



HAL
open science

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

Anne Forand, Antoine Muchir, Nathalie Mougenot, Caroline Sevoz-Couche, Cécile Peccate, Mégane Lemaitre, Mathew Wood, Stéphanie Lorain, France Pietri-Rouxel

► **To cite this version:**

Anne Forand, Antoine Muchir, Nathalie Mougenot, Caroline Sevoz-Couche, Cécile Peccate, et al.. PPMO pre-treatment is beneficial for AAV-based gene therapy in Duchenne Muscular Dystrophy. Myologie 2019, Mar 2019, Bordeaux, France. hal-03996969

HAL Id: hal-03996969

<https://hal.science/hal-03996969v1>

Submitted on 15 Mar 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

A. Forand¹, A. Muchir², N. Mougnot³, C. Sévoz-Couche⁴, C. Peccate², M. Lemaitre³, M. Wood⁵, S. Lorain² and F. Piétri-Rouxel²

¹Inovarian F-75013, Paris France. ²Sorbonne Université, Centre de Recherche en Myologie, UM76 /INSERM U974 Institut de Myologie, F-75013, Paris, France. ³Sorbonne Université-UPMC Paris 06-INSERM UMS28-Phénotypage du petit animal-Faculté de Médecine Pierre et Marie Curie, F-75013 Paris. ⁴Sorbonne Université-UPMC Univ Paris 06-INSERM UMR51158-Neurophysiologie Respiratoire Expérimentale et Clinique-Faculté de Médecine Pierre et Marie Curie, F-75013 Paris. ⁵Department of Physiology, Anatomy and Genetics, University of Oxford, United Kingdom, 6 Department of Paediatrics, University of Oxford, United Kingdom, 7 MDUK Oxford Neuromuscular Centre, University of Oxford, United Kingdom.



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.

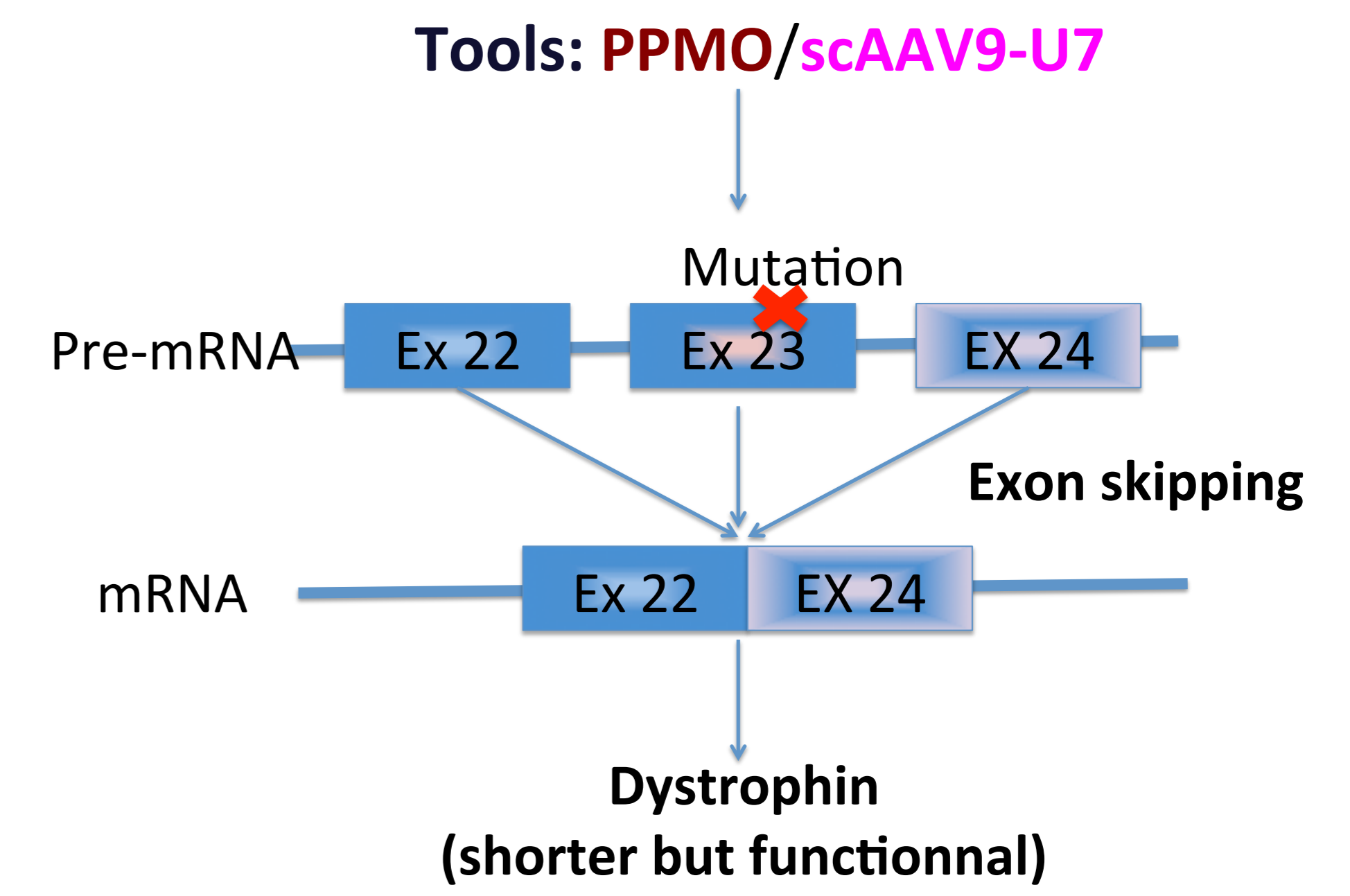
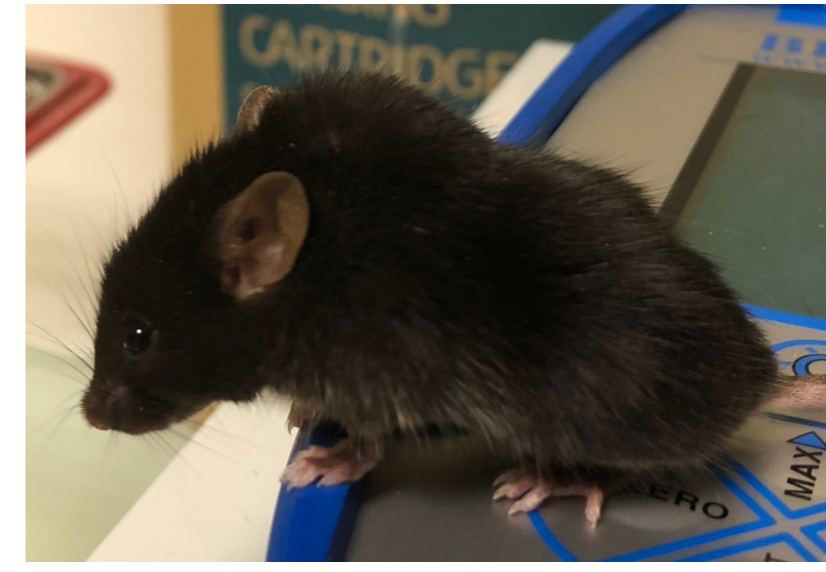
Here, we evaluated the synergistic effect of systemic injection of therapeutic PPMO and AAV-U7 vector to a severe DMD model (dystrophin/utrophin double-knockout mouse (dKO)). These mice suffer from a much more severe and progressive muscle wasting, heart and diaphragm functions, impaired mobility and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

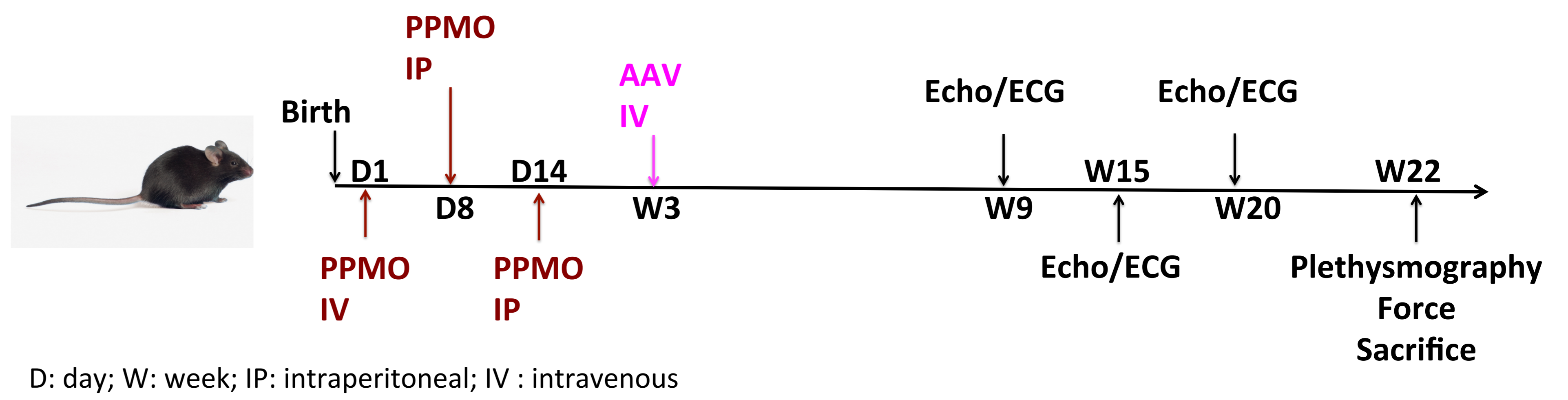
³Peccate et al., Hum Mol Genet. 2016 Aug 15;25(16):3555-3563.

MATERIALS AND METHODS

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



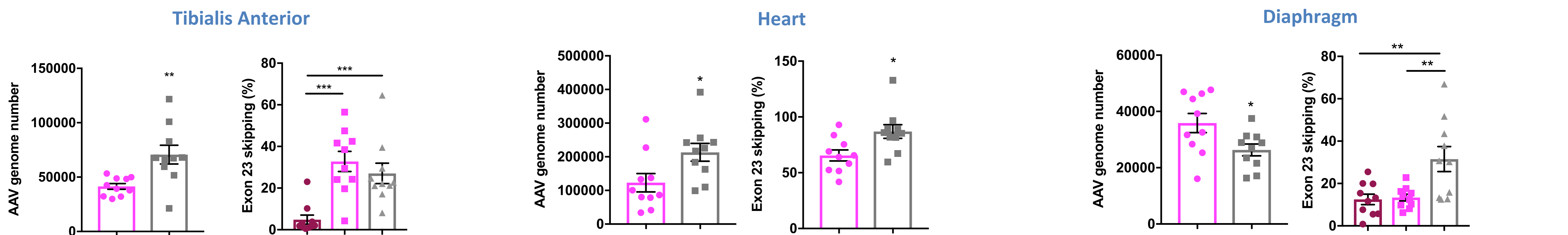
Protocol:



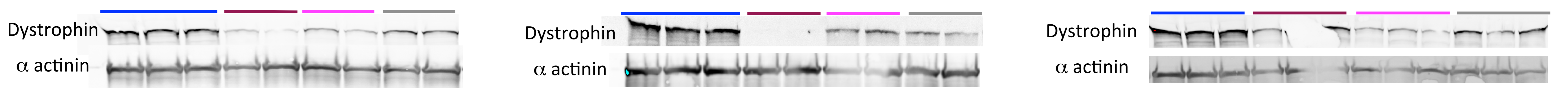
RESULTS

A. Molecular parameters (W22)

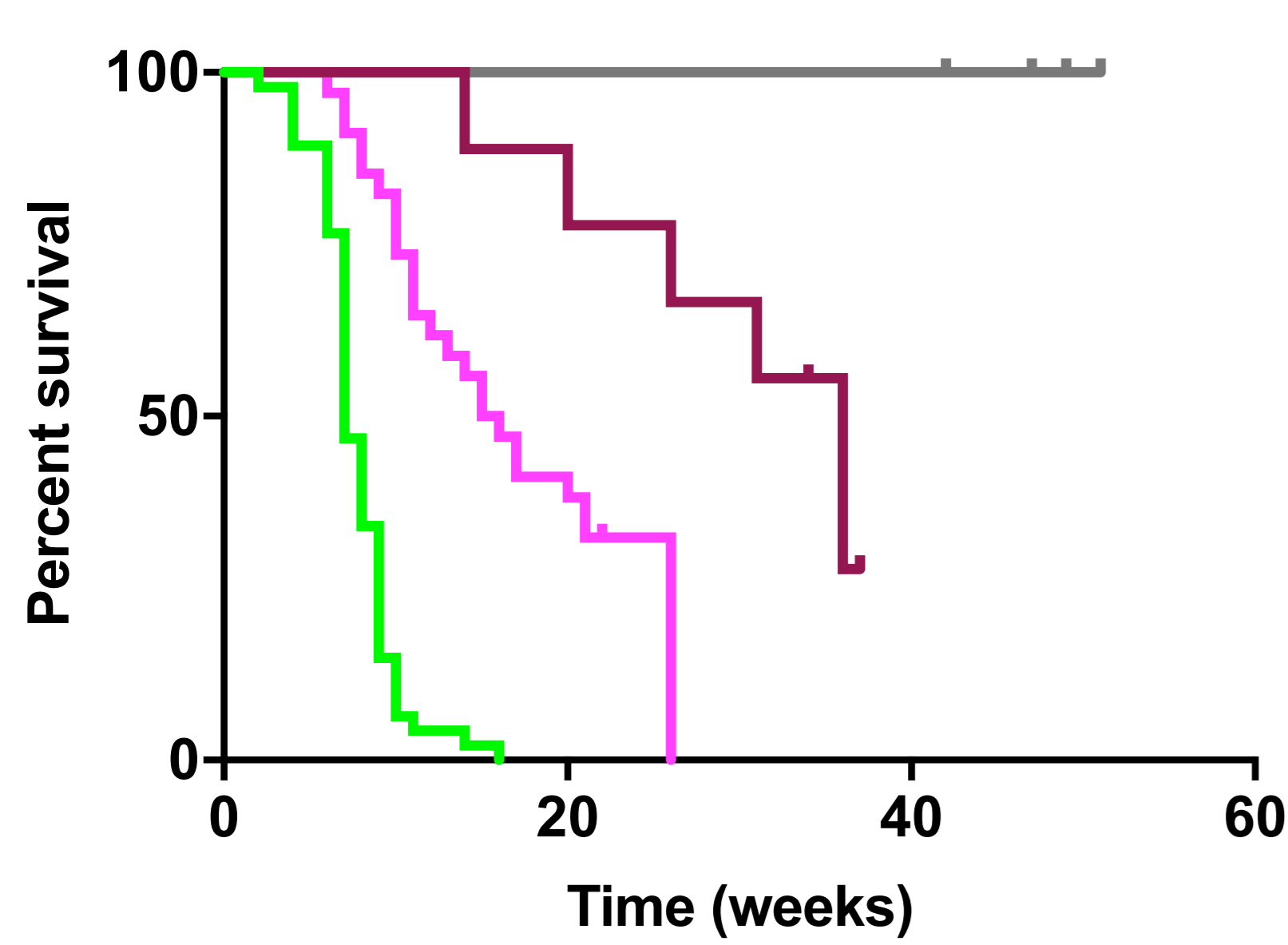
Viral genome number and exon skipping percentage quantification (Q-PCR)



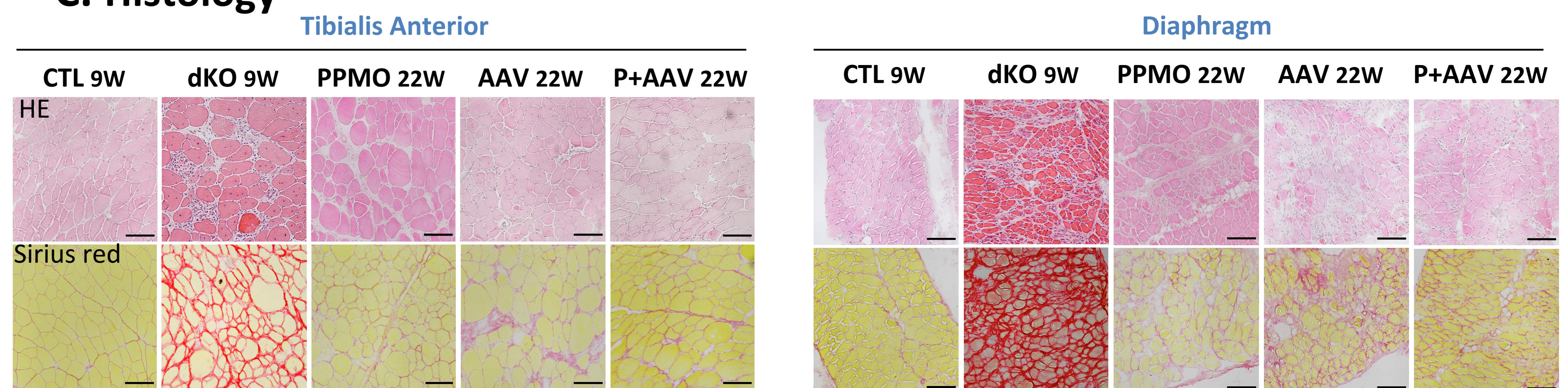
Dystrophin protein rescue (Western Blot)



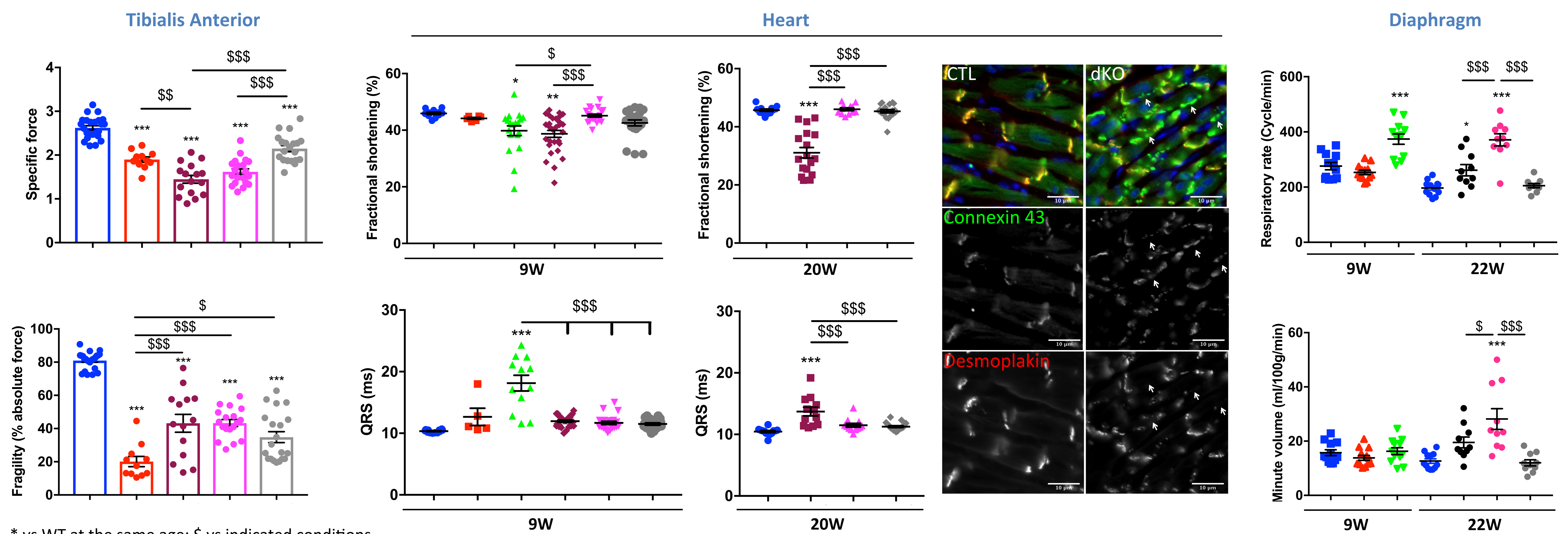
B. Survival



C. Histology



D. Function



* 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.