

**Supplementary Figure 1. Ex vivo detection of antigen-specific CD8 T-cells using pMHC multimer staining technology.** PBMC from HLA-A2<sup>+</sup> healthy donors were stained with tetramers bearing (A) the Cytomegalovirus pp65<sub>495-503</sub> peptide (NLVPMVATV, left), Epstein-Bar virus (EBV) LMP2A<sub>426-434</sub> peptide (CLGGLTMV, right), or (B) HLA-A2 BMLF1<sub>280-288</sub> either using tetramer alone (standard) or in combination with PKI and anti-fluorochrome antibody (optimized). Gates were set on lymphocytes and live CD3<sup>+</sup> CD14<sup>-</sup> CD19<sup>-</sup> cells. Irrelevant tetramer made with preproinsulin-derived peptide (PPI<sub>15-24</sub>, ALWGPDPA<sub>15-24</sub>) or Human Telomerase Reverse Transcriptase (hTERT<sub>540-548</sub>, ILAKFLHWL) were used to set the gates. Percentage of CD8<sup>+</sup> tetramer<sup>+</sup> 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 CLGGLTMV and GLCTLVAML peptides (10<sup>-5</sup> M, in duplicate) overnight. Response was quantified by IFN<sub>γ</sub> ELISpot. No peptide was used as negative control.

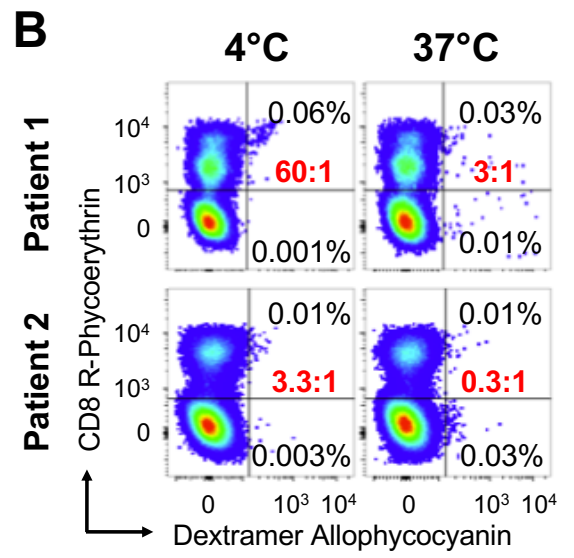
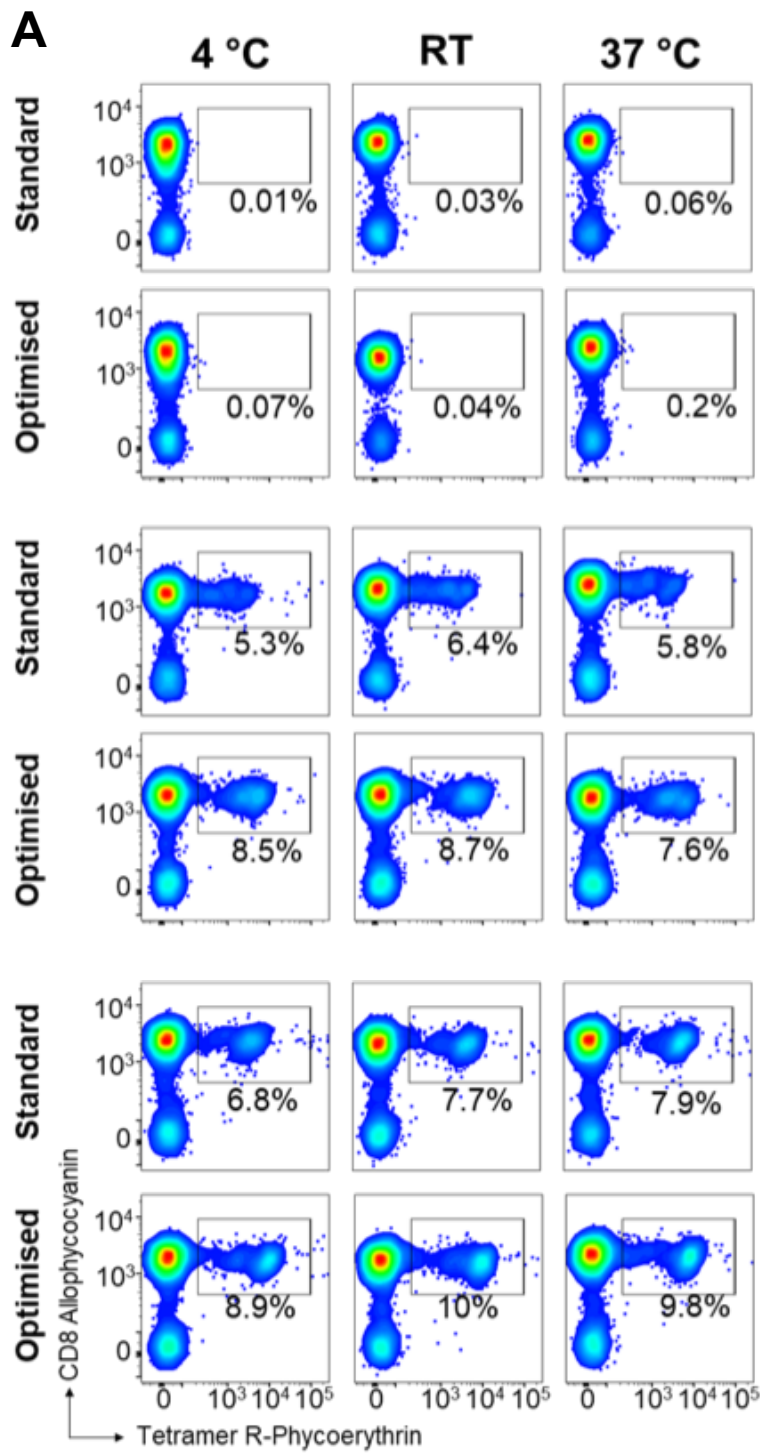
Donor 5  
EBV (GLC)Donor 0439  
EBV (GLC)Donor 0345  
YF (LLW)TIL  
MeI (ELA)Donor 0439  
IMP2 (NLS)Donor 0439  
IMP2 (NLS)

Key:

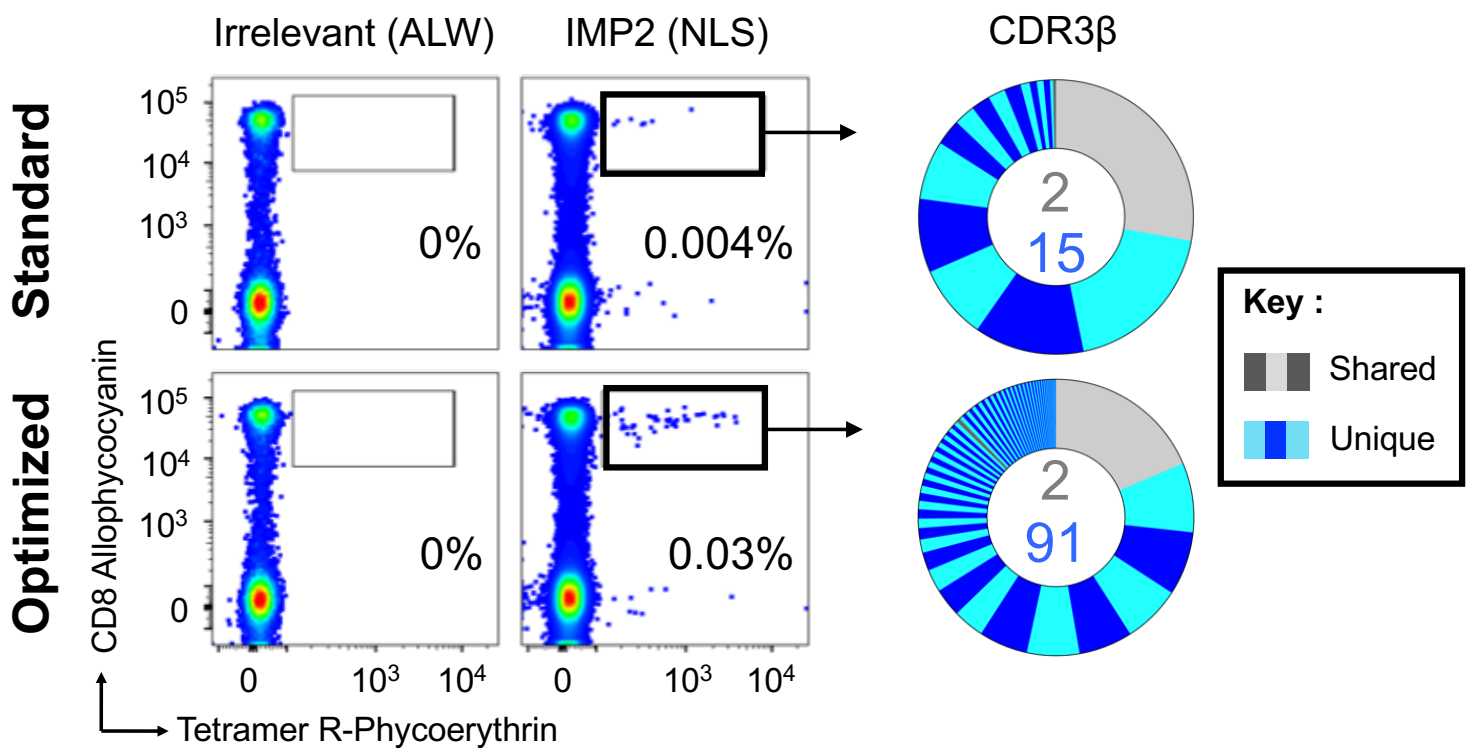
Standard Protocol  
Optimised Protocol

TRBV1										0.08
TRBV2	0.94								0.84	1.28
TRBV3-1		6.71	2.29	4.26				0.43	3.93	2.60
TRBV3-2										
TRBV4-1		2.41		2.19			1.10	15.91	5.42	15.43
TRBV4-2		0.48					1.00			
TRBV4-3										
TRBV5-1	14.95	14.87	2.13	0.92				2.59	0.73	2.02
TRBV5-2										
TRBV5-3										
TRBV5-4	2.30	1.16								0.07
TRBV5-5		4.22								0.35
TRBV5-6		0.80						0.41		0.57
TRBV5-7										
TRBV5-8										
TRBV6-1		2.83					0.60		0.55	2.64
TRBV6-2		0.06								0.35
TRBV6-3							5.30	1.10		
TRBV6-4										
TRBV6-5		4.45	0.71				8.80	1.10		9.43
TRBV6-6		5.90								
TRBV6-7										
TRBV6-8										
TRBV6-9										
TRBV7-1										
TRBV7-2		2.60						37.36	14.42	27.80
TRBV7-3		1.53		0.86						0.52
TRBV7-4										
TRBV7-5										
TRBV7-6		1.42					1.10		0.70	0.85
TRBV7-7										
TRBV7-8		3.29							0.17	2.42
TRBV7-9	35.53	5.56						5.46	16.61	3.07
TRBV8-1										
TRBV8-2										
TRBV9	0.65	5.49								2.61
TRBV10-1										0.40
TRBV10-2										6.93
TRBV10-3		2.02								7.43
TRBV11-1										0.99
TRBV11-2	5.62	3.34								0.48
TRBV11-3										
TRBV12-1										
TRBV12-2		5.05								6.15
TRBV12-3										0.11
TRBV12-4	6.46	2.00					5.30	3.10		1.09
TRBV12-5									8.28	2.94
TRBV13									1.43	1.08
TRBV14		0.11							0.17	0.37
TRBV15		0.75	38.83	44.91			0.70		0.17	1.24
TRBV16		0.24								0.09
TRBV17										
TRBV18		0.05							7.37	0.34
TRBV19	13.73	0.40							0.55	18.60
TRBV20-1	19.81	8.05	52.17	35.36			2.70	24.70	11.31	10.68
TRBV21-1										1.40
TRBV22										
TRBV23-1										
TRBV24-1		0.54					2.80		0.79	18.98
TRBV25-1		0.78								7.15
TRBV26										
TRBV27	0.04	0.08	4.46	3.87	6.38	68.40	64.20	12.12	13.84	8.70
TRBV28			2.49		2.07		9.70			4.91
TRBV29-1	99.96	99.92	5.91		2.65		2.40		14.57	2.24
TRBV30						12.30	5.00			6.85

**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<sup>+</sup> is displayed. Irrelevant HLA A2-ALWGPDPAAA (preproinsulin residues 15-24) was used to set the gates. **(B)** Peripheral blood mononuclear cells from HLA A2<sup>+</sup> 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<sup>+</sup> dextramer<sup>+</sup> (upper right) and CD8<sup>neg</sup> 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<sup>+</sup> cells of CD8<sup>+</sup> T-cells is shown for each gate. Irrelevant tetramer made with PPI was used to set the gates for sorting. CD8<sup>+</sup> Tetramer<sup>+</sup> cells were sorted for TCR sequencing and CDR3 analysis of  $\beta$  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.