Supplementary Figures

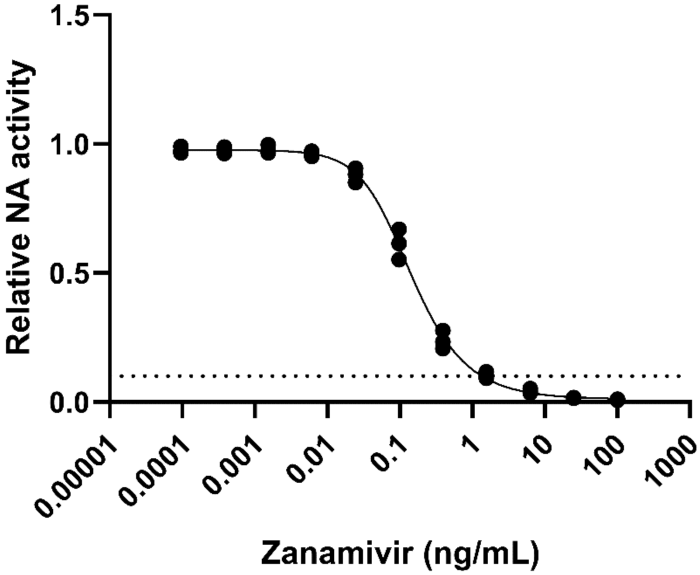
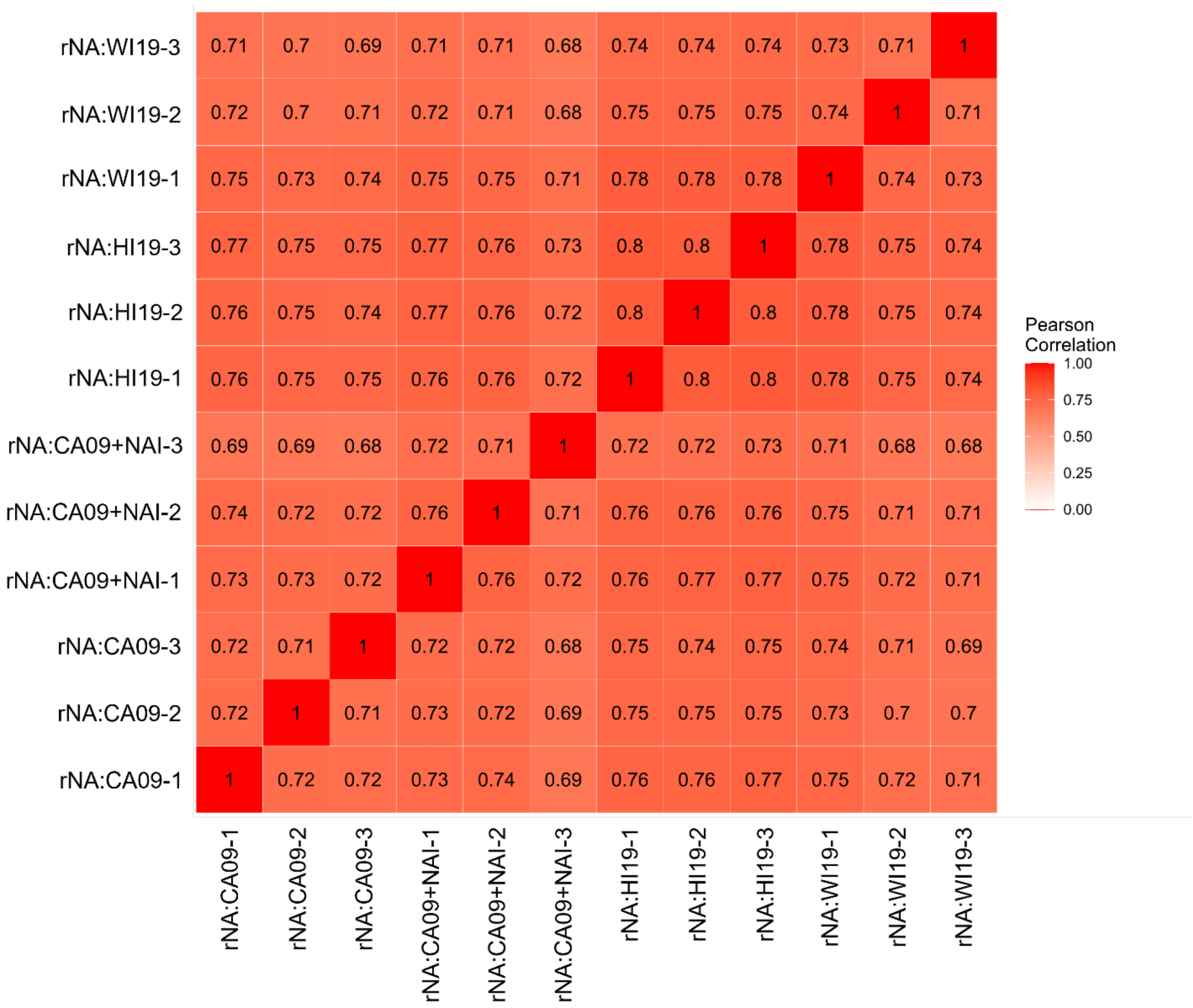


Figure S1: Inhibition curve of zanamivir against CA09 virus measured by MUNANA assay. *Nonlinear curve fits by asymmetric sigmoidal in GraphPad Prism, dot line indicates 90% inhibition of NA activity, n = 3.*



	rNA:CA09-1	rNA:CA09-2	rNA:CA09-3	rNA:CA09+NAI-1	rNA:CA09+NAI-2	rNA:CA09+NAI-3	rNA:HI19-1	rNA:HI19-2	rNA:HI19-3	rNA:WI19-1	rNA:WI19-2	rNA:WI19-3
rNA:WI19-3	0.71	0.7	0.69	0.71	0.71	0.68	0.74	0.74	0.74	0.73	0.71	1
rNA:WI19-2	0.72	0.7	0.71	0.72	0.71	0.68	0.75	0.75	0.75	0.74	1	0.71
rNA:WI19-1	0.75	0.73	0.74	0.75	0.75	0.71	0.78	0.78	0.78	1	0.74	0.73
rNA:HI19-3	0.77	0.75	0.75	0.77	0.76	0.73	0.8	0.8	1	0.78	0.75	0.74
rNA:HI19-2	0.76	0.75	0.74	0.77	0.76	0.72	0.8	1	0.8	0.78	0.75	0.74
rNA:HI19-1	0.76	0.75	0.75	0.76	0.76	0.72	1	0.8	0.8	0.78	0.75	0.74
rNA:CA09+NAI-3	0.69	0.69	0.68	0.72	0.71	1	0.72	0.72	0.73	0.71	0.68	0.68
rNA:CA09+NAI-2	0.74	0.72	0.72	0.76	1	0.71	0.76	0.76	0.76	0.75	0.71	0.71
rNA:CA09+NAI-1	0.73	0.73	0.72	1	0.76	0.72	0.76	0.77	0.77	0.75	0.72	0.71
rNA:CA09-3	0.72	0.71	1	0.72	0.72	0.68	0.75	0.74	0.75	0.74	0.71	0.69
rNA:CA09-2	0.72	1	0.71	0.73	0.72	0.69	0.75	0.75	0.75	0.73	0.7	0.7
rNA:CA09-1	1	0.72	0.72	0.73	0.74	0.69	0.76	0.76	0.77	0.75	0.72	0.71

Figure S2: Pearson correlations of normalized relative fitness score between samples in deep mutational scanning.

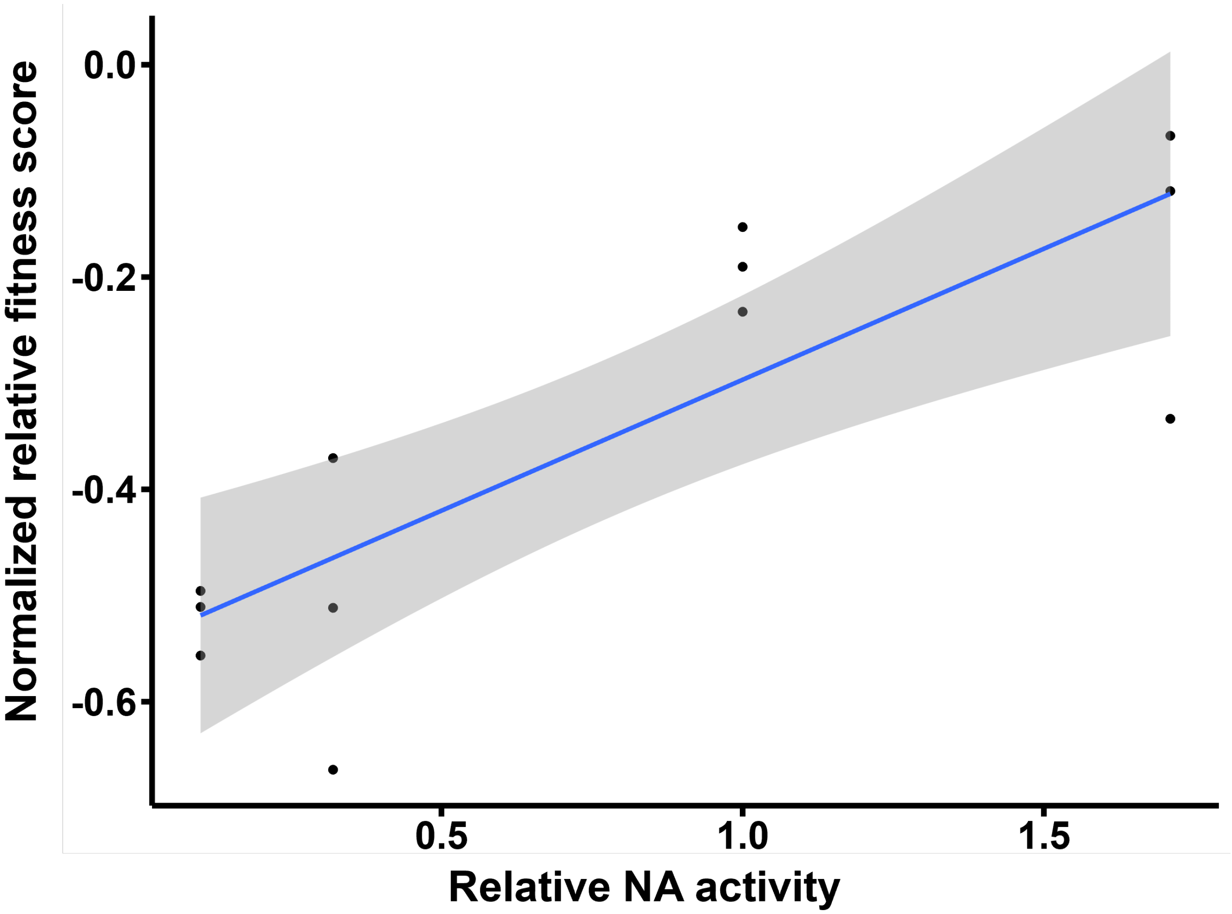


Figure S3: Example of linear regression of normalized relative fitness score against the relative NA activity for a given substitution. *Dots represent the normalized fitness scores measured in the given NA activity backgrounds in triplicates, blue line indicates the best-fit line by linear regression (lm() in R), gray area is the 95% interval of the regression.*

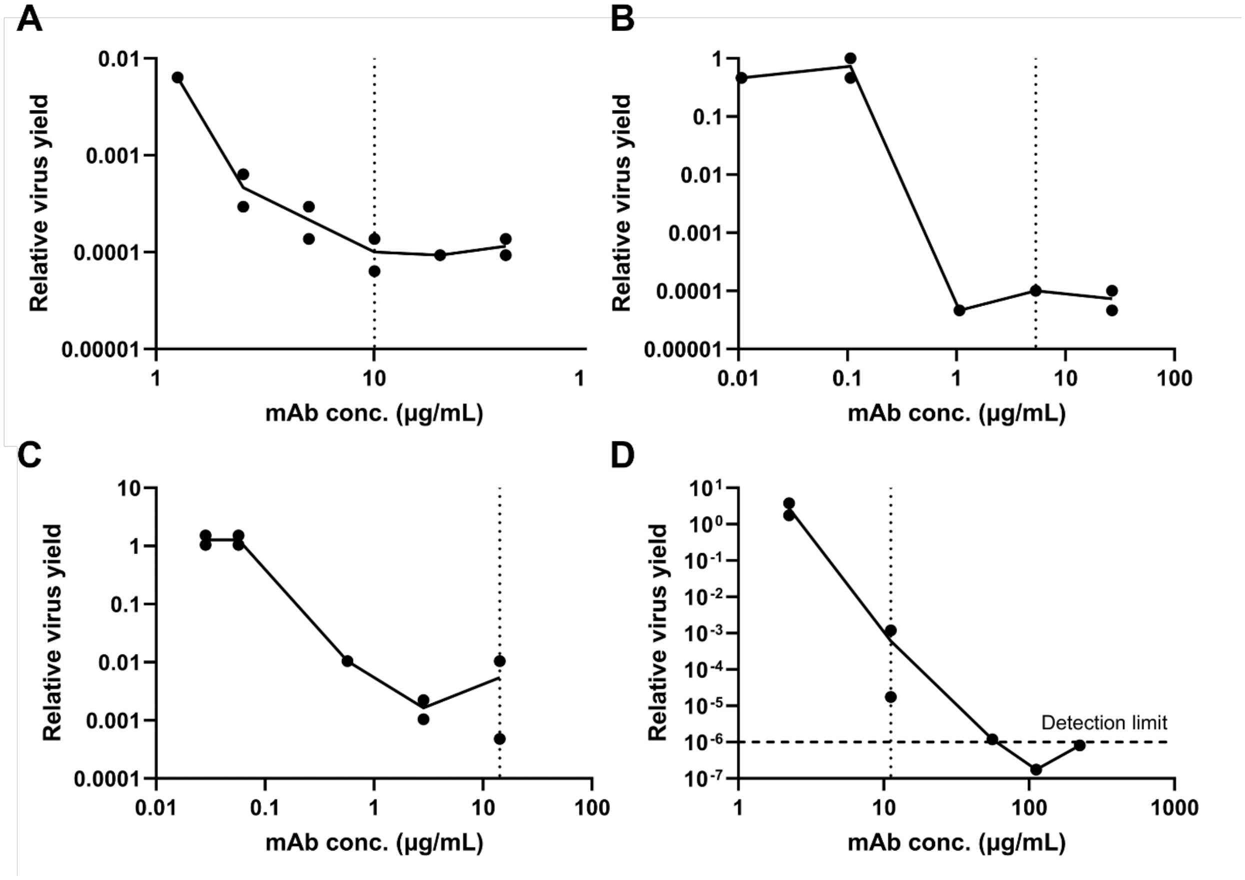


Figure S4: Saturated neutralization concentrations for the monoclonal antibodies used in this study. *10^7 TCID50 of rNA:CA09 was neutralized with (A) EM4-C04, (B) 2A05, (C) 2C05 and (D) CR9114 in the given concentration and infected the cells. Virus supernatants were collected 24 hours post infection (n = 2) and measured by TCID50 assay. Relative virus yield was normalized to the no antibody control group. Dot lines indicate the concentration used in selection.*

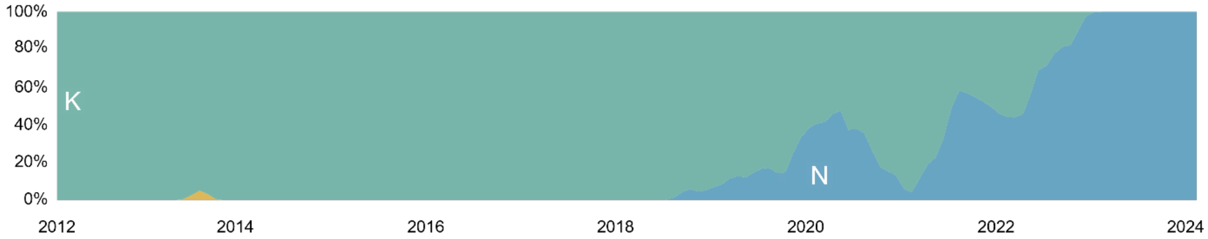


Figure S5: Normalized frequencies composition of pdmH1N1 HA position 133a over the years *colored by the amino acid: lysine(K, green), asparagine (N, blue), arginine (R, yellow) from 1472 genomes samples between February 2012 to February 2024 (Screenshot taken from Nextstrain[34,60]).*

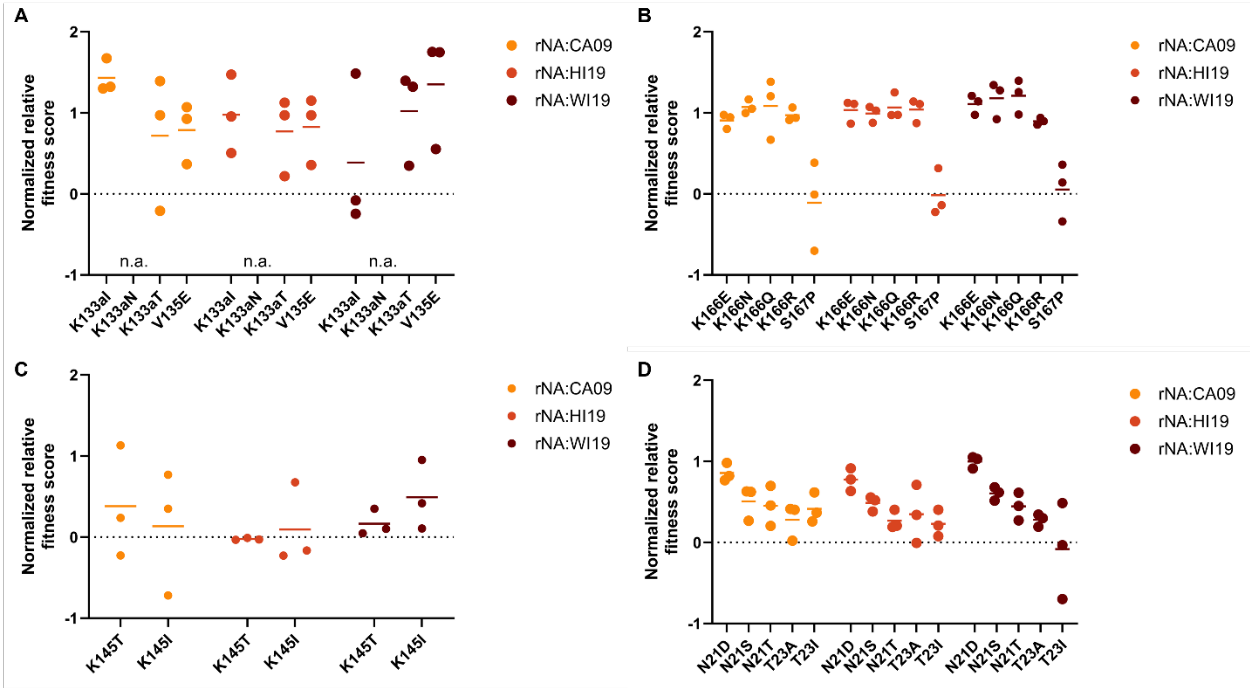


Figure S6: The normalized relative fitness score in deep mutational scanning of escape variants found in antibody selection *with (A) EM4-C04, (B) 2A05, (C) 2C05 and (D) CR9114 (excluding HA2 residues).*