



# Species-Specific Pathogenicity of Severe Fever with Thrombocytopenia Syndrome Virus Is Determined by Anti-STAT2 Activity of NSs

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ABSTRACT Severe fever with thrombocytopenia syndrome virus (SFTSV) is a novel emerging virus that has been identified in China, South Korea, and Japan, and it induces thrombocytopenia and leukocytopenia in humans with a high case fatality rate. SFTSV is pathogenic to humans, while immunocompetent adult mice and golden Syrian hamsters infected with SFTSV never show apparent symptoms. However, mice deficient for the gene encoding the  $\alpha$  chain of the alpha- and betainterferon receptor (Ifnar1-/- mice) and golden Syrian hamsters deficient for the gene encoding signal transducer and activator of transcription 2 ( $Stat2^{-/-}$  hamsters) are highly susceptible to SFTSV infection, with infection resulting in death. The nonstructural protein (NSs) of SFTSV has been reported to inhibit the type I IFN response through sequestration of human STAT proteins. Here, we demonstrated that SFTSV induces lethal acute disease in STAT2-deficient mice but not in STAT1deficient mice. Furthermore, we discovered that NSs cannot inhibit type I IFN signaling in murine cells due to an inability to bind to murine STAT2. Taken together, our results imply that the dysfunction of NSs in antagonizing murine STAT2 can lead to inefficient replication and the loss of pathogenesis of SFTSV in mice.

**IMPORTANCE** Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by SFTSV, which has been reported in China, South Korea, and Japan. Here, we revealed that mice lacking STAT2, which is an important factor for antiviral innate immunity, are highly susceptible to SFTSV infection. We also show that SFTSV NSs cannot exert its anti-innate immunity activity in mice due to the inability of the protein to bind to murine STAT2. Our findings suggest that the dysfunction of SFTSV NSs as an IFN antagonist in murine cells confers a loss of pathogenicity of SFTSV in mice.

**KEYWORDS** NSs, SFTSV, STAT2, animal model, mouse

**S**evere fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by the SFTS virus (SFTSV), which is a novel *Phlebovirus* of the *Phenuiviridae* family. SFTSV was first isolated in rural areas of central China in 2011 and subsequently identified in South Korea and Japan (1–4). Moreover, another emerging phlebovirus genetically close to SFTSV, Hartland virus, was found in the United States (5). SFTS is clinically characterized by fever, vomiting, diarrhea, thrombocytopenia, leukocytopenia, and elevated serum levels of enzymes, such as creatine kinase (CK), aspartate aminotransferase (AST), alanine transaminase (ALT), and lactate dehydrogenase (LDH) (6–8). However, the pathogenesis of SFTSV in humans is still poorly

Citation Yoshikawa R, Sakabe S, Urata S, Yasuda J. 2019. Species-specific pathogenicity of severe fever with thrombocytopenia syndrome virus is determined by anti-STAT2 activity of NSs. J Virol 93:e02226-18. https://doi .org/10.1128/JVI.02226-18.

**Editor** Rebecca Ellis Dutch, University of Kentucky College of Medicine

Copyright © 2019 American Society for Microbiology. All Rights Reserved. Address correspondence to Jiro Yasuda, j-yasuda@nagasaki-u.ac.jp. R.Y. and S.S. contributed equally to this work. Received 12 December 2018

Accepted 14 February 2019

Accepted manuscript posted online 27 February 2019 Published 1 May 2019 Yoshikawa et al.

understood, and no effective vaccines or antiviral drugs are currently available for treatment of SFTS.

The SFTSV genome is composed of three negative-strand RNA segments (S, M, and L). The L segment encodes the viral RNA-dependent RNA polymerase (L), the M segment encodes the glycoprotein precursors (Gn and Gc), and the S segment encodes the nucleocapsid protein (N) and nonstructural protein (NSs).

The innate immune response, including the type I interferon (IFN) response, is important for preventing viral infection (9). Antiviral innate immunity is initiated by the recognition of viral infection through cellular pattern recognition receptors (PRRs), such as transmembrane toll-like receptor 3 (TLR3), cytosolic RIG-I-like receptors, and MDA5 (10). Upon recognition, this signal cascade leads to the induction of type I IFN. The activation of the IFN signaling pathway by the binding of secreted IFN to IFN receptors results in the phosphorylation of STAT1 and STAT2. The heterodimer or homodimer of phosphorylated STAT forms heterotrimeric interferon-stimulated gene factor 3 (ISGF3) with IRF-9. The translocation of ISGF3 into the cell nucleus results in the activation of antiviral IFN-stimulated genes (ISGs) by its binding to an IFN-stimulated response element (ISRE) (11). However, during a phlebovirus infection, viral NSs is thought to play a major role in repressing the innate immune response by targeting the IFN response pathway as an IFN antagonist (12–15). Previous studies have reported that NSs of SFTSV inhibits type I and III IFN responses through sequestration of human STAT2 protein in viral replication complexes (13–15).

SFTSV infections do not cause severe disease in immunocompetent mice and golden Syrian hamsters, while type I IFN receptor knockout ( $Ifnar1^{-/-}$ ) mice, which lack the gene encoding the  $\alpha$  chain of the IFN- $\alpha$  and  $-\beta$  receptor, and STAT2-deficient golden Syrian hamsters are highly susceptible to SFTSV, with infection resulting in death (16–19). This suggests that efficient replication of SFTSV in mice and hamsters is prevented by antiviral innate immunity and that NSs of SFTSV does not inhibit IFN signaling in murine and hamster cells. STAT1 and STAT2 are important factors for antiviral innate immunity. However, the relationship between SFTSV pathogenicity and STAT2 in the pathogenesis and replication of SFTSV in mice, we examined the pathogenicity of SFTSV in *Stat1*<sup>-/-</sup> and *Stat2*<sup>-/-</sup> mice and measured the antagonistic activities of NSs against IFN signaling in murine cells.

# RESULTS

**SFTSV infection to** *lfnar1*<sup>-/-</sup> **mice.** It has been reported that *lfnar1*<sup>-/-</sup> mice are highly susceptible to SFTSV strains YL-1 and SPL010, with infection resulting in death (16–17). In this study, we used the YG-1 strain isolated from the first SFTS patient reported in Japan (4). Wild-type C57BL/6 mice and *lfnar1*<sup>-/-</sup> mice were intradermally (i.d.) inoculated with 10 focus-forming units (FFU) of the SFTSV (YG-1). All infected wild-type mice survived without any clinical signs (Fig. 1A). In contrast, all infected *lfnar1*<sup>-/-</sup> mice died 5 to 8 days after infection (Fig. 1A). Moreover, all *lfnar1*<sup>-/-</sup> mice infected with SFTSV showed severe body weight loss, leukocytopenia, and thrombocytopenia 1 to 7 days postinfection (p.i.) (Fig. 1B to D). The titers of SFTSV in the organs (brains, lungs, livers, spleens, kidneys, and intestines) and plasma of infected *lfnar1*<sup>-/-</sup> mice was observed in the spleen and plasma at 3 days p.i., all organs at 5 days p.i., and the spleen, kidney, and intestine at 7 days p.i. In contrast, we could not detect infectious SFTSV in the plasma or organs of wild-type mice.

The results show that SFTSV (YG-1) induces lethal acute infection accompanied by thrombocytopenia in *lfnar* $1^{-/-}$  mice.

**SFTSV** causes lethal infection in *Stat2*<sup>-/-</sup>mice but not *Stat1*<sup>-/-</sup> mice. To investigate the roles of STAT1 and STAT2 in SFTSV infection, *Stat1*<sup>-/-</sup>, *Stat2*<sup>-/-</sup>, and *Ifnar1*<sup>-/-</sup> mice next were infected with the YG-1 strain. None of the infected *Stat2*<sup>-/-</sup> mice survived, while all of the *Stat1*<sup>-/-</sup> mice infected with YG-1 survived (Fig. 1A). As



**FIG 1** Clinical pathologies of mice infected with SFTSV. Wild-type C57BL/6 (WT), *Ifnar1-/-*, *Stat1-/-*, and *Stat2-/-* mice were infected with 10 FFU of SFTSV (YG1). Survival (A) and body weight (B) changes were observed daily for 14 days p.i. (n = 10). For WBC and PLT counts, blood samples were collected from two male and three female mice at 0, 1, 3, 5, and 7 days p.i. The values are shown as means  $\pm$  standard deviations (SDs).

shown in Fig. 1B,  $Stat2^{-/-}$  mice lost body weight at 1 to 7 days p.i., while  $Stat1^{-/-}$  mice lost body weight at 1 to 5 days p.i. and then recovered. Unlike in  $Ifnar1^{-/-}$  mice, the number of white blood cells in  $Stat1^{-/-}$  and  $Stat2^{-/-}$  mice transiently decreased after infection and then recovered to normal values (Fig. 1C). Both  $Stat1^{-/-}$  and  $Stat2^{-/-}$  mice infected with SFTSV showed thrombocytopenia regardless of survival (Fig. 1D). This implies that the lethality of SFTSV is not associated with thrombocytopenia. We next measured the titers of SFTSV in organs (brains, lungs, livers, spleens, kidneys, and intestines) and plasma of infected  $Stat1^{-/-}$  mice, SFTSV was detected in the plasma, spleen, and kidney at 3 days p.i., the plasma and all organs at 5 days p.i., and the spleen and kidney at 7 days p.i. In  $Stat1^{-/-}$  mice, SFTSV replicated in the lung, spleen, kidney, intestine, and plasma; however, the maximum titers of SFTSV in these organs and plasma were lower than those in  $Stat2^{-/-}$  mice (Fig. 2).

These results indicate that  $Stat2^{-/-}$  and  $Ifnar1^{-/-}$  mice, but not  $Stat1^{-/-}$  mice, are highly susceptible to SFTSV infection, which suggests that STAT2 plays a critical role in the suppression of SFTSV replication in mice.

**SFTSV NSs cannot suppress type I IFN signaling in murine cells.** It has been reported that SFTSV suppresses type I IFN signaling in human cells (13). Therefore, we hypothesized that SFTSV cannot suppress type I IFN signaling in murine cells, and IFN-mediated innate immunity restricts SFTSV replication in mice. To address this possibility, the ISRE activation by SFTSV infection was examined in human-derived HEK293T cells and mouse-derived NIH 3T3 cells using dual-luciferase reporter (DLR) gene assay. As shown in Fig. 3A, SFTSV infection did not induce the ISRE activation in HEK293T cells, while the ISRE activation was induced by SFTSV infection in NIH 3T3 cells. These results suggest that SFTSV cannot inhibit type I IFN signaling in murine cells.

Recently, SFTSV NSs has been reported to function as an IFN antagonist (13–15). Therefore, the effects of NSs on ISRE activation in HEK293T cells and NIH 3T3 cells were



**FIG 2** Titers of SFTSV in mouse organs. Mice were inoculated with 10 FFU of SFTSV YG1. Three female mice were euthanized for virus titration at 3, 5, and 7 days p.i. The virus titers in the mouse brains, lungs, livers, spleens, kidneys, intestines, and plasma were measured by focus-forming assay. The values are shown as means  $\pm$  SDs (n = 3). \*, P < 0.05 for comparisons for each mouse strain.

examined by DLR gene assay. In this experiment, the VP40 protein of mouse-adapted Marburg virus (mMARV), which functions as an IFN signaling inhibitor in murine cells, was used as a positive control (20). As shown in Fig. 3B, IFN- $\alpha$ A/D treatment induced strong ISRE activation in both HEK293T and NIH 3T3 cells. As expected, the expression of SFTSV NSs significantly inhibited the ISRE activation driven by IFN- $\alpha$ A/D in HEK293T cells, while NSs expression did not suppress this activation in NIH 3T3 cells (Fig. 3B). We also confirmed that the ISRE activation driven by IFN- $\alpha$ A/D in NIH 3T3 cells was suppressed by the expression of mMARV VP40 (Fig. 3C). These results suggest that SFTSV NSs cannot interfere with type I IFN signaling in murine cells.

We also examined the effect of NSs on IFN- $\alpha$ A/D-induced expression of mRNA for two ISGs, ISG56 and oligoadenylate synthetase 1 (OAS1), by real-time quantitative PCR (qPCR). The induction of both ISGs by IFN in HEK293T cells was suppressed by NSs expression, whereas NSs did not suppress induction in NIH 3T3 cells (Fig. 3D).



FIG 3 Function of NSs as an IFN antagonist in human and murine cells. (A) HEK293T or NIH 3T3 cells transfected with the reporter plasmids were mock infected or infected with SFTSV. Two days after infection, cells were lysed to measure luciferase activity and to examine protein expression using immunoblotting. Relative light units (RLU) in mock-infected cells were set as 1. Fold activation by SFTSV infection is indicated. (B) The reporter plasmids were transfected into HEK293T or NIH 3T3 cells with or without the expression plasmid for NSs. Twenty-four h after transfection, cells were treated with  $IFN-\alpha A/D$  (500 U/ml) or left untreated for 18 h and then were lysed to measure luciferase activity and detect protein expression using immunoblotting. RLU in cells transfected with the empty vector or the indicated amount of NSs expression plasmid in the absence of IFN- $\alpha$ A/D were set as 1. Fold activation by IFN- $\alpha$ A/D is indicated. (C) The experiment depicted in panel B was repeated using the expression plasmid for mMARV VP40 in NIH 3T3 cells. (D) NSs expression plasmid (1  $\mu$ g or 1.5  $\mu$ g) or control plasmid was transfected into HEK293T or NIH 3T3 cells, respectively. Twenty-four h after transfection, the cells were treated with IFN- $\alpha$ A/D (200 U/ml) for 10 h or left untreated. Expression levels of *Isq56* and *Oas1* mRNAs in each cell line were measured by real-time qPCR. The mRNA expression levels of ISG56 and OAS1 in untreated cells were set as 1. Fold activation by IFN- $\alpha$ A/D is indicated. These assays were independently performed in triplicate. The data represent averages with SDs. \*\*, P < 0.05 versus no NSs.

**NSs does not interact with murine and hamster STAT2.** SFTSV NSs inhibits type I IFN signaling by the interaction with human STAT1 (hSTAT1) and STAT2 (hSTAT2) (13–15). However, the interaction with hSTAT1 is weaker than that with hSTAT2 (15). We also showed here that mice deficient for STAT2, but not STAT1, were highly susceptible



**FIG 4** Interaction of NSs with STAT1 and STAT2. (A) HEK293T or NIH 3T3 cells were transfected with the expression plasmid for HA-tagged NSs, and co-IP assays were performed. (B) NIH 3T3 cells were transfected with the expression plasmids for HA-tagged NSs and His-tagged hSTAT2 or hamSTAT2. The protein expression levels in cell lysates (left) and in co-IP assays (right) using an anti-HA or anti-His antibody are shown. (C) Colocalization of NSs with STAT2. HEK293T, NIH 3T3, or BHK-21 cells were transfected with the expression plasmid for HA-tagged NSs and the expression plasmid for His-tagged hSTAT2, mSTAT2, or hamSTAT2, respectively. IFA was also performed with NSs, STAT2, and the nuclei shown in green, red, and blue, respectively.

to SFTSV infection and progressed to severe disease (Fig. 1). Therefore, we suggest that NSs cannot interact with murine STAT2 and, thus, cannot antagonize IFN signaling in murine cells.

First, to examine whether NSs interacts with murine STAT2 (mSTAT2), we performed coimmunoprecipitation (co-IP) assays using lysates from cells transfected with an NSs expression plasmid. As shown in Fig. 4A, co-IP of STAT2 with NSs was observed in the lysates from HEK293T cells but not from NIH 3T3 cells, suggesting that NSs interact with hSTAT2 but not with mSTAT2. It also indicated that NSs bound to hSTAT1 but not to murine STAT1 (mSTAT1) (Fig. 4A).

We also examined the interaction of NSs with hamster STAT2 (hamSTAT2) by co-IP assay, since  $Stat2^{-/-}$  hamsters, like  $Stat2^{-/-}$  mice, are highly susceptible to SFTSV infection (19). As shown in Fig. 4B, the interaction of NSs with hamSTAT2, as well as mSTAT2, was not observed.

The interaction of NSs with STAT2 was also examined by subcellular colocalization of the proteins. The NSs expression plasmid was cotransfected with the expression plasmids for hSTAT2, mSTAT2, or hamSTAT2 into HEK293T, NIH 3T3, or BHK-21 cells, respectively, and subcellular localizations of proteins were observed. Cytoplasmic inclusion bodies (IBs), mainly formed by NSs, were also observed in HEK293T, NIH 3T3, and BHK-21 cells (Fig. 4C). In HEK293T cells, hSTAT2 colocalizes with NSs, consistent with previous reports (13–15). On the other hand, in NIH 3T3 and BHK-21 cells, colocalization of NSs with mSTAT2 or hamSTAT2 was not observed (Fig. 4C). These findings indicate that NSs interacts with hSTAT2 but not mSTAT2 and hamSTAT2.

The N-terminal region of hSTAT2 is important for binding to NSs. To investigate the difference in NSs binding between human and murine STAT2, we prepared a series of chimeric proteins from hSTAT2 and mSTAT2 (Fig. 5A). The interactions between NSs and the chimeric STAT2 proteins were examined by co-IP assay. All chimeric proteins were efficiently expressed in NIH 3T3 cells (Fig. 5B). As shown in Fig. 5B, NSs interacted with hSTAT2, HHM, HMM, H(101-315)MM, and H(101-315)HM but not mSTAT2, MHH, MMH, H(1-100)MM, H(1-221)MM, and H(222-315)MM.

We also confirmed the results by observation of colocalization of the proteins (Fig. 5C). NSs colocalized with hSTAT2, HHM, HMM, H(101-315)MM, and H(101-315)HM, while mSTAT2, MHH, MMH, H(1-100)MM, H(1-221)MM, and H(222-315)MM did not colocalize with NSs. These results are consistent with those from the co-IP assay. It has been reported that SFTSV NSs interacts with the DNA-binding domain (DBD; amino acid positions 316 to 485) of hSTAT2 (13). Taken together, these results suggest that region 101-315 of hSTAT2 is important for the binding to NSs in addition to the DBD, or that this region of mSTAT2 interferes with the binding to NSs.

To further examine whether MHH and MMH, which cannot bind to NSs, function as STAT2 proteins in the presence of NSs, ISRE activation by MHH or MMH in the presence of NSs was investigated by the DLR assay in HEK293T cells. ISRE activation was reduced by NSs in the absence of exogenous STAT2, and overexpression of exogenous hSTAT2 slightly compensated for this reduction induced by NSs (Fig. 6). In contrast, the suppression of ISRE activation by NSs was significantly recovered by mSTAT2, MMH, and MHH, suggesting that MMH and MHH, as well as mSTAT2, activate ISRE as a functional STAT2 protein in the presence of NSs in HEK293T cells.

**Type I IFN induces the phosphorylation of mSTAT2 in the presence of NSs.** Tyrosine phosphorylation of STAT2 is important for its function as a transcription factor in the type I IFN signaling pathway (21). To assess whether type I IFN signaling can phosphorylate mSTAT2 in the presence of NSs, HEK293T and NIH 3T3 cells were transfected with empty vector or the NSs-HA expression plasmid and then treated with IFN-αA/D. The expression levels of hSTAT2 and mSTAT2 were stable regardless of NSs expression (Fig. 7A). In the absence of NSs, IFN induced the phosphorylation of hSTAT2 and mSTAT2. hSTAT2 phosphorylation in HEK293T cells was significantly downregulated by NSs in a concentration-dependent manner. In contrast, mSTAT2 in NIH 3T3 cells was phosphorylated irrespective of NSs expression (Fig. 7A). These were also observed in SFTSV-infected cells (Fig. 7B). It is likely that NSs cannot interfere with the phosphorylation of mSTAT2, since NSs cannot bind to mSTAT2.

## DISCUSSION

Previous studies in animal models of SFTSV infection indicated the importance of the type I IFN response in mice and STAT2 in hamsters to prevent disease progression (16, 17, 19). Both STAT1 and STAT2 have been found to be key factors in the IFN signaling pathway (21). In this study, we demonstrated that STAT2-deficient mice, as well as type I IFN receptor-deficient mice, are more susceptible to SFTSV than STAT1-deficient mice and wild-type mice. Moreover, our results indicate that NSs has no ability



**FIG 5** Determination of the region of hSTAT2 important for binding to NSs. (A) Schematic representation of the chimeric mutants of hSTAT2 and mSTAT2. (B) NIH 3T3 cells were cotransfected with the expression plasmids for HA-tagged NSs and each of the STAT2 His-tagged chimeras. Representative results from the protein expression check in cell lysates (upper) and the co-IP assays using an anti-His antibody (lower) are shown. (C) IFA was also performed with NSs, STAT2, and the nuclei shown in green, red, and blue, respectively.

to suppress the IFN signaling pathway in murine cells because NSs, which binds hSTAT1 and hSTAT2, cannot interact with mSTAT1 and mSTAT2. SFTSV growth in the organs of  $Stat1^{-/-}$  mice was much less efficient than that in  $Stat2^{-/-}$  and  $Ifnar1^{-/-}$  mice, although SFTSV could grow in  $Stat1^{-/-}$  mice (Fig. 2). In addition, SFTSV infection induced milder symptoms in  $Stat1^{-/-}$  mice than in  $Stat2^{-/-}$  and  $Ifnar1^{-/-}$  mice (Fig. 1). These findings suggest that innate immunity dependent on STAT2, but not STAT1, strongly inhibits the replication of SFTSV in mice. In several human cell lines, the expression of some ISGs is upregulated by STAT2 independent of STAT1 (22). For example, the expression levels of several ISGs, including APOBEC3G, PKR, ISG15, and Mx1, are increased by type I IFN stimulation regardless of STAT1 expression in the human liver cell lines Huh7 and Hep3B. However, when the expression of STAT2 is



**FIG 6** Function of the NSs binding-deficient STAT2 chimera. The reporter plasmids were cotransfected into HEK293T cells with the expression plasmids for NSs and each STAT2 chimera. Twenty-four h after transfection, cells were treated with IFN- $\alpha$ A/D (500 U/ml) for 18 h or left untreated and then were lysed to measure luciferase activity (upper) or detect protein expression using immunoblotting (lower). The ISRE activity was calculated by dividing RLU in IFN- $\alpha$ A/D-treated cells by the units of cells not treated with IFN- $\alpha$ A/D. The ISRE activity in the absence of NSs was set as 100%. The assays were independently performed in triplicate. The data represent averages with SDs. \*\*, P < 0.05 versus hSTAT2.

suppressed, the expression levels of these ISGs are not increased after type I IFN treatment. These findings suggest that ISG expression mediated by STAT2, but not STAT1, mainly suppresses SFTSV infection in mice.

SFTSV causes severe disease in humans, while immunocompetent adult mice never show any apparent severe symptoms after SFTSV infection (18). In this study, we showed that mice lacking STAT2 are highly susceptible to SFTSV infection. Moreover, NSs can suppress the phosphorylation of hSTAT2, whereas the phosphorylation of mSTAT2 is not inhibited by NSs due to the inability of NSs to bind to mSTAT2. The data also indicate that NSs cannot interact with hamSTAT2. This result is consistent with a previous report that showed that STAT2-deficient hamsters are also highly susceptible to SFTSV infection (19).

The relationship between NSs and STAT2 is reminiscent of that of dengue virus NS5 and STAT2 (23–25). Previous studies reported that innate immunity mediated by mSTAT2 restricts dengue virus replication in mice (25, 26). To block the type I IFN signaling pathway in humans, dengue virus NS5 expression leads to the degradation of hSTAT2 (25). However, NS5 cannot suppress type I IFN signaling in mice, because mSTAT2 is resistant to NS5-mediated degradation (23–25). These results demonstrate that STAT2 may be one of the determinants for the species specificity of dengue virus. Here, we elucidated that SFTSV induces lethal disease in STAT2-deficient mice and that NSs cannot interact with mSTAT2. Thus, similar to dengue virus NS5, the anti-STAT2 activity of NSs appears to determine the species specificity of SFTSV infection.

In this study, chimeric mutants of hSTAT2 and mSTAT2 revealed that residues 101 to 315 of hSTAT2 are required for the interaction with NSs or that these residues in



**FIG 7** Suppression of STAT2 phosphorylation by NSs. (A) HEK293T or NIH 3T3 cells transfected with the expression plasmid for HA-tagged NSs were treated with IFN- $\alpha$ A/D (2,000 U/ml) for 45 min or left untreated and were then lysed for detection of expression of each protein by immunoblotting. (B) HEK293T or NIH 3T3 cells infected with SFTSV at an MOI of 10 were treated with IFN- $\alpha$ A/D (2,000 U/ml) for 45 min or left untreated and were then lysed for detection of expression of each protein by immunoblotting.

mSTAT2 interfere with the interaction with NSs. We also confirmed that mSTAT2 and all of the chimeric mutants of hSTAT2 and mSTAT2 used in this study can activate ISRE-mediated gene expression as functional STAT2 proteins. Previously, Ning et al. reported that the DBD region (316-485) of hSTAT2 is required for the interaction with NSs (13). However, we showed that mutants possessing the DBD region of mSTAT2, HMM, and H(101-315)MM can still bind to NSs (Fig. 5). At present, we cannot explain this result, although this discrepancy may be explained by the differences in the three-dimensional protein structure between our mutants and Ning et al.'s deletion mutants. Further analyses will be required to clarify this issue.

Taken together, we conclude that the anti-STAT2 activity of NSs determines the species specificity of SFTSV infection. In addition, we show that  $Stat2^{-/-}$  mice, as well as  $Ifnar1^{-/-}$  mice and  $Stat2^{-/-}$  hamsters, are highly susceptible to SFTSV infection, which causes lethal disease, suggesting that  $Stat2^{-/-}$  mice are useful as an animal model to develop antiviral drugs against SFTSV infection.

# **MATERIALS AND METHODS**

**Ethics statement.** Our research protocol for the use of mice follows the Nagasaki University Regulations for Animal Care and Use, which were approved by the Animal Experiment Committee of Nagasaki University (approval number 151110-1-5).

**Animals.** The B6.129-Dnase2a<tm10sa>lfnar1<tm1Agt> mouse strain (RBRC04021; *lfnar1<sup>-/-</sup> Dnase2a<sup>+/-</sup>*) (27) was provided by RIKEN BRC through the National BioResource Project of MEXT, Japan. *lfnar1<sup>-/-</sup>* mice were generated by crossing *Dnase2a<sup>+/-</sup> lfnar1<sup>-/-</sup>* parents. *Stat1<sup>-/-</sup>* mice were provided by Takayuki Yoshimoto (Tokyo Medical University). *Stat2<sup>-/-</sup>* mice were purchased from the Jackson Laboratory. The genetic background of all mice used in this study is C57BL/6.

**SFTSV infection in mice.** Six- to 8-week-old male or female mice were used in this study. In infection experiments, each mouse was infected with SFTSV by intradermal injection (50  $\mu$ l of virus solution for 10 FFU). Mouse survival and body weight changes were monitored daily for 14 days p.i. At 1, 3, and 7 days p.i., blood was collected and platelets and leukocytes were counted using a hematology analyzer (Sysmex pocH-100iV; Sysmex or VetScan HMII; Abaxis). Mice were euthanized, and plasma and organs (lungs, livers, spleens, kidneys, intestines, and brains) were collected. Viruses in plasma were titrated by focus-forming assay using Vero E6 cells. To determine the titer of SFTSV in organs, mouse organs were collected in a 9-fold volume of minimum essential medium (Sigma-Aldrich) and then disrupted through high-speed shaking using a TissueLyser II (Qiagen). After centrifugation (700  $\times$  g, 5 min, 4°C), the amounts of viruses in the homogenates (10%, wt/vol) were determined by focus-forming assay in Vero E6 cells. The limit of detection was 100 FFU/g.

**Cell culture and virus.** Human embryonic kidney (HEK) 293T (CRL-11268; ATCC), NIH 3T3 (CRL-1658; ATCC), BHK-21 (JCRB9020; Health Science Research Resources Bank [HSRRB]), and Vero E6 (CRL-1586; ATCC) cells were cultured in Dulbecco's modified Eagle's medium (Sigma-Aldrich) supplemented with 10% heat-inactivated fetal calf serum (FCS) and antibiotics (Thermo Fisher Scientific). SFTSV YG-1, a field isolate from an SFTS patient in Japan, was kindly provided by Ken Maeda, Yamaguchi University (4). The virus stocks were prepared from culture supernatants of Vero E6 cells.

**Focus-forming assay.** SFTSV titers were determined using a focus-forming assay. Briefly, confluent monolayers of Vero E6 cells were inoculated with 10-fold dilutions of SFTSV and incubated at 37°C for 1 h. The inoculum was removed, and cells were washed and overlaid with minimal essential medium (Sigma-Aldrich) containing 0.7% agarose and 0.7% FCS. After 4 days, cells were fixed with 4% paraformaldehyde and permeabilized with phosphate-buffered saline (PBS) containing 1% Triton X-100. Cells were blocked with PBS containing 0.1% Triton X-100 and 1% bovine serum albumin (BSA) and then incubated with anti-SFTSV N protein rabbit polyclonal antibody (28). After washing with PBS, the cells were incubated with PBS, SFTSV-infected cells were detected by using the peroxidase (HRP; Promega). After more washes with PBS, SFTSV-infected cells were detected by using the peroxidase stain DAB kit and metal enhancer for DAB stain (Nacalai Tesque). The number of SFTSV N-positive cells was determined and normalized as FFU per milliliter.

**Plasmids.** The open reading frame (ORF) encoding NSs was amplified by reverse transcription-PCR (RT-PCR) from SFTSV (YG-1) viral RNA and inserted into pcDNA3.1 (Invitrogen) with a hemagglutinin (HA) tag using the primers listed in Table 1 to produce pcDNA3.1/NSs-HA. To prepare the expression plasmids for  $6 \times$  histidine ( $6 \times$ His)-tagged hSTAT2 (pcDNA3.1/hSTAT2-His), mSTAT2 (pcDNA3.1/mSTAT2-His), and hamSTAT2 (pcDNA3.1/hamSTAT2-His), the desired genes were amplified by RT-PCR using the primers listed in Table 1 from the cDNA of HEK293T, NIH 3T3, and BHK-21 cells, respectively. The expression plasmids for the series of STAT2 chimeras were constructed using an In-Fusion HD cloning kit (TaKaRa) with the primers listed in Table 1. The expression plasmid for mMARV VP40 was constructed from MARV VP40 (29) using a KOD-Plus mutagenesis kit (Toyobo) using the primers listed in Table 1.

**Reporter gene assay.** HEK293T cells and NIH 3T3 cells were cotransfected with ISRE reporter plasmid (450 ng) (Promega) and pRL-TK plasmid (the *Renilla* luciferase control plasmid for the constitutively active herpes simplex virus [HSV]-thymidine kinase [TK] promoter) (100 ng) (Promega). Twenty-four h after transfection, the transfected cells were mock infected or infected with SFTSV. Two days after infection, luciferase activities were measured with a DLR assay kit (Promega) and a TriStar LB941 system (Berthold).

For the reporter gene assays with SFTSV NSs transfection, the ISRE reporter plasmid (500 ng) (Promega) and pRL-TK plasmid (100 ng) (Promega) were transfected into HEK293T and NIH 3T3 cells with or without the indicated amount of pcDNA3.1/NSs-HA plasmid or the expression plasmid for mMARV VP40 (800 ng) using LT-1 (Mirus) or Lipofectamine 3000 (Thermo Fisher Scientific) according to the manufacturer's instructions. Twenty-four h after transfection, cells were treated with IFN- $\alpha$ A/D (500 U/ml) (Sigma-Aldrich) or were left untreated for 18 h. Luciferase activities then were measured with a DLR assay kit (Promega) and a TriStar LB941 system (Berthold).

**Quantitative RT-PCR of IFN-treated cells.** Total RNA was extracted from HEK293T and NIH 3T3 cells using an RNeasy Minikit (Qiagen). Real-time RT-PCR was performed by using the one-step TB Green PrimeScript Plus RT-PCR kit (TaKaRa) according to the manufacturer's instructions, and the PCR primers used in this study are listed in Table 1. Relative mRNA levels were calculated by the  $2^{-\Delta\Delta CT}$  method with glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA as an internal control and are shown as relative fold changes normalized to the untreated control samples.

**Immunoblotting.** Protein samples were separated by SDS-PAGE and transferred to nitrocellulose membranes (Millipore). After blocking with 5% skim milk in Tris-buffered saline–Tween 20 (TBS-T), the membranes were incubated with each of the following antibodies: anti-HA (18850; QED Biosciences Inc.), anti-His (9F2; Wako), anti-hSTAT2 (A-9; Santa Cruz), anti-mSTAT2 (07-140; Merck), anti-STAT2 (phosphor Y690; ab53132; Abcam), anti-STAT1 (D19KY; Cell Signaling), and anti- $\beta$  actin (AC-15; Sigma-Aldrich). After washing with TBS-T, the membranes were incubated with horseradish peroxidase-labeled secondary antibodies, anti-mouse IgG-HRP (A2304; Sigma-Aldrich), or anti-rabbit IgG-HRP (WA4011; Promega) and then detected using ECL prime (GE Healthcare) according to the manufacturer's instructions. Bands were visualized using an image analyzer (LAS-4000 mini; GE Healthcare).

**Co-IP assay.** To examine the binding of NSs to endogenous STAT2, the pcDNA3.1/NSs-HA plasmid (15  $\mu$ g) was transfected into HEK293T and NIH 3T3 cells in 10-cm dishes (Thermo Fisher Scientific) using LT-1 or Lipofectamine 3000, respectively. To examine the binding of NSs to exogenous STAT2 or STAT2 derivatives, the pcDNA3.1/NSs-HA plasmid (3  $\mu$ g) was cotransfected into NIH 3T3 cells with each STAT2 expression plasmid (1  $\mu$ g) in 6-well plates (Thermo Fisher Scientific) using Lipofectamine 3000. Two days after transfection, cells were lysed in lysis buffer (25 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, and 1%

# TABLE 1 List of the primers used in this study

DDM. NSH4 RVF     ATCCACCATCTEGETCAGECANTEGETCANE     For construction of pcDNA3.1/N5S-HA       DDM. NSH4 RVF     ATCCACCATCTEGEGECATEGEGAACACCTATEGATAGAC     For construction of pcDNA3.1/N5S-HA       DUM. MSH4 RVF     ATCCACCACTGEGECATEGEGAACACCTGGATAGACA     For construction of pcDNA3.1/N5S-HA       DUMORSTAT2INF     CCTCGTGACCTCAATGGGAATGCTG     For construction of pcDNA3.1/N5TAT2-His       DUMORSTAT2INF     CCTCGTGACCTCAATGGGAATGGTGATGGATAGCATATTGAAG     For construction of pcDNA3.1/N5TAT2-His       DUMORSTAT2INF     CCTCGTGACCTCAATGGGAAGATGG     For construction of pcDNA3.1/NamSTAT2-His       DUMORSTAT2INF     CCTCGGACGCCCCCCAAAG     For construction of pcDNA3.1/NamSTAT2-His       DISTAT2-NDBN-MC     CCTCGCACAGCCCCAAAG     For construction of pcDNA3.1/NamSTAT2-His       DISTAT2-NDBN-MC     CCTCGCACAGCCCCAAAG     For construction of pHHM(#1)       NMMATHMI-NIR     ACCGGTATGCAATTGAAGACCCCGCCCG     For construction of pHHM(#2)       NMMATATEADBN-MC     ACCGGTATGCAATTGAAGACCCCGCCCGG     For construction of pHHM(#2)       NMM HMM-NR     ACCGGTATGCAATTGAAGACCCACCCCCCAAGG     For construction of pMHH(#4)       NMM HMM-NR     ACCGGTATGCAATGCCGCAGCCCCCCAAGG     For construction of pHHM(#2)       NMM HMM-NR     ACCGGTATGCAATGCCGCAGCCCCCCAAGG     For cons	Name	Sequence	Remark <sup>a</sup>
pcDM-MSH4Kpn-R     CCTTGGTACCTCAQCGTAATCGGAACACCGTATGGAATAGAC       humanSTAT2hink     ATCCACCATGGGCACACGGGAAATCGTG     For construction of pcDNA3.1/hSTAT2-His       mouseSTAT2hink     CATCGTGCACGGGAATGGGAATGGTG     For construction of pcDNA3.1/hSTAT2-His       mouseSTAT2hink     CCTTGGTACCTCAATGGGACACGGGGAAGCGTG     For construction of pcDNA3.1/hSTAT2-His       mouseSTAT2hink     CCTTGGTACCTCAATGGGACACGGGAGACGCGG     For construction of pcDNA3.1/hsTAT2-His       mamSTAT2hink     CCTTGGTACCTCAATGGGACACGGGAGACGCGG     For construction of pcDNA3.1/hsTAT2-His       mamSTAT2he     ATCCACCCAAGGGGAGACGCGG     For construction of pcDNA3.1/hsTAT2-His       mSTAT2-bBBVveF     GCTTGGTACGCAATGGGAGACGCGG     For construction of pcDNA3.1/hsTAT2-His       mSTAT2-bDBDveF     AACGGGTCATGCTATGGGAGAGCGGAGACGCGG     For construction of pcDNA3.1/hsTAT2-His       mSTAT2-bDBDveF     ACGGGGTCATGCTATGCAGGGTATC     For construction of pHMM(#1)       hmSTAT2bDDDveF     ACGGGGTCATGCATGCGAGGTATC     For construction of pHMM(#2)       hmSTAT2bDDveF     ACGGGGGTCATGCACGCC     For construction of pMMH(#3)       mSTAT2bDDveF     ACGGGGTCATGCACGCC     For construction of pMMH(#4)       mSTAT2bDDveF     ACGGGTCATGCCACGCCCCCCGGGGCAGC     For construction of pH(1-100)MM(#5)	pcDNA-NSsHA-RV-F	ATCCACCATGTCGCTGAGCAAATGCTCCAAC	For construction of pcDNA3.1/NSs-HA
Junnanistra     ATCCACCATGGCGCAGTGGGAATGCTG     For construction of pcDNA3.1/hSTAT2-His       Numensistra     ATCCACCATGGCGCAGTGGGAATGTG     For construction of pcDNA3.1/hSTAT2-His       mouseSTAT2hisR     ATCCACCATGGCGCAGTGGGAATGTG     For construction of pcDNA3.1/hSTAT2-His       massTAT2hisR     GCTTGGTACCTCAATGGTGATGGTGATGATGGACGCGATTGCATATTGAGG     For construction of pcDNA3.1/hSTAT2-His       hamSTAT2hBV     GCTTGGTACCTCAATGGTGATGGTGATGGCGATGCCATATTGCAT     For construction of pcDNA3.1/hsTAT2-His       hamSTAT2hDV     GCTTGGTACCTCAATGGTGATGGTGATGGCATATGGCATATTGCAT     For construction of pdHM(#1)       hamSTAT2hDV     GCTTGGTACCTCAATGGTGATGGCAGGG     For construction of pHMM(#1)       hMMHHM1in     ACAGGCCTTTGCATATGGAAGCCGCCCTG     For construction of pHMM(#2)       hMMHMHAININ     ACCGGTATGCATATGGAAGCCGACCCTG     For construction of pMHM(#2)       hMMHAININN     ACCGGTATGCATATGGAAGCCGACCCTG     For construction of pMHM(#2)       hMMATATTGAAGGGTATC     For construction of pMHM(#2)     ACCGGTATGCATATGGAAGCCGACCCTG       hMMHAINNIN     ACCGGTATGCATATGGAAGCCGACCCTG     For construction of pMHH(#3)       hMMATATGAAGCCATCCCCCCCAAG     For construction of pMHH(#3)     ACCGGTATGCATATGGAAGCCGACCGCCTG       hMMATATGCATACCGGCACTATAGCCAAGCCCAAGC     For construction	, pcDNA-NSsHA-Kpn-R	GCTTGGTACCTCAAGCGTAATCTGGAACATCGTATGGATAGAC	
humanSTAT2bBPreF ATATCACCATGGCGCATGGGGAGCGC mouseSTAT2bilsF ATCCACCATGGCGCAGGGGGAGCACTG GCTGGTACCTCAATGGTGATGGTGATGGTGATGGTGATGGCGATATGCATGTGAG mouseSTAT2bilsF ATCCACCATGGCGCAGGGGGAGCACTG GCTGGTACCTCAATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGAGGGGGG	humanSTAT2hisF	ATCCACCATGGCGCAGTGGGAAATGCTG	For construction of pcDNA3 1/bSTAT2-His
CAGAGEGET CAGAGEGATE CAGE     For construction of pcDNA3.1/mSTAT2+His       MOUSESTAT2hisF     ATCCACCATGGEGEAGAGEGEAGAGECCGA     For construction of pcDNA3.1/mSTAT2+His       MamSTAT2hisF     CACCATGGEGEAGAGECCGA     For construction of pcDNA3.1/mSTAT2+His       MamSTAT2hisF     CACCATGGEGEAGAGECGA     For construction of pcDNA3.1/mSTAT2+His       mSTAT2-bDBD-wF     GTTCGTCACTCAAGEGTCCCAA     For construction of pHHM(#1)       mSTAT2-bDBD-wF     GTTCGTCACCCAAGEGGAGEACCCGG     For construction of pHHM(#1)       mSTAT2-bDBD-wF     ACCAGECATTGGAAGAGECACCCGG     For construction of pHMM(#2)       mSTAT2-bDBD-wF     ACCAGECCTTGGTGGAGAACCCAGECCTG     For construction of pHMM(#2)       mSTAT2-bDBD-wF     ACCAGECCTTGTGGAGAACCCAGECCTG     For construction of pMHH(#3)       mSTAT2-bDBD-wF     ACCAGECCTTGTGGAGAACCCAGECCTG     For construction of pMHH(#3)       mSTAT2-bDBD-wF     ACCAGECCTTGTGGAGACGACGCC     For construction of pMHH(#4)       mSTAT2-bDBD-wF     ACCAGECCTTGTGGAGACGACGCC     For construction of pMHH(#4)       mSTAT2-bDBD-wF     ACCAGECCTTGTGGAGACGACGAC     For construction of pMHH(#4)       mSTAT2-bDBD-wF     ACCAGECTCTATCGAACCCAGECCCCCAGE     For construction of pH(1-100)MM(#5)       mSTAT2-bDBD-wF     ACCAGECTCTAC	humanSTAT2hisR	GCTTGGTACCTCAATGGTGATGGTGATGATGACCGGTATGCATATTGAAGT	
mouseSTAT2:hBPs ATCACCATGSCCCAGGGGGAGATGTG GATGATGGGTATGCATATTGAG GCTTGGTACCCAATGGGGAGACATCG ATCCAAGGGTCCATCCCCAA hamSTAT2:F ATCCACCATGSCCCATGGGGAGACACTG GCTTGGTACCTCAATGGTGATGGGAGACACTG GCTTGGTACCTCAATGGTGATGGTGATGGTGATGGTGATGGTGATGGTGATGTGATATTGTCAT TCGGAGAGATCAAGGGTCC MSTAT2:hBBN=rF GTTCTCTCCCCAAGGCGCCGGGGGCC MSTAT2:hBBN=rF GTTCTCTCCCCAAGGCGCGTGGTGCC MSTAT2:hBBN=rF GTTCTCTCCCCAAGGCCGCGTGGTCCGAAGG GCTTGGTCTCCCCGCGGTGCCGCG MSTAT2:hBBN=rF GCTTGGCGGAGAAGAACTGCTGGTGCGCG MSTAT2:hBBN=rF GCTGGCGGCGGCGCGCG MSTAT2:hBBN=rF GCTGGCGGGGGGGCGCGCG MSTAT2:hBBN=rF GCTGGCGGGGGGGGCGCGCG MSTAT2:hBBN=rF GCTGGCGGGGGGGGCGCGCGCGCGCGCGCGCGCGCGCGC		CAGAAGGCATCAAGGGTCC	
moueSTAT2bBP. GCTGCAAGGCCAAGGCAAGGAAGGCGAAGG hamSTAT2bBD.veF ACCGACCAAGGCACGCGAAGGCGAAGG STAT2-hDBD-veF ACCGACCAAGGCACGCGAAGGCGAAGG STAT2-hDBD-veF ACCGGCATGCCGAAGGACGCCCGAAGG STAT2-hDBD-veF ACCGGCATGCGCAGGCGCCGAAGG STAT2-hDBD-veF ACCGGCATGCGCGCAGGCGCGAGG STAT2-hDBD-veF ACCGGCATGCGCGCAGGCGCGAGG STAT2-hDBD-veF ACCGGCATGCGGCAGGCGCGCGAGG STAT2-hDBD-veF ACCGGCATGCGGCAGGCGCGCGCGCGCGCGCGCGCGCGCG	mouseSTAT2hisF	ATCCACCATGGCGCAGTGGGAGATGTTG	For construction of pcDNA3.1/mSTAT2-His
GTATCAAGASTCCATCCCAA   For construction of pcDNA3.1/hamSTAT2-His     ADVECTAATCGTACCTCAATCGTGGACGATGGACGATGGACGATGGCATATTGTCAT   Construction of pcDNA3.1/hamSTAT2-His     MATAT2-hDBD-veF   GTTCTTCTGCAAAGCTCCGAAAG   For construction of pHHM(#1)     MMM-HMM-IM   ACGGGTGCCATCATCACC   For construction of pHHM(#1)     MMTAT2-DBD-R   ATATGCATACCGGGTCATCATCAC   For construction of pHMM(#2)     MMM-HMM-IMA   ACCGGTATGCATATTGAGGTCGGTCCGGCCCGGCCCGGC	mouseSTAT2hisR	GCTTGGTACCTCAATGGTGATGGTGATGATGACCGGTATGCATATTGAAG	
hamSTAT2-bBB-veF GTCTCTGCCACGCCGAAGACCTG For construction of ptHMM(#1) hmSTAT2-bBB-veF GTCTCTCACCACGCGAAGACGTGCCGAAGACGTGCCGAAGACGTGCCGAAGACGTGCCGAAGACGTGCCGAAGACGTGCGAAGACGTGCGAAGACGTGCGAAGACGTGCGAAGACGTGCGAAGACGTGCGAAGACGTGCGAAGACGTGCGAAGACGTGCGAAGACGTGCGAGAGACGTGCGAGAGACGTGCGAGAGACGTGCGAGAGACGTGCGAGAGGGGCGAGGGGAGGGGCGGGGGGGG		GTATCAAGAGTCCATCCCAA	
hams/al2niskCCITICGIACULAAGGGAGGAGGAC CASCOPERFor construction of pHHM(#1)mSTAI2-hDBD-veFGTTCTTCIGCAAGGACTCAGGCAGGFor construction of pHMM(#2)hmSTAI2DBDVeFAATATGCATACCGGACAGGACCTCGGTTCGAAGGFor construction of pHMM(#2)hMSTAI2DBDVeFACAGAGCCTTGGTGGTGAGAAGGCCCGGGCCGGCFor construction of pHMM(#2)hMM-HMH-INAACGGTATGCATATGCAGGCCGGCCGGCCGGCGCGGCGCG	hamSTAT2F	ATCCACCATGGCGCAGTGGGAGACACTG	For construction of pcDNA3.1/hamSTAT2-His
CACAACUGAA (CAACUGAT (CCCCAAACGAACCCAAGCC)For construction of pHHM(#1)MTAT2-hDBD-veFACTCTTCCCCACCTCCCCAAACGACFor construction of pHHM(#1)MTAT2-hDBD-inFACAGAGCCTTTGAAGGTATCFor construction of pHMM(#2)MMTHM-HIM-inRACCCGGTATGCATATGAAGGCACCFor construction of pHMM(#2)MMTHM-HIM-inRACCCGGTATGCATATGAAGGTATCFor construction of pMHH(#3)MTAT2-hDBD-inFACAGAGCCTTTGTGATGAAAACCCCAGCCCAGCCCCFor construction of pMHH(#3)MMT-MIM-HIM-inRACCGGTATGCATATGAAGGCACFor construction of pMHH(#3)MMTAT2-hDBD-veRCTACAAAGGCTATGTGACACACAFor construction of pMHH(#4)MTAT2-hDBD-veRCTACAAAGGCACCTTGCACACACAFor construction of pMHH(#4)MMT-MIH-inRACCGGTATGCATATGAAGCACFor construction of pMHH(#4)MMT-MHH-inRACCGGTATGCATATGAAGCAGFor construction of pH(1-100)MM(#5)MTAT2-hDBD-veRCCTACAAAGGACCTGCTGGTCTCACACFor construction of pH(1-100)MM(#5)MTAT2-hDBD-veRACCGGTATGCATATGAAGGCATCFor construction of pH(1-221)MM(#6)MMT-MHH-inRACCGGTATGCATATGAAGGCATCFor construction of pH(1-221)MM(#6)MTAT2-DBDveFAATATGCATACCGCTCACCCAGGGCGGAATGFor construction of pH(1-221)MM(#6)MTAT2-DBDveFAATATGCATACCGCTCATCACACFor construction of pH(10-315)MM(#7)MTAT2-DBDveFAATATGCATACCGCCTGGCGATAGCCCCGGFor construction of pH(101-315)MM(#8)MTAT2-DBDveFAATAGCATCCTGGAGAGGCCTGFor construction of pH(101-315)MM(#8)MTAT2-DBDveFAATAGCACCCCCCTGGGAAAGGCCTGGAAGGCCCGFor construction of pH(101-315)MM(#8)MTAT2-DBDveFCTA	hamSTAT2hisR	GCTTGGTACCTCAATGGTGATGGTGATGATGACCGGTATGCATATTGTCAT	
mini Alz-DBD-VeF   GTC TIC TIC LOCAAGE LCCAAAGE   For construction of pHHM(#1)     hmS1 AT2-DBD-RR   GGTTGGCAATAGE CGTGGTGGCGTGGCGGTGGGGGGGGGGGGGGGGGGGG		CAGAAGGAATCAAGGGTCC	
HIMM-HIMM-IN HIMM-HIMM-IN HIMM-HIMM-IN HIMM-HIMM-IN ACCGGITAGCALTATIGAAGGALAC GCTTGGCAGAAGAGACICCTGCACACAGG HSTAT2-mDBD-uFF ACAGAGCCTTTGTGATGAGAACCCCAGCCCTG HMM-HIM-HIM-IN ACCGGTATGCATATIGAAGGACAG mSTAT2-hDBD-uFF ACAGGGTATGCATATIGAAGGACAG MMH-MIH-HIM-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-HIM-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-HIM-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-HIM-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-HIM-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-HIM-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-HIM-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-HIM-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-HIM-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-HIM-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-HIM-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-HIM-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATATIGAAGGACAG MMH-MIH-IN ACCGGTATGCATAGGACGCAG MMH-MIH-IN ACCGGTATGCATAGCACGCCCTG MMH-MIH-IN ACCGGTATGCATAGGACGCGCGATAACCACCCCCTG MMH-MIH-IN ACCGGTATGCATATGCATACCCCCCCCGGAGGCAG MMH-MIH-IN ACCGGTATGCATGCATGGCCATACCACGCCCTG MMH-MIH-IN ACCGGTATGCACCAGGCCCTTGGGAGGCGC MMH-MIH-IN ACCGGGTATGCACCCCCCCCCCCTACCCAGGCCCTG MMH-MIH-IN ACCGGGTATGCGCCATCGCGCGCAGGCAGGCAG MMH-MIH-IN ACCGGGTATGCGCCATCGGGCGCAGGCAGGCCG MMH-MIH-IN ACCGGGTATGCGCCATCGCGCGCCAGGCCCTG MMH-MIH-IN ACCGGGTATGCGCCATCGGGCGCAGGCGCC MMH-MIH-IN ACCGGGTAGGGCCATTGGGAAAGGGCCTGGAGAGGCC MMH-MIH-IN ACCGGGTATG	mSTAT2-hDBD-veF	GITCHTCHGCCAAGCTCCGAAAG	For construction of pHHM(#1)
Inns IA JUBBUVeF   ACIA ISCA IALCIGAT CALCAL     ISTAT2-bbBD-inF   ACAGAGCCTTTGTAGTAGAAACCCAGCCCTG     INSTAT2-bbBD-inF   ACAGAGCCTTTGTAGTAGAAACCCAGCCCTG     INMSTAT2-bbBD-veF   CTACAAAGGCTCTGTGTGAGGAAACCCAGCCCAGC     ISTAT2-bbBD-veF   CTACAAAGGCTCTGTGGAGAAACCCAGCCCC     INSTAT2-bbBD-veF   CTACAAAGGCTCTGTGGAGAAACCCAGCCC     INSTAT2-bbBD-veF   CTACAAAGGCTTGGATATGAAGCAG     INSTAT2-bbBD-veF   CTACAAAGGCTTGGGATATGAACCAC     INSTAT2-bbBD-veF   GTTCTTCCCAACCGGTATGCATCATCAC     INSTAT2-bbBD-veF   GTTCTTCCCAACCGGTATGCATCATCAC     INSTAT2-bbBD-veF   GTTCTTCCCAACCGGTATGGACAACC     INSTAT2-bbBD-veF   GTTCTTCCCAACCGGTATGGACAGC     INSTAT2-bbBD-veF   GTTCTTCCCAACCGGTATGGACAGC     INSTAT2-bbBD-veF   GTTCATGCAACCGGTATGCATCATCAC     INSTAT2-bbBD-veF   GTTCATGCAACCGGTATGCATCATCAC     INTSTAT2bBDVeF   AATATGCATACCGGTATGCATCATCACA     INSTAT2-bBDVeF   GGGTTGGAGAAAGGCTGTGGGCTGAACG     INTSTAT2bBDVeF   AATAGCATCCGGTATGCATCATCACA     INTSTAT2bBDVeF   AATAGCATCCGGTATGCATCATCACA     INTSTAT2bBDVeF   AATAGCATCGCGGATAACCACCCCTGG     INTSTAT2bBDVeF   CACGGTTGGCATATGCAACAGCCCTGGAACG     INTSTATDBDVeF   CACGGC	HMM-HHM-INK	ACCGGIAIGCAIAIIGAAGGIAIC	
INITIATIONED-INR   GCTTGGLAGAAGAAGAACTACTGCTGATGCAGGGCGG     INSTAT2-IDDED-INF   ACAGAGCCTTTGTAGTAGAAGCAGCCAGCCGG     INSTAT2-IDDED-INF   AAAGGTCCTTTGTAGTAGAAGCAGC     INSTAT2-IDDED-INF   AAAGGTCCTTTGTGGAGCAGC     INSTAT2-IDDED-INF   AAAGGTCCTTTGGAGCAGCAG     INSTAT2-IDDED-INF   AAAGGTCCTTTGGAGCAGCAG     INSTAT2-IDDED-INF   AAAGGTCCTTTGGAGCAGCAG     INSTAT2-IDDED-VEF   AATATGCATACCGGTCATCATCAG     INSTAT2-IDDED-VEF   AATATGCATACCGGTCATCATCAGC     INSTAT2-IDDED-VEF   AATATGCATACCGGTCATCATCAGC     INSTAT2-IDDED-VEF   AATATGCATACCGGTCATCATCAGC     INSTAT2-IDDED-VEF   AATATGCATACCGGTCATCATCAGC     INSTAT2-IDDED-VEF   AATATGCATACCGGTCATCATCAGC     INSTAT2-IDDED-VEF   AATATGCATACCGGTCATCATCACC     INSTAT2-IDDED-VEF   AATATGCATACCGGTCATCATCACC     INSTAT2-IDDED-VEF   AATATGCATACCGGTCATCATCACC     H(1-100)MMINF   CCCCAGGTATCACTCATCACC     H(1-100)MMINF   CCCCAGGTATCACTATCACC     H(1-100)MMINF   CCCCAGGTATCACTCATCACC     H(1-100)MMIVER   GGGTAGGCATCCTACCCAGCT     H(1-221)MMINF   CCCAGGTATCACTACCC     H(1-221)MMINF   CCCAGGTATGCACTACCCCTGCGGAAGGCCTC     H(1-221)M	hmSTAT2DBDveF	AATATGCATACCGGTCATCATCAC	
IsTAT2-mDBD-veF MMH-HH-inR mSTAT2:DBDveFACAGAGCCTTTGTAGTAGAAACCCAGCCCA AATATGCATACCGGTCATCATTGAAGGTATC AATATGCATACCGGTCATCATCAC CTACAAAGCCCTTGTGGAGAAACCAGCCAGCFor construction of pMHH(#2)mSTAT2-bDBD-veF mSTAT2:DBDveFAAAGGCCTTGTGGAGAAACCAGCCAGCFor construction of pMHH(#3)mSTAT2-bDBD-veF mSTAT2:DBDveFGTTCTTCTCCAACCCCCCCCAGG CACAAGGACCTTGGAGAAACCAGCCAFor construction of pMHH(#4)mSTAT2-bDBD-veF mSTAT2:DBDveFGTTCTTCTCCAACCCCCCCCAGG CACAAGGACCTTGGAGAAACCGGCTGACATCAC ACCGGTATGCATATGGAGAACCAGCFor construction of pMMH(#4)mSTAT2-bDBDveF mSTAT2:DBDveFGTTCTTCTCCAACCCCCCCCAGG CACAAGGACCTTGGAGAAGAACTGCTGGGTCATCATCAC CGGTTGGAGAAGGACTGCTGGGTCATCATCAC MIH-HH-HinR mSTAT2:DBDveFFor construction of pH(1-100)MM(#5)mSTAT2-bDDveF mSTAT2:DBDveFCCCAGGATCCATCACCAC CGGTTGGAGAAGGACTGCTGGGCGAATGFor construction of pH(1-221)MM(#6)mSTAT2:DBDveF mSTAT2:DBDveFCCCAGGATCCTGGGCGAAAGGGCTGAACG CGGTATGCATATGCATCCCGGTATCATCAC GGGTAGGCATCTGGAGAAAGGGCTGATCATCAC HI(1-100)MMINPF mSTAT2:DBDveFFor construction of pH(1-221)MM(#6)mSTAT2:DBDveF mSTAT2:DBDveFCACTGCTGGGCGATTACCCCGGGCACC CTCCAGGCCTGGCGCATCACCCGGCCGG AATGCATATGGAACCCGCCGG CTCCAAGGCCTTGGGAGCAGCCCTTGGGAGCAGC MH(10-1315)MminFCCCCAGGGCCCTGGCGCATCACCCAGCCCTG ACCGGCCATGGGACAAGGCCTGGGAGATGCFor construction of pH(101-315)MM(#8)mH(10-315)MminF mSTAT2:DBDveF mSTAT2:DBDVeF MCAGGCCCATGGGCAAGGCCTGGGAGATGC MH(101-315)MminFCCCCAATGGCCCTTGGGAGCAG CCCAATGGCCCACGCCTGGGAGATGCCCAGGCCTG GGTAGGGCCCATGGGAAAGGCTGGAATATCFor construction of pH(101-315)MM(#8)mH(10-315)MminF mSTAT2:DBDveF mSTAT2:DBDVeF MH(101-315)MMinFCCCCA	mSTAT2-nDBD-ink	GCTTGGCAGAAGAACTGCTGGTTCTGAAGG	
HMM-HMM-IR hmSTAT2DBDveFACCGGTATGCATATTGAAGGTATC hmSTAT2DBDveFCTACAAAGGCTCTTGTGGAGACAGmSTAT2-hDBD-vBF tSTAT2-mDBD-veFAAAGGTCCTTTGTGGTAGAAACCCAGCCC mSTAT2-hDBD-veFFor construction of pMHH(#3)mSTAT2-hDBD-veF mSTAT2-bDBD-veFAATATGCATACCGGTCATCATCAC ACCGGTATGCATCATCAGFor construction of pMHH(#4)mSTAT2-hDBD-veF mSTAT2-bDBD-veFGTTCTTTCTCAACCCCCCCAAG GCGTTGCATATTGAAGTCAGFor construction of pMMH(#4)mHM-HMH-INR mSTAT2-bDBD-veFGTTCTTTCTCAACCCCCCCAAG GGGTTGCATATTGAAGTCAGFor construction of pMMH(#4)mHM-HMH-INR mSTAT2-bDBD-veFGCCCAGAGCGTACCATCAC AATATGCATACCGGTCATCATCAC bTAT2-mDBD-inFGGGTGGAGAGGAGCGTGGGGTGGTGTGGGGmHI-100/MMICCCCAGGATGCAATTGGAGAGGTGCTGGGGTGAGATG HI-100/MMIFor construction of pH(1-100)MM(#5)HI-100/MMICCCCAGGTGCGCATTACCACCCTGG AATATGCATACCGGTCATCATCAC HI-100/MMWRFor construction of pH(1-221)MM(#6)HI-100/MMWRGGGTGGCGCATTACCACCCTGG AATATGCATACCGGCTGATCC HI-100/MMWRRFor construction of pH(1-221)MM(#6)HI-100/MMWRRGGTGGTGGCCGATTACCACCCTGG ACCGGCTAGCATGCTTGCACCCTG ATTGGACCACGCCTTGGAGAGGCCTGFor construction of pH(222-315)MM(#7)H1222-315/InF HSTAT2-mDBD-veFGACTGGTGGCCGATAACCACCCCTG ACGGCCATGCCAGCCTGGGAGAGGTCFor construction of pH(101-315)MM(#8)H110-1315/MMINF HCTACAAGGCCCTTGGGAGAAGGCCTTGGAGATG HSTAT2-mDBD-veFCCCAATGGCCCATGCGGAGAGGTCFor construction of pH(101-315)MM(#8)H110-1315/MMINF HCTACAGGCCCTTGGGAGAAGGCCTTGGATATC MH(101-315)MMINFCCCAATGGCCCATCCCCAGTTGGCTGAATATCFor construction of pH(101-315)MM(#8)H110-1315/MMINF HCTACAGGCCCTTGCCTACAC	hSTAT2-mDBD-inF	ACAGAGCCTTTGTAGTAGAAACCCAGCCCTG	For construction of pHMM(#2)
InstR122BDveF hSTA12DBDveF LSTA12CBDDveFAATATGCATACCGGTCATCACC CCACAAAGGCTCTTGTGGAGCAGSTA12-BDDveF mSTA12-BDDveFCCACAAAGGCTCTTGTGGAGCAGCSTA12-BDDveF mSTA12-BDDveFGTCTTCTCCAACCCCCCCAAG CCACAAAGGCCTCTTGGACAAGCACSTA12-BDDveF mSTA12-BDDveFGTCTTCTCCAACCCCCCCCAAG GGTTGGCATATTGAAGTCAGSTA12-BDDveF mSTA12-BDDveF AATATGCATATGCATCGCTGACACCFor construction of pMMH(#4)STA12-BDDveF mSTA12-BDDveF ATATGCATCGCTACCACCGCCCCAAG SGTTGGAGAAGAACTGCTGGTCATCACC STA12-DDDveF MSTA12-DDDveFFor construction of pH(1-100)MMI(#5)H(1-100)MminF H(1-100)MminF H(1-100)MMVeRCCCAGGATCCTGCGCAATGGCTGACACC GGGTTGGAGAGGCTCATGCACCCCTGG GCCTCTGGCAAAAGGGCTGACACCC CCCGGTATGCATATGAAGGGTATC ACTGGCCCAATAGCAGCCCTGGCAATAGCAGCCCGGATAACCGGCCGAATG H(1-100)MMVeRFor construction of pH(1-221)MM(#6)H(1-21)MMVeR MSTAT2DBDveF AATAGCATCACCGGTCATGCACCCCTGG HT222-315)MVeRFor construction of pH(1-221)MM(#6)H12-21)MMVeR MSTAT2DBDveF AATAGCATCCGGTCATCACCC H12-221)MMVeRFor construction of pH(122-315)MM(#7)STAT2-BDVeF H12-21)MMVeRGACTGGTGGCCGATTAACCACCCCTGG ACCGGCTATGGAAAGGCTCGACAG ACCGGCTATGGAAAGGCTCGAGATGCCFor construction of pH(101-315)MM(#8)H12-21)MMVeR H101-315JMminF H101-315JMminF H101-315JMMVeRCCCAATGGCCCTACCCAGTTGGCTGAGATG ACGGGCCATTGGAAAGGCTCGAAATGCFor construction of pH(101-315)MM(#8)MH(101-315JMminF H101-315JMMveRCCCCAATGGCCCTACCCAGTTGGCTGAGATG ACGGGCCATTGGAAAGGCTCGCAAATGCFor construction of pH(101-315)HM(#9)MH00-315JMmveR MATGCGGCCCTTGCGAAAGGGCTGGCAAAGGCTCGAAATGCFor construction of pH(101-315)HM(#9)MH101-315JMMveR MAT	HMM-HHM-inR	ACCGGTATGCATATTGAAGGTATC	· · · · · · · · · · · · · · · · · · ·
hsTAT2-mDBD-veRCTACAAAGGCTCTGTGGAGCAGmSTAT2-hDBD-inFAAAGGTCCTTGTGGAAAAACCCAGCCCMMH-MHH-inRACCGGTATGCATATTGAAGTCAGhsTAT2-bDBD-veFGTTCTTCCAACCCCCCCAAGhsTAT2-bDBD-veFGTTCTTCTCCAACCCCCCCCAAGhsTAT2-bDBD-veFGTTCTTCCAACCCCCCCCCAAGhsTAT2-bDBD-veFGTTCTTCCCAACCCCCCCCAAGhsTAT2-bDBD-veFGGGTTGGAAAAGACTGCTGGTCTTGGAGCAGAChsTAT2-bDBD-veFGGGTTGGAAAAGACTGCTGGTCTTGGGhsTAT2-bDBD-veFGGGTTGGAAAAGACTGCTGGTCTTGGGhsTAT2-bDBD-veFCCCACGAACGGCTCATCCCACGhsTAT2-bDBD-veFGGGTTGGAAAAGACTGCTGGTCTTGGGhsTAT2-bDBD-veFAATATGCATACCGGTCATCACCAhsTAT2-bDBD-veFACCGGTATGCATATCGAGGTCGTGAGATGhsTAT2-bDBD-veFCCCAGGATCGCATCACCACCTGGhsTAT2-bDBD-veFCCCAGGATCGCATCACCACCCTGGhsTAT2-bDBD-veFCCCAGGCGCGACTAACCACCCTGGhsTAT2-bDBD-veFCCCAGGTATGCATATCGACGCTCACCCChsTAT2-bDBD-veFCCCAGGTGGCCGATTAACCACCCCTGGhsTAT2-bDBD-veFCCCACATGGCCGATTAACCACCCCCGGhsTAT2-mDBD-veFCCCAATGGCCCTACCCCAGCCCTGhsTAT2-mDBD-veFCCCAATGGCCCTTGGGAACAGhsTAT2-mDBD-veFCCCAATGGCCCTTGGGAACAGCCCCTGhsTAT2-mDBD-veFCCCAATGGCCCTTGGGAACGGCCTGGAGGCGhsTAT2-mDBD-veFCCCAATGGCCCTTGGGAACGGCCCGGGCGGAGGCCGhsTAT2-mDBD-veFCCCAATGGCCCTTGGGAACGGCCCGGGAGGCCGhsTAT2-mDBD-veFCCCAATGGCCCTTGGGAACGGCCGGAGGCCGhsTAT2-mDBD-veFCCCAATGGCCCTACCCCGGGAGGGCCGAAGGCCGAGGCCGGAGGCCGGACGGCChsTAT2-mDBD-veFCCCAATGGCCCTACCCCGGGGAAGGCCGAAGGCCGGAAGGCCGGAAGGCCGAAGGCCGGACGCCGGCCGCGCGCGCG	hmSTAT2DBDveF	AATATGCATACCGGTCATCATCAC	
TAT2-IDBD-INF MMH-MHH-INRAAGGTCCTTTGGGTAGAAACCCAGCCCC ACCGGTATGCATATCGAGTATCGCAGFor construction of pMHH(#3)MMH-MHH-INR MSTAT2-IDBD-veFGTTCTTCTCCAACCCCCCCCAAG GCTCTTGCGACAAGGACCTTTGGAGCAGCFor construction of pMMH(#4)htsTAT2-bDD-veF MMM-MHH-INR MSTAT2-DBD-veFGTTCTTCTCCAACCCCCCCCAAG GGTTGGAGAAGAACTGCTGGTCATCACC hSTAT2-mDBD-inRFor construction of pMMH(#4)htsTAT2-bDD-veF MSTAT2-DBD-veF ACCGGTATGCATATGCATCCACCGGTCGTCTCACC hSTAT2-mDBD-inRCCCAGGATCCTGGCTGGTCGTCTCACC GGGTTGGAGAAGAACTGCTGGGTGGTCGACATCA CCCGGTATGCATCACC hSTAT2-mDBD-veF ACTGGCTAGCATCTGAGAGTATC hSTAT2-DBDveFFor construction of pH(1-100)MM(#5)H(1-100)MMIVER HIMA-HIM-inR ACCGGTATGCATCTTGAGGGCGATTACCCCGGTCGATCACCC H(1-100)MMVeRFor construction of pH(1-221)MM(#6)H(1-221)MmirF HC222315InF hSTAT2-mDBD-veF ACAGACCCTTGGAGCGCTTTGGAGGCATCFor construction of pH(1-221)MM(#7)hSTAT2-bDD-veF hSTAT2-mDBD-veR ACGGCCTACGCCATTACCGCTTGGAGCAGG ACGGCCTAGCAGCTCTTGGAGCAGGFor construction of pH(101-315)MM(#8)hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR CTACAAAGGCCTTGGAGCAGG CTACAAAGGCCTTGGAGCAGGFor construction of pH(101-315)MM(#8)hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR CTACAAAGGCCTTGGAGACGGCCTGGAGATGCFor construction of pH(101-315)MM(#8)hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT	hSTAT2-mDBD-veR	CTACAAAGGCTCTGTGGAGCAG	
mSTAT2-hDBD-inFAAAGGTCCTTTGTGTGAGAAACCCAGCCCFor construction of pMHH(#3)MMH-MHH-INAACCGGTATCCATATTGAAGTCAGFor construction of pMHH(#3)hmSTAT2-hDBD-veRCCACAAAGGACCTTTGGAGCAGACFor construction of pMMH(#4)hSTAT2-mDBD-veRGTTCTTCTCCAACCCCCCCAAGFor construction of pMMH(#4)hMH-MH-INAACCGGTATCCATATTGAAGTCAGFor construction of pH(1-100)MM(#5)hSTAT2-mDBD-refGGTTGGACAAAGACTGCTGGTCATCACFor construction of pH(1-100)MM(#5)hMT-MH-MH-INAACCGGTATGCATATTGAAGGTATCFor construction of pH(1-221)MM(#6)H(1-100)MminFCCCAGGATCCTACCCCAGTTGGCTGAGATGFor construction of pH(1-221)MM(#6)HMM-HHM-inAACCGGTATGCATATTGAAGGTATCFor construction of pH(1-221)MM(#6)HMT-TDBD-veRATATGCATACCGGTCATCACCFor construction of pH(1-221)MM(#6)H1-221)MminFCACTGCTAGGCGGTATCATCACFor construction of pH(1-221)MM(#6)H1-221)MMIVRRATATGCATACCGGTCATCATCACFor construction of pH(222-315)MM(#7)H1-221)MMIVRRCACTGGTTGGCCGATTAACTACCCACCTGFor construction of pH(101-315)MM(#8)H101-315)MMINFCCCAATGGCCCACCGGTGGGGGAGAGCCFor construction of pH(101-315)MM(#8)H101-315)MMINFCCCCAATGGCCCTACCCAGTGGGCTGAGATGFor construction of pH(101-315)MM(#8)H101-315)MMIVRRCCCAATGGCCCTACCCAGTGGGCGGAGATGFor construction of pH(101-315)MM(#8)HI01-315)MMIVRRCCCCAATGGCCTACCCCAGTGGGCGGAGATGFor construction of pH(101-315)MM(#8)HM101-315)MMIVRRCCCCATGGCCTACCCCAGTGGGCTGGAATGCFor construction of pH(101-315)MM(#8)HM101-315)MMIVRRCCCCATGGCCTACCCCAGTGGGCGGG			
MMH-MH-HinR IMMTAT2DBD-VeF ANTATGCATACGGTCATCATCGC mSTAT2-hDBD-VeF MMH-MHHinR ACCGGTATGCATATTGAAGTCAGFor construction of pMMH(#4)hSTAT2-mDBD-veF MMH-MHHinR ACCGGTATGCATATTGAAGTCAG ACCGGTATGCATATTGAAGTCAGFor construction of pMMH(#4)hSTAT2-mDBD-inRGGGTTGGAGAGAGACGTCGTGCTGTGGGGAGATG GGGTTGGAGAGAGACTGCGGTCATCATCAC GGGTTGGAGAGAGACTGGCGTATCATCAC GGGTTGGAGATGCATATTGAAGGTATC hmSTAT2DBDVeFFor construction of pH(1-100)MM(#5)H(1-100)MminF HMM-HHM-inR ACCGGTATGCATATTGAAGGTATC hmSTAT2DBDVeFCCCAGGAGAGAGACTGGCGGTATCATCAC GGGTGGGAGAGAGACTGGGGGATGCTGGGAGATG GGGTGGGAGAGACGGCTGATATGAAGGTATC H(1-20)MMVRRFor construction of pH(1-221)MM(#6)H(1-221)MminF HMM-HHM-inR ACCGGTATGCATATGGAGGCGATCC H(1-221)MMIVRRCACGGTAGCGATTACCACCTGG GGCTGGAGATGCGGGGGGCGGATG CTACAAAGGCTCTGGGAGAGGCAGCATC CTACAAAGGCTCTGGGAGAGGCAGCATC CTACAAAGGCTCTGGGAGAGGCGCATACCCCAATGG CTACAAAGGCTCTGGGAGAGGCGCATACCCCAATGC CTACAAAGGCTCTGGGAGAGGCGCATACCCCAATGCCCTG ACAGAGCCCTTGGGAGAGCGC ACCAGGCCCTTGGGAGAGGCGCATACCCCAAGTCCCTG CTACAAAGGCTCTGGGAGAGGCGCGATAGCCCAGCCCTG ACCAGGCCCTTGGGAGAGGCGCGATAGCCCAGCCCTG CCCCATGGCCCACCCCAGTGGCTGGGGAAAGGTCGAATACC MH(101-315)MMirPF CCCCAATGGCCCATCGCGCTGGCGGAGAGGCGGAAAGCGCCGAGGCGGCGGCGCGCGC	mSTAT2-hDBD-inF	AAAGGTCCTTTGTGGTAGAAACCCAGCCC	For construction of pMHH(#3)
hmsTat2bBbveF mSTAT2-bBB-veRATATGCATACCGGTCATCATCAC CCACAAAGGACCTTTGGAGCCAGAChSTAT2-mDBD-veF mMH-MH-Hin mACCGGTATCCATATTGAAGCACFor construction of pMMH(#4)hSTAT2-mDBD-veF mSTAT2-bBDveFGTTCTTCTCCAACCCCCCCAAG AATATGCATACCGGTCATCATCAC GGTTGGACAAGAACGACTGGTGGTCAGGATGFor construction of pH(1-100)MM(#5)H(1-100)MminF mSTAT22bBVeF mSTAT22bBVeFCCCAGGATCCTACCCAGTTGGCTGAGATG AATATGCATACCGGTCATCACCAC AATATGCATACCGGTCATCCACCACCACGGFor construction of pH(1-100)MM(#5)H(1-100)MMVeR mSTAT22bBVeF H1(-100)MWVeRCCCGGTAGCATCCTGGCAAAGGACTC AATATGCATACCGGTCATCACCAC ATATGCATACCGGTCATCACCACCCTGG H(1-221)MMVeRFor construction of pH(1-221)MM(#6)H(1-22)JMminF hSTAT2-mDBVeF AATATGCATACCGGTCATCACCACCCTGG H(1-221)MMVeRGACTGGTTGGCCGATTAACCAGCCTG CCGTAGCAGGTCTTGGAGGCATCFor construction of pH(1222-315)MM(#7)hSTAT2-mDBV-FF hSTAT2-mDBV-FF hSTAT2-mDBV-FF hSTAT2-mDBV-FF hSTAT2-mDBV-FF ACGGGCCAACCAGCCCTTGGGAGAGG hSTAT2-mDBV-FF hSTAT2-mDBV-FF ACGGGCCAACCAGCCCTTGGGAGAGG hSTAT2-mDBV-FF ACGGGCCAACCAGCCCTTGGGAGAGG CCCAATGGCCTACCCCAGTGGCGGAGATG CTACAAAGGCTTGTGGAGAGGCTGAATATCFor construction of pH(101-315)MM(#8)hSTAT2-BDBV-FF hSTAT2-mDBV-FF ACGGGCCATCGCCATCGCAGTGGCGGAGAGG hSTAT2-mDBV-FF ACGGGCCATCGGGAAGGCTGAATATCFor construction of pH(101-315)HM(#9)MH(101-315)MMinF hSTAT2-mDBV-FF hSTAT2-mDBV-FF ACGGGTAGGCCTACCCAGTTGGCAGAGGCCGAGAGG CTACAAAGGCCTTGGGAAGCGCGGAGAGG hSTAT2-mDBV-FF ACGGGTAGGCCTACCCAGTTGGCAGAGGCGGAAGG CTACAAAGGCCTGGGAGAGG ATTGCGCCTACCCCAGTTGGCAGAGGCGGAAGGCCGGAGAGG ACCGGTAGGCCTACCCAGTTGGCAGAGGCCGGAGAGG AATATGCATACCGGGTCGCTAGAAAGGCCGGAGAGG AATATGCATACCGGGTCGCTAGCAGGCGCGGAGGGCGCATGGGAATGGCCAGGGCCGCAGGGC	MMH-MHH-inR	ACCGGTATGCATATTGAAGTCAG	
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hSTAT2-mDBD-veF MMH-MHHinR MACGGATGGCCCCCCCCCAGG ATTATGCATCGGGTCAGCACCCCCCCCCAGG SGGTTGGAGAAGAACTGCTGGTCACC GGGTTGGAGAAGAACTGCTGGTGTCACC GGGTTGGAGAAGAACTGCTGGTGAGATG ACCGGTATGCATATTGAAGGTATC ATATGCATACCGGTCATCACC H(1-100)MminF H(1-21)MminF H(1-21)MminF H(1-21)MminF LC2CGGTAGGATGCATGCCCTGGGAAAAGGGCTGAATG H(1-221)MminF H(1-221)MminF LC2CGGTAGGATGCATGCACCCCTGG CCCCAGGCTGCACCCGGCATTAACCACCCCTGG HC1-221)MminF LC2CGGTAGGATGCATAGCAACCCCCGGCACCACCCCTGG HC22-315inF STAT2-mDBD-inF ACCGGCTTGGCCGATTAACCACCCCTGGGAGAGGC CCCCAGGCCCTGCCCAGCGGCCGATGCCCCGGCACCACCCCGGCACCACCCCGGCACCACCCCCGG ACCGGCTGGCCCGTTGGCGCGATAACCCCCCGCGCGCCGG ACCGGCTGGCCCGTTGGCGCGGTGGCCGATGCCCCGGCGCCGGCACCCACC	mSTAT2-hDBD-veR	CCACAAAGGACCTTTGGAGCAGAC	
Initial InductionChick ConstructionFor construction of philin (key)MMH-MHH-IARACCGGTTGCATATTGAAGTCAGFor construction of philin (key)hmSTAT2:mDBD-iRGGTTGGAGAAGACTGCATCATCAChSTAT2:mDBD-iRCCCCAGGATCCATCATCACCGTGGTGGTGGAGAGTGH(1-100)MminFCCCCAGGATCCATCATCATCACHMM-HMM-HIARACCGGTATGCATATTGAAGGTATChmSTAT2:mDBD-iRGGGTAGGACCTGGCAGATAACGGCCGATGAATGH(1-21)MminFCACTGCTAGGCCGATTAACCACCCTGGH(1-221)MminFCACTGCTAGGCCGATTAACCACCCTGGH(1-221)MminFCACTGCTAGGCCGATTAACCACCCTGGH(1-221)MminFGACTGGTTGGCCGATTAACCACCCTGGH(1-221)MWVeRATCGGCCTAGCAGTGCTTGGAGAGGCATCH(1-221)MWVeRACCGGCTATGACCATCATCACCH(1-221)MWVeRACCGGCCTAGCAGTGCTTGGAGAGCAGhSTAT2-mDBD-veRCTACAAAGGCTTGTGGAGACAGACCGGCCAACCAGTCCTTGAGAGACGCCTGFor construction of pH(101-315)MM(#7)hSTAT2-mDBD-veRCTACAAAGGCCTTGGCGAGAGGCCTGhSTAT2-mDBD-veRCTACAAAGGCCTTGGGAGAGAGTGCH1(101-315)MminFCCCAATGGCCCAACCAGTGGCTGAGAGTGACCGGCCATCGCCACCAGCTGGCGGAGAGGTCGAAATATCMH(101-315)MminFCCCAATGGCCCATCACCAGTGGCTGAGATGhSTAT2-mDBD-veRATATGCATCACCGGTGAGAGTGCTGAATATCMH(101-315)MminFCCCAATGGCCCATCACCAGTGGCTGAAATATCMH(101-315)MminFCCCCAATGGCCCTATCATCACCAGGMH(101-315)MminFCCCCAATGGCCCTATCATCACCAGGMH(101-315)MminFCCCCAATGGCCCTATCATCACCAGGMH(101-315)MminFCCCCAATGGCCCTATCATCACCAGGMH(101-315)MminFCCCCAATGGCCCTATCATCACCAGGAGTGCCMH(101-315)MminFCCC	hSTAT2-mDBD-veF	GTTCTTCTCCAACCCCCCCAAG	For construction of $pMMH(#4)$
InstitutionACCOGINACIONATIONMINITATIONAATATGCATACCGGTCATCACCAMINITATIONGGGTTGGAGAAGAACTGCTGGTCATCACMINITATIONGGGTTGGAGAAGAACTGCTGGTCATCACMINITATIONCCCGGTATGCATATCGAGTGGCTGAGATGMINITATIONACCCGGTATGCATATTGAAGGTATCMINITATIONACCCGGTATGCATATTGAAGGTATCH(1-100)MMVeRGGGTAGGATCCTGGGAAAAGGGCTGAATGH(1-221)MminFCACTGCTAGGCCGATTACCCACCCTGGH(1-221)MminFCACTGGTTGGCCGATTACCACCCCTGGH(1-221)MminFCACTGGTTGGCCGATTACCACCCCTGGH(1-221)MMVeRATCGGCCTACGGTCATCACH(1-221)MMVeRATCGGCCTACGGCTGATGCATATCGAAGGCATCH222-315inFGACTGGTTGGCCGATTAACTACCCCTACCCH222-315inFGACTGGTTGGCCGATTAACTACCCCTGCCCGH222-315iveRCTACAAAGGCCTGTGGGAGCAGH222-315veRCTACGAAGGCCTTGTGGAGCAGNSTAT2-DDBD-veRCTACAAAGGCCTGGGAGCAGH222-315veRCCCAATGGCCCTACCCAGTTGGCTGAGATGNH(10)-315)MminFCCCAATGGCCCTACCCAGTTGGCTGAGATGNCAGGGCCTAGCCAGCCTGGGAGCAGFor construction of pH(101-315)MM(#8)NSTAT2-DDBD-veRCTACAAAGGCTTGAGAAACCCAGCCCTGMH(10)-315)MmveRGGTAGGCCCATGGCGAGAGTGCAATATCMH(10)-315)MmveRACCGGTATGCATACTCACCAGGTGACAGATCMH(10)-315)MmveRCCCAATGGCCCATGTGGCTGAATATCMH(10)-315)MmveRCCCAATGGCCCCTCTTGGGAACGGCCMARV VP40 515PFCTTGAACCCCCCCCCTCTTAGCTGATCATACCMARV VP40 515PFCTGCAGGCAGCTGGATGGAATATGCAGACTGGACTGGAATGCCMARV VP40 5795RCCTCCTGGGTTGCGTCAAMARV VP40 5795RCCTCGGGGTGGCTCAAMARV VP40 57			
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hmSTAT2DBDveFAATATGCATACCGGTCATCATCAC GGGTAGGAATCCTGGGAAAAGGGCTGAATGH(1-100)MMVeRCACTGCTAGGCCGATTAACCACCCTGG ACCGGTAGCATATGCATATGCAGGTATGCATATGAAGGTATCHMM-HHM-INR hmSTAT2DBDveFAATATGCATACCGGTCATCATCAC AATAGCCATGCTGGCGATTGCAGGGCATCH222-315InF hSTAT2-mDBD-veRGACTGGTTGGCCGATTAACTACCCTAATCG CTACAAAGGCTTTGTGGAGCAG ACGAGGCCTTTGTGGAGCAGGH222-315InF hSTAT2-mDBD-veR hSTAT2-mDBD-veRGACTGGTTGGCCGATTAACTACCCTAATCG ACAGAGCCTTTGTAGTAGAAACCCAGCCCTG ATCGCCCACCCAGTTGAGTAGTAGAAACCCAGCCCTG ACGGCCATCGCCGAGCATGTTGGAGGAGTGTCCMH(101-315)MminF hSTAT2-mDBD-veR hSTAT2-mDBD-veR cTACAAAGGCTCTGTGGAGACG CTACAAAGGCTCTGTGGAGACGG CTACAAAGGCTCTGTGGAGACGG CTACAAAGGCTCTGTGGAGACGG CTACAAAGGCTCTGTGGAGACGG CTACAAAGGCTCTGTGGAGACGG CTACAAAGGCTCTGTGGAGACGG MH(101-315)MminF MCCGCAATGGCCATCGCAGTTGGCTGAGATG CCCCAATGGCCCTACCCAGTTGGCTGAGATG CCCCAATGGCCCTACCCAGTTGGCTGAGATGC MH(101-315)MmveRMH(101-315)MminF MSTAT2-DBD-veF ACAGGGCCATTGGGAAAGGTCTGAATATCMH(101-315)MmveR MATATGCATACTGGCAATGGCCATCGCAGCCGG MH(101-315)MMveRMH(101-315)MmveR MATATGCGTATGCATATGCAGGAAGGTCTGAATATCMARV VP40 515PF MARV VP40 515PF AATATGCATGCTGCTGCATCATCACCGG MARV VP40 G795FMARV VP40 G795F MARV VP40 G795FCTTCGGGTGCTCCTCACCAGTGGCAGCCGGAATGGCC MARV VP40 G795FCTCCTGGGTTGCTCACA GGCTGATATCTGGTGCACATAMSG56F MSG56F	HMM-HHM-inR	ACCGGTATGCATATTGAAGGTATC	
H(1-100)MMVeRGGGTAGGATCCTGGGAAAAGGGCTGAATGH(1-221)MminF hmSTAT2DBDveFCACTGCTAGGCCGATTAACCACCCTGG ACCGGTATGCATATTGAAGGTATC ATCGGCCTAGCAGGCGCTTGGAGGGCATCFor construction of pH(1-221)MM(#6)H222-315inF hSTAT2-mDBD-veR hSTAT2-mDBD-inF ACAGAGCCTTTGGAGCGATCACCCAGCCCTG ACCGGCCAACCAGCCCTGGAGCAGCFor construction of pH(222-315)MM(#7)MH(101-315)MminF hSTAT2-mDBD-veR hSTAT2-mDBD-inF ACAGAGCCTTTGGAGCAG ACCGGCCAACCAGTCCTTTGGAGCAGG CCTACAAAGGCTCTGTGGAGCAG hSTAT2-mDBD-veR hSTAT2-mDBD-inF hSTAT2-mDBD-inF ACAGAGCCTTTGGAGCAGA CCCAATGGCCCAACCAGTCCTGGGAGAGG GGTAGGCCCATTGGAGAAGACCCAGCCCTG GGTACGGCCCATTGGAAAAGCCCAGCCCTG GGTAGGCCCATTGGAAAAGCCCAGCCCTG MH(101-315)MminF hSTAT2-mDBD-veR GGTAGGCCCATTGGAAAGGTCTGAATATCFor construction of pH(101-315)MM(#8)MH(101-315)MminF hSTAT2-mDBD-veR hSTAT2-mDBD-veR hSTAT2-mDBD-veR GGTACGGCCCATTGGAAAGGTCTGAATATCFor construction of pH(101-315)HM(#9)MH(101-315)MminF MH(101-315)MmWeRCCCAATGGCCCATTGGAAAGGTCTGAATATCFor construction of pH(101-315)HM(#9)MH(101-315)MminF MMM-HHM-inR hmSTAT2DBDveF MARV VP40 S15PF MARV VP40 G795F MARV VP40 G795FCTTGAACCCCCCTCTTTGCTGATCACGG GCTTGGACATGTGGCCTATCGTCGCCTCTGGG GCTTTTGACTGTGTGTTATATGCAGAACTGGCCFor construction of pmMARV VP40MARV VP40 G795F MARV VP40 G795FCTCCTGGGTTCGTCTACA GGTGAGGCCCTTGGG GCTTTTGACTGTTGCGTCGTCTTAGCAGATATGCCFor real-time RT-PCR detection of humanhSS56FCCTCCTTGGGTTCGTCACACA GGCTGATATCTGGGGCCTASFOR eal-time RT-PCR detection of human	hmSTAT2DBDveF	AATATGCATACCGGTCATCATCAC	
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Immit Tunn ImmCoccuration of ph(222-315)HmSTAT2DBDveFAATATGCATACCGGTCATCATCACH(1-221)MMVeRATCGGCCTAGCAGTGCTTTGGAGGCAGH222-315inFGACTGGTTGGCCGATTAACTACCCTAATCGhSTAT2-mDBD-veRCTACAAAGGCTTTGTAGTAGAAACCCAGCCCTGhSTAT2-mDBD-inFACAGAGCCTTTGTAGTAGAAACCCAGCCCTGH222-315veRATCGGCCCAACCAGTCCTTTGGAGGAAGhSTAT2-mDBD-veRCTACAAAGGCTCTGTGGAGCAGhSTAT2-mDBD-veRCTACAAAGGCCTTGTAGTAGAAACCCAGCCCTGH(101-315)MminFCCCCAATGGCCCTACCAGTTGGCTGAGAATGhSTAT2-mDBD-inFACAGAGCCTTTGTAGTAGAAACCCAGCCCTGMH(101-315)MminFCCCCAATGGCCCTACCAGTTGGCAGAAGGTCTGAATATCMH(101-315)MminFCCCCAATGGCCCTACCCAGTTGGCTGAGAATGMH(101-315)MminFCCCCAATGGCCCTACCCAGTTGGCTGAGAATGMH(101-315)MMiveRGGTAGGGCCATTGGAAAGGTCTGAATATCMARV VP40 S15PFCTTGAACCCCCCTCCTTATGCTGATCACCAGGMARV VP40 S15PFCTTGAACCCCCTCCTTAGTGATCACGGCGCCMARV VP40 G79SFGTTTGGGTTGTTGTATATGCAGGCGCCMARV VP40 G79SFCCTCCTTGGGTTGTAATGCAGGAGTGGCCMARV VP40 G79SFCCTCCTTGGGTTGTAATGCAGGAGTAGCCMARV VP40 G79SFCCTCCTTGGGTTGTAATGCGTGCATATGCAGGACTGGCCMARV VP40 G79SFCCTCCTTGGGTTCGTCACAHSG56FCCTCCTTGGGTTCGTCACAHSG56FCCTCCTTGGGTTCGTCACAHSG56RCCTCCTTGGGTTCGTCACAHSG56RCCTCCTTGGGTTCGTCACAHSG56RCCTCCTTGGGTTCGTCACA	HMM_HHM_inB		
InitializationAntroduction of content of the ATCGGCCTAGCAGTGCTTTGGAGCAGTH222-315inFGACTGGTTGGCCGATTAACTACCCTAATCGFor construction of pH(222-315)MM(#7)hSTAT2-m0BD-veRCTACAAAGGCTCTGTGAGACGAGhSTAT2-m0BD-veRCTACAAAGGCTCTGTGGAGCAGhSTAT2-m0BD-veRCTACAAAGGCTCTGTGGAGCAGhSTAT2-m0BD-veRCTACAAAGGCTCTGTGGAGCAGhSTAT2-m0BD-veRCTACAAAGGCTCTGTGGAGACGhSTAT2-m0BD-veRCTACAAAGGCTCTGTGGAGACGhSTAT2-m0BD-veRCTACAAAGGCCTTGGGAAAGGTCTGAATATCMH(101-315)MMveRGGTAGGGCCATTGGGAAAGGTCTGAATATCMH(101-315)MMveRCCCAATGGCCCTACCCAGTTGGCTGAGATG GGTAGGGCCATTGGAAAGGTCTGAATATCMH(101-315)MminFCCCAATGGCCCTACCCAGTTGGCTGAGATG ACCGGTATGCATATTGAAGGTATCMH(101-315)MminFCCCCAATGGCCCTACCCAGTTGGCTGAGATG ACCGGTATGCGTACACTACCAC MH(101-315)MMveRMARV VP40 S15PFCTTGAACCCCCCCCTCTTATGCTGATCACAGG GTTCCGGCATGGCTGCCTGTGGAACTGGCC MARV VP40 S15PFMARV VP40 S15PFCTTGAACCCCCCCCTCTTATGCTGATCACGG GTTCCGGCATGGCTGCCTGTGTGAATATGCTGGAACTGGCC MARV VP40 G795FMARV VP40 G795FCTTGAACCCCCCCCTCTTATGCTGATCACGG GTTCCGGCATGGCTGCCTGTGTATATGCAGATTATGCAGGTTCGTCGTCATAATGCC MARV VP40 G795FhISG56FCCTCCTTGGGTTCGTCACAC GGCTGATATCTGGGTGCCTA	hmSTAT2DBDvoF	ΔΔΤΔΤGCΔΤΔCCGGTCΔTCΔC	
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hSTAT2-mDBD-inF H222-315veRACAGAGCCTTTGTAGTAGAAACCCAGCCCTG ATCGGCCAACCAGTCCTTTGGAGATGTCCMH(101-315)MminF hSTAT2-mDBD-veR hSTAT2-mDBD-inF MH(101-315)MMiveRCCCAATGGCCCTACCCAGTTGGCAGACG CTACAAAGGCCTTTGTAGTAGAAACCCAGCCCTG GGTAGGGCCATTGGGAAAGGTCTGAATATCFor construction of pH(101-315)MM(#8)MH(101-315)MminF HMM-HHM-inR hmSTAT2DBDveF MATATGCATACCGGTCATCGCAGATGCATGGCTGAGATG MH(101-315)MMveRFor construction of pH(101-315)HM(#9)MARV VP40 S15PF MARV VP40 S15PF MARV VP40 G795F MARV VP40 G795FCTTGAACCCCCTCCTTATGCTGATCACGG GTTCCGGCATGGCTGCTCTTATATGCAGATATGCCFor construction of pmMARV VP40hlSG56F hlSG56FCCTCCTTGGGTTCGTCACA GCTGATATCTGGGTGCCTAFor real-time RT-PCR detection of human ISG56 (Ref. 13)	hSTAT2-mDBD-veR	CTACAAAGGCTCTGTGGAGCAG	
H222-315veRATCGGCCAACCAGTCCTTTGGAGATGTCCMH(101-315)MminFCCCAATGGCCCTACCCAGTTGGCTGAGATG CTACAAAGGCTCTGTGGAGACAG STAT2-mDBD-veRFor construction of pH(101-315)MM(#8)STAT2-mDBD-vinFACAGAGCCTTTGTAGTAGAAACCCAGCCCTG GGTAGGCCCATTGGGAAAGGTCTGAATATCFor construction of pH(101-315)MM(#8)MH(101-315)MminFCCCAATGGCCCTACCCAGTTGGCTGAGATG ACCGGTATGCCATATGAAGGTATC hmSTAT2DBDveFFor construction of pH(101-315)HM(#9)MH(101-315)MminFCCCCAATGGCCCTACCCAGTTGGCTGAGATG GGTAGGCCATTGGGAAAGGTCTGAATATCFor construction of pH(101-315)HM(#9)MM(101-315)MMveRCCCCAATGGCCCTACCCAGTTGGCTGAGATG GGTAGGCCATTGGGAAAGGTCTGAATATCFor construction of pM(101-315)HM(#9)MARV VP40 S15PFCTTGAACCCCCCTCCTTATGCTGATCACGG GTTCCGGCATGGCTGCCTCTTGG GTTCCGGCATGGCTGCCTCTTGG GCTTTGACTGTTCGTCGCTCGTTATATGCCAGATATGTCFor construction of pmMARV VP40hISG56FCCTCCTTGGGTTCGTCTACA GGCTGATATCTGGGTGCCTAFor real-time RT-PCR detection of human ISG566 (Ref. 13)	hSTAT2-mDBD-inF	ACAGAGCCTTTGTAGTAGAAACCCAGCCCTG	
MH(101-315)MminF hSTAT2-mDBD-veR hSTAT2-mDBD-inF MH(101-315)MMveRCCCAATGGCCCTACCCAGTTGGCAGAGTG CTACAAAGGCTTTGTAGTAGAAACCCAGCCCTG GGTAGGGCCATTGGGAAAGGTCTGAATATCFor construction of pH(101-315)MM(#8)MH(101-315)MminF HMM-HHM-inR hmSTAT2DBDveF MATATGCATACCGGTATGCATATTGAAGGTATC AATATGCATACCGGTCATCACCAGTTGGCAGAAGGTCTGAATATCFor construction of pH(101-315)HM(#9)MRV VP40 S15PF MARV VP40 S15PF MARV VP40 G795F MARV VP40 G795F MARV VP40 G795F MARV VP40 G795FCTTGAACCCCCTCCTTATGCTGAACAGGCC GTTCCGGCATGGCTGCCTCTTGG GCTTTTGACTGTTCGTCGTCATAATGCAGAATATGTCFor construction of pmMARV VP40hlSG56F hlSG56FCCTCCTTGGGTTCGTCACAC GGCTGATATCTGGGTGCCTAFor real-time RT-PCR detection of human ISG56 (Ref. 13)	H222-315veR	ATCGGCCAACCAGTCCTTTGGAGATGTCC	
Min(101-515)/Min(#6)FOR CONStruction of ph(101-515)/Min(#6)hSTAT2-mDBD-veRCTACAAAGGCCTTGTAGTAGAAACCCAGCCCTGMH(101-315)MmveRGGTAGGGCCATTGGGAAAGGTCTGAATATCMH(101-315)MminFCCCCAATGGCCCTACCCAGTTGGCTGAGATGHMM-HHM-inRACCGGTATGCATATTGAAGGTATChmSTAT2DBDveFAATATGCATACCGGTCATCATCACMH(101-315)MMveRGGTAGGGCCATTGGGAAAGGTCTGAATATCMARV VP40 S15PFCTTGAACCCCCCTCCTTATGCTGATCACGGMARV VP40 S15PFCTTGAACCCCCCTCCTTATGCTGATCACGGMARV VP40 G79SFGTTCCGGCATGGCTGCTCTTGGMARV VP40 G79SFGTTCCGGCATGGCTGCTCTTAGCMARV VP40 G79SRGCTTTTGACTGTCGTCACAhISG56FCCTCCTTGGGTTCGTCTACAhISG56RGGCTGATATCTGGGTGCCTA	MU(101 215)MminF		For construction of pH(101 215)MM(#9)
Instatz-ImbBD-VerkCLACAAGGCCTGGGGGGGGGGGGGGGGGGGGGGGGGGGGG			For construction of pH(101-315)MM(#8)
INSTAT2-IIIDBD-IIIPACAGAGGCCTTIGTAGTAGAAACCCCAGCCCIGMH(101-315)MMiveRGGTAGGCCCATTGGGAAAGGTCTGAATATCMH(101-315)MminFCCCAATGGCCCTACCCAGTTGGCTGAGATG ACCGGTATGCATATTGAAGGTATCHMM-HHM-inRACCGGTATGCATATTGAAGGTATC hmSTAT2DBDveFAATATGCATACCGGTCATCATCAC GGTAGGCCATTGGGAAAGGTCTGAATATCMARV VP40 S15PFCTTGAACCCCCCTCCTTATGCTGATCACGG GGTAGGCCATTGGCATGTGTGTATGTGTTGTAATTGCTGGAACTGGCC MARV VP40 G79SFFor construction of pmMARV VP40MSG56FCCTCCTTGGGTTCGTCTACA GGCTGATATCTGGGTGCCTAFor real-time RT-PCR detection of human ISG56 (Ref. 13)	hSTAT2 mDBD inc		
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MH(101-315)MminF HMM-HHM-inR hmSTAT2DBDveF MH(101-315)MMveRCCCAATGGCCCTACCCAGTTGGCTGAGATG AATATGCATACCGGTCATCATCAC GGTAGGGCCATTGGGAAAGGTCTGAATATCFor construction of pH(101-315)HM(#9)MARV VP40 S15PF MARV VP40 S15PF MARV VP40 G79SF MARV VP40 G79SRCTTGAACCCCCCTCCTTATGCTGATCACGG TATTGCATGTGTGTGTATGTGTTGTAATTGCTGGAACTGGCC GTTCCGGCATGGCTGCCTCTTGG GCTTTTGACTGTTCGTCGTCATCACGATATGTCFor construction of pmMARV VP40hISG56F hISG56RCCTCCTTGGGTTCGTCTACA GGCTGATATCTGGGTGCCTAFor real-time RT-PCR detection of human ISG56 (Ref. 13)		GGTAGGGCCATTGGGAAAGGTCTGAATATC	
HMM-HHM-inR hmSTAT2DBDveF MH(101-315)MMveRACCGGTATGCATATTGAAGGTATC AATATGCATACCGGTCATCATCAC GGTAGGGCCATTGGGAAAGGTCTGAATATCMARV VP40 S15PF MARV VP40 S15PR MARV VP40 G79SF MARV VP40 G79SRCTTGAACCCCCCTCCTTATGCTGATCACGG GTTCCGGCATGGCTGCTCGTTATATGCTGGAACTGGCC GTTCCGGCATGGCTGCTCGTTATATGCAGATATGChISG56F hISG56RCCTCCTTGGGTTCGTCTACA GGCTGATATCTGGGTGCCTAFor real-time RT-PCR detection of human ISG56 (Ref. 13)	MH(101-315)MminF	CCCAATGGCCCTACCCAGTTGGCTGAGATG	For construction of pH(101-315)HM(#9)
hmSTAT2DBDveF MH(101-315)MMveRAATATGCATACCGGTCATCATCAC GGTAGGGCCATTGGGAAAGGTCTGAATATCMARV VP40 S15PF MARV VP40 S15PR MARV VP40 G79SF MARV VP40 G79SRCTTGAACCCCCCTCCTTATGCTGATCACGG CTTTGACTGTTGTATGTGTTGTAATTGCTGGAACTGGCC GCTTTTGACTGTTGTATATGCAGATATGTChISG56F hISG56RCCTCCTTGGGTTCGTCTACA GGCTGATATCTGGGTGCCTAFor real-time RT-PCR detection of human ISG56 (Ref. 13)	HMM-HHM-inR	ACCGGTATGCATATTGAAGGTATC	
MH(101-315)MMveRGGTAGGGCCATTGGGAAAGGTCTGAATATCMARV VP40 \$15PFCTTGAACCCCCCTCCTTATGCTGATCACGG TATTGCATGTATGTGTTGTAATTGCTGGAACTGGCC GTTCCGGCATGGCTGCCTCTTGG MARV VP40 G79SRFor construction of pmMARV VP40 For construction of pmMARV VP40hISG56F hISG56RCCTCCTTGGGTTCGTCACA GGCTGATATCTGGGTGCCTAFor real-time RT-PCR detection of human ISG56 (Ref. 13)	hmSTAT2DBDveF	AATATGCATACCGGTCATCAC	
MARV VP40 S15PF MARV VP40 S15PRCTTGAACCCCCCTCCTTATGCTGATCACGG TATTGCATGTATGTGTTGTAATTGCTGGAACTGGCC GTTCCGGCATGGCTGCCTCTTGG GCTTTTGACTGTTCGCCCGTTATATGCAGATATGTCFor construction of pmMARV VP40hISG56F hISG56RCCTCCTTGGGTTCGTCACA GGCTGATATCTGGGTGCCTAFor real-time RT-PCR detection of human ISG56 (Ref. 13)	MH(101-315)MMveR	GGTAGGGCCATTGGGAAAGGTCTGAATATC	
MARV VP40 S15F7   CFTGAACCCCCCTTATGCTGATCACGG   For construction of pmMARV VP40     MARV VP40 S15PR   TATTGCATGTATGTGTTGTAATTGCTGGAACTGGCC   MARV VP40 G79SF     MARV VP40 G79SR   GCTTTTGACTGTTCGCTCGTTATGCAGATATGTC   For real-time RT-PCR detection of human     hISG56F   CCTCCTTGGGTTCGTCACA   For real-time RT-PCR detection of human     hISG56R   GGCTGATATCTGGGTGCCTA   ISG56 (Ref. 13)			For construction of pmMADV/VD40
MARV VP40 5795F   GTTCCGGCATGGCTGCCTCTTGG     MARV VP40 G795F   GTTCCGGCATGGCTGCCTCTTGG     MARV VP40 G795R   GCTTTTGACTGTTCGCTCGTTATATGCAGATATGTC     hISG56F   CCTCCTTGGGTTCGTCGTCACA     hISG56R   GGCTGATATCTGGGTGCCTA			TO CONSTRUCTION OF PINIVIARY VP40
MARV VP40 G79SR GCTTTTGACTGTTCGCTCGTTATATGCAGATATGTC   hISG56F CCTCCTTGGGTTCGTCTACA   hISG56R GGCTGATATCTGGGTGCCTA	MARY VEAU SISPA		
hISG56F CCTCCTTGGGTTCGTCTACA For real-time RT-PCR detection of human   hISG56R GGCTGATATCTGGGTGCCTA ISG56 (Ref. 13)	MARV VP40 G793F	GCTTTTGACTGTTCGCTCGTTATATGCAGATATGTC	
hISG56FCCTCCTTGGGTTCGTCTACAFor real-time RT-PCR detection of humanhISG56RGGCTGATATCTGGGTGCCTAISG56 (Ref. 13)			
hISG56R GGCTGATATCTGGGTGCCTA ISG56 (Ref. 13)	hISG56F	CCTCCTTGGGTTCGTCTACA	For real-time RT-PCR detection of human
	hISG56R	GGCTGATATCTGGGTGCCTA	ISG56 (Ref. 13)

(Continued on next page)

### TABLE 1 (Continued)

Name	Sequence	Remark <sup>a</sup>
mISG56F	ACCATGGGAGAGAATGCTGAT	For real-time RT-PCR detection of mouse
mISG56R	GCCAGAGGTTGTGC	ISG56 (Ref. 30)
hOAS1realF	CATCCGCCTAGTCAAGCACTG	For real-time RT-PCR detection of human
hOAS1realR	CACCACCCAAGTTTCCTGTAG	OAS1 (Ref. 13)
mOAS1realF	GCCTGGTCACGCACTGGTA	For real-time RT-PCR detection of mouse
mOAS1realR	AAGCCCTGGGCTGTGTTG	OAS1 (Ref. 31)
hGAPDH-F	ATGGGGAAGGTGAAGGTCGG	For real-time RT-PCR detection of human
hGAPDH-R	TTACTCCTTGGAGGCCATGTG	GAPDH
mGAPDH-F	AGGTCGGTGTGAACGGATTTG	For real-time RT-PCR detection of mouse
mGAPDH-R	TGTAGACCATGTAGTTGAGGTCA	GAPDH (Ref. 32)

<sup>a</sup>Ref., reference.

Triton X-100) containing a protease inhibitor cocktail (Roche). To perform the co-IP assay, cell lysates were mixed with magnetic beads conjugated to an anti-His monoclonal antibody (OGHis; MBL) or anti-HA monoclonal antibody (5D8; MBL) and incubated at 4°C for 3 h or overnight, respectively. The magnetic beads then were washed with lysis buffer and wash buffer (50 mM Tris-HCl, 1% NP-40, 0.25% deoxycholic acid sodium salt, 150 mM NaCl, and 1 mM EDTA) and analyzed by immunoblotting as described above.

**Immunofluorescence assay (IFA).** The expression plasmids for His-tagged hSTAT2, mSTAT2, hamSTAT2, or a series of STAT2 chimeras were cotransfected into HEK293T, NIH 3T3, or BHK-21 cells with the pcDNA3.1/NSs-HA plasmid. Twenty-four h after transfection, transfected cells were fixed using 4% paraformaldehyde–PBS (Wako), and then the fixed cells were incubated in 1% Triton X-100 in PBS for permeabilization and blocked in 10% FCS in blocking buffer (3% BSA and 0.3% Triton X-100 in PBS). Cells then were treated with primary antibodies (anti-HA [18850; QED Biosciences Inc.] or anti-His [9F2; Wako]) overnight at 4°C and stained with secondary antibodies (anti-rabbit IgG-H&L [fluorescein isothiocyanate] [ab6009; Abcam] or anti-mouse IgG [whole molecule]-tetramethyl rhodamine isothiocyanate [T5393; Sigma-Aldrich]) for 2 h at room temperature with 4',6-diamidino-2-phenylindole (Roche) for visualization of nuclei. Image acquisition was performed with an LSM780 microscope (Carl Zeiss).

**Statistical analyses.** Significant differences in virus titers in mouse organs among mouse strains were determined by one-way analysis of variance following Tukey's multiple-comparison test using GraphPad Prism software. Statistically significant differences in the data of Fig. 3A to D and 6 were determined using Student's *t* test (Fig. 3A, C, and D) or Dunnett's test (Fig. 3B and 6).

#### ACKNOWLEDGMENTS

We thank K. Maeda (Yamaguchi University, Yamaguchi, Japan) and T. Yoshimoto (Tokyo Medical University, Tokyo, Japan) for providing the SFTSV YG1 strain and *Stat1*<sup>-/-</sup> mice, respectively. We also thank S. Morikawa and S. Fukushi (National Institute of Infectious Diseases, Tokyo, Japan) for providing the anti-SFTSV N antibody. We are grateful to all the members of the Department of Emerging Infectious Diseases, Institute of Tropical Medicine, Nagasaki University.

This research was supported by a grant from the Japan Agency for Medical Research and Development (AMED) under grant number JP18fk0108202 (J.Y.), the Japan Society for the Promotion of Science under grant number 15J06242 (R.Y.), and the Takeda Science Foundation (R.Y.).

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