Supplementary Figure S2. Nearly all (95%) scFvs from AML6691 Clone 2 affinity maturation demonstrate specificity for the exon 2/4 splice junction of CENP-A- Δ Exon3.

Cell supernatant ELISA graphs comparing splice junction specificity between the 19 affinity-matured Directed Evolution anti-NAT scFvs for AML6691 Clone 2. 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. Peptide antigens were used at 1 µg/mL (~400 nM). 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. 18 out of 19 clones (95%) were classified as splice junction-specific, demonstrating \geq 5-fold background-corrected signal against the NAT3 and/or NAT4 peptide over the Exon2 and Exon4 peptides.

- Exon 2/3 Junction ("Exon2" Peptide)
- Exon 3/4 Junction ("Exon4" Peptide)
- Exon 2/4 Junction ("NAT3" Peptide)
- Exon 2/4 Junction ("NAT4" Peptide)

