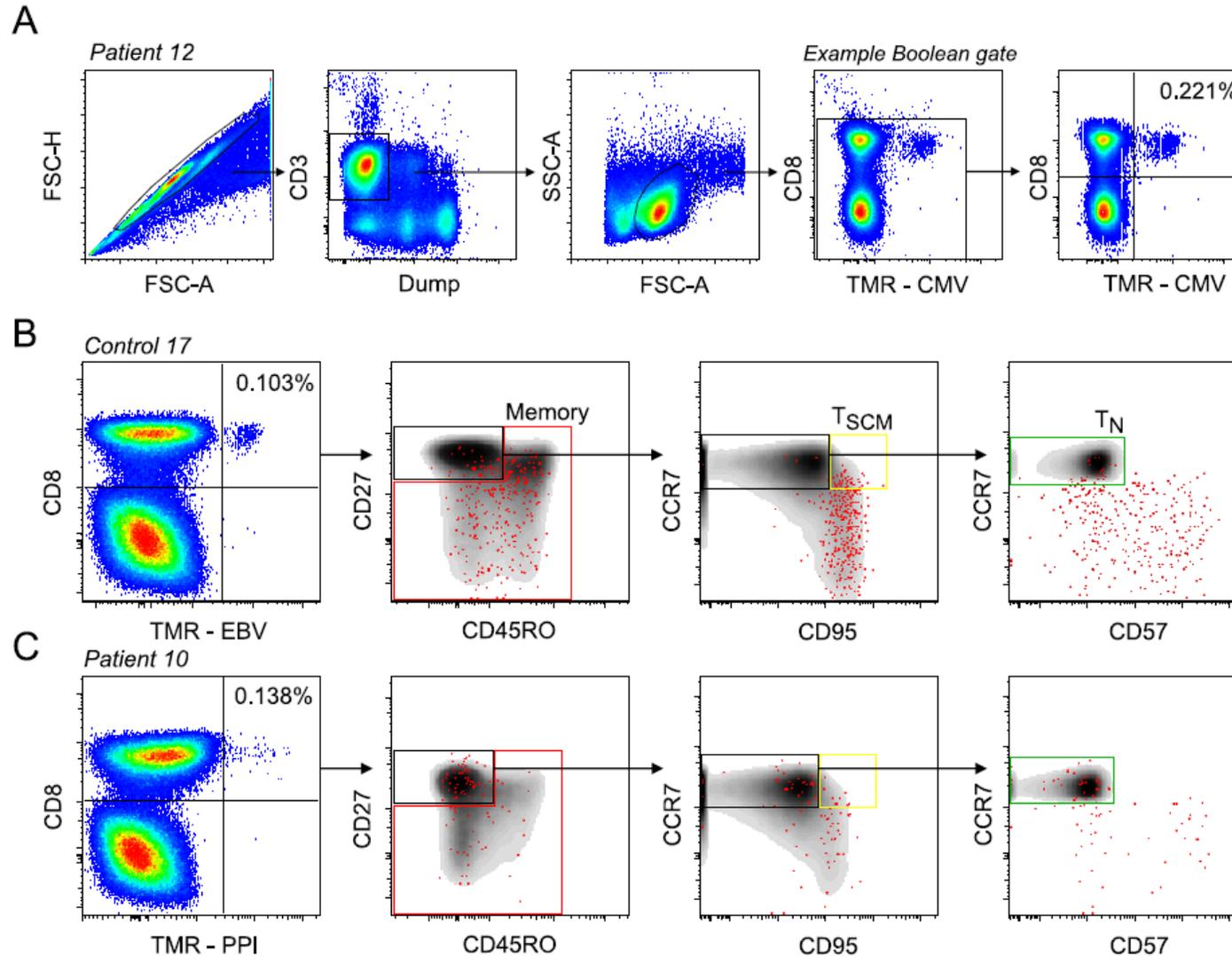


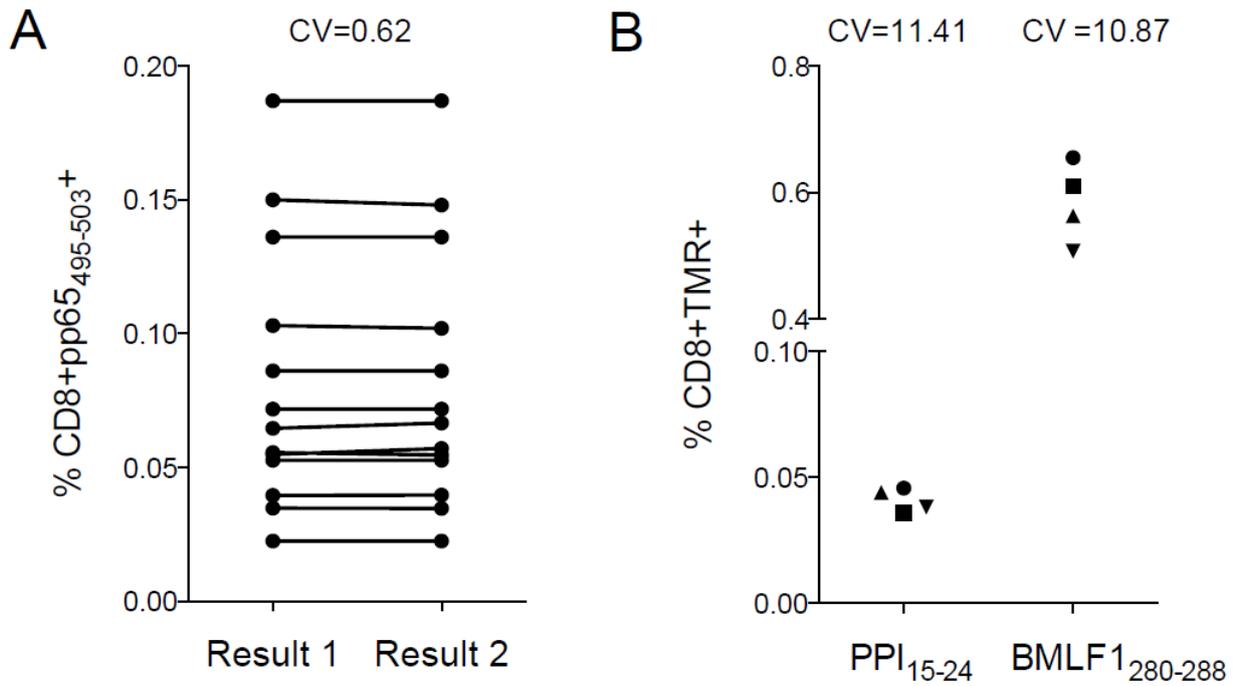
SUPPLEMENTARY DATA

Supplementary Figure 1. Representative flow cytometric data and gating strategy. *A*: Gates were set serially on singlets, live CD3⁺CD14⁻CD19⁻ cells and lymphocytes prior to exclusion of dye aggregates via Boolean gating and subsequent analysis as CD8 versus tetramer (TMR). *B&C*: Phenotypic analysis of tetramer-binding CD8 T cells to identify naïve (T_N - CD27⁺CD45RO⁻CD57⁻CD95⁻CCR7⁺) and stem cell memory (T_{SCM} - CD27⁺CD45RO⁻CD95⁺CCR7⁺) subsets. Antigen-specific events are shown as colored dots overlaid on cloud plots depicting the entire CD8 T cell population.



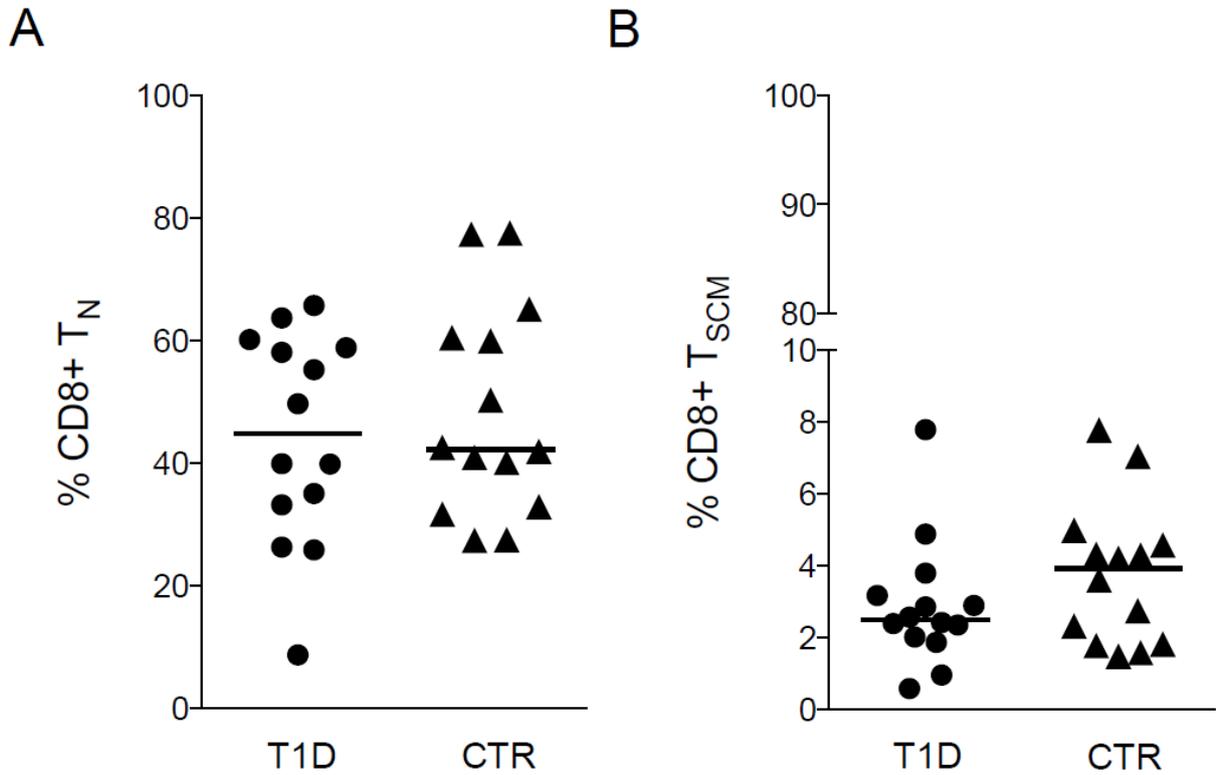
SUPPLEMENTARY DATA

Supplementary Figure 2. Quality control. *A*: Intra-assay variability was assessed by repeated measurements of tetramer-binding frequencies separated by intervals of more than one month. Different aliquots of cryopreserved PBMCs drawn from the same donor at the same time point were used for paired evaluations (n=13). *B*: Inter-assay variability was assessed across specificities as the coefficient of variation. Data points represent time-separated measurements (n=4) of tetramer-binding cells in different aliquots of cryopreserved PBMCs drawn from the same donor at the same time point.



SUPPLEMENTARY DATA

Supplementary Figure 3. Phenotypic analysis of whole CD8 T cell populations. *A:* Frequency of T_N cells as a percentage of the total CD8 T cell population in type 1 diabetes patients (T1D) and healthy controls (CTR). *B:* Frequency of T_{SCM} cells as a percentage of the total CD8 T cell population in type 1 diabetes patients (T1D) and healthy controls (CTR). Statistical comparisons were performed using the Mann-Whitney U-test.



SUPPLEMENTARY DATA

Supplementary Figure 4. Single marker analysis of antigen-specific CD8 T cells. *A-E*: Percent expression of CD95 (*A*), CD57 (*B*), CD27 (*C*), CCR7 (*D*) and CD45RO (*E*) is shown for type 1 diabetes patients (T1D) and healthy controls (CTR) across all CD8 T cells and the indicated specificities. Bars represent median values. Statistical analyses were performed using the Mann-Whitney U-test; *p* values ≤ 0.05 are shown.

