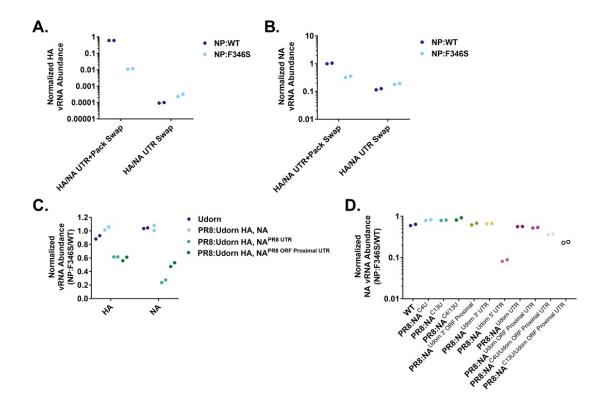


S1 Fig. Quantifying the abundance of newly synthesized, 4SU-labeled vRNAs using vRNA and segment-specific primers during the cDNA synthesis and qPCR steps. Normalized abundance of 4SU-labeled NA vRNA in MDCK cells infected with PR8 NP:WT/F346S at an MOI of 5 TCID₅₀/cell for 7hrs and pulsed with 500µM of 4SU for 1hr as determined by RT-qPCR using a tagged, vRNA and segment-specific primer during the cDNA synthesis step, and a primer pair consisting of a tag-specific primer and segment-specific primer for the qPCR step. Each data point represents a single cell culture well replicate from a single experiment.



S2 Fig. Quantification of gene segment ratios in viral RNA stocks. 140µL of viral RNA supernatant was treated with 0.25µg RNaseA, viral RNA was extracted and DNase-treated, and then viral gene segment abundance was quantified using RT-qPCR. A/B.) Normalized HA ORF (A) or NA ORF (B) containing viral RNA abundance in the viral stocks of the PR8 HA/NA UTR+Pack Swap NP:WT/F346S and PR8 HA/NA UTR Swap NP:WT/F346S viruses. C.) Normalized viral RNA abundance in the viral stocks of the Udorn NP:F346S, PR8:Udorn HA, NA NP:F346S, PR8:Udorn HA, NAPR8 UTR NP:F346S, and PR8:Udorn HA, NAPR8 ORF Proximal UTR NP:F346S viruses relative to Udorn NP:WT, PR8:Udorn HA, NA NP:WT, PR8:Udorn HA, NA NP:WT, and PR8:Udorn HA, NAPR8 ORF Proximal UTR NP:WT, and PR8:Udorn HA, NAPR8 ORF Proximal UTR NP:WT viruses respectively. D.) Normalized viral RNA abundance in the viral stocks of the indicated viruses with NP:F346S as determined by RT-qPCR normalized to the NP:WT versions of each virus. Each data point represents a qPCR technical replicate.