**Virologica Sinica**

**Supplementary Data**

**African swine fever virus S273R protein antagonizes type I interferon production by interfering with TBK1 and IRF3 interaction**

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**Supplementary Table S1** Primers used for PCR.

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| Primer | Sequence (5′–3′) |
| Myc-S273R-F | ATGGAGGCCCGAATTCGGATGTCTATATTAGAAAAAA |
| Myc-S273R-R | GCGGCCGCGGTACCTTTATGCGATGCGAAAC |
| Flag-S273R-F | GCTCTAGAATGTCTATATTAGAAAAAATTACGTC |
| Flag-S273R-R | GGGAAGCTTTTATGCGATGCGAAAC |
| GST-S273R-F | GGGCCCCTGGGATCCCCGATGTCTATATTAGAAAAAATTAC |
| GST-S273R-R | GTCAGTCACGATGCGGCCGTTATGCGATGCGAAACA |
| S273R-Arm-R | GCGGCCGCGGTACCTTTAGTTTCTGGGTCCT |
| S273R-Core-F | ATGGAGGCCCGAATTCGGATGAACACGGGTCTTCTTAC |
| S273R-H168A-F | CACTGGAAAAGCCTGGGTAGCCATC |
| S273R-H168A-R | CCCGTTGAAAAGTCCGTGTTTAAAA |
| S273R-N187A-F | CGAATATTTTGCTTCGACGGGAAATTCTCC |
| S273R-N187A-R | ATGCTCCAGCAGTCGCCCCGC |
| S273R-C232A-F | GCAGACCGAGGCCGGCCCCTACA |
| S273R-C232A-R | GACCGTTGGTGACGAATGTTGGTAAC |
| S273R-Core3M-F | AACACGGGTCTTCTTACAAAC |
| S273R-Core3M-R | CCGAATTCGGGCCTCCAT |
| HA-IRF3-F | CTGACTATGCGGGCGGATCC ATGGGAACTCAGAAG |
| HA-IRF3-R | GCCATGGCGGCCAAGCTT CTAGAAATCCATGTCCTC |
| Flag-IRF3-M1-F | ACGACGATGACAAGGGATCCGGGGACTTTCCTGAG |
| His-IRF3-F | ATGGCTGATATCGGATCCATGGGAACTCAGAAGCCT |
| His-IRF3-R | GTGCTCGAGTGCGGCCGCCTAGAAATCCATGTCCTCCAC |
| Flag-IRF3-M1-R | GGGCCATGGCGGCCAAGCTTCTAGAAATCCATGTCCTC |
| Flag-IRF3-M2-F | CGTGAAAACCCACTGAAG |
| Flag-IRF3-M2-R | AACTCCTGAGGTCACAAAC |
| Flag-IRF3-M3-F | GACGGGGGCGCCTCCTCC |
| Flag-IRF3-M3-R | GACTCCCAGGTTGGGGCAAGAAGC |
| Flag-IRF3-M4-F | ACGACGATGACAAGGGATCCATGGGAACTCAGAAG |
| Flag-IRF3-M4-R | GGGCCATGGCGGCCAAGCTTCCGCGCCATGTCTAC |

**Supplementary Table S2** Primers for real-time PCR.

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| Primer | Sequence (5′–3′) |
| Swine IFN-β-F | AGCACTGGCTGGAATGAAC |
| Swine IFN-β-R | TCCAGGATTGTCTCCAGGTC |
| Swine ISG54-F | TCTGTGGCTTTGCACCTCTT |
| Swine ISG54-R | GGGGTTTCAGCTCCATTCCA |
| Swine ISG56-F | TTCCGACACGCAGTCAAGTT |
| Swine ISG56-R | GTAGCAAAGCCTGTCTGGT |
| Human IFN-β-F | ACGCCGCATTGACCATCTAT |
| Human IFN-β-R | GTCTCATTCCAGCCAGTGCT |
| Human ISG54-F | CTGCAACCATGAGTGAGAA |
| Human ISG54-R | CCTTTGAGGTGCTTTAGATAG |
| Human ISG56-F | TACAGCAACCATGAGTACAA |
| Human ISG56-R | TCAGGTGTTTCACATAGGC |

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**Supplementary Fig. S1** 3D4/21 cells were transfected with empty or Myc-S273R (500 ng) plasmid for 12 h and then infected with PRV (MOI = 0.01) for the indicated times. The infected cells and supernatants were harvested to analyze *IFN-β* (**A**) and PRV *US6* (**B**) genes expression

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**Supplementary Fig. S2** HEK293T cells were transfected with Myc-S273R (1 μg) and Flag-TBK1 (1 μg) expression plasmids for 24 h. The interaction of TBK1 and pS273R was analyzed by co-immunoprecipitation assay and Western blotting.