



Gating strategy. Flow cytometry data of cross-reactive peptide mix I comprising HLA class I peptides for stimulation of CD8+T cells, positive and negative control at timepoint 2 are shown as an example to demonstrate the gating strategy of a T cell analysis. In a first step cell counts were selected in a time histogram. Lymphocytes were gated with forward (FSC) vs. sideward scatter (SSC). A forward scatter area vs forward scatter height dot plots are used to isolate single cells, and thereby remove any non-single cells (doublets, clumps or debris). Lymphocytes were then distinguished between viable and dead cells. Viable cells were then displayed in a CD56 vs CD8 plot, to discriminate between NK and T cells. CD8+ CD56- cells were further split into CD4+ helper and CD8+ cytotoxic T cells. To define functional markers for CD4+ and CD8+ T cells, a gate was applied for each cytokine. Boolean gates were then created based on these gates to identify cells expressing different combinations of markers. Since HLA class I peptides were used in this sample, no CD4 response but a clear CD8 response was detectable. This analysis example refers to figure 1 C, illustration on the right.