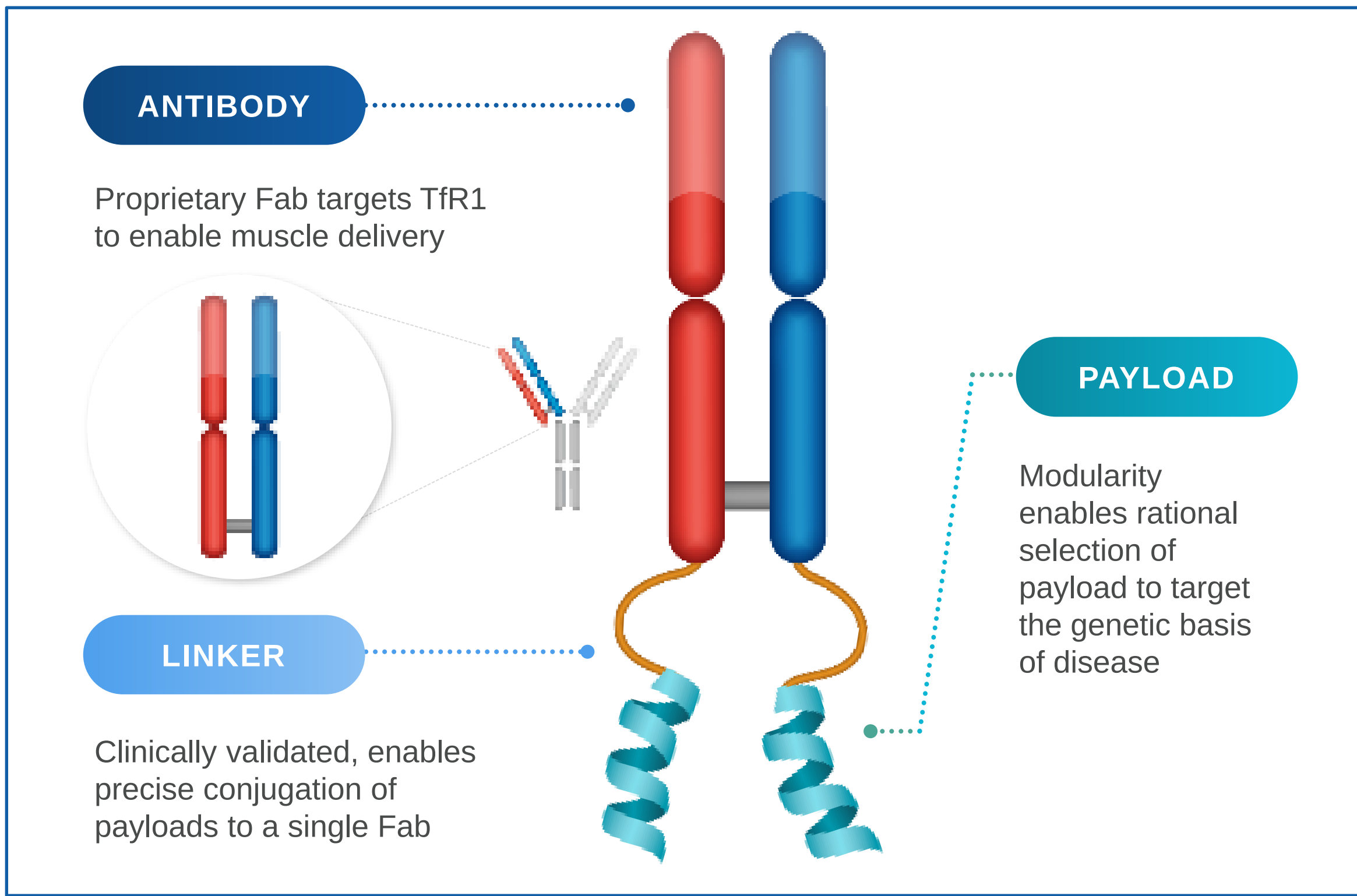


BACKGROUND

- Duchenne muscular dystrophy (DMD) is caused by mutations in the *DMD* gene resulting in the loss of functional dystrophin protein production
- Current therapies for DMD use phosphorodiamidate morpholino oligomers (PMOs) to induce exon skipping in the dystrophin pre-mRNA, enabling the translation of a shortened but functional dystrophin protein
- However, the efficacy of currently approved therapeutic approaches using systemic administration of exon skipping PMO chemistry is limited by poor muscle delivery secondary to inefficient cellular uptake and rapid renal clearance.¹⁻⁵ Thus, there is a need for new strategies to efficiently deliver exon-skipping oligonucleotides to muscle in patients with DMD
- The FORCE™ platform was developed to overcome the limitations of oligonucleotide delivery to muscle. It consists of an oligonucleotide payload conjugated to an antigen-binding fragment (Fab) targeting the extracellular region of the human transferrin receptor 1 (hTfR1) which is expressed in muscles.⁶⁻⁸ This allows for the rational selection of payloads to target the genetic basis of disease
- In this study, we used the FORCE platform to deliver an exon-skipping PMO to muscle in *mdx* mice, an established preclinical model of DMD, wild type cynomolgus monkeys, and myotubes isolated from patients with DMD

Figure 1. Dyne FORCE™ Platform: Modern Oligo Therapeutics for Muscle Diseases

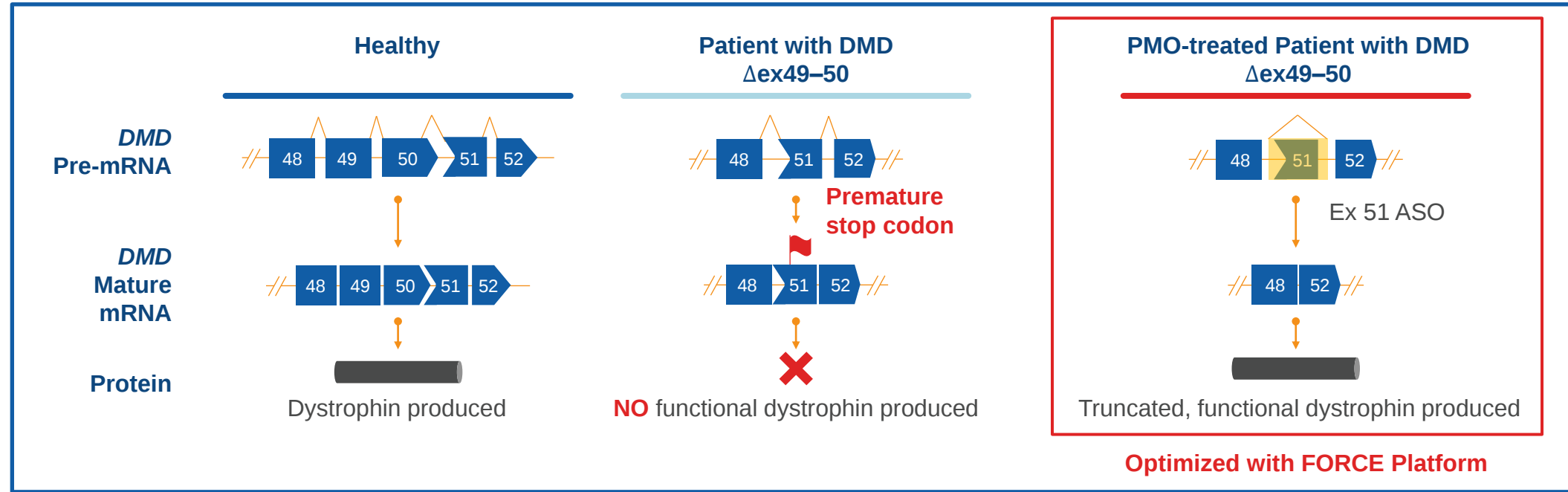


Fab, antigen-binding fragment; TfR1, transferrin receptor 1.

METHODS

- FORCE-M23D is a Fab-conjugated PMO designed to skip exon 23 of the mouse *Dmd* pre-mRNA. *Mdx* mice were administered with a single dose of FORCE-M23D containing the equivalent of 10 or 30 mg/kg PMO. Muscle PMO concentration, exon 23 skipping, and dystrophin protein were measured at multiple timepoints
- To determine translatability of mouse findings to higher species, Dyne's clinical candidate, DYNE-251, a Fab-conjugated PMO designed to skip exon 51, was evaluated for its ability to induce exon skipping in myotubes from patients with DMD amenable to exon 51 skipping and in wild-type non-human primates
- A GLP toxicology study was conducted in non-human primates (NHPs) to assess the safety profile of DYNE-251

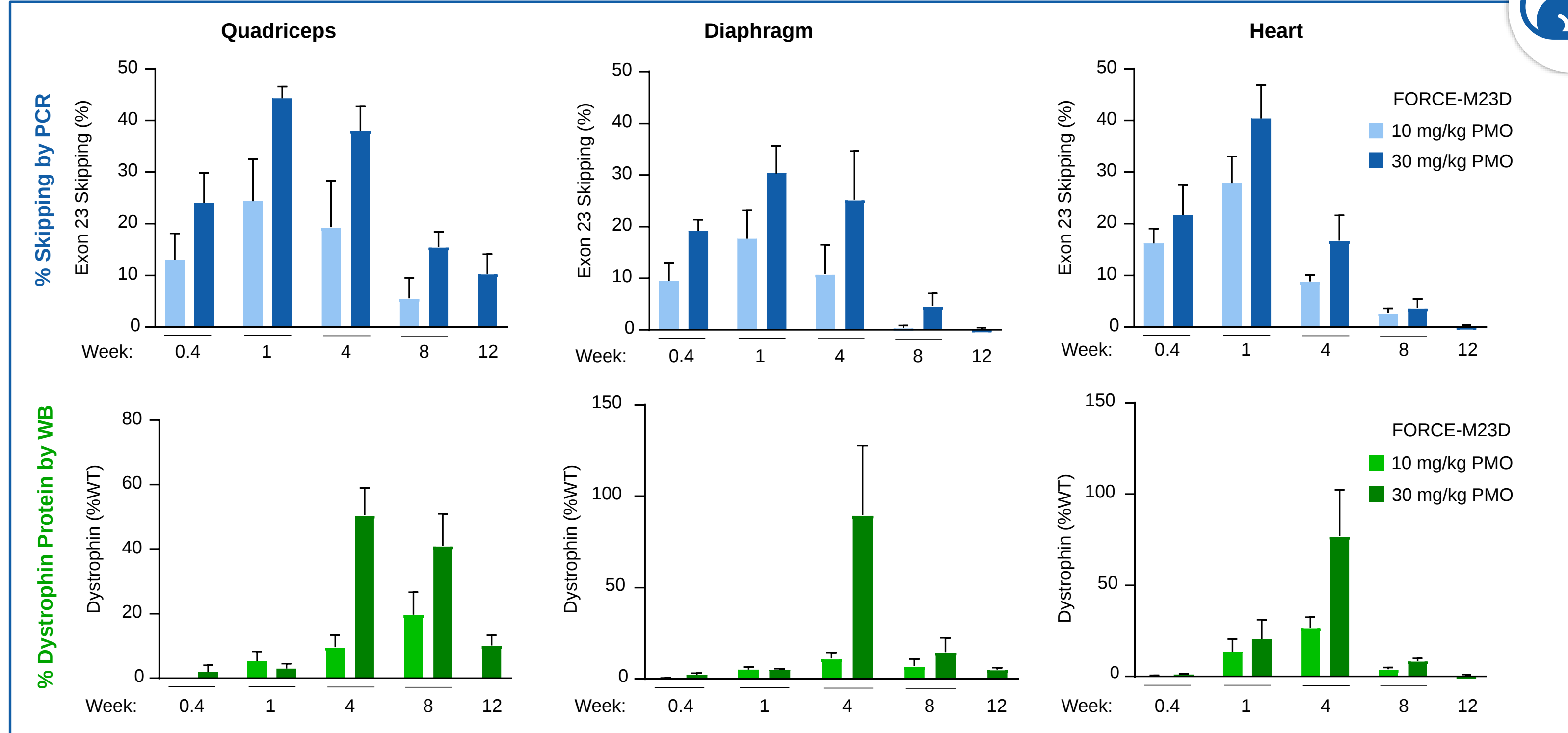
Figure 2. DYNE-251 Targets the Genetic Basis of DMD in Patients Amenable to Exon 51 Skipping



PMO, phosphorodiamidate morpholino oligomers; ASO, antisense oligonucleotide; DMD, Duchenne muscular dystrophy.

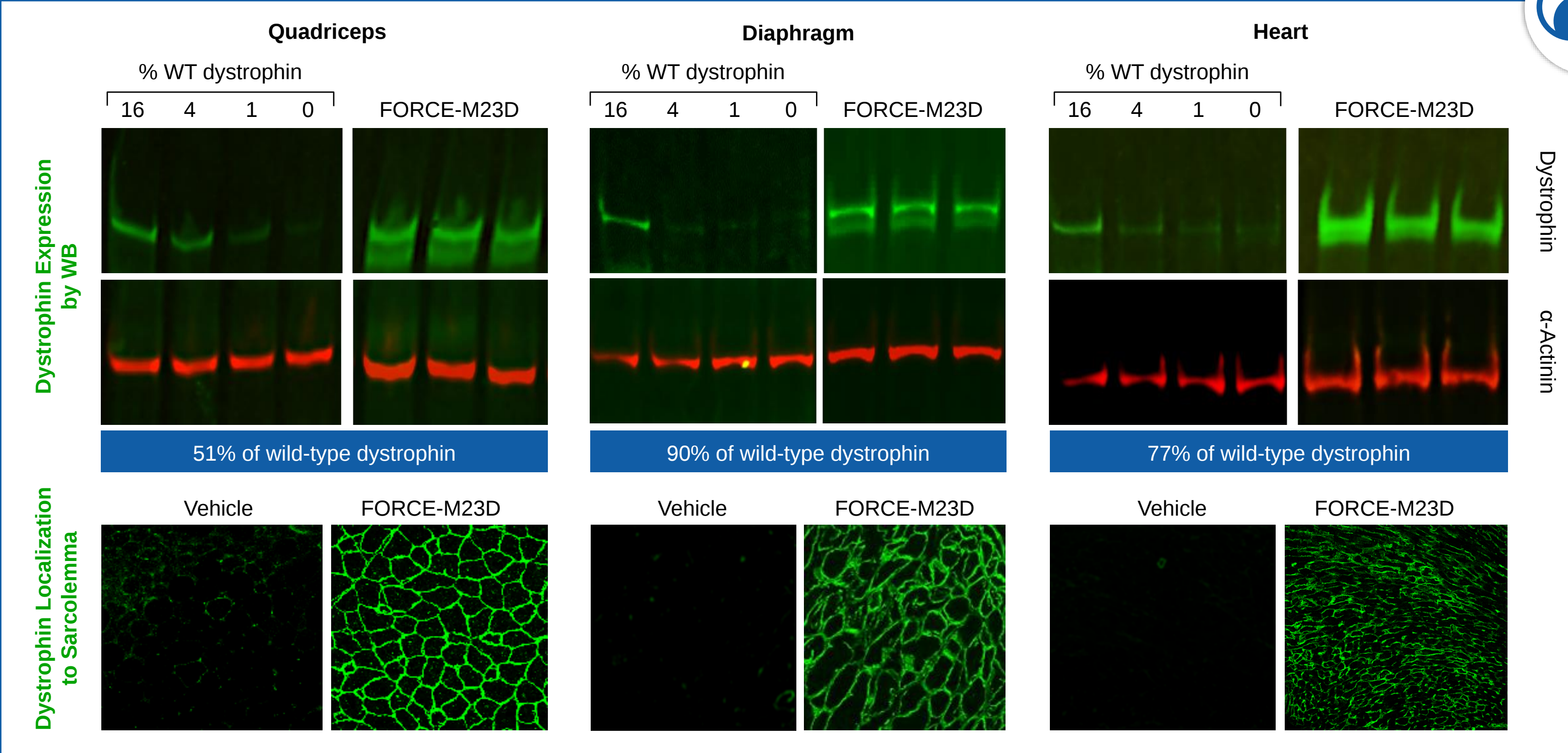
RESULTS

Figure 3: A Single Dose of FORCE-M23D Induces Dose-Dependent *Dmd* Exon 23 Skipping and Dystrophin Protein Expression in Heart and Skeletal Muscles of *mdx* Mice



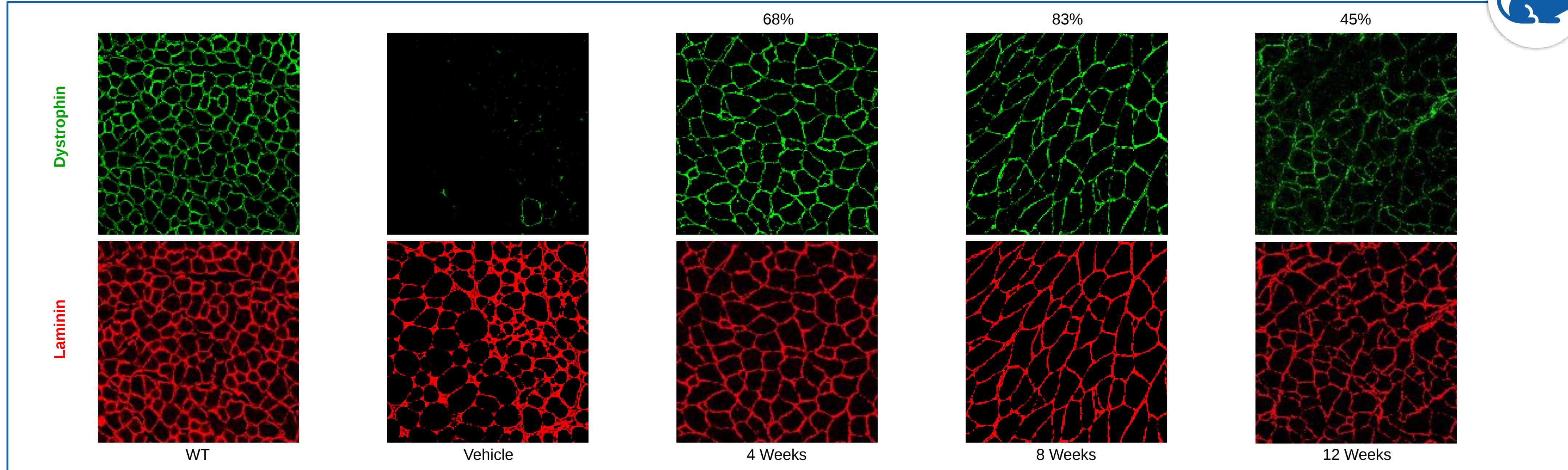
Exon 23 skipping was measured by RT-PCR and capillary electrophoresis. % Exon 23 Skipping = $\frac{\text{Molarity of Skipped Band}}{\text{Molarity of Skipped Band} + \text{Molarity of Unskipped Band}} \times 100$. Dystrophin levels were quantified by densitometry analysis of WBs. Data represent mean $\pm$ SD. PMO, phosphorodiamidate morpholino oligomer; RT-PCR, reverse transcription polymerase chain reaction; SD, standard deviation; WB, western blot; WT, wild type.

Figure 4: Single Dose of FORCE-M23D Achieves Durable Dystrophin Expression and Localization to Sarcolemma in Cardiac and Skeletal Muscle of *mdx* Mice



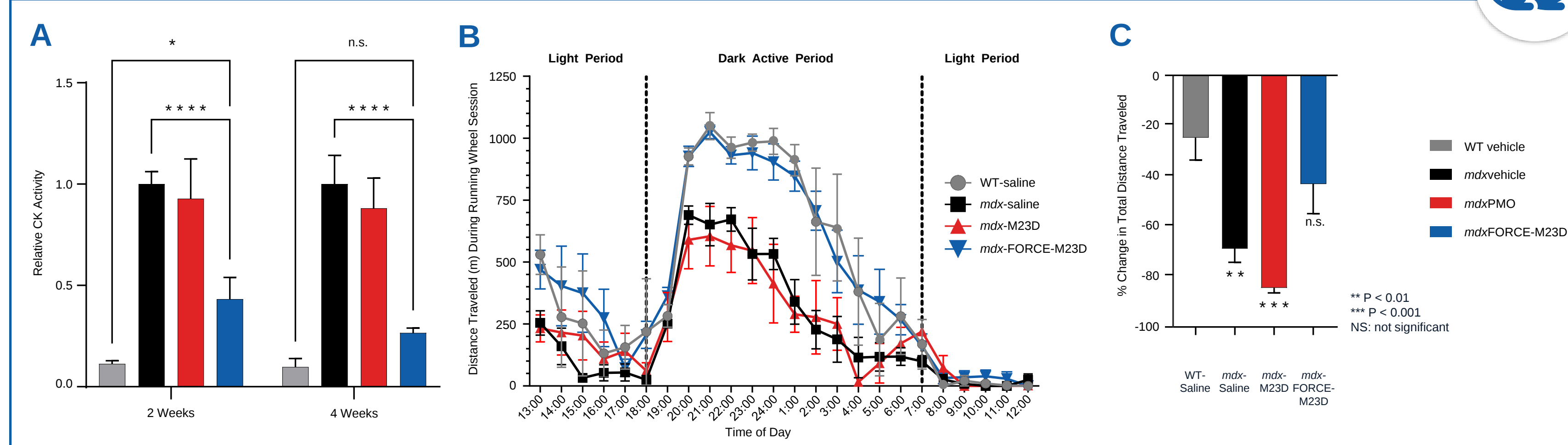
Representative WBs of dystrophin protein expression 28 days after a single 30-mg/kg dose of FORCE-M23D. Immunofluorescence images with dystrophin (green) staining of the same muscles isolated 28 days post-dose. WB, western blot; WT, wild type.

Figure 5: Up to 80% Dystrophin-Positive Fibers in Quadriceps Following Treatment With a Single Dose of FORCE-M23D



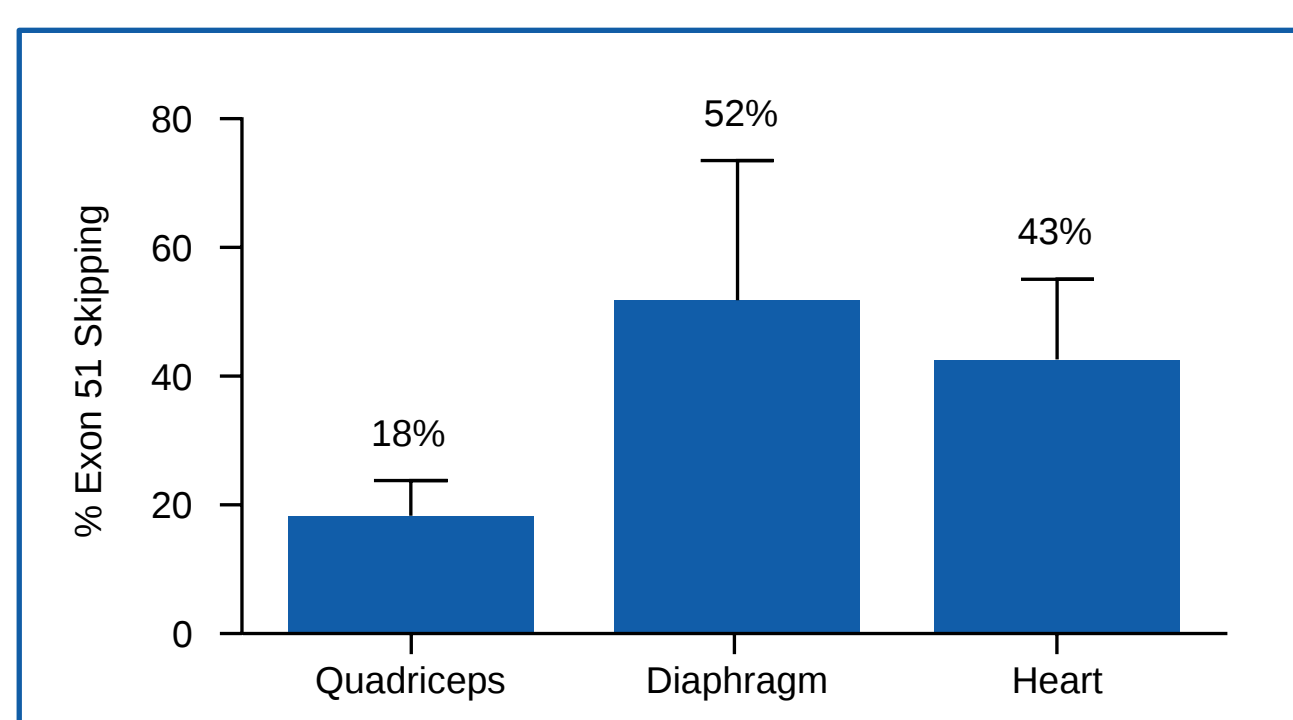
Immunofluorescence images with dystrophin (green) and laminin (red) staining of quadriceps cross-sections isolated from vehicle-treated WT or *mdx* mice 4 weeks post-dose or 4, 8, and 12 weeks post-dose from *mdx* mice treated with 30 mg/kg FORCE-M23D. WT, wild type.

Figure 6: Treatment With FORCE-M23D Leads to Improved Functional Outcomes in *mdx* Mice



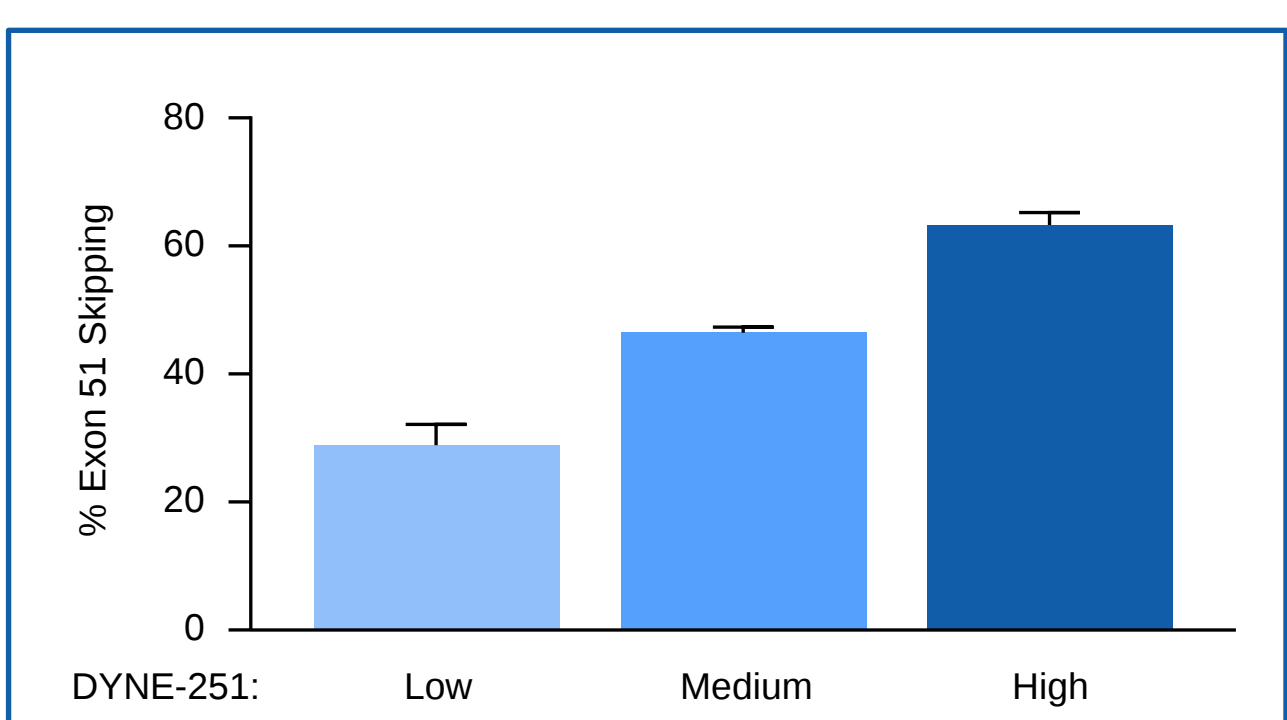
Functional assessments performed following administration of 5-week-old WT or *mdx* mice with vehicle or *mdx* mice injected with 30 mg/kg unjugated PMO or FORCE-M23D containing the equivalent of 30 mg/kg PMO. (A) Serum CK activity measured 2 or 4 weeks post-dose. (B) Total distance travelled on a running wheel for an uninterrupted 24-hour period 4 weeks post-dose. (C) Percent change in total distance travelled in an open field after hind limb fatigue challenge 2 weeks post-dose. Data are expressed as mean $\pm$ SD. * $P \leq .05$; ** $P \leq .01$; *** $P \leq .001$; **** $P \leq .0001$. CK, creatine kinase; n.s., not significant; PMO, phosphorodiamidate morpholino oligomer; WT, wild type; SD, standard deviation.

Figure 7: DYNE-251 Achieves Robust Exon Skipping in Cardiac and Skeletal Muscle of NHPs



NHPs received 5 weekly 30-mg/kg doses of DYNE-251 on weeks 0–4. Exon 51 skipping was measured on week 8. Data represent mean $\pm$ SD. NHP, non-human primate; SD, standard deviation.

Figure 8: DYNE-251 Achieves Dose-Dependent Exon Skipping in Patient-Derived Myotubes With DMD



Exon 51 skipping was in Myotubes of Patients with DMD after exposure to DYNE-251 for 10 days. Data represent mean $\pm$ SD. DMD, Duchenne muscular dystrophy; SD, standard deviation.

DYNE-251 Demonstrated a Favorable Safety Profile in a 5-Week GLP Toxicology Study in NHPs*

- No dose limiting toxicity observed after five weekly doses up to a maximally feasible dose
- No changes in cardiac, respiratory, neurologic, or ophthalmic endpoints
- No effect on kidney function
- No effect on liver function
- No effect on coagulation
- NOAEL was identified at the highest dose tested

NHP, non human primate; NOAEL, NOAEL: No-observed-adverse-effect level
*Based on conclusions of report from third-party CRO

CONCLUSIONS

- In the *mdx* mouse model of DMD, FORCE-M23D achieved: dose-dependent, robust, and durable exon skipping and dystrophin expression as well as long-lasting dystrophin sarcolemma localization and improvement in functional outcomes
- DYNE-251 demonstrated robust exon skipping in NHPs' cardiac and skeletal muscles and in myotubes bearing a DMD mutation amenable to exon 51 skipping
- The FORCE platform may provide a promising approach to addressing DMD

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DISCLOSURE INFORMATION All authors are employees of Dyne Therapeutics Inc. and may hold Dyne stock and/or stock options. These data were previously presented at the 2022 MDA Clinical and Scientific Conference (Desjardins C.A., et al. Poster 139) and at the 2021 Muscle Study Group Annual Meeting (Beskrovnaya O., et al.).