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*mdx* mice exhibit anxiety-induced freezing behavior in response to restraint<sup>6</sup>. To evaluate whether the FORCE platform can correct this phenotype, we generated the hTfR1/*mdx* mouse model expressing human TfR1. The hTfR1/*mdx* mouse recapitulates the anxiety-induced freezing behavior of the parental *mdx* strain. This model allowed us to test the efficacy of a human TfR1-targeting Fab and PMO conjugate designed to skip exon 23 of murine *Dmd* pre-mRNA (FORCE-M23D). A single intravenous dose of FORCE-M23D, but not unconjugated M23D or non-TfR1 targeting negative control conjugate, led to widespread PMO distribution to CNS in hTfR1/*mdx* mice. PMO delivery to CNS induced *Dmd* pre-mRNA exon skipping and restoration of dystrophin protein expression, leading to complete and durable resolution of anxiety-induced freezing behavior. To our knowledge, this is the only platform with demonstrated potential for a systemically-administered therapy to restore dystrophin protein in the brain.

**ANTIBODY**

Proprietary Fab targets Tfr1 to enable tissue delivery

**LINKER**

Cleavable valine-citrulline linker, enables precise conjugation of payloads to a single Fab

**PAYLOAD**

Modularity enables rational selection of payload to target the genetic basis of disease

**Tfr1-Mediated Transcytosis Across the BBB**

Tfr1  
FORCE

**hTfR1/mdx Mouse Model of DMD**

Uptakes human TfR1 targeting Fabs      Expresses mutant murine *DMD*

hTfR1      B10-*mdx*

hTfR1/mdx

5' 21 22 23 24 3'

Mouse *DMD* mRNA

**Mechanism of FORCE Delivery of Exon 23 Skipping PMO**

PMO      Linker      Fab

FORCE

TfR1

Cell Membrane

Nucleus

Reading frame disrupted

Dystrophin pre-mRNA      Exon 22      Exon 23      Exon 24

Splicing

Dystrophin mRNA      Exon 22      Exon 24

Timeline diagram of the behavioral experiment:

- Overall Trial Structure:**
  - Mouse Placed in Test Cage
  - Video Start
  - Total Trial Time (~5min)
  - Video Stop
  - Trial Start
  - Trial Stop
- Unrestrained (Motor Function Control):**
  - WT:** Shows a single blue block (Time frozen) around 2 minutes.
  - mdx:** Shows a single blue block (Time frozen) around 3 minutes.
- Restrained (Anxiety Assessment):**
  - WT:** Shows two blue blocks (Time frozen) around 2 minutes and 4 minutes.
  - mdx:** Shows multiple blue blocks (Time frozen) across the entire 5-minute period.
- Legend:**
  - Blue block: Time frozen
  - Grey line: Time active

**A.**

**Brain Vasculature**

Group	PMO Concentration (ng/g)
Vehicle	~0
Naked	~0
Control Fab	~150
FORCE	~1200

**B.**

**Brain Parenchyma**

Group	PMO Concentration (ng/g)
Vehicle	~0
Naked	~0
Control Fab	~30
FORCE	~230

**Legend:**

- Naked M23D
- Control Fab-M23D
- FORCE-M23D

**A. Tissue PMO Exposure (ng/g)**

Region	Vehicle	Naked	Control Fab	FORCE
Prefrontal Cortex	~0	~0	~150	~1050
Hippocampus	~0	~0	~100	~850
Cerebellum	~0	~0	~250	~1150

**B. Exon 23 Skipping (%)**

Region	Vehicle	Naked	Control Fab	FORCE
Prefrontal Cortex	~0	~0	~0	~2.3
Hippocampus	~0	~0	~0	~1.4
Cerebellum	~0	~0	~0	~9.0

Legend: Naked M23D (red), Control Fab-M23D (green), FORCE-M23D (blue). \*\*\*\* indicates p < 0.0001.

**A. hTfR1/*mdx* Mice Do Not Exhibit Freezing Behavior Without Restraint**

Condition	hTfR1 (%)	hTfR1/ <i>mdx</i> (%)
Unrestrained	~1	~1
Restrained	~3	~82

**B. Restraint-Induced Freezing is Durable Through 6 Months of Repeat Assessments**

Test Day	hTfR1 (%)	hTfR1/ <i>mdx</i> (%)
0	~48	~48
28	~0	~72
56	~0	~74
84	~0	~48
112	~0	~55
140	~1	~76
168	~4	~51

hTfR1 & hTfR1/*mdx* After Restraint

**A.** **Durable Resolution of Restraint-Induced Freezing Behavior in hTfR1/*mdx* Mice After a SD of FORCE-M23D**

Vehicle & FORCE Treated hTfR1/*mdx* Mice After Restraint

Time Frozen (%)

Days After Dosing

● Vehicle ■ FORCE-M23D

**B.** **Molecular Assessments 28 Days After a SD of FORCE-M23D**

PMO Exposure Exon 23 Skipping Dystrophin Protein

PMO Concentration (ng/g)

Exon 23 Skipping (%)

Dystrophin (% WT)

Cortex Deep Brain Cerebellum

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