

Systemically delivering dystrophin expressing chimeric (DEC) cells circumvents an immune response and improves various functional characteristics

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Abstract

Background: Over the past decade different stem cell (SC) based approaches were tested to treat Duchenne Muscular Dystrophy (DMD), a lethal X-linked disorder caused by mutations in dystrophin gene. Despite continued research efforts, there is no curative therapy for DMD. Allogeneic SC therapies aim to restore dystrophin in the affected muscles; however, they are challenged by immune rejection and limited engraftment. Thus, there is a need to develop new more efficacious SC therapies. Chimeric cells, created via ex vivo fusion of donor and recipient cells, represent a promising therapeutic option for tissue regeneration. Previously we have shown that by fusing normal myoblasts (dystrophin expressing) with the recipient's myoblasts (no dystrophin expression) the immune system was bypassed. We have also shown that local delivery strengthens the gastrocnemius muscle for at least 90 days after injection.

Objectives: Investigate the ability of fused myoblasts to systemically engraft into and functionally benefit mdx mice.

Approach: DMD and normal human myoblasts were fused and systemically injected – by intraosseous injection – into mdx/scid mice. Efficacy of myoblast fusion was confirmed by flow cytometry and dystrophin immunostaining, while proliferative and myogenic differentiation capacity of chimeric cells were assessed in vitro. The mice were assessed 90 days later for efficacy of transplants, signs of histologic benefits and functional benefits.

Results: Interosseous delivery of chimeric cells did increase multiple functional parameters 90 days after injection. Histology identified the presence of chimeric cells in multiple muscle tissues. The recipient mice demonstrated increased cardiac function by echocardiography. Plethysmography also demonstrated significant benefits for the recipient mice.

Conclusion: Our study confirmed feasibility and efficacy of chimeric cell therapy and represents a novel satellite cell based approach for treatment of MD.

Background: Dystrophin expressing chimeric cells engraft well and increase muscle strength.

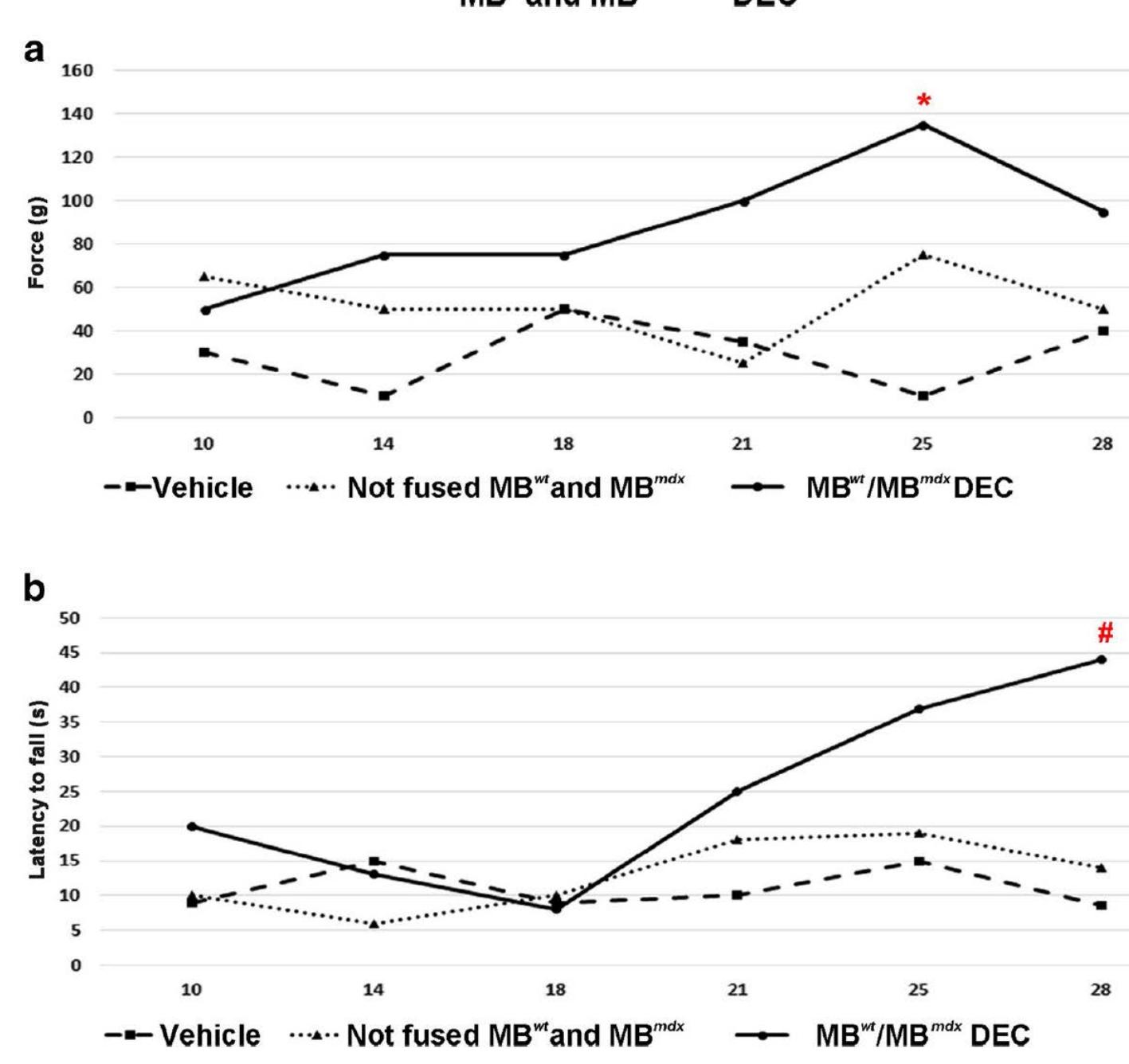
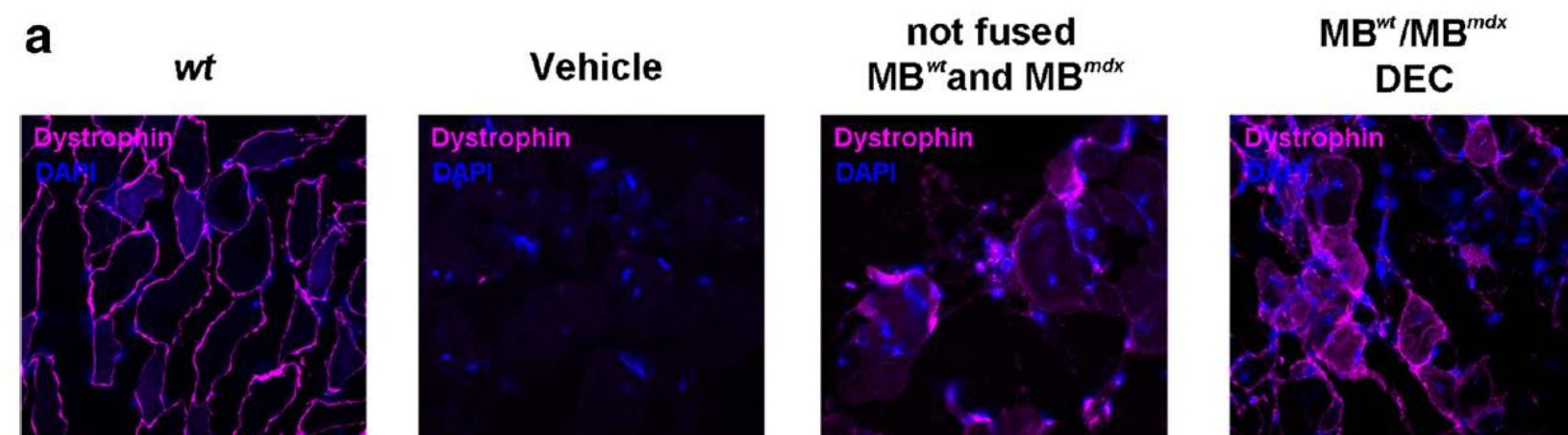
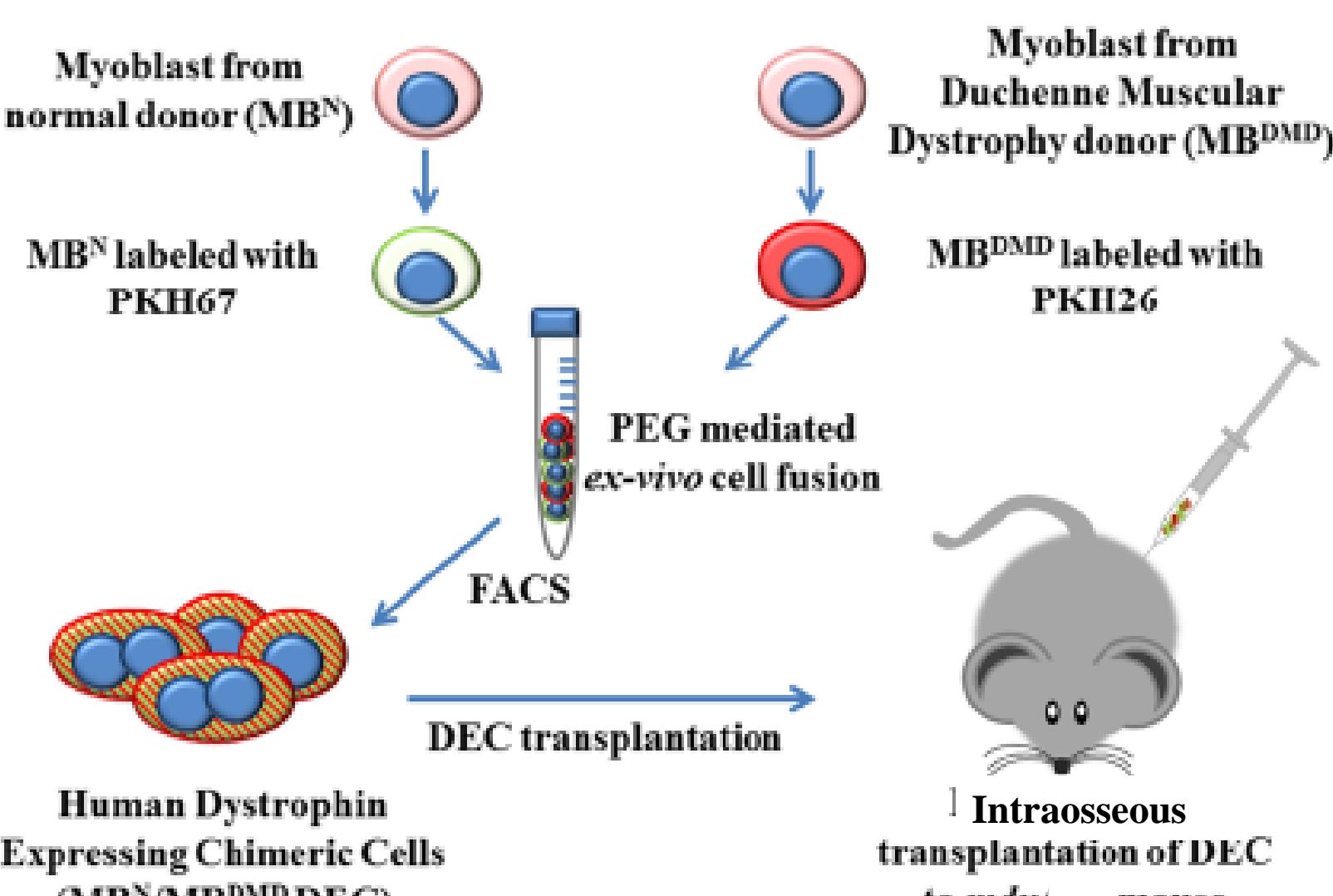


Figure 1. Dystrophin is expressed in gastrocnemius muscle after muscle direct injection of myoblasts cells. The fused cells engraft better and are most beneficial to muscle function. (N=6).

Methods:

Seven different cell populations were analyzed after interosseous injections.

- Vehicle
 - Myoblast normal 1 fused with normal 2; 500,000 cells (MBN1/MBN2)
 - Myoblast normal fused with myoblast DMD; 500,000 cells (MBN/MBDMD)
 - Myoblast normal fused with myoblast DMD; 1 mill cells (MBN/MBDMD 1M)
 - Myoblast normal 1 mixed with normal 2; 500,000 cells (MBN1+MBN2)
 - Myoblast normal mixed with myoblast DMD; 500,000 cells (MBN+MBDMD)
 - Myoblast normal mixed with myoblast DMD; 1 mill cells (MBN+MBDMD 1M)
- All cells were obtained from human normal or DMD patients (Lonza (Mapleton, IL)) and utilized between passages 5-7. Muscle-ness and differentiation ability were confirmed in vitro. Interosseous injections were performed under isoflurane anesthesia through the greater trochanter of 12wk-old mice. Non-terminal assessment procedures (echo and plethysmography) occurred at 30d, 60d and 90d post transplant. Terminal procedures (in vivo gastrocnemius and ex vivo gastrocnemius assessments) were done at 90d post transplant. In addition, serum and muscle tissues were acquired for post-mortem analyzes including: immunofluorescence, immunoblot, and creatine kinase assays.



Results

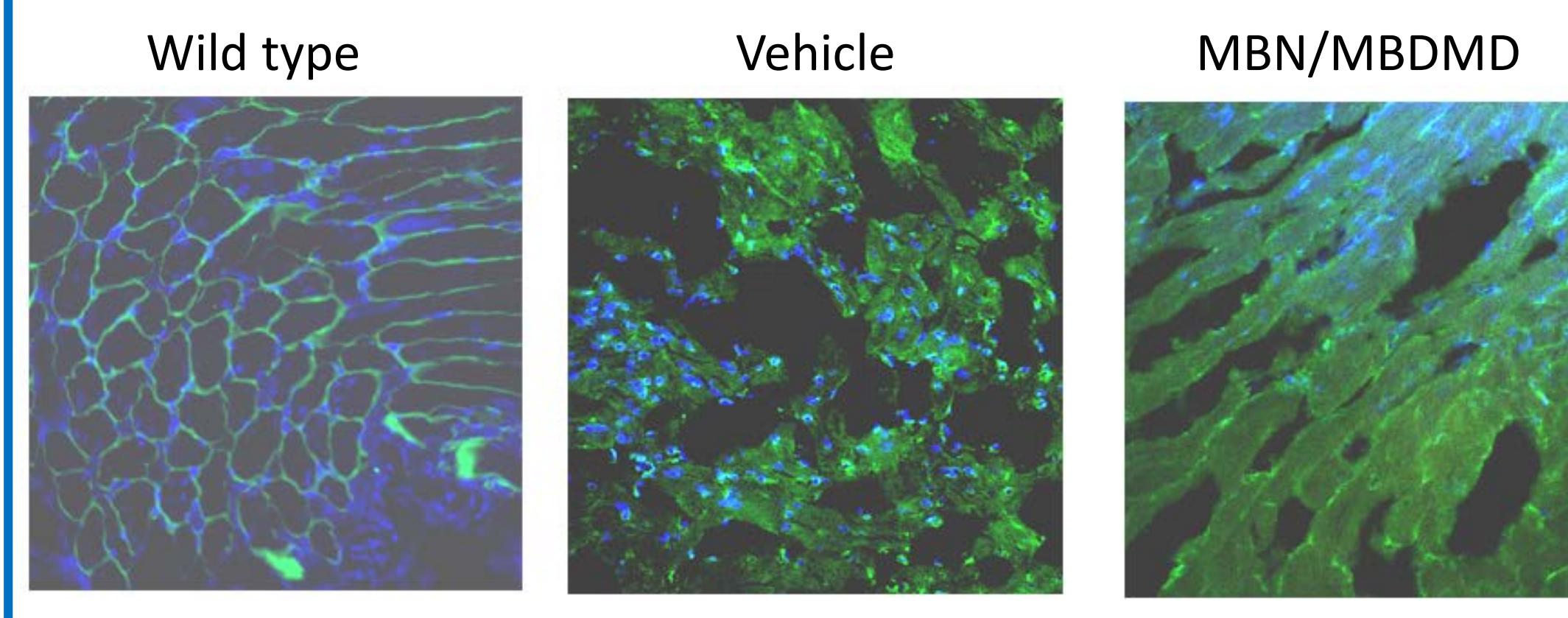


Figure 3. Dystrophin is expressed in cardiac muscle tissue after interosseous DEC transplantation. Immunofluorescence with primary dystrophin antibody from Abcam (ab15277, green) and DAPI (blue) counterstain. Magnification: 25x; Zeiss LSM 710 confocal microscope.

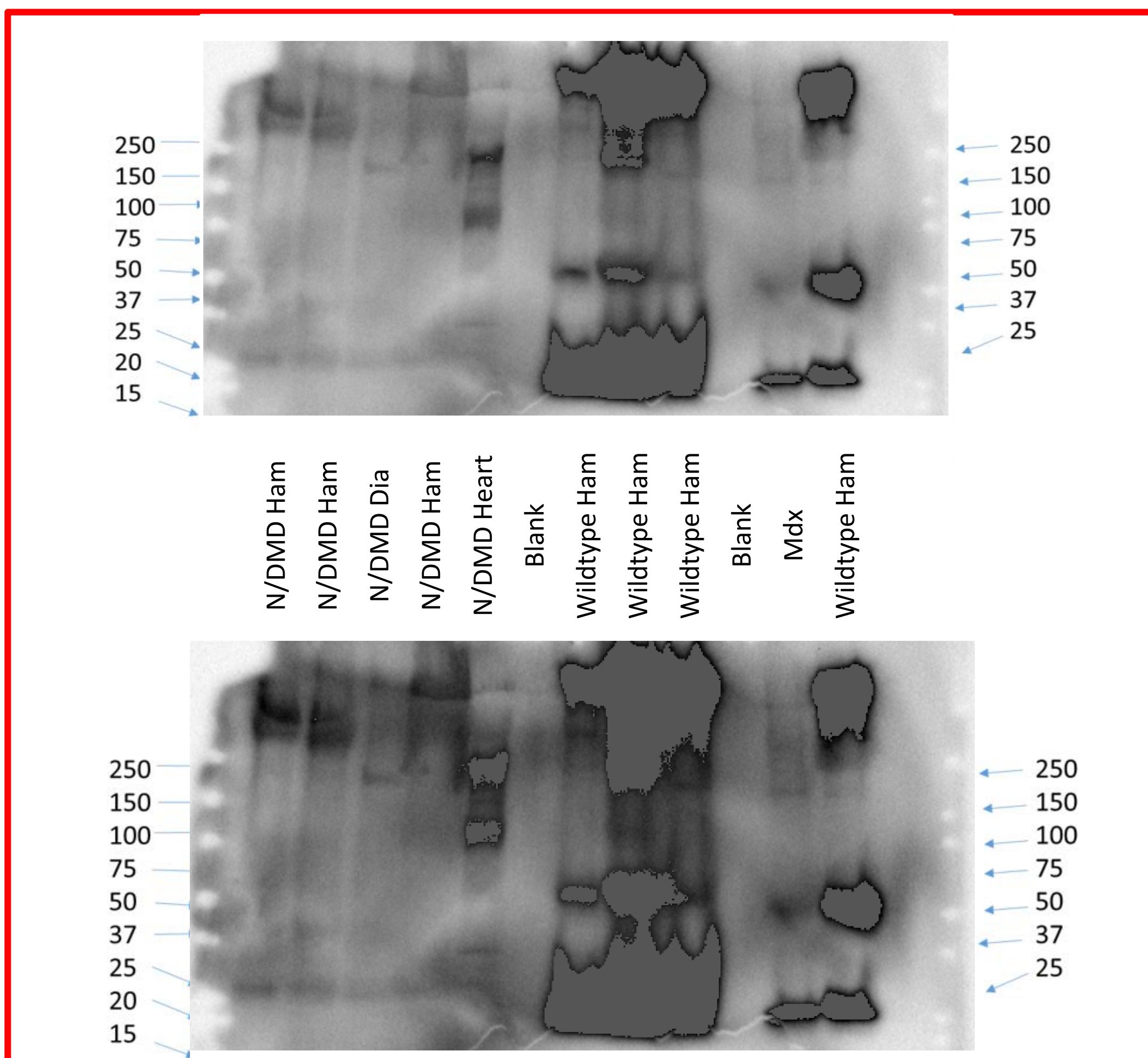


Figure 4. Dystrophin is expressed in hamstrings, maybe diaphragm and possibly cardiac tissues by WB. 60ug of protein/lane. Mandys antibody, Sigma.

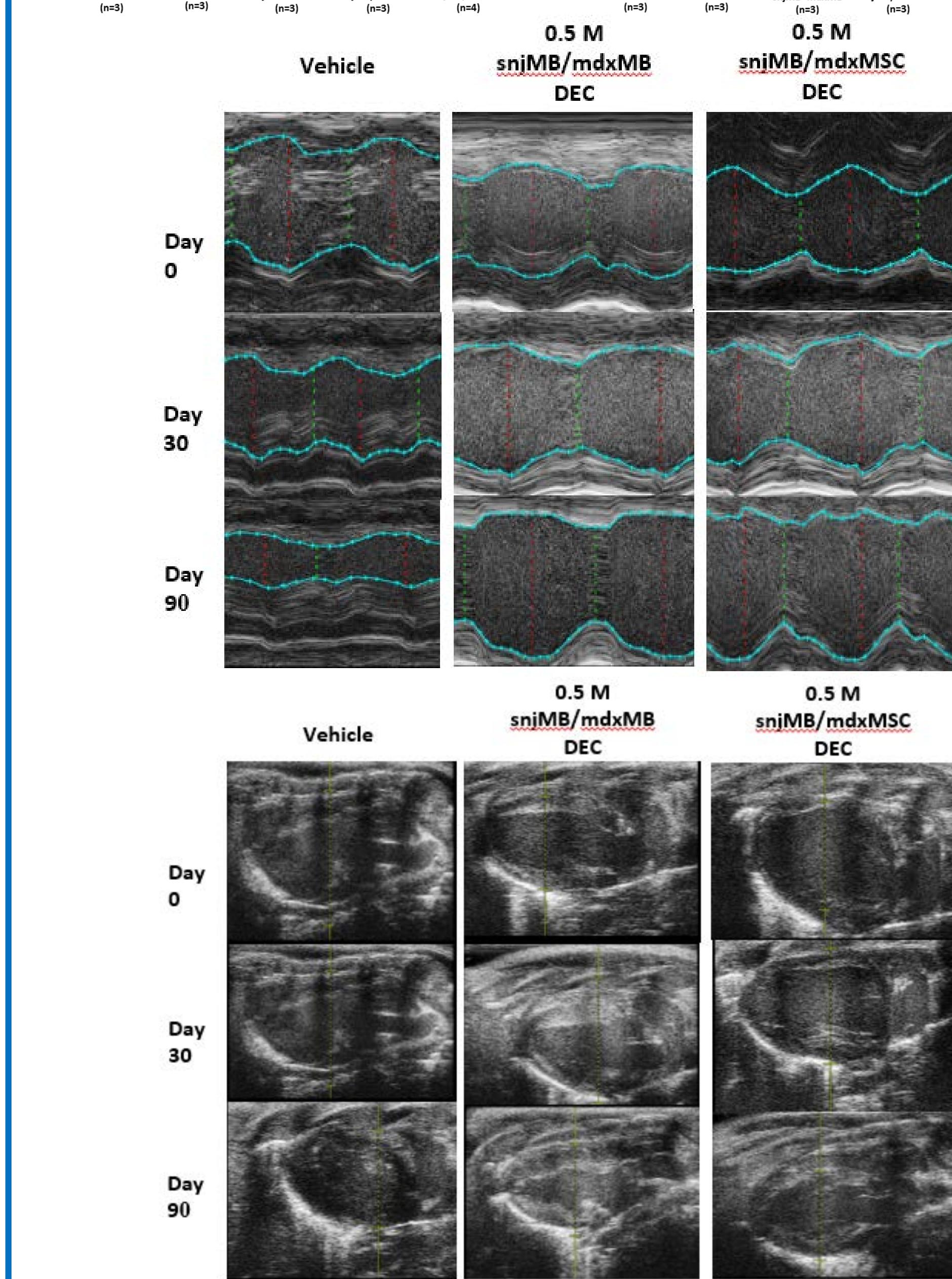
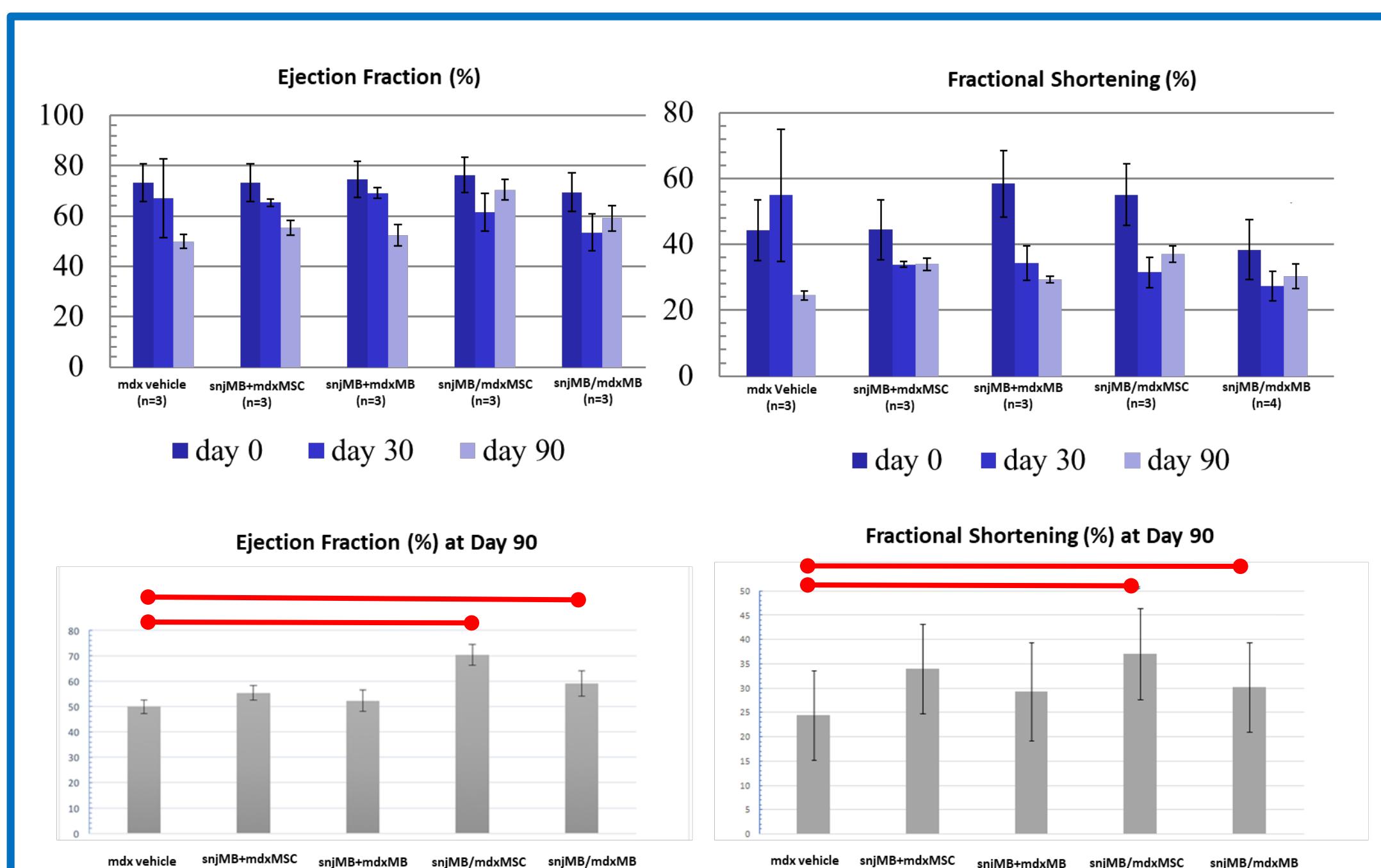


Figure 5. Reduced cardiac disease by echocardiography at 0, 30d and 90d post-transplant (Veo2100).

snj = normal = wildtype
MB = myoblasts
MSC = mesenchymal stem cells

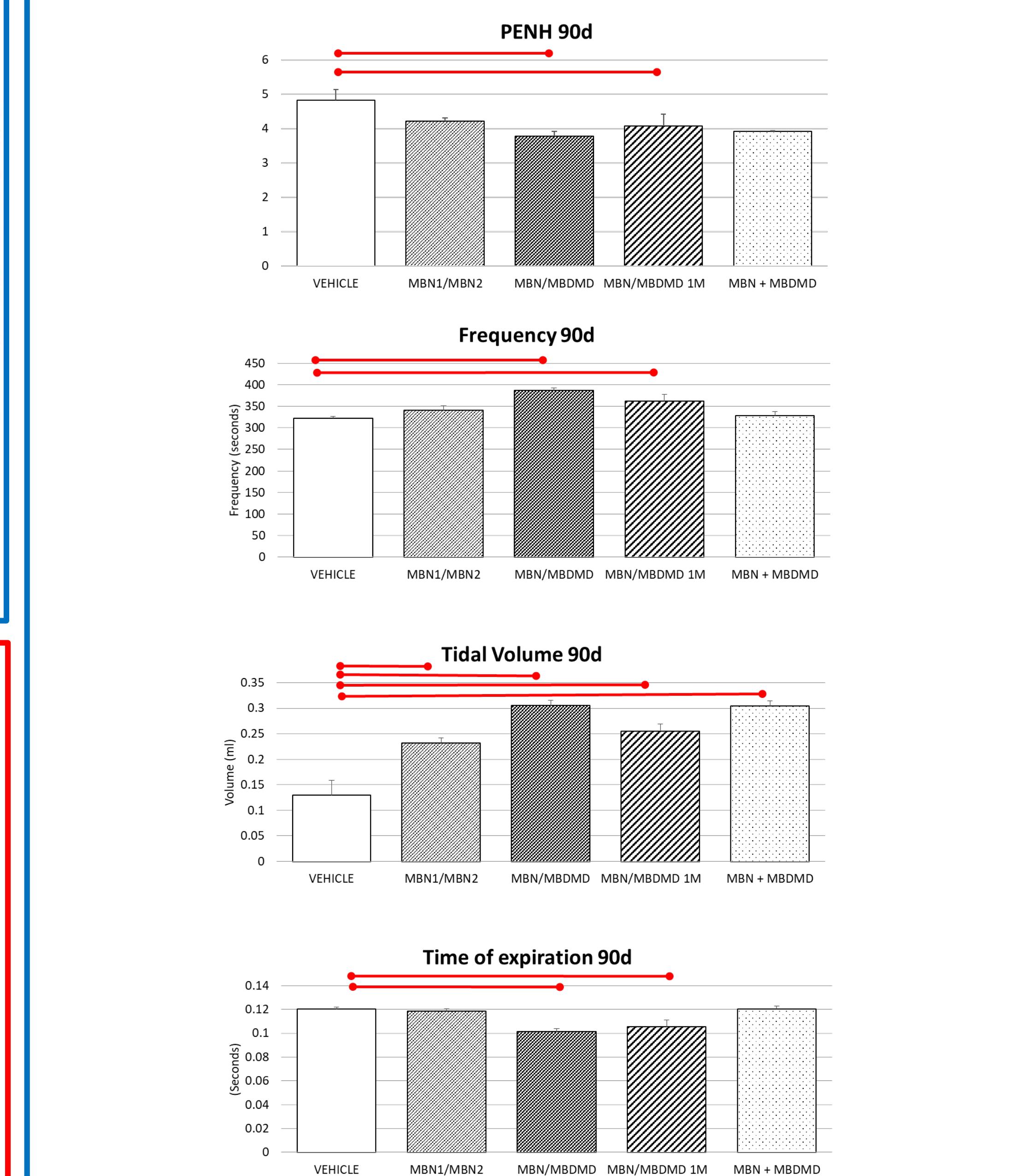


Figure 6. Reduced mdx-mediated respiratory disease assessed by plethysmography at 90d post transplant. Buxco Instrument (N>6).

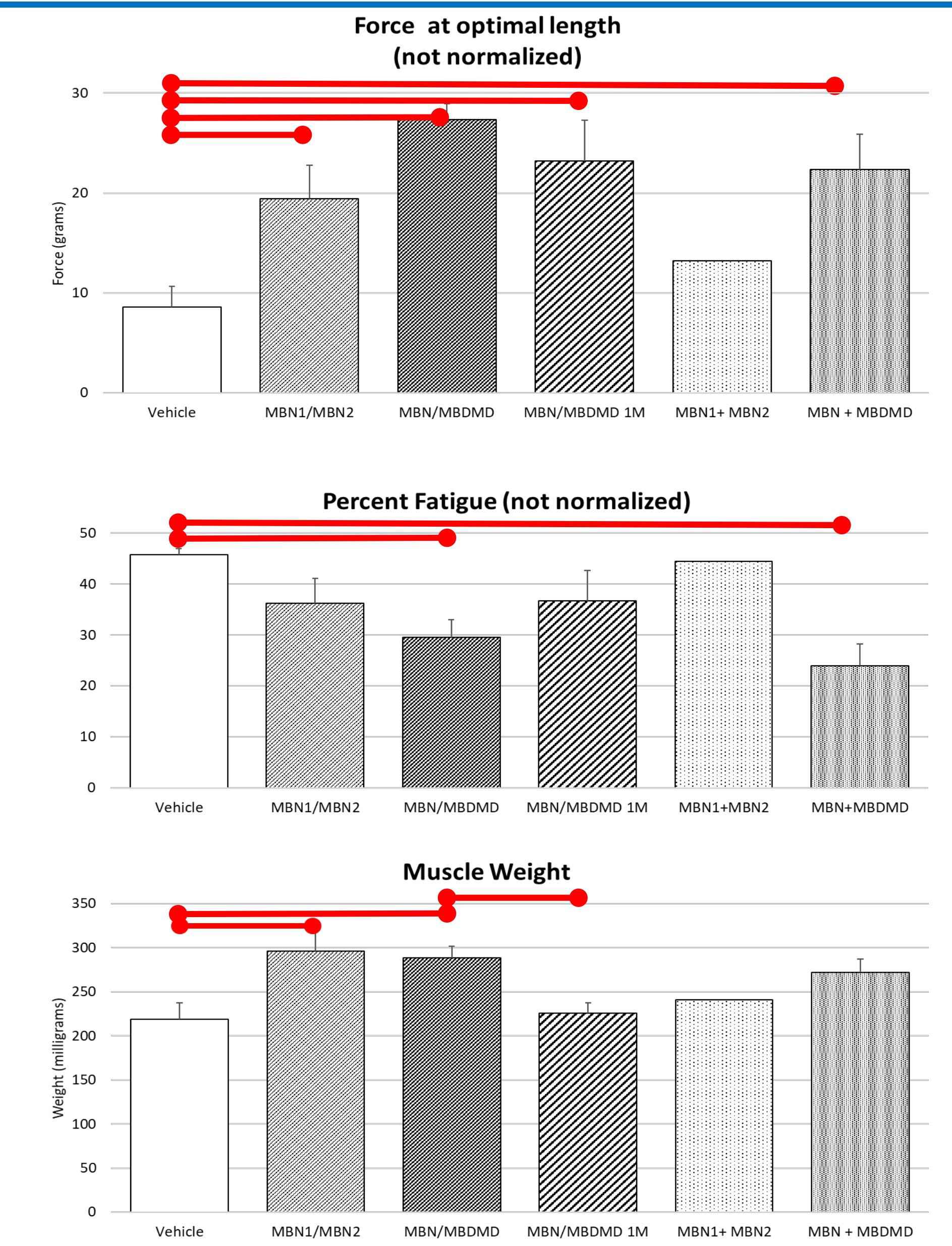


Figure 8. Reduced skeletal muscle disease assessed by improved ex vivo muscle force, reduced fatigue and increased muscle weight (N>3, except MBN1+MBN2 N=2)

Conclusions: So far we have demonstrated that chemically fused muscle cells:

- 1) When one donor is self, the immune system is circumvented
- 2) When one donor is wildtype, dystrophin is expressed
- 3) Engraft better than mixed, co-injected cells
- 4) Improve multiple functional characteristics
- 5) Interosseous injections deliver cells systemically
 - 1) Dystrophin is detected in diaphragm, heart and skeletal muscles.
 - 2) Confer benefits to diaphragm, heart and skeletal tissues

Future Directions

Additional immunofluorescence and western blot experiments are being conducted to attempt a quantification of dystrophin restoration. Additional animals are being analyzed.

References:

- Creation of Dystrophin Expressing Chimeric Cells of Myoblast Origin as a Novel Stem Cell Based Therapy for Duchenne Muscular Dystrophy. Siemionow M, Cwykiel J, Heydemann A, Garcia-Martinez J, Siemionow K, Szilagyi E. *Stem Cell Rev.* 2018 Apr. PMID: 29305755

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