Supplementary Data

Construction of Recombinant PRV Expressing PRRSV Antigens and Evaluation of Their Protective Efficacy in Pigs Against Homologous Challenge

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Figure S1

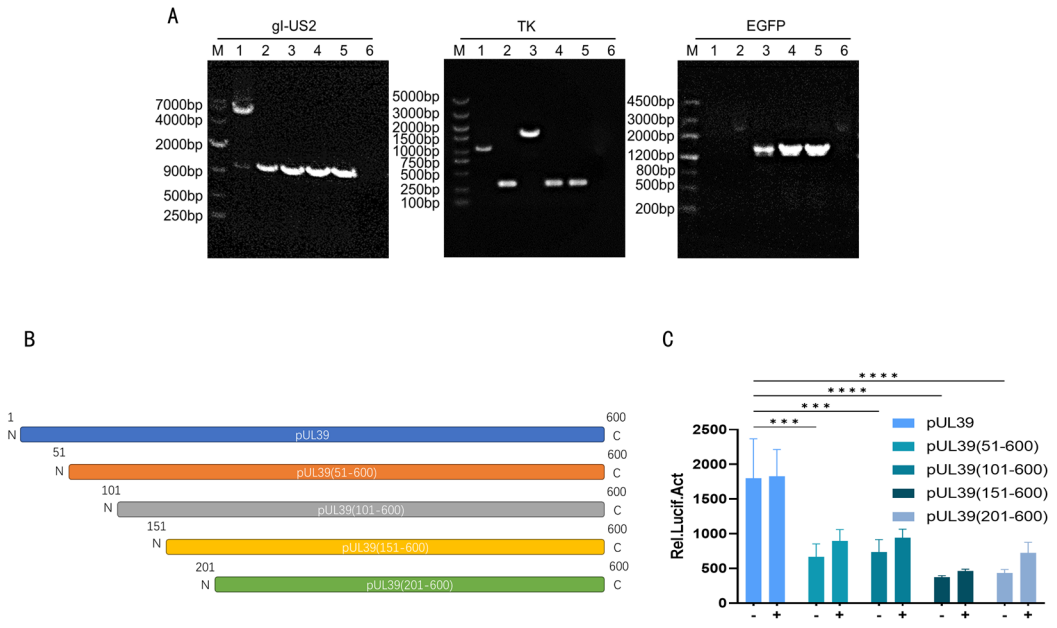


Figure S1. Identification of fluorescent virus and promoter truncation.

(A) PCR detection of recombinant fluorescent virus gI-US2 deletion, TK deletion, and EGFP fragment. Line 1: PRV-JM genome; Line 2: PRV-JM-DF genome; Line 3: PRV-JM-DF-EGFP-TK genome; Line 4: PRV-JM-DF-EGFP-gG-gD genome; Line 5: PRV-JM-DF-EGFP-gG-gD genome; Line 6: blank control. (B) Schematic diagram of pUL39 promoter truncation. (C) Activity detection of pUL39 promoter truncation (*** $p < 0.001$; **** $p < 0.0001$).

Figure S2

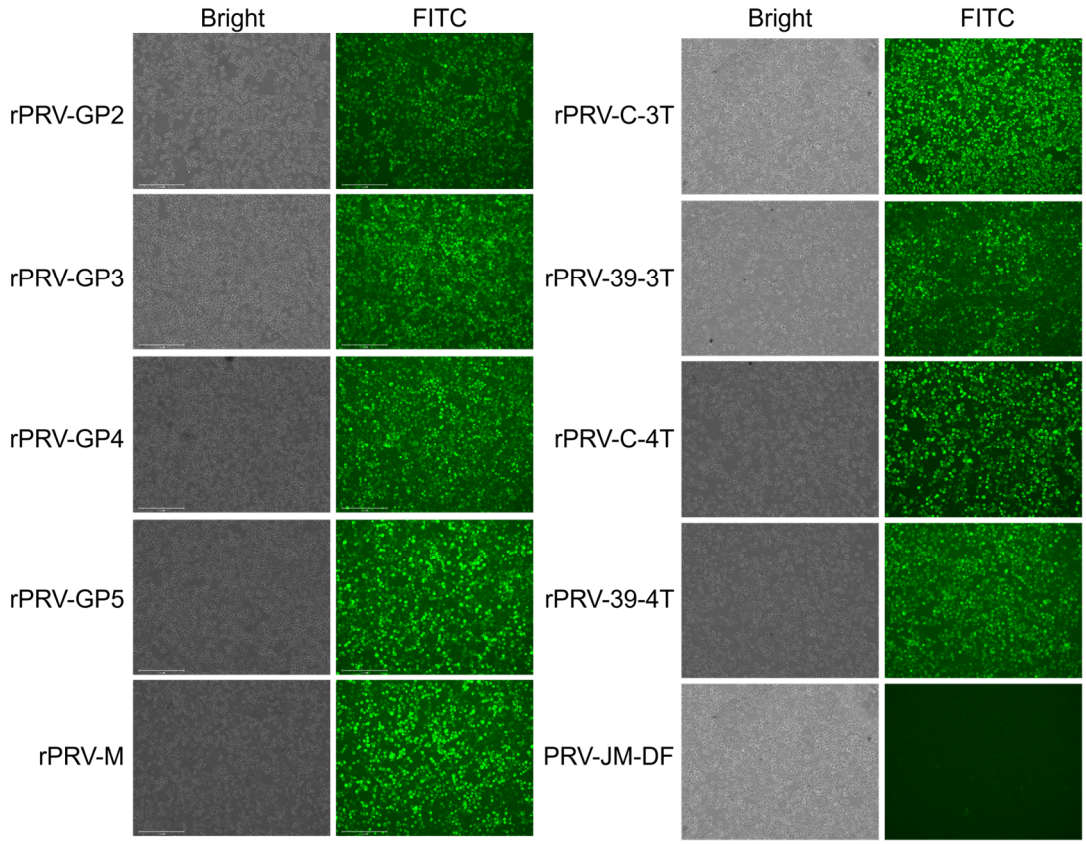


Figure S2. Identification of the expression of exogenous proteins of recombinant virus by IFA.

PK-15 cells in 12-well plate were infected with 500 $TCID_{50}$ of recombinant viruses for 48 h, and IFA was used to detect whether the exogenous proteins were expressed (the exogenous proteins are tagged with Flag, tested using Flag antibodies).

Figure S3

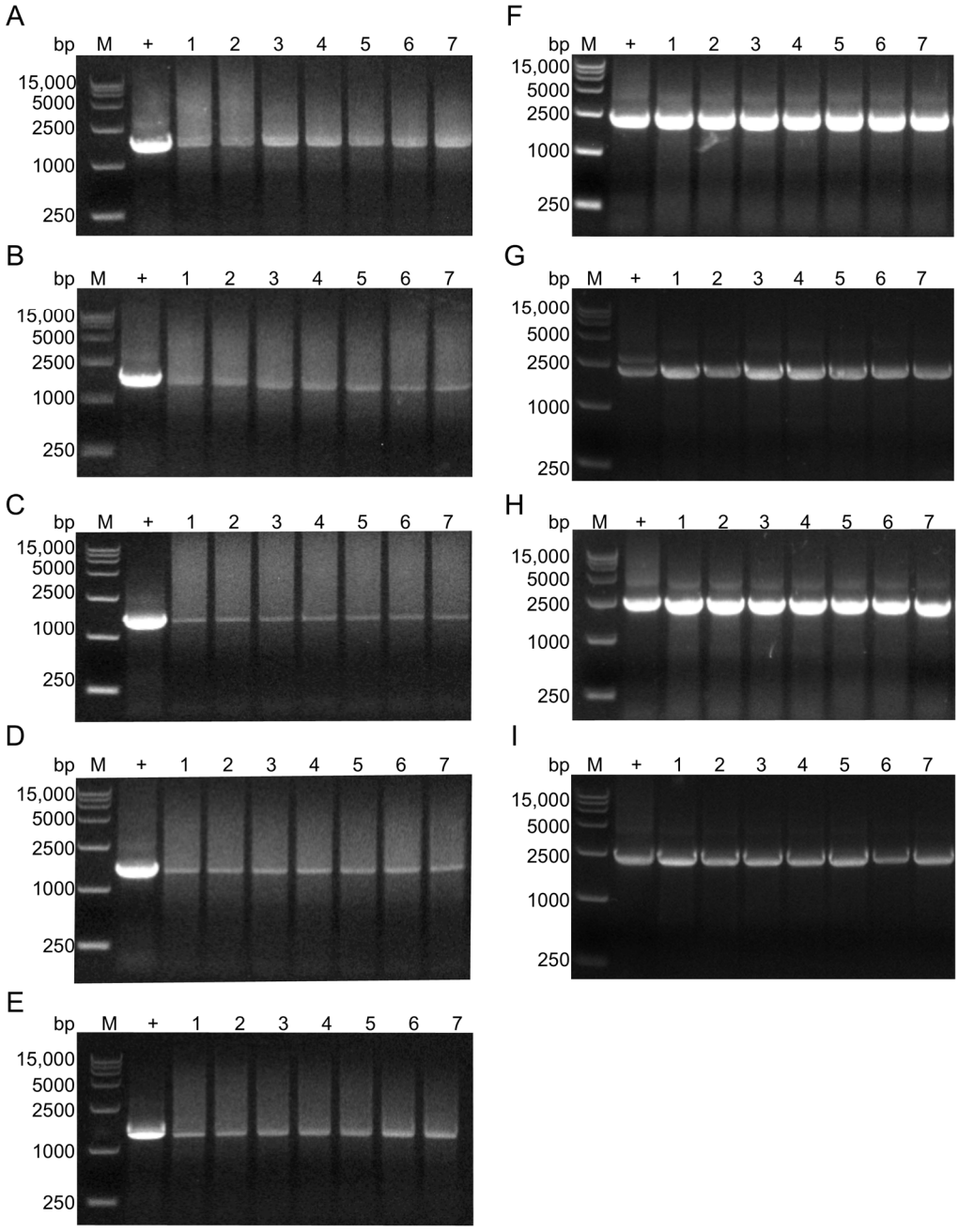


Figure S3. Identification of the genetic stability of recombinant viruses by PCR.

（A）rPRV-GP2；（B）rPRV-GP3；（C）rPRV-GP4；（D）rPRV-GP5；（E）rPRV-M；（F）rPRV-C-3T；（G）rPRV-39-3T；（H）rPRV-C-4T；（I）rPRV-39-4T；+: Positive control; Line 1: first generation viral genome; Line 2: fifth generation viral genome; Line 3: tenth generation viral genome; Line 4: fifteenth generation genome; Line 5: twentieth generation genome; Line 6: twenty-fifth generation genome; Line 7: thirtieth generation genome.

Figure S4

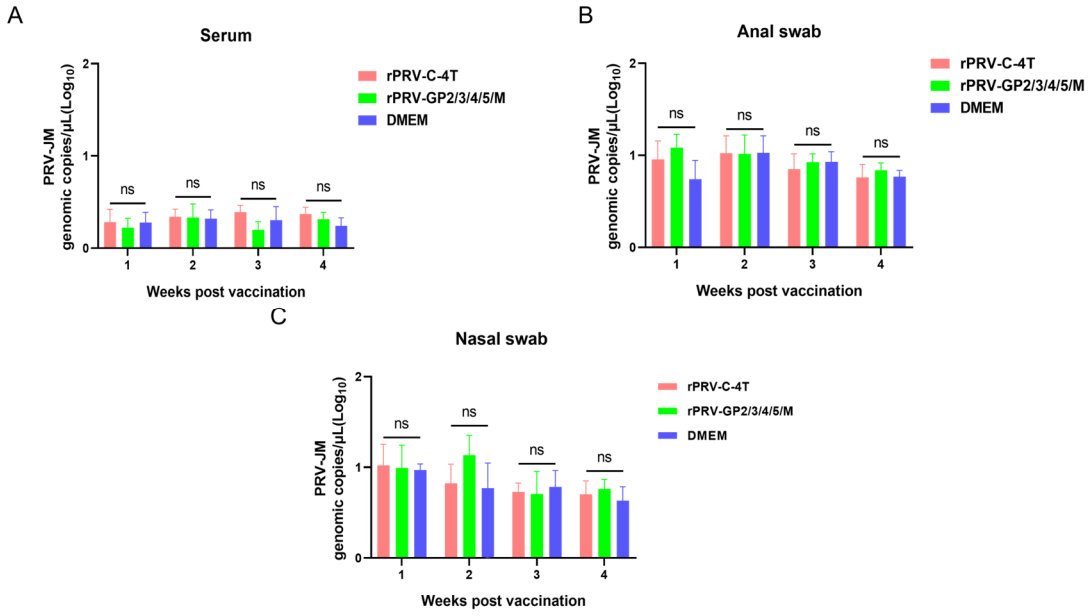


Figure S4. Virus load of PRV in piglet immune period.

The viral load of PRV in (A)serum, (B)anal swabs, and (C)nasal swabs four weeks after vaccination.

Figure S5

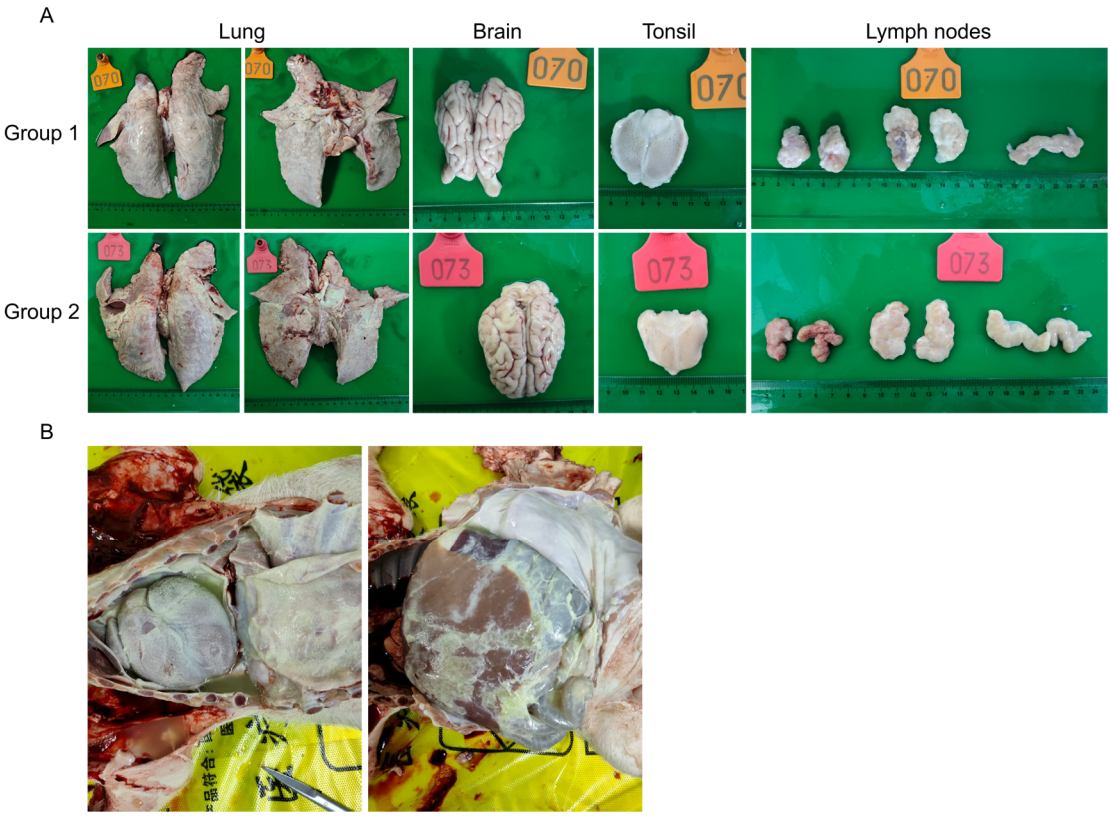


Figure S5. Tissue damage in piglets post-challenge.

(A) Group 1: The apparent pathological changes in surviving pigs of rPRV-GP2/3/4/5/M group; Group 2: The apparent pathological changes in pigs that died from secondary infection with Haemophilus parasuis in the PRRSV vaccine group; (B) The thoracic and abdominal cavities of pigs that died from secondary infection with Haemophilus parasuis in the PRRSV vaccine group.