Research Square

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Interferon Inducible Porcine 2', 5'-oligoadenylate Synthetase-Like Protein Limits Porcine Reproductive and Respiratory Syndrome Virus Infection via the MDA5-Mediated Interferon-Signaling Pathway

Mengmeng Zhao (zhaomengmeng2016@outlook.com)
Foshan University https://orcid.org/0000-0002-6589-4100
Weili Kong
University of California, San Francisco
Yinfeng Kang
Sun Yat-sen University Cancer Center
Huawei Li (547882336@qq.com)
Henan University of Animal Husbandry and Economy

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Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is a constant threat to the swine industry worldwide. Currently approved vaccines against PRRSV are losing effectiveness, as new viral strains are often refractory to conventional treatments. Thus, there is an urgent need to find new therapeutic targets to develop novel antiviral drugs. 2', 5'-oligoadenylate synthetase-like (OASL) protein has antiviral activity, but this has not been demonstrated for PRRSV and the mechanism is not well elucidated. In this study expression of porcine OASL (pOASL) in porcine alveolar macrophages (PAMs) induced by interferon (IFN) -β stimulation and PRRSV infection was examined by real-time polymerase chain reaction (RT-PCR). Exogenous expression and knockdown of pOASL were used to indicate the role of pOASL in the PRRSV replication cycle. The type I IFN signaling pathway was evaluated after pOASL overexpression. Results showed the expression of pOASL in PAMs was significantly increased by IFN-β stimulation or PRRSV infection. pOASL specific small interfering RNA (siRNA) promoted PRRSV replication, whereas exogenous expression of pOASL inhibited infection of PRRSV. The anti-PRRSV activity was lost after knockdown of the Melanoma differentiation-associated protein 5 (MDA5) RNA sensor. Taken together, pOASL inhibits PRRSV infection via the activation of MDA5.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) has been the most aggressive disease affecting the swine industry worldwide, resulting in economic losses of $664 million per year in the U.S [1, 2]. In 2006, the swine industry in China was greatly impacted by this severe infectious disease, which resulted in substantial economic losses [3]. Porcine reproductive and respiratory syndrome virus (PRRSV), the causative agent of PRRS, belongs to the Nidovirales order; *arteriviridae* family. The entire PRRSV genome contains ten open reading frames (ORFs), namely, ORF1a, ORF1b, ORF2a, ORF2b, ORF3, ORF4, ORF5, ORF5a, ORF6, and ORF7. ORF1a and ORF1b make up 80% of the PRRSV genome, encoding the main nonstructural proteins involved in viral replication and transcription, whereas the viral structural proteins GP2–GP5, M, N, E, and GP5a are encoded by ORF2–5, ORF6, ORF7, ORF2b, and ORF5a, respectively [4-6]. PRRSV strains are mainly subdivided into type I and type II according to their antigenicity, where the majority of China's epidemic strains are type II [7].

Current vaccines provide limited protection against PRRSV, finding new ways to control PRRSV is imperative. The host intrinsic restriction factors usually inhibit virus infection by direct interaction with viral proteins, and they are more promising because host intrinsic restriction factors are less likely to mutant under drug-mediated selective pressure [8, 9].

The genes coding for 2', 5'-oligoadenylate synthetase (OAS) proteins are interferon-inducible and play an important role in innate immunity as a host intrinsic restriction factor [10-13]. Upon induction by IFN, the expressed OAS protein up-regulates Ribonuclease L (RNase L) [14], leading to the degradation of viral and host RNAs [15] and inhibition of viral replication [16-18]. The OAS family comprises OAS1, OAS2, OAS3, and OASL. OAS1 has one functional OAS unit, OAS2 has two OAS units, and OAS3 has three OAS units,

whereas OASL has one tandem ubiquitin unit in its C-terminal domain and no oligoadenylates synthetase activity [16, 19-23]. Although OAS1 and OASL are widely distributed, OAS2 and OAS3 are found only in mammals [24]. All these OAS subtypes, except for OAS3, are found in pig genome [25].

OASL has been shown to inhibit replication of several viruses. For example, Newcastle disease virus replication in goose embryo fibroblasts is reduced significantly by overexpression of the goose OASL [26]. Human OASL has been shown to inhibit some specific DNA and RNA viruses, such as respiratory syncytial virus, vesicular stomatitis virus, dengue virus, and herpes simplex virus-1 [27, 28]. Nevertheless, human OASL does not protect against encephalomyocarditis virus infection [29]. Murine OASL2 strongly inhibits respiratory syncytial virus replication [27], whereas murine OASL1 fails to do so [27]. Instead, murine OASL1 inhibits the production of type I IFN, and *OASL1*$^{-/-}$ mice are more resistant to infection with encephalomyocarditis virus and herpes simplex virus-1 [30]. Chicken OASL was found to inhibit West Nile virus infection [31]. pOASL has been reported to inhibit the Japanese encephalitis virus infection in PK15 cells, this inhibition is not dependent on the OAS-RNase L pathway [32].

Previous transcriptomics results clarified that after PRRSV infection, OAS expression increased [33, 34]. The anti-PRRSV effects of porcine OAS1 (pOAS1) and porcine OAS2 (pOAS2) have been demonstrated [35, 36], but those of pOASL and the relationship between pOASL and IFN are not clear. Moreover, pOASL has a different sequence at its C terminus; it remains to be determined whether this feature has a different inhibitory effect on viral replication as compared with other OAS subtypes. Therefore, we evaluated the effect of pOASL on PRRSV replication *in vitro* and attempted to elucidate the mechanisms underlying its antiviral activity.

2. Materials And Methods

2.1. Cells and Viruses

Porcine alveolar macrophages (PAMs), isolated from lung lavage samples of seven-week old pigs which were free of PRRSV, pseudorabies virus, porcine circovirus type 2, and classical swine fever virus, were cultured in the Roswell Park Memorial Institute (RPMI) 1640 medium (Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Life Technologies) at 37 ℃, in a humidified atmosphere containing 5% CO_2.

CRL-2843-CD163, a stable porcine macrophage cell line that could be infected by PRRSV, was kindly provided by Prof. Enmin Zhou (Northwest A&F University, Lingyang, China) [37]. This cell line was grown in RPMI 1640 medium (Life Technologies) supplemented with 6% of fetal bovine serum (Sijiqing, ZhejiangTianhang Biotechnology Co. Ltd., China) at 37 ℃, in a humidified atmosphere containing 5% CO_2.

Marc-145 cells (American Type Culture collection, Manassas, VA, USA, (ATCC), #CRL-12231) were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Life Technologies) supplemented with 10% fetal bovine

serum (Sijiqing, ZhejiangTianhang Biotechnology Co. Ltd., China) at 37 ℃, in a humidified atmosphere containing 5% CO_2.

The type II PRRSV BJ-4 strain (GenBank accession no. AF331831) was kindly provided by Prof. Hanchun Yang (China Agricultural University, Beijing, China).

2.2. Expression of pOASL during PRRSV Infection of PAMs

PAMs were infected with PRRSV BJ-4 strain at a multiplicity of infection (MOI) of 1.0 for different times (0, 6, 12, 24, 36, and 48 h). The cells were then processed for reverse-transcription and real-time polymerase chain reaction (RT-PCR) analysis of *pOASL* mRNA expression and Western blotting for pOASL protein expression.

2.3. Expression of pOASL after Stimulation of PAMs with IFN

PAMs were stimulated with human IFN-β (Pepro-Tech, Rocky Hill, NJ, USA), which was diluted with PBS at a concentration of 1,000 IU/mL for different periods (0, 6, 12, 24, 36, and 48 h). The cells were then subjected for RT-PCR analysis of *pOASL* mRNA expression and Western blotting for pOASL protein expression.

2.4. Molecular Cloning

pOASL (GenBank accession no. NM_001031790.1) was cloned from the complementary DNA (cDNA) extracted from PAMs, using the following primer sequences: 5'-CCGGAATTCTGGAGCTATTTTACACCCCAGC-3' (OASL-For) and 5'-AAGGAAAAAAGCGGCCGCTCAGTCACAGCCTTTGGCTGAGA-3' (OASL-Rev). After double digestion, the purified products were ligated with the p3xFLAG-CMV™-7.1 vector (Sigma-Aldrich, St. louis, MO, USA, #E7533) to generate the pCMV-3xFLAG-7.1-OASL expression plasmid. Mix & Go! *E. coli* Transformation Kit and Zymopure Plasmid Midiprep Kit (Zymo Research, Irvine, CA, USA) were used for cloning and plasmid construct.

2.5. Small Interfering RNA (siRNA) Synthesis

SiRNAs were used to identify the genes or proteins involved in the antiviral mechanism of pOASL. The nontargeting control siRNA (si-NC), *OASL* siRNA (si-OASL), *RIG-I* siRNA (si-RIG-I), *RNase L* siRNA (si-RNase L), and *melanoma differentiation-associated protein 5* (*MDA5*) siRNA (si-MDA5) were all ordered from GenePharma Co., Ltd. (Suzhou, China). The siRNA sequences were listed in Table 1.

Table 1
Primers used in the research

primer	sequences
OASL- For	CCGGAATTCTGGAGCTATTTTACACCCCAGC
OASL- Rev	AAGGAAAAAAGCGGCCGCTCAGTCACAGCCTTTGGCTGAGA
qOASL-F	CTGGTGGCATTTCTGTGCT
qOASL-R	AGATGGTGAAGGCGATGG
qGAPDH-F	CTGCCGCCTGGAGAAACCT
qGAPDH-R	GCTGTAGCCAAATTCATTGTCG
qIRF3-F	AAGGTTGTCCCCATGTGTCTCCG
qIRF3-R	GGAAATGTGCAGGTCCACCGTG
qIRF7-F	TCCAGCCGAGATGCTAAGTG
qIRF7-R	GTCCAAGTCCTGCCCGATGT
qN-F	AAACCAGTCCAGAGGCAAGG
qN-R	GCAAACTAAACTCCACAGTGTAA
qIFN-beta -F	CTAGCACTGGCTGGAATGAGACT
qIFN-beta -R	GGCCTTCAGGTAATGCAGAATC
qTNF-alpha-F	CACCACGCTCTTCTGCCTAC
qTNF-alpha-R	ACGGGCTTATCTGAGGTTTGAG
qIL-8-F	GGCAGTTTTCCTGCTTTCT
qIL-8-R	CAGTGGGGTCCACTCTCAAT
qRIG-I-F	CAGAGCAGCGGCGGAATC
qRIG-I-R	ACTCAAGGTTGCCCAT
qTLR7-F	GAACTGTTTC TCTACAACA
qTLR7-R	AGACTTGTAATTCTGTCA
qTLR3-F	TACTGTACAC AACTTCTACC
qTLR3-R	TTAAATCCTCCATCCAAGG
qNF-κB-F	CCAGCACCTCCACTCCATTC
qNF-κB-R	ACATCAGCACCCAAAGACACC

primer	sequences
qMDA5-F	CGAATTAACAGGCACCGATT
qMDA5-R	CGTCCAGACTTGGCTGATCT
qMyD88-F	CTCCGGAGCG GAGTCCGCG
qMyD88-R	GCCAGCCCAGTCCAGTCC
qTBK1-F	CCAGTGGAT GTTCAAAT
qTBK1-R	CTCCCACATGGACAAAAT
Si-OASL	GGCACAUGAGCGUUUCCAUTT
Si-RIG-I	GCAGGUUAUUCUGGACUUUTT
Si-MDA-5	CCUCAGAUAUUGGGACUAATT
Si-RNase L	UGGAAGAGAUGAAUGCAUATT

2.6. Transfection and Infection

CRL-2843-CD163 cells were transfected with 800 ng of the pCMV-3xFLAG-7.1-OASL expression plasmid or control expression vector (pCMV-3xFLAG-7.1) via Lipofectamine 2000 Transfection Reagent (Life Technologies). After 24 h of incubation, the cells were infected with PRRSV (MOI of 0.1 and 1.0) for 24 h. RT-PCR was then carried out to determine the mRNA expression levels of various factors.

For the siRNA transfection experiments, CRL-2843-CD163 cells were transfected with 60 nM si-OASL, si-RNase L, si-RIG-I, si-MDA5, or si-NC via the Lipofectamine RNAiMAX Transfection Reagent (Life Technologies). At 24 h post-transfection, the cells were infected with PRRSV (MOI 1.0) for 24 h, PRRSV genomic copy number in the supernatant was determined by RT-PCR, and PRRSV titers were expressed as $TCID_{50}$.

For the siRNA and plasmid co-transfection experiments, CRL-2843-CD163 cells were transfected with 60 nM si-RNase L, si-RIG-I, si-MDA5, or si-NC and 800 ng pCMV-3xFLAG-7.1-OASL expression plasmid via the Lipofectamine 2000 Transfection Reagent (Life Technologies). At 24 h post-transfection, the cells were infected with PRRSV (MOI 1.0) for 24 h, PRRSV genomic copy number in the supernatant was determined by RT-PCR, and PRRSV titers were expressed as $TCID_{50}$.

2.7. RT-PCR

Total RNA from PAMs and CRL-2843-CD163 cells were extracted with the TRIzol Reagent (Life Technologies) and then subjected to reverse-transcriptase treatment by means of the First Strand cDNA Synthesis Kit (Takara, Dalian, China). RT-PCR was carried out on a 7500 Fast Real-time PCR system

(Applied Biosystems, Foster City, CA, USA) with the primers listed in Table 1. The *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) gene was analyzed as an internal control, and relative changes in the expression of the target genes were calculated by the $2^{-\triangle\triangle Ct}$ method [38].

2.8. Cell Survival Experiments

The toxicity of pOASL and various siRNAs toward PAMs and CRL-2843-CD163 cells was tested with the Enhanced Cell Counting Kit-8 (Solarbio, Beijing, China).

2.9. Western Blotting and Immunoprecipitation

Western blotting and Immunoprecipitation were carried out as described previously [39-42]. The primary antibodies were as follows: anti-FLAG monoclonal antibody (Bioss antibodies, Beijing, China, #bs-0879R), anti- Anti-β-actin Polyclonal Antibody (Solarbio, # K006153P), anti-GAPDH antibody (Solarbio, # K106389P), anti-OASL antibody (Abcam, Cambridge, MA. USA, # ab155422), anti-RNase L antibody (Santa Cruz Biotechnology, Dallas, TX, USA, # sc-74405), anti-RIG-I antibody (Cell Signaling Technology, Danvers, MA, USA, #3743), and anti-MDA5 antibody (Cell Signaling Technology, #5321). Secondary antibodies were horseradish peroxide-conjugated rabbit anti-mouse IgG antibody (Santa Cruz Biotechnology, #sc-358914), and mouse anti-rabbit IgG antibody (Santa Cruz Biotechnology, # sc-2357).

2.10. Luciferase Reporter Assay

CRL-2843-CD163 cells were transfected with 200 ng of reporter plasmid, 20 ng of pRL-TK, and 400 ng of the flag tagged OASL plasmid via the Lipofectamine 3000 Transfection Reagent (Life Technologies). After 24 h, 1.5 μg of poly (I: C) (InvivoGen, San Diego, CA, USA) treat the cells for 9 h. The cells were then subjected to luciferase reporter assay system (Promega, Madison, WI, USA) to test the promoter activity.

2.11. Virus Titers

Marc-145 cells were used to determine the PRRSV titers in the supernatants. PRRSV titers were expressed as $TCID_{50}$.

2.12. Statistical Analyses

All experiments were repeated three times, data were analyzed by Student's *t*-test. Differences were considered statistically significant when values of $p < 0.05$.

The sample size was sufficient for the data analysis using paired two-tailed Student's *t*-test. For all Statistical analyses, the differences were considered to be statistically significant at values of $p < 0.05$.

3. Results

3.1. pOASL Expression is increased by IFN-β Stimulation and PRRSV Infection

After 6 h of stimulation with 1,000 IU/mL IFN-β, *pOASL* mRNA expression level in PAMs increased quickly to a peak of 125 times than that in the untreated control cells (Fig. 1A). The *pOASL* mRNA expression level peak occurred at 12 h post stimulation. This protein level was also tested, showing increased pOASL protein levels (Fig. 1B). This suggests that *pOASL* is an interferon-stimulated gene (ISG).

The *OASL* mRNA expression level in the PRRSV-infected PAMs peaked at 36 h post-infection (Fig. 1C), showing a 15-fold increase compared to the untreated cells. This protein level was also tested, showing increased pOASL protein levels (Fig. 1D). This suggests PRRSV infection increases the pOASL expression.

3.2. pOASL Restricts PRRSV Replication

Western blotting results showed that pOASL was well expressed in the CRL-2843-CD163 cells transfected with p3xFLAG-CMV™-7.1-OASL for 48 h (Fig. 2A). Furthermore, pOASL exerted no cytotoxic activity toward CRL-2843-CD163 cells after transfection for 48 h (Fig. 2B).

After pOASL transfection for 24 h, PRRSV infected the CRL-2843-CD163 cells, and after 24 h post-infection, the PRRSV genomic mRNA level and viral titers were tested. In comparison with empty vector-transfected cells, the PRRSV genomic mRNA level decreased significantly in the CRL-2843-CD163 cells transfected with p3xFLAG-CMV™-7.1-OASL (Fig. 2C). PRRSV $TCID_{50}$ for the p3xFLAG-CMV™-7.1-OASL group was also lower (Fig. 2D). Above all, those results indicate that pOASL is a host restriction factor to PRRSV.

3.3. pOASL siRNA Enhances PRRSV Replication

The pOASL siRNA (si-OASL) transfection efficiently reduced the expression of pOASL compared with scrambled siRNA without affecting cell viability (Fig. 3A to C). After 60 nM si-OASL was transfected into CRL-2843-CD163, PRRSV infected the cells for 24 h, and the results showed that in the presence of si-OASL, the PRRSV genomic mRNA levels were higher than that in cells transfected with si-NC (Fig. 3D). The $TCID_{50}$ results are in line with the mRNA level results (Fig. 3E). This suggests pOASL siRNA enhances PRRSV infection.

3.4. Anti-PRRSV Activity is not dependent on RNase L

The si-RNase L (60 nM) was transfected into CRL-2843-CD163 for 48 h, resulting in efficient reduction of RNase L expression (Fig. 4A and 4B). The pCMV-3xFLAG-7.1-OASL expression plasmid (800 ng) and 60 nM si-RNase L were co-transfected into CRL-2843-CD163 cells, 24 h later, 1.0 MOI PRRSV infected the CRL-2843-CD163 cells for 24 h. In the 800 ng pCMV-3xFLAG-7.1-OASL expression plasmid and 60 nM si-RNase L co-transfected and PRRSV (MOI 1.0)-infected CRL-2843-CD163 cells, there were still significant decreases in both the PRRSV genomic mRNA level and viral titers relative to the control group (Fig. 4C and 4D). This suggests that anti-PRRSV activity is not dependent on RNase L.

3.5. pOASL Increases IFN Responses

Results above have showed that pOASL did not inhibit PRRSV replication via the classical RNase L pathway, so whether anti-PRRSV activity of pOASL is dependent on other pathways needs to further investigated. There are reports revealing that some interferon-stimulated genes (ISGs) have antiviral effects via different mechanisms [43, 44]. To investigate the mechanisms, dual-luciferase reporter assays were conducted .The results showed that reporter activities of IFN-β (Fig. 5A), ISRE (Fig. 5B), and NF-κB (Fig. 5C) were significantly increased, indicating that IFN-β, ISRE, and NF-κB pathway were enhanced by pOASL.

3.6. IFN Pathway was activated by pOASL

Report revealed that human OASL interacts with human RIG-I and increases IFN signaling pathway. Our results also show that pOASL enhances type I IFN responses. So we speculate that pOASL act its role via RIG-I or MDA5 RNA sensor. Then co-IP assay was carried out to investigate the interaction. In this regard, Flag-tagged pOASL interacted with porcine MDA5 (pMDA5), but not with porcine RIG-I (pRIG-I) (Fig. 6A).Moreover, the RNase A treat the cell lysates, co-IP results found the interaction between them was independent of RNA (Fig. 6B). Based on the results above, pOASL interacts with pMDA5. Then we speculated pMDA5 pathway mediates the function of pOASL, next mRNA level of IFN-β, myxovirus resistance protein 1 (Mx1) and interferon-stimulated gene 15 (ISG15) in CRL-2843-CD163 cells, which pOASL and pMDA5 were co-expressed, were tested by qRT-PCR. These data indicated that co-expression increased the mRNA levels of IFN-β, Mx1 and ISG15 (Fig. 6C to E). The data suggest that pMDA5-mediated IFN pathway was enhanced by pOASL.

3.7 Anti-PRRSV Activity is dependent on pMDA5

Si-RIG-I (60 nM) was transfected into CRL-2843-CD163 for 48 h, resulting in efficient reduction of pRIG-I expression (Fig. 7A, B). By contrast, in the 800 ng pCMV-3xFLAG-7.1-OASL expression plasmid and 60 nM si-RIG-I co-transfected and PRRSV (MOI 1.0)-infected CRL-2843-CD163 cells, there were still significant decreases in either the PRRSV genomic mRNA expression level or viral titers relative to the levels in the control group (Fig. 7C, D). This suggests that anti-PRRSV activity is not dependent on pRIG-I.

For the case of pMDA5, 60 nM si-MDA5 was transfected into CRL-2843-CD163 cells for 48 h, resulting in efficient reduction of pMDA5 expression (Fig. 7E, F). In the CRL-2843-CD163 cells which co-transfected with 800 ng pCMV-3xFLAG-7.1-OASL and 60 nM si-MDA5 and then infected with PRRSV (MOI 1.0), there were no decreases in both the PRRSV genomic mRNA expression level and viral titers relative to the control group (Fig. 7G, H). This suggests that anti-PRRSV activity is dependent on pMDA5.

4. Discussion

The pattern recognition receptors (PRRs) are the first line to defense against invading microorganisms in the innate immune system[45]. RIG-I and MDA5 identifies double-stranded RNA (dsRNA) or 5’ triphosphate RNA in the cytoplasm. After infection by viruses, IFN production was induced by RIG-I or MDA5 which activates IFN signal pathway [46-49]. Hundreds of ISGs were activated by type I IFN, for

example, Interferon Induced Transmembrane Protein 3(IFITM3) and Viperin. There are also some intrinsic host restriction factors that are constitutively expressed, they also have antiviral effects. These “pre-existed” host factors response more quickly and directly inhibit virus replication [50].For example, TRIM41 impeds Influenza A virus and some RNA virus infection via K48-mediated ubiquitination which lead to protein degradation [8, 51]. Here, we report that pOASL participates in MDA5-mediated IFN signal pathway. The schematic representation of the signaling pathway is presented in Fig. 8.

Reports have revealed that human OASL interacts with RIG-I and exerts an antiviral effect. Even though it has no enzymatic activity, OASL is usually maintained at low expression levels in cells. When viruses infect human cells, human OASL is notably upregulated by the double-stranded RNA, and IFN [29, 52, 53]. In the present study, after PRRSV infect the cells, pOASL was induced, whereupon viral replication was inhibited. In stark contrast to our results, Lee demonstrated that murine OASL1 downregulates IFN via IRF7 to impede its expression and therefore aids in viral replication [30]. The discrepancies could be explained that different OASL isoforms might have different regulatory mechanisms in the signaling pathway.

Since pOASL has a nucleotidyltransferase region (data not shown), we surmised that its antiviral activity was dependent on RNase L. Nevertheless, our results indicated that this was not the case; pOASL did not exert its action via the OAS-RNase L pathway, and there may be another critical factor influencing the antiviral effect. Similarly, one report revealed that pOASL also inhibits Japanese encephalitis virus replication but not through the OAS-RNase L signaling pathway [32]. Thus, our finding for PRRSV is the same as that for Japanese encephalitis virus. Another report showed that pOASL could inhibit replication of classical swine fever virus through the MDA5-dependent pathway [54], and in our study, pOASL also inhibit PRRSV via this pathway.

In line with other studies that have shown the inhibition of PRRSV replication by pOAS1 and pOAS2, our study proves that pOASL inhibits PRRSV replication as well. Besides, pOAS1 and pOAS2 inhibit the replication of Japanese encephalitis virus, whereas pOASL inhibits the replication of classical swine fever virus, thus confirming the antiviral effects of the OAS protein family.

On the other hand, the inhibition of PRRSV replication by pOAS2 is dependent on RNase L [36], whereas inhibition of PRRSV replication by pOASL is not. This phenomenon may be related to the structures difference between pOAS2 and pOASL, which need further experimental verification.

A limitation of this study is that testing of other virulent strains was not done. The NADC-30 strains responsible for the most recent epidemics are presumed to follow the same trends. Therefore, future studies should include these viruses. It was not defined which step pOASL targets in PRRSV replication, we assume that any step could be targeted for inhibition, this also need further investigation in the future.

In conclusion, we demonstrate that pOASL is a new restriction factor which dampens PRRSV infection via MDA5-mediated type I IFN signaling.

Upregulation of pOASL activity boosts host immunity to limit PRRSV infection. Knockout of pOASL increases the PRRSV titer during the virus production. Future investigation of pOASL activity might provide the insight and opportunities needed for the therapeutic developments and improved vaccine candidates.

5. Conclusions

Porcine OASL inhibits PRRSV replication *in vitro* through an MDA5-dependent signaling. This may point to future directions regarding new ways to target PRRSV. Further research regarding the regulation of pOASL may provide insight and new antiviral strategies for therapeutic developments.

Declarations

Consent for publication

Not applicable.

Author Contributions

MZ, WK performed the experiments and wrote the manuscript; YK analysed the data; HL conceived and designed the experiments; all authors read and approved the final manuscript.

Availability of data and materials

All datasets are available in the main manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Not applicable

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Figures

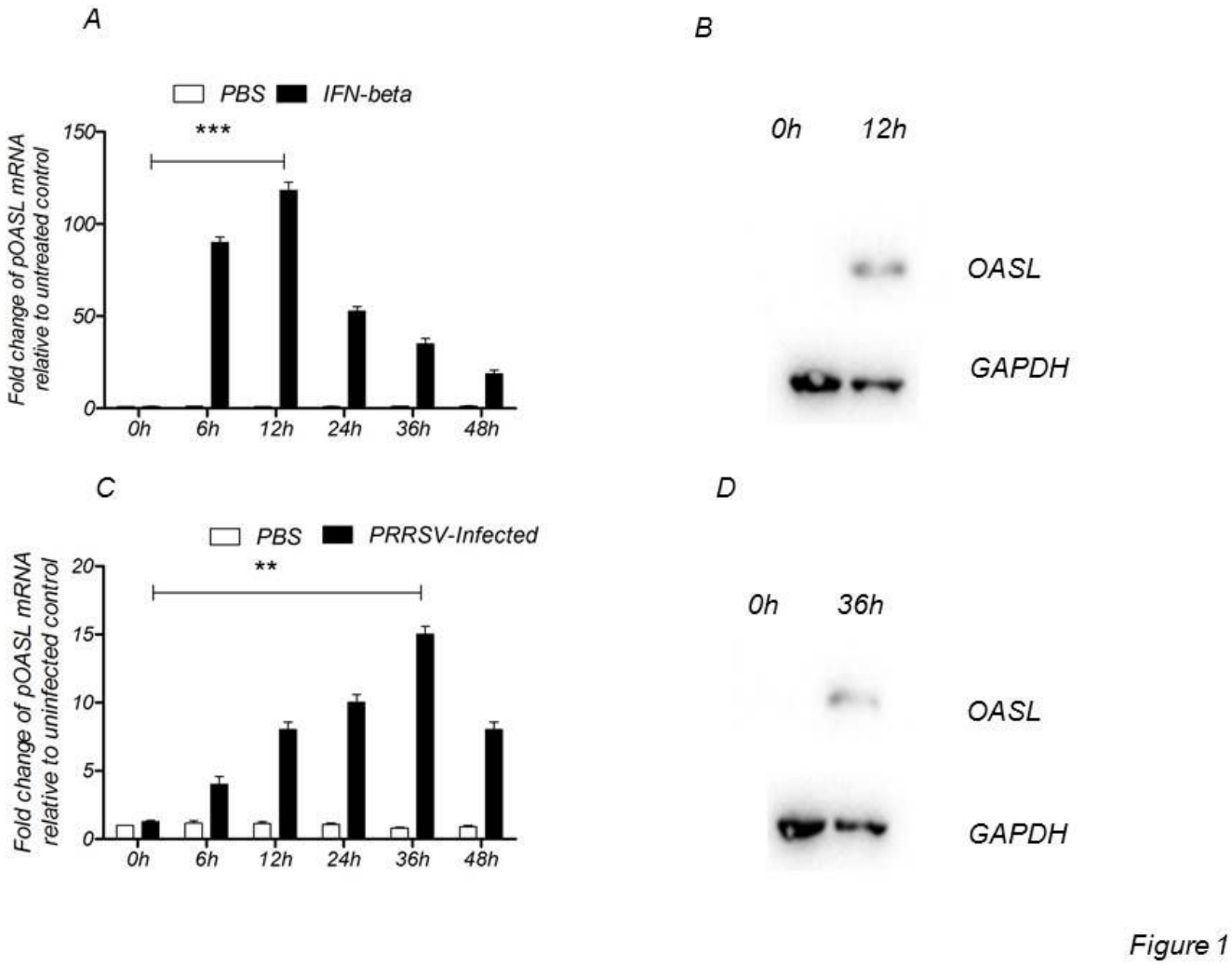

Figure 1

IFN-β stimulation and PRRSV infection induce porcine OASL (pOASL) expression. (A) IFN-β (1, 000 IU/mL) stimulates PAMs at the designated times (6, 12, 24, 36, and 48 h), then cells were harvested and subjected to mRNA extraction and real-time polymerase chain reaction (RT-PCR) analysis. (B) Protein level analysis of pOASL expression at 12 h post-stimulation with IFN-β. (C) PAMs were infected with PRRSV (MOI 1.0) for designated times (6, 12, 24, 36, and 48 h), mRNA was extracted, and RT-PCR was conducted to quantify pOASL mRNA expression. (D) Protein level analysis of pOASL expression at 36 h post infection with PRRSV. All experiments were biologically repeated three times, data represent means ± standard deviations. **P < 0.01; ***P < 0.001.

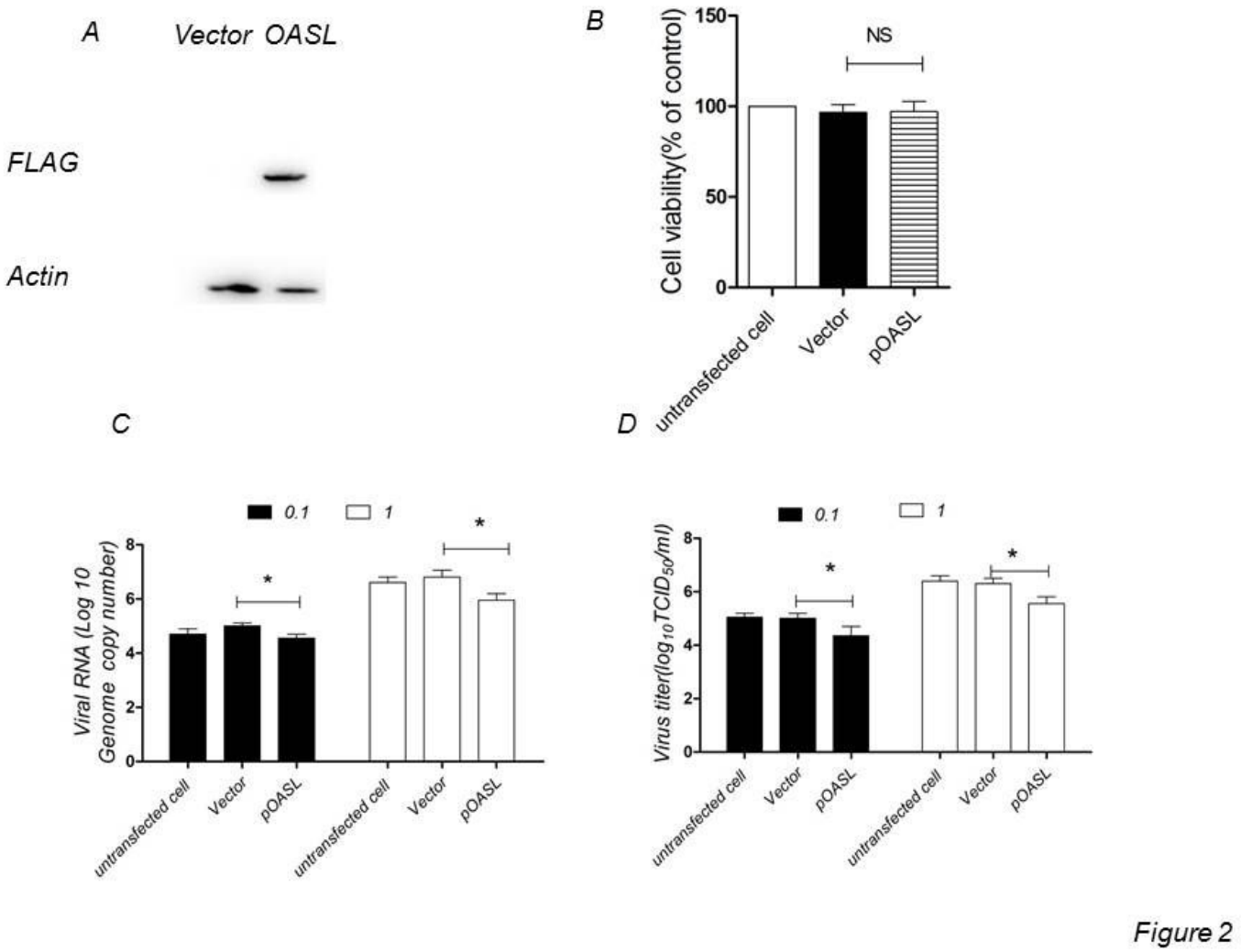

Figure 2

Figure 2

pOASL inhibits PRRSV replication. (A) 800 ng Flag-tagged pOASL plasmid was transfected into CRL-2843-CD163 cells. After 24 h, Western blotting analysis of pOASL expression was performed, and FLAG and GAPDH served as the primary antibodies. (B) A cell viability assay was used to quantify the toxicity of pOASL toward CRL-2843-CD163 cells. (C) After 800 ng of Flag-tagged pOASL plasmid transfection for 24 h, cells were infected with PRRSV (MOI 0.1 and 1.0), and RT-PCR quantitation of PRRSV genomic copy number in the supernatant was performed. (D) PRRSV titers were expressed as TCID50. All experiments were biologically repeated three times, data represent means ± standard deviations. *P < 0.05; NS, not significant.

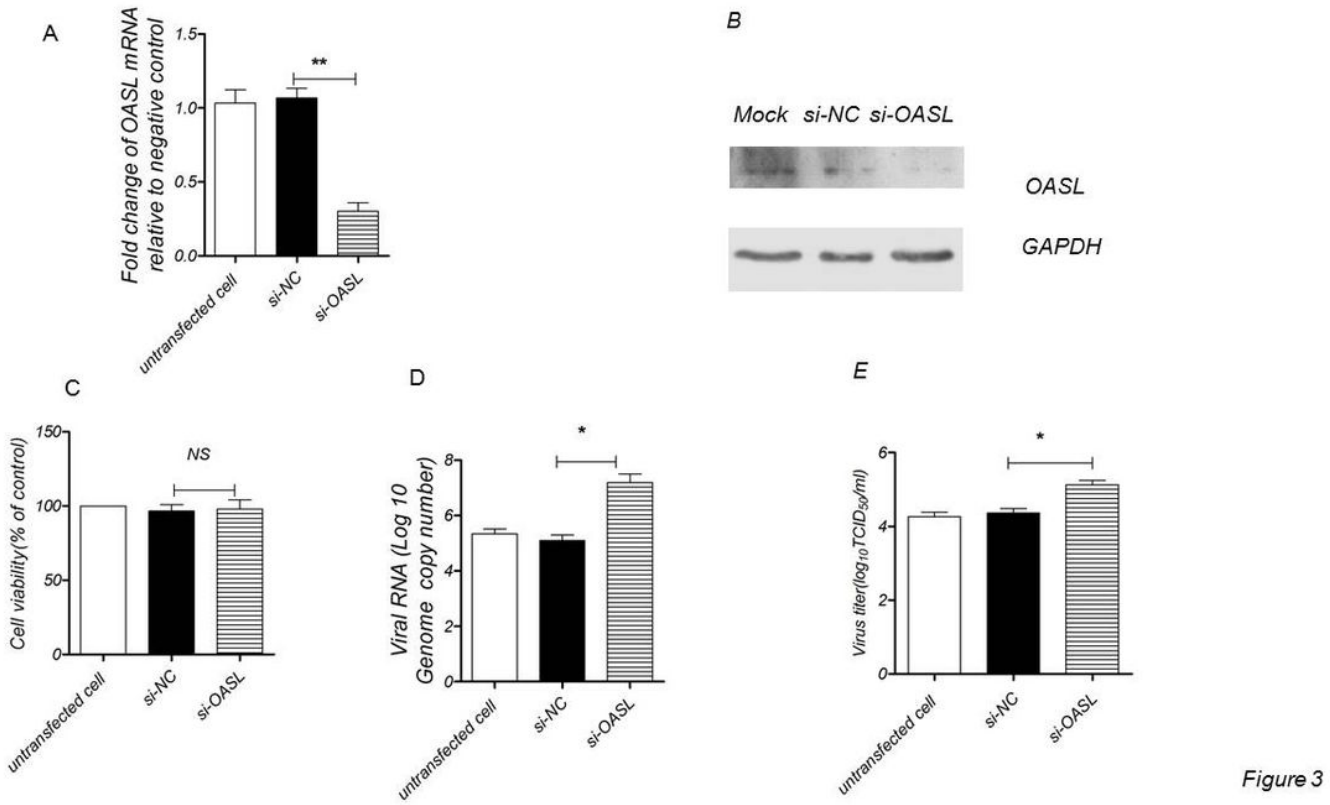

Figure 3

pOASL knockdown increases PRRSV growth. (A) RT-PCR quantitation of the pOASL mRNA in CRL-2843-CD163 cells transfected with 60 nM OASL siRNA (si-OASL). (B) Western blotting analysis of si-OASL transfections in CRL-2843-CD163 cells was presented. OASL and GAPDH served as the primary antibodies. (C) Cell viability assay to assess the toxicity of the siRNAs toward the CRL-2843-CD163 cells. (D) RT-PCR quantitation of the PRRSV genomic RNA level in the CRL-2843-CD163 cells transfected with 60 nM si-OASL and then infected with PRRSV (MOI 1.0). (E) PRRSV titers were expressed as TCID50. All experiments were biologically repeated three times, data represent means ± standard deviations. Each sample was run in triplicate. *P < 0.05; **P < 0.01; NS, not significant.

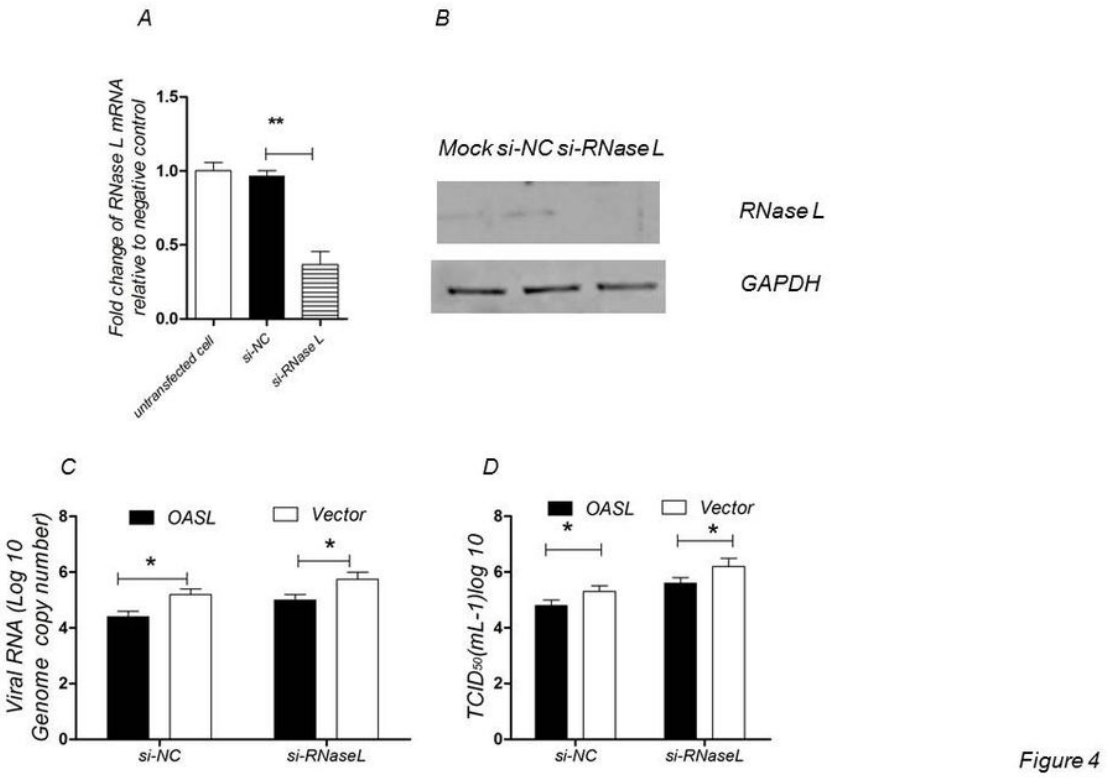

Figure 4

pOASL inhibition of PRRSV replication is not dependent on RNase L. (A) RT-PCR quantitation of RNase L mRNA in CRL-2843-CD163 cells transfected with 60 nM si-RNase L at 24 h post-transfection. (B) Western blotting results of si-RNase L transfections were presented. RNase L and GAPDH served as the primary antibodies. (C) RT-PCR quantitation of the PRRSV genomic copy number in the supernatants of CRL-2843-CD163 cells co-transfected with 60 nM si-RNase L siRNA and 800 ng pCMV-3xFLAG-7.1-OASL plasmids and then infected with PRRSV (MOI 1.0) at 24 h post-infection. (D) Viral titers were quantitated and expressed as TCID50/mL. All experiments were biologically repeated three times, data represent means ± standard deviations. *P < 0.05; **P < 0.01.

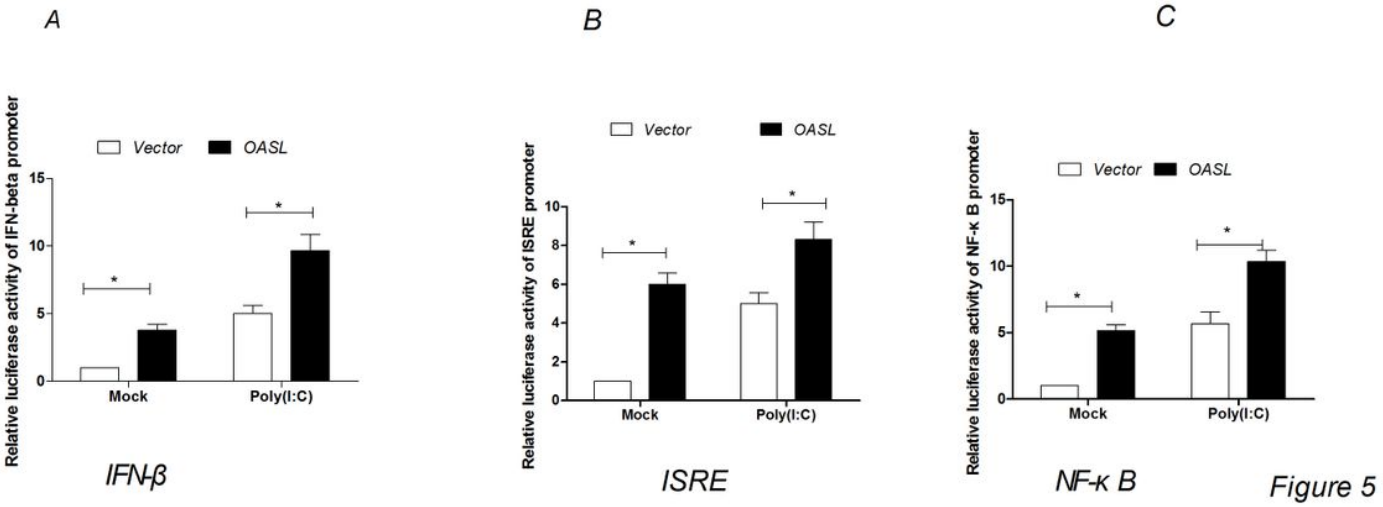

Figure 5

pOASL increases IFN responses. HEK293T cells were seeded in 24-well plate; on day one, 200 ng of pIFN-β-Luc (A), ISRE- Luc (B), or NF-κB- Luc (C), 20 ng of pRL-TK, and either the pCMV-3xFLAG-7.1 or pCMV-3xFLAG-7.1-OASL (400 ng) was transfected into the cells for 24h; On day two, cells were treated with 1.5 μg of poly (I: C) for 9 h, the promoter luciferase activity was tested. All experiments were biologically repeated three times, data represent means ± standard deviations. *P < 0.05.

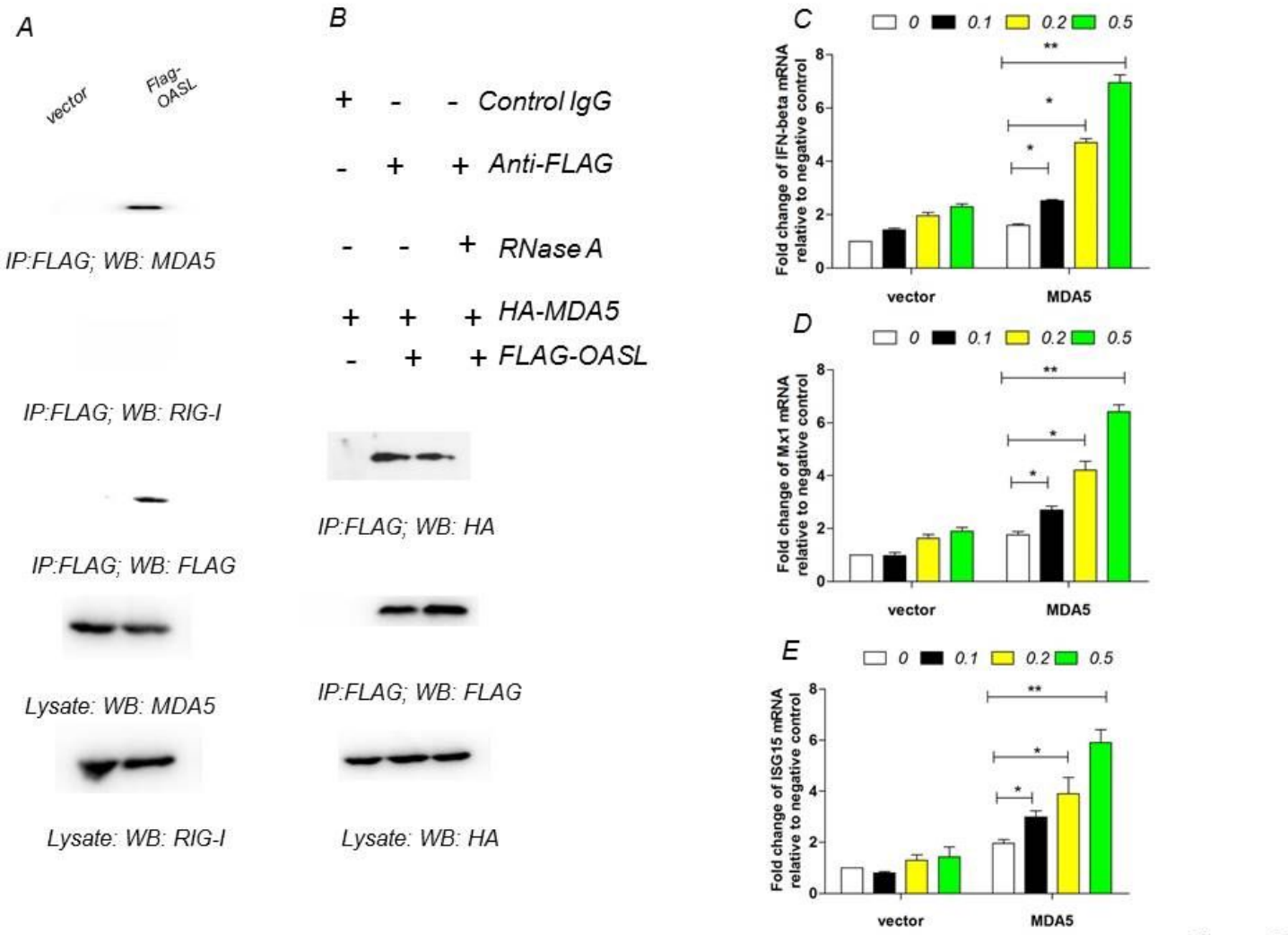

Figure 6

Figure 6

pOASL activates MDA5-mediated IFN-signaling pathway. (A) CRL-2843-CD163 cells were transfected with 800 ng Flag-tagged pOASL plasmid for 48 h. Cell lysates were immunoprecipitated with anti-FLAG antibody and blotted with indicated antibodies. (B) HA-MDA5 (1.25 μg) was transfected with 1.25 μg of pCMV-3xFLAG-7.1-OASL into HEK293 cells. Cell lysates were treated with or without RNase A and then immunoprecipitated with anti-FLAG antibody or control IgG, blotted as indicated. pOASL increases the mRNA level of IFN-β, Mx1 and ISG15 in CRL-2843-CD163 cells. CRL-2843-CD163 cells were transfected with different dose of pOASL plasmid (0, 100 ng, 200 ng, and 500 ng) plus HA-MDA5 (500 ng), or vector

(500 ng) for 24 h, and the mRNA levels of IFN-β(C), Mx1(D), ISG15(E) in the cells were quantified by RT-PCR assay. All experiments were biologically repeated three times, data represent means ± standard deviations. *P < 0.05; **P < 0.01; ***P < 0.001.

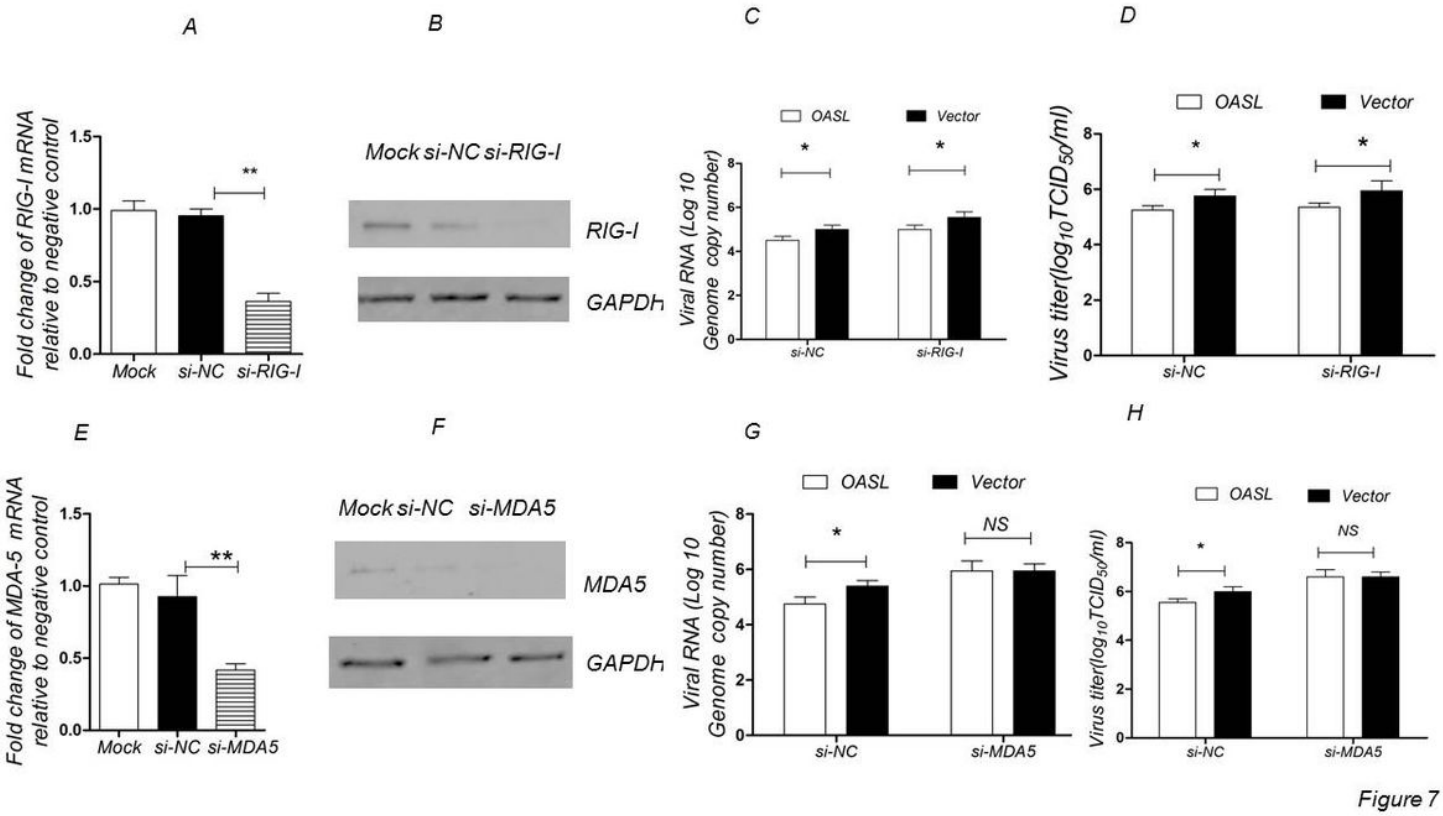

Figure 7

pOASL inhibition of PRRSV is dependent on pMDA5 but not on pRIG-I. (A) RT-PCR quantitation of the pRIG-I mRNA levels in CRL-2843-CD163 cells transfected with 60 nM si-RIG-I at 24 h post-transfection. (B) Western blotting results of si-RIG-I transfections. RIG-I and GAPDH served as the primary antibodies. (C) RT-PCR quantitation of the PRRSV genomic copy number in the supernatants in CRL-2843-CD163 cells co-transfected with 60 nM si-RIG-I and 800 ng pCMV-3xFLAG-7.1-OASL expression plasmid and then infected with PRRSV (MOI 1.0) at 24 h post-transfection. (D) PRRSV titers were quantitated and expressed as TCID50. (E) RT-PCR quantitation of the pMDA-5 mRNA levels in CRL-2843-CD163 cells transfected with 60 nM si-MDA-5 for 24 h. (F) Western blotting results of si-MDA5 transfections. MDA5 and GAPDH served as the primary antibodies. (G) RT-PCR quantitation of the PRRSV genomic copy number in the supernatants in CRL-2843-CD163 cells co-transfected with 60 nM si-MDA5 and 800 ng pCMV-3xFLAG-7.1-OASL plasmids and then infected with PRRSV (MOI 1.0) at 24 h post-infection. (H) PRRSV titers were quantitated and expressed as TCID50. *P < 0.05; **P < 0.01. NS, not significant.

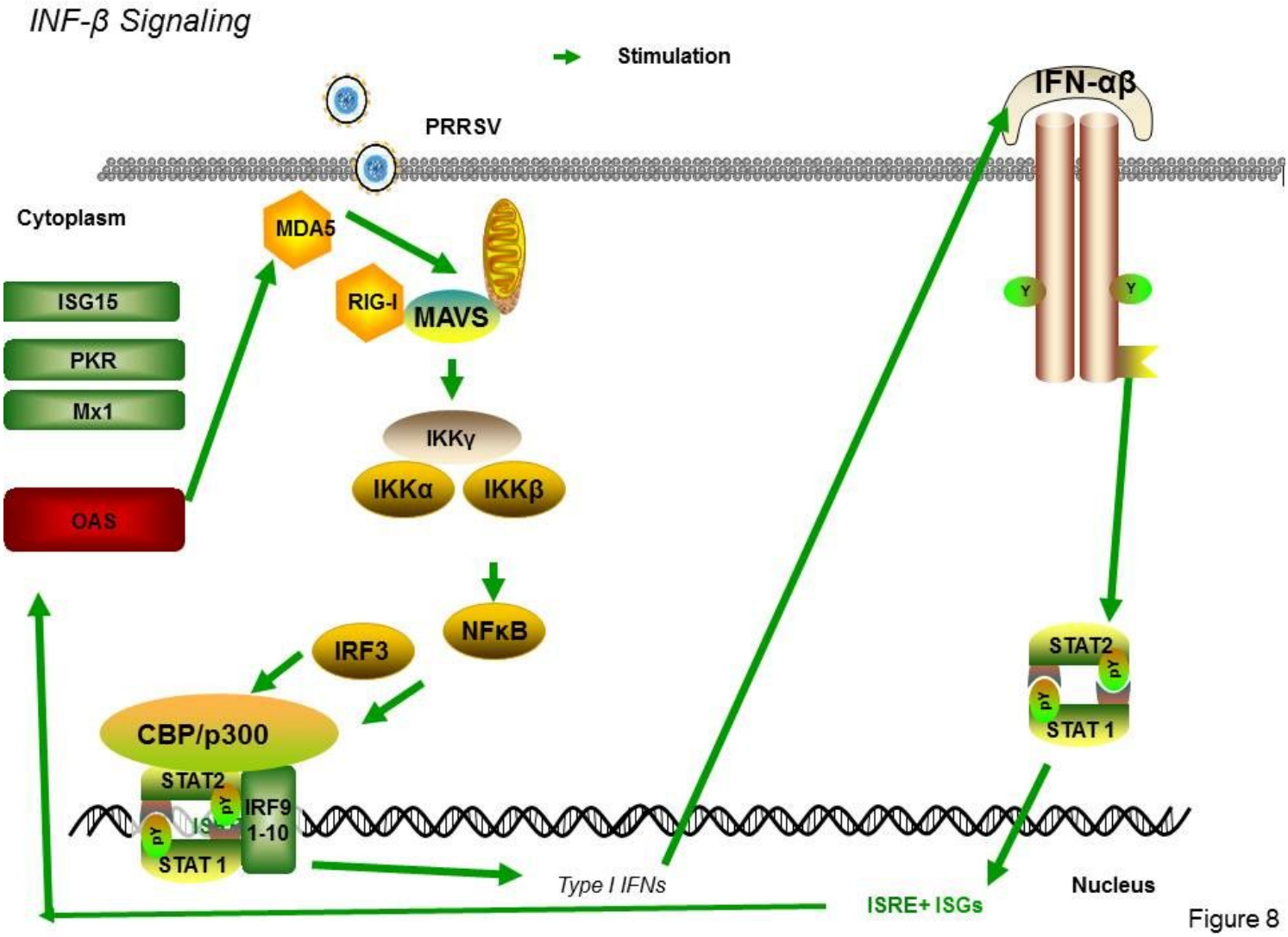

Figure 8

Schematic representation of the signaling pathways involved in pOASL-induced IFN expression. Human OASL interacts with RIG-I, thereby activating the IFN pathway. pOASL does not interact directly with pRIG-I but interacts with pMDA5, triggers the IFN pathway. IRF3 is a crucial protein in this pathway, IFNs up-regulate a series of IFN-stimulating genes, such as OAS, PKR, Mx1, and ISG15.