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Supplemental Information

Morpholino Oligomer-Induced Dystrophin Isoforms to Map the Functional Domains in the Dystrophin Protein

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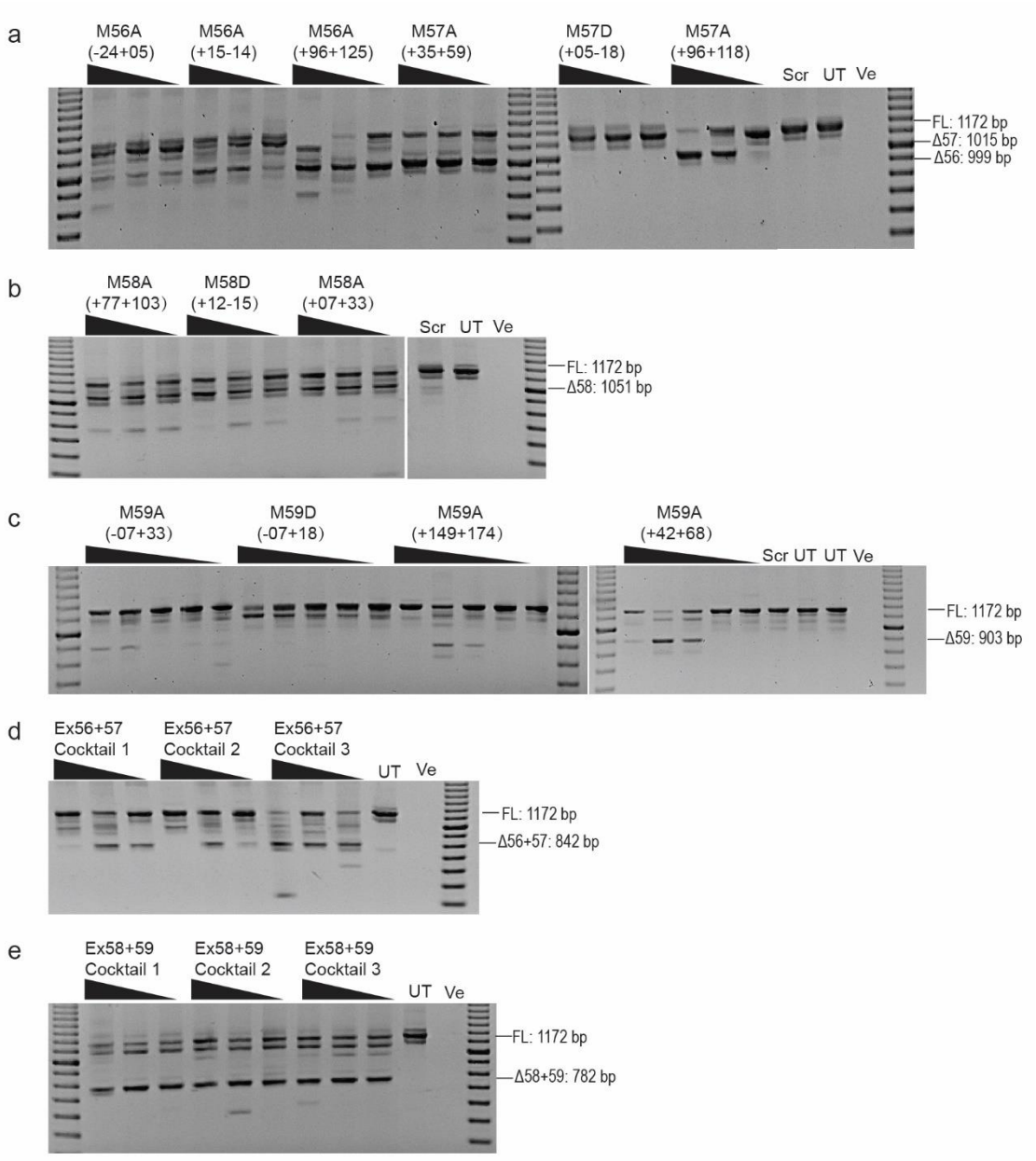

Figure S1. ***In vitro*** **screening of 2′-O-Methyl antisense oligonucleotides to induce dystrophin isoforms**. Nested RT-PCR analysis of DMD transcripts confirming the individual or dual skipping of exon 56, 57, 58 and 59. An RT-PCR no template negative control was loaded in the final lane. Transcript product size in base pairs (bp) are indicated by 100 bp DNA ladder. (a) Analysis of exon 56 or exon 57 skipping in vitro after 2′-O-Methyl AO treatment; (b) The level of exon 58 skipping detected by nested RT-PCR in mouse myogenic cells; (c) Analysis of exon 59 skipping; (d) The level of exons 56+57 skipping after 2′-O-Methyl AO cocktail treatment; (e) Analysis of exons 58+59 skipping in vitro. FL: full-length amplicon; Δ56: exon 56 skipped amplicon; Δ57: exon 57 excised amplicon; Δ58: exon 58 removed transcript; Δ59: exon 59 skipped transcript; Scr: scrambled sequence control AO; UT: untreated; Ve: no template negative control.

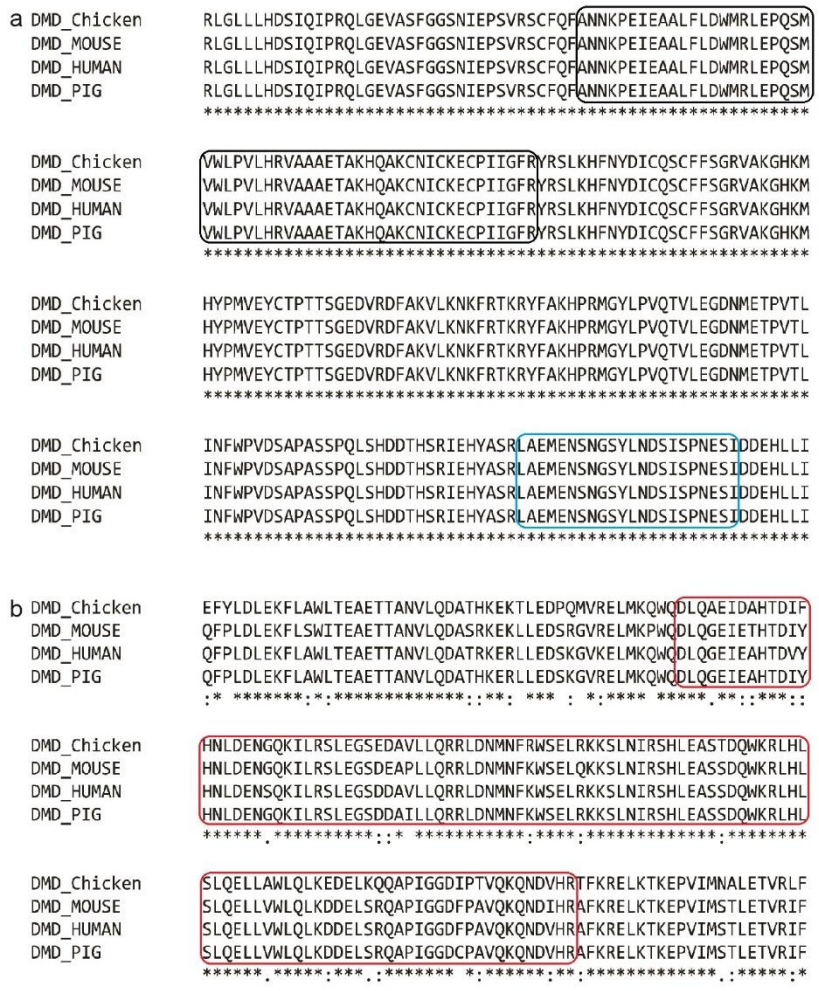

Figure S2. Alignment of dystrophin protein sequences across species. Human, pig, chicken and mouse dystrophin protein sequences were obtained from Ensembl (http://asia.ensembl.org/index.html) and aligned with Clustal Oemga program (https://www.ebi.ac.uk/Tools/msa/clustalo/). (a) Highly conserved sequences across species in the dystrophin C-terminal region, especially region encoded by exon 68 to exon 73; (b) Less conserved sequences encoded by DMD exons 56+57; Sequences encoded by DMD exon 68 are in black rectangle box, sequences encoded by exon 73 in blue, and sequences encoded by exons 56+57 are in red.

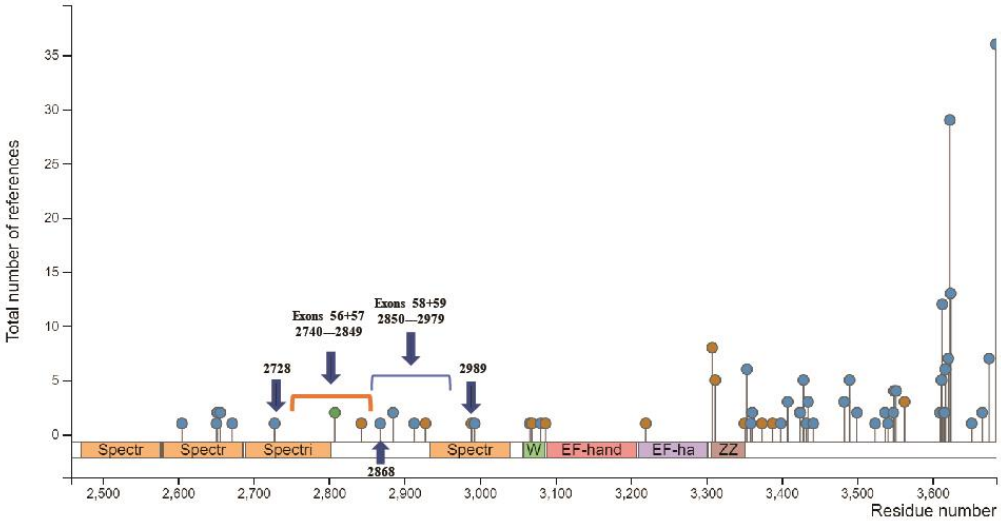

Figure S3. Predicted phosphorylation sites in the protein regions encoded by DMD exons 56+57 and exons 58+59. Absence of predicted phosphorylation sites in the exons 56+57 coding region (http://www.PhosphoSite.org), however there are three predicted phosphorylation sites in protein region encoded by the DMD exons 58+59.

Table S1. Potential exon skipping strategies to make in-frame dystrophin isoforms.

Exon/exons to be skipped	Known functional motifs involved
56/57	No known motifs
58/59	No known motifs
60	No known motifs
59-61	Hinge 4
62/63	WW domain
62-64	WW domain
64	No known motifs
65/66	EF-hands
64-66	EF-hands
62-66	WW domain and EF-hands
66-68	ZZ domain
68/69	ZZ domain
69/70	ZZ domain
70-75	Syntrophins binding sites and CC domain
71/72	No known motifs
72	No known motifs
71-73	Alpha 1-syntrophin binding site
72/73	Alpha 1-syntrophin binding site
73	No known motifs
71-74	Syntrophins binding sites and CC domain
73/74	Syntrophins binding sites and CC domain
74	Beta 1-syntrophin binding site
72-74	Syntrophins binding sites and CC domain
76-78	No known motifs
77	No known motifs

Table S2. Antisense oligonucleotide and primer sequences

PMO coordinates	Sequences (5′-3′)
M23D (+7-18)	GGCCAAACCTCGGCTTACCTGAAAT
M56A (+96 +125)	TATCCAAACGTCTTTGTAACAGGGGTGCTT
M57A (+96 +118)	CCACCGATGGGTGCCTGACGGCT
M58A (+07 +33)	AGGTTCTTTAGTTTTCAATTCCCTCTT
M59A (+42 +68)	GTTGACCTCTTCAGCCTGCTTTCGTAG
M70A (+04 +31)	CGAAGTCGCGAACATCTTCTCCGGATGT
Primer names	Sequences (5′-3′)
DmdEx20F	CCCAGTCTACCACCCTATCAGAGC
DmdEx26R	TTCTTCAGCTTGTGTCATCC
DmdEx51Fo	TCTCTGCTTGATCGAGTTATAA
DmdEx60Ro	TTCCAAAGTGCTGAGCTTATAAG
DmdEx53Fi	AAGGTCCTCACACAGTAGAT
DmdEx60Ri	CAAGGTCATTGACACGATTG
DmdEx65Fo	ATCTCTTGAGCCTGTCAGC
DmdEx78Ro	CTCTGCCCAAATCATCTGC
DmdEx66Fi	CACACTTGGAAGACAAGTACAG
DmdEx77Ri	CTCTTGAACTAGGGAAGGAGT

PMO: phosphorodiamidate morpholino oligomers; Ex: exon; F: forward primer; R: reverse primer; Fo: outer forward primer; Ro: outer reverse primer; Fi: inner forward primer; Ri: inner reverse primer.

Table S3. List of 2′-O-Methyl antisense oligonucleotides and antisense oligonucleotide cocktails for *in vitro* screening.

2′-O-Methyl antisense oligonucleotides	
AO coordinates	Sequences (5′-3′)
M56A (-24+05)	AGAUCCUACCAGAAAGCAAUAAAACACAU
M56D (+15-14)	UGAUCAUUGCCUACCUAAUGUUGAGAGAC
M56A (+96+125)	UAUCCAAACGUCUUUGUAACAGGGGUGCUU
M57A (+35+59)	GUUCCUGAAGAGAAAGAUGCAAACG
M57D (+05-18)	GUCUCUAAAGCAUCCUACCCUAU
M57A (+96+118)	CCACCGAUGGGUGCCUGACGGCU
M58A (+77+103)	UUUCUCUAGUCCUUCCAAAGGCUGCUC
M58D (+12-15)	UCCACAUUCAAUUACCUCUGGGCUCCU
M58A (+07+33)	AGGUUCUUUAGUUUUCAAUUCCCUCUU
M59A (-07+18)	UUUCUUCAGGAGGCAGUUCUAAAUU
M59D (+02-24)	GGAACAAAACAAAGCACACAGUACCU
M59A (+149+174)	GUCCAGUUCAUCGGCAGCUUCCUGAA
M59A (+42+68)	GUUGACCUCUUCAGCCUGCUUUCGUAG
2′-O-Methyl cocktails	
Names	AOs included
Ex56+57 cocktail 1	M56A (-24+05) and M57A (+96+118)
Ex56+57 cocktail 2	M56D (+15-14) and M57A (+96+118)
Ex56+57 cocktail 3	M56A (+96+125) and M57A (+96+118)
Ex58+59 cocktail 1	M58A (+77+103) and M59A (+42+68)
Ex58+59 cocktail 2	M58D (+12-15) and M59A (+42+68)
Ex58+59 cocktail 3	M58A (+07+33) and M59A (+42+68)