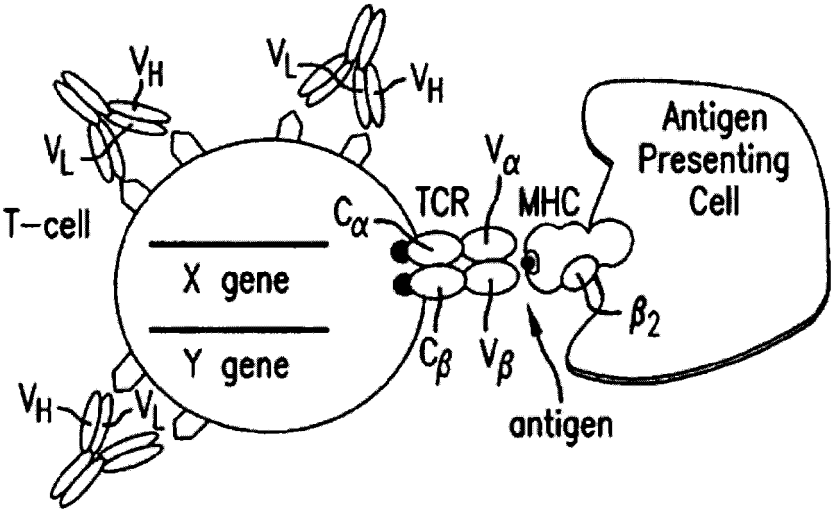


Figure 1

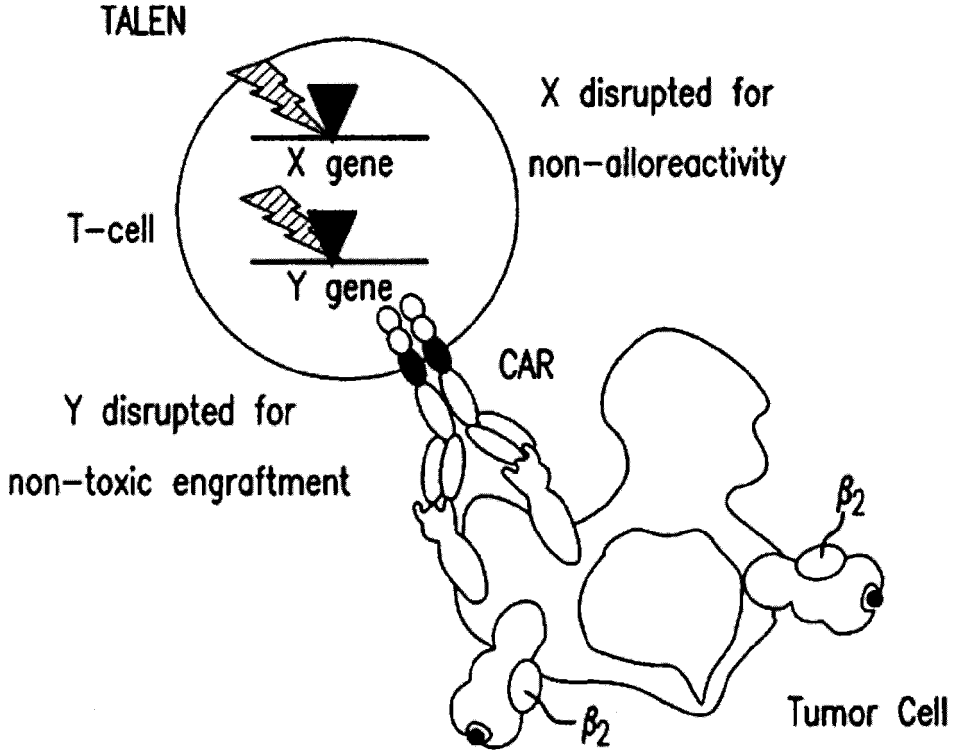


Figure 2

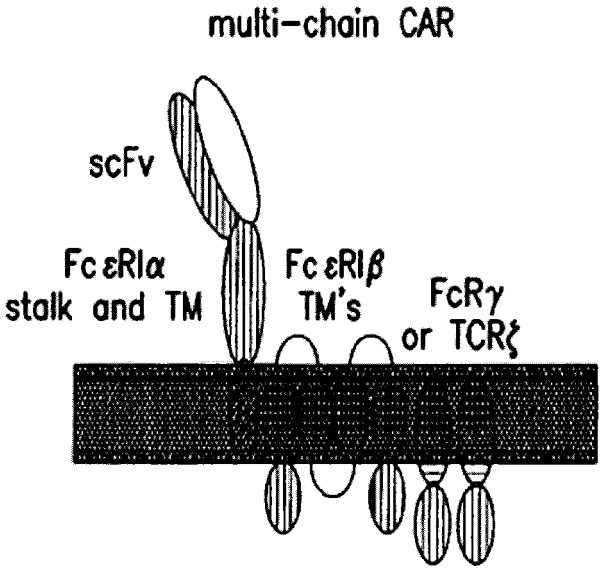

multi-chain CAR
scFv
FcεRIα
stalk and TM
FcεRIβ
TM's
FcRγ
or TCRζ
activating
signaling
domains
-beta chain allows all
signaling domains to be
in natural juxtamembrane
position
-beta chain already positioned
for interactions of signals
from FcRg or TCRzeta

Figure 3

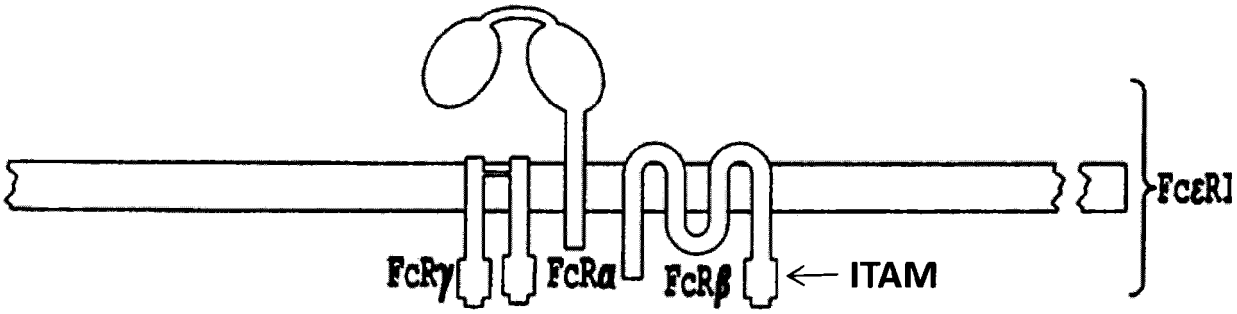


Figure 4A

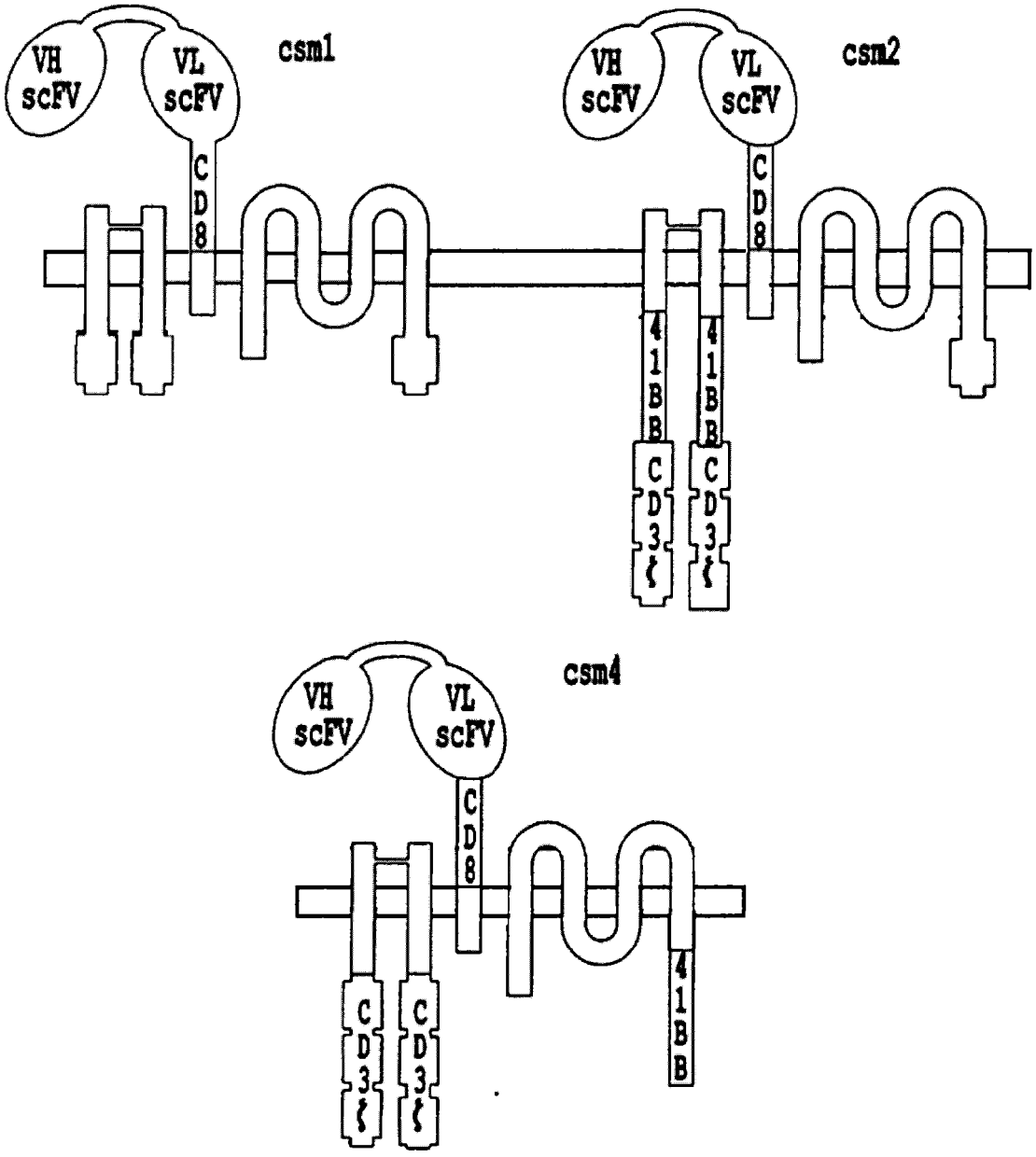


Figure 4B

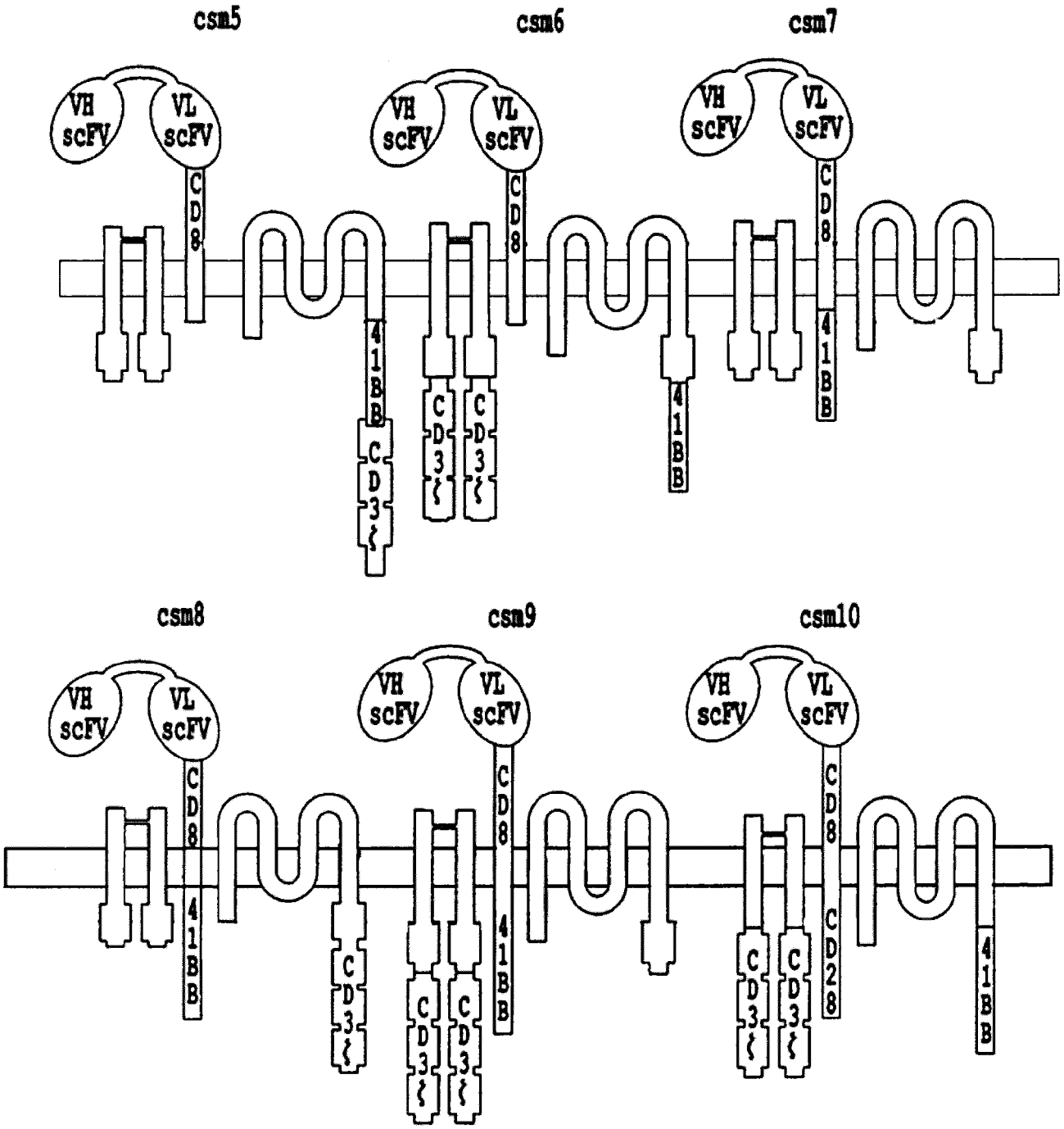
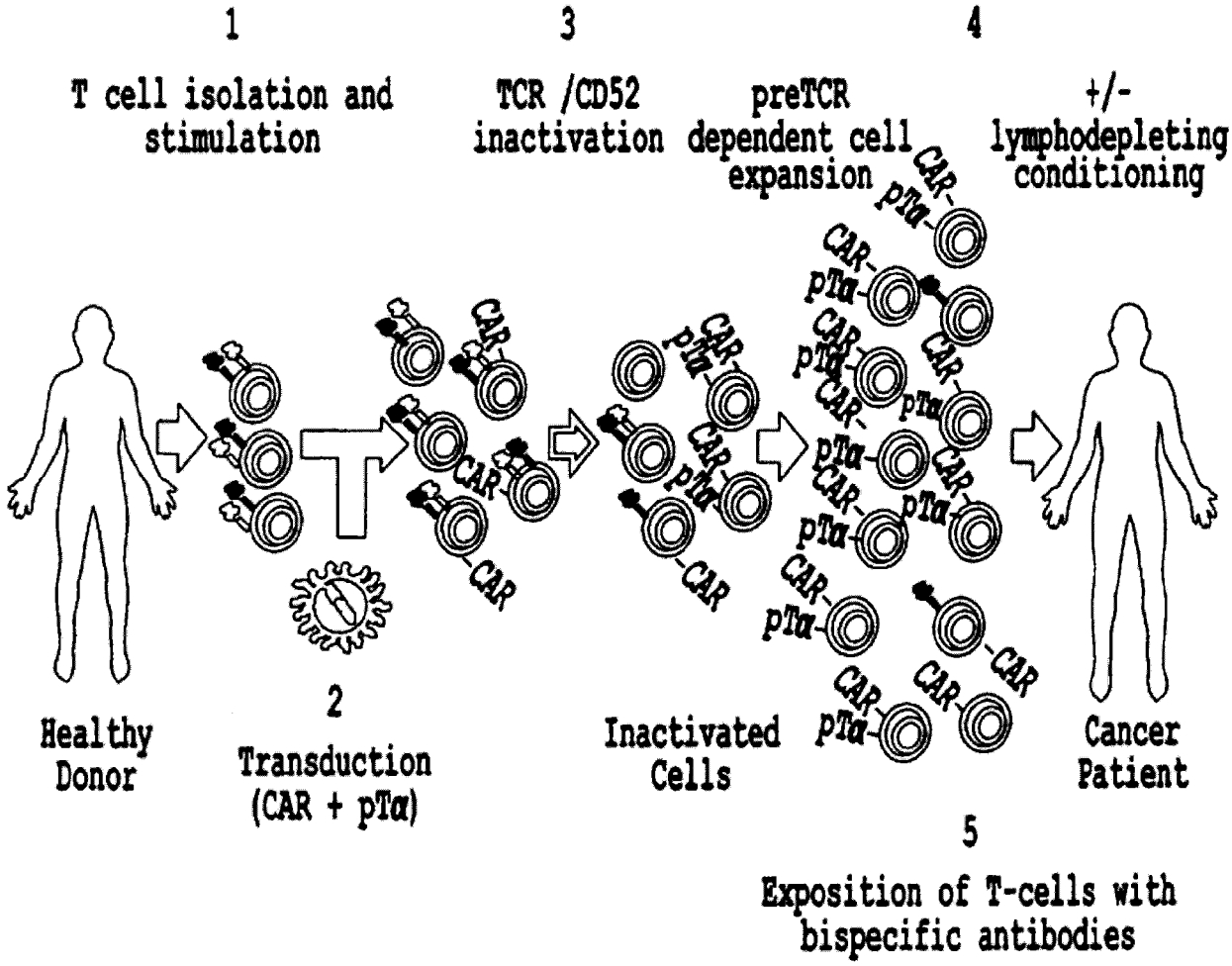

csm5
csm6
csm7
csm8
csm9
csm10
VH scFV
VL scFV
CD8
4 1BB
CD3ζ
CD28

Figure 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

Figure 5

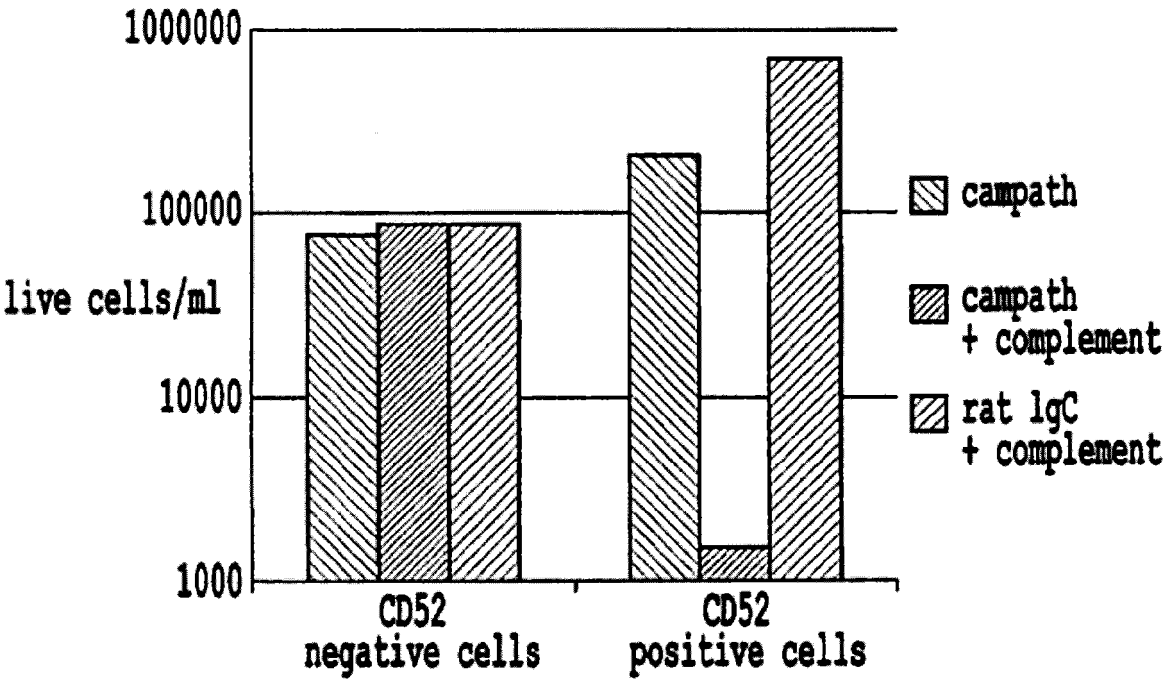

1000000
100000
10000
1000
live cells/ml
CD52
negative cells
CD52
positive cells
campath
campath
+ complement
rat IgC
+ complement

Figure 6

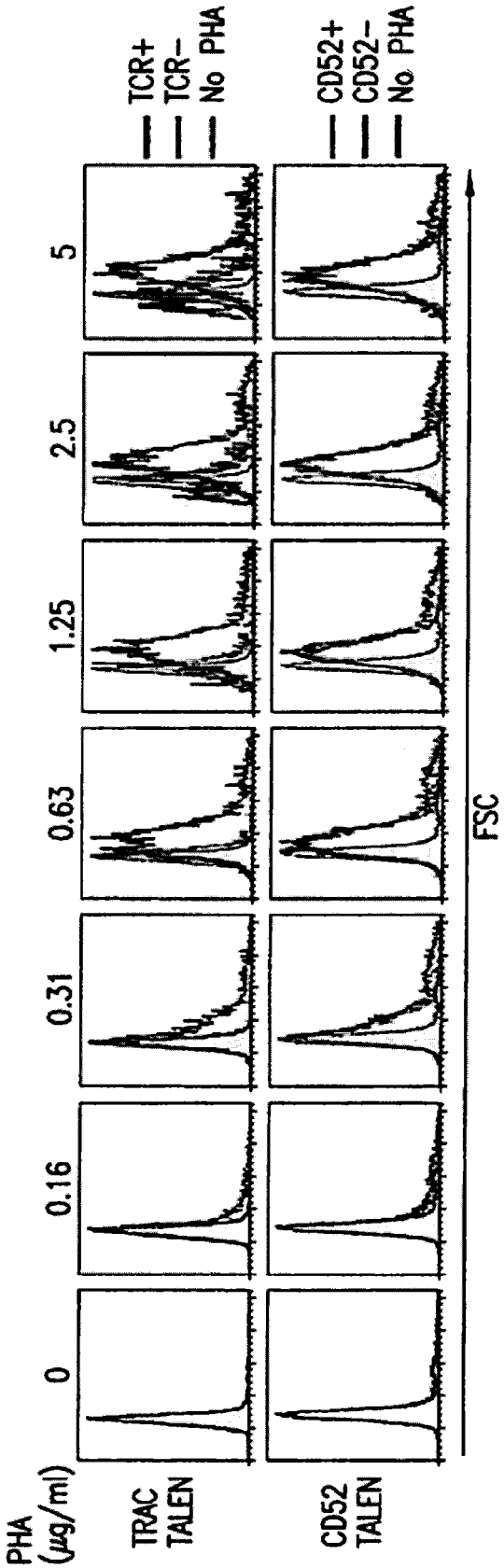

PHA (μg/ml)
0
0.16
0.31
0.63
1.25
2.5
5
TRAC TALEN
CD52 TALEN
TCR+
TCR−
No PHA
CD52+
CD52−
No PHA
FSC

Figure 7

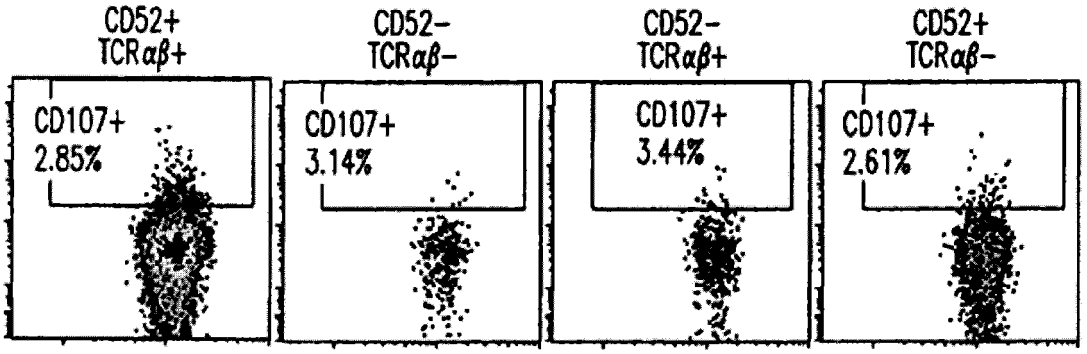


Figure 8A

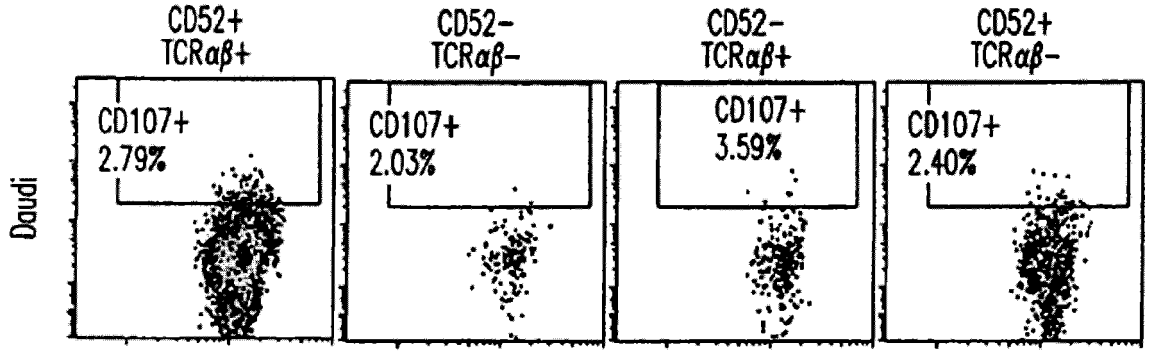


Figure 8B

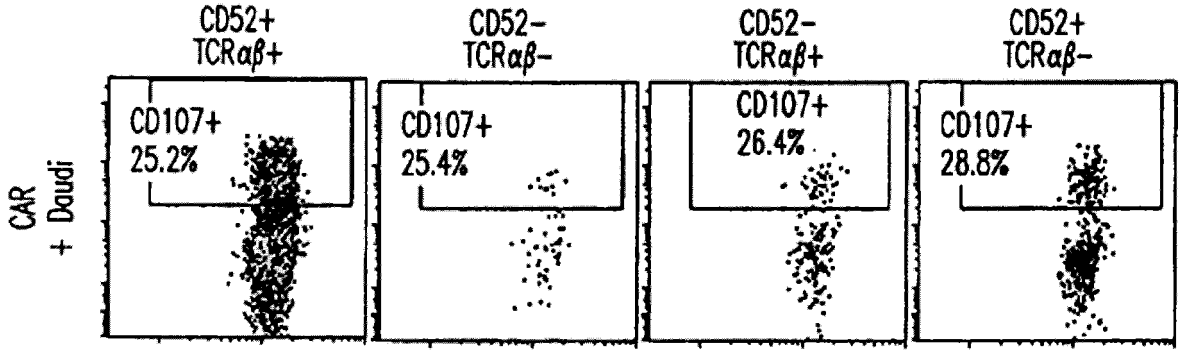


Figure 8C

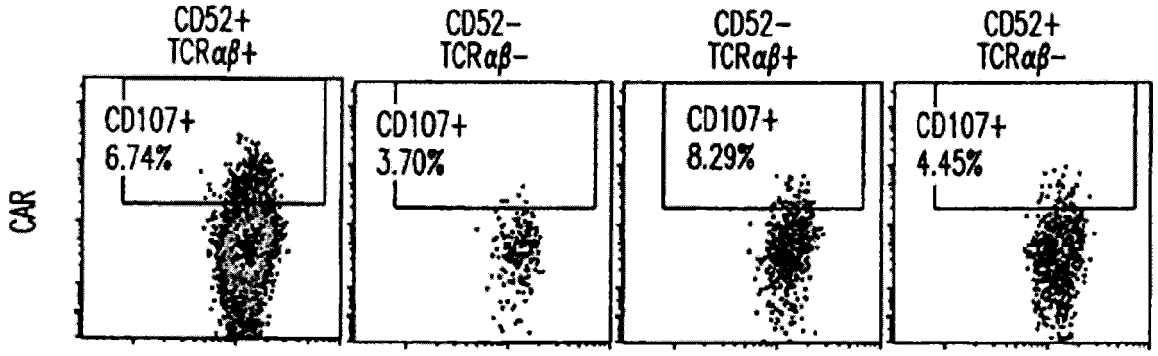


Figure 8D

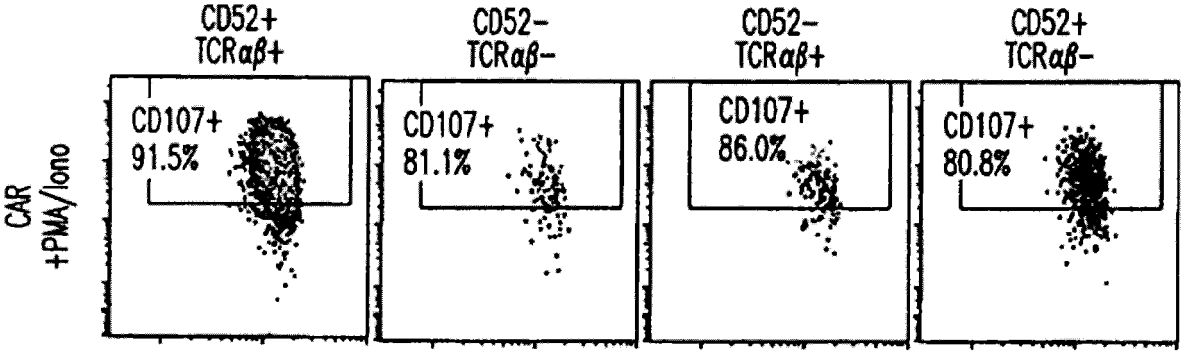


Figure 8E

	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

Figure 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

Figure 9B

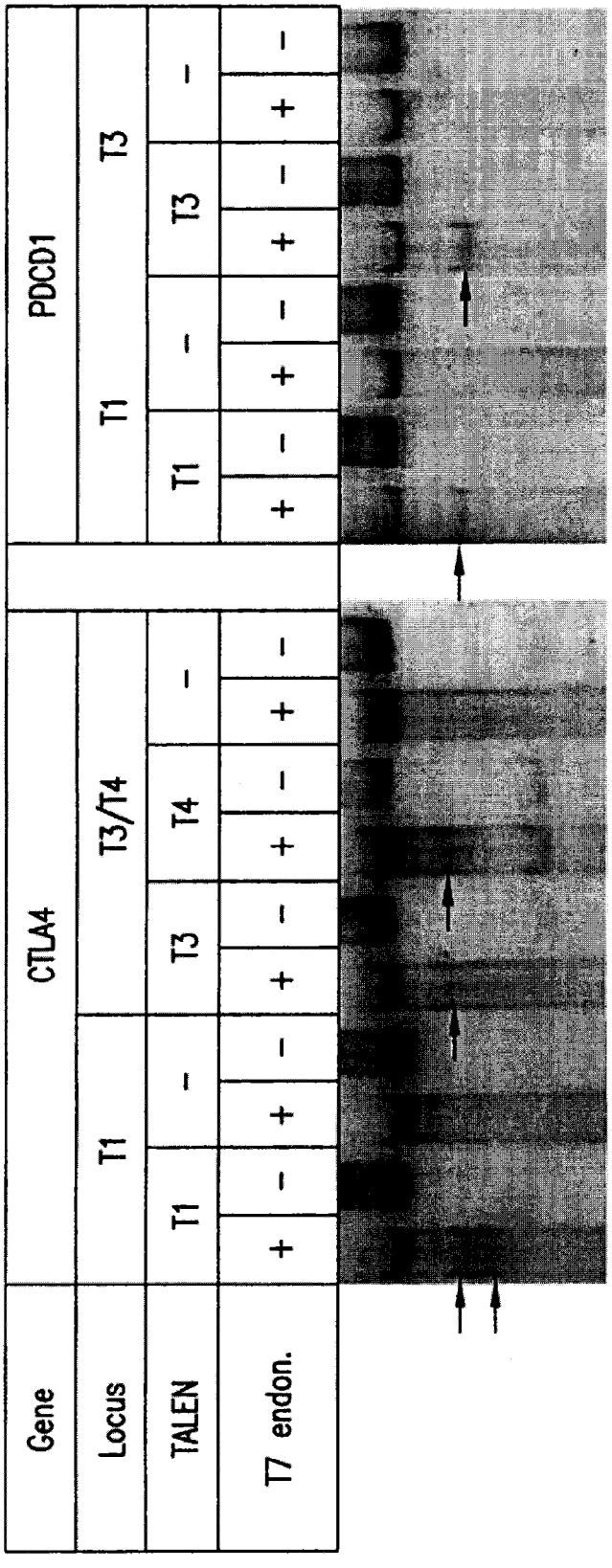

Gene
Locus
TALEN
T7 endon.
CTLA4
T1
T1
T3/T4
T1
T3
T4
T3
PDCD1
T1
T1
T3
T3
+
-

Figure 10

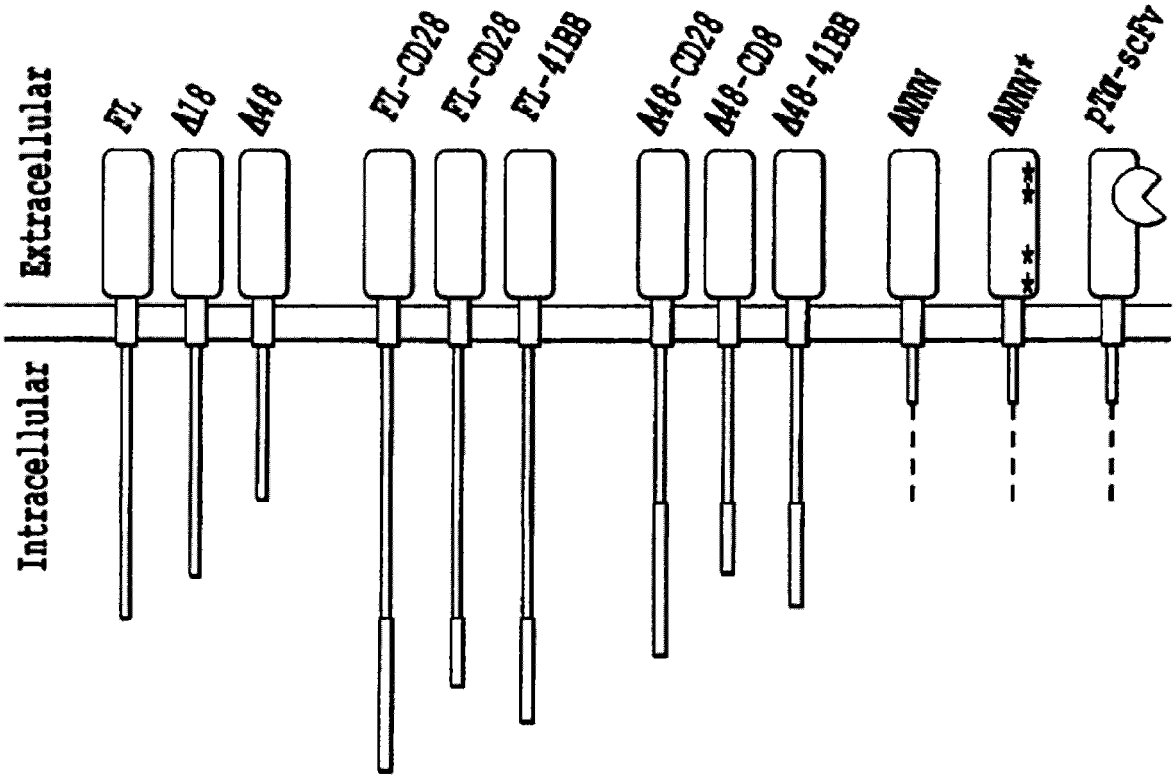


Figure 11



Figure 12

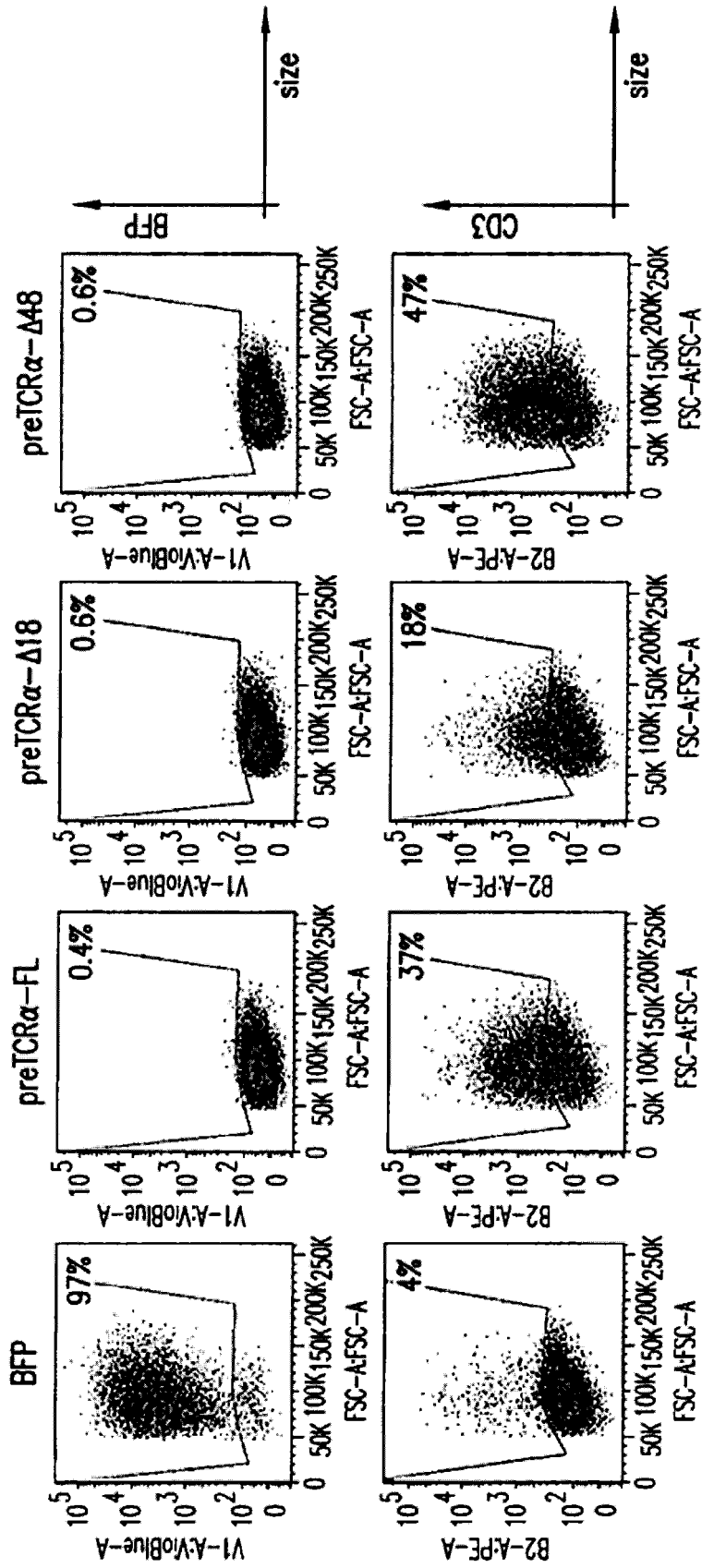

BFP
size
CD3
size
preTCRα−FL
preTCRα−Δ18
preTCRα−Δ48
97%
0.4%
0.6%
0.6%
4%
37%
18%
47%
V1-A:VioBlue-A
B2-A:PE-A
FSC-A:FSC-A
0
50K
100K
150K
200K
250K

Figure 13

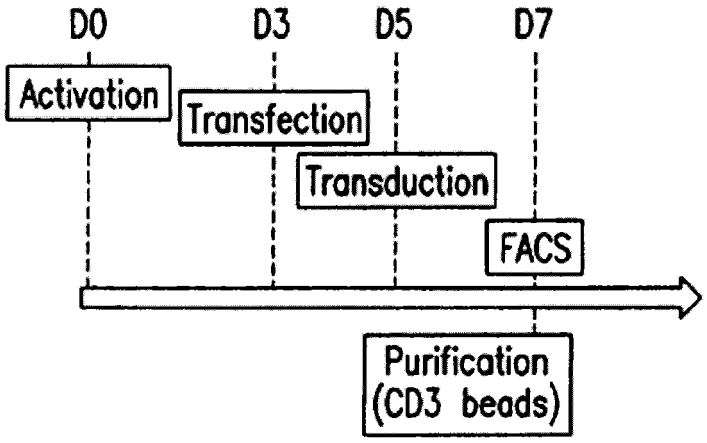

D0
D3
D5
D7
Activation
Transfection
Transduction
FACS
Purification
(CD3 beads)

Figure 14A

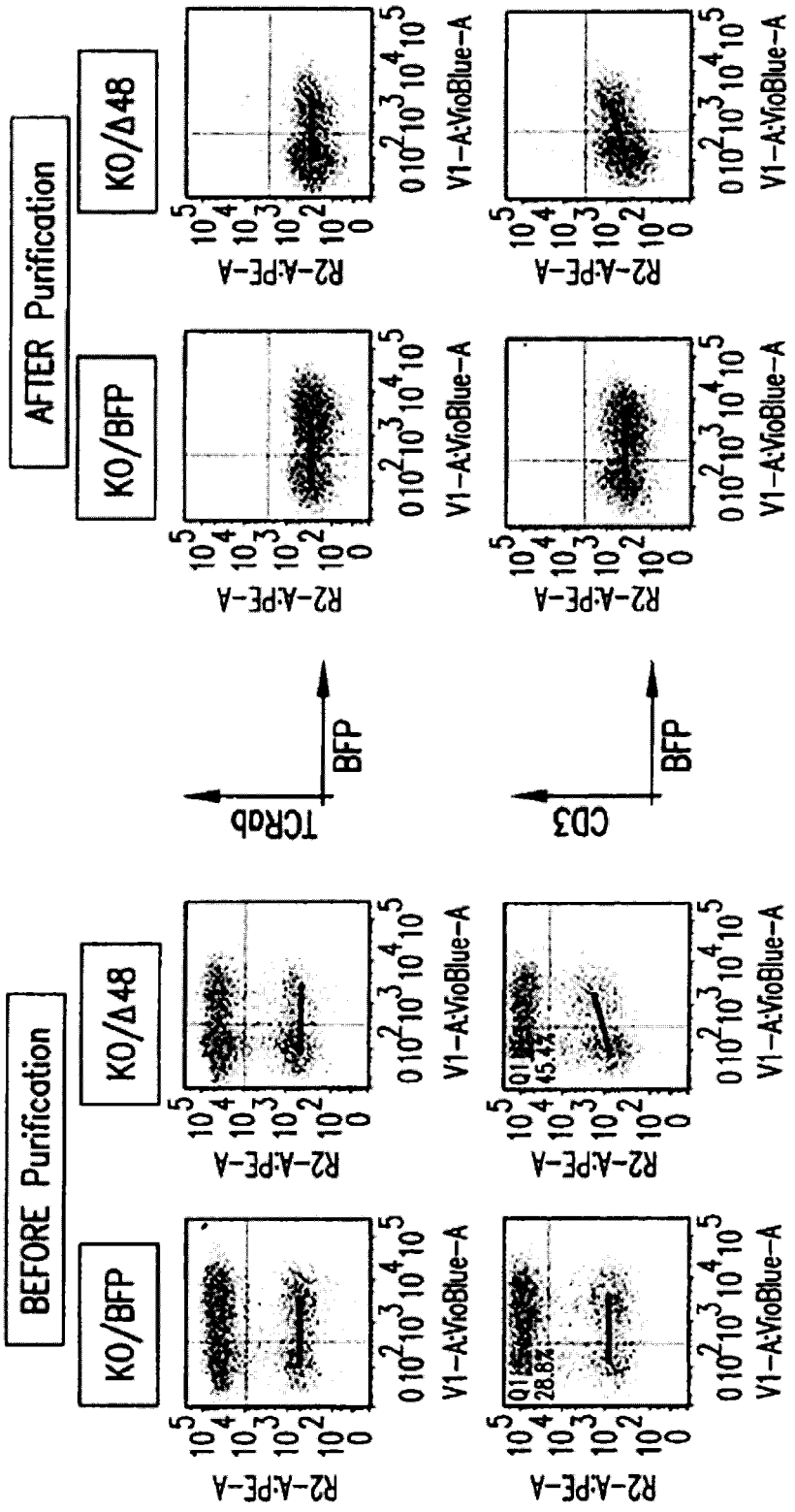

BEFORE Purification
AFTER Purification
KO/BFP
KO/Δ48
TCRαb
CD3
BFP
R2-A:PE-A
V1-A:VioBlue-A
28.8%
45.4%

Figure 14B

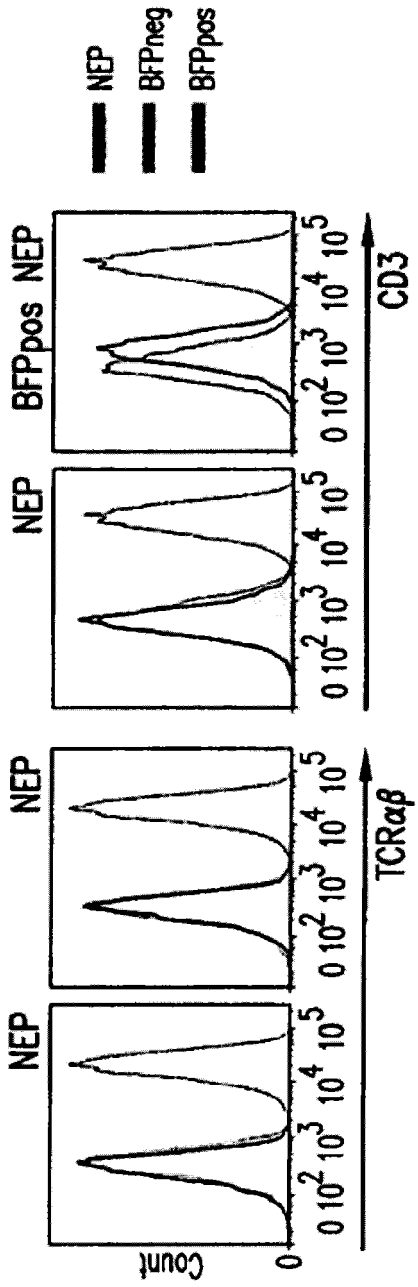

NEP
BFPneg
BFPpos
NEP
NEP
BFPpos
NEP
Count
0
0 10^2 10^3 10^4 10^5
TCRαβ
CD3

Figure 14C

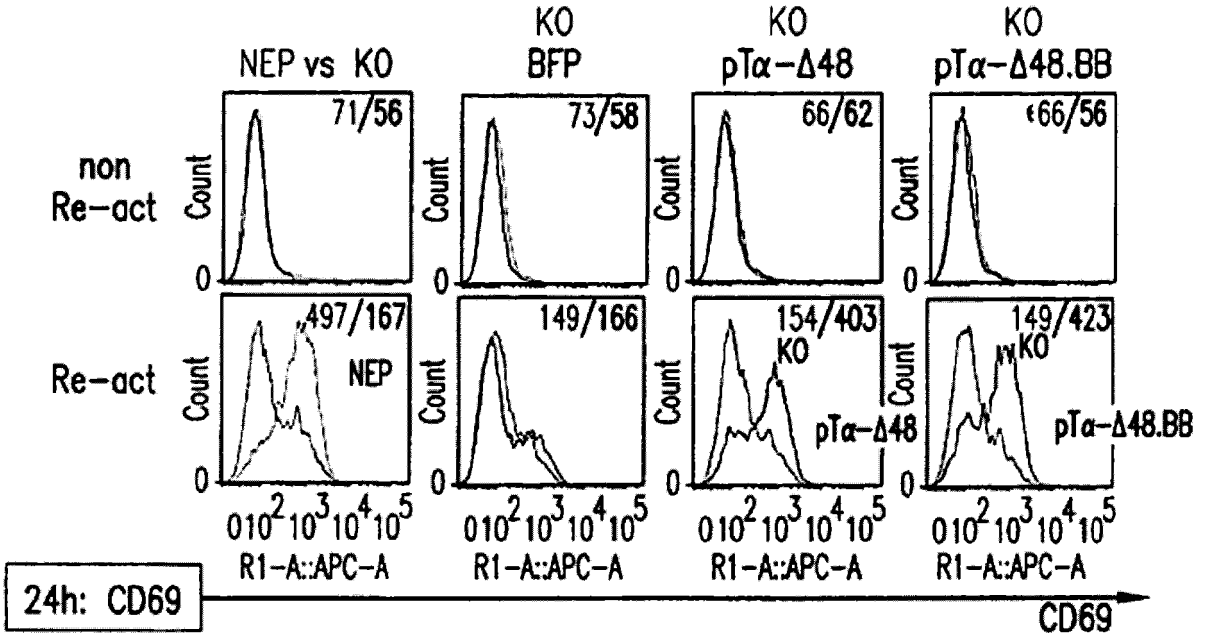


Figure 15A

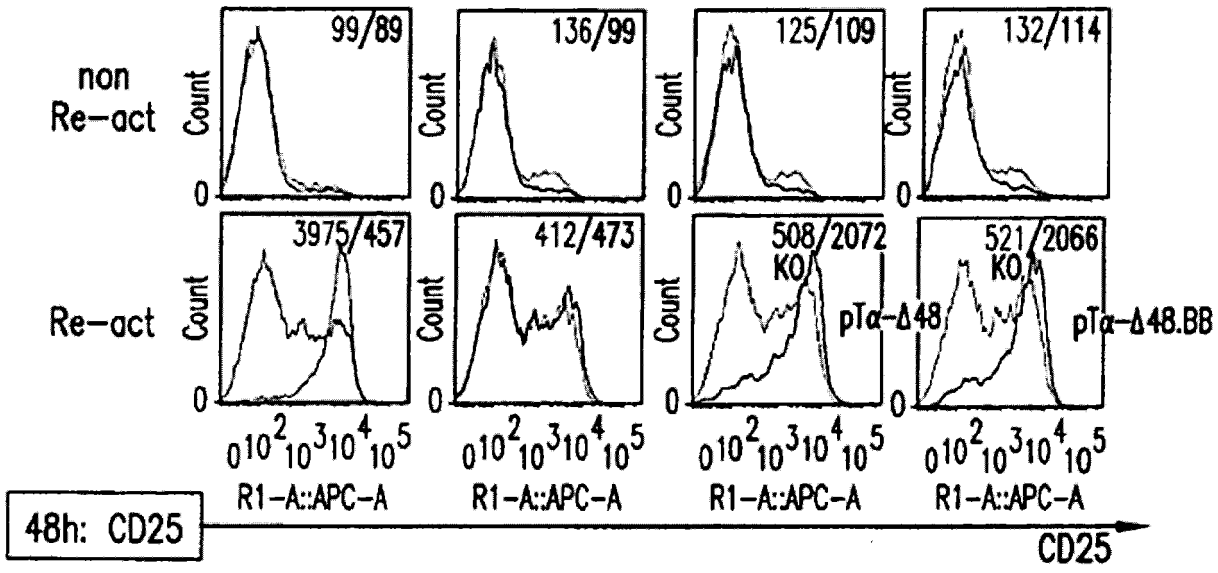


Figure 15B

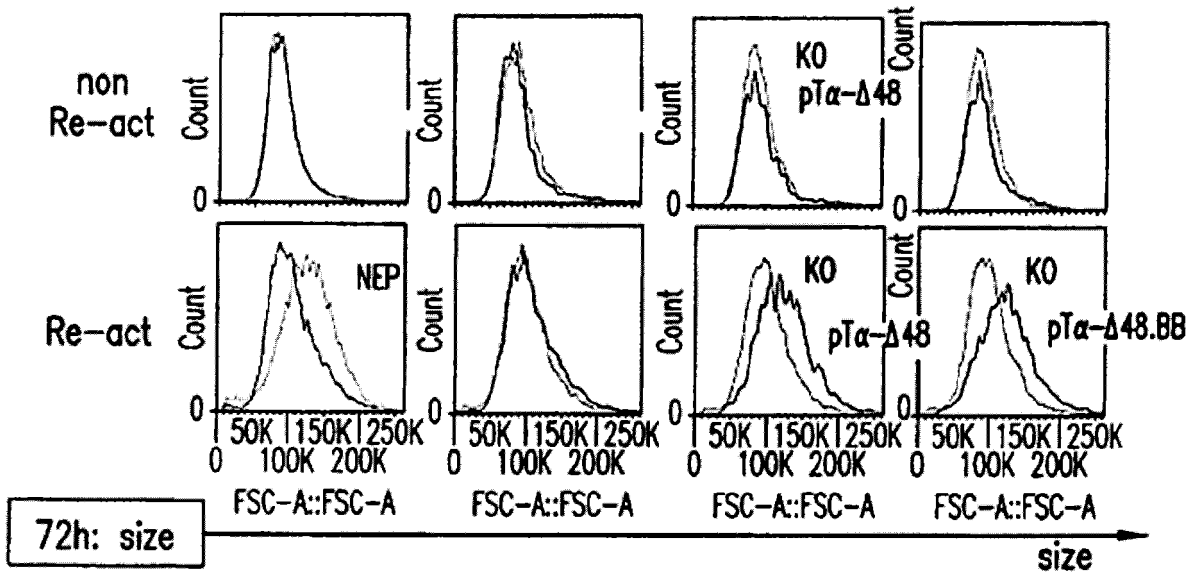

non
Re-act
Re-act
Count
0
NEP
KO
pTα-Δ48
KO
pTα-Δ48.BB
50K 150K 250K
0 100K 200K
FSC-A::FSC-A
72h: size
size

Figure 15C

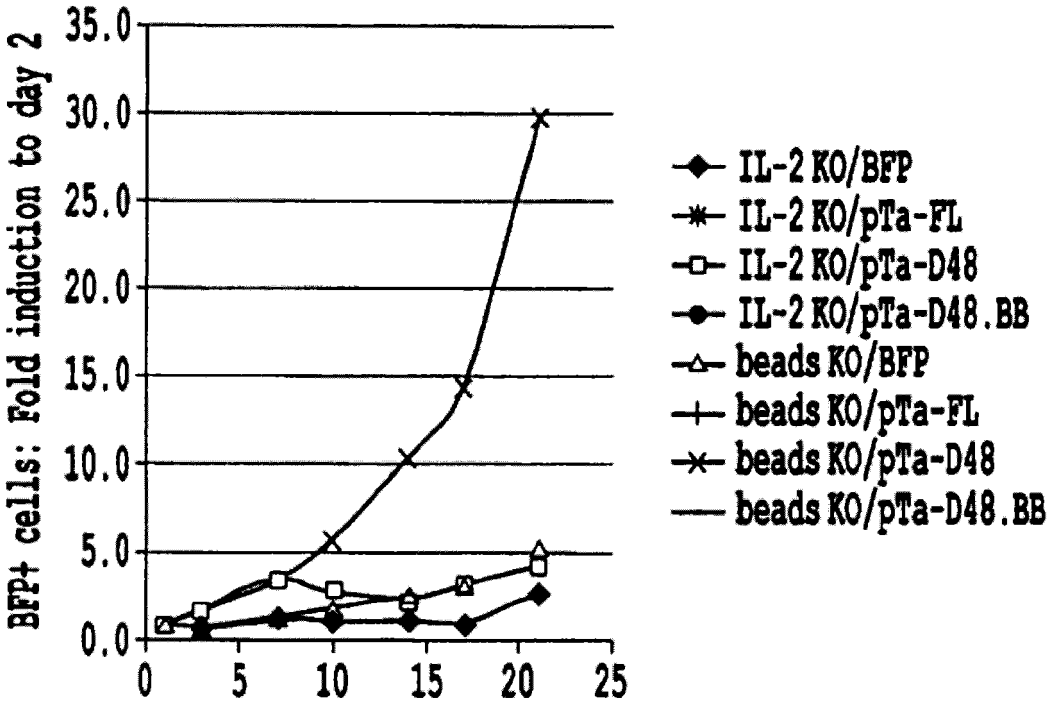


Figure 16A

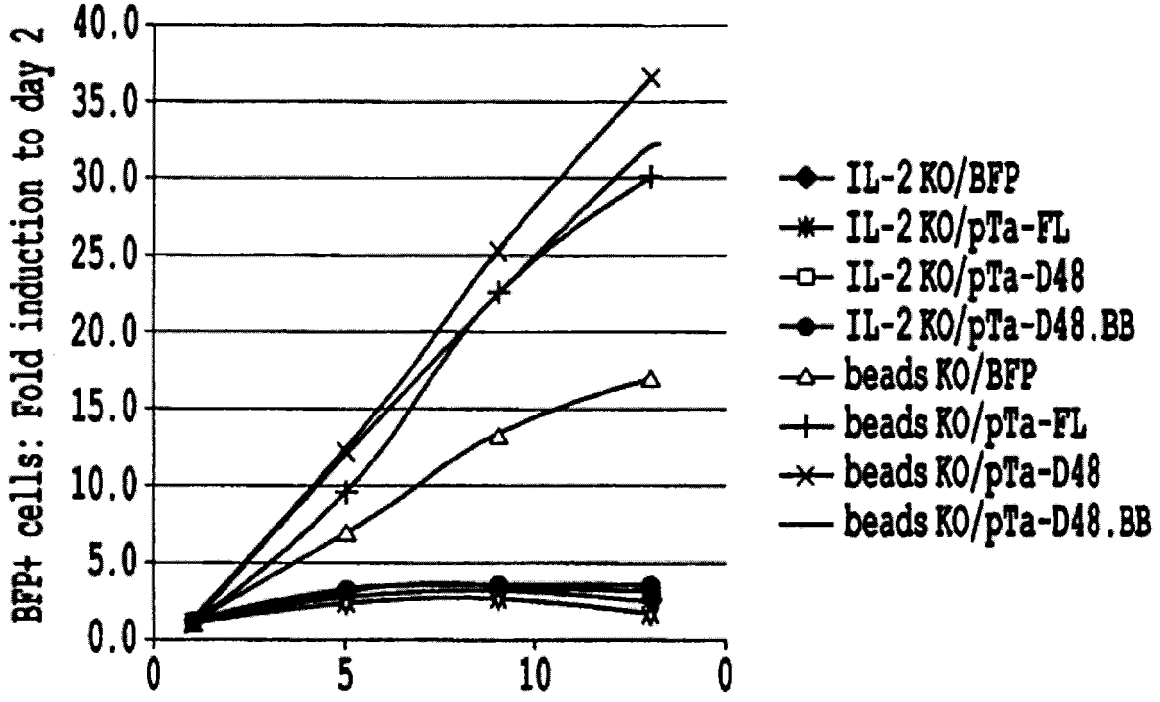


Figure 16B

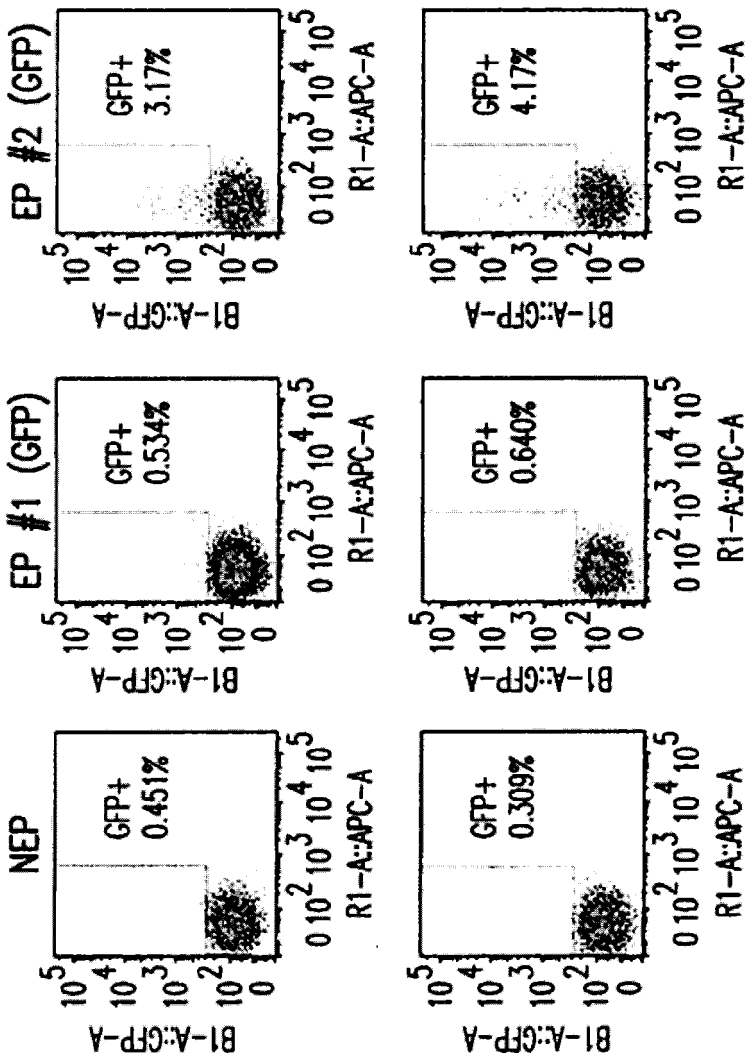

NEP
EP #1 (GFP)
EP #2 (GFP)
GFP+ 0.451%
GFP+ 0.534%
GFP+ 3.17%
GFP+ 0.309%
GFP+ 0.640%
GFP+ 4.17%
B1-A::GFP-A
R1-A::APC-A

Figure 17

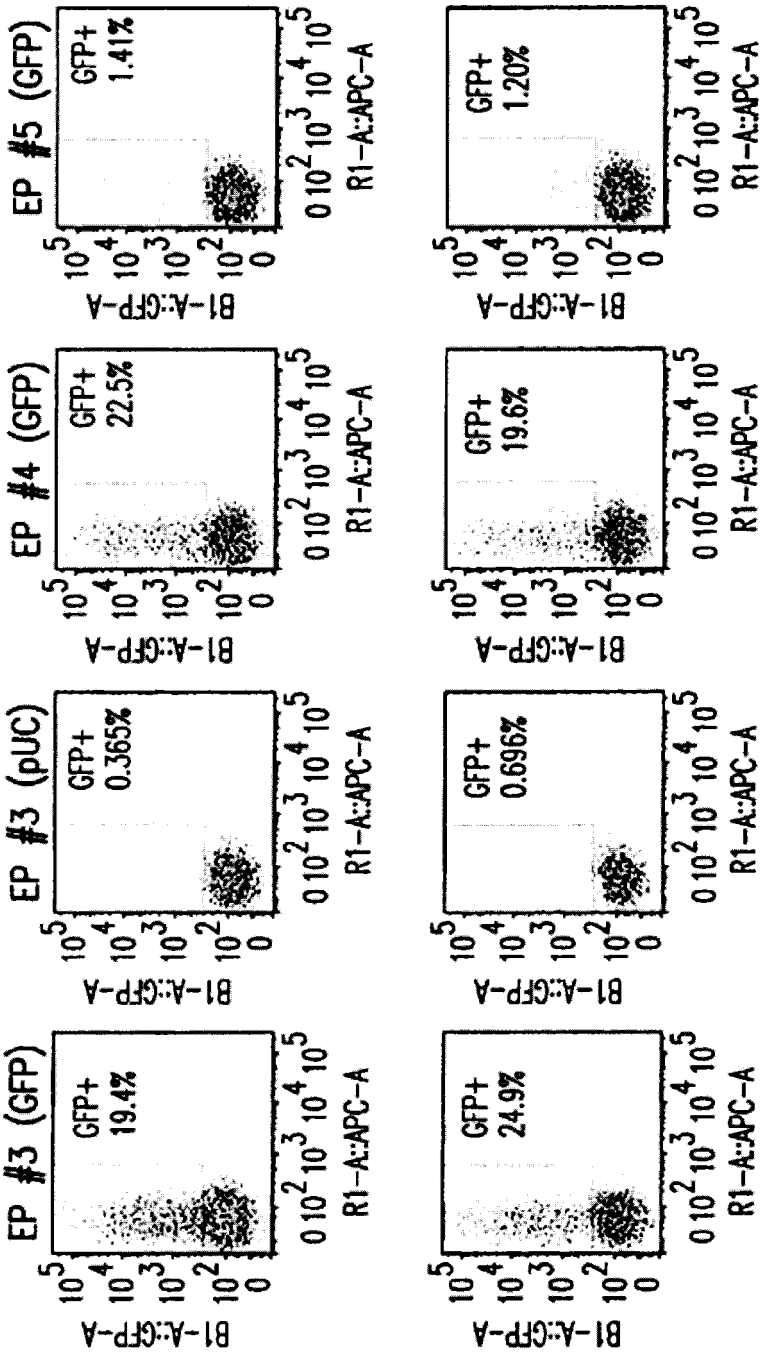

EP #3 (GFP)
GFP+ 19.4%
EP #3 (pUC)
GFP+ 0.365%
EP #4 (GFP)
GFP+ 22.5%
EP #5 (GFP)
GFP+ 1.41%
GFP+ 24.9%
GFP+ 0.696%
GFP+ 19.6%
GFP+ 1.20%
B1-A::GFP-A
R1-A::APC-A

Figure 17-1

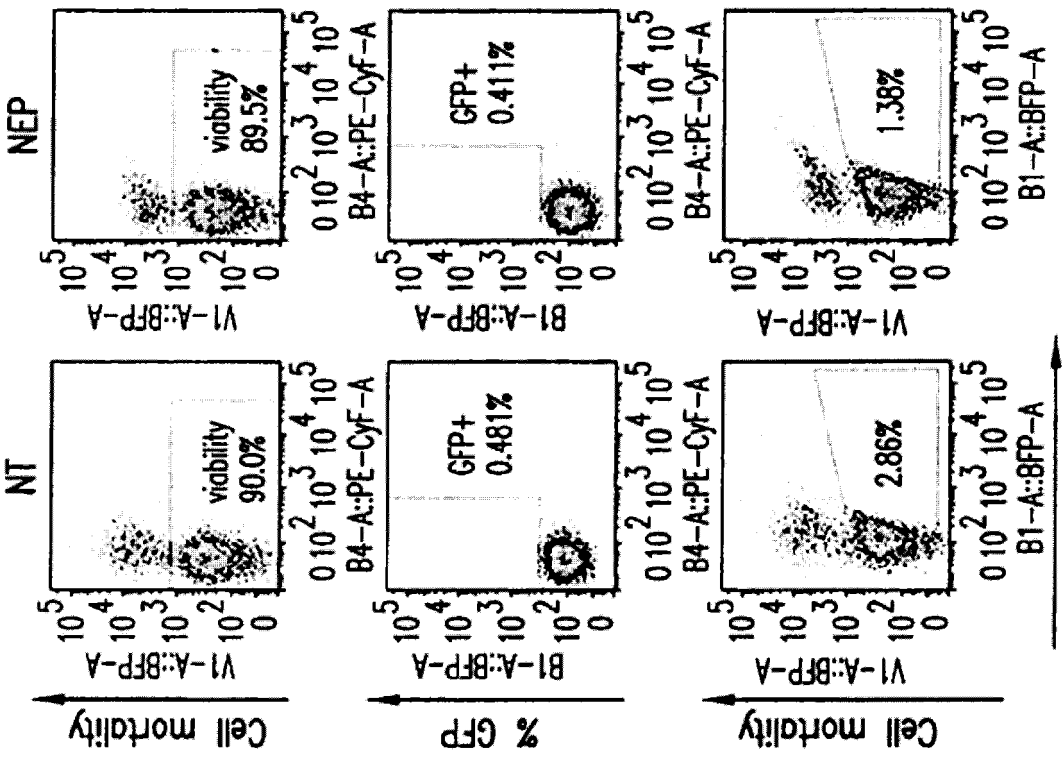

NEP
NT
viability
89.5%
viability
90.0%
GFP+
0.411%
GFP+
0.481%
1.38%
2.86%
V1-A::BFP-A
B4-A::PE-Cy5-A
B1-A::BFP-A
Cell mortality
% GFP
%GFP

Figure 18

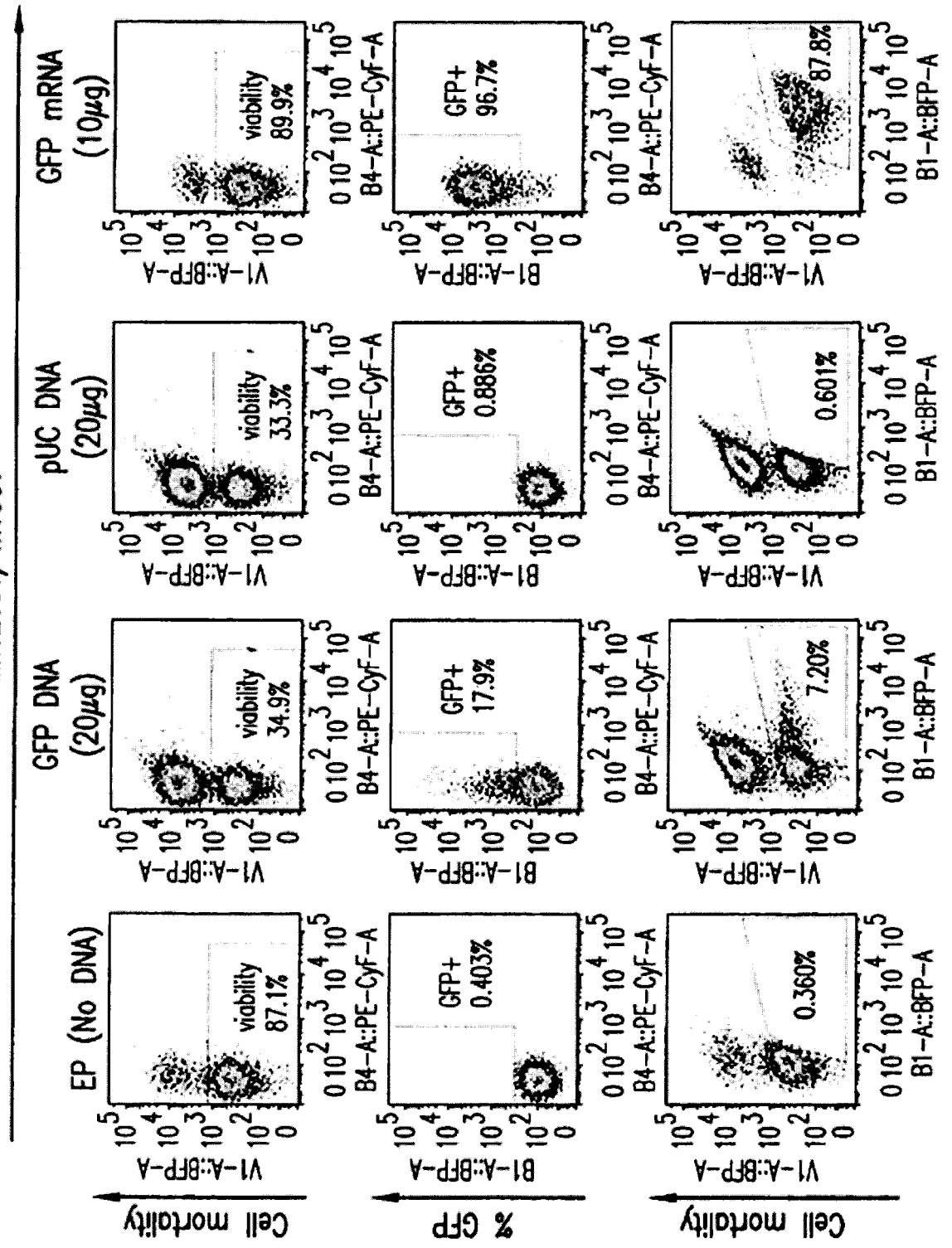

2x1200V/4x130V
EP (No DNA)
GFP DNA (20μg)
pUC DNA (20μg)
GFP mRNA (10μg)
Cell mortality
% GFP
Cell mortality
viability 87.1%
viability 34.9%
viability 33.3%
viability 89.9%
GFP+ 0.403%
GFP+ 17.9%
GFP+ 0.886%
GFP+ 96.7%
0.360%
7.20%
0.601%
87.8%
V1-A::BFP-A
B1-A::BFP-A
B4-A::PE-CyF-A
B1-A::BFP-A

Figure 18-1

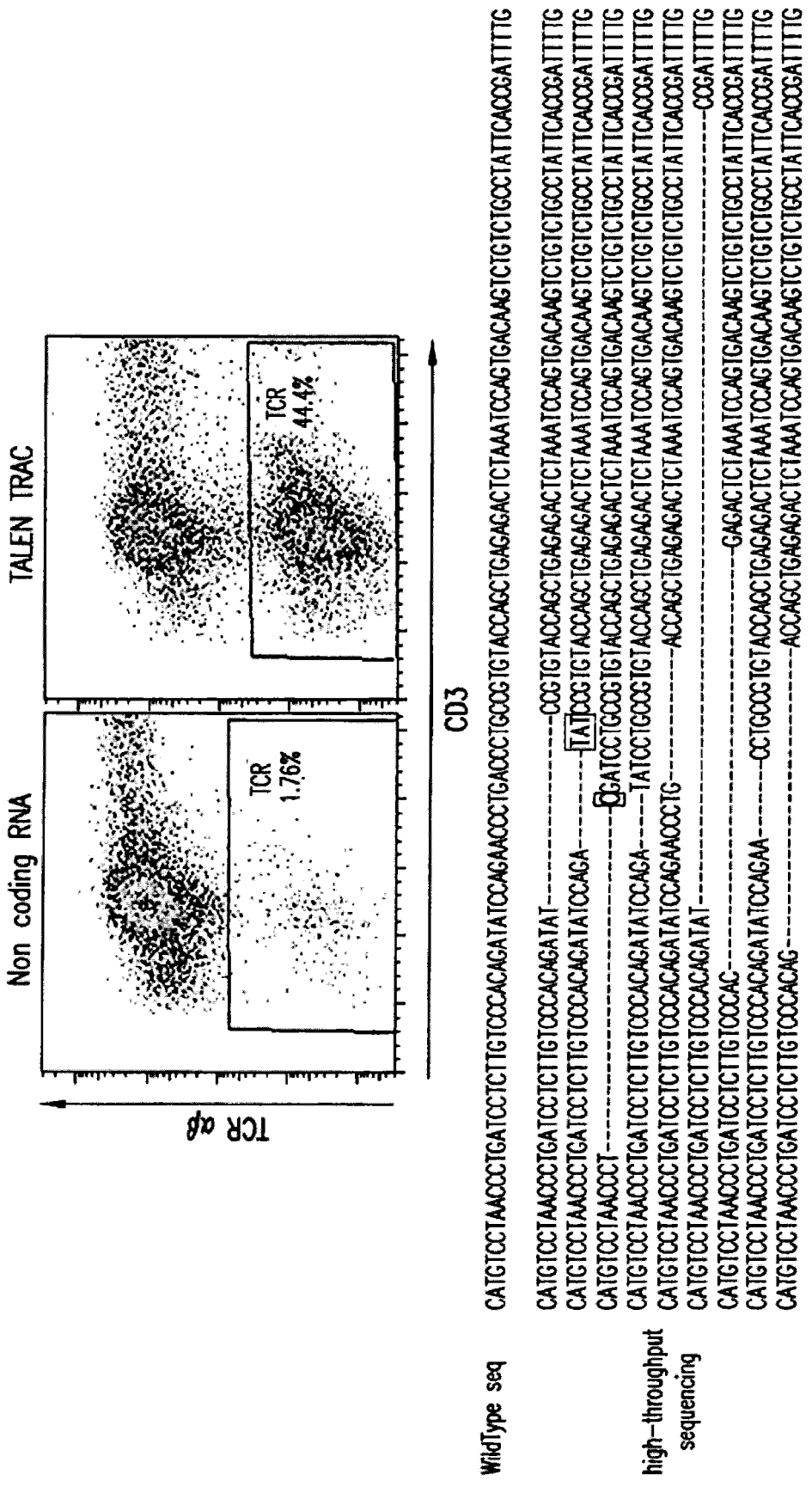

Non coding RNA
TALEN TRAC
TCR 1.76%
TCR 44.4%
TCR αβ
CD3
WildType seq
high-throughput sequencing

Figure 19

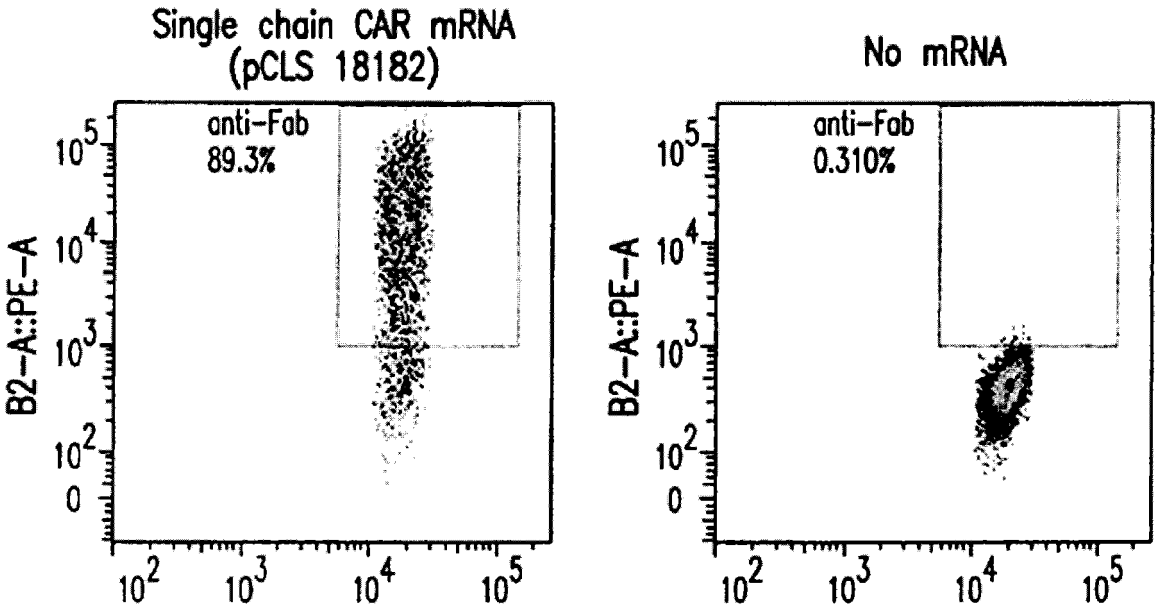


Figure 20 A

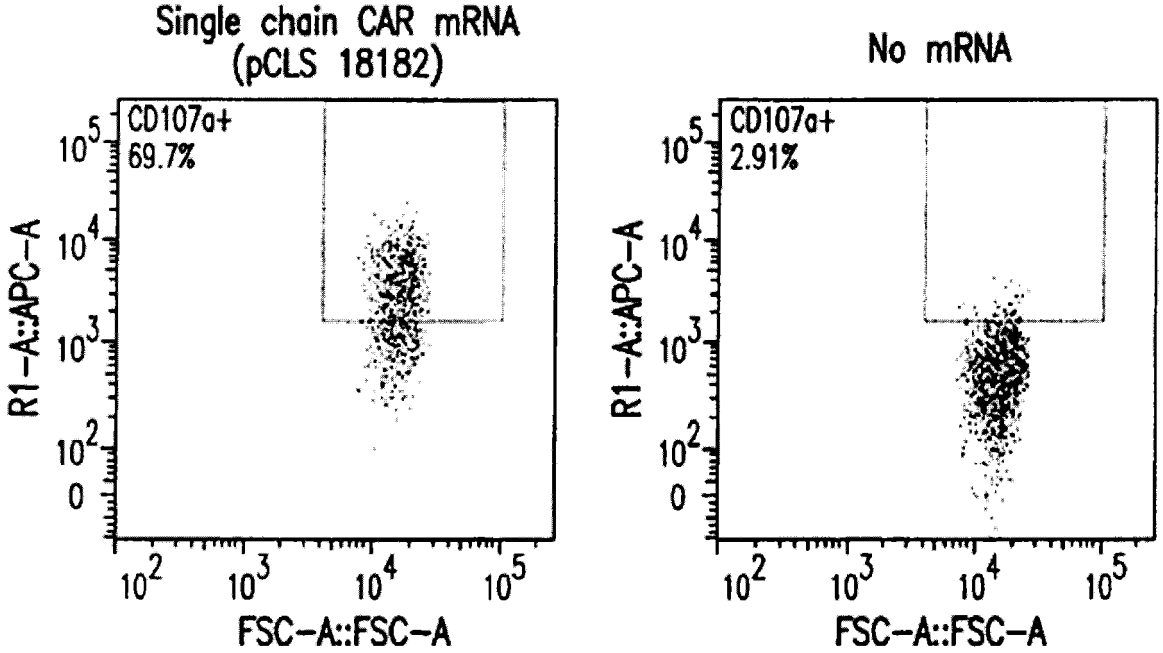


Figure 20 B

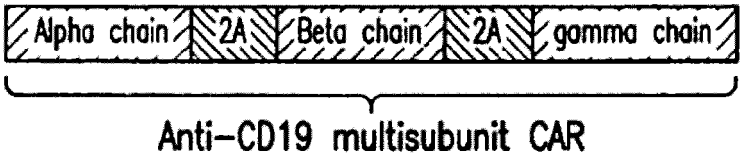


Figure 21 A

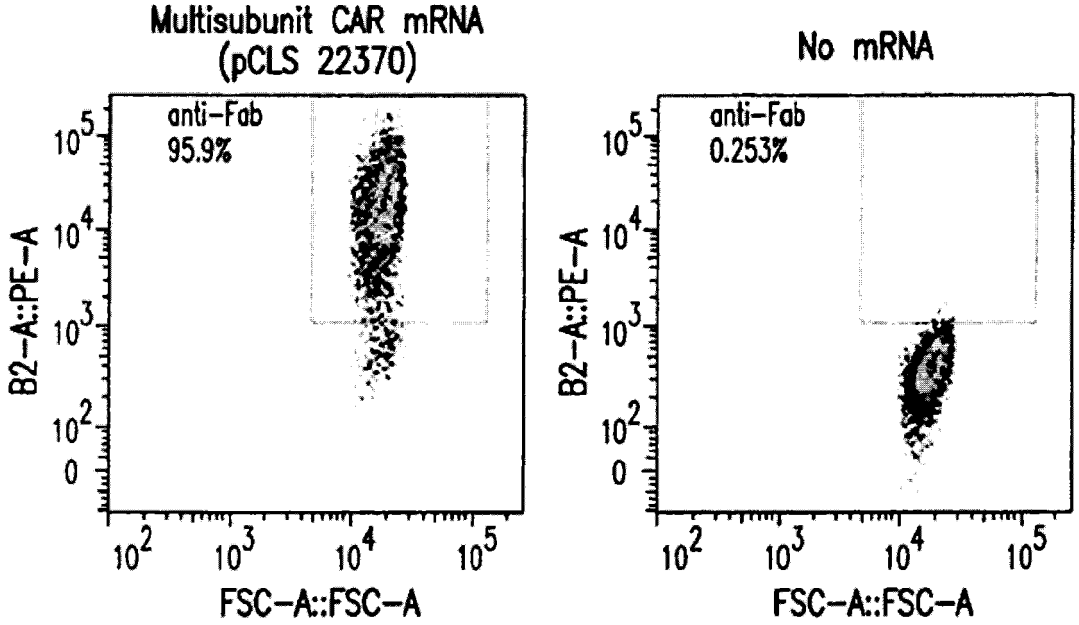


Figure 21 B

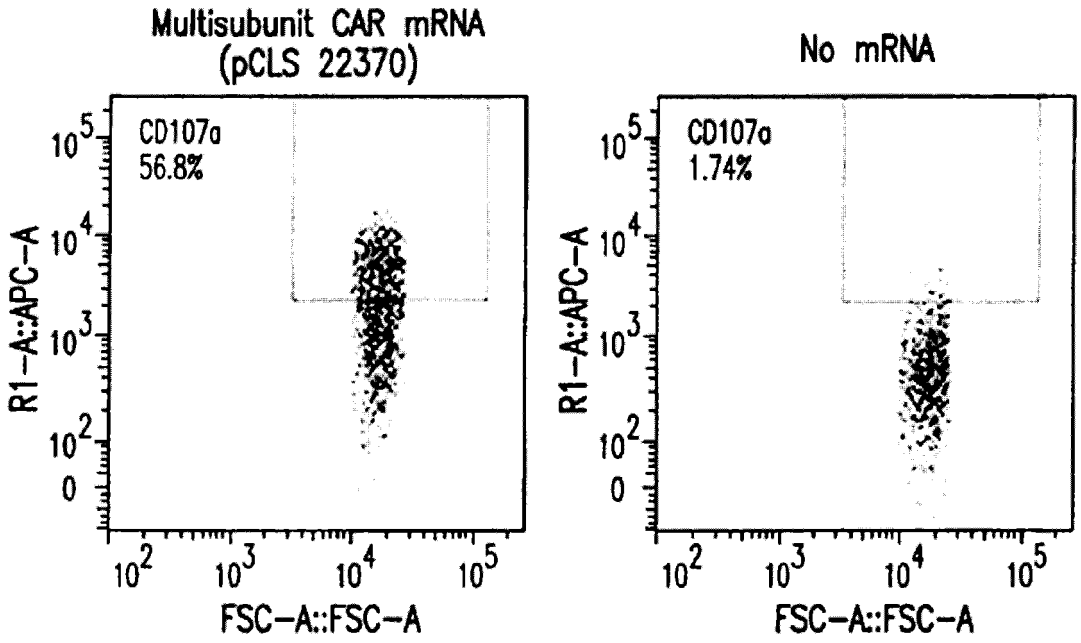


Figure 21 C

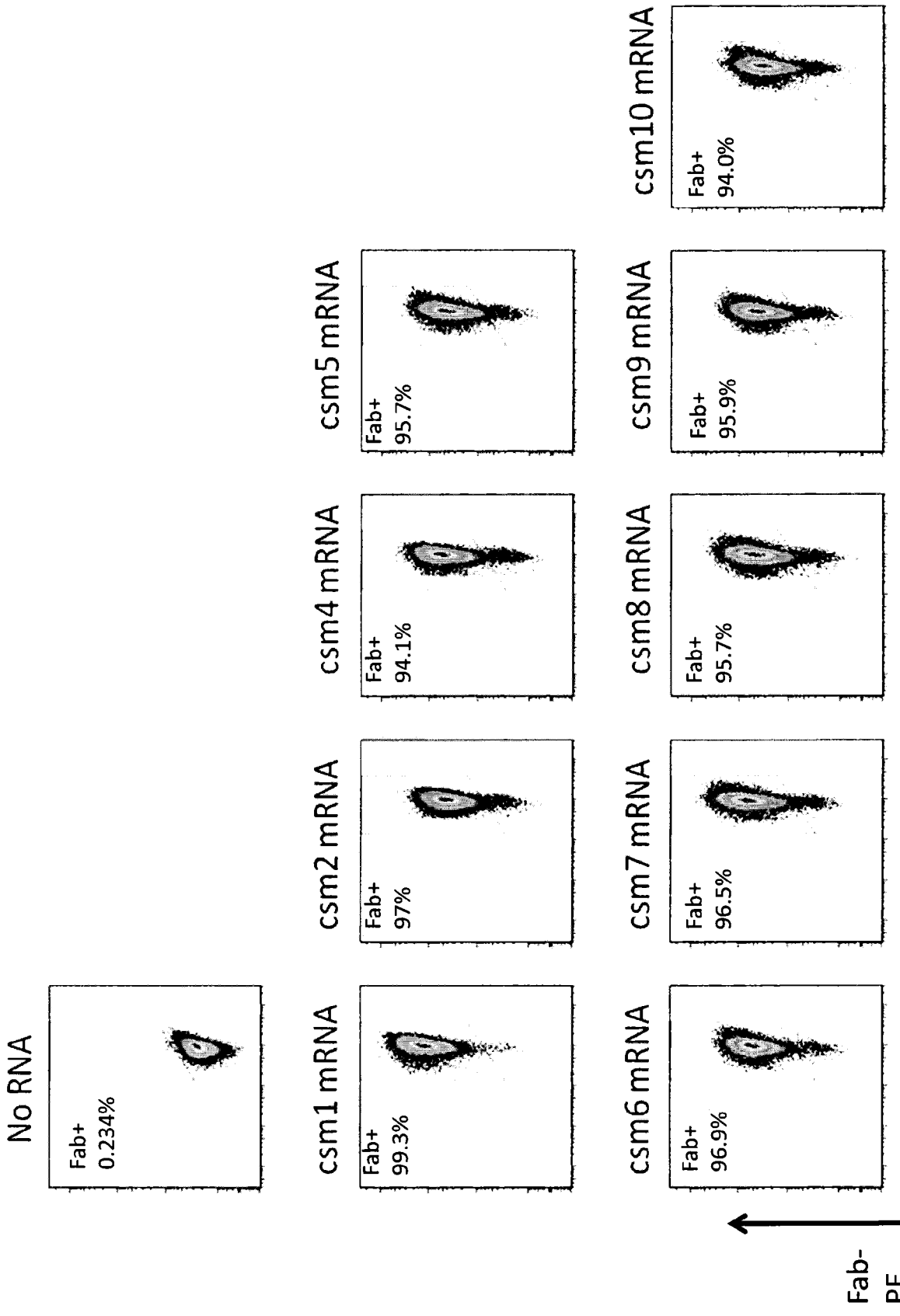

No RNA
Fab+
0.234%
csm1 mRNA
Fab+
99.3%
csm2 mRNA
Fab+
97%
csm4 mRNA
Fab+
94.1%
csm5 mRNA
Fab+
95.7%
csm6 mRNA
Fab+
96.9%
csm7 mRNA
Fab+
96.5%
csm8 mRNA
Fab+
95.7%
csm9 mRNA
Fab+
95.9%
csm10 mRNA
Fab+
94.0%
Fab-PE
FSC-A

Figure 22

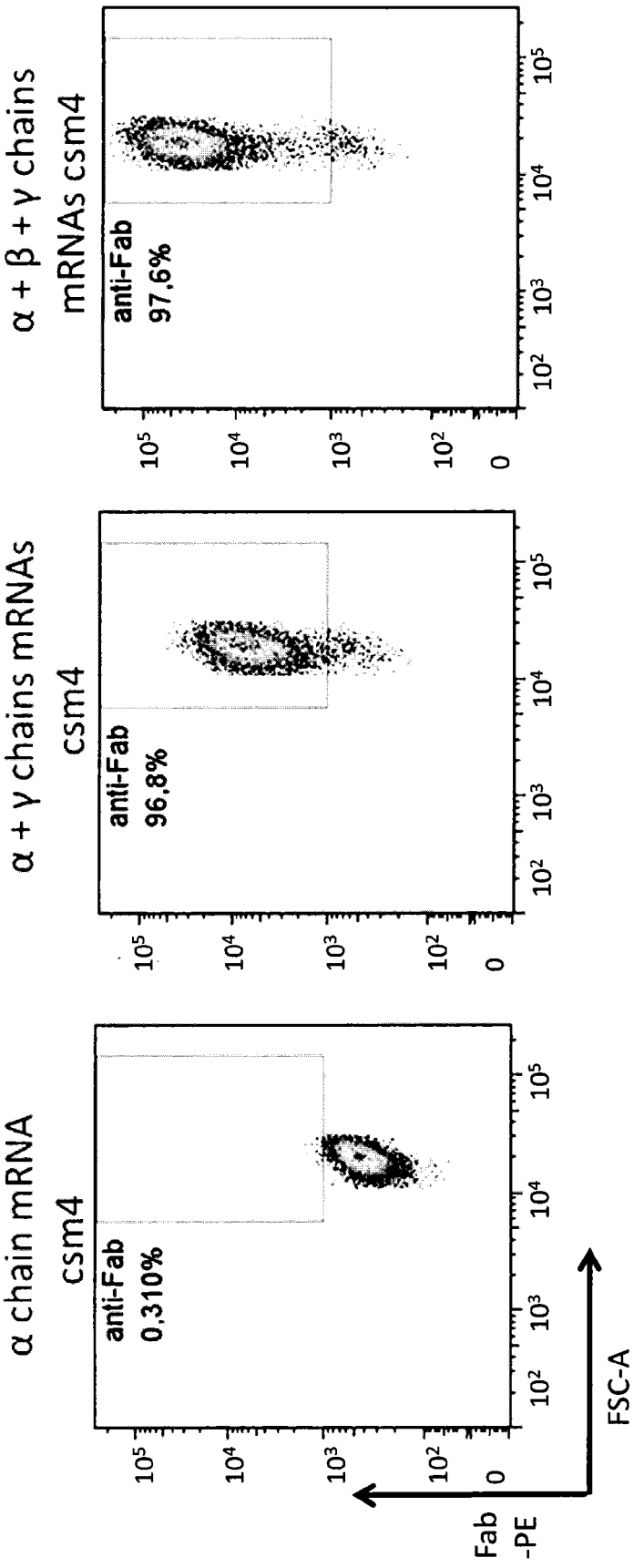

α chain mRNA csm4
anti-Fab 0,310%
α + γ chains mRNAs csm4
anti-Fab 96,8%
α + β + γ chains mRNAs csm4
anti-Fab 97,6%
Fab -PE
FSC-A
0
10²
10³
10⁴
10⁵

Figure 23

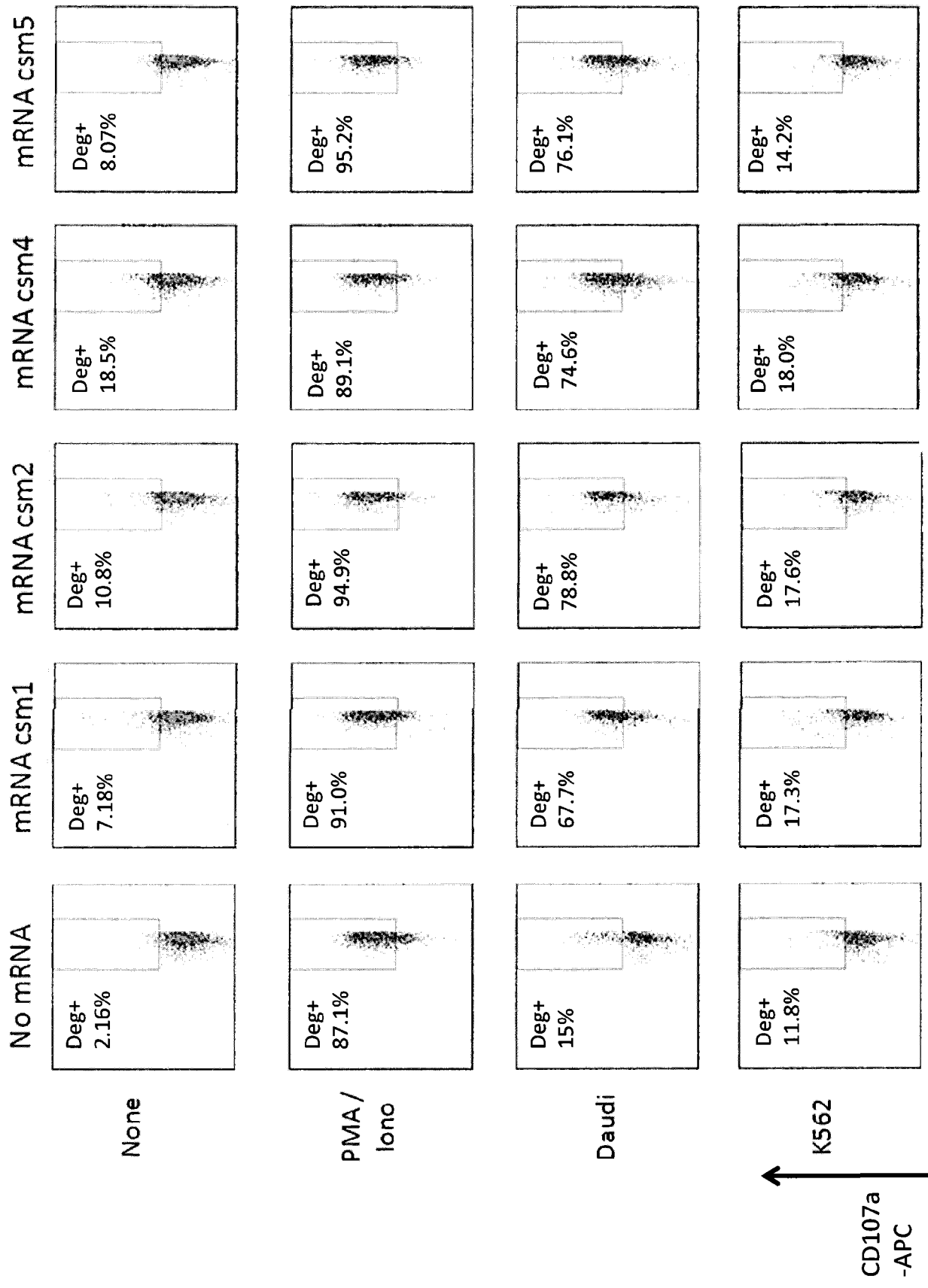

No mRNA
mRNA csm1
mRNA csm2
mRNA csm4
mRNA csm5
None
PMA / Iono
Daudi
K562
Deg+ 2.16%
Deg+ 7.18%
Deg+ 10.8%
Deg+ 18.5%
Deg+ 8.07%
Deg+ 87.1%
Deg+ 91.0%
Deg+ 94.9%
Deg+ 89.1%
Deg+ 95.2%
Deg+ 15%
Deg+ 67.7%
Deg+ 78.8%
Deg+ 74.6%
Deg+ 76.1%
Deg+ 11.8%
Deg+ 17.3%
Deg+ 17.6%
Deg+ 18.0%
Deg+ 14.2%
CD107a -APC
FSC-A

Figure 24A

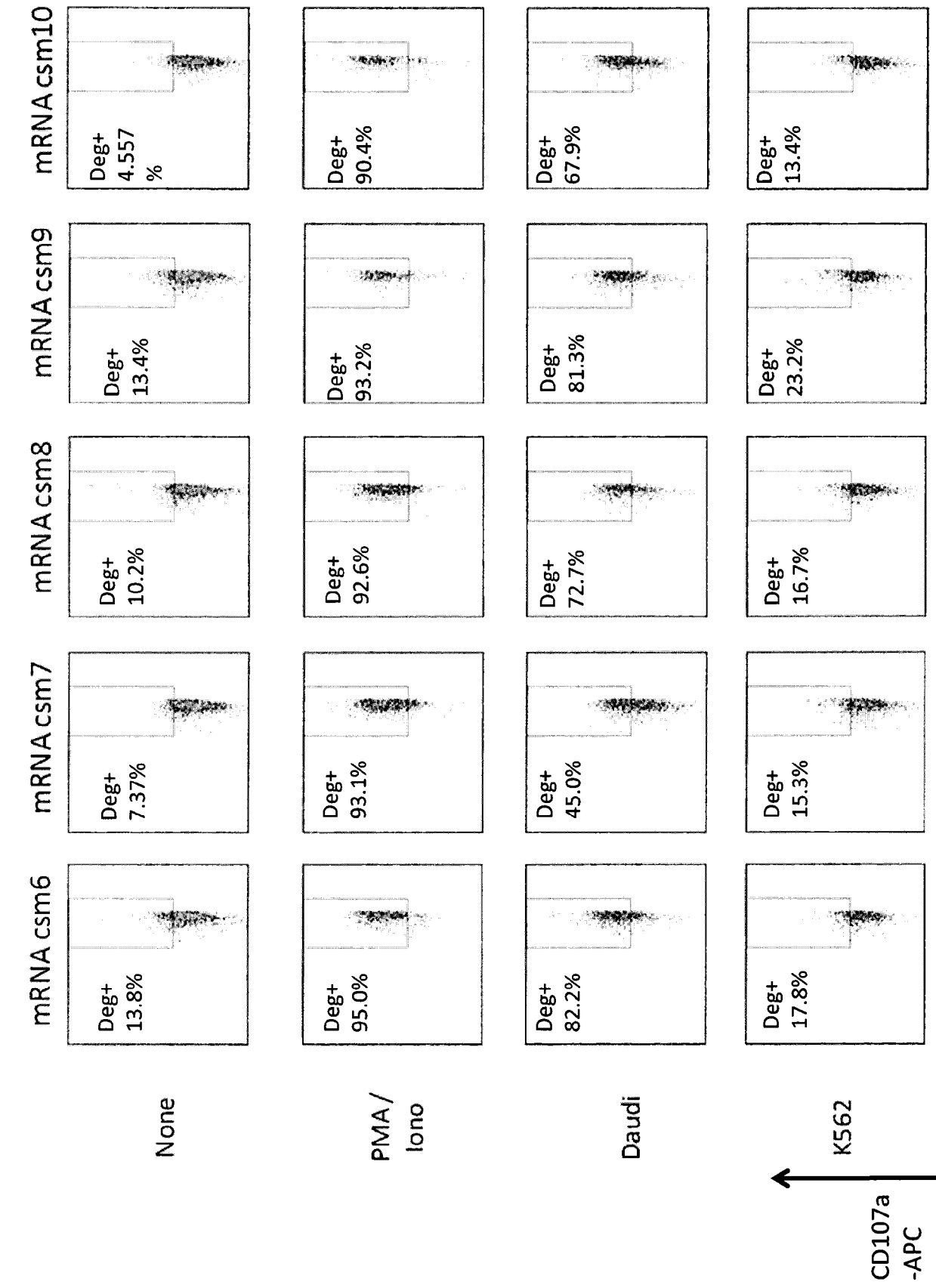

mRNA csm6
mRNA csm7
mRNA csm8
mRNA csm9
mRNA csm10
None
PMA/ Iono
Daudi
K562
Deg+ 13.8%
Deg+ 7.37%
Deg+ 10.2%
Deg+ 13.4%
Deg+ 4.557 %
Deg+ 95.0%
Deg+ 93.1%
Deg+ 92.6%
Deg+ 93.2%
Deg+ 90.4%
Deg+ 82.2%
Deg+ 45.0%
Deg+ 72.7%
Deg+ 81.3%
Deg+ 67.9%
Deg+ 17.8%
Deg+ 15.3%
Deg+ 16.7%
Deg+ 23.2%
Deg+ 13.4%
CD107a -APC
FSC-A

Figure 24B

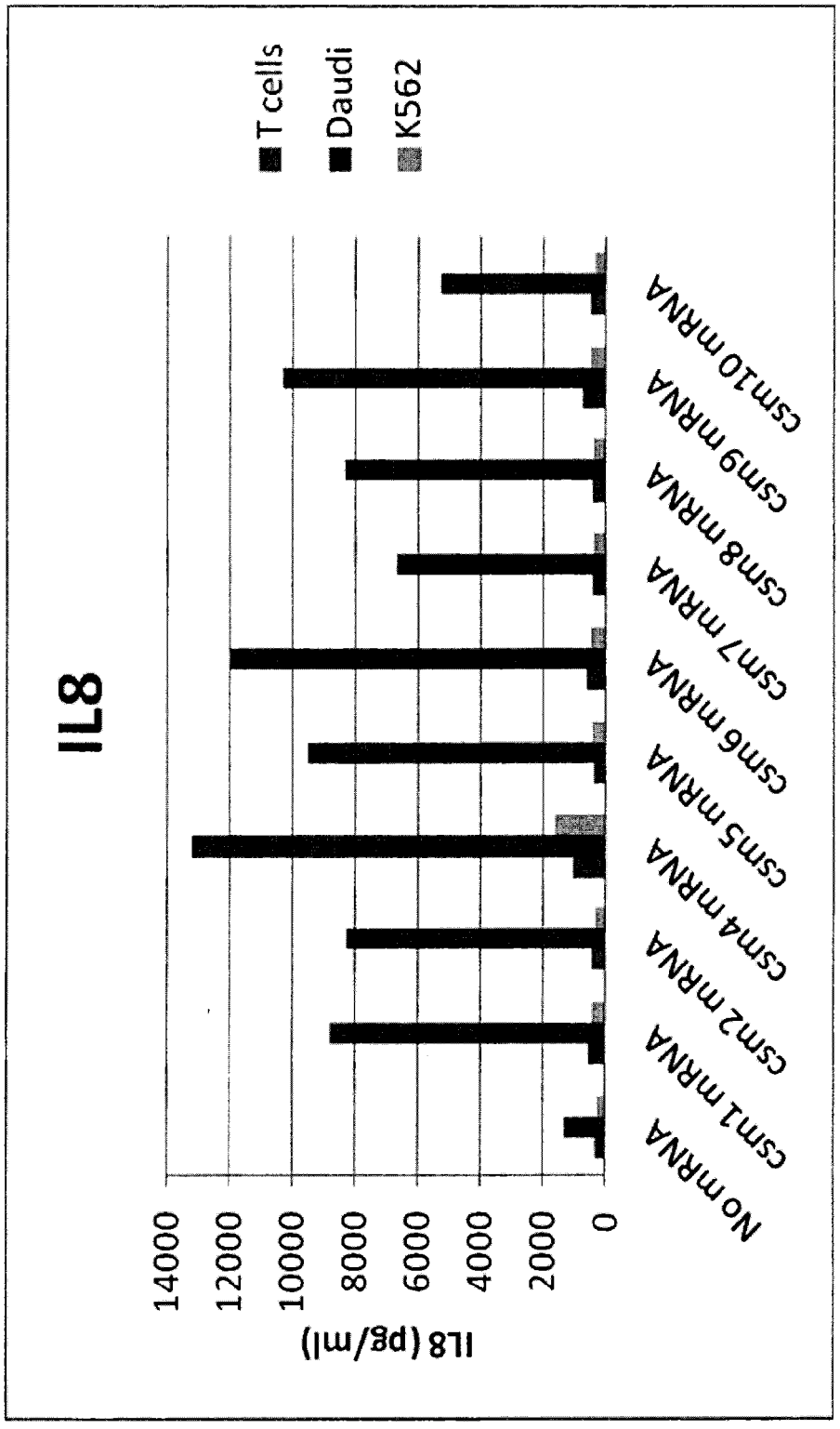

IL8
T cells
Daudi
K562
IL8 (pg/ml)
0
2000
4000
6000
8000
10000
12000
14000
No mRNA
csm1 mRNA
csm2 mRNA
csm4 mRNA
csm5 mRNA
csm6 mRNA
csm7 mRNA
csm8 mRNA
csm9 mRNA
csm10 mRNA

Figure 25A

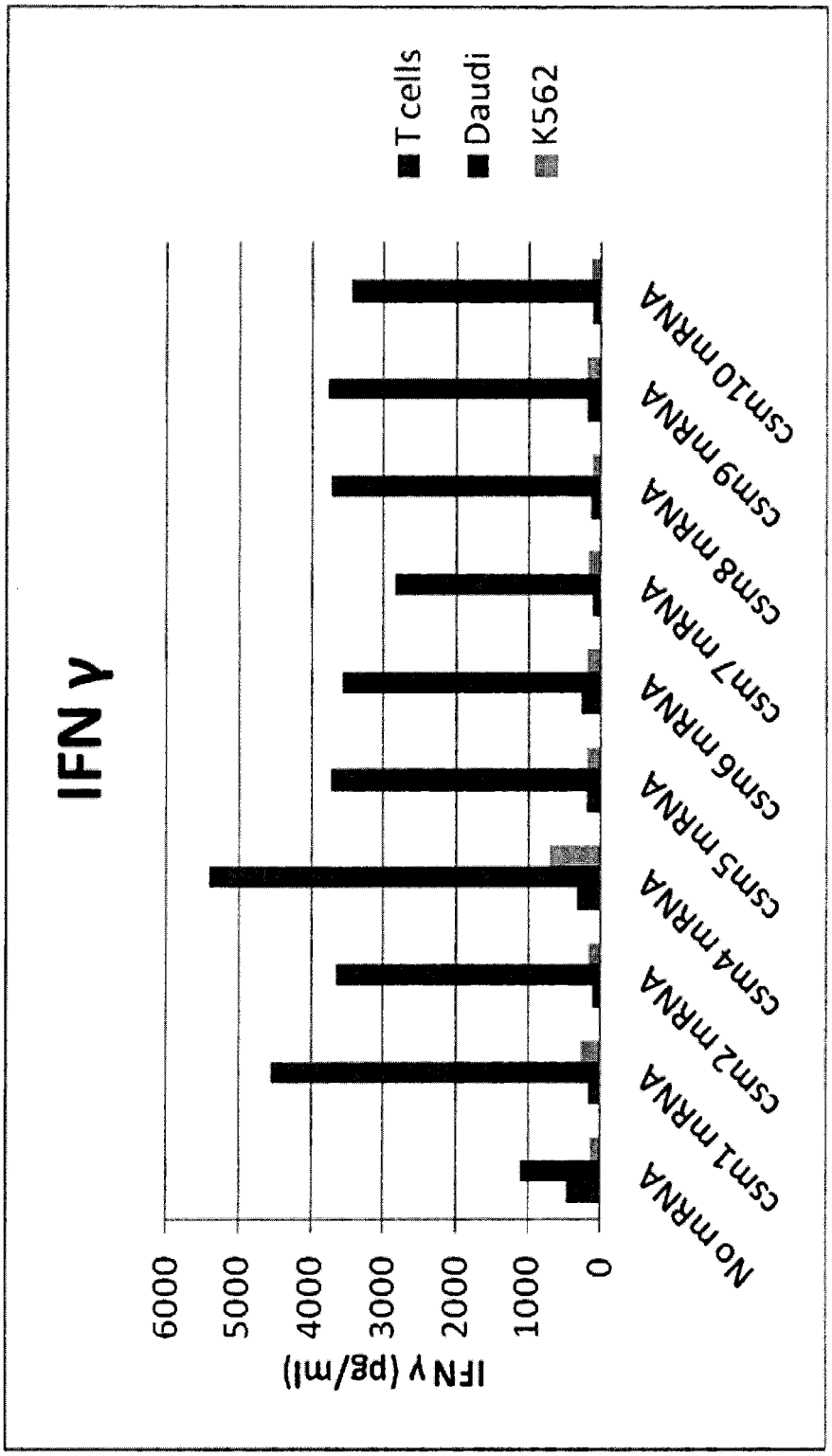

IFN γ
T cells
Daudi
K562
IFN γ (pg/ml)
6000
5000
4000
3000
2000
1000
0
No mRNA
csm1 mRNA
csm2 mRNA
csm4 mRNA
csm5 mRNA
csm6 mRNA
csm7 mRNA
csm8 mRNA
csm9 mRNA
csm10 mRNA

Figure 25B

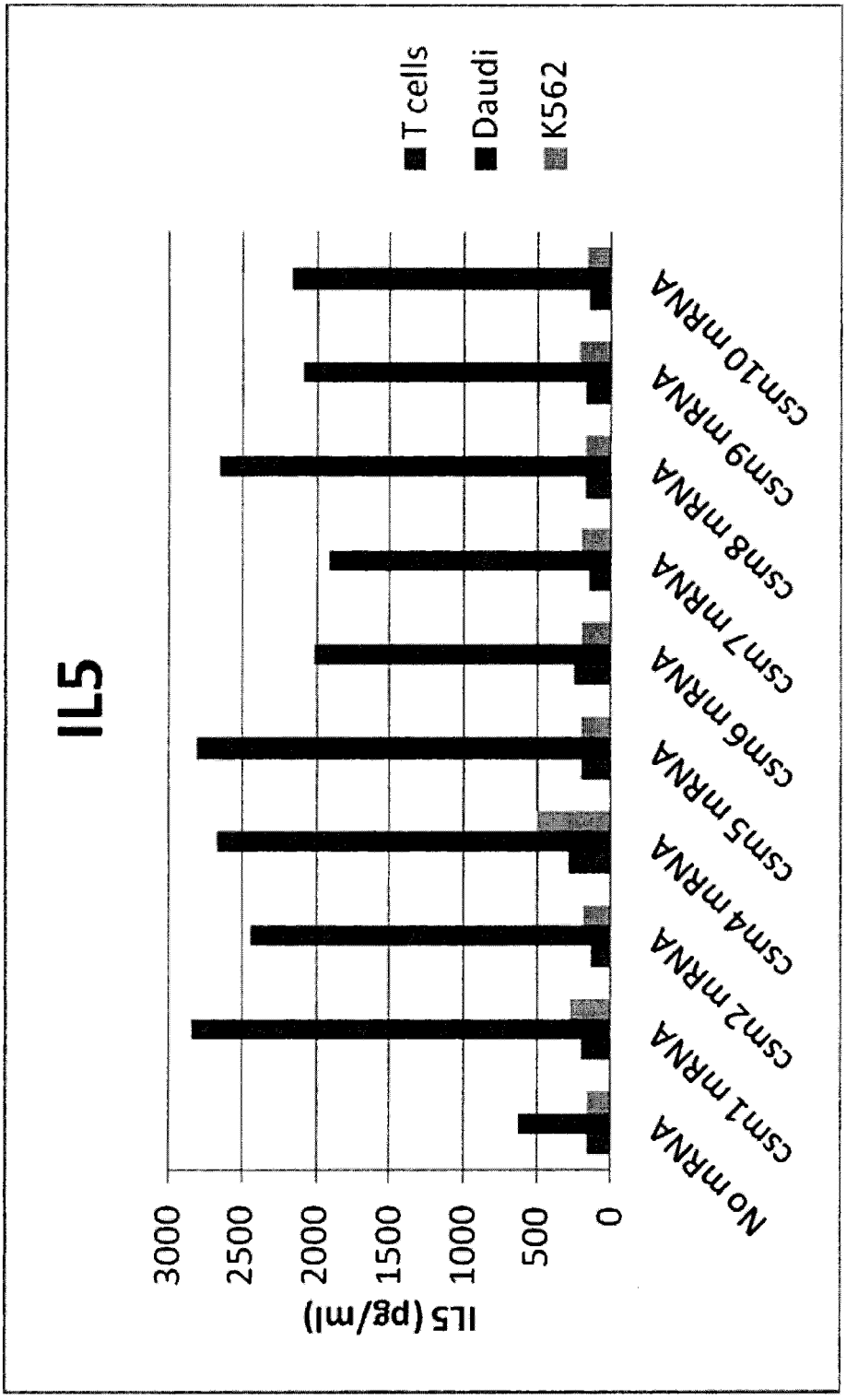

IL5
T cells
Daudi
K562
IL5 (pg/ml)
3000
2500
2000
1500
1000
500
0
No mRNA
csm1 mRNA
csm2 mRNA
csm4 mRNA
csm5 mRNA
csm6 mRNA
csm7 mRNA
csm8 mRNA
csm9 mRNA
csm10 mRNA

Figure 25C

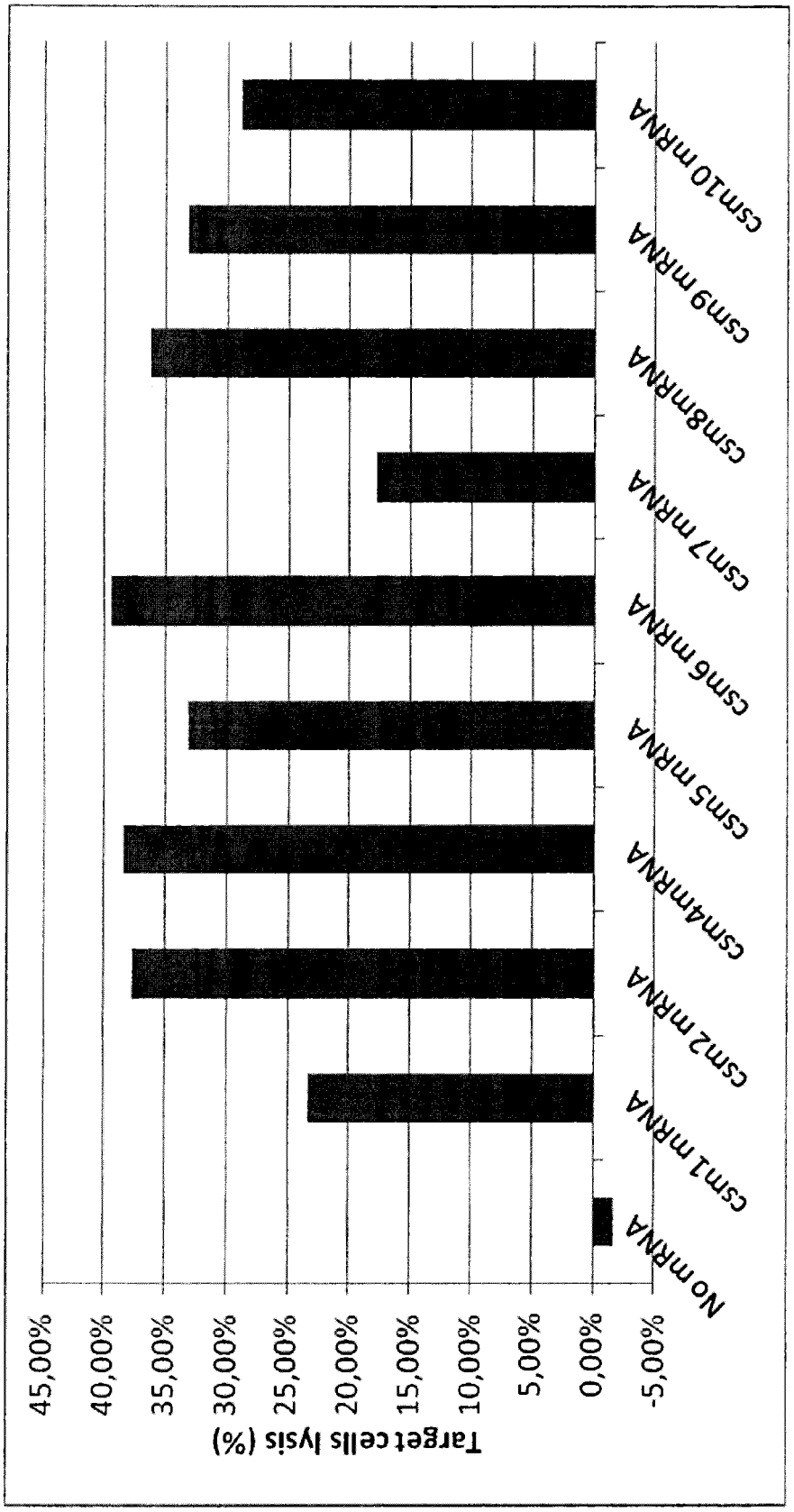

Target cells lysis (%)
45,00%
40,00%
35,00%
30,00%
25,00%
20,00%
15,00%
10,00%
5,00%
0,00%
-5,00%
No mRNA
csm1 mRNA
csm2 mRNA
csm4mRNA
csm5 mRNA
csm6 mRNA
csm7 mRNA
csm8mRNA
csm9 mRNA
csm10 mRNA

Figure 26