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ORIGINAL ARTICLE

Escherichia coli infection activates the production of IFN-α and IFN-β via the JAK1/STAT1/2 signaling pathway in lung cells

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Abstract

Escherichia coli infections can result in lung injury, which may be closely linked to the induction of interferon secretion. The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway is one of most important pathways that regulate interferon production. Thus, the present study aimed to dissect whether *E. coli* infections can regulate interferon production and the underlying mechanisms. For this aim, two lung cell lines, a human bronchial epithelial cell line transformed with Ad12-SV40 2B (BEAS-2b) and a human fetal lung fibroblast (HFL1) cell line, were used. The effects of *E. coli* infections on interferon production were studied using qRT-PCR, Western blot, and siRNA knockdown assays. *E. coli* infections remarkably promoted the expression levels of IFN-α, IFN-β, and ISGs. Major components of the JAK/STAT pathway, including JAK1, STAT1, and STAT2, were demonstrated to be regulated by *E. coli* infections. Importantly, knockdown of JAK1, STAT1, and STAT2 abolished the induction of IFN-α, IFN-β, and ISGs by *E. coli*. Therefore, experiments in the present study demonstrated that *E. coli* infections remarkably promoted interferon production in lung cells, which was closely regulated by the JAK/STAT signaling pathway. The findings in the present study are useful for further understanding the pathogenesis of *E. coli* infections in the lung and finding novel therapies to treat *E. coli*-induced lung injury.

Keywords *Escherichia coli* · IFN-α and IFN-β · JAK1/STAT1/2 signaling · Lung cell line · Protection

Introduction

The lung is one of the major organs in the body and is responsible for transferring oxygen from the atmosphere into the bloodstream (Mullassery and Smith 2015). Acute lung injury (ALI) is a common disease and a major cause of morbidity and mortality and is characterized by pulmonary inflammation and oxidative stress (Liu et al. 2018a). The incidence of ALI and its mortality are increasing (Rubenfeld et al. 2005). It was reported that mortality caused by ALI could be ~ 40%, with a more than 50% in-hospital mortality rate, although supportive care has been largely improved (Zhu et al. 2014). In the United States, it was reported that the incidence of ALI and acute respiratory distress syndrome (ARDS) was 78.9 and 58.7 cases per 100,000 individuals yearly in patients older than 15 years (Suresh et al. 2000). Thus, a greater understanding of ALI is beneficial for developing novel medicines to treat severe diseases.

The agents causing ALI are multitudinous and include smoking (Gotts et al. 2018), inflammation (Hamacher et al. 2017), immunity disruption (Grommes and Soehnlein 2011), viral infections (Gregory and Kobzik 2015), and bacterial infections (Wan et al. 2016). *Escherichia coli* is one of the most common pathogens causing intestinal diseases (Liu et al. 2018b). Recently, accumulating evidence has indicated that *E. coli* can cause acute lung injury (Liu et al. 2018b). *E. coli* infects the lung, which results in pulmonary capillary endothelial damage, thus leading to permeability edema (Ye and Liu 2020). The tissue damage caused by *E. coli* in the lung is mainly due to the induction of interferon production, especially inflammation (Aulakh et al. 2014). However, how *E. coli* regulates interferon production in the lung remains unclear.

The JAK/STAT pathway is one of most important innate immune signaling pathways and was discovered through

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a study that linked interferon-responsive genes to signal transduction (Coskun et al. 2013). Among them, IFN-α and IFN-β are the main components in innate immunity signaling. This pathway plays an important role in multiple physiological activities, including obesity and diabetes (Gurzov et al. 2016), rheumatoid arthritis (Malemud 2018), skeletal muscle pathophysiology (Moresi et al. 2019), and neuroinflammatory diseases (Yan et al. 2018). It was found that the regulation of innate immunity by *E. coli* is related to the JAK/STAT pathway (Ho 2017). Therefore, in the present study, we aimed to investigate the effects of *E. coli* infection on the production of IFN-α and IFN-β in lung cell lines. *E. coli* infection remarkably induced IFN-α and IFN-β production in lung cell lines via the JAK1/STAT1/2 signaling pathway. The findings in this study will provide useful information for studying the pathogenesis of *E. coli*-induced ALI and developing novel medicines to treat this difficult disease.

Materials and methods

Reagents

LB broth (catalog# ST156, Shanghai, China), BeyoECL Plus (catalog# P0018S), Bradford protein assay kit (catalog# P0006), BeyoFast™ SYBR Green qPCR Mix (2X, catalog# D7260), penicillin–streptomycin (catalog# C0222), fetal bovine serum (FBS, catalog# C0225), Beyozol (catalog# R0011), BeyoRT™ II cDNA synthesis reagent (catalog# D7168M), RIPA lysis buffer (catalog# P0013C), and bovine serum albumin (BSA, catalog# ST025-5 g) were purchased from Beyotime Biotechnology. Phosphate-buffered saline (PBS) was purchased from Thermo Fisher Scientific (pH 7.4, catalog# 10010072). Dulbecco's modified Eagle's medium (DMEM) was purchased from Thermo Fisher Scientific (catalog# 11995073). BEGM media with SingleQuot kit additives were purchased from Lonza (catalog# CC-3170, Walkersville, MD). Human fibronectin (catalog# PHE0023) was purchased from Thermo Fisher Scientific (catalog# PHE0023, Waltham, MA). Bovine collagen type I was purchased from STEMCELL (catalog# 07001, STEMCELL). Anti-IFN-α polyclonal antibody (catalog# 66162-1-Ig, Proteintech), Anti-IFN-β polyclonal antibody (catalog# 27506-1-AP, Proteintech), Anti-JAK1 (rabbit monoclonal, catalog# ab133666), anti-STAT1 (rabbit monoclonal, catalog# ab234400), anti-GAPDH (rabbit monoclonal, catalog# ab8245), and anti-STAT2 (rabbit monoclonal, catalog# ab32367) were purchased from Abcam.

Cell culture

The BEAS-2b cell line (a human nontumorigenic lung cell line) and human fetal lung fibroblast 1 (HFL1) cell line have been used in lung inflammation studies for a long time (Feng et al. 2012; Kikuchi et al. 2009). The two cell lines were obtained from the bioresource bank of the First Affiliated Hospital of China Medical University. The BEAS-2b cell line was maintained in BEGM media with SingleQuot kit additives in 75 cm^2 Falcon tissue culture-treated flasks (Thermo Fisher Scientific, Waltham, MA) at 37 °C and 5% CO_2. BEGM containing human fibronectin (0.01 mg/mL), bovine collagen type I (0.03 mg/mL), and BSA (0.01 mg/mL) was used to coat culture vessels for at least 2 h at 37 °C prior to seeding cells. The HFL1 cell line was cultured in DMEM supplemented with 1% penicillin–streptomycin (P/S) and 10% FBS at 37 °C and 5% CO_2. The medium was replaced every 2 or 3 days. Both cell lines were subcultured when the confluence reached more than 90%.

Bacteria culture and cell infection

Escherichia coli (strain O55:B5) was obtained from the Bioresource Center of the First Affiliated Hospital of China Medical University. *E. coli* was cultured in LB broth media at 37 °C overnight. The number of bacteria was counted spectrophotometrically at an optical density of 600 nm (OD600). For in vitro infections, bacteria were suspended in culture medium at a multiplicity of infection (MOI) of 100. After 3 h of infection, infected monolayers were washed 4 times with phosphate-buffered saline (PBS) and incubated in cell culture medium. *E. coli*-infected cells were incubated 24 h after invasion, followed by washing and lysis for subsequent assays.

Knockdown via siRNA

Small interfering RNAs (siRNAs) targeting JAK1, STAT1, and STAT2 were designed using the siDESIGN Center of Dharmacon, and they were synthesized by Biosyntech (Suzhou, China). BEAS-2b or HFL1 cells (2×10^5, 60–80% confluence) were added to a 12-well plate (Corning) in 1 mL of the corresponding media. After 24 h, when confluence reached 60–80%, control, JAK1, STAT1, and STAT2 siRNA knockdown assays (defined as si-scramble, siJAK1, siSTAT1, and STAT2, respectively) were carried out according to the manufacturer's protocol. The cells were harvested after 24 h of incubation for subsequent experiments. The sequences of siRNAs are listed in Table 1.

RNA isolation and RT–PCR

Total RNA was isolated from BEAS-2b and HFL1 cell lines using Beyozol reagent (Beyotime Biotechnology). The quantification of total RNA was performed using NanoDrop™ 2000/2000c spectrophotometers (Thermo Fisher Scientific). Subsequently, total RNA was converted to cDNA

Table 1 The sequences of siRNAs used in the present study

Gene name		Sequence (5′→3′)
Jak1	Sense	CCACAUAGCUGAUCUGAAAUU
	Anti-sense	UUUCAGAUCAGCUAUGUGGUU
STAT1	Sense	CCACAUAGCUGAUCUGAAAUU
	Anti-sense	UUUCAGAUCAGCUAUGUGGUU
STAT2	Sense	CCACAUAGCUGAUCUGAAAUU
	Anti-sense	UUUCAGAUCAGCUAUGUGGUU

using BeyoRT™ II cDNA synthesis reagent according to the manufacturer's protocol. qRT-PCRs were carried out in a total reaction volume of 10 μL containing 2 × BeyoFast™ SYBR Green qPCR Mix (2X), 8 μM each of sense and anti-sense primers and 1 μL of cDNA using the Roche LightCycler® 480 instrument (Roche Applied Science, Indianapolis, Ind.). Primers used for qRT–PCR are listed in Table 2. The relative expression of mRNA was calculated using the $2^{-\Delta\Delta}$ threshold cycle (Ct) (Livak) method. The RT-PCRs were performed in triplicate for each of the three independent samples.

Western blotting (WB) analysis

After treatment, the cells were washed and rinsed with cold PBS, followed by the addition of RIPA lysis buffer. Proteins were harvested in RIPA lysis buffer. Cell suspensions were centrifuged at 10,000 × *g* for 20 min at 4 °C. Protein was quantified using a Bradford protein assay kit. Subsequently, 30 μg protein samples were electrophoresed in 12% sodium dodecyl sulfate–polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Then, membranes were blocked using PBS containing 0.05% Tween-20 (Sigma, PBST) and 5% skim milk for 1 h at room temperature, followed by incubation with 1:1000 dilutions each of anti-IFN-α, anti-IFN-β, anti-JAK1, anti-STAT1, anti-STAT2, and anti-GAPDH at 4 °C overnight. Then, the membranes were washed with PBST three times and incubated with the appropriate secondary horseradish peroxidase-conjugated IgG antibody (R&D Systems) for 1 h at room temperature. The protein bands on the membrane were detected using an ECL-Plus Western blot detection system according to the manufacturer's instructions.

Statistical analysis

Data are expressed as the mean ± standard deviation (SD). Differences between 2 groups were assessed by a two-tailed unpaired Student's *t* test when data were distributed normally. Analysis of variance with Tukey's multiple-comparisons test was used to evaluate experiments involving multiple groups. P value < 0.05 was considered significant.

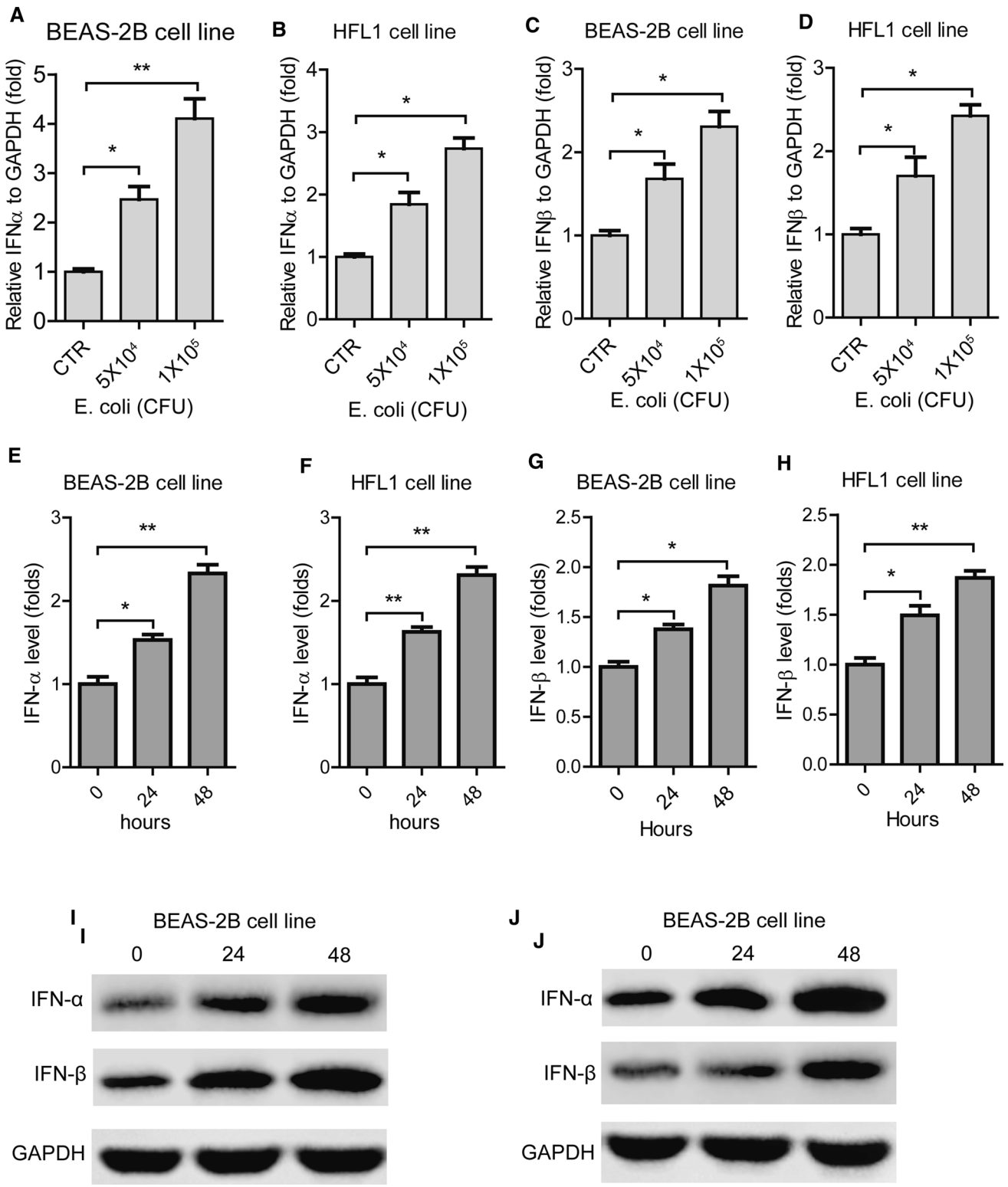
Results

E. coli infections increased the expression levels of IFN-α and IFN-β in lung cell lines

To investigate the effects of *E. coli* infections on interferon production in lung cells, we tested the mRNA levels of IFN-α and IFN-β using qRT-PCR. *E. coli* infections (5×10^4 and 1×10^5 CFU) significantly increased

Table 2 The sequences of primers used in the present study

Gene name		Sequence (5′→3′)	PCR product size (bp)	Tm
Jak1	Sense	GGAGGCGGGATGCATTTCTG	126	61.45
	Anti-sense	GAAGCGTGTGTCTCAGAAGC		59.21
STAT1	Sense	GCATCGAGCGCACAAAGTTA	82	59.56
	Anti-sense	ACCAGTAGGGTTGAGGGACA		59.81
STAT2	Sense	TTTACTCGCACAGCCTCCTG	128	60.04
	Anti-sense	GATCCTGAATGTCCCGGCAG		60.53
IFN-β	Sense	AGTAGGCGACACTGTTCGTG	173	55
	Anti-sense	GCCTCCCATTCAATTGCCAC		55
IFN-α	Sense	TTCGTATGCCAGCTCACCTT	167	50
	Anti-sense	GGATCAGTCAGCATGGTCCT		55
MX1	Sense	CCGAAACTGAATTGTCCGGG	123	55
	Anti-sense	TGAATGGGGAATCGGAAGGC		55
ISG20	Sense	ATCCCAGCCCTATTCCTGGT	517	55
	Anti-sense	CAGACCCTCCTCCCCATACA		60
IFIT1	Sense	AGTGGAGATGTGTACAAATGGTG	229	43
	Anti-sense	GCTCTTCAGGGCTTCCTCATT		52



the mRNA expression of IFN-α in the BEAS-2B cell line (Fig. 1A, $n=4$, $^{*}P<0.05$, $^{**}P<0.01$) and the HFL1 cell line (Fig. 1B, $n=4$, $^{*}P<0.05$). Similarly, *E. coli* infections (5×104 and 1×105 CFU) significantly increased the mRNA expression of IFN-β in the BEAS-2B cell line (Fig. 1C, $n=4$, $^{*}P<0.05$) and the HFL1 cell line (Fig. 1D, $n=4$, $^{*}P<0.05$). ELISAs were performed to further investigate the effects of *E. coli* infections on IFN-α and IFN-β secretion, which indicated that *E. coli* infections (5×10^4 CFU) significantly increased IFN-α secretion in the BEAS-2B cell

◂**Fig. 1** *E. coli* infections increased the expression levels of IFN-α and IFN-β in lung cells. **A** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) significantly increased mRNA expression of IFN-α in the BEAS-2B cell line ($n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$); *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) significantly increased mRNA expression of IFN-α in the HFL1 cell line ($n = 4$, $^{*}P < 0.05$); **C** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) significantly increased mRNA expression of IFN-β in the BEAS-2B cell line ($n = 4$, $^{*}P < 0.05$); **D** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) significantly increased mRNA expression of IFN-β in the HFL1 cell line ($n = 4$, $^{*}P < 0.05$). *E. coli* infections (5×10^4 CFU) significantly increased IFN-α secretion in the BEAS-2B cell line (**E**, $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$) and HFL1 cell line (**F**, $n = 4$, $^{**}P < 0.01$) over time (24 and 48 h). *E. coli* infections (5×10^4 CFU) significantly increased IFN-β secretion in the BEAS-2B cell line (**G**, $n = 4$, $^{*}P < 0.05$) and HFL1 cell line (**H**, $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$) over time (24 and 48 h). **I** *E. coli* infections (5×10^4 CFU) significantly increased IFN-α and IFN-β protein levels in the BEAS-2B cell line over time (24 and 48 h); **J** *E. coli* infections (5×10^4 CFU) significantly increased IFN-α and IFN-β secretion in the HFL1 cell line over time (24 and 48 h)

line (Fig. 1E, $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$) and the HFL1 cell line (Fig. 1F, $n = 4$, $^{**}P < 0.01$) over time. Similarly, we found that *E. coli* infection (5×10^4 CFU) significantly increased IFN-β secretion in the BEAS-2B cell line (Fig. 1G, $n = 4$, $^{*}P < 0.05$) and the HFL1 cell line (Fig. 1H, $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$) over time. WB assays indicated that *E. coli* infection (5×10^4 CFU) significantly increased IFN-α and IFN-β protein levels in the BEAS-2B cell line (Fig. 1I) over time, and *E. coli* infection (5×10^4 CFU) significantly increased IFN-α and IFN-β protein levels in the HFL1 cell line (Fig. 1J) over time. Thus, we confirmed that *E. coli* infection could increase the expression levels of IFN-α and IFN-β in lung cells.

E. coli infections increased the expression levels of the main components of the JAK1/STAT signaling pathway in lung cells

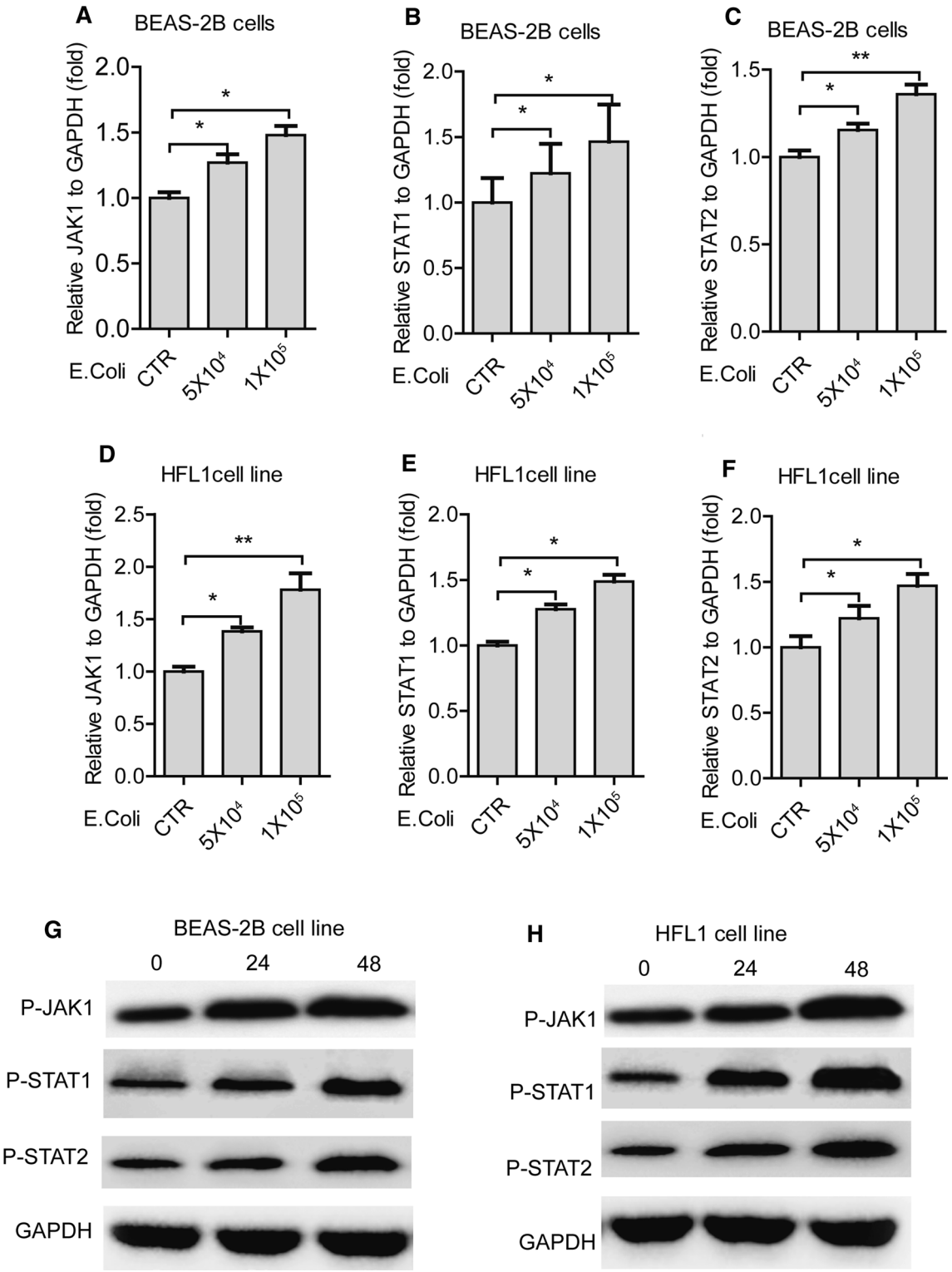
The JAK/STAT signaling pathway plays an important role in innate immunity (Yan et al. 2018). Thus, key genes, including JAK1, STAT1, and STAT2, in the JAK/STAT signaling pathways were studied. *E. coli* infections (5 × 104 and 1 × 105 CFU) significantly increased the mRNA expression of JAK1 in the BEAS-2B cell line (Fig. 2A, $n = 4$, $^{*}P < 0.05$). In parallel, *E. coli* infections (5 × 104 and 1 × 105 CFU) remarkably increased the mRNA levels of STAT1 (Fig. 2B, $n = 4$, $^{*}P < 0.05$) and STAT2 (Fig. 2C, $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$) in the BEAS-2B cell line. Similarly, *E. coli* infections (5 × 104 and 1 × 105 CFU) significantly increased the mRNA levels of JAK1 (Fig. 2D, $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$), STAT1 (Fig. 2E, $n = 4$, $^{*}P < 0.05$) and STAT2 (Fig. 2F, $n = 4$, $^{*}P < 0.05$) in the HFL1 cell line. Moreover, *E. coli* infection (5 × 104 CFU) increased phosphorylation level of JAK1, STAT1, and STAT2 in BEAS-2B cell line over time (Fig. 2G). *E. coli* infection (5 × 104 CFU) increased phosphorylation level of JAK1, STAT1, and STAT2 in BEAS-2B cell line over time (Fig. 2H). Taken together, these results demonstrated that *E. coli* infection could increase the expression levels of key genes of the JAK1/STAT signaling pathway in lung cells.

E. coli infections increased the expression levels of interferon-stimulated genes (ISGs) in lung cells

ISGs are downstream genes of the JAK/STAT signaling pathway (Ivashkiv and Donlin 2014). Therefore, we investigated the effects of *E. coli* infections on the expression of several ISGs, including ISG20, MX1, and IFIT1. *E. coli* infections (5×10^4 and 1×10^5 CFU) significantly increased the mRNA expression of ISG20 (Fig. 3A, $n = 4$, $^{**}P < 0.01$, $^{***}P < 0.001$), MX1 (Fig. 3B, $n = 4$, $^{**}P < 0.01$, $^{***}P < 0.001$), and IFIT1 (Fig. 3C, $n = 4$, $^{**}P < 0.01$, $^{***}P < 0.001$) in the BEAS-2B cell line. In parallel, *E. coli* infections (5×10^4 and 1×10^5 CFU) significantly increased the mRNA expression of ISG20 (Fig. 3D, $n = 4$, $^{***}P < 0.001$), MX1 (Fig. 3E, $n = 4$, $^{**}P < 0.01$, $^{***}P < 0.001$), and IFIT1 (Fig. 3F, $n = 4$, $^{**}P < 0.01$, $^{***}P < 0.001$) in the HFL1 cell line. Thus, the *E. coli* infections could increase the expression level of ISGs in lung cells.

JAK1 knockdown compromised the *E. coli* infection-mediated enhancement of the expression levels of IFN-α and IFN-β in lung cells

To further investigate the effects of JAK1/STAT signaling on IFN-α and IFN-β production by *E. coli* infections in lung cells, we performed a siRNA-based JAK1 knockdown assay. The qRT–PCR assay demonstrated successful knockdown of JAK1 in the BEAS-2B (Fig. 4A, $n = 4$, $^{**}P < 0.01$) and HFL1 cell lines (Fig. 4B, $n = 4$, $^{*}P < 0.05$). To further verify this hypothesis, we carried out a WB experiment, which indicated that siRNA against JAK1 could knock down the expression in the BEAS-2B cell line (Fig. 4C) and the HFL1 cell line (Fig. 4D) at the protein level. Interestingly, JAK1 knockdown abolished the *E. coli* infection (1×10^5 CFU)-mediated enhancement of the expression levels of IFN-α (Fig. 4E, $n = 4$, $^{**}P < 0.01$) and IFN-β (Fig. 4F, $n = 4$, $^{*}P < 0.05$, $^{**}P < 0.01$) in the BEAS-2B cell line. Similarly, JAK1 knockdown abolished the *E. coli* infection (1×10^5 CFU)-mediated enhancement of the expression levels of IFN-α (Fig. 4G, $n = 4$, $^{**}P < 0.01$) and IFN-β (Fig. 4H, $n = 4$, $^{**}P < 0.01$, $^{***}P < 0.001$) in the HFL1 cell line. Therefore, JAK1 knockdown could abolish the *E. coli* infection-mediated enhancement of the expression levels of IFN-α and IFN-β in lung cells.



STAT1 knockdown compromised the *E. coli* infection-mediated enhancement of the expression levels of IFN-α and IFN-β in lung cells

To further verify how JAK/STAT signaling influences *E. coli*-induced infections, we used siRNA against STAT1, which indicated that the siRNA significantly knocked down STAT1 in the BEAS-2B cell line (Fig. 5A, $n=4$, $^{**}P<0.01$) and the HFL1 cell line (Fig. 5B, $n=4$, $^{***}P<0.001$). Moreover, siRNA against STAT1 could reduce the protein

◂**Fig. 2** *E. coli* infections increased the expression levels of the main components of the JAK1/STAT signaling pathway in lung cells. **A** *E. coli* infections (55 × 104 and 1 × 105 CFU, 24 h) significantly increased mRNA expression of JAK1 in the BEAS-2B cell line ($n=4$, $^{*}P<0.05$); **B** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) remarkably increased the mRNA levels of STAT1 in the BEAS-2B cell line ($n=4$, $^{*}P<0.05$); **C** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) remarkably increased the mRNA levels of STAT2 ($n=4$, $^{*}P<0.05$, $^{**}P<0.01$); **D** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) significantly increased the mRNA levels of JAK1 in the HFL1 cell line ($n=4$, $^{*}P<0.05$, $^{**}P<0.01$); **E** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) significantly increased the mRNA levels of STAT1 in the HFL1 cell line ($n=4$, $^{*}P<0.05$); **F** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) significantly increased the mRNA levels of STAT2 in the HFL1 cell line ($n=4$, $^{*}P<0.05$). **G** *E. coli* infection (5 × 104 CFU) increased phosphorylation level of JAK1, STAT1, and STAT1 in BEAS-2B cell line over time (24 and 48 h). **H** *E. coli* infection (5 × 104 CFU) increased phosphorylation level of JAK1, STAT1, and STAT1 in HFL1 cell line over time (24 and 48 h)

levels of STAT1 in the BEAS-2B cell line (Fig. 5C) and the HFL1 cell line (Fig. 5D), as shown by WB assays. Moreover, STAT1 knockdown abolished the *E. coli* infection (1×10^5 CFU)-mediated enhancement of the expression levels of IFN-α (Fig. 5E, $n=4$, $^{**}P<0.01$) and IFN-β (Fig. 5F, $n=4$, $^{**}P<0.01$) in the BEAS-2B cell line. STAT1 knockdown also abolished the *E. coli* infection (1×10^5 CFU)-mediated enhancement of the expression levels of IFN-α (Fig. 5G, $n=4$, $^{**}P<0.01$) and IFN-β (Fig. 5H, $n=4$, $^{*}P<0.05$) in the HFL1 cell line. Therefore, STAT1 knockdown abolished the *E. coli* infection-mediated enhancement of the expression levels of IFN-α and IFN-β in lung cells.

STAT2 knockdown compromised the *E. coli* infection-mediated enhancement of the expression levels of IFN-α and IFN-β in lung cells

To further investigate how the JAK1/STAT pathway is involved in the effects of *E. coli* infections on IFN-α and IFN-β production, we used siRNA against STAT2 to knock down the gene expression in both lung cell lines; we found that the expression level of STAT2 was significantly reduced in the BEAS-2B cell line (Fig. 6A, $n=4$, $^{**}P<0.01$) and the HFL1 cell line (Fig. 6B, $n=4$, $^{**}P<0.01$). It was also found that siRNA against STAT2 could reduce the protein level of STAT1 in the BEAS-2B cell line (Fig. 6C) and the HFL1 cell line (Fig. 6D), as shown via WB assays. STAT1 knockdown abolished the *E. coli* infection (1×10^5 CFU)-mediated enhancement of the expression levels of IFN-α (Fig. 6E, $n=4$, $^{**}P<0.01$) and IFN-β (Fig. 6F, $n=4$, $^{**}P<0.01$) in the BEAS-2B cell line. Similarly, STAT1 knockdown abolished the *E. coli* infection (1×10^5 CFU)-mediated enhancement of the expression levels of IFN-α (Fig. 6G, $n=4$, $^{**}P<0.01$) and IFN-β (Fig. 6H, $n=4$, $^{*}P<0.05$) in the HFL1 cell line. Herein, it was demonstrated that STAT2 knockdown could abolish the *E. coli* infection-mediated enhancement of the expression level of IFN-α and IFN-β in lung cells.

Silencing JAK1, STAT1, and STAT2 abolished the *E. coli* infection-mediated promotion of the expression level of ISGs in lung cells

To further demonstrate how the JAK1/STAT pathway is involved in IFN-α and IFN-β production induced by *E. coli* infections, we investigated the effects of JAK1, STAT1, and STAT2 knockdown on the *E. coli* regulation of the expression level of ISGs in lung cells. JAK1 knockdown abolished the *E. coli* infection (1×10^5 CFU)-mediated enhancement of the expression of ISGs, including ISG20, MX1, and IFIT1, in the BEAS-2B cell line (Fig. 7A, $n=4$, $^{**}P<0.01$). Similarly, STAT1 (Fig. 7B, $n=4$, $^{*}P<0.05$, $^{**}P<0.01$) and STAT2 (Fig. 7C, $n=4$, $^{**}P<0.01$) knockdown abolished the *E. coli* infection (1×10^5 CFU)-mediated enhancement of the expression of ISGs, including ISG20, MX1, and IFIT1, in the BEAS-2B cell line. Similarly, knockdown of JAK1 (Fig. 7D, $n=4$, $^{*}P<0.05$, $^{**}P<0.01$), STAT1 (Fig. 7E, $n=4$, $^{*}P<0.05$, $^{**}P<0.01$), and STAT2 (Fig. 7F, $n=4$, $^{*}P<0.05$, $^{**}P<0.01$) abolished the *E. coli* infection (1×10^5 CFU)-mediated enhancement of the expression of ISGs, including ISG20, MX1, and IFIT1, in the HFL1 cell line. Thus, we confirmed that knockdown of JAK1, STAT1, and STAT2 could abolish the *E. coli* infection-mediated enhancement of the expression level of ISGs in lung cells.

Discussion

The lung is an organ that accesses air and contains many microorganisms (Lee et al. 2009). Among them, *E. coli* is one of the most common pathogens infecting lung tissues to cause lung injury (Masterson et al. 2018). It was reported that *E. coli* infections could induce innate immune responses in the lung tissue (Martin and Frevert 2005). However, it remains unclear how *E. coli* infections induce IFN-α and IFN-β production in lung cells. Thus, in this study, it was found that *E. coli* infection significantly increased the expression of IFN-α and IFN-β, ISGs, and key genes of JAK1/STAT signaling. Importantly, we found that knockdown of JAK1, STAT1, and STAT2 could abolish the *E. coli* infection-mediated promotion of the expression levels of IFN-α and IFN-β and ISGs. Taken together, these results demonstrated that *E. coli* infections induced interferon production in lung cells, which was regulated by the JAK1/STAT signaling pathway.

Lung cells are a critical source of innate immune molecules (Sturrock et al. 2018). It was found that the innate immune response is closely related to acute lung injury

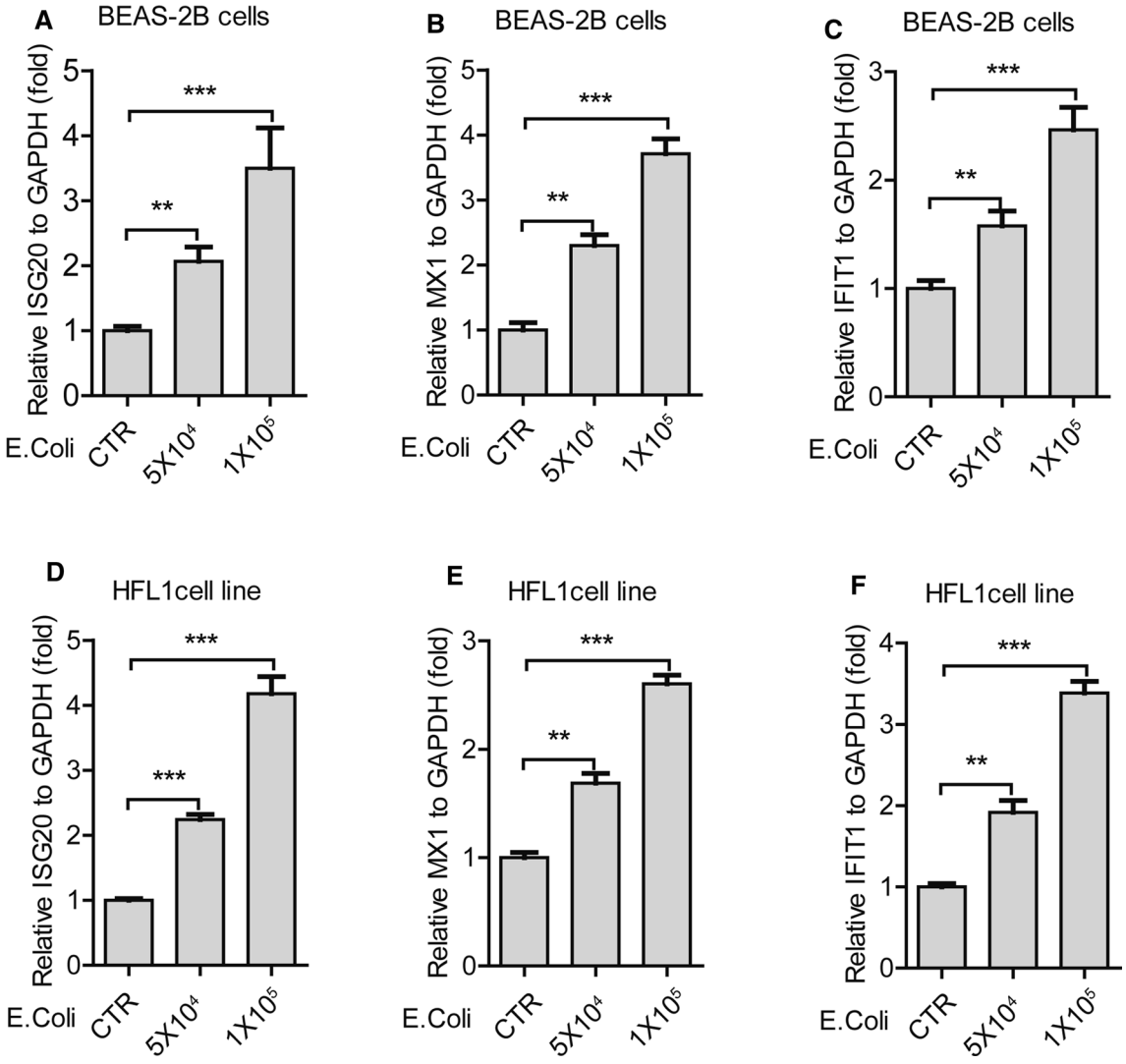


Fig. 3 *E. coli* infections increased the expression level of interferon-stimulated genes (ISGs) in lung cells. **A** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) significantly increased the mRNA expression of ISG20 in the BEAS-2B cell line ($n = 4$, $^{**}P < 0.01$, $^{***}P < 0.001$); **B** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) significantly increased the mRNA expression of MX1 in the BEAS-2B cell line ($n = 4$, $^{**}P < 0.01$, $^{***}P < 0.001$); **C** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) significantly increased the mRNA expression of IFIT1 in the BEAS-2B cell line ($n = 4$, $^{**}P < 0.01$, $^{***}P < 0.001$); **D** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) significantly increased the mRNA expression of ISG20 in the HFL1 cell line ($n = 4$, $^{***}P < 0.001$); **E** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) significantly increased the mRNA expression of MX1 in the HFL1 cell line ($n = 4$, $^{**}P < 0.01$, $^{***}P < 0.001$); **F** *E. coli* infections (5 × 104 and 1 × 105 CFU, 24 h) significantly increased the mRNA expression of IFIT1 in the HFL1 cell line ($n = 4$, $^{**}P < 0.01$, $^{***}P < 0.001$)

(Maijo et al. 2012; Wang et al. 2013). Thus, understanding innate immunity in the lung will help identify an effective approach to treating lung injury. Many bacteria have been confirmed to induce innate immunity, including IFN-α and IFN-β production; for example, it was reported that *Streptococcus pneumoniae* and *Staphylococcus aureus* induced the expression of IFN-γ mRNA and protein in the lungs of mice (Yamada et al. 2011). *Pseudomonas aeruginosa* was found to induce the type I interferon signaling pathway in the lung (Parker and Prince 2011). The levels of innate immunity-related genes, including TNF-α and IL-6, were increased by *E. coli* LPS treatment in the lungs of mice (Jeyaseelan et al. 2007). An avian *E. coli* strain named avian pathogenic *E. coli* (APEc) was reported to significantly increase the expression levels of the inflammatory cytokines IL-1β, IL-18, and TNF-α in the lung (Li et al. 2018). Similarly, in the present study, we found that *E. coli* infection induced type I innate immunity, including an increase in the expression levels of IFN-α and IFN-β and ISGs (Figs. 1 and 2).

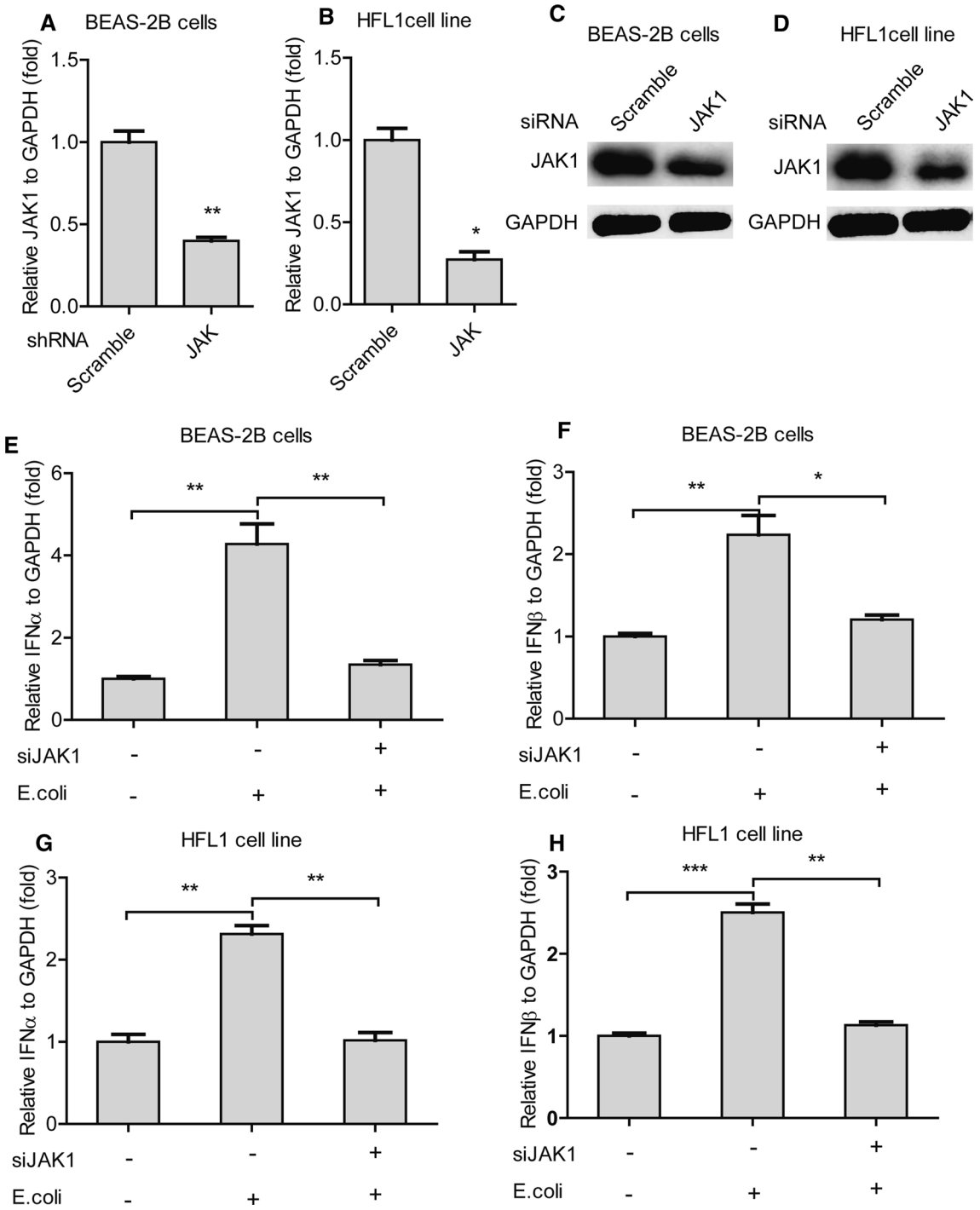


Fig. 4 JAK1 knockdown compromised the *E. coli* infection-mediated enhancement of on the expression levels of IFN-α and IFN-β in lung cells. **A** JAK1 was successfully knocked down in the BEAS-2B cell line, as shown using qPCR assays ($n=4$, $^{**}P<0.01$); **B** JAK1 was successfully knocked down in the HFL1 cell line, as shown using qPCR assays ($n=4$, $^{*}P<0.05$); **C** JAK1 was successfully knocked down in the BEAS-2B cell line, as shown using WB assays; **D** JAK1 was successfully knocked down in the HFL1 cell line, as shown using WB assays; **E** JAK1 knockdown abolished the *E. coli* infection (1 × 105 CFU, 24 h)-mediated enhancement of the expression level of IFN-α in the BEAS-2B cell line ($n=4$, $^{**}P<0.01$); **F** JAK1 knockdown abolished the *E. coli* infection (1 × 105 CFU, 24 h)-mediated enhancement of the expression level of IFN-β in the BEAS-2B cell line ($n=4$, $^{*}P<0.05$, $^{**}P<0.01$); **G** JAK1 knockdown abolished the *E. coli* infection (1 × 105 CFU, 24 h)-mediated enhancement of the expression level of IFN-α in the HFL1 cell line ($n=4$, $^{**}P<0.01$); **H** JAK1 knockdown abolished the *E. coli* infection (1 × 105 CFU, 24 h)-mediated enhancement of the expression level of IFN-β in the HFL1 cell line ($n=4$, $^{**}P<0.01$, $^{***}P<0.001$)

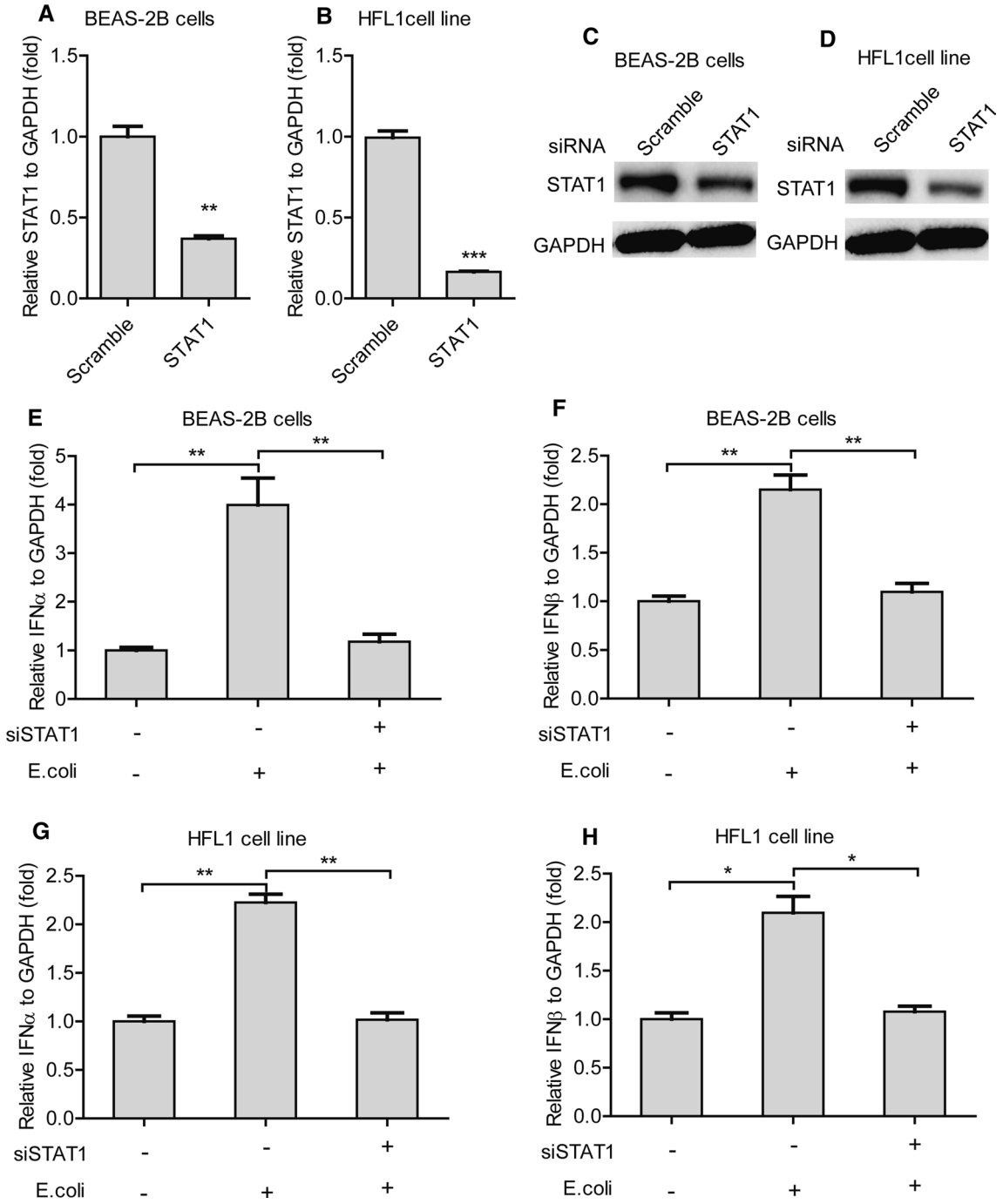


Fig. 5 STAT1 knockdown compromised the *E. coli* infection-mediated enhancement of the expression levels of IFN-α and IFN-β in lung cells. **A** STAT1 was successfully knocked down in the BEAS-2B cell line, as shown using qPCR assays ($n=4$, $^{**}P<0.01$); **B** STAT1 was successfully knocked down in the HFL1 cell line, as shown using qPCR assays ($n=4$, $^{**}P<0.01$); **C** STAT1 was successfully knocked down in the BEAS-2B cell line, as shown using WB assays; **D** STAT1 was successfully knocked down in the HFL1 cell line, as shown using WB assays; **E** STAT1 knockdown could abolish the *E. coli* infection (1×105 CFU, 24 h)-mediated enhancement of the expression level of IFN-α in the BEAS-2B cell line ($n=4$, $^{**}P<0.01$); **F** STAT1 knockdown could abolish the *E. coli* infection (1×105 CFU, 24 h)-mediated enhancement of the expression level of IFN-β in the BEAS-2B cell line ($n=4$, $^{*}P<0.05$, $^{**}P<0.01$); **G** STAT1 knockdown could abolish the *E. coli* infection (1×105 CFU, 24 h)-mediated enhancement of the expression level of IFN-α in the HFL1 cell line ($n=4$, $^{**}P<0.01$); **H** STAT1 knockdown could abolish the *E. coli* infection (1×105 CFU, 24 h)-mediated enhancement of the expression level of IFN-β in the HFL1 cell line ($n=4$, $^{**}P<0.01$, $^{***}P<0.001$)

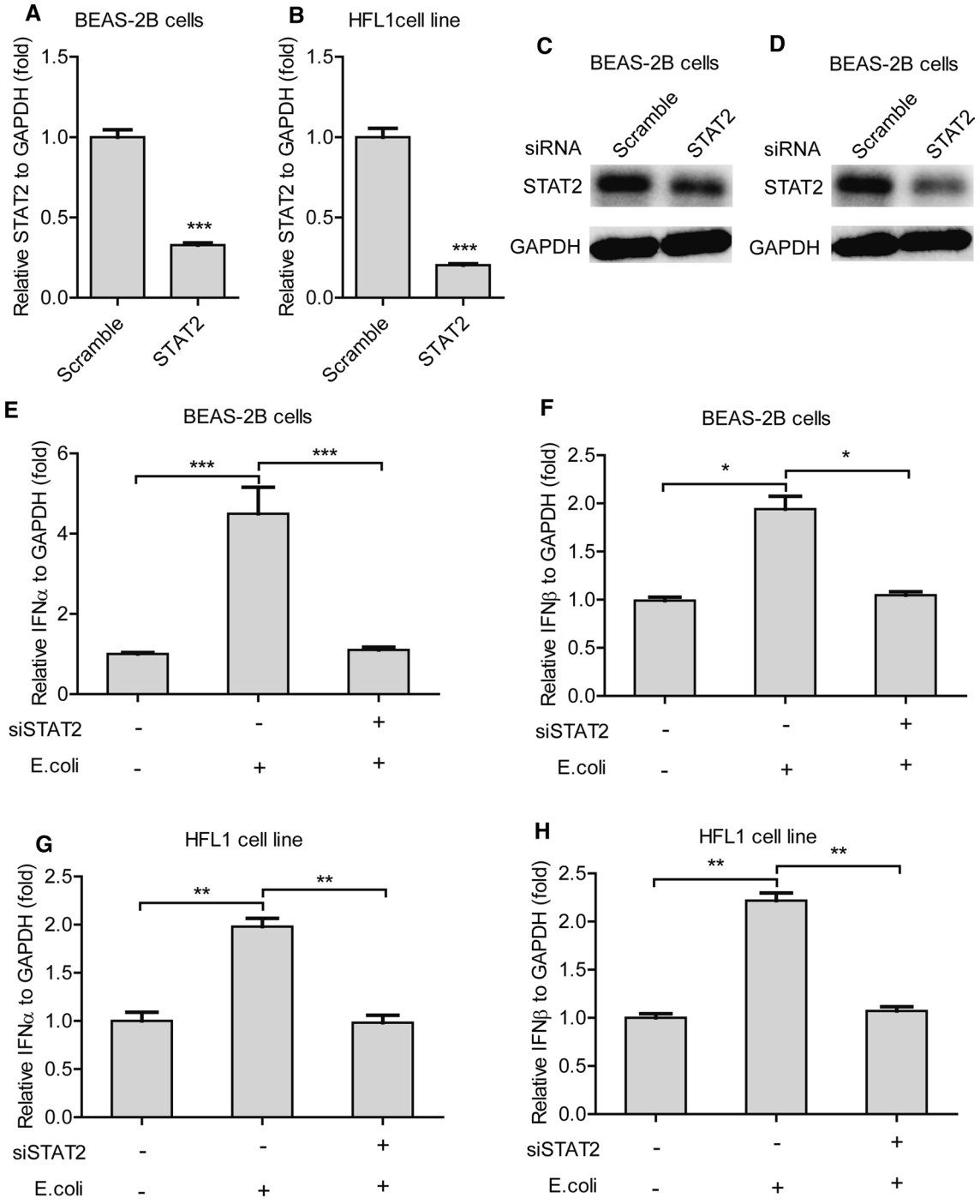


Fig. 6 STAT2 knockdown compromised the *E. coli* infection-mediated enhancement of the expression levels of IFN-α and IFN-β in lung cells. **A** STAT2 was successfully knocked down in the BEAS-2B cell line, as shown using qPCR assays ($n=4$, $^{**}P<0.01$); **B** STAT2 was successfully knocked down in the HFL1 cell line, as shown using qPCR assays ($n=4$, $^{**}P<0.01$); **C** STAT2 was successfully knocked down in the BEAS-2B cell line, as shown using WB assays; **D** STAT2 was successfully knocked down in the HFL1 cell line, as shown using WB assays; **E** STAT2 knockdown could abolish the *E. coli* infection (1×105 CFU, 24 h)-mediated enhancement of the expression level of IFN-α in the BEAS-2B cell line ($n=4$, $^{**}P<0.01$); **F** STAT2 knockdown could abolish the *E. coli* infection (1×105 CFU, 24 h)-mediated enhancement of the expression level of IFN-β in the BEAS-2B cell line ($n=4$, $^{*}P<0.05$, $^{**}P<0.01$); **G** STAT2 knockdown could abolish the *E. coli* infection (1×105 CFU, 24 h)-mediated enhancement of the expression level of IFN-α in the HFL1 cell line ($n=4$, $^{**}P<0.01$); (H) STAT2 knockdown could abolish the *E. coli* infection (1×105 CFU, 24 h)-mediated enhancement of the expression level of IFN-β in the HFL1 cell line ($n=4$, $^{*}P<0.05$)

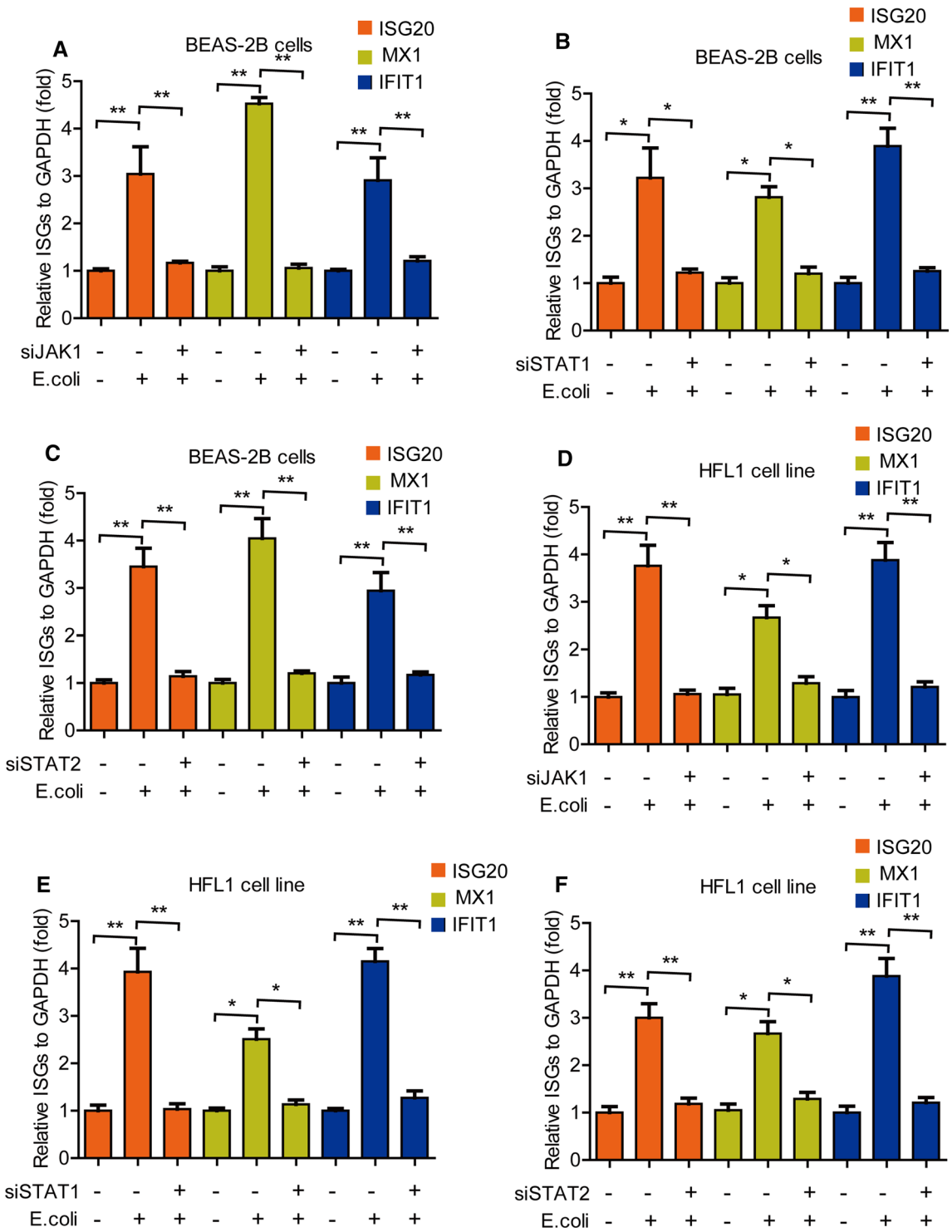


Fig. 7 Silencing JAK1, STAT1, and STAT2 abolished the *E. coli* infection-mediated promotion of the expression level of ISGs in lung cells. **A** JAK1 knockdown abolished the *E. coli*-mediated promotion of ISGs including ISG20, MX1, and IFIT1 in the BEAS-2B cell line ($n=4$, $^{**}P<0.01$) **B** STAT1 knockdown abolished the *E. coli*-mediated promotion of ISGs including ISG20, MX1, and IFIT1 in the BEAS-2B cell line ($n=4$, $^{*}P<0.05$, $^{**}P<0.01$); **C** STAT2 knockdown abolished the *E. coli*-mediated promotion of ISGs including ISG20, MX1, and IFIT1 in the BEAS-2B cell line ($n=4$, $^{**}P<0.01$); **D** the knockdown of JAK1 abolished the *E. coli*-mediated promotion (1×105 CFU, 24 h) of ISGs including ISG20, MX1, and IFIT1 in the HFL1 cell line ($n=4$, $^{*}P<0.05$, $^{**}P<0.01$); **E** the knockdown of STAT1 abolished the *E. coli*-mediated promotion (1×105 CFU, 24 h) of ISGs including ISG20, MX1, and IFIT1 in the HFL1 cell line ($n=4$, $^{*}P<0.05$, $^{**}P<0.01$); **F** the knockdown of STAT2 abolished the *E. coli*-mediated promotion (1×105 CFU, 24 h) of ISGs including ISG20, MX1, and IFIT1 in the HFL1 cell line ($n=4$, $^{*}P<0.05$, $^{**}P<0.01$)

The JAK/STAT pathway is one of the best understood signal transduction cascades to regulate innate immunity (Rawlings et al. 2004). JAK1 is a member of the JAK family, and its activation can phosphorylate the downstream major substrates STATs (Rawlings et al. 2004). JAK/STAT signaling directly translates an extracellular signal into a transcriptional response (Yan et al. 2018). The JAK/STAT pathway was reported to play an important role in inducing IFN-α and IFNγ (Kohlhuber et al. 1997). Interestingly, porcine reproductive and respiratory syndrome virus (PRRSV) infection facilitated type I IFN responses by targeting JAK1 (Zhang et al. 2016). Measles virus V protein was reported to inhibit JAK1-mediated phosphorylation of STAT1 to escape IFN-α/β signaling (Caignard et al. 2007). In the present study, we confirmed that *E. coli* infections significantly increased the expression of JAK1, STAT1, and STAT2 (Fig. 4), which led us to further investigate the role of JAK/STAT signaling in *E. coli*-induced IFN-α and IFN-β production. By silencing major JAK1, STAT1, and STAT2 genes involved in JAK/STAT signaling, we found that JAK/STAT signaling closely regulated *E. coli*-induced IFN-α and IFN-β production (Figs. 4, 5, and 6). Notably, knockdown of JAK1, STAT1, and STAT2 compromised the *E. coli* infection-mediated promotion of the expression of ISGs (Fig. 7). The aforementioned experiments strongly demonstrated the importance of JAK/STAT signaling in *E. coli*-induced IFN-α and IFN-β production. Similarly, previous studies have confirmed that mimicking *E. coli* infection using lipopolysaccharide (LPS) and poly I:C stimulation could remarkably promote the expression of STAT1 and STAT2 (Wu et al. 2017). Frendéus et al. found that *E. coli* infections potently induce the IFN-β response (Frendéus et al. 2001). Mancuso et al. also confirmed that several bacteria, including Group B *Streptococcus*, pneumococcus and *E. coli*, induced IFN-α and IFN-β, and type I IFN played an important role in protection from bacterial infection, since IFNAR$^{-/-}$ mice showed decreased survival and increased bacterial load (Mancuso et al. 2007). Thus, our study and others have confirmed that the IFN signaling pathway plays an essential role in *E. coli* infections.

In conclusion, we demonstrated that *E. coli* infections remarkably promoted IFN-α and IFN-β production in lung cells, which was closely regulated by the JAK/STAT signaling pathway. The findings in the present study are useful for further understanding the pathogenesis of *E. coli* infections in the lung and finding novel therapies to treat *E. coli*-induced lung injury.

Data availability statement

Raw data were generated at The First Affiliated Hospital of China Medical University. Derived data supporting the findings of this study are available from the corresponding author [Z.L.] on request.

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Author contributions YJ, JJ, ZJ, QC, and LG performed experiments; ZL designed the research; YJ and ZL wrote the manuscript; and ZL supervised the project.

Declarations

Conflict of interest The authors declare no competing financial interests.

Informed consent Not applicable.

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