Mutagenesis of beryllium-specific T cell receptors suggests an unusual binding topology for antigen recognition

Supplemental Data

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SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIGURE 1. Levels of TCR variants expressed on the cell surface of murine T cell hybridoma line, 5KC. Flow cytometry was used to measure cell surface expression of wild-type and variant TCR expressed on the murine T cell hybridoma line 5KC, designated 1332-28. Positive populations represent cells stained with a mAb specific for the murine T cell receptor Cβ domain while the negative population represents unstained cells.

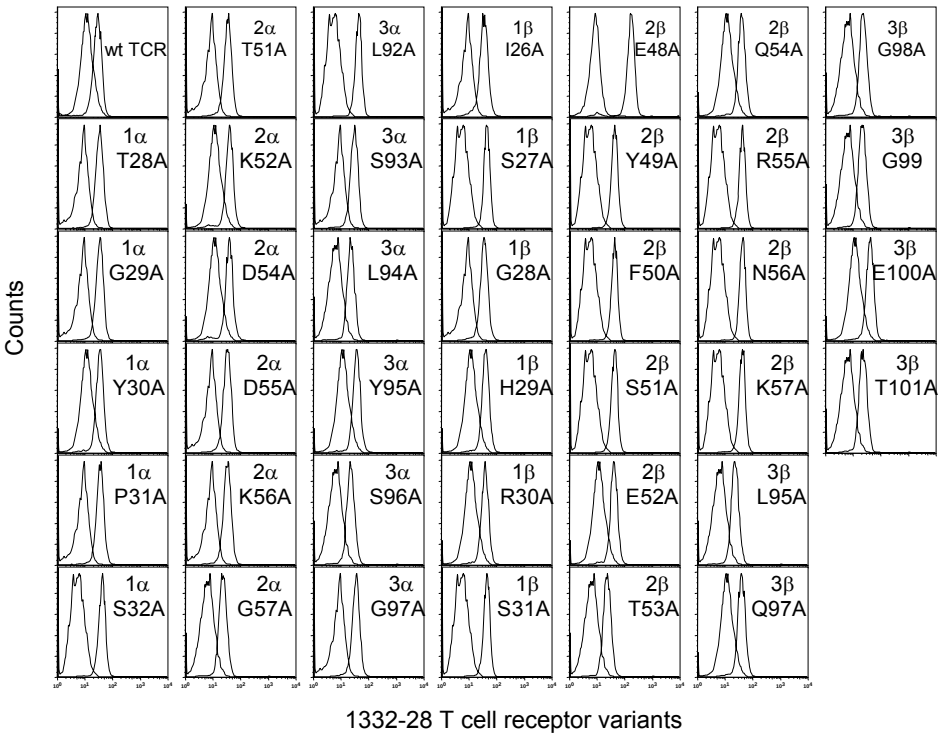
SUPPLEMENTAL FIGURE 2. Stimulation of Be-responsive $V\beta5^+$ T cell hybridomas expressing TCR variants. T cell hybridomas 1332-28, -22, and -2, expressing selected *TCRA* and *TCRB* variants, were stimulated with murine fibroblast line DAP.3 transfected with HLA-DP2, and various concentrations of $BeSO_4$. Hybridoma secretion of IL-2 was measured by ELISA, and activation curves were generated by plotting % maximal IL-2 release versus the concentration of $BeSO_4$. In order to quantitatively display the EC_{50} differences of the individual variant TCRs, the overall EC_{50} fold-change difference (mean ± SEM) for each TCR compared to the wild-type TCR for three separate experiments is shown. The dotted line set at y = 1 represents the response of the wild-type T cell hybridoma.

SUPPLEMENTAL FIGURE 3. Deduced amino acid sequence of the *TCRB* and *TCRA* CDR3 sequence of 1041-3.3. The T cell clone was isolated from the lung of CBD patient 1041 and expressed *TCRBV3* and *TCRAV1*. These sequence data are available from GenBank under accession numbers JN019785 - JN019786.

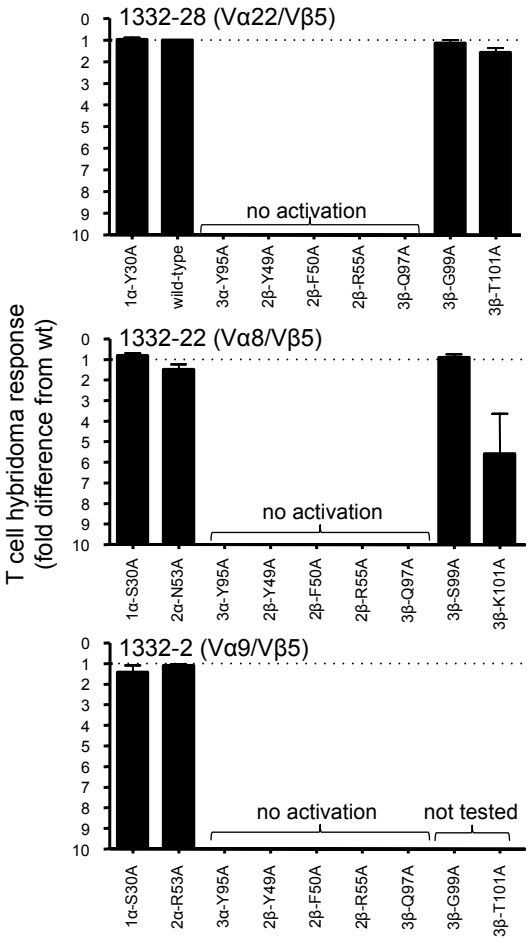
SUPPLEMENTAL FIGURE 4. Alternative docking footprints for Be-specific TCR recognition of antigen. *A*, Top-down view showing predicted interactions of TCR CDRs with HLA-DP2. Crystal

structure of HLA-DP2 with self-derived DR α-chain peptide (gray) as depicted with PyMol software (PDB ID code, 3LQZ) is shown. TCR CDRs are highlighted in yellow (CDR1α), orange (CDR2α), red (CDR3α), purple (CDR1β), green (CDR2β), and blue (CDR3β). Placement of TCR CDRs is speculative based upon the current studies involving site-directed mutagenesis of TCR and HLA-DP2. In *A*, the TCR docking footprint is shifted toward the β1 helix of HLA-DP2, with CDR1α and CDR2α making no MHCII contacts. *B*, The binding polarity for TCR recognition of HLA-DP2 is reversed, with TCR Vβ CDRs and β1 helix residues of HLA-DP2 dominating.

Supplemental Figure 1



Supplemental Figure 2

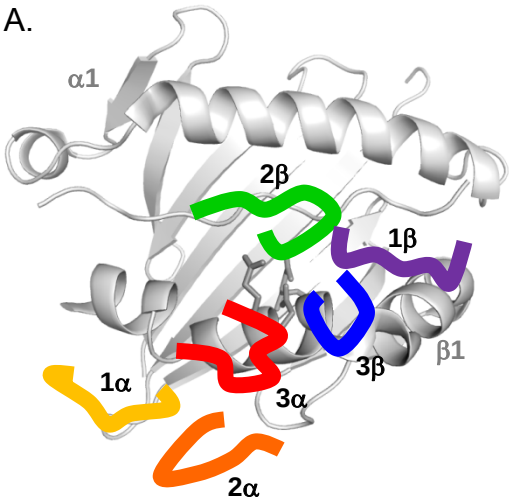


Supplemental Figure 3

	BV3	*NDN*	*BJ2.2*	*AV1S3*	*N*	*AJ4*
1041.-3.3	C A T G G	R A R G	T G E L F F G	C A	V S R V	S G G Y N K

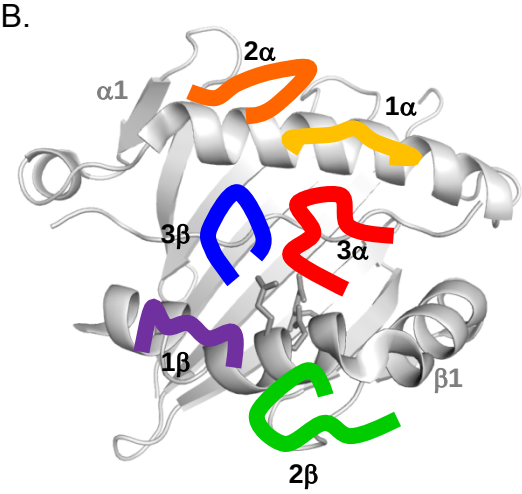
Supplemental Figure 4

A.



shifted footprint

B.



tilted and reversed polarity footprint