LincRNA-EPS impairs host antiviral immunity by antagonizing viral RNA–PKR interaction

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Abstract

LincRNA-EPS is an important regulator in inflammation. However, the role of lincRNA-EPS in the host response against viral infection is unexplored. Here, we show that lincRNA-EPS is downregulated in macrophages infected with different viruses including VSV, SeV, and HSV-1. Overexpression of lincRNA-EPS facilitates viral infection, while deficiency of lincRNA-EPS protects the host against viral infection in vitro and in vivo. LincRNA-EPS−/− macrophages show elevated expression of antiviral interferon-stimulated genes (ISGs) such as Mx1, Oas2, and Ifit2 at both basal and inducible levels. However, IFN-β, the key upstream inducer of these ISGs, is downregulated in lincRNA-EPS−/− macrophages compared with control cells. RNA pulldown and mass spectrometry results indicate that lincRNA-EPS binds to PKR and antagonizes the viral RNA–PKR interaction. PKR activates STAT1 and induces antiviral ISGs independent of IFN-I induction. LincRNA-EPS inhibits PKR-STAT1-ISGs signaling and thus facilitates viral infection. Our study outlines an alternative antiviral pathway, with downregulation of lincRNA-EPS promoting the induction of PKR-STAT1-dependent ISGs, and reveals a potential therapeutic target for viral infectious diseases.

Keywords lncRNA; lincRNA-EPS; type I interferon; antiviral immunity; PKR

Subject Categories Immunology; Microbiology; Virology & Host Pathogen Interaction; RNA Biology

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Introduction

The innate immune system is the first line of defense against pathogenic microbes including numerous life-threatening viruses. During viral infection, viral RNA or DNA are recognized by pattern recognition receptors (PRRs) to initiate complex signal transduction pathways, which ultimately leads to the induction of type I interferon (IFN-I) and proinflammatory cytokines (Akira et al, 2006; Goubau et al, 2013). Retinoic acid-inducible gene 1 (RIG-I) is one of the key cytosolic RNA sensors that recognize viral RNA from invaded viruses (Yoneyama et al, 2004). Viral RNA-triggered activation of RIG-I results in the phosphorylation of TBK1 and IRF3, which activates transcription factors including NF-κB, AP-1, and IRF3/7 to induce proinflammatory cytokines and IFN-I (Honda et al, 2006). IFN-I including IFN-α and IFN-β further trigger the phosphorylation of transcription factors STAT1 and STAT2 via the JAK-STAT pathway to induce multiple IFN-stimulated genes (ISGs) such as MX1, OAS2, ISG15, and IFIT2, which synergistically inhibit viral infection by targeting almost all the steps of viral life cycles (Sadler & Williams, 2008; Schneider et al, 2014). However, overproduction of IFN-I and hyperactivation of IFN-α/β receptor (IFNAR) downstream signaling lead to autoimmune diseases including systemic lupus erythematosus (SLE) and Aicardi-Goutières syndrome (AGS) (Chaussabel et al, 2008; Crow & Mannel, 2015). The innate immune signaling cascades during viral infection are precisely controlled by various negative feedback pathways, which protect the host by efficiently clearing invaded pathogens but avoiding autoimmunity (Wang et al, 2017b; Vierbuchen & Fitzgerald, 2021).

In addition to RIG-I, PKR is well-known as a nucleic acids receptor of viral dsRNA produced from replication or transcription intermediates of a wide range of virus families such as negative-strand RNA viruses VSV and Sendai virus (SeV) (Stojdl et al, 2000; Dauber & Wolff, 2009), positive-strand RNA virus Hepatitis C virus...
(Targett-Adams et al., 2008), and DNA virus Herpes simplex virus type 1 (HSV-1) (Jacquemont & Roizman, 1975). PKR is an autophosphorylated and activated following sensing viral dsRNA and then phosphorylates eukaryotic translation initiation factor 2 on its α subunit (eIF2α) to inhibit translation initiation of viral proteins (Dalet et al., 2015). Cellular non-coding RNAs, such as the inverted Alu repeats (IRAlus) elements located in the 3′-untranslated regions (3′-UTR) and mitochondrial RNAs (mRNAs) formed intermolecular dsRNA, also activate PKR through direct interaction to regulate cellular proliferation or metabolism (Kim et al., 2014, 2018). In addition to be activated by the cellular RNA, PKR is suppressed during binding with the cytoplasmic circular RNAs (circRNAs) that tend to form 16–26 bp imperfect RNA duplexes, and viral infection relieves this inhibition following circRNAs degradation by RNase L to activate PKR activity (Liu et al., 2019). However, it is unclear whether any linear long non-coding RNAs (lncRNAs) regulate PKR-dependent antiviral immunity.

PKR is also required for the activation of MAPK and IKK complex, as well as the transcriptional activities of IRF1 and STAT1 (Wong et al., 1997; García et al., 2006; Gal-Ben-Ari et al., 2018). As an IFN-I-inducible gene, PKR directly associates with STAT1 via the PKR dsRNA-binding domain (Tanaka & Samuel, 1994). PKR is essential for the phosphorylation of STAT1 on Ser727 and Tyr701 under the response to IFN-γ and LPS (Ramana et al., 2000; Lee et al., 2005; Karehed et al., 2007). LncRNA GRASLND acts to inhibit IFN-γ signaling by binding PKR and in turn inhibiting STAT1 activity during chondrogenesis (Huynh et al., 2020). However, it is unexplored whether any PKR-interacted lncRNAs contribute to the regulation of the IFN-I-JAK-STAT pathway during host innate immunity against viral infection.

The long intergenic noncoding RNA lincRNA-EPS was initially reported to inhibit apoptosis during erythroid cell differentiation in part through repressing the expression of the proapoptotic gene Pycard (Hu et al., 2011). During inflammatory responses, lincRNA-EPS is tightly regulated in macrophages to control the expression of immune response genes (IRGs) at the transcription level by interacting with hnRNPL (Atianand et al., 2016). The deficiency of lincRNA-EPS enhances inflammatory response and leads to death in the endotoxin-shock mouse model while protecting the host from Listeria monocytogenes infection (Atianand et al., 2016; Agliano et al., 2019). Furthermore, knockdown of lincRNA-EPS promoted autophagy in Bacillus Calmette-Guérin (BCG)-infected RAW264.7 macrophages by activating the JNK/MAPK pathway, and the downregulation of lincRNA-EPS was shown in active pulmonary tuberculosis (PTB) patients (Ke et al., 2020). Our previous study has described that lincRNA-EPS alleviates severe acute pancreatitis by suppressing HMGBI-triggered inflammation in pancreatic macrophages (Chen et al., 2021). Although lincRNA-EPS has been well identified as a key immunoregulatory lncRNA that restrains inflammatory responses, the role of lincRNA-EPS in antiviral immunity was not yet studied.

In this study, we have found that the expression of lincRNA-EPS is also precisely controlled during host antiviral immunity in IFN-1- and NF-kB-dependent manners. Downregulation of lincRNA-EPS protects the host against viral infection in vitro and in vivo. LincRNA-EPS binds to PKR and thus negatively regulates PKR-STAT1-dependent host antiviral immunity by antagonizing the interaction between viral RNA and PKR. Our study has indicated that lincRNA-EPS plays an important role in modulating host innate antiviral immunity.

Results

Downregulation of lincRNA-EPS by host antiviral immunity

To check the impact of viral infection on lincRNA-EPS expression, mouse bone marrow-derived macrophages (BMDMs) were infected with several viruses including RNA viruses VSV and SeV, and DNA virus HSV-1. All three viruses dramatically suppressed the expression of lincRNA-EPS (Fig 1A). Transfection of viral RNA mimics poly(C) and viral DNA mimics poly(dA:dT) also led to the downregulation of lincRNA-EPS expression in BMDMs (Fig 1B). To further confirm whether host antiviral immunity regulates lincRNA-EPS expression, BMDMs were treated with IFN-α and IFN-β, the key cytokines which are always induced during viral infection. Both IFN-Is significantly suppressed lincRNA-EPS expression at the very early stage (2 h post stimulation) and at the concentration as low as 20 U/ml (Fig 1C–F). Consistently, higher expression of lincRNA-EPS was detected in the Ifnar1−/− BMDMs than the WT BMDMs during cells were transfected with poly(C) or infected with SeV (Fig 1G and H). However, lincRNA-EPS expression was still downregulated in the poly(C)-transfected and SeV-infected Ifnar1−/− BMDMs (Fig 1G and H). In addition, there were no differences of lincRNA-EPS expression between Ifnar1−/− and WT BMDMs when the cells were infected with WSN and VSV (Fig EV1A), which suggest an IFN-I-independent pathway also contributed to suppress lincRNA-EPS expression during viral infection. Hence, we used inhibitors to specifically target NF-κB, p38, ERK, and JNK signaling pathways (Fig EV1B), which are also activated during viral infection. Inhibition of NF-κB rather than the MAPK pathways significantly reversed the downregulation of lincRNA-EPS triggered by VSV infection (Figs II and EV1C).

We further checked the cellular localization and abundance of lincRNA-EPS in macrophages. Similar to the LncRNA Neat1 which mainly localizes in the nucleus (Clemson et al., 2009), about 71% of lincRNA-EPS were detected in the nucleus. A more robust reduction of cytoplasmic lincRNA-EPS was observed than the nuclear lincRNA-EPS, although both nuclear and cytoplasmic lincRNA-EPS were significantly suppressed during VSV infection (Fig 1J). About 63, 23, and 8 copies per cell of lincRNA-EPS were detected in the RAW264.7, immortalized BMDMs (iBMMs), and BMDMs, respectively (Fig EV1D), indicating higher abundance of lincRNA-EPS in the macrophage cell lines than the primary macrophages.

Taken together, lincRNA-EPS was downregulated during host immunity against viral infection, in IFN-I- and NF-κB-dependent manners.

LincRNA-EPS facilitates viral infection in macrophages

Next, we sought to investigate the function of lincRNA-EPS during host innate immunity against viral infection by using macrophage cell lines. We stably overexpressed lincRNA-EPS in iBMMs (Fig 2A). More VSV, SeV, and HSV-1 infections were detected in the lincRNA-EPS-overexpressed iBMMs than the control cells (Fig 2B–D), which suggested that lincRNA-EPS broadly facilitated viral infection. Meanwhile, we used a pair of sgRNAs to efficiently knock down the lincRNA-EPS expression in RAW264.7 cells, a macrophage cell line that is susceptible to multiple viruses and expresses high level
lincRNA-EPS (Fig 2E). Less VSV-GFP-infected cells were observed in the lincRNA-EPS knockdown cells than the control cells (Fig 2F). Consistently, less VSV titer, fewer SeV and HSV-1 viral genes were detected in the lincRNA-EPS knockdown cells than the control cells (Fig 2G–I). These results indicated that lincRNA-EPS facilitated viral infection in macrophages.
Knockout of lincRNA-EPS enhances host antiviral ability

To further confirm the function of lincRNA-EPS in facilitating viral infection, we immortalized the lincRNA-EPS−/− and WT BMDMs (Fig 3A). Much less GFP-positive cells were observed in the VSV-GFP-infected lincRNA-EPS−/− iBMMs than the WT cells (Fig 3B). Plaque assay results also showed that lincRNA-EPS−/− iBMMs were more resistant to VSV than the WT iBMMs (Fig 3C).

Data information: Data of (A–E) and (G–I) are shown as the mean ± s.d. from three independent experiments. *P < 0.05 and **P < 0.01 by unpaired Student’s t-test. Data of (F) are representative images from three independent experiments, scale bar, 100 μm.

Figure 2. LincRNA-EPS facilitates viral infection in macrophages.
A RT-qPCR analysis of lincRNA-EPS transcripts in the lincRNA-EPS-stably-overexpressed iBMMs (lincRNA-EPS) and corresponding control cells (EV).
B LincRNA-EPS iBMMs were infected with VSV (MOI 1) for 8 h, VSV titers were measured by plaque assay.
C, D The EV and lincRNA-EPS iBMMs were infected with SeV (MOI 1) (C) or HSV-1 (MOI 5) (D) for 12 h. The viral RNA was measured by RT-qPCR.
E The transcripts level of lincRNA-EPS in control (sgCtrl) and lincRNA-EPS knockdown (sglincRNA-EPS) RAW264.7 cells were measured by RT-qPCR.
F, G sgCtrl and sglincRNA-EPS RAW264.7 cells were infected with VSV-GFP (MOI 0.1) for 6 h or infected with VSV (MOI 0.1) for 8 h. The fluorescence of GFP was checked by microscope (f) and the viral titer of VSV from the cell supernatant was measured by plaque assay (G).
H, I sgCtrl and sglincRNA-EPS RAW264.7 cells were infected with SeV (MOI 1) for 8 h (H) and HSV-1 (MOI 5) for 12 h (I). The viral RNA was measured by RT-qPCR.
Figure 3. Knockout of lincRNA-EPS enhances host antiviral ability.

A, B WT and lincRNA-EPS−/− iBMMS were infected with VSV-GFP (MOI 0.1) for 6 h, and the lincRNA-EPS transcripts were measured by RT–qPCR (A), and the fluorescence of GFP were checked by microscope (B).

C, D WT and lincRNA-EPS−/− iBMMS (MOI 0.1, 8 h) were collected and the viral titer were measured by plaque assay.

D, E WT and lincRNA-EPS−/− iBMMS were infected with SeV (MOI 1) for 8 h (D) or HSV-1 (MOI 1) for 12 h (E). The viral RNA was measured by RT–qPCR.

F Peritoneal macrophages isolated from WT and lincRNA-EPS−/− mice were infected with VSV (MOI 1) for 10 h, and the viral titer were measured by TCID50 assay.

G Eight weeks female lincRNA-EPS−/− mice (n = 12) and WT littersmates (n = 12) were injected (i.v) with VSV (lethal dose, 1 × 10⁸ pfu/g), and the survival situation was monitored for 120 h.

H–J Eight weeks female lincRNA-EPS−/− mice (n = 5) and WT littersmates (n = 5) were injected with VSV (sub-lethal dose, 6 × 10⁷ pfu/g) for 12 h, and negative control groups were injected with PBS (n = 3). Pathological section of liver and lung were harvested by H&E staining (H). Serum, liver, and lung from the VSV-infected mice were collected. The viral load of serum and tissue homogenate were measured by TCID50 assay (I), and the serum IFN-β protein level were checked by ELISA (J).

Data information: Data of (A, C–F) are shown as the mean ± s.d. from three independent experiments, data of (I, J) are shown as the mean ± s.d. of a typical representative result from three independent experiments, and one dot represents a mouse, *P < 0.05 and **P < 0.01 by unpaired Student’s t-test. Data of (C) are calculated with Log-rank (Mantel-Cox) test, **P < 0.01. Data of (B, H) are representative images from at least three independent experiments, scale bar, 100 µm.
the phenotypes observed in the lincRNA-EPS knockdown RAW264.7 cells, less SeV and HSV-1 viral genes were detected in the lincRNA-EPS /− iBMMs than the WT cells (Fig 3D and E). However, restored expression of lincRNA-EPS in the lincRNA-EPS /− iBMMs facilitated VSV and SeV infection (Fig EV2A–C), which indicated that knockout of lincRNA-EPS rather than the off-target effects of the sgRNAs regulated the host susceptibility to viral infection. Next, we isolated the primary peritoneal macrophages (PMs) to further validate the function of lincRNA-EPS. LincRNA-EPS /− PMs showed more resistant to the VSV than the WT cells (Fig 3F). In addition to the in vitro experiments, we further challenged the WT and lincRNA-EPS /− mice with VSV to investigate the function of lincRNA-EPS in vivo. The lincRNA-EPS /− mice exhibited a much higher survival rate than the WT group during the mice infection with a lethal dose of VSV intravenously (Fig 3G). Consistently, alleviated liver and lung injuries including fewer inflammatory cells infiltration, less liver fibrotic septa, less alveolar wall thickening, and less alveolar cavity atrophy were observed in the lincRNA-EPS /− mice than the WT group during the mice infected with a sublethal dose of VSV (Fig 3H). Moreover, less viral load in serum, livers, and lungs was detected in the VSV-infected lincRNA-EPS /− mice comparing to the WT mice (Fig 3I). Interestingly, lower serum IFN-β was detected in the VSV-infected lincRNA-EPS /− mice than in the WT group (Fig 3J). Together, these results demonstrated that lincRNA-EPS facilitated viral infection such as VSV infection in vitro and in vivo, likely in an IFN-I-independent manner.

Greater induction of antiviral ISGs in the lincRNA-EPS /− macrophages

To determine how lincRNA-EPS facilitates viral infection, we performed RNA sequencing and analyzed the transcriptomes of uninfected and VSV-infected iBMMs. Gene set enrichment analysis (GSEA) results showed that the interferon alpha response, the interferon gamma response, and the inflammatory response pathways were mostly affected by lincRNA-EPS deficiency (Figs 4A and EV3A). The majority of these genes in the IFN-I response gene sets were upregulated in the lincRNA-EPS /− iBMMs (Fig EV3B). We selected the upregulated differentially expressed genes (DEGs) in the lincRNA-EPS /− iBMMs and overlapped with the ISGs list (Hubel et al, 2019). Sixty-two ISGs were upregulated in the lincRNA-EPS /− iBMMs by comparing to the WT cells (Fig 4B). Most of these overlapped ISGs are antiviral genes (Fig 4B). We verified several key antiviral ISGs by RT-qPCR, and found that more induction of Mx1, Oas2, Ifit2, and Ifr7 were observed in the lincRNA-EPS /− iBMMs than the WT cells when the cells infected with VSV, SeV, HSV-1, or transfected with viral RNA mimics polyI:C or viral DNA mimics polydA:dT (Fig 4C). Basal expressions of multiple antiviral ISGs were also higher in the lincRNA-EPS /− iBMMs than the WT iBMMs (Fig 4B and C). In addition, Mx1, Oas2, Ifit2, and Ifr7 genes were also higher elevated in the VSV-infected lincRNA-EPS /− PMs and the viral mimics-transfected lincRNA-EPS knockdown RAW264.7 cells than their respective control cells (Figs 4D and EV3C). However, fewer Ifnb1 transcripts were detected in the VSV-infected iBMMs and primary PMs than their respective control cells (Fig 4B and D). Moreover, reduced VSV infection, enhanced ISGs expression, and repressed Ifnb1 transcription were observed in the VSV-infected lincRNA-EPS /− BMDMs (Fig EV4A), and in the liver and lung tissues from VSV-infected mice (Fig EV4B and C). These results suggested that lincRNA-EPS suppressed the induction of antiviral ISGs during viral infection in vitro and in vivo, and thus facilitated viruses such as VSV, SeV, and HSV-1 infection.

Stronger activation of IFNAR downstream signaling independent of IFN-β induction in the lincRNA-EPS /− macrophages

Induction of IFN-1 is essential for the host against acute viral infection (McNab et al, 2015). However, VSV infection-induced serum IFN-β and Ifnb1 transcripts were downregulated in the lincRNA-EPS /− mice and primary macrophages (Figs 3J, 4D, EV4A–C). In the VSV-infected iBMMs, we also confirmed that fewer Ifnb1 transcripts and supernatant IFN-β proteins were detected in the lincRNA-EPS /− cells than the WT cells (Fig 5A and B). To eliminate the influence of inhibited viral infection by deficiency of lincRNA-EPS on IFN-β upstream signaling, we activated iBMMs by transfecting polyI:C instead of VSV. Less Ifnb1 transcripts were detected in the lincRNA-EPS /− iBMMs than the WT cells (Fig EV5A). Rescued expression of lincRNA-EPS in the lincRNA-EPS /− iBMMs or overexpression of lincRNA-EPS in WT iBMMs significantly inhibited the induction of antiviral genes including Mx1 and Oas2 (Fig 5C–E), which was consistent with the results that lincRNA-EPS /− mice and macrophages exhibited stronger antiviral abilities than their respective controls. Knockout of lincRNA-EPS upregulated the basal expression level of numerous antiviral genes including Mx1, Oas2, Ifit2, and Ifr7 in BMDMs (Fig 5F). The histone modification, trimethylation of lysine 4 in histone H3 (H3K4me3) that localized to the 5′ regions of target genes, is associated with high transcription activity (Liang et al, 2004). Thus, we checked the H3K4me3 modification in the promoters of several antiviral ISGs by ChIP-qPCR. Transfection of polyI:C dramatically induced the H3K4me3 modifications in the promoters of Mx1 and Oas2 in both WT and lincRNA-EPS /− iBMMs, while knockout of lincRNA-EPS with or without polyI:C transfection induced higher level of H3K4me3 modifications in the promoters of these genes (Fig 5G).

Figure 4. Greater induction of antiviral ISGs in the lincRNA-EPS /− macrophages.

A Overview of GSEA analysis using the whole transcriptome of RNA-seq from biological duplicates of VSV (MOI 0.1)-infected WT and lincRNA-EPS /− iBMMs for 6 h.
B The mean RPKM values of the biological duplicates of Mock or VSV-infected WT and lincRNA-EPS /− iBMMs were calculated, and the upregulated antiviral genes of lincRNA-EPS /− iBMMs comparing to WT iBMMs were listed by heatmap.
C RT–qPCR analysis of Mx1, Oas2, Ifit2, Ifr7 mRNA level in the WT and lincRNA-EPS /− iBMMs transfected with 1 μg/ml polyI:C, polydA:dT or infected with VSV (MOI 0.1) and SeV (MOI 1) for 6 h, or HSV (MOI 5) for 12 h.
D RT–qPCR analysis of Mx1, Oas2, Ifit2, Ifr7, Ifnb1 mRNA level and lincRNA-EPS transcripts in the WT and lincRNA-EPS /− PMs infected with VSV (MOI 1) for 6 h and 10 h.

Data information: Data of (C, D) are shown as the mean ± s.d. from three independent experiments. *P < 0.05 and **P < 0.01 by unpaired Student’s t-test.
Figure 4.
Figure 5. Stronger activation of IFNAR downstream signaling independent of IFN-β induction in the lincRNA-EPS−/− macrophages.

A, B RT-qPCR analysis of Ifnb1 mRNA level in the WT and lincRNA-EPS−/− iBMMs infected with VSV (MOI 0.1) for 6 h (A). ELISA analysis of supernatant IFN-β protein after infecting for 8 h (B).

C, D Mx1 (C) and Oas2 (D) mRNA level were measured in the control (EV) and lincRNA-EPS-rescued (lincRNA-EPS) iBMMs after infecting with VSV with different titer for 6 h.

E The basal level of ISGs and proinflammatory genes in the WT and lincRNA-EPS−/− BMDMs (Datasets from the ArrayExpress database under the accession number E-MTAB-4088) were listed by heatmap.

F, I WT and lincRNA-EPS−/− iBMMs were infected with VSV (MOI 0.1) for 6 or 10 h (H) or transfected with polyI:C (1 μg/ml) for 4 or 8 h (I), phosphorylation and total protein expression were analyzed by Western blot with α-tubulin and β-Actin as loading controls.

Data information: data of (A–E) and (G) are shown as the mean ± s.d. from three independent experiments, *P < 0.05 and **P < 0.01 by unpaired Student’s t-test. Data of (H, I) are representative results from three independent experiments.
Moreover, upon VSV infection, activation of IFN-β upstream signaling including phosphorylation of TBK1 and IFR3 was attenuated while IFN-β downstream activation including STAT1 phosphorylation was elevated in the lincRNA-EPS−/− iBMMs, which was further verified in the poly(I:C)-transfected macrophages to eliminate the indirect effect of inhibited viral load on the IFN-β upstream signaling (Fig 5H and I).

Taken together, these results demonstrated that knockout of lincRNA-EPS suppressed IFN-I upstream signaling activation and IFN-β induction. However, IFNAR downstream signaling activation and antiviral ISGs were highly induced in the lincRNA-EPS−/− macrophages.

**LincRNA-EPS and viral RNA competitively interact with PKR**

LincRNA-EPS tends to function through recruiting the RNA-binding protein hnRNPL in the nucleus to restrain chromatin accessibility at the promoters of interferon regulated genes in macrophages (Atianand et al., 2016; Agliano et al., 2019). To study how lincRNA-EPS inhibits IFNAR downstream signaling and thus restricts host antiviral immunity, we checked all the lincRNA-EPS-interacted proteins in the whole cell lysates of macrophages by RNA pulldown and mass spectrometry technology. Among the numerous candidates, lincRNA-EPS strongly interacted with the dsRNA-binding protein PKR and the positive control protein hnRNPL (Fig 6A, Table EV1). The interaction between PKR and lincRNA-EPS was confirmed by using biotinylated lincRNA-EPS and poly(I:C) RNA pulldown assay (Fig 6B). Next, we validated the native interaction between PKR and lincRNA-EPS in macrophages by pulling down the endogenous PKR proteins to analyze the associated RNA molecules by RT–qPCR (Fig 6C). To further explore whether PKR is involved in the lincRNA-EPS-mediated regulation of antiviral immunity, we knocked down PKR by RNAi technology in both WT and lincRNA-EPS−/− iBMMs. Once the expression of PKR was efficiently downregulated (Figs 6D and EV5B), the reduction of VSV infection in the lincRNA-EPS−/− iBMMs was significantly reversed, although knockout of lincRNA-EPS still enhanced the host antiviral immunity against invaded VSV in both siNC and siPKR iBMMs (Fig 6E and F).

PKR was well-known as a viral RNA sensor to recognize virus-derived RNA molecules including VSV genomic RNA (Stojdl et al., 2000; Dauber & Wolff, 2009). VSV transcripts include the leader region at the 3′ genomic promoter, N-P-M-G-L encoding viral genes, and the 5′ trailer region (Villarreal et al., 1976). The VSV viral RNA fragment of leader/N junction is able to bind and trigger RNA sensor RIG-I (Linder et al., 2021). We used VSV RNA fragments to pulldown PKR and found that VSV-N but not VSV-G strongly interacted with PKR (Fig 6C). To investigate whether VSV viral RNA and lincRNA-EPS competitively bind with PKR, we activated iBMMs with different doses of VSV or poly(I:C), and extracted cell lysates for lincRNA-EPS RNA-pulldown assay. The Western blot results indicated that VSV infection and poly(I:C) transfection hindered the interaction between lincRNA-EPS and PKR in a dose-dependent manner (Fig 6H and I). The RIP–RT–qPCR assay for testing the ability of PKR in binding with lincRNA-EPS and VSV viral RNA demonstrated that overexpression of lincRNA-EPS could antagonize the interaction between PKR and VSV viral RNA (Fig 6J), which further confirmed that lincRNA-EPS inhibited antiviral immunity through restraining PKR sensing viral RNA.

**LincRNA-EPS inhibits PKR-STAT1-dependent induction of ISGs**

PKR is required for phosphorylation and activation of STAT1 under the response to IFN-γ and LPS (Ramana et al., 2000; Lee et al., 2005; Karehed et al., 2007). During VSV infection, phosphorylation of STAT1 was also attenuated in the PKR knockdown iBMMs (Fig 7A). Overexpression of PKR upregulated the phosphorylation of STAT1 as the IFN-β treatment (Fig 7B), which suggested a potential IFN-I-independent pathway favoring PKR-trigged phosphorylation of STAT1. In addition, overexpression of lincRNA-EPS repressed STAT1 phosphorylation and total STAT1 protein level which were induced by ectopic expressed PKR in MEF cells (Fig EV5C).

To investigate whether lincRNA-EPS involves in the regulation of PKR-STAT1 signaling, we checked the basal expression of antiviral ISGs including Mx1, Ifit2, and Isg15 after PKR knockdown in iBMMs. Upregulation of Mx1, Ifit2, and Isg15 in lincRNA-EPS−/− iBMMs were attenuated after PKR knockdown (Figs 7C–H and EV5D and E). Consistently, induction of Mx1,
Ifit2 and Isg15 were also attenuated after PKR knockdown in the VSV-infected and polyC-transfected lincRNA-EPS−/− iBMMS (Figs 7C and E, and G, and EV5F). Moreover, knockdown of STAT1, the key transcription factor of the PKR-STAT1 axis, significantly decreased the basal expression of lincRNA-EPS-regulated ISGs Mx1 and Oas2 (Fig EV5G).

In summary, our study has demonstrated that lincRNA-EPS negatively regulated PKR-STAT1-dependent antiviral immunity. In addition to the inhibitory role of lincRNA-EPS on restraining chromatin accessibility by associating with hnRNPL in nucleus (Atianand et al., 2016), the cytosolic lincRNA-EPS is able to repress PKR-STAT1 axis and downregulate ISGs expression in the resting cells. Downregulation of lincRNA-EPS during viral infection or genetic knockout of lincRNA-EPS facilitates PKR-STAT1 signaling axis induced antiviral genes expression, such as Mx1, Oas2, Ifit2, and Irf7 (Fig 7I). PKR-mediated STAT1 activation and ISGs induction directly inhibit viral infection, which may result in less RIG-I-TBK1-IRF3 signaling activation and less IFN-β production.
Discussion

LncRNAs are widely expressed in immune cells and precisely regulated during innate immunity against viral infection (Ouyang et al., 2016; Atianand et al., 2017). Here, our study has indicated that downregulation of lincRNA-EPS in macrophages significantly facilitates host antiviral innate immunity in vitro and in vivo. LincRNA-EPS not only acts as a transcriptional brake to restrain PAMP- and DAMP-triggered inflammation (Atianand et al., 2016; Chen et al., 2021), but also negatively regulates host antiviral immune responses. Decreased expression of lincRNA-EPS at the early stage of viral infection benefits the host cells by enhancing the ability to clear the invaded viruses. Higher expression of lincRNA-EPS in the resting macrophages or recovered expression of lincRNA-EPS after clearance of viruses also helps the host to maintain homeostasis and avoid autoimmunity.

Knockout of lincRNA-EPS changes the repressed chromatin state to a more accessible chromatin state at the promoters of selected IRGs including key antiviral genes (Atianand et al., 2016). However, it is well accepted that accessibility of promoter DNA is not sufficient to drive gene transcription (Ernst & Kellis, 2013; Chereti et al., 2019). Our study describes the cytoplasmic function of lincRNA-EPS in the regulation of antiviral immunity. Therefore, it is possible that cytoplasmic and nuclear lincRNA-EPS synergistically suppress ISG expression at both resting and viral infected conditions. Nuclear lincRNA-EPS-hnRNPL complex determines chromatin accessibility of the ISG promoters, and the cytoplasmic lincRNA-EPS–PKR–STAT1 signaling axis controls ISG transcription. Transcriptome analysis of resting WT and lincRNA-EPS−/− macrophages with PKR knockdown did not show any specific genes regulated by lincRNA-EPS–PKR–STAT1 axis comparing to lincRNA-EPS-hnRNPL axis (Fig EV6), which suggest that cytoplasmic and nuclear lincRNA-EPS may have synergistic functions in regulating host antiviral immunity.

Multiple lncRNAs have been reported to inhibit viral infection via the IFN-1-dependent pathways. Lnczcc3h7a associates with TRIM25 and serves as a molecular scaffold to stabilize the interaction between TRIM25 and RIG-I to strengthen IFN-1 production and antiviral response (Lin et al., 2019). LncRNA-GM enhances IFN-1 production and thus inhibits viral replication by reducing GSTM-mediated S-glutathionylation of TBK1 (Wang et al., 2020). LncLrrc55-AS promotes IRF3 phosphorylation to enhance IFN-1 signaling (Zhou et al., 2019). However, there are also numerous lncRNAs that are hijacked by viruses to suppress host immune responses for facilitating viral escape. For instance, LncRNA-ACOD1 interacts with glutamic-oxaloacetic transaminase (GOT2) and supports virus infection through modulating cellular metabolic networks (Wang et al., 2017a). NRAV, which represses the transcription of ISGs by affecting histone modification, is downregulated once sensing viruses to benefit host antiviral ability (Li et al., 2020). LncRNA-CMPK2 negatively regulates the activation of IFN-1 signaling and induction of ISGs to promote HCV replication in vitro and in vivo (Kambara et al., 2014). Here, we have discovered a new regulator, lincRNA-EPS, which inhibits antiviral immunity by attenuating the PKR-STAT1-dependent induction of antiviral ISGs, while not affecting IFN-1 production. PKR recognizes viral dsRNA from replication or transcription intermediates of a wide range of viruses (Dauber & Wolff, 2009), mRNAs formed intermolecular dsRNA (Kim et al., 2018), cytoplasmic circRNAs formed 16-26 bp imperfect RNA duplexes (Liu et al., 2019), and lncRNA GRASLND (Huynh et al., 2020). We have found that PKR also interacts with lincRNA-EPS, which potentially antagonizes the interaction between PKR and viral RNA.

Although there are only 20–30% of lincRNA-EPS located at the cytoplasm and ~23 copies per cell of lincRNA-EPS in the iBMMs. However, 6 copies of circPOLR2A in Hela cells are sufficient to suppress PKR activation significantly. Reduced circPOLR2A to ~1 copy per cell during polyIC stimulation still inhibits the PKR activation (Liu et al., 2019). Therefore, it is possible that 4-6 copies of cytoplasmic lincRNA-EPS are sufficient to interact with PKR, block viral RNA, and thus suppress host antiviral immunity.

PKR plays a vital antiviral role through recognizing viral dsRNA, which is derived both from RNA viruses (e.g. VSV and SeV) and DNA viruses (e.g. HSV-1) (Dauber & Wolff, 2009). Although the classical downstream signaling of PKR is activating eIF2α to suppress viral protein translation and then inhibit virus replication (Dalet et al., 2015). However, we did not observe the elevated phosphorylation of eIF2α in the virus-infected lincRNA-EPS−/− macrophages. Interestingly, knockdown of PKR by RNAi attenuates STAT1 phosphorylation in the VSV-infected iBMMs, and overexpression of PKR facilitates STAT1 phosphorylation as IFN-1 treatment in A549 cells, which suggests that PKR is able to activate STAT1 independently of IFN-1 production. Direct interaction between PKR and STAT1 was observed (Wong et al., 1997), although it is not thoroughly verified whether this interaction favors phosphorylation of STAT1 and induces STAT1-targeted antiviral genes. Further studies are required to elucidate the mechanism for PKR-mediated STAT1 activation after PKR recognizing exogenous or endogenous dsRNA.

RIG-I is also an important cytoplasmic RNA sensor for most RNA viruses including VSV (Yoneyama et al., 2004). RIG-I-MAVS-TBK1-IRF3-IFN-β signaling axis is essential for host antiviral
immunity (Honda et al., 2006). However, our results show that viral infection or genetic knockout of lincRNA-EPS facilitates PKR-STAT1 signaling axis induced antiviral genes such as Mx1, Oas2, Ifit2, and Irf7, while inhibits phosphorylation of TBK1 and IRF3, as well as the IFN-β induction. One explanation is that PKR-mediated STAT1 activation and ISGs induction directly inhibits
viral infection, which may result in less activation of RIG-I-TBK1-IRF3 signaling and less IFN-β production. Another possibility is that activation of PKR-STAT1 signaling induces negative regulators of RIG-I such as Inc-Lsm3b, an inducible host lncRNA that binds to RIG-I monomers and inactivates the RIG-I innate function (Jiang et al., 2018).

In the virus-infected macrophages, we found that the repression of lincRNA-EPS is not totally dependent on IFN-I downstream pathway, although both IFN-α and IFN-β significantly inhibit lincRNA-EPS expression. NF-xB activity is also required for downregulating lincRNA-EPS during viral infection, which is consistent with the phenotypes observed in the LPS-stimulated macrophages (Atlanand et al., 2016; Chen et al., 2021). Detailed molecular mechanism for dramatic downregulation of lincRNA-EPS expression within 2 h is unexplored. Whether any transcriptional repressor inhibits lincRNA-EPS transcriptionally or any RNA-binding proteins promote lincRNA-EPS degradation post-translationally will be investigated in our future studies.

In conclusion, our study has expanded the physiological function of lincRNA-EPS in host antiviral immunity and certified that host lincRNA-EPS degradation post-transcriptionally will be investigated in our future studies.

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Materials and Methods

Mice

C57BL/6N lincRNA-EPS−/− mice were generated by Biocytogen (Beijing, China) by using the CRISPR/Cas9 technology as described previously (Chen et al., 2021). Briefly, the whole chromosomal region (about 4 kb) of gene Ttc39aos1 (Gene ID: 102635290) was deleted by a pair of sgRNAs: 5′-CCGCCCGCTTCCGGCCCTTGG-3′ and 5′-CCATTACCTTGGACACGCCCTTGG-3′. Genotyping primers for WT mice were 5′-TCATGTAATACAGGTGTCGCAA-3′ (lincRNA-EPS-F1) and 5′-CGGTGAATCCGCTTCTCTGCAA-3′ (lincRNA-EPS-R). PCR products for WT and KO bands were 456 bp and 417 bp, respectively. All the mice were maintained in the specific pathogen-free (SPF) environment at Suzhou Institute of Systems Medicine (ISM) under a controlled temperature (25°C) and a 12 h day-night cycle. All animal experiments were conducted according to the US National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Animal Service Center of ISM (AUP no. ISM-IACUC-0011-R).

Reagents and antibodies

Anti-α-tubulin antibody (#432516) were purchased from Sigma-Aldrich (St. Louis, MO). Polycl:C, biotin-poly:C, polyDA:dT, NF-xB inhibitor BAY11-7082, and MAPK inhibitors including SB203580, PD98059, and SP600125 were from InvivoGen (San Diego, CA). Recombinant mouse IFN-α and IFN-β were from PBL Assay Science (Piscataway, NJ). Recombinant human IFN-β was from R&D system (Minneapolis, MN). Primary antibodies against GAPDH (#5174), β-Actin (#3700), pSTAT1 (Tyr701, #9167), STAT1 (#14994), pTBK1 (Ser172, #5483), TBK1 (#3504), pIRF3 (Ser396, #29047), IRF3 (#4302), pelfF2x (Ser51, #3398), elfF2x (#5324), Flag (#14793), and HRP-linked secondary antibodies anti-rabbit IgG (#7074) and antimouse IgG (#7076) were from Cell Signaling Technology (Danvers, MA). Anti-PKR (18244-1-AP) was purchased from Proteintech (Wuhan, China), anti-IFIT2 (sc-398610) was from Santa Cruz (Santa Cruz, CA). The fluorescence secondary antibodies were purchased from LI-COR (Lincoln, NE).

Primary cells and cell lines

BMDMs were harvested and differentiated from bone marrow cells of mice in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin/streptomycin (P/S), and 1% M-CSF conditioned medium for 7 days. WT and lincRNA-EPS−/− iBMMs were infected and immortalized by the J2 virus produced from cell line GG2EE (Palleroni et al., 1991; Ma et al., 2014), and cultured in RPMI1640 (10 mM HEPES pH 7.8, 10% heat-inactivated FBS, 1% P/S, 1% M-CSF conditioned medium). Conditioned medium containing M-CSF was collected from the supernatant of GMG14-12 cells (Gifted by Genhong Cheng Laboratory, University of California, Los Angeles). HEK293T, A549, and RAW264.7 were obtained from American Type Culture Collection (ATCC, Manassas, VA) and cultured in DMEM supplemented with 10% FBS and 1% P/S. For all experiments, cells were plated overnight, and the medium was replaced before stimulation, infection, or transfection. LincRNA-EPS-overexpressed and lincRNA-EPS-rescued iBMM cell lines were constructed using retroviruses packaged in HEK293T cells that transfected with pEco and pMSCV-PIG-lincRNA-EPS (Gifted by Katherine A. Fitzgerald Laboratory, University of Massachusetts Medical School, Worcester). LincRNA-EPS-KD RAW264.7 cells were generated by using CRISPR/Cas9 technology. Briefly, a pair of sgRNAs (5′-AGGCTCGAGGATGTCAGAAGG-3′ and 5′-ATGTTAAACTACGTGTCAAAGG-3′) targeting lincRNA-EPS were cloned into the lentCRISPRv2 vectors (Addgene, #52961). The constructed vectors were co-transfected into WT RAW264.7 cell lines via electroporation using program D-032 of Nucleofector 2b device (Lonza), following selected with 5 μg/ml puromycin at 24 h post-transfection. The selected cells were cultured in the medium with 0.5 μg/ml puromycin and the expression of lincRNA-EPS was confirmed by RT–qPCR.

Cell transfection and stimulation

pMSCV-PIC-lincRNA-EPS, pcDNA3.1-PKR, and the corresponding empty vectors were transfected into HEK293T by using polyethyleneimine (Merck). Plasmids were transfected into A549 cells and MEF by using Lipofectamine 2000 (Life Technologies) and JetPrime (Polyplus Transfection) according to the manufacturer’s instructions, respectively. siRNAs targeting PKR and negative control were delivered into iBMMs with INTERFERin (Polyplus Transfection) according to the manufacturer’s instructions. PolyI:C and polyDA:dT were transfected into RAW264.7, BMDMs, or iBMMs by Lipofectamine 2000. The ratio of transfection reagent to ligands was 2.5 μl/μg. Cells were stimulated with LPS, IFN-α, and IFN-β as indicated in the figure legends.
Viral infection and titer assay

The viruses VSV, VSV-GFP, SeV, HSV-1 (strain KOS), and Influenza (A/WSN) were gifted from Genhong Cheng Laboratory (University of California, Los Angeles). Before VSV, VSV-GFP, SeV, and HSV-1 infection, cultured cells in the medium were changed with medium containing 2% FBS, and then infected with different MOI as indicated in the figure legends. WSN was infected using specific medium containing 1 mg/ml TPCK-E, 0.075% BSA, 1% P/S. 1 h post infection, the medium was replaced with regular medium containing 10% FBS and cultured for indicated times. For in vivo studies, littermate mice that age and sex-matched were infected with VSV (6 × 10^7 pfu/g, i.v., 12 h) for detection of IFN-β, virus titer in serum, and organs injuries. For survival study, littermate mice that age and sex-matched were infected with VSV (1 × 10^8 pfu/g, i.v.). The titer of VSV was measured using the methods of plaque assay for cell culture supernatant and TCID₅₀ for serum (Ma et al., 2014; Lei et al., 2021).

RNA extraction and real-time quantitative PCR

Total RNA was extracted from cells using TRIzol (Thermo Fisher Scientific) according to the manufacturer’s instructions. After assessing RNA quality and concentration using NanoDrop 2000, equal amounts of RNA were reversed transcribed using PrimeScript RT Reagent Kit (Takara). RT-qPCR was performed using SYBR RT-PCR kits (Takara). Gene expression levels were normalized to Rpl32 as internal control genes by 2^−ΔΔCt cycle threshold method (Schmittgen & Livak, 2008). Primer sequences for RT-qPCR and ChiP-qPCR are listed in Table EV2.

RNA sequencing and data analysis

Total RNA for RNA sequencing was extracted from WT and lincRNA-EPS/−/− iBMMs in biological duplicates. After analyzed the quantity and quality of samples, RNA libraries were constructed using a TruSeq Stranded mRNA Sample Prep Kit (Illumina, San Diego, CA) according to the manufacturer’s guidelines. Then the libraries were sequenced on the HiSeq X10 using the paired-end 2 × 150 bp, dual-index format. For data analysis, CLC Genomics Workbench (Qiagen) was used to remove Illumina sequencing adapters within raw reads of every sample, and the clean reads were mapped to mouse mm 10 reference genome. Finally, differential genes (P ≤ 0.01, Fold change ≥ 2 or ≤ −2) were screened by CLC based on raw read counts. Heatmap analysis for RPKM of sequenc- ing data was performed by R language using packages of heatmap. RPKM of VSV-infected WT and lincRNA-EPS/−/− iBMMs were analyzed by GSEA software to measure enrichment score for Hallmark data sets (Mootha et al., 2003; Subramanian et al., 2005). RNA sequencing raw data have been deposited in Gene Expression Omnibus (Accession no. GSE193326).

Absolute copies quantification of lincRNA-EPS

The RNA molecules of lincRNA-EPS were produced by in vitro transcription according to the instructions of in vitro Transcription T7 Kit (Takara). LincRNA-EPS was quantified by Nanodrop 2000 and subsequently diluted to perform RT-qPCR. The standard curve was illustrated based on RNA quantity, transcript length, and Ct value. Total RNA from a certain number of cells was isolated using TRIzol (Thermo Fisher Scientific) to determine the copy numbers of lincRNA-EPS according to the standard curve.

Western blot and RNA immunoprecipitation (RIP)

Cell lysates were prepared in lysis buffer containing 50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% (v/v) NP-40, and 0.5 mM EDTA supplemented with 1× complete protease inhibitor cocktail (Roche) and phosphatase inhibitor PhosSTOP (Roche). Equal total protein amounts from clarified lysates were resolved on SDS-PAGE, and transferred to 0.2 μm PVDF membranes (Millipore) for Western blot analysis. The target proteins were visualized using ECL chemiluminescent substrate (Millipore) or Odyssey Imaging Systems (LI-COR Biosciences). For RIP experiments, cell lysates were harvested in lysis buffer with 10 U RNase Inhibitor (Thermo Fisher Scientific), then performed as the introduction of Magna RIP Kit (Millipore) by 5 μg of anti-PKR or 2 μg of anti-flag antibodies with equal amount of isotype anti-IgG, and using 20 μl Protein A/G Magnetic beads. Protein-RNA complexes were treated with Proteinase K at 55°C for 30 min to elute and extract binding RNA. RNA samples were reverse transcribed and analyzed by qPCR. Results were normalized to input RNA extracted from cell lysates for RIP groups and isotype anti-IgG groups.

RNA pulldown assay and mass spectrometry

For RNA pulldown assay, RNA molecules were transcribed in vitro from amplified DNA fragments of lincRNA-EPS flanked with T7 promoter sequence (5’-TAATACGACTCACTATAGGG-3’) at the 5’-terminal according to the manufacturer’s instructions of in vitro Transcription T7 Kit (Takara). Transcribed RNA molecules of about 2,500 nt were confirmed by agarose gel electrophoresis and then ligated with a desthiobiotin linker using Pierce™ RNA 3’ End Desthiobiotinyltination Kit (Thermo Fisher Scientific). The non-labeled RNA in this kit was used as a negative control to ligate with the same linker. 50 pmol biotin-lincRNA-EPS, 1 μg biotin-polyI:C, and matched amount of negative control molecules were performed to pulldown interacted proteins extracted from iBMM cell lysates with Pierce™ Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Scientific). Binding proteins that pulled down were loaded on SDS-PAGE for silver staining (Thermo Fisher Scientific). The differential protein bands relative to negative control were sliced for mass spectrometry analysis by PTM BIO (China, Hangzhou).

Chromatin immunoprecipitation (ChIP)

iBMM cells plated on 10-cm dish were cross-linked with 1% formaldehyde for 10 min at room temperature and quenched with 125 mM Glycine for 5 min. Nuclear pellets were isolated in hypotonic lysis buffer as indicated in the study of Atianand et al. (2016), and vortex for 10 s every 5 min during 15 min incubation on ice. The nuclear pellets were suspended in the sonication buffer to incubate for 10 min on ice and then sonicated using Covaris S220 Focused-ultrasonicator (peak incident power of 140 W, duty factor of 10%, treatment time of 60 s) to generate 200–500 bp chromatin
fragments which were confirmed by agarose gel electrophoresis. Equal quantities of sheared chromatin (10 μg per IP) were used for IP assay with 2 μg anti-H3K4me3 or isotype control IgG antibodies at 4°C overnight. The 20 μl Protein G Dynabeads (Invitrogen) were added in chromatin complexes at 4°C for 1 h and washed as indicated in the study of Atianand et al (2016). Purified DNA was analyzed by qPCR using primers designed at the transcriptional regulatory region of genes as described previously (Shen et al., 2012).

**Nuclear and cytoplasmic RNA fraction**

A 5 × 10^7 iBMM cell pellets were collected after washing with ice-cold PBS twice and resuspended and lysed in 0.5 ml ice-cold lysis buffer containing 0.15% NP-40 and fractionated with 2.5 volumes of a chilled sucrose cushion (24% sucrose in lysis buffer) (Pandya-Jones & Black, 2009). The nuclear and cytoplasmic fraction were catted in the study of Atianand et al (2016). Purified RNA was dissolved in TRIzol-LS (ThermoFisher Scientific) for RNA extraction and RT–qPCR.

**Statistical analysis**

The data represent the mean of at least three independent experiments, and error bars represent the s.d. of the mean. Statistical analysis was performed by unpaired two-tailed Student’s t-test using GraphPad Prism (version 7; GraphPad Software Inc.). Survival data were analyzed using Log-rank (Mantel-Cox) test. P < 0.05 was considered as a statistically significant difference.

**Data availability**

The primary dataset of RNA-seq have been deposited in Gene Expression Omnibus under the accession number GSE193326 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc = GSE193326). Sequence information for gene cloning and RNAi targets are available upon request.

**Expanded View** for this article is available online.

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**Author contributions**

**Jingfei Zhu:** Data curation; Investigation; Writing – original draft. **Sheng-chuan Chen:** Investigation. **Li-Qiong Sun:** Investigation. **Siying Liu:** Data curation; Software; Investigation. **Xue Bai:** Investigation. **Dapei Li:** Investigation. **Fan Zhang:** Investigation. **Zigang Qiao:** Investigation. **Liang Li:** Investigation. **Haiping Yao:** Investigation. **Yu Xia:** Resources. **Ping Xu:** Resources; Funding acquisition. **Xiaohui Jiang:** Investigation. **Zhengrong Chen:** Resources. **Yongdong Yan:** Conceptualization; Supervision. **Feng Ma:** Conceptualization; Supervision; Funding acquisition; Writing – original draft; Writing – review & editing.

In addition to the CRediT author contributions listed above, the contributions in detail are:

FM and YY conceived the idea and designed the experiments. JZ, SC, LQS, SL, XB, DL, FZ, QZ, LL, HY, and XJ performed all the experiments. YX, PX, and ZC provided the reagents and suggestions. FM and JZ analyzed the data and wrote the manuscript.

**Disclosure and competing interests statement**

The authors declare that they have no conflict of interest.

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