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Supplemental Data

Expanding the Boundaries of RNA Sequencing as a Diagnostic Tool for Rare Mendelian Disease

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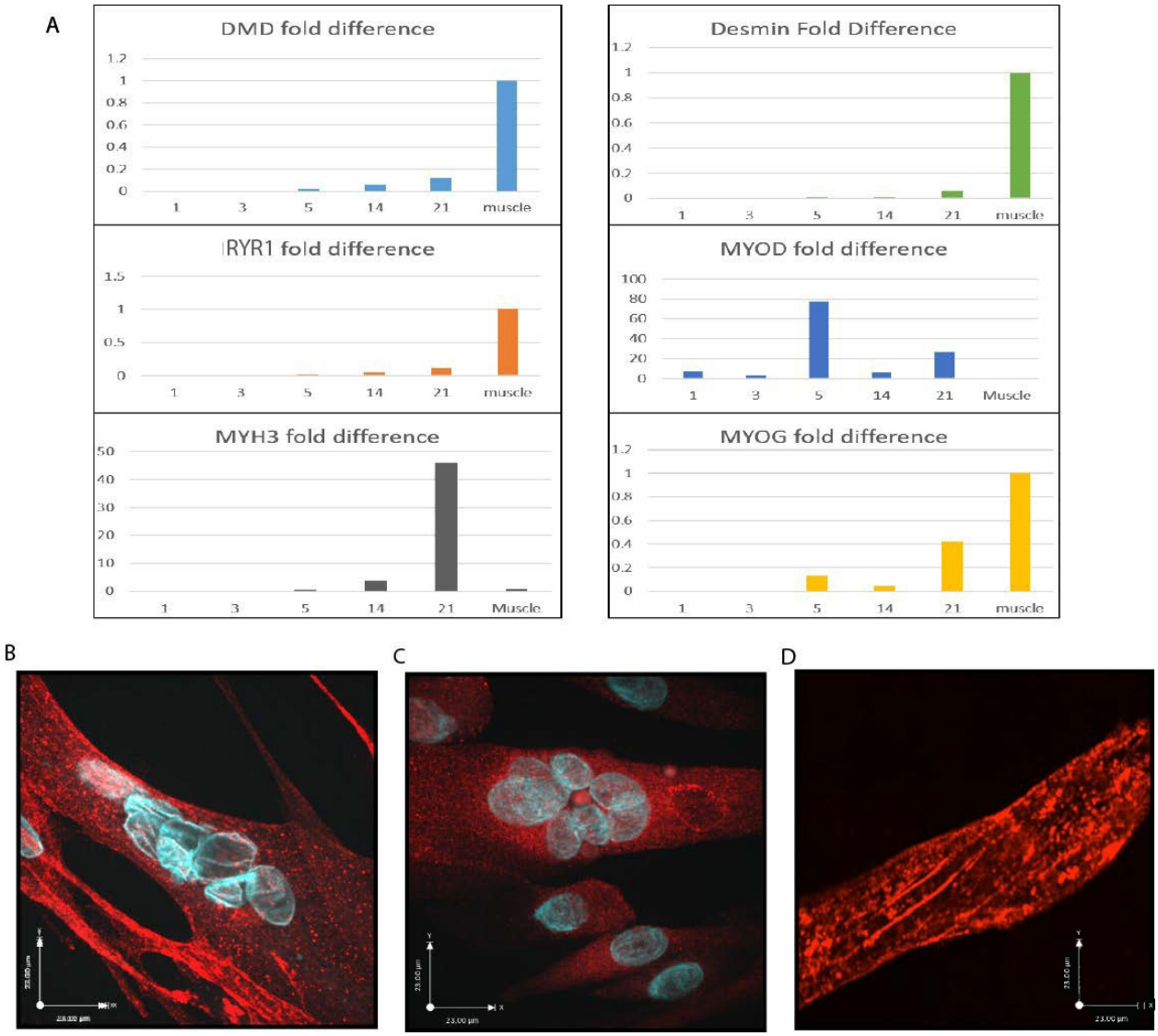
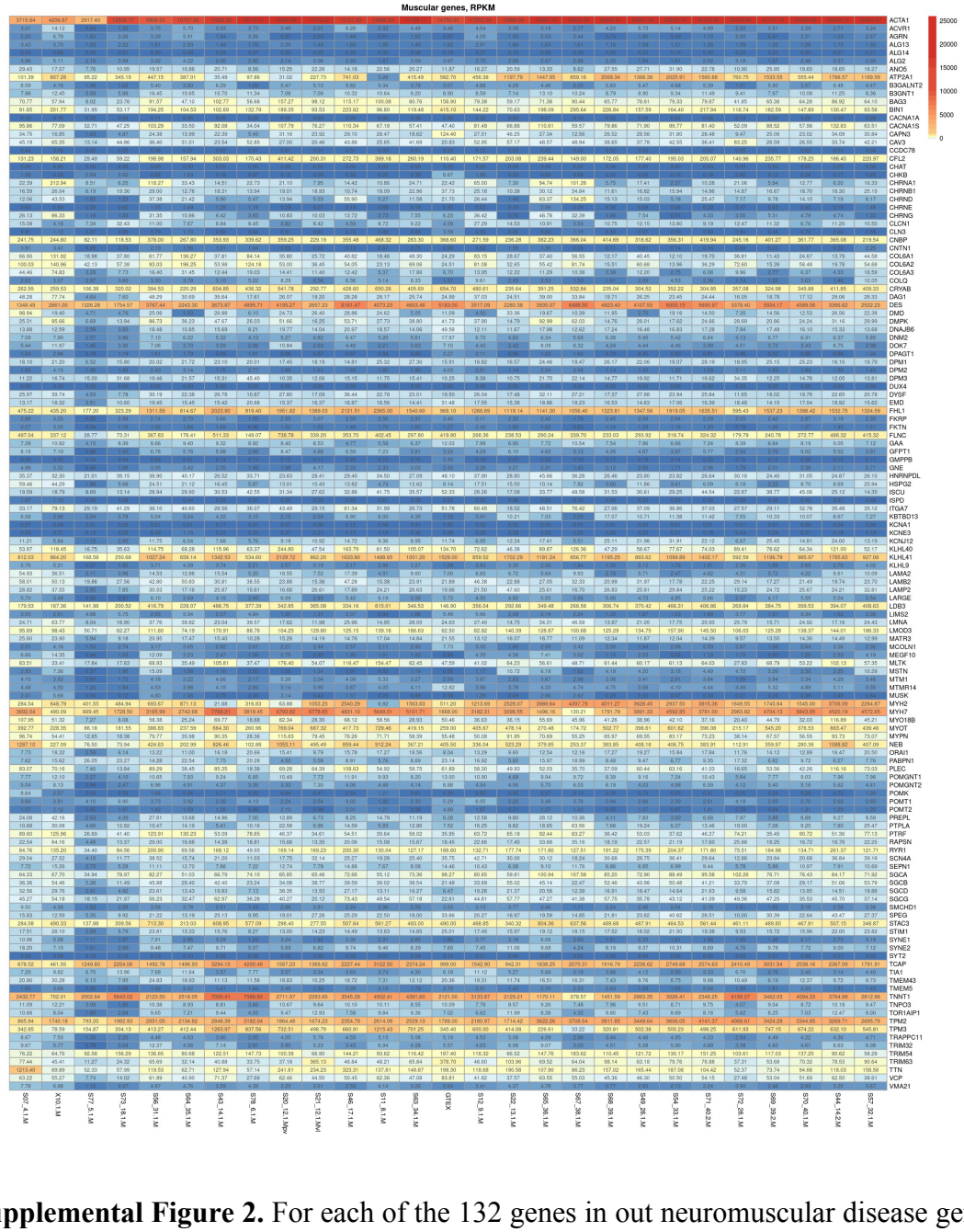


Supplemental Figure 1. **A.** We carried out qRT-PCR to quantify expression for 6 genes at 5 different time points (days 1, 3, 5, 14 and 21) after direct trans-differentiation into myotubes and compared this to their expression in muscles. *MYOD1* showed maximum expression on day 5 after infection while *MYOG*, a myogenic transcription factor known to be stimulated by *MYOD1,* was expressed at increasing levels beginning day 5. The expression of *MYH3* steadily increased until the end of the experiment (day 21) and reached a level of 50x higher than skeletal muscle. The expression of terminal differentiation markers such as *DMD*, *DES,* and *RYR1* steadily increased through the end of the time course (day 21), with easily detectable expression starting at day 5. For each of these genes the final expression on day 21 was still lower than their expression in skeletal muscle samples. **B-D.** Morphological assessment revealed that most of transdifferentiated myotubes had characteristics of early myotubes. The majority were mononucleated, with less than 2% having multiple nuclei. Consistent with the qPCR data, t-myotubes stained positive for Dystrophin **(B)**, ryanodine receptor type 1 (RyR1) **(C)**, and skeletal muscle alpha-actinin **(D)**.

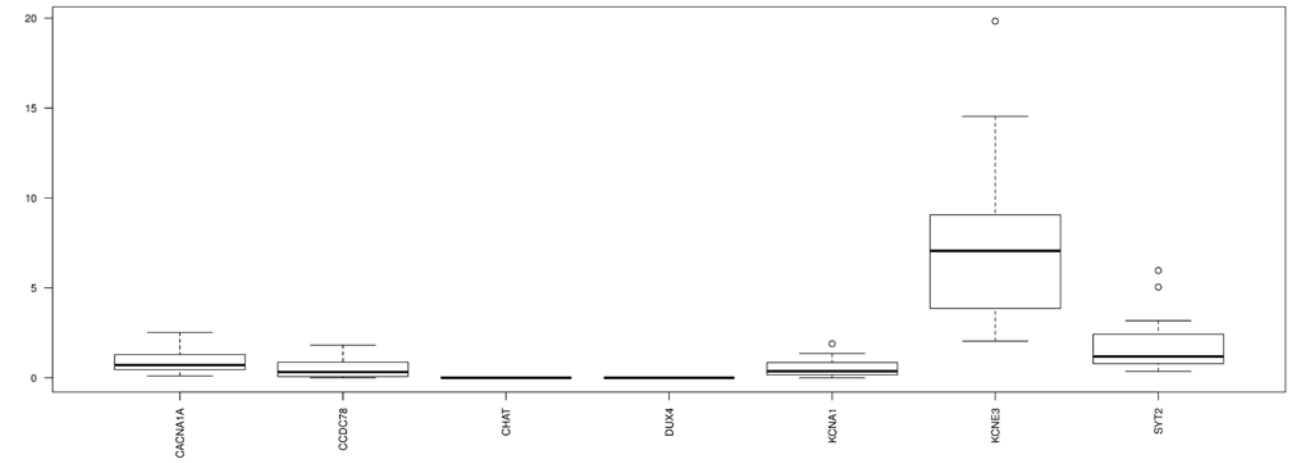


Supplemental Figure 2. For each of the 132 genes in out neuromuscular disease gene panel, we report the expression values (RPKM) across all the skeletal muscle samples in our cohort as well as GTEx data. Each box in the table represents the expression of a gene (rows) in a muscle sample (columns) and colours associated with each value representing the expression level, with blue representing low expression, red representing high expression and yellow representing intermediate expression levels.

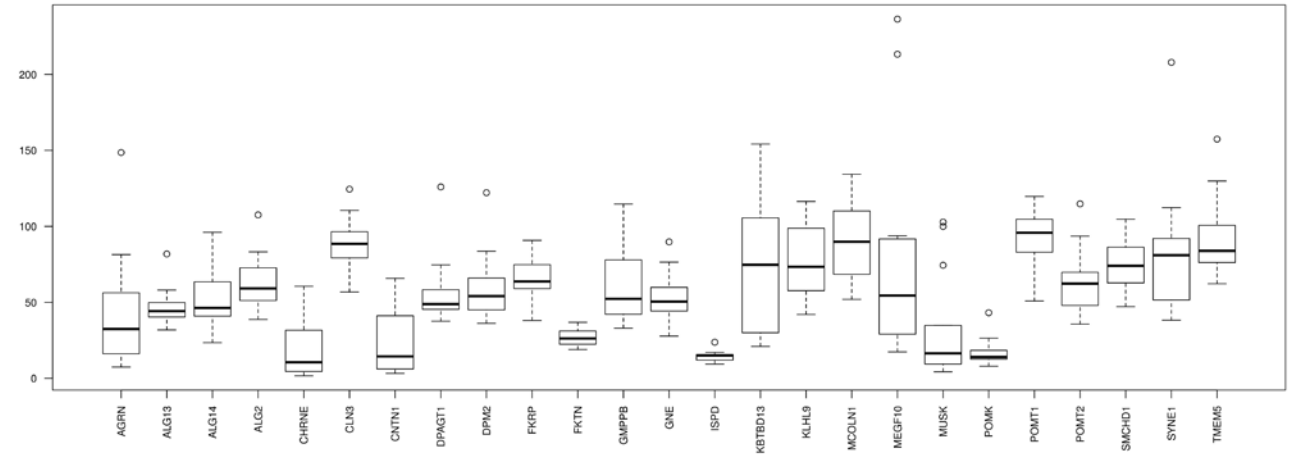
Supplemental figure 3. In each of the 8 plots (**A-H –** corresponding to the 8 gene panels) we compare the expression of 132 genes in muscle (green), myotubes (red), and fibroblasts (orange). Each dot represents a sample of each tissue type. The eight panels are **(A)** channelopathies, **(B)** congenial muscular dystrophies, **(C)** congenital myasthenic syndromes, **(D)** congenital myopathies, **(E)** distal myopathies, **(F)** limb girdle muscular dystrophies, **(G)** muscular dystrophies and **(H)** vacuolar and other myopathies.

A. Muscle

A1 (<10x)



A2 (10x-100x)



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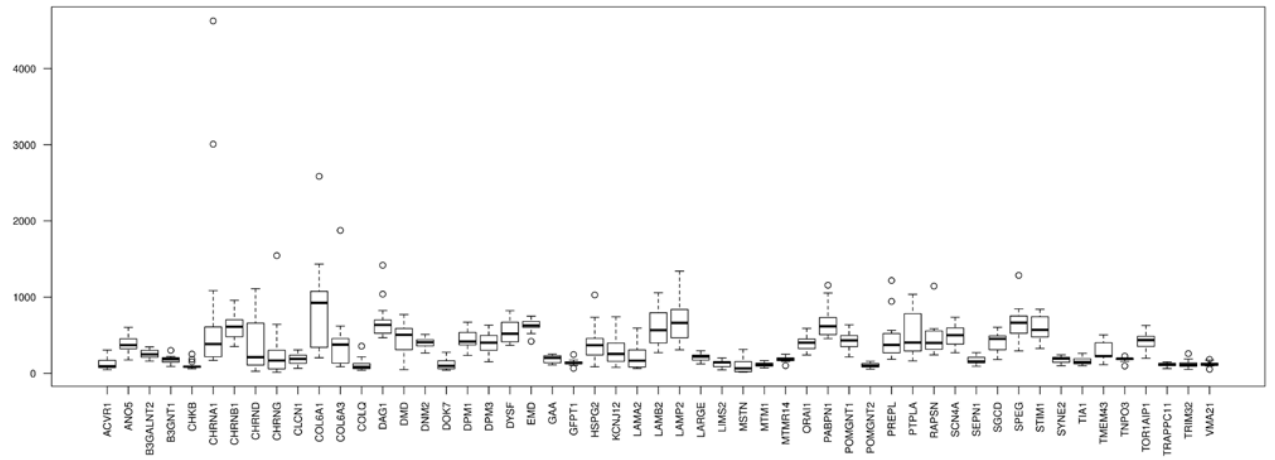
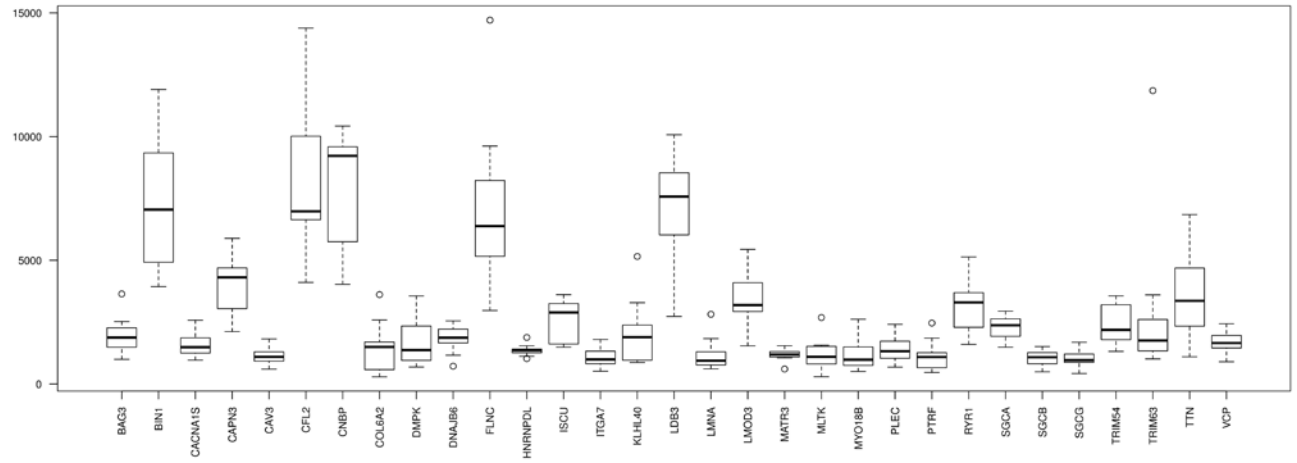
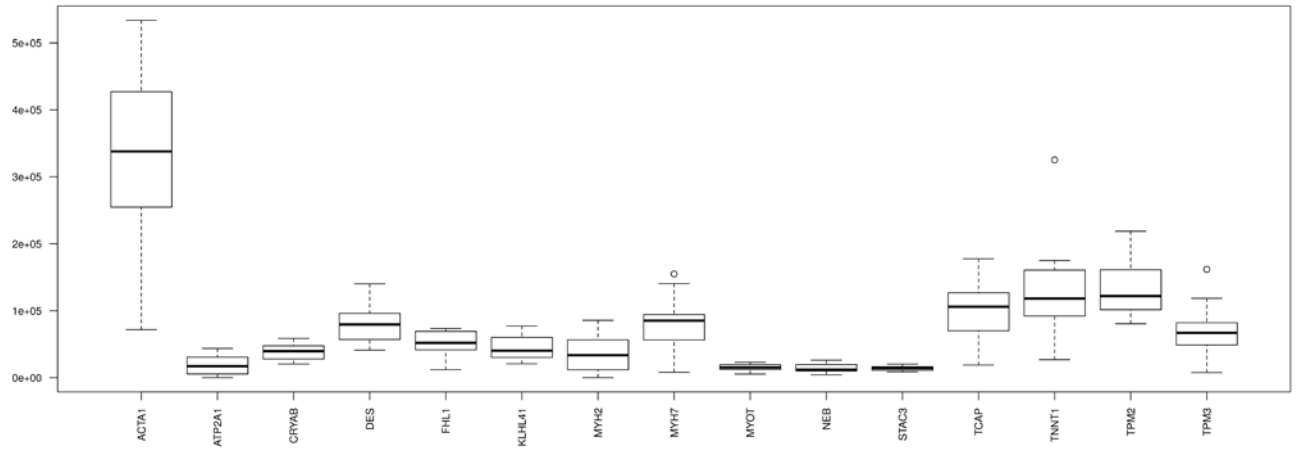


A4 (1,000x-10,000x)

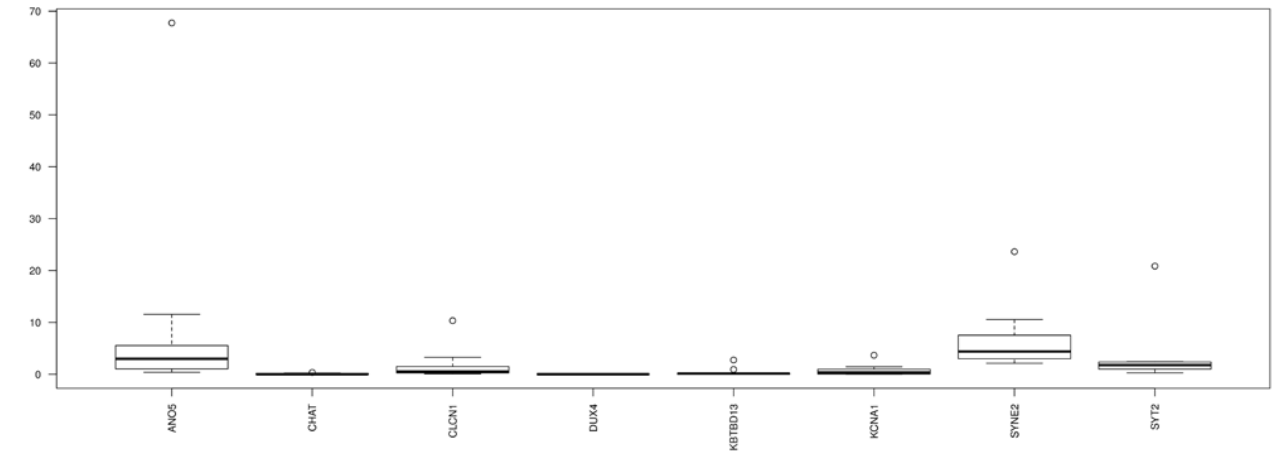


A5 (>10,000x)



B. Myotubes

B1 (<10x)



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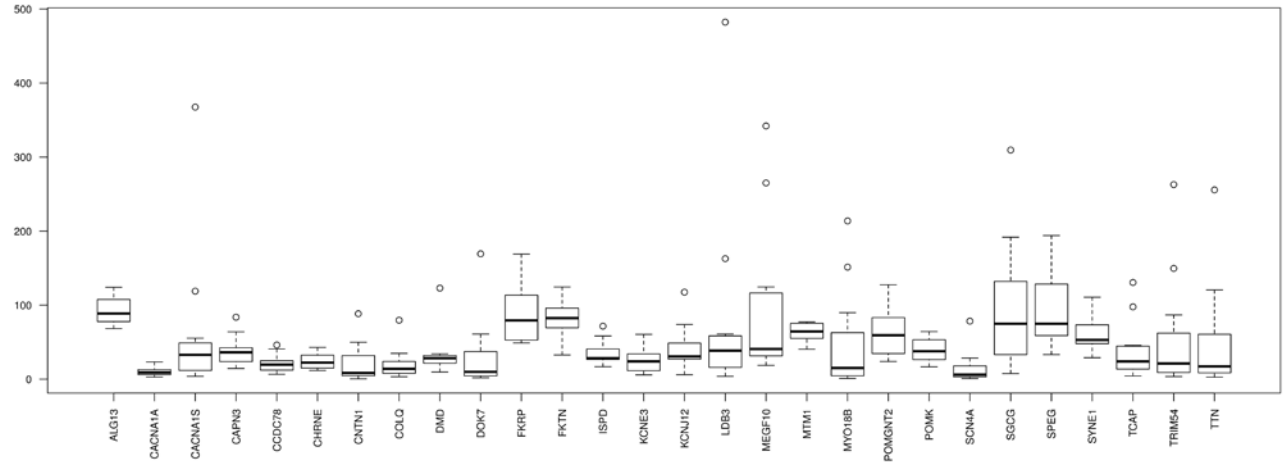
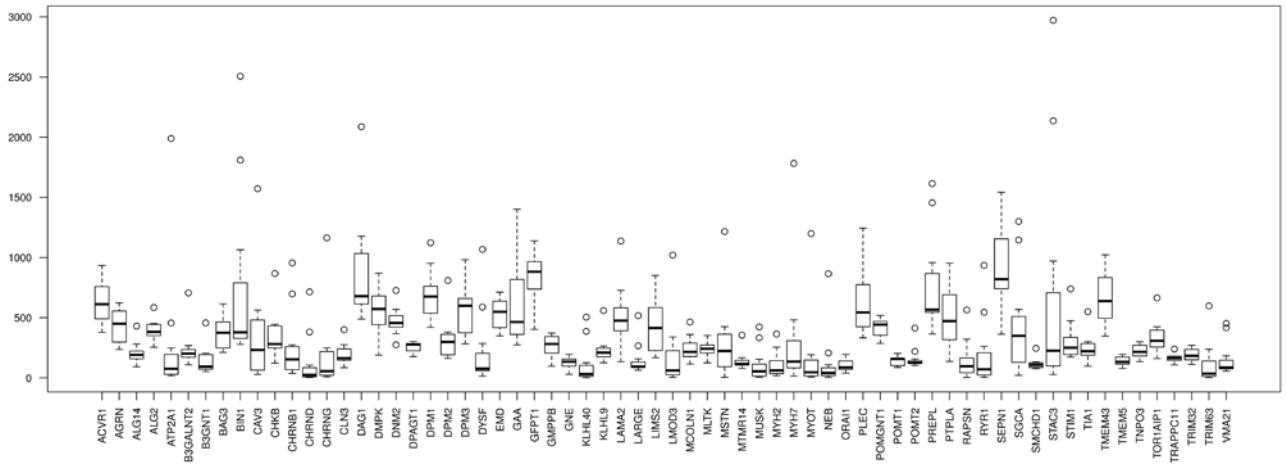


B3 (100x-1,000x)



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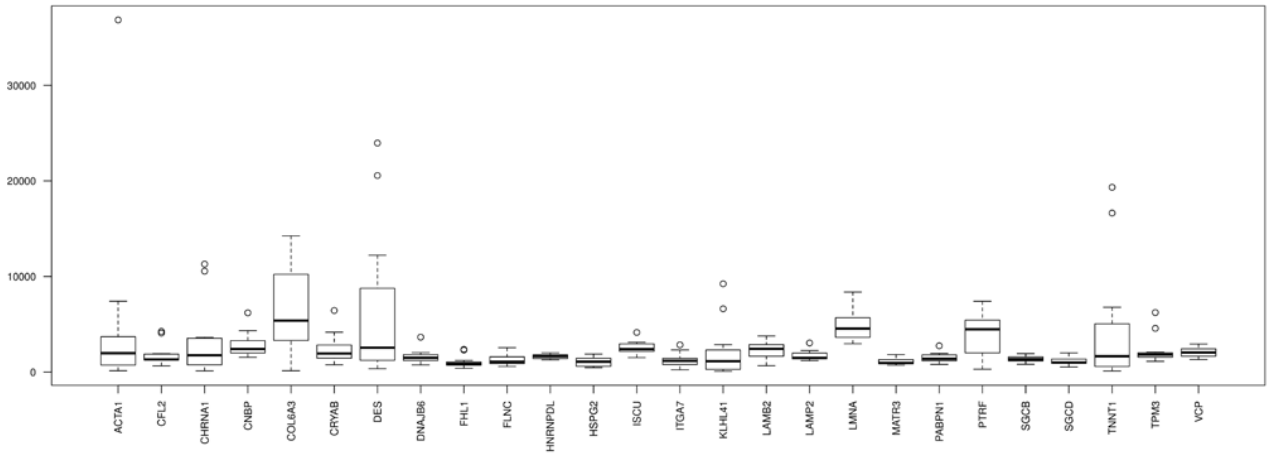
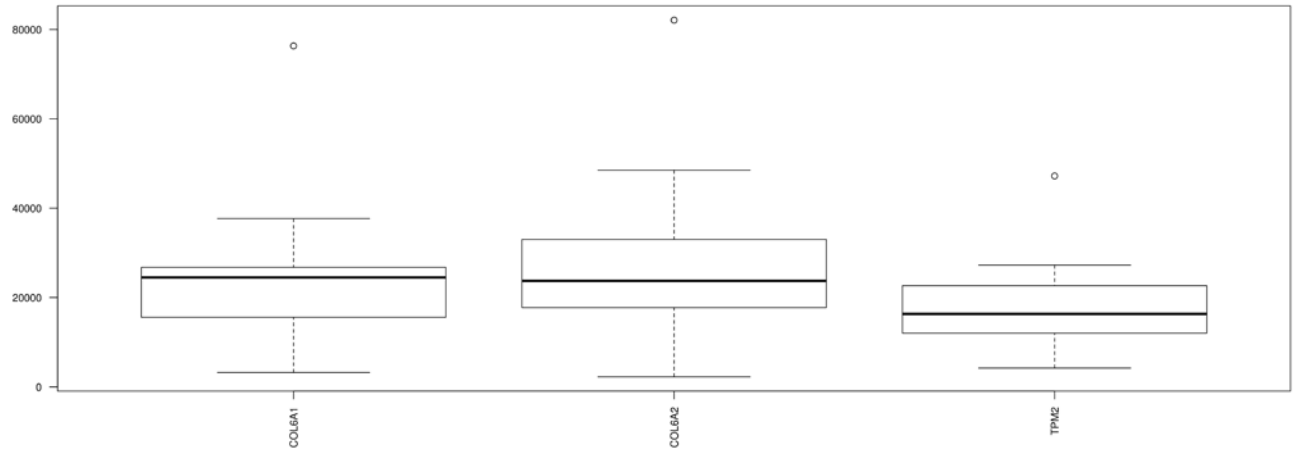
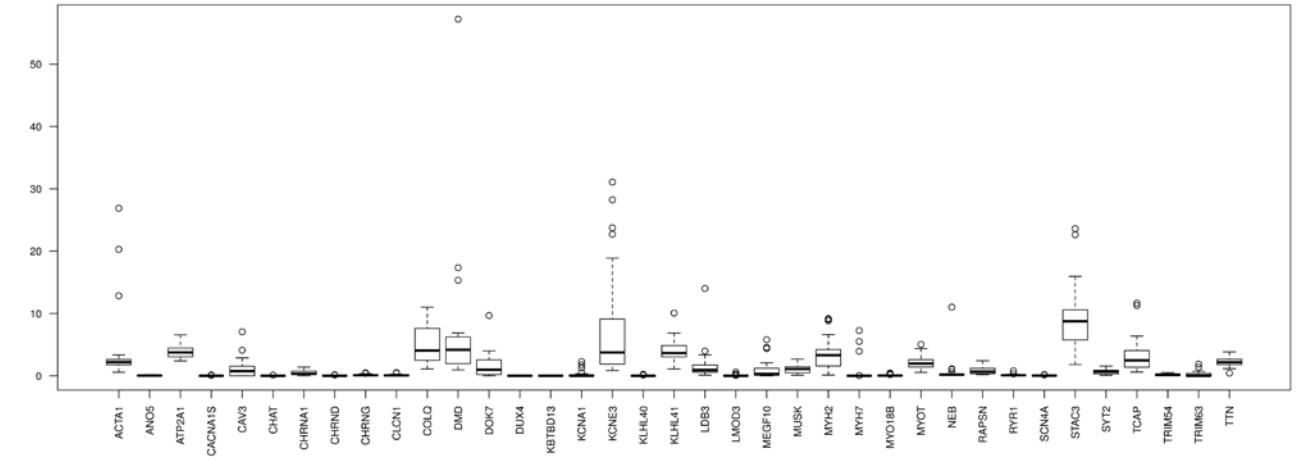


B5 (>10,000x)

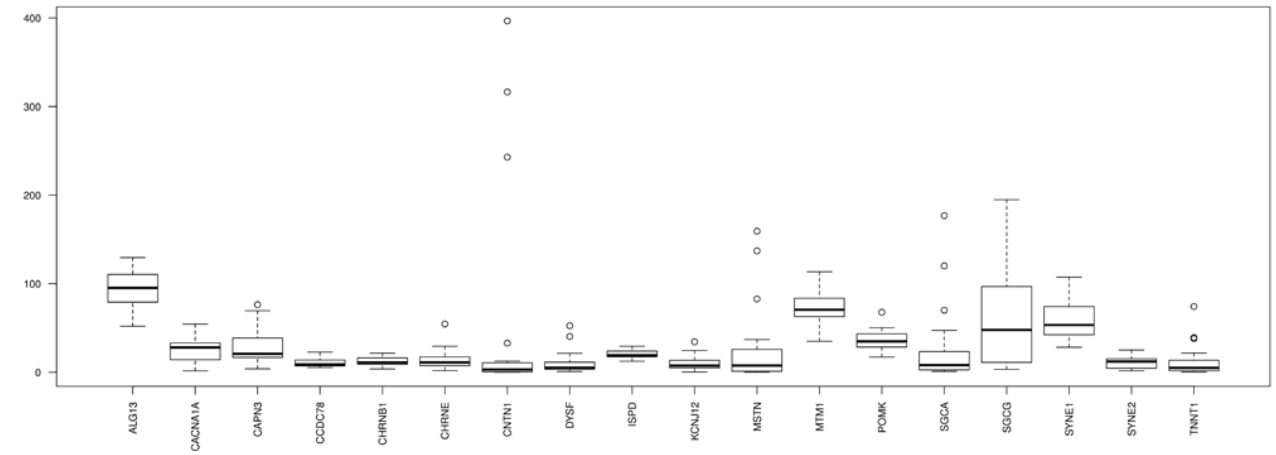


C. Fibroblasts

C1 (<10x)



C2 (10x-100x)



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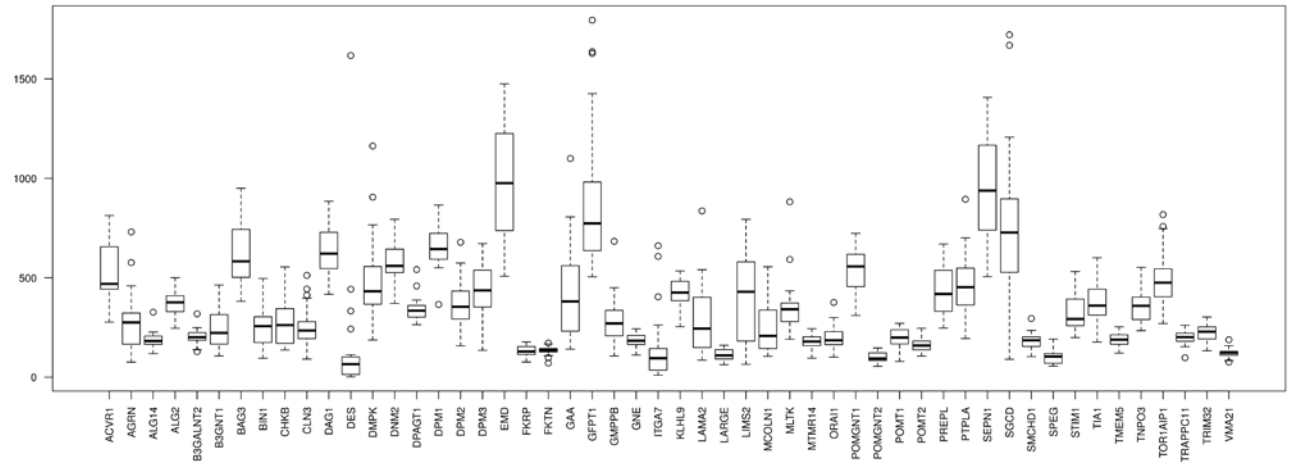

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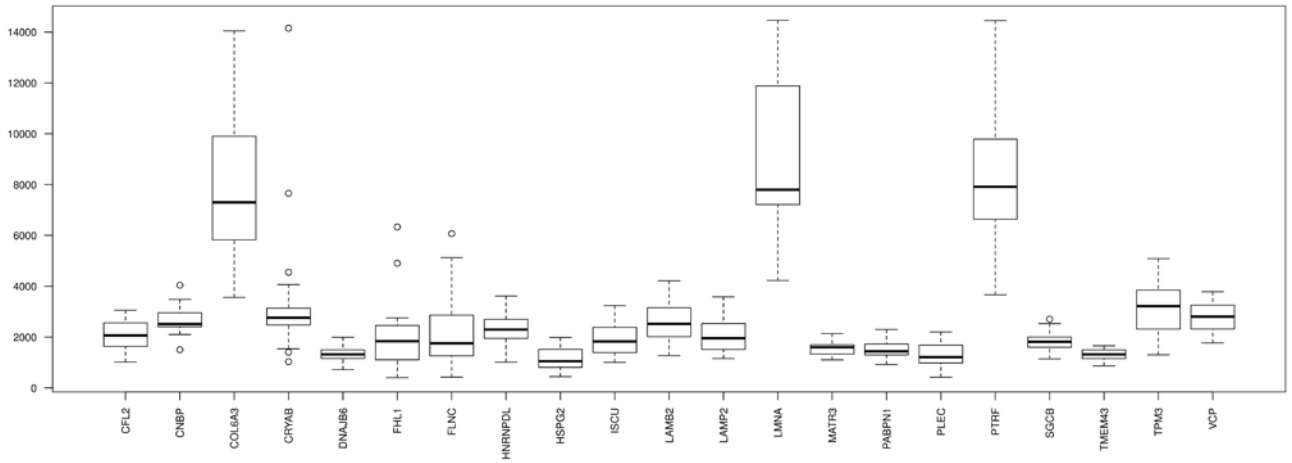

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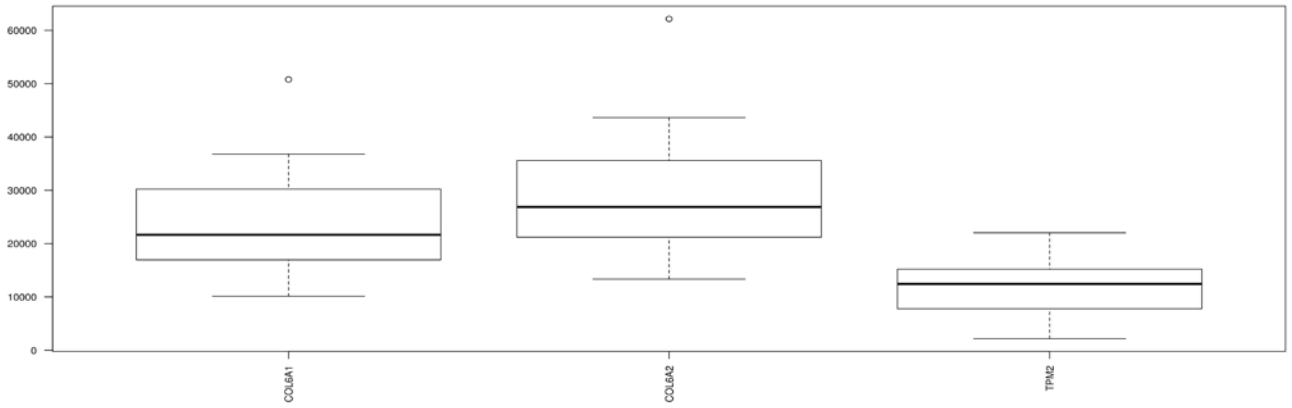

Supplemental figure 4. Each of the 132 genes in our neuromuscular gene panel were placed into 5 bins according to their mean coverage across the samples in each tissue (A) Muscle, (B) Myotubes, and (C) Fibroblasts. The 5 bins represent (1) coverage <10X, (2) coverage between10x-100x, (3) coverage between 100x-1,000x, (4) coverage between 1,000x- 10,000x, and (5) coverage >10,000x.