



C Retrogenic Thymocyte Data

DE-Loop (170-175)

WT: MKAMDS

DE1: MAAAAS

C-Strand (147-158)

WT: DSQINVPKTMES

C4: DSQIDVPETMES

F-Strand (185-203)

WT: NQTSFTCQDIFKETN

F4: NQTSETCQDIFAETN

	% SP	% DP	n
WT	13.0% ± 2.6%	68.3% ± 7.0%	7
DE1	17.7% ± 0.2%	59.8% ± 3.8%	7
C4	93.7% ± 2.0%	0.2% ± 0.1%	3
F4	89.4% ± 4.7%	5.4% ± 1.5%	2

WT vs DE1 %SP $p=0.1840$, WT vs DE1 %DP $p=0.4992$,

WT vs C4 %SP $p<0.0001$, WT vs C4 %DP $p<0.0001$

DE1 vs C4 %SP $p<0.001$, DE1 vs C4 %DP $p<0.0001$

SFigure 1: Functional sidedness of the $\alpha\beta$ TCR.

(A) Cartoon representations of the $\alpha\beta$ TCR showing the side of the TCR with the unusual C α surface (left) and the side where CD3 $\gamma\epsilon$ and CD3 $\delta\epsilon$ dock (right) (PDB: 1TCR). (B) Cryogenic electron microscopy (cryo-EM) spacefill representation of $\alpha\beta$ TCR-CD3 complex showing the free side of the $\alpha\beta$ TCR with the exposed C α surface (left) and the side with docked CD3 modules (right) (PDB: 6JXR). For both (A) and (B), structural features are colored as followed: TCR α (grey), TCR β (magenta). The C-strand (yellow), F-strand (red) and DE-loop (cyan) are highlighted on the TCR α constant region (C α). CD3 modules are colored as CD3 δ (cyan), CD3 $\zeta\zeta$ (red), CD3 ϵ (blue), and CD3 γ (orange) are highlighted. (C) Amino acid sequences for the DE loop, C-strand and F-strand are shown. DP (CD8⁺ CD4⁺) and SP (CD8⁻ CD4⁺) thymocyte percentages are shown for retrogenic mice with the mutated 2B4 TCR. Values are shown as percentages \pm SEM, n is number of mice per group. Statistical analysis was done using One-way ANOVA and Tukey's post-test with exact p values shown. F4 was not included in the analysis due to insufficient n. Values were determined after pre-gating on FSC vs SSC, V α 11⁺, then CD8 vs CD4 and then gating on CD4⁺ SP and CD4⁺CD8⁺ DP.