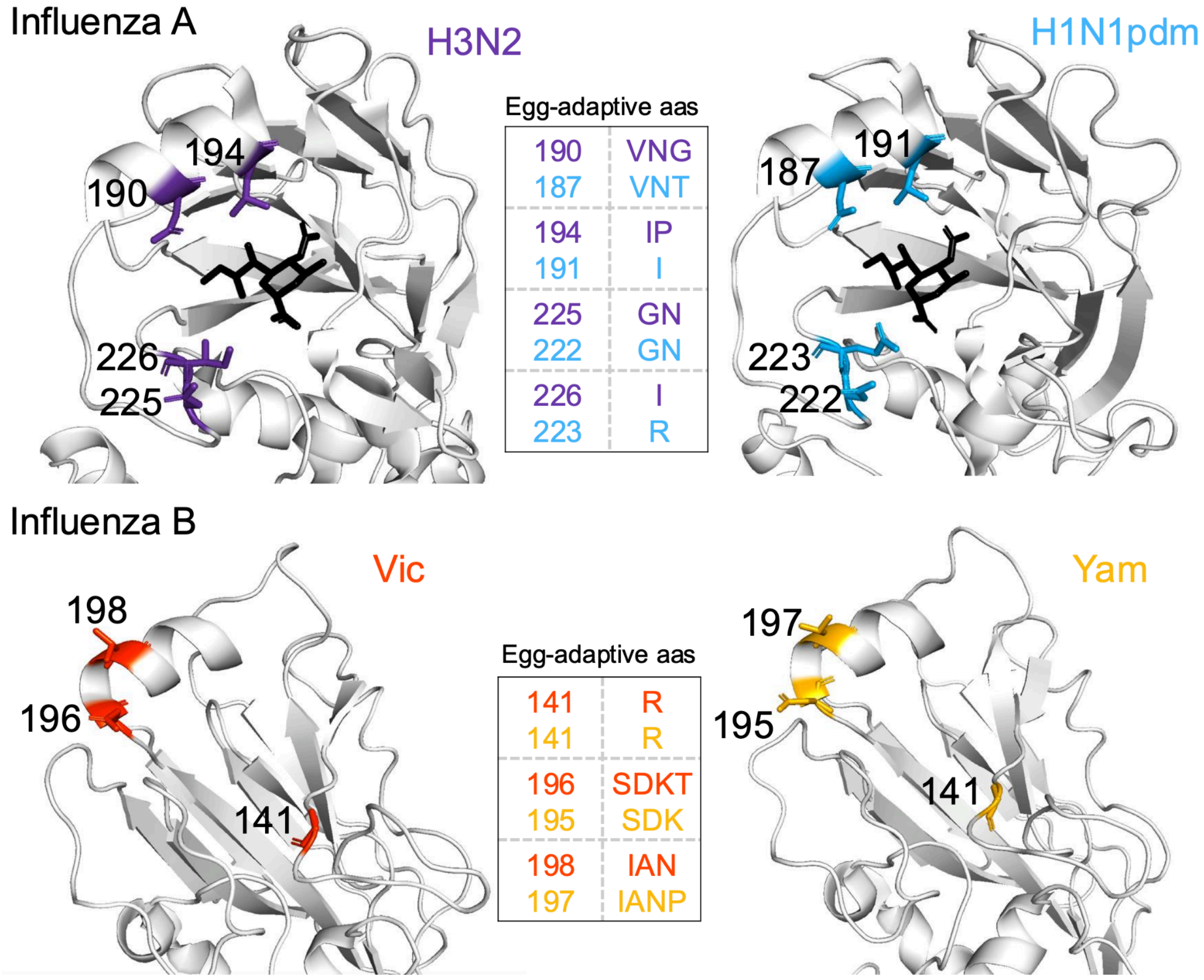
Supplements



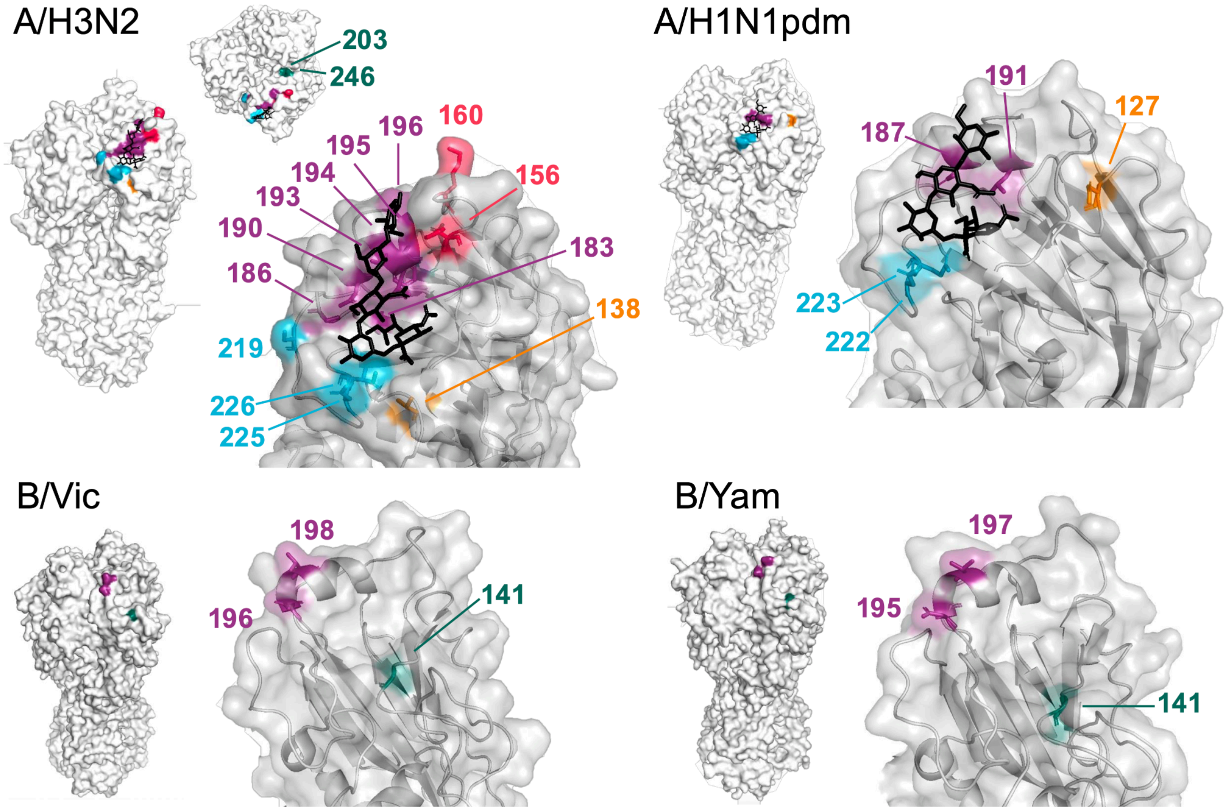
Egg-adaptive aas

190	VNG
187	VNT
194	IP
191	I
225	GN
222	GN
226	I
223	R

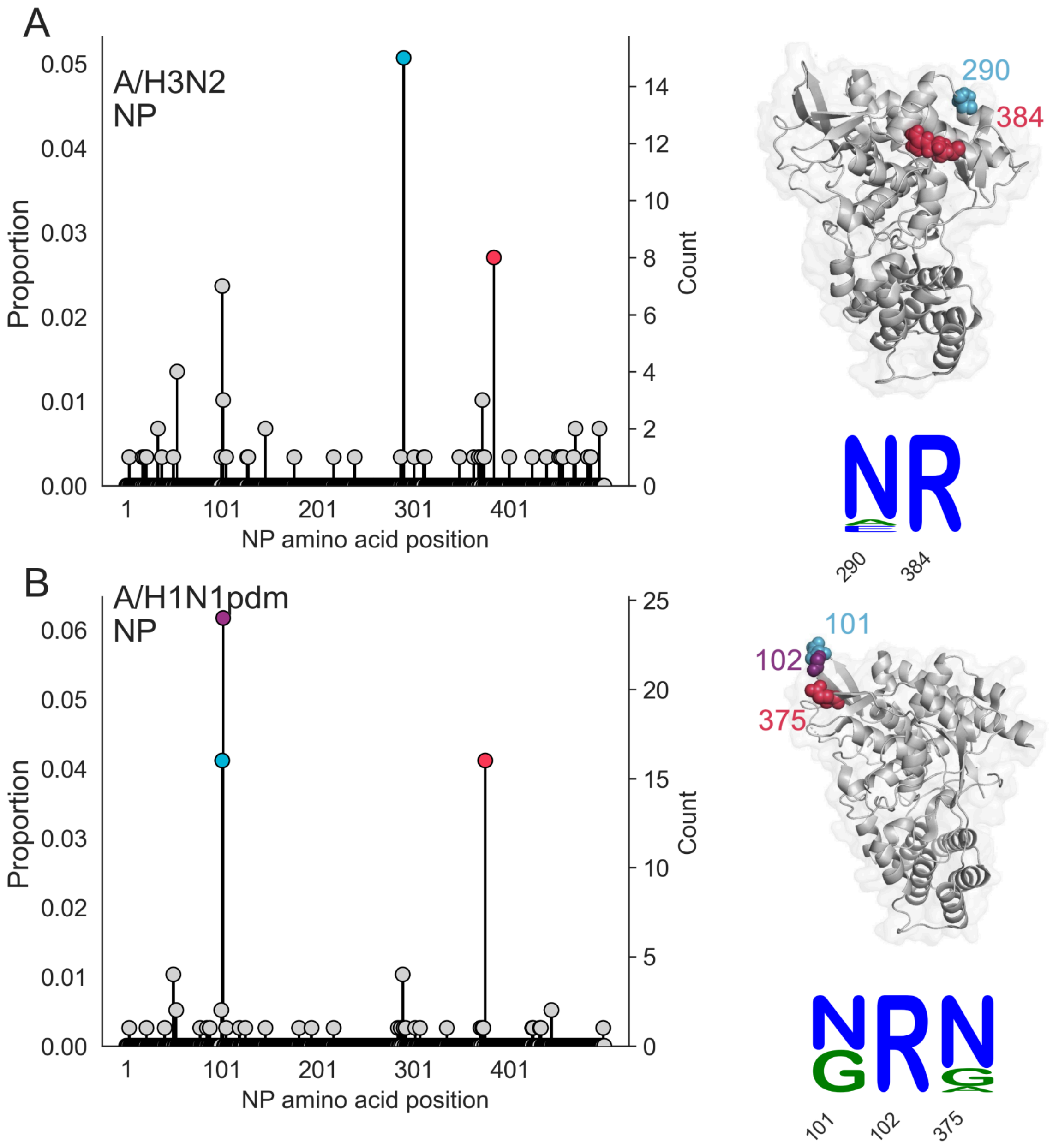
Egg-adaptive aas

141	R
141	R
196	SDKT
195	SDK
198	IAN
197	IANP

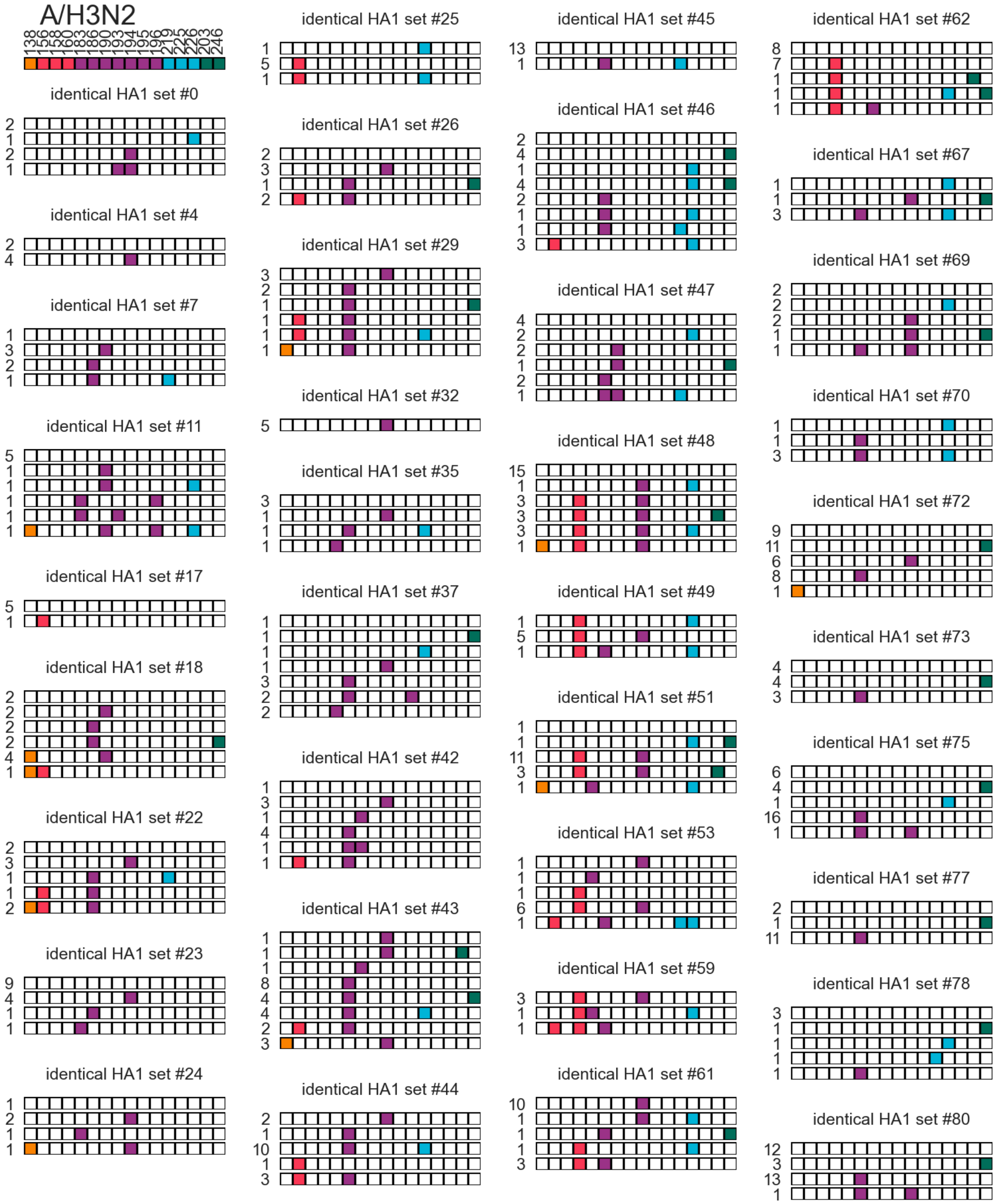
Supplemental Figure 1. Structurally homologous locations of egg-adaptive mutations in HA1. Residues that get egg-adaptive mutations in both influenza A viruses (top row) or both influenza B viruses (bottom row) are highlighted on the structure of HA. Egg-adaptive amino acids observed at these homologous sites in both viruses are listed in the table. Influenza A viruses are shown with the terminal moeity of an α-2,6 sialic acid analog (black) for context. The PDB for structures are H3N2 (2YP4), H1N1pdm (3UBE), Vic (4FQM), Yam (4M40).



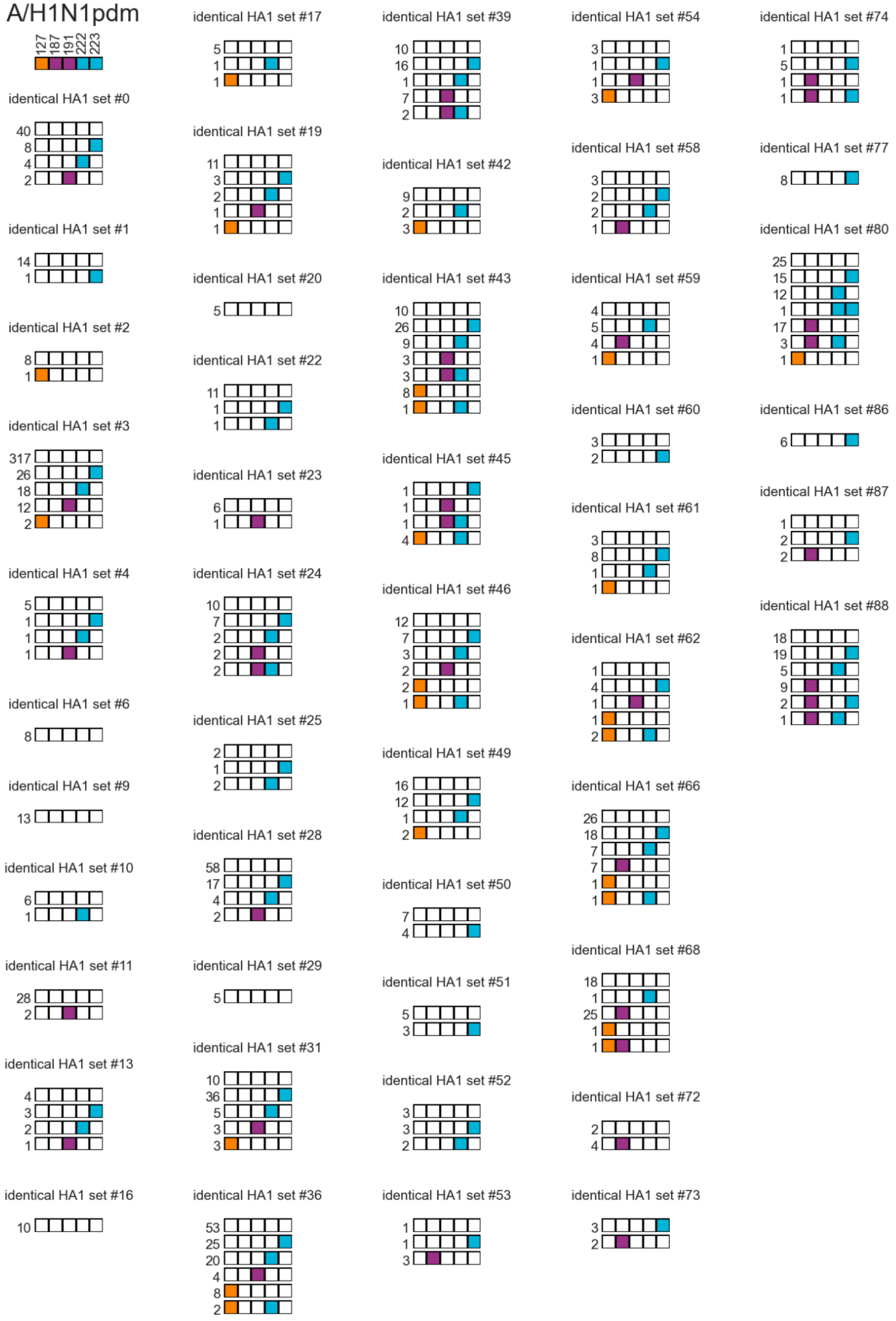
Supplemental Figure 2. Locations of all HA1 egg-adaptive mutations. The position of all egg-adaptive mutations in HA are highlighted on the protein structure for each influenza subtype. The PDB for structures are H3N2 (2YP4), H1N1pdm (3UBE), Vic (4FQM), Yam (4M40).



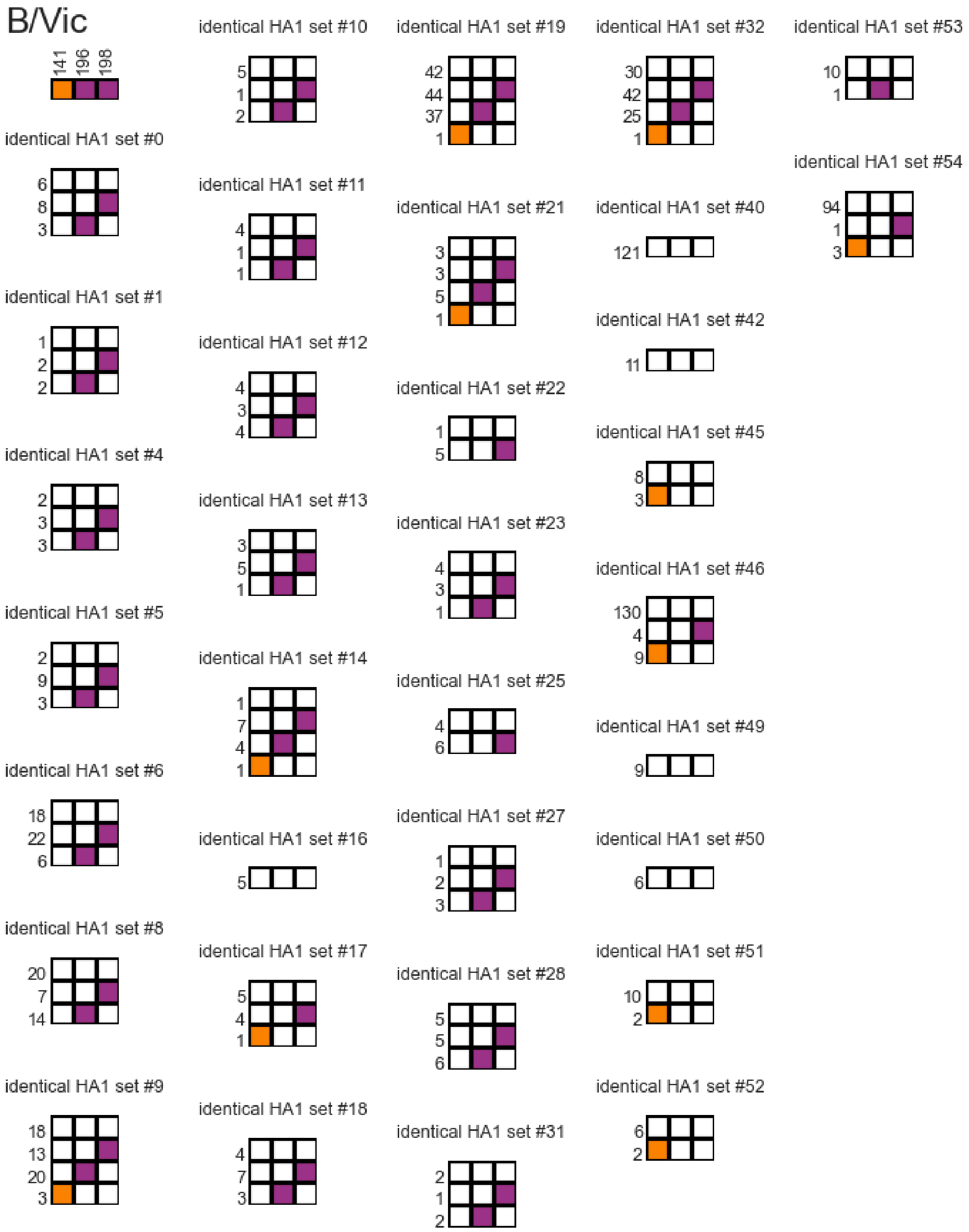
Supplemental Figure 3. Egg-adaptive mutations in nucleoprotein of influenza A viruses. Proportion of egg-passaged strains with a mutation at each residue in the NP protein for A) H3N2, and B) H1N1pdm. Inferred egg-adaptive mutations are colored and these same colors are used to indicate the position of each mutation on the structure of NP. The H3N2 structure is from a 1968 strain (PDB 7nt8), and the H1N1 structure is from the 1933 WSN H1N1 strain (PDB 3zdp). Logo plots show the amino acid substitutions at each egg-adaptive site in NP, in proportions.



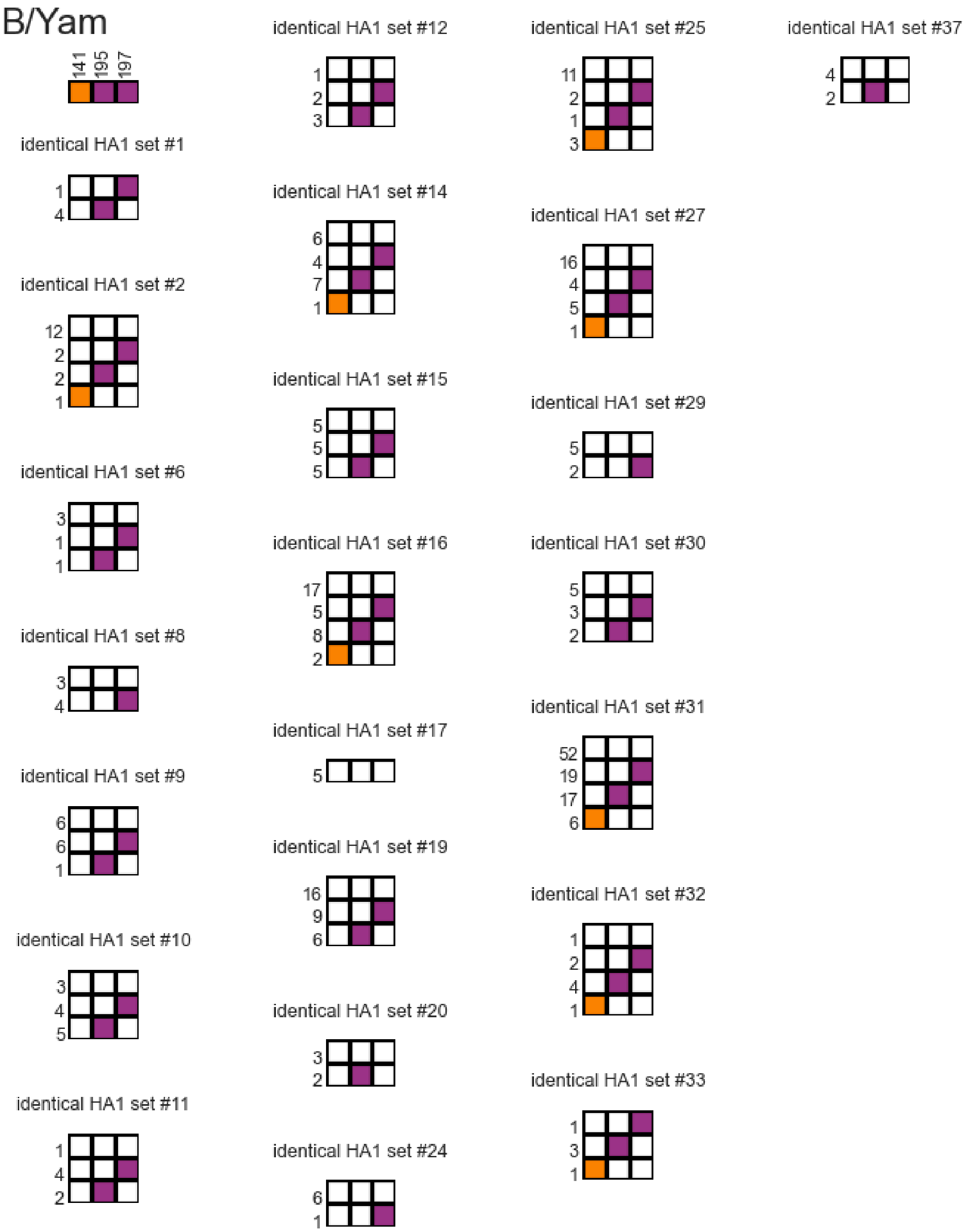
Supplemental Figure 4. All sets of A/H3N2 egg-passaged strains derived from identical HA1 sequences.



Supplemental Figure 5. All sets of A/H1N1pdm egg-passaged strains derived from identical HA1 sequences.

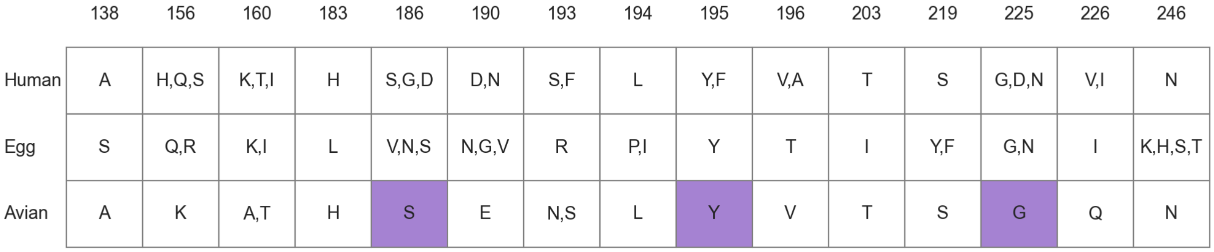


Supplemental Figure 6. All sets of B/Vic egg-passaged strains derived from identical HA1 sequences.



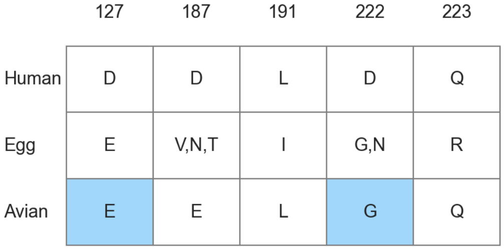
Supplemental Figure 7. All sets of B/Yam egg-passaged strains derived from identical HA1 sequences.

A/H3N2



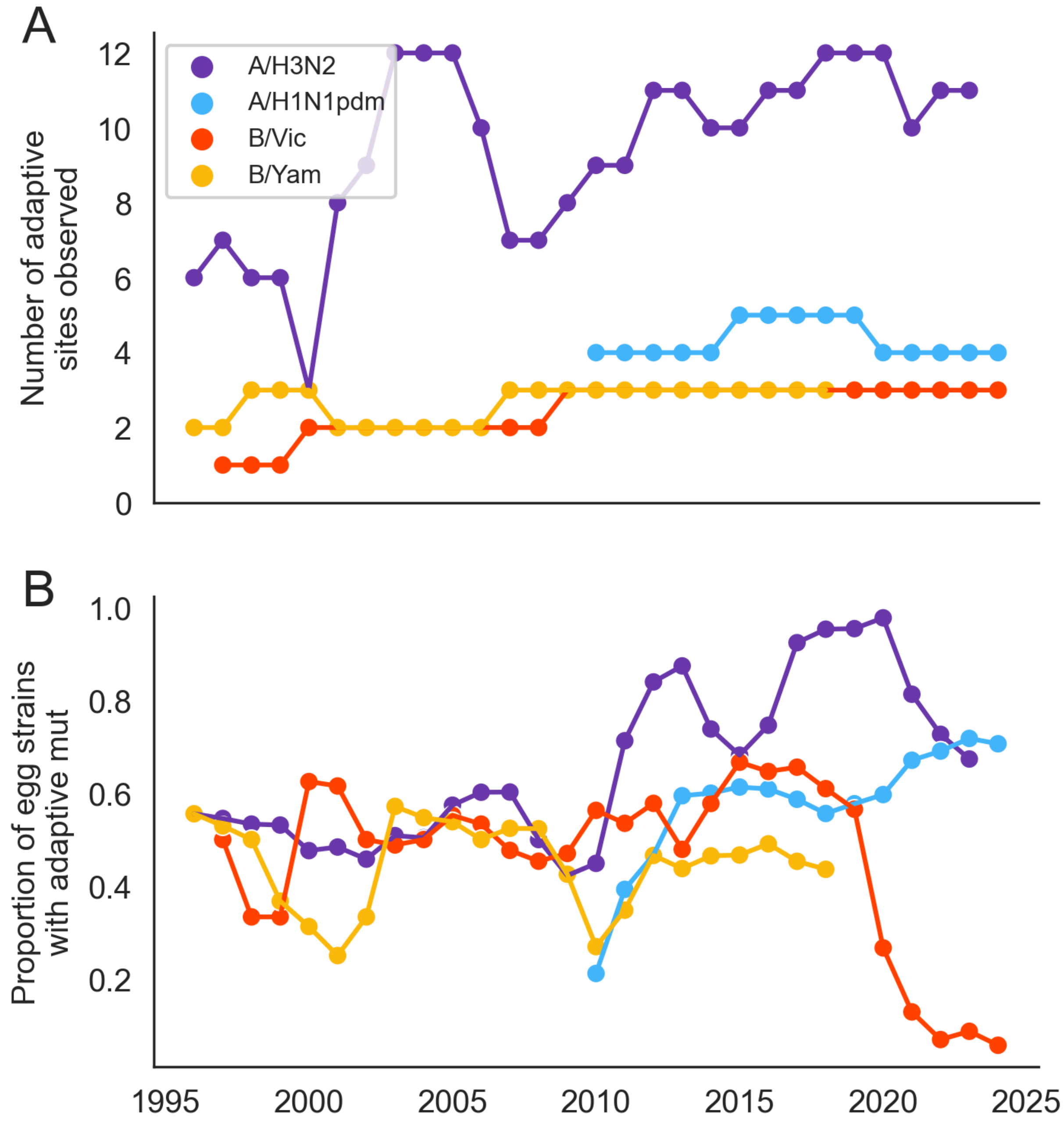
	138	156	160	183	186	190	193	194	195	196	203	219	225	226	246
Human	A	H,Q,S	K,T,I	H	S,G,D	D,N	S,F	L	Y,F	V,A	T	S	G,D,N	V,I	N
Egg	S	Q,R	K,I	L	V,N,S	N,G,V	R	P,I	Y	T	I	Y,F	G,N	I	K,H,S,T
Avian	A	K	A,T	H	S	E	N,S	L	Y	V	T	S	G	Q	N

A/H1N1pdm



	127	187	191	222	223
Human	D	D	L	D	Q
Egg	E	V,N,T	I	G,N	R
Avian	E	E	L	G	Q

Supplemental Figure 8. Egg-adaptive mutations make human viruses more avian-like at some, but not all, adaptive sites. Amino acids observed at each egg-adaptive site in the human HA, egg-adapted human HA, and avian HA. For the human and avian HA, all amino acids observed in 20% or more of the sequences are listed.



Supplemental Figure 9. Total number of egg-adaptive mutations differs more over time for H3N2 than for H1N1pdm or influenza B viruses. A) The number of egg-adaptive sites (residues) in HA where a mutation is observed in at least 2 strains or at least 10% of strains during a 3-year window. B) The proportion of egg-passaged strains within a 3-year window that have an adaptive mutation in HA. Both panels are calculated in sliding 3-year windows, plotted at the midpoint year.