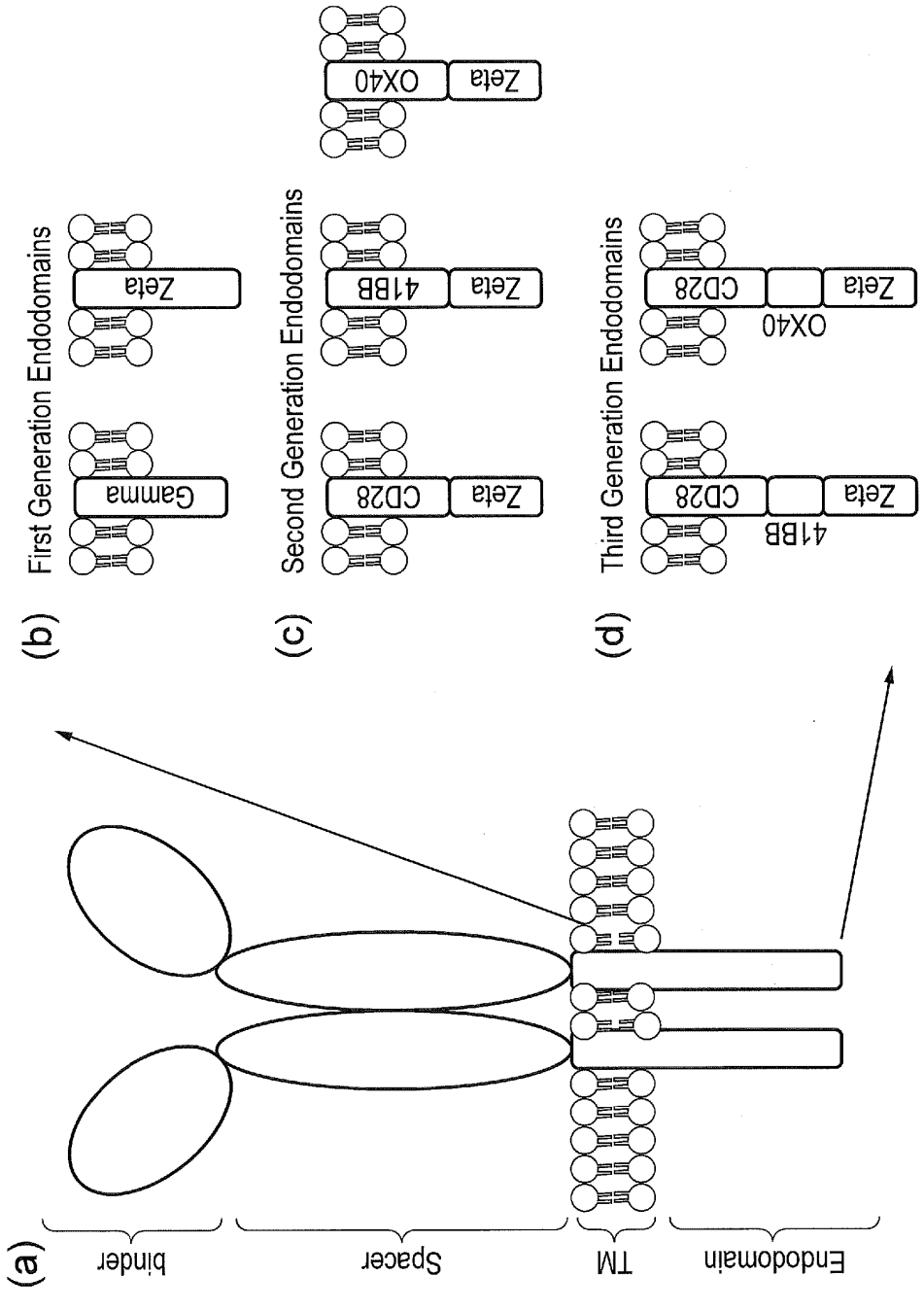

(a)
binder
Spacer
TM
Endodomain
(b)
First Generation Endodomains
Zeta
Gamma
(c)
Second Generation Endodomains
Zeta
41BB
Zeta
CD28
Zeta
OX40
(d)
Third Generation Endodomains
Zeta
CD28
OX40
Zeta
CD28
41BB

FIG. 1

(a) WTWNAYAFAAPS-GGGS-[Protein]

(b)

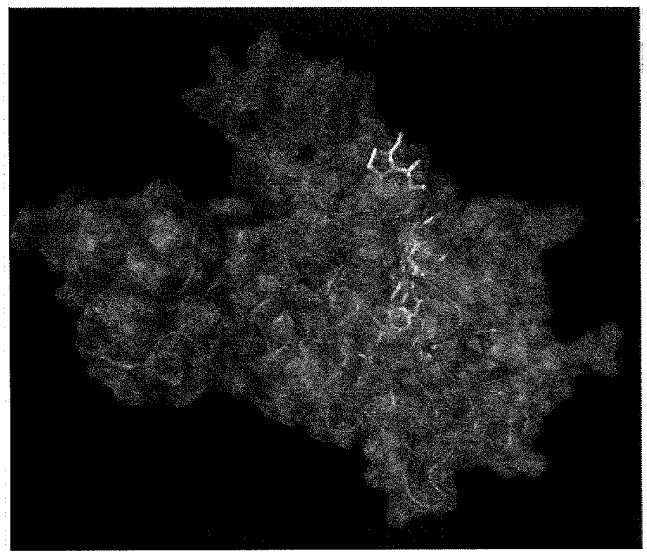


FIG. 2

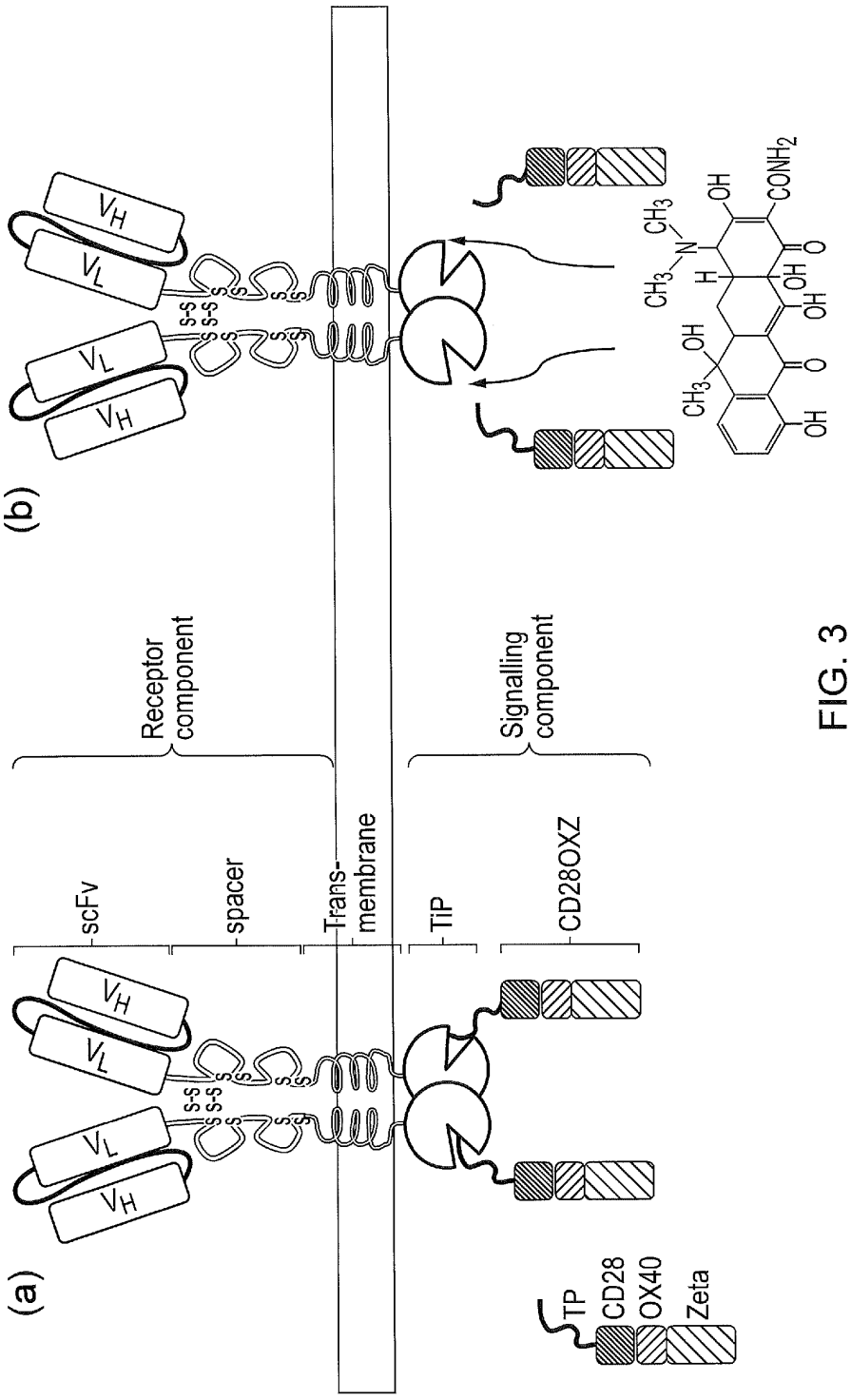
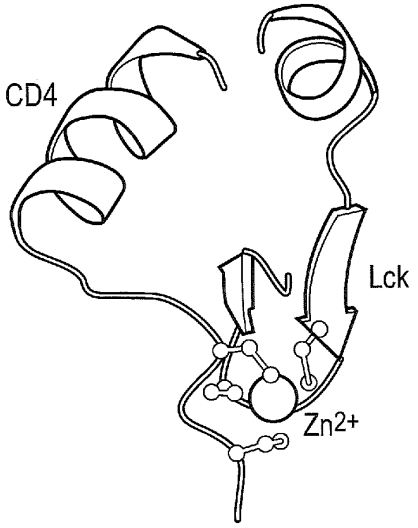

(a)
(b)
Receptor component
Signalling component
scFv
spacer
Trans-membrane
TiP
CD28OXZ
TP
CD28
OX40
Zeta
VH
VL
S-S
CONH2
OH
CH3
N
H
O

FIG. 3

(a)



(b)

>WT_CD4
LSDSGQVLLESNIKVLPTWSTPVQPMALIVLGGVAGLLLFIGLGIFFCVRCRHRRRQAERMSQIKRLLSEKKTCQCPHRFQKTCSPI

>CD4_endodomain_optimized_for
...ALIVLGGVAGLLLFIGLGIFFCVRCRHRRRQAERMAQIKRVVSEKKTAQAPHRFQKTCSPI

[transmembrane domain]
[internalization Sering]
[clathryn pit dileucine]
[zinc clasp cysteines]

FIG. 4

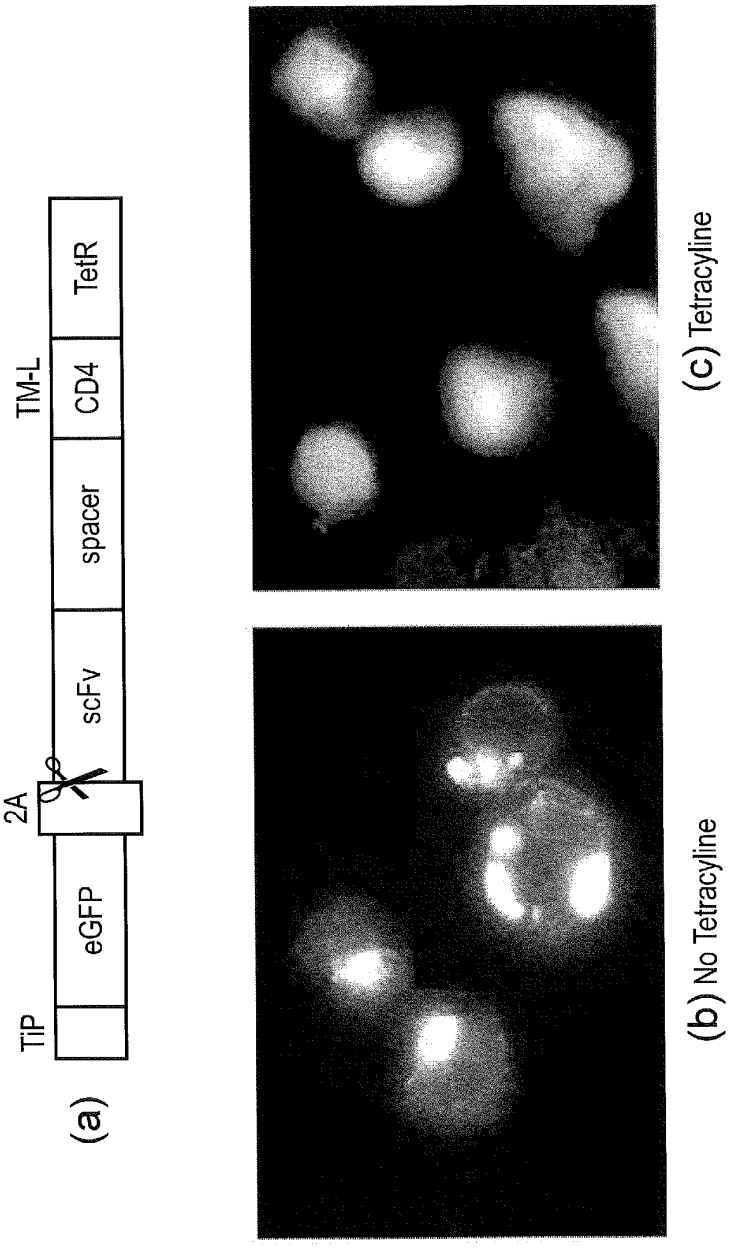
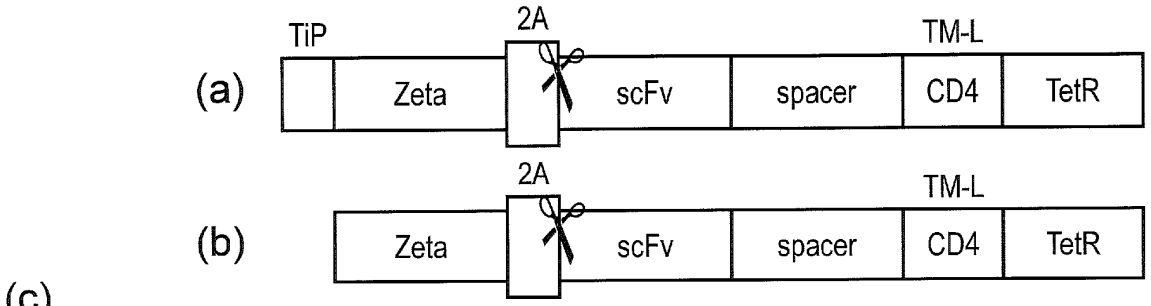

(a)
TiP
eGFP
2A
scFv
spacer
TM-L
CD4
TetR
(b) No Tetracyline
(c) Tetracyline

FIG. 5



(c)

MWTWNAYAFAAPSGGGSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGL
YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRRAEGRGSLLTCGDV
EENPGPMAVPTQVLGLLLLWLTDARCDIQMTQSPSSLSASVGDRVTITCRASEDIYFNLVWYQQKPGK
APKLLIYDTNRLADGVPSRFSGSGSGTQYTLTISSLQPEDFATYYCQHYKNYPLTEGQGTKLEIKRSG
GGGSGGGGSGGGGSGGGGSRSEVQLVESGGGLVQPGGSLRLSCAASGFTLSNYGMHWIRQAPGKGLEW
VSSISLNGGSTYYRDSVKGRETISRDNAKSTLYLQMNSLRAEDTAVYYCAAQDAYTGGYFDYWGQGTL
VTVSSMDPAEPKSPDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKF
NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP
REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPMALIVLGGVAGLLLFIGLGIFFCVRCRHR
RRQAERMAQIKRVVSEKKTAQAPHRFQKTCSPISGGGGSMSRLDKSKVINSALELLNEVGIEGLTTRK
LAQKLGVEQPTLYWHVKNKRALLDALAIEMLDRHHTHFCPLEGESWQDFLRNNAKSFRCALLSHRDGA
KVHLGTRPTEKQYETLENQLAFLCQQGFSLENALYALSAVGHFTLGCVLEDQEHQVAKEERETPTTDS
MPPLLRQAIELFDHQGAEPAFLFGLELIICGLEKQLKCESGS

[TiP][CD3Zeta][2A][scFv][HCH2CH3pvaa][CD4tm-endo][linker][TetR]

FIG. 6

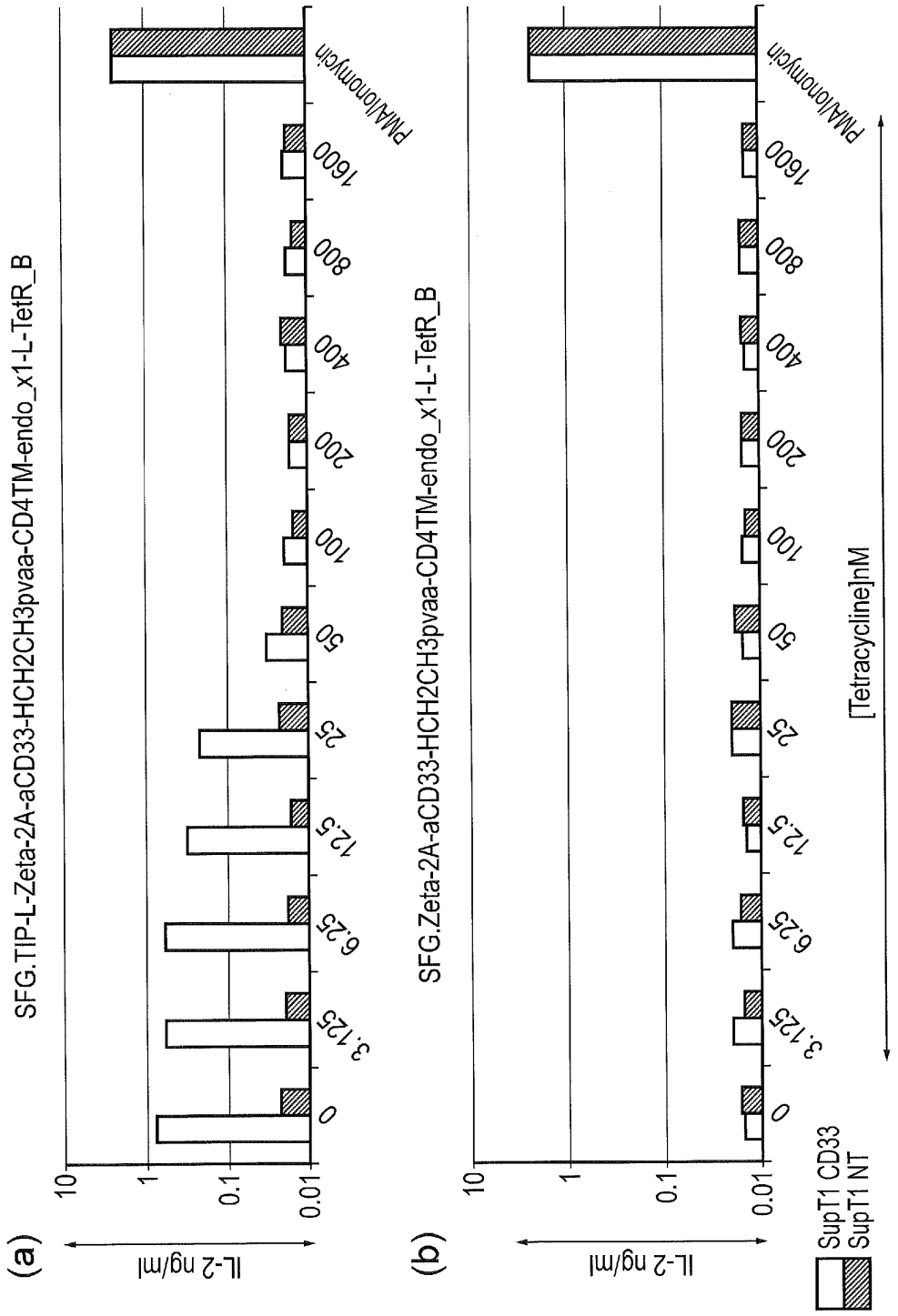

(a)
SFG.TIP-L-Zeta-2A-aCD33-HCH2CH3pvaa-CD4TM-endo_x1-L-TetR_B
IL-2 ng/ml
10
1
0.1
0.01
0
3.125
6.25
12.5
25
50
100
200
400
800
1600
PMA/Ionomycin
(b)
SFG.Zeta-2A-aCD33-HCH2CH3pvaa-CD4TM-endo_x1-L-TetR_B
IL-2 ng/ml
10
1
0.1
0.01
0
3.125
6.25
12.5
25
50
100
200
400
800
1600
PMA/Ionomycin
[Tetracycline]nM
SupT1 CD33
SupT1 NT

FIG. 7

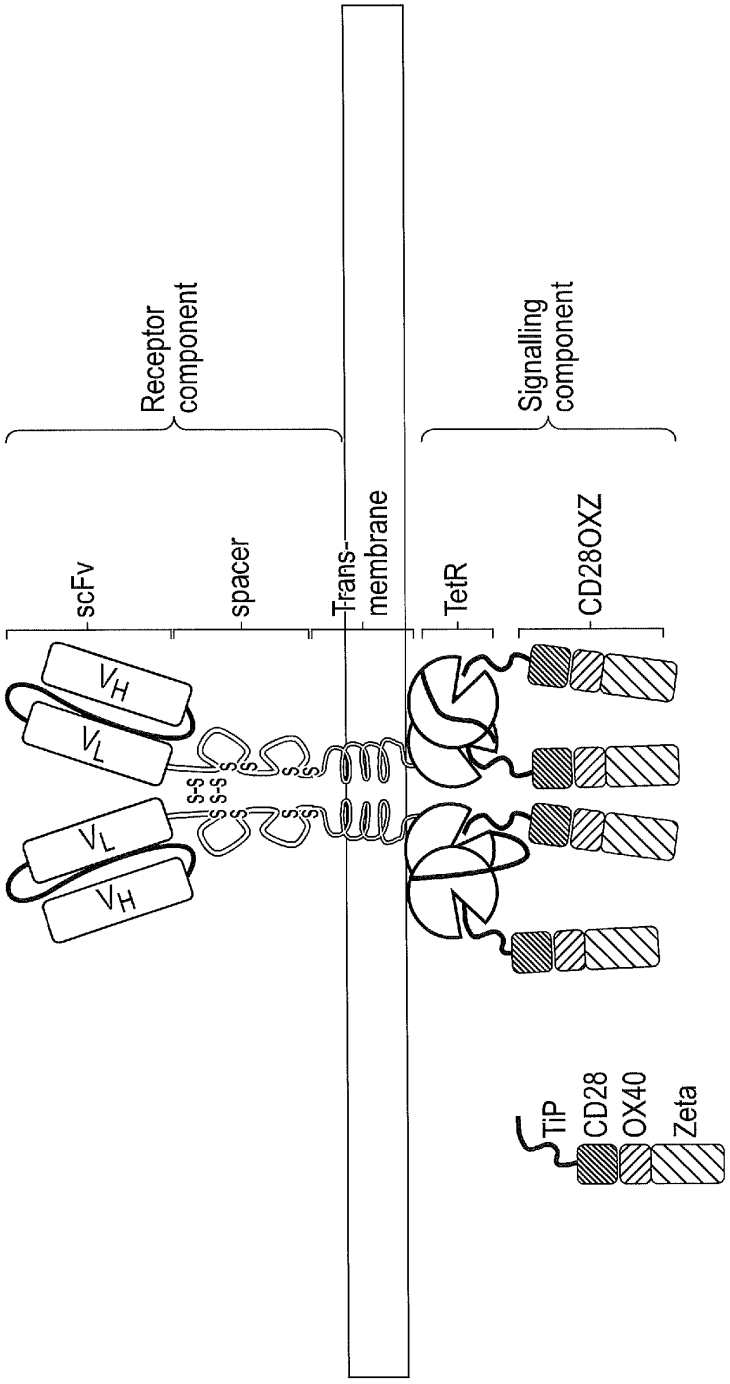

Receptor component
Signalling component
scFv
spacer
Trans-membrane
TetR
CD28OXZ
V_H
V_L
V_L
V_H
S-S
TiP
CD28
OX40
Zeta

FIG. 8

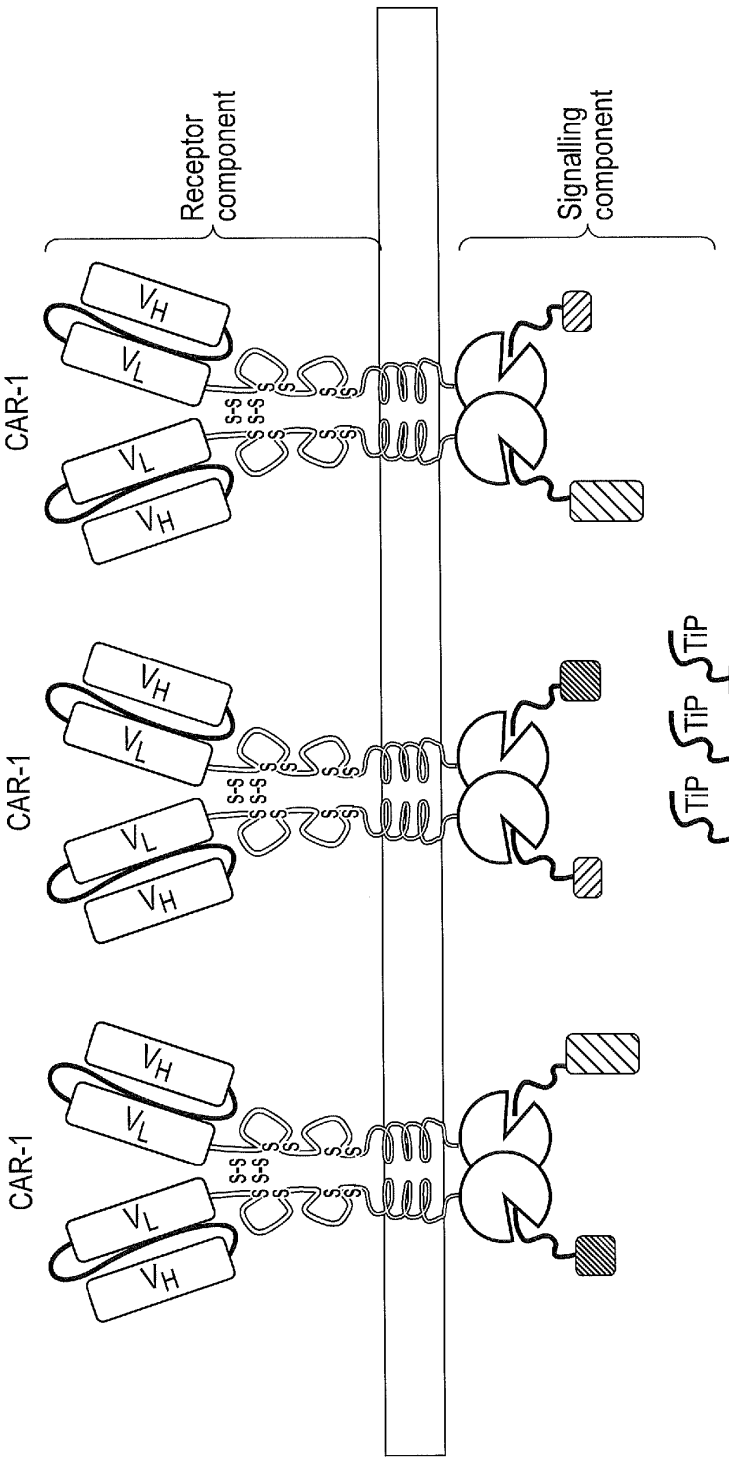

Receptor component
Signalling component
CAR-1
CAR-1
CAR-1
V_H
V_L
V_L
V_H
S-S
TiP
TiP
TiP
CD28
OX40
Zeta

FIG. 9

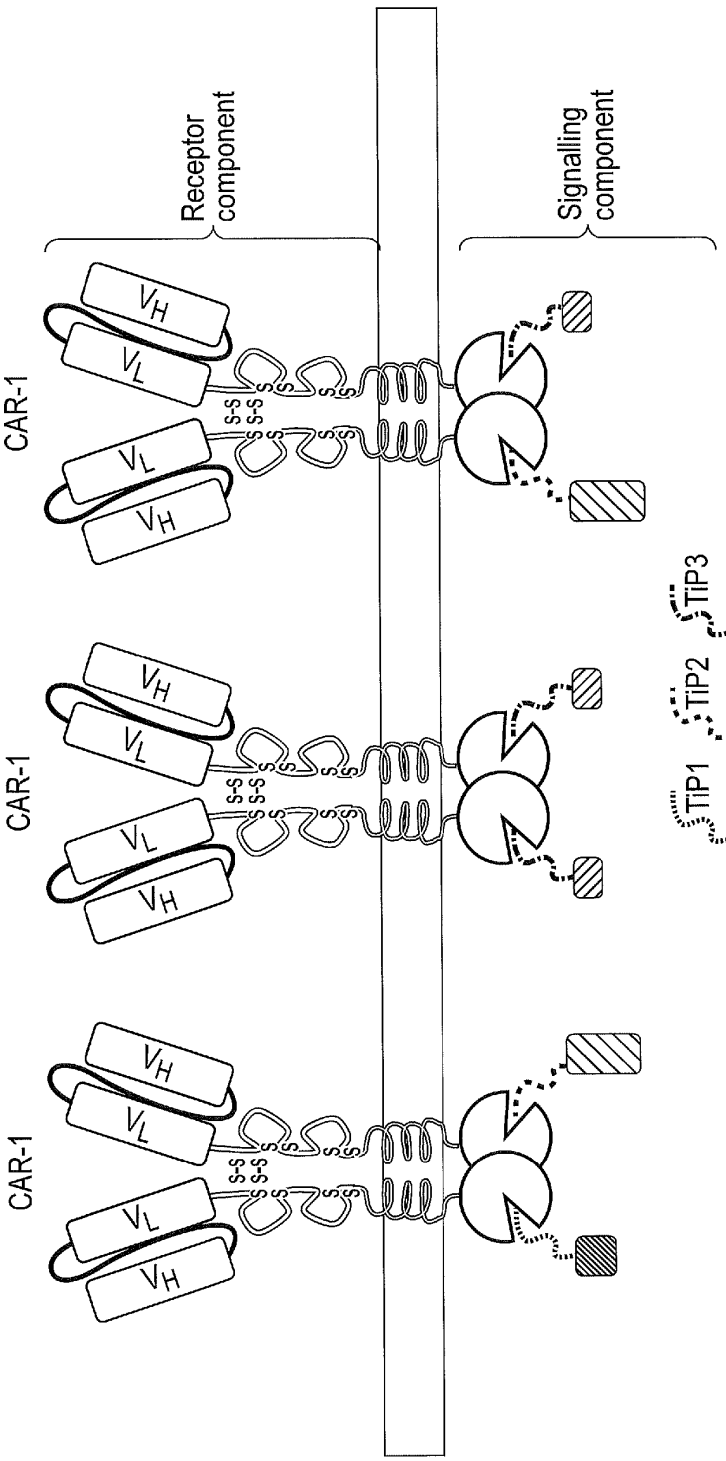

Receptor component
Signalling component
CAR-1
CAR-1
CAR-1
VH
VL
VL
VH
S-S
TiP1
TiP2
TiP3
CD28
OX40
Zeta

FIG. 10

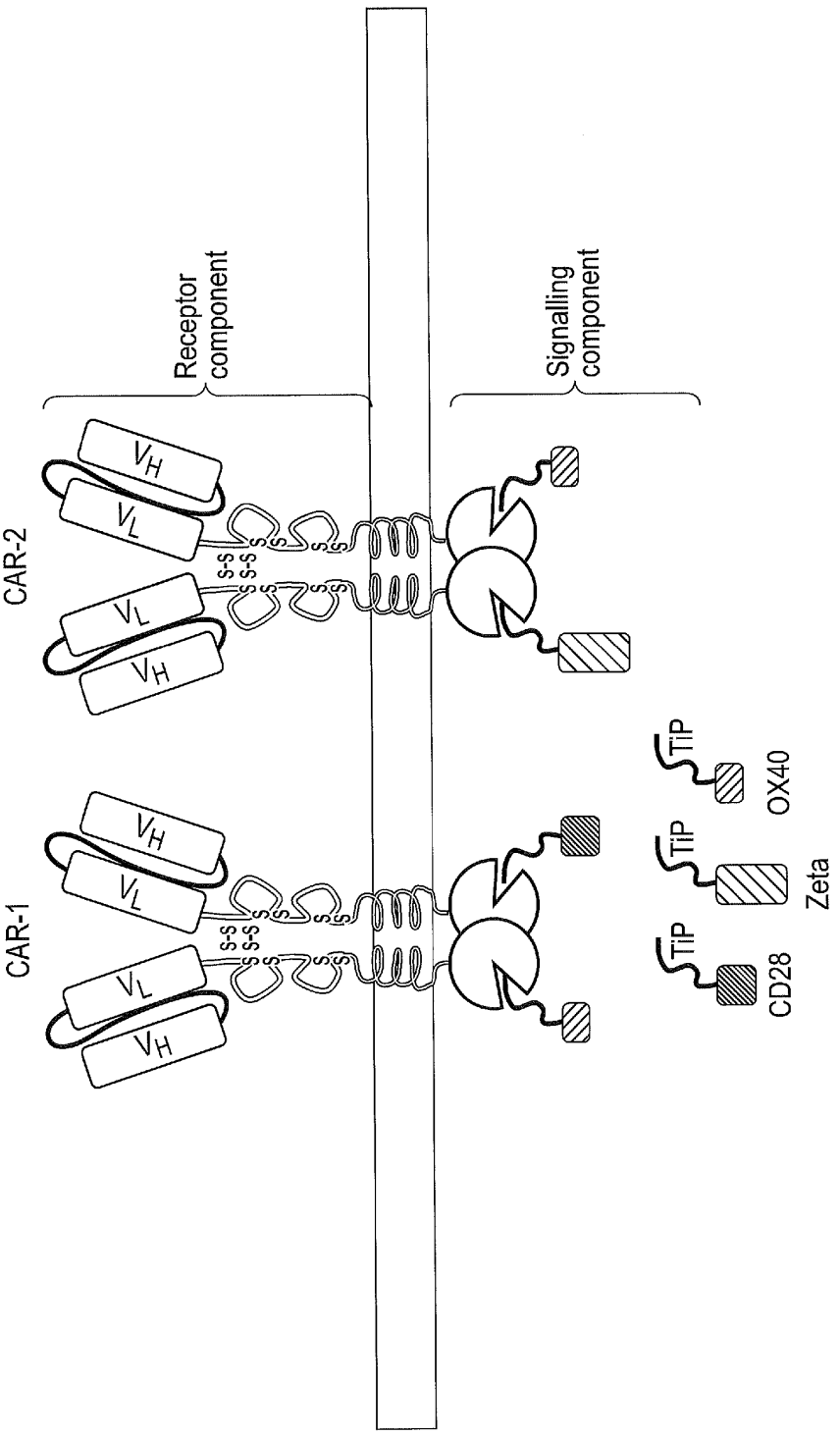

Receptor component
Signalling component
CAR-2
CAR-1
V_H
V_L
S-S
TiP
CD28
OX40
Zeta

FIG. 11

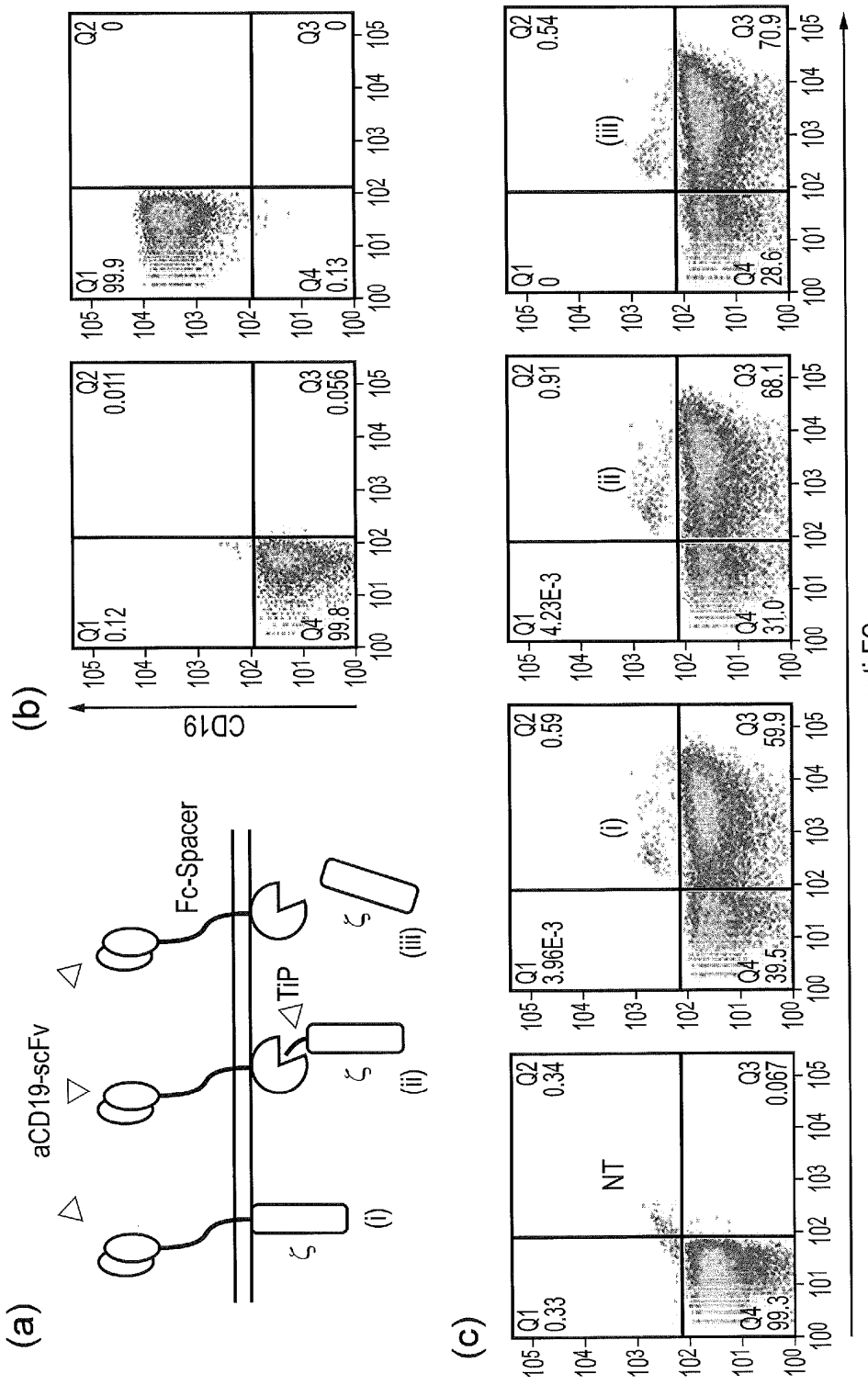

(a)
aCD19-scFv
Fc-Spacer
TiP
ζ
(i)
(ii)
(iii)
(b)
CD19
Q1 0.12
Q2 0.011
Q4 99.8
Q3 0.056
Q1 99.9
Q2 0
Q4 0.13
Q3 0
(c)
NT
Q1 0.33
Q2 0.34
Q4 99.3
Q3 0.067
(i)
Q1 3.96E-3
Q2 0.59
Q4 39.5
Q3 59.9
(ii)
Q1 4.23E-3
Q2 0.91
Q4 31.0
Q3 68.1
(iii)
Q1 0
Q2 0.54
Q4 28.6
Q3 70.9
anti-FC

FIG. 12

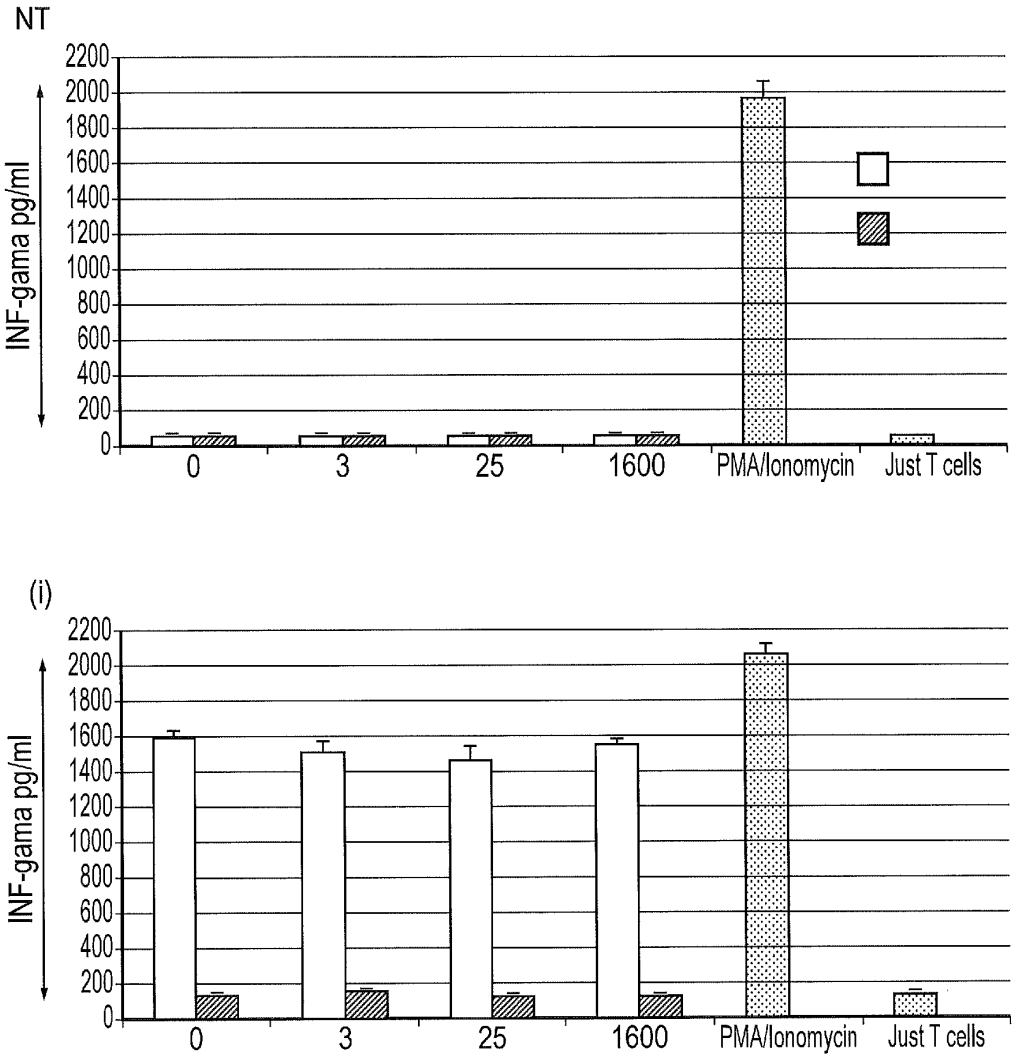

NT
INF-gama pg/ml
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
3
25
1600
PMA/Ionomycin
Just T cells
(i)
INF-gama pg/ml
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
3
25
1600
PMA/Ionomycin
Just T cells

FIG. 13

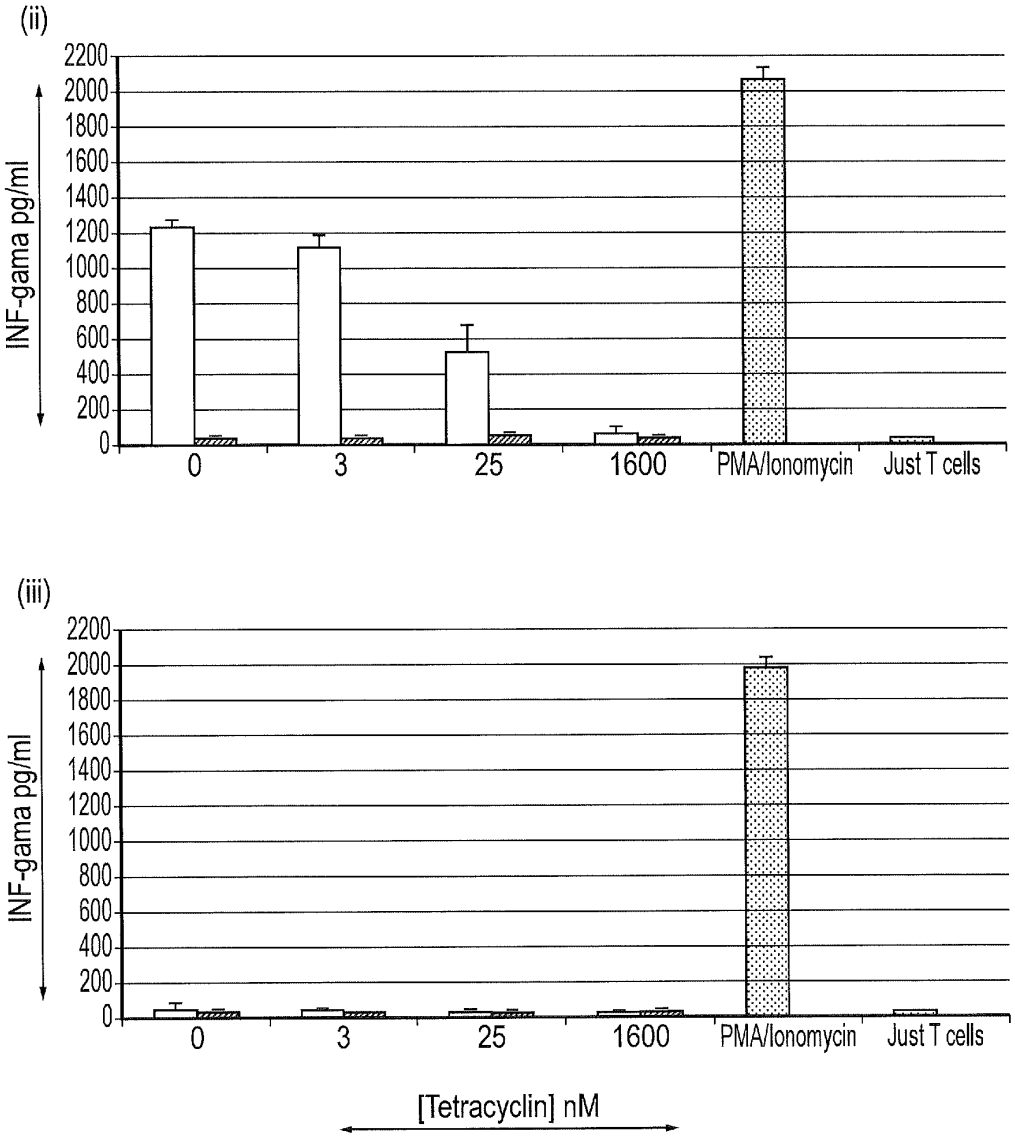


FIG. 13 (Continued)

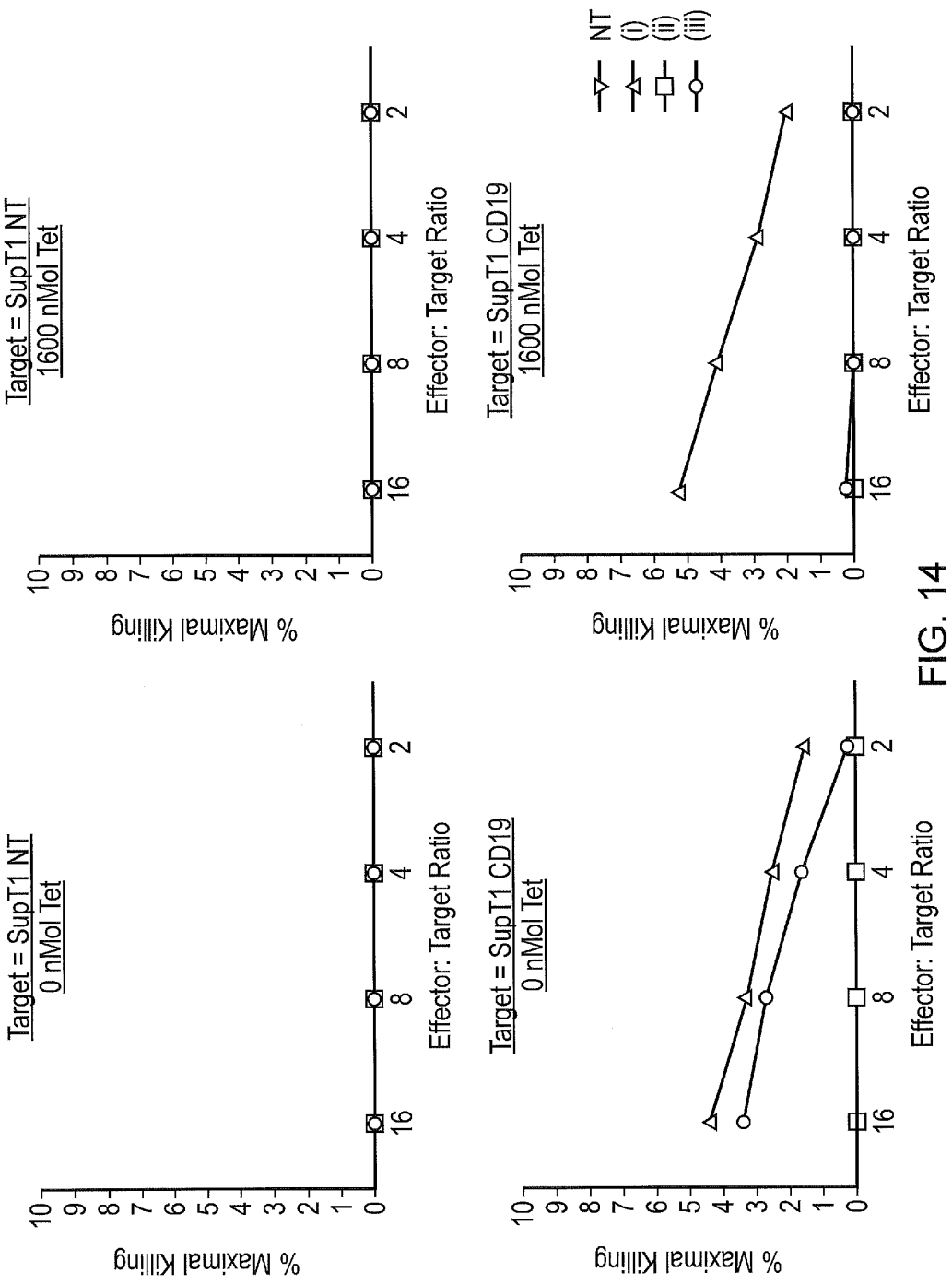

Target = SupT1 NT
0 nMol Tet
Target = SupT1 NT
1600 nMol Tet
Target = SupT1 CD19
0 nMol Tet
Target = SupT1 CD19
1600 nMol Tet
% Maximal Killing
Effector: Target Ratio
NT
(i)
(ii)
(iii)

FIG. 14