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PPMO pre-treatment is beneficial for AAV-based gene therapy in Duchenne Muscular Dystrophy

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INTRODUCTION

Duchenne muscular dystrophy (DMD) is a X-linked disorder characterized by progressive skeletal muscle degeneration and weakness associated with cardiac dysfunction. DMD is caused by mutations in the *DMD* gene, resulting in the absence of the dystrophin protein at the sarcolemma of muscle fibers.

Exon-skipping therapy intends to restore an in-frame sequence leading to the expression of a shorter but functional dystrophin. However, in preclinical DMD models, dystrophin restoration by adeno-associated virus (AAV)-U7-mediated exon-skipping therapy was shown to significantly decrease after one year in treated animals^{1,2}.

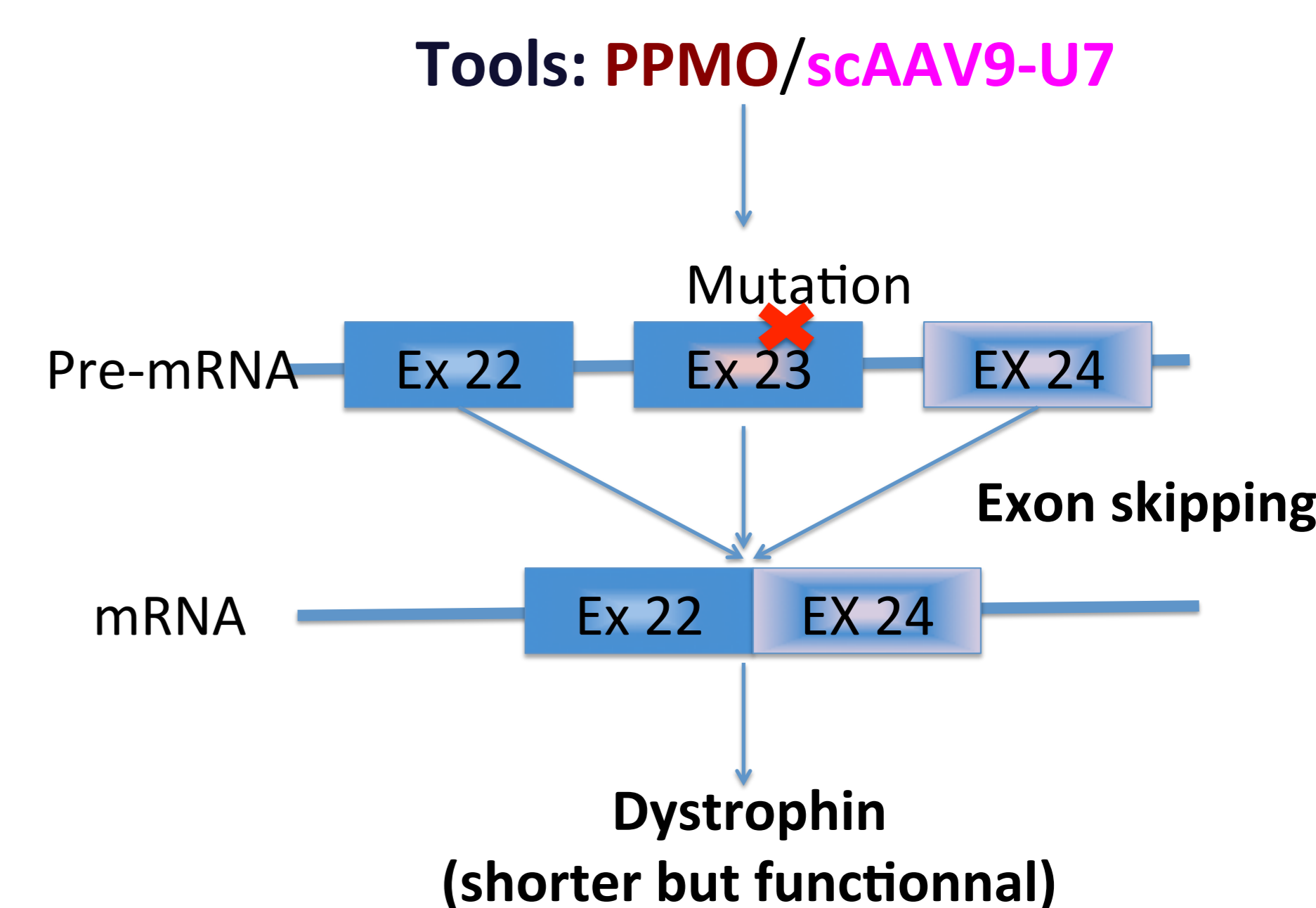
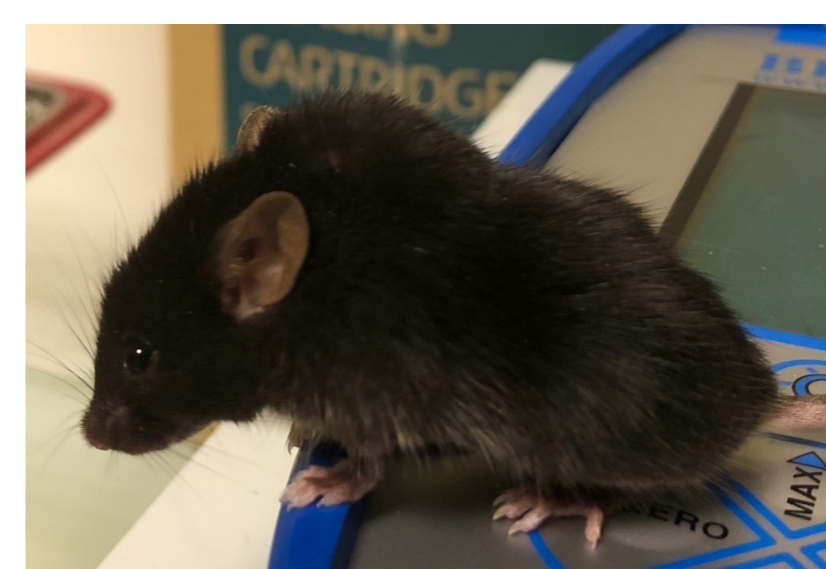
We recently showed that pre-treating *mdx* mice (a model of DMD) muscle with a single dose of peptide-phosphorodiamidate morpholino (PPMO) antisense oligonucleotides led to temporary dystrophin expression at the sarcolemma, allowing efficient maintenance of AAV genomes and therefore enhancing the long-term effect of AAV-U7 therapy³.

Here, we evaluated the synergistic effect of systemic injection of therapeutic PPMO and AAV-U7 vector to a severe DMD model (dystrophin/utrophin double-knockout mouse (dKO)). These mice suffer from a much more severe and progressive muscle wasting, heart and diaphragm functions, impaired mobility and premature death, mimicking pathophysiology of DMD patients.

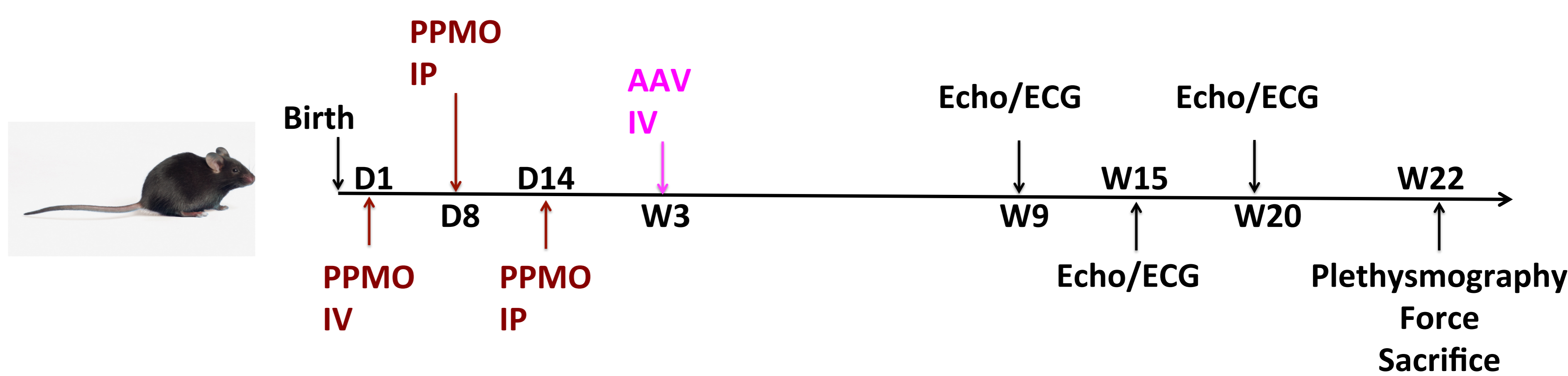
¹Vulin *et al.*, *Mol Ther.* 2012 Nov;20(11):2120-33. ²Le Hir *et al.*, *Mol Ther.* 2013 Aug;21(8):1551-8. ³Peccate *et al.*, *Hum Mol Genet.* 2016 Aug 15;25(16):3555-3563.

MATERIALS AND METHODS

Mouse model: *dKO dystrophin^{-/-} / Utrophin^{-/-}*



Protocol:

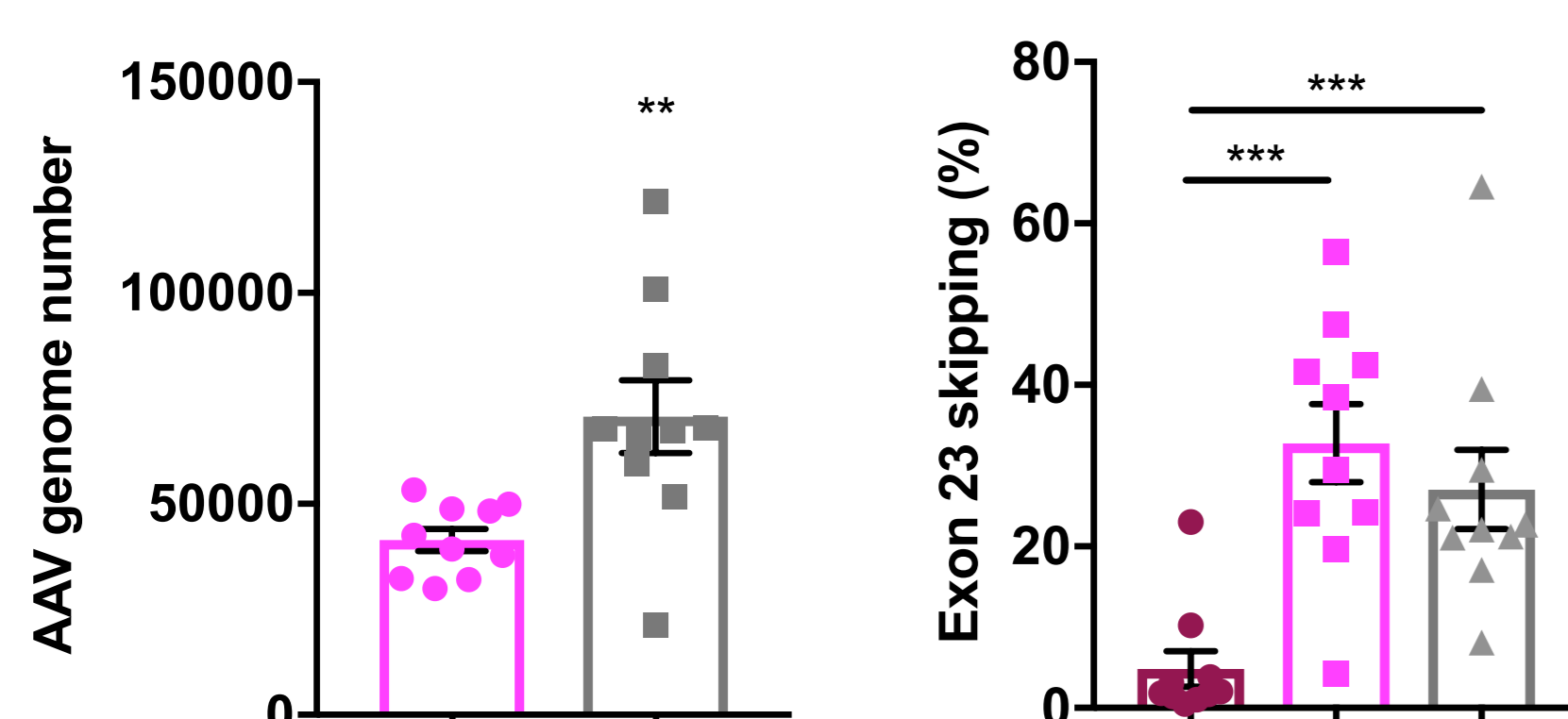


RESULTS

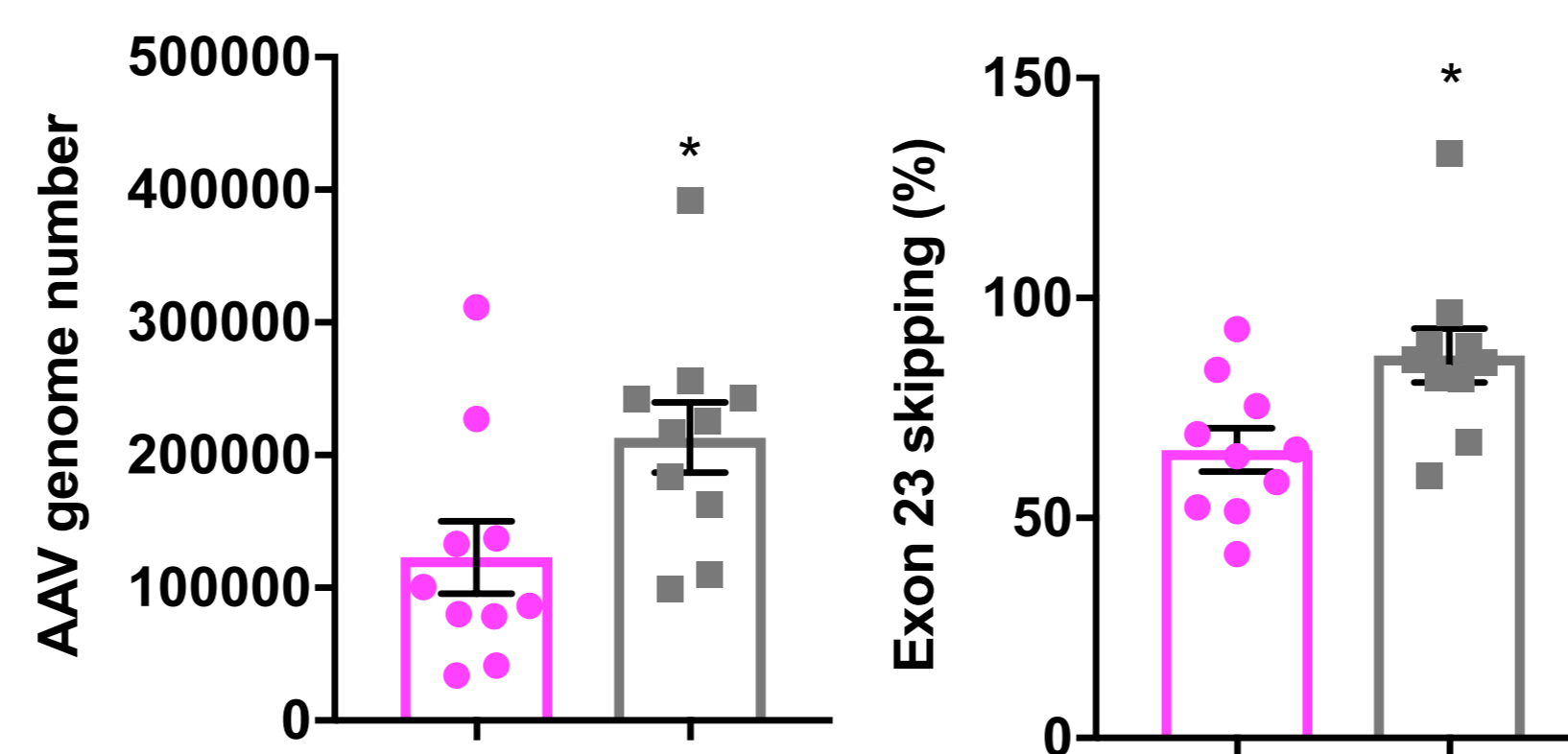
A. Molecular parameters (W22)

Viral genome number and exon skipping percentage quantification (Q-PCR)

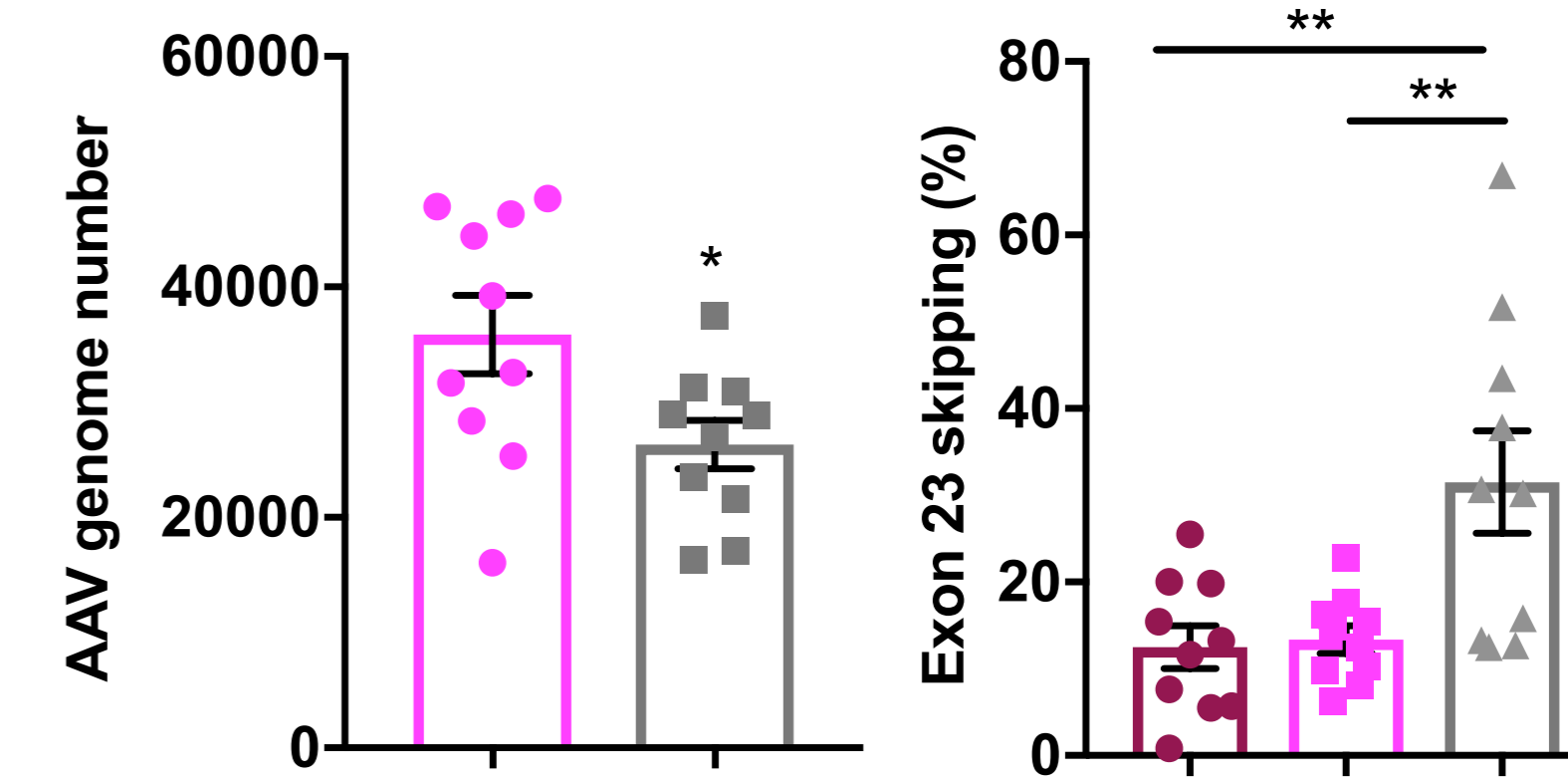
Tibialis Anterior



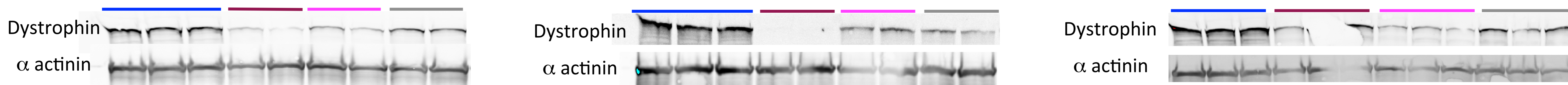
Heart



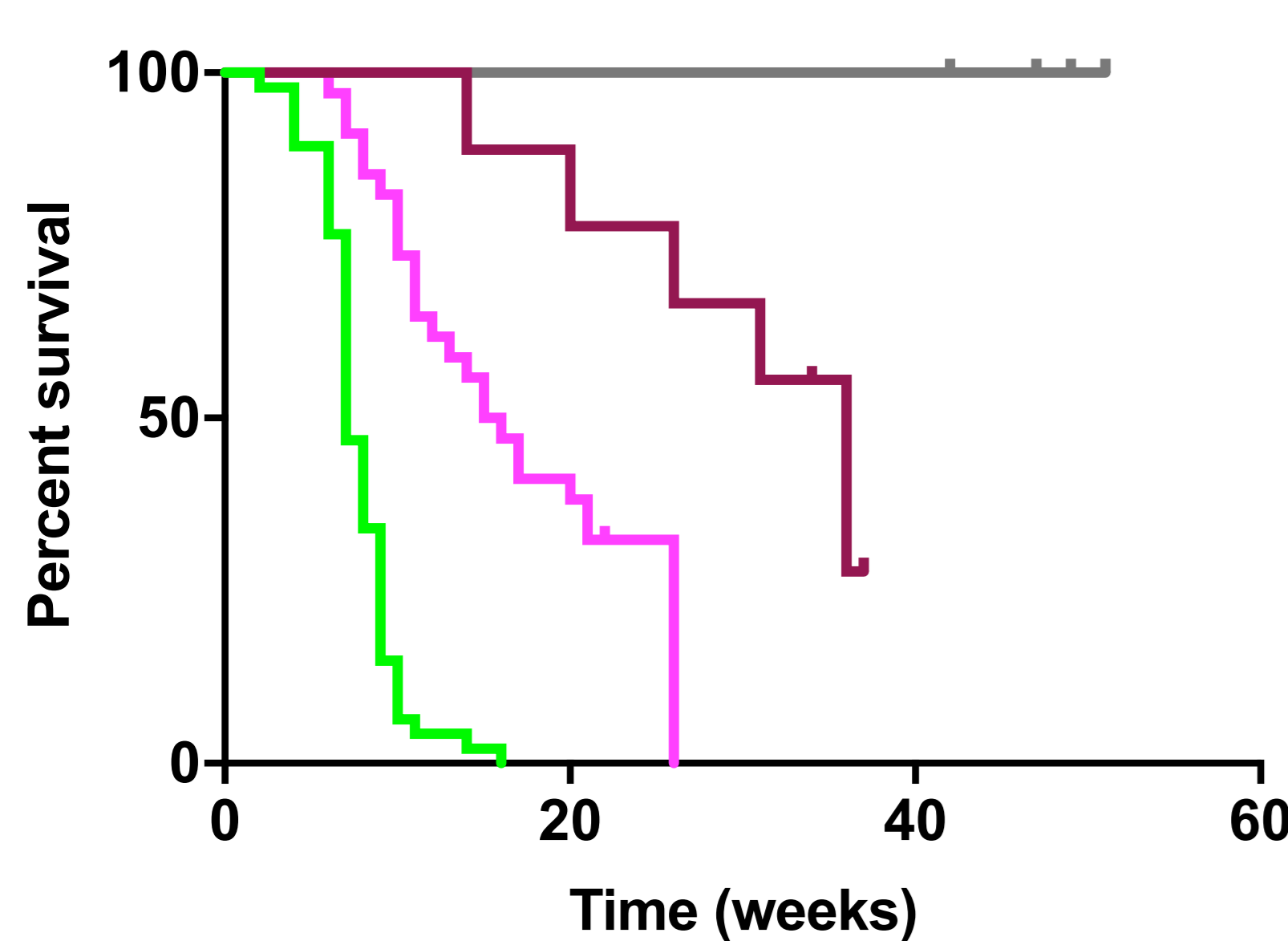
Diaphragm



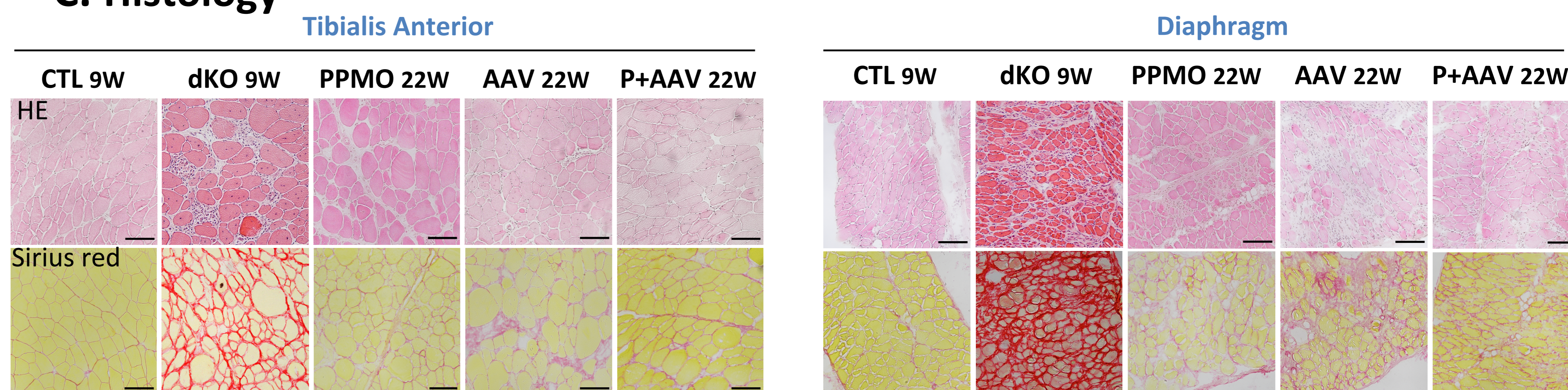
Dystrophin protein rescue (Western Blot)



B. Survival

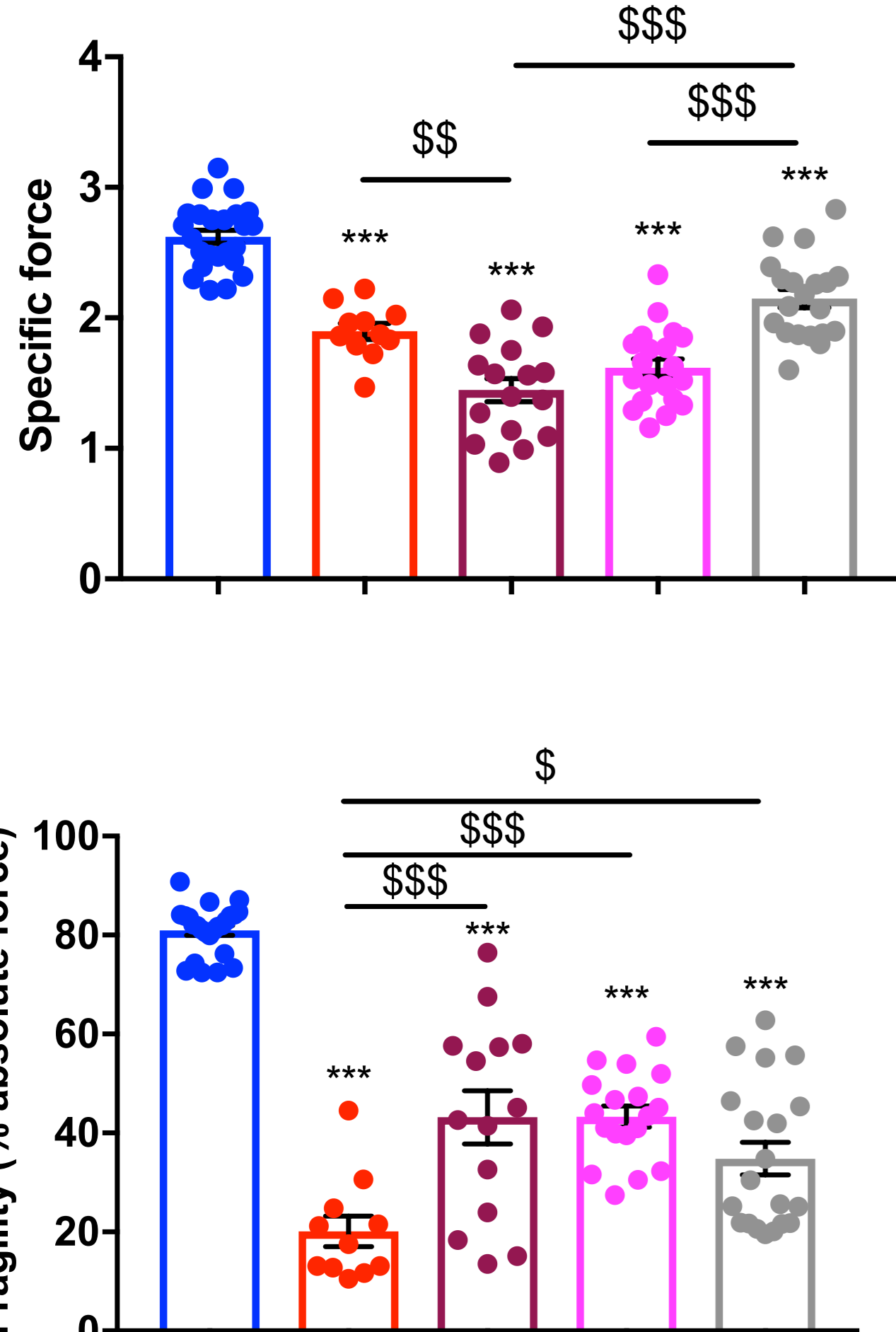


C. Histology

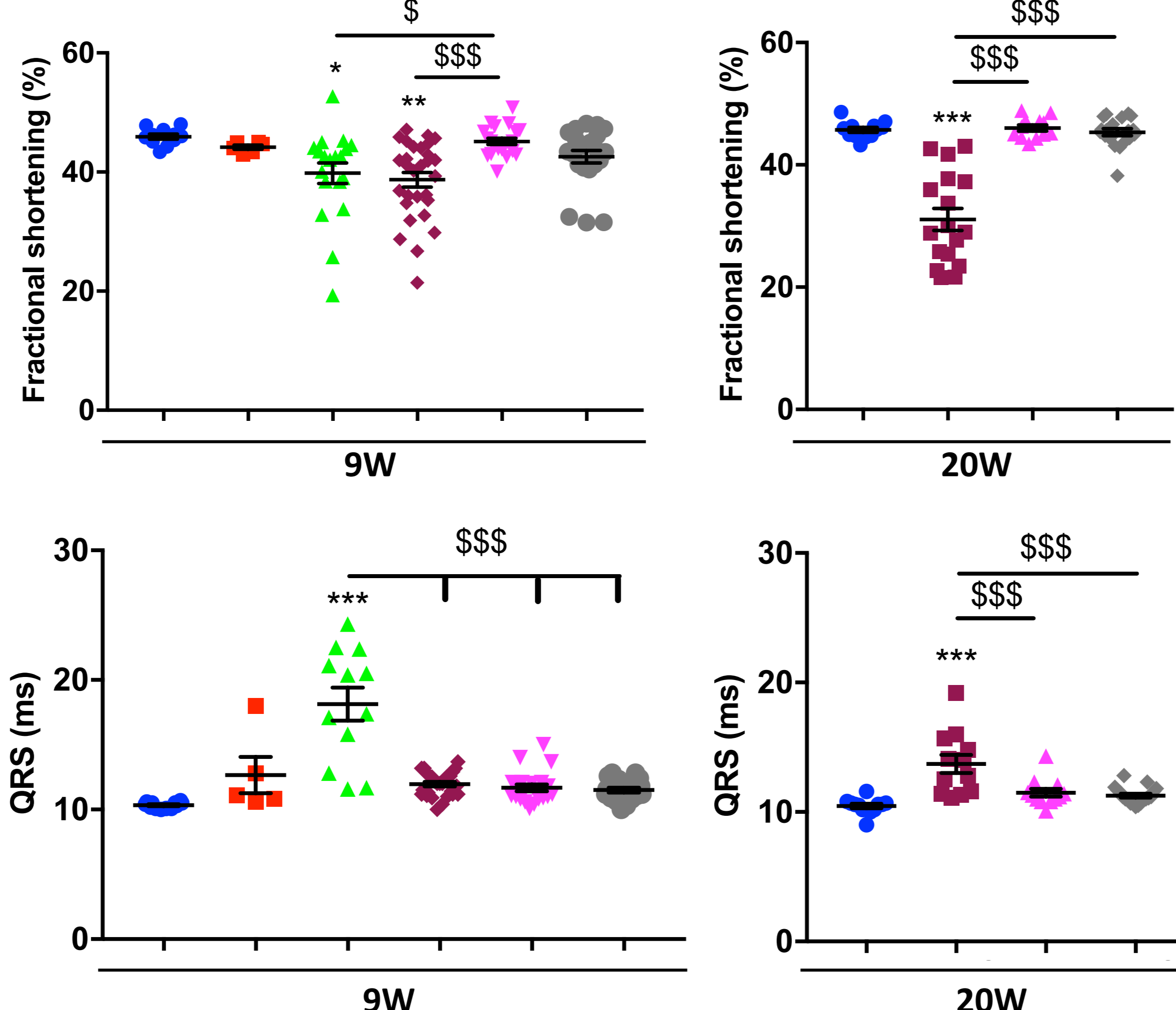


D. Function

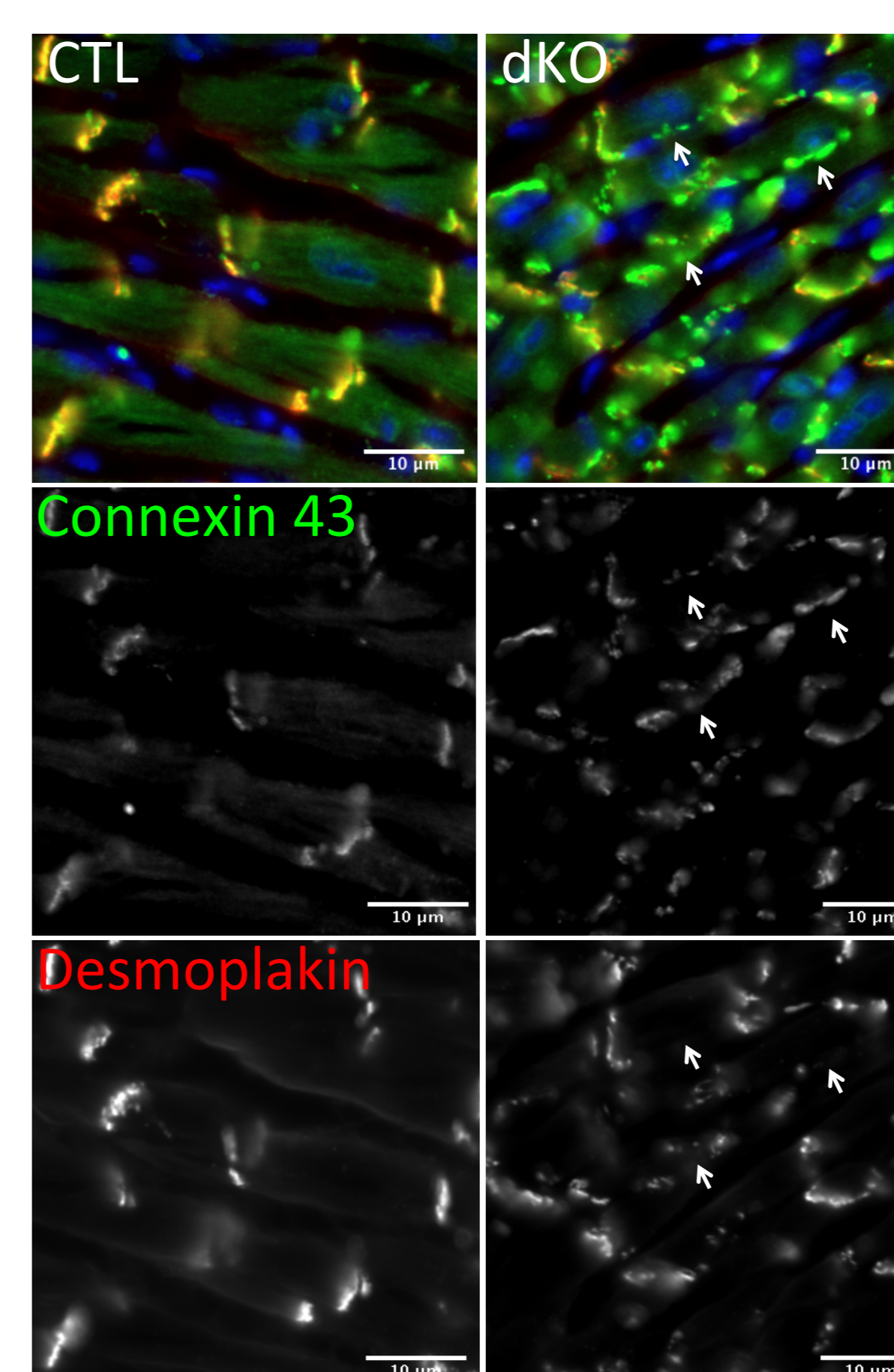
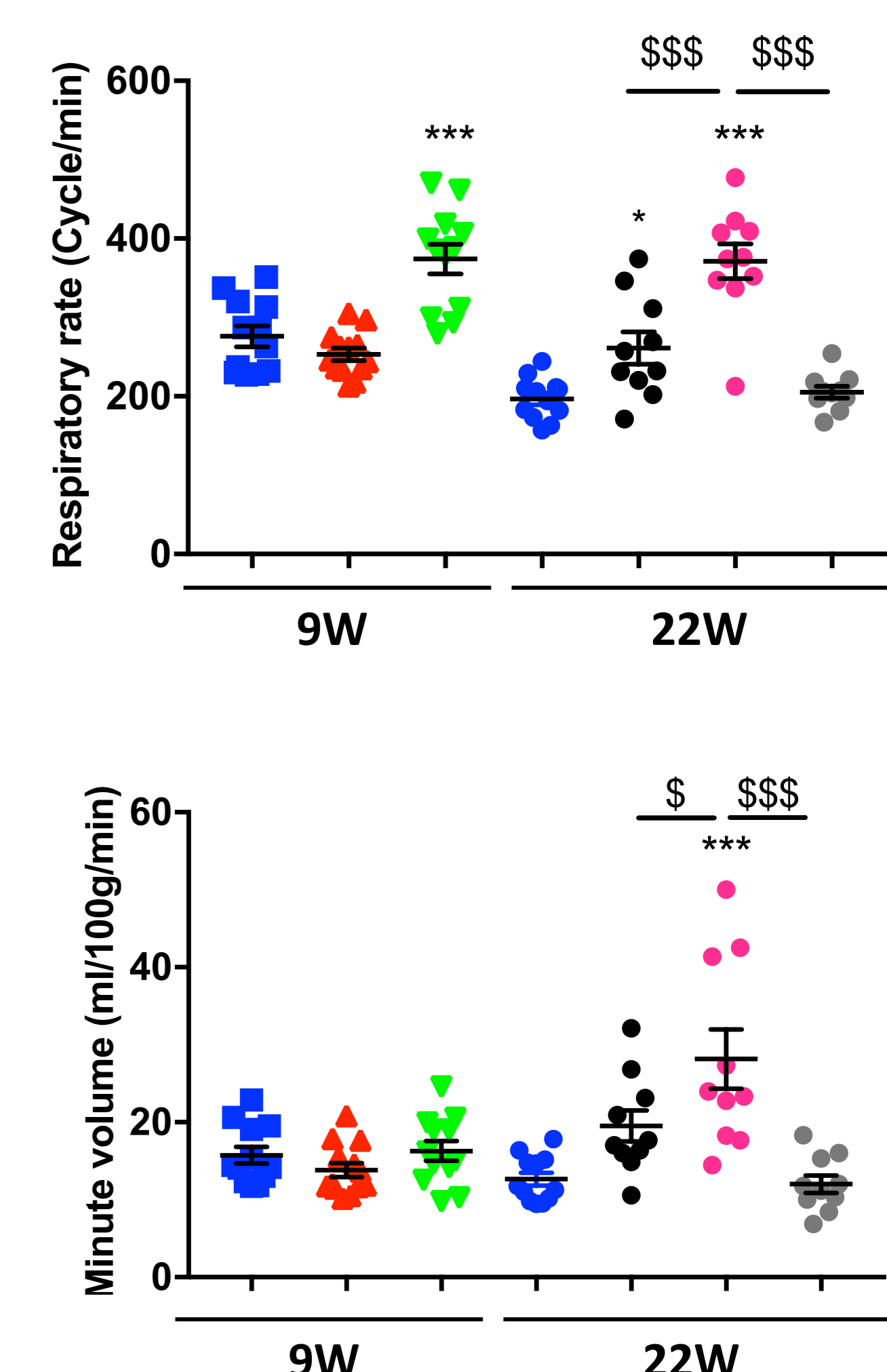
Tibialis Anterior



Heart



Diaphragm



* vs WT at the same age; \$ vs indicated conditions

CONCLUSIONS / PERSPECTIVES

The systemic delivery of a combined treatment, PPMO+AAV-U7, in severe DMD model presents a great benefit on skeletal and cardiac muscle functions; and on recovery of respiratory function with significant improvement of survival over the single therapeutic (to AAV-U7 or PPMO) approaches.

Whereas AAV-U7 and PPMO treatment alone rescued structure and function of heart and diaphragm, respectively; the combined treatment allows global improvement of the whole striated muscle functions. We need now i/ to determine if the therapeutic benefit will persist over time, and ii/ how to improve the combined treatment protocol. This work opens novel therapeutic perspectives for DMD patients.