

Figure S1. Cells from swine muscle explants support BoHV-4 transduction and replication. **A)** Representative pictures of BoHV-4-EFGP Δ TK-infected fibroblastic-like primary cell line with 1 M.O.I. at 24, 48 and 72 hours post-infection. Picture at 72 hours post infection was expanded to better monitor the CPE. (all panels). **B)** Viral titer was measured and expressed as \log_{10} TCID₅₀ per mL of viral particles released at 24, 48 and 72 hours post-infection, when infected with a M.O.I. of 1. Values are the means \pm standard errors of three independent experiments.

A)

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G V T R K Y K I K S N P L T K D I V I K
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B)



C)

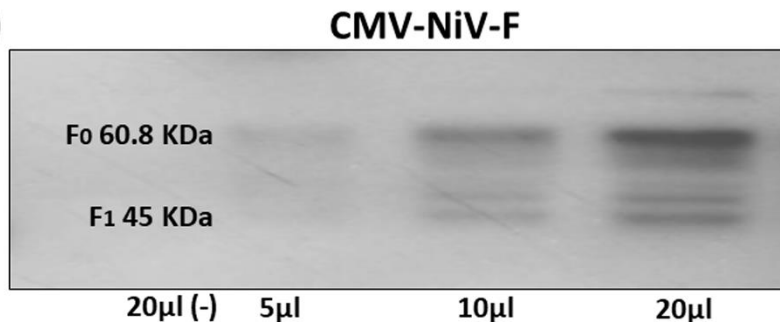


Figure S2. Design and expression of Nipah virus glycoprotein F. NiV-F AU1 peptide tagged ORFs full length sequence with the deduced amino acid composition along with the diagram (not on scale) of their expression cassette driven by CMV (blue) promoter. Western immunoblotting of cells, transfected with CMV-NiV-F. The lanes were loaded with different amounts of total protein cell extract (5, 10 and 20 μ l [1 μ g/ μ l]). Negative control was established with pEGFP-1 transfected cells (20 μ g (-)).

A)

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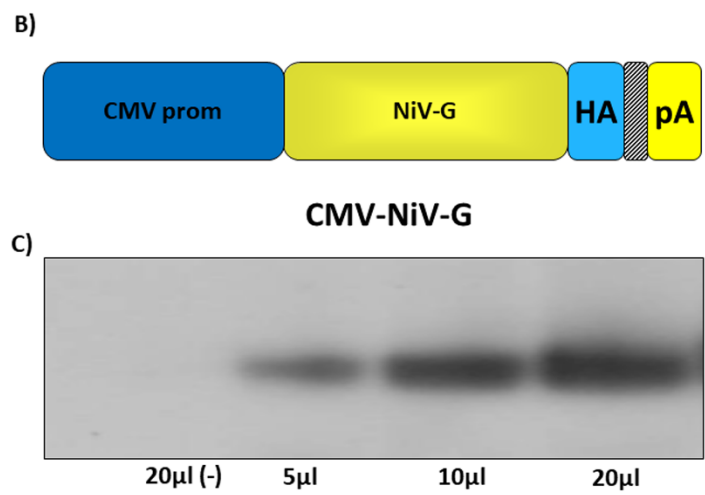


Figure S3. Design and expression of Nipah virus glycoprotein G. NiV-G HA peptide tagged ORFs full length sequence with the deduced amino acid composition along with the diagram (not on scale) of their expression cassette driven by CMV (blue) promoter. Western immunoblotting of cells, transfected with CMV-NiV-G. The lanes were loaded with different amounts of total protein cell extract (5, 10 and 20 μ l [1 μ g/ μ l]). Negative control was established with pEGFP-1 transfected cells (20 μ g (-)).

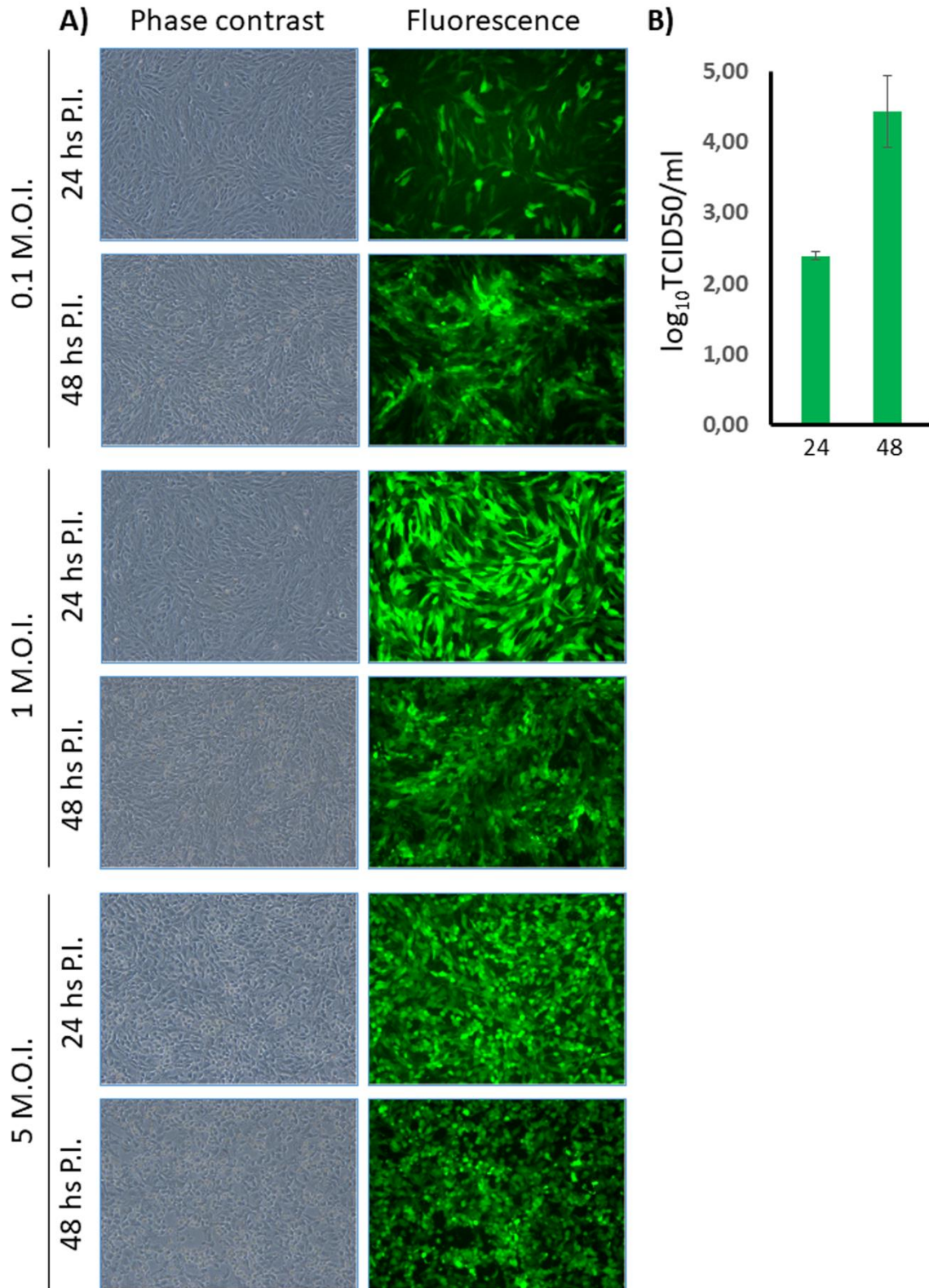


Figure S4. BoHV-4 adaptation and replication in PECs. **A)** Representative pictures of BoHV-4-EFGP Δ TK-infected PECs with different M.O.I. at 24 and 48 hours post-infection. Magnification, $\times 10$ (all panels). **B)** Viral titer was measured and expressed as \log_{10} TCID₅₀ per ml of viral particles released at 24 and 48 hours post-infection when infected with 0.1 M.O.I.. Values shown are the means \pm standard errors of three independent experiments.

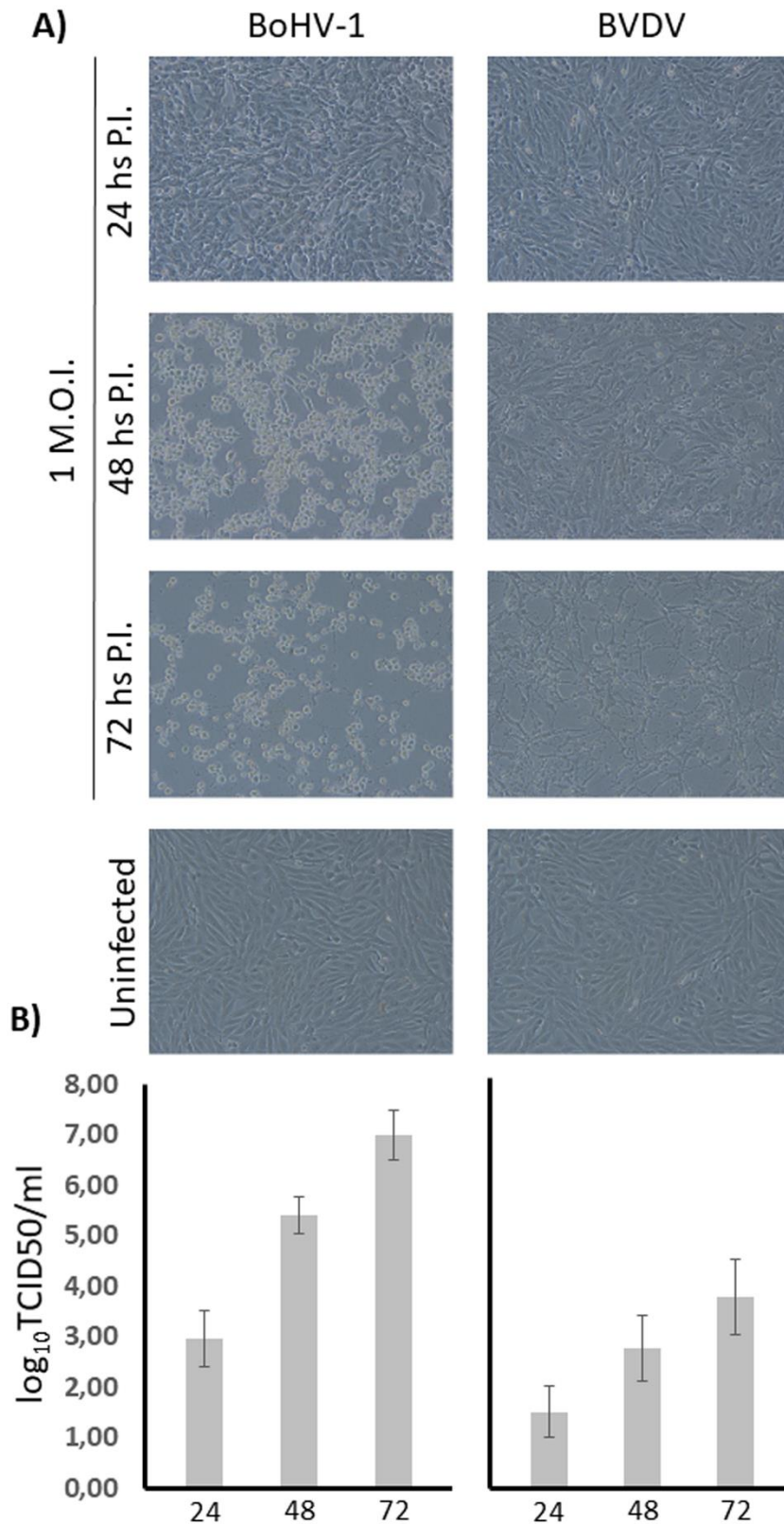


Figure S5. PECs are permissive for BoHV-1 and BVDV. **A)** Representative pictures of BoHV-1 and BVDV infected PECs with 1 M.O.I. at 24, 48, 72 hours post-infection and uninfected control. Magnification, $\times 10$ (all panels). **B)** Viral titer was measured and expressed as \log_{10} TCID₅₀ per mL of viral particles released at different times (24, 48 and 72 hours). Values are the means \pm standard errors of three independent experiments.

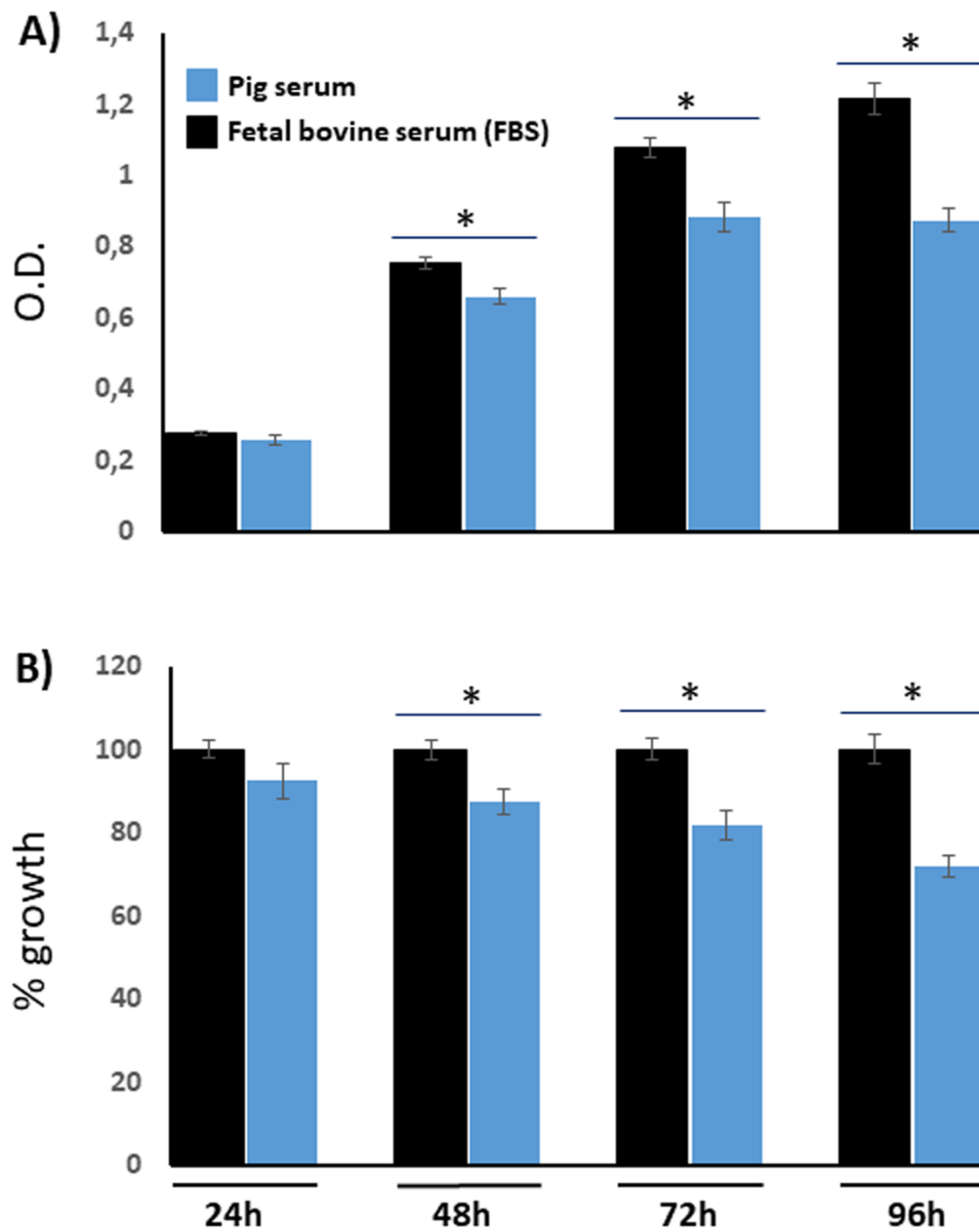


Figure S6. Pig serum reduces PECs growth. MTT assay comparing PECs growth with medium containing 10% of FBS and the same number of PECs growth with medium containing 10% of PS, at different time points (24, 48, 72 and 96 hours). In **A)** absolute values are expressed as optical density (O.D.), whereas in **B)** the same data were normalized and expressed as percentage of cell growth reduction, where the cell growth with FBS was considered equal to 100% of growth. The data presented are the means \pm standard errors of triplicate measurements ($P > 0.05$ (*) as measured by Student's *t* test).

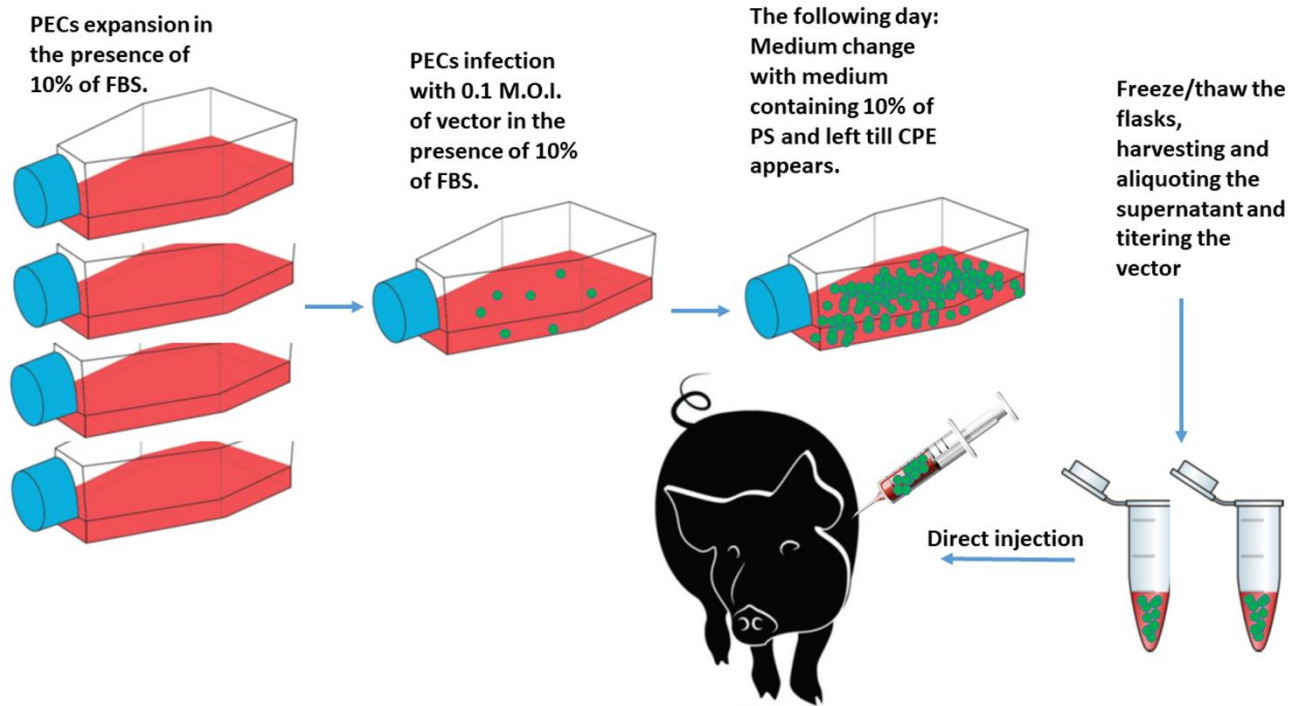


Figure S7. Flow chart of the strategy used to produce BoHV-4-A-CMV-NiV-F Δ TK and BoHV-4-A-CMV-NiV-G Δ TK free of bovine antigens.

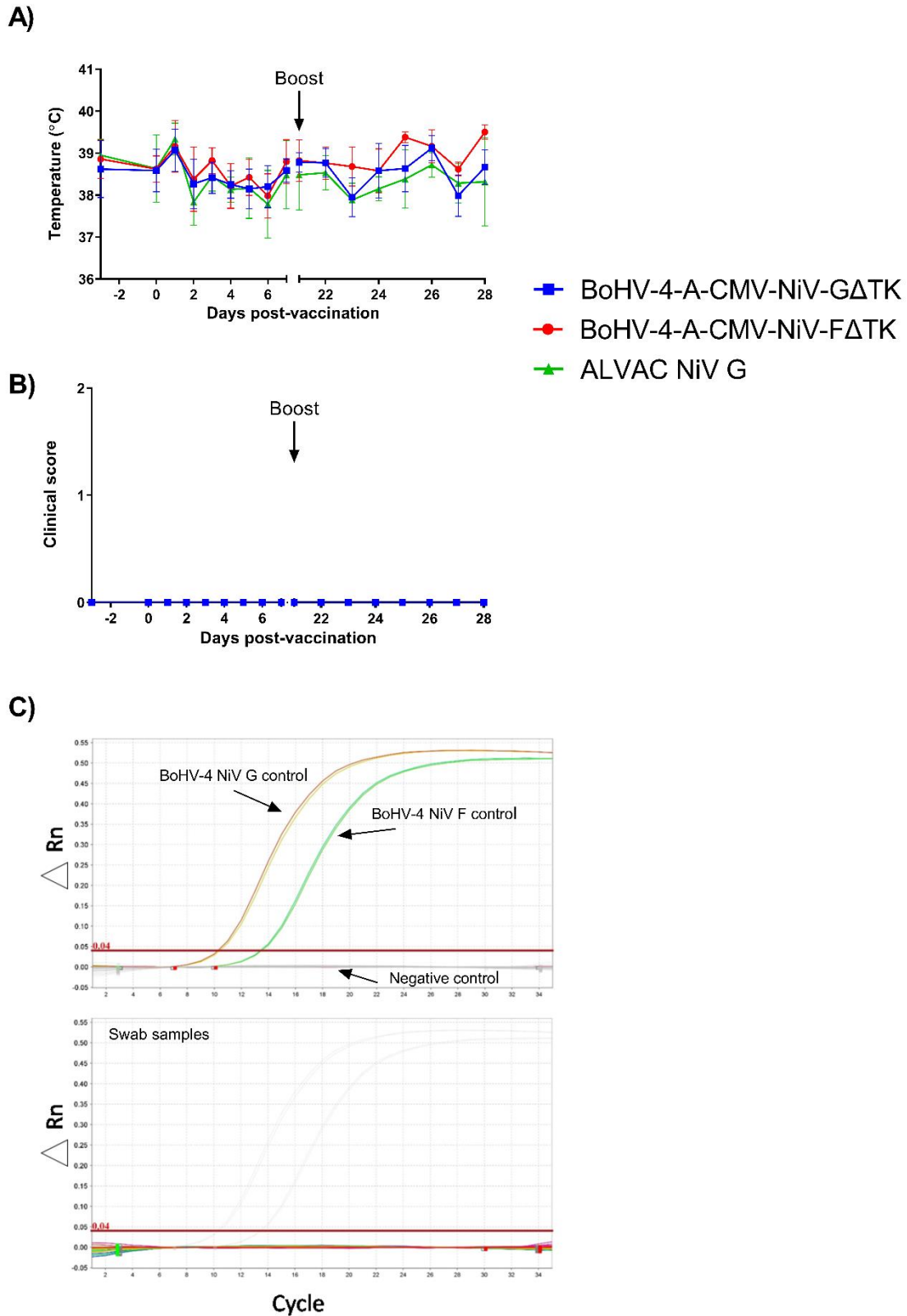


Figure S8. Evaluation of post-vaccinal reactions of immunized pigs and assessment of BoHV-4 vector shedding. Rectal temperatures (A) and clinical scores (B) were monitored for 7 days following prime and boost immunization with BoHV-4-A-CMV-NiV-G Δ TK, BoHV-4-A-CMV-NiV-F Δ TK or ALVAC NiV G. Quantitative PCR was used to assess the presence of BoHV-4 in rectal and nasal swabs following primary immunization (C).

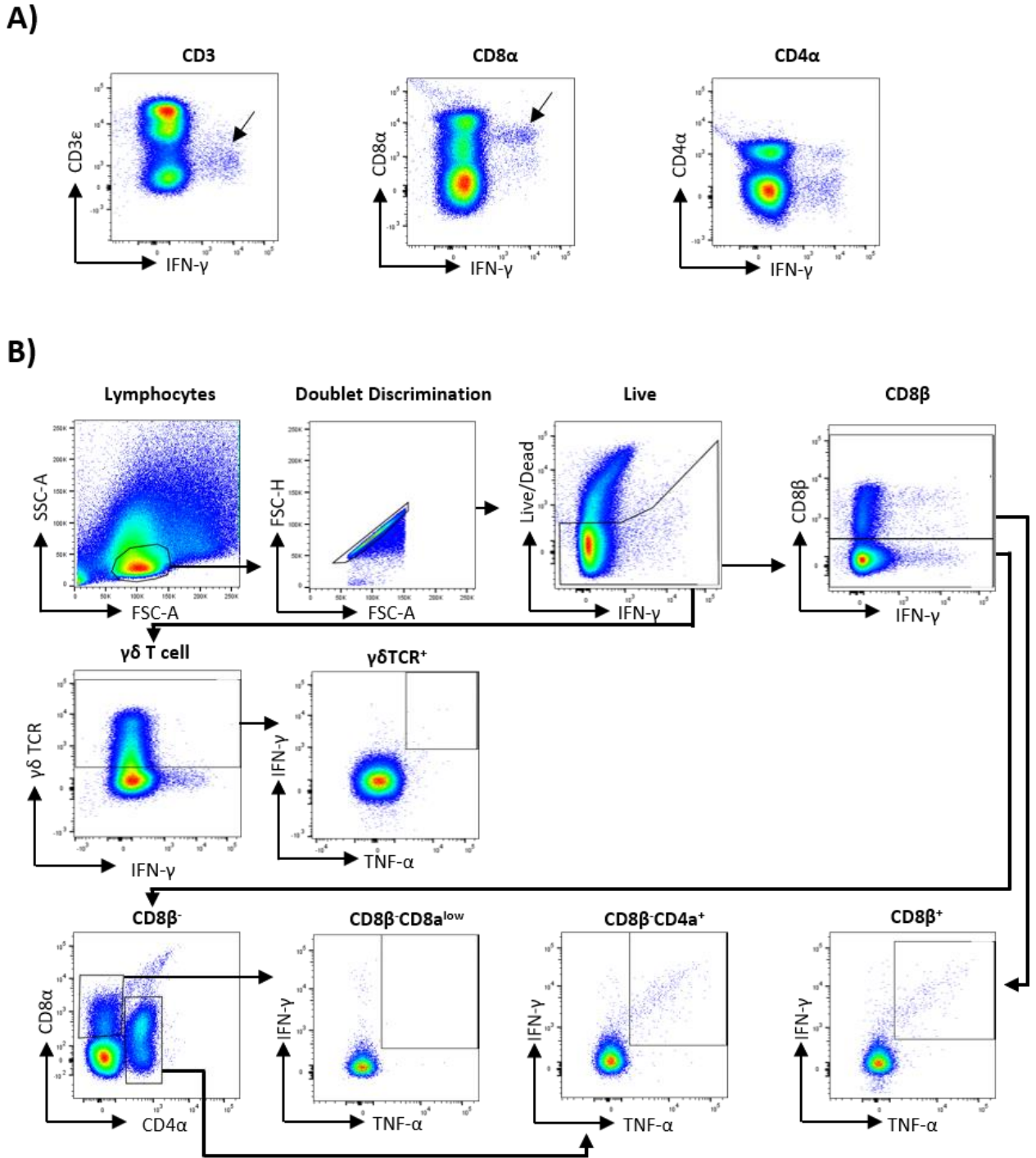


Figure S9. Evaluation of gating strategies for ICS analysis of NiV-specific CD8 T cell responses. Representative ICS data from NiV G peptide-stimulated PBMC from a BoHV-4-A-CMV-NiV-G Δ TK immunised pig showing an apparent down-regulation of CD3 and CD8 α on IFN- γ ⁺ cells – indicated by black arrows (A). To discount responses from $\gamma\delta$ T cells or NK cells, NiV G peptide stimulated PBMC were stained with CD4, CD8 α , CD8 β and $\gamma\delta$ Tcr mAbs (B).

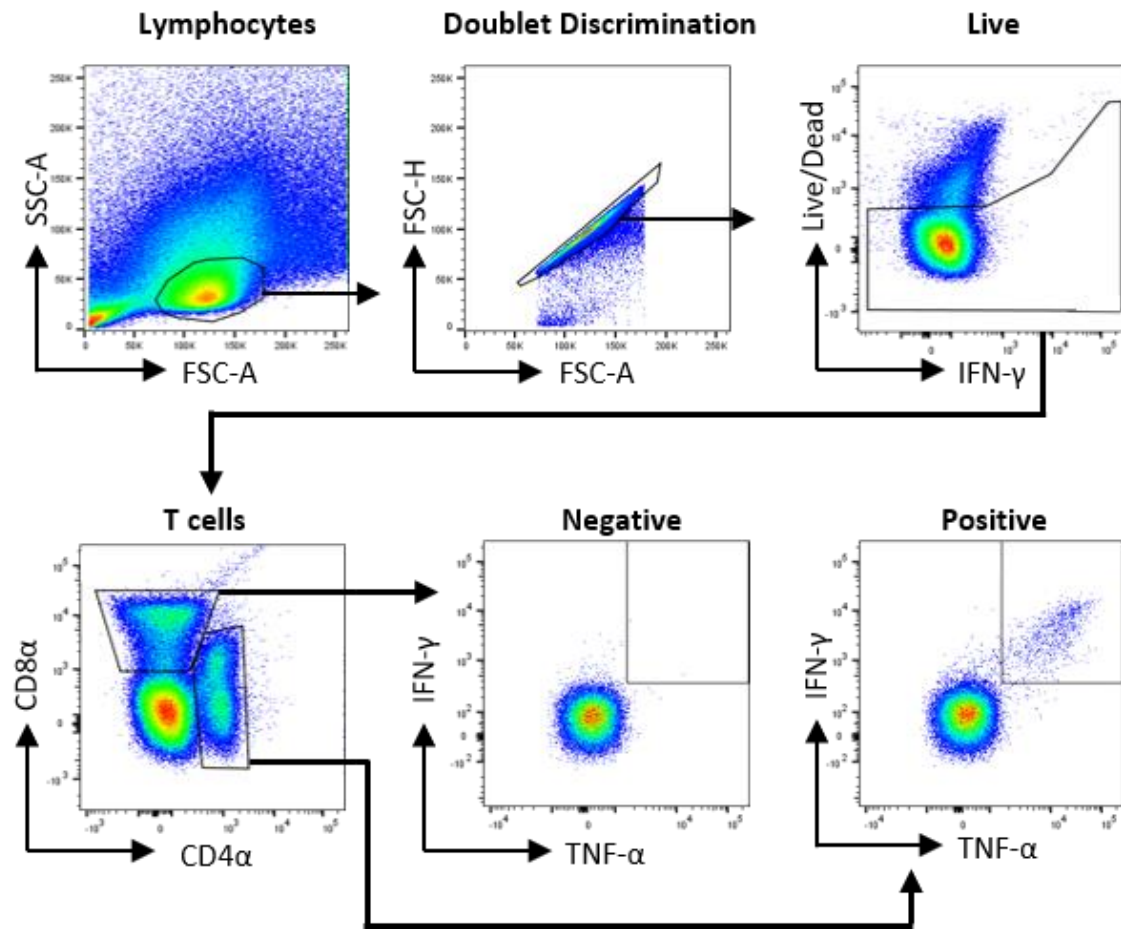


Figure S10. Flow cytometric gating for ICS analysis of longitudinal PBMC datasets. Singlet, live lymphocytes were gated as either CD8 α^+ CD4 $^-$ and CD8 α^- CD4 $^+$ cells and the % of cells co-expressing IFN- γ and TNF- α determined.