Supplementary tables

Table S1. Amino acid sequences of RA14 CDR3 variants isolated from round 3 sorting

Variant	CDR3α	CDR3β
IMGT #	104 105 106 107 108 109 114 115	105 106 107 108 109 110 111 112 113 114 115 116 117
Wild-Type	**CARNTGNQ**	**ASSPVTGGIYGYT**
Library	**CAR***N***	**ASS**T***Y*YT**
CDR3α1	CARN**S**GN**P**	ASSPVTGGIYGYT
CDR3α2	CARN**Y**GN**H**	ASSPVTGGIYGYT
CDR3α3	CARN**YI**N**T**	ASSPVTGGIYGYT
CDR3α4	CAR**SF**GN**P**	ASSPVTGGIYGYT
CDR3α5	CAR**SV**GN**S**	ASSPVTGGIYGYT
CDR3α6	CAR**LLA**N**L**	ASSPVTGGIYGYT
CDR3α7	CAR**GSW**NQ	ASSPVTGGIYGYT
CDR3β1	CARNTGNQ	ASSPVTGG**V**Y**L**YT
CDR3β2	CARNTGNQ	ASS**R**VTGG**V**Y**L**YT
CDR3β3	CARNTGNQ	ASSP**I**TG**ST**Y**I**YT
CDR3β4	CARNTGNQ	ASSP**I**TG**AP**Y**L**YT
CDR3β5	CARNTGNQ	ASSPVTG**SS**YGYT
CDR3β6	CARNTGNQ	ASS**WI**TG**S**IY**T**YT
CDR3β7	CARNTGNQ	ASSPVTG**SS**Y**W**YT
CDR3β8	CARNTGNQ	ASS**L**VTGG**V**Y**L**YT
CDR3β9	CARNTGNQ	ASS**L**VTG**IP**Y**L**YT
CDR3β10	CARNTGNQ	ASS**R**VTG**SV**YGYT

Supplementary figures

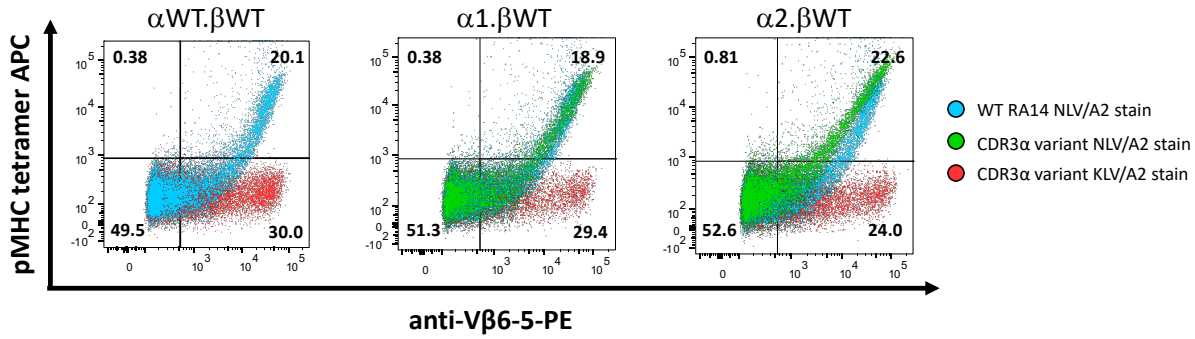

Figure S1. Comparison of selected CDR3α variants with the wild-type RA14 β chain. Alpha and beta chain combinations were transfected into CHO-T cells and stained with NLV/ A2 (green) or control KLV/ A2 tetramers (red) and analyzed by flow cytometry. Shown in each panel are cells expressing the wild-type RA14 alpha and beta chains (blue) for comparison.

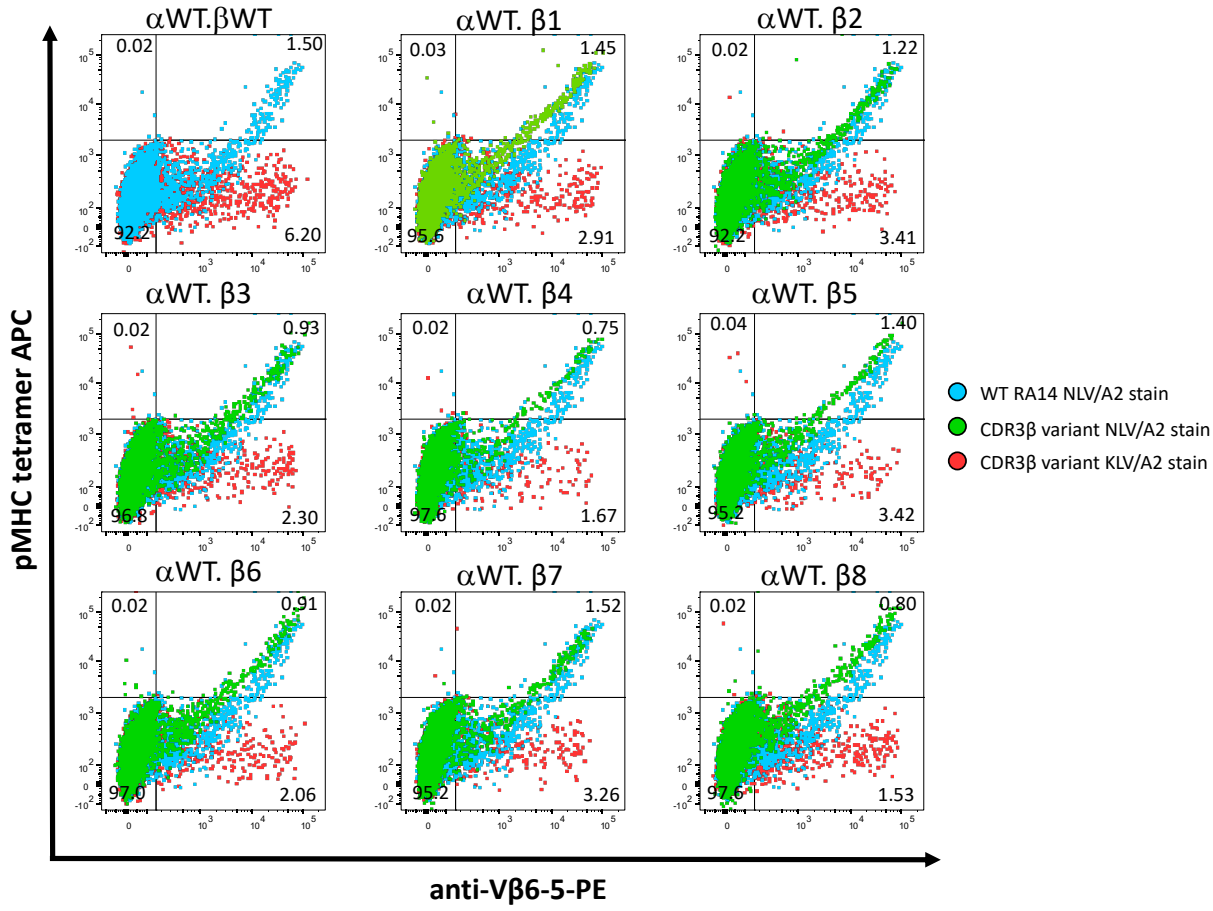

Figure S2. Comparison of selected CDR3β variants with the wild-type RA14 α chain. Alpha and beta chain combinations were transfected into CHO-T cells and stained with NLV/ A2 (green) or control KLV/ A2 tetramers (red) and analyzed by flow cytometry. Shown in each panel are cells expressing the wild-type RA14 alpha and beta chains (blue) for comparison.

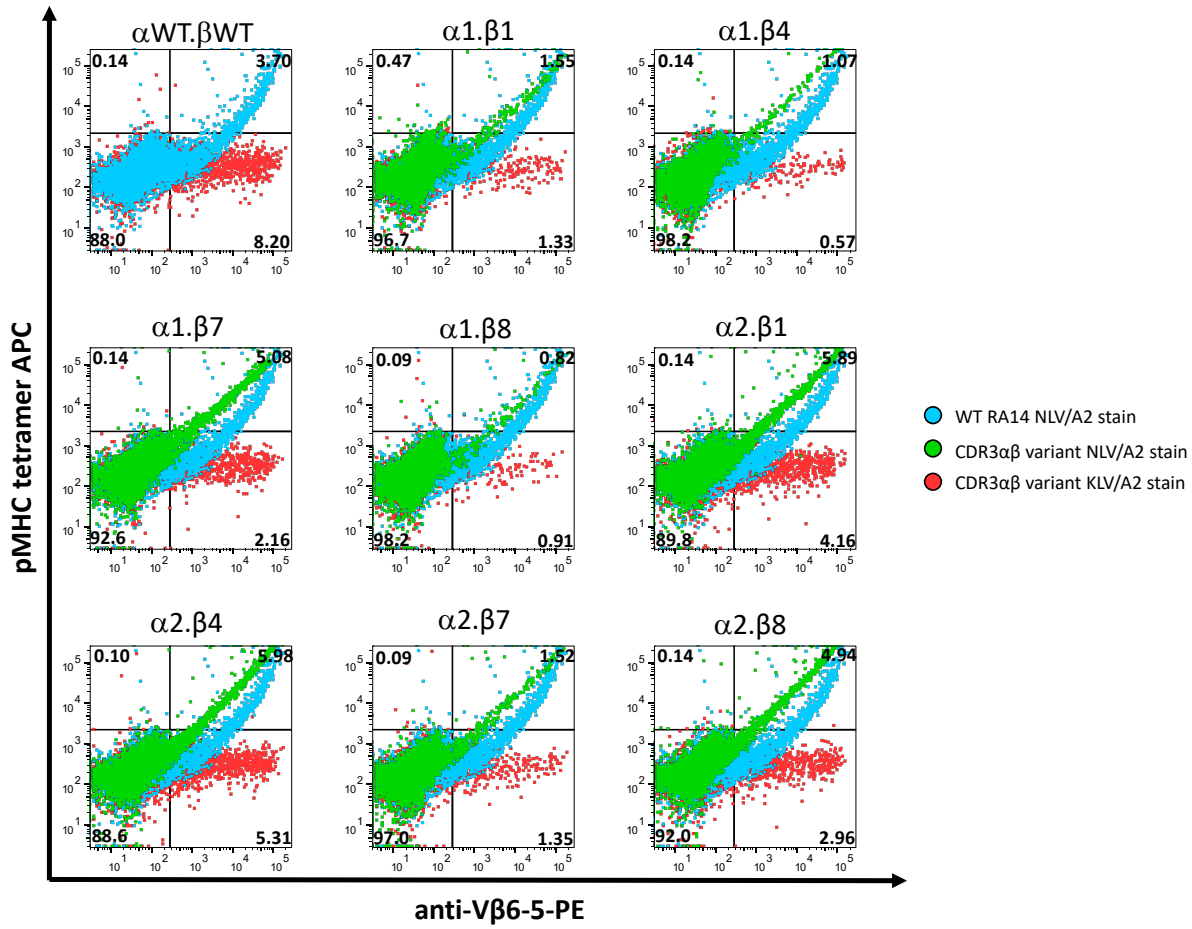

Figure S3. Screening of selected α and β CDR3 variants. The wild-type and improved alpha-chain variants (α1 and α2) were cloned in combination with the wild-type and improved beta-chain variants (β1, β4, β7, β8) in the display format and expressed in CHO-T cells. After two days, cells were stained with NLV/ A2 (green) or control KLV/ A2 tetramers (red) and analyzed by flow cytometry. Shown in each panel are cells expressing the wild-type RA14 alpha and beta chains (blue) for comparison.

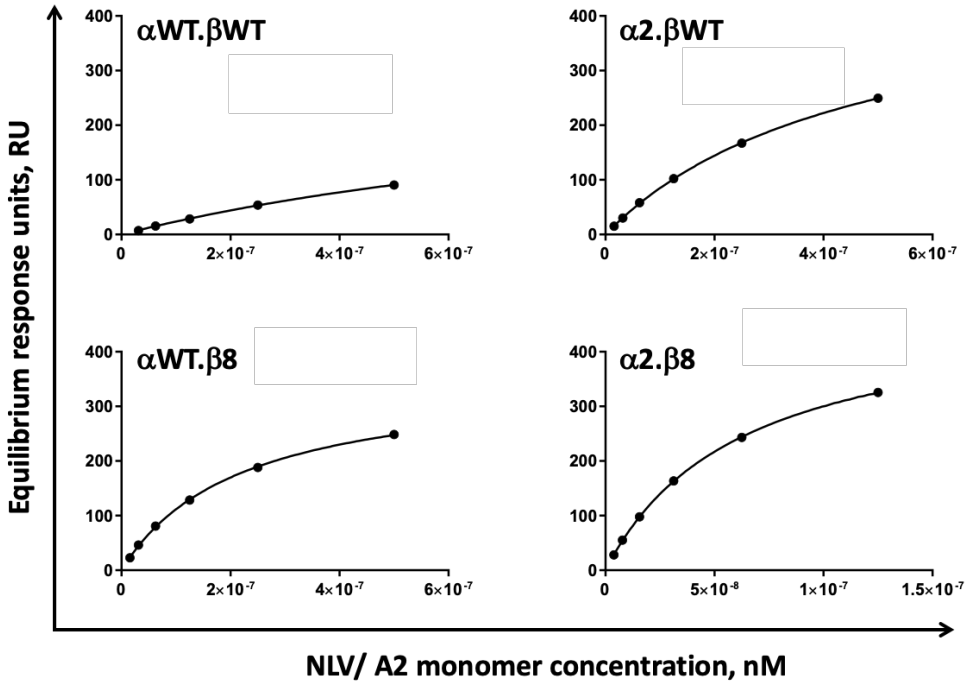

Figure S4. Equilibrium binding affinity of RA14 and variants for NLV/ A2. The wild-type RA14 TCR2ds-huFc fusion and variants were immobilized on a CM5 chip at 2000-5000 RUs before injecting monomeric NLV/ A2 at concentrations ranging from 7.9 to 500 nM at a flowrate of 30 ul/min. The observed equilibrium response values were plotted against concentration using BIAevaluation 3.0 software. The equilibrium affinity K_d was calculated using a least-squares fit to the data, assuming a 1:1 stoichiometry; the numerical values are listed in Table 2. No binding was observed with the control HCV/ A2 monomer at the highest concentrations used for each variant (shown in Figure 6).

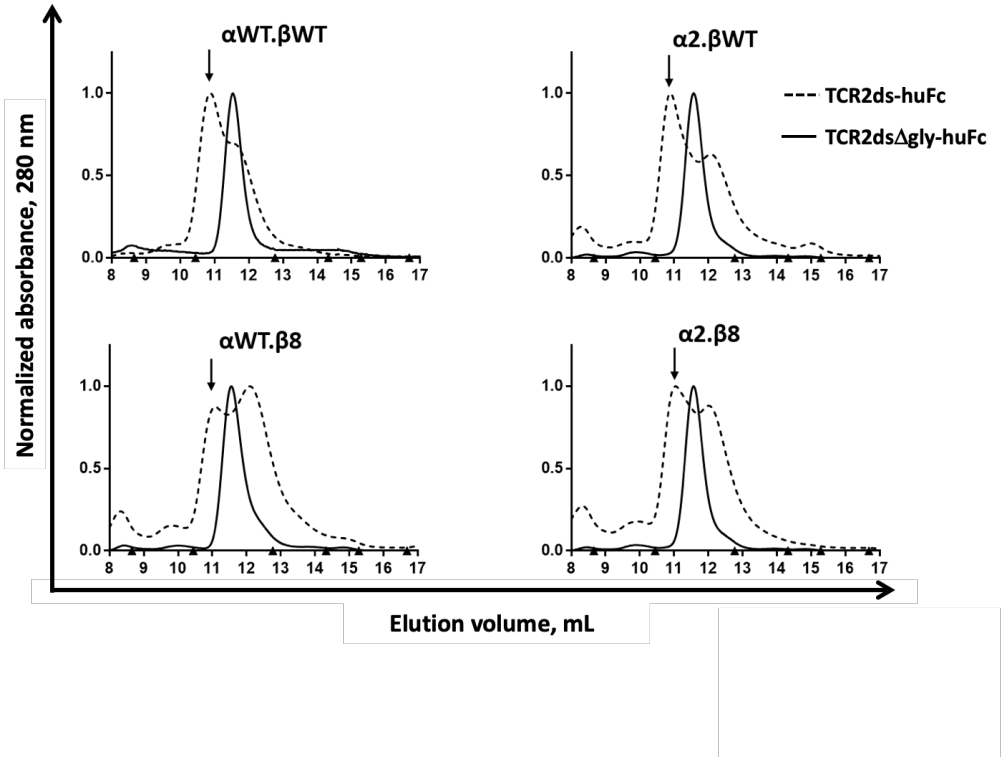

Figure S5. Genetic de-glycosylation of TCR2ds-huFc fusion proteins yields monodisperse protein. The purity of TCR2dsΔgly-huFc (solid lines) and TCR2ds-huFc (dashed lines) proteins for affinity matured RA14 variants was analyzed by size exclusion chromatography. Protein A purified protein was injected (100 μg in 100 μl) onto a Superdex S200 column on an Åkta FPLC. Triangles indicate elution volumes for the molecular weight standards: thyroglobulin (669 kDa), ferritin (440 kDa), aldolase (158 kDa),conalbumin (75 kDa), ovalbumin (44 kDa), andcarbonic anhydrase (29 kDa). Representative data are shown for each TCR variant in each format.

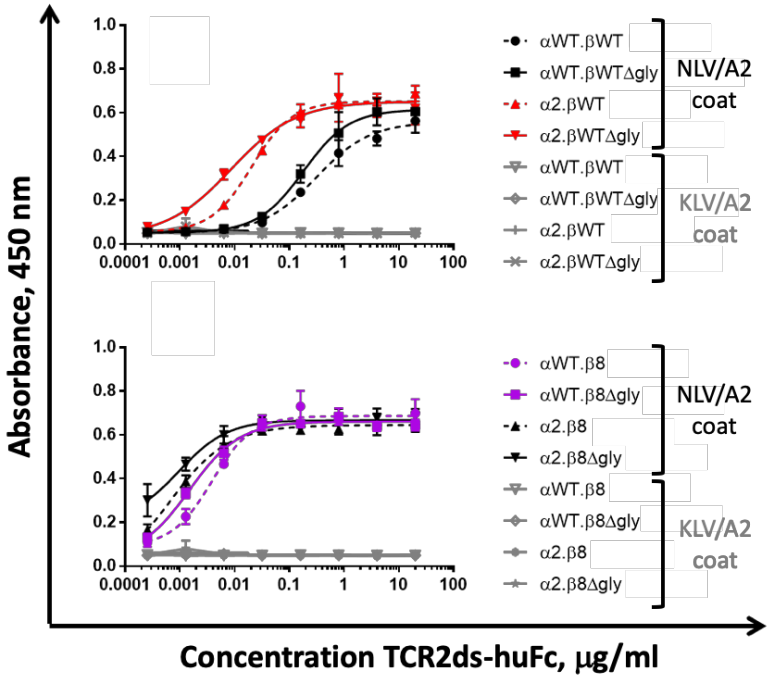

Figure S6. NLV/A2 binding is retained in deglycosylated TCR2ds-huFc variants. The tetramer binding activities of RA14 variants genetically deglycosylated (TCR2dsΔgly-huFc) versus unmodified (TCR2ds-huFc) were compared by ELISA. Plates were coated with NLV/ A2 or KLV/A2 tetramer at 1 µg/mL, followed by serially diluted, SEC-purified TCR2ds-huFc or TCR2dsΔgly-huFc and 1:1000 goat-anti-human Fc-HRP. Data shown are the average and range of duplicate series; the experiment was repeated several times with similar results.