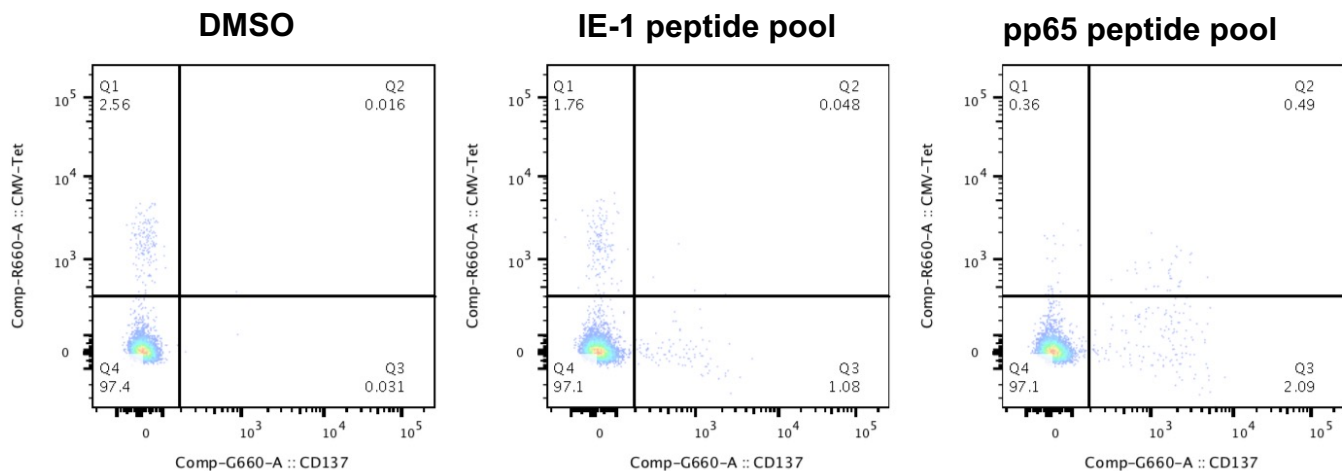
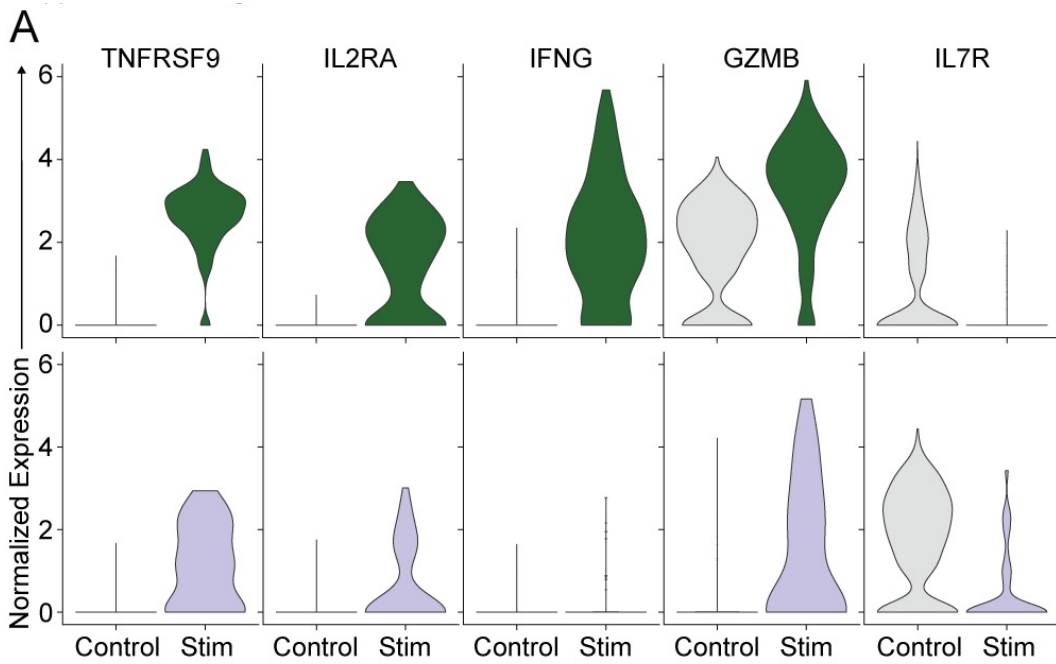


Supplemental Figure 1. The transcriptional profile of CMV-Tetramer+ CD8+ T cells in context of all CD8+ T cells. To compare the transcriptional profile of CMV-Tetramer+ CD8+ T to all CD8+ T cells, we merged CMVtet objects with CD8 objects, and integrated these two datasets using Harmony (PMID: 31740819). The merged data (for all 4 patients) are shown as UMAP plots using SingleR cell state classifications to assign T cell subset identities in (A). The transcriptional shared space between CMVtet+ and bulk CD8 T cells for all 4 patients is shown in (B). To illustrate patient-specific contributions, the CMVtet+ population for each patient is shown in (C). The plots in the left column are color-coded to show SingleR classifications and the plots in the right column are color-coded to show the contribution for each time point (see color scheme on right hand side for days 30, 60 and 90). CMVtet+ CD8s appear to be mainly “terminally differentiated effectors” (as assigned by SingleR) for patient 2 and 4, and in patient 3 some CMVtet+ CD8s additionally also fall into the “effector memory” space.



Supplemental Figure 2. CMV-NLV Tetramer+ CD8+ T cell staining following ex vivo stimulation with CMV peptide pools. Shown is the CMV-NLV tetramer (y-axis) vs. CD137 staining (x-axis) for the ex vivo stimulation experiment. CMV-NLV tetramer+ CD8+ T cells were detected at comparable frequencies in our no stimulation (DMSO) control and the IE-1 peptide pool stimulation. Stimulation with the pp65 peptide pool led to a concomitant strong decrease in tetramer staining, which is expected and a result of TCR downregulation.



B

Peptide pool	Cell count (Stim)	Cell Count (ex vivo)	CDR3b	Literature match
pp65	70	0	CASSRQTGAAYGYTF	pp65_NLV
	5	0	CASSRQTGAAYGYTF	pp65_NLV
	3	0	CASSQEEGPGHQPHF	pp65_NLV
	2	0	CASSADWKRETQYF	N/A
	2	0	CASSVNEQFF	pp65_NLV
IE-1	18	123	CASSLDAAYEQYF	N/A
	5	9	CASSFTSGYNEQFF	N/A
	3	65	CAWSATGPYEQYF	N/A
	2	33	CATSTGRRGVGQETQYF	N/A
	2	9	CAWSVSGPYEQYF	N/A

Supplemental Figure 3. Additional analysis of peptide-pool stimulated CD137+ CD8+ T cells compared to unstimulated CD8 T cells (control). (A) The gene expression profile of recipient-derived (green) and donor-derived (purple) CD8 T cells compared to CD8 T cells from the DMSO (no stim) control group (grey). (B) CD137+ sorted T cells were analyzed by 5' scRNA-seq to determine TCR gene usage and CDR3 sequences. Cell counts for each clone are shown from the peptide pool stimulation experiment (stim) and compared to the direct ex vivo sequencing data (shown in Fig. 3).