Supplementary Materials

Erythrocyte elution assay

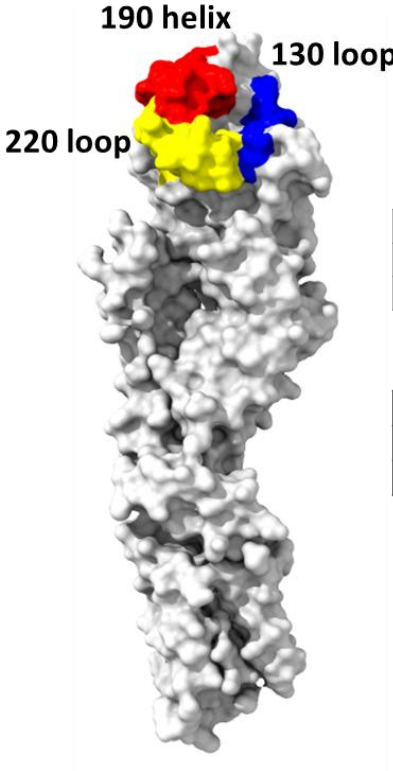
The erythrocyte elution assay was performed as previously described[1]. In brief, 50 µL of virus was serially diluted in PBS in 96-well V-bottom plates and mixed with 1% chicken or guinea pig RBCs. The plate was incubated at 4 °C for 1 h, and the HA titre was recorded. Subsequently, the plate was transferred to a 37 °C incubator for 6 h, with the HA titre recorded hourly.

Stable cell line production

Pseudotyped lentiviruses expressing the Sda epitope (which modifies α2,3-linked SA to become inaccessible to bind HA), were produced by transfecting HEK 293T cells in a six well plate with lipofectamine 3000 (Invitrogen), 0.4µg of HIV packaging plasmid (pGAG-POL), 0.4µg VSV-G, and 0.4µg B4GALNT2 containing plasmid, (which catalyses the last step of the Sda antigen synthesis; provided by Professor Nicholas S. Heaton)[2]. Supernatant with pseudoviruses was collected after 48hrs and filtered through a sterile 0.45-µm Millex®-HA filter unit (Millipore, SLHA033SB).

Pseudoviruses were diluted 1:10 in DMEM with 10% FBS, 1% pen-strep, 1% NEAA and added to each well. Cells were treated with 4µg/ml of puromycin (selection antibiotic) at 48hrs and 96hrs post transfection. Cells were then treated with 0.05% Trypsin-EDTA (1X) (25300-054) and seeded into 25cm^2 cell culture flask (Grenier, 690175) with 1µg/ml of puromycin. Stable cell lines were validated by immunofluorescence in duplicate (Supplemental Fig S9).

Cells were stained as described previously[3]. In brief, cells were seeded in a twelve well plate. After 24hrs, media was removed from the cells. Cells were fixed with 200µL 4% paraformaldehyde in PBS for 30min, and permeabilized for 10min with 200µL 1% Triton X in PBS. Cells were washed with 1X PBS and stained with 20ug/mL lectins conjugated to fluorescein (FITC) in 1X OptiMEM, either Maackia amurensis lectin I (MALI; binds α2,3-linked SA; Vector Laboratories, FL-1311-2), Sambucus nigra lectin (SNA; preferentially binds α2,6-linked SA; Vector Laboratories, FL-1301-2), or Dolichos biflorus lectin (DBA; binds the Sda epitope, Sigma-Aldrich). Cells were incubated with the lectins for 1hr at 4°C, then washed once with 200µl OptiMEM. The DBA lectin was conjugated to biotin, so after 1hr these wells were washed and stained with streptavidin-conjugated Alexa Fluor™ 488nm (Invitrogen, S11223) for 1hr. Cells were stored in OptiMEM and visualised with an EVOS M3000 Imaging Auto fluorescent microscope.

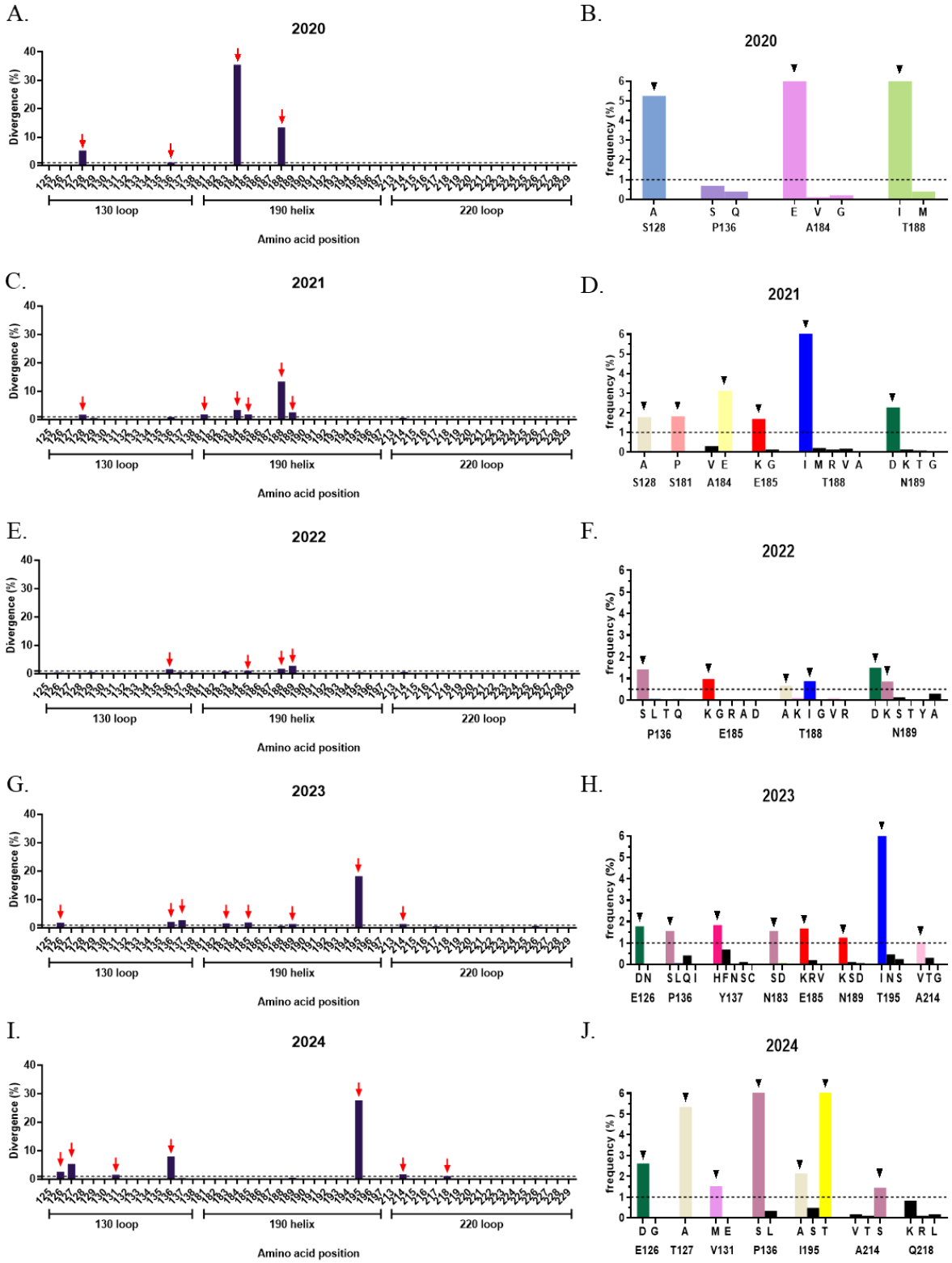


130 loop (amino acid positions)													
125	126	127	128	129	130	131	132	133	134	135	136	137	138
H	E	T	S	L	G	V	S	A	A	C	P	Y	Q

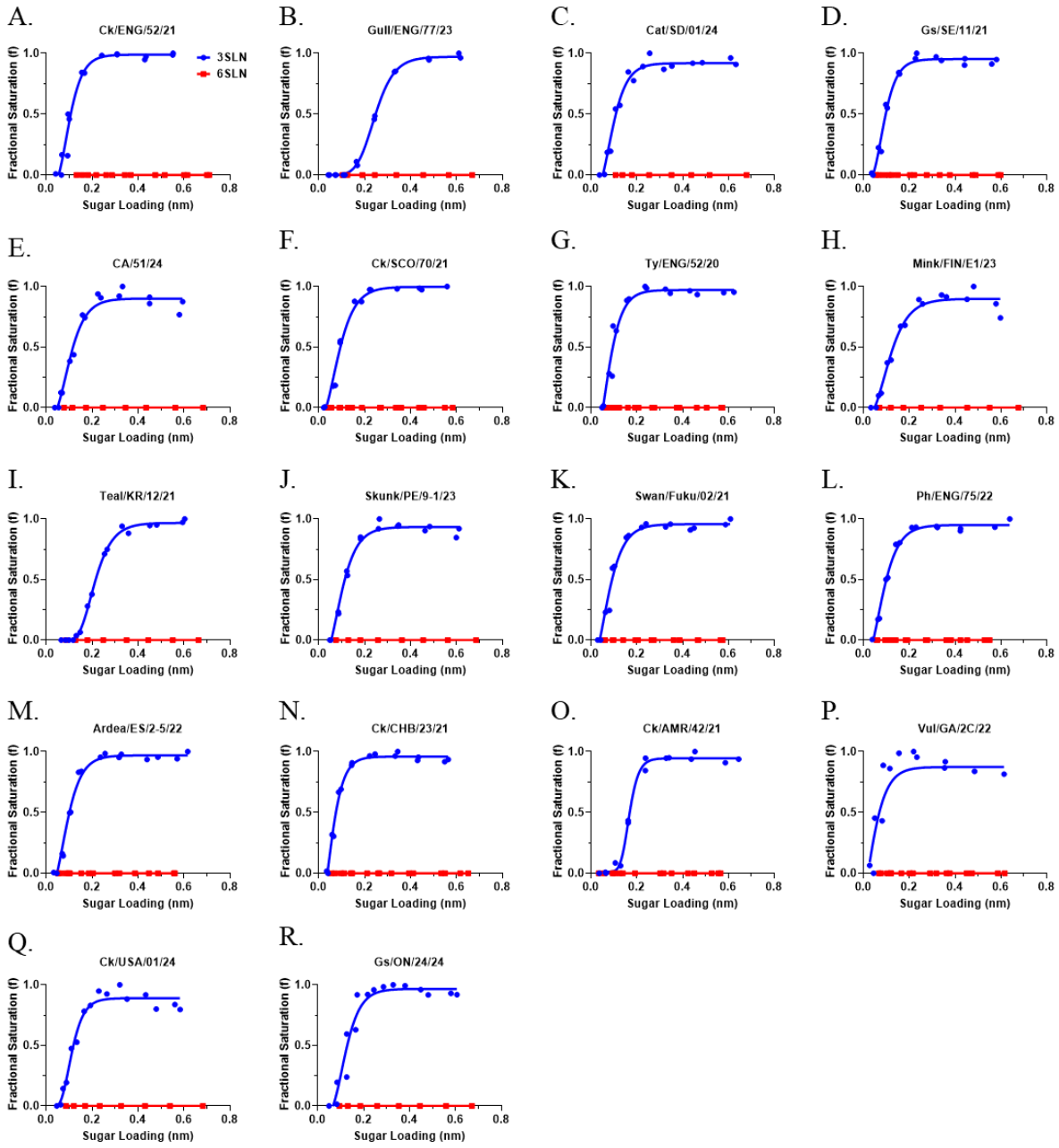
190 helix (amino acid positions)															
181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197
S	N	N	A	E	E	Q	T	N	L	Y	K	N	P	T	T	Y

220 loop (amino acid positions)																
213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229
I	A	T	R	S	Q	V	N	G	Q	R	G	R	M	D	F	F

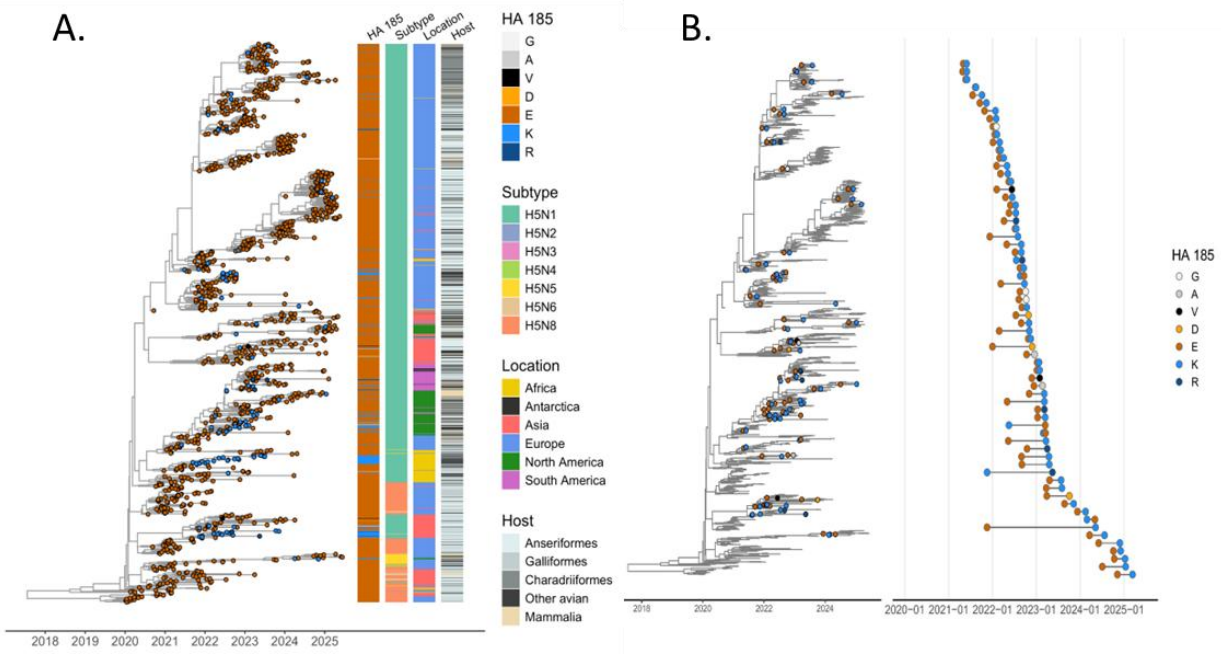
Supplementary Figure S1. Conserved amino acid residues at the three domains of clade 2.3.4.4b HA. The three receptor binding domains, 130 loop, 190 helix, and 220 loop are highlighted in blue, red, and yellow, respectively. The conserved amino acid residues of the three domains are indicated in the tables next to the HA structure. Based on the available clade 2.3.4.4b H5Nx HA sequences from GISAID, the consensus sequences of these regions from 2020 to 2024 were HETSLGVSAACPYQ for the 130-loop, SNNAEEQTNLYKNPTTY for the 190-helix, and IATRSQVNGQRGRMDFF for the 220-loop.H5 HA crystal structure was acquired from Protein Data Bank (https://www.rcsb.org/) with accession number 4JUL.



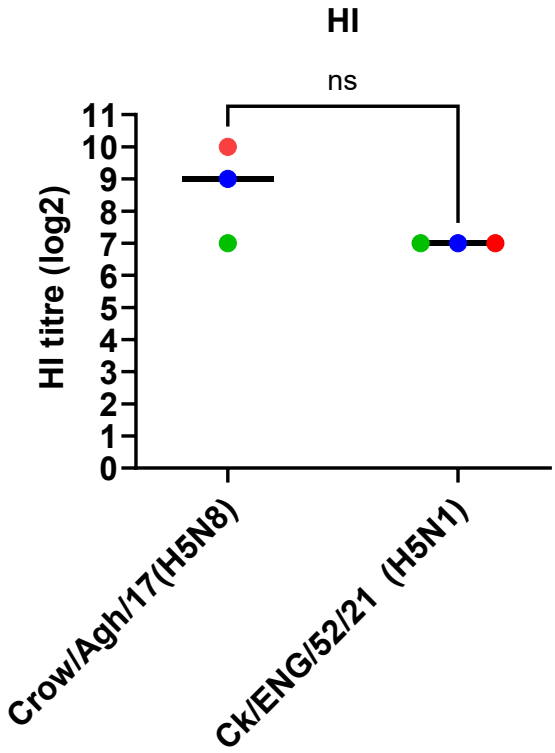
Supplementary Figure S2. Identification of less conserved amino acid residues from 2020-2024. Setting divergence level at 1% as threshold, the less conserved amino acid positions within the three domains of year (A) 2020, (C) 2021, (E) 2022, (G) 2023, and (I) 2024, are identified and indicated with red arrows. The identified amino acid mutations of the less conserved amino acid positions of year (B) 2020, (D) 2021, (F) 2022, (H) 2023, and (J) 2024 are then identified and indicated with black arrows. 1,011, 2,381, 7,438, 3,612, and 2,917 HA sequences from 2020, 2021, 2022, 2023 and 2024 were analysed, respectively.



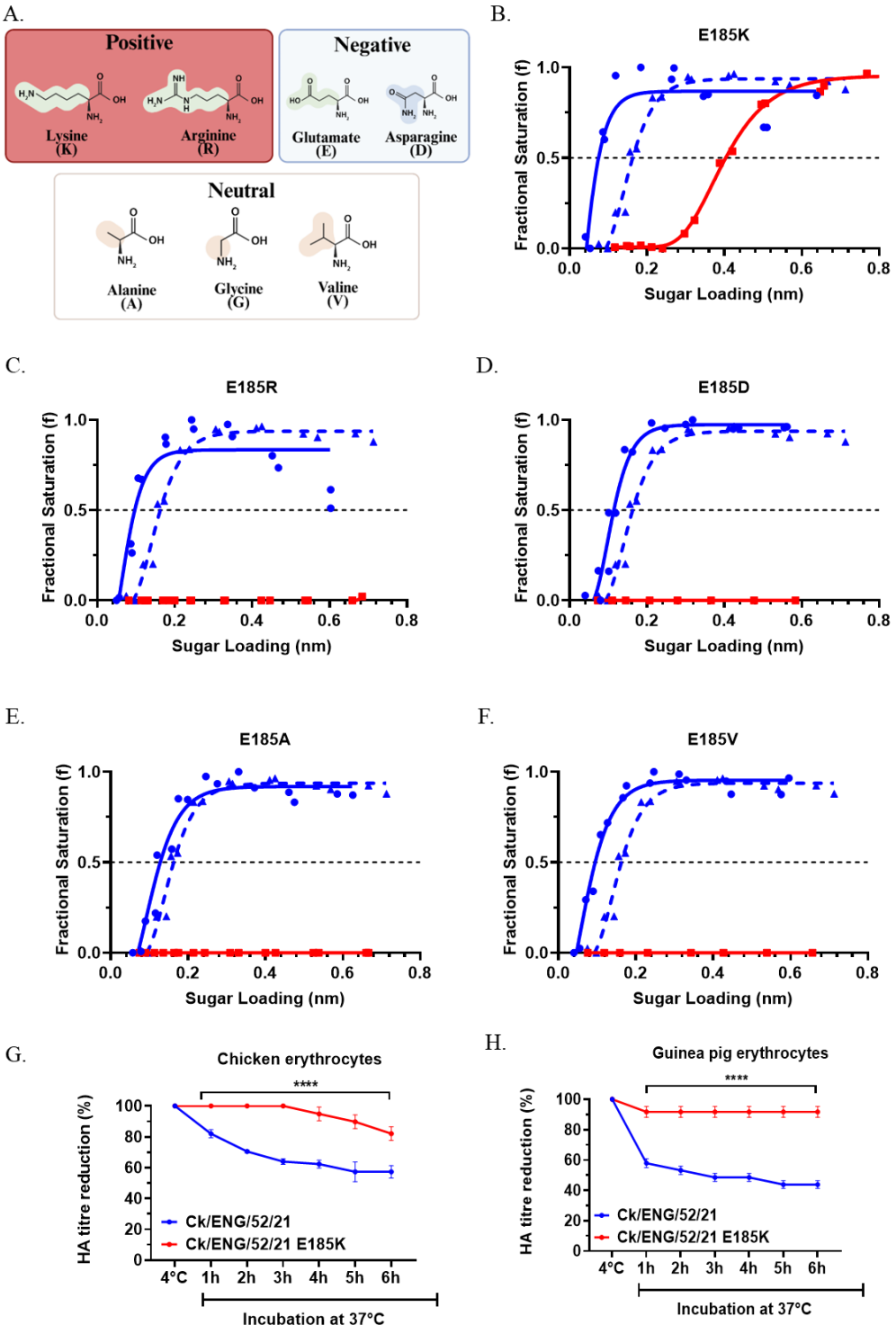
Supplementary Figure S3. Receptor binding profiles of H5Nx viruses. BLI was used to assess the receptor binding of the H5Nx viruses with avian-like receptor analogue 3SLN (blue) and human-like receptor analogue 6SLN (red). Data points represent normalized virus binding across eight concentrations of each sugar analogue, with two replicates per concentration. Curves were fitted using an asymmetric five-parameter logistic (5PL) model. Panels (A to R) correspond to wild-type–like viruses.



Supplementary Figure S4. Phylodynamics of HA from clade 2.3.4.4b H5Nx viruses. (A) Time resolved HA phylogeny generated from stratified sample of 1,144 sequences. Tips are coloured by amino acid at position 185 and alongside coloured bars also indicate subtype, sampling location and host category. (B) To the left, branches of the phylogeny in **A** to which substitutions at position 185 are highlighted with nodes at each end of such branches coloured by amino acid. To the right, these branches are extracted from the tree and replotted evenly distributed on the y-axis.

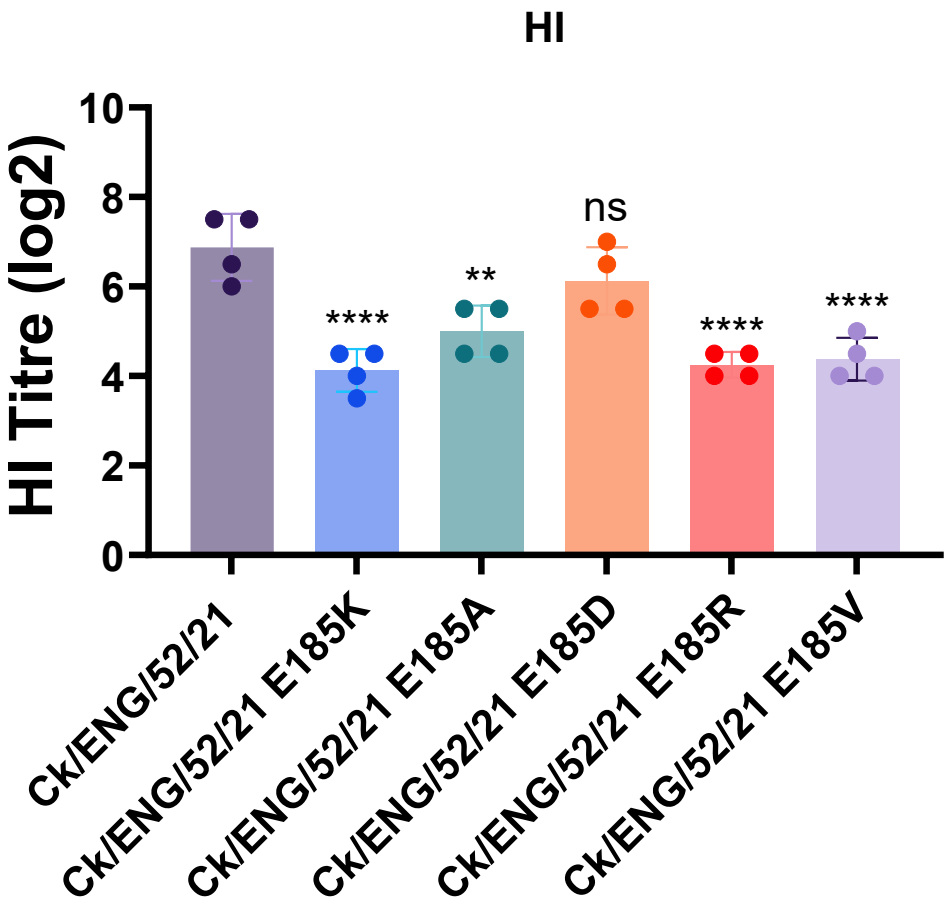


Supplementary Figure S5. Haemagglutination inhibition (HI) titres comparison between chicken Crow/Agh/17 (H5N8) post-vaccination antiserum and duck Ck/ENG/52/21 (H5N1) post-infection antiserum. Data points represent three biological replicates. Statistical significance was assessed by *t*-test. HI titres are shown as $\log_2$ fold values. Statistical significance was assessed by t test indicated by asterisks: ns, not significant 0.05>P; *, 0.05 ≥ P > 0.01; **, 0.01 ≥ P > 0.001; ***, 0.001 ≥ P > 0.0001; ****, P ≤ 0.0001; ****, P ≤ 0.0001.

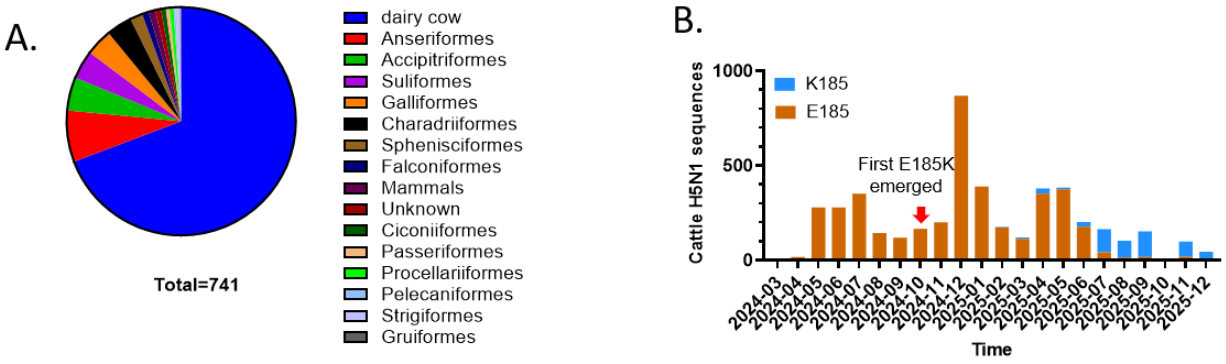


Supplementary Figure S6. Determination of receptor-binding avidity of HA E185X viruses. Bio-layer interferometry (BLI) was used to assess receptor binding of mutant viruses to avian-like receptor sugar analogue 3SLN (blue) and human-like receptor sugar analogue 6SLN (red). (A) Amino acid charge properties of natural variants at position 185, including positively charged lysine (K) and arginine (R); negatively charged glutamate (E) and aspartate (D); and neutral alanine (A), glycine (G), and valine (V). Data points represent normalized virus binding across eight sugar analogue concentrations, with two replicates per concentration. Curves

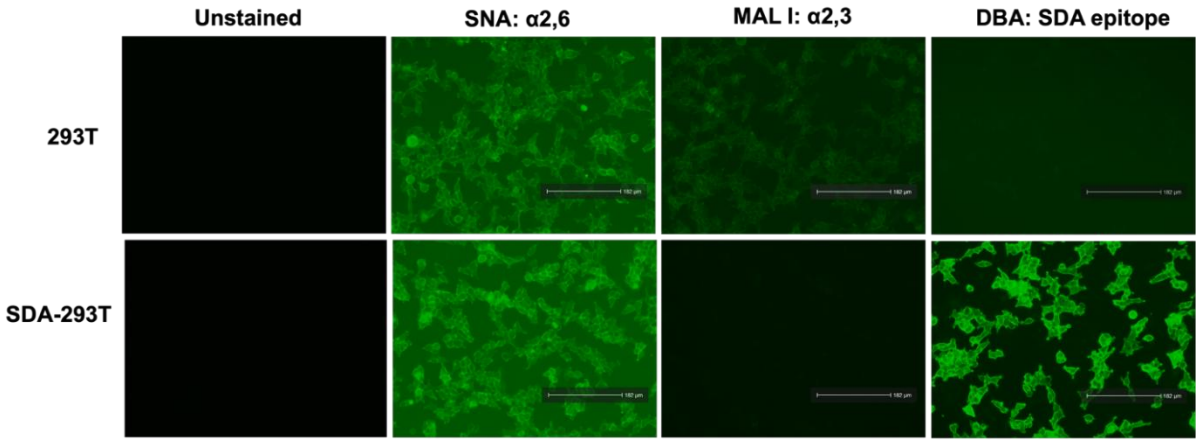
were fitted using an asymmetric five-parameter logistic (5PL) model. Solid lines indicate E185X viruses, and dotted lines indicate E185 (Ck/ENG/52/21). (B–F): Receptor binding avidity of mutant viruses: (B) E185K; (C) E185R; (D) E185D; (E) E185A; (F) E185V. (G, H): Elution profiles of the E185K mutant virus from erythrocytes at 37 ° C using (G) chicken erythrocytes and (H) guinea pig erythrocytes. Elution assays were repeated three times independently. Statistical significance was assessed by two-way ANOVA and indicated by asterisks: ns, not significant ($P > 0.05$); *, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $0.001 \geq P > 0.0001$; ****, $P \leq 0.0001$.



Supplementary Figure S7. The antigenicity of E185X mutant viruses. HI assay was performed using chicken post-vaccination antiserum raised against Ck/ENG/52/21 (H5N1) post-infection duck antisera using serum samples from four biological replicates. Data points indicate biological replicates. The mutations were made based on de-engineered Ck/ENG/52/21 virus. Statistical significance was assessed by one-way ANOVA indicated by asterisks: ns, not significant 0.05>P; *, $0.05 \geq P > 0.01$; **, $0.01 \geq P > 0.001$; ***, $0.001 \geq P > 0.0001$; ****, $P \leq 0.0001$; ****, $P \leq 0.0001$.



Supplementary Figure S8. HA E185K mutation in different host species. (A) Host distribution of clade 2.3.4.4b H5Nx isolates carrying lysine (K) at HA position 185 (N = 741) based on GISAID data. All available HA sequences from this clade in GISAID collected between 1 January 2020 and 31 December 2025 were analysed. A total of 2.1% of all 2.3.4.4b sequences, (741/34495) possessed lysine (K) at HA position 185. (B) Distribution of HA E185 (glutamic acid) and K185 (lysine) among available cattle H5N1 isolates. K185 was enriched in cattle sequences where 10.8% (513/4753) contained 185K. The earliest cattle sequence with HA E185K is from October 2024. After a brief absence, E185K-positive H5N1 viruses re-emerged in February 2025 and became increasingly dominant from July 2025 onwards.



Supplementary Figure S9. SDA-293T cells expressing SDA with limited α2,3 expression. SDA expressing cells were generated by transducing 293Ts with lentivirus containing the B4GALNT2 gene. Cells were fixed with 4% PBS in 1X PBS and permeabilized with 1% Triton X in PBS. Cells were stained as with either OptiMEM only or 20ug/mL SNA, MAL I, or DBA lectins. DBA-stained wells were then washed with PBS and stained with Alexa Fluor™ 488nm. Results were visualised with the EVOS M3000 Imaging System at 20X objective. Immunofluorescence was run in duplicate.

Table S1. Metadata details of the de-engineered H5Nx viruses used in this study.

Isolate Name	GISAID Isolate ID/NCBI	HA Segment ID	NA Segment ID	Submitting_Lab	Originating_Lab	Authors
A/turkey/England/039352/2020 (H5N8)	EPI_ISL_766053	EPI1839510	EPI1839512	Animal and Plant Health Agency (APHA)	Animal and Plant Health Agency (APHA)	Byrne, A.
A/Herring_Gull/England/123277/2023 (H5N5)	EPI_ISL_18378733	EPI2773672	EPI2773671	Animal and Plant Health Agency (APHA)	Animal and Plant Health Agency (APHA)	Byrne, A.
A/pheasant/England/331075/2022 (H5N1)	EPI_ISL_16384125	EPI2277809	EPI2277808	Animal and Plant Health Agency (APHA)	Animal and Plant Health Agency (APHA)	Byrne, A.
A/long-tailed_skua/Scotland/22/2022 (H5N1)	EPI_ISL_14864652	EPI2158728	EPI2158727	Animal and Plant Health Agency (APHA)	Animal and Plant Health Agency (APHA)	Byrne, A.
A/Ardea_cinerea/Spain/863-1_22VIR6312-5/2022 (H5N1)	EPI_ISL_13990743	EPI2102625	EPI2102624	Istituto Zooprofilattico Sperimentale delle Venezie	Laboratorio Central de Veterinaria	Ruano, M.J.; Rocha, A.; Sanchez, A.; Aguero, M; Barbierato, G.; Zecchin, B.; Fusaro, A.; Schivo, A.; Salviato, A.; Palumbo, E.; Giussani, E.; Monne, I.; Terregino, C.
A/Striped_Skunk/PE/FAV-0089-1/2023 (H5N1)	NCBI	PV319248.1	PV319250.1	Canadian Food Inspection Agency	Atlantic Veterinary College, University of Prince Edward Island	Signore, A.; Erdelyan, C.; Hisanaga, T.; Ayilara, I.; Pama, L.; Fisher, M.; Kruczkiewicz, P.; Sullivan, D.; Lung, O.; Berhane, Y.
A/white mink/Finland/UH-357-E1/2023 (H5N1)	EPI_ISL_18771244	EPI2917759	EPI2917761	Finnish Institute for Health and Welfare, THL	University of Helsinki, Department of Virology	Altan, E.; Avelin, V.; Lindh, E.; Savolainen-Kopra, C.; Sironen, T.; Österlund, P.
A/cat/SD/24-038917-001-original/2024 (H5N1)	NCBI	PV160247.1	PV214577.1	National Veterinary Services Laboratories, USDA Animal Plant Health Inspection Service-National Veterinary Services Laboratories	National Veterinary Services Laboratories, USDA Animal Plant Health Inspection Service-National Veterinary Services Laboratories	Norris,C.; Tod,S.; Jessica,H.; Matthew,A.; Kerrie,F.; Mary,K.; Steven,L.; and Kris,L.
A/Canada Goose/ON/FAV-0056-4-2024/2024(H5N1)	NCBI	PV306512.1	PV306514.1	National Centre for Foreign Animal Disease, Canadian Food Inspection Agency	National Centre for Foreign Animal Disease, Canadian Food Inspection Agency	Signore,A.V.; Erdelyan,C.; Hisanaga,T.; Ayilara,I.; Pama,L.; Fisher,M.; Kruczkiewicz,P.; Sullivan,D.; Lung,O.; and Berhane,Y.
A/black vulture/Georgia/W22-722C/2022 (H5N1)	NCBI	OQ584606.1	OQ584607.1	CEIRR Influenza Data Processing and Communication Center, St. Jude Center of Excellence for Influenza Research and Response (CEIRR)	CEIRR Influenza Data Processing and Communication Center, St. Jude Center of Excellence for Influenza Research and Response (CEIRR)	Poulson,R.; Stallknecht,D.; Rubrum,A.; Jeevan,T.; Walker,D.; Webby,R.
A/chicken/Aomori/TU9-4142/2021 (H5N1)	NCBI	LC699176.1	LC699178.1	Kosuke Soda Tottori University, Avian Zoonosis Research Center, Faculty of Agriculture	Kosuke Soda Tottori University, Avian Zoonosis Research Center, Faculty of Agriculture	Soda,K.; Usui,T.; Ito,H.; Yamaguchi,T.; and Ito,T.
A/chicken/USA/24-031166-001/2024 (H5N1)	NCBI	PQ687319.1	PQ687321.1	National Veterinary Services Laboratories, USDA Animal Plant Health Inspection Service-National Veterinary Services Laboratories	National Veterinary Services Laboratories, USDA Animal Plant Health Inspection Service-National Veterinary Services Laboratories	Aufderhar,M.; Franzen,K.; Killian,M.; Lantz,K.; Stuber,T.; Hicks,J.; Norris,C.; and Lakin,S.
A/chicken/Chiba/T50-23/2021 (H5N8)	NCBI	LC633395	LC633397	Kosuke Soda Tottori University, Avian Zoonosis Research Center, Faculty of Agriculture	Kosuke Soda Tottori University, Avian Zoonosis Research Center, Faculty of Agriculture	Soda,K.; Usui,T.; Ito,H.; Yamaguchi,T.; and Ito,T
A/California/151/2024(H5N1)	NCBI	PQ615353.1	PQ615347.1	WHO Collaborating Center for Surveillance, Epidemiology and Control of Influenza, Influenza Division, Centers for Disease Control and Prevention	WHO Collaborating Center for Surveillance, Epidemiology and Control of Influenza, Influenza Division, Centers for Disease Control and Prevention	Kirby,M.K.; Steel,J.; Garten,R.; Davis,T.; Dugan,V.; DaSilva,J.; Liddell,J.; Frederick,J.C.; Di,H.; Sheffield,S.; Radford,K.; De La Cruz,J.; Keong,L.; Lacek,K.A.; Shu,B.; Lopez,D.; Seliskar,L.; Le,H.; Cadiz,V.; Hua,C.; Hok,S.; and Millena,S.
A/goose/Sweden/SVA210301SZ0005/KN049802-IP11/2021 (H5N8)	EPI_ISL_1236901	EPI1850319	EPI1850321	Swedish Veterinary Agency (SVA)	Swedish Veterinary Agency (SVA)	Zohari, S.
A/chicken/Scotland/068270/2021 (H5N1)	EPI_ISL_13370562	EPI2071680	EPI2071679	Animal and Plant Health Agency (APHA)	Animal and Plant Health Agency (APHA)	Byrne, A.
A/chicken/England/053052/2021 (H5N1)	EPI_ISL_9012457	EPI1963312	EPI1963311	Animal and Plant Health Agency (APHA)	Animal and Plant Health Agency (APHA)	Byrne, A.
A/whooper swan/Fukushima/0701B002/2021(H5N8)	NCBI	MZ235326.1	MZ235328.1	School of Veterinary Medicine, Hokkaido University	School of Veterinary Medicine, Hokkaido University	Sakoda,Y.; Isoda,N.; and Hiono,T.
A/Common Teal/South Korea/W812/2021 (H5N1)	EPI_ISL_8405663	EPI1950423	EPI1950425	Chungbuk National University	Chungbuk National University, College of Medicine and Medical Research Institute	Kim, Y.-I.

Table S2. Amino acid differences of the panel viruses in this study.

Virus	Amino acid														
	36	51	53	83	86	88	104	109	115	120	126	127	128	131	136
Ck/ENG/52/21	T	I	K	A	A	D	L	N	L	S	E	T	S	V	P
Gull/ENG/77/23	T	I	K	A	A	D	L	N	L	G	D	T	S	V	P
Cat/SD/01/24	T	I	K	A	A	D	M	N	Q	S	E	A	S	V	P
Gs/SE/11/2021	T	I	K	A	A	D	L	N	L	S	E	T	A	V	P
CA/51/24	T	I	K	A	A	G	M	N	Q	S	E	T	S	M	P
Ck/SCT/70/21	T	I	K	A	A	D	L	N	L	S	E	T	S	V	Q
Ty/ENG/52/20	T	I	K	A	A	D	L	N	L	S	E	T	S	V	S
Mink/FIN/E1/23	T	I	K	A	A	G	L	N	L	S	E	T	S	V	P
Teal/KR/12/21	T	I	K	A	T	D	L	N	L	N	E	T	S	V	P
Skunk/PE/9-1/23	T	I	K	A	V	D	M	N	L	S	E	T	S	V	P
Swan/Fuku/02/21	A	V	N	T	A	D	L	K	L	S	E	T	S	V	P
Ph/ENG/75/22	T	I	K	A	A	D	L	N	L	S	E	T	S	V	P
Skua/SCT/22/22	T	I	K	A	A	D	L	N	L	S	E	T	S	V	P
Ardea/ES/2-5/22	T	I	K	A	A	D	L	N	L	S	E	T	S	V	P
Ck/CHB/23/21	T	I	K	A	A	D	L	N	L	S	E	T	S	V	P
Ck/AMR/42/21	T	I	K	A	T	D	L	N	L	N	E	T	S	V	P
Vul/GA/2C/22	T	I	K	A	A	D	M	N	L	S	E	T	S	V	P
Ck/USA/01/24	T	I	K	A	A	G	M	N	Q	S	E	T	S	V	P
Gs/ON/24/24	T	I	K	A	A	D	M	N	Q	S	E	T	S	V	P

Virus	Amino acid														
	137	140	156	163	178	181	183	184	185	188	189	195	210	214	236
Ck/ENG/52/21	Y	T	A	S	I	S	N	A	E	T	N	T	V	A	D
Gull/ENG/77/23	Y	A	T	S	I	S	N	A	E	T	N	T	V	A	G
Cat/SD/01/24	Y	A	A	S	I	S	N	A	E	T	N	I	A	A	D
Gs/SE/11/2021	Y	A	A	S	V	S	N	A	E	T	N	T	V	A	D
CA/51/24	Y	A	A	S	I	S	N	A	E	T	N	I	A	A	D
Ck/SCT/70/21	Y	A	A	S	I	S	N	A	E	T	N	T	V	A	D
Ty/ENG/52/20	Y	A	A	S	I	S	N	A	E	T	N	T	V	A	D
Mink/FIN/E1/23	H	A	A	S	I	S	N	A	E	T	N	T	V	A	D
Teal/KR/12/21	Y	A	A	S	I	P	N	A	E	T	N	T	V	A	D
Skunk/PE/9-1/23	Y	V	A	S	I	S	S	A	E	T	N	T	A	A	D
Swan/Fuku/02/21	Y	T	A	N	I	S	N	E	E	T	N	T	V	A	N
Ph/ENG/75/22	Y	A	A	S	I	S	N	V	E	T	N	T	V	A	D
Skua/SCT/22/22	Y	A	A	S	I	S	N	A	K	T	N	T	V	A	D
Ardea/ES/2-5/22	Y	A	A	S	I	S	N	A	E	A	N	T	V	A	D
Ck/CHB/23/21	Y	A	A	S	I	S	N	A	E	I	N	T	V	A	D
Ck/AMR/42/21	Y	A	A	S	I	S	N	A	E	T	D	T	V	A	D
Vul/GA/2C/22	Y	A	A	S	I	S	N	A	E	T	K	T	A	A	D
Ck/USA/01/24	Y	A	A	S	I	S	N	A	E	T	N	I	A	S	D
Gs/ON/24/24	Y	A	A	S	I	S	N	A	E	T	N	T	A	V	D

Virus	Amino acid												
	238	268	282	314	320	321	372	473	476	495	496	508	518
Ck/ENG/52/21	A	G	V	A	S	P	D	D	Q	S	I	A	M
Gull/ENG/77/23	S	G	V	A	S	P	D	D	Q	S	I	A	M
Cat/SD/01/24	A	G	V	A	S	P	D	D	Q	S	V	A	M
Gs/SE/11/2021	A	G	V	A	S	P	D	D	Q	S	I	A	M
CA/51/24	A	G	V	A	N	P	D	D	Q	S	V	A	M
Ck/SCT/70/21	A	G	V	A	S	P	D	D	Q	S	I	A	M
Ty/ENG/52/20	A	G	V	A	N	P	D	D	Q	S	I	A	M
Mink/FIN/E1/23	A	G	I	V	S	P	D	D	Q	S	I	A	I

Teal/KR/12/21	A	G	V	A	S	P	D	D	Q	S	I	A	M
Skunk/PE/9-1/23	A	G	V	A	S	P	D	D	**H**	S	I	**V**	M
Swan/Fuku/02/21	A	**E**	V	A	S	P	**N**	**Y**	Q	S	I	**V**	**V**
Ph/ENG/75/22	A	G	V	A	S	P	D	D	Q	S	I	A	**I**
Skua/SCT/22/22	A	G	V	A	S	P	D	D	Q	S	I	A	**I**
Ardea/ES/2-5/22	A	G	V	A	S	P	D	D	Q	S	I	A	**I**
Ck/CHB/23/21	A	G	V	A	S	P	D	D	Q	S	I	A	M
Ck/AMR/42/21	A	G	V	A	S	P	D	D	Q	S	I	A	M
Vul/GA/2C/22	A	G	V	A	S	**R**	D	D	Q	S	I	A	M
Ck/USA/01/24	A	G	V	A	**N**	P	D	D	Q	S	**V**	A	M
Gs/ON/24/24	A	G	V	A	S	P	D	D	Q	**T**	**V**	A	M

Table S3. Binding profiles of the tested H5 viruses to 3SLN.

Virus	Estimated K_d values to 3SLN (relative to Ck/ENG/52/21)
Gull/ENG/77/23	-55.2
Cat/SD/01/24	1.4
Gs/SE/11/2021	2.3
CA/51/24	1.2
Ck/SCT/70/21	2.3
Ty/ENG/52/20	3.5
Mink/FIN/E1/23	-1.6
Teal/KR/12/21	-29.2

Skunk/PE/9-1/23	1.3
Swan/Fuku/02/21	4.6
Ph/ENG/75/22	2.7
Skua/SCT/22/22	37.2
Ardea/ES/2-5/22	1.8
Ck/CHB/23/21	13.7
Ck/AMR/42/21	-9.9
Vul/GA/2C/22	347.5
Ck/USA/01/24	-1.0
Gs/ON/24/24	-2.3
Skua/SCO/22/22 K185E	1.0

Table S4. Binding profiles of E185X viruses to 3SLN and 6SLN.

Virus	Estimated K_d values to 3SLN (relative to Ck/ENG/52/21)	Estimated K_d values to 6SLN (relative to K185)
E185K	528.2	/
E185D	3.2	/
E185R	18.3	-13.5
E185A	3.4	/
E185V	21.4	/

Table S5. Metadata details of the full H5N1 HPAIVs used in this study.

Isolate Name	Isolate ID	PB2	PB1	PA	HA	NP	NA	MP	NS	Submitting Lab	Originating Lab	Authors
A/dairy cattle/Texas/24-008749-001/2024 (H5N1)	EPI_ISL_19014384	EPI3158670	EPI3158671	EPI3158668	EPI3158678	EPI3158664	EPI3158672	EPI3158667	EPI3158665	National Veterinary Services Laboratories - USDA	National Veterinary Services Laboratories - USDA	Aufderhar,M., Franzen,K., Love,E., Killian,M.L., Lantz,K., Stuber,T., Hicks,J. and Norris,C.
A/dairy cow/California/010998-001/2025 (H5N1)	EPI_ISL_19851131	EPI4294111	EPI4294109	EPI4294106	EPI4294107	EPI4294108	EPI4294105	EPI4294112	EPI4294110	USDA, APHIS, Veterinary Services, Diagnostic Virology Laboratory, National Veterinary Services Laboratories	USDA, APHIS, Veterinary Services, Diagnostic Virology Laboratory, National Veterinary Services Laboratories	Aufderhar,M., Franzen,K., Killian,M.L., Lantz,K., Stuber,T., Hicks,J. and Lakin,S.

Reference

1 Chang, P. *et al.* Immune Escape Adaptive Mutations in the H7N9 Avian Influenza Hemagglutinin Protein Increase Virus Replication Fitness and Decrease Pandemic Potential. *J Virol* **94** (2020). https://doi.org/10.1128/JVI.00216-20

2 Heaton, B. E. *et al.* A CRISPR Activation Screen Identifies a Pan-avian Influenza Virus Inhibitory Host Factor. *Cell Rep* **20**, 1503–1512 (2017). https://doi.org/10.1016/j.celrep.2017.07.060

3 Sanchez-Felipe, L., Villar, E. & Munoz-Barroso, I. alpha2-3- and alpha2-6- N-linked sialic acids allow efficient interaction of Newcastle Disease Virus with target cells. *Glycoconj J* **29**, 539–549 (2012). https://doi.org/10.1007/s10719-012-9431-0