

Supplementary Figure 1 | Weight and body temperature of ferrets inoculated with A/Anhui/1/2013 (H7N9) influenza virus. (a) Body temperature and (b) weight change of ferrets after intranasal inoculation with 10^6 TCID50 of A/Anhui/1/2013 (H7N9) influenza virus, direct contact, and aerosol contact (n = 4 per group). Body temperature is shown for only 3 direct contact ferrets, as one temperature probe was faulty.



Supplementary Figure 2 | Mean variation frequencies of HA and NA genes after virus replication in chickens and ferrets. "Round 1 ferrets" were inoculated with parental A/Anhui/1/13 (H7N9) influenza virus; "all ferrets" include donor and contact ferrets in rounds 1 and 2. Statistical comparisons were performed by using two-tailed Mann-Whitney U test, *P < 0.05.

а

Parental -Donor1_day1_round1 -Donor2_day1_round1 Donor3_day1_round1 -Donor4 day1 round1 -Donor1_day4_round1 -Donor3_day4_round1 -Donor4_day4_round1 -DC1_day6_round1 -DC2_day6_round1 -DC4 day6 round1 DC4_day8_round1 -AC4_day8_round1 -Donor1_day2_round2 -Donor2_day2_round2 -Donor3_day2_round2 -Donor2_day4_round2 -Donor3_day4_round2 -



b

Pare

Donor1_day1_round1 Donor2 day1 round1 Donor3 dav1 round1 · Donor4 day1 round1 Donor1 dav4 round1 Donor3_day4_round1 Donor4 day4 round1 DC1_day6_round1 DC2_day6_round1 DC4_day6_round1 · DC4_day8_round1 AC4_day8_round1 · Donor1_day2_round2 Donor2_day2_round2 -Donor3_day2_round2 · Donor2_day4_round2 · Donor3_day4_round2



С

Parenta Donor1 day1 round1 Donor2_day1_round1 Donor3_day1_round1 Donor4_day1_round1 Donor1_day4_round1 Donor3_day4_round1 Donor4_day4_round1 DC1_day6_round1 DC2_day6_round1 DC4_day6_round1 DC4_day8_round1 AC4_day8_round1 Donor1 day2 round2 Donor2 day2 round2 -Donor3_day2_round2 Donor2_day4_round2 Donor3 dav4 round2





22 AS N254 d

Non-synonymous variant frequencies for segment HA





Non-synonymous variant frequencies for segment NP





var_freq 0.6

0.4

0.2

0.0







Supplementary Figure 3 | **Heat map of variations in the whole genome of A/Anhui/1/2013** (H7N9) influenza virus in ferrets. Non-synonymous variant sequences for segments PB2 (a); PB1, PB1-F2 (b); PA, PA-X (c); HA (d); NP (e); NA (f); M1, M2 (g); and NS1, NEP (h) are shown. Parental virus is compared with viruses obtained in experimental rounds 1 and 2. Rows represent viruses from donor, direct contact (DC) and aerosol contact (AC) ferrets. Columns represent amino acid positions (counted from the start codon). Mutations in PA, M1, and NS1 genes are shown in black while those in PA-X, M2, and NS2 are in purple. Mutations with frequency > 0.01 are shown.



Supplementary Figure 4 | Effect of identified amino acid substitutions in the NA, PB1, and NP on replication kinetics of A/Anhui/1/2013 (H7N9) influenza viruses *in vitro*. Growth curves of the wild-type and the NA_{E73K} (a), NA_{I300V} (b), PB1_{D76N} (c), and NP_{I365V} (d) rg-A/Anhui/1/2013 (H7N9) influenza viruses using MOI 1 TCID₅₀ per cell in MDCK cells at 37°C. Values are mean and SD from two independent experiments (n= 4). *<0.05 compared to WT virus by 2-way ANOVA.



Supplementary Figure 5 | Activation pH of the HA proteins of H7N9 influenza viruses.

Vero cells were inoculated with the indicated viruses at an MOI of 5 TCID_{50/cell}. Cells were then treated with pH buffers at the indicated pH values, and syncytium formation was monitored. The highest pH at which syncytia were observed (boxed in red) was recorded as the pH of HA activation. Data is representative of two independent experiments.

Supplementary Table 1 | Homology sequence analysis of A/Anhui/1/2013 (H7N9) influenza virus in comparison with H7N9 influenza viruses from birds and environment isolated during the first wave of the outbreak in China, 2013.

H7N9 virus	Date of	Segment ^a	Accession	% Nucleotide sequence	% Amino acid
(isolated form birds and environment)	isolation	_	No. ^b	homology with	sequence homology
				A/Anhui/1/2013 ^c	with A/Anhui/1/2013
A/Chicken/Shanghai/S1053/2013	2013-04-03	PB2	EPI440682	99.8	99.8
-		PB1	EPI440683	100.0	100.0
		PA	EPI440681	99.8	99.6
		HA	EPI440685	99.3	98.7
		NP	EPI440678	100.0	100.0
		NA	EPI440684	99.8	100.0
		MP	EPI440680	99.9	100.0
		NS	EPI440679	99.9	100.0
A/Chicken/Zhejiang/SD033/2013	2013-04-11	PB2	EPI457746	99.9	99.7
		PB1	EPI457747	100.0	100.0
		PA	EPI457745	100.0	100.0
		HA	EPI457749	99.9	100.0
		NP	EPI457742	100.0	100.0
		NA	EPI457748	99.9	99.8
		MP	EPI457744	98.3	99.3
		NS	EPI457743	99.9	100.
A/Environment/Shanghai/S1088/2013	2013-04-03	PB2	EPI440690	99.9	99.8
		PB1	EPI440691	100.0	100.0
		PA	EPI440689	99.4	98.7
		HA	EPI440693	99.4	99.1
		NP	EPI440686	100.0	100.
		NA	EPI440692	99.9	100.0
		MP	EPI440688	99.7	99.8
		NS	EPI440687	99.9	100.0
A/Pigeon/Shanghai/S1069/2013	2013-04-02	PB2	EPI440698	99.8	99.7
		PB1	EPI440699	100.0	100
		PA	EPI440697	99.8	99.6
		HA	EPI440701	99.8	100.0

		NP NA	EPI440694 EPI440700	98.8 99.8	99.7 99.8
		MP	EPI440696	99.8	100
		NS	EPI440695	96.9	99.4
A/Pigeon/Shanghai/S1423/2013	2013-04-03	PB2	EPI457626	99.9	99.8
		PB1	EPI457627	100.0	100.0
		PA	EPI457625	97.8	98.7
		HA	EPI457629	99.8	99.7
		NP	EPI457622	100.0	100.0
		NA	EPI457628	99.8	100.0
		MP	EPI457624	99.9	99.8
		NS	EPI457623	99.9	100.0

^a PB2: RNA polymerase basic subunit 2; PB1: RNA polymerase basic subunit 1; PA: RNA polymerase acidic subunit; HA:

6 haemagglutinin; NP: nucleoprotein; NA: neuraminidase; MP: matrix gene; NS: non-structural gene.

⁷ ^b Sequence data used in this table were downloaded from GISAID, Global Initiative on Sharing Avian Influenza Data

8 (<u>http://platform.gisaid.org</u>)

9 ^c Homology analysis was performed using BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html) Sequence Identity

10 Matrix tool.

Animal	Dni				Gene segm	ent			
number	ърг	PB2	PB1	PA	HA	NP	NA	Μ	NS
(Parental virus)	N/A	b	_	_	N141D (0.4211), A143T (01250), N167D (0.5824), L235Q (0.2182), Y501N (0.1454), X501C	A373T (0.1277)	T10I (0.2283)		
1	3	N100D (0.4203), K627R (0.1687), L665P (0.125530625	N346S (0.125), V421A (0.3125), V609A (0.1355)	T210N (0.4737), A255G (0.3125), E377G (0.100)	Y 501C (0.1457) N141D (0.6923), N167D (0.7945), Q444L (0.1477), Y 501N (0.1874), Y 501C (0.1884)	R26G (0.4809), R98K (0.1094), A284T (0.1975), A428T (0.1407)	T50K (0.1250), K139N (0.1677)	a	_
	5	R70G (0.1102)		P28L (0.1070), S184N (0.7272), Q654stop (0.1698), R663G (0.1709)	N141D (0.4201), A143T (0.1203), G151E (0.1761), N167D (0.4350), Y501N (0.1935), Y501C (0.1935)	R99K (0.2261), A373T (0.1207), R446K (0.1058)			

12 Supplementary Table 2 | Genetic diversity of A/Anhui/1/2013 (H7N9) influenza viruses in chickens.

2	3	K48R (0.1020), W98stop (0.72), D275E (0.125)		E623K (0.3325)	N141D (0.4225), A143T (0.1509), N167D (0.5347), N208D (0.1339), A310T (0.1724), G475D (0.36951), Y501N (0.1598), Y501C (0.1598)				
	5	_	_		N141D (0.6972), N167D (0.5634), Y501Y (0.1596), Y501C (0.1586)	_	T10I (0.2241)	M165I (0.1438), M192I (0.1846), A193V (0.1969)	
3	3	M90T (0.2526)	K54E (0.6257), M523I (0.6470), Q569K (0.1161)	Y110H (0.1724)	N141D (0.7217), N167D (0.6614), L235Q (0.8971), Y501N (0.2537), Y501C (0.2529), G520V (0.1451)	A284T (0.1267),	_		

5	 F94L (0.1726),	 N141D	A373T	S119P	_	
	L108F	(0.2786),	(0.1265)	(0.1097)		
	(0.1274)	N167D				
		(0.3247),				
		L235Q				
		(0.1111),				
		K414R				
		(0.1111),				
		Y501N				
		(0.46939),				
		Y501C				
		(0.4694)				

14 Three 6-week-old, specific pathogen–free chickens were inoculated with 10^6 EID₅₀ of virus in 0.5 ml PBS via natural routes (nares,

15 trachea, eyes). RNA was extracted from oropharyngeal swabs on the indicated days post-inoculation (dpi) and influenza virus genes

16 were amplified and sequenced. Shown are all amino acid variations that differed from the consensus parental sequence and were

17 present at a frequency ≥ 0.1 .

18 —, no nonsynonymous mutations were observed at a frequency ≥ 0.1 .

A / A					Seru	m HI tite	er (HAU s	50 μl ⁻¹)				
A/ANNU/1/2015 (117N0)		After ir	noculati	on	A	After dire	ect contac	t	Af	ter aeros	sol conta	ict
(H7N9) virus	D1	D2	D3	D4	DC1	DC2	DC3	DC4	AC1	AC2	AC3	AC4
Parental	160	80	40	160	160	320	160	40	<	<	10	40
AC	80	160	<	ND	ND	ND	ND	ND	<	<	<	ND

Supplementary Table 3 | Ferret serum hemagglutination inhibition titers. 19

Titers are reciprocal values. HAU, hemagglutinating units. D, donor; DC, direct contact; AC aerosol contact; <, below the lower limit of detection (10 HAU/50 μ l); ND, not determined. 20

Supplementary Table 4 | Variation frequency of A/Anhui/1/2013 (H7N9) influenza virus after inoculation of ferrets and 23 chickens. 24

Animal model	Experiment round	Group	Animal no.	DPI	Total variation frequency (log)	HA variation frequency (log)	NA variation frequency (log)
N/A	N/A	Parental virus	N/A	N/A	-2.905440162	-2.338603516	-2.972306897
Ferrets	1	Donor	D1	1	-2.806927772	-2.457879004	-2.833868038
				4	-2.742696441	-2.453907496	-2.805941954
			D2	1	-2.816327184	-2.387386966	-2.783279964
			D3	1	-2.769769832	-2.394822726	-2.81441596
				4	-2.776612542	-2.292597553	-2.807442559
			D4	1	-2.802506048	-2.24905896	-2.832470569
				4	-2.774978978	-2.313737707	-2.839594171
		Direct	DC1	6	-2.772057833	-2.438035302	-2.756583119
		Contact	DC2	6	-2.748001965	-2.439238638	-2.773588496
			DC4	6	-2.841064242	-2.811259698	-2.637607146
				8	-2.779612706	-2.787387123	-2.696347657
		Aerosol Contact	AC4	8	-2.837547661	-2.349990787	-2.809618722
	2	Donor	D1-2	2	-2.832660393	-2.617216784	-2.660533406
			D2-2	2	-2.846847365	-2.575942667	-2.687619251
				4	-2.847458857	-2.694908903	-2.673835236
			D3-2	2	-2 851934700	-2 801427075	-2 681704602

	_			4	-2.841801531	-2.799644372	-2.647563697
Chicken	N/A	N/A	1	3	-2.634408252	-2.233465376	-2.841465687
S				5	-2.675060293	-2.127884799	-2.891189157
			3	3	-2.67762697	-2.209500848	-2.697862833
				5	-2.736429542	-2.342424772	-2.822763484
			4	3	-2.659243758	-2.335177799	-2.695671659
				5	-2.764034125	-2.266838626	-2.789758423

26 Three- to 4-month-old male ferrets were inoculated intranasally with 10⁶ TCID₅₀ of virus. Four 6-week-old, specific-pathogen-free

27 chickens were inoculated with 10^6 50% egg infectious doses (EID₅₀). Viral genes were amplified and sequenced from RNA extracted

28 from nasal washes (ferrets) or oropharyngeal swabs (chickens) on the indicated day pi. Mean variation frequency for each sample was

29 calculated using positions that are variable in at least one of the examined samples. If a site has a minor variant frequency above zero

30 in any of the samples this was included in the nominator and the denominator of the proportion was the total number of variable sites

31 that was variable in all samples. Variation frequency was expressed as the negative log.

32 DPI, day post-inoculation when mutation was detected; N/A, not applicable.

34	Supplementary Table 5 Genetic diversification of A/Anhui/1/2013 (H7N9) influenza viruses during two rounds of
35	experiments in ferrets.

Experiment	Ferret	Ferre	DDI				Gene seg	ment			
round	group	t no.	DPI -	PB2	PB1	РА	НА	NP	NA	MP	NS
1	(Parenta	N/A	N/A	_	_	_	N141D (0.4211),	A373T	T10I	_	_
	l virus)						A143T (0.1250),	(0.1277)	(0.2283)		
	,						N167D (0.5824),	. ,			
							L235Q (0.2182),				
							Y501N (0.1454),				
							Y501C (0.1457)				
	Donor	D1	1	_	_	_	A143T (0.2322),	A373T	T10I	_	_
							N167D (0.2251)	(0.1036)	(0.1838)		
			4	_	_	N409S	N167D (0.1619).	A373T	T10I	_	_
						(0.1130)	L2350 (0.3133)	(0.1530)	(0.1019)		
		D2	1	_	_		N141D (0.1936),	A373T	T10I	_	_
							A143T (0.1922),	(0.1123)	(0.1822)		
							N167D (0.3266)		· · · ·		
		D3	1	_	_	_	N141D (0.1561),	A373T	T10I	_	_
							A143T (0.2241),	(0.1078)	(0.2023)		
							N167D (0.2254).	()			
							Y501N (0.1688),				
							Y501C (0.1628)				
			4	_	_	_	N141D (0.2861),	A373T	T10I	_	_
							A143T (0.1873).	(0.1371)	(0.2098)		
							N167D (0.3769),		· · · ·		
							L235Q (0.3691),				
							Y501N (0.1782),				
							Y501C (0.1782)				
		D4	1	_	_	_	N141D (0.2087),	A373T	T10I	_	_
							N167D (0.3380)	(0.1199)	(0.2392)		
			4	_	_	_	N141D (0.2391),	A373T	` _ ´	_	_
							N167D (0.4117),	(0.1408)			
							L235Q (0.2562)	. ,			
	Direct	DC1	6	A674T	_	_	_ `` `	_	_	_	_
	Contact			(0.9712)							
	Direct	DC2	6	` ´	_	_	A228E (0.9880)	_	T10I	_	_
	Contact								(0.6308),		

		DC4	6	_	D76N (0.5316)	_	N141D (0.1818), N167D (0.4178), A228E (0.1173), L235Q (0.1737)	L193M (0.1111), M352I (0.1887)	R103K (0.9576) T10I (0.2404), I26M (0.1266), N340I (0.1511)	_	-
			8	_	D76N (0.2793)	_	N141D (0.2464), N167D (0.5005), A228E (0.1004), L235Q (0.1174)	_		_	_
A C	Aerosol Contact	AC4	8	_	D76N (0.4320)	_	_	I365V (0.6387)	E73K (0.4058), I300V (0.4103)	_	_
2 I	Donor	D1-2	2	_	D76N (0.3177)	_	K414E (0.1563), Y501N (0.3182), Y501C (0.3182)	I365V (0.6163)	E73K (0.3941), I300V (0.3451)	_	_
I	Donor	D2-2	2	_	D76N (0.3887)	_	Y501N (0.3137), Y501C (0.3137)	I365V (0.6236)	E73K (0.4348), I300V (0.3739)	_	_
			4	_	D76N (0.2604)	_	_	1365V (0.4976)	E73K (0.2933), I300V (0.2848)	_	_
		D3-2	2	_	D76N (0.2993)	_	K414E (0.1455), Y501N (0.1783), Y501C (0.1719)	I365V (0.6174)	E73K (0.3309), I300V (0.4053)	_	_
			4		D76N (0.2016)		Y501N (0.1449), Y501C (0.1439)	1365V (0.5547)	E73K (0.4471), I300V (0.4426)		

- 37 Three to 4-month-old male ferrets were inoculated intranasally with 10^6 TCID₅₀ of virus. Viral genes were amplified and sequenced
- 38 from RNA extracted from nasal washes on the indicated day pi. Shown are all amino acid variations that differed from the consensus
- 39 parental sequence and were present at a frequency of ≥ 0.1 . Numbers in parentheses indicate the frequency at which each mutation
- 40 occurred under each condition.
- 41 DPI, day post inoculation when mutation was detected; NA, not applicable; –, no nonsynonymous mutations were detected at a
- 42 frequency ≥ 0.1 .
- 43
- 44

Primer	Description	Sequence $(5^{\circ} \rightarrow 3^{\circ})$
N9 E73K F	E73K site-directed mutagenesis	CAAACATCACCAACATCCAAATGAAAGAGAGAACAAG
N9 E73K R	E73K site-directed mutagenesis	GAAATTCCTGCTTGTTCTCTCTTTCATTTGGATGTTG
N9 I300V F	1300V site-directed mutagenesis	GCAGGGCTCAAATAGACCAGTGGTTCAGATAGACCC
N9 I300V R	1300V site-directed mutagenesis	GTGTCATTGCTACTGGGTCTATCTGAACCACTGGTCTATTTG
N9 ClaI/- F	ClaI site deletion	GTGGACCAGCAATAGTATAGTTTCGATGTGTTCCAGTACAG
N9 ClaI/- R	ClaI site deletion	CCAGGAATTCTGTACTGGAACACATCGAAACTATACTAT
PB2 K627E F	K627E site-directed mutagenesis	CCATTTGCAGCAGCCCGGCGGAGCAGAGTAGG
PB2 K627E R	K627E site-directed mutagenesis	CGGCGGGGCTGCTGCAAATGGTAATAGCTT
PB1 D76N F	D76N site-directed mutagenesis	ATTGATGGACCATTACCTGAGAACAACGAGCCGA
PB1 D76N R	D76N site-directed mutagenesis	CTCAGGTAATGGTCCATCAATTGGATTGAGT
I365V F	1365V site-directed mutagenesis	GCTATCCACTAGAGGGGTTCAAGTTGCTTCAAAT
I365V R	1365V site-directed mutagenesis	TTGAACCCCTCTAGTGGATAGCTGTCCTCTT
N9 ClaI F	N-terminal ClaI restriction site insertion for pCAAGS cloning	TTACCATCGATATGAATCCAAATCAGAAGATTC
N9 NheI HA-tag R	C-terminal NheI restriction site and HA- tag insertion	TTTCGCGCTAGCTTAAGCGTAATCTGGAACATCGTATGGGTAGAGGAAGTACT CTATTTTAGCC
N9 NheI R	C-terminal NheI restriction site insertion	TTTCGCGCTAGCTTAGAGGAAGTACTCTATTTTAGCC

45 Supplementary Table 6 | List of primers used in the study.

47 Supplementary Table 7 | List of sialic acid-containing glycans used in glycan arrays

Chart number	Name	NeuAcα-	Туре	Sulfated
1	$Gal\beta(1-4)$ -GlcNAc β -ethyl-NH ₂	_	Type 2	
2	$\label{eq:Galbachar} \begin{split} Gal\beta(1-4)-GlcNAc\beta(1-2)-Man\alpha(1-3)-[Gal\beta(1-4)-GlcNAc\beta(1-2)-Man\alpha(1-6)]-Man\beta(1-4)-GlcNAc\beta(1-4)-GlcNAc\beta-Asn-NH_2 \end{split}$	_	Ν	
3	$NeuAca(2-3)$ -Gal $\beta(1-4)$ -6-O-sulfo-GlcNAc β -propyl-NH ₂	3	Type 2	6S-GlcNAc
4	$NeuAca(2-3)$ -Gal $\beta(1-4)$ -[Fuca(1-3)]-6-O-sulfo-GlcNAc β -propyl-NH ₂	3	Type 2	6S-GlcNAc
5	$NeuAca(2-3)-6-O-sulfo-Gal\beta(1-4)-GlcNAc\beta-ethyl-NH_2$	3	Type 2	6S-Gal
6	$NeuAc\alpha(2-3)-6-O-sulfo-Gal\beta(1-4)-[Fuc\alpha(1-3)]-GlcNAc\beta-propyl-NH_2$	3	Type 2	6S-Gal
7	$NeuAca(2-3)$ -Gal $\beta(1-3)$ -6-O-sulfo-GlcNAc β -propyl-NH ₂	3	Type 1	6S-GlcNAc
8	$NeuAca(2-3)$ - $Gal\beta(1-4)$ - $Glc\beta$ -ethyl- NH_2	3	Lacto	
9	$NeuAca(2-3)$ - $Gal\beta(1-4)$ - $GlcNAc\beta$ -ethyl- NH_2	3	Type 2	
10	$NeuAca(2-3)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta-ethyl-NH_2$	3	Type 2	
11	$NeuAca(2-3)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4$	3	Type 2	
12	NeuAca(2-3)-GalNAc β (1-4)-GlcNAc β -ethyl-NH ₂	3	LacDiNAc	
13	$NeuAca(2-3)$ - $Gal\beta(1-3)$ - $GlcNAc\beta$ -ethyl- NH_2	3	Type 1	
14	NeuAca(2-3)-Gal β (1-3)-GlcNAc β (1-3)-Gal β (1-4)-GlcNAc β -ethyl-NH ₂	3	Type 2	

15	$NeuAca(2-3)-Gal\beta(1-3)-GlcNAc\beta(1-3)-Gal\beta(1-3)-GlcNAc\beta-ethyl-NH_2$	3	Type 1
16	$NeuAca(2-3)-Gal\beta(1-3)-GalNAc\beta(1-3)-Gala(1-4)-Gal\beta(1-4)-Glc\beta-ethyl-NH_2$	3	Lacto
17	NeuAca(2-3)-Gal β (1-3)-GalNAca-Thr-NH ₂	3	Core 1
18	NeuAca(2-3)-Gal β (1-3)-[GlcNAc β (1-6)]-GalNAca-Thr-NH ₂	3	Core 2
19	$NeuAc\alpha(2-3)-Gal\beta(1-4)-GlcNAc\beta(1-6)-[Gal\beta(1-3)]-GalNAc\alpha-Thr-NH_2$	3	Core 2
20	$NeuAc\alpha(2-3)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta(1-6)-[Gal\beta(1-3)]-GalNAc\alpha-Thr-NH_2$	3	Core 2
21	NeuAca(2-3)-Gal β (1-4)-GlcNAc β (1-3)-GalNAca-Thr-NH ₂	3	Core 3
22	$NeuAc\alpha(2-3)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta(1-3)-GalNAc\alpha-Thr-NH_2$	3	Core 3
23	NeuAca(2-3)-Gal β (1-4)-GlcNAc β (1-3)-[NeuAca(2-3)-Gal β (1-4)-GlcNAc β (1-6)]-GalNAca The NU	3	Core 4
24	NeuAca(2-3)-Gal β (1-4)-GlcNAc β (1-3)-Gal β (1-4)-GlcNAc β (1-3)-[NeuAca(2-3)-Gal β (1-4)-GlcNAc β (1-3)-Gal β (1-4)-GlcNAc β (1-6)]-GalNAc α -Thr-NH ₂	3	Core 4
25	$\label{eq:label} NeuAca(2-3)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Mana(1-3)-[NeuAca(2-3)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Mana(1-6)]-Man\beta(1-4)-GlcNAc\beta(1-4)-GlcNAc\beta-Asn-NH_2$	3	N
26	$\label{eq:label} \begin{split} NeuAc\alpha(2-3)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Man\alpha(1-3)-\\ [NeuAc\alpha(2-3)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Man\alpha(1-6)]-Man\beta(1-4)-GlcNAc\beta(1-4)-GlcNAc\beta-Asn-NH_2 \end{split}$	3	Ν
27	$\label{eq:second} \begin{split} \text{NeuAca(2-3)-Gal}\beta(1-4)-\text{GlcNAc}\beta(1-3)-\text{Gal}\beta(1-4)-\text{GlcNAc}\beta(1-2)-\text{GlcNAc}\beta(1-3)-\text{Gal}\beta(1-4)-\text{GlcNAc}\beta(1-3)-\text{Gal}\beta(1-4)-\text{GlcNAc}\beta(1-3)-\text{Gal}\beta(1-4)-\text{GlcNAc}\beta(1-3)-\text{Gal}\beta(1-4)-\text{GlcNAc}\beta(1-2)-\text{Man}\alpha(1-6)]-\text{Man}\beta(1-4)-\text{GlcNAc}\beta(1-4)-\text{GlcNAc}\beta-\text{Asn-NH}_2 \end{split}$	3	Ν

28	$NeuAca(2-3)-[GalNAc\beta(1-4)]-Gal\beta(1-4)-GlcNAc\beta-ethyl-NH_2$	3	Type 2	
29	$NeuAca(2-3)-[GalNAc\beta(1-4)]-Gal\beta(1-4)-Glc\beta-ethyl-NH_2$	3	Ganglio	
30	$Gal\beta(1-3)-GalNAc\beta(1-4)-[NeuAca(2-3)]-Gal\beta(1-4)-Glc\beta-ethyl-NH_2$	3	Ganglio	
31	$NeuAca(2-3)$ - $Gal\beta(1-4)$ -[Fuca(1-3)]- $GlcNAc\beta$ -propyl- NH_2	3	Type 2	
32	$NeuAca(2-3)-Gal\beta(1-3)-[Fuca(1-4)]-GlcNAc\beta(1-3)-Gal\beta(1-4)-[Fuca(1-3)]-GlcNAc\beta-ethyl-NH_2$	3	Type 2	
33	$NeuAca(2-3)-Gal\beta(1-4)-[Fuca(1-3)]-GlcNAc\beta(1-3)-Gal\beta(1-4)-[Fuca(1-3)]-GlcNAc\beta-ethyl-NH_2$	3	Type 2	
34	$\label{eq:linear} \begin{split} NeuAca(2-3)-Gal\beta(1-4)-[Fuca(1-3)]-GlcNAc\beta(1-3)-Gal\beta(1-4)-[Fuca(1-3)]-GlcNAc\beta(1-3)-Gal\beta(1-4)-[Fuca(1-3)]-GlcNAc\beta-ethyl-NH_2 \end{split}$	3	Type 2	
35	$NeuGca(2-3)$ -Gal $\beta(1-4)$ -GlcNAc β -ethyl-NH ₂	3-Gc	Type 2	
36	$NeuAca(2-6)$ -Gal $\beta(1-4)$ -6-O-sulfo-GlcNAc β -propyl-NH ₂	6	Type 2	6S-GlcNAc
37	$NeuAca(2-6)$ - $Gal\beta(1-4)$ - $Glc\beta$ -ethyl- NH_2	6	Lacto	
38	$NeuAca(2-6)$ -Gal $\beta(1-4)$ -GlcNAc β -ethyl-NH ₂	6	Type 2	
39	$NeuAca(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta-ethyl-NH_2$	6	Type 2	
40	$NeuAca(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta-ethyl-NH_2$	6	Type 2	
41	$NeuAc\alpha(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-3)-[NeuAc\alpha(2-6)]-Gal\beta(1-4)-GlcNAc\beta-ethyl-NH_2$	6	Type 2	
42	$NeuAca(2-6)$ -GalNAc $\beta(1-4)$ -GlcNAc β -ethyl-NH ₂	6	LacDiNAc	

43	NeuAca(2-6)-[Gal β (1-3)]-GalNAca-Thr-NH ₂	6	Core 1
44	$NeuAc\alpha(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-6)-[Gal\beta(1-3)]-GalNAc\alpha-Thr-NH_2$	6	Core 2
45	$NeuAc\alpha(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta(1-6)-[Gal\beta(1-3)]-GalNAc\alpha-Thr-NH_2$	6	Core 2
46	$NeuAca(2-6)$ -Gal $\beta(1-4)$ -GlcNAc $\beta(1-3)$ -GalNAc α -Thr-NH ₂	6	Core 3
47	$NeuAc\alpha(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta(1-3)-GalNAc\alpha-Thr-NH_2$	6	Core 3
48	NeuAca(2-6)-Gal β (1-4)-GlcNAc β (1-3)-[NeuAca(2-6)-Gal β (1-4)-GlcNAc β (1-6)]-GalNAca-Thr-NH ₂	6	Core 4
49	$eq:loss_start_s$	6	Core 4
50	$\label{eq:Galback} \begin{split} Gal\beta(1-4)-GlcNAc\beta(1-2)-Man\alpha(1-3)-[NeuAc\alpha(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Man\alpha(1-6)]-Man\beta(1-4)-GlcNAc\beta(1-4)-GlcNAc\beta-Asn-NH_2 \end{split}$	6	N
51	$\label{eq:linear} \begin{split} NeuAc\alpha(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Man\alpha(1-3)-[Gal\beta(1-4)-GlcNAc\beta(1-2)-Man\alpha(1-6)]-Man\beta(1-4)-GlcNAc\beta(1-4)-GlcNAc\beta-Asn-NH_2 \end{split}$	6	N
52	$\label{eq:GlcNAc} GlcNAc\beta(1-2)-Man\alpha(1-3)-[NeuAc\alpha(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Man\alpha(1-6)]-Man\beta(1-4)-GlcNAc\beta(1-4)-GlcNAc\beta-Asn-NH_2$	6	N
53	$\label{eq:label} NeuAca(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Mana(1-3)-[NeuAca(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Mana(1-6)]-Man\beta(1-4)-GlcNAc\beta(1-4)-GlcNAc\beta-Asn-NH_2$	6	N
54	$\label{eq:second} \begin{split} \text{NeuAca(2-6)-Gal}\beta(1-4)-\text{GlcNAc}\beta(1-3)-\text{Gal}\beta(1-4)-\text{GlcNAc}\beta(1-2)-\text{Man}\alpha(1-3)-\\ [\text{NeuAc}\alpha(2-6)-\text{Gal}\beta(1-4)-\text{GlcNAc}\beta(1-3)-\text{Gal}\beta(1-4)-\text{GlcNAc}\beta(1-2)-\text{Man}\alpha(1-6)]-\text{Man}\beta(1-4)-\text{GlcNAc}\beta(1-4)-\text{GlcNAc}\beta-\text{Asn-NH}_2 \end{split}$	6	Ν

55	$\label{eq:alpha} \begin{split} NeuAc\alpha(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Man\alpha(1-3)-[NeuAc\alpha(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta(1-3)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Man\alpha(1-6)]-Man\beta(1-4)-GlcNAc\beta(1-4)-GlcNAc\beta-Asn-NH_2 \end{split}$	6	Ν	
56	$NeuGca(2-6)$ -Gal $\beta(1-4)$ -GlcNAc β -ethyl-NH ₂	6-Gc	Type 2	
57	$\label{eq:alpha} \begin{split} NeuAc\alpha(2-3)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Man\alpha(1-3)-[NeuAc\alpha(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Man\alpha(1-6)]-Man\beta(1-4)-GlcNAc\beta(1-4)-GlcNAc\beta-Asn-NH_2 \end{split}$	3/6	Ν	
58	$NeuAca(2-6)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Mana(1-3)-[NeuAca(2-3)-Gal\beta(1-4)-GlcNAc\beta(1-2)-Mana(1-6)]-Man\beta(1-4)-GlcNAc\beta(1-4)-GlcNAc\beta-Asn-NH_2$	3/6	Ν	