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Supplemental Information

Identifying and Avoiding tcDNA-ASO Sequence-Specific Toxicity for the Development of DMD Exon 51 Skipping Therapy

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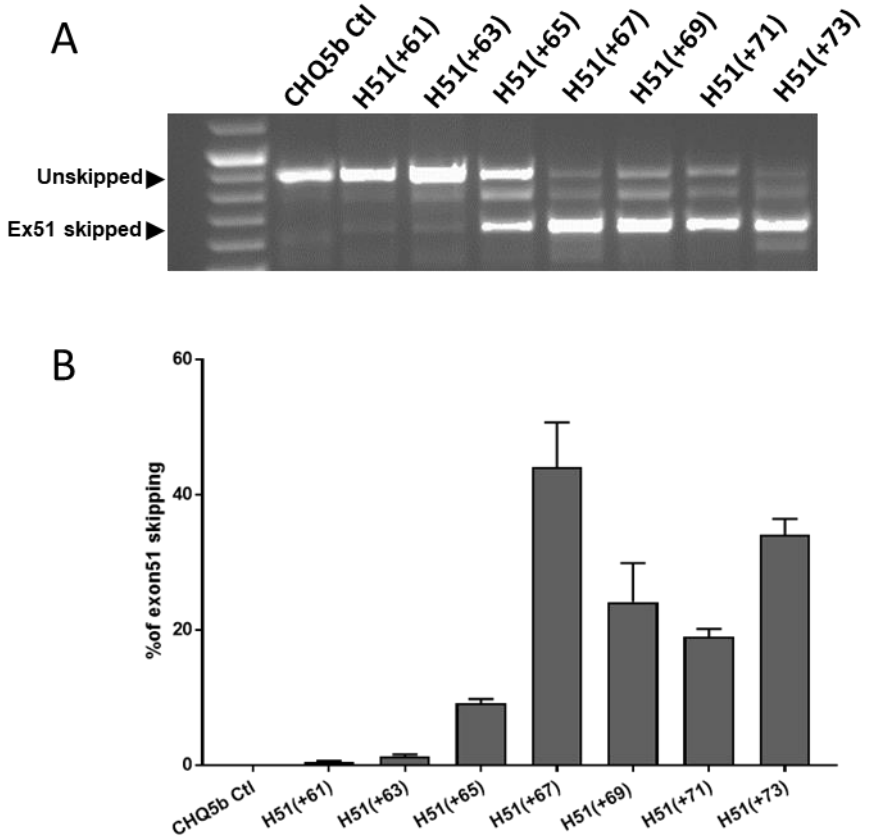
SUPPLEMENTAL MATERIAL

Figure S1



C

name	chemistry	sequence
H51(+61)	Full tcDNA-PS	5'-G*C*A*T*T*T*C*T*A*G*T*T*T*G*G-3'
H51(+63)	Full tcDNA-PS	5'-T*G*G*C*A*T*T*T*C*T*A*G*T*T*T-3'
H51(+65)	Full tcDNA-PS	5'-G*A*T*G*G*C*A*T*T*T*C*T*A*G*T-3'
H51(+67)	Full tcDNA-PS	5'-A*A*G* A*T*G* G*C*A* T*T*T* C*T*A-3'
H51(+69)	Full tcDNA-PS	5'-G*G*A*A*G*A*T*G*G*C*A*T*T*T*C-3'
H51(+71)	Full tcDNA-PS	5'-A*A*G*G*A*A*G*A*T*G*G*C*A*T*T-3'
H51(+73)	Full tcDNA-PS	5'-T*C*A*A*G*G*A*A*G*A*T*G*G*C*A-3'

Figure S1: In vitro screening of tcDNA efficacy. 300nm of the different tcDNA-ASOs were transfected in the human-derived skeletal muscle cell line CHQ5b and levels of exon 51 skipping were determined by qRT-PCR. A- representative gel of a transfection experiment. B- quantification by qRT-PCR of 3 transfection experiments. Results are expressed as Mean ± SEM. C- Sequence and chemistry of the tested compounds.

Figure S2

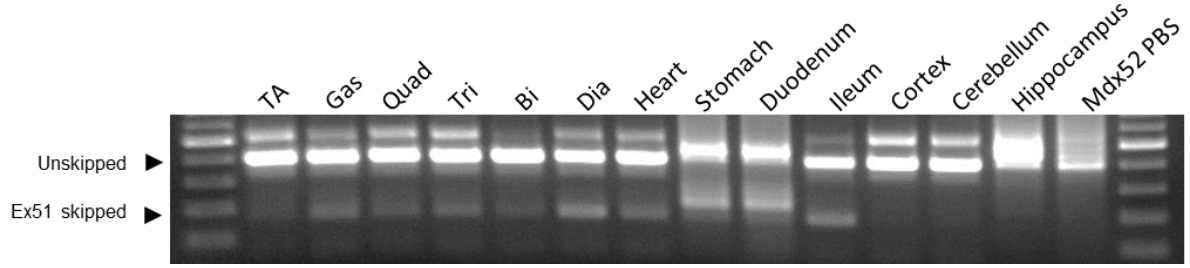


Figure S2: Evaluation of exon 51 skipping following H51(+67)W treatment. Representative gel showing exon 51 levels evaluated by RT-PCR in treated *mdx52* tissues 2 weeks after the end of the 12-week treatment at 200mg/kg/week. TA: tibialis anterior, Gas: Gastrocnemius, Quad: quadriceps, Tri: triceps, Bi: biceps, Dia: diaphragm.

Figure S3

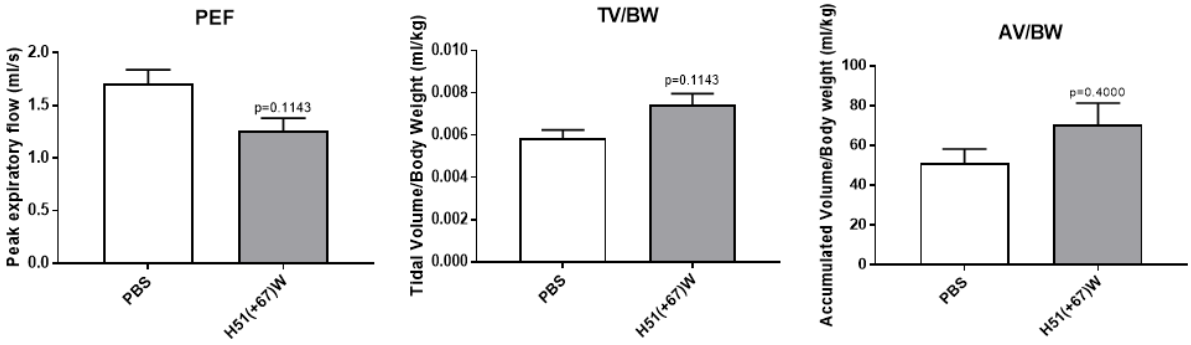


Figure S3: H51(+67)W treatment tends to improve respiratory function. Whole Body plethysmography was performed on PBS treated *mdx52* (n=3) and H51(+67)W treated *mdx52* (n=4) one week after the end of treatment. Different parameters were measured: Peak Expiratory Flow (PEF) (left), Tidal Volume normalized on Body Weight (TV/BW) (center) and Accumulated Volume Normalized on Body Weight (AV/BW) (right). Results are expressed as Mean ± SEM. P values are indicated for H51(+67)W treated *mdx52* compared to PBS.

Figure S4

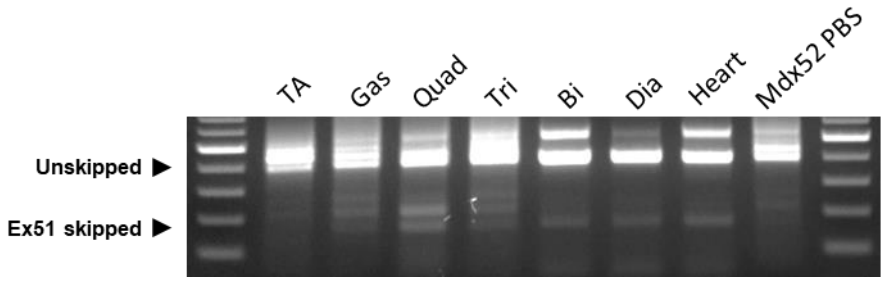


Figure S4: Evaluation of exon 51 skipping after 16 weeks of washout following H51(+67)W treatment. Representative gel showing exon 51 levels evaluated by RT-PCR in treated *mdx52* tissues 16 weeks after the end of the 12-week treatment at 200mg/kg/week. TA: tibialis anterior, Gas: Gastrocnemius, Quad: quadriceps, Tri: triceps, Bi: biceps, Dia: diaphragm.

Table S1

Amount for 1% Efficacy (1%EA)		TA	Gas	Quad	Tri	Dia	Heart	Mean
Analysis **2wks** WO	Quantity (µg/g)	47,5	23,3	47,6	43,6	100,6	57,2	
	Efficacy (%ex51 skipping)	4,6	12,3	10,4	5,1	15,1	12,6	
	1%EA *(Quantity/Efficacy)*	**10,3**	**1,9**	**4,6**	**8,6**	**6,7**	**4,5**	**6,1**
Analysis **16wks** WO	Quantity (µg/g)	1,7	5,2	5,9	3,3	11,3	14,2	
	Efficacy (%ex51 skipping)	1,9	5,4	3,3	2,0	4,7	2,0	
	1%EA *(Quantity/Efficacy)*	**0,9**	**1,0**	**1,8**	**1,6**	**2,4**	**7,1**	**2,5**

Table S1: 1%EA calculation. The 1%EA corresponds to the amount of H51(+67)W ASO required to achieve 1% of exon 51 skipping and is calculated based on the values measured after 2 weeks or 16 weeks of washout (WO).