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Supplemental information

The evolutionary potential of influenza A virus hemagglutinin is highly constrained by epistatic interactions with neuraminidase

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Supplementary Material:

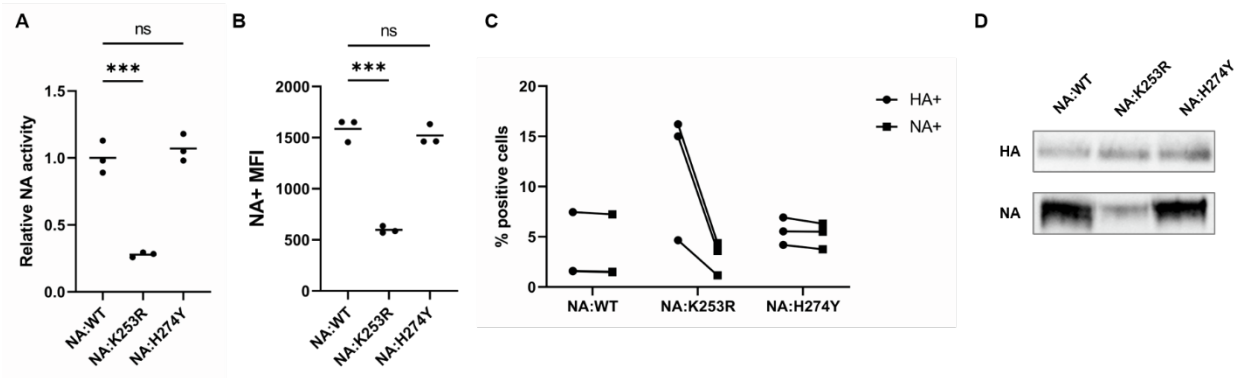

Figure S1: NA:K253R reduces NA surface expression and virion-associated NA activity, related to Figures 1 and 2 and STAR Methods. (A) *The normalized V_{max} of NA activities from three independently generated virus stocks for each NA variant measured by MUNANA assay. The results were normalized by NP genome equivalents as determined by RT-qPCR. *** indicates $p < 0.01$ and ns indicates $p > 0.05$, based on t tests.* ***(B)*** *NA surface expression levels represented by mean fluorescence intensities (MFI) of NA positive cells at 16 hpi as measured by flow cytometry on MDCK cells infected at MOI = 0.05 TCID50/cell. *** indicates $p < 0.01$ and ns indicates $p > 0.05$, based on t tests.* ***(C)*** *Percentages of positive cells by surface staining of HA and NA, as determined by flow cytometry.* ***(D)*** *Western blotting for HA and NA protein in purified virions. The input amounts of purified virions in the western blot were normalized based on the mean gray value of HA signal in a previous Western blot of the same samples.*

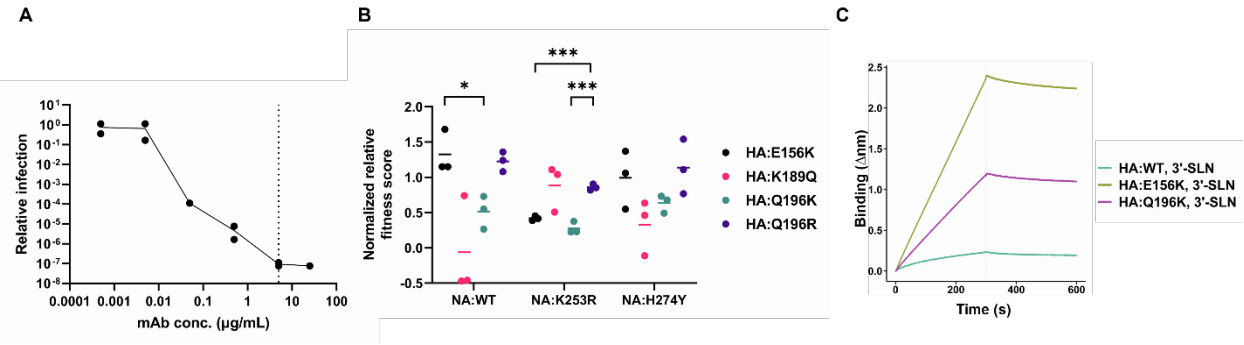

Figure S2: Escape variants in H36-26 selection, related to Figure 2. (A) *Saturated neutralization concentration of H36-26. 10^7 TCID50 of NA:WT virus was neutralized by the given concentration of the antibody and infected a well of 6-well plate. The supernatant was collected 16 hours post infection. The output titer was measured by TCID50 assay and normalized by the titer of no antibody controls. The dash line indicates the concentration used in the selection experiment.* ***(B)*** *The normalized relative fitness scores (measured in the DMS experiment) of the escape variants found in H36-26 antibody selection. *** indicates $p < 0.01$ and * indicates $p < 0.05$, based on Welch t tests (all pairwise comparisons without an asterisk were $P>0.05$).* ***(C)*** *Binding kinetics to the receptor was measured by biolayer interferometry. The input was normalized by the protein concentration of the purified virion. Streptavidin sensors were coated with 3'-SLN-PEG3-biotin (3'-Sialyllactosamine-PEG3-Biotin (Single Arm). Separated by the dashed line, the first 300 seconds was the association period of the virion to the receptor, the next 300 seconds showed the dissociation period. 10 μM zanamivir was present during the assay to inhibit NA activity.*

Table S1: Correlations between the replicates in deep mutational scanning, related to Figure 1

	NA:WT-1	NA:WT-2	NA:K253R-1	NA:K253R-2	NA:H274Y-1	NA:H274Y-2
NA:WT-2	0.702					
NA:WT-3	0.682	0.665				
NA:K253R-2			0.756			
NA:K253R-3			0.756	0.815		
NA:H274Y-2					0.679	
NA:H274Y-3					0.725	0.719

Table S3. Primers and adaptors for barcode sequencing of HA1, related to Figure 1.

Name	Sequence
HA1_1-F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNGCACTTGCAGCTGCAGATGCA
HA1_2-F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNAGGGAGCAATTGAGCTCAGTG
HA1_3-F	CACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNNNACCCCGGAAATAGCAGAAAGA
HA1_1-R	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNNTTCGAATCTTTCGAATGATGA
HA1_2-R	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNNAGCTTGATCTCTTACTTTGGG
HA1_3-R	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNNAATGGCTCCAAATAGACCTCT
TruSeq_UDI0001-F	AATGATACGGCGACCACCGAGATCTACACAGCGCTAGACACTCTTTCCCTACACGACGCT
TruSeq_UDI0002-F	AATGATACGGCGACCACCGAGATCTACACGATATCGAACACTCTTTCCCTACACGACGCT
TruSeq_UDI0003-F	AATGATACGGCGACCACCGAGATCTACACCGCAGACGACACTCTTTCCCTACACGACGCT
TruSeq_UDI0004-F	AATGATACGGCGACCACCGAGATCTACACTATGAGTAACACTCTTTCCCTACACGACGCT
TruSeq_UDI0005-F	AATGATACGGCGACCACCGAGATCTACACAGGTGCGTACACTCTTTCCCTACACGACGCT
TruSeq_UDI0006-F	AATGATACGGCGACCACCGAGATCTACACGAACATACACACTCTTTCCCTACACGACGCT
TruSeq_UDI0007-F	AATGATACGGCGACCACCGAGATCTACACACATAGCGACACTCTTTCCCTACACGACGCT
TruSeq_UDI0008-F	AATGATACGGCGACCACCGAGATCTACACGTGCGATAACACTCTTTCCCTACACGACGCT
TruSeq_UDI0009-F	AATGATACGGCGACCACCGAGATCTACACCCAACAGAACACTCTTTCCCTACACGACGCT
TruSeq_UDI0010-F	AATGATACGGCGACCACCGAGATCTACACTTGGTGAGACACTCTTTCCCTACACGACGCT
TruSeq_UDI0011-F	AATGATACGGCGACCACCGAGATCTACACCGCGGTTCACACTCTTTCCCTACACGACGCT
TruSeq_UDI0012-F	AATGATACGGCGACCACCGAGATCTACACTATAACCTACACTCTTTCCCTACACGACGCT
TruSeq_UDI0001-R	CAAGCAGAAGACGGCATACGAGATAACCGCGGGTGACTGGAGTTCAGACGTGTGCT
TruSeq_UDI0002-R	CAAGCAGAAGACGGCATACGAGATGGTTATAAGTGACTGGAGTTCAGACGTGTGCT
TruSeq_UDI0003-R	CAAGCAGAAGACGGCATACGAGATCCAAGTCCGTGACTGGAGTTCAGACGTGTGCT
TruSeq_UDI0004-R	CAAGCAGAAGACGGCATACGAGATTTGGACTTGTGACTGGAGTTCAGACGTGTGCT
TruSeq_UDI0005-R	CAAGCAGAAGACGGCATACGAGATCAGTGGATGTGACTGGAGTTCAGACGTGTGCT
TruSeq_UDI0006-R	CAAGCAGAAGACGGCATACGAGATTGACAAGCGTGACTGGAGTTCAGACGTGTGCT
TruSeq_UDI0007-R	CAAGCAGAAGACGGCATACGAGATCTAGCTTGGTGACTGGAGTTCAGACGTGTGCT
TruSeq_UDI0008-R	CAAGCAGAAGACGGCATACGAGATTCGATCCAGTGACTGGAGTTCAGACGTGTGCT
TruSeq_UDI0009-R	CAAGCAGAAGACGGCATACGAGATCCTGAACTGTGACTGGAGTTCAGACGTGTGCT
TruSeq_UDI0010-R	CAAGCAGAAGACGGCATACGAGATTTCAGGTCGTGACTGGAGTTCAGACGTGTGCT
TruSeq_UDI0011-R	CAAGCAGAAGACGGCATACGAGATAGTAGAGAGTGACTGGAGTTCAGACGTGTGCT
TruSeq_UDI0012-R	CAAGCAGAAGACGGCATACGAGATGACGAGAGGTGACTGGAGTTCAGACGTGTGCT