

# Insights into the antigenic advancement of influenza A(H3N2) viruses, 2011-2018

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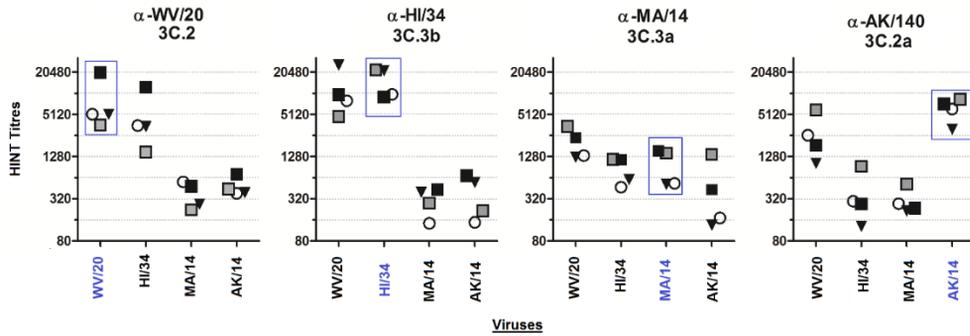
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# Figure S1



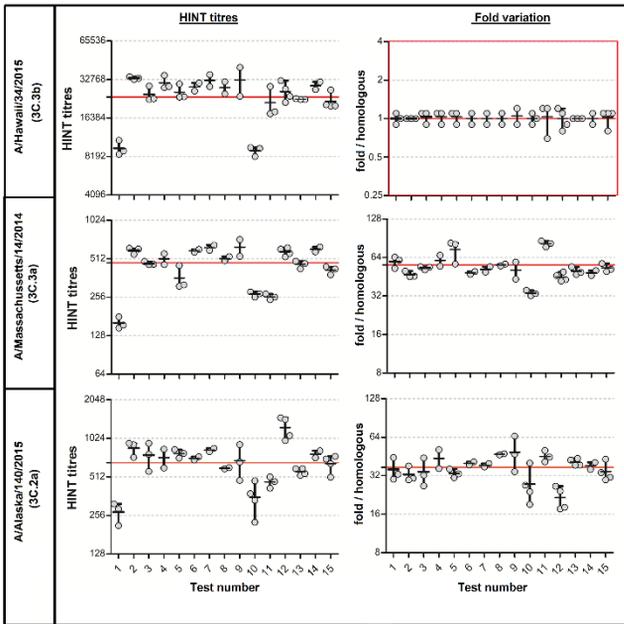
Ferret antiserum	clade	ferret	Viruses, HINT titres (fold)			
			A/WV/20/2012	A/HI/34/2015	A/MA/14/2014	A/AK/140/2015
$\alpha$ -WV/20	3C.2	1	<a href="#">3612 (1)</a>	1483 (2)	222 (16)	438 (8)
		2	<a href="#">5123 (1)</a>	3430 (1)	269 (19)	393 (13)
		3	<a href="#">5146 (1)</a>	3549 (1)	557 (9)	384 (13)
		4*	<a href="#">20165 (1)</a>	12430 (2)	479 (42)	713 (28)
		Geometric Mean	<a href="#">6620 (1)</a>	3870 (1.7-fold)	355 (19-fold)	467 (14-fold)
$\alpha$ -HI/34	3C.3b	5*	25833 (1)	<a href="#">21682 (1)</a>	397 (55)	547 (40)
		6	9751 (1)	<a href="#">9047 (1)</a>	433 (21)	683 (13)
		7	4780 (5)	<a href="#">22045 (1)</a>	278 (79)	214 (103)
		8	8007 (1)	<a href="#">9850 (1)</a>	143 (69)	147 (67)
		Geometric Mean	9909 (1.5-fold)	<a href="#">14366 (1)</a>	288 (50-fold)	329 (44-fold)
$\alpha$ -MA/14	3C.3a	9*	2388 (1)	1168 (1)	<a href="#">1550 (1)</a>	434 (4)
		10	1252 (0.4)	603 (1)	<a href="#">516 (1)</a>	135 (4)
		11	3463 (0.4)	1167 (1)	<a href="#">1440 (1)</a>	1366 (1)
		12	1320 (0.4)	469 (1)	<a href="#">531 (1)</a>	170 (3)
		Geometric Mean	1923 (0.5-fold)	788 (1.1-fold)	<a href="#">884 (1)</a>	341 (2.6-fold)
$\alpha$ -AK/140	3C.2a	13*	1851 (4)	273 (26)	236 (31)	<a href="#">7224 (1)</a>
		14	2579 (2)	295 (21)	273 (22)	<a href="#">6098 (1)</a>
		15	5929 (1)	926 (9)	520 (16)	<a href="#">8423 (1)</a>
		16	1022 (3)	129 (24)	214 (15)	<a href="#">3123 (1)</a>
		Geometric Mean	2319 (2.5-fold)	313 (19-fold)	291 (20-fold)	<a href="#">5834 (1)</a>

**Sup. Figure 1: HINT titres for ferret antisera collected at day 14 post infection with human respiratory specimens.** Homologous response is shown in blue. Individual values for each ferret serum is shown below the graphs. Antisera chosen for further analysis are indicated with an asterisk (ferrets 4, 5, 9 and 13).

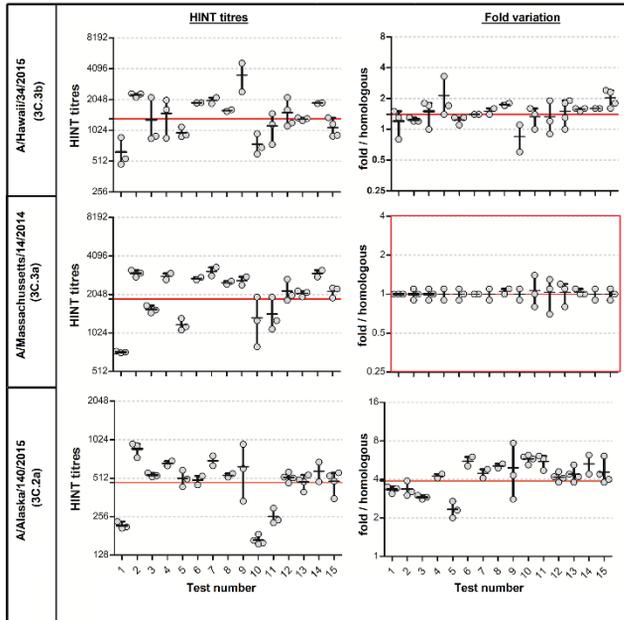
# Figure S2

**Sup.Fig.2: HINT reproducibility.** Reference viruses and ferret antisera were tested in 15 independent tests. Data are presented as HINT neutralization titres and fold variation in HINT titre compared to homologous geometric mean titre. The horizontal red line indicates the geometric mean value for the entire population. Plots with a red frame indicate homologous pairs.

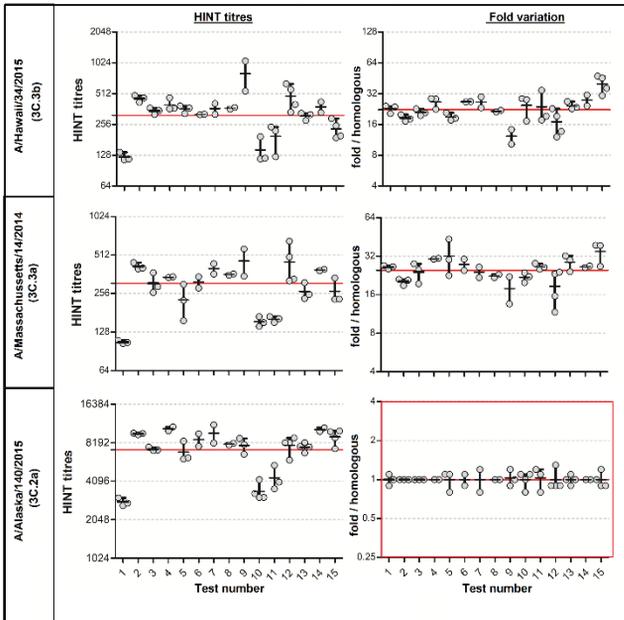
anti-3C.3b  
(TX/2012-like)



anti-3C.3a  
(SW/2013-like)

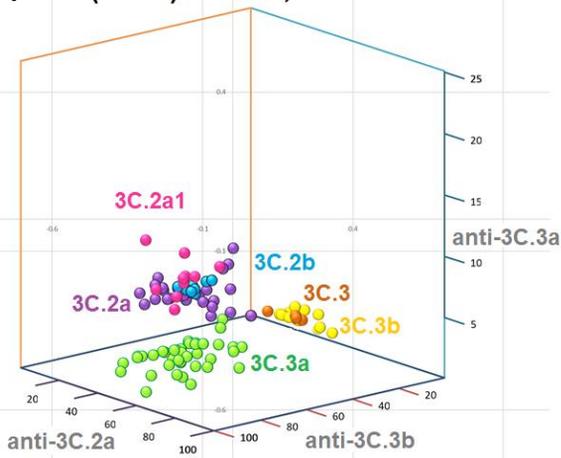


anti-3C.2a  
(HK/2014-like)

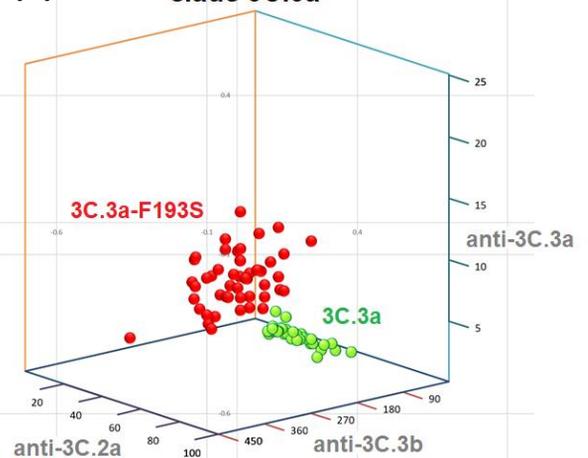


# Figure S3

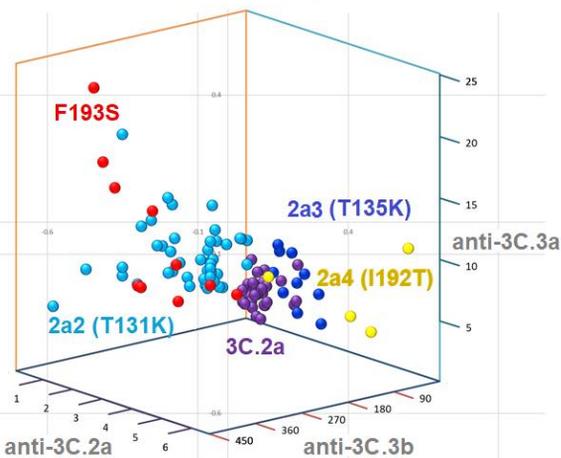
(A) A(H3N2) viruses, 2014-2016



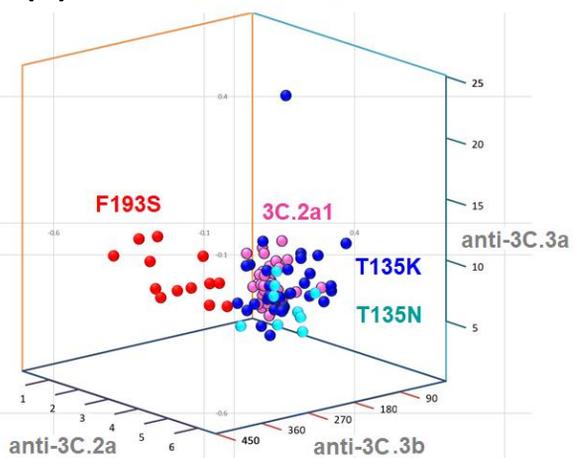
(B) clade 3C.3a



(C) clade 3C.2a

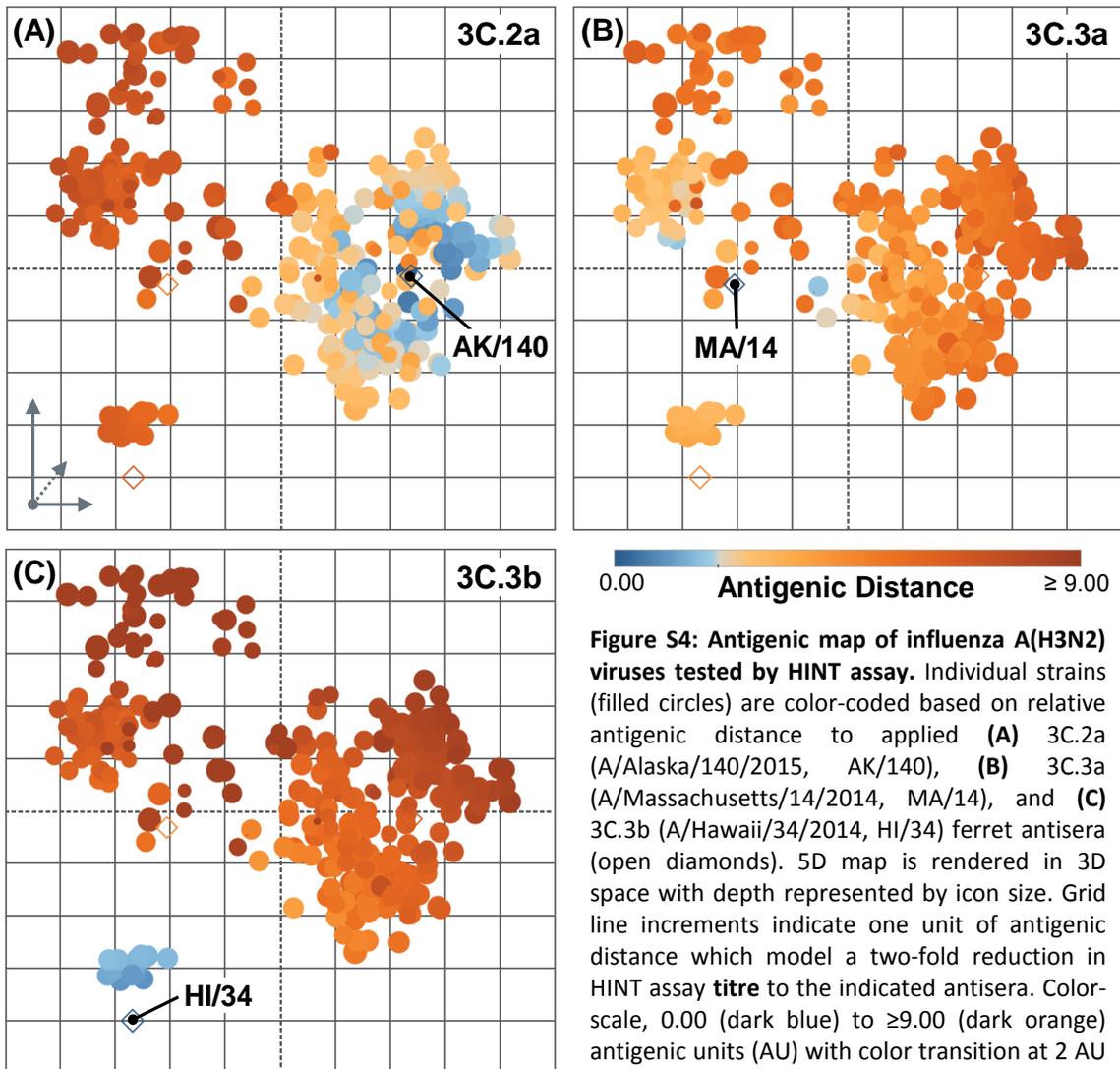


(D) subclade 3C.2a1

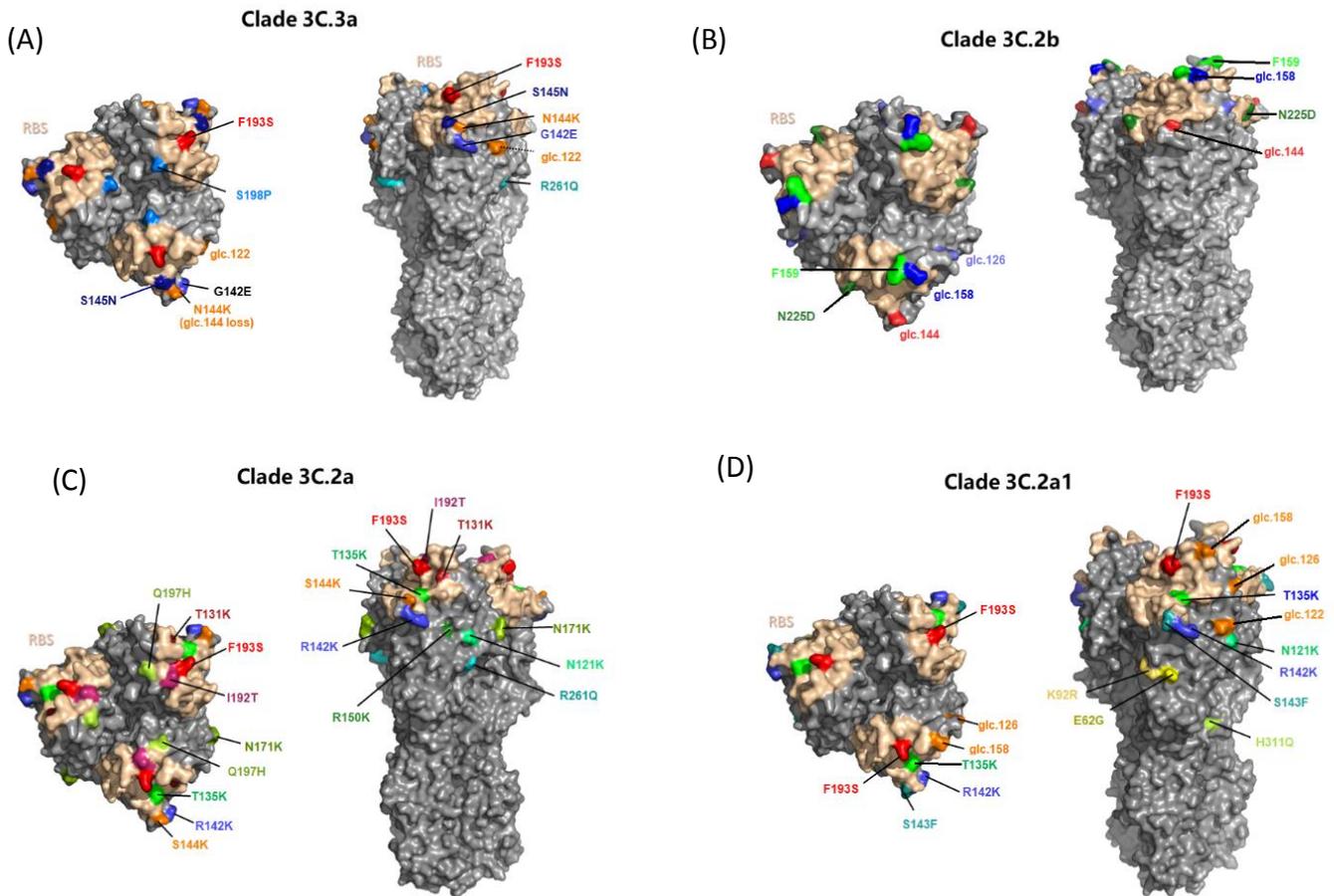


**Sup. Fig. 3. Neutralization profile of influenza A(H3N2) viruses tested with three reference antisera.** Individual groups are color-coded for an easier visualization. **(A)** Earlier Influenza A(H3N2) viruses circulating during Jan/2014-Dec/2016. **(B)** clade 3C.3a viruses isolated in Oct/2014-Jun/2017 ; **(C)** clade 3C.2a isolated in Nov/2014-Nov/2017, and **(D)** clade 3C.2a1 isolated in Sept/2015- Dec/2017. Axes indicate the fold reduction in HINT titres by the clade-specific antisera.

## Figure S4

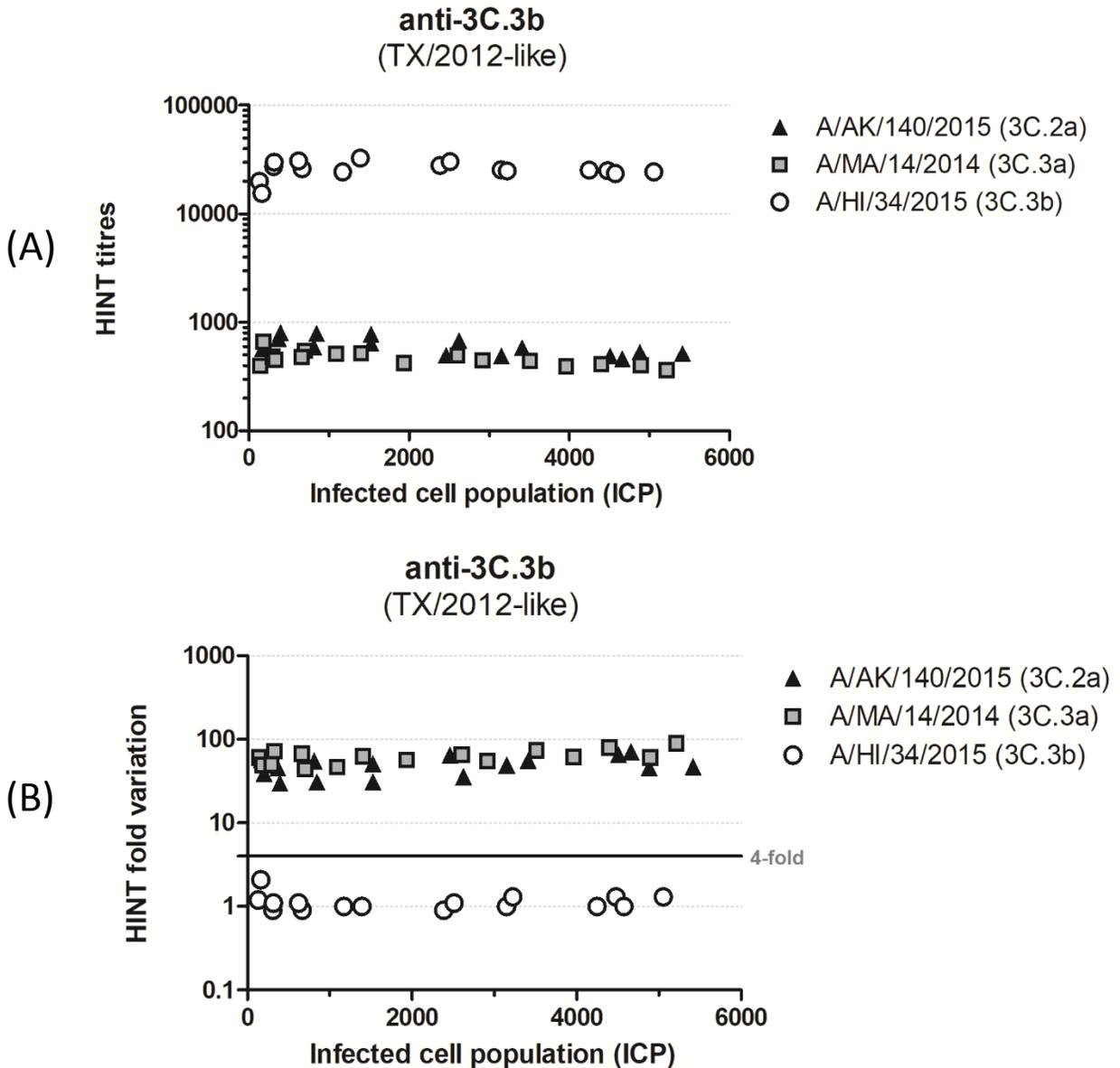


## Figure S5



**Fig. S5: 3D visualization of the HA trimer highlighting HA1 AA changes detected in A(H3N2) viruses.** The H3 HA protein structures were presented using the user-sponsored PyMOL molecular graphic system, Version 1.8.6 (Schrödinger, LLC). The individual AA substitutions seen in clade A) 3C.3a, B)3C.2b, C)3C.2a and D)3C.2a1 were mapped onto the 3-dimensional structure of the A/Victoria/361/2011 (H3N2) virus HA protein (RCSB Protein Data Bank, accession number: 4WE8).

# Figure S6



**Sup. Fig.6: Effect of ICP on neutralization by anti-3C.3b reference antiserum.** Increasing amounts of reference viruses (100 to 6000 ICP/well) were tested in HINT using the anti-3C.3b reference antiserum as source of neutralizing antibodies. Data is presented as HINT titres (A) or HINT fold variation (B) versus infectious dose. HINT fold variation were calculated based to 1000 ICP of A/HI/34/2015. The ability of anti-3C.3b serum to neutralize the reference viruses was not affected by the inoculation dose, showing linear inhibition between 100 to 6000 ICP.