```
                                  10        20        30        40        50
                                  ....|....|....|....|....|....|....|....|....|
Human\TIGIT\NP_776160.2           ------MMTGTIETTGNISAEKGGSIILQCHLSSTTAQVTQVNWEQQDQ-
Cynomolgus\TIGIT\XP_005548157.1   ------..............K....V.........M..........H.HS
Mouse\TIGIT\NP_001139797.1        AFLATGATA...D.KR.....E...V.....F..D..E....D.K....-
Rat\TIGIT\XP_008766987.1          AFLAAGATA..M..K......E...VV....F..D..E........R..-
Clustal Consensus                       :**::*. ****:.***::****:**  *:****:*:*:*:

                                  60        70        80        90        100
                                  ....|....|....|....|....|....|....|....|....|
Human\TIGIT\NP_776160.2           LLAICNADLGWHISPSFKDRVAPGPGLGLTLQSLTVNDTGEYFCIYHTYP
Cynomolgus\TIGIT\XP_005548157.1   ....R..E.....Y.A.....................M........T.....
Mouse\TIGIT\NP_001139797.1        ....YSV.....VASV.S...V...S....F....M........T.....
Rat\TIGIT\XP_008766987.1          ...VYSV....YVPSV.S...V...S....F....T........T.....
Clustal Consensus                 ***: ..:***::   *.***.***.****:**** ******** *****

                                  110       120
                                  ....|....|....|....|....|..
Human\TIGIT\NP_776160.2           DGTYTGRIFLEVLESSVAEHGARFQIP
Cynomolgus\TIGIT\XP_005548157.1   ....R..............S......
Mouse\TIGIT\NP_001139797.1        G.I.K.....K.Q.....QFQTAP---
Rat\TIGIT\XP_008766987.1          ..I.K.....K.Q...AH-FQIA----
Clustal Consensus                  * * *****:* ***.  .
```

FIG. 1A

```
Human\PVRL4\ECD\Q96NY8          ---GELETSDVVTVVLGQDAKLPCFYRGDSGEQVGQVAWARVDAGEGAQELALLHSKYGL
Human\TIGIT\ECD\NP_776160.2     MMTGTIETTGNISAEKGGSIILQCHLSS-TTAQVTQVNWEQQ-----DQLLAICNADLGW
Clustal Consensus                  * :**:  ::.  * .  * *.  . :  ** ** * :       * **: .:. *

Human\PVRL4\ECD\Q96NY8          HVSPAYEGRVEQPPPPRNPLDGSVLLRNAVQADEGEYECRVSTFPAGSFQARLRLRVLVP
Human\TIGIT\ECD\NP_776160.2     HISPSFKDRVAPGPGLG-------LTLQSLTVNDTGEYFCIYHTYPDGTYTGRIFLEVLES
Clustal Consensus               *:**::: **   *          : *:. .  * *** *   *:* *:: .*: *.**

Human\PVRL4\ECD\Q96NY8          PLPSLNPGPALEEGQGLTLAASCTAEGSPAPSVTWDTEVKGTTSSRSFKHSRSAAVTSEF
Human\TIGIT\ECD\NP_776160.2     SVAEHGARFQIP-----------------------------------------------
Clustal Consensus                : .      :

Human\PVRL4\ECD\Q96NY8          HLVPSRSMNGQPLTCVVSHPGLLQDQRITHILHVSFLAEASVRGLEDQNLWHIGREGAML
Human\TIGIT\ECD\NP_776160.2     ------------------------------------------------------------
Clustal Consensus

Human\PVRL4\ECD\Q96NY8          KCLSEGQPPPSYNWTRLDGPLPSGVRVDGDTLGFPPLTTEHSGIYVCHVSNEFSSRDSQV
Human\TIGIT\ECD\NP_776160.2     ------------------------------------------------------------
Clustal Consensus

Human\PVRL4\ECD\Q96NY8          TVDVLDPQEDSGKQVDLVSAS
Human\TIGIT\ECD\NP_776160.2     ---------------------
```

FIG. 1B

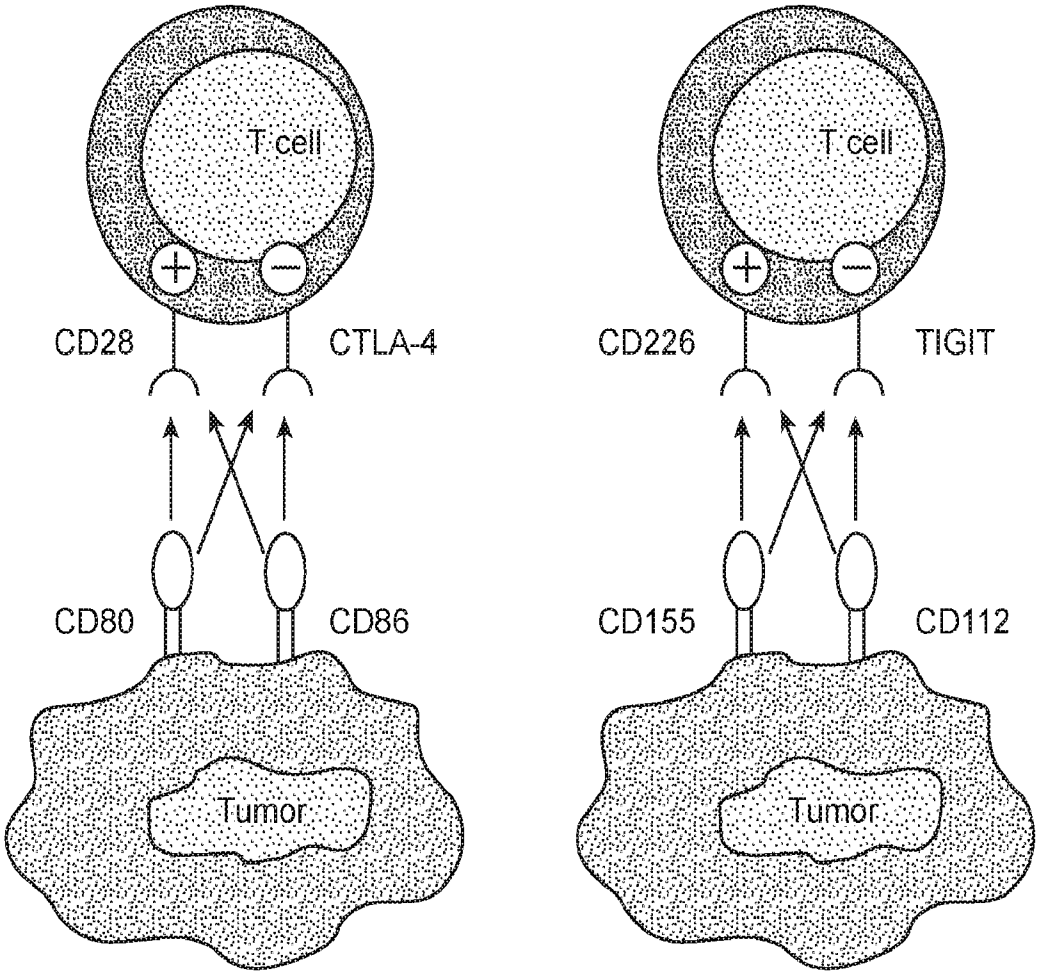
T cell
CD28
CTLA-4
CD80
CD86
Tumor
T cell
CD226
TIGIT
CD155
CD112
Tumor

FIG. 2

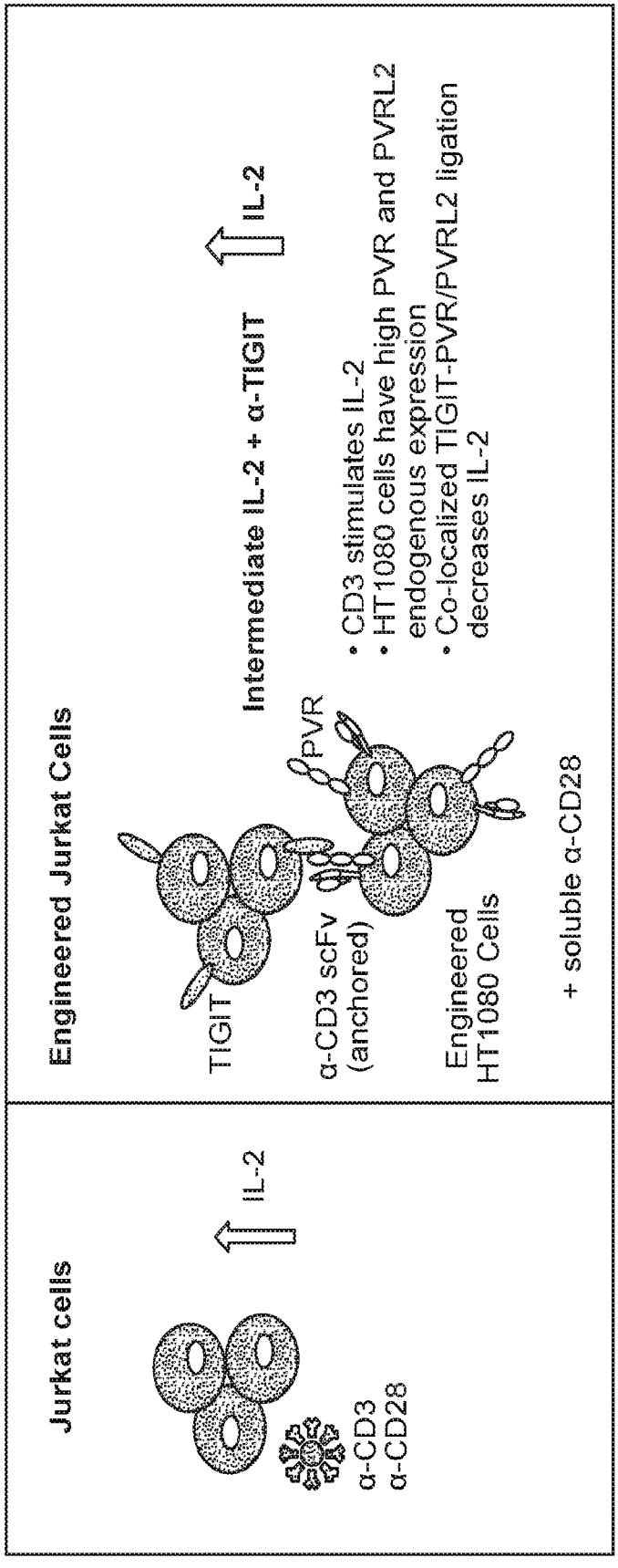
Jurkat cells
IL-2
α-CD3
α-CD28
Engineered Jurkat Cells
Intermediate IL-2 + α-TIGIT
IL-2
TIGIT
PVR
α-CD3 scFv
(anchored)
Engineered
HT1080 Cells
+ soluble α-CD28
• CD3 stimulates IL-2
• HT1080 cells have high PVR and PVRL2 endogenous expression
• Co-localized TIGIT-PVR/PVRL2 ligation decreases IL-2

FIG. 3

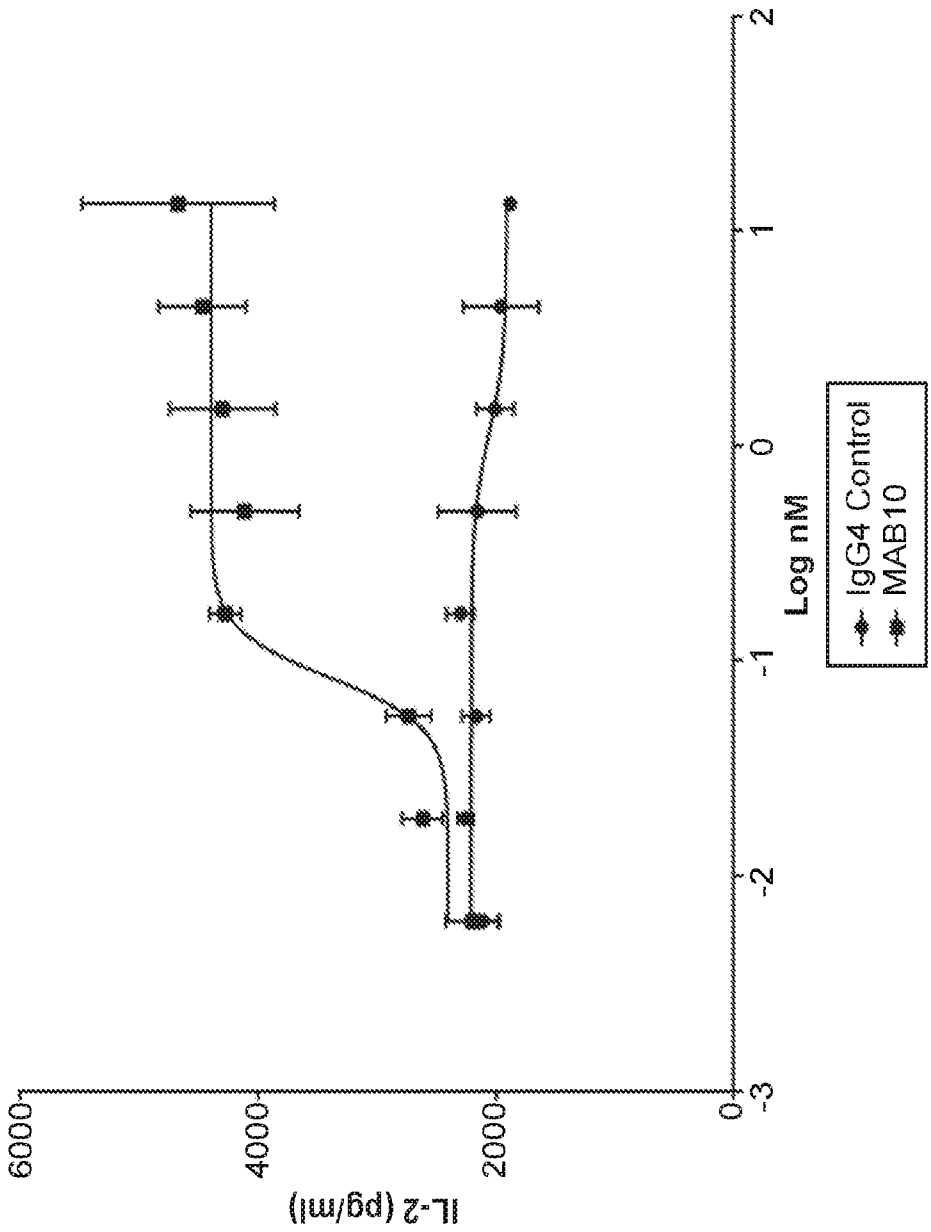

FIG. 4A

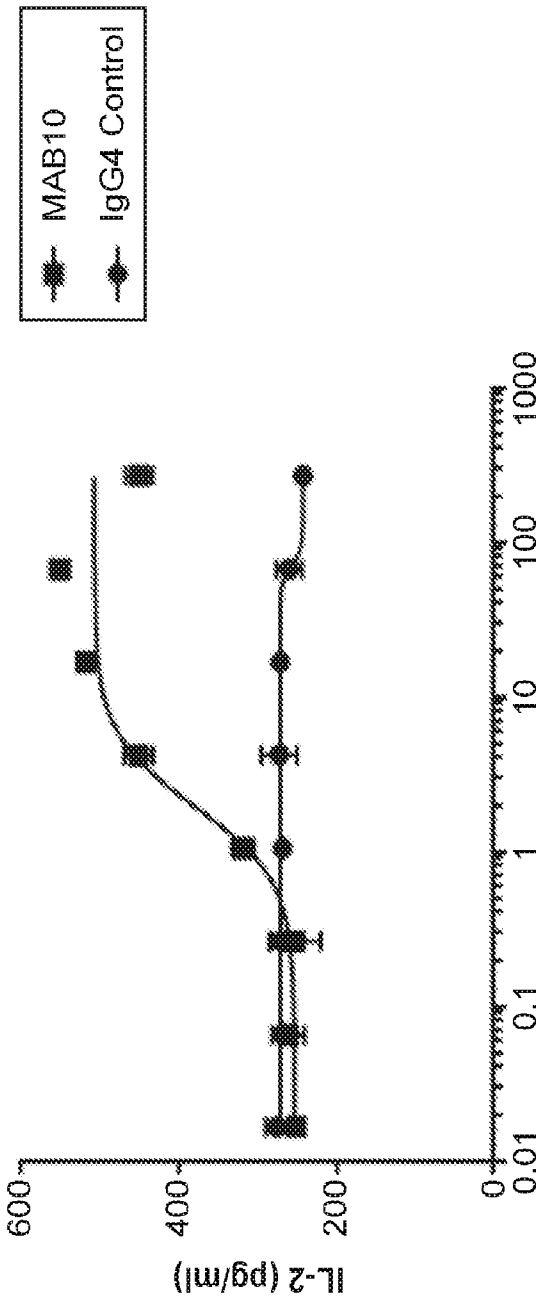
MAB10
IgG4 Control
IL-2 (pg/ml)
600
400
200
0
0.01
0.1
1
10
100
1000
Log (nM)

FIG. 4B

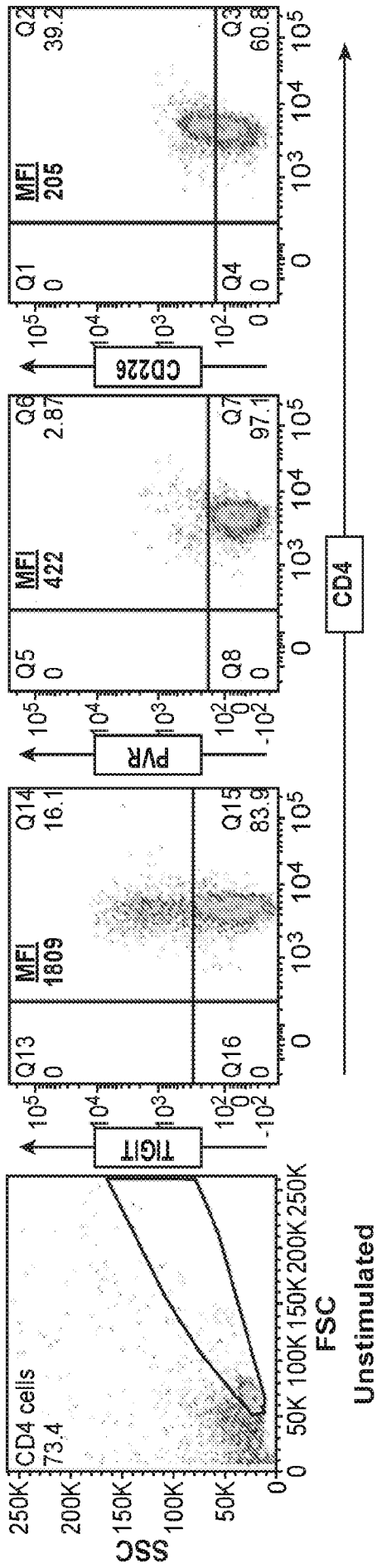
SSC
FSC
CD4 cells
73.4
TIGIT
PVR
CD226
CD4
Unstimulated
MFI
1809
MFI
422
MFI
205
Q13
Q14
Q15
Q16
Q5
Q6
Q7
Q8
Q1
Q2
Q3
Q4
16.1
83.9
2.87
97.1
39.2
60.8

FIG. 5A

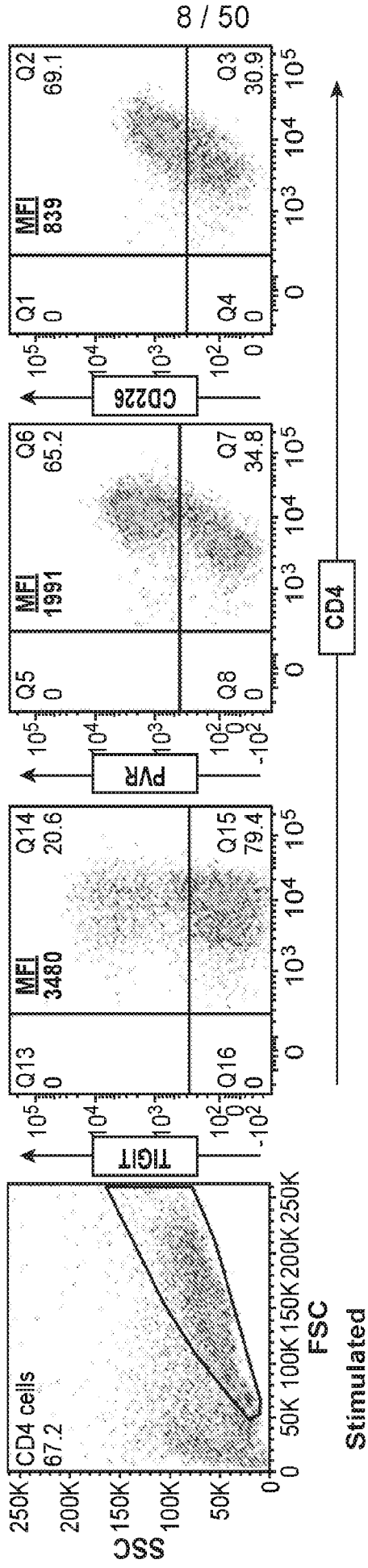
SSC
FSC
CD4 cells
67.2
TIGIT
PVR
CD226
CD4
MFI
3480
1991
839
Q13
Q14
Q15
Q16
Q5
Q6
Q7
Q8
Q1
Q2
Q3
Q4
20.6
79.4
65.2
34.8
69.1
30.9
Stimulated
1 µg/mL α-CD3 (coated)
2 µg/mL α-CD28

FIG. 5B

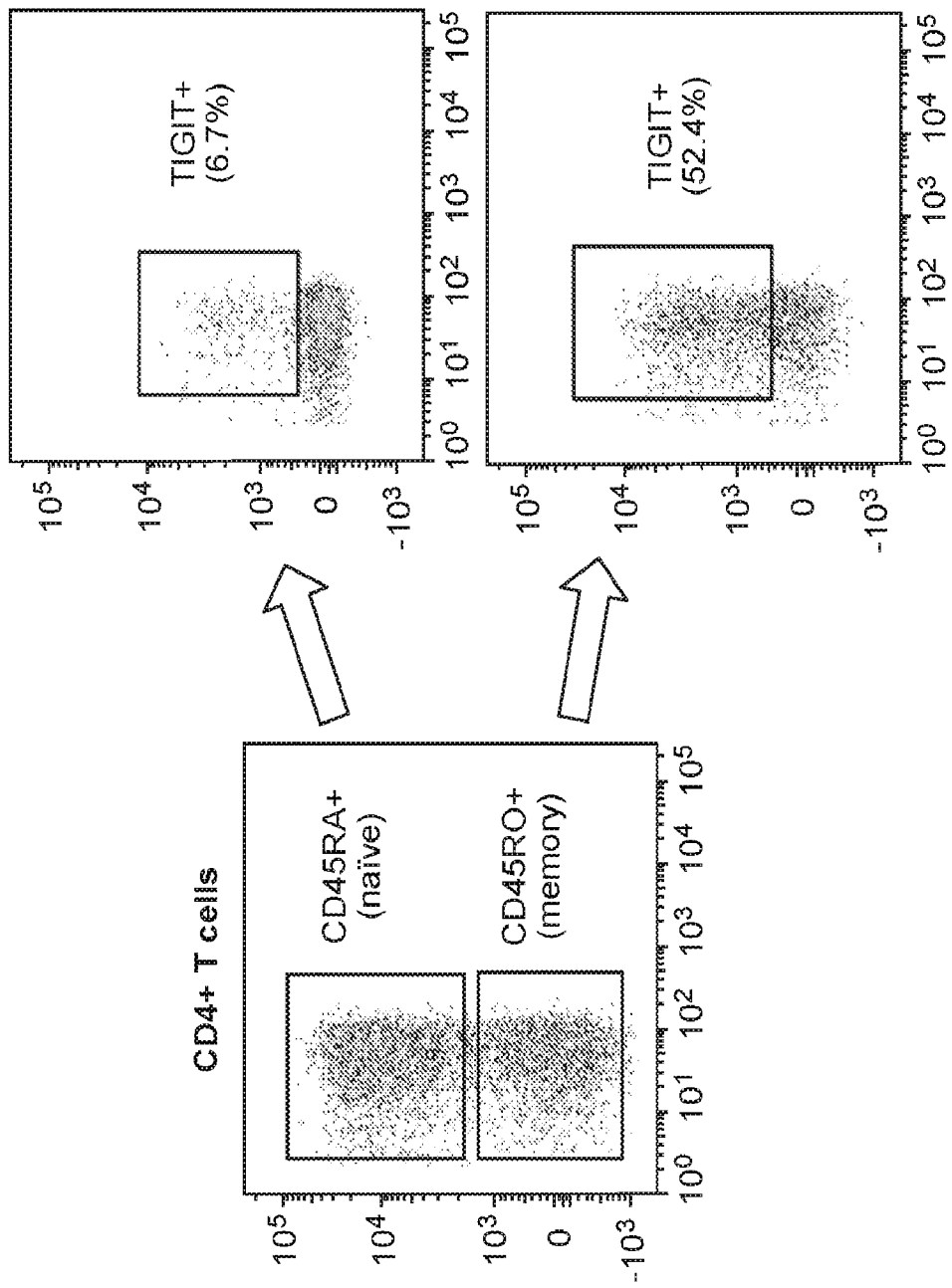

FIG. 5C

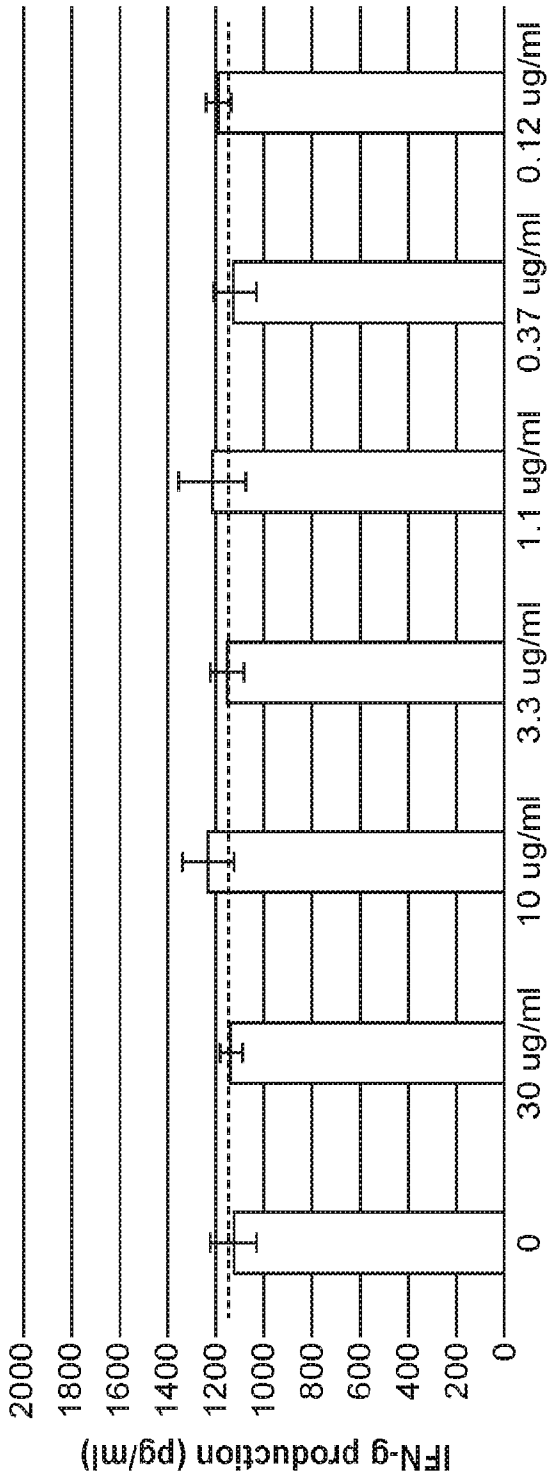
Control IgG4
IFN-g production (pg/ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
30 ug/ml
10 ug/ml
3.3 ug/ml
1.1 ug/ml
0.37 ug/ml
0.12 ug/ml
Control IgG4

FIG. 6A

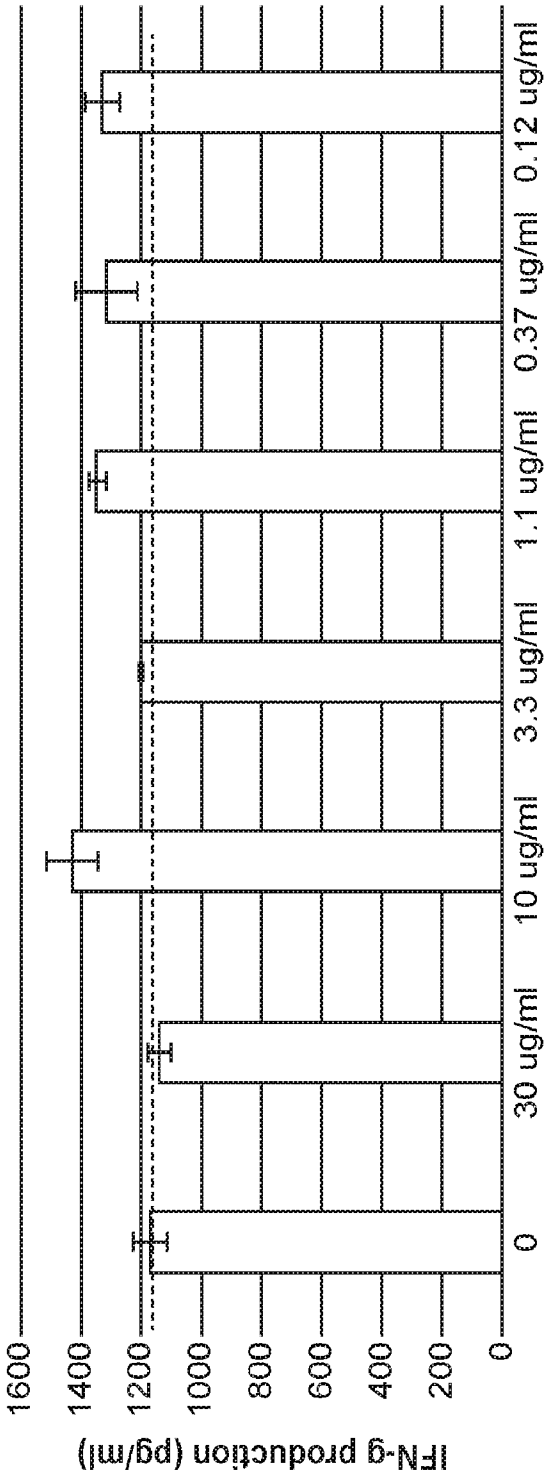
MAB2
IFN-g production (pg/ml)
1600
1400
1200
1000
800
600
400
200
0
0
30 ug/ml
10 ug/ml
3.3 ug/ml
1.1 ug/ml
0.37 ug/ml
0.12 ug/ml
MAB2

FIG. 6B

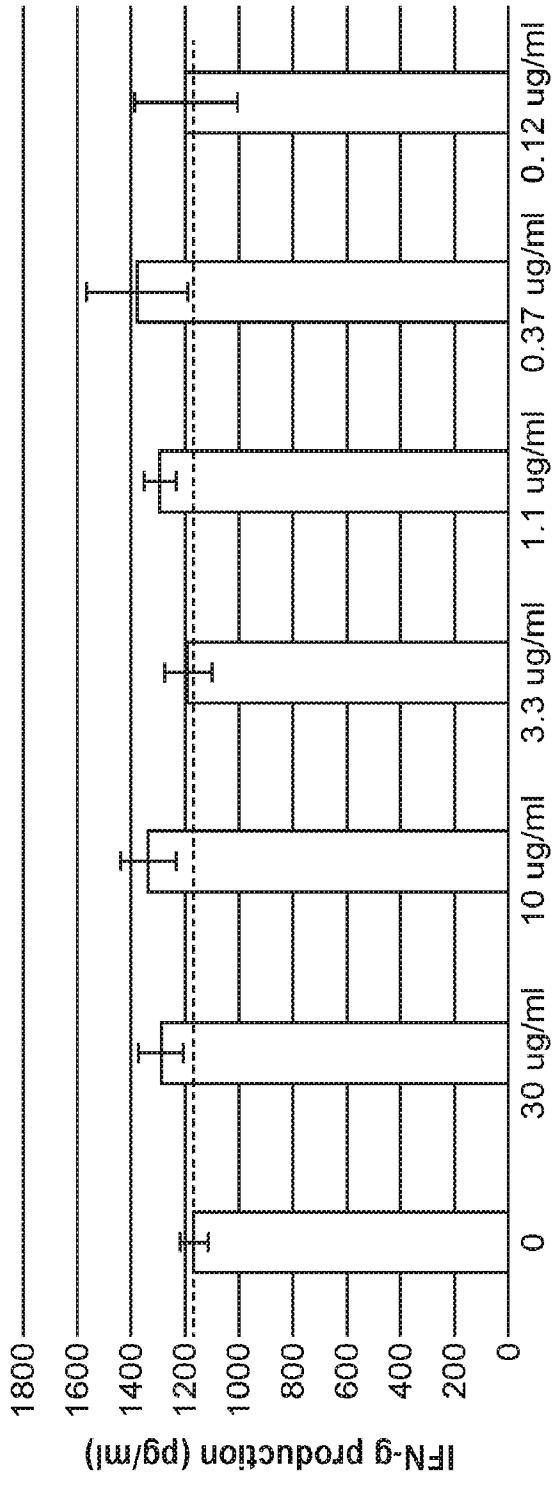
MAB3
IFN-g production (pg/ml)
1800
1600
1400
1200
1000
800
600
400
200
0
0
30 ug/ml
10 ug/ml
3.3 ug/ml
1.1 ug/ml
0.37 ug/ml
0.12 ug/ml
MAB3

FIG. 6C

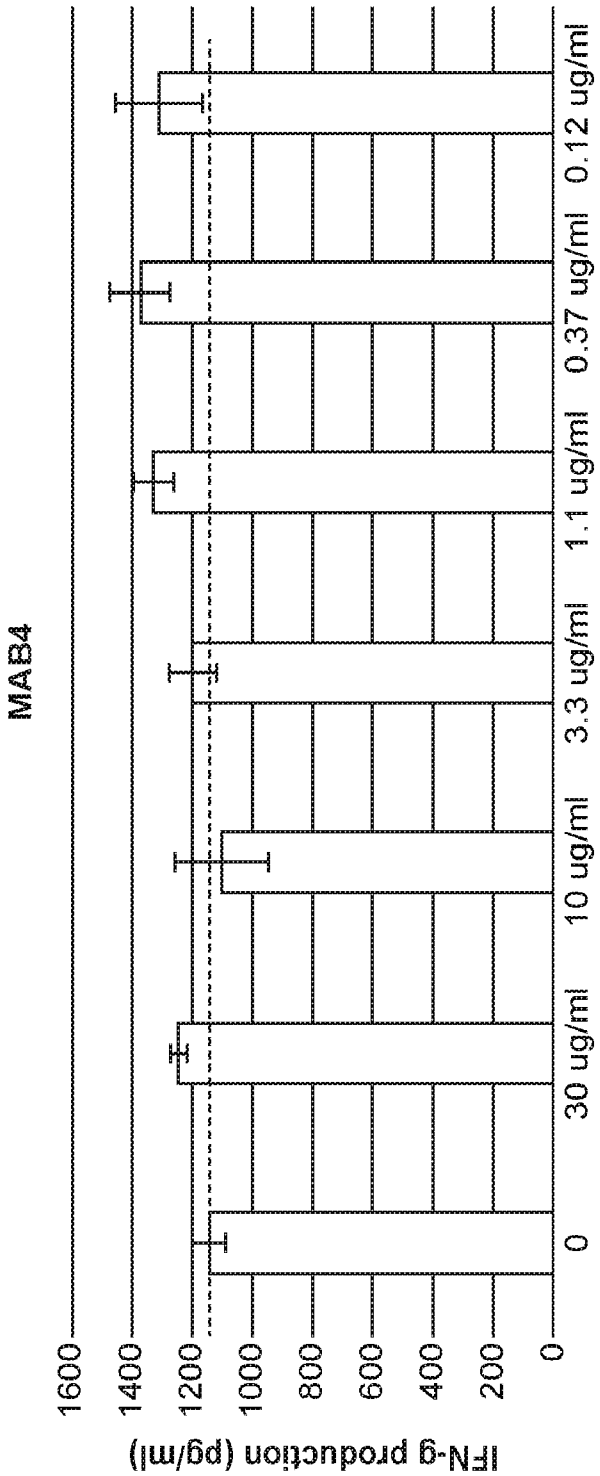
MAB4
IFN-g production (pg/ml)
1600
1400
1200
1000
800
600
400
200
0
0
30 ug/ml
10 ug/ml
3.3 ug/ml
1.1 ug/ml
0.37 ug/ml
0.12 ug/ml
MAB4

FIG. 6D

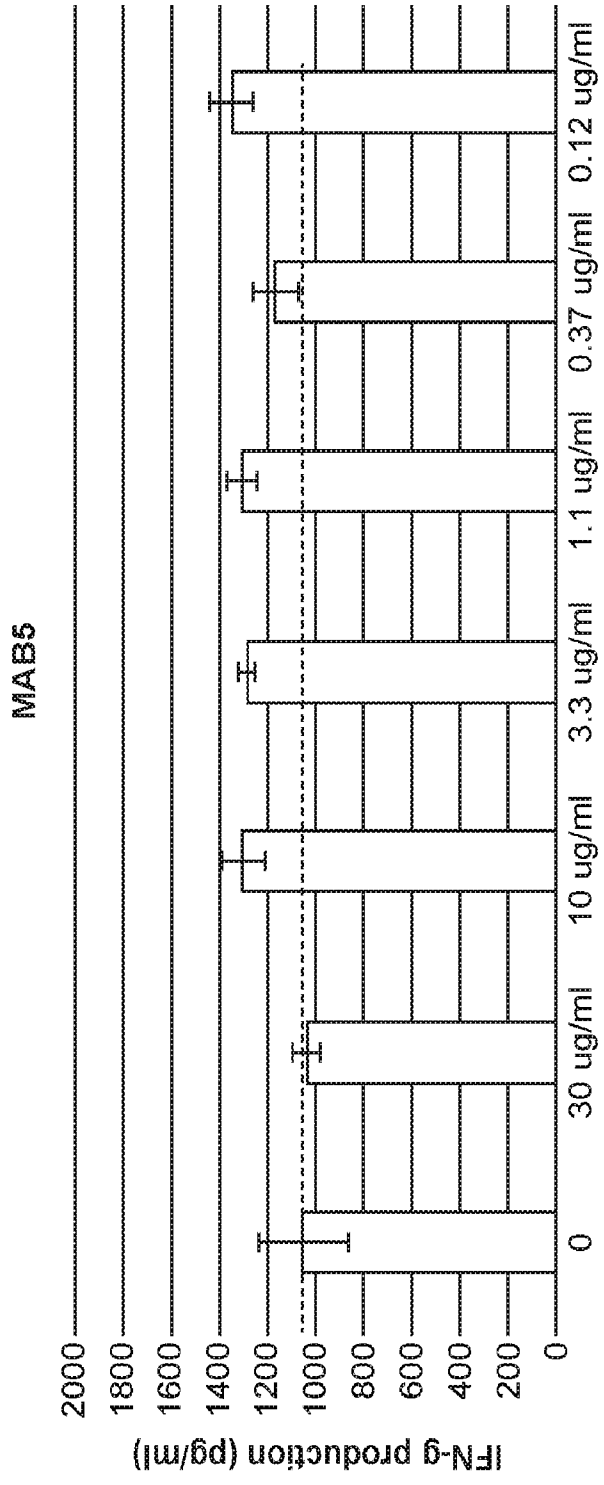
MAB5
IFN-g production (pg/ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
30 ug/ml
10 ug/ml
3.3 ug/ml
1.1 ug/ml
0.37 ug/ml
0.12 ug/ml
MAB5

FIG. 6E

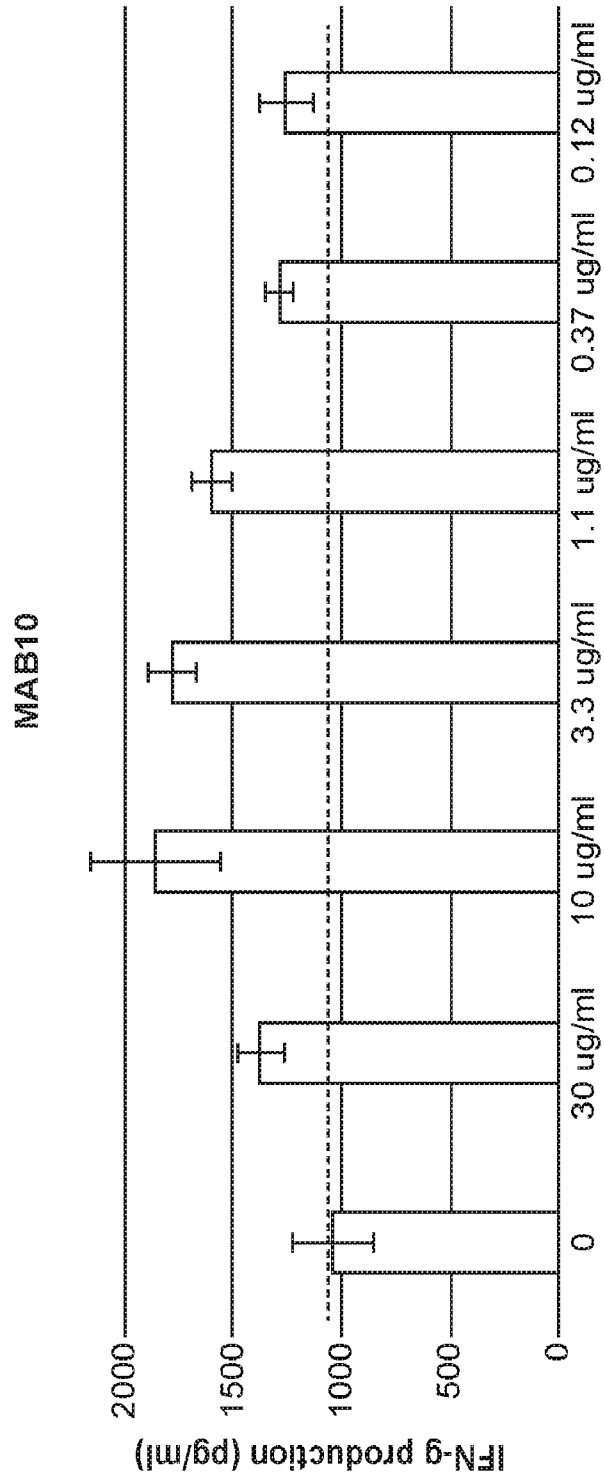
MAB10
IFN-g production (pg/ml)
2000
1500
1000
500
0
0
30 ug/ml
10 ug/ml
3.3 ug/ml
1.1 ug/ml
0.37 ug/ml
0.12 ug/ml
MAB10

FIG. 6F

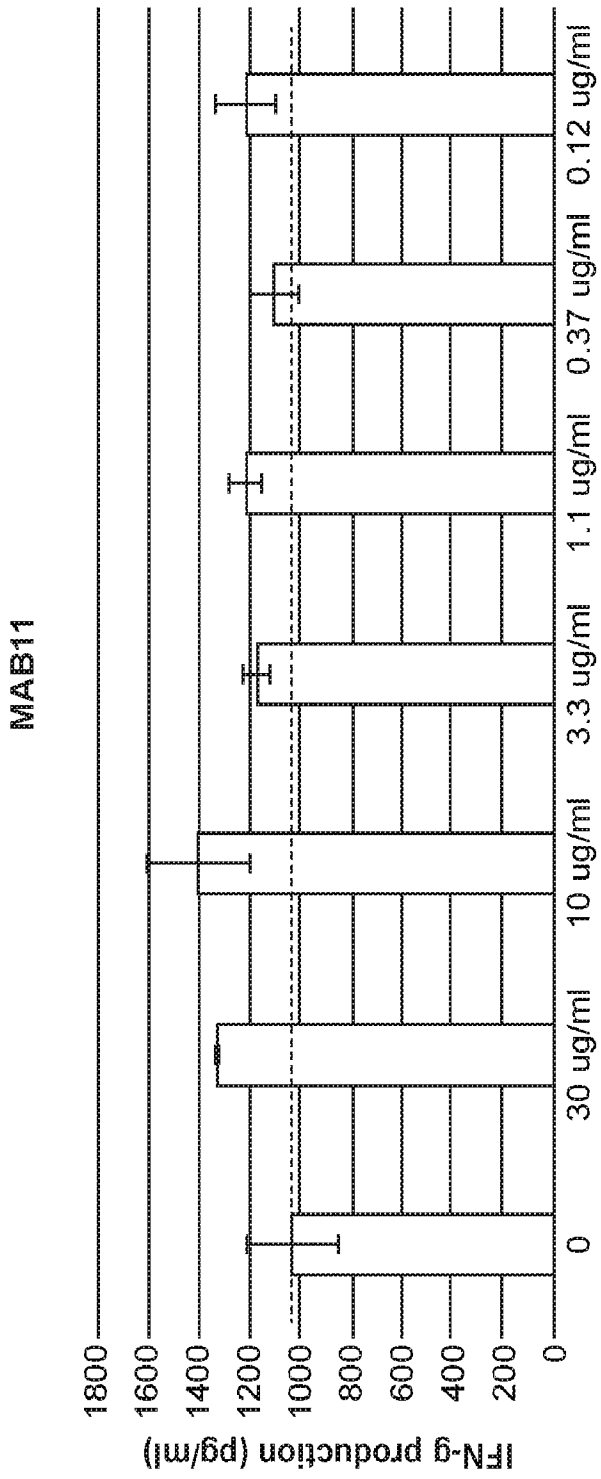
MAB11
IFN-g production (pg/ml)
1800
1600
1400
1200
1000
800
600
400
200
0
0
30 ug/ml
10 ug/ml
3.3 ug/ml
1.1 ug/ml
0.37 ug/ml
0.12 ug/ml
MAB11

FIG. 6G

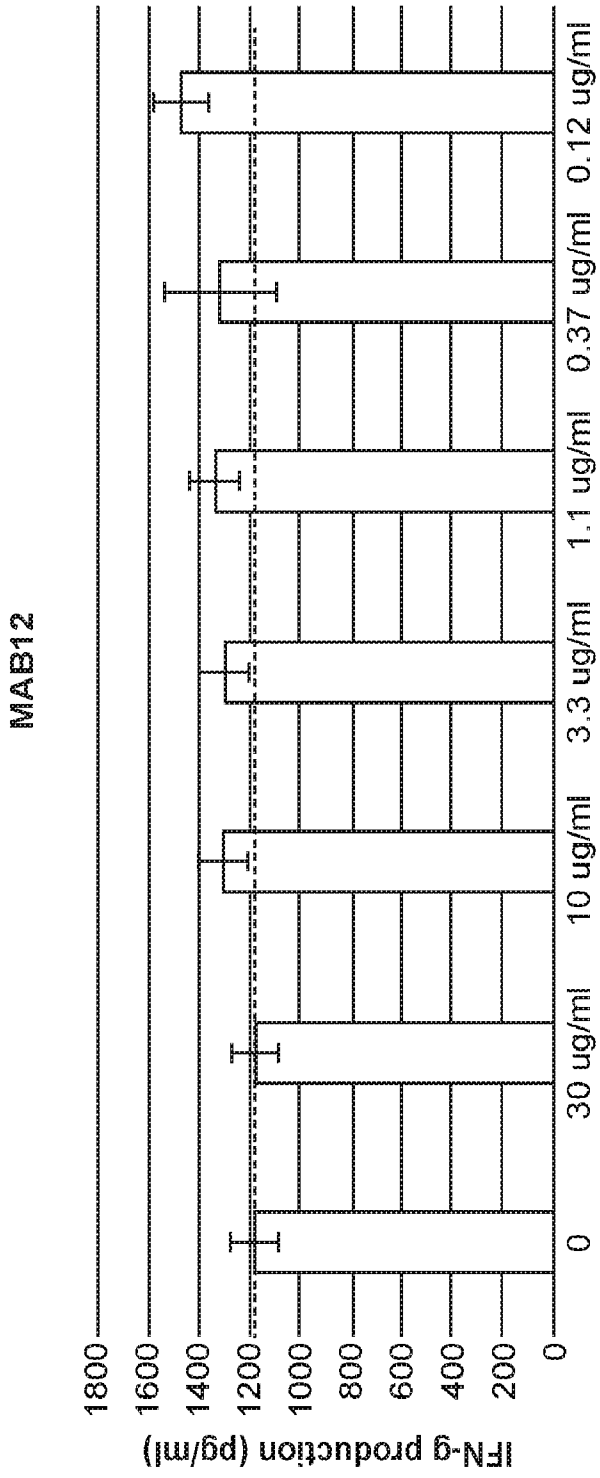
MAB12
IFN-g production (pg/ml)
1800
1600
1400
1200
1000
800
600
400
200
0
0
30 ug/ml
10 ug/ml
3.3 ug/ml
1.1 ug/ml
0.37 ug/ml
0.12 ug/ml
MAB12

FIG. 6H

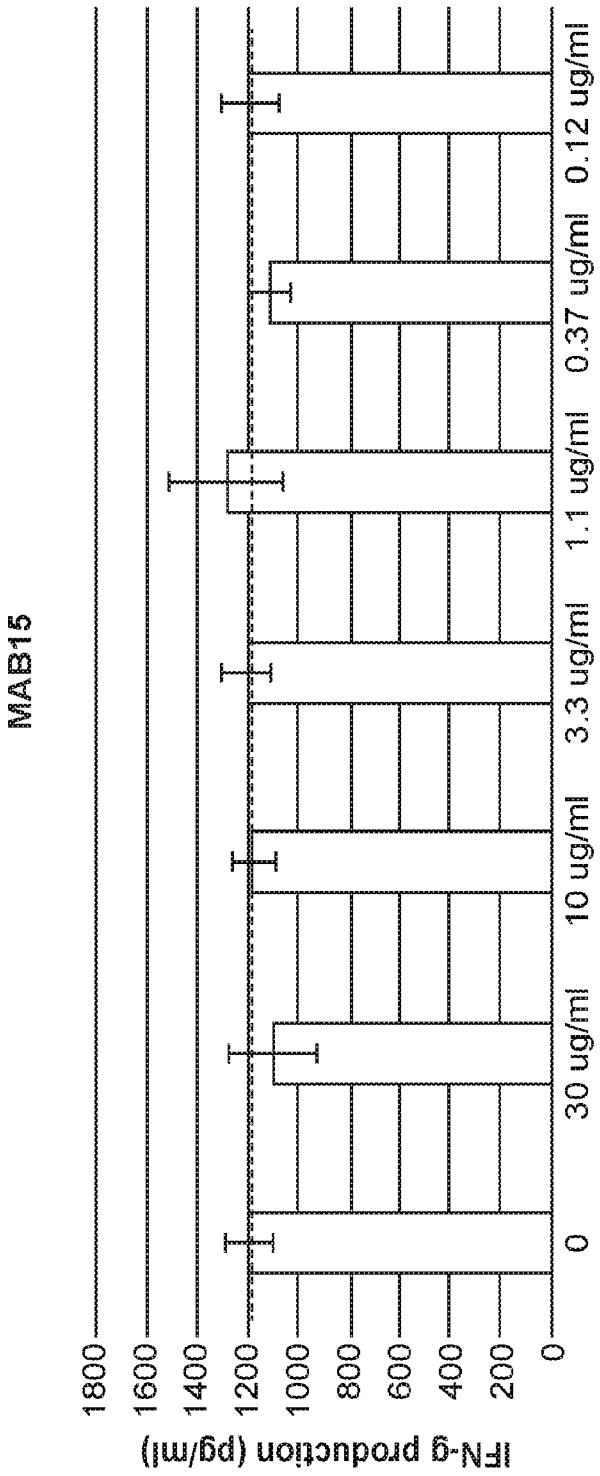
MAB15
IFN-g production (pg/ml)
1800
1600
1400
1200
1000
800
600
400
200
0
0
30 ug/ml
10 ug/ml
3.3 ug/ml
1.1 ug/ml
0.37 ug/ml
0.12 ug/ml
MAB15

FIG. 6I

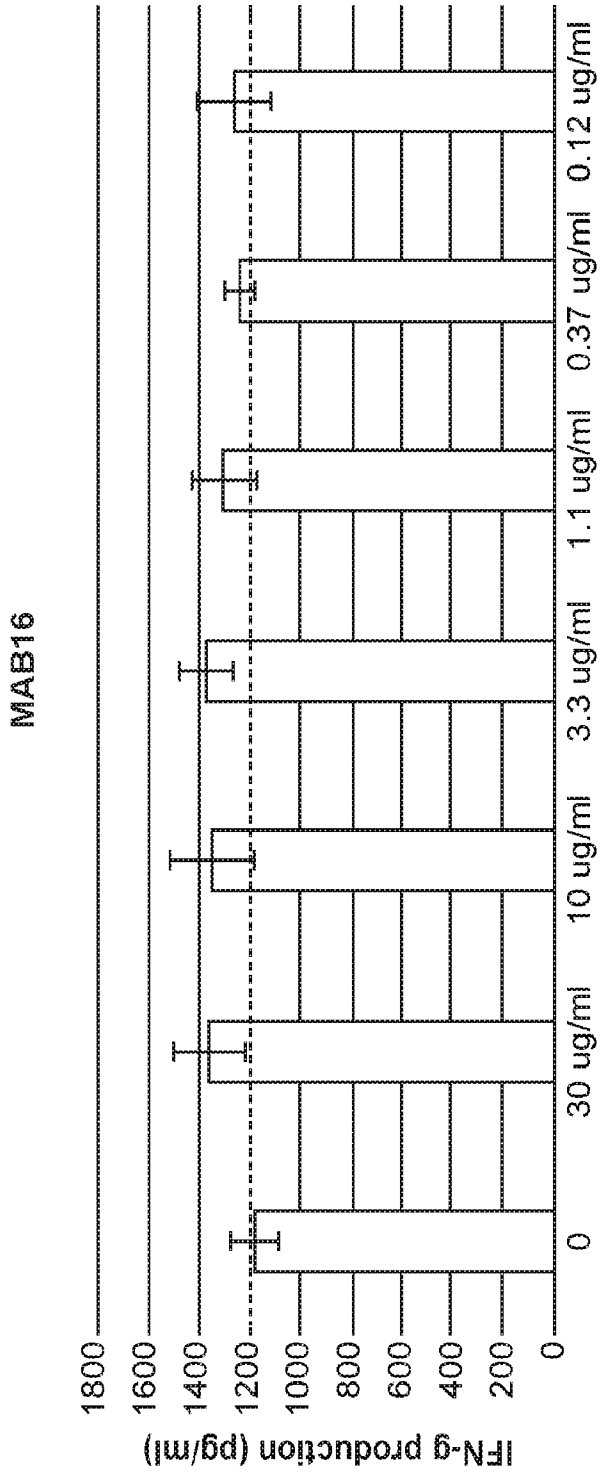
MAB16
IFN-g production (pg/ml)
1800
1600
1400
1200
1000
800
600
400
200
0
0
30 ug/ml
10 ug/ml
3.3 ug/ml
1.1 ug/ml
0.37 ug/ml
0.12 ug/ml
MAB16

FIG. 6J

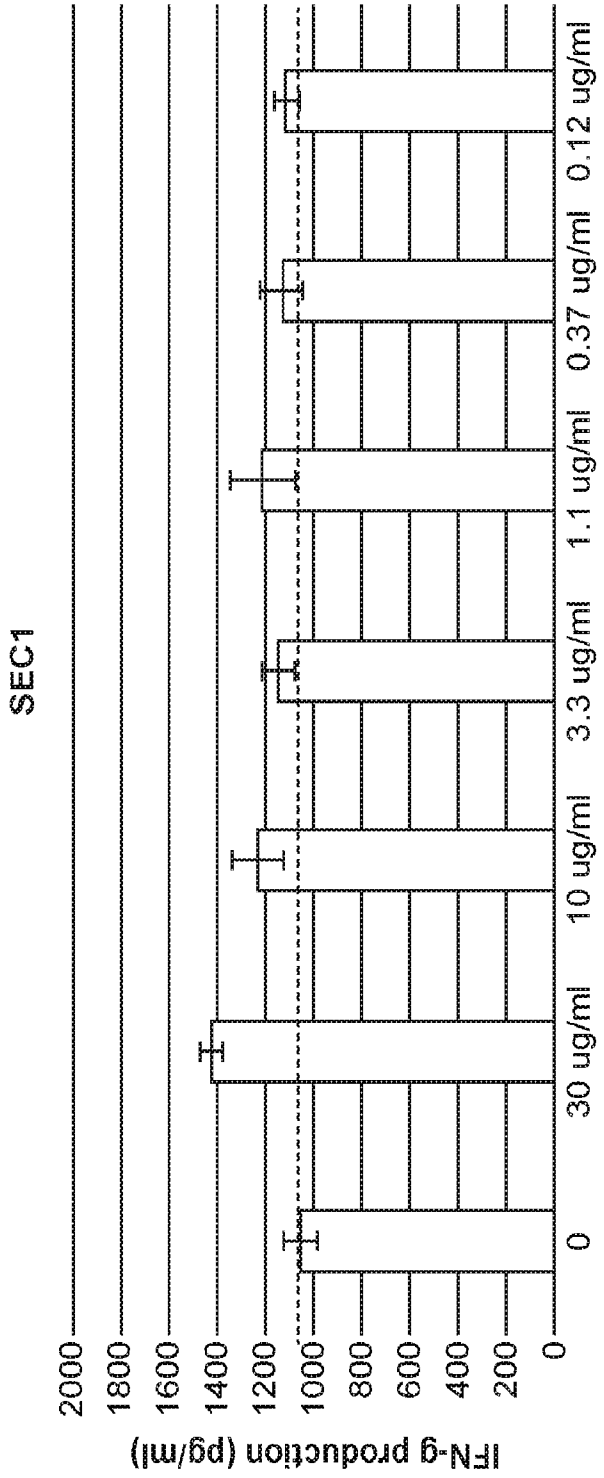
SEC1
IFN-g production (pg/ml)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
30 ug/ml
10 ug/ml
3.3 ug/ml
1.1 ug/ml
0.37 ug/ml
0.12 ug/ml
SEC1

FIG. 6K

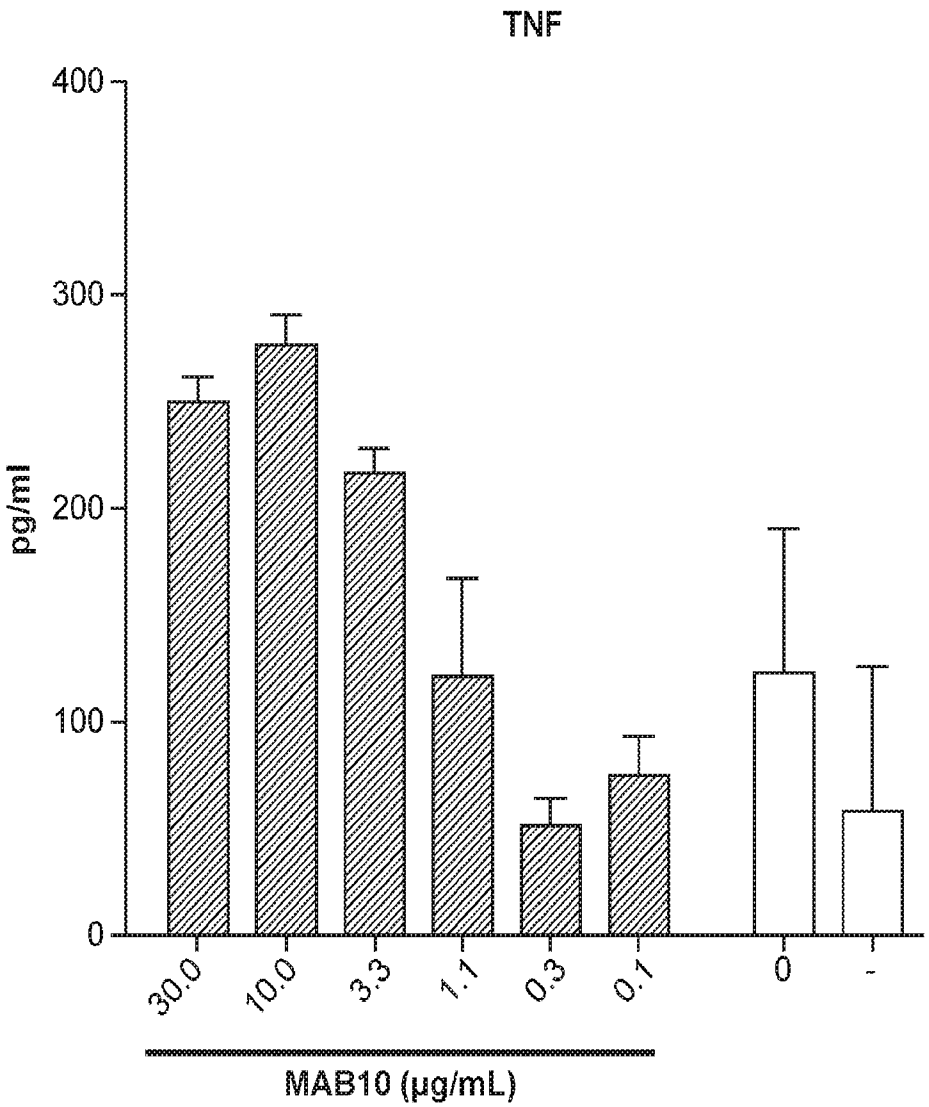

FIG. 6L

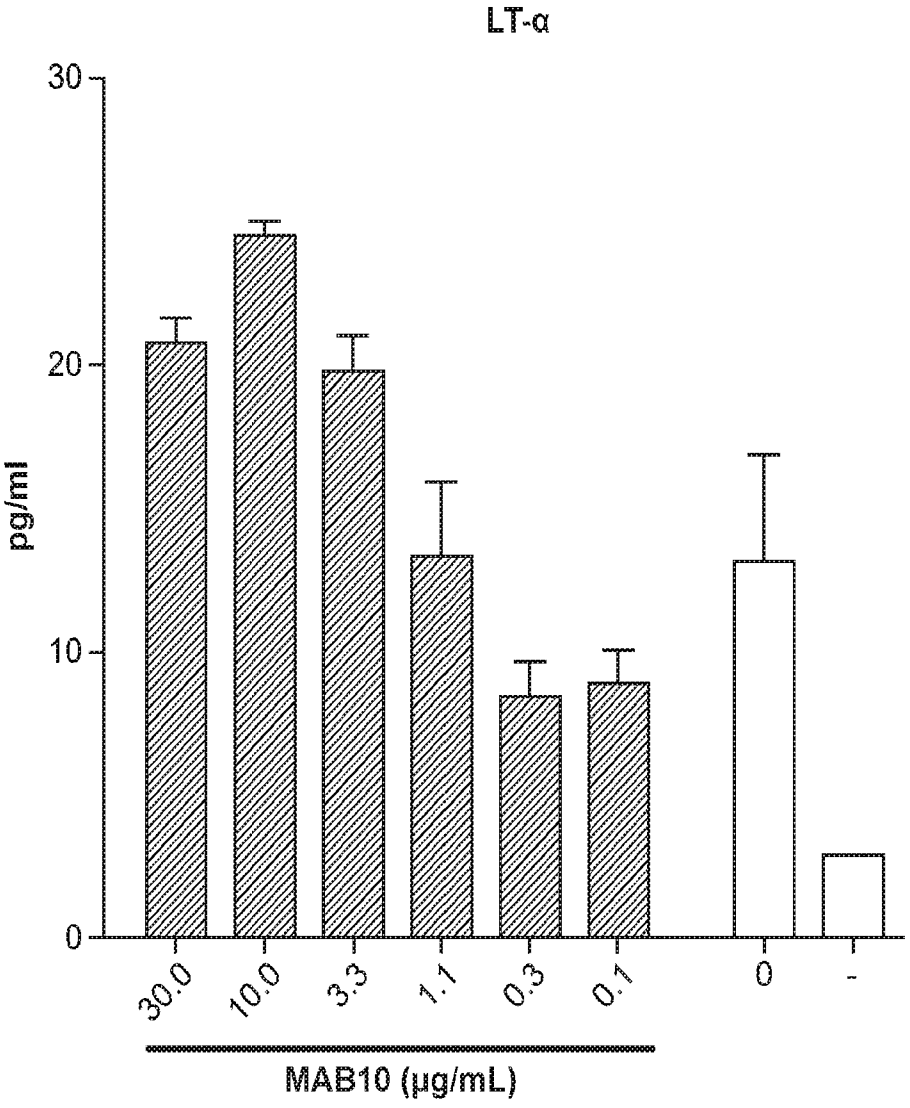

FIG. 6M

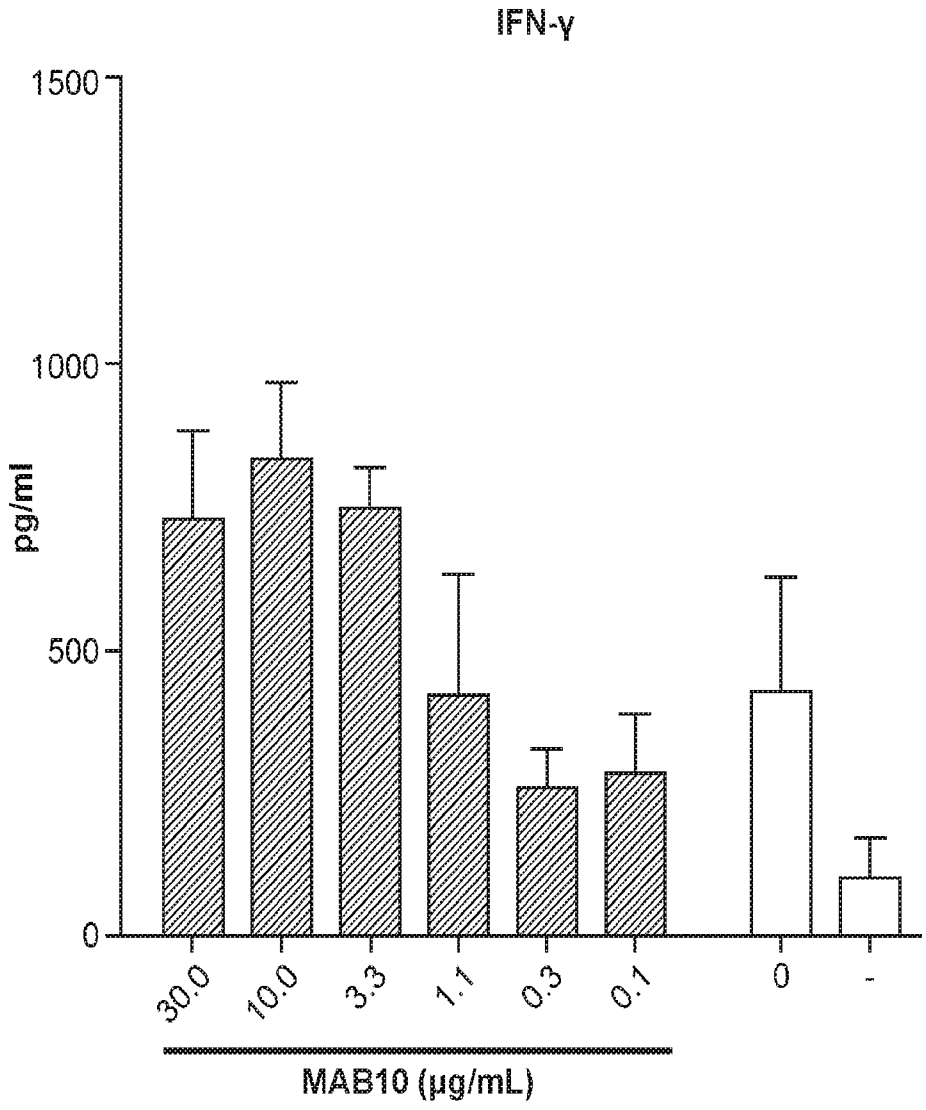

FIG. 6N

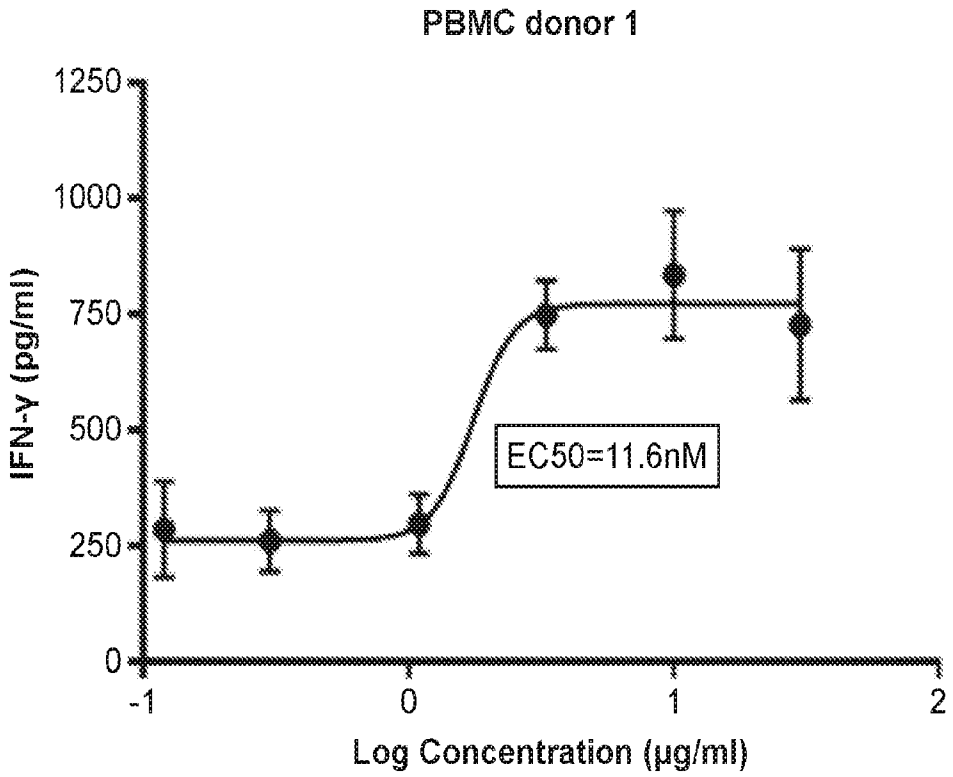
PBMC donor 1
1250
1000
750
500
250
0
IFN-γ (pg/ml)
EC50=11.6nM
-1
0
1
2
Log Concentration (µg/ml)

FIG. 6O

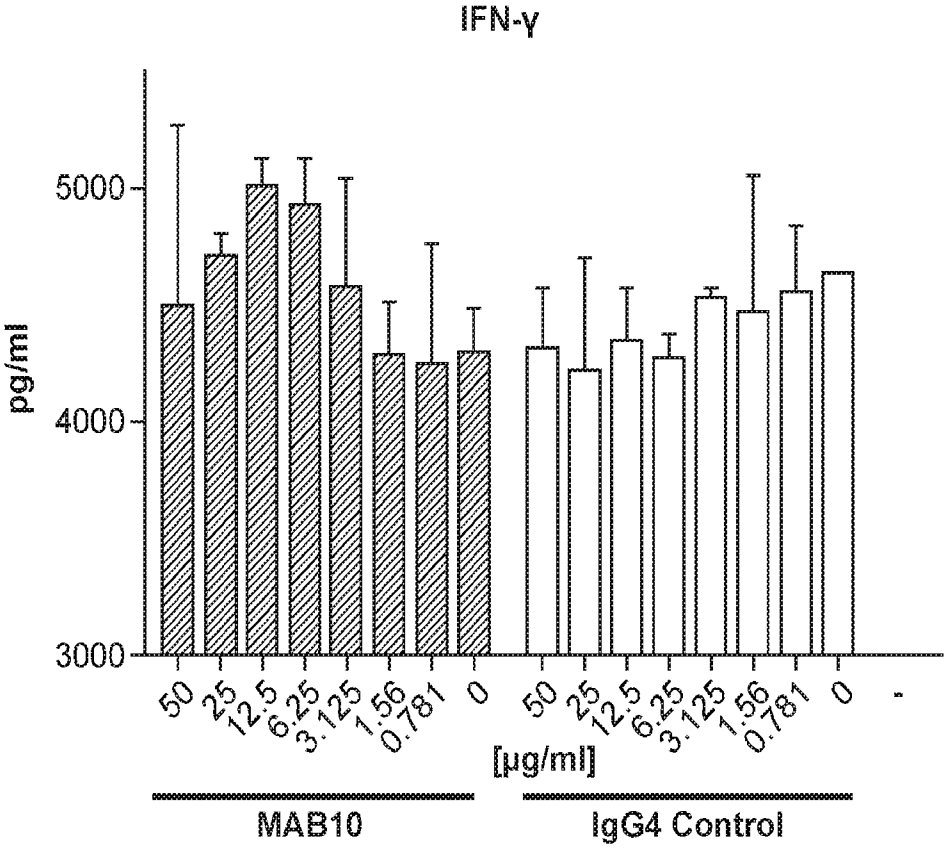
IFN-γ
pg/ml
5000
4000
3000
50
25
12.5
6.25
3.125
1.56
0.781
0
50
25
12.5
6.25
3.125
1.56
0.781
0
-
[µg/ml]
MAB10
IgG4 Control

FIG. 7A

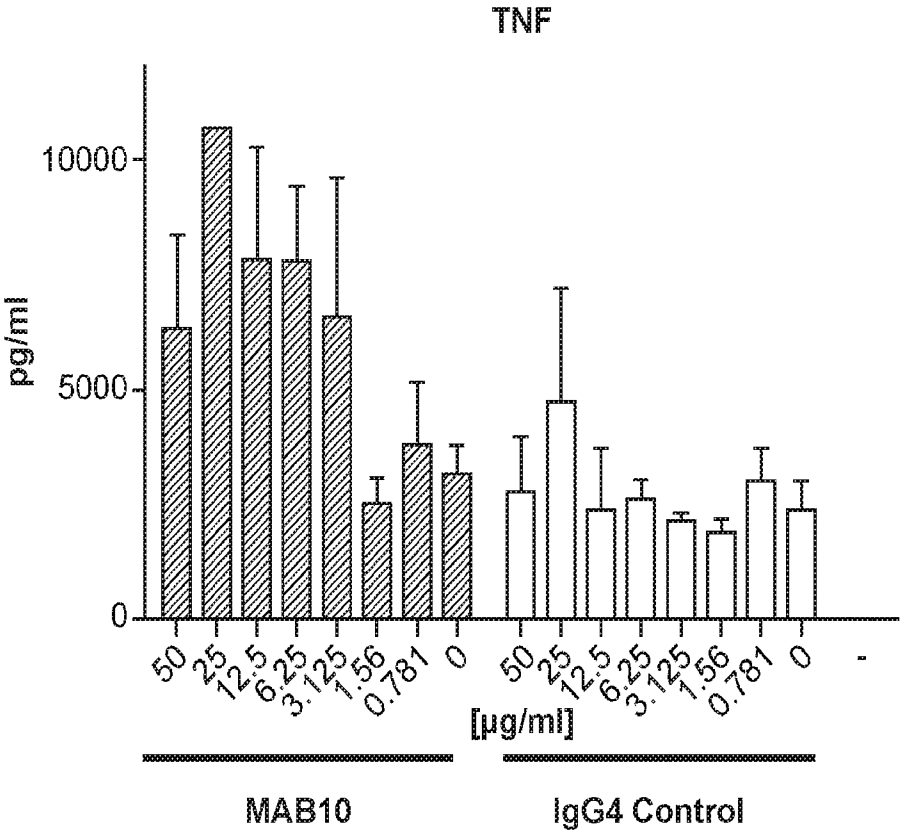
TNF
10000
5000
0
pg/ml
50
25
12.5
6.25
3.125
1.56
0.781
0
50
25
12.5
6.25
3.125
1.56
0.781
0
-
[µg/ml]
MAB10
IgG4 Control

FIG. 7B

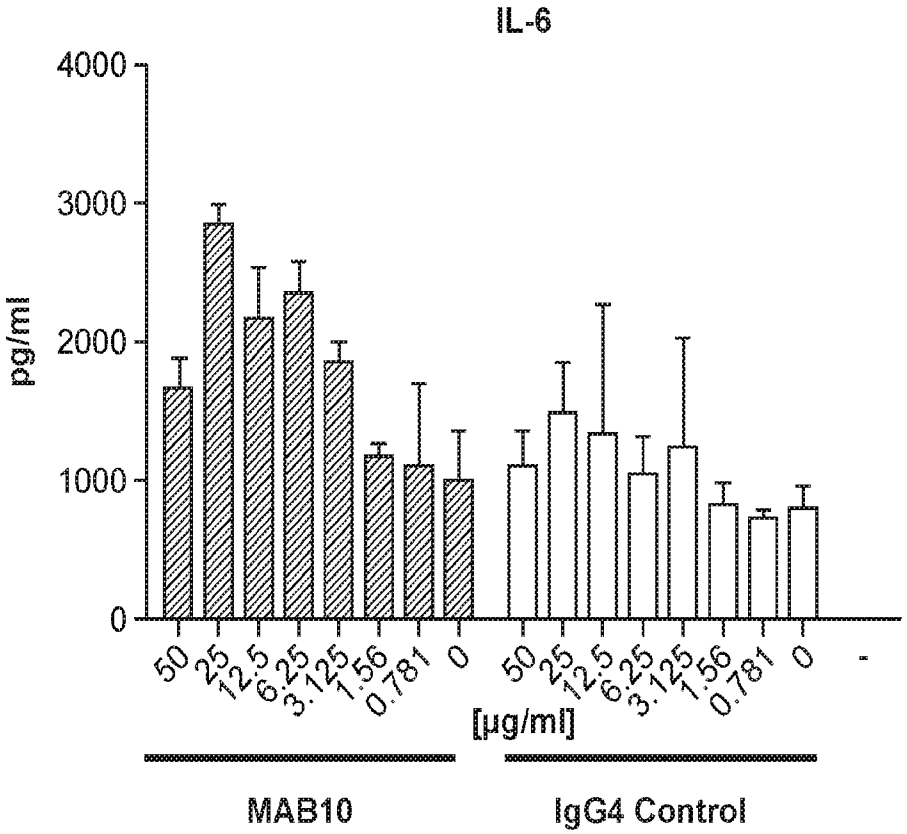
IL-6
4000
3000
2000
1000
0
pg/ml
50
25
12.5
6.25
3.125
1.56
0.781
0
50
25
12.5
6.25
3.125
1.56
0.781
0
-
[µg/ml]
MAB10
IgG4 Control

FIG. 7C

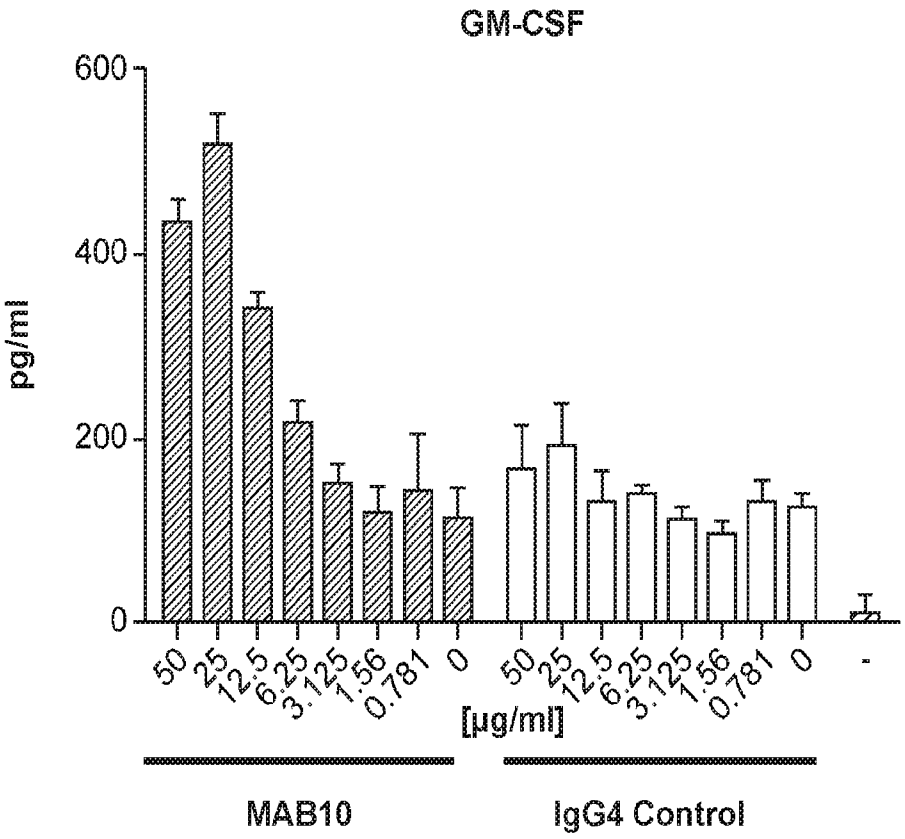
GM-CSF
600
400
200
0
pg/ml
50
25
12.5
6.25
3.125
1.56
0.781
0
50
25
12.5
6.25
3.125
1.56
0.781
0
-
[µg/ml]
MAB10
IgG4 Control

FIG. 7D

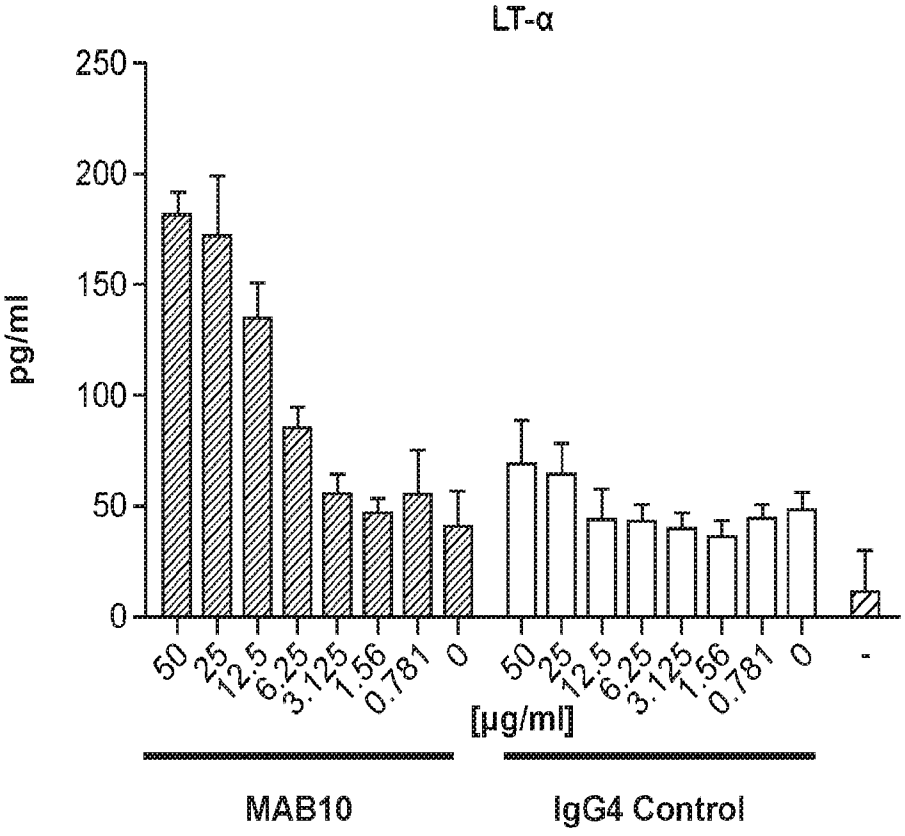
LT-α
250
200
150
100
50
0
pg/ml
50
25
12.5
6.25
3.125
1.56
0.781
0
50
25
12.5
6.25
3.125
1.56
0.781
0
-
[µg/ml]
MAB10
IgG4 Control

FIG. 7E

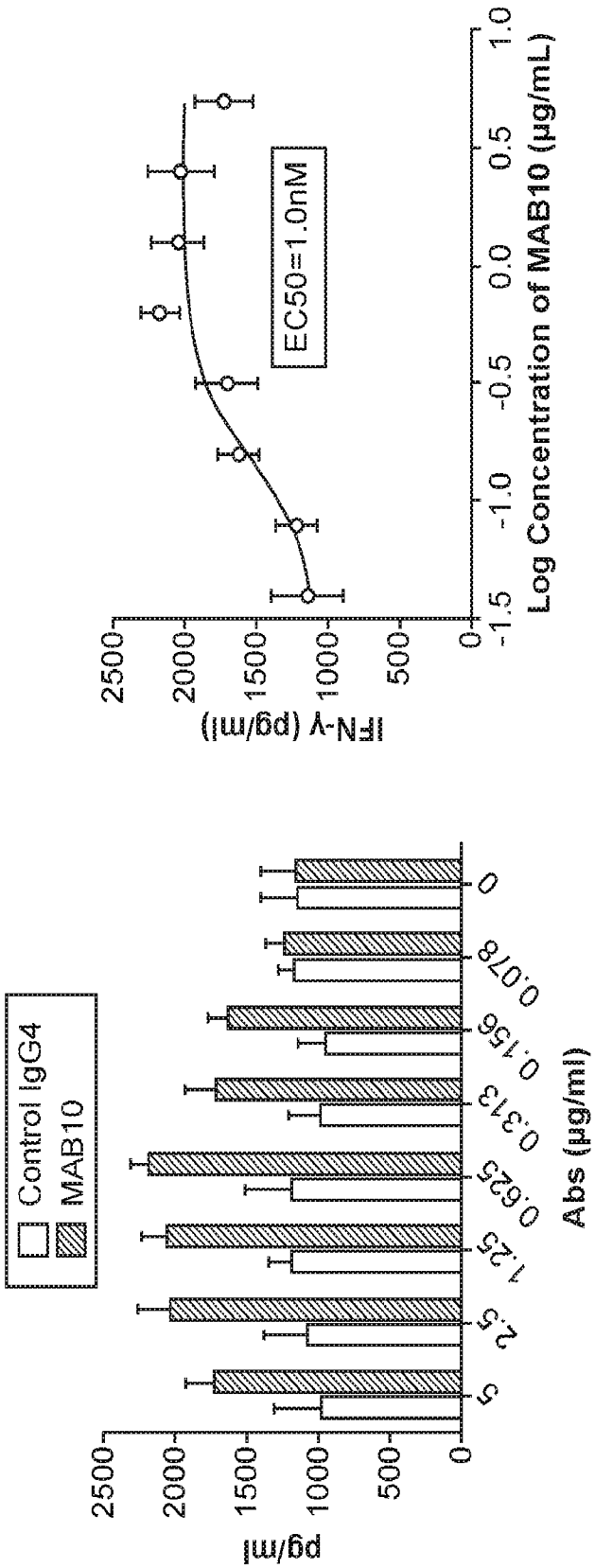
Control IgG4
MAB10
pg/ml
2500
2000
1500
1000
500
0
5
2.5
1.25
0.625
0.313
0.156
0.078
0
Abs (µg/ml)
IFN-γ (pg/ml)
EC50=1.0nM
Log Concentration of MAB10 (µg/mL)
1.0
0.5
0.0
-0.5
-1.0
-1.5

FIG. 8A

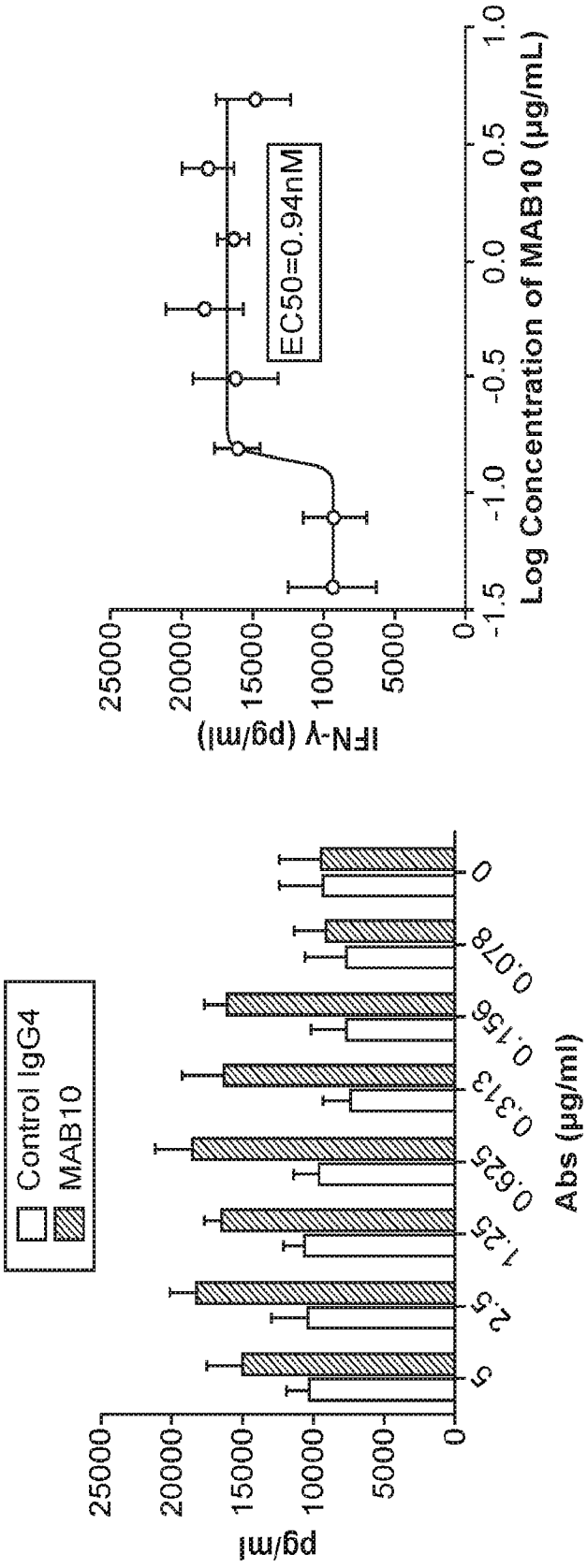
IFN-γ (pg/ml)
25000
20000
15000
10000
5000
0
EC50=0.94nM
-1.5
-1.0
-0.5
0.0
0.5
1.0
Log Concentration of MAB10 (µg/mL)
pg/ml
Control IgG4
MAB10
0
0.078
0.156
0.313
0.625
1.25
2.5
5
Abs (µg/ml)

FIG. 8B

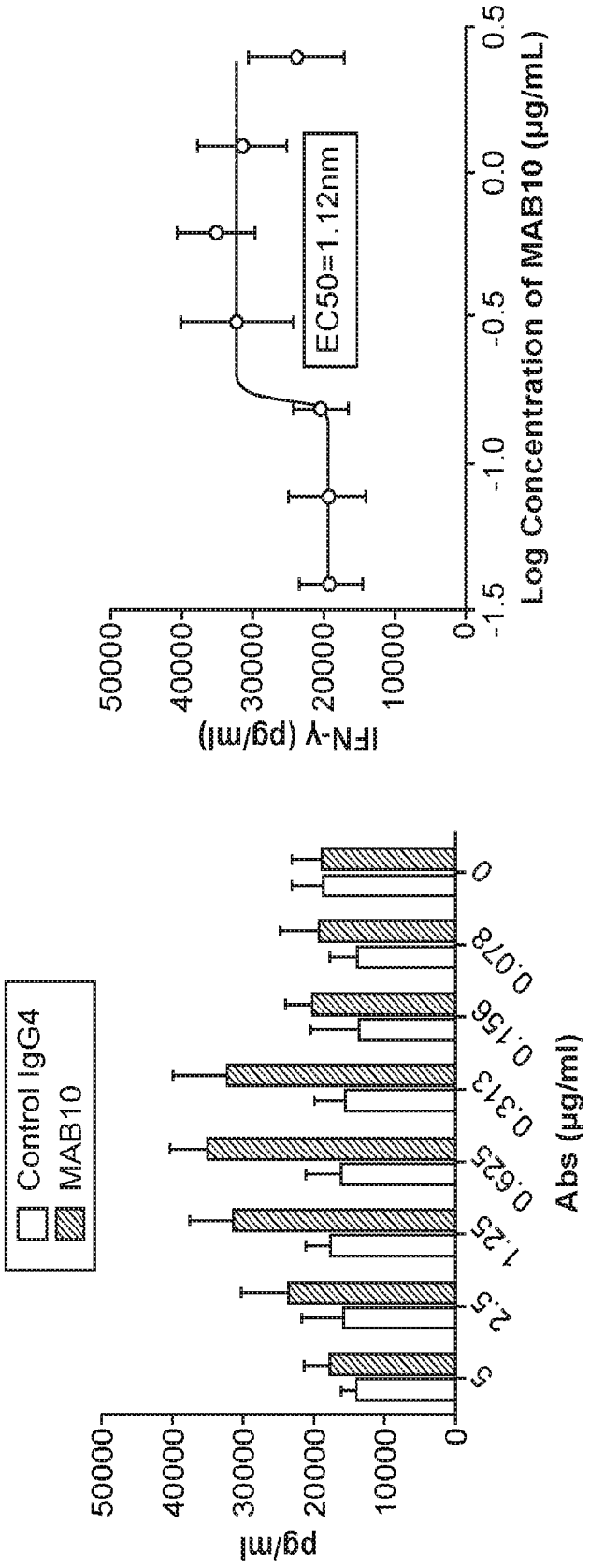
Control IgG4
MAB10
pg/ml
50000
40000
30000
20000
10000
0
5
2.5
1.25
0.625
0.313
0.156
0.078
0
Abs (µg/ml)
IFN-γ (pg/ml)
50000
40000
30000
20000
10000
0
EC50=1.12nm
-1.5
-1.0
-0.5
0.0
0.5
Log Concentration of MAB10 (µg/mL)

FIG. 8C

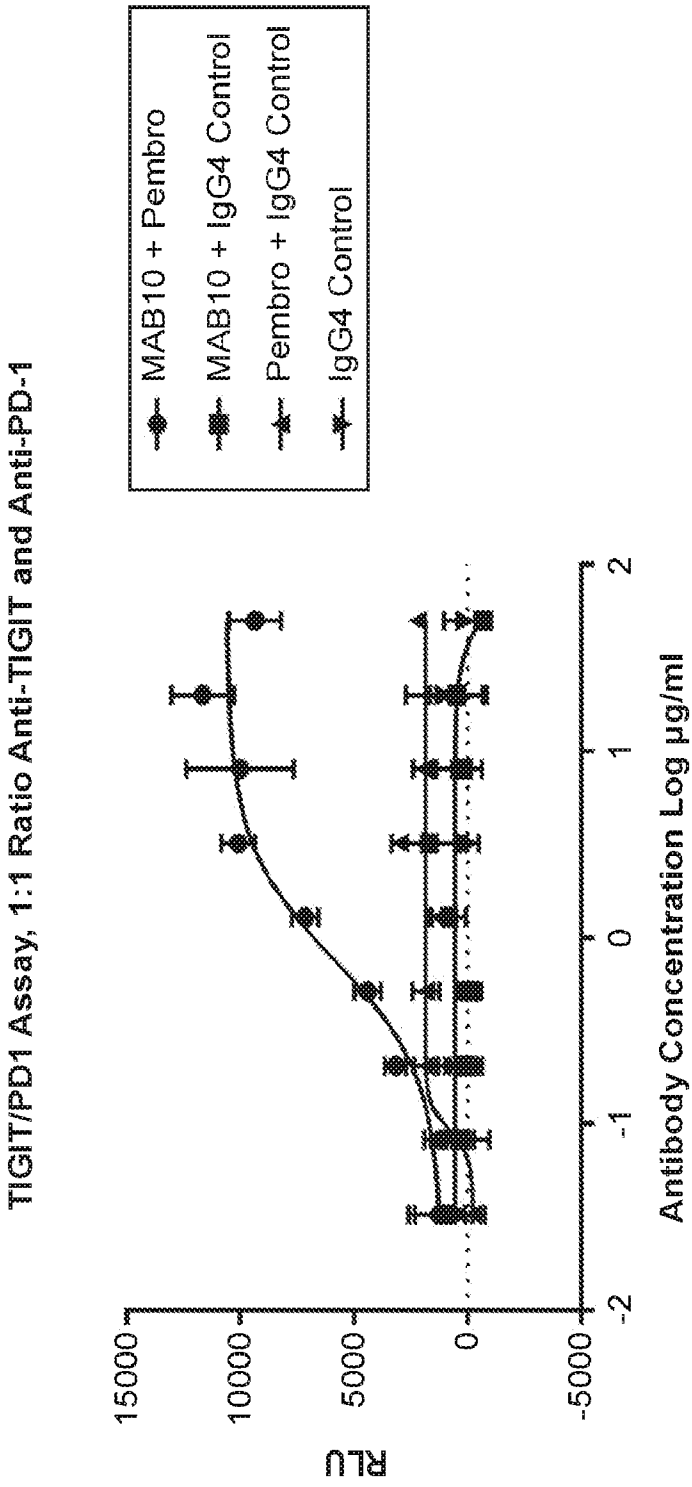
TIGIT/PD1 Assay, 1:1 Ratio Anti-TIGIT and Anti-PD-1
MAB10 + Pembro
MAB10 + IgG4 Control
Pembro + IgG4 Control
IgG4 Control
RLU
15000
10000
5000
0
-5000
-2
-1
0
1
2
Antibody Concentration Log µg/ml

FIG. 9A

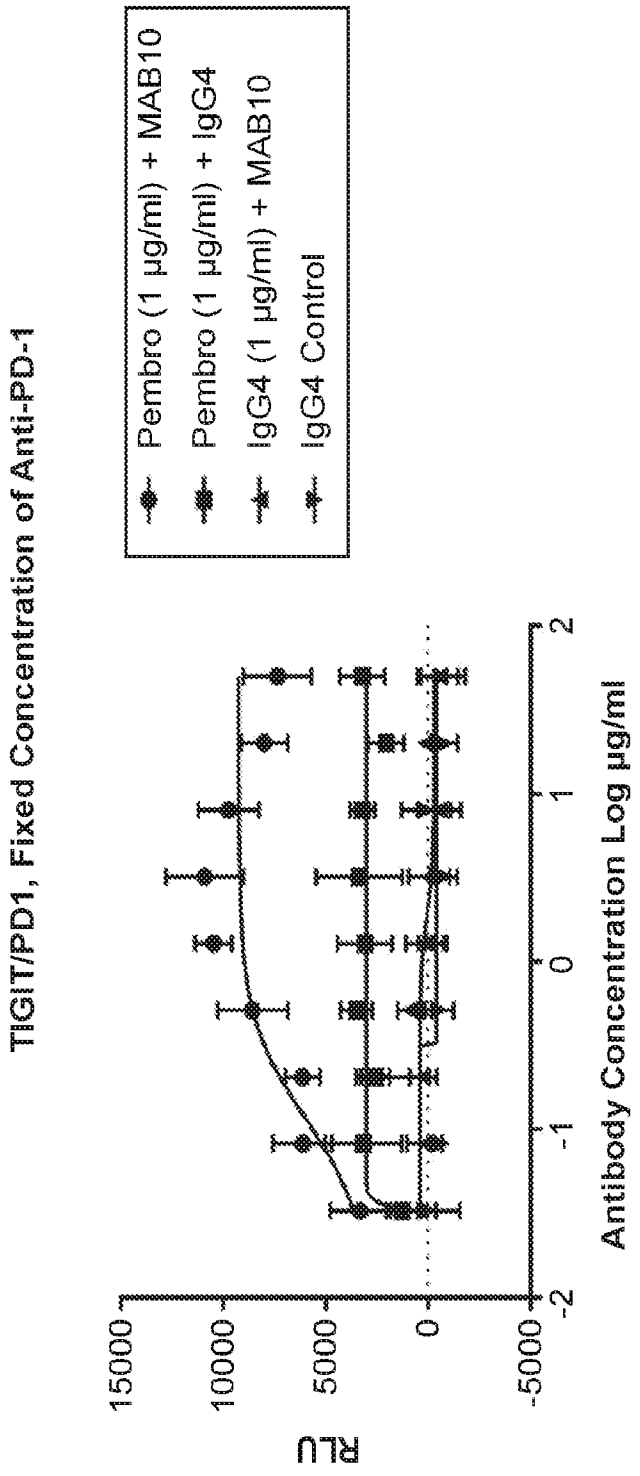
TIGIT/PD1, Fixed Concentration of Anti-PD-1
Pembro (1 µg/ml) + MAB10
Pembro (1 µg/ml) + IgG4
IgG4 (1 µg/ml) + MAB10
IgG4 Control
RLU
15000
10000
5000
0
-5000
-2
-1
0
1
2
Antibody Concentration Log µg/ml

FIG. 9B

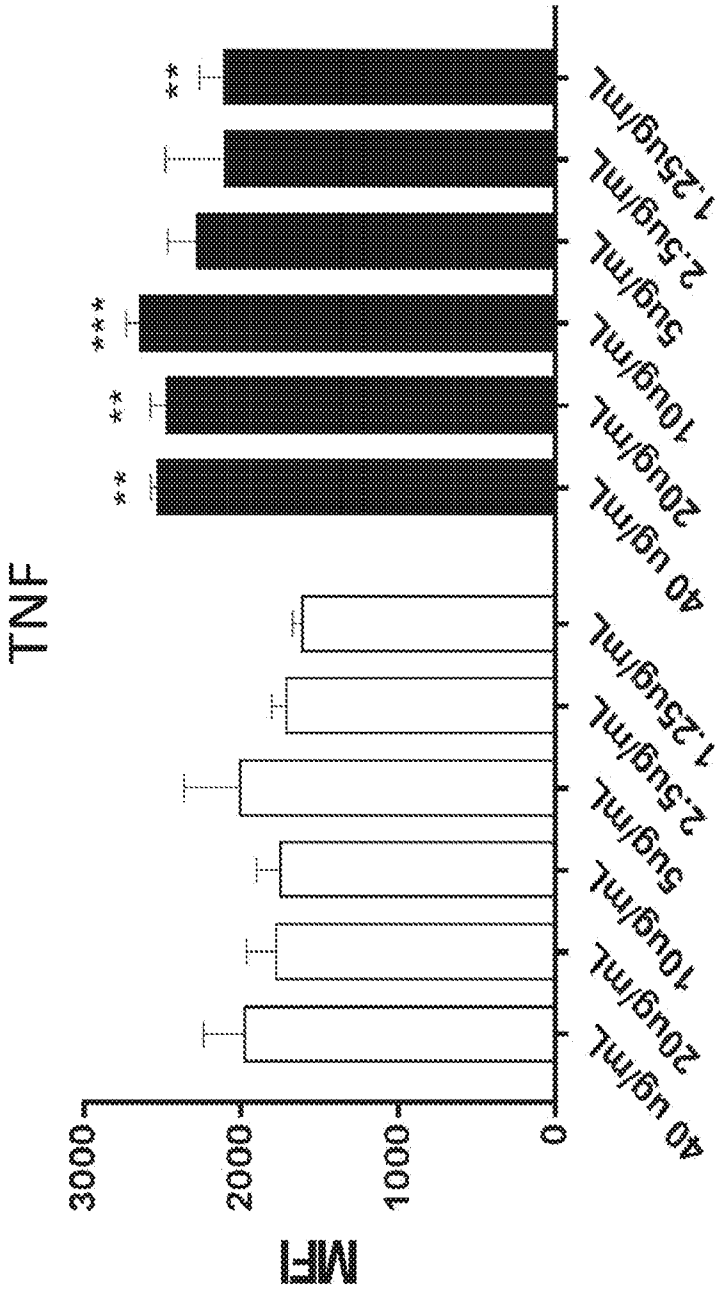
TNF
MFI
3000
2000
1000
0
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
**
**
***
**
**

FIG. 10A

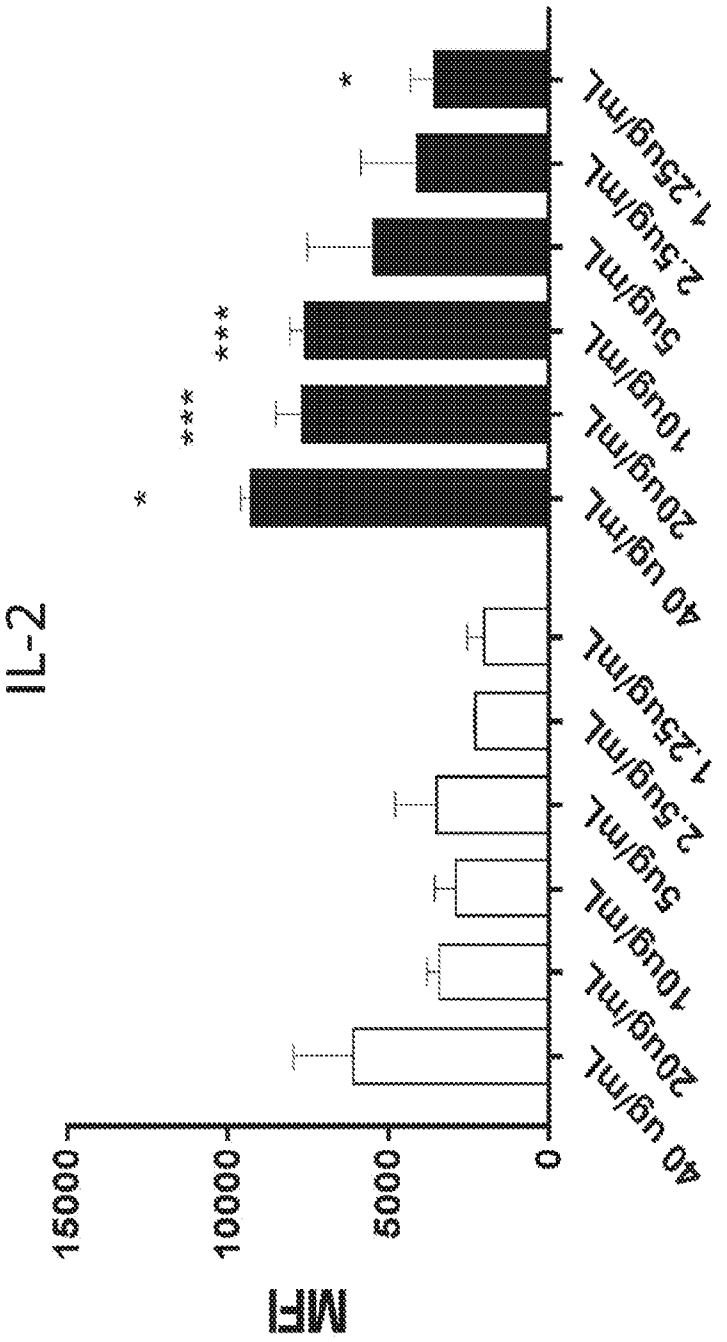
IL-2
MFI
15000
10000
5000
0
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
*
***
***
*

FIG. 10B

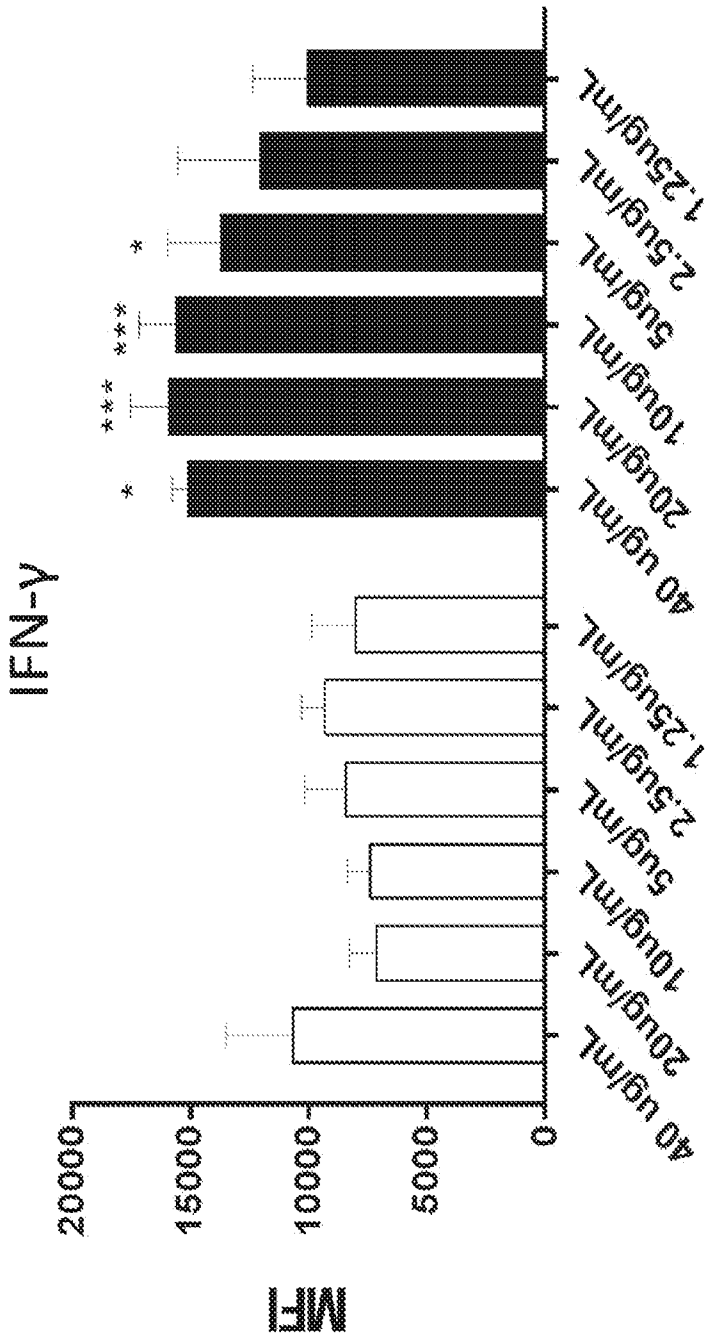
IFN-γ
MFI
20000
15000
10000
5000
0
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
*
***
***
*

FIG. 10C

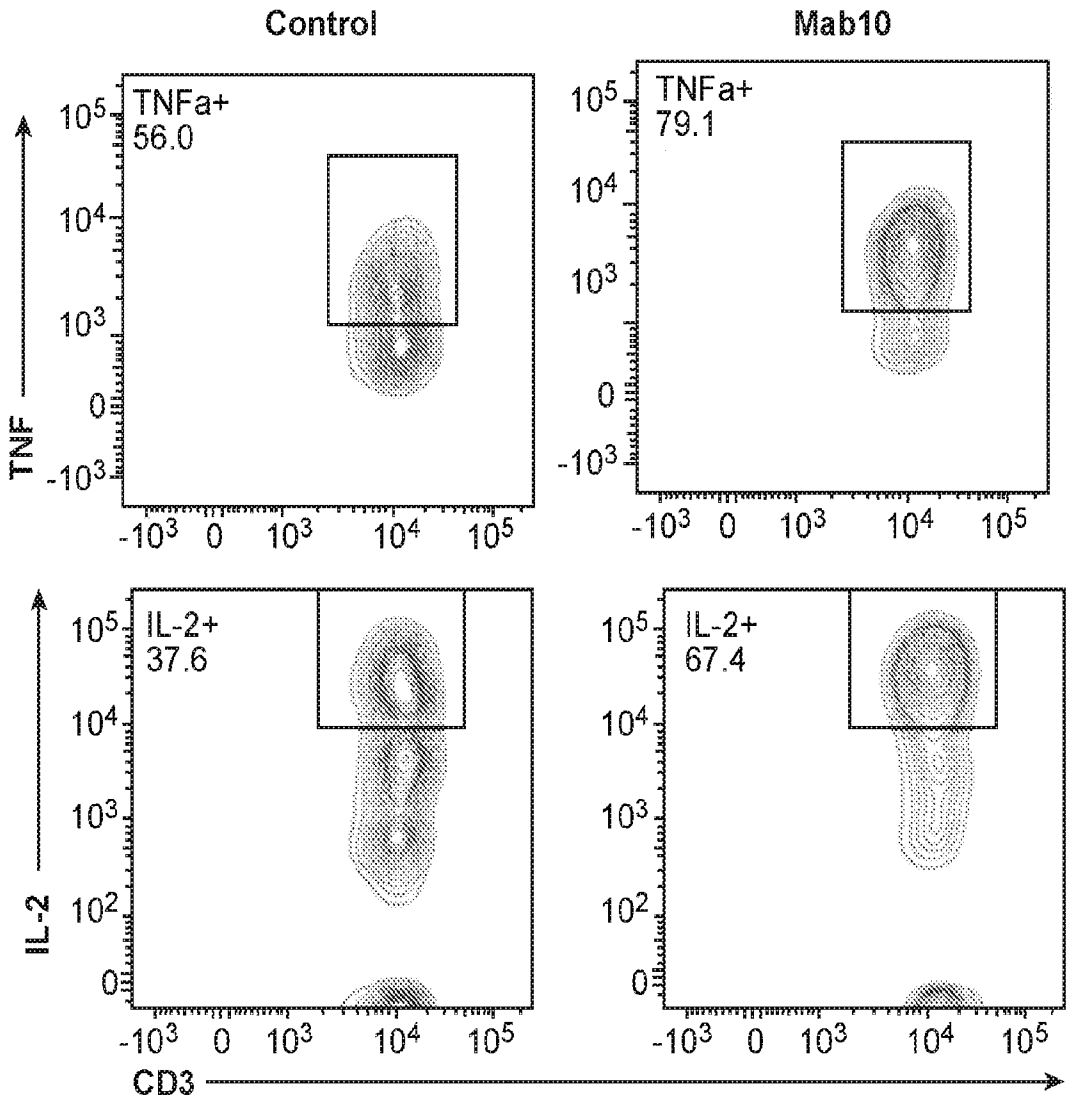
Control
Mab10
TNFa+
56.0
TNFa+
79.1
IL-2+
37.6
IL-2+
67.4
TNF
IL-2
CD3
10^5
10^4
10^3
10^2
0
-10^3

FIG. 10D

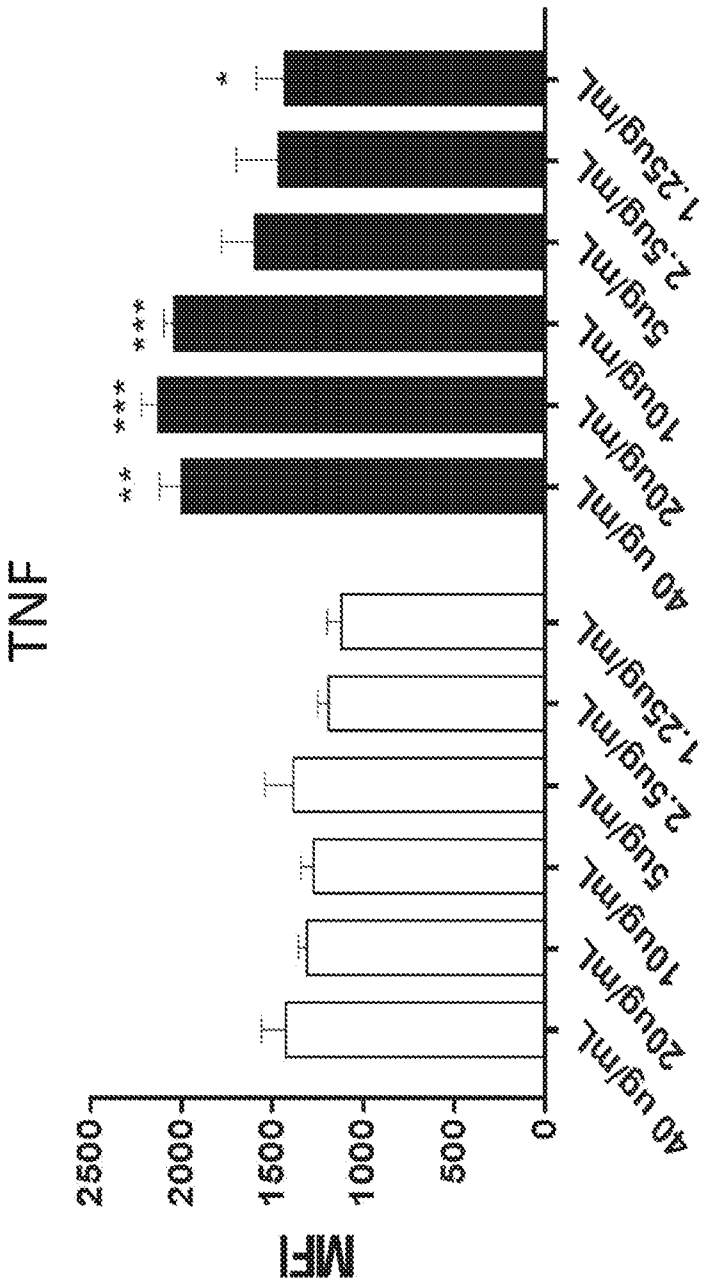
TNF
MFI
2500
2000
1600
1000
500
0
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
**
***
***
*

FIG. 11A

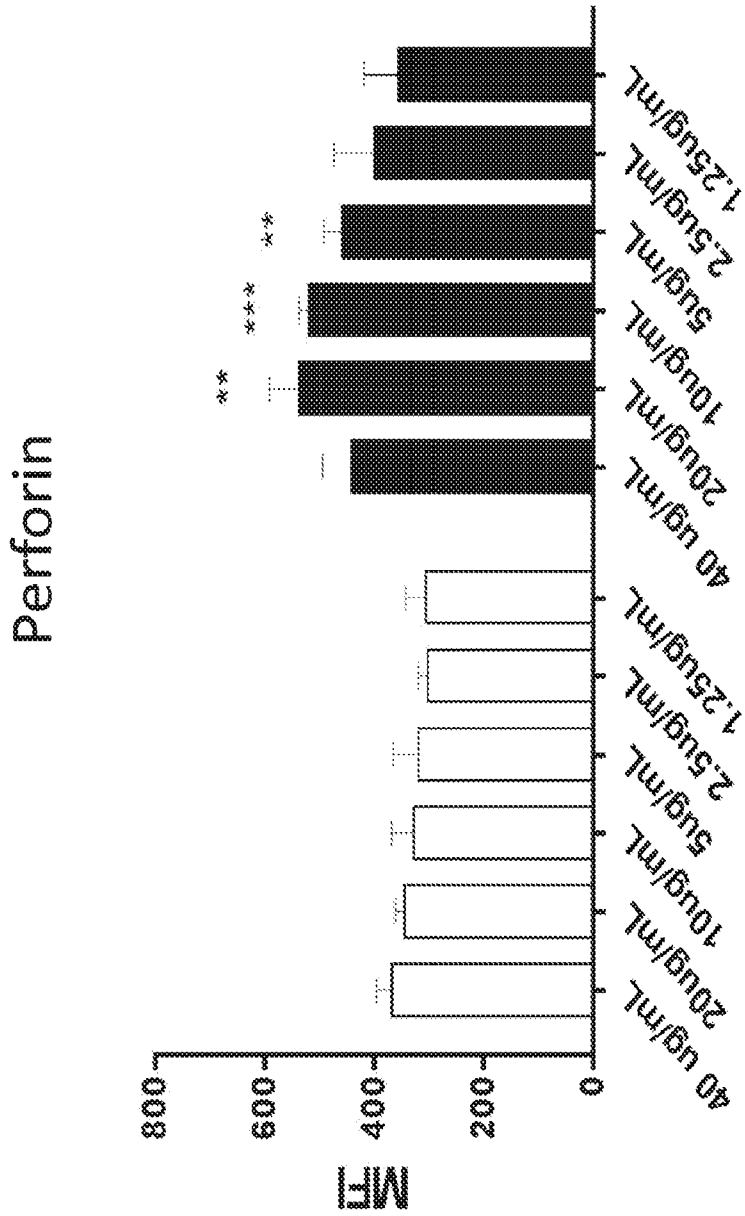
Perforin
MFI
800
600
400
200
0
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
**
***
**

FIG. 11B

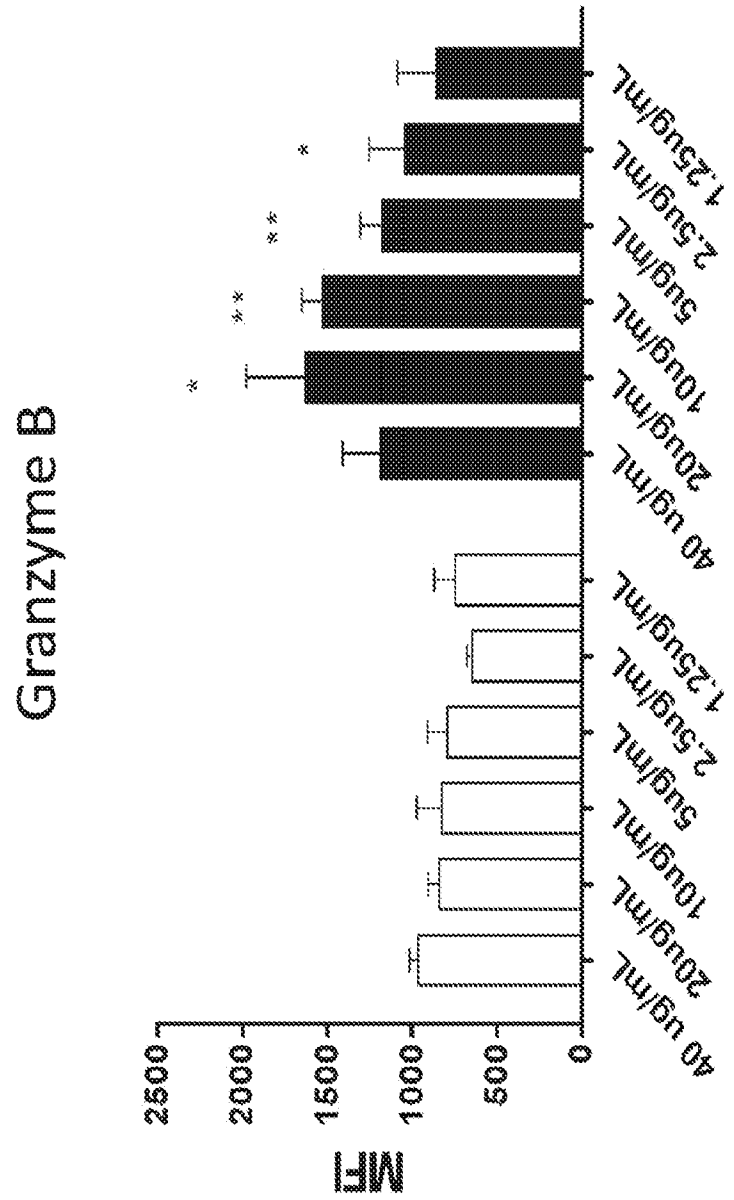
Granzyme B
MFI
2500
2000
1500
1000
500
0
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
*
**
***
*

FIG. 11C

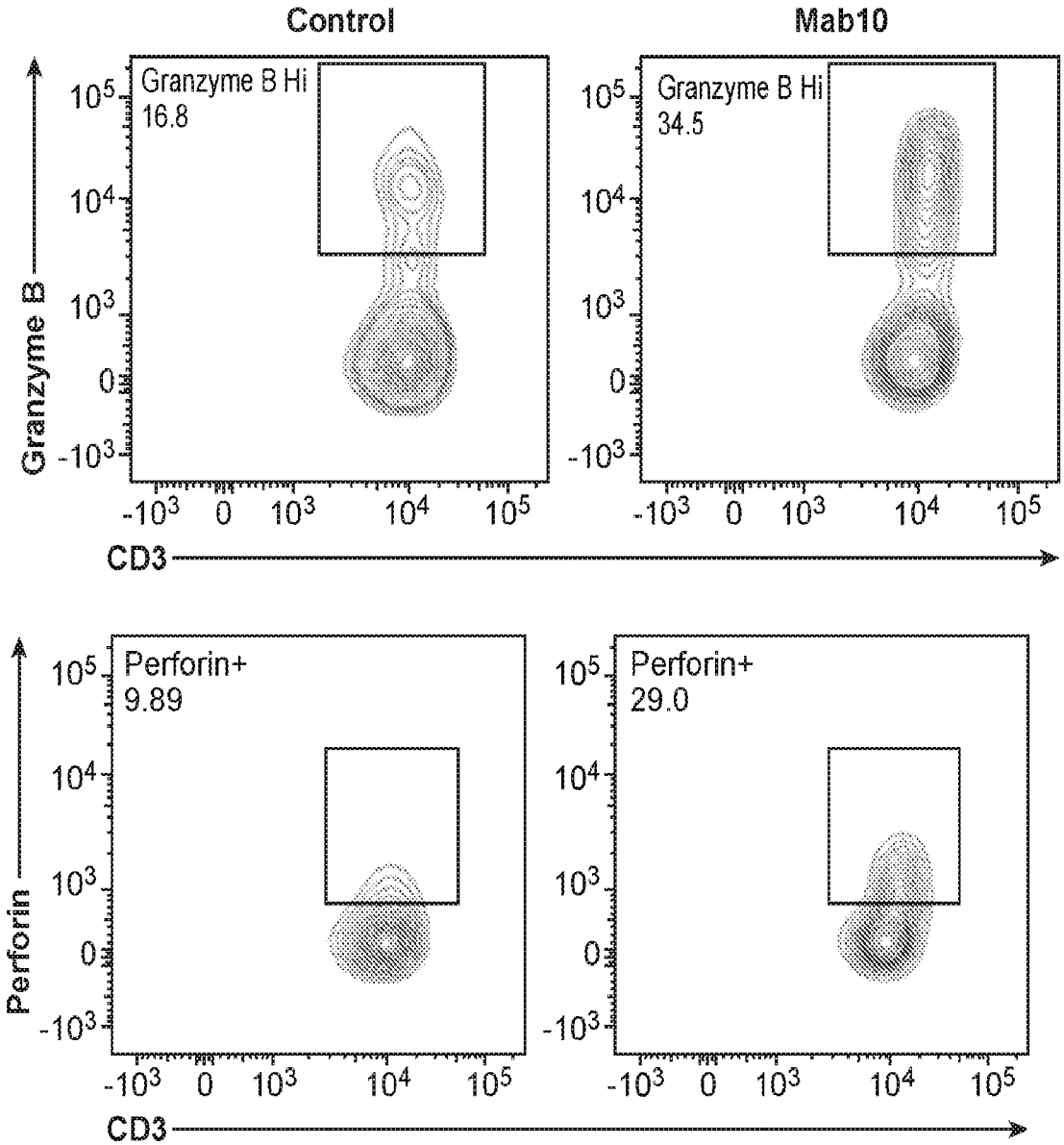
Control
Mab10
Granzyme B Hi
16.8
Granzyme B Hi
34.5
Granzyme B
CD3
Perforin+
9.89
Perforin+
29.0
Perforin
CD3
10^5
10^4
10^3
0
-10^3

FIG. 11D

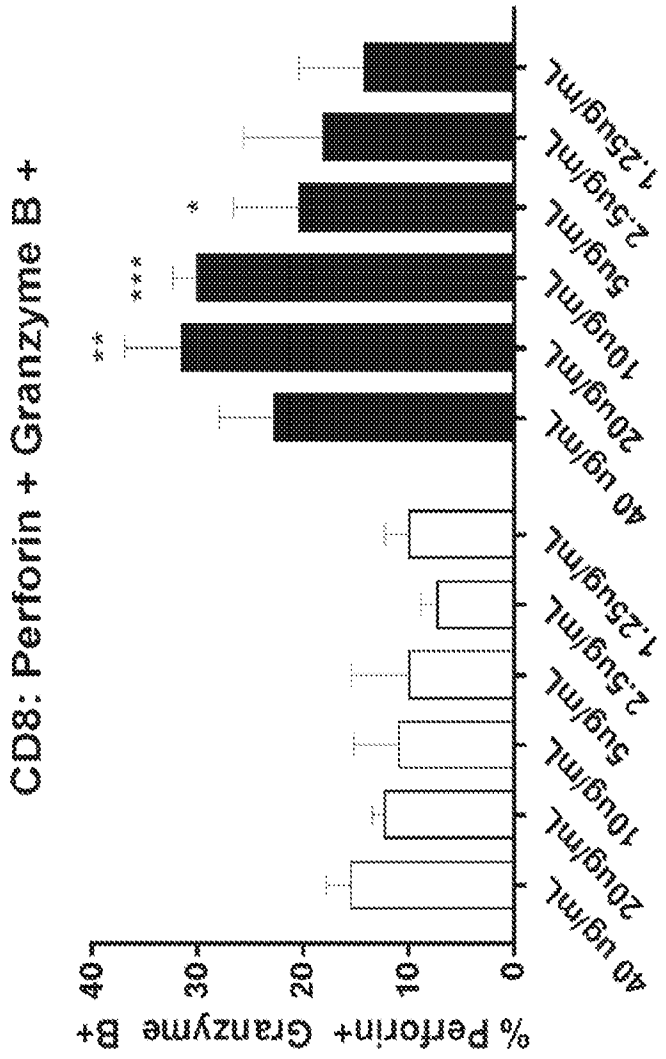
CD8: Perforin + Granzyme B +
% Perforin+ Granzyme B+
0
10
20
30
40
**
***
*
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL

FIG. 12A

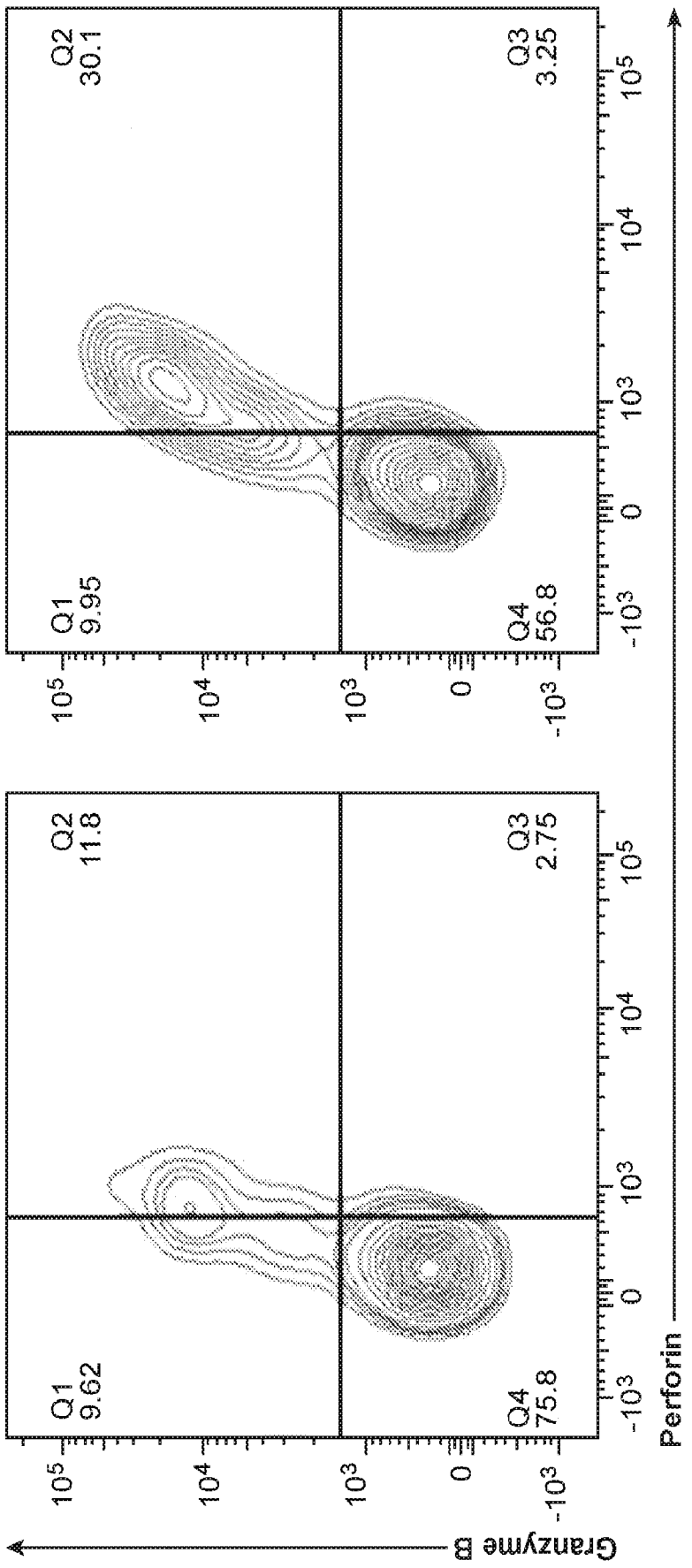
Q2
30.1
Q3
3.25
Q1
9.95
Q4
56.8
Q2
11.8
Q3
2.75
Q1
9.62
Q4
75.8
10^5
10^4
10^3
0
-10^3
Granzyme B
Perforin

FIG. 12B

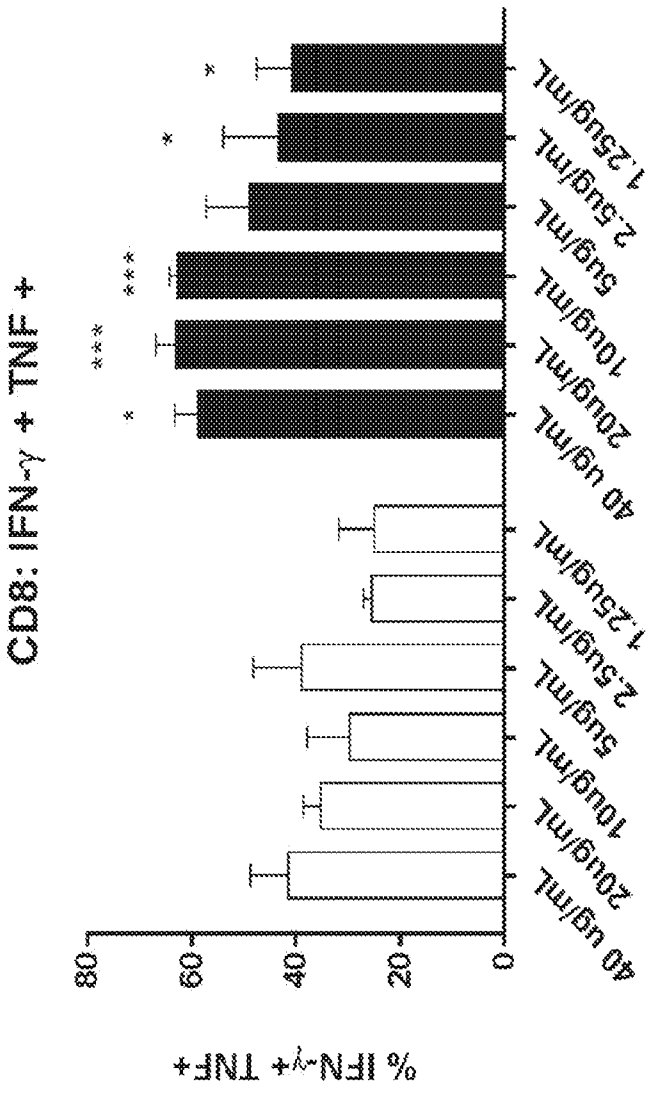
CD8: IFN-γ + TNF +
% IFN-γ+ TNF+
0
20
40
60
80
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
40 ug/mL
20ug/mL
10ug/mL
5ug/mL
2.5ug/mL
1.25ug/mL
*
***
***
*
*
*

FIG. 12C

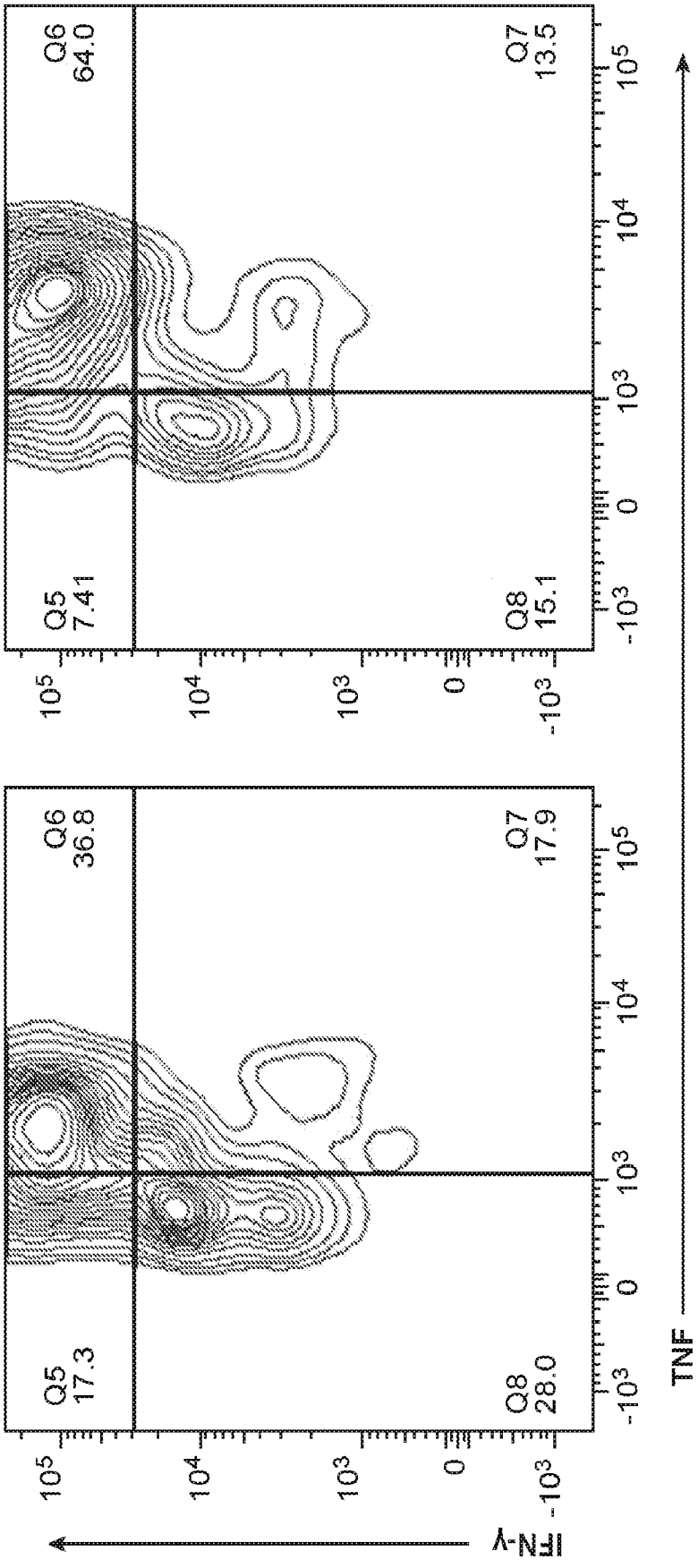
Q6
64.0
Q7
13.5
Q5
7.41
Q8
15.1
Q6
36.8
Q7
17.9
Q5
17.3
Q8
28.0
10^5
10^4
10^3
0
-10^3
IFN-γ
TNF

FIG. 12D

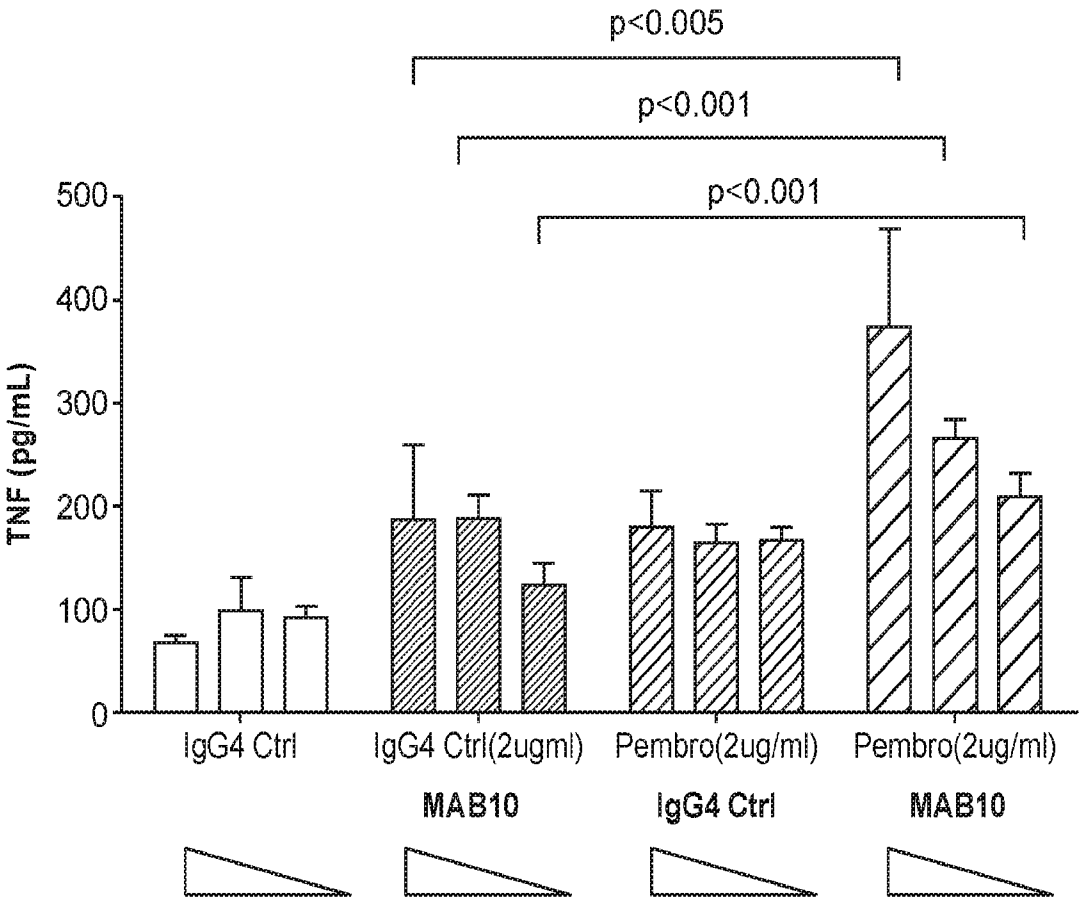
p<0.005
p<0.001
p<0.001
500
400
300
200
100
0
TNF (pg/mL)
IgG4 Ctrl
IgG4 Ctrl(2ugml)
Pembro(2ug/ml)
Pembro(2ug/ml)
MAB10
IgG4 Ctrl
MAB10

FIG. 13

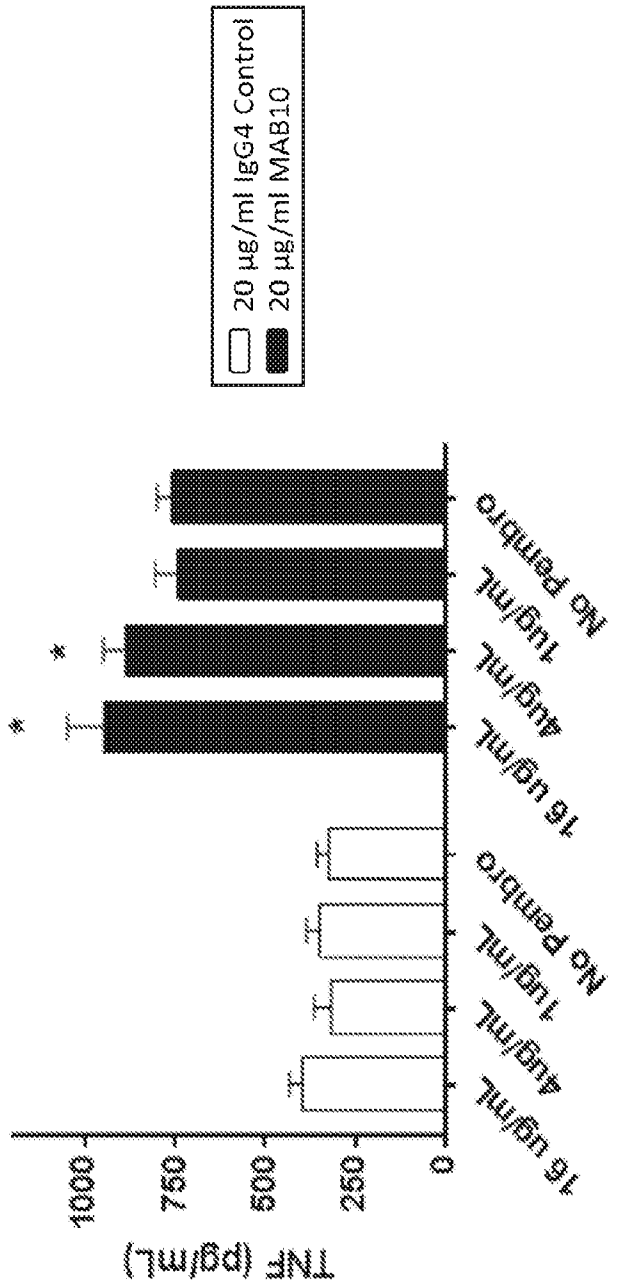
20 µg/ml IgG4 Control
20 µg/ml MAB10
TNF (pg/mL)
1000
750
500
250
0
16 ug/mL
4ug/mL
1ug/mL
No Pembro
16 ug/mL
4ug/mL
1ug/mL
No Pembro
*
*

FIG. 14A

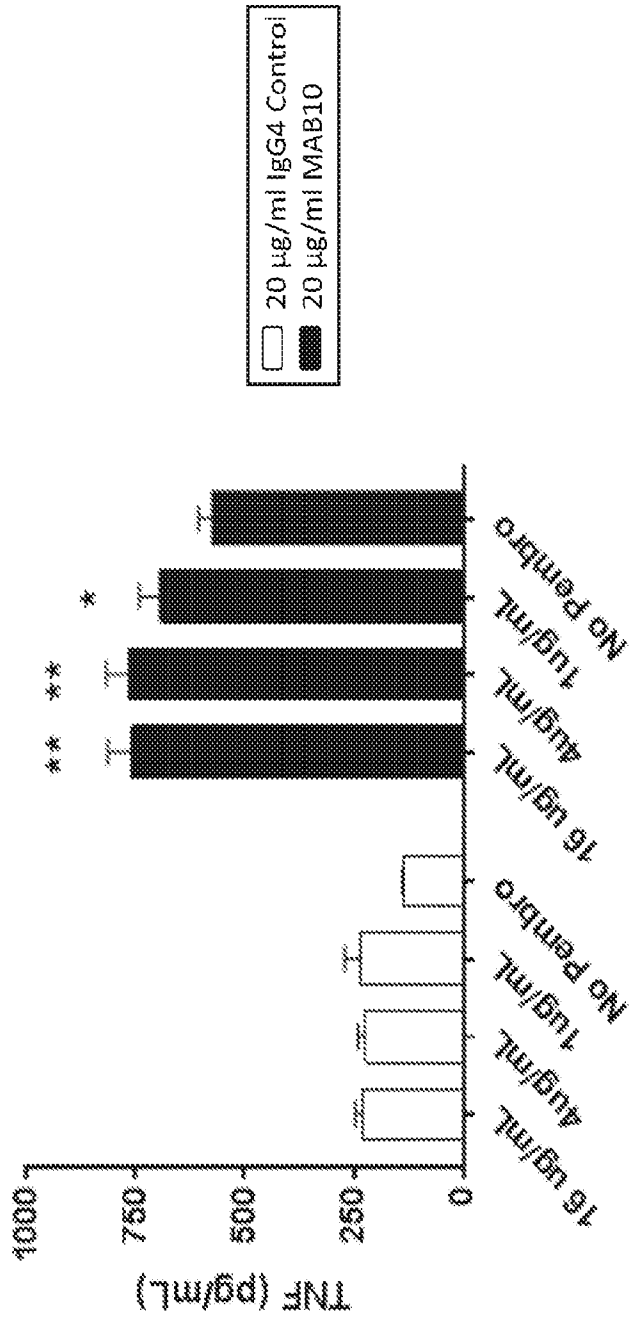
20 µg/ml IgG4 Control
20 µg/ml MAB10
TNF (pg/mL)
1000
750
500
250
0
16 ug/mL
4ug/mL
1ug/mL
No Pembro
16 ug/mL
4ug/mL
1ug/mL
No Pembro
**
**
*

FIG. 14B

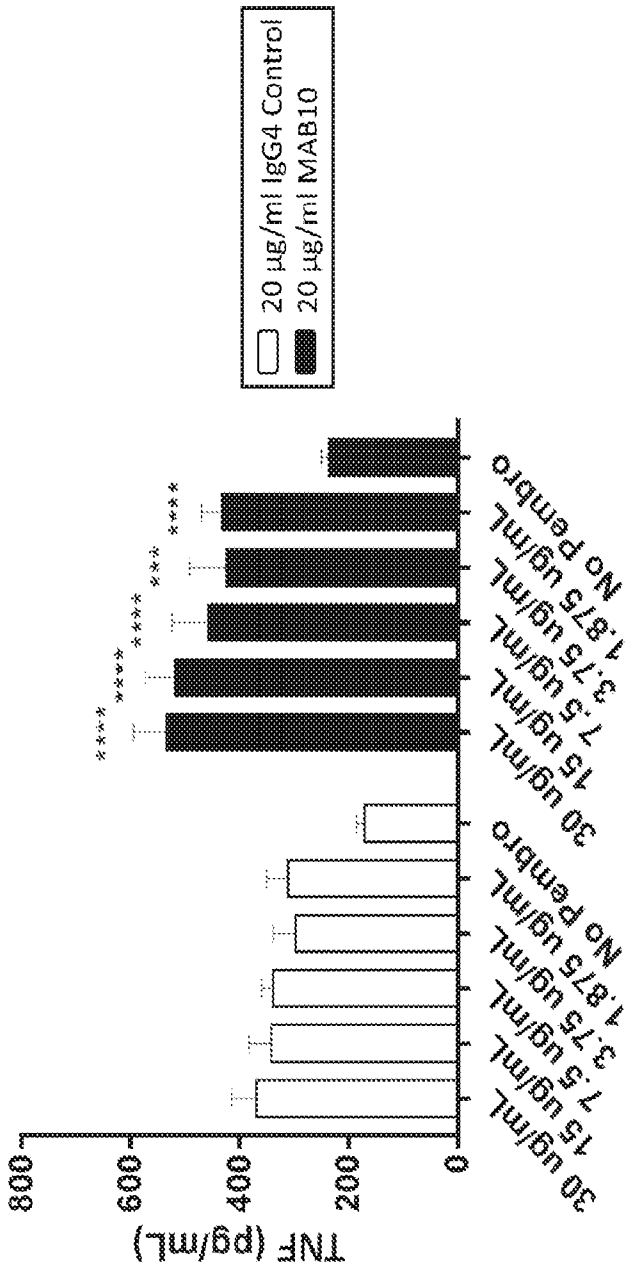
20 µg/ml IgG4 Control
20 µg/ml MAB10
TNF (pg/mL)
800
600
400
200
0
30 ug/mL
15 ug/mL
7.5 ug/mL
3.75 ug/mL
1.875 ug/mL
No pembro
30 ug/mL
15 ug/mL
7.5 ug/mL
3.75 ug/mL
1.875 ug/mL
No pembro
****
****
****
***
****

FIG. 14C