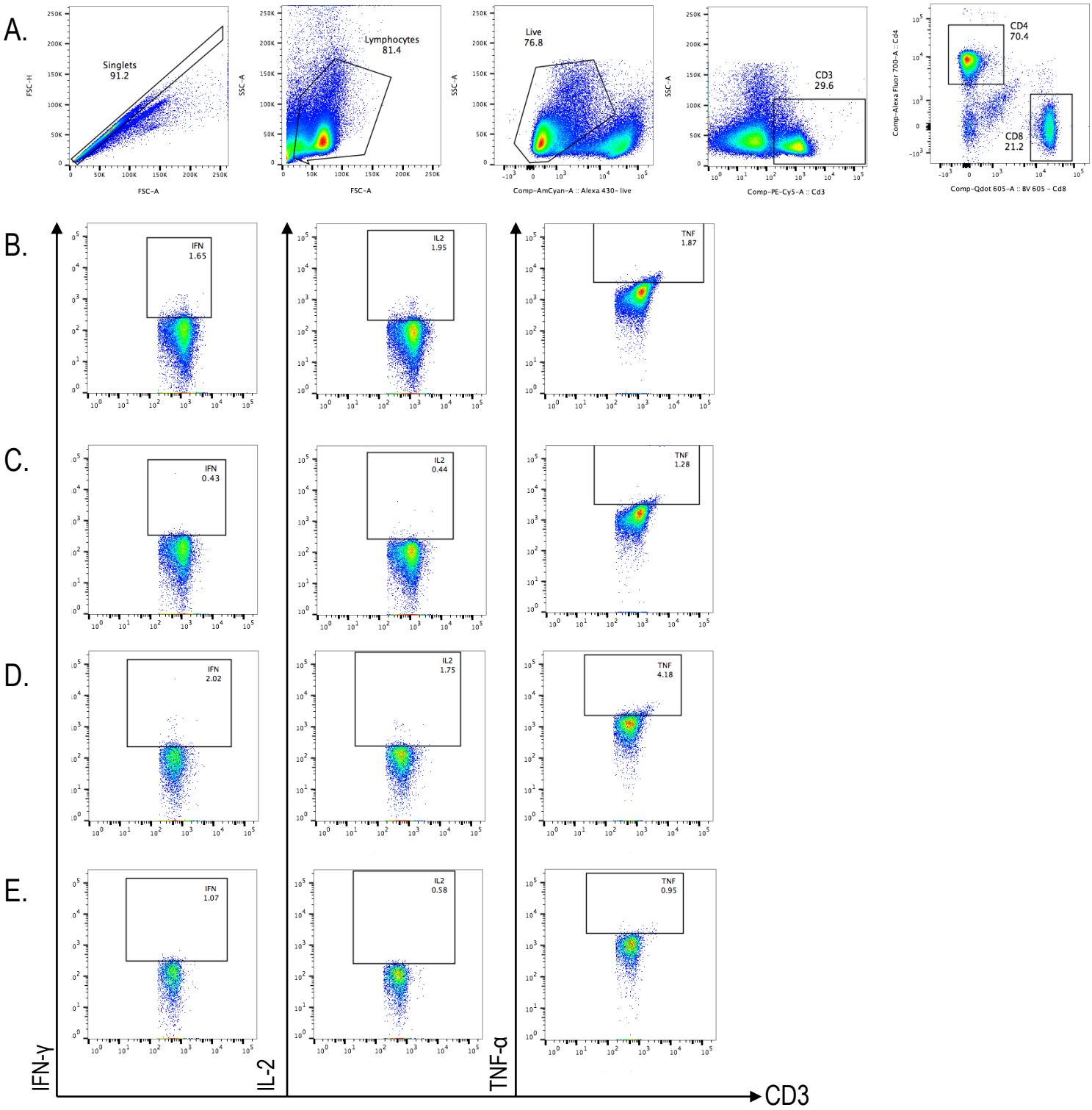
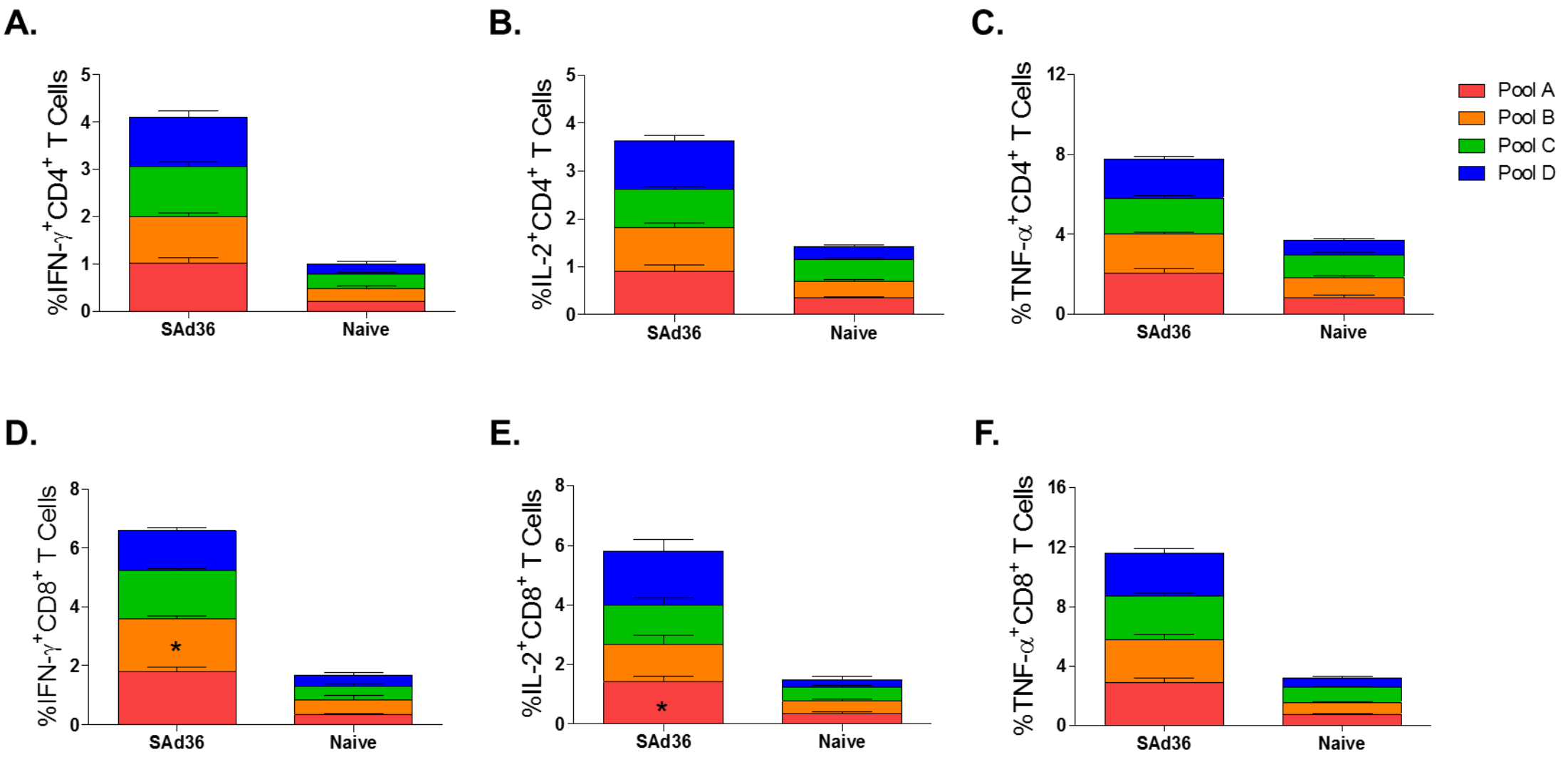
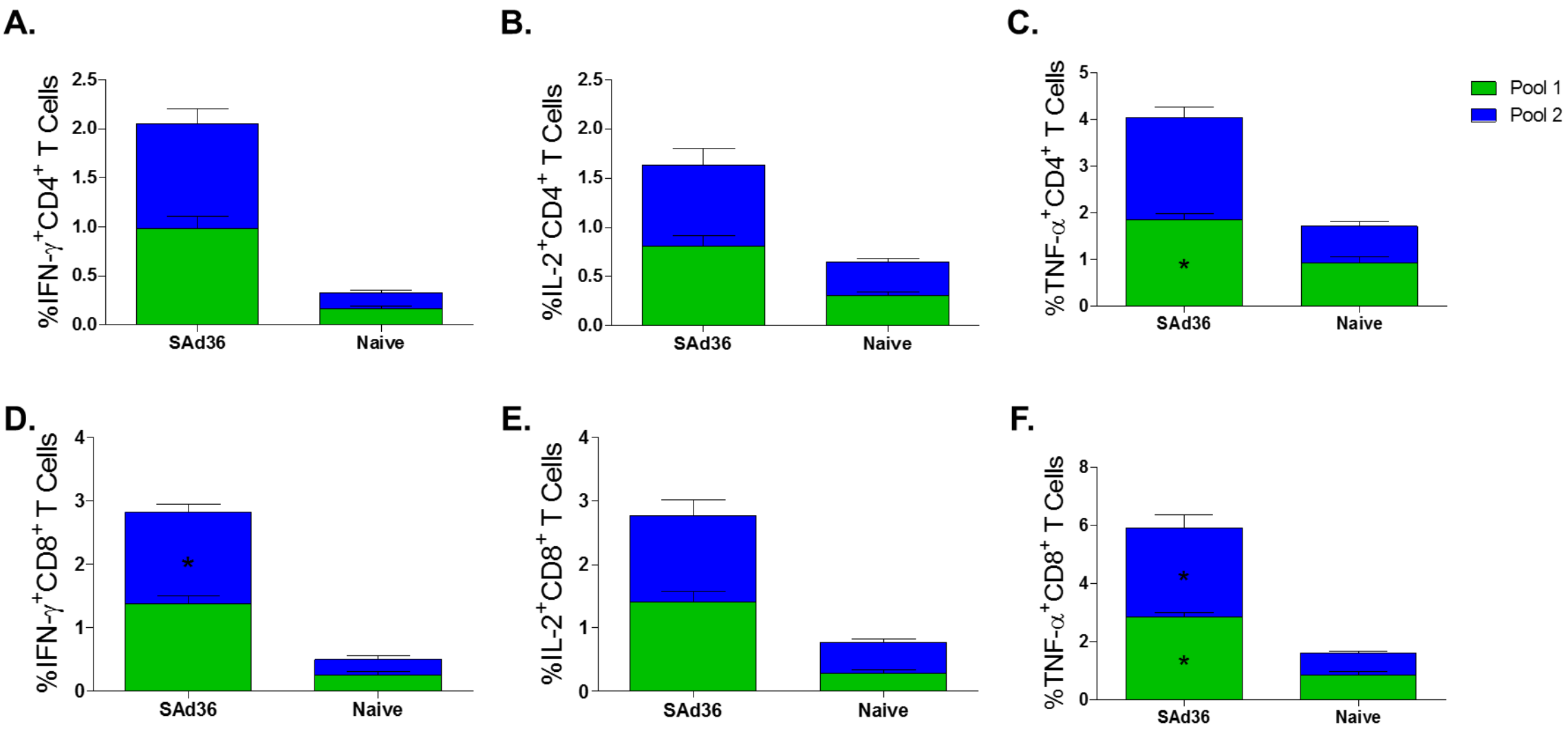


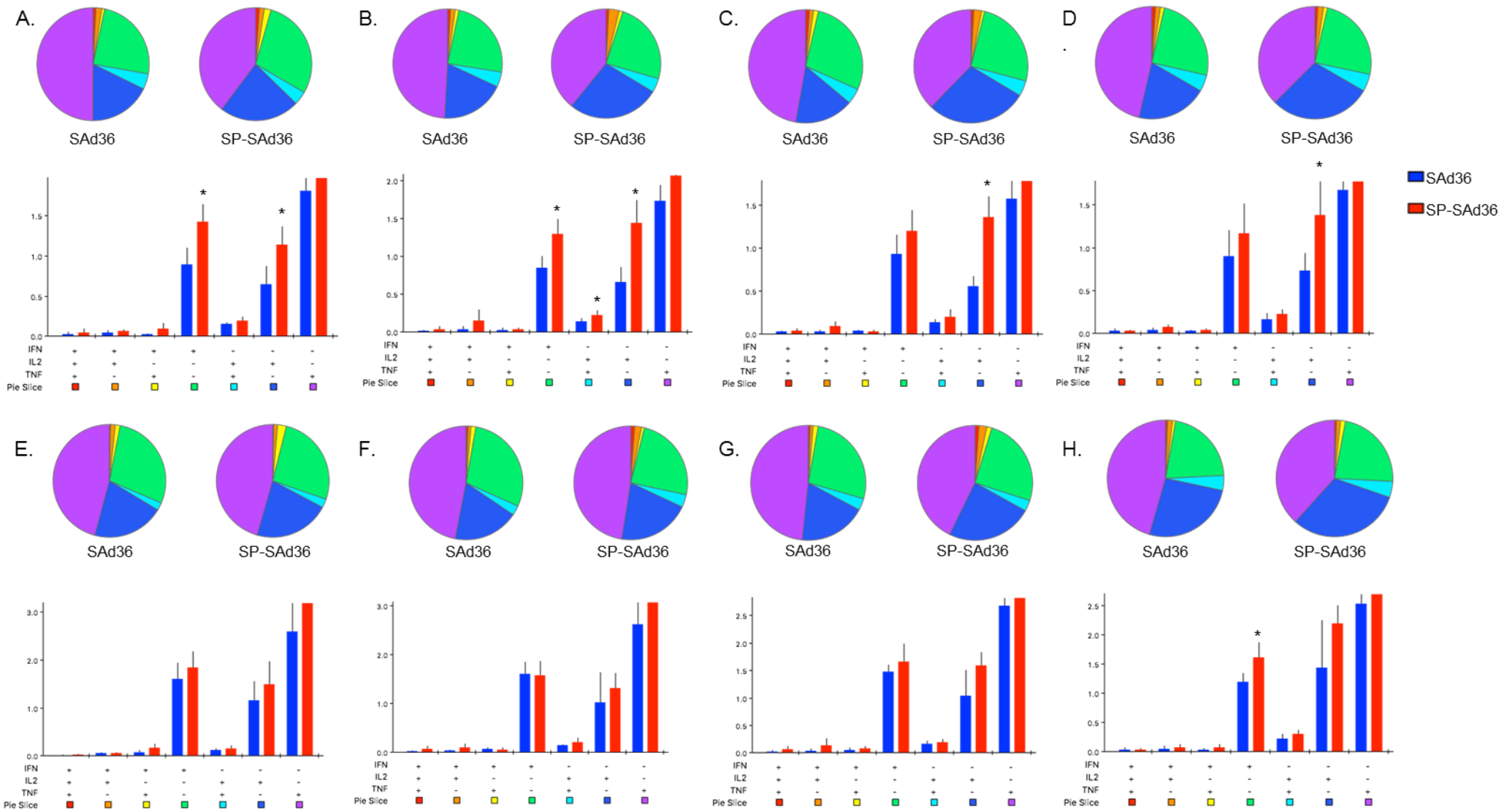
Supplementary Figure 1. Sample Gating Strategy. Splenocytes from immunized and naïve animals were obtained 5 days after the final immunization and stimulated with a single peptide pool from either cPvCSP or cPvMSP1. Cells were then stained with fluorescent antibodies and gated based on size for singlets and lymphocytes, then on live cells based on negative staining for viability dye. Live cells were further gated on CD3+, then on either CD4+ or CD8+ (A). CD4+ and CD8+ populations were then gated on interferon-γ, interleukin-2, and tumor necrosis factor-α positive populations. Plots from a representative mouse from the SP-SAd36-*cPvCSP/cPvMSP1* and naïve groups following stimulation with Pool B of cPvCSP are shown: Cytokine production by the CD4+ T cells from the representative SP-SAd36 mouse (B) and naïve mouse (C), and cytokine production by CD8+ T cells from the representative SP-SAd36 mouse (D) and naïve mouse (E).



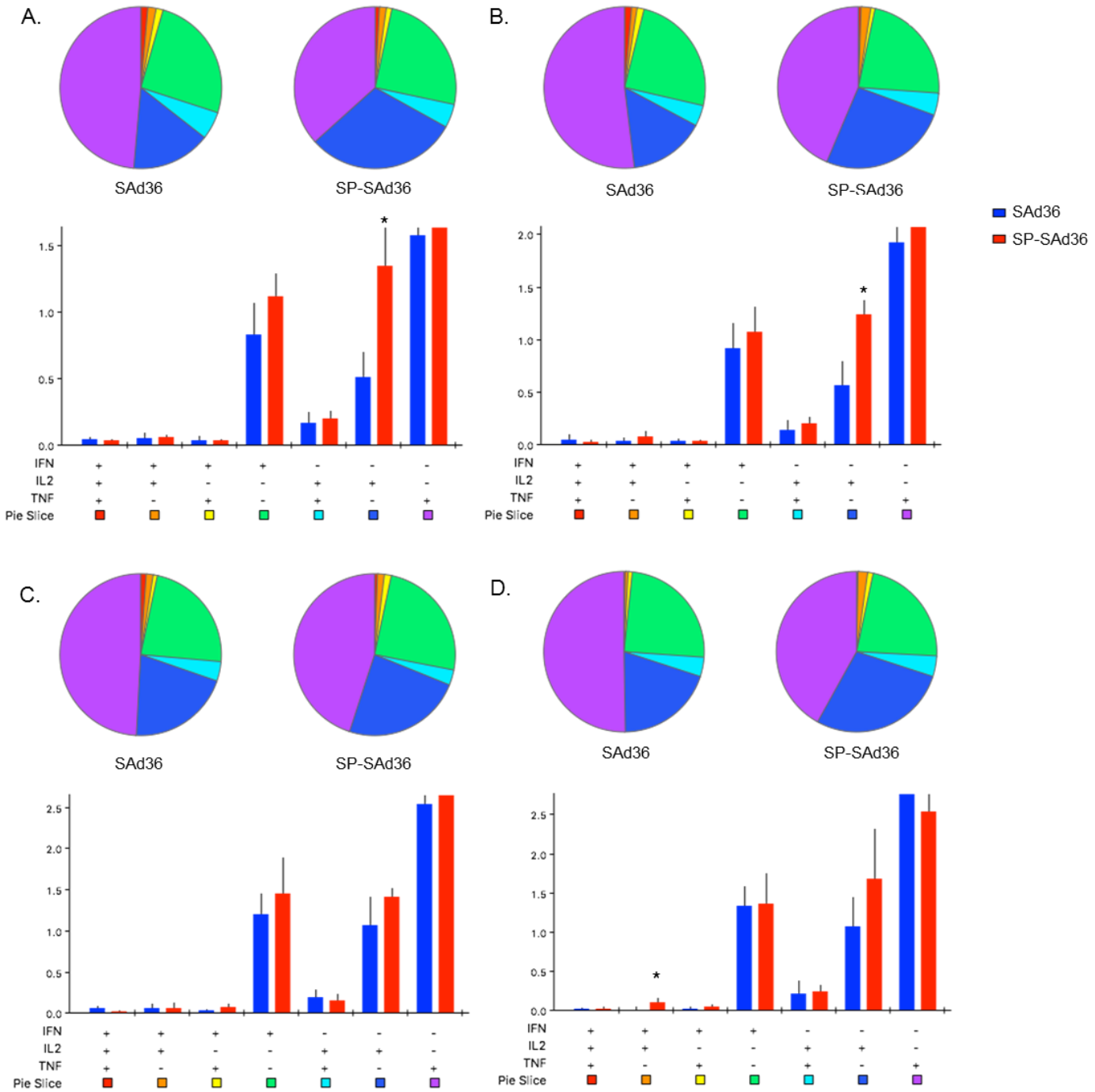
Supplementary Figure 2. Cytokine-secreting T cells after ex vivo stimulation with cPvCSP peptide pools 5 days after the final immunization of mice primed with SAd36-*cPvCSP/cPvMSP1* and assessed by flow cytometry. A-C) Cytokine responses of CD4+ T cells following stimulation with cPvCSP peptide pools A, B, C, or D. D-F) Cytokine responses of CD8+ T cells following stimulation with cPvCSP peptide pools A, B, C, or D. Interferon-γ responses are shown in figures A and D. Interleukin-2 responses are shown in B and E. Tumor necrosis factor-α responses are shown in C and F. Values presented represent the percentage of either CD4+ or CD8+ T cells positive for the cytokine. Statistical analysis was conducted using Mann Whitney test to determine differences in the production of cytokines in response to stimulus with an individual pool between the regimen that included priming with SAd36-*cPvCSP/cPvMSP1* and naïve mice. Statistically significant differences between groups in response to individual pools are denoted by *($p < 0.05$) within the SAd36 bar.



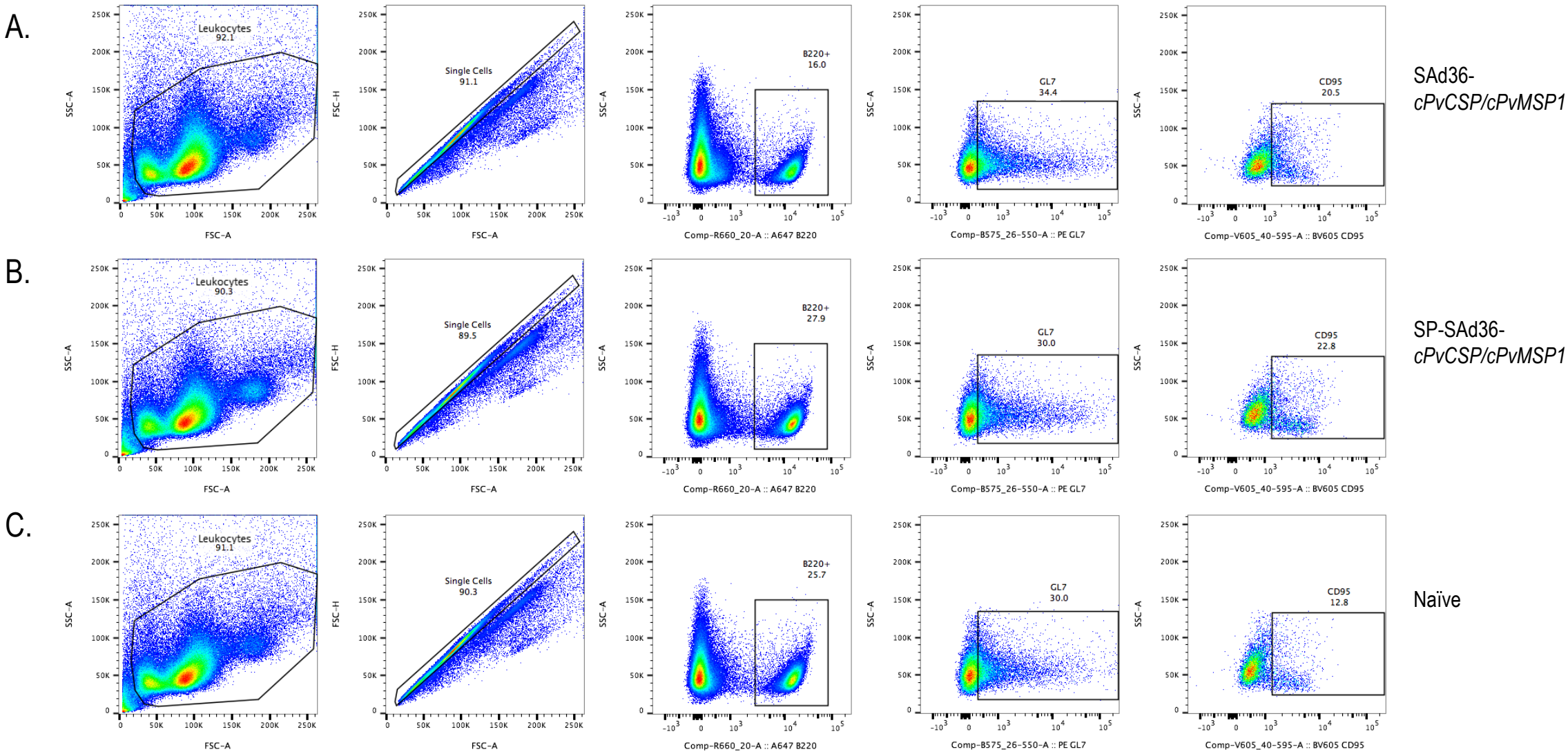
Supplementary Figure 3. Cytokine-secreting T cells after ex vivo stimulation with cPvMSP1 peptide pools 5 days after the final immunization of mice primed with SAd36-*cPvCSP/cPvMSP1* and assessed by flow cytometry. A-C) Cytokine responses of CD4+ T cells following stimulation with cPvMSP1 peptide pools 1 or 2. D-F) Cytokine responses of CD8+ T cells following stimulation with cPvMSP1 peptide pools 1 or 2. Interferon-γ responses are shown in figures A and D. Interleukin-2 responses are shown in B and E. Tumor necrosis factor-α responses are shown in C and F. Values presented represent the percentage of either CD4+ or CD8+ T cells positive for the cytokine. Statistical analysis was conducted using Mann Whitney test to determine differences in the production of cytokines in response to stimulus with an individual pool between the regimen that included priming with SAd36-*cPvCSP/cPvMSP1* and naïve mice. Statistically significant differences between groups in response to individual pools are denoted by *($p < 0.05$) within the SAd36 bar.



Supplementary Figure 4. Secretion of multiple cytokines by T cells in response to ex vivo stimulation with cPvCSP peptide pools 5 days after the final immunization and assessed by flow cytometry. Pies represent the percentage of multifunctional and single cytokine producing CD4+ or CD8+ T cells. Bar graphs represent the percentage of CD4+ or CD8+ T cells producing three, two or one cytokines out of the total CD4+ or CD8+ T cell population. The multifunctionality of CD4+ T cells in response to stimulation with cPvCSP (A) Pool A, (B) Pool B, (C) Pool C, or (D) Pool D are shown in the top four panels. The multifunctionality of CD8+ T cells in response to stimulation with cPvCSP (E) Pool A, (F) Pool B, (G) Pool C, or (H) Pool D, are shown in the bottom four panels. Statistically significant differences between mice immunized with a regimen that included a priming with SAd36-*cPvCSP/cPvMSP1* (blue bars) or priming with SP-SAd36-*cPvCSP/cPvMSP1* (red bars) in response to individual pools were determined using Student's T test are denoted by *($p < 0.05$) above the SP-SAd36 bar. Graphs were produced using SPICE software [57].



Supplementary Figure 5. Secretion of multiple cytokines by T cells in response to ex vivo stimulation with cPvMSP1 peptide pools 5 days after the final immunization and assessed by flow cytometry. Pies represent the percentage of multifunctional and single cytokine producing CD4+ or CD8+ T cells. Bar graphs represent the percentage of CD4+ or CD8+ T cells producing three, two or one cytokines out of the total CD4+ or CD8+ T cell population. The multifunctionality of CD4+ T cells in response to stimulation with (A) Pool 1 of cPvMSP1 and (B) Pool 2 of cPvMSP1, as well the multifunctionality of CD8+ T cells in response to stimulation with (C) Pool 1 of cPvMSP1 and (D) Pool 2 of cPvMSP1 are shown. Statistically significant differences between mice immunized with a regimen that included a priming with SAd36-*cPvCSP/cPvMSP1* (blue bars) or priming with SP-SAd36-*cPvCSP/cPvMSP1* (red bars) in response to individual pools were determined using Student's T test are denoted by *($p < 0.05$) above the SP-SAd36 bar. Graphs were produced using SPICE software [57].



Supplementary Figure 6. Sample Gating Strategy for Germinal Center B cells. Leukocytes from the draining inguinal lymph nodes of immunized and naïve animals were obtained 9 days after priming with either the unmodified SAd36 or SP-SAd36 vector or left unvaccinated. Cells were then stained with fluorescent antibodies and gated based on size for leukocytes and singlets, then on live cells based on negative staining for viability dye. Live cells were further gated on B220+, and GL7+ and CD95+. Plots from representative mice from the SAd36-*cPvCSP/cPvMSP1* (A), SP-SAd36-*cPvCSP/cPvMSP1* (B), and naïve groups (C) are shown.