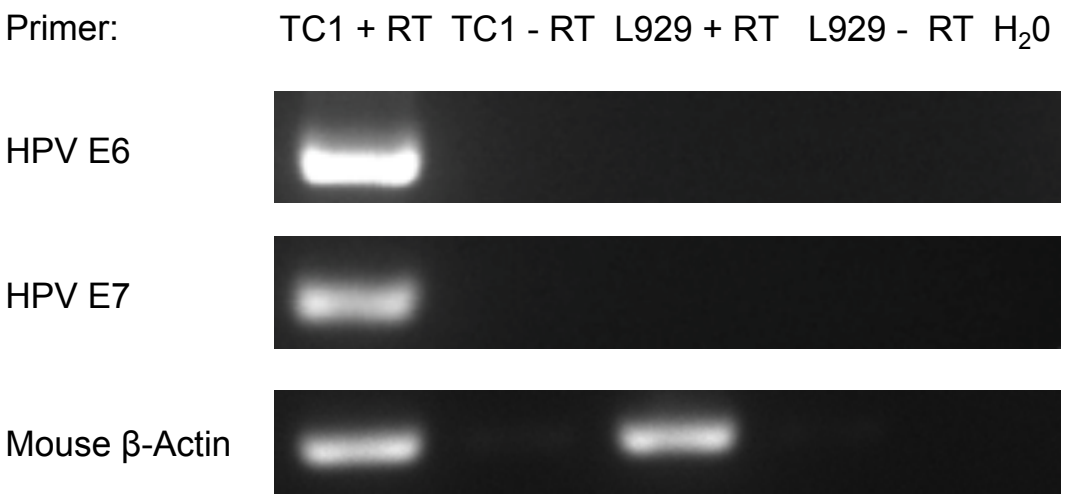
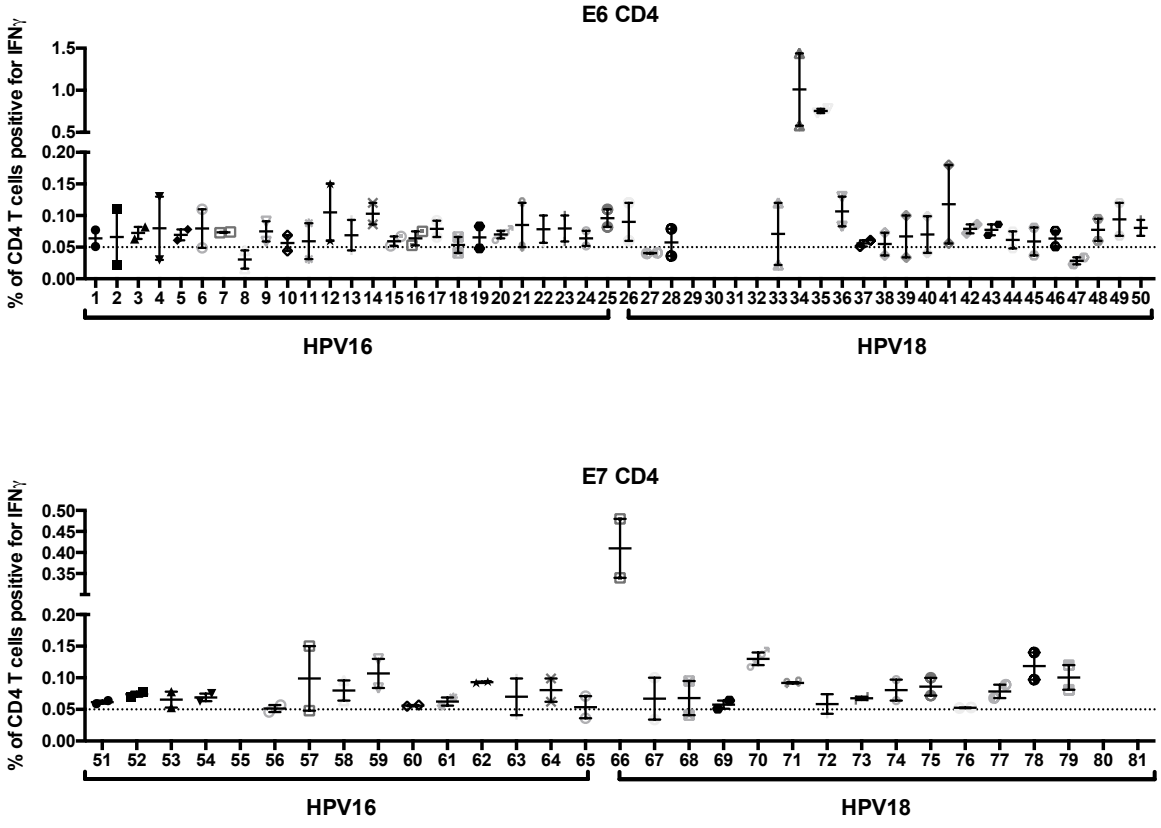


Supplementary Figure 1

Supplementary Figure 1: RT-PCR confirms expression of E6 and E7 in TC1

RNA was extracted from TC1 cells and murine fibroblast L929 cells as a negative control; standard RT-PCR was performed to amplify cDNA (reactions were also performed without reverse transcriptase to confirm subsequent products were not amplified from genomic DNA contamination and a reaction containing water in place of cDNA was also performed). Primers for E6, E7, and murine β-actin were used to complete standard PCR and products were run out on agarose gel and subsequently imaged to confirm gene expression.

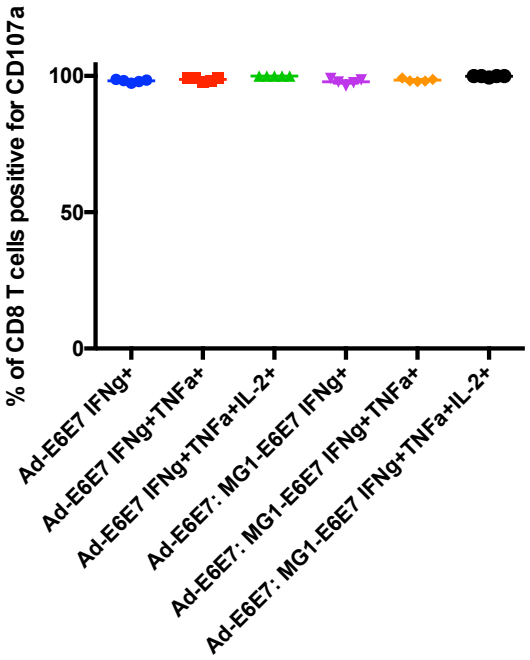


Supplementary Figure 2

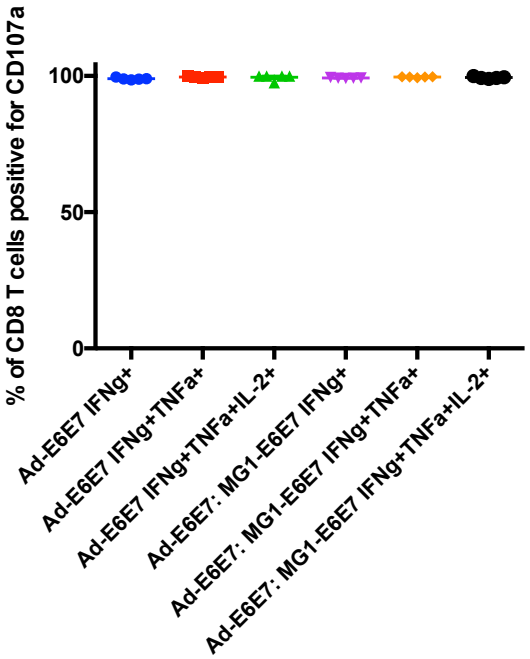
Supplementary Figure 2: CD4+ T cell responses after E6E7 vaccination

Spleens were harvested from 2 mice treated with Ad-E6E7 prime and MG1-E6E7 boosting. Splenocytes were re-stimulated with a library of peptides from the WT sequences of HPV16 and 18, E6 and E7 (15mer peptides with 9mer overlap, splenocytes were re-stimulated with 15 μg/ml of each individual peptide). Splenocytes were then stained for CD4+ and ICS was performed for IFNg prior to analysis using flow cytometry (mean and SEM displayed,some peptides were not synthesizeable and in this case the corresponding column for these peptides is blank, dotted line intercepting y-axis represents lower limit of detection).

A



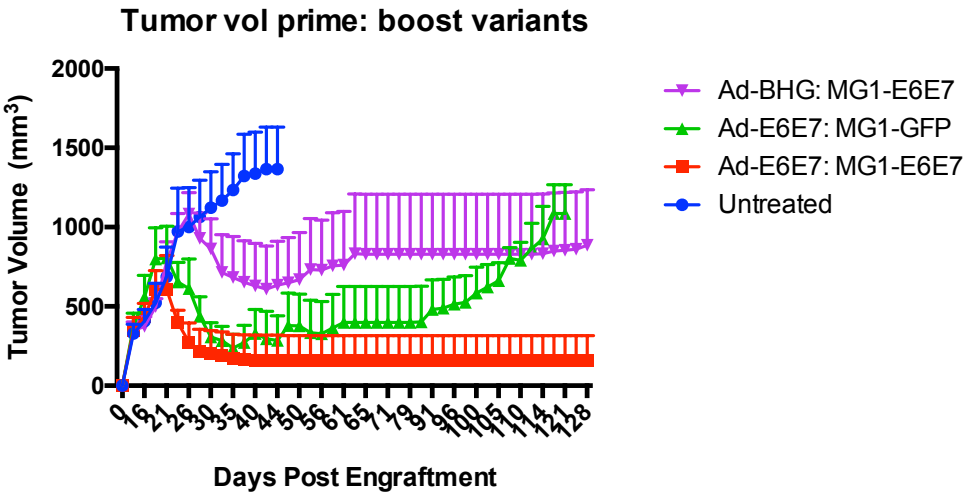
B



Supplementary Figure 3

Supplementary Figure 3: E6E7 vaccination induces T-cell degranulation

Tumor free C57BL/6 mice were treated with Ad-E6E7 (n=5) alone or boosted with MG1-E6E7 (n=5). Frequency of CD107a+ CD8+ T cells in single positive (IFNg), double positive (IFNg & TNFα) and triple positive (IFNg, TNFα & IL2) populations in (A) the blood and (B) the spleen are shown (mean and SEM displayed).

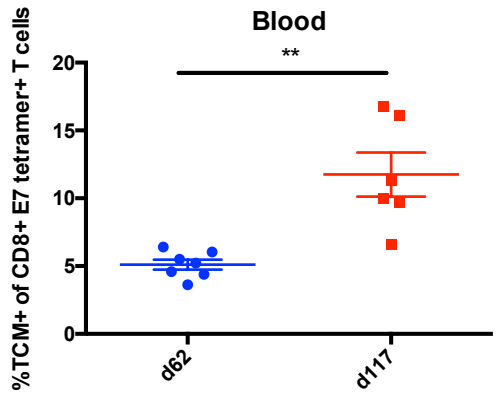


Supplementary Figure 4

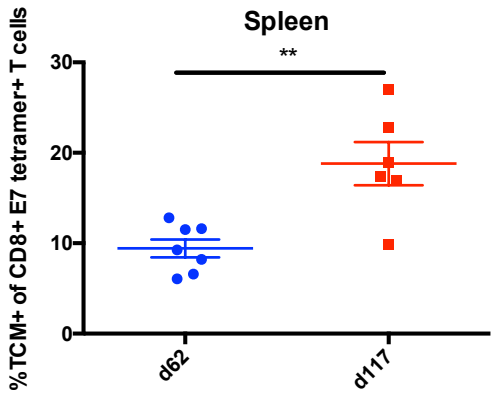
Supplementary Figure 4: Tumor volume curves in E6E7 vaccinated mice

TC1 tumor volume curves of mice treated with adenoviral prime and MG1-E6E7 boosting (n=4), sham boosted (n=4), sham primed mice (n=5) and untreated control mice (n=5). Mice were primed at day 14 when tumors reached a mean volume of approximately 300mm^3 and were boosted with MG1 Maraba at day 23. Tumor growth curves end once all animals from the respective group have reached end point (mean and SEM displayed).

A



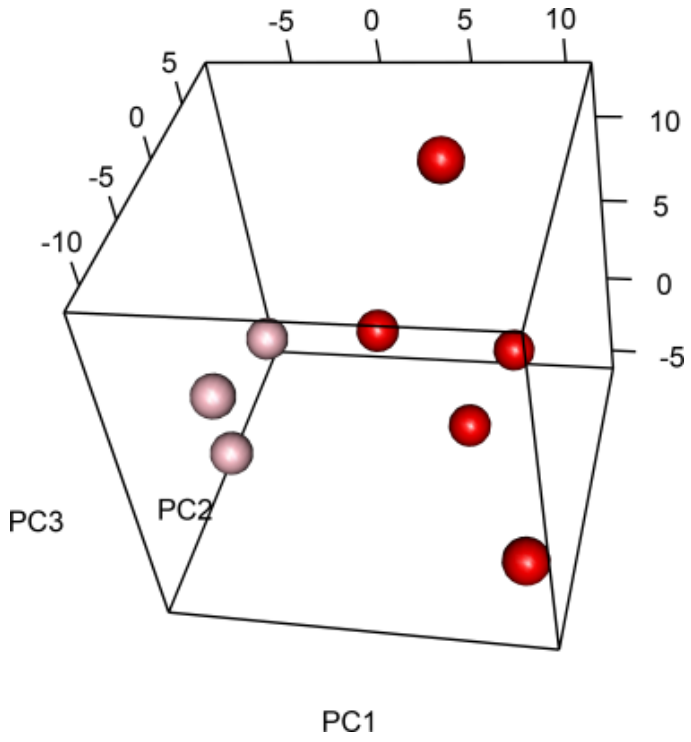
B



Supplementary Figure 5

Supplementary Figure 5: TCM frequency in mice after TC1 tumor clearance

Frequency of central memory T cells (TCM) of E7 tetramer+ CD8+ T cells in (A) the blood and (B) the spleen, 62 days and 117 days post MG1-E6E7 boosting. Mean and SEM displayed; comparisons performed using unpaired (Student's) two-tailed t-tests, $^{**}p \leq 0.01$.

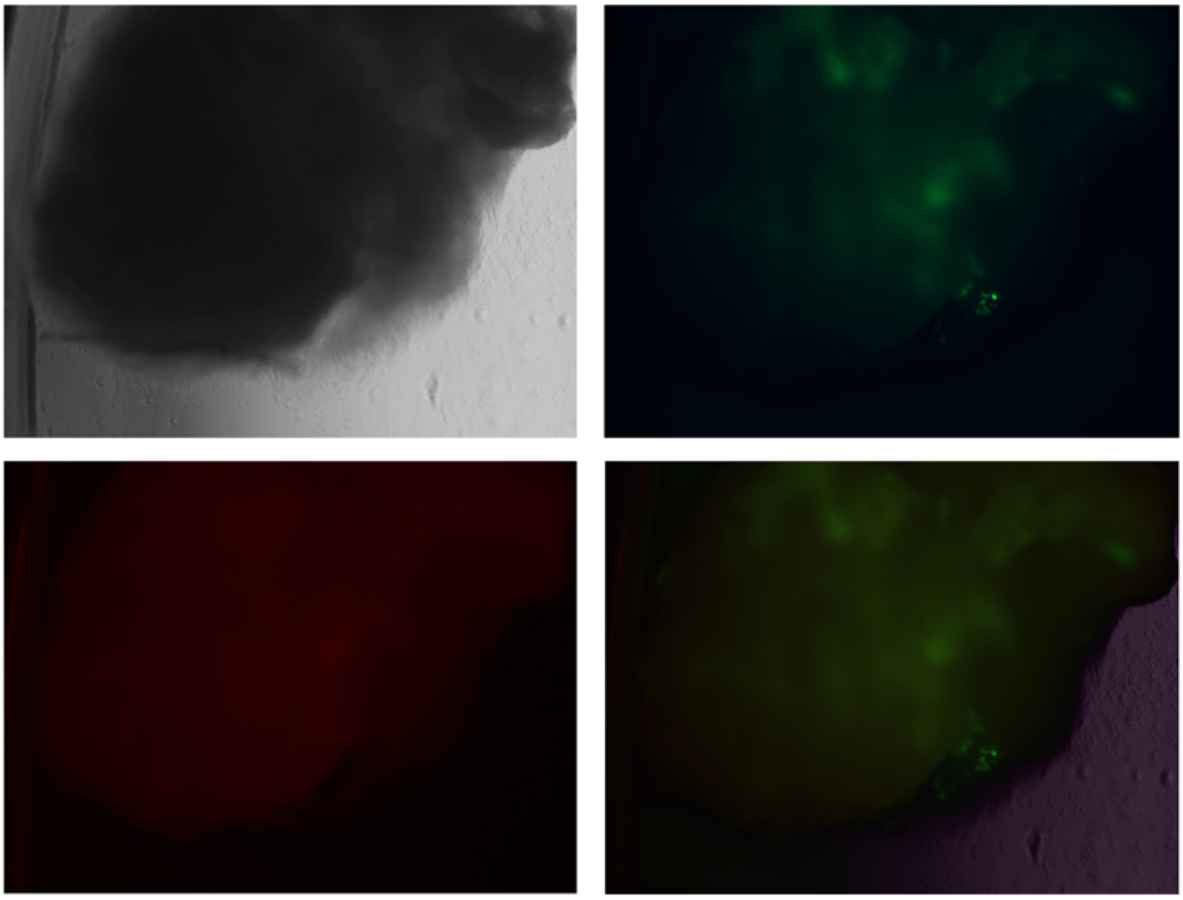


Untreated
MG1-E6E7

Supplementary Figure 6

Supplementary Figure 6: PCA analysis of TC1 tumors

Sample distribution (principal component analysis (PCA) plot). Visualization of the first 3 components of PCA. The analysis was performed based on all 561 genes profiled on the NanoString platform from untreated tumors (n=3) and tumors treated with MG1-E6E7 (n=5).



Supplementary Figure 7

Supplementary Figure 7: Images of MG1-GFP infected HPV+ primary tumor

Bright field, GFP (specific), RFP (background) and fluorescent overlay images from a representative HPV+ tumor biopsy following *ex vivo* infection with MG1-GFP, 4X magnification.