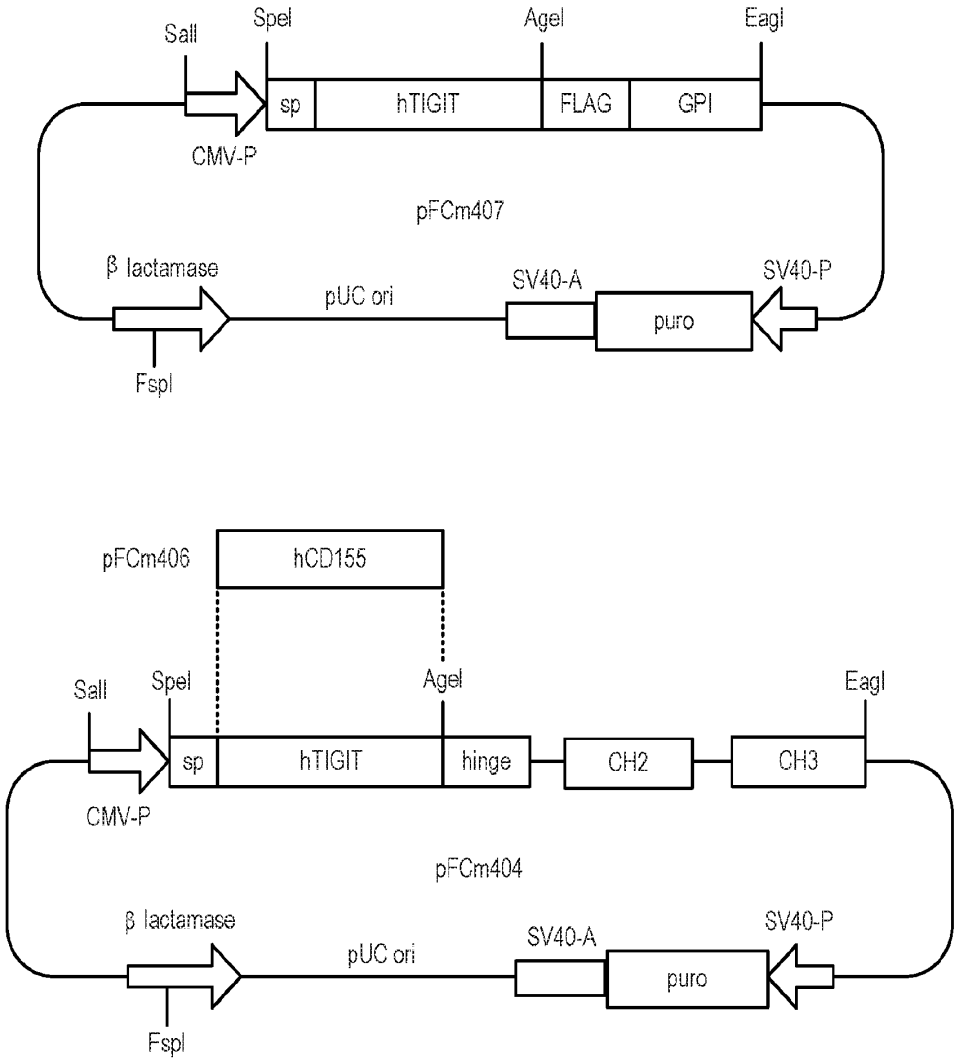

SalI
SpeI
AgeI
EagI
sp
hTIGIT
FLAG
GPI
CMV-P
pFCm407
β lactamase
pUC ori
SV40-A
SV40-P
puro
FspI
pFCm406
hCD155
SalI
SpeI
AgeI
EagI
sp
hTIGIT
hinge
CH2
CH3
CMV-P
pFCm404
β lactamase
pUC ori
SV40-A
SV40-P
puro
FspI

FIG. 1

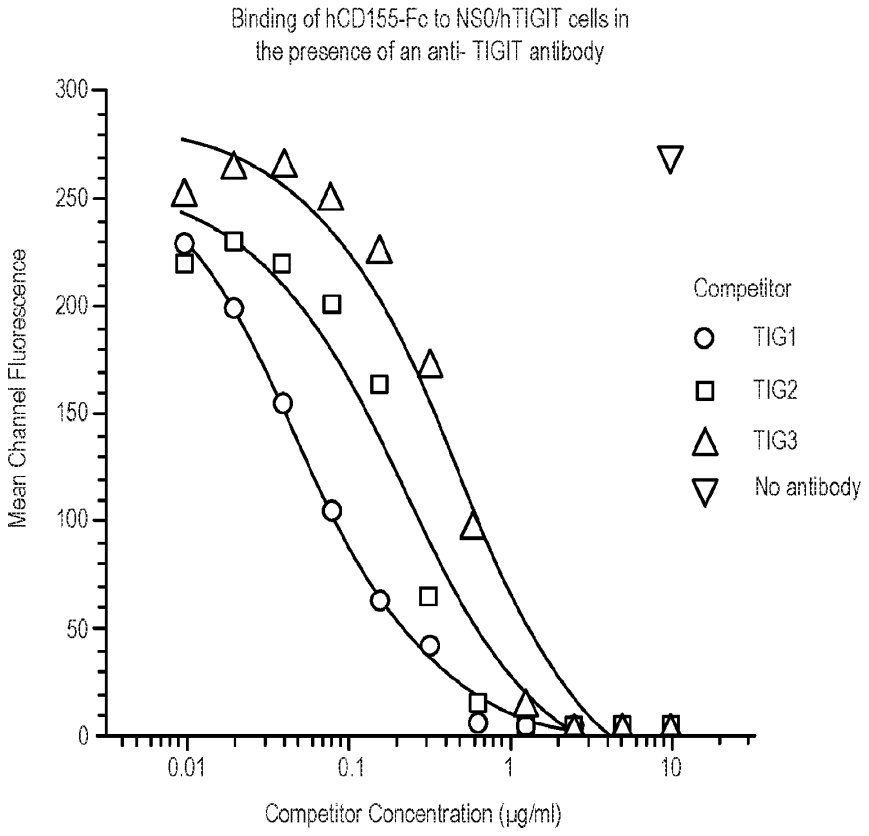

Binding of hCD155-Fc to NS0/hTIGIT cells in the presence of an anti- TIGIT antibody
Mean Channel Fluorescence
300
250
200
150
100
50
0
0.01
0.1
1
10
Competitor Concentration (µg/ml)
Competitor
TIG1
TIG2
TIG3
No antibody

FIG. 2

TIG1 VH

```
            1           2           3
123456789 0123456789 0123456789 0123456789
DVQLVESGG GLVQPGGSRK LSCAASGFTF SNFGMHWVRQ
                                  CDR1

4           5           6           7
0123456789 01223456789 0123456789 0123456789
             a
APEKGLEWVA FISSGSSSIYY ADTVKGRFTI SRDNPKNTLF
                CDR2

                                1           1
8               9               0           1
0122223456789 0123456789 000123456789 0123
   abc                        ab
LQMTSLRSEDTAM YYCARMRLDY YAMDYWGQGTSV TVSS
                CDR3
```

FIG. 3

TIG1 VL

```
          1          2          3
123456789 0123456789 0123456789 0123456789
DVQITQSPS YLAAS PGETI TINCRASKSI SKYLAWYQEK
                                CDR1

4          5          6          7
0123456789 0123456789 0123456789 0123456789
PGKTNKLLIY SGSTLQSGIP SRFSGSGSGT DFTLTISSLE
           CDR2

                                1
8          9                    0
0123456789 0123456789 01234567
PEDFAMYYCQ QHNEYPWTFG GGTKLEIK
           CDR3
```

FIG. 4

TIG2 VH

```
                1                2                3
123456789  0123456789  0123456789  0123456789
EVQLQQSGP  ELVKPGASVK  ISCKTSGYTF  TEYTMHWVKQ
                                       CDR1

4                5                6                7
0123456789  01223456789  0123456789  0123456789
              a
SHGKNLEWIG  GINPNNGGTSY   NQKFKGRATL  TVDKSSSTAY
                    CDR2

                                   1                1
8                9                0                1
0122223456789  0123456789  000123456789  0123
   abc                         ab
MELRSLTSDDSAV  YYCARPGWYN  YAMDYWGQGTSV  TVSS
                      CDR3
```

FIG. 5

TIG2 VL

```
                1           2           3
123456789 0123456789 0123456789 0123456789
DIVMTQSHK FMSTSVGDRV NITCKASQGV STAVAWYQQK
                                 CDR1

4           5           6           7
0123456789 0123456789 0123456789 0123456789
PGQSPKLLIY SASYRYTGVP DRFTGSGSGT DFTFTISSVQ
           CDR2

                                1
8           9           0
0123456789 0123456789 01234567
AEDLAVYHCQ QHYITPWTFG GGTKLEIK
           CDR3
```

FIG. 6

TIG3 VH

```
                 1              2              3
123456789  0123456789  0123456789  0123456789
EVQLVESGG  GLVKPGGSLK  LSCAASGFAF  SDYDMSWVRQ
                                       CDR1

4            5              6            7
0123456789 0123456789   0123456789 0123456789
             a
TPEKRLEWVA YISDGGYNTYY   PDTVKGRFTI SRDNAKNTLY
                  CDR2

                                 1            1
8              9                 0            1
0122223456789 0123456789  000123456789 0123
   abc                        ab
LQMSSLKSEDTAI YYCARQILLR  YYFDYWGQGTTL TVSS
                     CDR3
```

FIG. 7

TIG3 VL

```
                1                2                    3
123456789 0123456789 0123456789777777789 0123456789
                                  abcdef
DIVMSQSPS SLAVSVGEKV TMTCKSSQSLLYSSNQ KNYLAWYQQK
                                                CDR1

4          5          6          7
0123456789 0123456789 0123456789 0123456789
PGQSPKLLIY WASTRESGVP DRFTGSGSGT DFTLTISSVK
             CDR2

                                  1
8          9          0
0123456789 0123456789 01234567
AEDLAVYYCQ QYHSYPWTFG GGTKLEIK
             CDR3
```

FIG. 8

HuTIG1 VH gene

ACTAGTACCACCATGGACTCCAGGCTCAATCTGGTTTTCCTTGTCCTTATTCTGAAAGGC

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

GTCCAGTGTGAAGTGCAGCTGGTGGAGTCTGGGGGAGGCCTGGTGCAGCCTGGAGGGTCC

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

CTGAGACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAACTTTGGAATGCACTGGGTT

L R L S C A A S G F T F S N F G M H W V

CGACAGGCTCCAGGGAAGGGGCTGGAGTGGGTCGCATTCATTAGTAGTGGCAGTAGTTCC

R Q A P G K G L E W V A F I S S G S S S

ATCTACTATGCAGACACAGTGAAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAAC

I Y Y A D T V K G R F T I S R D N A K N

AGCCTGTACCTGCAAATGAACAGTCTGAGGGCTGAGGACACTGCCGTGTATTACTGTGCA

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

AGAATGAGACTGGATTACTATGCTATGGACTACTGGGGTCAAGGAACCATGGTCACCGTC

R M R L D Y Y A M D Y W G Q G T M V T V

TCCTCAGGTAAGTATGGCCTCTAAGCTT (SEQ ID NO:38)

S S (SEQ ID NO:34)

FIG. 9A

HuTIG1 VL gene

GCTAGCACCACCATGAGGTTCCAGGTTCAGGTTCTGGGGCTCCTTCTGCTCTGGATCTCA

M R F Q V Q V L G L L L L W I S

GGAGCCCAGTGTGATATCCAGATGACCCAGTCTCCATCTTCTCTTTCTGCATCTGTTGGA

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

GATAGAGTCACTATTACTTGCAGGGCAAGTAAGAGCATTAGCAAATATCTGGCCTGGTAT

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

CAACAGAAACCTGGGAAAGCTCCTAAGCTGCTTATCTACTCTGGGTCCACTTTGCAATCT

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

GGAGTTCCATCAAGATTCAGTGGCAGTGGATCTGGTACAGATTTCACTCTCACCATCAGT

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

AGCCTGCAGCCTGAAGATTTTGCAACCTATTACTGTCAACAGCATAATGAATACCCCTGG

S L Q P E D F A T Y Y C Q Q H N E Y P W

ACCTTCGGCGGAGGCACCAAAGTCGAAATCAAACGTAAGTAGAATCCAAAGAATTC (SEQ ID NO:39)

T F G G G T K V E I K (SEQ ID NO:36)

FIG. 9B

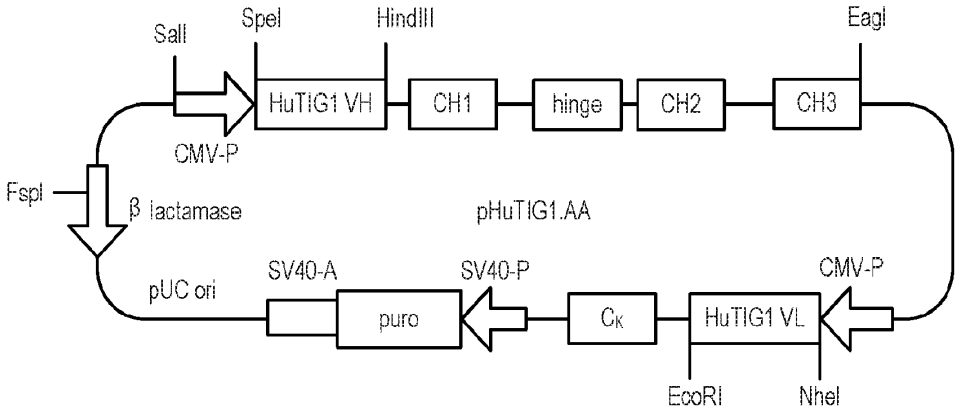

SalI
SpeI
HindIII
EagI
HuTIG1 VH
CH1
hinge
CH2
CH3
CMV-P
FspI
β lactamase
pHuTIG1.AA
pUC ori
SV40-A
SV40-P
CMV-P
puro
Cκ
HuTIG1 VL
EcoRI
NheI

FIG. 10

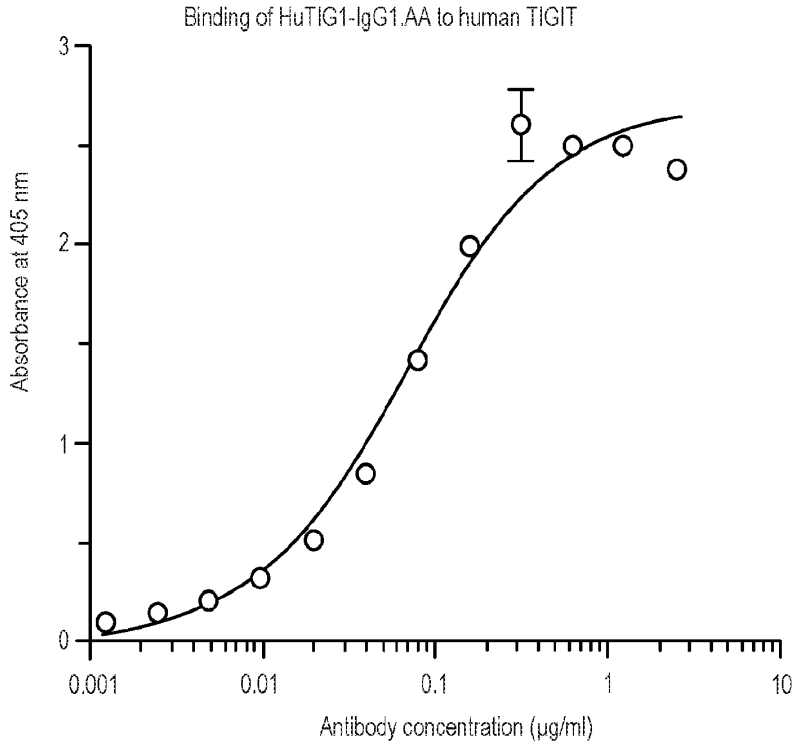

Binding of HuTIG1-IgG1.AA to human TIGIT
Absorbance at 405 nm
3
2
1
0
0.001
0.01
0.1
1
10
Antibody concentration (µg/ml)

FIG. 11

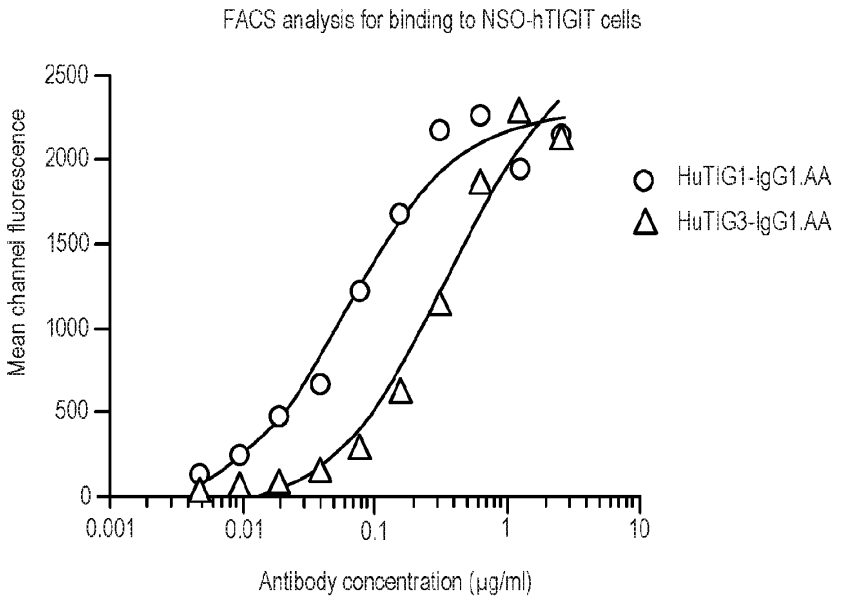

FACS analysis for binding to NSO-hTIGIT cells
Mean channel fluorescence
2500
2000
1500
1000
500
0
0.001
0.01
0.1
1
10
Antibody concentration (µg/ml)
HuTIG1-IgG1.AA
HuTIG3-IgG1.AA

FIG. 12

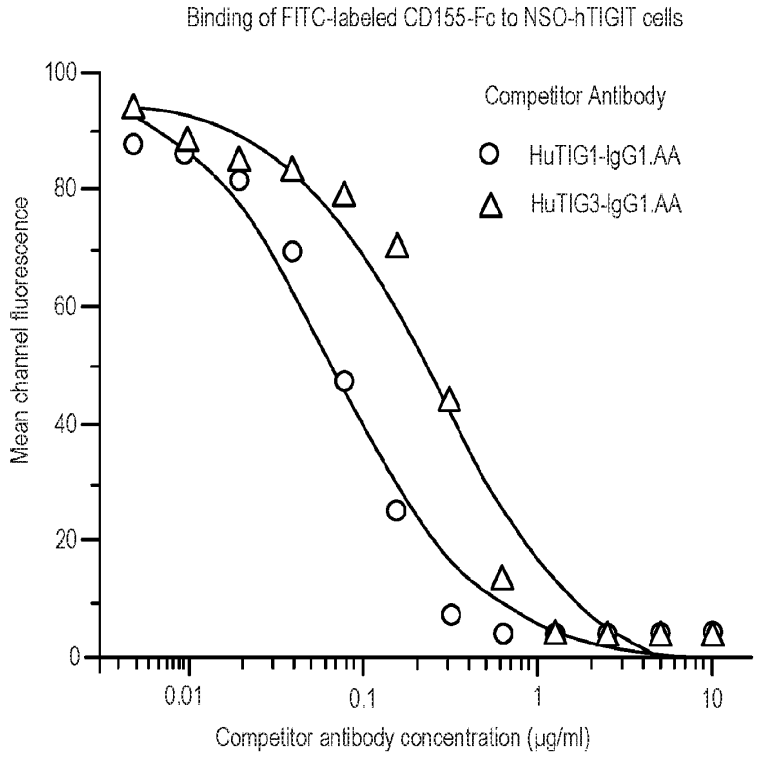

Binding of FITC-labeled CD155-Fc to NSO-hTIGIT cells
Competitor Antibody
HuTIG1-IgG1.AA
HuTIG3-IgG1.AA
Mean channel fluorescence
100
80
60
40
20
0
0.01
0.1
1
10
Competitor antibody concentration (µg/ml)

FIG. 13

HuTIG3 VH gene

ACTAGTACCACCATGAACTTTGGGCTCAGATTGATTTTCCTTGTCCTTACTCTGAAAGGC

M N F G L R L I F L V L T L K G

GTGAACTGTGAAGTCCAGCTCGTGGAGTCTGGGGGAGGCCTTGTGCAGCCTGGAGGGTCC

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

CTGAGACTCTCCTGTGCAGCCTCTGGATTCGCTTTCAGTGACTATGACATGTCTTGGGTT

L R L S C A A S G F A F S D Y D M S W V

CGCCAGGCTCCTGGCAAGGGGCTGGAGTGGGTCGCATACATTAGTGATGGCGGTTATAAC

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

ACCTACTATCCAGACACTGTGAAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAAC

T Y Y P D T V K G R F T I S R D N A K N

TCCCTGTACCTGCAAATGAACAGTCTGAGGGCTGAGGACACAGCCGTCTATTACTGTGCA

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

AGACAAATTCTGCTGCGGTACTACTTTGACTACTGGGGCCAAGGCACCACTGTCACAGTC

R Q I L L R Y Y F D Y W G Q G T T V T V

TCCTCAGGTGAGTCCTTAAAACAAGCTT (SEQ ID NO:46)

S S (SEQ ID NO:42)

FIG. 14A

HuTIG3 VL gene

GCTAGCACCACCATGGATTCACAGGCCCAGGTTCTTATGCTGCTGCTGCTCTGGGTTTCT

M D S Q A Q V L M L L L L W V S

GGAACCTGTGGGGACATTCAGATGACACAGTCTCCATCCTCCCTGTCTGCCTCAGTTGGA

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

GACAGGGTTACTATCACCTGCAAGTCCAGTCAGAGTCTTCTGTATAGTAGCAATCAAAAG

D R V T I T C K S S Q S L L Y S S N Q K

AACTACTTGGCCTGGTACCAGCAGAAACCAGGGAAGGCTCCTAAACTGCTGATTTACTGG

N Y L A W Y Q Q K P G K A P K L L I Y W

GCATCCACTAGGGAATCTGGGGTCCCTAGTCGCTTCTCAGGCAGTGGATCTGGGACAGAT

A S T R E S G V P S R F S G S G S G T D

TTCACTCTCACCATCAGCAGTCTGCAGCCTGAAGACTTCGCAGTTTATTACTGTCAGCAA

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

TATCATAGCTATCCCTGGACCTTCGGCGGAGGCACCAAGGTGGAAATCAAACGTAAGTAG

Y H S Y P W T F G G G T K V E I K (SEQ ID NO:44)

AATCCAAAGAATTC (SEQ ID NO:47)

FIG. 14B

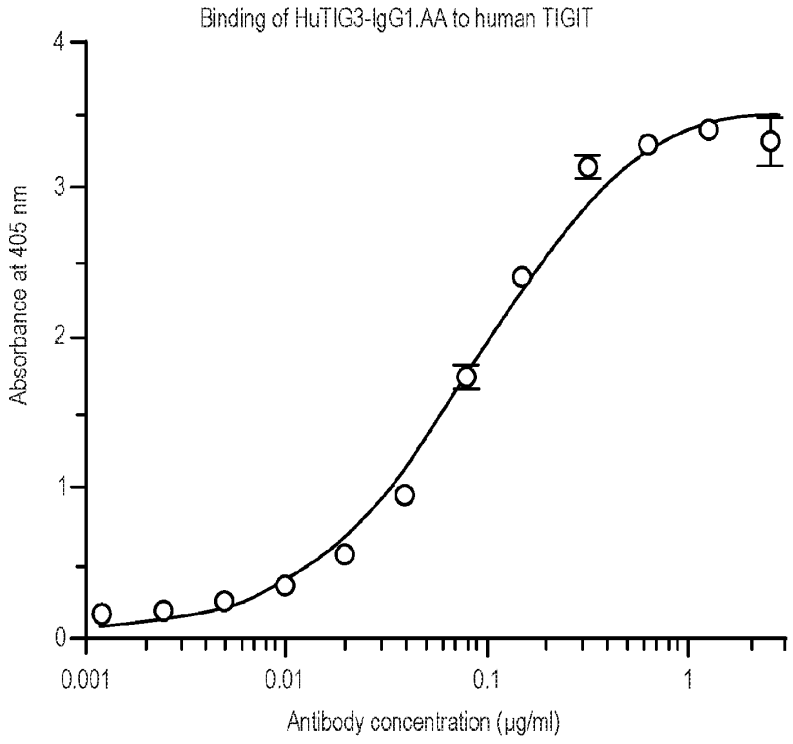

Binding of HuTIG3-IgG1.AA to human TIGIT
Absorbance at 405 nm
0
1
2
3
4
0.001
0.01
0.1
1
Antibody concentration (µg/ml)

FIG. 15

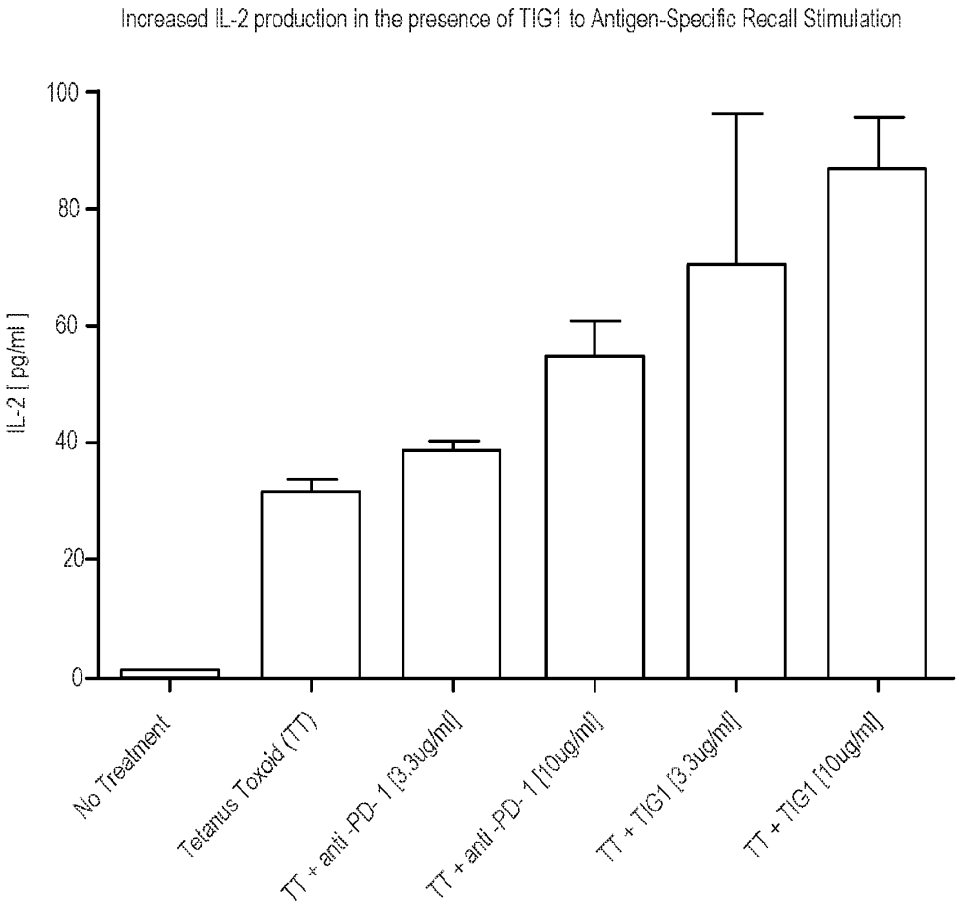

Increased IL-2 production in the presence of TIG1 to Antigen-Specific Recall Stimulation
IL-2 [pg/ml]
100
80
60
40
20
0
No Treatment
Tetanus Toxoid (TT)
TT + anti-PD-1 [3.3ug/ml]
TT + anti-PD-1 [10ug/ml]
TT + TIG1 [3.3ug/ml]
TT + TIG1 [10ug/ml]

FIG. 16

Increased CD4+ & CD8+ T cell proliferation by TIG1 to Antigen-Specific Recall Stimulation.

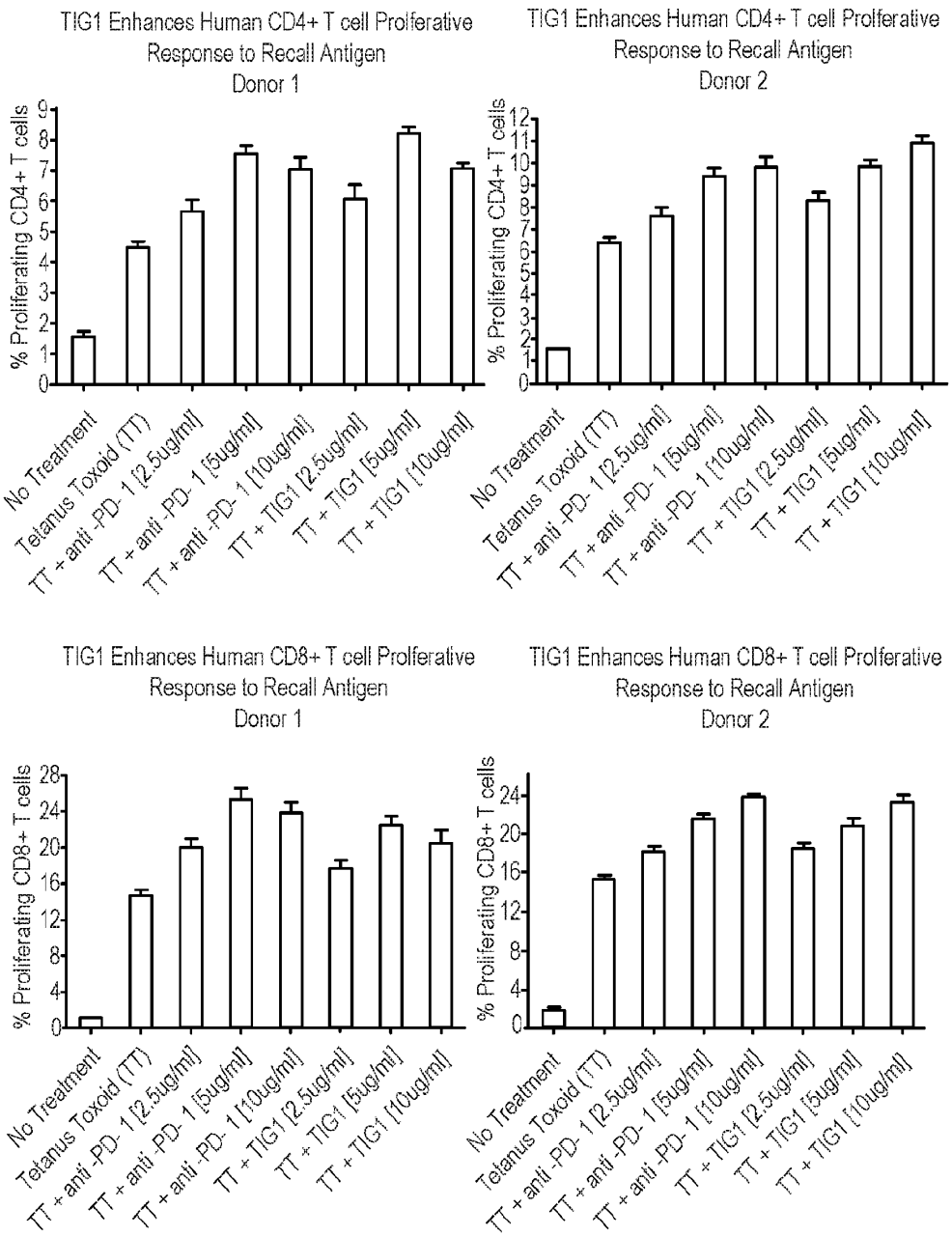


FIG. 17

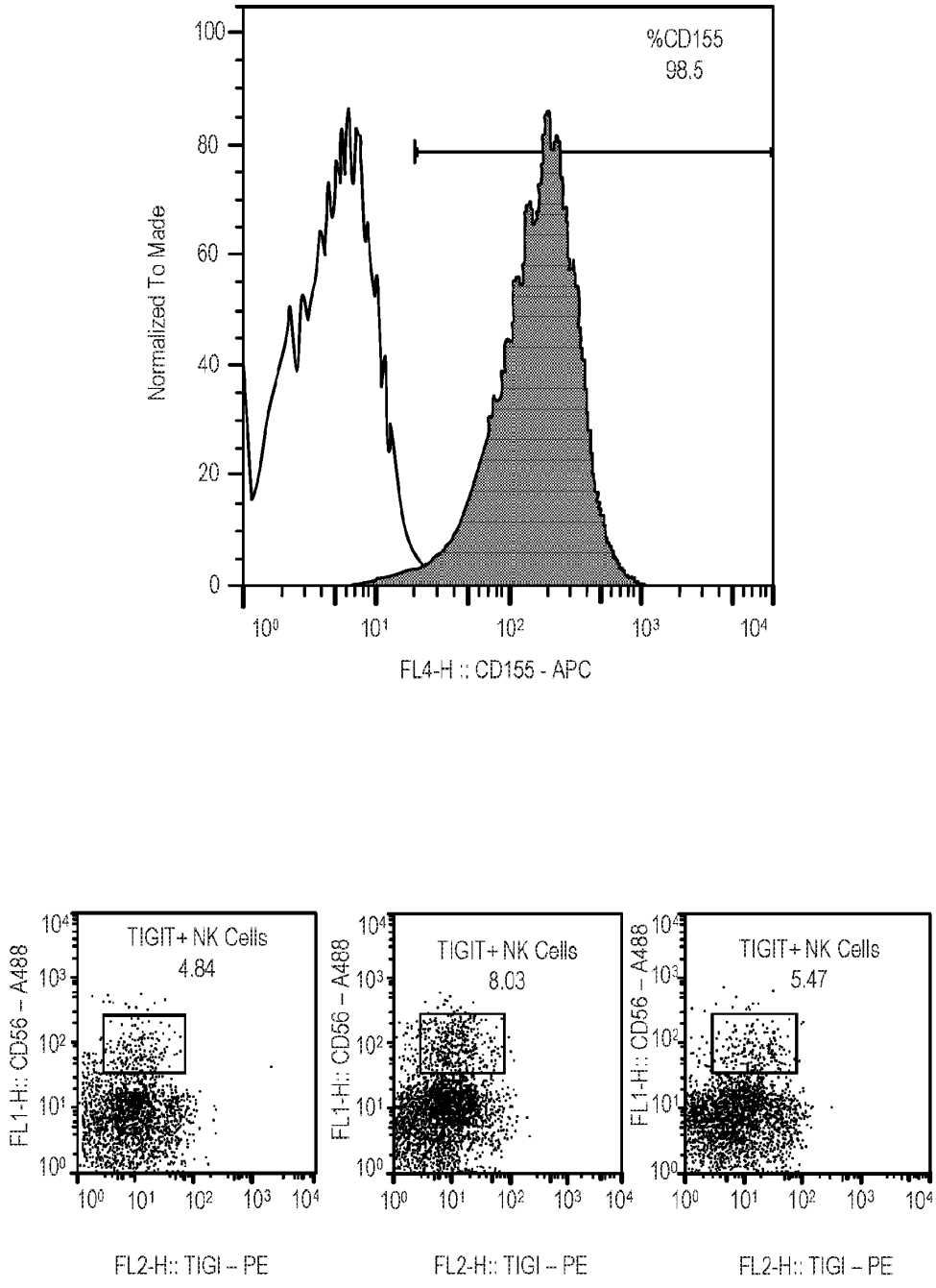

%CD155
98.5
Normalized To Mode
FL4-H :: CD155 - APC
TIGIT+ NK Cells
4.84
TIGIT+ NK Cells
8.03
TIGIT+ NK Cells
5.47
FL1-H:: CD56 – A488
FL2-H:: TIGI – PE

FIG. 18

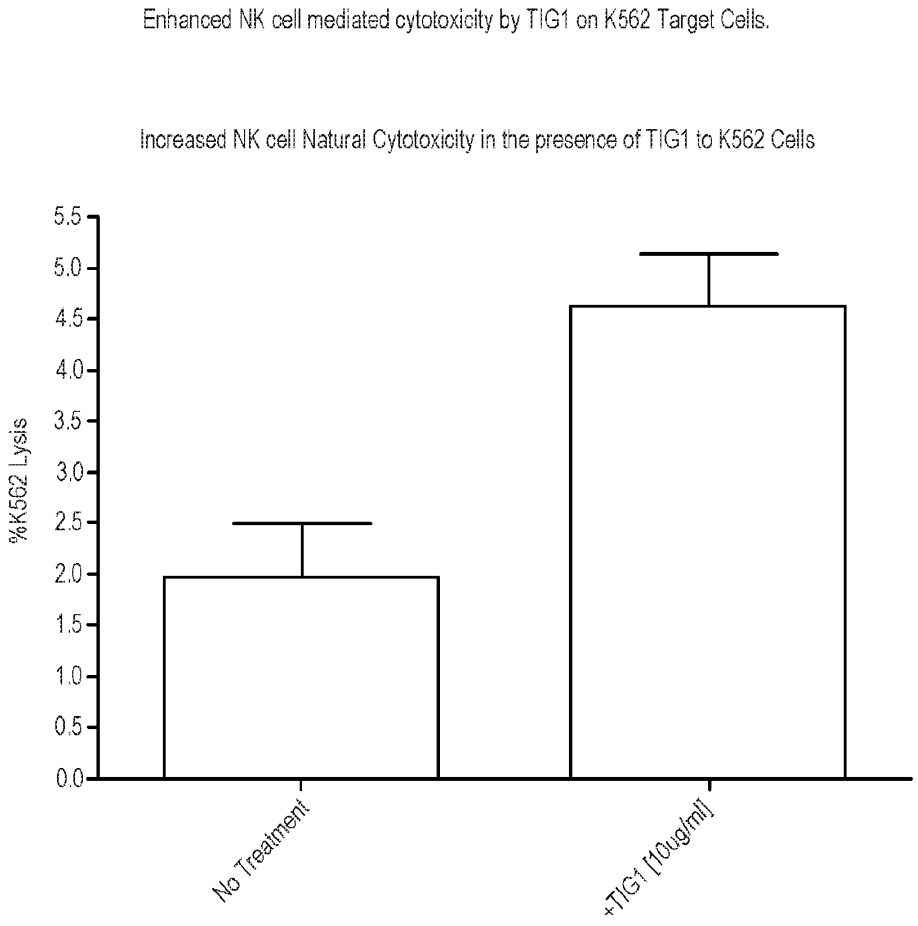

Enhanced NK cell mediated cytotoxicity by TIG1 on K562 Target Cells.
Increased NK cell Natural Cytotoxicity in the presence of TIG1 to K562 Cells
%K562 Lysis
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
No Treatment
+TIG1 [10ug/ml]

FIG. 19

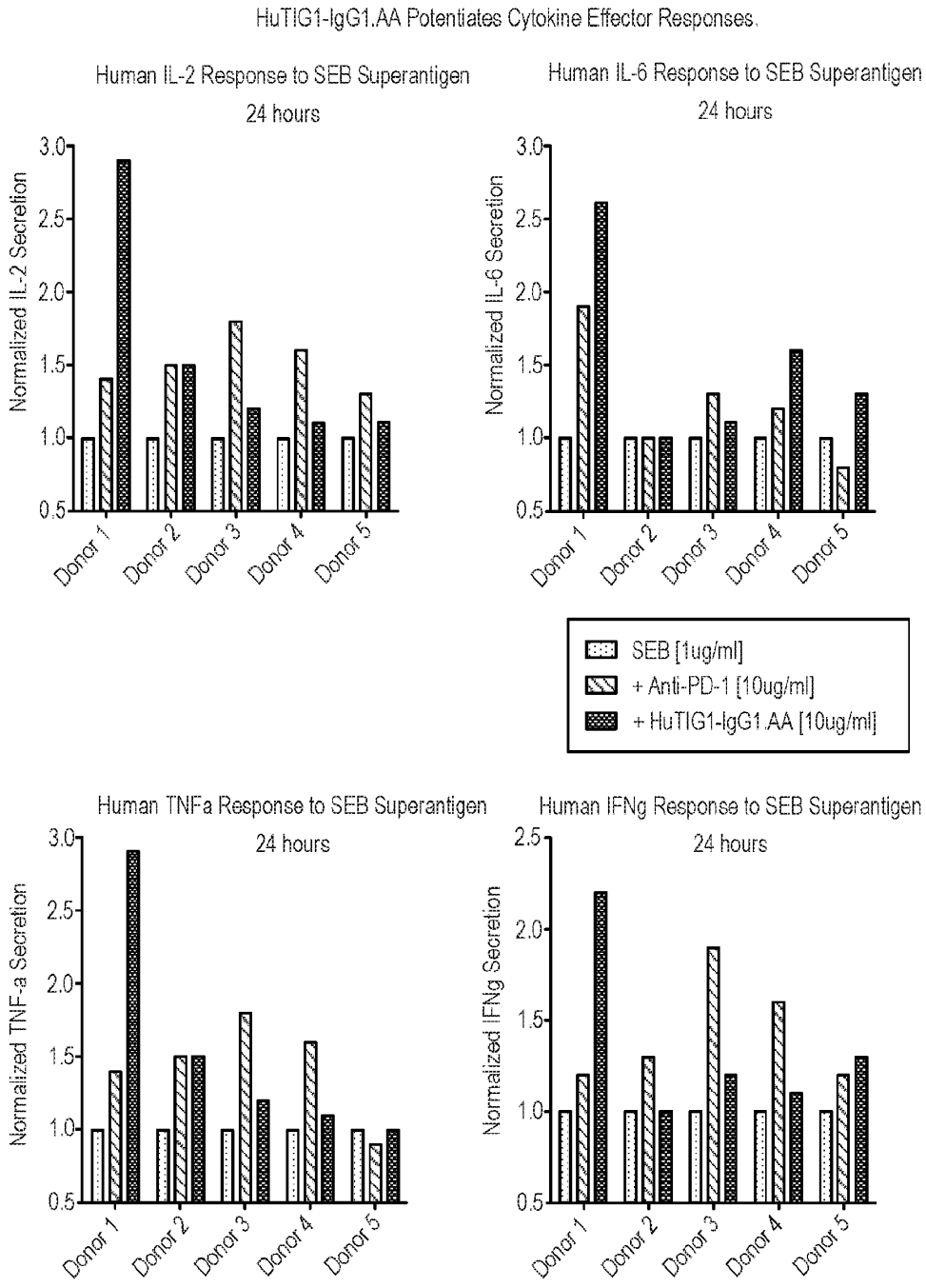

HuTIG1-IgG1.AA Potentiates Cytokine Effector Responses.
Human IL-2 Response to SEB Superantigen
24 hours
Normalized IL-2 Secretion
Human IL-6 Response to SEB Superantigen
24 hours
Normalized IL-6 Secretion
Donor 1
Donor 2
Donor 3
Donor 4
Donor 5
SEB [1ug/ml]
+ Anti-PD-1 [10ug/ml]
+ HuTIG1-IgG1.AA [10ug/ml]
Human TNFa Response to SEB Superantigen
24 hours
Normalized TNF-a Secretion
Human IFNg Response to SEB Superantigen
24 hours
Normalized IFNg Secretion

FIG. 20

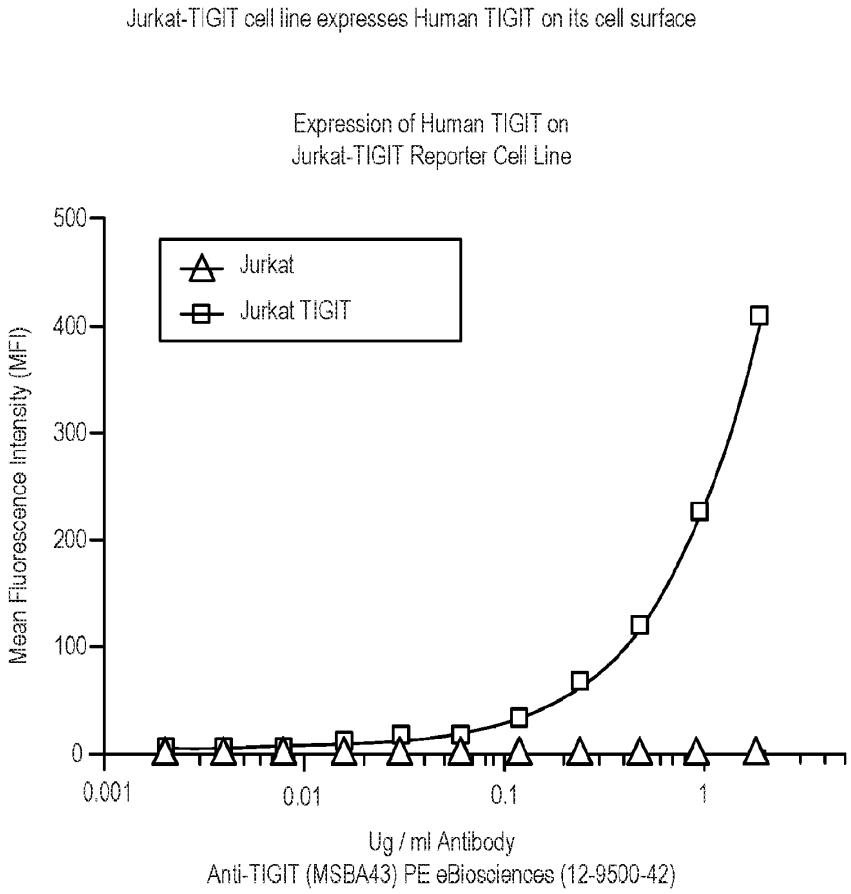

Jurkat-TIGIT cell line expresses Human TIGIT on its cell surface
Expression of Human TIGIT on
Jurkat-TIGIT Reporter Cell Line
Jurkat
Jurkat TIGIT
Mean Fluorescence Intensity (MFI)
500
400
300
200
100
0
0.001
0.01
0.1
1
Ug / ml Antibody
Anti-TIGIT (MSBA43) PE eBiosciences (12-9500-42)

FIG. 21

HuTIG1-IgG1.AA & HuTIG3-IgG1.AA do not elicit CDC Activity.

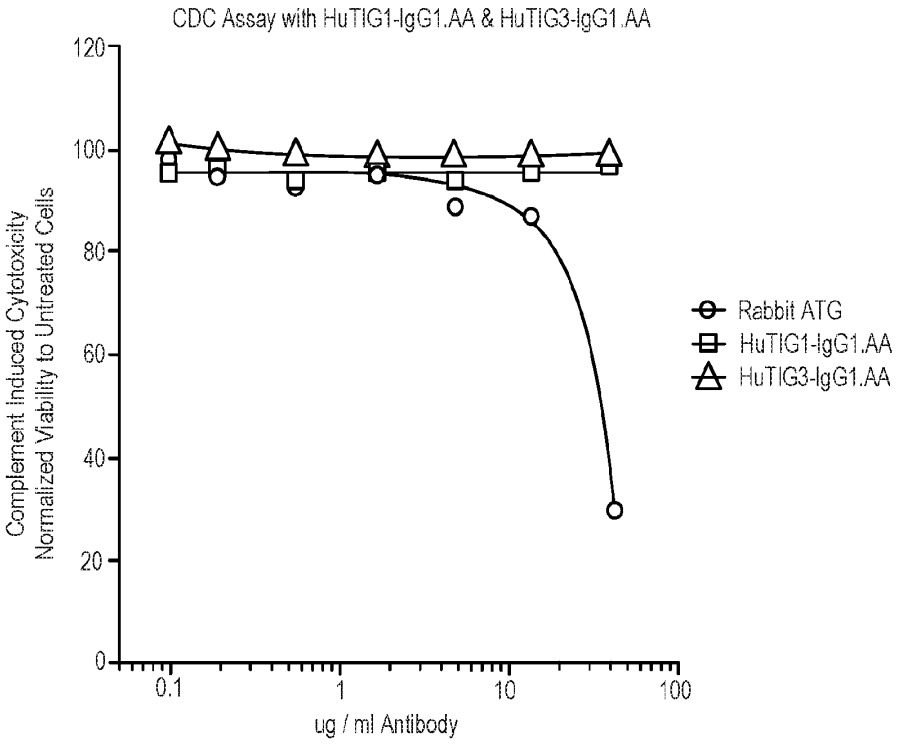


FIG. 22

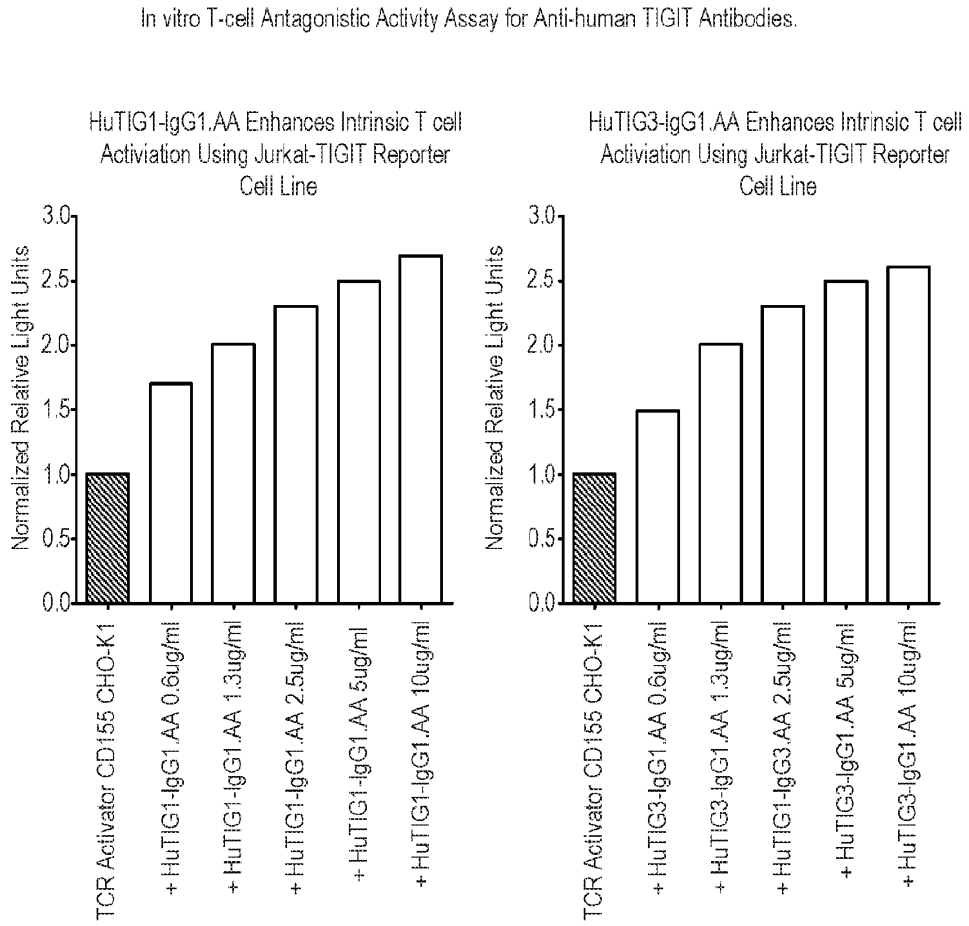

In vitro T-cell Antagonistic Activity Assay for Anti-human TIGIT Antibodies.
HuTIG1-IgG1.AA Enhances Intrinsic T cell Activiation Using Jurkat-TIGIT Reporter Cell Line
Normalized Relative Light Units
3.0
2.5
2.0
1.5
1.0
0.5
0.0
TCR Activator CD155 CHO-K1
+ HuTIG1-IgG1.AA 0.6ug/ml
+ HuTIG1-IgG1.AA 1.3ug/ml
+ HuTIG1-IgG1.AA 2.5ug/ml
+ HuTIG1-IgG1.AA 5ug/ml
+ HuTIG1-IgG1.AA 10ug/ml
HuTIG3-IgG1.AA Enhances Intrinsic T cell Activiation Using Jurkat-TIGIT Reporter Cell Line
Normalized Relative Light Units
3.0
2.5
2.0
1.5
1.0
0.5
0.0
TCR Activator CD155 CHO-K1
+ HuTIG3-IgG1.AA 0.6ug/ml
+ HuTIG3-IgG1.AA 1.3ug/ml
+ HuTIG1-IgG3.AA 2.5ug/ml
+ HuTIG3-IgG1.AA 5ug/ml
+ HuTIG3-IgG1.AA 10ug/ml

FIG. 23

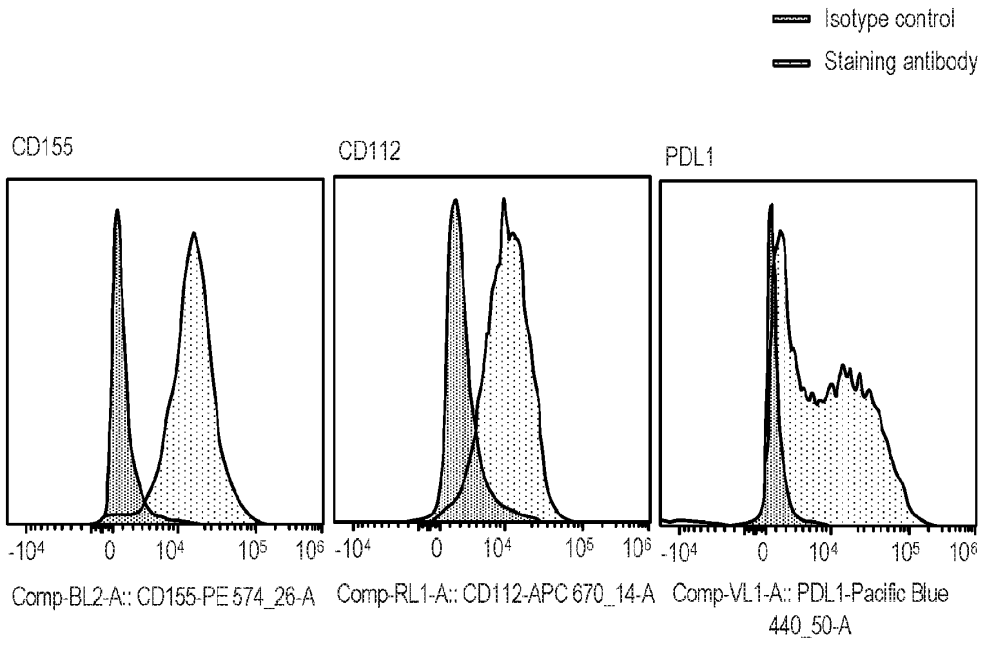

Isotype control
Staining antibody
CD155
CD112
PDL1
-10^4
0
10^4
10^5
10^6
Comp-BL2-A:: CD155-PE 574_26-A
Comp-RL1-A:: CD112-APC 670_14-A
Comp-VL1-A:: PDL1-Pacific Blue 440_50-A

FIG. 24

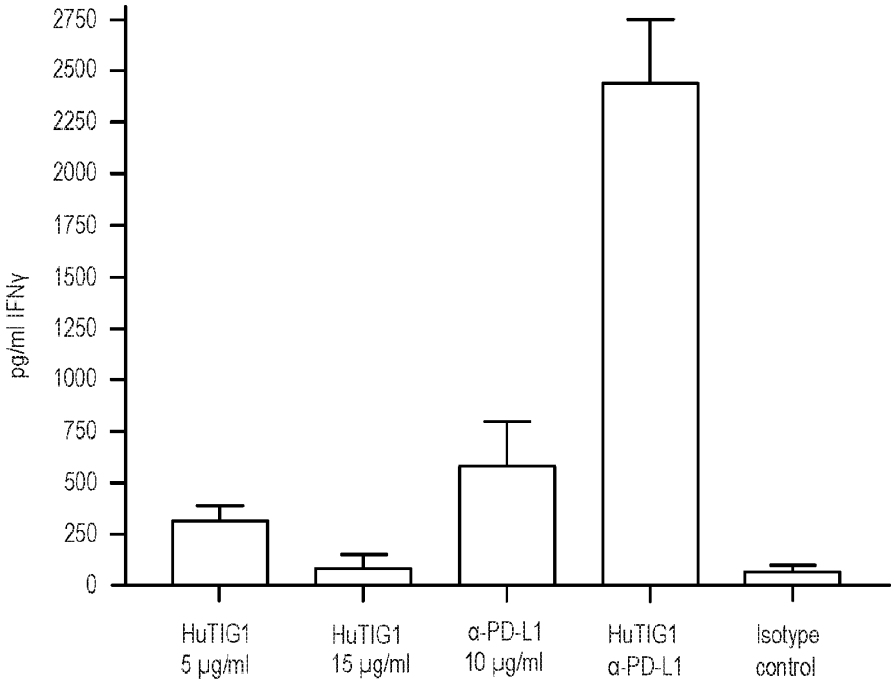

pg/ml IFNγ
2750
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
HuTIG1 5 µg/ml
HuTIG1 15 µg/ml
α-PD-L1 10 µg/ml
HuTIG1 α-PD-L1
Isotype control

FIG. 25

				α-TIGIT		
		Subsets	Cell Surface Markers	HuTIG1	HuTIG3	α -CD96
Lymphoid	B cells	B1	$CD19^+ IgD^+ CD27^+$	-	-	-
		Naïve	$CD19^+ IgD^+ CD27^-$	-	-	-
		Memory/Plasma	$CD19^+ IgD^- CD27^+$	-	-	-
	NK cells	CD56hi	$CD56^+ CD16^-$	-	-	+
		CD56lo	$CD56^+ CD16^+$	+++	+++	-
		γδ T cells	$TCR\gamma\delta^+$	-	-	+
	CD4 T cells	Naïve	$CD4^+ CD45RA^+ CCR7^+$	-	-	+/-
		Effector	$CD4^+ CD45RA^+ CCR7^-$	-	-	-
		EM	$CD4^+ CD45RA^- CCR7^-$	+/-	+/-	+
		CM	$CD4^+ CD45RA^- CCR7^+$	+/-	+/-	+
		CD25+ Treg	$CD4^+ CD25RA^+ CD127^-$	+++	+++	+
	CD8 T cells	Naïve	$CD8^+ CD45RA^+ CCR7^+$	-	-	-
		Effector	$CD8^+ CD45RA^+ CCR7^-$	+++	+++	-
		EM	$CD8^+ CD45RA^- CCR7^-$	+/-	+/-	+
Myeloid	Dendritic cell	Plasmacytoid	$CD56^- CD141^- CLEC9a^- CD14^- CD123^+ HLA\text{-}DR^+$	-	-	-
		BDCA3+	$CD56^- CLEC9a^+ CD141^+ HLA\text{-}DR^+$	-	-	-
		Myeloid	$CD56^- CD141^- CLEC9a^- CD14^{-/lo} CD33^- HLA\text{-}DR^+$	-	-	-
	Monocyte	Classical	$CD56^- CD141^- CLEC9a^- CD14^{hi} CD16^- HLA\text{-}DR^+$	-	-	+
		Inflammatory	$CD56^- CD141^- CLEC9a^- CD14^{hi} CD16^+ HLA\text{-}DR^+$	+	+	+
		CD16hi CD14lo	$CD56^- CD141^- CLEC9a^- CD14^{-/lo} CD33^- HLA\text{-}DR^+$	+	+	+

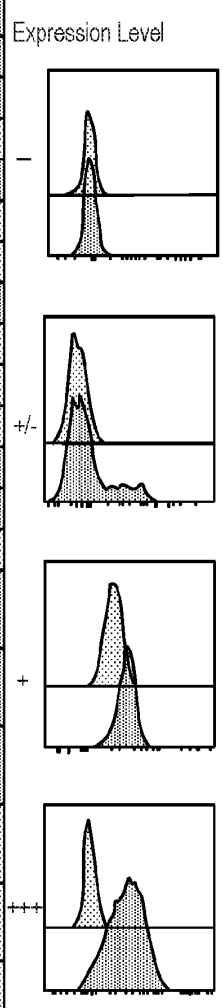
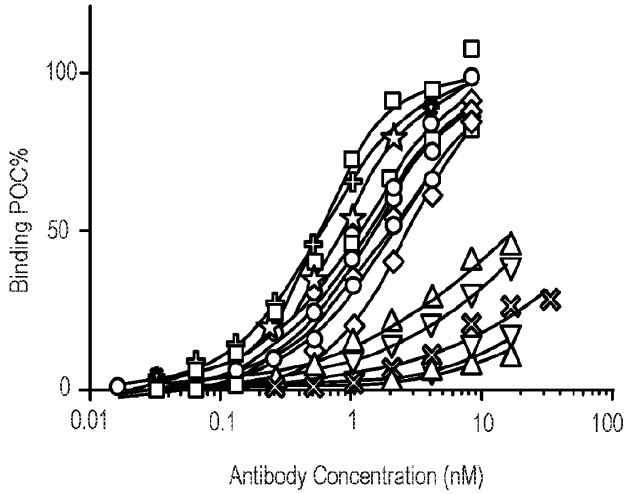
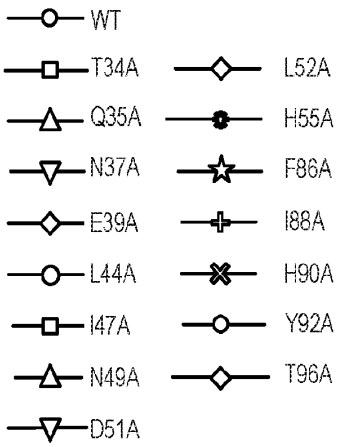


FIG. 26A FIG. 26B





	EC50
WT	1.28
T34A	0.577
Q35A	176
N37A	126
E39A	1.58
L44A	1.23
I47A	1.27
N49A	17.7
D51A	30.5
L52A	2.67
H55A	1.19
F86A	0.844
I88A	0.591
H90A	104
Y92A	2.12
T96A	1.36

FIG. 27

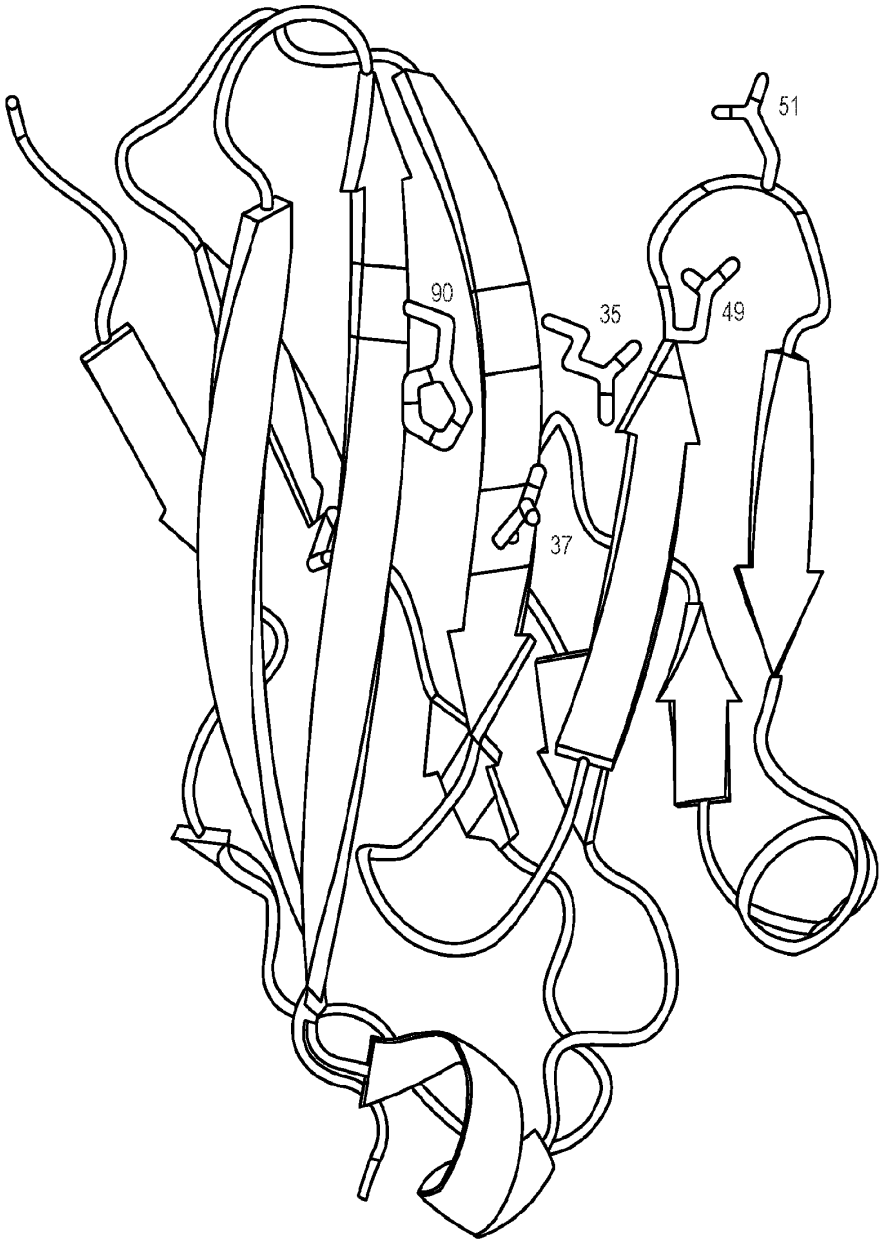


FIG. 28

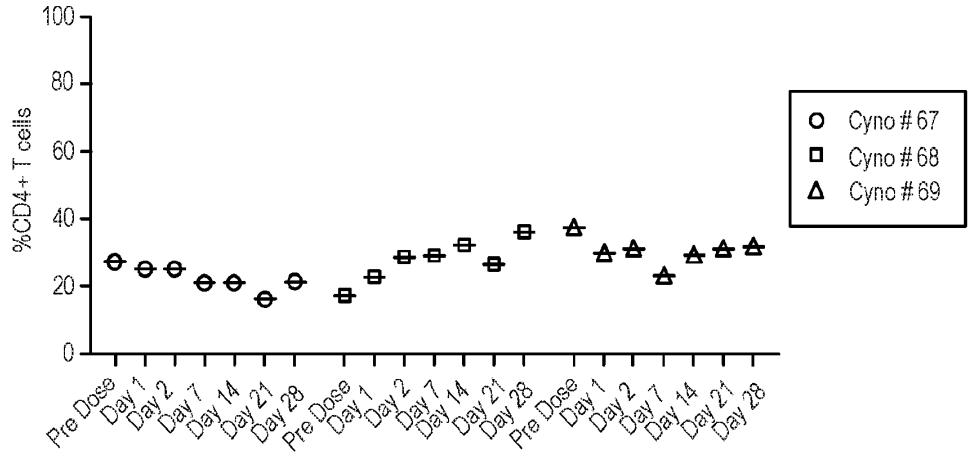


FIG. 29A

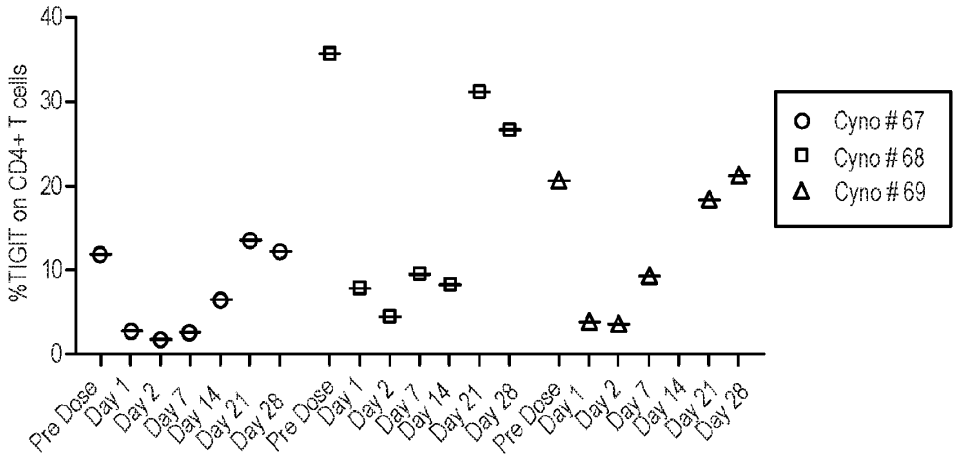


FIG. 29B

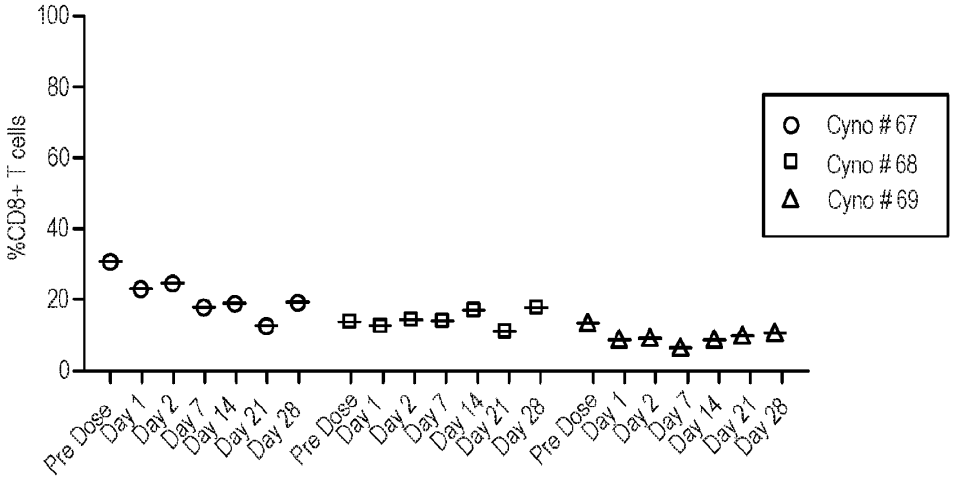


FIG. 30A

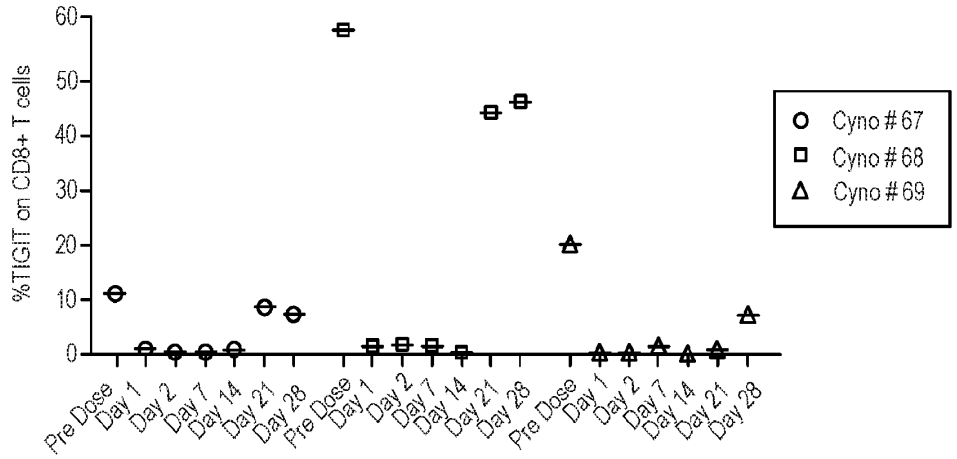


FIG. 30B

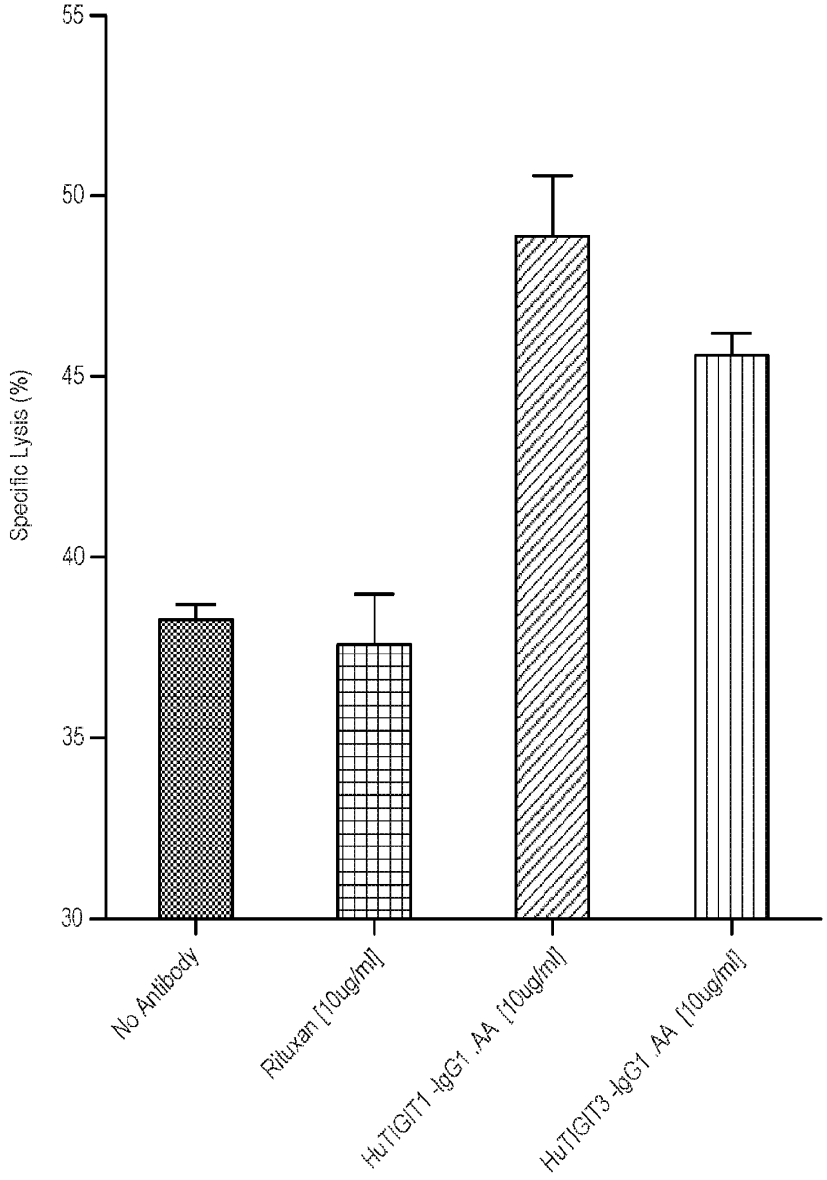

55
50
45
40
35
30
Specific Lysis (%)
No Antibody
Rituxan [10ug/ml]
HuTIGIT1-IgG1_AA [10ug/ml]
HuTIGIT3-IgG1_AA [10ug/ml]

FIG. 31

		Cell Surface Markers	HuTIG1
Melanoma	T cells	$TCR\beta^+ CD4^+ CD25^-$	+/-
		$TCR\beta^+ CD4^+ CD25^+$	++++
		$TCR\beta^+ CD8^+$	++++
	Antigen Presenting Cells	$CD11b^+ CD14^+ HLA\text{-}DR^+$	-
		$CD11b^- CD14^- HLA\text{-}DR^+$	-
Colorectal Cancer	T cells	$TCR\beta^+ CD4^+ CD25^-$	+/-
		$TCR\beta^+ CD4^+ CD25^+$	++++
		$TCR\beta^+ CD8^+$	++++
	Antigen Presenting Cells	$CD11b^+ CD14^+ HLA\text{-}DR^+$	-
		$CD11b^- CD14^- HLA\text{-}DR^+$	-
Non Small Cell Lung Carcinoma	T cells	$TCR\beta^+ CD4^+ CD25^-$	+/-
		$TCR\beta^+ CD4^+ CD25^+$	++++
		$TCR\beta^+ CD8^+$	++++
	Antigen Presenting Cells	$DC11b^+ CD14^+ HLA\text{-}DR^+$	-
		$DC11b^- CD14^- HLA\text{-}DR^+$	-
Renal Clear Cell Carcinoma	T cells	$TCR\beta^+ CD4^- CD25^-$	+/-
		$TCR\beta^+ CD4^+ CD25^+$	++++
		$TCR\beta^+ CD8^+$	++++
	Antigen Presenting Cells	$CD11b^+ CD14^+ HLA\text{-}DR^+$	-
		$CD11b^- CD14^- HLA\text{-}DR^+$	-

FIG. 32A

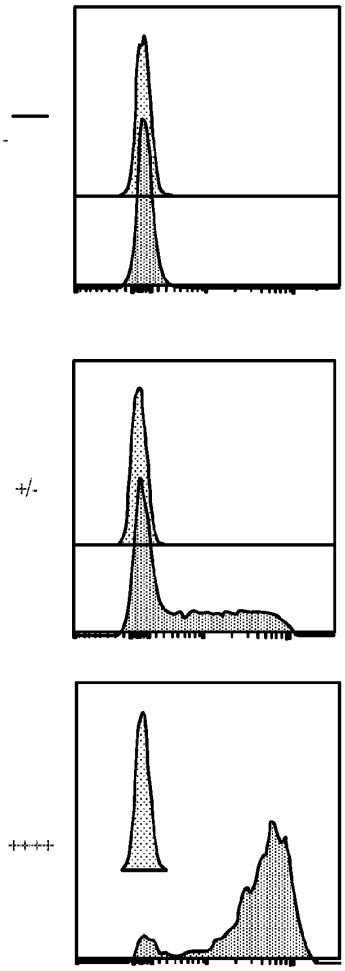


FIG. 32B