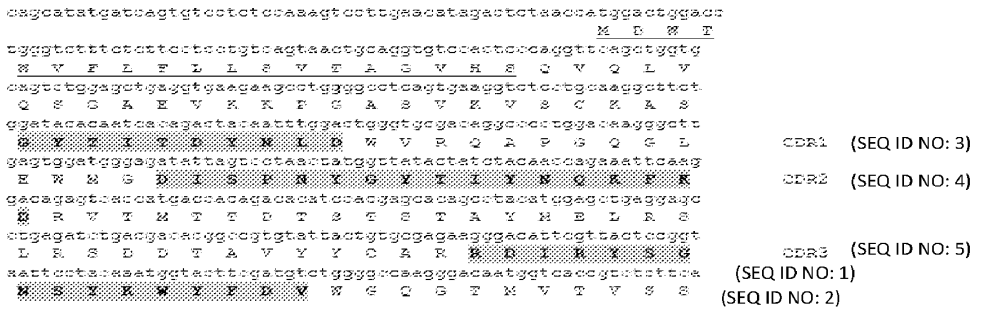
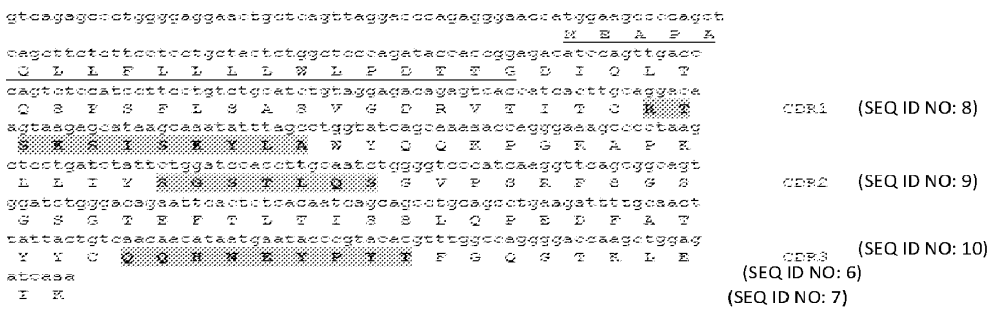
humanized 2G1 VH-11801



Humanized 2G1 VL-1901



Parent mAb (humanized 2G1 VH-11801 and VL-1901)

Figure 1

humanized 2G1 VH-11801

```
cagcatatgatcagtgtcctctccaaagtccttgaacatagactctaaccatggactggacc
                                                  M  D  W  T
tgggtctttctcttcctcctgtcagtaactgcaggtgtccactcccaggttcagctggtg
 W  V  F  L  F  L  L  S  V  T  A  G  V  H  S  Q  V  Q  L  V
cagtctggagctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggcttct
 Q  S  G  A  E  V  K  K  P  G  A  S  V  K  V  S  C  K  A  S
ggatacacaatcacagactacaatttggactgggtgcgacaggcccctggacaagggctt
 G  Y  T  I  T  D  Y  N  L  D  W  V  R  Q  A  P  G  Q  G  L
gagtggatgggagatattagtcctaactatggttatactatctacaaccagaaattcaag
 E  W  M  G  D  I  S  P  N  Y  G  Y  T  I  Y  N  Q  K  F  K
gacagagtcaccatgaccacagacacatccacgagcacagcctacatggagctgaggagc
 D  R  V  T  M  T  T  D  T  S  T  S  T  A  Y  M  E  L  R  S
ctgagatctgacgacacggccgtgtattactgtgcgagaagggacattcgttactccggt
 L  R  S  D  D  T  A  V  Y  Y  C  A  R  R  D  I  R  Y  S  G
aattcctacaaatggtacttcgatgtctggggccaagggacaatggtcaccgtctcttca
 N  S  Y  K  W  Y  F  D  V  W  G  Q  G  T  M  V  T  V  S  S
```



CDR1 (SEQ ID NO: 3)

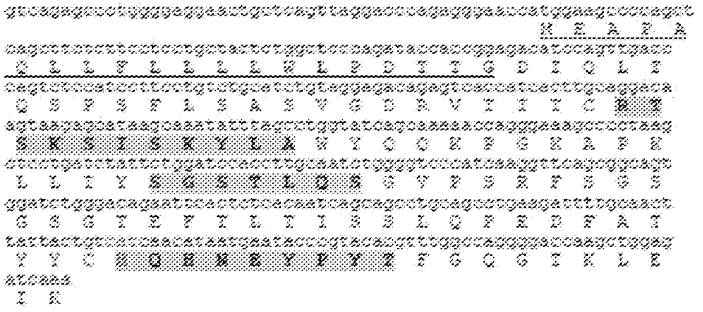
CDR2 (SEQ ID NO: 4)

CDR3 (SEQ ID NO: 5)

(SEQ ID NO: 1)
(SEQ ID NO: 2)

Humanized 2G1 VL-1901 variant

```
gtcagagccctggggaggaactgctcagttaggacccagagggaaccatggaagccccagct
                                                  M  E  A  P  A
cagcttctcttcctcctgctactctggctcccagataccaccggagacatccagttgacc
 Q  L  L  F  L  L  L  L  W  L  P  D  T  T  G  D  I  Q  L  T
cagtctccatccttcctgtctgcatctgtaggagacagagtcaccatcacttgcaggaca
 Q  S  P  S  F  L  S  A  S  V  G  D  R  V  T  I  T  C  R  T
agtaagagcataagcaaatatttagcctggtatcagcaaaaaccagggaaagcccctaag
 S  K  S  I  S  K  Y  L  A  W  Y  Q  Q  K  P  G  K  A  P  K
ctcctgatctattctggatccaccttgcaatctggggtcccatcaaggttcagcggcagt
 L  L  I  Y  S  G  S  T  L  Q  S  G  V  P  S  R  F  S  G  S
ggatctgggacagaattcactctcacaatcagcagcctgcagcctgaagattttgcaact
 G  S  G  T  E  F  T  L  T  I  S  S  L  Q  P  E  D  F  A  T
tattactgtcatcaacataatgaatacccgtacacgtttggccaggggaccaagctggag
 Y  Y  C  H  Q  H  N  E  Y  P  Y  T  F  G  Q  G  T  K  L  E
atcaaa
 I  K
```



CDR1 (SEQ ID NO: 8)

CDR2 (SEQ ID NO: 9)

CDR3 (SEQ ID NO: 11)
(SEQ ID NO: 12)
(SEQ ID NO: 13)

Engineered mAb L3-1 (humanized 2G1 comprising VH-11801and VL-1901 with Q →H mutation in CDR3)

Figure 2

humanized 2G1 VH-11801



CDR1 (SEQ ID NO: 3)

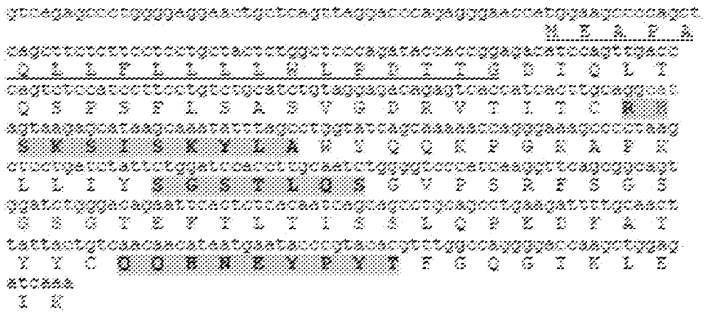
CDR2 (SEQ ID NO: 4)

CDR3 (SEQ ID NO: 5)

(SEQ ID NO: 1)

(SEQ ID NO: 2)

Humanized 2G1 VL-1901 variant



CDR1 (SEQ ID NO: 14)

CDR2 (SEQ ID NO: 9)

CDR3 (SEQ ID NO: 10)

(SEQ ID NO: 15)

(SEQ ID NO: 16)

Engineered mAb L1-2 (humanized 2G1 comprising VH-11801 and VL-1901 with T→H mutation in CDR1)

Figure 3

humanized 2G1 VH-11801 variant



CDR1 (SEQ ID NO: 17)

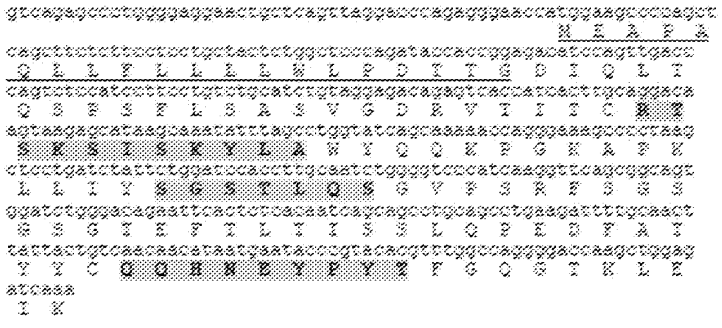
CDR2 (SEQ ID NO: 4)

CDR3 (SEQ ID NO: 5)

(SEQ ID NO: 18)

(SEQ ID NO: 19)

Humanized 2G1 VL-1901



CDR1 (SEQ ID NO: 8)

CDR2 (SEQ ID NO: 9)

CDR3 (SEQ ID NO: 10)

(SEQ ID NO: 6)

(SEQ ID NO: 7)

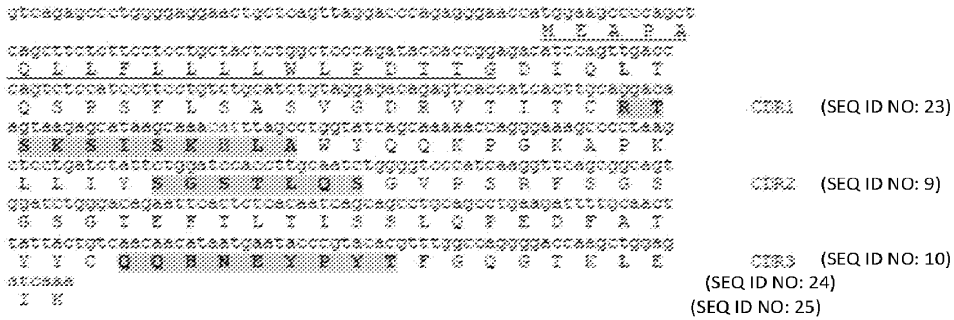
Engineered mAb H1-4 (humanized 2G1 comprising VH-11801with I to ℋ mutation in CDR1 and VL-1901)

Figure 4

humanized 2G1 VH-11801 variant



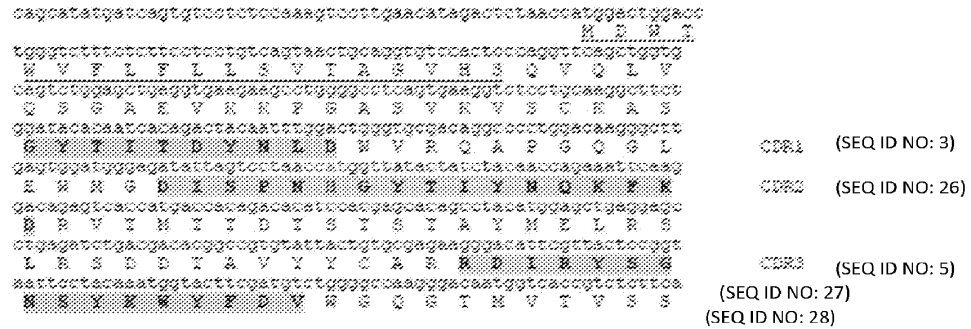
Humanized 2G1 VL-1901 variant



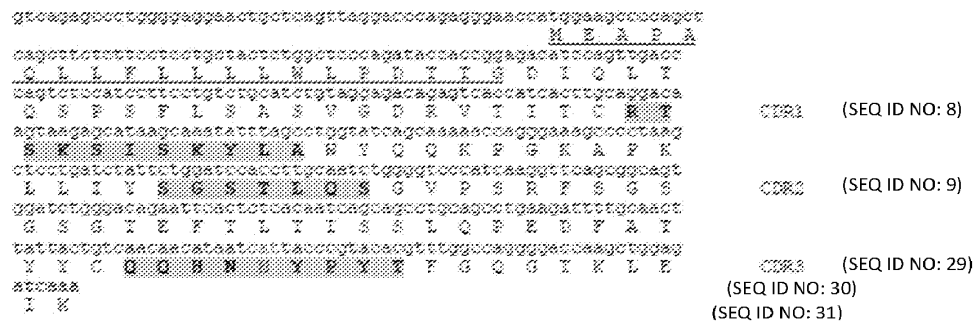
Engineered mAb H1-8/L1-9 (humanized 2G1 comprising VH-11801with N → ⁇ mutation in CDR1 and VL-1901 with Y→⁇ mutation in CDR1)

Figure 5

humanized 2G1 VH-11801 variant

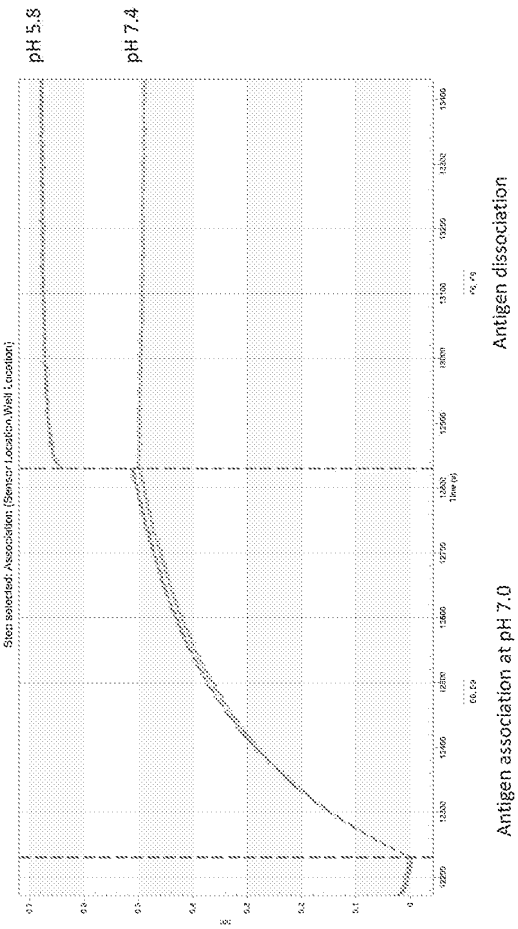


Humanized 2G1 VL-1901 variant



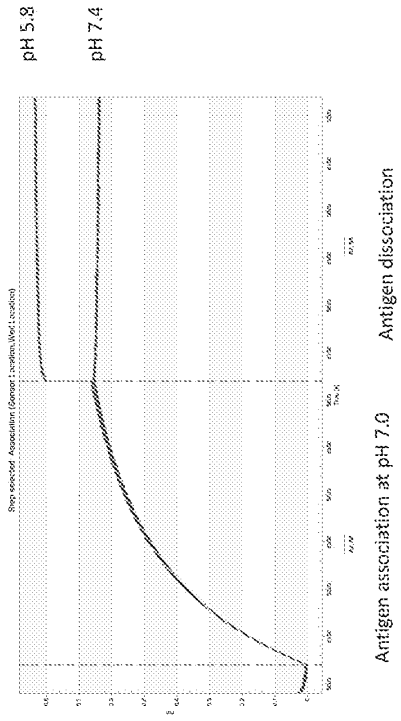
Engineered mAb H2-6/L3-5 (humanized 2G1 comprising VH-11801with Y → ℋ mutation in CDR2 and VL-1901 with E→ℋ mutation in CDR3)

Figure 6



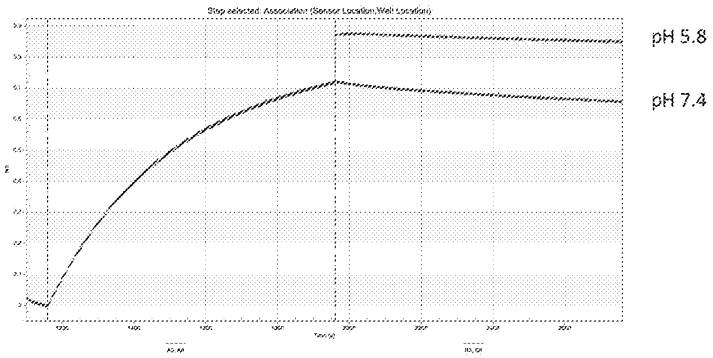
Parent mAb 11801 (VH-11801 and VL-1901)

Figure 7



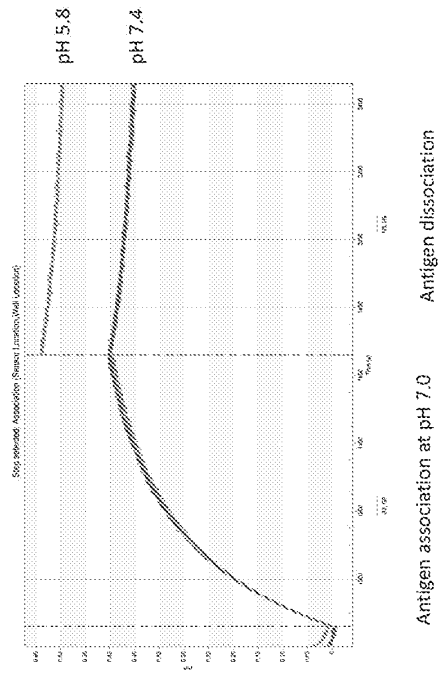
mAb L3-1

Figure 8



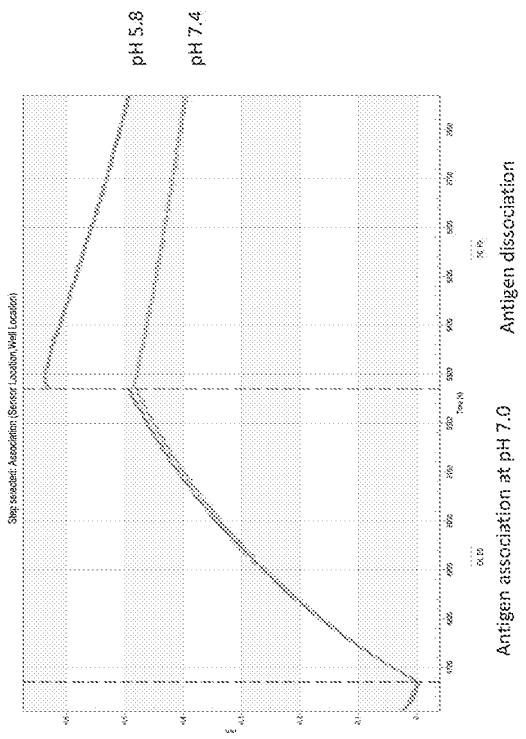
mAb L1-2

Figure 9



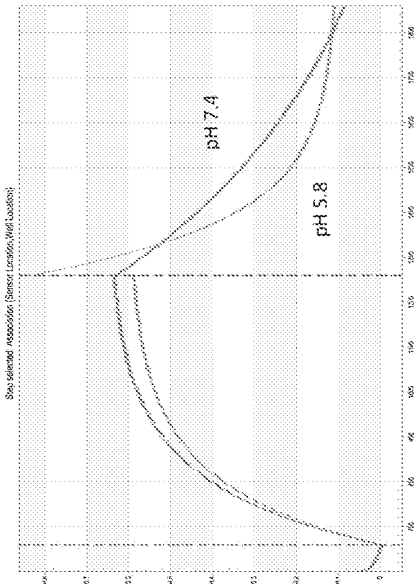
mAb H1-4

Figure 10



mAb H2-6/L3-5

Figure 11



Antigen association at pH 7.0 Antigen dissociation

mAb H1-8/L1-9

Figure 12

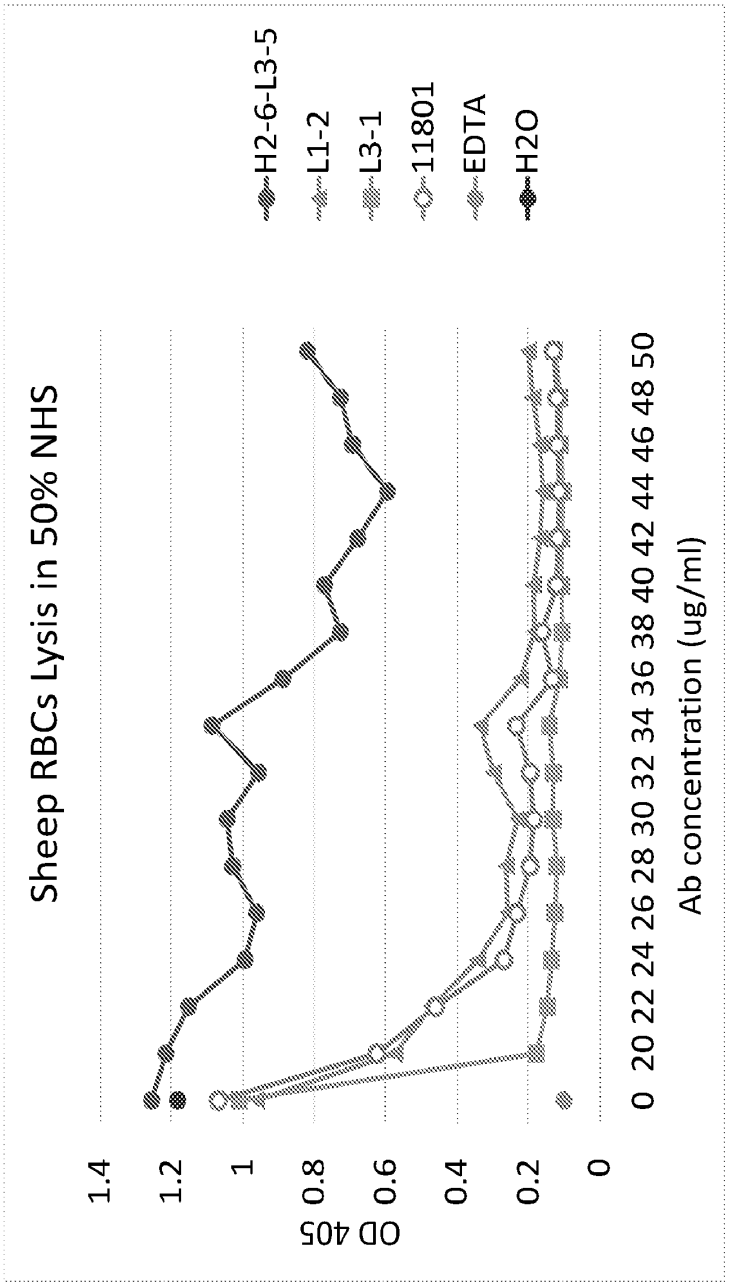

Sheep RBCs Lysis in 50% NHS
OD 405
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
Ab concentration (ug/ml)
H2-6-L3-5
L1-2
L3-1
11801
EDTA
H2O

Figure 13

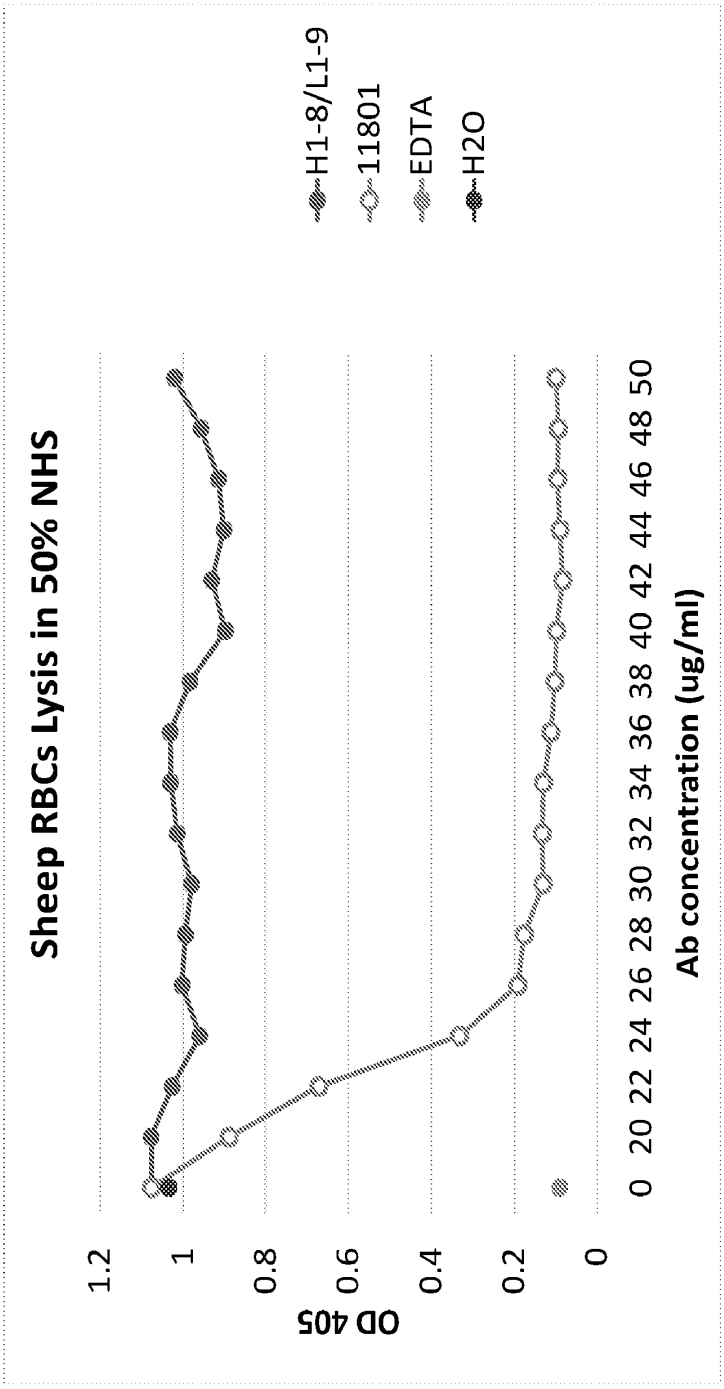

Sheep RBCs Lysis in 50% NHS
OD 405
1.2
1
0.8
0.6
0.4
0.2
0
0 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
Ab concentration (ug/ml)
H1-8/L1-9
11801
EDTA
H2O

Figure 14

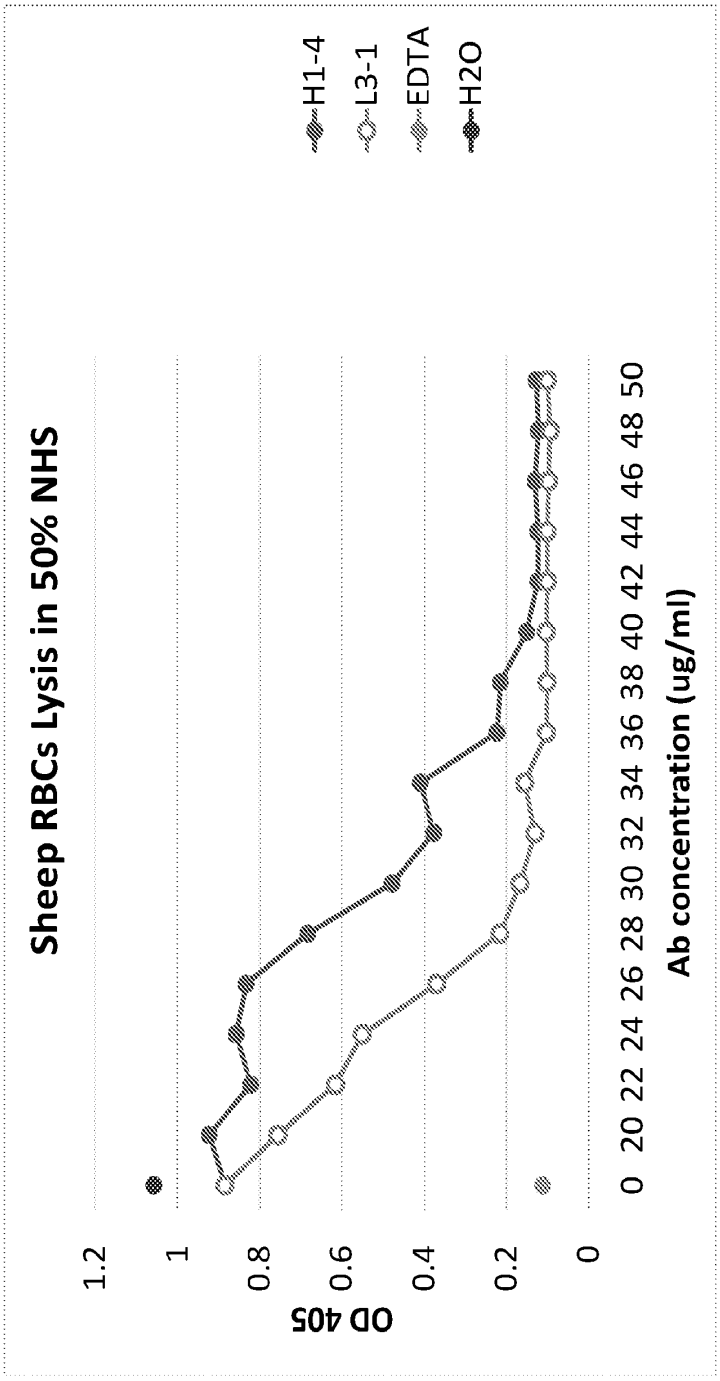

Sheep RBCs Lysis in 50% NHS
OD 405
1.2
1
0.8
0.6
0.4
0.2
0
0 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
Ab concentration (ug/ml)
H1-4
L3-1
EDTA
H2O

Figure 15

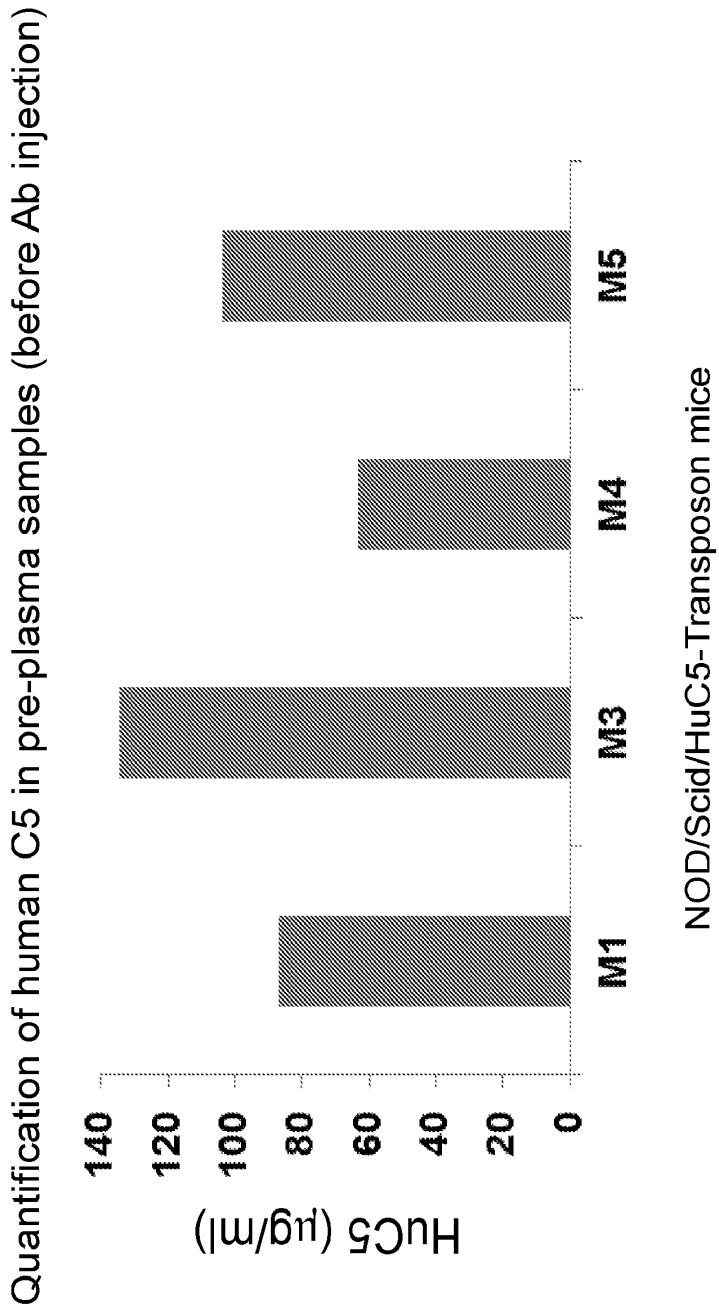

Quantification of human C5 in pre-plasma samples (before Ab injection)
HuC5 (µg/ml)
140
120
100
80
60
40
20
0
M1
M3
M4
M5
NOD/Scid/HuC5-Transposon mice

Figure 16

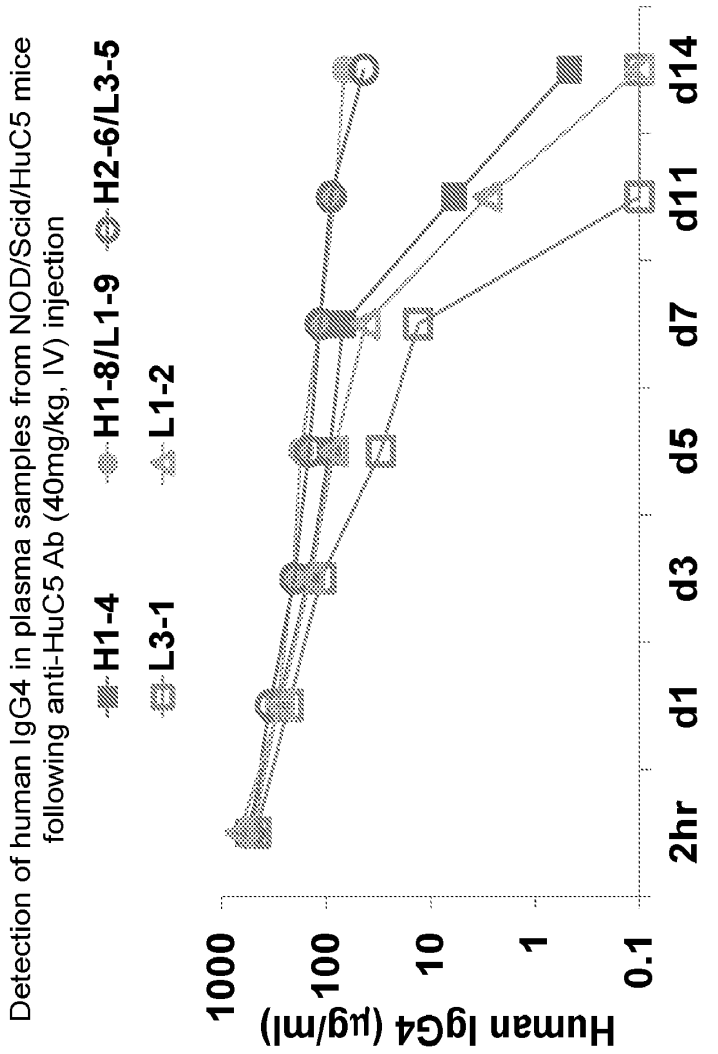

Detection of human IgG4 in plasma samples from NOD/Scid/HuC5 mice following anti-HuC5 Ab (40mg/kg, IV) injection
H1-4
H1-8/L1-9
H2-6/L3-5
L3-1
L1-2
Human IgG4 (µg/ml)
1000
100
10
1
0.1
2hr
d1
d3
d5
d7
d11
d14

Figure 17

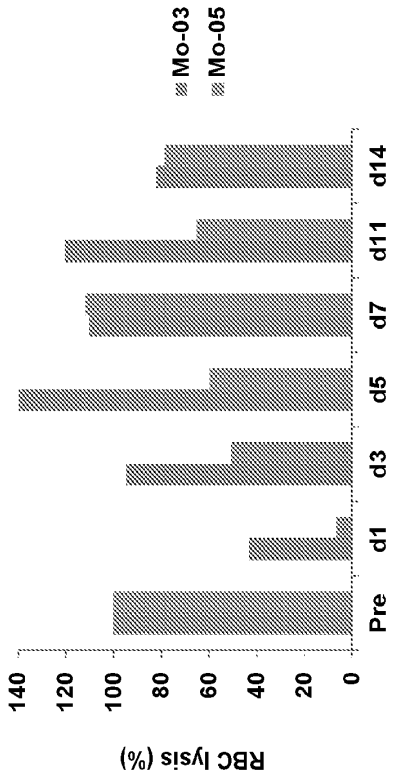

Mo-03
Mo-05
RBC lysis (%)
140
120
100
80
60
40
20
0
Pre
d1
d3
d5
d7
d11
d14

Figure 18

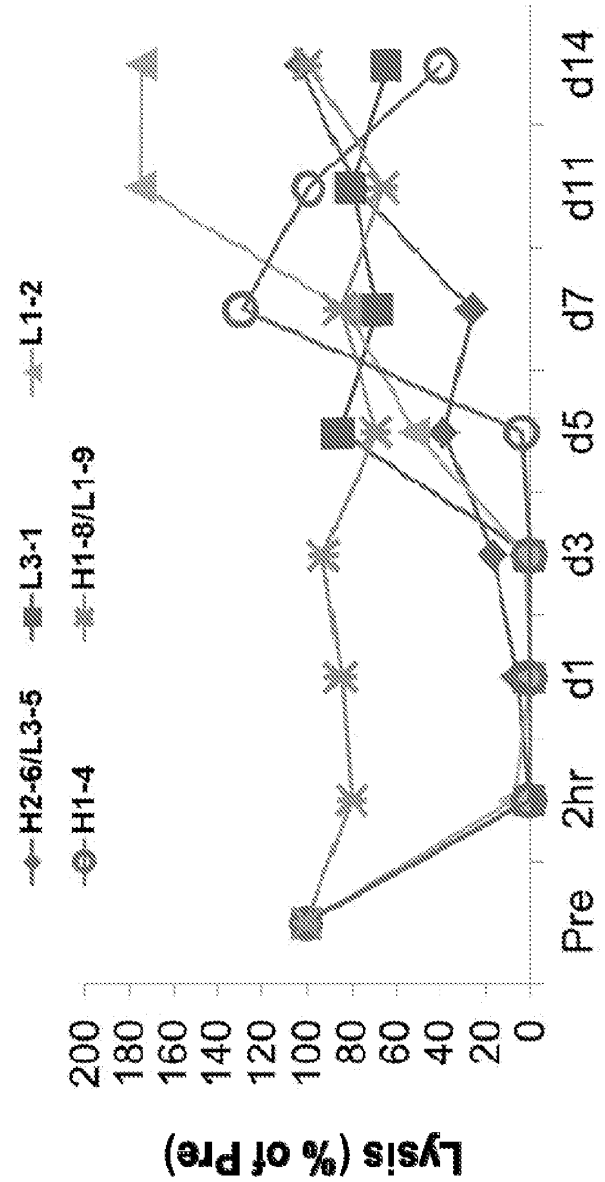

Lysis (% of Pre)
200
180
160
140
120
100
80
60
40
20
0
Pre
2hr
d1
d3
d5
d7
d11
d14
H2-6/L3-5
H1-4
L3-1
H1-8/L1-9
L1-2

Figure 19

Improved binding by parsimonious mutagenesis of 11801 CDRs

Row Number	Name	Score	Score Percent	Variant	Sequence	Variant Sequence	Mutations	Mutations	Number of Mutations
1	3H3	3.0637	4.3559				0.2558/0.2317	0.7345/0.8944	1
2	3C8	3.0752	4.4095	.K.			0.2558/0.2318	0.7345/0.8945	1
3	9H5	3.0776	4.4208	.K.			0.2558/0.2319	0.7345/0.8946	1
4	7A6	2.8504	3.4048	.T.			0.2558/0.2709	0.7345/0.8852	1
5	4H3	2.8461	2.9207	...W.			0.2489/0.2712	0.9404/0.8853	3
6	4D4	1.2810	2.3447	...I.			0.2489/0.2713	0.9404/0.8854	4
7	4C7	0.8506	1.6622	...V.			0.2489/0.2714	0.9404/0.8855	5
8	4E8	2.3868	2.9387	...Y.			0.2489/0.2715	0.9404/0.8856	2
9	4H8	3.1231	3.1701	...F.			0.2489/0.2716	0.9404/0.8857	2
10	7E4	0.6606	0.7436	....	A.....		0.2761/0.2591	0.8839/0.8995	2
11	7E8	0.5483	0.5708	....	S.....		0.2761/0.2592	0.8839/0.8996	1
12	8G2	0.8151	1.2935	.NM.	..G.....		0.2858/0.2812	0.9807/0.9483	1
13	9H1	2.2982	2.7148	....	...F.....		0.2924/0.2751	0.9218/0.8886	4
14	9C3	1.5171	2.1394	....	...I.....		0.2924/0.2752	0.9218/0.8887	5
15	9C7	1.3710	1.5117	....	...M.....		0.2924/0.2753	0.9218/0.8888	2
16	9A8	1.7092	2.3499	....	...W.....		0.2924/0.2754	0.9218/0.8889	1
17	9C10	0.6719	0.8583	....	...I.....		0.2924/0.2755	0.9218/0.8890	2
18	10F11	1.9052	0.4797	....	....R.....		0.2936/0.2883	0.6144/0.7944	1
19	14A2	0.4319	0.5778	....	.....F.....		0.2800/0.2549	0.8784/0.8436	1
20	14C6	0.4365	0.5194	....	.....H.....		0.2800/0.2550	0.8784/0.8437	1
21	17G5	0.4400	0.3667	....	.....R.....		0.3356/0.3126	1.0286/1.0008	1
22	18C6	0.5507	0.2480	....	.....K.....		0.2729/0.2776	0.9286/0.918	1
23	18D6	0.7387	0.5179	....	.....N.....		0.2729/0.2777	0.9286/0.919	1
24	18E8	0.5596	0.3071	....	.....M.....		0.2729/0.2778	0.9286/0.920	1
25	19F4	0.5757	0.3362	....	.....R.....		0.2974/0.3081	0.9396/0.9645	1
26	27F1	0.6690	0.5984	....	.....	...I.....	0.2738/0.3205	1.094/1.0993	2
27	27D4	0.5677	0.6202	....	.....	...M.....	0.2738/0.3206	1.094/1.0994	3
28	36G4	1.6856	0.6896	....	.....	.....N..	0.3805/0.3257	1.3192/1.3187	1
29	38H2	0.7111	0.7221	....	.....	.....F	0.3003/0.3012	1.097/1.11121	8
30	38B5	1.7269	2.6637	....	.....	.....E	0.3003/0.3013	1.097/1.11122	2
31	38D7	1.1011	2.0612	....	.....	.....W	0.3003/0.3014	1.097/1.11123	2
32	38G9	0.7032	0.4669	....	.....	.....Q	0.3003/0.3015	1.097/1.11124	1

	Distance
L9 in CDR1	0.3392
P4 in CDR2	1.0356
V16 in CDR3	

Figure 20

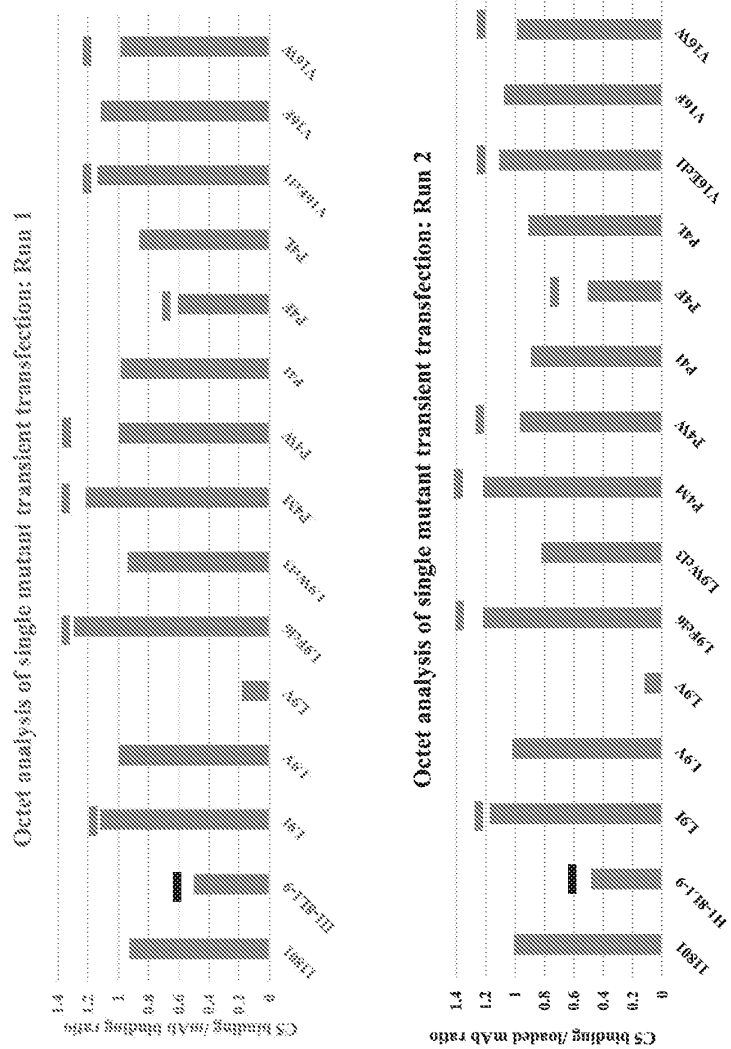
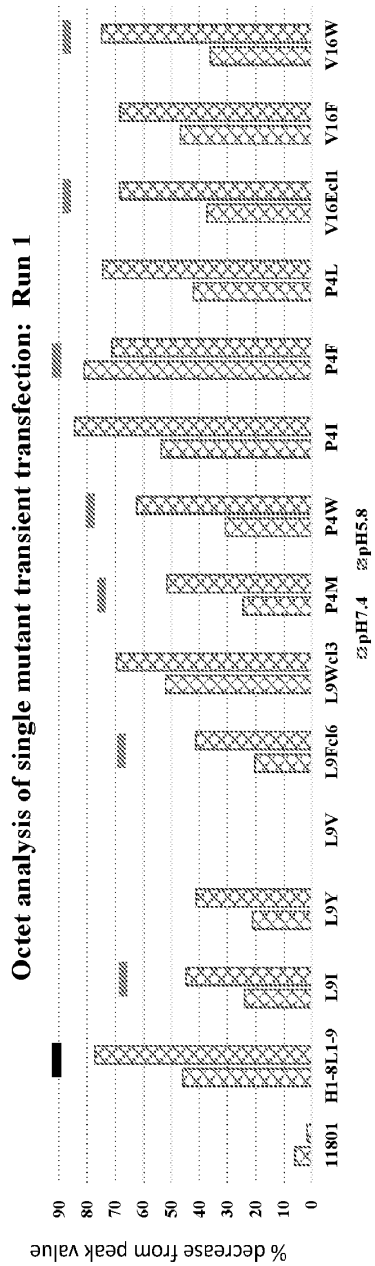

Octet analysis of single mutant transient transfection: Run 1
C5 binding /mAb binding ratio
1.4
1.2
1
0.8
0.6
0.4
0.2
0
11801
H1-8L1-9
L9I
L9Y
L9V
L9Fc16
L9Wc13
P4M
P4W
P4I
P4F
P4L
V16Ecl1
V16F
V16W
Octet analysis of single mutant transient transfection: Run 2
C5 binding /loaded mAb ratio
1.4
1.2
1
0.8
0.6
0.4
0.2
0
11801
H1-8L1-9
L9I
L9Y
L9V
L9Fc16
L9Wc13
P4M
P4W
P4I
P4F
P4L
V16Ecl1
V16F
V16W

Figure 21


Octet analysis of single mutant transient transfection: Run 1
% decrease from peak value
0
10
20
30
40
50
60
70
80
90
11801
H1-8L1-9
L9I
L9Y
L9V
L9Fcl6
L9Wcl3
P4M
P4W
P4I
P4F
P4L
V16Ecl1
V16F
V16W
pH7.4
pH5.8

Octet analysis of single mutant transient transfection: Run 2
% decrease from peak value
0
10
20
30
40
50
60
70
80
90
100
11801
H1-8L1-9
L9I
L9Y
L9V
L9Fcl6
L9Wcl3
P4M
P4W
P4I
P4F
P4L
V16Ecl1
V16F
V16W
pH7.4
pH5.8

Figure 22

L9I/P4M
L9I/P4W
L9I/P4F

L9F/P4M
L9F/P4W
L9F/P4F

L9I/P4M/V16W
L9I/P4W/V16W
L9I/P4F/V16W

L9F/P4M/V16W
L9F/P4W/V16W
L9F/P4F/V16W
L9I/P4M/V16E
L9I/P4W/V16E
L9I/P4F/V16E

L9F/P4M/V16E
L9F/P4W/V16E
L9F/P4F/V16E

Figure 23

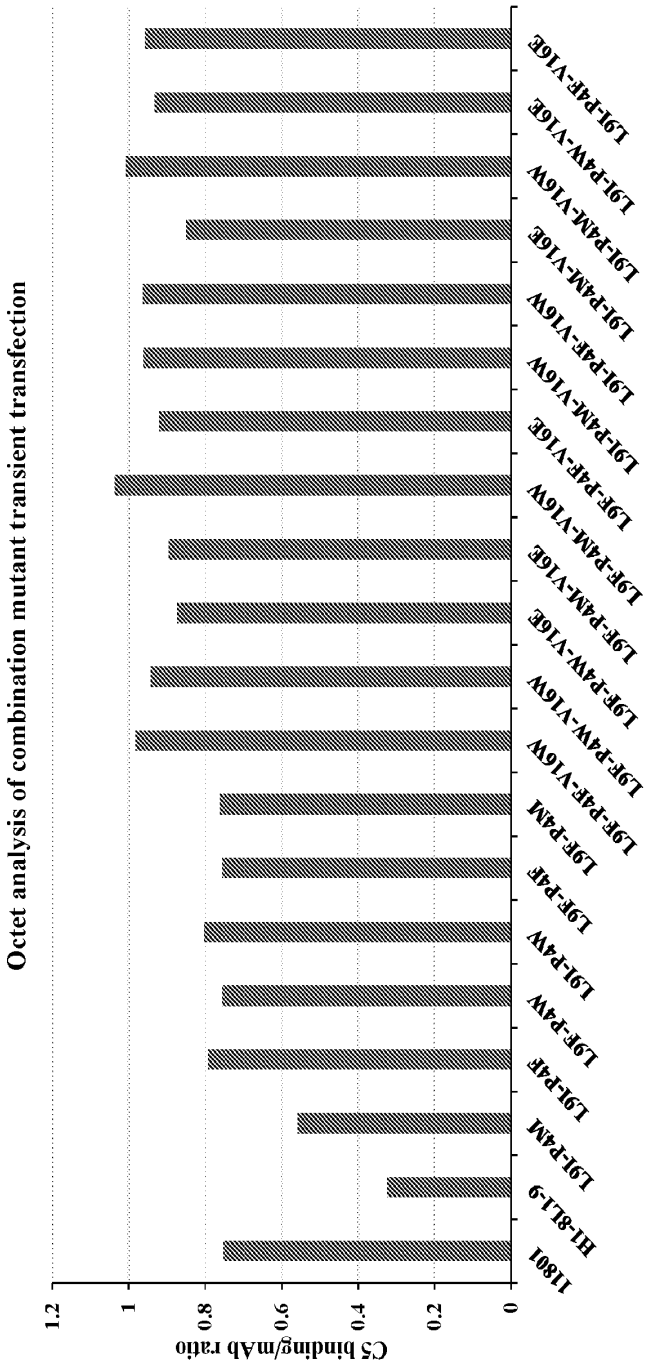

Octet analysis of combination mutant transient transfesction
C5 binding/mAb ratio
1.2
1
0.8
0.6
0.4
0.2
0
I1601
HI-8L1-9
L9I-P4M
L9I-P4F
L9F-P4W
L9I-P4W
L9F-P4F
L9F-P4M
L9F-P4F-V16W
L9F-P4W-V16W
L9F-P4W-V16E
L9F-P4M-V16E
L9F-P4M-V16W
L9F-P4F-V16E
L9I-P4M-V16W
L9I-P4F-V16W
L9I-P4M-V16E
L9I-P4M-V16W
L9I-P4W-V16E
L9I-P4F-V16E

Figure 24

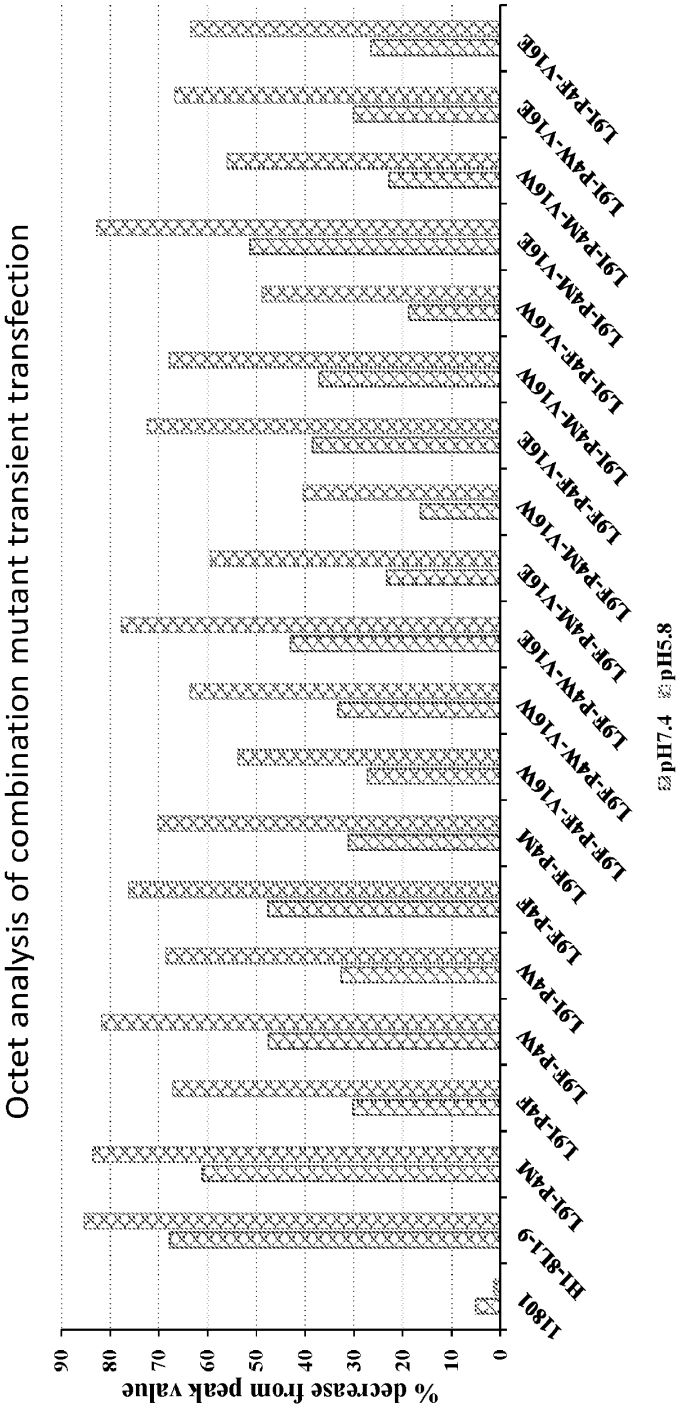
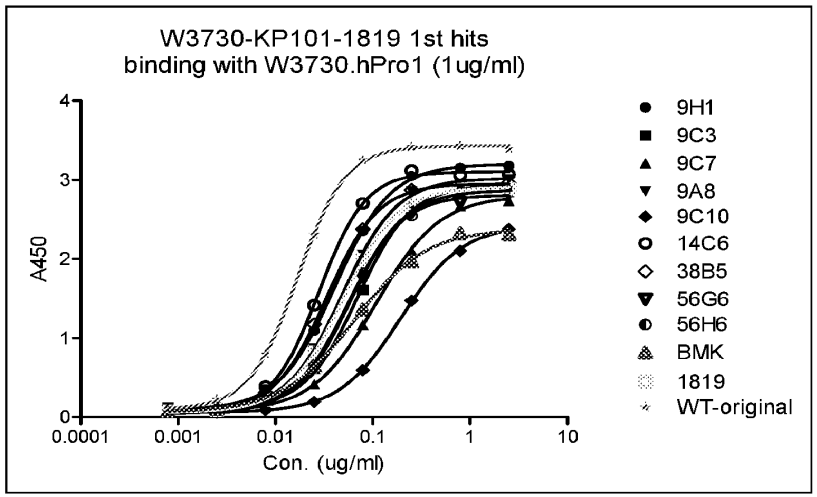

Octet analysis of combination mutant transient transfection
% decrease from peak value
0
10
20
30
40
50
60
70
80
90
pH7.4
pH5.8
I1601
HL-8L1-9
L9L-P4M
L9L-P4F
L9F-P4W
L9L-P4W
L9F-P4F
L9F-P4M
L9F-P4F-V16W
L9F-P4W-V16W
L9F-P4W-V16E
L9F-P4M-V16W
L9F-P4M-V16E
L9F-P4F-V16E
L9L-P4F-V16W
L9L-P4M-V16E
L9L-P4W-V16W
L9L-P4W-V16E
L9L-P4F-V16E

Figure 25





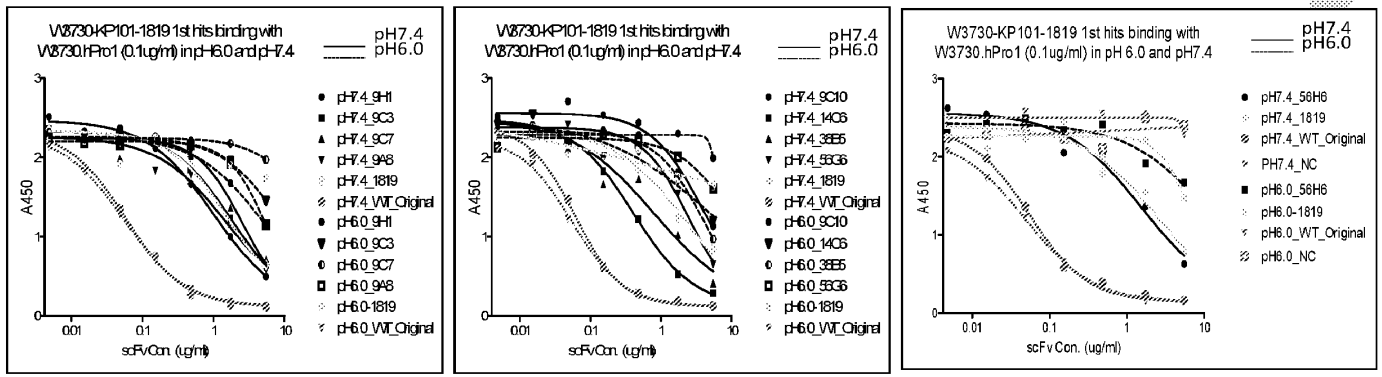
	9H1	9C3	9C7	9A8	9C10	14C6	38B5	56G6	56H6	WT-1819	BMK	WT-original
Top	3.1980	2.9380	2.8030	3.0220	2.4320	3.1040	2.9520	2.8110	2.8720	2.9120	2.3800	3.4320
EC50	0.0406	0.0703	0.1076	0.0486	0.1923	0.0280	0.0335	0.0568	0.0569	0.0491	0.0645	0.0169

Figure 26

W3730-KP101 Affinity Maturation - *Competition KD Elisa*

scFv after dialysis

Coat:W3730 hIgG 0.1ug/ml, 4 degrees C, ON → Blocking: RT 1h → Add scFv (diluted 3.16 fold with buffer) and W3730 hIgG (0.2ug/ml), 4 degrees C, ON → Add Goat x-human Ig G-Fc HRP, RT, 1h → OD450



Blocking & dilution buffer: 1xPBS with 2% BSA in pH7.4 and pH6.0, respectively

	pH7.4						pH6.0					
	9H1	9C3	9C7	9A8	1819	WT_Original	9H1	9C3	9C7	9A8	1819	WT_Original
Top	2.469	2.336	2.244	2.260	2.338	2.345	2.257	2.260	2.245	2.203	Not converged	2.315
EC50	0.9884	1.759	2.529	1.493	1.622	0.05641	5.005	9.065	28.46	5.459	Not converged	0.05447
	9C10	14C6	38B5	56G6	1819	WT_Original	9C10	14C6	38B5	56G6	1819	WT_Original
Top	2.561	2.481	2.484	2.382	2.340	2.396	2.283	2.474	2.326	2.248	2.680	2.262
EC50	3.937	0.4040	0.7818	2.430	2.081	0.06109	~ 6.808	4.970	3.911	11.41	42.34	0.05461
	56H6	NC	1819	WT_Original			pH6.0_56H6	pH6.0_NC	1819	WT_Original		
Top	2.564	2.499	2.390	2.384			2.428	252.3	2.266	2.264		
EC50	1.541	~ 6.968	2.325	0.05478			11.55	/	6.921	0.04870		

Figure 27

W3730-KP101 Affinity Maturation_1819

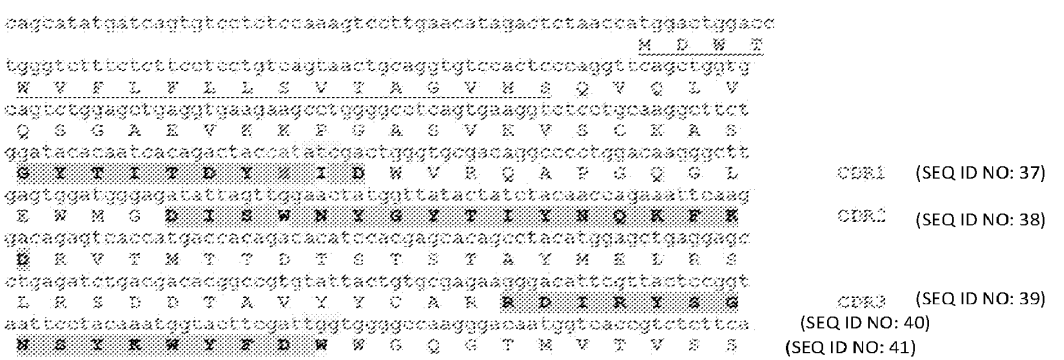
Binding with W3730.hPro1 in pH 6.0 and pH 7.4_Ranking in top

	pH 6.0		pH 7.4				CDRH1	CDRH2	CDRH3	CDRL1	CDRL2	CDRL3	Ranking
No.	KD (M)	koff (1/s)	KD (M)	koff (1/s)	KD ratio (pH6.0/pH7.4)	koff ratio (pH6.0/pH7.4)	SEQ ID NO:	SEQ ID NO:	SEQ ID NO:	SEQ ID NO:	SEQ ID NO:	SEQ ID NO:	
14C6	5.1260	0.0719	1.2940	0.03234	0.4335	0.5191	.....	..H.....	.....	.....	.....	.....	1
38H2	2.5670	0.4361	2.8360	0.2265	1.1769	2.6637	.....	.....	.....E....	.....	.....	.....	2
1442	2.3890	0.0234	2.1710	0.0158	0.4319	0.5773	.....	.....F....	.....	.....	.....	.....	1
4H8	2.1560	0.0293	3.4520	0.0141	3.1231	3.1781	....F.	.....	.....	.....	.....	.....	2
27F1	2.0660	0.0311	2.0310	0.0150	0.6690	0.5984	.....	.....	....L.....	.....	.....	.....	2
27D4	1.9960	0.0278	2.1800	0.0212	0.5677	0.6202	.....	.....	....M.....	.....	.....	.....	3
4E8	1.9810	0.0474	3.2410	0.0350	2.3868	2.9397	....Y.	.....	.....	.....	.....	.....	2
4H3	1.9770	0.0584	2.3220	0.0195	2.3461	2.9207	....V.	.....	.....	.....	.....	.....	3
7E1	1.9610	0.0285	3.3180	0.0271	0.6606	0.7435	.....	.A.....	.....	.....	.....	.....	2
18D6	1.8350	0.0264	3.2600	0.0948	0.7367	0.5179	.....	.....N....	.....	.....	.....	.....	1
7E8	1.7110	0.0358	3.1310	0.0452	0.5485	0.5705	.....	.S.....	.....	.....	.....	.....	1
4D4	1.7030	0.0389	3.0110	0.029-	1.2610	2.5447	....I.	.....	.....	.....	.....	.....	4
47D8	1.7020	0.0208	2.3480	0.0156	0.6901	0.6812	.....	.....	.....	.....F..	.....	.....	1
9H1	1.6960	0.0744	2.0120	0.0213	2.5263	2.7143	.....	I.....	.....	.....	.....	.....	4
47G2	1.6630	0.0186	2.3760	0.0230	0.7948	0.6582	.....	.....	.....	.....Y..	.....	.....	3
58E9	1.6270	0.0264	2.3560	0.0256	0.6406	0.6647	.....	.....	.....	.....	.....E	.....	1
17G2	1.6140	0.0332	1.8910	0.0250	0.4400	0.5667	.....	.....K....	.....	.....	.....	.....	1
4C7	1.5990	0.0427	2.9450	0.0296	0.8506	1.6622	....Y.	.....	.....	.....	.....	.....	5
58H5	1.5970	0.0531	2.4100	0.0397	0.7511	0.7291	.....	.....	.....	.....F..	.....	.....	6
59D6	1.4600	0.2494	2.6090	0.2231	0.7078	0.8630	.....	.....	.....	.....	.....	...A.....	2
18E8	1.4120	0.0478	1.2590	0.0413	0.6596	0.5071	.....	.....M....	.....	.....	.....	.....	1
9A8	1.3930	0.0878	0.4707	0.0284	1.7092	2.3499	.....	W.....	.....	.....	.....	.....	3
18F11	1.3780	0.0750	1.8250	0.0676	1.9052	0.4797	.....	....R.....	.....	.....	.....	.....	1
36D7	1.3550	0.0396	1.6690	0.0463	1.1011	2.0612	.....	.....	.....F	.....	.....	.....	2
18C6	1.3470	0.0568	1.4720	0.0409	0.5307	0.2480	.....	.....K....	.....	.....	.....	.....	1
19F1	1.2690	0.0531	1.5430	0.0463	0.5757	0.5382	.....	.....R..	.....	.....	.....	.....	1
47B2	1.1750	0.0344	1.9290	0.029-	2.1031	0.2944	.....	.....	.....	.....V..	.....	.....	1
41F10	1.1620	0.1357	1.0990	0.126-	1.5068	0.2536	.....	.....	.....	..?.....	.....	.....	1
36G9	1.1530	0.0372	1.4810	0.08248	0.7032	0.4669	.....	.....	.....Q	.....	.....	.....	1
9C8	1.1420	0.0936	1.7530	0.4360	1.5171	2.1391	.....	I.....	.....	.....	.....	.....	5
51E1	1.0660	0.1289	1.1580	0.1918	1.1804	0.2489	.....	.....	.....	.....	.E.....	.....	1
9C7	0.9630	0.1437	0.2635	0.1102	1.3710	1.3117	.....	W.....	.....	.....	.....	.....	2
58C6	0.9429	0.0492	0.9382	0.09237	0.8178	0.5493	.....	.....	.....	.....	.....T	.....	2
58D8	0.9099	0.0869	0.7286	0.08248	0.9288	0.5081	.....	.....	.....	.....	I	.....	1
51F10	0.7982	0.0486	0.9446	0.0465	0.8805	0.2894	.....	.....	.....	.....	.S.....	.....	1
9C10	0.7432	0.1260	0.8394	0.8363	0.8719	0.8583	.....	I.....	.....	.....	.....	.....	2
51F1	0.3924	0.2587	0.1808	0.3201	1.2885	0.1519	.....	.....	.....	.....	.Y.....	.....	1
51G1	0.2274	0.1020	/	/	0.3782	0.1315	.....	.....	.....	.....	.F.....	.....	2

Gray highlight: Originally selected hits. Yellow highlight: Newly selected hits

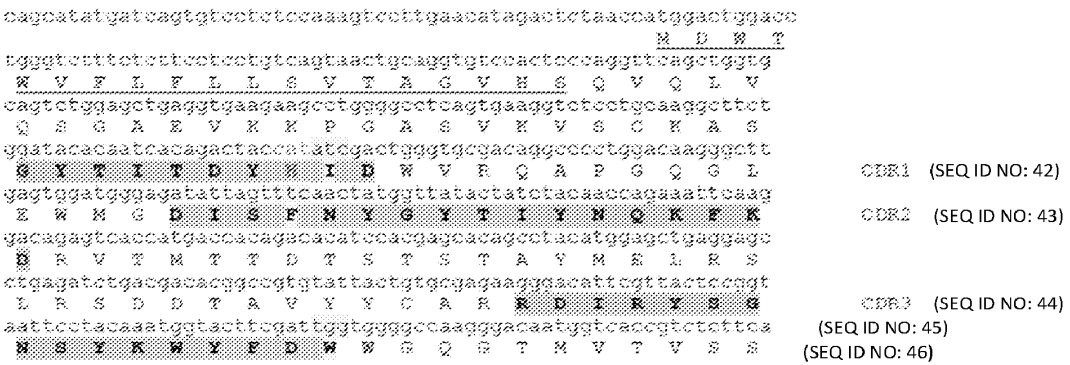
Figure 28

IWW-VH



Heavy chain sequence of mAb H1-8/L1-9 variant IWW

IFW-VH



Heavy chain sequence of mAb H1-8/L1-9 variant IFW

Figure 29

FME-VH

```
cagcatatgatcagtgtcctctccaaagtccttgaacatagactctaaccatggactggacc
                                                M  D  W  T
tgggtctttctcttcctcctgtcagtaactgcaggtgtccactcccaggttcagctggtg
 W  V  F  L  F  L  L  S  V  T  A  G  V  H  S  Q  V  Q  L  V
cagtctggagctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggcttct
 Q  S  G  A  E  V  K  K  P  G  A  S  V  K  V  S  C  K  A  S
ggatacacaatcacagactaccatttcgactgggtgcgacaggcccctggacaagggctt
 G  Y  T  I  T  D  Y  H  F  D  W  V  R  Q  A  P  G  Q  G  L        CDR1  (SEQ ID NO: 47)
gagtggatgggagatattagtatgaactatggttatactatctacaaccagaaattcaag
 E  W  M  G  D  I  S  M  N  Y  G  Y  T  I  Y  N  Q  K  F  K        CDR2  (SEQ ID NO: 48)
gacagagtcaccatgaccacagacacatccacgagcacagcctacatggagctgaggagc
 D  R  V  T  M  T  T  D  T  S  T  S  T  A  Y  M  E  L  R  S
ctgagatctgacgacacggccgtgtattactgtgcgagaagggacattcgttactccggt
 L  R  S  D  D  T  A  V  Y  Y  C  A  R  R  D  I  R  Y  S  G        CDR3  (SEQ ID NO: 49)
aattcctacaaatggtacttcgatgagtggggccaagggacaatggtcaccgtctcttca      (SEQ ID NO: 50)
 N  S  Y  K  W  Y  F  D  E  W  G  Q  G  T  M  V  T  V  S  S       (SEQ ID NO: 51)
```

Heavy chain sequence of mAb H1-87.1-9 variant FME

FMW-VH

```
cagcatatgatcagtgtcctctccaaagtccttgaacatagactctaaccatggactggacc
                                                M  D  W  T
tgggtctttctcttcctcctgtcagtaactgcaggtgtccactcccaggttcagctggtg
 W  V  F  L  F  L  L  S  V  T  A  G  V  H  S  Q  V  Q  L  V
cagtctggagctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggcttct
 Q  S  G  A  E  V  K  K  P  G  A  S  V  K  V  S  C  K  A  S
ggatacacaatcacagactaccatttcgactgggtgcgacaggcccctggacaagggctt
 G  Y  T  I  T  D  Y  H  F  D  W  V  R  Q  A  P  G  Q  G  L        CDR1  (SEQ ID NO: 52)
gagtggatgggagatattagtatgaactatggttatactatctacaaccagaaattcaag
 E  W  M  G  D  I  S  M  N  Y  G  Y  T  I  Y  N  Q  K  F  K        CDR2  (SEQ ID NO: 53)
gacagagtcaccatgaccacagacacatccacgagcacagcctacatggagctgaggagc
 D  R  V  T  M  T  T  D  T  S  T  S  T  A  Y  M  E  L  R  S
ctgagatctgacgacacggccgtgtattactgtgcgagaagggacattcgttactccggt
 L  R  S  D  D  T  A  V  Y  Y  C  A  R  R  D  I  R  Y  S  G        CDR3  (SEQ ID NO: 54)
aattcctacaaatggtacttcgatgagtggggccaagggacaatggtcaccgtctcttca      (SEQ ID NO: 55)
 N  S  Y  K  W  Y  F  D  W  W  G  Q  G  T  M  V  T  V  S  S       (SEQ ID NO: 56)
```

Heavy chain sequence of mAb H1-87.1-9 variant FMW

Figure 30

FMEH-VH

```
cagcatatgatcagtgtcctctccaaagtccttgaacatagactctaaccatggactggacc
                                                  M  D  W  T
tgggtctttctcttcctcctgtcagtaactgcaggtgtccactcccaggttcagctggtg
 W  V  F  L  F  L  L  S  V  T  A  G  V  H  S  Q  V  Q  L  V
cagtctggagctgaggtgaagaagcctggggcctcagtgaaggtctcctgcaaggcttct
 Q  S  G  A  E  V  K  K  P  G  A  S  V  K  V  S  C  K  A  S
ggatacacaatcacagactaccatttcgactgggtgcgacaggcccctggacaagggctt
 G  Y  T  I  T  D  Y  H  F  D  W  V  R  Q  A  P  G  Q  G  L
gagtggatgggagatattagtatgaactatggttatcatatctacaaccagaaattcaag
 E  W  M  G  D  I  S  M  N  Y  G  Y  H  I  Y  N  Q  K  F  K
gacagagtcaccatgaccacagacacatccacgagcacagcctacatggagctgaggagc
 D  R  V  T  M  T  T  D  T  S  T  S  T  A  Y  M  E  L  R  S
ctgagatctgacgacacggccgtgtattactgtgcgagaagggacattcgttactccggt
 L  R  S  D  D  T  A  V  Y  Y  C  A  R  R  D  I  R  Y  S  G
aattcctacaaatggtacttcgatgagtggggccaagggacaatggtcaccgtctcttca
 N  S  Y  K  W  Y  F  D  E  W  G  Q  G  T  M  V  T  V  S  S
```

CDR1 (SEQ ID NO: 47)

CDR2 (SEQ ID NO: 57)

CDR3 (SEQ ID NO: 49)

(SEQ ID NO: 58)

(SEQ ID NO: 59)

Heavy chain sequence of mAb H1-8/L1-9 variant FMEH

Figure 31

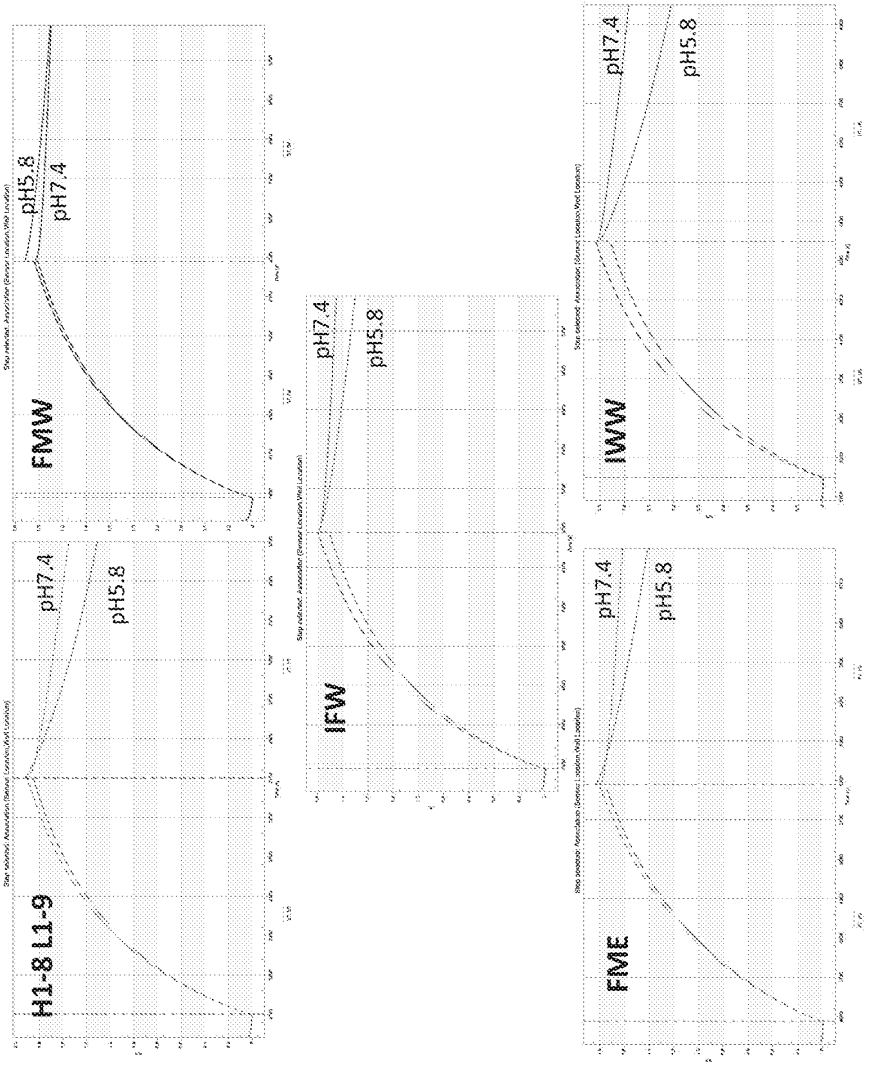

H1-8 L1-9
pH7.4
pH5.8
FMW
pH5.8
pH7.4
IFW
pH7.4
pH5.8
FME
pH7.4
pH5.8
IWW
pH7.4
pH5.8

Figure 32

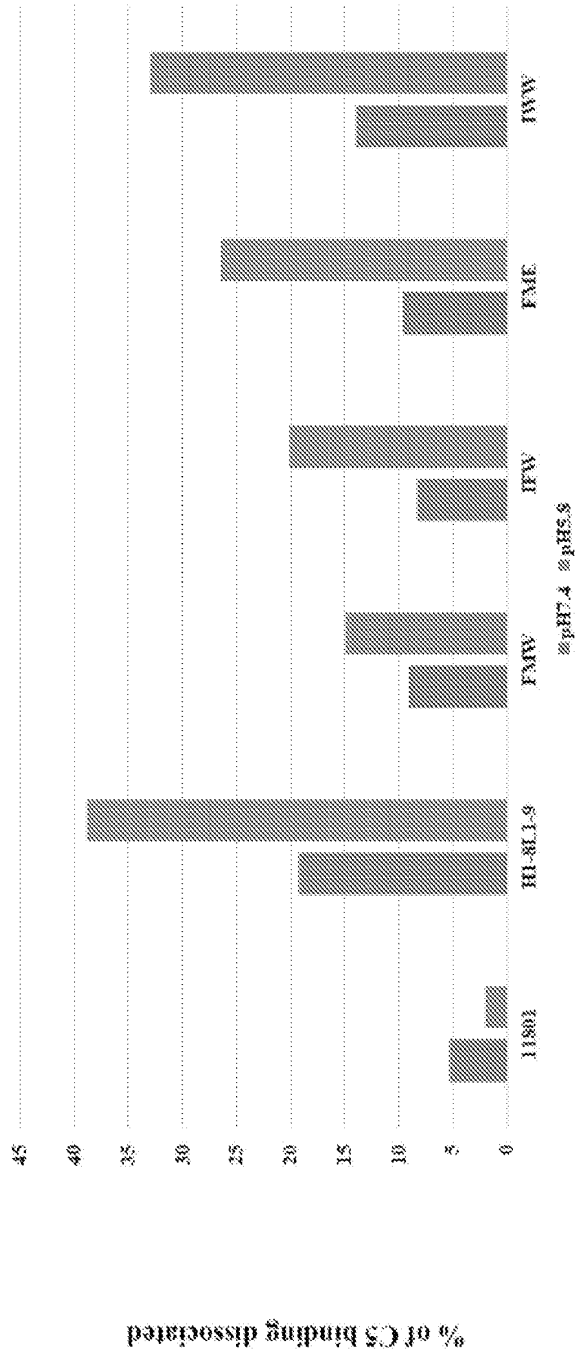

% of C5 binding dissociated
45
40
35
30
25
20
15
10
5
0
H1-8L1-9
FMW
IFW
FME
IWW
pH7.4
pH5.9

Figure 33

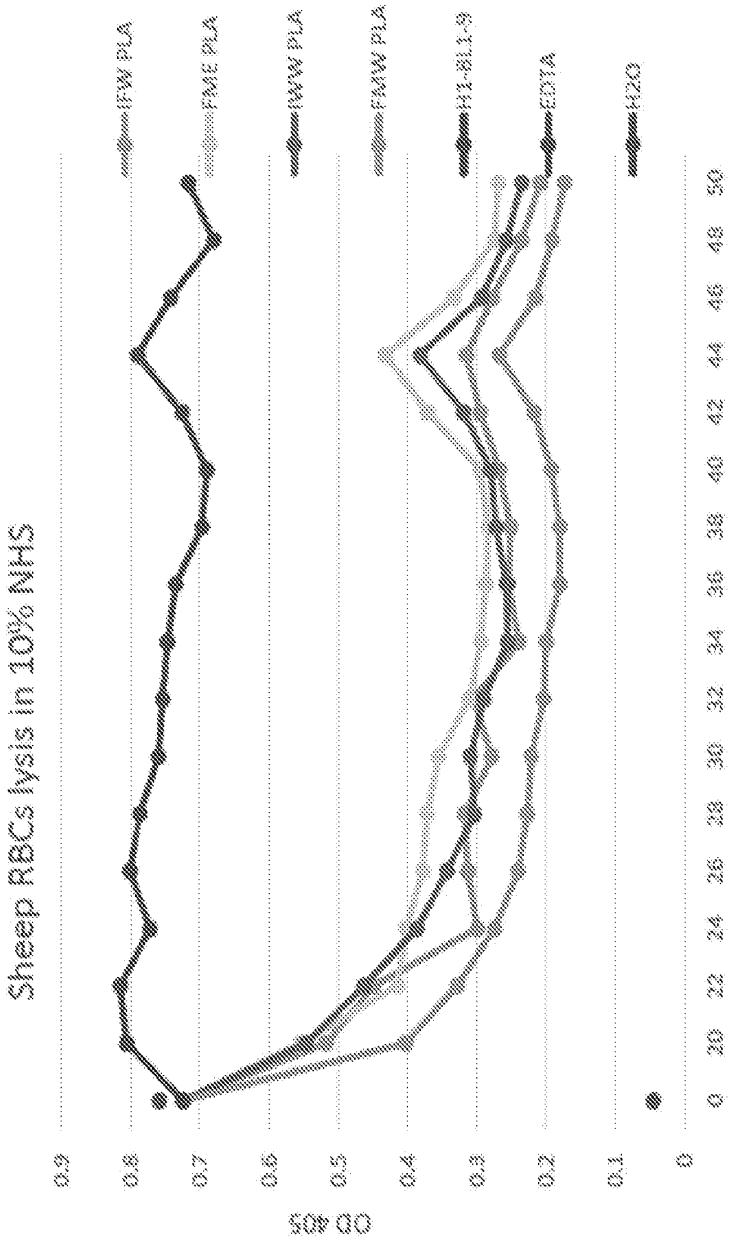

Sheep RBCs lysis in 10% NHS
OD 405
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
Ab concentration (ug/ml)
IFW PLA
FME PLA
IWW PLA
FAAW PLA
H1-BL1-9
EDTA
H2O

Figure 34

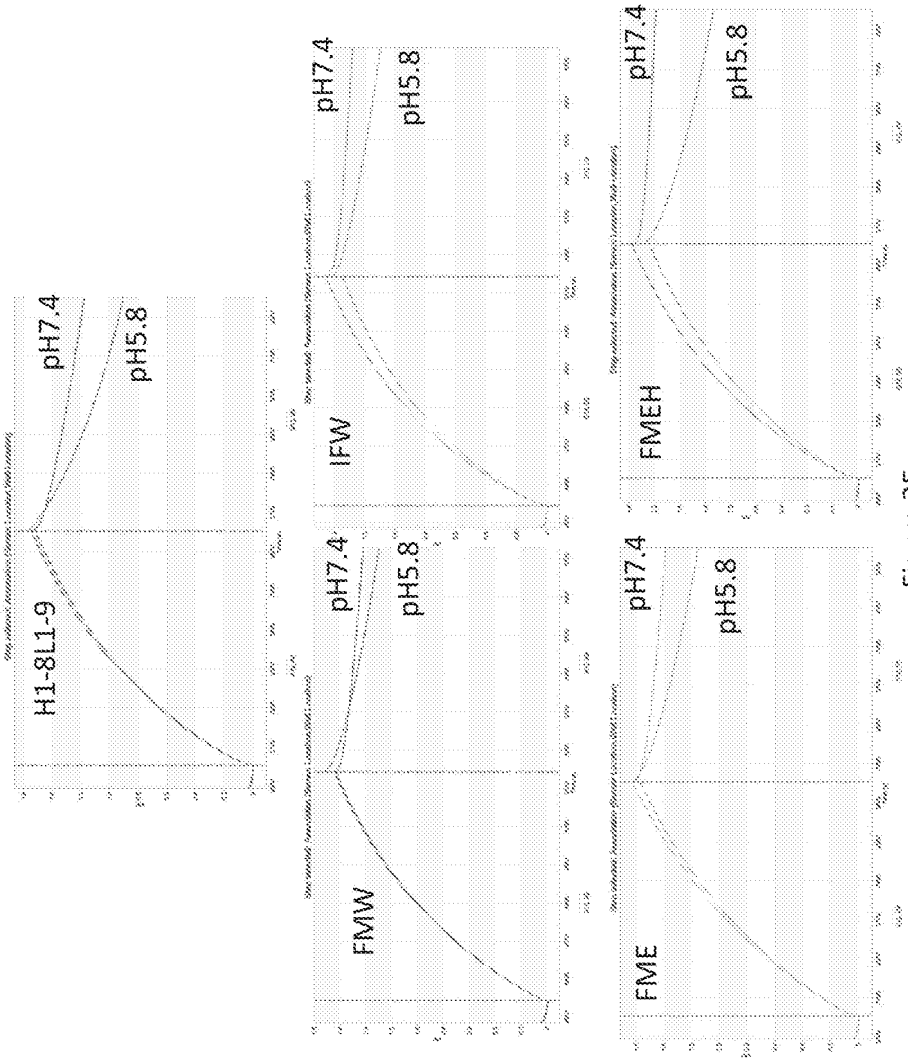

H1-8L1-9
pH7.4
pH5.8
FMW
pH7.4
pH5.8
IFW
pH7.4
pH5.8
FME
pH7.4
pH5.8
FMEH
pH7.4
pH5.8

Figure 35

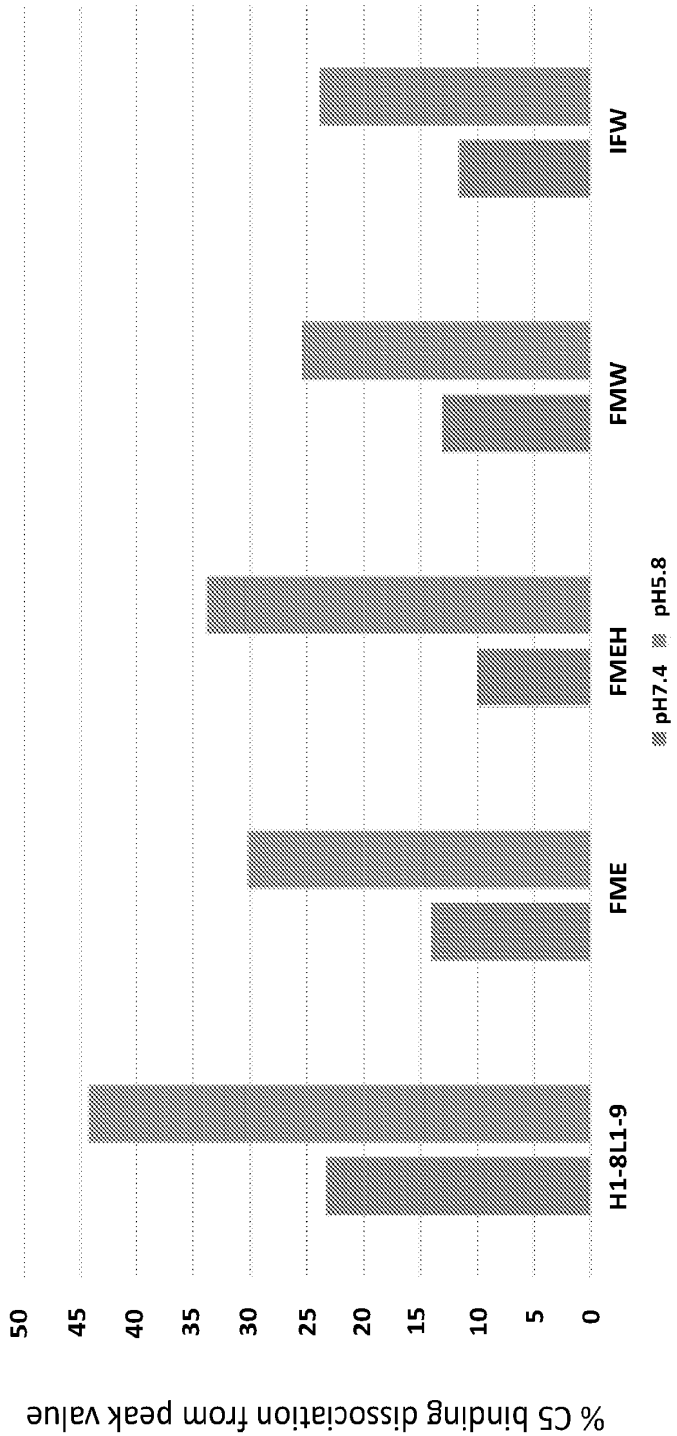

% C5 binding dissociation from peak value
50
45
40
35
30
25
20
15
10
5
0
H1-8L1-9
FME
FMEH
FMW
IFW
pH7.4
pH5.8

Figure 36

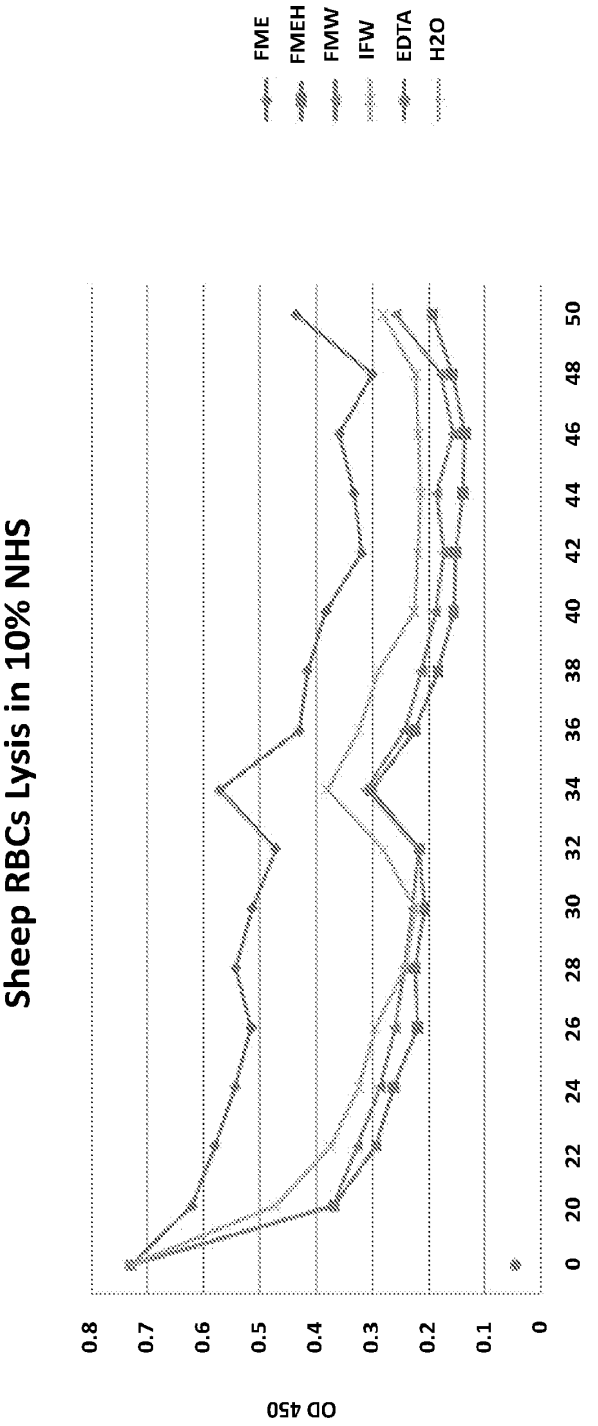
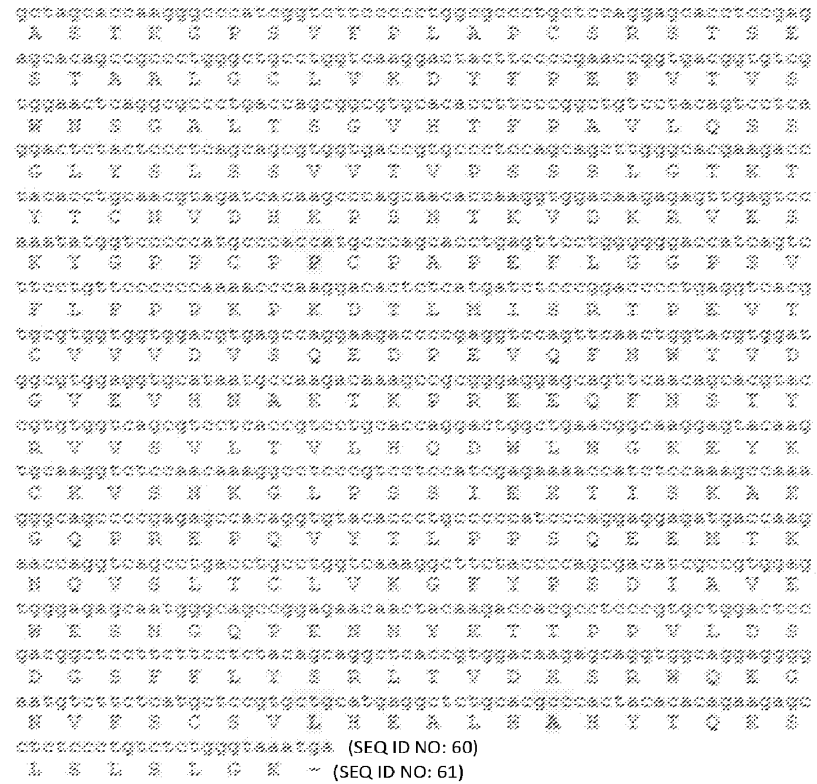

Sheep RBCs Lysis in 10% NHS
OD 450
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48 50
Ab concentration (ug/ml)
FME
FMEH
FMW
IFW
EDTA
H2O

Figure 37

Human IgG4-Fc- PLA mutant



(SEQ ID NO: 60)

(SEQ ID NO: 61)

Human IgG4 Fc domain mutations

Figure 38

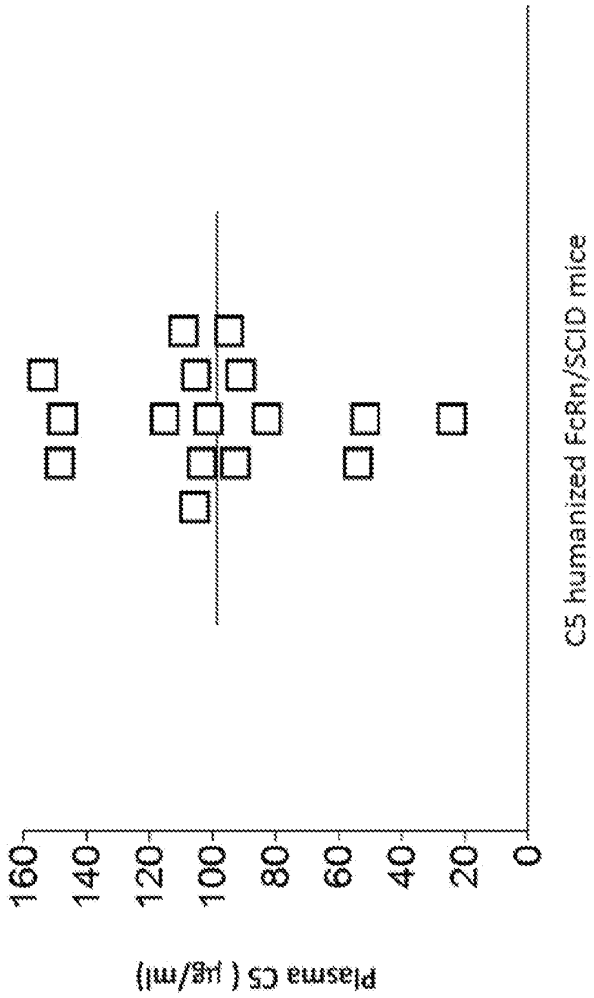

Plasma C5 (μg/ml)
160
140
120
100
80
60
40
20
0
C5 humanized FcRn/SCID mice

Figure 39

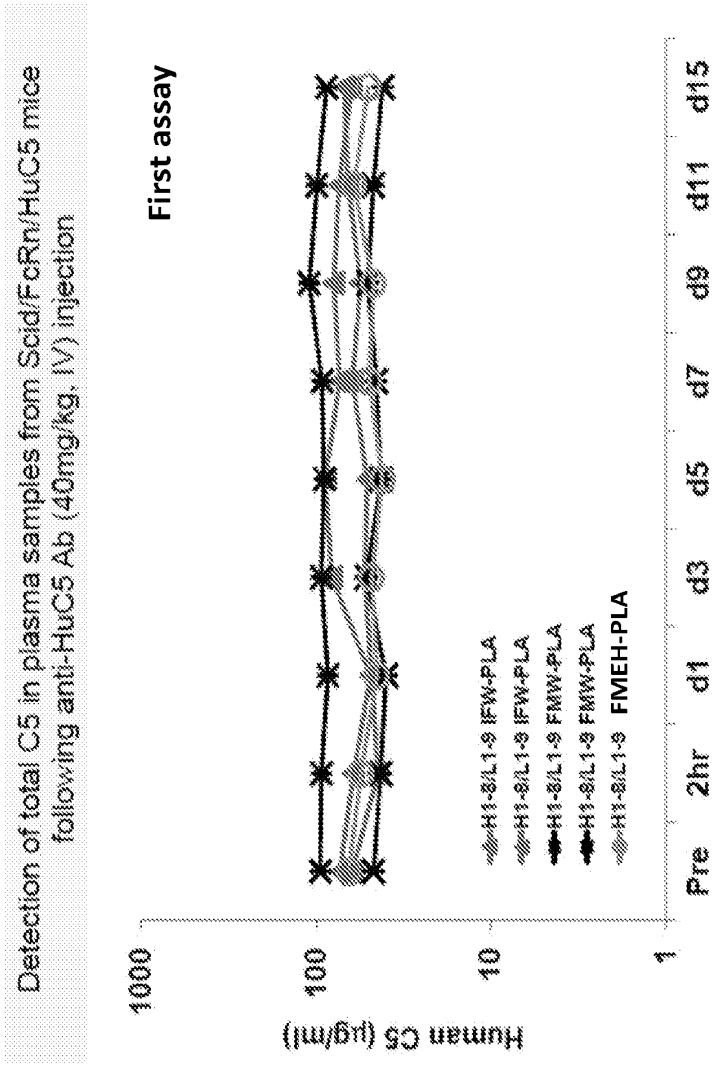

Detection of total C5 in plasma samples from Scid/FcRn/HuC5 mice following anti-HuC5 Ab (40mg/kg, IV) injection
First assay
Human C5 (µg/ml)
1000
100
10
1
Pre
2hr
d1
d3
d5
d7
d9
d11
d15
H1-8/L1-9 IFW-PLA
H1-8/L1-9 IFW-PLA
H1-8/L1-9 FMW-PLA
H1-8/L1-9 FMW-PLA
H1-8/L1-9 FMEH-PLA

Figure 40

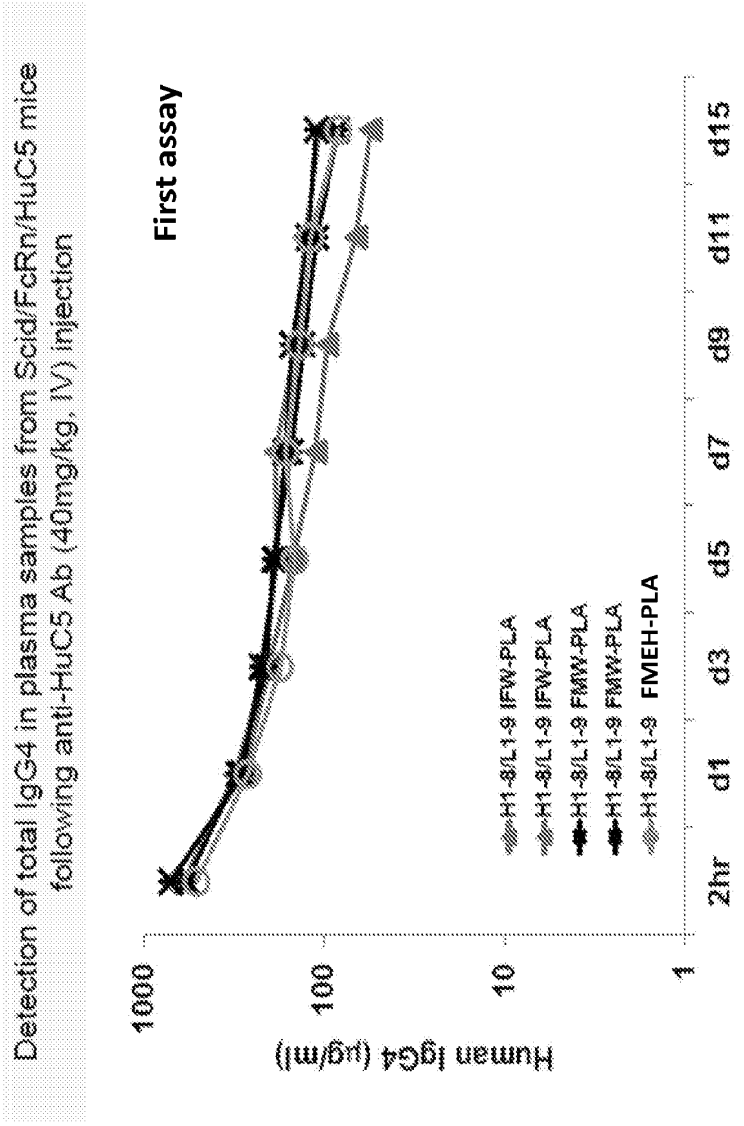

Detection of total IgG4 in plasma samples from Scid/FcRn/HuC5 mice following anti-HuC5 Ab (40mg/kg, IV) injection
First assay
Human IgG4 (µg/ml)
1000
100
10
1
2hr
d1
d3
d5
d7
d9
d11
d15
H1-8/L1-9 IFW-PLA
H1-8/L1-9 IFW-PLA
H1-8/L1-9 FMW-PLA
H1-8/L1-9 FMW-PLA
H1-8/L1-9 FMEH-PLA

Figure 41

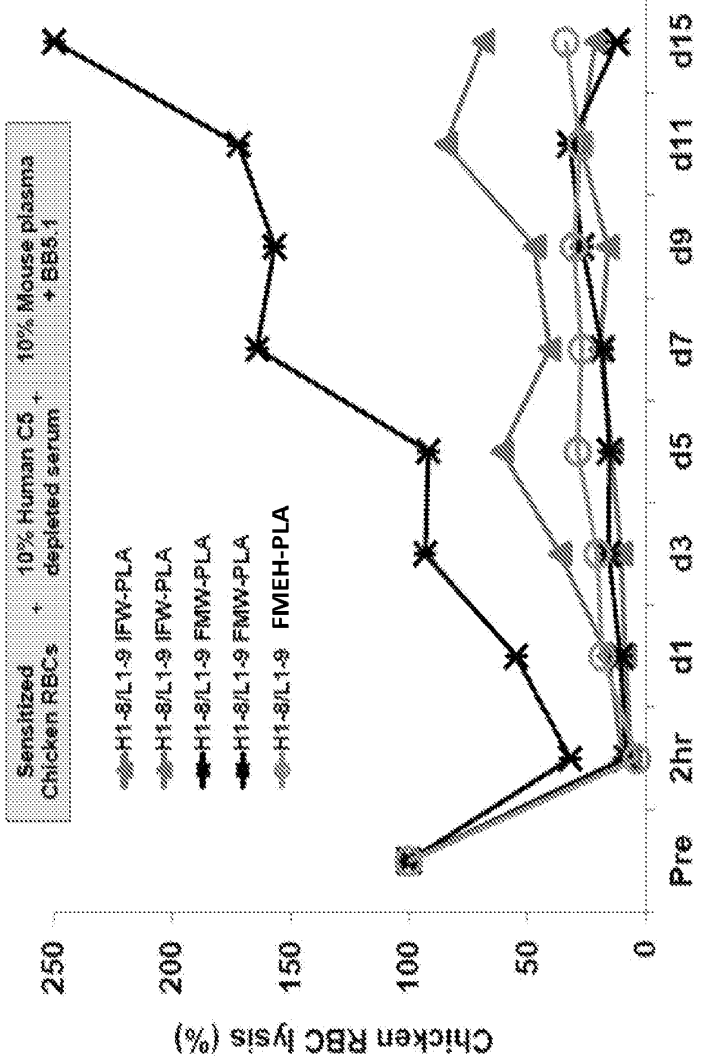

Sensitized Chicken RBCs + 10% Human C5 depleted serum + 10% Mouse plasma + BB5.1
H1-8/L1-9 IFW-PLA
H1-8/L1-9 IFW-PLA
H1-8/L1-9 FMW-PLA
H1-8/L1-9 FMW-PLA
H1-8/L1-9 FMEH-PLA
Chicken RBC lysis (%)
250
200
150
100
50
0
Pre
2hr
d1
d3
d5
d7
d9
d11
d15

Figure 42

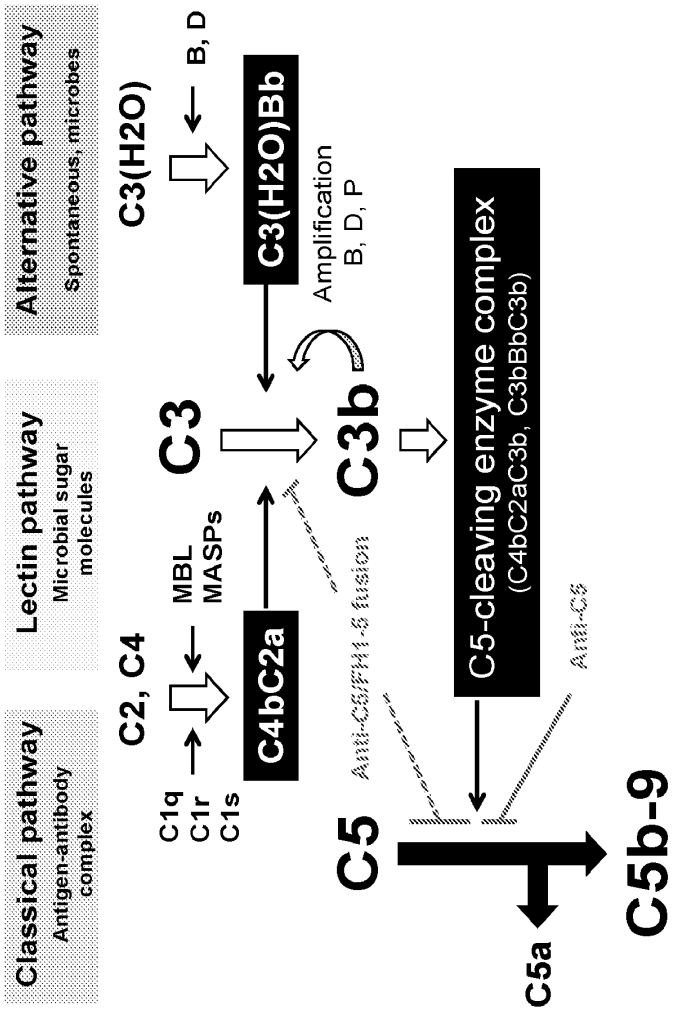

Classical pathway
Antigen-antibody complex
Lectin pathway
Microbial sugar molecules
Alternative pathway
Spontaneous, microbes
C1q
C1r
C1s
C2, C4
MBL
MASPs
C4bC2a
C3
C3(H2O)
B, D
C3(H2O)Bb
Amplification
B, D, P
C3b
Anti-C5/FH1-5 fusion
C5
C5-cleaving enzyme complex
(C4bC2aC3b, C3bBbC3b)
Anti-C5
C5a
C5b-9

Figure 43

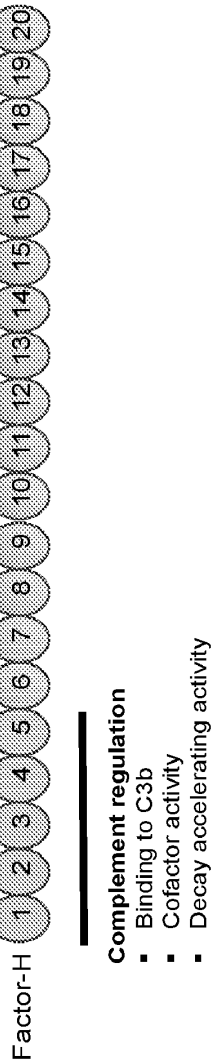

SCR 1-5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
Factor-H
Complement regulation
Binding to C3b
Cofactor activity
Decay accelerating activity
IgG4
FH SCR1-5

Figure 44

VH sequence of FMEH-IgG4PLA-FH1-5

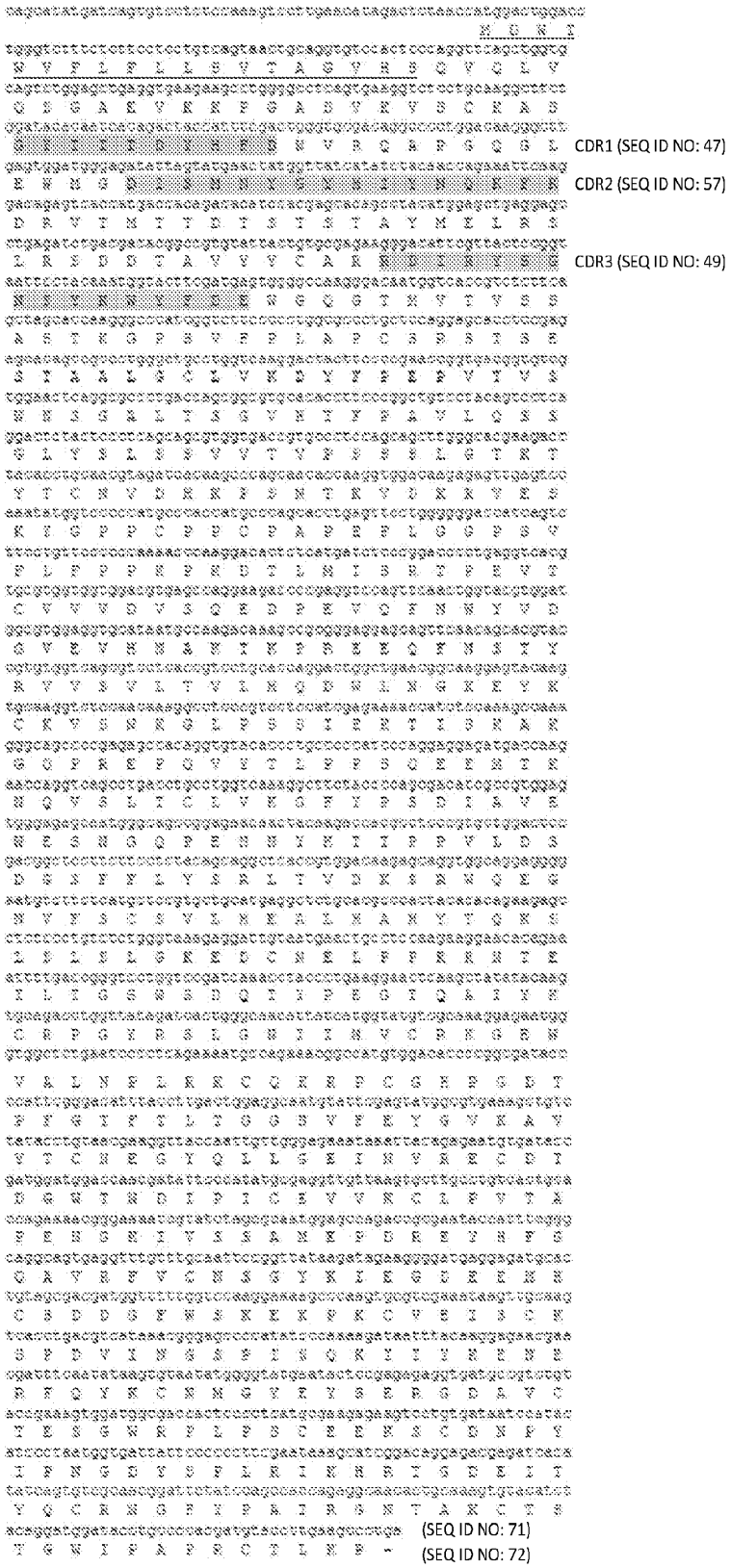


Figure 45

VL sequence of FMEH-IgG4-FH1-5

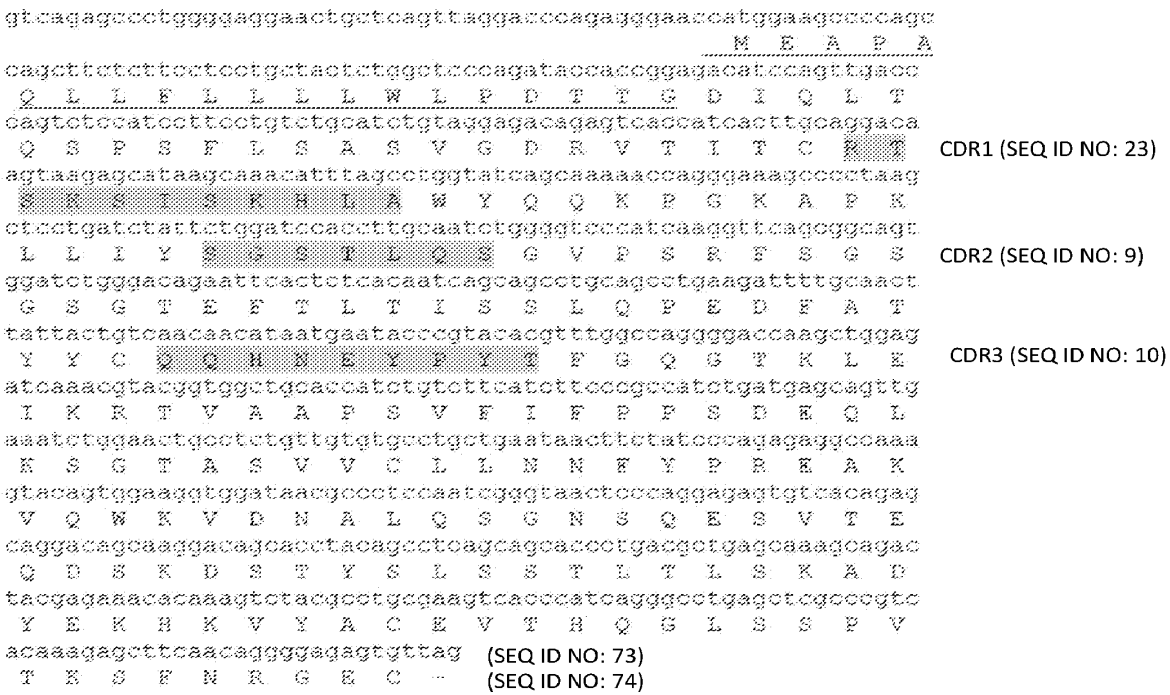
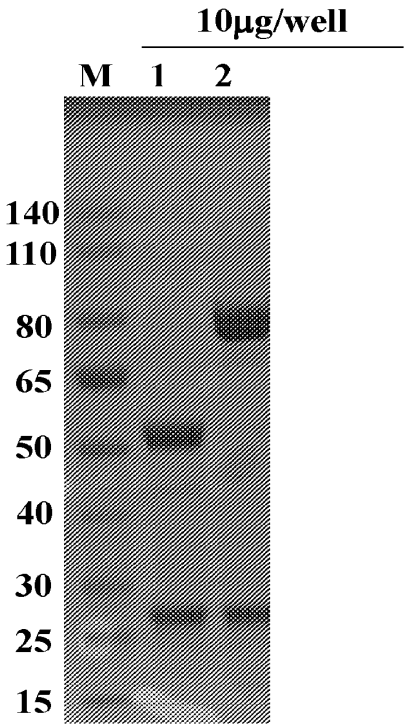


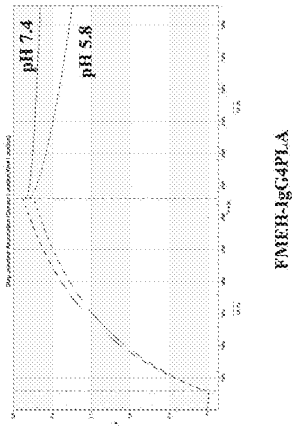
Figure 46



M: Mw marker (kDa)
1:FMEH-IgG4PLA
2:FMEH-IgG4PLA-FH1-5

Figure 47

Figure 48



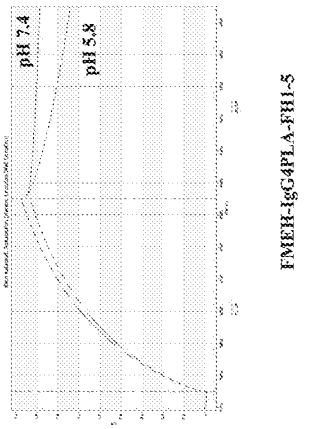


Figure 49

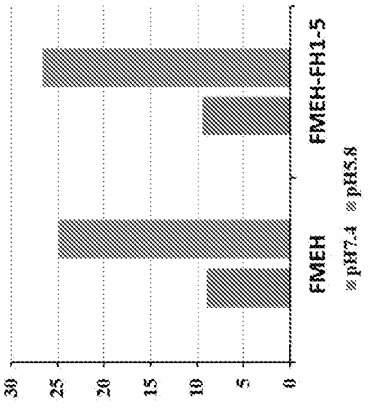


Figure 48 and Figure 49

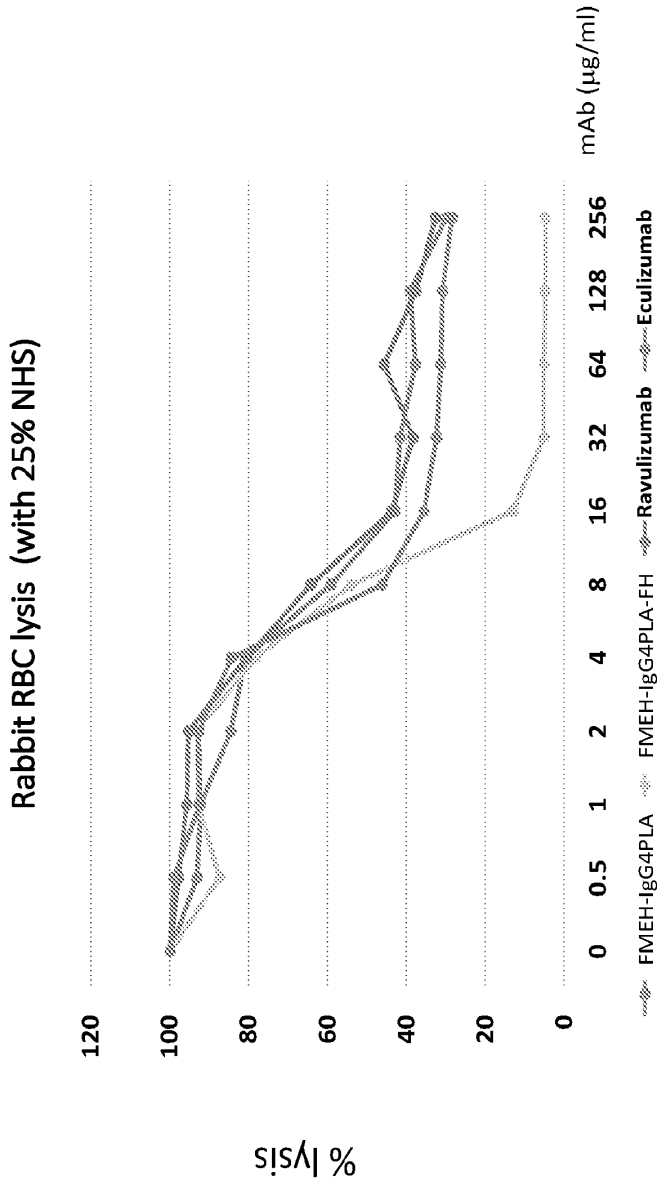

Rabbit RBC lysis (with 25% NHS)
% lysis
120
100
80
60
40
20
0
0
0.5
1
2
4
8
16
32
64
128
256
mAb (µg/ml)
FMEH-IgG4PLA
FMEH-IgG4PLA-FH
Ravulizumab
Eculizumab

Figure 50

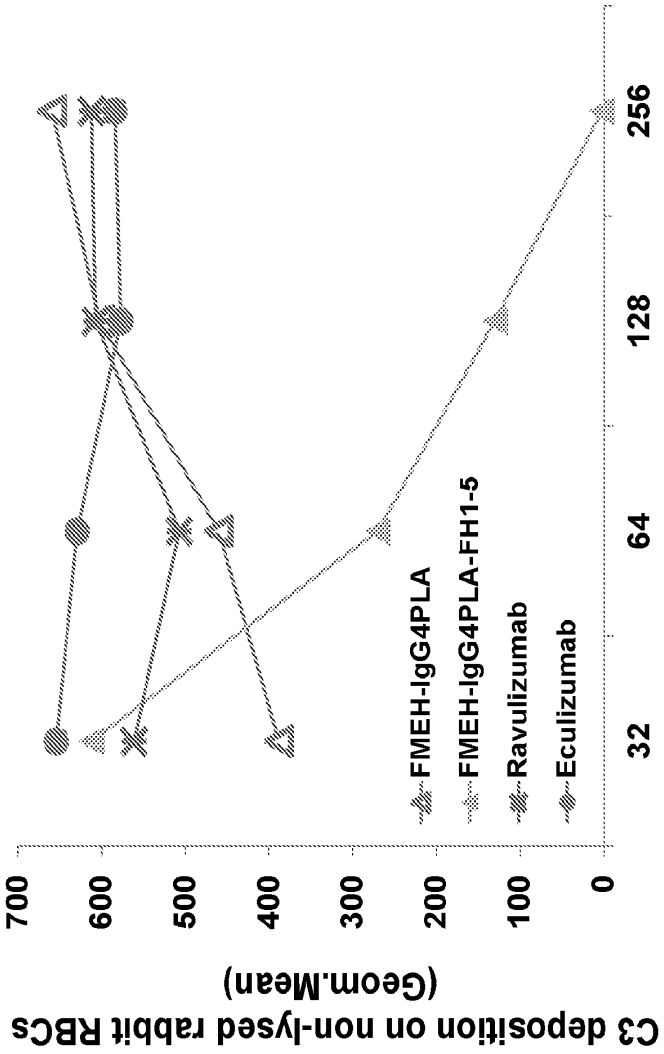

C3 deposition on non-lysed rabbit RBCs
(Geom.Mean)
700
600
500
400
300
200
100
0
32
64
128
256
Antibody (µg/ml)
FMEH-IgG4PLA
FMEH-IgG4PLA-FH1-5
Ravulizumab
Eculizumab

Figure 51

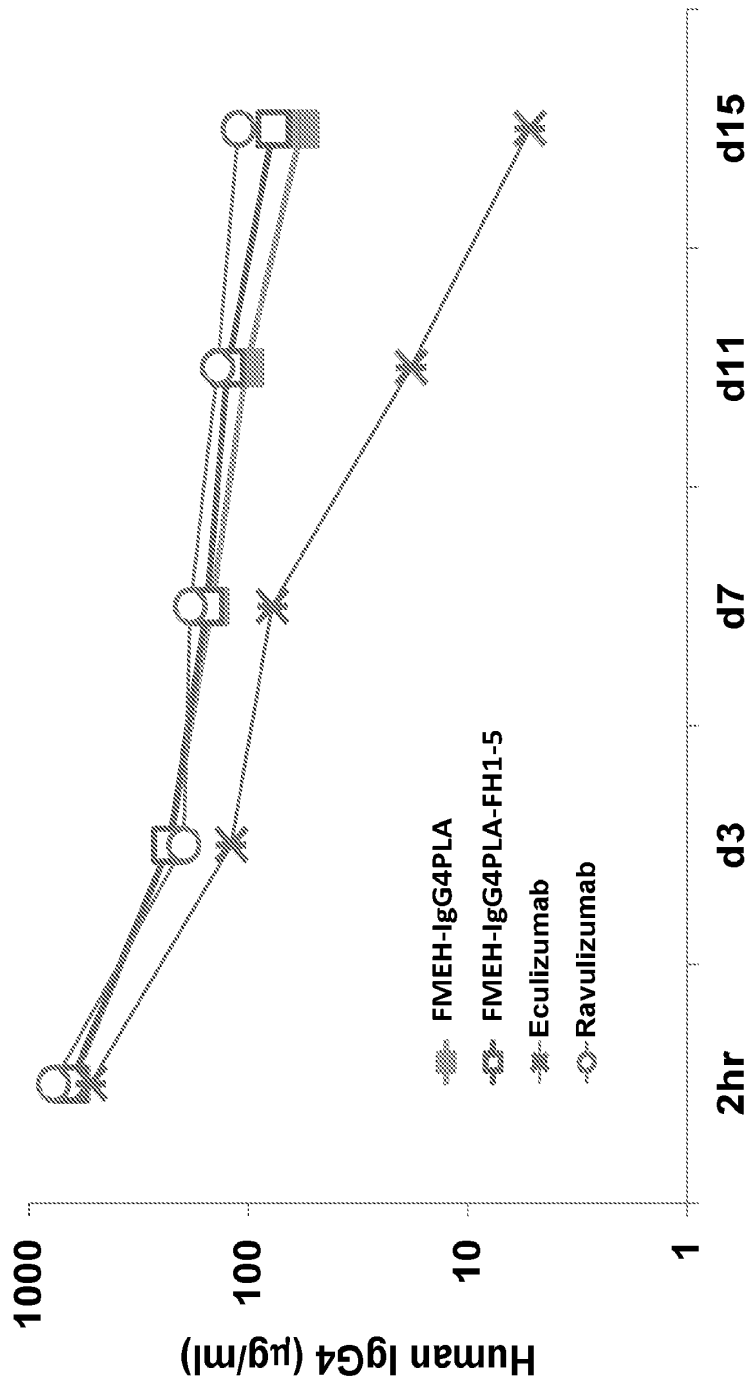

Human IgG4 (µg/ml)
1000
100
10
1
2hr
d3
d7
d11
d15
FMEH-IgG4PLA
FMEH-IgG4PLA-FH1-5
Eculizumab
Ravulizumab

Figure 52

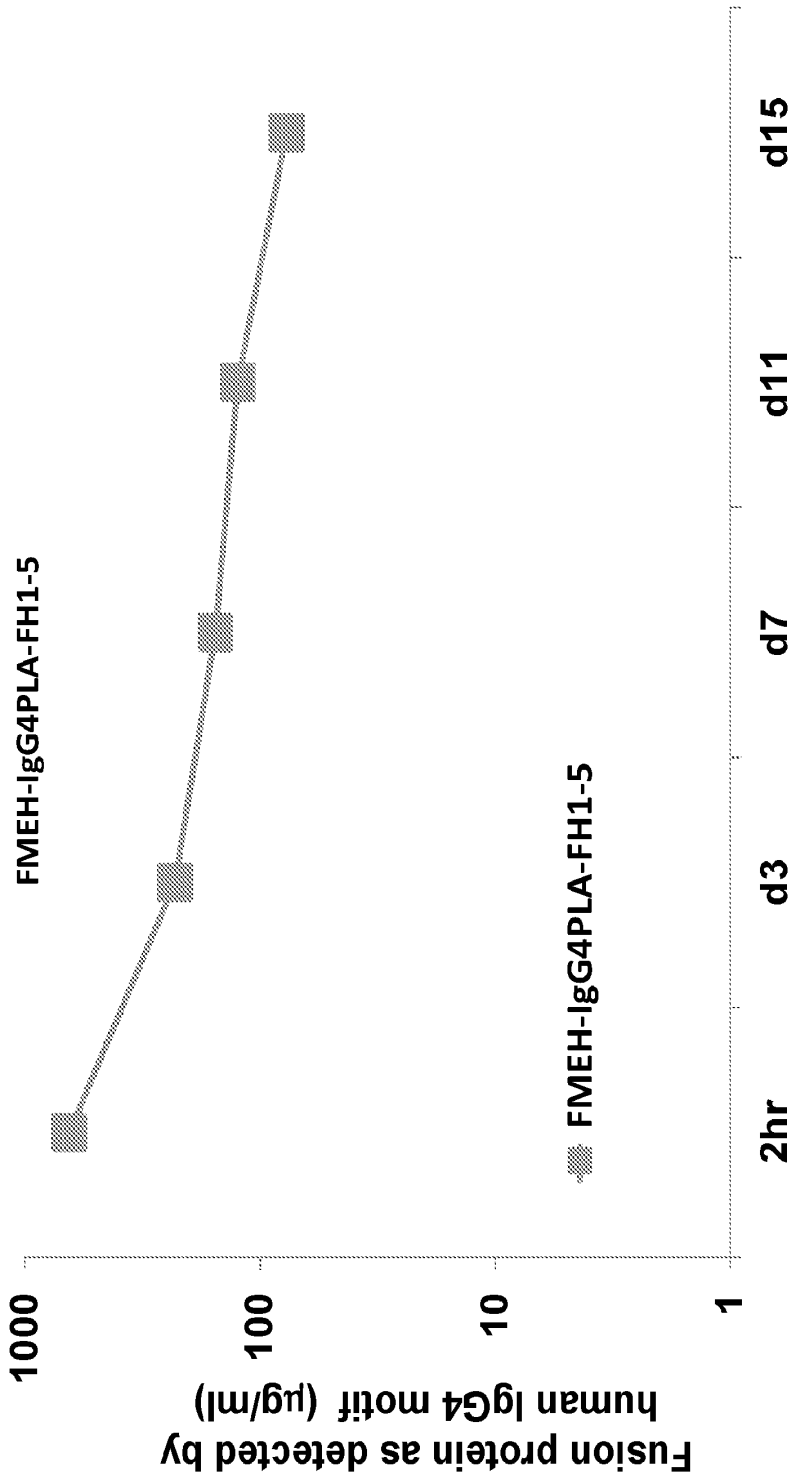

Fusion protein as detected by
human IgG4 motif (μg/ml)
1000
100
10
1
2hr
d3
d7
d11
d15
FMEH-IgG4PLA-FH1-5
FMEH-IgG4PLA-FH1-5

Figure 53

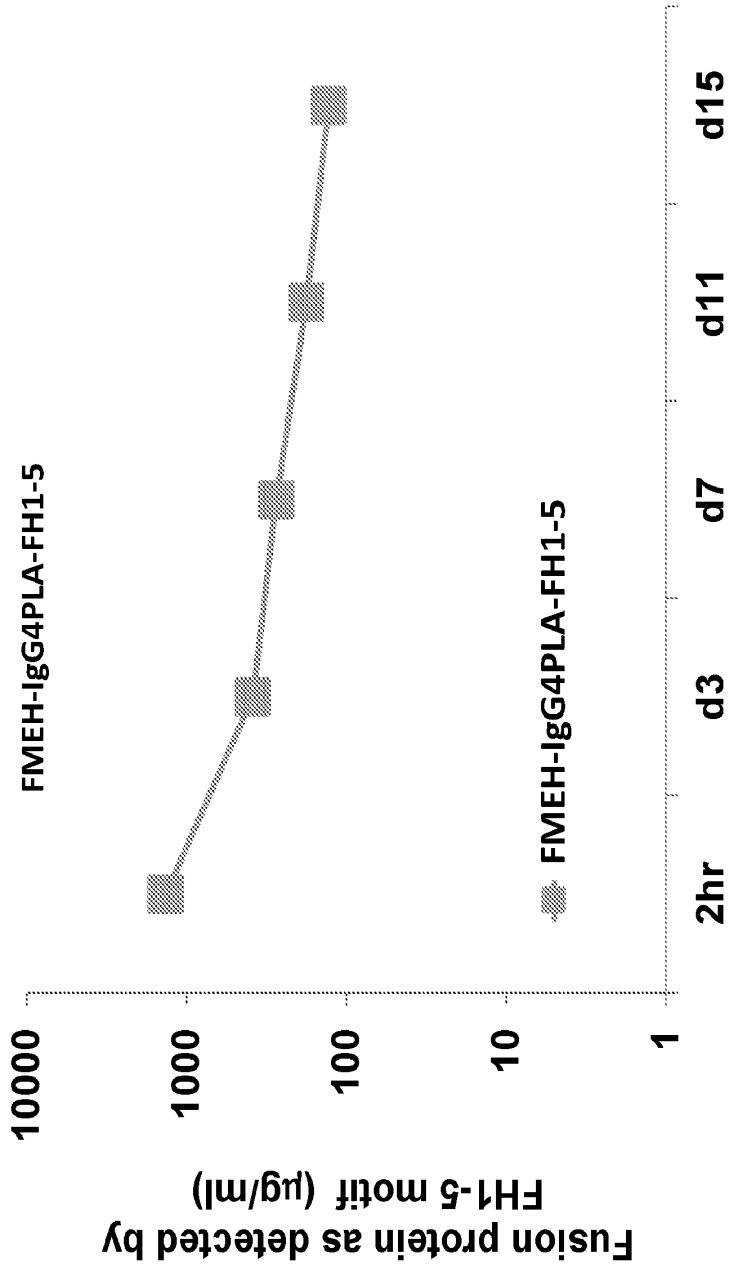

Fusion protein as detected by
FH1-5 motif (µg/ml)
10000
1000
100
10
1
2hr
d3
d7
d11
d15
FMEH-IgG4PLA-FH1-5
FMEH-IgG4PLA-FH1-5

Figure 54

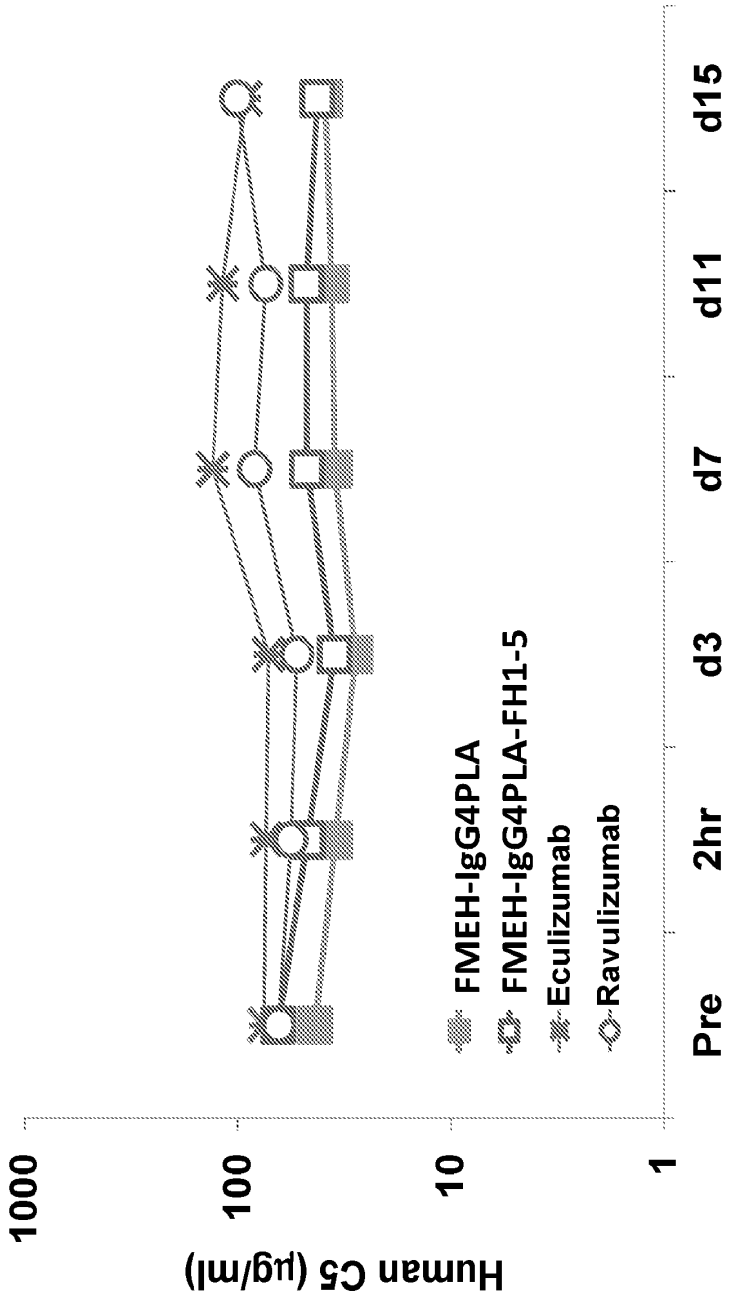

Human C5 (µg/ml)
1000
100
10
1
Pre
2hr
d3
d7
d11
d15
FMEH-IgG4PLA
FMEH-IgG4PLA-FH1-5
Eculizumab
Ravulizumab

Figure 55

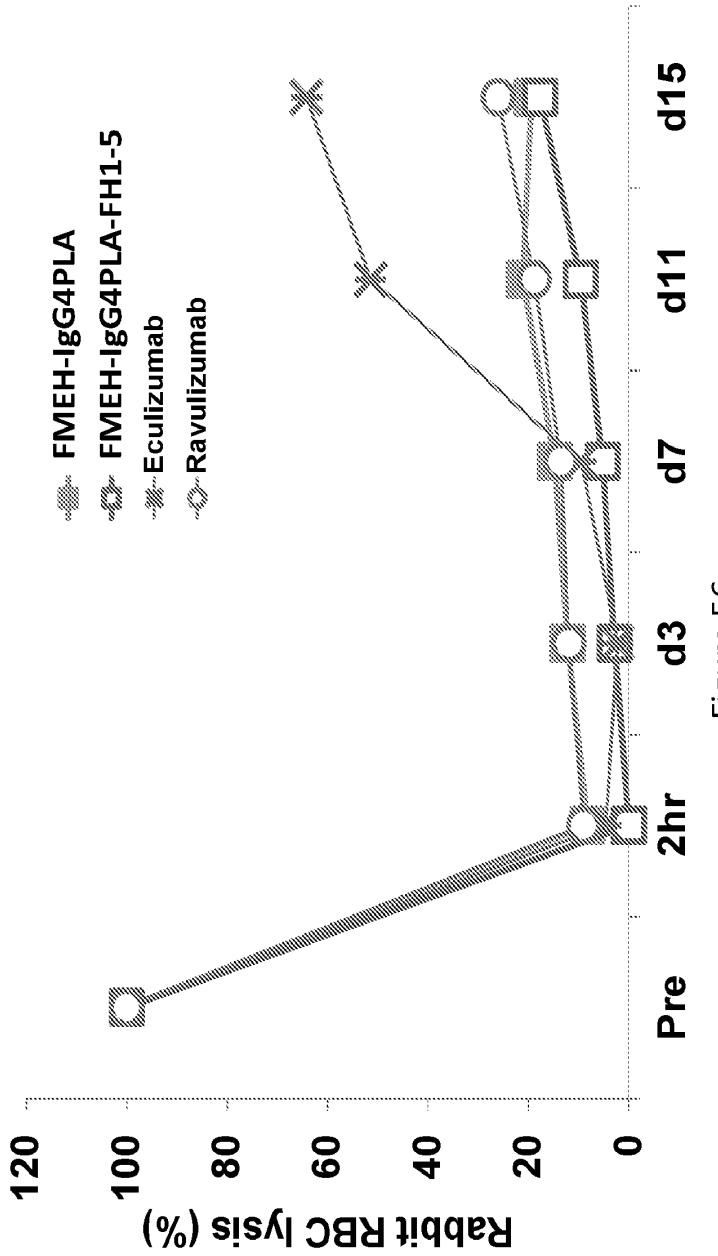
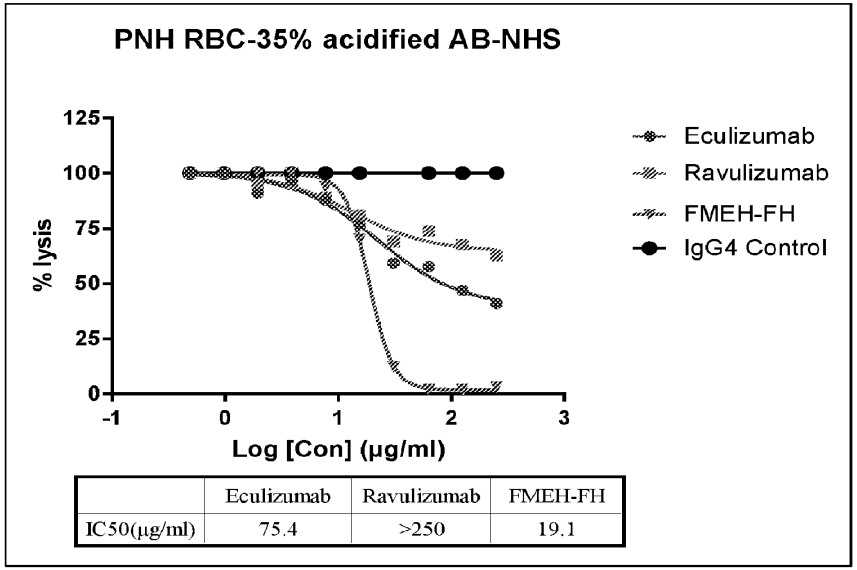

FMEH-IgG4PLA
FMEH-IgG4PLA-FH1-5
Eculizumab
Ravulizumab
Rabbit RBC lysis (%)
120
100
80
60
40
20
0
Pre
2hr
d3
d7
d11
d15

Figure 56



	Eculizumab	Ravulizumab	FMEH-FH
IC50(µg/ml)	75.4	>250	19.1

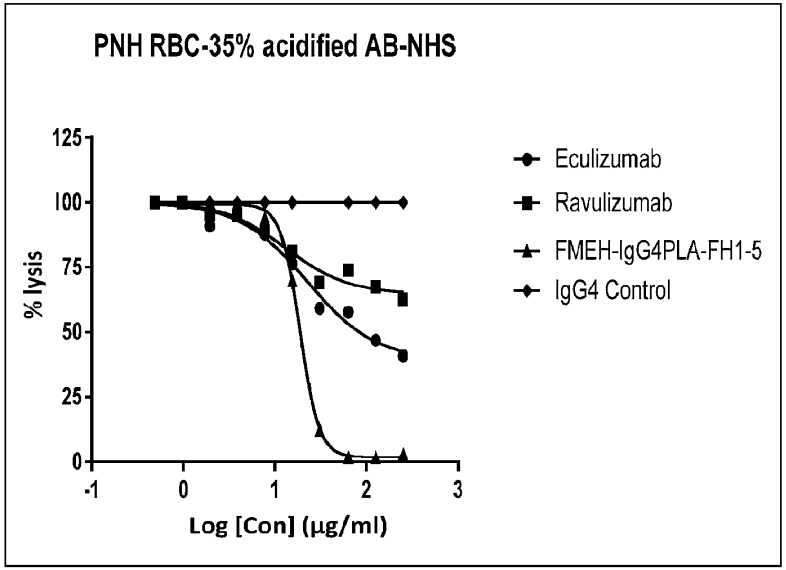


Figure 57

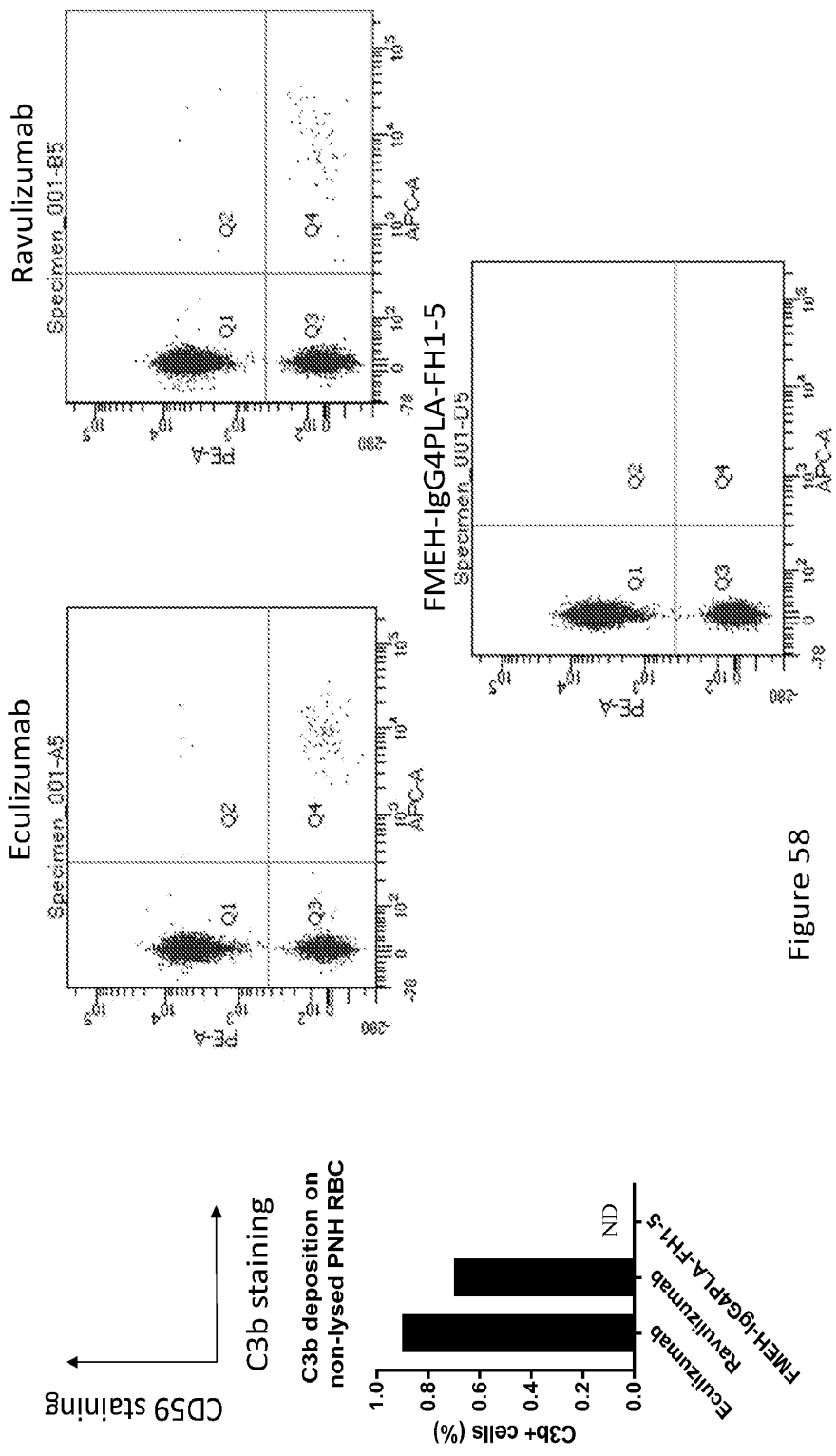

Eculizumab
Ravulizumab
FMEH-IgG4PLA-FH1-5
Specimen_001-A5
Specimen_001-B5
Specimen_001-D5
Q1
Q2
Q3
Q4
PE-A
APC-A
CD59 staining
C3b staining
C3b deposition on non-lysed PNH RBC
C3b+ cells (%)
1.0
0.8
0.6
0.4
0.2
0.0
ND
Eculizumab
Ravulizumab
FMEH-IgG4PLA-FH1-5

Figure 58

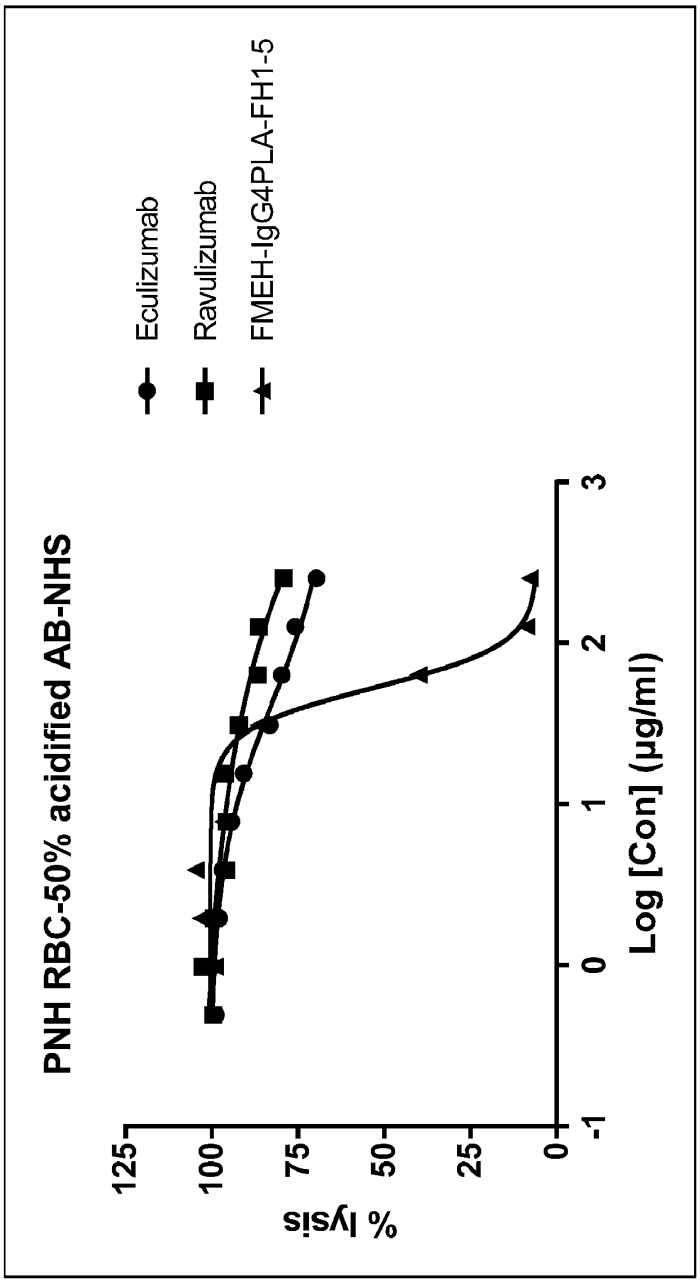
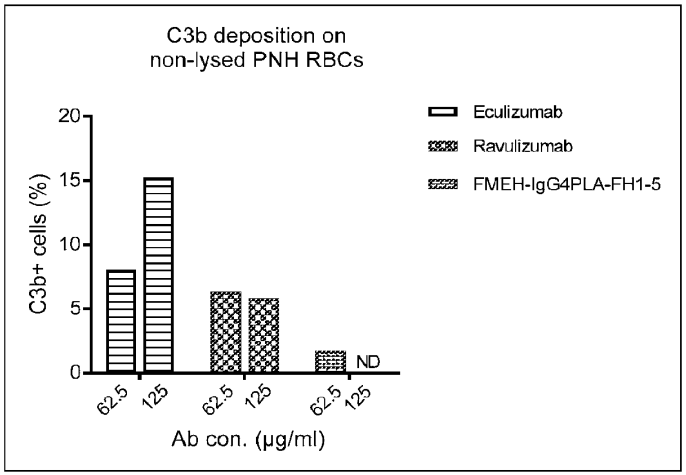
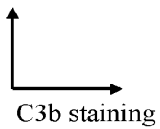

PNH RBC-50% acidified AB-NHS
Eculizumab
Ravulizumab
FMEH-IgG4PLA-FH1-5
% lysis
125
100
75
50
25
0
-1
0
1
2
3
Log [Con] (µg/ml)

Figure 59


C3b deposition on
non-lysed PNH RBCs
C3b+ cells (%)
20
15
10
5
0
Eculizumab
Ravulizumab
FMEH-IgG4PLA-FH1-5
ND
62.5
125
62.5
125
62.5
125
Ab con. (µg/ml)


62.5µg/ml
C3b staining

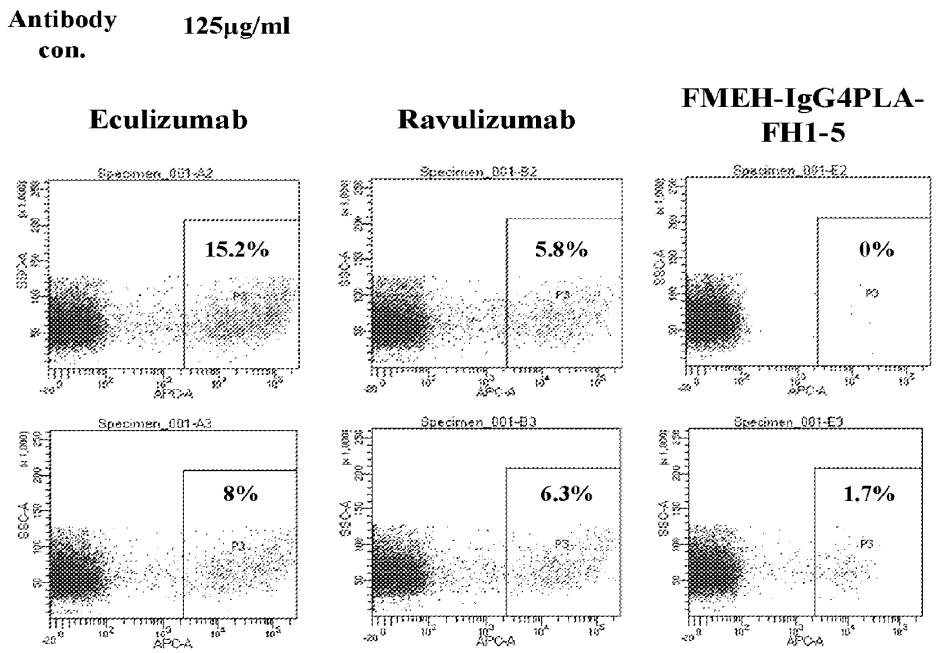

Antibody con.
125µg/ml
Eculizumab
Ravulizumab
FMEH-IgG4PLA-FH1-5
15.2%
5.8%
0%
8%
6.3%
1.7%
APC-A
SSC-A

Figure 60

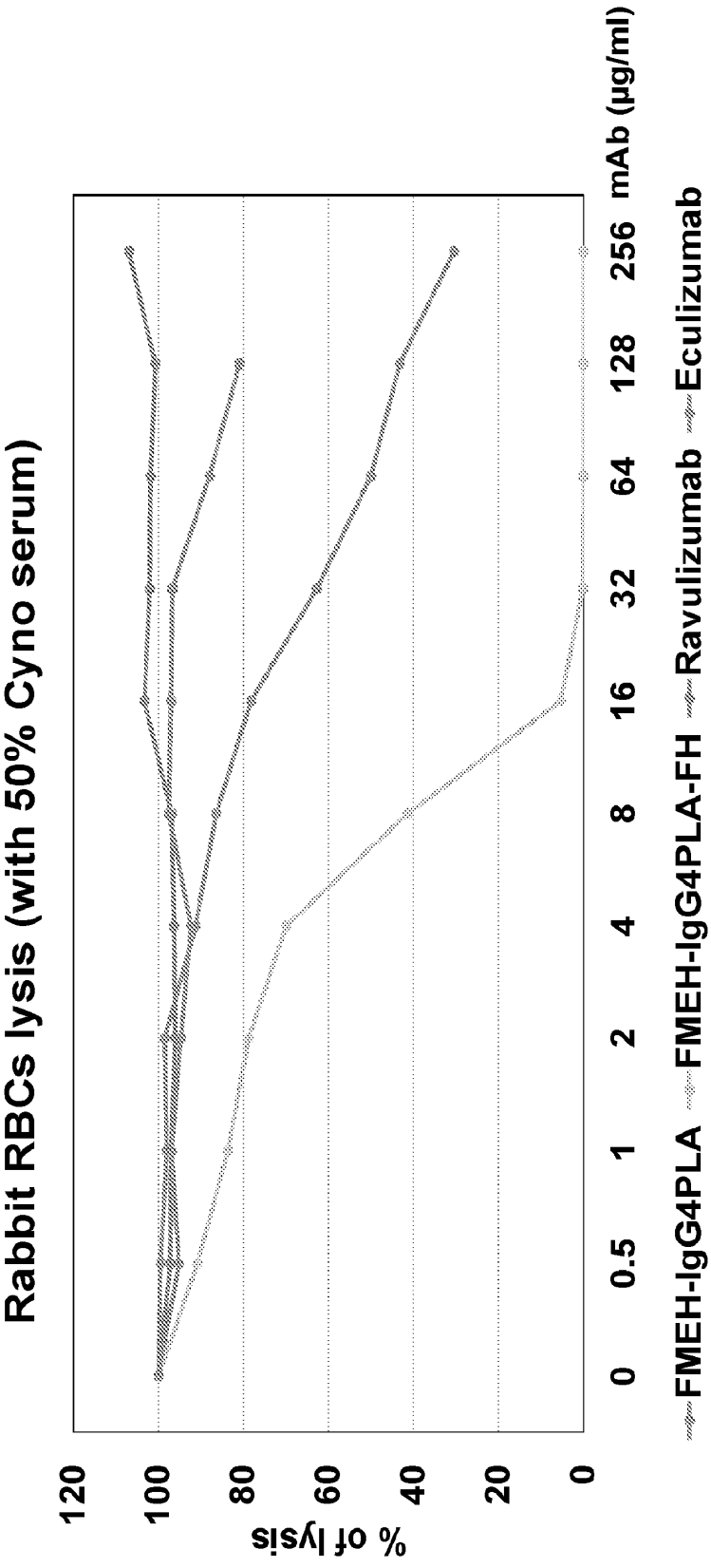

Rabbit RBCs lysis (with 50% Cyno serum)
% of lysis
120
100
80
60
40
20
0
0
0.5
1
2
4
8
16
32
64
128
256
mAb (µg/ml)
FMEH-IgG4PLA
FMEH-IgG4PLA-FH
Ravulizumab
Eculizumab

Figure 61

Figure 62A

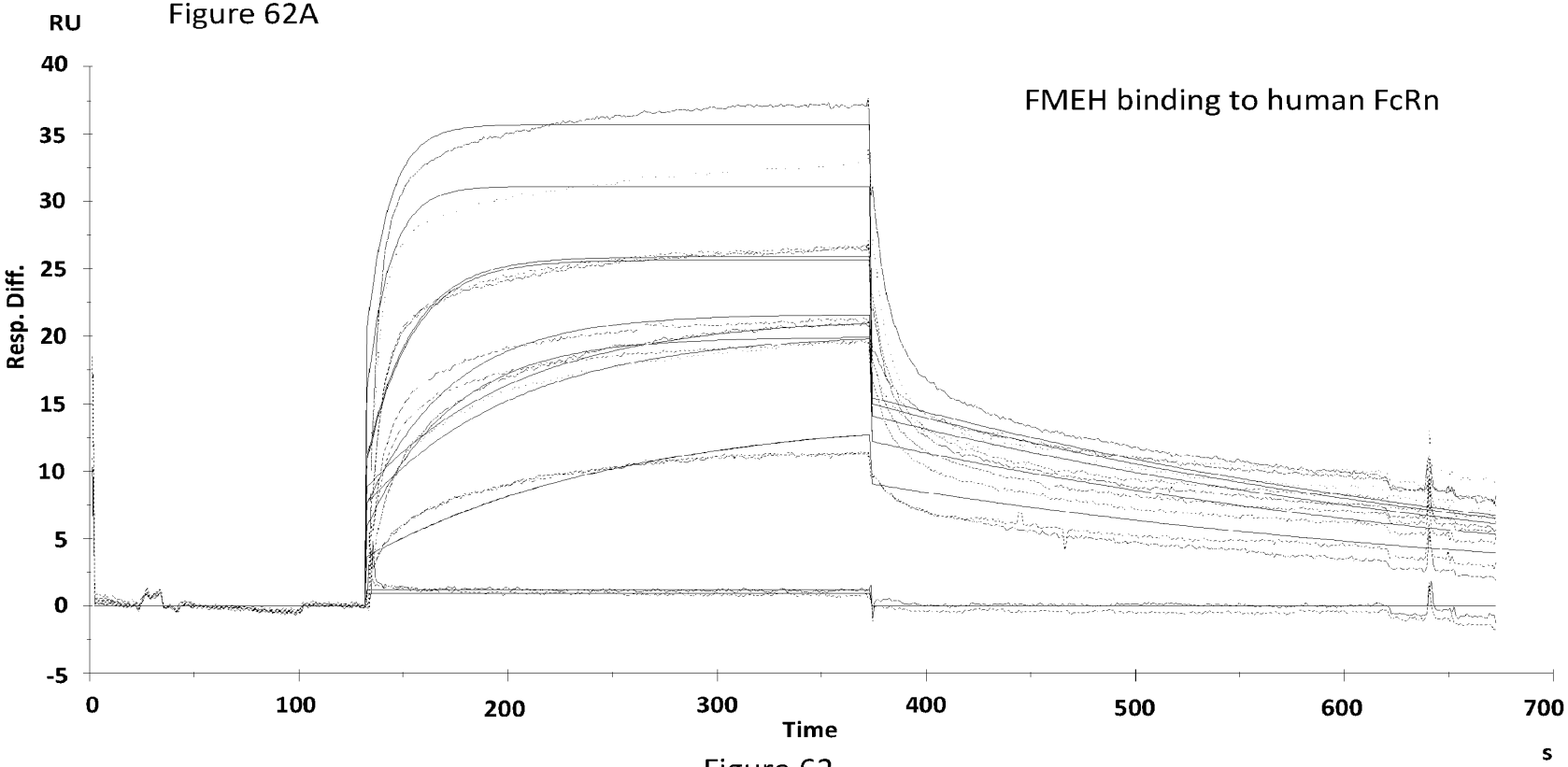


Figure 62

Figure 62B

	ka (1/Ms)	kd (1/s)	Rmax (RU)	RI (RU)	Conc of analyte	KA (1/M)	KD (M)	Req (RU)	kobs (1/s)	Chi2
	4.32E+05	2.80E-03	16			1.54E+08	6.47E-09			2.33
26-7-2019	FMHEPLA Ki Fc=2-1 - 1			20.2	2.00E-07			15.5	0.0892	
26-7-2019	FMHEPLA Ki Fc=2-1 - 2			8.76	2.50E-08			12.7	0.0136	
26-7-2019	FMHEPLA Ki Fc=2-1 - 3			3.66	1.25E-08			10.5	8.20E-03	
26-7-2019	FMHEPLA Ki Fc=2-1 - 4			7.48	5.00E-08			14.2	0.0244	
26-7-2019	FMHEPLA Ki Fc=2-1 - 5			0.955	0			0	2.80E-03	
26-7-2019	FMHEPLA Ki Fc=2-1 - 6			10.9	1.00E-07			15	0.046	
26-7-2019	FMHEPLA Ki Fc=2-1 - 7			10.6	1.00E-07			15	0.046	
26-7-2019	FMHEPLA Ki Fc=2-1 - 8			3.67	1.25E-08			10.5	8.20E-03	
26-7-2019	FMHEPLA Ki Fc=2-1 - 9			5.8	5.00E-08			14.2	0.0244	
26-7-2019	FMHEPLA K Fc=2-1 - 10			7.6	2.50E-08			12.7	0.0136	
26-7-2019	FMHEPLA K Fc=2-1 - 11			15.6	2.00E-07			15.5	0.0892	
26-7-2019	FMHEPLA K Fc=2-1 - 12			1.21	0			0	2.80E-03	

Figure 62 (cont.)

Figure 63A

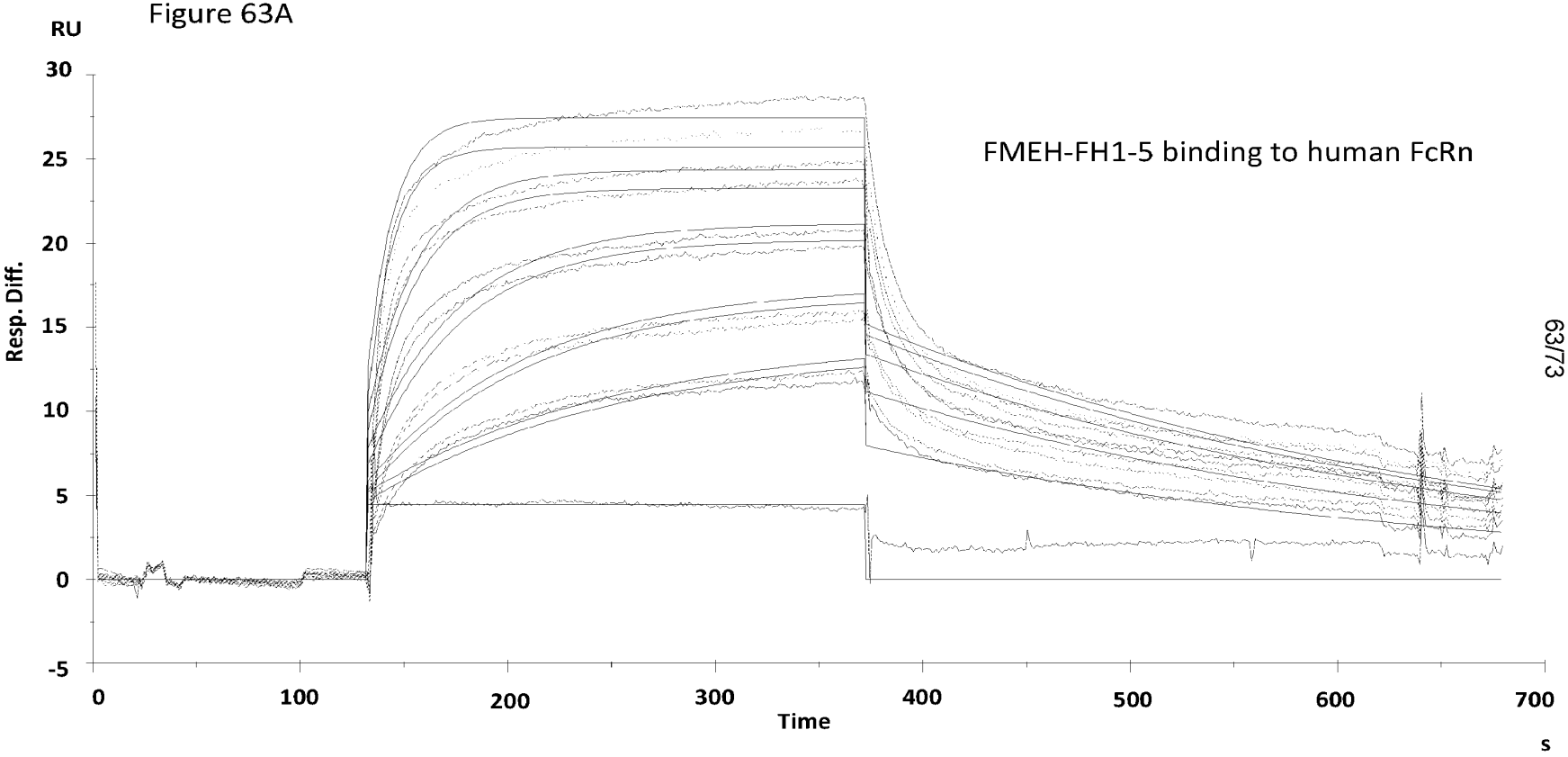


Figure 63

Figure 63B

	ka (1/Ms)	kd (1/s)	Rmax (RU)	RI (RU)	Conc of analyte	KA (1/M)	KD (M)	Req (RU)	kobs (1/s)	Chi2
	3.80E+05	3.37E-03	15.9			1.13E+08	8.88E-09			1.09
27-7-2019 FMHEfHPLA Fc=2-1 - 1				4.46	0			0	3.37E-03	
27-7-2019 FMHEfHPLA Fc=2-1 - 2				7.72	5.00E-08			13.5	0.0224	
27-7-2019 FMHEfHPLA Fc=2-1 - 3				12.3	2.00E-07			15.2	0.0793	
27-7-2019 FMHEfHPLA Fc=2-1 - 4				5.19	1.25E-08			9.27	8.12E-03	
27-7-2019 FMHEfHPLA Fc=2-1 - 5				9.79	1.00E-07			14.6	0.0413	
27-7-2019 FMHEfHPLA Fc=2-1 - 6				5.82	2.50E-08			11.7	0.0129	
27-7-2019 FMHEfHPLA Fc=2-1 - 7				6.75	5.00E-08			13.5	0.0224	
27-7-2019 FMHEfHPLA Fc=2-1 - 8				8.68	1.00E-07			14.6	0.0413	
27-7-2019 FMHEfHPLA Fc=2-1 - 9				5.28	2.50E-08			11.7	0.0129	
27-7-2019 FMHEfHPLA Fc=2-1 - 10				10.5	2.00E-07			15.2	0.0793	
27-7-2019 FMHEfHPLA Fc=2-1 - 12				4.68	1.25E-08			9.27	8.12E-03	

Figure 63 (cont.)

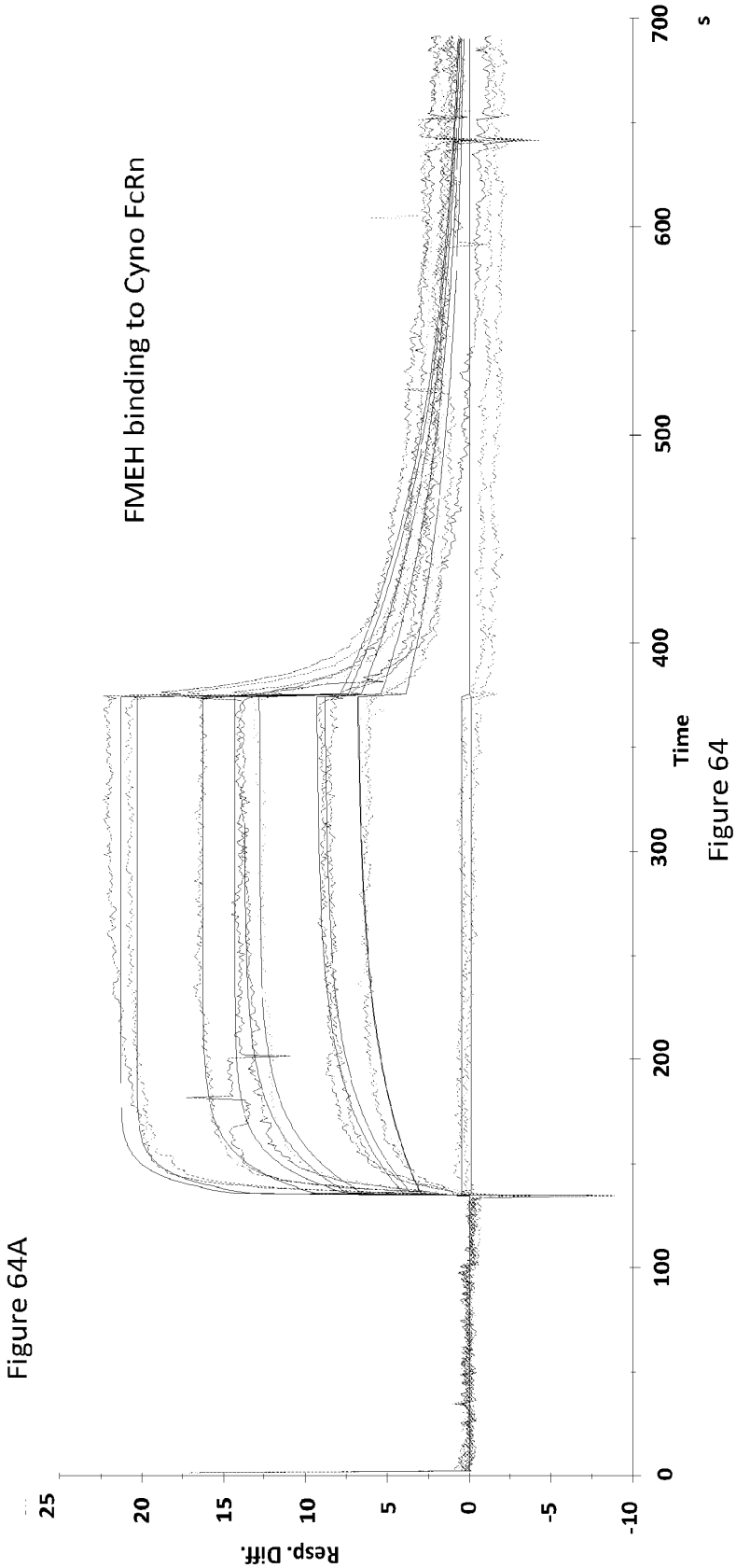


Figure 64A

Figure 64

Figure 64B

	ka (1/Ms)	kd (1/s)	Rmax (RU)	RI (RU)	Conc of analyte	KA (1/M)	KD (M)	Req (RU)	kobs (1/s)	Chi2
	5.59E+05	7.85E-03	8.48			7.13E+07	1.40E-08			1.06
25-7-2019 FMHEPLA Ki	Fc=4-3 - 1			7.07	5.00E-08			6.63	0.0358	
25-7-2019 FMHEPLA Ki	Fc=4-3 - 2			3.87	2.50E-08			5.44	0.0218	
25-7-2019 FMHEPLA Ki	Fc=4-3 - 3			13.3	2.00E-07			7.93	0.12	
25-7-2019 FMHEPLA Ki	Fc=4-3 - 4			2.89	1.25E-08			4	0.0148	
25-7-2019 FMHEPLA Ki	Fc=4-3 - 5			8.82	1.00E-07			7.44	0.0638	
25-7-2019 FMHEPLA Ki	Fc=4-3 - 6			0.427	0			0	7.85E-03	
25-7-2019 FMHEPLA Ki	Fc=4-3 - 7			3.37	2.50E-08			5.44	0.0218	
25-7-2019 FMHEPLA Ki	Fc=4-3 - 8			12.4	2.00E-07			7.93	0.12	
25-7-2019 FMHEPLA Ki	Fc=4-3 - 9			-0.0948	0			0	7.85E-03	
25-7-2019 FMHEPLA K	Fc=4-3 - 10			6.19	5.00E-08			6.63	0.0358	
25-7-2019 FMHEPLA K	Fc=4-3 - 11			2.92	1.25E-08			4	0.0148	
25-7-2019 FMHEPLA K	Fc=4-3 - 12			6.87	1.00E-07			7.44	0.0638	

Figure 64 (cont.)

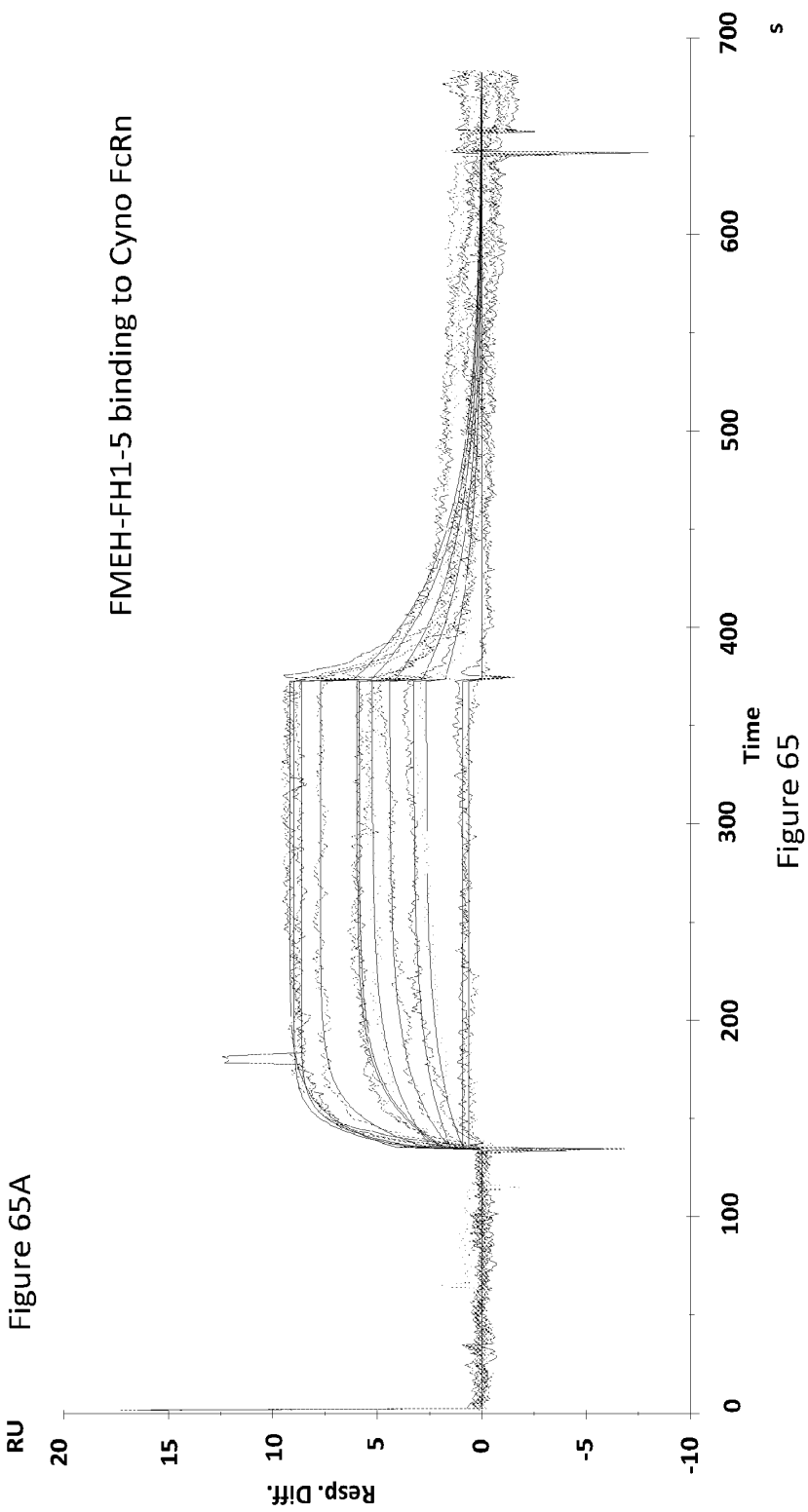


Figure 65A

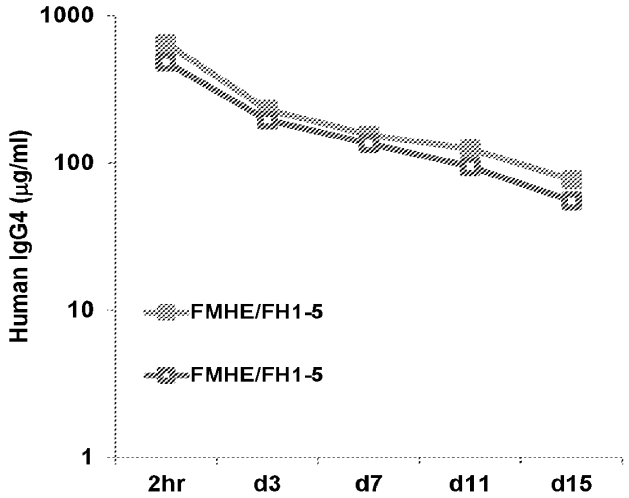
Figure 65

Figure 65B

	ka (1/Ms)	kd (1/s)	Rmax (RU)	RI (RU)	Conc of analyte	KA (1/M)	KD (M)	Req (RU)	kobs (1/s)	Chi2
	4.33E+05	0.0169	7.48			2.56E+07	3.91E-08			0.374
26-7-2019 FMHEfH PLA Fc=4-3 - 1				2.74	2.00E-07			6.25	0.104	
26-7-2019 FMHEfH PLA Fc=4-3 - 2				1.47	1.25E-08			1.81	0.0223	
26-7-2019 FMHEfH PLA Fc=4-3 - 3				1.78	5.00E-08			4.19	0.0386	
26-7-2019 FMHEfH PLA Fc=4-3 - 4				0.608	0			0	0.0169	
26-7-2019 FMHEfH PLA Fc=4-3 - 5				1.48	2.50E-08			2.91	0.0278	
26-7-2019 FMHEfH PLA Fc=4-3 - 6				3.85	1.00E-07			5.37	0.0602	
26-7-2019 FMHEfH PLA Fc=4-3 - 7				2.41	2.00E-07			6.25	0.104	
26-7-2019 FMHEfH PLA Fc=4-3 - 8				1.67	5.00E-08			4.19	0.0386	
26-7-2019 FMHEfH PLA Fc=4-3 - 9				2.36	1.00E-07			5.37	0.0602	
26-7-2019 FMHEfH PL Fc=4-3 - 10				2.33	2.50E-08			2.91	0.0278	
26-7-2019 FMHEfH PL Fc=4-3 - 11				0.866	1.25E-08			1.81	0.0223	
26-7-2019 FMHEfH PL Fc=4-3 - 12				0.918	0			0	0.0169	

Figure 65 (cont.)

BW		28g	22g
Injected Ab (40mg/kg)		1.12mg	0.88mg
		Mo-42	Mo-45
		FMHE/FH1-5	FMHE/FH1-5
2hr		847.1	483.5996
d3		229.9444	194.8892
d7		164.7872	136.6932
d11		124.3184	94.3632
d15		77.0168	55.2248



BW		28g	22g
Injected Ab (40mg/kg)		1.12mg	0.88mg
		Mo-42	Mo-45
		FMHE/FH1-5	FMHE/FH1-5
2hr		1350.5024	1209.2416
d3		386.1504	417.9456
d7		274.7008	215.2064
d11		176.2016	140.9488
d15		129.1872	83.35788687

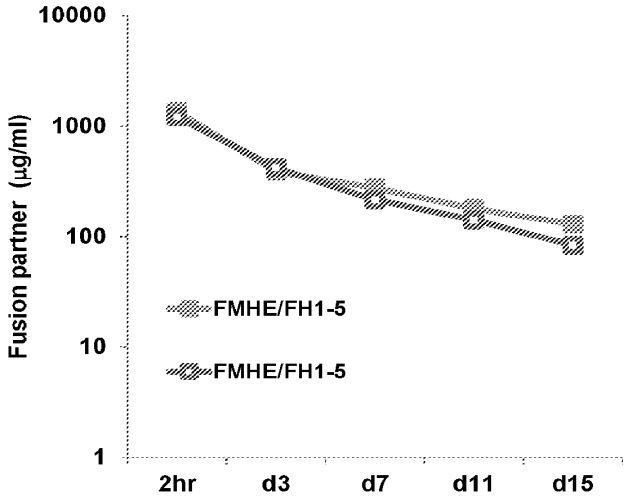


Figure 66

Figure 67A

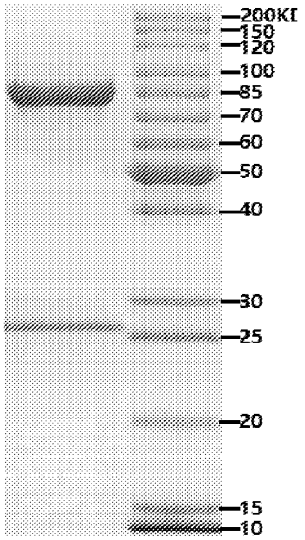


Figure 67B

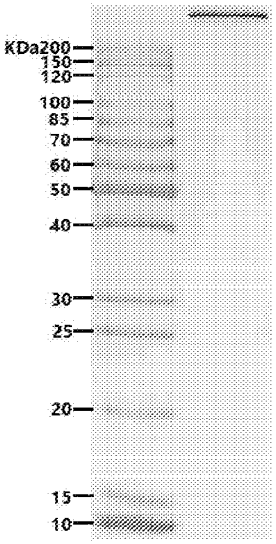


Figure 67C

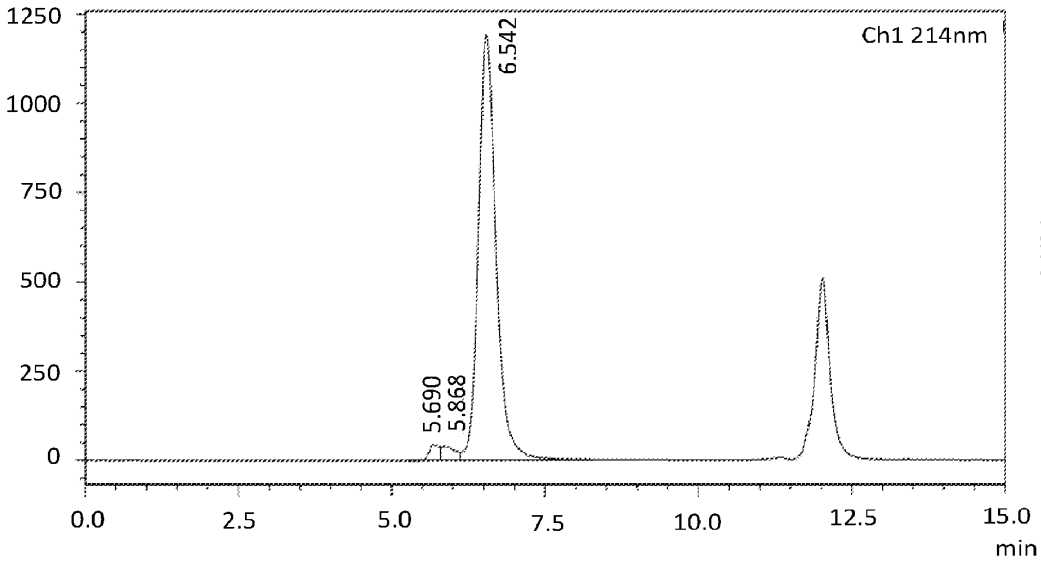


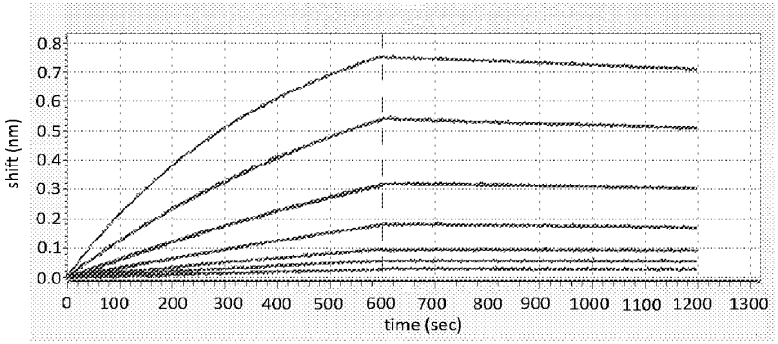
Figure 67

Antibody	Theoretical Average Mass (Da)	Measured Mass (Da)
FMEH-IgG4PLA-FH1-5	219235.9	219228.2
FMEH-IgG4PLA-FH1-5-Light Chain	23520.2	23517.6 (Monoisotopic)
FMEH-IgG4PLA-FH1-5-Heavy Chain	86117.8	86119.1
KFMEH-IgG4PLA-FH1-5 -Deglyco	216347.3	216346.3
FMEH-IgG4PLA-FH1-5-Deglyco-LC	23520.2	23517.7 (Monoisotopic)
KFMEH-IgG4PLA-FH1-5-Deglyco-HC	84673.5	84672.3

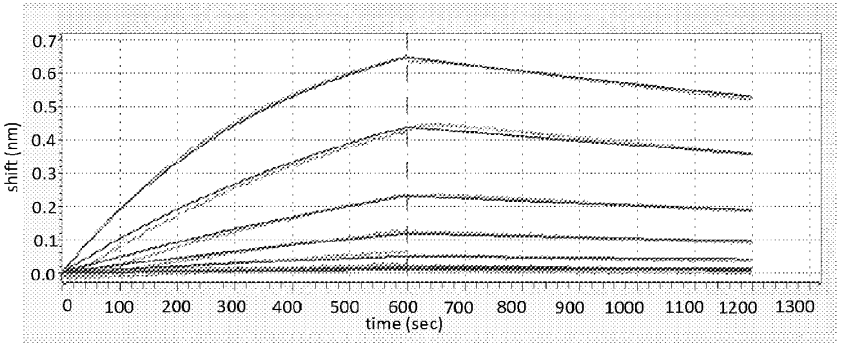
Figure 68

Figure 69A

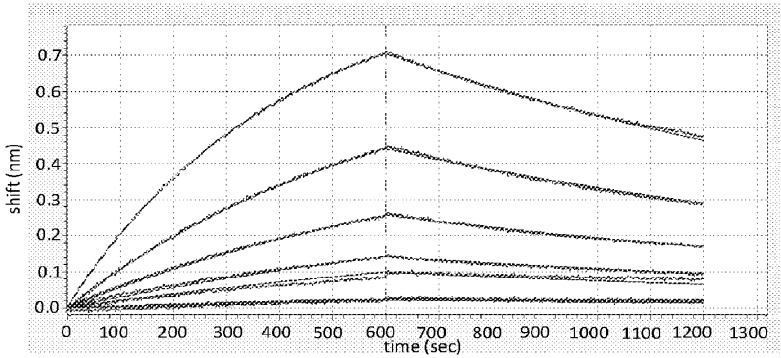
FMEH-IgG4PLA-FH1-5, human C5, pH7.4



FMEH-IgG4PLA-FH1-5, cyno C5, pH7.4



FMEH-IgG4PLA-FH1-5, human C5, pH5.8



FMEH-IgG4PLA-FH1-5, cyno C5, pH5.8

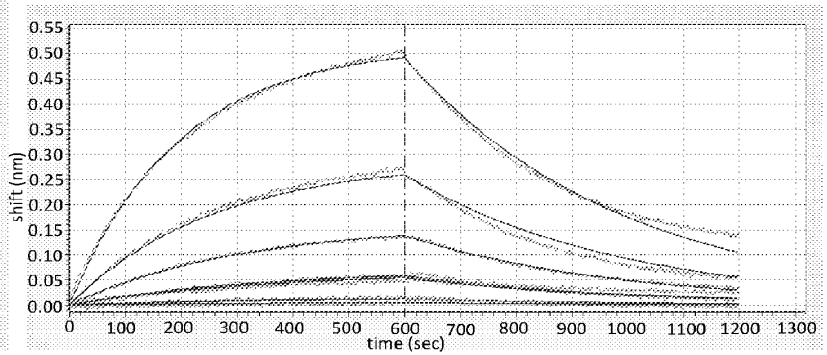


Figure 69

Figure 69B

Antibody	Antigen	Association pH, Dissociation pH	koff(1/s)	kon(1/Ms)	KD (M)
FMEH-IgG4PLA-FH1-5	human C5	7.4,7.4	9.10E-05	6.02E+04	1.51E-09
FMEH-IgG4PLA-FH1-5	cyno C5	7.4,7.4	3.36E-04	5.56E+04	6.04E-09
FMEH-IgG4PLA-FH1-5	human C5	5.8,5.8	6.99E-04	4.66E+04	1.50E-08
FMEH-IgG4PLA-FH1-5	cyno C5	5.8,5.8	2.59E-03	6.31E+04	4.11E-08

Figure 69 (cont.)