Supplementary Table S4. Affinity Maturation phage display screening results for improving the binding of Directed Evolution scFvs to the native exon 2/4 splice junction of CENP-A-Δ Exon3.

(A) Mutagenic scFv-displaying phage libraries (AML6691 Clone 1 and AML6691 Clone 2) were generated for two NAT-improved scFvs (see Fig. 2A, right and Supplementary Table S3B). Recombinant rates, mutation rates and theoretical diversities were determined using 12 library clone sequences as per Supplementary Table S3A. Note that 50% of the library clones sequenced for AML6691 Clone 2 were empty phagemids containing no scFv sequence, thus lowering the recombinant rate which is mitigated during library generation by performing more transformations to achieve a greater theoretical diversity. (B) Each Affinity Maturation library was screened against the nonphosphorylated, native exon 2/4 splice junction of CENP-A-ΔExon3 under stringent conditions. 88 single clones per screen were expressed as secretory scFvs and tested in cell supernatant ELISAs against the NAT3 or NAT4 peptide and NeutrAvidin alone. "# of Hits" was determined by OD450 ≥ 0.2 against NAT3 or NAT4 with ≥ 2-fold signal over NeutrAvidin. "# of NAT-Improved Hits" was determined by ≥ 2-fold, ≥ 10-fold, or ≥ 40-fold signal against NAT3 or NAT4 over the Directed Evolution parental signal against the same peptide. A subset of NAT-improved hits was sequenced and unique clones were identified within and between screens. "Total # of Uniques with CDR Mutations Identified" includes the 12 or 9 unique clones identified within the AML6691 Clone 1 or Clone 2 screens, respectively, as well as clones identified in parallel screens. A subset of unique clones was expressed as secretory scFvs and tested in cell supernatant ELISAs against NeutrAvidin alone and four splice junction-spanning peptides as per Supplementary Figs. S1 and S2. 28 clones (~97%) with ≥ 5-fold background-corrected signal against the NAT3 and/or NAT4 peptide over the Exon2 and Exon4 peptides were classified as splice junction-specific.

A

Affinity Maturation Library QC

Library	Recombinant Rate	Mutation Rate	Theoretical Diversity
AML6691 Evolved Clone 1	83%	1.0%	7.1×10^7
AML6691 Evolved Clone 2	42%	1.5%	6.3×10^7

B

Affinity Maturation Screening Results

Affinity Maturation Library #	Affinity Maturation Screen #	ELISA Antigen Name	# of Hits (out of 88)	# of NAT-Improved Hits (≥ 2-Fold)	# of NAT-Improved Hits (≥ 10-Fold)	# of NAT-Improved Hits (≥ 40-Fold)	# of NAT-Improved Hits Sequenced	# of Unique NAT-Improved Hits (within screen)	# of Uniques with CDR Mutations (%)	Total # of Uniques with CDR Mutations Identified	# of Uniques Tested in Split-Splice Junction ELISA	# of Splice Junction-Specific Clones (over Exon2)	# of Splice Junction-Specific Clones (over Exon4)
AML6691 Clone 1	p4344	NAT3	61	61	61	47	49	12	12 (100%)	15	10	10	10
AML6691 Clone 2	p4345	NAT4	20	20	1	0	19	15	9 (60%)	28	19	19	18