

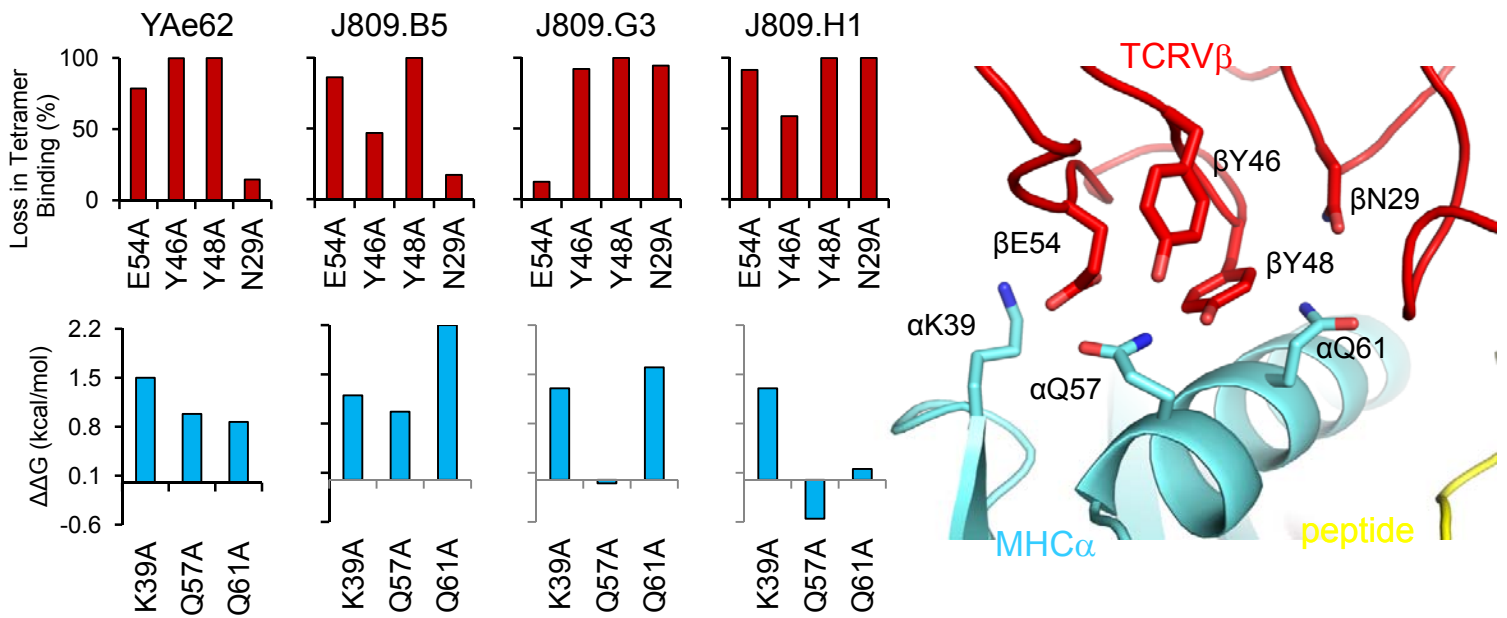
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**Supplemental Information**

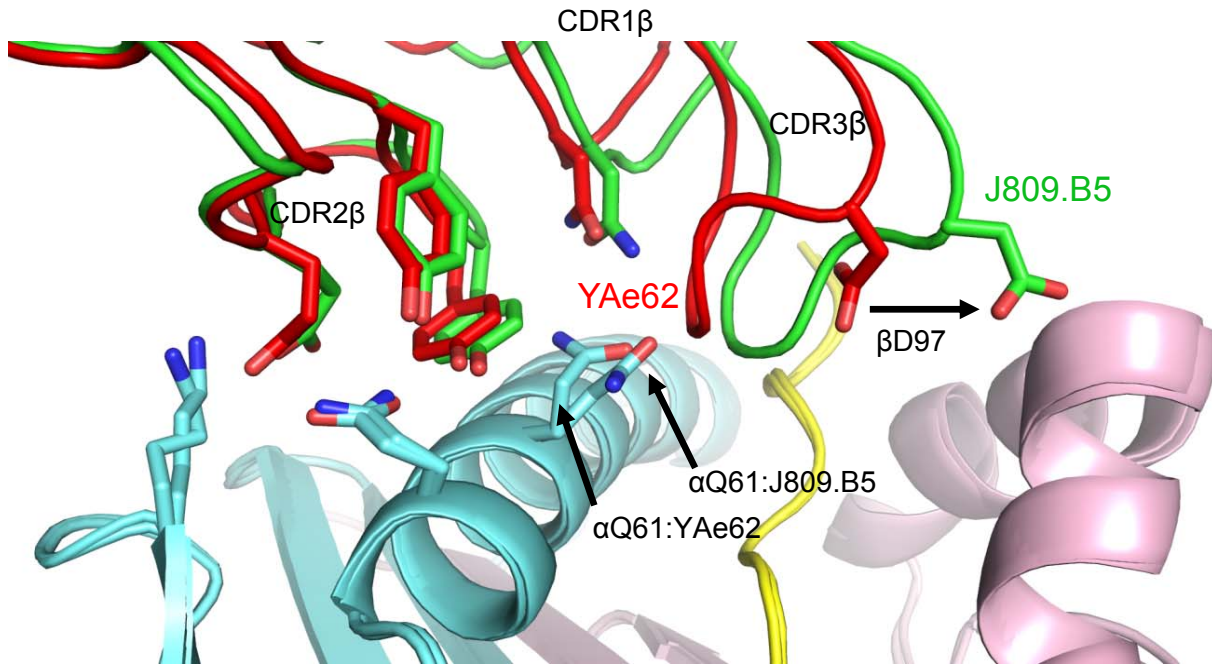
**Response to Garcia et al.**

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A.



B.



**Figure 1. Alteration of TCR $\beta$  interactions to IA<sup>b</sup>-3K.**

(A) The YAe62, J809.B5, J809.G3 and J809.H1 TCRs, and TCRs containing alanine substitutions at  $\beta$ E54  $\beta$ Y46  $\beta$ Y48 or  $\beta$ N29 were expressed on insect cells and stained with IA<sup>b</sup>-3K tetramers (upper panel). Data are percent loss in binding as compared to the wild-type TCR. The  $\Delta\Delta G$  values of YAe62, J809.B5, J809.G3 and J809.H1 TCRs binding IA<sup>b</sup>-3K containing alanine substitutions at IA<sup>b</sup>  $\alpha$ K39,  $\alpha$ Q57 or  $\alpha$ Q61 as calculated from SPR measurements ( $\alpha$ Q57 and  $\alpha$ Q61) or TCR multimer staining ( $\alpha$ K39) (lower panel). Positions of YAe62 (red) CDR2 $\beta$  residues  $\beta$ E54,  $\beta$ Y46,  $\beta$ Y48 and CDR1 $\beta$  residue  $\beta$ N29 and IA<sup>b</sup>  $\alpha$  (cyan) side chains  $\alpha$ K39,  $\alpha$ Q57,  $\alpha$ Q61 in YAe62:IA<sup>b</sup>-3K complex (right panel).

(B) Overlay of J809.B5 (green) and YAe62 (red) TCR $\beta$  chains binding IA<sup>b</sup>-3K. IA<sup>b</sup> $\alpha$  chain (cyan) IA<sup>b</sup> $\beta$  chain (magenta) and the 3K peptide (yellow) are shown, while the TCR $\alpha$  chains were removed for clarity. The alternate rotamer of IA<sup>b</sup>  $\alpha$ Q61 in the J809.B5:IA<sup>b</sup>-3K complex versus the YAe62:IA<sup>b</sup>-3K complex is labeled. Alteration of CDR3 loop structure is highlighted by the CDR3 $\beta$  residue D97, which forms interactions with the IA<sup>b</sup> $\beta$  chain in the J809.B5 TCR:IA<sup>b</sup>-3K complex.