AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology

Immunology | Full-Length Text

Deacetylase Sirtuin 1 mitigates type I IFN- and type II IFN-induced signaling and antiviral immunity

Shuang-Shuang Yu,[1] Rong-Chun Tang,[1] Ao Zhang,[1] Shijin Geng,[1] Hengxiang Yu,[1] Yan Zhang,[1] Xiu-Yuan Sun,[1] Jun Zhang[1]

AUTHOR AFFILIATION See affiliation list on p. 18.

ABSTRACT Type I and type II IFNs are important immune modulators in both innate and adaptive immunity. They transmit signaling by activating JAK-STAT pathways. Sirtuin 1 (SIRT1), a class III NAD^+-dependent deacetylase, has multiple functions in a variety of physiological processes. Here, we characterized the novel functions of SIRT1 in the regulation of type I and type II IFN-induced signaling. Overexpression of SIRT1 inhibited type I and type II IFN-induced interferon-stimulated response element activation. In contrast, knockout of *SIRT1* promoted type I and type II IFN-induced expression of ISGs and inhibited viral replication. Treatment with SIRT1 inhibitor EX527 had similar positive effects. SIRT1 physically associated with STAT1 or STAT3, and this interaction was enhanced by IFN stimulation or viral infection. By deacetylating STAT1 at K673 and STAT3 at K679/K685/K707/K709, SIRT1 downregulated the phosphorylation of STAT1 (Y701) and STAT3 (Y705). *Sirt1*$^{+/-}$ primary peritoneal macrophages and *Sirt1*$^{+/-}$ mice exhibited enhanced IFN-induced signaling and antiviral activity. Thus, SIRT1 is a novel negative regulator of type I and type II IFN-induced signaling through its deacetylase activity.

IMPORTANCE SIRT1 has been reported in the precise regulation of antiviral (RNA and DNA) immunity. However, its functions in type I and type II IFN-induced signaling are still unclear. In this study, we deciphered the important functions of SIRT1 in both type I and type II IFN-induced JAK-STAT signaling and explored the potential acting mechanisms. It is helpful for understanding the regulatory roles of SIRT1 at different levels of IFN signaling. It also consolidates the notion that SIRT1 is an important target for intervention in viral infection, inflammatory diseases, or even interferon-related therapies.

KEYWORDS SIRT1, interferon, deacetylation, STAT1, STAT3

Type I IFN (IFN-I; e.g., IFNα and IFNβ) and type II IFN (IFNγ) have a wide range of functions in both innate and adaptive immunity (1, 2). To initiate the signaling cascade, IFN-I binds to IFN-I receptors (IFNAR1 and IFNAR2). The IFN-I receptor IFNAR1 or IFNAR2 is constitutively associated with the JAK family kinases, TYK2 or JAK1. When IFN-I binds to the IFN-I receptors, it brings together TYK2 and JAK1, which phosphorylate and activate each other, promoting the phosphorylation of STAT1 and STAT2. Phosphorylated STAT1, phosphorylated STAT2, and IRF9 form a complex called interferon-stimulated gene factor 3 (ISGF3). ISGF3 then translocates into the nucleus and binds to the interferon-stimulated response element (ISRE) to promote the transcription of a series of interferon-stimulated genes (ISGs) (3). In addition to STAT1 and STAT2, type I IFN signaling can activate other members of the STAT family, including STAT3, STAT4, STAT5A/5B, or STAT6 under certain circumstances (4, 5).

IFNγ, the member of type II IFN, binds to IFNGR (IFNGR1 and IFNGR2) to initiate the signaling cascade. Receptor binding facilitates phosphorylation of JAK1 and JAK2. Activated JAK1/JAK2 then phosphorylates the IFNGR1 chain and recruits STAT1 to bind to IFNGR1. Following phosphorylation by JAK1/2, phosphorylated STAT1 then assembles

Editor J.-H. James Ou, University of Southern California, Los Angeles, California, USA

Address correspondence to Jun Zhang, junzhang@bjmu.edu.cn.

The authors declare no conflict of interest.

See the funding table on p. 18.

Received 17 January 2024
Accepted 31 January 2024
Published 22 February 2024

into a homodimer, translocates to the nucleus, and binds to the γ-interferon-activated sequence region of the ISG promoters (3). In addition to STAT1, STAT3 is also activated by IFNγ treatment (6). Aberrantly activated type I and/or type II IFN signaling pathways are associated with excessive inflammation and the development of autoimmune diseases (7–10). Therefore, both the type I IFN and type II IFN signaling pathways are under strict regulation to ensure that appropriate responses are elicited.

Acetylation is an important type of post-translational modification that is a reversible, dynamic process mediated by lysine acetyltransferases and lysine deacetylases. Both histone and non-histone proteins can be acetylated (11). Acetylation modification can affect gene transcription, enzyme activity, protein stability, or subcellular localization of the protein (12). It may crosstalk with other post-translational modifications, synergizing or antagonizing them and then exerting the corresponding biological effects (13–17). Acetylation and deacetylation have been documented to modulate type I or type II IFN-mediated signaling (18), but there are still unknown targets and mechanisms worth exploring. Sirtuin 1 (SIRT1), the most studied member of the class III NAD^+-dependent deacetylases, is involved in various physiological and pathological processes by deacetylating a variety of histones or non-histone proteins (19). By regulating the acetylation balance, and the crosstalk with other post-translational modifications, SIRT1 modulates cell proliferation and differentiation, apoptosis, autophagy, oxidative stress, metabolism, and inflammation. Known non-histone targets of SIRT1 include p53, FOXO1/3/4, HIF1α, p65, p300, TIP60, IRF3, IRF7, IFI16, etc. (20–26). It is closely associated with several diseases such as autoimmune diseases and even cancer (27, 28). The agonists or inhibitors of SIRT1 have been developed to be potential treatment strategies for inflammation or autoimmune diseases (29). However, it is possible that there are still unknown targets or pathways for SIRT1 in the regulation of inflammation or inflammation-related pathways.

In this study, we characterized the novel regulatory functions of SIRT1 in type I and type II IFN-induced pathways. It attenuated the signaling pathways by deacetylating STAT1 and STAT3 and reducing their phosphorylation levels, which then promoted viral replication.

RESULTS

SIRT1 negatively modulates type I IFN-induced signaling

To assess whether SIRT1 is involved in type I IFN-induced signaling, we first performed Dual-Luciferase reporter assays stimulated by IFNα or IFNβ. As shown in Fig. 1A and B, overexpression of SIRT1 suppressed IFNα- or IFNβ-induced ISRE activation. Treatment with the potent and selective SIRT1 inhibitor, EX527 (30), resulted in enhanced IFNα- or IFNβ-induced ISRE activation (Fig. 1C and D). Then we attempted to examine the effects of knockout of endogenous *SIRT1* on type I IFN-induced transcription of downstream genes by real-time PCR. Compared to that in WT cells, the expression of downstream genes such as *IFIT1*, *ISG12*, and *OAS1* induced by IFNα or IFNβ was increased in *SIRT1-KO* cells (Fig. 1E and F). Almost all STAT family members are activated in type I IFN-induced activation. STAT1 and STAT2 are the characteristic STAT family members activated by type I IFN treatment (4). STAT3 is another important STAT member, which is also activated by type I IFN treatment (5). Therefore, we monitored the effects of SIRT1 expression on the phosphorylation state of STAT1, STAT2, and STAT3. As shown in Fig. 2A and B, knockout of *SIRT1* enhanced IFNα- or IFNβ-induced phosphorylation of STAT1 (Y701) and STAT3 (Y705). As shown in Fig. 2C and D, inhibition of SIRT1 by EX527 enhanced the phosphorylation of STAT1 (Y701) or STAT3 (Y705) induced by type I IFNs such as IFNα or IFNβ. In contrast, knockout of *SIRT1* or treatment with EX527 had no effects on the phosphorylation of STAT2 (Y690) induced by IFNα or IFNβ (Fig. 2E through H). Taken together, these data suggest that SIRT1 may be a novel negative modulator in type I IFN-induced JAK-STAT signaling.

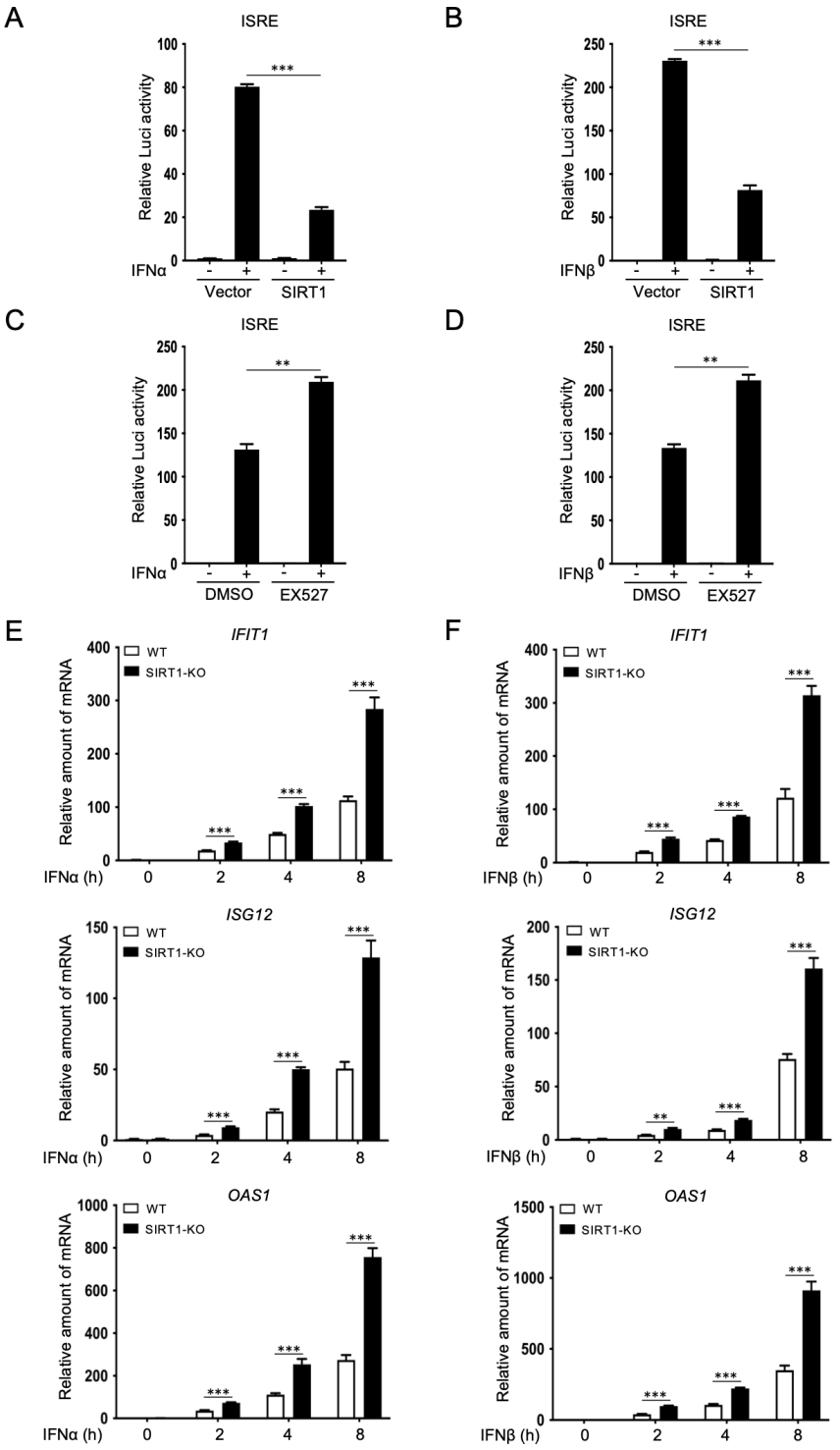

FIG 1 SIRT1 attenuates type I IFN-mediated response. (A and B) Luciferase activity analysis of an ISRE reporter in human embryonic kidney (HEK) 293T cells transfected with control vector or SIRT1 expressing plasmid, followed by treatment with 10 ng/mL IFNα (A) or IFNβ (B) for 12 h. (C and D) Luciferase activity analysis of an ISRE reporter in HEK293T cells pretreated with DMSO or 20 µM EX527 for 2 h, then treated with 10 ng/mL IFNα (C) or IFNβ (D) for 12 h. (E and F) qPCR analysis of *IFIT1*, *ISG12*, or *OAS1* mRNA in WT and *SIRT1-KO* HCT116 cells treated with 10 ng/mL IFNα (C) or IFNβ (D) for indicated time points. $^{**}P < 0.01$, $^{***}P < 0.001$.

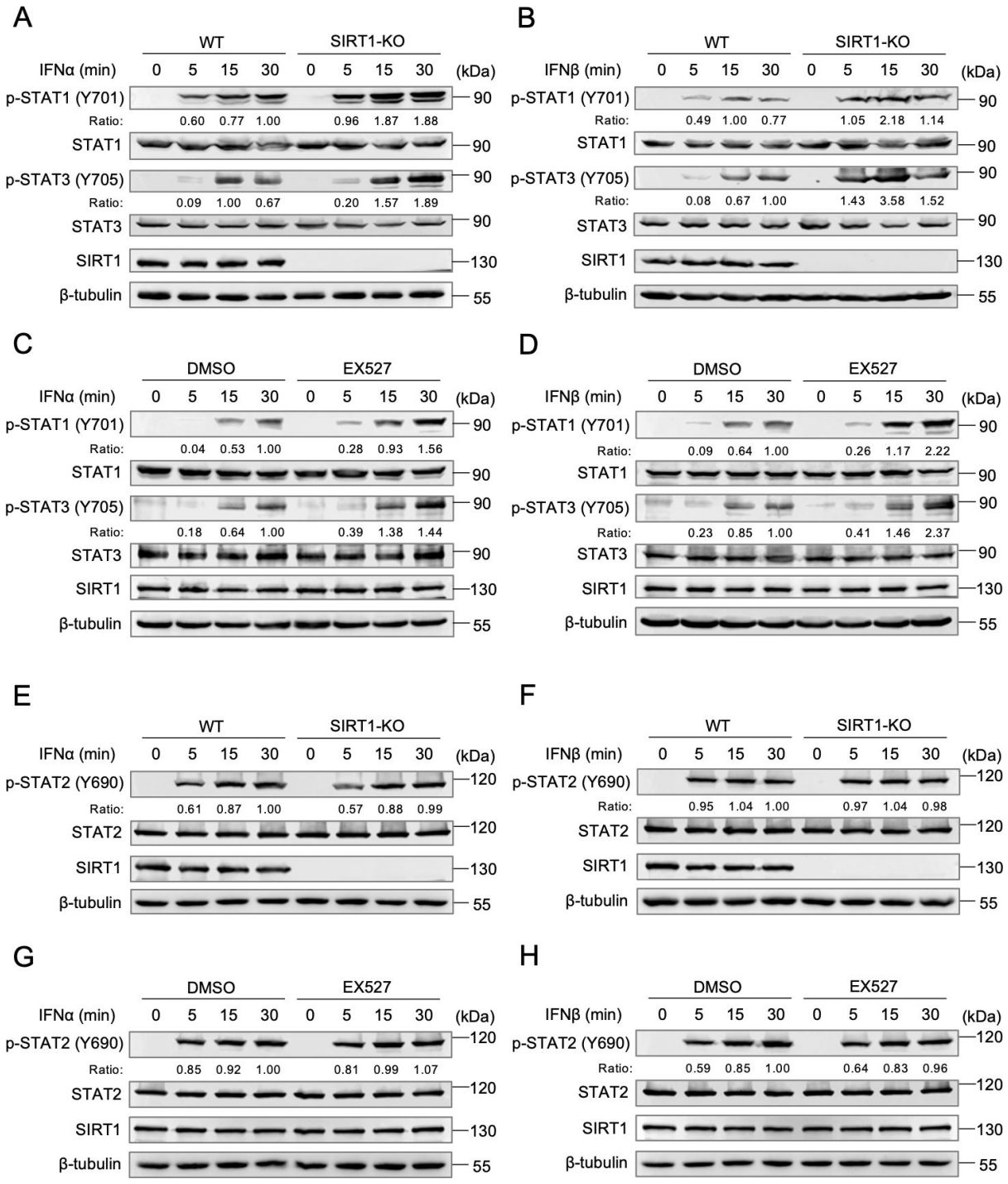

FIG 2 SIRT1 suppresses type I IFN-induced signaling. (A and B) Immunoblot analysis of phosphorylated (p-) STAT1 (Y701) and p-STAT3 (Y705) in WT and *SIRT1-KO* HCT116 cells treated with 10 ng/mL IFNα (A) or IFNβ (B) for indicated time points. (C and D) Immunoblot analysis of p-STAT1 (Y701) and p-STAT3 (Y705) in HEK293T cells pretreated with DMSO or 20 µM EX527 for 8 h, then treated with 10 ng/mL IFNα (C) or IFNβ (D) for indicated time points. (E and F) Immunoblot analysis of p-STAT2 (Y690) in WT and *SIRT1-KO* HCT116 cells treated with 10 ng/mL IFNα (E) or IFNβ (F) for indicated time points. (G and H) Immunoblot analysis of p-STAT2 (Y690) in HEK293T cells pretreated with DMSO or 20 µM EX527 for 8 h, then treated with 10 ng/mL IFNα (G) or IFNβ (H) for indicated time points.

SIRT1 negatively modulates type II IFN-induced signaling

The JAK-STAT signaling pathway is also used by other cytokines such as type II IFN as well (31, 32). We then investigated whether SIRT1 is also involved in type II IFN-induced signaling. In Dual-Luciferase assays, overexpression of SIRT1 inhibited IFNγ-induced ISRE activation (Fig. 3A). Treatment with SIRT1 inhibitor led to increased IFNγ-induced ISRE activation (Fig. 3B). Consistently, knockout of *SIRT1* increased IFNγ-induced downstream genes such as *IFIT2*, *ISG12,* and *OAS1* (Fig. 3C). IFNγ-stimulated phosphorylated STAT1 (Y701) and STAT3 (Y705) were also examined. As it was shown in Fig. 3D, knockout of *SIRT1* enhanced IFNγ-induced phosphorylation of STAT1 (Y701) and STAT3 (Y705). Coherently, treatment with the SIRT1 inhibitor EX527 enhanced IFNγ-induced phosphorylation of STAT1 (Y701) and STAT3 (Y705) (Fig. 3E). Thus, these findings suggest that SIRT1 also inhibited type II IFN-induced signaling.

SIRT1 impairs type I and type II IFN-induced antiviral immunity

It is well documented that type I IFN and type II IFN are important in antiviral immunity (33). We have demonstrated that SIRT1 is a negative regulator in both type I and type II IFN-induced signaling. We then sought to answer the question whether SIRT1 is involved in viral replication. We used the RNA virus, vesicular stomatitis virus (VSV), and DNA virus, herpes simplex virus 1 (HSV-1), to investigate the functions of SIRT1 in the antiviral activity of type I or type II IFN. Real-time PCR, immunoblot, and fluorescence microscopy were used to monitor viral infection. As it was shown in Fig. 4A through C, the VSV replication was significantly inhibited in *SIRT1-KO* cells pretreated with IFNα, IFNβ, or IFNγ. Similarly, the HSV-1 replication was also inhibited in *SIRT1-KO* cells pretreated with IFNα, IFNβ, or IFNγ (Fig. 4D through F). Taken together, SIRT1 negatively regulates type I and type II IFN-induced signaling and promotes viral replication.

SIRT1 physically interacts with STAT1 and STAT3

As demonstrated above, SIRT1 inhibited both type I and type II IFN-induced signaling and the phosphorylation of both STAT1 and STAT3. We then tried to understand how SIRT1 exerts its suppressive functions. First, we tested whether SIRT1 can associate with STAT1 or STAT3. Initially, we detected the interaction between SIRT1 and STAT1 or STAT3 in human embryonic kidney (HEK) 293T cells. SIRT1 physically interacted with STAT1 and STAT3, and this interaction was enhanced by IFNα, IFNβ, or IFNγ stimulation in HEK293T cells (data not shown). Then we used the monocyte-like cell line THP-1 to examine the interaction. The interaction was similarly enhanced by IFNα, IFNβ, or IFNγ stimulation in THP-1 cells (Fig. 5A). It is well known that both RNA virus such as Sendai virus (SeV) and DNA virus such as HSV-1 infection promote the production of type I IFNs, then we detected the interaction between SIRT1 and STAT1 or STAT3 after RNA or DNA virus infection. As expected, the interaction between SIRT1 and STAT1 or SIRT1 and STAT3 was also enhanced after SeV or HSV-1 infection (Fig. 5B and C). Then we tried to map the interaction domain between SIRT1 and STAT1 or STAT3. As it was shown in Fig. 5D through G, the C-terminal domain of STAT1 (amino acids 489–750) or STAT3 (amino acids 495–770) was responsible for the interaction with SIRT1. These data suggest that SIRT1 can associate with STAT1 and STAT3, and this interaction was enhanced by type I or type II IFN stimulation and viral infection.

SIRT1 deacetylates STAT1

The above data showed that SIRT1 inhibited type I or type II IFN-induced STAT1 phosphorylation, SIRT1 physically interacted with STAT1. It was previously reported that STAT1 can be acetylated (34). SIRT1 is a known class III deacetylase. We next attempted to determine whether SIRT1 can deacetylate STAT1. As shown in Fig. 6A, the acetyltransferase CREB-binding protein (CBP) can promote the acetylation of STAT1. Whereas, SIRT1 indeed reduced the acetylation level of STAT1 upon IFNα treatment. In contrast, the SIRT1 inhibitor EX527 processing increased the acetylation level of STAT1 upon IFNα treatment

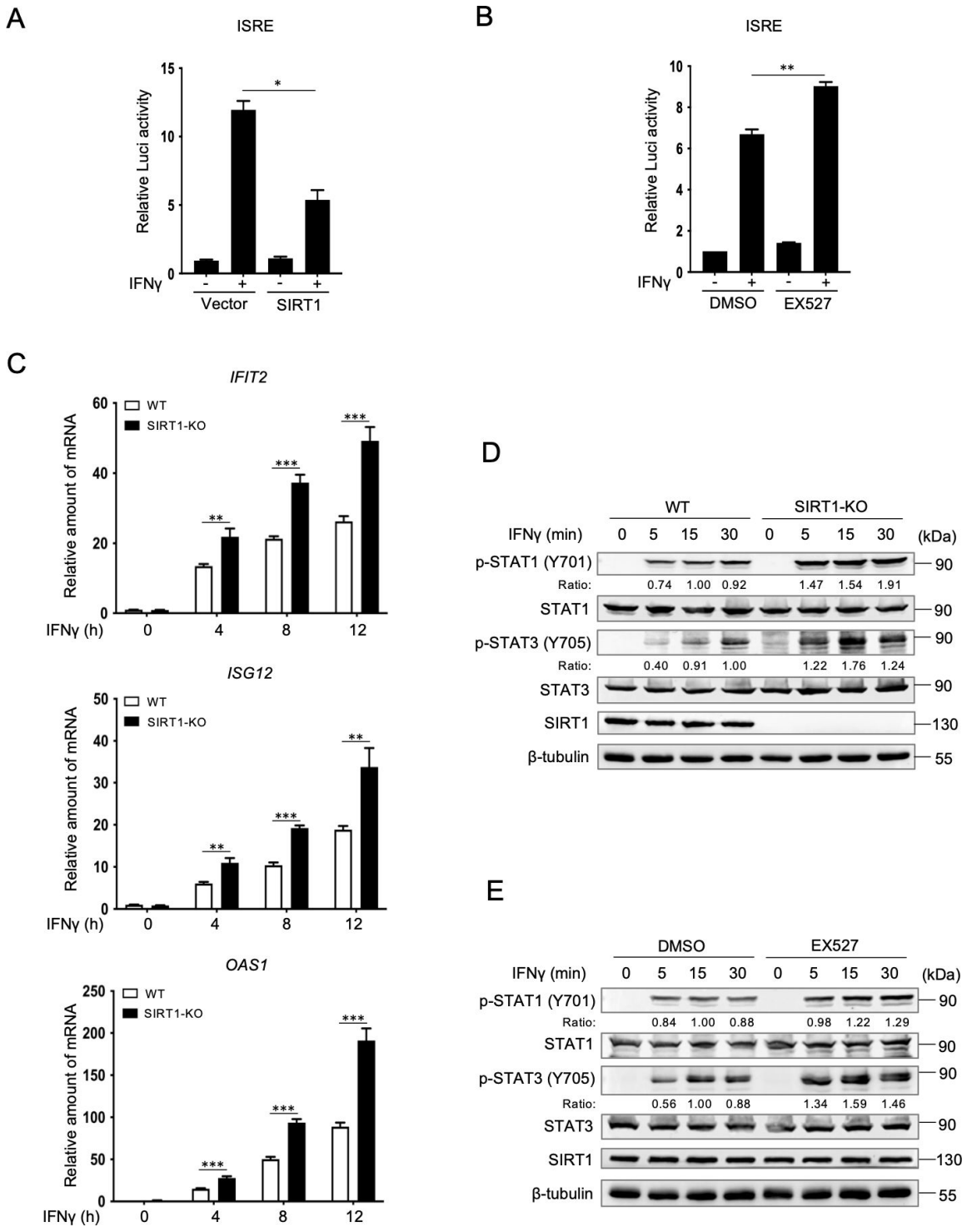

FIG 3 SIRT1 negatively modulates type II IFN-induced signaling. (A) Luciferase activity analysis of an ISRE reporter in HEK293T cells transfected with control vector or SIRT1 expressing plasmid, followed by treatment with 50 ng/mL IFNγ for 12 h. (B) Luciferase activity analysis of an ISRE reporter in HEK293T cells pretreated with DMSO or 20 μM EX527 for 2 h, then treated with 50 ng/mL IFNγ for 12 h. (C) qPCR analysis of *IFIT2*, *ISG12,* or *OAS1* mRNA in WT and *SIRT1-KO* HCT116 cells treated with 50 ng/mL IFNγ for indicated time points. (D) Immunoblot analysis of p-STAT1 (Y701) and p-STAT3 (Y705) in WT and *SIRT1-KO* HCT116 cells treated with 50 ng/mL IFNγ for indicated time points. (E) Immunoblot analysis of p-STAT1 (Y701) and p-STAT3 (Y705) in HEK293T cells pretreated with DMSO or 20 μM EX527 for 8 h, then treated with 50 ng/mL IFNγ for indicated time points. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

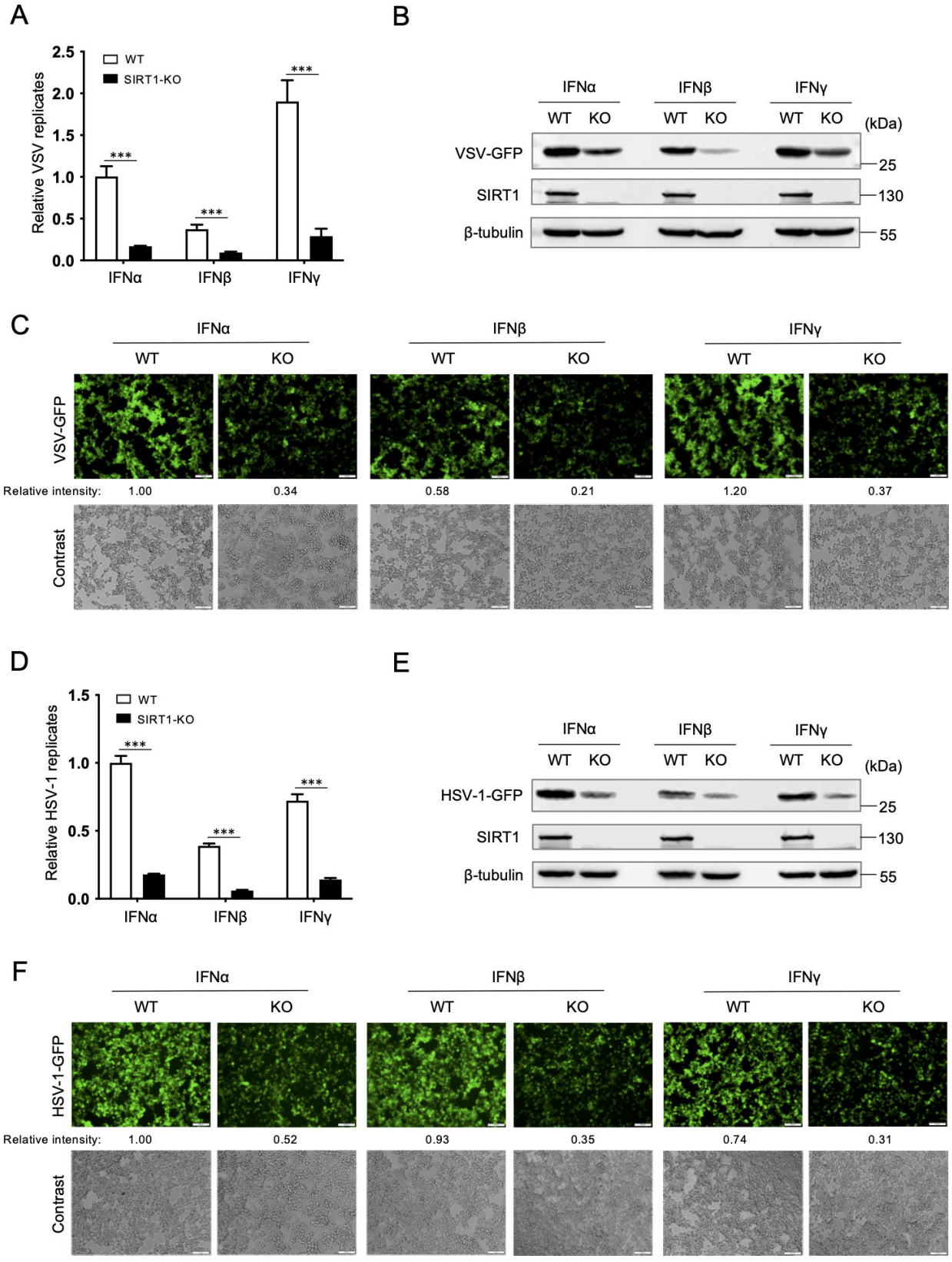

FIG 4 SIRT1 impairs type I and type II IFN-induced antiviral immunity. (A–C) WT and *SIRT1-KO* HCT116 cells pretreated with 10 ng/mL IFNα/IFNβ or 50 ng/mL IFNγ for 8 h were infected with VSV-GFP (MOI = 1) for 24 h, then detected by qPCR (A), immunoblot (B), and fluorescence analysis (C). Scale bars, 100 µm. (D–F) WT and *SIRT1-KO* HCT116 cells pretreated with 10 ng/mL IFNα/IFNβ or 50 ng/mL IFNγ for 8 h were infected with HSV-1-GFP (MOI = 10) for 24 h, then detected by qPCR (D), immunoblot (E), and fluorescence (F) analysis. Scale bars, 100 µm. ***$P < 0.001$.

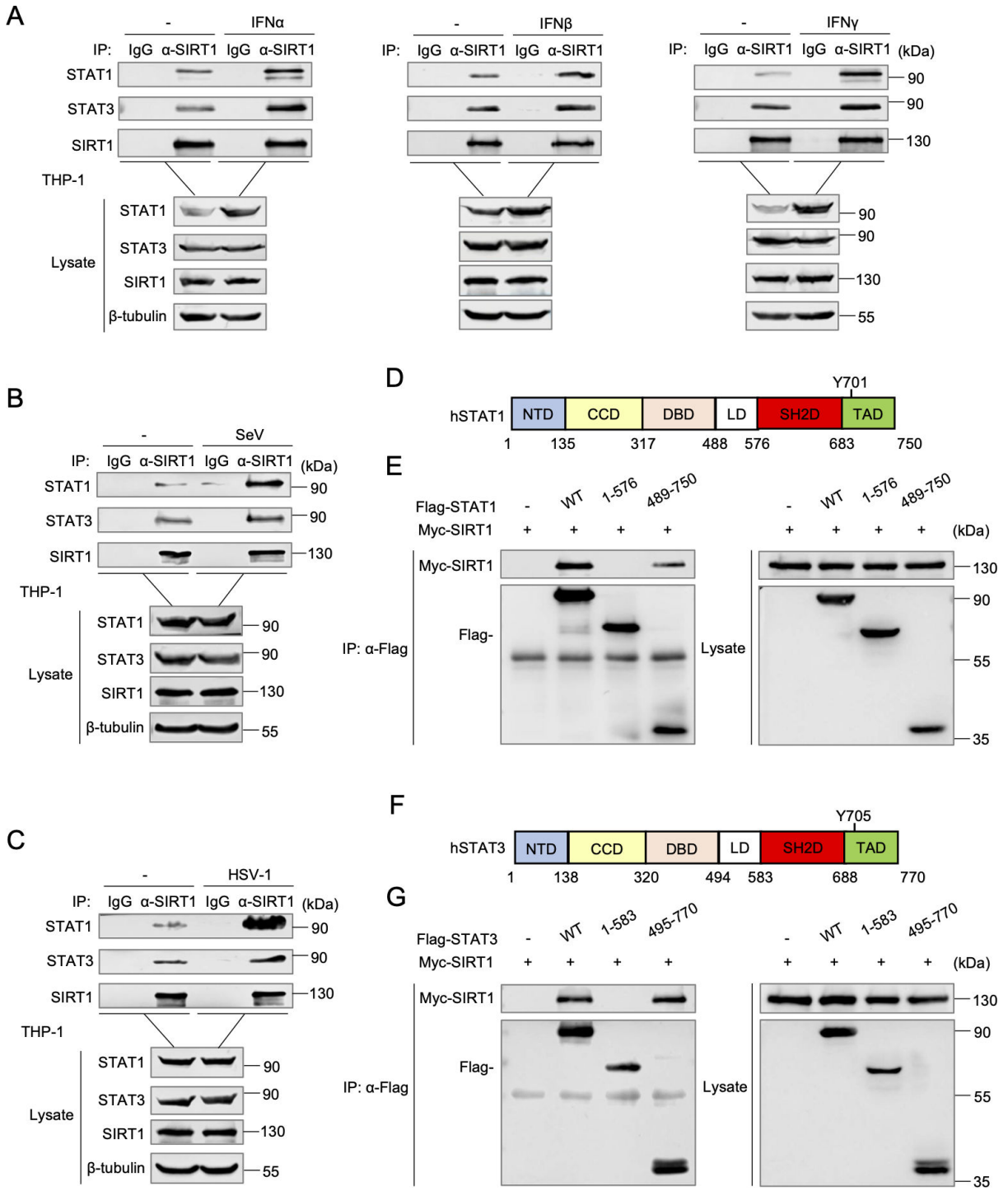

FIG 5 SIRT1 physically interacts with STAT1 and STAT3. (A) Co-immunoprecipitation analysis of the interaction of endogenous SIRT1 with STAT1 and STAT3 in THP-1 cells, untreated (left) or treated (right) with 10 ng/mL IFNα/IFNβ or 50 ng/mL IFNγ for 8 h. (B and C) Co-immunoprecipitation analysis of the interaction of endogenous SIRT1 with STAT1 and STAT3 in THP-1 cells, uninfected (left) or infected (right) with SeV (MOI = 1, C) or HSV-1 (MOI = 10, D) for 8 h. (D, F) Structure domain of human STAT1 (D) and STAT3 (F). NTD, N-terminal domain; CCD, coiled-coil domain; DBD, DNA-binding domain; LD, linker domain; SH2D, SH2 domain; TAD, transactivation domain. (E) Co-immunoprecipitation analysis of HEK293T cells transfected with plasmids encoding Myc-SIRT1 and Flag-STAT1 or its truncation mutants for 24 h. (G) Co-immunoprecipitation analysis of HEK293T cells transfected with plasmids encoding Myc-SIRT1 and Flag-STAT3 or its truncation mutants for 24 h.

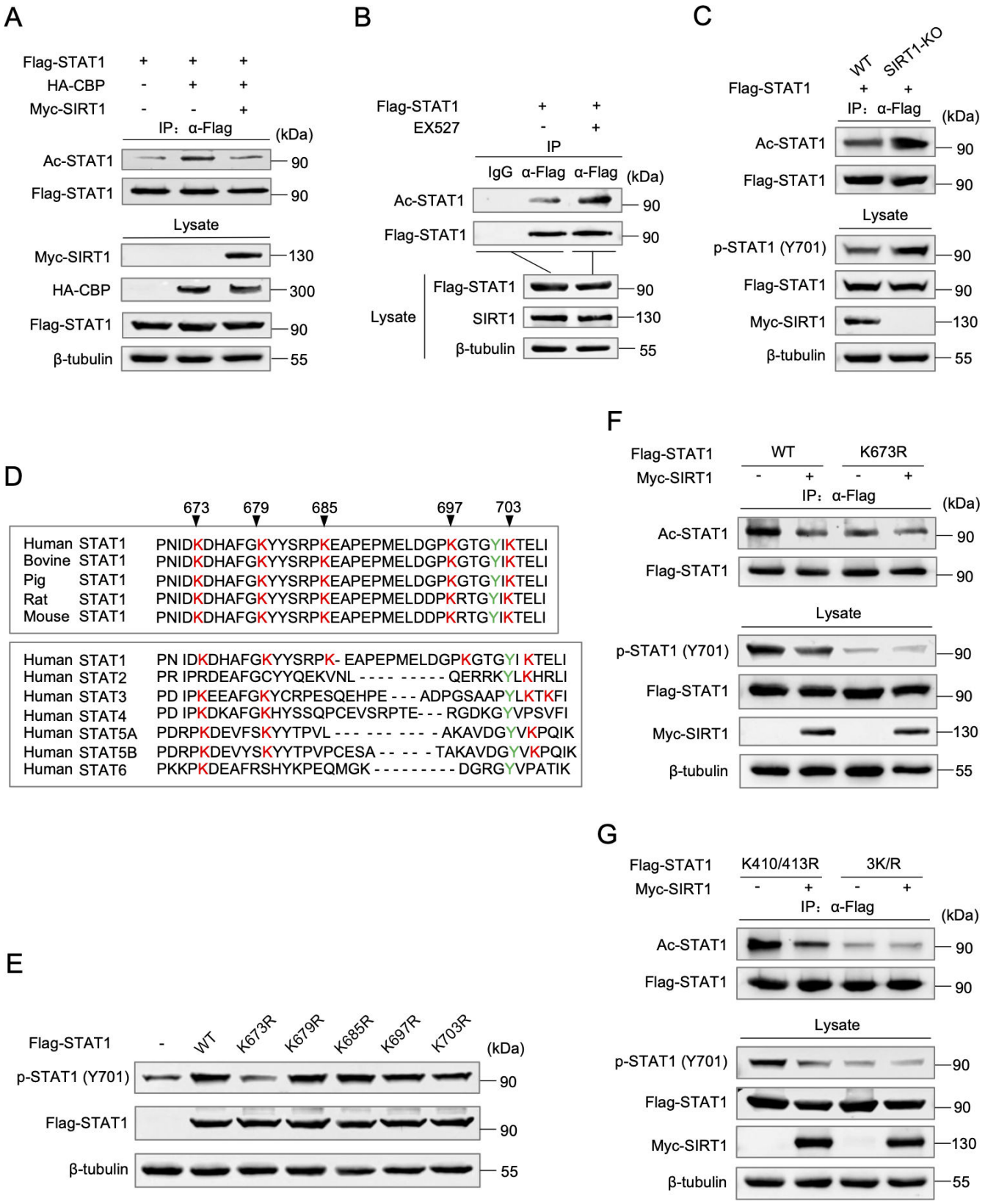

FIG 6 SIRT1 deacetylates STAT1. (A) Immunoprecipitation analysis of STAT1 acetylation in HEK293T cells transfected with plasmids encoding Flag-STAT1, HA-CBP, and Myc-SIRT1 for 24 h, then treated with 10 ng/mL IFNα for 0.5 h. (B) Immunoprecipitation analysis of STAT1 acetylation in HEK293T cells transfected with plasmids encoding Flag-STAT1 for 24 h, dealed with DMSO or 20 μM EX527 for 8 h, then treated with 10 ng/mL IFNα for 0.5 h. (C) Immunoprecipitation

(Continued on next page)

FIG 6 (Continued)

analysis of STAT1 acetylation in WT and *SIRT1-KO* HCT116 cells transfected with plasmids encoding Flag-STAT1 for 24 h, then treated with 10 ng/mL IFNα for 0.5 h. (D) Sequence conservation analysis of STAT1 from different species (top) and sequence alignment of the human STAT family (bottom). (E) Immunoblot analysis of p-STAT1 (Y701) in HEK293T cells transfected with plasmids encoding Flag-STAT1 WT or mutant for 24 h, then treated with 10 ng/mL IFNα for 0.5 h. (F) Immunoprecipitation analysis of STAT1 acetylation in HEK293T cells transfected with plasmids encoding Flag-STAT1 WT or K673R and Myc-SIRT1 for 24 h, then treated with 10 ng/mL IFNα for 0.5 h. (G) Immunoprecipitation analysis of STAT1 acetylation in HEK293T cells transfected with plasmids encoding Flag-STAT1 K410/413R or 3 K/R and Myc-SIRT1 for 24 h, then treated with 10 ng/mL IFNα for 0.5 h. 3 K/R, K410/413/673R.

(Fig. 6B). Consistently, the acetylation level of STAT1 was increased in *SIRT1-KO* cells upon IFNα treatment (Fig. 6C).

The next question is which lysine site of STAT1 is acetylated and mediates the crosstalk between SIRT1 and STAT1. We have demonstrated that SIRT1 interacted with the C-terminal of STAT1 (amino acids 489–750). The lysine sites of STAT1 near Y701 were analyzed. STAT1 K673, K679, K685, K697, and K703 are conserved in human, bovine, pig, mouse, and rat (Fig. 6D). These lysine residues were mutated to arginine. Upon IFNα treatment, the phosphorylation state of these mutants was detected. The STAT1 K673R mutant showed a dramatic reduction in the phosphorylation level of STAT1 (Y701), which was comparable to the endogenous phosphorylation in cells (Fig. 6E), suggesting that the modification of STAT1 K673 is critical for STAT1 (Y701) phosphorylation. This lysine site is also highly conserved in the human STAT family (Fig. 6D). We then tested whether K673 is the acetylation/deacetylation site in IFN-induced signaling. SIRT1 inhibited STAT1 acetylation upon IFNα treatment. In contrast, the acetylation level of STAT1 K673R mutant was very weak and not altered by overexpressed SIRT1 (Fig. 6F). Previously, the acetylation of STAT1 at K410, K413 sites was reported (34). We then constructed the STAT1 K410/413R mutant. In the presence of SIRT1, the STAT1 K410/413R can still be deacetylated by SIRT1 (Fig. 6G). Then we further mutated K673 on the K410/413R mutant and obtained the STAT1 3 K/R (K410/413/673R) mutant. In-line with the data about STAT1 K673R, the acetylation level of STAT1 3 K/R was barely detectable and not altered by SIRT1 (Fig. 6G). These data suggest that SIRT1 can remove the acetyl moiety from STAT1 K673, which is critical for the phosphorylation of STAT1 (Y701).

SIRT1 deacetylates STAT3

The above data suggested that SIRT1 can physically interact with STAT3; we next tried to explore whether STAT3 was another deacetylation target of SIRT1. As shown in Fig. 7A, the acetyltransferase CBP promoted the acetylation of STAT3. As expected, SIRT1 reduced the acetylation level of STAT3 upon IFNγ treatment. The SIRT1 inhibitor EX527 processing resulted in an increased acetylation level of STAT3 upon IFNγ treatment (Fig. 7B). Consistently, the acetylation level of STAT3 was increased in *SIRT1-KO* cells upon IFNγ treatment (Fig. 7C).

In an early report on gluconeogenesis, four lysine acetylation residues, K679, K685, K707, and K709, of STAT3 were reported to be deacetylated by SIRT1. The 4 K/R STAT3 mutant was no longer sensitive to stimulation by IL-6 (35). These four lysines are adjacent to the phosphorylation site of STAT3 Y705 and locate at the C-terminal of STAT3 (amino acids 495–770), which interacts with SIRT1. Here, we investigated whether SIRT1 can deacetylate these four lysines under IFN stimulation. STAT3 K679, K685, K707, and K709 are highly conserved among different species (Fig. 7D). These lysines were then mutated into arginine alone or altogether. The K679, K685, K707, and K709 sites were all shown to be critical for the phosphorylation of STAT3 (Y705). Consistently, the STAT3 4 K/R mutant was barely phosphorylated upon IFNγ stimulation (Fig. 7E). Furthermore, the STAT3 4 K/R mutant was barely acetylated, and the acetylation level was not altered by overexpression of SIRT1 upon IFNγ treatment (Fig. 7F). The acetylation of STAT3 at K49, K87 was previously reported (36). We constructed the STAT3 K49/87R mutant. The STAT3 K49/87R mutant can still be deacetylated by SIRT1 (Fig. 7G). In-line with the data about STAT3 4 K/R mutant, the acetylation level of STAT3 6 K/R (K49/87/679/685/707/709R) was barely detectable and not changed by SIRT1 (Fig. 7G).

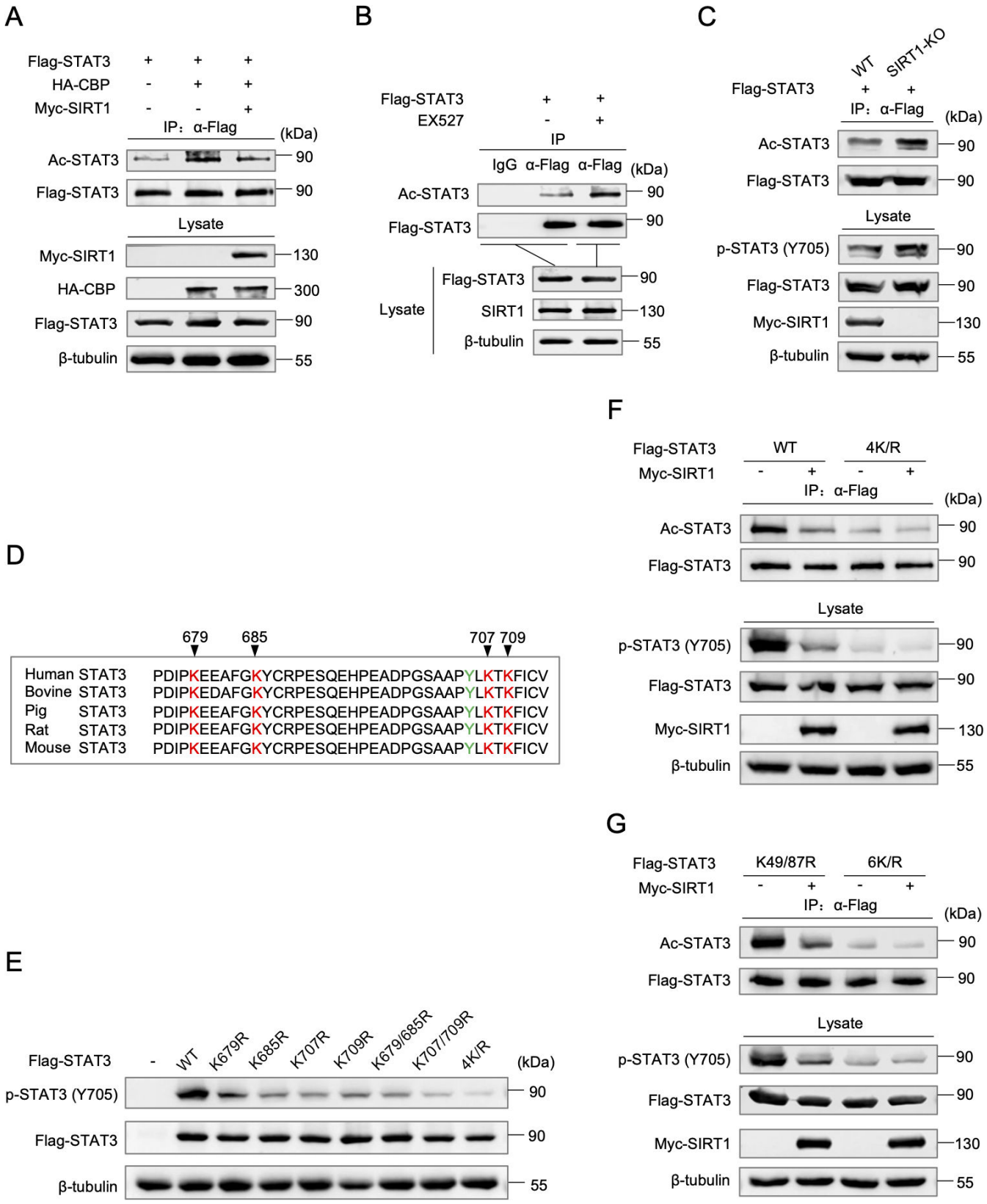

FIG 7 SIRT1 deacetylates STAT3. (A) Immunoprecipitation analysis of STAT3 acetylation in HEK293T cells transfected for 24 h with plasmids encoding Flag-STAT3, HA-CBP, and Myc-SIRT1, then treated with 50 ng/mL IFNγ for 0.5 h. (B) Immunoprecipitation analysis of STAT3 acetylation in HEK293T cells transfected with plasmids encoding Flag-STAT3 for 24 h, dealed with DMSO or 20 μM EX527 for 8 h, then treated with 50 ng/mL IFNγ for 0.5 h. (C) Immunoprecipitation analysis

(Continued on next page)

FIG 7 (Continued)

of STAT3 acetylation in WT and *SIRT1-KO* HCT116 cells transfected with plasmids encoding Flag-STAT3 for 24 h, then treated with 50 ng/mL IFNγ for 0.5 h. (D) Sequence conservative analysis of STAT3 from different species. (E) Immunoblot analysis of p-STAT3 (Y705) in HEK293T cells transfected with plasmids encoding Flag-STAT3 WT or mutant for 24 h, then treated with 50 ng/mL IFNγ for 0.5 h. (F) Immunoprecipitation analysis of STAT3 acetylation in HEK293T cells transfected with plasmids encoding Flag-STAT3 WT or 4 K/R and Myc-SIRT1 for 24 h, then treated with 50 ng/mL IFNγ for 0.5 h. (G) Immunoprecipitation analysis of STAT3 acetylation in HEK293T cells transfected with plasmids encoding Flag-STAT3 K49/87R or 6 K/R and Myc-SIRT1 for 24 h, then treated with 50 ng/mL IFNγ for 0.5 h. 4 K/R, K679/685/707/709R; 6 K/R, K49/87/679/685/707/709R.

Taken together, our results indicate that both STAT1 and STAT3 are targets for the deacetylase SIRT1. In response to interferon stimulation, SIRT1 can decrease the acetylation level of both STAT1 and STAT3, leading to reduced activation of STAT1 and STAT3.

Sirt1$^{+/-}$ primary macrophages and *Sirt1*$^{+/-}$ mice exhibit enhanced IFN-induced signaling

We then sought to investigate the functions of SIRT1 in primary cells. We were unable to obtain *Sirt1*$^{-/-}$ mice in our breeding stock, possibly due to the reproductive problems caused by the loss of *Sirt1*. We used *Sirt1*$^{+/-}$ primary peritoneal macrophages and *Sirt1*$^{+/-}$ mice for further studies instead. We harvested primary peritoneal macrophages from WT and *Sirt1*$^{+/-}$ mice and stimulated these cells with IFNα or IFNγ. In response to IFNα stimulation, *Sirt1*$^{+/-}$ primary macrophages had increased production of downstream genes of *Ifit1* and *Ifit2* (Fig. 8A) and increased phosphorylation levels of STAT1 (Y701) and STAT3 (Y705) (Fig. 8C). Similar results were obtained upon stimulation with IFNγ. In response to IFNγ stimulation, *Sirt1*$^{+/-}$ primary macrophages had increased production of downstream genes of *Ifit1* and *Ifit2* (Fig. 8B) and increased phosphorylation levels of STAT1 (Y701) and STAT3 (Y705) (Fig. 8D) as well.

We next investigated the antiviral activity of *Sirt1*$^{+/-}$ mice upon IFN treatment. The mice were pretreated with IFNα for 8 h, then infected with VSV for 24 h, and the organs were harvested to monitor the viral replication or inflammation. As shown in Fig. 8E, the VSV replication was significantly suppressed in *Sirt1*$^{+/-}$ mice compared to WT mice. HE staining showed less infiltration of inflammatory cells, destruction of pulmonary alveoli, and tissue damage in the lungs of *Sirt1*$^{+/-}$ mice after VSV infection, compared to those of WT mice (Fig. 8F). Taken together, our data demonstrate that SIRT1 is a negative regulator of type I and type II IFN-induced signaling, which attenuates antiviral innate immunity or inflammation.

DISCUSSION

SIRT1, a member of the class III deacetylases, is primarily regarded as an anti-inflammatory deacetylase and has been extensively studied in inflammatory diseases (21). SIRT1 controls the level of acetylation of histones or non-histone proteins, including p65, HIF1α, AP-1, and p38, to exert its anti-inflammatory effects (37, 38). SIRT1 can reduce the expression of inflammatory cytokines by regulating the acetylation level of histones. For example, SIRT1 inhibits the production of IL-6 and TNF-α by removing the acetylation of the H3K9 on the corresponding promoter region (39). In addition, SIRT1 can reduce the acetylation level of K310 of p65, which affects its nuclear localization and downstream inflammatory cytokine production, thereby exerting anti-inflammatory effects (40). In addition, SIRT1 indirectly regulates the NF-κB signaling pathway by modulating the activity of AMPK and PPARs. SIRT1 interacts with and activates AMPK, which then indirectly inhibits NF-κB. SIRT1 enhances the interaction between PPARα and p65, thereby attenuating the activation of p65 (41). SIRT1 is able to directly interact with HIF1α and reduce its acetylation level, thereby inhibiting the activity of HIF1α. In necrotizing enterocolitis, SIRT1 can alleviate the intestinal epithelial barrier dysfunction by inhibiting the expression and activity of HIF1α and downregulating the expression of inflammatory cytokines such as IL-6, IL-8, and TNF-α (42). Moreover, in recent years, the

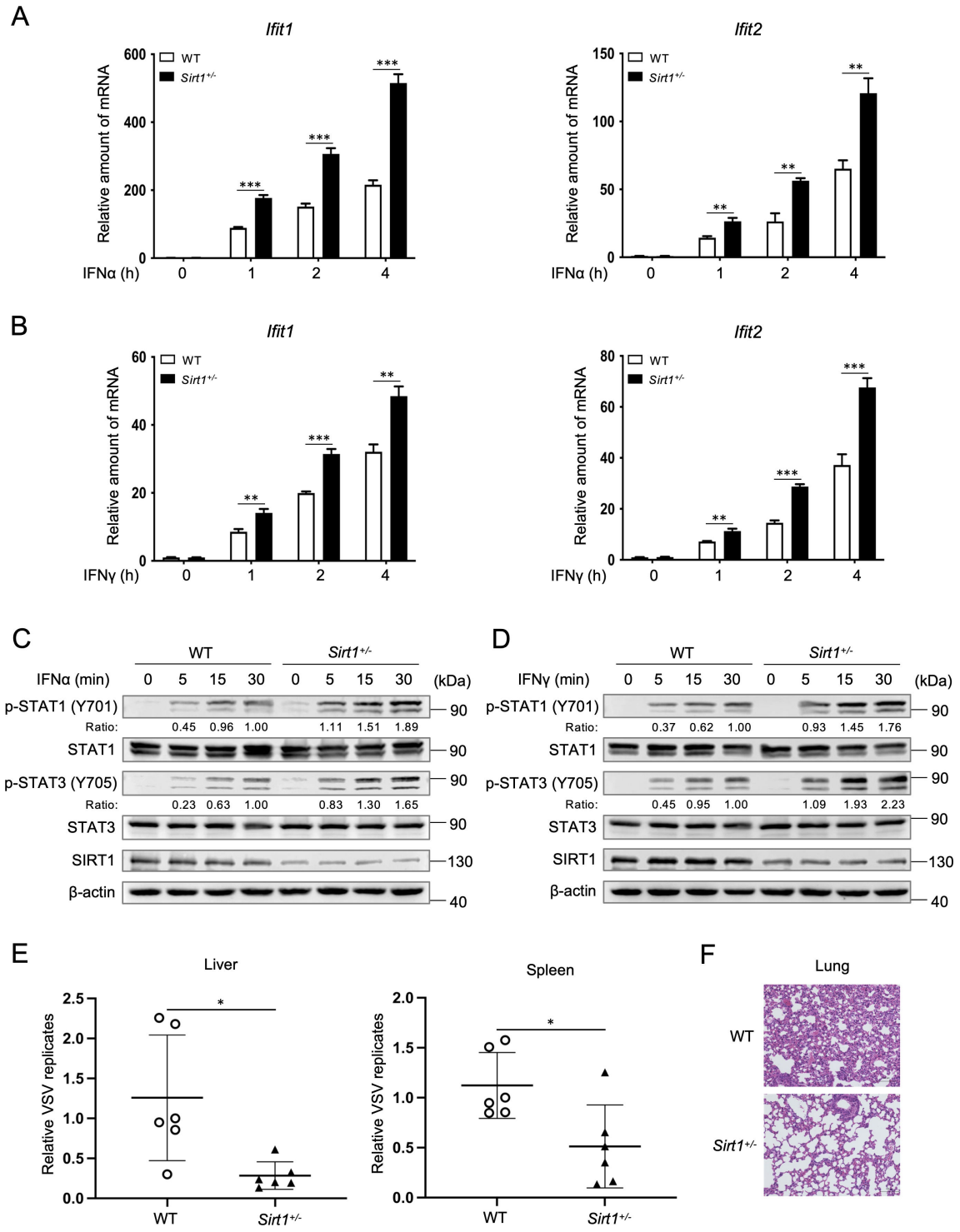

FIG 8 *Sirt1*$^{+/-}$ mice exhibit enhanced type I and type II IFN-induced signaling and antiviral activity. (A and B) qPCR analysis of *Ifit1* or *Ifit2* mRNA in WT and *Sirt1*$^{+/-}$ peritoneal macrophages treated with 50 ng/mL IFNα (A) or IFNγ (B) for indicated time points. (C and D) Immunoblot analysis of p-STAT1 (Y701) and p-STAT3 (Y705) in WT and *Sirt1*$^{+/-}$ peritoneal macrophages treated with 50 ng/mL IFNα (C) or IFNγ (D) for indicated time points. (E) qPCR analysis of VSV RNA in the liver and spleen of WT and *Sirt1*$^{+/-}$ mice (n = 6 per group) pretreated with IFNα (50 ng/g, i.p.) for 8 h and then treated with VSV (5×10^7 pfu/g, i.p.) for 24 h. (F) HE staining of lung sections from mice in (E). Scale bars, 100 µm. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

regulatory functions of SIRT1 in innate immunity have also been documented, such as RNA virus- or DNA virus-induced type I IFN signaling, which can target IRF3, IRF7, or IFI16

for deacetylation (25, 26). Thus, although SIRT1 has broad regulatory functions in diverse physiological conditions, its functions in type I and type II IFN-induced downstream signaling are still unclear.

Type I and type II IFNs are important mediators in both innate and adaptive immunity (1, 2). They are under tight control. Acetylation and deacetylation of key molecules in JAK-STAT signaling will ultimately affect the strength of these pathways (43, 44). Acetylation of STAT1 at K410 and K413 results in reduced expression of anti-apoptotic NF-κB target genes (34). However, it is not yet known whether there exit the acetylation sites at the C-terminal domain of STAT1. STAT3 is known to play an important role downstream of IL-6 family cytokines. In addition, STAT3 is also implicated in the type I IFN-induced signaling pathway as well as the type II IFN-induced signaling pathway. The acetyltransferase CBP/p300 can promote the acetylation modification of STAT3 at K685, and this modification promotes the nuclear translocation, DNA-binding ability, and the transactivation activity of STAT3 (45). Furthermore, it was reported that STAT3 K679, K685, K707, and K709 were acetylated under IL-6 stimulation, which is critical for the phosphorylation of STAT3 (35). In addition, the acetyltransferase p300 promotes the acetylation modification of STAT3 at N-terminal K49 and K87. The STAT3 K49/87R mutant retains the ability to translocate to the nucleus, but it loses the ability to bind to p300, and the acetylation of STAT3 at other sites is affected, thereby attenuating the expression of downstream target genes (36).

Members of the deacetylase HDAC family are known to be important in the regulation of JAK-STAT signaling. HDACs can deacetylate STAT1 (46, 47). For example, it has been reported that HDAC4 can act as a STAT1 deacetylase and alter the effect of cisplatin on STAT1 signaling in ovarian cancer (48). In addition, HDAC1, 2, and 3 can remove the acetyl moiety from STAT3 and inhibit the transcription of downstream genes. Inhibition of both HDAC1 and HDAC2 augments the STAT3-dependent expression of downstream genes. HDACi can increase the acetylation level of STAT3, e.g., TSA and LBH589 treatment can enhance the acetylation level of STAT3, accompanied by dephosphorylation (49–51).

In this report, we sought to dissect the functions of SIRT1 in the type I and type II IFN signaling pathways. Dual-Luciferase reporter assays showed that SIRT1 indeed negatively modulated the type I and type II IFN signaling pathways. To consolidate the suppressive functions of SIRT1, the downstream genes of both type I and type II IFN-induced signaling and the phosphorylation levels of STAT1 and STAT3 were determined in *SIRT1-KO* cells. Knockout of *SIRT1* resulted in increased expression of the downstream target genes of type I and type II IFN signaling and enhanced antiviral activity. Besides, the phosphorylation levels of STAT1 and STAT3 were increased in *SIRT1-KO* cells. *Sirt1*$^{+/-}$ primary macrophages and *Sirt1*$^{+/-}$ mice also showed improved IFN-induced signaling and enhanced antiviral activity. EX527, a SIRT1 inhibitor, also enhanced type I and type II IFN-induced signaling, implying that the suppressive functions of SIRT1 are dependent of its deacetylase activity. This as well increases the potential application of SIRT1 agonists or antagonists in the treatment of viral infection, inflammatory diseases, or even interferon-related therapies.

The endogenous co-immunoprecipitation assays showed that SIRT1 indeed physically interacted with STAT1 or STAT3. This interaction was enhanced by type I IFN or type II IFN treatment. RNA virus SeV or DNA virus HSV-1 infection also increased the association between SIRT1 and STAT1 or STAT3. These data implied that SIRT1 may play its suppressive functions at the late phase of the cytokine stimulation or viral infection and contribute to the homeostasis maintenance. This is consistent with previous reports that SIRT1 is an important anti-inflammatory regulator. Dysregulation of SIRT1 is closely related with the occurrence and progression of autoimmune diseases (29, 38). Domain mapping suggested that SIRT1 interacted with the C-terminal of STAT1 or STAT3, where the key phosphorylation site of STAT1 or STAT3 is located.

Cross-regulation between phosphorylation and acetylation is well documented. In colorectal cancer cells, PGAM5 dephosphorylates ME1 at S336 and then promotes

increased acetylation of ME1 at the adjacent K337 (52). Here, we found that SIRT1 can remove the acetylation moiety from STAT1 at K673, which is located at the SH2 domain of STAT1. STAT1 K673 is very critical for the STAT1 Y701 phosphorylation. STAT1 K673R has a reduced phosphorylation state, suggesting that the acetylation at K673 may help the phosphorylation and activation of STAT1. We also tested whether the known acetylation sites of STAT1 K410 and K413 were also affected by SIRT1. The interaction between SIRT1 and STAT1 was mapped to the C-terminal of STAT1. The acetylation level of STAT1 K410/413R mutant was still decreased by SIRT1, suggesting that SIRT1 deacetylates STAT1 at the sites in the C-terminus. The STAT1 3 K/R (K410/413/673R) had similar results to STAT1 K673R. Previously, it was reported that two cases of autosomal-dominant Mendelian susceptibility to mycobacterial disease patients had SH2 domain-negative mutants including K673R (53). This indicates that STAT1 K673 is important for STAT1 activation. The STAT1 K673R mutant is not only important for antiviral immunity but also for antimycobacterial immunity. In our study, we provide evidence that the acetylation of STAT1 at K673 is essential for the activation of type I or type II IFN-induced STAT1 activation.

As mentioned above, the acetylation status near Y705 also affects the phosphorylation level of STAT3, while we stimulated with IFNγ and previous researchers treated with IL-6. STAT3 C-terminal 4 K/R mutant almost loses the ability to be phosphorylated, suggesting that all these four lysines are essential for STAT3 phosphorylation and activation. Other reported acetylation sites of STAT3 include K49 and K87. We also examined whether the acetylation level of STAT3 K49 and K87 was regulated by SIRT1. The interaction between SIRT1 and STAT3 was mapped to the C-terminal of STAT3, instead of the N-terminal of STAT3. The acetylation level of STAT3 K49/87R was still decreased by SIRT1, suggesting that SIRT1 deacetylates STAT3 at the sites in the C-terminus. The STAT3 6 K/R had similar results to STAT3 4 K/R. Why acetylation state of lysines close to STAT1 Y701 or STAT3 Y705 affects the phosphorylation requires future studies. One possible explanation is that the acetylation status may affect the recruitment and association of phosphatases or kinases with STAT1 or STAT3. Intriguingly, in our data, we found that SIRT1 only reduced type I and type II IFN-induced phosphorylation of STAT1 and STAT3. SIRT1 had no effects on type I IFN-induced STAT2 (Y690) activation. We have not figured out the exact mechanisms to explain this. One possible explanation is that STAT1 and STAT3 are more phylogenetically related.

In addition to acting as a deacetylase, SIRT1 has also been suggested to be an eraser of post-translational modifications such as lactylation or crotonylation (54–57). However, in this study, we clearly show that SIRT1 can remove the acetyl moiety from STAT1 and STAT3. Further studies are required to dissect whether novel post-translational modifications such as lactylation and crotonylation are also involved in the precise modulation of type I and type II IFN-induced signaling. The acetyltransferases and deacetylases may have a variety of targets. It is possible that in addition to STAT1 and STAT3, SIRT1 may act synergistically on other key signaling molecules or histones to regulate type I or type II IFN-induced signaling. Type I and type II IFN-induced signaling are very important for antiviral immunity and tumor therapies. In this study, we tested the effects of SIRT1 or its inhibitor EX527 in multiple cell lines, such as HEK293T cells, HCT116 cells, THP-1 cells, and primary macrophages, suggesting that the suppressive functions of SIRT1 on type I or type II IFN-induced signaling are extensively existed. It extends the potential applications of SIRT1 agonists or antagonists in the future.

Here, we show that the class III deacetylase SIRT1 can remove the acetyl moiety from STAT1 and STAT3, thereby reducing STAT1 and STAT3 activation, and leading to reduced type I and type II IFN signaling (Fig. 9). Our study reinforces the notion that SIRT1 is an important target for intervention in inflammatory diseases. Inhibition or activation of SIRT1 by antagonists or agonists will have promising applications in antiviral immunity, inflammation, and the related diseases in the future.

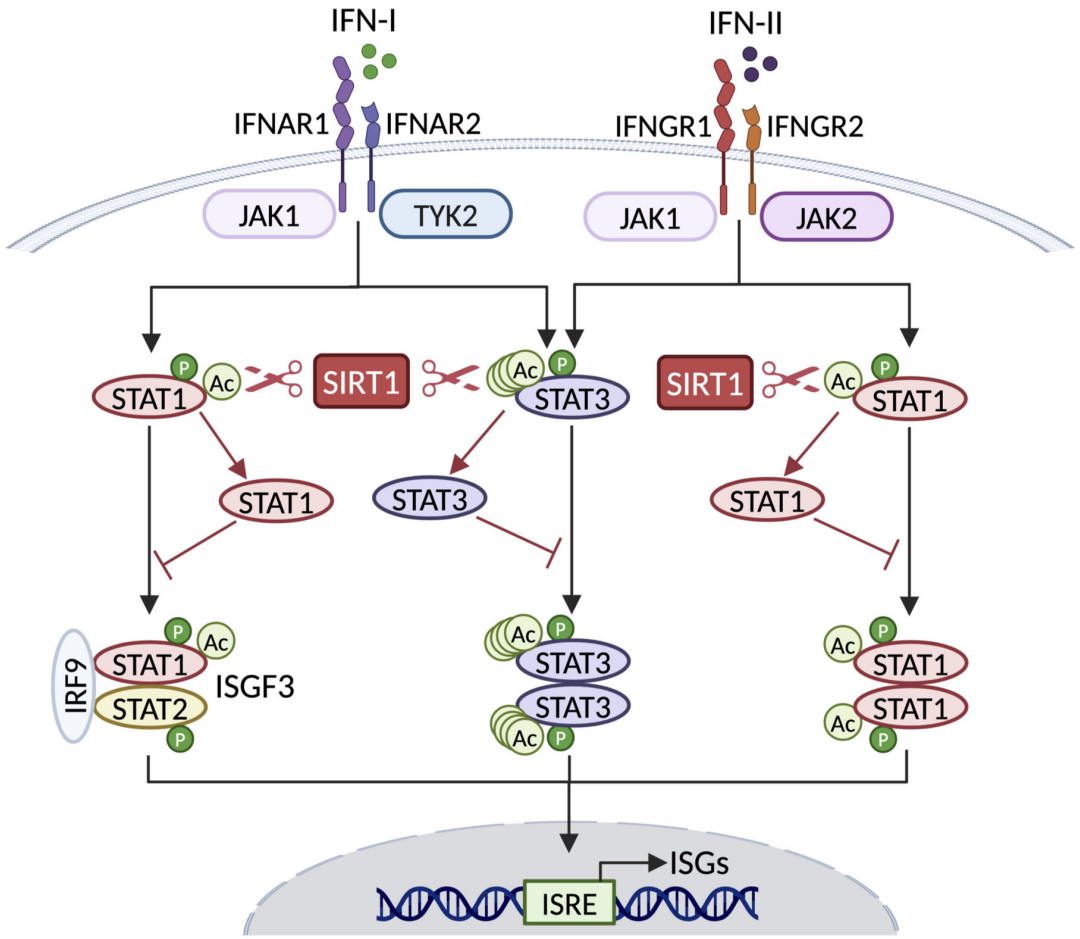

FIG 9 Proposed working model for SIRT1 as a negative regulator in type I and type II IFN-induced signaling. The deacetylase SIRT1 interacts with STAT1 and STAT3 to remove the acetyl moiety from the C-terminus of STAT1 and STAT3, thereby inhibiting their phosphorylation and activation. Image created with BioRender.com, with permission.

MATERIALS AND METHODS

Cell lines, viruses, and plasmids

HEK293T, HCT116, Vero, and L929 cells were cultured in DMEM (Thermo-Fisher), supplemented with 10% heat-inactivated FBS (ExCell), 100 U/mL penicillin, and 100 µg/mL streptomycin (Thermo-Fisher). THP-1 cells were cultured in RPMI 1640 medium (Thermo-Fisher) supplemented with 10% FBS and penicillin/streptomycin. WT and *SIRT1-KO* HCT116 cells were kindly provided by Dr. Wei Yu (Fudan University, China). SeV was amplified by inoculation of chick embryos, while VSV, VSV-GFP, and HSV-1-GFP were amplified by infection of Vero cells. The viral titers were determined by TCID50 in L929 cells. The Myc-SIRT1 and HA-CBP plasmids were kindly provided by Dr. Jianyuan Luo (Peking University, China). The pRL-SV40 and ISRE luciferase reporter plasmids were gifts from Dr. Hong-Bing Shu (Wuhan University, China). The plasmid pRK-Flag-STAT3 was from Dr. Dan Lu (Peking University, China). The plasmids of pRK-Flag-STAT1, STAT1 mutants, and STAT3 mutants were constructed using standard molecular techniques and verified by sequencing.

Reagents and antibodies

Recombinant human IFNα (C005), human IFNγ (C014), mouse IFNα (CK83), and mouse IFNγ (C746) were from Novoprotein. Human IFNβ (10704-HNAS) was from Sino Biological. SIRT1 inhibitor EX527 (S1541) was purchased from Selleck Chemicals. Antibodies

against STAT1 (14995S), phospho-STAT1 (Tyr701) (9167S), phospho-STAT2 (Tyr690) (4441S), and phospho-STAT3 (Tyr705) (9145S) were from Cell Signaling Technology. The STAT3 antibody (A19566) was from ABclonal. The SIRT1 (13161-1-AP), STAT2 (16674-1-AP), and β-actin (66009-1-Ig) antibodies were from Proteintech. The acetylated-lysine antibody (PTM-105RM) was from PTM Biolabs. The anti-HA tag (M20003S) and β-tubulin (M30109S) antibodies were from Abmart. The anti-Flag tag (M185-3L) and GFP (MBL598) antibodies were from MBL. The anti-Myc tag antibody (TA150121) was from Origene. Mouse IgG (I5381) and rabbit IgG (I5006) were from Sigma-Aldrich. HRP-conjugated goat anti-mouse IgG (BF03001) and HRP-conjugated goat anti-rabbit IgG (BF03008) were from Biodragon.

Cell transfection and Luciferase reporter assays

Polyethyleneimine (PEI, Sigma-Aldrich) was used for transfection of HEK293T and HCT116 cell lines. Dual-Luciferase assays were performed to monitor type I or type II IFN-induced transcriptional activity. Briefly, HEK293T cells were cotransfected by PEI with 100 ng of ISRE luciferase reporter plasmid and 1 ng of pRL-SV40 plasmid, as well as control vector or SIRT1 overexpressing vector. Twenty-four hours after transfection, the cells were treated with IFNα (10 ng/mL) or IFNβ (10 ng/mL) or IFNγ (50 ng/mL) for 12 h. The cells were then harvested, and the luciferase activity was quantified using the Dual-Luciferase Reporter Assay Kit (Promega).

RT-PCR and quantitative real-time PCR

Total RNAs were extracted by TRIzol reagent (Gene-Protein Link). The cDNAs were synthesized by Hifair III 1st Strand cDNA Synthesis Kit (Yeasen). Hieff qPCR SYBR Green Master Mix (Yeasen) was used for the subsequent quantitative real-time PCR. The ABI Q5 Detection System was used for real-time PCR analysis.

Gene-specific primer sequences were as follows:

Human *GAPDH* forward: 5′-ACCCACTCCTCCACCTTTGA-3′, reverse: 5′-CTGTTGCTGTAG CCAAATTCGT-3′; Human *IFIT1* forward: 5′-GCCATTTTCTTTGCTTCCCCTA-3′, reverse: 5′-T GCCCTTTTGTAGCCTCCTTG-3′; Human *IFIT2* forward: 5′-CACCTCTGGACTGGCAATAGC-3′, reverse: 5′-GTCAGGATTCAGCCGAATGG-3′; Human *ISG12* forward: 5′-TGCCATGGGCTTCA CTGCGG-3′, reverse: 5′-CTGCCCGAGGCAACTCCACC-3′; Human *OAS1* forward: 5′-CATC CGCCTAGTCAAGCACTG-3′, reverse: 5′-CCACCACCCAAGTTTCCTGTAG-3′; Mouse *β-actin* forward: 5′-AGAGGGAAATCGTGCGTGAC-3′, reverse: 5′-CAATAGTGATGACCTGGCCGT-3′; Mouse *Ifit1* forward: 5′-GAACCCATTGGGGATGCACAACCT-3′, reverse: 5′-CTTGTCCAGGTA GATCTGGGCTTCT-3′; Mouse *Ifit2* forward: 5′-CGGAAAGCAGAGGAAATCAA-3′, reverse: 5′-T GAAAGTTGCCATACCGAAG-3′; VSV forward: 5′-ACGGCGTACTTCCAGATGG-3′, reverse: 5′-C TCGGTTCAAGATCCAGGT-3′; HSV-1-UL30 forward: 5′-CATCACCGACCCGGAGAGGGAC-3′, reverse: 5′-GGGCCAGGCGCTTGTTGGTGTA-3′.

Immunoblot analysis

Cells were harvested and lysed in RIPA lysis buffer (Beyotime Biotechnology) with protease and phosphatase inhibitors (Roche) on ice for 1 h. The protein concentration was determined using a BCA Quantification Kit (Beyotime), followed by the addition of SDS loading buffer (5×) and boiling at 100°C for 10 min, and subjected to SDS-PAGE. The membrane was blocked in 5% non-fat milk at room temperature for 1 h and incubated with diluted antibodies at 4°C with moderate shaking overnight. The membrane was then washed three times with TBST. HRP-conjugated secondary antibody was then added. Finally, the membrane was washed three times again and imaged using ImageQuant LAS 500.

Immunoprecipitation

HEK293T cells or THP-1 cells treated as indicated were harvested and lysed in IP lysis buffer (Beyotime) with protease inhibitor and phosphatase inhibitors at 4°C for 1 h.

The lysates were sonicated for 20 s, and the supernatants were collected. Each sample was co-incubated with control IgG or antibody at 4°C overnight. On the second day, protein A agarose beads were added and co-incubated for 3–4 h. The samples were then centrifuged and washed for several times, followed by boiling in SDS loading buffer (1×) at 100°C for 10 min for further immunoblot analysis. The acetylation modification was analyzed with acetylated-lysine antibody.

Mice and animal experiments

The *Sirt1*$^{+/-}$ mice were kindly provided by Dr. Michael W. McBurney (University of Ottawa, Canada) (58). The mice were crossed with C57BL/6 mice over eight generations. The mice were bred under specific pathogen-free conditions. Age- and sex-matched 6–8-week-old mice were used in our experiments. All animal experiments were approved by the Institutional Animal Care and Use Committee of Peking University Health Science Center.

WT and *Sirt1*$^{+/-}$ mice were treated with IFNα (50 ng/g, i.p.) for 8 h and infected with VSV (5×10^7 pfu/g, i.p.) for 24 h. Then the organs were harvested and detected by qPCR and HE staining analysis.

Preparation of primary peritoneal macrophages

Peritoneal macrophages were harvested from WT and *Sirt1*$^{+/-}$ mice 3 days after intraperitoneal injection of 4% thioglycolate (Sigma-Aldrich), and the cells were cultured in RPMI 1640 medium (Thermo-Fisher) supplemented with 10% FBS and penicillin/streptomycin.

Statistical analysis

Data were analyzed by the unpaired two-tailed Student *t* tests using GraphPad Prism 9.0. Results were presented as mean ± SD in this study. Statistical significance was set at $P < 0.05$.

ACKNOWLEDGMENTS

We thank Dr. Jianyuan Luo (Peking University, China), Dr. Hong-Bing Shu (Wuhan University, China), and Dr. Dan Lu (Peking University, China) for plasmids. We thank Dr. Xiangmei Chen and Danying Chen (Peking University, China) for reagents. We thank Dr. Wei Yu (Fudan University, China) for *SIRT1-KO* HCT116 cells. We thank Dr. Michael W. McBurney (University of Ottawa, Canada) for *Sirt1*$^{+/-}$ mice.

AUTHOR AFFILIATION

[1]Department of Immunology, School of Basic Medical Sciences, NHC Key Laboratory of Medical Immunology, Medicine Innovation Center for Fundamental Research on Major Immunology-related Diseases, Peking University, Beijing, China

AUTHOR ORCIDs

Shuang-Shuang Yu http://orcid.org/0009-0006-8482-8738
Jun Zhang http://orcid.org/0000-0001-5147-6963

FUNDING

Funder	Grant(s)	Author(s)
MOST | National Natural Science Foundation of China (NSFC)	82271796, 82071786	Jun Zhang

AUTHOR CONTRIBUTIONS

Shuang-Shuang Yu, Data curation, Methodology, Writing – original draft | Rong-Chun Tang, Investigation, Methodology | Ao Zhang, Validation | Shijin Geng, Validation | Hengxiang Yu, Investigation | Yan Zhang, Resources | Xiu-Yuan Sun, Resources | Jun Zhang, Conceptualization, Funding acquisition, Writing – review and editing

DATA AVAILABILITY

The data generated or analyzed during this study are available from the corresponding author upon reasonable request.

ETHICS APPROVAL

Animal experiments were approved by the Institutional Animal Care and Use Committee of Peking University Health Science Center.

REFERENCES

1. Ivashkiv LB, Donlin LT. 2014. Regulation of type I interferon responses. Nat Rev Immunol 14:36–49. https://doi.org/10.1038/nri3581
2. Ivashkiv LB. 2018. IFNγ: signalling, epigenetics and roles in immunity, metabolism, disease and cancer immunotherapy. Nat Rev Immunol 18:545–558. https://doi.org/10.1038/s41577-018-0029-z
3. 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
4. Barrat FJ, Crow MK, Ivashkiv LB. 2019. Interferon target-gene expression and epigenomic signatures in health and disease. Nat Immunol 20:1574–1583. https://doi.org/10.1038/s41590-019-0466-2
5. Mazewski C, Perez RE, Fish EN, Platanias LC. 2020. Type I interferon (IFN)-regulated activation of canonical and non-canonical signaling pathways. Front Immunol 11:606456. https://doi.org/10.3389/fimmu.2020.606456
6. Hu X, Ivashkiv LB. 2009. Cross-regulation of signaling pathways by interferon-gamma: implications for immune responses and autoimmune diseases. Immunity 31:539–550. https://doi.org/10.1016/j.immuni.2009.09.002
7. Billiau A, Matthys P. 2009. Interferon-gamma: a historical perspective. Cytokine Growth Factor Rev 20:97–113. https://doi.org/10.1016/j.cytogfr.2009.02.004
8. Baccala R, Kono DH, Theofilopoulos AN. 2005. Interferons as pathogenic effectors in autoimmunity. Immunol Rev 204:9–26. https://doi.org/10.1111/j.0105-2896.2005.00252.x
9. Chen K, Liu J, Cao X. 2017. Regulation of type I interferon signaling in immunity and inflammation: a comprehensive review. J Autoimmun 83:1–11. https://doi.org/10.1016/j.jaut.2017.03.008
10. Jiang J, Zhao M, Chang C, Wu H, Lu Q. 2020. Type I interferons in the pathogenesis and treatment of autoimmune diseases. Clin Rev Allergy Immunol 59:248–272. https://doi.org/10.1007/s12016-020-08798-2
11. Narita T, Weinert BT, Choudhary C. 2019. Functions and mechanisms of non-histone protein acetylation. Nat Rev Mol Cell Biol 20:156–174. https://doi.org/10.1038/s41580-018-0081-3
12. Sapountzi V, Côté J. 2011. MYST-family histone acetyltransferases: beyond chromatin. Cell Mol Life Sci 68:1147–1156. https://doi.org/10.1007/s00018-010-0599-9
13. Li M, Luo J, Brooks CL, Gu W. 2002. Acetylation of p53 inhibits its ubiquitination by Mdm2. J Biol Chem 277:50607–50611. https://doi.org/10.1074/jbc.C200578200
14. Yang X-J, Seto E. 2008. Lysine acetylation: codified crosstalk with other posttranslational modifications. Mol Cell 31:449–461. https://doi.org/10.1016/j.molcel.2008.07.002
15. Kurash JK, Lei H, Shen Q, Marston WL, Granda BW, Fan H, Wall D, Li E, Gaudet F. 2008. Methylation of p53 by Set7/9 mediates p53 acetylation and activity *in vivo*. Mol Cell 29:392–400. https://doi.org/10.1016/j.molcel.2007.12.025
16. Liu X, Wang D, Zhao Y, Tu B, Zheng Z, Wang L, Wang H, Gu W, Roeder RG, Zhu W-G. 2011. Methyltransferase Set7/9 regulates p53 activity by interacting with Sirtuin 1 (SIRT1). Proc Natl Acad Sci U S A 108:1925–1930. https://doi.org/10.1073/pnas.1019619108
17. Ito A, Lai CH, Zhao X, Saito S, Hamilton MH, Appella E, Yao TP. 2001. p300/CBP-mediated p53 acetylation is commonly induced by p53-activating agents and inhibited by MDM2. EMBO J 20:1331–1340. https://doi.org/10.1093/emboj/20.6.1331
18. Tang X, Gao J-S, Guan Y, McLane KE, Yuan Z-L, Ramratnam B, Chin YE. 2007. Acetylation-dependent signal transduction for type I interferon receptor. Cell 131:93–105. https://doi.org/10.1016/j.cell.2007.07.034
19. Imai S, Armstrong CM, Kaeberlein M, Guarente L. 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature 403:795–800. https://doi.org/10.1038/35001622
20. Finkel T, Deng C-X, Mostoslavsky R. 2009. Recent progress in the biology and physiology of sirtuins. Nature 460:587–591. https://doi.org/10.1038/nature08197
21. Yang Y, Liu Y, Wang Y, Chao Y, Zhang J, Jia Y, Tie J, Hu D. 2022. Regulation of SIRT1 and its roles in inflammation. Front Immunol 13:831168. https://doi.org/10.3389/fimmu.2022.831168
22. Chang H-C, Guarente L. 2014. SIRT1 and other sirtuins in metabolism. Trends Endocrinol Metab 25:138–145. https://doi.org/10.1016/j.tem.2013.12.001
23. Rada P, Pardo V, Mobasher MA, García-Martínez I, Ruiz L, González-Rodríguez Á, Sanchez-Ramos C, Muntané J, Alemany S, James LP, Simpson KJ, Monsalve M, Valdecantos MP, Valverde ÁM. 2018. SIRT1 controls acetaminophen hepatotoxicity by modulating inflammation and oxidative stress. Antioxid Redox Signal 28:1187–1208. https://doi.org/10.1089/ars.2017.7373
24. Xu C, Wang L, Fozouni P, Evjen G, Chandra V, Jiang J, Lu C, Nicastri M, Bretz C, Winkler JD, Amaravadi R, Garcia BA, Adams PD, Ott M, Tong W, Johansen T, Dou Z, Berger SL. 2020. SIRT1 is downregulated by autophagy in senescence and ageing. Nat Cell Biol 22:1170–1179. https://doi.org/10.1038/s41556-020-00579-5
25. Qin Z, Fang X, Sun W, Ma Z, Dai T, Wang S, Zong Z, Huang H, Ru H, Lu H, Yang B, Lin S, Zhou F, Zhang L. 2022. Deactylation by SIRT1 enables liquid-liquid phase separation of IRF3/IRF7 in innate antiviral immunity. Nat Immunol 23:1193–1207. https://doi.org/10.1038/s41590-022-01269-0
26. Wang J, Qin X, Huang Y, Zhang G, Liu Y, Cui Y, Wang Y, Pei J, Ma S, Song Z, Zhu X, Wang H, Yang B. 2023. Sirt1 negatively regulates cellular antiviral responses by preventing the cytoplasmic translocation of interferon-inducible protein 16 in human cells. J Virol 97:e0197522. https://doi.org/10.1128/jvi.01975-22
27. Haigis MC, Sinclair DA. 2010. Mammalian sirtuins: biological insights and disease relevance. Annu Rev Pathol 5:253–295. https://doi.org/10.1146/annurev.pathol.4.110807.092250
28. Zhao B, Li X, Zhou L, Wang Y, Shang P. 2019. SIRT1: a potential tumour biomarker and therapeutic target. J Drug Target 27:1046–1052. https://doi.org/10.1080/1061186X.2019.1605519

29. Shen P, Deng X, Chen Z, Ba X, Qin K, Huang Y, Huang Y, Li T, Yan J, Tu S. 2021. SIRT1: a potential therapeutic target in autoimmune diseases. Front Immunol 12:779177. https://doi.org/10.3389/fimmu.2021.779177
30. Gertz M, Fischer F, Nguyen GTT, Lakshminarasimhan M, Schutkowski M, Weyand M, Steegborn C. 2013. Ex-527 inhibits Sirtuins by exploiting their unique NAD+-dependent deacetylation mechanism. Proc Natl Acad Sci U S A 110:E2772–E2781. https://doi.org/10.1073/pnas.1303628110
31. Banerjee S, Biehl A, Gadina M, Hasni S, Schwartz DM. 2017. JAK-STAT signaling as a target for inflammatory and autoimmune diseases: current and future prospects. Drugs 77:521–546. https://doi.org/10.1007/s40265-017-0701-9
32. Luo Y, Alexander M, Gadina M, O'Shea JJ, Meylan F, Schwartz DM. 2021. JAK-STAT signaling in human disease: from genetic syndromes to clinical inhibition. J Allergy Clin Immunol 148:911–925. https://doi.org/10.1016/j.jaci.2021.08.004
33. Samuel CE. 2001. Antiviral actions of interferons. Clin Microbiol Rev 14:778–809. https://doi.org/10.1128/CMR.14.4.778-809.2001
34. Krämer OH, Baus D, Knauer SK, Stein S, Jäger E, Stauber RH, Grez M, Pfitzner E, Heinzel T. 2006. Acetylation of Stat1 modulates NF-kappaB activity. Genes Dev 20:473–485. https://doi.org/10.1101/gad.364306
35. Nie Y, Erion DM, Yuan Z, Dietrich M, Shulman GI, Horvath TL, Gao Q. 2009. STAT3 inhibition of gluconeogenesis is downregulated by SirT1. Nat Cell Biol 11:492–500. https://doi.org/10.1038/ncb1857
36. Ray S, Boldogh I, Brasier AR. 2005. STAT3 NH2-terminal acetylation is activated by the hepatic acute-phase response and required for IL-6 induction of angiotensinogen. Gastroenterology 129:1616–1632. https://doi.org/10.1053/j.gastro.2005.07.055
37. Gu X, Cai Z, Cai M, Liu K, Liu D, Zhang Q, Tan J, Ma Q. 2018. AMPK/SIRT1/p38 MAPK signaling pathway regulates alcohol-induced neurodegeneration by resveratrol. Mol Med Rep 17:5402–5408. https://doi.org/10.3892/mmr.2018.8482
38. Qiu Y, Zhou X, Liu Y, Tan S, Li Y. 2021. The role of Sirtuin-1 in immune response and systemic lupus erythematosus. Front Immunol 12:632383. https://doi.org/10.3389/fimmu.2021.632383
39. Zhang Y, Li Y, Li J, Li B, Chong Y, Zheng G, Sun S, Feng F. 2019. SIRT1 alleviates isoniazid-induced hepatocyte injury by reducing histone acetylation in the IL-6 promoter region. Int Immunopharmacol 67:348–355. https://doi.org/10.1016/j.intimp.2018.11.054
40. Chen L, Mu Y, Greene WC. 2002. Acetylation of RelA at discrete sites regulates distinct nuclear functions of NF-kappaB. EMBO J 21:6539–6548. https://doi.org/10.1093/emboj/cdf660
41. Liu X, Xu Q, Long X, Liu W, Zhao Y, Hayashi T, Hattori S, Fujisaki H, Ogura T, Tashiro S-I, Onodera S, Yamato M, Ikejima T. 2019. Silibinin-induced autophagy mediated by PPARα-sirt1-AMPK pathway participated in the regulation of type I collagen-enhanced migration in murine 3T3-L1 preadipocytes. Mol Cell Biochem 450:1–23. https://doi.org/10.1007/s11010-018-3368-y
42. Bai M, Lu C, An L, Gao Q, Xie W, Miao F, Chen X, Pan Y, Wang Q. 2020. SIRT1 relieves necrotizing enterocolitis through inactivation of hypoxia-inducible factor (HIF)-1a. Cell Cycle 19:2018–2027. https://doi.org/10.1080/15384101.2020.1788251
43. Wieczorek M, Ginter T, Brand P, Heinzel T, Krämer OH. 2012. Acetylation modulates the STAT signaling code. Cytokine Growth Factor Rev 23:293–305. https://doi.org/10.1016/j.cytogfr.2012.06.005
44. Icardi L, De Bosscher K, Tavernier J. 2012. The HAT/HDAC interplay: multilevel control of STAT signaling. Cytokine Growth Factor Rev 23:283–291. https://doi.org/10.1016/j.cytogfr.2012.08.002
45. Wang R, Cherukuri P, Luo J. 2005. Activation of Stat3 sequence-specific DNA binding and transcription by p300/CREB-binding protein-mediated acetylation. J Biol Chem 280:11528–11534. https://doi.org/10.1074/jbc.M413930200
46. Krämer OH, Heinzel T. 2010. Phosphorylation-acetylation switch in the regulation of STAT1 signaling. Mol Cell Endocrinol 315:40–48. https://doi.org/10.1016/j.mce.2009.10.007
47. Krämer OH, Knauer SK, Greiner G, Jandt E, Reichardt S, Gührs K-H, Stauber RH, Böhmer FD, Heinzel T. 2009. A phosphorylation-acetylation switch regulates STAT1 signaling. Genes Dev 23:223–235. https://doi.org/10.1101/gad.479209
48. Stronach EA, Alfraidi A, Rama N, Datler C, Studd JB, Agarwal R, Guney TG, Gourley C, Hennessy BT, Mills GB, Mai A, Brown R, Dina R, Gabra H. 2011. HDAC4-regulated STAT1 activation mediates platinum resistance in ovarian cancer. Cancer Res 71:4412–4422. https://doi.org/10.1158/0008-5472.CAN-10-4111
49. Icardi L, Lievens S, Mori R, Piessevaux J, De Cauwer L, De Bosscher K, Tavernier J. 2012. Opposed regulation of type I IFN-induced STAT3 and ISGF3 transcriptional activities by histone deacetylases (HDACS) 1 and 2. FASEB J 26:240–249. https://doi.org/10.1096/fj.11-191122
50. Gupta M, Han JJ, Stenson M, Wellik L, Witzig TE. 2012. Regulation of STAT3 by histone deacetylase-3 in diffuse large B-cell lymphoma: implications for therapy. Leukemia 26:1356–1364. https://doi.org/10.1038/leu.2011.340
51. Ray S, Lee C, Hou T, Boldogh I, Brasier AR. 2008. Requirement of histone deacetylase1 (HDAC1) in signal transducer and activator of transcription 3 (STAT3) nucleocytoplasmic distribution. Nucleic Acids Res 36:4510–4520. https://doi.org/10.1093/nar/gkn419
52. Zhu Y, Gu L, Lin X, Liu C, Lu B, Cui K, Zhou F, Zhao Q, Prochownik EV, Fan C, Li Y. 2020. Dynamic regulation of ME1 phosphorylation and acetylation affects lipid metabolism and colorectal tumorigenesis. Mol Cell 77:138–149. https://doi.org/10.1016/j.molcel.2019.10.015
53. Tsumura M, Okada S, Sakai H, Yasunaga S, Ohtsubo M, Murata T, Obata H, Yasumi T, Kong XF, Abhyankar A, Heike T, Nakahata T, Nishikomori R, Al-Muhsen S, Boisson-Dupuis S, Casanova JL, Alzahrani M, Shehri MA, Elghazali G, Takihara Y, Kobayashi M. 2012. Dominant-negative STAT1 SH2 domain mutations in unrelated patients with Mendelian susceptibility to mycobacterial disease. Hum Mutat 33:1377–1387. https://doi.org/10.1002/humu.22113
54. Moreno-Yruela C, Zhang D, Wei W, Bæk M, Liu W, Gao J, Danková D, Nielsen AL, Bolding JE, Yang L, Jameson ST, Wong J, Olsen CA, Zhao Y. 2022. Class I histone deacetylases (HDAC1-3) are histone lysine delactylases. Sci Adv 8:eabi6696. https://doi.org/10.1126/sciadv.abi6696
55. Hao S, Wang Y, Zhao Y, Gao W, Cui W, Li Y, Cui J, Liu Y, Lin L, Xu X, Wang H. 2022. Dynamic switching of crotonylation to ubiquitination of H2A at lysine 119 attenuates transcription-replication conflicts caused by replication stress. Nucleic Acids Res 50:9873–9892. https://doi.org/10.1093/nar/gkac734
56. Sun Y, Chen Y, Xu Y, Zhang Y, Lu M, Li M, Zhou L, Peng T. 2022. Genetic encoding of ε-N-L-lactyllysine for detecting delactylase activity in living cells. Chem Commun (Camb) 58:8544–8547. https://doi.org/10.1039/d2cc02643k
57. Bao X, Wang Y, Li X, Li X-M, Liu Z, Yang T, Wong CF, Zhang J, Hao Q, Li XD. 2014. Identification of 'erasers' for lysine crotonylated histone marks using a chemical proteomics approach. Elife 3:e02999. https://doi.org/10.7554/eLife.02999
58. McBurney MW, Yang X, Jardine K, Hixon M, Boekelheide K, Webb JR, Lansdorp PM, Lemieux M. 2003. The mammalian SIR2alpha protein has a role in embryogenesis and gametogenesis. Mol Cell Biol 23:38–54. https://doi.org/10.1128/MCB.23.1.38-54.2003