

Supplementary Materials

Supplementary Table 1. The list of H5N1 and H1N1 viruses from which the codon optimized H5 and H1 HA were derived and used to make the influenza HA and NA pseudotype panel.

Original strains	(Sub)clades	Accession Number
A/Hongkong/156/97	0	AF028709
A/Thailand/1 (KAN-1)/04	1	AY555150
A/Vietnam/1203/04	1	EF541403
A/Indonesia/5/05	2. 1	EU146622
A/Turkey/65596/2006	2. 2	EF619998
A/Xingjaing/1/2006	2. 2	FJ492886
A/Common Magpie/Hong Kong/5052/2007	2. 3. 2	CY036173
A/Anhui/1/05	2. 3	DQ371928
A/Shenzhen/406H/06	2. 3	EF137706
A/Chichen/Guangxi/12/2004	2. 4	DQ366330
A/Chicken/Korea/es/2003	2. 5	AY676035
A/Silk Chicken/Hongkong/SF189/2001	3	AF509021
A/Goose/Guiyang/337/2006	4	DQ992765
A/Duck/Guangxi/1378/2004	5	DQ320884
A/Blackbird/Hunan/1/2004	6	AY741213
A/Duck/Hubei/wg/02	6	DQ997094
A/Beijing/01/2003	7. 1	EF587277
A/Chicken/Vietnam/NCVD-016/2008	7. 2	FJ842476
A/Chicken/Henan/16/2004	8	AY950234
A/Goose/Shantou/1621/2005	9	DQ095628
A/WSN/1933		CY034132

Supplementary Table 2. The list of primers for amplifying constant region sequences of $\gamma 1$, $\kappa 1$ and $\lambda 1$ and variable region sequences of VH, V κ and V λ of human immunoglobulin

Primer to amplify $\gamma 1$ -constant, $\kappa 1$ -constant and $\lambda 1$ -constant region

Pmt- $\gamma 1$ -constant-forward	GATACC AGATCT TACA GGTACC CCTCCACCAAG GGGCC CATC
Pmt- $\gamma 1$ -constant-reverse	CAGCGG TCTAGA GTTTAAACT TTATCA TTTACCCGGAGACAGGGAGAGG
Pmt- $\kappa 1$ -constant-forward	GATACC AGATCT CAC GGTACC GTACGG TGGCTGCACCATCTGTC
Pmt- $\kappa 1$ -constant-reverse	CAGCGG TCTAGA GTTTAAACT TTATCA ACACTCTCCCCTGTTGAAGCTC
pmt- $\lambda 1$ -constant-forward	GTTCCCGCC CTCGAG TGAGGAGCTCC
pmt- $\lambda 1$ -constant-reverse	GG TCTAGA GTTTAAAC TTATCA TGAACATTCTGTAGGGGCCACTG

Primer to amplify the variable region of immunoglobulin heavy and light chain

		Primer to amplify the variable region of immunoglobulin heavy		
primary PCR	forward primer	5' L-VH 1	ACAGGTGCCCACTCCCAGGTGCAG	
		5' L-VH 3	AAGGTGCCAGTGTGARGTGCAG	
		5' L-VH 4/6	CCCAGATGGGTCTGTCCCAGGTGCAG	
		5' L-VH 5	CAAGGAGTCTGTTCCGAGGTGCAG	
		reverse primer	3' C γ CH1	GGAAGGTGTGCACGCCGCTGGTC
			3' C μ CH1	GGGAATTCTCACAGGAGACGA
nest PCR	forward primer	5' Sfil VH1	TACTACT GGCCGTCGTGGC CTTTGTTGGCCTCTCGCTCGGG GTACATTCC CAGGTGCAGCTGGTGCAG	
		5' Sfil VH1/5	TACTACT GGCCGTCGTGGC CTTTGTTGGCCTCTCGCTCGGG GTACATTCC GAGGTGCAGCTGGTGCAG	
		5' Sfil VH3	TACTACT GGCCGTCGTGGC CTTTGTTGGCCTCTCGCTCGGG GTACATTCT GAGGTGCAGCTGGTGGAG	
		5' Sfil VH3-23	TACTACT GGCCGTCGTGGC CTTTGTTGGCCTCTCGCTCGGG GTACATTCT GAGGTGCAGCTGTTGGAG	
		5' Sfil VH4	TACTACT GGCCGTCGTGGC CTTTGTTGGCCTCTCGCTCGGG GTACATTCC CAGGTGCAGCTGCAGGAG	
		5' Sfil VH 4-34	TACTACT GGCCGTCGTGGC CTTTGTTGGCCTCTCGCTCGGG GTACATTCC CAGGTGCAGCTACAGCAGTG	
		5' Sfil VH 1-18	TACTACT GGCCGTCGTGGC CTTTGTTGGCCTCTCGCTCGGG GTACATTCC CAGGTTACAGCTGGTGCAG	
		5' Sfil VH 1-24	TACTACT GGCCGTCGTGGC CTTTGTTGGCCTCTCGCTCGGG GTACATTCC CAGGTCCAGCTGGTACAG	
		5' Sfil VH3-33	TACTACT GGCCGTCGTGGC CTTTGTTGGCCTCTCGCTCGGG GTACATTCT CAGGTGCAGCTGGTGGAG	
		5' Sfil VH 3-9	TACTACT GGCCGTCGTGGC CTTTGTTGGCCTCTCGCTCGGG GTACATTCT GAAGTGCAGCTGGTGGAG	
		5' Sfil VH4-39	TACTACT GGCCGTCGTGGC CTTTGTTGGCCTCTCGCTCGGG GTACATTCC CAGCTGCAGCTGCAGGAG	
		5' Sfil VH 6-1	TACTACT GGCCGTCGTGGC CTTTGTTGGCCTCTCGCTCGGG GTACATTCC CAGGTACAGCTGCAGCAG	
		reverse primer	3' Apal JH 1/2/4/5	GACCGAT GGGCC CTTGGTGGAG GCTGAGGAGACGGTGACCAG
			3' Apal JH 3	GACCGAT GGGCC CTTGGTGGAG GCTGAAGAGACGGTGACCATTG
			3' Apal JH 6	GACCGAT GGGCC CTTGGTGGAG GCTGAGGAGACGGTGACCCTG

Primer to amplify the variable region of immunoglobulin kappa chain

primary PCR	forward primer	5' L Vk 1/2	ATGAGGSTCCCYGCTCAGCTGCTGG			
		5' L Vk 3	CTCTTCTCCTGCTACTCTGGCTCCCAG			
		5' L Vk 4	ATTTCTCTGTTGCTCTGGATCTCTG			
	reverse primer	3' Ck 543	GTTTCTCGTAGTCTGCTTTGCTCA			
1st nest PCR	forward primer	5' Pan Vk	ATGACCCAGWCTCCABYCWCCCTG			
	reverse primer	3' Ck 494	GTGCTGTCCTTGCTGTCCTGCT			
2nd nest PCR	forward primer	5'Sfil Vk 1-5	TCGCCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	GTACATTCTGACATCCAGATGACCCAGTC		
		5'Sfil Vk 1-9	TCGCCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	GTACATTGACATCCAGTTGACCCAGTCT		
		5'Sfil Vk 1D-43	TCGCCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	GTACATTGTGCCATCCGGATGACCCAGTC		
		5'Sfil Vk 2-24	TCGCCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	GTACATGGGGATATTGTGATGACCCAGAC		
		5'Sfil Vk 2-28	TCGCCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	GTACATGGGGATATTGTGATGACTCAGTC		
		5'Sfil Vk 2-30	TCGCCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	GTACATGGGGATGTTGTGATGACTCAGTC		
		5'Sfil Vk 3-11	TCGCCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	GTACATTAGAAATGTTGACACAGTC		
		5' Sfil Vk 3-15	TCGCCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	GTACATTAGAAATGATGACGACAGTC		
		5' Sfil Vk 3-20	TCGCCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	GTACATTAGAAATGTTGACGACAGTC		
		5' Sfil Vk 4-1	TCGCCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	GTACATTGACATCGTATGACCCAGTC		
			reverse primer	3' BsiWI Jk 1/4	<i>GCCACCGTACG</i> TTTGATYTCCACCTTGGTC	
				3' BsiWI Jk 2	<i>GCCACCGTACG</i> TTTGATCTCCAGCTTGGTC	
				3' BsiWI Jk	<i>GCCACCGTACG</i> TTTGATATCCACTTTGGTC	
				3' BsiWI Jk 5	<i>GCCACCGTACG</i> TTTAATCTCCAGTCGTGTC	

Primer to amplify the variable region of immunoglobulin lambda chain

primary PCR	forward primer	5' L Vλ 1	GGTCTGGGCCCAGTCTGTGCTG	
		5' L Vλ 2	GGTCTGGGCCCAGTCTGCCCTG	
		5' L Vλ 3	GCTCTGTGACCTCCTATGAGCTG	
		5' L Vλ 4/5	GGTCTCTCTCSCAGCYTGCTG	
		5' L Vλ 6	GTTCTTGGGCAATTTTATGCTG	
		5' L Vλ 7	GGTCCAATTCYAGGCTGTGGTG	
		5' L Vλ 8	GAGTGGATTCTCAGACTGTGGTG	
			reverse primer	3' Cλ
nest PCR	forward primer	5'Sfil Vλ 1	CCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	TCCTGGGCCAGTCTGTGCTGACKCAG
		5' Sfil Vλ 2	CCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	TCCTGGGCCAGTCTGCCCTGACTCAG
		5' Sfil Vλ 3	CCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	TCTGTGACCTCCTATGAGCTGACWCAG
		5' Sfil Vλ 4/5	CCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	TCTCTCTCSCAGCYTGCTGACTCA
		5' Sfil Vλ 6	CCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	TCTTGGGCAATTTTATGCTGACTCAG
		5' Sfil Vλ 7/8	CCT <i>GGCCGTCGTGGCC</i> TTTGTGGCCTCTCGCTCGGG	TCCAATTCYAGRCTGTGGTGACYCAG
			reverse primer	3' XhoI Cλ

Restriction sites are indicated in bold and italic.

Supplementary Fig. 1. Protein sequences of VH and VL of antibodies 65C6, 3C11 and 100F4 and their VH and VL germline usages.

100F4 –LV

<u>ID%</u>			<-----FWR1----->	<---CDR1--->	<-----FWR2--->	<-CDR2>	<----->	
	100F4-LV	1	QSVLTQPPSVSGAPGQRVTISC	TGGSSNIGAGYSVH	WYQQLPGTAPKLLIY	GSNSRPS	GVPDRFSGSKSG	70
93.0 (93/100)	IGLV1-40*01	1S.....D..NSN...	70
<u>ID%</u>			---FWR3----->					
	100F4-LV	71	TSASLAITGLRPEDEADYYC	QSYDSSLSGS	100			
93.0 (93/100)	IGLV1-40*01	71QA.....	100			

100F4-HV

<u>ID%</u>			<-----FWR1----->	<-CDR1>	<-----FWR2--->	<-----CDR2----->	<---	
	100F4-HV	1	QLQLQESGLGLVKPSETLSLCTVSGDSVS	SGSYYS	WLRQPPGKLEWIG	NMHGSGHTNYNPSLKS	RVT	70
87.9 (87/99)	IGHV4-61*03	1	.V.....P.....G...I.....	YIYY..S.....	...	70
<u>ID%</u>			-----FWR3----->					
	100F4-HV	71	ITPDTSKNHFSRLSSVTAADTAVYYCAR	99				
87.9 (87/99)	IGHV4-61*03	71	.SV.....K.....	99				

65C6-LV

<u>ID%</u>		<-----FWR1----->	<---CDR1--->	<----FWR2--->	<--CDR2>	<----->	
	65C6-LV	1	EIVLTQSPLTSLVSPGERATLSC	RASQSVSSN-LA	WYQQMPGQAPRLLIY	GASTRAT	GIPARLSGSASGT 69
94.7 (90/95)	IGKV3D-15*01	1	...M...A.....-..	...K.....F...G...	69
<u>ID%</u>			--FWR3----->				
	65C6-LV	70	EFTLTISSLQSEDFAVYYC	QQYNNWP			95
94.7 (90/95)	IGKV3D-15*01	70			95

65C6-HV

<u>ID%</u>		<-----FWR1----->	<CR1>	<----FWR2--->	<-----CDR2----->	<---	
	65C6-HV	1	EVQLVQSGAEVKKPGESLRISCKGFAYSST	YFWIS	WVRQMPGKGPEWMG	RIDPTDSYINYSFSFG	HVTI 70
88.7 (86/97)	IGHV5-a*03	1SG..F. SY...L....	...S...T.....	70
<u>ID%</u>			-----FWR3----->				
	65C6-HV	71	SVDRSISTVYLVWSSLKASDTAMYYCA-				97
88.7 (86/97)	IGHV5-a*03	71	.A.K...A.....	R			98

3C11-KV

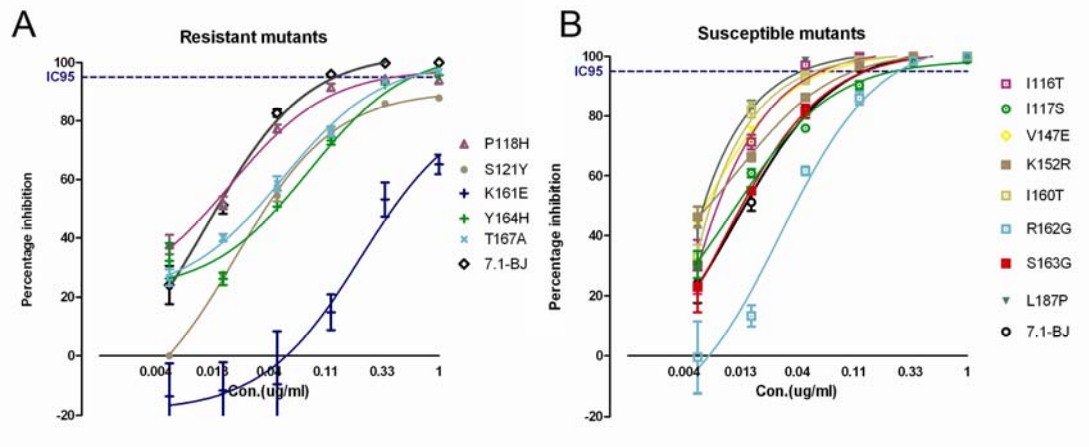
<u>ID%</u>			<-----FWR1----->	<----CDR1----->	<----FWR2-->	<-CDR2>	<-----	
	<u>3C11-KV</u>	1	EIVMTQSPLTLPVTPGAPASIS	RSSQSLHSD-G-YNYLD	WYLQKPGQSPQLLIY	LGSHRAS	GVPDRFS	68
95.0 (95/100)	<u>IGKV2D-28*01</u>	1	D.....S.....E.....N-.....N...	68
<u>ID%</u>			-----FWR3----->					
	<u>3C11-KV</u>	69	GSGSGTDFTLKISRVEAEDVGY	YQC	MQALQTP			100
95.0 (95/100)	<u>IGKV2D-28*01</u>	69				100

3C11-HV

<u>ID%</u>			<-----FWR1----->	<CR1>	<----FWR2-->	<-----CDR2----->	<---	
	<u>3C11-HV</u>	1	QVQLVQSGAEVKETGESLNISCKV	SGNFP	SYIIS	WVRQMPGNLEWMG	RIDPSDSDTNYRPSFQG	HVTI 70
85.7 (84/98)	<u>IGHV5-a*03</u>	1	E.....KP....R....G..YS.T	..W..K.....Y...S.....	70
<u>ID%</u>			-----FWR3----->					
	<u>3C11-HV</u>	71	SADKSTSTAYLQWRSLKASDTAMY	CAR				98
85.7 (84/98)	<u>IGHV5-a*03</u>	71I.....S.....				98

Results were obtained from IgbLAST(<http://www.ncbi.nlm.nih.gov/projects/igblast/>) using the mAbs Vh and Vh amino acids sequence. amino acid identities are shown as dots.

Supplementary Fig. 2. Titration of antibody 65C6 against 13 single surface mutants of HA as compared to parental subclade 7.1 HA by PN assay. A. Resistant mutants. B. Susceptible mutants.



Supplementary Fig. 3. Sequence comparison of amino acid residues 118, 121, 161, 164 and 167 (highlight by red frame) involved in neutralization epitope of antibody 65C6.

Consensus sequence	NDL C YPGDFNDY E ELKHL L SRIN H FEK I Q I PKES S WSNHEASSGVSSAC P YQ G KSS F FR
CAA24269 A/Aichi/2/1968 (H3N2)	SN...Y.VP..AS.RS.VASSGTL.---F.TEGFT.TGVTQNG.-.N..KRGPG.G..S
ACF54598 A/WSN/1933 (H1N1)	.GA.....I.....REQ..SVSSL.RFE.F.....P..TFN-.TAS.SHR.....Y.
ACQ55359 A/California/07/2009 (H1N1)	.GT.....I.....REQ..SVSS..RFE.F..T...P..DSNK..TA...HA.AK...YK
AAA64366 A/Singapore/1/1957 (H2N2)	DG.....S.....SVK...VK.L...-DR.TQ.TTTG.-.R..AVS.NP....
ABD28180 A/Anhui/1/2005 (H5N1)N.....-.....D.....TP...
ABQ58979 A/Beijing/01/2003 (H5N1)-.....L.RP...
ABE68931 A/Goose/Shantou/1621/05 (H5N1)-.....L.P...
AAC40508 A/Hong Kong/156/97 (H5N1)N.....D.....L.R...
ABW06108 A/Indonesia/5/2005 (H5N1)S.....D.....L.SP...
ABO36644 A/Shenzhen/406H/2006 (H5N1)N.....D.....TP...
AAO52864 A/Silky Chicken/Hong Kong/SF189/01 (H5N1)-.....L.....
AAS65615 A/Thailand/1 (KAN-1)/2004 (H5N1)S.....L.....R.....
ABQ58925 A/Turkey/65596/2006 (H5N1)N.....D.....R.....
ABP51977 A/Viet Nam/1203/2004 (H5N1)-.....S.....L.....
ACJ68614 A/Xinjiang/1/2006 (H5N1)N.....D.....R.....
AAW19638 A/blackbird/Hunan/1/2004 (H5N1)-.....D.....NP...
ABD14809 A/chicken/Guangxi/12/2004 (H5N1)I.....D.....
AAK53508 A/chicken/Henan/16/2004 (H5N1)	.G.....-.....
AAV97603 A/chicken/Korea/ES/03 (H5N1)N.....D.....R.....
ACO07033 A/chicken/Vietnam/NCVD-016/2008 (H5N1)	.G.....L.K..K...-Y.....A..S.L.EP...
ACJ26242 A/common magpie/Hong Kong/5052/2007 (H5N1)N.....D.....L.....N.....
ABC66526 A/duck/Guangxi/1378/2004 (H5N1)-.....L.RP...
ABI94747 A/duck/Hubei/wg/2002 (H5N1)-.....A.....SP...
ABJ96698 A/goose/Guiyang/337/2006 (H5N1)-.....P.....L.....L.E...

Consensus sequence	NVV W L I K N S T Y P T I K R S Y N N T N Q E D L L V L W G I H H P N D A E Q T K L Y Q N P T T Y I S V G T S T L
CAA24269 A/Aichi/2/1968 (H3N2)	RLN..T.SG...VINDVTFP..NDNF.K.YI.....STNQ...S..VQASGRVT.S.RRS
ACF54598 A/WSN/1933 (H1N1)	.LL..T..GDS..KLTN..V..KKGKEV.....V...SSSD..QS..S.GNA.V..AS.NY
ACQ55359 A/California/07/2009 (H1N1)	.LI..V..GNS..KLSK..I..KKGKEV.....STS.D.OS...ADA.VF..S.RI
AAA64366 A/Singapore/1/1957 (H2N2)	.M..T..E.N..VA..G...SG.QM.II..V...ET..RT...VG..V.....
ABD28180 A/Anhui/1/2005 (H5N1)N.....I.....S.....
ABQ58979 A/Beijing/01/2003 (H5N1)N.....E..I.....
ABE68931 A/Goose/Shantou/1621/05 (H5N1)A.....
AAC40508 A/Hong Kong/156/97 (H5N1)A.....V.....
ABW06108 A/Indonesia/5/2005 (H5N1)K.....R.....I.....
ABO36644 A/Shenzhen/406H/2006 (H5N1)	.I.....N.....I.....S.....
AAO52864 A/Silky Chicken/Hong Kong/SF189/01 (H5N1)-.....
AAS65615 A/Thailand/1 (KAN-1)/2004 (H5N1)-.....
ABQ58925 A/Turkey/65596/2006 (H5N1)DNA.....R.....
ABP51977 A/Viet Nam/1203/2004 (H5N1)-.....
ACJ68614 A/Xinjiang/1/2006 (H5N1)N.....R.....
AAW19638 A/blackbird/Hunan/1/2004 (H5N1)A.....A.....
ABD14809 A/chicken/Guangxi/12/2004 (H5N1)A.....I.....R.....
AAK53508 A/chicken/Henan/16/2004 (H5N1)-.....R.....
AAV97603 A/chicken/Korea/ES/03 (H5N1)A.....R.....
ACO07033 A/chicken/Vietnam/NCVD-016/2008 (H5N1)N..P..VN..T.....EK..IRI...N.S.....
ACJ26242 A/common magpie/Hong Kong/5052/2007 (H5N1)GNA...K.....E.....R.....I.....
ABC66526 A/duck/Guangxi/1378/2004 (H5N1)A.....
ABI94747 A/duck/Hubei/wg/2002 (H5N1)A.....
ABJ96698 A/goose/Guiyang/337/2006 (H5N1)S.....I.....N.....