

Synergy of two low-affinity NLSs determines the high avidity of influenza A virus nucleoprotein NP for human importin α isoforms

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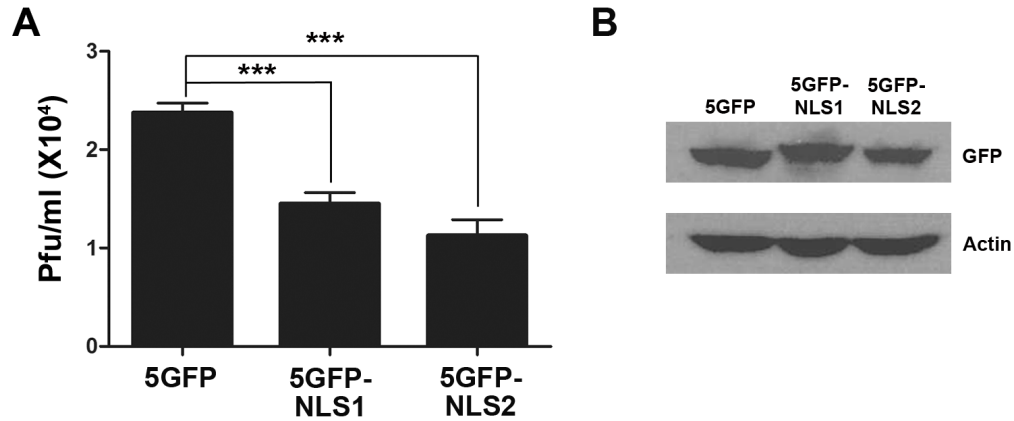


Figure S1: Infection with influenza A virus strain PR8 is reduced in cells expressing NLS1 or NLS2. (A) HeLa cells expressing 5GFP, 5GFP-NLS1, or 5GFP-NLS2 were infected with influenza A virus (strain A/PR8/1934/H1N1). At 24 h post-infection, the supernatant was collected and the viral titer was determined by infecting MDCK cells and counting plaques 3 days post-infection. Bar graphs show the mean $\pm$ standard error of the mean from three independent experiments (** $p < 0.001$, one-way ANOVA followed by Tukey's tests). (B) Western blots of whole cell lysates from cells transfected with 5GFP, 5GFP-NLS1, or 5GFP-NLS2 for 24 h and infected with influenza A virus (strain A/PR8/1934/H1N1) for 24 h. Antibodies used were anti-GFP (Invitrogen, A11121, 1:10000 dilution) and anti-beta actin (Sigma, A4700, 1:5000 dilution).

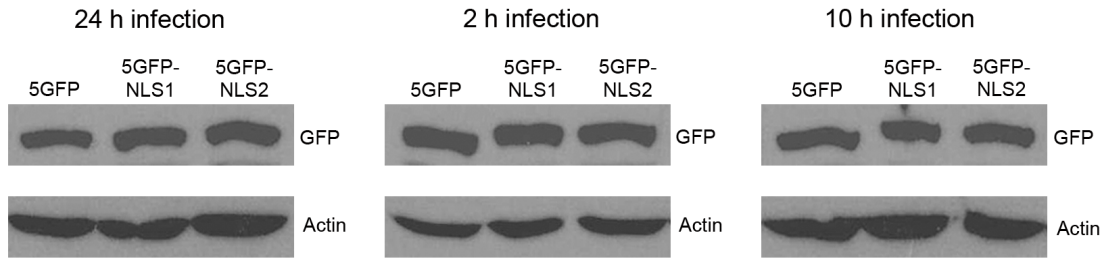


Figure S2: Levels of expression of 5GFP, 5GFP-NLS1, and 5GFP-NLS2 for experiments showed in Figs. 1B and 1C. Western blots of whole cell lysates from cells transfected with 5GFP, 5GFP-NLS1, or 5GFP-NLS2 for 24 h and infected with influenza A virus (strain X-31, A/Aichi/68 (H3N2)) for 24 h, 2 h, or 10 h. Antibodies used were anti-GFP (Invitrogen, A11121, 1:10000 dilution) and anti-beta actin (Sigma, A4700, 1:5000 dilution).

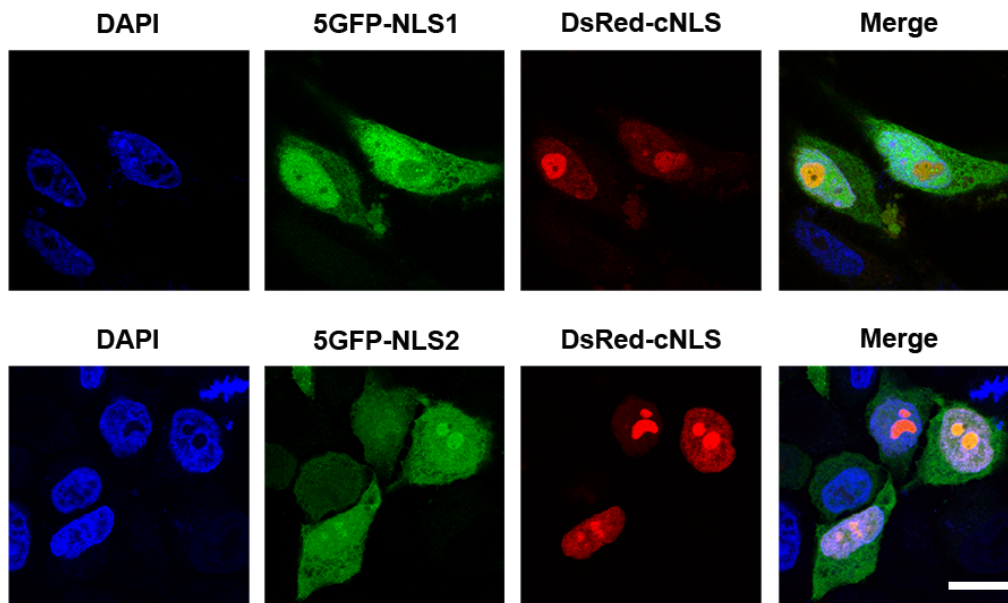


Figure S3: 5GFP-NLS1 and 5GFP-NLS2 do not affect the nuclear import of DsRed-cNLS. HeLa cells were co-transfected with plasmids expressing DsRed-cNLS (DsRed fused to three copies of the classical NLS of SV40 T antigen; Clontech Laboratories) and 5GFP-NLS1 or 5GFP-NLS2. The cells were visualized by confocal microscopy 24 h post transfection. DNA was detected by staining with DAPI. Scale bar, 10 μ m. DAPI, blue; 5GFP-NLSs, green; DsRed-cNLS, red.

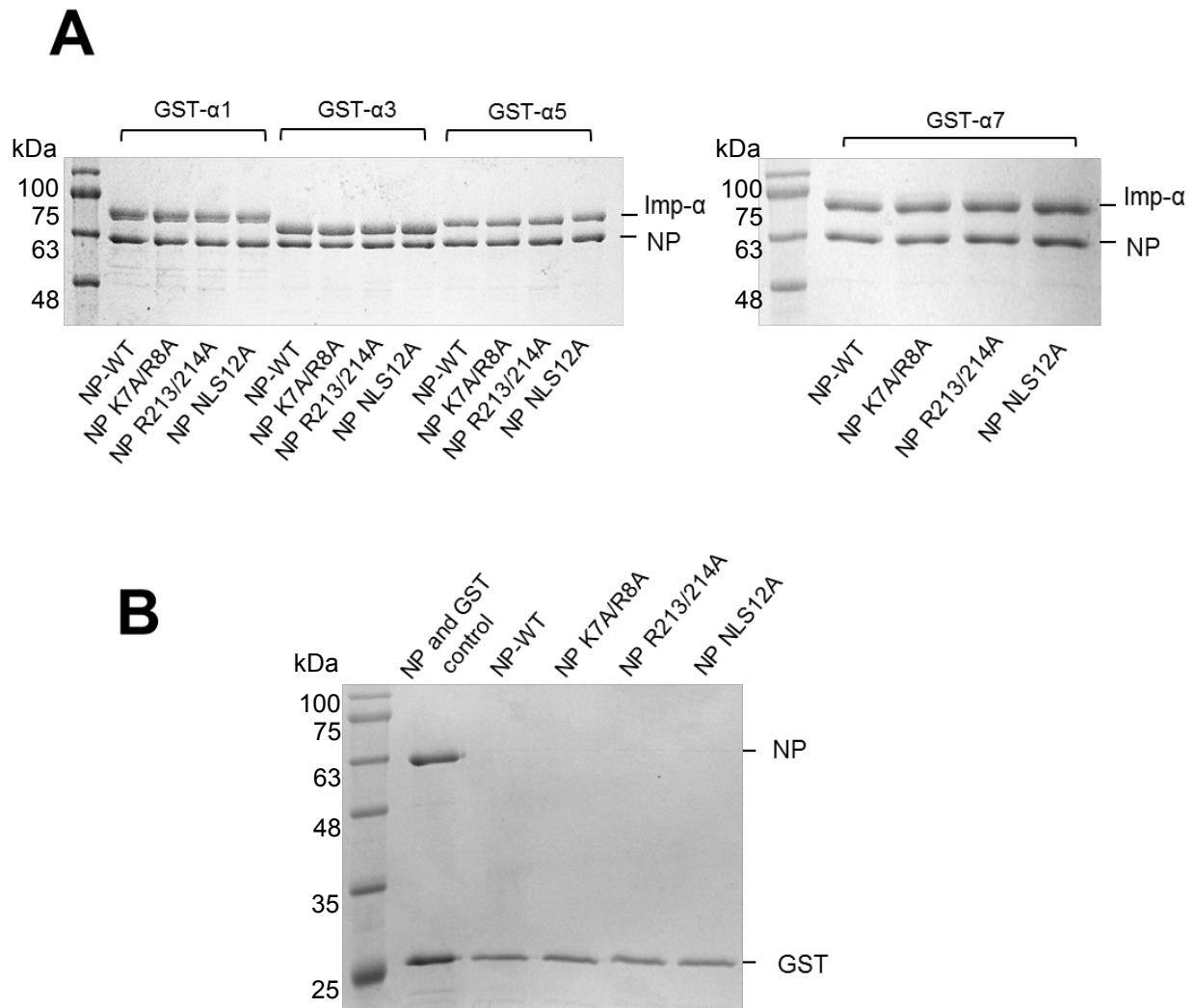


Figure S4: Control pull-downs. (A) 1 μ M GST-tagged importin α isoforms 1, 3, 5, and 7 were used to pull down 0.75 μ M His-tag NP and mutants. (B) 1 μ M free GST was used to pull down 0.75 μ M His-tagged NP and mutants.