers correspond to reference list in main article)**


11. Percival JM, Anderson KN, Gregorevic P, *et al.* Functional deficits in nNOSmu-deficient skeletal muscle: myopathy in nNOS knockout mice. *PLoS One* 2008; **3:** e3387.

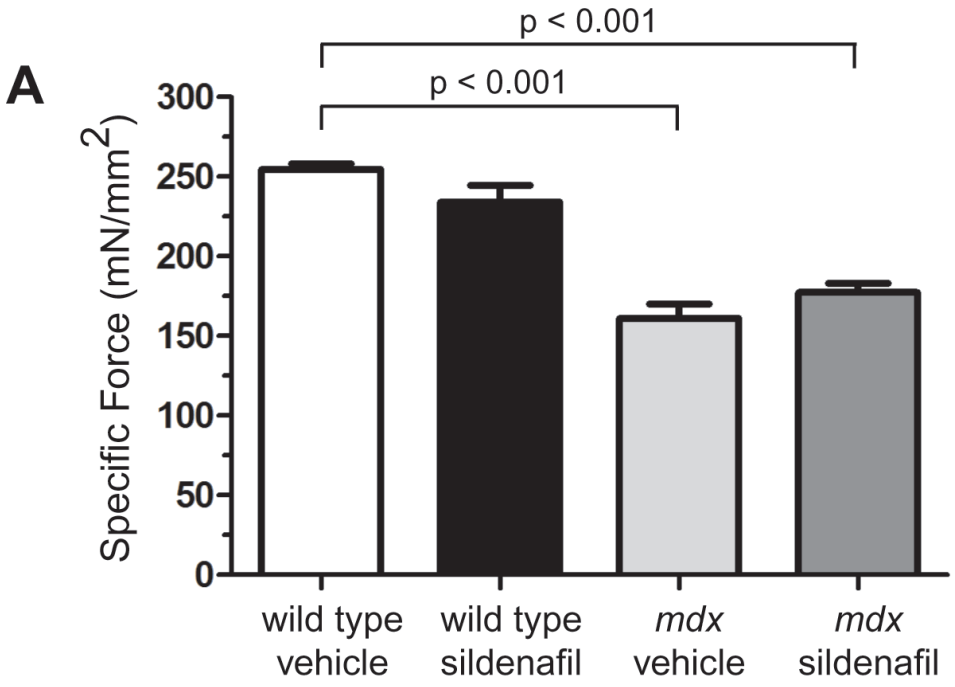
12. Percival JM, Anderson KN, Huang P, *et al*. Golgi and sarcolemmal neuronal NOS differentially regulate contraction-induced fatigue and vasoconstriction in exercising mouse skeletal muscle. *J Clin Invest* 2010; **120:** 816-826.

**Supplementary Figure Legends**

**Supplementary Figure 1.** Chronic sildenafil has no significant impact on *mdx* tibialis anterior muscle strength. Specific force output was measured in tibialis anterior *in situ* in untreated and sildenafil treated wild type and *mdx* mice using previously described methods [11,12]. **A,** Mean specific force output of tibialis anterior hindlimb muscles from untreated and sildenafil-treated wild type and *mdx* mice. As expected, *mdx* tibialis anterior muscles were significantly weaker than wild type controls. However, in contrast to the *mdx* diaphragm, sildenafil treatment had no effect on the specific force output of wild type or *mdx* tibialis anterior muscle. Wild type untreated (n = 5), wild type sildenafil-treated (n = 4), *mdx* untreated (n = 5) and sildenafil-treated *mdx* (n = 6).

**Supplementary Figure 2.** Chronic sildenafil treatment did not impact the numbers of central nucleated muscle cells in the *mdx* tibialis anterior hindlimb muscle. **A**, Hematoxylin & eosin stained tibialis anterior muscle sections from vehicle- and sildenafil-treated wild type (top row) and *mdx* (bottom row) mice. *Mdx* tibialis anterior showed no overt differences in inflammatory cell infiltration, muscle necrosis or numbers of centrally nucleated myofibers. **B**, Quantitation of the fraction of muscle fibers with centrally localized nuclei or degeneration/regeneration index. The fraction of centrally nucleated muscle cells was *mdx* very high (~ 75 %) in *mdx* tibialis anterior muscles relative to wild type controls (~1 %). As observed in the diaphragm, sildenafil treatment did not impact the degeneration/regeneration index of *mdx* tibialis anterior hindlimb muscle sections. Scale bar = 100 μm. Wild type untreated (n = 4), wild type sildenafil-treated (n = 3), *mdx* untreated (n = 4) and sildenafil-treated *mdx* (n = 4).

**Supplementary Figure 1**
Percival et al., 2012



**Supplementary Figure 2**
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