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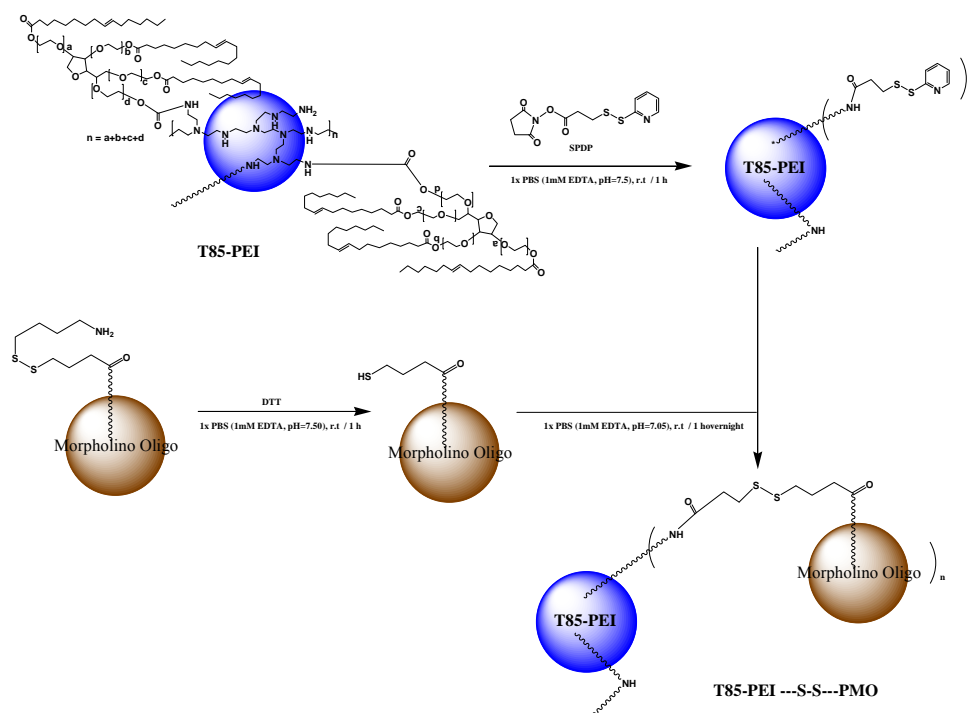
Supplemental Information

Tween 85-Modified Low Molecular Weight PEI Enhances Exon-Skipping of Antisense Morpholino Oligomer In Vitro and in *mdx* Mice

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Z8-PMO conjugation was prepared as the previously reported by Ming X et al., with a little modification (**Scheme-S1**).³⁷

The amino groups of Polymer (Z8) react with the bifunctional crosslinker N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) at a 1:25 molar ratio of polymer to linker in PBS with 1 mM EDTA (pH 7.5) at room temperature for 2 hours. The 2-pyridyldithio linked Z8 was obtained after removing the excess amount of SPDP by gel filtration column. The disulfide PMO-S2 [Disulfide amide functionalized oligonucleotide (PMO, 5'-GGCCAAACCTCGGCTTACCTGAAAT-3') at the 3' position was ordered from Gene Tools, LLC (Philomath, OR, USA)] was reduced with 10 mM DTT at room temperature for 1 hour to get the free sulfhydryl PMO (PMO-SH). The above obtained 2-pyridyldithio linked Z8 was then reacted with PMO-SH, conjugating at a 1/5 molar of Z8 to PMO in PBS with 1mM EDTA (pH 7.0) overnight at room temperature. The product was purified by gel filtration using a column with Sephadex G-100 gel (GE Healthcare), Amicon centrifuge MWCO 10,000 to remove the unconjugated PMO or other small molecules, followed by lyophilizing and characterized by Shimadzu UPLC (Acquity C18, 2.0 x 150 mm, 1.7 μ m) with buffer A, 5% acetonitrile in 0.05 % trifluoroacetic acid (TFA), and buffer B, 100% acetonitrile in 0.05 % TFA with detection at UV 254 nm (Supplementary Figure 1a). The number of PMOs linked to the Z8 polymer was determined by the formation of the colored product pyridine-2-thione was monitored as the 3-thiol PMO reacted with the SPDP-conjugated Z8 polymer, which indicated about three PMO were grafted onto each Z8 polymer.



Scheme-S1. Synthetic Scheme of Z-PMO conjugate

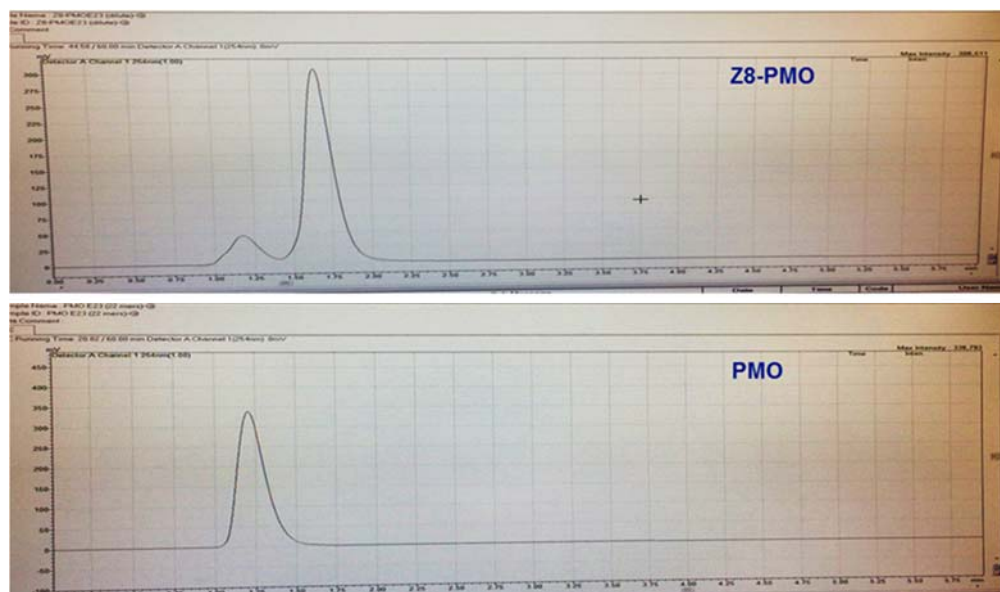


Figure -S1. UPLC of Z-PMO and PMO only condition, (A+30%B, rate: 0.3, 25 mins, buffer A: H₂O/Acetonitrile/TFA =95/5/0.05; Buffer B: H₂O/Acetonitrile/TFA =0/100/0.05).