

Figure S1. The comparison of replication efficiency between parental and chimeric viruses in PAMs. (A, B, C, D) The growth kinetics between parental HuN4, CH-1a and their chimeric viruses in PAMs. The parental and mutant viruses are infected with PAMs at a multiplicity of infection (MOI) of 0.01. The cell supernatants are harvested at 0, 24, 48, 72 and 96 hours post-infection (hpi). Virus titers from 24 h to 96 h are determined by microtitration infectivity assays, respectively. The data are presented as the mean standard deviation (shown by error bars) from three independent experiments. LOD: limit of detection. Asterisk (*) indicates a significant difference between HuN4, CH-1a and HC₃, CH₃, respectively. (*, $p < 0.05$). And (&) indicates a significant difference between HuN4, CH-1a and HC₁₁, CH₁₁, respectively. (&&, $p < 0.01$). Epsilon (ε) indicates a significant difference between HuN4, CH-1a and HC_{GP2}, CH_{GP2}, respectively. (ε, $p < 0.05$; εεε, $p < 0.001$). Phi (φ) indicates a significant difference between HuN4, CH-1a and HC_{GP4}, CH_{GP4}, respectively. (φφφ, $p < 0.001$).

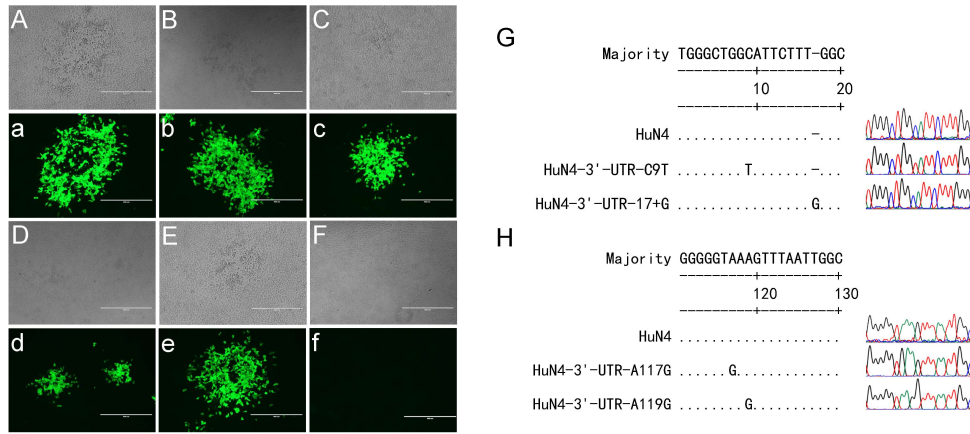


Figure S2. Identification of mutant viruses. (A, B, C, D, E, F) The cytopathic effect of HuN4, HuN4-3'-UTR-C9T, HuN4-3'-UTR-19+G, HuN4-3'-UTR-A117G, HuN4-3'-UTR-A119G and negative control under microscope. The parental and mutant viruses are infected with Marc-145 cells at 80 % confluence and observed under light microscope after 4 or 5 days. (a, b, c, d, e, f) Identification of HuN4, HuN4-3'-UTR-C9T, HuN4-3'-UTR-19+G, HuN4-3'-UTR-A117G, HuN4-3'-UTR-A119G and negative control by IFA. The parental and mutant viruses are infected with Marc-145 cells at 80 % confluence and observed under inverted fluorescence microscope. (G, H) The viruses of HuN4, HuN4-3'-UTR-C9T, HuN4-3'-UTR-19+G, HuN4-3'-UTR-A117G, HuN4-3'-UTR-A119G are sequenced and analyzed using MegAlign and Chromas.



Lineage 1 NADC30-like PRRSV

Lineage 3 QYYZ-like PRRSV

Lineage 4 EDRD-1-like PRRSV

Lineage 5 VR-2332-like PRRSV

Lineage 6 P129-like PRRSV

Lineage 7 SP-like PRRSV

Lineage 8 HP-PRRSV and CH-1a-like PRRSV

Lineage 9 NC16845-like PRRSV

Figure S3. The 3'-UTR alignment of 765 PRRSV-2 strains. The purple, cyan, buff, orange, light green, jacinth, blue and black represent the L1-NADC30-like PRRSV, L3-QYYZ-like PRRSV, L4-EDRD-1-like PRRSV, L5-VR-2332-like PRRSV, L6-P129-like PRRSV, L7-SP-like PRRSV, L8-HP-PRRSV and CH-1a-like PRRSV, and L9-NC16845-like PRRSV strains, respectively. The four conserved nucleotides are highlighted in yellow.