AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology®

VIRUS-CELL INTERACTIONS

Herpes Simplex Virus 1 UL36USP Antagonizes Type I Interferon-Mediated Antiviral Innate Immunity

Hui Yuan,[a,c] Jia You,[b] Hongjuan You,[d] Chunfu Zheng[a,e]

[a]Department of Immunology, School of Basic Medical Sciences, Fujian Medical University, Fuzhou, China
[b]The First Affiliated Hospital of Fujian Medical University, Fuzhou, China
[c]Center of Clinical Laboratory, The Fifth People's Hospital of Wuxi, Affiliated with Jiangnan University, Wuxi, China
[d]Jiangsu Key Laboratory of Immunity and Metabolism, Department of Pathogenic Biology and Immunology, Xuzhou Medical University, Xuzhou, China
[e]Department of Microbiology, Immunology and Infectious Diseases, University of Calgary, Calgary, AB, Canada

ABSTRACT Type I interferons (IFNs), as major components of the innate immune system, play a vital role in host resistance to a variety of pathogens. Canonical signaling mediated by type I IFNs activates the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway through binding to the IFN-α/β receptor (IFNAR), resulting in transcription of IFN-stimulated genes (ISGs). However, viruses have evolved multiple strategies to evade this process. Here, we report that herpes simplex virus 1 (HSV-1) ubiquitin-specific protease (UL36USP) abrogates the type I IFN-mediated signaling pathway independent of its deubiquitinase (DUB) activity. In this study, ectopically expressed UL36USP inhibited IFN-β-induced activation of ISRE promoter and transcription of ISGs, and overexpression of UL36USP lacking DUB activity did not influence this effect. Furthermore, UL36USP was demonstrated to antagonize IFN-β-induced activation of JAKs and STATs via specifically binding to the IFNAR2 subunit and blocking the interaction between JAK1 and IFNAR2. More importantly, knockdown of HSV-1 UL36USP restored the formation of JAK1-IFNAR2 complex. These findings underline the roles of UL36USP-IFNAR2 interaction in counteracting the type I IFN-mediated signaling pathway and reveal a novel evasion mechanism of antiviral innate immunity by HSV-1.

IMPORTANCE Type I IFNs mediate transcription of numerous antiviral genes, creating a remarkable antiviral state in the host. Viruses have evolved various mechanisms to evade this response. Our results indicated that HSV-1 encodes a ubiquitin-specific protease (UL36USP) as an antagonist to subvert type I IFN-mediated signaling. UL36USP was identified to significantly inhibit IFN-β-induced signaling independent of its deubiquitinase (DUB) activity. The underlying mechanism of UL36USP antagonizing type I IFN-mediated signaling was to specifically bind with IFNAR2 and disassociate JAK1 from IFNAR2. For the first time, we identify UL36USP as a crucial suppressor for HSV-1 to evade type I IFN-mediated signaling. Our findings also provide new insights into the innate immune evasion by HSV-1 and will facilitate our understanding of host-virus interplay.

KEYWORDS HSV-1, UL36USP, IFNAR2, type I IFN-mediated signaling

Type I interferons (IFNs) are a group of secreted cytokines induced immediately upon viral infection. These cytokines are an extremely powerful tool for the host to resist virus invasion and replication. Type I IFNs are a large family that consists of more than 13 different IFN-α subtypes and single subtypes of IFN-β, IFN-ω, IFN-κ, IFN-ε, IFN-ζ, IFN-δ, and IFN-τ (1, 2). The signaling induced by type I IFNs emanates from a heterodi-

Received 4 July 2018 **Accepted** 9 July 2018
Accepted manuscript posted online 11 July 2018
Citation Yuan H, You J, You H, Zheng C. 2018. Herpes simplex virus 1 UL36USP antagonizes type I interferon-mediated antiviral innate immunity. J Virol 92:e01161-18. https://doi.org/10.1128/JVI.01161-18.
Editor Rozanne M. Sandri-Goldin, University of California, Irvine

Address correspondence to Chunfu Zheng, zheng.alan@hotmail.com.
H.Y. and J.Y. contributed equally to this article.

meric receptor complex, known as the IFN-α/β receptor (IFNAR), which appears to be ubiquitously expressed on all cell lines (3). The IFNAR is composed of two subunits, IFNAR1 and IFNAR2, which will dimerize upon binding of type I IFNs (4, 5). Once infected by virus, all type I IFN family members are induced and bind to IFNAR at the cell surface, leading to the dimerization of IFNAR1 and IFNAR2. Subsequently, the tyrosine kinases, including Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2), which previously were associated with IFNAR2 and IFNAR1, respectively, are activated and transphosphorylated with each other. In the canonical pathway, the phosphorylated JAK1 and TYK2 then phosphorylate the tyrosine residues of receptors on the cytoplasmic side, causing the recruitment and phosphorylation of signal transducers and activators of transcription 1 and 2 (STAT1 and STAT2). Once phosphorylated, STAT1 and STAT2 disassociate from the receptor and heterodimerize, further recruiting interferon regulatory factor 9 (IRF9) and forming IFN-stimulated gene factor 3 (ISGF3) complex. ISGF3 then translocates into the nucleus and promotes transcription of genes by binding to interferon-stimulated response elements (ISRE) of IFN-stimulated genes (ISGs) (6, 7). Consequently, these ISG-encoded proteins act directly at different stages of the viral life cycle, establishing a remarkable antiviral state for the host to combat various pathogens (8).

While the signaling cascade mediated by type I IFNs, also called the JAK-STAT pathway, confers on the host antiviral ability, viruses in turn can employ various strategies to circumvent type I IFN-mediated signaling for effective infection and replication in the host. So far, multiple viruses have been reported to antagonize type I IFN-mediated signaling at different levels, such as blocking IFN binding to receptors, targeting essential molecules involved in JAK-STAT signaling, and blocking ISGF3 formation and nuclear translocation (9).

Herpes simplex virus 1 (HSV-1) is characterized as a typical member of the alphaherpesvirus subfamily, with a large double-stranded DNA that encodes over 80 viral proteins (10, 11). Previous studies provide evidence that HSV-1 is capable of modulating type I IFN-mediated signaling. Several proteins encoded by HSV-1 have been discovered to exert different inhibitory effects on type I IFN response. HSV-1 protein ICP27 inhibits type I IFN-mediated signaling at or before JAK1 phosphorylation (12). Moreover, HSV-1 downregulates the protein levels of JAK1 and STAT2 through the virion host shutoff (vhs) protein at a relatively high multiplicity of infection (MOI) (13). HSV-1 infection also upregulates the expression of type I IFN-mediated signaling antagonism of SOCS1 in keratinocytes (14). These effects of viral proteins allow HSV-1 to establish lifelong latency. However, the underlying molecular mechanisms are still elusive. Whether other HSV-1-encoded proteins also contribute to the evasion of type I IFN response still remains to be determined.

UL36 (VP1/2), the largest tegument protein of HSV-1, has been discovered to have a ubiquitin-specific protease (USP) embedded within its N-terminal 500 residues (15). This fragment, termed UL36USP, will be cleaved from full-length UL36 and display deubiquitinase (DUB) activity after viral infection (16). For HSV-1 F strain, residue Cys40 in UL36USP is the essential point responsible for DUB activity (17). In the current study, we found that ectopic expression of UL36USP inhibited the activation of the ISRE promoter and the transcription of ISGs in response to IFN-β, and C40A, which was designed to mutate Cys40 to Ala to abrogate the deubiquitinase activity of UL36USP, also exhibited similar inhibitory effects on IFN-β-induced activation of the ISRE promoter and the accumulation of ISG mRNA. Moreover, we explored the inhibitory mechanism of UL36USP, which antagonized type I IFN-mediated signaling via competitively binding to IFNAR2 and blocking JAK1-IFNAR2 association independent of its DUB activity. In short, for the first time we revealed that HSV-1 UL36USP plays a pivotal role in evading type I IFN-mediated signaling.

RESULTS

UL36USP abrogates type I IFN-mediated signaling independent of DUB activity. Previous studies have discovered that several viral proteins of HSV-1 antagonize the

innate immune response and facilitate viral replication. Here, we try to identify if any HSV-1 proteins could inhibit type I IFN-mediated antiviral innate immune response. Thus, we have done a screen assay for viral proteins that could inhibit the activation of ISRE promoter induced by IFN-β (Fig. 1A). HEK293T cells were cotransfected with empty vector or expression vectors encoding HSV-1 viral proteins in the presence of ISRE promoter plasmid (ISRE-Luc) and *Renilla* luciferase reporter (pRL-TK) plasmid, and then we subjected samples to Dual-Luciferase reporter (DLR) assay. As shown in Fig. 1B, UL36USP significantly inhibited IFN-β-triggered ISRE reporter activation. Furthermore, ectopic expression of the C40A mutant lacking DUB activity of UL36USP also inhibited the activation of the ISRE promoter at a level comparable to that of UL36USP. Moreover, real-time quantitative PCR (qPCR) confirmed that overexpression of UL36USP or C40A mutant strongly suppressed ISG54 and ISG56 gene expression after IFN-β treatment (Fig. 1C and D). These data suggested that UL36USP blocked the IFN-β-mediated downstream signaling in a DUB-independent manner.

To determine whether UL36USP affected IFN-β-induced transcription of ISGs during HSV-1 infection, we constructed the UL36USP stable-knockdown HEK293T cells. Three different small hairpin RNAs specific for UL36USP (shUL36USP) or the scrambled small hairpin RNA (shNC) (NC stands for negative control) were cloned into pSUPER.retro.puro vector to yield pSUPER-shUL36USP and pSUPER-shNC plasmids. The knockdown efficiency of each UL36USP RNA interference plasmid was evaluated by Western blot (WB) analysis. HEK293T cells were cotransfected with UL36USP-Flag and pSUPER-shNC or the indicated pSUPER-shUL36USP plasmids, and then cells were harvested and subjected to WB analysis (Fig. 1E). The pSUPER-shUL36USP#2 and pSUPER-shUL36USP#3 plasmids were selected to generate UL36USP stable-knockdown HEK293T cells (HEK293T-shUL36USP). As shown in Fig. 1F, a relatively low level of UL36USP was detected in HEK293T-shUL36USP#2 and HEK293T-shUL36USP#3 cells compared with that of HEK293T-shNC cells during HSV-1 infection. In addition, the protein levels of UL42 and UL46 were similar in shUL36USP and shNC cells, indicating that viral replication was not influenced in UL36USP knockdown cells. To verify whether DUB activity was required for UL36USP antagonizing IFN-β-mediated signaling, we also utilized a recombinant HSV-1, called C40A HSV-1, in which Cys40 of UL36 was mutated to Ala (17). The stably transfected HEK293T-shNC and HEK293T-shUL36USP (#2 and #3) cells then were infected with wild-type (WT) HSV-1 or C40A HSV-1 for 12 h before IFN-β treatment. Cells were harvested and subjected to qPCR to analyze the mRNA levels of ISG54 and ISG56. As a consequence, infection with WT HSV-1 and C40A HSV-1 in HEK293T-shNC cells both suppressed the ISG54 and ISG56 mRNA expression induced by IFN-β, while infection with WT HSV-1 and C40A HSV-1 in HEK293T-shUL36USP (#2 and #3) cells partially restored ISG54 and ISG56 mRNA expression (Fig. 1G and H). Collectively, these results indicated that UL36USP was sufficient to inhibit IFN-β-triggered signaling and that the DUB activity was dispensable for its inhibitory ability. Meanwhile, HEK293T-shUL36USP#2 cells were selected for the following experiments.

UL36USP inhibits IFN-β-induced phosphorylation of JAKs and STATs. To investigate the effect of UL36USP on type I IFN-mediated signaling, we next tested whether UL36USP affected the activation of STATs and JAKs induced by IFN-β. HEK293T cells were transfected with UL36USP or C40A expression plasmid for 24 h, followed by IFN-β stimulation. WB analysis showed that overexpression of either UL36USP or C40A impaired the phosphorylation of endogenous STAT1 and STAT2 in response to IFN-β without affecting their protein expression (Fig. 2A and B). This result implied that the inhibition site of UL36USP was on STATs or the upstream level of STATs. Due to the JAK activation being the initial event in IFN-β-activated signaling, we then examined whether UL36USP had an impact on the phosphorylation of JAK1 and TYK2 in the presence of IFN-β. Interestingly, cells transiently expressing either UL36USP or C40A also reduced endogenous JAK1 and TYK2 phosphorylation levels triggered by IFN-β while the protein levels were not affected (Fig. 2C and D). These results indicated that UL36USP inhibited IFN-β-induced phosphorylation of JAKs and STATs in a manner that

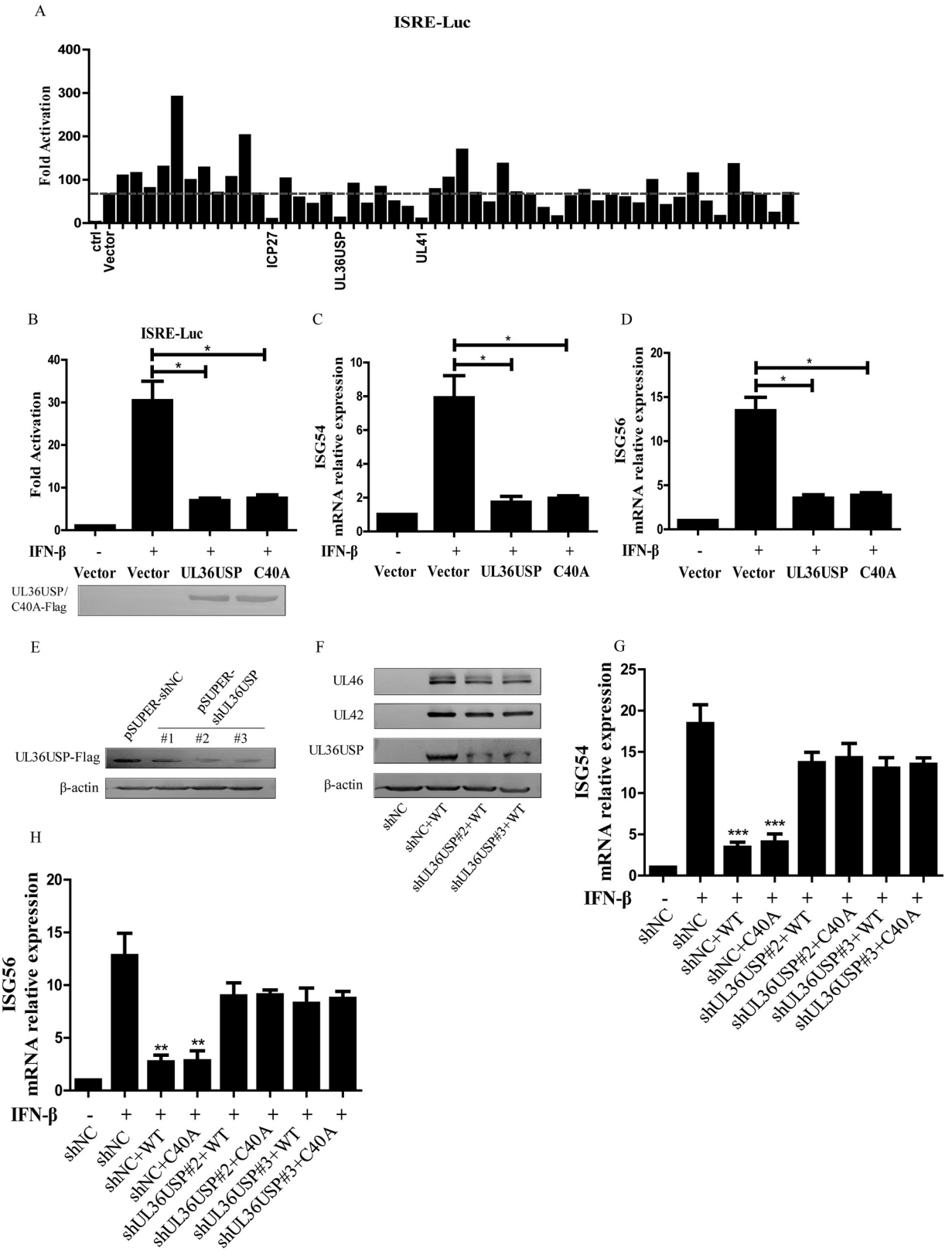

FIG 1 HSV-1 UL36USP inhibits IFN-β induced activation of ISRE promoter and transcription of ISGs independent of DUB activity. (A) HEK293T cells were cotransfected with ISRE-Luc reporter plasmid, pRL-TK control plasmid, and empty vector or expression plasmids of HSV-1 proteins. At 24 h posttransfection, cells were mock treated or treated with exogenous IFN-β (1,000 U/ml) for 8 h and then subjected to Dual-Luciferase reporter (DLR) assay, and the fold activation was determined compared to that of the empty vector with mock-treated cells. (B) HEK293T cells were cotransfected with ISRE-Luc reporter plasmid, pRL-TK control plasmid, and empty vector, UL36USP-Flag, or C40A-Flag. At 24 h posttransfection,

(Continued on next page)

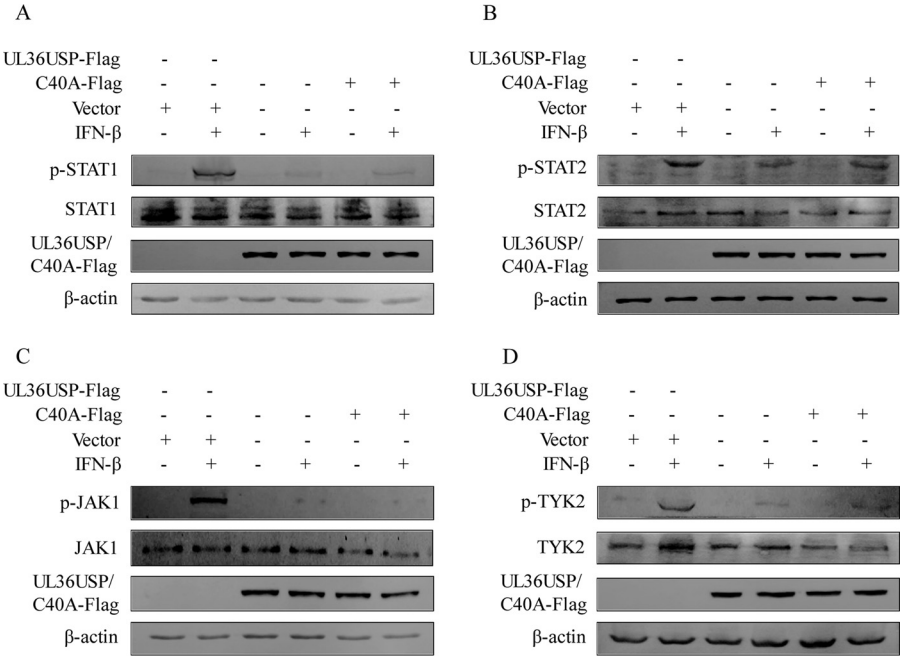

FIG 2 UL36USP inhibits IFN-β-induced phosphorylation of STATs and JAKs. (A, B, C, and D) HEK293T cells were transfected with empty vector, UL36USP-Flag, or C40A-Flag for 24 h and then mock treated or treated with IFN-β (1,000 U/ml) for 30 min. Cells were then harvested and subjected to WB to assess the phosphorylation and expression levels of endogenous STAT1 (A), STAT2 (B), JAK1 (C), and TYK2 (D). The data represent results from one of three independent experiments.

did not rely on its DUB activity. It also suggested that UL36USP counteracted type I IFN-mediated signaling most probably at or before JAKs.

UL36USP antagonizes type I IFN-mediated signaling via specifically binding with IFNAR2 subunit. To further clarify the mechanism by which UL36USP inhibits type I IFN-mediated signaling, a coimmunoprecipitation (co-IP) assay was applied to examine the potential target molecules of UL36USP. HEK293T cells were cotransfected with UL36USP expression plasmid along with STAT1, STAT2, JAK1, TYK2, IFNAR1, or IFNAR2 expression plasmid. Cells were harvested after 36 h and then subjected to co-IP experiments. As shown in Fig. 3A to F, UL36USP specifically associated with IFNAR2, while other indicated proteins were not able to interact with UL36USP. To confirm the interaction between UL36USP and IFNAR2 at the physiological level, the endogenous co-IP assay was performed under conditions of HSV-1 infection. As a result, the data presented in Fig. 3G ascertained that UL36USP physically associated with endogenous IFNAR2. Meanwhile, the interaction between C40A and IFNAR2 was also detected at the transient expression level and the physiological level (Fig. 3H and I). We therefore concluded that UL36USP specifically bound with IFNAR2 to exert an inhibitory effect on type I IFN-mediated signaling.

FIG 1 Legend (Continued)
cells were mock treated or treated with exogenous IFN-β (1,000 U/ml) for 8 h and then subjected to DLR assay. The fold activation was determined compared to that of the empty vector with mock-treated cells. (C and D) HEK293T cells were transfected with empty vector, UL36USP-Flag, or C40A-Flag. After 24 h, cells were mock treated or treated with exogenous IFN-β (1,000 U/ml) for 8 h, and qPCR analysis was applied to detect ISG54 (C) or ISG56 (D) mRNA. (E) HEK293T cells were cotransfected with UL36USP-Flag and pSUPER-shNC or the indicated pSUPER-shUL36USP plasmids (#1, #2, and #3), and cells were harvested at 48 h posttransfection and subjected to WB analysis. (F) HEK293T-shNC and HEK293T-shUL36USP (#2 and #3) cells were infected with WT HSV-1 for 20 h at an MOI of 1. Cells were harvested and subjected to WB to analyze the protein levels of UL36USP, UL42, and UL46. (G and H) HEK293T-shNC and HEK293T-shUL36USP (#2 and #3) cells were infected with WT HSV-1 or C40A HSV-1 for 12 h at an MOI of 1, and then IFN-β (1,000 U/ml) was added for another 8 h. Cells were harvested and subjected to qPCR to detect ISG54 (G) or ISG56 (H) mRNA. Asterisks indicates a significant difference from the IFN-β-treated control. Error bars represent standard deviations (SD) from three independent experiments. Statistical analysis was performed using Student's *t* test (*, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.0001$).

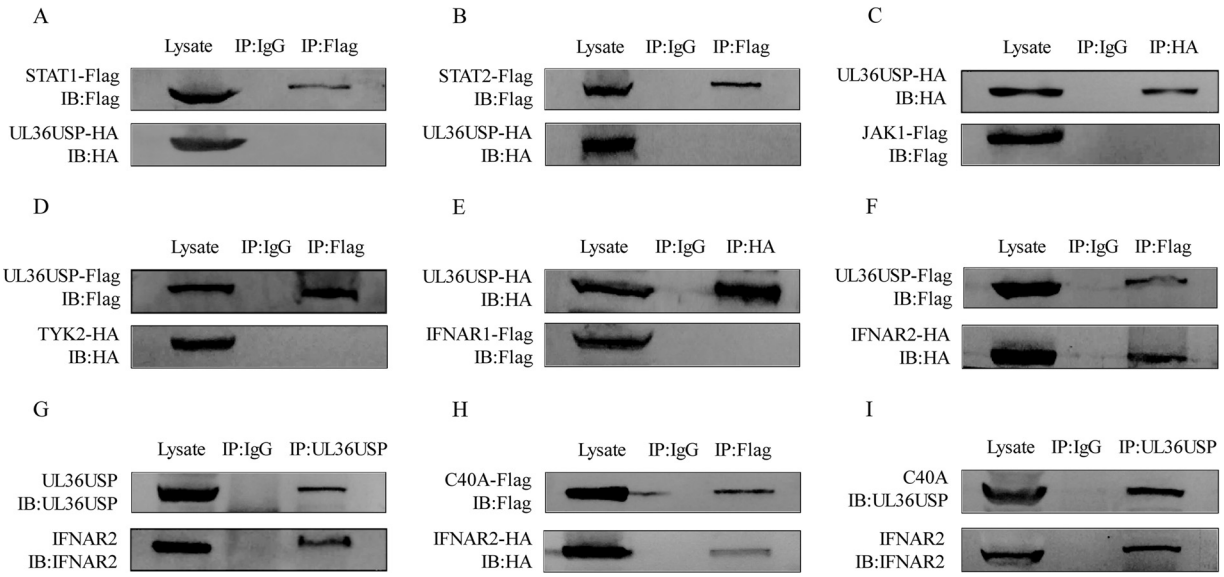

FIG 3 UL36USP specifically binds with IFNAR2 subunit. (A to F) HEK293T cells were transfected with UL36USP plasmid, along with STAT1 (A), STAT2 (B), JAK1 (C), TYK2 (D), IFNAR1 (E), and IFNAR2 (F) plasmids separately. After 36 h, cells were harvested and subjected to co-IP assays. Cell lysates from panels A, B, D, and F were immunoprecipitated with anti-Flag antibody, and lysates from panels C and E were immunoprecipitated with anti-HA antibody. All lysates then were subjected to immunoblotting with anti-HA or anti-Flag antibody to analyze the interaction between UL36USP plasmid and the aforementioned plasmids. (G and I) HEK293T cells were infected with WT HSV-1 (G) or C40A HSV-1 (I) for 20 h at an MOI of 1. Cell lysates were then extracted and immunoprecipitated with anti-UL36USP antibody and immunoblotted with anti-IFNAR2 antibody. (H) HEK293T cells were transfected with C40A-Flag and IFNAR2-HA plasmids for 36 h. Cell lysates were then immunoprecipitated with anti-Flag antibody and subjected to immunoblotting with anti-HA antibody. The data represent results from one of three independent experiments.

UL36USP competes with JAK1 for binding to IFNAR2. It is well established that JAK1 associates with IFNAR2 inside the cell membrane in advance prior to activation (6). Thus, we assumed that the interaction between UL36USP and IFNAR2 would disrupt JAK1-IFNAR2 association. To test this assumption, we coexpressed JAK1 and IFNAR2 in HEK293T cells in the absence or presence of UL36USP and then subjected them to co-IP analysis. As expected, cells expressing UL36USP showed a notable impairment in JAK1-IFNAR2 association (Fig. 4A). Meanwhile, we also detected JAK1-IFNAR2 interaction in C40A-expressing cells, and similar suppression was observed (Fig. 4A). These results were in agreement with our assumption that UL36USP antagonized type I IFN-mediated signaling through binding to IFNAR2 and interfering with JAK1-IFNAR2 interaction.

To further confirm the role of UL36USP in hampering JAK1-IFNAR2 complex formation, we next examined JAK1-IFNAR2 interaction under increasing amounts of UL36USP in HEK293T cells. As shown in Fig. 4B, with the expression of UL36USP increased, the lower level of interaction between JAK1 and IFNAR2 was measured. These data suggested that UL36USP was capable of interfering with JAK1-IFNAR2 interaction in a dose-dependent manner. To examine whether this inhibition of UL36USP could affect the receptor recruitment of STAT1, we then performed co-IP experiments to detect STAT1-IFNAR2 interaction in UL36USP-overexpressed cells after IFN-β treatment. In the presence of UL36USP, a significant impairment of STAT1-IFNAR2 interaction was observed in IFN-β-stimulated cells, correlated with the inhibitory effect on JAK1-IFNAR2 interaction (Fig. 4C). These data suggested that UL36USP competed with JAK1 for binding to IFNAR2 and thus blocked the receptor recruitment of STAT1.

To further support that UL36USP utilized this pattern to downregulate type I IFN-mediated signaling, we subsequently evaluated the endogenous JAK1-IFNAR2 interaction in the context of HSV-1 infection. HEK293T-shNC and HEK293T-shUL36USP cells were infected with WT HSV-1 or C40A HSV-1 in the absence or presence of IFN-β and then subjected to co-IP assay. As presented in Fig. 4D, either WT HSV-1 or C40A HSV-1 infection significantly suppressed the interaction between endogenous JAK1 and

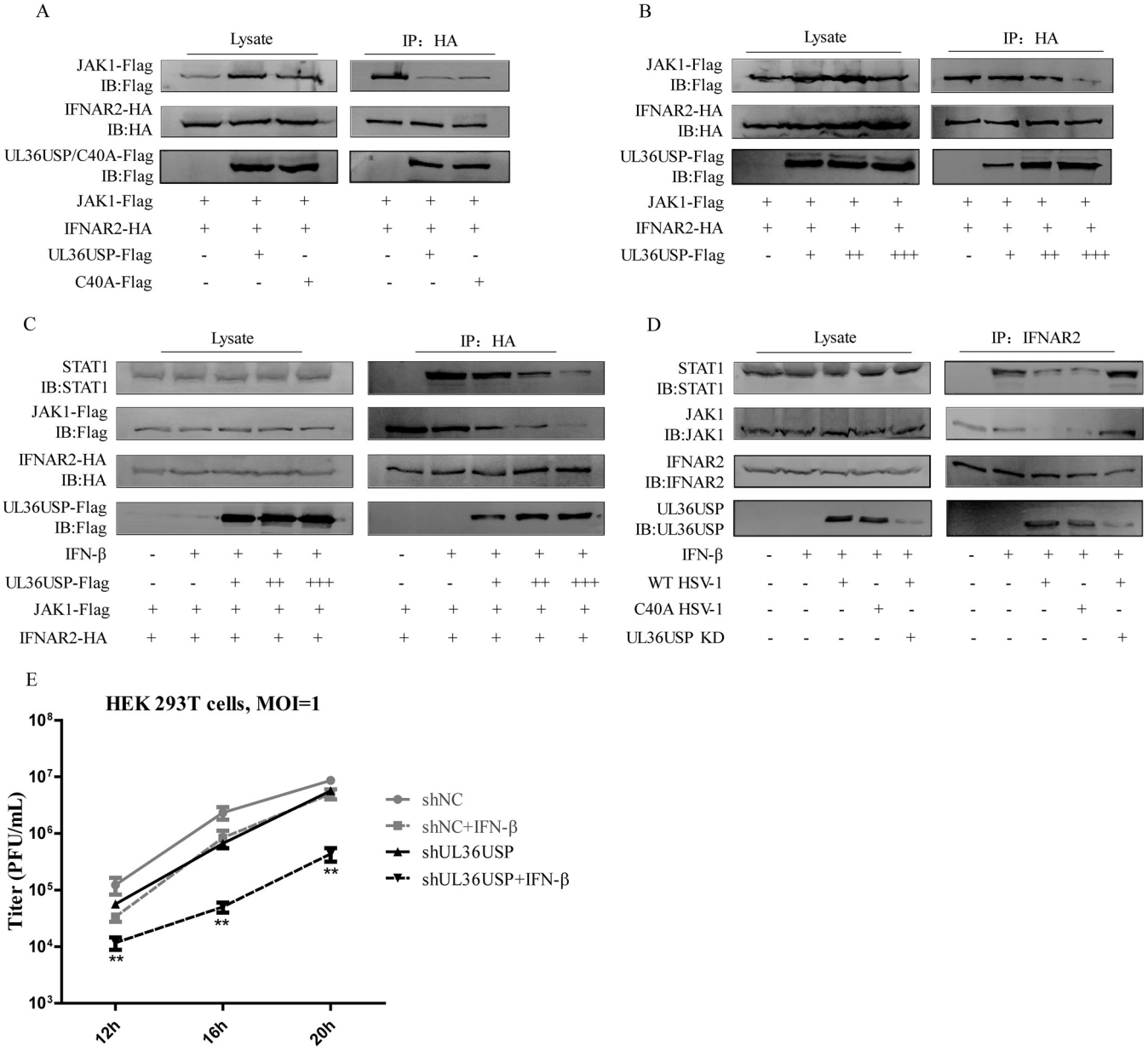

FIG 4 UL36USP blocks the interaction between JAK1 and IFNAR2. (A) HEK293T cells were cotransfected with JAK1-Flag, IFNAR2-HA, and UL36USP-Flag or C40A-Flag. Immunoprecipitations (IP) were performed using anti-HA antibody, and then samples were subjected to immunoblotting with anti-HA or anti-Flag antibody. (B) HEK293T cells were cotransfected with JAK1-Flag or IFNAR2-HA in the absence or presence of increasing concentrations of UL36USP-Flag for 36 h, followed by immunoprecipitation with anti-HA antibody and immunoblotting with anti-HA or anti-Flag antibody. (C) HEK293T cells were cotransfected with JAK1-Flag or IFNAR2-HA in the absence or presence of increasing concentration of UL36USP-Flag for 36 h and then stimulated with IFN-β (1,000 U/ml) for another 30 min. Cell lysates were extracted and immunoprecipitated with anti-HA antibody and immunoblotted with anti-HA, anti-Flag, or anti-STAT1 antibody. (D) HEK293T-shNC and HEK293T-shUL36USP cells were infected with WT HSV-1 or C40A HSV-1 for 20 h at an MOI of 1 and stimulated with IFN-β (1,000 U/ml) for another 30 min. Cell lysates were extracted and immunoprecipitated with anti-IFNAR2 antibody and then immunoblotted with anti-IFNAR2, anti-JAK1, anti-UL36USP, or anti-STAT1 antibody. (E) HEK293T-shNC and HEK293T-shUL36USP cells were pretreated with IFN-β (1,000 U/ml) or with medium only. After 8 h, cells were infected with WT HSV-1 at an MOI of 1 and then harvested at the indicated time points postinfection. Viral titers were determined by plaque assay in Vero cells. Asterisks indicate a significant difference of WT HSV-1 titer in HEK293T-shNC and HEK293T-shUL36USP cells in the context of IFN-β treatment. The data represent results from one of three independent experiments. Statistical analysis was performed using Student's *t* test (**, $0.001 < P < 0.01$).

IFNAR2, thereby blocking STAT1 docking at IFNAR2, whereas knockdown of UL36USP restored their interaction to a certain extent. These data indicated that endogenous viral UL36USP was competent to competitively inhibit JAK1 binding to IFNAR2.

To better delineate the activity of UL36USP that contributes to HSV-1 replication, we infected HEK293T-shNC and HEK293T-shUL36USP cells with WT HSV-1 at an MOI of 1 in the absence or presence of IFN-β. As shown in Fig. 4E, there was slightly impaired

replication of WT HSV-1 in shUL36USP cells compared with that in shNC cells when not stimulated with IFN-β. However, the viral protein levels of UL42 and UL46 were similar in shNC and shUL36USP cells at 20 h postinfection (Fig. 1F). With IFN-β treatment, the replication of WT HSV-1 was affected in both shNC cells and shUL36USP cells, and viral replication was inhibited more significantly in shUL36USP cells than in shNC cells when treated with IFN-β. These data indicated that UL36USP evades the IFN-β-induced antiviral activity and promotes viral replication.

Taken together, the findings described above demonstrated that HSV-1 UL36USP abrogated type I IFN-mediated signaling via specifically binding to IFNAR2 and blocking the interaction between JAK1 and IFNAR2.

DISCUSSION

In this study, we present the first evidence that HSV-1 UL36USP is capable of modulating type I IFN-mediated signaling. It is noteworthy that UL36USP antagonizes type I IFN-mediated signaling independent of its DUB activity. Both UL36USP and C40A were shown to inhibit the activation of the ISRE promoter and transcription of ISGs in response to IFN-β when transiently expressed in HEK293T. Specifically, UL36USP was found to interact with type I IFN receptor subunit IFNAR2 and disrupt the integrity of the JAK1-IFNAR2 complex. Moreover, knockdown of UL36USP diminished the impact on blocking JAK1-IFNAR2 association and induced much higher levels of ISG mRNA. Our results revealed a novel role of UL36USP, which competitively interacts with IFNAR2 and interrupts downstream activation, thereby enabling HSV-1 to retreat from type I IFN-mediated defense.

IFNs act as representative mediators of innate immune responses that are induced rapidly upon viral infection. In order to achieve a stable antiviral environment in the host, a large array of ISGs will be induced by IFNs during viral invasion and target different steps of the viral replication cycle (8). However, as Fensterl et al. summarized that there is no love lost between viruses and IFNs, many viruses, including herpesviruses, have evolved various mechanisms to antagonize IFN system, such as blocking IFN synthesis, disrupting IFN-mediated signaling, and directly targeting ISG-encoded proteins (18). For HSV-1, a widespread and important human pathogen, several viral proteins have been reported to counteract IFN defenses at multiple levels (19). For instance, HSV-1 tegument protein kinase Us3 and other HSV-1 proteins (ICP0, Us11, UL36, ICP34.5, VP16, and vhs) are able to abrogate IFN-β production (17, 20–26). In addition, HSV-1 can also act directly against ISG proteins, like viperin, ZAP, tetherin, IFIT3, and CH25H, mainly via its UL41 gene product (vhs) (27–31). In summary, much progress has been made in figuring out how HSV-1 escapes from innate immunity, especially the upstream level of IFN response. Even so, there is still a quantity of IFN-β that can be detected under HSV-1 infection, resulting in activation of IFN-β downstream signaling. Therefore, investigating the mechanisms of how HSV-1 prevails against IFN-triggered defenses will be conducive to understanding HSV-1-mediated immune evasion and providing new insights into prevention of HSV-1 infection.

Given that the antiviral effects of type I IFNs play a powerful role in innate immunity, it is well worth identifying the evasion strategies of HSV-1 in the battle between viruses and interferons. Chee and Roizman found that HSV-1 infection altered several essential molecules in type I IFN-mediated signaling at a relatively high MOI, and vhs might be responsible for the rapid decrease of JAK1 and STAT2 expression levels (13). Since then, growing amounts of research have aimed at discovering HSV-1 antagonisms in type I IFN downstream signaling. ICP27, the immediate-early gene of HSV-1, has been reported to downregulate STAT1 phosphorylation and interfere with nuclear accumulation of STAT1 (32). HSV-1 infection also upregulates the expression of the type I IFN-mediated signaling inhibitor SOCS1 in a cell-specific manner (14). Nevertheless, our current understanding of the countermeasures of HSV-1 against type I IFN-mediated signaling is still limited, and more potential antagonisms between HSV-1 and this pathway remain to be discovered. Therefore, DLR assays have been performed to screen for HSV-1 viral proteins that could inhibit the activation of the ISRE promoter

induced by IFN-β. As a result, several viral proteins possess the ability to downregulate IFN-β-mediated activation of the ISRE promoter (Fig. 1A). UL36USP was selected for conducting further research on its potent repression ability in DLR assays.

UL36 is the largest and most essential tegument protein of HSV-1, participating in several steps of the viral life cycle, such as virus entry, capsid transport, capsid routing and subsequent uncoating, virion assembly, etc. (33–37). It has been discovered that UL36 has a deubiquitinase domain in its N terminus, denoted UL36USP, for UL36 ubiquitin-specific protease (16). UL36USP was found to be active on cleavage of both Lys-48- and Lys-63-linked polyubiquitin chains (38). Our previous study demonstrated that UL36USP deubiquitinates TRAF3, thereby inhibiting IRF3 dimerization and IFN-β transcription (17). In addition, UL36USP also targets IκBα, restricting its degradation and leading to the inhibition of NF-κB activation and IFN-β induction (39). Given that UL36USP blocks signal transduction, mainly depending on its DUB activity, it is plausible that DUB activity is also involved in the inhibition of IFN-β downstream signaling. Thus, we utilized the C40A mutant plasmid and virus, with the catalytic activity of UL36USP mutated in the HSV-1 F strain to determine if the DUB activity was required for UL36USP antagonizing IFN-β downstream signaling. However, the results depicted in Fig. 1 demonstrated that either UL36USP or C40A suppressed IFN-β-induced activation of the ISRE promoter and transcription of ISGs at a comparable level. This suggested that DUB activity was not required for UL36USP antagonizing IFN-β downstream signaling.

Since the phosphorylation of key molecules in type I IFN downstream signaling is the essential step leading to the activation of the pathway, many viruses encode antagonists to block their phosphorylation (9). It is reasonable to hypothesize that UL36USP interferes with the phosphorylation of STAT proteins or JAKs. To test our hypothesis, we examined the phosphorylation levels of STATs and JAKs stimulated by IFN-β in the absence or presence of UL36USP. The data shown in Fig. 2 indicated that UL36USP downregulated the phosphorylation levels of STAT1, STAT2, JAK1, and TYK2 in response to IFN-β. It also reminded us that UL36USP might counteract type I IFN-mediated signaling, most probably at or before JAKs. However, we could not rule out the possibility that UL36USP acted at STAT levels or between JAKs and STATs simultaneously. Thus, the co-IP experiments were applied to identify the molecular target of UL36USP. From Fig. 3, a specific interaction between UL36USP or C40A and IFNAR2 at exogenous and endogenous levels was identified.

The first step for type I IFNs toward activating downstream signaling is to bind to a cognate receptor (IFNAR) at the cell surface, which is made of two subunits, IFNAR1 and IFNAR2 (4). A number of studies utilizing gene-targeted cells or mice to exploit the actions of IFNAR have highly validated its critical role in initiating type I IFN downstream signaling. Accordingly, many viruses subvert type I IFN-mediated signaling via targeting IFNAR. Vaccinia virus has been reported to encode a soluble protein, B18R, that is homologous to IFNAR1 and presents at the cell surface, thereby restricting IFN-α/β binding to IFNAR and attenuating IFN-α/β-mediated antiviral effects (40, 41). Measles virus suppresses IFN-α signaling through its accessory proteins C and V, which are able to form a complex with IFNAR1 and block JAK1 phosphorylation (42). Similarly, viral protein RIF of Kaposi's sarcoma-associated herpesvirus blocks IFN signaling by forming an inhibitory complex involving IFNAR, JAK1, TYK2, and STAT2 (43). In addition, Epstein-Barr virus-encoded membrane proteins LMP2A and LMP2B modulate IFN responses by targeting IFNAR1 for degradation (44). Moreover, hemagglutinin protein of influenza A virus is capable of decreasing IFNAR1 expression (45). Recently, pseudorabies virus, a swine alphaherpesvirus, has been uncovered in which viral protein UL50 accelerates IFNAR1 degradation to avoid type I IFN responses. It also demonstrated that HSV-1 UL50 possesses similar activity, although the activity is milder (46). In fact, a previous study has already provided evidence that HSV-1 infection induces the degradation of IFNAR1 (13). However, it is unknown whether HSV-1 can target IFNAR2 to evade type I IFN responses. Here, we identified for the first time that HSV-1 UL36USP specifically bound with IFNAR2 and disassociated JAK1 from IFNAR2, thereby blocking

receptor recruitment of STAT1 (Fig. 4). Furthermore, the interaction of UL36USP-IFNAR2 makes a large contribution to HSV-1 replication, since knockdown of UL36USP significantly impaired viral growth after IFN-β treatment (Fig. 4E).

In summary, we have explored a novel regulatory mechanism of HSV-1 in evading type I IFN-mediated signaling that encoded UL36USP as an antagonist acting on IFNAR2. Our results also revealed an entirely new model of UL36USP to exert inhibitory impacts on IFN defenses independent of its enzymatic activity. These findings will facilitate our understanding of the interplay between HSV-1 and the innate immune response and contribute to vaccine design.

MATERIALS AND METHODS

Cells, viruses, and antibodies. HEK293T cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained in Dulbecco's modified Eagle medium (DMEM) (Gibco-BRL) containing 10% fetal bovine serum (FBS) and 100 U/ml of penicillin and streptomycin. The WT HSV-1 F strain and its derivative UL36USP mutant HSV-1 strain (C40A HSV-1) were propagated in Vero cells and titrated as described previously (47). The protease inhibitor mixture cocktail was purchased from Thermo Fisher Scientific (MA, USA). Radioimmunoprecipitation assay (RIPA) lysis buffer was purchased from Beyotime (Shanghai, China). Mouse monoclonal anti-HA and anti-Flag antibodies (MAbs) were purchased from Abmart (Shanghai, China). Rabbit anti-STAT1 polyclonal antibody (pAb) and mouse anti-β-actin MAb were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit anti-pSTAT1 at Tyr-701, anti-pTYK2 at Tyr-1054/1055, and anti-TYK2 MAbs were purchased from CST (Boston, MA). Rabbit anti-JAK1, anti-pJAK1 at Tyr-1022, anti-STAT2, and anti-pSTAT2 at Tyr-690 pAbs were purchased from ImmunoWay (Plano, TX, USA). Rabbit anti-IFNAR2 pAb was purchased from Proteintech (Wuhan, China). Rabbit anti-UL36USP, anti-UL42, and anti-UL46 pAbs were made by GL Biochem, Ltd. (Shanghai, China).

Plasmid construction. All enzymes used for cloning procedures were purchased from Thermo Fisher Scientific (Waltham, MA), except for T4 DNA ligase, from Vazyme (Nanjing, China). To construct STAT2-Flag plasmid, the STAT2 gene was amplified from human cDNA and cloned into pCMV-Flag vector (Beyotime, Shanghai, China). UL36USP and C40A were cloned into pCMV-Flag and pCMV-HA vectors (Beyotime, Shanghai, China) as previously described (17). Small hairpin RNA specific for UL36USP (shUL36USP) or scrambled small hairpin RNA (shNC) (NC stands for negative control) was cloned into pSUPER.retro.puro vector (Oligoengine, Seattle, WA, USA) to yield pSUPER-shUL36USP and pSUPER-shNC plasmids, respectively, as described in our previous study (25). The primer sets used for knocking down UL36USP were the following: For shUL36USP#1, 5′-GATCCCCACCAAGAAGAAATCCACCCTTCAAGAGAGGGTGGATTTCTTCTTGGTTTTTTA-3′ and 5′-AGCTTAAAAAACCAAGAAGAAATCCACCCTCTCTTGAAGGGTGGATTTCTTCTTGGTGGG-3′; For shUL36USP#2, 5′-GATCCCCTCCATACCTGACGTATTACTTCAAGAGAGTAATACGTCAGGTATGGATTTTTA-3′ and 5′-AGCTTAAAAATCCATACCTGACGTATTACTCTCTTGAAGTAATACGTCAGGTATGGAGGG-3′; For shUL36USP#3, 5′-GATCCCCGAAGATGACGACGACATGCTTCAAGAGAGCATGTCGTCGTCATCTTCTTTTTA-3′ and 5′-AGCTTAAAAAGAAGATGACGACGACATGCTCTCTTGAAGCATGTCGTCGTCATCTTCGGG-3′. Commercial reporter plasmid pRL-TK (RL stands for *Renilla* luciferase and TK stands for thymidine kinase) was purchased from Promega Corporation (Madison, WI, USA). Gift plasmids included the following: IFNAR1-Flag, IFNAR2-HA (48), JAK1-Flag (49), TYK2-HA, STAT1-Flag, and ISRE-Luc (50).

Establishment of UL36USP stable-knockdown HEK293T cells. HEK293T cells were transfected with pSUPER-shUL36USP or pSUPER-shNC plasmid for 36 h, and then puromycin was added to cells at a concentration of 1 μg/ml and screened for a week. The stably transfected HEK293T-shNC and HEK293T-shUL36USP cells were then maintained with puromycin (500 ng/ml).

RNA isolation and qPCR. Total RNA was extracted with TRIzol (Invitrogen, CA) according to the manufacturer's manual. Samples were digested with DNase I and subjected to reverse transcription (RT) as described in our previous study. The cDNA was used as a template for qPCR to detect the mRNA levels of ISG54 and ISG56, and 18S rRNA was used as an internal reference as previously described (51).

Transfection and DLR assay. HEK293T cells were cotransfected with ISRE-Luc reporter plasmid and an internal control plasmid, pRL-TK, with or without expression plasmids, by standard calcium phosphate coprecipitation (52). At 24 h posttransfection, luciferase assays were performed with a luciferase assay kit (Promega, Madison, WI) as previously described (51).

Co-IP and WB analysis. Co-IP assays and WB analysis were performed as previously described (47). Briefly, cells ($\sim 5 \times 10^6$) were transfected with the indicated plasmids or infected with WT HSV-1 or C40A HSV-1 as indicated in the figure legends. Harvested cells were lysed on ice with 1 ml of lysis buffer. The lysates were incubated with the antibodies referred to in the figure legends and 20 μl of a 1:1 slurry of protein A/G plus agarose (Santa Cruz Biotechnology) overnight at 4°C. The beads were washed three times with phosphate-buffered saline, and WB analysis was performed to detect the interaction of proteins. The co-IP assays were repeated three times, and similar data were obtained.

Statistical analysis. Data are presented as means ± standard deviations where indicated. A two-tailed unpaired Student's *t* test was used to determine differences. A *P* value of <0.05 was considered statistically significant, and values are marked with asterisks (*, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.0001$) in the figures.

ACKNOWLEDGMENTS

We thank Rongtuan Lin for ISRE-Luc plasmid, Zhao Yuanhou for JAK1-Flag plasmid, John J. Krolewski for IFNAR2-HA plasmid, and Hui Zheng for IFNAR1-Flag, STAT1-Flag, and TYK2-HA plasmids.

REFERENCES

1. Honda K, Takaoka A, Taniguchi T. 2006. Type I interferon gene induction by the interferon regulatory factor family of transcription factors. Immunity 25:349–360. https://doi.org/10.1016/j.immuni.2006.08.009.
2. Takaoka A, Yanai H. 2006. Interferon signalling network in innate defence. Cell Microbiol 8:907–922. https://doi.org/10.1111/j.1462-5822.2006.00716.x.
3. Borden EC, Sen GC, Uze G, Silverman RH, Ransohoff RM, Foster GR, Stark GR. 2007. Interferons at age 50: past, current and future impact on biomedicine. Nat Rev Drug Discov 6:975–990. https://doi.org/10.1038/nrd2422.
4. de Weerd NA, Nguyen T. 2012. The interferons and their receptors–distribution and regulation. Immunol Cell Biol 90:483–491. https://doi.org/10.1038/icb.2012.9.
5. Gonzalez-Navajas JM, Lee J, David M, Raz E. 2012. Immunomodulatory functions of type I interferons. Nat Rev Immunol 12:125–135. https://doi.org/10.1038/nri3133.
6. Platanias LC. 2005. Mechanisms of type-I- and type-II-interferon-mediated signalling. Nat Rev Immunol 5:375–386. https://doi.org/10.1038/nri1604.
7. Majoros A, Platanitis E, Kernbauer-Holzl E, Rosebrock F, Muller M, Decker T. 2017. Canonical and non-canonical aspects of JAK-STAT signaling: lessons from interferons for cytokine responses. Front Immunol 8:29. https://doi.org/10.3389/fimmu.2017.00029.
8. Yan N, Chen ZJ. 2012. Intrinsic antiviral immunity. Nat Immunol 13:214–222. https://doi.org/10.1038/ni.2229.
9. Nan Y, Wu C, Zhang YJ. 2017. Interplay between Janus kinase/signal transducer and activator of transcription signaling activated by type I interferons and viral antagonism. Front Immunol 8:1758. https://doi.org/10.3389/fimmu.2017.01758.
10. McGeoch DJ, Dalrymple MA, Davison AJ, Dolan A, Frame MC, McNab D, Perry LJ, Scott JE, Taylor P. 1988. The complete DNA sequence of the long unique region in the genome of herpes simplex virus type 1. J Gen Virol 69(Part 7):1531–1574.
11. McGeoch DJ, Cook S. 1994. Molecular phylogeny of the alphaherpesvirinae subfamily and a proposed evolutionary timescale. J Mol Biol 238:9–22. https://doi.org/10.1006/jmbi.1994.1264.
12. Johnson KE, Knipe DM. 2010. Herpes simplex virus-1 infection causes the secretion of a type I interferon-antagonizing protein and inhibits signaling at or before Jak-1 activation. Virology 396:21–29. https://doi.org/10.1016/j.virol.2009.09.021.
13. Chee AV, Roizman B. 2004. Herpes simplex virus 1 gene products occlude the interferon signaling pathway at multiple sites. J Virol 78:4185–4196. https://doi.org/10.1128/JVI.78.8.4185-4196.2004.
14. Frey KG, Ahmed CM, Dabelic R, Jager LD, Noon-Song EN, Haider SM, Johnson HM, Bigley NJ. 2009. HSV-1-induced SOCS-1 expression in keratinocytes: use of a SOCS-1 antagonist to block a novel mechanism of viral immune evasion. J Immunol 183:1253–1262. https://doi.org/10.4049/jimmunol.0900570.
15. Kattenhorn LM, Korbel GA, Kessler BM, Spooner E, Ploegh HL. 2005. A deubiquitinating enzyme encoded by HSV-1 belongs to a family of cysteine proteases that is conserved across the family Herpesviridae. Mol Cell 19:547–557. https://doi.org/10.1016/j.molcel.2005.07.003.
16. Bolstad M, Abaitua F, Crump CM, O'Hare P. 2011. Autocatalytic activity of the ubiquitin-specific protease domain of herpes simplex virus 1 VP1-2. J Virol 85:8738–8751. https://doi.org/10.1128/JVI.00798-11.
17. Wang S, Wang K, Li J, Zheng C. 2013. Herpes simplex virus 1 ubiquitin-specific protease UL36 inhibits beta interferon production by deubiquitinating TRAF3. J Virol 87:11851–11860. https://doi.org/10.1128/JVI.01211-13.
18. Fensterl V, Chattopadhyay S, Sen GC. 2015. No love lost between viruses and interferons. Annu Rev Virol 2:549–572. https://doi.org/10.1146/annurev-virology-100114-055249.
19. Su C, Zhan G, Zheng C. 2016. Evasion of host antiviral innate immunity by HSV-1, an update. Virol J 13:38. https://doi.org/10.1186/s12985-016-0495-5.
20. Peri P, Mattila RK, Kantola H, Broberg E, Karttunen HS, Waris M, Vuorinen T, Hukkanen V. 2008. Herpes simplex virus type 1 Us3 gene deletion influences toll-like receptor responses in cultured monocytic cells. Virol J 5:140. https://doi.org/10.1186/1743-422X-5-140.
21. Wang S, Wang K, Lin R, Zheng C. 2013. Herpes simplex virus 1 serine/threonine kinase US3 hyperphosphorylates IRF3 and inhibits beta interferon production. J Virol 87:12814–12827. https://doi.org/10.1128/JVI.02355-13.
22. Melroe GT, DeLuca NA, Knipe DM. 2004. Herpes simplex virus 1 has multiple mechanisms for blocking virus-induced interferon production. J Virol 78:8411–8420. https://doi.org/10.1128/JVI.78.16.8411-8420.2004.
23. Melroe GT, Silva L, Schaffer PA, Knipe DM. 2007. Recruitment of activated IRF-3 and CBP/p300 to herpes simplex virus ICP0 nuclear foci: potential role in blocking IFN-beta induction. Virology 360:305–321. https://doi.org/10.1016/j.virol.2006.10.028.
24. Xing J, Wang S, Lin R, Mossman KL, Zheng C. 2012. Herpes simplex virus 1 tegument protein US11 downmodulates the RLR signaling pathway via direct interaction with RIG-I and MDA-5. J Virol 86:3528–3540. https://doi.org/10.1128/JVI.06713-11.
25. Su C, Zheng C. 2017. Herpes simplex virus 1 abrogates the cGAS/STING-mediated cytosolic DNA-sensing pathway via its virion host shutoff protein, UL41. J Virol 91:e02414-16. https://doi.org/10.1128/JVI.02414-16.
26. Xing J, Ni L, Wang S, Wang K, Lin R, Zheng C. 2013. Herpes simplex virus 1-encoded tegument protein VP16 abrogates the production of beta interferon (IFN) by inhibiting NF-kappaB activation and blocking IFN regulatory factor 3 to recruit its coactivator CBP. J Virol 87:9788–9801. https://doi.org/10.1128/JVI.01440-13.
27. Shen G, Wang K, Wang S, Cai M, Li ML, Zheng C. 2014. Herpes simplex virus 1 counteracts viperin via its virion host shutoff protein UL41. J Virol 88:12163–12166. https://doi.org/10.1128/JVI.01380-14.
28. Su C, Zhang J, Zheng C. 2015. Herpes simplex virus 1 UL41 protein abrogates the antiviral activity of hZAP by degrading its mRNA. Virol J 12:203. https://doi.org/10.1186/s12985-015-0433-y.
29. Zenner HL, Mauricio R, Banting G, Crump CM. 2013. Herpes simplex virus 1 counteracts tetherin restriction via its virion host shutoff activity. J Virol 87:13115–13123. https://doi.org/10.1128/JVI.02167-13.
30. Jiang Z, Su C, Zheng C. 2016. Herpes simplex virus 1 tegument protein UL41 counteracts IFIT3 antiviral innate immunity. J Virol 90:11056–11061. https://doi.org/10.1128/JVI.01672-16.
31. You H, Yuan H, Fu W, Su C, Wang W, Cheng T, Zheng C. 2017. Herpes simplex virus type 1 abrogates the antiviral activity of Ch25h via its virion host shutoff protein. Antiviral Res 143:69–73. https://doi.org/10.1016/j.antiviral.2017.04.004.
32. Johnson KE, Song B, Knipe DM. 2008. Role for herpes simplex virus 1 ICP27 in the inhibition of type I interferon signaling. Virology 374:487–494. https://doi.org/10.1016/j.virol.2008.01.001.
33. Luxton GW, Lee JI, Haverlock-Moyns S, Schober JM, Smith GA. 2006. The pseudorabies virus VP1/2 tegument protein is required for intracellular capsid transport. J Virol 80:201–209. https://doi.org/10.1128/JVI.80.1.201-209.2006.
34. Sandbaumhuter M, Dohner K, Schipke J, Binz A, Pohlmann A, Sodeik B, Bauerfeind R. 2013. Cytosolic herpes simplex virus capsids not only require binding inner tegument protein pUL36 but also pUL37 for active transport prior to secondary envelopment. Cell Microbiol 15:248–269. https://doi.org/10.1111/cmi.12075.
35. Abaitua F, Hollinshead M, Bolstad M, Crump CM, O'Hare P. 2012. A Nuclear localization signal in herpesvirus protein VP1-2 is essential for infection via capsid routing to the nuclear pore. J Virol 86:8998–9014. https://doi.org/10.1128/JVI.01209-12.
36. Roberts AP, Abaitua F, O'Hare P, McNab D, Rixon FJ, Pasdeloup D. 2009. Differing roles of inner tegument proteins pUL36 and pUL37 during entry of herpes simplex virus type 1. J Virol 83:105–116. https://doi.org/10.1128/JVI.01032-08.
37. Desai PJ. 2000. A null mutation in the UL36 gene of herpes simplex virus

type 1 results in accumulation of unenveloped DNA-filled capsids in the cytoplasm of infected cells. J Virol 74:11608–11618. https://doi.org/10.1128/JVI.74.24.11608-11618.2000.
38. Kim ET, Oh SE, Lee YO, Gibson W, Ahn JH. 2009. Cleavage specificity of the UL48 deubiquitinating protease activity of human cytomegalovirus and the growth of an active-site mutant virus in cultured cells. J Virol 83:12046–12056. https://doi.org/10.1128/JVI.00411-09.
39. Ye R, Su C, Xu H, Zheng C. 2017. Herpes simplex virus 1 ubiquitin-specific protease UL36 abrogates NF-kappaB activation in DNA sensing signal pathway. J Virol 91:e02417-16. https://doi.org/10.1128/JVI.02417-16.
40. Symons JA, Alcami A, Smith GL. 1995. Vaccinia virus encodes a soluble type I interferon receptor of novel structure and broad species specificity. Cell 81:551–560. https://doi.org/10.1016/0092-8674(95)90076-4.
41. Alcami A, Symons JA, Smith GL. 2000. The vaccinia virus soluble alpha/beta interferon (IFN) receptor binds to the cell surface and protects cells from the antiviral effects of IFN. J Virol 74:11230–11239. https://doi.org/10.1128/JVI.74.23.11230-11239.2000.
42. Yokota S, Saito H, Kubota T, Yokosawa N, Amano K, Fujii N. 2003. Measles virus suppresses interferon-alpha signaling pathway: suppression of Jak1 phosphorylation and association of viral accessory proteins, C and V, with interferon-alpha receptor complex. Virology 306:135–146. https://doi.org/10.1016/S0042-6822(02)00026-0.
43. Bisson SA, Page AL, Ganem D. 2009. A Kaposi's sarcoma-associated herpesvirus protein that forms inhibitory complexes with type I interferon receptor subunits, Jak and STAT proteins, and blocks interferon-mediated signal transduction. J Virol 83:5056–5066. https://doi.org/10.1128/JVI.02516-08.
44. Shah KM, Stewart SE, Wei W, Woodman CB, O'Neil JD, Dawson CW, Young LS. 2009. The EBV-encoded latent membrane proteins, LMP2A and LMP2B, limit the actions of interferon by targeting interferon receptors for degradation. Oncogene 28:3903–3914. https://doi.org/10.1038/onc.2009.249.
45. Xia C, Vijayan M, Pritzl CJ, Fuchs SY, McDermott AB, Hahm B. 2015. Hemagglutinin of influenza A virus antagonizes type I interferon (IFN) responses by inducing degradation of type I IFN receptor 1. J Virol 90:2403–2417. https://doi.org/10.1128/JVI.02749-15.
46. Zhang R, Xu A, Qin C, Zhang Q, Chen S, Lang Y, Wang M, Li C, Feng W, Zhang R, Jiang Z, Tang J. 2017. Pseudorabies virus dUTPase UL50 induces lysosomal degradation of type I interferon receptor 1 and antagonizes the alpha interferon response. J Virol 91:e01148-17. https://doi.org/10.1128/JVI.01148-17.
47. Xing J, Wang S, Lin F, Pan W, Hu CD, Zheng C. 2011. Comprehensive characterization of interaction complexes of herpes simplex virus type 1 ICP22, UL3, UL4, and UL205. J Virol 85:1881–1886.
48. Saleh AZ, Fang AT, Arch AE, Neupane D, El Fiky A, Krolewski JJ. 2004. Regulated proteolysis of the IFNaR2 subunit of the interferon-alpha receptor. Oncogene 23:7076–7086. https://doi.org/10.1038/sj.onc.1207955.
49. Jia H, Song L, Cong Q, Wang J, Xu H, Chu Y, Li Q, Zhang Y, Zou X, Zhang C, Chin YE, Zhang X, Li Z, Zhu K, Wang B, Peng H, Hou Z. 2017. The LIM protein AJUBA promotes colorectal cancer cell survival through suppression of JAK1/STAT1/IFIT2 network. Oncogene 36:2655–2666. https://doi.org/10.1038/onc.2016.418.
50. Lin R, Yang L, Nakhaei P, Sun Q, Sharif-Askari E, Julkunen I, Hiscott J. 2006. Negative regulation of the retinoic acid-inducible gene I-induced antiviral state by the ubiquitin-editing protein A20. J Biol Chem 281:2095–2103. https://doi.org/10.1074/jbc.M510326200.
51. Zhu H, Zheng C, Xing J, Wang S, Li S, Lin R, Mossman KL. 2011. Varicella-zoster virus immediate-early protein ORF61 abrogates the IRF3-mediated innate immune response through degradation of activated IRF3. J Virol 85:11079–11089. https://doi.org/10.1128/JVI.05098-11.
52. Jordan M, Schallhorn A, Wurm FM. 1996. Transfecting mammalian cells: optimization of critical parameters affecting calcium-phosphate precipitate formation. Nucleic Acids Res 24:596–601. https://doi.org/10.1093/nar/24.4.596.