Supporting Information

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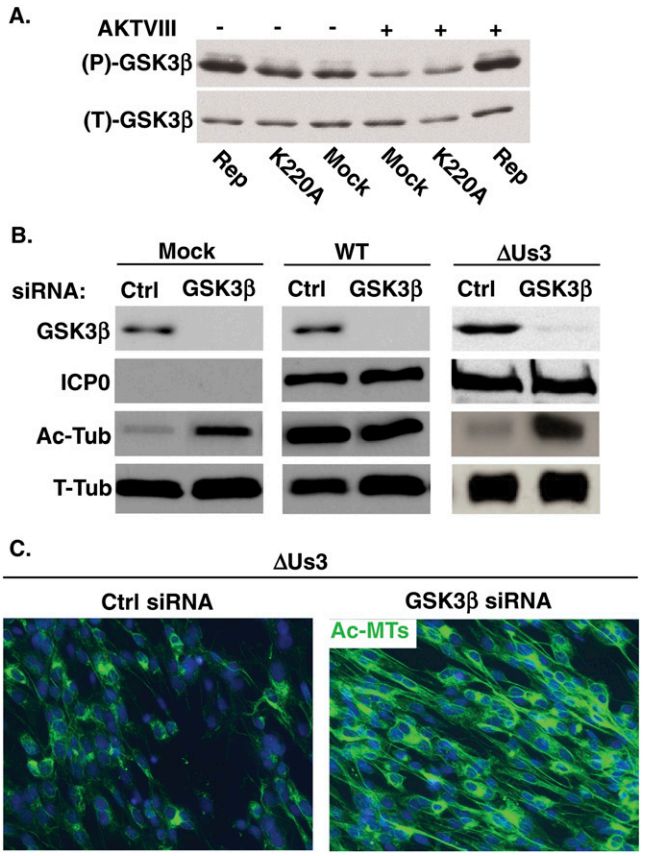

Fig. S1. Us3 inactivates glycogen synthase kinase 3beta (GSK3β) to induce microtubule (MT) stabilization. (*A*) Normal human dermal fibroblasts (NHDFs) were mock-infected or infected at multiplicity of infection (m.o.i.) 10 with the indicated viruses in the presence of DMSO (–) or AKTVIII (+). Whole-cell extracts were analyzed by WB with the indicated antibodies. (P)-GSK3β, Ser9 phosphorylated GSK3β; (T)-GSK3β, total GSK3β. (*B*) NHDFs were treated with Ctrl or GSK3β siRNAs and then mock-infected or infected with herpes simplex virus type 1 (HSV-1) wild-type (WT) or HSV-1 ΔUs3 at m.o.i. 10 for 16 h. Western blot analysis of whole-cell lysates, using the indicated antibodies, demonstrates an increase in Ac-tubulin accumulation on GSK3β depletion in mock-infected or ΔUs3-infected cells but not in cells infected with WT HSV-1. Ac-Tub, acetylated tubulin; T-Tub, total tubulin. (*C*) NHDFs were treated with Ctrl or GSK3β siRNAs and then infected with HSV-1 ΔUs3 at m.o.i. 10 for 16 h. Fixed samples were probed with anti–Ac-Tubulin (green), illustrating the enhanced formation of stable MTs in GSK3β-depleted cultures. Nuclei were stained using Hoechst 33342.

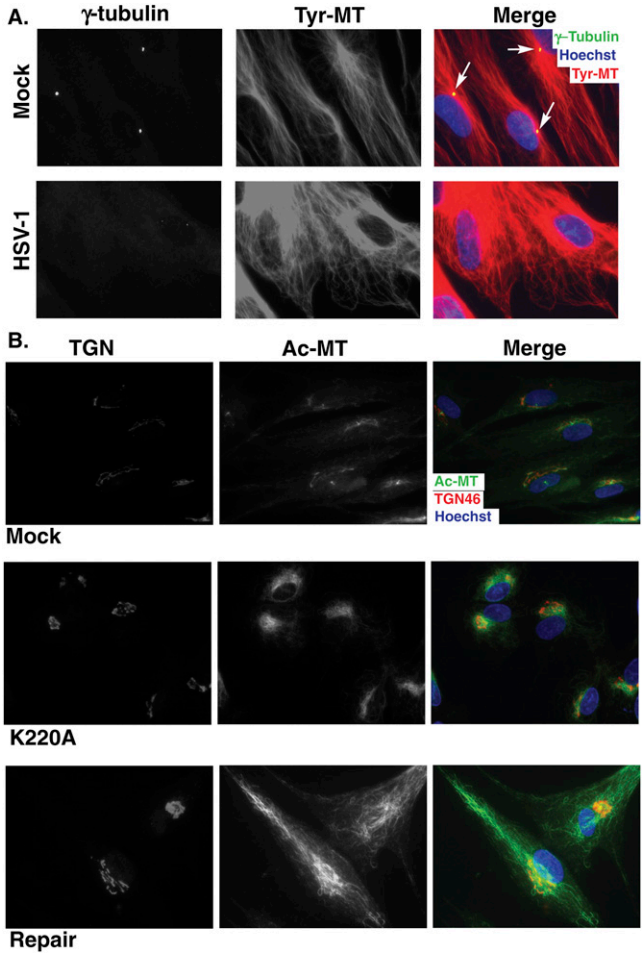

Fig. S2. HSV-1 infection disrupts the centrosome. NHDFs were mock-infected or infected with the indicated viruses at m.o.i. 10 for 9 h. (*A*) Fixed samples were stained for Tyr-MTs (red) and γ-tubulin (green). Arrows point to centrosomes. (*B*) Fixed samples were stained for the trans-Golgi network marker TGN46 (red) and Ac-MTs (green). Ac-MTs cluster proximal to the TGN in cells infected with Us3 mutant virus.

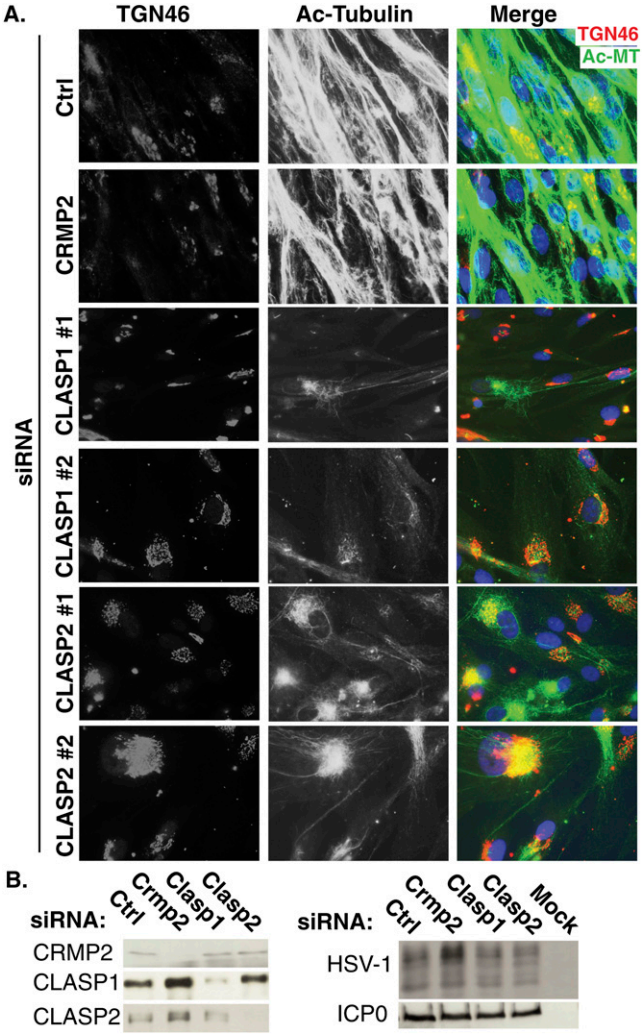

Fig. S3. Cytoplasmic linker-associated protein 1 (CLASP1) and CLASP2 are required for stable MT formation in HSV-1-infected cells. NHDFs were treated with Ctrl, collapsin response-mediating protein 2 (CRMP2), CLASP1, or CLASP2 siRNAs and then infected at m.o.i. 10 for 16 h. #1 and #2 indicate independent-targeting siRNAs. (*A*) Fixed samples were stained for TGN46 (red) and Ac-tubulin (green). Acquisition settings to image CLASP-depleted samples frequently results in overexposed images for Ctrl and CRMP2 samples as a result of high levels of Ac-MT staining. (*B*) WB analysis of whole-cell extracts demonstrates depletion of CLASP1, CLASP2, and CRMP2 in infected samples and levels of infected cell protein ICP0 (early) and HSV-1 virion (late structural) proteins in each sample.

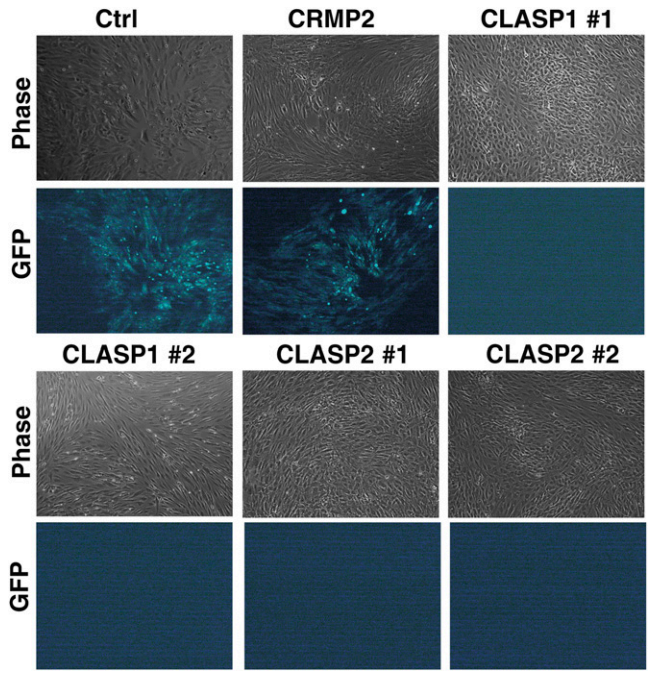

Fig. S4. CLASP1 or CLASP2 silencing suppresses HSV-1 spread and plaque formation. NHDFs were treated with Ctrl, CRMP2, CLASP1, or CLASP2 siRNAs and then infected with HSV-1-GFP-Us11 at m.o.i. 0.0005 for 3 d. Representative cytopathic effect and plaque sizes are shown in phase and fluorescent (GFP) images.