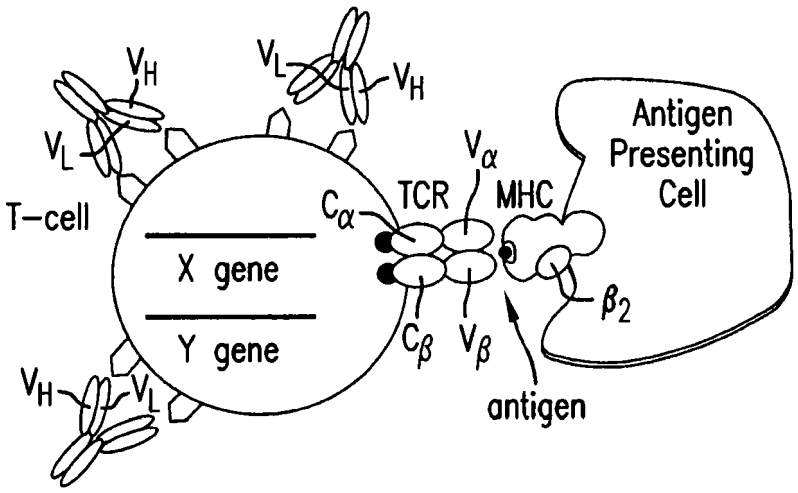

V_H
V_L
V_L
V_H
V_L
V_H
T-cell
X gene
Y gene
V_H
V_L
C_α
TCR
V_α
MHC
Antigen Presenting Cell
β_2
C_β
V_β
antigen

Fig.1

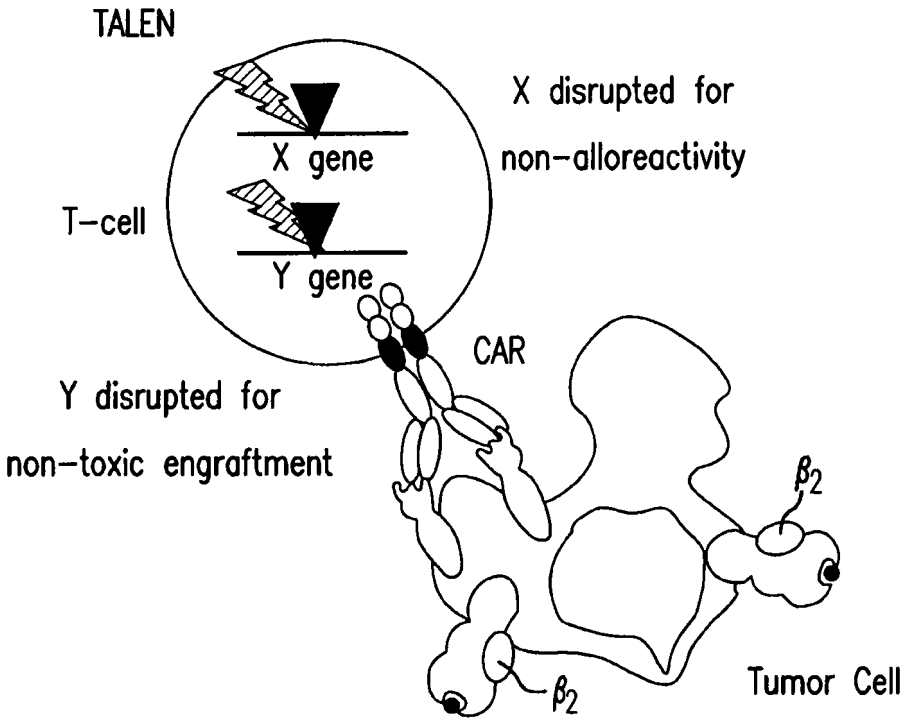

TALEN
X gene
Y gene
T-cell
X disrupted for non-alloreactivity
Y disrupted for non-toxic engraftment
CAR
β_2
β_2
Tumor Cell

Fig.2

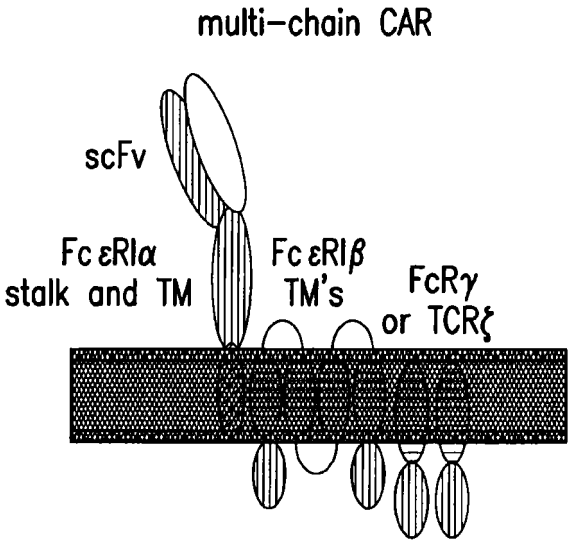

multi-chain CAR
scFv
Fc εRIα
stalk and TM
Fc εRIβ
TM's
FcRγ
or TCRζ
activating
signaling
domains
-beta chain allows all
signaling domains to be
in natural juxtamembrane
position
-beta chain already positioned
for interactions of signals
from FcRg or TCRzeta

Fig.3

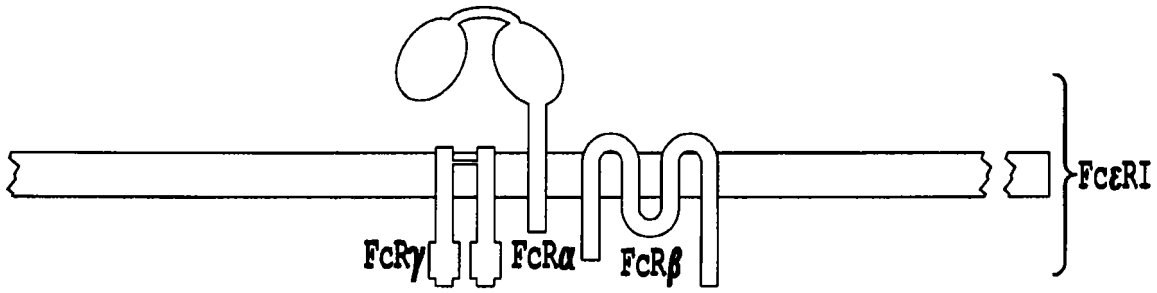

FcεRI
FcRγ
FcRα
FcRβ

Fig. 4A

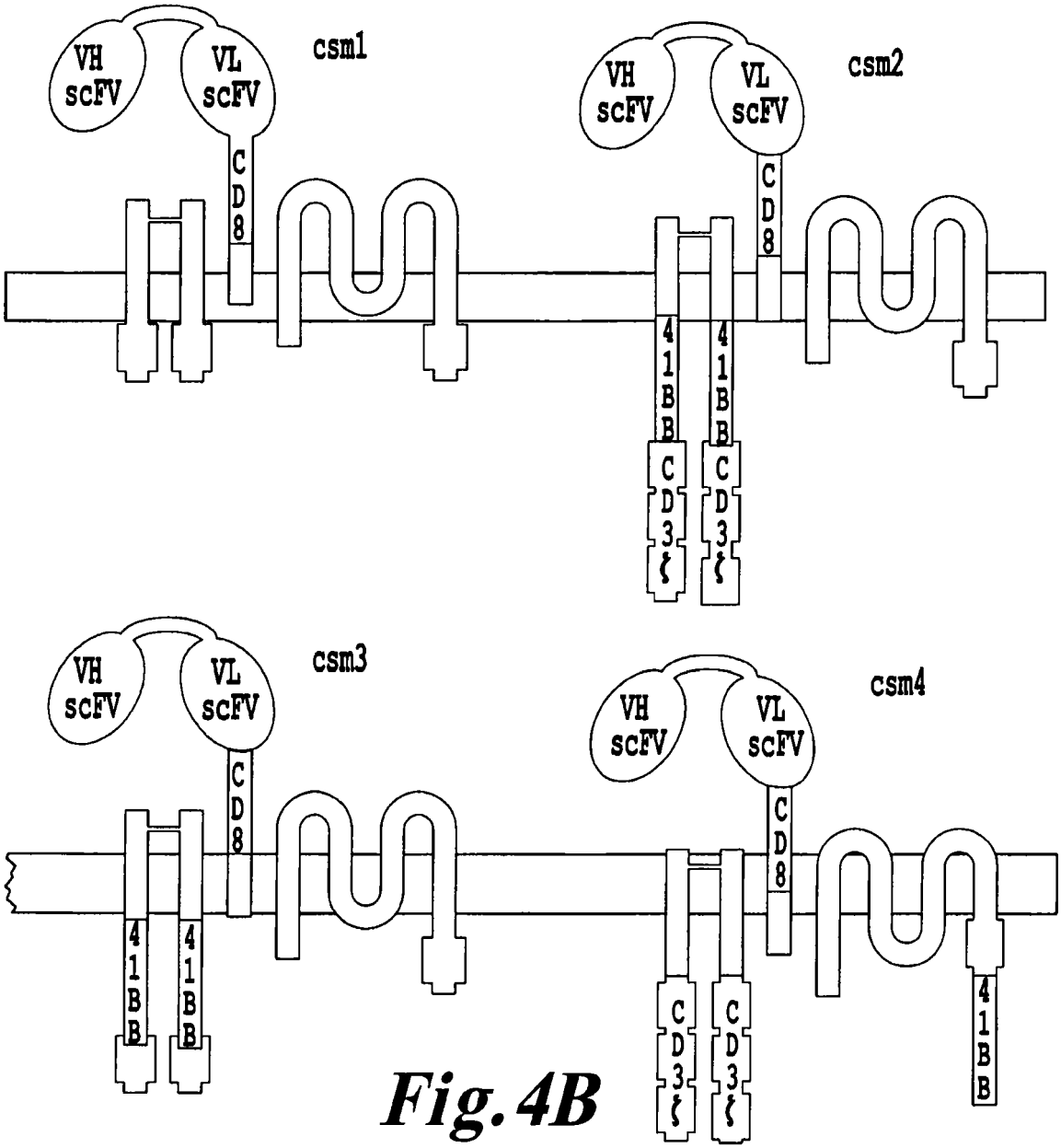

csm1
VH scFV
VL scFV
CD8
csm2
VH scFV
VL scFV
CD8
41BB
CD3ζ
41BB
CD3ζ
csm3
VH scFV
VL scFV
CD8
41BB
41BB
csm4
VH scFV
VL scFV
CD8
CD3ζ
CD3ζ
41BB

Fig. 4B

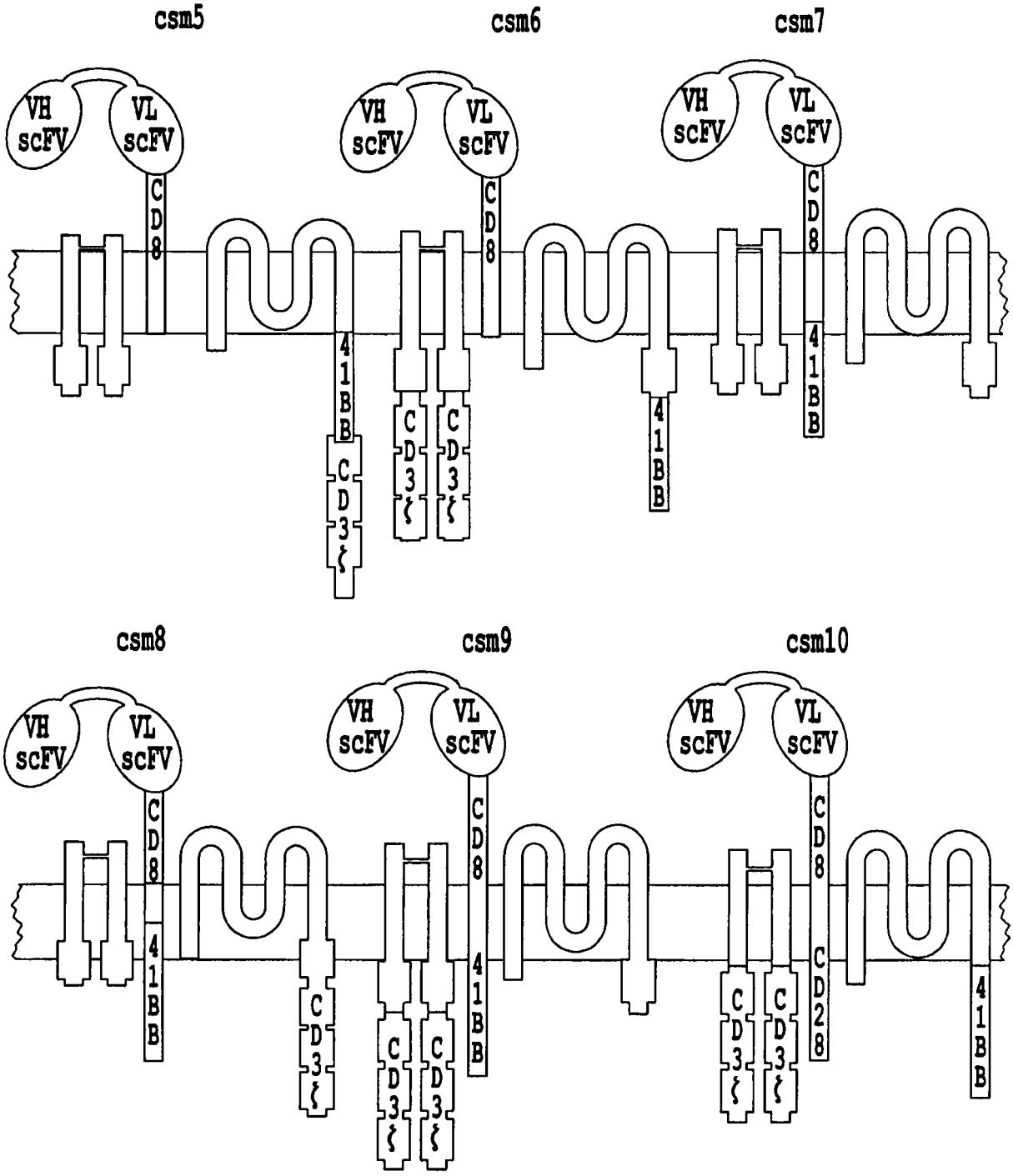
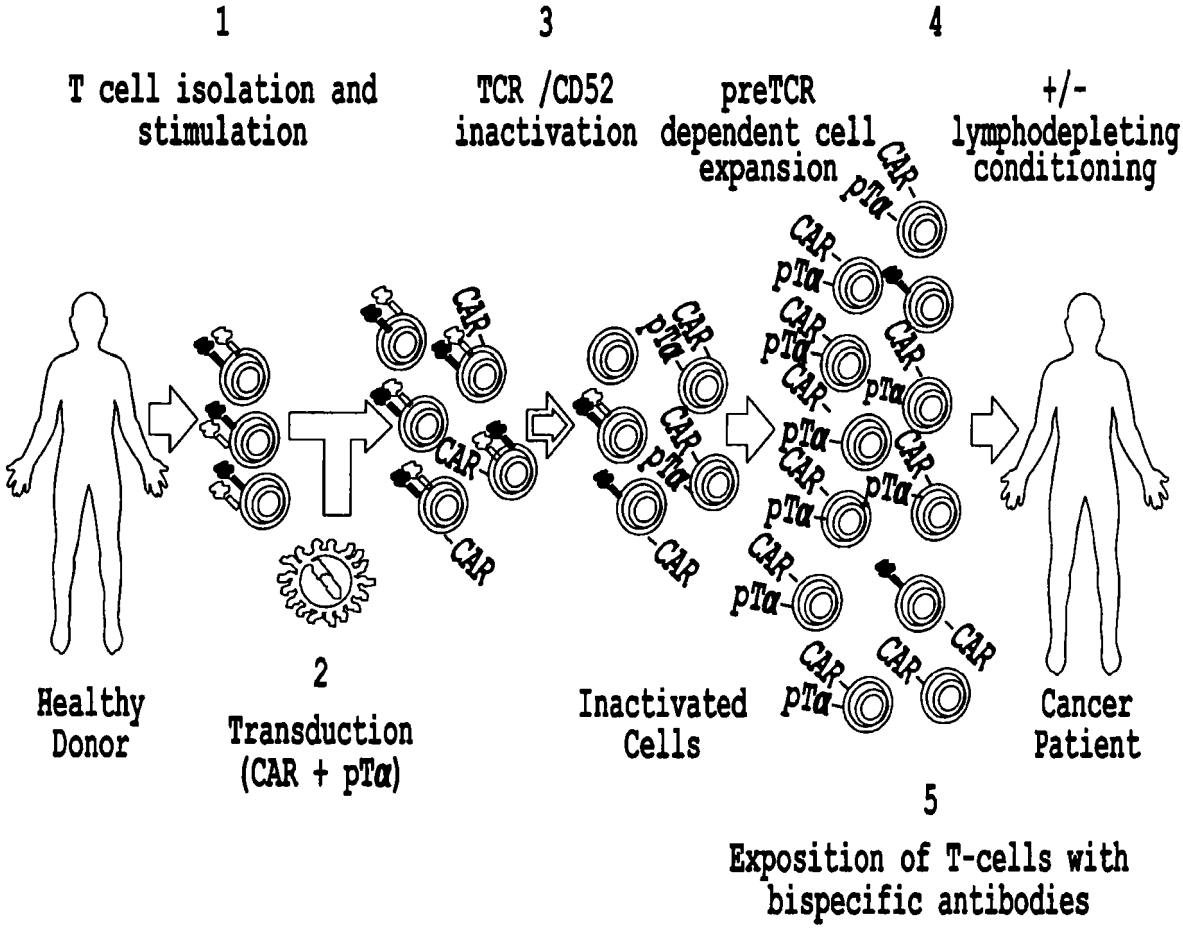

csm5
csm6
csm7
csm8
csm9
csm10
VH scFV
VL scFV
CD8
4 1BB
CD3ζ
CD28

Fig. 4C



	CD52	CD52 disruption provides chemotherapy resistance
	TCR	TCRα disruption removes alloreactivity (GvHD)
	CAR	T-cell redirection / tumor recognition
	PTα	pre-TCRα(pTα) drives cell proliferation

Fig.5

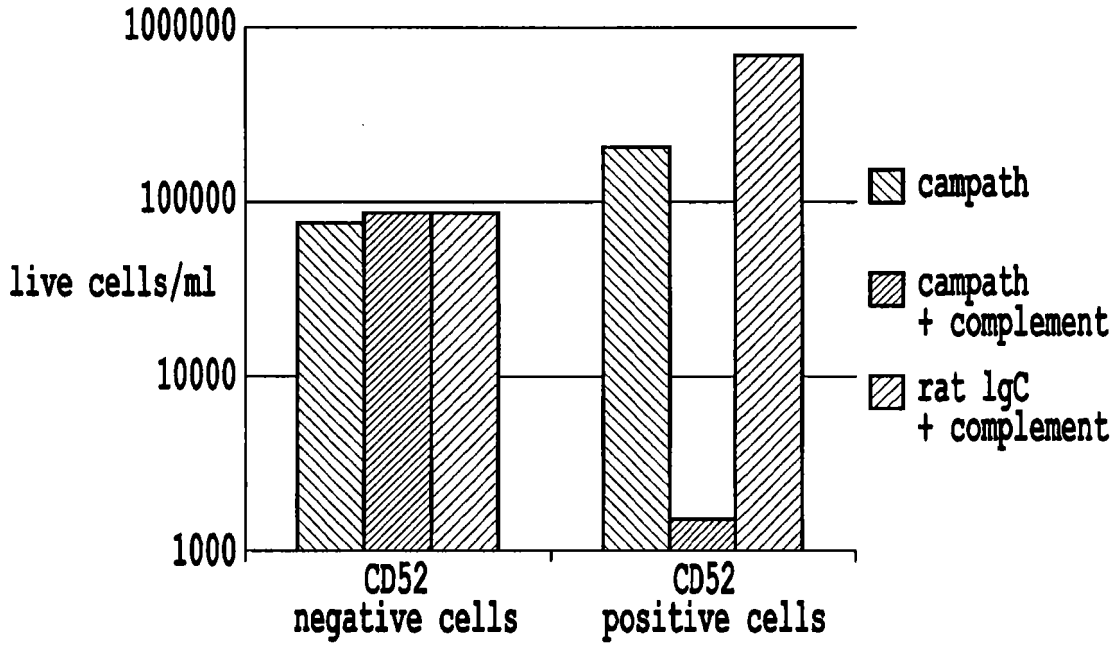

1000000
100000
10000
1000
live cells/ml
CD52
negative cells
CD52
positive cells
campath
campath
+ complement
rat IgC
+ complement

Fig. 6

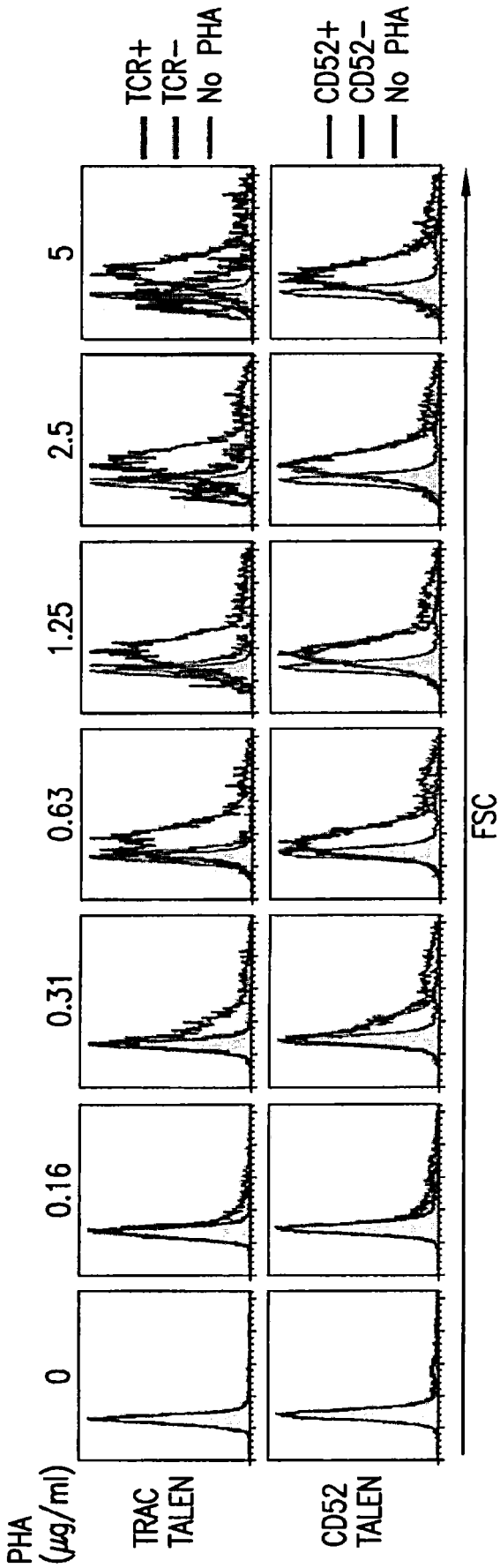

PHA (μg/ml)
0
0.16
0.31
0.63
1.25
2.5
5
TRAC TALEN
CD52 TALEN
TCR+
TCR−
No PHA
CD52+
CD52−
No PHA
FSC

Fig. 7

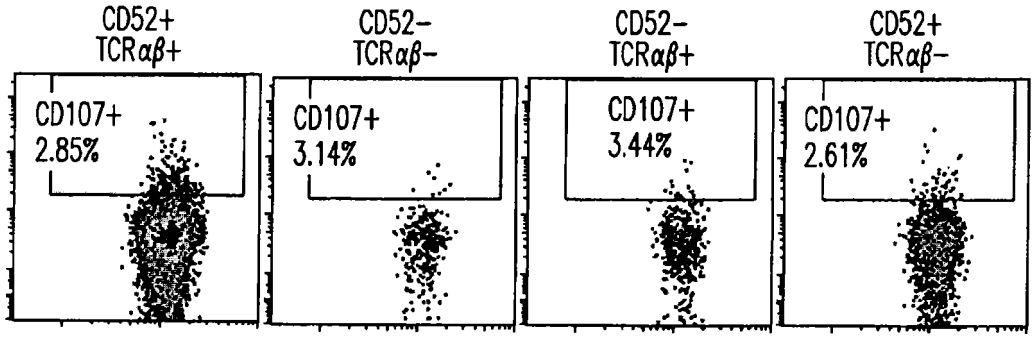


Fig.8A

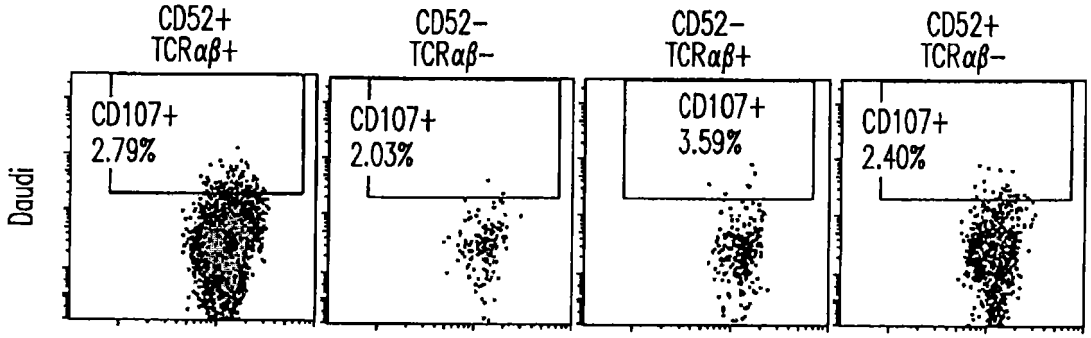


Fig.8B

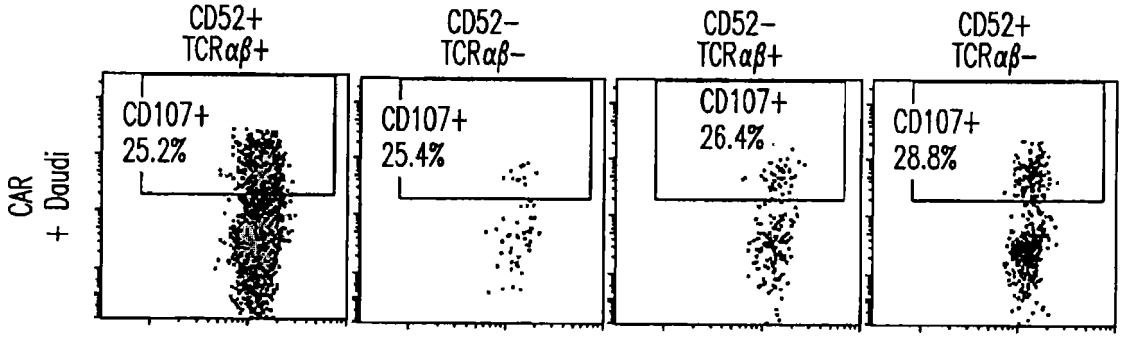


Fig.8C

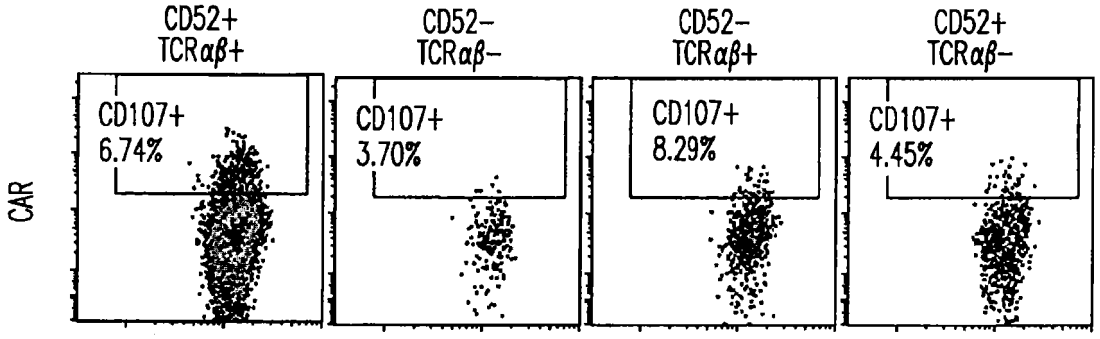

CD52+
TCRαβ+
CD52−
TCRαβ−
CD52−
TCRαβ+
CD52+
TCRαβ−
CAR
CD107+
6.74%
CD107+
3.70%
CD107+
8.29%
CD107+
4.45%

Fig.8D

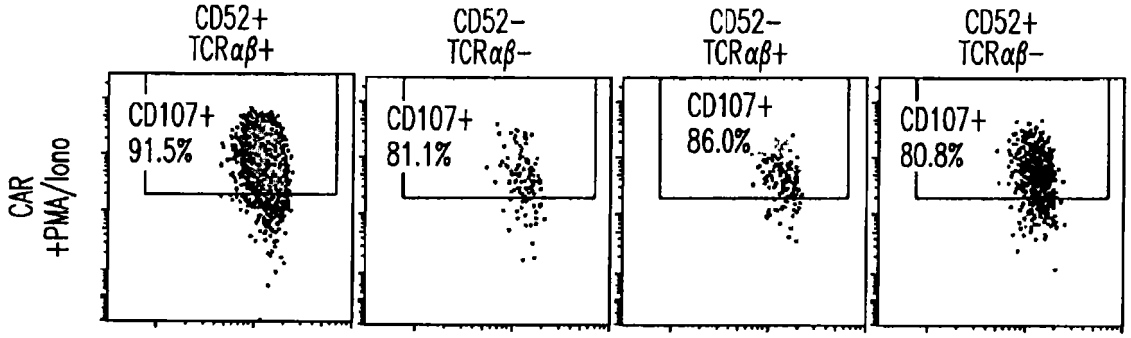

CD52+
TCRαβ+
CD52−
TCRαβ−
CD52−
TCRαβ+
CD52+
TCRαβ−
CAR
+PMA/Iono
CD107+
91.5%
CD107+
81.1%
CD107+
86.0%
CD107+
80.8%

Fig.8E

	LEFT HALF TARGET	Spacer size (bp)	RIGHT HALF TARGET	
TRAC	TTGTCCCACAGATATCC	15	CCGTGTACCCAGCTGAGA	
CD52	TTCCTCCTACTCACCAT	15	GGTACAGGTAAGAGCA A	
Potential offsite targets	Left matched sequence	Spacer size (bp)	Right matched sequence	Mis-matc hes
1	ttgctctCaccAgtaTA	25	TTtTcaggtaagTgcaa	8
2	tCActcttacctgGacc	19	CCtacaggttaagGgcCa	7
3	tctcagAtgAtacacCC	24	AgtacaggCaTgagcCa	8
4	tGAtcccacagaAatAc	18	gCatTtctgtgggaTCa	8
5	ttCctctAacctgtaTT	25	gAtCcaggtaagGTcaa	8
6	tAgtcccCcagatatGA	19	aAggtgTgGaTgaggaa	8
7	ttgtcAcacaTataCcG	21	TgGtatTtgtgTgacaa	8
8	tAActcttacctgtaGT	16	AgatTtctCtgggGcaa	8
9	ttActccAactAacTat	16	ccgtTtaccGgctTaga	7
10	tGgctcAtacctgtaGT	14	aGgAtgagGTggaggaa	8
11	ttgctcAtacAtgtGcA	21	atgCtgTgtaggTggTa	8
12	ttgtcccacagaCatTc	18	ccACgtaGcagctgGga	6
13	tcAcaCctggtacaTAg	27	GtgTtTagtaggGggaa	8
14	ttgtcccacagCtaCcc	29	gAgtCtTtgtAggacaa	6
15	tctcaActgAAacaAgg	23	TgtaAtgTCaagagcaa	8

Fig. 9A

	Control transfection (no RNA)			CD52-TALEN+TRAC+TALEN transfection		
	Nb seq analyzed	Nb indels	Frequency indels (less than)	Nb seq analyzed	Nb indels	Frequency indels (less than)
	3965	0	2.52E-04	7560	3371	0.44
	1046	0	9.56E-04	2266	1056	0.47
Matched sequence						
CD52-R_TRAC-R	7132	0	1.4E-04	7644	1	1.3E-04
CD52-R_TRAC-R	6431	0	1.6E-04	7377	2	2.7E-04
CD52-R_TRAC-R	2771	0	3.6E-04	2704	80	3.7E-04
TRAC-L_CD52-L	5525	0	1.8E-04	4739	0	2.1E-04
CD52-R_TRAC-R	27958	0	3.6E-05	16646	0	6.0E-05
TRAC-L_CD52-L	22456	0	4.5E-05	32912	10	3.0E-04
TRAC-L_CD52-L	8275	0	1.2E-04	5629	0	1.8E-04
TRAC-L_CD52-R	23253	0	4.3E-05	22054	16	7.3E-04
CD52-L_TRAC-R	13371	0	7.5E-05	13688	1	7.3E-05
CD52	22856	0	4.4E-05	31292	0	3.2E-05
CD52	3238	1	3.1E-04	3064	0	3.3E-04
TRAC	4530	0	2.2E-04	4652	0	2.1E-04
CD52-L_TRAC-R	17361	0	5.8E-05	14454	0	6.9E-05
TRAC-L_CD52-L	32823	0	3.0E-05	33911	1	2.9E-05
CD52-R_TRAC-R	6479	0	1.5E-04	6088	0	1.6E-04

Fig.9B

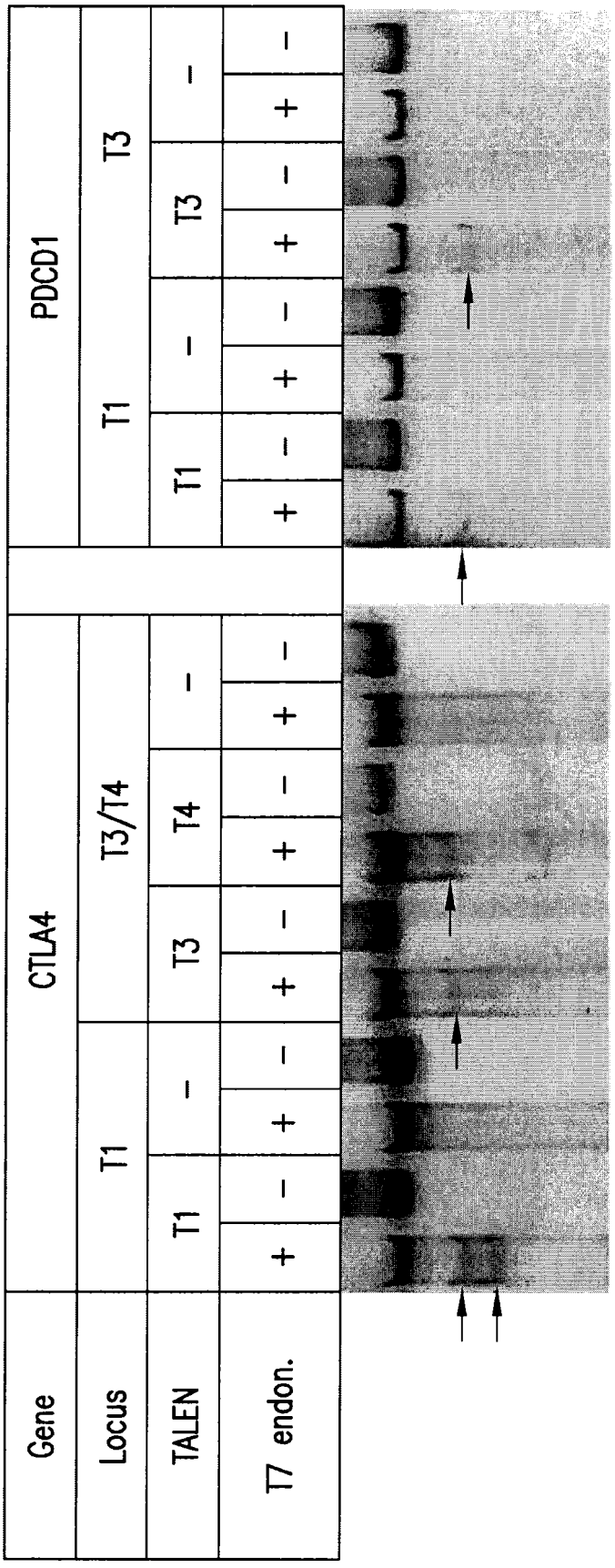


Fig.10

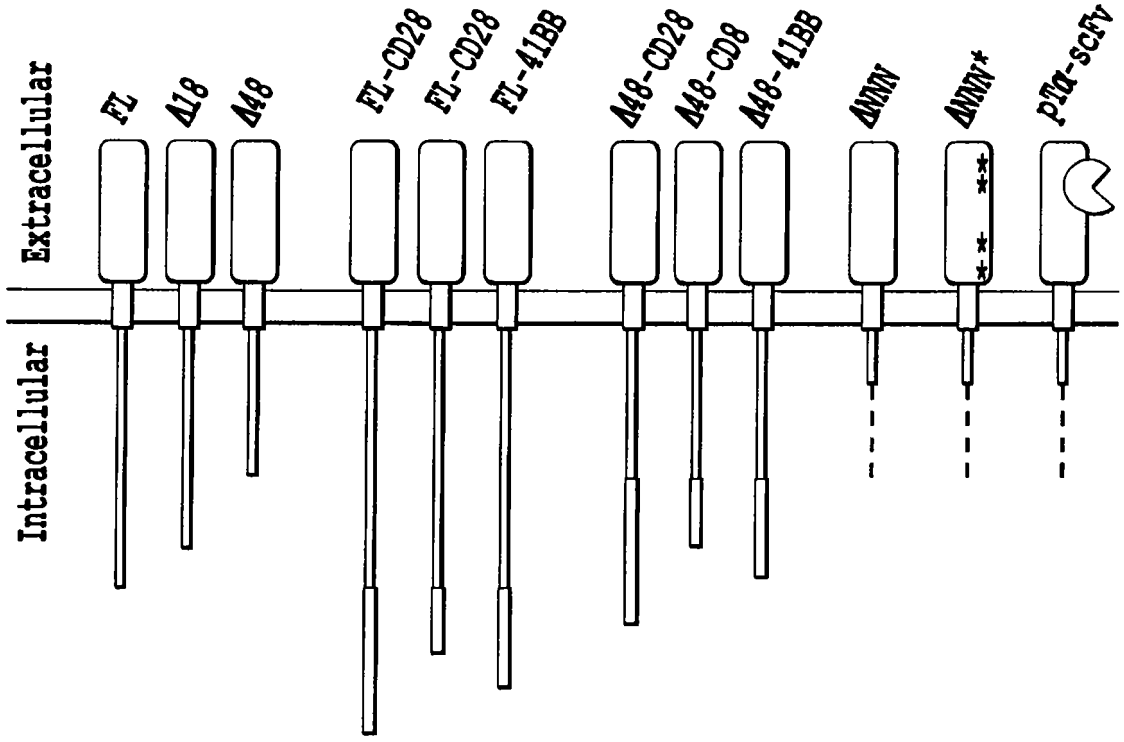

Extracellular
Intracellular
FL
Δ18
Δ48
FL-CD28
FL-CD28
FL-41BB
Δ48-CD28
Δ48-CD8
Δ48-41BB
ΔNNN
ΔNNN*
pTα-scFv

Fig. 11


ΔU3
R
U5
SFFVpro
BFP
2A
preTCRα
U3
R
U5

Fig. 13

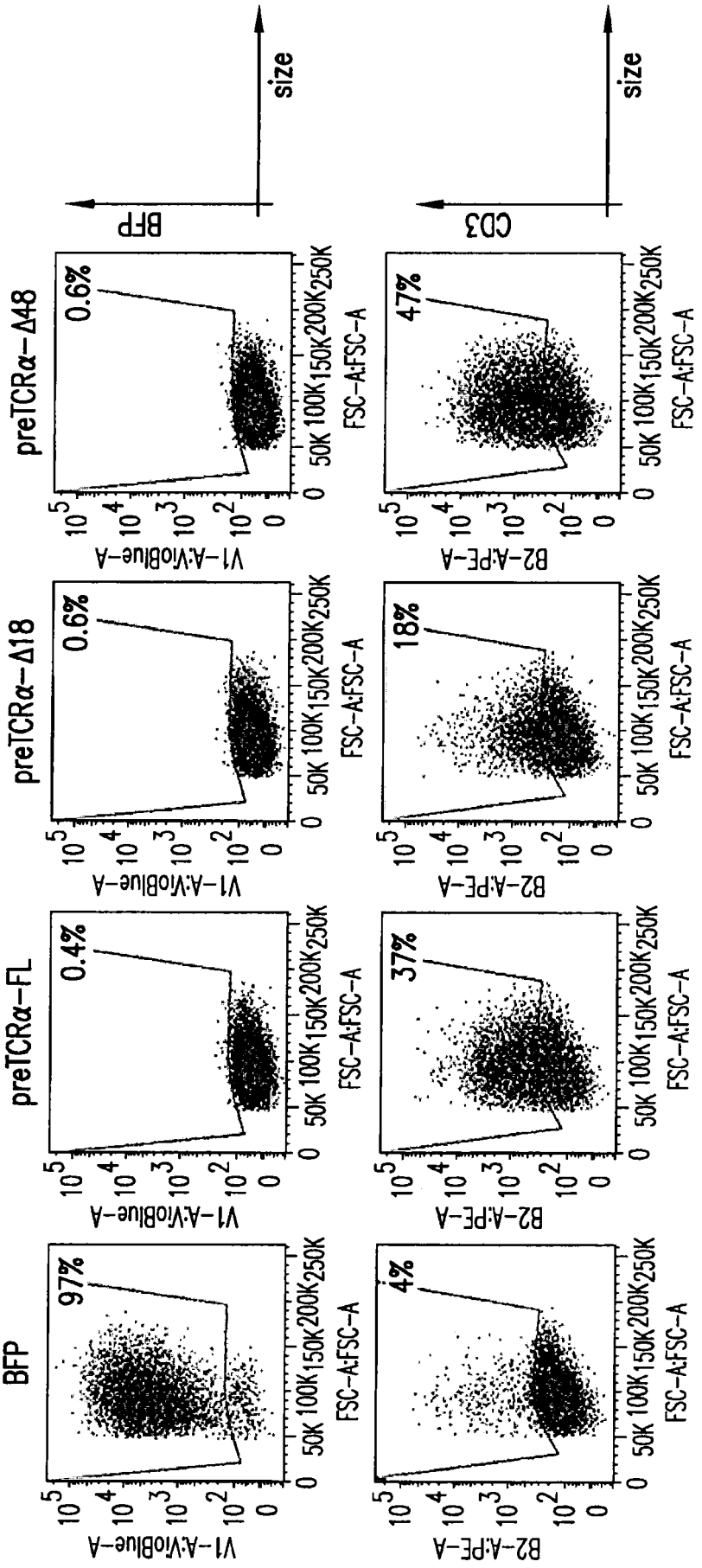

BFP
preTCRα–FL
preTCRα–Δ18
preTCRα–Δ48
97%
0.4%
0.6%
0.6%
4%
37%
18%
47%
V1–A:VioBlue–A
B2–A:PE–A
FSC–A:FSC–A
0
50K
100K
150K
200K
250K
size
CD3

Fig. 12

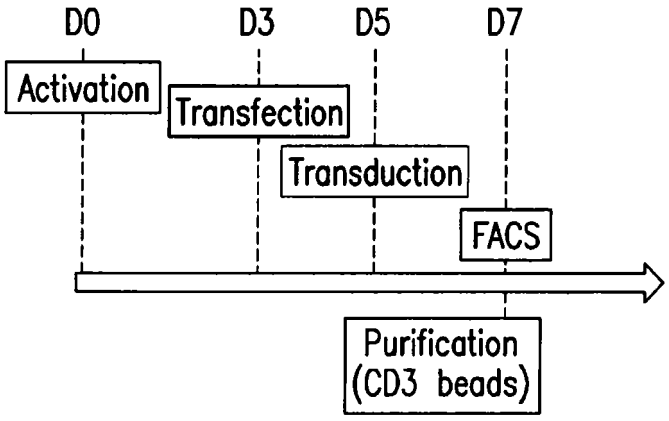

D0
D3
D5
D7
Activation
Transfection
Transduction
FACS
Purification
(CD3 beads)

Fig.14A

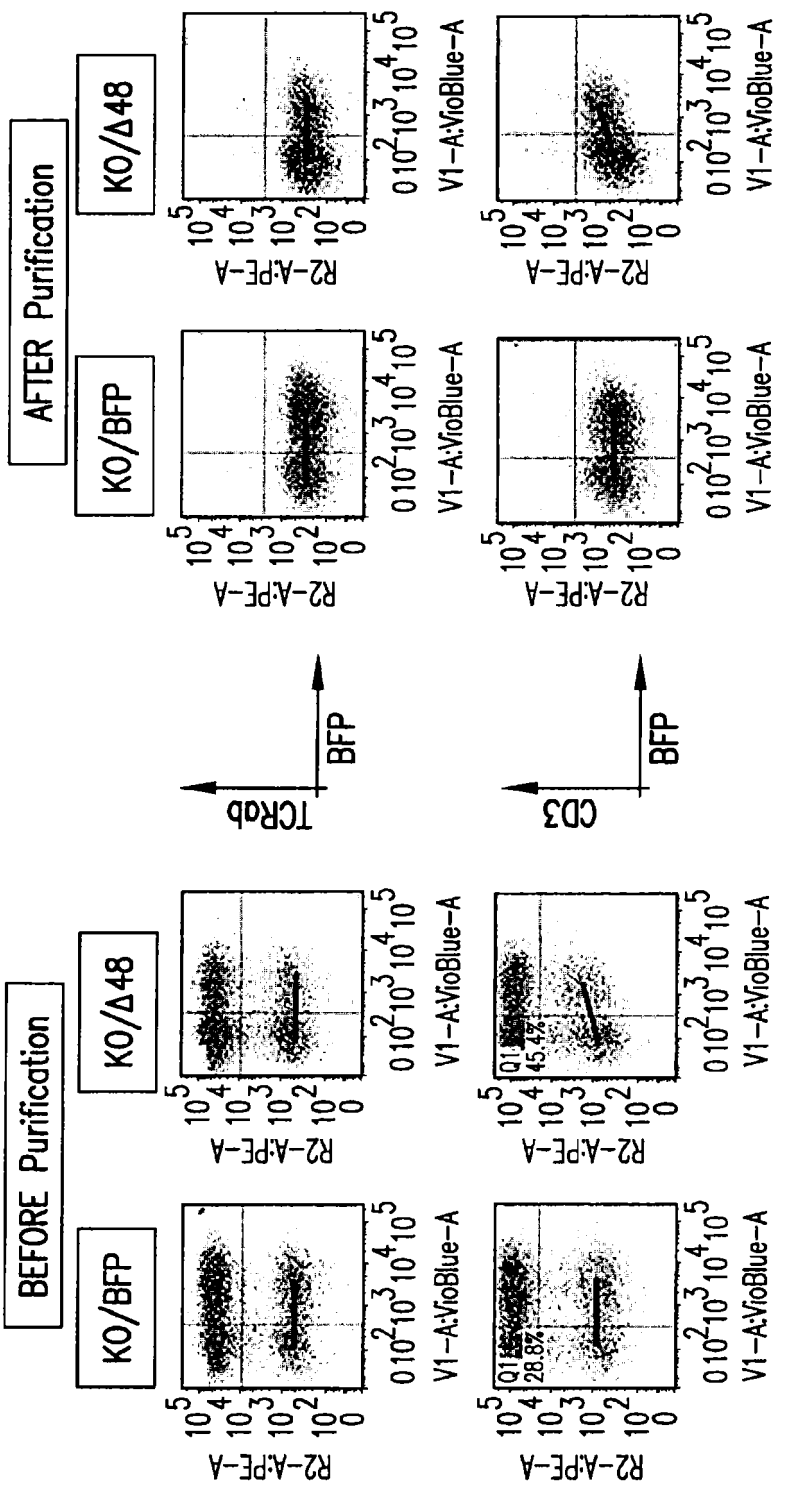

BEFORE Purification
AFTER Purification
KO/BFP
KO/Δ48
TCRαb
CD3
BFP
R2-A:PE-A
V1-A:VioBlue-A
28.8%
45.4%

Fig. 14B

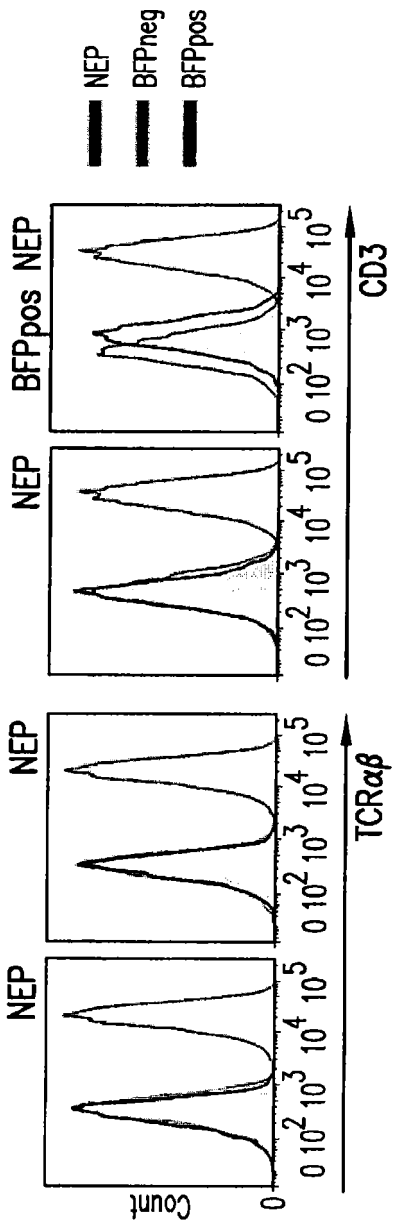

NEP
BFPneg
BFPpos
NEP
NEP
BFPpos NEP
Count
0
0 10^2 10^3 10^4 10^5
TCRαβ
CD3

Fig.14C

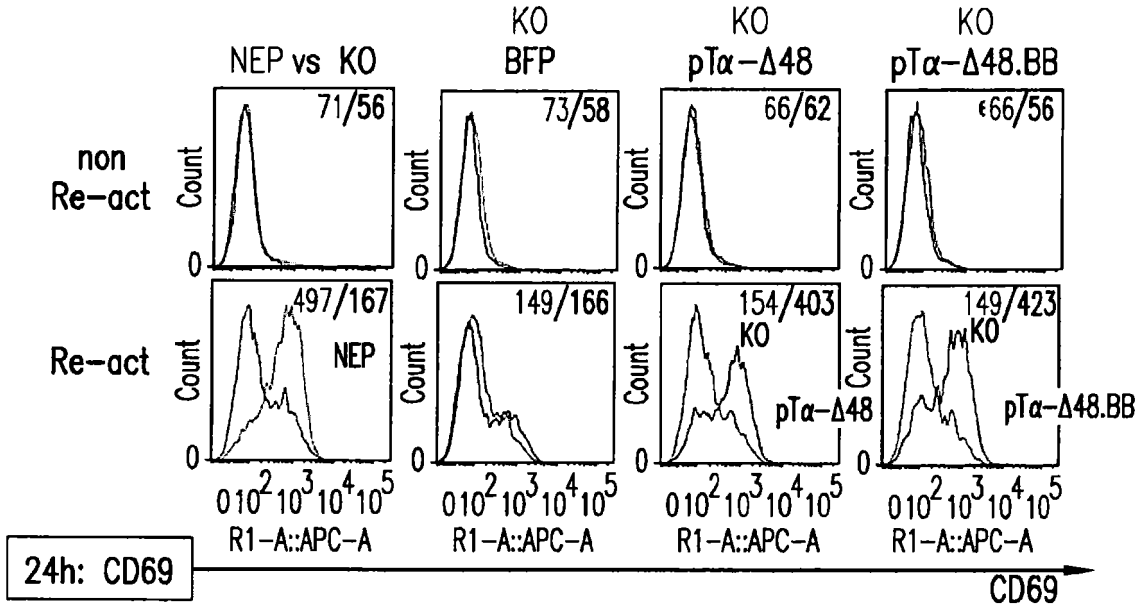


Fig.15A

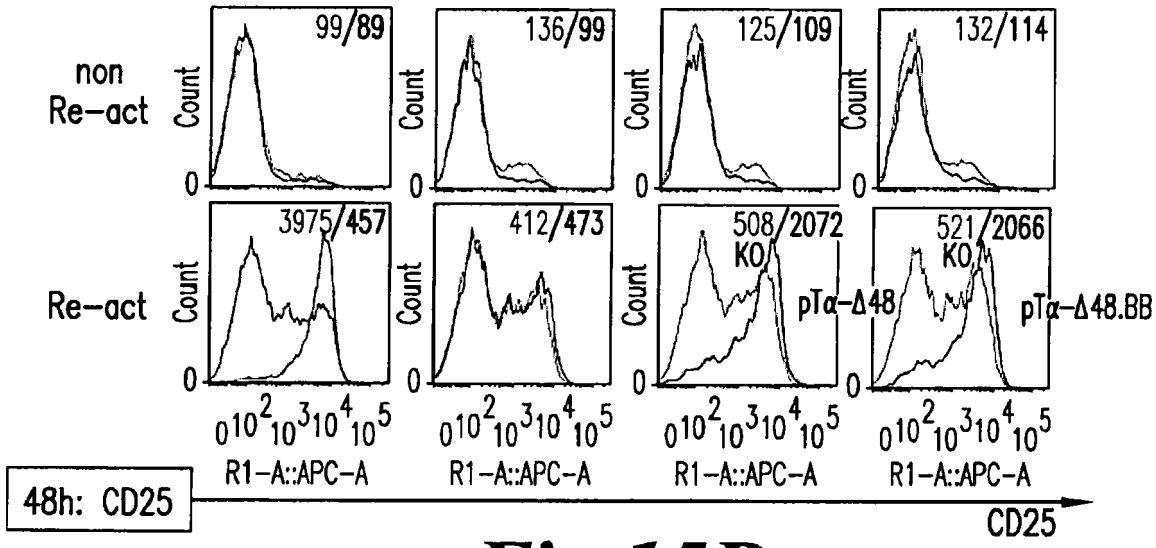


Fig.15B

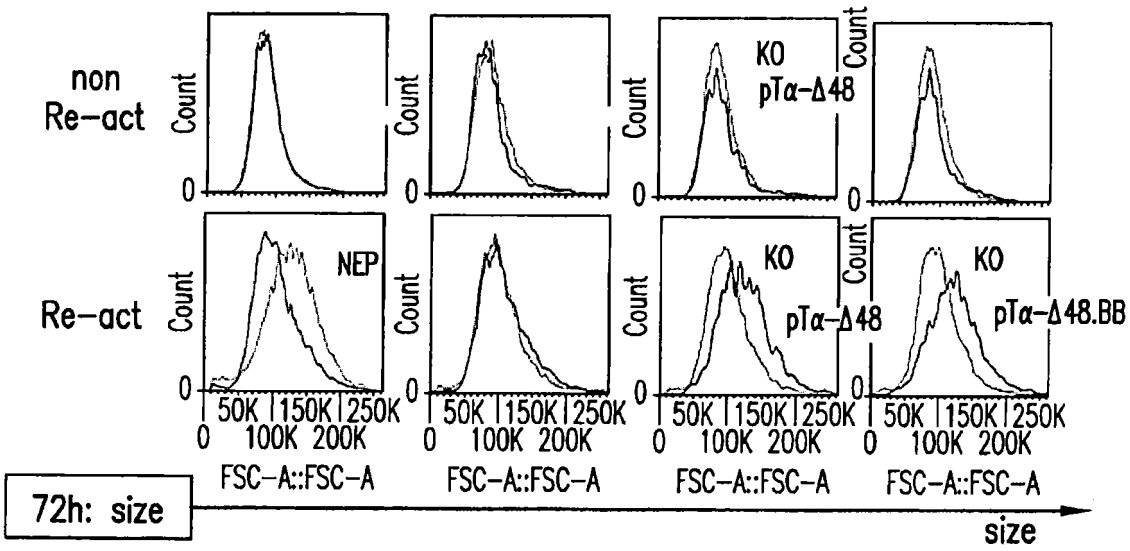

non
Re-act
Re-act
Count
0
NEP
KO
pTα-Δ48
KO
pTα-Δ48
KO
pTα-Δ48.BB
50K 150K 250K
0 100K 200K
FSC-A::FSC-A
72h: size
size

Fig.15C

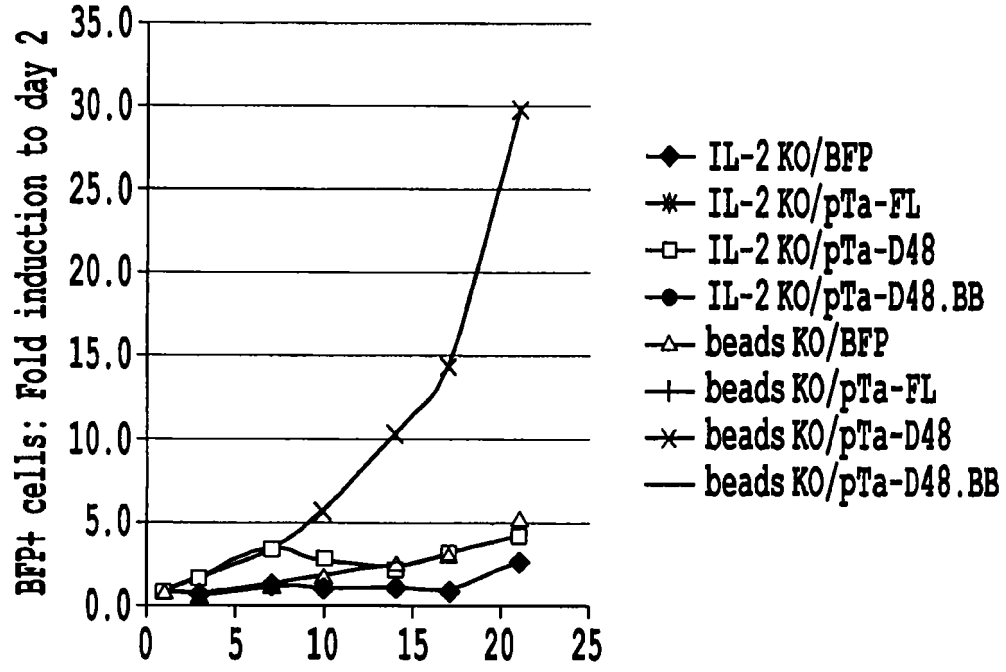


Fig. 16A

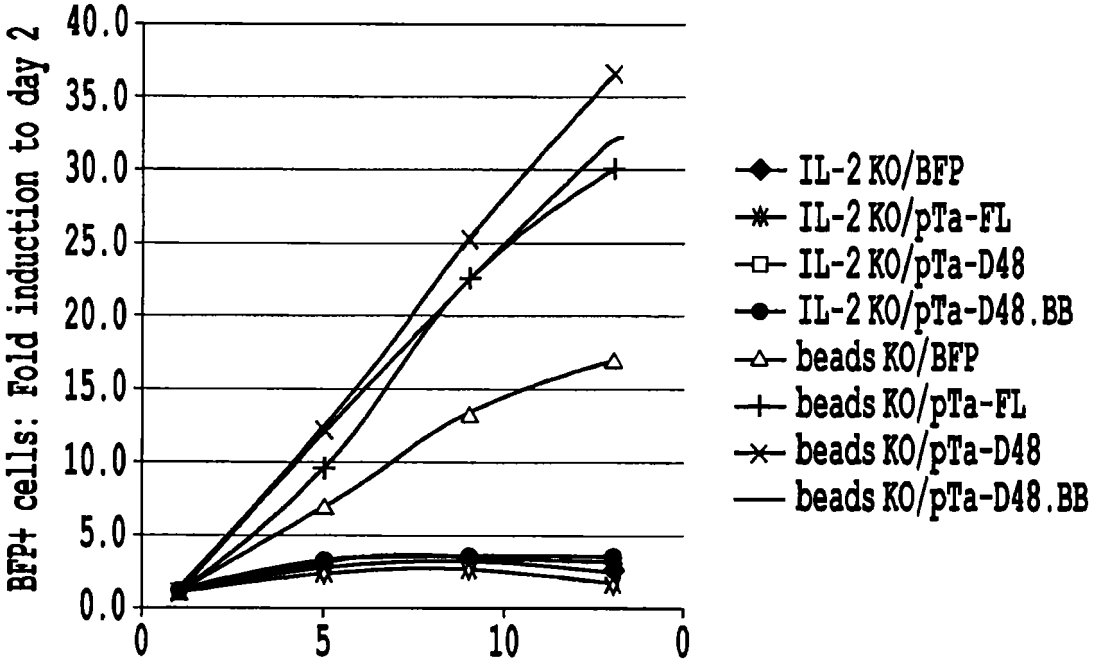


Fig. 16B

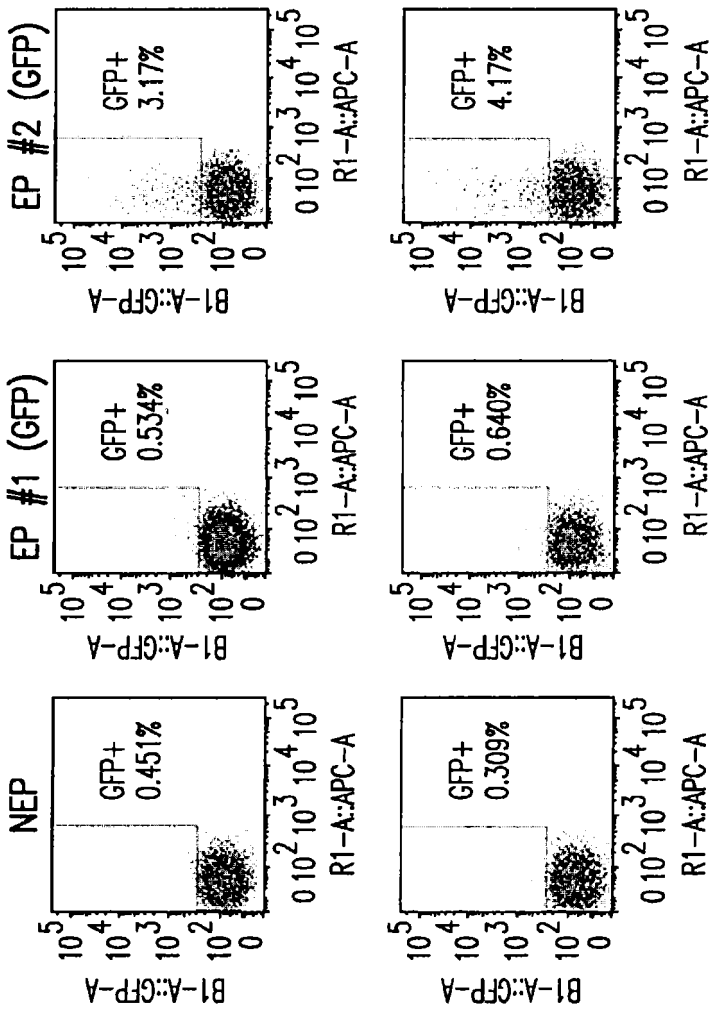

NEP
EP #1 (GFP)
EP #2 (GFP)
GFP+ 0.451%
GFP+ 0.534%
GFP+ 3.17%
GFP+ 0.309%
GFP+ 0.640%
GFP+ 4.17%
B1-A::GFP-A
R1-A::APC-A

Fig. 17

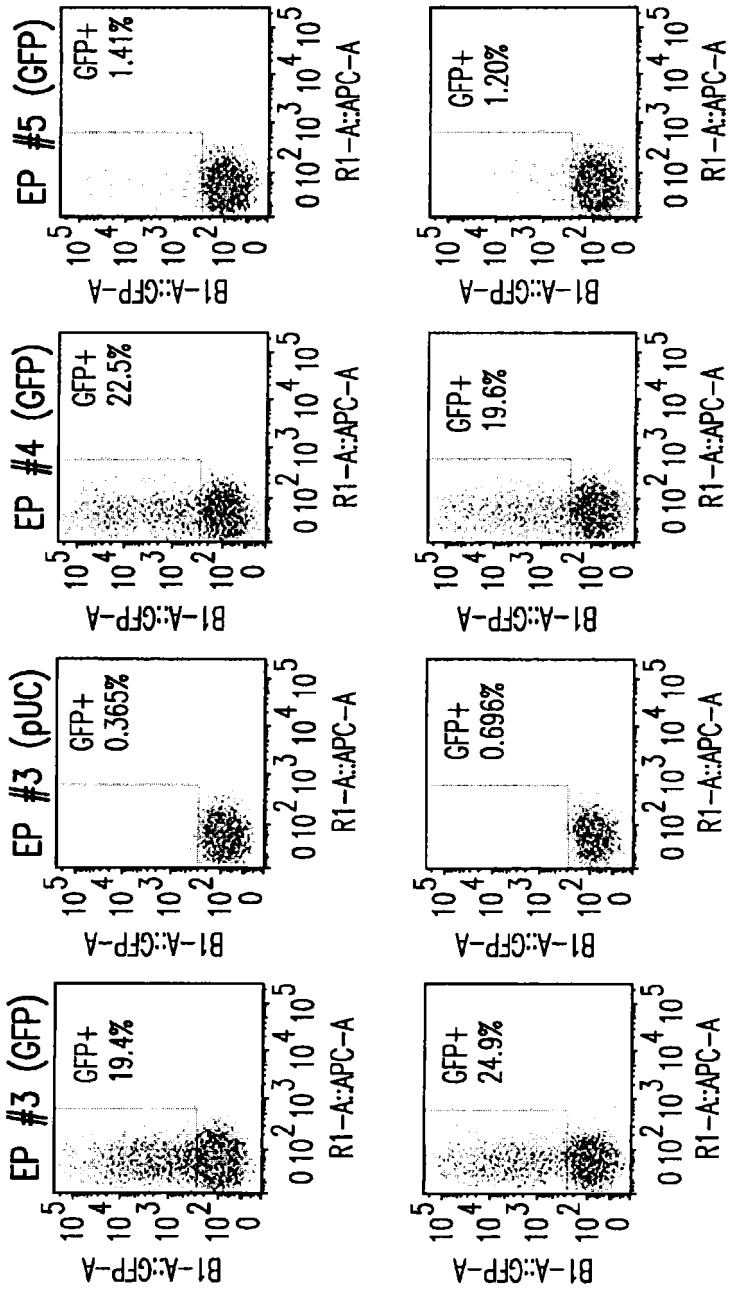

EP #3 (GFP)
GFP+ 19.4%
EP #3 (pUC)
GFP+ 0.365%
EP #4 (GFP)
GFP+ 22.5%
EP #5 (GFP)
GFP+ 1.41%
GFP+ 24.9%
GFP+ 0.696%
GFP+ 19.6%
GFP+ 1.20%
B1-A::GFP-A
R1-A::APC-A

Fig.17-1

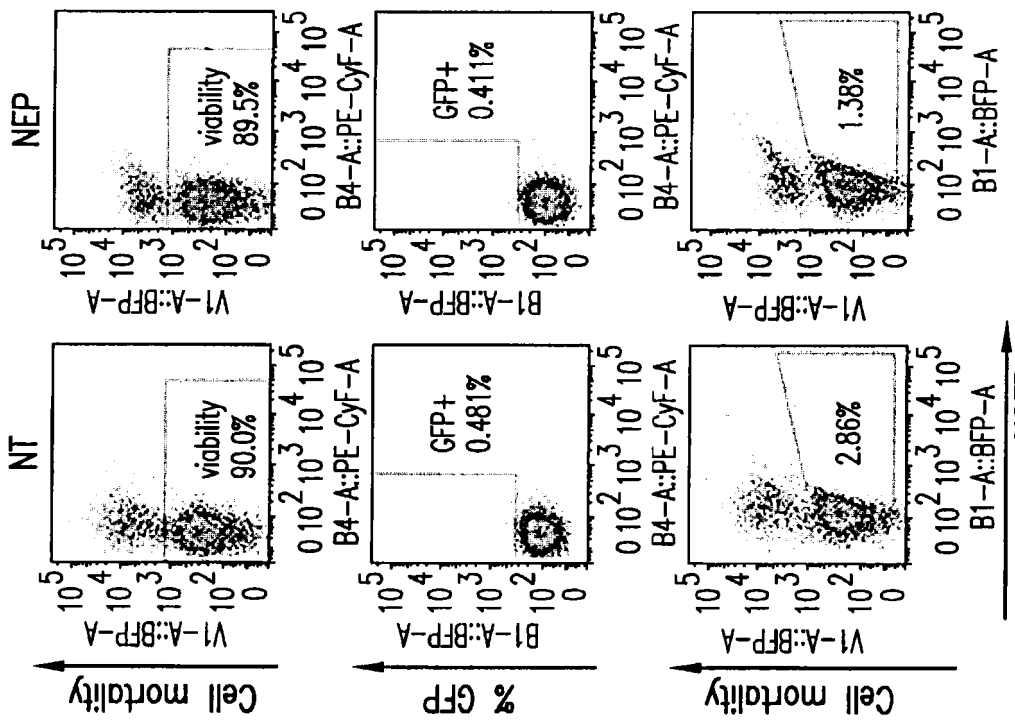

NT
NEP
viability 90.0%
viability 89.5%
GFP+ 0.481%
GFP+ 0.411%
2.86%
1.38%
Cell mortality
% GFP
V1-A::BFP-A
B4-A::PE-Cy5-A
B1-A::BFP-A
%GFP

Fig. 18

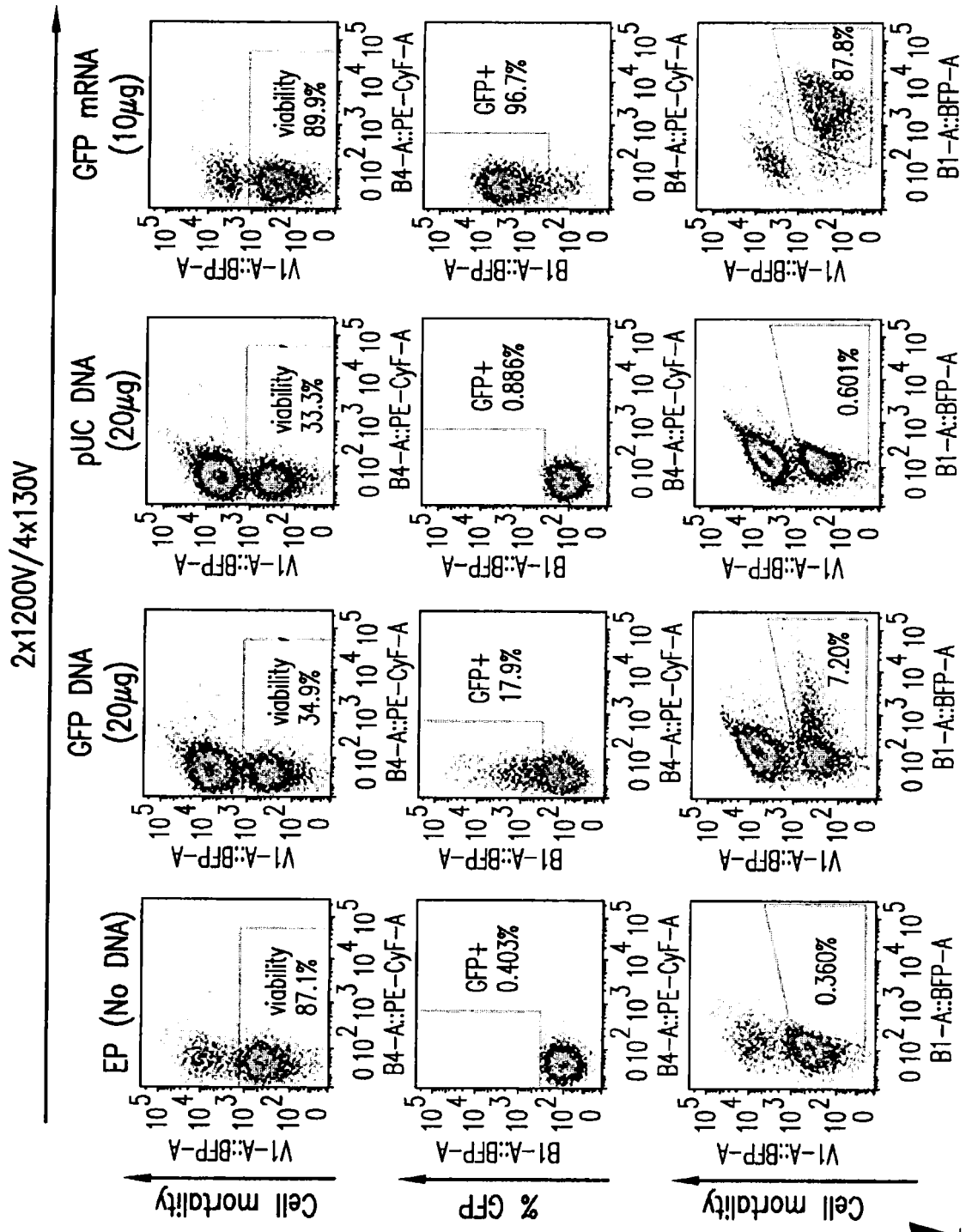
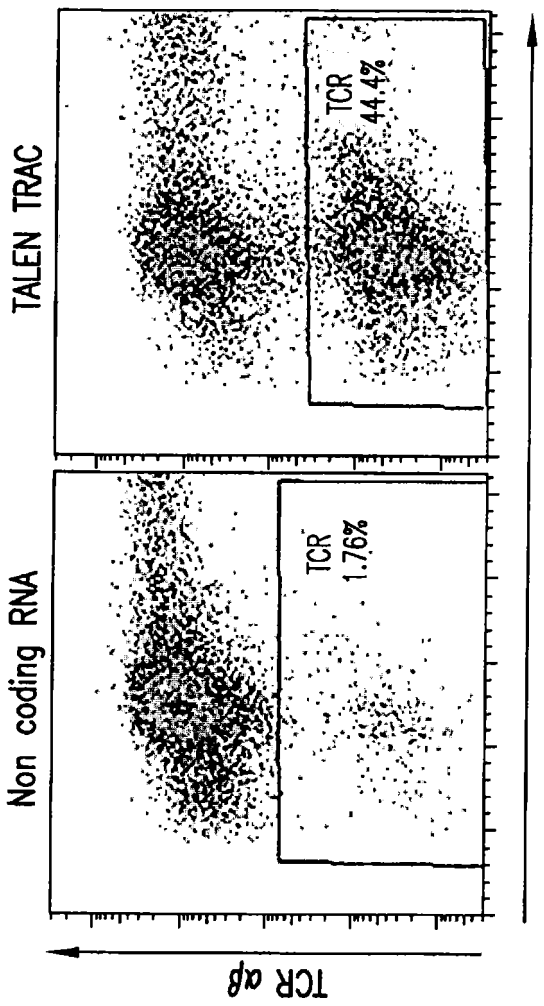

2x1200V/4x130V
EP (No DNA)
GFP DNA (20μg)
pUC DNA (20μg)
GFP mRNA (10μg)
Cell mortality
% GFP
Cell mortality
viability 87.1%
viability 34.9%
viability 33.3%
viability 89.9%
GFP+ 0.403%
GFP+ 17.9%
GFP+ 0.886%
GFP+ 96.7%
0.360%
7.20%
0.601%
87.8%
V1-A::BFP-A
B1-A::BFP-A
B4-A::PE-CyF-A
B1-A::BFP-A

Fig. 18-1



WildType seq CATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTG

high-throughput sequencing

CATGTCCTAACCCTGATCCTCTTGTCCCACAGATAT----------CCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTG
CATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCAGA----TATCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTG
CATGTCCTAACCCT----------GATCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTG
CATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCAGA----TATCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTG
CATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCAGAACCCTG----------ACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTG
CATGTCCTAACCCTGATCCTCTTGTCCCACAGATAT----------CCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTG
CATGTCCTAACCCTGATCCTCTTGTCCCAC----------GAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTG
CATGTCCTAACCCTGATCCTCTTGTCCCACAGATATCCAGAA----CCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTG
CATGTCCTAACCCTGATCCTCTTGTCCCACAG----------ACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTG

Fig. 19

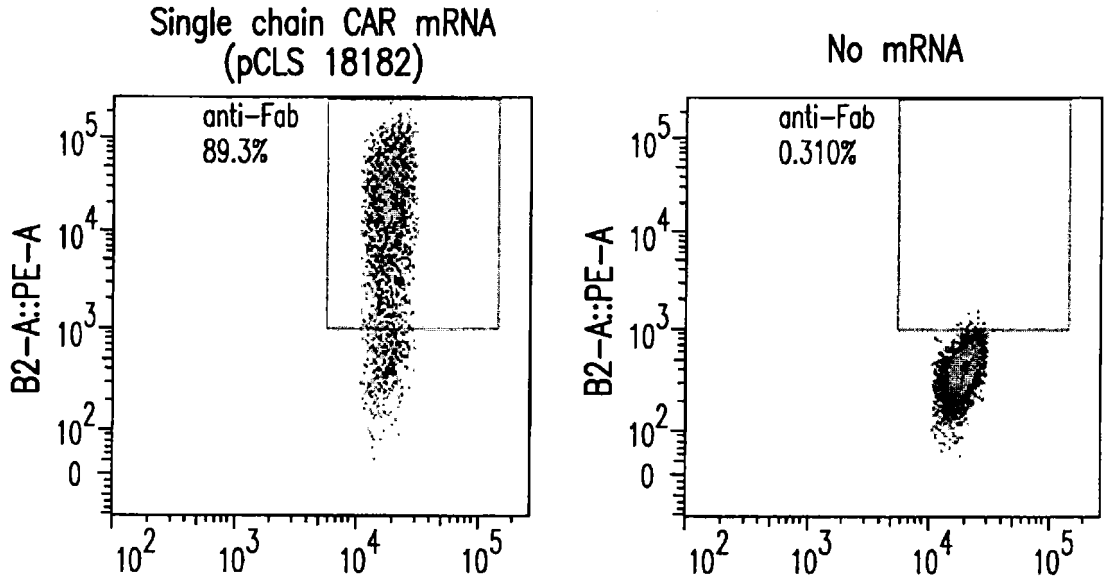


Fig.20A

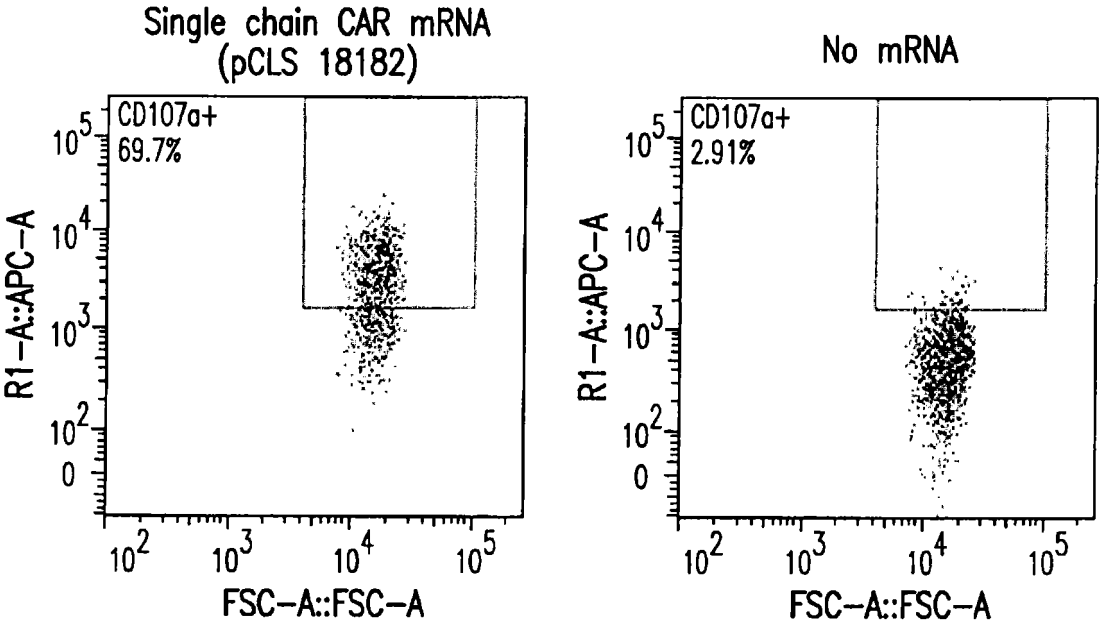


Fig.20B

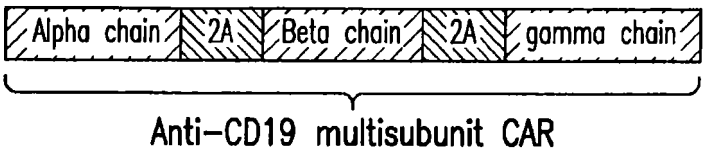


Fig.21A

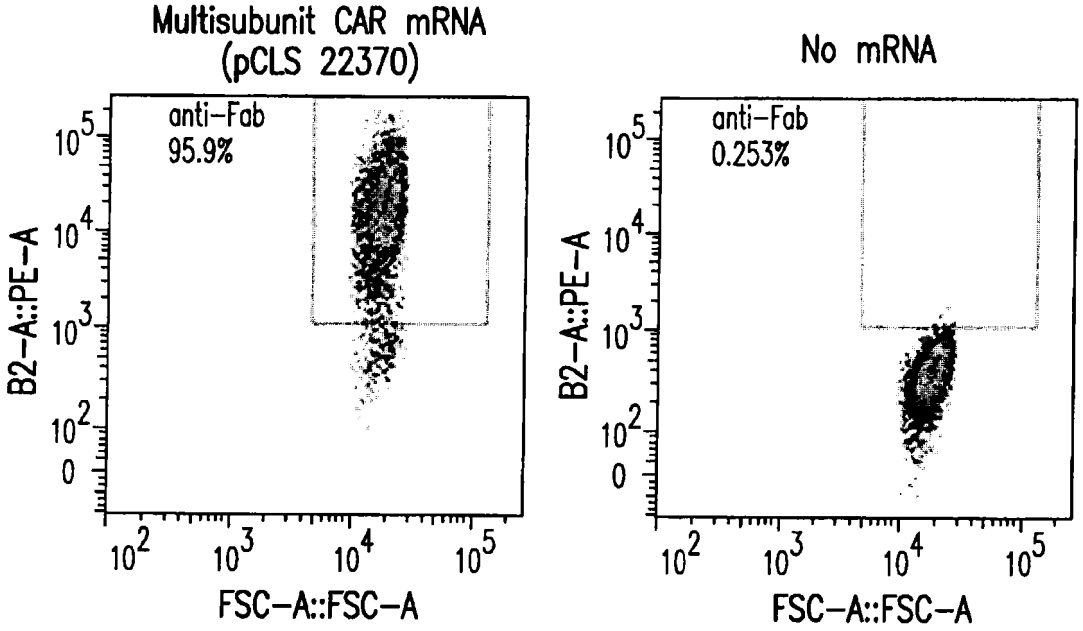


Fig.21B

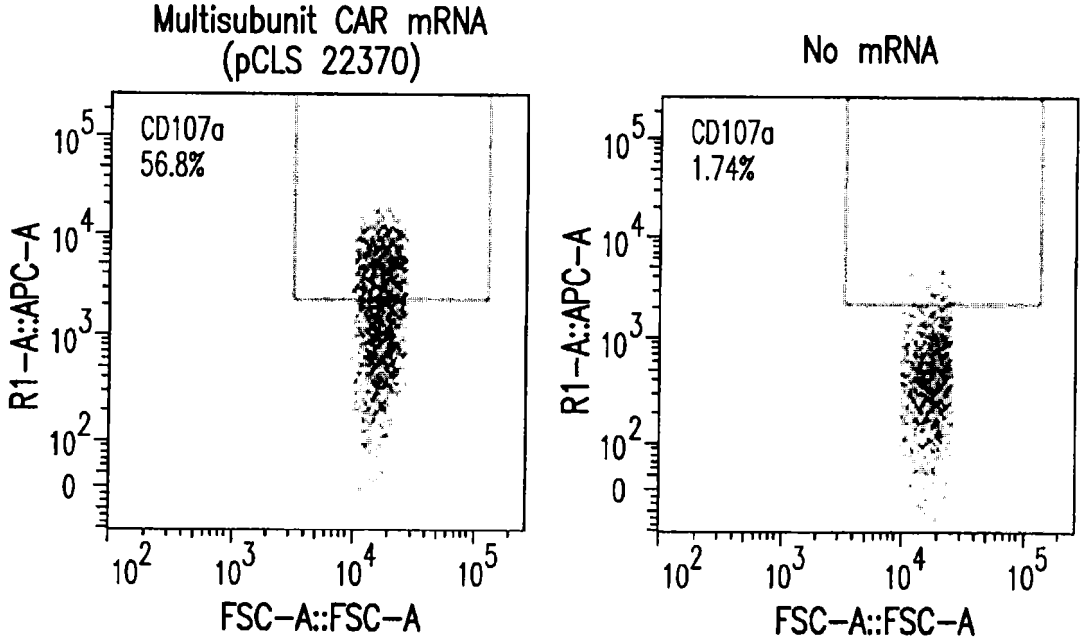


Fig.21C