

The host cellular protein Ndufaf4 interacts with the vesicular stomatitis virus M protein and affects viral propagation

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Abstract

Background: Vesicular stomatitis virus (VSV) is an archetypal member of Mononegavirales which causes important diseases in cattle, horses and pigs. The matrix protein (M) of VSV plays critical roles in the replication, assembly/budding and pathogenesis of VSV. To further investigate the role of M during viral growth, we used a two-hybrid system to screen for host factors that interact with the M protein.

Results: Here, NADH: ubiquinone oxidoreductase complex assembly factor 4 (Ndufaf4) was identified as an M-binding partner, and this interaction was confirmed by yeast cotransformation and GST pulldown assays. The globular domain of M was mapped and shown to be critical for the M-Ndufaf4 interaction. Two double mutations (E156A/H157A, D180A/E181A) in M impaired the M-Ndufaf4 interaction. Overexpression of Ndufaf4 inhibited VSV propagation, and knockdown of Ndufaf4 by short hairpin RNA (shRNA) markedly promoted VSV replication. Finally, we also demonstrate that the anti-VSV effect of Ndufaf4 is independent of activation of the type I IFN response. These results indicated that Ndufaf4 might exploit other mechanisms to affect VSV replication.

Conclusions: In summary, we identify Ndufaf4 as a potential target for the inhibition of VSV propagation. These results provided further insight into the study of VSV pathogenesis.

Background

VSV is the pathogen of vesicular stomatitis (VS), an important disease in bovine, horses and swine. VS presents as widely erosive vesicles on the surfaces of the tongue, oral tissues, gums and feet and causes significant economic losses to the livestock industry[1]. VSV is a member of the *Vesiculovirus* genus, which belongs to the *Rhabdoviridae* family. The VSV genome is composed of a single strand of negative-polarity RNA that encodes five proteins: the nucleocapsid (N), phosphoprotein (P), matrix (M) protein, surface glycoprotein (G) and RNA-dependent RNA polymerase (L). N encapsidates the viral RNA to form the nucleocapsid (NC), which with the P and L proteins constitutes viral ribonucleoprotein (RNP) particles that are involved in genomic RNA replication and transcription [2]. The G protein of VSV is essential for virus attachment to specific receptors and entry into the cell.

The M protein of VSV is a multifunctional protein that plays a pivotal role in modulation of the host antiviral immune response[3, 4], suppression of host transcription/translation[5, 6], virion assembly/budding[7-10], and the induction of apoptosis[11, 12]. To perform numerous functions, viral proteins may need to interact with specific host proteins. Thus, further identification of host cellular protein(s) that interact with M is essential to better understand the roles of M in the replication and pathogenesis of VSV. In this study, we identified the host protein Ndufaf4 as a novel M-interacting partner that regulates the growth of VSV.

Ndufaf4 is an assembly factor of mitochondrial respiratory chain complex I that associates with assembly intermediates of the Q-module[13]. The mitochondrial respiratory chain has been shown to affect the propagation and pathogenesis of many viruses. For example, mitochondrial redox state and

electron transport activity can be restored by reducing HCV replication in full genomic HCV replicon cells[14]. The phosphoproteins of lyssaviruses, such as rabies virus (RABV) and Mokola (MOK) virus, interact with mitochondrial respiratory chain complex I to cause mitochondrial dysfunction and increase the generation of ROS and oxidative stress[15]. Ebermann et al. demonstrated that increasing the activity of mitochondrial respiratory chain complexes I and III inhibited coxsackievirus B3 (CVB3) replication[16]. Qu et al found that pharmacological inhibition of mitochondrial respiratory chain complex III potently restricted hepatitis E virus (HEV) replication[17]. However, no studies have investigated the role of the mitochondrial respiratory chain in the VSV life cycle. Here, we found that a subunit of mitochondrial respiratory chain complex I, Ndufaf4, is not only a VSV M-binding partner but also a cellular antiviral protein against VSV replication.

Results

The VSV M protein interacts with Ndufaf4

To identify potential cellular proteins that interact with the VSV M protein, yeast two-hybrid screening was performed using the VSV M protein as a bait against a normalized HeLa cDNA library. Putative positive clones were isolated and sequenced. Among these clones, Ndufaf4 was identified as a potential cellular partner of VSV M. The M-Ndufaf4 interaction was confirmed by yeast cotransformation assay. All the yeasts cotransformed with the indicated constructs grew well on SD/-2 medium, but only BD-M/AD-Ndufaf4 and BD-p53/AD-T (the positive control) grew on SD/-4 medium (Fig. 1A). Therefore, Ndufaf4 may be a binding partner of VSV M.

To further confirm the M-Ndufaf4 interaction, a GST pulldown assay was performed with purified GST-tagged M protein expressed in *E. coli* and Flag-tagged Ndufaf4 protein expressed in BSR-T7/5 cells. The presence of GST-M, but not GST alone, resulted in an interaction with Flag-Ndufaf4 (Fig. 1 B). Purified GST-Ndufaf4 protein expressed in *E. coli* and Flag-M expressed in BSR-T7/5 cells were also used in the pulldown assay. The results showed that GST-Ndufaf4, but not GST, interacted with Flag-M (Fig. 1C). Overall, these results indicated that M interacts with Ndufaf4.

Identification of critical amino acids in the M protein involved in the M-Ndufaf4 interaction

To determine the critical regions within the M protein that mediate its interaction with the Ndufaf4 protein, the carboxy-terminal globular domain and amino-terminal flexible domain of M were individually fused to the DNA-binding domain of GAL4, and the ability of the fusion proteins to interact with the Ndufaf4 protein was assessed using the Y2H system. Yeast transformed with BD-M/AD-Ndufaf4 and BD-M1/AD-Ndufaf4 grew well on SD/-2 and SD/-4 media; however, yeast transformed with BD-M2/AD-Ndufaf4 did not grow on SD/-4 medium (Fig. 2 A). The above results showed that the carboxy-terminal globular domain of M participates in the M-Ndufaf4 interaction.

To further identify the critical residues in the M protein involved in the M-Ndufaf4 interaction, all amino acids at the surface of the globular domain of the M protein were chosen for mutagenesis analysis and

the following 27 point or double point mutations were designed: R73A, N75A, R79A/T80A, H93A/M94A, I96A, M98A, M98R, V122A/L123A, D125A/Q126A, E136A, P149A/P150A, L152D, V154Y, V154A/P155A, E156A, E156A/H157A, R159A/R160A, G165A/L166A, D180A/E181A, L183A/E184A, P187A/M188A, S199A/D200A, K214A/K215A, S217A, G218A, D223A, and V225A/S226A[18, 19]. As shown by the results, all cells expressing the mutants grew as well as cells expressing wild-type M on SD/-4 medium, except for cells expressing E156A, E156A/H157A, or D180A/E181A M (Fig. 2 B). These results indicate that the E156/H157 and D180/E181 amino acids in M are critical for the M-Ndufaf4 interaction.

Ndufaf4 inhibits VSV replication

To investigate the role of Ndufaf4 in VSV propagation, HeLa cells were transfected with pCMV-Flag-Ndufaf4 or pCMV-Flag-N empty vector and then infected with VSV at 48 hours posttransfection. VSV G protein levels were detected by Western blotting, and the VSV titer was determined by TCID₅₀ assay at 4, 8 and 12 hours postinfection. As shown by the results, overexpression of Ndufaf4 decreased expression of the VSV G protein at 12 hours postinfection and the VSV titer at 8 and 12 hours postinfection (Fig. 3 A and B). These results show that overexpression of Ndufaf4 inhibits the growth of VSV.

To further analyze the effect of Ndufaf4 knockdown on VSV replication, HeLa cells were transfected with plasmids to deliver two independent short hairpin RNA (shRNA) targeting the Ndufaf4 gene. Western blot analyses demonstrated that the specific shRNA efficiently decreased the expression level of Ndufaf4 (Fig. 3 C). Furthermore, transfection of HeLa cells with the plasmid resulted in the efficient knockdown of Ndufaf4 expression, which promoted expression of the G protein and the VSV titer (Fig. 3 D and E). Collectively, these results suggest that Ndufaf4 is a cellular antiviral protein against VSV replication.

The anti-VSV effect of Ndufaf4 is independent of the type I IFN response

To further explore the mechanism exploited by Ndufaf4 to affect VSV replication, we detected the effect of Ndufaf4 overexpression or knockdown on the type I IFN response. HeLa cells were transfected with Ndufaf4-overexpression or Ndufaf4-knockdown plasmid and then infected with VSV at an MOI of 5. The mRNA levels of IFN- β and the IFN-stimulated genes ISG56, IFITM3 and MX1 were detected by real-time RT-PCR at 4, 8, 12 hours post-infection. Compared with their transcription in the control group, neither the overexpression nor knockdown of Ndufaf4 affected the transcription of these genes (Fig. 4 A and B). These results indicated that Ndufaf4 inhibits VSV replication independent of the type I IFN response.

Discussion

Ndufaf4, an assembly factor of mitochondrial respiratory chain complex I, is essential for normal mitochondrial function. Mutation of this gene is associated with a reduction in the mature form of complex I in muscle and causes Leigh syndrome and developmental delay[20]. However, the exact role of Ndufaf4 in complex I biogenesis is not yet fully understood. Furthermore, no studies have investigated the role of Ndufaf4 in the viral life cycle. In this study, Ndufaf4, a component of mitochondrial respiratory chain complex I, was identified as a binding partner of the VSV M protein using the yeast two-hybrid

system. To preclude possible autoactivation of Ndufaf4 and confirm the M-Ndufaf4 interaction, a yeast cotransformation assay was performed. This interaction was further demonstrated by GST pulldown assay. The VSV M protein is composed of two domains, an amino-terminal flexible domain and a carboxy-terminal globular domain. The domains play different roles during viral replication and assembly. The flexible domain of M can interact with dynamin to promote viral assembly[8]. This domain is also involved in the viral budding process by recruiting cellular partners. The globular domain of M can inhibit the host antiviral response to promote viral replication by inhibiting host gene expression or impairing the type I IFN system[3]. To determine which domain of M participates in the M-Ndufaf4 interaction, each domain of M was cloned into the PGBKT7 plasmid, and its ability to bind Ndufaf4 was determined in the Y2H system. The results showed that the globular domain, but not the flexible domain, of the M protein interacted with Ndufaf4. To further confirm the site of M that interacts with Ndufaf4, exposed residues at the surface of the globular domain were selected for mutagenesis analysis. Residues E156/H157 and D180/E181 were demonstrated to be important for the M-Ndufaf4 interaction. To test the role of the M-Ndufaf4 interaction, we determined the effect of Ndufaf4 silencing and overexpression on VSV replication. Overexpression of Ndufaf4 reduced the VSV titer and G protein expression, whereas knockdown of Ndufaf4 by shRNA dramatically promoted CSFV replication. These results demonstrated that Ndufaf4 is a cellular factor that acts against VSV infection.

Mitochondrial proteins, such as mitochondrial antiviral signaling (MAVS), have been reported to be essential for the antiviral immunity response[21]. Furthermore, some studies provide evidence for the critical role of the mitochondrial respiratory chain in innate immunity. NLRX1, a regulator of mitochondrial antiviral immunity, was suggested to associate with UQCRC2, a subunit of mitochondrial respiratory chain complex III[22, 23]. Mitochondrial complex I deficiency triggers innate immune and inflammatory responses in the retina[24]. GRIM-19, a mitochondrial respiratory chain complex I protein, plays an important role in antibacterial immunity[25]. As type I IFN- β plays a critical role in the host response against VSV infection, we detected the effects of overexpression and knockdown of Ndufaf4 on the I IFN response. The results showed that neither the overexpression nor the knockdown of Ndufaf4 affected the type I IFN response during VSV infection. Mitochondrial respiratory chain is the main site that produces ATP and reactive oxygen species (ROS). Given the critical antiviral roles of ROS, further study was needed to investigate whether Ndufaf4 affect VSV propagation by regulating production of ROS.

Conclusion

In summary, our study indicate that Ndufaf4 interacts with the VSV M protein and inhibits VSV replication. Although the precise mechanism of this effect needs to be further elucidated, the present study identifies Ndufaf4 as a potential target for the inhibition of VSV replication.

Methods

Cells and virus

BSR-T7/5 and HeLa cells were maintained at 37°C in DMEM medium, supplemented with 2 mM L-glutamine and 10% fetal bovine serum (FBS; HyClone). Vesicular stomatitis virus serotype Indiana (VSV-IND) (GenBank accession no. AM690336.1) was propagated in HeLa cells. The titer of VSV was determined by TCID₅₀ assays. Briefly, HeLa cells seeded in plates with 96 wells were infected with tenfold serial dilutions of virus sample using serum-free medium, which resulted in 11 concentrations, including a control without virus. CPE of HeLa cells were monitored at 4-5 days post infection and the number of positive and negative wells were recorded. TCID₅₀ titer was calculated using the Reed–Muench method.

Construction of expression vectors

The Nduf4 gene (GenBank accession no. NM_014165.4) was amplified by using cDNA extracted from HeLa cells and cloned into the pCMV-flag vector (catalog No. 635688; Clontech) to generate the pCMV-flag-Nduf4 plasmid. The M gene expressing the 229-amino acid long full length M protein, the flexible amino-terminal domain comprising the 57 first residues and the globular carboxy-terminal domain comprising the 58-229 residues were amplified by using cDNA extracted from Vesicular stomatitis virus serotype Indiana (VSV-IND) (GenBank accession no. AM690336.1) and cloned into the pGBKT7 vector [18, 19]. A eukaryotic Flag-M expression vector designated pT-Flag-M was constructed as previously described [26]. The Nduf4 and M ORFs were cloned at the C-terminus of GST in the pGEX-4T-1 vector to construct prokaryotic expression plasmids. The primers sequences are listed in Table 1. M point-mutation expression constructs were generated by PCR using pGBKT7-M plasmid as template, and then digested with Dpn I enzyme and transformed into *E. coli* DH5a and cultured on LB agar plate containing 100 µg/ml kanamycin. The vector used to deliver short hairpin RNA (shRNA) targeting the Nduf4 gene was constructed using small interfering RNA sequences targeting the Nduf4 gene (sh Nduf4-1: 5'-GGGAAATCAGCAAGATGAAGCCTCGAGGCTTCATCTTGCTGATTTCCC-3', sh Nduf4-2: 5'-GCACTAGTGATTCGCGGTATCCTCGAGGATACCGCGAATCACTAGTGC-3'), and a negative control sequence (sh NC: 5'-ACGUGACACGUUCGGAGAA-3') was designed, synthesized and cloned into the pYr-Lvsh lentiviral vector as previously described [27, 28].

Yeast two-hybrid assay

The VSV M gene expressing the 229-amino acid long full length M protein was cloned into the pGBKT7 plasmid to construct the bait vector and transformed into the yeast strain AH109 purchased from Clontech (catalog no. 630444; Clontech) using LiAc. The concentrated yeast strain AH109 transformed with pGBKT7-M was mated with the yeast strain Y187 containing a pretransformed HeLa cDNA library purchased from Clontech (catalog no. 630479; Clontech). Heterozygotes were selected on DDO/X/A (SD/-Leu/-Trp/X-a-Gal/AbA) plates. Blue colonies that grew on DDO/X/A were transferred to QDO/X/A (SD/-Ade/-His/-Leu/-Trp/X-a-Gal/AbA) plates. All Blue colonies grew on QDO/X/A plates were selected to extract prey plasmids using the Easy Yeast Plasmid Isolation Kit (catalog no. 630467; Takara). The putative positive prey plasmids were transformed into *E. coli* DH5a and cultured on LB agar plate containing 100 µg/ml ampicillin, and then sequenced and analyzed through NCBI BLAST searches. To eliminate false positive hits, the pGBKT7-M and prey plasmids were cotransformed into the AH109 yeast

strain. For mutagenesis analysis, M point-mutation and pGADT7-Nduf4 plasmid were cotransformed into the AH109 yeast strain and cultured on SD/4 (SD/-Ade/-His/-Leu/-Trp) plate.

Plasmid transfection

Briefly, the HeLa cells seeded in plates with 6 wells were transfected with 2 ml of DMEM containing 2% FBS, 2 µg of plasmids and 6 µl of Lipofectamine 3000 transfection reagent. At 24 hours post-transfection, the transfection mixture was replaced with new DMEM supplemented with 2% FBS and incubated for an additional 24 hours before being assayed.

GST Pulldown Assay

GST pulldown assay was performed as previously described[26].

Western blot

Western blotting analysis was performed as previously described[29].

Real-Time RT-PCR

Total RNA was isolated from HeLa cells using TriPure reagent (catalog No. 11667165001; Roche). The RNA was quantified by NanoDrop and then used for cDNA synthesis using M-MLV reverse transcriptase (catalog No. 2641A; TaKaRa, Dalian, China) and oligo(dT) primers. The qRT-PCR assay was performed using a Premix Ex Taq kit (TaKaRa, Japan). The primer sequences used were as follows: Actin sense, 5'-TGACGTGGACATCCGCAAAG-3'; Actin antisense, 5'-CTGGAAGGTGGACAGCGAGG-3'; IFNB1 sense, 5'-TGTC AACATGACCAACAAGTGTC T-3'; IFNB1 antisense, 5'-GCAAGTTGTAGCTCATGGAAAGAG-3'; ISG56 sense, 5'-AACACCCACTTCTGTCTTACTGCAT-3'; ISG56 antisense, 5'-GATTTGGATCATTGTGCCTTGT -3'; IFITM3 sense, 5'-GCTGATCTTCCAGGGCTA TGG -3'; IFITM3 antisense, 5'-GCGAGGAATGGAAGTTGGAGT-3'; MX1 sense, 5'-GGCCAGCAAGCGCATCT -3'; and MX1 antisense, 5'-TGGAGCATGAAGAACTGGATGA -3'.

Statistical analysis

The data were analyzed for statistical significance by using Student's t-test.

Abbreviations

VSV: vesicular stomatitis virus; Nduf4: NADH: ubiquinone oxidoreductase complex assembly factor 4; shRNA: short hairpin RNA; HCV: Hepatitis C virus; ROS: reactive oxygen species; RABV: rabies virus; MOK: Mokola; CVB3: Coxsackievirus B3; HEV: Hepatitis E virus.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declared that there are no potential conflicts of interest in this study, authorship, and/or publication of this article.

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Authors' contributions

Wei Pan and Hongbing He conceived and designed the study. Wei Pan performed the experiments. Hongmei Wang wrote the manuscript. All authors read and approved the final version of the manuscript.

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Table

TABLE 1 Primers used in this study

Primer	Sequence(5' to 3') ^a	Purpose
BD-M-F	CCG <u>GAATTC</u> CGGATGAGTTCCTTAAAGAAGATTCTC	Amplification and cloning of M in PGBKT7 vector
BD-M-R	CGC <u>GGATCC</u> CGTCATTTGAAGTGGCTGACAG	Amplification and cloning of M in PGBKT7 vector
AD-Ndufaf4-F	CG <u>GGATCC</u> GATGGGAGCACTAGTGATTC	Amplification and cloning of Ndufaf4 in pGADT7 vector
AD-Ndufaf4-R	CCG <u>CTCGAG</u> CGGTCATTTTGATCGTATTGCTTTC	Amplification and cloning of Ndufaf4 in pGADT7 vector
GST-M-F	CGC <u>GGATCC</u> CGGATGAGTTCCTTAAAGAAGATTCTC	Amplification and cloning of M in PGEX-4T-1 vector
GST-M-R	CCG <u>GAATTC</u> CGGTCATTTGAAGTGGCTGACAG	Amplification and cloning of M in PGEX-4T-1 vector
GST-Ndufaf4-F	CGC <u>GGATCC</u> CGGATGGGAGCACTAGTGATTC	Amplification and cloning of Ndufaf4 in pGEX-4T-1 vector
GST-Ndufaf4-R	CCG <u>CTCGAG</u> CGGTCATTTTGATCGTATTGCTTTC	Amplification and cloning of Ndufaf4 in PGEX-4T-1 vector
Flag-Ndufaf4-F	CCC <u>AAGCTT</u> GGGATGGGAGCACTAGTGATTC	Amplification and cloning of Ndufaf4 in pcmv-flag vector
Flag-Ndufaf4-R	GGG <u>TACCC</u> CTCATTTTGATCGTATTGCTTTC	Amplification and cloning of Ndufaf4 in pcmv-flag vector
BD-M _N -F	CCG <u>GAATTC</u> CGGATGAGTTCCTTAAAGAAGATTCTC	Amplification and cloning of M _N in PGBKT7 vector
BD-M _N -R	CGC <u>GGATCC</u> CGTCGAATTCGGATCATAGGTGTCCAT	Amplification and cloning of M _N in PGBKT7 vector
BD-M _C -F	CCG <u>GAATTC</u> CGGCAATTAAGATATGAGAAATTC	Amplification and cloning of M _C in PGBKT7 vector
BD-M _C -R	CGC <u>GGATCC</u> CGTCATTTGAAGTGGCTGACAG	Amplification and cloning of M _C in PGBKT7 vector

^aUnderlined sequences indicate restriction sites

Figures

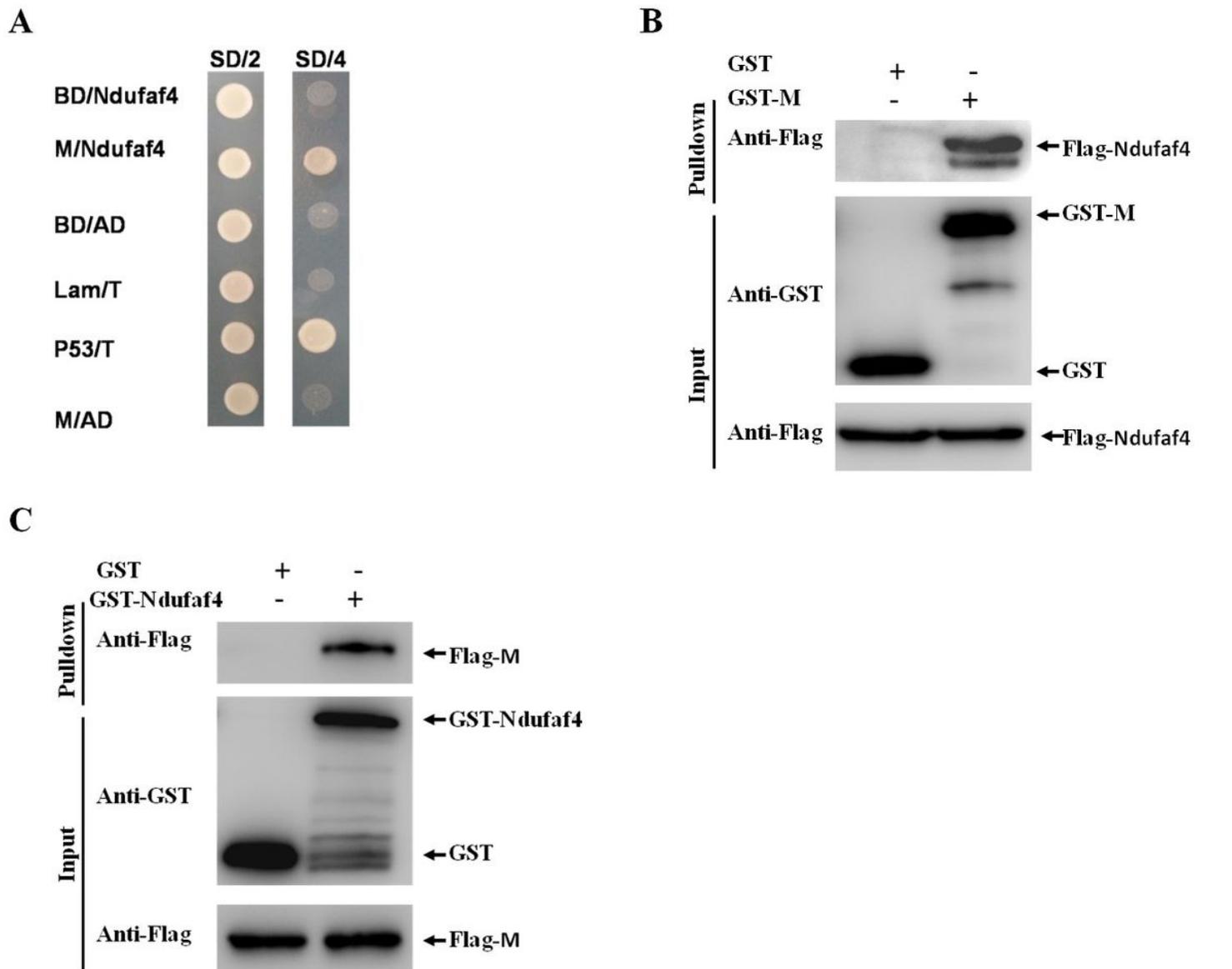


Figure 1

The VSV M protein interacts with Ndufaf4 (A) The M-Ndufaf4 interaction was examined using a Y2H system. M/Ndufaf4: Yeast strain AH109 was co-transformed with a bait plasmid (BD-M) which encodes the full-length M fused to the GAL4 binding domain and a prey plasmid (AD-Ndufaf4) which encodes the full-length Ndufaf4 fused to the GAL4 activation domain. BD/Ndufaf4: The empty vector BD was co-transformed with AD-Ndufaf4 and M/AD: the bait plasmid (BD-M) co-transformed with empty vector AD to exclude self-activation. Lam/T: AH109 yeast cotransformed with BD-Lam (human lamin C protein)/AD-T (simian virus 40 [SV40] large T antigen) and BD/AD were used as negative control. P53/T: AH109 yeast cotransformed with BD-p53 (murine p53)/AD-T was used as the positive control. All the yeasts cotransformed with the indicated constructs were streaked onto SD/2 (SD/-Leu/-Trp) and SD/4 (SD/-Ade/-His/-Leu/-Trp) plates (B) GST-M pulldown assay. GST and GST-M proteins expressed in *E. coli* BL21 (DE3) were purified with glutathione Sepharose 4B beads (catalog no.17075601; GE Healthcare) and incubated with recombinant Flag-Ndufaf4 protein. After washing with cold PBS, the bound proteins were

separated by SDS-PAGE (12%) and detected by western blotting. (C) GST-Ndufaf4 pulldown assay. GST and GST-Ndufaf4 proteins expressed in *E. coli* BL21 (DE3) were purified with glutathione Sepharose 4B beads and incubated with recombinant Flag-Ndufaf4 protein. After washing with cold PBS, the bound proteins were separated by SDS-PAGE (12%) and detected by western blotting. Each experiment was repeated at least three times.

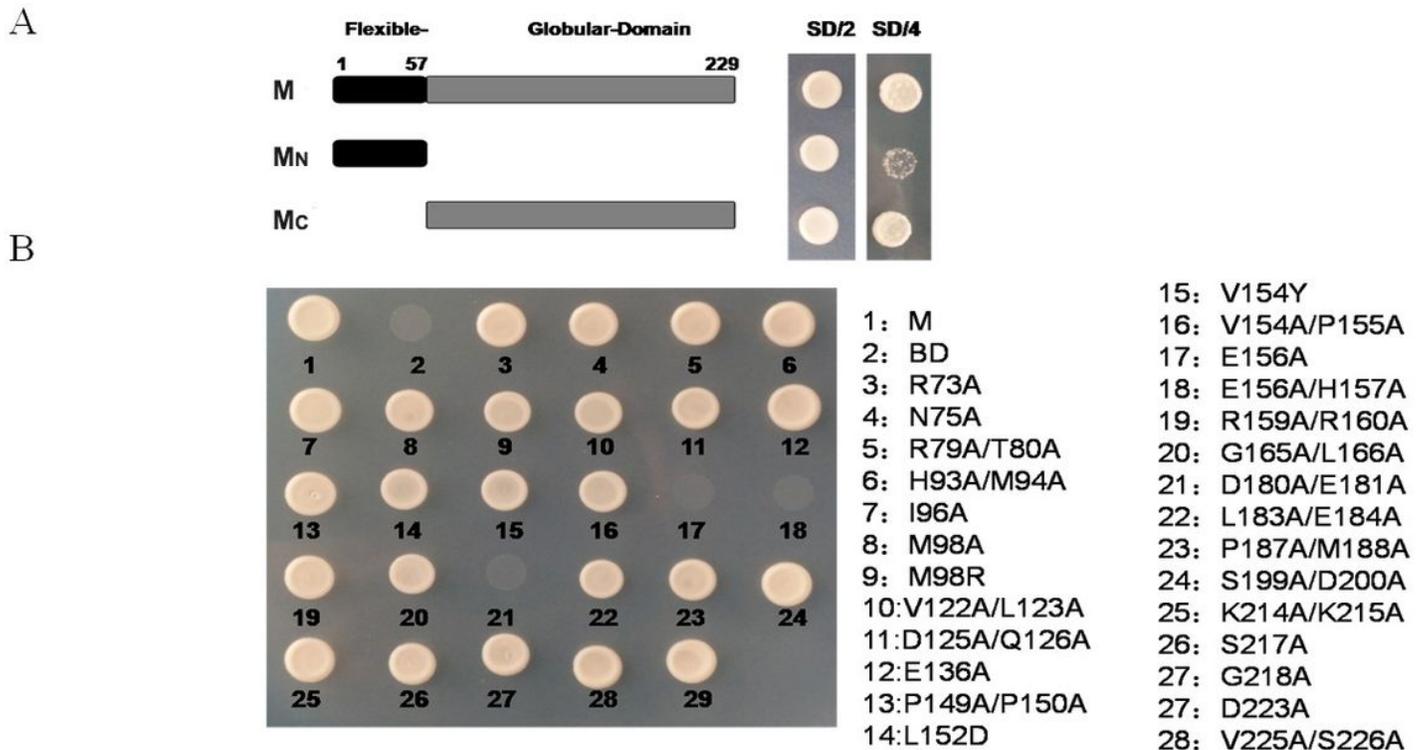


Figure 2

Identification of critical amino acids in the M protein involved in the M-Ndufaf4 interaction (A)
 Identification of the M protein domain that interacts with Ndufaf4. Y2H screens were performed to assess the interaction between the Ndufaf4 protein and the domains of the M protein. M1 and M2 refer to the flexible amino-terminal domain and the globular carboxy-terminal domain respectively. (B) Y2H screens were performed to confirm the interaction between mutant M proteins and Ndufaf4. All the experiments were repeated three times.

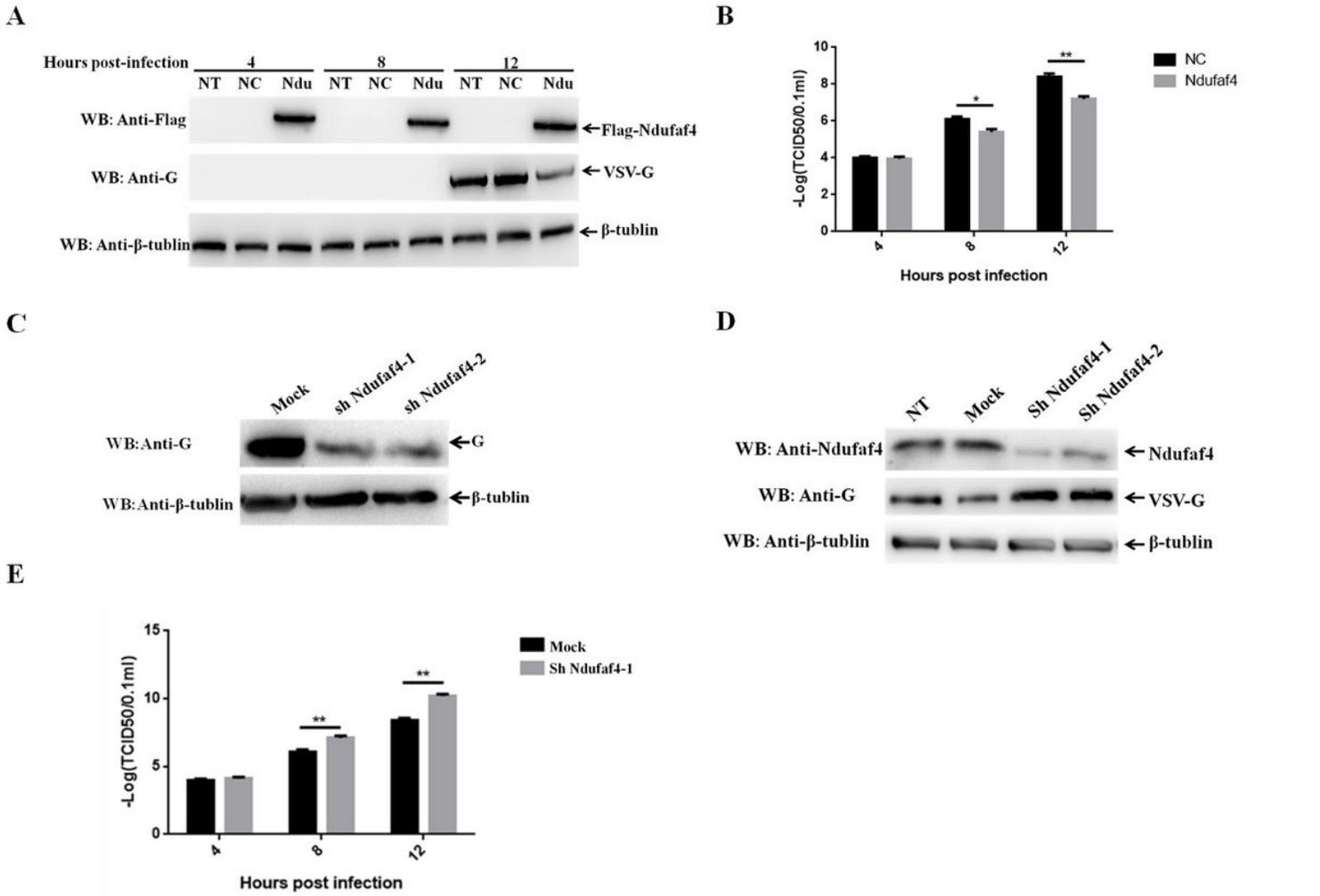


Figure 3

Ndufaf4 affects VSV replication (A) HeLa cells were transfected with pCMV-Flag-Ndufaf4 or pCMV-Flag-N empty vector and then infected with VSV at an MOI of 0.1. At 4, 8 and 12 hours postinfection, VSV G and Ndufaf4 protein levels were detected by Western blotting with anti-VSV-G Mab (1:2000) (catalog no. AB0053; Abways) and anti-Flag MAb (1:2000) (catalog no. 66008-2-Ig; Proteintech) respectively. NT: HeLa cells with non-transfection; NC: HeLa cells transfected with pCMV-Flag-N empty vector; Ndu: HeLa cells transfected with pCMV-Flag-Ndufaf4 vector. (B) HeLa cells were transfected with pCMV-Flag-Ndufaf4 or pCMV-Flag-N empty vector and then infected with VSV at an MOI of 0.1. At 4, 8 and 12 hours postinfection, VSV titer were detected by TCID50 assay at 4, 8 and 12 hours postinfection. (C) HeLa cells were transfected with plasmid to deliver short hairpin RNA (shRNA) targeting the Ndufaf4 gene. At 48 h posttransfection, levels of the Ndufaf4 protein were determined by Western blotting with anti-Ndufaf4 Rabbit Polyclonal antibody (1:2000) (catalog no. 26003-1-AP; Proteintech). Mock: HeLa cells transfected with plasmid to deliver control shRNA; sh Ndufaf4-1: HeLa cells transfected with plasmid to deliver sh Ndufaf4-1; sh Ndufaf4-2: HeLa cells transfected with plasmid to deliver sh Ndufaf4-2. (D) HeLa cells were transfected with plasmid to deliver control shRNA or shRNA targeting the Ndufaf4 gene. At 48 hours posttransfection, the cells were infected with VSV at an MOI of 0.1. At 12 hours postinfection, the VSV G

and Ndufaf4 protein level was detected by Western blotting with anti-VSV-G Mab (1:2000) (catalog no. AB0053; Abways) and anti-Ndufaf4 Rabbit Polyclonal antibody (1:2000) (catalog no. 26003-1-AP; Proteintech). NT: HeLa cells with Non-transfection; Mock: HeLa cells transfected with plasmid to deliver control shRNA; sh Ndufaf4-1: HeLa cells transfected with plasmid to deliver sh Ndufaf4-1. (E) HeLa cells were transfected with plasmid to deliver control shRNA or shRNA targeting the Ndufaf4 gene. At 48 hours posttransfection, the cells were infected with VSV at an MOI of 0.1. At 4, 8, and 12 hours postinfection, the VSV titer was detected by TCID50 assay. The experiments were repeated three times for (A), (C) and (D) and β -tubulin utilized as the load control. The data were averaged from three replicates of three independent experiments and analyzed for statistical significance by using Student's t-test for (B) and (E). The data are shown as the means \pm SD (** $p < 0.01$; * $p < 0.05$).

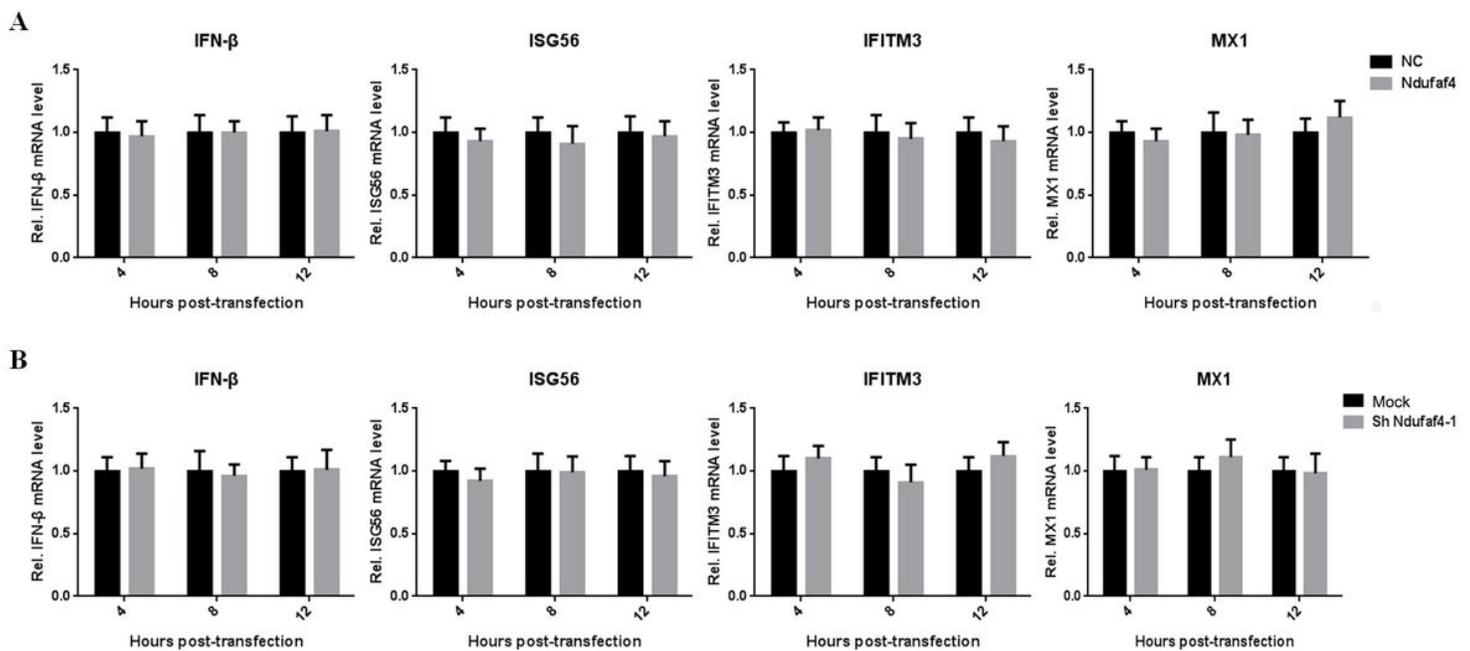


Figure 4

The anti-VSV effect of Ndufaf4 is independent of the type I IFN response (A) HeLa cells were transfected with pCMV-Flag-Ndufaf4 or pCMV-Flag-N empty vector and then infected with VSV at an MOI of 5. The mRNA levels of type I IFN pathway-related genes at 12 hours postinfection were detected by real-time RT-PCR. (B) HeLa cells were transfected with plasmid to deliver control shRNA or shRNA targeting the Ndufaf4 gene. At 48 hours posttransfection, the cells were infected with VSV at an MOI of 5. The mRNA levels of IFN- β and the IFN-stimulated genes at 4, 8 and 12 hours postinfection were detected by real-time RT-PCR. The data were averaged from three replicates of three independent experiments and analyzed for statistical significance by using Student's t-test. The data are shown as the means \pm SD.

Supplementary Files

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