Supporting information

High-affinity CD8 variants enhance the sensitivity of pMHCI antigen recognition via low-affinity TCRs

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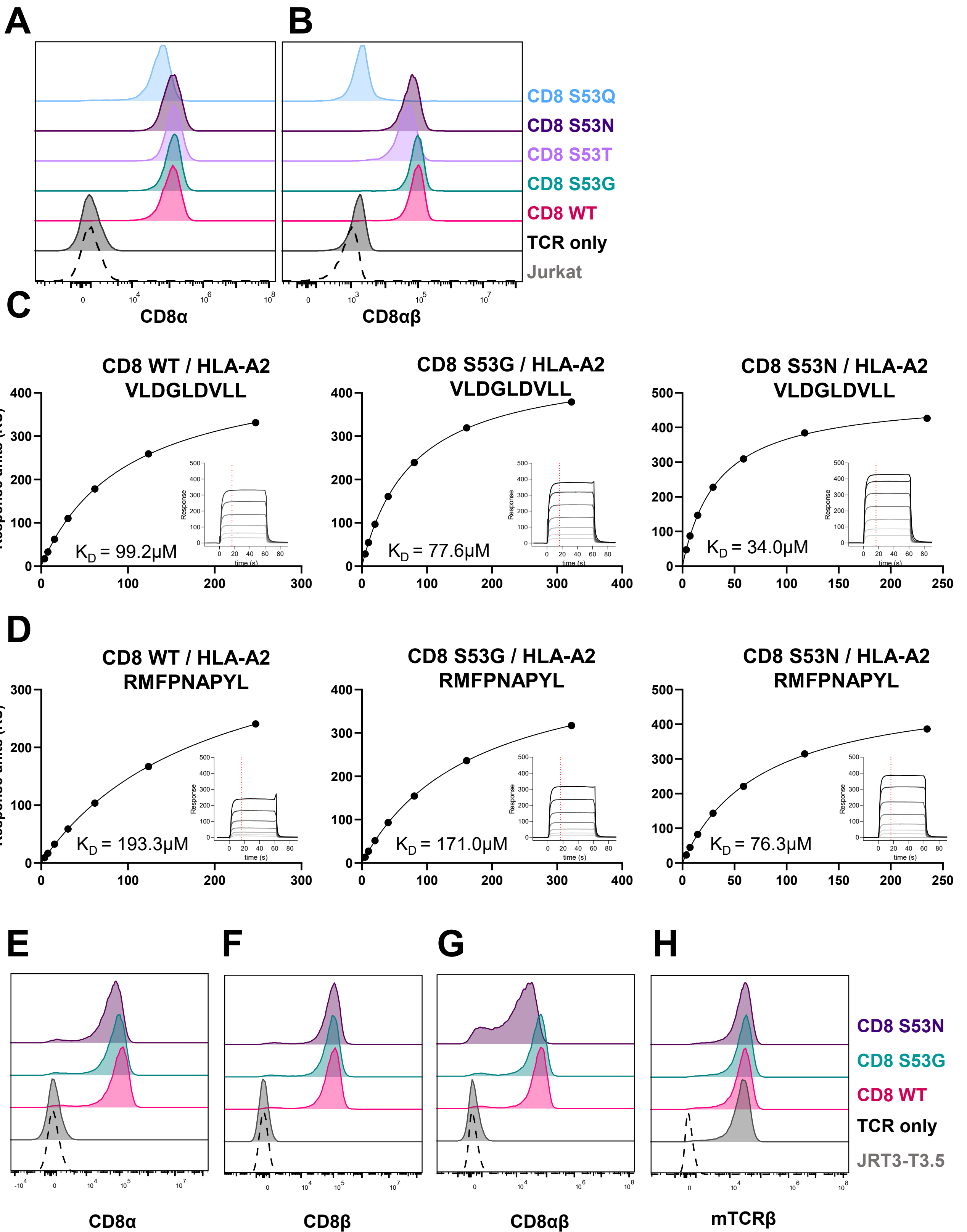
The supporting information contains the following figures:

Figure S1 Biophysical and cellular characterization of CD8 variants.

Figure S2. Sequencing and biophysical characterization of the RLA TCR

Figure S3 Functional analysis of primary $CD4^+$ and $CD8^+$ T cells transduced with tumor-targeting TCRs.

S1 Phenotyping of transgenic Jurkat cells and affinity measurements of CD8 variants



Supporting Figure S1 Biophysical and cellular characterization of CD8 variants.

A-B Expression of CD8α (A) and CD8αβ (B) on Jurkat cells transduced with the RLA TCR and CD8αβ containing either wild-type (WT) CD8α or mutated forms (S53G, S53T, S53N, or S53Q) of CD8α. **C- D** Representative surface plasmon resonance affinity measurements of wild-type (WT) CD8αα and the most functionally potent variants of CD8αα, namely S53G and S53N, versus VLD/HLA-A*0201 or RMF/HLA-A*0201. **E–H** Expression of CD8α (E), CD8β (F), CD8αβ (G), and TCRβ (H) on MEL5 TCR^{+} $CD8\alpha\beta^{+}$ JRT3-T3.5 cells transduced with CD8αβ containing either wild-type (WT) CD8α (red) or mutated forms of CD8α, namely S53G (teal) or S53N (purple).

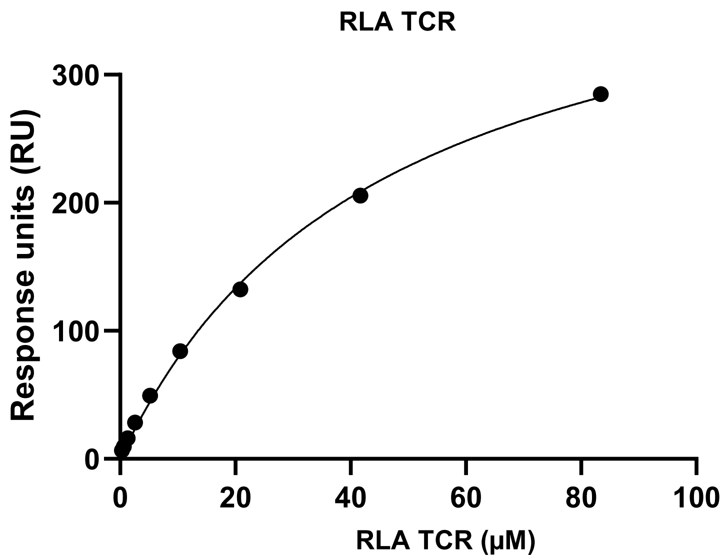
S2 RLA TCR sequencing and affinity measurement

A

RLA TCR Sequence

TRAV	CDR3	TRAJ
19	CALSEAVTDSSYKLI	12
TRBV	**CDR3**	**TRBJ**
10-3	CATGQGEYNEQF	2-1

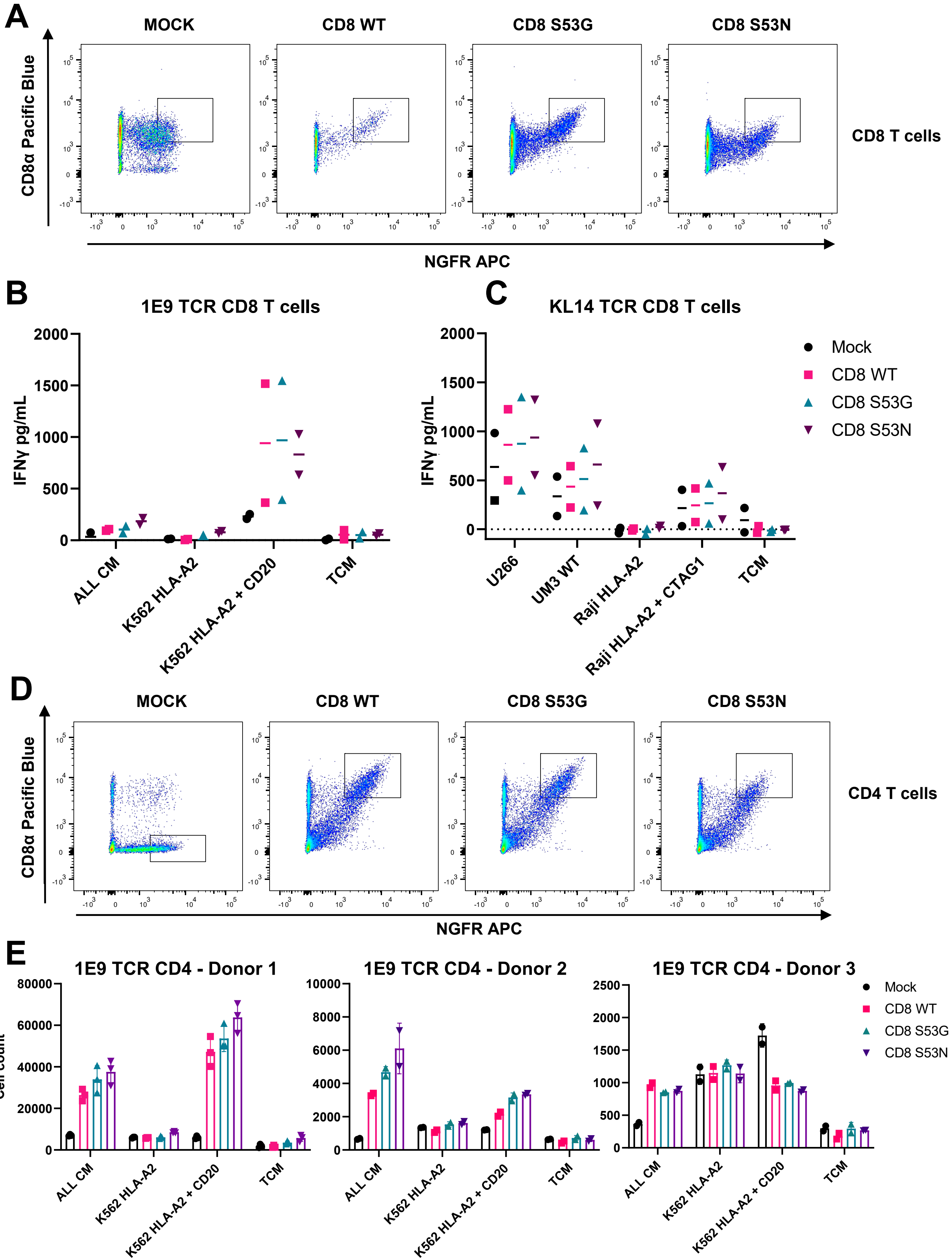
B



Supporting Figure S2. Sequencing and biophysical characterization of the RLA TCR

A TCRα and TCRβ sequences of the RLA TCR. Gene use was assigned according to the ImMunoGeneTics (IMGT) information system (http://www.imgt.org). **B** Surface plasmon resonance measurements of the RLA TCR versus RLA/HLA-A*0201.

S4 Sorting strategy for primary T-cells and further functional data in primary CD8 and CD4 T-cells



Supporting Figure S3 Functional analysis of primary $CD4^+$ and $CD8^+$ T cells transduced with tumor-targeting TCRs.

A Gating strategy for the purification of primary $CD8^+$ T cells expressing CD8αβ containing either wild-type (WT) CD8α or mutated forms of CD8α, namely S53G or S53N, alongside the 1E9 TCR via FACS. **B** Primary $CD8^+$ T cells expressing CD8αβ containing either wild-type (WT) CD8α (red) or mutated forms of CD8α, namely S53G (teal) or S53N (purple), alongside the 1E9 TCR were cocultured with a panel of cell lines lacking or expressing CD20. The panel shows mean IFN-γ production from each of two donors. **C** Gating strategy for the purification of primary $CD4^+$ T cells expressing CD8αβ containing either wild-type (WT) CD8α or mutated forms of CD8α, namely S53G or S53N, alongside the 1E9 TCR via FACS. **D** Primary $CD8^+$ T cells expressing CD8αβ containing either wild-type (WT) CD8α (red) or mutated forms of CD8α, namely S53G (teal) or S53N (purple), alongside the KL14 TCR were cocultured with a panel of cell lines lacking or expressing CTAG1. The panel shows mean IFN-γ production from each of two donors. **E** Primary $CD4^+$ T cells expressing CD8αβ containing either wild-type (WT) CD8α (red) or mutated forms of CD8α, namely S53G (teal) or S53N (purple), alongside the 1E9 TCR were cocultured with a panel of cell lines lacking or expressing CD20. Cells were quantified after 5 days via flow cytometry ($n = 3$ donors). TCM, T cell medium. Data are shown as mean ± SD from duplicate or triplicate measurements per donor.