Supplementary Table 1. H3N2 components of WHO-recommended influenza vaccines for influenza seasons between 2008 and 2020.

SEASON	STRAIN NAME	REFERENCE
2008-2010	A/Brisbane/10/2007	https://www.who.int/influenza/vaccines/200809Recommendation.pdf
2010-2011	A/Perth/16/2009 (NIB-64)	https://www.who.int/influenza/vaccines/virus/recommendations/201002_Recommendation.pdf
2011-2012		https://www.who.int/influenza/vaccines/2011_02_recommendation.pdf
2012-2013	A/Victoria/361/2011 (X-217, IVR-165)	https://www.who.int/influenza/vaccines/virus/recommendations/201202_recommendation.pdf
2013-2014		https://www.who.int/influenza/vaccines/virus/recommendations/201302_recommendation.pdf
2014-2015	A/Texas/50/2012 (X-223)	https://www.who.int/influenza/vaccines/virus/recommendations/201402_recommendation.pdf
2015-2016	A/Switzerland/9715293/2013 (NIB-88, X-247, IVR-176)	https://www.who.int/influenza/vaccines/virus/recommendations/201502_recommendation.pdf
2016-2017	A/Hong Kong/4801/2014 (X-263)	https://www.who.int/influenza/vaccines/virus/recommendations/201602_recommendation.pdf
2017-2018		https://www.who.int/influenza/vaccines/virus/recommendations/201703_recommendation.pdf
2018-2019	A/Singapore/INFIMH-16-0019/2016 (IVR-186, NYMC-X-307, NIB-104)	https://www.who.int/influenza/vaccines/virus/recommendations/201802_recommendation.pdf
2019-2020 (Northern hemisphere)	A/Kansas/14/2017 (X-327, IVR-195)	https://cdn.who.int/media/docs/default-source/influenza/cvvs/cvv-northern-hemisphere-2019-2020/summary-a-h3n2-cvv-egg-nh1920.pdf
2019 (Southern hemisphere)	A/Switzerland/8060/2017 (NIB-112)	https://www.who.int/influenza/vaccines/virus/recommendations/201809_recommendation.pdf
2020-2021	A/Hong Kong/2671/2019 (NIB-121)	https://www.who.int/influenza/vaccines/virus/recommendations/202002_recommendation.pdf

Supplementary Table 2. H3N2 genes that were used in this study.

PARENTAL VIRUS	STRAIN NAME	CLADE	GENE	EGG-ADAPTIVE MUTATION	GISAID ACCESSION NO.
A/Kansas/14/2017	X-327	3C.3a1	HA	G186V, D190N, S219Y	EPI1490170
			NA	-	EPI1504534
A/Switzerland/8060/2017	NIB-112	3C.2a2	HA	T160K, L194P	EPI1313524
			NA	-	EPI1313525
A/Singapore/INFIMH-16-0019/2016	WT	3C.2a1	HA	-	EPI1381186
			NA	-	EPI1381185
A/Italy/11871/2020	WT	3C.2a1b.1a	HA	-	EPI1735610
			NA	-	EPI1735609
A/Victoria/22/2020	WT	3C.2a1b.1a	HA	-	EPI1721444
			NA	-	EPI1721443

‘-‘: no egg-adaptive mutation.

Supplementary Table 3. Frequencies of mutations in egg-passaged viruses that showed an average frequency of >10% in the fifth passage.

INOCULUM	MUTATION	BIOLOGICAL REPLICATE	FREQUENCY DURING EGG-PASSAGING				
			E1	E2	E3	E4	E5
Kansas17 V186/L194/N190/Y219 (X-327)	-	Rep1	-	-	-	-	-
		Rep2	-	-	-	-	-
		Rep3	-	-	-	-	-
Switz17 K160/G186/P194	T203I	Rep1	0.01	0.62	0.97	0.98	0.98
		Rep2	0.06	0.14	0.32	0.53	0.85
		Rep3	0.08	0.13	0.23	0.57	0.75
Sing16 K160/G186/P194	I140K	Rep1	0.00	0.00	0.00	0.00	0.00
		Rep2	0.00	0.03	0.49	0.48	0.73
		Rep3	0.00	0.00	0.00	0.00	0.00
	T203I	Rep1	0.01	0.01	0.07	0.21	0.27
		Rep2	0.45	0.16	0.43	0.49	0.24
		Rep3	0.01	0.01	0.02	0.57	0.90
Kansas17 X-327 V186G/L194P/N190D	I140K	Rep1	0.00	0.00	0.00	0.02	0.06
		Rep2	0.15	0.44	0.71	0.82	0.87
		Rep3	0.02	0.05	0.14	0.19	0.28
	T203I	Rep1	0.37	0.70	0.88	0.86	0.93
		Rep2	0.00	0.01	0.01	0.01	0.00
		Rep3	0.03	0.07	0.17	0.22	0.21
	D225N	Rep1	0.00	0.00	0.02	0.10	0.28
		Rep2	0.01	0.02	0.02	0.01	0.01
		Rep3	0.02	0.10	0.25	0.34	0.39

Continued

INOCULUM	MUTATION	BIOLOGICAL REPLICATE	FREQUENCY DURING EGG-PASSAGING				
			E1	E2	E3	E4	E5
Switz17 K160/V186/L194	-	Rep1	-	N/A	N/A	N/A	N/A
		Rep2	-	N/A	N/A	N/A	N/A
		Rep3	-	N/A	N/A	N/A	N/A
Sing16 K160/V186/L194	H156R	Rep1	0.00	0.00	N/A	N/A	N/A
		Rep2	0.00	0.98	N/A	N/A	N/A
		Rep3	0.00	0.02	N/A	N/A	N/A
	D190N	Rep1	0.00	0.00	N/A	N/A	N/A
		Rep2	0.00	0.00	N/A	N/A	N/A
		Rep3	0.00	0.94	N/A	N/A	N/A
	D225N	Rep1	0.12	0.45	N/A	N/A	N/A
		Rep2	0.00	0.00	N/A	N/A	N/A
		Rep3	0.40	0.98	N/A	N/A	N/A

‘-’: no mutation found. N/A: copy number of viral RNA was insufficient for deep sequencing analysis.

Supplementary Table 4. Information of HA from human H3N2 strains with or without egg-passaging.

VIRUS NAME	NON-EGG-PASSAGED		EGG-PASSAGED		MUTATION IN EGG-PASSAGED					
	PASSAGE HISTORY	HA ACCESSION NO.	PASSAGE HISTORY	HA ACCESSION NO.	160	186	190	194	219	225
A/Bangladesh/3005/2020	Original	EPI1838357	E3	EPI1844062	I	N	N	L	S	G
			E3+E6	EPI1857491	I	N	N	L	S	G
A/Bangladesh/3011/2020	Original	EPI1838349	E3	EPI1844060	I	N	N	L	S	G
A/Bangladesh/4002/2020	Original	EPI1838416	E3	EPI1844058	I	N	N	L	S	G
A/Bangladesh/911009/2020	Original	EPI1838408	E3+E6	EPI1857489	I	N	N	L	S	G
A/Beijing-Daxin/33/2020	C2	EPI1753671	E7	EPI1753663	K	D	N	L	S	D
A/Beijing-Miyun/51/2020	C1	EPI1753655	E7+E2	EPI1805765	A	D	N	L	Y	G
			E9	EPI1801276	I	D	N	L	F	G
A/Beijing-Miyun/53/2020	C1	EPI1753639	E5+E2	EPI1806767	I	D	N	L	F	G
A/Beijing-Miyun/54/2020	C1	EPI1753623	E4	EPI1753615	T	V	N	L	S	G
A/Belgium/G0023/2019	SIAT1	EPI1436935	E3	EPI1592043	K	G	D	L	S	G
A/Brisbane/148/2019	SIAT1+SIAT1	EPI1658656	E5	EPI1740888	K	V	D	L	Y	D
A/Brunei/39/2020	SIAT1	EPI1797893	E5	EPI1804363	K	D	N	L	S	D
A/California/194/2019	Original	EPI1630686	E5	EPI1713739	K	V	D	L	S	D
A/Cambodia/e0826360/2020	Original	EPI1837753	E5+E1+E8	EPI1877927	K	R	D	L	F	D
A/Canberra/407/2019	SIAT1	EPI1671923	E4	EPI1713737	K	G	D	P	S	D
A/Christchurch/515/2019	SIAT2	EPI1484393	E3+E2	.2a1b.2b	T	G	D	P	F	D
A/Christchurch/516/2019	SIAT1	EPI1484453	E3	EPI1491158	K	V	D	L	F	D
A/Darwin/1/2021	Original	EPI1851811	E8	EPI1859990	K	S	D	L	S	G
A/Darwin/11/2021	Original	EPI1859986	E3	EPI1859998	I	D	N	L	S	D
A/Darwin/17/2021	SIAT1	EPI1888120	E3	EPI1924410	I	V	N	L	S	D
A/Darwin/2/2021	Original	EPI1851819	E3	EPI1859970	K	R	D	L	S	D
A/Darwin/22/2021	SIAT1	EPI1888104	E3	EPI1923164	I	N	N	L	S	G

Continued

VIRUS NAME	NON-EGG-PASSAGED		EGG-PASSAGED		MUTATION IN EGG-PASSAGED					
	PASSAGE HISTORY	HA ACCESSION NO.	PASSAGE HISTORY	HA ACCESSION NO.	160	186	190	194	219	225
A/Darwin/24/2021	Original	EPI1888096	E3	EPI1923174	I	N	N	L	S	G
A/Darwin/402/2019	SIAT1	EPI1508629	E3	EPI1584616	T	G	D	P	F	N
A/Darwin/6/2021	Original	EPI1857216	E3+E7+E1	EPI1885098	I	N	N	L	S	G
			E5+E2	EPI1925255	I	D	N	L	F	G
A/Darwin/726/2019	SIAT1	EPI1658695	E6	EPI1675460	A	V	E	L	S	D
A/Darwin/9/2021	SIAT1	EPI1883349	E4	EPI1888006	I	N	N	L	S	G
A/Delaware/01/2021	Original	EPI1869534	E2	EPI1940656	I	D	N	L	S	G
A/Finland/183/2020	SIAT1	EPI1753460	E6	EPI1847912	K	S	D	L	S	D
A/Hong Kong/2671/2019	MDCK1	EPI1543098	E9	EPI1843071	I	V	D	L	F	N
A/Kansas/14/2017	SIAT2	EPI1504535	E17	EPI1415371	K	V	N	L	Y	D
A/KANAGAWA/ZC1841/2019	SIAT1	EPI1478189	E7	EPI1696421	K	V	D	L	S	D
A/Michigan/173/2020	Original	EPI1843859	E4+E2	EPI1922037	I	N	N	L	S	G
A/Netherlands/00007/2021	Original	EPI1885138	E3	EPI1924781	I	N	N	L	S	G
A/Newcastle/42/2019	SIAT1	EPI1430423	E3	EPI1444940	K	V	D	L	F	D
A/Newcastle/623/2019	SIAT1	EPI1430438	E2	EPI1444941	K	G	D	L	S	G
A/Norway/16606/2021	SIAT1	EPI1922181	E3	EPI1940648	I	N	N	L	S	G
A/Norway/2279/2019	SIAT1	EPI1619464	E4	EPI1719268	K	G	D	P	S	D
A/Oregon/28/2019	Original	EPI1627961	E6	EPI1713733	K	V	D	L	S	D
A/Paris/2554/2019	Original	EPI1638885	E4+E7	EPI1794629	I	D	N	L	S	G
A/Pennsylvania/01/2021	Original	EPI1858654	E5	EPI1924787	I	G	N	L	S	G
			E4	EPI1924789	I	D	N	L	S	G

Continued

VIRUS NAME	NON-EGG-PASSAGED		EGG-PASSAGED		MUTATION IN EGG-PASSAGED					
	PASSAGE HISTORY	HA ACCESSION NO.	PASSAGE HISTORY	HA ACCESSION NO.	160	186	190	194	219	225
A/Pennsylvania/1025/2019	Original	EPI1630907	E3+E1	EPI1796140	I	V	D	L	S	N
			E3+D8+E1	EPI1804937	I	V	E	L	S	D
			E3	EPI1713729	I	V	D	L	S	D
A/Pennsylvania/1026/2019	Original	EPI1631152	E5+E8	EPI1794631	K	V	D	L	Y	D
			E5+E2+E9	EPI1843569	K	V	D	L	Y	D
A/Perth/20/2020	MDCK1+SIAT1	EPI1733852	E3+E2+E9	EPI1848094	K	D	N	L	Y	G
			E3	EPI1740884	K	D	N	L	Y	D
			E3+E7	EPI1794633	K	D	N	L	Y	N
A/Saitama/92/2020	MDCK1+hMDCK1	EPI1847848	E4	EPI1847862	K	R	D	L	S	D
A/Singapore/INFKK0001/2021	Original	EPI1883806	E4	EPI1889199	I	N	N	L	S	G
A/Singapore/INFKK0002/2021	Original	EPI1883814	E4	EPI1924397	I	N	N	L	S	G
A/Singapore/KK0001/2020	Original	EPI1750786	E3	EPI1804361	K	D	D	L	S	D
A/South Africa/R06421/2019	MDCK1+SIAT1	EPI1582733	E4	EPI1694137	K	V	D	L	F	D
A/South Australia/2/2019	SIAT1	EPI1387412	E4+E2	EPI1698481	I	D	D	L	S	D
A/South Australia/320/2019	SIAT1	EPI1484436	E4	EPI1526498	T	G	D	P	F	D
A/South Australia/34/2019	SIAT1	EPI1387331	E5	EPI1703041	K	I	D	L	F	D
A/South Australia/36/2019	SIAT1	EPI1387334	E4	EPI1440496	T	G	D	P	F	D
A/South Australia/39/2019	SIAT1	EPI1387337	E4	EPI1440498	K	G	D	P	S	D
A/South Australia/4/2019	SIAT1	EPI1371913	E4+E2	EPI1698473	K	D	D	L	S	D
A/Sydney/53/2019	MDCK-SIAT1+SIAT1	EPI1430420	E3+E2	EPI1588472	K	V	D	L	F	D
A/Tasmania/503/2020	SIAT1	EPI1752480	E5	EPI1848147	K	R	D	L	S	D
			EX	EPI1868371	K	R	E	L	F	D
			EX	EPI1868373	K	R	D	L	F	D

Continued

VIRUS NAME	NON-EGG-PASSAGED		EGG-PASSAGED		MUTATION IN EGG-PASSAGED					
	PASSAGE HISTORY	HA ACCESSION NO.	PASSAGE HISTORY	HA ACCESSION NO.	160	186	190	194	219	225
A/Vermont/11/2019	Original	EPI1428265	E2	EPI1439232	K	G	N	L	S	D
A/Vermont/14/2019	Original	EPI1428013	E2	EPI1439224	K	G	N	L	S	D
A/Vermont/25/2019	Original	EPI1618933	E3	EPI1713735	K	V	D	L	S	D
A/Victoria/223/2019	SIAT1	EPI1584570	E3	EPI1610406	T	G	D	P	F	D
A/Victoria/703/2019	SIAT1	EPI1430441	E2	EPI1444936	K	G	D	L	S	G

For passage history, ‘C’: passaged in cells. ‘E’: passaged in eggs. The passage number is indicated as the suffix. ‘X’ indicates missing information. Egg-adaptive mutations are highlighted in yellow.