Supplementary Table S3. Directed Evolution phage display screening results for evolving SEP-specific scFvs to the native exon 2/4 splice junction of CENP-A-Δ Exon3.

(A) Mutagenic scFv-displaying phage libraries (DEL6691, DEL6695 and DEL6698) were generated for three SEP-specific scFvs (see Fig. 1B and Supplementary Table S2). Recombinant rate is the fraction of library clones that contain mutation(s) in a full scFv sequence compared to the parental scFv, based on the sequencing results for 10 library clones and omitting poor sequence quality reads. Mutation rate is the average frequency of nucleotide mutations in a single library clone, based on the same sequencing results used to calculate the recombinant rate. Theoretical diversity is based on the recombinant rate, transformation efficiency, and number of transformations performed to generate the final library. (B) Each Directed Evolution library was screened against the nonphosphorylated, native exon 2/4 splice junction of CENP-A-Δ Exon3 using two strategies, as per Fig. 1C. 88 single clones per screen (44 clones per strategy) were expressed as secretory scFvs and tested in cell supernatant ELISAs against the NAT4 peptide and NeutrAvidin alone. "# of Hits" was determined by OD450 ≥ 0.2 against NAT4 with ≥ 2-fold signal over NeutrAvidin. "# of NAT-Improved Hits" was determined by ≥ 2-fold signal against NAT4 over the Discovery parental NAT4 signal. A subset of NAT-improved hits was sequenced and three unique clones were identified. All unique scFvs were purified and tested in scFv titration ELISAs against the NAT4 peptide and NeutrAvidin alone. Clones with ≥ 2-fold background-corrected signal against the NAT4 peptide over the parental NAT4 corrected signal for at least two titration points were classified as NAT-improved (as shown in Fig. 2A-B, right).

A

Directed Evolution Library QC

Library	Recombinant Rate	Mutation Rate	Theoretical Diversity
DEL6691	67%	1.6%	1.0×10^8
DEL6695	89%	1.8%	1.7×10^8
DEL6698	100%	1.9%	1.0×10^8

B

Directed Evolution Screening Results

Directed Evolution Library #	Directed Evolution Screen #	Discovery Screen Clonal Origin	ELISA Antigen Name	# of Hits (out of 88)	# of NAT-Improved Hits	# of NAT-Improved Hits Sequenced	# of Unique NAT-Improved Hits	% of Uniques with CDR Mutations	# of Uniques Tested in scFv Protein ELISA	# of NAT-Improved scFvs in Titration ELISA
DEL6691	p4260	p3496	NAT4	78	76	33	2	100%	2	2
DEL6695	p4261	p3498	NAT4	12	0	N/A	N/A	N/A	N/A	N/A
DEL6698	p4264	p3502	NAT4	2	2	2	1	100%	1	1