

# Diversity of Influenza A (H5N1) Viruses in Infected Humans

## Technical Appendix

**Technical Appendix Table 1.** Primer sequences used in this study

vRNA segment	Nucleotide positions of RT-PCR products*	Forward primer		Reverse primer	
		Name	Sequence	Name	Sequence
PB2	1–1784	PB2–1-24F	AGCGAAAGCAGGTCAAATATATTC	PB2–1762–1784R	TTGGGTACCAAGGATTGGAACGG
	532–2341	PB2–532–558F	CCAAATGAAGTGGGAGCTAGAATATTG	PB2–2318–2341R	AGTAGAAACAAGGTCGTTTTTAAA
	167–2277	PB2–167–186F	TGAAATGGATGATGGCAATG	PB2–2258–2277R	CGCTGTCTGGCTGTCAGTAA
PB1	1–1829	PB1–1-18F	AGCGAAAGCAGGCAAACC	PB1–1804–1829R	CGGATATTGTATAGATTTGGTCCTCC
	501–2341	PB1–501–522F	ATCGGGACGGCTAATAGATTTTC	PB1–2317–2341R	AGTAGAAACAAGGCATTTTTTTCACG
	106–2214	PB1–106–125F	CCTCCATACAGCCATGGAAC	PB1–2195–2214R	GAAATCAATTCGTGCGTCAA
PA	1–1668	PA-1–21F	AGCGAAAGCAGGTAATGATCC	PA-1646–1668R	CATGTCTCCTATCTCGAGGACAC
	565–2233	PA-565–587F	GAAATGGCCAGTAGGGGTCTATG	PA-2208–2233R	AGTAGAAACAAGGTACTTTTTTGGAC
	74–2169	PA-74–93F	CGGAAAAGGCAATGAAAGAA	PA-2150–2169R	CAGTGCATGTGTGAGGAAGG
HA <sup>†</sup>	1–1776(1779) <sup>‡</sup>	HA-1–21F-T	AGCAAAAGCAGGGGTTCATC	HA-1754–1776R-T	AGTAGAAACAAGGGTGTTTTTAACTA
	1–1776	HA-1–21F-T	AGCAAAAGCAGGGGTTCATC	HA-1754–1776R-C	AGTAGAAACAAGGGTGTTTTTCACTA
	1–1776	HA-1–21F-C	AGCAAAAGCAGGGGTTCATC	HA-1754–1776R-T	AGTAGAAACAAGGGTGTTTTTAACTA
	96–793	HA-96–115F	ACCATGCAAACAACCTCGCAC	HA-774–793R	GATTGCATCGTCCGGTTTTA
NP	1–1565	NP-1–24F	AGCAAAAGCAGGGTAGATAATCAC	NP-1541–1565R	AGTAGAAACAAGGGTATTTTTCTTTA ATTG
	51–1487	NP-51–70F	GTCTCAAGGCACCAAACGAT	NP-1468–1487R	ATGTCAAAGGAAGGCACGAT
NS	1–875	NS-1–23F	AGCAAAAGCAGGGTGACAAAAC	NS-852–875R	AGTAGAAACAAGGGTGTTTTTTAT
	83–772	NS-83–102F	CAAACGATTTGCAGACCAAG	NS-752–772R	TCTGTTCGAAGCTGTTTTCTG

\*All numbers listed refer to positive-sense orientation.

<sup>†</sup>To amplify the full-length HA segment, we used three different combinations of primers, based on the predetermined terminal sequences of the vRNA segments of each virus: HA-1–21F-T/HA-1754–1776R-T were used for UT3040I/II, UT31312I/II/III, UT31394I/II, and UT36250I/II; HA-1–21F-T/HA-1754–1776R-C were used for UT31413I/II; and HA-1–21F-C/HA-1754–1776R-T were used for UT36282I/II and UT36285I/II.

<sup>‡</sup>The HA genes of UT3040I and UT36250I are 1779 nt in length because of three additional nucleotides at the multibasic cleavage site.

**Technical Appendix Table 2.** Summary of nonsynonymous and synonymous single-nucleotide polymorphisms (SNPs) detected at  $\geq 1\%$

Virus isolate	Date of sample collection	Viral RNA segment											
		PB2		PB1		PA		HA		NP		NS	
		N*	S†	N	S	N	S	N	S	N	S	N	S
UT3040I	2004 Jan 6	8	2	6	10	10	0	1	4	1	3	1	0
UT3040II	2004 Jan 7	NA‡	NA	0	0	0	1	1	0	NA	NA	0	0
UT31312I	2007 Jul 25	3	2	0	1	0	0	1	3	1	0	0	0
UT31312II	2007 Jul 26	4	1	0	0	0	0	5	0	1	0	1	0
UT31312III	2007 Jul 25	4	0	0	0	0	0	6	2	0	1	0	0
UT31394I	2008 Jan 17	12	3	6	10	5	3	11	8	5	0	2	1
UT31394II	2008 Jan 17	6	1	3	2	4	0	4	3	4	0	1	0
UT31413I	2008 Feb 13	0	0	9	2	5	5	1	0	6	3	4	0
UT31413II	2008 Feb 13	0	1	0	3	0	1	5	8	0	0	2	0
UT36250I	2010 Mar 10	12	8	9	7	11	4	14	1	7	0	3	0
UT36250II	2010 Mar 11	0	0	NA	NA	NA	NA	6	2	NA	NA	1	0
UT36282I	2010 Apr 1	3	3	2	5	1	2	3	2	2	1	0	0
UT36282II	2010 Apr 3	2	2	1	3	2	2	5	7	3	3	8	4
UT36285I	2010 Apr 4	0	1	NA	NA	0	1	0	0	0	0	0	0
UT36285II	2010 Apr 8	3	2	NA	NA	3	2	NA	NA	1	0	0	0

\*N, nonsynonymous single-nucleotide polymorphism; NA, not applicable because of low sequence coverage; S, synonymous single-nucleotide polymorphism.

**Technical Appendix Table 3.** Genetic variability among influenza A(H5N1) virus samples isolated from infected persons, northern Vietnam, 2004–2010

Viral protein, aa position	Virus isolate	Major variant in sample		Minor variant in sample		Consensus of top 25 blastn hits of avian H5 viruses†
		Amino acid	Percentage	Amino acid	Percentage	
<b>PB2</b>						
15	UT36250I	R	69.7	C	26.9	R
	UT36250II	C	100.0	R	0.0	R
<b>627</b>	UT3040I	K	92.6	E	7.4	E
	UT31312I	E	67.9	K	32.1	E
	UT31312II	E	72.1	K	27.9	E
	UT31312III	K	64.7	E	35.3	E
	UT31394I	E	50.5	K	49.5	E

Viral protein, aa position	Virus isolate	Major variant in sample		Minor variant in sample		Consensus of top 25 blastn hits of avian H5 viruses†
		Amino acid	Percentage	Amino acid	Percentage	
	UT31394II	K	51.7	E	48.3	E
	UT36250I	E	74.3	K	25.7	E
<b>PB1</b>						
123	UT36250I	T	95.0	A	5.0	T
213	UT36282I	N	92.3	D	7.7	N
	UT36282II	N	94.4	D	5.6	N
254	UT31394I	F	94.3	L	5.7	F
	UT31394II	F	93.2	L	6.8	F
538	UT31394II	D	84.9	G	15.1	D
598	UT36250I	L	68.8	P	31.2	L
<b>PA</b>						
85	UT3040I	T	95.2	M	4.8	T
	UT3040II	M	100.0	T	0.0	T
90	UT3040I	V	94.8	M	5.2	V
142	UT3040I	K	87.2	E	12.8	K
260	UT31413I	F	94.5	S	5.5	F
325	UT31413I	P	94.9	S	5.1	P
387	UT3040I	V	94.2	I	5.8	V
417	UT3040I	L	91.4	V	8.6	L
	UT3040II	V	100.0	L	0.0	L
651	UT31394I	A	89.0	S	11.0	A
	UT31394II	A	90.1	S	9.9	A
<b>HA<sup>‡</sup></b>						
54(+1) <sup>§</sup>	UT31394I	E	66.5	D	33.5	D
	UT31394II	D	54.5	E	45.5	D
67	UT31394II	A	91.7	T	8.3	A
92	UT31312II	N	60.7	K	39.3	N
<b>138</b>	UT3040I	A	94.7	V	5.3	A
	UT36250I	A	72.0	V	28.0	A
	UT36250II	A	80.2	V	19.8	A
	UT36282I	A	93.9	V	6.1	A
	UT36282II	A	94.7	V	5.3	A
<b>186</b>	UT3040II	N	88.6	K	11.4	N

Viral protein, aa position	Virus isolate	Major variant in sample		Minor variant in sample		Consensus of top 25 blastn hits of avian H5 viruses†
		Amino acid	Percentage	Amino acid	Percentage	
	UT31312III	N	86.8	K	13.2	N
203	UT31312III	S	85.4	P	14.6	S
207	UT31394I	S	79.0	T	21.0	S
	UT31394II	S	52.8	T	47.2	S
226	UT36250II	Q	86.0	K	14.0	Q
456	UT31312III	M	54.9	R	45.1	R
486	UT31413II	Y	94.7	H	5.3	Y
511	UT31413I	T	90.4	I	9.6	T
<b>NP</b>						
77	UT31394I	R	92.3	K	7.7	R
	UT31394II	R	79.1	K	20.9	R
430	UT36282II	A	92.5	V	7.5	T <sup>#</sup>
454	UT31394I	E	91.2	G	8.8	E
	UT31394II	E	79.0	G	21.0	E
<b>NS1</b>						
124	UT36250I	I	85.8	M	14.2	I
	UT36250II	I	78.2	M	21.8	I

\*Listed are single-nucleotide polymorphisms detected in 5%–50% of the sequence reads directly derived from viral RNAs (single-nucleotide polymorphisms detected in more than half of the sequence reads were considered the major variant); also listed is 1 variant (at position 85 of PA) that was found in <5% of the reads at the first sampling time, but increased substantially in frequency at the second sampling time. HA, hemagglutinin; NP, nucleocapsid; NS, nonstructural; PA, polymerase acidic; PB, polymerase basic. Boldface indicates amino acid changes detected in samples isolated from several patients.



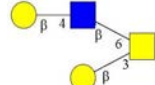

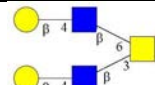

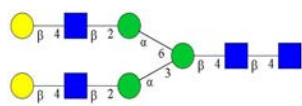
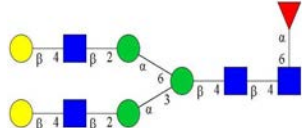
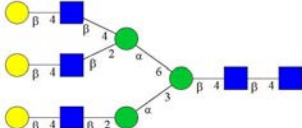
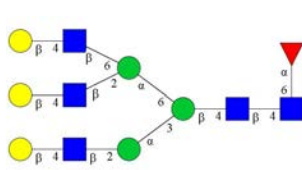
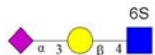
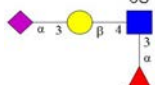
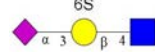
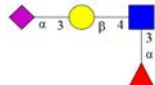
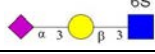
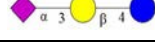
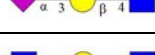

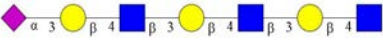
†To compare the amino acid sequences of the human H5N1 virus isolates tested here with those of closely related avian H5 viruses, each human H5N1 viral gene sequence was first BLASTn searched with avian H5N1 viruses. Then, the consensus amino acids of the top 25 BLASTn hits were retrieved.

‡For HA, the numbers refer to the amino acid positions in mature H3 HA.

§After position 54, H5 HA proteins possess an additional amino acid not found in H3 HA proteins.

#For the top 5 BLASTn (<https://www.fludb.org/brc/blast.spg?method=ShowCleanInputPage&decorator=influenza>) hits (i.e., the most closely related viruses), the consensus amino acid at this position is A.

**Technical Appendix Table 2. List of sialosides used for glycan arrays**

Glycan #	Common Name	Structure
1	Gal $\beta$ (1-4)GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
2	Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
3	Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[Gal $\beta$ (1-3)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
4	Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
5	Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)[Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
6	Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
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	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)[Fuc $\alpha$ (1-6)]-GlcNAc $\beta$ -Asn-Ser-Thr-NH <sub>2</sub>	
9	Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3){Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)[Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)]-Man $\alpha$ (1-6)}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)GlcNAc $\beta$ -Asn-Lys-NH <sub>2</sub>	
10	Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3){Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)[Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)]-Man $\alpha$ (1-6)}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)[Fuc $\alpha$ (1-6)]-GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
11	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)6-O-sulfo-GlcNAc $\beta$ -propyl-NH <sub>2</sub>	
12	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-6-O-sulfo-GlcNAc $\beta$ -propyl-NH <sub>2</sub>	
13	NeuAc $\alpha$ (2-3)6-O-sulfo-Gal $\beta$ (1-4)GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
14	NeuAc $\alpha$ (2-3)6-O-sulfo-Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ -propyl-NH <sub>2</sub>	
15	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-3)6-O-sulfo-GlcNAc $\beta$ -propyl-NH <sub>2</sub>	
16	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)Glc $\beta$ -ethyl-NH <sub>2</sub>	
17	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
18	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
19	NeuAc $\alpha$ (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>	

Glycan #	Common Name	Structure
20	NeuAc $\alpha$ (2-3)GalNAc $\beta$ (1-4)GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
21	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-3)GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
22	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-3)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
23	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-3)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
24	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-3)GalNAc $\beta$ (1-3)Gal $\alpha$ (1-4)Gal $\beta$ (1-4)Glc $\beta$ -ethyl-NH <sub>2</sub>	
25	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
26	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
27	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
28	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
29	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
30	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
31	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-3)[GlcNAc $\beta$ (1-6)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
32	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[Gal $\beta$ (1-3)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
33	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[Gal $\beta$ (1-3)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
34	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[Gal $\beta$ (1-3)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
35	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[Gal $\beta$ (1-3)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
36	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[Gal $\beta$ (1-3)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	









Glycan #	Common Name	Structure
58	NeuAc $\alpha$ (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)Man $\alpha$ (1-3)[NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-6)]-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
59	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3)[NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-6)]-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
60	NeuAc $\alpha$ (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)Man $\alpha$ (1-3)[NeuAc $\alpha$ (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)Man $\alpha$ (1-6)]-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)[Fuc $\alpha$ (1-6)]-GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
61	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3)[NeuAc $\alpha$ (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)Man $\alpha$ (1-6)]-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)[Fuc $\alpha$ (1-6)]-GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
62	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3){NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)[NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)Man $\alpha$ (1-6)]}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
63	NeuAc $\alpha$ (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)Man $\alpha$ (1-3){NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)[NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)Man $\alpha$ (1-6)]}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	

Glycan #	Common Name	Structure
64	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3){NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)[NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)Man $\alpha$ (1-6)]}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
65	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3){NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)[NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)Man $\alpha$ (1-6)]}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)[Fuc $\alpha$ (1-6)]-GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
66	NeuAc $\alpha$ (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)Man $\alpha$ (1-3){NeuAc $\alpha$ (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)[NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)Man $\alpha$ (1-6)]}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)[Fuc $\alpha$ (1-6)]-GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
67	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3){NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)[NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)Man $\alpha$ (1-6)]}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)[Fuc $\alpha$ (1-6)]-GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
68	Gn/3'SLN/3'SLN-TriN	
69	NeuAc $\alpha$ (2-3)[GalNAc $\beta$ (1-4)]-Gal $\beta$ (1-4)Glc $\beta$ -ethyl-NH <sub>2</sub>	
70	NeuAc $\alpha$ (2-3)[GalNAc $\beta$ (1-4)]-Gal $\beta$ (1-4)Glc $\beta$ -ethyl-NH <sub>2</sub>	
71	Gal $\beta$ (1-3)GalNAc $\beta$ (1-4)[NeuAc $\alpha$ (2-3)]-Gal $\beta$ (1-4)Glc $\beta$ -ethyl-NH <sub>2</sub>	

Glycan #	Common Name	Structure
72	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ -propyl-NH <sub>2</sub>	
73	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-3)[Fuc $\alpha$ (1-4)]-GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
74	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
75	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
76	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
77	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
78	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)[NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-6)-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
79	NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3)[NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4)[Fuc $\alpha$ (1-3)]-GlcNAc $\beta$ (1-2)Man $\alpha$ (1-6)]-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)GlcNAc $\beta$ -(Lys-Val-Ala)Asn-(Lys-Thr)NH <sub>2</sub>	
80	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)(6S)GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
81	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)6-O-sulfo-GlcNAc $\beta$ -propyl-NH <sub>2</sub>	
82	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)Glc $\beta$ -ethyl-NH <sub>2</sub>	
83	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
84	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
85	NeuAc $\alpha$ (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>	
86	NeuAc $\alpha$ (2-6)GalNAc $\beta$ (1-4)GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	
87	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	

Glycan #	Common Name	Structure
88	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
89	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
90	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
91	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
92	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[Gal $\beta$ (1-3)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
93	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[Gal $\beta$ (1-3)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
94	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[Gal $\beta$ (1-3)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
95	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[Gal $\beta$ (1-3)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
96	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[Gal $\beta$ (1-3)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
97	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
98	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-3)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
99	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
100	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
101	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	



Glycan #	Common Name	Structure
102	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
103	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
104	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
105	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
106	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
107	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
108	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)]-GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
109	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
110	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)GalNAc $\alpha$ -Thr-NH <sub>2</sub>	
111	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[NeuAc $\alpha$ (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>	
112	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-3)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-3)GlcNAc $\beta$ (1-3)]Gal $\beta$ (1-4)GlcNAc $\beta$ -ethyl-NH <sub>2</sub>	

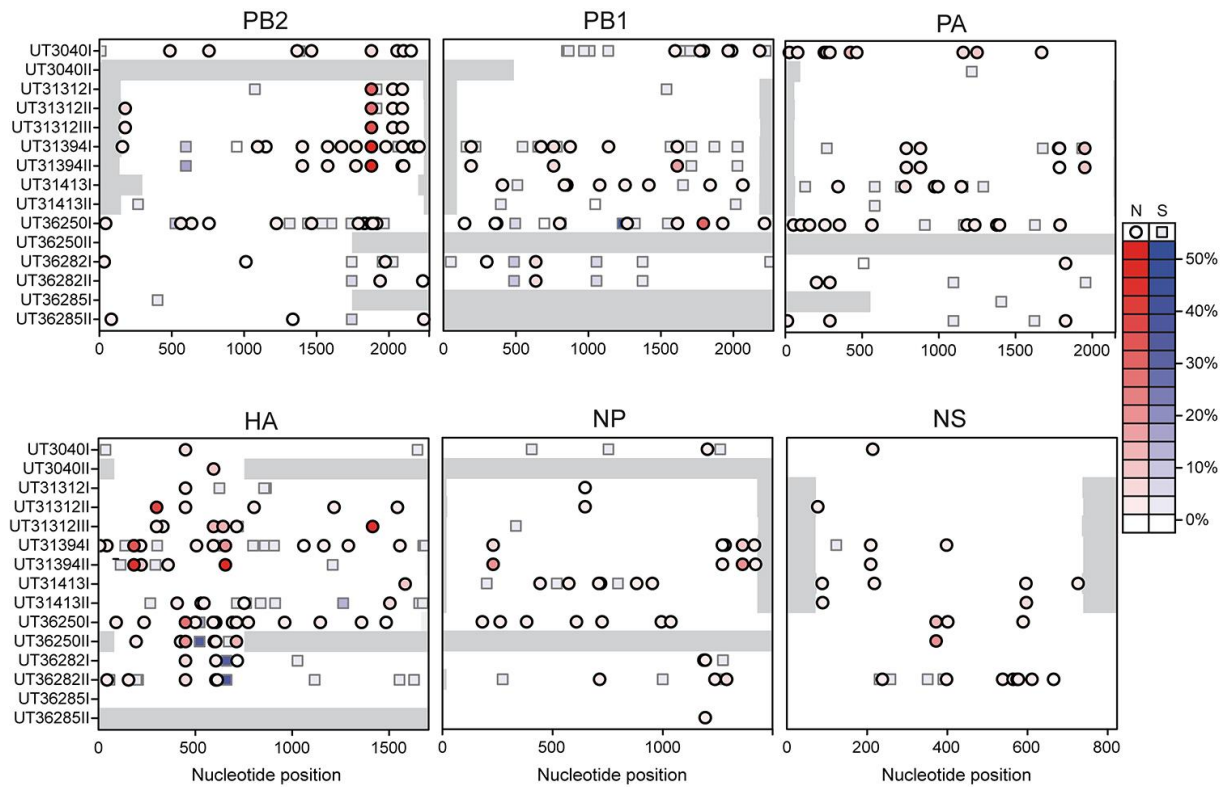


Glycan #	Common Name	Structure
122	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-6)]-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
123	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-6)]-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)[Fuc $\alpha$ (1-6)]-GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
124	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-6)]-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)[Fuc $\alpha$ (1-6)]-GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
125	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-6)]-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)[Fuc $\alpha$ (1-6)]-GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
126	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3){NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)Man $\alpha$ (1-6)]}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
127	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3){NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)Man $\alpha$ (1-6)]}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	

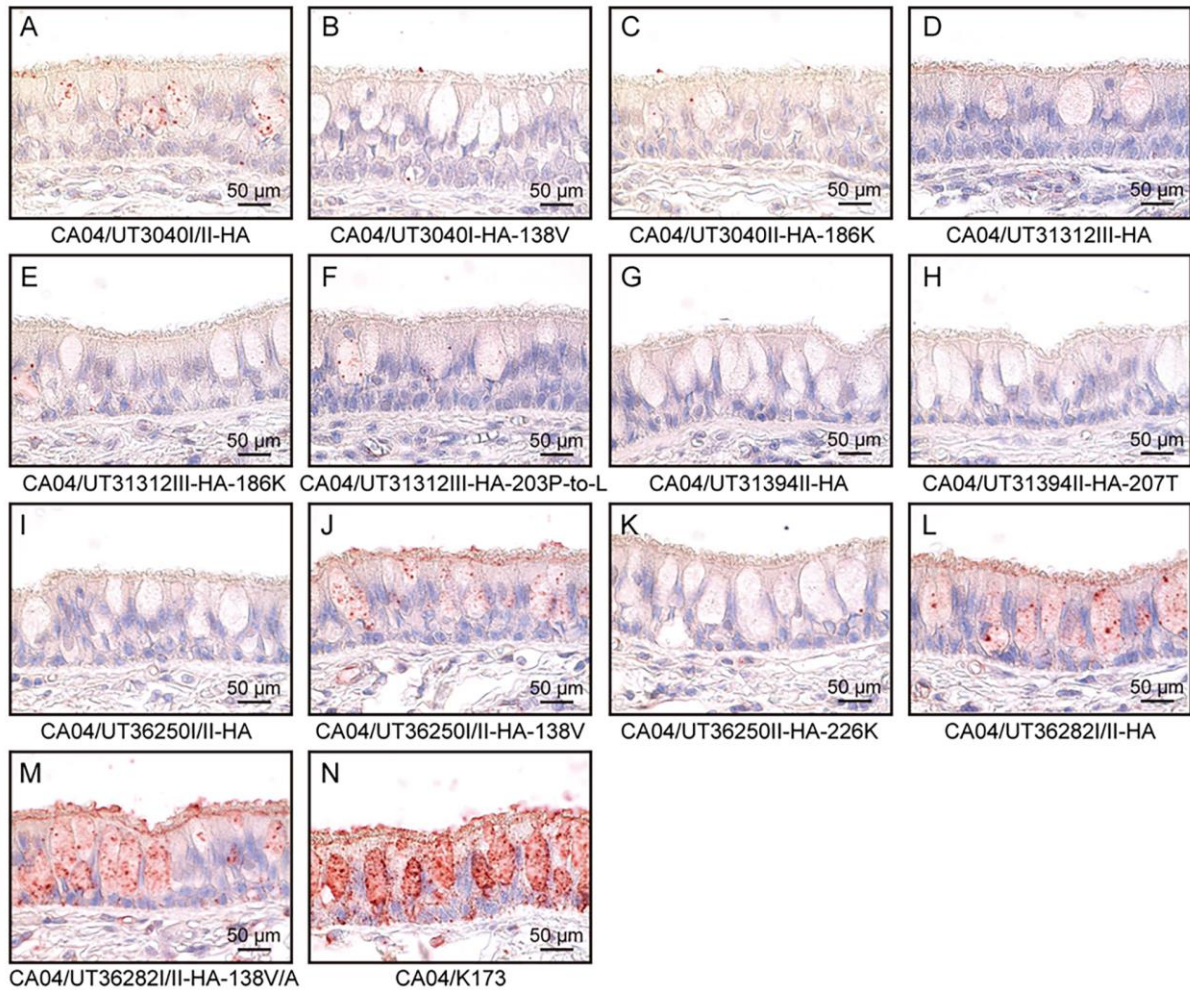


Glycan #	Common Name	Structure
128	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3){NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)Man $\alpha$ (1-6)]}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
129	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3){NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)Man $\alpha$ (1-6)]}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)[Fuc $\alpha$ (1-6)]-GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
130	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3){NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)Man $\alpha$ (1-6)]}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)[Fuc $\alpha$ (1-6)]-GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
131	NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)Man $\alpha$ (1-3){NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-2)[NeuAc $\alpha$ (2-6)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-3)Gal $\beta$ (1-4)GlcNAc $\beta$ (1-6)Man $\alpha$ (1-6)]}-Man $\beta$ (1-4)GlcNAc $\beta$ (1-4)[Fuc $\alpha$ (1-6)]-GlcNAc $\beta$ -(Lys-Val-Ala)Asn-Lys-Thr-NH <sub>2</sub>	
132	LN/6'SLN/6'SLN-TriN	
133	6'SLNLN/6'SLN/6'SLN-TriN	



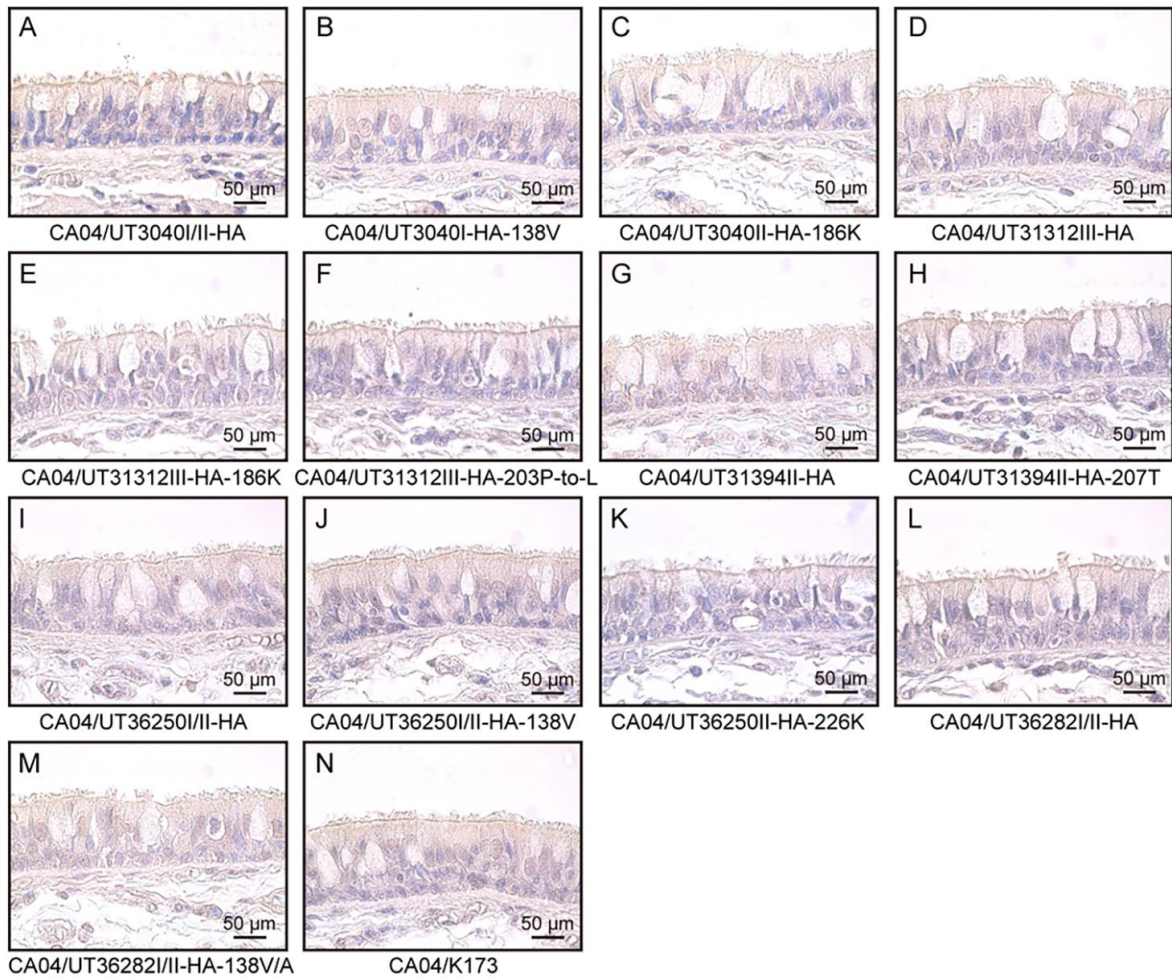


**Technical Appendix Figure 2.** Deep-sequencing reveals H5N1 influenza sequence variation among humans infected with H5N1 viruses. The PB2, PB1, PA, HA, NP, and NS vRNAs were amplified from throat swabs or tracheal aspirates and deep-sequenced. Single-nucleotide polymorphisms (SNPs) detected in  $\geq 1\%$  of sequence reads were identified by comparing sequence reads with the consensus sequence assembled for each sample. Grey bars indicate gene regions with insufficient sequence coverage (<100 sequence reads) to call variants. Squares and circles indicate synonymous and nonsynonymous SNPs, respectively. SNP frequencies are represented as a heatmap with synonymous and nonsynonymous mutations represented in blue and red, respectively. Since SNPs were called relative to their consensus nucleotide, the maximum frequency of each SNP is <50%.

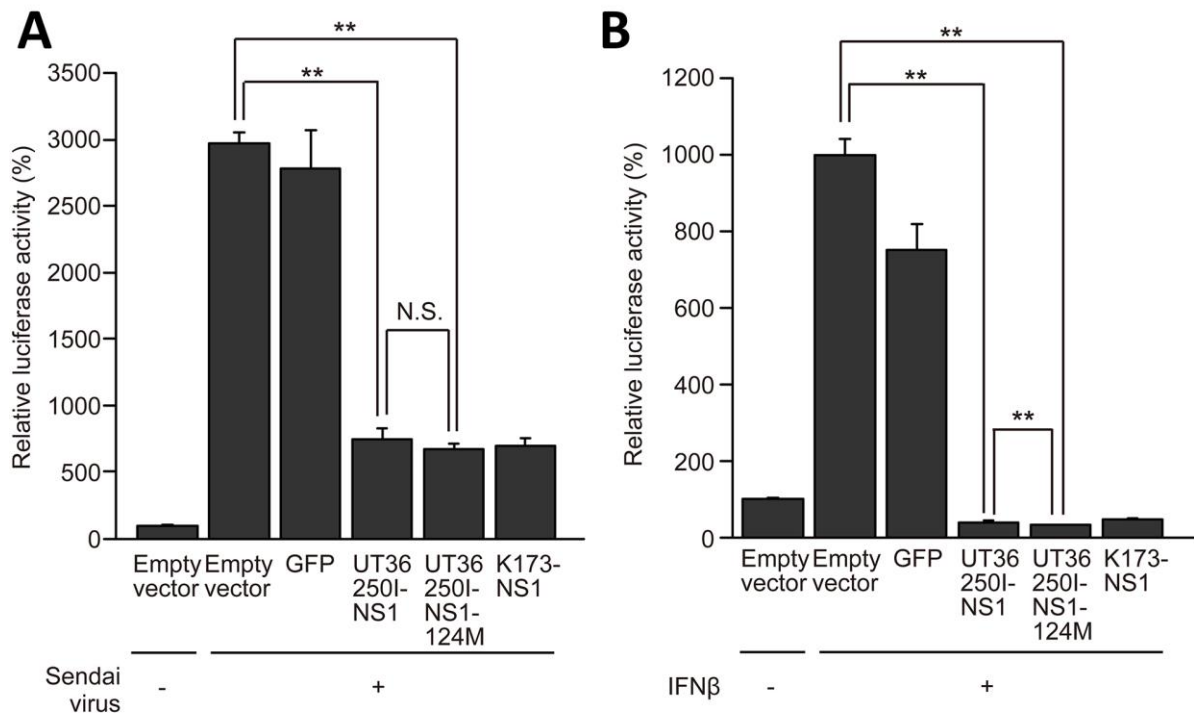


**Technical Appendix Figure 3.** Effect of amino acid variations in HA on tissue binding ability. Binding of viruses to human trachea sections, on which  $\alpha$ 2,6-linked sialic acids are present, was assessed. If two isolate numbers are listed (such as CA04/UT3040I/II), we tested the major sequence variant, which is identical between the two samples. FITC-labeled viruses were incubated with deparaffinized tissue sections and virus-tissue binding was detected by means of immunohistochemistry. Signals were observed as a dark-red color; images were obtained by using the upright microscope, Axio Imager.A2 (Zeiss, Oberkochen, Germany) with a 100 X objective lens. Nuclei were counterstained with Mayer's hematoxylin (Sigma Aldrich, St. Louis, MO, USA). CA04/K173 virus was used as a control.





**Technical Appendix Figure 4.** Confirmation of virus binding specificity to sialic acids. To confirm virus binding specificity to sialic acids, we pretreated tracheal tissues with  $\alpha(2\rightarrow3,6,8,9)$  neuraminidase from *Arthrobacter ureafaciens* (Sigma Aldrich), which removes terminal sialic acids. Binding of viruses to the pretreated sections was assessed. If two isolate numbers are listed (such as CA04/UT3040I/II), we tested the major sequence variant, which is identical between the two samples. FITC-labeled viruses were incubated with the pretreated tissue sections and virus-tissue binding was detected by means of immunohistochemistry. Nuclei were counterstained with Mayer's hematoxylin (Sigma Aldrich).



**Technical Appendix Figure 5.** Effect of an amino acid variation in NS1 on interferon antagonistic properties. (A) Effect of NS1 on interferon production. 293T cells were transfected with a plasmid encoding the wild-type or mutant NS gene and with a plasmid encoding the firefly luciferase gene under the control of the interferon $\beta$  promoter. An empty vector and a plasmid expressing GFP served as negative controls. A plasmid expressing the human influenza A virus K173 NS gene was used as a positive control. Twenty-four hours after transfection, cells were infected with  $10^6$  fluorescence-forming units of Sendai virus to stimulate interferon synthesis. The ability of NS1 to interfere with interferon production was measured 48 h later. (B) Effect of NS1 on interferon signaling. Cells were transfected with control plasmids or plasmids encoding the wild-type or mutant NS gene, and with a plasmid expressing luciferase under the control of an interferon-regulated stimulatory element (ISRE). The cells were incubated for 24 h and then treated with 100 units of human interferon $\beta$  for 6 h. In both assays, firefly luciferase activities were measured by using Luciferase Assay Reagent II (Promega). The experiments (each in triplicate) were repeated twice independently. Mean relative interferon antagonistic activities with standard deviations are shown. The activity of cells transfected with the pCAGGS empty vector (nontreated with Sendai virus or nonincubated with interferon $\beta$ ) was set to 100%. The asterisks indicate a significant difference in interferon antagonistic activities between the 'empty' vector, UT36250I-NS1, and/or UT36250I-NS1–124M (\*\* $p < 0.01$ ; one-way ANOVA followed by Tukey's post-hoc test; N.S.: not significant).