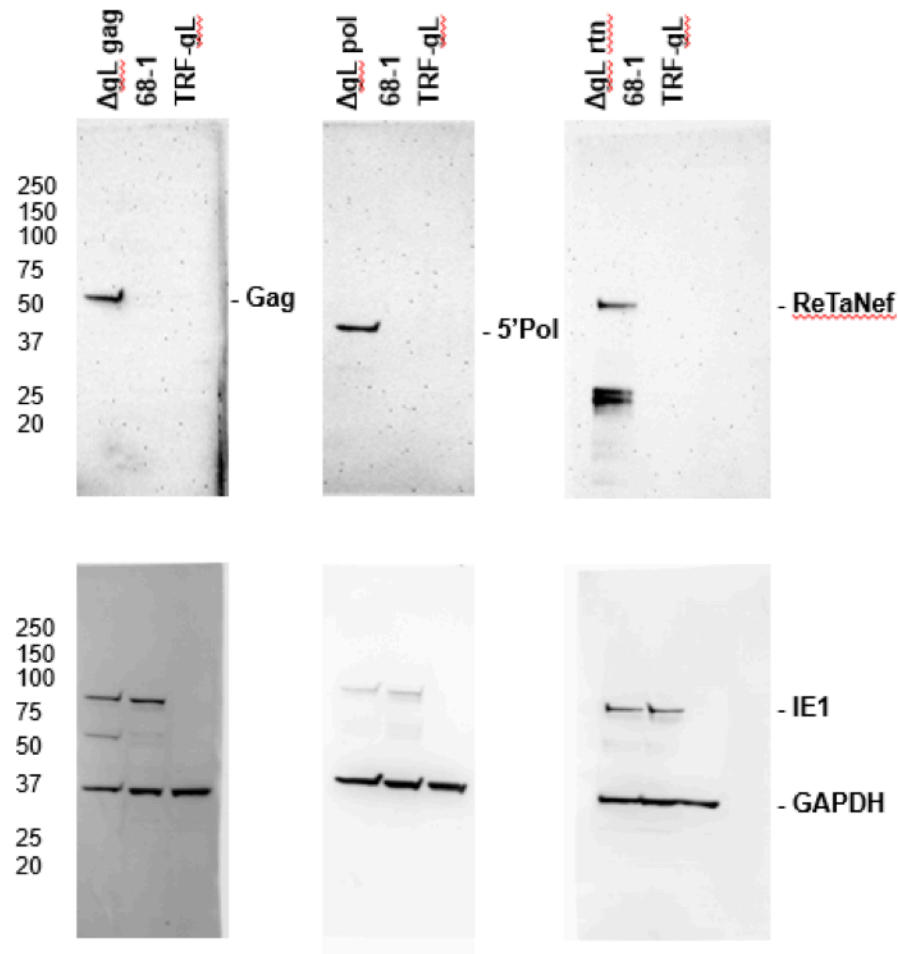
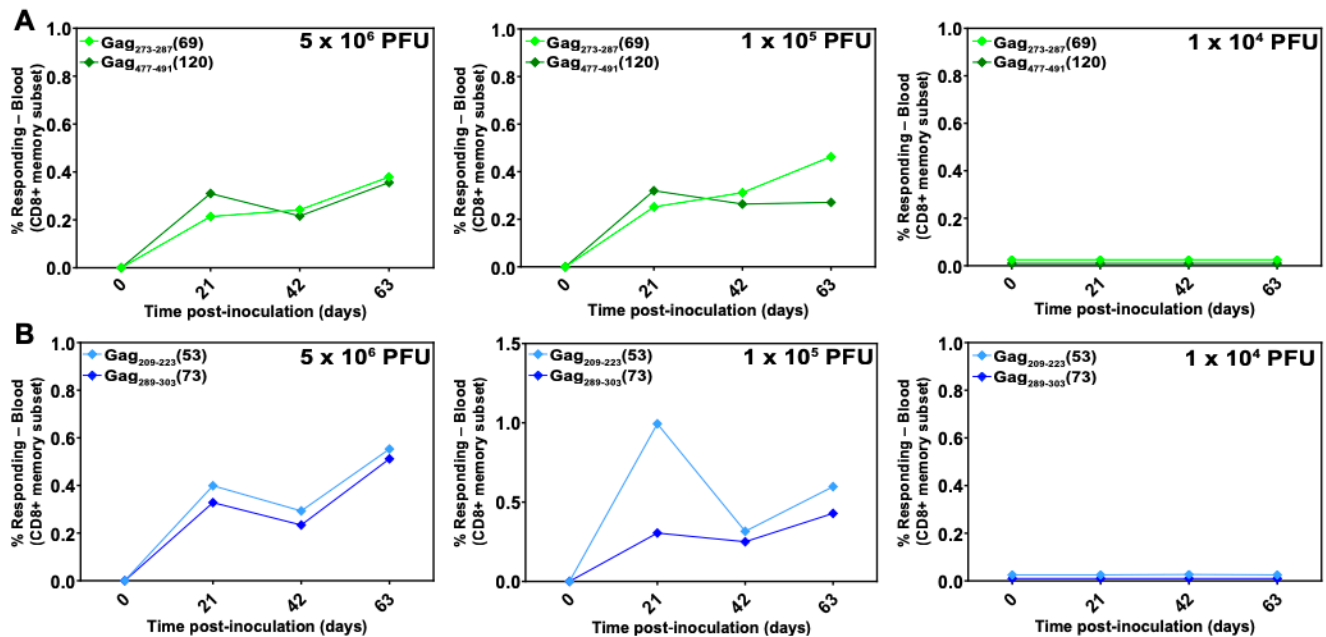


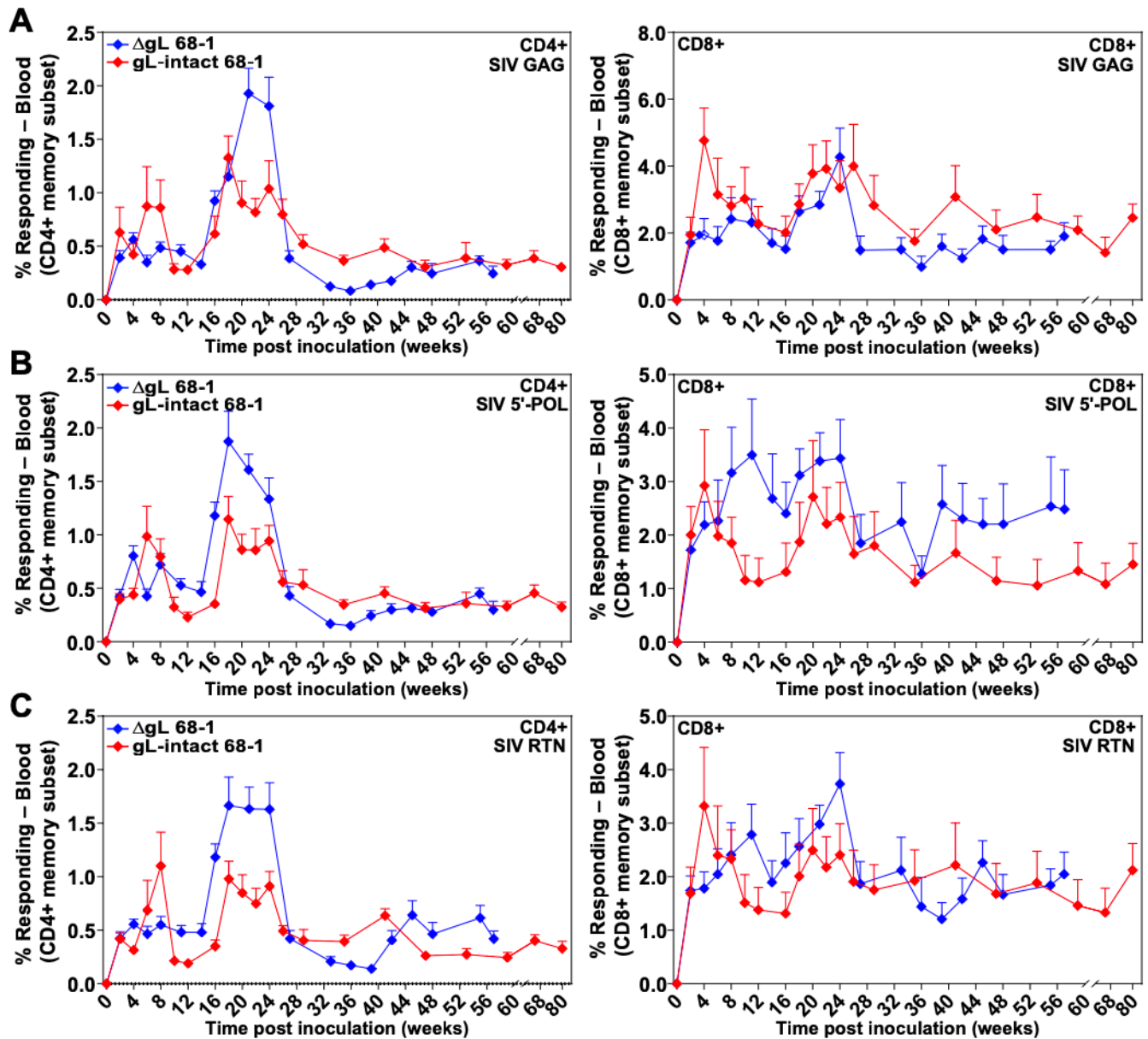
Supplemental Figures



Supplemental Figure 1. SIV antigen expression by Δ gL 68-1 RhCMV/SIV. TRF-gL were infected with a multiplicity of infection = 1 of either parental 68-1 RhCMV (68-1) or the indicated Δ gL constructs for 48 hours. Cells were lysed and electrophoretically separated by SDS PAGE prior to immunoblot for epitope tags fused to SIV antigens: anti-FLAG (Gag), Anti-V5 (Retanef), Anti-HA (5'Pol). Immunoblots were then stripped and re-probed with for RhCMV IE1 and for GAPDH.

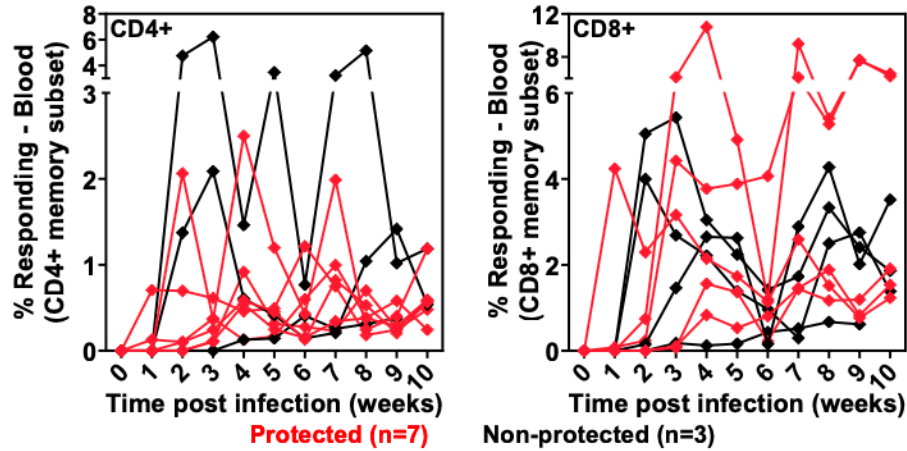


Supplemental Figure 2. Induction of CD8⁺ T cell responses to unconventionally restricted supertopes with administration of above-threshold doses of Δ gL 68-1 RhCMV/Gag. Longitudinal analysis of peripheral blood CD8⁺ T cell responses to two MHC-E-restricted (**A**, green) and two MHC-II-restricted (**B**, blue) SIV Gag supertopes after inoculation of 3 RM with 5 x 10⁶, 10⁵ and 10⁴ PFU, respectively, of Δ gL 68-1 RhCMV/Gag. Responses to these individual supertope 15mers were measured by intracellular cytokine staining (ICS) analysis as described in **Fig. 1**. The frequency of IFN- γ and/or TNF- α positive memory T cells is shown for each of the indicated post-inoculation timepoints.

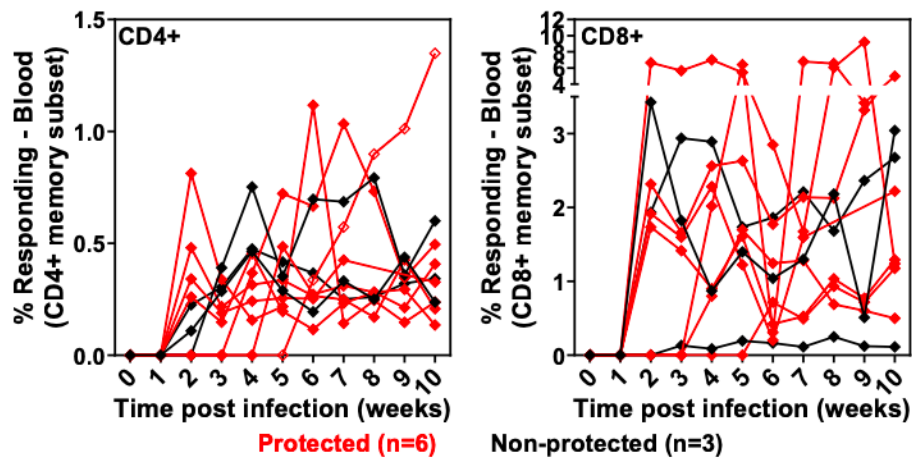


Supplemental Figure 3. Magnitude and durability of SIV ORF-specific T cell responses elicited by Δ gL vs. gL-intact 68-1 RhCMV/SIV vaccine vectors. Longitudinal analysis of the individual SIV ORF insert-specific CD4⁺ and CD8⁺ T cell responses in peripheral blood of RMs from Group 1 (Δ gL 68-1 RhCMV/SIV) and Group 2 (gL-intact 68-1 RhCMV/SIV). Longitudinal analysis of total responses (sum of the individual ORF insert-specific responses at each timepoint) are shown in **Fig. 2**. Longitudinal analysis of the constituent SIV Gag, SIV Rev/Nev/Tat, and SIV 5'-Pol responses are shown in this figure, with statistical analysis described in **Fig. 2**.

Group 1 – Δ gL 68-1:



Group 2 – gL-intact 68-1:



Supplemental Figure 4. *De novo* induction of SIV Env-specific T cell responses after effective SIVmac239 challenge during efficacy analysis of Δ gL vs. gL-intact 68-1 RhCMV/SIV vaccine vectors. CD4⁺ and CD8⁺ T cell responses to overlapping SIV Env 15mer peptide mixes were determined by ICS analysis in parallel with SIV Vif-specific responses during repeated limiting dose SIVmac239 challenge of Group 1 and Group 2 RM. SIV Env, like SIV Vif, was not expressed by the RhCMV/SIV vaccines and responses were not identified prior to challenge. Thus, the induction of SIV Env-specific T cell responses during challenge, like SIV Vif, indicates take of infection, even in the absence of measurable plasma SIV in protected RM.