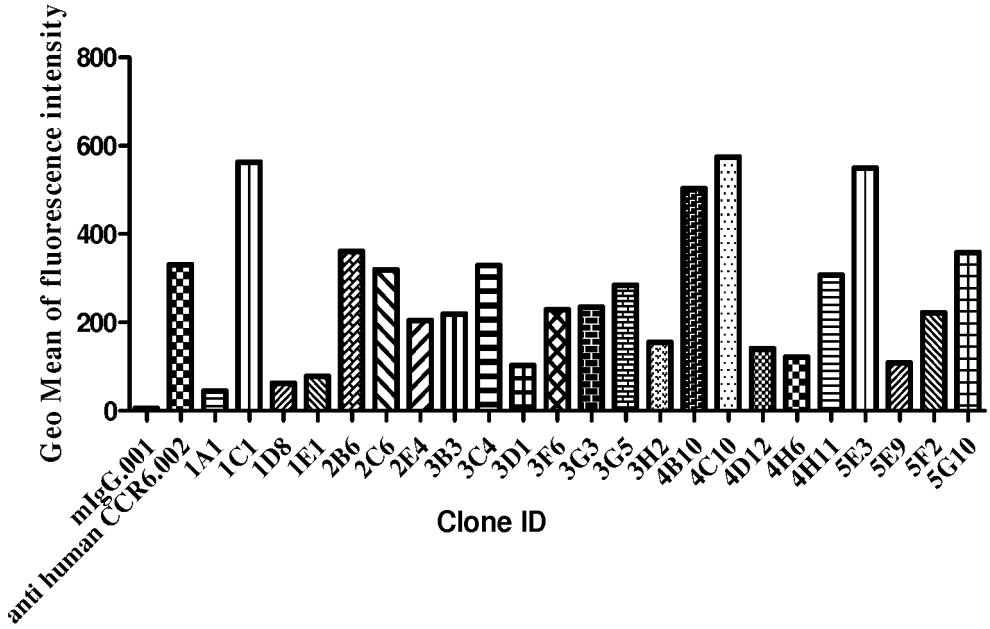
FIG. 1

A


Geo Mean of fluorescence intensity
800
600
400
200
0
mIgG.001
anti human CCR6.002
1A1
1C1
1D8
1E1
2B6
2C6
2E4
3B3
3C4
3D1
3F6
3G3
3G5
3H2
4B10
4C10
4D12
4H6
4H11
5E3
5E9
5F2
5G10
Clone ID

B

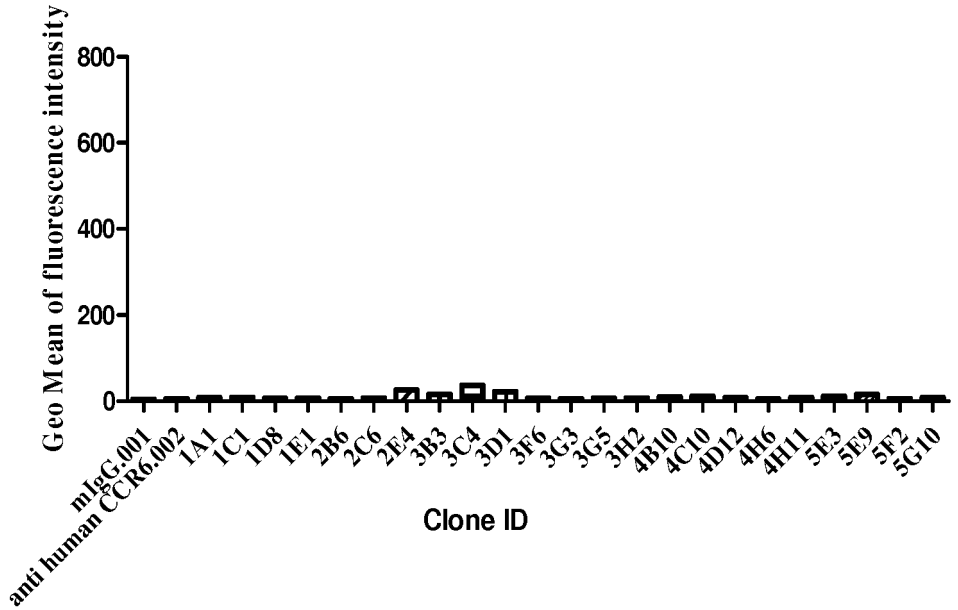

Geo Mean of fluorescence intensity
800
600
400
200
0
mIgG.001
anti human CCR6.002
1A1
1C1
1D8
1E1
2B6
2C6
2E4
3B3
3C4
3D1
3F6
3G3
3G5
3H2
4B10
4C10
4D12
4H6
4H11
5E3
5E9
5F2
5G10
Clone ID

FIG. 2

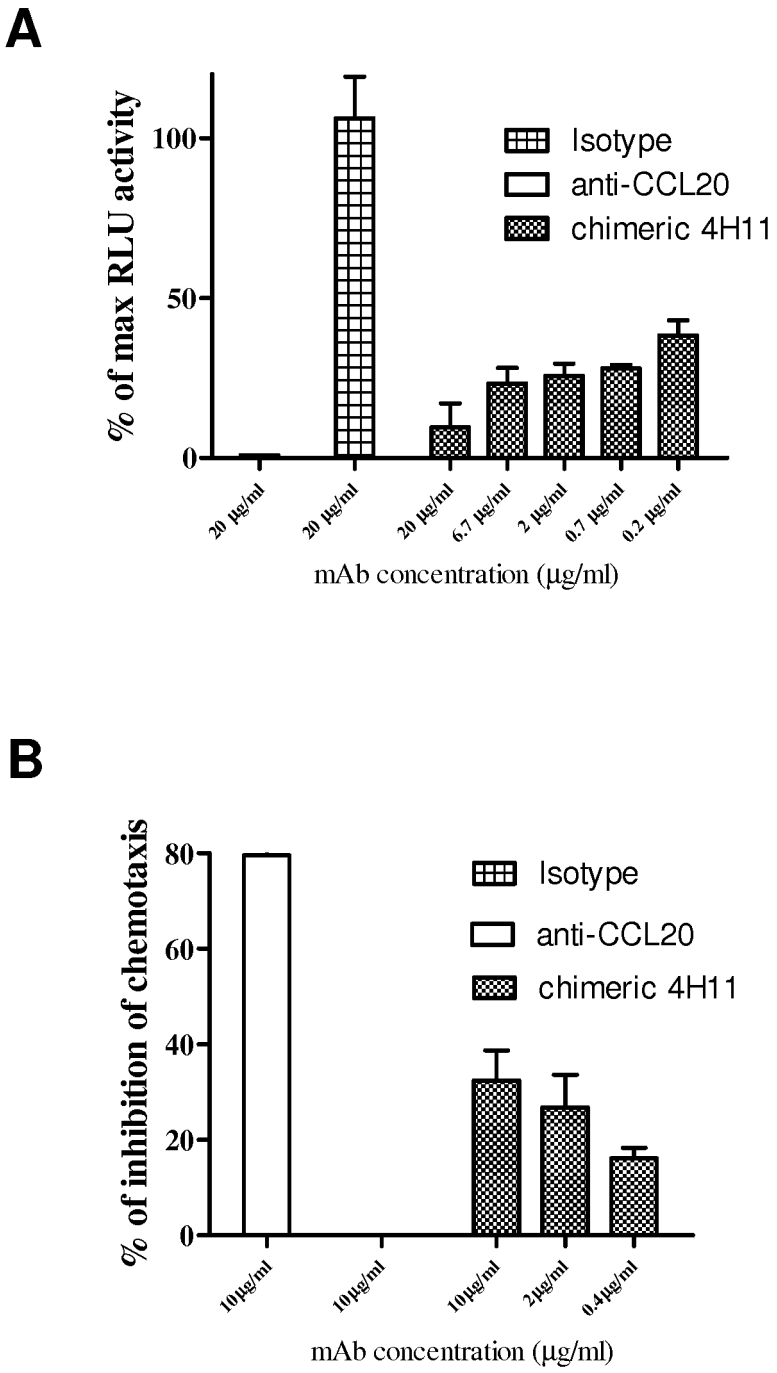
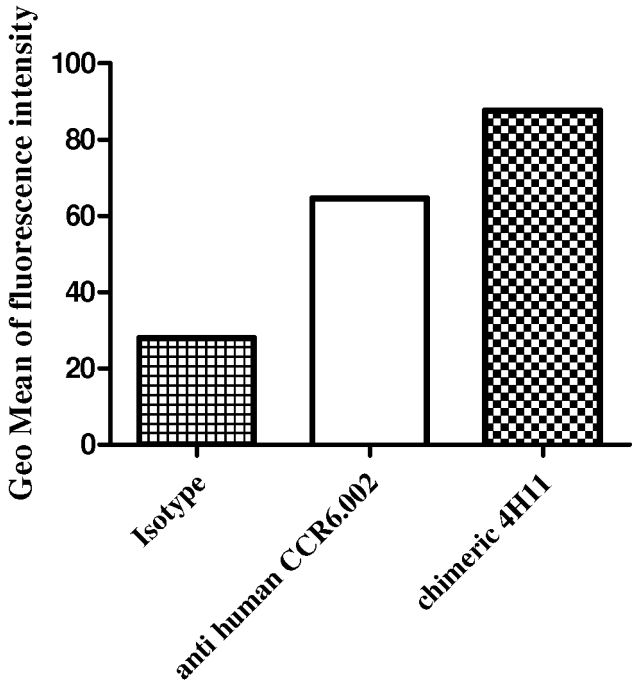

A
% of max RLU activity
100
50
0
Isotype
anti-CCL20
chimeric 4H11
20 µg/ml
20 µg/ml
20 µg/ml
6.7 µg/ml
2 µg/ml
0.7 µg/ml
0.2 µg/ml
mAb concentration (µg/ml)
B
% of inhibition of chemotaxis
80
60
40
20
0
Isotype
anti-CCL20
chimeric 4H11
10µg/ml
10µg/ml
10µg/ml
2µg/ml
0.4µg/ml
mAb concentration (µg/ml)

FIG. 3

A


Geo Mean of fluorescence intensity
100
80
60
40
20
0
Isotype
anti human CCR6.002
chimeric 4H11

B

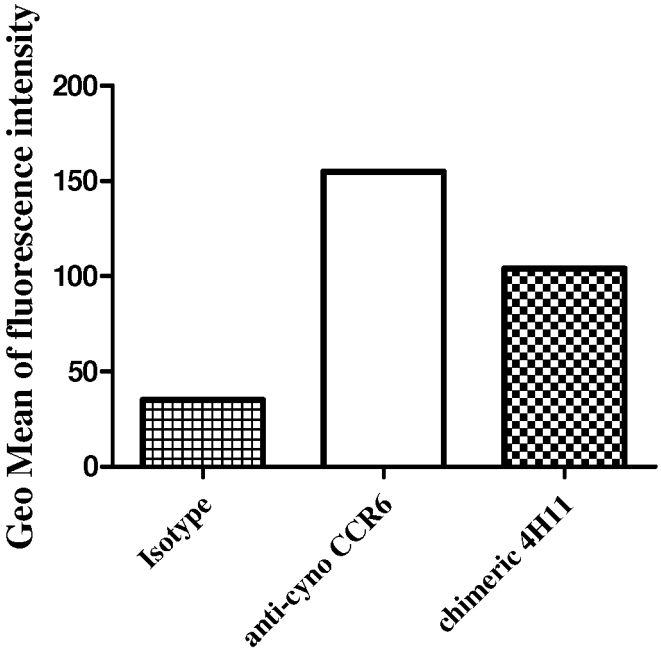

Geo Mean of fluorescence intensity
200
150
100
50
0
Isotype
anti-cyno CCR6
chimeric 4H11

FIG. 4

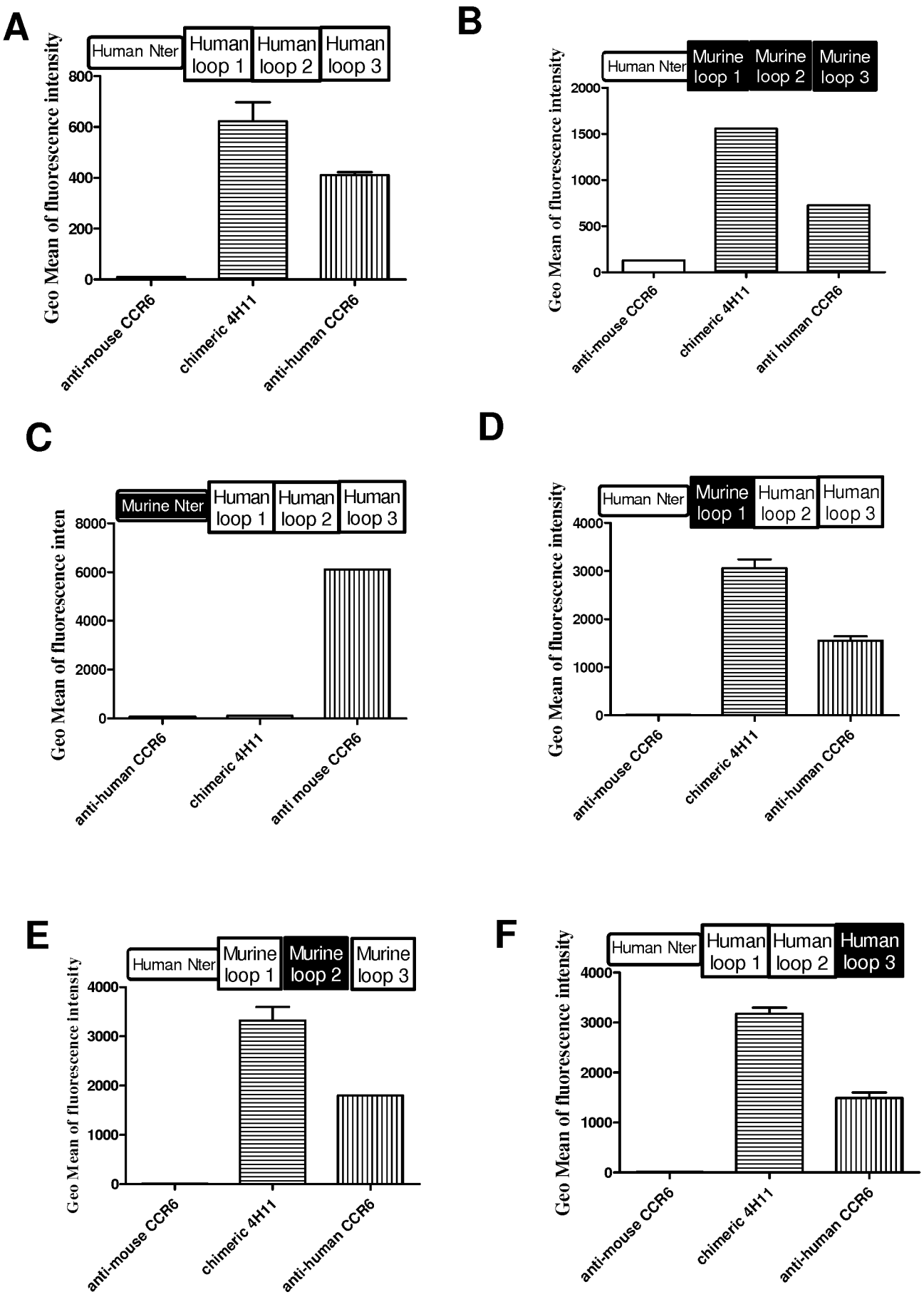

A
Human Nter
Human loop 1
Human loop 2
Human loop 3
Geo Mean of fluorescence intensity
800
600
400
200
0
anti-mouse CCR6
chimeric 4H11
anti-human CCR6
B
Human Nter
Murine loop 1
Murine loop 2
Murine loop 3
Geo Mean of fluorescence intensity
2000
1500
1000
500
0
anti-mouse CCR6
chimeric 4H11
anti human CCR6
C
Murine Nter
Human loop 1
Human loop 2
Human loop 3
Geo Mean of fluorescence inten
8000
6000
4000
2000
0
anti-human CCR6
chimeric 4H11
anti mouse CCR6
D
Human Nter
Murine loop 1
Human loop 2
Human loop 3
Geo Mean of fluorescence intensity
4000
3000
2000
1000
0
anti-mouse CCR6
chimeric 4H11
anti-human CCR6
E
Human Nter
Murine loop 1
Murine loop 2
Murine loop 3
Geo Mean of fluorescence intensity
4000
3000
2000
1000
0
anti-mouse CCR6
chimeric 4H11
anti-human CCR6
F
Human Nter
Human loop 1
Human loop 2
Human loop 3
Geo Mean of fluorescence intensity
4000
3000
2000
1000
0
anti-mouse CCR6
chimeric 4H11
anti-human CCR6

FIG.5

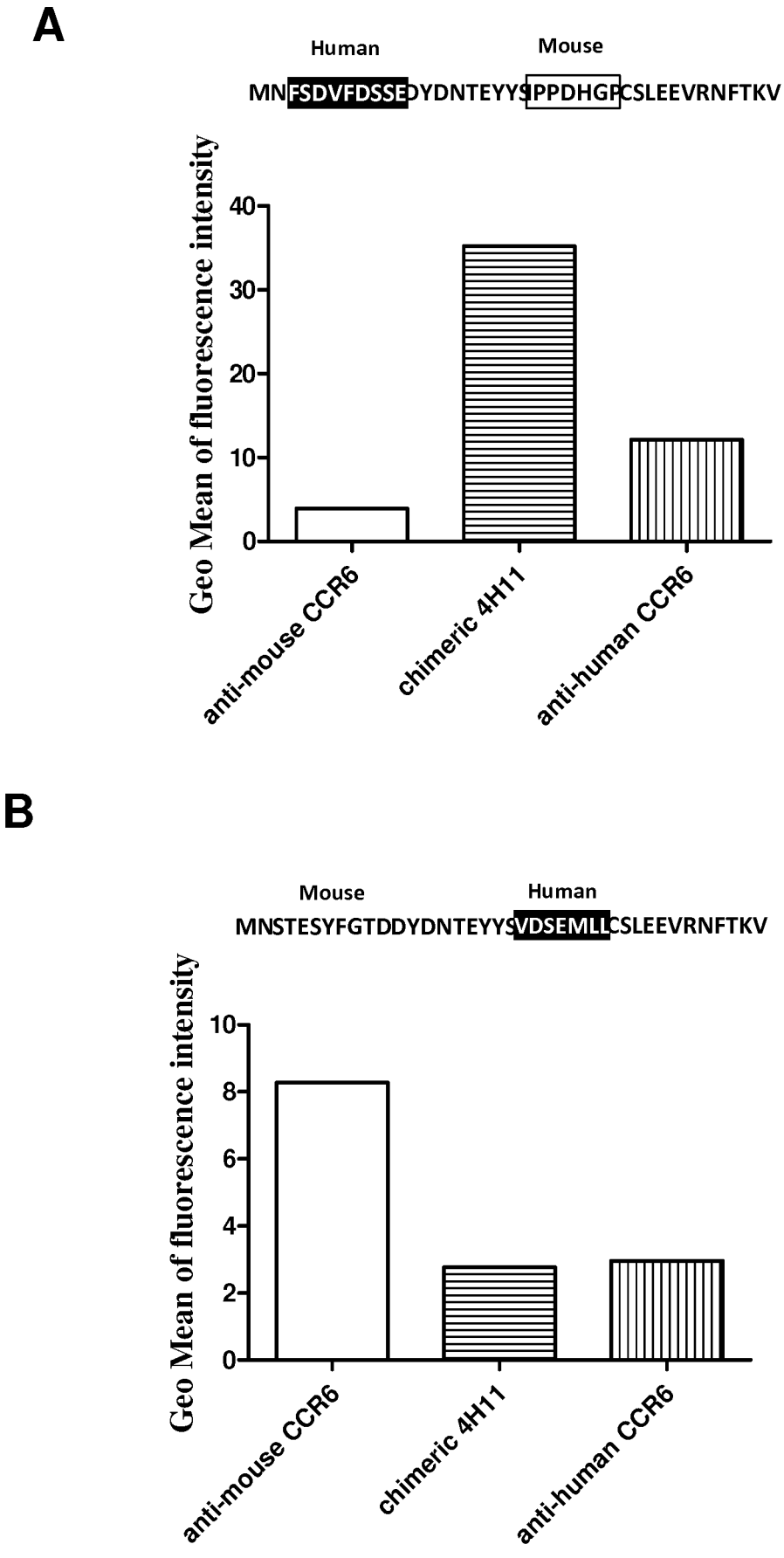

A
Human
Mouse
MNFSDVFDSSEDYDNTEYYSPPDHGPCSLEEVRNFTKV
Geo Mean of fluorescence intensity
40
30
20
10
0
anti-mouse CCR6
chimeric 4H11
anti-human CCR6
B
Mouse
Human
MNSTESYFGTDDYDNTEYYSVDSEMLLCSLEEVRNFTKV
Geo Mean of fluorescence intensity
10
8
6
4
2
0
anti-mouse CCR6
chimeric 4H11
anti-human CCR6

FIG.6

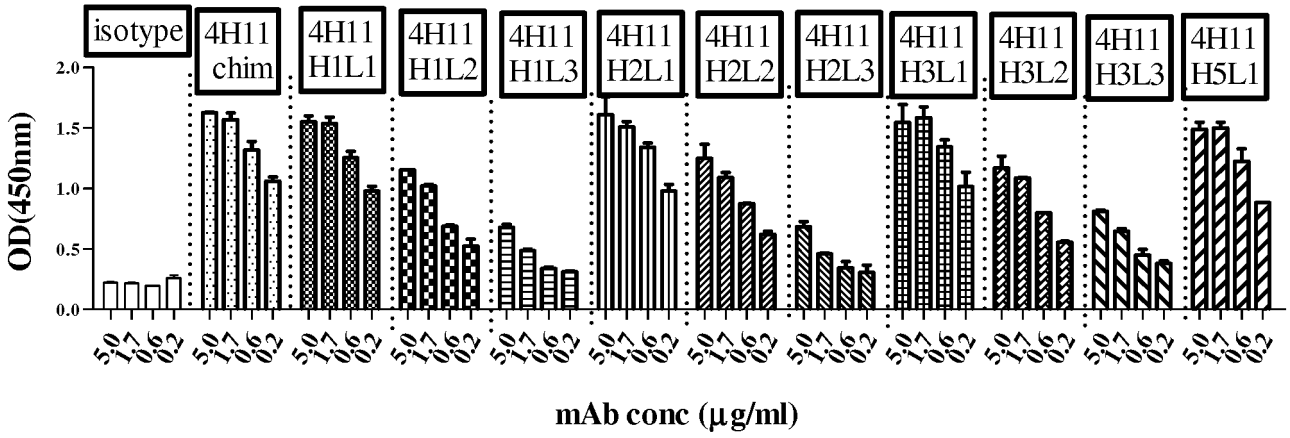

isotype
4H11 chim
4H11 H1L1
4H11 H1L2
4H11 H1L3
4H11 H2L1
4H11 H2L2
4H11 H2L3
4H11 H3L1
4H11 H3L2
4H11 H3L3
4H11 H5L1
OD(450nm)
2.0
1.5
1.0
0.5
0.0
5.0 1.7 0.6 0.2
mAb conc (µg/ml)

FIG. 7

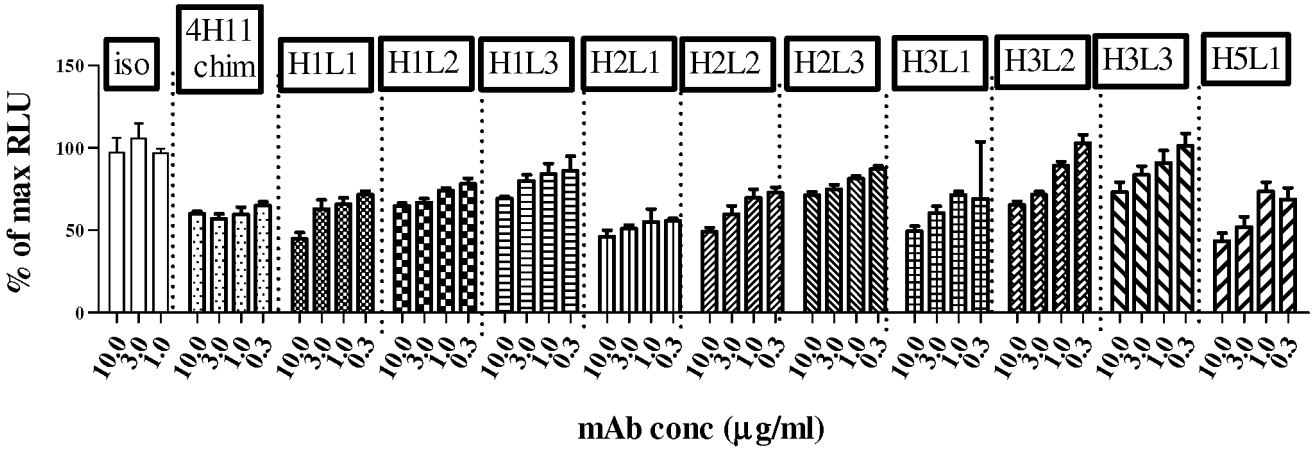
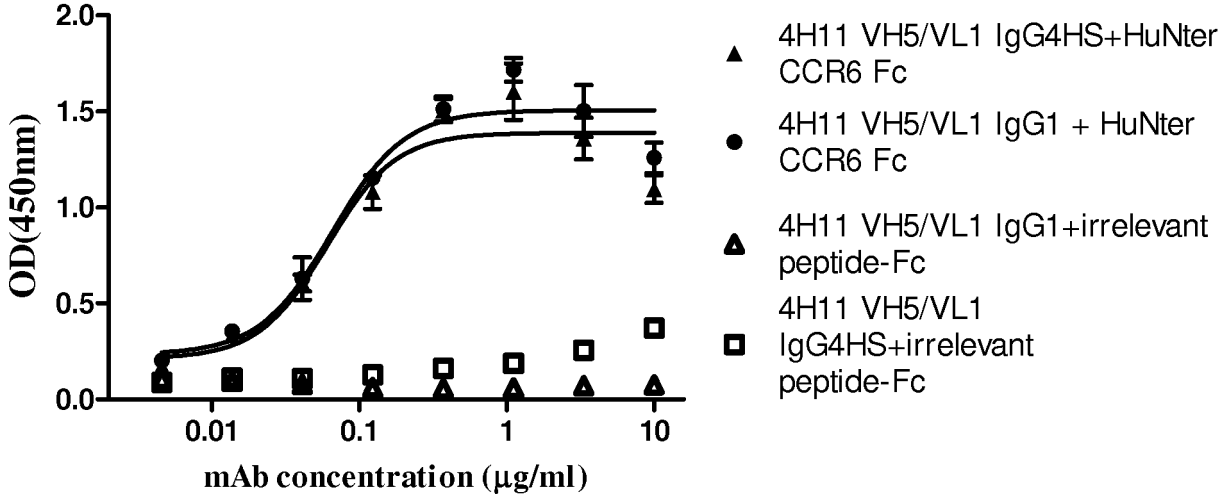

iso
4H11 chim
H1L1
H1L2
H1L3
H2L1
H2L2
H2L3
H3L1
H3L2
H3L3
H5L1
% of max RLU
150
100
50
0
10.0
3.0
1.0
0.3
mAb conc (µg/ml)

FIG.8

A


2.0
1.5
1.0
0.5
0.0
OD(450nm)
0.01
0.1
1
10
mAb concentration (µg/ml)
4H11 VH5/VL1 IgG4HS+HuNter CCR6 Fc
4H11 VH5/VL1 IgG1 + HuNter CCR6 Fc
4H11 VH5/VL1 IgG1+irrelevant peptide-Fc
4H11 VH5/VL1 IgG4HS+irrelevant peptide-Fc

B

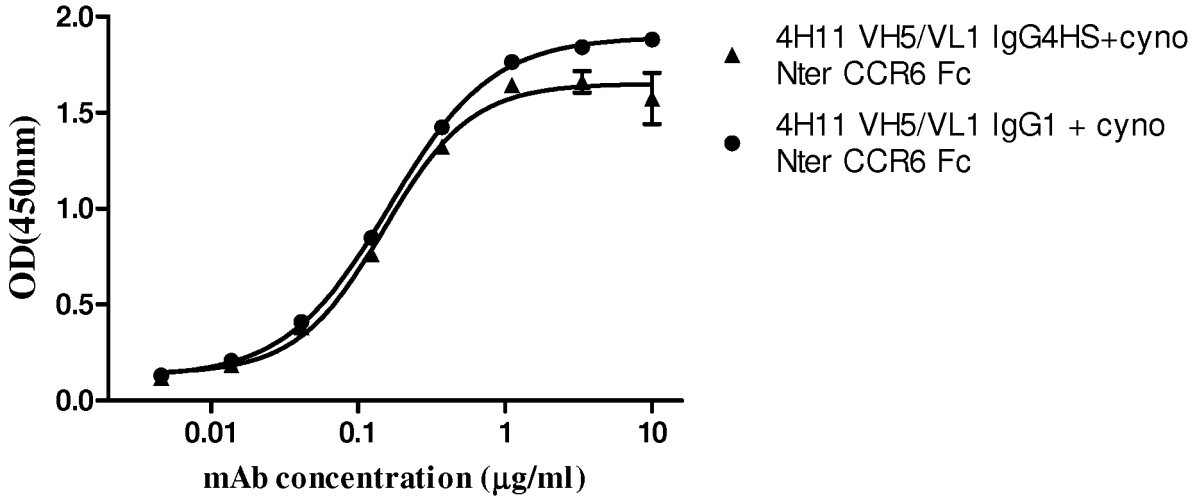

2.0
1.5
1.0
0.5
0.0
OD(450nm)
0.01
0.1
1
10
mAb concentration (µg/ml)
4H11 VH5/VL1 IgG4HS+cyno Nter CCR6 Fc
4H11 VH5/VL1 IgG1 + cyno Nter CCR6 Fc

FIG.9

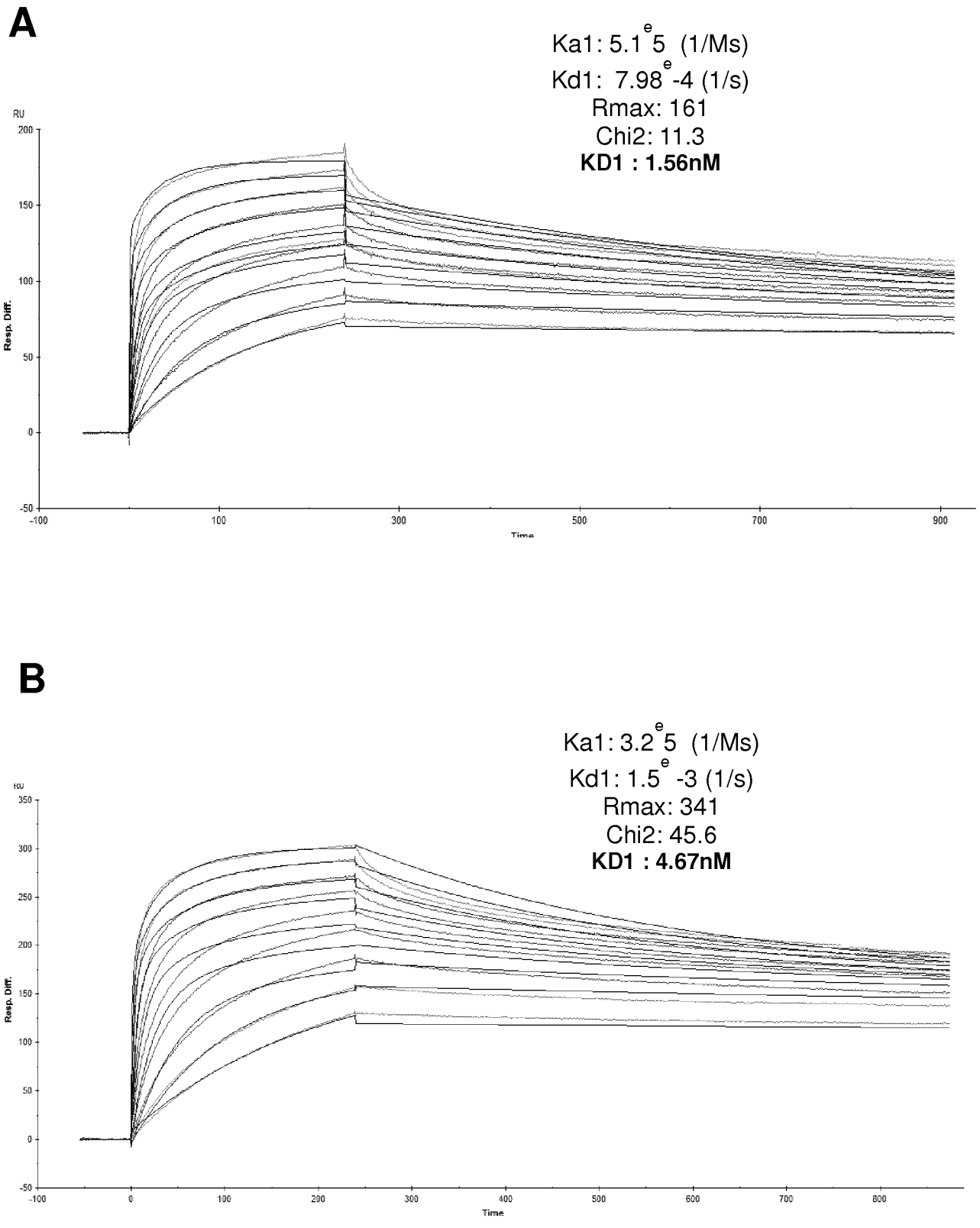

A
Ka1: 5.1e5 (1/Ms)
Kd1: 7.98e-4 (1/s)
Rmax: 161
Chi2: 11.3
KD1 : 1.56nM
RU
Resp. Diff.
Time
B
Ka1: 3.2e5 (1/Ms)
Kd1: 1.5e-3 (1/s)
Rmax: 341
Chi2: 45.6
KD1 : 4.67nM
RU
Resp. Diff.
Time

FIG.10

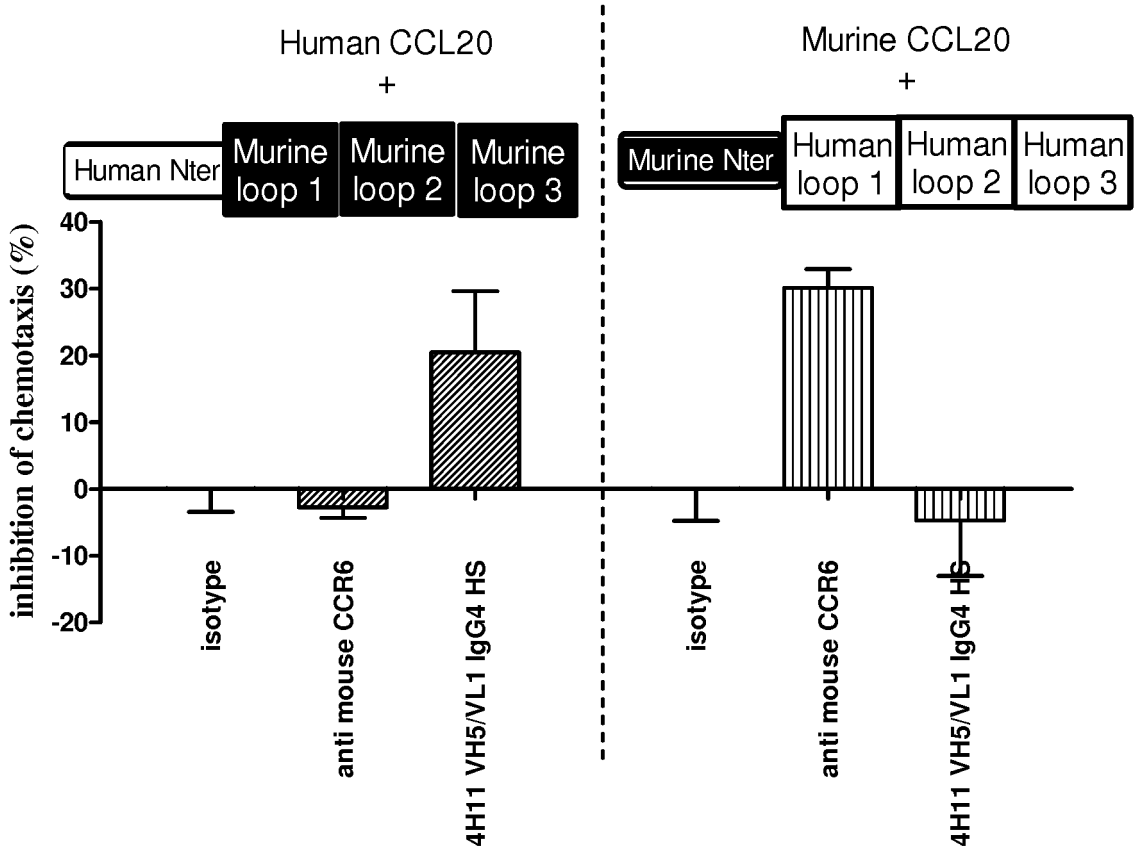

Human CCL20
+
Human Nter
Murine loop 1
Murine loop 2
Murine loop 3
Murine CCL20
+
Murine Nter
Human loop 1
Human loop 2
Human loop 3
inhibition of chemotaxis (%)
40
30
20
10
0
-10
-20
isotype
anti mouse CCR6
4H11 VH5/VL1 IgG4 HS
isotype
anti mouse CCR6
4H11 VH5/VL1 IgG4 HS

FIG.11

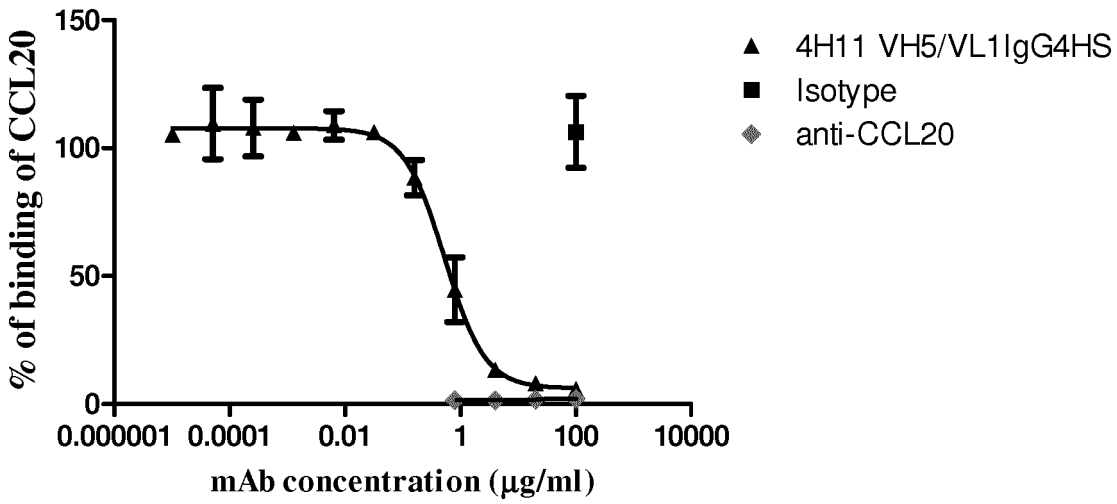

150
100
50
0
% of binding of CCL20
0.000001
0.0001
0.01
1
100
10000
mAb concentration (µg/ml)
4H11 VH5/VL1IgG4HS
Isotype
anti-CCL20

FIG.12

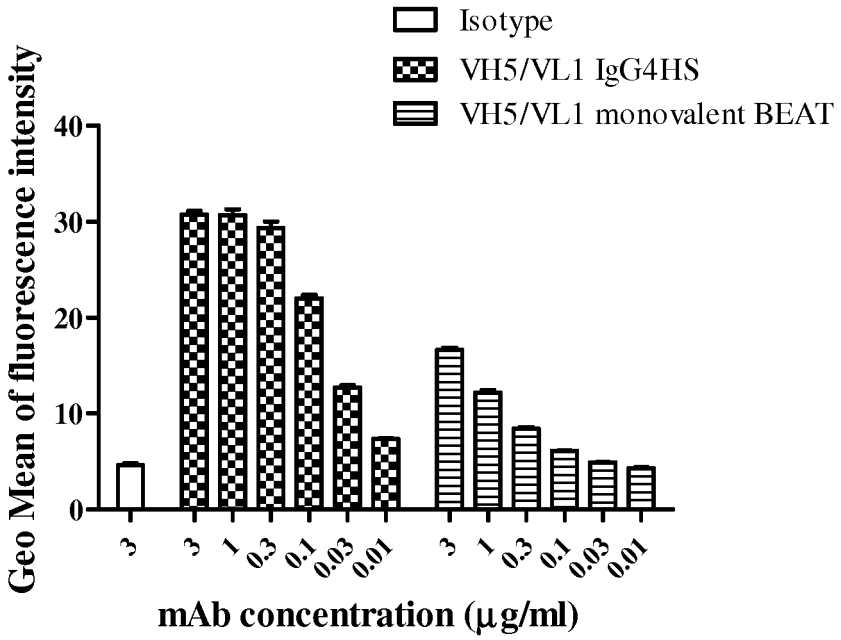

Isotype
VH5/VL1 IgG4HS
VH5/VL1 monovalent BEAT
40
30
20
10
0
Geo Mean of fluorescence intensity
3
3
1
0.3
0.1
0.03
0.01
3
1
0.3
0.1
0.03
0.01
mAb concentration (µg/ml)

FIG.13

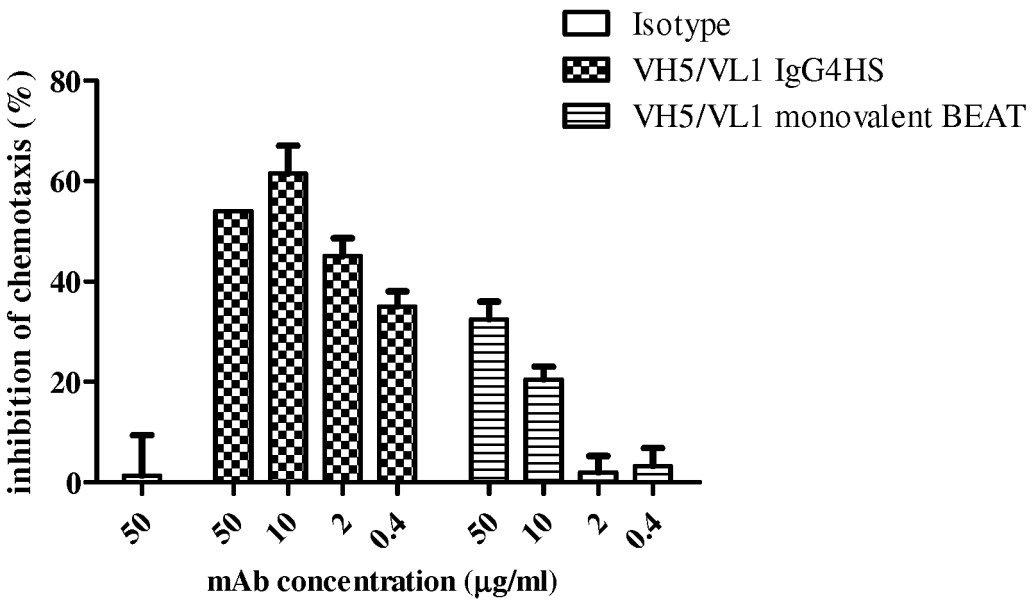

Isotype
VH5/VL1 IgG4HS
VH5/VL1 monovalent BEAT
inhibition of chemotaxis (%)
80
60
40
20
0
50
50
10
2
0.4
50
10
2
0.4
mAb concentration (µg/ml)

FIG. 14A

	Kabat:	H52	H52a	H53	H54	H55	H56	H57	H58	Biacore binding
H2:		T	-	N	G	G	I	T	Y	
Diversification		X	-	X	G	G	X	T	X	
scFv clones:										
VH5/VL1-H2-H1		S					K			=
VH5/VL1-H2-D1				S			K			-
VH5/VL1-H2-B1				M			K			-
VH5/VL1-H2-C3				R			K			-
VH5/VL1-H2-A3							K			=/+
VH5/VL1-H2-C1				T			D			-
VH5/VL1-H21-C2		N		T			G			=
VH5/VL1-H2-G2				T			T			=
VH5/VL1-H2-B3				**T**			**R**			**=/+**
VH5/VL1-H2-E1				T			S			-
VH5/VL1-H2-G1				K			K		F	-
VH5/VL1-H2-E3		N		A			S		L	0

KEYS:

(-) weaker off-rate
(=) off-rate similar to H5/L1 parental scFv
(+) better off-rate
(++) significantly better off-rate
(0) no binding
(ND) not determined
(X) diversity: NNK – all 20 amino-acids

FIG.14B

	Kabat:	L92	L93	L94	L95	L96	Biacore binding
L3:		S	H	V	P	L	
Diversification:		X	X	X	P	x	
ScFv clones:							
VH5/VL1-L3-H7			L	L			++
VH5/VL1-L3-F9		T	Y	M			++
VH5/VL1-L3-B8		T	Y	N			++
VH5/VL1-L3-G8		**T**	**Y**	**L**			**++**
VH5/VL1-L3-D8		T	K	L			+
VH5/VL1-L3-E8			Y	L			+
VH5/VL1-L3-A9			L	M		I	ND
VH5/VL1-L3-B9			Y	I			ND
VH5/VL1-L3-C9		**T**	**Y**	**Y**			**++**
VH5/VL1-L3-G7		T	F	L			++
VH5/VL1-L3-F8			Y	S			0
VH5/VL1-L3-C8		T	Y	L		I	++
VH5/VL1-L3-B10			K	L			+
VH5/VL1-L3-D9		S	X	M		L	++

KEYS:
(-) weaker off-rate
(=) off-rate similar to H5/L1 parental scFv
(+) better off-rate
(++) significantly better off-rate
(0) no binding
(ND) not determined

FIG.15

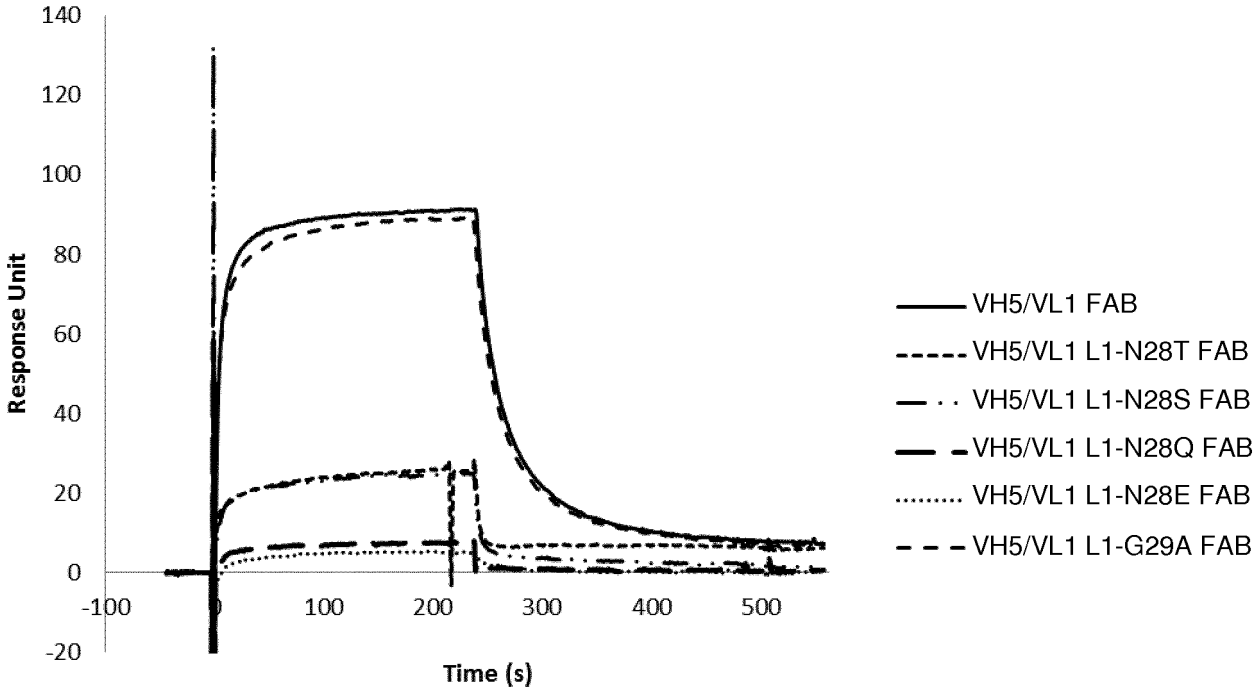

140
120
100
80
60
40
20
0
-20
-100
100
200
300
400
500
Response Unit
Time (s)
VH5/VL1 FAB
VH5/VL1 L1-N28T FAB
VH5/VL1 L1-N28S FAB
VH5/VL1 L1-N28Q FAB
VH5/VL1 L1-N28E FAB
VH5/VL1 L1-G29A FAB

FIG.16

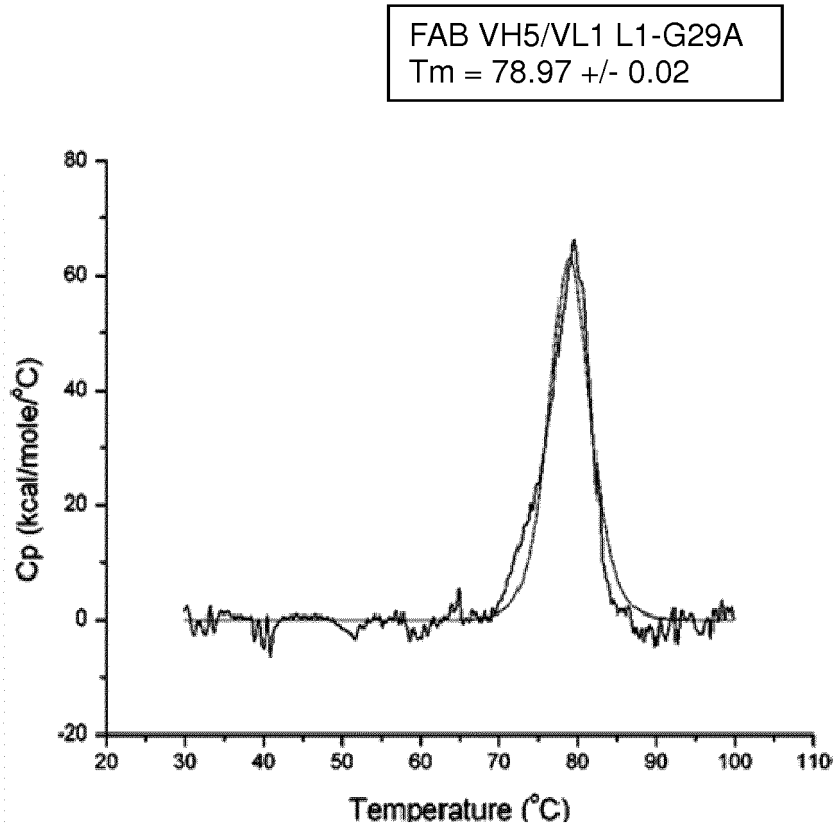

FAB VH5/VL1 L1-G29A
Tm = 78.97 +/- 0.02
Cp (kcal/mole/°C)
80
60
40
20
0
-20
20
30
40
50
60
70
80
90
100
110
Temperature (°C)

FIG.17

	Human FAB HC/LC SEQ ID NO:	Human IgG1 HC/LC SEQ ID NO:	Human monovalent BEAT® HC1/HC2/LC SEQ ID NO:	Human IgG4 HC/LC SEQ ID NO:
VH5/VL1-B3G8-G29A	206/207	198/199	227/228/229	214/215
VH5/VL1-B3C9-G29A	208/209	200/201	230/231/232	216/217
VH5/VL1-G8-G29A	202/203	194/195	221/222/223	210/211
VH5/VL1-C9-G29A	204/205	196/197	224/225/226	212/213
VH5/VL1	233/234	10/30	218/219/220	235/236

FIG.18A

FAB	Ka human CCR6	Kd human CCR6	KD human CCR6	Ka cynomolgus CCR6	Kd cynomolgus CCR6	KD cynomolgus CCR6
VH5/VL1-B3G8-G29A	6.46e6	3.39e-3	526 pM	1.72e6	2.02e-3	1.17 nM
VH5/VL1-B3C9-G29A	6.96e6	3.79e-3	544 pM	1.83e6	2.25e-3	1.23 nM
VH5/VL1-G8-G29A	2.65e6	2.66e-3	1.0 nM	8.97e5	1.96e-3	2.19 nM
VH5/VL1-C9-G29A	2.66e6	3.41e-3	1.28 nM	8.75e5	2.44e-3	2.79 nM
VH5/VL1	1.58e6	3.11e-2	19.6 nM	7.44e5	2.50e-2	33.6 nM

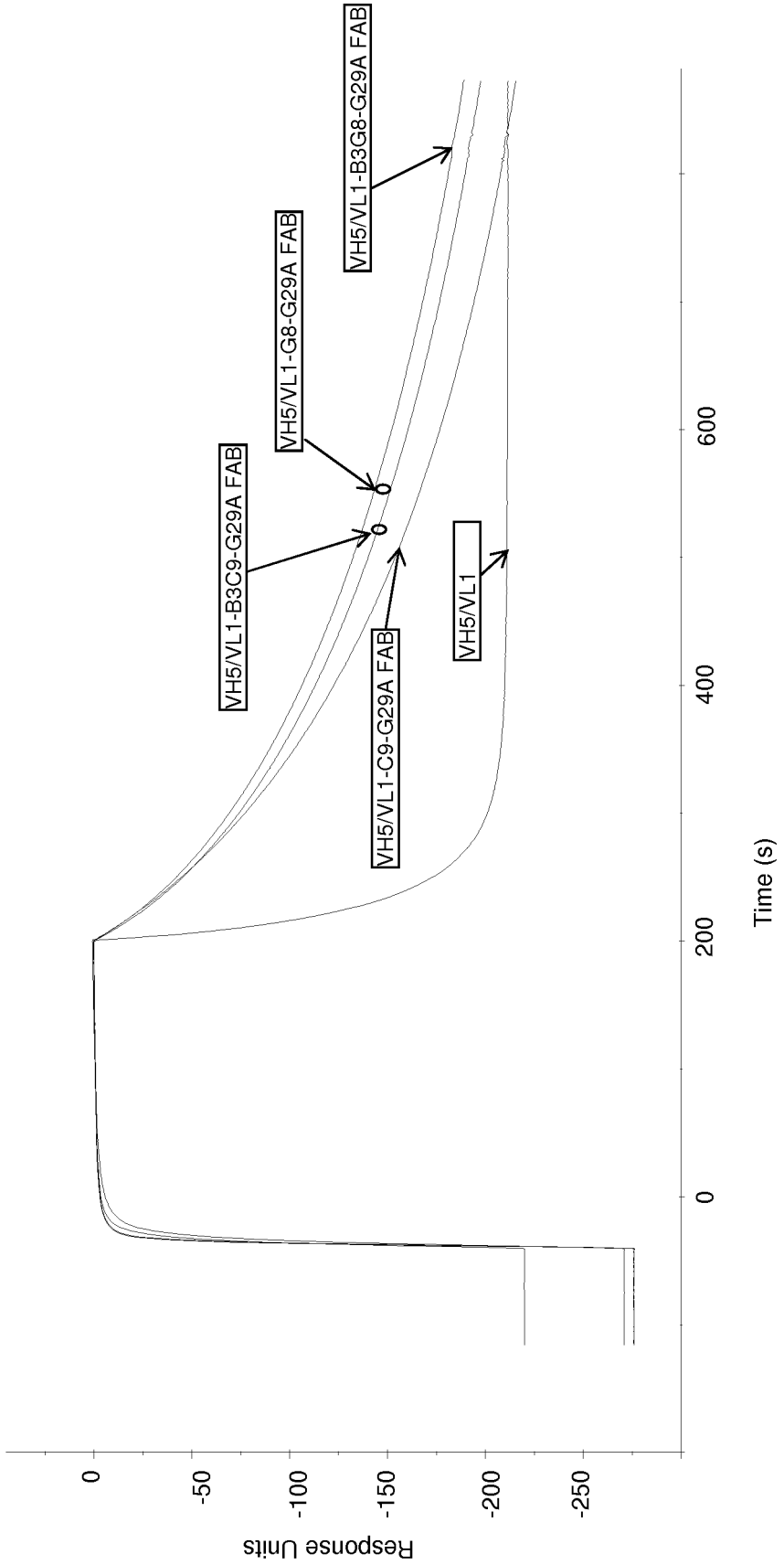
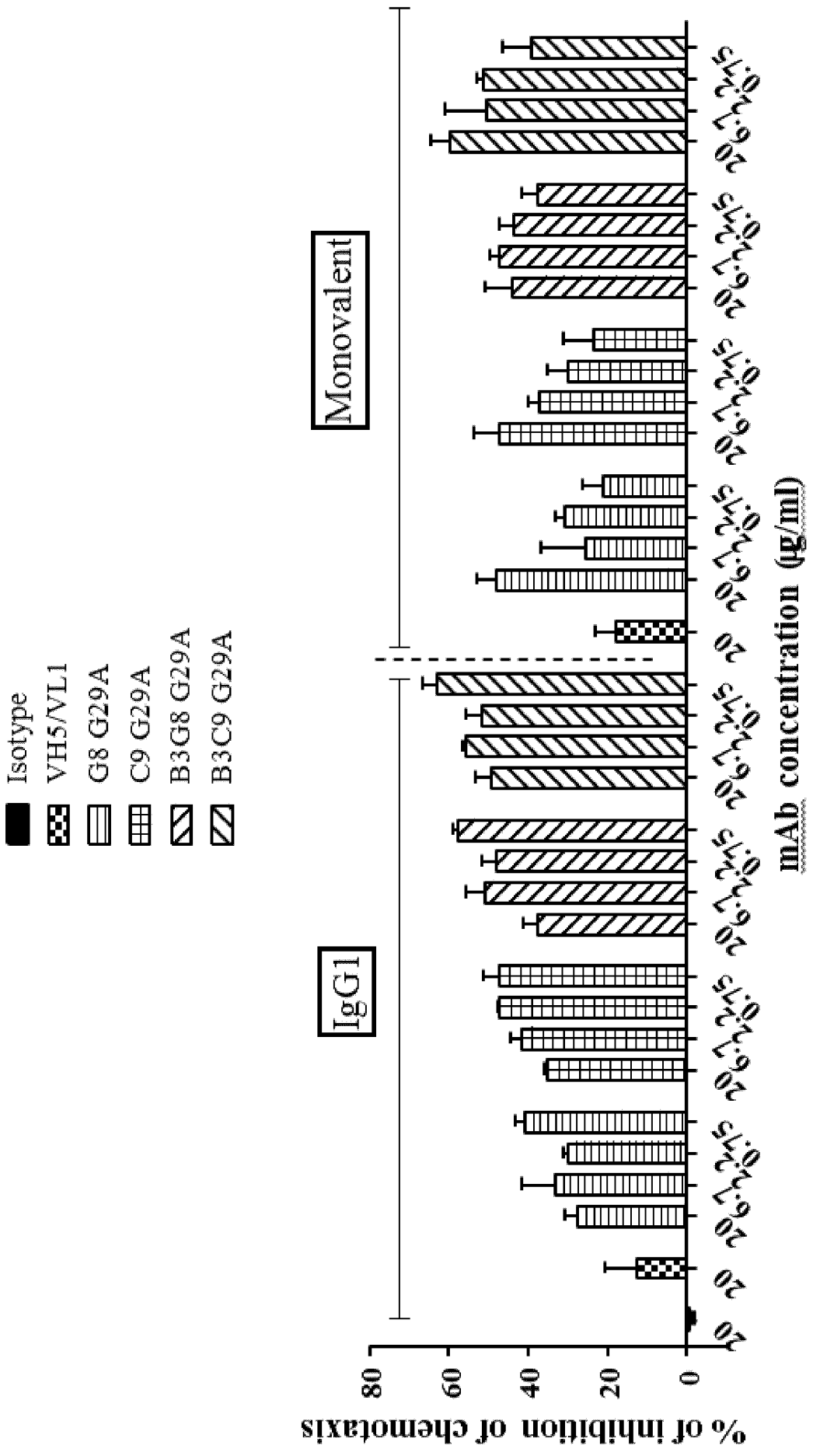

VH5/VL1-B3G8-G29A FAB
VH5/VL1-G8-G29A FAB
VH5/VL1-B3C9-G29A FAB
VH5/VL1-C9-G29A FAB
VH5/VL1
Response Units
0
-50
-100
-150
-200
-250
0
200
400
600
Time (s)

FIG.18B

FIG.19


Isotype
VH5/VL1
G8 G29A
C9 G29A
B3G8 G29A
B3C9 G29A
IgG1
Monovalent
% of inhibition of chemotaxis
0
20
40
60
80
mAb concentration (µg/ml)
20
20
20+0.2
20+0.1
20+0.05
20
20+0.2
20+0.1
20+0.05
20
20+0.2
20+0.1
20+0.05
20
20+0.2
20+0.1
20+0.05
20
20+0.2
20+0.1
20+0.05