**Virologica Sinica**

**Supplementary Data**

**FoxJ1 inhibits African swine fever virus replication and viral S273R protein decreases the expression of FoxJ1 to impair its antiviral effect**

Caina Maa, Shasha Lib, Fan Yangb, Weijun Caob, Huisheng Liub, Tao Fengb, Keshan Zhangb, Zixiang Zhub, Xiangtao Liub, Yonghao Hua\*, Haixue Zhengb\*

1. *College of Veterinary Medicine, Gansu Agricultural University, Lanzhou, 730070, China*
2. *State Key Laboratory of Veterinary Etiological Biology, National Foot and Mouth Diseases Reference Laboratory, Key Laboratory of Animal Virology of Ministry of Agriculture, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, 730046, China*

Corresponding author:

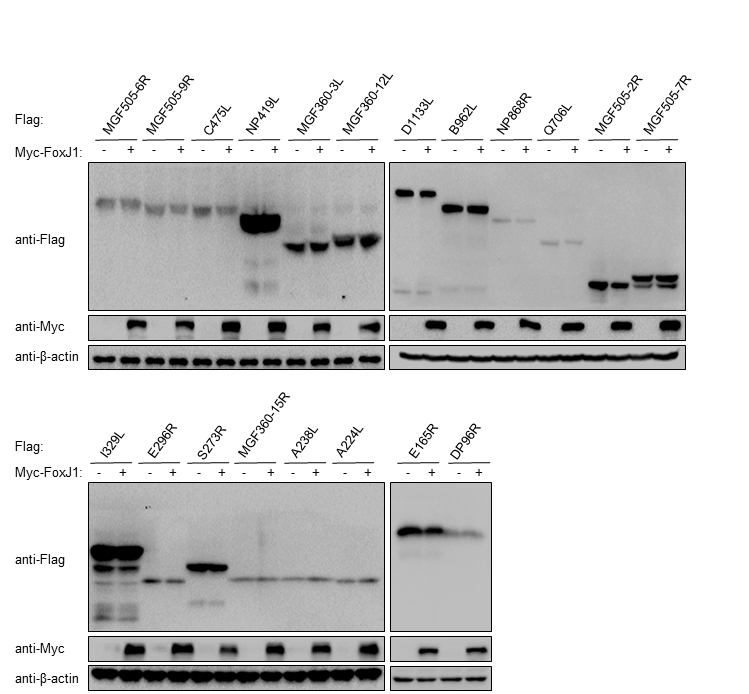
[haixuezheng@163.com](mailto:haixuezheng@163.com) (H. Zheng)

**Supplementary Table S1.** TheqPCR primers and siRNA sequences used in this study.

|  |  |  |
| --- | --- | --- |
| Primer | Sequence (5’–3’) | Target gene |
| pIFN-β-F | GCTAACAAGTGCATCCTCCAAA | Porcine *IFN-β* gene |
| pIFN-β-R | AGCACATCATAGCTCATGGAAAGA |  |
| pISG15-F | GATCGGTGTGCCTGCCTTC | Porcine *ISG15* gene |
| pISG15-R | CGTTGCTGCGACCCTTGT |  |
| pISG54-F | CTGGCAAAGAGCCCTAAGGA | Porcine *ISG54* gene |
| pISG54-R | CTCAGAGGGTCAATGGAATTCC |  |
| pISG56-F | TTAGAAAACAGGGTCTTGGAGGAG | Porcine *ISG56* gene |
| pISG56-R | CGTAAGGTAATACAGCCAGGCATA |  |
| pGAPDH-F | ACATGGCCTCCAAGGAGTAAGA | Porcine *GAPDH* gene |
| pGAPDH-R | GATCGAGTTGGGGCTGTGACT |  |
| pFoxJ1-F  pFoxJ1-R | TACTCCTATGCCACGCTCAT  GCGGAAGTAGCAGAAGTTGTC | Porcine *FoxJ1* gene |
| mFoxJ1-F  mFoxJ1-R | GTGGACTACGCCACCAATCC  TAGATGGCCGACAGGGTGAT | Monkey *FoxJ1* gene |
| mGAPDH-F  mGAPDH-R | CCTGCACCACCAACTGCTTA  CATGAGTCCTTCCACGATACCA | Monkey *GAPDH* gene |
| mISG15-F  mISG15-R | TGGACAAATGCGACGAACC  CCCGCTCACTTGCTGCTT | Monkey *ISG15* gene |
| mISG54-F  mISG54-R | ACCGAACAATGCCTACCTG  TGAGCCACAGCGTGTCCTA | Monkey *ISG54* gene |
| mISG56-F  mISG56-R | TGTATTACCACATGGGCAGAC  ATCCAGGCGATAGGCAGAG | Monkey *ISG56* gene |
| mIFN-β-F  mIFN-β-R | GACATCCCTGAGGAGATTAAG  ATGTTCTGGAGCATCTCATAG | Monkey *IFN-β* gene |
| ASFV p72 (B646L)-F | TGCGATGATGATTACCTT | ASFV *B646L* gene |
| ASFV p72(B646L)-R | ATTCTCTTGCTCTGGATAC |  |
| ASFV p30(CP204L)-F | CTCCGATGAGGGCTCTTGCT | ASFV *CP204L* gene |
| ASFV p30(CP204L)-R | AGACGGAATCCTCAGCATCTTC |  |
| FoxJ1-siRNA-911(FoxJ1-siRNA)-F  FoxJ1-siRNA-911(FoxJ1-siRNA)-R | CUCAAAGGCAACUUCGACUTT  AGUCGAAGUUGCCUUUGAGTT | Porcine *FoxJ1* gene |
| FoxJ1-siRNA-911(FoxJ1-siRNA1)-F  FoxJ1-siRNA-911(FoxJ1-siRNA1)-R | CUCAAAGGCAACUUCGACUTT  AGUCGAAGUUGCCUUUGAGTT | Monkey *FoxJ1* gene |

**Supplementary Table S2.** The top ten genes that were most significantly down-regulated at each time point after ASFV infection.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Gene category | 12 hpi | | 24 hpi | | 48 hpi | |
| Gene | log2Fold Change | Gene | log2Fold Change | Gene | log2Fold Change |
| Downregulated | *LOC100522201* | -12.873 | *LOC100522201* | -12.992 | *LOC100522201* | -12.735 |
| *FOSB* | -9.033 | *FOSB* | -11.965 | *FOXJ1* | -12.464 |
| *FOXJ1* | -8.936 | *FOXJ1* | -10.863 | *FOSB* | -11.374 |
| *LOC100038328* | -8.756 | *RRAD* | -9.777 | *RRAD* | -10.521 |
| *FOS* | -8.446 | *ID1* | -9.281 | *LOC396781* | -9.973 |
| *EGR4* | -8.065 | *MT1D* | -9.072 | *CLEC12A* | -9.371 |
| *KIF18B* | -7.779 | *FAM83D* | -9.008 | *METTL24* | -9.138 |
| *PLIN1* | -7.772 | *ARC* | -8.940 | *LOC110258822* | -9.120 |
| *LOC102162420* | -7.567 | *KIF18B* | -8.861 | *LOC100038328* | -8.619 |
| *E2F8* | -7.523 | *THBD* | -8.775 | *PLIN1* | -8.596 |



Supplementary Figure S1. Screening of ASFV proteins that interacted with FoxJ1. iPAM cells were co-transfected with empty vector (2 µg/well) or Myc-FoxJ1 (2 µg/well) expressing plasmids and the indicated ASFV protein-expressing plasmids (2 µg/well) for 24 h. The expression of the indicated viral proteins and FoxJ1 was then detected by Western blotting.



Supplementary Figure S2. Working concentration of MG132, Z-VAD-FMK, CQ, and NH4Cl in iPAM cells. **A** The effects of inhibitors on the MGF-505-7R-mediated destabilization of JAK2. iPAM cells were transfected with MGF505-7R expressing plasmids or empty vector, and JAK2 expressing plasmids. MG132 (50 μmol/L) or NH4Cl (20 mmol/L) was added after transfection for 18 h. After 6 h incubation, the expression of HA-JAK2 was detected by Western blotting. **B** iPAM cells were transfected with MGF505-2R or E165R expressing plasmids, and vector or FoxJ1 expressing plasmids. MG132 (50 μmol/L) or NH4Cl (20 mmol/L) was added after18 h transfection. After 6 h treatment, the expression of Flag-MGF505-2R or Flag-E165R was detected by Western blotting. **C** iPAM cells were transfected with SVA-2B expressing plasmids or empty vector plasmids, and MAVS expressing plasmids. Z-VAD-FMK (50 μmol/L) and CQ (100 μmol/L) was added after 18 h transfection. After 6 h treatment, the expression of HA-MAVS was detected by Western blotting. **D** iPAM cells were transfected with MGF505-2R or E165R expressing plasmids, and vector or FoxJ1 expressing plasmids. Z-VAD-FMK (50 μmol/L) was added after 18 h transfection. After 6 h incubation, the expression of Flag-MGF505-2R or Flag-E165R was detected by Western blotting.