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Supplemental Information

Efficacy and Safety Profile of Tricyclo-DNA Antisense Oligonucleotides in Duchenne Muscular Dystrophy Mouse Model

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Supplementary materials

Fig. S1. Evaluation of 13mer-tcDNA following intramuscular injection.

Fig. S2. *In vitro* complement activation analysis.

Fig. S3. Creatinine normalized renal injury biomarkers.

Fig. S4. Hybridization mediated-Off Target effects (OTEs) on mRNA mouse transcript.

Table S1. RT-qPCR primer sequences

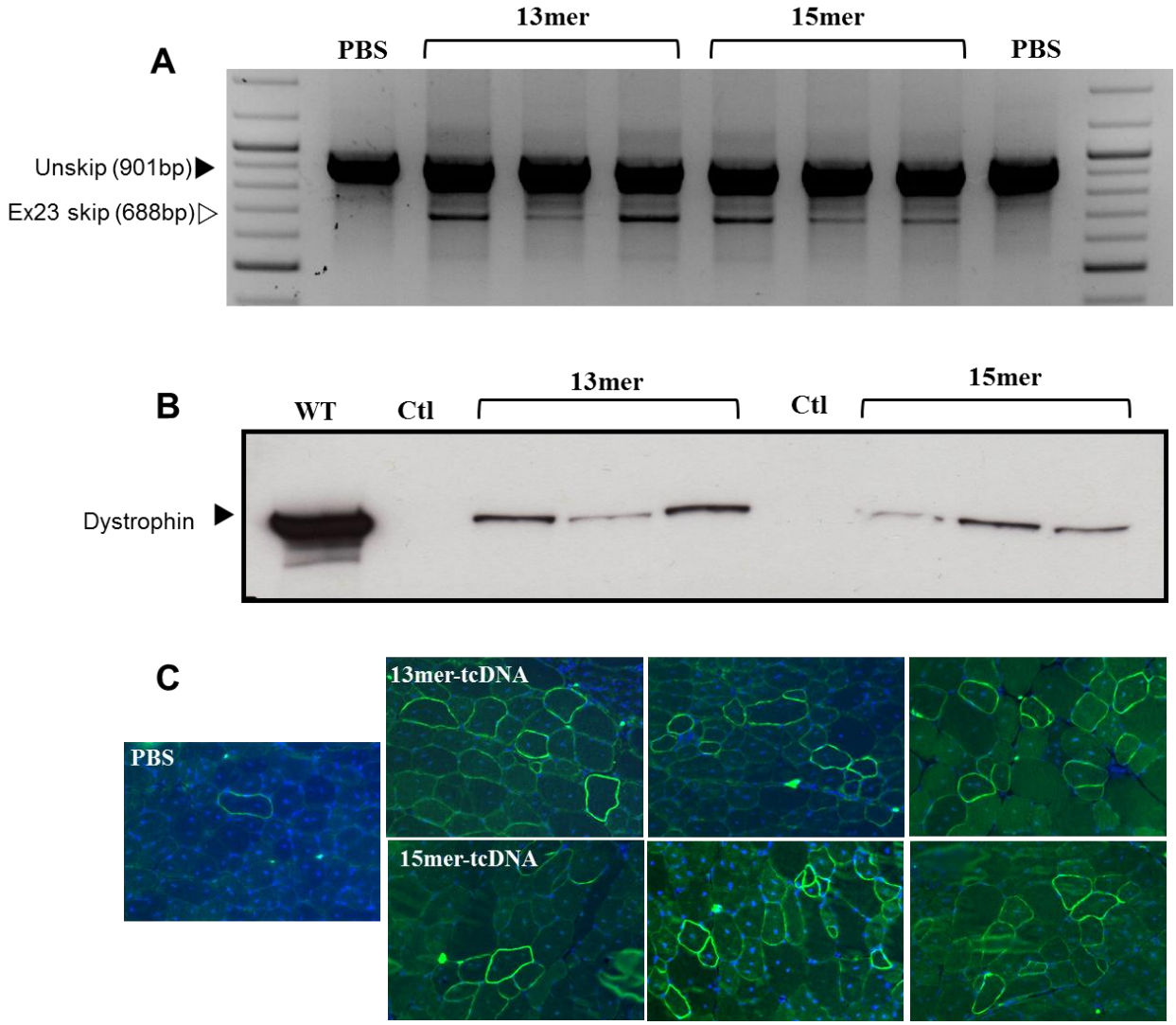


Fig. S1. Evaluation of 13mer-tcDNA following intramuscular injection. Adult *mdx* mice were injected intramuscularly (IM) in the tibialis anterior (TA) muscle with 30 µg of tcDNA-13mer or tcDNA-15mer (previously described) and muscles were collected 3 weeks after the injection for analysis (n=3 per group). **(A)** Detection of exon 23–skipped dystrophin mRNA in *mdx* muscles after IM delivery of tcDNA 13mer or tcDNA-15mer for comparison. The lower 688-bp fragment corresponding to the exon 23–skipped mRNA is detected by nested RT-PCR. **(B)** Western blot showing dystrophin expression in TA muscles injected with 30ug of tcDNA-13mer or tcDNA-15mer compared to *mdx* control (Ctl) and *WT* control mice. 100 µg of total protein were loaded for all samples. **(C)** Dystrophin immunostaining on transverse sections from *mdx* treated muscles. Nuclei, blue (DAPI); Dystrophin, green.

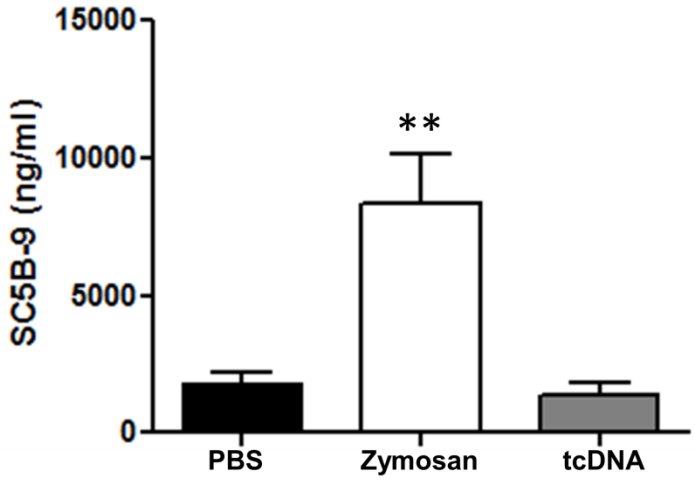
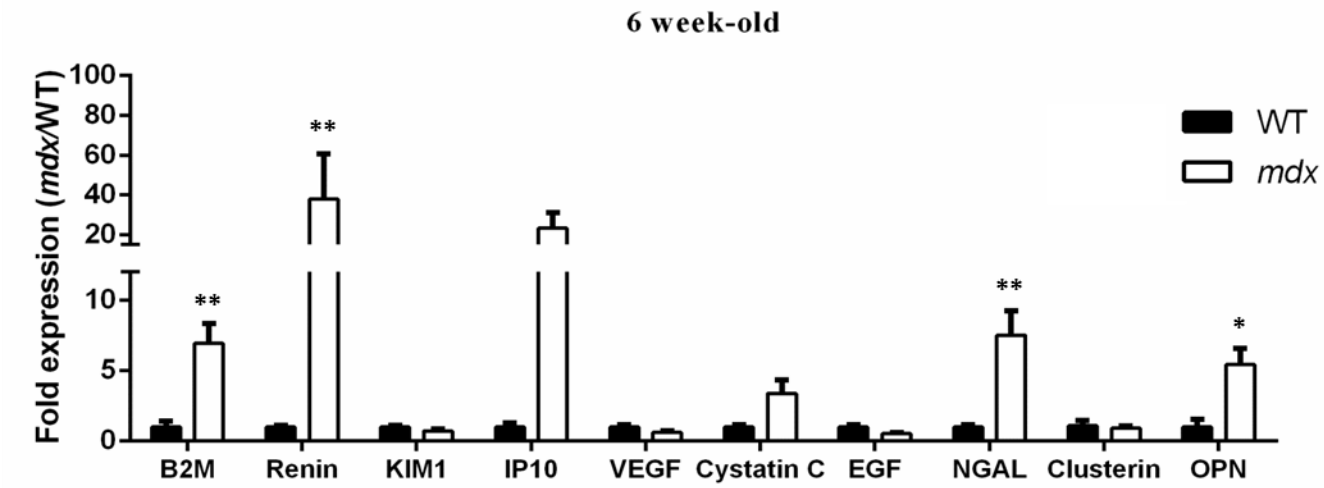
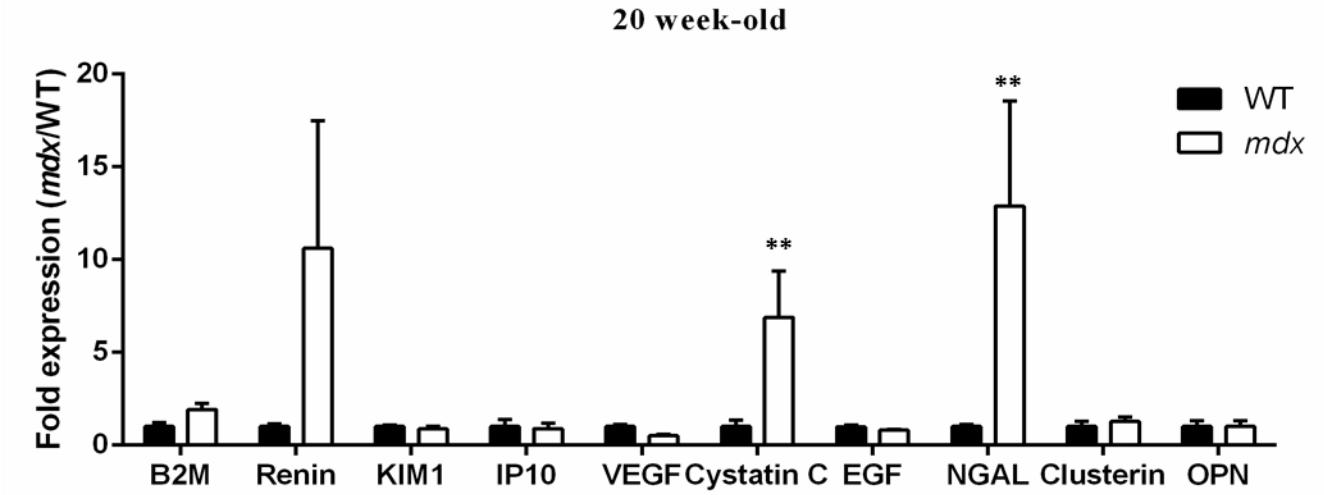


Fig. S2. *In vitro* complement activation analysis. SC5b-9 levels were measured in normal human serum samples at 45 minutes after PBS or tcDNA incubation. Zymosan was used as a positive control. (Data are expressed as means ± SEM). P <0.05 are significant (Mann-Whitney U tests).



	B2M (µg/mg)	Renin (µg/mg)	Kim1 (µg/mg)	IP10 (µg/mg)	VEGF (µg/mg)	Cystatin C (ng/mg)	EGF (µg/mg)	NGAL (ng/mg)	Clusterin (µg/mg)	OPN (ng/mg)
WT	7.81±3.41	0.06±0.01	0.32±0.04	0.09±0.03	0.14±0.02	263.70±49.39	5.46±0.95	102.30±17.51	5.19±0.60	263.36±142.67
mdx	50,79±16.71	2.45±1.46	0.25±0.05	2.27±0.79	0.09±0,02	827.63±389.08	3.21±0.32	729.04±279.23	4.62±0.59	1360.39±412.65
P value	<0.05	<0.05	0.85	0.057	0.11	0.55	0.19	<0.05	0.68	0.06

Creatinine normalised renal injury biomarkers in 6 week-old WT and mdx mice. (mean ± SEM). P-values <0.05 are significant (Mann-Whitney tests).



	B2M (µg/mg)	Renin (µg/mg)	Kim1 (µg/mg)	IP10 (µg/mg)	VEGF (µg/mg)	Cystatin C (ng/mg)	EGF (µg/mg)	NGAL (ng/mg)	Clusterin (µg/mg)	OPN (ng/mg)
WT	27.55±5.55	0.09±0.02	0.34±0.03	0.80±0.30	0.24±0.03	104.26±37.02	4.89±0.44	110.74±13.54	6.32±1.80	327.24±100.7
mdx	53.19±9.28	1.00±0.65	0.30±0.05	0.72±0.23	0.12±0.02	718.37±259.01	3.95±0.16	1427.38±626.43	8.02±1.54	1818.24±698.74
P value	0.05	0.09	0.66	0.94	<0.05	<0.05	0.15	<0.05	0.66	0.22

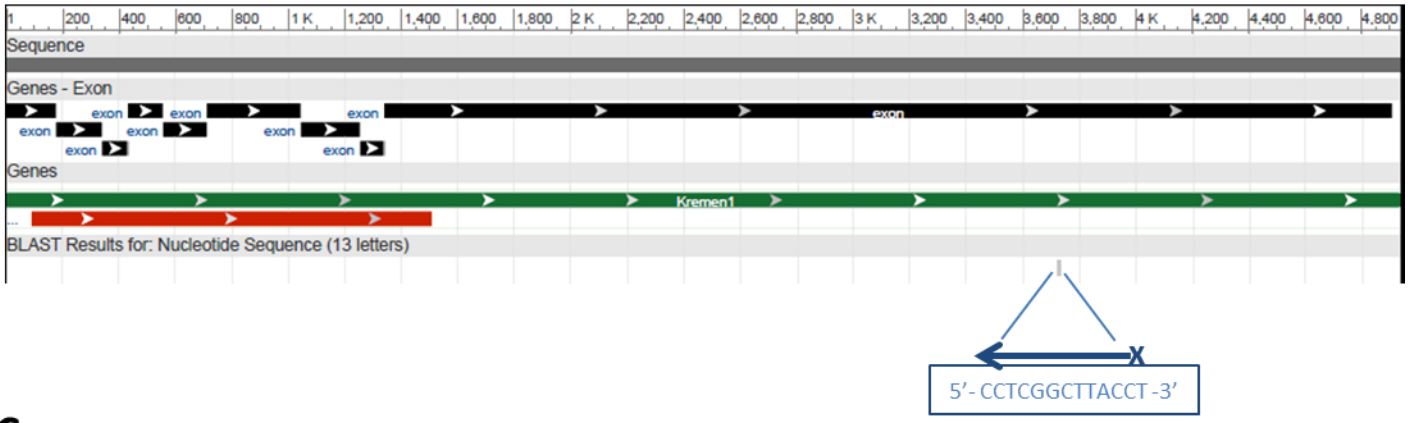
Creatinine normalised renal injury biomarkers in 20 week-old WT and mdx mice. (mean ± SEM). P-values <0.05 are significant (Mann-Whitney tests).

Fig. S3. Creatinine normalized renal injury biomarkers in 6 and 20 week-old *WT* and *mdx* mice. (Data are expressed as means ± SEM). P <0.05 are significant (Mann-Whitney U tests).

A Nucleotide Blast (blastn) on mouse transcript on NCBI: https://blast.ncbi.nlm.nih.gov/Blast.cgi
Sequence query (tcDNA-13mer) : 5'- CCTCGGCTTACCT -3'

Genes	Score	Identities	Strand	Match on mRNA	Tissue expression	Kidney expression
Kremen 1	24.3	12/12 (100%)	Plus/Minus	Yes (last Exon)	Ubiquitous	High
Kif13a	24.3	12/12 (100%)	Plus/Plus	No	Ubiquitous	High
Hopx	24.3	12/12 (100%)	Plus/Plus	No	Limited	Very low
Ucn3	24.3	12/12 (100%)	Plus/Plus	No	Limited	Very low

B **Sequence alignement on Kremen 1**



C **Expression of Kremen1 and Kif13a in kidney**

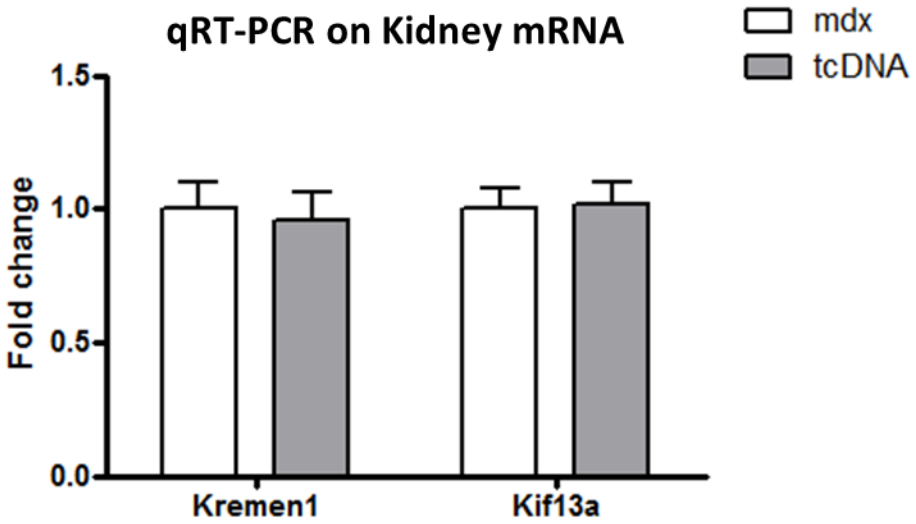


Fig. S4. Hybridization mediated-Off Target effects (OTEs) on mRNA mouse transcript. (A) Nucleotide blast analysis identifies 4 mRNA targets, Kremen1, Kif13a, Hopx and Ucn3 t with (12nt/13nt) homology (100% homology 12/12). The tcDNA hybridization can only occur on Kremen1 mRNA as the strand are Plus/Minus. **(B)** Sequence alignement of Kremen1 gene with the tcDNA sequence to visualize the localization of hybridization (last exon). Hybridization of 12nt on 13nt (the last nucleotide (T) of the tcDNA sequence does not match. **(C)** Expression of Kremen1 and Kif13a in the kidney of *mdx* control and tcDNA-treated *mdx*. Kidney was chosen to evaluate OTEs because of the high expression of the 2 target genes and the high amount of tcDNA detected in kidneys following 12 weeks of injections at 200mg/kg/wk as shown in figure 1D. No significant differences are observed following the treatment. Data are mean ±SEM (n=3 for mdx control and n=4 for tcDNA mouse). Primers were also designed to evaluate the expression of Hopx and Ucn3, but their expression levels were below the detection threshold limit and could therefore not be analysed.

Gene Name	Forward	Reverse
IFNg	GCGTCATTGAATCACACCTG	TGAGCTCATTGAATGCTTGG
IL6	CAAAGCCAGAGTCCTTCAGAG	GCCACTCCTTCTGTGACTCC
Gzmb	TCGACCCTACATGGCCTTAC	TCCTTCACAGTGAGCAGCAG
IP10	AAGTGCTGCCGTCATTTTCT	CCTATGGCCCTCATTCTCAC
TNF	CCACCACGCTCTTCTGTCTA	AGGGTCTGGGCCATAGAACT
Ccl2	CCCAATGAGTAGGCTGGAGA	TCTGGACCCATTCCTTCTTG
Ccl3	ATGAAGGTCTCCACCACTGC	GATGAATTGGCGTGGAATCT
B2m	GAGCCCAAGACCGTCTACTG	GCTATTTCTTTCTGCGTGCAT
Kim1	AGCTACAGGAAGACCCACGA	TGTCACAGTGCCATTCCAGT
Ren1	ATCTTTGACACGGGTTCAGC	TGATCCGTAGTGGATGGTGA
EGF	GAACTGTCAGCCAGGTCCTC	CACCAATTGCTGGTGATTTG
Kremen1	GCGAGCACAATTATTGCAGA	TGGGTTTCCATGATCCTTGT
Hopx	GCCAGCAGGCTATTTAAGCA	GGGTGCTTGTTGACCTTGTT
Ucn3	AAGCTGCAACCCTGAACAGT	AGTAGGTGGGCATCAGCATC
Kif13a	TGGGAAGAGAAGCTGAGGAA	TGACGAGGTAGCACTTGTCG

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