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STRAP upregulates antiviral innate immunity against PRV by targeting TBK1

Wenfeng He[1†], Hongtao Chang[2†], Chen Li[1†], Chenlong Wang[1], Longxi Li[1], Guoqing Yang[1], Jing Chen[1] and Huimin Liu[1*]

Abstract
Serine/threonine kinase receptor-associated protein (STRAP) serves as a scaffold protein and is engaged in a variety of cellular activities, although its importance in antiviral innate immunity is unknown. We discovered that STRAP works as an interferon (IFN)-inducible positive regulator, facilitating type I IFN signaling during pseudorabies virus infection. Mechanistically, STRAP interacts with TBK1 to activate type I IFN signaling. Both the CT and WD40 7−6 domains contribute to the function of STRAP. Furthermore, TBK1 competes with PRV-UL50 for binding to STRAP, and STRAP impedes the degradation of TBK1 mediated by PRV-UL50, thereby increasing the interaction between STRAP and TBK1. Overall, these findings reveal a previously unrecognized role for STRAP in innate antiviral immune responses during PRV infection. STRAP could be a potential therapeutic target for viral infectious diseases.

Keywords STRAP, TBK1, Pseudorabies virus, Antiviral immunity, Type I interferon

Introduction

Pseudorabies virus (PRV), also known as suid herpesvirus 1 or Aujeszky's illness, belongs to the alphaherpesvirus subfamily and infects a variety of species, including its native host, pigs [1]. PRV infection in swine can cause severe sickness and significant economic losses internationally [2]. Animals pose a risk to human health [3, 4]. PRV is an encapsulated virus with a large linear double-strand DNA genome encoding more than 70 functional proteins [5]. Although vaccines are available to prevent PRV infection, there is still a pressing need for novel preventive treatments to combat PRV infection successfully.

The innate immune system is the initial line of defence against viral infections, identifying pathogen-associated molecular patterns (PAMPs). Cytosolic DNA sensors such as cyclic GAMP synthase (cGAS) detect pathogen-derived DNA by directly binding to it and producing cyclic GMP-AMP (cGAMP) [6, 7]. cGAMP activates the stimulator of interferon gene (STING), which recruits TANK-binding kinase 1 (TBK1) and promotes the phosphorylation of downstream interferon regulatory factor 3 (IRF3), leading to the expression of type I interferon (IFN-I) and downstream antiviral IFN-stimulated genes (ISGs) [8, 9]. TBK1 is an important regulator of IFN-I production, which helps prevent harmful bacteria from invading the body. Increasing data suggest that activation of the cGAS-STING axis is necessary for innate antiviral responses [10, 11]. For example, investigations have shown that the herpes simplex virus 1 (HSV-1) protein ICP27 interacts with the STING-TBK1 complex

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to reduce IRF3 phosphorylation [12], whereas the tegument proteins UL41 and UL46 of HSV-1 directly degrade cGAS mRNA or limit TBK1 activation [13, 14]. Similarly, the human cytomegalovirus (HCMV) tegument protein UL82 has been shown to disrupt STING trafficking and attract TBK1 or IRF3 to STING [15]. HCMV US9 has been confirmed to impair the STING-TBK1 interaction and impede IRF3 nuclear translocation [16]. PRV UL13 also acts as an antagonist of IFN signaling, targeting STING to prevent host antiviral responses [1]. Despite these findings, the functions and molecular mechanisms of TBK1-mediated antiviral responses to PRV infection are largely unknown.

Serine-threonine kinase receptor-associated protein (STRAP) is a scaffolding protein that mediates a variety of cellular processes, including signal transduction, protein transport, transcription control, and RNA processing [17–20]. STRAP suppresses the TGF-β signaling pathway by interacting with Smad7 [21]. The inhibitory effect of STRAP on TGF-β signaling promotes carcinogenesis, as evidenced by its overexpression in breast and lung cancer [22, 23]. Recent research has shown that STRAP regulates signal transduction pathways such as the TGF-β, PI3K/PDK, ASK1, and p53 pathways, which regulate cell growth and apoptosis [20, 24–26]. Furthermore, STRAP acts as a scaffold protein, positively regulating TLR-mediated NF-κB signaling [27]. Acetylation of STRAP regulates p53 activity and stability [26]. STRAP has seven WD40 repeat domains that promote TLR2/4-mediated cytokine production through TAK1-IKKα-p65 interactions [27]. However, the role of STRAP in the host antiviral innate immune response has yet to be studied.

This work revealed that overexpressed STRAP interacts with TBK1 to positively regulate the type I IFN-mediated antiviral response during PRV infection. The connection between STRAP and TBK1 promotes IRF3 phosphorylation and IFN-I production in response to PRV infection. Mechanistically, STRAP inhibits PRV-UL50-mediated TBK1 degradation, enhancing the STRAP-TBK1 interaction in the IFN-I signaling pathway. Our findings reveal the new role of STRAP as a positive regulator of innate immune responses to PRV and suggest that STRAP could be a therapeutic target for viral infectious illnesses.

Materials and methods

Cells, viruses and antibodies

PK15 and BHK21 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (vol/vol) fetal bovine serum (FBS) (Gibco), penicillin and streptomycin. The PRV strain QXX was preserved in our laboratory as previously described [28]. Prof. Tang Jun of Chinese Agricultural University kindly supplied the recombinant PRV UL50 knockout virus (PRV UL50 KO). Viral aliquots were stored at -80 °C until use. Santa Cruz Biotechnology supplied mouse β-actin, anti-HA, anti-Myc, and anti-Flag monoclonal antibodies. Proteintech Group Inc. (Shanghai, China) supplied rabbit anti-STRAP, anti-IRF3, anti-TBK1, anti-cGAS, and anti-STING polyclonal antibodies. Phospho-TBK1 and phospho-IRF3 (Ser396) were purchased from Cell Signaling Technology (Danvers, Massachusetts, USA).

Virus infection and plaque assay

PRV WT, EMCV, and PRV-UL50 KO viruses were cultured and titrated in PK15 cells. For infection, the cells were treated with PRV or PRV-UL50 KO for 1 h, rinsed with PBS, and then incubated in DMEM supplemented with 5% FBS for the durations specified. PK15 cells were treated with either 10 μM MG132 or 0.2 μM bafilomycin A1 (BafA1) for 2 h before being infected with PRV (MO = 1) for 24 h.

Tittering was used to measure viral yield in PK15 cells. Briefly, the supernatants of PRV-infected cells were collected and diluted at a ratio of 1:10 to 1:109. After 1 h, the supernatants were withdrawn, and the cells were covered with media containing 1% agar. At 72 hpi, the cells were fixed for 20 min with 4% formaldehyde and stained with 0.2% crystal violet, and the plaques in each well were counted. The data were averaged and multiplied by the dilution factor to calculate virus titers (PFU/mL).

Plasmid construction

PRV ORFs were amplified from the PRV genome, and swine STRAP (XM_003355564.4), STING (NM_001142838.1), TBK1 (XM_021090852.1), and IRF3 (NM_213770.1) genes were amplified from PK15 cells before being cloned and inserted into pCMV-Myc or pCMV-Flag plasmids. Multiple truncation mutants of STRAP were amplified from the templates of full-length STRAP, which were subsequently cloned and inserted into the pCAGGS-HA plasmid. The luciferase reporter plasmids for IFN-β and NF-κB were stored in our laboratory. DNA sequencing was used to analyze and verify all of the generated plasmids. The plasmids were transfected into PK15 cells via Lipofectamine 3000 (Thermo Scientific) following the manufacturer's instructions.

RNA extraction and real-time quantitative PCR (RT-qPCR)

Total RNA was extracted from BHK21 and PK15 cells via TRIzol reagent (Invitrogen, Carlsbad, CA) and reverse transcribed into cDNA according to the manufacturer's protocol. The ABI QuantStudio 7 qPCR

System and SYBR Green real-time PCR Master Mix were used to perform RT-qPCR. The β-actin gene served as an internal control. The relative expression of mRNA was determined via the comparative cycle threshold (2-ΔΔCT) method. The sequences of the qPCR primers used were as follows: swine PRV-gE-F: GACACGTTCGACCTGATGCC, R: TGGTAGATGCA GGGCTCGTA; swine STRAP-F: TGCTACGCCAGG GAGATACA, R: CAGCATCCCATACTTTGGCTG T; swine IFNα-F: CACCTCAGCCAGGACAGAAGC, R: ATGAGGGGATCCAAAGTCCCT; swine IFN-β β-F: T GATGGGCAGATGGATGACC, R: AGGCACAGCTT CTGT ACTCC; swine Mx1-F: GTCATCGGGGACCA GAGTTC, R: TCCCGGTAACTGAC TTTGCC; swine OAS1-F: GTTTCCGAACGCAGGTCAAG, R: GGAA GACGACGAGGTCAGCATC; swine IFIT1-F: GACT CACAGCAACCATGAGTAATA, R: CCTCATTCTG GCCTTTCAGGT; swine ISG15-F: GGTGAGGAACG ACAAGGGT C, R: GGCTTGAGGTCATACTCCCC; swine β-actin-F: TGGAACGGTGAAGGTGA CAG, R: CTTTTGGGAAGGCAGGGACT.

Coimmunoprecipitation (Co-IP) and Western blotting

At appropriate time points, the cells were collected and lysed in a lysis buffer containing a protease inhibitor cocktail. The cell lysates were then immunoprecipitated with the relevant antibodies, and the coimmunoprecipitation samples were separated via SDS-PAGE before being transferred to PVDF membranes. h at room temperature (RT) and then incubated with the specified primary antibodies for 6–8 h at 4 °C. The membranes were washed three times with TBST before they were incubated with horseradish peroxidase (HRP)-labeled goat anti-mouse IgG or anti-rabbit antibody for one h at room temperature. Chemiluminescence detection reagents from Thermo Fisher Scientific were used to view the antibody-antigen complexes.

Knockdown of STRAP via siRNA

Tsingke Biological Technology (Wuhan, China) developed and produced the small interfering RNAs (siRNAs) employed in this work. STRAP siRNA was transfected into PK15 cells to knock down endogenous STRAP, with NC siRNA serving as a negative control. Lipofectamine 3000 was used for siRNA transfection, as specified by the manufacturer's procedure. The swine STRAP siRNA sequence used was GCACUUCC CACCUGAUAA.

Luciferase reporter assay

PK15 cells were plated in 24-well plates and cotransfected with IFN-β-Luc, ISRE-Luc, or NF-κB-Luc; pRL-TK Renilla luciferase reporter plasmid; or additional plasmids. Twenty-four h later, the firefly and Renilla luciferase activities of the cell lysates were tested via the Dual-Luciferase Reporter Assay Kit (Promega) according to the manufacturer's guidelines.

ELISA

Porcine IFN-α and IFN-β ELISA kits (Solarbio) were used to assess the expression of these proteins in the supernatants of PRV-infected cells. The measured values were compared to the standard according to the manufacturer's instructions.

Nuclear and cytoplasmic extraction

PK15 cells were transfected with several plasmids and then infected with PRV (MOI = 1). The cells were collected, and subcellular fractions were extracted via a nucleus and cytoplasm extraction kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was conducted via Student's *t*-test or analysis of variance (ANOVA) on at least three independent replicates via GraphPad Prism software. A significance level of $P < 0.05$ was used to determine statistical significance for each test. The data from at least three independent experiments are presented as the means ± SDs.

Results

PRV infection upregulates STRAP expression

To explore the function of STRAP in PRV infection, we first examined whether PRV infection affects STRAP expression in cells, including PK15 and BHK21 cells. We detected increases in both the mRNA and protein levels of STRAP in PRV-infected cells compared with those in uninfected cells (Fig. 1A). These findings suggest that PRV infection led to an increase in endogenous STRAP in both PK15 and BHK21 cells. However, an RNA virus (encephalomyocarditis virus, EMCV) failed to induce STRAP mRNA expression in PK15 cells (Fig. 1B).

Next, we sought to evaluate whether endogenous STRAP is required for PRV replication. PK15 cells were infected with ultraviolet-inactivated PRV (UV-PRV) or PRV at an MOI of 1 for 24 h. Notably, UV-PRV infection did not elicit STRAP expression in PK15 cells (Fig. 1C), suggesting that the failure of STRAP induction was possibly due to the inability of PRV gene expression. We previously reported that UV inactivation prevents PRV gene expression after viral entry [28]; thus, STRAP upregulation may be attributed to PRV infection and viral gene expression. Furthermore, STRAP was shown to be localized mainly in the

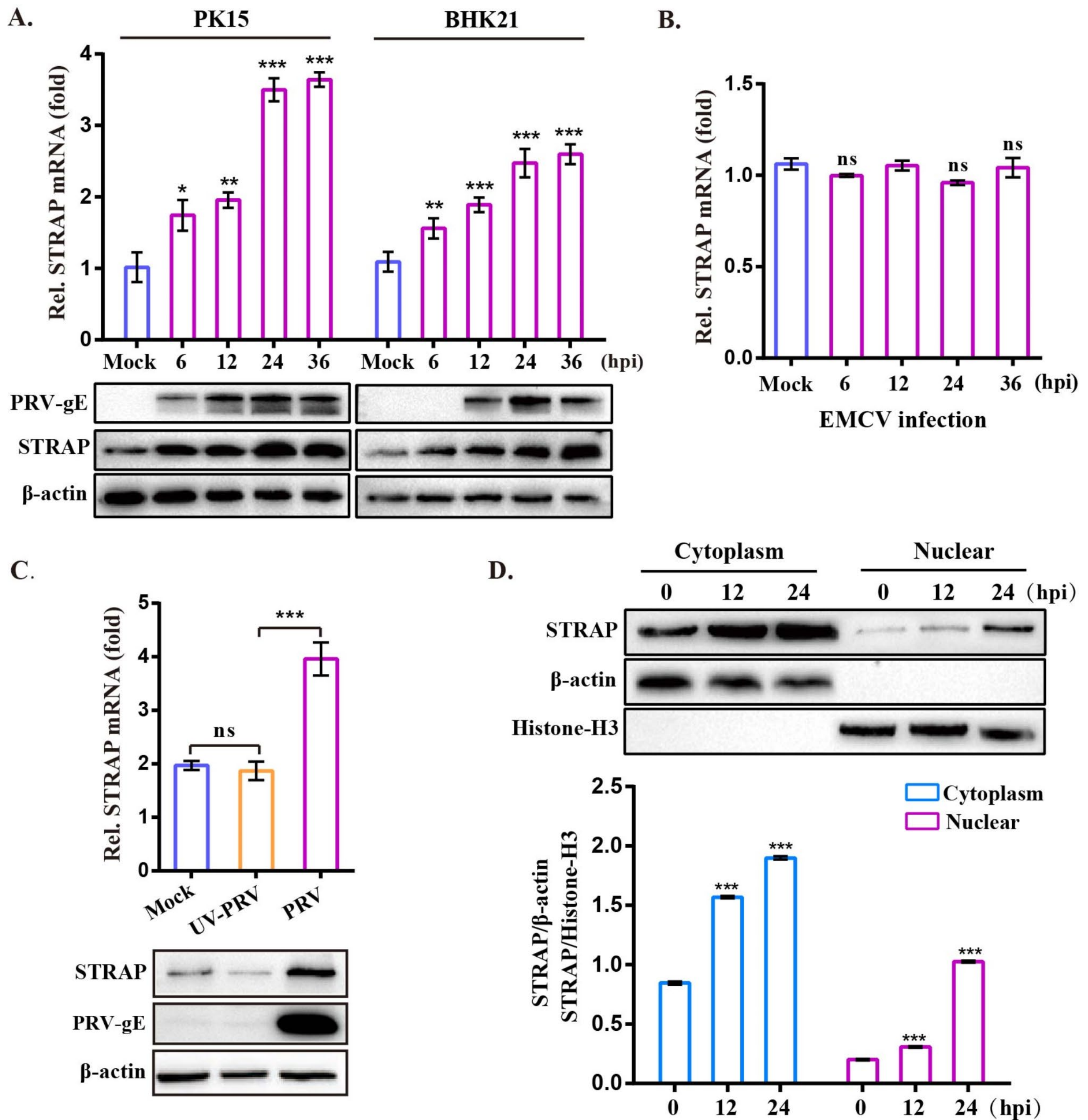

Fig. 1 PRV replication induces STRAP upregulation in PK15 cells. PK15 and BHK21 cells were infected with PRV or EMCV at the indicated time points (MOI = 1). (**A**) The mRNA and protein levels of STRAP were analyzed via RT-qPCR and Western blotting, respectively. (**B**) RT-qPCR was used to examine STRAP mRNA expression in ECMV-infected PK15 cells. (**C**) PK15 cells were infected with PRV or UV-PRV (MOI = 1) for 24 h, after which STRAP and PRV-gE expression levels were detected. (**D**) Nuclear and cytoplasmic extracts from PRV-infected PK15 cells were prepared at the identified times, and the STRAP expression levels were analyzed via Western blotting. The means ± SDs are plotted from triplicate experiments. Statistical significance was analyzed by Student's t test with GraphPad Prism 6.0 software. *, $P < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

cytoplasm, whereas PRV infection caused an increase in STRAP in both the cytoplasm and the nucleus (Fig. 1D). Thus, we concluded that STRAP functions mostly in the cytoplasm. In conclusion, these findings indicate that PRV infection causes an increase in STRAP expression in host cells, suggesting that STRAP plays a crucial role in PRV infection.

STRAP can suppress PRV replication

Given the role of STRAP in PRV infection, we further examined whether STRAP affects PRV replication in vitro. We transfected the STRAP plasmid (HA-STRAP) into PK15 cells. After transfection for 24 h, the cells were infected with PRV at an MOI of 1. We collected infected cell culture supernatants and cell pellets at the indicated time points and measured PRV viral titers and PRV glycoprotein E (gE) expression. When STRAP was overexpressed, we observed reduced PRV-gE mRNA and protein levels (Fig. 2A). Consistent results were observed for the viral titers in the supernatants of the PRV-infected cells (Fig. 2B). Moreover, we designed siRNAs targeting STRAP, transfected the STRAP siRNA into PK15 cells, and detected that the interference efficiency of the STRAP siRNA reached approximately 91% (Fig. 2C). As anticipated, silencing STRAP obviously promoted PRV replication in PK15 cells (Fig. 2D and E). These data indicated that STRAP restricted PRV replication in vitro.

Type I IFN (IFN-I) is crucial for creating an antiviral state and regulating viral infection [29]. As a result, we determined whether the STRAP reduction in PRV replication was due to an IFN-I response and evaluated the effects of STRAP on IFN-I production in response to PRV infection. IFN-I mRNA expression and protein secretion were significantly increased when STRAP was overexpressed but decreased when STRAP was

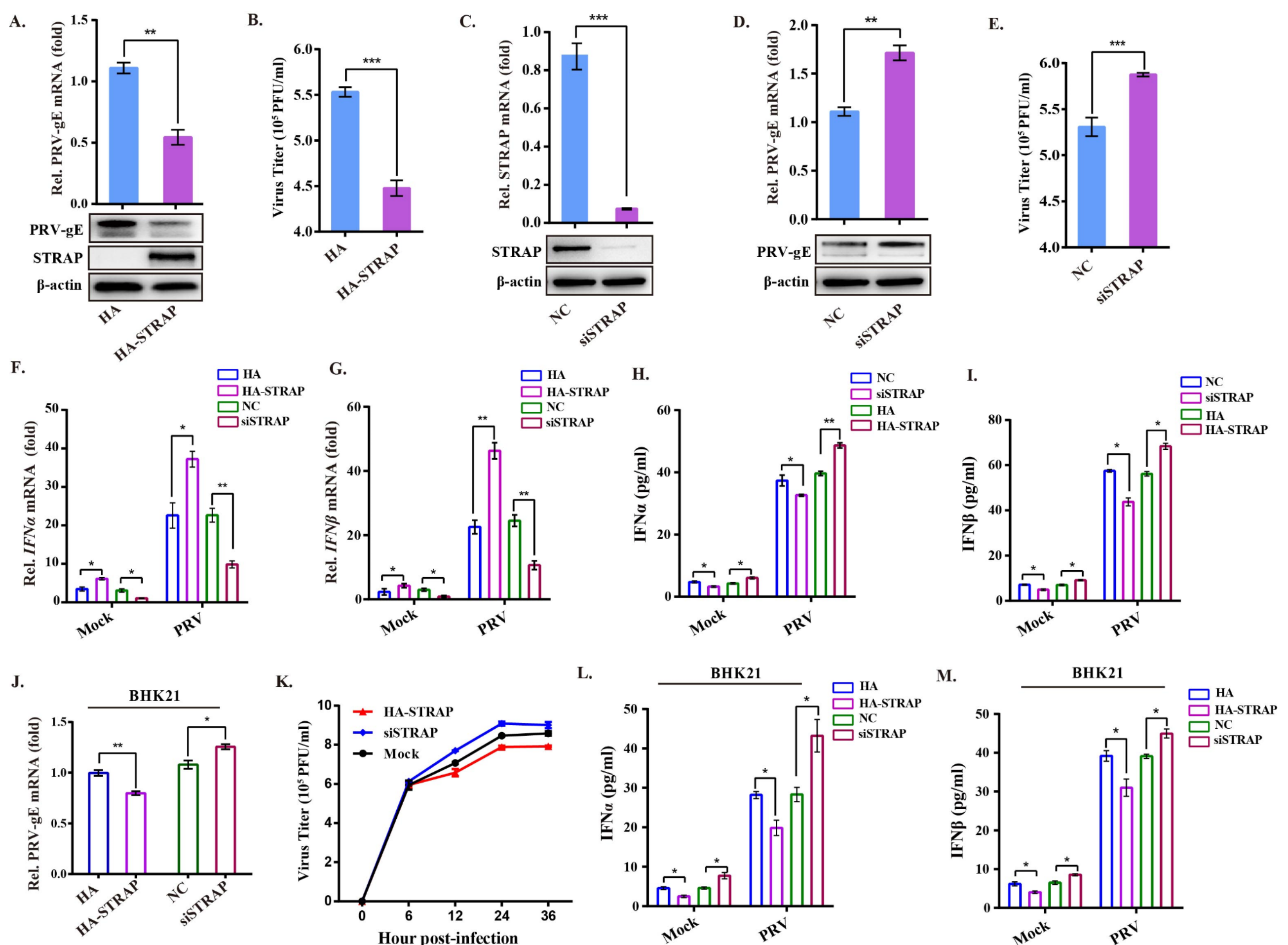

Fig. 2 The overexpression of STRAP promotes the innate immune response to PRV infection. PK15 cells and BHK21 cells were transfected with plasmids encoding HA, HA-STRAP, NC, or siSTRAP. At 24 hpt, the cells were infected with PRV at an MOI of 1, and the mRNA and protein levels of PRV-gE were analyzed by RT-qPCR and Western blotting, respectively (**A** and **D**). The virus titers were measured as the number of viral plaques at 24 hpi (**B**, **E** and **K**). The mRNA and protein levels of IFN-α and IFN-β were examined by RT-qPCR (**F** and **G**) and enzyme-linked immunosorbent assays (ELISAs) (**H**, **I**, **L** and **M**). The means ± SDs are plotted from triplicate experiments. Statistical significance was analyzed by Student's t test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

knocked down (Fig. 2F and I). STRAP thus plays a crucial role in promoting the IFN-I production induced by PRV. To rule out the possibility of cell specificity, we performed the above-described experiments in BHK21 cells and obtained similar results (Fig. 2J and M). Collectively, these data provide evidence that STRAP promotes the IFN-I production triggered by PRV and exhibits antiviral activity.

TBK1 is a potential target of STRAP

Given that STRAP expression increases PRV-induced type I IFN production, we hypothesize that STRAP targets one or more components of the type I IFN signaling pathway. To identify possible targets controlled by STRAP, we first investigated PRV-induced activation of the IFN-β and NF-κB promoters. The overexpression of STRAP increased the PRV-induced activation of the IFN-β promoter but not the NF-κB promoter (Fig. 3A and B). PK15 cells were cotransfected with a Flag-STRAP expression plasmid and plasmids expressing each component of the innate immune signaling pathway (including cGAS, STING, TBK1, and IRF3), along with the IFN-β-Luc or ISRE-Luc and pRL-TK plasmids, to test whether STRAP induces the IFN-I response through the STING-TBK1-IRF3 pathway. A dual-luciferase reporter assay was used to assess the activity of the IFN-β and ISRE promoters. STRAP overexpression significantly increased the activation of the IFN-β and ISRE promoters caused by all agents except IRF3 (Fig. 3C and D). Western blotting revealed that TBK1 and IRF3 phosphorylation were clearly increased in STRAP-overexpressing PK15 cells but decreased in STRAP-silenced cells (Fig. 3E).

In contrast, no changes in the protein expression of cGAS, STING, or the downstream effectors total TBK1 and IRF3 were detected (Fig. 3E). Moreover, our findings indicated that STRAP did not interact with STING or IRF3 (Fig. 3F). As a result, it was anticipated that STRAP might target TBK1 to increase type I IFN production.

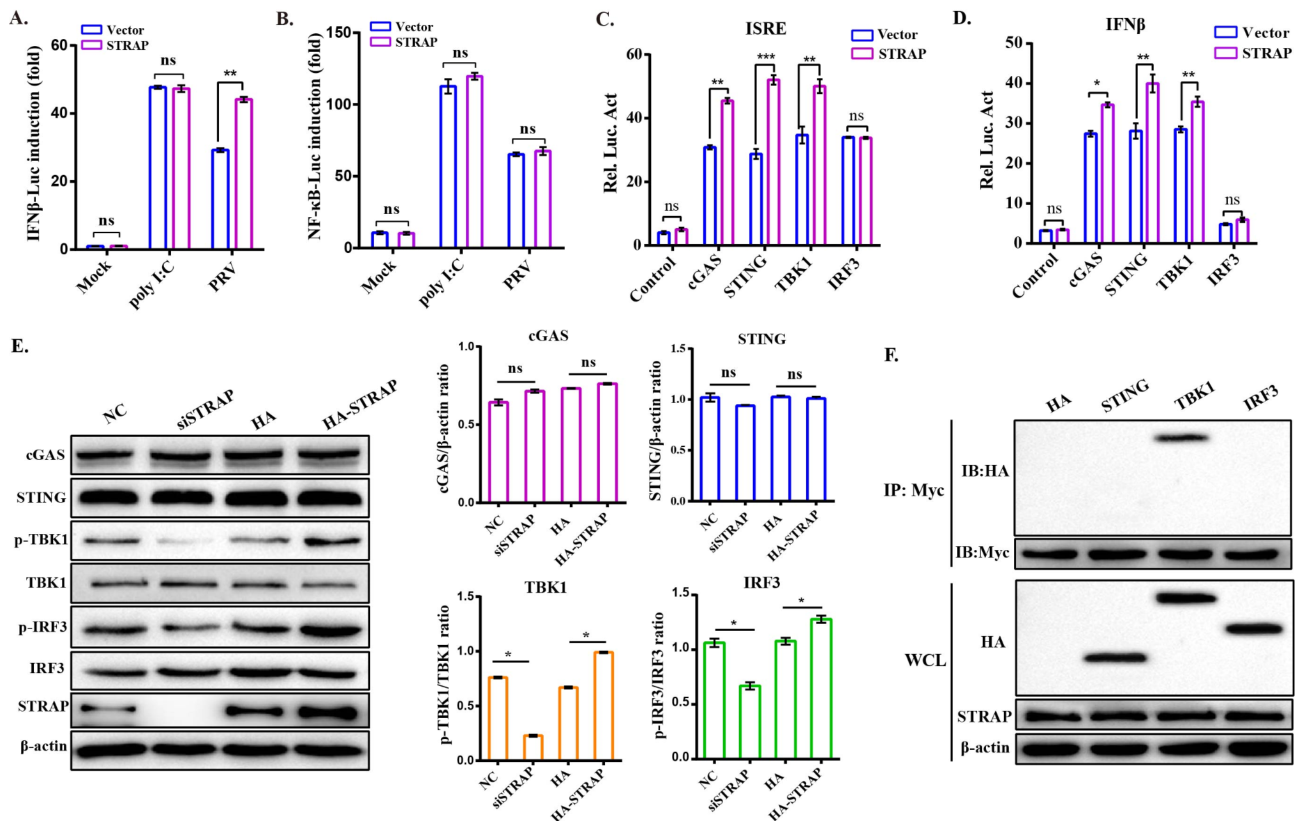

Fig. 3 STRAP promoted type I IFN production by targeting TBK1. PK15 cells were transfected with 0.1 μg of IFN-β-Luc, ISRE-Luc, or 0.01 μg of the pRL-TK plasmid along with the Flag empty vector (EV) or Flag-STRAP expression plasmid. At 24 hpt, the cells were mock-infected, infected with PRV (MOI = 1) or transfected with poly (**I**: **C**). The promoter activation of IFN-β (**A**) and NF-κB (**B**) was determined via a dual-luciferase assay kit. (**C-D**) Luciferase activity of lysates of cells transfected for 24 h with IFN-β-Luc or ISRE-Luc plus STING, TBK1 or IRF3 along with STRAP and EV was analyzed. (**E**) PK15 cells were transfected with EV, HA-STRAP, NC or siSTRAP and then infected with PRV (MOI = 1) for 24 h. Immunoblotting assays were performed with the indicated antibodies. (**F**) PK15 cells were cotransfected with the Myc-STRAP expression plasmid along with the HA empty vector, HA-tagged STING, TBK1 or IRF3. Immunoprecipitation and Western blotting were performed with the identified antibodies. The means ± SDs are plotted from triplicate experiments. Statistical significance was analyzed by Student's t test. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

STRAP interacts with TBK1

To elucidate how STRAP promoted IFN-I production via TBK1, we next explored the possible interaction between STRAP and TBK1. PK15 cells were transfected with the Myc empty vector, Myc-TBK1 expression plasmids, and/or HA-STRAP expression plasmid. After 24 h of transfection, the cells were infected with PRV for 24 h, and the cell lysates were immunoprecipitated with an anti-HA antibody and analyzed via SDS-PAGE. HA-STRAP downregulated Myc-TBK1 (Fig. 4A). To confirm the endogenous interaction between STRAP and TBK1, PK15 cells were infected with PRV, and the cell lysates were immunoprecipitated with anti-TBK1 and anti-IgG antibodies, followed by Western blotting. Endogenous STRAP was shown to pull down TBK1 (Fig. 4B), and endogenous TBK1 did the same in PK15 cell lysates (Fig. 4C). These results support our observation that STRAP targets and interacts with TBK1.

We next addressed which domain of STRAP is essential for its interaction with TBK1. These proteins are thought to stabilize its structure and perform regulatory functions in diverse cellular processes [30]. We created four plasmids expressing different truncated segments of STRAP: STRAP ΔCT (aa 1–293), STRAPΔ7–6 (aa 1–221), STRAPΔ5–4 (aa 1–141), and STRAPΔ3 (aa 1–103) (Fig. 4D). PK15 cells were transfected with Myc-tagged TBK1 together with the HA empty vector, HA-STRAP, and HA-STRAP mutant expression plasmids. The cell lysates were precipitated with an anti-Myc antibody and then probed with an HA antibody. The results demonstrated that the ability of STRAP to bind to TBK1 was clearly affected by the deletion of its C-terminal (CT) region, whereas the deletion of the CT and 6–7 domains eliminated the ability of STRAP to interact with TBK1 (Fig. 4E). These findings suggest that both the CT and WD40 6–7 domains of STRAP are critical for its binding to TBK1.

STRAP potentiates antiviral immunity by interacting with TBK1

These data provide evidence that STRAP overexpression facilitates the phosphorylation of IRF3 upon PRV infection. To assess the impact of the STRAP-TBK1 interaction on IFN-I production and signaling, we used an IFN-β-luc reporter assay in STRAP-overexpressing PK15 cells. Exogenous STRAP expression led to dose-dependent activation of the IFN-β promoter (Fig. 5A). To establish the enhancing effect of STRAP on IFN-β induction, IFN-β transcription was evaluated by RT-qPCR after PRV infection. Similar to the results of the IFN-β reporter assay, STRAP-TBK1 binding increased IFN-β mRNA levels (Fig. 5B). Furthermore, the STRAP-TBK1 interaction increased IRF3 activation (Fig. 5C). These data suggest that STRAP promotes type I IFN signaling by targeting TBK1.

The STING-TBK1-IRF3 pathway is essential for IFN-I production, and activated STING recruits the kinase TBK1 to promote IRF3 phosphorylation [31]. To investigate the influence of STRAP on the STING-TBK1-IRF3 complex, PK15 cells were transfected with plasmids expressing STING, TBK1, and IRF3 along with Flag-STRAP or siSTRAP. We discovered that STRAP overexpression promoted the formation of TBK1-IRF3 dimers (Fig. 5D) and STING-TBK1-IRF3 trimeric complexes (Fig. 5E), but STRAP knockdown prevented complex formation (Fig. 5D and E). We also investigated the effect of the STRAP-TBK1 connection on the transcription of IFN-I and downstream antiviral genes, including IFIT1, OAS1, ISG15, and Mx1. As shown in Fig. 5F and G, the combination of exogenous TBK1 and STRAP dramatically increased IFN-I production and downstream antiviral gene transcription in response to PRV infection. In contrast, the knockdown of STRAP caused a significant decrease in these reactions. Taken together, these findings suggest that STRAP works as a scaffold protein, facilitating the recruitment of STING, TBK1, and IRF3 and resulting in the activation of the type I IFN signaling pathway.

Both the CT and WD40 7 – 6 domains contribute to the antiviral activity of STRAP

As mentioned above, the CT and WD40 7 – 6 domains of STRAP are important for the interaction between STRAP and TBK1. Consistent with this result, the inhibitory effect of the CT deletion mutant on PRV replication was impaired, but the different WD40 truncations of STRAP completely lost the ability to inhibit PRV replication (Fig. 6A and B). Our findings showed that STRAP plays an antiviral role by positively regulating the IFN-I signaling pathway. We studied how different STRAP truncations affect IFN-β induction and ISG expression. The data are shown in Fig. 6C and F. Compared with those in cells transfected with the empty vector (EV), the luciferase activities of IFN-β and ISRE did not obviously change in PK15 cells overexpressing different STRAP truncation mutants. Moreover, the mRNA levels of relevant ISGs in different STRAP truncations were normalized to those in EV-transfected PK15 cells. Inconsistent with a previous report [27], these observations indicated that both the CT and WD40 7 – 6 domains of STRAP are necessary for its antiviral activity.

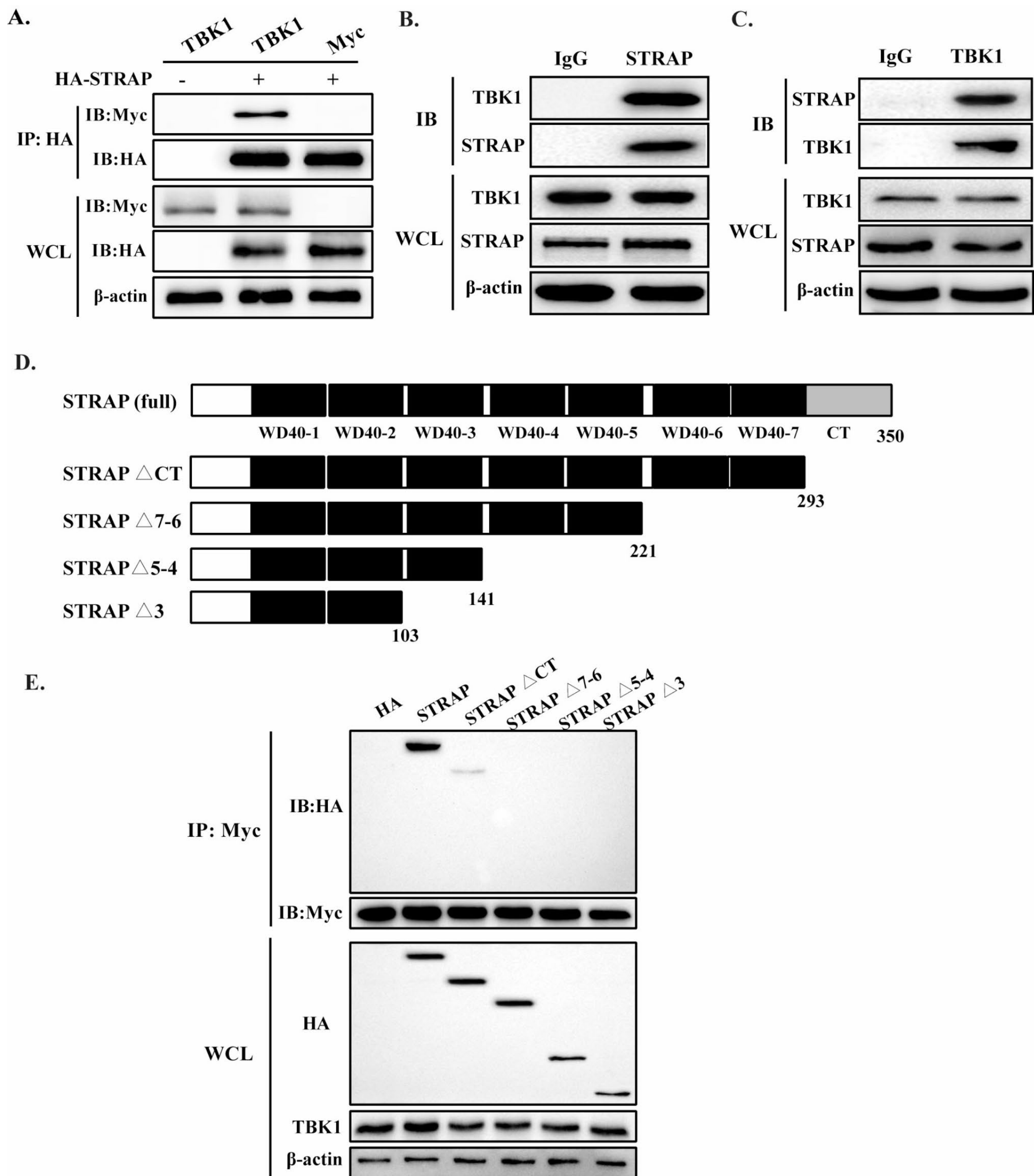

Fig. 4 STRAP interacts with TBK1. (**A**) PK15 cells were transfected with HA-STRAP expression plasmids along with Myc-tagged empty vector or Myc-TBK1 expression plasmids. The cell lysates were immunoprecipitated with an anti-HA antibody and subjected to Western blotting. (**B** and **C**) PK15 cells were infected with PRV for 12 h, and the cell lysates were immunoprecipitated with anti-STRAP and anti-IgG or anti-TBK1 antibodies and subjected to Western blotting. The lysate was immunoprecipitated with IgG as a negative control. (**D**) Schematic illustration of STRAP and its different truncations. (**E**) PK15 cells were cotransfected with plasmids encoding Myc-TBK1 and full-length HA-STRAP or four STRAP mutants for 12 h and then infected with PRV for 24 h. Co-IP and immunoblotting were performed with the indicated antibodies. The means ± SDs are plotted from triplicate experiments. Statistical significance was analyzed by Student's t test with GraphPad Prism 6.0 software. ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

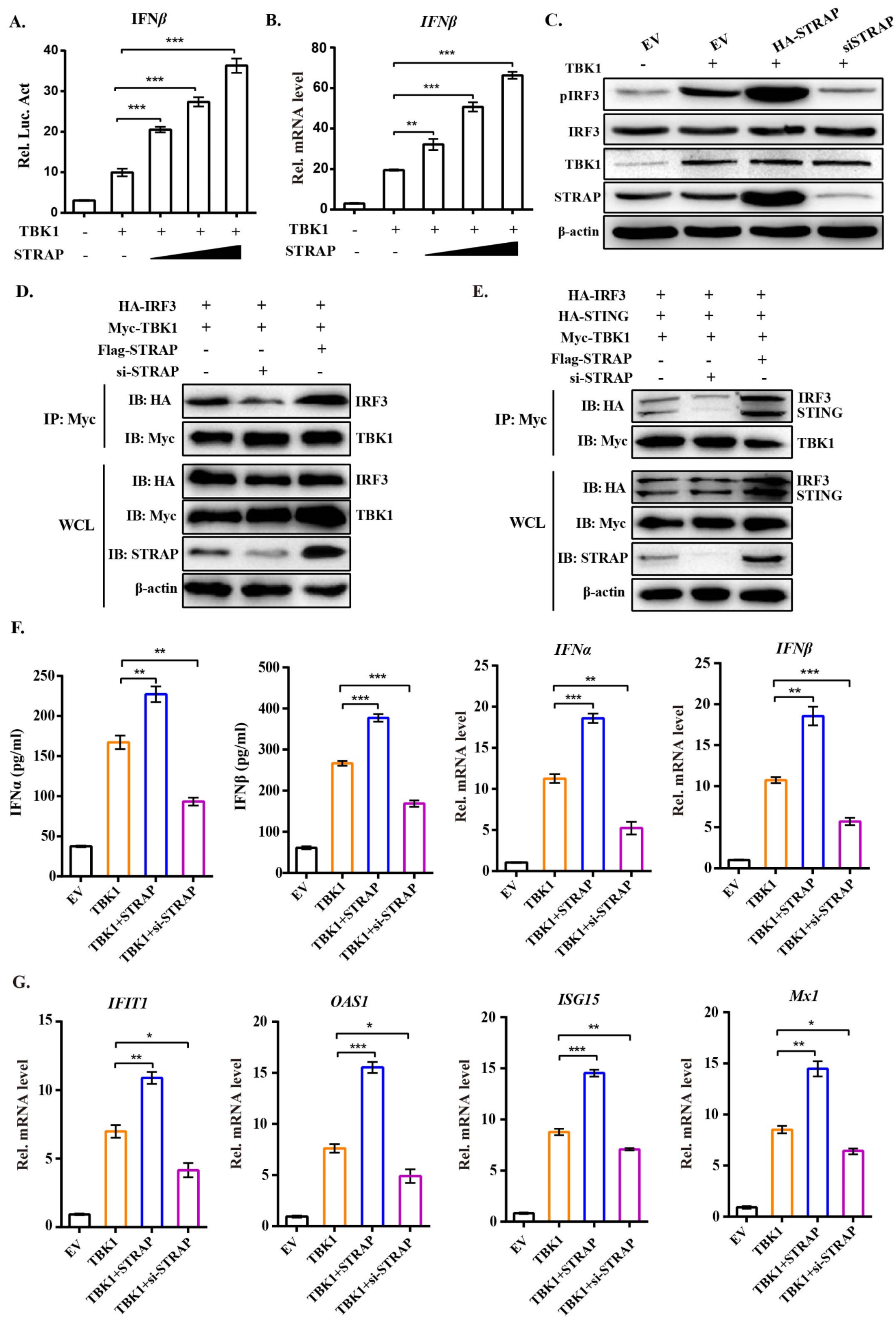

Fig. 5 (See legend on next page.)

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Fig. 5 The STRAP-TBK1 interaction promotes cellular antiviral responses. (**A** and **B**) PK15 cells were transfected with 0.1 μg/well of IFN-β-Luc or 0.01 μg/well of pRL-TK plasmids together with TBK1 or EV with increasing doses of STRAP-expressing plasmid. At 24 hpt, the activity of the IFN-β promoter and the mRNA level of IFN-β were evaluated via a dual-luciferase assay kit and RT-qPCR assays, respectively. (**C**) PK15 cells were cotransfected with TBK1 and plasmids encoding EV, STRAP, or siSTRAP after PRV infection for 24 h. Immunoprecipitation and Western blotting were performed with the identified antibodies. (**D** and **E**) PK15 cells were cotransfected with the indicated plasmids along with Flag-STRAP or si-STRAP for 24 h, followed by PRV infection for 24 h. (**F**) The mRNA and protein levels of IFN-α and IFN-β in PK15 cells transfected with TBK1, TBK1 and STRAP, or TBK1 and siSTRAP were detected by RT-qPCR and ELISA, respectively. (**G**) As in panel F, the mRNA levels of ISGs (IFIT1, OAS1, ISG15 and Mx1) were analyzed by RT-qPCR. The means ± SDs are plotted from triplicate experiments. Statistical significance was analyzed by Student's t test with GraphPad Prism 6.0 software. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

PRV-UL50 can interact with STRAP and induce TBK1 degradation

To further explore the mechanism through which STRAP hinders PRV replication, we analyzed whether STRAP inhibits PRV replication by regulating PRV tegument proteins (UL56, UL50, UL24, UL13, and US3), PRV glycoprotein E (PRV-gE), and PRV thymidine kinase (PRV-TK) proteins. A co-IP assay was performed using the indicated antibodies in PK15 cells subjected to specific plasmid and Myc-STRAP plasmid cotransfection. The results revealed that PRV-UL50 was precipitated via Myc-STRAP (Fig. 7A), indicating that the PRV-UL50 protein directly interacts with STRAP. Furthermore, the CT and WD40 7 – 6 domains of STRAP are required for its interaction with the PRV-UL50 protein (Fig. 7B).

Given that STRAP has antiviral efficacy against PRV through its interaction with TBK1, we investigated the effect of PRV-UL50 on TBK1 expression. PK15 cells were infected with PRV-WT or PRV-UL50-knockout virus (PRV-UL50 KO), and endogenous TBK1 and phosphorylated IRF3 levels were determined. Western blot analysis revealed that UL50 knockout led to increased TBK1 expression and subsequently increased the level of IRF3 phosphorylation (Fig. 7C), indicating that PRV-UL50 could induce TBK1 degradation. Protein breakdown in eukaryotic cells occurs via two major mechanisms: the ubiquitin-proteasome and autolysosome processes [32]. To better understand how PRV-UL50 affects TBK1 stability, PK15 cells were cotransfected with the HA-UL50 plasmid for 24 h and treated with several protein degradation pathway inhibitors. The results demonstrated that TBK1 expression was significantly decreased by ectopic expression of PRV-UL50 (lane 1 and lane 2, Fig. 7D-E), which was consistent with the results shown in Fig. 7C.

Furthermore, no significant difference was observed in the UL50-untransfected group without inhibitors compared with the UL50-transfected group with inhibitors (lane 1 and lane 4, Fig. 7D-E). Notably, ectopic expression of UL50 resulted in partial restoration of TBK1 expression following inhibitor treatment compared with that in the UL50-untransfected group. These data suggest that TBK1 degradation is partially independent of PRV-UL50.

STRAP impairs TBK1 degradation induced by PRV-UL50

Based our findings above, we hypothesized that TBK1 and PRV-UL50 might competitively bind to STRAP. To confirm the role of PRV-UL50 in the interaction between STRAP and TBK1, the endogenous STRAP-TBK1 interactions between PRV WT and UL50 KO-infected PK15 cells were analyzed. Co-IP results demonstrated that PRV UL50 deficiency markedly promoted the STRAP-TBK1 interaction (Fig. 8A-B), indicating that STRAP can competitively interact with TBK1 and PRV-UL50. The next step was to determine whether the STRAP-TBK1 interaction affected the binding of STRAP and PRV-UL 50. PK15 cells were cotransfected with Myc-TBK1 and Flag-STRAP, followed by increasing dosages of HA-UL50. The immunoblot results revealed that TBK1 expression decreased progressively, accompanied by an increase in PRV-UL50, but STRAP expression remained unchanged (Fig. 8C). These findings indicate that the binding of STRAP and UL50 suppresses the STRAP-TBK1 interaction. To further validate this, PK15 cells were cotransfected with HA-UL50 and Flag-STRAP, as well as escalating dosages of TBK1. This result further confirmed that the STRAP-TBK1 interaction hindered the interaction between STRAP and UL50 (Fig. 8D). Together, these findings support the hypothesis that TBK1 and PRV-UL50 may competitively interact with STRAP.

We found that STRAP exerts its anti-PRV activity by promoting IFN-I production, while PRV-UL50 can inhibit the IFN-I signaling pathway [33]. Therefore, we postulate that the STRAP-TBK1 interaction may hinder the interaction of STRAP and PRV-UL50, thereby impairing the inhibitory effect of UL50 on the IFN-I response. To validate this hypothesis, PK15 cells were transfected with Flag-STRAP and HA-UL50 plasmids in the presence or absence of BafA1 or MG132, and the levels of endogenous TBK1 protein were assessed. Notably, STRAP overexpression partially restored the inhibition of TBK1 (lanes 1 and 2 to 5 and 6, Fig. 7D and E). Importantly, the UL50-induced degradation of TBK1 was restored by the overexpression of STRAP, mainly via Baf A1 treatment, but not MG132 (lanes 3 and 4 to lanes 7 and 8, Fig. 7D and E). These data demonstrated that STRAP could block the TBK1 degradation induced by PRV-UL50 partially via the autophagy

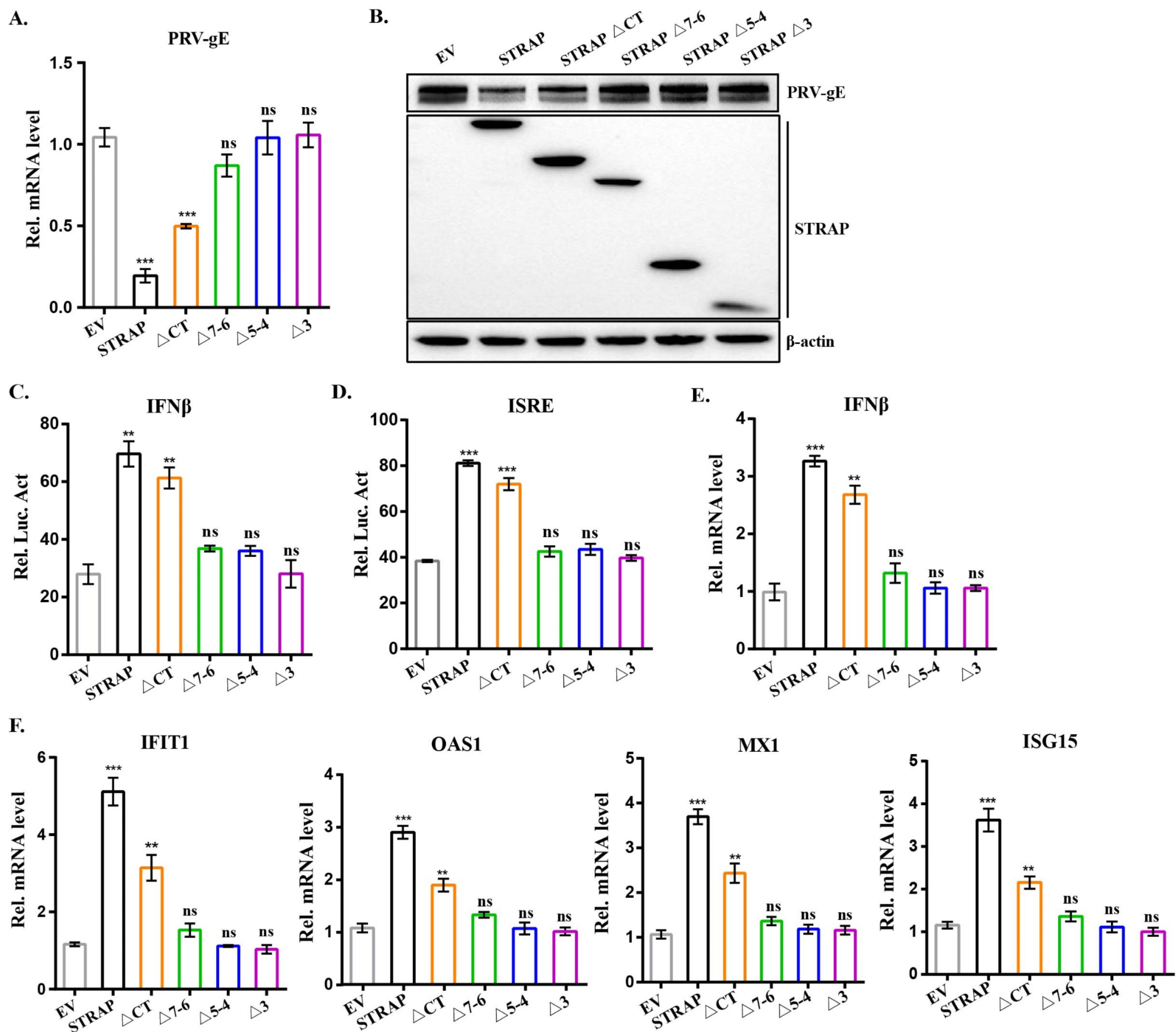

Fig. 6 The CT and WD40 7 – 6 domains of STRAP are essential for its antiviral activity. (**A** and **B**) PK15 cells were cotransfected with STRAP and its four truncation mutants, and the mRNA and protein levels of PRV-gE were detected by RT-qPCR and Western blotting, respectively. (**C** and **D**) Luciferase activities of IFN-β, IFN-β and ISRE were detected via a dual-luciferase assay. (**E** and **F**) PK15 cells were transfected with EV, STRAP or their different mutants, and the mRNA levels of ISGs (IFN-β, IFN-β, IFIT1, OAS1, Mx1 and ISG15) were detected via RT-qPCR. The data are representative of three independent experiments. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, no significant difference according to Student's test

pathway. This finding was further confirmed in PK15 cells treated with Baf A1 at different time points (Fig. 8E). As shown in Fig. 8E, the expression level of TBK1 decreased with prolonged UL50 transfection time in the absence of Baf A1 (lanes 1–4), providing additional evidence of UL50-mediated TBK1 degradation. However, this degradation was restored by the overexpression of STRAP. However, this mechanism needs to be investigated further.

On the basis of these observations, we propose that STRAP, as a scaffold protein, prevents the TBK1 degradation induced by UL50 to enhance the STRAP-TBK1 interaction, thereby promoting type I IFN-mediated antiviral activity.

Discussion

The cGAS-mediated innate immune response forms the first line of defence that protects hosts from invasion by DNA viruses. Viral infection activates the IFN-β signaling pathway, leading to the production of IFN-β and ISGs and the initiation of an adaptive immune response. Investigating the processes underlying the innate immune response has enormous potential for improving disease control and developing effective vaccines. In the present study, we investigated

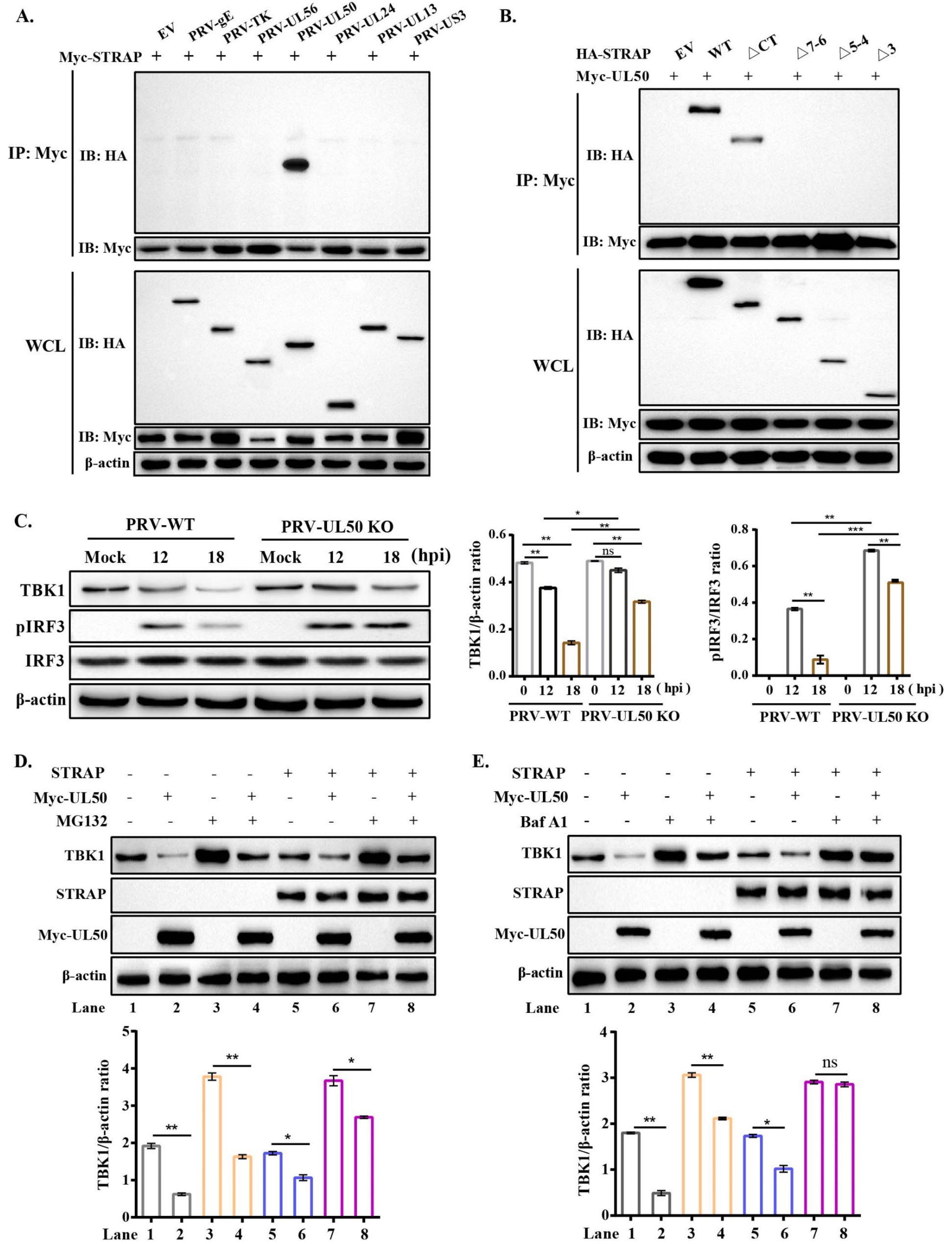

Fig. 7 (See legend on next page.)

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Fig. 7 PRV-UL50 can induce TBK1 degradation. (**A**) PK15 cells were cotransfected with Myc-STRAP and HA-tag vectors encoding the following PRV proteins: gE, TK, UL56, UL50, UL24, UL13 or US3. At 24 hpt, the cells were lysed in an IP lysis buffer, and whole-cell lysates (WCLs) were loaded as input. WCLs were incubated with the indicated antibody and protein A+G, and the precipitates were fractionated by SDS-PAGE. Western blotting was performed with the appropriate antibody. (**B**) The interactions between Myc-UL50 and plasmids encoding full-length STRAP and four STRAP truncation mutants were also analyzed by Western blotting. (**C**) PK15 cells were infected with either wild-type PRV (PRV WT) or a recombinant PRV UL50-knockout virus (PRV UL50 KO) (MOI=1). At 12 and 18 hpi, the protein expression of total TBK1 and phosphorylated IRF3 was detected by Western blotting. (**D** and **E**) PK15 cells were cotransfected with the indicated plasmids for 24 h, infected with PRV, and treated with BafA1 or MG132 for an additional 6 h. The experiments were independently repeated two or three times, with similar results

the involvement of STRAP in the type I IFN-mediated innate immune response to PRV. The overexpression of STRAP dramatically decreased IFN-β promoter activation and induction in response to PRV, whereas STRAP knockdown had the opposite effect. This finding highlights the important function of STRAP in the innate immune response.

While STRAP is a scaffold protein involved in a variety of cellular processes, its involvement in regulating innate immunity is not well recognized [18, 19, 34, 35]. In this study, we presented five lines of evidence demonstrating that STRAP has a favorable regulatory influence on the type I IFN signaling response to PRV infection. First, we found that STRAP expression increased significantly in response to PRV infection, suggesting that STRAP plays an important role during PRV infection. Second, we demonstrated that the overexpression or silencing of STRAP leads to increased or decreased production of IFN-I triggered by PRV infection, respectively, underscoring the critical role of STRAP in promoting the innate immune response against PRV. Third, we revealed that STRAP facilitates the IFN-I signaling pathway against PRV infection by targeting the kinase TBK1. Fourth, we revealed that both the CT and WD40 7−6 domains contribute to the function of STRAP in the IFN-I signaling pathway. Finally, we showed that STRAP impairs the ability of PRV-UL50 to degrade TBK1, thereby promoting the interaction between STRAP and TBK1. These findings confirm that STRAP is a positive regulator of IFN-I signaling and emphasize its role in host innate immunity against PRV infection, which may extend to other viral infections.

Previous research has indicated that STRAP positively regulates the TLR-mediated signaling pathway [27] but negatively regulates the TGF-β signaling pathway [21]. We discovered that STRAP is a positive regulator of the IFN-I signaling pathway, contributing to the host antiviral response against PRV through interactions with TBK. STRAP has been demonstrated to interact with PDK1 and p53, regulating ASK1 and p53 activities [20, 25]. STRAP interacts directly with Smad proteins to inhibit TGF-β signaling [21]. signaling pathways. Furthermore, we discovered that STRAP is found primarily in the cytoplasm, with only a tiny amount in the nucleus (Fig. 1D). These findings indicate that STRAP is largely active in the cytoplasm.

WD40 repeat proteins play regulatory roles in a variety of biological processes [30]. Despite their lack of intrinsic enzymatic activity, the WD40 domain of STRAP plays an important role in mediating protein-protein interactions. Our findings demonstrate that the WD40 domain of STRAP is critical for its interaction with TBK1 and the antiviral response. To study the importance of the WD40 region, we created four STRAP truncations by removing one or two WD40 repeats from the C-terminus, with or without intervening regions. These four truncations have fewer interactions with TBK1 and anti-PRV activity than the wild-type STRAP and STRAP with C-terminal deletions do. Both the CT and WD40 7−6 domains of STRAP may play important roles in attracting other cellular proteins in IFN-I signaling. This regulation is similar to the synergistic effect of the STRAP-Smad7 interaction in suppressing TGF-β signaling [21] and aligns with the favorable involvement of STRAP in regulating the MyD88-dependent TLR2/4 signaling pathway [27]. However, contrary to our findings, a prior investigation revealed that the C-terminal domain is necessary for functional activity in TLR3-mediated cytokine generation [36]. Finally, our findings identify a previously unknown role for STRAP in host resistance against PRV infection.

TBK1, a key kinase for IFN production, is phosphorylated after virus infection and is required for its activation and the production of type I IFNs [10]. The data presented in this study provide evidence that the overexpression of STRAP enhances TBK1 phosphorylation, whereas STRAP knockdown decreases TBK1 phosphorylation following PRV infection (Fig. 3E). These findings support the critical role of STRAP in TBK1 activation and IFN-I signaling. However, the precise mechanism by which STRAP regulates TBK1 kinase activity needs to be further investigated. Additionally, TBK1 stability is required for its ability to control type I IFN signaling. Several regulators, including DTX4, NLRP4, TRIM27, USP38, TRIP, and TRAF3IP3, can degrade TBK1 via the ubiquitin-proteasome pathway [37–41]. We demonstrated that UL50, encoded by PRV, can induce TBK1 degradation through both the proteasome and autophagy pathways. Notably, the

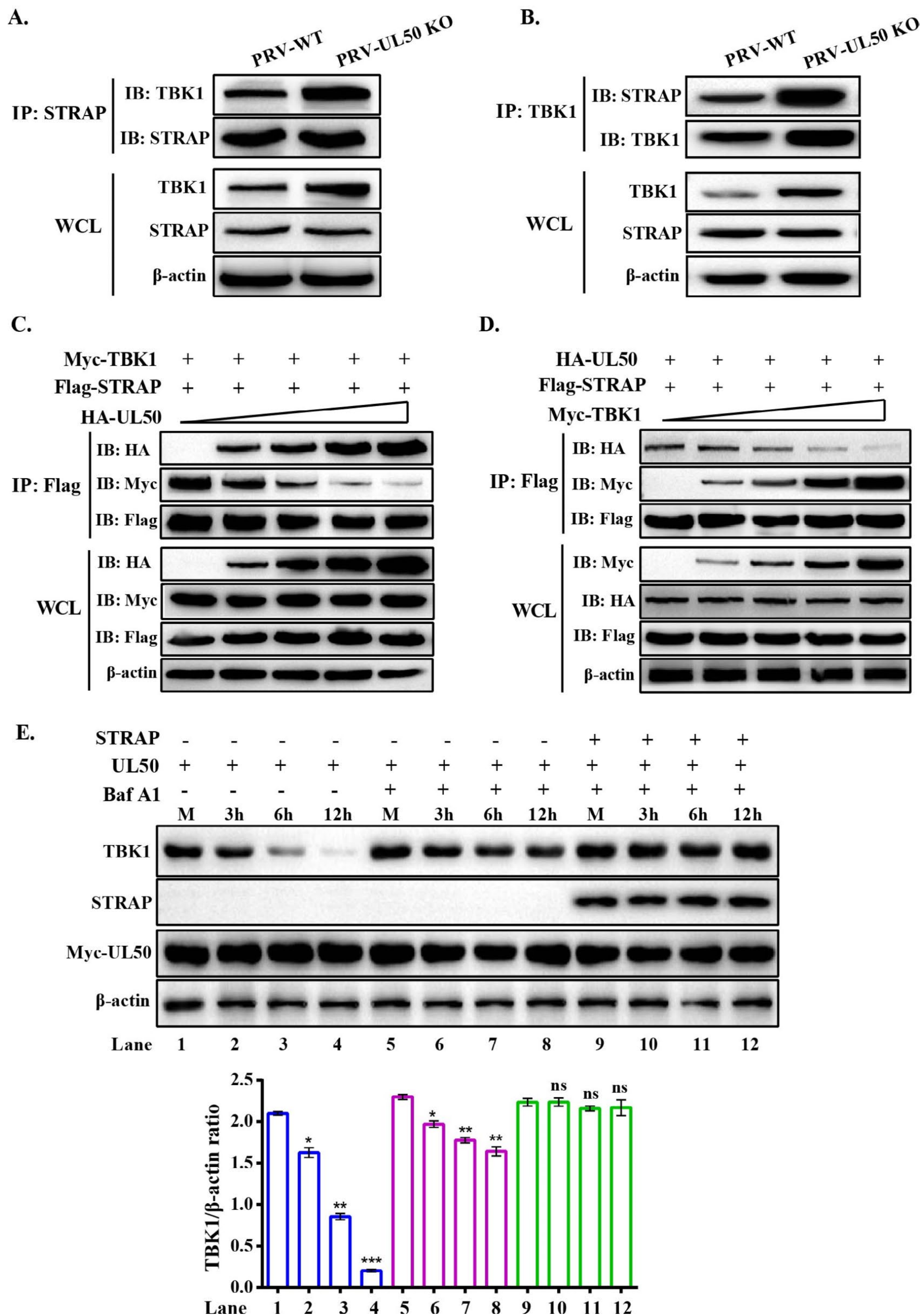

Fig. 8 STRAP blocks TBK1 degradation. (**A** and **B**) PK15 cells were infected with either PRV WT or PRV-UL50 KO (MOI = 1) for 24 h, and the interaction between endogenous STRAP and TBK1 was detected via Co-IP. (**C** and **D**) PK15 cells were cotransfected with the indicated plasmids with increasing doses of UL50 or TBK1 plasmids for 24 h and infected with PRV (MOI = 1). (**E**) PK15 cells were transfected with the indicated plasmids for 24 h and then infected with PRV with or without BafA1 for different durations. An immunoblot assay was performed with the indicated antibodies. The experiments were independently repeated two or three times, each yielding similar results

TBK1 degradation induced by PRV-UL50 was counteracted by STRAP overexpression. Thus, STRAP might play an essential role in the maintenance of TBK1 stability.

Herpesviruses, including HCMV and HSV-1, have evolved diverse mechanisms to circumvent host antiviral immunity and promote viral infection. However, research on the role of PRV proteins in altering the cGAS-STING signaling pathway is rare compared with that of other herpesviruses [12, 14, 15]. We found that STRAP can interact with PRV-UL50, a tegument protein encoded by PRV. Additionally, we determined that UL50 decreases TBK1 expression, thereby impairing the phosphorylation of IRF3, which supports the notion that PRV inhibits type I IFN signaling to establish persistent infection. Importantly, our data revealed that STRAP interacts competitively with TBK1, interrupting the STRAP–UL50 connection, increasing TBK1 stability, and hence driving IFN-I production. These findings provide more information regarding the regulatory mechanism of STRAP in the IFN-I signaling pathway, as well as a potential method by which UL50 reduces IFN-I production. This finding offers a more comprehensive explanation for why STRAP promotes cellular antiviral activity in response to PRV.

On the basis of our findings, we developed a model that explains the role of STRAP in antiviral innate immune repositories (Fig. 9). STRAP positively regulates the PRV-triggered innate immune response by interacting with TBK1 and inhibiting TBK1 degradation produced by PRV-UL50, increasing the synthesis of IFN-I and its downstream ISGs, which inhibits PRV replication. The function of STRAP is determined by its CT and WD40 7–6 domains. Finally, our findings reveal an underlying mechanism by which STRAP positively regulates type I IFN signaling by targeting TBK1, which contributes to a better understanding of the positive regulation of host innate immune responses and the role of STRAP during PRV infection.

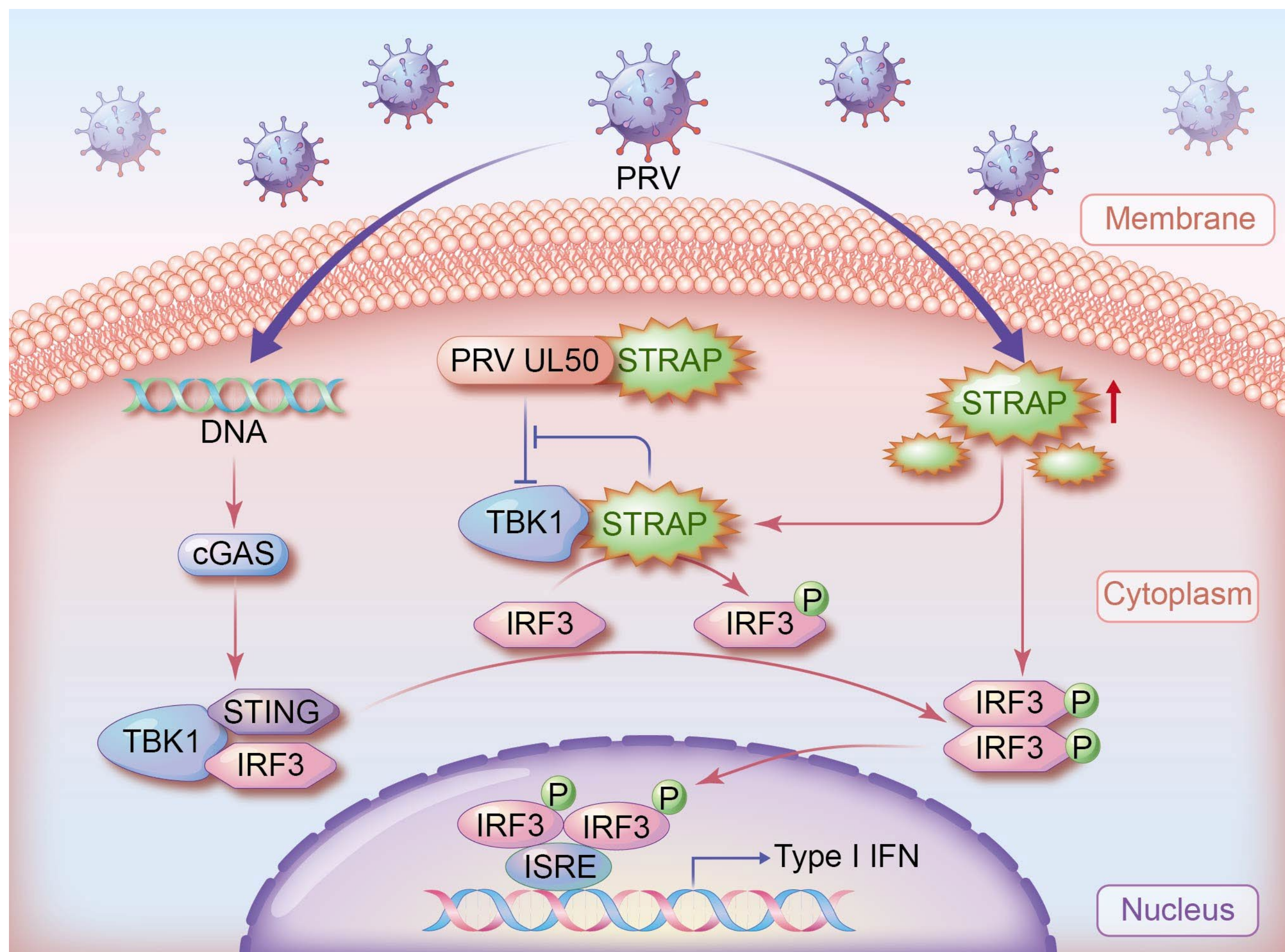

Fig. 9 Schematic representation of the proposed model. In this model, STRAP positively regulates the PRV-triggered innate immune response. STRAP interacts with TBK1 to block the binding of PRV-UL50 to STRAP, resulting in increased production of type I IFN, which in turn suppresses PRV replication

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12985-024-02474-z.

Supplementary Material 1

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Author contributions

H. WF, C.HT. and L. C. wrote the main manuscript text and W.CL. and L.LX. prepared Fig. 9. L.HM. wrote and review manuscript. All authors reviewed the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

All methods and experimentations are performed in accordance with the relevant guidelines and regulations of the Animal Care and Use Committee of Henan Agricultural University, China.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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