**Virologica Sinica**

**Supplementary Data**

**PRRSV degrades MDA5 via dual autophagy receptors P62 and CCT2 to evade antiviral innate immunity**

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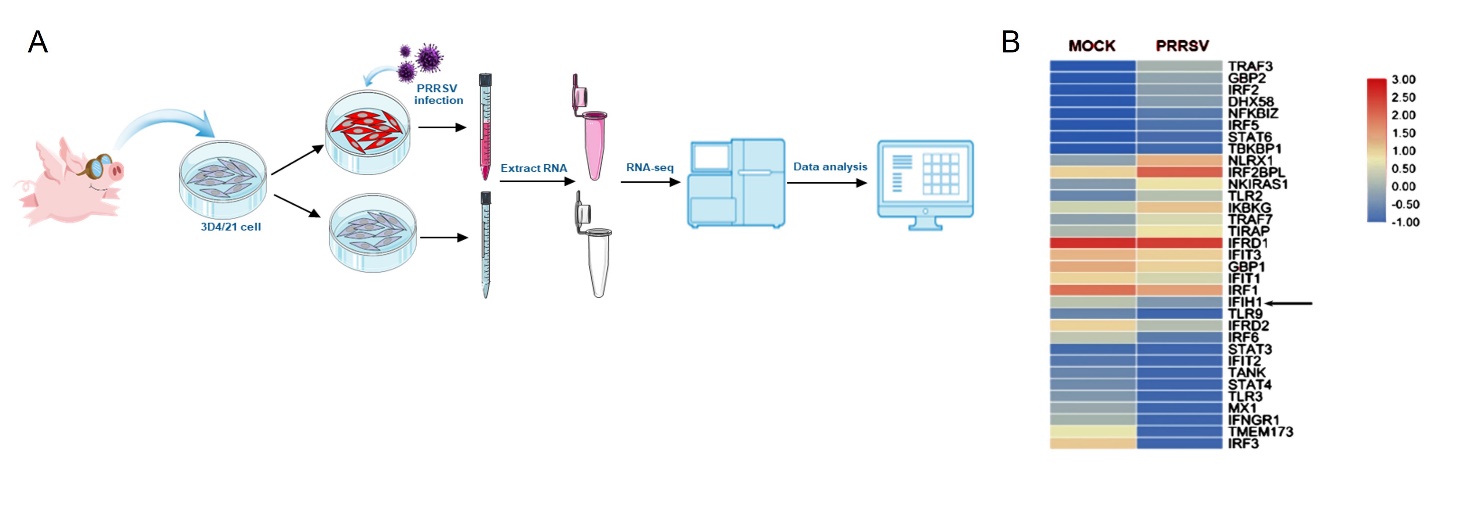
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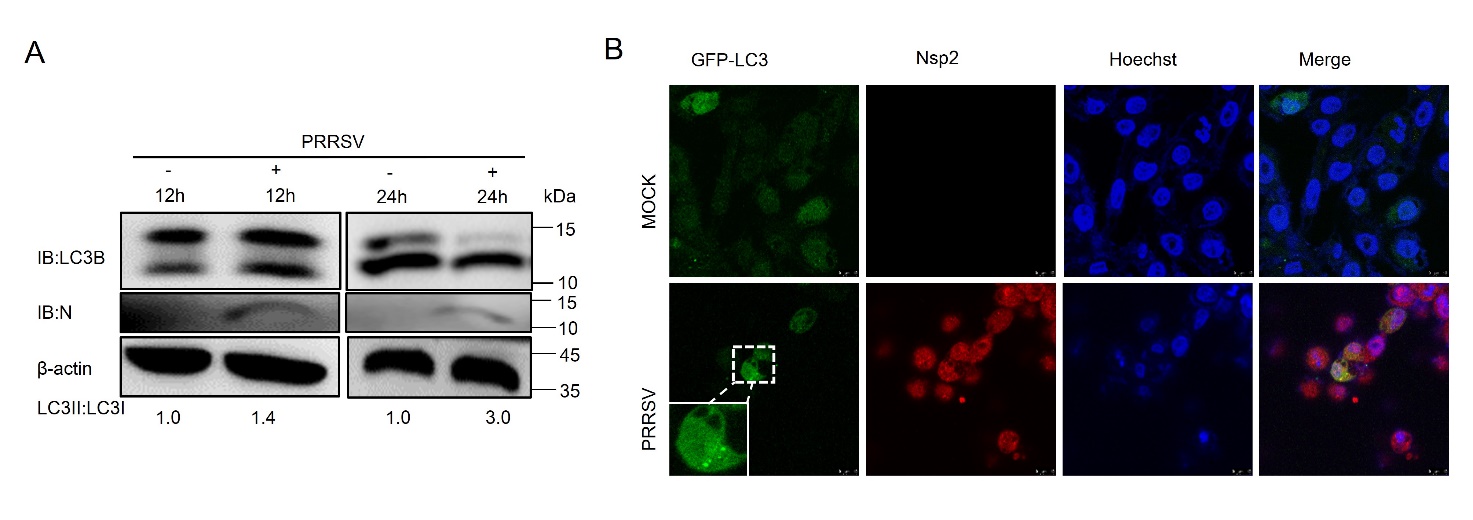
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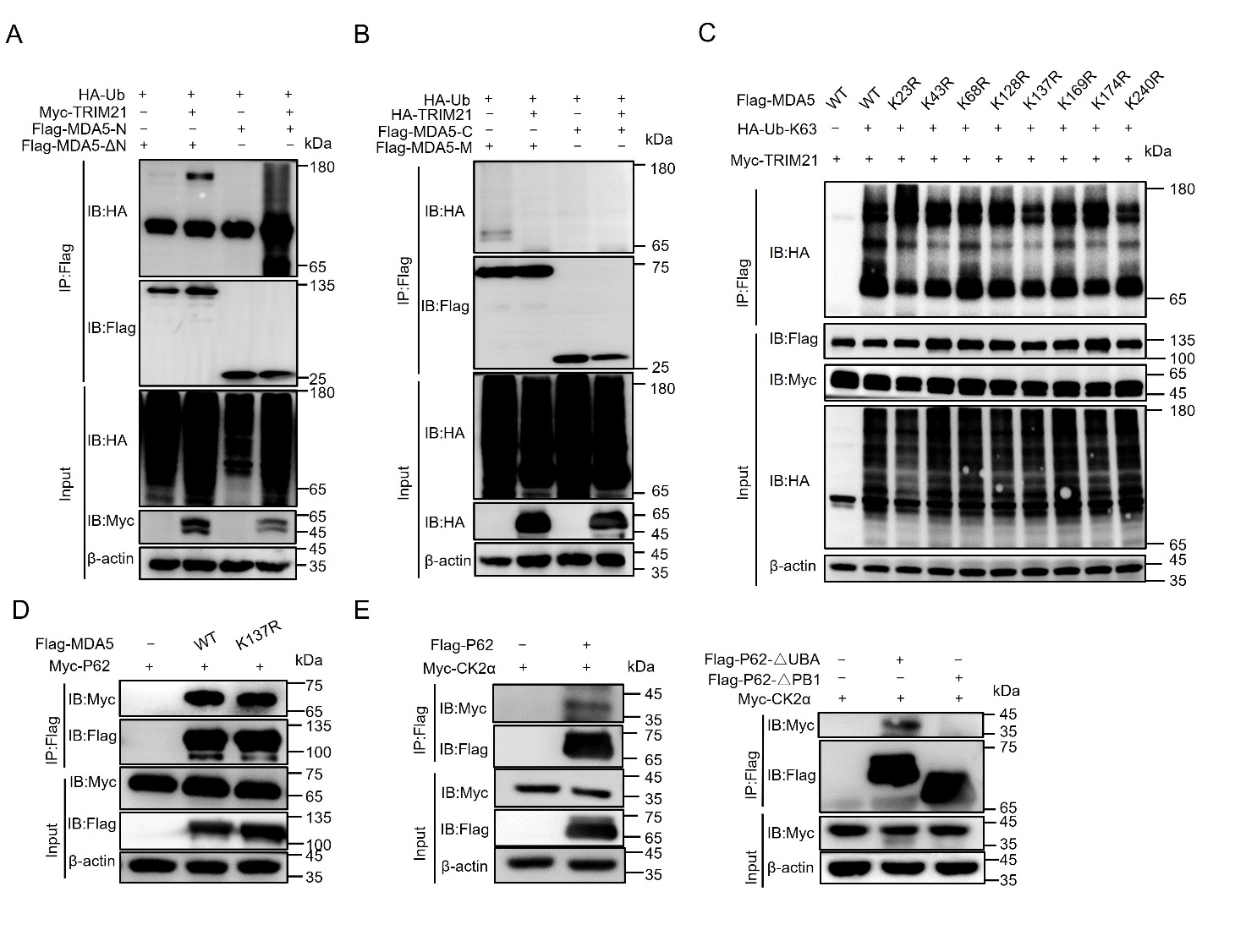
1 Ruiqi Sun, Yanyu Guo, Lilin Zhang contributed equally to this work.

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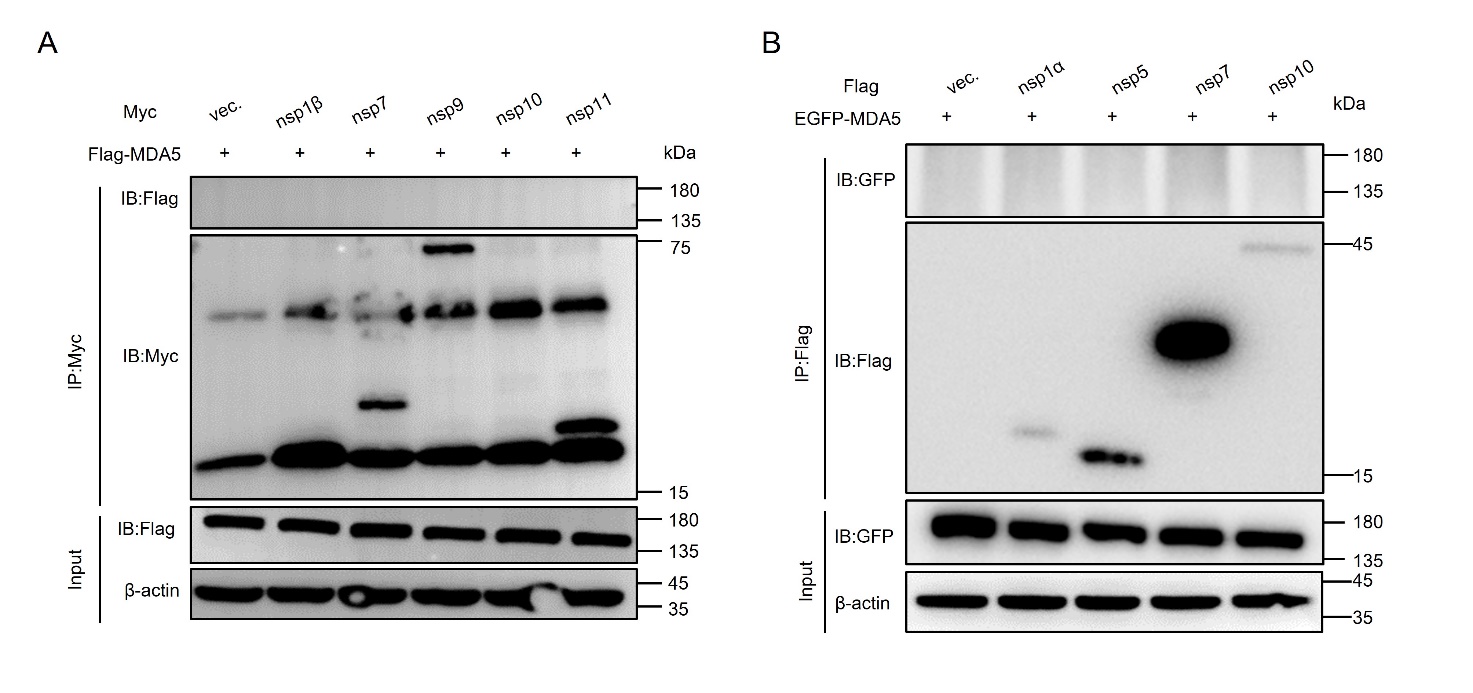
**Figure. S1 A** Diagram representation of transcriptome sequencing of 3D4/21CD163 cells uninfected and infected with PRRSV strain JXwn06 (MOI of 0.5). **B** Heat map with differential expression of molecules in the interferon signaling pathway upon PRRSV infection.

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**Figure. S2** PRRSV can induce autophagy. **A** 3D4/21CD163 cells were uninfected or infected with PRRSV for 12 and 24 h. The cell samples were harvested and lysed, and the protein expression levels of LC3 were detected by WB analysis. Quantification of band density in WB was done using ImageJ, and the ratio of the intensity of LC3-II to LC3-I was calculated to present the autophagic level. **B** 3D4/21CD163 cells were transfected with EGFP-LC3 plasmids and uninfected or infected with PRRSV for 24 h. The fixed cells were observed under confocal laser scanning microscope. Virions were stained with anti-PRRSV-Nsp2 protein pAb (red), and nuclei were stained with Hoechst (blue). Scale bars: 10 μm.

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**Figure. S3** Phosphorylase CK2α and ubiquitinase TRIM21 are involved in the selective autophagic degradation of MDA5. **A-B** Flag-MDA5 mutants and HA-Ub were cotransfected with or without Myc-TRIM21 into HEK 293T cells for 24 h. Cell lysates were subjected to immunoblot analysis of ubiquitination of MDA5 domains. **C** Immunoblot analysis of MDA5 ubiquitination in HEK 293T cells transfected with Myc-TRIM21, HA-K63-Ub, Flag-MDA5, or the K-to-R mutants of MDA5 N terminal domain. **D** Coimmunoprecipitation and immunoblot assays of extracts of HEK 293T cells transfected with Flag-MDA5 or Flag-MDA5 (K137R) and Myc-P62. **E** Coimmunoprecipitation and immunoblot analysis of the relation between Myc-CK2α with Flag-P62 or Flag-P62 (ΔPB1) or Flag-P62 (ΔUBA) in the lysates of cells.



**Figure. S4** No direct interaction of MDA5 with partial viral proteins of PRRSV was found. **A-B** Coimmunoprecipitation and immunoblot analysis of the relation between nsp1α, nsp1β, nsp5, nap7, nsp9, nsp10 or nsp11 with MDA5 in the lysates of HEK 293T cells, using anti-Myc beads or Flag beads.

**Table S1 The primers used for PCR amplification**

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| --- | --- |
| Primer name | Sequence of primer (5’-3’) |
| Flag-MDA5(sus)-F | CCAGTCGACTCTAGAGGATCCATGTCGTCGGATGGGTATTCC |
| Flag-MDA5(sus)-R | CAGGGATGCCACCCGGGATCCTCAGTCCTCATCACTAGACAAAC |
| EGFP-MDA5-F | TCGAGCTCAAGCTTCGAATTCTATGTCGTCGGATGGGTATTCC |
| EGFP-MDA5-R | GGATCCCGGGCCCGCGGTACCTCAGTCCTCATCACTAGACAAAC |
| Flag-MDA5-N-F | CCAGTCGACTCTAGAGGATCCATGTCGTCGGATGGGTATTC |
| Flag-MDA5-N-R | CAGGGATGCCACCCGGGATCCATCGGTGCCTGTTAATTCGC |
| Flag-MDA5-M-F | CCAGTCGACTCTAGAGGATCCGCATCCCCTGAGCCAGAGC |
| Flag-MDA5-M-R | CAGGGATGCCACCCGGGATCCGGCAACCAGGACGTAGGTGC |
| Flag-MDA5-C-F | CCAGTCGACTCTAGAGGATCCATGTACGTCCTGGTTGCCCA |
| Flag-MDA5-C-R | CAGGGATGCCACCCGGGATCCTCAGTCCTCATCACTAGACAAAC |
| Flag-MDA5-△C-F | AAGCAATGCAAGGACGAATATTGTTTGTCTAGT |
| Flag-MDA5-△C-R | GTCCTTGCATTGCTTTGCAAT |
| Flag-MDA5-△N-F | TCGGATGGGTATTCCTGCTGTGAAAGCAATGCAGAATCT |
| Flag-MDA5-△N-R | GGAATACCCATCCGACGACATGGATCC |
| PGL3-basic-MDA5-luc-F | ATCTGCGATCTAAGTAAGCTTCCTCAGAGCACTAATCCTCACC |
| PGL3-basic-MDA5-luc-R | CAGTACCGGAATGCCAAGCTTCTTTCCTTCTGAGAGGAGGG |
| PGL3-basic-MDA5-1-luc-F | CTAATCCTCACCGGGCGGATTGAATCCGAGCTGCAG |
| PGL3-basic-MDA5-2-luc-F | CTAATCCTCACCGGGCCAATCAGGCTCACCTGCAAG |
| PGL3-basic-MDA5-3-luc-F | CTAATCCTCACCGGGCCCAGGCTTCCCTGGCCTATC |
| PGL3-basic-MDA5-4-luc-F | CTAATCCTCACCGGGCCAGTCACCCAGGGCTGCAGA |
| PGL3-basic-MDA5-5-luc-F | CTAATCCTCACCGGGCCATTTTTCAGTGTGGGTGTCT |
| PGL3-basic-MDA5-6-luc-F | CTAATCCTCACCGGGCCTAGCTGCTTCAGCCAAAACT |
| PGL3-basic-MDA5-7-luc-F | CTAATCCTCACCGGGCGGTTCTTGGGGTTACACATC |
| PGL3-basic-MDA5-8-luc-F | CTAATCCTCACCGGGCCGTAGCGACGGCGTCTTG |
| PGL3-basic-MDA5-9-luc-F | CTAATCCTCACCGGGCCACTTCTCACCTGTGGAGC |
| PGL3-basic-MDA5-mutant-R | GCCCGGTGAGGATTAGTGCTCTGAG |
| Flag/HA-P62(sus)-F | CCAGTCGACTCTAGAGGATCCATGAGGGGCGGGGCTTC |
| Flag/HA-P62(sus)-R | CAGGGATGCCACCCGGGATCCTCACAAGGGCGGTGGGTG |
| Myc-P62-F | GAGGATCTGGGTACCGGATCCATGAGGGGCGGGGCTTC |
| Myc-P62-R | ATGCTCGAGGAATTCGGATCCTCACAAGGGCGGTGGGTG |
| Flag-P62-△PB1-F | GTCATGGCGTCGAAGGAGTGCCGGCGGGAC |
| Flag-P62-△PB1-R | CGACGCCATGACCACCAGTTG |
| Flag-P62-△UBA-F | ACAGGGCTGAAGGGATCCCGGGTGGCATCC |
| Flag-P62-△UBA-R | CTTCAGCCCTGTGGGTCCTTC |
| Flag-P62-△LIR-F | AGACATGGGCACCTCCAGTCTCTACAGATG |
| Flag-P62-△LIR-R | GTGCCCATGTCTCAGCTTCCG |
| HA-CCT2(sus)-F | CCAGTCGACTCTAGAGGATCCATGGCGTCCCTTTCCCTG |
| HA-CCT2(sus)-R | CAGGGATGCCACCCGGGATCCTTAACAGGGGTGGTGATC |
| Myc-CCT2-F | GAGGATCTGGGTACCGGATCCATGGCGTCCCTTTCCCTG |
| Myc-CCT2-R | ATGCTCGAGGAATTCGGATCCTTAACAGGGGTGGTGATC |
| Flag-CCT2-△D1-F | GACTCTAGAGGATCCGGCTTTCTGTTGGATAAA |
| Flag-CCT2-△D1-R | GGATCCTCTAGAGTCGACTGGTAC |
| Flag-CCT2-△D2-F | TCCTATTTAGATGAATCTGGGGTAGCCCTCGGT |
| Flag-CCT2-△D2-R | TTCATCTAAATAGGAATCTGCCAG |
| Flag-CCT2-△D3-F | GTAGCCCTCGGTGGATCCCGGGTGGCATCC |
| Flag-CCT2-△D3-R | ACCGAGGGCTACCCCAGAGAA |
| Flag/HA-TRIM21(sus)-F | CCAGTCGACTCTAGAGGATCCATGGCCTCAGCACTGCCC |
| Flag/HA-TRIM21(sus)-R | CAGGGATGCCACCCGGGATCCTCAGGGGGGGTGCATTTC |
| Myc-TRIM21-F | GAGGATCTGGGTACCGGATCCATGGCCTCAGCACTGCCC |
| Myc-TRIM21-R | ATGCTCGAGGAATTCGGATCCTCAGGGGGGGTGCATTTC |
| Myc-CSNK2A1(sus)-F | CTGGGTACCGGATCCGAATTCATGTCGGGACCCGTGCCA |
| Myc-CSNK2A1(sus)-R | AGATGCATGCTCGAGGAATTCTTACTGCTGAGCGCCAGCG |
| Myc-CSNK2A1(K68M)-F | AGTTGTTGTTATGATTCTCAAGCCAGTAAAG |
| Myc-CSNK2A1(K68M)-R | CATAACAACAACTTTTTCATTATTTGTGATG |
| Flag/Myc-TFAP2C(sus)-F | CCAGTCGACTCTAGAGGATCCATGTTGTGGAAAATAACCGATAATG |
| Flag/Myc-TFAP2C(sus)-R | CAGGGATGCCACCCGGGATCCTTACTTCCTGTGCTTCTCCAGC |
| PGL3-basic-MDA5-4-1-luc-F | ACCGGGCCAGTCACCCAGCTATGCAGAGCATC |
| PGL3-basic-MDA5-4-1-luc-R | TAGCTGGGTGACTGGCCCGGTGAGGATTAG |
| PGL3-basic-IFN-β(sus)-F | ATCTGCGATCTAAGTAAGCTTACTTGGCTTATGGTGG |
| PGL3-basic-IFN-β(sus)-R | CAGTACCGGAATGCCAAGCTTTGATGAAAACGGAAACACGG |
| Flag-nsp1α-F | CAAGCTTGCGGCCGCGAATTCAATGTCTGGGATACTTGATCG |
| Flag-nsp1α-R | CAGGGATGCCACCCGGGATCCTCAAGCACACTCAAAAGGGC |
| Myc-nsp1β-F | GAGGATCTGGGTACCGGATCCGCTGACGTCTATGACATTGG |
| Myc-nsp1β-R | ATGCTCGAGGAATTCGGATCCTCAACCGTACCACTTATGAC |
| Myc-nsp3-F | GAGGATCTGGGTACCGGATCCGGCCCACACCTCATTGCTG |
| Myc-nsp3-R | ATGCTCGAGGAATTCGGATCCTCACTCAAGGAGGGACCCG |
| Myc-nsp4-F | GAGGATCTGGGTACCGGATCCGGCGCTTTCAGAACTCAAAAG |
| Myc-nsp4-R | ATGCTCGAGGAATTCGGATCCTCATTCCAGTTCGGGTTTGG |
| Flag-nsp5-F | CAAGCTTGCGGCCGCGAATTCAATGGGAGGCCTTTCCACAGT |
| Flag-nsp5-R | CAGGGATGCCACCCGGGATCCTCACTCGGCAAAGTATCGCA |
| Myc-nsp7-F | GAGGATCTGGGTACCGGATCCTCGCTGACTGGTGCCCTCG |
| Myc-nsp7-R | ATGCTCGAGGAATTCGGATCCTCATTCCCACTGAGCTCTTC |
| Myc-nsp9-F | GAGGATCTGGGTACCGGATCCTTTAAACCTGCTAGCCGCCA |
| Myc-nsp9-R | ATGCTCGAGGAATTCGGATCCTCACTCATGATTGGACCTGAG |
| Myc-nsp10-F | GAGGATCTGGGTACCGGATCCGGGAAGAAGTCCAGAATGTG |
| Myc-nsp10-R | ATGCTCGAGGAATTCGGATCCTCATTCCAGGTCTGCGCAA |
| Myc-nsp11-F | GAGGATCTGGGTACCGGATCCGGGTCGAGCTCCCCGCTC |
| Myc-nsp11-R | ATGCTCGAGGAATTCGGATCCTCATTCAAGTTGAAAATAGG |
| Myc-N-F | GAGGATCTGGGTACCGGATCCCCAAATAACAACGGCAAGCA |
| Myc-N-R | ATGCTCGAGGAATTCGGATCCTCATGCTGAGGGTGATGCTGT |

**Table S2 The primers used for qRT-PCR amplification**

|  |  |
| --- | --- |
| Primer name | Sequence of primer (5’-3’) |
| MDA5(sus)-F | TCAGACACAAGTTTGGCAGAAGG |
| MDA5(sus)-R | CATGGTGCCTGAATCACTGCC |
| IFN-β(sus)-F | AGCACTGGCTGGAATGAAACCG |
| IFN-β(sus)-R | CAGGTCATCCATCTGCCCATCAAG |
| CK2α(sus)-F | GGTGAGGATAGCCAAGGTTCTG |
| CK2α(sus)-R | TCACTGTGGACAAAGCGTTCCC |
| TFAP2C(sus)-F | CACCTGTTGCTGCACGATCAGA |
| TFAP2C(sus)-R | AGGAGCGACAATCTTCCAGGGA |
| TRIM21(sus)-F | CCAGACTCCCCTCTACCCT |
| TRIM21 (sus)-R | GCCGTGGTCACTGATGTTG |
| CCT2(sus)-F | TTTGGTGCTGCTGGTGTTATGG |
| CCT2(sus)-R | ACCACCTGTGACAAGAGCAAGG |
| PRRSV-N-F | CAGTCAATCAGCTGTGCCAAA |
| PRRSV-N-R | ATCTGACAGGGCACAAGTTCCA |
| β-actin(sus)-F | GAATCCTGCGGCATCCACGA |
| β-actin (sus)-F | CTCGTCGTACTCCTGCTTGCT |