Supplementary material

N-glycosylation of rotavirus NSP4 protein affects viral replication and pathogenesis

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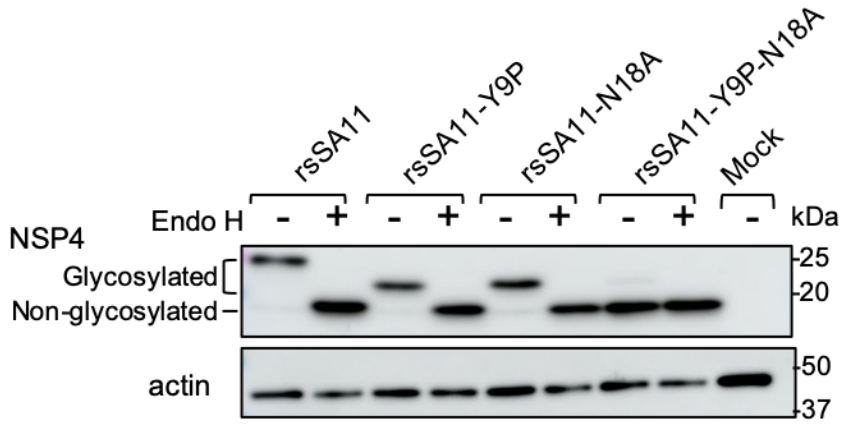
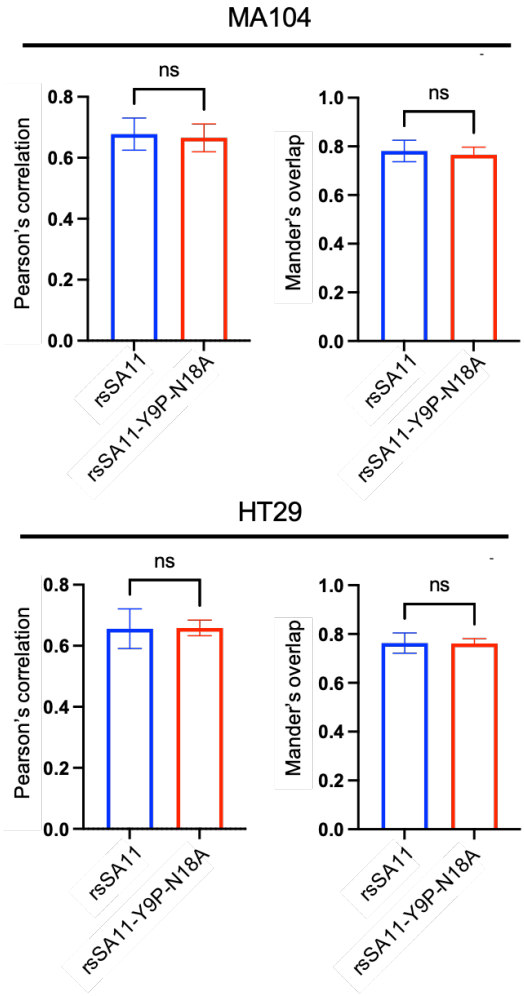
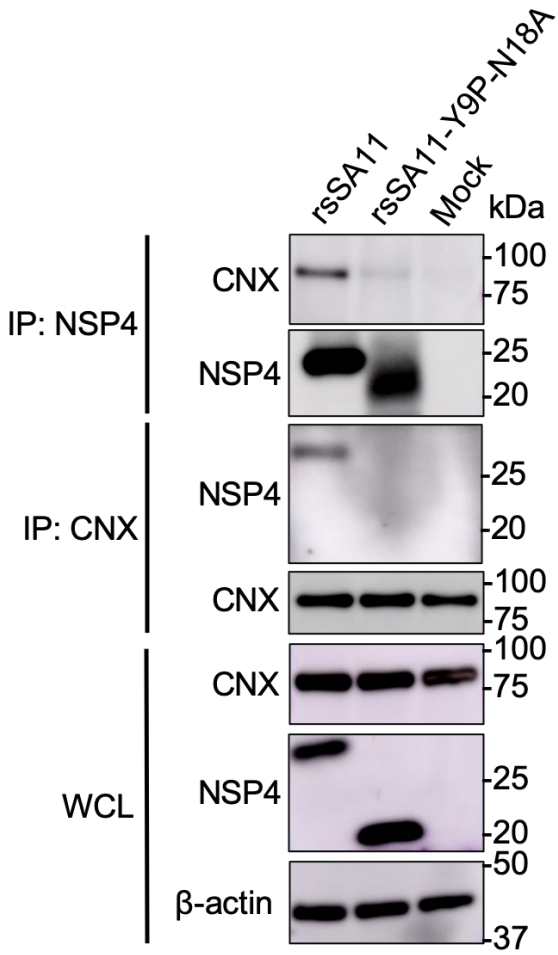


Supplementary Figure S1. N-glycosylation patterns of recombinant NSP4 in MA104 cells.

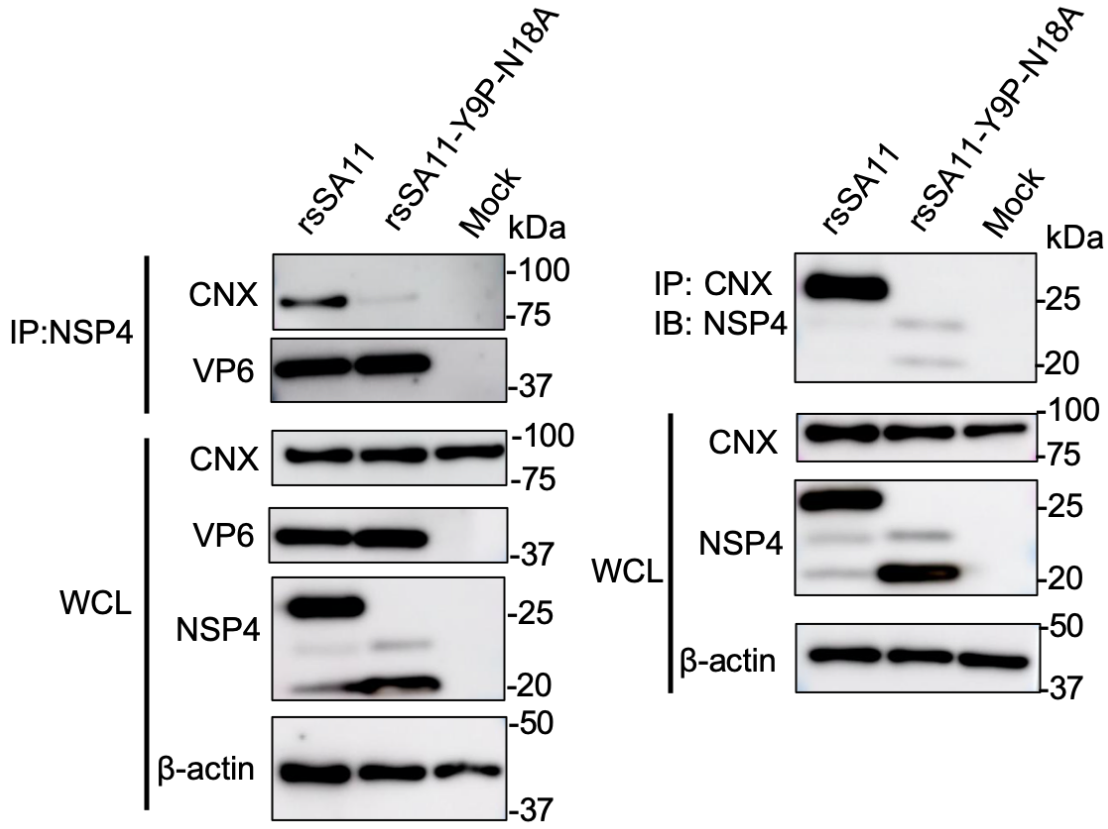
MA104 cells were infected with rsSA11, rsSA11-Y9P, rsSA11-N18A, or rsSA11-Y9P-N18A at an MOI of 10 in the absence of trypsin. Cell lysates were collected at 10 h post-infection, followed by treatment with Endo-H or distilled water.



Supplementary Figure S2. Co-localization of NSP4 and an ER marker. Pearson's correlation coefficient and Mander's overlap were calculated. Data are expressed as the mean ± SD from 7–10 cells. Statistical significance was determined using a t-test, with $p < 0.05$ considered significant (*$p < 0.05$).



Supplementary Figure S3. Validation of co-immunoprecipitation results using HT29. HT29 cells were infected with rsSA11 or rsSA11-Y9P-N18A at an MOI of 5. Cell lysates were collected at 18 h post-infection and subjected to co-immunoprecipitation using anti-calnexin and anti-NSP4 antibodies. Molecular weight (kDa) is shown on the right. (IP = immunoprecipitation; WCL = whole cell lysate; CNX = calnexin).



Supplementary Figure S4. Co-immunoprecipitation assay using MA104. MA104 cells were infected with rsSA11 or rsSA11-Y9P-N18A at an MOI of 5. Cell lysates were collected at 10 h post-infection and subjected to co-immunoprecipitation using anti-calnexin and anti-NSP4 antibodies. Molecular weight (kDa) is shown on the right. (IP = immunoprecipitation; IB = immunoblotting; WCL = whole cell lysate; CNX = calnexin).