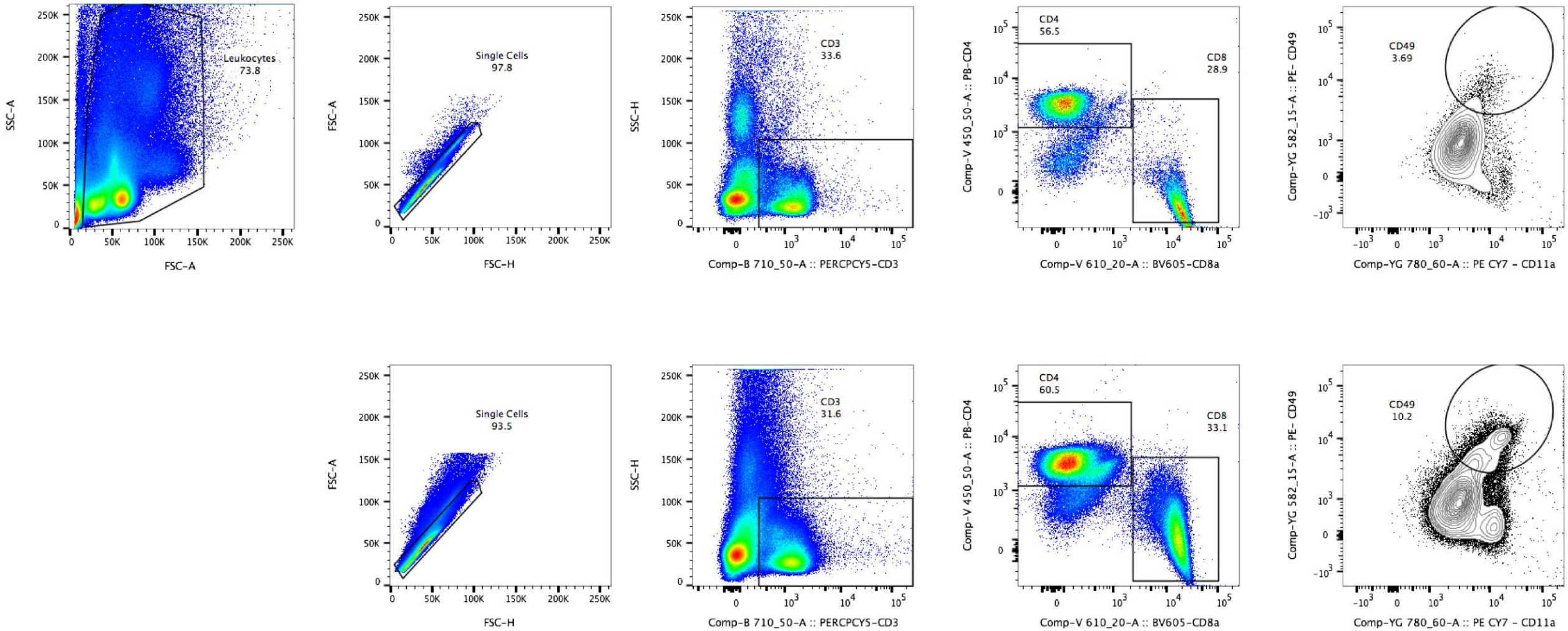
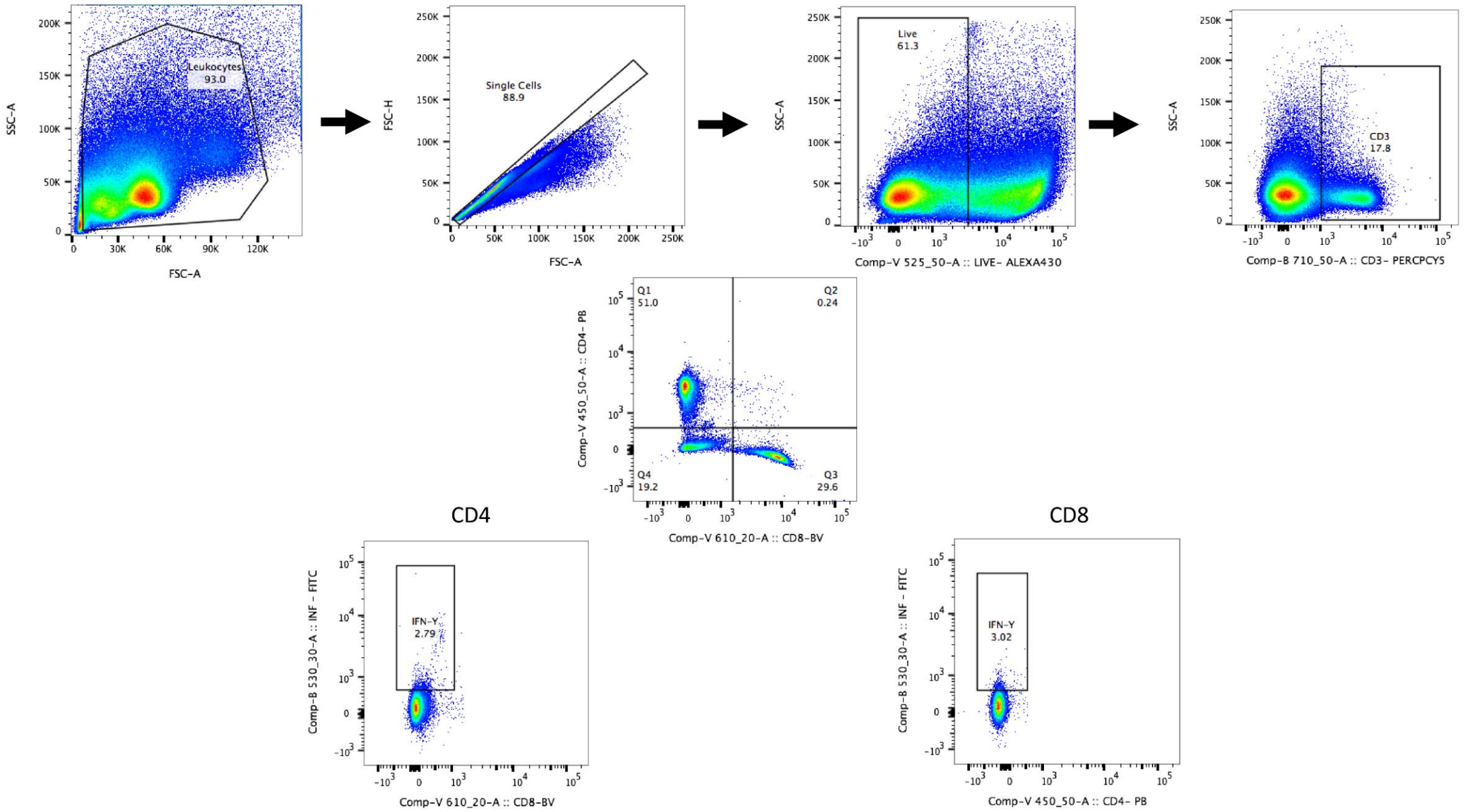
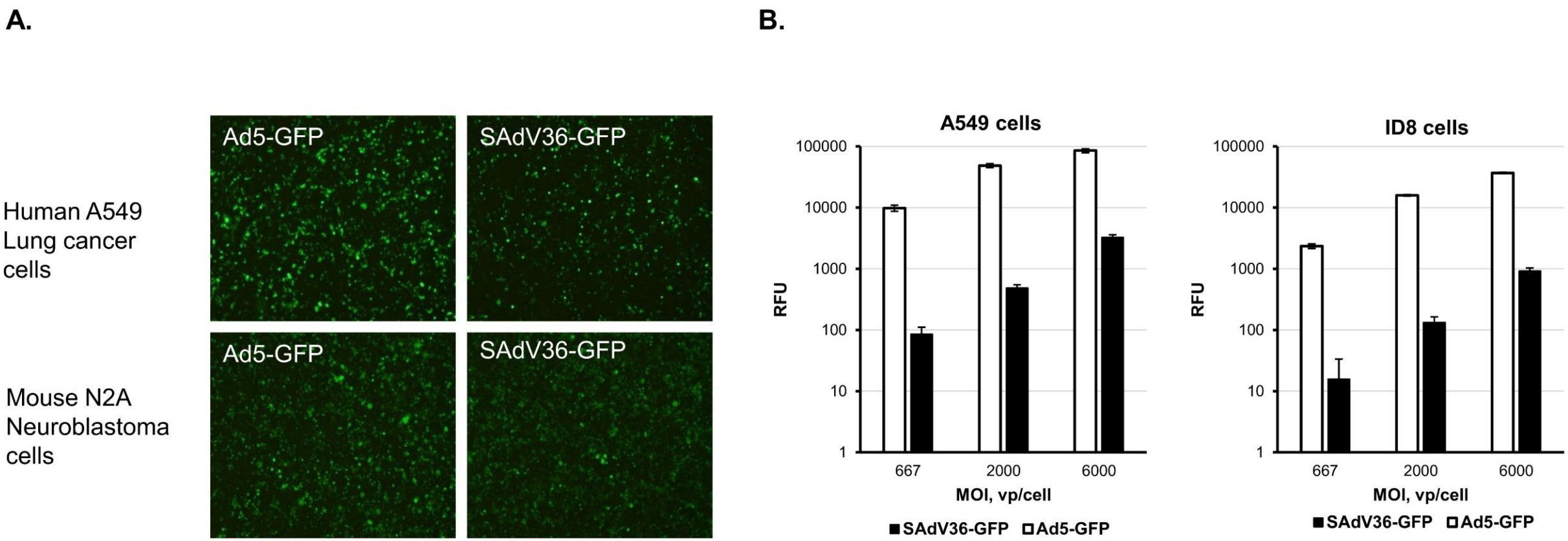


Supplementary Figure 1. Gating strategy for the definition of antigen-specific CD4⁺ T cells. In this sample gating strategy Leukocytes were gated in SSC-A vs FSC-A. In the leukocyte population CD3+ cells were gated to select lymphocytes, and the surface expression of CD4 and CD8 was posteriorly identified. On the CD4+ T cells the expression of CD49d and CD11a was assessed as it is associated to antigen-experienced CD4+ T cells. The top row shows the gating of a representative naïve mouse and the bottom row shows the strategy for a mouse receiving SAd36 at a 10^{10} dose.



Supplementary Figure 2. Gating Strategy For Flow Cytometry Analysis. In this sample gating cells were first gated as leukocytes (SSC-A vs. FSC-A) and then for singlets (FSC-H vs. FSC-A). The singlets gate is further analyzed for their uptake of the Live/Dead stain to determine live versus dead cells. After Selection of the live population the CD3 expression was then analyzed to select the T cell population. CD4 and CD8 surface expression was then determined. Intracellular expression of cytokines (IFN-γ, TNF-α, IL-2) was analyzed in each T cell subset.



Supplementary Figure 3. Characterization of the Infective Capacity of Ad5 and SAd36 vectors. A. Monolayers A549 (human epithelial lung cancer cell line) or N2A (mouse neuroblastoma cell line) cells were incubated for 1 hour with either SAd36-GFP or Ad5-GFP at a multiplicity of infection (MOI) of 300 vp/cell. After incubation, infection medium (DMEM/F-12, 1:1) containing virus and 2% FBS was replaced with culture medium containing 5% FBS and cells were incubated at 37°C and 5% CO_2 for at least 20 hours to allow eGFP reporter gene expression. The infected cells expressing eGFP were detected using an epifluorescent microscope, and images were taken at 10X magnification. **B.** Monolayers of A549 (human epithelial lung cancer cell line) and ID8 (mouse epithelial ovarian cancer cell line) cells were infected with either SAd36-GFP or Ad5-GFP vectors using the indicated multiplicities of infection (MOI). The infection efficiencies of SAd36-GFP or Ad5-GFP vectors were determined based on the levels of fluorescent signal intensity detected with multi-mode plate reader in infected and uninfected cells 48 hours post-infection. The average values of relative fluorescent units (RFU) are presented after subtracting background signal detected in uninfected cell monolayers that served as negative control. Each bar represents the cumulative mean of triplicate measurements ± SD.