

## Electronic Supplementary Material

### C19orf66 Inhibits Japanese Encephalitis Virus Replication by Targeting -1 PRF and the NS3 Protein

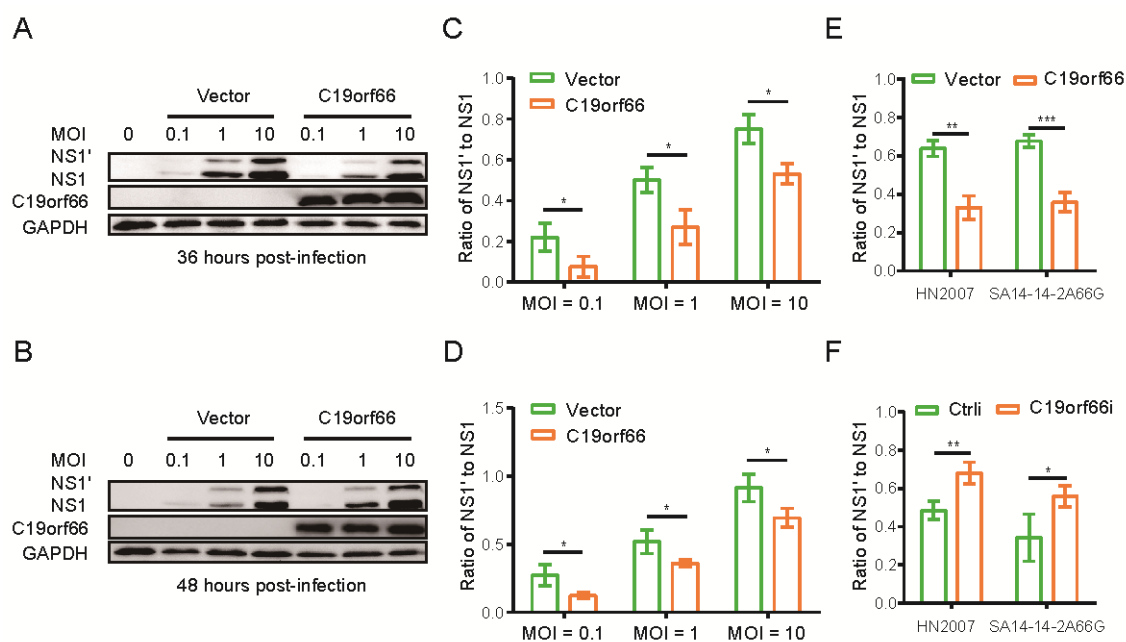
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**Table S1** Sequences of primers used in this work

| Oligos for      | Oligos name   | Primers              | Sequence (5'-3')                   |                                    |
|-----------------|---------------|----------------------|------------------------------------|------------------------------------|
| p3xFLAG-CMV-7.1 | NS3           | Forward              | CGGAATTCAATGGGGGGCGTGTTTTGGGACAC   |                                    |
|                 |               | Reverse              | GCTCTAGATTATCTCTTTTCCTGCTGCGAAGT   |                                    |
|                 | NS1-NS2A      | Forward              | CGCTCGAGACCATGTCAATTGCTTTGGCCTTCT  |                                    |
|                 |               | Reverse              | GCGAATTCTCATCTTTTTGTTGTTTTTAAAGTG  |                                    |
|                 | FLAG-C19orf66 | Forward              | GCCTCGAGATGGATTACAAGGATGACGACGATAA |                                    |
|                 |               | Reverse              | GTCTCAGGAAGGTGTG                   |                                    |
|                 | FLAG-C19orf53 | Forward              | CGGAATTCTGTCTGGTTTGTATCTGC         |                                    |
|                 |               | Reverse              | GCCTCGAGATGGATTACAAGGATGACGACGATAA |                                    |
|                 | pcDNA3.1(-)   | FLAG-ISG15           | Forward                            | GATGGCGCAGGGGCAGCGCAA              |
|                 |               |                      | Reverse                            | CGGAATTCTCAGGAAGGTGTCTTGGAGG       |
|                 |               | FLAG-PKR             | Forward                            | GCCTCGAGATGGATTACAAGGATGACGACGATAA |
|                 |               |                      | Reverse                            | GATGGGCTGGGACCTGACGGT              |
| FLAG-OAS1       |               | Forward              | CGGGATCCTTAGCTCCGCCCGCCAGGCT       |                                    |
|                 |               | Reverse              | GCCTCGAGATGGATTACAAGGATGACGACGATAA |                                    |
| qNS1-NS2A       |               | Forward              | GATGGCTGGTGATCTTTCAGC              |                                    |
|                 |               | Reverse              | CGGGATCCCTAACATGTGTGTCGTTTCAT      |                                    |
| qNS3            |               | Forward              | GCCTCGAGATGGATTACAAGGATGACGACGATAA |                                    |
|                 |               | Reverse              | GATGATGGATCTCAGAAATAC              |                                    |
| qPCR            |               | qE                   | Forward                            | CGGAATTCTCAAGCTTCATGGAGAGGGGCAGGGA |
|                 |               |                      | Reverse                            | TGAA                               |
| qPCR            | qNS1-NS2A     | Forward              | ATCACTGACTGGTGCTGTGCG              |                                    |
|                 |               | Reverse              | TCAACCTGCGATCTGACGAG               |                                    |
|                 | qNS3          | Forward              | GTGATCGTGGTAGAACCGGG               |                                    |
|                 |               | Reverse              | ATGTTCTCGCGGGTAATCC                |                                    |
|                 | qC19orf66     | Forward              | GGCAAACGACAAACCAACATT              |                                    |
|                 |               | Reverse              | ATCAGCTCGCTTCTCGTTGTG              |                                    |
| qGAPDH          | Forward       | GGTGTGGAGCTGGAGAAGAG |                                    |                                    |
|                 | Reverse       | GCCGAATTTCTCATCAGAG  |                                    |                                    |
|                 |               | Forward              | TCGGAGTCAACGGATTTG                 |                                    |
|                 |               | Reverse              | GCATCGCCCCACTTGATT                 |                                    |



**Fig. S1** C19orf66 significantly affected the ratio of NS1' and NS1 under different circumstances. **A, B** 293T cells transfected with a plasmid encoding C19orf66 (0.5  $\mu$ g) or an empty vector (0.5  $\mu$ g) were inoculated with JEV NJ2008 strain (MOI = 0.1, 1 or 10). Cell lysates were collected at 36 and 48 hours post-infection, the expression levels of C19orf66, NS1', NS1 and GAPDH were analyzed by Western blotting. **C, D** The gray value of NS1' and NS1 were analyzed and the ratios were calculated using ImageJ. **E, F** The NS1' and NS1 grayscale values are quantized to calculate the ratios from Fig. 2B and 3C. The data were expressed as the means  $\pm$  SDs from three repeat experiments using *t*-test. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .