TABLE S1. Arithmetic mean normalized HI titers.
TABLE S2. Arithmetic mean normalized MN titers.
TABLE S3. Antigenic distance changes between mutant influenza viruses and their respective parent influenza viruses using microneutralization data.

position [a]			Mutant [b]	Antigenic Distance Change [c]	Position [a]			Mutant [b]	Antigenic Distance Change [c]
H5	H3	H1			H5	H3	H1		
45	54(C)	48	04-VNM-D45N	2.7781	140	144(A)	144	04-VNM-K140N	2.9008
			07-HKG-N45D	0.1475	141	145(A)	145(Ca2)	04-VNM-S141F	2.9572
83	91(E)	87	04-VNM-A83I	2.8002				04-VNM-S141Y	3.0131
			05-MNG-I83A	2.4771	282	285	286	04-VNM-M282I	2.1379
129	133(A)	133	04-VNM-L129S	3.0389				05-MNG-I282M	2.3785

[a] The positions are numbered based on the amino-acid position in the HA of H5N1 HPAIV and corresponding positions in HA of H3N2 influenza A virus, respectively. All of these positions were predicted as antigenicity associated sites except for position 129; [b] The 6+2 mutants generated by using site-directed mutagenesis and reverse genetics were named according to HA/NA donor-mutation, 04-VNM denotes A/Vietnam/1203/04(H5N1), 05-MNG A/whooper swan/Mongolia/244/05(H5N1), and 07-HKG A/common magpie/Hong Kong/5052/07(H5N1); [c] These antigenic distances were measured by using antigenic cartography (http://sysbio.cvm.msstate.edu/AntigenMap) [23, 24, 25] using microneutralization (MN) data. One grid unit corresponds to a 2-fold change in the MN assay–measured titer.

Legends for Supplementary Figure

FIG. S1. The antigenic cartography of H5N1 HPAIVs and their mutants (listed in TABLE 4) made by using AntigenMap (http://sysbio.cvm.msstate.edu/AntigenMap) (5, 6). The mutant positions were numbered based on the corresponding residue number in HA of H5N1 HPAIVs. Cartography based on the results from microneutralization assays. The parental viruses are labeled with heavily colored circles, and the mutants with lightly colored circles. The parental viruses are also encircled (circle = 2-unit diameter). A symbol (p) is appended after the name of each parent strain. One unit (grid) corresponds to a 2-fold change in the MN assay measured titer.

TABLE S1. Arithmetic mean normalized HI titers.

Titers obtained with various ferret antisera

Antigens	An	Ab	Ac	Ad	Ba	Bb	Be	Bd	Ca	Cb	Cc	Cd	Du	Db	Dc	Dd	1-04-VNa	1-04-VNb	1-04-VNc	1-04-VNd	2.2-05-WSa	2.2-05-WSb	2.2-05-WSc	2.2-05-WSd	2.1-02-Hma	2.1-02-Hmb	2.1-02-Hmc	2.1-02-Hmd	2.3.4-06-Laoa	2.3.4-06-Laob	2.3.4-06-Laoc	2.3.4-06-Laod	2.3.4-06-JWEa	2.3.4-06-JWEb	2.3.4-06-JWEc	2.3.4-06-JWEd	4-06-GGa	4-06-GGb	4-06-GGc	4-06-GGd	1-03-HK213a	1-03-HK213b	1-03-HK213c	0-97-HK156	2.3.4-08-AP	2.3.2-07-CMP	2.3.2-08-gh	2.2-05-F5	2.2-07-F7
A	1(40)	1(40)	1(20)	1(80)	0.5	0.5	0.25	0	0.25	0.5	0.5	0.25	0.333333333	0.166666667	0.5	0.125	1	1	1	1	0.25	0.333333333	0.25	0.5	0.25	1	1	0.5	0	0.25	0.166666667	0	0	0.125	0.125	0.25	0.0625	0	0	0	0	0	0	0	0.5	0	0	1	0.5

TABLE S2. Arithmetic mean normalized MN titers

Antigens	Titers obtained with various ferret antisera															
	Aa	**Ab**	**Ac**	**Ad**	**Ba**	**Bb**	**Bc**	**Bd**	**Ca**	**Cb**	**Cc**	**Cd**	**Da**	**Db**	**Dc**	**Dd**
A	1(640)	1(480)	1(640)	1(640)	0.375	1.333333333	1	1	0.166666667	0.25	0.25	0.166666667	0.5	0.375	1	1
B	0.75	2	1	1	1(1280)	1(480)	1(640)	1(640)	0.083333333	0.375	0.25	0.166666667	0.75	0.375	0.375	0.333333333
C	1.5	2.666666667	2	2	1	1.333333333	0.5	4	1(960)	1(320)	1(1280)	1(960)	2	0.5	1	1.333333333
D	0.75	1	0.5	1	0.25	0.333333333	0.5	0.5	0.333333333	0.25	0.25	0.083333333	1(320)	1(640)	1(320)	1(240)
04-VNM	0.125	0.25	0.125	0.125	0.0625	0.083333333	0.125	0.125	0.125	0.125	0.03125	0.166666667	0.125	0.0625	0.25	0.083333333
05-MNG	0.125	0.333333333	0.125	0.25	0.125	0.333333333	0.1875	0.125	0.083333333	0.125	0.0625	0.166666667	1	0.375	2	1.333333333
02-CHN	0.125	0.166666667	0.0625	0.125	0.0625	0.166666667	0.09375	0.125	0.083333333	0.125	0.046875	0.0625	0.125	0.125	0.25	0.333333333
06-LAO	0.015625	0.041666667	0.015625	0.0078125	0.00390625	0.010416667	0.0234375	0.0234375	0	0	0	0.005208333	0	0	0	0
06-HKG	0.0625	0.166666667	0.125	0.125	0.046875	0.083333333	0.09375	0.09375	0.083333333	0.0625	0.03125	0.041666667	0.1875	0.125	0.1875	0.166666667
06-CHN	0.125	0.25	0.125	0.25	0.0625	0.125	0.125	0.125	0.041666667	0.1875	0.0234375	0.083333333	0.125	0.0625	0.25	0.25
03-HKG	1	1.333333333	1	1	0.5	0.666666667	0.5	2	1	0.25	1	1.333333333	1.5	0.75	3	4
97-HKG	0.015625	0.020833333	0.0078125	0.046875	0.00390625	0.020833333	0	0	0	0	0	0	0	0	0	0
08-HKG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
07-HKG	0	0	0.0078125	0	0	0	0	0.015625	0	0	0	0	0.015625	0	0	0.020833333
08-HKG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
05-SAU	0.03125	0.041666667	0.03125	0.015625	0.03125	0.0625	0.0078125	0.015625	0	0	0.015625	0.010416667	0.1875	0.046875	0.25	0.25
07-SAU	0.375	0.5	0.25	0.125	0.0625	0.166666667	0.125	0.375	0.041666667	0.046875	0.046875	0.083333333	0.5	0.375	0.5	2
07-KHM	0	0	0	0	0	0	0	0	0	0	0	0	0.015625	0.0078125	0	0
07-EGY	0.0625	0.125	0.046875	0.046875	0.03125	0.03125	0.125	0.0625	0.010416667	0	0.015625	0.005208333	0.1875	0.1875	0.1875	0.333333333
06-NGA	0	0.03125	0.015625	0.03125	0.01171875	0	0	0	0	0	0.01171875	0	0.0625	0.015625	0.25	0.083333333
08-EGY	0	0	0	0	0	0	0.0078125	0	0	0	0.00390625	0	0	0	0.015625	0
04-VNM2	0.015625	0.020833333	0	0.0234375	0.0078125	0	0	0	0	0	0.00390625	0.03125	0	0	0	0
07-VNM	0	0	0	0	0	0	0	0	0	0	0	0.020833333	0	0	0	0
07-LAO	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
05-KHM	0.0078125	0.03125	0	0.0078125	0.0078125	0	0	0	0.005208333	0	0.00390625	0.010416667	0	0	0	0
04-CHN	0.25	0.333333333	0.25	0.125	0.125	0.166666667	0.5	0.1875	0.041666667	0.03125	0.03125	0.020833333	0.25	0.5	0.5	1.333333333
05-CHM	0.0078125	0.020833333	0	0.0078125	0.00390625	0	0	0	0	0	0	0	0	0	0.0625	0
VN1203	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 D45N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 A83I	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 M282I	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CMP N45D	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
WS244 I83A	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
WS244 I282M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 K140N	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 S141F	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 S141Y	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 L129S	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Antigens	**1-04-VNa**	**1-04-VNb**	**1-04-VNc**	**1-04-VNd**	**2.2-05-WSa**	**2.2-05-WSb**	**2.2-05-WSc**	**2.2-05-WSd**	**2.1-02-Huna**	**2.1-02-Hunb**	**2.1-02-Hunc**	**2.1-02-Hund**
A	0.5	0.5	0.5	0.25	0.03125	0.0625	1.333333333	2	0.125	0.333333333	0.1875	0.1875
B	0	0	0.0625	0	0.03125	0.0625	1.333333333	2	1	1.333333333	0.5	1
C	0.25	0.75	0.375	0.375	0.03125	0.09375	2	4	1	1	0.75	0.5
D	0.03125	0.25	0.03125	0	0.0625	0.125	4	4	1.5	2	1	2
04-VNM	1(320)	1(80)	1(160)	1(40)	0.046875	0.0625	1	1	0.0625	0.333333333	0.125	0.125
05-MNG	0.25	0.5	0.25	1	1(640)	1(640)	1(30)	1(20)	1	1.333333333	0.25	1
02-CHN	0.0625	0.25	0.0625	0.125	0.03125	0.0625	2.666666667	1	1(1280)	1(480)	1(1280)	1(640)
06-LAO	0	0	0	0	0	0	0	0	0.015625	0.03125	0.015625	0.03125
06-HKG	0.03125	0	0.0625	0	0	0.0078125	0.666666667	0	0.0078125	0.0625	0.015625	0.0234375
06-CHN	0.5	0.5	0.5	1	0.03125	0.0234375	0.666666667	1	0.0625	0.083333333	0.125	0.125
03-HKG	0.5	0.25	0.5	0.375	0.015625	0.125	5.333333333	6	0.375	0.25	0.125	0.0625
97-HKG	0.0625	0	0.09375	0	0	0	0.166666667	0.25	0	0	0	0
08-HKG	0	0	0	0	0	0	0	0	0	0	0	0
07-HKG	0	0	0	0	0	0.015625	0	0	0.00390625	0	0.015625	0.0078125
08-HKG	0	0	0	0	0.0625	0.0078125	0.666666667	0.5	0	0	0.0078125	0
05-SAU	0.03125	0.125	0	0	0.125	0.125	5.333333333	4	0.125	0.333333333	0.0625	0.125
07-SAU	0.015625	0.125	0.03125	0	0.0625	0.125	5.333333333	2	0.1875	0.166666667	0.0625	0.25
07-KHM	0.03125	0	0.03125	0	0	0	0	0	0	0	0.015625	0
07-EGY	0.015625	0	0	0	0.015625	0.046875	0.666666667	0.75	0.0625	0.083333333	0.03125	0.09375
06-NGA	0.03125	0	0	0	0.125	0.1875	10.66666667	4	0.1875	0.0625	0.0234375	0.1875
08-EGY	0	0	0	0	0.09375	0.046875	2.666666667	1.5	0.01171875	0.010416667	0	0.0234375
04-VNM2	0.1875	0.375	0.1875	0.25	0	0.0078125	0	0	0	0.041666667	0.015625	0.015625
07-VNM	0.09375	0.0625	0.0625	0.25	0	0	0	0	0	0.041666667	0.015625	0.015625
07-LAO	0	0	0	0	0	0.0078125	0.666666667	0	0.0078125	0.041666667	0.0234375	0.03125
05-KHM	0.0625	0.25	0.09375	0.25	0	0.0078125	0	0	0.0078125	0.041666667	0.015625	0.0234375
04-CHN	0.015625	0	0.03125	0	0.0078125	0.046875	1.333333333	0.75	0.1875	0.333333333	0.125	0.375
05-CHM	0.09375	0.125	0.0625	0.25	0	0.015625	0.5	0	0	0.083333333	0.03125	0.0625
VN1203	1	1	1	1	0.0469	0.0625	1	1	0.33333	0.5	0.125	ND
VN1203 D45N	0.25	0.25	0.25	0.25	0	0.015625	0.333333333	0	0.020833333	0.03125	0.03125	ND
VN1203 A83I	0.25	0.5	0.125	0	0	0	0.333333333	0	0.041666667	0.03125	0.015625	ND
VN1203 M282I	1	1	1	0.5	0	0.0625	0.333333333	0	0.166666667	0.0625	0.125	ND
CMP N45D	0	0.125	0.0625	0.25	0.015625	0	0.666666667	0	0	0.015625	0.015625	ND
WS244 I83A	0.03125	0	0	0	0.03125	0.125	5.333333333	1	0.083333333	0.0625	0.25	ND
WS244 I282M	0.03125	0	0.0625	0.25	0.25	0.125	5.333333333	4	0.166666667	0.0625	0.125	ND
VN1203 K140N	0.0625	0.125	0.0625	0.25	0	0	0.333333333	0	0	0	0	ND
VN1203 S141F	0.03125	0.125	0.125	0.25	0	0	0	0	0	0	0	ND
VN1203 S141Y	0.0625	0.125	0.0625	0	0	0	0	0	0.020833333	0.015625	0.015625	ND
VN1203 L129S	0	0	0	0	0	0	0	0	0.041666667	0	0	ND

Antigens	**2.3.4-06-Laoa**	**2.3.4-06-Laob**	**2.3.4-06-Laoc**	**2.3.4-06-Laod**	**2.3.4-06-JWEa**	**2.3.4-06-JWEb**	**2.3.4-06-JWEc**	**2.3.4-06-JWEd**	**4-06-GGa**	**4-06-GGb**	**4-06-GGc**	**4-06-GGd**
A	4	4	4	4	0.5	0.333333333	0.083333333	0.333333333	0.03125	0.0078125	0.083333333	0.01171875
B	2	1	1.5	2	0.25	0.125	0.0625	0.333333333	0.0078125	0.005859375	0.041666667	0.0078125
C	4	2	1.5	2	1	0.666666667	0.166666667	0.666666667	0.0234375	0.0078125	0.083333333	0.015625
D	4	1.5	3	2	0.5	0.333333333	0.166666667	0.333333333	0.00390625	0.0078125	0.041666667	0.005859375
04-VNM	4	3	2	2	0.0625	0.083333333	0.083333333	0.166666667	0.125	0.0625	0.25	0.0625
05-MNG	4	1.5	4	2	0.1875	0.125	0.333333333	0.5	0.046875	0.0625	0.25	0.0625
02-CHN	4	2	4	2	0.0625	0.083333333	0.083333333	0.25	0.0078125	0.00390625	0.0625	0.015625
06-LAO	1(20)	1(40)	1(40)	1(40)	0	0	0.03125	0	0	0	0	0
06-HKG	1	0.5	2	1	1(160)	1(480)	1(480)	1(120)	0	0	0.020833333	0.001953125
06-CHN	3	2	2	4	0.125	0.083333333	0.166666667	0.666666667	1(1280)	1(2560)	1(480)	1(2560)
03-HKG	1	0	0.5	1	2	1.333333333	0.166666667	0.666666667	0	0	0.083333333	0.0078125
97-HKG	0	0	0	0	0	0	0	0	0	0.001953125	0	0
08-HKG	0	0	0	0	0	0	0	0	0	0	0	0
07-HKG	0	0	0	0	0	0	0	0	0	0	0	0
08-HKG	0	0	0	0	0	0	0	0	0	0	0	0
05-SAU	0.25	0	0.25	0.125	0	0	0	0	0.00390625	0	0	0.001953125
07-SAU	0.25	0	0.25	0	0.25	0.166666667	0.03125	0.041666667	0	0	0	0
07-KHM	0	0	0	0	0	0.010416667	0	0	0.0078125	0.0078125	0.010416667	0.005859375
07-EGY	0.25	0	0	0.125	0.125	0.083333333	0	0	0.01171875	0.00390625	0	0.001953125
06-NGA	0	0	0.25	0	0	0	0.020833333	0	0	0	0	0
08-EGY	0	0.125	0.125	0.375	0.0625	0.020833333	0.041666667	0.083333333	0	0	0	0
04-VNM2	0	0.125	0	0	0	0	0	0	0.015625	0.01171875	0.020833333	0.0078125
07-VNM	0	0	0	0	0	0	0	0	0.01171875	0.015625	0.041666667	0.0078125
07-LAO	1.5	1	0.375	0.375	0	0	0.010416667	0	0	0.00390625	0	0
05-KHM	0	0.125	0	0	0	0	0	0	0.015625	0.00390625	0.020833333	0.0078125
04-CHN	0	0	0.375	0	0.125	0.0625	0.03125	0.041666667	0	0	0	0
05-CHM	0.5	0.375	0.375	0	0	0	0	0	0	0.00390625	0.010416667	0.00390625
VN1203	1	3	2	2	0.0625	0.0833	0.0833	0.1667	0.125	0.0625	0.25	0.0625
VN1203 D45N	0	0	0	0	0	0	0	0	0.015625	0.015625	0.020833333	0.0078125
VN1203 A83I	0	0	0	0	0	0	0	0	0.03125	0.00390625	0.020833333	0.015625
VN1203 M282I	0	0.25	0	0.25	0	0	0	0	0.03125	0.015625	0.041666667	0.03125
CMP N45D	0	0	0	0	0	0	0	0	0	0	0	0.00390625
WS244 I83A	0	0	0	0	0	0.020833333	0	0	0	0	0	0
WS244 I282M	0	0	0	0	0	0.020833333	0	0	0	0	0	ND
VN1203 K140N	0	0	0	0	0	0	0	0	0	0.00390625	0	0.015625
VN1203 S141F	0	0	0	0	0	0	0	0	0	0	0	0
VN1203 S141Y	0	0	0	0	0	0	0	0	0	0	0	0
VN1203 L129S	0	0.25	0	0	0	0	0	0	0	0	0	0

Antigens	**1-03-HK213a**	**1-03-HK213b**	**1-03-HK213c**	**0-97-HK156**	**2.3.4-08-AP**	**2.3.2-07-CMP**	**2.3.2-08-gh**	**2.2-05-F5**	**2.2-07-F7**
A	0	0.125	0.25	8	0	0	0	0.09375	0.375
B	0	0	0	2	0	0	6	0.125	0.125
C	0.666666667	1	3	2.666666667	0.25	0.166666667	8	0.1875	0.5
D	0	0	0	1	0.125	0.125	12	0.09375	0.25
04-VNM	0	0	0	2.666666667	0	0.041666667	3	0.03125	0
05-MNG	0.333333333	0.25	0.125	0	0.25	0.125	16	0.5	0.0625
02-CHN	0	0	0	0.333333333	0.25	0.083333333	12	0.0625	0
06-LAO	0	0	0	0	0	0	0	0.0078125	0
06-HKG	0	0.25	0.125	0	0	0	0	0.0078125	0.125
06-CHN	0.333333333	0.125	0.5	1.333333333	0	0	0	0	0
03-HKG	1(30)	1(40)	1(40)	2.666666667	0.375	0.333333333	32	0.375	1
97-HKG	0	0	0	1(15)	0	0	0	0	0
08-HKG	0	0	0	0	1(40)	0	0	0	0
07-HKG	0	0	0	0	0	1(120)	8	0	0
08-HKG	0	0	0	0	0	0.333333333	1(5)	0.03125	0
05-SAU	0	0	0	0.666666667	0	0.25	0	1(640)	0.125
07-SAU	0	0.125	0	1.333333333	0.25	0.083333333	0	0.75	1(640)
07-KHM	ND	ND	ND	ND	ND	ND	ND	ND	ND
07-EGY	ND	ND	ND	ND	ND	ND	ND	ND	ND
06-NGA	ND	ND	ND	ND	ND	ND	ND	ND	ND
08-EGY	ND	ND	ND	ND	ND	ND	ND	ND	ND
04-VNM2	ND	ND	ND	ND	ND	ND	ND	ND	ND
07-VNM	ND	ND	ND	ND	ND	ND	ND	ND	ND
07-LAO	ND	ND	ND	ND	ND	ND	ND	ND	ND
05-KHM	ND	ND	ND	ND	ND	ND	ND	ND	ND
04-CHN	ND	ND	ND	ND	ND	ND	ND	ND	ND
05-CHM	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 D45N	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 A83I	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 M282I	ND	ND	ND	ND	ND	ND	ND	ND	ND
CMP N45D	ND	ND	ND	ND	ND	ND	ND	ND	ND
WS244 I83A	ND	ND	ND	ND	ND	ND	ND	ND	ND
WS244 I282M	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 K140N	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 S141F	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 S141Y	ND	ND	ND	ND	ND	ND	ND	ND	ND
VN1203 L129S	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note: Homologous normalized titers (in parentheses) were assigned a value of “1.” ND means not done and ″0″ means antigen and antibody does not react.
Antisera from four animals were tested against each antigen.

FIG. S1