**IMMUNOLOGY**

**LATS1 is a central signal transmitter for achieving full type-I interferon activity**

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Interferons (IFNs) have broad-spectrum antiviral activity to resist virus epidemic. However, IFN antiviral efficacy needs to be greatly improved. Here, we reveal that LATS1 is a vital signal transmitter governing full type-I IFN (IFN-I) signaling activity. LATS1 constitutively binds with the IFN-I receptor IFNAR2 and is rapidly tyro-phosphorylated by Tyk2 upon IFN-I engagement. Tyro-phosphorylation of LATS1 promotes LATS1 activation and YAP degradation, thereby promoting IFN-mediated antiproliferation activity. Moreover, activated LATS1 translocates into the nucleus and induces CDK8-Ser62 phosphorylation, which in turn phosphorylates STAT1 at Ser727 and induces full IFN-I antiviral activity. LATS1 deficiency restricts in vivo IFN-I signaling and attenuates host antiviral immune response. Our study identifies IFN-I as a previously unidentified extracellular diffusible ligand signal for activation of the Hippo core LATS1 pathway and reveals Tyk2-LATS1-CDK8 as a complete signaling cascade controlling full IFN-I activity.

**INTRODUCTION**

Interferons (IFNs) are a superfamily of cytokines that crucially regulate both innate and adaptive immune responses and modulate normal cell growth and tumor cell proliferation (1, 2). Accordingly, IFNs exhibit not only broad-spectrum antiviral activity but also therapeutic potential for numerous human tumors. All three classes of IFNs, including type-I (IFN-α), type-II (IFN-γ), and type-III (IFN-λ) IFNs, trigger intracellular signaling through the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway. The JAK-STAT pathway is recognized as the canonical IFN signaling. In addition, a few IFN-related noncanonical signaling pathways, such as p38/extracellular signal–regulated kinase (ERK) and phosphotyrosininositol 3-kinase (PI3K), have been identified (3–6). Thus far, the precise signaling cascades that critically control the full activity of the canonical IFN signaling remain to be explored.

IFN-I initiates signaling by recruiting IFN-I receptors (IFNAR1 and IFNAR2) to form a heterodimeric complex, which brings together JAK family members (JAK1 and Tyk2) that constitutively bind with either IFNAR2 or IFNAR1. JAK1 and Tyk2 phosphorylate each other and, in turn, result in STAT1 Tyr701 phosphorylation. Phosphorylated STAT1 and STAT2, together with interferon regulatory factor 9 (IRF9), form the interferon-stimulated gene factor 3 (ISGF3) complex, which translocates into the nucleus to bind to the IFN-stimulated response element (ISRE) for transcriptional activation of interferon-stimulated genes (ISGs) (7). In addition to Tyr701 phosphorylation, Ser727 phosphorylation has been documented to be essential to STAT1 transcriptional activity and full activation of IFN-I signaling (8–11). Several protein kinases, including p38, protein kinase C (PKC)–δ, and CDK8, were reported to mediate different stimulant-induced STAT1 Ser727 phosphorylation (12–14). However, unlike that leading to STAT1 Tyr701 activation, the IFN-I signaling cascade leading to STAT1 Ser727 activation has not yet been clearly established.

The Hippo pathway has been demonstrated to regulate other cellular signaling pathways (15). As an essential signal in organ size control and tumor suppression, the Hippo core LATS1/2 (large tumor suppressor 1 and 2)–YAP (Yes-associated protein) pathway is activated by contact inhibition, mechanic stress, cytoskeletal rearrangement, and other factors. However, the diffuse extracellular signals regulating the Hippo pathway remained elusive until a recent study that identified G protein–coupled receptor–mediated regulation of the Hippo pathway (16). Further studies on extracellular ligand/receptor signals activating the Hippo core pathway are needed to define the biological significance of the Hippo pathway. In addition, two recent reports demonstrated that the YAP component of the Hippo pathway can regulate some signaling proteins in immune-related pathways: YAP/TAZ (transcriptional coactivator with PDZ binding motif) interacts with TBK1 and inhibits virus-induced TBK1 and IRF3 activation (17); YAP interacts with IRF3 and blocks IRF3-dimer formation and IRF3 nuclear translocation (18). However, whether and how immune-related signaling activates LATS1 and induces specific immune responses remain unknown.

Here, we revealed that IFN-I activates the Tyk2-LATS1-CDK8 pathway, which is an essential component and an indispensable booster of the canonical IFN-I signaling. IFN-I induces tyro-phosphorylation of LATS1, which promotes LATS1 activation. IFN-activated LATS1, on the one hand, induces YAP phosphorylation and degradation that promotes IFN-mediated antiproliferation effects and, on the...
other hand, translocates into the nucleus to phosphorylate CDK8, which in turn induces STAT1 Ser\(^{277}\) phosphorylation to achieve full IFN-mediated antiviral activity. We uncovered that Lats1 deficiency restricted in vivo antiviral activity of IFN-I signaling in mice. Our study established a complete signaling cascade linking IFN-I to STAT1 Ser\(^{277}\) activation and discovered the central role of LATS1 in controlling host IFN-mediated antiviral innate immunity.

**RESULTS**

**IFN-I activates LATS1-YAP signaling to inhibit cell proliferation**

The antiviral efficacy and broad-spectrum applications of IFNs are severely limited by negative regulation from both the host and viruses. During the exploration of strategies for enhancing IFN-I antiviral efficacy, we unexpectedly found that IFN-I could activate LATS1 signaling. We observed that IFN-\(\alpha\) rapidly induced LATS1 phosphorylation at Ser\(^{909}\), an activated form of LATS1, in various types of human cell lines (Fig. 1A and fig. S1A). Likewise, another type of IFN-I, IFN-\(\beta\), strongly induced LATS1 phosphorylation in mouse embryonic fibroblast (MEF) cells (Fig. 1B). However, IFN-II (IFN-\(\gamma\)) and IFN-III (IFN-\(\lambda\)) were not able to activate LATS1 (Fig. 1, C and D). Furthermore, deficiency of IFN-I receptor 1 (IFNAR1) abolished IFN-\(\beta\)-induced LATS1 activation (Fig. 1E). Knockout or knockdown of MST1/2, a well-known protein kinase of LATS1, did not attenuate IFN-induced LATS1 phosphorylation (fig. S1, B and C), suggesting that MST1/2 could be dispensable for IFN-induced LATS1 activation. We further tested several other immunomodulatory cytokines, including tumor necrosis factor-\(\alpha\) (TNF\(\alpha\)) and interleukin-6 (IL-6). The results showed that these cytokines did not noticeably stimulate LATS1 phosphorylation at Ser\(^{909}\) (Fig. 1F).

Activated LATS1 has been clearly demonstrated to induce YAP phosphorylation, which ultimately results in YAP degradation and the inhibition of cell proliferation (19). Given that LATS1 is activated in IFN-I signaling, we further determined whether IFN-I could induce YAP phosphorylation and degradation. The results showed that IFN-\(\alpha\) treatment markedly stimulated YAP phosphorylation at Ser\(^{219}\) (Fig. 1G). IFN treatment largely reduced cellular levels of YAP in both a time-dependent and dose-dependent manner (Fig. 1H). Knockdown of LATS1 restricted YAP phosphorylation induced by IFN-\(\alpha\), whereas LATS2 knockdown did not affect IFN-induced YAP phosphorylation (Fig. 1I). Furthermore, LATS1/2 deficiency blocked YAP down-regulation induced by IFN-I (Fig. 1J), suggesting that IFN-I induces YAP phosphorylation and down-regulation through LATS1.

The findings above indicated that YAP-mediated cell proliferation signaling could be inhibited by IFN-I. Thus, we further investigated the transcriptional expression of two classic YAP targets (Cyr61 and Ctgf). Our data showed that IFN-\(\beta\) treatment significantly restricted the expression of both Cyr61 and Ctgf (Fig. 1K), whereas LATS1 deficiency abolished IFN-\(\beta\)-induced regulation of Cyr61 and Ctgf (Fig. 1K). Consistently, LATS1 deficiency largely attenuated IFN-\(\beta\)-induced inhibitory effects on cell proliferation (Fig. 1, L and M, and fig. S1D), whereas LATS1 overexpression enhanced IFN-I-induced antiproliferative activity (fig. S1E). Similarly, YAP deficiency also attenuated IFN-I-induced inhibition of cell proliferation (fig. S1F). Together, these findings demonstrated that LATS1-YAP signaling largely contributes to the antiproliferation potency of IFN-Is.

**Tyk2 induces Tyr\(^{200/277}\) phosphorylation of LATS1 that promotes LATS1 activation**

We further explored how IFN-I signaling activates LATS1. Given that LATS1 is phosphorylated very rapidly, even within 10 min (Fig. 1B), we first observed the signaling molecules upstream of IFN-I signaling. By immunoprecipitation (IP) analysis, we found that endogenous LATS1 constitutively binds with endogenous IFNAR2 but not IFNAR1 in not only human embryonic kidney (HEK) 293T cells (fig. S2A) but also mouse primary splenocytes (Fig. 2A). Exogenously expressed HA-IFNAR2 can also interact with LATS1 (fig. S2B). Consistently, endogenous LATS1 also interacts with endogenous JAK1 (fig. S2C), a physiological interactor of IFNAR2 (7), whereas knockout of IFNAR2 abolished the interaction between LATS1 and JAK1 (fig. S2D).

Binding of LATS1 to the IFN-I receptor could facilitate rapid activation of LATS1 at the early stage of IFN-I signaling. Similar to JAK1 and Tyk2, which undergo tyrosine phosphorylation and activation upon IFN-I treatment, LATS1 but not LATS2 can be rapidly tyrosine phosphorylated by IFN-\(\alpha\) in a time-dependent manner (Fig. 2B and fig. S2E). We further determined how LATS1 tyrosine phosphorylation is induced by IFN-I signaling. We noticed that knockdown of Tyk2 but not JAK1 largely blocked IFN-induced tyrosine phosphorylation of LATS1 (Fig. 2C). Moreover, Tyk2 overexpression markedly stimulated tyrosine phosphorylation of LATS1 (Fig. 2D and fig. S2F), suggesting that Tyk2 could be responsible for LATS1 tyrosine phosphorylation in IFN-I signaling.

Next, by analyzing the putative tyrosine phosphorylation residues of LATS1 from both the PhosphoSitePlus database and our mass spectrometry observations (fig. S2G), we noticed 12 tyrosine residues that could have phosphorylation modifications (Fig. 2E). Thus, we mutated these tyrosine (Y) residues to phenylalanine (F) residues one at a time. The results showed that mutation of some of these tyrosine residues affected Tyk2-induced tyrosine phosphorylation of LATS1 (Fig. 2F). In particular, mutating either Tyr\(^{200}\) or Tyr\(^{277}\) largely attenuated LATS1 tyrosine phosphorylation induced by Tyk2, compared with mutating the other tyrosine residues of LATS1 (Fig. 2E). Furthermore, the double mutations of LATS1 at Tyr\(^{200}\) and Tyr\(^{277}\) (Y200F/277F and YF/YF) abolished Tyk2-induced tyrosine phosphorylation of LATS1 (Fig. 2F and fig. S2H), suggesting that Tyr\(^{200}\) and Tyr\(^{277}\) of LATS1 are the major residues for tyrosine phosphorylation induced by Tyk2. Consistently, when LATS1 and Tyk2 were overexpressed in cells, we observed by mass spectrometry analysis that Tyr\(^{200}\) and Tyr\(^{277}\) residues of LATS1 can undergo phosphorylation modifications (Fig. 2G). An in vitro kinase assay confirmed that recombinant Tyk2 can directly phosphorylate LATS1 on tyrosine residues, whereas mutating Tyr\(^{200/277}\) largely restricted Tyk2-induced tyrosine phosphorylation of LATS1 (Fig. 2H), suggesting that Tyk2 could induce LATS1 tyrosine phosphorylation mostly at Tyr\(^{200/277}\) in the IFN-I signaling pathway. In line with this speculation, mutation of Tyr\(^{200/277}\) of LATS1 largely inhibited IFN-\(\alpha\)-stimulated tyrosine phosphorylation of LATS1 (Fig. 2I).

We noticed that IFN-\(\alpha\) also cannot stimulate LATS1-Y200F/Y277F phosphorylation at Ser\(^{909}\) (Fig. 2I), suggesting that Tyr\(^{200/277}\) phosphorylation could be critical for IFN-\(\alpha\)-stimulated Ser\(^{909}\) phosphorylation and activation of LATS1. To further reveal the correlation between LATS1 Tyr\(^{200/277}\) phosphorylation and its activation, we constructed Y200E, Y277E, and Y200/277E mutants of LATS1, which mimic LATS1 tyrosine phosphorylation at the corresponding residue(s). The results showed that LATS1 Tyr\(^{200/277}\) phosphorylation...
Fig. 1. IFN-I activates LATS1-YAP signaling to inhibit cell proliferation. (A and B) Immunoblotting (IB) analysis of Ser909 phosphorylation of LATS1 (pS-LATS1) in 2fTGH cells treated with IFN-α (1000 IU/ml) (A) or in MEFs treated with mouse IFN-β (mIFNβ; 1000 IU/ml) (B). (C to E) IB analysis of pS-LATS1 in 2fTGH treated with IFN-γ (3000 IU/ml) (C) or IFN-λ (100 ng/ml) (D) or in Ifnar1<sup>−/−</sup> and Ifnar1<sup>+/+</sup> MEFs treated with mIFNβ (1000 IU/ml) (E). (F) IB analysis of pS-LATS1 in 2fTGH treated with tumor necrosis factor-α (TNFα) (20 ng/ml) or interleukin-6 (IL-6) (100 ng/ml). (G and H) IP or IB analysis of pYAP (G) and YAP (H) in HEK293T treated with IFN-α as indicated. (I) IP-IB analysis of pYAP in HEK293T transfected with control short hairpin RNAs (shRNAs; shCON) or shLATS1 or shLATS2 and then treated with IFN-α (1000 IU/ml, 1 hour). (J) IB analysis of YAP in Lats1/2<sup>+/+</sup> or Lats1/2<sup>−/−</sup> MEFs treated with mIFNβ (1000 IU/ml, 4 hours). (K) RT-qPCR analysis of Cyr61 and Cfgf in Lats1/2<sup>+/+</sup> or Lats1/2<sup>−/−</sup> MEFs treated with mIFNβ (1000 IU/ml). (L) Cell counting kit 8 assay for analyzing the proliferation of Lats1/2<sup>+/+</sup> and Lats1/2<sup>−/−</sup> MEFs treated with mIFNβ (500 IU/ml, 48 hours). (M) Cell numbers were counted in Lats1/2<sup>+/+</sup> and Lats1/2<sup>−/−</sup> MEFs treated with mIFNβ (500 IU/ml) for 1, 2, and 3 days. IFN-mediated inhibition rate of cell proliferation was calculated. Data are representative of three independent experiments (A to J) or are shown as means and SD of three biological replicates (K to M). N.S, not significant (P > 0.05), *P < 0.05, **P < 0.01, and ***P < 0.001 (two-tailed unpaired Student’s t test).
Fig. 2. Tyk2 induces Tyr200/277 phosphorylation of LATS1 that promotes LATS1 activation. (A) IP-IB analysis of endogenous LATS1-IFNAR1 or LATS1-IFNAR2 interaction in mouse primary splenocytes. (B) IP-IB analysis of pan-tyrosine phosphorylation of LATS1 (Pan-pY) in HEK293T treated with IFN-α (1000 IU/ml). (C) IP-IB analysis of pan-pY-LATS1 in HEK293T transfected with shCON or shRNAs against JAK1 or Tyk2 (shJAK1 or shTyk2) and then treated with IFN-α (1000 IU/ml, 30 min). The first lane represents an IgG control for IP. (D) IP-IB analysis of pan-pY-LATS1 in HEK293T cotransfected with Flag-LATS1 and increasing amounts of HA-Tyk2. (E and F) IP-IB analysis of pan-pY-LATS1 in HEK293T cotransfected with HA-Tyk2 and Flag-LATS1 mutants (E) or in Lats1/2−/− MEFs transfected with LATS1 [wild type (WT) or Y200F/Y277F] and HA-Tyk2 (F). (G) Flag-LATS1 proteins were immunoprecipitated from HEK293T cotransfected with Flag-LATS1 and HA-Tyk2 by Flag agarose. The red “y” represents Y200/277. m/z is the mass/charge ratio. The “b”s and “y”s indicate mass spectrometry–identified fragment ions from the N termini (b) and C termini (y) of the peptides after fragmentation. The presented diagrams provided the tandem mass spectra of the identified peptides. (H) In vitro kinase assay using Flag-LATS1 pulled down from HEK293T transfected with Flag-LATS1 (WT or YF/YF) and recombinant Tyk2. (I) IP-IB analysis of LATS1-pY-LATS1 (pY-LATS1) and pS909 (pS-LATS1) in Lats1/2−/− MEFs transfected with LATS1 (WT or Y200F/Y277F) and treated with IFN-α (1000 IU/ml, 30 min). (J) IP-IB analysis of pS909-LATS1 in HEK293T transfected with Flag-LATS1 (WT or its phosphomimetic mutant: Y200E, Y277E, or Y200E/Y277E). Data are representative of three independent experiments (A to F and H to J).
mimic mutants harbored much higher levels of Ser\textsuperscript{909} phosphorylation in cells, as compared with wild-type (WT) LATS1 (Fig. 2J). Collectively, these findings suggest that IFN-I signaling induces tyrosine phosphorylation at Tyr\textsuperscript{200/277} of LATS1 through Tyk2, which promotes Ser\textsuperscript{909} phosphorylation and activation of LATS1.

**LATS1 promotes Ser\textsuperscript{727} phosphorylation of STAT1 to enhance IFN-I antiviral activity**

We next sought to determine the effects of LATS1 activation on IFN-I signaling activity. To this end, we performed RNA sequencing (RNA-seq) analyses of differential gene expression in fibroblast 2iTGH cells transfected with either shCON or shLATS1 and then treated with or without IFN-α (Fig. S3, A and B). We noticed that there were 1687 genes down-regulated and 1789 genes up-regulated in IFN-treated LATS1-deficient cells, as compared with IFN-treated shCON cells (Fig. 3A). The Top 10 Biological Process Gene Ontology (BPGO) showed that these 1687 down-regulated genes are mainly related to IFN-I signaling and response to viruses (Fig. 3B), suggesting that LATS1 knockdown could down-regulate IFN-I antiviral signaling. We further analyzed IFN-induced genes by comparing IFN-α-treated (IFN-α) and IFN-α-untreated (Mock) groups in LATS1-sufficient cells. From these IFN-induced genes, we noticed that there are a large number of identified ISGs, including many well-recognized ISGs (such as Ifi1f1/2/3, Ifitm3, Ifi6, Ifih1, Mx1, Oas1/2/3, Oasl, Ifi15, Ifg20, Rasa2, Agpe3g, Cxcl10/11, Ifr1/27, Tlr3, Ddx58, Tnfaip3, and so on) and other reported ISGs, which were strongly up-regulated in LATS1-sufficient cells (Fig. 3C, left). However, when LATS1 was knocked down, the induction of these ISGs by IFN-I was markedly restricted (Fig. 3C, middle). Further analysis of the fold induction of these ISGs by IFN-I in the shCON and shLATS1 groups showed that LATS1 deficiency significantly attenuates IFN-induced ISG transcriptional expression (Fig. 3C, right). Consistently, overexpression of LATS1 but not LATS2 significantly enhanced transcriptional expression of Ifi1f1 (Fig. 3D), a widely used representative ISG (20–22), whereas LATS1 mutants, LATS1-S909A and -Y200/277F (YF/YF), lost the ability to promote Ifi1f1 expression (Fig. 3, E and F). Knockdown of LATS1 remarkably inhibited the activity of the ISRE promoter of ISGs induced by IFN-I (Fig. 3G). In line with the decreased activity of the ISRE promoter, IFN-I–induced ISG expression was significantly inhibited by LATS1 knockdown (Fig. 3H). These findings suggested that LATS1 could be a positive regulator of IFN-I–induced expression of ISGs.

LATS1 did not affect the expression levels of Jak1, Tyk2, Stat1, Stat2, and Ifi9 (Fig. 4, A to D) and did not affect IFN-α–induced activation of Jak1, Tyk2, and Stat2 (Fig. 4, A and D). In addition, knockdown of LATS1 did not affect Tyr\textsuperscript{701} phosphorylation (pY701) of Stat1 induced by IFN-I (Fig. 4C). However, knockdown (Fig. 4, E and F) or knockout (Fig. 4G) of LATS1 strongly inhibited IFN-α/β–induced Ser\textsuperscript{727} phosphorylation of Stat1. Conversely, overexpression of LATS1 promoted Stat1 Ser\textsuperscript{727} phosphorylation induced by IFN-I (Fig. 4C). Furthermore, overexpression of LATS1 but not LATS2 in Lats1/2−/− MEF cells rescued IFN-β–induced Ser\textsuperscript{727} phosphorylation of Stat1 (Fig. 4H). These results, LATS1 overexpression cannot promote IFN-α–induced Ser\textsuperscript{727} phosphorylation of Stat1 (Fig. 4I), lost the ability to promote Stat1 Ser\textsuperscript{727} phosphorylation induced by IFN-I signaling. These findings suggested that LATS1 mediates IFN-I–stimulated Stat1 Ser\textsuperscript{727} phosphorylation, whereas LATS2 does not affect Stat1 Ser\textsuperscript{727} activation in IFN-I signaling. Although LATS1 and LATS2 share high sequence similarity within their kinase domains, they have very lower conservation at their N terminus and show critical differences in their in vivo functions in knockout mice (23), which could affect the regulation of LATS1 but not LATS2 on IFN-I signaling.

We further found that LATS1 deficiency inhibited not only IFN-I–induced expression of Ifi1f1 but also another two representative ISGs, Tap1 and Gbp2 (Fig. S4, A, B, and C), which are relatively specific for reflecting Stat1 Ser\textsuperscript{727} phosphorylation–mediated transcriptional activity (12). Consistently, knockdown of LATS1 cannot inhibit IFN-I–induced transcriptional expression of ISGs in Stat1−/− mice any longer (Fig. 4J and fig. S4D), suggesting that LATS1 regulates IFN-I full activity via Stat1. Ser\textsuperscript{727}. In addition, the decreased levels of pSer\textsuperscript{277} Stat1 and ISGs in LATS1-deficient cells was not due to the impaired binding of the Stat1-containing transcription complex (ISGF3) with the ISRE promoter, because knockout of LATS1 did not affect enrichment of the ISGF3 complex on the ISRE promoter of ISG genes (Fig. S4E). These findings supported the role of LATS1 in regulating Stat1 Ser\textsuperscript{727} phosphorylation induced by IFN-I. In line with the regulation of ISG expression, knockdown of LATS1 restricted IFN-α–mediated antiviral activity against vesicular stomatitis virus (VSV) infection (Fig. 4K and fig. S4, F and G). Likewise, LATS1 knockout largely inhibited IFN-β–mediated antiviral activity to fight against Sendai virus (SeV) infection (fig. S4H). These findings suggest that LATS1 promotes Stat1 Ser\textsuperscript{727} phosphorylation and IFN-I antiviral activity.

**IFN-I stimulates LATS1 nuclear import to target CDK8 for Stat1 Ser\textsuperscript{727} phosphorylation**

To further study how LATS1 regulates Stat1 Ser\textsuperscript{727} phosphorylation, we observed three protein kinases, Pck8, p38, and Cdk8, which were reported to induce Ser\textsuperscript{727} phosphorylation of Stat1 (12–14). The results showed that two widely used inhibitors, a pan-PKC inhibitor (Go 6983) and a p38 inhibitor (SB202190), did not attenuate LATS1-mediated regulation of Stat1 Ser\textsuperscript{727} phosphorylation induced by IFN-I signaling (Fig. S5, A and B). However, a Cdk8 inhibitor (MSC2530818, MSC), which displays excellent kinase selectivity to Cdk8 but not to other Cdk family members and has good biochemical and cellular potency (24), strongly inhibited both IFN-α–induced Ser\textsuperscript{727} phosphorylation of Stat1 and LATS1-mediated up-regulation of Stat1 Ser\textsuperscript{727} phosphorylation (Fig. 5A). Consistently, the Cdk8 inhibitor also significantly inhibited LATS1-mediated regulation of ISG expression induced by IFN-α (fig. S5C). Knockdown of Cdk8 markedly restricted LATS1-mediated regulation of Stat1 Ser\textsuperscript{727} phosphorylation in IFN-I signaling (Fig. 5B), suggesting that LATS1 could regulate Ser\textsuperscript{727} phosphorylation of Stat1 through Cdk8. As a matter of fact, LATS1 was repeatedly observed among the potential Cdk8–interacting proteins in mass spectrometry analysis (Fig. 5C, left, and fig. S5D). IP analysis also confirmed the interaction between HA-LATS1 and Flag-Cdk8 (Fig. 5C, right). Consistently, Cdk8 did not affect IFN-I–induced activation of Stat2 (fig. S5E). Together, we speculated that LATS1 could interact with Cdk8 to regulate IFN-I–induced pSer\textsuperscript{727} Stat1.
Fig. 3. LATS1 regulates IFN-I–induced transcriptional expression of ISGs. (A and B) RNA-seq analysis in 2fTGH cells transfected with shCON or shLATS1 and then treated with IFN-α (1000 IU/ml, 8 hours). A total of 1687 genes were significantly down-regulated (blue) and 1789 genes were significantly up-regulated (red) in the shLATS1 + IFN-α group, comparing with the shCON + IFN-α group. Differential expressed genes were defined as the genes with the changes ≥2 folds and P value < 0.05 between two groups (A). Top 10 BPGO terms that are enriched in (A) were analyzed using the R package cluster Profiler (B). (C) The relative expression levels of the recognized ISGs in the shCON + Mock, shCON + IFN-α, shLATS1 + Mock, and shLATS1 + IFN-α groups are shown in the left (orange to white). The fold induction (IFN-α versus Mock) of ISGs in shCON and shLATS1 groups were analyzed in the right (red to blue). (D to F) RT-qPCR analysis of Ifit1 in HEK293T transfected with empty vectors (CON) or Myc-LATS1/2 (D), or in 2fTGH transfected with HA-LATS1 (WT or S909A) (E), or in HEK293T transfected with Flag-LATS1 (WT or YF/YF) (F), followed by IFN-α treatment (1000 IU/ml, 4 hours). (G) Dual-luciferase reporter assay for the ISRE activity in HEK293T cotransfected with shLATS1, ISRE-luciferase, and Renilla and then stimulated with IFN-α (1000 IU/ml). Data are shown as means and SD of three biological replicates (D to H). N.S (P > 0.05), *P < 0.05, **P < 0.01, and ***P < 0.001 (two-tailed unpaired Student’s t test).
Fig. 4. LATS1 promotes STAT1 Ser\(^{727}\) phosphorylation to enhance IFN-I antiviral activity. (A) IB analysis of IFN-I signaling in HEK293T transfected with HA-LATS1 and treated with IFN-\(\alpha\) (1000 IU/ml). (B) IB analysis of IRF9 in Lats1/2\(^{+/+}\) and Lats1/2\(^{-/-}\) MEFs. (C) IB analysis of pY701-STAT1 in HEK293T transfected with shLATS1 and treated with IFN-\(\alpha\) (1000 IU/ml). (D) IB analysis of pY690-STAT2 (pSTAT2), STAT2, and IRF9 in Lats1/2\(^{+/+}\) and Lats1/2\(^{-/-}\) MEFs treated with mIFN\(\beta\) (1000 IU/ml). (E and F) IB analysis of pS727-STAT1 in HeLa cells transfected with shLATS1 (E) or in HEK293T transfected with shLATS1 (F) and then treated with IFN-\(\alpha\) (1000 IU/ml). (G and H) IB analysis of pS727-STAT1 in Lats1/2\(^{+/+}\) or Lats1/2\(^{-/-}\) MEFs treated with mIFN\(\beta\) (500 or 1000 IU/ml, 1 hour) (G) or transfected with Flag-LATS1 or Flag-LATS2 and treated with mIFN\(\beta\) (1000 IU/ml, 1 hour) (H). (I) IB analysis of pS727-STAT1 in HEK293T transfected with Flag-LATS1 (WT or YF/YF) and treated with IFN-\(\alpha\) (1000 IU/ml, 1 hour). (J) RT-qPCR analysis of the representative ISG (\(Ifit1\)) in STAT1-WT or STAT1-S727A-knockin 2FTHG cells transfected with shCON or shLATS1, followed by IFN-\(\alpha\) treatment (1000 IU/ml, 4 hours) (right). S727A-knockin was confirmed by sequencing (left). (K) Median tissue culture infectious dose (TCID\(_{50}\)) assay to analyze VSV titers in supernatants from HEK293T transfected with shLATS1 and stimulated with IFN-\(\alpha\) (60 IU/ml), followed by VSV infection (multiplicity of infection = 0.1, 24 hours). Data are shown as means and SD of three biological replicates (J to K) or are representative of three independent experiments (A to I). N.S (\(P > 0.05\)), *\(P < 0.05\), **\(P < 0.01\), and ***\(P < 0.001\) (two-tailed unpaired Student’s t test).
Fig. 5. IFN-I stimulates LATS1 nuclear import to induce CDK8-mediated Ser^{727} phosphorylation of STAT1. (A) HEK293T cells transfected with or without HA-LATS1 were pretreated with MSC2530818 (1 μM) for 24 hours and then stimulated with IFN-α (1000 IU/ml, 30 min). STAT1-pS727 levels were analyzed by IB. (B) IB analysis of pS727-STAT1 in HEK293T transfected with HA-LATS1 and (or) shRNAs against CDK8 (shCDK8), followed by IFN-α treatment (1000 IU/ml, 30 min). (C) Flag-CDK8 and its binding proteins were immunoprecipitated from Flag-CDK8–overexpressing HEK293T by a Flag antibody and then subjected to mass spectrometry analysis (left). IP-IB analysis of the interaction between Flag-CDK8 and HA-LATS1 in HEK293T cells (right). (D and E) IB analysis of LATS1 (D) or pS909-LATS1 (E) in the cytoplasm and nucleus of HEK293T treated with IFN-α (1000 IU/ml). (F) Cellular pSer^{909}-LATS1 was immunoprecipitated from HEK293T treated with IFN-α (1000 IU/ml, 30 min) using a specific pSer909-LATS1 antibody. The supernatant after IP of pSer909-LATS1 was used to pull down non–pSer909-LATS1 using a total LATS1 antibody. Then, IB was used to analyze the interaction of CDK8. (G) Confocal microscopy was used to observe the localization of LATS1 proteins in HeLa treated with or without IFN-α (1000 IU/ml, 30 min). 4′,6-Diamidino-2-phenylindole was used for the nucleus. Scale bars, 5 μm. (H) Confocal microscopy was used to observe the colocalization of pS909-LATS1 with CDK8 in HeLa treated with IFN-α (1000 IU/ml, 30 min). Scale bar, 5 μm. Data are shown as representative of three independent experiments (A to F).
in the cytoplasm and simultaneously increased in the nucleus (Fig. 5D), suggesting nuclear import of LATS1 stimulated by IFN-I. Moreover, as soon as pS-LATS1 was induced by IFN-I, it rapidly disappeared in the cytoplasm. Accordingly, pS-LATS1 rapidly accumulated in the nucleus (Fig. 5E). We then immunoprecipitated pSer909-LATS1 from whole-cell lysates from HEK293T cells treated with IFN-I and then used the supernatant after IP of pSer909-LATS1 to pull down non–pSer909-LATS1 using a total LATS1 antibody. We found that pSer909-LATS1 but not non–pSer909-LATS1 interacted with CDK8 (Fig. 5F). Given that IFN-induced pS-LATS1 accumulated in the nucleus and only pS-LATS1 interacts with CDK8, we speculated that under IFN-I treatment, LATS1 could interact with CDK8 in the nucleus. Consistent with this speculation, our data showed that the interaction between LATS1 and CDK8 occurred only in the nucleus after IFN-I treatment (fig. S5F).

To directly observe the colocalization of LATS1 and CDK8 in IFN-I signaling, confocal microscopy was performed. The results showed that without IFN-I treatment, LATS1 mainly locates in the cytoplasm, whereas under IFN-I stimulation, nuclear LATS1 can be clearly observed (Fig. 5G). In addition, we also observed clear colocalization of IFN-activated pS-LATS1 with CDK8 in the nucleus (Fig. 5H). Together, these findings suggest that IFN-activated pS-LATS1 translocates into the nucleus to target CDK8 for the regulation of STAT1 Ser\(^{727}\) phosphorylation.

**LATS1 promotes CDK8 phosphorylation at Ser\(^{62}\)**

We next wondered how LATS1 regulates CDK8. By mass spectrometry analysis, we noticed two phosphorylated serine residues of CDK8, Ser\(^{60}\) and Ser\(^{62}\), which can be easily observed only when LATS1 was overexpressed in cells (Fig. 6A and fig. S6A). Thus, we mutated both Ser\(^{60}\) and Ser\(^{62}\) of CDK8 to test their roles in regulating STAT1 Ser\(^{727}\) phosphorylation. The results showed that the CDK8-S62A but not CDK8-S60A mutant lost the ability to induce Ser\(^{727}\) phosphorylation of STAT1 (Fig. 6B), suggesting that Ser\(^{62}\) of CDK8 could be essential for CDK8-induced Ser\(^{727}\) phosphorylation of STAT1. Furthermore, by an in vitro kinase assay, we confirmed that mutation of the Ser\(^{62}\) residue disrupted the ability of CDK8 to phosphorylate STAT1 at Ser\(^{727}\) (Fig. 6C). On the basis of these findings, we then made a specific antibody targeting phosphorylated Ser\(^{62}\) of CDK8 (fig. S6, B and C). We noticed that overexpression of LATS1, but not the LATS1 S909A/T1079A (SA/TA) inactive mutants, induced Ser\(^{62}\) phosphorylation of both exogenously expressed CDK8 (Fig. 6D) and endogenous CDK8 (Fig. 6E). Likewise, overexpression of LATS1 Y200/277F (YF/YF) also did not induce CDK8 Ser\(^{62}\) phosphorylation (Fig. 6F and fig. S6D). An in vitro kinase assay demonstrated that recombinant LATS1 proteins can induce CDK8 phosphorylation at Ser\(^{62}\) (Fig. 6G). CDK8 was phosphorylated at the Ser\(^{62}\) residue by IFN-I signaling (Fig. 6H). When LATS1 was knocked down, IFN-I did not induce CDK8 Ser\(^{62}\) phosphorylation any longer (Fig. 6I). LATS1 deficiency completely abolished IFN-I–induced Ser\(^{62}\) phosphorylation of CDK8 (Fig. 6I). Furthermore, mutation of Ser\(^{62}\) of CDK8 inhibited CDK8–mediated regulation of ISG expression induced by IFN-I signaling (Fig. 6K). We noticed that the Ser\(^{62}\) motif seems to be inconsistent with either the H-x-(H/R/K)-x-x-S/T consensus motif for LATS1 or the preferred R-x-x-S phosphorylation motif for the LATS1 homolog Dbf2 (25, 26). However, recent studies proved that LATS1 can phosphorylate some Ser/Thr residues that either do not match or only partially match the LATS1 consensus (27). In fact, three-dimensional (3D) structure, which could be important and sufficient in determining phosphorylation accessibility of protein substrates (28, 29), showed that the CDK8 Ser\(^{62}\) residue is located in the kinase domain of CDK8 and has an exposed R\(_{55-x-x-S_{62}}\) motif in 3D structure (fig. S6E). In summary, we think that LATS1 promotes CDK8 Ser\(^{62}\) phosphorylation to enhance IFN-I signaling activity.

**LATS1 deficiency attenuates IFN-I signaling and antiviral activity in vivo**

We further determined the roles of LATS1 in regulating in vivo IFN-I antiproliferative and antiviral functions. To this end, we first established a B16 melanoma transplantation model to observe the in vivo proliferation of cells with or without IFN-I treatment. We found that IFN-I treatment significantly inhibited B16 melanoma cell proliferation as expected (Fig. 7A and fig. S7A). In addition, we noticed that knockdown of LATS1 restricted B16 cell growth to some extent (Fig. 7A and fig. S7A), which is consistent with a previous observation (30). We found that LATS1 knockdown largely attenuated IFN-I–induced in vivo antiproliferation activity in B16 melanoma mouse model (Fig. 7, A and B), suggesting that LATS1 can regulate in vivo IFN-I antiproliferative activity.

IFN-α is a routine treatment drug for patients with hepatitis B virus (HBV). We found that IFN-α administration in HBV patients strongly stimulated LATS1 activation at Ser\(^{909}\) and CDK8 phosphorylation at Ser\(^{62}\) in the peripheral blood mononuclear cells (PBMCs) of patients (Fig. 7C and fig. S7B). In addition, both mouse IFN-β (mIFNβ) injection and virus infection markedly induced LATS1-Ser909 phosphorylation in mouse spleen tissues (Fig. 7D). Consistently, LATS1 activation in mouse liver and lung tissues was also clearly observed under virus infection (fig. S7C). Furthermore, we found that mIFNβ injection in mice induced the interaction of pS-LATS1 and CDK8 in the nucleus (Fig. 7E). These findings demonstrated that IFN-I activates the LATS1-CDK8 pathway in both humans and mice.

It has been reported that Lats1\(^{−/−}\) mice exhibit severe defects in mammary gland development, fertility, and growth (31). Thus, we next used Lats1\(^{−/−}\) mice to study the effect of LATS1 deficiency on virus infection. We found that LATS1 levels were substantially reduced in Lats1\(^{−/−}\) mice, as compared with Lats1\(^{+/−}\) mice (Fig. 7F). In line with the previous observations, we found that virus-induced STAT1 activation at Ser\(^{727}\) was markedly inhibited in Lats1\(^{−/−}\) mice (Fig. 7F). Consistently, the expression levels of the representative ISGs, including Ifit1, Viperin, and Gbp2, were significantly lower in the different organs of Lats1\(^{−/−}\) mice than Lats1\(^{+/−}\) mice during virus infection (Fig. 8, A and B, and fig. S7, D to F). As a consequence, the viral loads in the organs of Lats1\(^{−/−}\) mice were much higher than those of WT mice (Fig. 8C). Lats1\(^{−/−}\) mice showed higher mortality when challenged with viruses, as compared with Lats1\(^{+/−}\) mice (Fig. 8D). Together, these findings revealed biological importance of activation of LATS1-CDK8 signaling by IFN-I in the host during virus infection.

**DISCUSSION**

In this study, we demonstrated that IFN-I via its receptors rapidly activates the Tyk2-LATS1-CDK8 pathway, which is required for full IFN-I activity to inhibit cell proliferation and fight against viral infection. IFN-activated Tyk2 induces tyrosine phosphorylation of LATS1, which promotes LATS1 activation and nuclear translocation for...
Fig. 6. LATS1 promotes CDK8 phosphorylation at Ser62. (A) HEK293T cells were transfected with Flag-CDK8 and Flag-LATS1. Mass spectrometry was used to detect serine phosphorylation sites of Flag-CDK8. (B) IB analysis of pS727-STAT1 in HEK293T transfected with increasing amounts of Flag-CDK8 (WT, S60A, or S62A). (C) Flag-CDK8 and Flag-STAT1 were pulled down from HEK293T transfected with Flag-CDK8 (WT/S60A/S62A) or Flag-STAT1 and then subjected to extensive high-salt washing. In vitro kinase assay was performed to analyze the phosphorylation effect of Flag-CDK8 or whole-cell lysates (WCL) on Flag-STAT1. (D) IB analysis of pS62-CDK8 in HEK293T cotransfected with Flag-CDK8 and HA-LATS1-WT or -SA/TA (WT or S909A/T1079A) using a specific pSer62-CDK8 antibody. (E and F) IB analysis of pS62-CDK8 in HEK293T transfected with HA-LATS1 (WT or SA/TA) (E) or Flag-LATS1 (WT or YF/YF) (F). (G) Flag-CDK8 was immunoprecipitated from Flag-CDK8–overexpressing HEK293T and subjected to extensive high-salt washing. In vitro kinase assay was performed using recombinant glutathione S-transferase (GST)–LATS1. (H) IB analysis of pS62-CDK8 in HEK293T treated with IFN-α (1000 IU/ml). (I and J) IB analysis of pS62-CDK8 in HEK293T transfected with Flag-CDK8 and shLATS1 and treated with IFN-α (1000 IU/ml, 1 hour) (I) or in Lats1/2−/− or Lats1/2−/− MEFs treated with miFNβ (1000 IU/ml, 1 hour) (J). (K) RT-qPCR analysis of Ifi17mRNA (fold) in IFN-α (1000 IU/ml, 4 hours). Data are shown as means and SD of three biological replicates (K) or are representative of three independent experiments (B to J). N.S (P > 0.05), *P < 0.05, and **P < 0.01 (two-tailed unpaired Student’s t test).
CDK8-Ser62 phosphorylation. Consequently, this Tyk2-LATS1-CDK8 signaling cascade mediates IFN-I–induced STAT1 Ser727 phosphorylation and activation (Fig. 8E).

IFNs were initially discovered to be an antiviral factor released from chick chorio-allantoic membranes infected with influenza viruses (32). More than 30 years later, STAT1, STAT2, and IRF9 were identified as transcription factors of IFN-I signaling (33, 34). Further studies revealed the JAK-STAT pathway as the canonical IFN signaling. In addition, IFNs can activate other signaling pathways to regulate IFN activity, but the detailed mechanisms remain elusive (35). For example, IFN-I can activate the p38 signaling pathway, which promotes the transcriptional activity of ISGs. However, p38-mediated regulation of IFN-I signaling is independent of STAT1 activation and the STAT1-STAT2-IRF9 complex (6, 35, 36). Thus, the p38 pathway is considered as the noncanonical IFN signaling pathway. In this study, we revealed that IFN-I activates the LATS1-CDK8 pathway, which is triggered by Tyk2 upon IFN-I engagement and targets STAT1 at Ser727. The LATS1-CDK8 pathway is essential for full STAT1 activation and controls IFN-I–induced antiviral activity. In addition, IFN-activated LATS1 signaling participates in the regulation of IFN-I–induced antiproliferation effects. Thus, the Tyk2-LATS1-CDK8 pathway is indeed an essential component of canonical IFN-I signaling.

In the Hippo pathway, MST1/2 induces Ser909/Thr1079 phosphorylation of the hydrophobic motif of LATS1, which results in LATS1 activation. However, MST1/2 are not the sole protein kinases for LATS1 activation. MAP4K (mitogen-activated protein kinase kinase kinase) family members also phosphorylate the LATS1 hydrophobic motif (37). In addition, TAOKs (thousand-and-one amino acid kinases) can induce phosphorylation of the LATS1
hydrophobic motif (38). MST1/2, MAP4K, and TAOks are all the members of the Ste20-like family of kinases. In addition to Ser\textsuperscript{909}/Thr\textsuperscript{1079} phosphorylation of LATS1, the Ser\textsuperscript{613}/Thr\textsuperscript{490} residues of LATS1 can be phosphorylated by CDK1/CDC2 (39). Ser\textsuperscript{464} phosphorylation of LATS1 can be induced by NUAK1 (40). All these studies reported the Ser/Thr phosphorylation situation of LATS1. Here, we identified the Tyr\textsuperscript{200/277} residues of LATS1 that are phosphorylated by Tyk2 in the IFN-I signaling pathway. LATS1 Tyr\textsuperscript{200/277} phosphorylation promotes its Ser\textsuperscript{909}/Thr\textsuperscript{1079} phosphorylation, thus linking LATS1 tyrosine phosphorylation to its activation. Therefore, our findings revealed a Tyr-phosphorylated form of LATS1 for its activation. LATS1 has been reported to exhibit different subcellular localizations. In the classical Hippo pathway, LATS1 is activated and, in turn, phosphorylates YAP/TAZ in the cytoplasm. LATS kinases were also detected on centrosomes, which could regulate mitosis (41). In addition, LATS1 activation could occur in the nucleus. Derepressed CRL\textsubscript{4}DAF1 can ubiquitinate and inhibit LATS1 in the nucleus (42).
MST1 can translocate into the nucleus to phosphorylate LATS1 in the nucleus for epithelial differentiation (43). In this study, we found that LATS1 colocalizes with IFNAR2. Upon IFN-1 engagement, LATS1 is tyr phosphorylated and activated by Tyk2. Then, activated LATS1 translocates into the nucleus to further execute its function. Together, this study uncovered a previously unknown mechanism of action of LATS1.

Despite the importance of STAT1 Ser727 phosphorylation, the kinases targeting STAT1 Ser727 remain to be illuminated. Although p38 was identified as a protein kinase for stress-induced STAT1 Ser727 phosphorylation, most studies demonstrated that IFN-stimulated Ser27 phosphorylation of STAT1 is independent of p38 (13, 44). In addition, two important studies uncovered that PKC- and CDK8 can phosphorylate STAT1 at Ser727 (12, 14). It has been documented that STAT1 Ser727 phosphorylation is restricted to promoter-bound transcription factors, suggesting that the kinases targeting STAT1 Ser727 are located in the nucleus (12). Although several protein kinases have been reported to induce Ser727 phosphorylation of STAT1, how IFN-1 stimulates the nuclear translocation of those cytoplasmic kinases to target STAT1 Ser727, or how those nuclear kinases targeting STAT1 Ser727 are activated by IFN-1 signaling, is still unknown. In this study, we revealed that IFN-1-stimulated formation of the IFNAR1/IFNAR2 heterodimer enables LATS1, which binds constitutively with IFNAR2, to interact with and be tyr-phosphorylated by Tyk2 that physiologically binds with IFNAR1. Activated LATS1 then translocates into the nucleus for nuclear CDK8 Ser62 phosphorylation, which lastly induces STAT1 Ser727 phosphorylation. Together, this study revealed a complete signaling cascade that links IFN-1 stimulation to STAT1 Ser727 activation and full IFN-I activity.

**Materials and Methods**

**Patients and sample collection**

This study included samples from seven patients (male; age, 32.66 ± 6.03) with HBV infection who were treated with peg–IFN-α–2b in the Affiliated Infectious Diseases Hospital of Soochow University in 2020–2021. Peripheral blood samples were collected in patients with HBV before initial peg–IFN-α–2b treatment and 1 hour after IFN injection. Human PBMC was then isolated from peripheral blood by Ficoll. Informed consent was obtained from each person, and these studies were approved by the Ethics Committee of Soochow University.

**Mice**

Lats1+/− mice were generated by Cyagen Biosciences Inc. (Guangzhou). Ifnar1−/− mice were gifts from C. Dong (Soochow University). Mst1−/− mice were gifts from Y. Liu (Soochow University). WT C57BL/6 mice were purchased from Shanghai SLAC Laboratory Animals. Following procedures previously described (21), we maintained all mice under specific pathogen–free conditions in the animal facility of Soochow University and used 6- to 8-week-old mice in all experiments. Animal care and use protocol adhered to the National Regulations for the Administration of Affairs Concerning Experimental Animals. All animal experiments have received ethical approval by the Ethics Committee of the Soochow University and were carried out in accordance with the Laboratory Animal Management Regulations with approval of the Scientific Investigation Board of Soochow University, Suzhou.

**Mouse primary cells and gene knockout MEF**

Lats1+/−, Mst1−/− or WT mouse (C57BL/6) tissues were prepared from 6- to 8-week-old adult mice. Briefly, mouse tissues were cut into small pieces and then grinded to cell suspension. Mouse primary liver, spleen, heart, kidney, and lung cells were collected and prepared for further experiments. Ifnar1−/− MEFs were isolated from the embryo tissues of Ifnar1−/− mice that were pregnant for 13 to 14 days, which were digested with collagenase.

**Cell culture and reagents**

HEK293T, A549, HeLa, MEF, Vero, and HT1080 cells were obtained from the American Type Culture Collection. Lats1/2−/− MEF cells were provided by L. Chen (Xiamen University, China). 2fTGH and U3A cells were described previously (21). B16 melanoma cells were gifts from L. Hu (Soochow University, China). Following procedures previously described (21), we cultured all cells at 37°C under 5% CO2 in Dulbecco’s modified Eagle’s medium (DMEM; HyClone) supplemented with 10% fetal bovine serum (FBS; Gibco, Life Technologies), penicillin (100 U/ml), and streptomycin (100 μg/ml). Recombinant human IFN-α was from PBL Interferon Source. Recombinant mIFNβ, human IFN-γ, and IFN-λ were purchased from R&D Systems. Recombinant Tyk2 proteins were purchased from Abcam. The CDK8 inhibitor MSC2530818 was purchased from Selleck. Non–phospho-peptide (NP-Pep) and phospho-peptide (P-Pep) were from GL Biochem (Shanghai). Recombinant glutathione S-transferase (GST)–LATS1, Flag peptides (F3290), Puromycin, and other chemicals were purchased from Sigma-Aldrich. The polyclonal antibody to phosphorylated Ser727 of CDK8 was generated by GL Biochem (Shanghai).

**Plasmids and transfection**

Flag-LATS1 and HA-LATS1 plasmids were gifts from Z. Wang (Soochow University). Myc-LATS2 was a gift from Y. Hata (Addgene, no. 66852). Flag-STAT1 was generated using polymerase chain reaction (PCR) amplified from pIND-STAT1-V5 from S. Johnson. Flag-His-CDK8 was purchased from Vigene Biosciences. Myc-LATS1 was generated using PCR amplification from Flag-LATS1. HA-IFNAR2, ISRE-Luc, HA-Tyk2, shJAK1, shTyk2, and Renilla plasmids were gifts from S. Y. Fuchs (University of Pennsylvania). The shCDK8 and shLATS1 plasmids were purchased from GENECHEM (Shanghai, China). All mutations were generated by the QuickChange Lightning Site-Directed Mutagenesis Kit (Stratagene, 210518). Transient transfections for different cell lines were carried out using LongTrans (Ucallm, TF/07). Transient transfections for mouse primary cells were performed using GenePORTER2 (Genlantis, T202015).

**Mass spectrometry analysis**

HEK293T cells were transfected with either Flag-LATS1/HA-Tyk2 or Flag-CDK8/HA-LATS1. Forty-eight hours after transfection, cells were harvested by the NP-40 lysis buffer [150 mM NaCl, 1% NP-40, tris-HCl (20 mM, pH 7.4), phenylmethylsulfonyl fluoride (PMSF; 50 mg/ml), 0.5 mM EDTA, and protease inhibitors mixtures]. M2 affinity gel (Sigma-Aldrich, A2220) was used to pull down Flag-LATS1 or Flag-CDK8. Following procedures previously described (21), we performed the mass spectrometry analysis as follows: SDS–polyacrylamide gel electrophoresis (SDS-PAGE) gels were minimally stained with Coomassie brilliant blue and then cut into 1 × 1-mm gel block, followed by digestion with trypsin. Next, the resulting tryptic peptides were identified using a mass spectrometer.
peptides were purified using the C18 Zip Tip. Then, the peptides were analyzed by an Orbitrap Elite hybrid mass spectrometer (Thermo Fisher Scientific) coupled with a Dionex LC, and tandem mass spectra were collected for the selected precursor ion within a 0.02-Da mass isolation window. Then, spectral data were searched using the Proteome Discoverer 1.4 against a UniProt protein database. The peptide spectrum matches for LATS1 or CDK8 were obtained by an Orbitrap Elite hybrid mass spectrometer.

**Immunoblotting and IP**

Cells were harvested using the lysis buffer containing 1% NP-40, 150 mM NaCl, tris-HCl (20 mM, pH 7.4), 0.5 mM EDTA, PMSF (50 μg/ml), and protease inhibitor mixtures (Sigma-Aldrich). Proteins from whole-cell lysates were first subjected to SDS-PAGE and then transferred to polyvinylidene difluoride membranes (Millipore). After blocking with 5% nonfat milk or 5% bovine serum albumin (BSA; for the antibodies targeting phosphorylated proteins) for 0.5 hour, the membranes were incubated with the corresponding primary antibodies overnight, followed by incubation with the secondary antibodies (Bioworld or Abbkine). All immunoreactive bands were visualized with SuperSignal West Dura Extended kits (Thermo Fisher Scientific).

IP was carried out using specific antibodies at 4°C. Protein G agarose beads (Millipore, #16-266) were incubated with the samples on a rotor at 4°C. After washing five times with the lysis buffer, the immunoprecipitates were eluted by heating at 95°C with the loading buffer containing β-mercaptoethanol for 10 min and then analyzed by SDS-PAGE gels and subsequent immunoblotting (IB).

The antibodies with the indicated dilutions were as follows: anti-pY701 (STAT1) (Cell Signaling Technology, 9167; 1:1000), anti-pYAP (Cell Signaling Technology, 13008; 1:1000), anti-pJAK1 (Cell Signaling Technology, 3331S; 1:1000), anti-pTyk2 (Cell Signaling Technology, 9321; 1:1000), anti-Flag (Sigma-Aldrich, F7425; 1:5000), anti-HA (Abcam, ab9110; 1:3000), anti-JAK1 (Santa Cruz Biotechnology, sc-1677; 1:1000), anti-STAT1 (Cell Signaling Technology, 9172; 1:1000), anti-Tyk2 (Cell Signaling Technology, 14193; 1:1000), anti-pS909-LATS1 (Cell Signaling Technology, 9157; 1:1000), anti-Myc (Abmart, M20002H; 1:1000), anti-–VSV-G (Abcam, ab1874; 1:1000), anti-β-actin (Viperin), anti-IFNAR1 (Sino Biological, 13222-T20; 1:1000), anti-IFN-αR1 (Sino Biological, 13222-T20; 1:1000), anti-STAT1 (Cell Signaling Technology, 8826; 1:1000), anti-VSV (Abcam, ab9110; 1:3000), anti-JAK1 (Santa Cruz Biotechnology, sc-1677; 1:1000), anti-STAT1 (Cell Signaling Technology, 9172; 1:1000), anti-Tyk2 (Cell Signaling Technology, 14193; 1:1000), anti-pS909-LATS1 (Cell Signaling Technology, 9157; 1:1000), anti-Myc (Abmart, M20002H; 1:1000), anti-–VSV-G (Abcam, ab1874; 1:1000), anti-β-actin (Viperin), anti-IFNAR1 (Sino Biological, 13222-T20; 1:1000), anti-IFN-αR1 (Sino Biological, 13222-T20; 1:1000), anti-STAT1 (Cell Signaling Technology, 8826; 1:1000), anti-VSV (Abcam, ab9110; 1:3000), anti-JAK1 (Santa Cruz Biotechnology, sc-1677; 1:1000), anti-STAT1 (Cell Signaling Technology, 9172; 1:1000), anti-Tyk2 (Cell Signaling Technology, 14193; 1:1000), anti-pS909-LATS1 (Cell Signaling Technology, 9157; 1:1000), anti-Myc (Abmart, M20002H; 1:1000), anti-–VSV-G (Abcam, ab1874; 1:1000), anti-β-actin (Viperin), anti-IFNAR1 (Sino Biological, 13222-T20; 1:1000), anti-IFN-αR1 (Sino Biological, 13222-T20; 1:1000), anti-STAT1 (Cell Signaling Technology, 8826; 1:1000), anti-VSV (Abcam, ab9110; 1:3000), anti-JAK1 (Santa Cruz Biotechnology, sc-1677; 1:1000), anti-STAT1 (Cell Signaling Technology, 9172; 1:1000), and anti-β-actin (Viperin).

**RNA iso-seq analysis**

Human fibroblast 2TGH cells were transfected with either control short hairpin RNAs (shRNAs; shCON) or shRNAs against LATS1 (shLATS1). Seventy-two hours after transfection, cells were treated with IFN-α (1000 IU/ml) for 8 hours. Total RNAs were isolated from cells using a TRIzol reagent (Invitrogen). The RNA samples were sent to the Suzhou Institute of Systems Medicine in China for further sequencing analysis. The cDNA libraries were made for sequencing with Universal Plus mRNA-Seq with NuQuant (TECAN GENOMICS, 0520B-A01). Then, the libraries were sequenced on the NovaSeq 6000 platform (Illumina). The pair-end sequencing data were treated by fastp software to filter reads with bad quality and then mapped to Ensembl hg19 reference genome by CLC genomics workbench 12.0 (Qiagen). Differential expressed genes were defined as the genes whose fold changes (increase and decrease) are more than 2, with a P value <0.05 between two groups. The R package cluster Profiler and org.Hs.eg.db are the tool for Gene Ontology enrichment. All RNA-seq data have been deposited in the Gene Expression Omnibus (GSE193088).

**CRISPR-Cas9-mediated genome editing**

The lenti-CRISPRv2 vector was a gift from F. Zhou (Soochow University, China). For gene knockout, small guide RNAs (sgRNAs) were first cloned into the lenti-CRISPRv2 vector and were then transfected into the cells.
into HEK293T cells. Forty-eight hours after transfection, the cells were cultured under puromycin (1.5 μg/ml) selection for 2 weeks, and then cells were identified by IB analysis. After that, cells were transferred to 96-well plates and cultured for further experiments.

For gene knockin, two CRISPR gRNA sequences near the S727 codon were chosen on the basis of their specificity scores to create gene-targeted alleles encoding STAT1(S727A) in cells. The sgRNA sequences were cloned into the lenti-CRISPRv2 plasmid. The constructs were then transfected into 2TGH cells together with a corresponding single-stranded DNA oligonucleotide (Donor) containing sequence (TC1→GCT) encoding the S727A substitution. The cells were cultured under puromycin (1 μg/ml) selection, and single cells were seeded into separate wells of 96-well plates. After clonal expansion, genomic DNA was purified and the PCR products were then validated to ensure complete coverage of all alleles by sequencing.

The gRNA sequences are as following: human Ifnar2, 5′-CATATGGAAATCACAACACG-3′; human Lats1, 5′-GCGACGCACTCTGTCGTCG-3′; human Yap1, 5′-TGGGGGCTGTGAGCTCATC-3′; human Stati1(sgRNA1), 5′-CTGTGGTCTGACTGTTAGA-3′; human Stati1(sgRNA2), 5′-TGACGAGGTCTCAGGATAG-3′.

Cell counting kit 8 assay
Lats1/2+/+, or Lats1/2−/− cells were seeded in 96-well plate at a density of 3 × 10^3 cells per well. Then, the Lats1/2+/+ and Lats1/2−/− cells were treated with or without mIFNβ for 1 to 3 days. To measure cell viability, cells in each well were firstly washed with 1× phosphate-buffered saline (PBS). Then, the fresh complete (10% FBS) medium containing 10 μl of cell counting kit 8 reagent was added. The plate was further incubated for 2 hours at 37°C. The number of living cells was evaluated at the absorbance of 420 nm.

Cell proliferation assay
HEK293T, Lats1/2+/+, or Lats1/2−/− cells were treated with IFN-α or mIFNβ and then were harvested in different days (days 1, 2, and 3). The number of cells was counted under an upright microscope, and then the data were analyzed by GraphPad Prism 5 (San Diego, CA, USA).

Tumor transplantation
B16 cells (1 × 10^6) stably transfected with shCON or shLATS1 were subcutaneously transplanted into the back flanks of C57BL/6 mice. After 5 days, mice were subcutaneously injected with mIFNβ (1000 IU/g, once a day) around the tumor sites. Tumor height and width were measured with a caliper every day to calculate tumor volume. Mice were euthanized after 10 days, and tumor weight was determined.

In vitro kinase assay
Recombinant Tyk2 and recombinant GST-LATS1 proteins were purchased from Sigma-Aldrich and Abcam, respectively. Flag-LATS1, Flag-STAT1, and Flag-CDK8 proteins were pulled down by a Flag antibody from HEK293T cells transfected with Flag-LATS1 or Flag-STAT1 or Flag-CDK8 and then were subjected to extensive washing for five times using high-salt (500 mM NaCl) washing buffer. The in vitro kinase assays were performed in the kinase buffer [50 mM tris-HCl, pH 7.4; 2 mM dithiothreitol (DTT); and 10 mM MgCl₂] containing 0.2 mM adenosine triphosphate for 6 hours at 37°C. The reaction products were analyzed by SDS-PAGE and detected by IB using the corresponding antibodies.

Cytoplasmic and nuclear proteins extraction
As described previously (22), cells were scraped in cold PBS and harvested in lysis buffer. This buffer contains 10 mM Heps (pH 7.9), 0.5% Triton X-100, 0.5 mM sucrose, 0.1 mM EDTA, 50 mM NaCl, 1 mM DTT, 0.5 M NaF, 10 mM sodium pyrophosphate decahydrate, 0.2 M Na₃VO₄, 1 mM PMSF, and protease inhibitors mixtures (Sigma-Aldrich). Then, the supernatant was collected after centrifugation at 1000 rpm for 10 min. The pellet was collected and resuspended in buffer A containing 10 mM Heps (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM PMSF, 1 mM DTT, and protease inhibitors mixtures. Then, the pellet was treated by centrifuging at 1000 rpm for 5 min in swinging bucket rotor. After removing the supernatant, 4 volume of buffer C containing 10 mM Heps (pH 7.9), 0.1% NP-40, 500 mM NaCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM PMSF, 1 mM DTT, and protease inhibitors mixtures was added, followed by the vortex at 4°C for 15 min and centrifugation at 14,000 rpm for 10 min. The supernatant was collected for the nuclear extract.

Viruses and viral infection in vitro
VSV, SeV, and VSV-GFP were described previously (21). Following procedures previously described (21), we first transfected cells with the corresponding plasmids. Cells were then treated with IFN-α (60 IU/ml) overnight. After washing twice, cells were infected by either VSV-GFP, SeV, or VSV at a multiplicity of infection of 0.1 or 1.0 in the serum-free medium for 2 hours for virus entry. Then, the infection medium was removed by washing twice by 1× PBS. Cells were fed with fresh medium (10% FBS) for 24 hours. Then, cells were analyzed by immunofluorescence, RT-qPCR, TCID₅₀, or Western blot.

Viral infection in vivo
The procedures for the in vivo viral infection were described previously (21). Briefly, 8-week-old mice were given intraperitoneal injections of VSV [1 × 10⁶ plaque-forming units (PFU) per gram body mouse]. Twenty-four hours after infection, lung, kidney, liver, spleen, and heart, were obtained. Then, RT-qPCR was used to analyze VSV viral RNAs. To detect in vivo activation of LATS1, CDK8, and STAT1, Lats1+/+ and Lats1−/− mice were injected with mIFNβ [1500 IU/g, 2 hours, intraperitoneally (ip)] or infected with VSV [1 × 10⁶ PFU per gram body mouse] for 6 hours. Next, Western blot was carried out using the whole-cell lysates from mouse spleen, lung, and liver tissues. For mouse survival curves, the mice were monitored for survival until 14 days.

TCID₅₀ assay
VSV viral titers were analyzed by a standard 50% tissue culture–infective dose (TCID₅₀) assay. Briefly, we first infected cells with VSV viruses. The supernatants from VSV-infected cells were collected and diluted serially using the DMEM. Next, the diluted supernatants were added on the monolayer of Vero cells in a 96-well plate. The TCID₅₀ was calculated by the Spearman–Karber algorithm.

Immunofluorescence microscopy
Following procedures previously described (21), we first washed HeLa cells by 1× PBS and fixed the cells in 4% paraformaldehyde at ice. Then, cells were permeabilized with Triton X-100 (0.5%) and blocked with BSA (5%). The cells were incubated overnight with an anti-CDK8 antibody and an anti–pS909-LATS1 antibody in 0.5% BSA. After washing three times with 1× PBS, cells were stained with

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