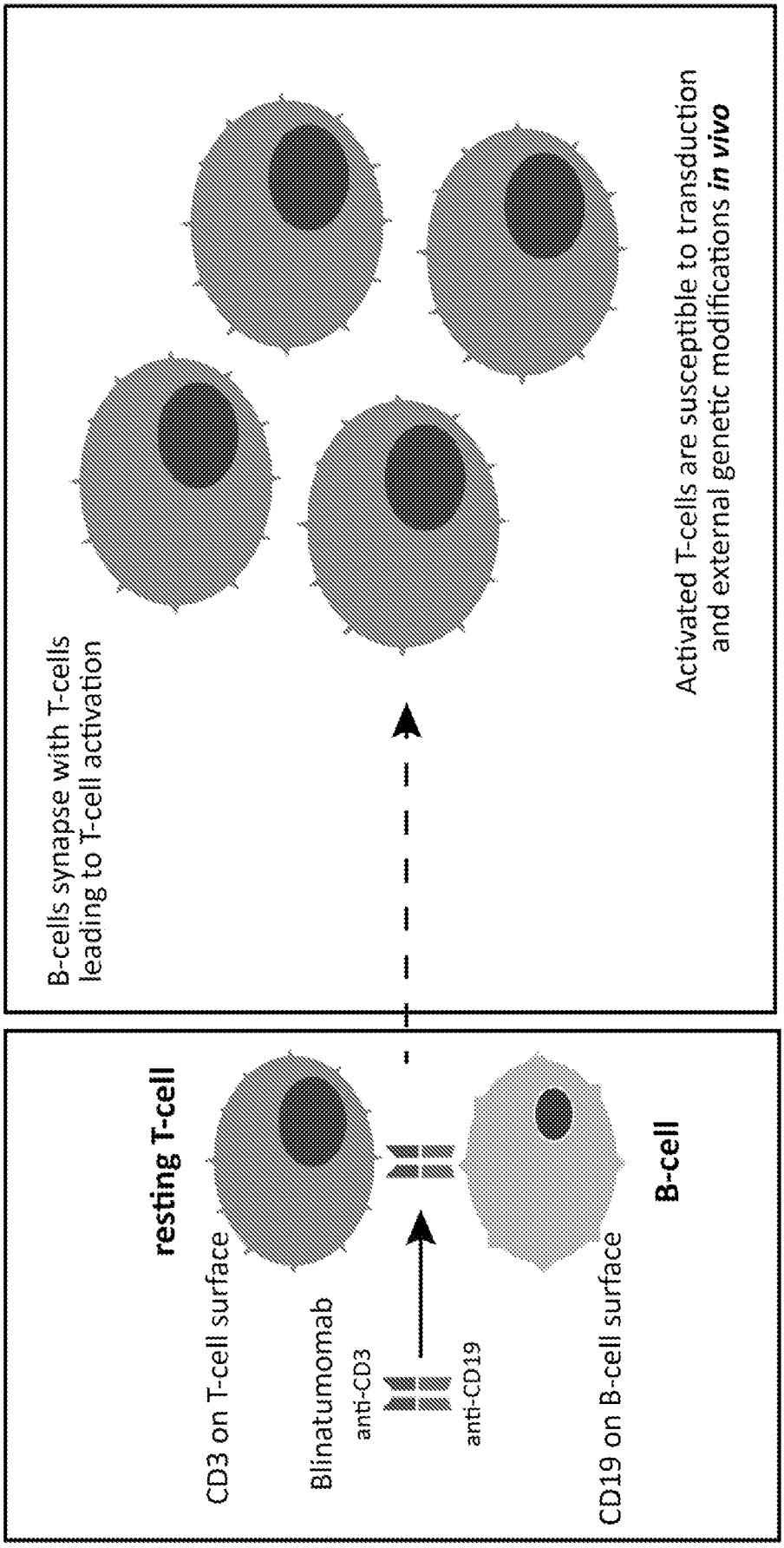
resting T-cell
CD3 on T-cell surface
Blinatumomab
anti-CD3
anti-CD19
CD19 on B-cell surface
B-cell
B-cells synapse with T-cells
leading to T-cell activation
Activated T-cells are susceptible to transduction
and external genetic modifications *in vivo*

FIG. 1

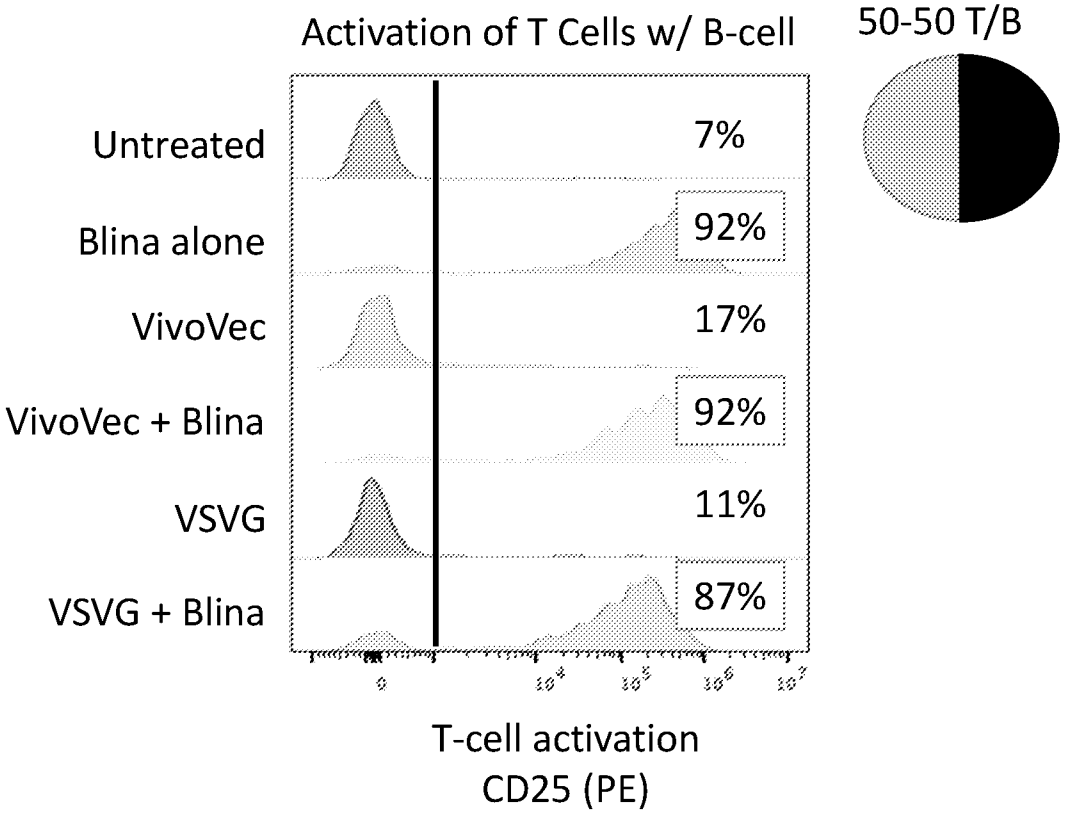
Activation of T Cells w/ B-cell
50-50 T/B
Untreated
Blina alone
VivoVec
VivoVec + Blina
VSVG
VSVG + Blina
7%
92%
17%
92%
11%
87%
0
10^4
10^5
10^6
10^7
T-cell activation
CD25 (PE)

FIG. 2A

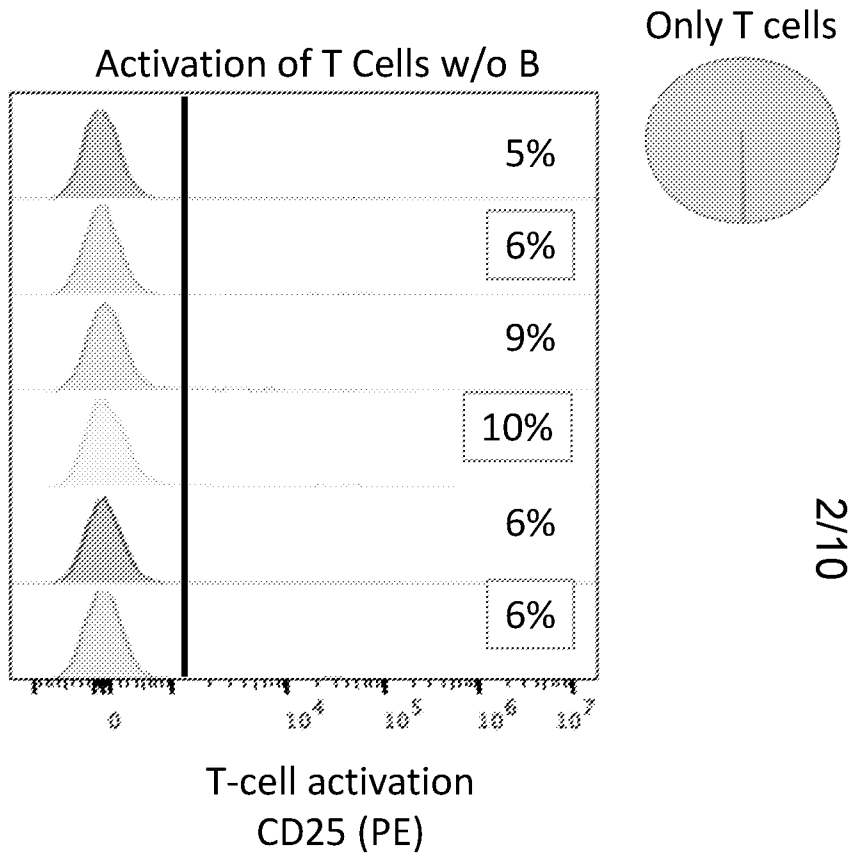
Activation of T Cells w/o B
Only T cells
5%
6%
9%
10%
6%
6%
0
10^4
10^5
10^6
10^7
T-cell activation
CD25 (PE)

FIG. 2B

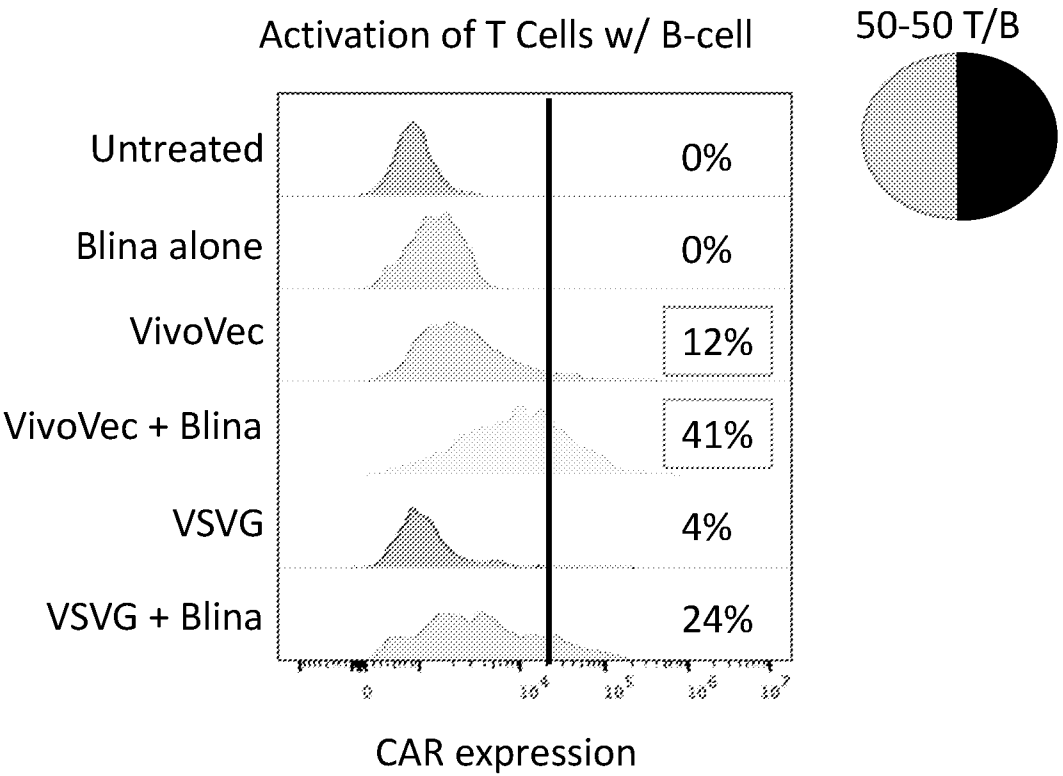
Activation of T Cells w/ B-cell
50-50 T/B
Untreated
Blina alone
VivoVec
VivoVec + Blina
VSVG
VSVG + Blina
0%
0%
12%
41%
4%
24%
0
10^4
10^5
10^6
10^7
CAR expression

FIG. 3A

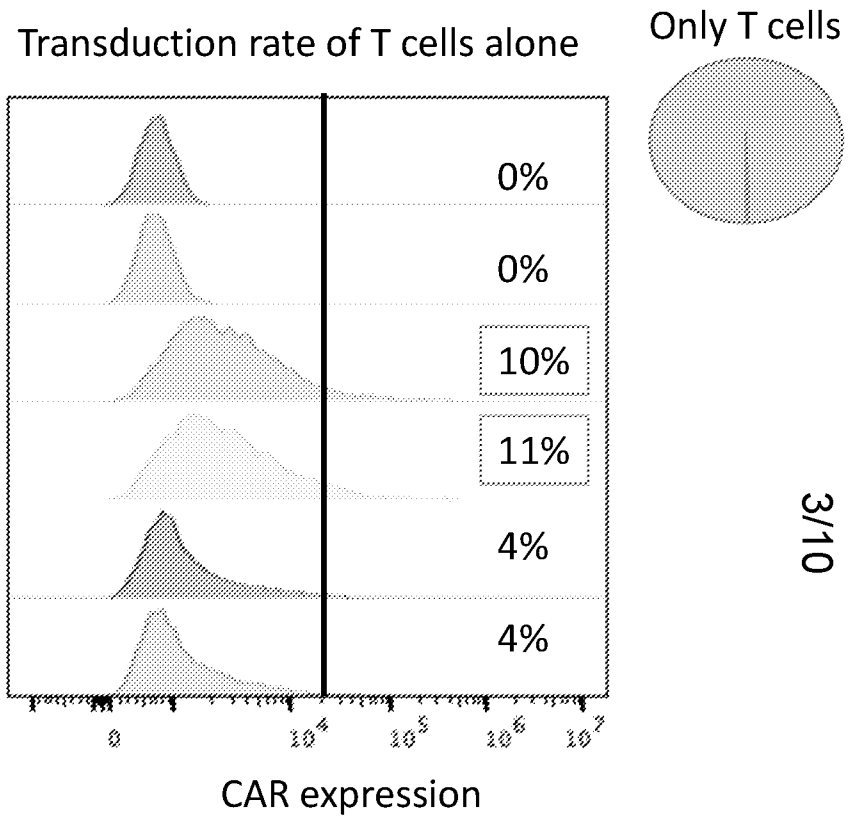
Transduction rate of T cells alone
Only T cells
0%
0%
10%
11%
4%
4%
0
10^4
10^5
10^6
10^7
CAR expression

FIG. 3B

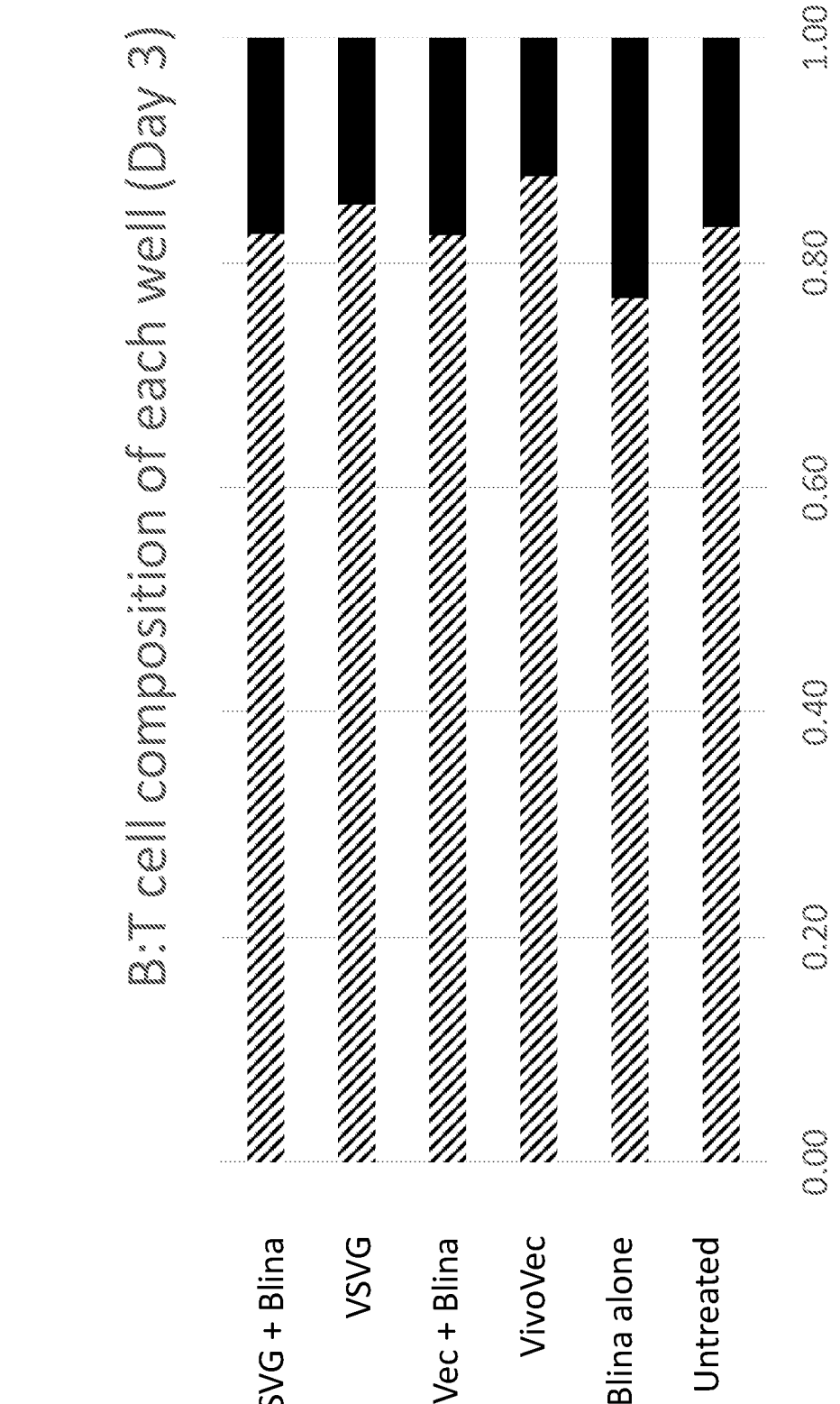
B:T cell composition of each well (Day 3)
VSVG + Blina
VSVG
VivoVec + Blina
VivoVec
Blina alone
Untreated
0.00
0.20
0.40
0.60
0.80
1.00
B cells
T cells

FIG. 4

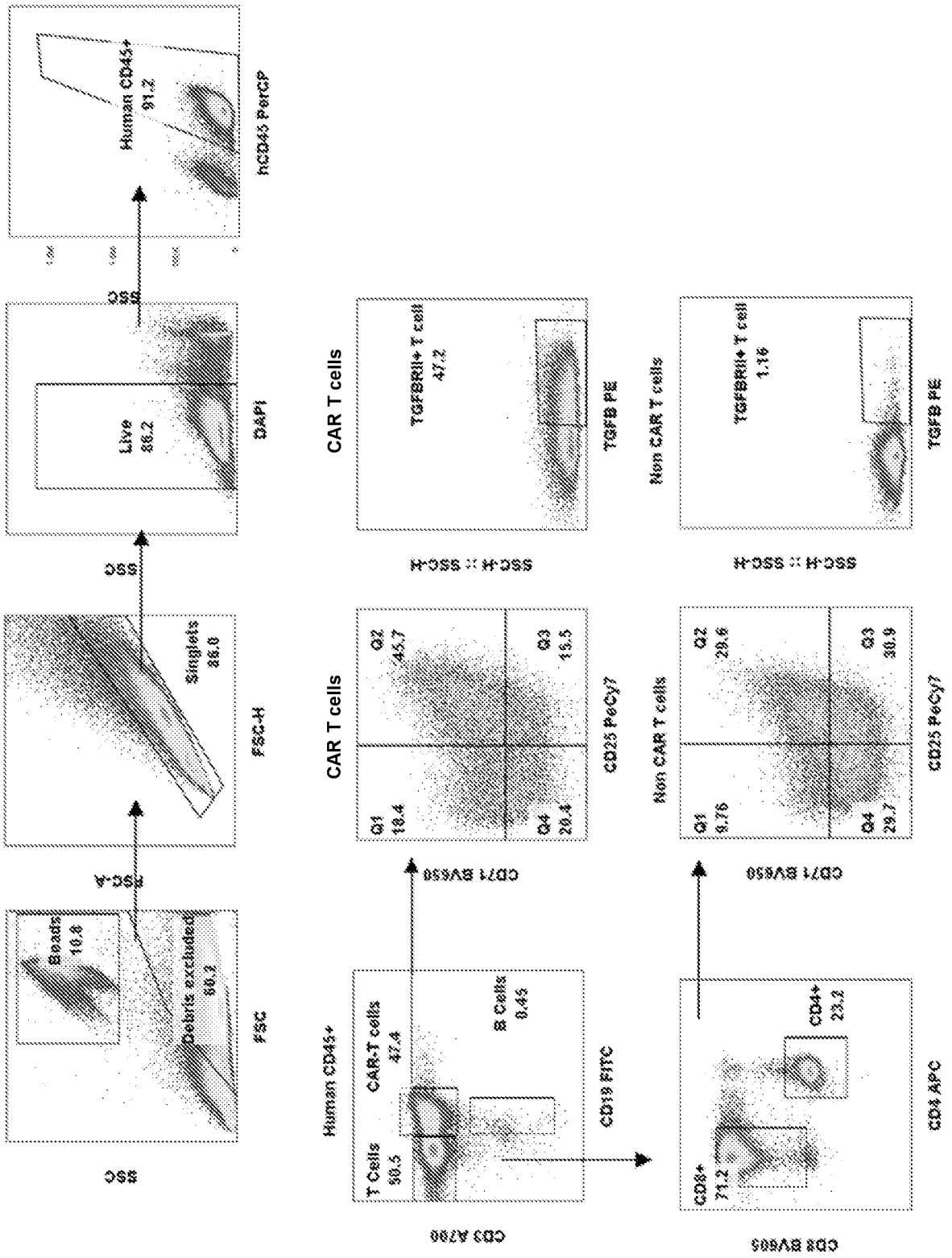
SSC
FSC
Beads
10.8
Debris excluded
60.2
FSC-A
FSC-H
Singlets
86.0
SSC
DAPI
Live
86.2
SSC
hCD45 PerCP
Human CD45+
91.2
CD3 A700
Human CD45+
T Cells
50.5
CAR-T cells
47.4
B Cells
0.45
CD19 FITC
CD71 BV650
CAR T cells
Q1
18.4
Q2
45.7
Q4
20.4
Q3
15.5
CD25 PeCy7
SSC-H :: SSC-H
CAR T cells
TGFBRII+ T cell
47.2
TGFB PE
CD8 BV605
CD8+
71.2
CD4+
23.2
CD4 APC
CD71 BV650
Non CAR T cells
Q1
9.76
Q2
29.6
Q4
29.7
Q3
30.9
CD25 PeCy7
SSC-H :: SSC-H
Non CAR T cells
TGFBRII+ T cell
1.16
TGFB PE

FIG. 5A

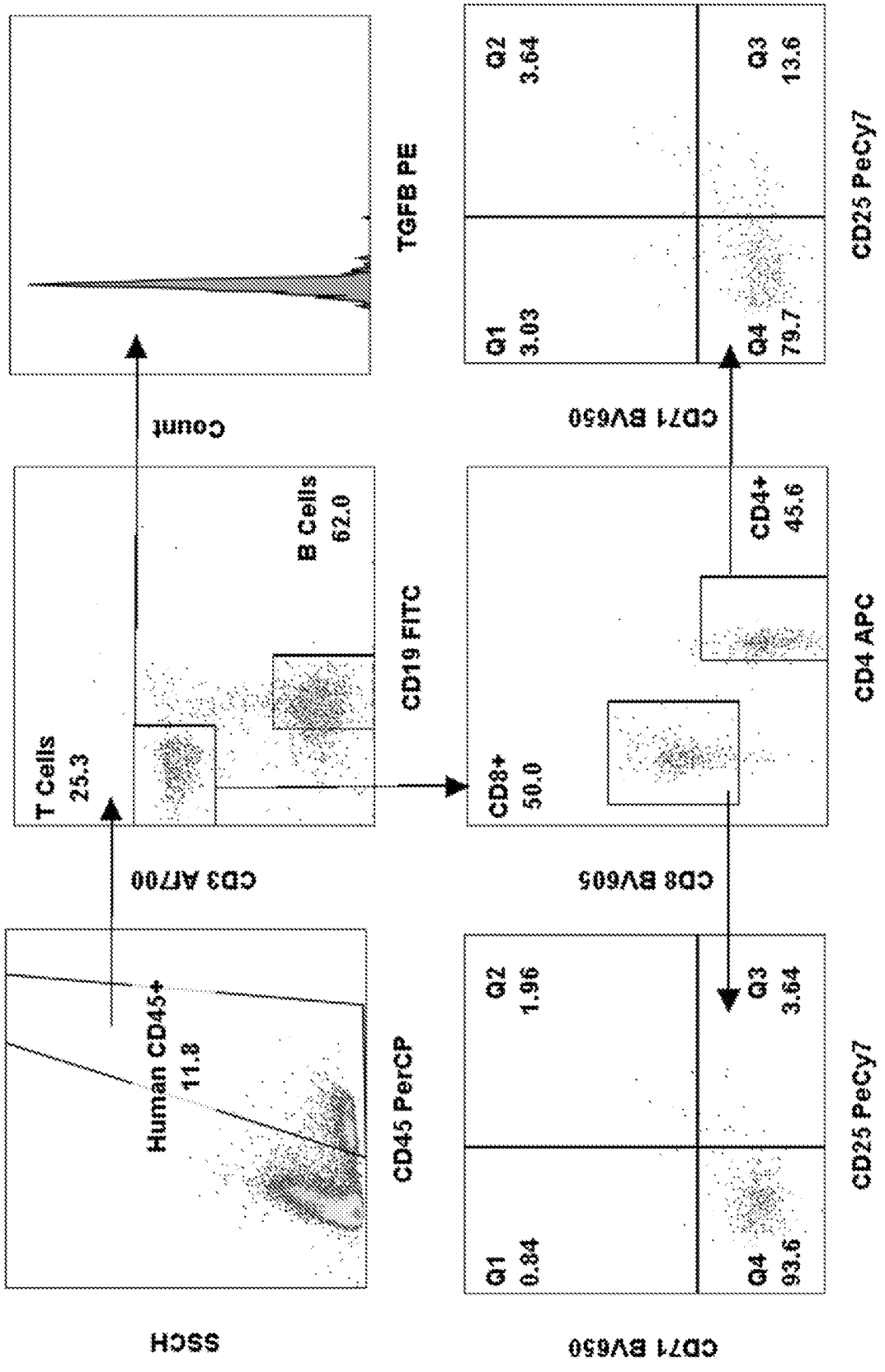
SSCH
Human CD45+
11.8
CD45 PerCP
CD3 AF700
T Cells
25.3
B Cells
62.0
CD19 FITC
Count
TGFB PE
CD71 BV650
Q1
0.84
Q2
1.96
Q3
3.64
Q4
93.6
CD25 PeCy7
CD8 BV605
CD8+
50.0
CD4+
45.6
CD4 APC
CD71 BV650
Q1
3.03
Q2
3.64
Q3
13.6
Q4
79.7
CD25 PeCy7

FIG. 5B

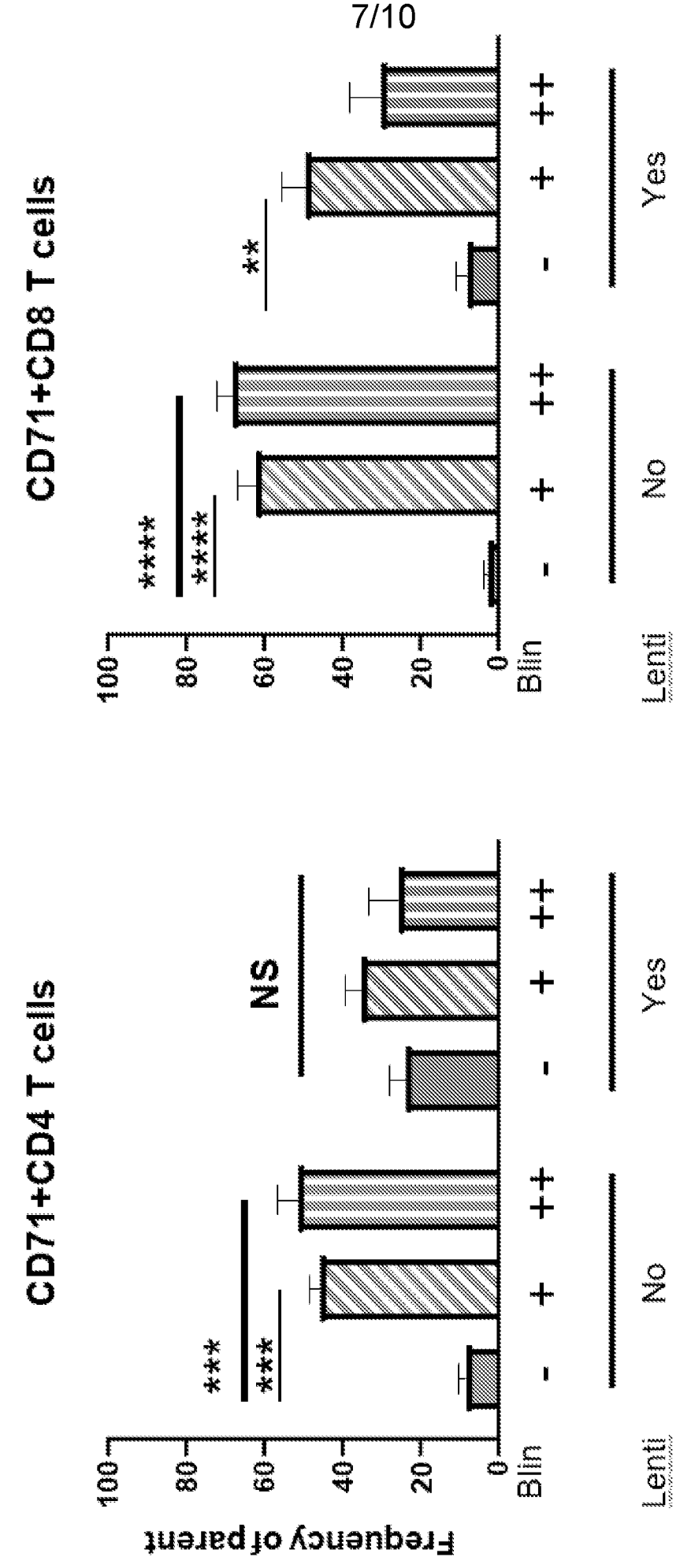
CD71+CD4 T cells
CD71+CD8 T cells
Frequency of parent
100
80
60
40
20
0
***
***
NS
****
****
**
Blin
-
+
++
-
+
++
Lenti
No
Yes

FIG. 6

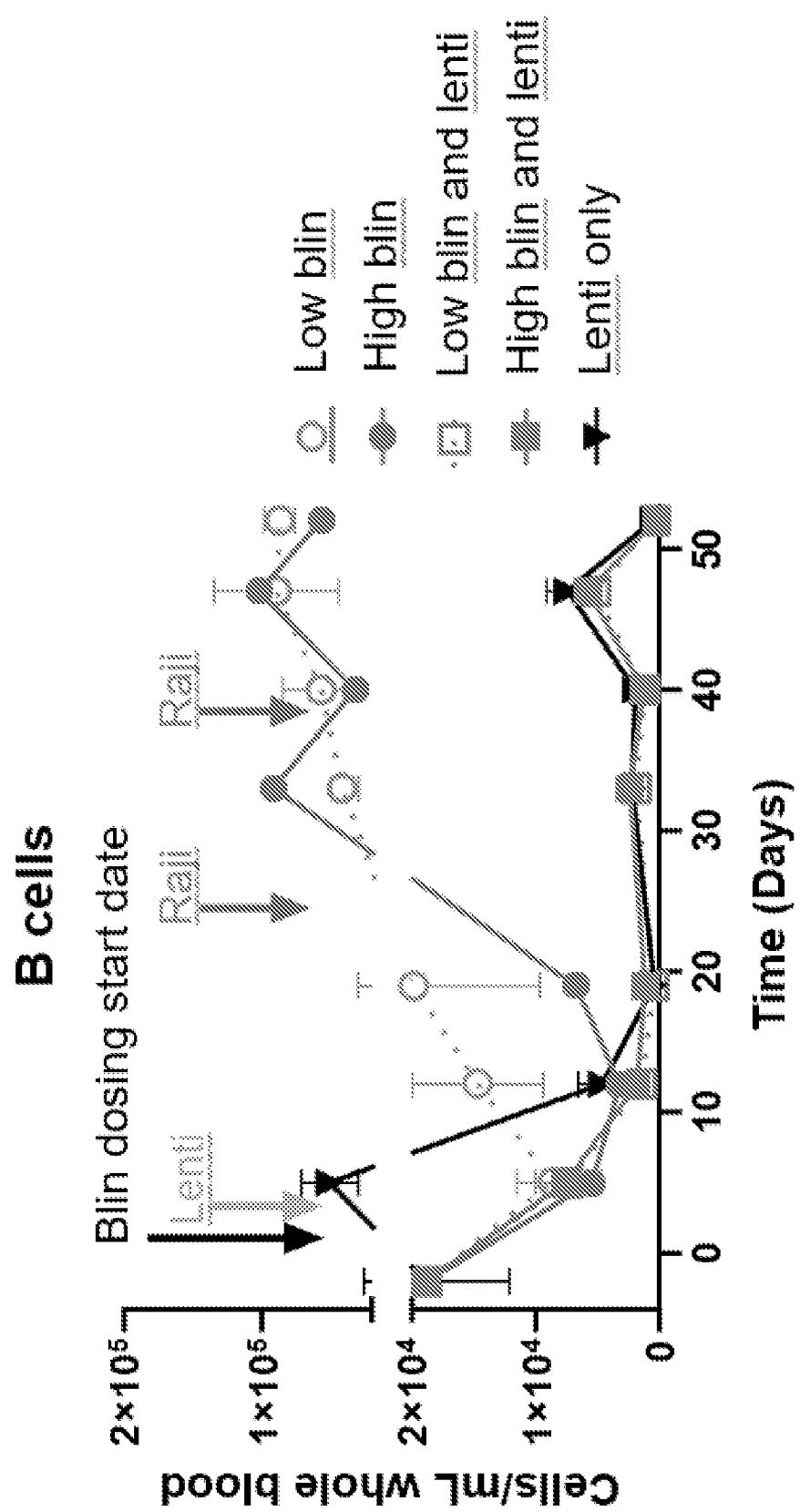
B cells
Blin dosing start date
Lenti
Rail
Rail
Low blin
High blin
Low blin and lenti
High blin and lenti
Lenti only
Cells/mL whole blood
2×10^5
1×10^5
2×10^4
1×10^4
0
0
10
20
30
40
50
Time (Days)

FIG. 7

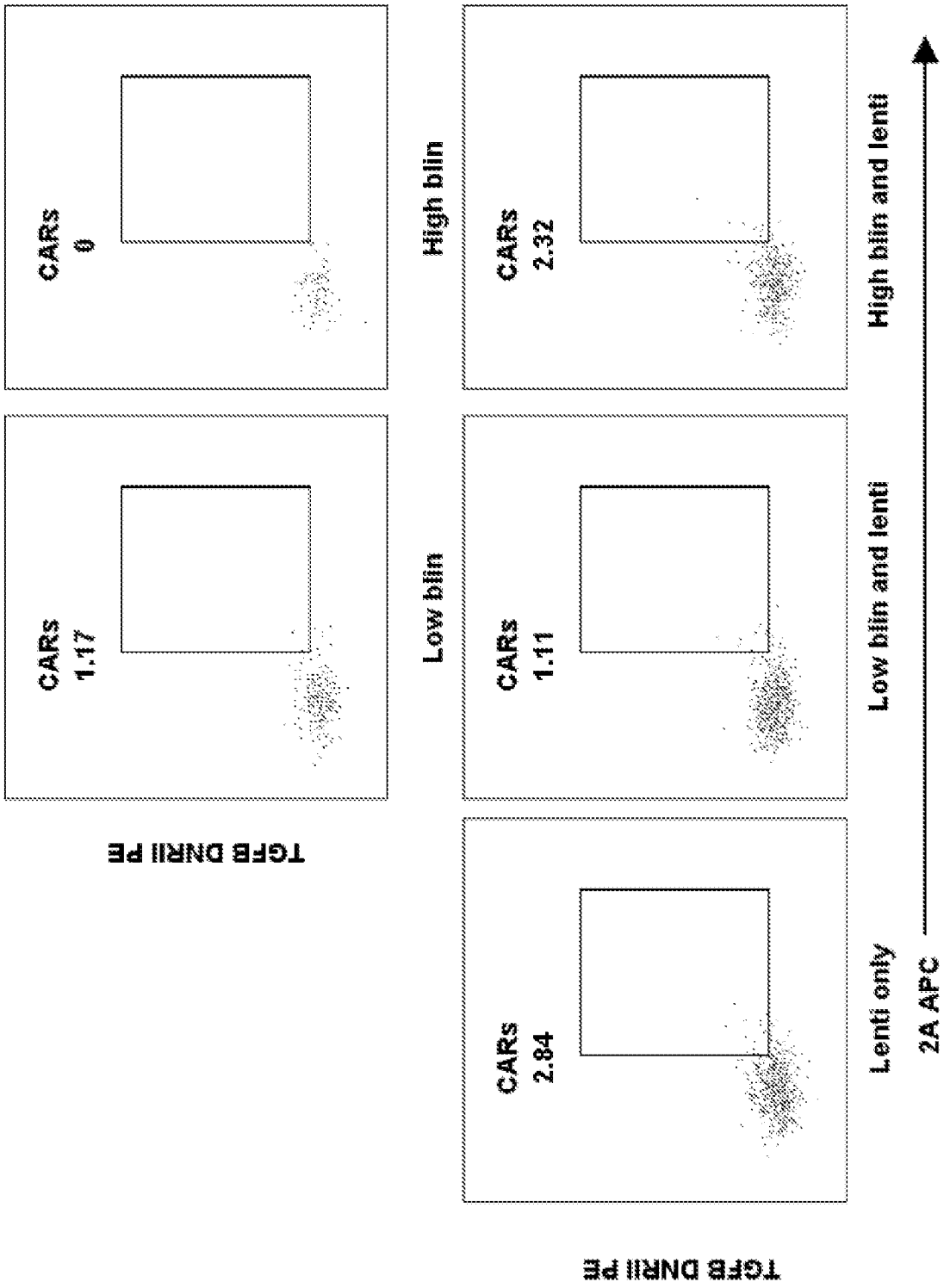
CARs
0
CARs
2.32
High blin
High blin and lenti
CARs
1.17
CARs
1.11
Low blin
Low blin and lenti
CARs
2.84
Lenti only
2A APC
TGFB DNRII PE
TGFB DNRII PE

FIG. 8

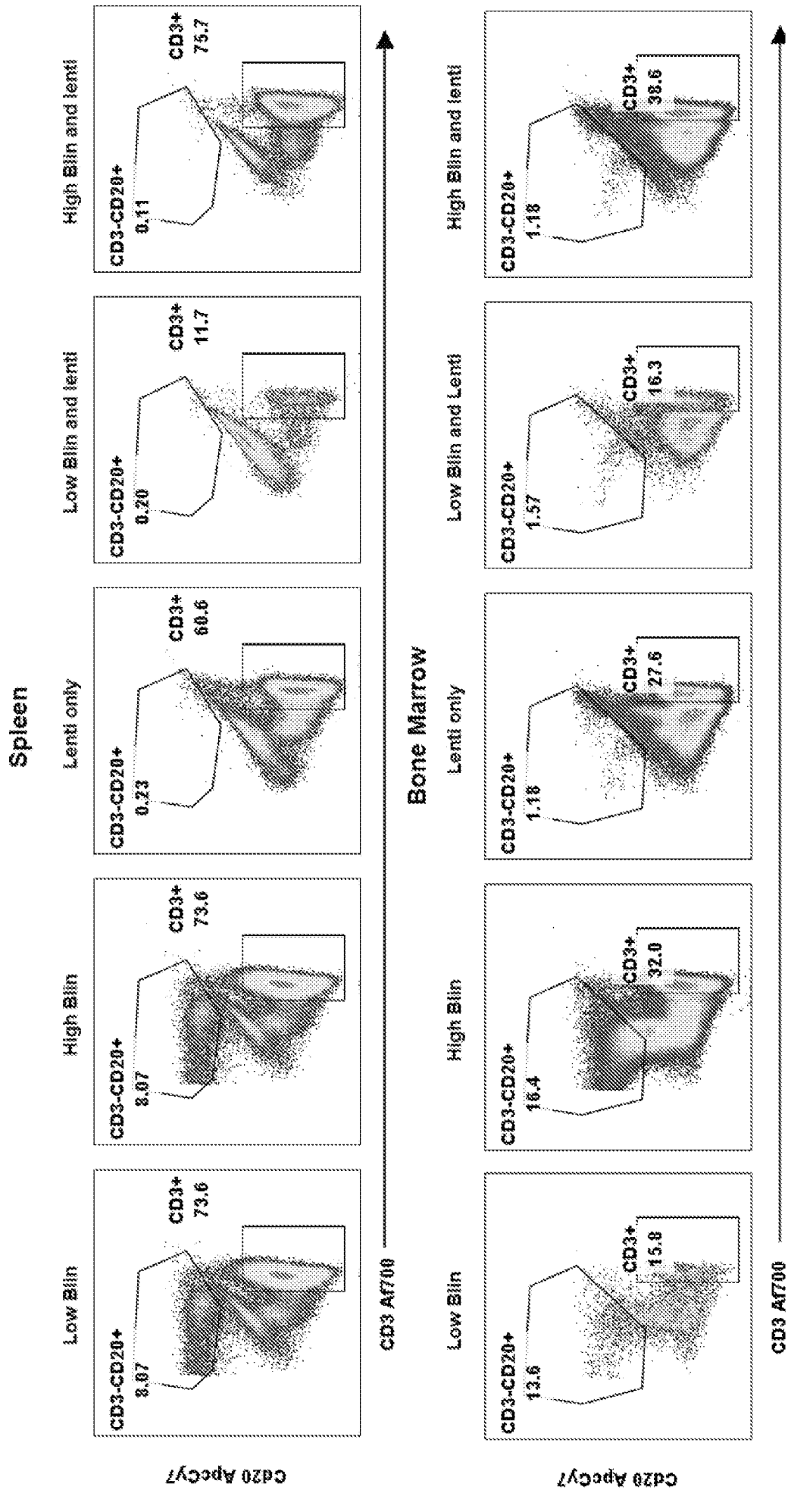
Spleen
Bone Marrow
Low Blin
High Blin
Lenti only
Low Blin and lenti
High Blin and lenti
CD3-CD20+
8.07
CD3+
73.6
CD3-CD20+
8.07
CD3+
73.6
CD3-CD20+
0.23
CD3+
60.6
CD3-CD20+
0.20
CD3+
11.7
CD3-CD20+
0.11
CD3+
75.7
CD3-CD20+
13.6
CD3+
15.8
CD3-CD20+
16.4
CD3+
32.0
CD3-CD20+
1.18
CD3+
27.6
CD3-CD20+
1.57
CD3+
16.3
CD3-CD20+
1.18
CD3+
38.6
CD3 Af700
cd20 ApcCy7
CD3 Af700
cd20 ApcCy7

FIG. 9