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Supplemental information

Vpr attenuates antiviral immune responses and is critical for full pathogenicity of SIV_{mac239} in rhesus macaques

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Supplemental figures

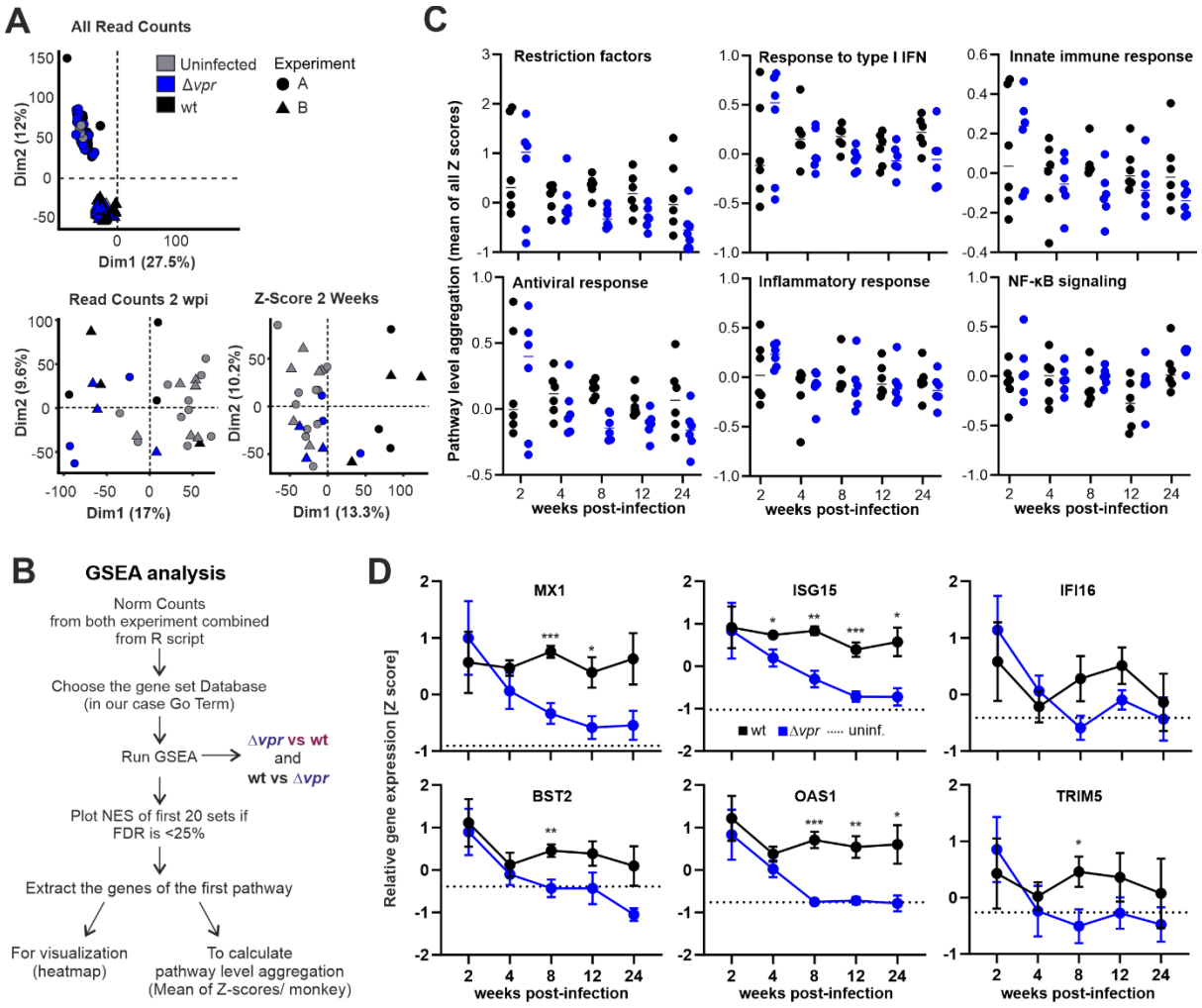

Figure S1 (related to Figure 2). RNAseq analysis of PBMCs from wt and Δ*vpr* SIVmac-infected macaques.

(**A**) Upper, the PCA score plots show first 2 dimensions (Dim1 and Dim2) with symbols denoting samples from experiment A (circles) or B (triangles) and SIV i.e uninfected (grey), Δ*vpr* (blue) and wt (black). PCA shows normalized read counts from all timepoints of PBMCs from wt, *Δvpr* and uninfected controls. Lower, PCA on normalized read counts (left) and z-Score (right) from 2 wpi of PBMC of wt and *Δvpr* and uninfected controls. As outlined in the methods section, infections were performed at two time-points in groups of six animals, referred to as experiments A and B. To account for the inherent variability of performing sample preparations and sequencing in two separate batches, data were normalized by calculates Z scores separately for each experiment before combining. Normalization allowed the meaningful combination of both experiments and biases of sampling, handling and analyses at different time periods. (**B**) Work-flow for GSEA analysis. (**C**) Pathway level aggregation scores for the indicated sets involved in innate antiviral immune defense and activation. (**D**) Relative expression of indicated antiviral genes in PBMC of wt and *Δvpr* SIVmac-infected animals and uninfected controls.

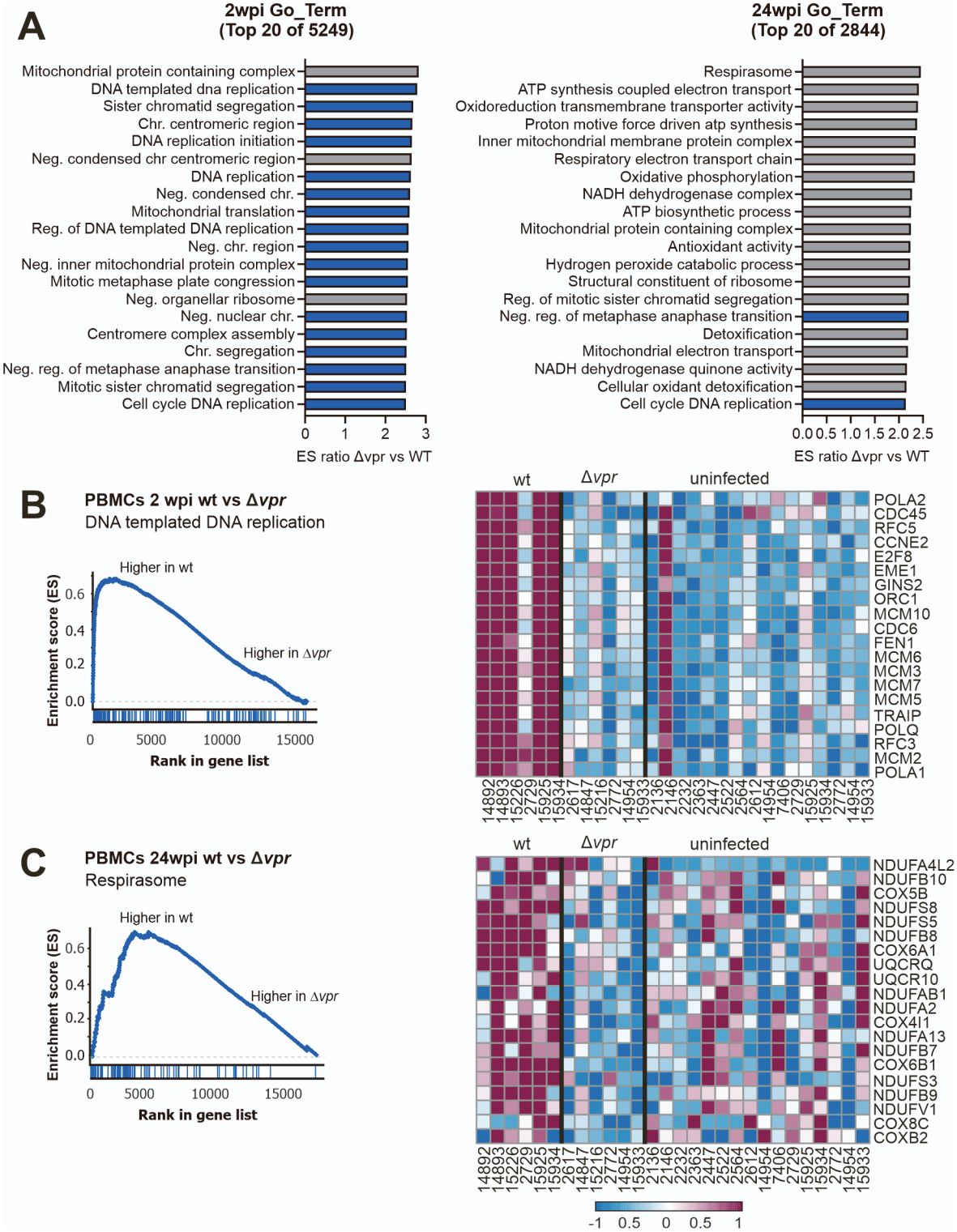

Figure S2 (related to Figure 3). Genes enriched in wt vs Δ*vpr* SIV$_{mac239}$ infection. (**A**) Top 20 Go_term pathways enriched in wt compared to Δ*vpr* SIV$_{mac}$ infection at 2 (left) or 24 (right) wpi. Pathways involved in mitochondrial function and respiratory stress (light blue), DNA replication (orange) and cell cycling (yellow) are highlighted. (**B**) GSEA plot (left) and heat map (right) of wt vs Δ*vpr* in PBMC collected at 2 wpi for DNA templated DNA replication gene set. The running enrichment score (y-axis) is indicated for each gene ordered by their rank in the whole data set for that specific comparison (shown by the bars below the x-axis). **C**) GSEA plot (left) and heat map (right) for the leading-edge genes enriched in wt SIVmac infection at 24 wpi. The color scale is shown at the bottom, with lowest to highest gene expression across all animals represented by the blue to red color gradient.

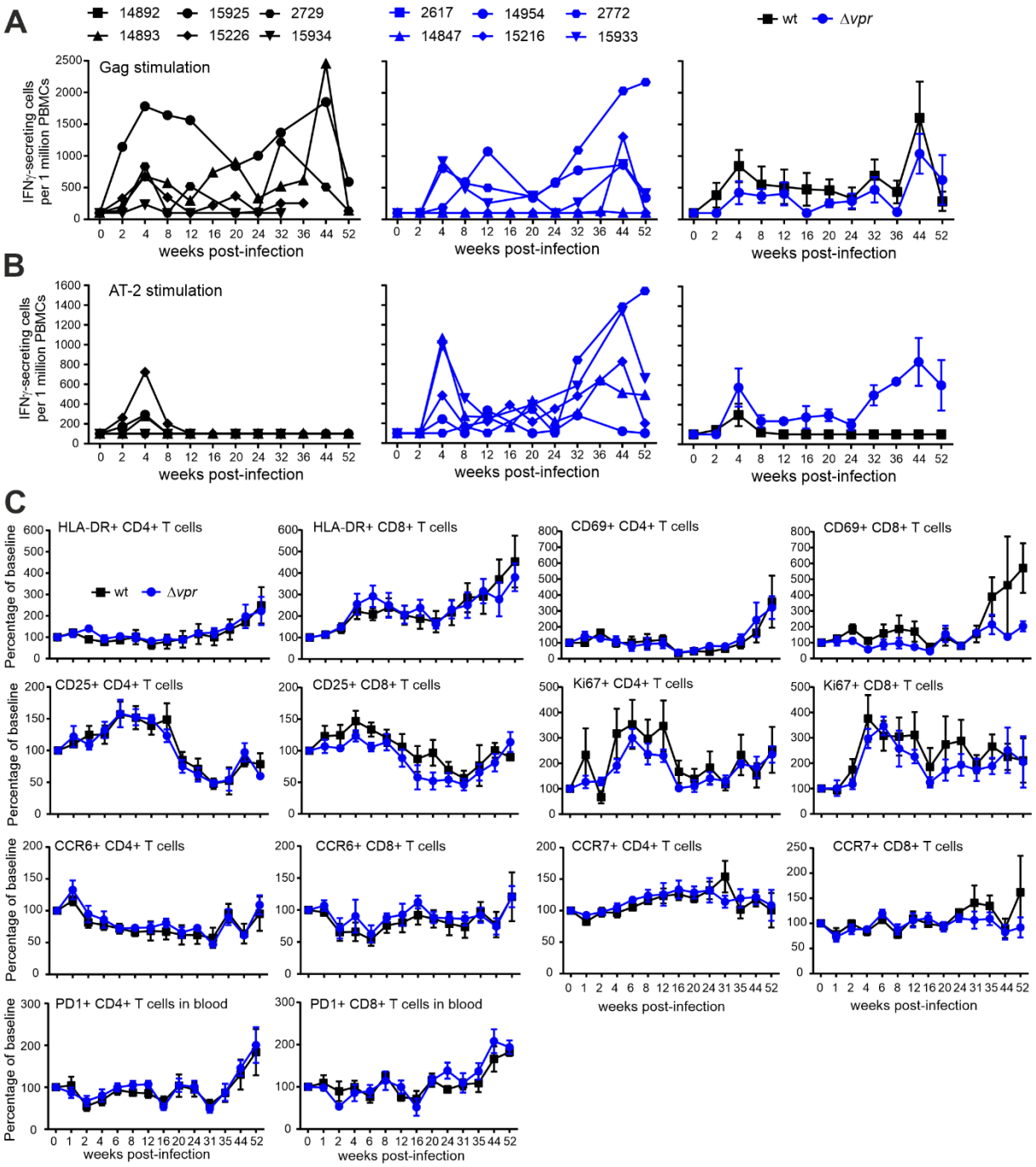

Figure S3 (related to Figure 4). Cellular activation in wt and Δ*vpr* SIVmac infection. (**A, B**) IFN-γ secreting lymphocytes as determined by ELISpot assay are shown as the number of spot-forming cells (SFC) per 10^6 PBMC after stimulation with (A) p27 Gag peptide pool or (B) AT-2 whole antigen. (**C**) Changes in the proportion of activated (CD69+, HLA-DR+, CD25+), exhausted (PD1+), proliferating (Ki67+) and chemokine receptor (CCR6+, CCR7+) expressing CD4+ and CD8+ T cells compared to the average percentages detected prior to infection (set to 100%). Shown are mean values (±SEM) measured for the wt (black) and Δ*vpr* (blue) groups of animals. Symbols specifying individual animals are indicated in the top left panel.

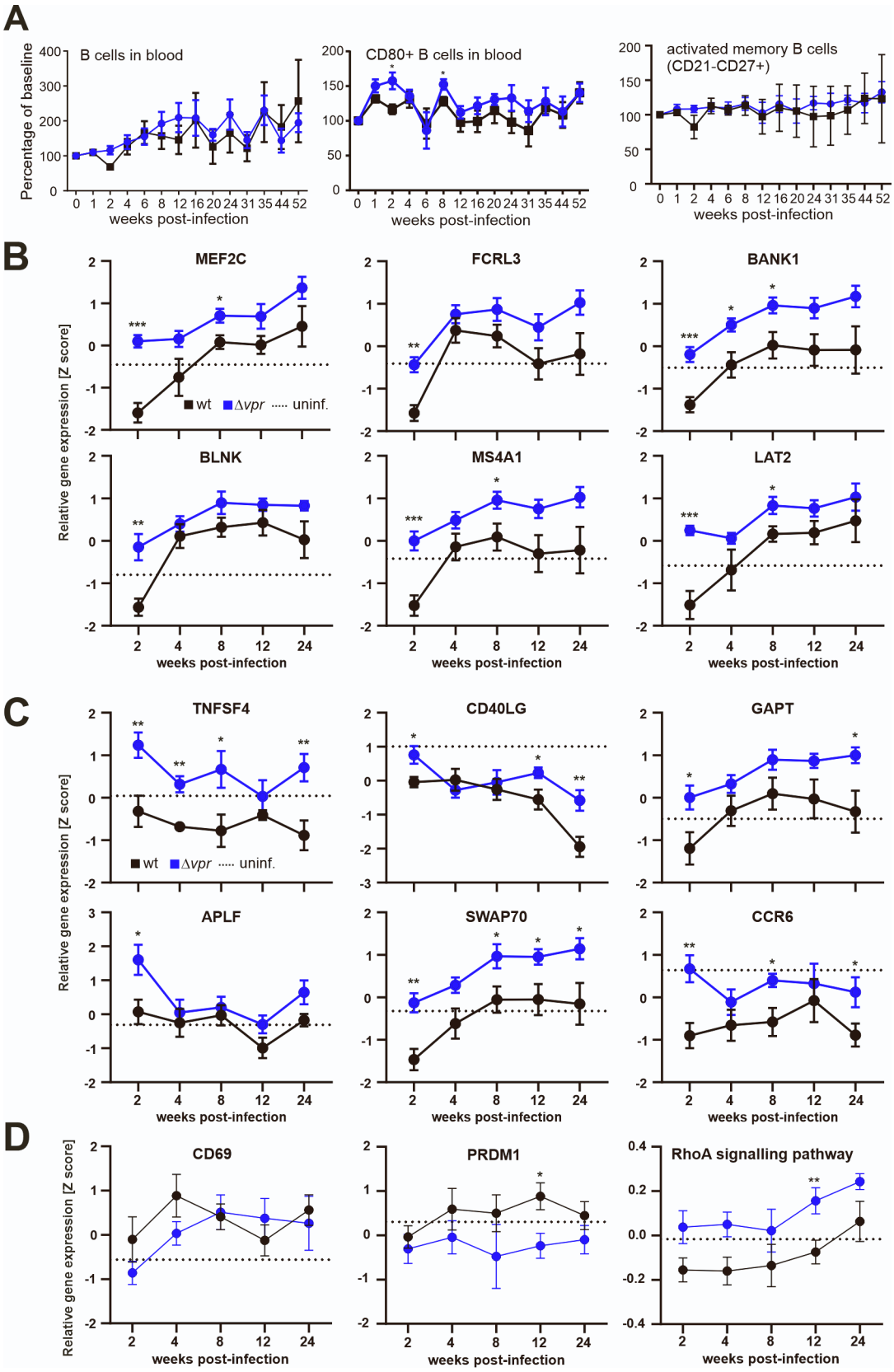

Figure S4 (related to Figure 4). B cell activation in wt and Δ*vpr* SIVmac infection. (**A**) Percentages of B cell populations in blood at indicated wpi relative to the baseline levels (100%). (**B**) Relative activation of B cell pathways in uninfected and infected animals. Shown are mean values of Z scores. (**C, D**) Relative induction of individual genes involved in BCR activation, signaling or antibody productions or infection in resting $CD4^+$ T cells.

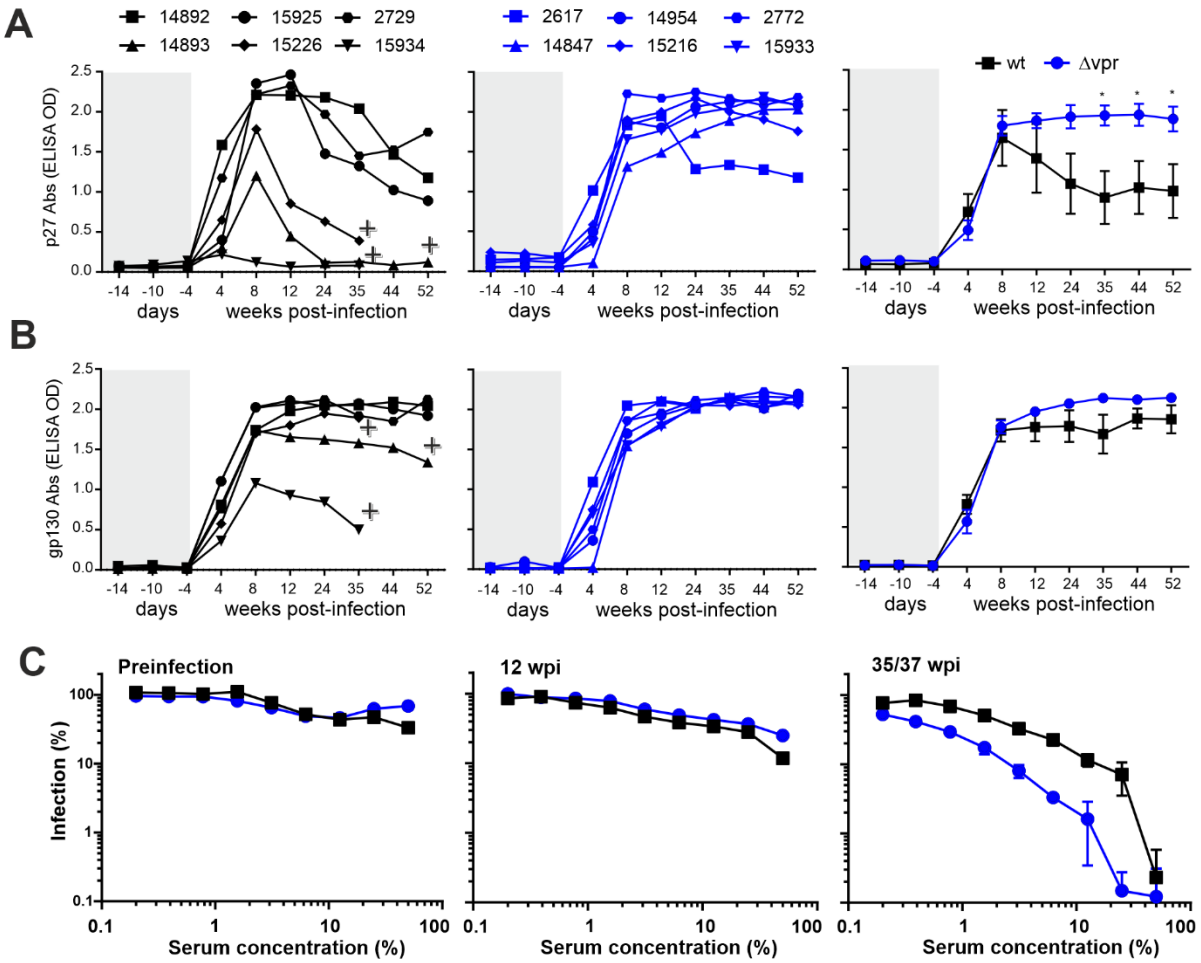

Figure S5 (related to Figure 4). Antibody detection and neutralizing activity of sera from wt or Δ*vpr* SIV$_{mac}$-infected animals. (**A, B**) Antibody levels against the (A) SIV-gp130 Env and (B) SIV-p27 capsid protein determined in serum of animals infected with wt or Δ*vpr* SIV$_{mac239}$ by ELISA. *, $p<0.05$. Note that saturating effect may be observed at O.D. values >2.0. (**C**) Neutralization of SIV$_{mac239}$ by pooled sera obtained from wt or Δ*vpr* infected animals prior to infection or at 12 or 35/37 wpi. Serially diluted pooled sera were used to determine their neutralizing capacity in wt and Δ*vpr* infection. Shown are mean values (±SD) derived from triplicate experiments.