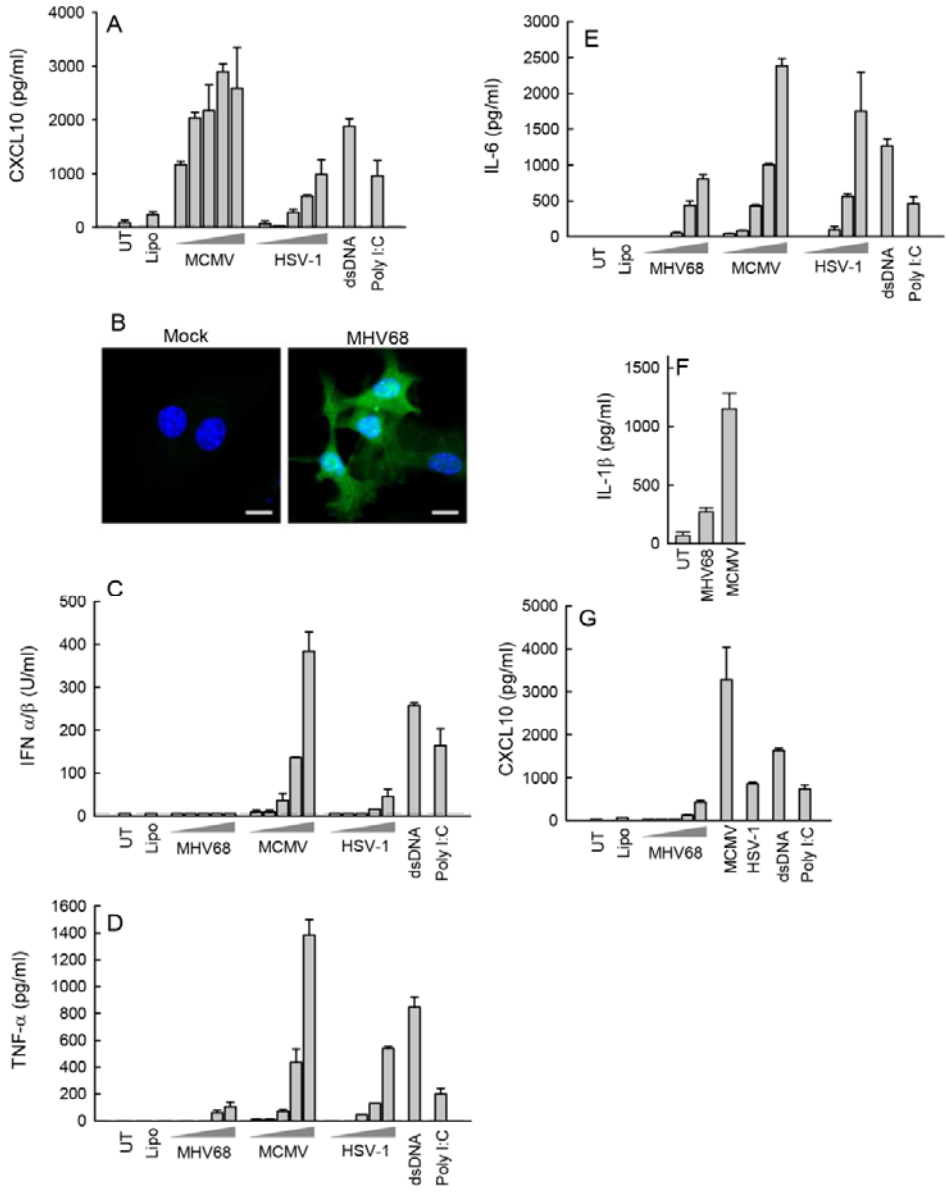
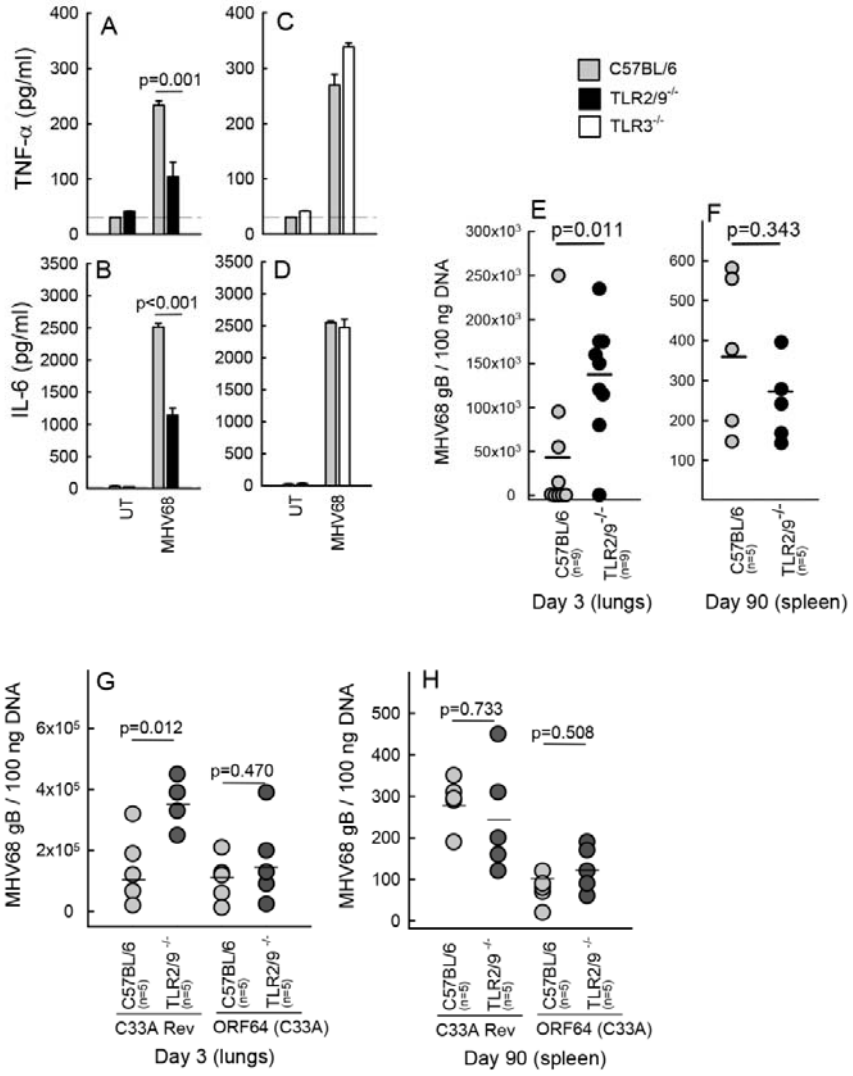
Data supplement

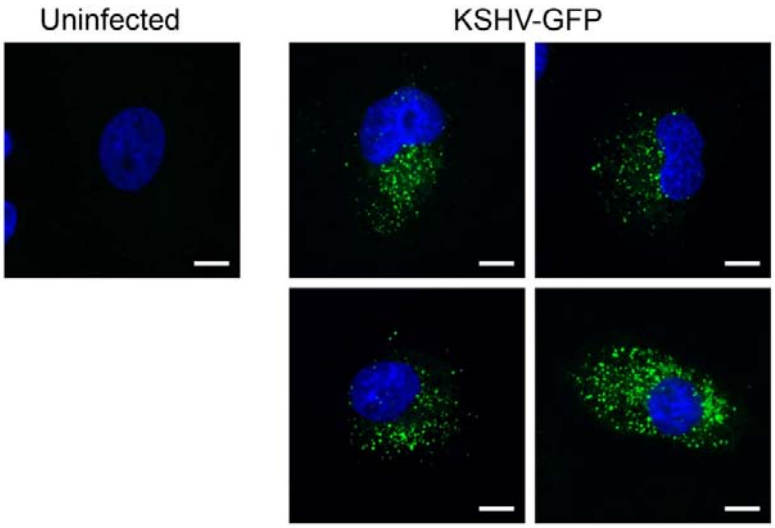
Table of Content



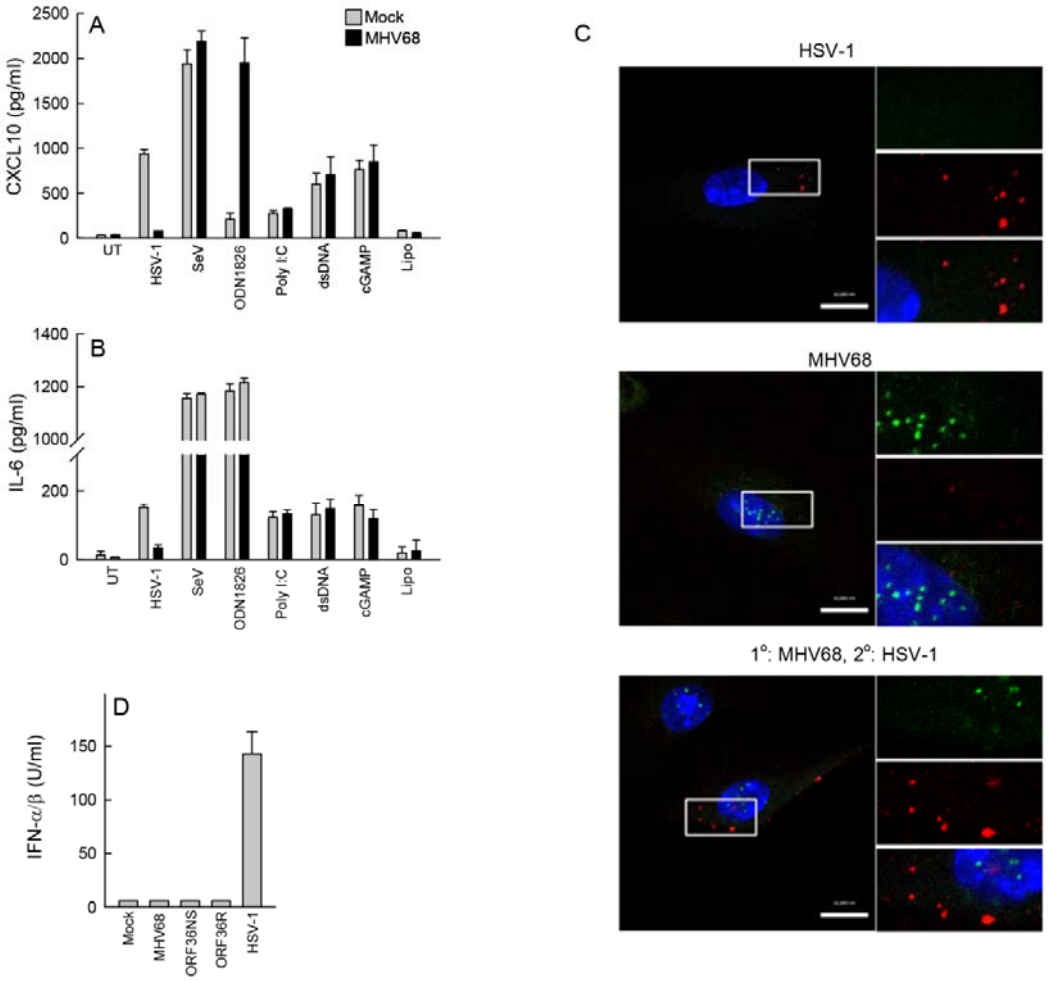
Supplemental Figure 1. MHV68 stimulates a blunted innate immune response. (A) BMDCs from C57BL/6 were infected with MCMV or HSV-1 (both MOI 0.1 - 10) or transfected with dsDNA or polyIC (both 2 μg/ml). Culture supernatants were harvested 24 h post treatment and analyzed for levels of CXCL10 by ELISA. (B) BMDCs were infected with MHV68-GFP at an MOI of 5 for 24 h, and infection was visualized by detection of GFP expression (green). Blue, DAPI. Scale bar: 10 μm. (C-F) BMMs from C57BL/6 were infected with MHV68 (MOI 1 - 100), MCMV or HSV-1 (both MOI 0.1 - 10) or transfected with dsDNA or polyIC (both 2 μg/ml). Culture supernatants were harvested 24 h post treatment and analyzed for levels of (C) type I IFN bioactivity, and protein levels of (D) TNF-α and (E) IL-6. (F) Cells were stimulated with LPS (200 ng/ml) for 3 h prior to treatment with virus. Levels of IL-1β in the culture supernatants (18 h) were measured by ELISA. (G) BMDCs from C57BL/6 were infected with MHV68 (MOI 1 - 100), MCMV or HSV-1 (both MOI 3) or transfected with dsDNA or polyIC (both 2 μg/ml). Culture supernatants were harvested 24 h post treatment and analyzed for levels of CXCL10. Data are presented as means of measurements from triplicate cultures +/- st.dev. Data are representative of two independent experiments.



Supplemental Figure 2. TLR2 and 9 mediate expression of inflammatory cytokines and control acute gammaherpesvirus infection. (A-D) BMDCs from C57BL/6, TLR2/9$^{-/-}$ and TLR3$^{-/-}$ mice were infected with MHV68, MOI 100. Supertantants were harvested 18 h post treatment for measurement of levels of (A, C) TNF-α and (B, D) IL-6 by ELISA. Data are presented as means of measurements from triplicate cultures +/- st.dev. (E-H) C57BL/6 and TLR2/9$^{-/-}$ mice were infected intranasally with 5x10^4 pfu of (E, F) MHV68 or (G, H) MHV68 C33A mutant and revertant virus as indicated. (E, G) Lungs were isolated from mice infected for 3 days and (F, H) spleens were isolated from mice infected for 90 days. Organs were homogenized and analyzed for the presence of MHV68 DNA by PCR. Each data point presented represents one individual mouse.



Supplemental Figure 3. KSHV is capable of infecting PMA-differentiated THP1 cells. PMA-differentiated THP1 cells were infected with KSHV-GFP at an MOI of 20. After 48 hours of infection, the cells were fixed, stained with DAPI and subjected to confocal microscopy. Green, GFP; Blue, DAPI. Scale bar: 10 μm.



Supplemental Figure 4. MHV68 antagonizes herpesvirus-mediated signaling. (A, B) BMDCs from C57BL/6 mice were infected with MHV68 (MOI 10) for 1 h before treatment with HSV-1 (MOI 3), Sendai virus, SeV (MOI 1), ODN1826 (1 μM), polyI:C (2.0 μ/ml), dsDNA (2.0 μ/ml), cGAMP (2.5 μM), or lipofectamine. Supernatants were harvested 20 h post-stimulation and levels of CXCL10 and IL-6 were measured by ELISA. Data are presented as means of measurements from triplicate cultures +/- st.dev. (C) BMMs were left unreated or infected with EdC-MHV68 at an MOI of 20. One hour later, the medium was exchanged and cells were infected with HSV-1 at an OMI of 10. Two hours post HSV infection, the cells were fixed and stained using anti-VP5 (HSV-2 capsid), click-it imaging kit (MHV68 genomes) and DAPI (nuclei). Scale bar, 10 μm. (D) BMDCs from C57BL/6 mice were infected with WT MHV68 the ORF36NS mutant, a revertant virus (all MOI 100) and HSV-1 (MOI 3).