Supplementary Note

The experimental mice used in this study were originally purchased from the Jackson Laboratory (Bar Harbor, Maine). The colonies were subsequently established by in-house breeding at the University of Missouri and mice were housed in a specific-pathogen-free animal facility at 20-23 ºC with a 12 hr-12 hr light-dark cycle. To minimize inter-animal differences, we have intentionally performed a paired study between the left (co-infected with both donor and acceptor viruses) and the right (infected with only acceptor virus or mock-infected with HEPES saline) EDL muscles of the same mouse to evaluate the physiological effect of *trans*-splicing vector therapy. Statistical analysis revealed a significant improvement in the specific force and a stronger resistance to eccentric contraction-induced injury in the left EDL muscles of adult *mdx* mice (Fig. 4). Interestingly, the overall specific forces in this group of experimental mice were significantly lower than what we have seen previously in age and sex matched *mdx* mice 1.

To investigate this difference, we examined the muscle pathology in untreated muscles (the right EDL muscles) from both studies. We first compared the number of myofibers carrying centrally localized nuclei. Surprisingly, the EDL muscles from this study showed 85.09 ± 4.37 % central nucleation, a value significantly higher than what we have observed in our previous study (70.02 ± 2.30 %) (Supplementary Fig. 1; two-tail, unpaired t test, $P < 0.023$). Another hallmark of *mdx* pathology is sarcolemma injury. This can be assessed by the uptake of either vital dye (such as Evans blue) or serum proteins (such as immunoglobin) by muscle cells 2, 3. To estimate the extent of myofiber injury, we performed immunostaining with Texas-red conjugated anti-mouse IgG (1:100, Jackson ImmunoResearch Laboratories, Inc, West Grove, Pennsylvania). The IgG infiltration in damaged fibers results in cytosolic staining. Consistent with the central nuclear quantification result, muscles from the current study were more severely injured. On average, the IgG infiltration reached 23.54 ± 7.34 % in this study. However, the IgG infiltration was only 8.00 ± 0.71% in the previous study (Supplementary Fig. 1; two-tail, unpaired t test, $P < 0.049$). Taken together, the adult *mdx* mice used in this study seemed to have a more severe pathology than the adult mice we used before. Since our sentinel mouse

monitoring did not show any sign of secondary infection by rodent virus and/or bacteria, the exact mechanism(s) underlying our observation remain to be investigated. Nevertheless, the difference in muscle pathology dose not undermine the significant protection we have observed following *trans*-splicing vector therapy.

References

1. Liu, M. et al. Adeno-associated Virus-mediated Micro-dystrophin Expression Protects Young Mdx Muscle from Contraction-induced Injury. *Mol Ther* **11**, 245-256 (2005).

2. Straub, V., Rafael, J.A., Chamberlain, J.S. & Campbell, K.P. Animal models for muscular dystrophy show different patterns of sarcolemmal disruption. *J Cell Biol* **139**, 375-385. (1997).

3. Hainsey, T.A., Senapati, S., Kuhn, D.E. & Rafael, J.A. Cardiomyopathic features associated with muscular dystrophy are independent of dystrophin absence in cardiovasculature. *Neuromuscul Disord* **13**, 294-302 (2003).