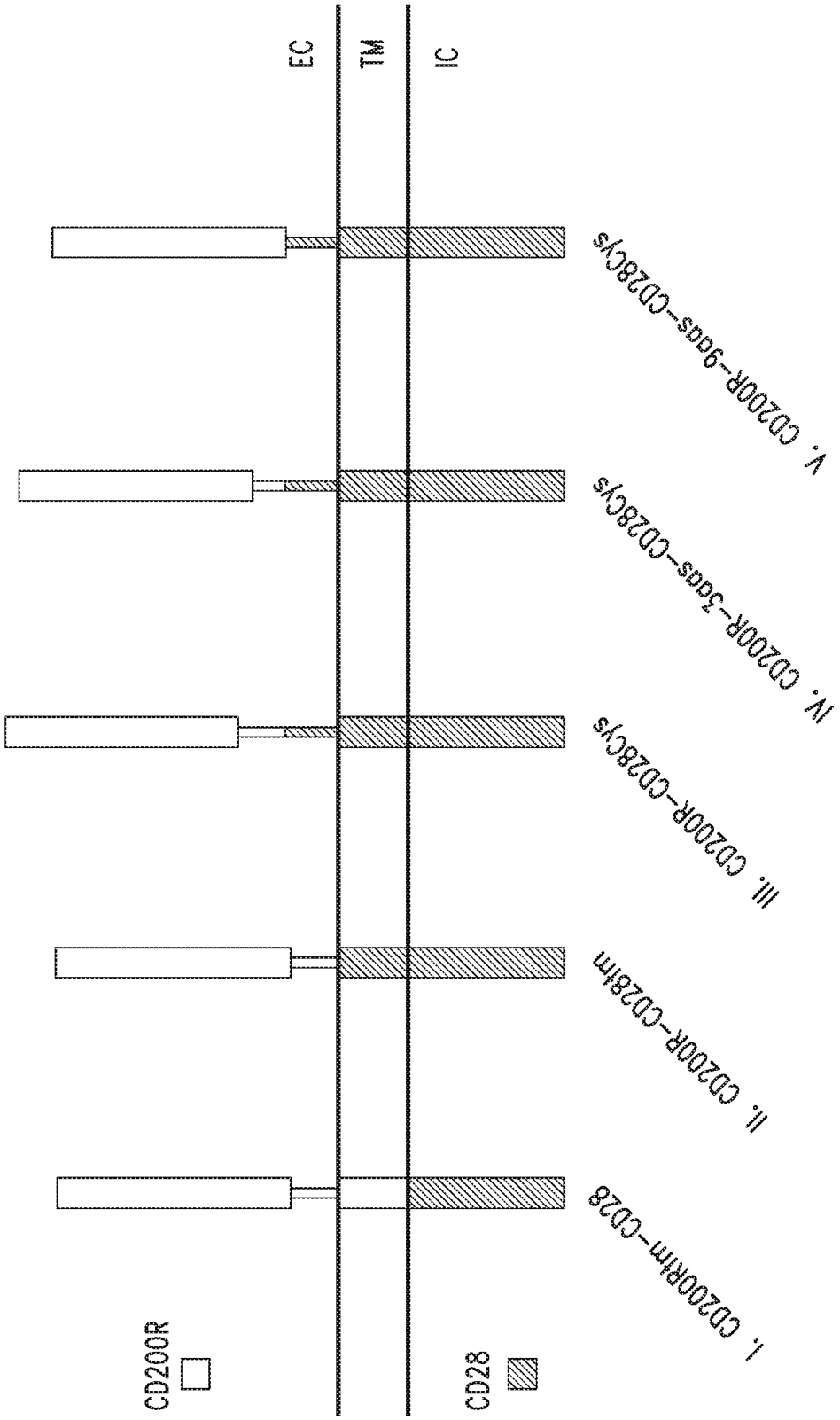

EC
TM
IC
CD200R
CD28
I. CD200Rtm-CD28
II. CD200R-CD28tm
III. CD200R-CD28Cys
IV. CD200R-3Gas-CD28Cys
V. CD200R-9Gas-CD28Cys

FIG. 1A

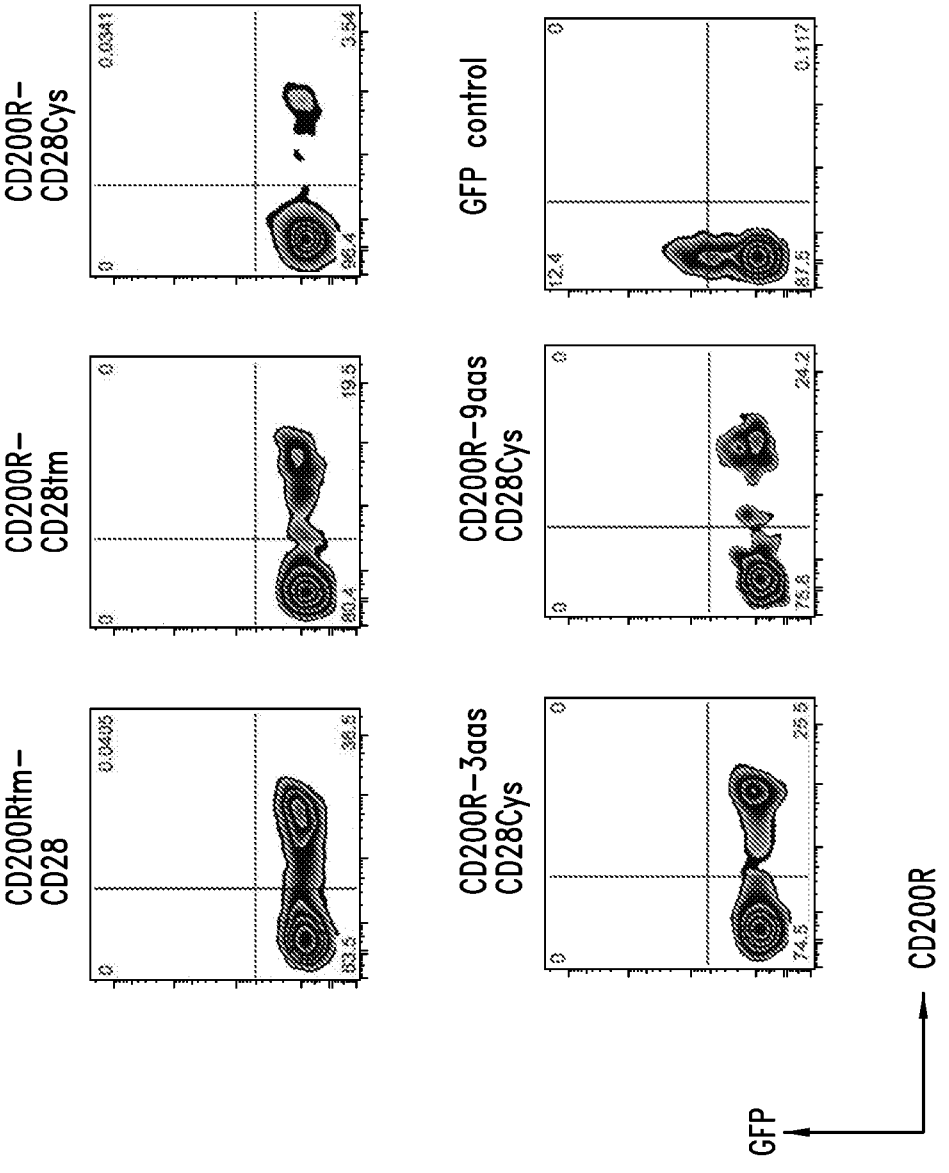

CD200Rtm-CD28
CD200R-CD28tm
CD200R-CD28Cys
CD200R-3aas CD28Cys
CD200R-9aas CD28Cys
GFP control
GFP
CD200R

FIG. 1B

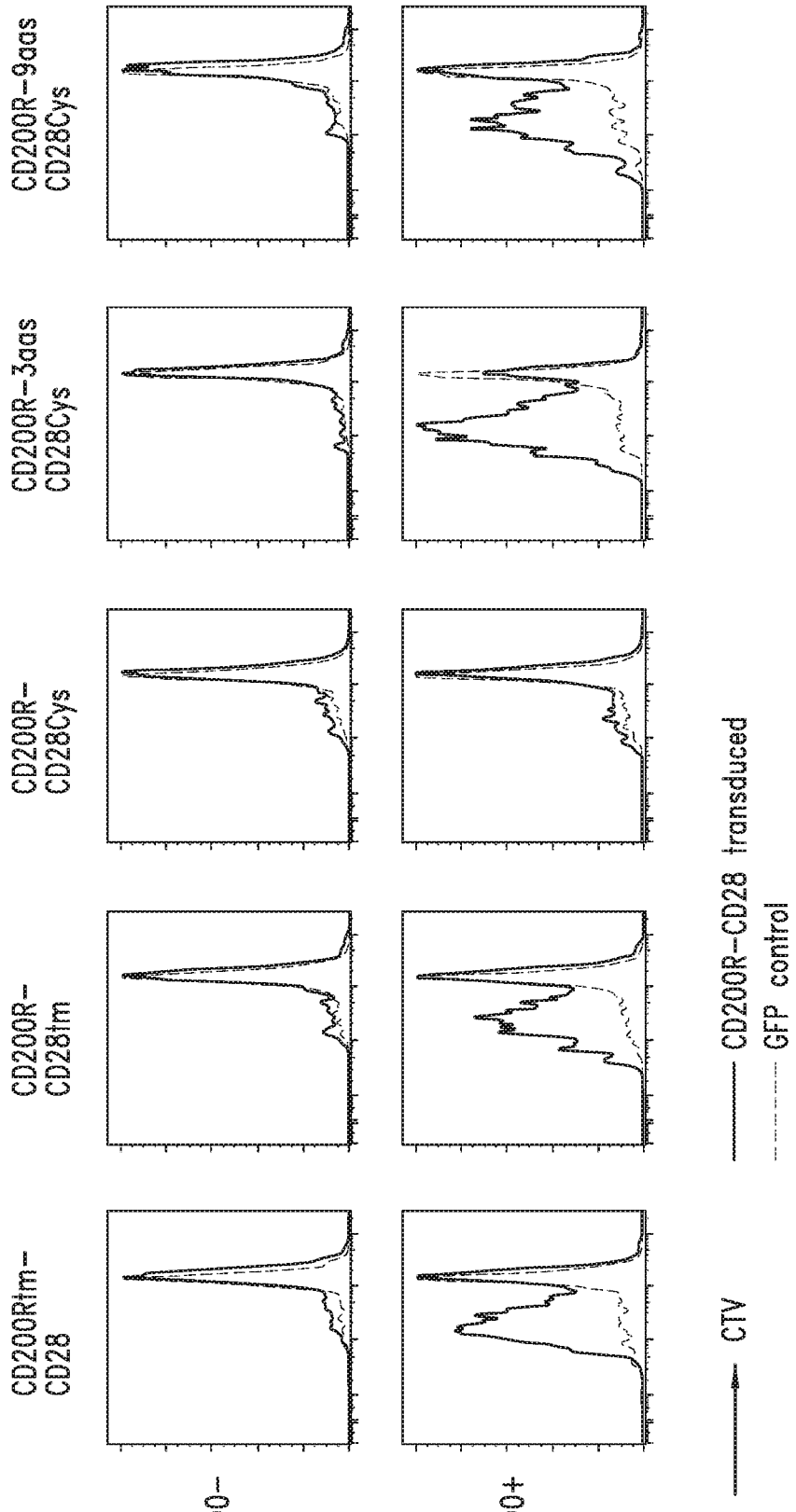

CD200Rtm-CD28
CD200R-CD28tm
CD200R-CD28Cys
CD200R-3aas CD28Cys
CD200R-9aas CD28Cys
CD200- FBL
CD200+ FBL
CTV
CD200R-CD28 transduced
GFP control

FIG. 2A

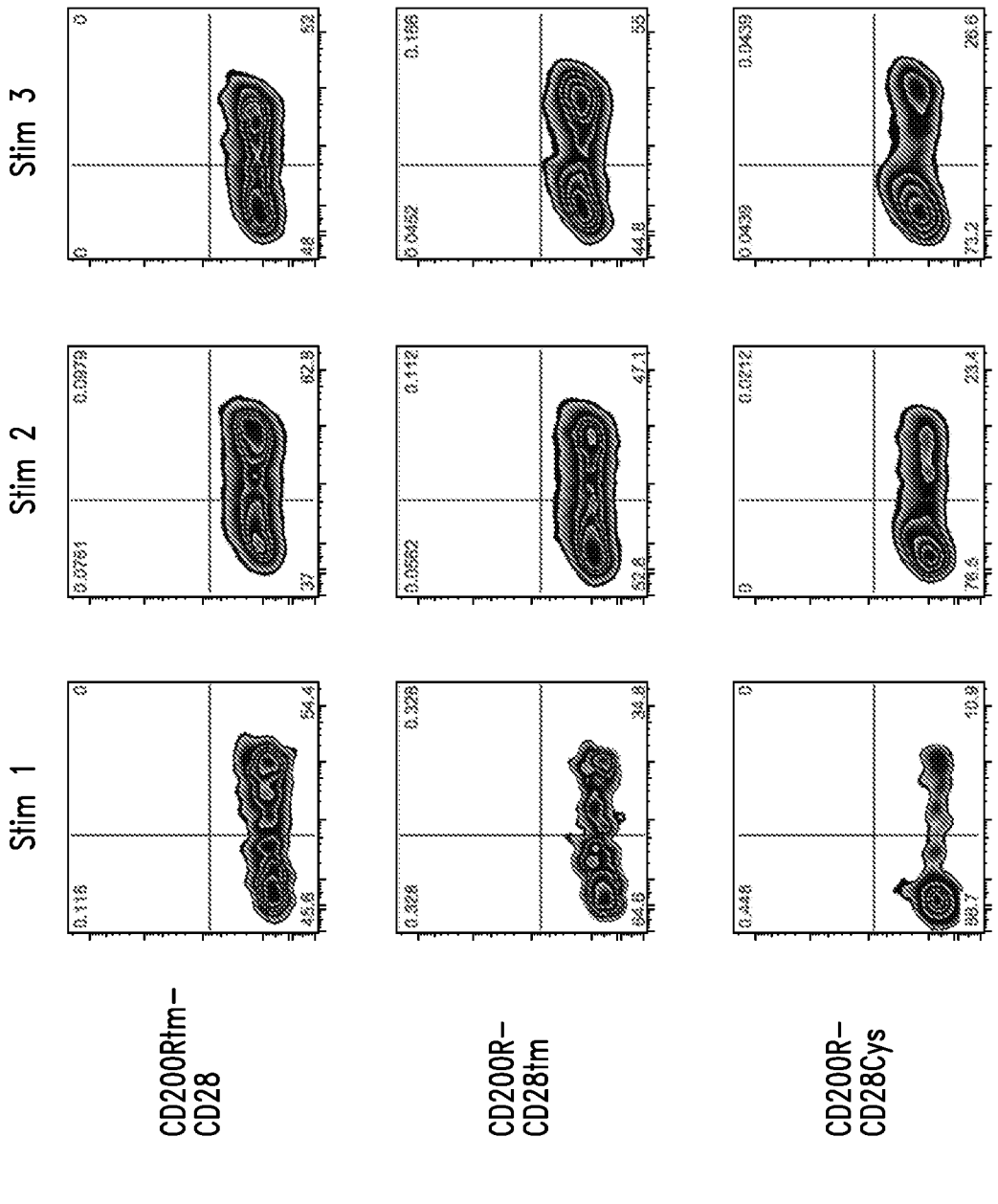

Stim 1
Stim 2
Stim 3
CD200Rtm-CD28
CD200R-CD28tm
CD200R-CD28Cys
CD200R

FIG. 2B

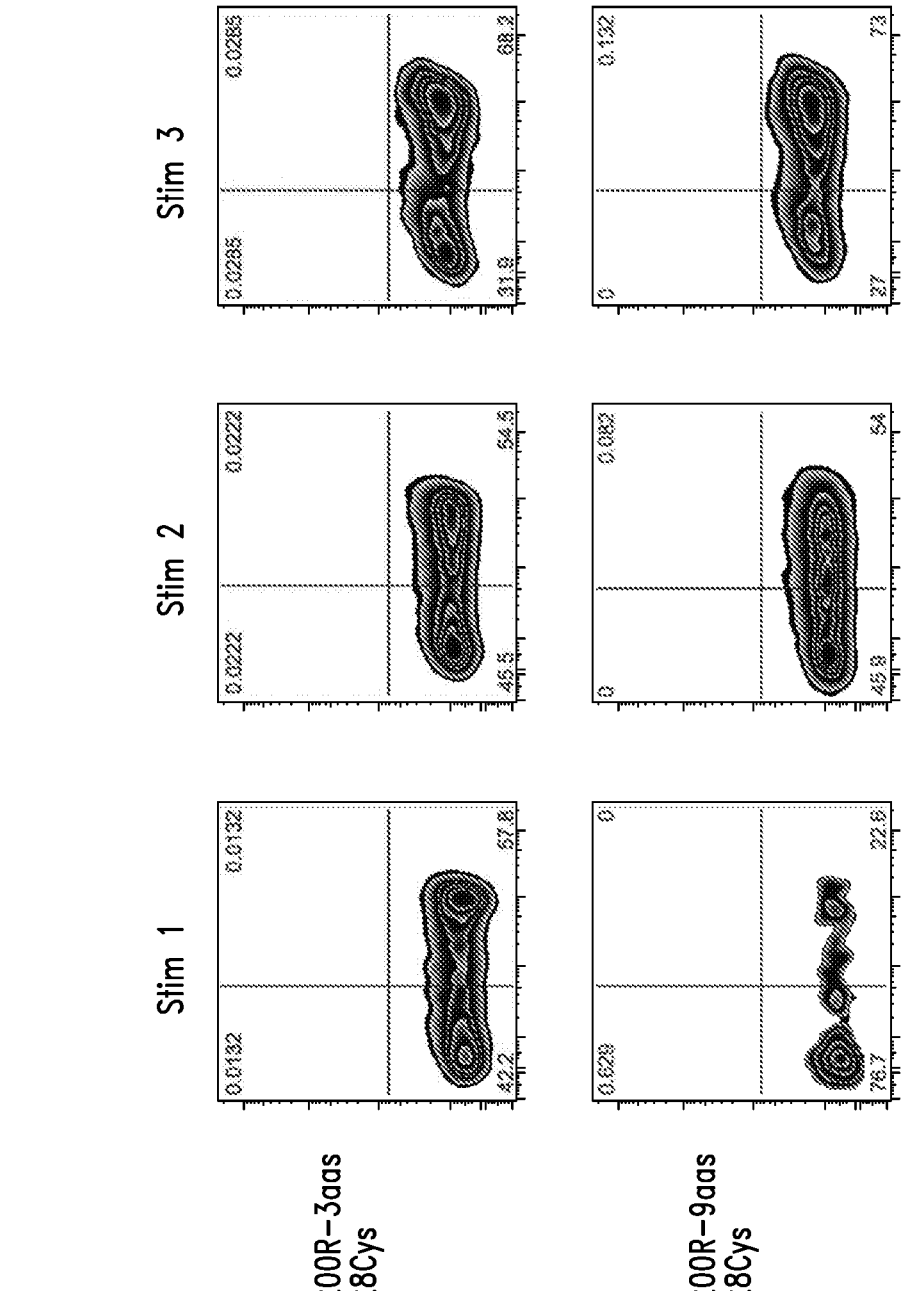

Stim 1
Stim 2
Stim 3
CD200R-3aas CD28Cys
CD200R-9aas CD28Cys
CD200R
0.0132
0.0132
57.8
42.2
0.0222
0.0222
54.5
45.5
0.0285
0.0285
68.2
31.9
0.629
0
22.8
76.7
0
0.082
54
45.8
0.192
0.192
73
27

FIG. 2B (Cont.)

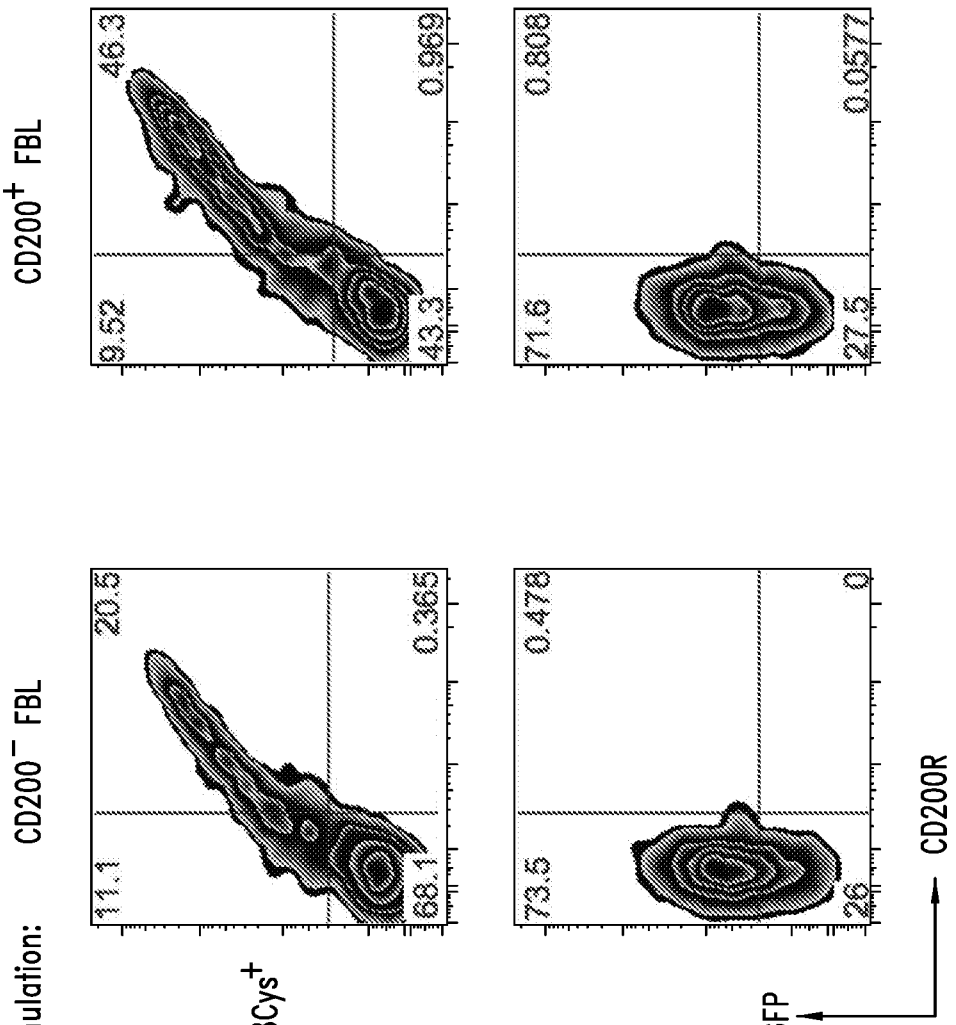

Restimulation:
CD200⁻ FBL
CD200⁺ FBL
CD200R-9aas-CD28Cys⁺
Empty vector control
11.1
20.5
68.1
0.365
9.52
46.3
43.3
0.969
73.5
0.478
26
0
71.6
0.808
27.5
0.0577
GFP
CD200R

FIG. 2C

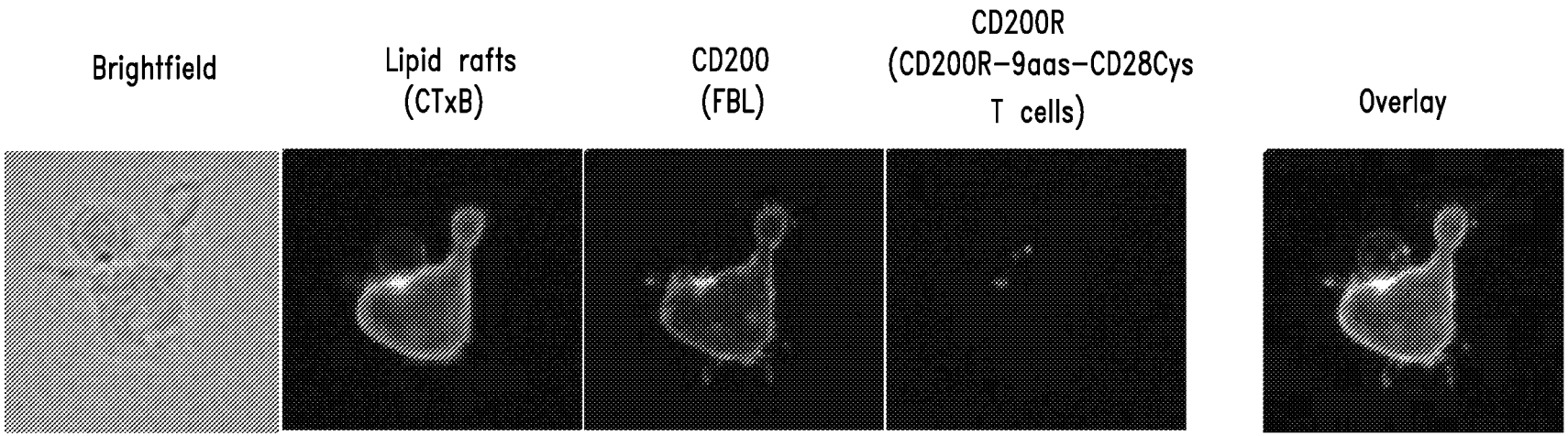

Brightfield
Lipid rafts
(CTxB)
CD200
(FBL)
CD200R
(CD200R-9aas-CD28Cys
T cells)
Overlay
I
II
III
IV
Lipid rafts are
enriched
at synapse
10X, DeltaVision

FIG. 2D

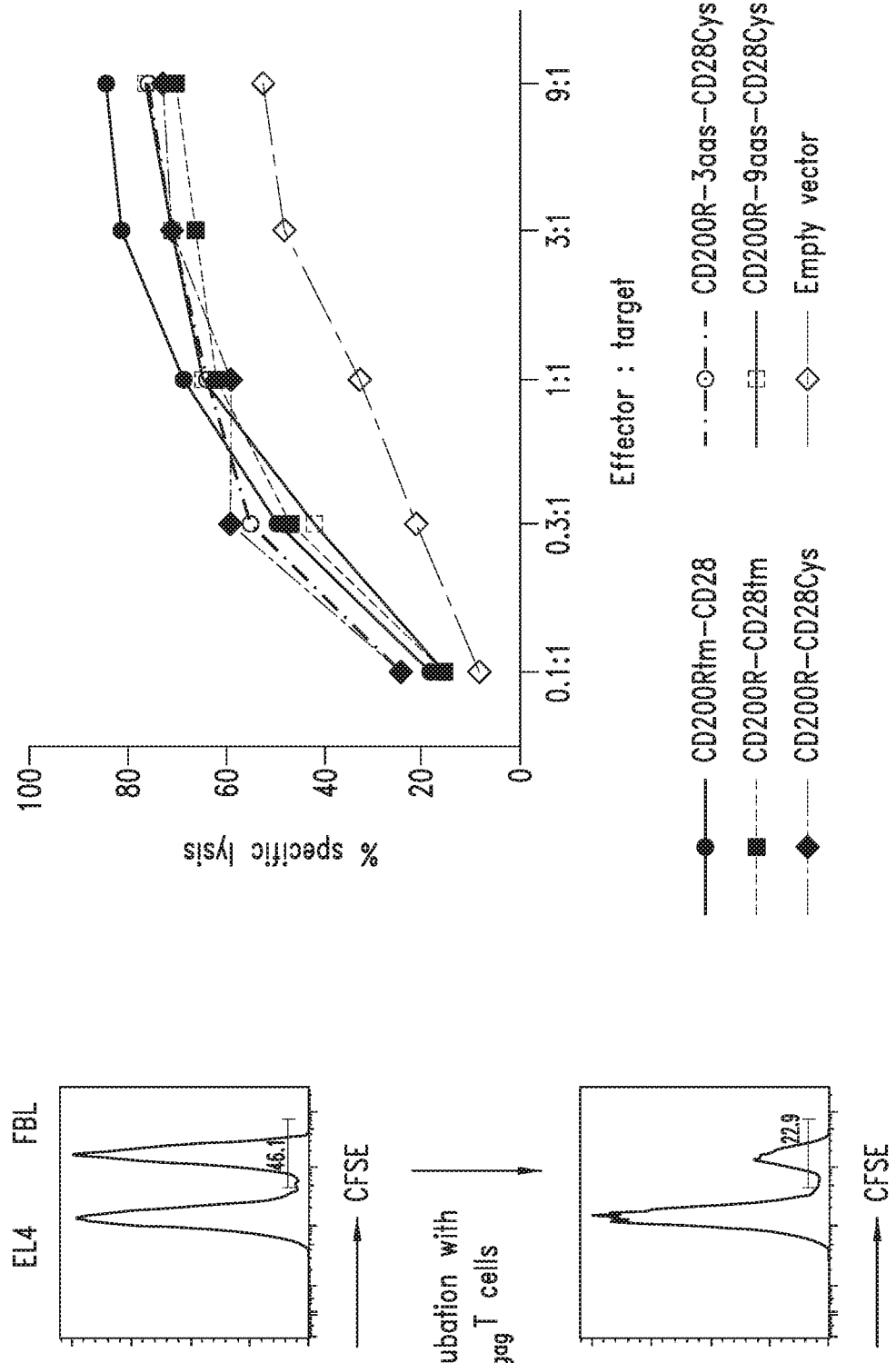

% specific lysis
100
80
60
40
20
0
0.1:1
0.3:1
1:1
3:1
9:1
Effector : target
CD200Rtm-CD28
CD200R-CD28tm
CD200R-CD28Cys
CD200R-3aas-CD28Cys
CD200R-9aas-CD28Cys
Empty vector
EL4
FBL
46.1
22.9
CFSE
CFSE
5h incubation with
TCR$_{gag}$ T cells

FIG. 2E

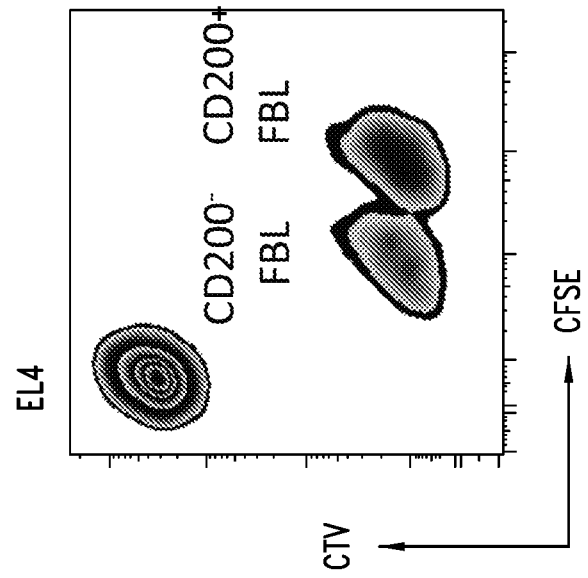


FIG. 2F

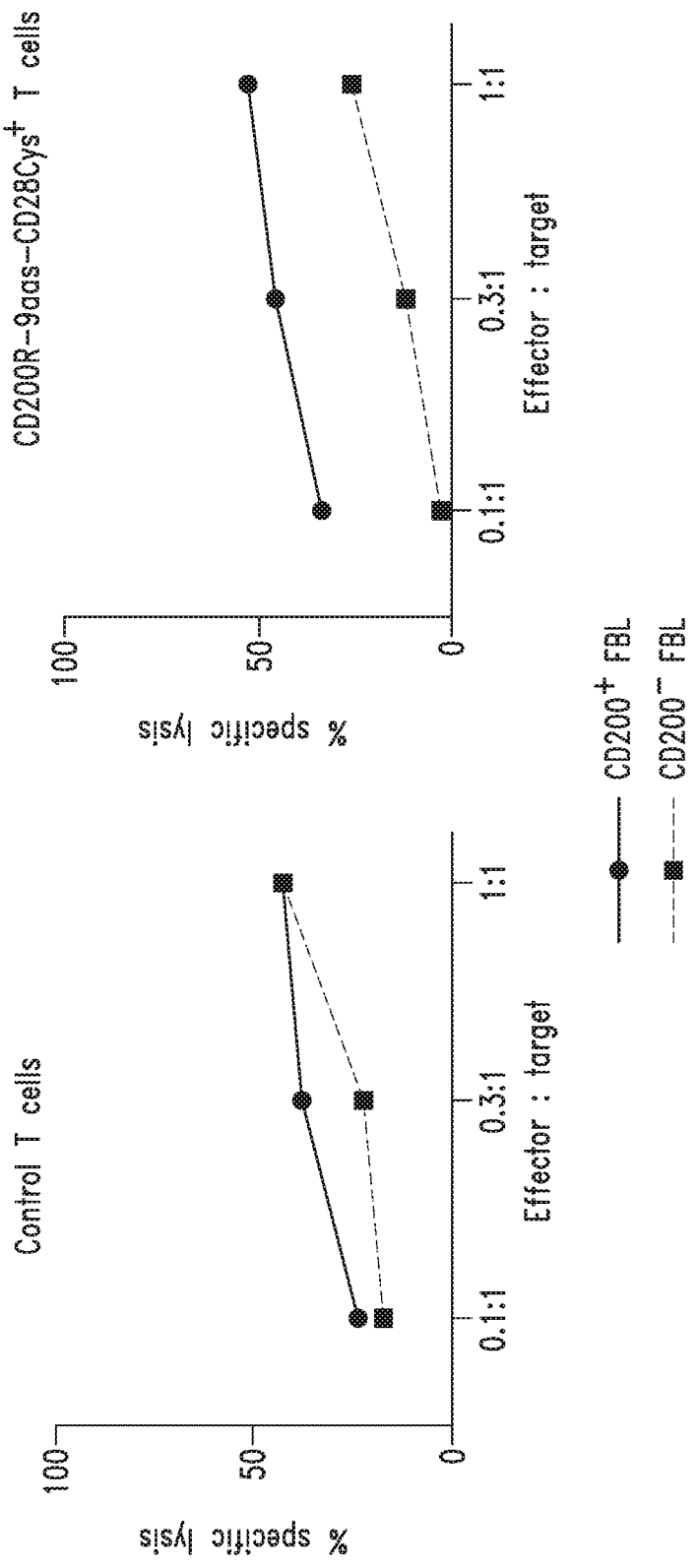

CD200R-9aas-CD28Cys+ T cells
Control T cells
% specific lysis
100
50
0
0.1:1
0.3:1
1:1
Effector : target
CD200+ FBL
CD200− FBL

FIG. 2G

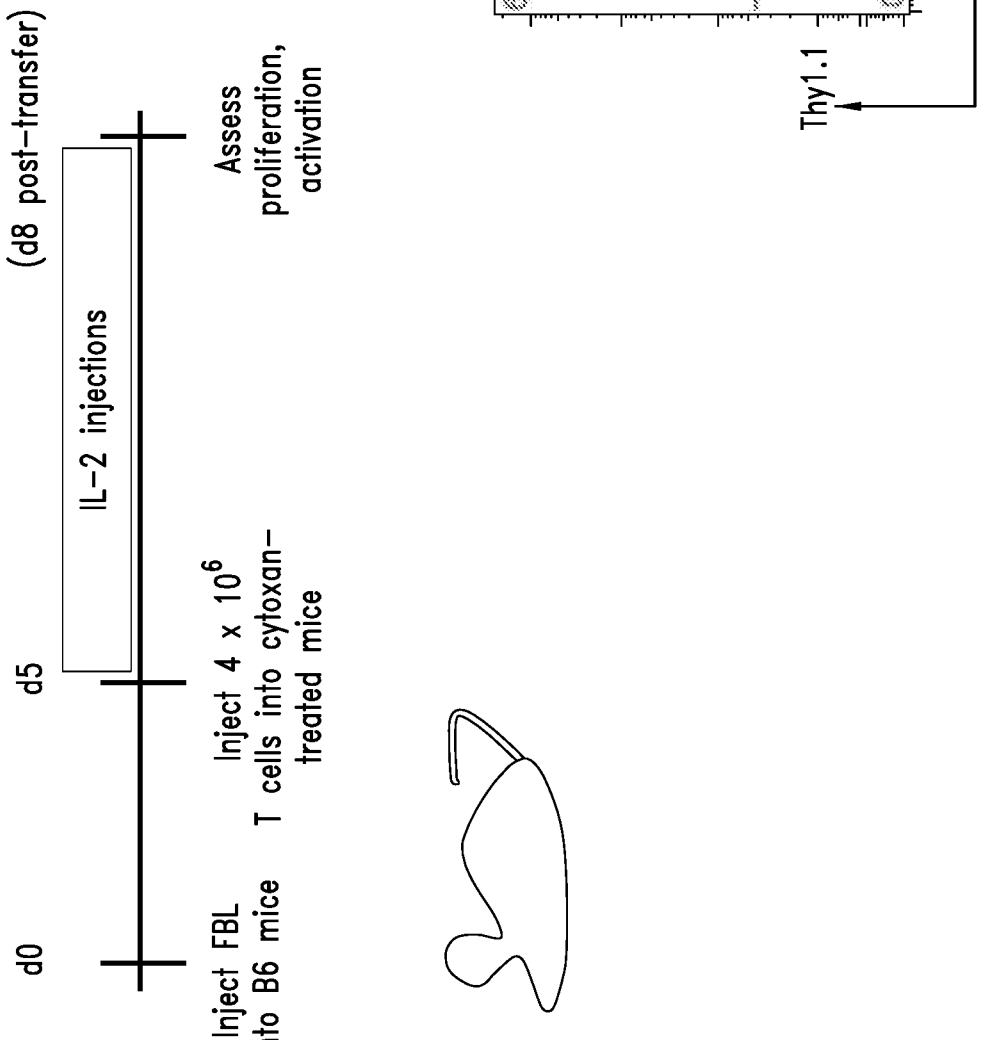

d0
d5
d13
(d8 post-transfer)
IL-2 injections
Inject FBL into B6 mice
Inject 4 x 10^6 T cells into cytoxan-treated mice
Assess proliferation, activation
Injected:
CD200R construct
Empty vector
23.7
36.3
0
0
Thy1.1
Thy1.2

FIG. 3A

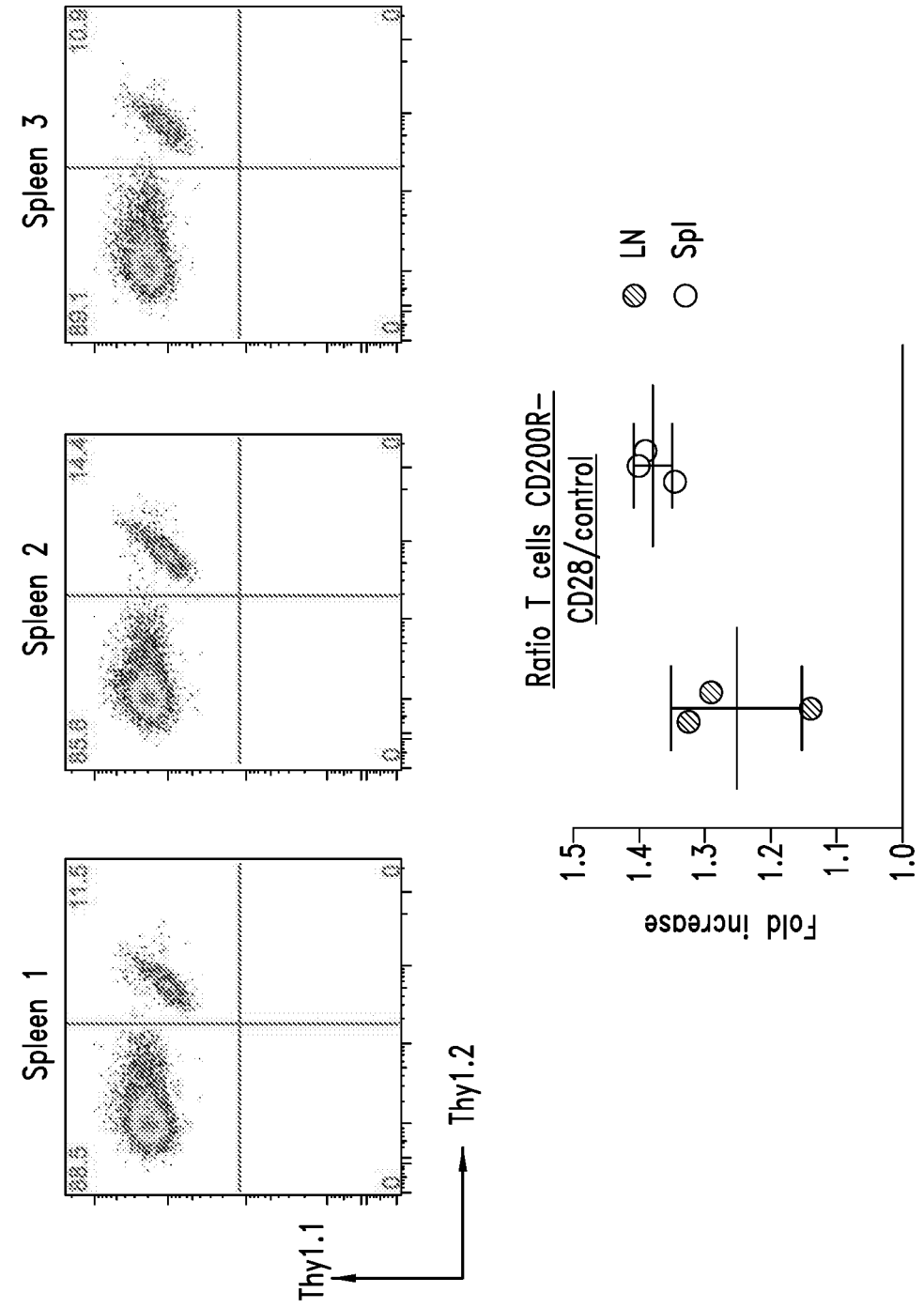

Spleen 1
Spleen 2
Spleen 3
Thy1.1
Thy1.2
Ratio T cells CD200R-
CD28/control
LN
Spl
Fold increase
1.5
1.4
1.3
1.2
1.1
1.0

FIG. 3B

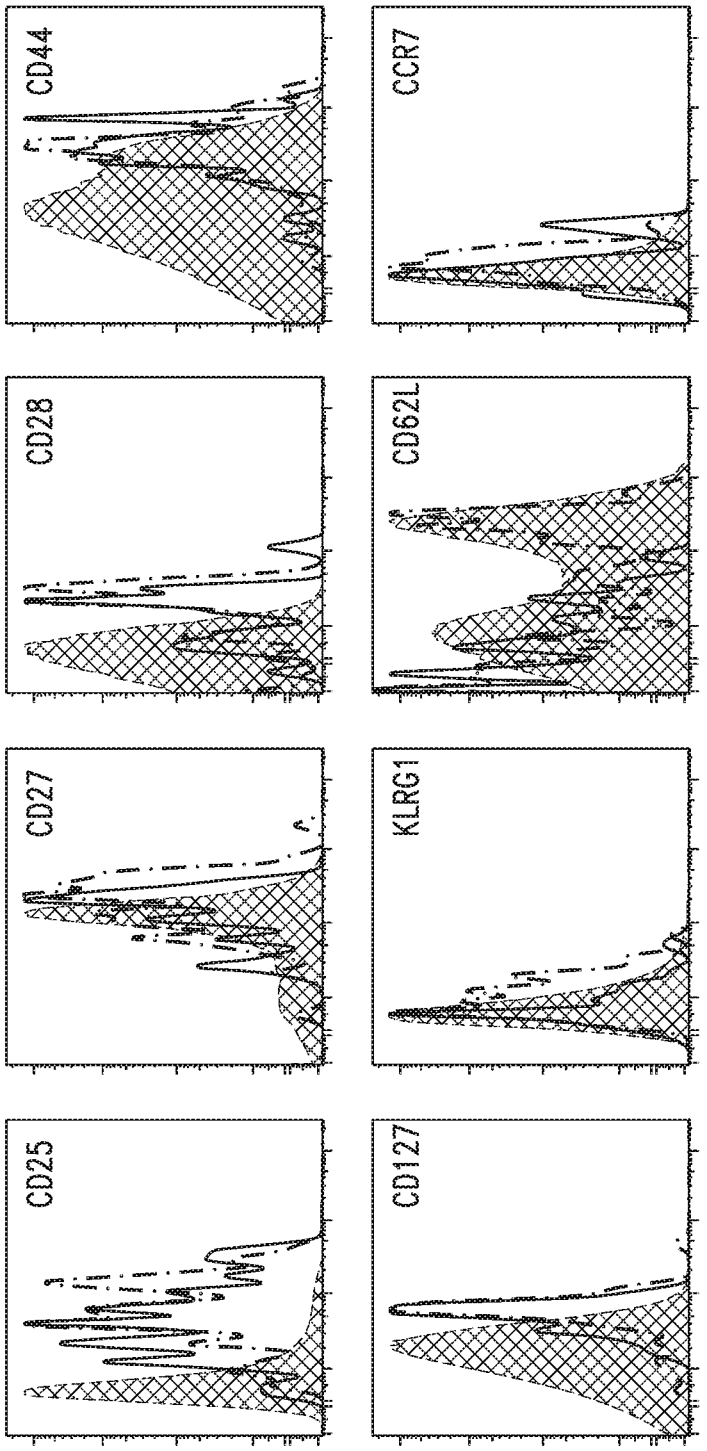

CD25
CD27
CD28
CD44
CD127
KLRG1
CD62L
CCR7
CD200R−9aas−CD28Cys+ T cells
Empty vector T cells
Endogenous T cells

FIG. 3C

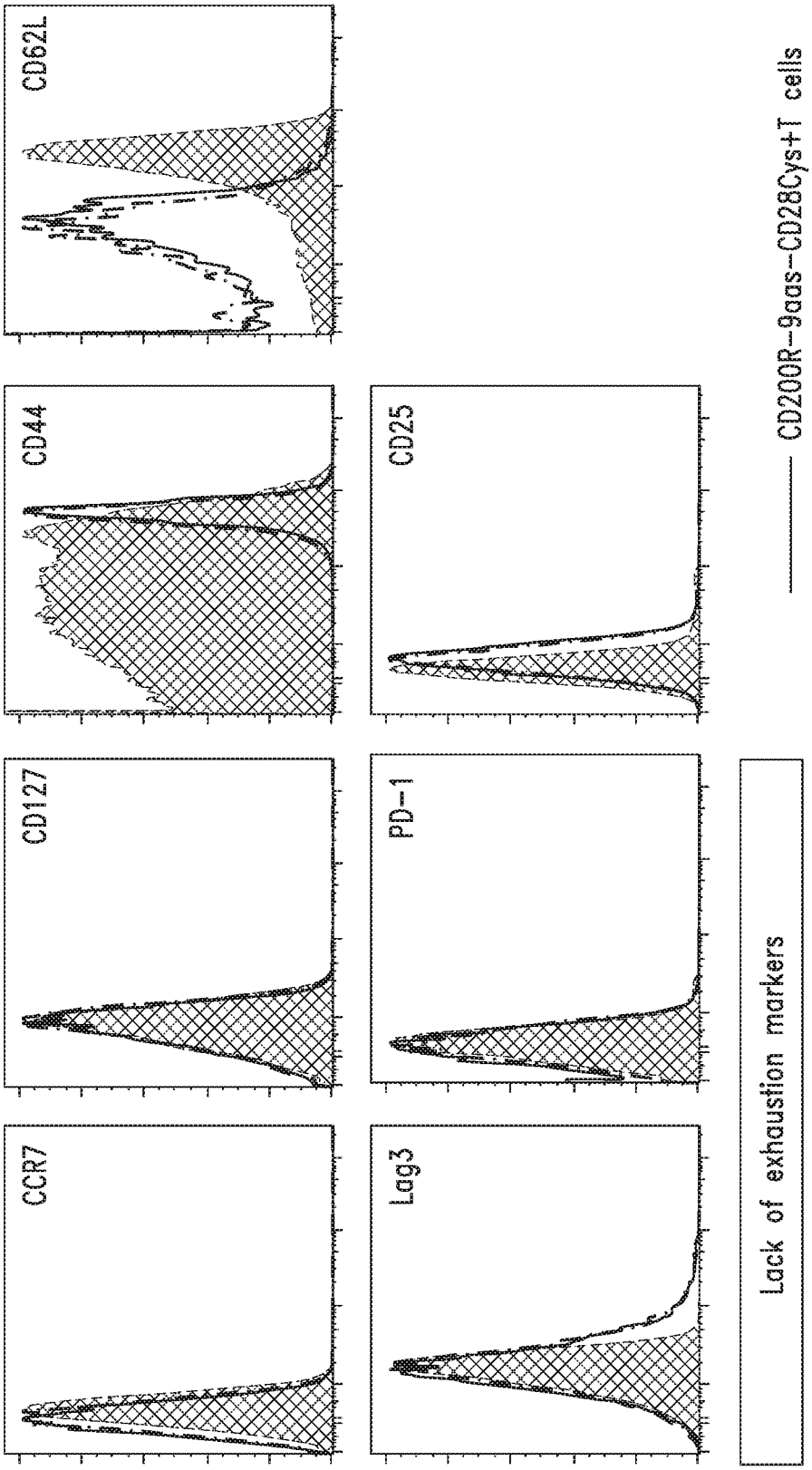

CCR7
CD127
CD44
CD62L
Lag3
PD-1
CD25
Lack of exhaustion markers
CD200R-9aas-CD28Cys+T cells
Empty vector T cells
Endogenous T cells

FIG. 3D

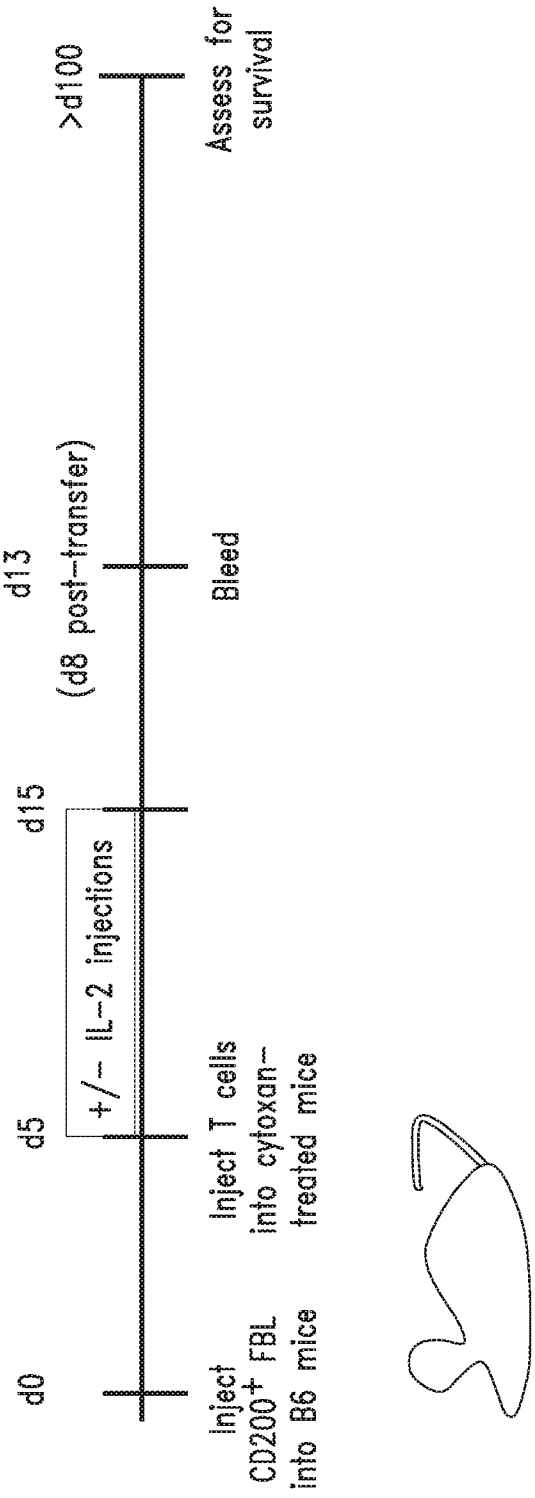

d0
d5
d15
d13
(d8 post-transfer)
>d100
+/- IL-2 injections
Inject CD200+ FBL into B6 mice
Inject T cells into cytoxan-treated mice
Bleed
Assess for survival

FIG. 4A

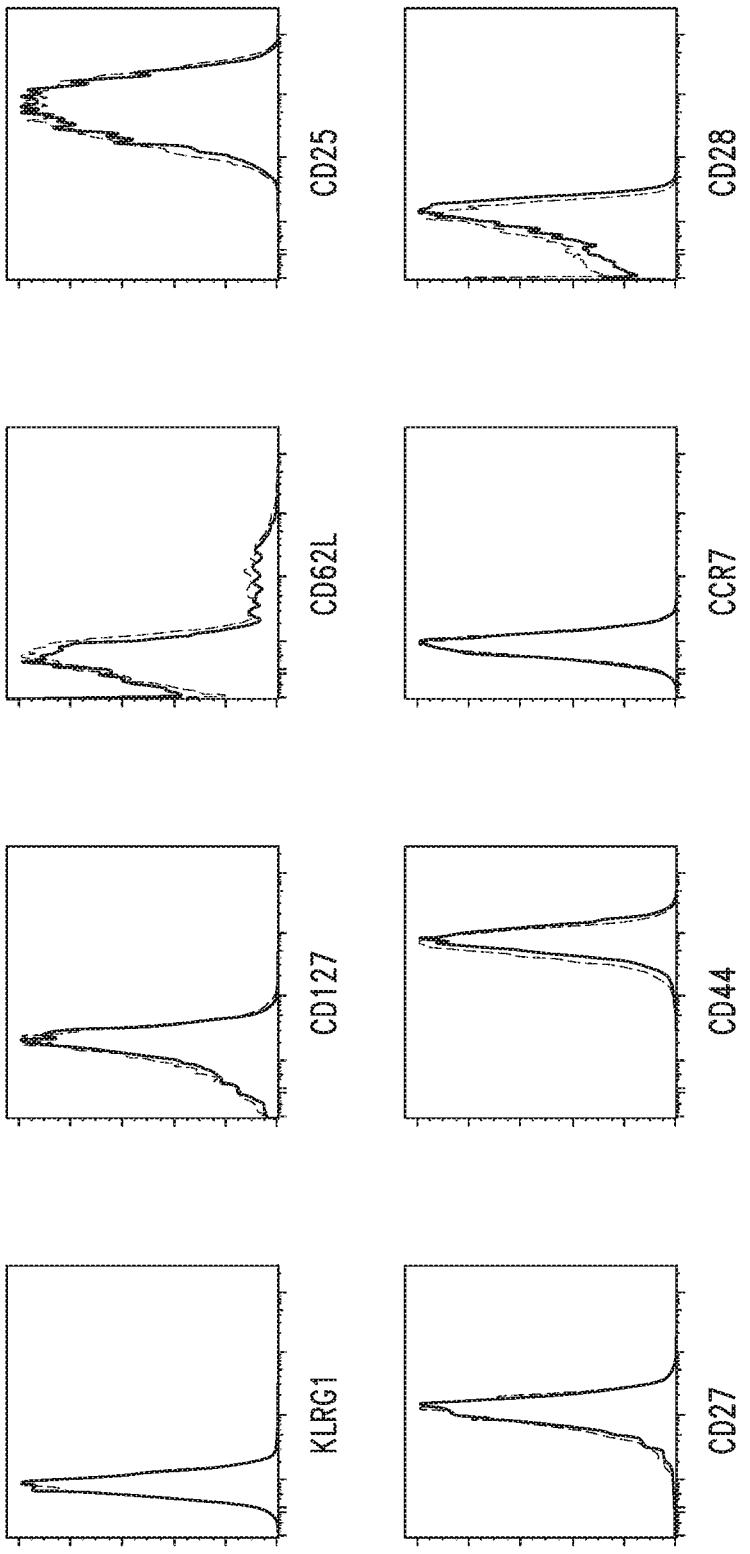

KLRG1
CD127
CD62L
CD25
CD27
CD44
CCR7
CD28
CD200R-CD28+
CD200R-CD28-
d5 post-stim 3

FIG. 4B

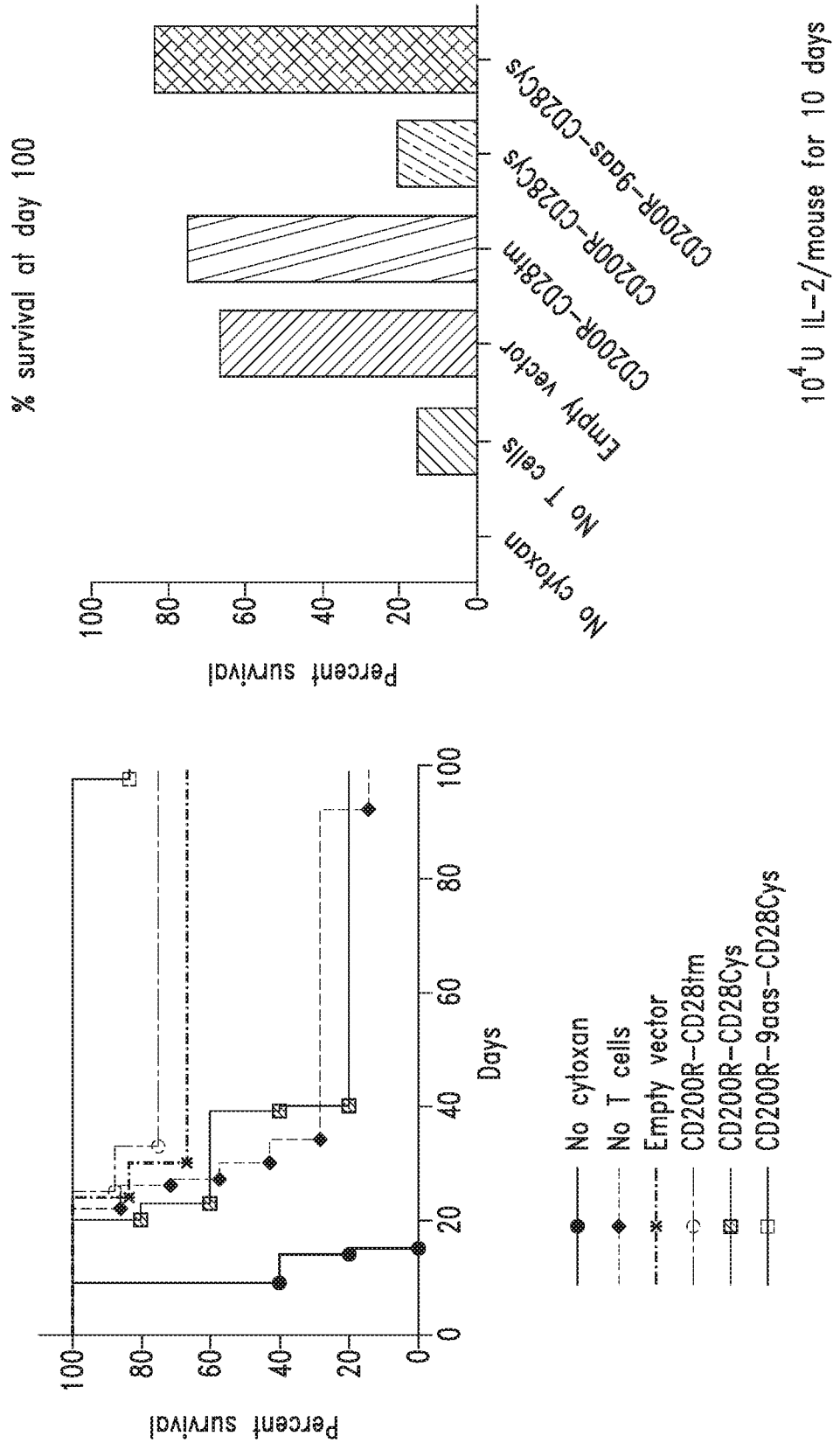
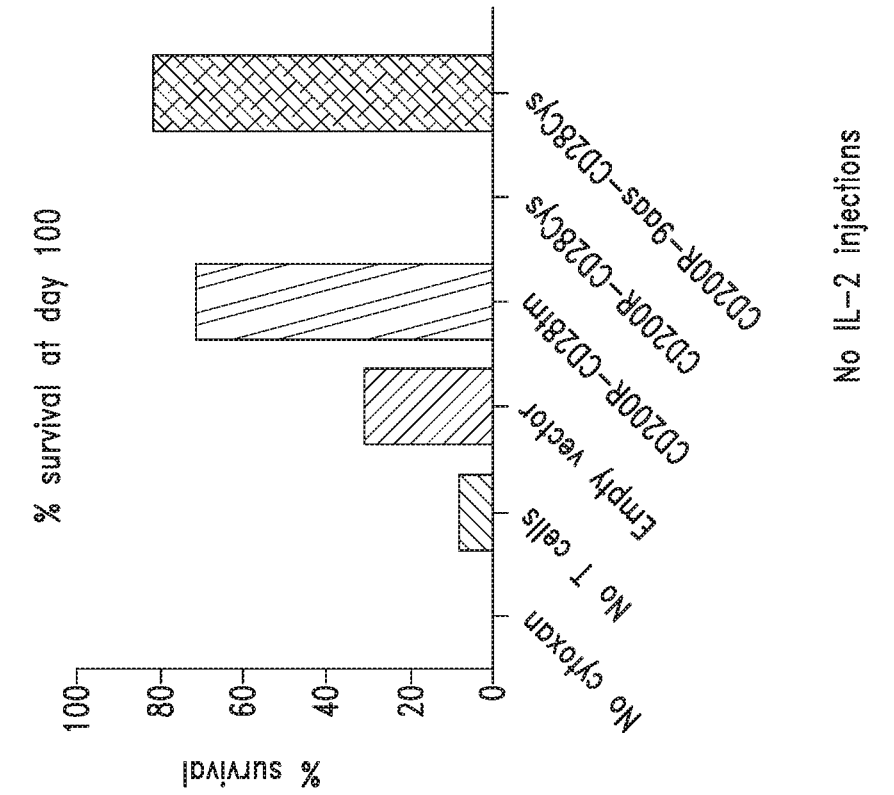

Percent survival
Days
0
20
40
60
80
100
No cytoxan
No T cells
Empty vector
CD200R-CD28tm
CD200R-CD28Cys
CD200R-9aas-CD28Cys
% survival at day 100
10^4 U IL-2/mouse for 10 days

FIG. 4C


% survival at day 100
100
80
60
40
20
0
No cytoxan
No T cells
Empty vector
CD200R-CD28tm
CD200R-CD28Cys
CD200R-9aas-CD28Cys
No IL-2 injections

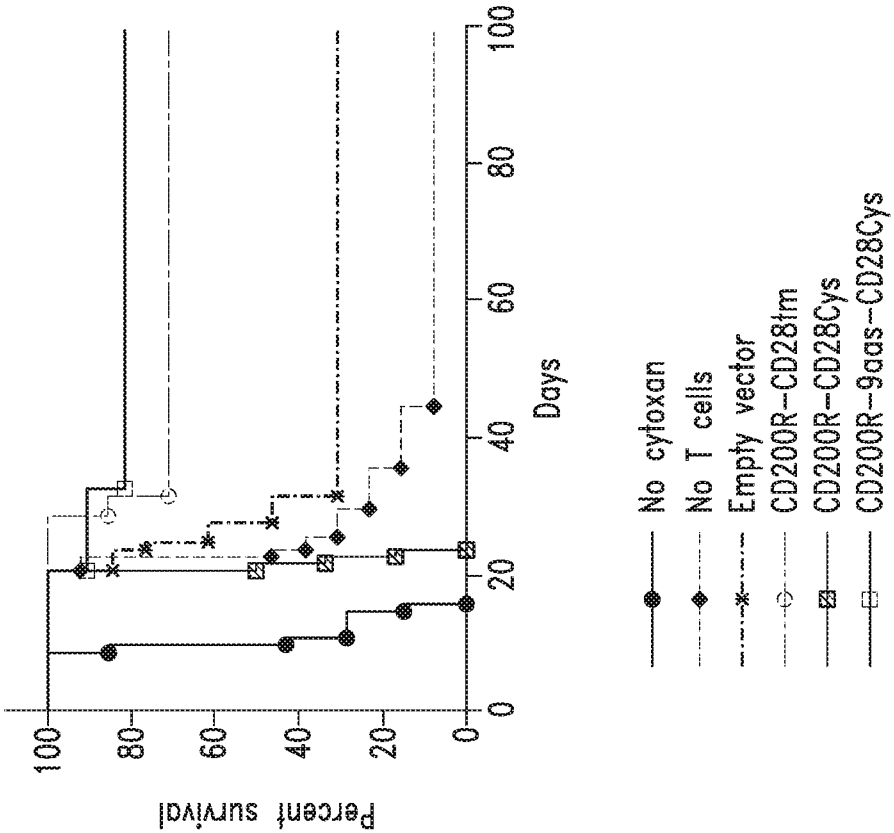

Percent survival
% survival
100
80
60
40
20
0
0
20
40
60
80
100
Days
No cytoxan
No T cells
Empty vector
CD200R-CD28tm
CD200R-CD28Cys
CD200R-9aas-CD28Cys

FIG. 4D

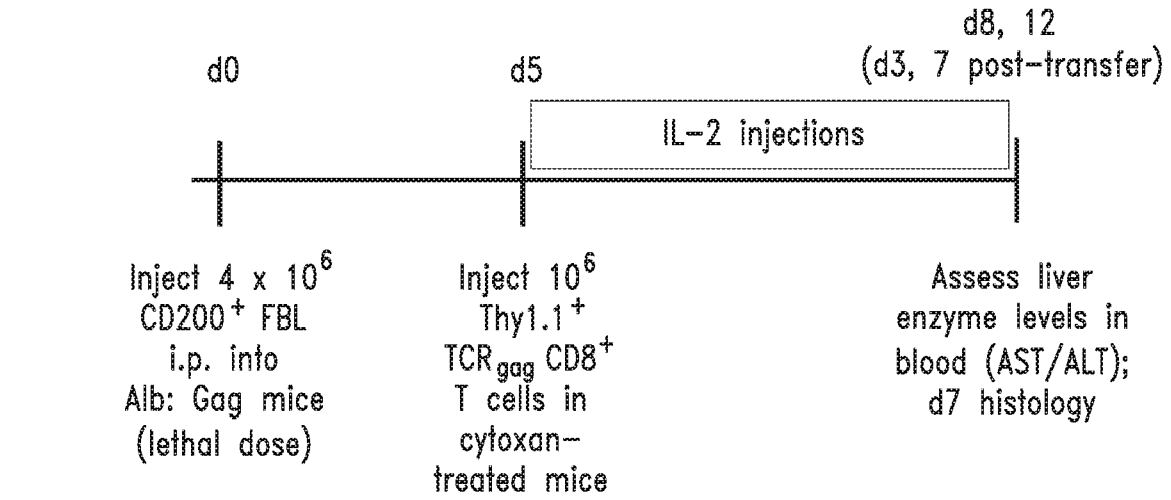

d0
d5
d8, 12
(d3, 7 post-transfer)
IL-2 injections
Inject 4 x 10^6 CD200+ FBL i.p. into Alb: Gag mice (lethal dose)
Inject 10^6 Thy1.1+ TCRgag CD8+ T cells in cytoxan-treated mice
Assess liver enzyme levels in blood (AST/ALT); d7 histology



FIG. 5A

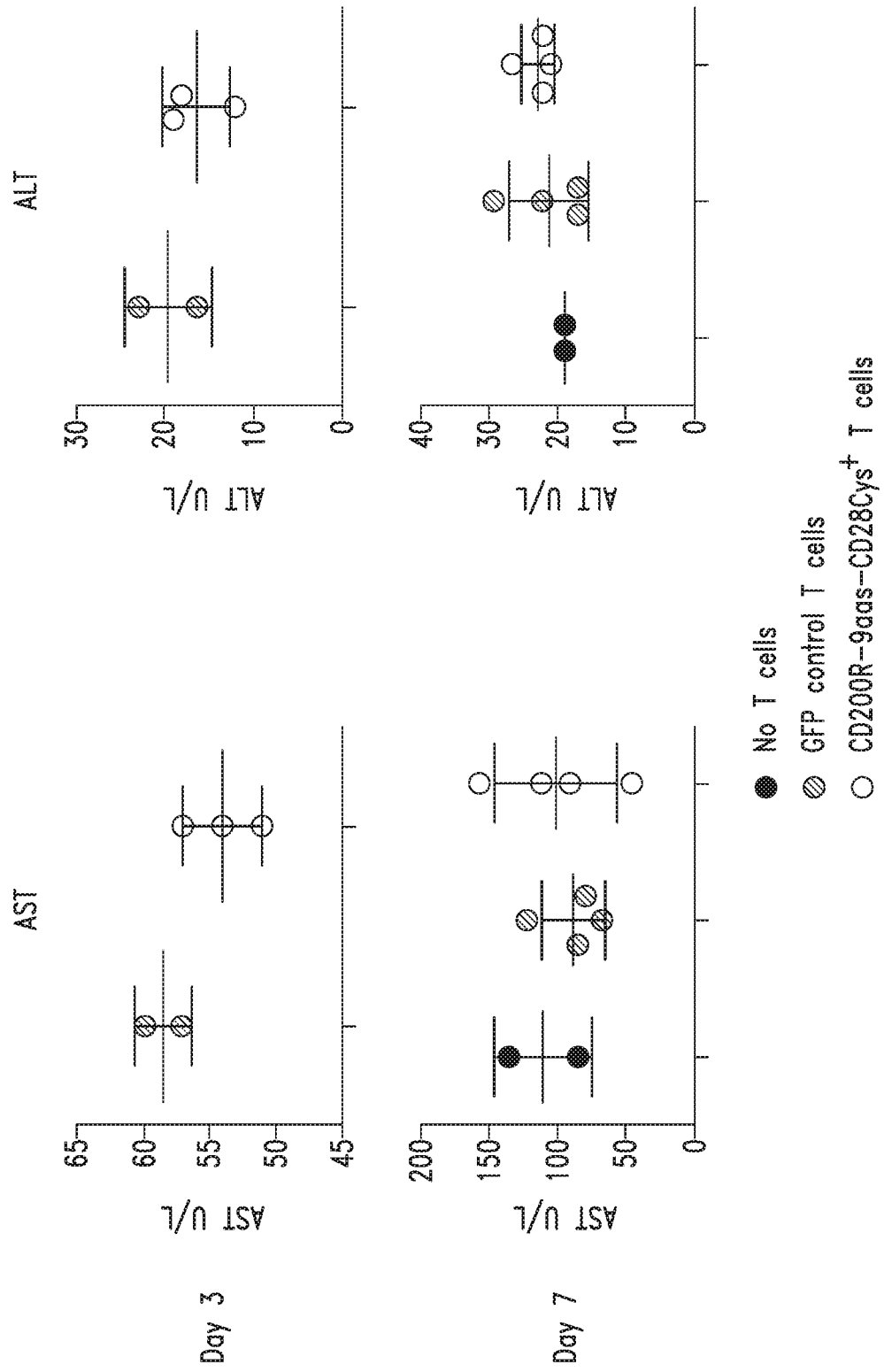

AST
ALT
Day 3
Day 7
AST U/L
ALT U/L
65
60
55
50
45
200
150
100
50
0
40
30
20
10
No T cells
GFP control T cells
CD200R-9aas-CD28Cys+ T cells

FIG. 5B

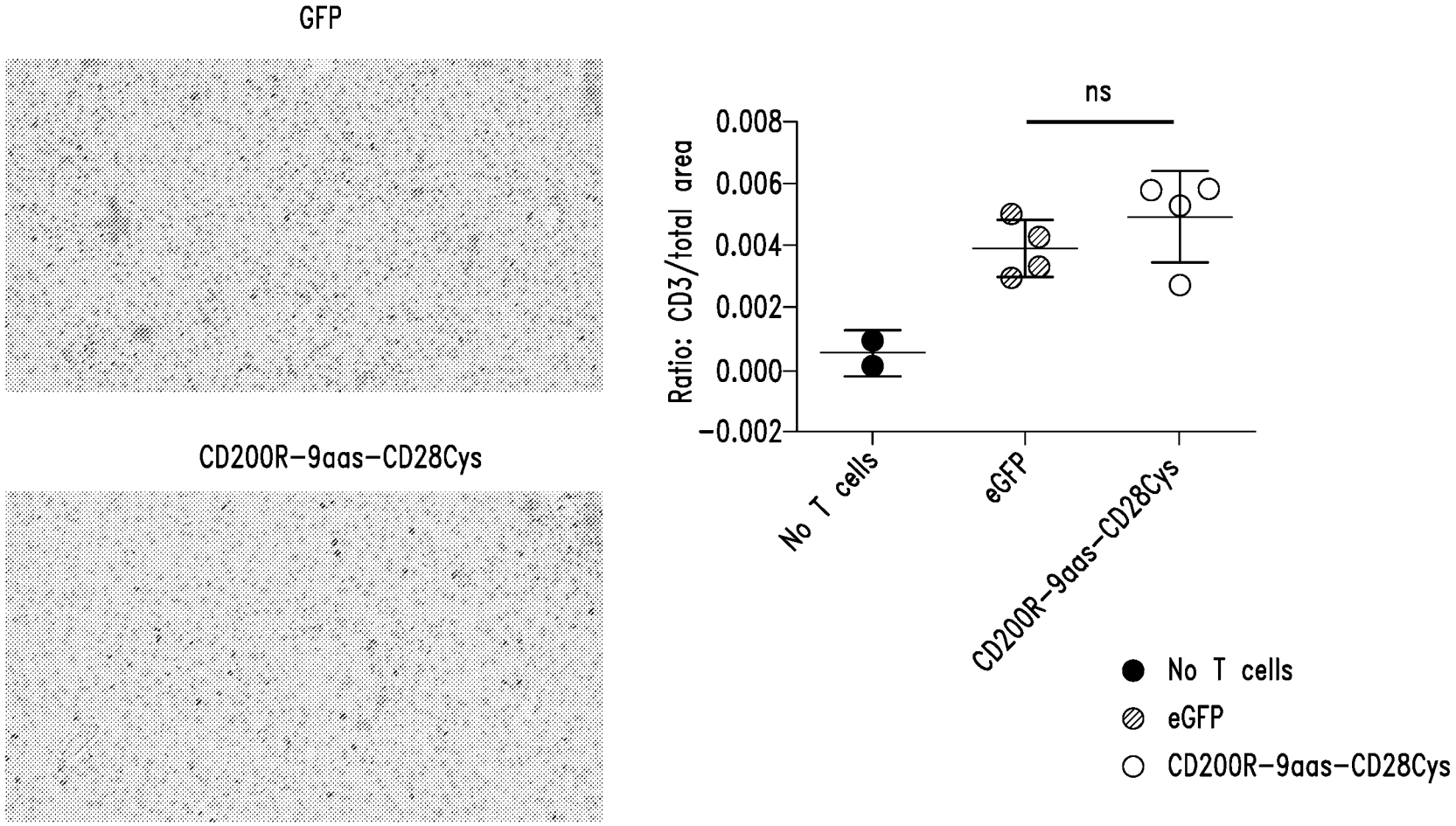

GFP
CD200R-9aas-CD28Cys
ns
Ratio: CD3/total area
0.008
0.006
0.004
0.002
0.000
-0.002
No T cells
eGFP
CD200R-9aas-CD28Cys
No T cells
eGFP
CD200R-9aas-CD28Cys

FIG. 5C

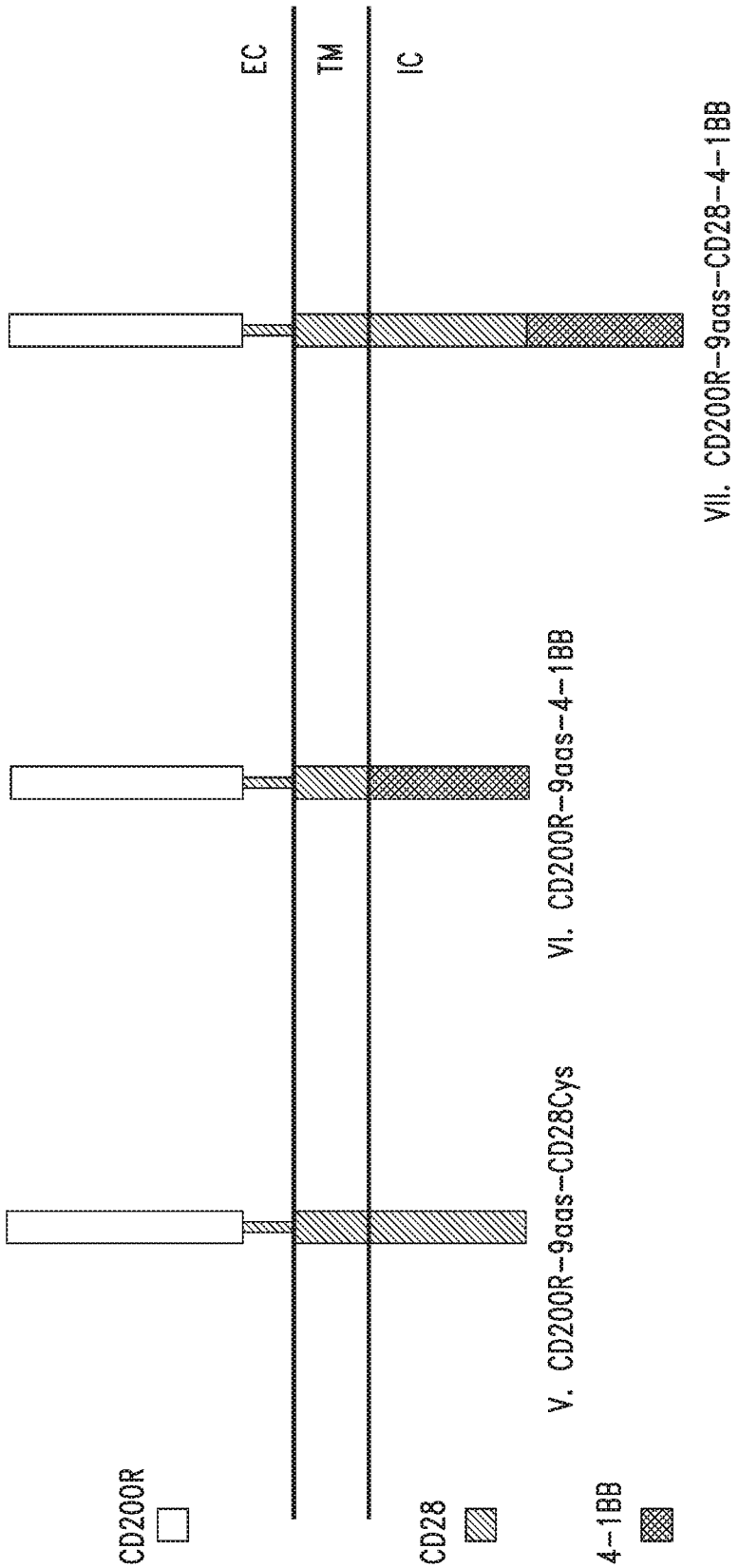

EC
TM
IC
VII. CD200R-9aas-CD28-4-1BB
VI. CD200R-9aas-4-1BB
V. CD200R-9aas-CD28Cys
CD200R
CD28
4-1BB

FIG. 6A

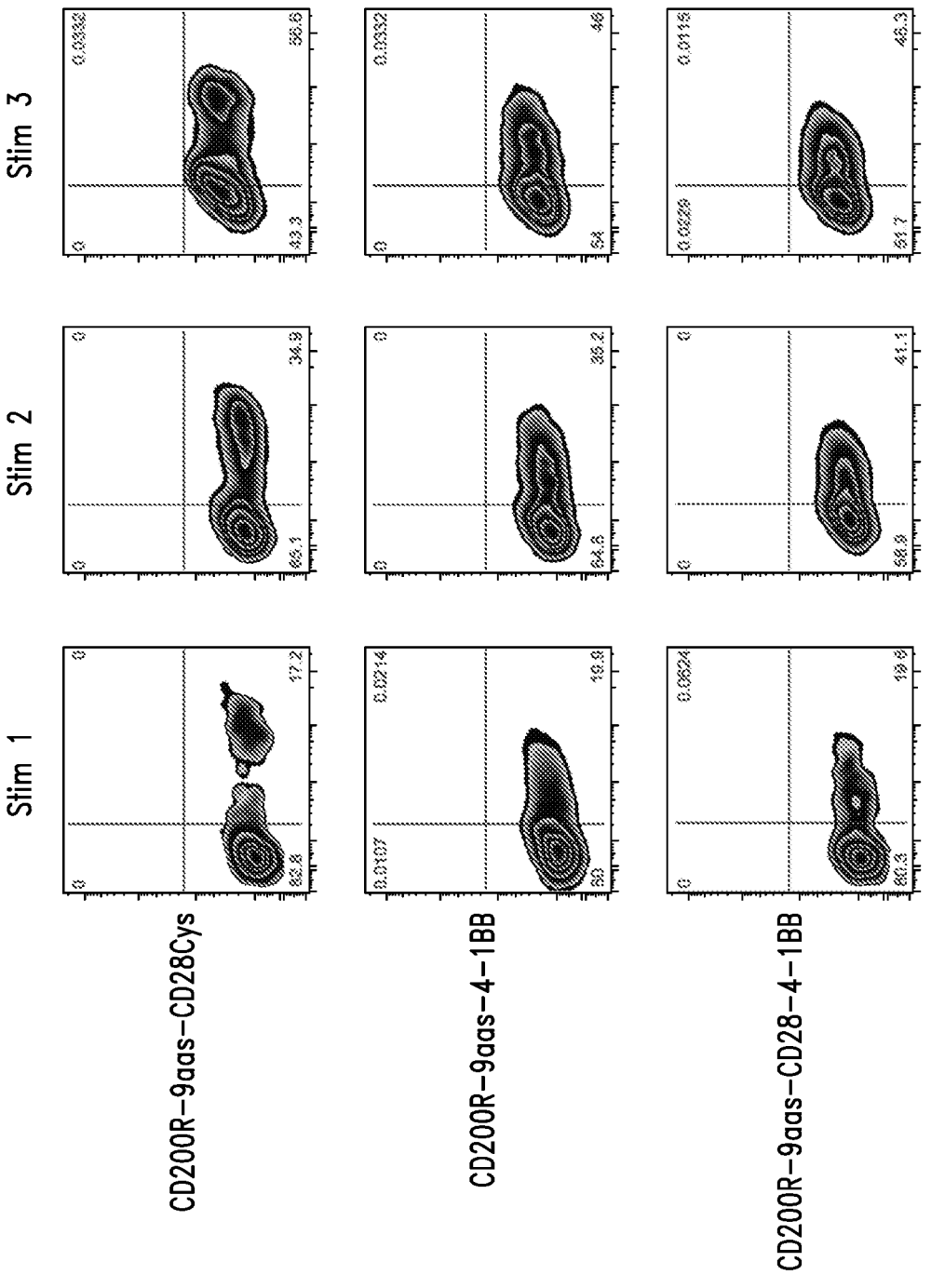

Stim 1
Stim 2
Stim 3
CD200R-9aas-CD28Cys
CD200R-9aas-4-1BB
CD200R-9aas-CD28-4-1BB
CD200R

FIG. 6B

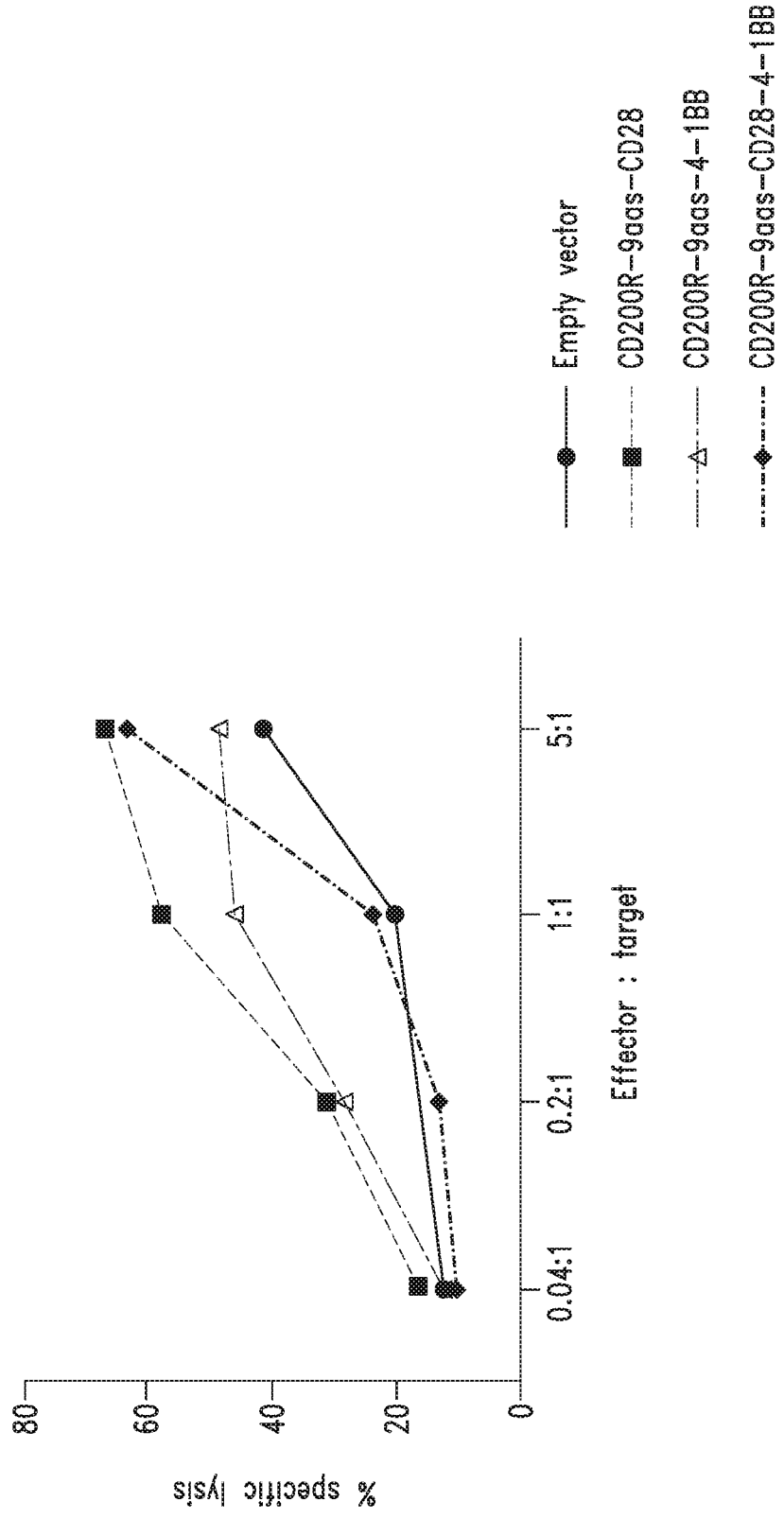

% specific lysis
80
60
40
20
0
0.04:1
0.2:1
1:1
5:1
Effector : target
Empty vector
CD200R-9aas-CD28
CD200R-9aas-4-1BB
CD200R-9aas-CD28-4-1BB

FIG. 6C

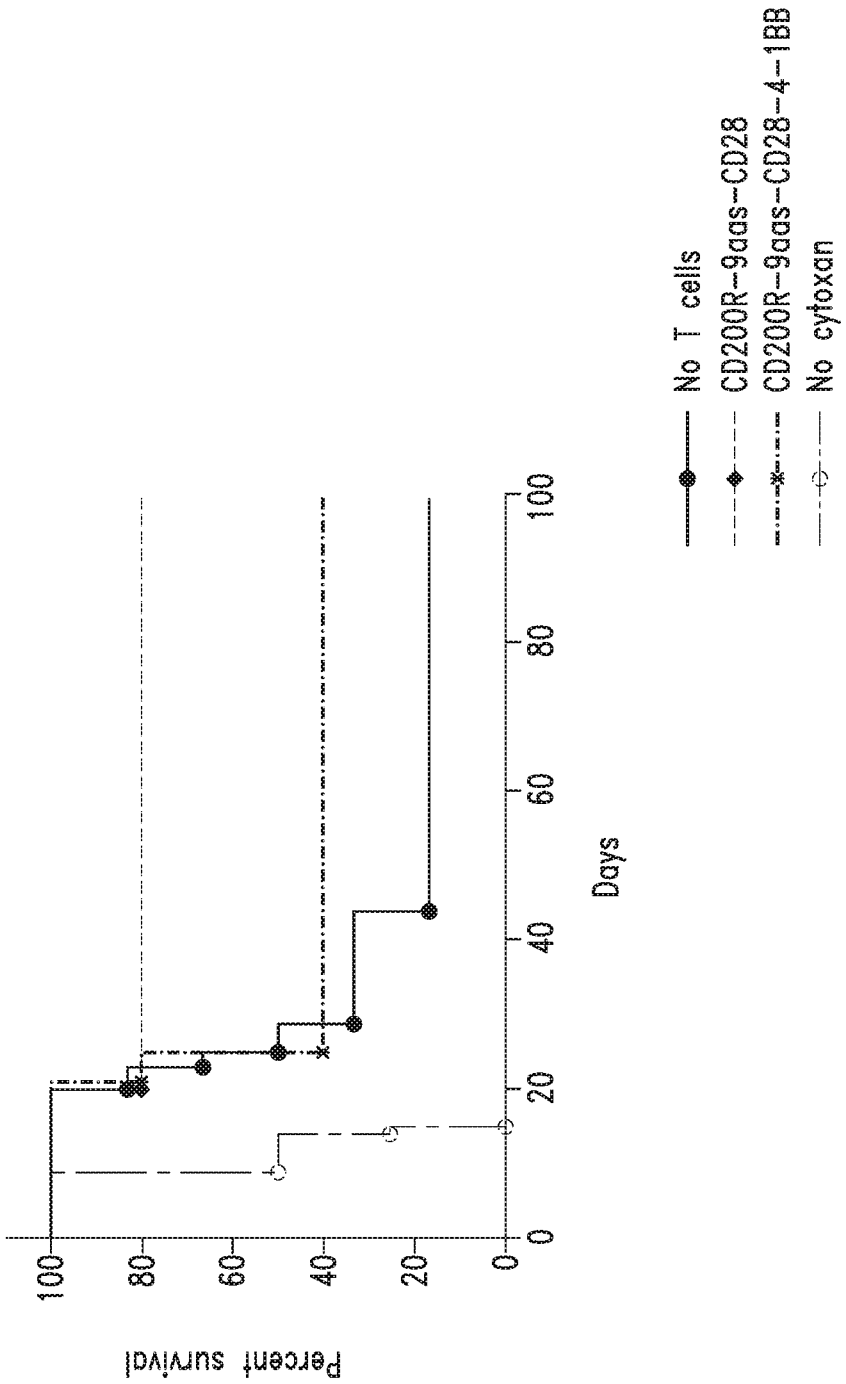

Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
Days
No T cells
CD200R-9aas-CD28
CD200R-9aas-CD28-4-1BB
No cytoxan

FIG. 6D

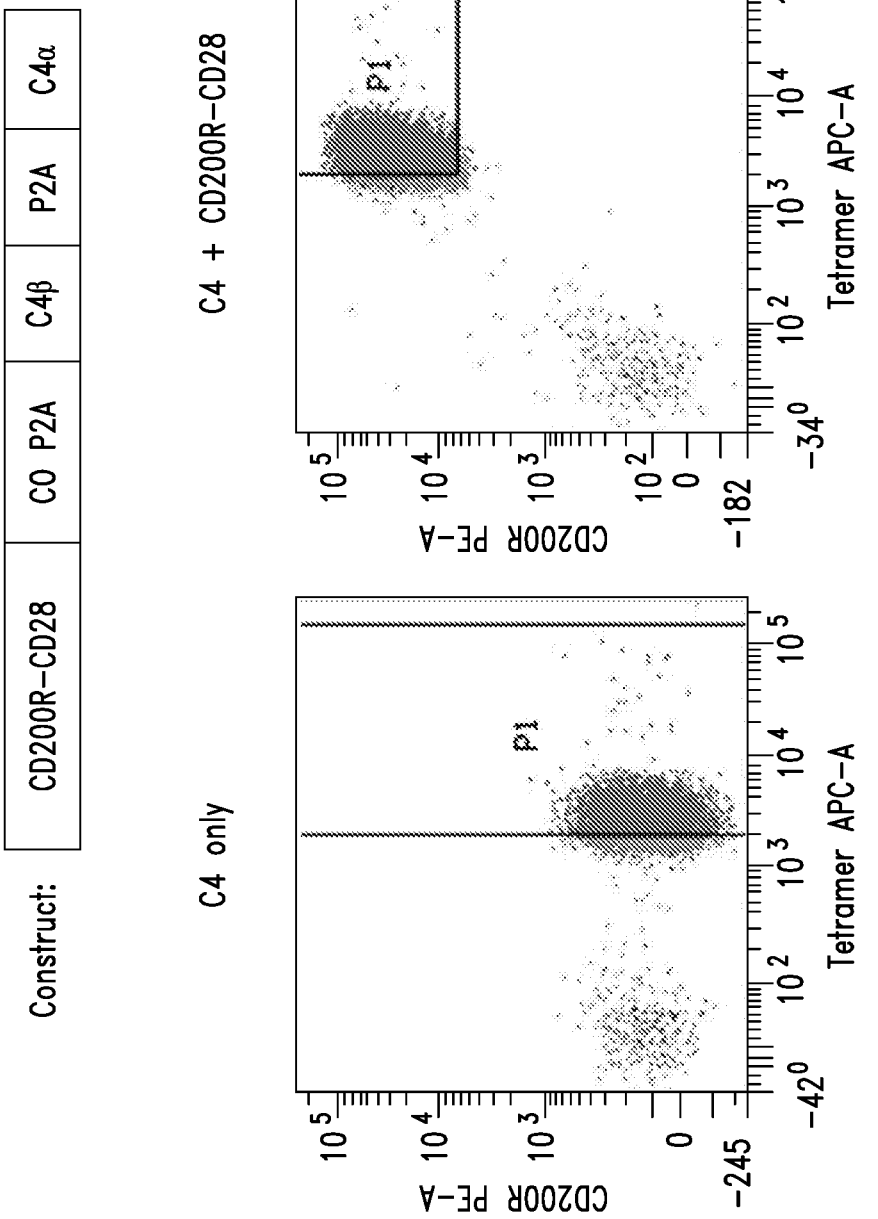

Construct:
CD200R-CD28
CO P2A
Cβ
P2A
Cα
C4 only
C4 + CD200R-CD28
P1
CD200R PE-A
Tetramer APC-A
-245
-42
-182
-34

FIG. 7A

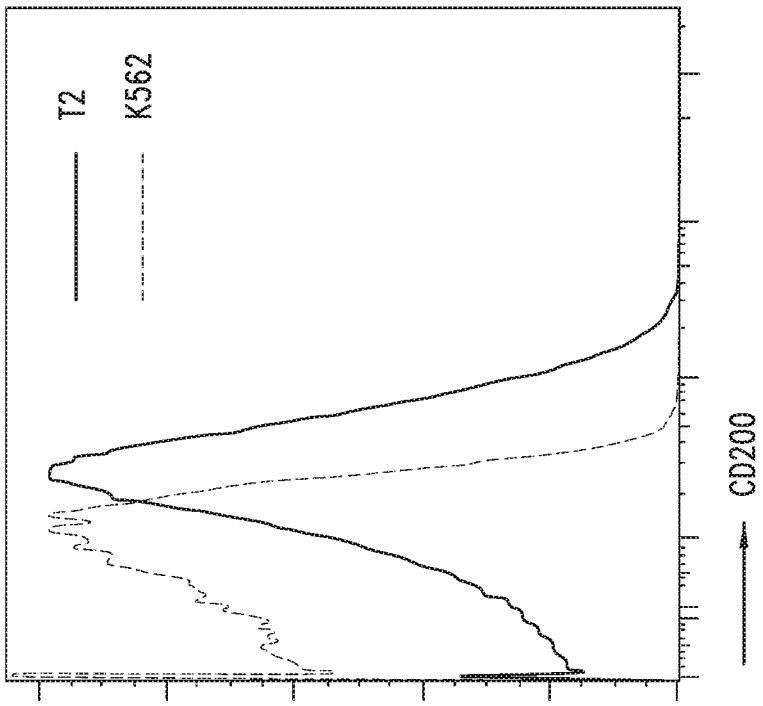


FIG. 7B

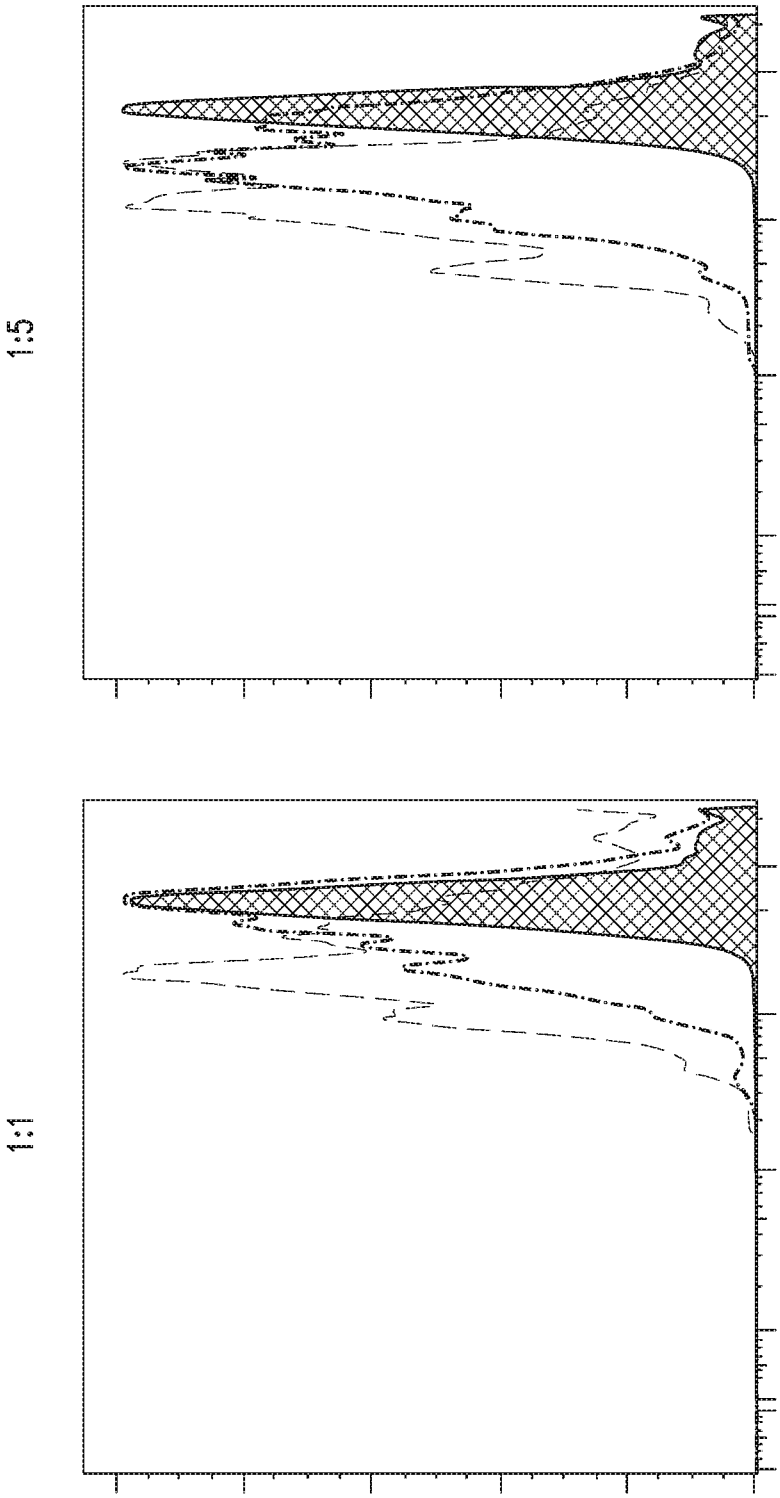


FIG. 7C

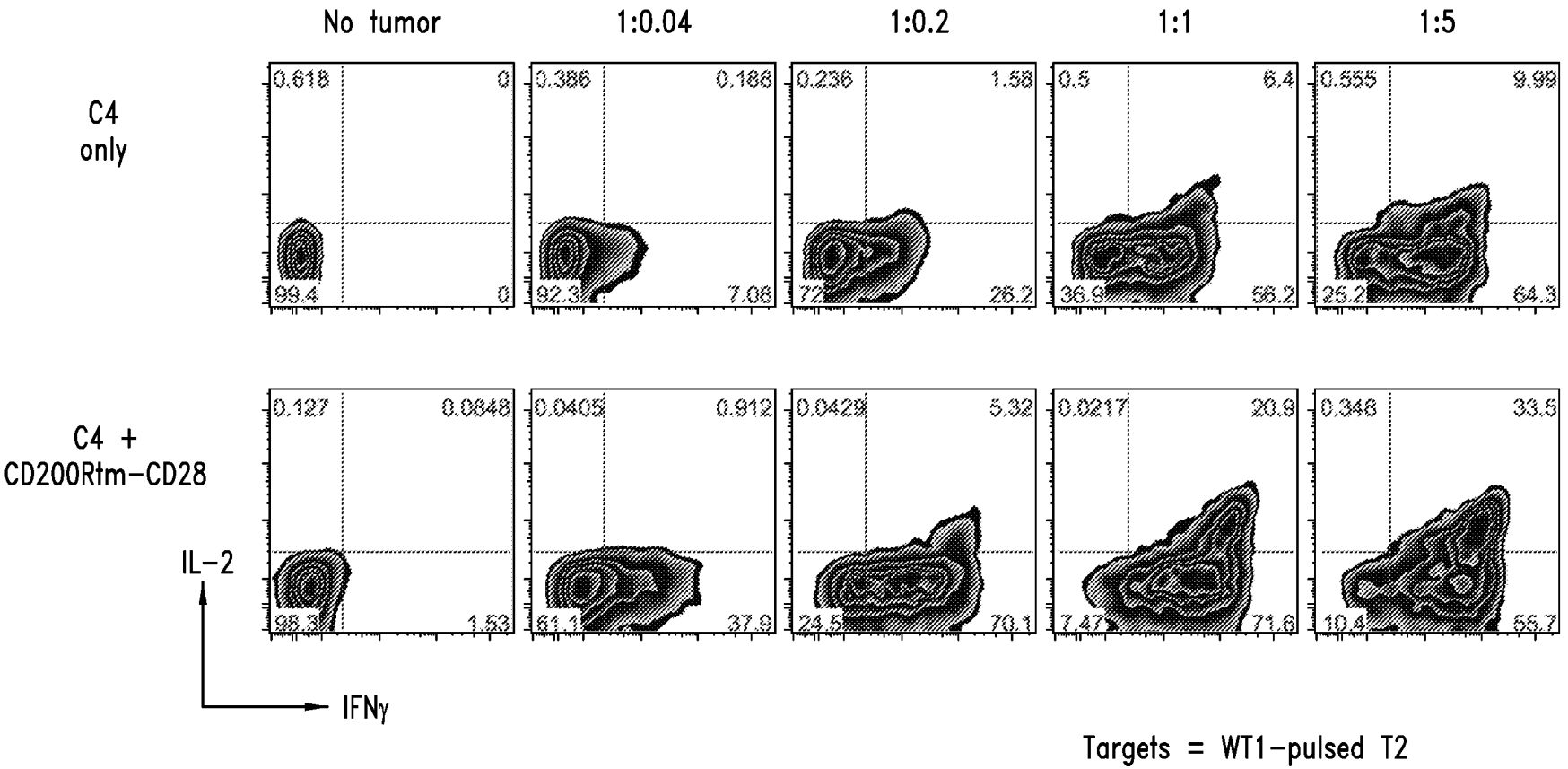

No tumor
1:0.04
1:0.2
1:1
1:5
C4
only
C4 +
CD200Rtm-CD28
0.618
0
99.4
0
0.386
0.186
82.3
7.08
0.236
1.58
72
26.2
0.5
6.4
36.9
56.2
0.555
9.99
25.2
64.3
0.127
0.0848
96.3
1.53
0.0405
0.912
61.1
37.9
0.0429
5.32
24.5
70.1
0.0217
20.9
7.47
71.6
0.346
33.5
10.4
55.7
IL-2
IFNγ
Targets = WT1-pulsed T2

FIG. 7D

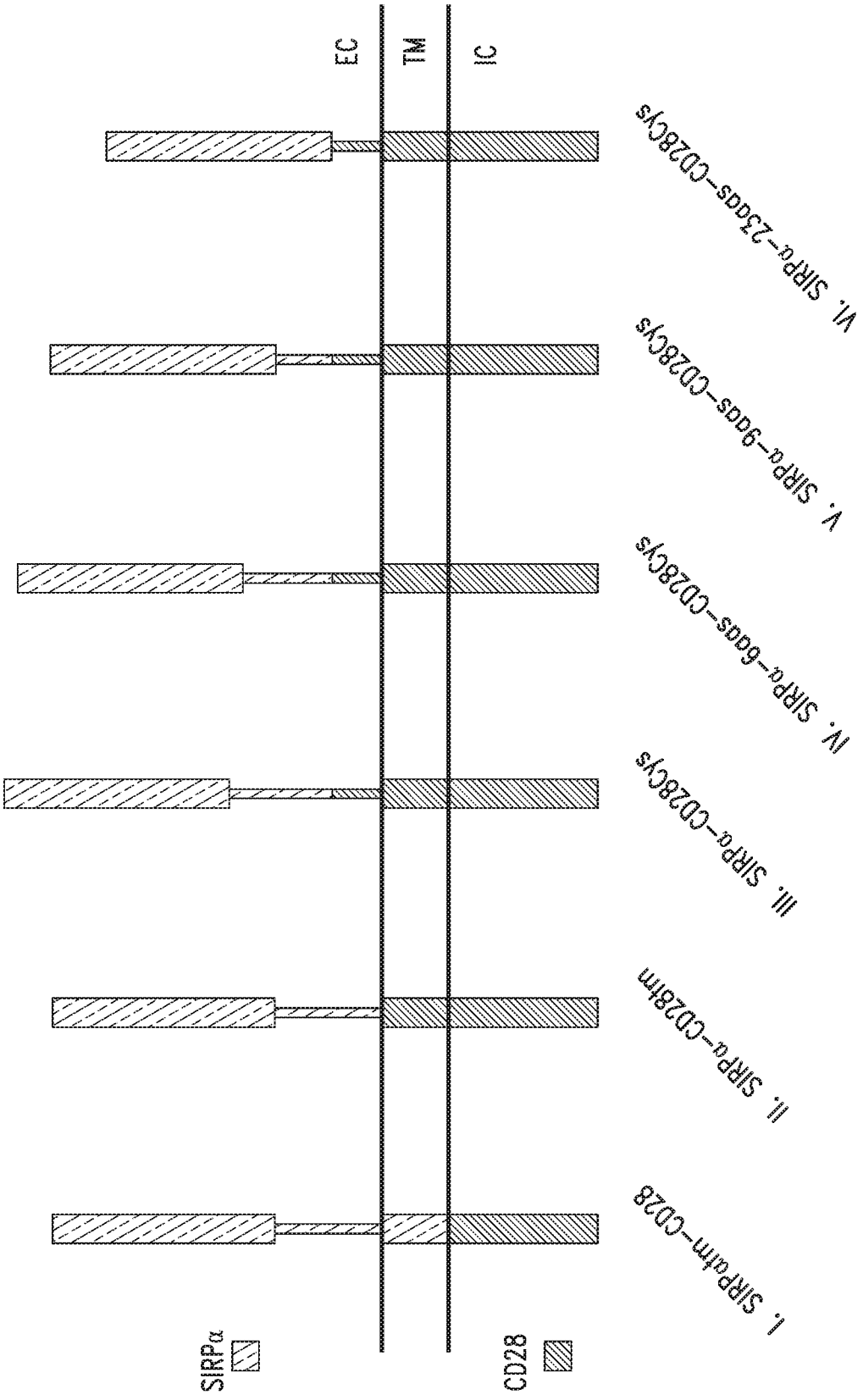

EC
TM
IC
I. SIRPαtm-CD28
II. SIRPα-CD28tm
III. SIRPα-CD28Cys
IV. SIRPα-6aas-CD28Cys
V. SIRPα-9aas-CD28Cys
VI. SIRPα-23aas-CD28Cys
SIRPα
CD28

FIG. 8A

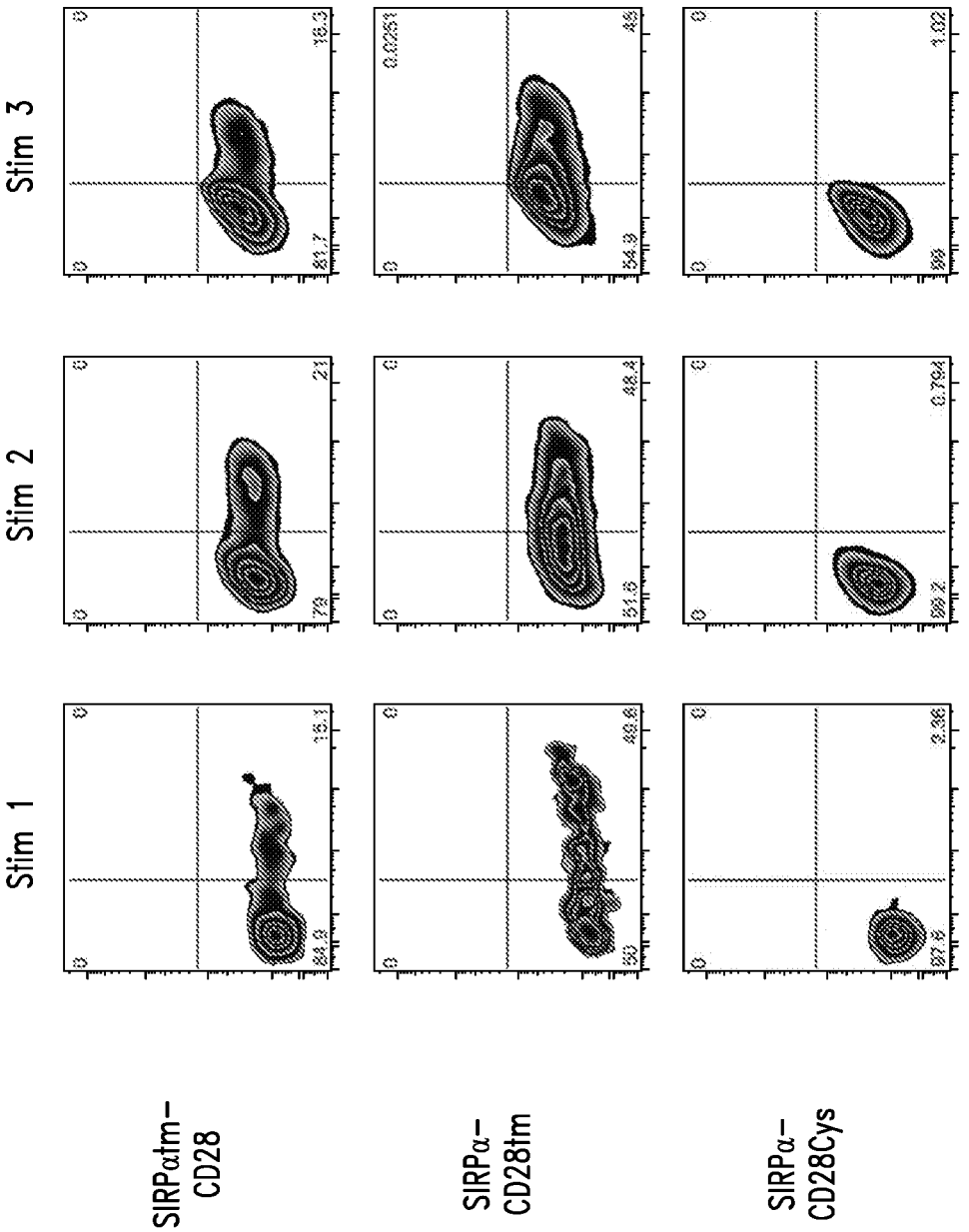

Stim 1
Stim 2
Stim 3
SIRPαtm–CD28
SIRPα–CD28tm
SIRPα–CD28Cys
SIRPα

FIG. 8B

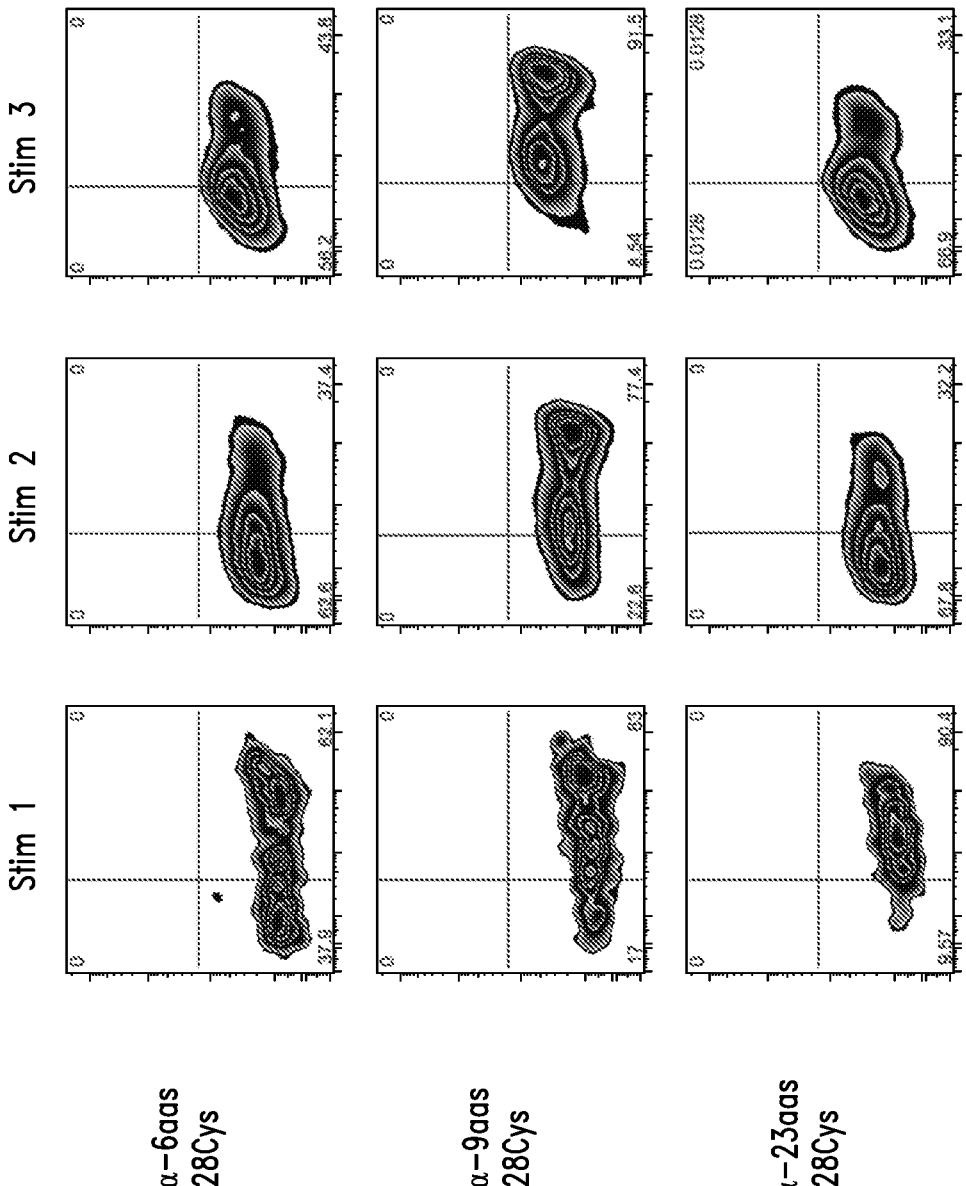

Stim 1
Stim 2
Stim 3
SIRPα−6aas CD28Cys
SIRPα−9aas CD28Cys
SIRPα−23aas CD28Cys
SIRPα

FIG. 8B (Cont.)

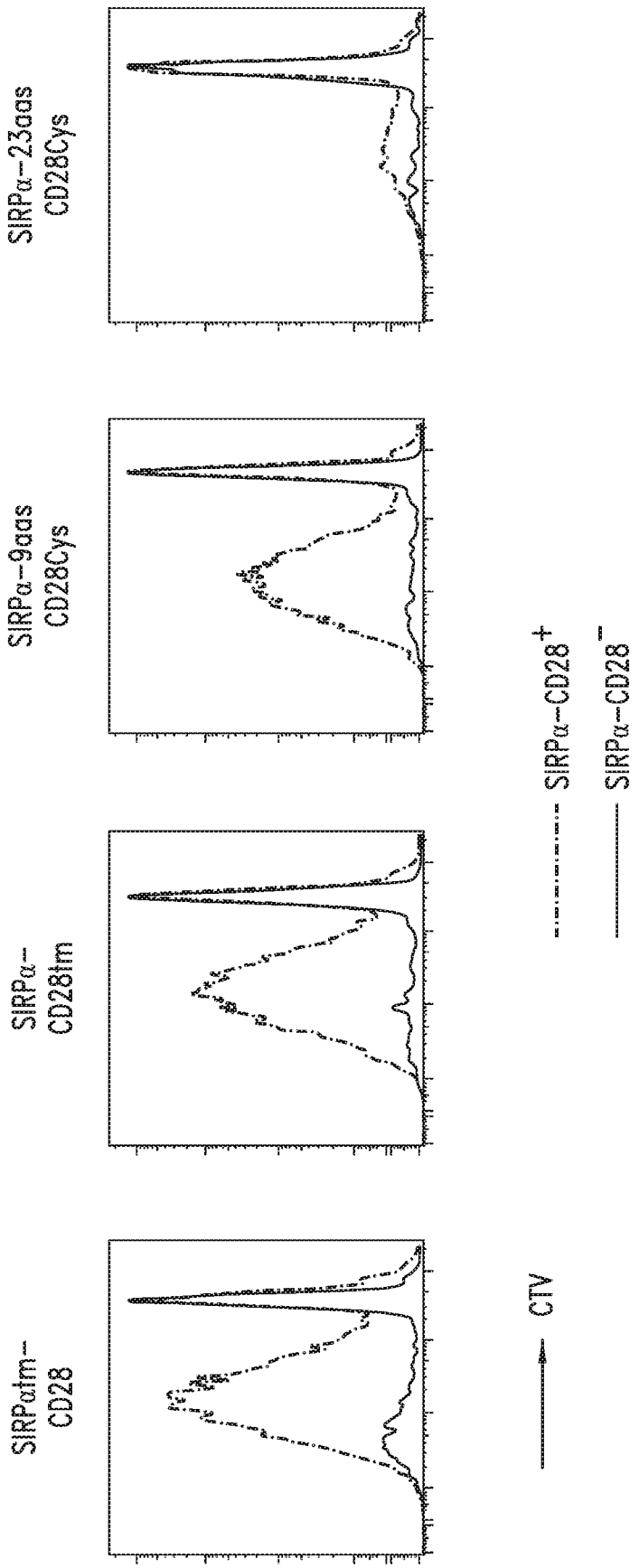

SIRPαtm–CD28
SIRPα–CD28tm
SIRPα–9aas CD28Cys
SIRPα–23aas CD28Cys
CTV
SIRPα–CD28+
SIRPα–CD28–

FIG. 8C

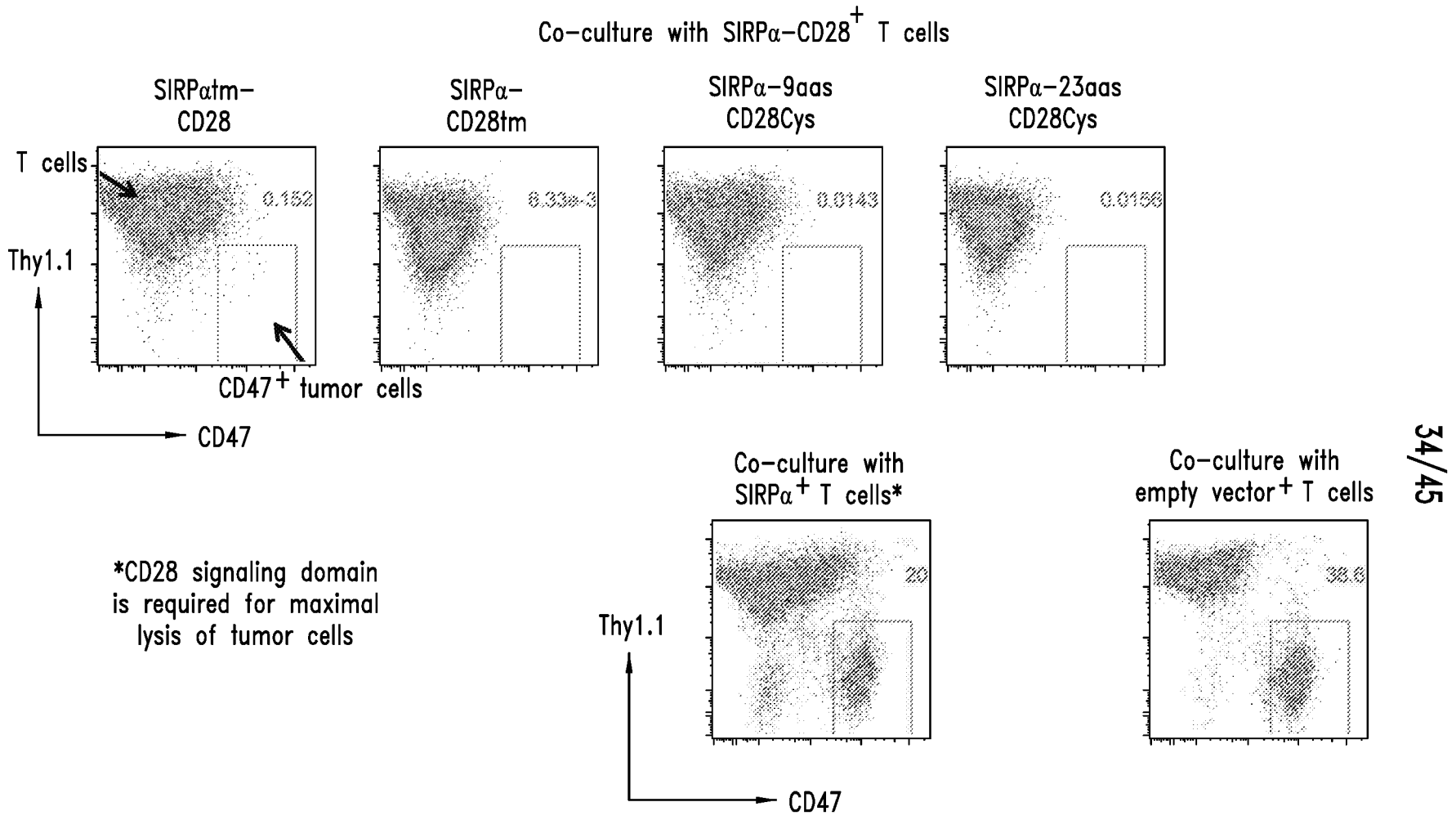

Co-culture with SIRPα-CD28+ T cells
SIRPαtm-CD28
SIRPα-CD28tm
SIRPα-9aas CD28Cys
SIRPα-23aas CD28Cys
T cells
Thy1.1
0.152
8.33e-3
0.0143
0.0156
CD47+ tumor cells
CD47
Co-culture with SIRPα+ T cells*
Co-culture with empty vector+ T cells
20
36.6
Thy1.1
CD47
*CD28 signaling domain is required for maximal lysis of tumor cells

FIG. 8D

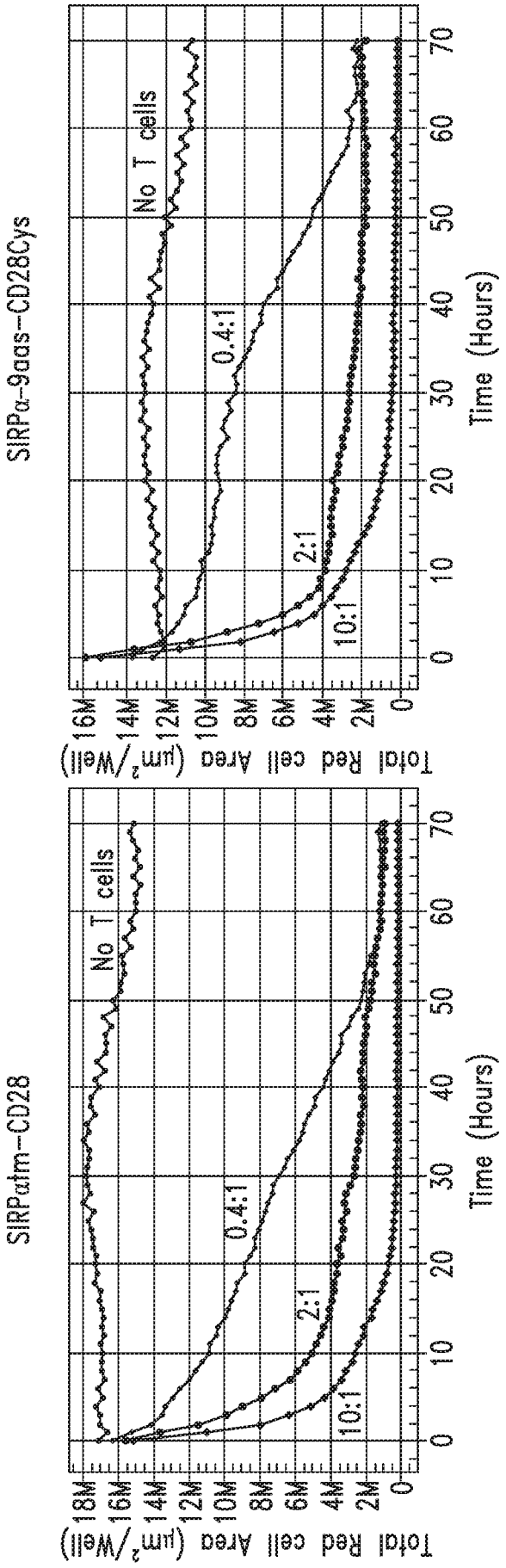

SIRPα-9aas-CD28Cys
Total Red cell Area (μm²/Well)
Time (Hours)
No T cells
0.4:1
2:1
10:1
SIRPαtm-CD28
Total Red cell Area (μm²/Well)
Time (Hours)
No T cells
0.4:1
2:1
10:1

FIG. 8E

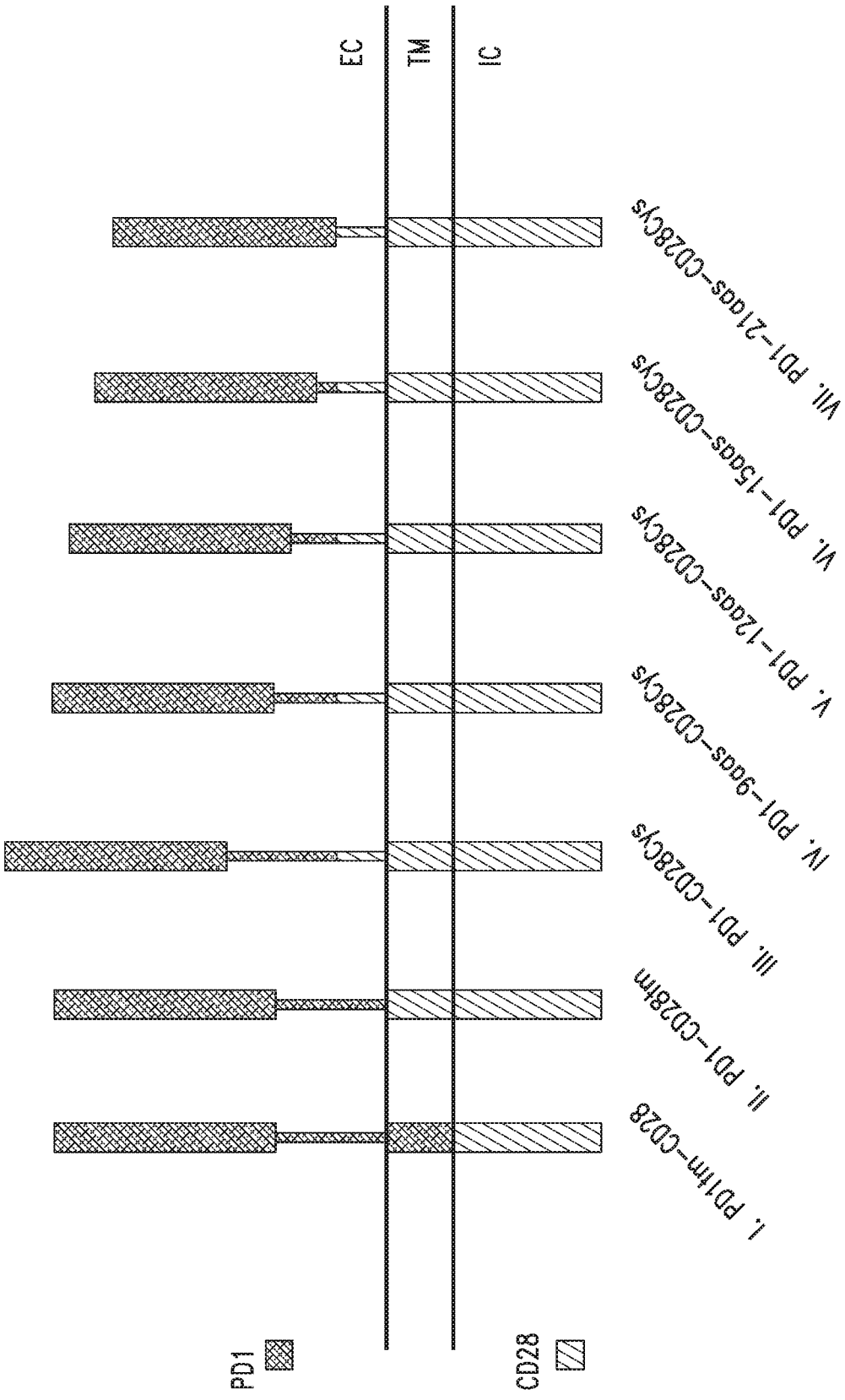

EC
TM
IC
I. PD1tm-CD28
II. PD1-CD28tm
III. PD1-CD28cys
IV. PD1-9aas-CD28cys
V. PD1-12aas-CD28cys
VI. PD1-15aas-CD28cys
VII. PD1-21aas-CD28cys
PD1
CD28

FIG. 9A

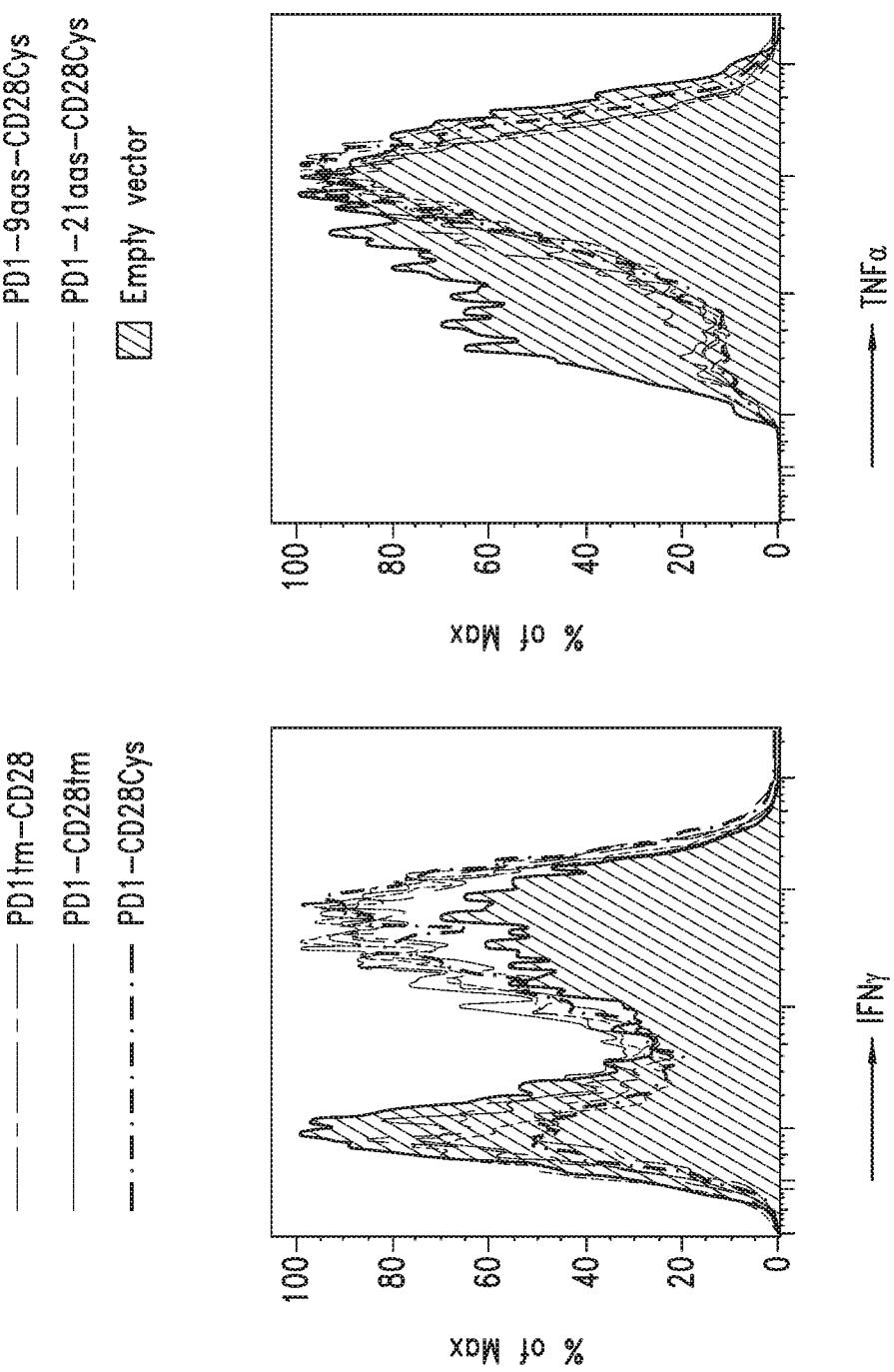

PD1tm-CD28
PD1-CD28tm
PD1-CD28Cys
PD1-9aas-CD28Cys
PD1-21aas-CD28Cys
Empty vector
% of MAX
100
80
60
40
20
0
IFNγ
TNFα

FIG. 9B

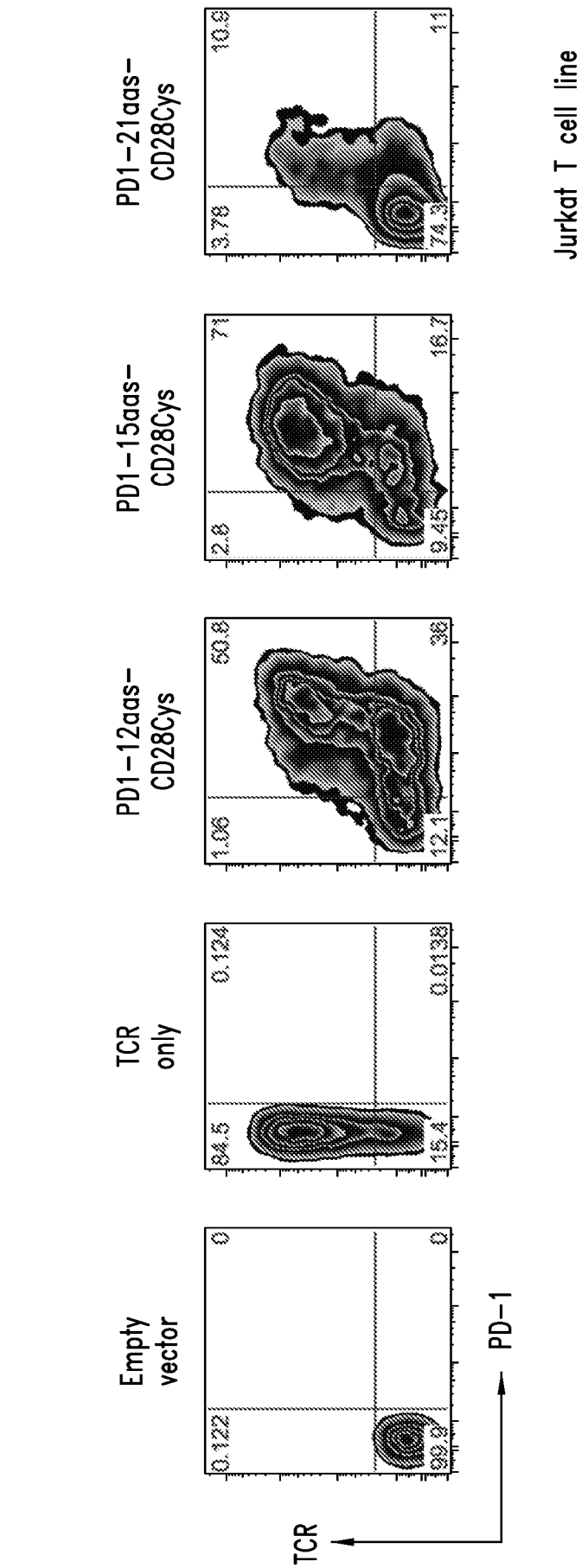

Empty vector
TCR only
PD1-12aas-CD28Cys
PD1-15aas-CD28Cys
PD1-21aas-CD28Cys
Jurkat T cell line
TCR
PD-1
0.122
99.9
0
0
84.5
15.4
0.124
0.0138
1.06
12.1
60.8
38
2.8
0.45
71
16.7
3.78
74.3
10.8
11

FIG. 10

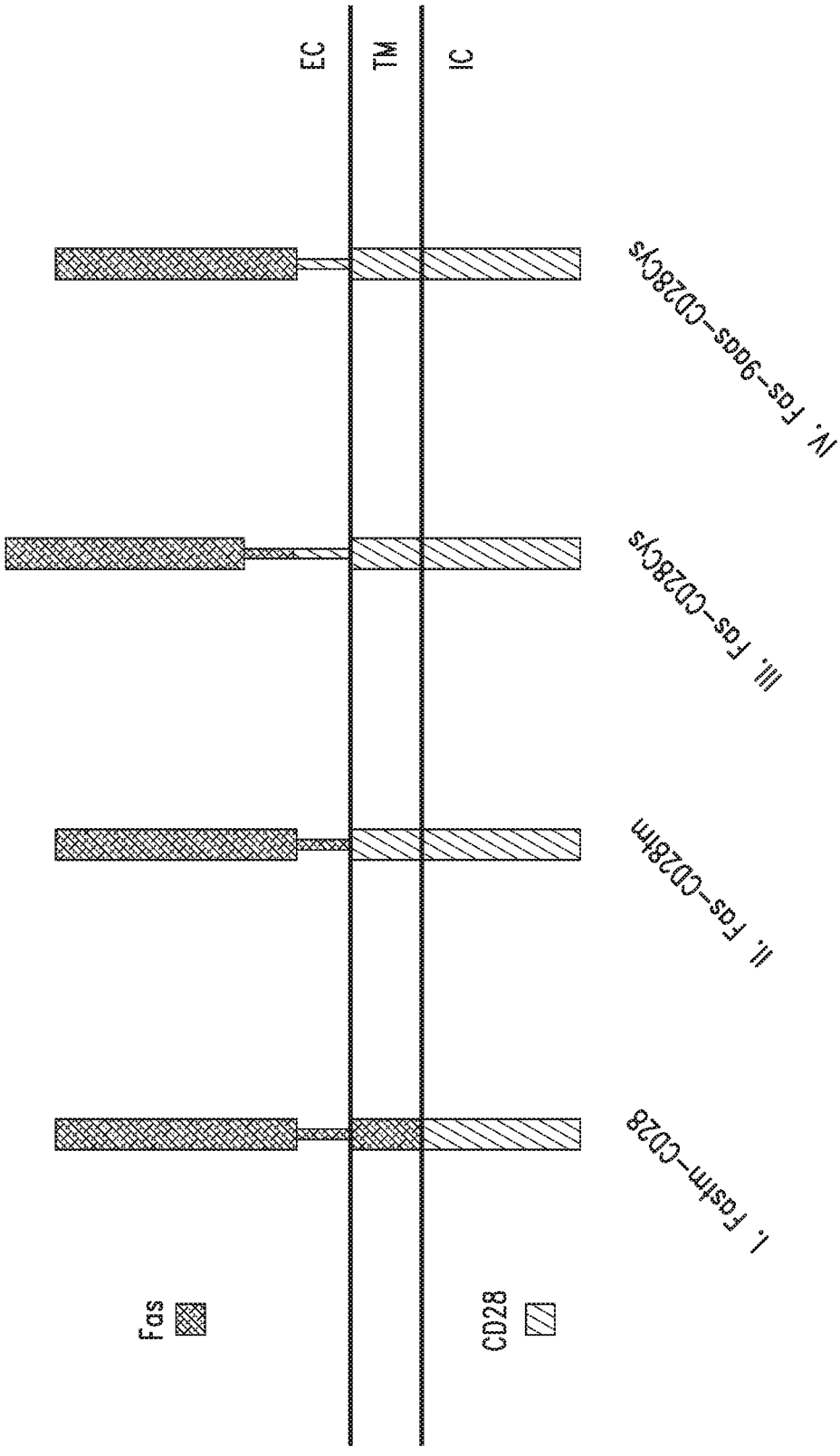
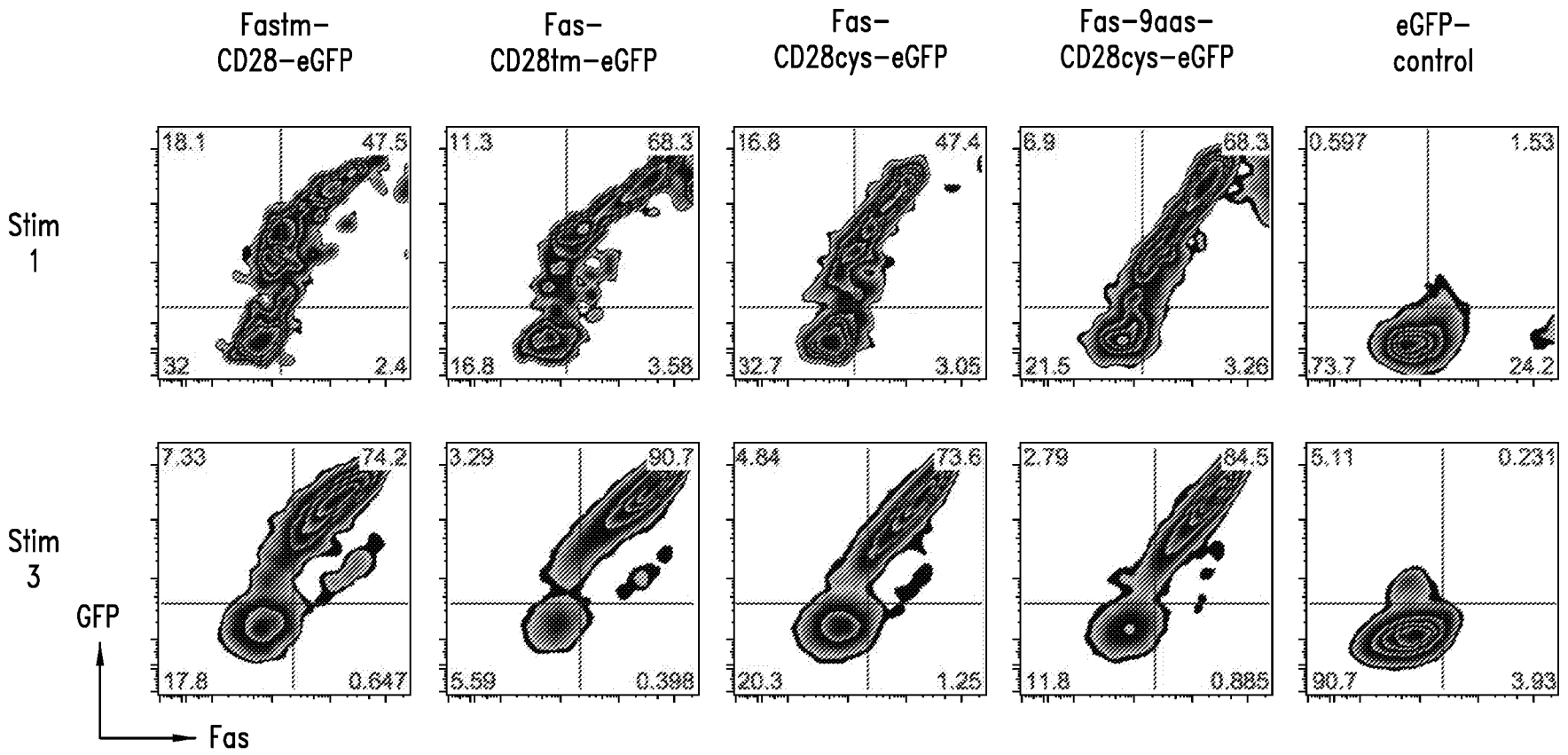

EC
TM
IC
I. fastm-CD28
II. Fas-CD28tm
III. Fas-CD28Cys
IV. Fas-9aas-CD28Cys
Fas
CD28

FIG. 11A



Transduced TCR_{gag} T cells, 5 days post-stimulation

FIG. 11B

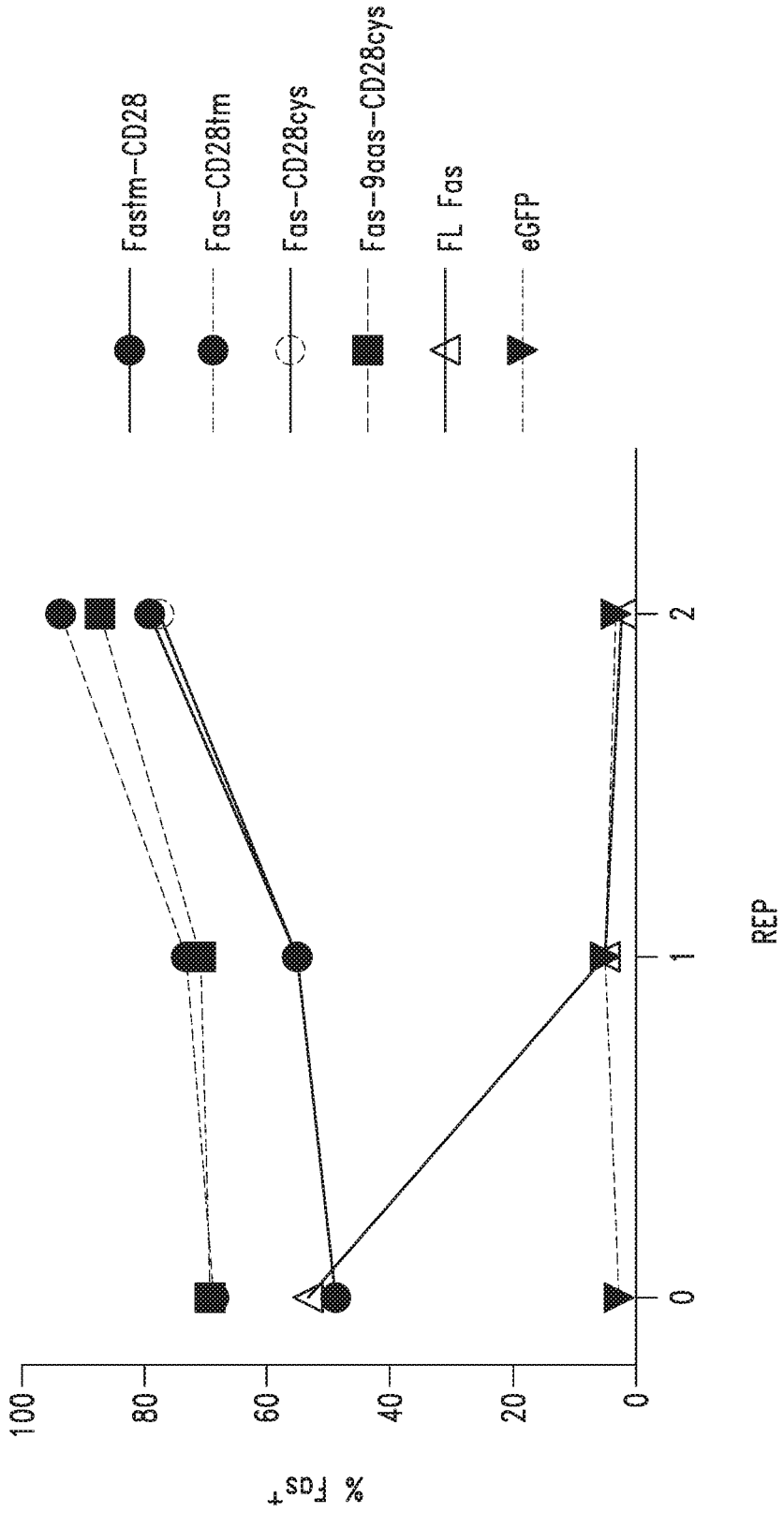

Fastm-CD28
Fas-CD28tm
Fas-CD28cys
Fas-9aas-CD28cys
FL Fas
eGFP
% FAS+
100
80
60
40
20
0
0
1
2
REP

FIG. 11C

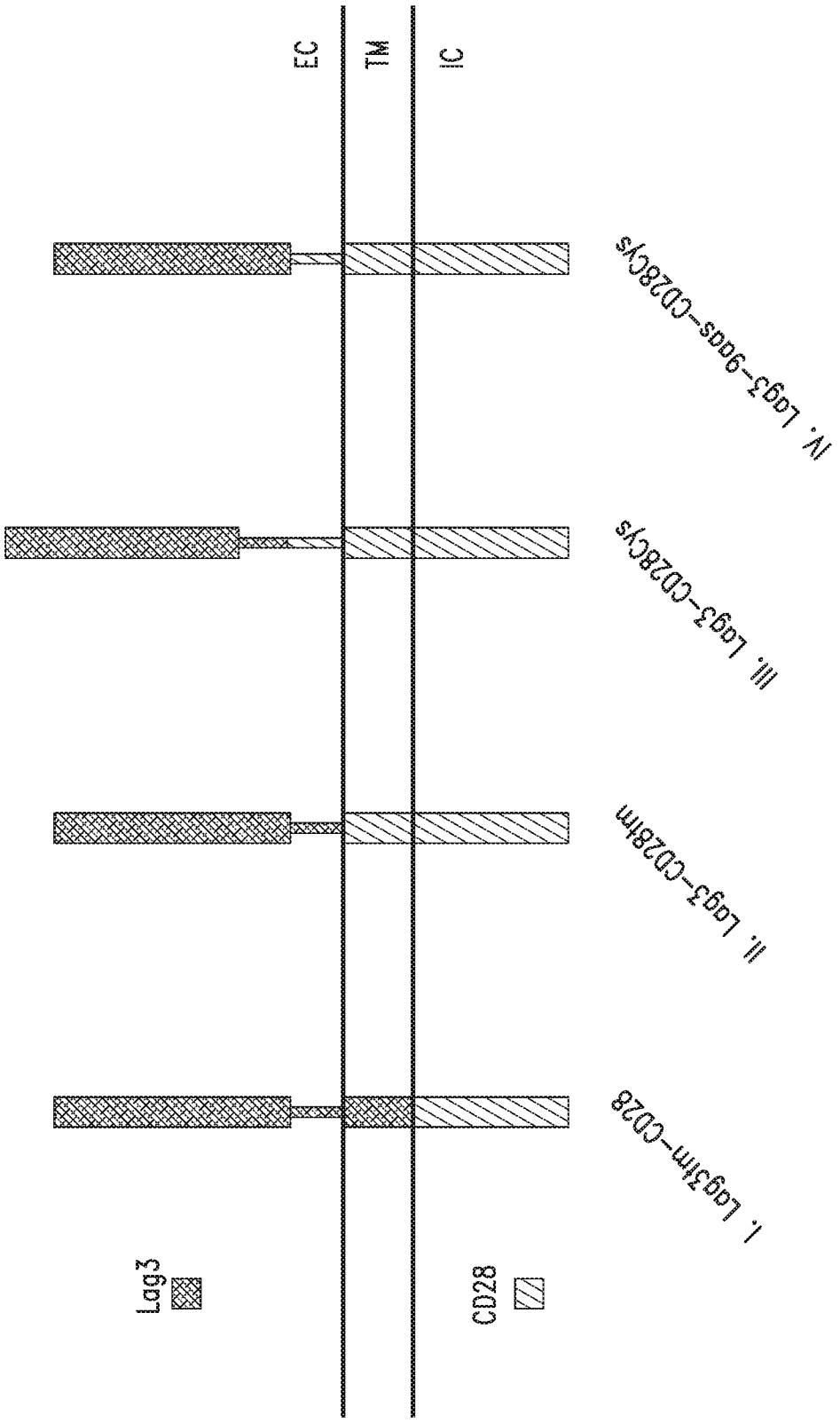

EC
TM
IC
I. Lag3tm-CD28
II. Lag3-CD28tm
III. Lag3-CD28cys
IV. Lag3-9aas-CD28cys
Lag3
CD28

FIG. 12A

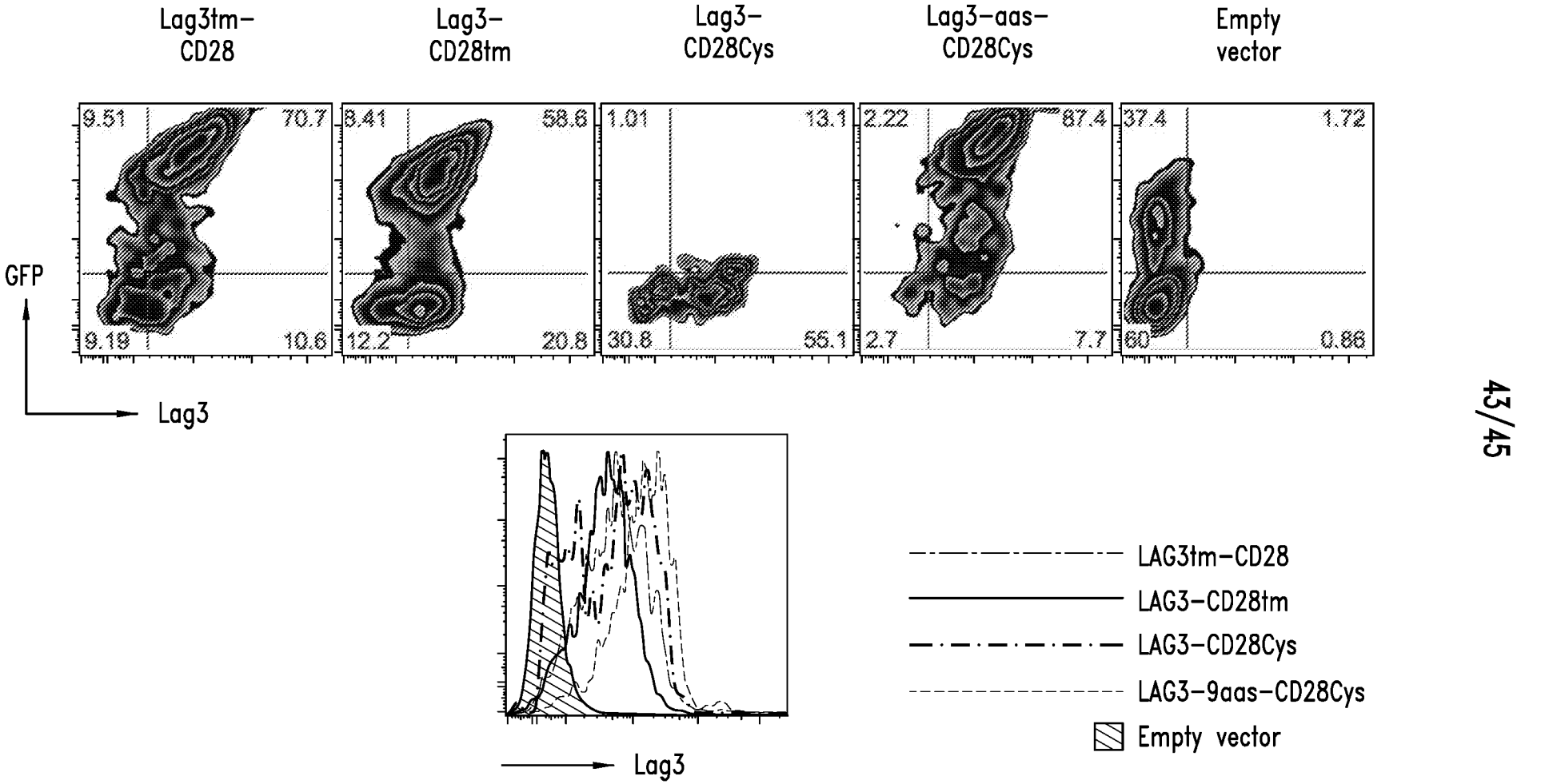

Lag3tm-CD28
Lag3-CD28tm
Lag3-CD28Cys
Lag3-aas-CD28Cys
Empty vector
9.51
70.7
9.19
10.6
8.41
58.6
12.2
20.8
1.01
13.1
30.8
55.1
2.22
87.4
2.7
7.7
37.4
1.72
60
0.86
GFP
Lag3
Lag3
LAG3tm-CD28
LAG3-CD28tm
LAG3-CD28Cys
LAG3-9aas-CD28Cys
Empty vector

FIG. 12B

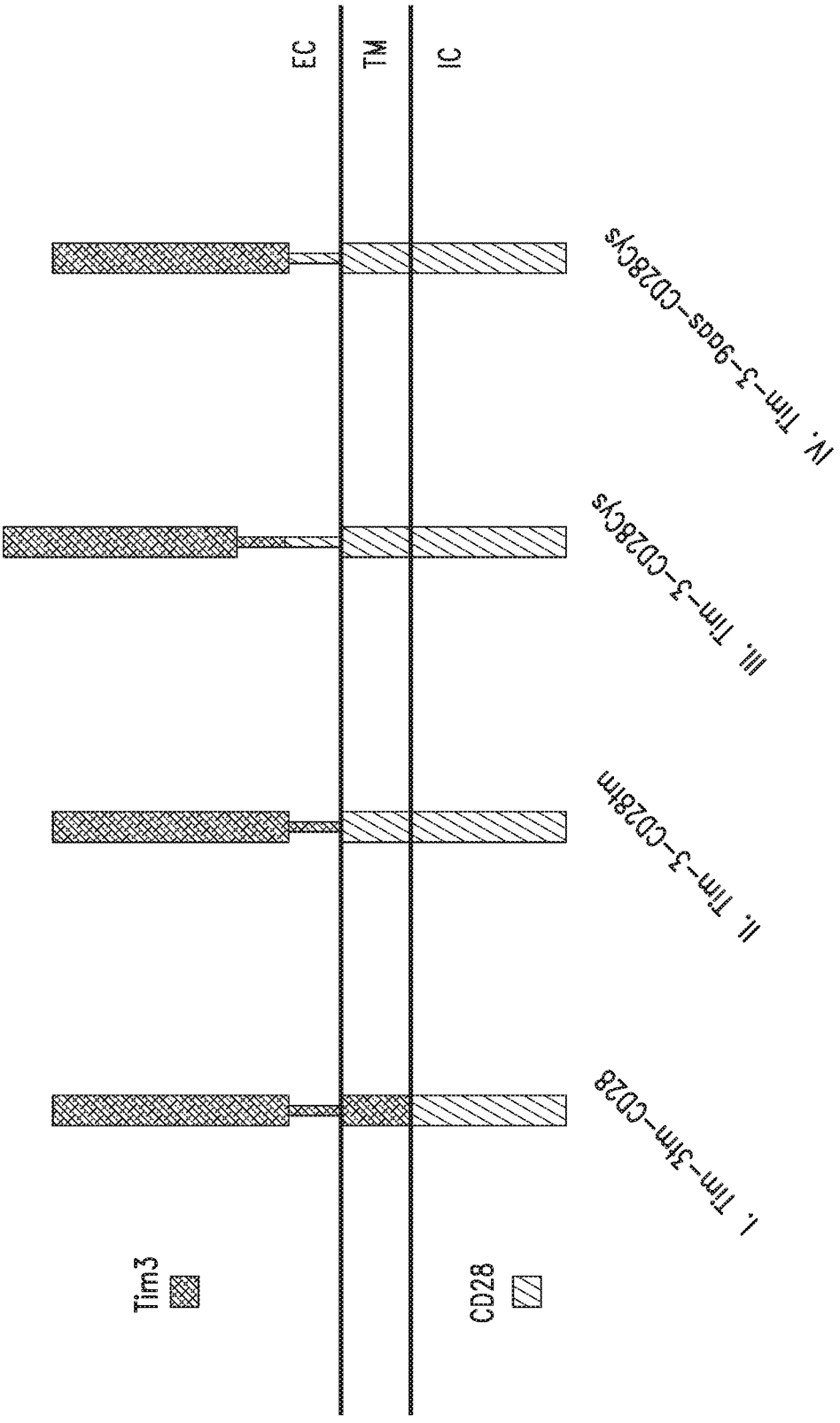

EC
TM
IC
I. Tim-3m-CD28
II. Tim-3-CD28m
III. Tim-3-CD28cys
IV. Tim-3-gas-CD28cys
Tim3
CD28

FIG. 13A

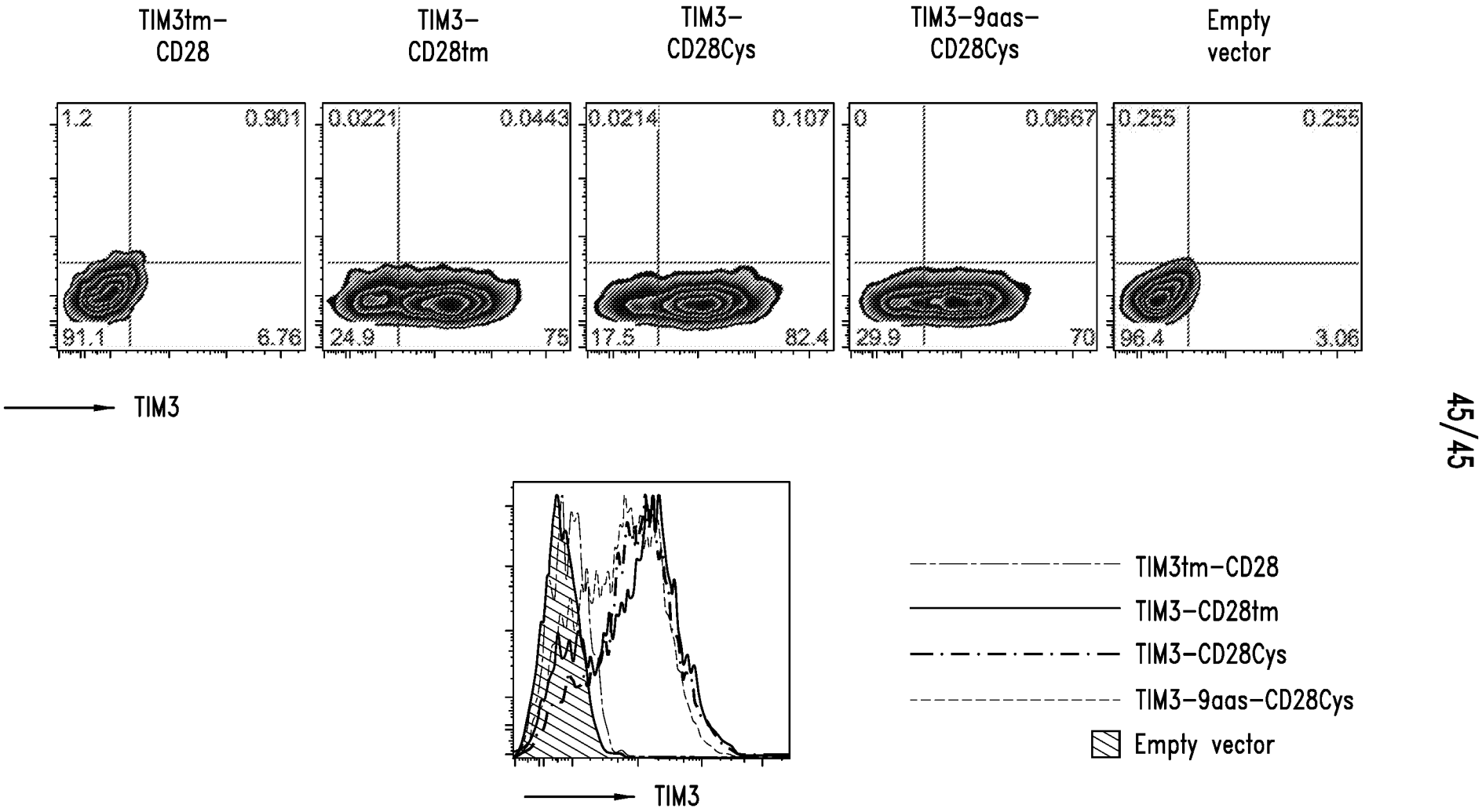

TIM3tm–CD28
TIM3–CD28tm
TIM3–CD28Cys
TIM3–9aas–CD28Cys
Empty vector
1.2
0.901
91.1
6.76
0.0221
0.0443
24.9
75
0.0214
0.107
17.5
82.4
0
0.0667
29.9
70
0.255
0.255
96.4
3.06
TIM3
TIM3
TIM3tm–CD28
TIM3–CD28tm
TIM3–CD28Cys
TIM3–9aas–CD28Cys
Empty vector

FIG. 13B