

Supplemental information for

Characterization of a live-attenuated HCMV-based vaccine platform

by

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Supplemental Figures

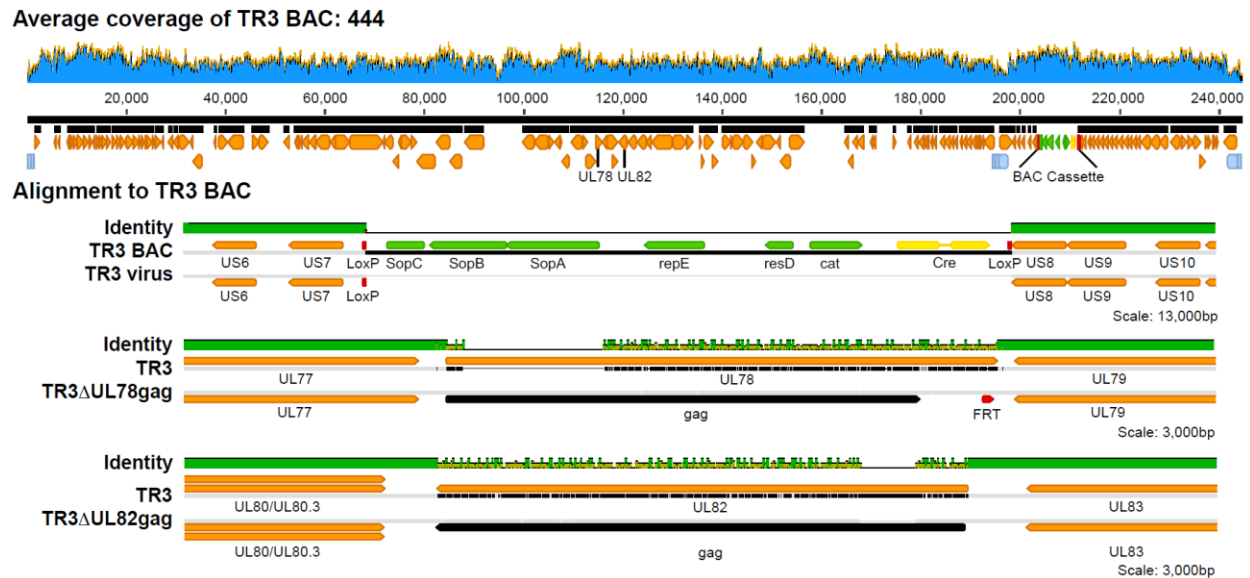


Figure S1: Sequence analysis of TR3 (BAC and Virus), TR3 Δ UL78gag and TR3 Δ UL82gag.

The top panel shows the sequencing coverage map for the TR3 (BAC) graphically depicted as number of reads per nucleotide position. The ORF map is shown below with the BAC cassette highlighted in green and cre-gene in yellow. Genome alignments of TR3 (BAC) with TR3-virus as well as TR3 (BAC) with TR3 Δ UL78gag (BAC) and TR3 Δ UL82gag (BAC) are shown below. Only the regions that differ between TR3 (BAC) and the indicated recombinants are shown. The green bar indicates the percentage of nucleotide identity between both virus sequences with green being 100% identical. The black bar represents the HIVgag ORF. The residual FRT site in TR3 Δ UL78gag is shown in red.

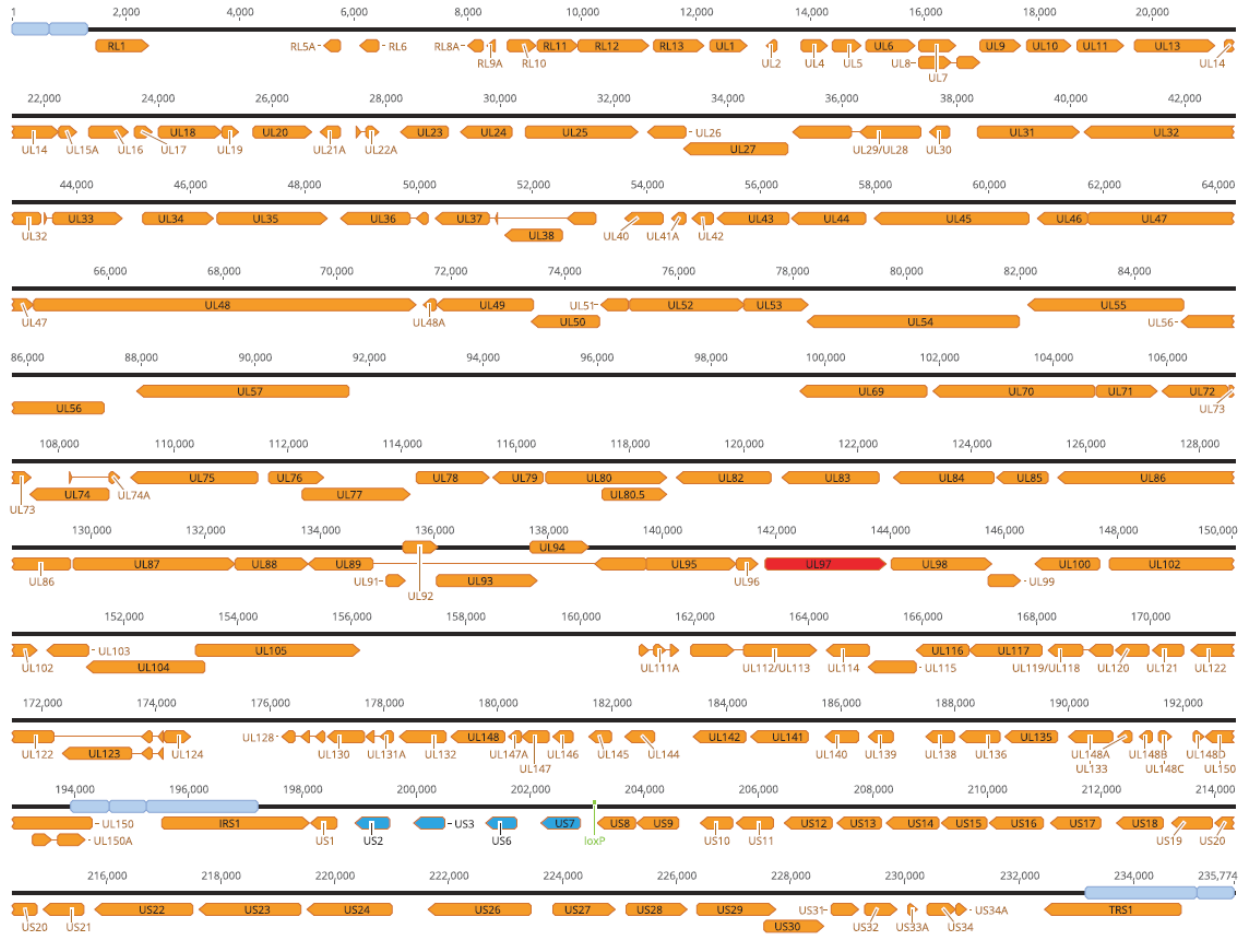


Figure S2: ORF map of HCMV TR3 (BAC). Orange: viral ORFs, light blue: repeat region, red: UL97 from HCMV AD169, dark blue: US2-US7 from HCMV AD169.

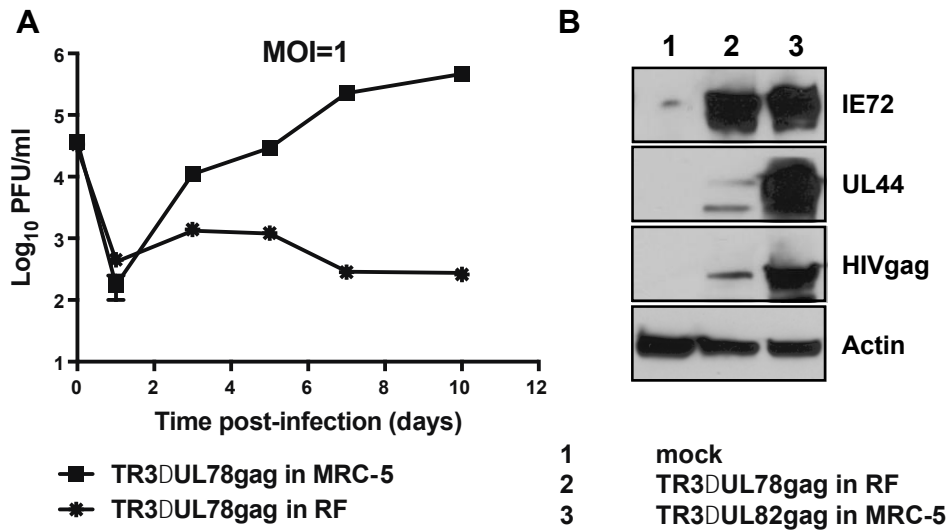


Figure S3: HCMV TR3 does not replicate in rhesus fibroblasts. A) Growth curve of TR3 in human versus rhesus fibroblasts. MRC-5 cells or primary rhesus fibroblasts were infected with TR3 Δ UL78gag at an MOI=1. Supernatants were collected at the indicated days and the viral titer determined. B) Viral protein expression in human versus rhesus fibroblasts. MRC-5 cells or primary rhesus fibroblasts were infected with TR3 Δ UL78gag (MOI=1) and infected cells were harvested at 96h post-infection. Cell lysates were electrophoretically separated and probed for the indicated proteins by immunoblot.

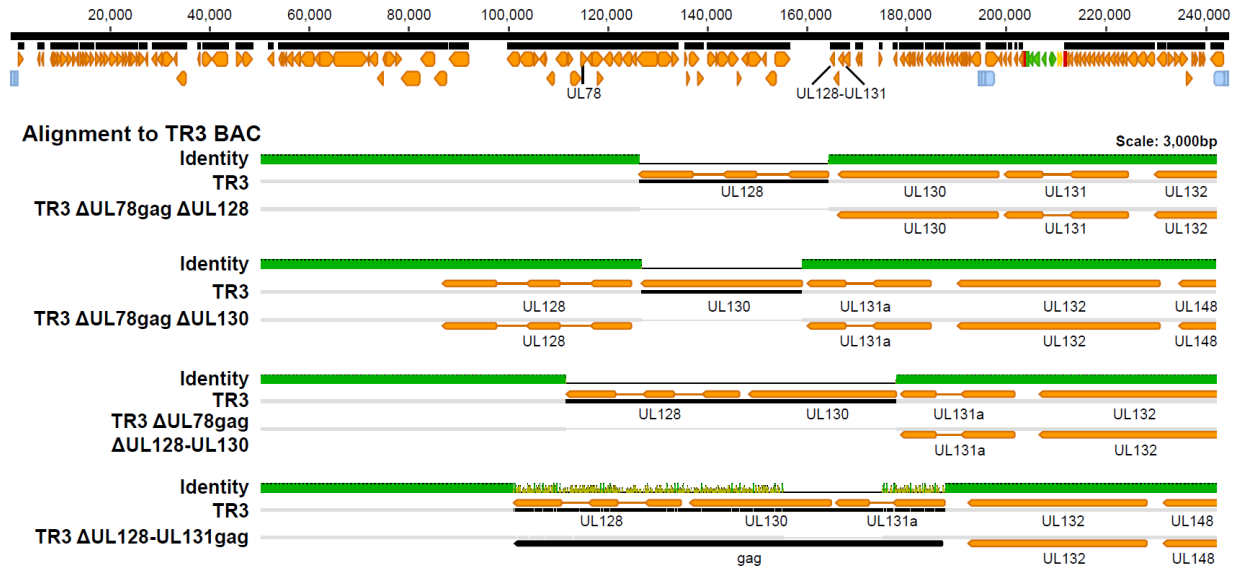


Figure S4: Sequence analysis of PC-deficient HCMV TR3 vectors. The top panel shows the ORF map of TR3 (BAC) with the BAC cassette and modified ORFs highlighted. Genome alignments of TR3 (BAC) with the indicated recombinants are shown below. Only the region spanning the UL128-131 ORFs is shown. The green bar indicates the percentage of nucleotide identity between both virus sequences with green being 100% identical. TR3 Δ UL78gag was used to delete UL128 and/or UL130 to generate TR3 Δ UL78gag Δ UL128, TR3 Δ UL78gag Δ UL130 and TR3 Δ UL78gag Δ UL128-130, whereas HIV/gag replaces the UL128-131 region in TR3 Δ UL128-131gag as indicated.

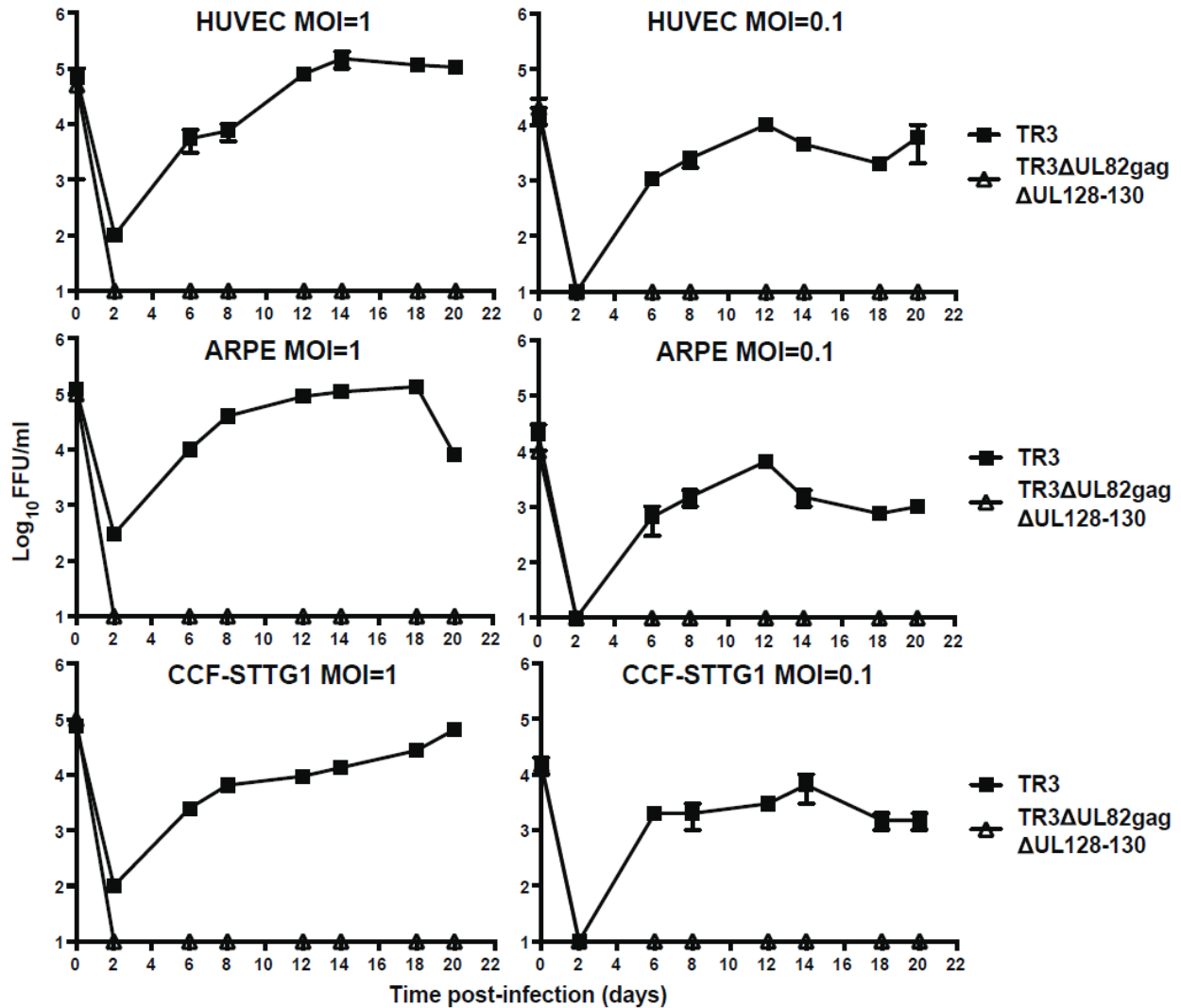


Figure S5: Pentamer-and pp71-deficient TR3 does not grow in non-fibroblast cells.

Endothelial cells (HUVEC), epithelial cells (ARPE-19) and astrocytic cells (CCF-STGG1) were infected with the indicated MOI of TR3 or TR3ΔUL82gag. The supernatants were harvested at the indicated days and the virus titer determined on BJ-pp71 cells.

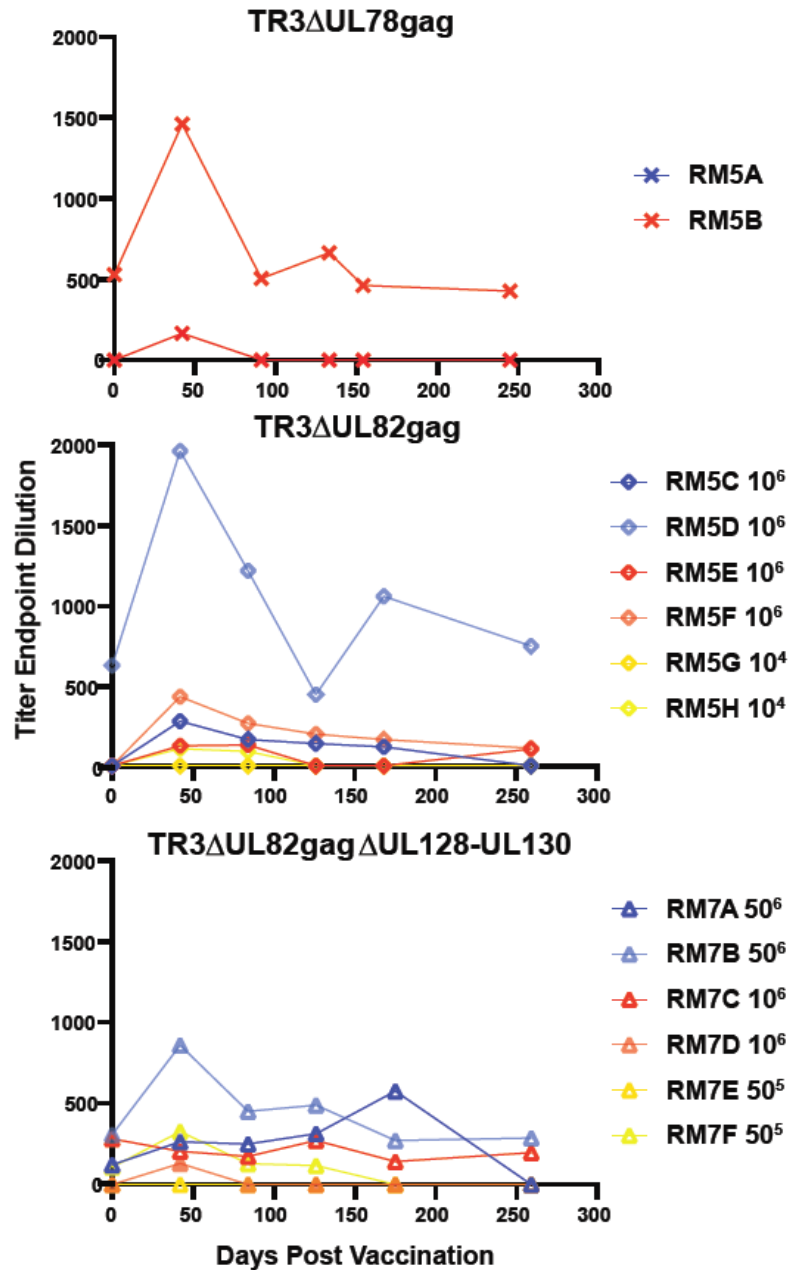


Figure S6: Anti-HCMV antibody responses elicited by PC-intact and PC-deficient UL82-deleted TR3. As shown in Figures 5 and 7, RM were inoculated with TR3 Δ UL78gag, TR3 Δ UL82gag or TR3 Δ UL82gag Δ UL128-130 at the indicated doses. Shown are end-point titers for the indicated days post-inoculation and determined by ELISA using HCMV-infected cell lysate.

Supplemental Data: Immunoblots used in this study

Figure 1C

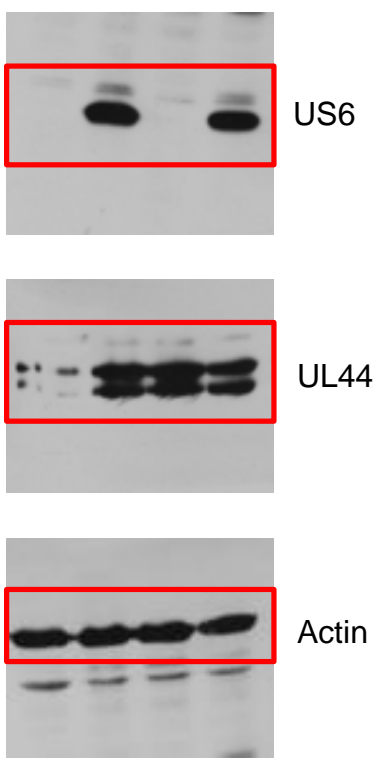


Figure 1E

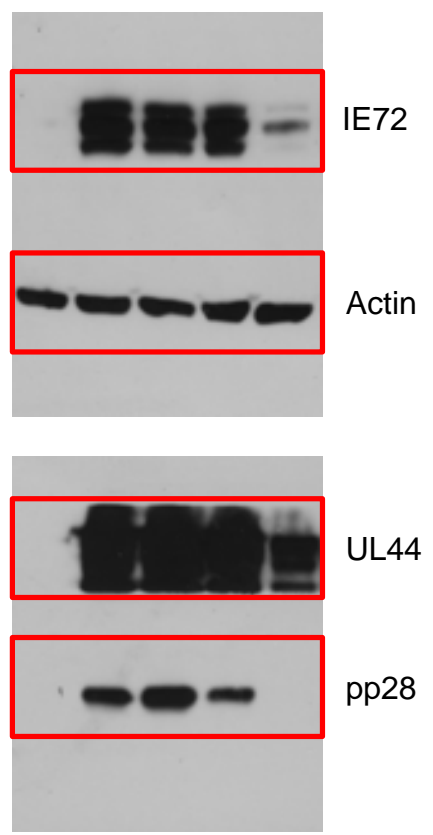


Figure 3A

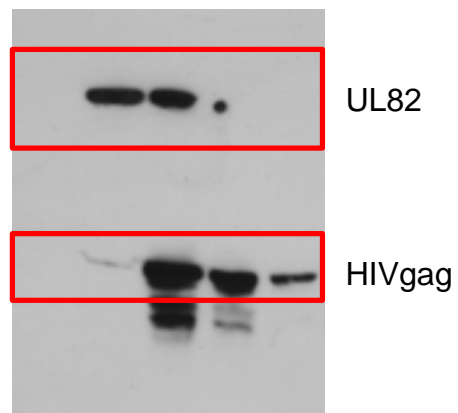
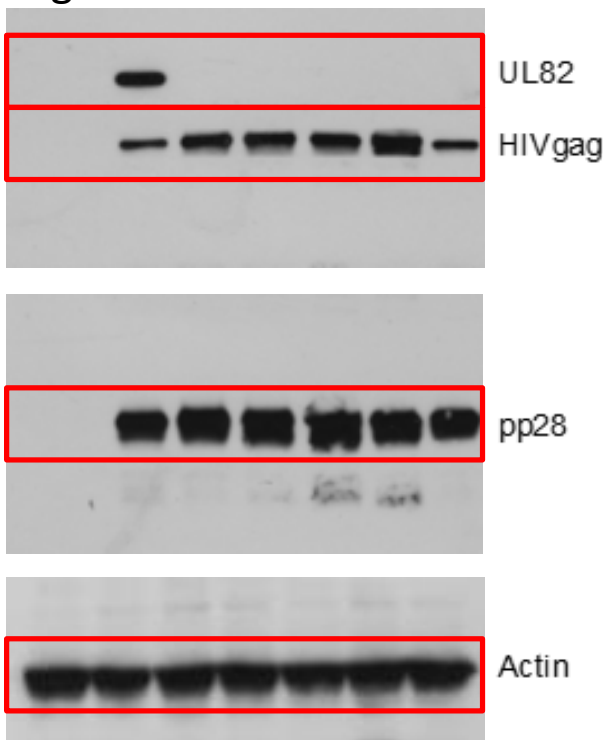


Figure 7A

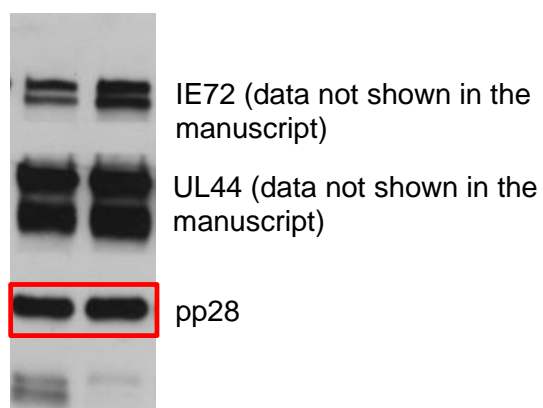
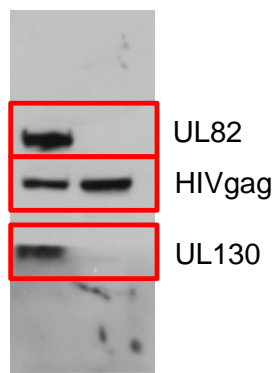


Figure S3

