Supplemental Figure Legends

Figure S1. Real time RT-PCR analysis of firefly luciferase (Fluc) transcript levels. (A) Amplification plot of Fluc RNA standards derived from making serial dilutions of RNA purified from Vero cells transfected with pGL3-pol5'UTR and pRL-SV40. Serial dilutions were prepared by diluting sample RNA in RNA purified from mock transfected Vero cells. Dilutions of the samples assayed were assigned arbitrary levels based on the amount of dilution. The highest concentration of Fluc mRNA was set to 100,000 a.u. (red line), and several 10-fold dilutions prepared: 1:10 (orange line), 1:100 (yellow line), 1:1000 (green line), and 1:10,000 (blue line). (B) Amplification plot of Fluc mRNA samples purified from Vero cells transfected with either pGL3-MCS (red line), pGL3-pol5'UTR (orange line), pGL3-pol1-57 (yellow line), pGL3-pol58-208 (green line), or pGL3-pol-uORFmut (blue line) and pRL-SV40. (C) Standard curve of Fluc RNA standards (black data points with black trend line) plotted as Ct versus arbitrary transcript levels. Fluc mRNA samples are shown on the trend line with the same color as the corresponding sample in (B).

Figure S2. Real time RT-PCR analysis of Renilla luciferase (Rluc) transcript levels. (A) Amplification plot of Rluc RNA standards from Fig. 1S. The highest concentration of Rluc mRNA was set to 100,000 a.u. (red line), and several 10-fold dilutions prepared: 1:10 (orange line), 1:100 (yellow line), 1:1000 (green line), and 1:10,000 (blue line). (B) Amplification plot of Rluc mRNA samples purified from Vero cells transfected with either pGL3-MCS (red line), pGL3-pol5'UTR (orange line), pGL3-pol1-57 (yellow line), pGL3-pol58-208 (green line), or pGL3-pol-uORFmut (blue line) and pRL-SV40. (C) Standard curve of Rluc RNA standards (black data points with black trend line) plotted as

Ct versus arbitrary transcript levels. Rluc mRNA samples are shown on the trend line with the same color as the corresponding sample in (B).

Figure S3. Analysis of Pol and ICP8 levels by western blot. (A) Western blot analyses of Pol (top panel) and ICP8 (bottom panel) were performed on samples from Vero cells infected with polΔ54.x, wt.x, or KOS at an MOI of 10 for the time indicated. A 2-fold dilution series was also included from a sample of Vero cells infected with KOS at an MOI of 10 for 24 hours. (B) Pol (left graphs) and ICP8 (right graphs) dilution series were quantified by measuring the intensity of the bands of a scanned film using Quantity One software (BioRad). The left dilutions series were used to generate standard curves to quantify the 6 hours post infection samples (blue line for Pol and light blue line for ICP8) and the right dilution series were used to generate standard curves to quantify the 9 hours post infection samples (red line for Pol and orange line for ICP8). The 2:1 samples were arbitrarily assigned a value of 16 and the subsequent dilutions were assigned values accordingly.