Legends for Supplemental Figures:

SUPPLEMENTAL FIGURE S1: Comparison of UL97 Expression Levels in AD169rv, NTAP97, FLAG97 and Δ97-infected cells. Protein extracts of HFF at 72 h post infection were resolved by SDS-PAGE, transferred to PVDF membranes, and probed with polyclonal rabbit serum raised against recombinant GST-UL97 fusion protein. Number of cell equivalents loaded per lane is indicated, in thousands of cells, below the Western blot image. Mock-infected HFF are represented in lane 1, WT AD169rv-infected HFF in lanes 2-3, NTAP97-infected cells in lanes 4-5, FLAG97 in lanes 6-7, and Δ97 in lanes 8-9. Protein extracts of NTAP97-infected cells were pre-treated with TEV protease to remove the TAP tag, which otherwise would promiscuously bind IgG molecules and confound quantitative comparisons of protein expression; in parallel mock, AD169rv, FLAG97 and Δ97-infected cell extracts were incubated in identical AcTEV protease buffer conditions but without TEV protease.

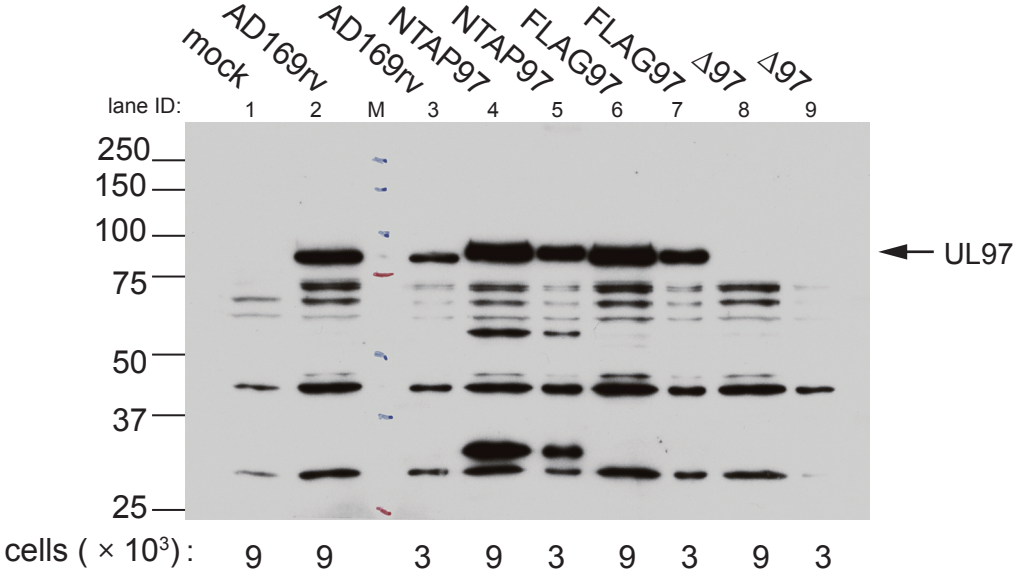
SUPPLEMENTAL FIGURE S2: Comparison of pp65 Expression Levels in AD169rv, NTAP97, FLAG97 and Δ97-infected cells. Western blot of lysates of HFF which were mock-infected (mock, lanes 1-2), infected with AD169rv (lanes 3-5), Δ97 (lanes 6-8), FLAG97 (lanes 9-11) or NTAP97 (lanes 12-14) and harvested at 72 h post-infection, were probed with anti-pp65 monoclonal antibody (mAb) and detected with Alexa 680 conjugated secondary antibody using an digital infrared imaging system (Li-Cor Odyssey). Asterisks above lanes 2, 5, 8, 11, 14 indicate lysate samples prior to TEV protease treatment (NTAP97) or mock-treatment (mock, AD169rv, & FLAG97). Number of cell equivalents loaded per lane is indicated below each lane, in thousands. An arrow to the left of the blot indicates a prominent immunoreactive band corresponding to the expected relative mobility of pp65. Note: pp65 was quantified (Li-Cor Odyssey 2.1 software) to be approximately 35% - 45% less abundant in lysate of Δ97-infected cells than in that of AD169rv-infected cells (data not shown). Note: Neither TEV protease treatment, nor mock-treatment, appeared to result in significant losses of detectable pp65.

SUPPLEMENTAL FIGURE S3: Western Blot to Determine Fraction of UL97 Bound by pp65 in AD169rv-Infected Cell Lysate. Proteins immunoprecipitated from AD169rv-infected cell lysate harvested at 72 h post-infection were compared to defined quantities of input cell lysate in a Western blot. Lysate was subjected to IP using either normal mouse serum (ms IgG) as a negative control, or using an anti-pp65 mouse mAb (anti-pp65) to capture pp65 and associated proteins. The signal intensity of a prominent band (indicated by a labeled arrow), which was immunoreactive to anti-UL97 rabbit serum, and which matched the expected relative mobility of UL97, was compared between input lysate and IP eluate lanes using densitometry (Quantity One Software version 4.5, Bio-Rad, data not shown). Note: a duplicate blot probed with anti-pp65 mAb indicated that pp65 was abundant in the anti-pp65 IP eluate and not in that of the control IP using mouse serum (data not shown). Note: pp65 was detected in the flow-

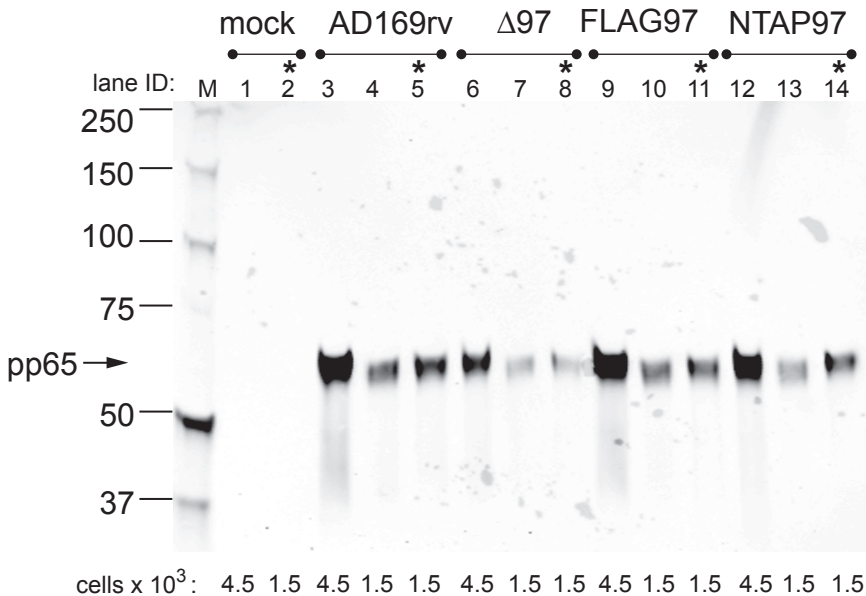
throughs after IP in both control and anti-pp65 IP reactions, indicating that not all pp65 was captured during the IP (data not shown).

SUPPLEMENTAL FIGURE S4: Quantification of in vitro pull down results shown in Figure 5. Quantity One Software Version 4.5 was used to analyze phosphorscreen data captured from a dried SDS-PAGE gel following a GST-pull down experiment. Signal intensity was specifically compared between the ^{35}S-labeled UL97 band bound by GST-US11 and that bound by GST-pp65.

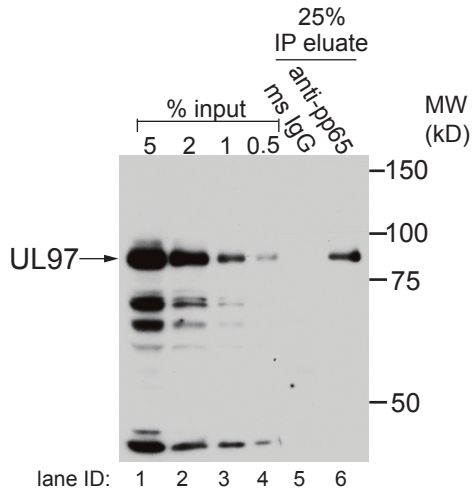
SUPPLEMENTAL FIGURE S1: Comparison of UL97 expression levels in AD169rv, NTAP97, FLAG97 and Δ97-infected cells



SUPPLEMENTAL FIGURE S2: Comparison of pp65 expression levels in AD169rv, NTAP97, FLAG97 and Δ97-infected cells.



SUPPLEMENTAL FIGURE S3: Western Blot to Determine Fraction of UL97 Bound by pp65 in AD169rv-Infected Cell Lysate

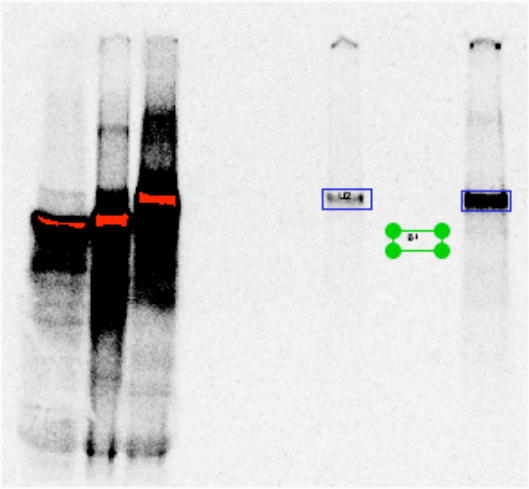


Supplemental Figure S4: Analysis of GST-pp65 pull down experiment

Description	Name	Area (mm2)	Density (counts/mm^2)	Density after Substracting Background (counts/mm$^{2)}$
GST-pp65:UL97 signal	U1	17.81999969	286663.5717	278222.1578
GST-US11:UL97 signal	U2	17.81999969	41443.76981	33002.35592
Background signal	B1	17.81999969	8441.41389	0

fold-difference U1/U2 (background subtracted) = 8.430372622

Gel name : phosscrn35gstpp6501182007 v3 (Raw 1-D Image)



Index	Name	Area mm2	Density CNT/mm2
1	U1	17.820000	286663.5717
2	U2	17.820000	41443.76981
3	B1	17.820000	8441.413890

Background Subtraction Method: Local
data units: Counts (CNT)

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