Sodium Tanshinone IIA Sulfonate Promotes RSV Replication by Type I Interferon-Independent Mechanism in Vitro

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Research

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Abstract

Background

Respiratory syncytial virus (RSV) is a leading cause of viral respiratory tract infection in young children and elderly worldwide. Sodium tanshinone IIA sulfonate (STS) is reported to have antiviral effect on some viruses. However, the effect of STS on RSV replication is still unknown.

Methods

To investigate the effect of STS on RSV replication, RSV infected A549 cell model was first established and treated with different concentration of STS (12.5、25、50、100 and 200μg/ml).CPE, virus titer and RSV N protein were detected. Cytotoxicity of STS on A549 cells were performed. Then Different cell lines (16HBE and BEAS-2B) were also used to investigate the effect of STS on RSV replication. The Type I-IFN-associated protein (IFN-α, IFN-β and IP-10) were also detected by ELISA, and IFN-deficient Vero cells were also used to investigate the role of IFN response in STS-modulated RSV replication.

Results

Low concentration of STS potently promoted RSV replication in vitro by increasing virus titers and the expression of RSV N protein, and 25μg/ml STS reached the maximum effect. While high concentration of STS (200μg/ml) effectively inhibited virus replication but it may due to the effect of cytotoxicity. STS treatment (25μg/ml) also showed the same effect in 16HBE and BEAS-2B cells. STS treatment did not impaired Type I interferon (IFN) induction and also promoted RSV replication in IFN-deficient Vero cells.

Conclusion

STS promoted RSV replication in vitro by IFN-independent mechanism. Our results indicated that STS treatment should be avoided in clinical RSV-related diseases.

Introduction

Respiratory syncytial virus (RSV) is a leading cause of viral respiratory tract infection in young children and elderly people worldwide, causing more deaths than influenza each year. RSV infects nearly all children during the first 2 years of life[1, 2]. Severe RSV infection in childhood may be associated with recurrent wheezing and asthma[3]. The RSV genome contains 10 genes encoding 11 proteins. Helical nucleocapsids is the template for RSV polymerase transcription and replication, which are formed by viral RNA encapsidated by the nucleocapsid(N) protein [4]. The heightened viral load caused by the massive replication of RSV is closely related to the disease severity[5-7]. However, there is no available vaccine nor antiviral treatment against RSV[4, 5].Therefore, identification of drug that regulates viral replication may contribute to clinical management of disease.

Sodium tanshinone IIA sulfonate (STS) is a derivative of tanshinone IIA (Tan IIA), which is a main active lipophilic constitute extracted from the dried roots of traditional Chinese medicine Danshen (*Salvia miltiorrhiza Bge*)[8]. STS presents a variety of pharmacological activities, such as anti-inflammation, anti-oxidant, anti-apoptosis and anti-coagulation et al. It has been approved and widely used for treatment of cardiovascular diseases[8-11]. Respiratory viral infections may cause to thrombocytosis and even coagulation dysfunction in children, and the most common virus is RSV[12-14].Moreover, RSV is also considered to be an important cause of lower respiratory tract infection in the elderly and patients with underlying chronic cardiopulmonary diseases[2, 15], and patients with cardiopulmonary disease had higher utilization of healthcare for RSV-related diseases and worse outcomes[2]. Moreover，adults hospitalized due to RSV infection is complicated by cardiovascular events in 14-22%, including acute coronary syndrome, worsening congestive heart failure, and arrhythmias[2].Therefore, STS is also used clinically in adults and children with RSV-related diseases.

In recent years, STS was reported to have the antiviral activity for viruses such as Marek's disease virus (MDV)[16-19], Porcine reproductive and respiratory syndrome virus (PRRSV)[20-22]. However, no studies have focused on the effects of STS on RSV replication so far. Type I interferon (IFN) response is the critical pathway that defense cells against virus infection by inducing the expression of hundreds of IFN-stimulated genes(ISGs) such as interferon gamma-induced protein，IP-10，[23, 24]. IFN in the response to RSV infection is related to viral load，rate of virus clearance and the severity of disease[5-7].

Thus，we aimed in the study to investigate the effect of STS on RSV replication in vitro and the relationship between the IFN response. We first studied the antiviral activity of different concentration of STS by applying it to RSV infected A549 cells. We found that low concentration of STS promoted RSV replication mainly at the late stage of infection. Then we demonstrated the same effect of STS in other cell lines such as 16HBE and BEAS-2B. Finally, we provided evidence that STS promoted RSV replication in an IFN-independent mechanism.

Materials And Methods

Virus and cells

Cell lines of 16HBE (human bronchial epithelial cells), BEAS-2B (human bronchial epithelial transformed cell) and Vero cells (African green monkey kidney cells) were obtained from ATCC. HEp-2 (laryngeal squamous cell carcinoma cells) and A549 (lung adenocarcinoma cells) cells were obtained from Prof. Enmei Liu，Children's Hospital of Chongqing Medical University，. The cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (AusGeneX).

All RSV infections were carried out by RSV A2 strain, which was obtained from Prof. Enmei Liu，Children's Hospital of Chongqing Medical University，. The RSV A2 strain was prepared in HEp-2 cells as previously described [7]. Briefly，HEp-2 cells inoculated with RSV A2 were incubated with DMEM containing 2% FBS at 37 °C (5% CO2) until the cells exhibited >90% cytopathic effect (CPE). The cultures were then frozen and thawed, and the cell lysates containing viral particles were collected and centrifuged at 4 °C，at 12000

rpm for 10 minutes to remove the cell debris. The viral stocks were titrated by agarose plaque assay, and then stored at -80℃ in small aliquots.

STS treatment

In STS treatment assays, virus at a multiplicity of infection (MOI) of 0.01 was added to 80% confluent cells and left to adhere at 37℃ for 2h，followed by washing twice with PBS to remove unabsorbed virus. Then the cells were cultured in DMEM supplemented with different concentration of sodium tanshinone IIA sulfonate (STS, MedChem Express, New Jersey, USA) (12.5，25，50，100 and 200μg/ml or only 25μg/ml) [17, 19, 21] and 2% FBS, cells or supernatants samples were collected at 12h, 24h, 36h,48h or 60h post RSV infection. In STS efficiency assay (percent of inhibition or promotion), virus infection was similar, except the cells were covered with the mixture consisted with 1% agar and 2×DMEM supplemented with 5% FBS containing different concentration of STS (0-200μg/ml). STS efficiency was defined as the ratio of plaques formed on treated versus untreated cells[25].

Virus titrations

Supernatants of RSV infected cells were collected, and tittered by plaque assay on HEp-2 monolayers with neutral red staining. Viral titers were calculated as plaque-forming units (PFU) as previously described[25].

Cell Viability tests

Cell viability testing was done by using a Cell Counting Kit-8 (CCK8) according to the manufacturer's instructions. Briefly, 1×10^5 A549 cells were seeded in 96-well plates. They were left to adhere overnight and then treated with STS (0-200μg/ml) for 48 h, then the supernatants were removed and cells were washed with PBS for three times. CCK8 reagent was added to the wells for 1 h at 37 °C. Then the OD values were measured at 450nm with a microplate reader and cell viability was calculated.

Immunofluorescence tests

Cells were grown in 24-well plates to about 80% confluence, then infected with RSV and treated with STS as described above. The infected cells were washed three times with PBS and fixed in 4% formaldehyde for 20 min, followed by 1% BSA (Solarbio, Beijing, China) with 0.3% Triton X-100 (Solarbio, Beijing, China) in PBS treatment for 30min. Cells were then stained with a mouse monoclonal antibody against RSV N (1:200, mouse; Abcam, Cambridge, UK) in 1% BSA at 37℃ for 2h. Cells were washed with PBS for three times, then FITC 488-conjugated goat anti-mouse secondary antibody (1:300; Bioss, China) were incubated at 37℃ for 1h. Images were obtained using a Olympus inverted immunofluorescence microscope.

ELISA analysis

The levels of IFN-α, IFN-β and IP-10 in supernatants of infected cells were measured with commercial ELISA kits of IFN-α, IFN-β and IP-10 (Neobioscience, Wuhan, China) according to the manufacturer's instructions.

Western blots analysis

Cells in 6-well plates were washed twice with PBS, then protein was extracted using a total protein extraction kit (KeyGEN, Nanjing, China). After protein quantification using a BCA assay reagent (Solarbio, Beijing, China), equal amounts of protein (60 μg) were separated on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA). The membranes were blocked and then incubated with a mouse antibody against RSV N (1:1000, mouse; Abcam, Cambridge, UK) or GAPDH (1:5,000, rabbit; Bioss, China) at 4°C overnight. Then, an HRP-conjugated goat anti-mouse secondary antibody (1:5,000; Bioss, China) or a goat anti-rabbit secondary antibody (1:5,000; Bioss, China) were used to detect the presence of the respective protein bands. Signals were quantified using the Quantity One software (Bio-Rad, Hercules, CA) and normalized relative to GAPDH.

Statistical Analysis

The GraphPad Prism 5.0 software was used for data analysis. All results are expressed as the mean ± SEM unless otherwise stated. Statistical significance was analyzed by a student's t-test and ANOVA. Differences were considered significant at $P < 0.05$.

Ethics statement

The study was approved by the Ethics Committees of the China Guizhou Provincial People's Hospital (Approve number 2018058).

Results

STS affects CPE and virus titer in RSV-infected A549 cells

In order to investigate the impact of STS on RSV replication and to screen the best concentration of STS, RSV infected A549 cell model was established (MOI=0.01). After adsorption for 2h, the cells were washed twice with PBS, followed by treatment with different concentration of STS (12.5、25、50、100 and 200μg/ml) in DMEM with 2% FBS. Then the cultures were left to grow for 48h and obvious CPE can be observed. RSV infection of confluent A549 monolayers produced plaques of about 1.5-2.5 mm in diameter after 48h of incubation. STS treatment significantly induced the numbers and size of plaques when the concentration was lower than 100μg/ml, and the 25μg/ml STS gave a maximum effect. However, both CPE and the numbers and size of plaques were obviously suppressed when STS was at the concentration of 200μg/ml (Table 1). To further determine the virus titers, the supernatants from 25 and 200μg/ml of STS-treated cells were tested. The virus recovered from only RSV infected cells titered at 7.7*10^6 PFU/ml. However, the titers reached 3.7 *10^8 PFU/ml when treated with 25μg/ml STS (p 0.05

Figure 1). While treated with 200μg/ml of STS significantly suppressed the virus yield and titered at only 1.3*10^3 PFU/ml (p 0.001Figure 1). The results indicated that the low concentration of STS effectively promoted RSV replication in A549 cells while the high concentration of STS (200μg/ml) potently suppressed virus replication.

STS affects the expression of RSV N protein in A549 cells

To investigate the effect of different STS on the expression of RSV N protein, the RSV N protein was detected by Western Blot at 48h post RSV infection. The results demonstrated that the levels of RSV N protein were significantly increased when the concentration of STS was lower than 100μg/ml (p 0.05-0.001 Figure 2A), and the 25μg/ml STS gave a maximum effect (p 0.001Figure 2A). However, RSV N protein was greatly suppressed when STS was at the concentration of 200μg/ml (p 0.05Figure 2A). The immunofluorescence staining further demonstrated that the expression of RSV N protein was significantly increased and the virus particles were released diffusely to form more infected cells when STS was at the concentration of 25μg/ml compared with RSV control group. While RSV N protein was clearly decreased and only limited infected cells were found when treated with 200μg/ml STS (Figure 2B). The results indicated that STS affected the express of RSV N protein, which were consistent with the increase of CPE and virus titer. Therefore, the expression of RSV N protein may represent the virus titer.

Cytotoxicity of STS on A549 cells

We identified that 200μg/ml STS significantly suppressed RSV replication. To examine whether STS had cytotoxicity effect on A549 cells, different concentration of STS (12.525501002000 and 200μg/ml) were added into the culture medium for 48 hours. As revealed in the CCK8 test, cell growth was not affected when the concentration of STS was lower than 50μg/ml. However, high concentration of STS (100 and 200μg/ml) treatment significantly showed cytotoxicity on the growth of A549 cells (p 0.01-0.001, Figure 3A) and the effect of 200μg/ml STS is more obvious (p0.001, Figure 3A). Moreover, it was observed that 200μg/ml STS caused an increase in cell size (Figure 3B). Therefore, we supposed that the antiviral effect of high concentration of STS was most probably due to the obvious cytotoxicity. Thus, 25μg/ml STS was selected for the further study due to no cytotoxicity and had the best effect in promoting RSV replication.

Kinetics of STS-regulated RSV replication in A549 cells

Then Western Blot and indirect immunofluorescence staining were used to analyze the effect of 25μg/ml STS on the expression of RSV N protein at 24-48 h post RSV infection. As shown in Western Blot, there was almost no detectable band (N protein) at 24h, and STS didn’t significantly increase the expression of RSV N protein at 36 h. However, the expression of RSV N protein was obviously increased at 48 h post RSV infection (p 0.001Figure 4A). Immunofluorescence staining also showed only limited infected cells were found in both groups at 24 and 36h. However, STS significantly increased the expression of RSV N protein and more infected cells and virus particles were found at 48h post RSV infection (Figure 4B).

Therefore, the promotion effect of STS on RSV replication in A549 cells is mainly at the late stage of infection.

STS promotes RSV replication in different cell lines

Due to A549 is a tumor-derived airway epithelial cell, we then investigate whether STS also had the same effect in other normal airway-derived cell lines that commonly used in RSV studies, such as 16HBE and BEAS-2B. As the results shown, at 60h post RSV infection in both groups, STS treatment significantly induced more obvious CPE both in 16HBE (Figure 4A) and BEAS-2B cells (Figure 4D). Western blot demonstrated increased expression of RSV N protein in both16HBE (p 0.001, Figure 4B) and BEAS-2B cells (p 0.05, Figure 4E). Immunofluorescence staining also showed the consistent results and more infected cells both in 16HBE (Figure 4C) and BEAS-2B cells (Figure 4F). Thus, the effect of promotion RSV replication by STS in vitro was independent of epithelial cell types.

The effect of promotion RSV replication of STS is IFN-independent

Innate antiviral responses characterized by the induction of IFN-α, IFN-β is the critical first line of defense against virus infection[23, 24]. We asked whether the promotion of RSV replication by STS is due to the suppression of IFN signaling. To answer this question, 25μg/ml STS treated supernatants of 12-48h post RSV infection were collected, and IFN-α IFN -β and IP-10 were examined by ELISA. Results showed that although STS treatment increased the level of both IFN-α (p 0.05Figure 6 A) and IFN -β (p 0.01Figure 6 B) in supernatants at 48h post RSV infection, which were opposite to our hypothesis, though IP-10, the critical ISG induced by IFN, was reduced due to STS treatment (p 0.05-0.01Figure 6 C), suggested that STS treatment did not inhibit the induction of IFN. We further infected Vero cells that are naturally lacking type I-IFN genes[26]. Results showed at 60h post RSV infection, STS treatment also significantly induced more obvious CPE in Vero cells (Figure 6D), Western blot demonstrated increased expression of RSV N protein (p 0.01, Figure 6E) and immunofluorescence staining showed the consistent results and more infected cells (Figure 6F).The above results clearly demonstrated that the promotion effect of STS on RSV replication is not due to impaired IFN response.

Discussion

In this current study, we confirmed that STS affected RSV replication in vitro. Low concentration of STS potently promoted RSV replication in A549 cells mainly at the late stage of infection, while high concentration of STS effectively inhibited virus replication. However, high concentration of STS was detrimental to cell growth, while low concentration of STS had no cytotoxicity. Low concentration of STS showed the same effect in other cell lines such as 16HBE BEAS-2B and even the IFN-deficient Vero cells. We demonstrated that STS promoted RSV replication in vitro by IFN-independent mechanism.

STS is one of the derivatives of tanshinone IIA (Tan IIA), which is a main lipophilic component of traditional Chinese medicine Danshen. Due to the multiple activities, there are several available drugs which contain STS or were developed from Danshen, including Tanshinone IIA Sulfonate injections [8].

These drugs have been widely used in China to treat various diseases, particularly cardiovascular disease and cerebral vascular diseases with few side effects [8]. STS is also used clinically in adults and children with RSV-related diseases, such as those with cardiovascular underlying diseases or complications in adults [2, 15], and those with thrombocytosis or coagulation dysfunction in children[12-14].

Many compounds present in Danshen have been showed to have potent antiviral activity against Enterovirus 71 infection[27]、Human Immunodeficiency Virus[28]、Hepatitis B virus[29], Human papilloma virus[30]. Sun N et al. reported that STS had the antiviral activity effect against MDV both *in vivo* and *in vitro*[16-19]. They found that STS at 31.25-250μg/ml could effectively inhibit the MDV replication in chicken embryo fibroblasts cells in a dose-dependent manner, mainly by inhibition the expression of several virus genes and proteins. Meanwhile, the same researchers also demonstrated the anti-PRRSV effect of STS by using Marc-145 cells and found 62.5 μg/ml STS showed the antiviral effect, and the potential mechanism may due to the anti-apoptosis and anti-autophagy[20-22].

In this study, by using RSV infected A549 cell model, we identified the effect of STS on RSV replication. But STS showed completely opposite effects on RSV replication between high and low concentrations. When treated with high concentration（200μg/ml）of STS, RSV infected A549 cells showed reduced CPE, decreased virus titers, decreased expression of N protein and limited infected cells, suggested that high concentration（200μg/ml）of RSV effectively inhibited RSV replication in A549 cells (Table1、Figures 1 and 2). When treated with low concentration（≤100μg/ml）of STS, especially with 25μg/ml, RSV infected A549 cells showed more obvious CPE, increased virus titers, increased expression of N protein and diffused release of virus particles and more infected cells (Table1、Figs 1 and 2), suggested that low concentration （≤100μg/ml）of RSV significantly promoted RSV replication in A549 cells. Moreover, we found that the expression of N protein was consistent with the CPE and virus titers, suggested that N protein may represent the viral replication, which was consistent with others results[31]. We demonstrated that high concentration of STS（100-200μg/ml）showed cytotoxicity to cell growth, which may be the reason for the antiviral effect of 200μg/ml STS. However, though 100μg/ml STS also showed certain cytotoxicity to cell growth, increased viral replication was still observed. So, we concluded that STS promoted RSV replication in general. Our results were different with that of Sun N et al. [16-22], implying that the effect of STS may differ amongst viruses, which need to be further studied.

We demonstrated that low concentration especially 25μg/ml of STS had no cytotoxicity and had the best effect in promoting RSV replication. Then 25μg/ml of STS was selected for further studies. Kinetics of RSV replication study showed the difference of viral replication was only observed in 48h post RSV infection, suggested STS promoted RSV replication possibly in the late stage of RSV infection.

Cell lines may alter some of the structural patterns of the yield virus, which may in turn affect virus infectivity[32-34]. Some receptors associated with viral infection may differ between tumor cells and well-differentiated ciliated epithelial cells[35]. Due to A549 is a tumor-derived airway epithelial cell, other normal airway-derived cell lines were used to further clarify the effect of STS on promotion RSV

replication. By using 16HBE and BEAS-2B cells, we demonstrated that the promotion effect of STS on RSV replication is independent of cell lines.

Antiviral response characterized by the induction of IFN-α, IFN-β and ISGs such as IP-10 are critical in inhibition viral replication [23, 24], and are known in related to viral load and disease severity in RSV infection[5-7]. However, we found that STS treatment oppositely increased the induction of both IFN-α, IFN-β at 48 hours in response to RSV. However, IP-10, which directly acts as an antiviral ISG was not increased, suggested STS did not impaired IFN induction in response to RSV. STS treated IFN-deficient Vero cells also showed more obvious CPE, increased expression of RSV N protein, further suggested that the promotion effect of STS on RSV replication was IFN-independent.

Conclusions

This study is the first report to demonstrate that low concentration of STS promoted RSV replication, which was cell lines-independent and IFN- independent. Treatment with STS in RSV infection substantially promoted RSV replication. Our results suggested that STS should be avoided in clinical RSV-related diseases.

Abbreviations

RSV: Respiratory syncytial virus; STS: Sodium tanshinone IIA sulfonate;

IFN: Interferon; MDV: Marek's disease virus; PRRSV: Porcine reproductive and respiratory syndrome virus; ISGs: IFN-stimulated genes;IP-10:Interferon gamma-induced protein;16HBE: Human bronchial epithelial cells; BEAS-2B:Human bronchial epithelial transformed cell;HEp-2: laryngeal squamous cell carcinoma cells;A549: Lung adenocarcinoma cells; DMEM: Dulbecco's modified Eagle medium; FBS: Fetal bovine serum; MOI：Multiplicity of infection；CPE: Cytopathic effect; PFU: Plaque-forming units; CCK8: Cell Counting Kit-8

Declarations

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Authors' contributions

ZY,XJ,YL,YL,LS and LW performed experiments and analyses. FY,LF,EL

and YC conceived the study, designed and analysed experiments. ZY and XJ wrote the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. STS affects RSV induced CPE in A549 cells

STS(μg/ml)	Plaque increment or reduction (%) a	Relative plaque size b
200	-78.57	±
100	33.03	++
50	81.25	+++
25	103.57	++++
12.5	83.93	+++
0	0	+

STS efficiency was defined as the ratio of plaques formed on treated versus untreated cells.

STS: sodium tanshinone IIA sulfonate

a Values represent the average of two experiments, each done with n=4.

b Plaques in the present of STS averaged about 2 mm in diameter. ±,+,++,+++,++++ (describe what each stands for .)

Figures

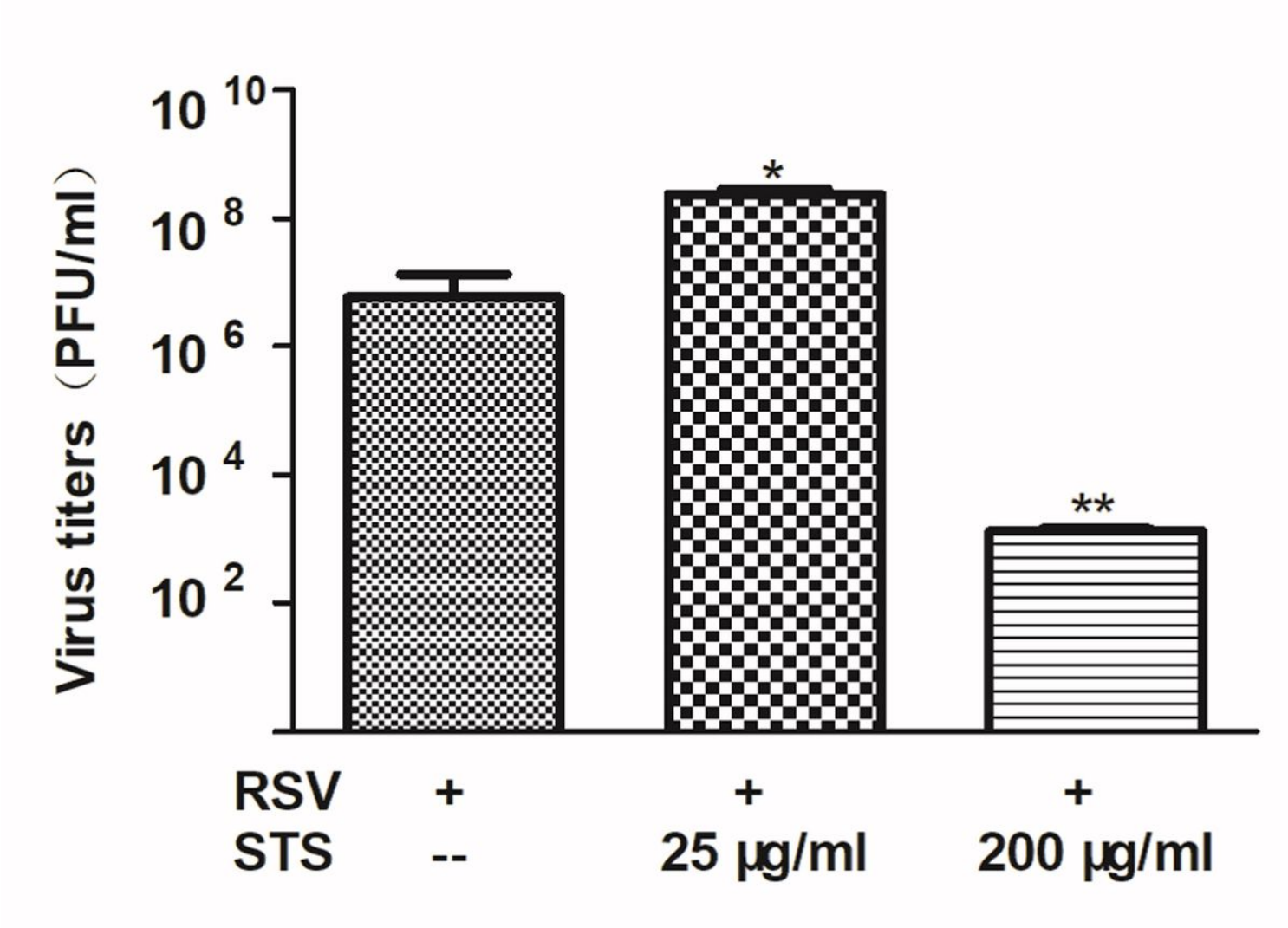
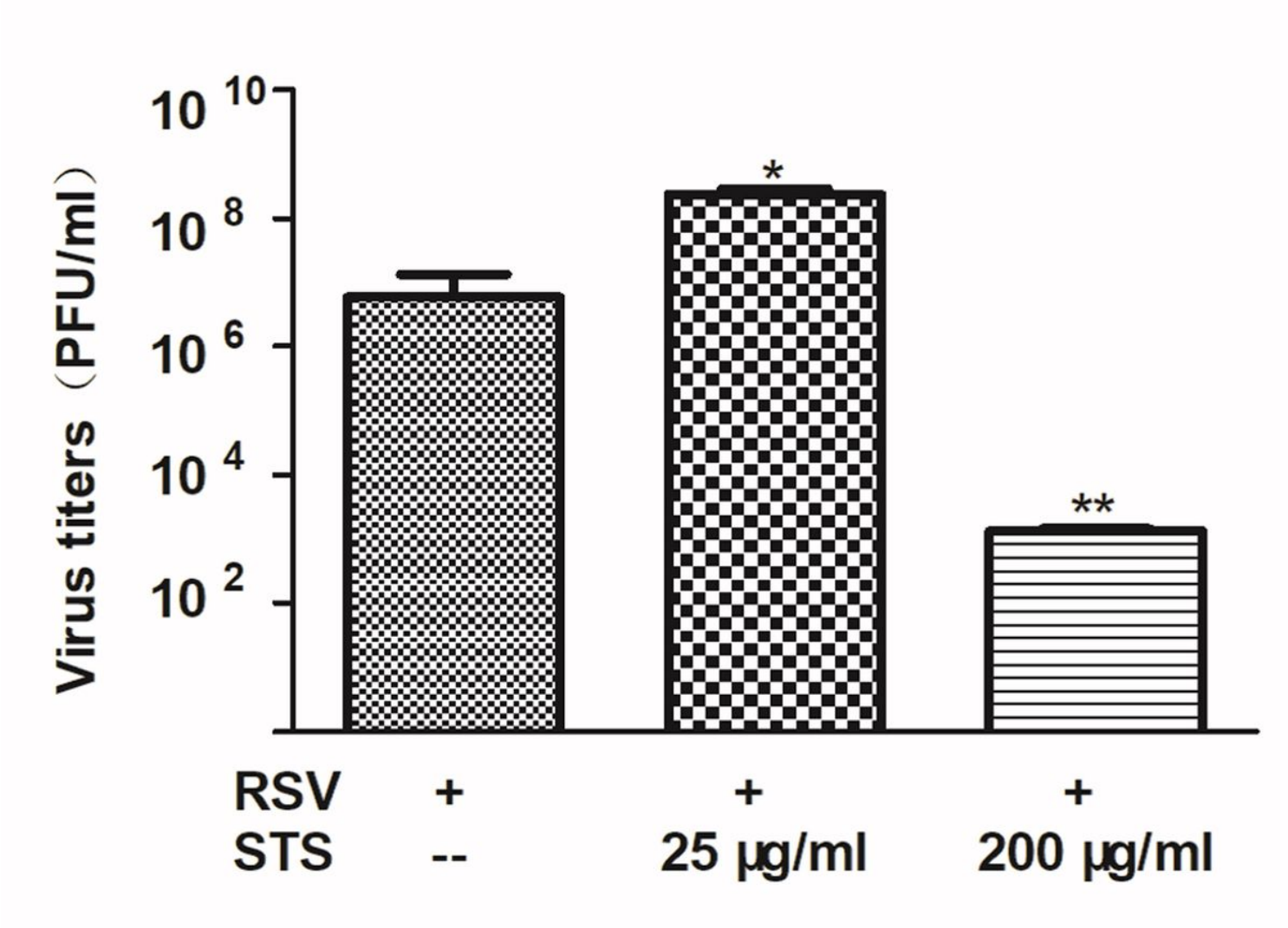


Figure 1

STS treatment affects viral titers post RSV infection in A549 cells. Supernatants from 25 and 200μg/ml of STS-treated cells were titered by plaque assay. high concentration（200μg/ml）of STS significantly decreased virus titers (p 0.01), while low concentration（25μg/ml） of STS effectively increased virus titers (p 0.05). STS: sodium tanshinone IIA sulfonate. The results represent twice independent experiments (n=4), and all data are presented as the mean ± SEM. (*) $p < 0.05$, (**) $p < 0.01$.



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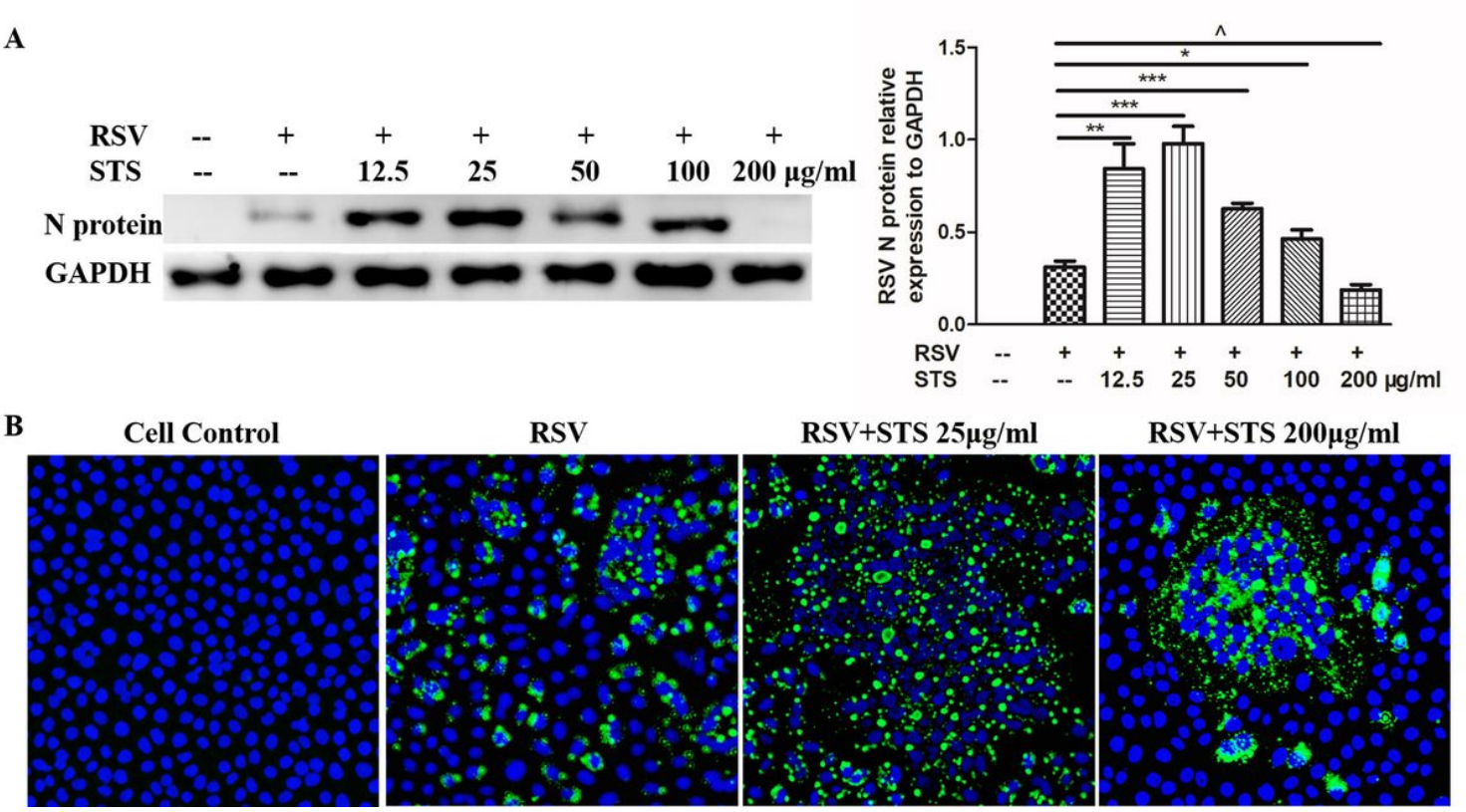
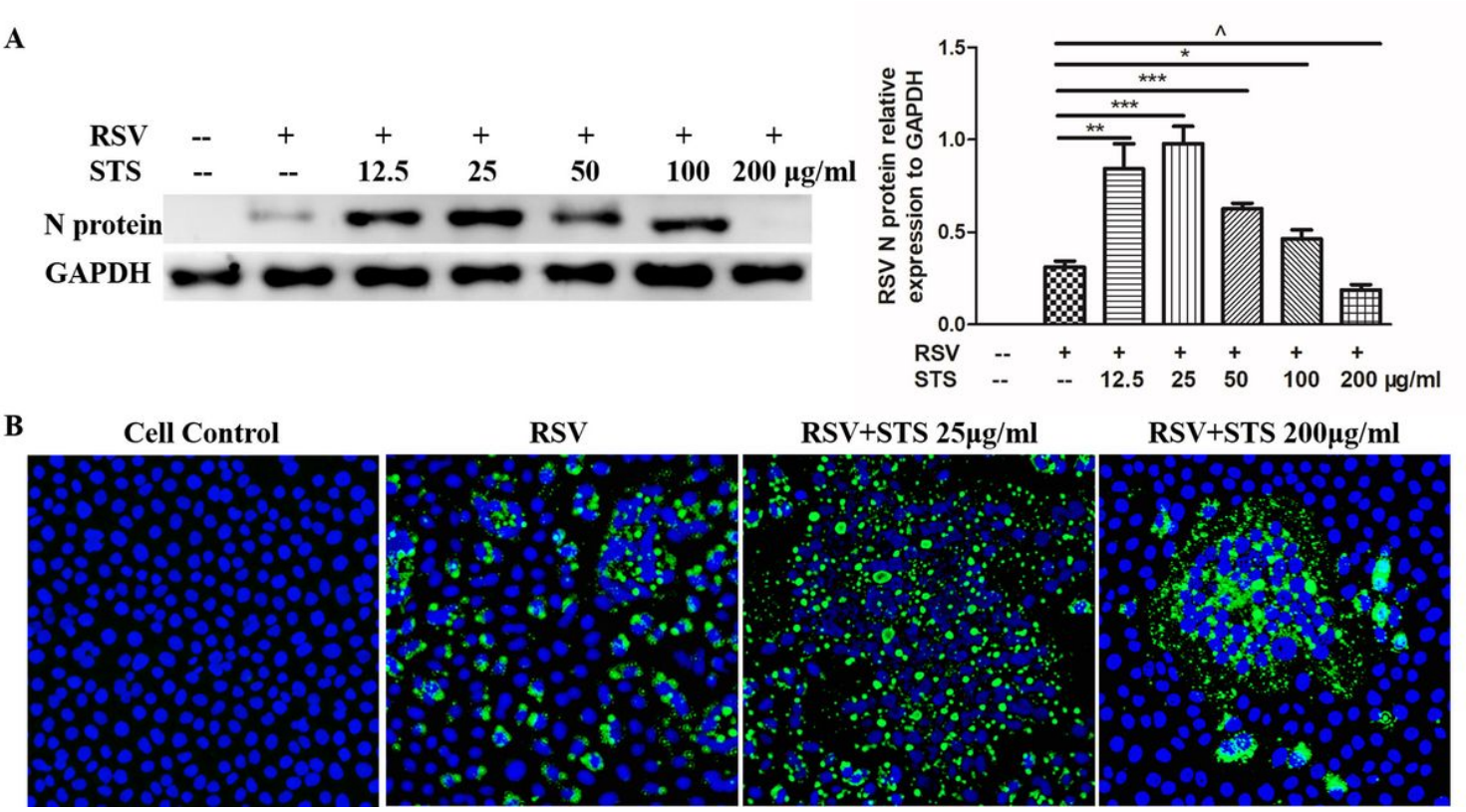


Figure 2

STS affects the expression of RSV N protein in A549 cells. RSV N protein was detected by Western Blot and immunofluorescence staining at 48h post RSV infection. (A) RSV N protein examined by Western Blot. (B) RSV N protein examined by Immunofluorescence staining. bar=75 μm. The results represent three independent experiments (n=4), and all data are presented as the mean ± SEM. (*) $p < 0.05$, (^) $p < 0.05$, (**) $p < 0.01$, and (***) $p < 0.001$.



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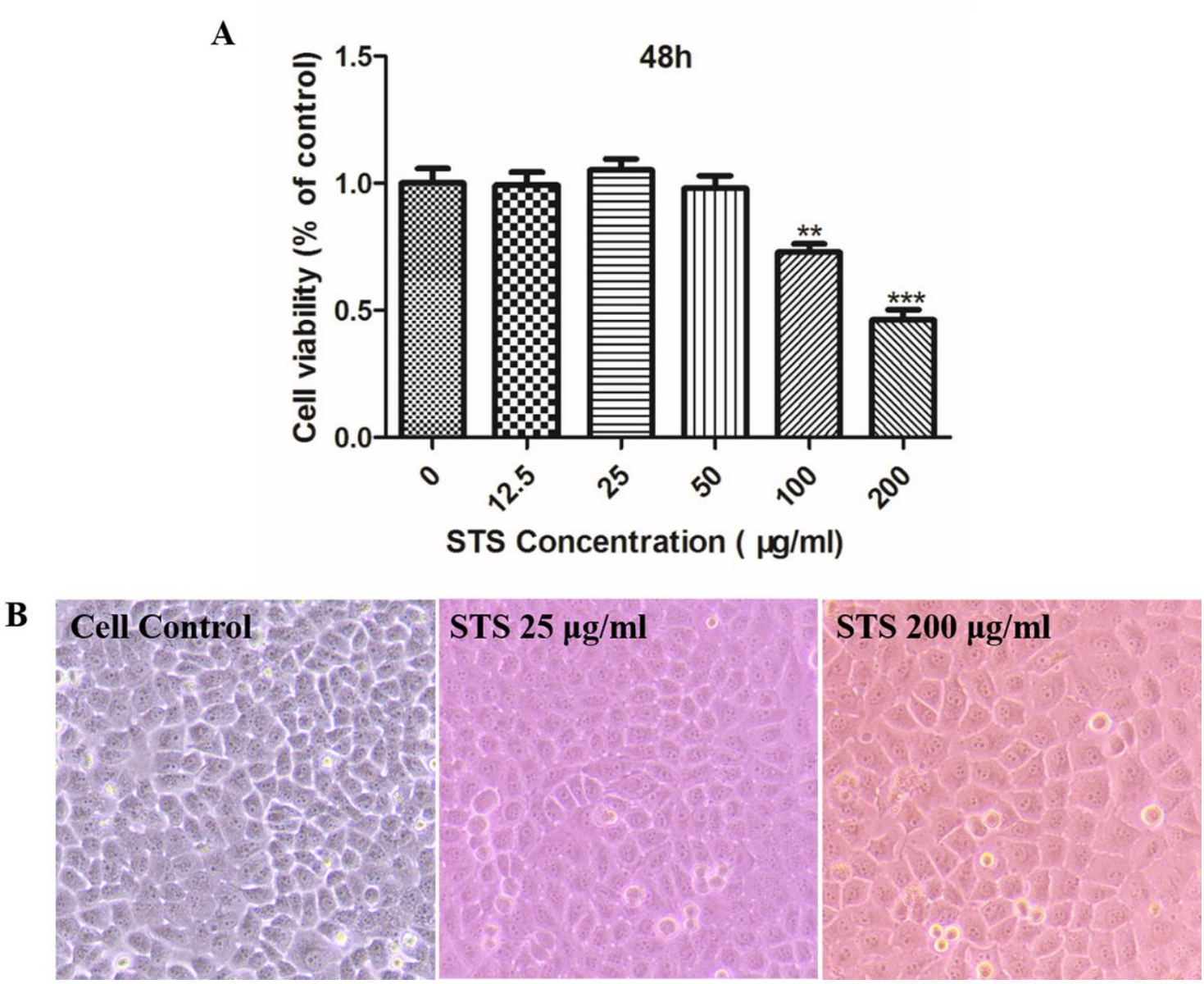
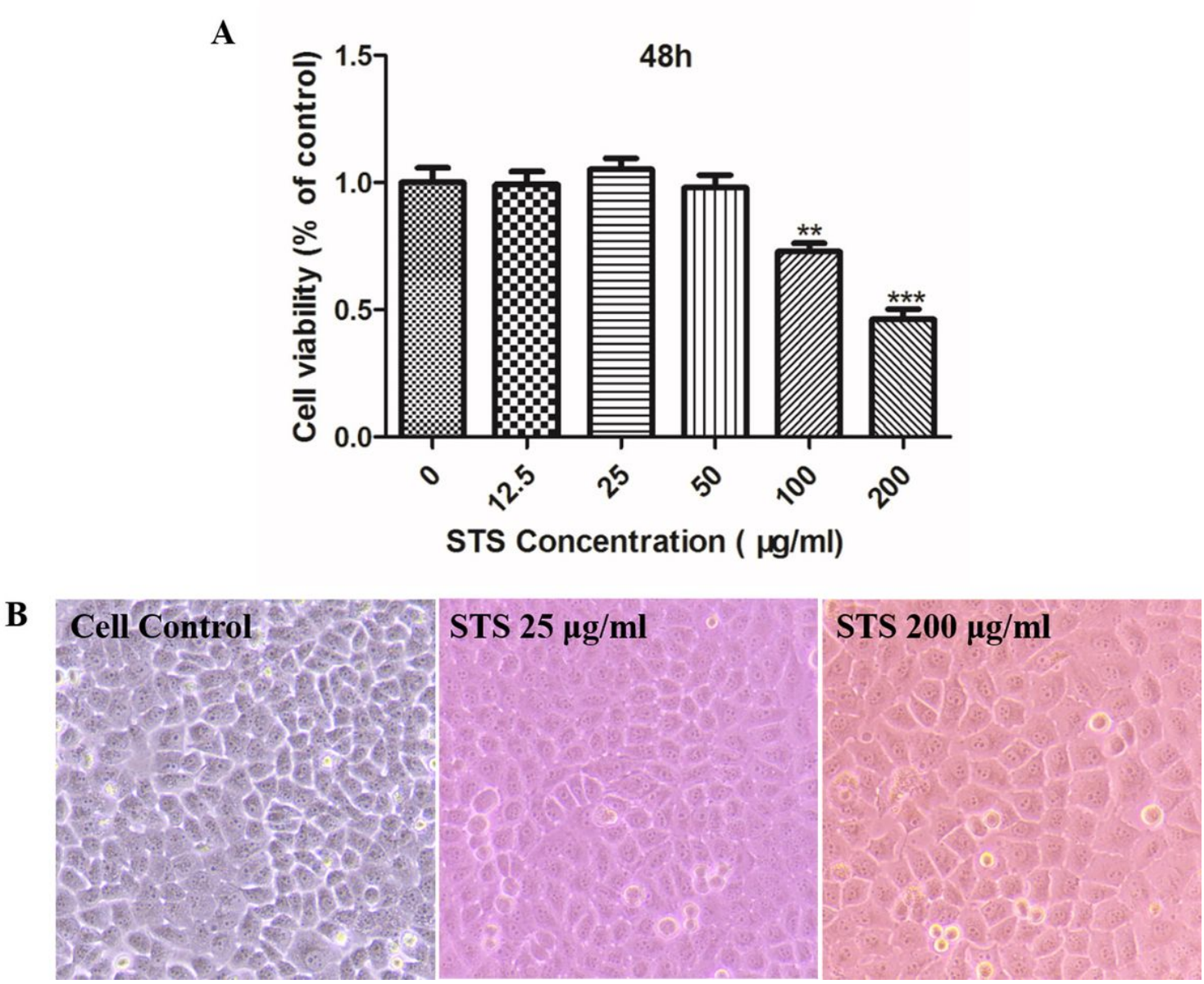


Figure 3

Cytotoxicity of STS on A549 cells. Different concentration of STS (0-200μg/ml) were added into the medium of A549 cells for 48 hours. CCK8 test was performed to investigate the cytotoxicity and morphology of the cells was observed by microscope. (A) CCK8 test showed high concentration of STS (100 and 200μg/ml) treatment significantly showed cytotoxicity and the effect of 200μg/ml STS is more obvious. (B) 200μg/ml STS treatment caused an increase in cell size. bar=150 μm. The results represent three independent experiments (n=6), and all data are presented as the mean ± SEM. (**) $p < 0.01$, and (***) $p < 0.001$.



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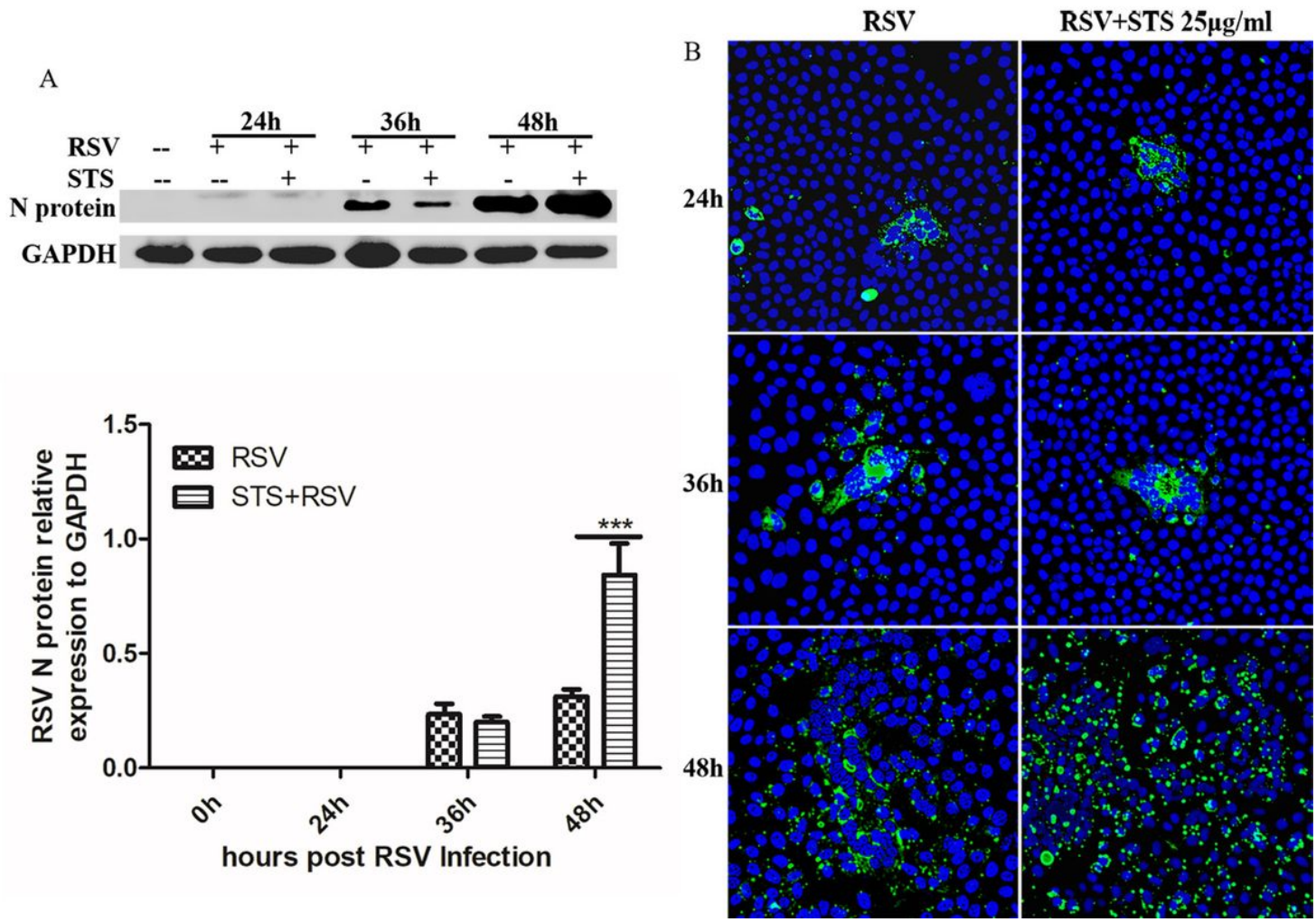
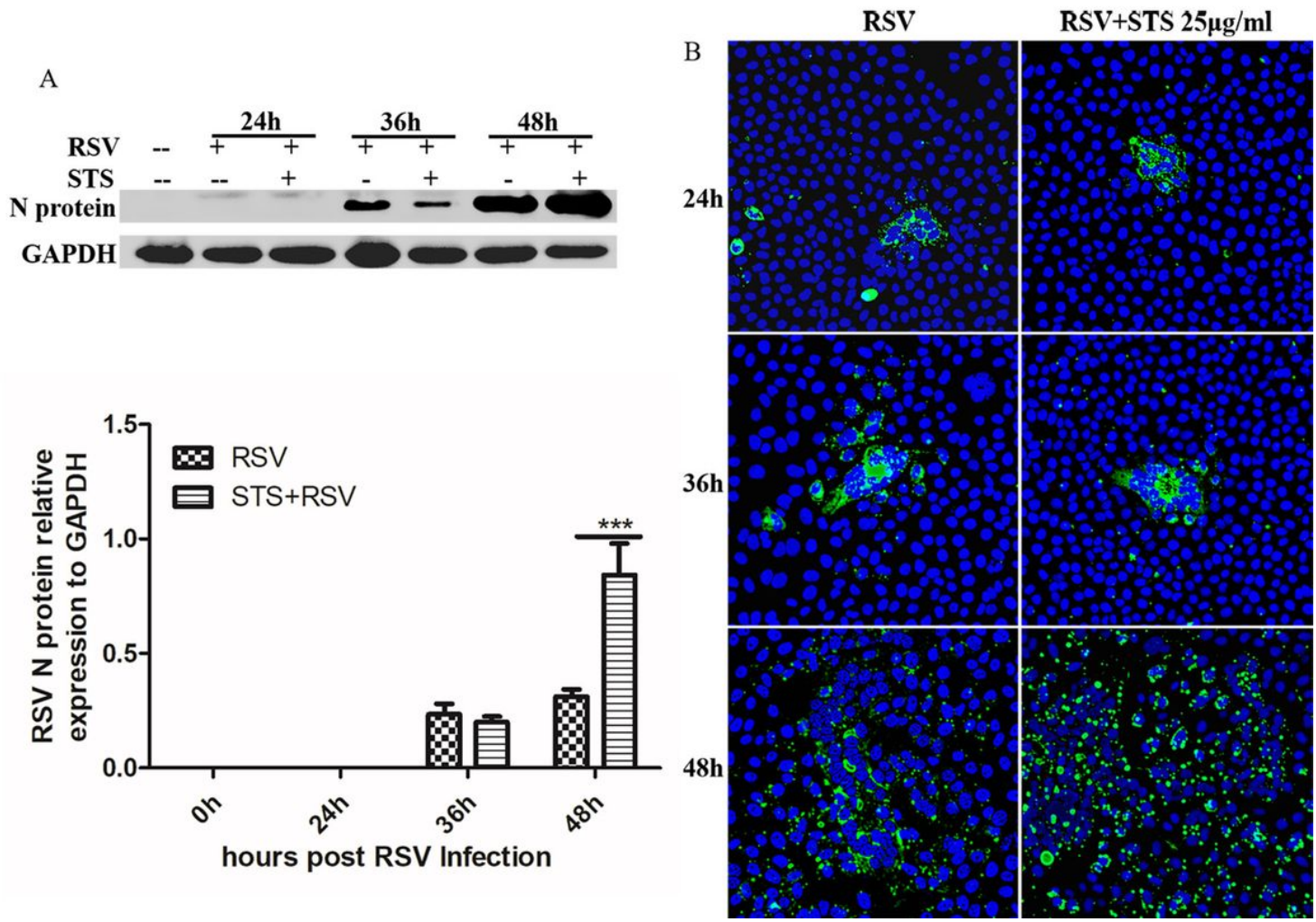


Figure 4

Kinetics of STS-regulated RSV replication in A549 cells. Western Blot and immunofluorescence staining were used to analyze the effect of 25μg/ml STS on the expression of RSV N protein at 24-48 h post RSV infection. (A) RSV N protein examined by Western Blot.□B□RSV N protein examined by Immunofluorescence staining. bar=75 μm. The results are representative of three independent experiments (n=4), and all data are presented as the mean ± SEM. (***) $p < 0.001$.



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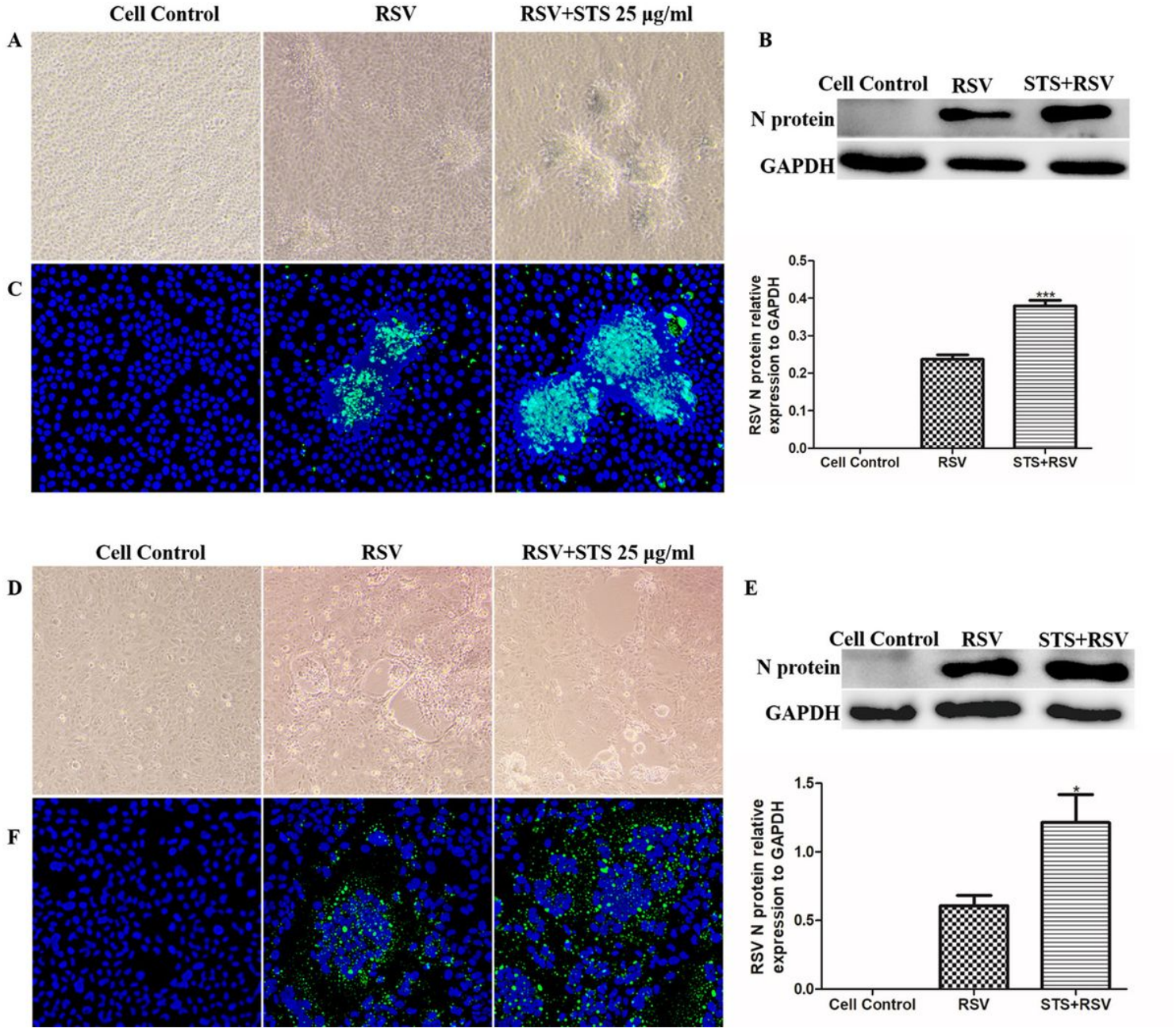
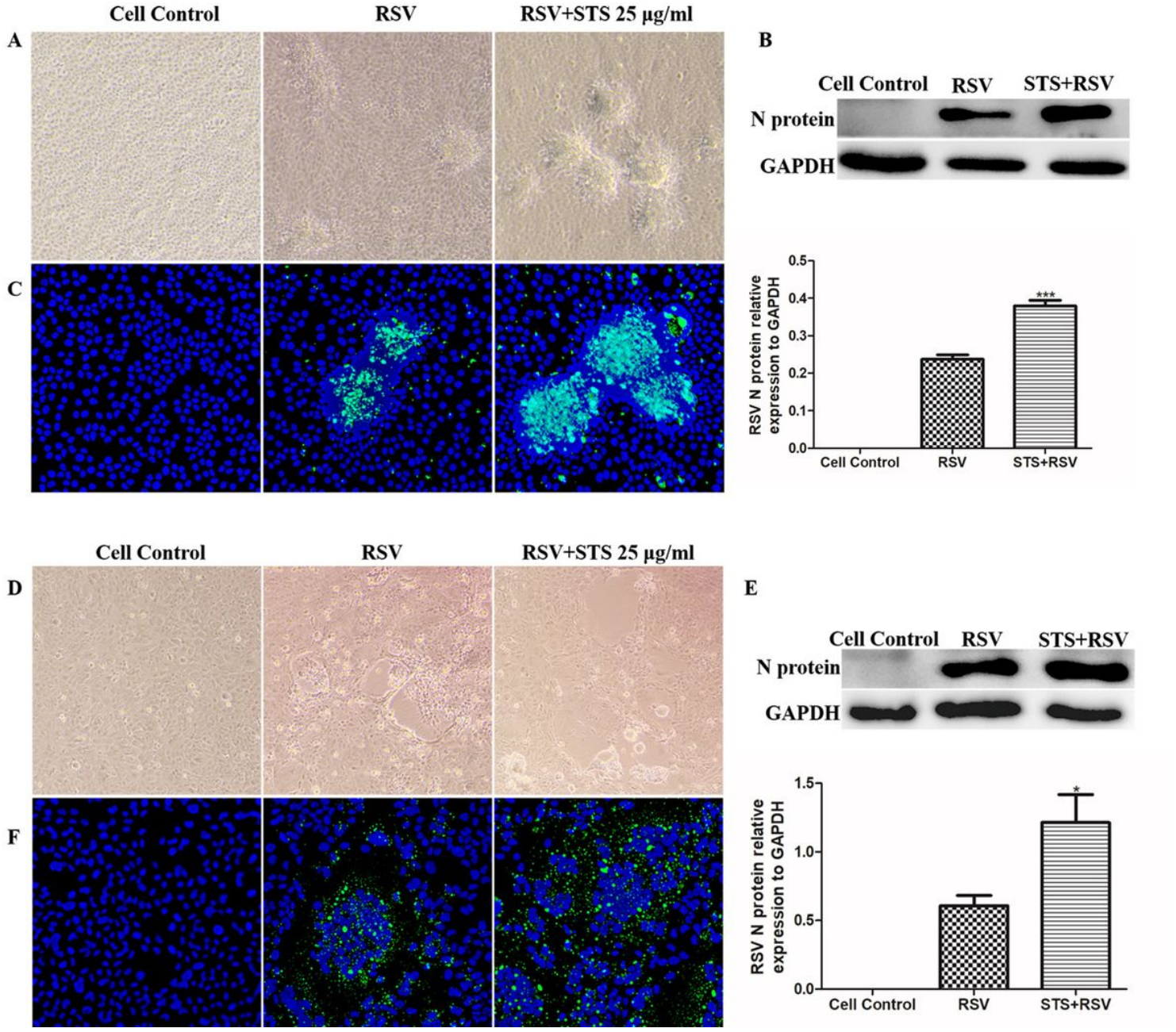


Figure 5

STS promotes RSV replication in different cell lines. 16HBE and BEAS-2B were used to investigate the effect of 25μg/ml STS. CPE was observed and RSV N protein was detected by Western Blot and immunofluorescence staining at 60h post RSV infection. (A) CPE in 16HBE. bar=25 μm. (B) RSV N protein in 16HBE detected by Western Blot. (C) RSV N protein in 16HBE detected by Immunofluorescence staining. bar=75 μm. (D) CPE in BEAS-2B. bar=25 μm. (E) RSV N protein in BEAS-2B detected by Western Blot. (F) RSV N protein in BEAS-2B detected by Immunofluorescence staining. bar=75 μm. The results are representative of three independent experiments (n=4), and all data are presented as the mean ± SEM. (*) $p < 0.05$, and (***) $p < 0.001$.



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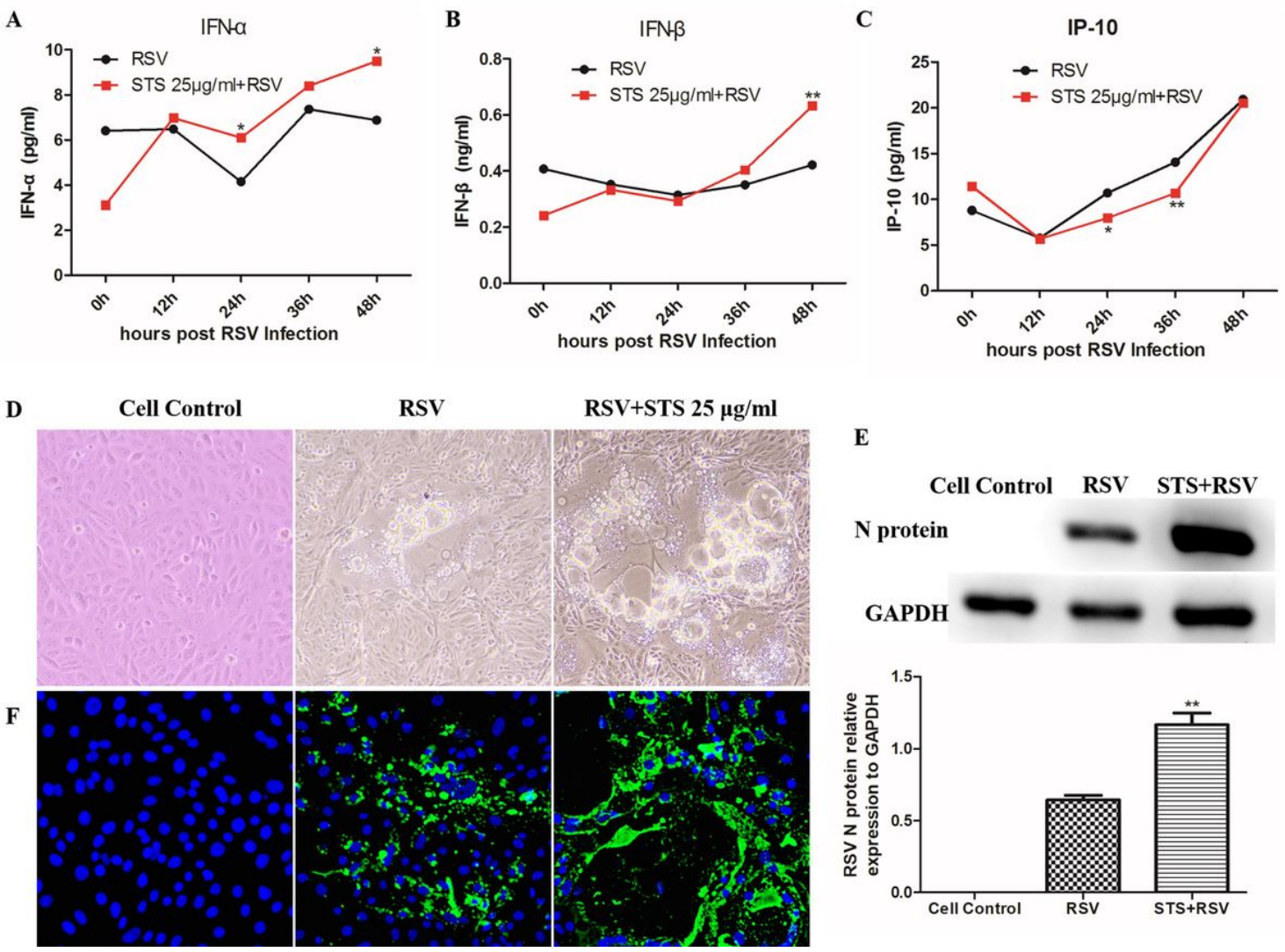
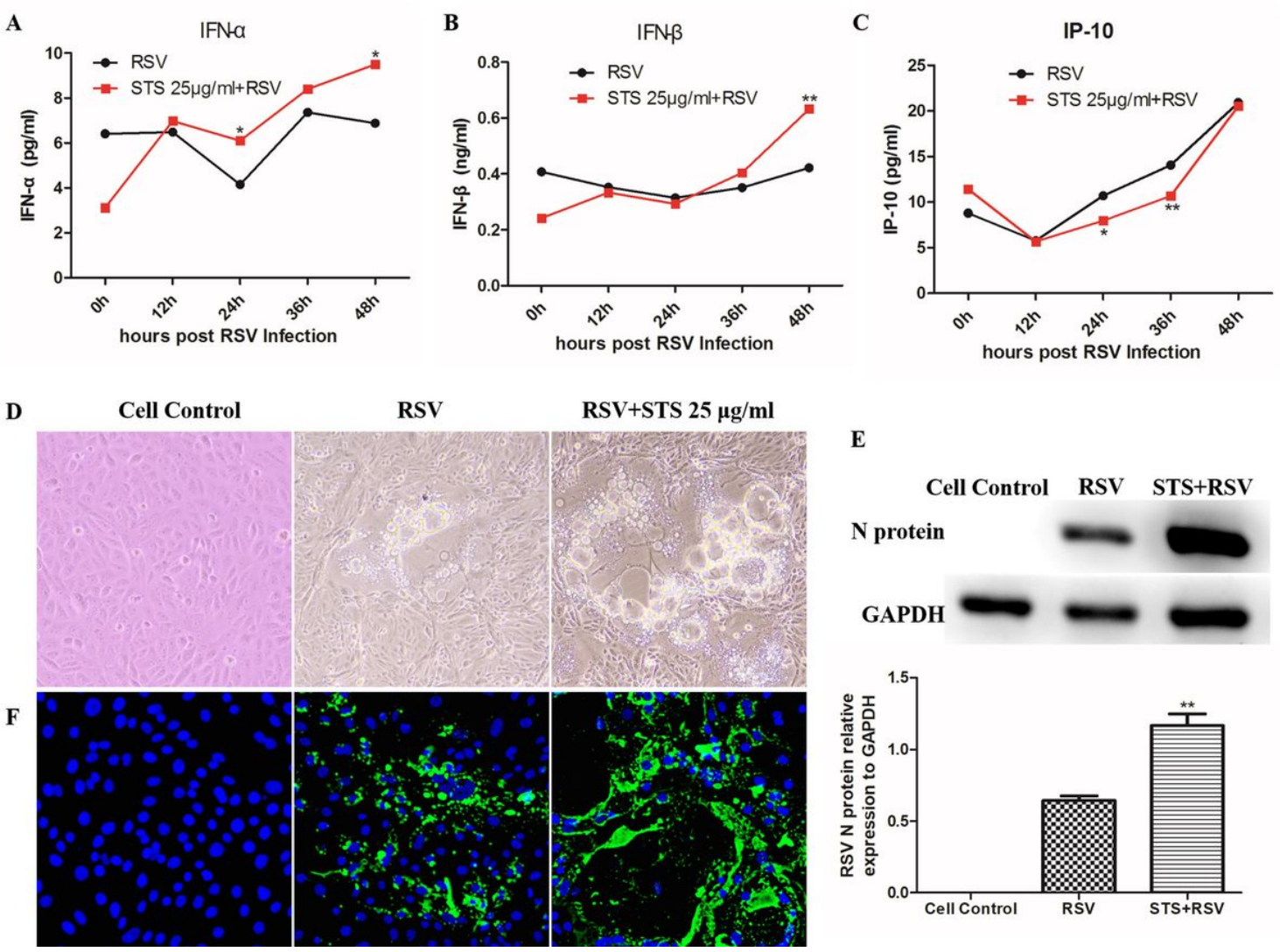


Figure 6

The effect of promotion RSV replication of STS is IFN-independent. IFN-α、IFN -β and IP-10 from supernatants of 25μg/ml STS treated cells (12-48h post RSV infection) were examined by ELISA and Vero cells were used to investigate the effect of 25μg/ml STS. CPE was observed and RSV N protein was detected by Western Blot and immunofluorescence staining at 60h post RSV infection. (A) IFN-α. (B) IFN-β. (C) IP-10. (D) CPE in Vero cells. bar=25 μm. (E) RSV N protein in Vero cells detected by Western Blot. (F) RSV N protein in Vero cells detected by Immunofluorescence staining. bar=75 μm. The results are representative of three independent experiments (n=4), and all data are presented as the mean ± SEM. (*) $p < 0.05$, and (**) $p < 0.01$.



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