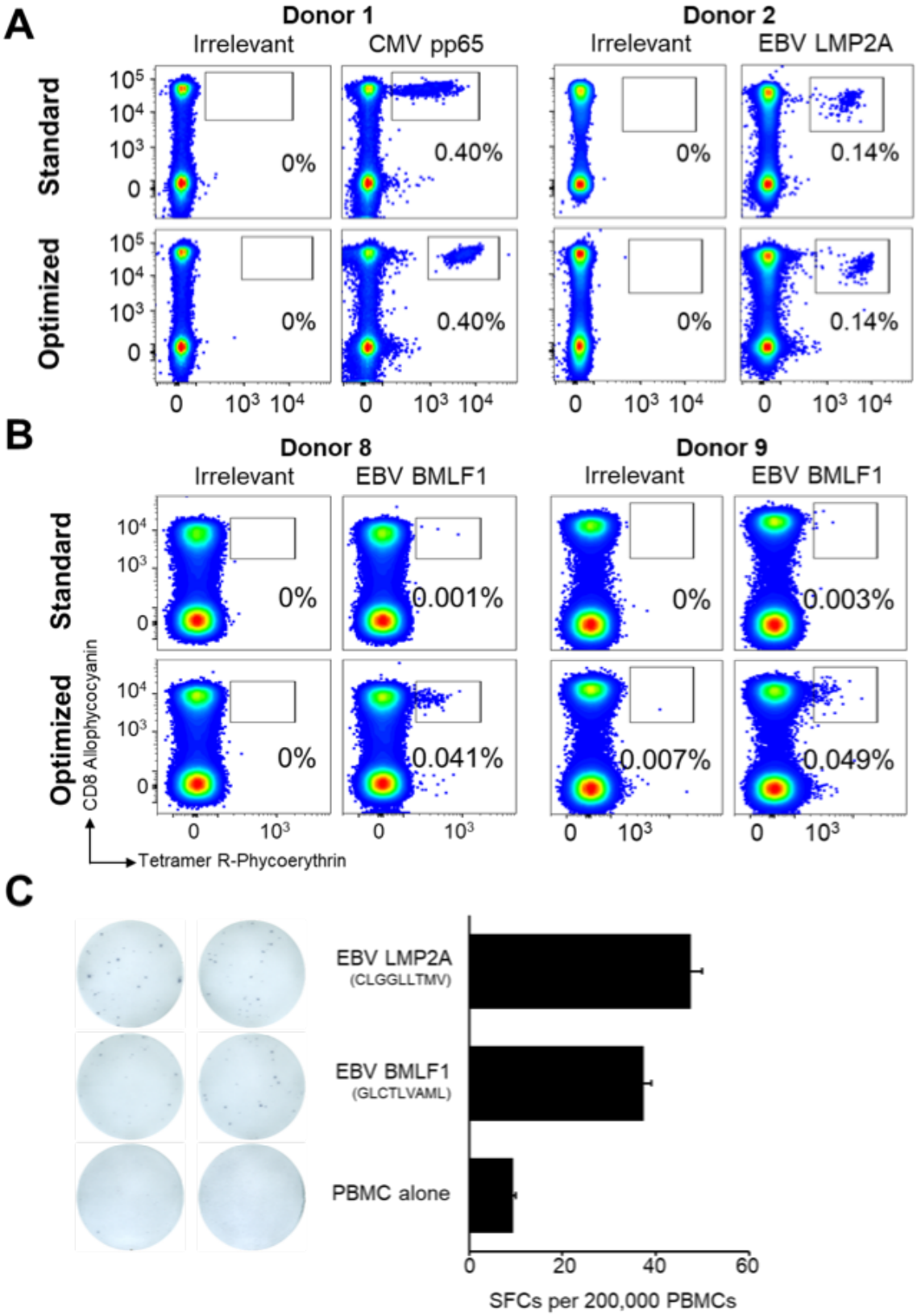
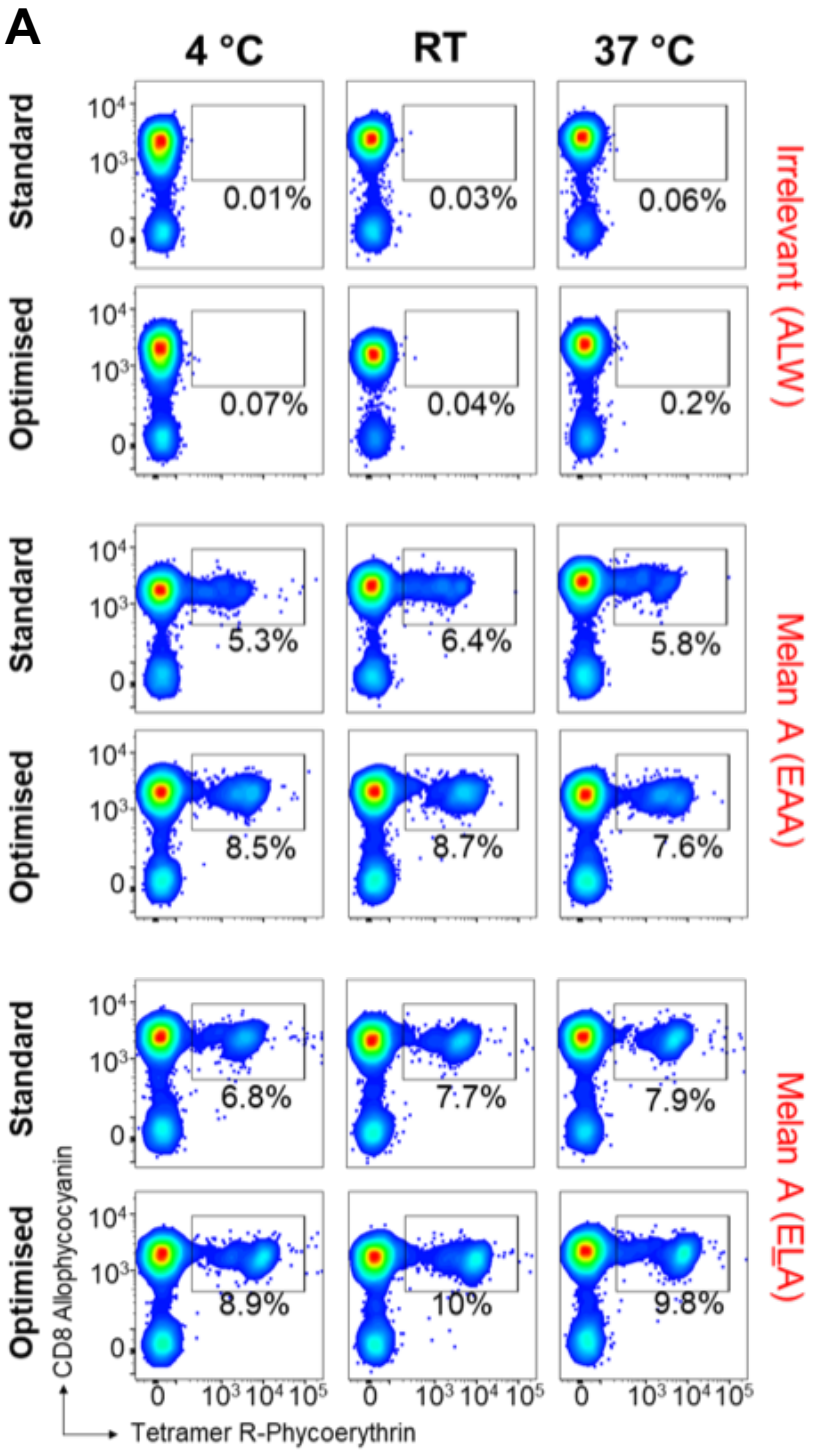
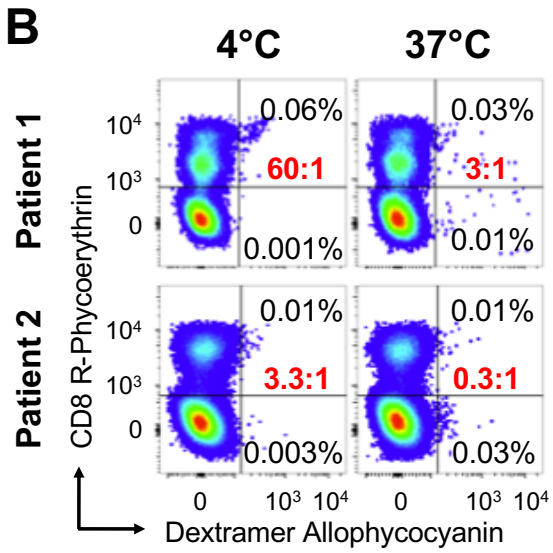


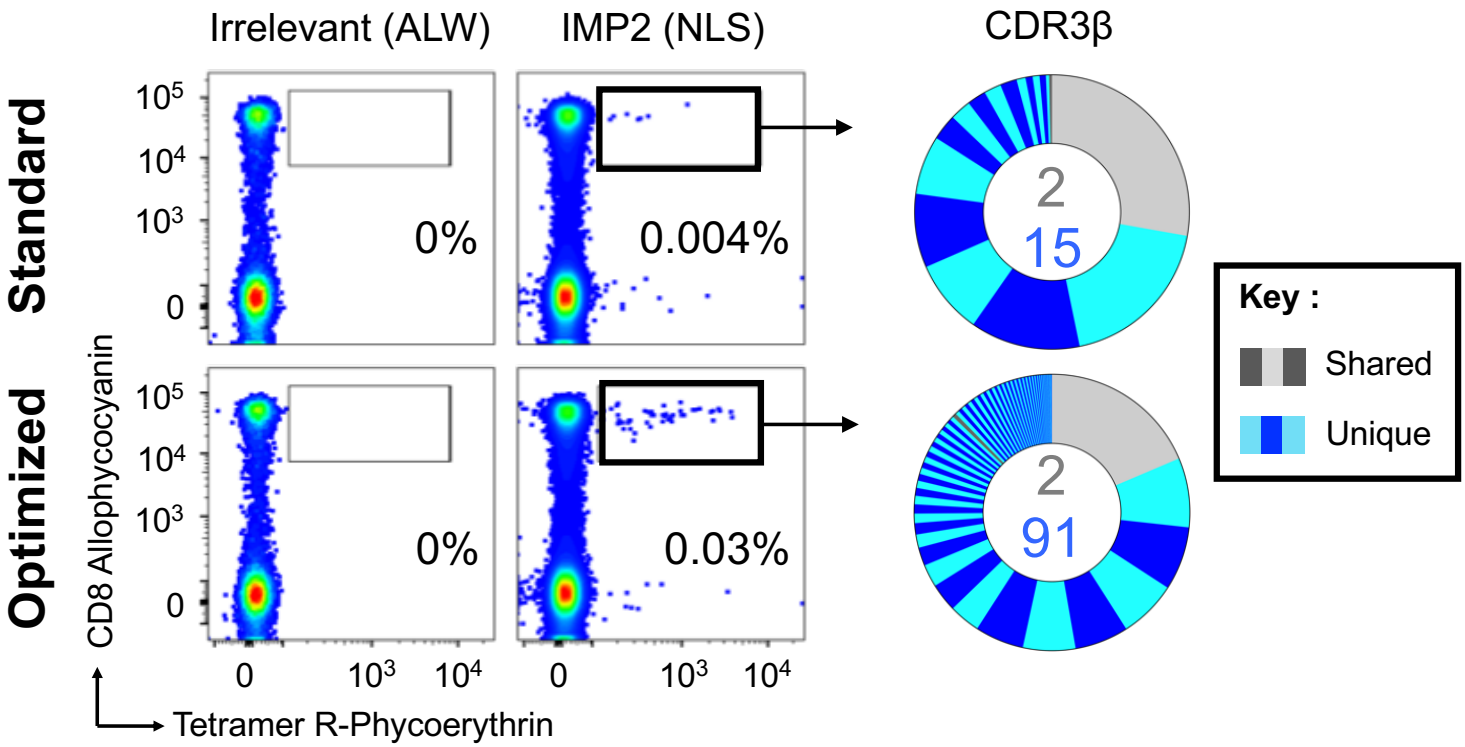
Supplementary Figure 1. *Ex vivo* detection of antigen-specific CD8 T-cells using pMHC multimer staining technology. PBMC from HLA-A2+ healthy donors were stained with tetramers bearing **(A)** the Cytomegalovirus $pp65_{495\text{-}503}$ peptide (NLVPMVATV, left), Epstein-Bar virus (EBV) $LMP2A_{426\text{-}434}$ peptide (CLGGLLTMV, right), or **(B)** HLA-A2 $BMLF1_{280–288}$ either using tetramer alone (standard) or in combination with PKI and anti-flurorochrome antibody (optimized). Gates were set on lymphocytes and live $CD3^+$ $CD14^-$ $CD19^-$ cells. Irrelevant tetramer made with preproinsulin-derived peptide ($PPI_{15\text{-}24}$, ALWGPDPAAA) or Human Telomerase Reverse Transcriptase ($hTERT_{540\text{-}548}$, ILAKFLHWL) were used to set the gates. Percentage of $CD8^+$ $tetramer^+$ T-cells is shown for each gate. **(C)** 0439 donor was pre-screened for T-cell activation against virus stimulation. *Ex vivo* PBMC were incubated with EBV-derived CLGGLLTMV and GLCTLVAML peptides (10^{-5} M, in duplicate) overnight. Response was quantified by IFNγ ELISpot. No peptide was used as negative control.

Supplementary Figure 2. Cumulative %TRBV gene usage for standard (Yellow) and optimized (blue) staining protocols, with predominant gene usages indicated by darker colors in the heatmap. All human genes listed on the Y-axis for completeness.





Supplementary Figure 3. (A) Melan A specific TILs were stained using HLA A2-EAAGILGILTV and HLA A2-ELAGILGILTV standard or optimized tetramer staining at 4°C, 37°C or room temperature (~22 °C). The percentage of tetramer$^+$ is displayed. Irrelevant HLA A2-ALWGPDPAAA (preproinsulin residues 15-24) was used to set the gates. **(B)** Peripheral blood monocular cells from HLA A2$^+$ donors with type I diabetes were thawed and stained with HLA A2-ALWGPDPAAA (preproinsulin residues 15-24) allophycocyanin conjugated dextramer. Staining was performed on ice (4°C) or at 37°C. The percentage of viable CD3 cells is shown for the CD8$^+$ dextramer$^+$ (upper right) and CD8^{neg} control (lower left) gate, with the signal to noise ratio in red.



Supplementary Figure 4. Optimized pMHC multimer staining results are reproducible. (A) PBMC from donor 0439 were *ex vivo* stained in parallel using either IMP2 standard or optimized tetramer protocols Percentage tetramer⁺ cells of CD8⁺ T-cells is shown for each gate. Irrelevant tetramer made with PPI was used to set the gates for sorting. CD8⁺ Tetramer⁺ cells were sorted for TCR sequencing and CDR3 analysis of β chains (right). Clonotypes are displayed as sort-shared (grey) or sort-unique (blue) sections of a donut pie, with each section for each sort corresponding to a different CDR3. The number of shared (grey) and unique (blue) CDR3s for the respective sorts are shown in the center of each pie.