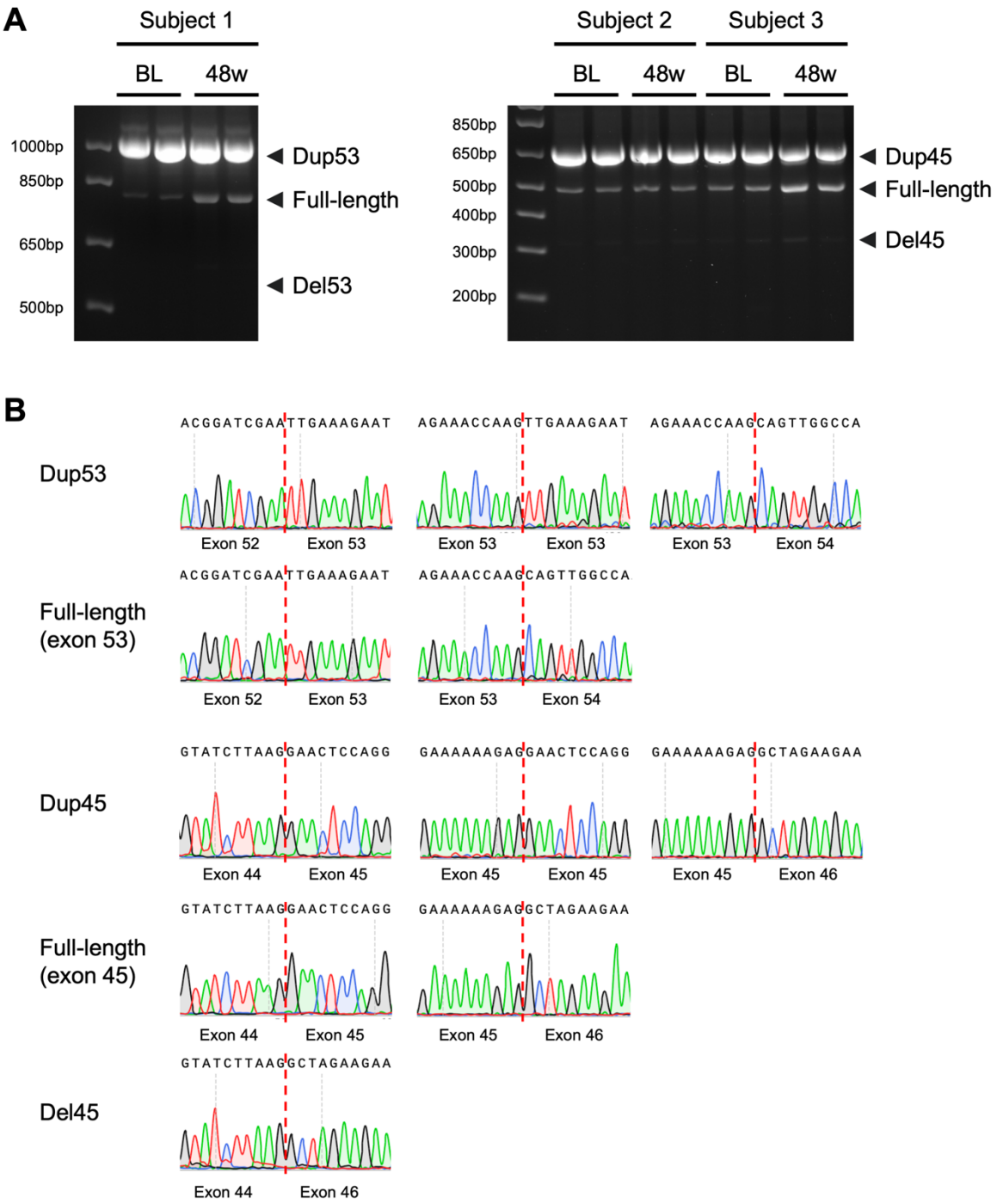
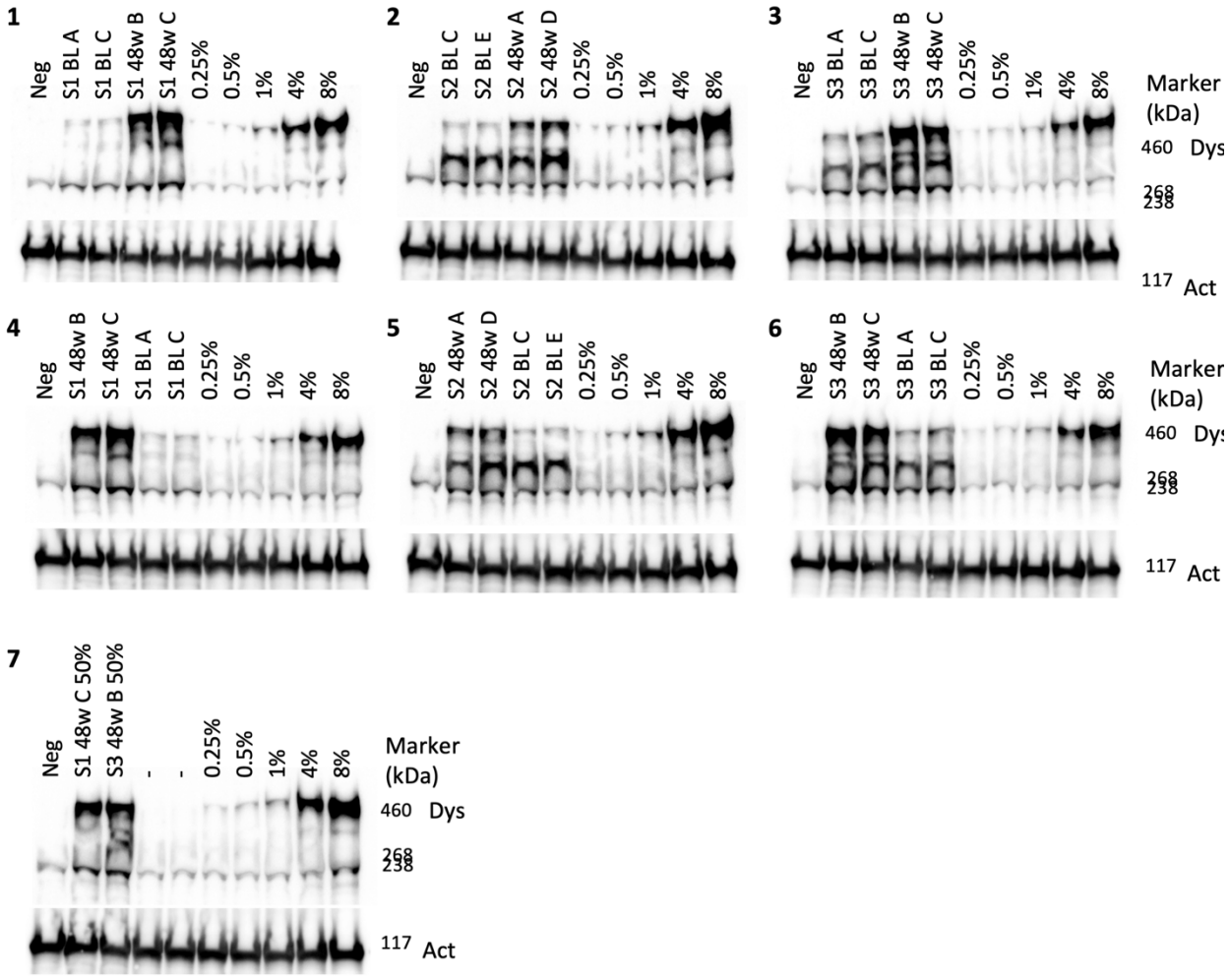
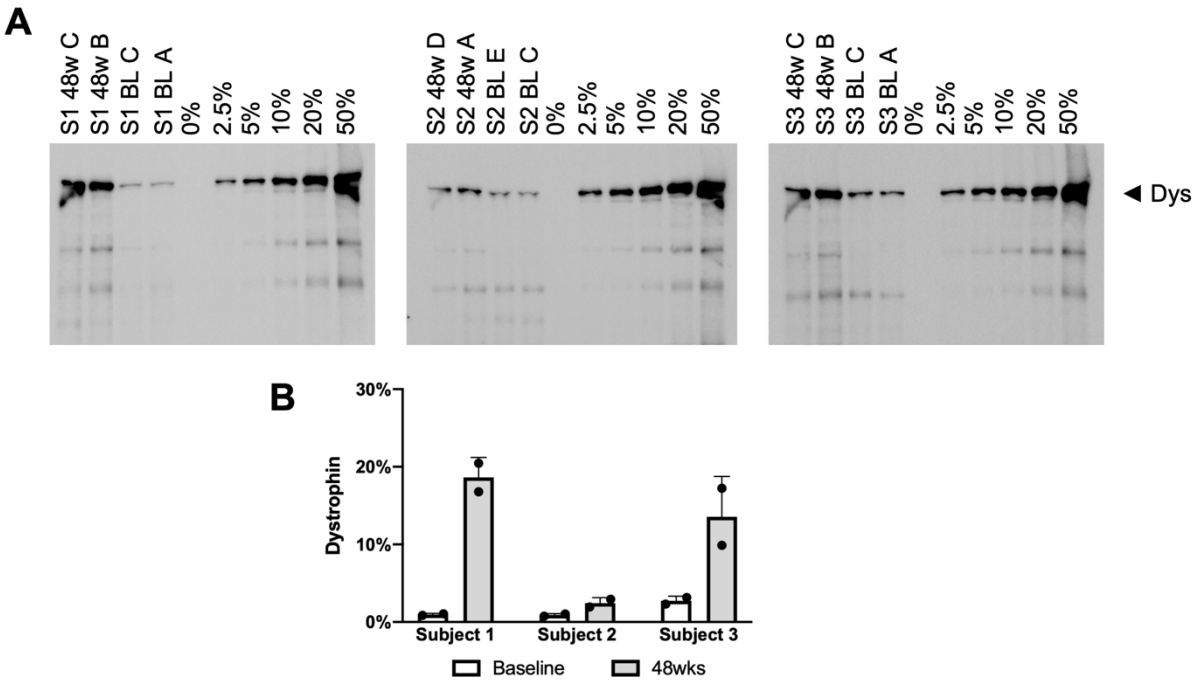


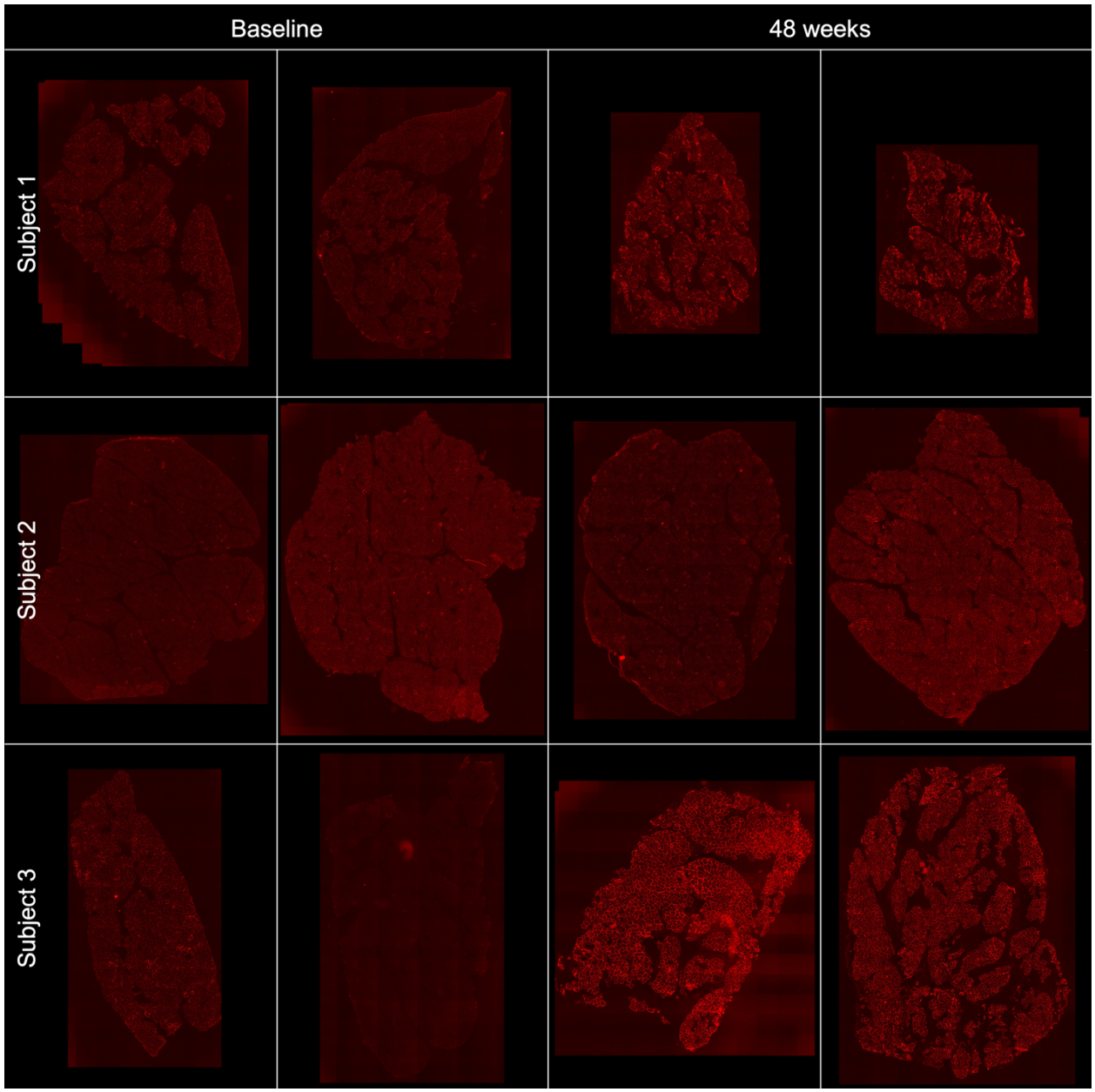
Supplementary figure 1 – RT-PCR analysis of exon skipping induced by antisense oligonucleotide therapy. A. RT-PCR identified 2 transcript isoforms in samples from subject 1, and 3 isoforms in samples from subjects 2 and 3. **B.** Sanger sequencing confirmed that each band corresponds to two, one, or no copies of the target exon. 48w = 48 weeks; BL = baseline



Supplementary figure 2 – Uncropped images of the primary western blot analysis. Each blot contains up to 4 subject samples, along with a standard curve prepared from healthy control tissue and a negative control. Two blocks (designated A, B, C, D, or E) were quantified for each subject and time point, and two technical replicates were performed for each block. Two samples that exceeded the upper limit of the standard curve were diluted by half and re-quantified on blot 7. 48w = 48 weeks; BL = baseline; S1, S2, S3 = Subject 1, 2, 3



Supplementary figure 3 – Supplementary western blot analysis. A. A second western blot analysis was independently performed to confirm dystrophin expression findings. Two blocks (designated A, B, C, D, or E) were quantified for each subject and time point. For this analysis, protein was extracted using radioimmunoprecipitation assay buffer (Cell Signaling Technology). Briefly, 150 µL of lysis buffer was added to 10 sections of 20 µm thick tissue. Tissue was lysed using a metal bead for 2 min at 30Hz in a TissueLyser II bead mill (Qiagen), followed by 30 min incubation at room temperature (RT), and a second lysis step for 1 min at 30Hz. The lysate was centrifuged at 14,000g for 20 min and the supernatant was collected for analysis. The protein concentration was quantified using the BCA assay kit (BioRad). The lysate was mixed with 4x Laemmli sample buffer and denatured for 5 min at 95°C. An on-blot standard curve was prepared from 5 healthy control lysates. 30 µg total protein was loaded on a precast 3–8% Tris-Acetate NuPage gel (Invitrogen) and run for 1h at 80V, followed by 2h at 120V. Protein was transferred from to a 0.45µm PVDF membrane (BioRad) at a constant 55 mA current overnight at 4°C. Membranes were probed with the ab154168 rabbit monoclonal dystrophin C-terminal antibody (Abcam) at 1:1000 dilution in 5% non-fat dry milk in PBS buffer with 0.1% Tween-20 (PBST) for 2h. Membranes were then washed 5 × 5 min with PBST and exposed to the goat anti-rabbit HRP secondary antibody (1:5000) for 1h at RT, followed by 5 x 5 min washes with PBST and 1 x 5 min wash with PBS. Membranes were incubated with 2 mL of ECL reagent (Thermo Scientific) prior to visualization on the Chemidoc MP Imaging System (BioRad). Dystrophin signal was quantified using densitometric analysis in Image Lab (BioRad). A linear regression curve was fitted to the standard curve on each gel and individual samples were quantified according to that curve. **B.** This analysis confirmed an increase in dystrophin expression in each subject. The mean dystrophin level at baseline was 1.5% of normal, rising to 11.5% of normal at 48 weeks. 48w = 48 weeks; BL = baseline; S1, S2, S3 = Subject 1, 2, 3



Supplementary figure 4 – Immunofluorescence analysis of dystrophin expression. Whole slide images used for automated immunofluorescence analysis of dystrophin expression are shown.

Supplementary Table 1 – Primer sequences

Assay	Target	Primer location	Sequence
Genomic PCR	Exon 53	Intron 52 (F)	GGCAAAGCAAACTCCTGTGG
		Intron 53 (R)	TGACATTAGCTGTCAATTTTCCTCT
	Exon 45	Intron 44 (F)	TGGAACATCCTTGTGGGGACA
		Intron 45 (R)	TGTGGTGAAACTAGAGGTAAGTGA
RT-PCR	Exon 53	Exon 51 (F)	ATTTCAACCGGGCTTGGACA
		Exon 55/56 (R)	CACCTTGGAGGTCTTGCCAT
	Exon 45	Exon 44 (F)	GTGGCTAACAGAAGCTGAAC
		Exon 47 (R)	GGGCTTATGGGAGCACTTAC

Supplementary Table 2 – Adverse events

Adverse event	Severity	Study drug/procedure relationship
Bruising at biopsy site	Mild	Definitely related to study procedure
Bruising at port site	Mild	Definitely related to study procedure
Cutaneous erythema	Mild	Unrelated
Decreased Hemoglobin	Mild	Probably related to study procedure
Discomfort at biopsy site (2)	Mild	Definitely related to study procedure
Finger laceration	Mild	Unrelated
Frenulectomy surgery	Mild	Unrelated
Functional constipation	Mild	Unlikely related to study drug
Headache (2)	Mild	Unrelated
Intermittent Headache	Mild	Possibly related to study drug
Intermittent urinary incontinence	Mild	Unlikely related to study drug
IV infiltration	Mild	Definitely related to study procedure
Leukocytosis	Mild	Unrelated
Mild persistent asthma without complication	Mild	Unrelated
Nasal congestion	Mild	Unrelated
Pain at biopsy site	Moderate	Definitely related to study procedure
Pain at biopsy site (2)	Mild	Definitely related to study procedure
Pain at IV site	Moderate	Definitely related to study procedure
Pain at port site	Mild	Definitely related to study procedure
Pain at vaccination site (2)	Mild	Unrelated
Pruritus	Moderate	Unrelated
Rash	Mild	Possibly related to study drug
Renal calculi passage	Moderate	Possibly related to study drug
Wound dehiscence	Mild	Definitely related to study procedure