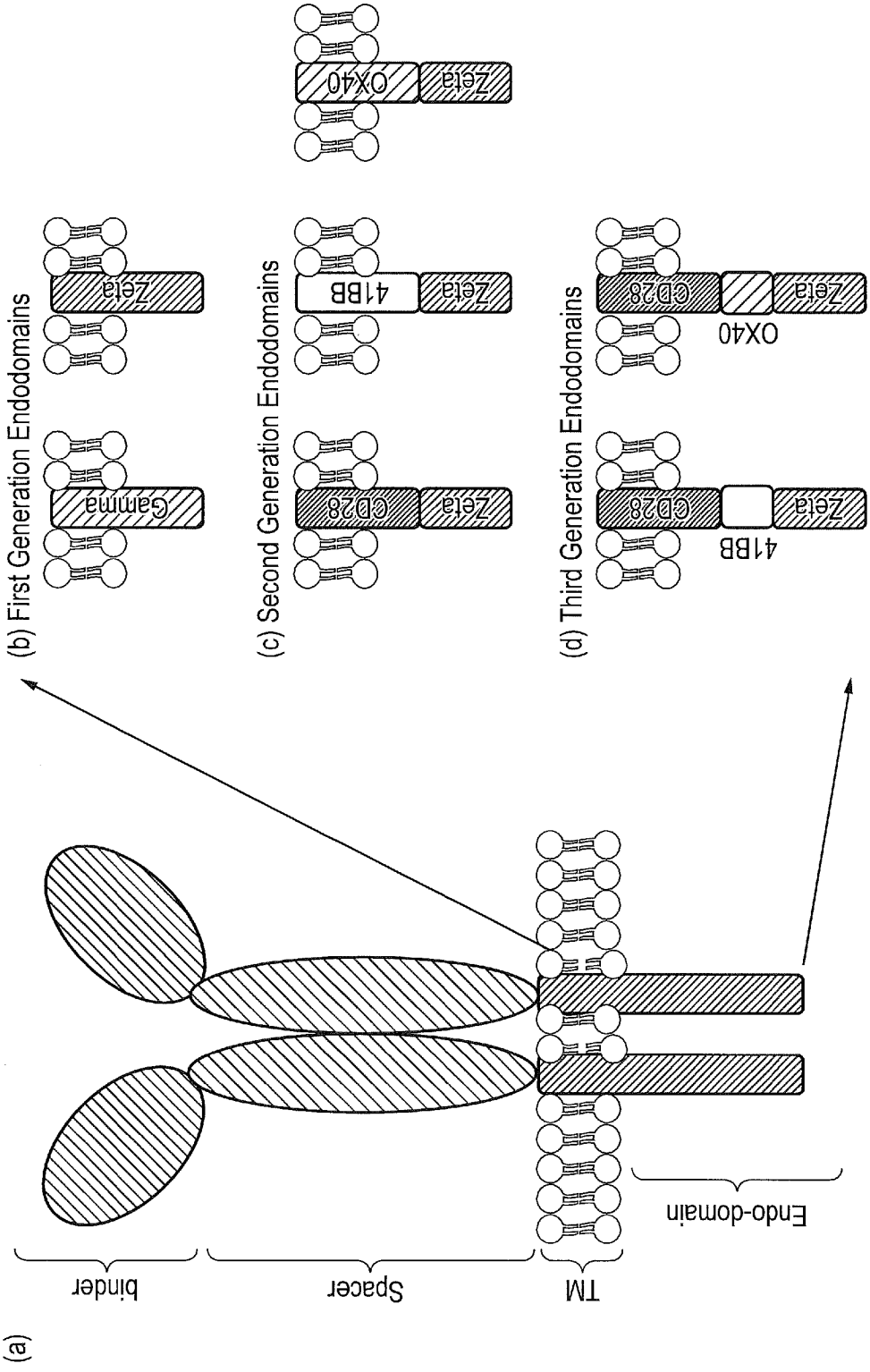

(a)
binder
Spacer
TM
Endo-domain
(b) First Generation Endodomains
Gamma
Zeta
(c) Second Generation Endodomains
CD28
41BB
Zeta
OX40
(d) Third Generation Endodomains
CD28
41BB
Zeta
CD28
OX40
Zeta

FIG. 1

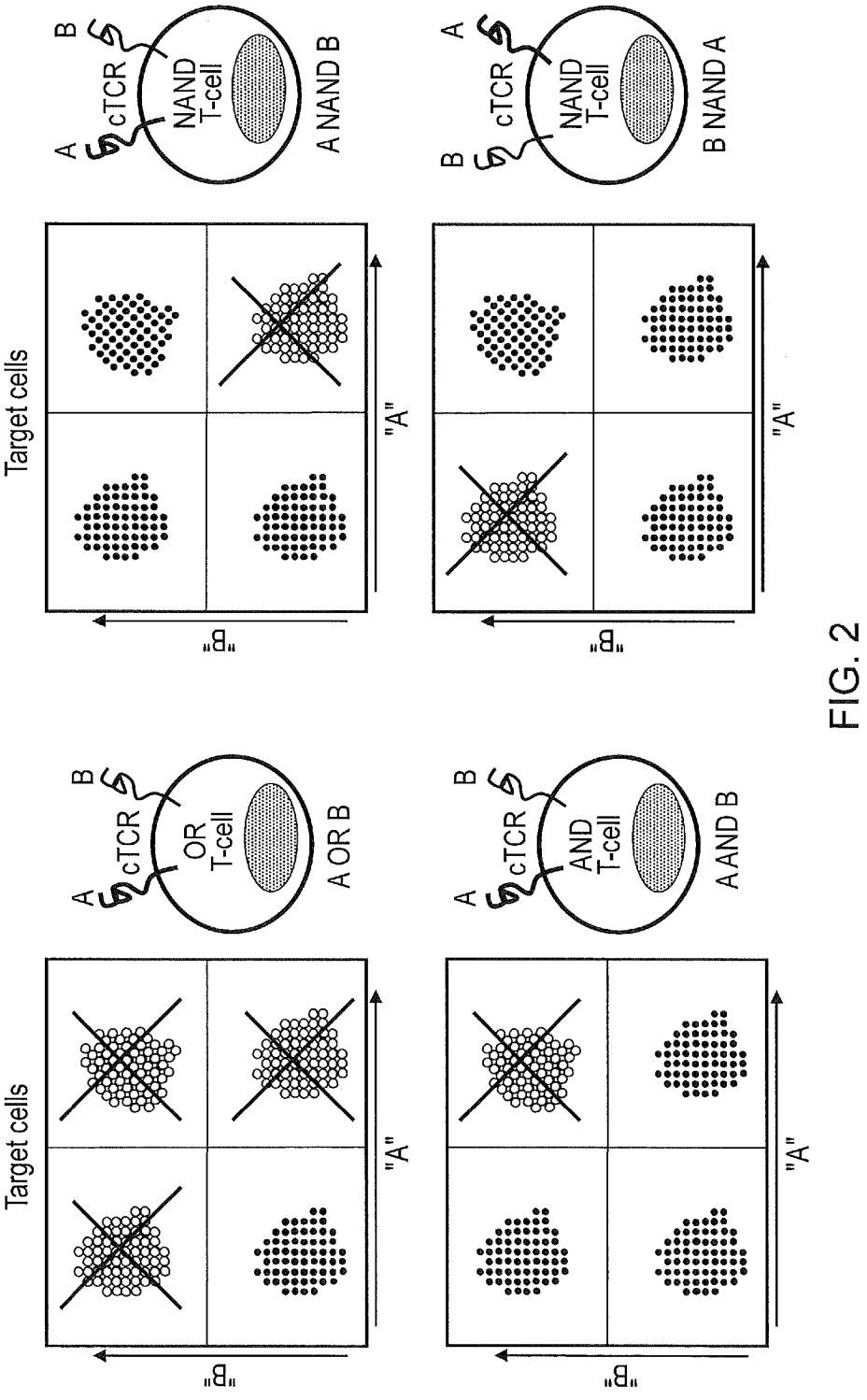

Target cells
"B"
"A"
A
B
cTCR
OR
T-cell
A OR B
AND
A AND B
NAND
A NAND B
B NAND A

FIG. 2

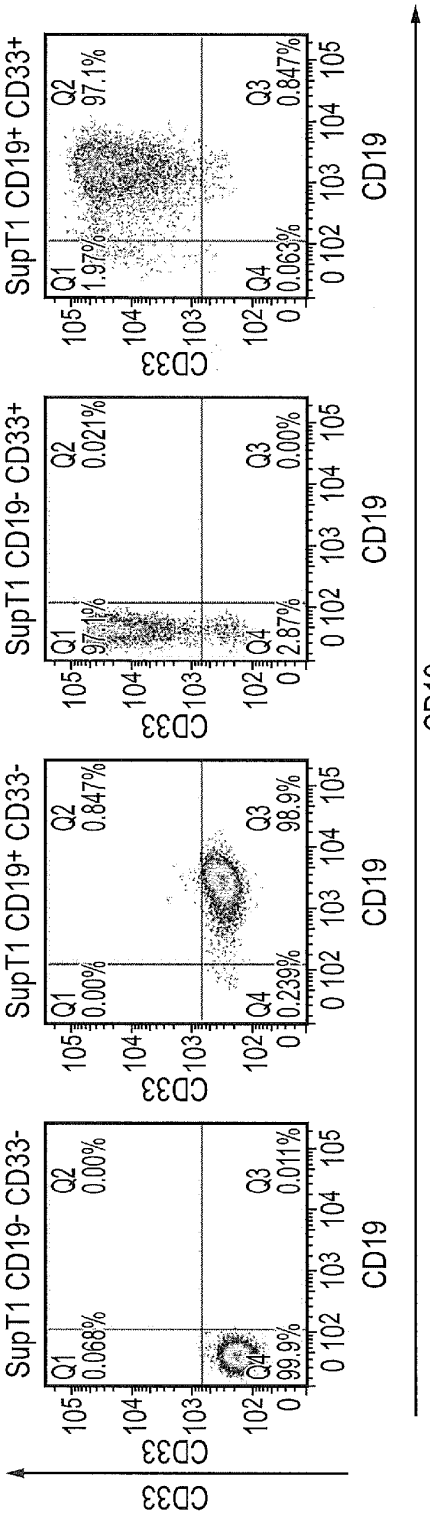

SupT1 CD19- CD33-
Q1 0.068%
Q2 0.00%
Q4 99.9%
Q3 0.011%
SupT1 CD19+ CD33-
Q1 0.00%
Q2 0.847%
Q4 0.239%
Q3 98.9%
SupT1 CD19- CD33+
Q1 97.1%
Q2 0.021%
Q4 2.87%
Q3 0.00%
SupT1 CD19+ CD33+
Q1 1.97%
Q2 97.1%
Q4 0.063%
Q3 0.847%
CD33
CD19
0 102 103 104 105

FIG. 3

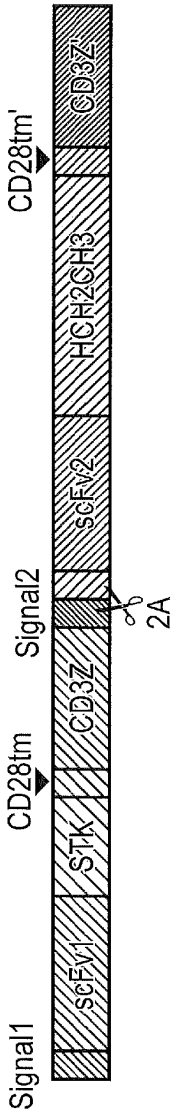

Signal1
scFv1
STK
CD28tm
CD3Z
Signal2
2A
scFv2
HCH2CH3
CD28tm'
CD3Z'

FIG. 4

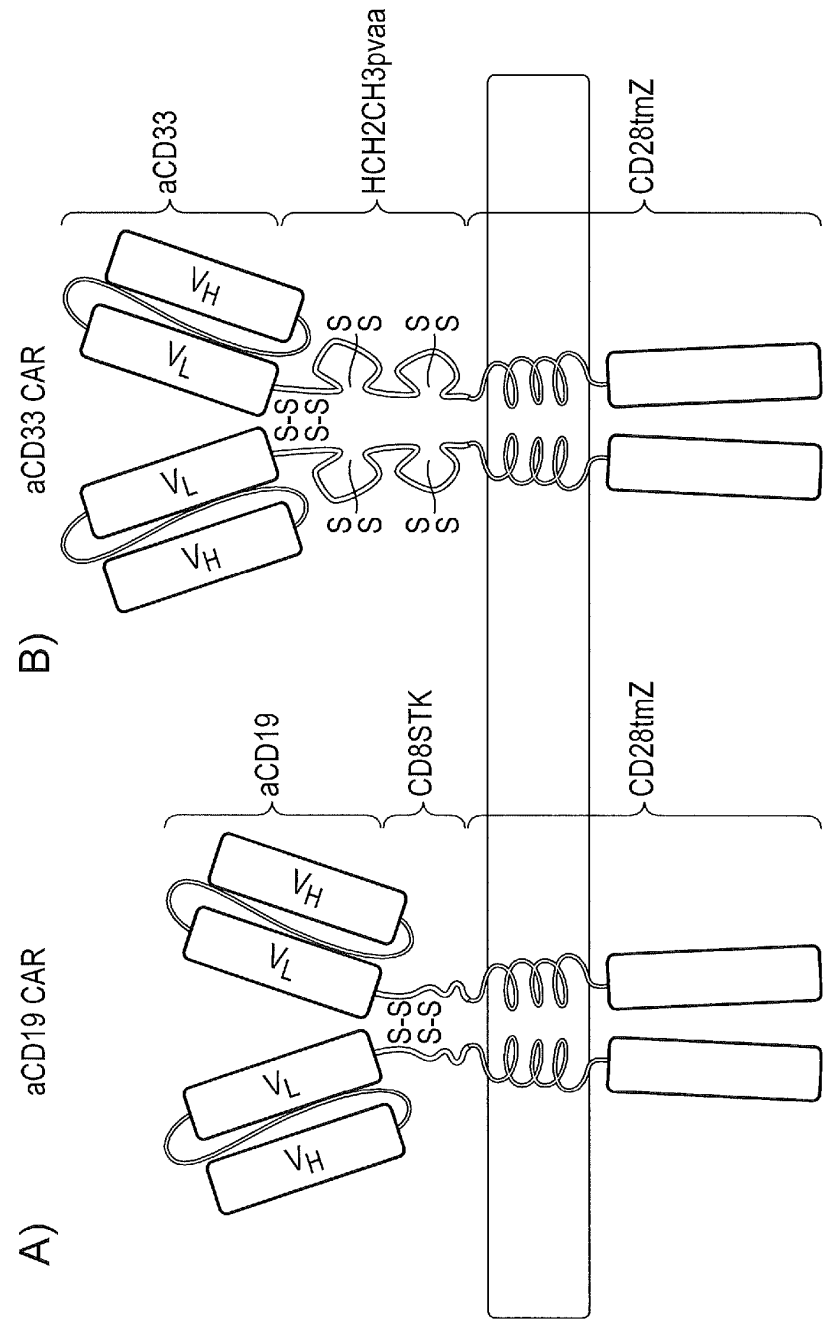

A)
aCD19 CAR
aCD19
CD8STK
CD28tmZ
V_H
V_L
V_L
V_H
S-S
S-S
B)
aCD33 CAR
aCD33
HCH2CH3pvaa
CD28tmZ
V_H
V_L
V_L
V_H
S-S
S-S
S
S
S
S
S
S
S
S

FIG. 5

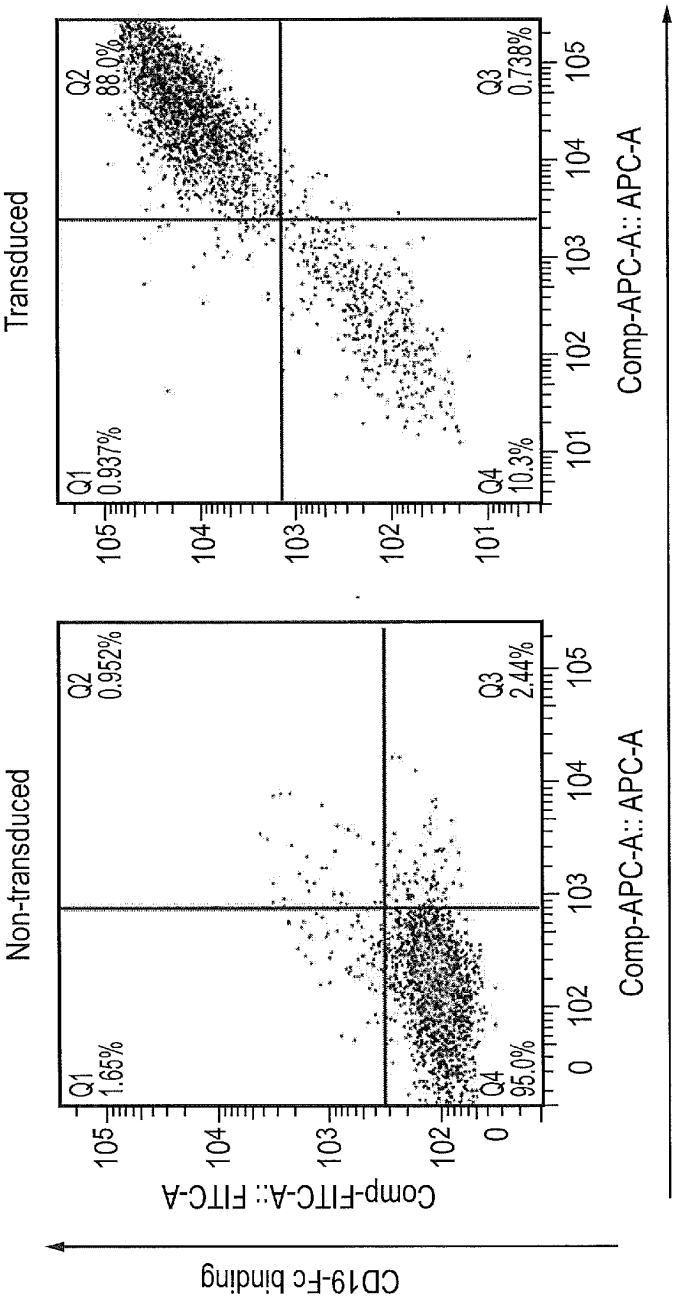

Non-transduced
Transduced
Q1
1.65%
Q2
0.952%
Q4
95.0%
Q3
2.44%
Q1
0.937%
Q2
88.0%
Q4
10.3%
Q3
0.738%
Comp-FITC-A:: FITC-A
CD19-Fc binding
Comp-APC-A:: APC-A
CD33-Fc binding

FIG. 6

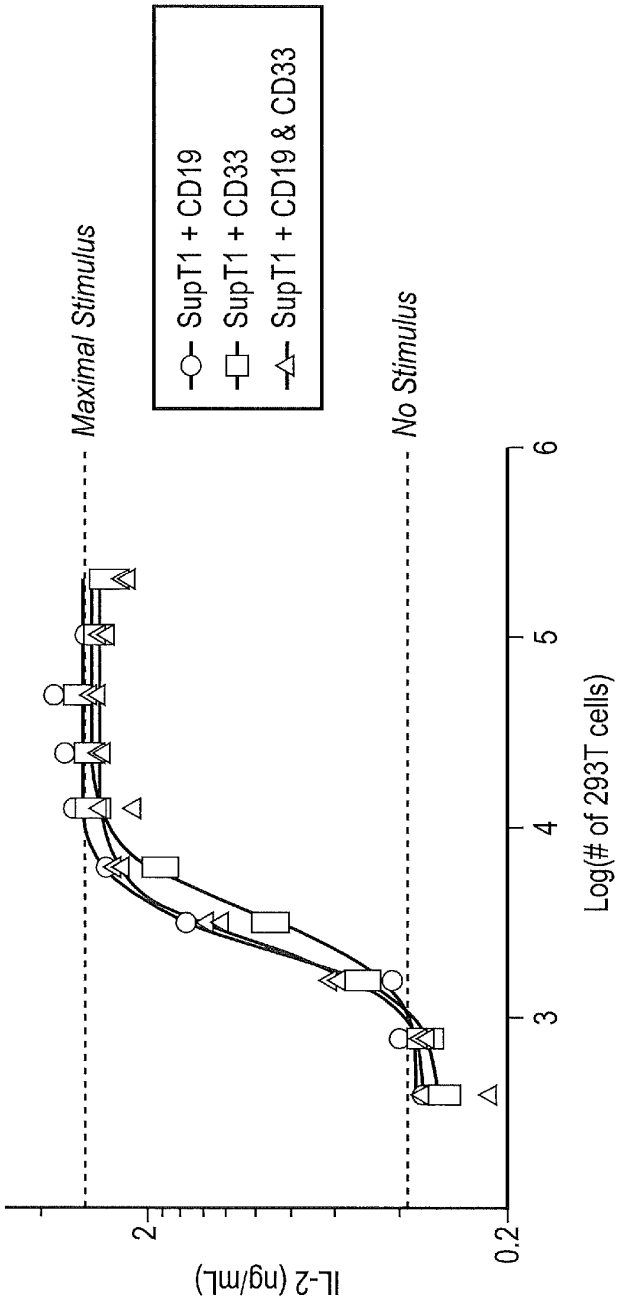

SupT1 + CD19
SupT1 + CD33
SupT1 + CD19 & CD33
Maximal Stimulus
No Stimulus
IL-2 (ng/mL)
2
0.2
3
4
5
6
Log(# of 293T cells)

FIG. 7

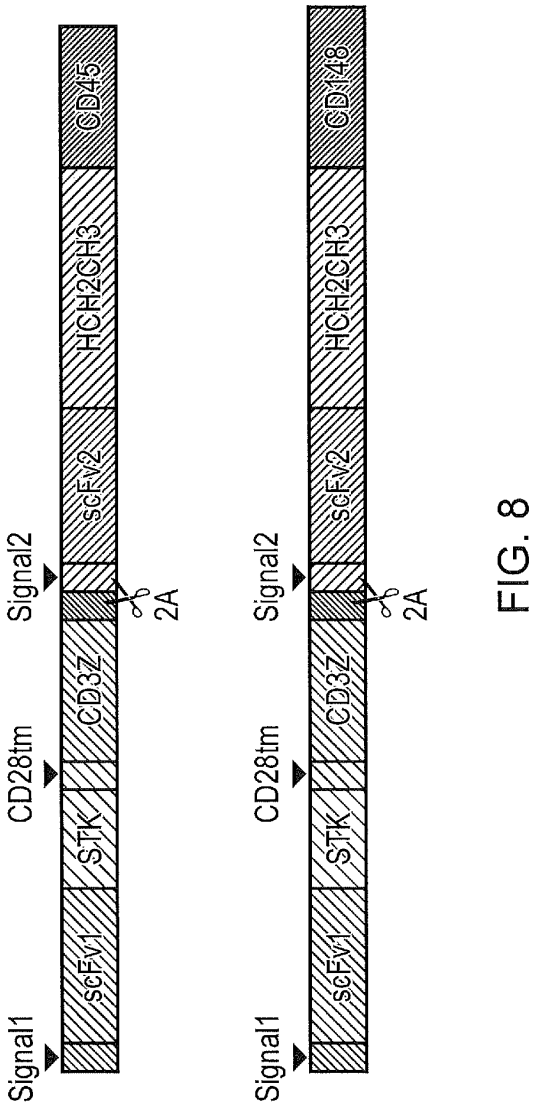

Signal1
scFv1
STK
CD28tm
CD3Z
Signal2
scFv2
HCH2CH3
2A
CD45
Signal1
scFv1
STK
CD28tm
CD3Z
Signal2
scFv2
HCH2CH3
2A
CD148

FIG. 8

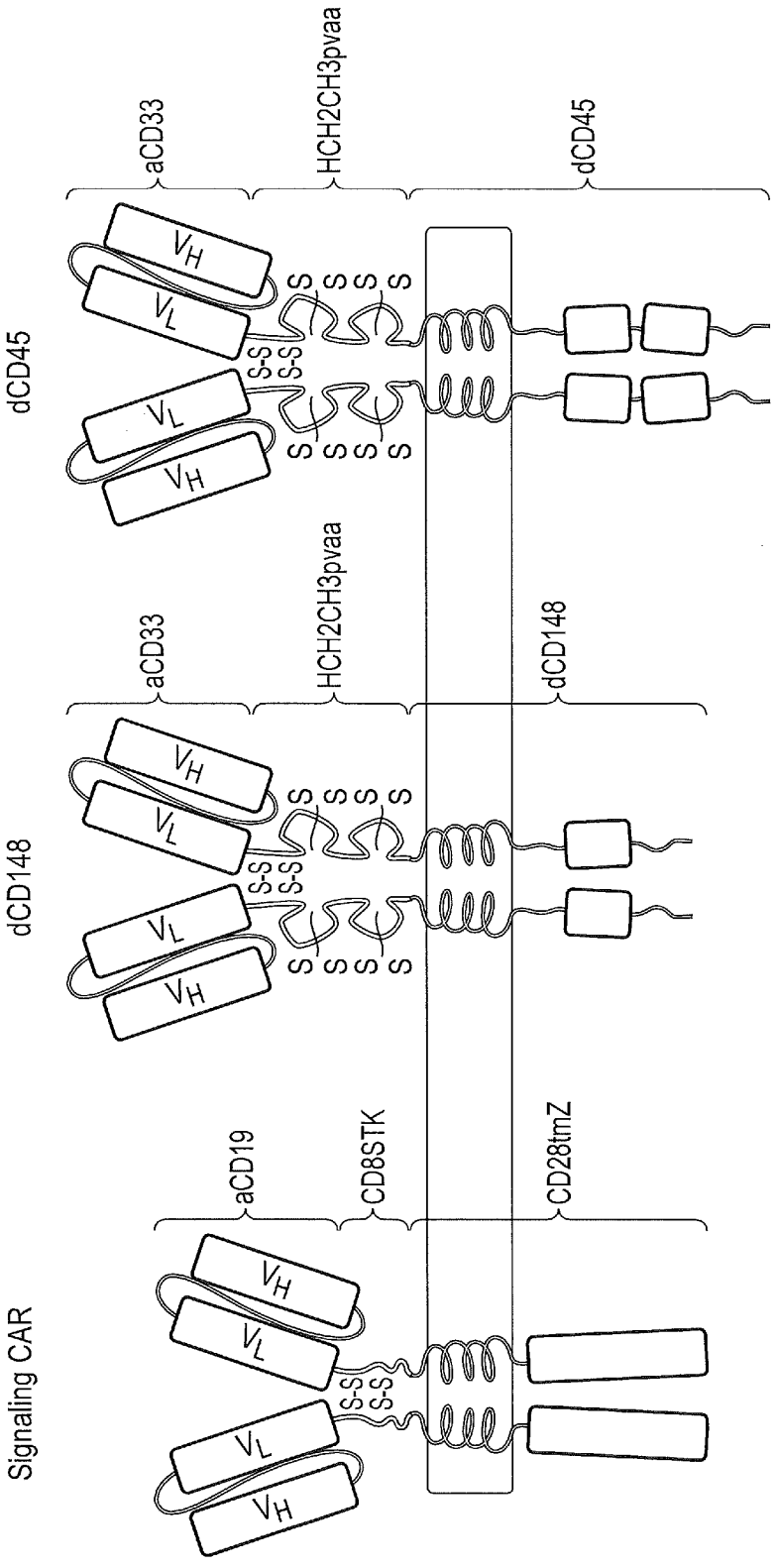

Signaling CAR
aCD19
CD8STK
CD28tmZ
dCD148
aCD33
HCH2CH3pvaa
dCD148
dCD45
aCD33
HCH2CH3pvaa
dCD45
VH
VL
S-S
S

FIG. 9

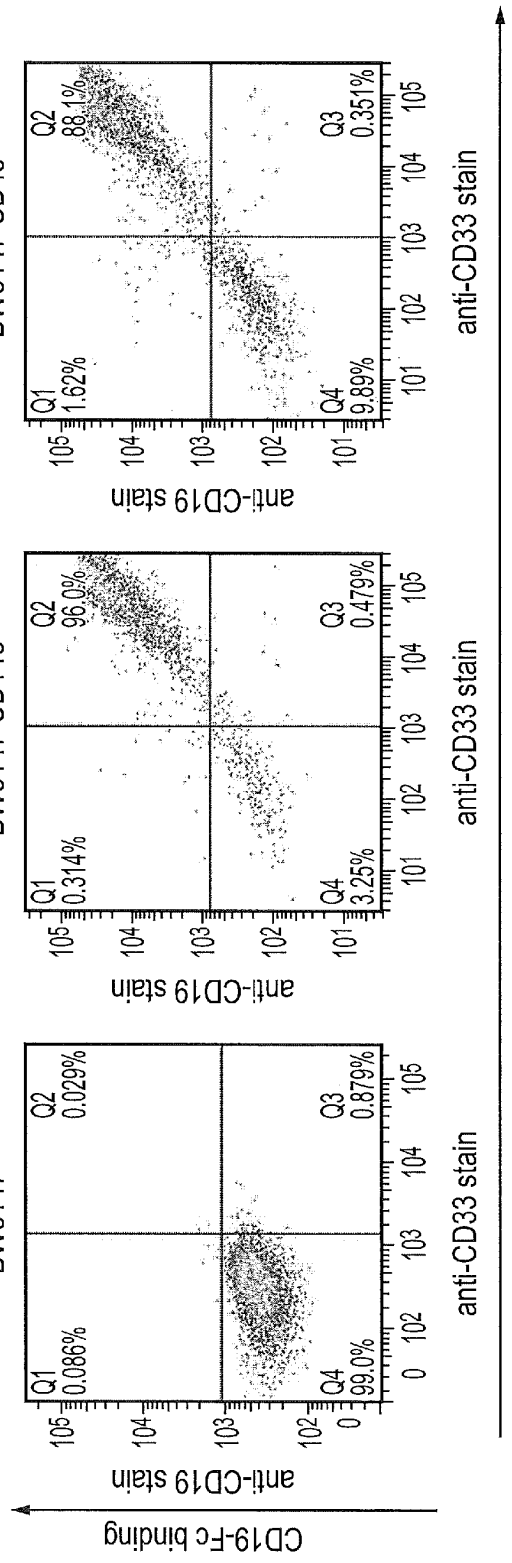

BW5147
BW5147 CD148+
BW5147 CD45+
Q1
0.086%
Q2
0.029%
Q4
99.0%
Q3
0.879%
Q1
0.314%
Q2
96.0%
Q4
3.25%
Q3
0.479%
Q1
1.62%
Q2
88.1%
Q4
9.89%
Q3
0.351%
anti-CD19 stain
anti-CD33 stain
CD19-Fc binding
CD33-Fc binding

FIG. 10

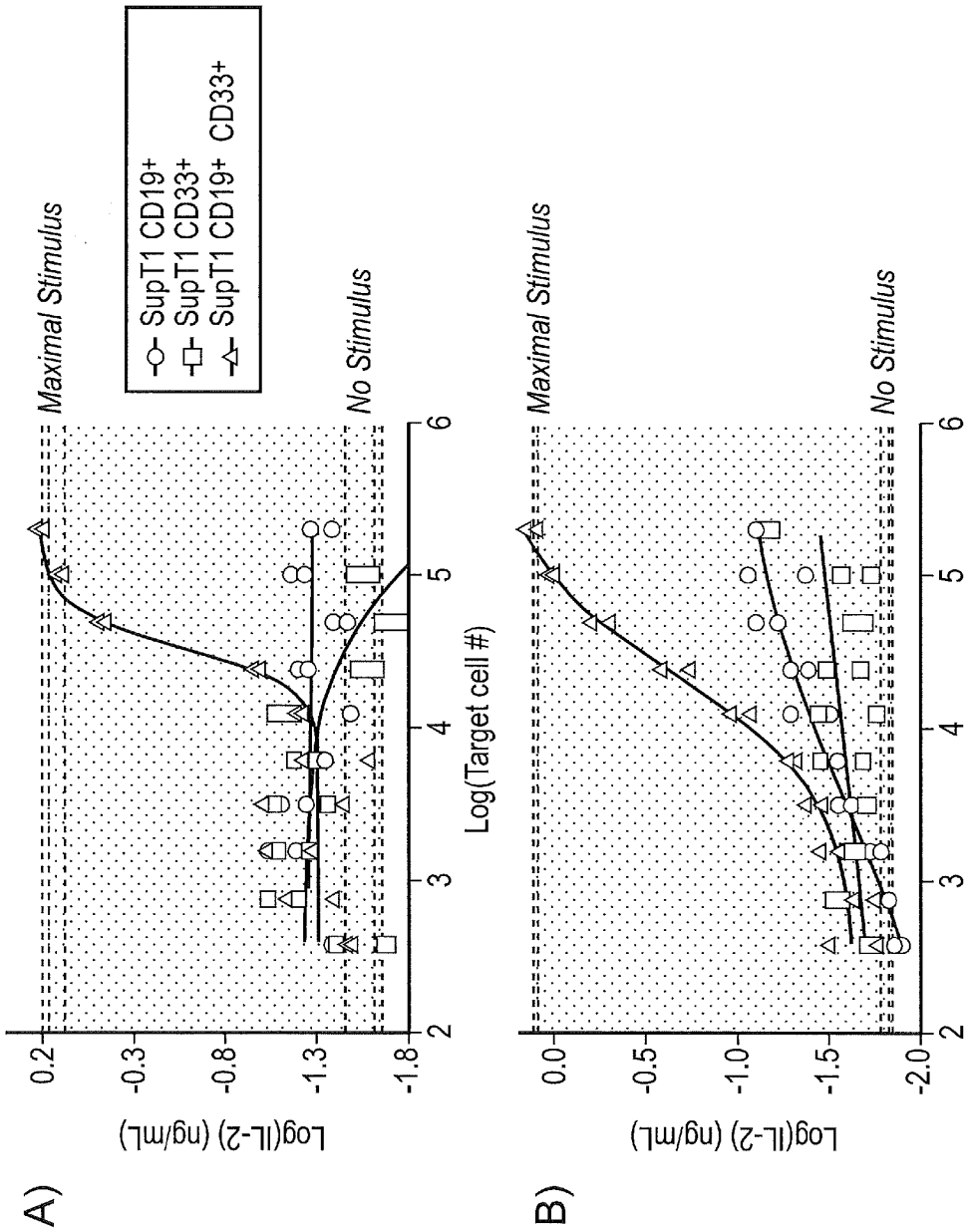

A)
B)
SupT1 CD19+
SupT1 CD33+
SupT1 CD19+ CD33+
Maximal Stimulus
No Stimulus
Log(IL-2) (ng/mL)
Log(Target cell #)
0.2
-0.3
-0.8
-1.3
-1.8
0.0
-0.5
-1.0
-1.5
-2.0
2
3
4
5
6

FIG. 11

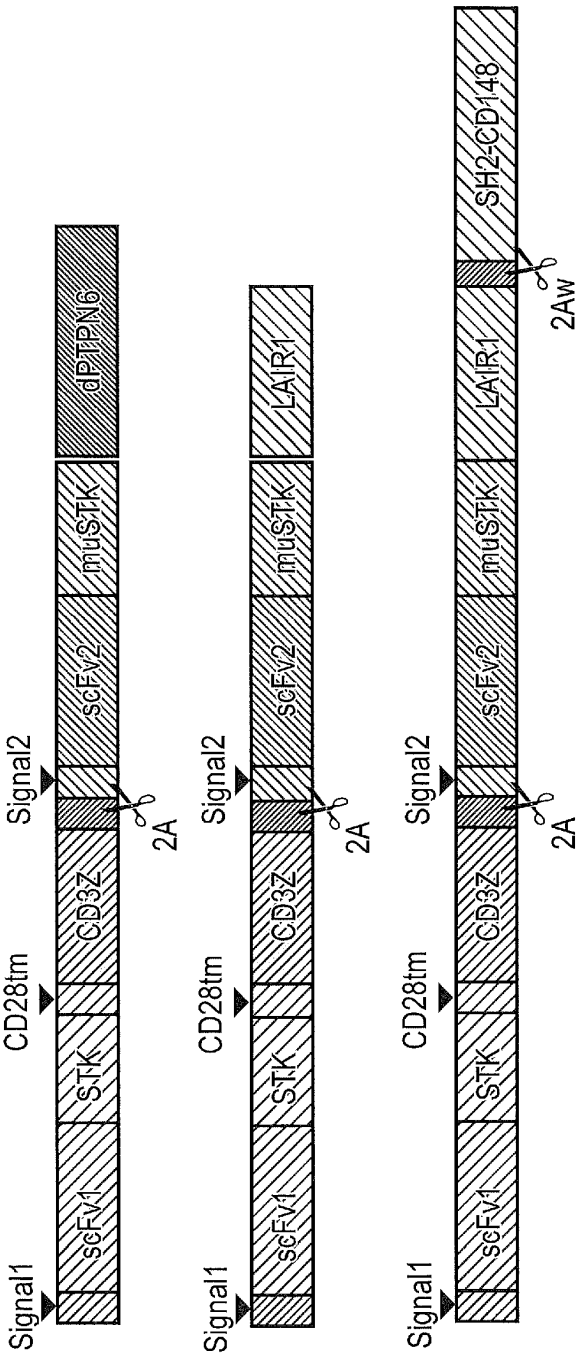

Signal1
scFv1
STK
CD28tm
CD3Z
Signal2
scFv2
muSTK
dPTPN6
2A
Signal1
scFv1
STK
CD28tm
CD3Z
Signal2
scFv2
muSTK
LAIR1
2A
Signal1
scFv1
STK
CD28tm
CD3Z
Signal2
scFv2
muSTK
LAIR1
SH2-CD148
2A
2Aw

FIG. 12

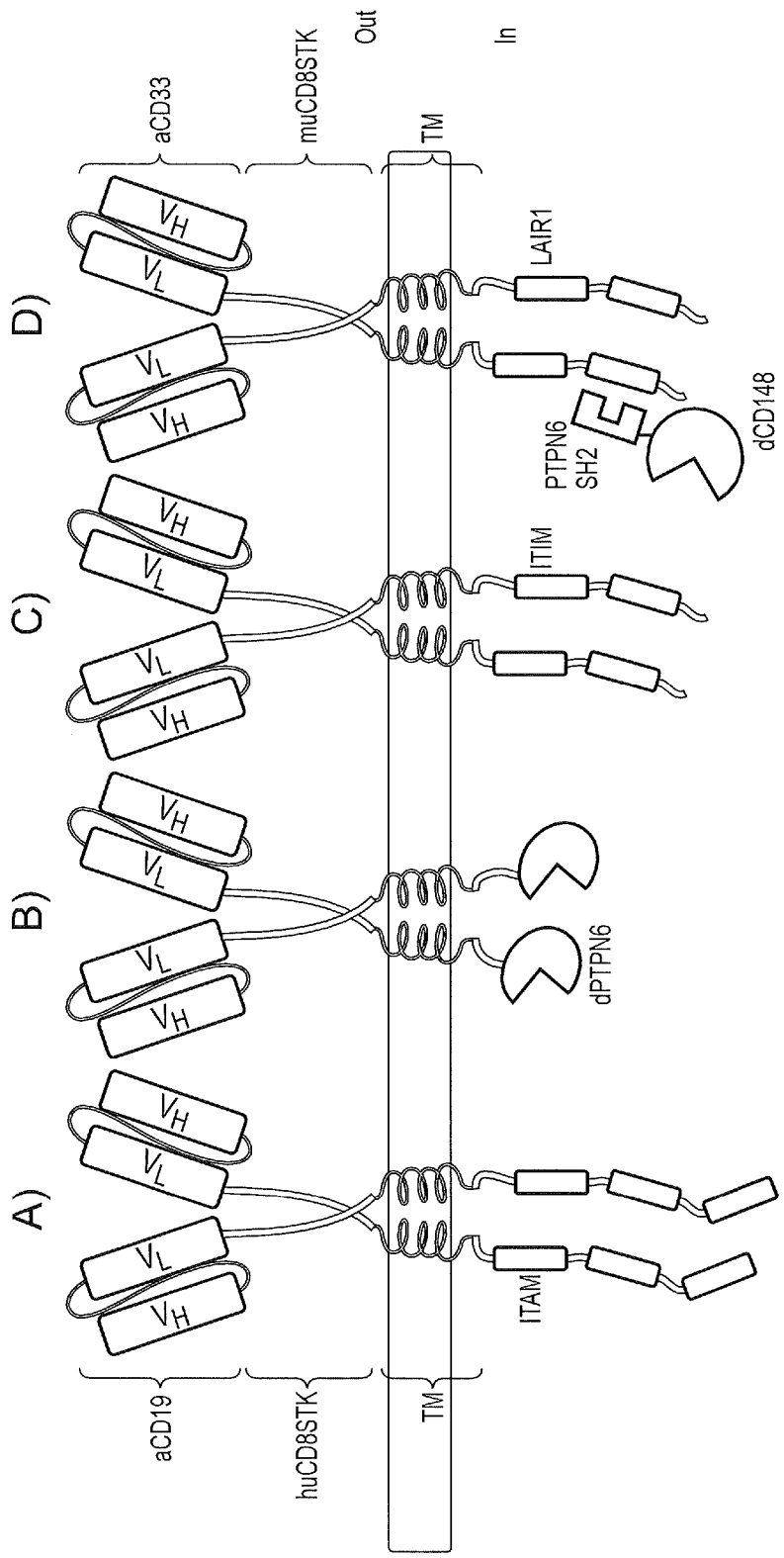

A)
B)
C)
D)
aCD19
huCD8STK
TM
aCD33
muCD8STK
TM
Out
In
ITAM
dPTPN6
ITIM
LAIR1
PTPN6
SH2
dCD148

FIG. 13

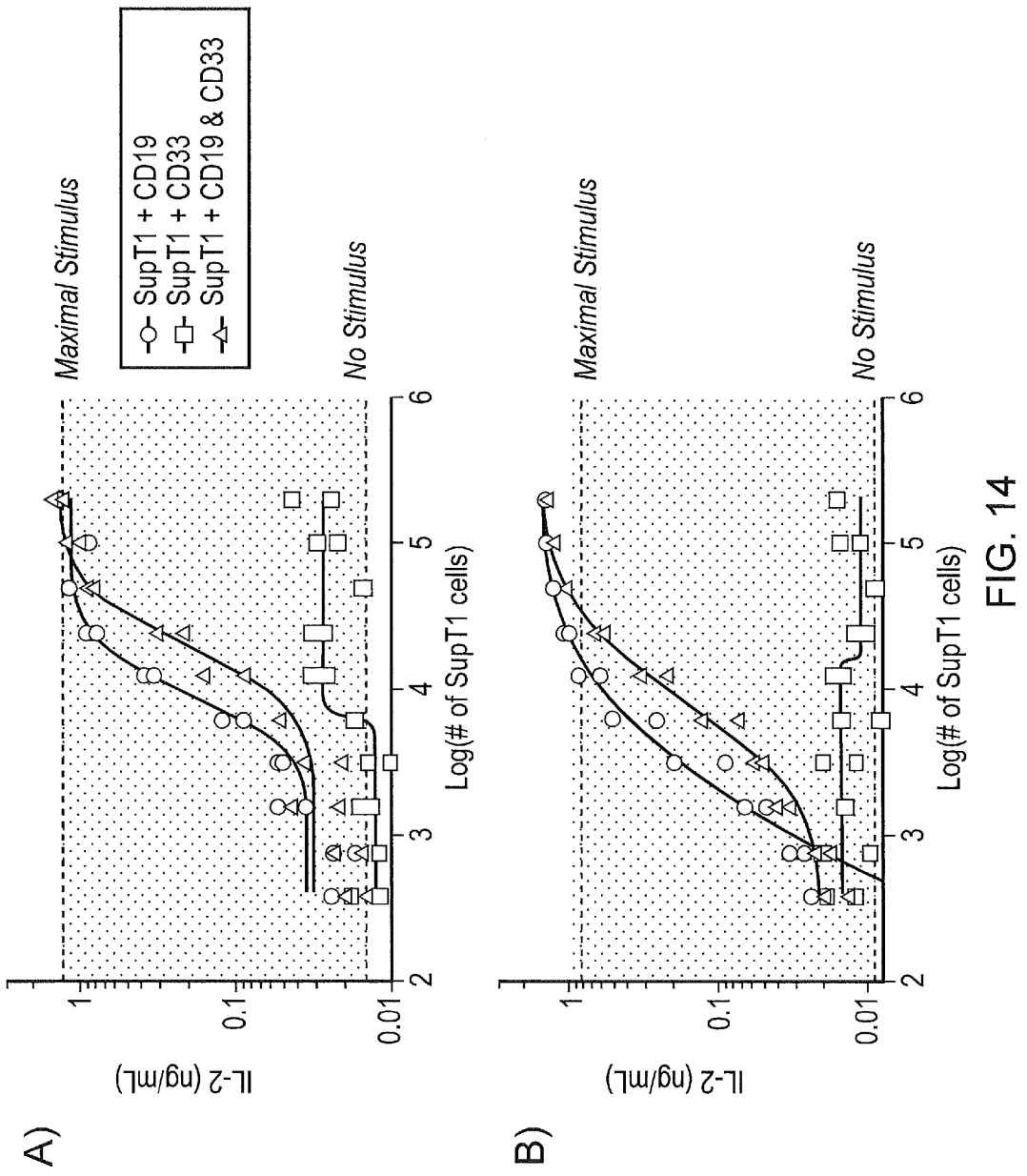
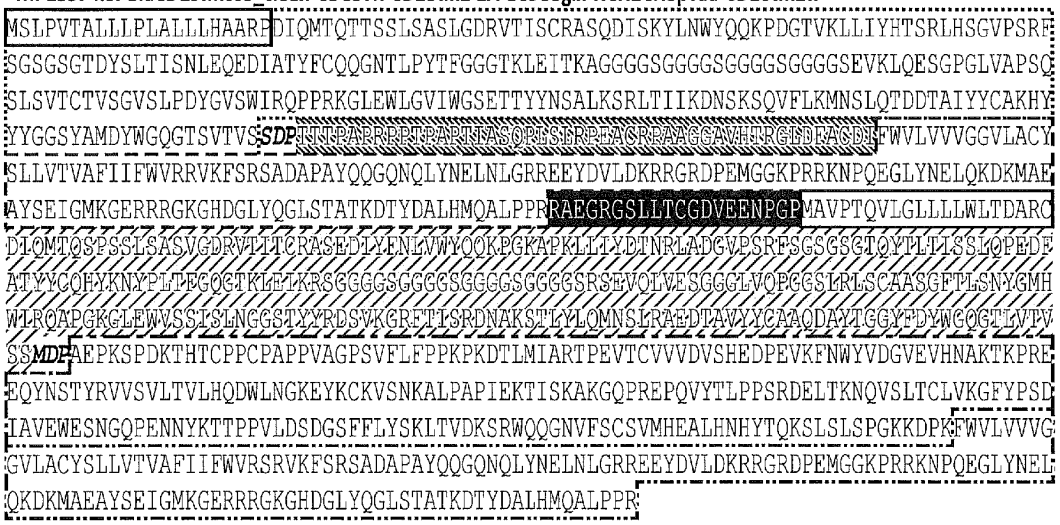

A)
B)
IL-2 (ng/mL)
Log(# of SupT1 cells)
Maximal Stimulus
No Stimulus
SupT1 + CD19
SupT1 + CD33
SupT1 + CD19 & CD33
1
0.1
0.01
2
3
4
5
6

FIG. 14

>MP13974.SFG.aCD19fmc63_clean-CD8STK-CD28tmZ-2A-aCD33glx-HCH2CH3pvaa-CD28tmZw



Region	*Description*
Signal1	Signal peptide 1
scFv1	scFv 1 – anti-CD19
SDP	Linker and chain break
STK	CD8alpha stalk
CD28tmZ	CD28 transmembrane domain and CD3 Zeta endodomain
FMD-2A	Foot-and-mouth disease 2A peptide
Signal2	Signal peptide 2
scFv2	scFv 2 – anti-CD33
MDP	Linker and chain break
HCH2CH3	Hinge, CH2 and CH3 of human IgG1
CD28tmZ'	CD28 transmembrane domain and CD3 Zeta endodomain

FIG. 15

>MP14801.SFG.aCD19fmc63_clean-CD8STK-CD28tmZ-2A-aCD33glx-HCH2CH3pvaa-dCD148

MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRF
SGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQ
SLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHY
YYGGSYAMDYWGQGTSVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDFWVLVVVGGVLACY
SLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARC
DIQMTQSPSSLSASVGDRVTITCRASEDIYFNLVWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDF
ATYYCQHYKNYPLTFGQGTKLEIKRSGGGGSGGGGSGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMH
WIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISRDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYEDYWGQGTLVTV
SSMDPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKAVFGCIFG
ALVIVTVGGFIFWRKKRKDAKNNEVSFSQIKPKKSKLIRVENFEAYFKKQQADSNCGFAEEYEDLKLVGISQPKYAAELAENR
GKNRYNNVLPYDISRVKLSVQTHSTDDYINANYMPGYHSKKDFIATQGPLPNTLKDFWRMVWEKNVYAIIMLTKCVEQGRTKC
EEYWPSKQAQDYGDITVAMTSEIVLPEWTIRDFTVKNIQTSESHPLRQFHFTSWPDHGVPDTTDLLINFRYLVRDYMKQSPPE
SPILVHCSAGVGRTGTFIAIDRLIYQIENENTVDVYGIVYDLRMHRPLMVQTEDQYVFLNQCVLDIVRSQKDSKVDLIYQNTT
AMTIYENLAPVTTFGKTNGYIA

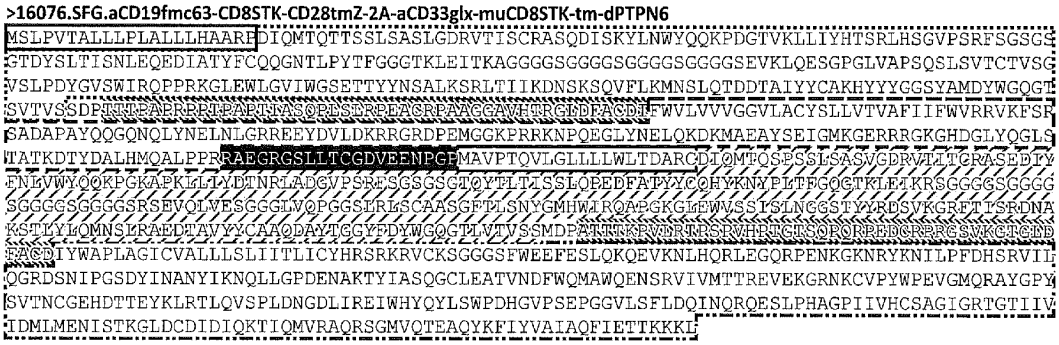
MP14802. SFG.aCD19fmc63_clean-CD8STK-CD28tmZ-2A-aCD33glx-HCH2CH3pvaa-dCD45

MSLPVTALLLPLALLLHAARPDIQMTQTTSSLSASLGDRVTISCRASQDISKYLNWYQQKPDGTVKLLIYHTSRLHSGVPSRF
SGSGSGTDYSLTISNLEQEDIATYFCQQGNTLPYTFGGGTKLEITKAGGGGSGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQ
SLSVTCTVSGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNSALKSRLTIIKDNSKSQVFLKMNSLQTDDTAIYYCAKHY
YYGGSYAMDYWGQGTSVTVSSDPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDFWVLVVVGGVLACY
SLLVTVAFIIFWVRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAE
AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAEGRGSLLTCGDVEENPGPMAVPTQVLGLLLLWLTDARC
DIQMTQSPSSLSASVGDRVTITCRASEDIYFNLVWYQQKPGKAPKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDF
ATYYCQHYKNYPLTFGQGTKLEIKRSGGGGSGGGGSGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMH
WIRQAPGKGLEWVSSISLNGGSTYYRDSVKGRFTISRDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYEDYWGQGTLVTV
SSMDPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKALIAFLAF
LIIVTSIALLVVLYKIYDLHKKRSCNLDEQQELVERDDEKQLMNVEPIHADILLETYKRKIADEGRLFLAEFQSIPRVFSKFP
IKEARKPFNQNKNRYVDILPYDYNRVELSEINGDAGSNYINASYIDGFKEPRKYIAAQGPRDETVDDFWRMIWEQKATVIVMV
TRCEEGNRNKCAEYWPSMEEGTRAFGDVVVKINQHKRCPDYIIQKLNIVNKKEKATGREVTHIQFTSWPDHGVPEDPHLLLKL
RRRVNAFSNFFSGPIVVHCSAGVGRTGTYIGIDAMLEGLEAENKVDVYGYVVKLRRQRCLMVQVEAQYILIHQALVEYNQFGE
TEVNLSELHPYLHNMKKRDPPSEPSPLEAEFQRLPSYRSWRTQHIGNQEENKSKNRNSNVIPYDYNRVPLKHELEMSKESEHD
SDESSDDDSDSEEPSKYINASFIMSYWKPEVMIAAQGPLKETIGDFWQMIFQRKVKVIVMLTELKHGDQEICAQYWGEGKQTY
GDIEVDLKDTDKSSTYTLRVFELRHSKRKDSRTVYQYQYTNWSVEQLPAEPKELISMIQVVKQKLPQKNSSEGNKHHKSTPLL
IHCRDGSQQTGIFCALLNLLESAETEEVVDIFQVVKALRKARPGMVSTFEQYQFLYDVIASTYPAQNGQVKKNNHQEDKIEFD
NEVDKVKQDANCVNPLGAPEKLPEAKEQAEGSEPTSGTEGPEHSVNGPASPALNQGS

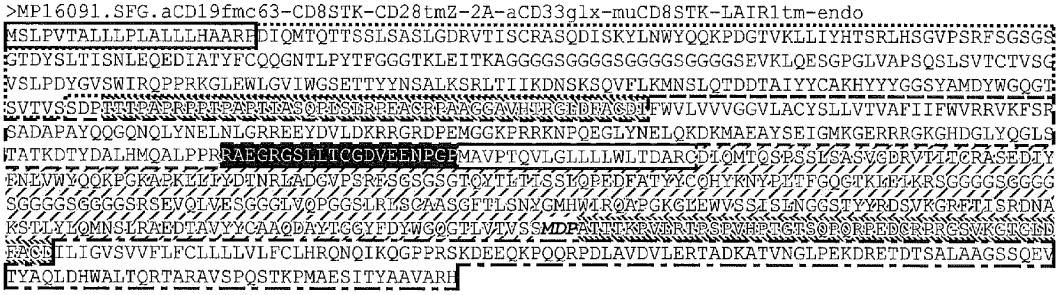
Region	*Description*
Signal1	Signal peptide 1
scFv1	scFv 1 – anti-CD19
SDP	Linker and chain break
STK	CD8alpha stalk
CD28tmZ	CD28 transmembrane domain and CD3 Zeta endodomain
FMD-2A	Foot-and-mouth disease 2A peptide
Signal2	Signal peptide 2
scFv2	scFv 2 – anti-CD33
MDP	Linker and chain break
HCH2CH3	Hinge, CH2 and CH3 of human IgG1
dCD148 / dCD45	Trans-membrane and endo-domains of CD148 and CD45

FIG. 16

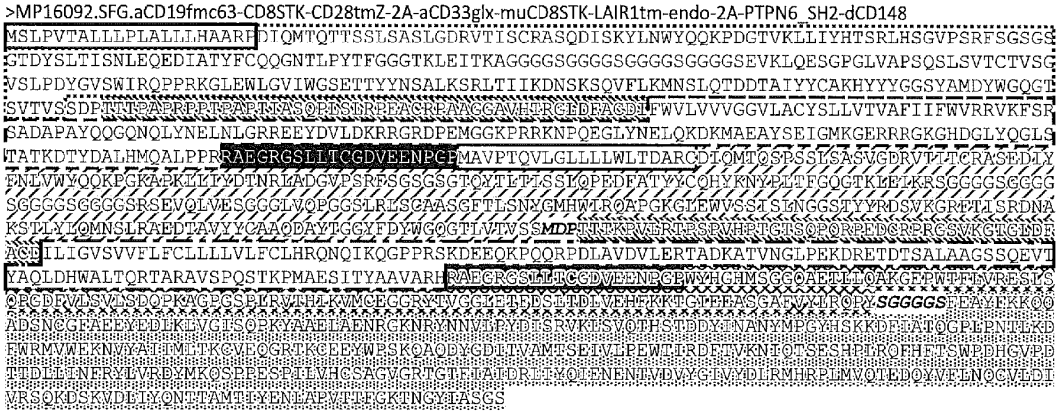
>16076.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33glx-muCD8STK-tm-dPTPN6



>MP16091.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33glx-muCD8STK-LAIR1tm-endo



>MP16092.SFG.aCD19fmc63-CD8STK-CD28tmZ-2A-aCD33glx-muCD8STK-LAIR1tm-endo-2A-PTPN6_SH2-dCD148



Region	*Description*
Signal1	Signal peptide 1
scFv1	scFv 1 – anti-CD19
SDP	Linker and chain break
STK	Human CD8alpha stalk
CD28tmZ	CD28 transmembrane domain and CD3 Zeta endodomain
FMD-2A	Foot-and-mouth disease 2A peptide
Signal2	Signal peptide 2
scFv2	scFv 2 – anti-CD33
MDP	Linker and chain break
STK	Mouse CD8alpha stalk
dLAIR1	Hinge, CH2 and CH3 of human IgG1
dPTPN6	Phosphatase domain of PTPN6
FMD-2A'	Foot-and-mouth disease 2A peptide codon wobbled
PTPN6-SH2	SH2 domain of PTPN6
SGGGGS	Serine glycine linker and chain break
dCD148	Phosphatase domain of CD148

FIG. 17

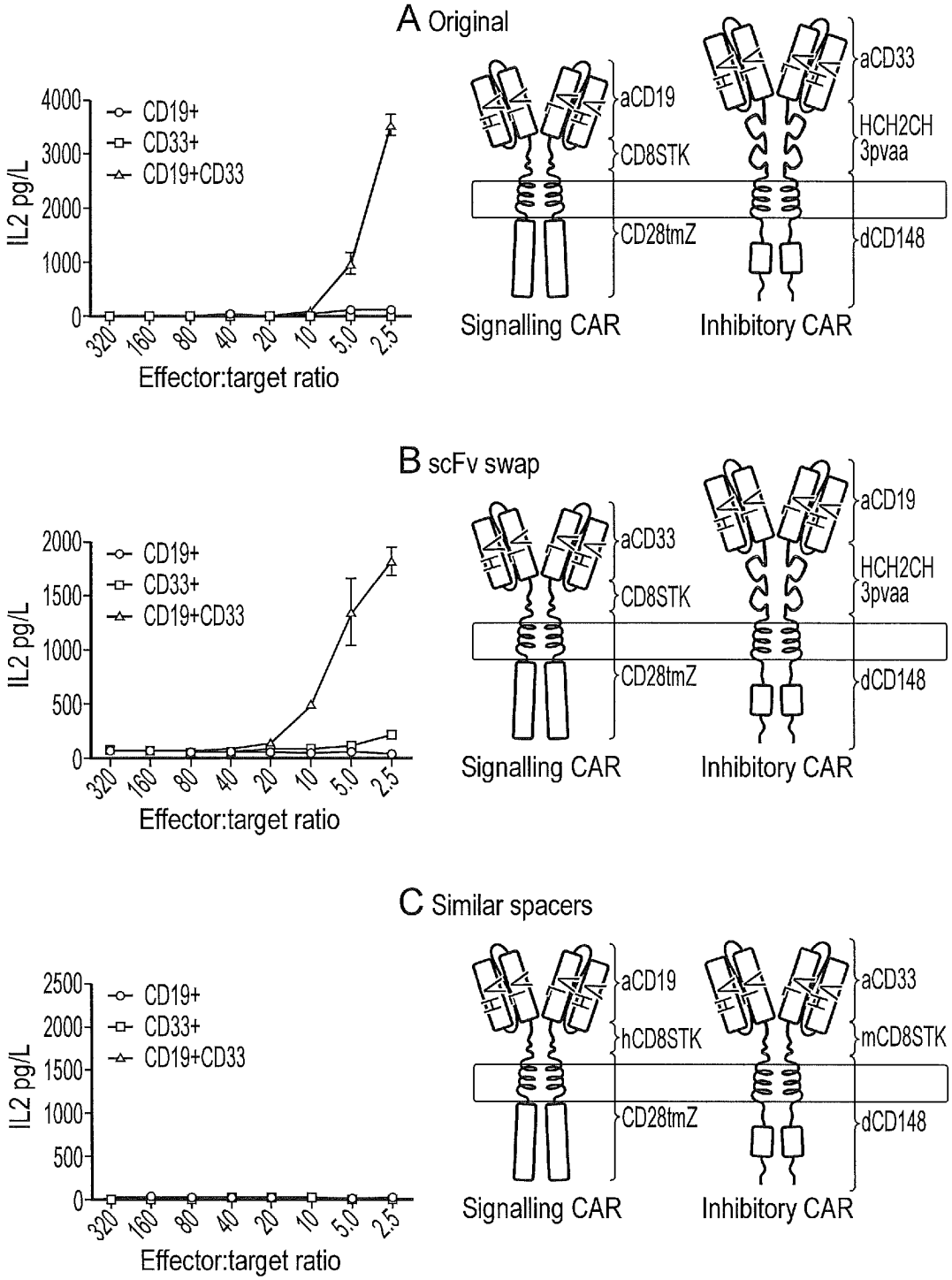

A Original
4000
3000
2000
1000
0
IL2 pg/L
CD19+
CD33+
CD19+CD33
320
160
80
40
20
10
5.0
2.5
Effector:target ratio
aCD19
CD8STK
CD28tmZ
aCD33
HCH2CH
3pvaa
dCD148
Signalling CAR
Inhibitory CAR
B scFv swap
2000
1500
1000
500
0
IL2 pg/L
CD19+
CD33+
CD19+CD33
320
160
80
40
20
10
5.0
2.5
Effector:target ratio
aCD33
CD8STK
CD28tmZ
aCD19
HCH2CH
3pvaa
dCD148
Signalling CAR
Inhibitory CAR
C Similar spacers
2500
2000
1500
1000
500
0
IL2 pg/L
CD19+
CD33+
CD19+CD33
320
160
80
40
20
10
5.0
2.5
Effector:target ratio
aCD19
hCD8STK
CD28tmZ
aCD33
mCD8STK
dCD148
Signalling CAR
Inhibitory CAR

FIG. 18

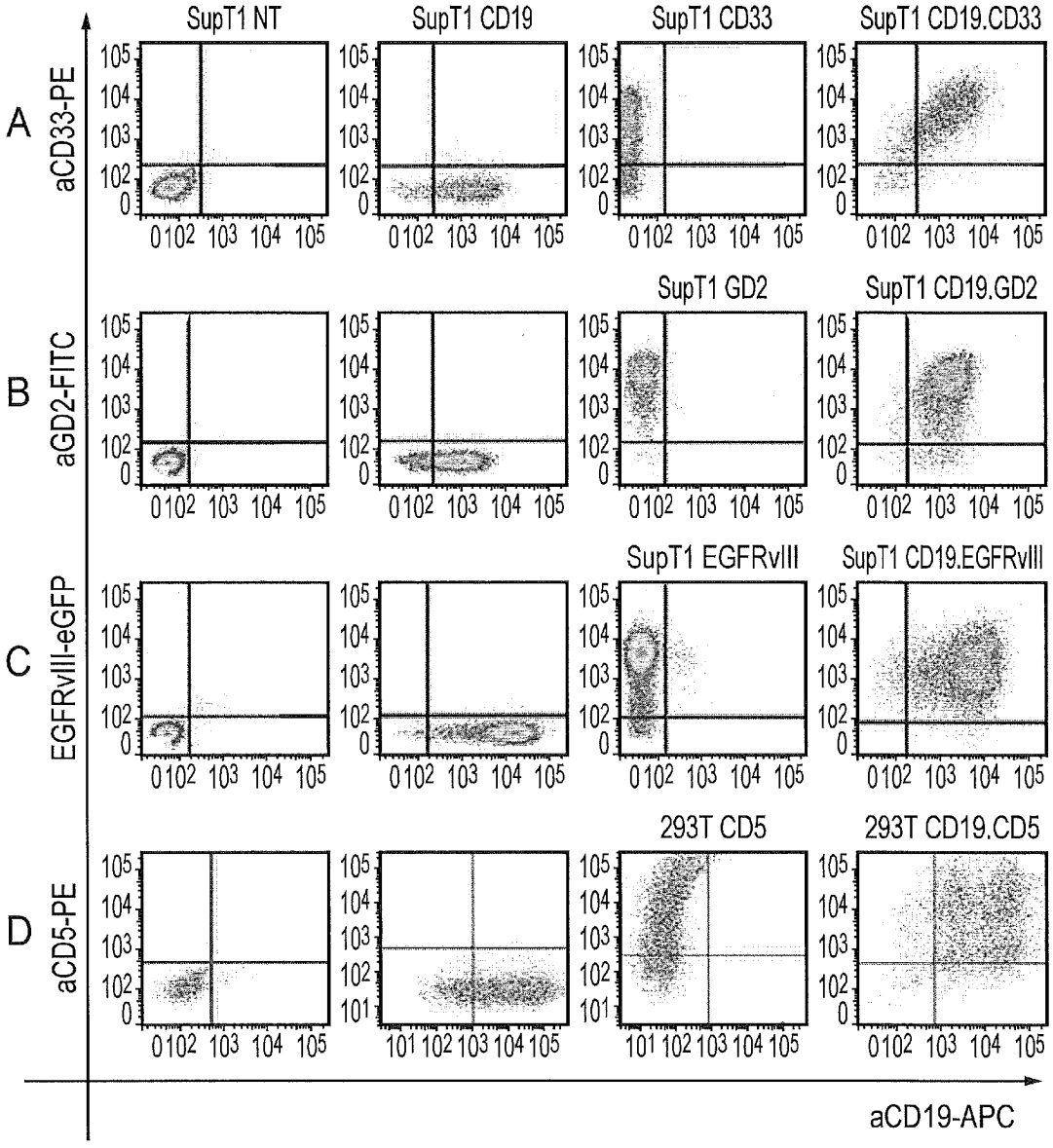

SupT1 NT
SupT1 CD19
SupT1 CD33
SupT1 CD19.CD33
A
aCD33-PE
SupT1 GD2
SupT1 CD19.GD2
B
aGD2-FITC
SupT1 EGFRvIII
SupT1 CD19.EGFRvIII
C
EGFRvIII-eGFP
293T CD5
293T CD19.CD5
D
aCD5-PE
aCD19-APC

FIG. 19

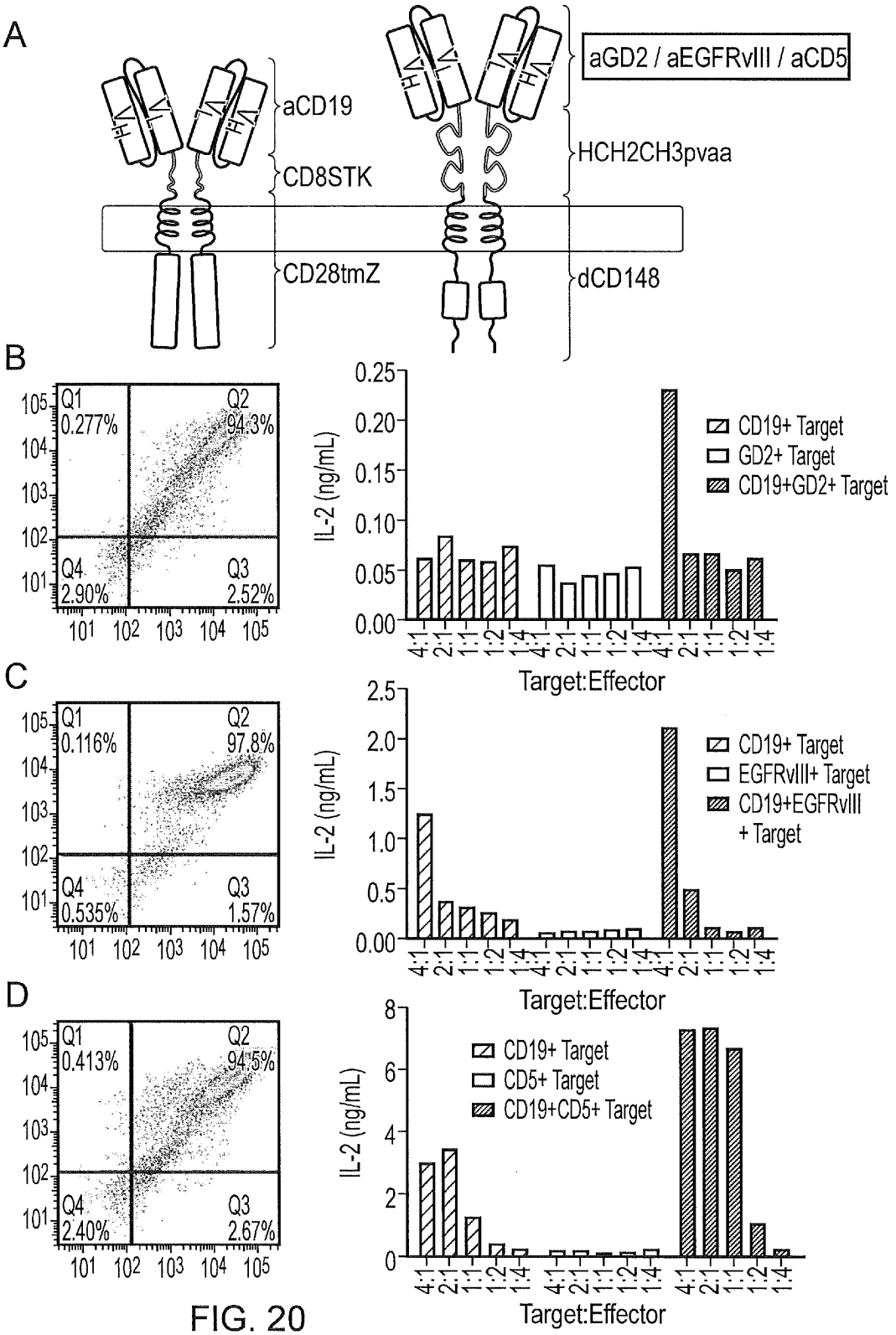

A
aCD19
CD8STK
CD28tmZ
aGD2 / aEGFRvIII / aCD5
HCH2CH3pvaa
dCD148
B
Q1 0.277%
Q2 94.3%
Q4 2.90%
Q3 2.52%
IL-2 (ng/mL)
CD19+ Target
GD2+ Target
CD19+GD2+ Target
Target:Effector
C
Q1 0.116%
Q2 97.8%
Q4 0.535%
Q3 1.57%
IL-2 (ng/mL)
CD19+ Target
EGFRvIII+ Target
CD19+EGFRvIII + Target
Target:Effector
D
Q1 0.413%
Q2 94.5%
Q4 2.40%
Q3 2.67%
IL-2 (ng/mL)
CD19+ Target
CD5+ Target
CD19+CD5+ Target
Target:Effector

FIG. 20

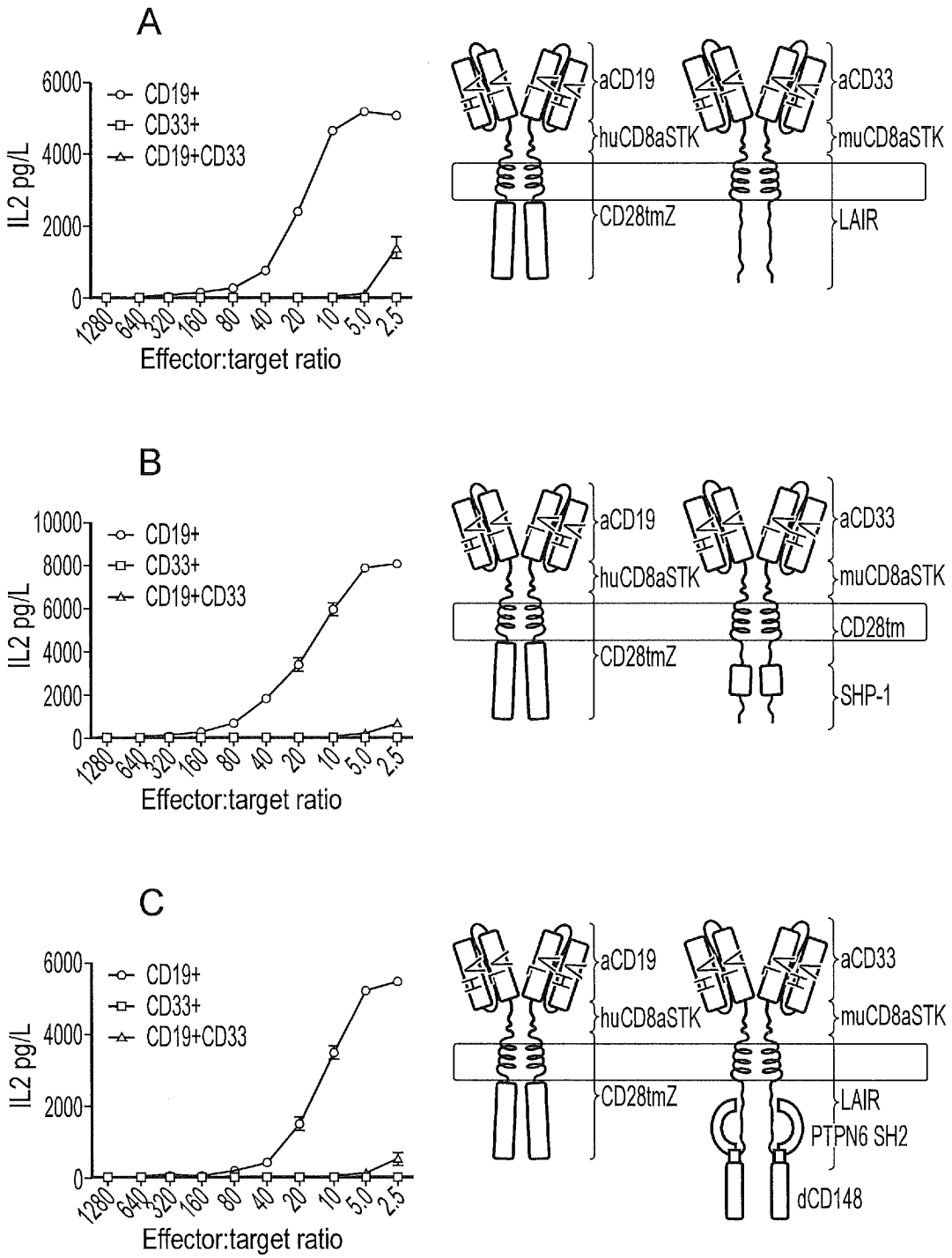

A
6000
4000
2000
0
IL2 pg/L
CD19+
CD33+
CD19+CD33
1280
640
320
160
80
40
20
10
5.0
2.5
Effector:target ratio
aCD19
huCD8aSTK
CD28tmZ
aCD33
muCD8aSTK
LAIR
B
10000
8000
6000
4000
2000
0
IL2 pg/L
CD19+
CD33+
CD19+CD33
Effector:target ratio
aCD19
huCD8aSTK
CD28tmZ
aCD33
muCD8aSTK
CD28tm
SHP-1
C
6000
4000
2000
0
IL2 pg/L
CD19+
CD33+
CD19+CD33
Effector:target ratio
aCD19
huCD8aSTK
CD28tmZ
aCD33
muCD8aSTK
LAIR
PTPN6 SH2
dCD148

FIG. 21

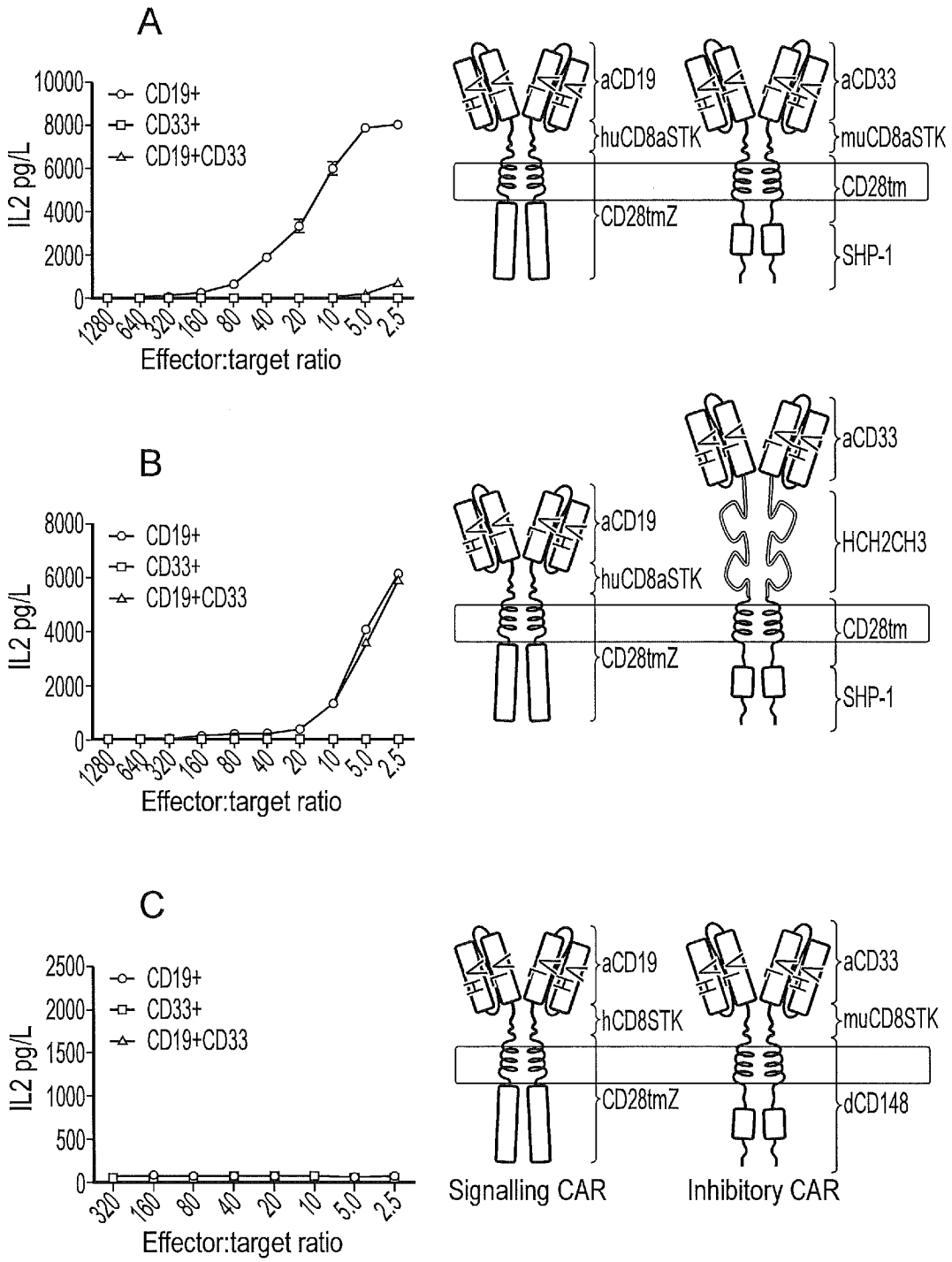

A
IL2 pg/L
10000
8000
6000
4000
2000
0
CD19+
CD33+
CD19+CD33
1280
640
320
160
80
40
20
10
5.0
2.5
Effector:target ratio
aCD19
huCD8aSTK
CD28tmZ
aCD33
muCD8aSTK
CD28tm
SHP-1
B
IL2 pg/L
8000
6000
4000
2000
0
CD19+
CD33+
CD19+CD33
Effector:target ratio
aCD19
huCD8aSTK
CD28tmZ
aCD33
HCH2CH3
CD28tm
SHP-1
C
IL2 pg/L
2500
2000
1500
1000
500
0
CD19+
CD33+
CD19+CD33
320
160
80
40
20
10
5.0
2.5
Effector:target ratio
aCD19
hCD8STK
CD28tmZ
aCD33
muCD8STK
dCD148
Signalling CAR
Inhibitory CAR

FIG. 22

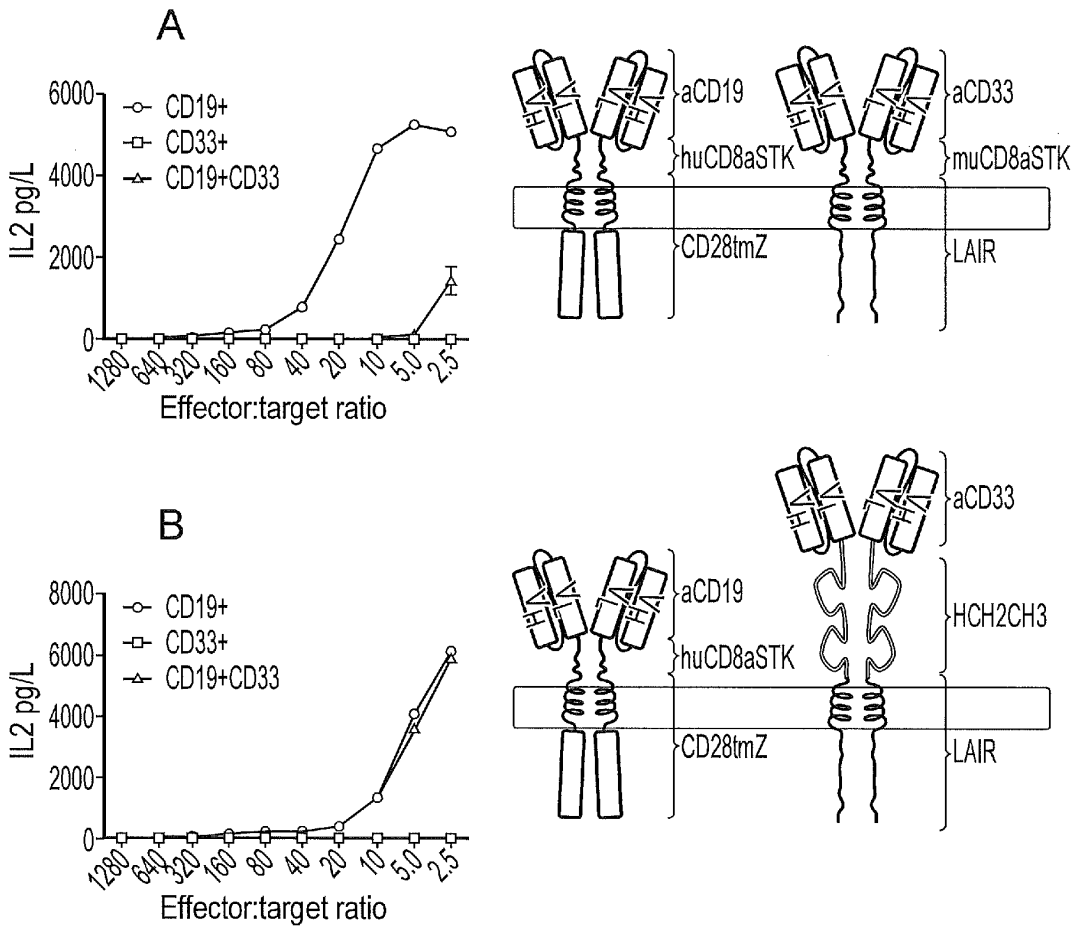

A
6000
4000
2000
0
IL2 pg/L
CD19+
CD33+
CD19+CD33
1280
640
320
160
80
40
20
10
5.0
2.5
Effector:target ratio
aCD19
huCD8aSTK
CD28tmZ
aCD33
muCD8aSTK
LAIR
B
8000
6000
4000
2000
0
IL2 pg/L
CD19+
CD33+
CD19+CD33
1280
640
320
160
80
40
20
10
5.0
2.5
Effector:target ratio
aCD19
huCD8aSTK
CD28tmZ
aCD33
HCH2CH3
LAIR

FIG. 23

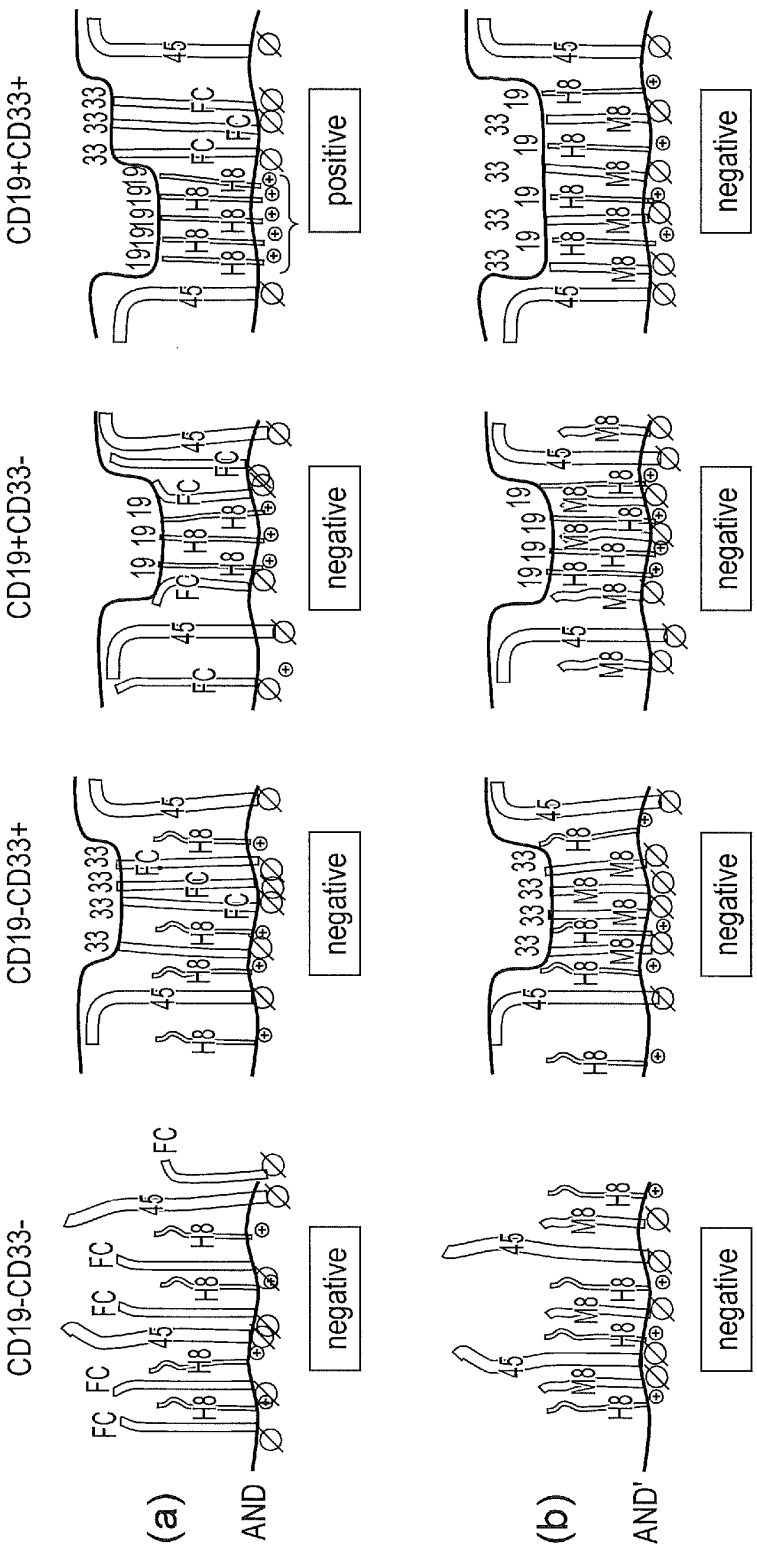

CD19-CD33-
CD19-CD33+
CD19+CD33-
CD19+CD33+
(a)
AND
(b)
AND'
negative
positive

FIG. 24

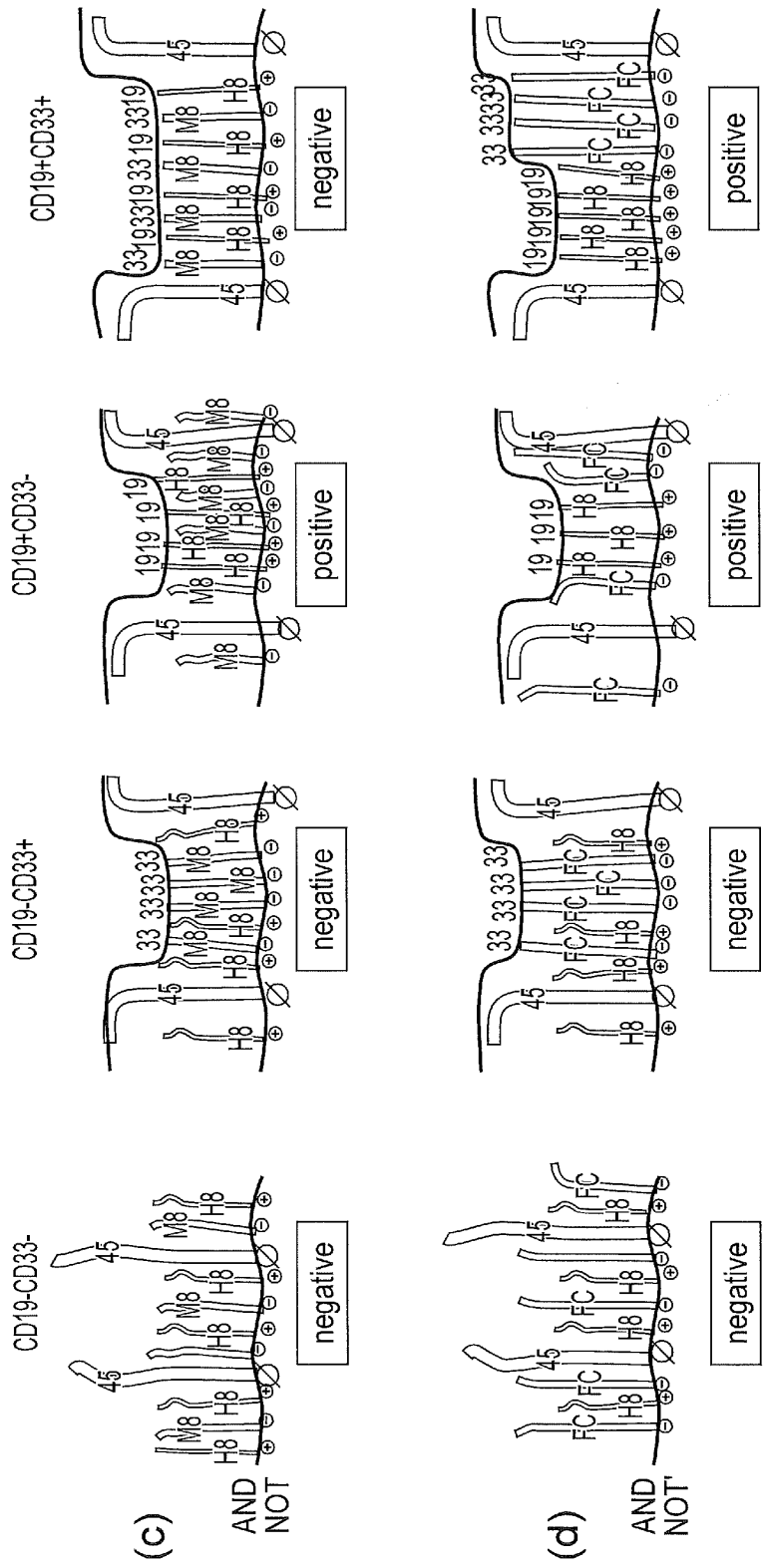


FIG. 24 (Continued)

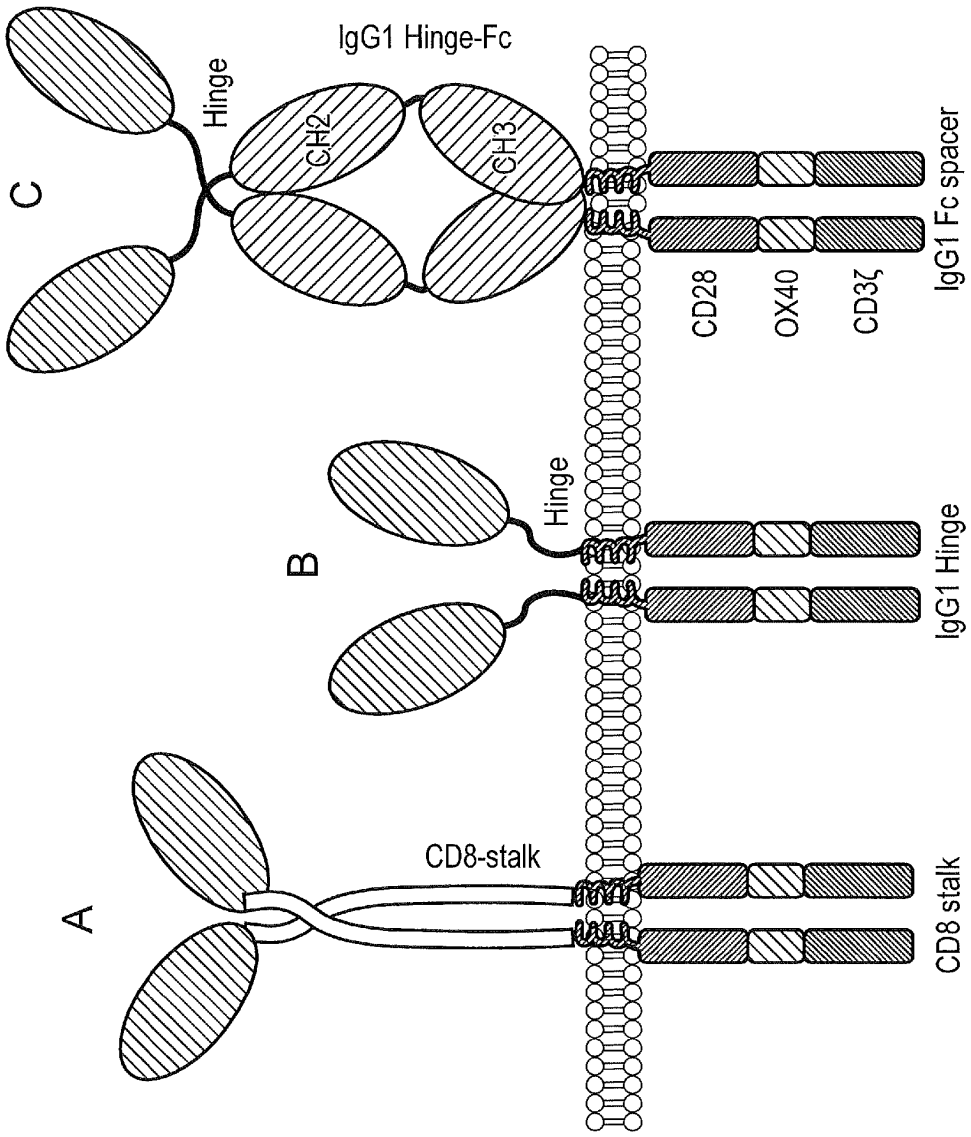
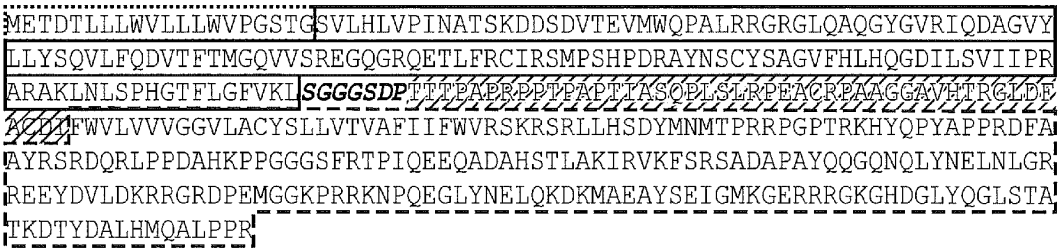

IgG1 Hinge-Fc
Hinge
CH2
CH3
C
CD28
OX40
CD3ζ
IgG1 Fc spacer
Hinge
B
IgG1 Hinge
CD8-stalk
A
CD8 stalk

FIG. 25

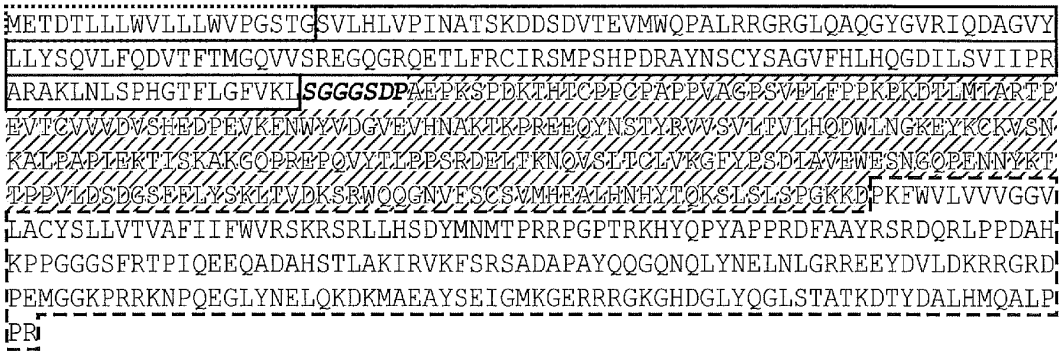
A



B



C



Signal Peptide	Efficient signal peptide
dAPRIL	Truncated APRIL
Spacer	Either hinge-CH2CH3 of human IgG1, human CD8α stalk and human IgG1 hinge
TM and endodomain	Compound endodomain comprising of the CD28TM domain, CD28 endodomain and OX40 and CD3-Zeta endodomains

FIG. 26

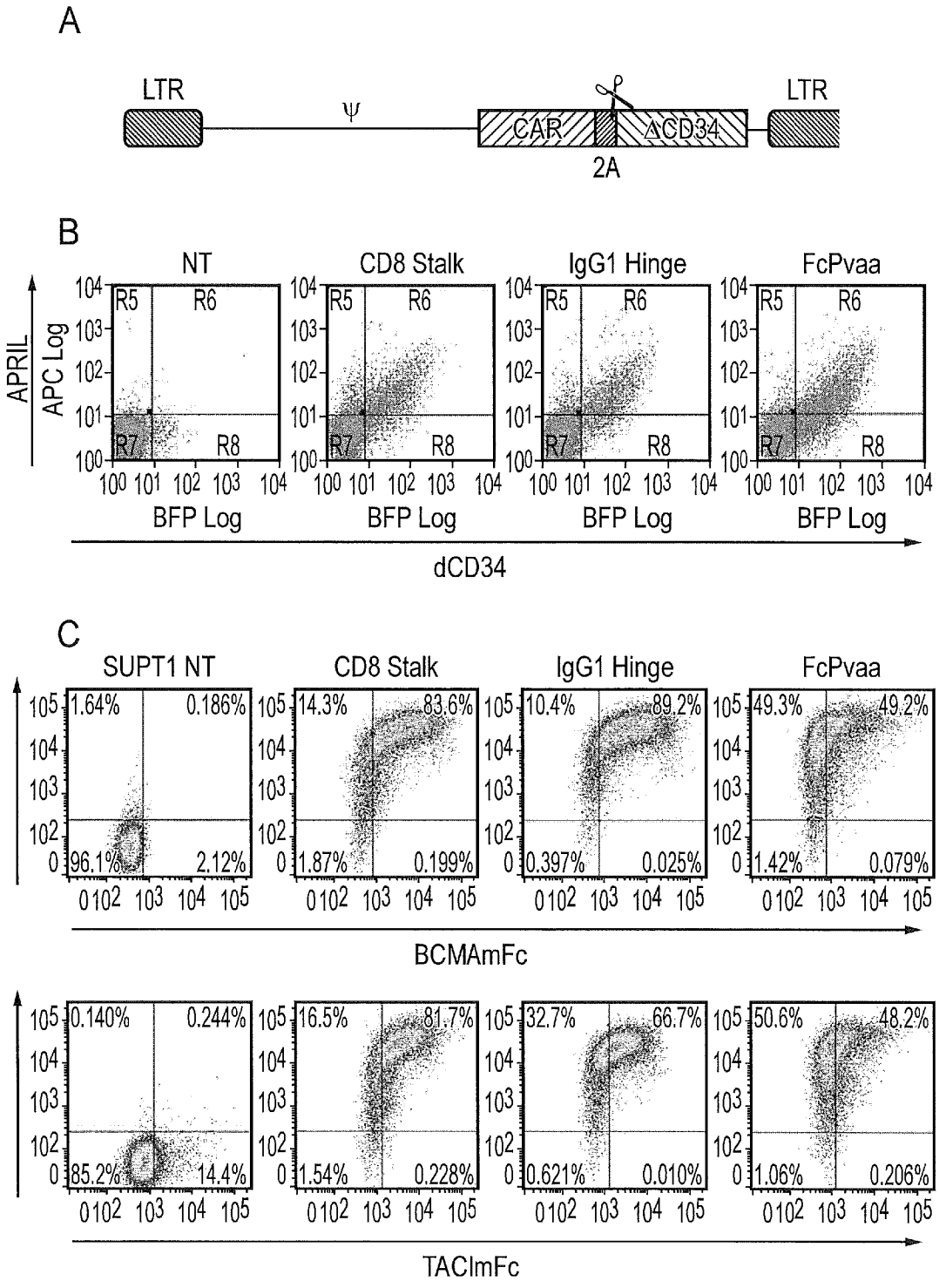

A
LTR
ψ
CAR
ΔCD34
LTR
2A
B
NT
CD8 Stalk
IgG1 Hinge
FcPvaa
APRIL
APC Log
R5
R6
R7
R8
BFP Log
dCD34
C
SUPT1 NT
CD8 Stalk
IgG1 Hinge
FcPvaa
1.64%
0.186%
96.1%
2.12%
14.3%
83.6%
1.87%
0.199%
10.4%
89.2%
0.397%
0.025%
49.3%
49.2%
1.42%
0.079%
BCMAmFc
0.140%
0.244%
85.2%
14.4%
16.5%
81.7%
1.54%
0.228%
32.7%
66.7%
0.621%
0.010%
50.6%
48.2%
1.06%
0.206%
TACImFc

FIG. 27

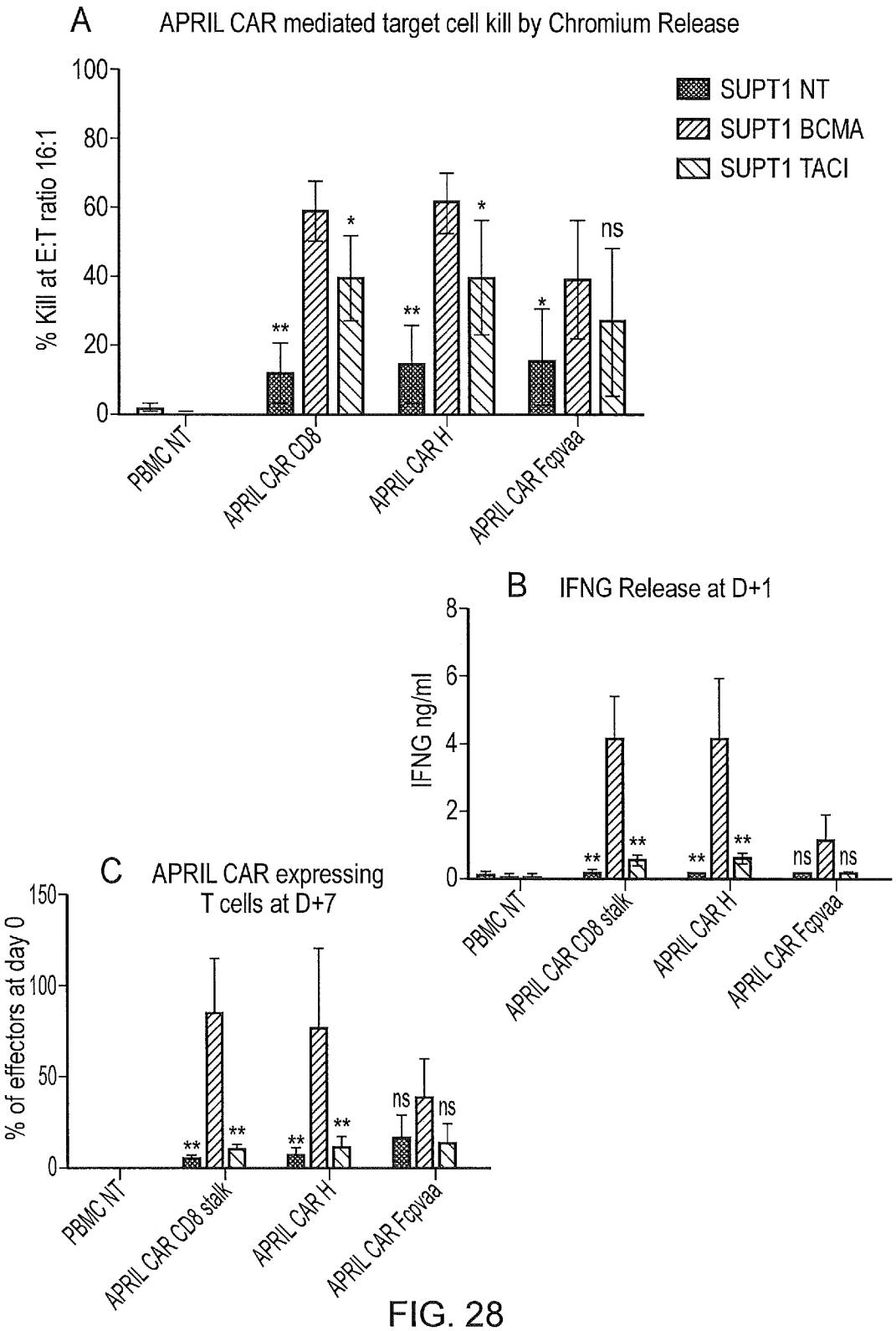

A
APRIL CAR mediated target cell kill by Chromium Release
% Kill at E:T ratio 16:1
100
80
60
40
20
0
SUPT1 NT
SUPT1 BCMA
SUPT1 TACI
**
*
**
*
*
ns
PBMC NT
APRIL CAR CD8
APRIL CAR H
APRIL CAR Fcpvaa
B
IFNG Release at D+1
IFNG ng/ml
8
6
4
2
0
**
**
**
**
ns
ns
PBMC NT
APRIL CAR CD8 stalk
APRIL CAR H
APRIL CAR Fcpvaa
C
APRIL CAR expressing
T cells at D+7
% of effectors at day 0
150
100
50
0
**
**
**
**
ns
ns
PBMC NT
APRIL CAR CD8 stalk
APRIL CAR H
APRIL CAR Fcpvaa

FIG. 28

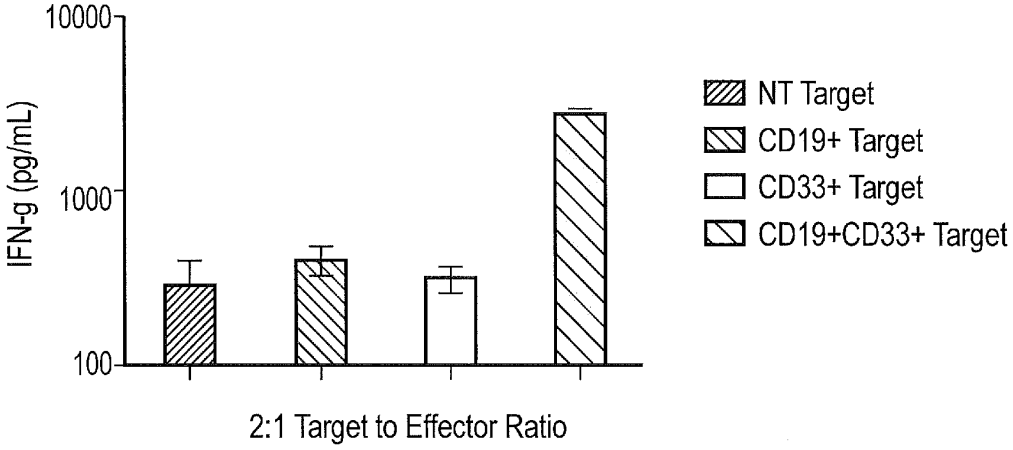

10000
1000
100
IFN-g (pg/mL)
2:1 Target to Effector Ratio
NT Target
CD19+ Target
CD33+ Target
CD19+CD33+ Target

FIG. 29

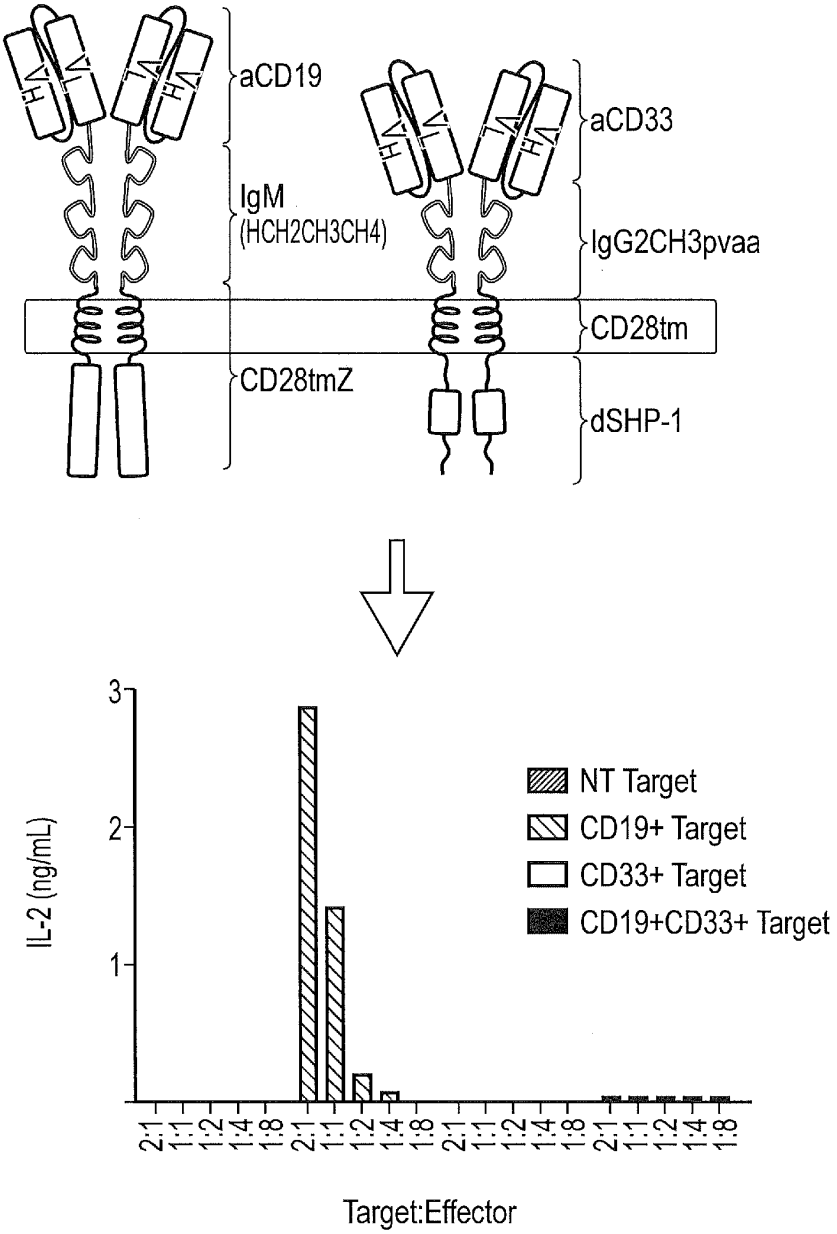

aCD19
IgM
(HCH2CH3CH4)
CD28tmZ
aCD33
IgG2CH3pvaa
CD28tm
dSHP-1
IL-2 (ng/mL)
3
2
1
NT Target
CD19+ Target
CD33+ Target
CD19+CD33+ Target
2:1
1:1
1:2
1:4
1:8
Target:Effector

FIG. 30

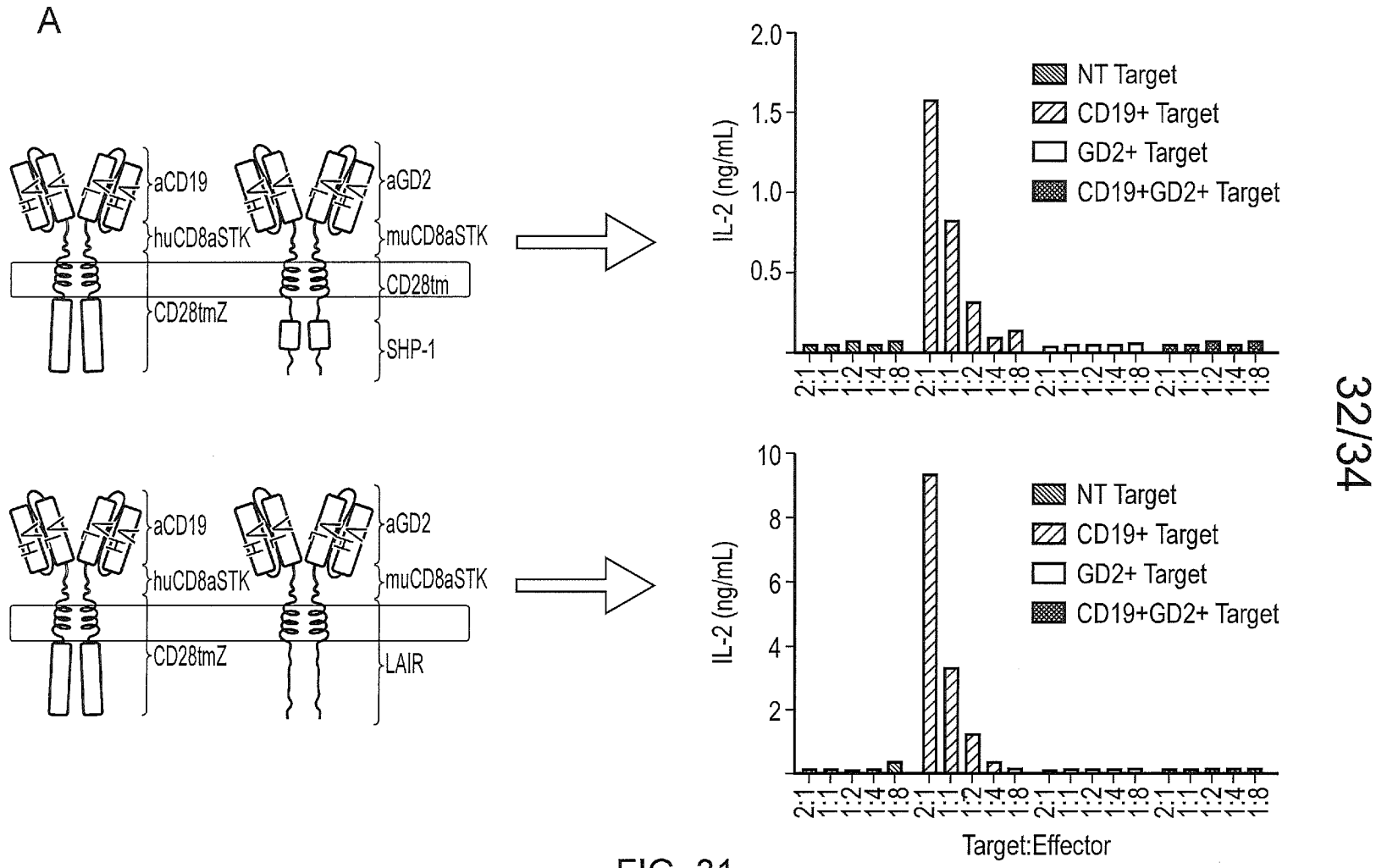
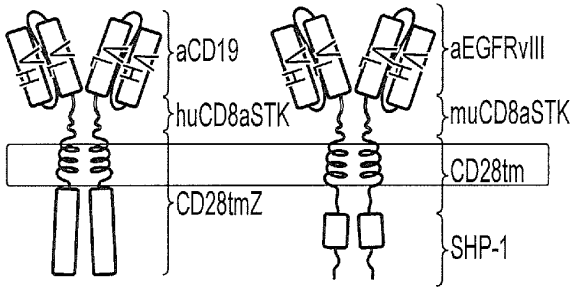
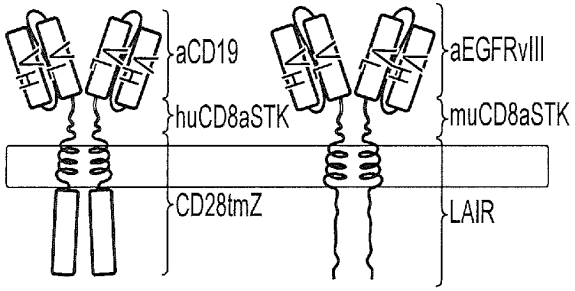
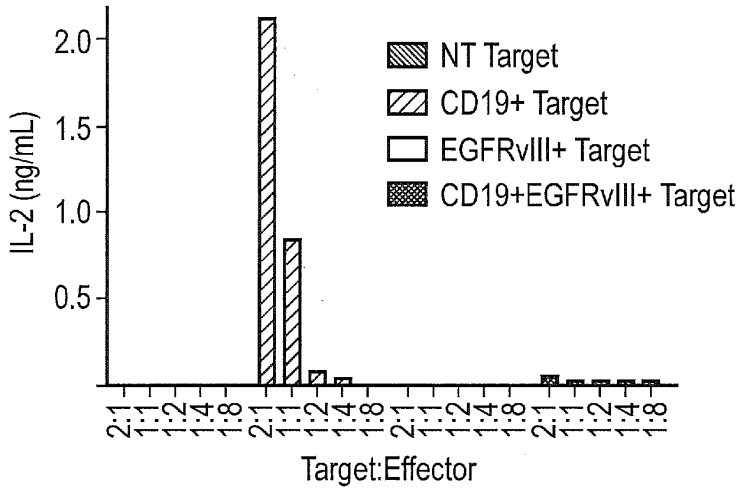

A
aCD19
huCD8aSTK
CD28tmZ
aGD2
muCD8aSTK
CD28tm
SHP-1
aCD19
huCD8aSTK
CD28tmZ
aGD2
muCD8aSTK
LAIR
IL-2 (ng/mL)
2.0
1.5
1.0
0.5
NT Target
CD19+ Target
GD2+ Target
CD19+GD2+ Target
2:1
1:1
1:2
1:4
1:8
IL-2 (ng/mL)
10
8
6
4
2
NT Target
CD19+ Target
GD2+ Target
CD19+GD2+ Target
Target:Effector

FIG. 31

B







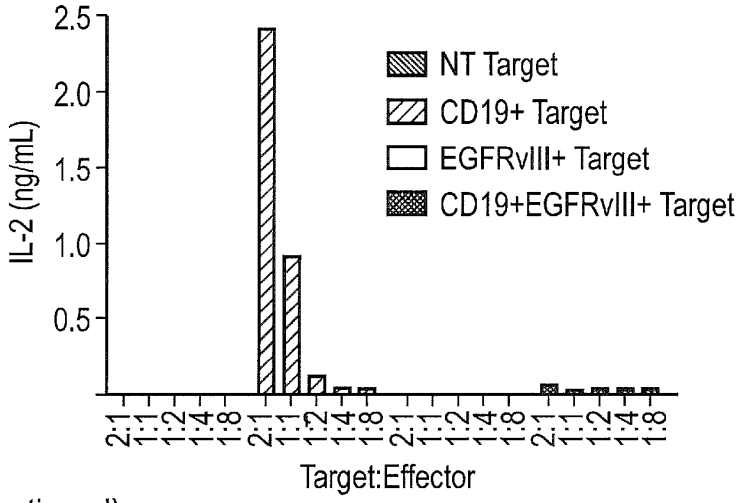


FIG. 31 (Continued)

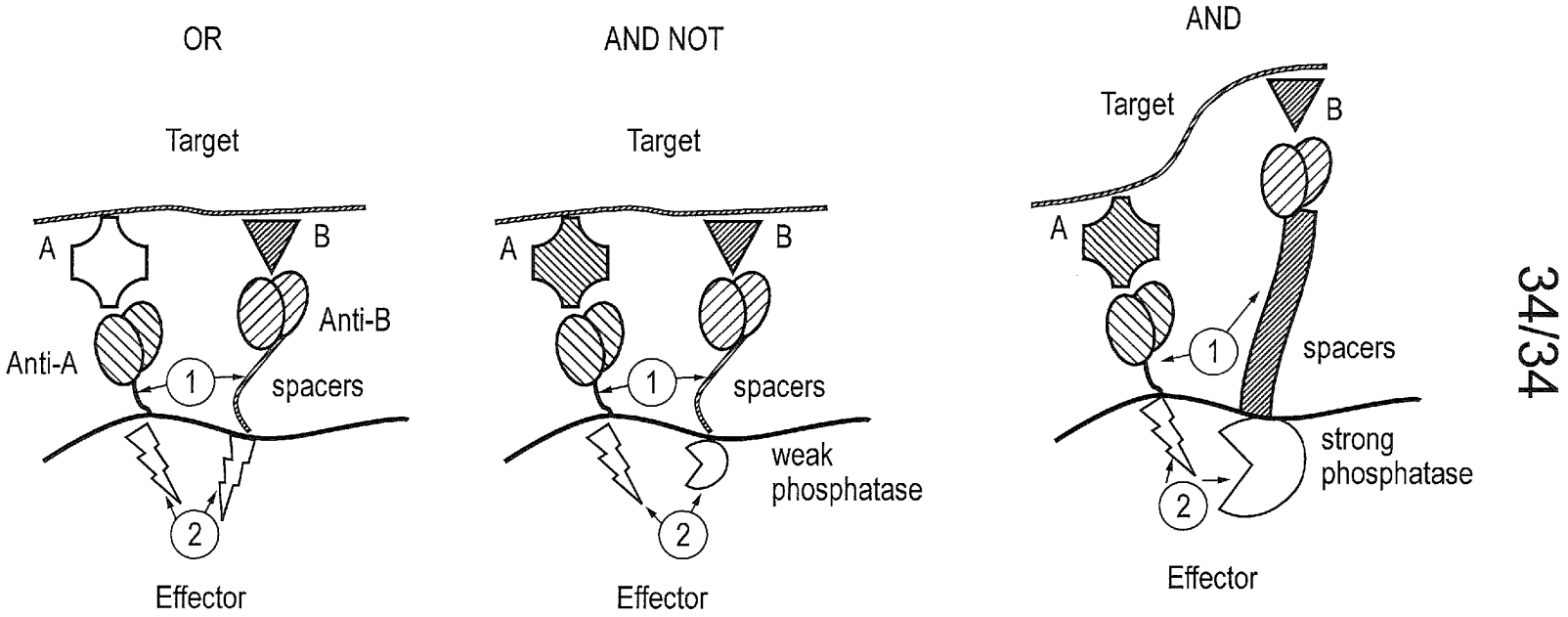

OR
Target
A
B
Anti-B
Anti-A
1
spacers
2
Effector
AND NOT
Target
A
B
1
spacers
weak
phosphatase
2
Effector
AND
Target
B
A
1
spacers
strong
phosphatase
2
Effector

FIG. 32