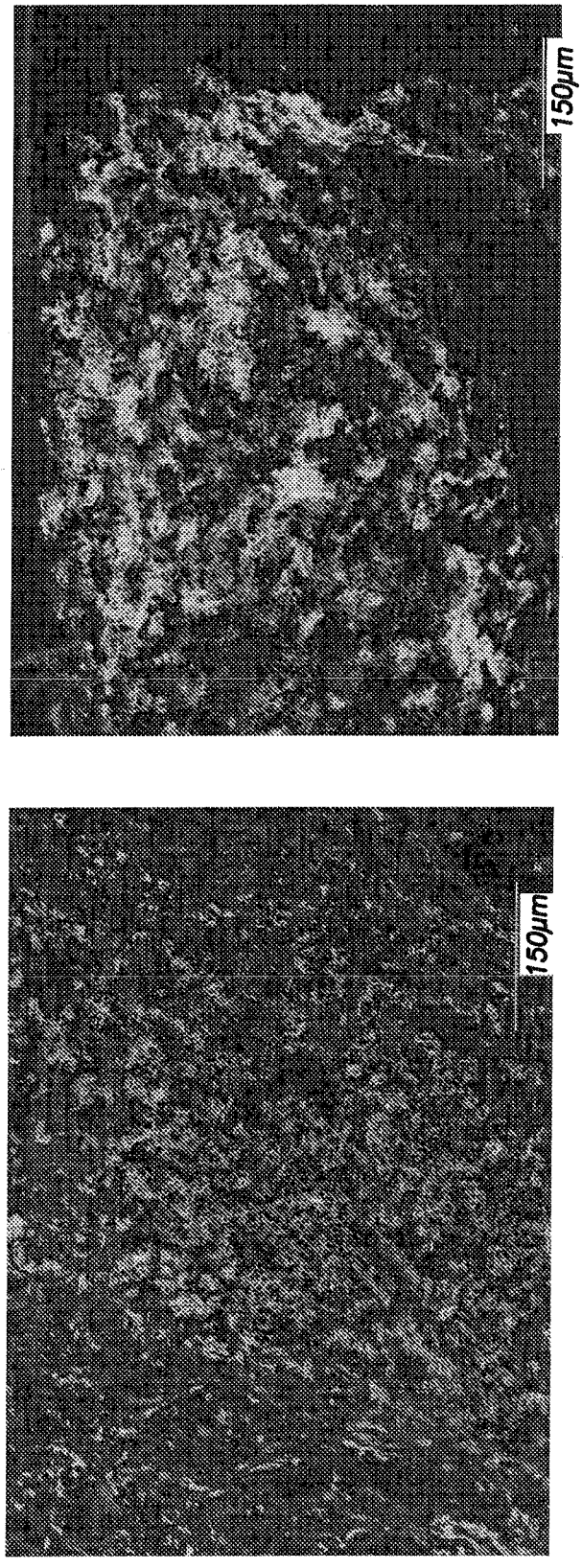


FIG. 1

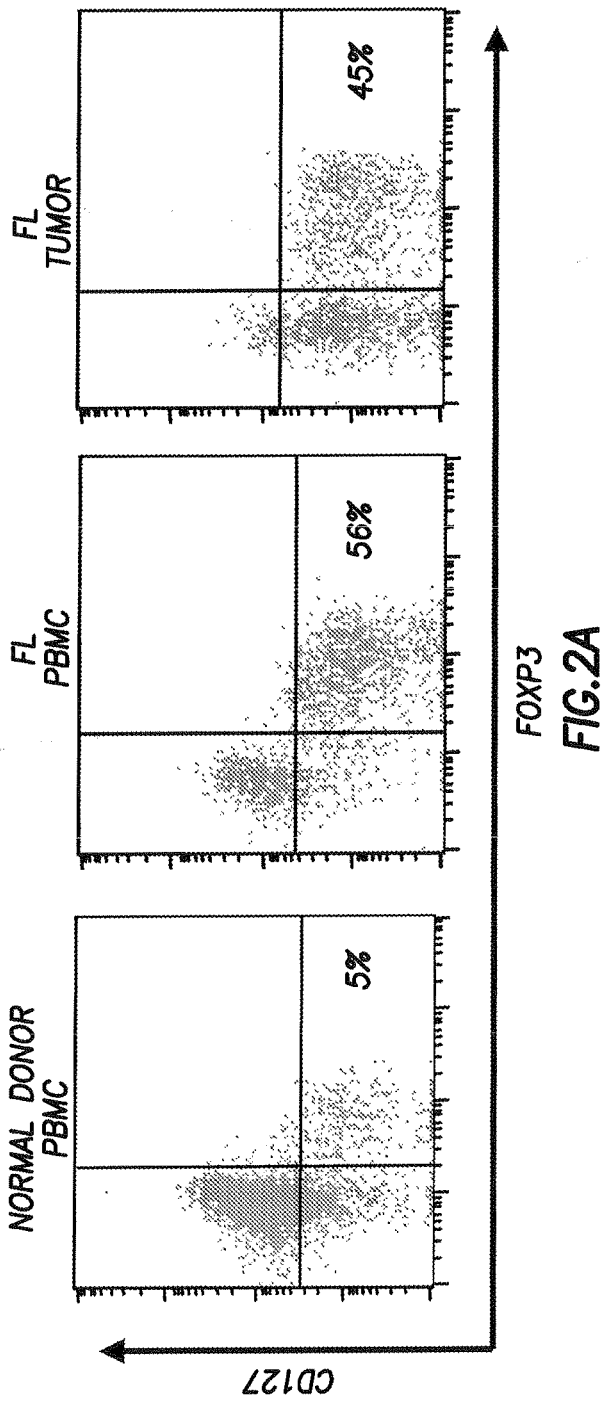

NORMAL DONOR PBMC
5%
FL PBMC
56%
FL TUMOR
45%
CD127
FOXP3

FIG.2A

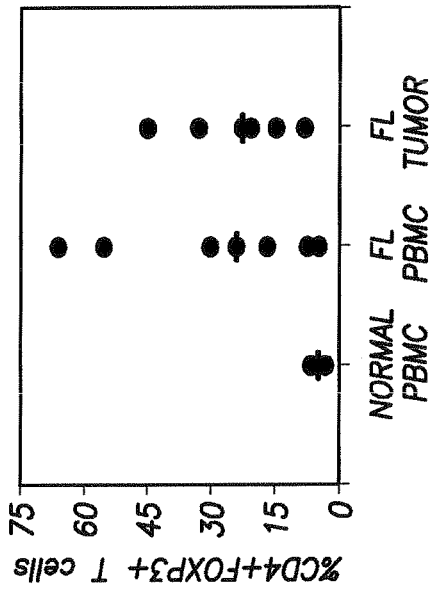

%CD4+FOXP3+ T cells
75
60
45
30
15
0
NORMAL PBMC
FL PBMC
FL TUMOR

FIG.2B

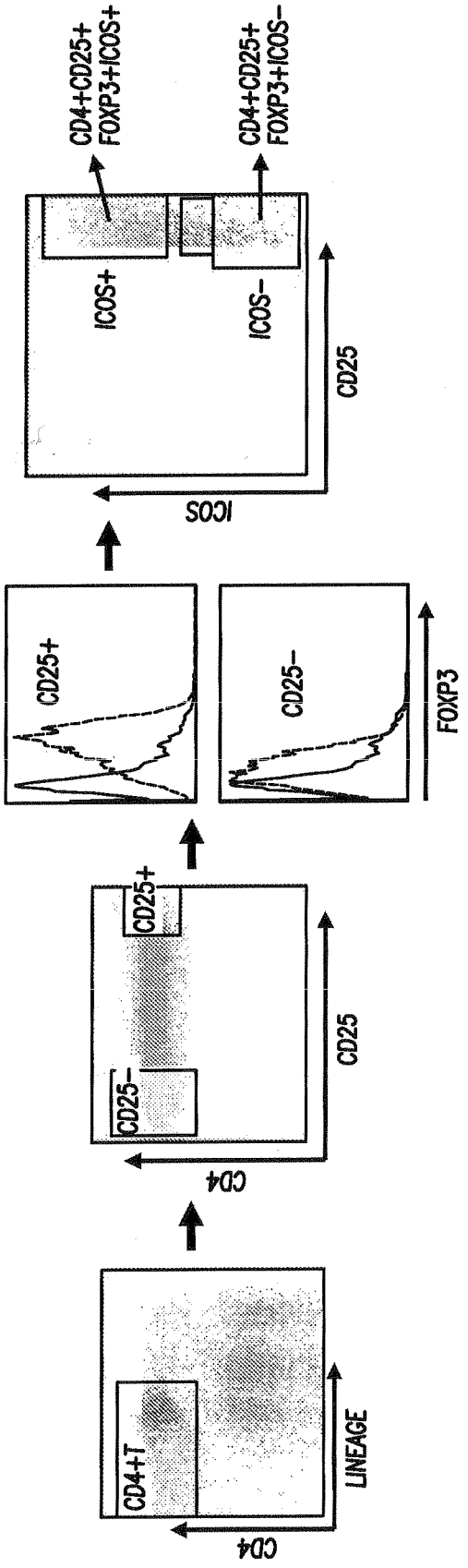

CD4+CD25+
FOXP3+ICOS+
CD4+CD25+
FOXP3+ICOS−
ICOS+
ICOS−
CD25
ICOS
CD25+
CD25−
FOXP3
CD25+
CD25−
CD25
CD4
CD4+T
LINEAGE
CD4

FIG.3

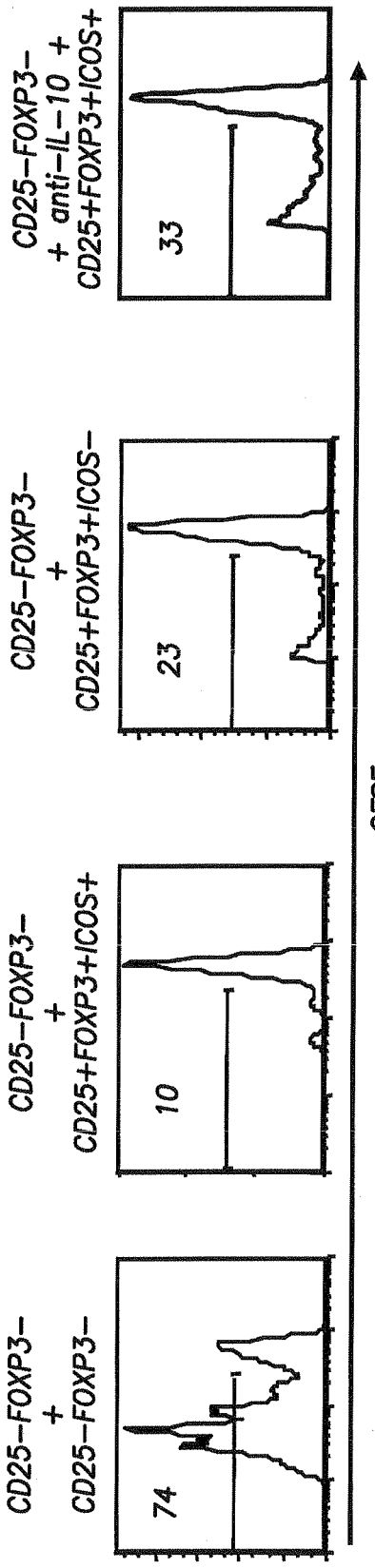

CD25−FOXP3−
+
CD25−FOXP3−
74
CD25−FOXP3−
+
CD25+FOXP3+ICOS+
10
CD25−FOXP3−
+
CD25+FOXP3+ICOS−
23
CD25−FOXP3−
+ anti−IL−10 +
CD25+FOXP3+ICOS+
33
CFSE

FIG.4

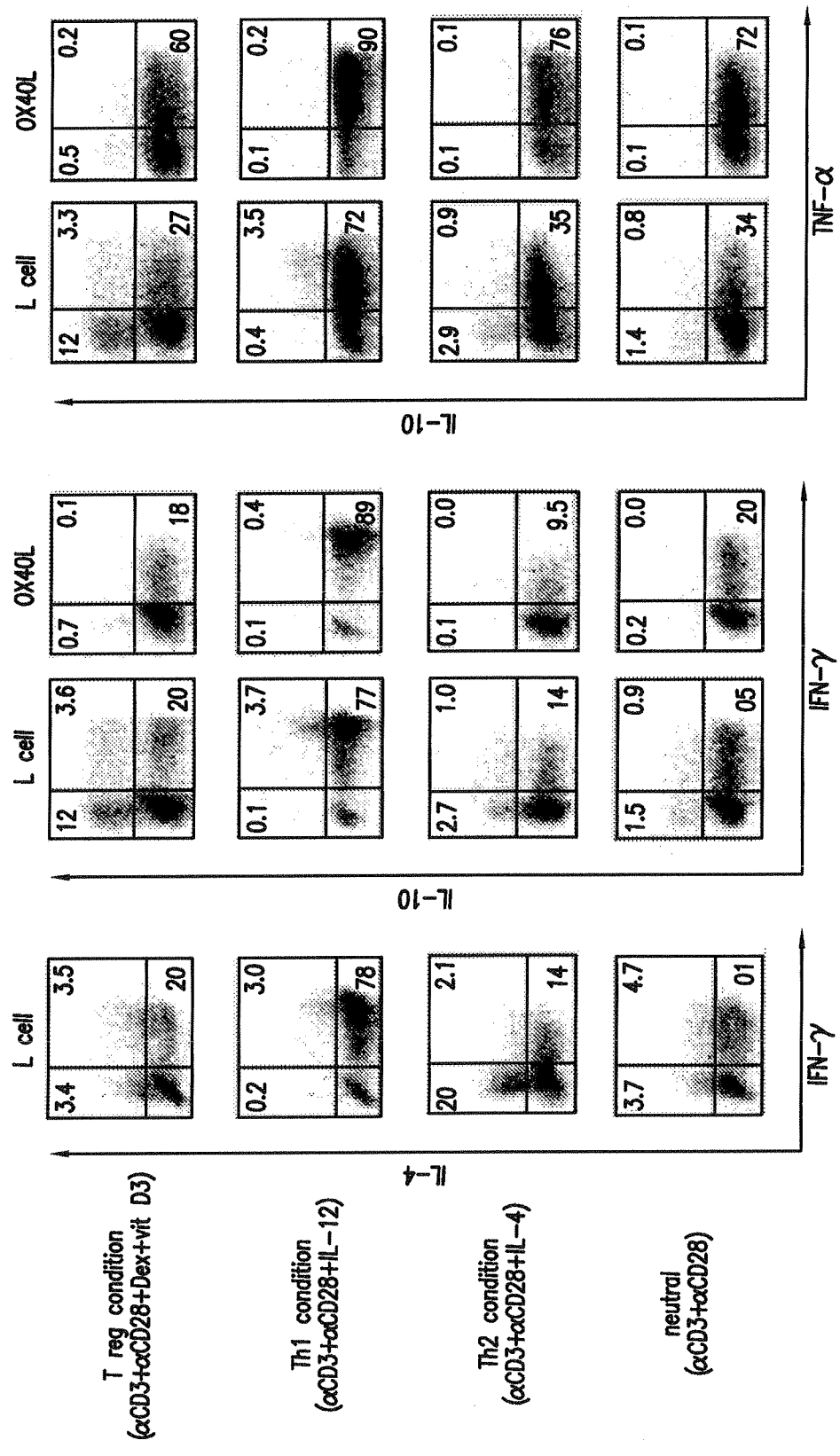

T reg condition (αCD3+αCD28+Dex+vit D3)
Th1 condition (αCD3+αCD28+IL-12)
Th2 condition (αCD3+αCD28+IL-4)
neutral (αCD3+αCD28)
IL-4
IFN-γ
IL-10
IFN-γ
IL-10
TNF-α
L cell
OX40L

FIG.5A

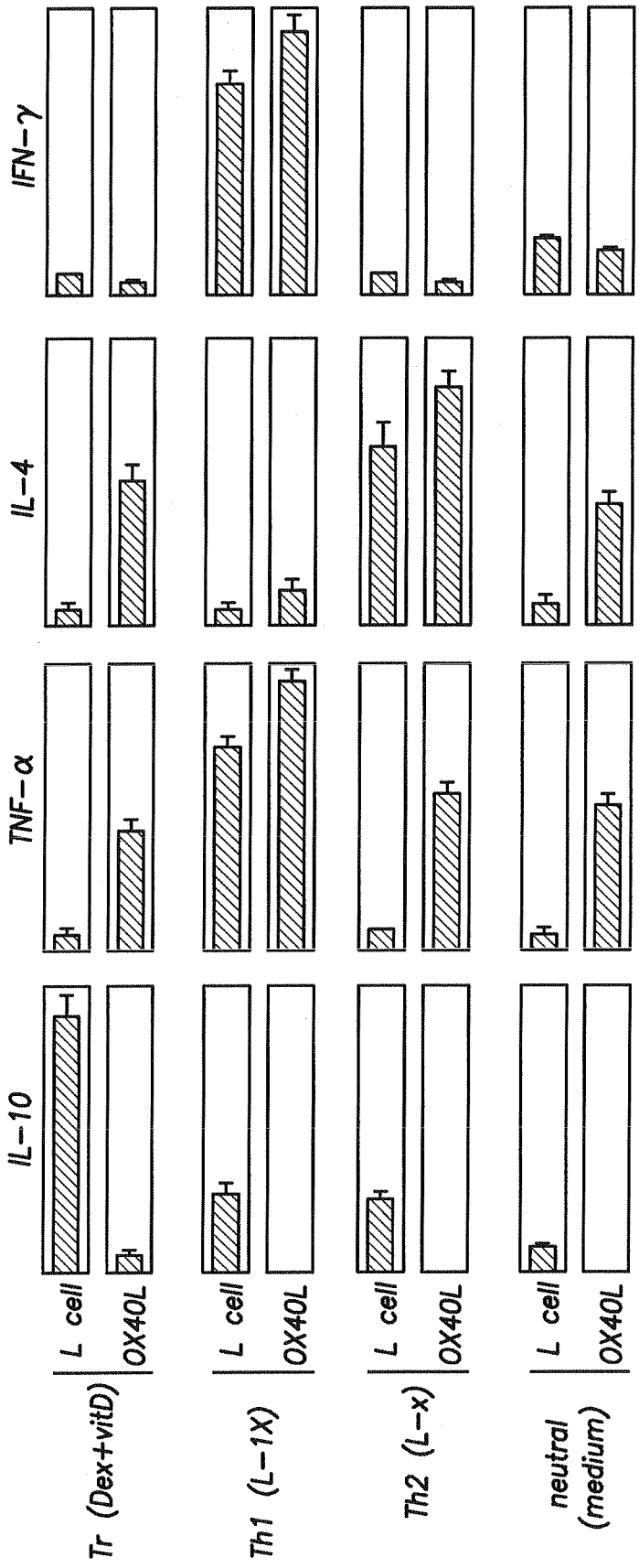

IL-10
TNF-α
IL-4
IFN-γ
L cell
OX40L
Tr (Dex+vitD)
Th1 (L-1X)
Th2 (L-x)
neutral (medium)

FIG.5B

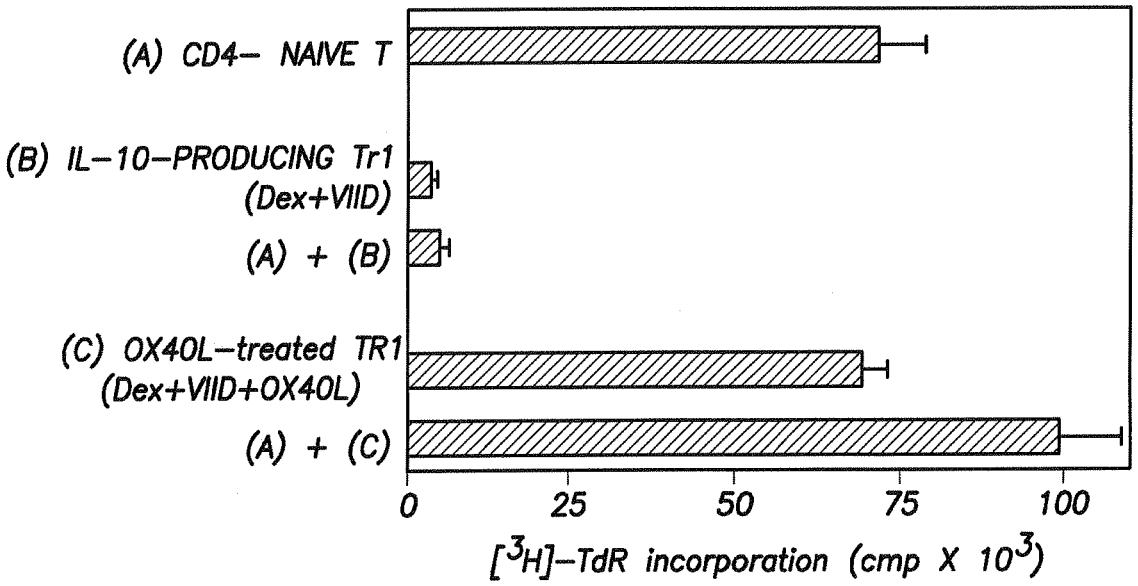

(A) CD4– NAIVE T
(B) IL–10–PRODUCING Tr1 (Dex+VIID)
(A) + (B)
(C) OX40L–treated TR1 (Dex+VIID+OX40L)
(A) + (C)
0
25
50
75
100
[3H]–TdR incorporation (cmp X 10^3)

FIG.5C

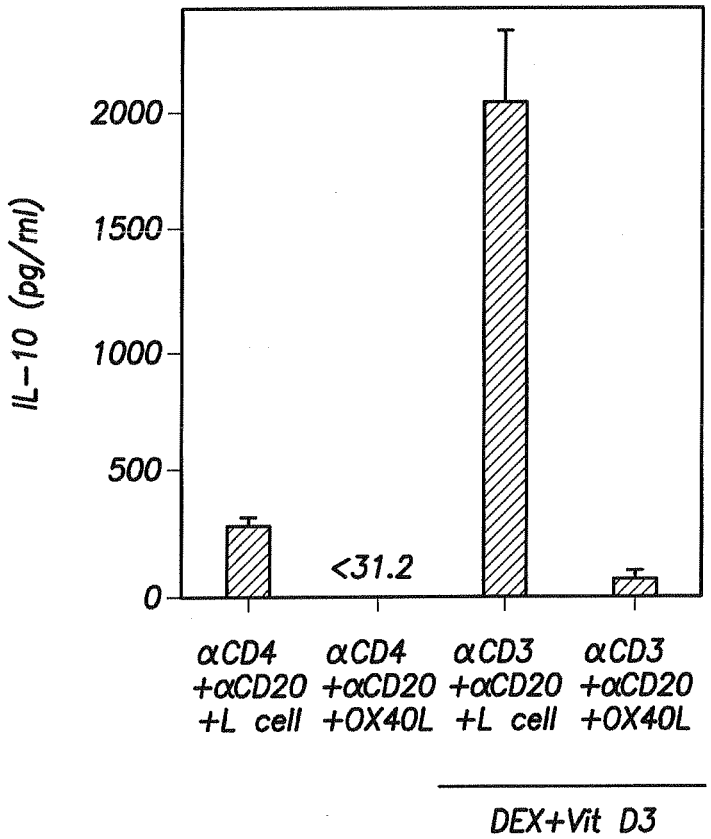

IL–10 (pg/ml)
2000
1500
1000
500
0
<31.2
αCD4 +αCD20 +L cell
αCD4 +αCD20 +OX40L
αCD3 +αCD20 +L cell
αCD3 +αCD20 +OX40L
DEX+Vit D3

FIG.6B

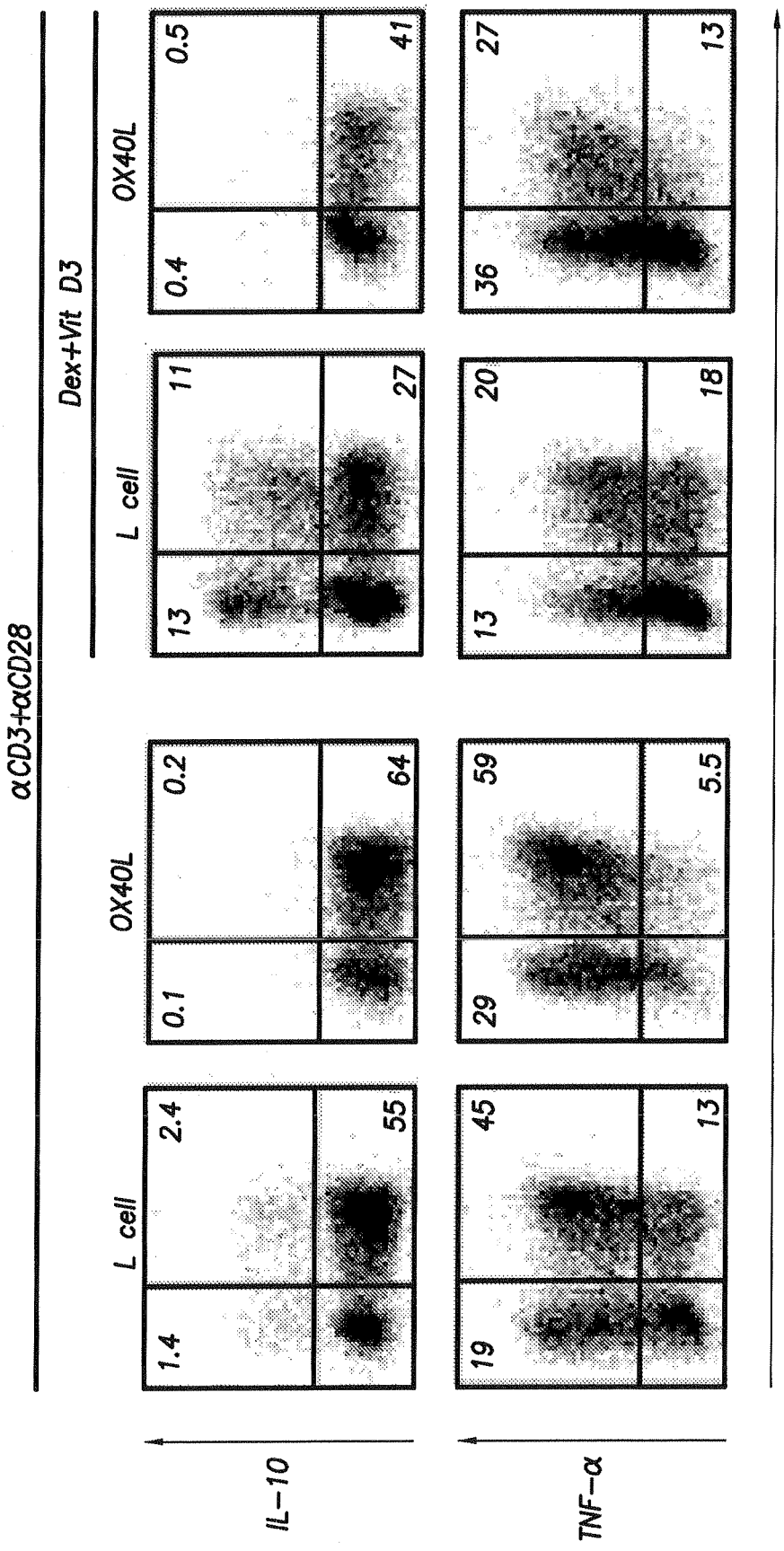

αCD3+αCD28
Dex+Vit D3
L cell
OX40L
L cell
OX40L
1.4
2.4
55
0.1
0.2
64
19
45
13
29
59
5.5
IL-10
TNF-α
IFN-γ
13
11
27
0.4
0.5
41
13
20
18
36
27
13

FIG. 6A

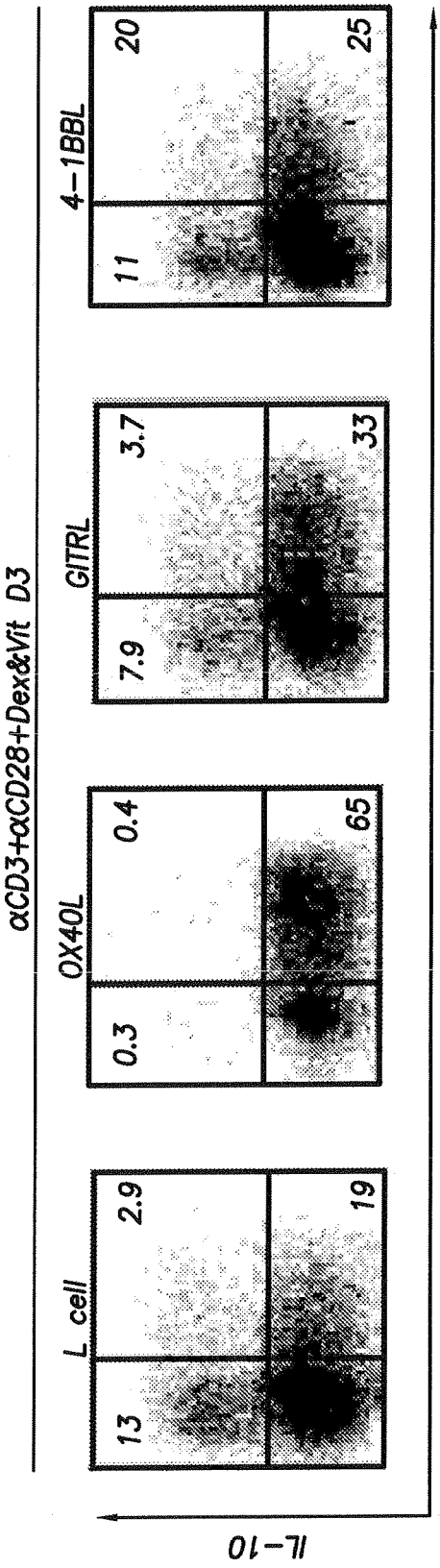


FIG. 7A

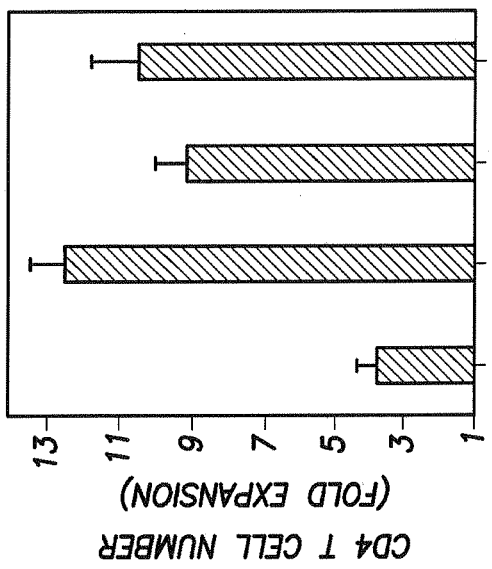


FIG. 7C

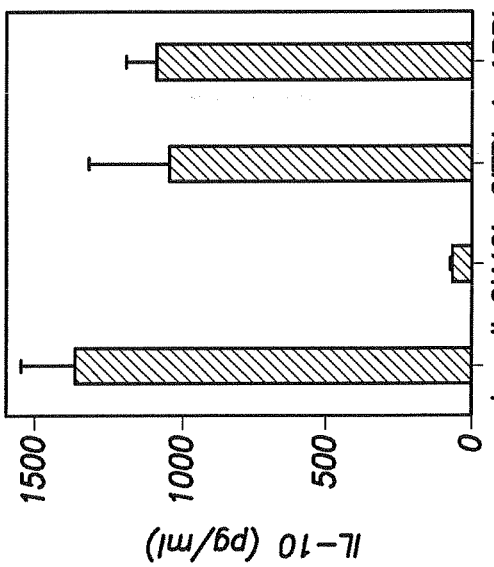


FIG. 7B

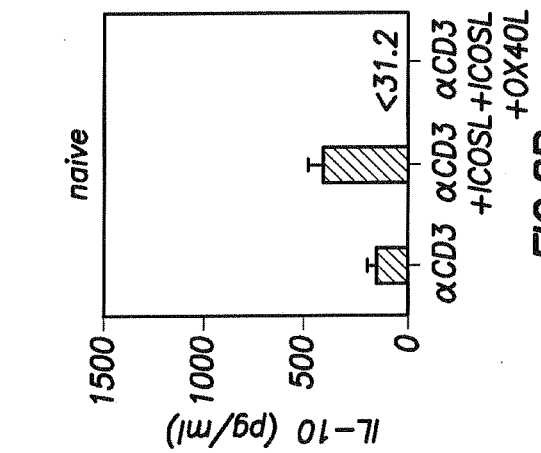


FIG.8B

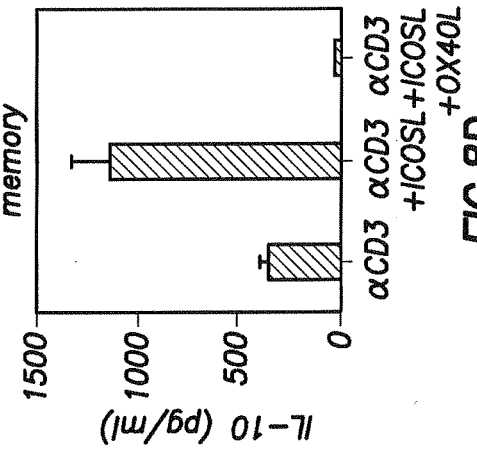


FIG.8D

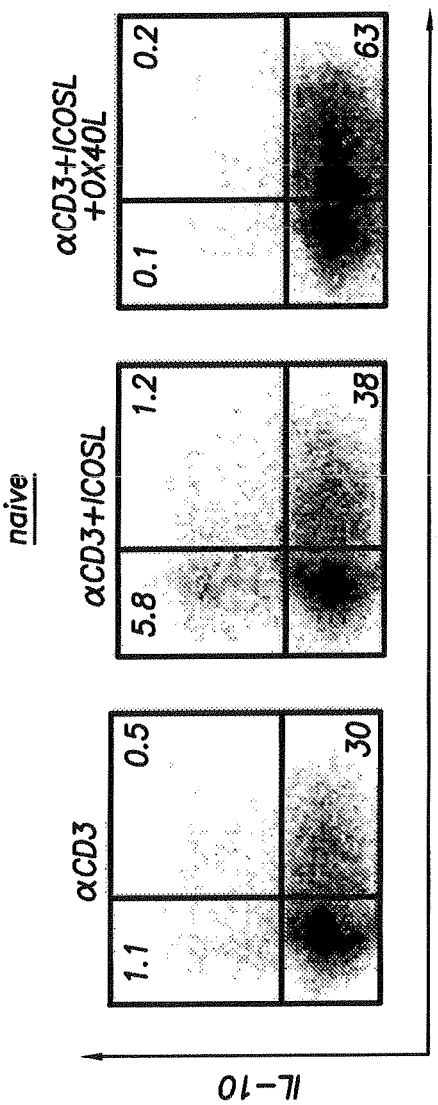


FIG.8A

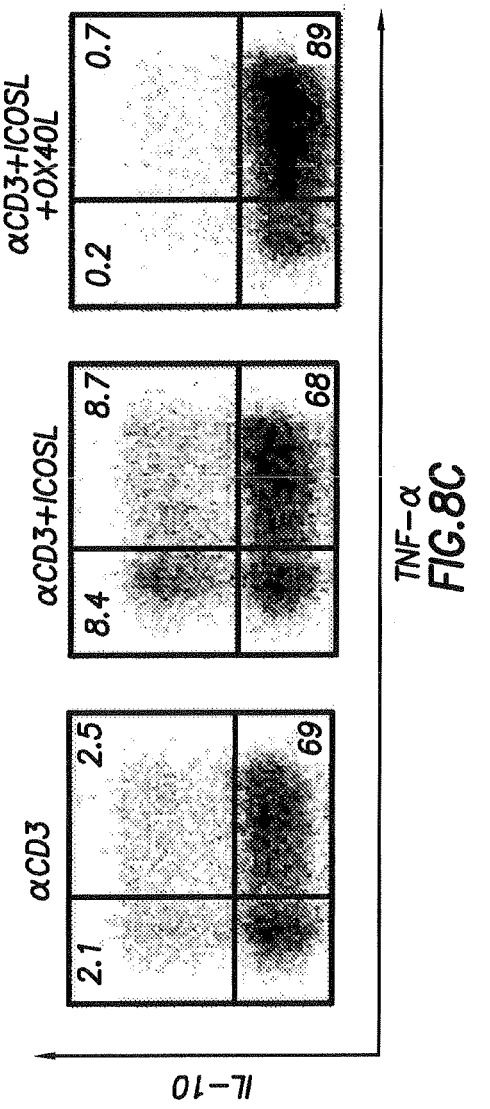


FIG.8C

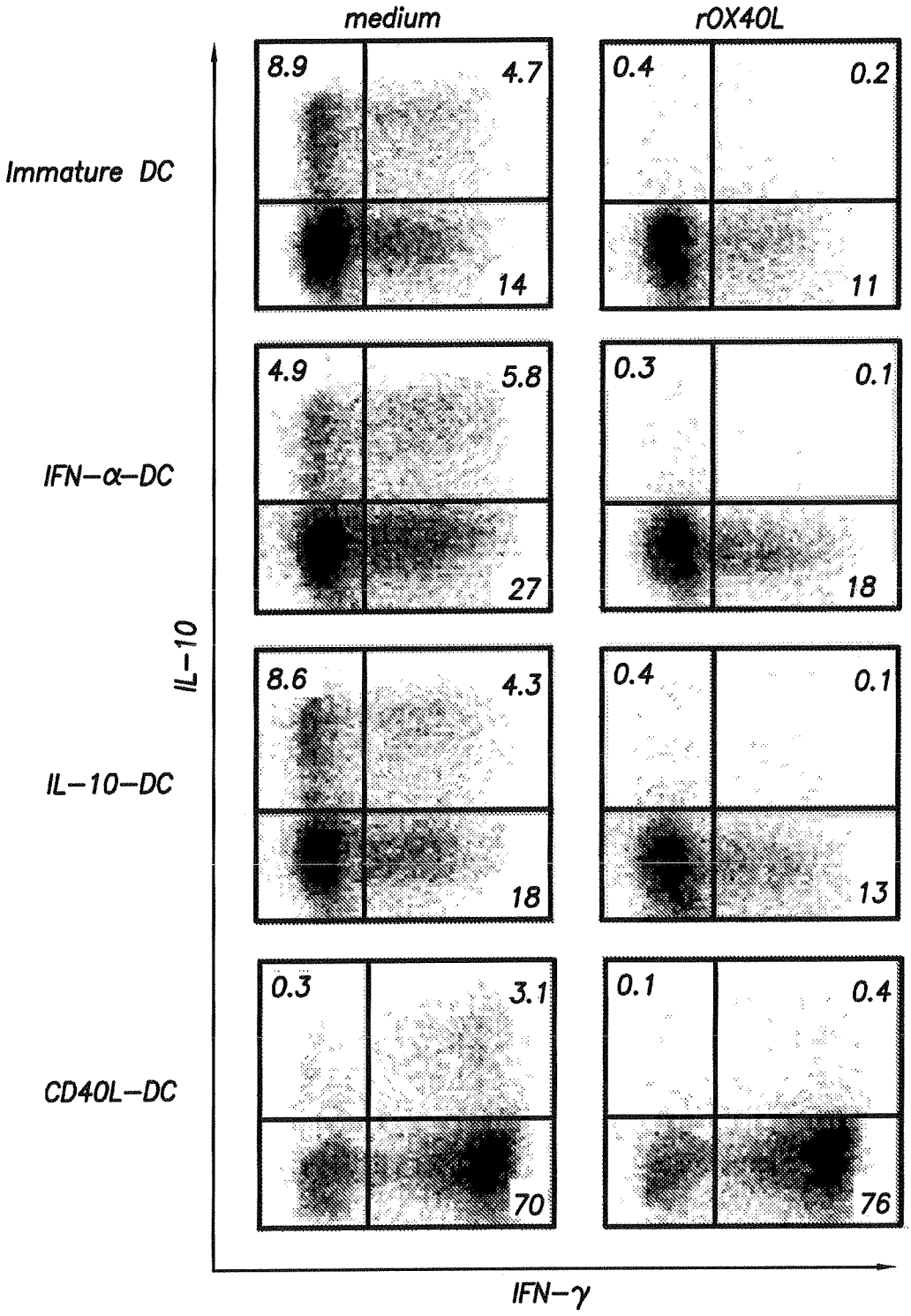

medium
rOX40L
Immature DC
IFN–α–DC
IL–10–DC
CD40L–DC
IL–10
IFN–γ
8.9
4.7
14
0.4
0.2
11
4.9
5.8
27
0.3
0.1
18
8.6
4.3
18
0.4
0.1
13
0.3
3.1
70
0.1
0.4
76

FIG.8E

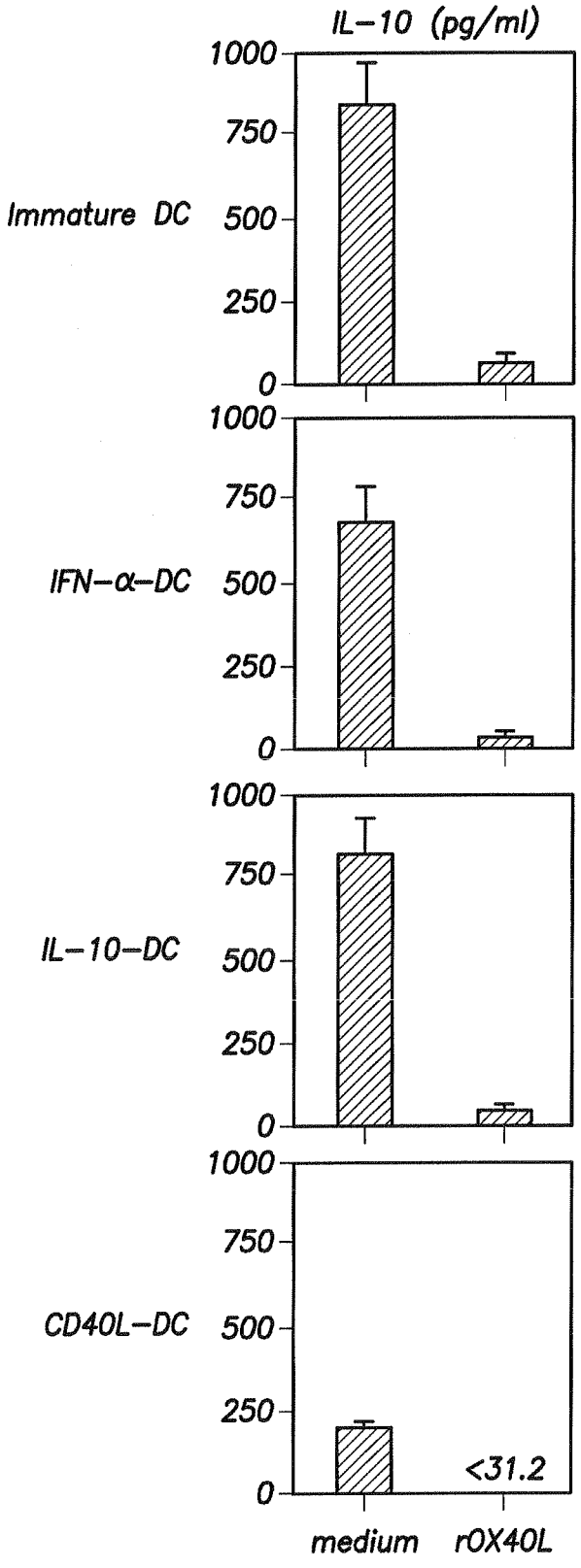

IL-10 (pg/ml)
Immature DC
IFN-α-DC
IL-10-DC
CD40L-DC
1000
750
500
250
0
<31.2
medium
rOX40L

FIG.8F

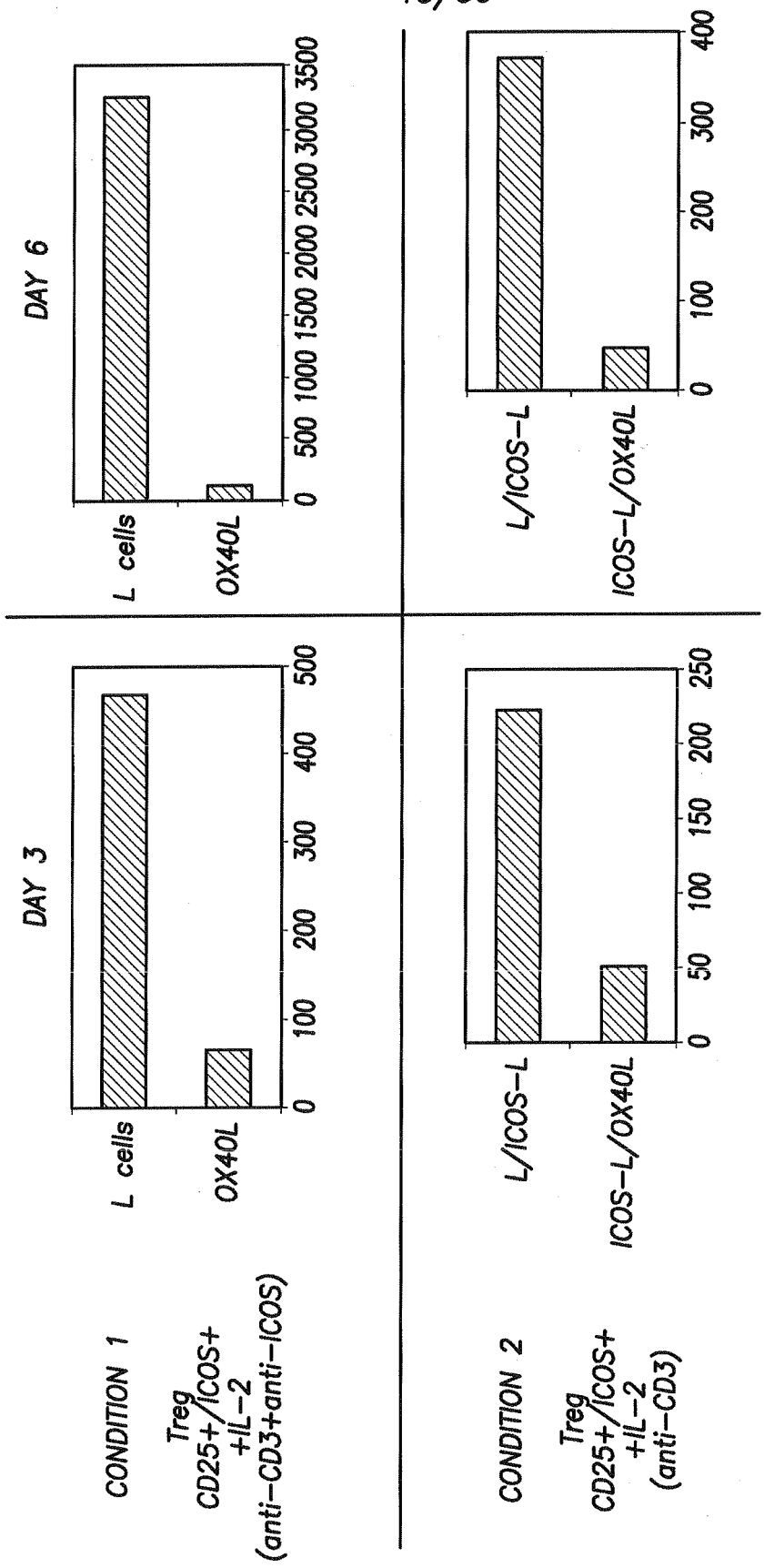

DAY 6
DAY 3
L cells
OX40L
L/ICOS-L
ICOS-L/OX40L
0
500
1000
1500
2000
2500
3000
3500
400
300
200
100
50
150
250
CONDITION 1
Treg
CD25+/ICOS+
+IL-2
(anti-CD3+anti-ICOS)
CONDITION 2
Treg
CD25+/ICOS+
+IL-2
(anti-CD3)
IL-10 (pg/ml)

FIG.9

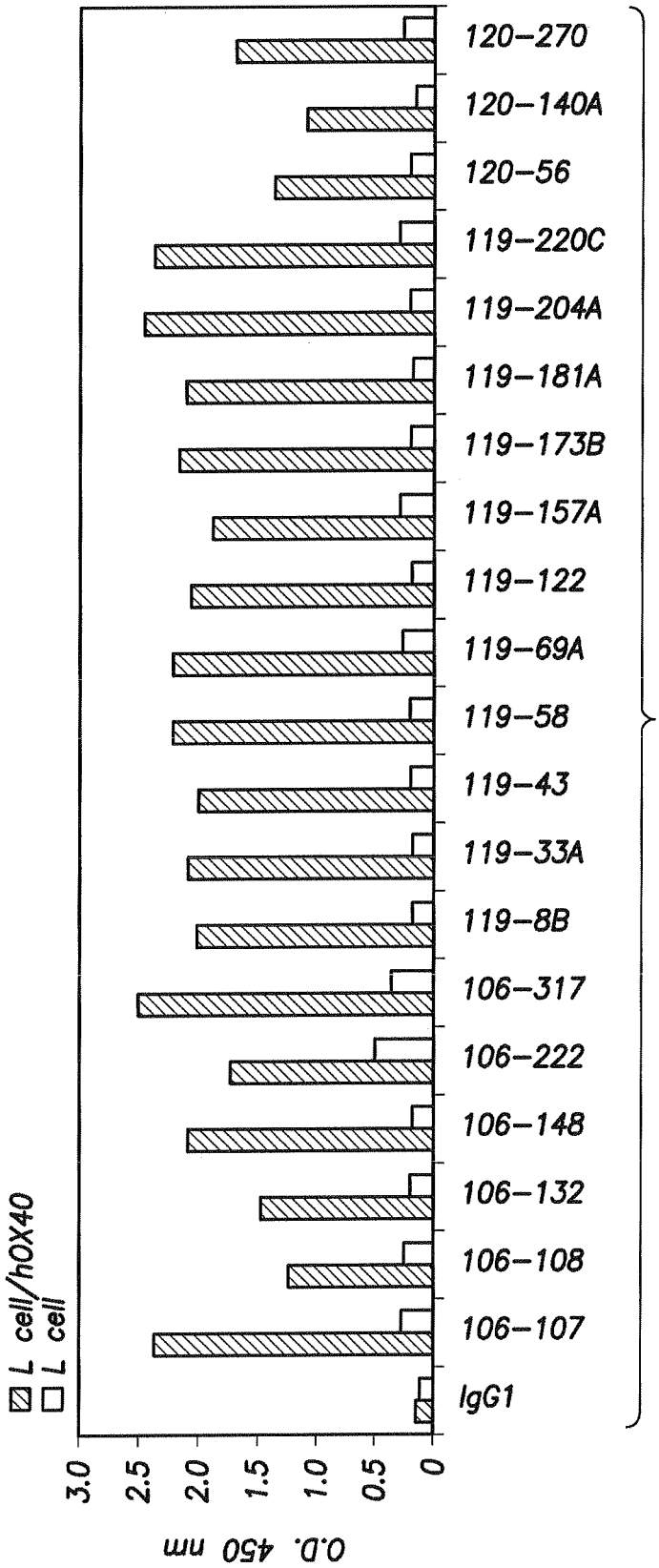

120-270
120-140A
120-56
119-220C
119-204A
119-181A
119-173B
119-157A
119-122
119-69A
119-58
119-43
119-33A
119-8B
106-317
106-222
106-148
106-132
106-108
106-107
IgG1
Anti-hOX40 hybridoma clones
L cell/hOX40
L cell
3.0
2.5
2.0
1.5
1.0
0.5
0
O.D. 450 nm

FIG. 10

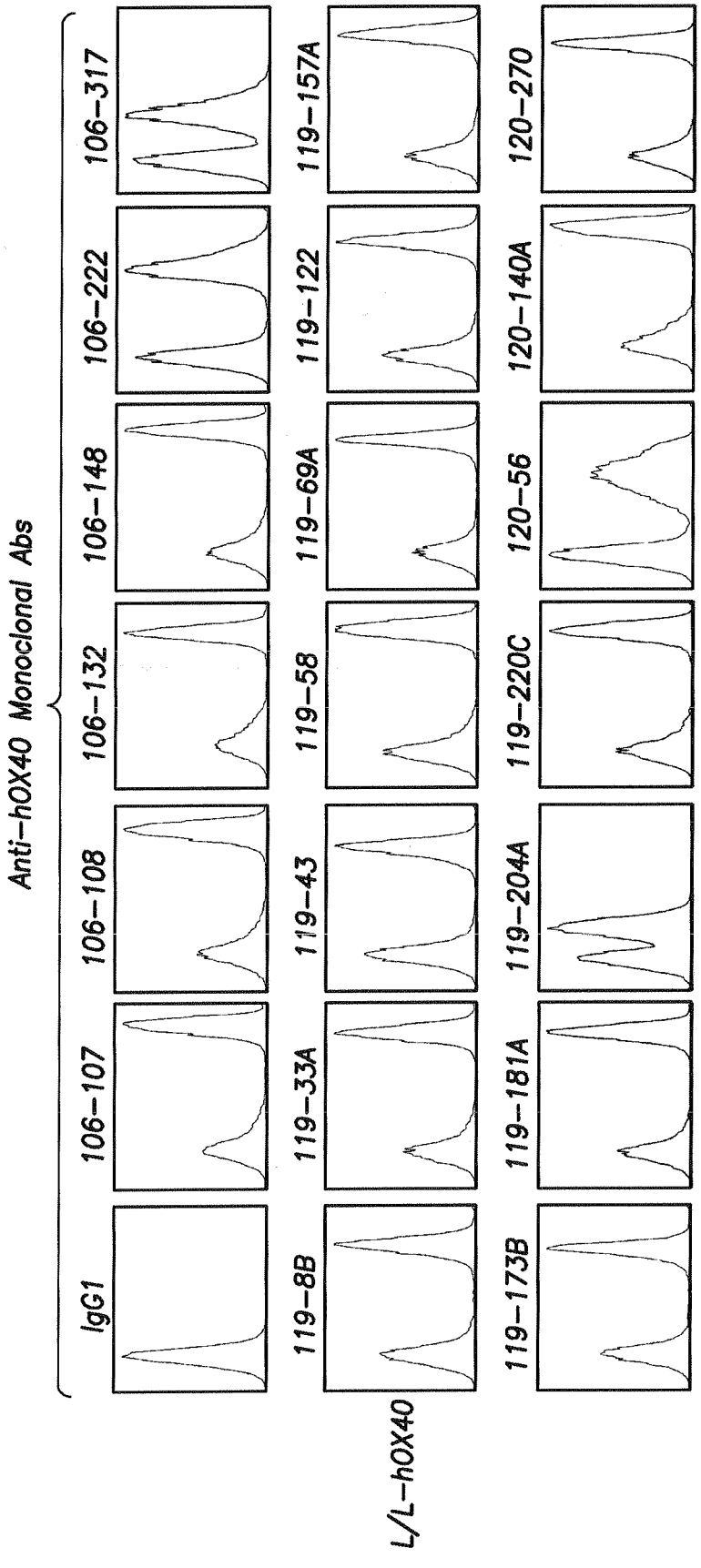

Anti-hOX40 Monoclonal Abs
IgG1
106-107
106-108
106-132
106-148
106-222
106-317
119-8B
119-33A
119-43
119-58
119-69A
119-122
119-157A
119-173B
119-181A
119-204A
119-220C
120-56
120-140A
120-270
L/L-hOX40

FIG. 11

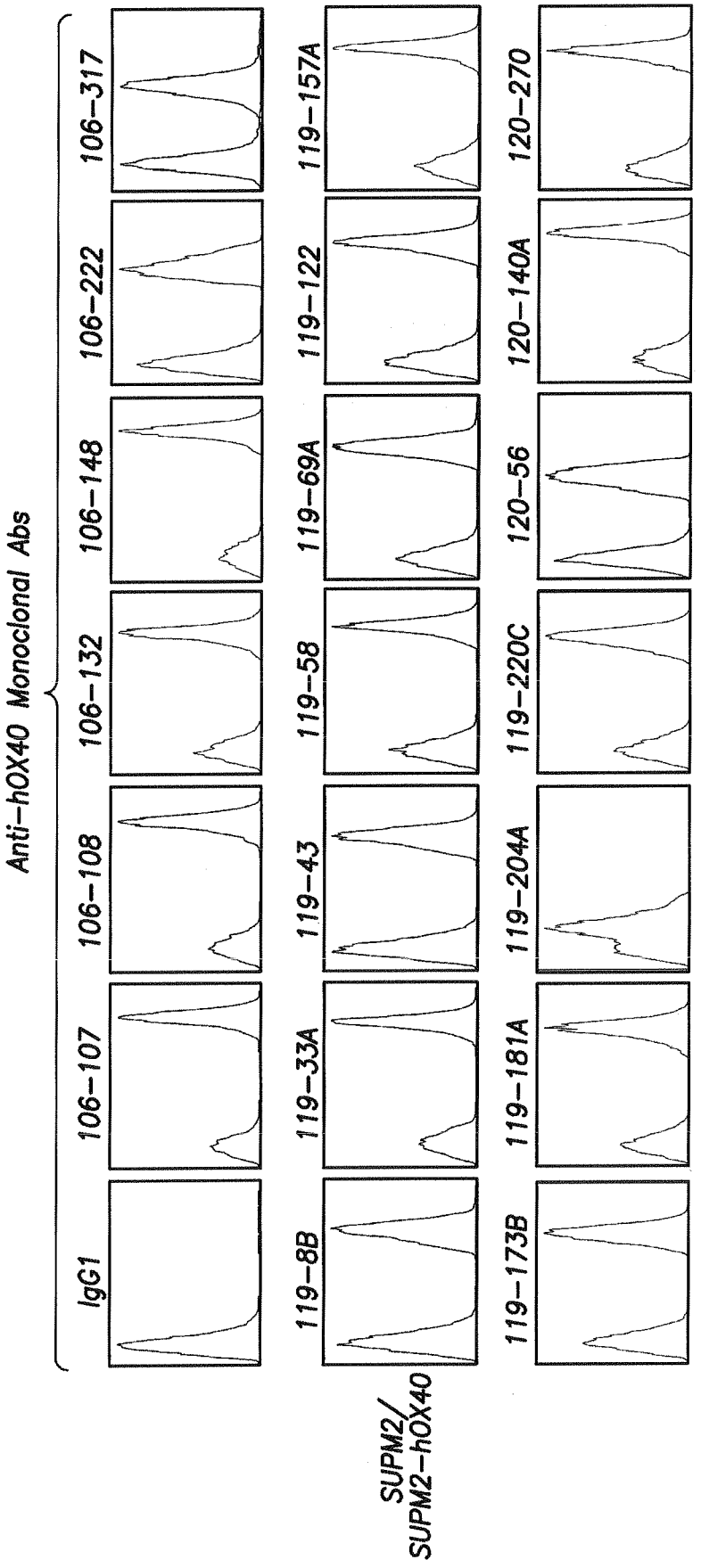

Anti-hOX40 Monoclonal Abs
IgG1
106-107
106-108
106-132
106-148
106-222
106-317
119-8B
119-33A
119-43
119-58
119-69A
119-122
119-157A
119-173B
119-181A
119-204A
119-220C
120-56
120-140A
120-270
SUPM2/
SUPM2-hOX40

FIG. 12

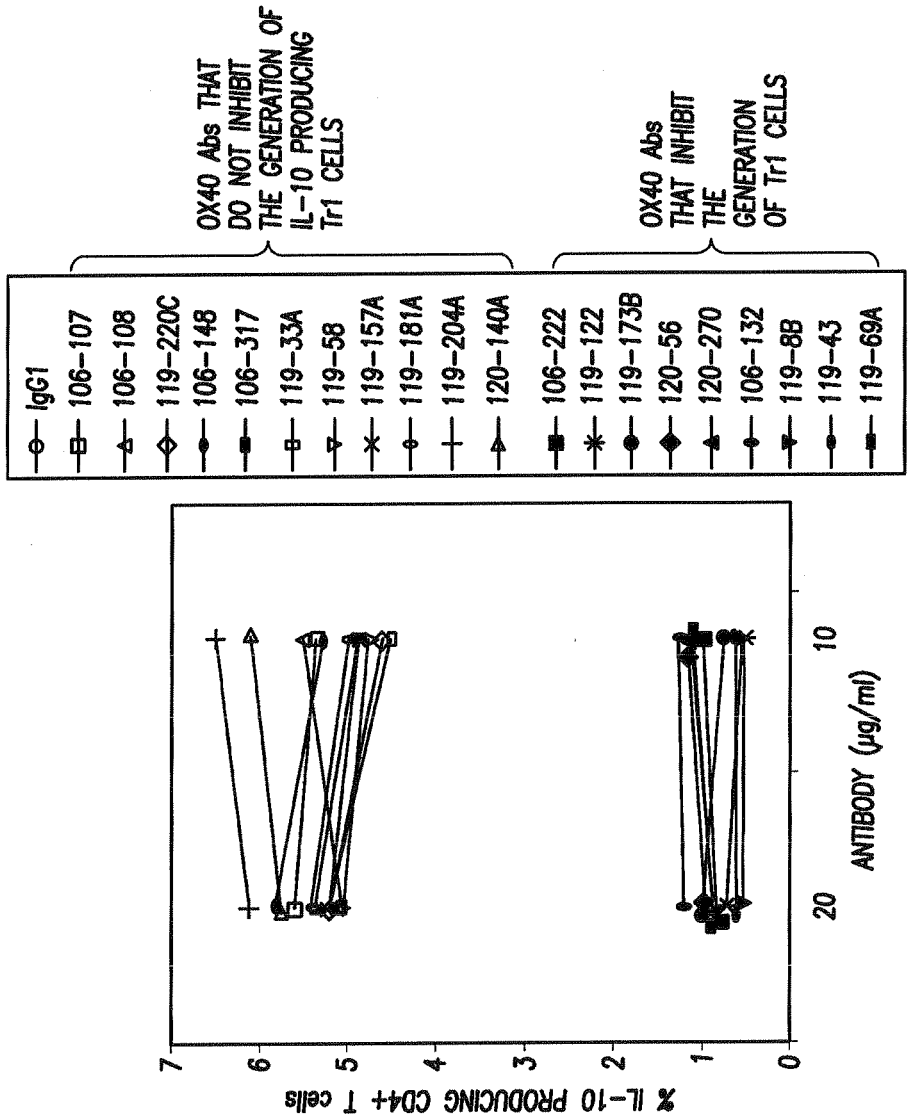


FIG.13B

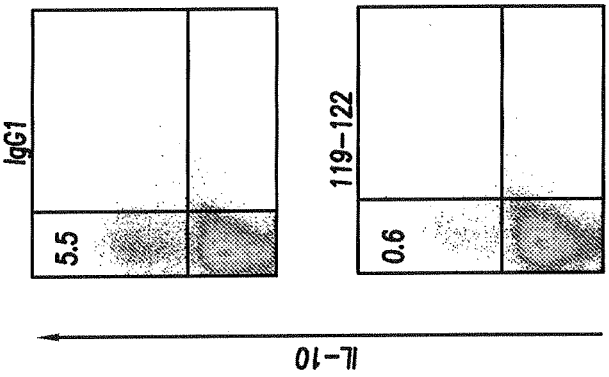


FIG.13A

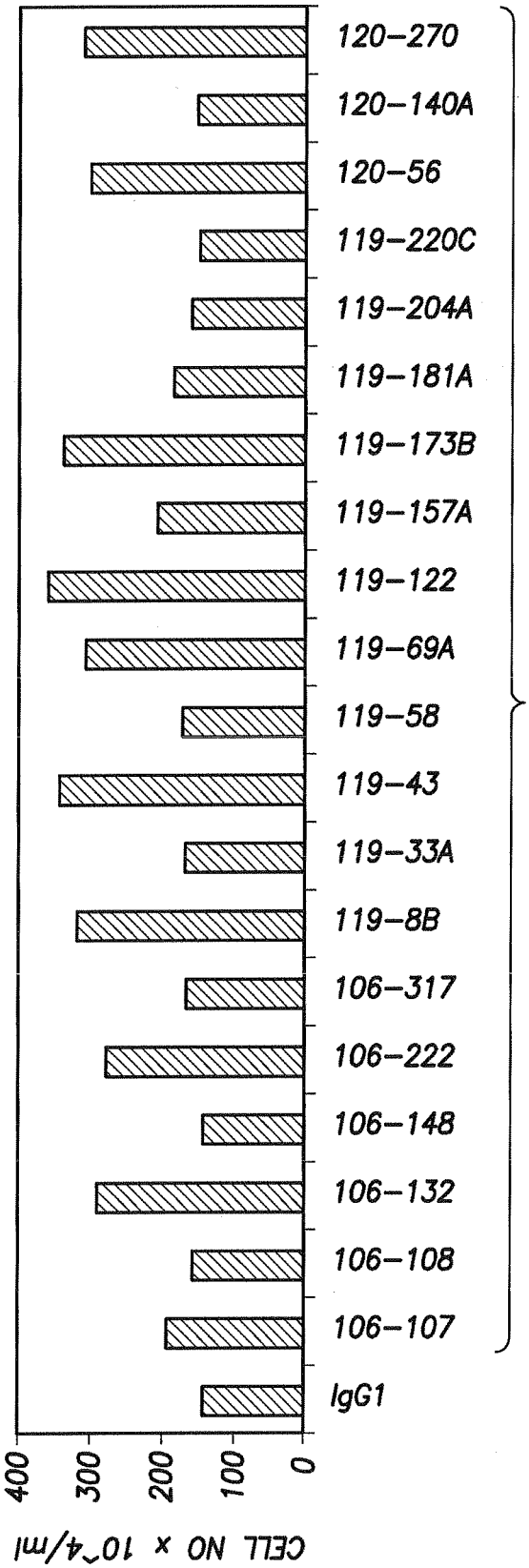

120-270
120-140A
120-56
119-220C
119-204A
119-181A
119-173B
119-157A
119-122
119-69A
119-58
119-43
119-33A
119-8B
106-317
106-222
106-148
106-132
106-108
106-107
IgG1
Anti-hOX40 MAbs
400
300
200
100
0
CELL NO x 10^4/ml

FIG. 14

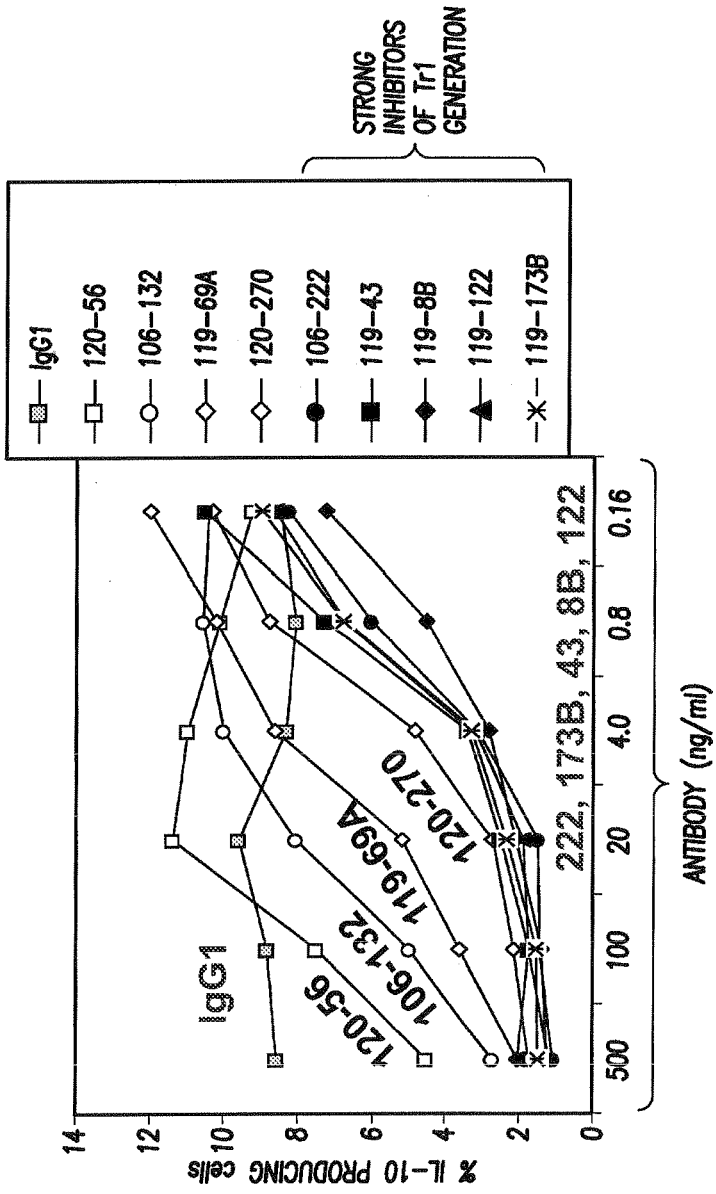


FIG. 15B

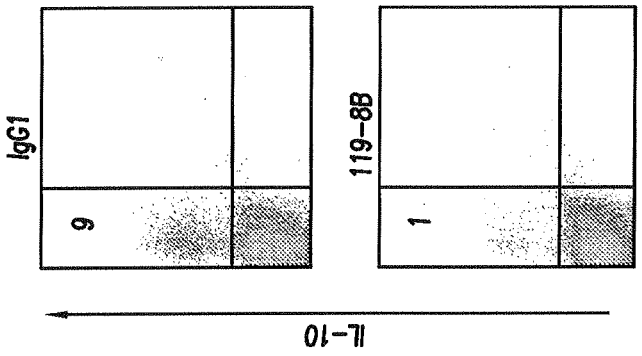


FIG. 15A

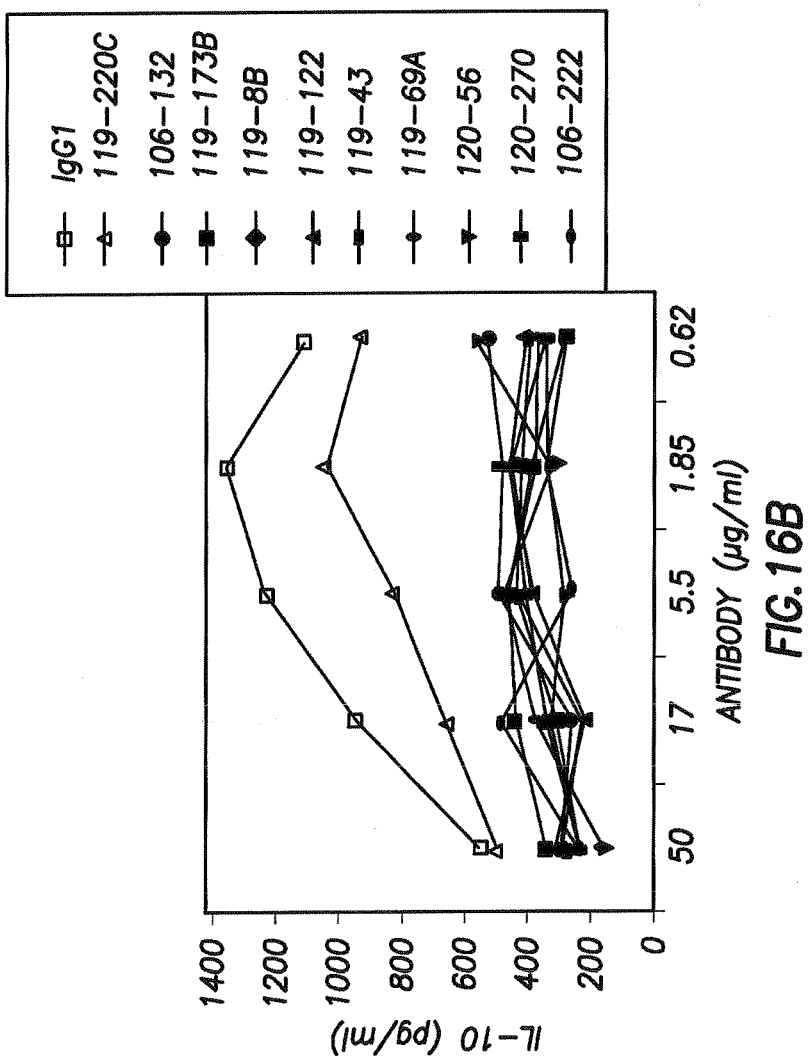


FIG. 16B

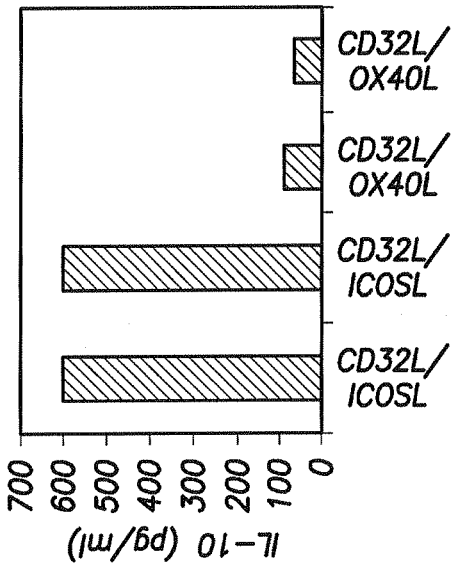


FIG. 16A

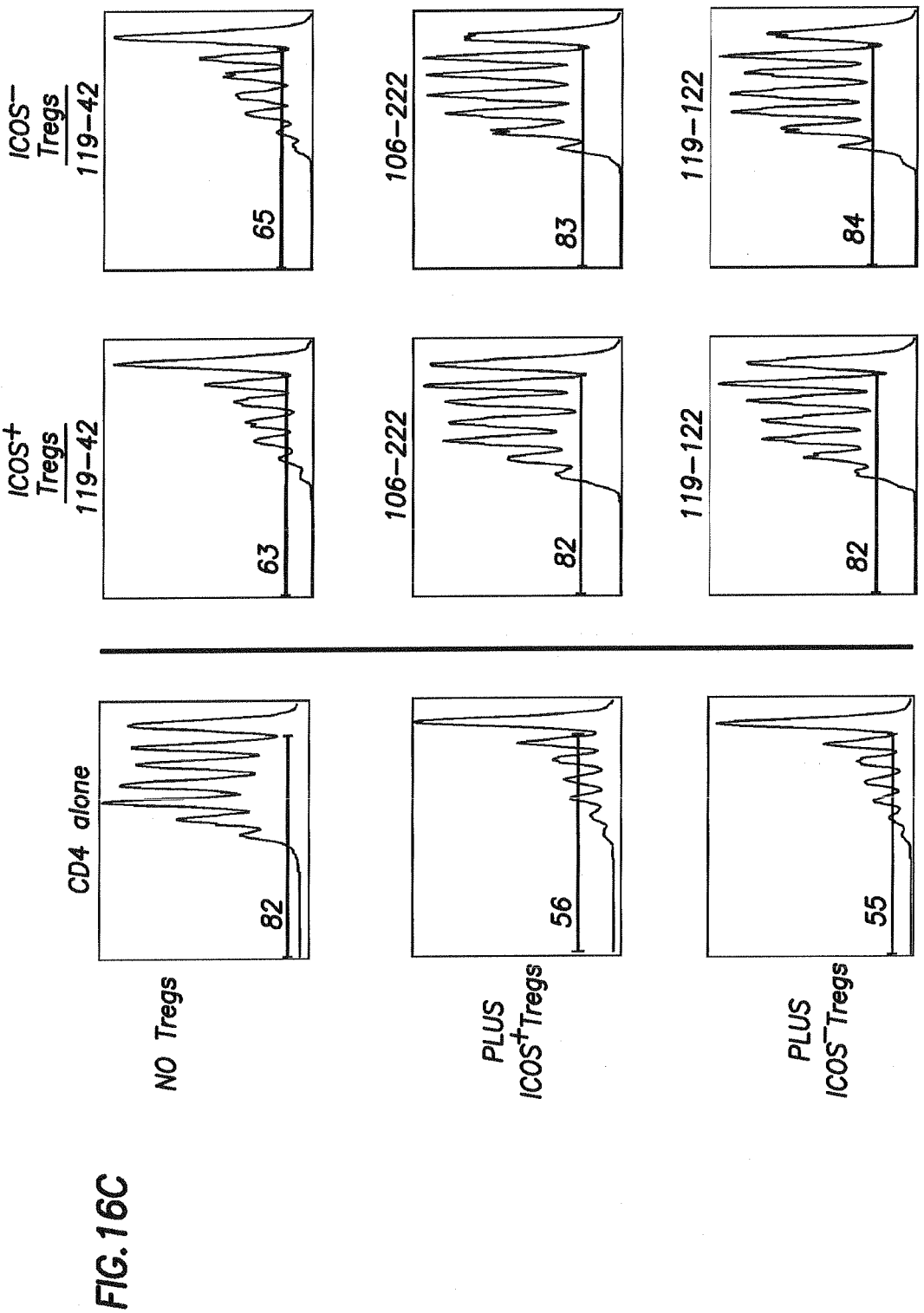

ICOS− Tregs 119−42
106−222
119−122
ICOS+ Tregs 119−42
106−222
119−122
CD4 alone
NO Tregs
PLUS ICOS+Tregs
PLUS ICOS−Tregs
65
83
84
63
82
82
82
56
55

FIG.16C

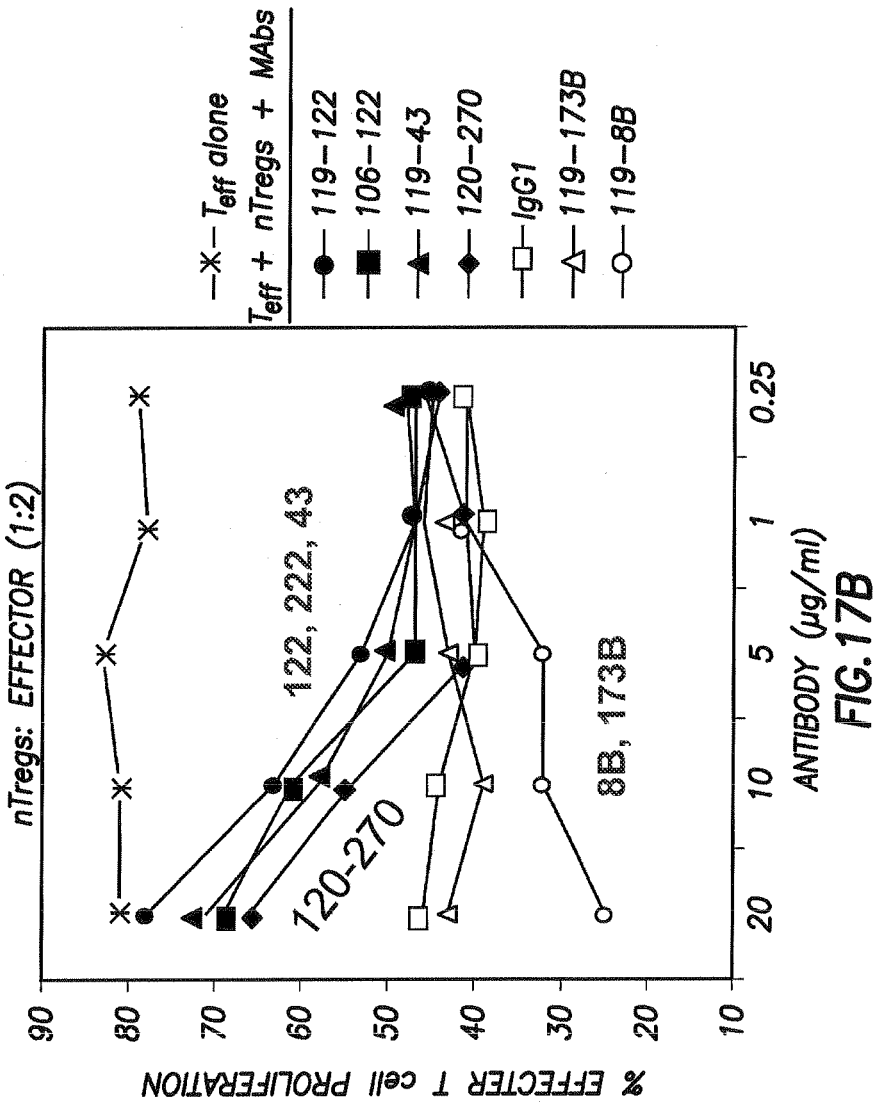


FIG. 17B

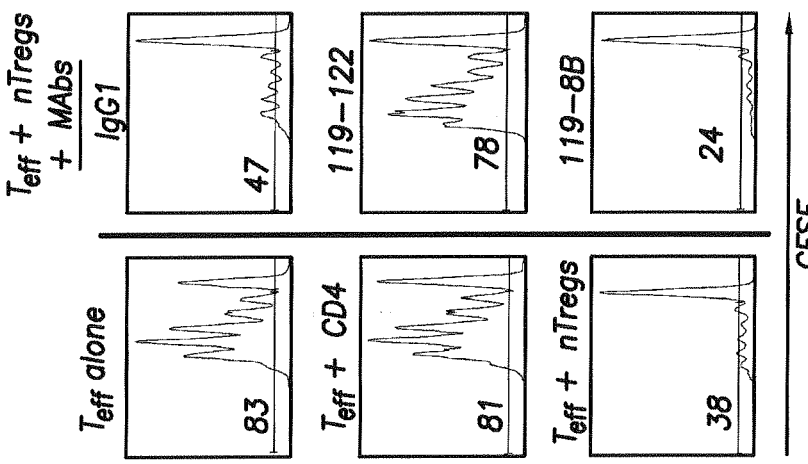


FIG. 17A

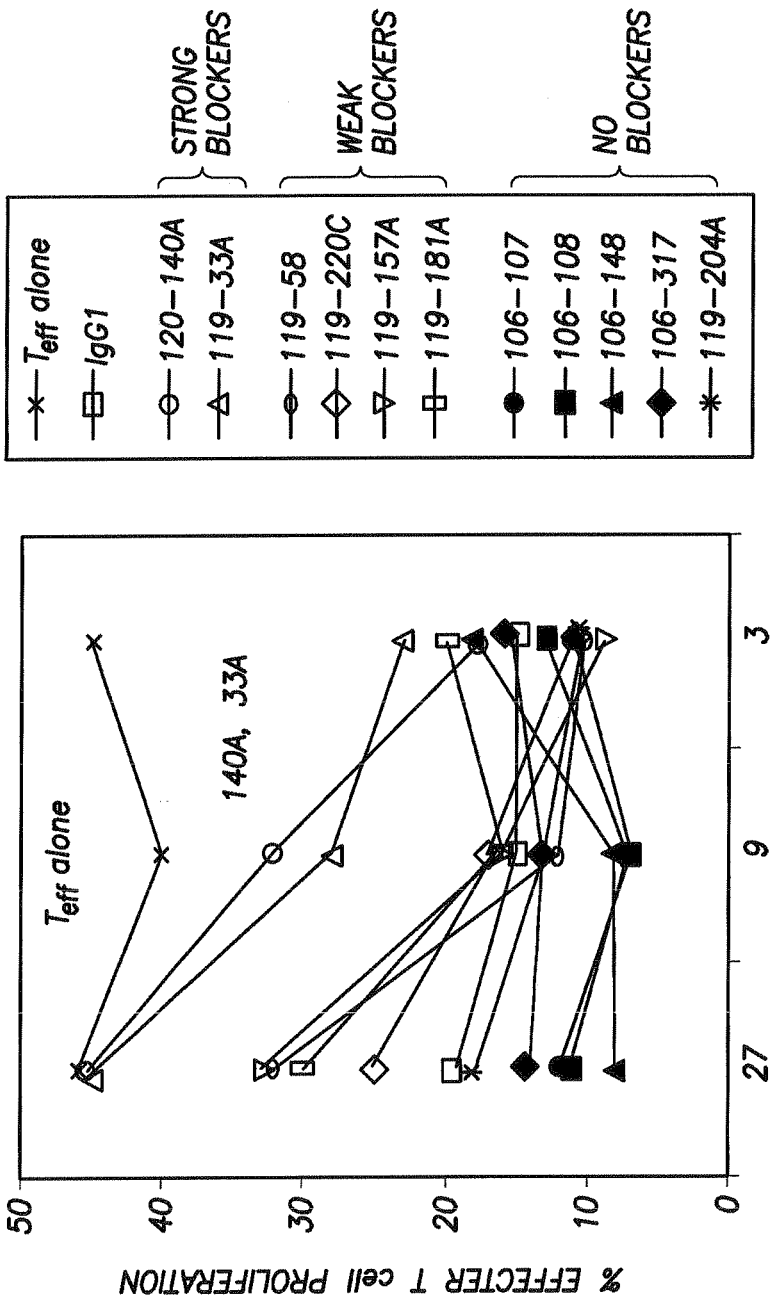

STRONG BLOCKERS
WEAK BLOCKERS
NO BLOCKERS
Teff alone
IgG1
120-140A
119-33A
119-58
119-220C
119-157A
119-181A
106-107
106-108
106-148
106-317
119-204A
140A, 33A
Teff alone
% EFFECTER T cell PROLIFERATION
0
10
20
30
40
50
27
9
3
ANTIBODY (µg/ml)

FIG. 18

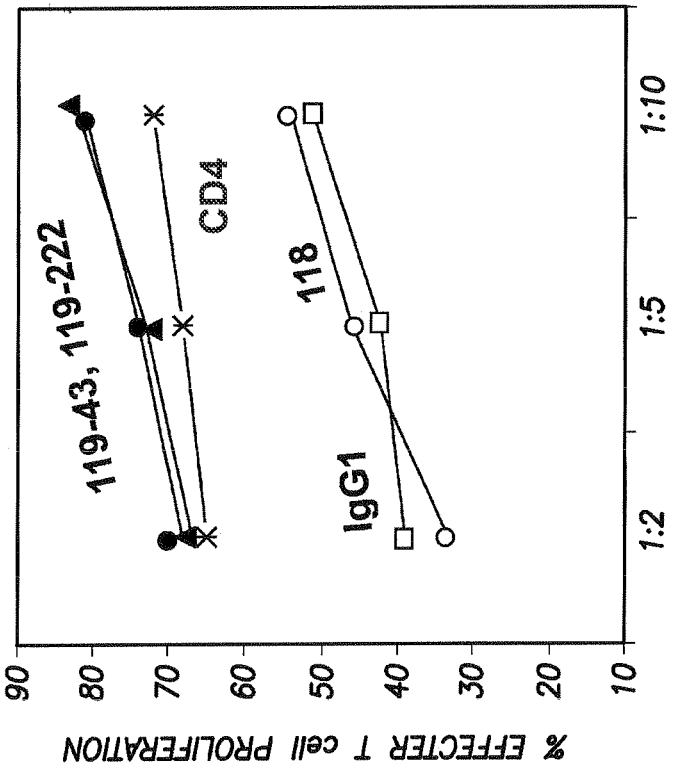


FIG. 19B

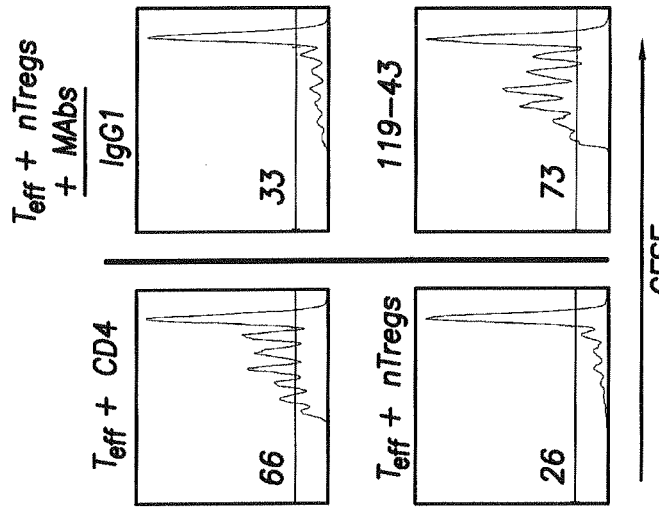


FIG. 19A

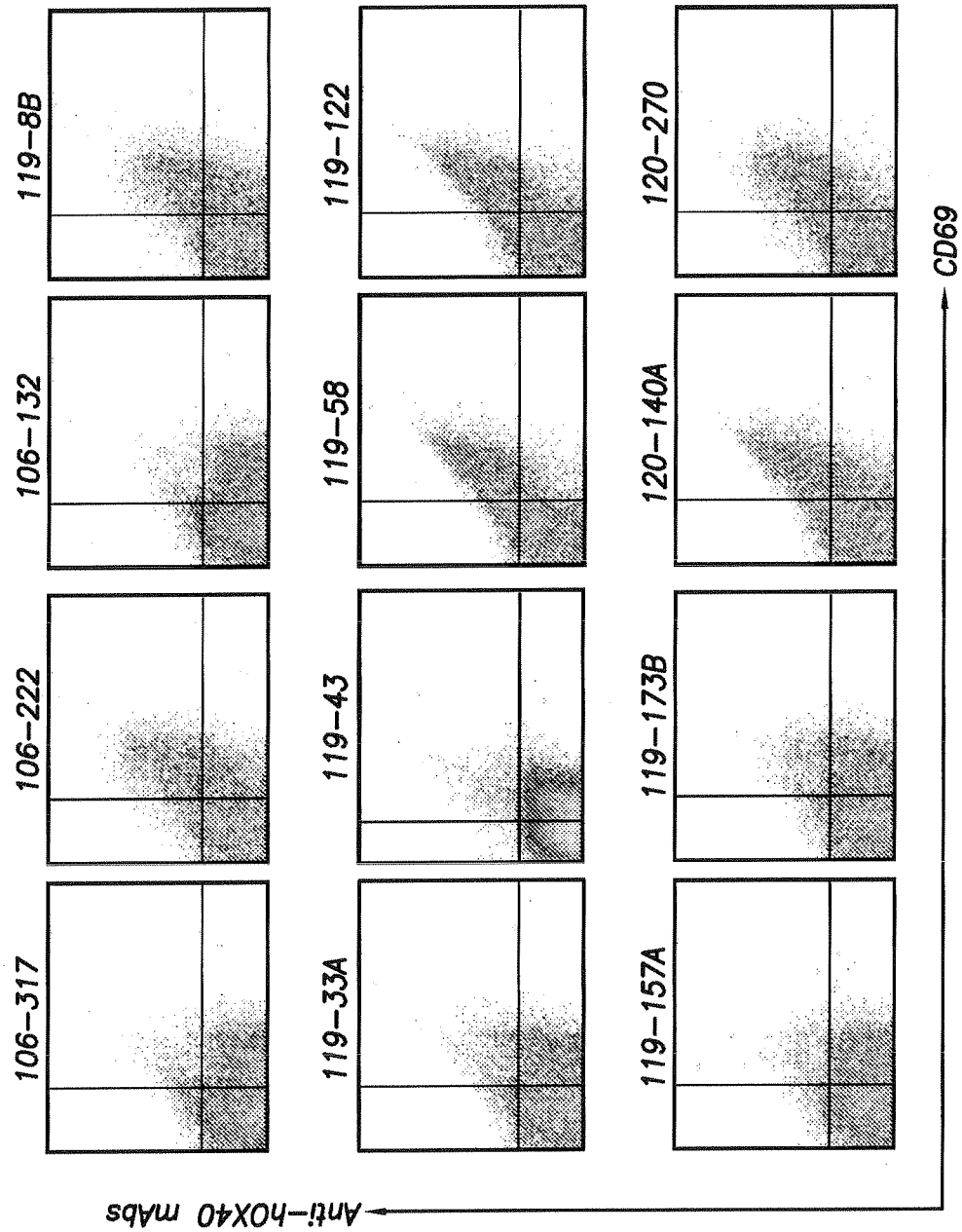

119-8B
119-122
120-270
106-132
119-58
120-140A
106-222
119-43
119-173B
106-317
119-33A
119-157A
CD69
Anti-hOX40 mAbs

FIG.20

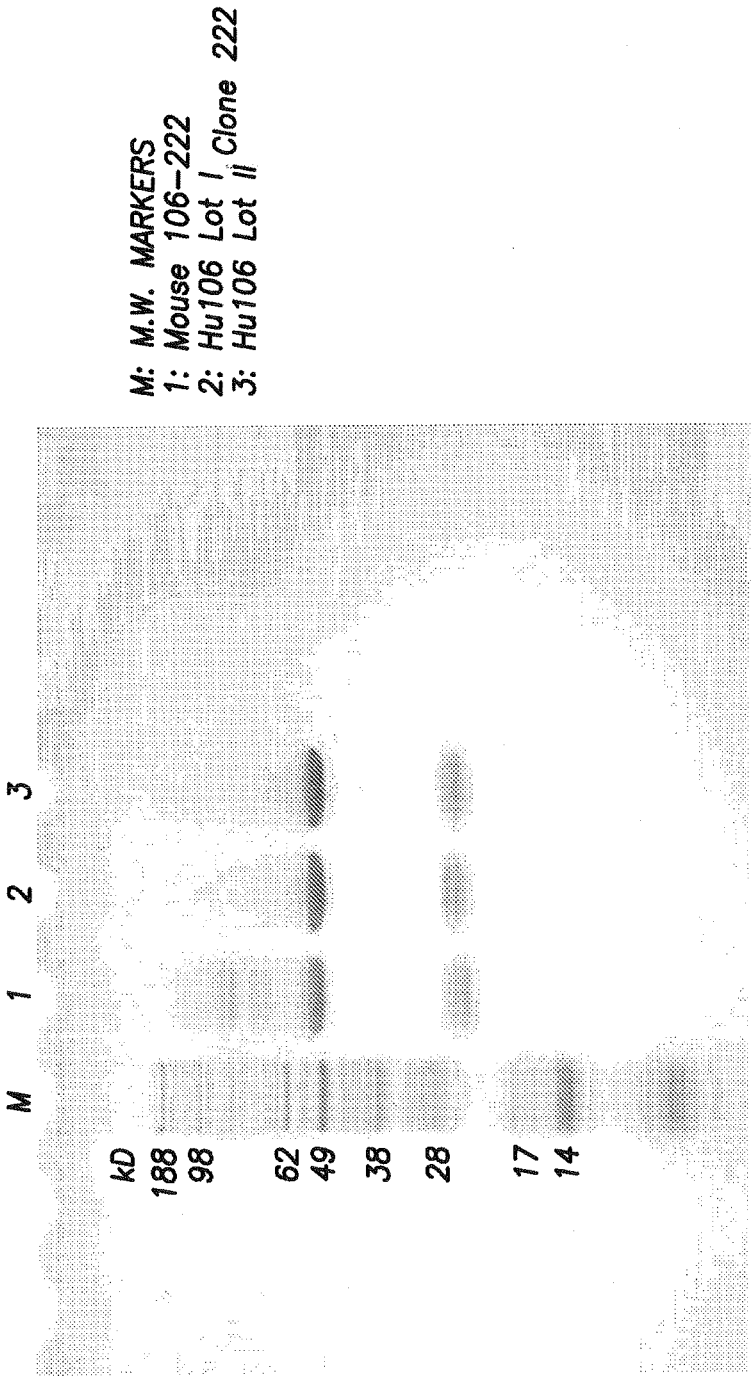

M: M.W. MARKERS
1: Mouse 106-222
2: Hu106 Lot I Clone 222
3: Hu106 Lot II
M
1
2
3
kD
188
98
62
49
38
28
17
14

FIG.21

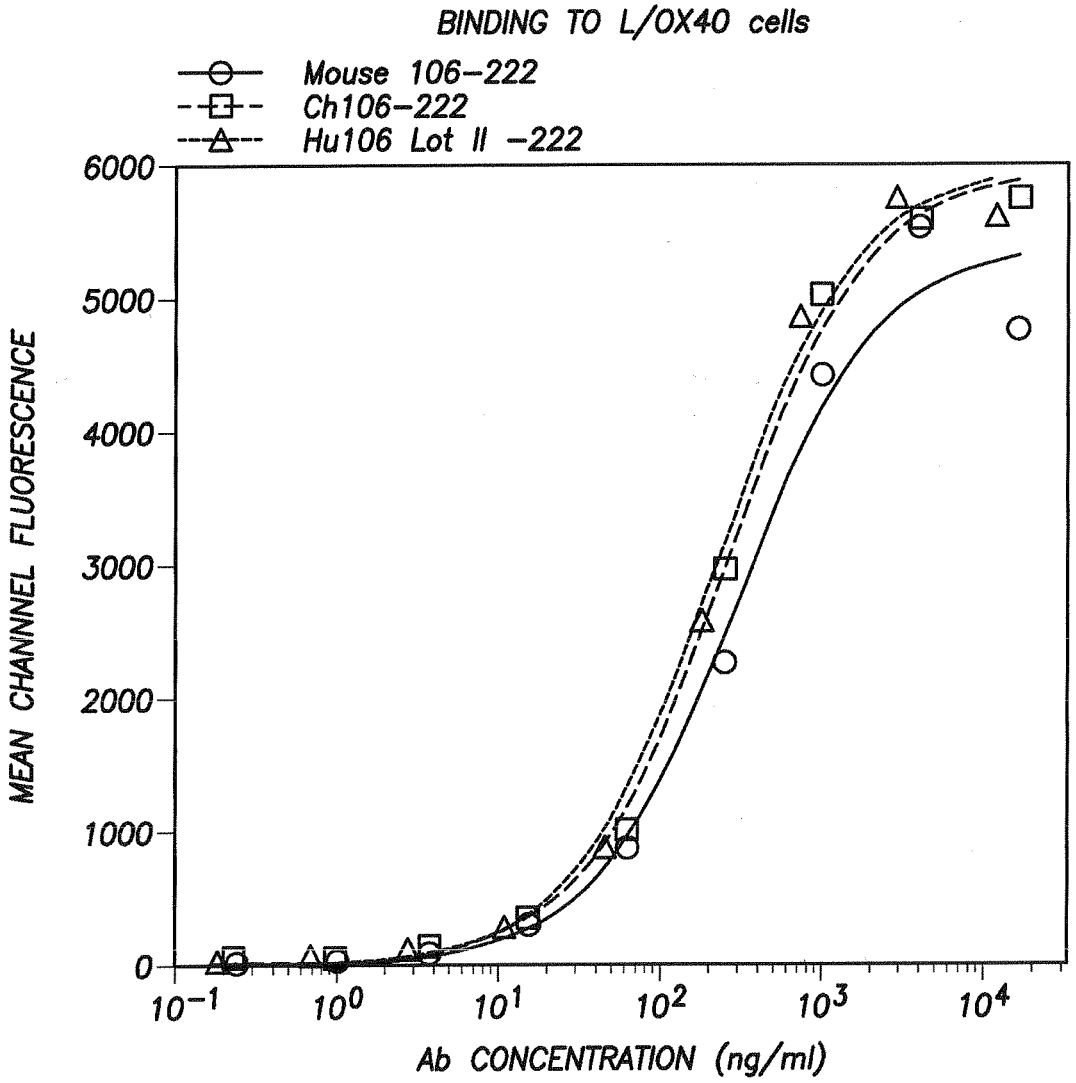

BINDING TO L/OX40 cells
Mouse 106-222
Ch106-222
Hu106 Lot II -222
MEAN CHANNEL FLUORESCENCE
6000
5000
4000
3000
2000
1000
0
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
10^{4}
Ab CONCENTRATION (ng/ml)

FIG.22

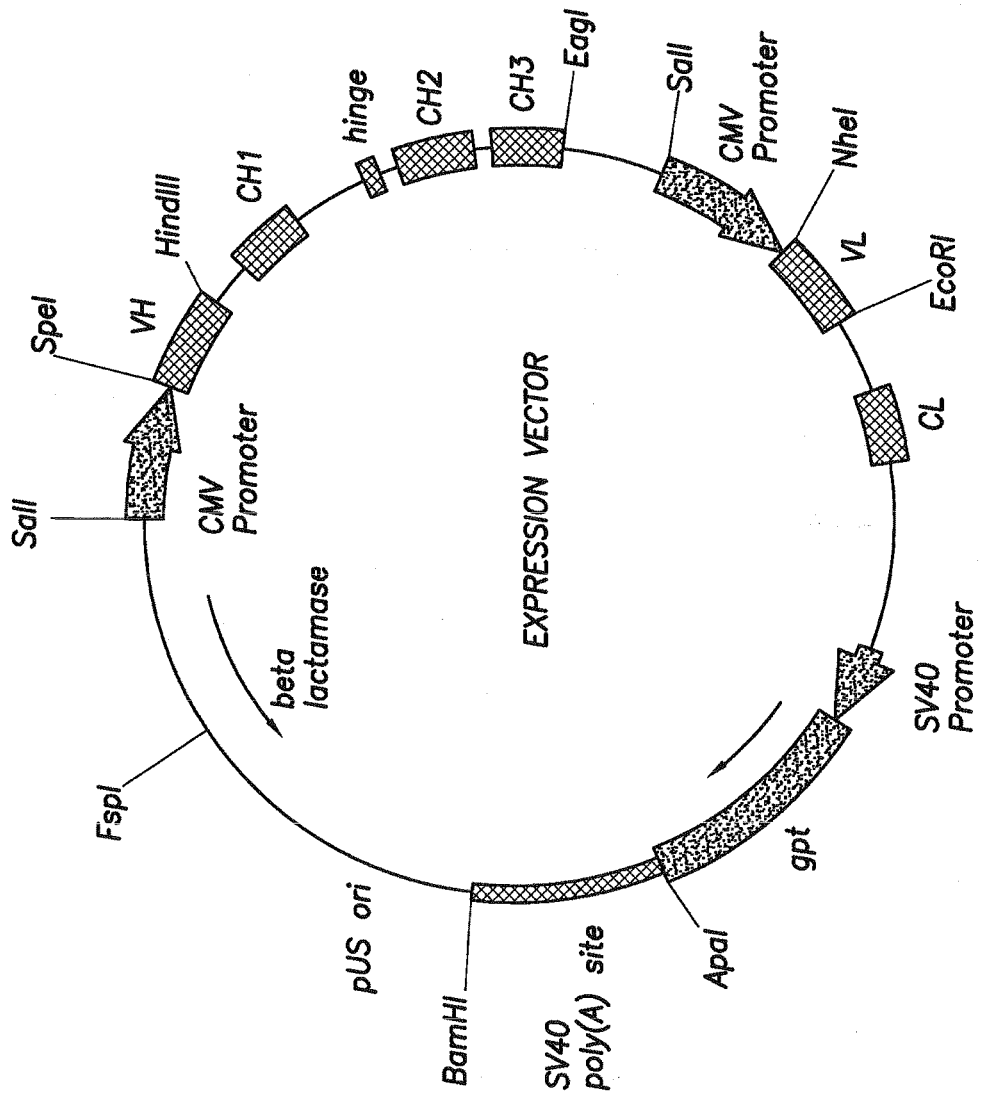

EXPRESSION VECTOR
CMV Promoter
beta lactamase
FspI
pUS ori
BamHI
SV40 poly(A) site
ApaI
gpt
SV40 Promoter
CL
EcoRI
VL
NheI
CMV Promoter
SalI
EagI
CH3
CH2
hinge
CH1
HindIII
VH
SpeI
SalI

FIG.23

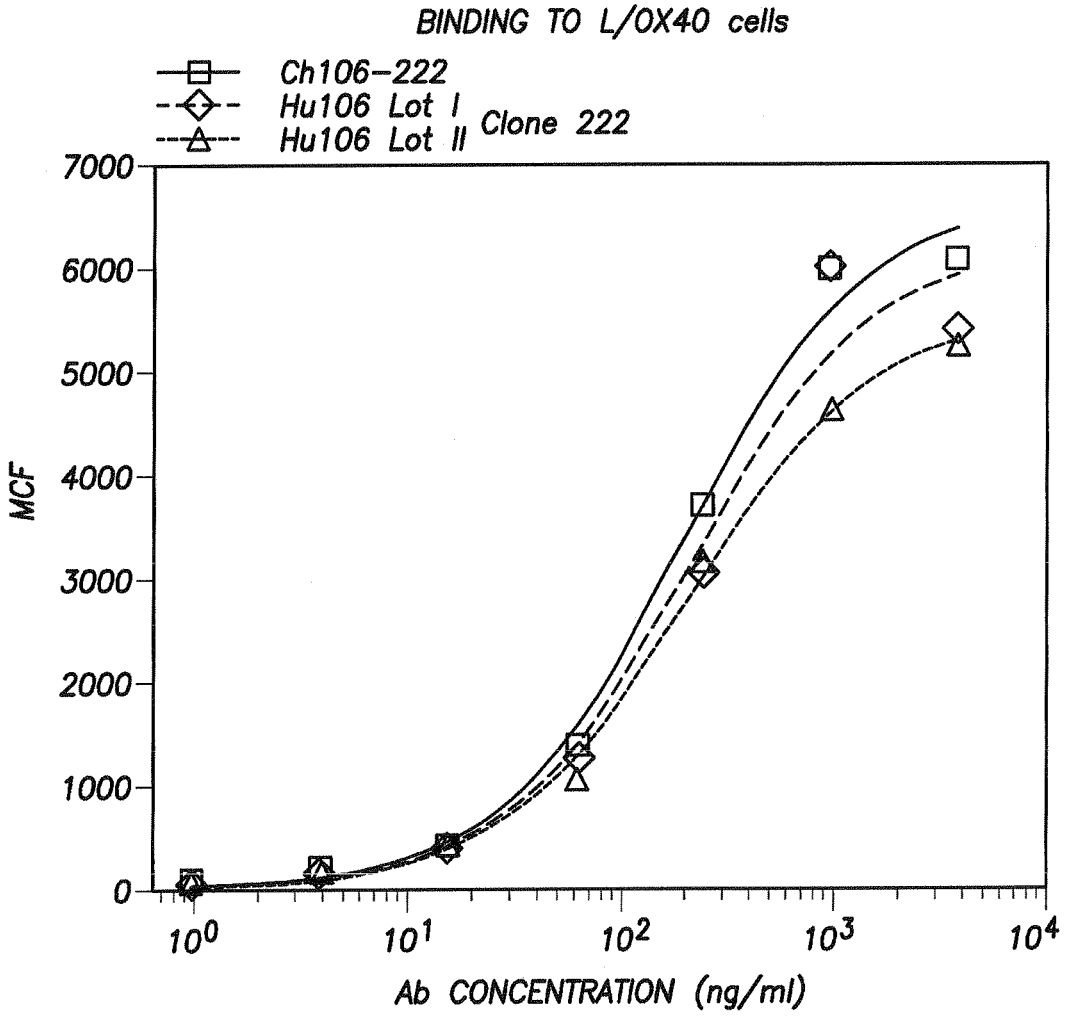

BINDING TO L/OX40 cells
Ch106-222
Hu106 Lot I
Hu106 Lot II
Clone 222
7000
6000
5000
4000
3000
2000
1000
0
MCF
10^0
10^1
10^2
10^3
10^4
Ab CONCENTRATION (ng/ml)

FIG.24

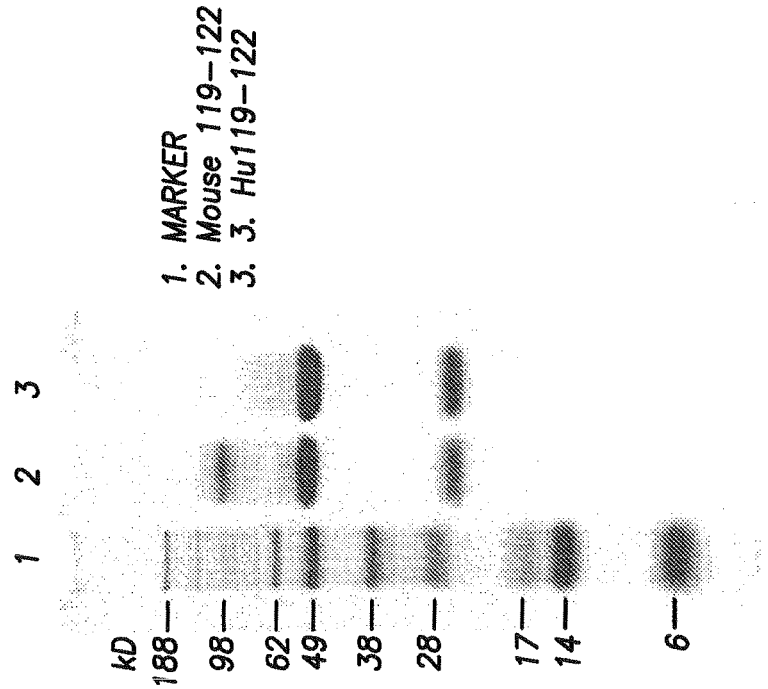

1. MARKER
2. Mouse 119-122
3. 3. Hu119-122
1 2 3
kD
188
98
62
49
38
28
17
14
6

FIG.25

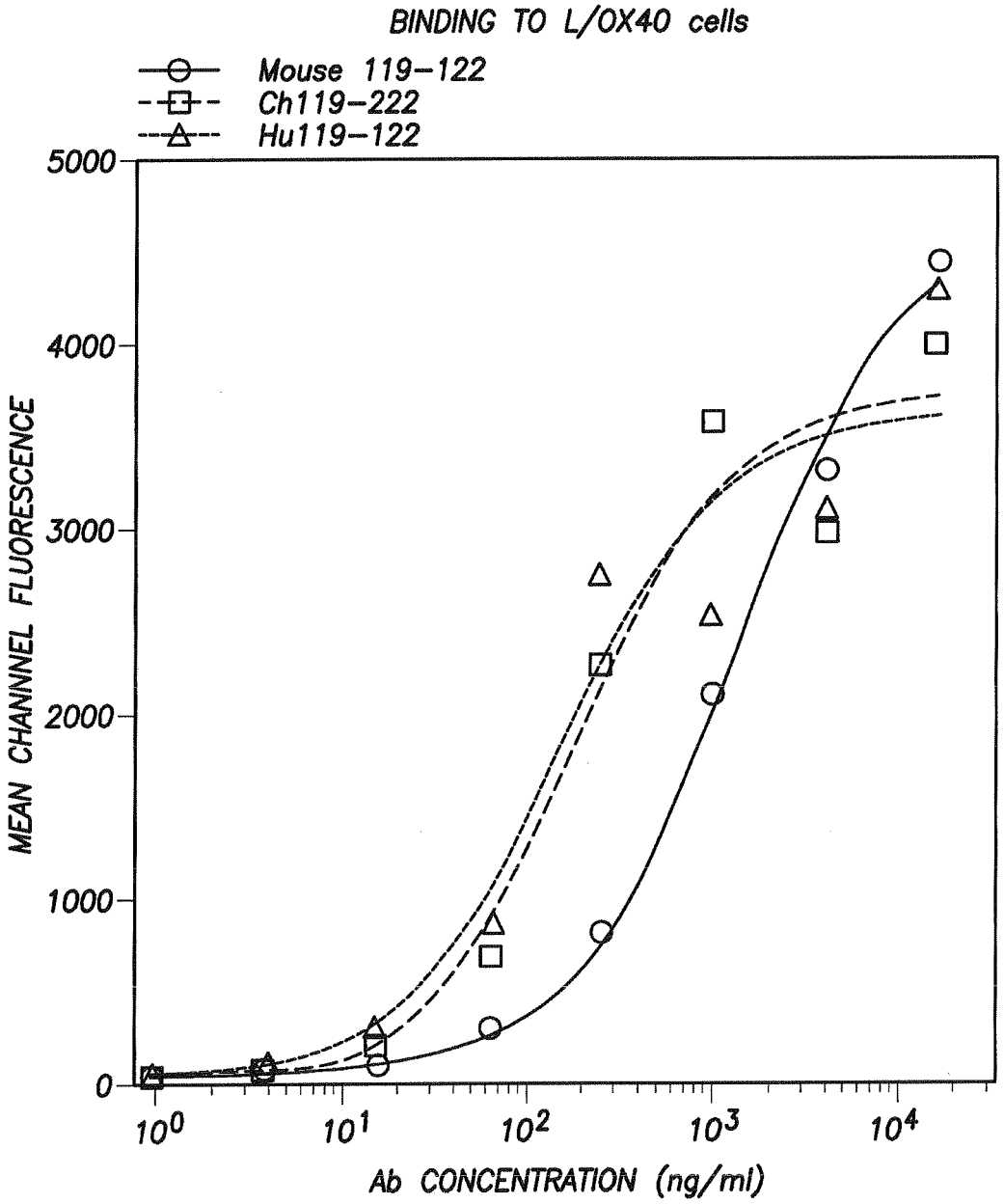

BINDING TO L/OX40 cells
Mouse 119-122
Ch119-222
Hu119-122
MEAN CHANNNEL FLUORESCENCE
5000
4000
3000
2000
1000
0
10^0
10^1
10^2
10^3
10^4
Ab CONCENTRATION (ng/ml)

FIG.26

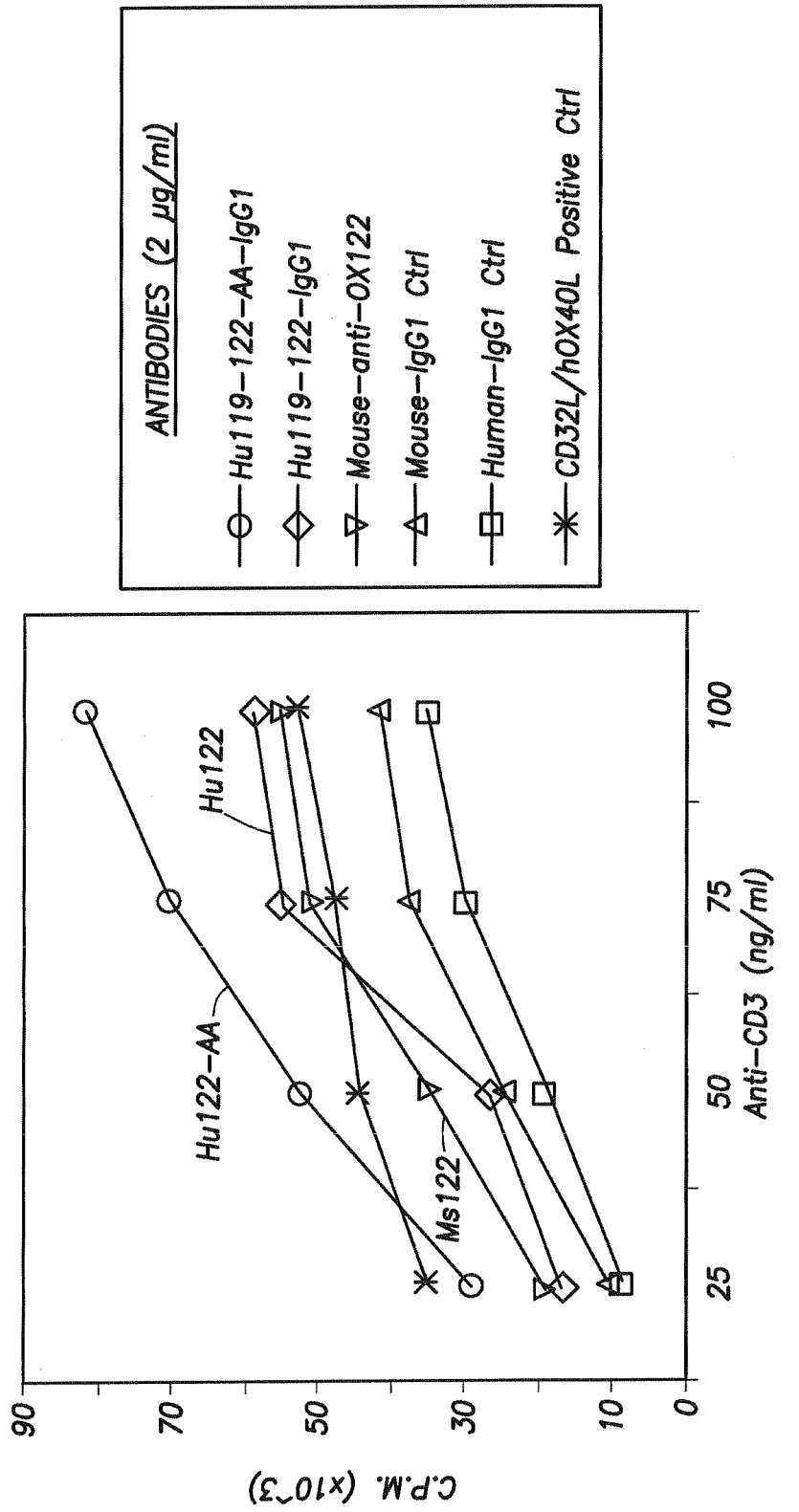

ANTIBODIES (2 µg/ml)
Hu119-122-AA-IgG1
Hu119-122-IgG1
Mouse-anti-OX122
Mouse-IgG1 Ctrl
Human-IgG1 Ctrl
CD32L/hOX40L Positive Ctrl
L cell SYSTEM
C.P.M. (x10^3)
0
10
30
50
70
90
25
50
75
100
Anti-CD3 (ng/ml)
Hu122
Hu122-AA
Ms122

FIG.27

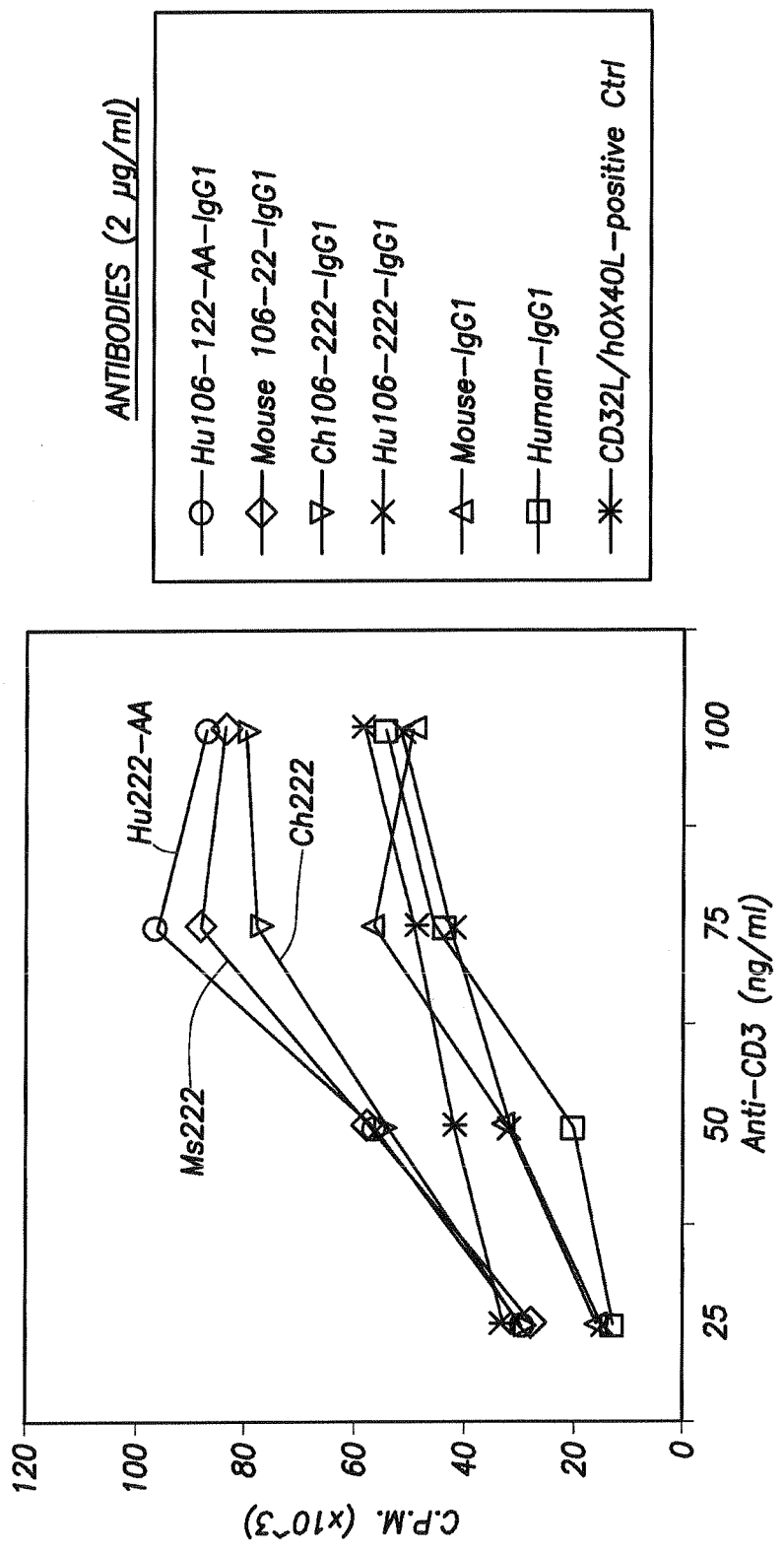

ANTIBODIES (2 µg/ml)
Hu106-122-AA-IgG1
Mouse 106-22-IgG1
Ch106-222-IgG1
Hu106-222-IgG1
Mouse-IgG1
Human-IgG1
CD32L/hOX40L-positive Ctrl
L cell SYSTEM
C.P.M. (x10^3)
120
100
80
60
40
20
0
25
50
75
100
Anti-CD3 (ng/ml)
Hu222-AA
Ch222
Ms222

FIG.28

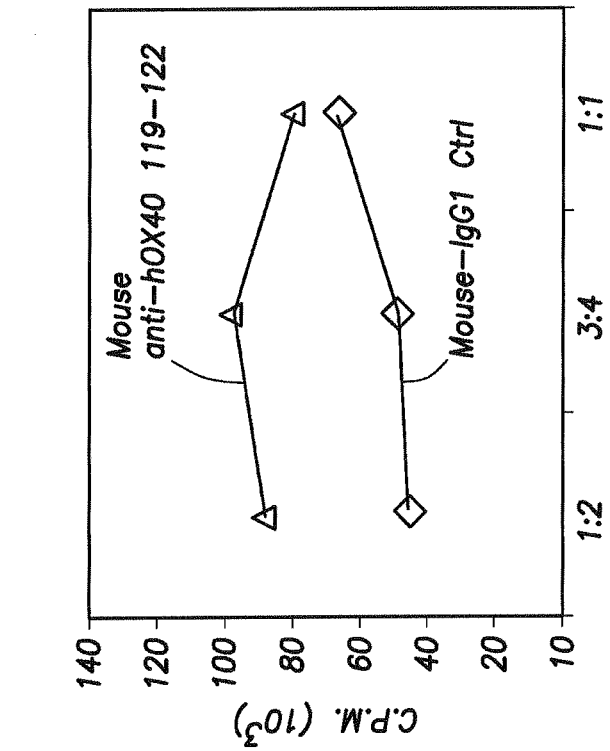


FIG.29B

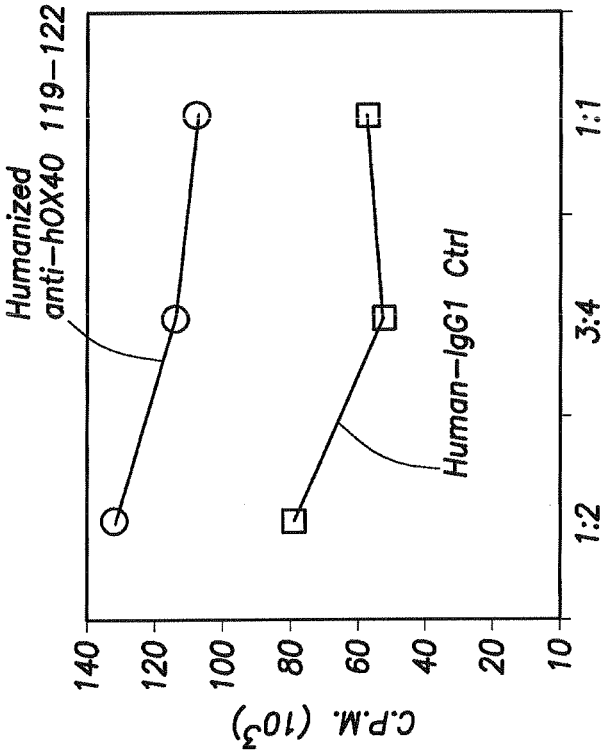


FIG.29A

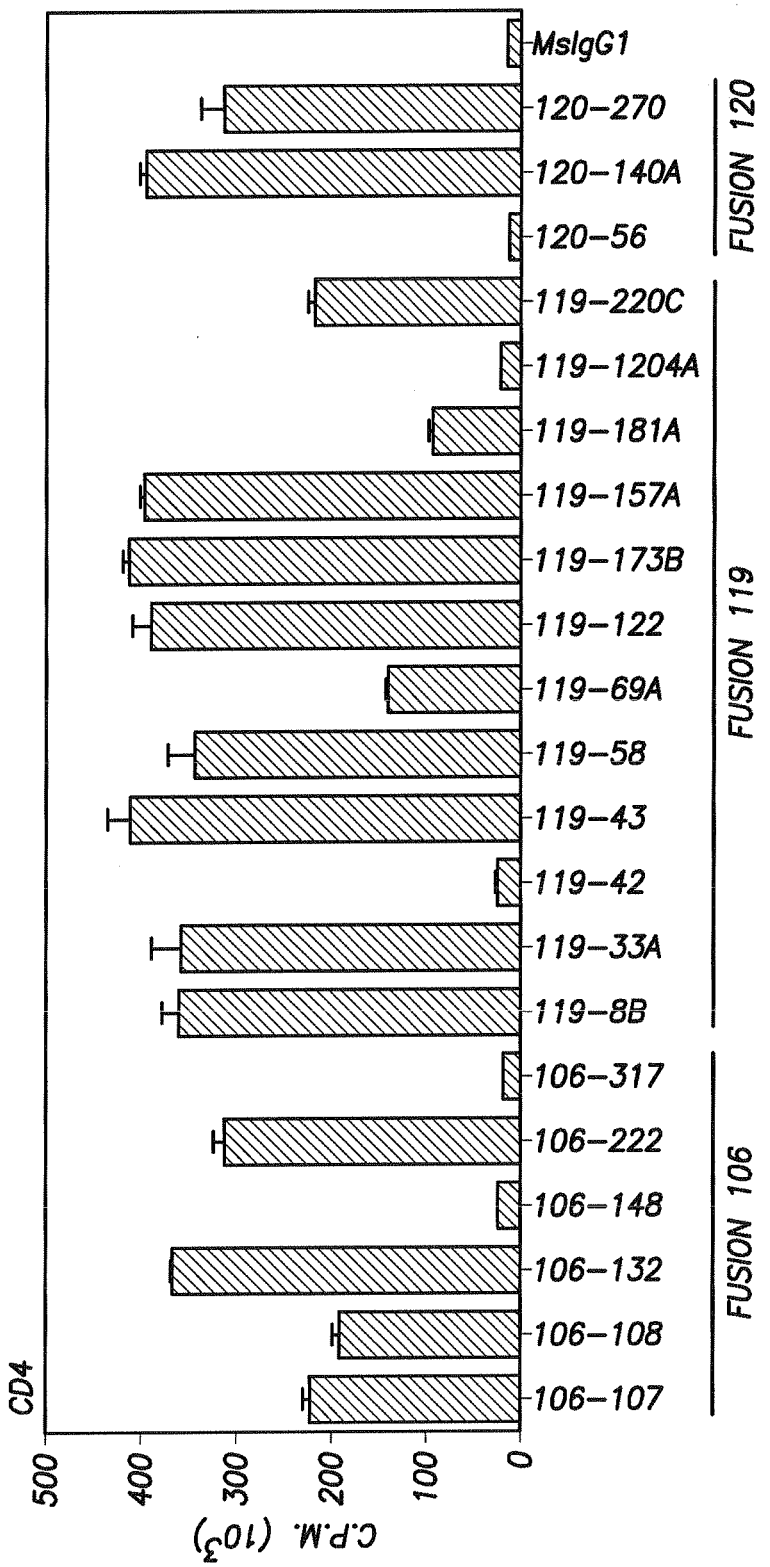

MsIgG1
120-270
120-140A
120-56
119-220C
119-1204A
119-181A
119-157A
119-173B
119-122
119-69A
119-58
119-43
119-42
119-33A
119-8B
106-317
106-222
106-148
106-132
106-108
106-107
FUSION 120
FUSION 119
FUSION 106
CD4
C.P.M. (10^3)
500
400
300
200
100
0

FIG.30A

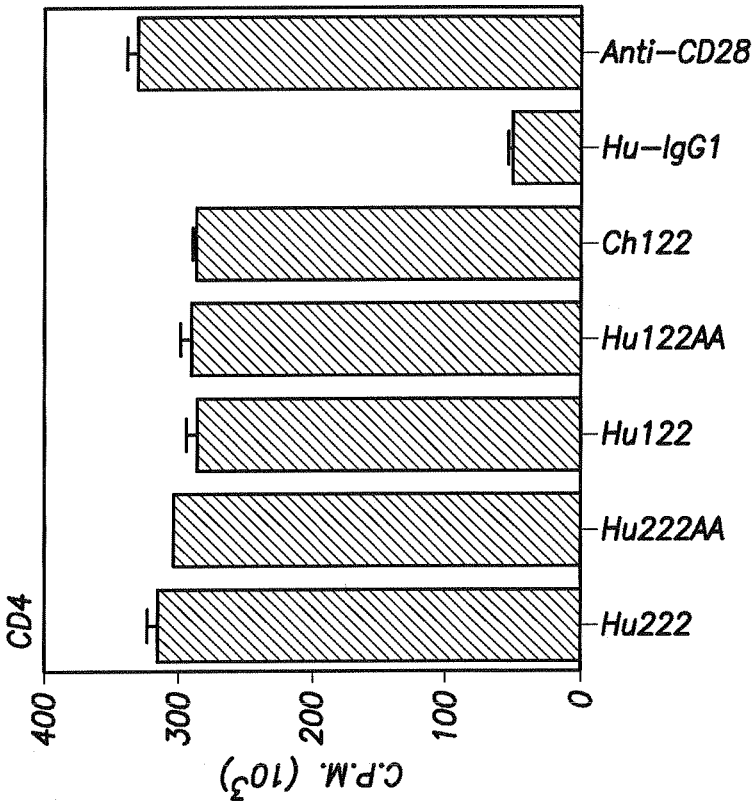


FIG.30C

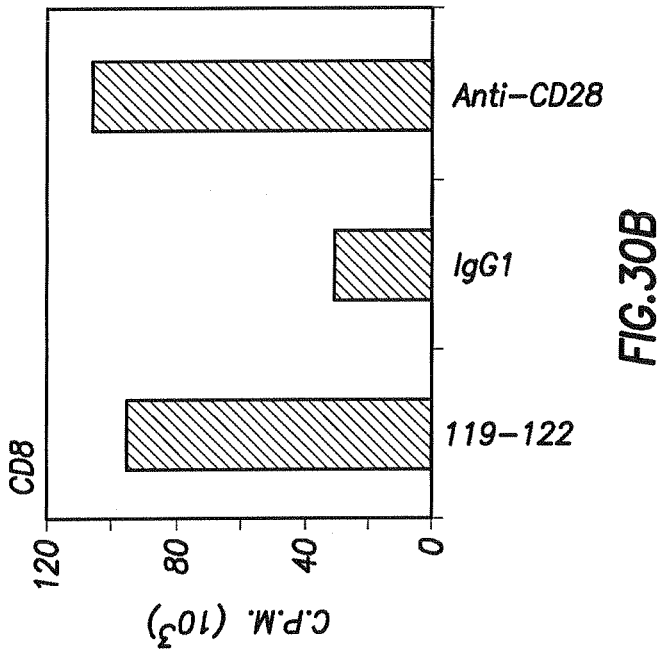


FIG.30B

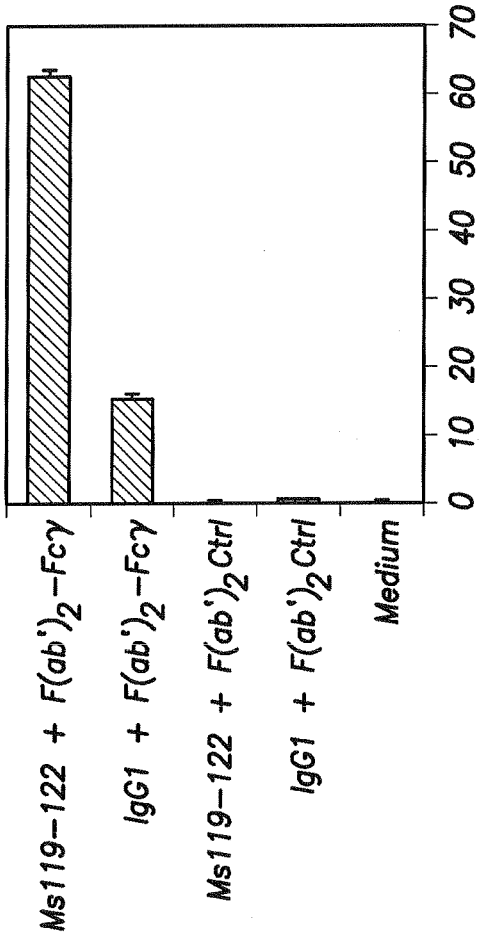


FIG.31B

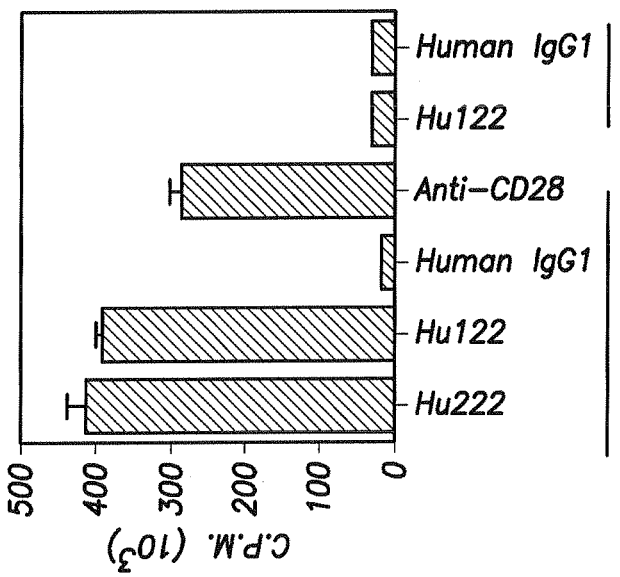


FIG.31A

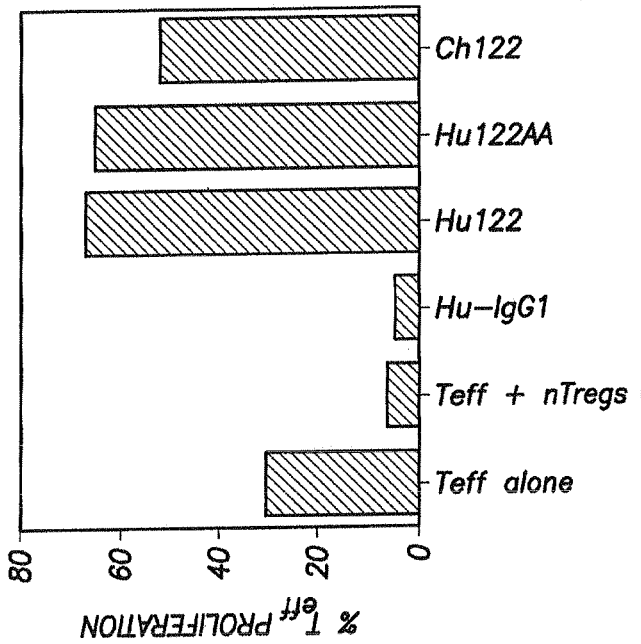


FIG.32C

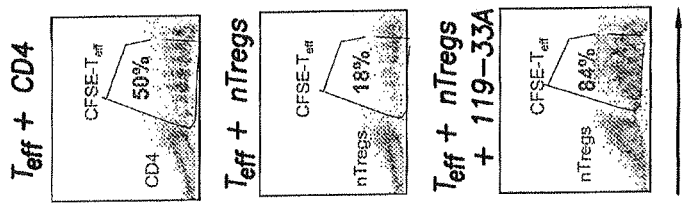


FIG.32A

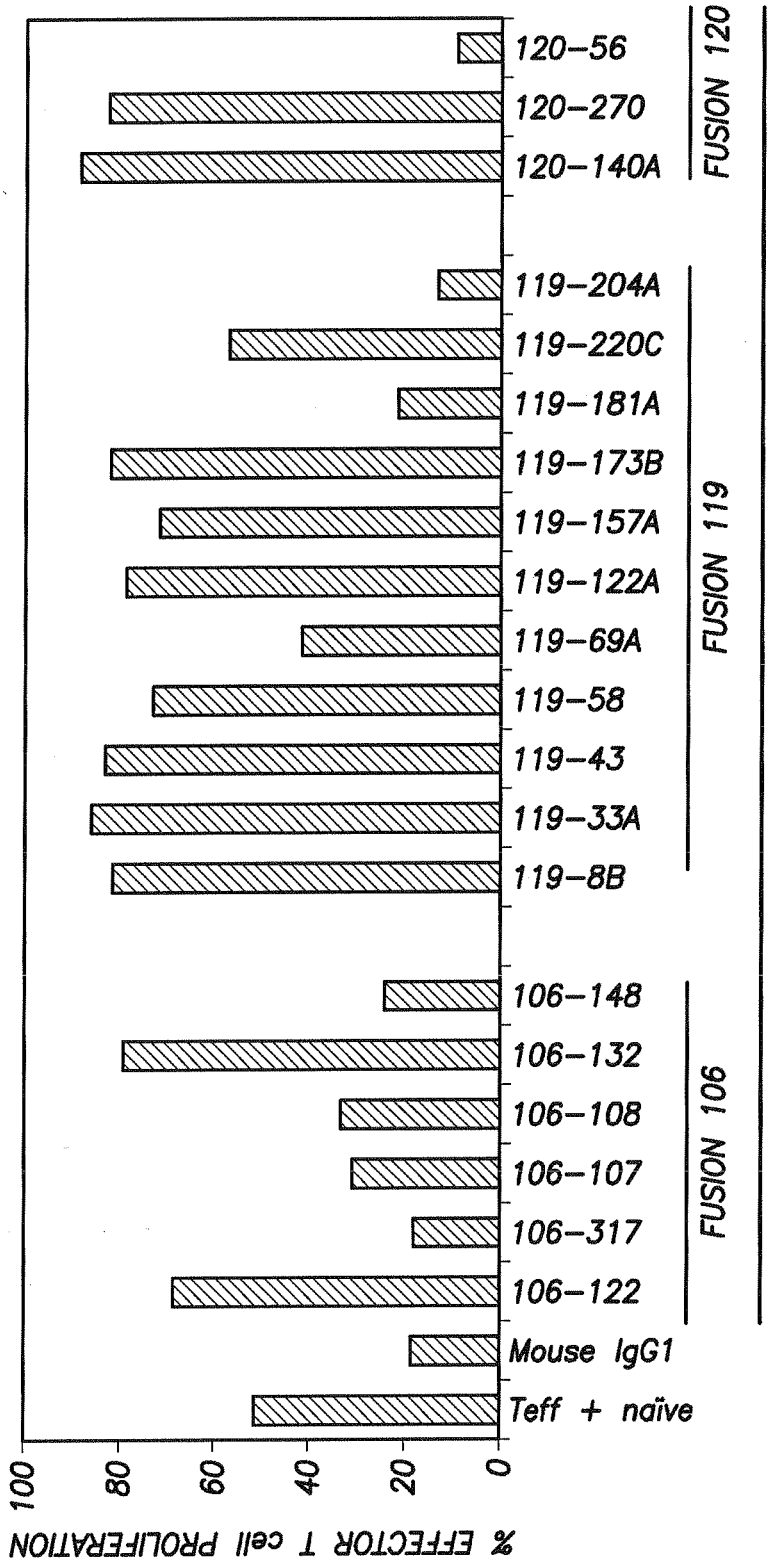

120–56
120–270
120–140A
119–204A
119–220C
119–181A
119–173B
119–157A
119–122A
119–69A
119–58
119–43
119–33A
119–8B
106–148
106–132
106–108
106–107
106–317
106–122
Mouse IgG1
Teff + naïve
FUSION 120
FUSION 119
FUSION 106
Teff + nTregs + mouse anti–hOX40 mAbs
100
80
60
40
20
0
% EFFECTOR T cell PROLIFERATION

FIG.32B

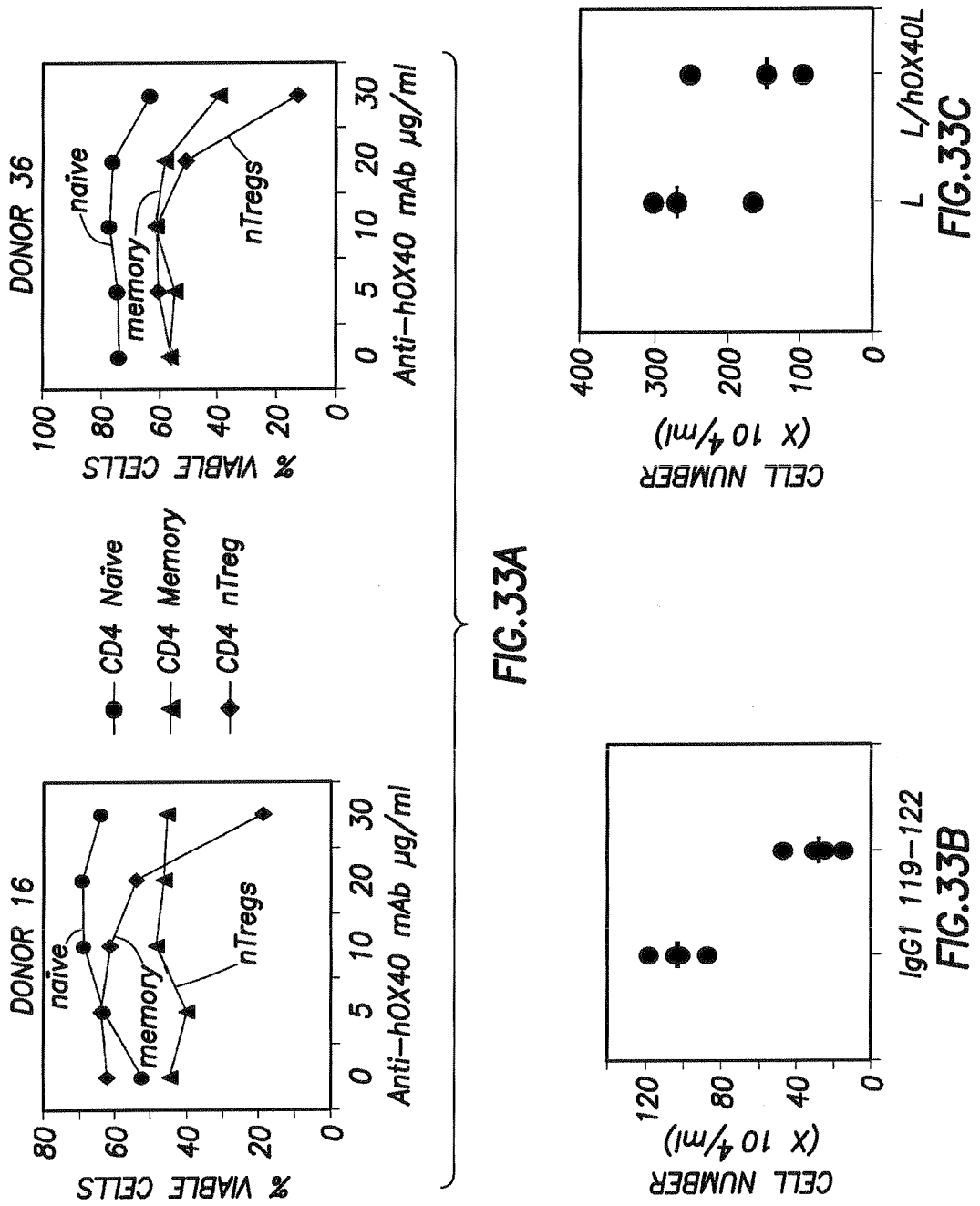


FIG.33A

FIG.33B

FIG.33C

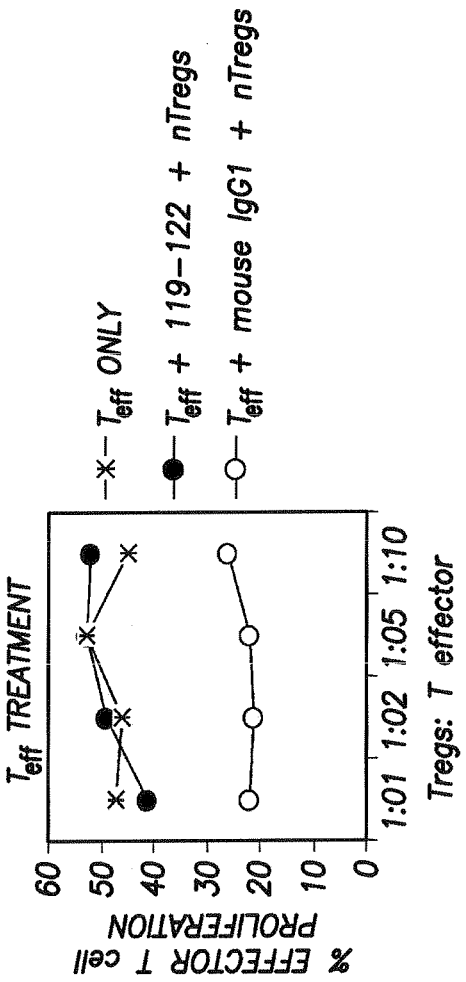


FIG.34A

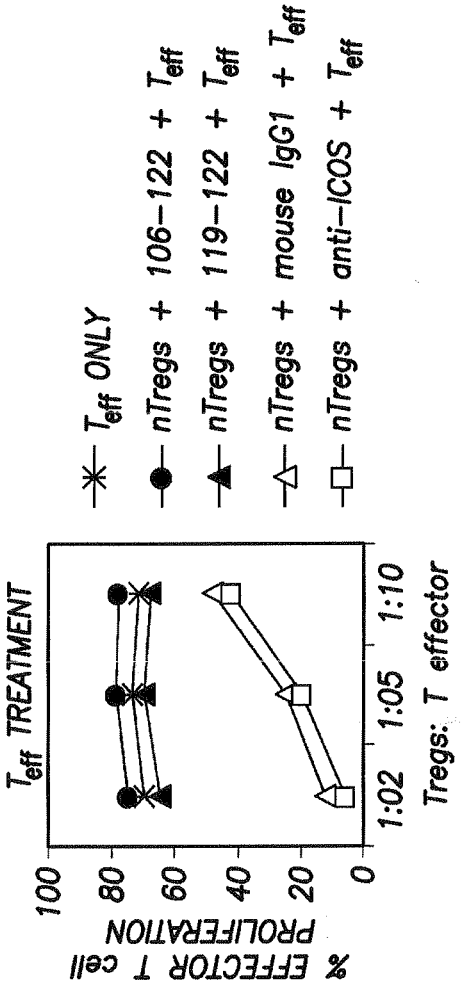


FIG.34B

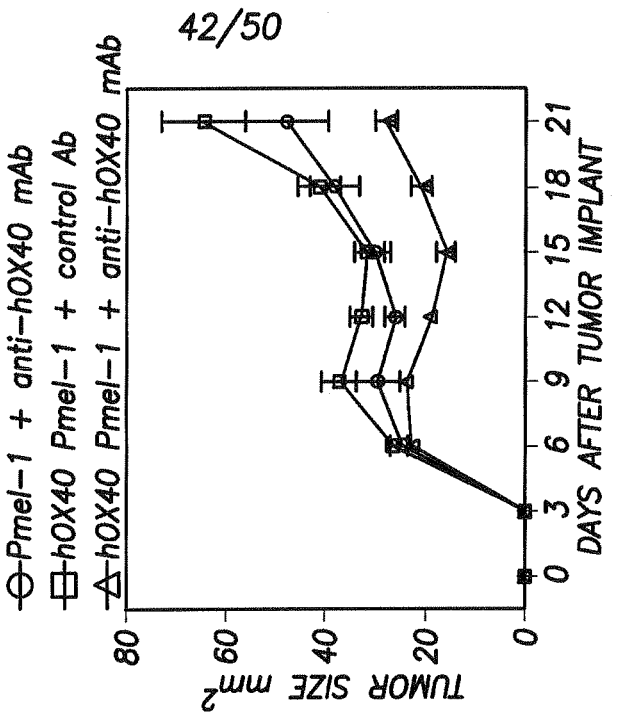


FIG.35C

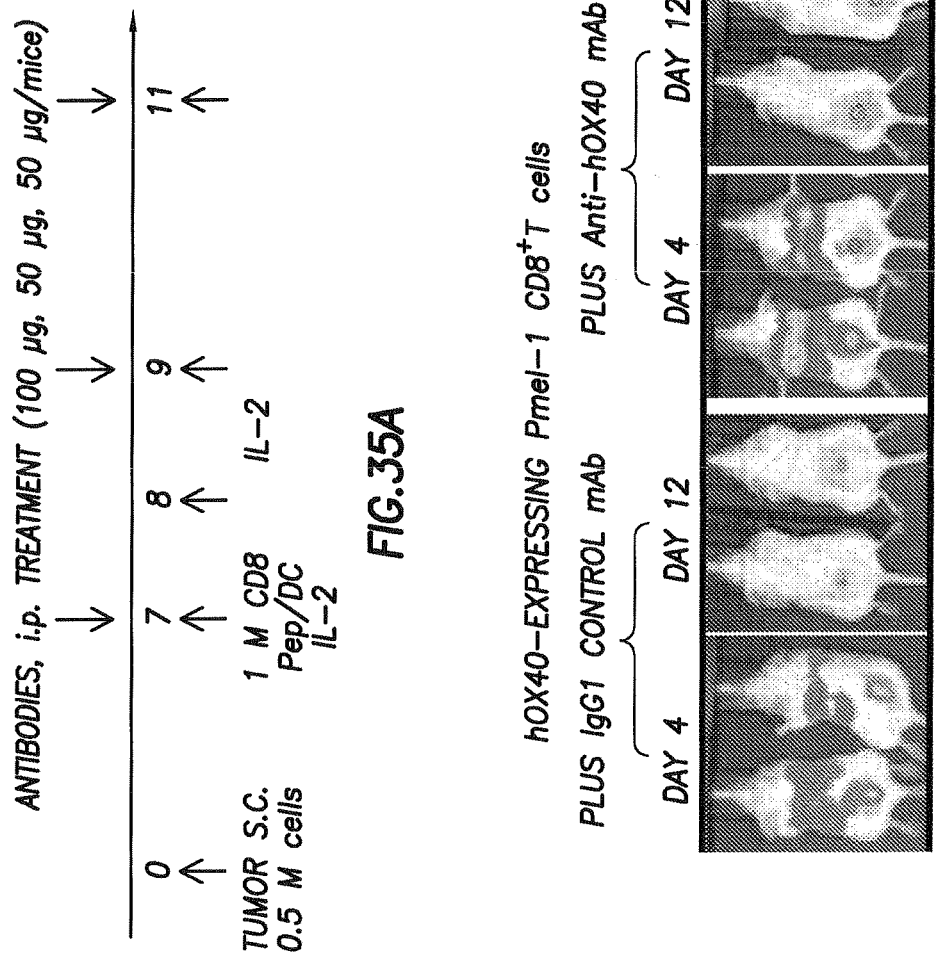


FIG.35A

FIG.35B

106–222 VH

```
                    1         2         3
          123456789 0123456789 0123456789      CDR1 SEQ ID No.1
SEQ ID No.4 106-222 VH QIQLVQSGP ELKKPGETVK ISCKASGYTF TDYSMHWVKQ
SEQ ID No.5 Hu106 VH QVQLVQSGS ELKKPGASVK VSCKASGYTF TDYSMHWVRQ
            X61012   QVQLVQSGS ELKKPGASVK VSCKASGYTF T----WVRQ

                    4         5         6         7
          0123456789 0122345678 9012345678 9012345678
                       a      CDR2 SEQ1D No.2
106-222 VH APGKGLKWMG WINTETGEPTY ADDFKGRFAF SLETSASTAY
Hu106 VH  APGQGLKWMG WINTETGEPTY ADDFKGRFVF SLDTSVSTAY
X61012    APGQGLEWMG ---------- -------RFVF SLDTSVSTAY

                              1         1         1
          8         9         0         1         1
          0122223456789 0123456789 0000001234567890123
             abc              abcde      CDR3 SEQ ID No.3
106-222 VH LQINNLKNEDTAT YFCANPYYDY VSYYAMDYWGHGTSV TVSS
Hu106 VH  LQISSLKAEDTAV YYCANPYYDY VSYYAMDYWGQGTTV TVSS
X61012    LQISSLKAEDTAV YYCAR------ ---------WGKGTTV TVSS
```

FIG.36

106-222 VL

CDR1 SEQ ID No.7

```
                                1         2         3
                      123456789 0123456789 0123456789
SEQ ID No.10 106-222 VL DIVMTQSHK FMSTSVRDRV SITCKASQDV STAVAWYQQK
SEQ ID No.11 Hu106 VL   DIQMTQSPS SLSASVGDRV TITCKASQDV STAVAWYQQK
             AJ388641   DIQMTQSPS SLSASVGDRV TITC------ -----WYQQK
```

CDR2 SEQ ID No.8

```
                      4         5         6         7
                      0123456789 0123456789 0123456789 0123456789
106-222 VL            PGQSPKLLIY SASYLYTGVP DRFTGSGSGT DFTFTISSVQ
Hu106 VL              PGKAPKLLIY SASYLYTGVP SRFSGSGSGT DFTFTISSLQ
AJ388641              PGKAPKLLIY --------GVP SRFSGSGSGT DFTFTISSLQ
```

CDR3 SEQ ID No.9

```
                                1         2
                      123456789 0123456789 01234567
106-222 VL            AEDLAVYYCQ QHYSTPRTFG GGTKLEIK
Hu106 VL              PEDIATYYCQ QHYSTPRTFG QGTKLEIK
AJ388641              PEDIATYYC- --------FG QGTKLEIK
```

FIG.37

SEQ ID No.6 *Hu106-222 VH*

SpeI

ACTAGTACCACCATGGCTTGGGTGTGGACCTTGCTATTCCTGATGGCAGCTGCCCAAAGT

M A W V W T L L F L M A A A Q S

ATCCAAGCACAGGTTCAGTTGGTGCAGTCTGGATCTGAGCTGAAGAAGCCTGGAGCCTCA

I Q A Q V Q L V Q S G S E L K K P G A S

GTCAAGGTTTCCTGCAAGGCTTCTGGTTATACCTTCACAGACTATTCAATGCACTGGGTG

V K V S C K A S G Y T F T D Y S M H W V

CGACAGGCTCCAGGACAAGGTTTAAAGTGGATGGGCTGGATAAACACTGAGACTGGTGAG

R Q A P G Q G L K W M G W I N T E T G E

CCAACATATGCAGATGACTTCAAGGGACGGTTTGTCTTCTCTTTGGACACCTCTGTCAGC

P T Y A D D F K G R F V F S L D T S V S

ACTGCCTATTTGCAGATCAGCAGCCTCAAAGCTGAGGACACGGCTGTGTATTACTGTGCT

T A Y L Q I S S L K A E D T A V Y Y C A

AATCCCTACTATGATTACGTCTCTTACTATGCTATGGACTACTGGGGTCAGGGAACCACG

N P Y Y D Y V S Y Y A M D Y W G Q G T T

HindIII

GTCACCGTCTCCTCAG*GTAAGAATGGCCTCTCAAGCTT*

V T V S S

FIG.38

SEQ ID No.12 Hu106-222 VL

NheI

GCTAGCCACCACCATGGAGTCACAGATTCAGGTCTTTGTATTCGTGTTTCTCTGGTTGTCT

M E S Q I Q V F V F V F L W L S

GGTGTTTGACGGAGACATTCAGATGACCCAGTCTCCATCCTCCCTGTCCGCATCAGTGGGA

G V D G D I Q M T Q S P S S L S A S V G

GACAGGGTCACCATCACCTGCAAGGCCAGTCAGGATGTGAGTACTGCTGTAGCCTGGTAT

D R V T I T C K A S Q D V S T A V A W Y

CAACAGAAACCAGGAAAAGCCCCTAAACTACTGATTTACTCGGCATCCTACCTCTACACT

Q Q K P G K A P K L L I Y S A S Y L Y T

GGAGTCCCTTCACGCTTCAGTGGCAGTGGATCTGGGACGGATTTCACTTTCACCATCAGC

G V P S R F S G S G S G T D F T F T I S

AGTCTGCAGCCTGAAGACATTGCAACATATTACTGTCAGCAACATTATAGTACTCCTCGG

S L Q P E D I A T Y Y C Q Q H Y S T P R

EcoRI

ACGTTCGGTCAGGGCACCAAGCTGGAAATCAAACGTAAGTAGAATCCAAAGAATTC

T F G Q G T K L E I K

FIG.39

119–122 VH

```
                  1         2         3
         123456789 0123456789 0123456789 0123456789
SEQ ID No.16 119-122 VH EVQLVESGG GLVQPGESLK LSCESNEYEF PSHDMSWVRK CDR1 SEQ ID No.13
SEQ ID No.17 Hu119 VH   EVQLVESGG GLVQPGGSLR LSCAASEYEF PSHDMSWVRQ
             Z14189     EVQLVESGG GLVQPGGSLR LSCAASGFTF S----WVRQ

                  4         5         6         7
         0123456789 0122345678 9012345678 9012345678
                       a          CDR2 SEQ ID No.14
119-122 VH TPEKRLELVA AINSDGGSTYY PDTMERRFII SRDNTKKTLY
Hu119 VH   APGKGLELVA AINSDGGSTYY PDTMERRFTI SRDNAKNSLY
Z14189     APGKGLEWVA ---------- -------RFTI SRDNAKNSLY

                  1         1         1
         8901234567 8901234567 8901234567 8901234567 0123
                  abc       abc       CDR3 SEQ ID No.15
119-122 VH LQMSSLRSEDTAL YYCARHYDDY YAWFAYWGQGTLV TVSA
Hu119 VH   LQMNSLRAEDTAV YYCARHYDDY YAWFAYWGQGTMV TVSS
Z14189     LQMNSLRAEDTAV YYCAR----- ------WGQGTMV TVSS
```

FIG.40

119–122 VL

```
                    1               2               3
          123456789 0123456789 01234567777789 0123456789
                                      abcd     CDR1 SEQ ID No.19
SEQ ID No.22 119-122 VL DIVLTQSPA SLAVSLGQRA TISCRASKSVSTSG YSYMHWYQQK
SEQ ID No.23 Hu119 VL   EIVLTQSPA TLSLSPGERA TLSCRASKSVSTSG YSYMHWYQQK
             M29469     EIVLTQSPA TLSLSPGERA TLSC---------- -----WYQQK
                                CDR2 SEQ ID No.20
                                 4       5       6       7
                                 0123456789 0123456789 0123456789
             119-122 VL         PGQPPKLLIY LASNLESGVP ARFSGSGSGT DFTLNIHPVE
             Hu119 VL           PGQAPRLLIY LASNLESGVP ARFSGSGSGT DFTLTISSLE
             M29469             PGQAPRLLIY -------GVP ARFSGSGSGT DFTLTISSLE
                                CDR3 SEQ ID No.21
                                         8       9       0       1
                                         0123456789 0123456789 01234567
             119-122 VL                 EEDAATYYCQ HSRELPLTFG AGTKLELK
             Hu119 VL                   PEDFAVYYCQ HSRELPLTFG GGTKVEIK
             M29469                     PEDFAVYYC- ---------FG GGTKVEIK
```

FIG.41

SEQ ID No.18 Hu119-122 VH

SpeI

ACTAGTACCACCATGGACTTCGGGCTCAGCTTGGTTTTCCTTGTCCTTATTTTAAAAAGT

M D F G L S L V F L V L I L K S

GTACAGTGTGAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCC

V Q C E V Q L V E S G G G L V Q P G G S

CTGAGACTCTCCTGTGCAGCCTCTGAATACGAGTTCCCTTCCCATGACATGTCTTGGGTC

L R L S C A A S E Y E F P S H D M S W V

CGCCAGGCTCCGGGGAAGGGGCTGGAGTTGGTCGCAGCCATTAATAGTGATGGTGGTAGC

R Q A P G K G L E L V A A I N S D G G S

ACCTACTATCCAGACACCATGGAGAGACGATTCACCATCTCCAGAGACAATGCCAAGAAC

T Y Y P D T M E R R F T I S R D N A K N

TCACTGTACCTGCAAATGAACAGTCTGAGGGCCGAGGACACAGCCGTGTATTACTGTGCA

S L Y L Q M N S L R A E D T A V Y Y C A

AGACACTATGATTACTACGCCTGGTTTGCTTACTGGGGCCAAGGGACTATGGTCACT

R H Y D D Y Y A W F A Y W G Q G T M V T

HindIII

GTCTCTTCAGGTGAGTCCTAACTTCAAGCTT

V S S

FIG.42

SEQ ID No.24 119–122 VL

NheI
GCTAGCACCACCATGGAGACAGACACACTCCTGTTATGGGTACTGCTGCTCTGGGTTCCA
M E T D T L L L L W V L L L W V P

GGTTCCACTGGTGAAATTGTGCTGACACAGTCTCCTGCTACCTTATCTTTGTCTCCAGGG
G S T G E I V L T Q S P A T L S L S P G

GAAAGGGCCACCCTCTCATGCAGGGCCAGCAAAAGTGTCAGTACATCTGGCTATAGTTAT
E R A T L S C R A S K S V S T S G Y S Y

ATGCACTGGTACCAACAGAAACCAGGACAGGCTCCCAGACTCCTCATCTATCTTGCATCC
M H W Y Q Q K P G Q A P R L L I Y L A S

AACCTAGAATCTGGGGTCCCTGCCAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCACC
N L E S G V P A R F S G S G S G T D F T

CTCACCATCAGCAGCCTAGAGCCTGAGGATTTTGCAGTTTATTACTGTCAGCACAGTAGG
L T I S S L E P E D F A V Y Y C Q H S R

GAGCTTCCGCTCACGTTCGGCGGAGGGACCAAGGTCGAGATCAAACGTAAGTACACTTTT
E L P L T F G G G T K V E I K

EcoRI
CTGAATTC

FIG.43