

**Supplemental information**

**Antigenic and virological properties of an H3N2  
variant that continues to dominate the 2021–22  
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## **Cell Reports**

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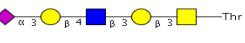
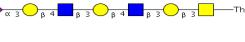
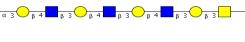
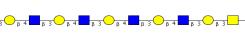
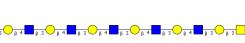
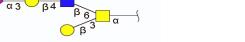
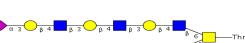
#### **Antigenic and virological properties of an H3N2 variant that continues to dominate the 2021-2022 Northern Hemisphere influenza season**

Marcus J. Bolton, Jordan T. Ort, Ryan McBride, Nicholas J. Swanson, Jo Wilson, Moses Awofolaju, Colleen Furey, Allison R. Greenplate, Elizabeth M. Drapeau, Andrew Pekosz, James C. Paulson, Scott E. Hensley

Supplementary Table 1

No	Common Name	Structure
1	Gal $\beta$ (1-4)-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
2		
3		
4		
5		
6		
7	Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-2)-Man $\alpha$ (1-3)-[Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-2)-Man $\alpha$ (1-6)]-Man $\beta$ (1-4)-GlcNAc $\beta$ (1-4)-GlcNAc $\beta$ -Asn-NH <sub>2</sub>	
8		
9		
10		
11	NeuAca(2-3)-Gal $\beta$ (1-4)-6-O-sulfo-GlcNAc $\beta$ -propyl-NH <sub>2</sub>	
12	NeuAca(2-3)-Gal $\beta$ (1-4)-[Fuca(1-3)]-6-O-sulfo-GlcNAc $\beta$ -propyl-NH <sub>2</sub>	

13	NeuAca(2-3)-6-O-sulfo-Gal $\beta$ (1-4)-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
14	NeuAca(2-3)-6-O-sulfo-Gal $\beta$ (1-4)-[Fuca(1-3)]-GlcNAc $\beta$ -propyl-NH <sub>2</sub>	
15	NeuAca(2-3)-Gal $\beta$ (1-3)-6-O-sulfo-GlcNAc $\beta$ -propyl-NH <sub>2</sub>	
16	NeuAca(2-3)-Gal $\beta$ (1-4)-Glc $\beta$ -ethyl-NH <sub>2</sub>	
17	NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
18	NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
19	NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
20	NeuAca(2-3)-GalNAc $\beta$ (1-4)-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
21	NeuAca(2-3)-Gal $\beta$ (1-3)-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
22	NeuAca(2-3)-Gal $\beta$ (1-3)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
23	NeuAca(2-3)-Gal $\beta$ (1-3)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-3)-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
24	NeuAca(2-3)-Gal $\beta$ (1-3)-GalNAc $\beta$ (1-3)-Gala(1-4)-Gal $\beta$ (1-4)-Glc $\beta$ -ethyl-NH <sub>2</sub>	
25	NeuAca(2-3)-Gal $\beta$ (1-3)-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	

26	3' NeuAc LN Core 1 (1163)	
27	3' NeuAc DiLN Core 1 (1528)	
28	3' NeuAc TriLN Core 1 (1894)	
29	3' NeuAc TetraLN Core 1 (2259)	
30	3' NeuAc PentaLN Core 1 (2624)	
31	NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-6)[Galβ(1-3)]-GalNAca-Thr-NH <sub>2</sub>	
32	NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-6)[Galβ(1-3)]-GalNAca-Thr-NH <sub>2</sub>	
33	3' NeuAc TriLN Core 2 (1894)	
34	3' NeuAc TetraLN Core 2 (2259)	
35	3' NeuAc PentaLN Core 2 (2624)	
36	3' NeuAc TetraLN TriLN Core 2 (3645)	
37	3' NeuAc PentaLN TetraLN Core 2 (4376)	
38	NeuAcα(2-3)-Galβ(1-4)-GlcNAcβ(1-3)-GalNAca-Thr-NH <sub>2</sub>	

39	NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-GalNAca-Thr-NH <sub>2</sub>	
40	3' NeuAc TriLN Core 3 (1732)	
41	3' NeuAc TetraLN Core 3 (2097)	
42	3' NeuAc PentaLN Core 3 (2462)	
43	NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-[NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-6)]-GalNAca-Thr-NH <sub>2</sub>	
44	NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-[NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-6)]-GalNAca-Thr-NH <sub>2</sub>	
45	3' NeuAc TrLN Core4 (3118)	
46	3' NeuAc TetraLN Core4 (3848)	
47	3' NeuAc PentaLN Core4 (4579)	
48	3' NeuAc TetraLN Core6 (2097)	
49	3' NeuAc PentaLN Core6 (2462)	
50	3' NeuAc LecLN I-Antigen(2104)	
51	NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-2)-Mano(1-3)-[NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-2)-Mano(1-6)]-Man $\beta$ (1-4)-GlcNAc $\beta$ (1-4)-GlcNAc $\beta$ -Asn-NH <sub>2</sub>	

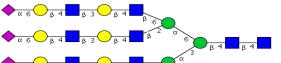
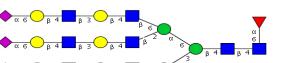
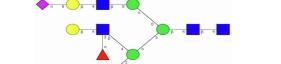
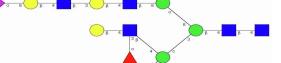
52	NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-2)-Mano(1-3)-[NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-2)-Mano(1-6)]-Man $\beta$ (1-4)-GlcNAc $\beta$ (1-4)-GlcNAc $\beta$ -Asn-NH <sub>2</sub>	
53	NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-2)-Mano(1-3)-[NeuAca(2-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-2)-Mano(1-6)]-Man $\beta$ (1-4)-GlcNAc $\beta$ (1-4)-GlcNAc $\beta$ -Asn-NH <sub>2</sub>	
54	3' NeuAc DiLN Bi-(3594)	
55	3' NeuAc TriLN Bi-(4324)	
56	3' NeuAc TetraLN Bi-(4828)	
57	3' NeuAc TriLN Bi-CF(4470)	
58	3' NeuAc TetraLN Bi-CF(5200)	
59	3' NeuAc DiLN Tri-(4615)	
60	3' NeuAc DiLN Tri-CF(4761)	
61	Gn/3'SLN/3'SLN-TriN	
62	NeuAca(2-3)-[GalNAc $\beta$ (1-4)]-Gal $\beta$ (1-4)-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
63	NeuAca(2-3)-[GalNAc $\beta$ (1-4)]-Gal $\beta$ (1-4)-Glc $\beta$ -ethyl-NH <sub>2</sub>	
64	Gal $\beta$ (1-3)-GalNAc $\beta$ (1-4)-[NeuAca(2-3)-Gal $\beta$ (1-4)-Glc $\beta$ -ethyl-NH <sub>2</sub> ]	

65	NeuAca(2-3)-Galβ(1-4)-[Fuca(1-3)]-GlcNAcβ-propyl-NH <sub>2</sub>	
66	NeuAca(2-3)-Galβ(1-4)-[Fuca(1-4)]-GlcNAcβ(1-3)-Galβ(1-4)-[Fuca(1-3)]-GlcNAcβ-ethyl-NH <sub>2</sub>	
67	NeuAca(2-3)-Galβ(1-4)-[Fuca(1-3)]-GlcNAcβ(1-3)-Galβ(1-4)-[Fuca(1-3)]-GlcNAcβ-ethyl-NH <sub>2</sub>	
68	NeuAca(2-3)-Galβ(1-4)-[Fuca(1-3)]-GlcNAcβ(1-3)-Galβ(1-4)-[Fuca(1-3)]-GlcNAcβ(1-3)-Galβ(1-4)-[Fuca(1-3)]-GlcNAcβ-ethyl-NH <sub>2</sub>	
69	3' SLeX TrilN Core 1(2332)	
70	3' SLeX TrilN Core 3(2170)	
71	3' SLeX TrilN Core 4(3994)	
72		
73	NeuAc(2-6)-Galβ(1-4)-(6S)GlcNAcβ-ethyl-NH <sub>2</sub>	
74	NeuAca(2-6)-Galβ(1-4)-6-O-sulfo-GlcNAcβ-propyl-NH <sub>2</sub>	
75	NeuAca(2-6)-Galβ(1-4)-Glcβ-ethyl-NH <sub>2</sub>	
76	NeuAca(2-6)-Galβ(1-4)-GlcNAcβ-ethyl-NH <sub>2</sub>	
77	NeuAca(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ-ethyl-NH <sub>2</sub>	

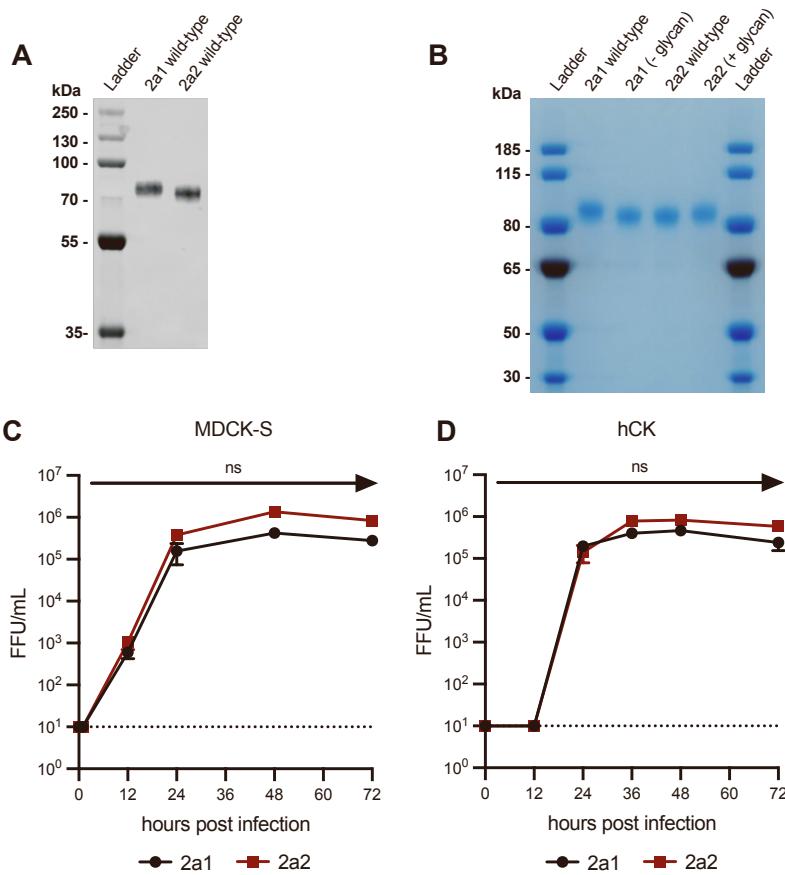
78	NeuAca(2-6)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
79	NeuAca(2-6)-GalNAc $\beta$ (1-4)-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
80	6' NeuAc LN Core 1 (1163)	
81	6' NeuAc DiLN Core 1 (1528)	
82	6' NeuAc TrILN Core 1 (1894)	
83	6' NeuAc TetraLN Core 1 (2259)	
84	6' NeuAc PentaLN Core 1 (2624)	
85	NeuAca(2-6)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-6)-[Gal $\beta$ (1-3)]-GalNAca-Thr-NH <sub>2</sub>	
86	NeuAca(2-6)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-3)-Gal $\beta$ (1-4)-GlcNAc $\beta$ (1-6)-[Gal $\beta$ (1-3)]-GalNAca-Thr-NH <sub>2</sub>	
87	6' NeuAc TrILN Core 2 (1894)	
88	6' NeuAc TetraLN Core 2 (2259)	
89	6' NeuAc PentaLN Core 2 (2624)	
90	6' NeuAc TetraLN TrILN Core 2 (3645)	

91	6' NeuAc PentaLN TetraLN Core 2 (4376)	
92	NeuAca(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-GalNAca-Thr-NH <sub>2</sub>	
93	NeuAca(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-3)-GalNAca-Thr-NH <sub>2</sub>	
94	6' NeuAc TriLN Core 3 (1732)	
95	6' NeuAc TetraLN Core 3 (2097)	
96	6' NeuAc PentaLN Core 3 (2462)	
97	NeuAca(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-[NeuAca(2-6)-Galβ(1-4)-GlcNAcβ(1-6)]-GalNAca-Thr-NH <sub>2</sub>	
98	NeuAca(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-3)-[NeuAca(2-6)-Galβ(1-4)-GlcNAcβ(1-6)]-GalNAca-Thr-NH <sub>2</sub>	
99	6' NeuAc TriLN Core4 (3118)	
100	6' NeuAc TetraLN Core4 (3848)	
101	6' NeuAc PentaLN Core4 (4579)	
102	6' NeuAc TetraLN Core6 (2097)	
103	6' NeuAc PentaLN Core6 (2462)	

104	6' NeuAc TriLN I-Antigen (2856)	
105	6' NeuAc DiLN I-Antigen (2104)	
106	Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAco(2-6)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ-Asn-NH <sub>2</sub>	
107	NeuAco(2-6)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ-Asn-NH <sub>2</sub>	
108	GlcNAcβ(1-2)-Manα(1-3)-[NeuAco(2-6)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ-Asn-NH <sub>2</sub>	
109	NeuAco(2-6)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAco(2-6)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH <sub>2</sub>	
110	NeuAco(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAco(2-6)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH <sub>2</sub>	
111	6' NeuAc DiLN Bi-(3594)	
112	NeuAco(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-3)-[NeuAco(2-6)-Galβ(1-4)-GlcNAcβ(1-3)-Galβ(1-4)-GlcNAcβ(1-2)-Manα(1-6)]-Manβ(1-4)-GlcNAcβ(1-4)-GlcNAcβ-Asn-NH <sub>2</sub>	
113	6' NeuAc TriLN Bi-(4324)	
114	6' NeuAc TetraLN Bi-(4828)	
115	6' NeuAc DiLN Bi-CF(3740)	
116	6' NeuAc TriLN Bi-CF(4470)	

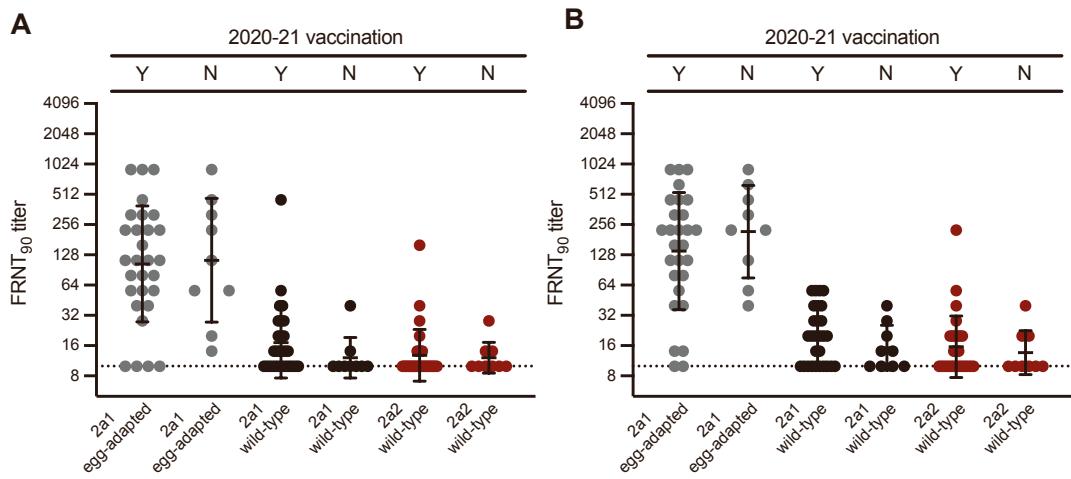
117	6' NeuAc TetraLN Bi-CF(5200)	
118	6' NeuAc DiLN Tri-(4615)	
119	6' NeuAc DiLN Tri-CF(4761)	
120	LN/6'SLN/6'SLN-TriN	
121	6'SLN/LeX/LeX-TriN	
122	6'SLNLN/LeX/LeX-TriN	

**Supplementary Table 1. List of glycan moieties used in this study.** Key for glycan moieties: purple diamond; sialic acid, yellow circle; galactose, green circle; mannose, blue square; N-acetylglucosamine, red triangle; fucose, yellow square; N-acetylgalactosamine.



**Figure S1. Characterization of 2a1 and 2a2 H3N2 viruses, Related to Figure 1.**

- a** Western blot analysis of HA proteins from wild-type 2a1 and 2a2 viruses.
- b** SDS-PAGE analysis of wild-type and mutant recombinant HA proteins. Mutant 2a1 HA proteins had N159 and I160 substitutions to remove glycosylation motif, and mutant 2a2 HA proteins had Y159 and T160 substitutions to add glycosylation motif.
- c,d** Infectious virus production following infection with virus at MOI of 0.0001 in MDCK-S (**c**) and hCK (**d**) cells incubated at 37°C. Virus titers in supernatant were measured by focus forming unit assay on MDCK-S cells. Statistical comparison of viruses was completed using a Welch's t-test on log<sub>10</sub>-transformed titers at each timepoint post infection (\*p≤0.05).



**Figure S2. Prior year vaccination does not affect antibody magnitude, Related to Figure 2.**

Pre-vaccination (**a**) and post-vaccination (**b**) neutralization titers stratified by previous year (2020-21) vaccination status. Two vaccine recipients from Figure 2 had unknown vaccination status in 2020-21, and so were excluded from this analysis.