Supplementary Figure S1. All 10 scFvs from AML6691 Clone 1 affinity maturation demonstrate specificity for the exon 2/4 splice junction of CENP-A-ΔExon3.
(A) Cell supernatant ELISA graphs comparing splice junction specificity between the 10 affinity-matured Directed Evolution anti-NAT scFvs for AML6691 Clone 1. Two distinct binding profiles were observed (1a and 1b, as per Fig. 3) which differ in their ELISA signal for the Exon4 peptide at 1 µg/mL (~400 nM) antigen. White circles within each bar graph represent a biological replicate for scFv expression and ELISA result; each bar height represents the average ELISA result of biological replicates. P-values (* = < 0.05, ** = < 0.01, *** = < 0.001, **** = < 0.0001) were calculated using an unpaired t test with Welch's correction on square root OD450 values in GraphPad Prism on samples with at least two replicates. Error bars correspond to standard deviations. NC = No Change. (B) The eight affinity-matured scFvs with "Binding Profile 1b" were tested in cell supernatant ELISAs as in (A) but against 0.05 µg/mL peptide (20-fold less). All 10 clones were classified as splice junction-specific, demonstrating ≥ 5-fold background-corrected signal against the NAT3 and/or NAT4 peptide over the Exon2 and Exon4 peptides (A and B).