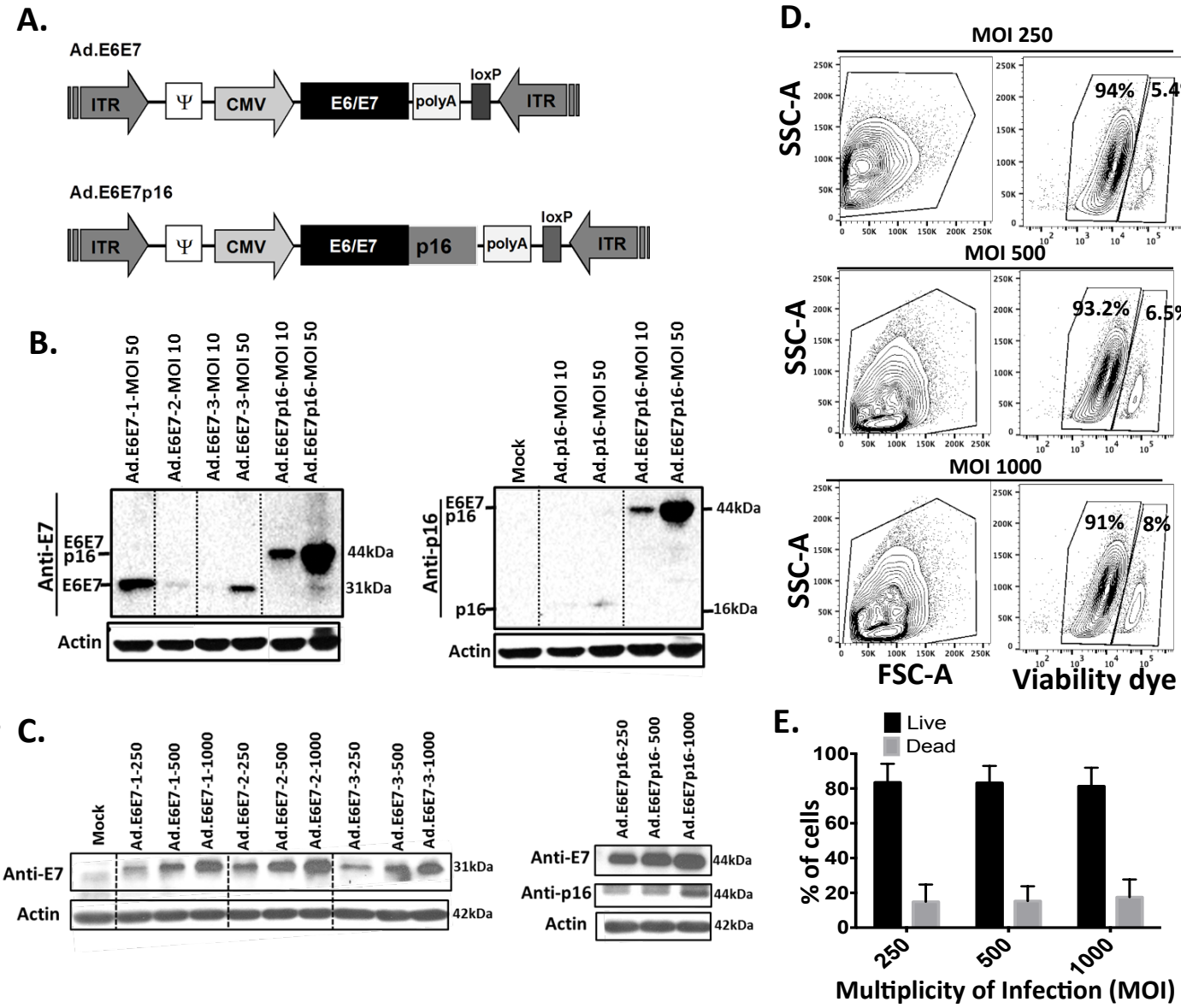
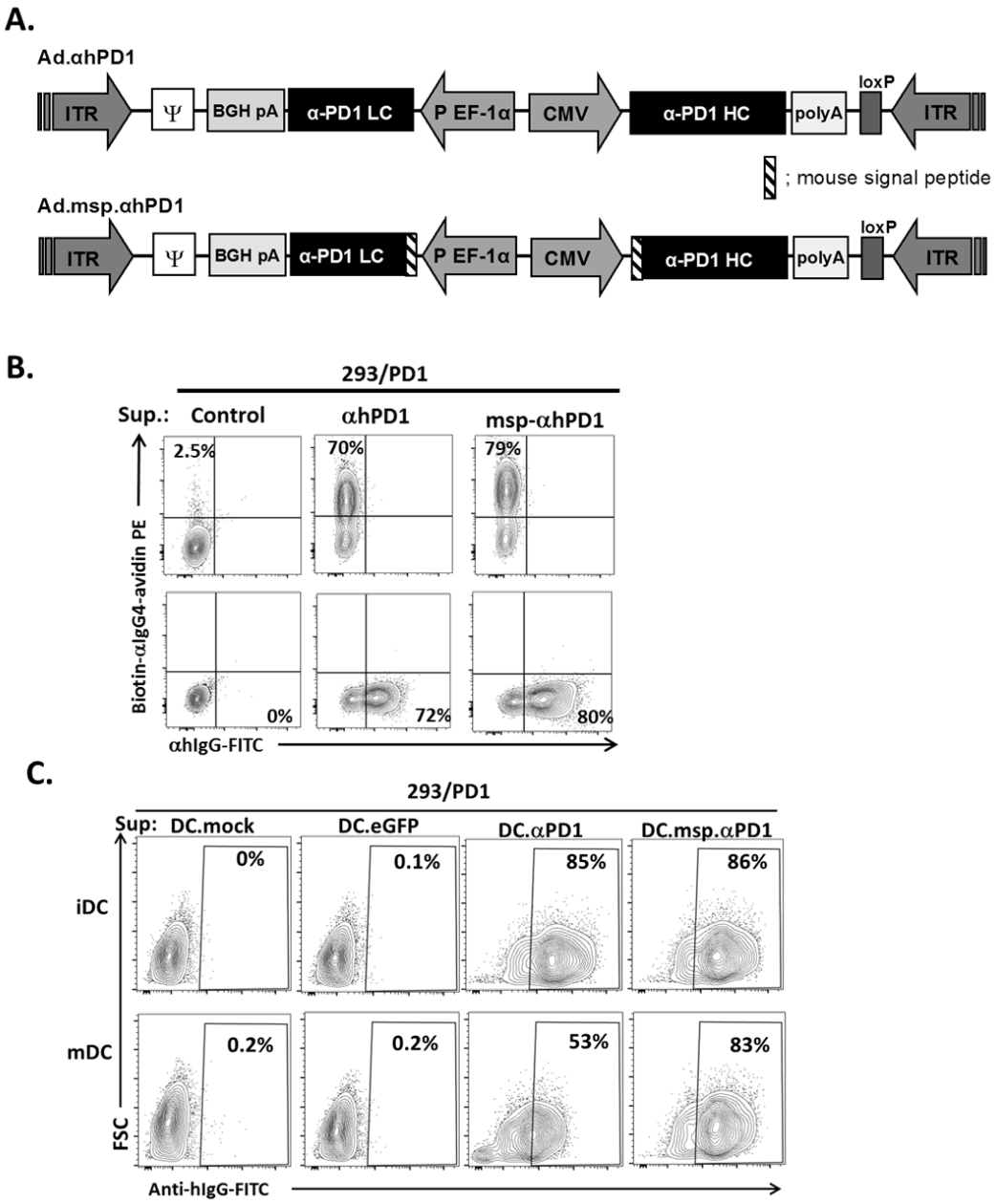
Garcia-Bates, Supplementary Figure 1



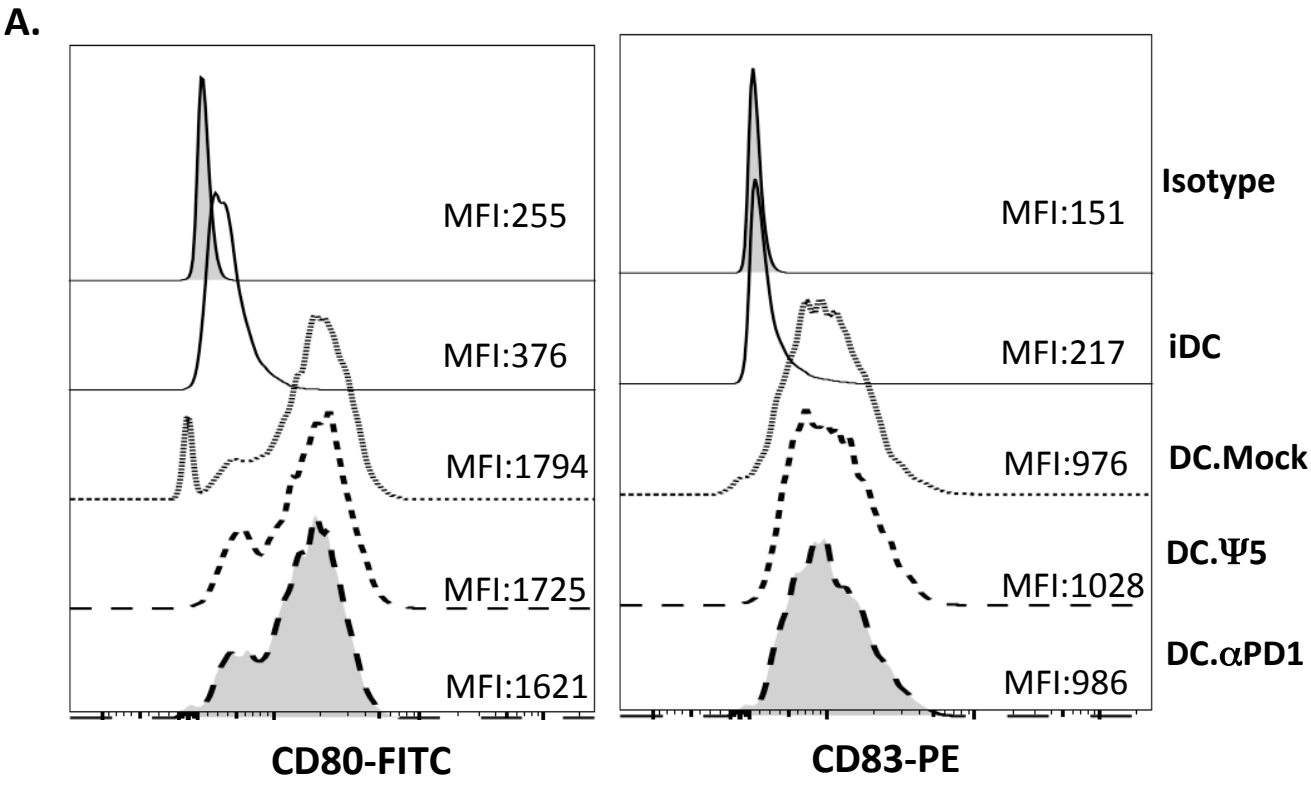
Supplementary Figure 1. Design of adenoviral vectors encoding human codon-optimized HPV 16 E6 and E7 alone or fused with p16^{INK4} as DC vaccine vectors for immunotherapy. A) Schematic diagram of Ad.E6E7 and Ad.E6E7p16 vectors. Non-oncogenic mutated E6 and E7 proteins were cloned in an Ad-Ψ5 vector under CMV promoter (Ad.E6E7) or were also fused with p16^{INK4} (Ad.E6E7p16). As a control, the backbone adenoviral vector was used (Ad.Ψ5) or the adenovirus vector with p16 only was used (Ad.p16). **B)** Transgene expression was first evaluated in an HPV negative SCCHN cell line PCI-13. Cells were mock infected or infected with three different codon-optimized Ad.E6E7 vectors (Ad.E6E7-1, Ad.E6E7-2 and Ad.E6E7-3) or Ad.E6E7p16 or Ad.p16 at MOI of 10 or 50. Expression of E7 and p16 were evaluated by western blot 48 hrs after infection. **C)** Transgene expression on monocyte-derived dendritic cells (DC). DC were left untreated (mock) or were infected with the three Ad.E6E7 (Ad.E6E7-1, Ad.E6E7-2 and Ad.E6E7-3) orAd.E6E7p16 or Ad.p16 at MOI of 250, 500 and 1000 for 48 hours. Expression of E7 and p16 was evaluated by western blot. **D)** Representative flow cytometry contour plots illustrating forward and size scatter (left) and the overall viability (right) of mDC transduced with Ad-E6E7 at MOIs of 250, 500 and 1000. **E)** Percent of cells that are viable (black bars) or dead (grey bars) for each MOI tested.

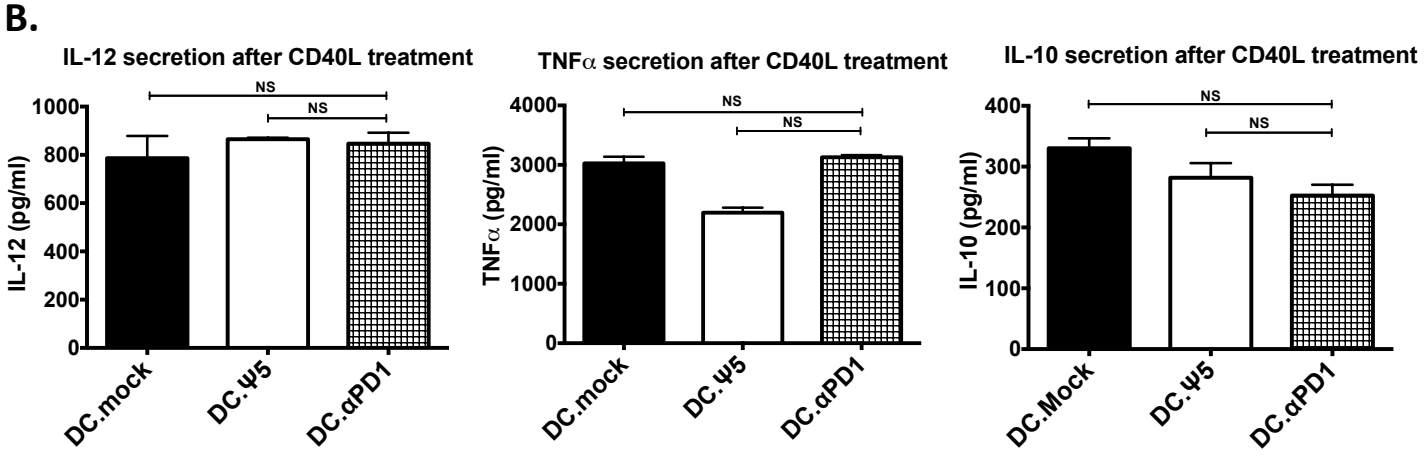
Garcia-Bates, Supplementary Figure 2



Supplementary Figure 2. Characterization of an adenoviral vector that expresses human antibody against PD1 and its expression and secretion by human dendritic cells. A) Schematic diagram of Ad-αhPD1 and Ad.msp.αPD1. The light chain and heavy chain of the anti-PD1 antibody were cloned under a CMV promoter and an EF-1a promoter respectively (Ad.αhPD1). We also constructed the same vector but with a mouse signal peptide sequence right before the light or heavy chain sequence (Ad.msp.αhPD1). **B)** Human embryonic kidney 293 cells that express PD1 (293/PD1) were used to test the binding of adenovirus-encoded anti-PD1. HEK293 cells were transfected with either a control DNA vector or αhPD1 or msp-αhPD1 DNA constructs and after 72 hours the supernatants were collected from all three different conditions. These supernatants were then added to 293/PD1 cells and binding of the anti-PD1 antibody to PD1 was tested by the addition of two secondary antibodies, one against human IgG4 (Biotin-αIgG4-avidin PE) and one against whole human IgG conjugated to FITC (αhIgG FITC). **C)** Monocyte derived iDCs and mDCs were transduced at an MOI of 500 for 72 hours and supernatants were collected. These supernatants were then added to 293/PD1 cells to test binding by flow cytometry using an anti-hIgG-FITC.

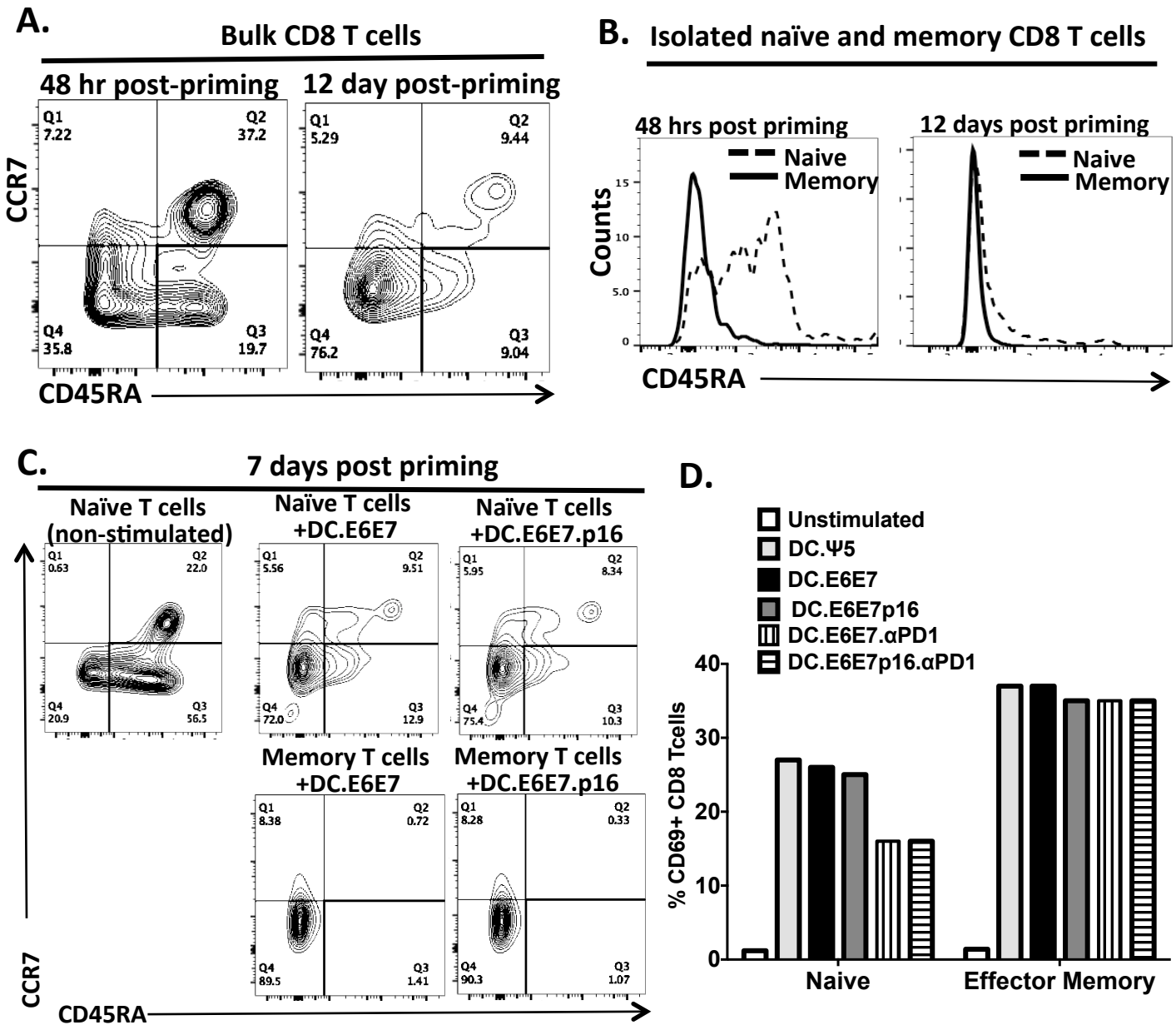
Garcia-Bates, Supplementary Figure 3





Supplementary Figure 3. Phenotype and cytokine secretion of mDC after Adenovirus transduction. A) Flow cytometry histograms analyzing the expression of CD80 (left) and CD83 markers after 72 hr post-infection in immature DC (iDC), αDC1-mock infected (DC.Mock), αDC1 infected with either Ad.Ψ5 (DC.Ψ5) or Ad-αPD1 (DC.αPD1). The mean fluorescence intensity (MFI) is shown on the right of each histogram. **B)** Mature αDC1 were mock-infected (DC.Mock), or infected with Ad.Ψ5 (DC.Ψ5) or Ad.αPD1 (DC.αPD1) for 24 hours and then harvested, counted and plated in a 96 well plate at the same cell density. After cells were seeded, they were exposed to CD40L-expressing J588 cells for 24 hours and supernatant were harvested. IL-12, TNFα and IL-10 cytokine secretion were then measured by ELISA (n=3. Mean ± SD). NS= Non-significant.

Garcia-Bates, Supplementary Figure 4



Supplementary Figure 4. Phenotype of CD8⁺ T cells overtime after priming with Adenovirus-infected mDC. **A)** Representative flow cytometry contour plots showing expression of CD45RA and CCR7 markers on bulk CD8+ T cells 48 hours post-priming (left) and after 12 days post-priming (right) with DC.E6E7p16. Naïve CD8+ T cells are considered to be CD45RA+/CCR7+ (upper right quadrant) and Effector Memory CD8+ T cells are considered to be $CD45RA^-/CCR7^-$ (Lower left quadrant) **B)** $CD8^+$ Naïve and Memory T cells were isolated using a negative selection kit and the levels of CD45RA were evaluated 48 hours post-priming (left) and 12 days post-priming (right). At 48 hours the levels of CD45RA are still high on the naïve $CD8^+$ T cells, but after 12 days, the naïve T cells lose CD45RA expression as they become effector memory ($CD45RA^-$). The levels of CD45RA on memory $CD8^+$ T cells stayed negative during the course of 12 days. Results are shown for the CD8 T cells stimulated with DC.E6E7p16 as a representative **C)** CD45RA and CCR7 expression were evaluated on the $CD8^+$ T cells stimulated under different conditions. Non-stimulated Naïve $CD8^+$ T cells and Naïve $CD8^+$ T cells stimulated with either DC.E6E7 or DC.E6E7p16 were analyzed 7 days post-priming. **D)** Naïve and Effector memory $CD8^+$ T cells were analyzed for expression of CD69 after 48 hr post-priming with either DC.Ψ5, DC.E6E7, DC.E6E7p16, DC.E6E7.αPD1 or DC.E6E7p16.αPD1. Results are shown as percent $CD69^+$ cell of $CD8^+$ T cells.