Supporting Information

Jaworska et al. 10.1073/pnas.0909951107

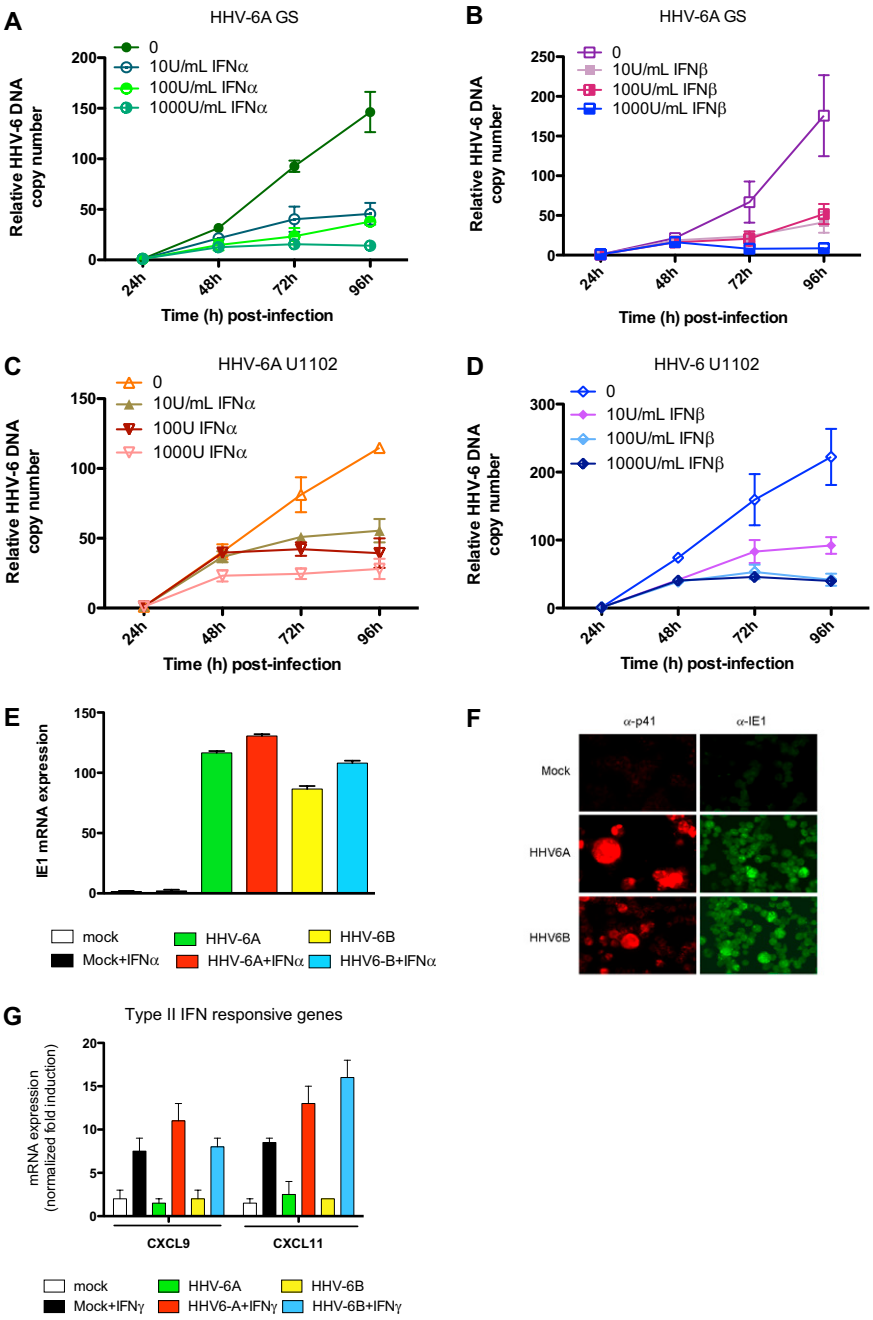

Fig. S1. (*A–D*) Peripheral blood mononuclear cells (PBMC) were infected with HHV-6A GS (*A* and *B*) or U1102 (*C* and *D*) for 24 h at a multiplicity of infection (m.o.i.) of 0.1. The next day, IFN-α (*A*, *C*) or IFN-β (*B*, *D*) was added (10–1,000 U/mL) and viral DNA was isolated every day for 4 d. Viral DNA was assessed by qPCR. Results are expressed as mean (triplicate) ± SDs of HHV-6 DNA copy number after normalization with GAPDH. (*E*) One-day-old infected SupT1 cells ± IFNα were analyzed for immediate-early 1 (IE1) expression by RT-qPCR to demonstrate similar efficiencies of infection by both HHV-6 variants. Results are expressed as mean (triplicate) ± SD. IE1 mRNA expression after normalization of samples with GAPDH. Results are representative of two independent experiments. (*F*) Infected cells (as in *E*) were analyzed for IE1 and p41 protein expression by immunofluorescence using specific anti-IE1 and anti-p41 antibodies. Images were acquired on a BX 51 Olympus microscope. (*G*) SupT1 cells were mock-infected or infected with HHV-6 at a m.o.i. of 1. At 48 h postinfection, exogenous IFN-γ (50 ng/mL) was added to the culture medium and 16 h later levels of mRNA transcripts for CXCL9 and CXCL11 genes were assessed by RT-qPCR. Results are expressed as mean (triplicate) inductions (*n*-fold) ± SD relative to mock-infected and nonstimulated cells after normalization of samples with GAPDH expression. Results are representative of two independent experiments.

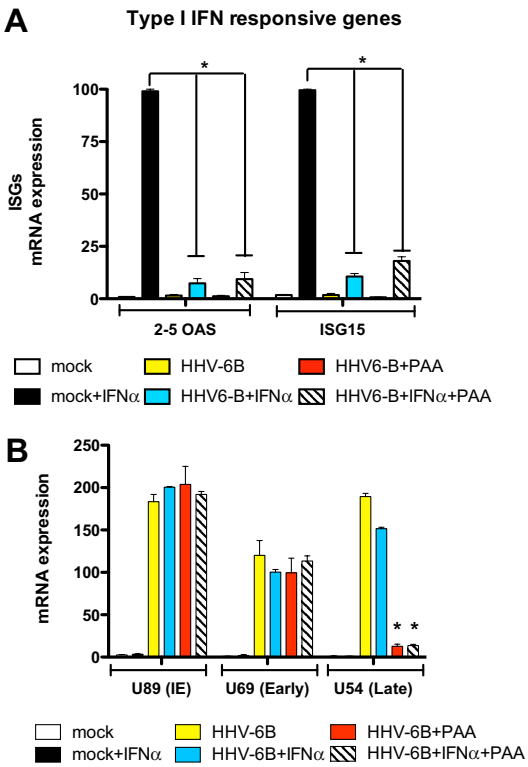

Fig. S2. (*A*) SupT1 cells were infected with HHV-6B (m.o.i. = 1) and 5 h postinfection cells were stimulated with IFN-α in the absence or presence of phosphonoacetic acidn (PAA). After 24 h, total RNA was isolated and analyzed for ISG mRNA by RT-qPCR. All samples were normalized by using GAPDH housekeeping gene expression. The values represent the average of two independent experiments with variability shown by the error bars. (*B*) IE, E, and L gene expression in 1-day-old HHV-6B-infected and IFN-α-stimulated SupT1 cells in the absence or presence of phosphonoacetic acid as determined by RT-qPCR. Results are expressed as mean (triplicate) mRNA expression ± SD and are representative of two separate experiments. *, $P < 0.01$.

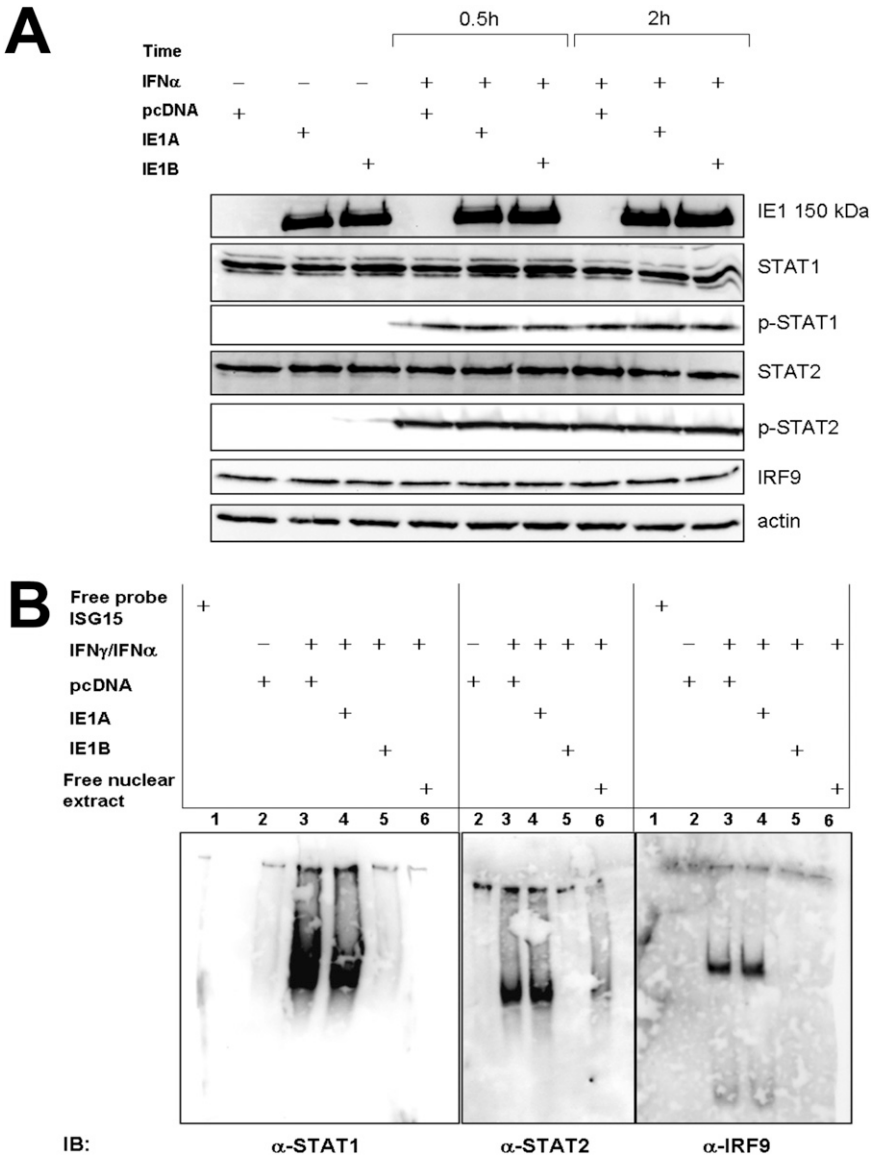

Fig. S3. (*A*) 293T cells were transfected with a control or IE1-expression vectors, followed 48 h later by IFN-α (1,500 U/mL) stimulation. At 0.5 and 2 h after IFN stimulation, cells were collected and protein levels were determined by Western blot using specific antibodies against IE1, STAT1, phospho (P)-STAT1, STAT2, P-STAT2, IRF9, and actin. (*B*) Western blots of mobility-shift gels. Nuclear extracts were reacted with the ISG15 probe, subjected to nondenaturing polyacrylamide gel electrophoresis, and subsequently transferred to nitrocellulose. After incubation of the blots with antisera specific to the ISGF3 components STAT1, STAT2, and IRF9 (p48), the positions of stained bands were determined with control lanes that had been processed for autoradiography. Nuclear extracts free of ISG15 probe were used as negative controls.

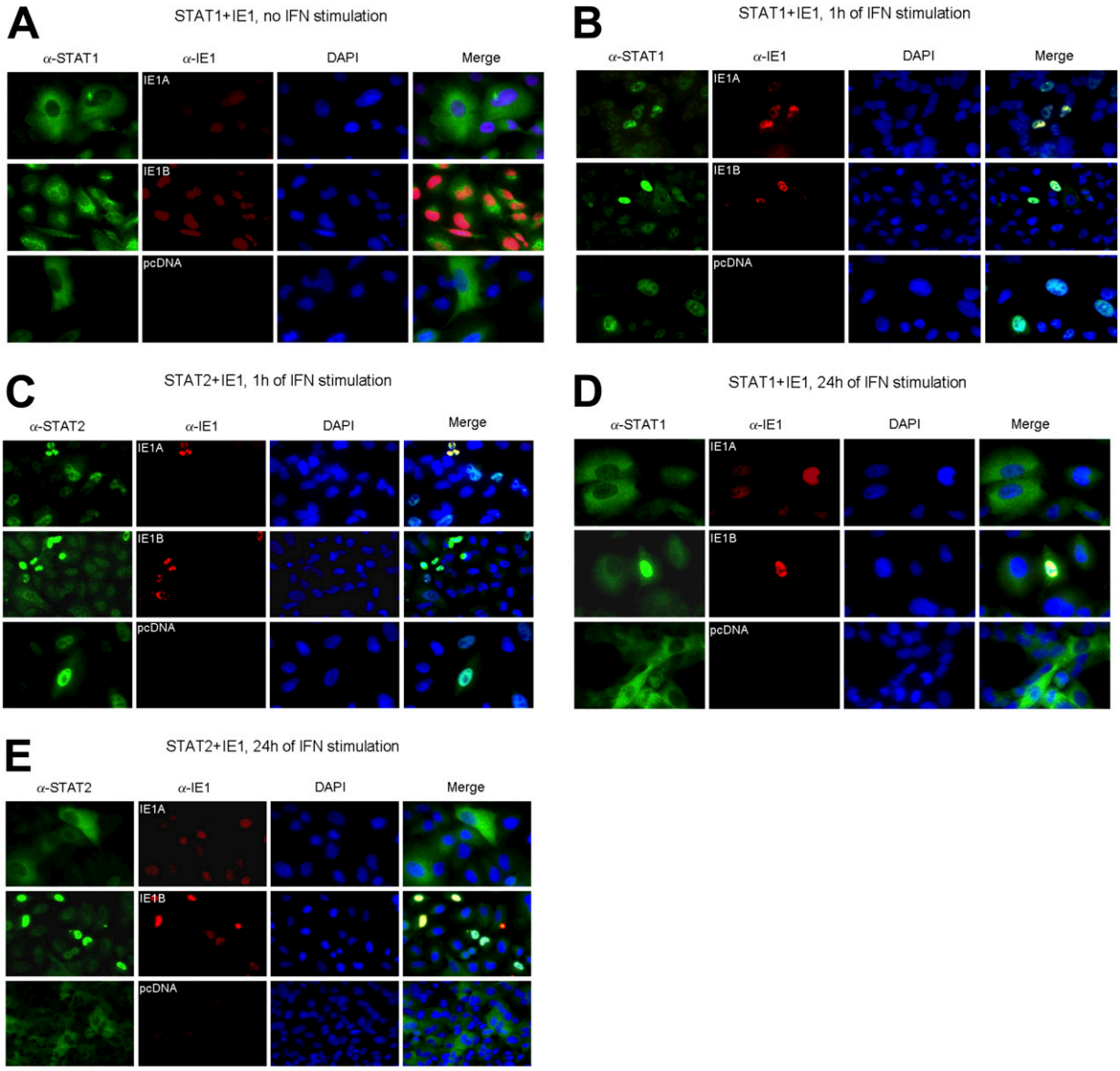

Fig. S4. A549 cells were nucleofected with pcDNA, IE1A, or IE1B expression vectors. Forty-eight hours posttransfection, cells were either left unstimulated (*A*), stimulated with IFN-α for 1 h (*B* and *C*), or stimulated with IFN-α for 24 h (*D* and *E*) before being analyzed by immunofluorescence for STAT1 [FITC-conjugated secondary antibody (green)] (*A*, *B*, *D*), STAT2 [FITC-conjugated secondary antibody (green)] (*C* and *E*), or IE1 [anti-IE1 directly conjugated with Alexa 568 (red)] (all panels) expression. Nuclei were stained with DAPI (blue). The localization of the proteins relative to the nucleus is presents in the merged pictures. Results are representative of three independent experiments.

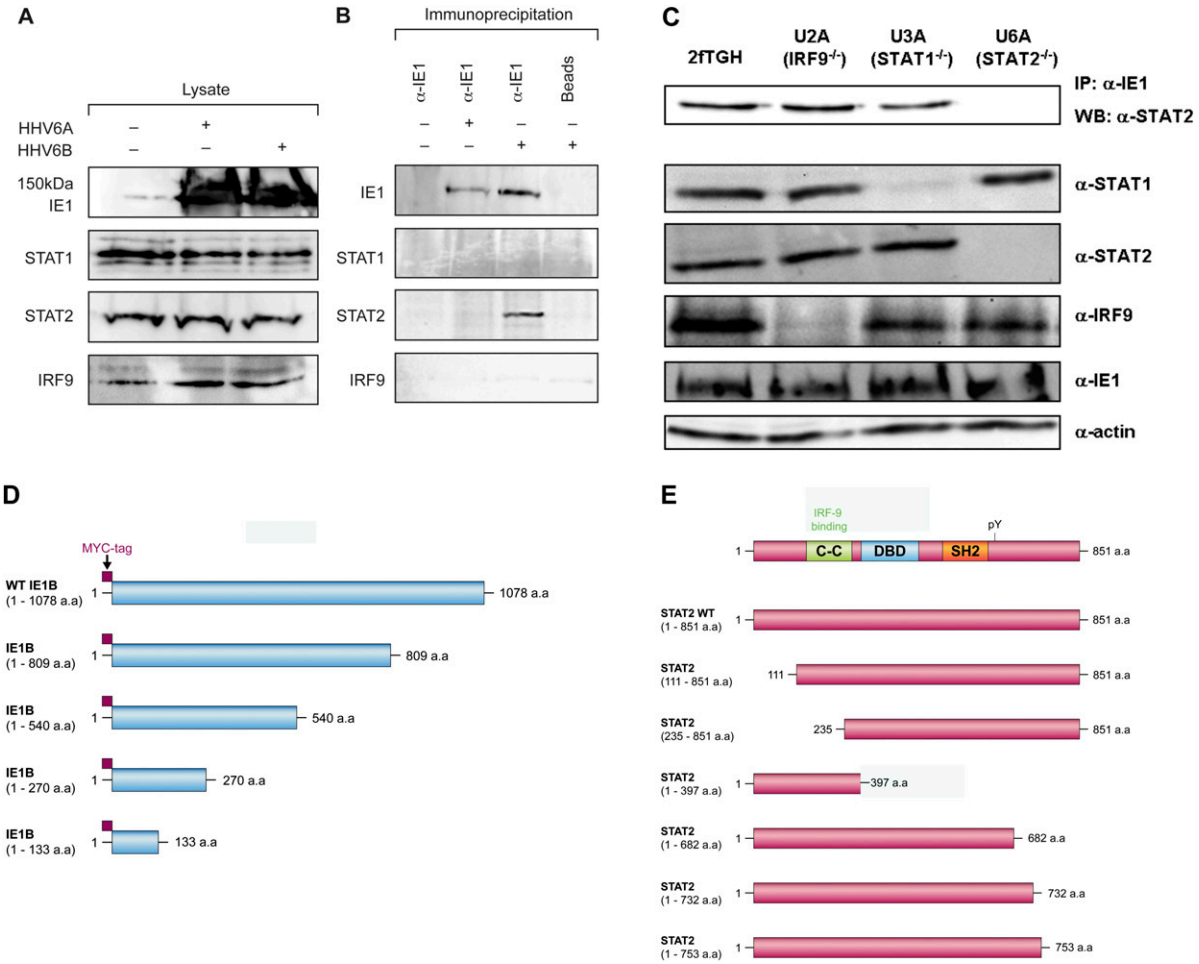

Fig. S5. (*A*) SupT1 cells were mock-infected or infected with HHV-6A or HHV-6B (m.o.i. = 1). Two days later, cells were harvested and lysed in RIPA buffer. Whole-cell extracts from control and HHV-6-infected cells were separated in 10% SDS/PAGE and relevant proteins detected by Western blot with antibodies, as indicated. Results are representative of two independent experiments. +, present; –, absent. (*B*) Whole-cell extracts were used for immunoprecipitation (IP) using anti-IE1 antibodies or empty Sepharose beads as control. Immunoprecipitates were resolved by SDS/PAGE followed by Western blot analysis using specific α-IE1, α-STAT1, α-STAT2, and α-IRF9 antibodies. (*C*) 2fTGH, U2A, U3A, and U6A cells were transfected with IE1B expression vector, followed 48 h later by IP using α-IE1 antibodies and Western blot analyses for STAT2. Lower panels show expression of indicated proteins in whole cell extracts as determined by Western blot. (*D*) Schematic representation of myc-tagged IE1B mutants generated. (*E*) Schematic representation of GFP-tagged STAT2 mutants. Various domains and transcription binding site are presented.

Table S1. Primers, probes, and shRNA used in this study

	Forward primer	Reverse primer	Probe
ISG15	5′-CATGGGCTGGGACCTGACG-3′	5′-CGCCAATCTTCTGGGTGATCTG-3′	N/A
ADAR-1	5′-TGCTGCTGAATTCAAGTTGG-3′	5′-TCGTTCTCCCCAATCAAGAC-3′	N/A
2′–5′ OAS	5′-CAAGCTCAAGAGCCTCATCC-3′	5′-TGGGCTGTGTTGAAATGTGT-3′	N/A
IRF7	5′-TACCATCTACCTGGGCTTCG-3′	5′-GCTCCATAAGGAAGCACTCG-3′	N/A
CXCL9	5′-GCATCATCTTGCTGGTTCTG-3′	5′-TAGGTGGATAGTCCCTTGG-3′	N/A
CXCL11	5′-CTTGGCTGTGATATTGTGTGC-3′	5′-GGGTACATTATGGAGGCTTTC-3′	N/A
GAPDH	5′-CGAGATCCCTCCAAAATCAA-3′	5′-TTCACACCCATGACGAACAT-3′	5′-JOE-TGGAGAAGGCT-GGGGCTCAT-BHQ1-3′
HHV-6 U54	5′-CGACCGTGGTTAGACTTGGT-3′	5′-CGCATCAATTACTCGCAAGA-3′	N/A
HHV-6 U65/66	5′-GACAATCACATGCCTGGATAATG-3′	5′-TGTAAGCGTGTGGTAATGGACTAA-3′	5′-FAM-AGCAGCTGGCGAA-AAGTGCTGTGC-BHQ1-3′
HHV-6 U69	5′-AGACTCGCCGATGAAACTGT-3′	5′-GCTTTGCACGTCAAACAAAA-3′	N/A
HHV-6 U89	5′-GGCGGTGTCT(G/C)AATTTGCATC-3′	5′-CA(C/T)TGGATCGGGA(C/T)GGTAGT(C/T)TT-3′	5′-FAM-ACCCTCTGGAA-ACAACATGG(A/G)ATCCAA-BHQ1-3′
shIE1A	5′-GATCCCCTTGGTGCGAACCTAGATTCTTCAAGAGAGAATCTAGGTTCGCACCAATTTTTGGAAA-3′		
shIE1B	5′-GATCCCCGGCCCTGATAACTTTAAATTTCAAGAGAATTTAAAGTTATCAGGGCCTTTTTA-3′		