Supplementary Material

NS Segment Reassortment Can Increase Growth Kinetics and Pathogenicity of the 2009 Pandemic H1N1 Influenza A Virus *In Vitro* and *In Vivo*

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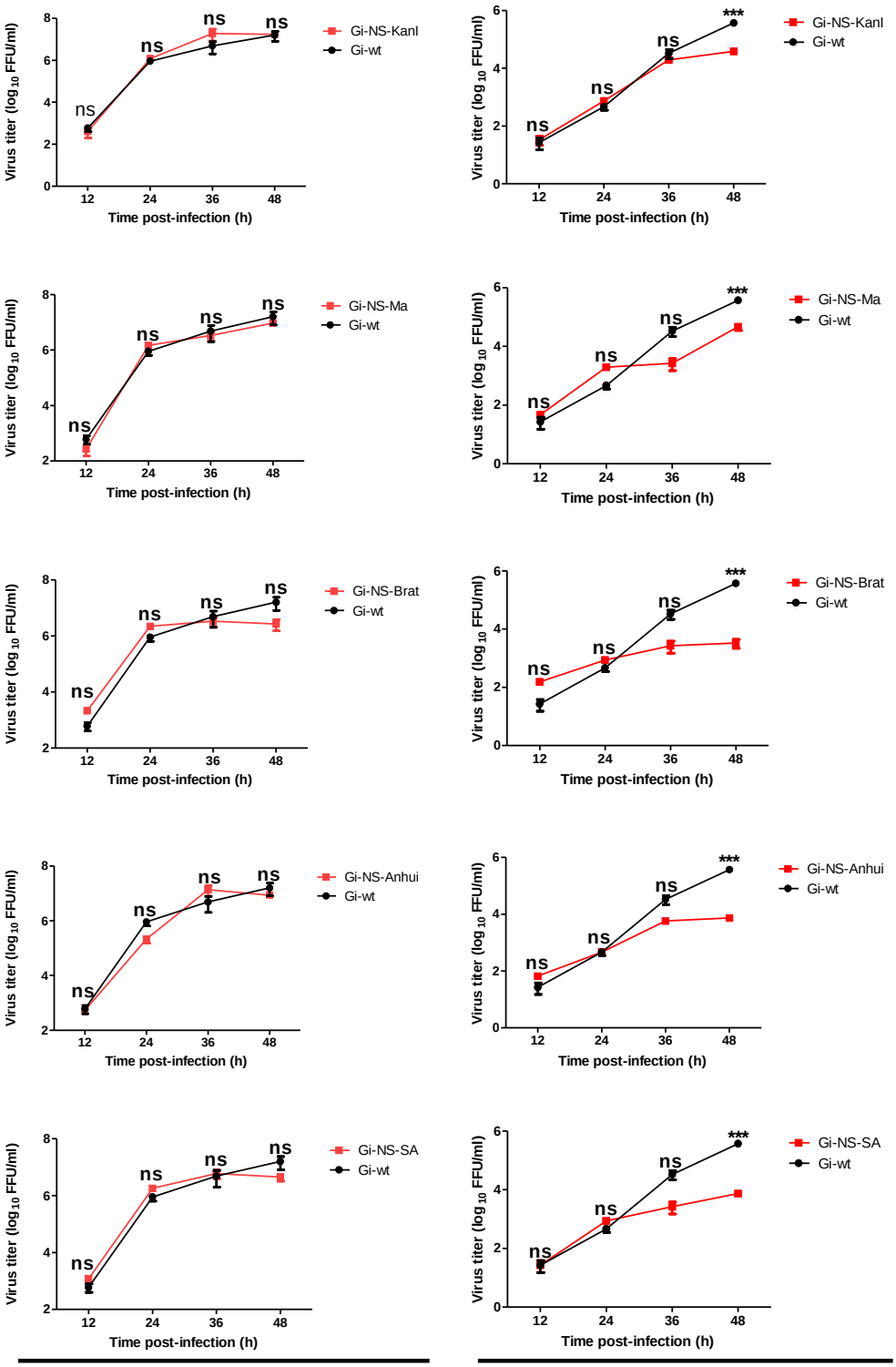
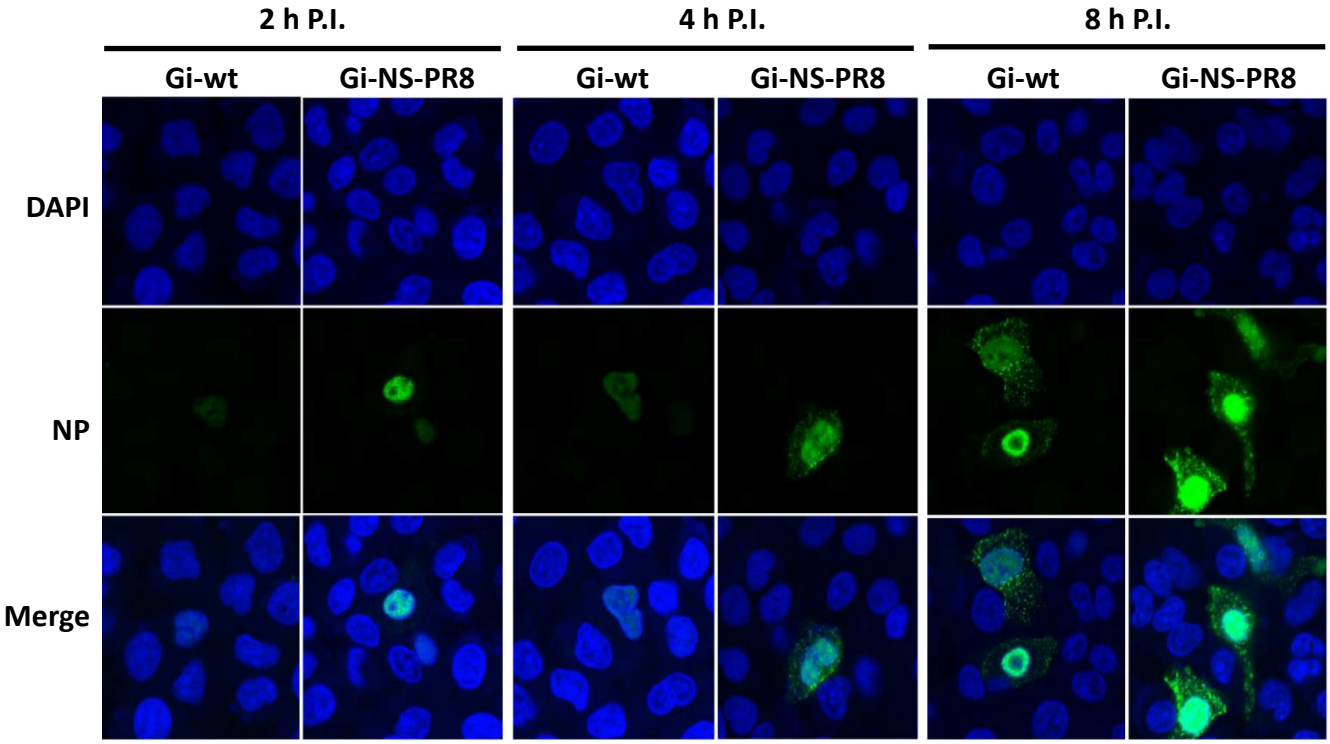
1 Supplementary Figures

1.1 Supplementary Figure 1



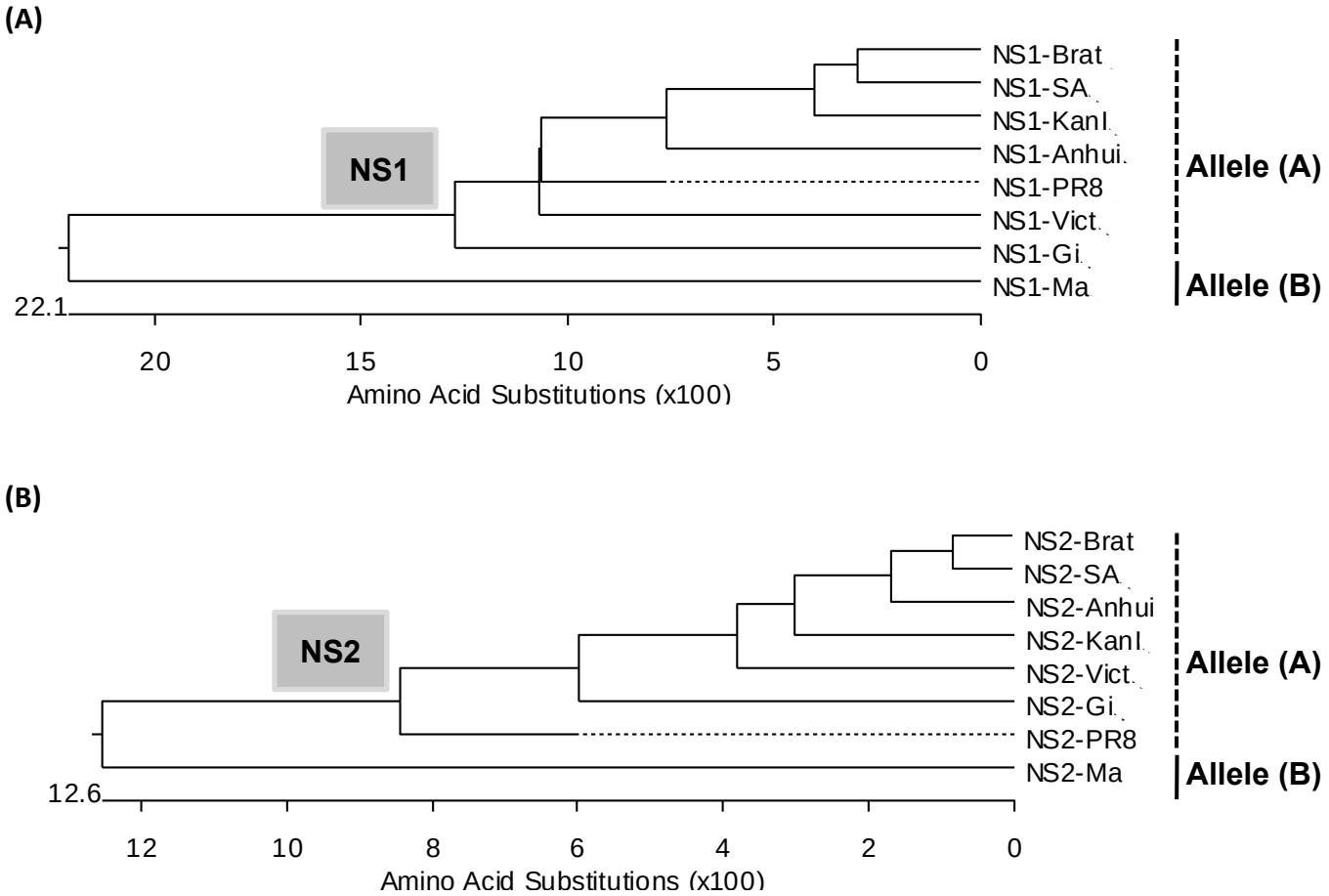
Supplementary Figure 1. Growth kinetics of different Gi-NS-reassortants and Gi-wt in human A549 and swine NPTr cells. Cells were infected with different Gi-NS-reassortants and recombinant Gi-wt at an MOI of 0.001 FFU/cell. Supernatants were collected at 12, 24, 36, and 48 h p.i.. Collected samples were then titrated using foci assay (FFU/ml) in MDCK cells. The titers were calculated for three independent experiments; error bars indicate the standard deviation (SD). Statistical analysis was performed using "repeated measures ANOVA", followed by "Bonferroni post hoc" test. (*) $p < 0.05$; (**) $p < 0.001$; (***) $p < 0.0001$; ns, non-significant. Error bars represent standard deviation (SD).

1.2 Supplementary Figure 2



Supplementary Figure 2. vRNP export of recombinant Gi-wt and reassortant Gi-NS-PR8. A549 cells were infected with recombinant viruses (MOI=1). After 2, 4, 8 h p.i., A549 cells were processed for immunofluorescence. The viral NP protein (green) indicates the RNP localisation; nuclei were stained with DAPI (blue).

1.3 Supplementary Figure 3



(C)

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                  190        200        210        220        230
NS1-Brat.pro      NTVRVSETLQRFAWRSSNEDRRPPLPPKQKRKMARTIESEV                  230 aa
NS1-Gi.pro        NGNTVRVSENIQRFAWRNCDENGRPSLPPEQK                           219 aa
NS1-KanI.pro      NDNTVRVTETIQRFAWRSSDEDGRLPLPPNQKRKMARTIESEV                225 aa
NS1-Ma.pro        NDNSIRASENIQRFAWGIRDENGGPPLPPKQKRYMARRVESEV                230 aa
NS1-PR8.pro       NDNTVRVSETLQRFAWRSSNENGRPPLTPKQKREMAGTIRSEV                230 aa
NS1-SA.pro        NDNTVRVSETLQRFAWRGSNEDGRPPLPPKQKRKMARTIESEV                230 aa
NS1-Vict.pro      NDNTVRVSKTLQRFAWGSSNENGGPPLTPKQKRKMARTARSKVRRDKMAD        237 aa
NS1-Anhui.pro     NDNTVRVSETLQRFAWRSSDEDGRSPLSTK                             217 aa
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Supplementary Figure 3. Phylogenetic analysis of the different NS1 and NS2 proteins encoded by the different NS segments. (A) The NS1 aa sequences were aligned by ClustalW program in the DNA Star software to generate the phylogenetic tree (Lasergene, WI). (B) Phylogenetic tree of the studied NS2 proteins. (C) Truncations and prolongation of disordered tails of different NS1 used.