Conserved Function of Bat IRF7 in Activating Antiviral Innate Immunity Insights into the Innate Immune Response in Bats

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Abstract

Bats are natural hosts for various highly pathogenic viruses, these viruses pose a huge threat to humans and animals. Yet they rarely display signs of disease infection from these viruses. The expression of IRF7-induced IFN-β plays a crucial role in against viral infections. However, the role of batIRF7 during viral infection remains unclear. In this study, we cloned the Tadarida brasiliensis IRF7 and discovered that its amino acid sequence showed poor conservation among species. Next, we investigated the expression of bat IRF7 mRNA in TB1 Lu cells infected with RNA viruses such as Newcastle disease virus (NDV), avian influenza virus (AIV), vesicular stomatitis virus (VSV), and double-stranded RNA (dsRNA) analogs poly (I:C) and demonstrated that these viral infections significantly upregulated the expression of bat IRF7 mRNA. Furthermore, the overexpression of IRF7 in TB1 Lu cells activated the expression of bat innate immune-related genes and inhibited virus replication. Importantly, we observed that bat IRF7 function is highly conserved in avian and mammalian species. Structurally, we identified that the IRF domain of bat IRF7 was essential for activating IFN-β. In summary, our findings indicate that bat IRF7 possesses a conserved ability to activate bat antiviral innate immunity. This study provides a theoretical foundation for further understanding the innate immune response in bats.

Introduction

Bats, belonging to the order Chiroptera, are a diverse group of mammals comprising a significant portion of the total mammal species[1]. With over 1,400 known species, bats are unique not only in that they are the only mammals capable of sustained flight, but they are also natural reservoirs of viruses[2]. Bats harbor a number of deadly viruses such as henipaviruses (Hendra and Nipah), rabies, ebola virus, and coronaviruses (54% of those known are associated with bats): severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and the recently emerged SARS-CoV-2 (CoVID-19)[3–6]. Despite being carriers of these deadly viruses, bats rarely exhibit symptoms of the disease[7]. This suggests that bats possess a unique immune system that allows them to coexist with these pathogens. Therefore, the antiviral innate immune regulatory mechanism of bats has attracted great attention.

Upon viral infection, pattern recognition receptors (PRRs) recognize the pathogen-associated molecular patterns (PAMPs) of the invading virus and trigger a series of signaling cascade reactions that activate the expression of interferons (IFNs) to control viral replication[8]. IFN expression is regulated by interferon regulatory factors (IRFs). IRFs are a large family of transcription factors, which consists of nine members[9]. They through a helix-turn-helix DNA-binding motif bind to the IFN-stimulated consensus response element (ISRE) in the promoter region of IFN genes to regulate IFN transcription[10]. Among them, IRF1, IRF3, IRF5, and IRF7 have been identified as positive regulators of type I IFN transcription[11, 12]. IRF7 is considered a master regulator of type I IFN production[13]. It was initially cloned within the biological context of Epstein-Barr virus (EBV) latency and discovered to have an intimate relationship with the EBV primary oncogenic protein, latent membrane protein-1 (LMP-1)[14]. Studies using mice deficient in the IRF7 gene have shown that IRF7 is essential for the induction of IFN-alpha/beta genes, while IRF3 also plays a role in these pathways, its contribution is minimal in the absence of IRF7[13]. In humans,

IRF7 predominantly acts in plasmacytoid dendritic cells (pDCs) through the activation of TLR7/9 and the MyD88-dependent signaling pathway[15]. In chickens, where IRF3 is absent, IRF7 is utilized to reconstitute the corresponding IFN signaling pathway in response to viral infections[16]. These findings underscore the significance of IRF7 in innate immunity and its crucial role in regulating the production of type I IFNs.

Studies have shown that bats IRF1/3/7 have a high basal expression[17], this means that bats are able to rapidly and effectively initiate an innate immune response upon viral infection. Our previous studies have found that bat IRF1 can activate IFNβ expression and inhibit viral replication[18]. Another study found that IRF3-mediated signaling limits Middle-East Respiratory Syndrome (MERS) coronavirus propagation in cells from an insectivorous Bat[19]. This mechanism helps prevent excessive replication of MERS-CoV, which is crucial for the host's health and contributes to the ability of bats to coexist with these viruses. However, it remains unclear whether bat IRF7 also plays a conserved role in antiviral innate immunity.

In this study, we cloned the IRF7 gene from the Tadarida brasiliensis cell and discovered that the amino acid sequence of bat IRF7 showed limited conservation among species. However, we observed that RNA viruses were able to significantly increase the expression of IRF7 mRNA in TB1Lu cells. Furthermore, overexpression of bat IRF7 not only activated the bat IFNβ promoter but also activated the IFNβ promoters of chicken and humans. Additionally, overexpression of bat IRF7 was found to enhance the expression of genes related to innate immunity in TB1Lu cells and inhibit virus replication. Overall, these findings suggest that bat IRF7 plays a conserved role in antiviral innate immunity. This will enhance our understanding of the innate immunity in bats and shed light on the mechanisms underlying the coexistence of bats and viruses.

Materials and Methods

Cell Culture and Virus

Chicken embryonic fibroblast cell line DF1, human 293T cells, and bat TB1 Lu cells were obtained from ATCC and cultured in DMEM supplemented with 10% FBS and cells were incubated at 37°C in a 5% CO2 incubator. Newcastle disease virus (NDV-GFP) was a low virulent strain LaSota named NDV-GFP. Avian influenza virus (AIV) was A/Chicken/Shanghai/010/2008(H9N2) virus (SH010) was isolated from chicken in Shanghai, China, in 2008 and identified as H9N2 avian influenza A virus. The GFP tagged vesicular stomatitis virus (VSV) VSV-GFP were stored in our Laboratory. The viruses were purified, propagated, and stored as described in our previous study[20].

Virus Infection

The TB1Lu cells were plated, washed twice with PBS. Each well was seeded with 1 MOI of NDV-GFP or AIV or VSV-GFP. After 3h, 6h, 12h and 24h of infection, samples were collected for subsequent experiments.

Cloning and Bioinformatics Analysis of Bat IRF7

Based on the Molossus molossus IRF7 sequence (XM_036281692.1) obtained from the National Center for Biotechnology Information (NCBI), the primers batIRF7-F and batIRF7-R (Supplementary Table 1) were designed and used to amplify batIRF7 from TB 1 Lu cells cDNA. The PCR product was ligated into a pTOPO-Blunt vector (Vazyme Biotech co.,ltd) for sequencing, and the positive colonies were sent to the Beijing Genomics Institute (Beijing, China) for sequencing. The amino acid sequence of batIRF7 was aligned with the other animal IRF7 proteins from chicken, ducks, pigs, cattles, dogs, cats, mice, humans, zebrafishes, and salmons using Clustal W and edited with ESPript 3.0 (https://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi). Sequence homology and phylogenetic analysis of the IRF7 amino acid sequences were conducted using DNASTAR. A phylogenetic tree was constructed based on the IRF7 from 15 different species, including mammals, birds, fish. Different domains in the IRF7 amino acid sequences were predicted using the simple modular architecture research tool (SMART) program (http://smart.embl-heidelberg.de/). Homology modeling for IRF7 was conducted using the online protein-modeling server Swiss Model (https://swissmodel.expasy.org/).

Plasmid construction

pcDNA3.1-bat-IRF7 plasmids were constructed by inserting full- length TbIRF7 into the Hind III, and EcoR I sites pcDNA3.1 of the expression vector using a ClonExpress II one-step cloning kit (Yeasen, Shanghai, China). The primers used in the PCR are listed in Supple-mentary Table 1. The truncated plasmids of bat IRF7, including deleting amino acid 123–223(dAA123-223), amino acid 223–335(dAA223-335), insulin growth factor-binding protein homologues (IB), interferon regulatory factor (IRF), interferon-regulatory factor 3 (IRF-3), and repeat of unknown function (DUF), were con-structed using a modified homologous recombination method and the primers were listed in Supplementary Table 1. The DH5α Chemically Competent Cell (Tsingke Biology Technology, Beijing, China) was used for plasmid transformation. The pGL-IFN-β-Luc plasmid was constructed in our previous study (23).

Cells transfection

293T, DF1and TB1Lu cells were seeded in 12-well or 24-well plates (NEST Biotechnology, Wuxi, China) at 5×10^5/mL or 1×10^6/mL. And the plasmid was transfected 250 ng/well in 24-well or 500 ng/well in 12-well transfected with plasmid were performed with Nulen Plus-Trans™ Transfection Reagent (Nulen, Shanghai, China) according to the manufacturer's protocol.

Luciferase Reporter Assay

The DF-1, 293T, and TB 1 Lu cells were plated in 24-well plates and were transiently transfected with the reporter plasmid pGL-chIFN-β-Luc or pGL-huIFN-β-Luc or pGL-batIFN-β-Luc (120 ng/well) and the control Renilla luciferase (pRL-TK, 60 ng/well). According to the manufacturer's instructions, the cells were lysed 24 hours after transfection, and luciferase activity was detected using a Dual-Luciferase Reporter Assay System kit (Promega, Madison,WI). Renilla luciferase activity was used for normalization.

RNA Extraction and Quantitative Real-Time PCR

Cells' total RNAs were extracted with AG RNAex Pro Reagent (Ac-curate Biology, Hunan, China). mRNA was reverse-transcribed to cDNA using a two-step reverse transcription kit, the first reaction removed genomic DNA by adding gDNA wiper enzyme, and the second step reverse transcribed mRNA to cDNA. The specific operation is carried out according to the instructions provided by the kit (Vazyme, Nanjing, China), and the cDNA was analyzed using the SYBR green PCR mix (Vazyme, Nanjing, China) with the Applied Biosystems machine (ABI 7500; Thermo Fisher Scientific). Relative gene expression was analyzed using the $2^{-\Delta\Delta Ct}$ method. The β-actin was the internal reference when examining the level of genes. The primer sequences for the genes are shown in the Supplementary Table 1.

Western Blot Analysis

The cells' total proteins were extracted by radioimmunoprecipitation assay (Beyotime, Shanghai, China) containing a protease cocktail (Yeasen, Shanghai, China) and phenylmethylsulfonyl fluoride (PMSF) (Yeasen, Shanghai, China). The lysate was centrifuged at 13,000 rpm for 10 min to obtain the supernatant, and a 5 × SDS loading buffer was added before the lysates were boiled for 10 min. The proteins isolated from the cell lysates were separated via SDS-PAGE and analyzed using Western blot. The antibody included ant-GFP (Yeasen, Shanghai, China) and β-tubulin overnight at 4°C. The membrane was washed 3 times with tris buffered saline andTween-20 (TBST) (Sangon Biotech Co., Ltd, Shanghai, China). Then, the secondary antibody was added for 1 h incubation at 4°C shaker Images were obtained using the Tanon 5200 imaging system (Tanon, Shanghai, China).

Statistical Analysis

Results are expressed as the mean ± SD. GraphPad Prism 8.0 was utilized to graph the results. Data were analyzed by using a two-tailed independent the Student's t-test. $P < 0.05$ was considered statistically significant, and $P < 0.\ 0\ 1$ was considered highly statistically significant (*$P < 0.05$; **$P < 0.01$).

Result

Upregulation of batIRF7 Expression in Response to RNA Viral Infection

Mammalian and avian cells infected with RNA viruses exhibit a significant up-regulation of IRF7 expression, which plays a crucial role in controlling virus replication[21, 22]. However, it remains unclear whether RNA virus infection can also induce the up-regulation of IRF7 in bats. To investigate the role of bat IRF7 in RNA virus infection, we infected bat TB1Lu cells with Newcastle disease virus (NDV), avian influenza virus (AIV), and vesicular stomatitis virus (VSV-GFP). The expression of batIRF7 mRNA was assessed at 0h, 3h, 12h, and 24h of post-infection. Our findings that all three RNA viruses significantly up-regulated the expression of batIRF7 after 3 hours of infection (Figs. 1A-C). To further confirm the universality of this effect, we transfected TB1Lu cells with a RNA virus nucleic acid mimic, ploy(I:C), and observed that it also induced the expression of IRF7 in bats (Fig. 1D). These results provide evidence that virus infection in bat cells can indeed up-regulate the expression of IRF7.

3.2 Bioinformatic analysis of batIRF7

To elucidate the biological function of bat IRF7 in antiviral innate immunity, we cloned Tadarida brasiliensis bat IRF7 using the Tadarida brasiliensis 1 lung (TB1Lu) cell line cDNA. The open reading frame (ORF) of IRF7 was found to be 1758 bp long, encoding 586 amino acids. Our analysis of multiple sequence alignments revealed that bat IRF7 exhibits low conservation among different species. The similarities of bat IRF7 with various species, including chicken (AJS11515.1), mice (NP_058546.1), pigs (NP_001090897.1), ducks (AYI50403.1), humans (AAI36556.1), zebrafishes (AAH65902.1), horse (XP_023510519.1), baboon (XP_031509712.1), chimpanzee (JAA38191.1), salmons (NP_001165321.1), goats (XP_017898523.1), cattles (AAI51519.1), dogs (XP_038279968.1) and cats (XP_011285476.2), ranged from 3.7–21.3% (Figs. 2A and B). Furthermore, we performed structural analysis and found that the four characteristic domains in bat IRF7: factor-binding protein homologues domain (IB), interferon regulatory factor domain (IRF), interferon-regulatory factor 3 domain (IRF-3), and repeat of unknown function (DUF) domain (Figure. 2C). To explore the evolutionary relationship of bat IRF7 with other species, we conducted a phylogenetic analysis based on multiple alignments of IRF7 from mammals, birds, and fishes. Our analysis revealed that bat IRF7 belongs to a subgroup with other mammals, while birds and fishes have their own distinct subgroups (Figure. 2D). Additionally, we used the Swiss Model to predict the three-dimensional structure of bat IRF7, which showed the presence of 4 α helices and 11 β folds (Figure. 2E). Overall, our study provides a comprehensive characterization and evolutionary analysis of bat IRF7, shedding light on its potential regulatory effects in antiviral innate immunity.

3.3 Overexpression of batIRF7 activates the innate immune response of bats

To further explores the role of bat IRF7 in antiviral innate immunity, we were transfected pcDNA3.1-batIRF7 plasmids into TB1Lu and 293T cells. Dual luciferase reporter assays were performed to assess the activity of the IFN-β promoter. The results showed that the overexpression of bat IRF7 significantly activated the IFN-β promoter activity in a dose-dependent manner in both 293T and TB1Lu cells (Figs. 3A and B). Additionally, we overexpression of the batIRF7 in DF1 cells, it was found that batIRF7 can also significantly activate chicken IFNβ promoter (Fig. 3C), indicating that batIRF7 has a conserved role in activating IFN-β in both mammals and birds.

Furthermore, we examined the effect of batIRF7 on the bat innate immunity response. Overexpressing batIRF7 in bat TB1Lu cells, we observed a significant activation of the expression of IFNβ and OAS1 both before and after VSV-GFP virus infection (Fig. 3D-F). This suggests that bat IRF7 can activate the innate immune response in bat.

3.4 Bat IRF7 Inhibits Vesicular Stomatitis Virus (VSV-GFP) Replication

To investigate the impact of batIRF7 on virus replication, we overexpressed batIRF7 in bat TB1Lu cells and infected with Vesicular Stomatitis Virus (VSV-GFP). The replication of the virus was observed at 12

and 24 hours post-infection, and the expression of GFP fluorescent protein was used as an indicator of virus replication. Fluorescence microscopy analysis revealed that the overexpression of batIRF7 obviously reduced the fluorescence intensity of virus (Fig. 4A). Furthermore, quantification of the fluorescence intensity confirmed that the overexpression of batIRF7 significantly inhibited the replication of VSV-GFP virus (Fig. 4B). Additionally, as the concentration of overexpressed batIRF7 increased, the fluorescence intensity of VSV-GFP gradually weakened (Fig. 4C). Western blot analysis further demonstrated a concentration-dependent decrease in GFP protein levels with increasing batIRF7 concentration (Fig. 4D). These results suggest that batIRF7 can activate the innate immune response in bats to inhibition of virus replication.

3.5 Essential Domains of batIRF7 in IFNβ Activation

The above studies indicate that bat IRF7 has a conserved ability to activate innate immunity. To further analyze its important functional domains, we constructed a series of plasmids with deletions of different functional domains, including IRF7 AA123-223, AA223-335, IB domain, IRF domain, IRF3 domain, and DUF domain (Fig. 5A). These plasmids were transfected into 293T and TB1Lu cells, and it was found that the deletion of IRF7 AA123-223, AA223-335, IB domain, and DUF domain weakened the activation of IRF7 to the IFN-β promoter activity. Conversely, the deletion of the IRF3 domain significantly enhanced its activation of IFN-β promoter activity in both 293T and TB1Lu cells (Fig. 5B and C). These findings suggest that the IRF domain, AA123-223, AA223-335, and IB domain are essential for batIRF7 to activate IFNβ, while the IRF3 domain acts as an inhibitor.

Discussion

As natural reservoirs of numerous highly pathogenic viruses, bats pose a significant threat to human health and survival. However, what is intriguing is that bats themselves do not exhibit any apparent signs of disease despite carrying such viruses[23]. This phenomenon suggests that bats possess specific and unique innate immune characteristics. Multiple studies have revealed that bats have a higher basal expression of interferons (IFNs) and interferon-stimulated genes (ISGs) compared to other animals. This heightened immune response may play a crucial role in bats' ability to effectively respond to viral infections and maintain a virus tolerance phenotype[24]. But the precise regulatory mechanisms underlying this high basal expression and the potential effects it has on bats' immune response require further investigation.

Members of the IRFs family play an important role in regulating the expression of IFNs and ISGs. Especially IRF3 and IRF7 can significantly induce IFN-α/β gene transcription[25]. The difference is that in mammals, IRF3 is a constitutive gene and is expressed in various tissues and organs, while IRF7 is an inducible gene and is strongly induced by IFN by activating ISGF3[9]. In birds, IRF3 is naturally missing, and IRF7 plays a major role[16, 22]. However, both IRF3 and IRF7 have higher basal expression in bats. This suggests that bat IRF7 has unique functions that remain to be defined.

In this study, we cloned Tadarida brasiliensis IRF7. Analysis of the bat IRF7 amino acid sequence found middle region sequence of IRF7 shows extremely low conservation. But the N-terminal DNA binding domain (DBD) and C-terminal sequence are relatively conserved. The DBD domain at the N-terminal is particularly important for IRF to function as a transcription factor[26]. DBD contains a unique cluster of five well-spaced tryptophan residues, Fish possess only four of the five conserved tryptophan residues[27, 28]. This region form a helix-turn-helix motif that latches onto DNA recognizing elements containing GAAA repeats[29]. These five tryptophan sites in bat IRF7 are conserved. The C-terminal region of mammalian IRF7 holds a conserved serine-rich domain important for virus-induced phosphorylation. Both human and mouse IRF7 have two serine sites that are phosphorylated in this region[30]. The serine-rich domain is also conserved in bats. These shows that the key functional domains of bat IRF7 are highly conserved among species. This indicates that the function of batIRF7 may also be conserved during species evolution.

To explore the function of batIRF7, we detected the basal expression of batIR7 and the induced expression after virus infection. It was found that three RNA viruses: Newcastle disease virus (NDV), avian influenza virus (AIV) and vesicular stomatitis virus (VSV) could significantly up-regulate batIRF7 mRNA expression after infecting TB 1Lu cells; in addition, overexpression of batIRF7 could also significantly up-regulate the expression of bat innate immunity-related genes and inhibit virus replication. Similar results were found in Australian Black Flying Fox: When knocking down bat IRF7, Pulau virus replicated to a titer more than four-fold higher than in mock-transfected cells(40). This suggests that bat IRF7, like other animals IRF7s, also has typical features of interferon stimulation-related genes (ISGs). In this study, we also found that batIRF7 not only activates the bat IFNβ promoter, but also activates the chicken and human IFNβ promoters. This suggests that bat IRF7 has conserved functions of other animal IRF7s. What is unique about bat IRF7 may be its higher basal expression.

Structurally, IRF7 contains a C-terminal IRF3 domain (AA366-546), which may be the reason why IRF7 has higher homology with IRF3 than with other IRFs. After deletion of the IRF3 domain, the ability of IRF7 to activate IFN-β was greatly improved. Indicating that IRF3 domain is an inhibitory domain. In humans, the C-terminal region of IRF-7 also contains an inhibitory domain (ID) that interferes with the transactivation function of IRF-7. This is consistent with what we mentioned earlier: The C-terminal of IRF7 is relatively conservative among species. When the N-terminal IRF domain was deleted, the activation of IFN-β by bat IRF7 was completely lost. This indicates the N-terminal IRF7 domain is crucial for IRF7 to function as a transcription factor. The conserved cluster of five well-spaced tryptophan residues which is associated with DNA binding are located in this domain.

In conclusion, we found that the amino acid sequence of bat IRF7 is less conserved among species and only retains the key conserved functional domain that activates IFNβ expression. Mutation of the remaining functional domain amino acids may give bat IRF7 more functions and promote its coexistence with viruses. However, the specific mechanism still needs further study. Overall, in this study, we found that bat IRF7 has a conserved antiviral innate immune function. This study helps us further understand the innate immune system of bats.

Declarations

Availability of data and materials

The data analyzed during the current study are available from the corresponding author on reasonable request.

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Contributions

JW, QJL, JHS and YQC designed the research and analyzed the data. JW, QJL, YQC, FYF, QS, CXX, YPF, ZFW, JJM, HAW, YXY and JHS conducted the experiments and collected the data. JW and QJL wrote the paper. All authors approved the final version of the manuscript.

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Ethics declarations

Competing interests

The authors declare that they have no competing interests.

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Figures

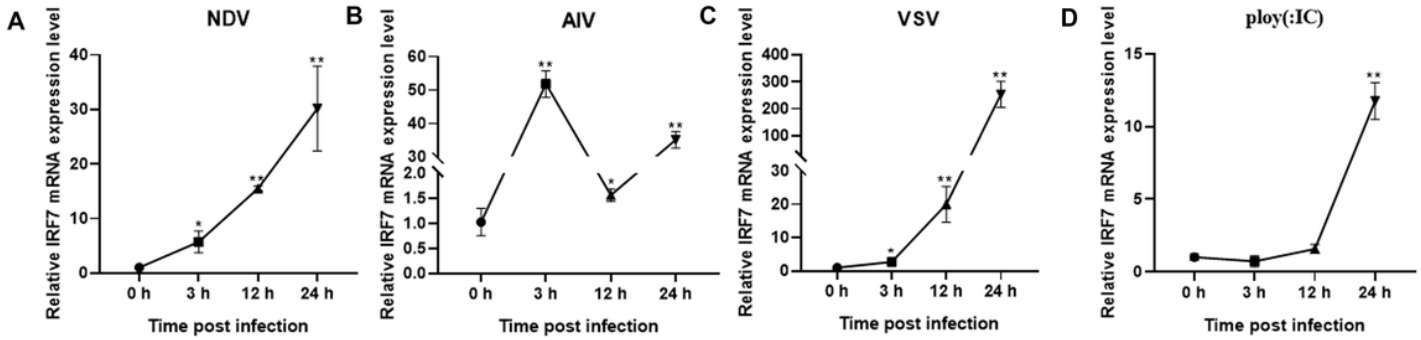

Figure 1

Upregulation of batIRF7 Expression in Response to RNA Viral Infection. (A) The mRNA expression level of batIRF7 was detected by RT-qPCR in TB1Lu cells infected with NDV at 1.0 MOI for 0, 3, 12, or 24 hours. (B) The mRNA expression level of batIRF7 was detected by RT-qPCR in TB1Lu cells infected with AIV at 1.0 MOI for 0, 3, 12, or 24 hours. (C) The mRNA expression level of batIRF7 was detected by RT-qPCR in TB1Lu cells infected with VSV at 1.0 MOI for 0, 3, 12, or 24 hours. (D) The mRNA expression level of batIRF7 was detected by RT-qPCR in TB1Lu cells stimulated with poly(I:C) at 0.1 μg/mL for 0, 3, 12, or 24 hours. Data are expressed as the means ± SD of three independent experiments. *$P < 0.05$; **$P < 0.01$.

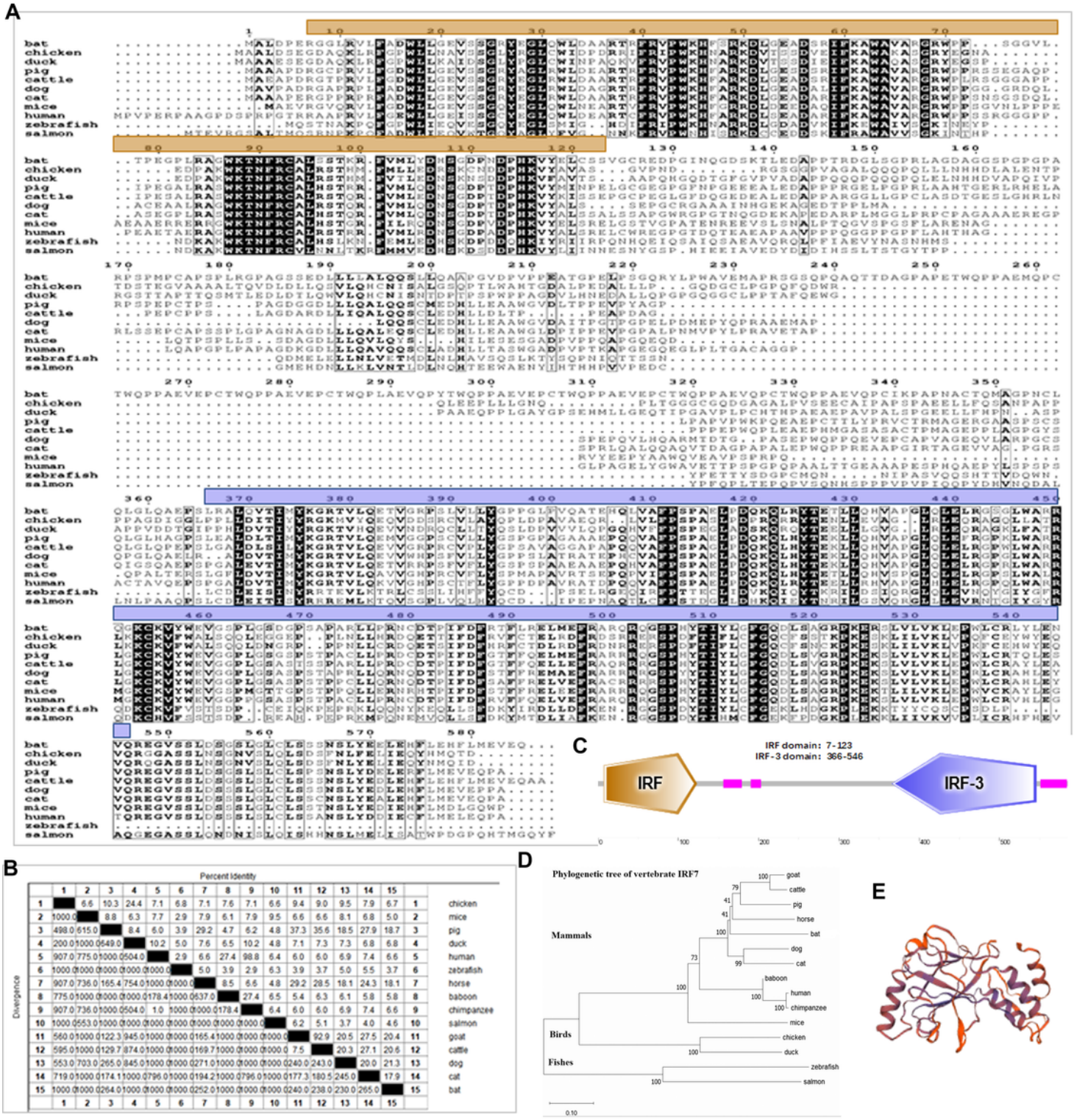

Figure 2

Bioinformatic analysis of batIRF7. (A) Alignment of IRF7 amino acid sequences in different species including bats (Tadarida brasiliensis), chicken (AJS11515.1), ducks (AYI50403.1), pigs (NP_001090897.1), cattles(AAI51519.1), dogs(XP_038279968.1), cats(XP_011285476.2), mice(NP_058546.1), humans (AAI36556.1), zebrafishes(AAH65902.1) and salmons (NP_001165321.1). The amino acid sequences of different animals were aligned using ClustalW and edited with ESPript 3.0.

(B) Homology analysis of IRF7 amino acid sequences in different species. (C) Protein domains of bat IRF7 predicted by SMART. (D) Phylogenetic tree of vertebrate IRF7. A neighbor-joining phylogenetic tree of vertebrate IRF7 was generated with MegAlign software using IRF7 sequences from the following animals: chicken (AJS11515.1), mice (NP_058546.1), pigs (NP_001090897.1), ducks (AYI50403.1), humans (AAI36556.1), zebrafishes (AAH65902.1), horse (XP_023510519.1), baboon (XP_031509712.1), chimpanzee (JAA38191.1), salmons (NP_001165321.1), goats (XP_017898523.1), cattles (AAI51519.1), dogs (XP_038279968.1), cats (XP_011285476.2) and bats (Tadarida brasiliensis). (E) Threedimensional structure of bat IRF7 predicted using SWISS-MODEL.

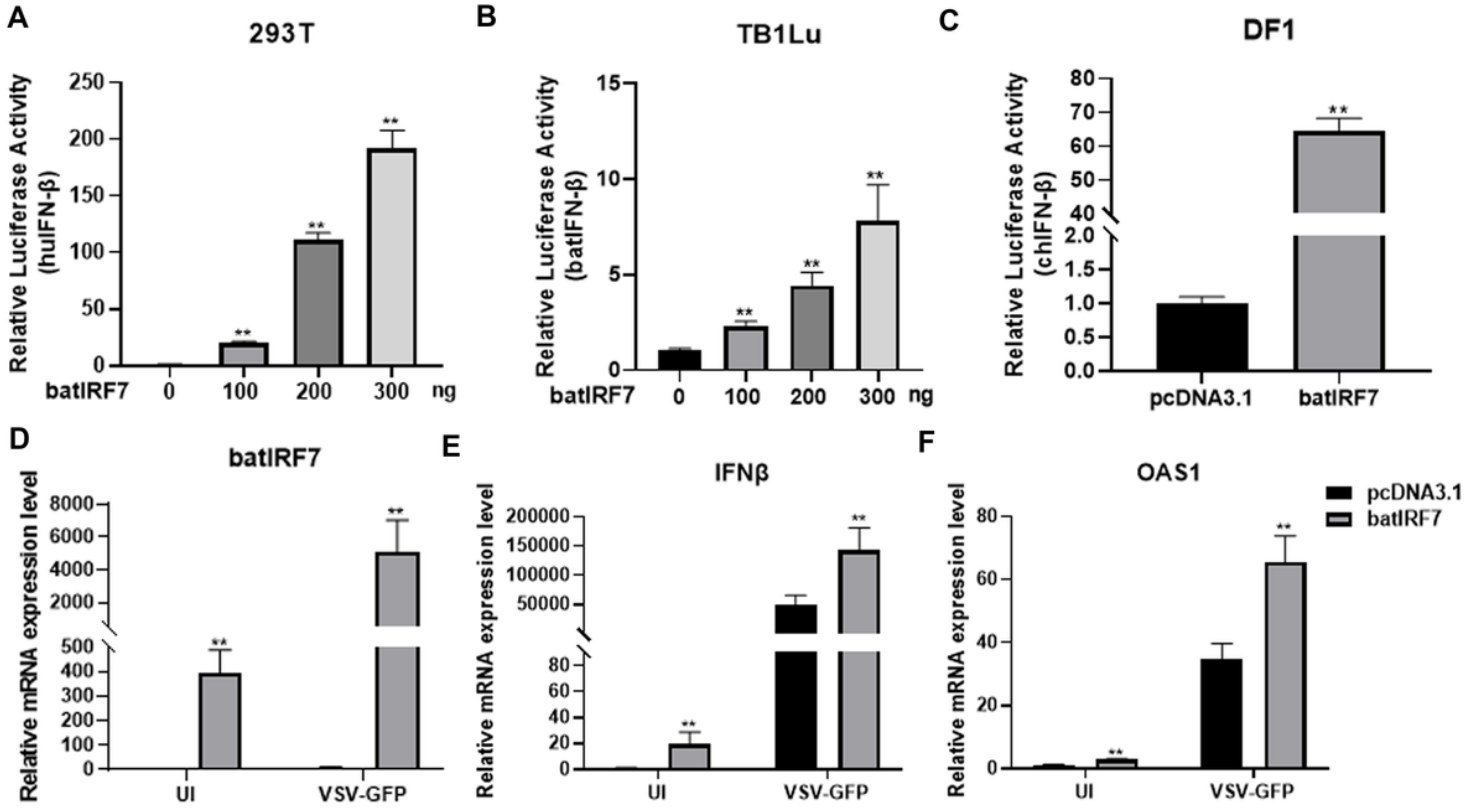

Figure 3

Overexpression of bat IRF7 significantly activated antiviral innate immunity. (A) 293T cells were transiently transfected with IFN-β reporter plasmid with increasing amounts of batIRF7 plasmid (0 ng/well, 100 ng/well, 200 ng/well or 300 ng/well) analyzed for luciferase activity. (B) TB 1Lu cells were transiently transfected with IFN-β reporter plasmid with increasing amounts of batIRF7 plasmid (0 ng/well, 100 ng/well, 200 ng/well or 300 ng/well) analyzed for luciferase activity. (C) DF1 cells were transiently transfected with IFN-β reporter plasmid with batIRF7 plasmid analyzed for luciferase activity. (D) The efficiency of batIRF7 overexpression in TB1Lu cells was assessed by RT-qPCR. Cells were transfected with 500 ng/well of pcDNA3.1 or pcDNA3.1-batIRF7 and then uninfected (UI) or infected with VSV-GFP at 1.0 MOI. (E-F) The expression levels of IFNβ and OAS1 were detected by RT-qPCR in TB1Lu cells after overexpression of pcDNA3.1-batIRF7. Cells were uninfected (UI) or infected with VSV-GFP at 1.0 MOI. Data are presented as means ± SD of three independent experiments. *P< 0.05; **P < 0.01.

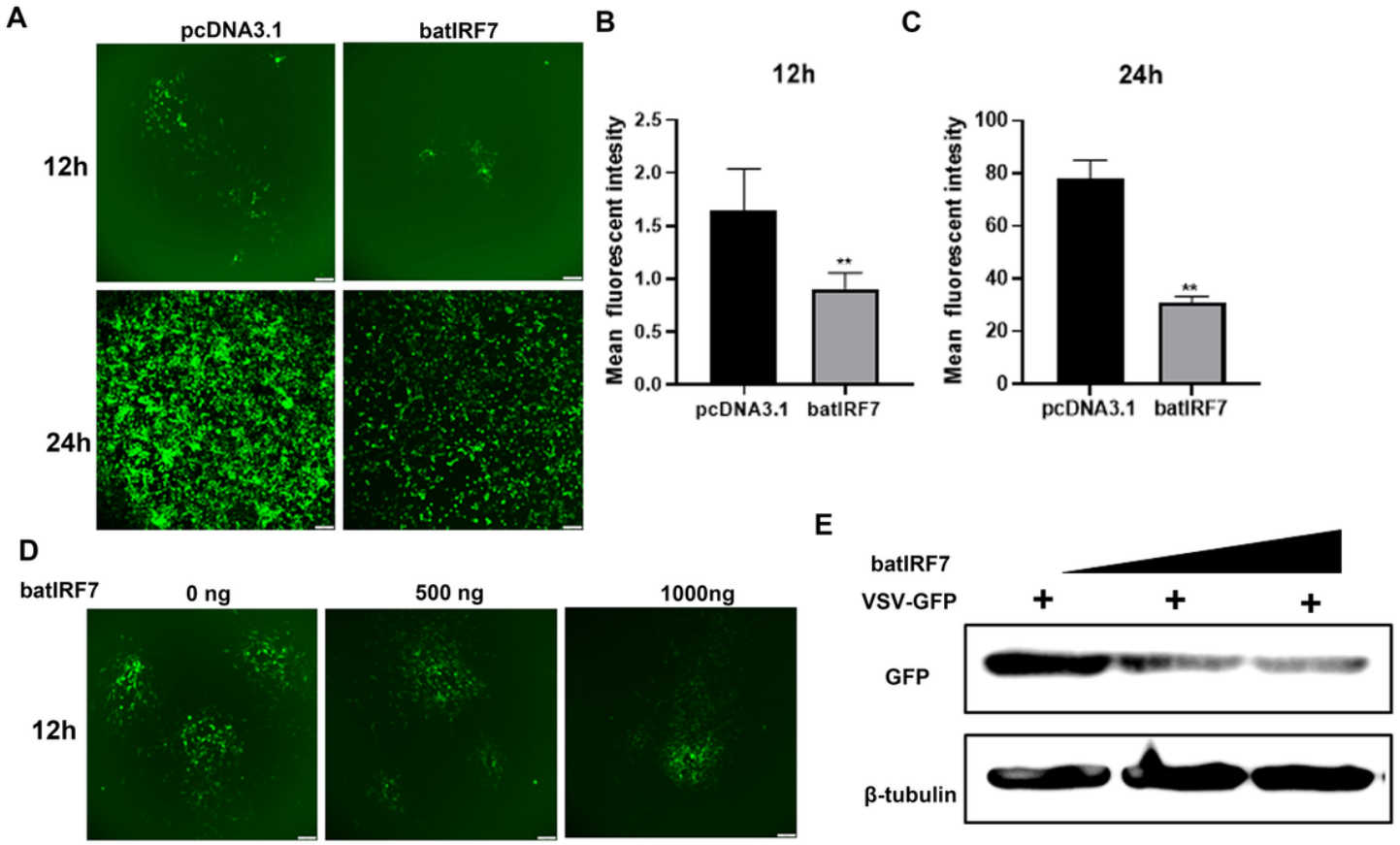

Figure 4

Overexpression of batIRF7 Inhibits Viral Replication. (A) Viral fluorescence in TB1Lu cells after overexpression of pcDNA3.1 or pcDNA3.1-batIRF7 and infection with VSV-GFP at 1.0 MOI for 12 and 24 hours. (B, C) Mean fluorescent intensity of VSV-GFP in TB1Lu cells after overexpression of pcDNA3.1 or pcDNA3.1-batIRF7 and infection with VSV-GFP at 1.0 MOI for 12 hours (B) and 24 hours (C). (D) Viral fluorescence in TB1Lu cells after overexpression of batIRF7 at 0 ng/well, 500 ng/well, or 1000 ng/well and infection with VSV-GFP at 1.0 MOI for 12 hours. (E) Western blot analysis was used to detect the expression of GFP, which reflects virus replication, after overexpression of batIRF7 at 0 ng/well, 500 ng/well, or 1000 ng/well and infection with VSV-GFP at 1.0 MOI for 12 hours. Data are expressed as means ± SD of three independent experiments. *$P < 0.05$; **$P < 0.01$.

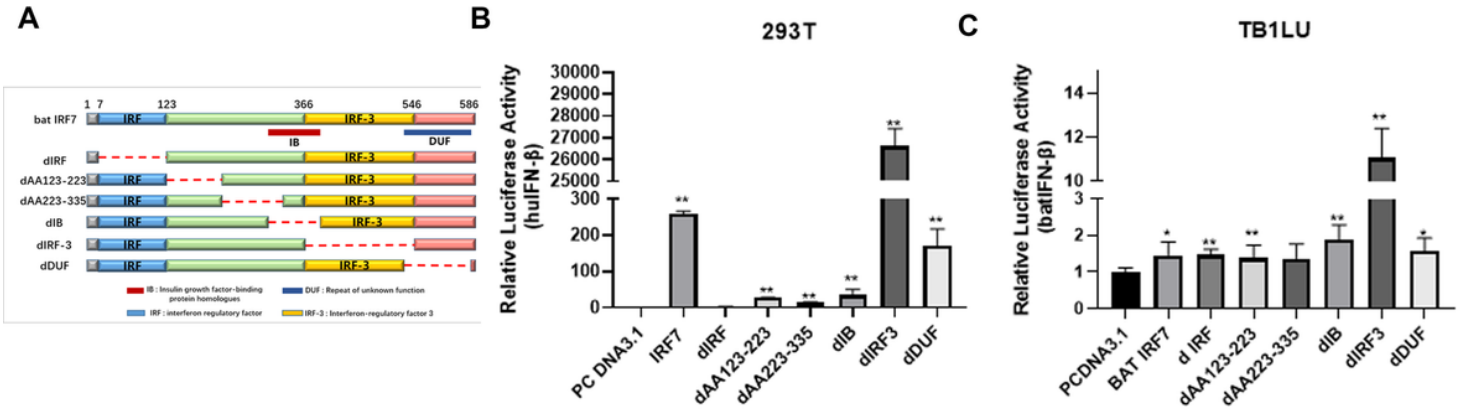

Figure 5

Essential functional domains of bat IRF7. (A) Schematic structure of bat IRF7 mutants lacking AA123-223, AA223-335, IB, IRF, IRF3, or DUF domains. (B) Luciferase reporter assay was performed to measure the IFN-β promoter activity in 293T cells. Cells were co-transfected with luciferase reporter plasmids (pRL-TK and pGL-huIFN-β-Luc) along with bat IRF7 mutant plasmid or pcDNA3.1 control. After 6 hours of transfection, cells were infected with VSV-GFP at 1.0 MOI for 16 hours. The double luciferase reporting assay was used to detect the IFN-β promoter activity. (C) Luciferase reporter assay was performed to measure the IFN-β promoter activity in TB1LU cells. Cells were co-transfected with luciferase reporter plasmids (pRL-TK and pGL-batIFN-β-Luc) along with bat IRF7 mutant plasmid or pcDNA3.1 control. After 6 hours of transfection, cells were infected with VSV-GFP at 1.0 MOI for 16 hours. The double luciferase reporting assay was used to detect the IFN-β promoter activity. Data are presented as means ± SD of three independent experiments. *$P < 0.05$; **$P < 0.01$.

Supplementary Files

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