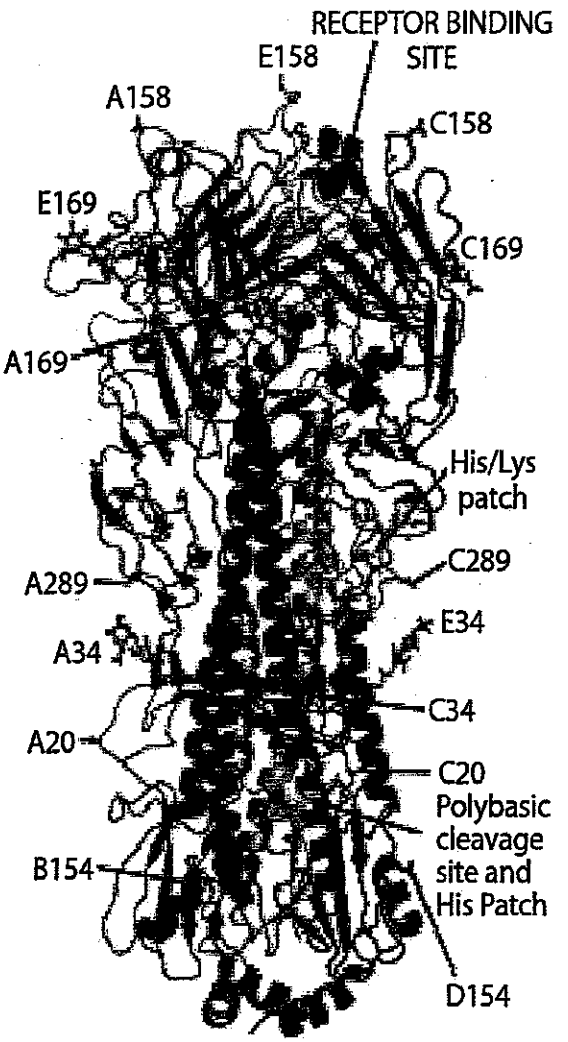
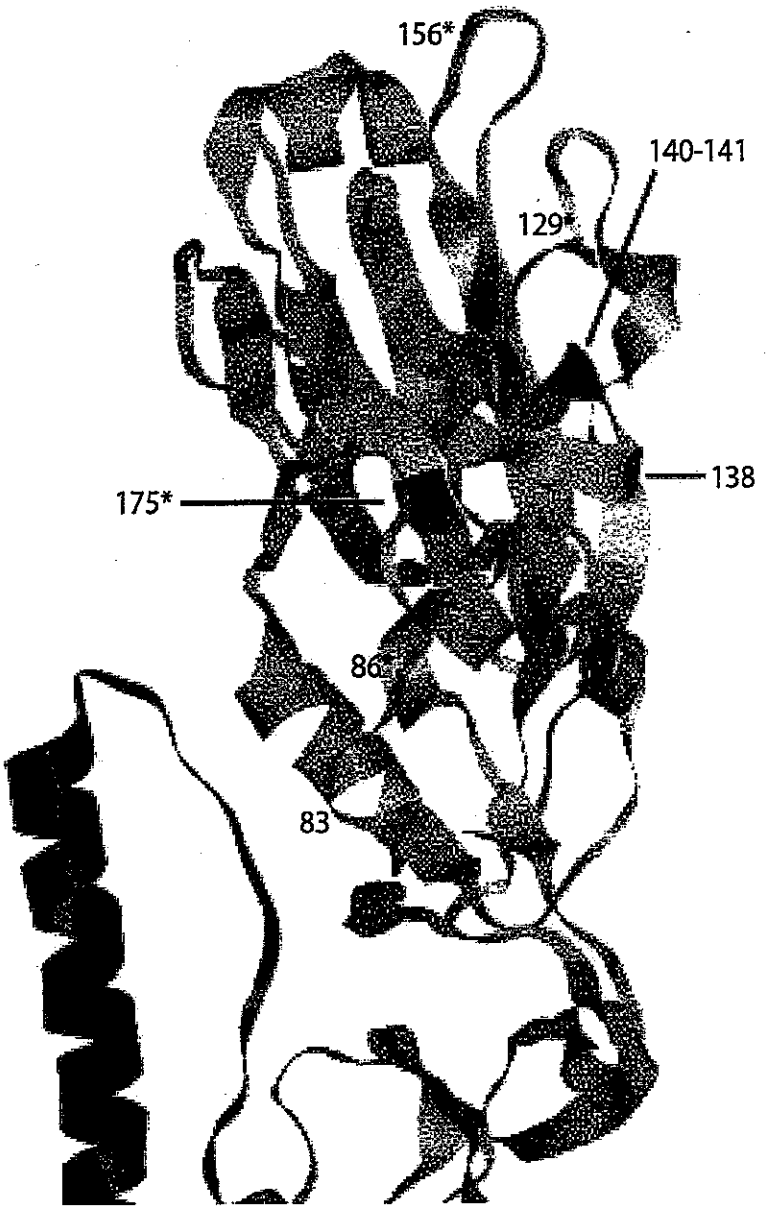


Structure overview of the A/Vietnam 1203/04 trimer, receptor binding site and antigenic variation sites are highlighted on the monomer (cited from Stevens, 2006).

Fig. 1A



Location of amino acid residues in the HA of H5N1 influenza viruses that are under positive selection (cited from Smith, 2006).

Fig. 1B

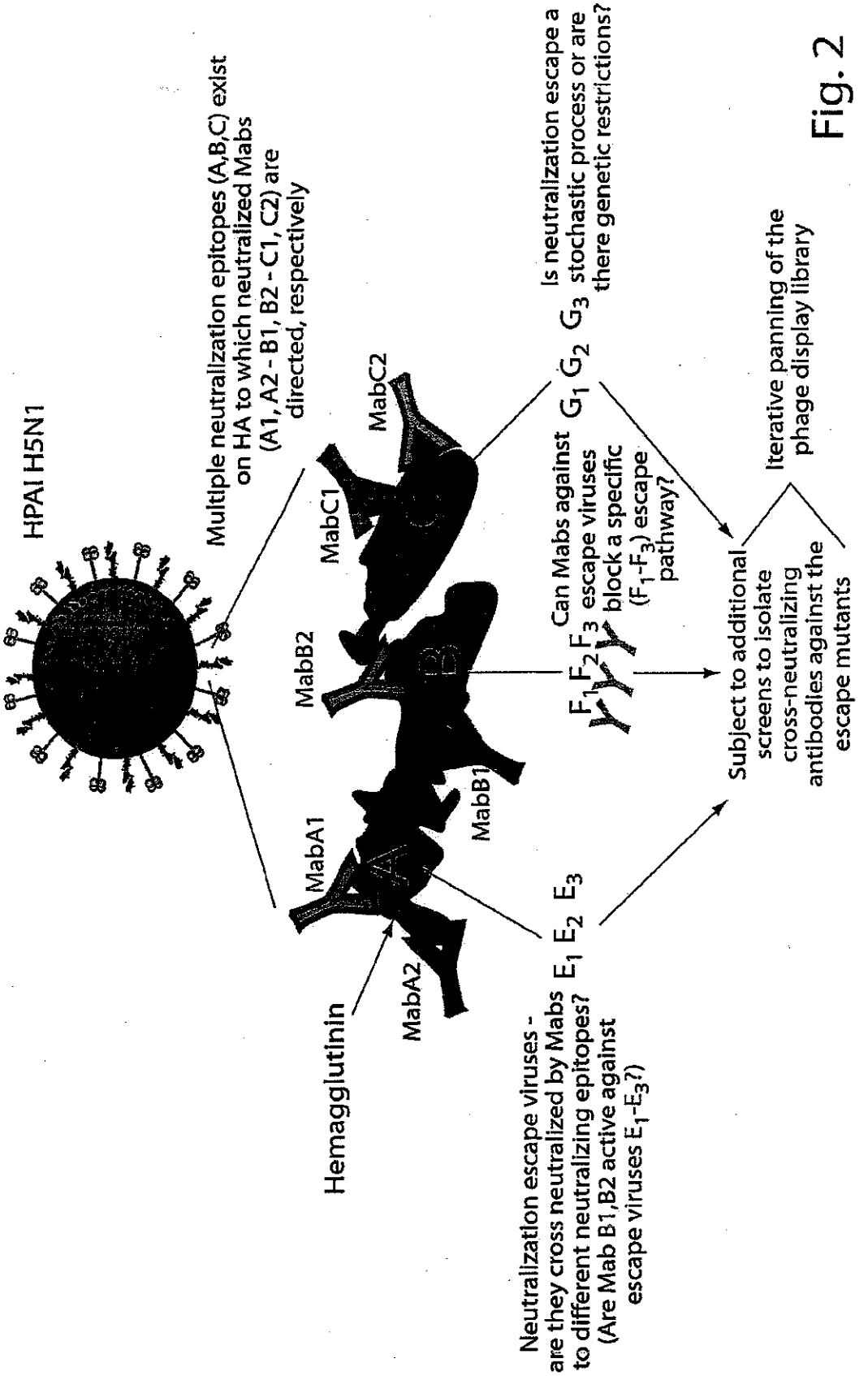

HPAI H5N1
Multiple neutralization epitopes (A,B,C) exist on HA to which neutralized Mabs (A1, A2 - B1, B2 - C1, C2) are directed, respectively
MabA1
MabA2
MabB1
MabB2
MabC1
MabC2
Hemagglutinin
Neutralization escape viruses - are they cross neutralized by Mabs to different neutralizing epitopes? (Are Mab B1,B2 active against escape viruses E_1-E_3?)
E_1 E_2 E_3
Can Mabs against escape viruses block a specific (F_1-F_3) escape pathway?
F_1 F_2 F_3
Is neutralization escape a stochastic process or are there genetic restrictions?
G_1 G_2 G_3
Subject to additional screens to isolate cross-neutralizing antibodies against the escape mutants
Iterative panning of the phage display library

Fig. 2

Amino Acid sequences of variable regions of anti-H5 mAbs

VH	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4	V GENE	
Cons.	KKPGSSVKVSCKAS	GGTFSSYA	ISWVRQAPGQGLEWMGG	IIPMFGTP	NYAQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYC	AR		WGQGTL	
IGHV	....	.GT.SS..	.....	...IF.T.	.....	.----------		------	1-69
F10/E90	.....TSS	EVT.SSF.	.....L..	.S.MF.T.	.....Q..R....D.R.....	..---SPSYIDSGGTCVFDH		.....	1-69
D8/D80	.....	.GT.SA..	FT.....	..GMF.TA	.....L.....T.....L...	..---GLYYYESS----FDY		.....	1-69
A66/E88	.....	.GF.SMT.	FT.L.....	.S.IFRT.	K.....N..N...T..K.....	..--TLSSYQPNND--AFAI		.....M	1-69
G17	....A.....T.	.VT.SS..	.....	..GVF.V.	K.....KP...V....N...A.....	..---EPGAYVGKN--GFDV		.....M	1-69
D7/H98	.....P	.GI.NTN.	F.....V..	V..LFRTA	S...NV.....N.....T....A.....	..---SSGYHFRSH---FDS		..L...	1-69
H40	R...A.....I.	.FT.TG.Y	.H.....W	.N.MT.GT	.....VW..M.R.T.IN.....VTR.T.D.....	..GASVLRYFDWQP-EALDI		..L..T	1-2

VL	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4	GENE	
Cons.	QPVLTQPPS-ASGSPGQRVTISCTGS		VAWYQQKPGQAPKLLIY		DRPSGIPDRFSGSR--SGTTASLTISGLQPEDEADYYC			FGGGTKLTVL	
D7Vλ	NFM....H.-V.A...KT.....	SGNIAANY	.Q....R..S..TTV..	EDD	R....V.....IDR.SNS.....KT.....	QTYDTNNHAV		.....H....	LV6-57
D8Vλ	.S.....-.....S.....T	SSDVGGYNS	.S....H..K....M..	EVT	K....V.....A.K--..N.....V....A.....F.	CSYAGHSAYV		..T...V...	VL2-11
F10Vλ	..G.....-V.KGLR.TA.LT...N	SNNVGNQG	A..L..HQ.HP....S.	RNN	.....SE...A..--..N.....T.....	STWDSSLSAVV		.....	LV10-54
G17Vλ	SYE.....-V.KGLR.TAILT...D	SNNVGHQG	T..L..HQ.HP....S.	RNG	N.....SE...A..--..N.....I.....	SVWDSSLSAWV		.....	LV10-54
H40Vλ	.....-V.VA...TAS.P.G.N	NIGGYS--	.H.....L.V..	DDK	.....E....AN--..S..T....RVEAG..G....	QVWDSGNDRPL		.....	VL3-21
A66Vκ	EI....S.ATL.L...E.A.L..RA.	QSVSSY--	L.....R....	DAS	N.AT...A.....G--...DFT....R.E...F.V.F.	QQYGSSPQ---		..Q..R.EIK	KV3-20
D80Vκ	EI....S.GTL.L...E.A.L..RA.	QSLSSKY-	L.....R....	GAS	S.AT.....G--...DFT....R.E...F.V.S.	QQYDGVPRT--		..Q..TVEIK	KV3-20
E88Vλ	L.....-...T.....I..S..	SSNIGSNT	.N....L..T.....	SNN	Q....V.....--...S...A.I..R.....	QSYDSRLSASL		..T..TV...	LV1-44
E90Vκ	DIQM..S..SL.A.V.D....T.RA.	QSISSY--	LN.....K.....	AAS	SLQR.V.S.....G--...DFT....S.....F.V.Y.	QQYDSSPYT--		..Q...VEIK	KV1-39
H98Vλ	SYE.....-...KH.....S.G	TSNIGRNH	.N....L..T.....	SNE	Q....V.....K--...S...AV....S.....	ASWDDNLSGWV		.....	LV1-44

Framework regions 1-4 (FR1-4) and complementarity-determining regions 1-3 (CDR1-3) for VH and VL are shown. FR, CDR and gene names are defined using the IMGT database. For VH, the first 11 residues of FR1 (QVQLVQSGAEV) and last 5 residues of FR4 (VTVSS) are invariant and not shown. Dots denote identity with the consensus sequence, hyphens denote gaps. Six different VH and 10 different VL genes were found. 4 out of 6 antibodies share the same VH gene. 5 out of the 6 VH belong to one gene family, IGHV1-69*01. The VL genes are much more diverse than the VH genes, consistent with the lack of contacts observed between F10 and H5; three out of the 10 VL are k chain. The 3 VH chains used to make IgG1s are coloured red. H5 contact residues to F10 are highlighted, along with corresponding residues in other VHs, in cyan (critical residues as defined by FastContact energy calculations) and grey (other contacts < 4.5 Å).

Fig. 3

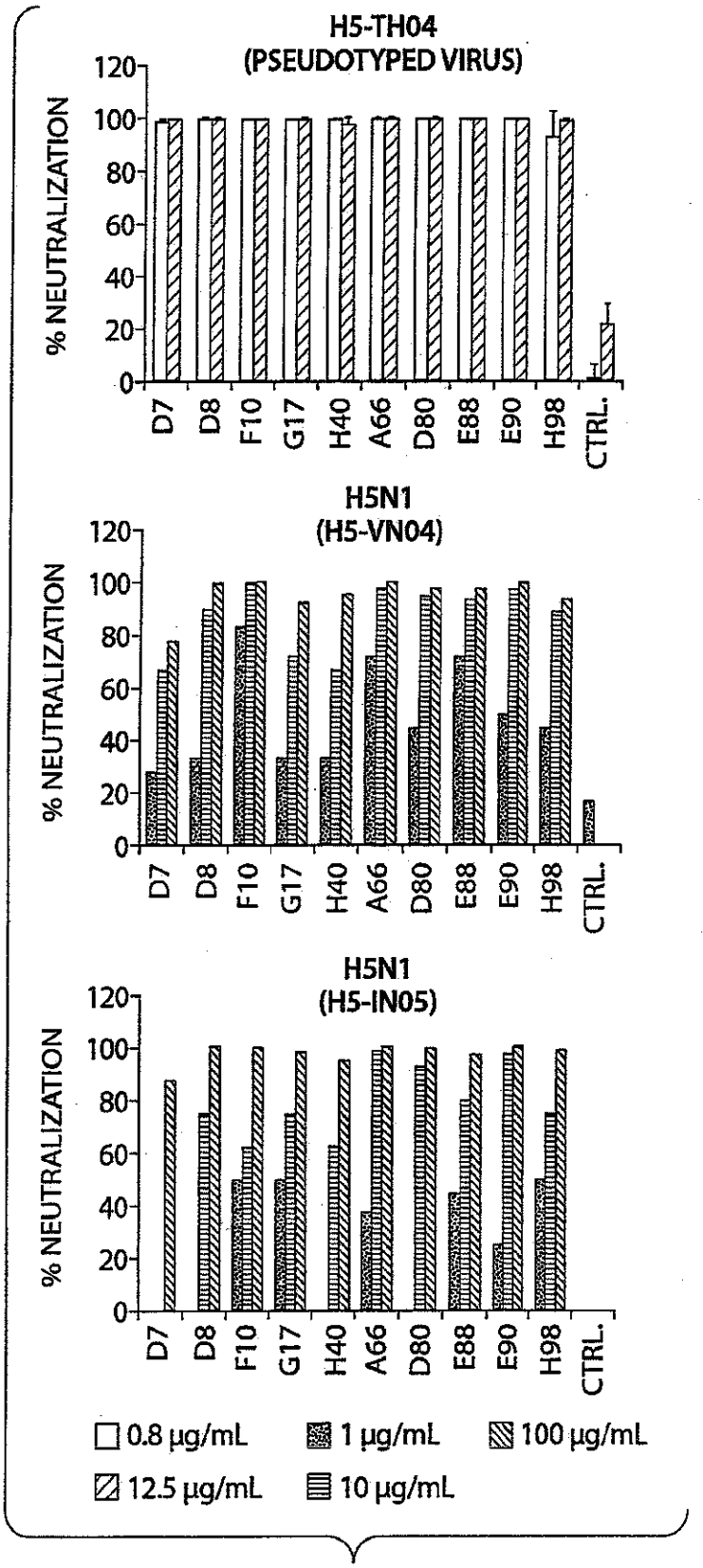

H5-TH04
(PSEUDOTYPED VIRUS)
% NEUTRALIZATION
120
100
80
60
40
20
0
D7
D8
F10
G17
H40
A66
D80
E88
E90
H98
CTRL.
H5N1
(H5-VN04)
% NEUTRALIZATION
H5N1
(H5-IN05)
% NEUTRALIZATION
0.8 µg/mL
1 µg/mL
100 µg/mL
12.5 µg/mL
10 µg/mL

Fig. 4A

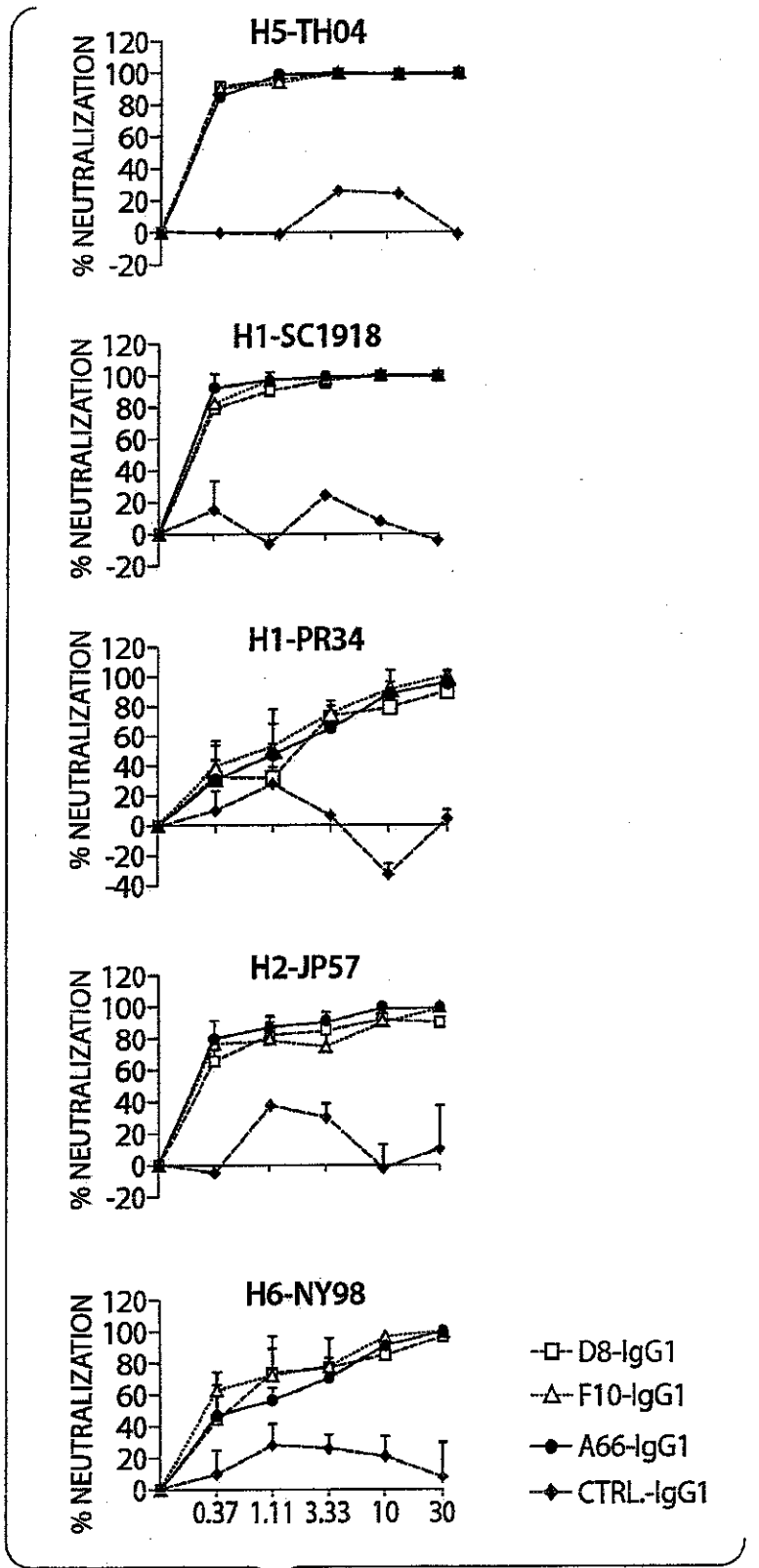

H5-TH04
H1-SC1918
H1-PR34
H2-JP57
H6-NY98
% NEUTRALIZATION
120
100
80
60
40
20
0
-20
-40
0.37 1.11 3.33 10 30
D8-IgG1
F10-IgG1
A66-IgG1
CTRL.-IgG1

Fig. 4B

VIRUS	HUMAN mAb F10	MURINE mAb 21G8.6	CTRL. 80R
H5-TH04 (H5N1)	40	40	<
H5-HK97 (H5N1)	160	40	<
H1-OH83 (H1N1)	40	<	<
H1-PR34 (H1N1)	320	<	<
H2-AA60 (H2N2)	20	<	<

Fig. 4C

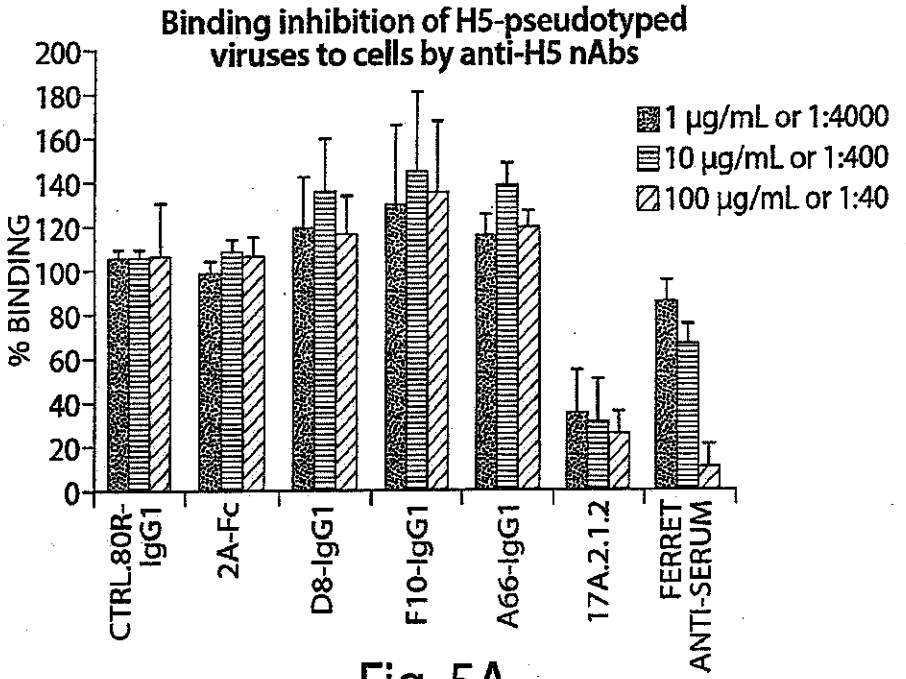

Binding inhibition of H5-pseudotyped viruses to cells by anti-H5 nAbs
% BINDING
200
180
160
140
120
100
80
60
40
20
0
1 µg/mL or 1:4000
10 µg/mL or 1:400
100 µg/mL or 1:40
CTRL.80R-IgG1
2A-Fc
D8-IgG1
F10-IgG1
A66-IgG1
17A.2.1.2
FERRET ANTI-SERUM

Fig. 5A

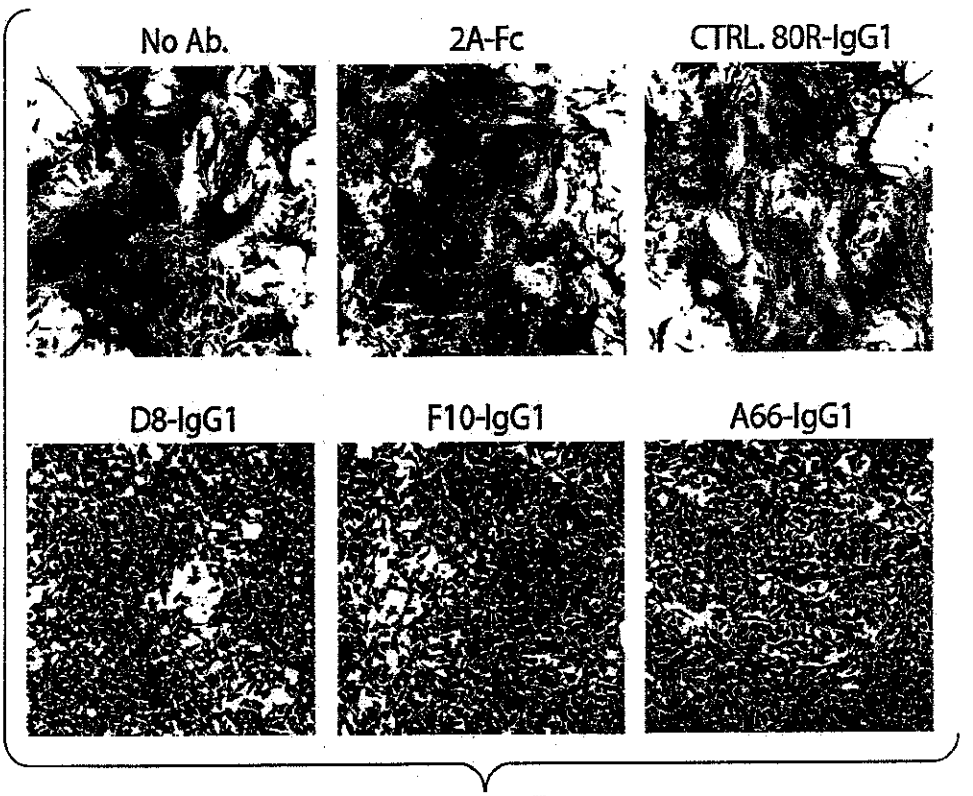

No Ab.
2A-Fc
CTRL. 80R-IgG1
D8-IgG1
F10-IgG1
A66-IgG1

Fig. 5B

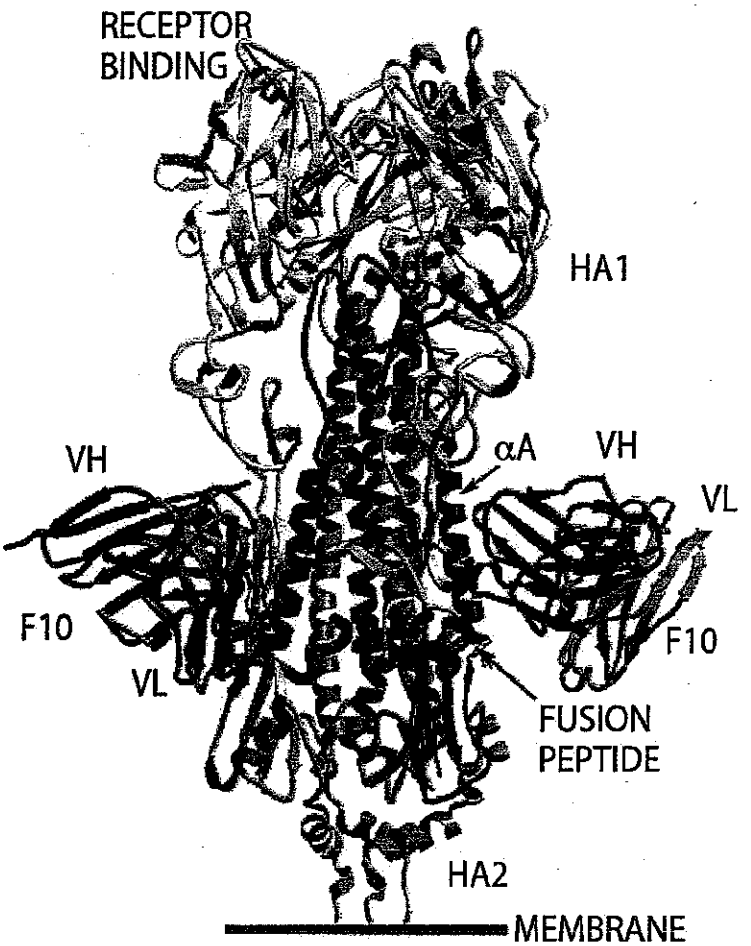

RECEPTOR
BINDING
HA1
VH
αA
VH
VL
F10
F10
VL
FUSION
PEPTIDE
HA2
MEMBRANE

Fig. 6A

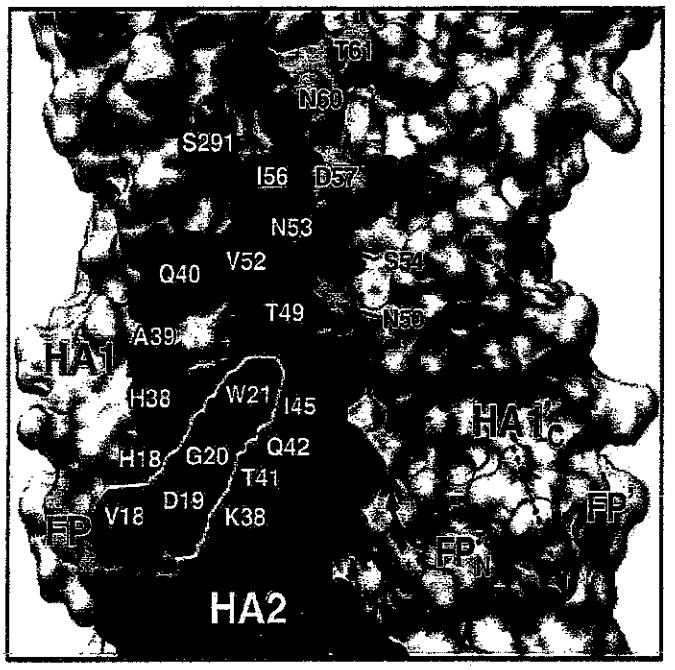

T61
N60
S291
I56
D57
N53
V52
Q40
S54
T49
N50
A39
HA1
H38
W21
I45
HA1c
H18
G20
Q42
T41
D19
V18
K38
FP
FP
FPN
HA2

Fig. 6B

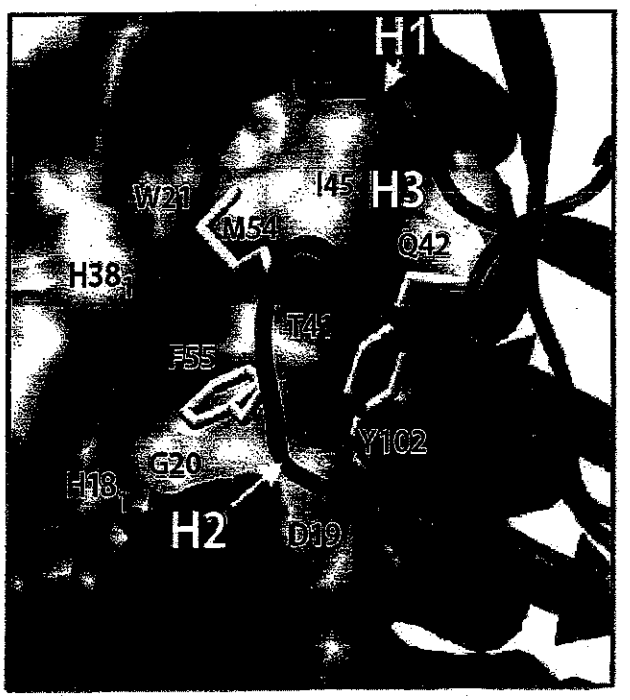

H1
W21
I45
H3
M54
Q42
H38₁
T41
F55
Y102
G20
H18₁
H2
D19

Fig. 6C

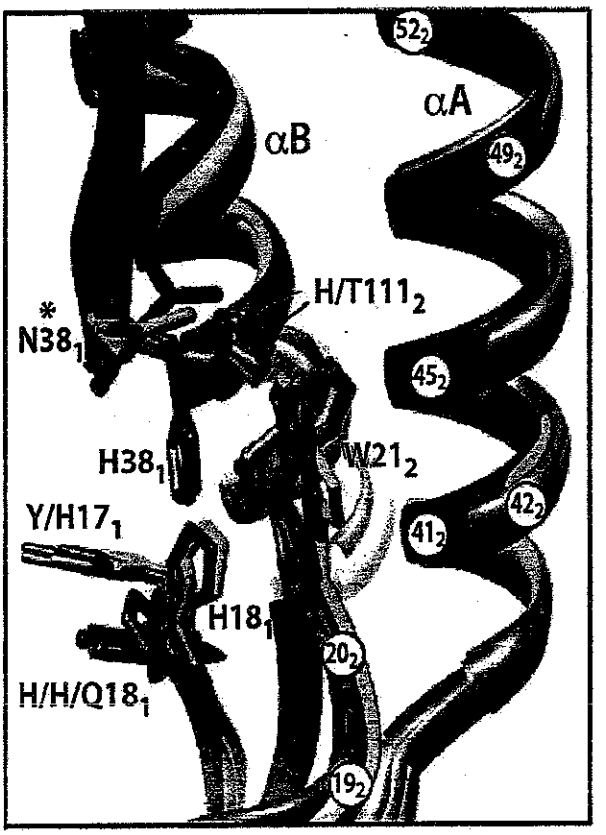

52₂
αA
αB
49₂
H/T111₂
*
N38₁
45₂
H38₁
W21₂
42₂
Y/H17₁
41₂
H18₁
20₂
H/H/Q18₁
19₂

Fig. 6D

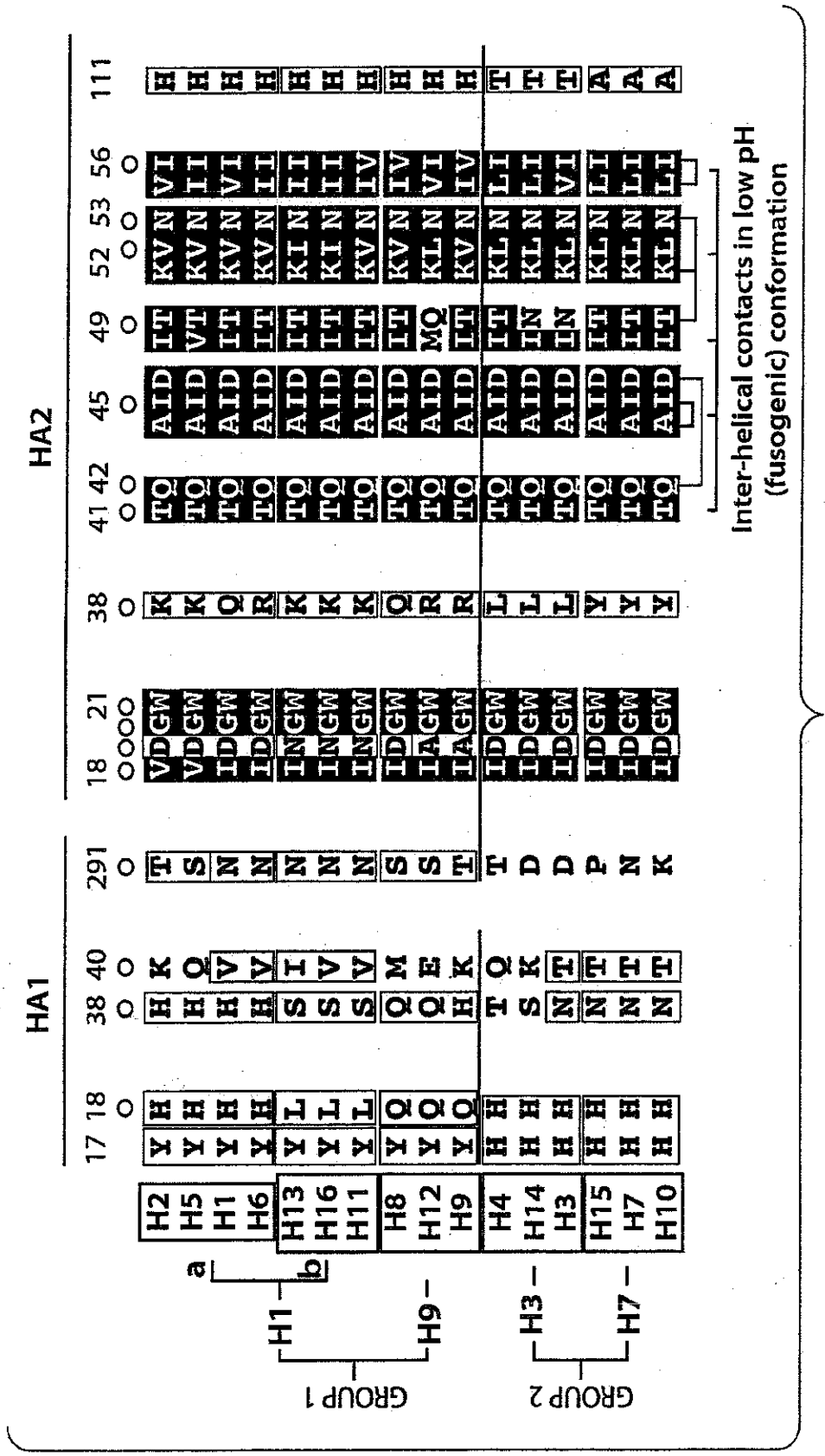

HA1
HA2
17 18 38 40 291 18 21 38 41 42 45 49 52 53 56 111
H2 H5 H1 H6 H13 H16 H11 H8 H12 H9 H4 H14 H3 H15 H7 H10
a
b
H1
H9
H3
H7
GROUP 1
GROUP 2
Inter-helical contacts in low pH (fusogenic) conformation

Fig. 6E

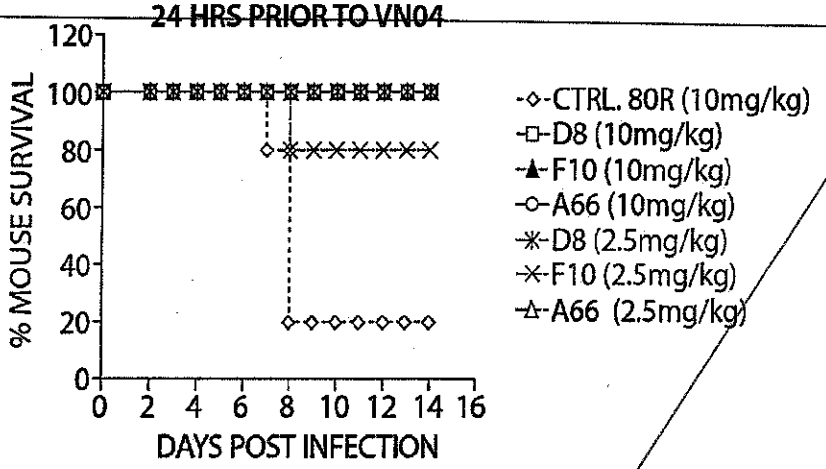


Fig. 7A

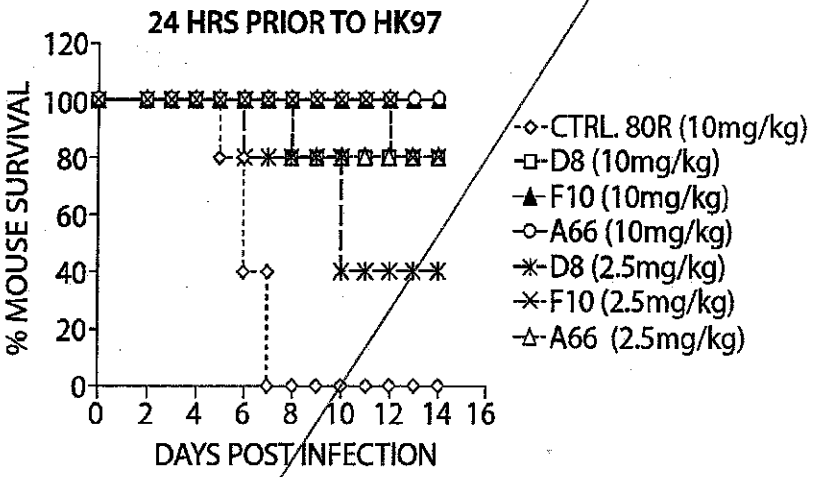


Fig. 7B

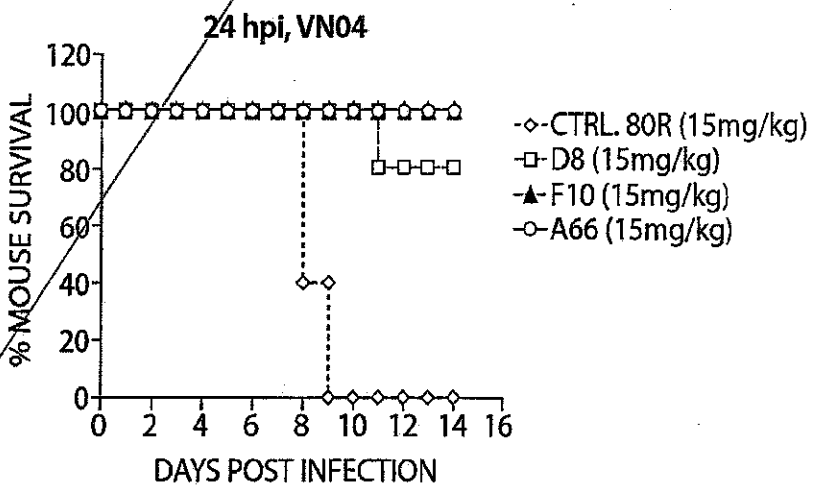


Fig. 7C

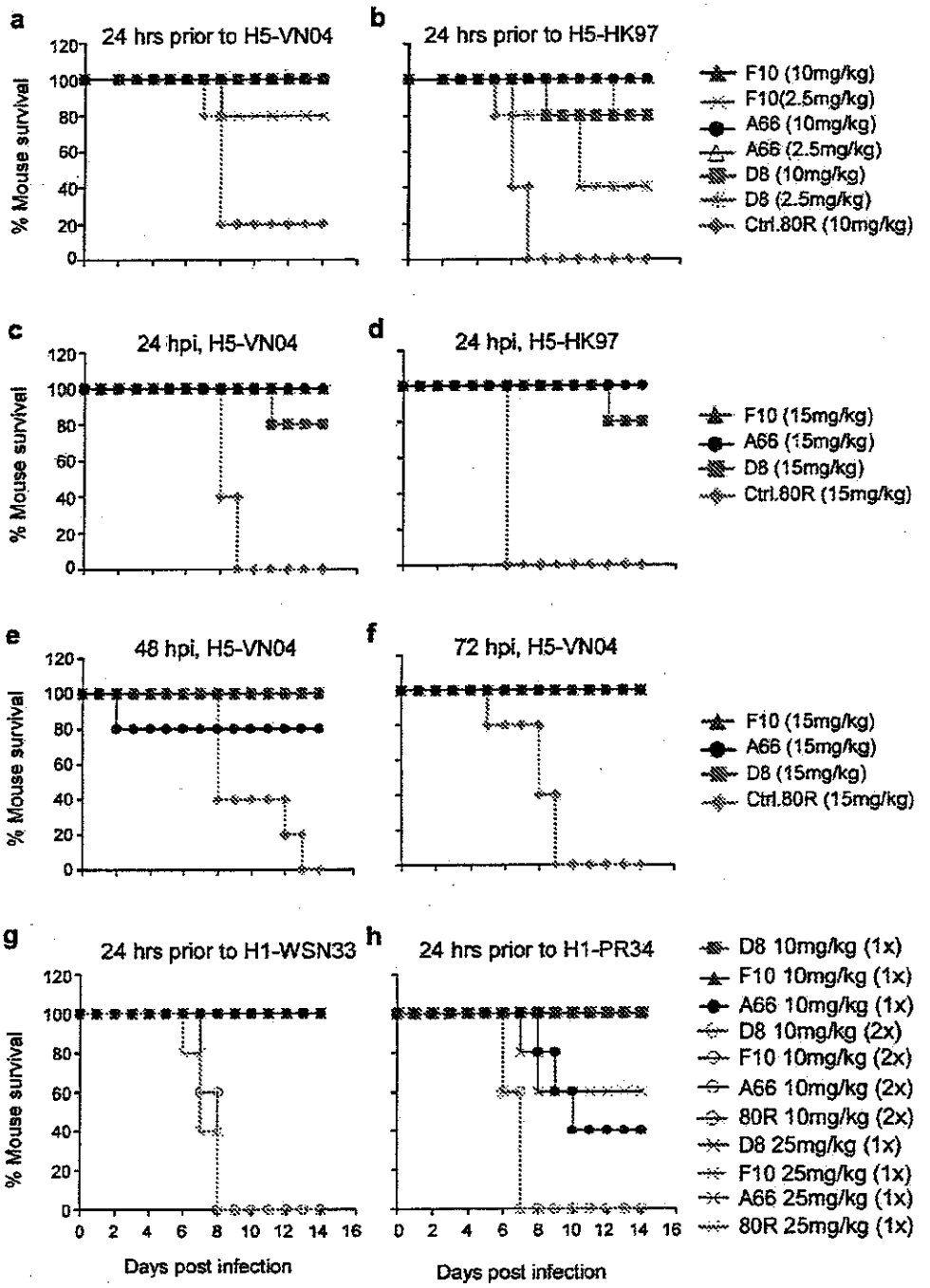

a
24 hrs prior to H5-VN04
b
24 hrs prior to H5-HK97
% Mouse survival
F10 (10mg/kg)
F10(2.5mg/kg)
A66 (10mg/kg)
A66 (2.5mg/kg)
D8 (10mg/kg)
D8 (2.5mg/kg)
Ctrl.80R (10mg/kg)
c
24 hpi, H5-VN04
d
24 hpi, H5-HK97
F10 (15mg/kg)
A66 (15mg/kg)
D8 (15mg/kg)
Ctrl.80R (15mg/kg)
e
48 hpi, H5-VN04
f
72 hpi, H5-VN04
g
24 hrs prior to H1-WSN33
h
24 hrs prior to H1-PR34
D8 10mg/kg (1x)
F10 10mg/kg (1x)
A66 10mg/kg (1x)
D8 10mg/kg (2x)
F10 10mg/kg (2x)
A66 10mg/kg (2x)
80R 10mg/kg (2x)
D8 25mg/kg (1x)
F10 25mg/kg (1x)
A66 25mg/kg (1x)
80R 25mg/kg (1x)
Days post infection
Days post infection

Figure 7

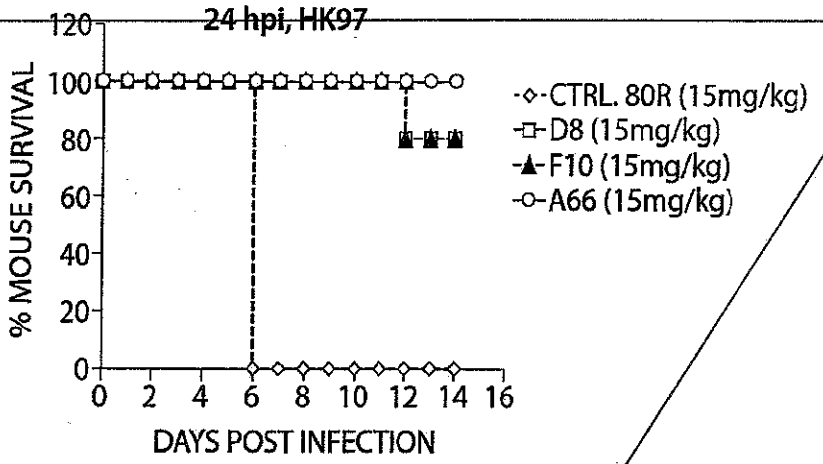


Fig. 7D

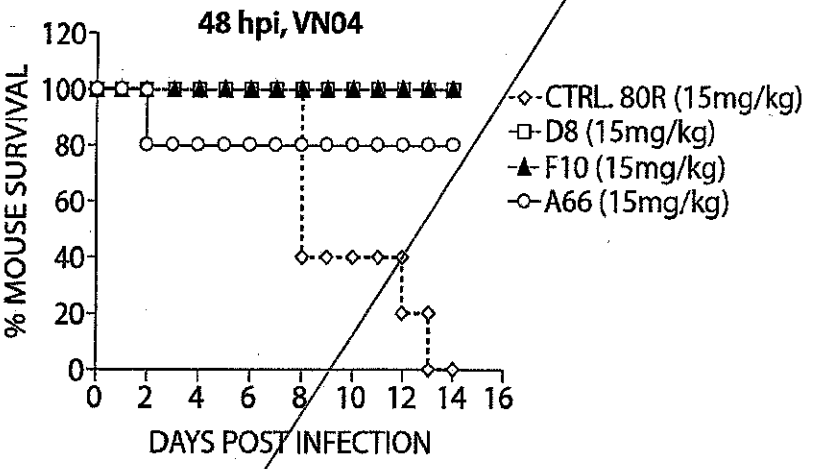


Fig. 7E

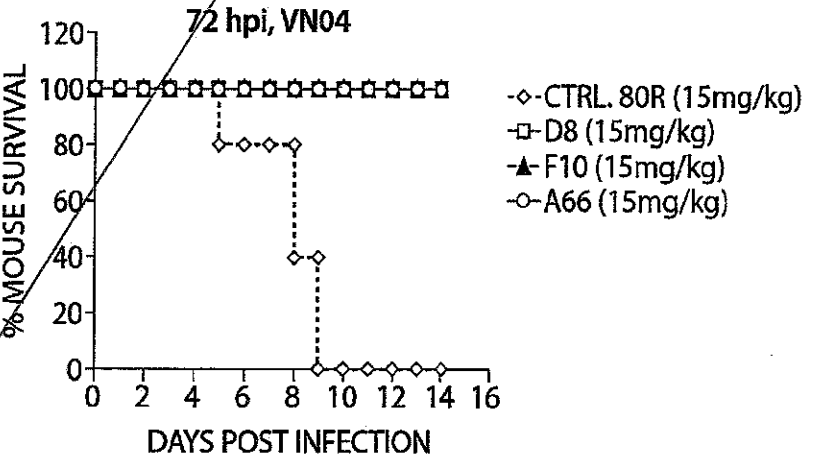


Fig. 7F

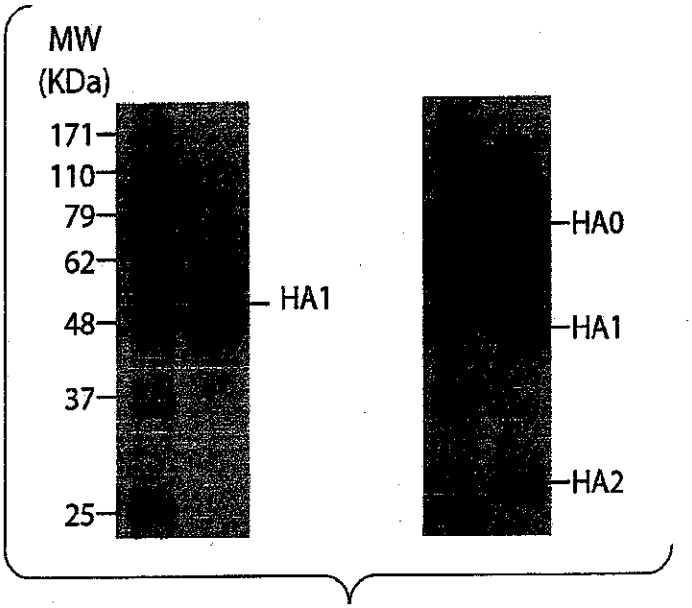

MW
(KDa)
171
110
79
62
48
37
25
HA1
HA0
HA1
HA2

Fig. 8A

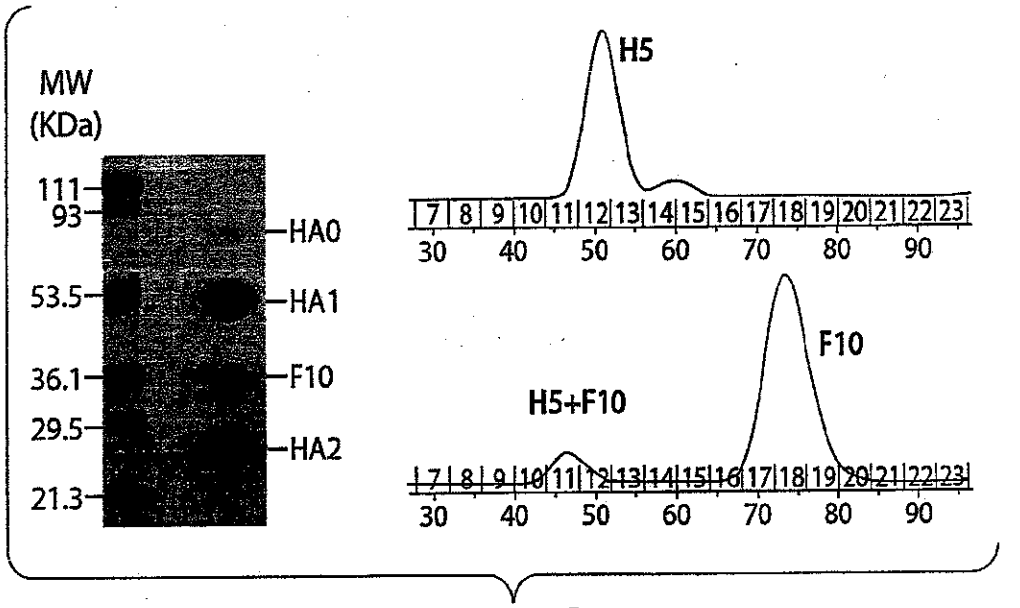

MW
(KDa)
111
93
53.5
36.1
29.5
21.3
HA0
HA1
F10
HA2
H5
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
30 40 50 60 70 80 90
F10
H5+F10
7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
30 40 50 60 70 80 90

Fig. 8B

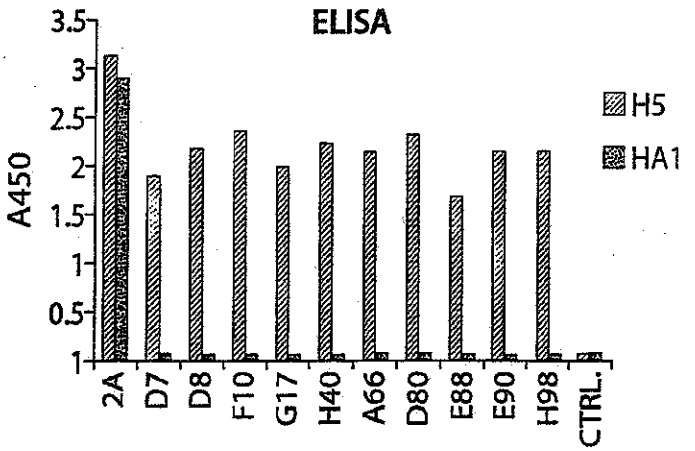

ELISA
A450
3.5
3
2.5
2
1.5
1
0.5
1
H5
HA1
2A
D7
D8
F10
G17
H40
A66
D80
E88
E90
H98
CTRL.

Fig. 9A

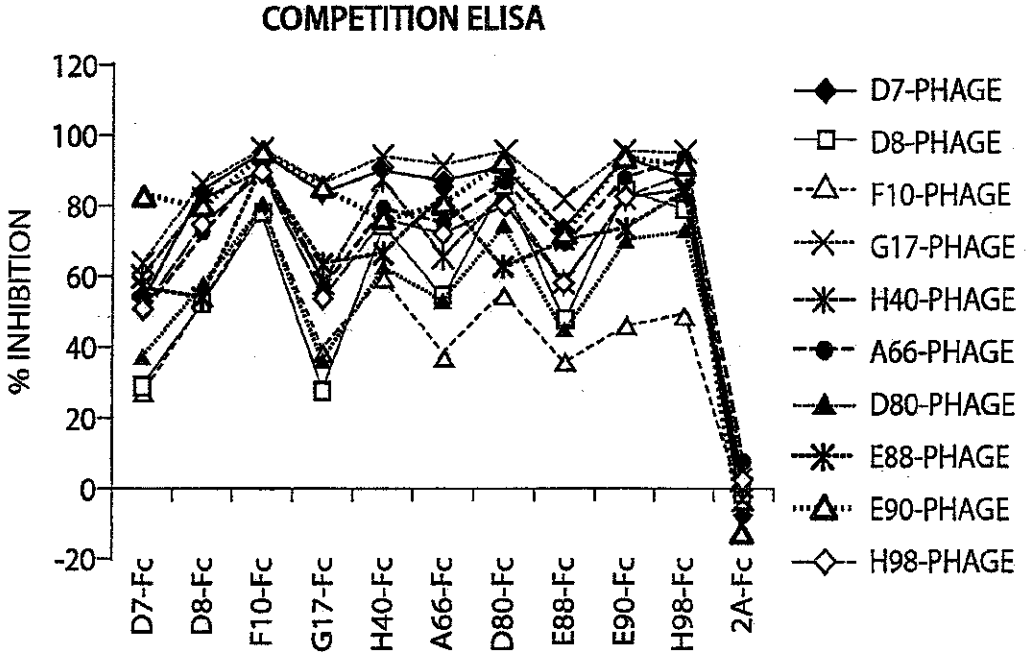
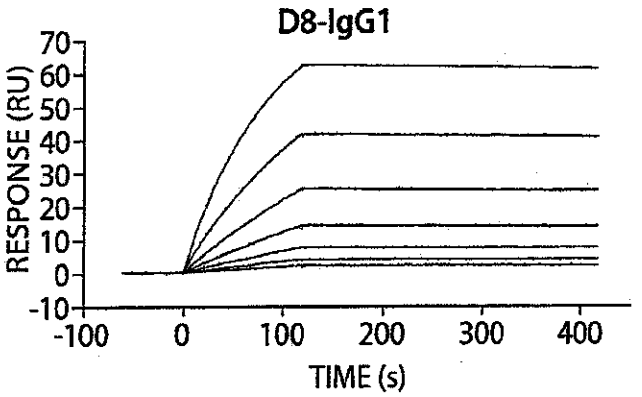
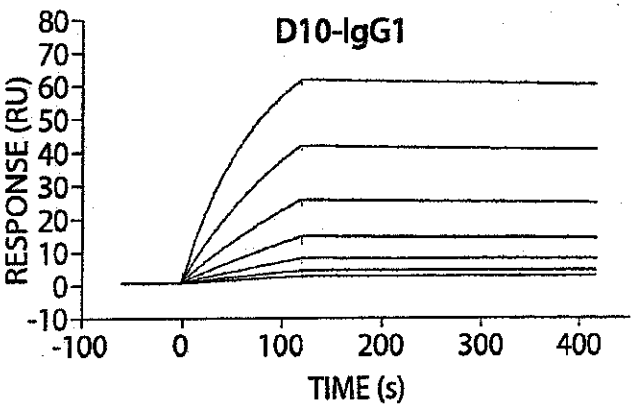
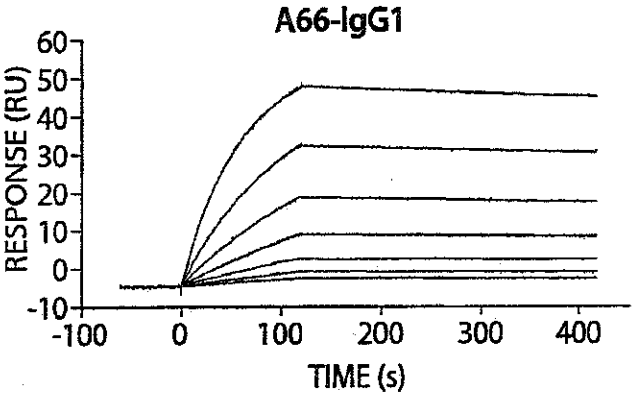
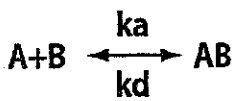

COMPETITION ELISA
% INHIBITION
120
100
80
60
40
20
0
-20
D7-Fc
D8-Fc
F10-Fc
G17-Fc
H40-Fc
A66-Fc
D80-Fc
E88-Fc
E90-Fc
H98-Fc
2A-Fc
D7-PHAGE
D8-PHAGE
F10-PHAGE
G17-PHAGE
H40-PHAGE
A66-PHAGE
D80-PHAGE
E88-PHAGE
E90-PHAGE
H98-PHAGE

Fig. 9B









	ka (M^{-1} s^{-1} x 10^5)	*kd* (s^{-1}x10^{-4})	K_D (pM)
D8-IgG1	5.98±0.08	0.88±0.11	148±19
F10-IgG1	6.52±0.92	0.84±0.19	130±28
A66-IgG1	7.75±0.63	1.80±0.31	233±47

Fig. 10

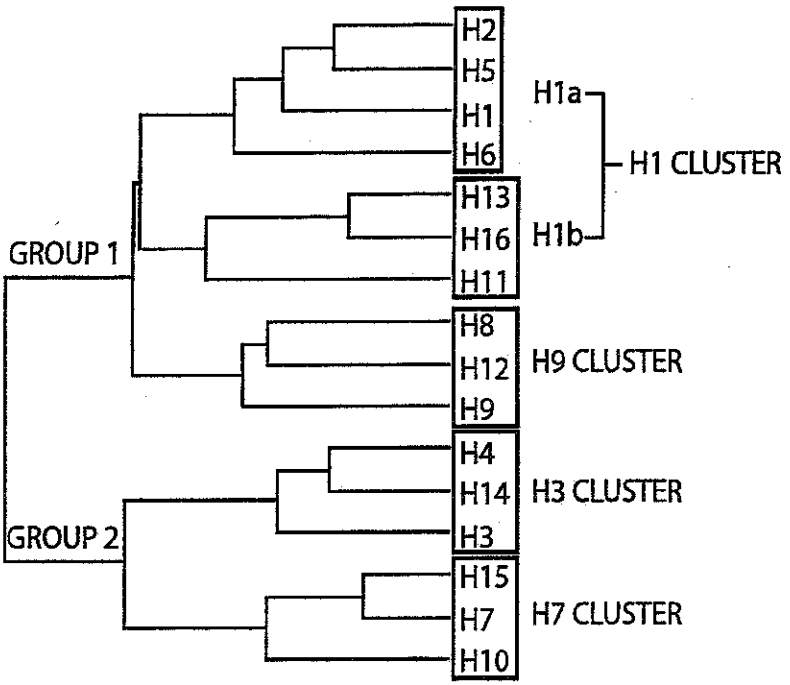

H2
H5
H1
H6
H1a
H1 CLUSTER
H13
H16
H11
H1b
GROUP 1
H8
H12
H9
H9 CLUSTER
H4
H14
H3
H3 CLUSTER
GROUP 2
H15
H7
H10
H7 CLUSTER

Fig. 11

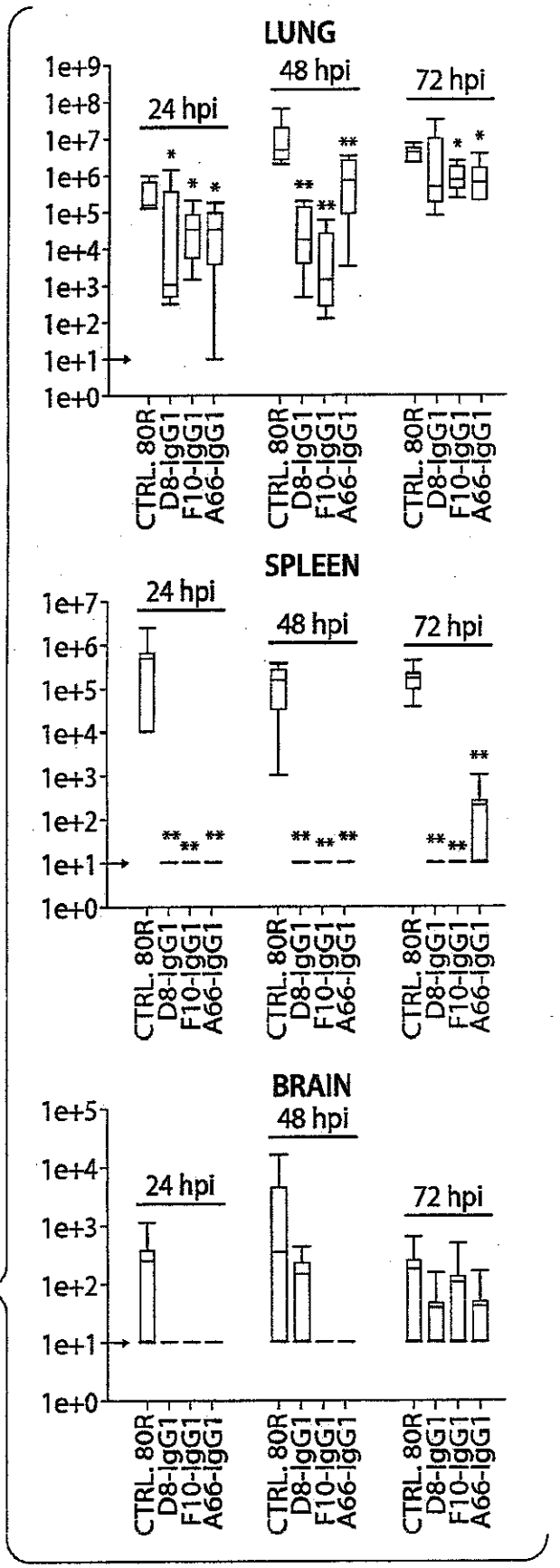

LUNG
24 hpi
48 hpi
72 hpi
1e+9
1e+8
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
1e+0
CTRL. 80R
D8-IgG1
F10-IgG1
A66-IgG1
SPLEEN
24 hpi
48 hpi
72 hpi
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
1e+0
CTRL. 80R
D8-IgG1
F10-IgG1
A66-IgG1
BRAIN
48 hpi
24 hpi
72 hpi
1e+5
1e+4
1e+3
1e+2
1e+1
1e+0
CTRL. 80R
D8-IgG1
F10-IgG1
A66-IgG1

Fig. 12

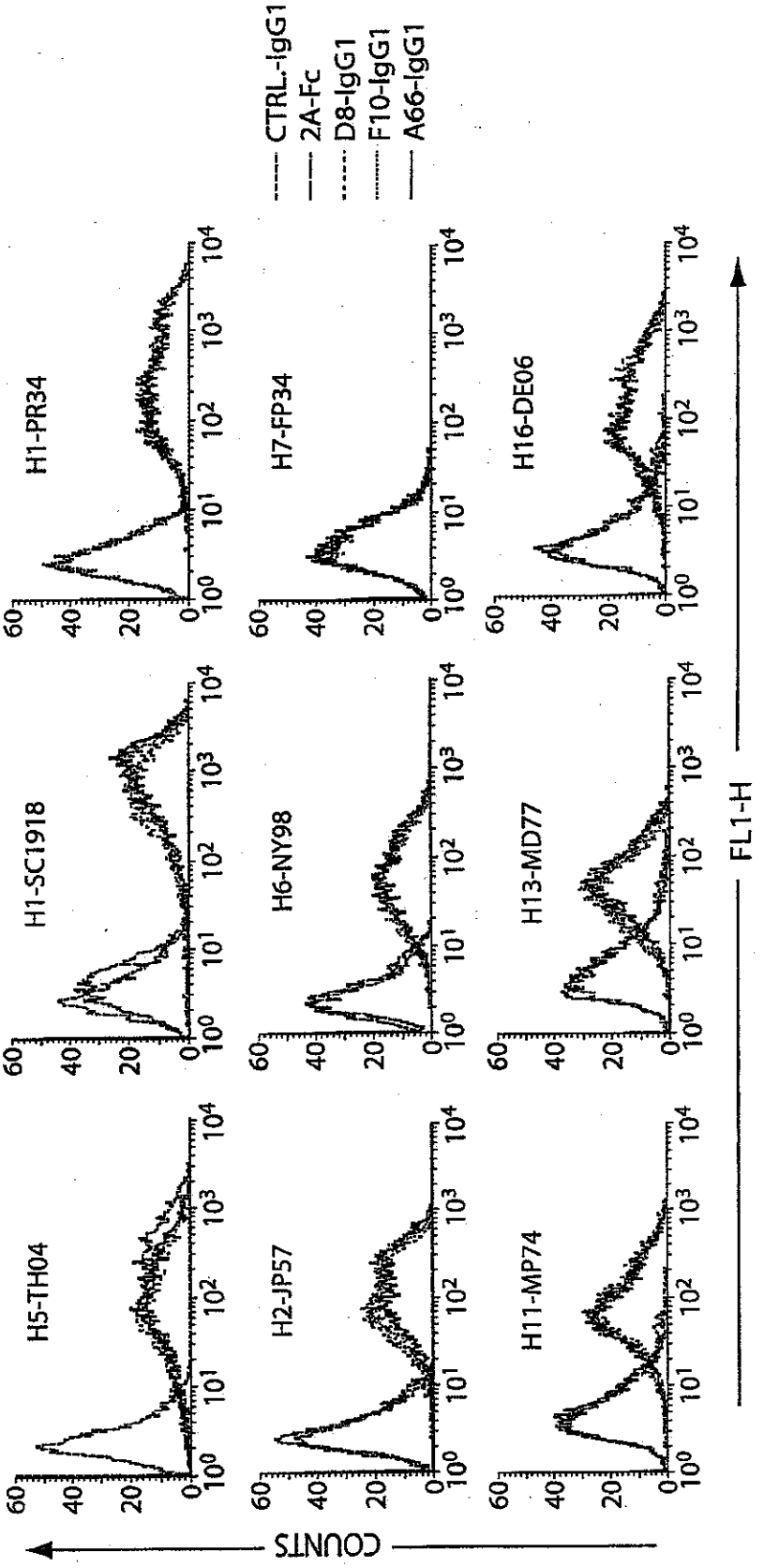

CTRL.-IgG1
2A-Fc
D8-IgG1
F10-IgG1
A66-IgG1
H1-PR34
H7-FP34
H16-DE06
H1-SC1918
H6-NY98
H13-MD77
H5-TH04
H2-JP57
H11-MP74
COUNTS
FL1-H

Fig. 13