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Supplemental Information

Antibody-Antisense Oligonucleotide Conjugate Downregulates a Key Gene in Glioblastoma Stem Cells

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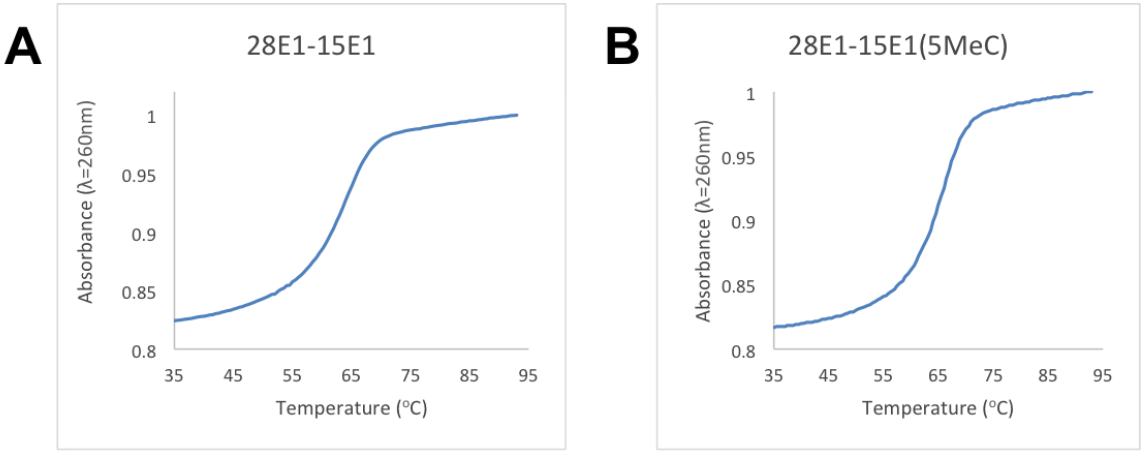
Supplemental Methods

Melting Point Determination

Equimolar amounts (1.5 nmol) of complementary sequences were combined and dried. To the resulting pellet was added 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl and 0.1 mM EDTA (1 mL). They were then transferred into cuvettes in a Varian UV spectrophotometer. The samples were heated to 90 °C and then cooled to 5 °C. The change in absorbance at 260 nm was then monitored upon heating from 5 to 90 °C at a rate of 0.5 °C/min. The dissociation temperatures were calculated as the midpoint of the transition ($T_{1/2}$) using the first derivative of the melting curve.



C

Duplex ID	Description	Duplex Sequence	T_m (°C)
28E1-15E1	Gapmer + DRR Sense strand	5′- (DBCO-TEG) – d(CCA GAG GGA GCG GGC AGA CAT TGG T) - (Cy3) -3′ 5′ - d(**AAT GTC** TGC CCG CTC **CCT CT**) -3′	64.0
28E1-15E1 5MeC	Gapmer MeC + DRR Sense strand	5′- (DBCO-TEG) – d(CCA GAG GGA GCG GGC AGA CAT TGG T) - (Cy3) -3′ 5′ - d(**AAT GTC** TGC C(C^{Me})G CTC **CCT CT**) -3′	66.0

Figure S1. Melting temperature (T_m) comparison between the DRR sense strand and the Gapmer and Gapmer MeC antisense strands. 28E1 is the DRR sense strand. 15E1 is the Gapmer antisense strand. 15E1 5MeC is the Gapmer MeC strand. (A) Melting curve of 28E1-15E1. (B) Melting curve of 28E1-15E1(5MeC). (C) Table with duplex IDs, descriptions, sequences, and calculated T_m.

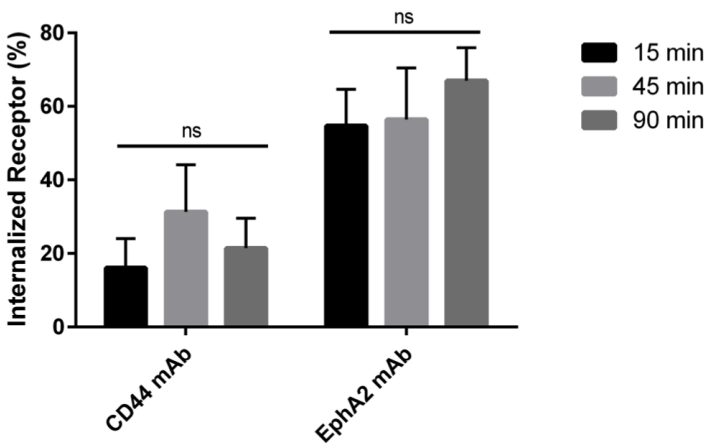


Figure S2. Internalization of CD44 and EphA2 mAbs at 15, 45, and 90 mins determined via flow cytometry. No significant differences between time points were observed. 45 mins was chosen as the comparison point between CD44 mAb, EphA2 mAb, and CTL as there is a trend towards the most internalization at 45 min for the CD44 mAb (Figure 3). Data was analyzed using two-way-ANOVA followed by Sidak's post-hoc test. Data is shown as mean+SD, n=3.

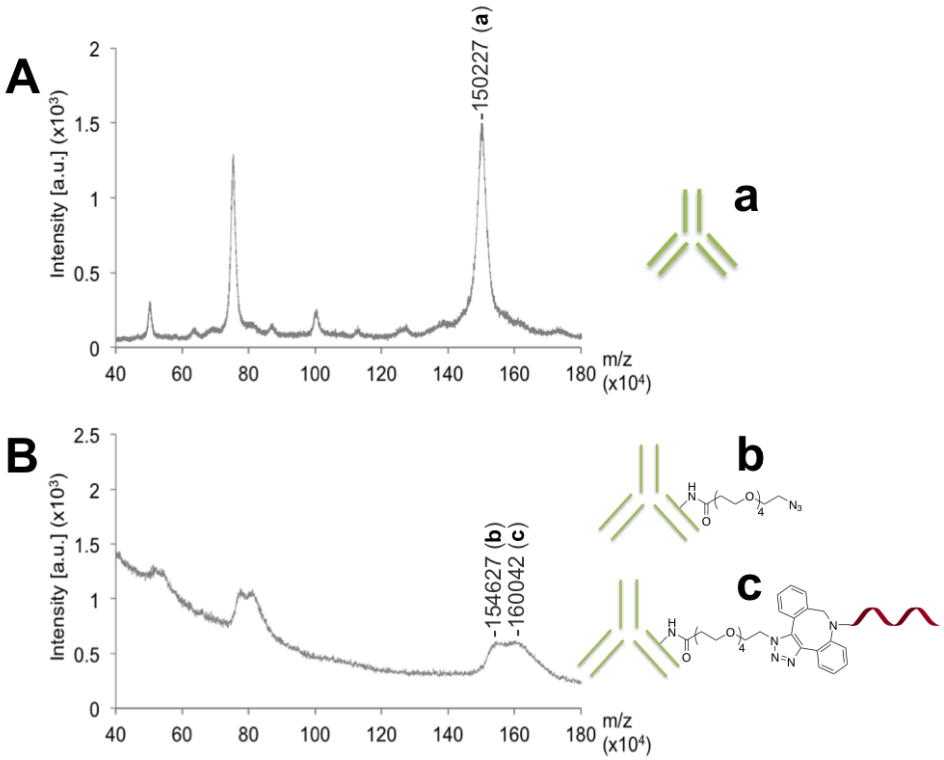


Figure S3. (A) MALDI-TOF mass spectrometry analysis of CD44 mAb (M_{obt} = 150.2 kDa vs M_{th} = 150.0 kDa). (B) MALDI-TOF mass spectrometry analysis of CD44 mAb-DRR sense strand conjugate. For CD44-DRR sense strand, two shifted peaks were obtained: the first represents the azide-modified CD44 (M_{obt} = 154 kDa vs. M_{th} = 150.8 kDa) and the second for the CD44-DRR sense strand (M_{obt} = 160.0 kDa vs. M_{th} = 160.2 kDa).

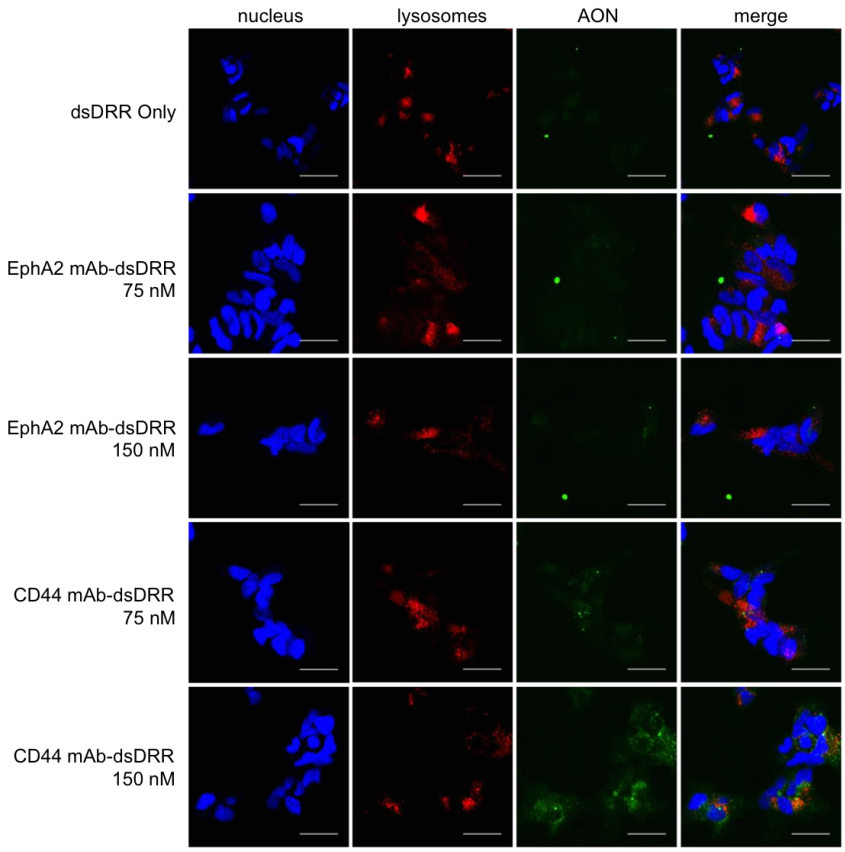


Figure S4. Comparison of uptake at 75 nM vs. 150 nM for EphA2 mAb-dsDRR and CD44 mAb-dsDRR. DsDRR only is shown as a negative control. A significant increase in uptake was observed for both EphA2 mAb-dsDRR and CD44 mAb-dsDRR at 150 nM. Cell nucleus (Hoechst, blue); AON (Cy3, green); lysosome (Dextran647, red). Representative z-stack images shown. All scale bars are 50 μM.

Table S1. Mass analysis of the oligonucleotide strands used in this study. High-resolution mass spectrometry (HRMS) acquisition was performed in negative ion mode using electrospray ionization (ESI).

Oligo ID	Expected Mass	Obtained Mass
Gapmer	6476.9	6478.3
Gapmer MeC	6490.9	6492.2
Altmer	6476.9	6478.3
Altmer MeC	6490.9	6492.2
Scrambled	6431.1	6430.5
Scrambled (Sense)	8689.4	8690.3
DRR (Sense)	8870.5	8869.8