**868Z11**

Matched Kd data is acquired from the same source and same supplementary data sheet (“Figure 6b”) as the activation values of mutants.

**TIL1383I**

Kd values of mutants are derived from the DDG heatmap of Fig. 5b. The raw data were not available, neither are the heatmaps in vector graphics format. So we saved the heatmaps as webp converted to jpeg file, read pixel-wise RGB values using a Python code and then map the RGB values to DDG values using the colorbar. From DDG data, we calculate Kd by using Kd = exp(DG/RT), with DG = DG\_WT + DDG, DG of WT peptide is -6.25 kcal/mol from Fig. 6 table and T is assumed to be 298K, so RT=0.592 (this also matches Fig. 6 DG and Kd conversions).

**c259**

1. Kd value is taken from Table S2 (Geometric mean) of “[Generation of T cells with reduced off-target cross-reactivities by engineering co-signalling receptors](https://www.biorxiv.org/content/10.1101/2024.10.25.620274v1.full)”. Full mutational scan of c259 around epitope SLLMWTQ**C** is recorded elsewhere from PMID: 37607971 (also the source of the TCR sequence), where the TCR is recorded as “1G4”. The activation data is from “[Generation of T cells with reduced off-target cross-reactivities by engineering co-signalling receptors](https://www.biorxiv.org/content/10.1101/2024.10.25.620274v1.full)”, Fig 5C killing heatmap, where the scan is around SLLMWITQ**V.**
2. For activation, We record the killing data corresponding to Fig 5C in the paper. The raw data is not available, neither are the heatmaps in vector graphics format. So we save the heatmaps as jpg files, read pixel-wise RGB values using a Python code and then map the RGB values to killing values using the colorbar. Cysteine is excluded in the scan at all positions. Killing data for mutations at position 9 broadly matches with the IFN values for the same mutants found in the other source (e.g., higher activity for substituted amino acids A, I, L, M).

**3K-TCRs**

TCRs name are slightly changed to match PMID: 16051149 TCR activity data: 2W20.4 is recorded as TCR2W1S12-20.4 and Yae62.8 is recorded as YAe5-62.8. For a subset of these peptides, the Kd values are explicitly denoted in Table 1, which we record directly. For the rest, we extracted the DDG data from the bar heights in Fig. 3 and converted them to Kd values using Kd = exp(DG/RT), with DG = DG\_WT + DDG, with DG\_WT recorded from Table 1, and T is assumed to be 298K, so RT=0.592.