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Supplementary Data

Herpes Simplex Virus Type I Induces an Incomplete Autophagic Response in Human Neuroblastoma Cells

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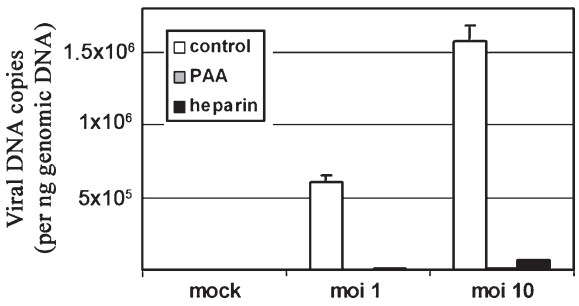

Supplementary Figure 1. Heparin and PAA efficiently inhibit viral DNA replication. Quantification of viral DNA by real-time quantitative PCR for HSV-1-infected SK-N-MC cells at a moi of 1 or 10 pfu/cell for 18 h. The data shown represent the mean ± SD for triplicate samples from one of three independent experiments.

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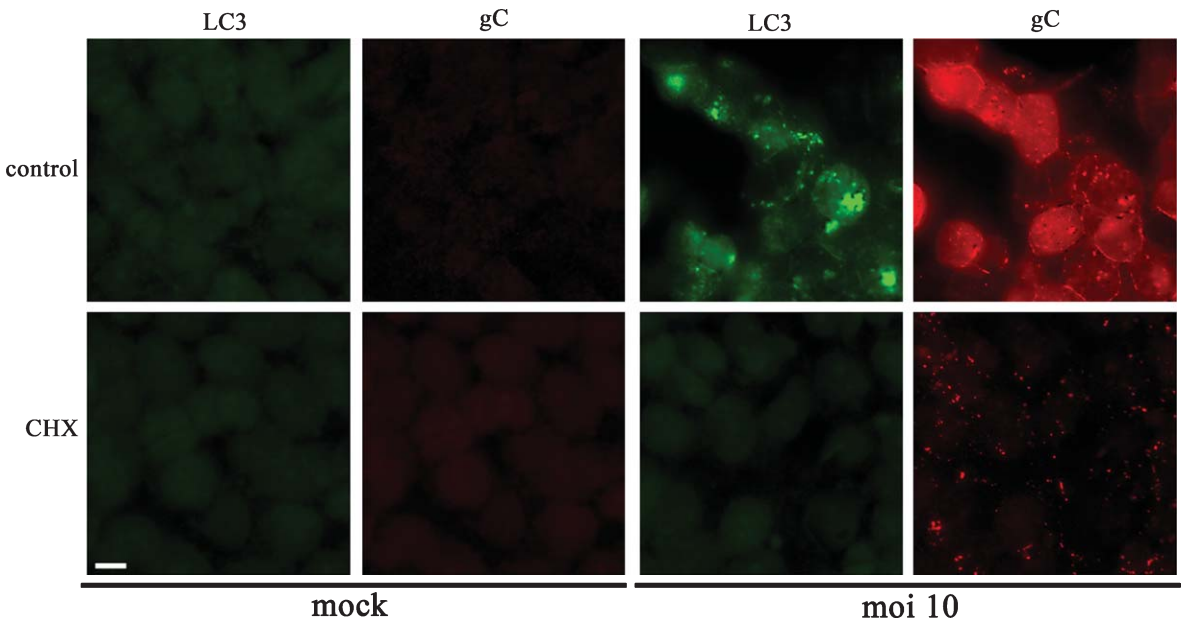
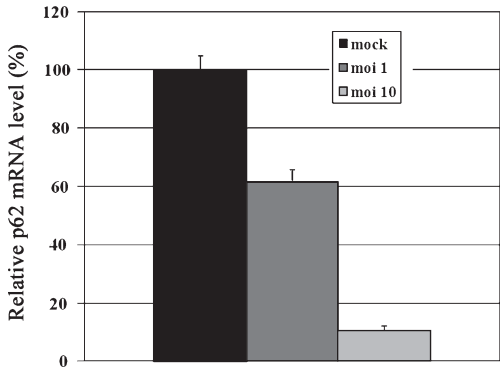
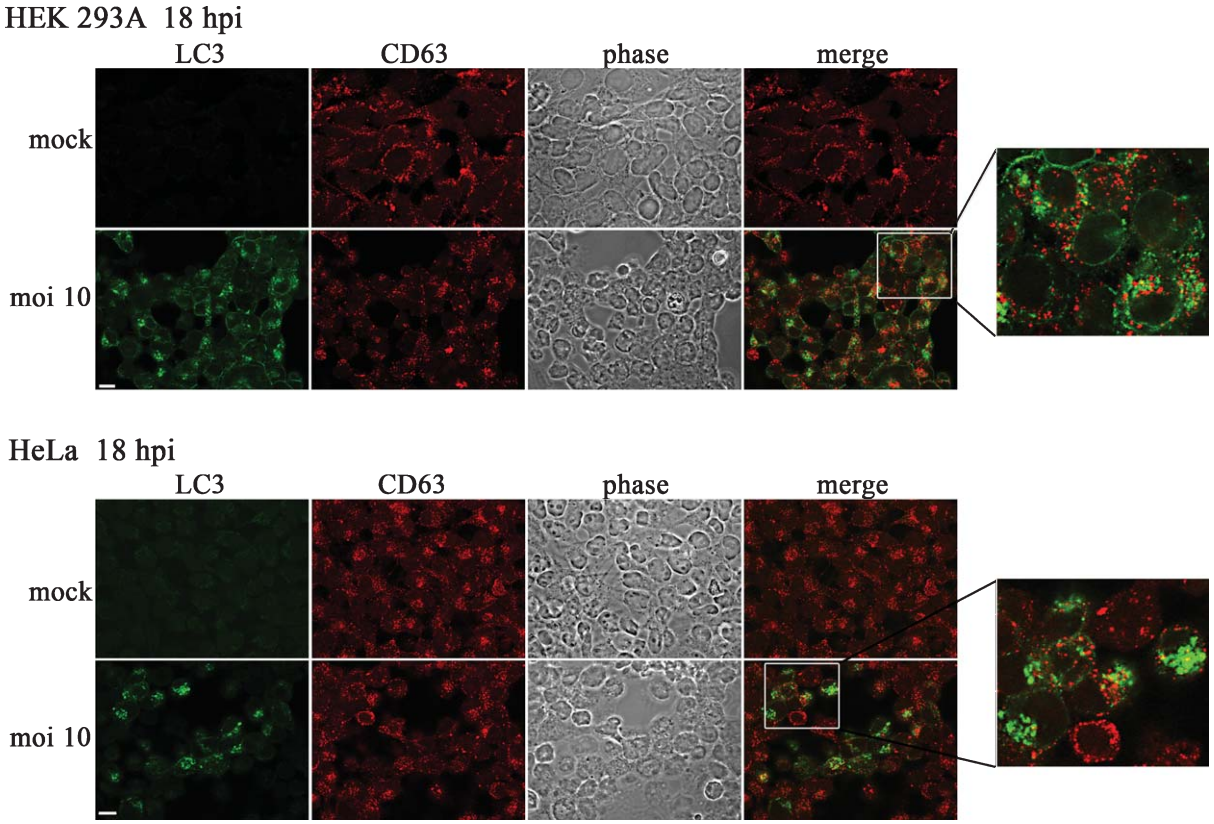


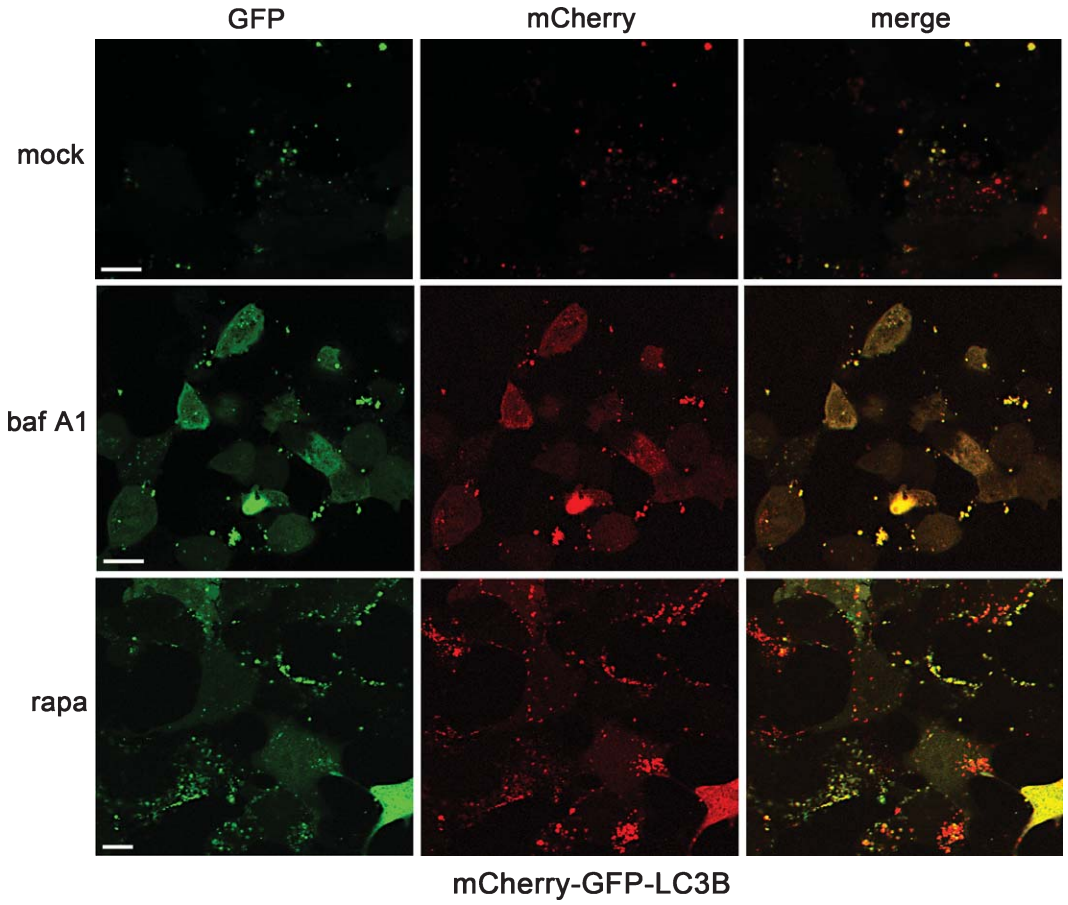
Supplementary Figure 2. Cycloheximide blocks autophagosome accumulation induced by HSV-1. Immunofluorescence analysis of SK-N-MC cells treated with cycloheximide (CHX) and simultaneously exposed to HSV-1 at a moi of 10 pfu/cell for 18 h. Cells were labeled with anti-LC3 and anti-gC antibodies. Exposure to cycloheximide inhibited gC expression indicating that cycloheximide treatment was effective. Original magnification: ×63. Scale bar: 10 μm.



Supplementary Figure 3. HSV-1 infection decreases the p62 mRNA levels. Quantitative RT-PCR of p62 mRNA levels in SK-N-MC cells exposed to HSV-1 at a moi of 1 and 10 pfu/cell for 18 h. The percentages of p62 mRNA levels with respect to non-infected cells are represented. The data shown represent the mean ± SD for triplicate samples from one of three independent experiments.



Supplementary Figure 4. HSV-1 induces autophagosome accumulation in different cell types. HEK 293A (upper panels) and HeLa (lower panels) cells were mock-infected or infected with HSV-1 at a moi of 10 pfu/cell for 18 h and subjected to confocal microscopy analysis employing anti-CD63 and anti-LC3 antibodies. Phase contrast micrographs are also shown. The merged panels combine the CD63 (green) and LC3 (red) signals. The boxed area is enlarged to show the low rate of colocalization between autophagosomes and lysosomes. Original magnification: ×63. Scale bars: 10 μm.



Supplementary Figure 5. Effects of autophagy modulators on autophagosome-lysosome fusion. SK-N-MC cells were transfected with mCherry-GFP-LC3 and then treated with bafilomycin A1 (baf A1) or rapamicin (rapa) for 18 h. Representative images of confocal microscopy are shown and GFP (green) and mCherry (red) channels were merged. Original magnification: $\times 63$. Scale bars: 10 μm.