Supporting Information

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SI Materials and Methods

Antibodies used in Western blot experiments were mouse anti-IFI16 (ab55328; 1:2,000; Abcam), mouse anti-GAPDH (G041; 1:5,000; Applied Biological Materials), rabbit anti-TMEM173 (ab92650; 1:2,000; Abcam), mouse anti-ICP0 (1:1,000; EastCoast Bio), mouse anti–β-tubulin (clone JDR.3B8; 1:2,000; Sigma-Aldrich), mouse anti-Myc (9E10; 1:2,000; Santa Cruz Biotechnology), mouse anti-large T antigen (pab419; 1:50) (1). HRP-conjugated goat antibodies were used at 1:5,000 to 1:20,000 (Santa Cruz Biotechnology).

Antibodies used for indirect immunofluorescence studies were mouse anti-IFI16 (ab55328; 1:200; Abcam), rabbit anti-ICP8 (3-83; 1:500) (2), rabbit anti-PML (1:100; Santa Cruz Biotechnology), and mouse anti-ICP4 (39S; 1:200) (3). Goat anti-mouse Alexa-488 (Jackson ImmunoResearch) and anti-rabbit Alexa 594 (Jackson ImmunoResearch) were used at 1:500 for secondary detection.

1. Harlow E, Crawford LV, Pim DC, Williamson NM (1981) Monoclonal antibodies specific for simian virus 40 tumor antigens. *J Virol* 39(3):861–869.
2. Knipe DM, Senechek D, Rice SA, Smith JL (1987) Stages in the nuclear association of the herpes simplex virus transcriptional activator protein ICP4. *J Virol* 61(2):276–284.
3. Showalter SD, Zweig M, Hampar B (1981) Monoclonal antibodies to herpes simplex virus type 1 proteins, including the immediate-early protein ICP 4. *Infect Immun* 34(3): 684–692.

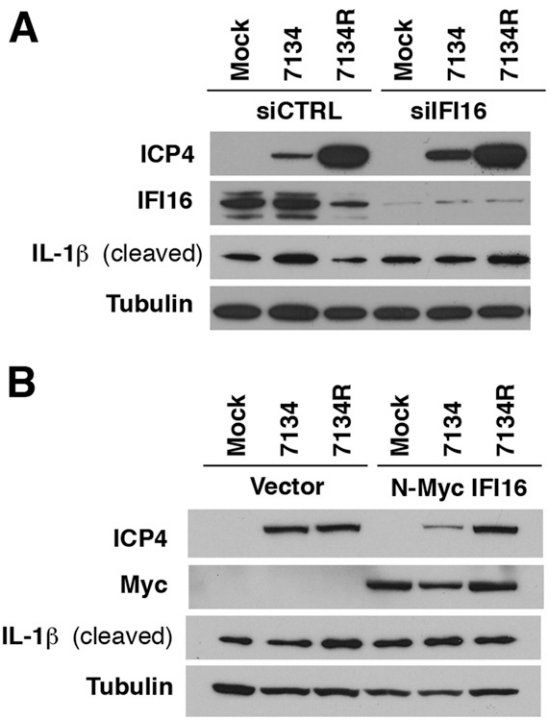

Fig. S1. Depletion or overexpression of IFI16 does not result in increased IL-1β cleavage. (*A*) Nontargeting control siRNA (siControl)- or siRNA specific for *IFI16* (siIFI16)-transfected human foreskin fibroblasts (HFFs) were infected with the 7134 virus and its corresponding rescued virus (7134R) at a multiplicity of infection (MOI) of 1. Protein lysates were examined for IL-1β cleavage at 6 h postinfection (hpi). IFI16 and ICP4 protein levels were examined to confirm knockdown efficiency and restrictive phenotype. (*B*) U2OS cells transfected with a vector or Myc-IFI16 construct were infected with the 7134 and 7134R viruses at MOI of 0.1. Lysates were harvested at 6 hpi and analyzed for the indicated proteins. Tubulin was used as a control for protein loading.

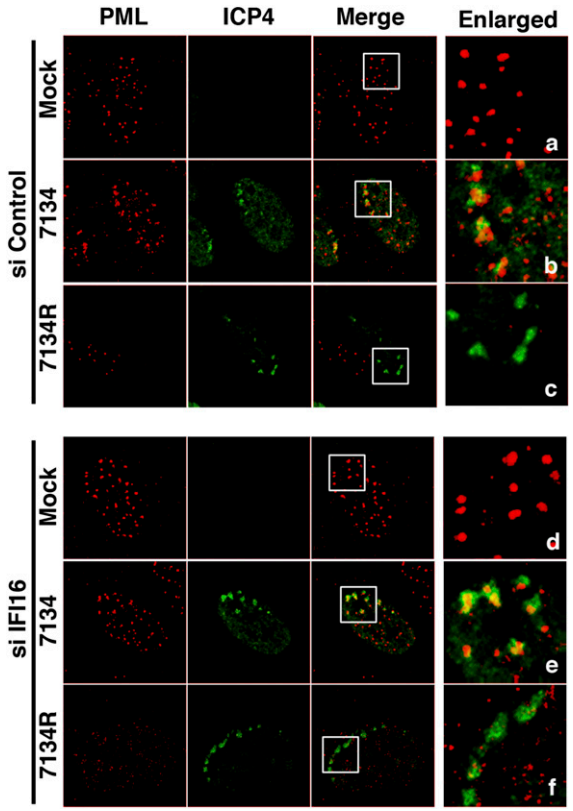

Fig. S2. ND10 component PML is recruited to sites associated with viral genomes in IFI16-depleted cells. HFFs were treated with indicated siRNA for 72 h before infection with an ICP0-null (7134) or rescued virus (7134R) at an MOI of 1 or 0.001, respectively. Cells were fixed and simultaneously stained at 24 hpi with mouse anti-ICP4 and rabbit anti-PML antibodies, followed by Alexa Fluor 488-conjugated goat anti-mouse and Alexa Fluor 594-conjugated goat-anti rabbit secondary antibodies. Representative immunofluorescence images of nontargeting control or IFI16 siRNA transfected HFFs infected with HSV-1 are shown.

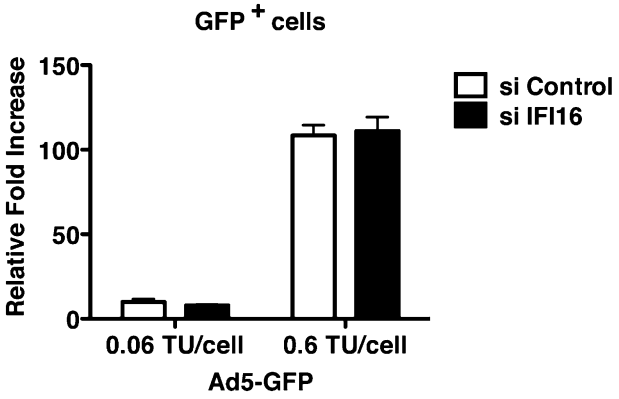

Fig. S3. Depletion of IFI16 does not affect GFP expression from an Ad5 vector. IFI16 siRNA-treated cells were mock-infected or infected with Ad5-GFP at 0.6 or 0.06 transducing units (TU) per cell. GFP^+ cells were determined by flow cytometry at 24 hpi and normalized to mock-infected cells.

Table S1. Primer sequences

Target	Purpose	Forward	Reverse
hISG54	qRT-PCR	5′-ACGGTATGCTTGGA ACGATTG-3′	5′-AACCCAGAGTGT GGCTGATG-3′
h18S RNA	qRT-PCR	5′-GCATTCGTATTGCG CCGCTA-3′	5′-AGCTGCCCGGC GGGT-3′
IFI16	qRT-PCR	5′-ACTGAGTACAACAA AGCCATTTGA-3′	5′-TTGTGACATTGTC CTGTCCCCAC-3′
STING	qRT-PCR	5′-CCTGAGCAGAACA ACTGC-3′	5′-GGTCTTCAAGCT GCCCACAGT-3′
ICP4	qRT-PCR	5′GCGTCGTCGAGGT CGT-3′	5′-CGCGGAGACGGA GGAG-3′
ICP27	qRT-PCR	5′-GCATCCTTCGTGTT TGTCATT-3′	5′-GCATCTTCTCTCC GACCCCG-3′
ICP8 promoter	qPCR	5′-GCCCGGGCGCTGC TTGTTCTCC-3′	5′-CGTCCGCCGTCG CAGCCGTATC-3′
ICP4 promoter	qPCR	5′-GCCGTCGACGCGG AACT-3′	5′-CCTTTTTCCCACC CAAGCAT-3′
ICP27 promoter	qPCR	5′-CCGCCGGCCTGGA TGTGACG-3′	5′-CGTGGTGGCCGG GGTGGTGCTC-3
GAPDH	qPCR	5′-TTCGACAGTCAGC CGCATCTTCTT-3′	5′-CAGGCGCCCAAT ACGACCAAATC-3′
γ-actin	qPCR	5′-CACCGCCGCATC CTCCTCTTC-3′	5′-GTGGTGCCGCCC GACAGC-3′

qPCR, quantitative PCR.