Clone	KD (pM)	kon(1/Ms)	kdis(1/s)
3C3	10.7	8.13E+05	8.67E-06
3G5	3.3	9.05E+05	2.97E-06
1B4	10.5	8.32E+05	8.76E-06
3B6	7.9	7.82E+05	6.14E-06
6H6	2.8	9.05E+05	2.49E-06

FIG. 1

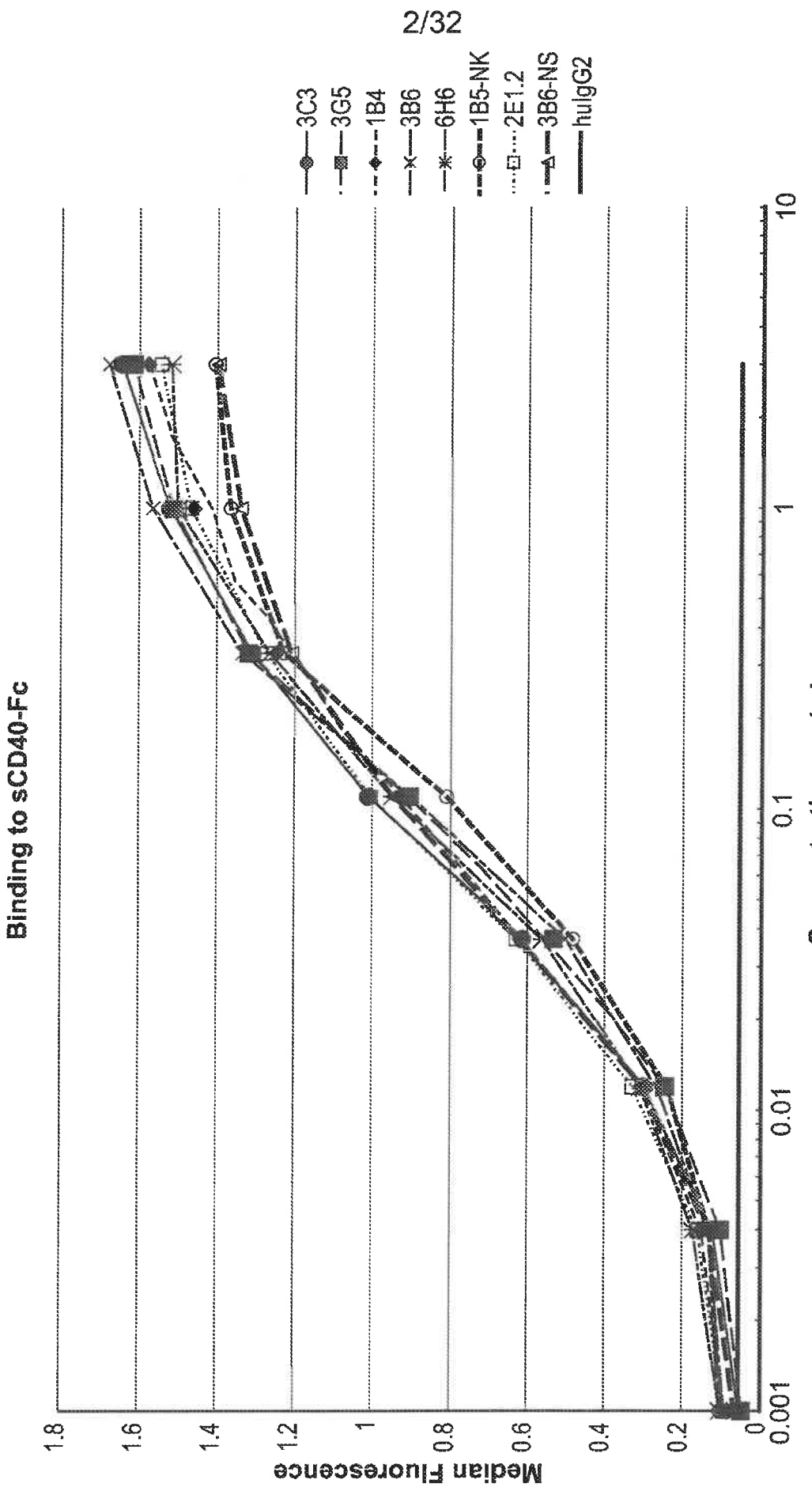

Binding to sCD40-Fc
Median Fluorescence
Concentration, ug/mL
0.001
0.01
0.1
1
10
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
3C3
3G5
1B4
3B6
6H6
1B5-NK
2E1.2
3B6-NS
huIgG2

FIG. 2

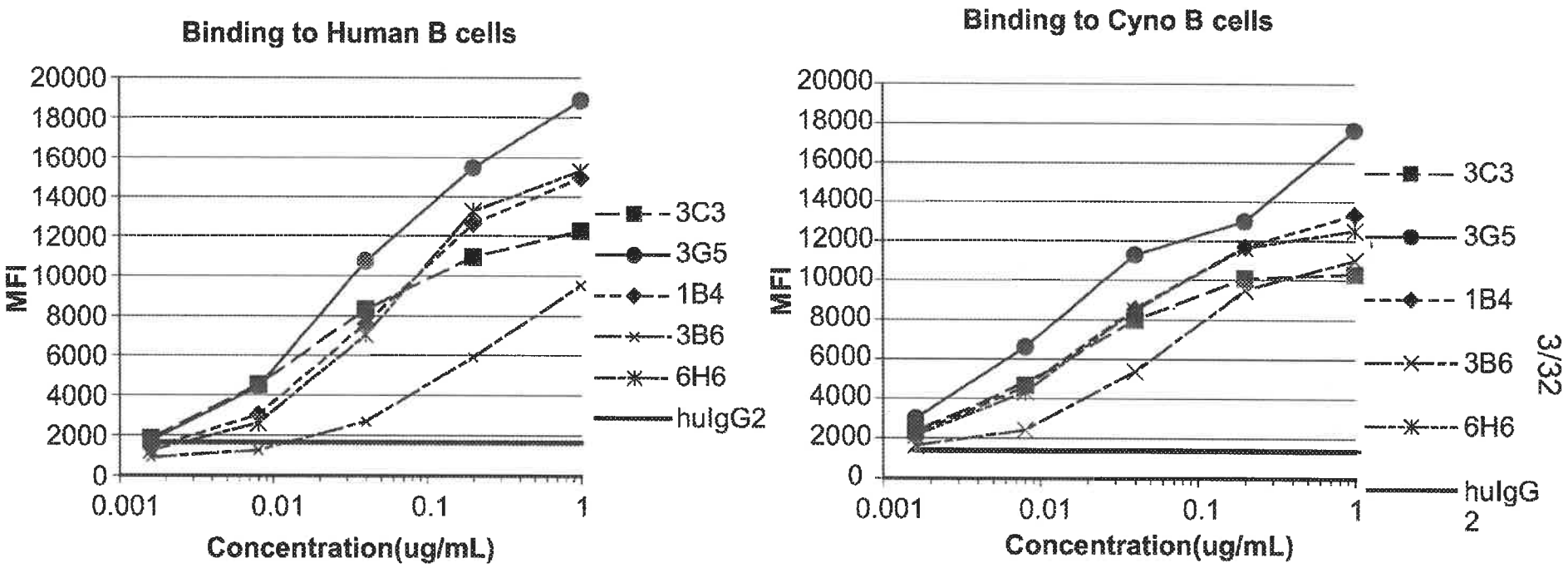

Binding to Human B cells
MFI
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0.001
0.01
0.1
1
Concentration(ug/mL)
3C3
3G5
1B4
3B6
6H6
huIgG2
Binding to Cyno B cells
MFI
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0.001
0.01
0.1
1
Concentration(ug/mL)
3C3
3G5
1B4
3B6
6H6
huIgG2

FIG. 3

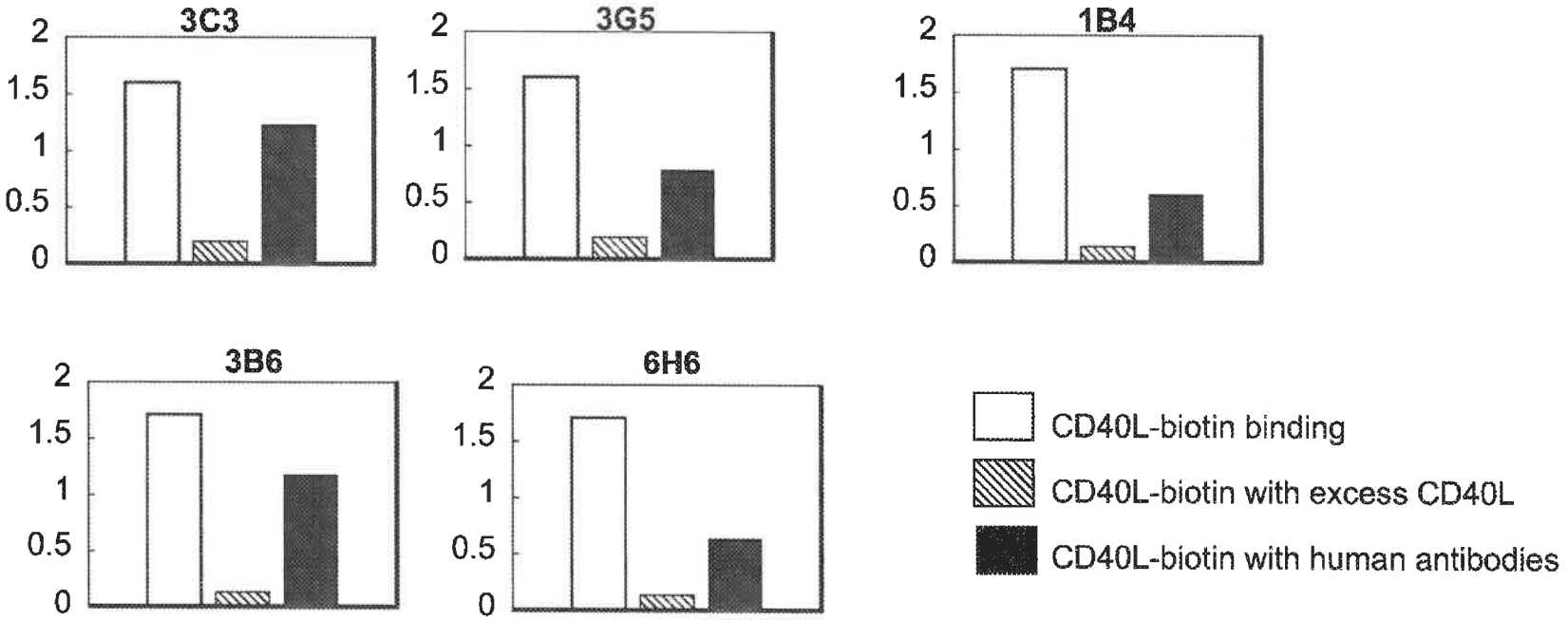
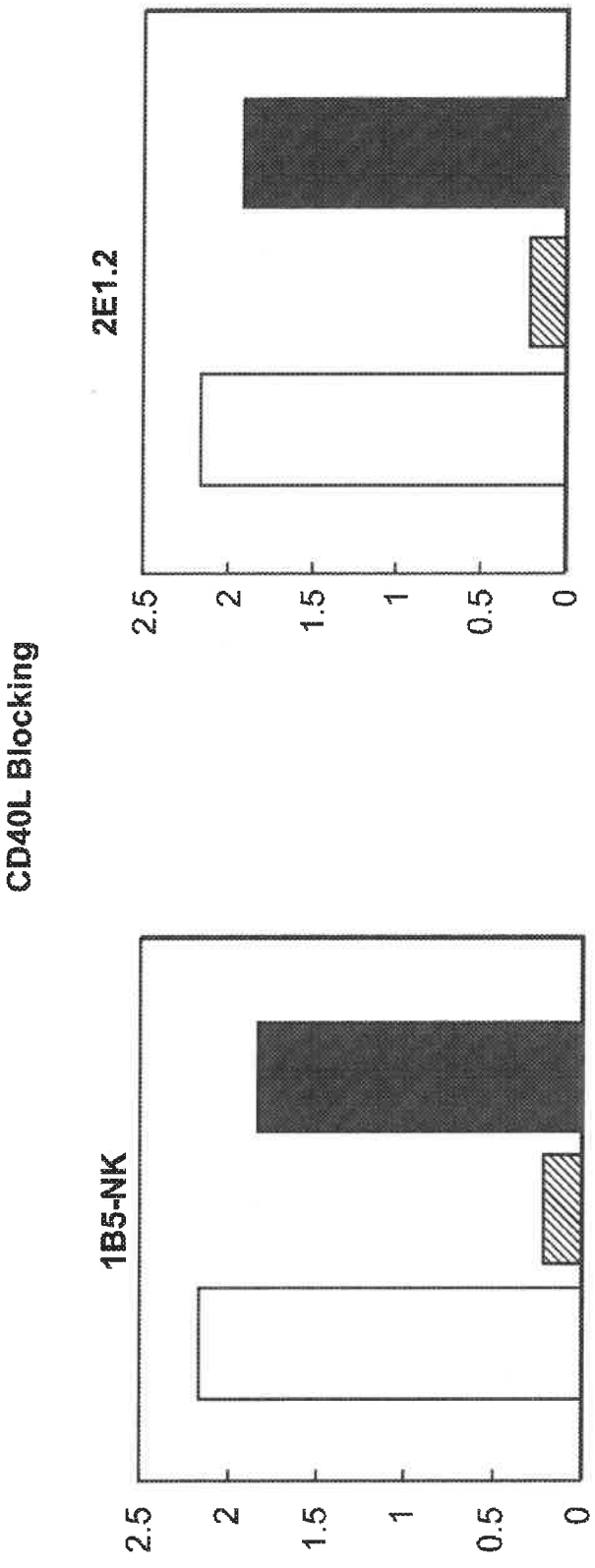

3C3
3G5
1B4
3B6
6H6
2
1.5
1
0.5
0
CD40L-biotin binding
CD40L-biotin with excess CD40L
CD40L-biotin with human antibodies

FIG. 4A

FIG. 4B



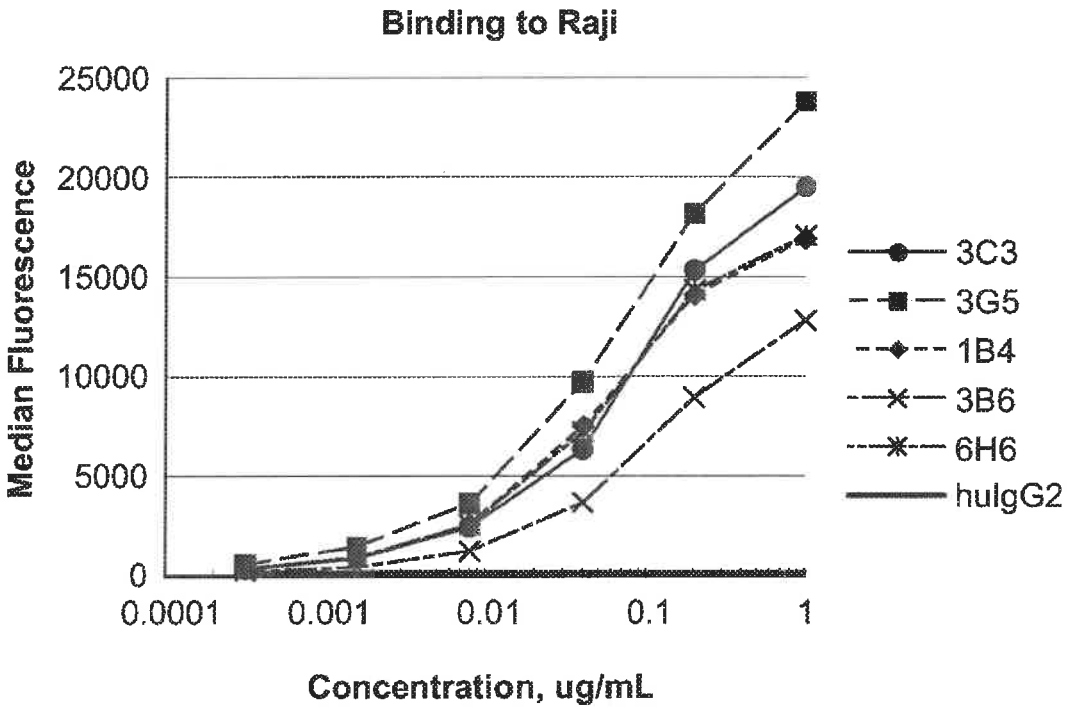

Binding to Raji
Median Fluorescence
25000
20000
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
Concentration, ug/mL
3C3
3G5
1B4
3B6
6H6
hulgG2

FIG. 5

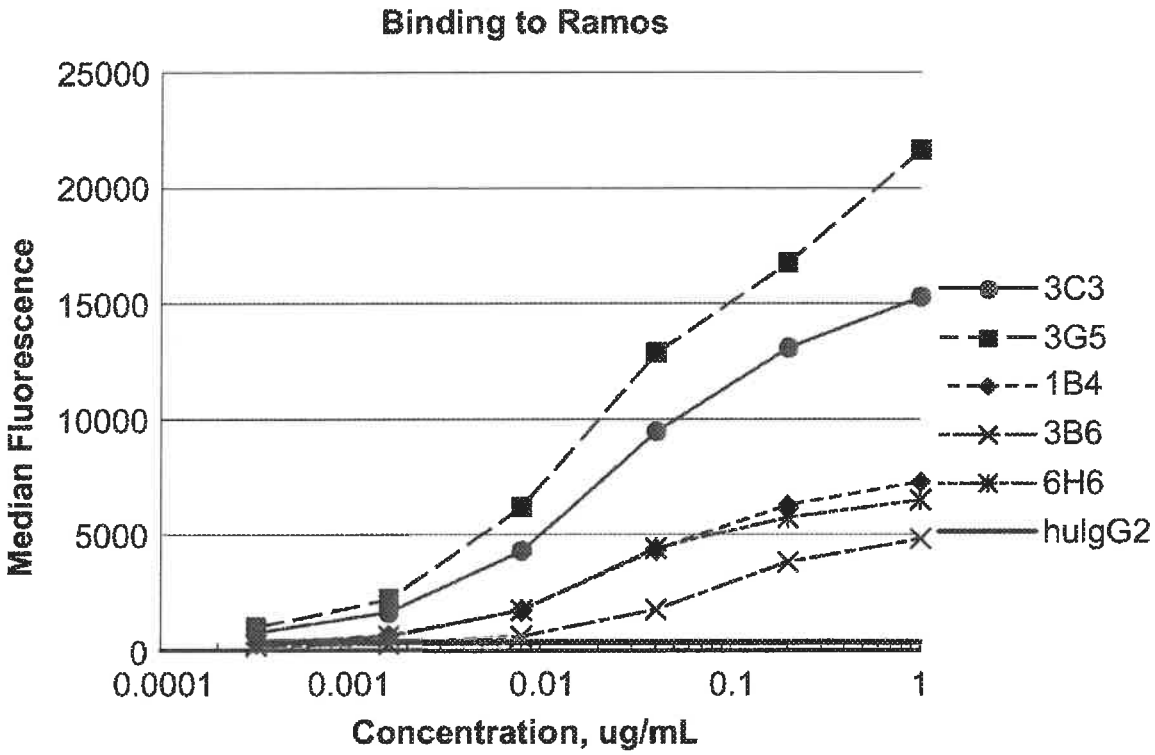

Binding to Ramos
Median Fluorescence
25000
20000
15000
10000
5000
0
0.0001
0.001
0.01
0.1
1
Concentration, ug/mL
3C3
3G5
1B4
3B6
6H6
hulgG2

FIG. 6

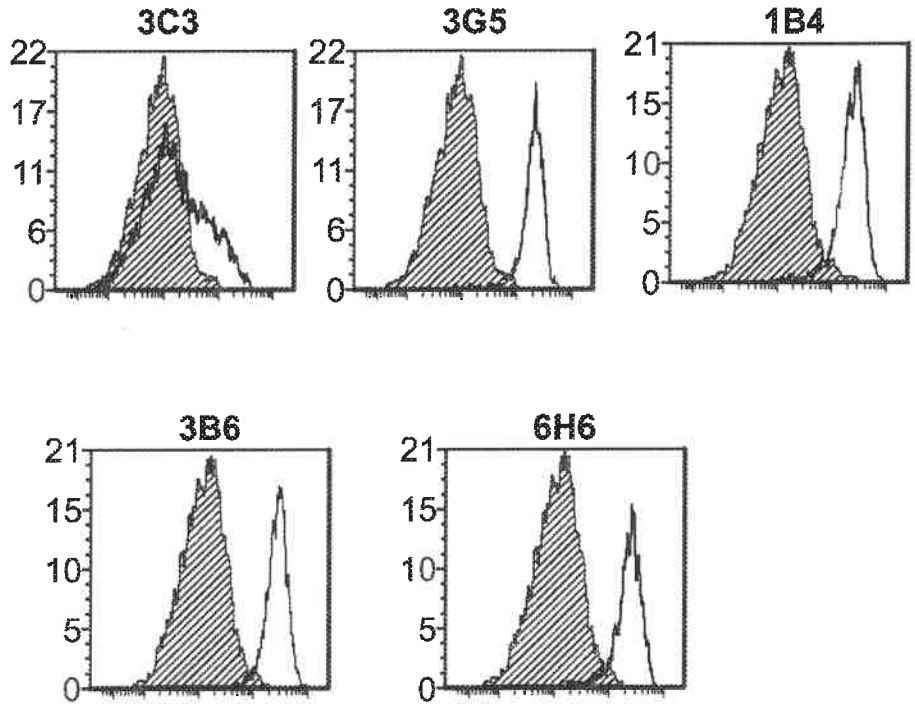
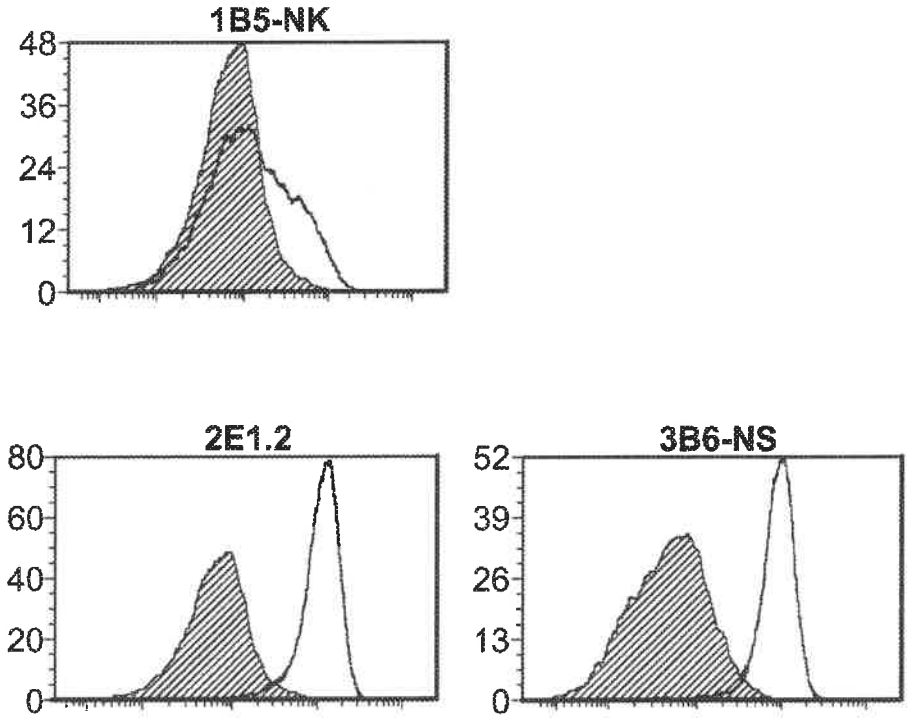
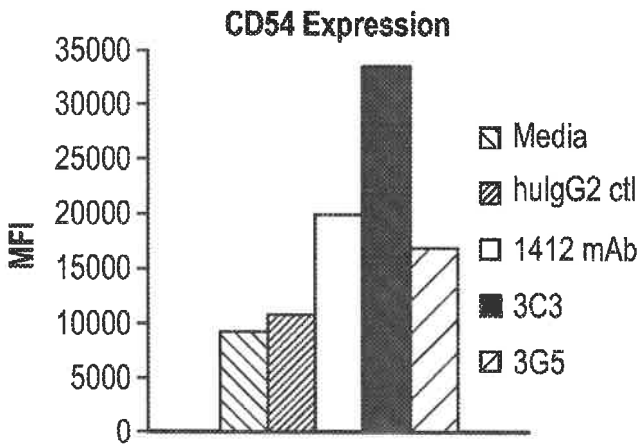
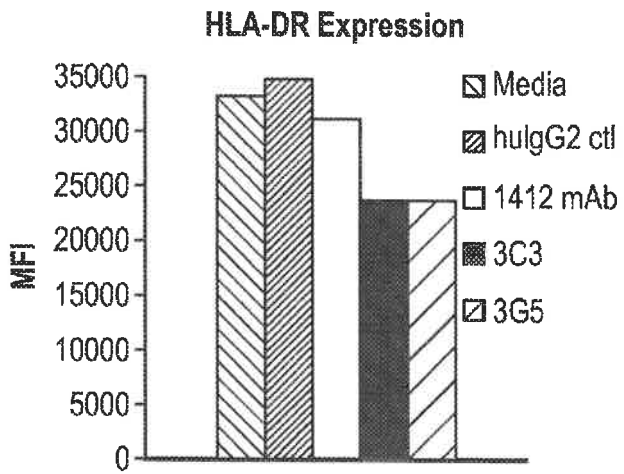

3C3
22
17
11
6
0
3G5
22
17
11
6
0
1B4
21
15
10
5
0
3B6
21
15
10
5
0
6H6
21
15
10
5
0

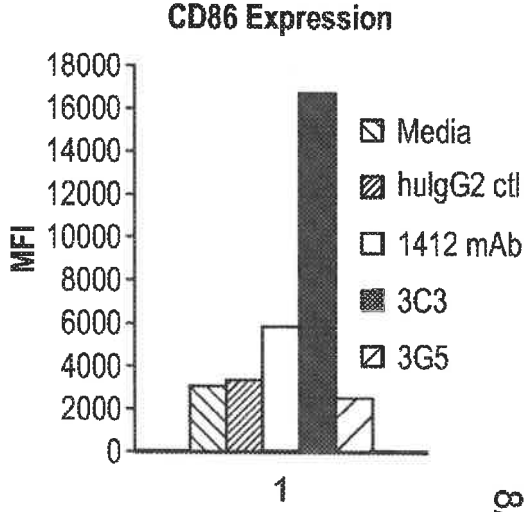
FIG. 7A

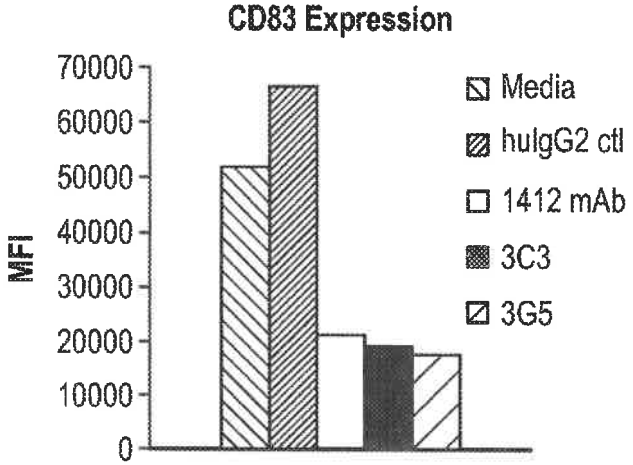
FIG. 7B


1B5-NK
48
36
24
12
0
2E1.2
80
60
40
20
0
3B6-NS
52
39
26
13
0


CD54 Expression
MFI
35000
30000
25000
20000
15000
10000
5000
0
Media
hulgG2 ctl
1412 mAb
3C3
3G5


HLA-DR Expression
MFI
35000
30000
25000
20000
15000
10000
5000
0
Media
hulgG2 ctl
1412 mAb
3C3
3G5


CD86 Expression
MFI
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Media
hulgG2 ctl
1412 mAb
3C3
3G5
1


CD83 Expression
MFI
70000
60000
50000
40000
30000
20000
10000
0
Media
hulgG2 ctl
1412 mAb
3C3
3G5

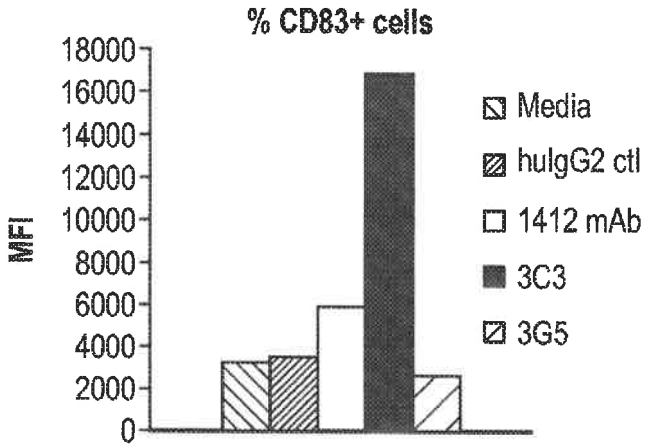

% CD83+ cells
MFI
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
Media
hulgG2 ctl
1412 mAb
3C3
3G5

FIG. 8A

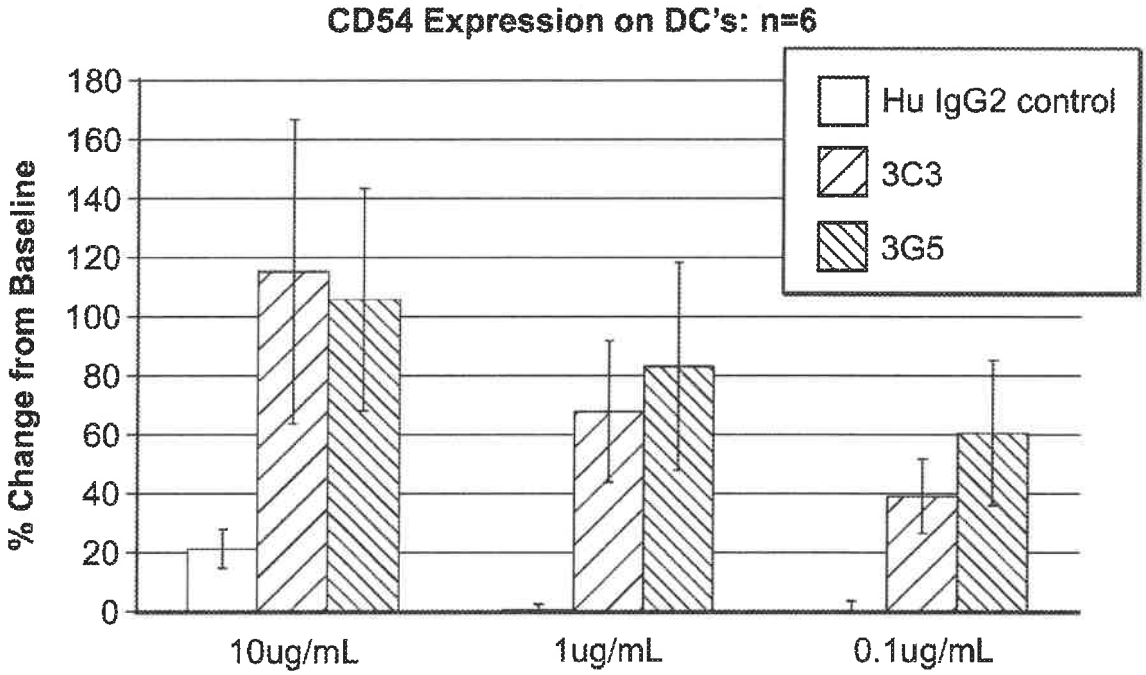

CD54 Expression on DC's: n=6
% Change from Baseline
180
160
140
120
100
80
60
40
20
0
10ug/mL
1ug/mL
0.1ug/mL
Hu IgG2 control
3C3
3G5

FIG. 8B

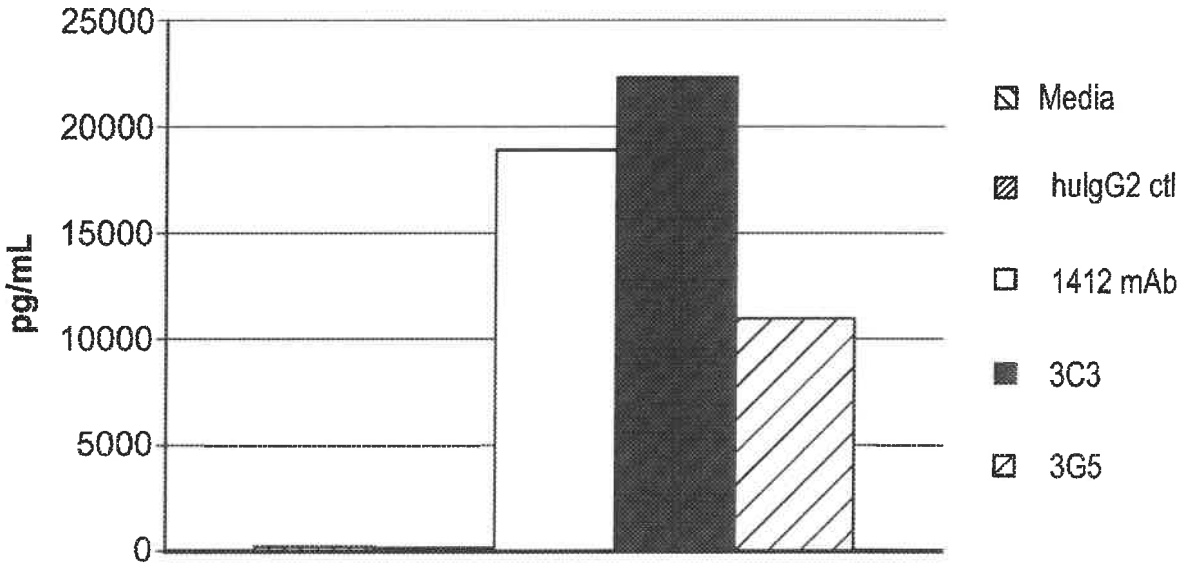

25000
20000
15000
10000
5000
0
pg/mL
Media
hulgG2 ctl
1412 mAb
3C3
3G5

FIG. 9A

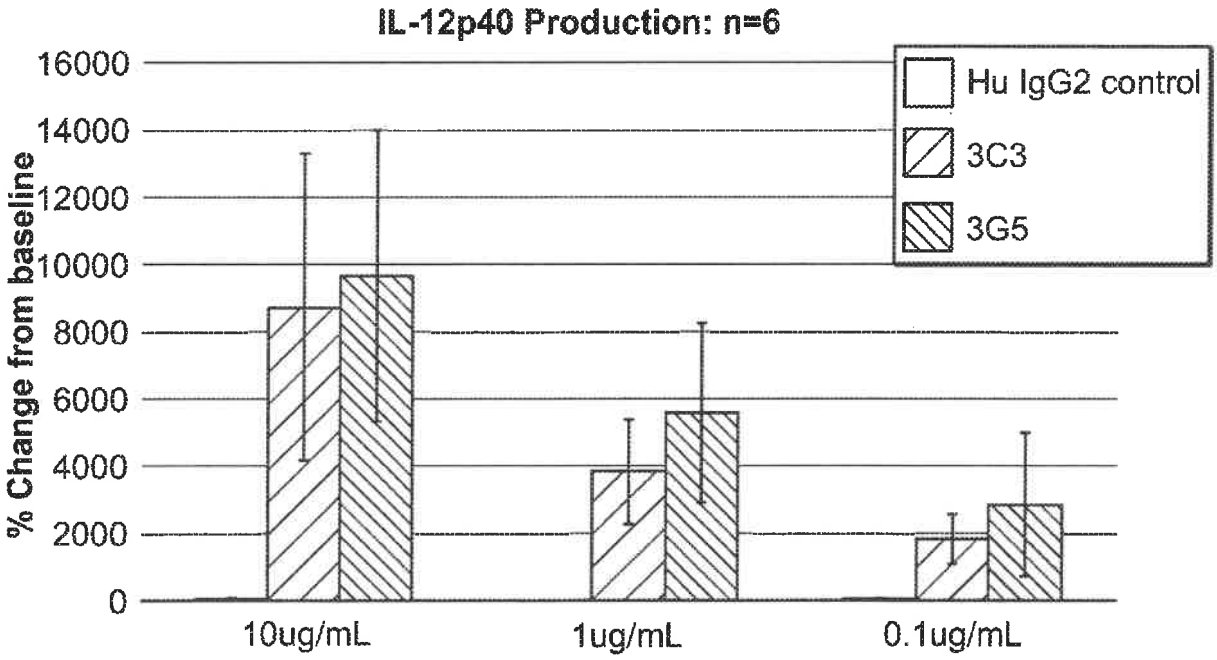

IL-12p40 Production: n=6
16000
14000
12000
10000
8000
6000
4000
2000
0
% Change from baseline
Hu IgG2 control
3C3
3G5
10ug/mL
1ug/mL
0.1ug/mL

FIG. 9B

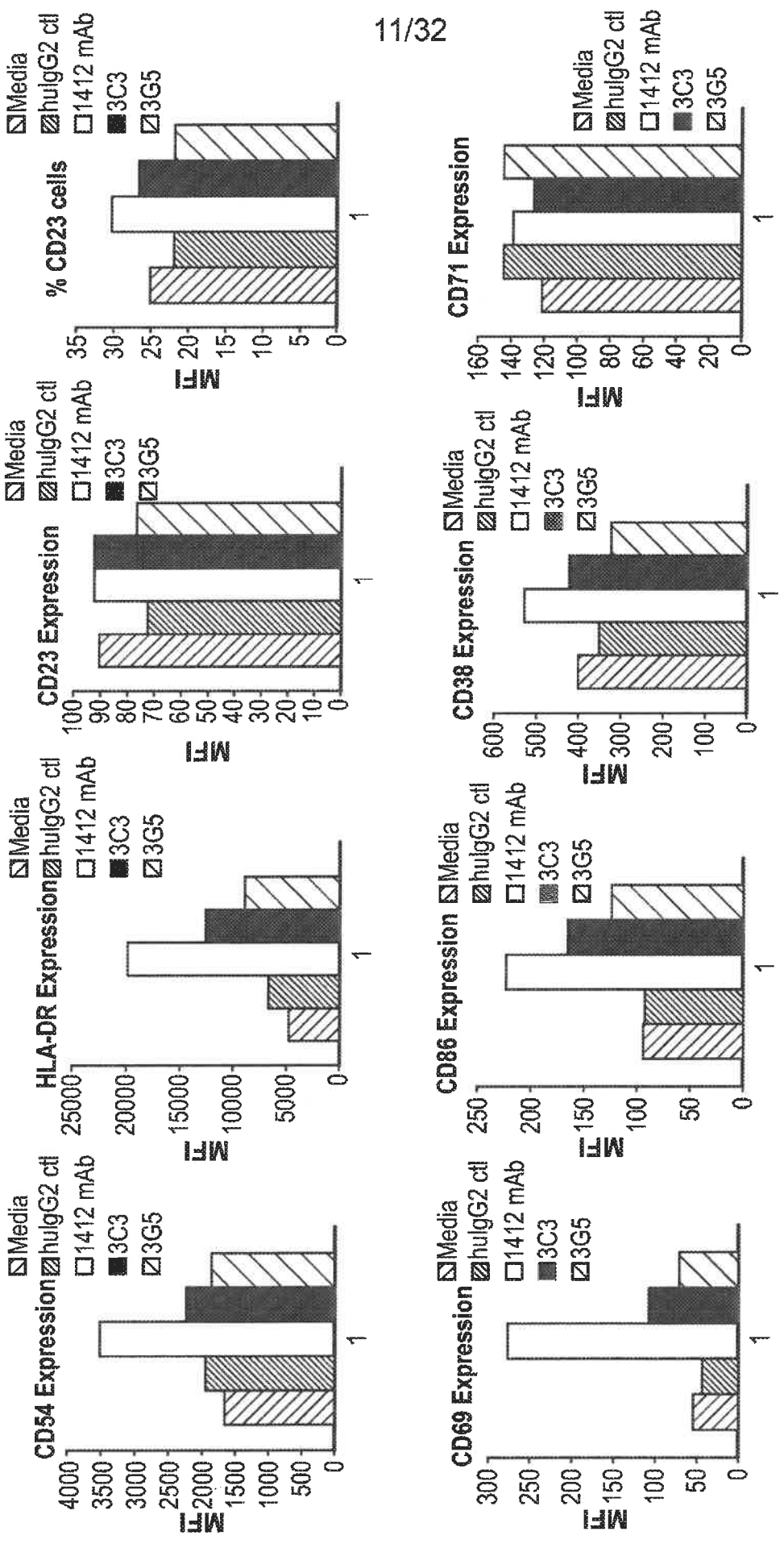

CD54 Expression
HLA-DR Expression
CD23 Expression
% CD23 cells
CD69 Expression
CD86 Expression
CD38 Expression
CD71 Expression
MFI
Media
hulgG2 ctl
1412 mAb
3C3
3G5
1

FIG. 10A

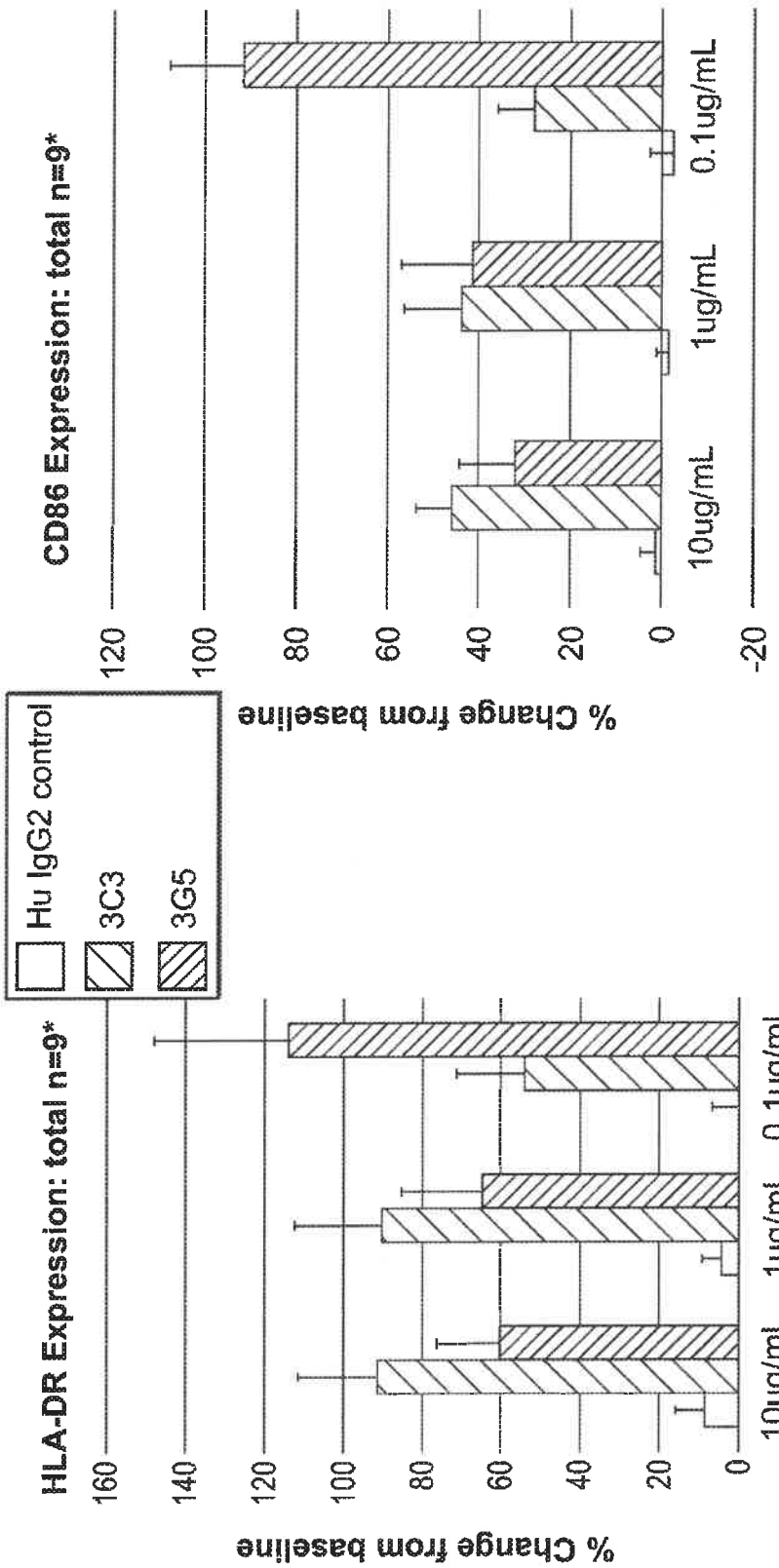

HLA-DR Expression: total n=9*
% Change from baseline
160
140
120
100
80
60
40
20
0
10ug/mL
1ug/mL
0.1ug/mL
Hu IgG2 control
3C3
3G5
CD86 Expression: total n=9*
% Change from baseline
120
100
80
60
40
20
0
-20
10ug/mL
1ug/mL
0.1ug/mL

FIG. 10B

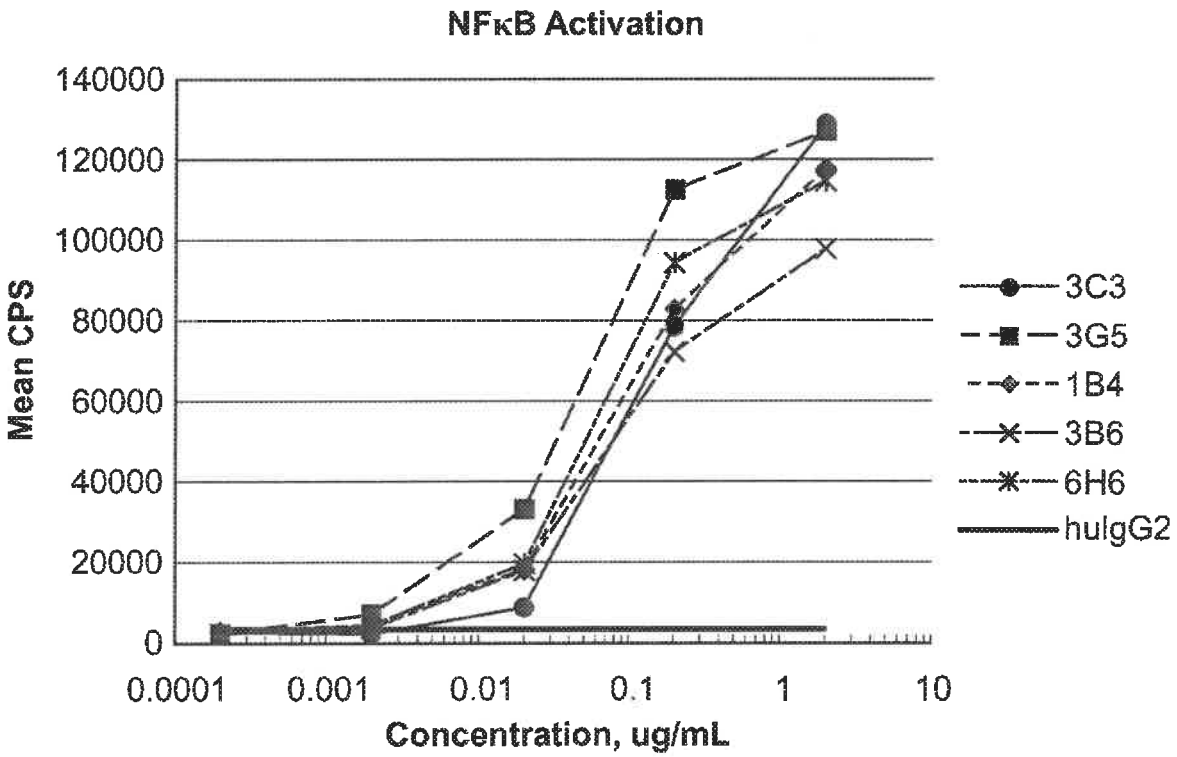

NFκB Activation
Mean CPS
140000
120000
100000
80000
60000
40000
20000
0
0.0001
0.001
0.01
0.1
1
10
Concentration, ug/mL
3C3
3G5
1B4
3B6
6H6
hulgG2

FIG. 11A

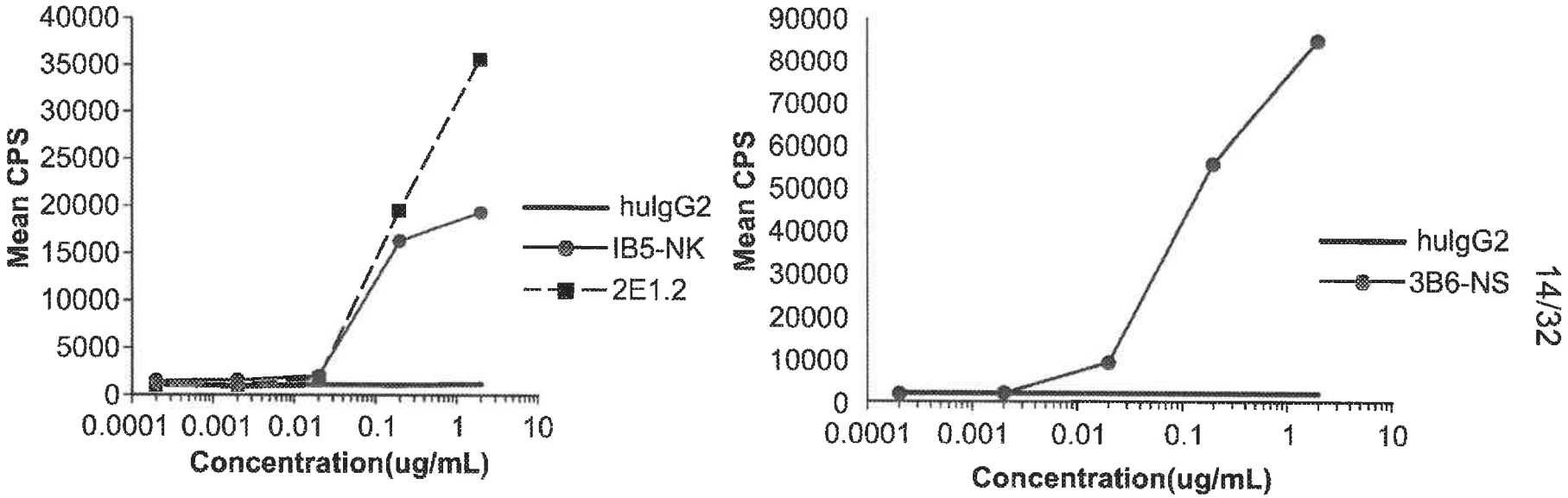

40000
35000
30000
25000
20000
15000
10000
5000
0
Mean CPS
0.0001 0.001 0.01 0.1 1 10
Concentration(ug/mL)
hulgG2
IB5-NK
2E1.2
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
Mean CPS
0.0001 0.001 0.01 0.1 1 10
Concentration(ug/mL)
hulgG2
3B6-NS

FIG. 11B

FIG. 12

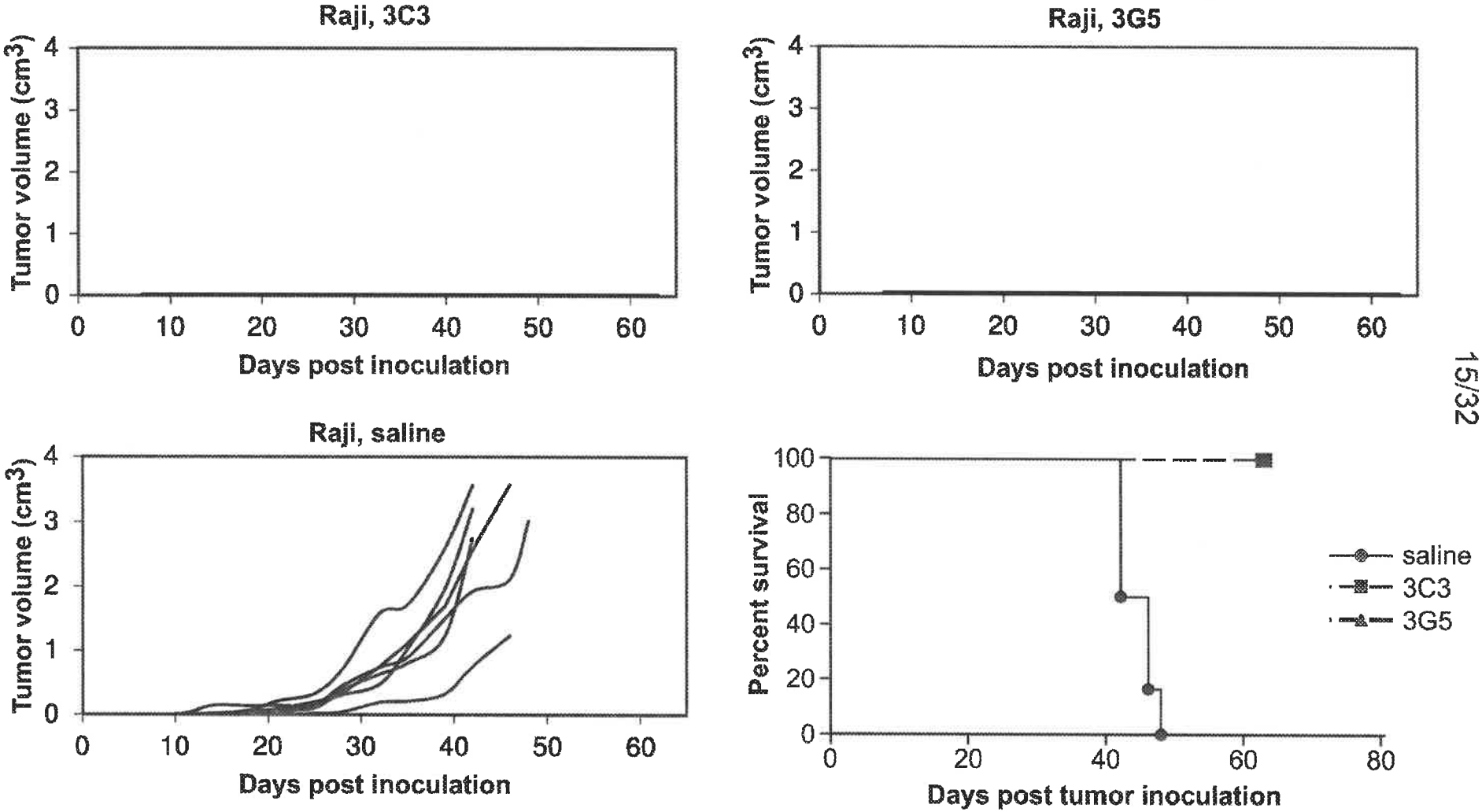
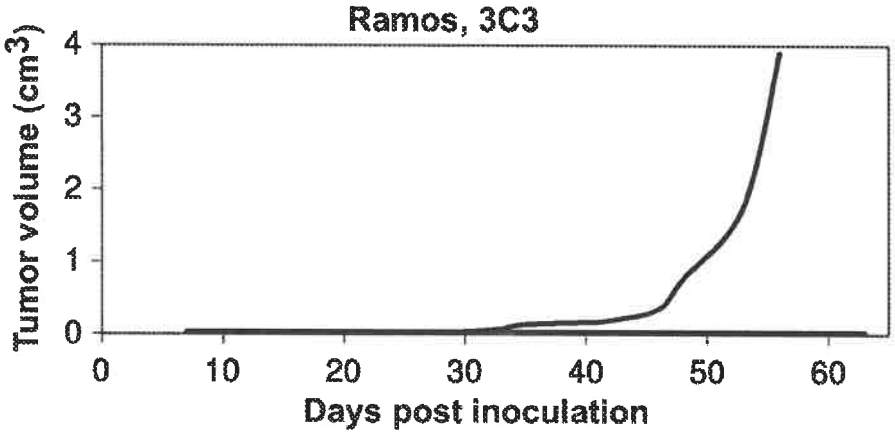
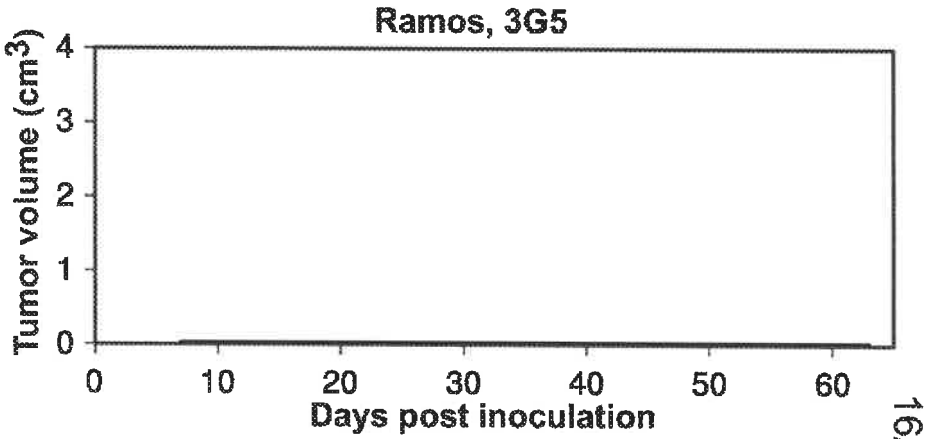
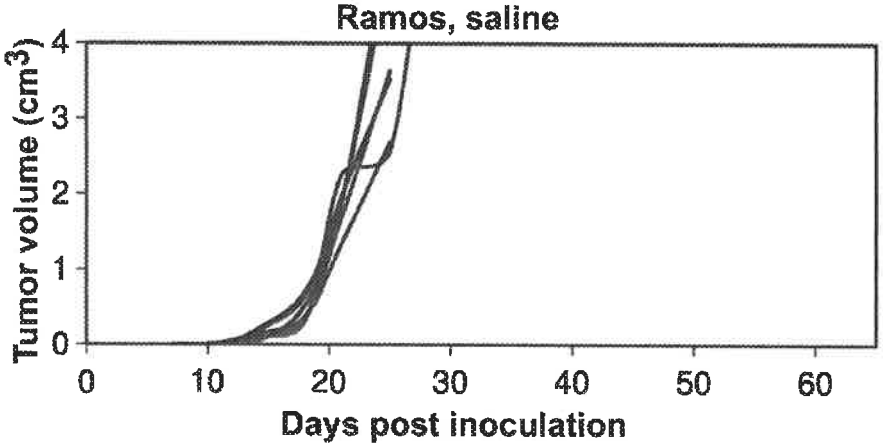
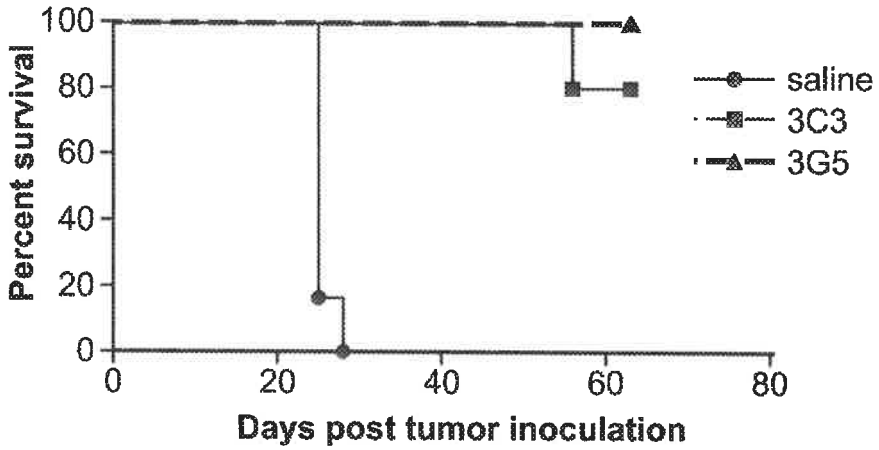

Raji, 3C3
Tumor volume (cm3)
Days post inoculation
Raji, 3G5
Tumor volume (cm3)
Days post inoculation
Raji, saline
Tumor volume (cm3)
Days post inoculation
Percent survival
Days post tumor inoculation
saline
3C3
3G5

FIG. 13


Ramos, 3C3
Tumor volume (cm3)
0
1
2
3
4
0
10
20
30
40
50
60
Days post inoculation


Ramos, 3G5
Tumor volume (cm3)
0
1
2
3
4
0
10
20
30
40
50
60
Days post inoculation


Ramos, saline
Tumor volume (cm3)
0
1
2
3
4
0
10
20
30
40
50
60
Days post inoculation


Percent survival
0
20
40
60
80
100
0
20
40
60
80
Days post tumor inoculation
saline
3C3
3G5

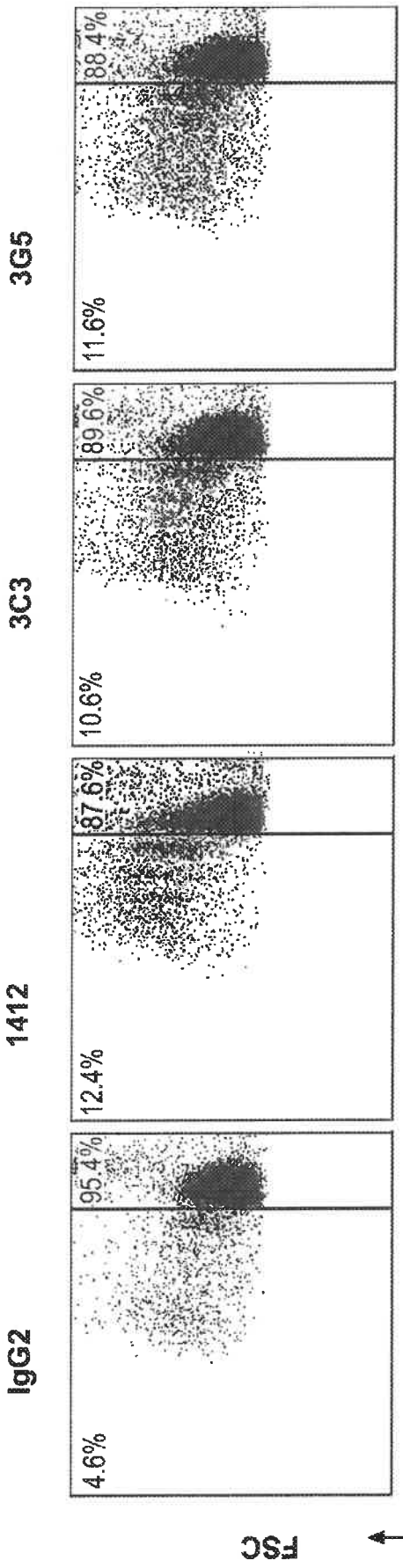


FIG. 14A

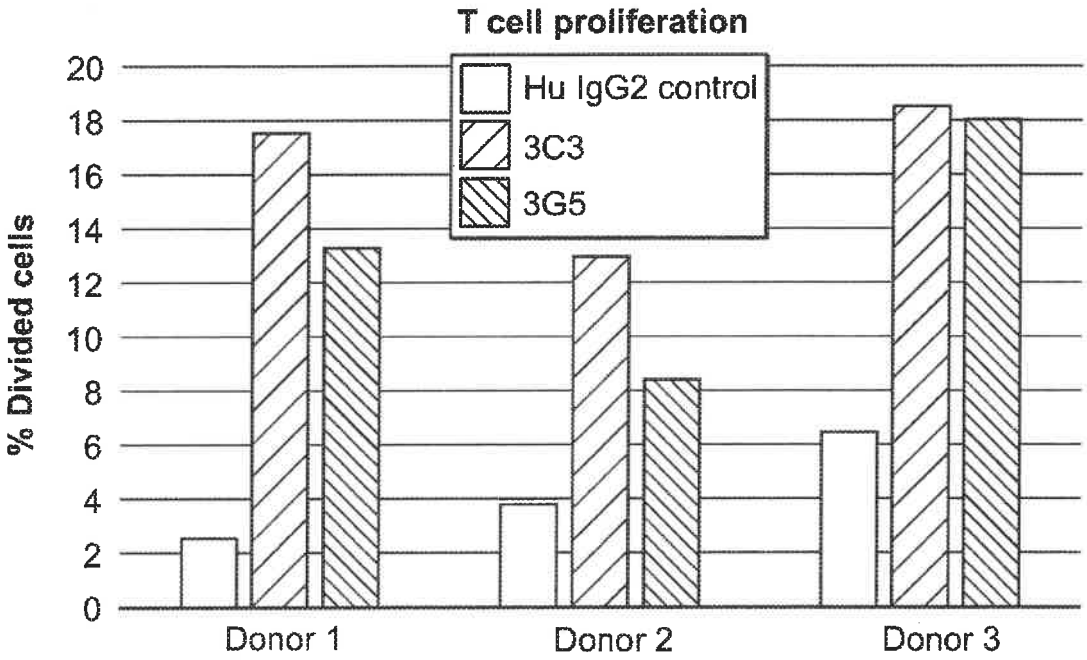


FIG. 14B

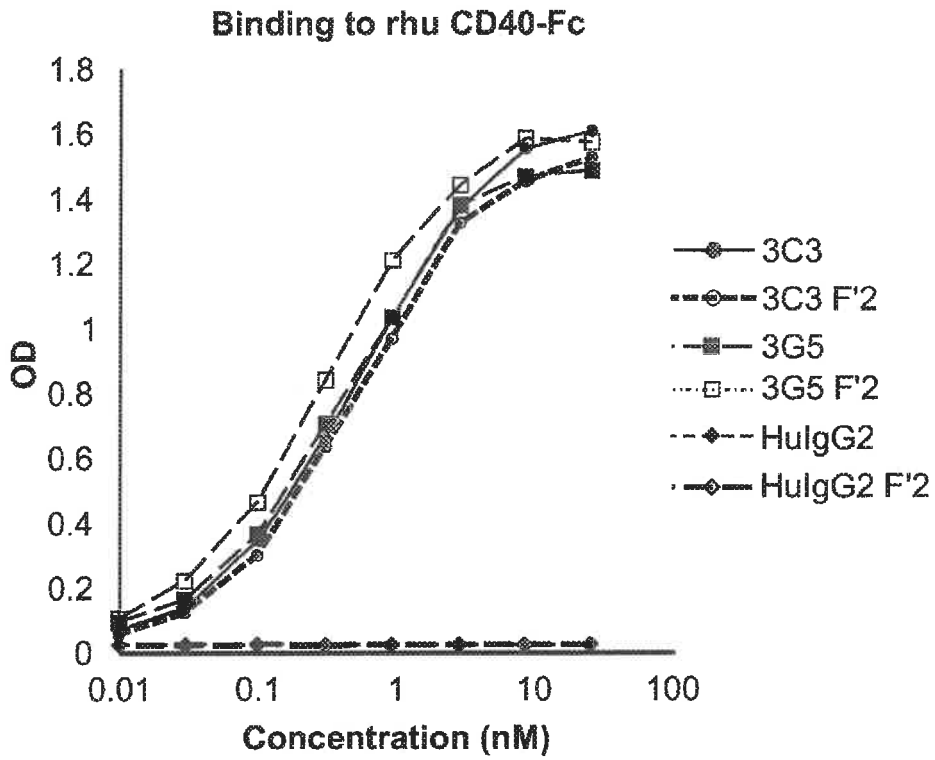


FIG. 15

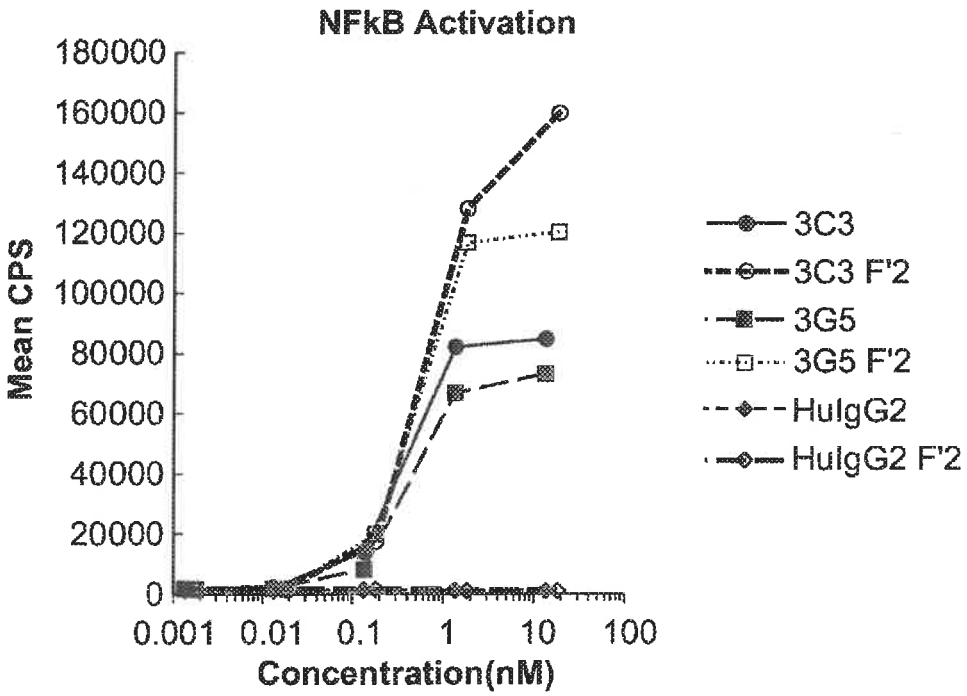

NFkB Activation
Mean CPS
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
0.001
0.01
0.1
1
10
100
Concentration(nM)
3C3
3C3 F'2
3G5
3G5 F'2
HuIgG2
HuIgG2 F'2

FIG. 16

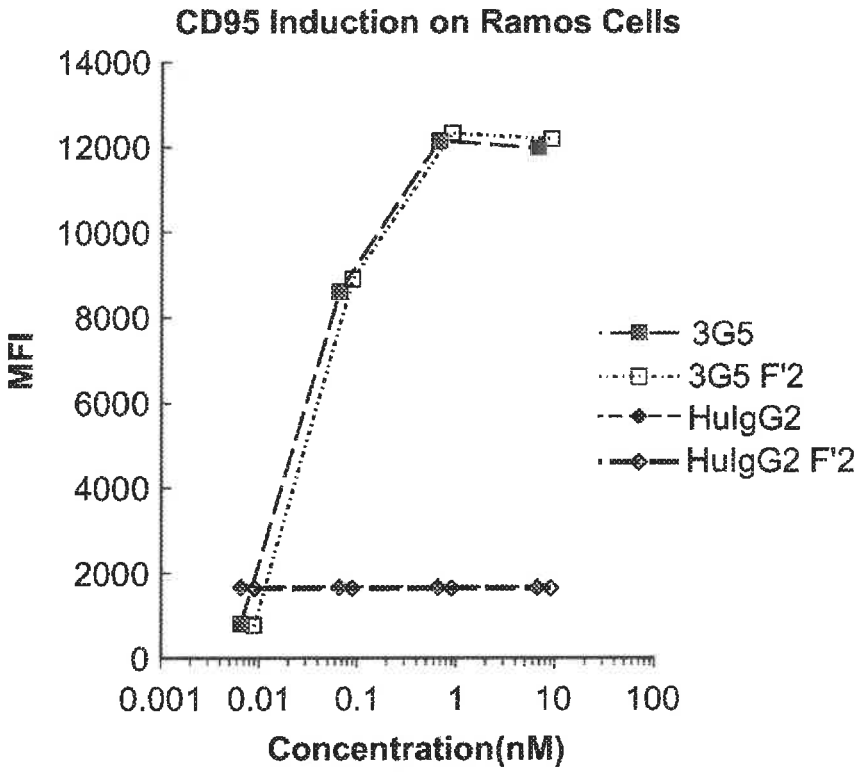

CD95 Induction on Ramos Cells
MFI
14000
12000
10000
8000
6000
4000
2000
0
0.001
0.01
0.1
1
10
100
Concentration(nM)
3G5
3G5 F'2
HuIgG2
HuIgG2 F'2

FIG. 17

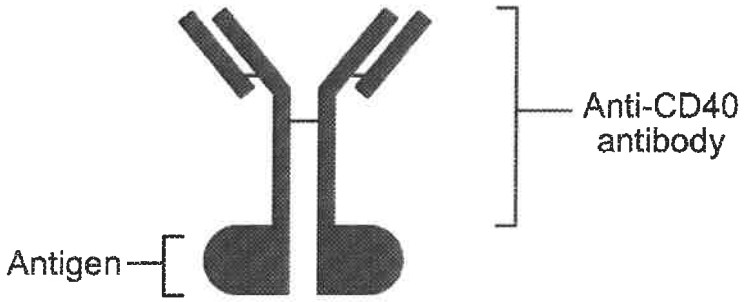

Anti-CD40
antibody
Antigen

FIG. 18

FIG. 19

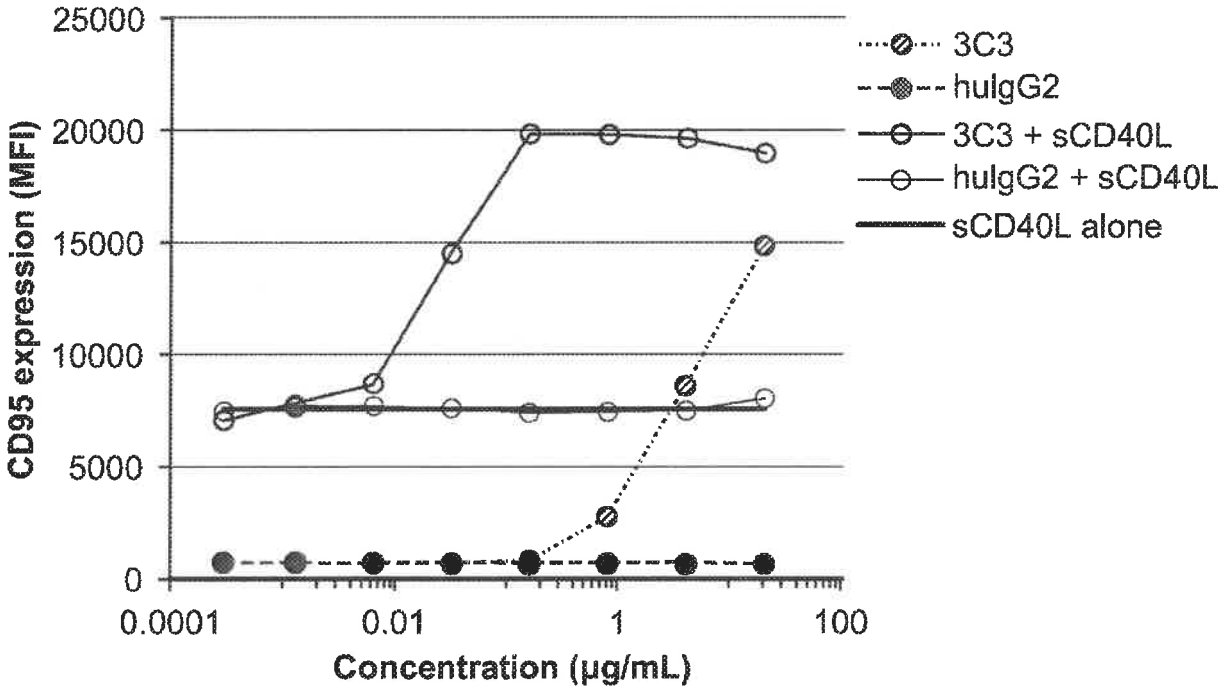

25000
20000
15000
10000
5000
0
CD95 expression (MFI)
0.0001
0.01
1
100
Concentration (µg/mL)
3C3
hulgG2
3C3 + sCD40L
hulgG2 + sCD40L
sCD40L alone

FIG. 20

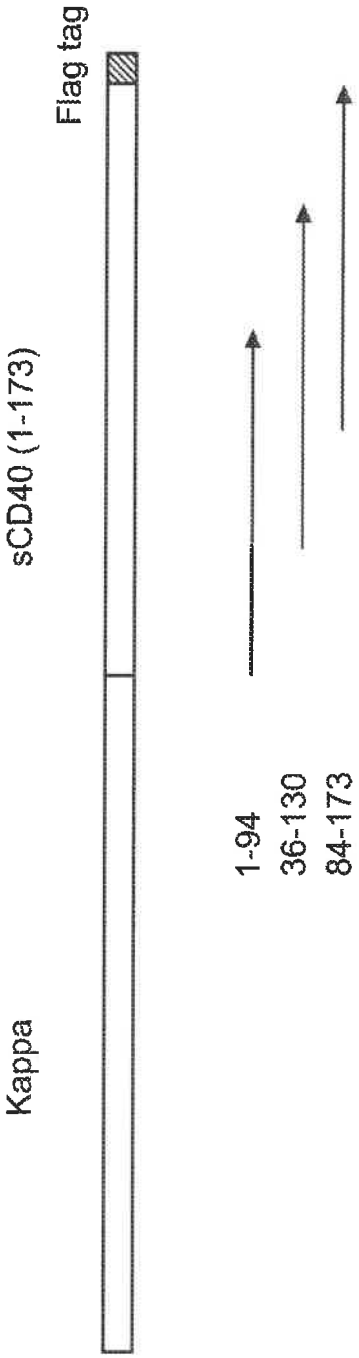
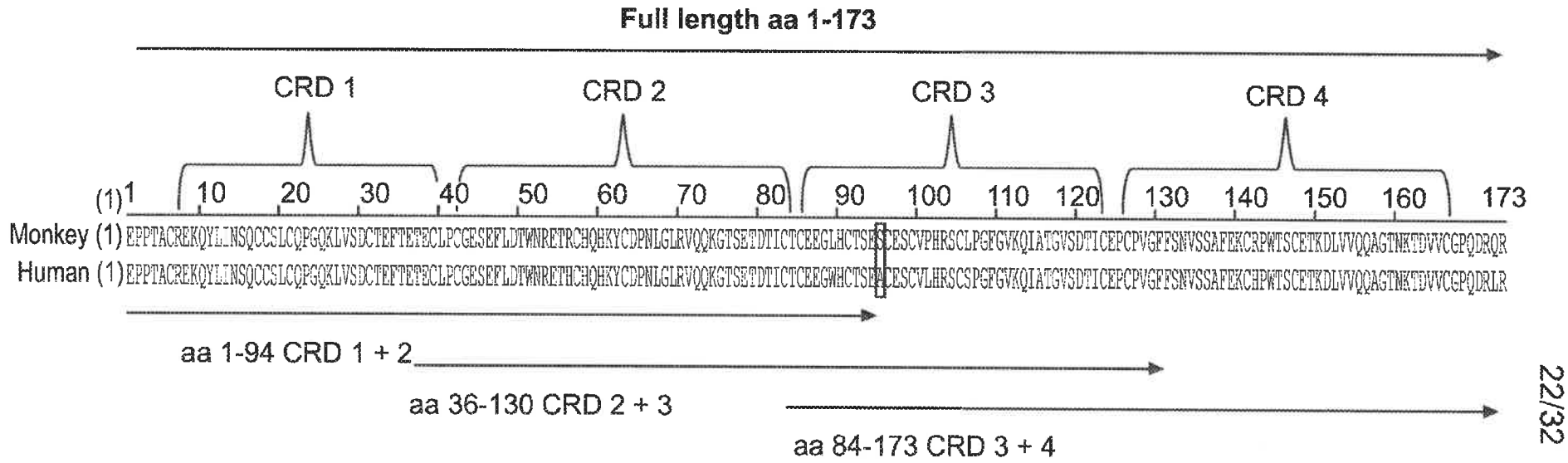


FIG. 21


Full length aa 1-173
CRD 1
CRD 2
CRD 3
CRD 4
(1) 1 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 173
Monkey (1) EPPTACREKQYLINSQCCSLCQPGQKLVSDCTEFTETECLPCGESEFLDTWNRETRCHQHKYCDPNLGLRVQQKGTSETDTICTCEEGLHCTSEDCESCVPHRSCLPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCRPWTSCETKDLVVQQAGTNKTDVVCGPQDRQR
Human (1) EPPTACREKQYLINSQCCSLCQPGQKLVSDCTEFTETECLPCGESEFLDTWNRETHCHQHKYCDPNLGLRVQQKGTSETDTICTCEEGWHCTSEECESCVLHRSCSPGFGVKQIATGVSDTICEPCPVGFFSNVSSAFEKCHPWTSCETKDLVVQQAGTNKTDVVCGPQDRLR
aa 1-94 CRD 1 + 2
aa 36-130 CRD 2 + 3
aa 84-173 CRD 3 + 4

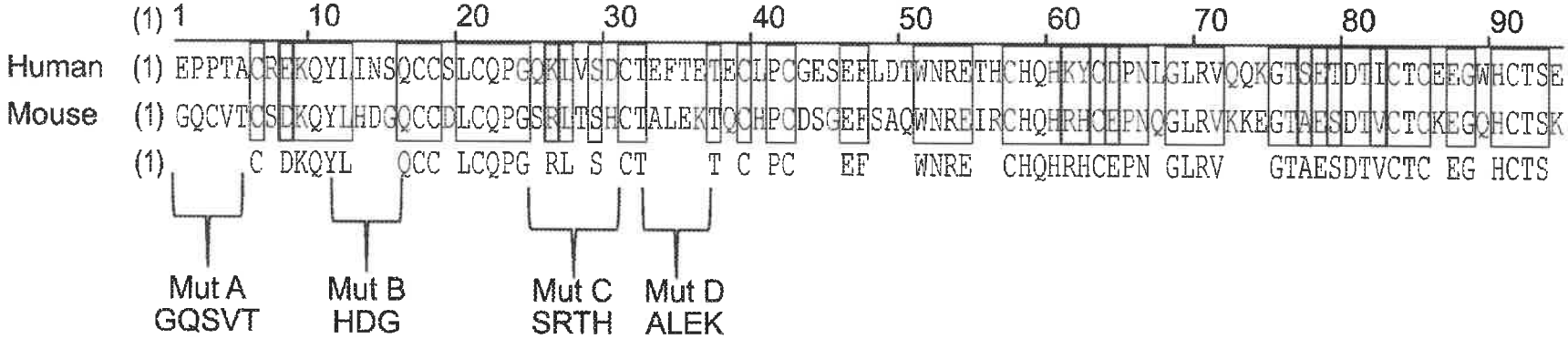
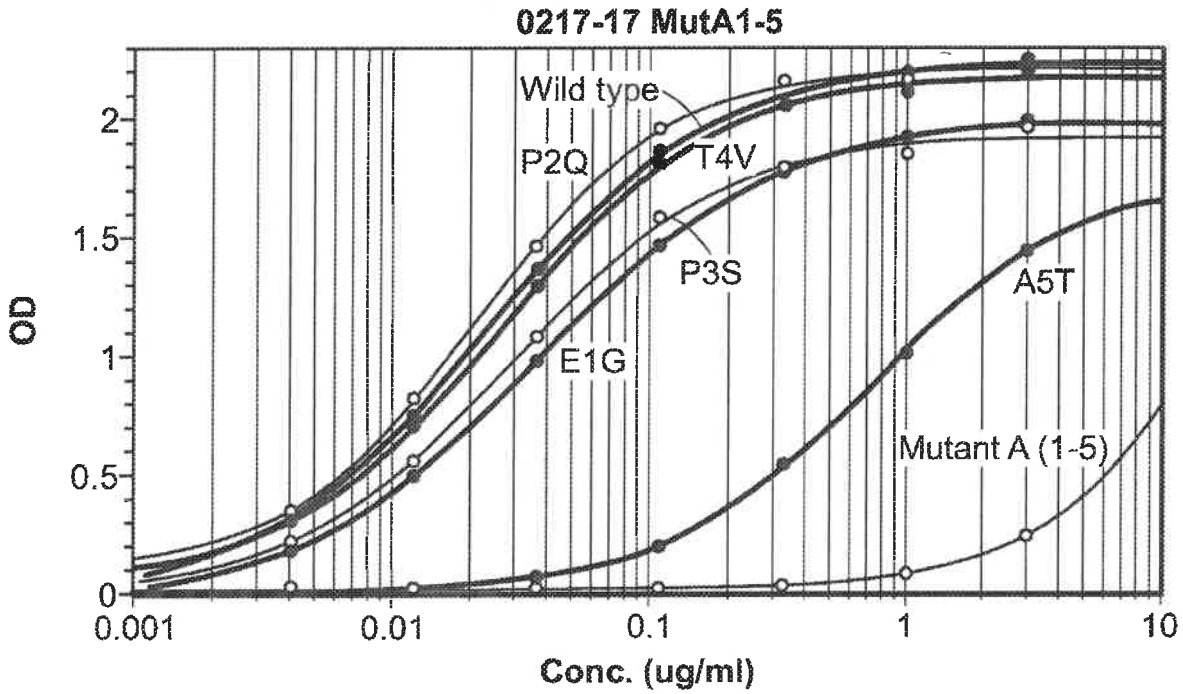

(1) 1 10 20 30 40 50 60 70 80 90
Human (1) EPPTACREKQYLINSQCCSLCQPGQKLVSDCTEFTETECLPCGESEFLDTWNRETHCHQHKYCDPNLGLRVQQKGTSETDTICTCEEGWHCTSE
Mouse (1) GQCVTCSDKQYLHDGQCCDLCQPGSRLTSHCTALEKTQCHPCDSGEFSAQWNREIRCHQHRHCEPNQGLRVKKEGTAESDTVCTCKEGQHCTSK
(1) C DKQYL QCC LCQPG RL S CT T C PC EF WNRE CHQHRHCEPN GLRV GTAESDTVCTC EG HCTS
Mut A GQSVT
Mut B HDG
Mut C SRTH
Mut D ALEK

FIG. 22


0217-17 MutA1-5
Wild type
P2Q
T4V
P3S
A5T
E1G
Mutant A (1-5)
OD
0
0.5
1
1.5
2
0.001
0.01
0.1
1
10
Conc. (ug/ml)

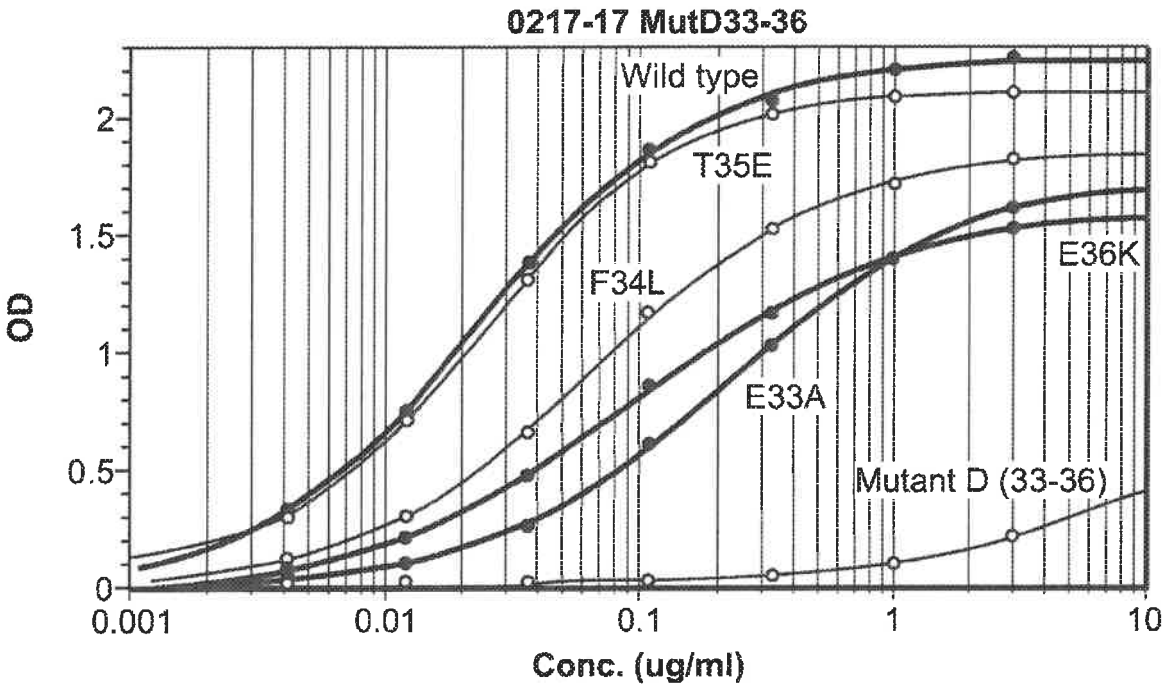

0217-17 MutD33-36
Wild type
T35E
F34L
E36K
E33A
Mutant D (33-36)
OD
0
0.5
1
1.5
2
0.001
0.01
0.1
1
10
Conc. (ug/ml)

FIG. 23A

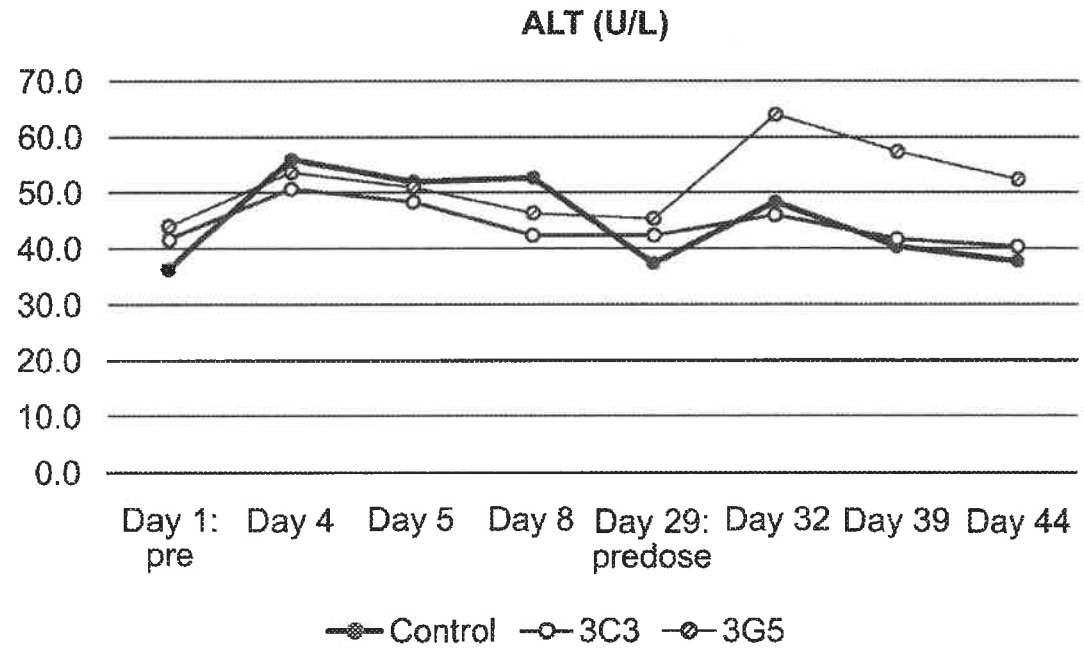


FIG. 23B

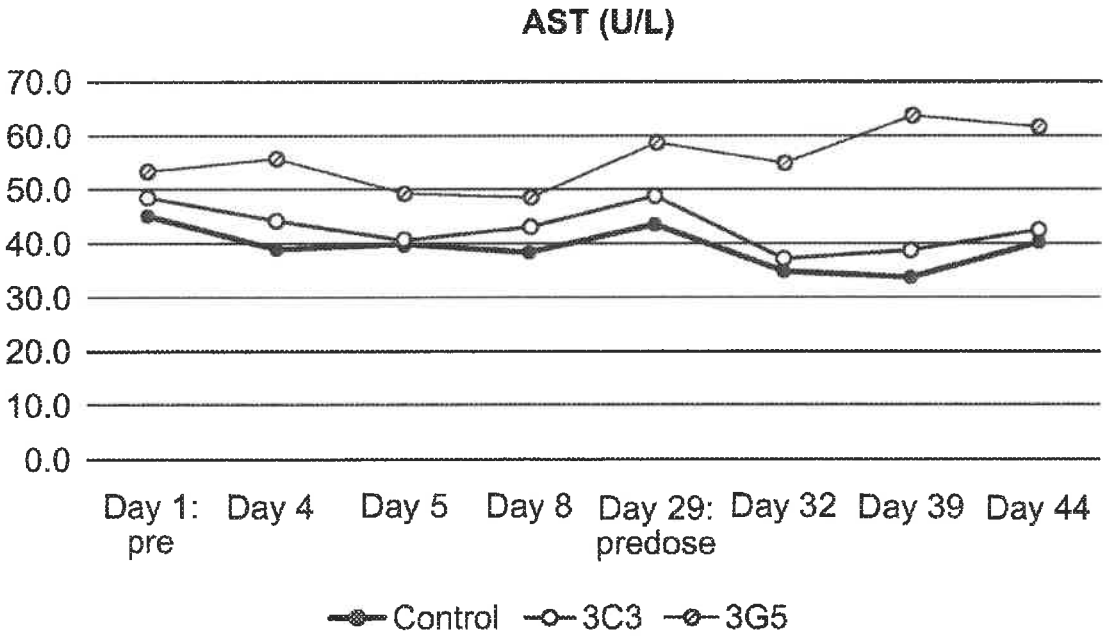


FIG. 23C

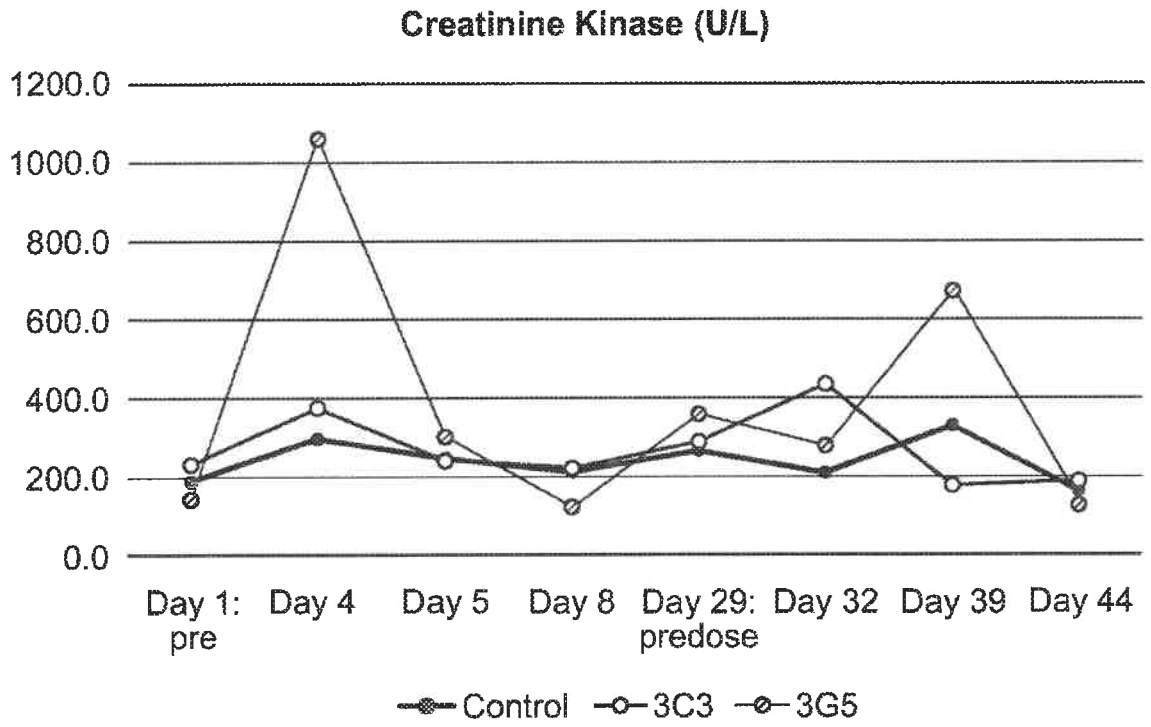

Creatinine Kinase (U/L)
1200.0
1000.0
800.0
600.0
400.0
200.0
0.0
Day 1: pre
Day 4
Day 5
Day 8
Day 29: predose
Day 32
Day 39
Day 44
Control
3C3
3G5

FIG. 24

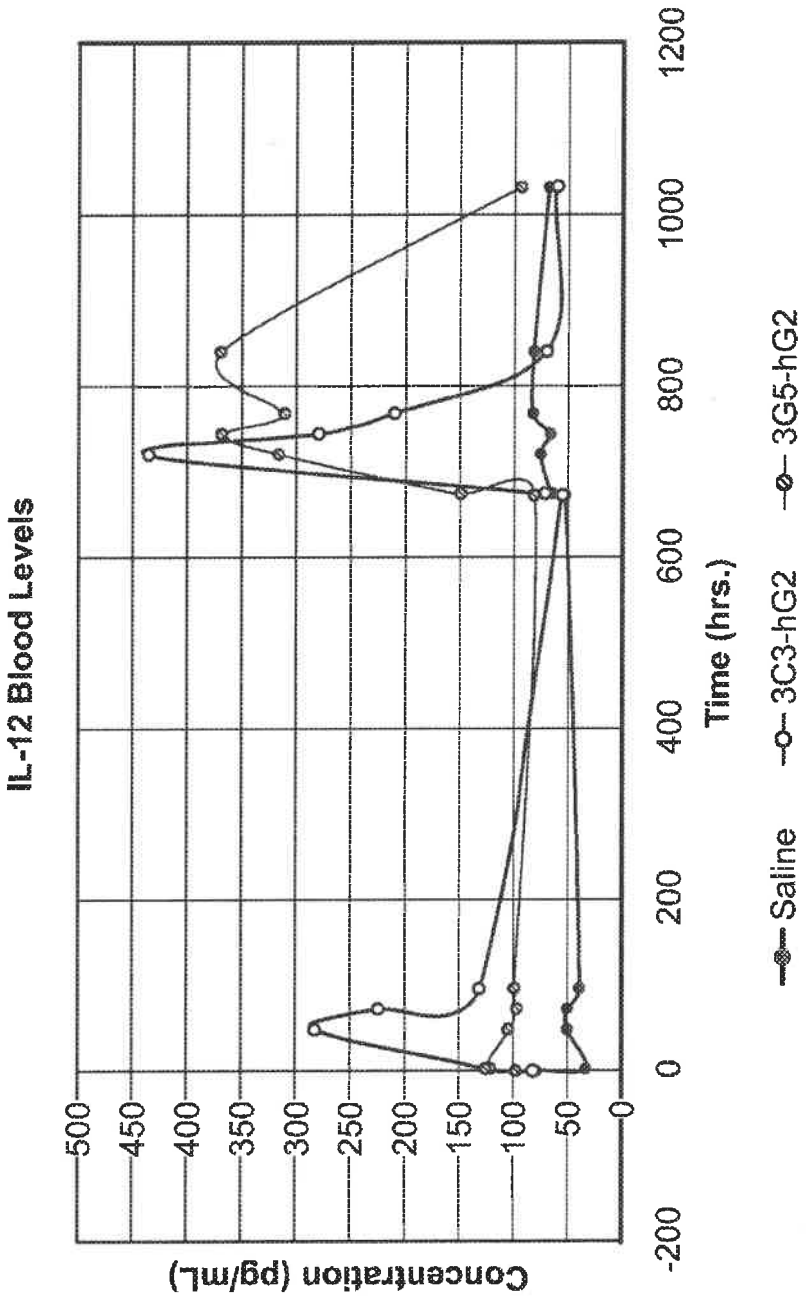

IL-12 Blood Levels
Concentration (pg/mL)
500
450
400
350
300
250
200
150
100
50
0
-200
0
200
400
600
800
1000
1200
Time (hrs.)
Saline
3C3-hG2
3G5-hG2

FIG. 25A

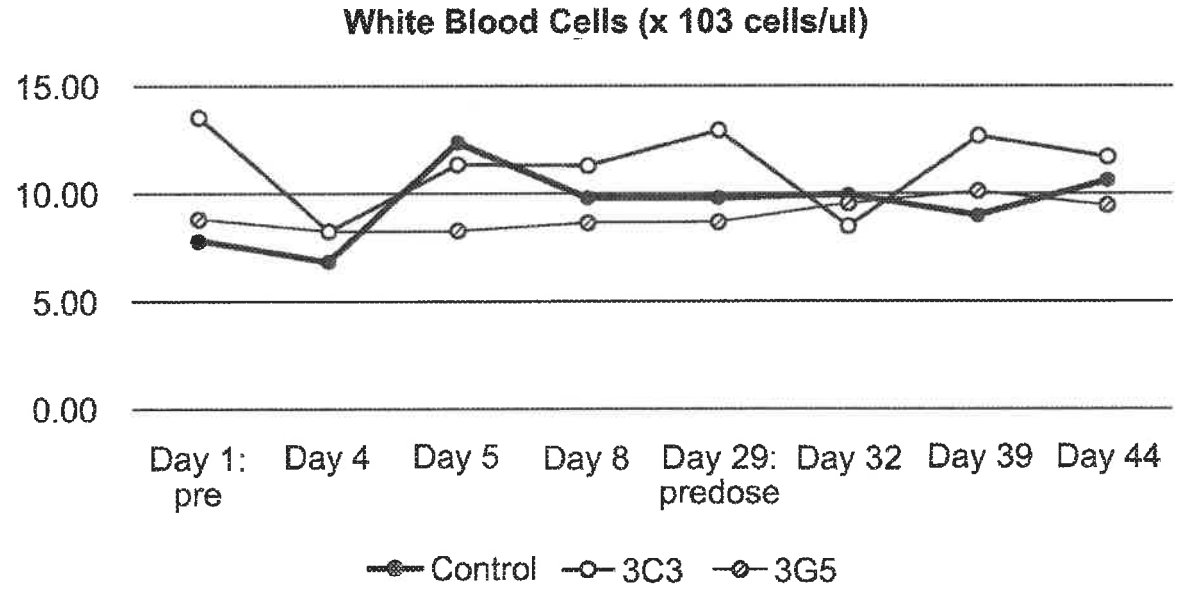


FIG. 25B

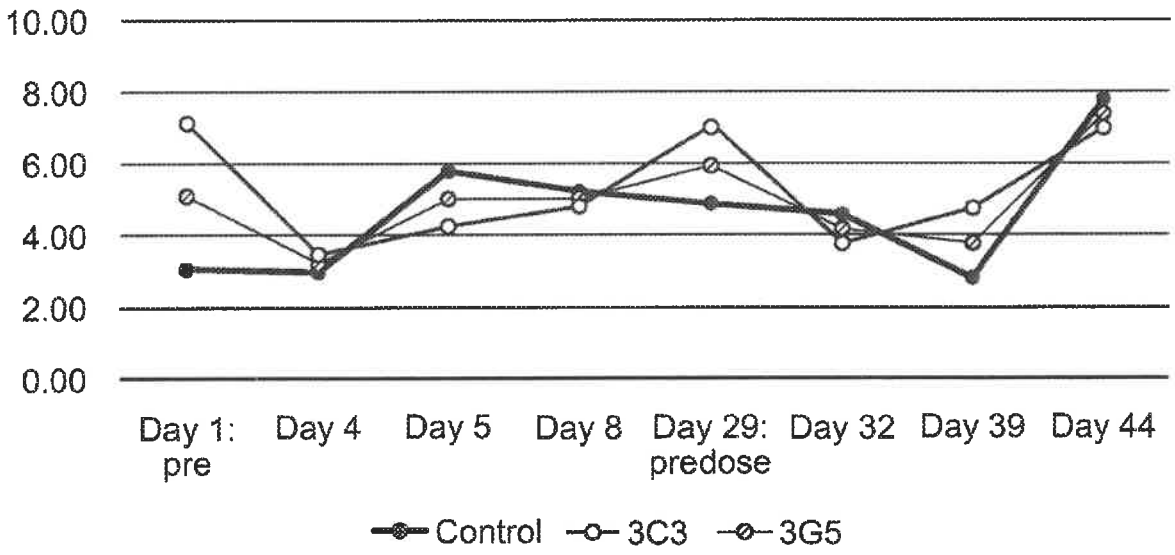


FIG. 25C

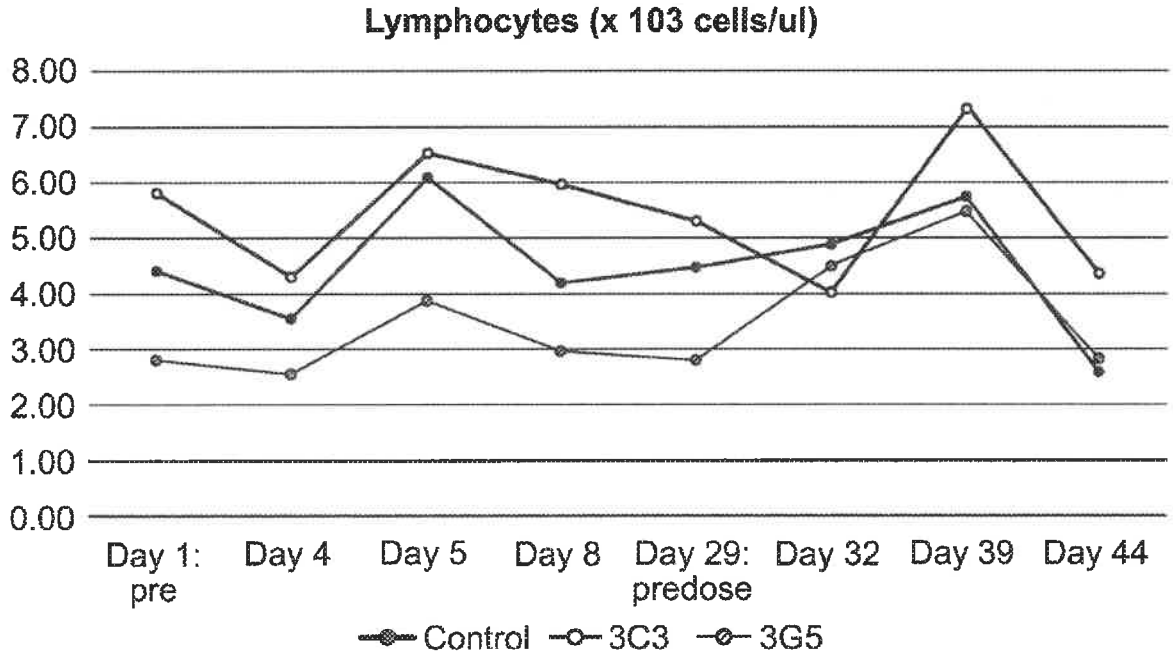


FIG. 26

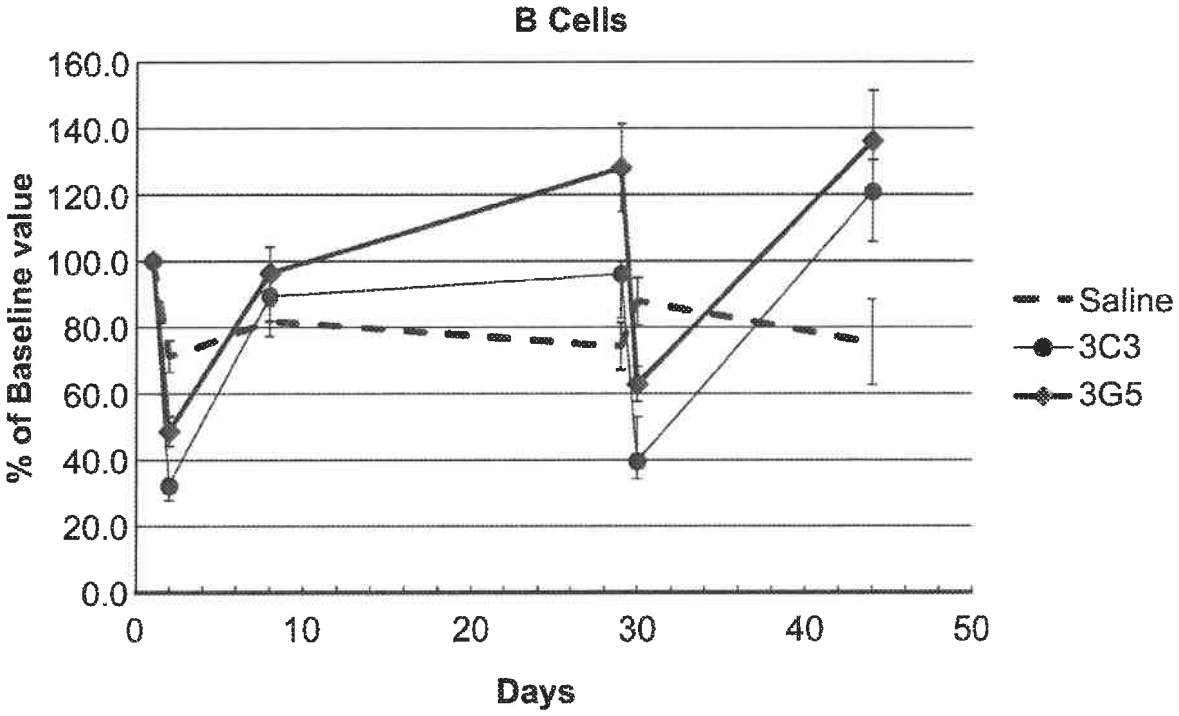
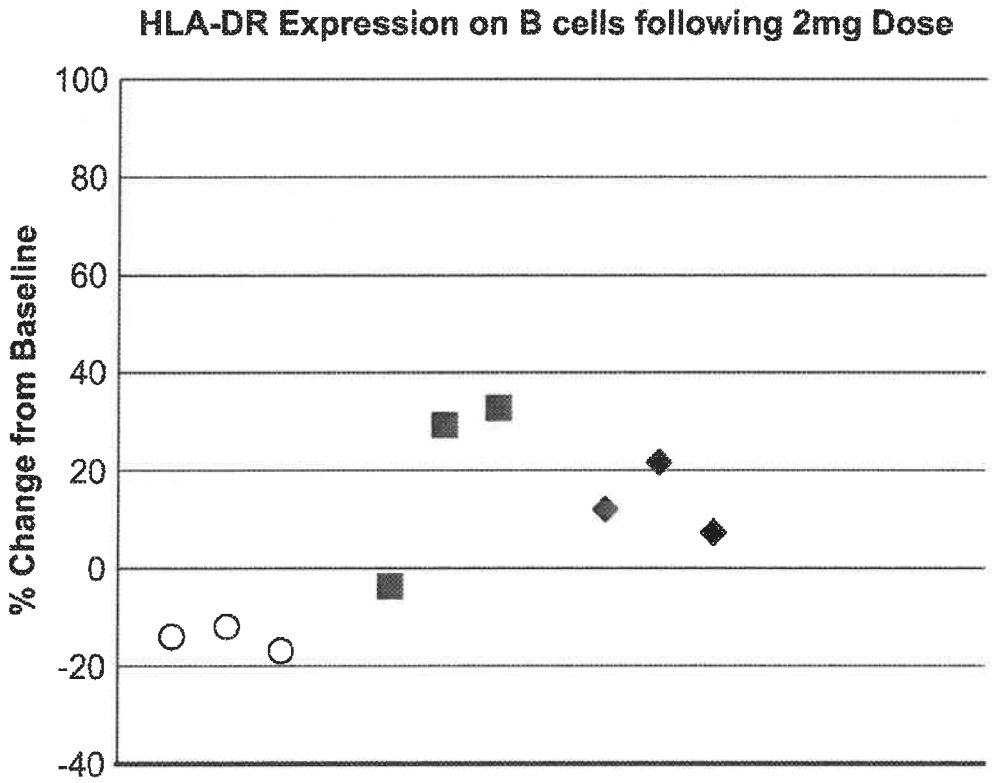
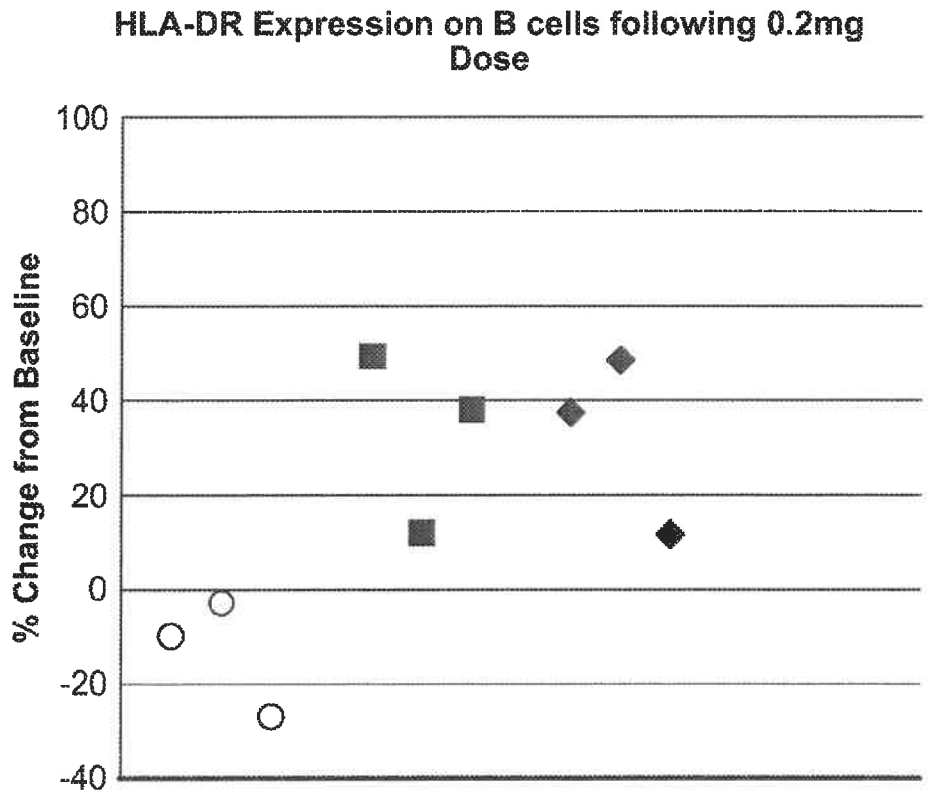


FIG. 27


HLA-DR Expression on B cells following 2mg Dose
% Change from Baseline
100
80
60
40
20
0
-20
-40


HLA-DR Expression on B cells following 0.2mg Dose
% Change from Baseline
100
80
60
40
20
0
-20
-40

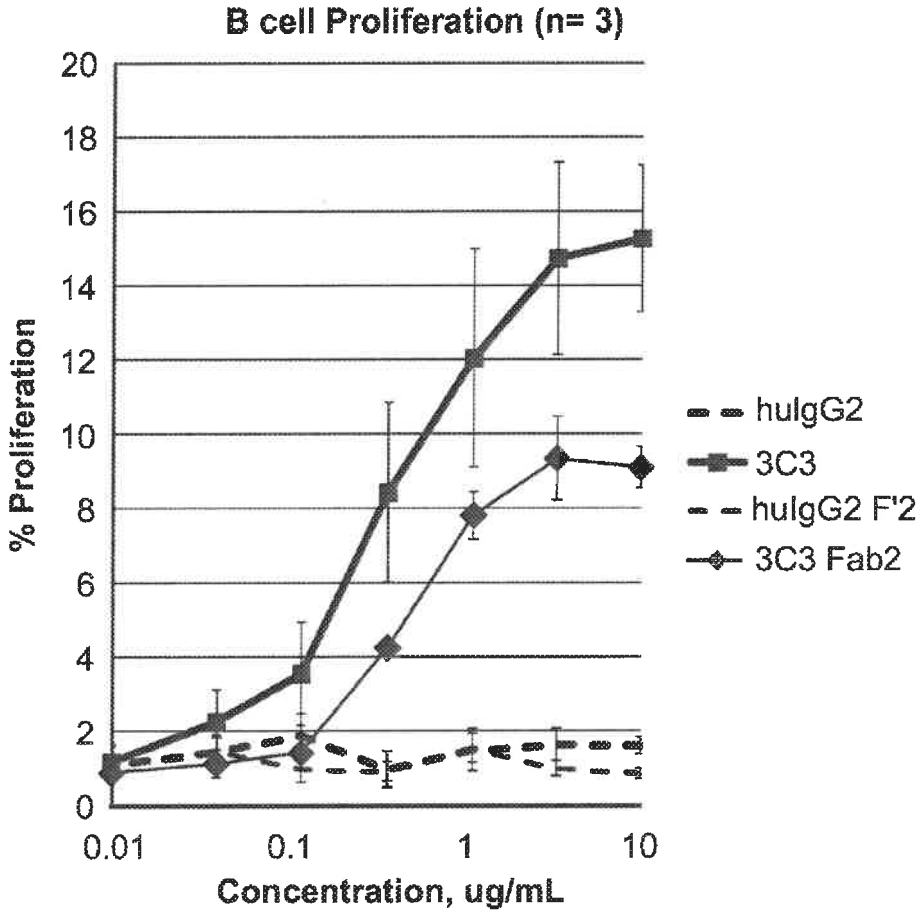

B cell Proliferation (n= 3)
% Proliferation
20
18
16
14
12
10
8
6
4
2
0
0.01
0.1
1
10
Concentration, ug/mL
hulgG2
3C3
hulgG2 F'2
3C3 Fab2

FIG. 28

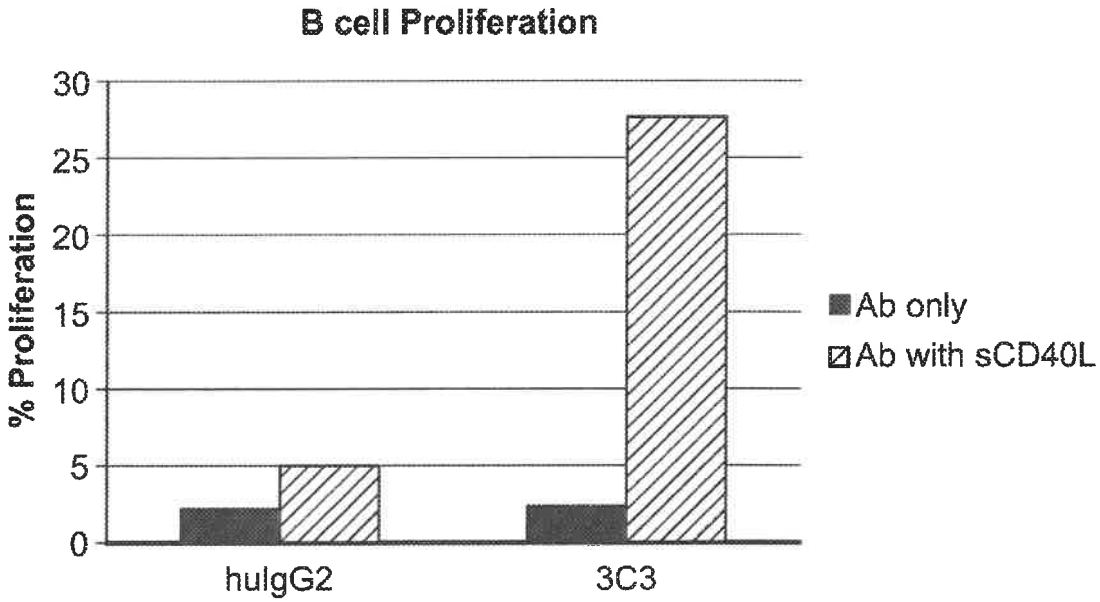

B cell Proliferation
% Proliferation
30
25
20
15
10
5
0
hulgG2
3C3
Ab only
Ab with sCD40L

FIG. 29

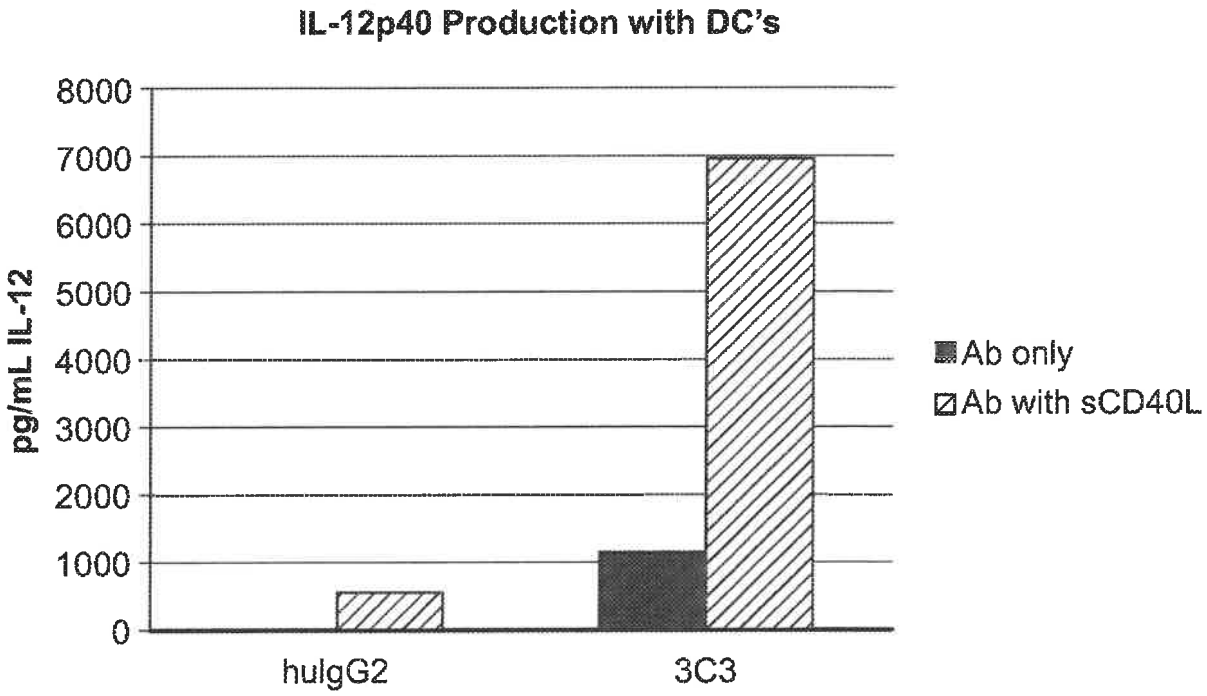

IL-12p40 Production with DC's
pg/mL IL-12
8000
7000
6000
5000
4000
3000
2000
1000
0
hulgG2
3C3
Ab only
Ab with sCD40L

FIG. 30

Cytokine response in whole blood assay

	IL-1β				
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
huIgG2 control	0.2	1.4	1.6	13	4.5
LPS	682	697	885	882	858
3C3	0.1	1.3	1	11.3	5.6
	IL-6				
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
huIgG2 control	2.1	1	12.3	1.3	2.9
LPS	12.5	12.6	11.6	11.7	16
3C3	1.9	0.9	11	1.2	2.5
	TNFα				
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
huIgG2 control	0.6	0.7	1.4	1	1.3
LPS	45.2	43.9	48.7	45.6	27.5
3C3	0.7	1.2	1.5	1.1	1.6
	IFNγ				
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5
huIgG2 control	BD	BD	BD	BD	0.5
LPS	BD	BD	BD	49.8	BD
3C3	BD	BD	BD	BD	1.3

FIG. 31