

Supporting Information

Kyratsous and Silverstein 10.1073/pnas.0810656105

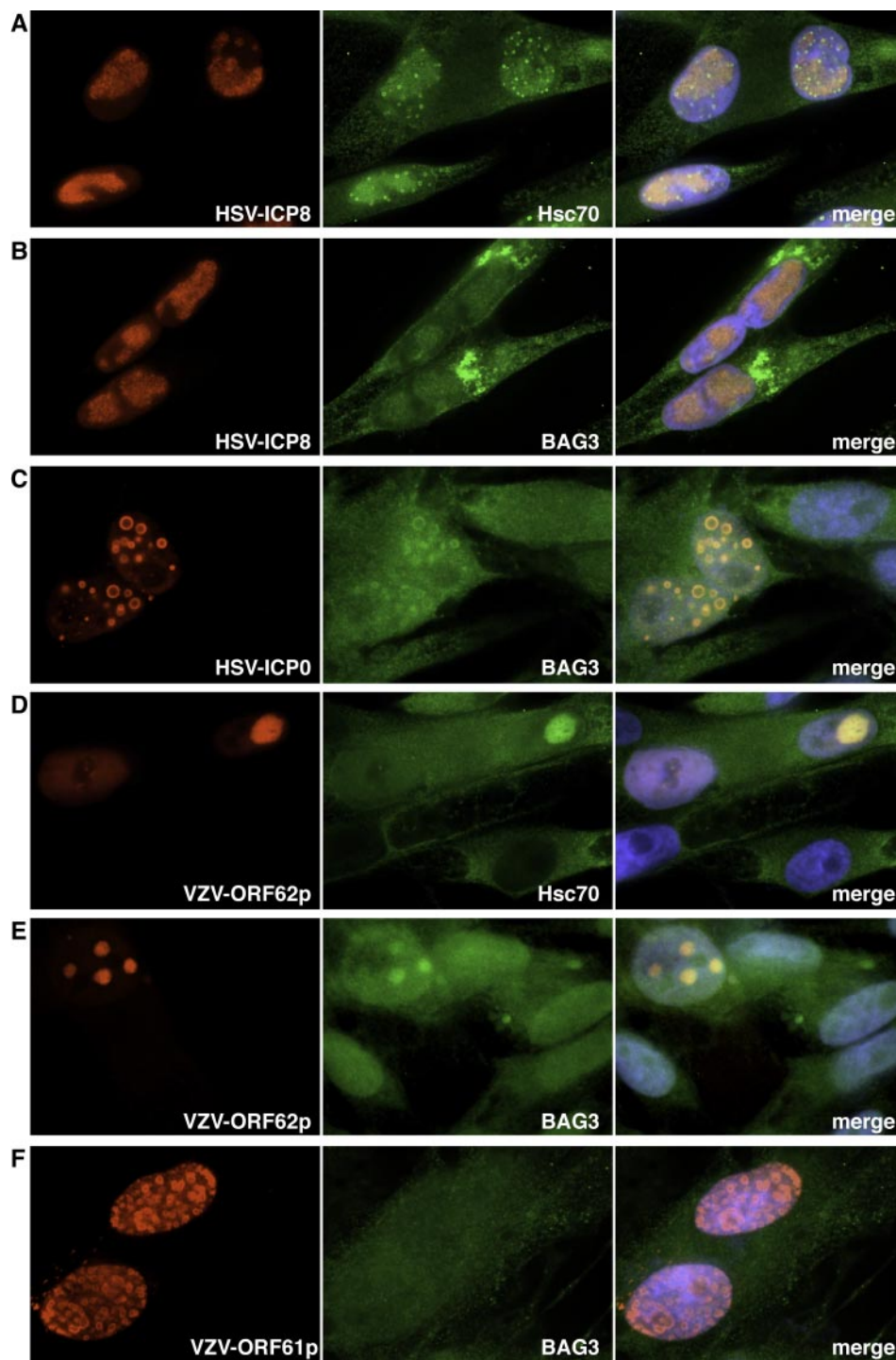


Fig. S1. Localization of Hsc70 and BAG3 during VZV and HSV infection or ICP0 and ORF61 transfection. MeWo cells were infected with HSV (A and B) or cell-free VZV (D and E) or transfected with a plasmid expressing HSV-ICP0 (C) or VZV-ORF61p (F). Cells were fixed at 6, 24, or 48 hpi respectively and the indicated proteins were visualized by indirect immunofluorescence microscopy. Images were captured with a 100x objective and analyzed by volume deconvolution.

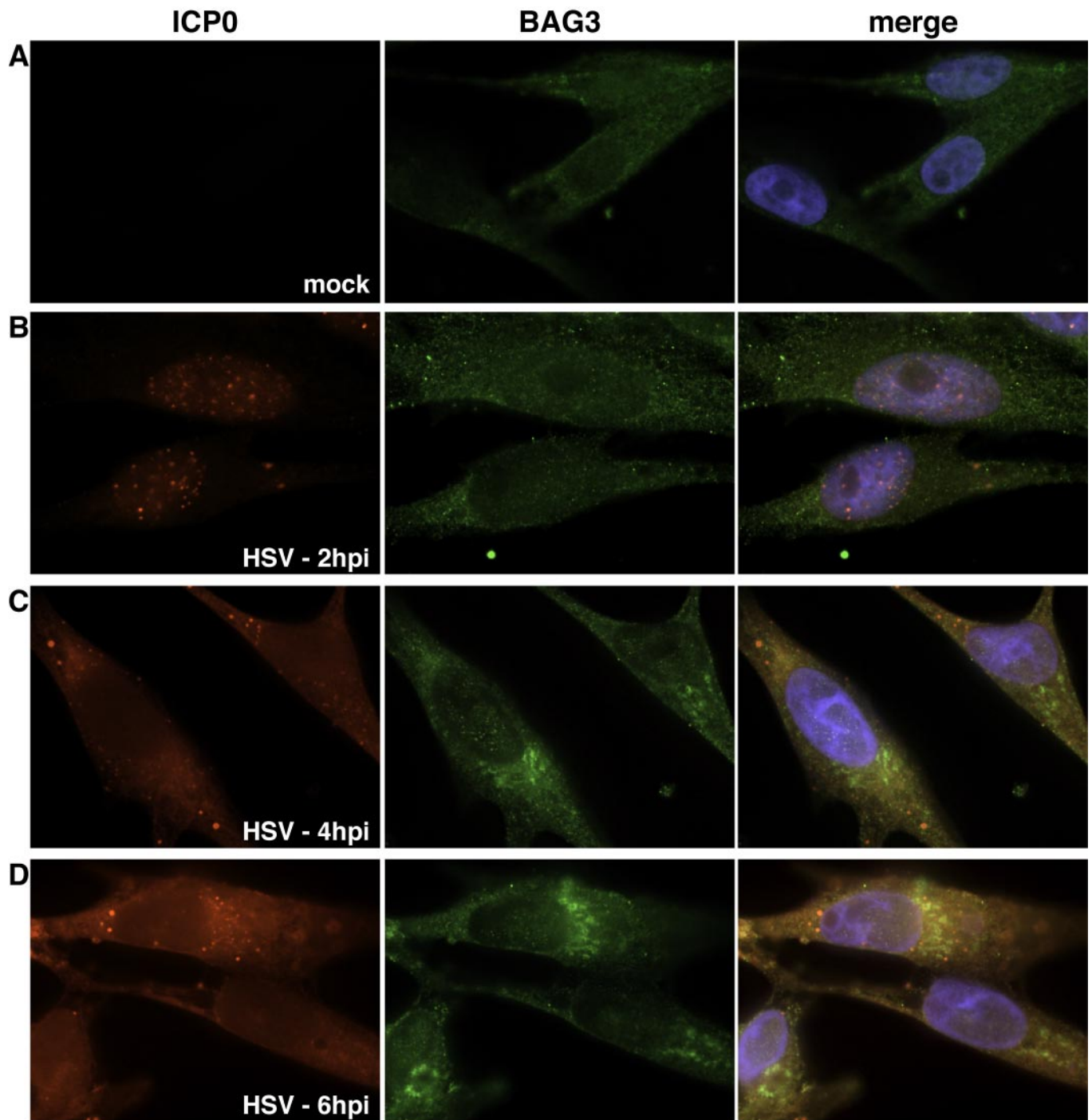


Fig. S2. Localization of BAG3 during a time course of HSV infection. MeWo cells were either mock treated (A) or infected with HSV (B–D). At the indicated times post infection cells were fixed and ICP0 and BAG3 were visualized by indirect immunofluorescence microscopy. Images were captured with a 100 \times objective and analyzed by volume deconvolution.

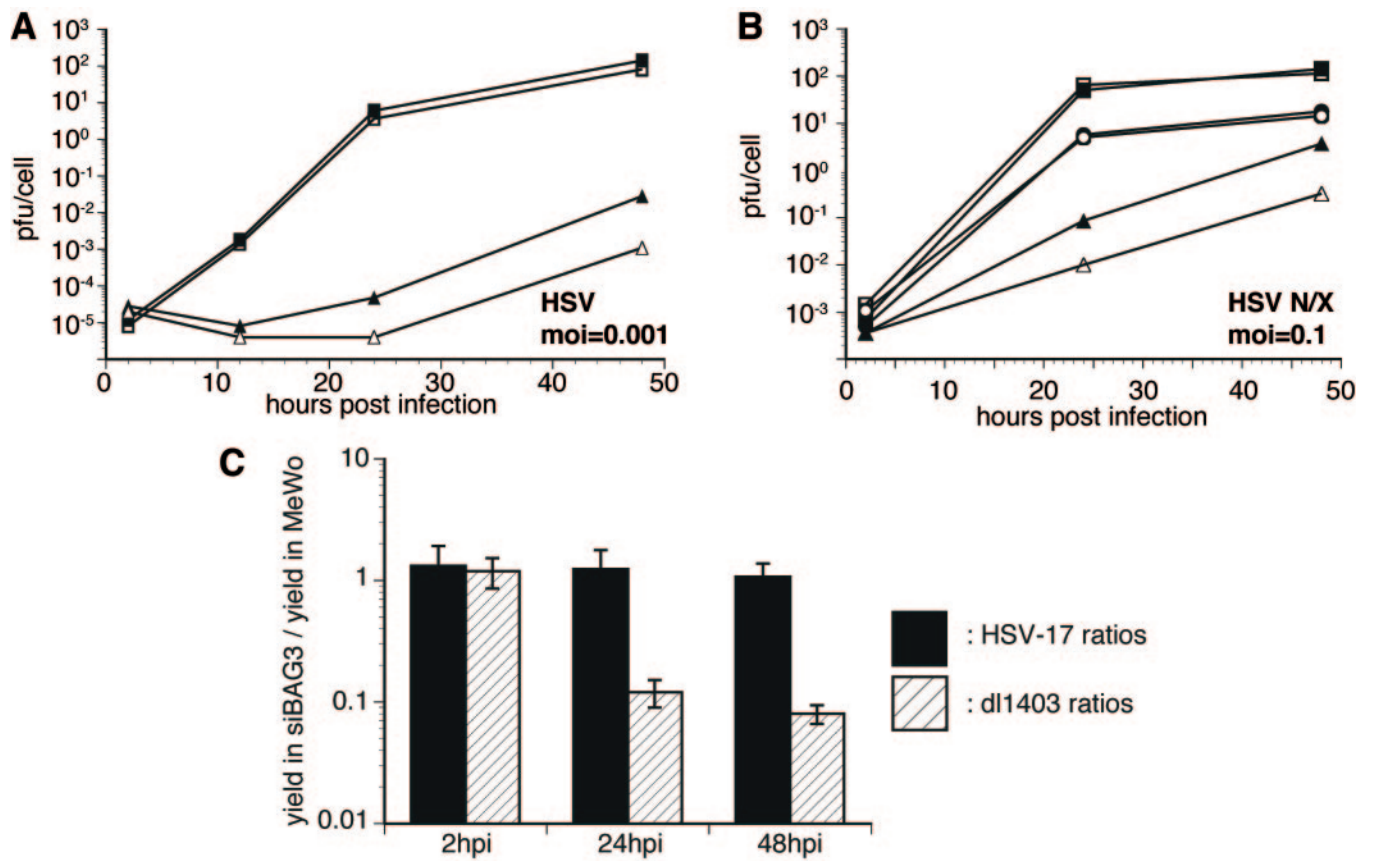


Fig. S3. Growth of wild type, N/X mutant and ICP0 null HSV viruses in the presence and absence of BAG3. (A) MeWo (closed shapes) or siBAG3 (open shapes) were infected with HSV (squares) or ICP0 null (triangles) at an MOI of 0.001. At the indicated times post infection viruses were harvested and titrated on L7 cells. (B) MeWo (closed shapes) or siBAG3 (open shapes) cells were infected with HSV-17 (squares), ICP0 null mutant (triangles) or N/X, an ICP0 mutant lacking the N-terminal activator sequences, (circles) at an MOI of 0.1. At the indicated times post infection viruses were harvested and titrated on L7 cells. (C) MeWo or siBAG3 were infected with HSV (solid bars) or ICP0 null virus (striped bars) at an MOI of 0.1. At the indicated times post infection viruses were harvested and titrated on L7 cells. The bars represent virus $\frac{\text{yield in siBAG3 cells}}{\text{yield in MeWo cells}}$ for each time point. Error bars depict standard deviation.

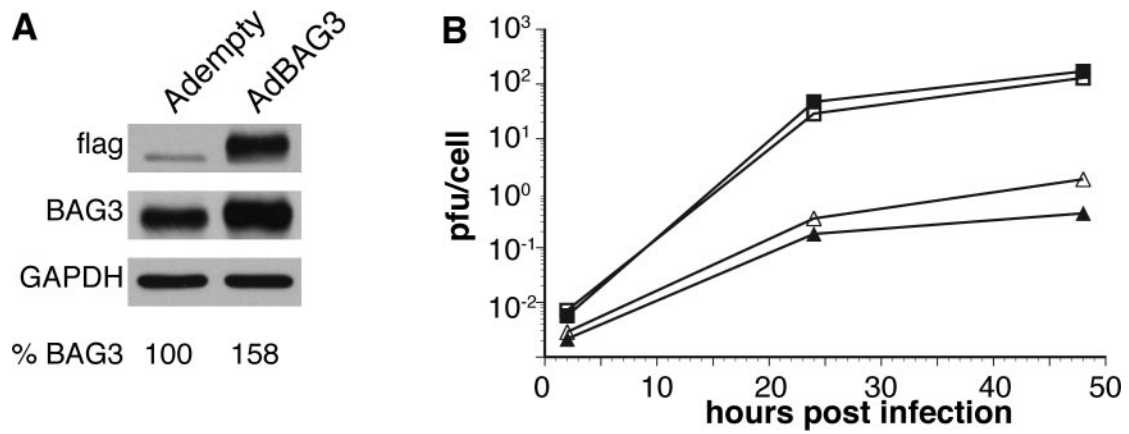


Fig. S4. Growth of wild type and ICP0 null HSV viruses in cells overexpressing BAG3. (A) MeWo cells were infected with either an empty replication deficient or an adenovirus expressing flag-tagged BAG3. Thirty-six hours post infection the cells were harvested and protein levels were determined by Western blotting. Quantification of the signal intensity was performed using NIH ImageJ. (B) MeWo cells were infected with either an empty replication deficient adenovirus (closed shapes) or an adenovirus expressing flag-tagged BAG3 (open shapes). Thirty-six hours post infection the cells were superinfected with either wild type (squares) or ICP0 null (triangles) HSV viruses at an moi of 0.1. At the indicated times post infection viruses were harvested and titrated on L7 cells.

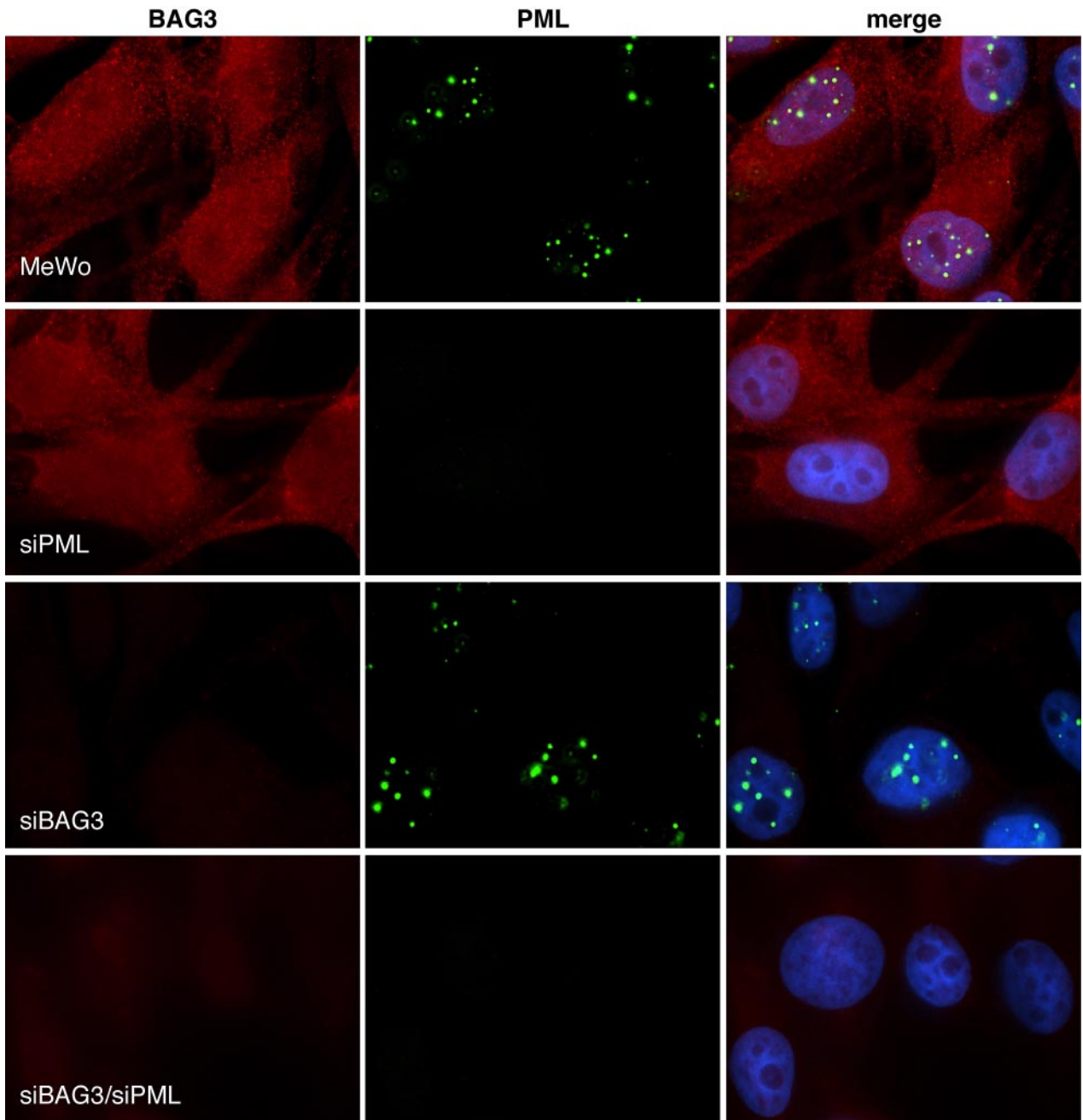


Fig. S5. Localization of BAG3 and PML in the presence or absence of each other. MeWo, siPML, siBAG3 and siPML/siBAG3 cells were fixed and BAG (red) and PML (green) were visualized by indirect immunofluorescence microscopy. Images were captured with a 100x objective and analyzed by volume deconvolution.

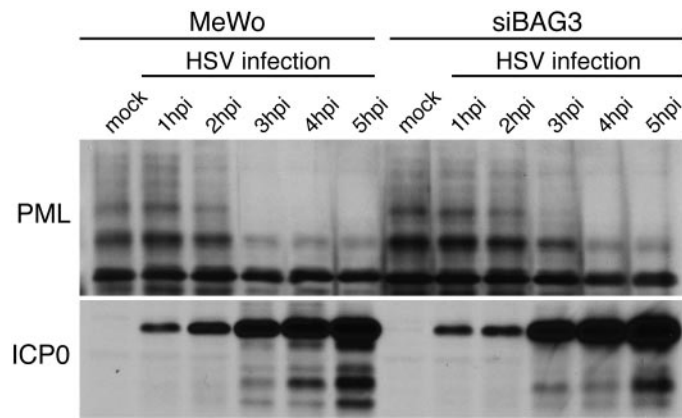


Fig. S6. Degradation of PML in the presence or absence of BAG3. MeWo or siBAG3 cells were either mock treated or infected with wild type HSV at an MOI of 5. At the indicated times post infection the cells were harvested and the indicated proteins were analyzed by Western blotting.