

Supplementary Figure 1: Gag-specific T cell responses post vaccination. (A) Gag CM9 tetramer-specific CD8 T cells following vaccination. (B) Gag-specific proliferative CD4 and CD8 T cell responses in blood 4 weeks prior to SIV challenge measured using CFSE dilution assay. Key to animal names is presented in Figure 3B.

Supplementary Figure 2. Cytokine co-expression profiles of SIV Gag-specific CD4 T cells at one week post the 1st MVA boost. (A) Schematic for the analysis of cytokine co-expression profiles of CD4T cells following stimulation with Gag peptide pool. Lymphocytes were gated based on forward and side scatter and CD4 T cells (CD3+, CD8-, CD4+) were then analyzed for IFN- γ , IL-2 and TNF- α expression. Cytokine co-expression profiles were determined using Boolean gating function of FlowJo software. (B) Frequency of triple (TP), double (DP) and single (SP) producers expressed as a percent of total CD4 T cells. Bars represent mean \pm SEM (n=8 for each group). (C) Pie chart represent the quality of the response with the subsets (TP, DP and SP) expressed as percent of total cytokine positive CD4 T cells (n=8 for each group). I, IFN γ ; L, IL-2 and T, TNF α .

Supplementary Figure 3. VV-specific humoral and cellular immunity contributes to diminished SIV-specific CD8 T cell response. Correlation between VV-specific CD8 T cell or neutralizing antibody responses prior to 2nd MVA boost and the magnitude of SIV-specific CD8 T cell responses at one week following the 2nd MVA boost. Each symbol represents an individual macaque.

Supplementary Figure 4. Analysis of plasma viral load by A*01 status. Temporal Plasma viral RNA (A) median for the group and (B) for individual animals in Mamu A*01+ animals. Temporal Plasma viral RNA (C) median for the group and (D) for individual animals in Mamu A*01- animals. The sensitivity of viral load assay was 80 copies of RNA/ml, and animals with levels of virus below 80 were scored at 100. Viral loads from an additional 5 unvaccinated rhesus macaques (2 Mamu A*01 and 3 non-Mamu A*01) challenged intrarectally with the same virus and dose(50) were included for these subgroup analyses. ‘*’ denote significant differences from control animals at the indicated timepoints.

Supplementary Figure 5. Correlation between colorectal virus and depletion of gut CD4 T cells post challenge. (A) Frequency of total central memory (CD28+ CD95+) CD4 T cells in blood at 24 weeks following SIV251 challenge. Correlation between colorectal virus and percent CD4 T cells in colorectal tissue at (B) week 3 and (C) week 24 post challenge. DN, Dryvax-naïve; DI, Dryvax-immune; C, Controls. r_s , Spearman’s rank correlation.