Supplemental Tables and Figures

Table SI. Focus reduction assay of reference viruses and contemporary test viruses.

		Reference Ferret Antisera			
		A/Michigan /15/2014	A/Singapore /INFIMH-16-0019/2016	A/Nebraska /2/2017	A/California /179/2016
Reference Virus	**Clade**	3c2.A	A1	A2	A3
A/Michigan/15/2014	3c2.A	**640**	320	1600	320
A/Singapore/Infimh-16-0019/16	A1	640	**1280**	800	1280
A/Nebraska/02/2017	A2	640	1280	**12800**	1280
A/California/179/2016	A3	640	2560	800	**1280**
Test Virus					
A/Haiti/5464/2017	A1b	640	1280	1600	1280
A/Maryland/13/2018	A1b	320	1280	400	640
A/Kuwait/922/2018	A1b	320	1280	400	640
A/Kazakhstan/079/2018	A2	640	1280	12800	640
A/Kazakhstan/031/2018	A2	640	640	12800	1280
A/Alberta/Rv2371/2017	A2	320	640	6400	640
A/Tennessee/70/2017	A2/re	640	2560	25600	1280
A/Puerto Rico/09/2018	A2/re	640	2560	25600	1280
A/Peru/3817/2017	A2/re	640	640	12800	640
A/Cambodia/949/2017	A3	320	640	800	640
A/New Jersey/23/2018	3c3.A	80	320	<200	80
A/Delaware/20/2018	3c3.A	20	160	<200	40
A/Massachusetts/18/2018	3c3.A	40	80	<200	40

Table SII. Sites showing signatures of positive selection in HA as determined by dN/dS analysis. Site 160 in particular shows evidence of positive selection when analyzed by this method.

Codon	Partition	S	N	dS	dN	Selection detected?
53	1	1.000	13.000	1.312	5.812	Pos. p = 0.095
128	1	0.000	8.000	0.000	4.000	Pos. p = 0.039
160	1	2.000	36.000	2.058	17.807	Pos. p = 0.000
193	1	1.000	12.000	1.285	5.400	Pos. p = 0.112
197	1	0.000	6.000	0.000	3.997	Pos. p = 0.084
198	1	0.000	11.000	0.000	6.209	Pos. p = 0.007
214	1	1.000	12.000	1.007	5.979	Pos. p = 0.040
261	1	1.000	7.000	0.938	5.967	Pos. p = 0.047

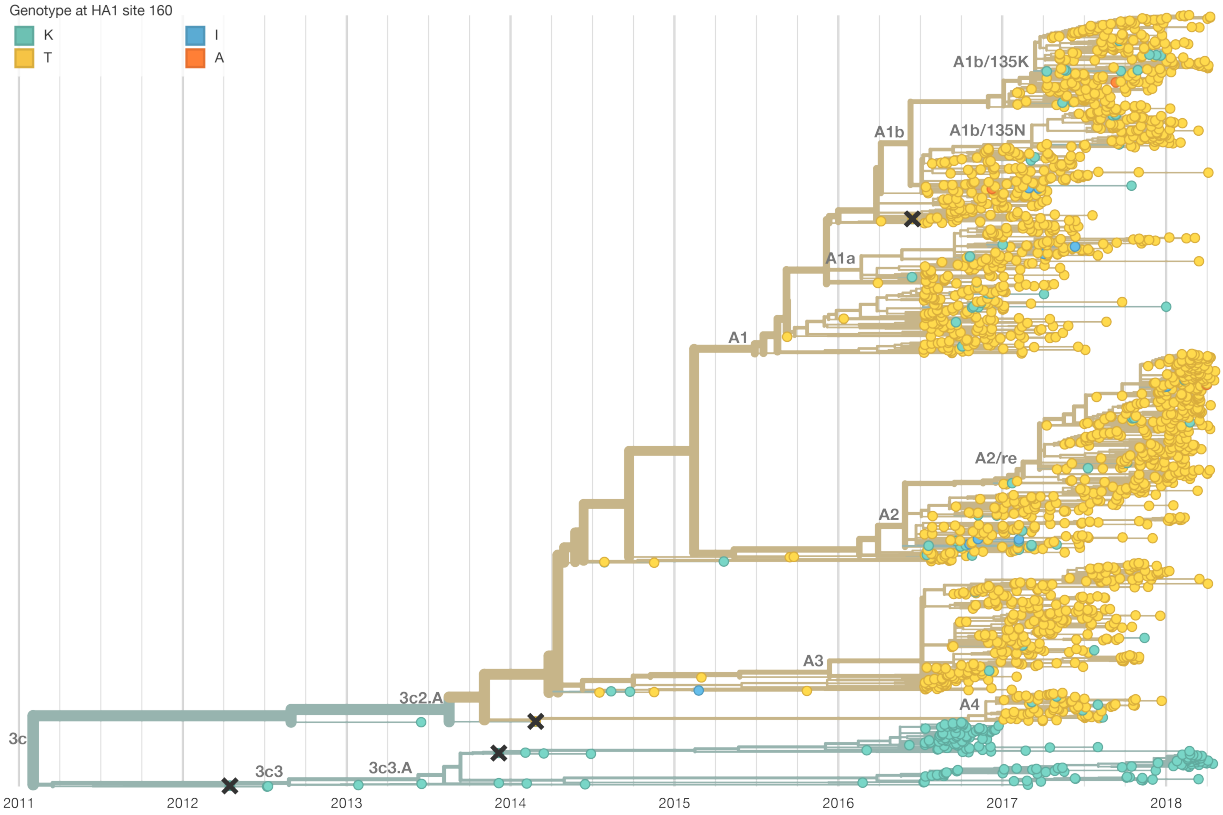

Figure S1. Phylogeny of HA colored by genotype at site HA:160. The frequent recurrent mutations of T to K, without any sustained growth suggest that this mutation is not adaptive, despite showing evidence of positive selection when analyzed by dN/dS methods.

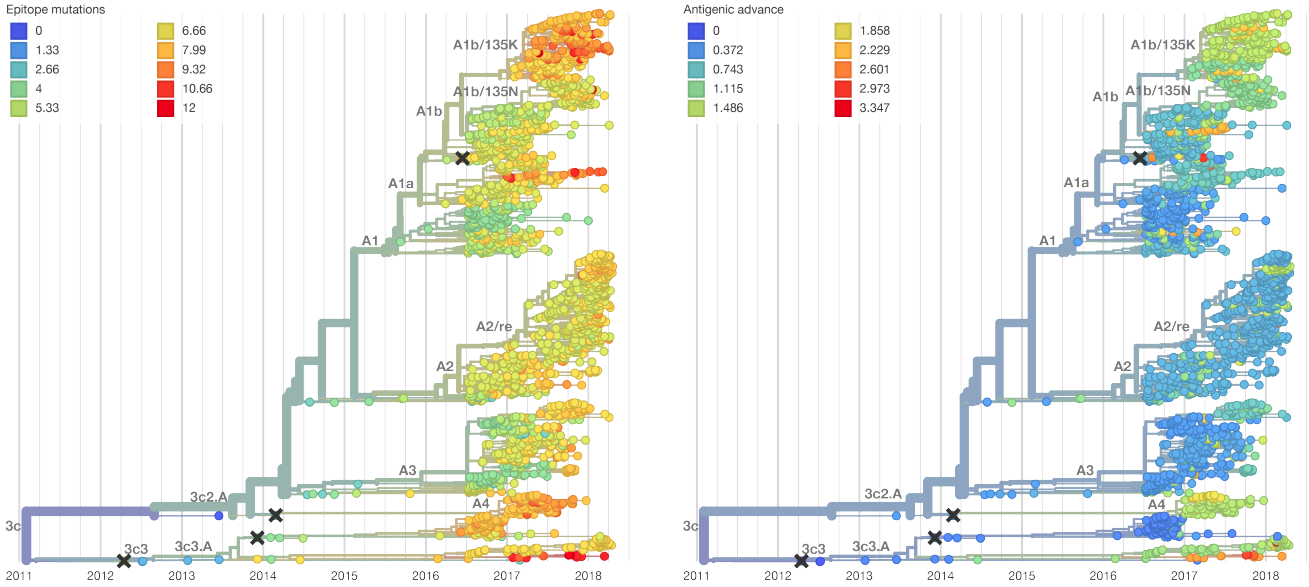

Figure S2. *(left)* Temporally resolved haemagglutinin phylogenetic tree colored by amino acid mutations in epitope sites Wolf et al. (2006) of HA. *(right)* The same tree, colored by antigenic advance—a measure of how much antigenic drift viruses have undergone based on related viruses' HI titers. Both measures normally indicate that a clade may be prone to grow rapidly, due to increased ability to escape immune pressure. In both trees A2/re does not demonstrate any change from A2, making its sudden growth unusual.

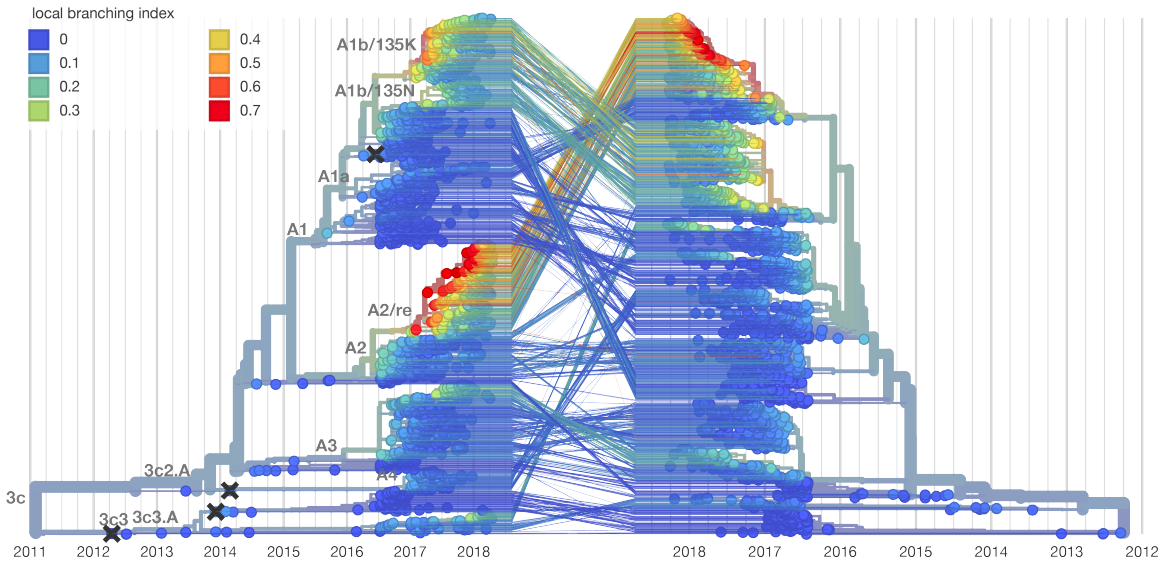

Figure S3. Tangle tree matching phylogenetic trees of HA and NA, colored by local branching index (LBI)—a measure that captures rapid expansion of clades. Here, LBI reveals that there is an inflection point where rapid clade growth begins at the same time that A2/re differentiates from A2 in the HA tree. Similarly, LBI is highest in the matched viruses of the NA tree, indicating that the reassortant virus saw much greater success than either of the background viruses from which it arose.

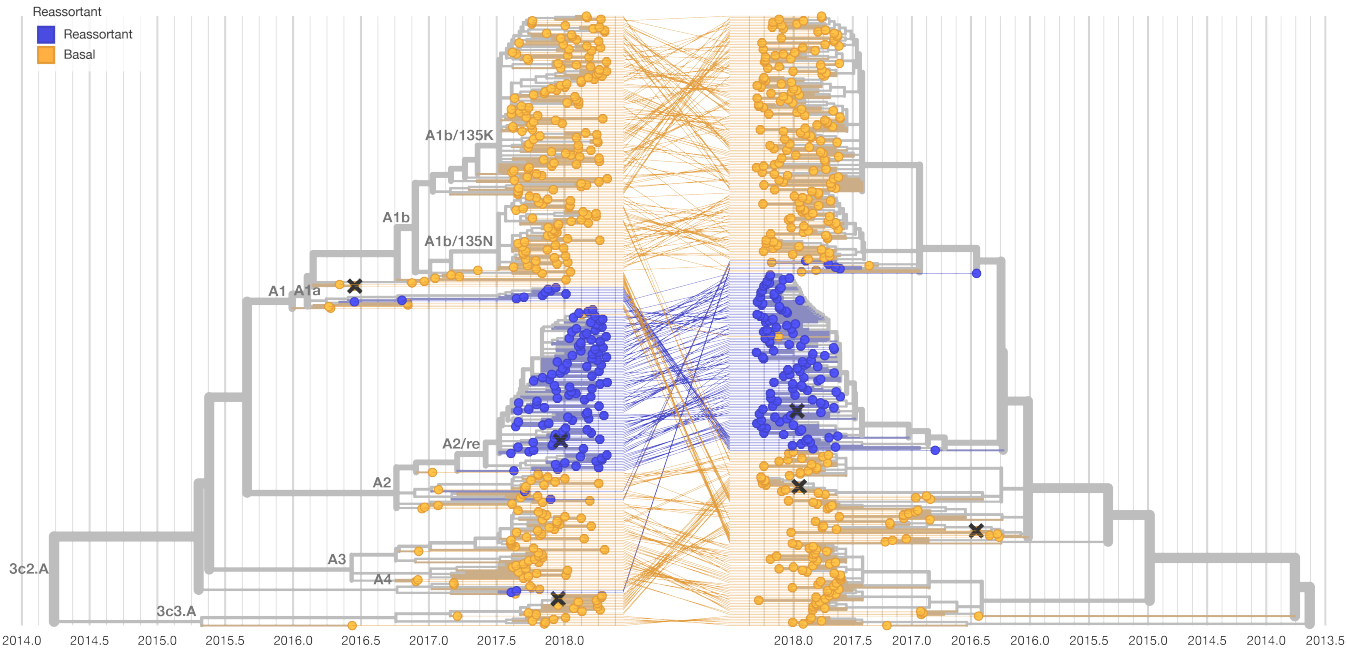

Figure S4. Tangle tree matching the phylogenies of HA and NA, colored by reassortment status as measured by GiRaF. This coloring shows all tips determined to be part of the reassortment (blue) from both the HA and NA trees.

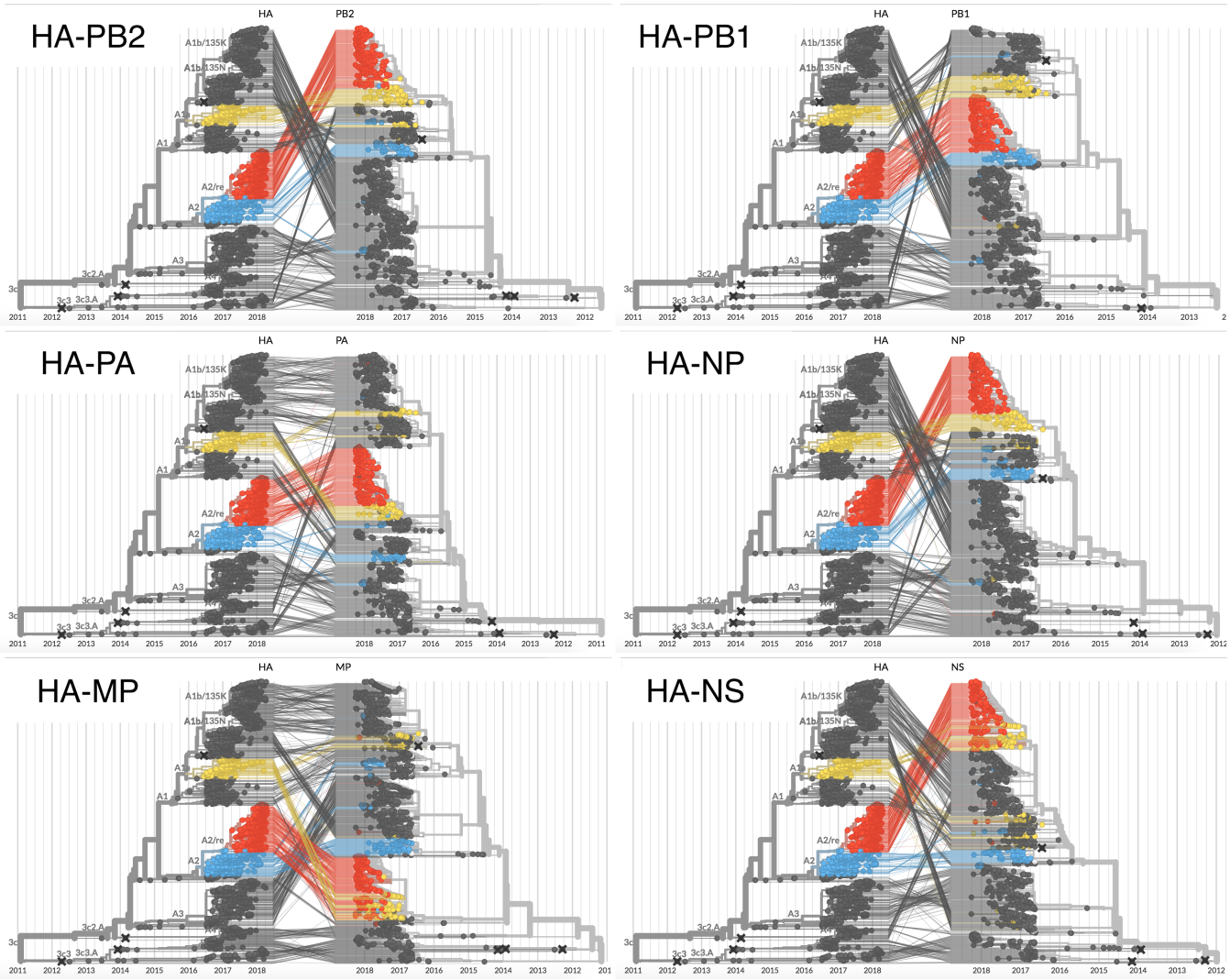

Figure S5. Tangle trees matching the HA phylogeny (always on left) with the six other non-NA segments. Clade A2 is colored in blue, A1b is colored in yellow, and A2/re is in red. In all segments other than PB1 (top right) A2 and A2/re lie in different parts of the tree in the second segment than in HA, indicating that reassortment has taken place.