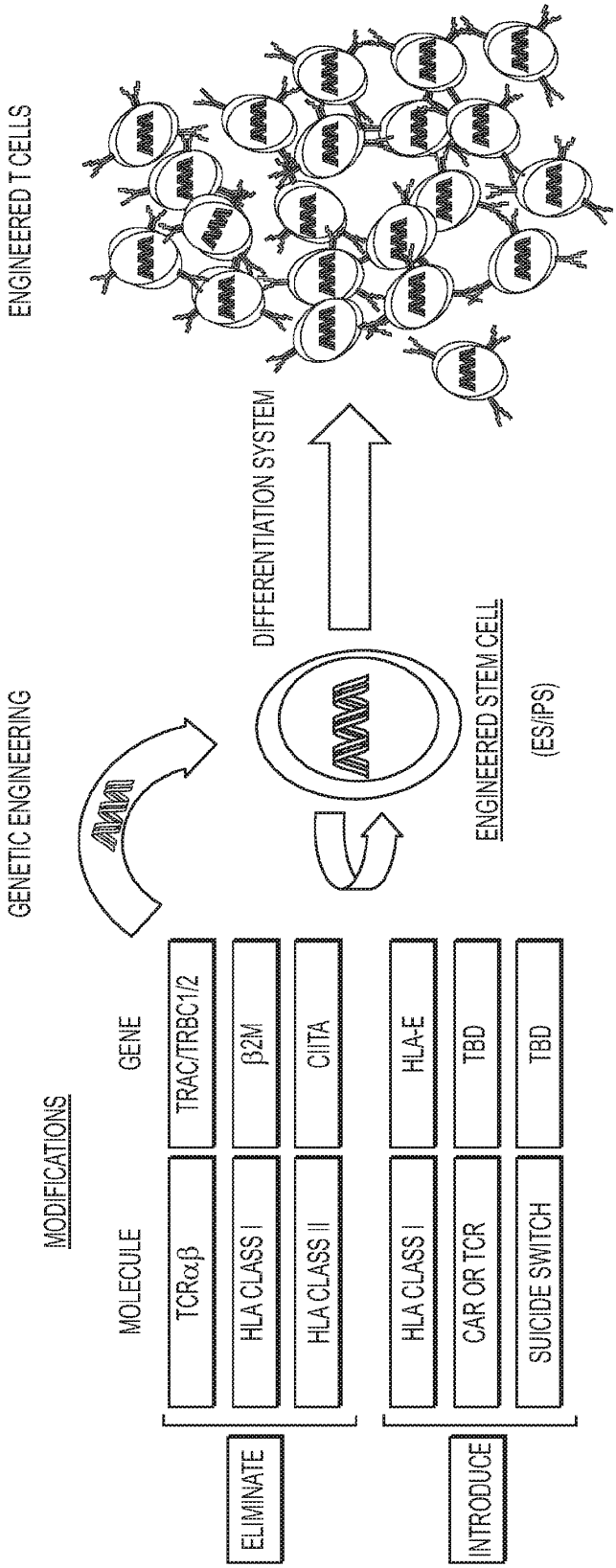
ENGINEERED T CELLS
DIFFERENTIATION SYSTEM
GENETIC ENGINEERING
ENGINEERED STEM CELL
(ES/IPS)
MODIFICATIONS
MOLECULE
GENE
TCRαβ
TRAC/TRBC1/2
HLA CLASS I
β2M
HLA CLASS II
CIITA
HLA CLASS I
HLA-E
CAR OR TCR
TBD
SUICIDE SWITCH
TBD
ELIMINATE
INTRODUCE

FIG. 1

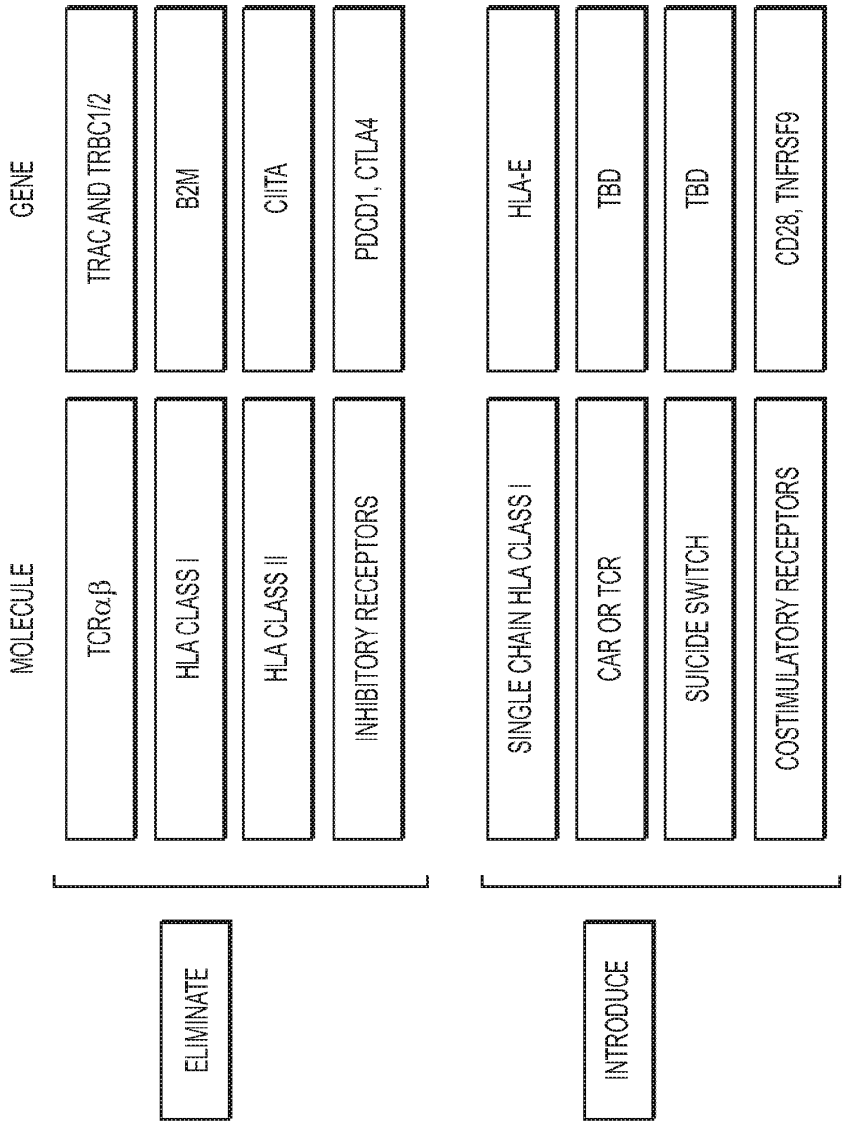
MOLECULE
GENE
TCRαβ
HLA CLASS I
HLA CLASS II
INHIBITORY RECEPTORS
TRAC AND TRBC1/2
B2M
CIITA
PDCD1, CTLA4
SINGLE CHAIN HLA CLASS I
CAR OR TCR
SUICIDE SWITCH
COSTIMULATORY RECEPTORS
HLA-E
TBD
TBD
CD28, TNFRSF9
ELIMINATE
INTRODUCE

FIG. 2

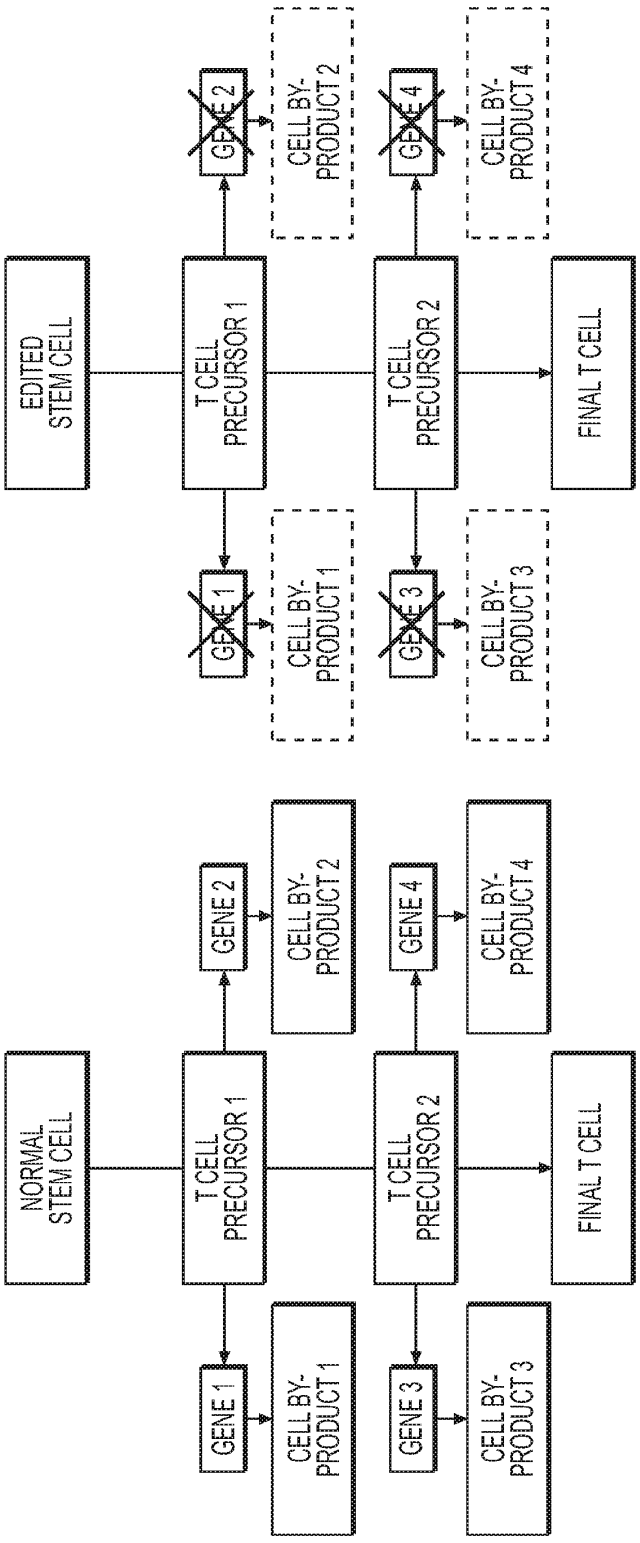
EDITED STEM CELL
T CELL PRECURSOR 1
T CELL PRECURSOR 2
FINAL T CELL
GENE 1
GENE 2
GENE 3
GENE 4
CELL BY-PRODUCT 1
CELL BY-PRODUCT 2
CELL BY-PRODUCT 3
CELL BY-PRODUCT 4
NORMAL STEM CELL
T CELL PRECURSOR 1
T CELL PRECURSOR 2
FINAL T CELL
GENE 1
GENE 2
GENE 3
GENE 4
CELL BY-PRODUCT 1
CELL BY-PRODUCT 2
CELL BY-PRODUCT 3
CELL BY-PRODUCT 4

FIG. 3

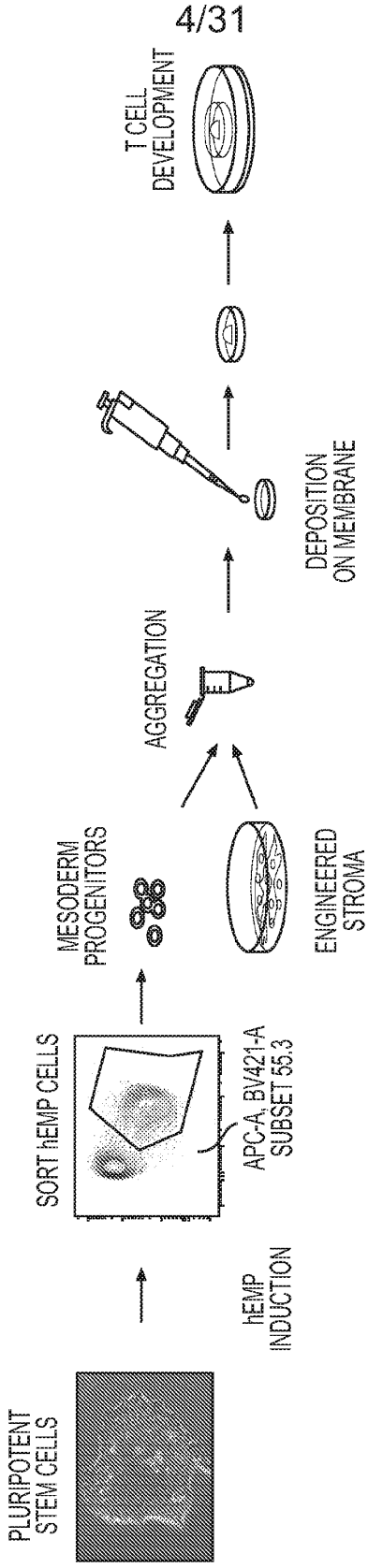
PLURIPOTENT STEM CELLS
hEMP INDUCTION
SORT hEMP CELLS
APC-A, BV421-A SUBSET 55.3
MESODERM PROGENITORS
ENGINEERED STROMA
AGGREGATION
DEPOSITION ON MEMBRANE
T CELL DEVELOPMENT

FIG. 4

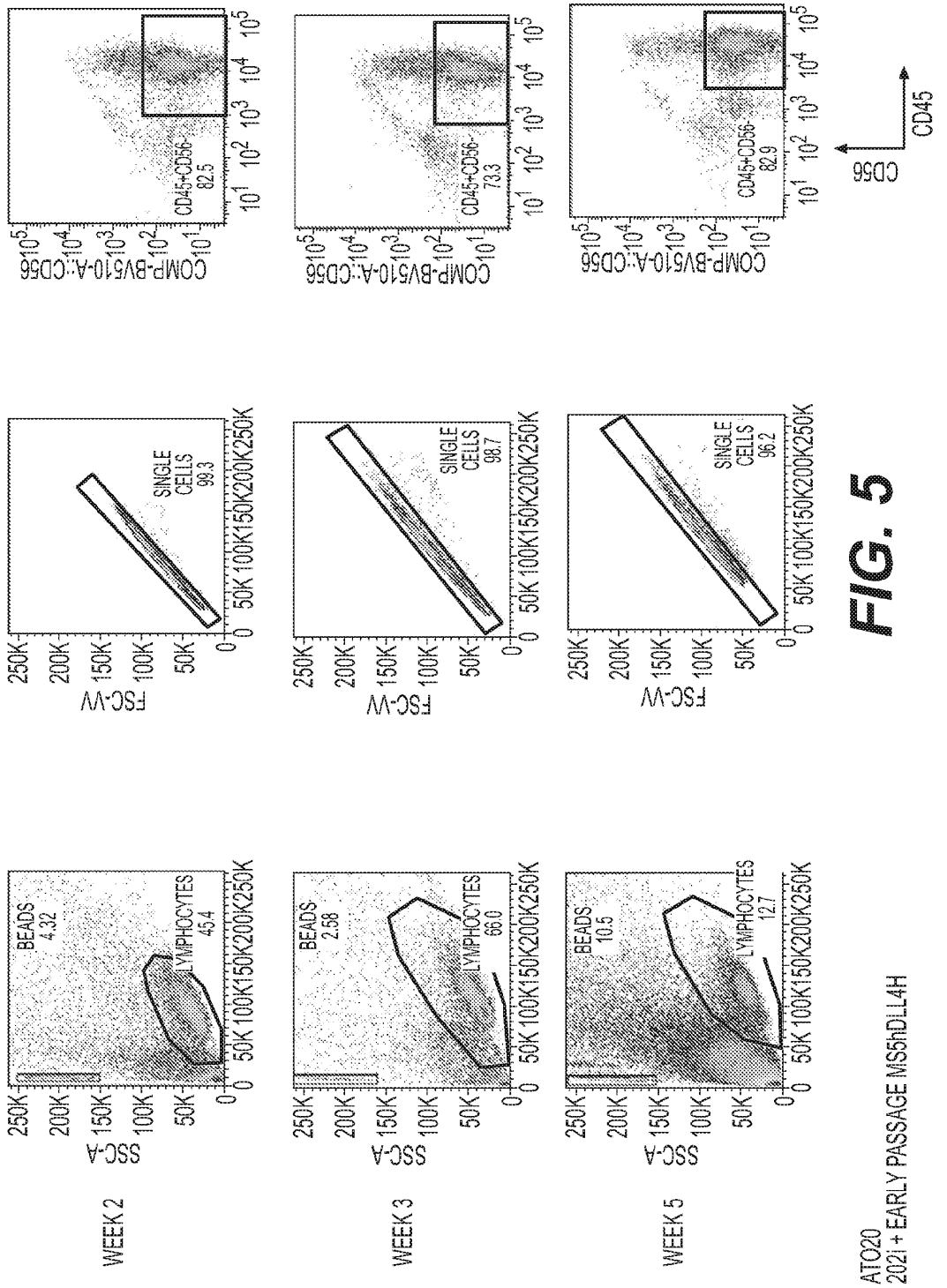

FIG. 5

ATO20
2021 + EARLY PASSAGE MS5hDLL4H

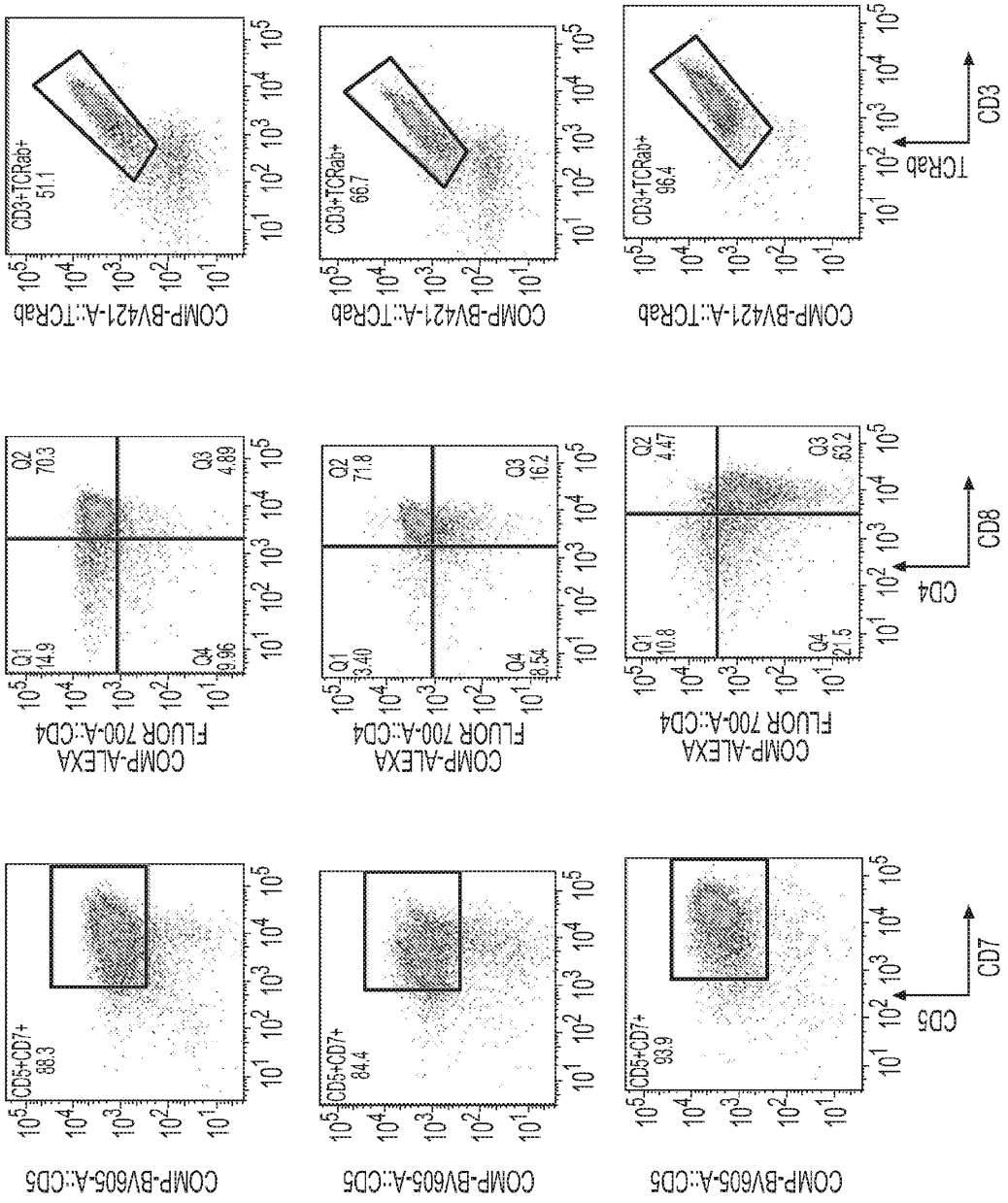
CD3+TCRab+
51.1
CD3+TCRab+
66.7
CD3+TCRab+
96.4
COMP-BV421-A::TCRab
TCRab
CD3
Q1
14.9
Q2
70.3
Q3
4.89
Q4
9.96
Q1
33.40
Q2
71.8
Q3
16.2
Q4
8.54
Q1
10.8
Q2
4.47
Q3
63.2
Q4
21.5
COMP-ALEXA FLUOR 700-A::CD4
CD4
CD8
CD5+CD7+
88.3
CD5+CD7+
84.4
CD5+CD7+
93.9
COMP-BV605-A::CD5
CD5
CD7

FIG. 5 (CONT.-1)

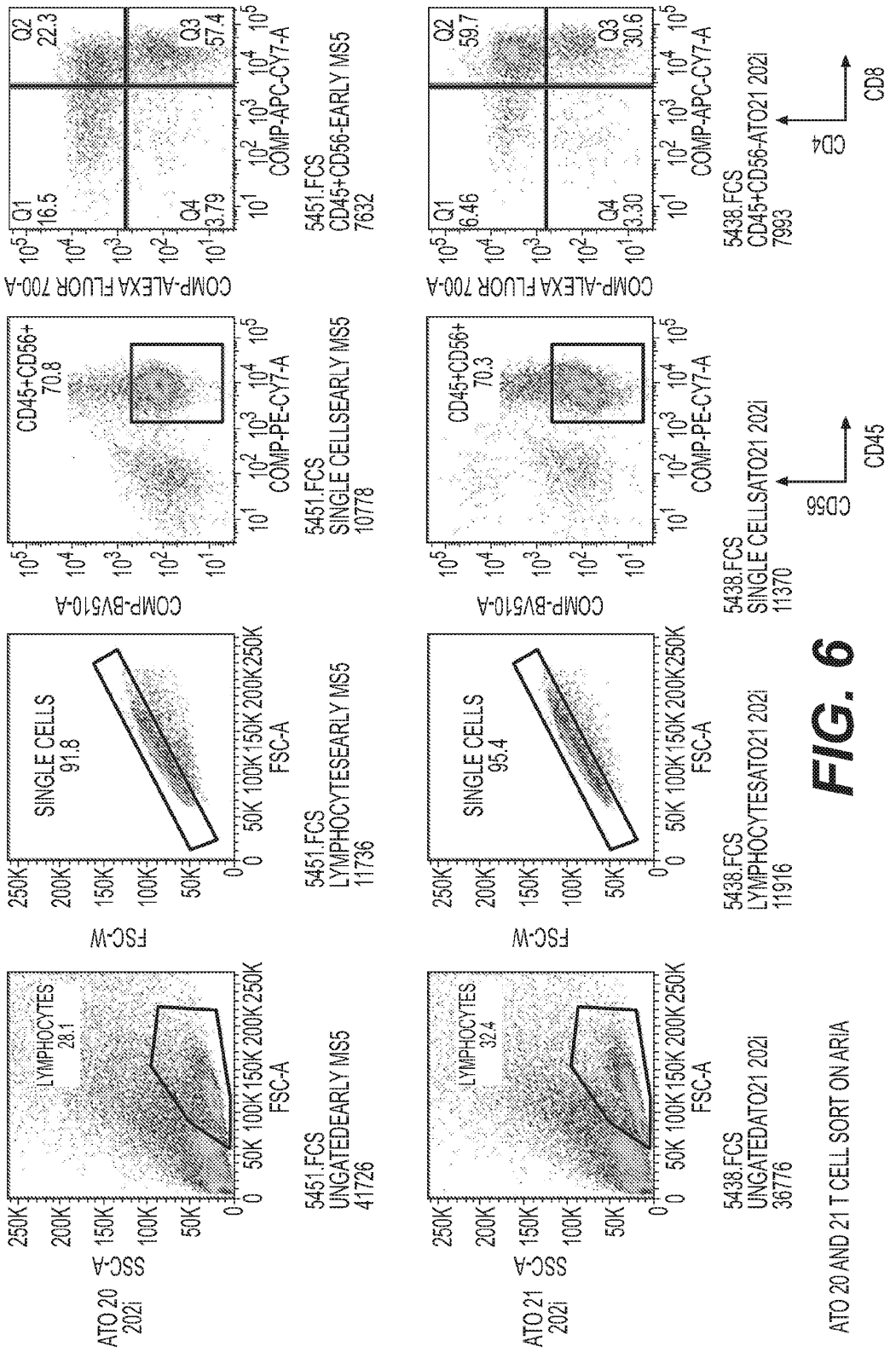
ATO 20 202i
ATO 21 202i
SSC-A
FSC-A
LYMPHOCYTES 28.1
LYMPHOCYTES 32.4
5451.FCS
UNGATEDEARLY MS5
41726
5438.FCS
UNGATEDATO21 202i
36776
FSC-W
SINGLE CELLS 91.8
SINGLE CELLS 95.4
5451.FCS
LYMPHOCYTESEARLY MS5
11736
5438.FCS
LYMPHOCYTESATO21 202i
11916
COMP-BV510-A
CD45+CD56+ 70.8
CD45+CD56+ 70.3
COMP-PE-CY7-A
5451.FCS
SINGLE CELLSEARLY MS5
10778
5438.FCS
SINGLE CELLSATO21 202i
11370
CD56
CD45
COMP-ALEXA FLUOR 700-A
Q1 16.5
Q2 22.3
Q4 3.79
Q3 57.4
COMP-APC-CY7-A
5451.FCS
CD45+CD56-EARLY MS5
7632
Q1 6.46
Q2 59.7
Q4 3.30
Q3 30.6
5438.FCS
CD45+CD56-ATO21 202i
7993
CD4
CD8
ATO 20 AND 21 T CELL SORT ON ARIA

FIG. 6

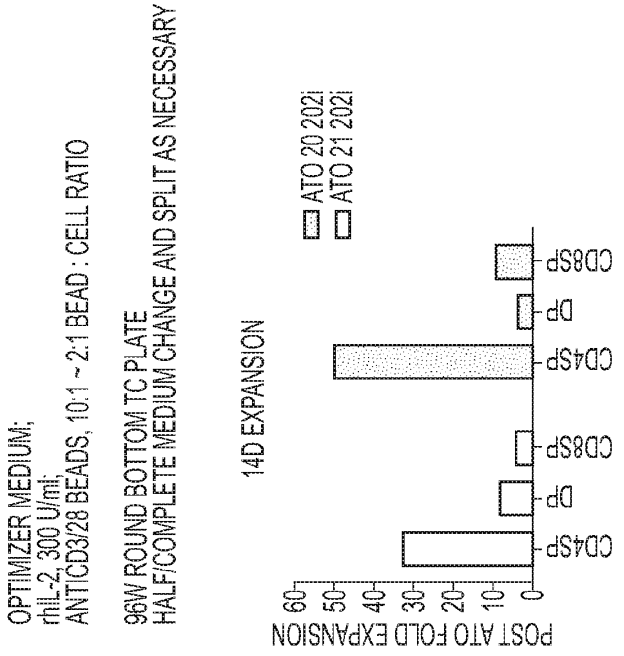
OPTIMIZER MEDIUM;
rhIL-2, 300 U/ml;
ANTICD3/28 BEADS, 10:1 ~ 2:1 BEAD : CELL RATIO
96W ROUND BOTTOM TC PLATE
HALF/COMPLETE MEDIUM CHANGE AND SPLIT AS NECESSARY
14D EXPANSION
ATO 20 202i
ATO 21 202i
POST ATO FOLD EXPANSION
0
10
20
30
40
50
60
CD4SP
DP
CD8SP
CD4SP
DP
CD8SP

FIG. 6
(CONT.-1)

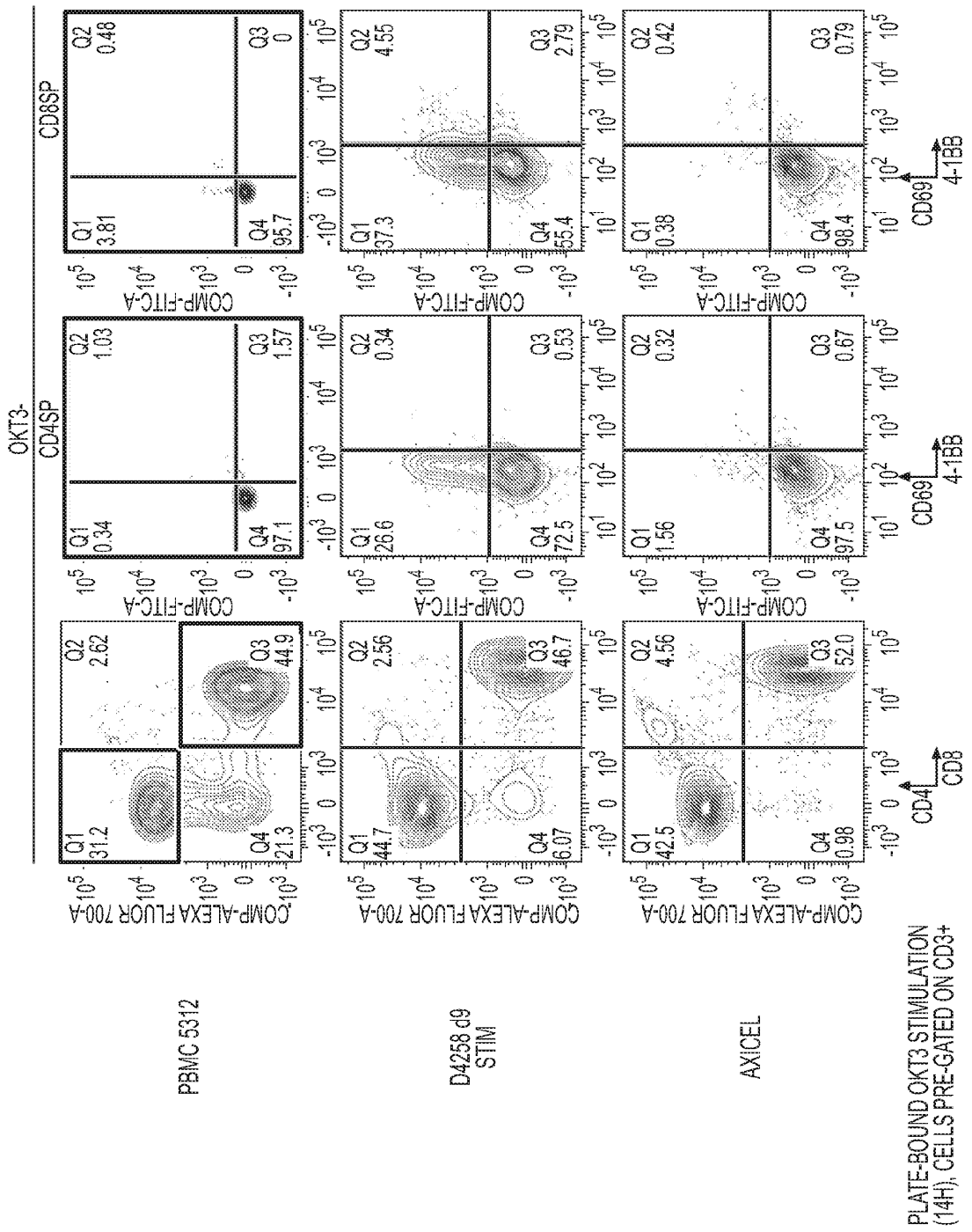
OKT3-
CD4SP
CD8SP
PBMC 5312
D4258 d9 STIM
AXICEL
COMP-ALEXA FLUOR 700-A
COMP-FITC-A
CD4
CD8
CD69
4-1BB
Q1 31.2
Q2 2.62
Q4 21.3
Q3 44.9
Q1 0.34
Q2 1.03
Q4 97.1
Q3 1.57
Q1 3.81
Q2 0.48
Q4 95.7
Q3 0
Q1 44.7
Q2 2.56
Q4 6.07
Q3 46.7
Q1 26.6
Q2 0.34
Q4 72.5
Q3 0.53
Q1 37.3
Q2 4.55
Q4 55.4
Q3 2.79
Q1 42.5
Q2 4.56
Q4 0.98
Q3 52.0
Q1 1.56
Q2 0.32
Q4 97.5
Q3 0.67
Q1 0.38
Q2 0.42
Q4 98.4
Q3 0.79
PLATE-BOUND OKT3 STIMULATION (14H), CELLS PRE-GATED ON CD3+

FIG. 7

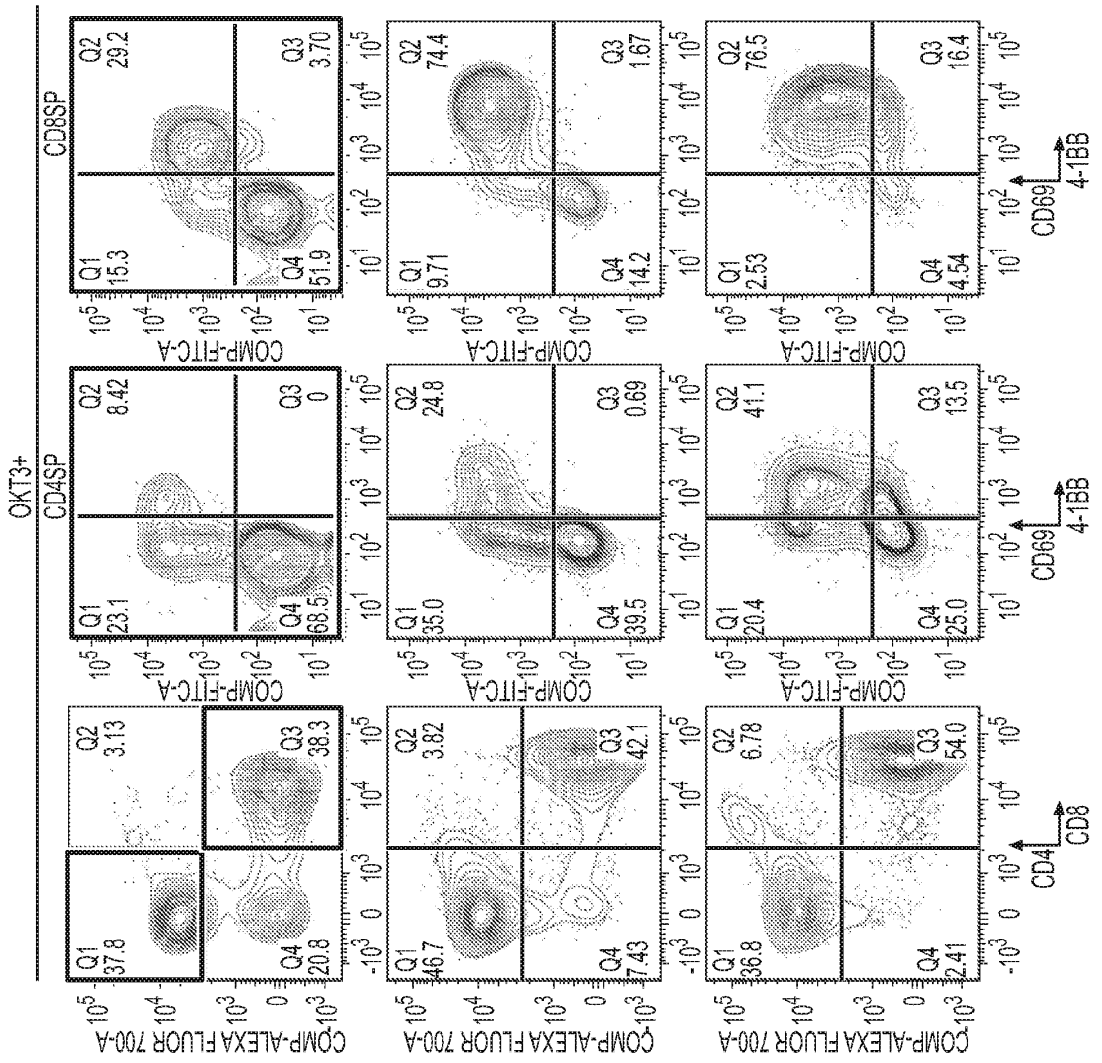
OKT3+
CD4SP
CD8SP
COMP-FITC-A
COMP-ALEXA FLUOR 700-A
CD4
CD8
CD69
4-1BB
Q1 37.8
Q2 3.13
Q3 38.3
Q4 20.8
Q1 23.1
Q2 8.42
Q3 0
Q4 68.5
Q1 15.3
Q2 29.2
Q3 3.70
Q4 51.9
Q1 46.7
Q2 3.82
Q3 42.1
Q4 7.43
Q1 35.0
Q2 24.8
Q3 0.69
Q4 39.5
Q1 9.71
Q2 74.4
Q3 1.67
Q4 14.2
Q1 36.8
Q2 6.78
Q3 54.0
Q4 2.41
Q1 20.4
Q2 41.1
Q3 13.5
Q4 25.0
Q1 2.53
Q2 76.5
Q3 16.4
Q4 4.54

FIG. 7
(CONT.-1)

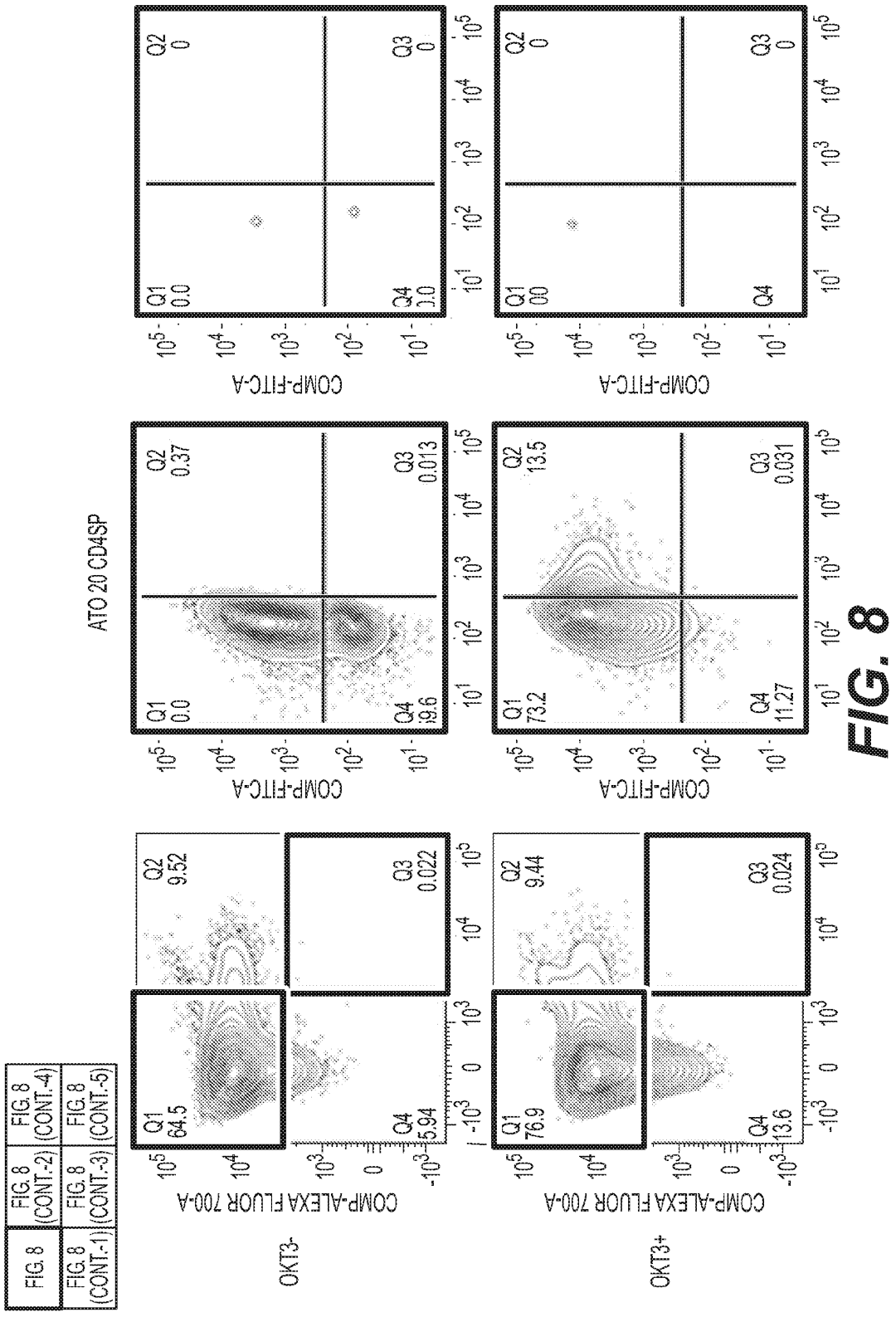
FIG. 8
FIG. 8 (CONT.-1)
FIG. 8 (CONT.-2)
FIG. 8 (CONT.-3)
FIG. 8 (CONT.-4)
FIG. 8 (CONT.-5)
OKT3-
OKT3+
ATO 20 CD4SP
COMP-ALEXA FLUOR 700-A
COMP-FITC-A
Q1 84.5
Q2 9.52
Q3 0.022
Q4 5.94
Q1 76.9
Q2 9.44
Q3 0.024
Q4 13.6
Q1 0.0
Q2 0.37
Q3 0.013
Q4 19.6
Q1 73.2
Q2 13.5
Q3 0.031
Q4 11.27
Q1 0.0
Q2 0
Q3 0
Q4 0.0
Q1 00
Q2 0
Q3 0
Q4

FIG. 8

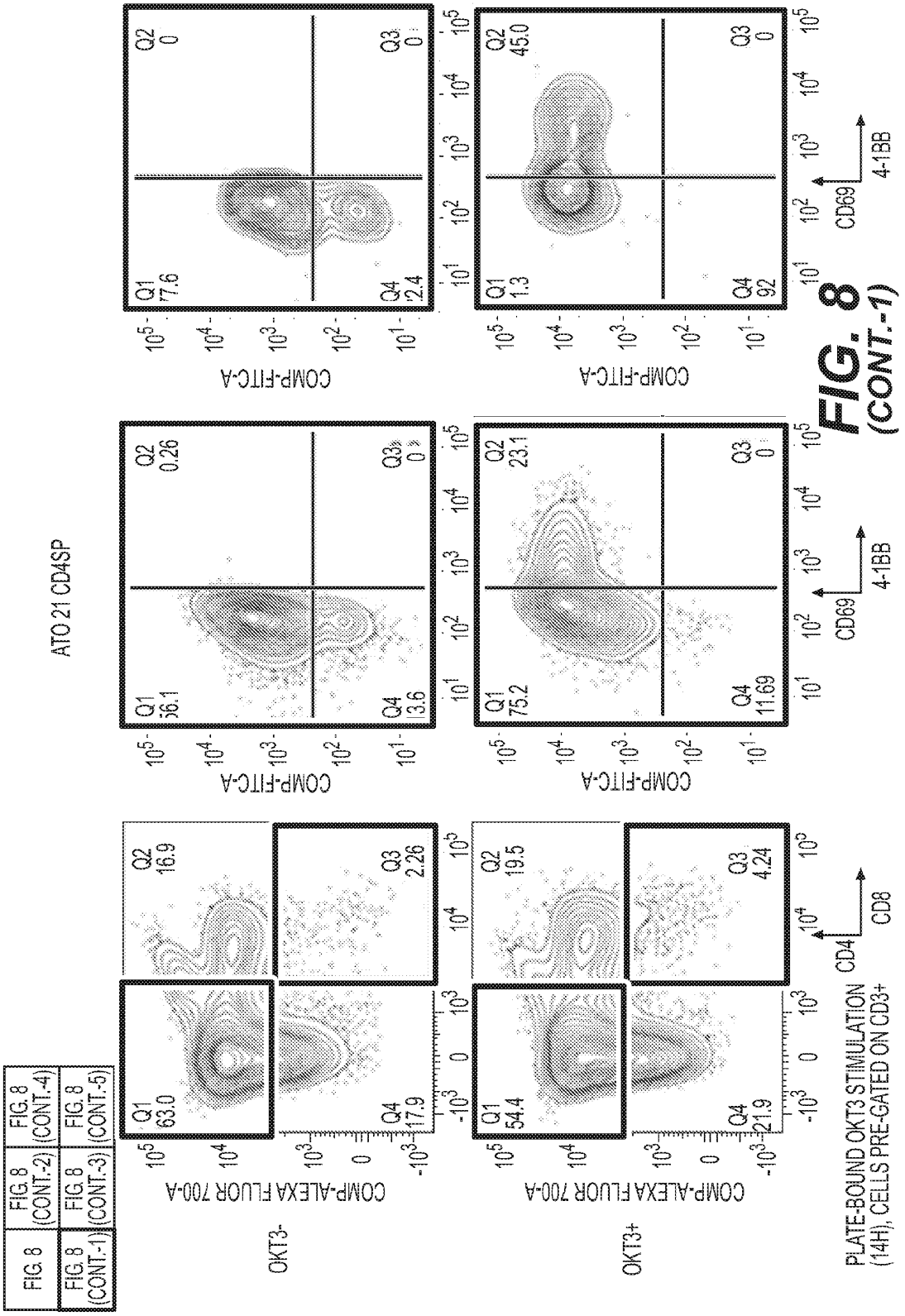
FIG. 8
FIG. 8 (CONT.-1)
FIG. 8 (CONT.-2)
FIG. 8 (CONT.-3)
FIG. 8 (CONT.-4)
FIG. 8 (CONT.-5)
ATO 21 CD4SP
OKT3-
OKT3+
COMP-ALEXA FLUOR 700-A
COMP-FITC-A
CD4
CD8
CD69
4-1BB
Q1 63.0
Q2 16.9
Q3 2.26
Q4 17.9
Q1 54.4
Q2 19.5
Q3 4.24
Q4 21.9
Q1 16.1
Q2 0.26
Q3 0
Q4 3.6
Q1 75.2
Q2 23.1
Q3 0
Q4 11.69
Q1 17.6
Q2 0
Q3 0
Q4 2.4
Q1 1.3
Q2 45.0
Q3 0
Q4 92
PLATE-BOUND OKT3 STIMULATION (14H), CELLS PRE-GATED ON CD3+

FIG. 8 (CONT.-1)

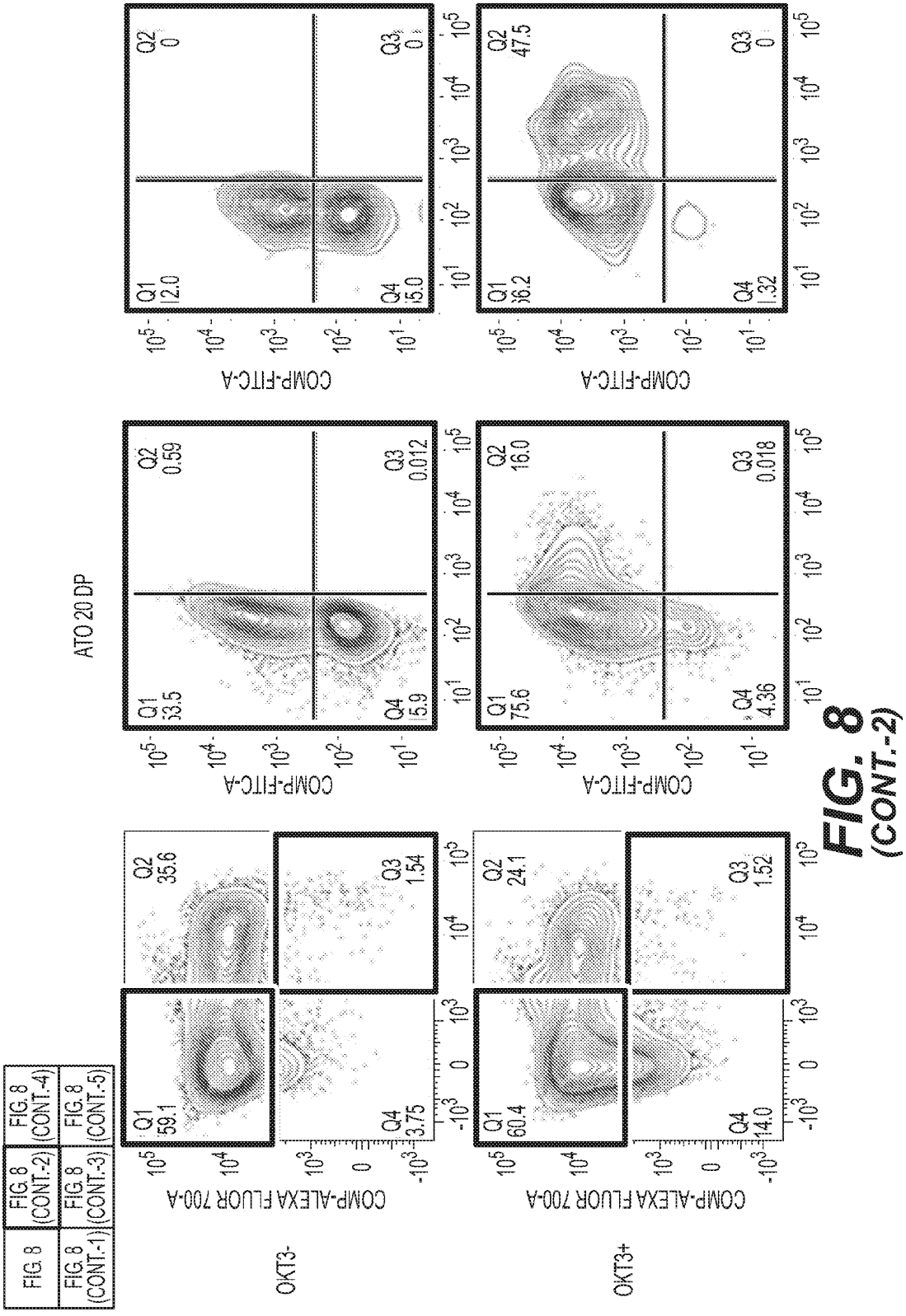
FIG. 8
FIG. 8 (CONT.-1)
FIG. 8 (CONT.-2)
FIG. 8 (CONT.-3)
FIG. 8 (CONT.-4)
FIG. 8 (CONT.-5)
OKT3-
OKT3+
ATO 20 DP
COMP-ALEXA FLUOR 700-A
COMP-FITC-A
Q1 59.1
Q2 35.6
Q3 1.54
Q4 3.75
Q1 60.4
Q2 24.1
Q3 1.52
Q4 14.0
Q1 13.5
Q2 0.59
Q3 0.012
Q4 5.9
Q1 75.6
Q2 16.0
Q3 0.018
Q4 4.36
Q1 12.0
Q2 0
Q3 0
Q4 5.0
Q1 16.2
Q2 47.5
Q3 0
Q4 1.32

FIG. 8 (CONT.-2)

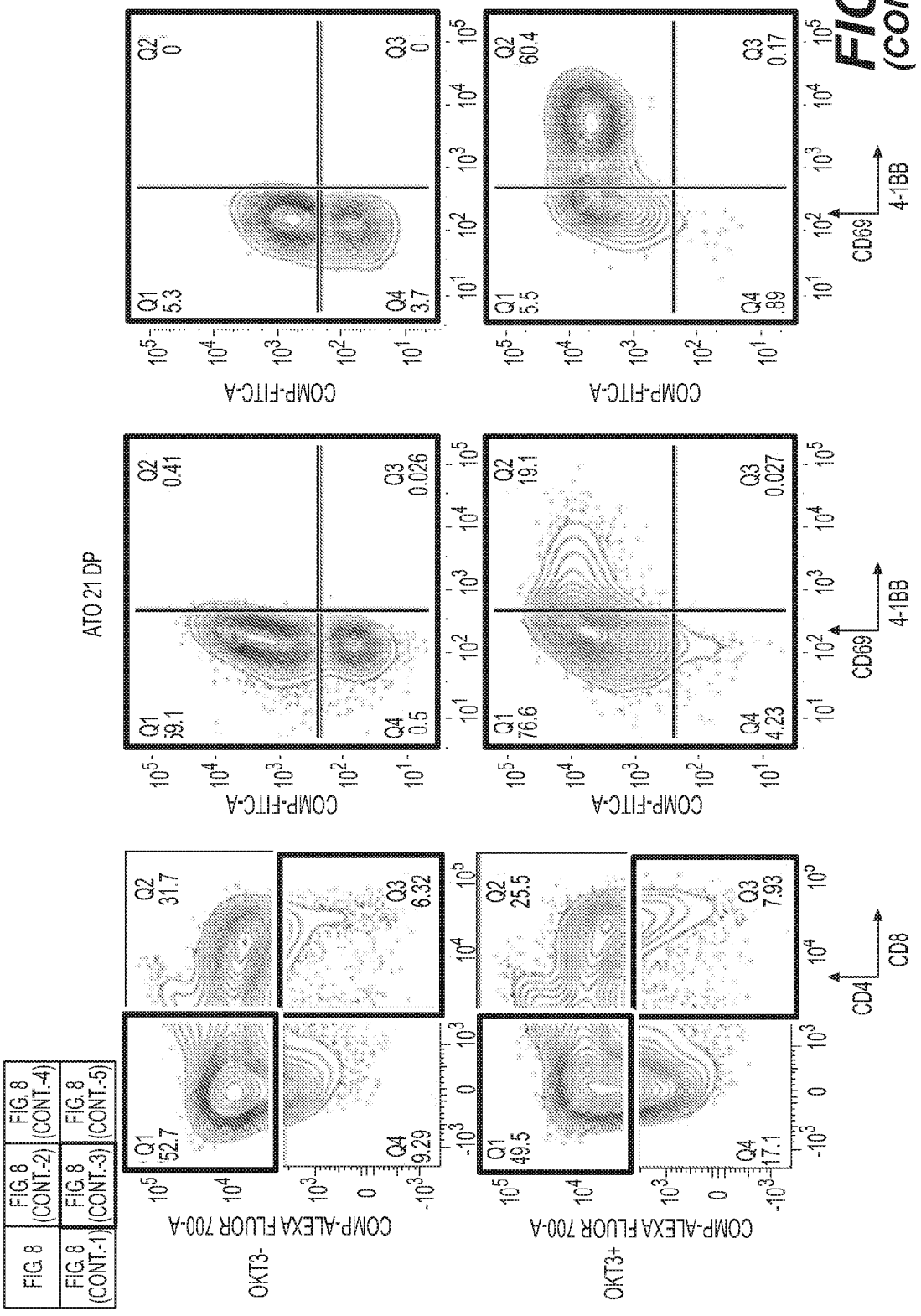
FIG. 8
FIG. 8 (CONT.-1)
FIG. 8 (CONT.-2)
FIG. 8 (CONT.-3)
FIG. 8 (CONT.-4)
FIG. 8 (CONT.-5)
OKT3+
OKT3-
ATO 21 DP
CD4
CD8
CD69
4-1BB
COMP-ALEXA FLUOR 700-A
COMP-FITC-A
Q1 49.5
Q2 25.5
Q3 7.93
Q4 17.1
Q1 52.7
Q2 31.7
Q3 6.32
Q4 9.29
Q1 76.6
Q2 19.1
Q3 0.027
Q4 4.23
Q1 59.1
Q2 0.41
Q3 0.026
Q4 0.5
Q1 5.5
Q2 60.4
Q3 0.17
Q4 .89
Q1 5.3
Q2 0
Q3 0
Q4 3.7

FIG. 8 (CONT.-3)

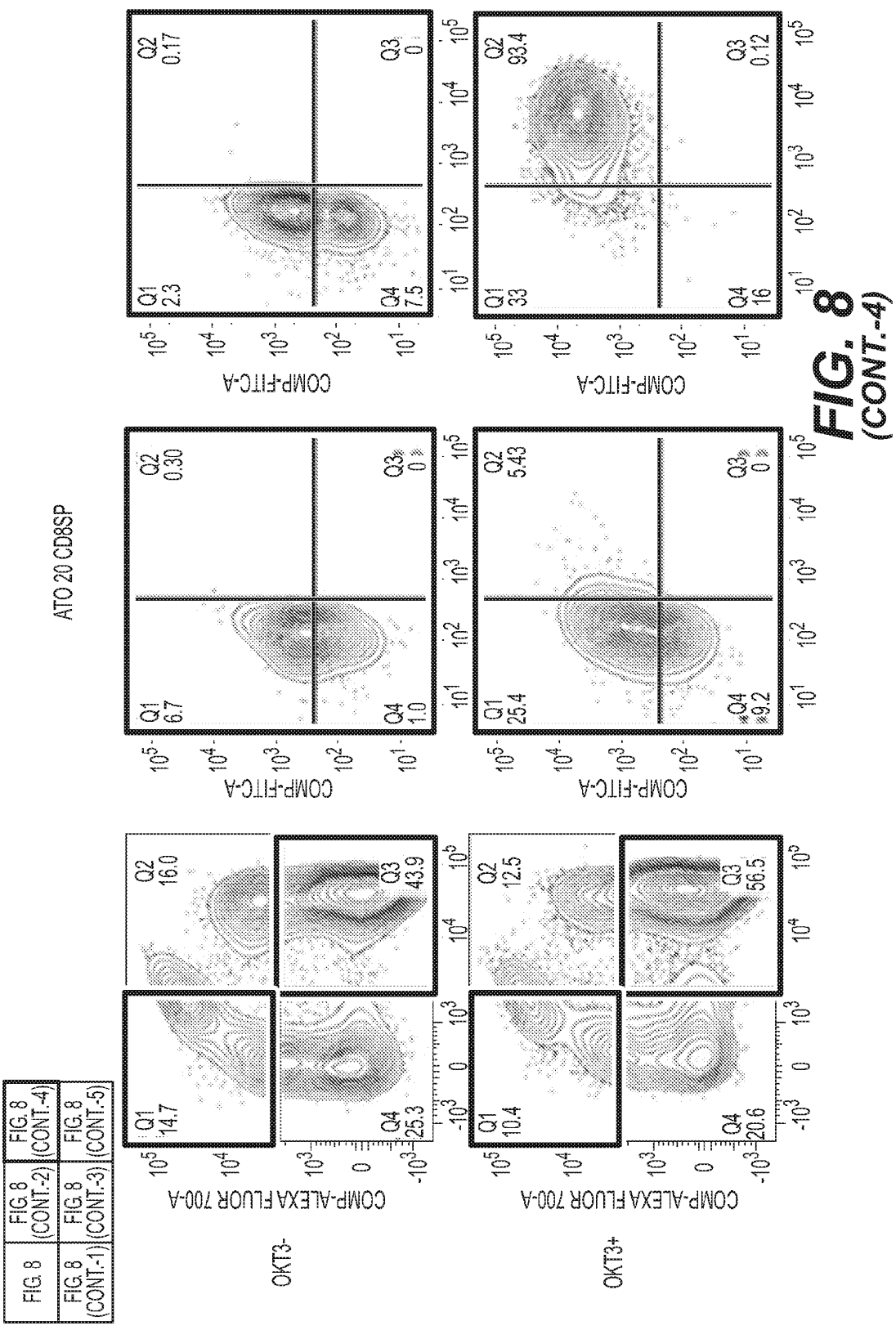
FIG. 8
FIG. 8 (CONT.-1)
FIG. 8 (CONT.-2)
FIG. 8 (CONT.-3)
FIG. 8 (CONT.-4)
FIG. 8 (CONT.-5)
ATO 20 CD8SP
OKT3-
OKT3+
COMP-ALEXA FLUOR 700-A
COMP-FITC-A
Q1 14.7
Q2 16.0
Q3 43.9
Q4 25.3
Q1 10.4
Q2 12.5
Q3 56.5
Q4 20.6
Q1 6.7
Q2 0.30
Q3 0.18
Q4 1.0
Q1 25.4
Q2 5.43
Q3 0.18
Q4 9.2
Q1 2.3
Q2 0.17
Q3 0
Q4 7.5
Q1 33
Q2 93.4
Q3 0.12
Q4 16

FIG. 8 (CONT.-4)

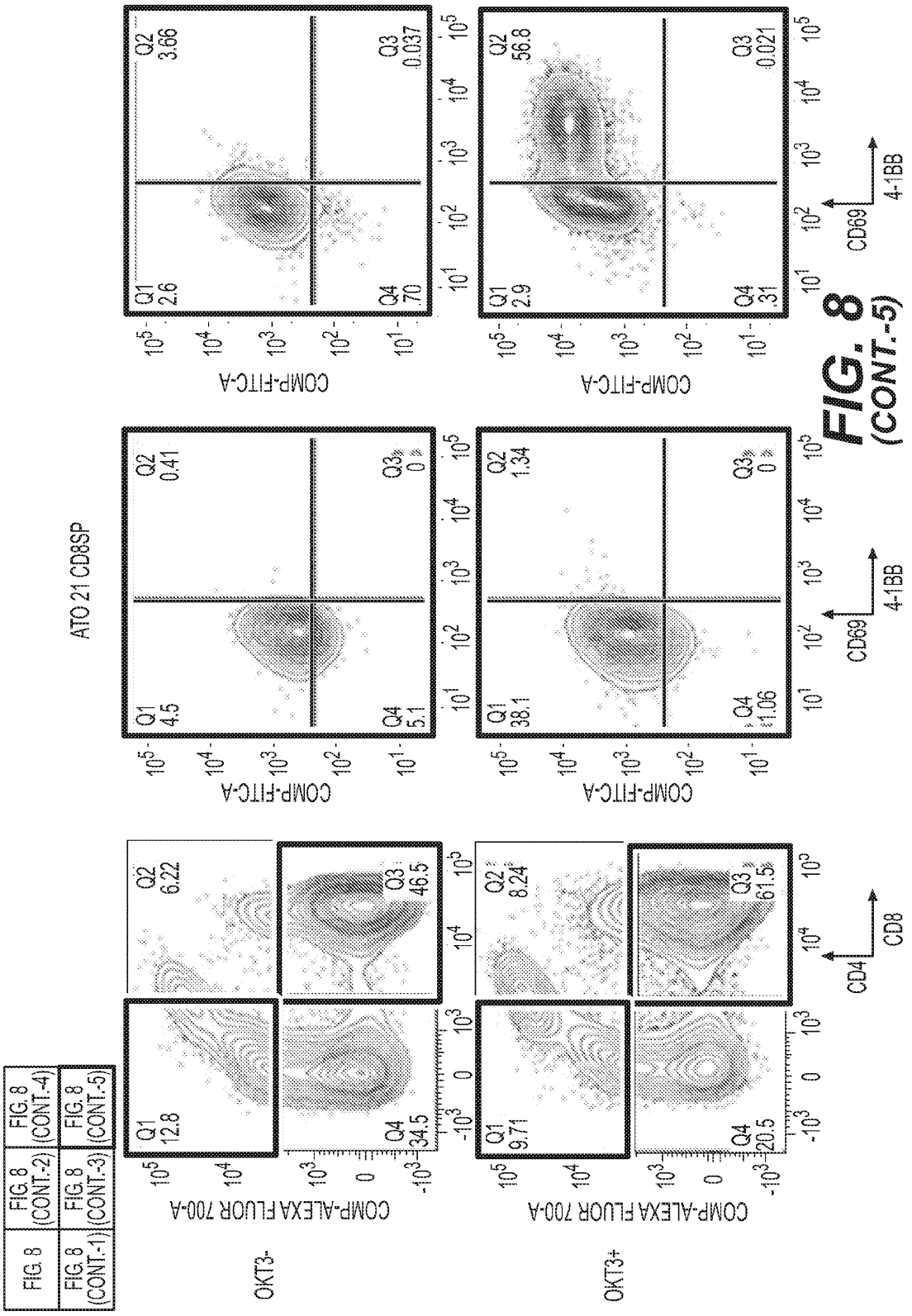
FIG. 8
FIG. 8 (CONT.-1)
FIG. 8 (CONT.-2)
FIG. 8 (CONT.-3)
FIG. 8 (CONT.-4)
FIG. 8 (CONT.-5)
ATO 21 CD8SP
OKT3-
OKT3+
COMP-ALEXA FLUOR 700-A
COMP-FITC-A
CD4
CD8
CD69
4-1BB
Q1 12.8
Q2 6.22
Q3 46.5
Q4 34.5
Q1 9.71
Q2 8.24
Q3 61.5
Q4 20.5
Q1 4.5
Q2 0.41
Q3 0
Q4 5.1
Q1 38.1
Q2 1.34
Q3 0
Q4 1.06
Q1 2.6
Q2 3.66
Q3 0.037
Q4 70
Q1 2.9
Q2 56.8
Q3 0.021
Q4 31

FIG. 8 (CONT.-5)

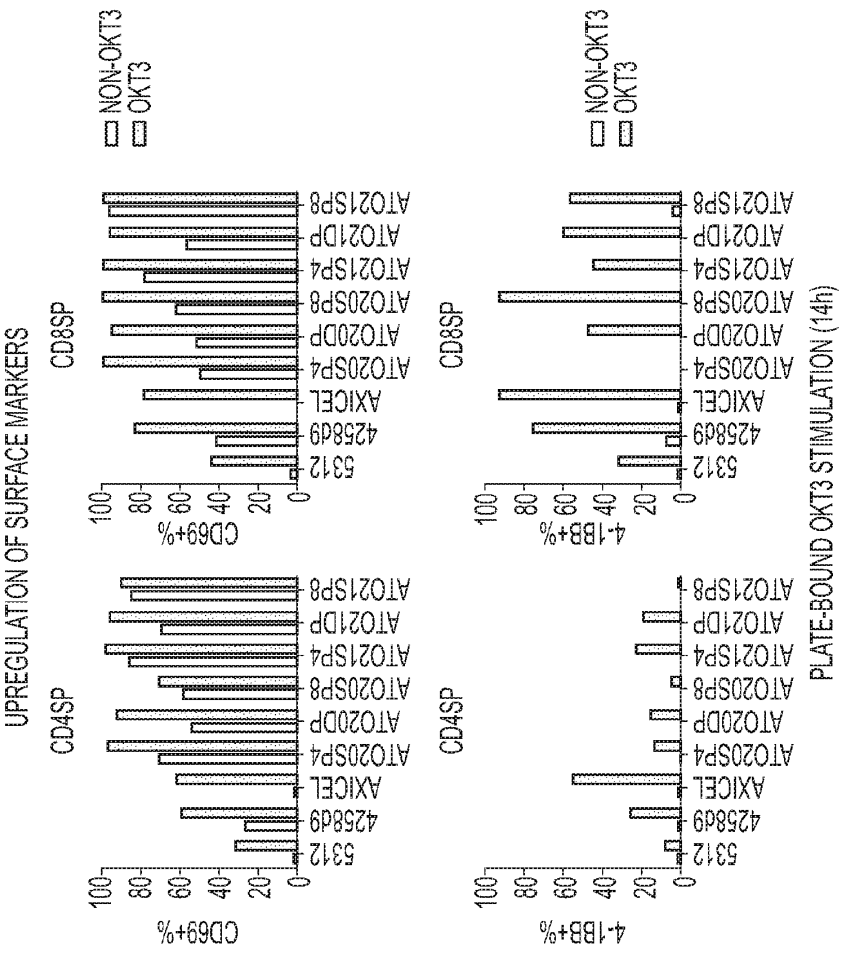
UPREGULATION OF SURFACE MARKERS
CD4SP
CD8SP
CD69+%
4-1BB+%
NON-OKT3
OKT3
5312
4258d9
AXICEL
ATO20SP4
ATO20DP
ATO20SP8
ATO21SP4
ATO21DP
ATO21SP8
PLATE-BOUND OKT3 STIMULATION (14h)

FIG. 9

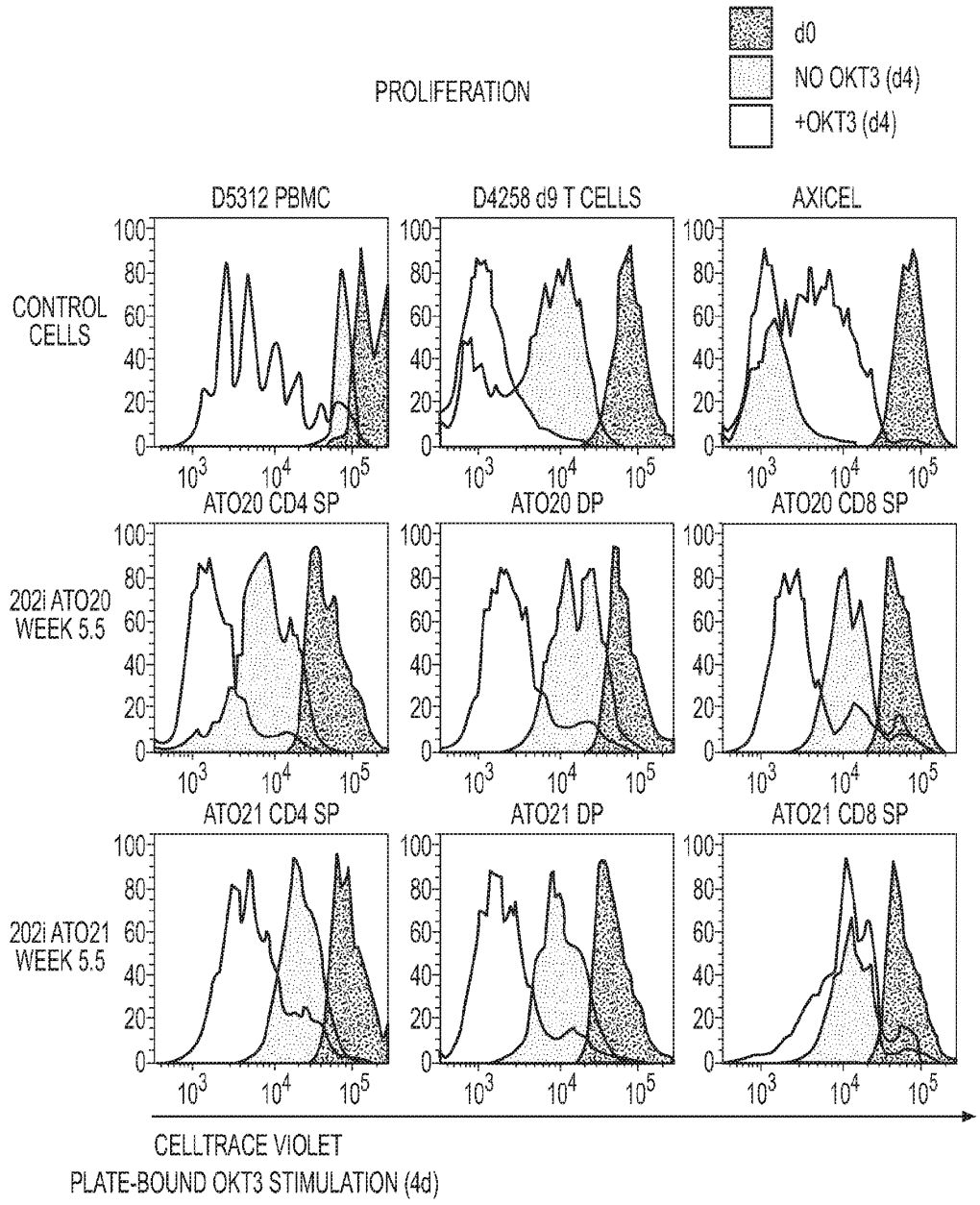
PROLIFERATION
d0
NO OKT3 (d4)
+OKT3 (d4)
D5312 PBMC
D4258 d9 T CELLS
AXICEL
CONTROL CELLS
ATO20 CD4 SP
ATO20 DP
ATO20 CD8 SP
202i ATO20 WEEK 5.5
ATO21 CD4 SP
ATO21 DP
ATO21 CD8 SP
202i ATO21 WEEK 5.5
CELLTRACE VIOLET
PLATE-BOUND OKT3 STIMULATION (4d)

FIG. 9
(CONT.-1)

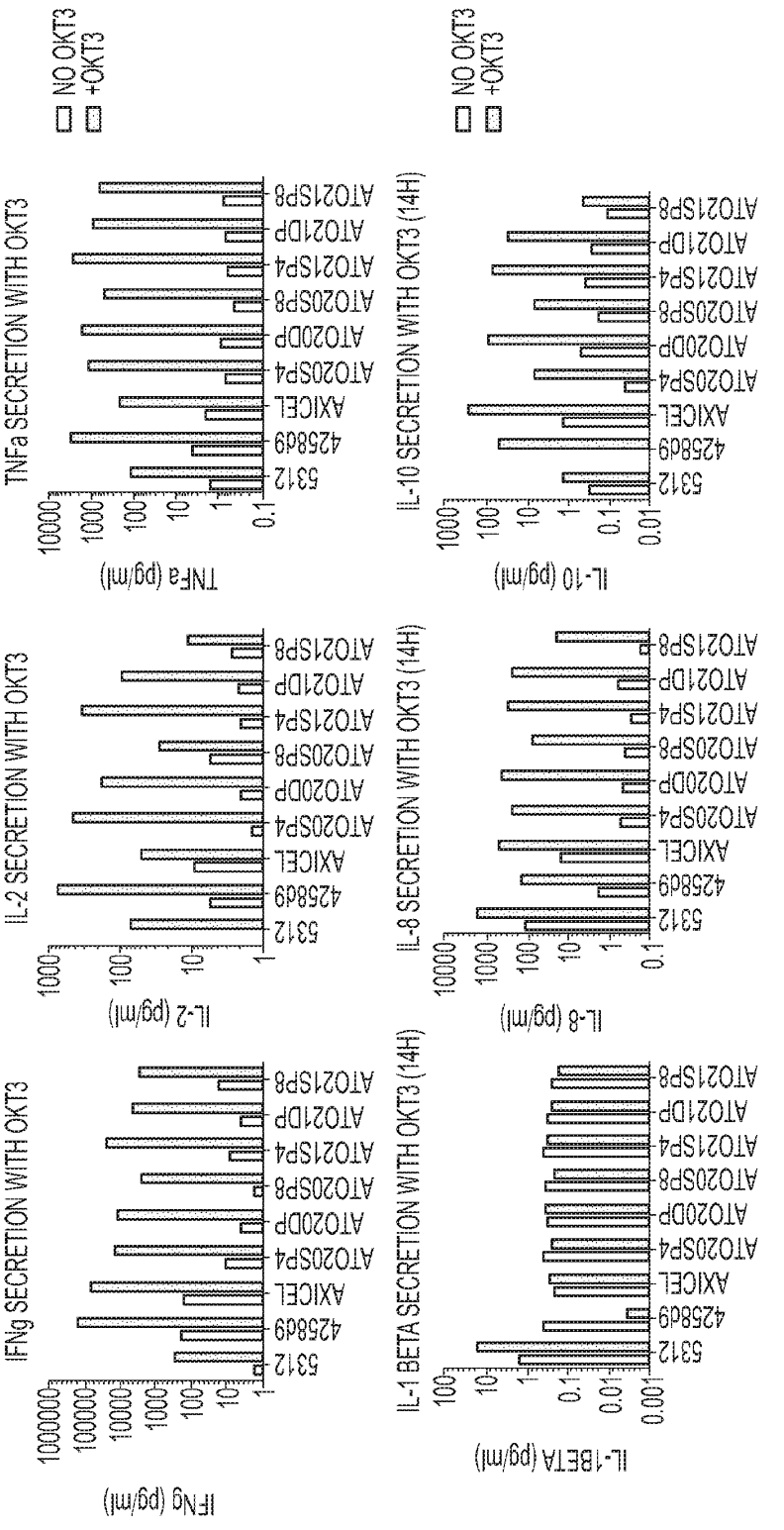
NO OKT3
+OKT3
TNFa SECRETION WITH OKT3
TNFa (pg/ml)
IL-10 SECRETION WITH OKT3 (14H)
IL-10 (pg/ml)
IL-2 SECRETION WITH OKT3
IL-2 (pg/ml)
IL-8 SECRETION WITH OKT3 (14H)
IL-8 (pg/ml)
IFNg SECRETION WITH OKT3
IFNg (pg/ml)
IL-1 BETA SECRETION WITH OKT3 (14H)
IL-1BETA (pg/ml)
5312
425809
AXICEL
ATO20SP4
ATO20DP
ATO20SP8
ATO21SP4
ATO21DP
ATO21SP8
MSD TEST OF ATO 20/21 DERIVED T CELL OKT3 STIMULATION (14h)

FIG. 10

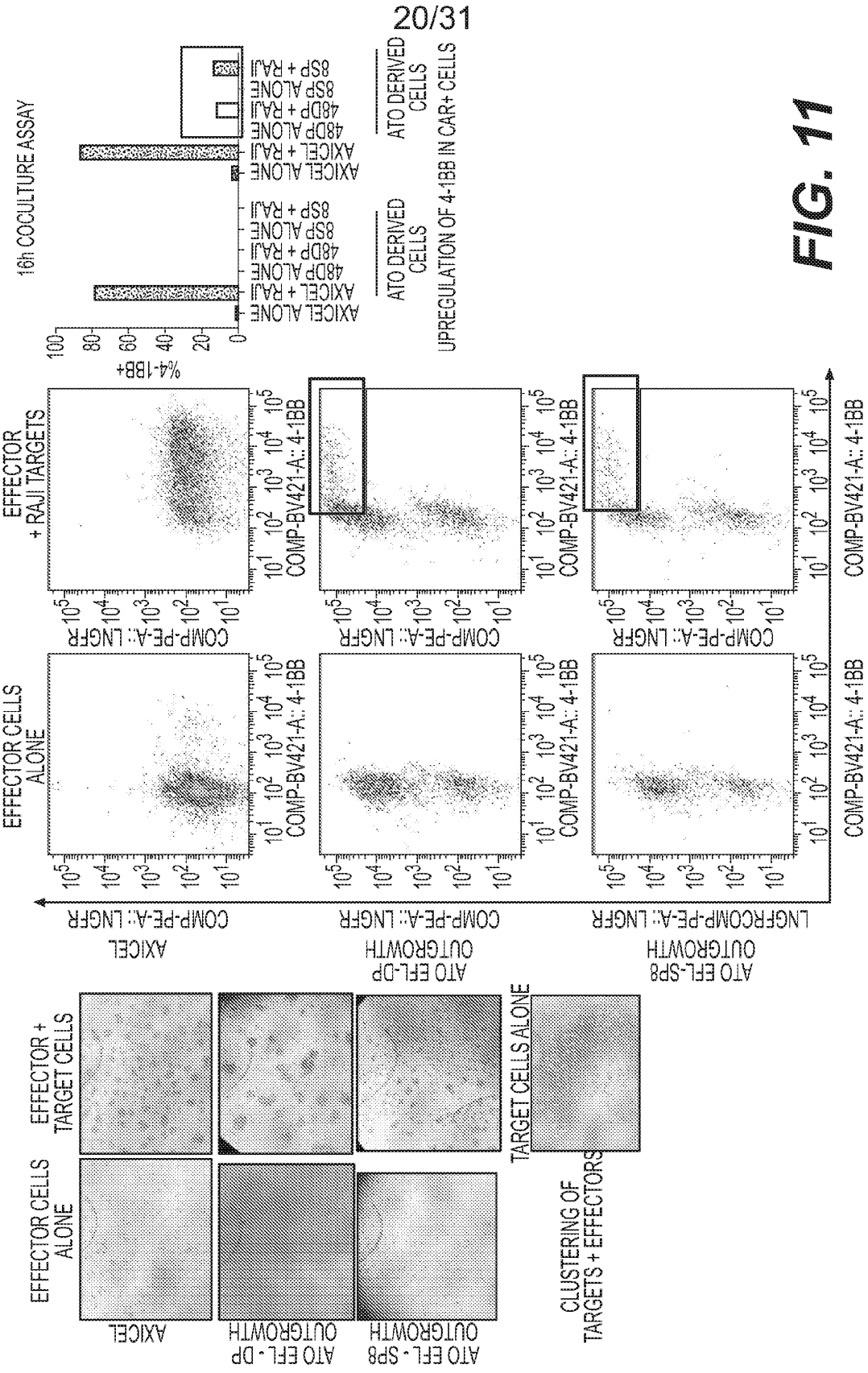
16h COCULTURE ASSAY
%4-1BB+
AXICEL ALONE
AXICEL + RAJI
4BDP ALONE
4BDP + RAJI
8SP ALONE
8SP + RAJI
ATO DERIVED CELLS
ATO DERIVED CELLS
UPREGULATION OF 4-1BB IN CAR+ CELLS
EFFECTOR + RAJI TARGETS
EFFECTOR CELLS ALONE
COMP-PE-A:: LNGFR
COMP-BV421-A:: 4-1BB
AXICEL
ATO EFL-DP OUTGROWTH
ATO EFL-SP8 OUTGROWTH
EFFECTOR + TARGET CELLS
EFFECTOR CELLS ALONE
TARGET CELLS ALONE
CLUSTERING OF TARGETS + EFFECTORS

FIG. 11

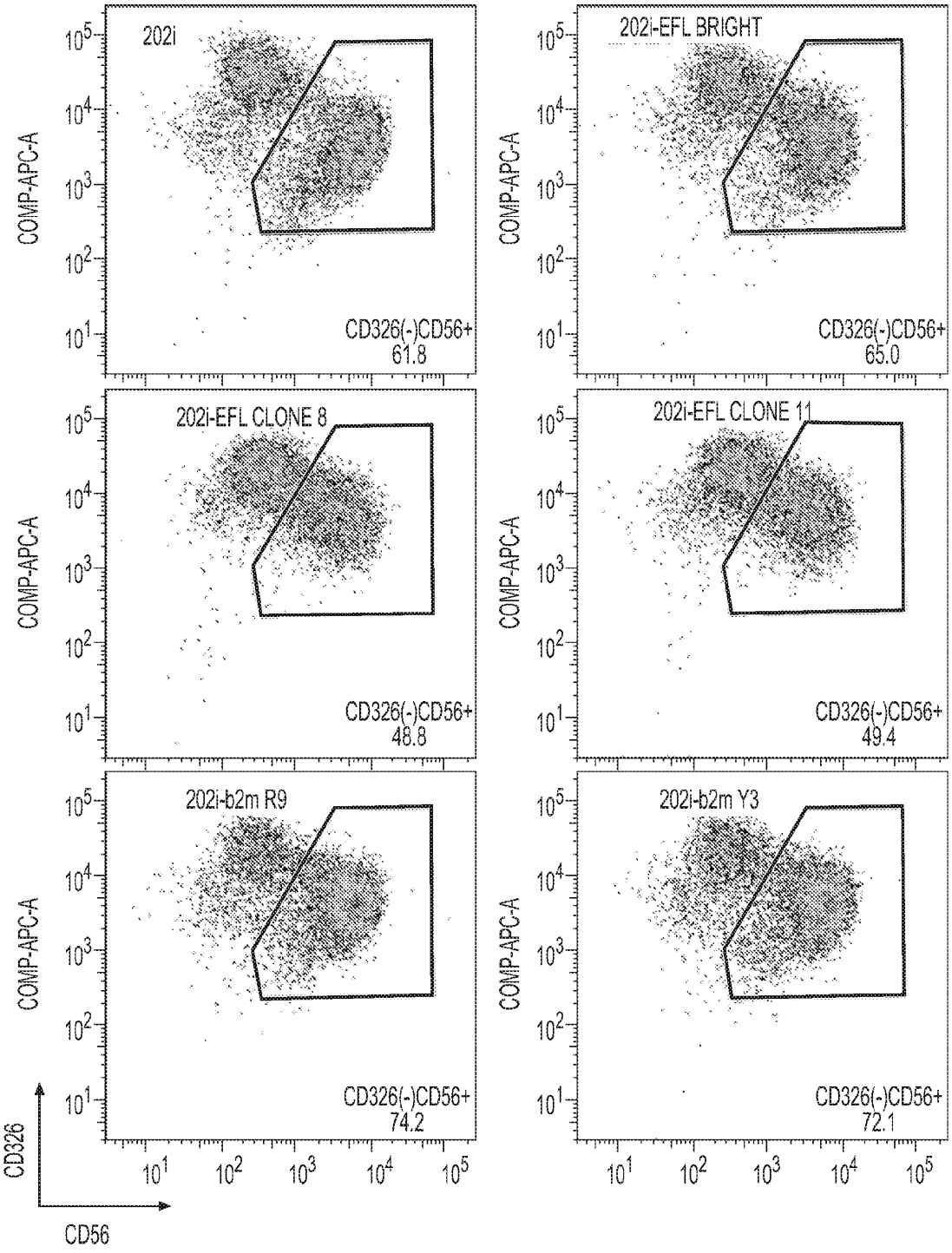
202i
CD326(-)CD56+
61.8
202i-EFL BRIGHT
CD326(-)CD56+
65.0
202i-EFL CLONE 8
CD326(-)CD56+
48.8
202i-EFL CLONE 11
CD326(-)CD56+
49.4
202i-b2m R9
CD326(-)CD56+
74.2
202i-b2m Y3
CD326(-)CD56+
72.1
COMP-APC-A
CD326
CD56

FIG. 12

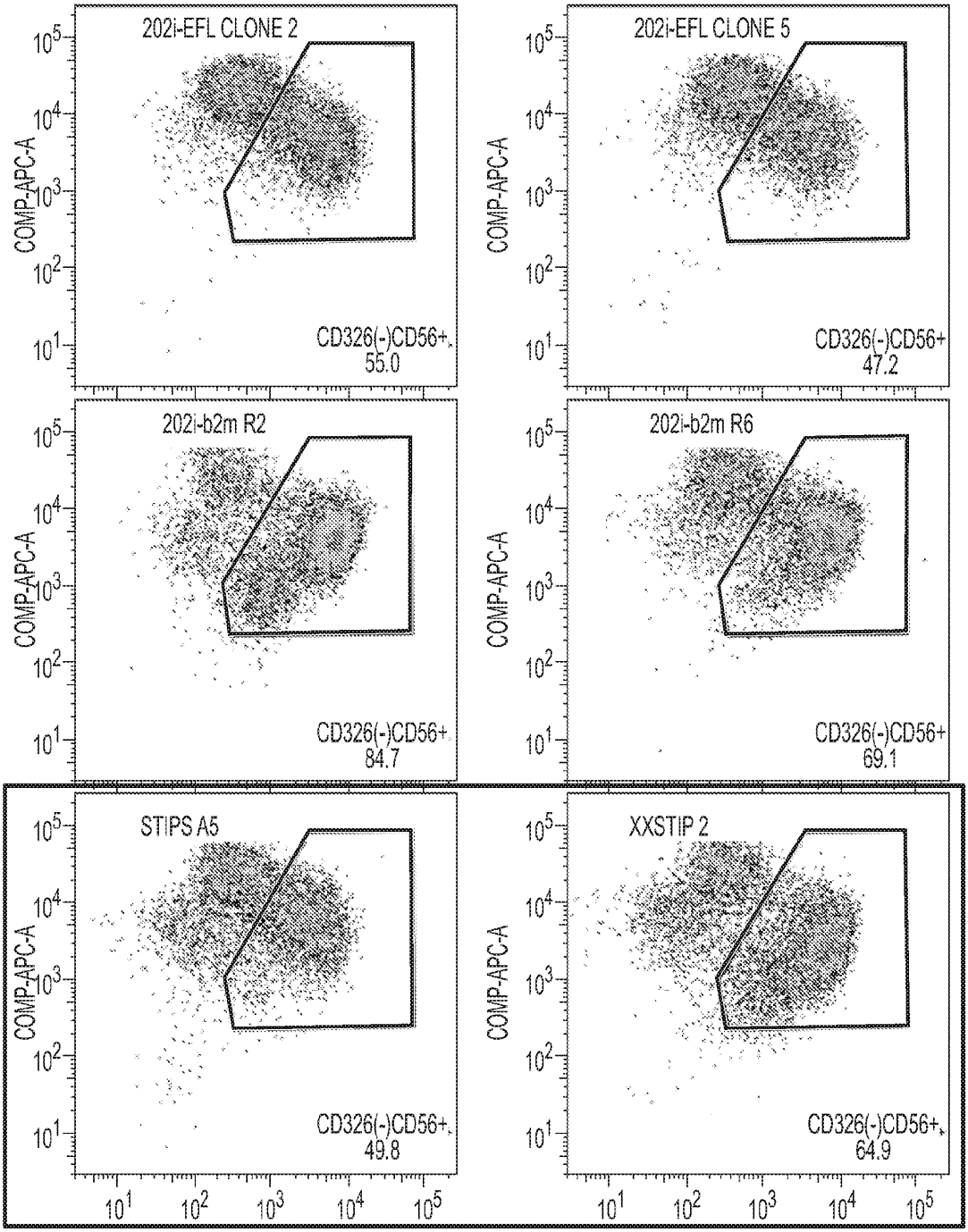
202i-EFL CLONE 2
CD326(-)CD56+
55.0
202i-EFL CLONE 5
CD326(-)CD56+
47.2
202i-b2m R2
CD326(-)CD56+
84.7
202i-b2m R6
CD326(-)CD56+
69.1
STIPS A5
CD326(-)CD56+
49.8
XXSTIP 2
CD326(-)CD56+
64.9
COMP-APC-A
10^1
10^2
10^3
10^4
10^5

FIG. 12
(CONT.-1)

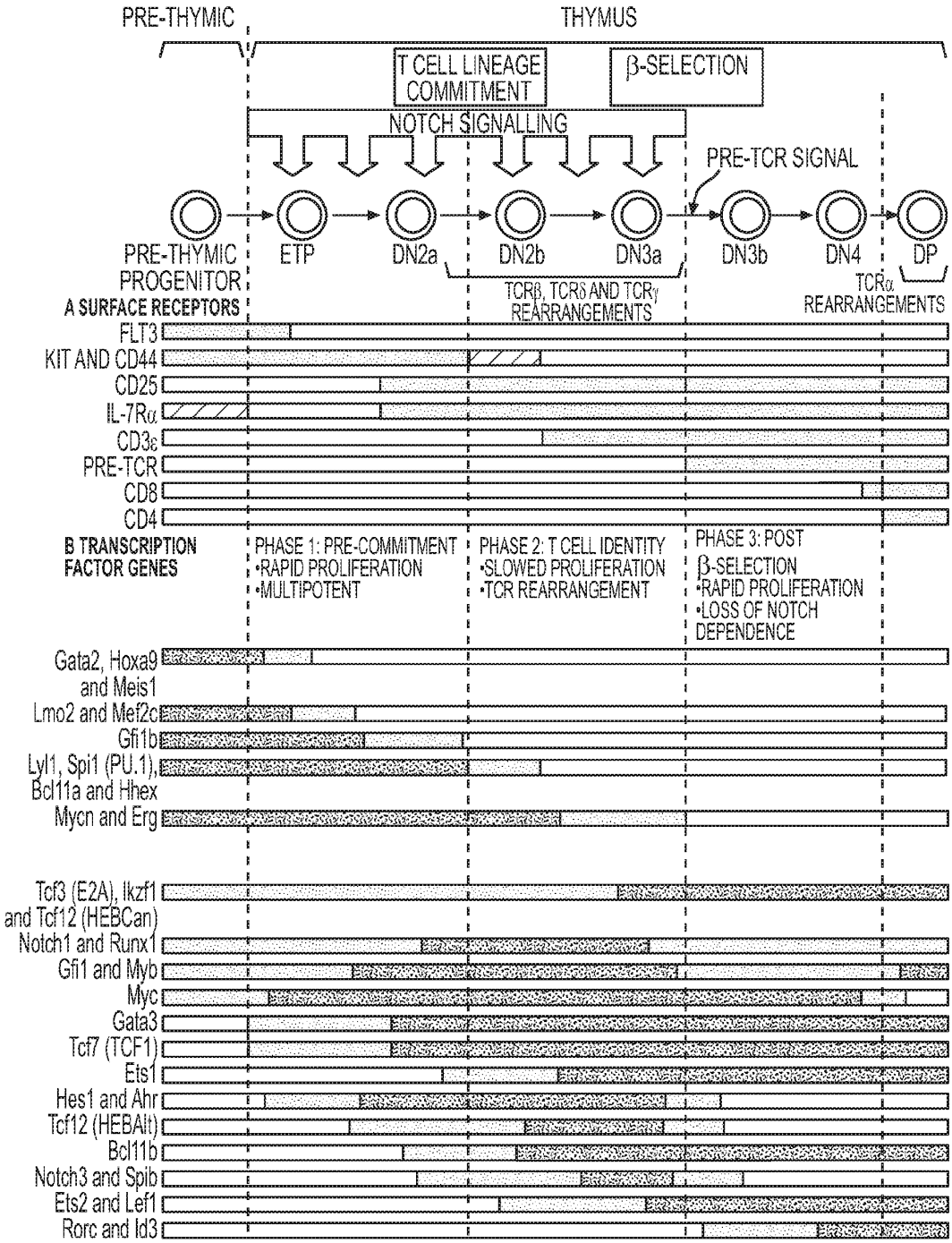
PRE-THYMIC
THYMUS
T CELL LINEAGE COMMITMENT
β-SELECTION
NOTCH SIGNALLING
PRE-TCR SIGNAL
PRE-THYMIC PROGENITOR
ETP
DN2a
DN2b
DN3a
DN3b
DN4
DP
TCRβ, TCRδ AND TCRγ REARRANGEMENTS
TCRα REARRANGEMENTS
A SURFACE RECEPTORS
FLT3
KIT AND CD44
CD25
IL-7Rα
CD3ε
PRE-TCR
CD8
CD4
B TRANSCRIPTION FACTOR GENES
PHASE 1: PRE-COMMITMENT
•RAPID PROLIFERATION
•MULTIPOTENT
PHASE 2: T CELL IDENTITY
•SLOWED PROLIFERATION
•TCR REARRANGEMENT
PHASE 3: POST β-SELECTION
•RAPID PROLIFERATION
•LOSS OF NOTCH DEPENDENCE
Gata2, Hoxa9 and Meis1
Lmo2 and Mef2c
Gfi1b
Lyl1, Spi1 (PU.1), Bcl11a and Hhex
Mycn and Erg
Tcf3 (E2A), Ikzf1 and Tcf12 (HEBCan)
Notch1 and Runx1
Gfi1 and Myb
Myc
Gata3
Tcf7 (TCF1)
Ets1
Hes1 and Ahr
Tcf12 (HEBAlt)
Bcl11b
Notch3 and Spib
Ets2 and Lef1
Rorc and Id3

FIG. 13

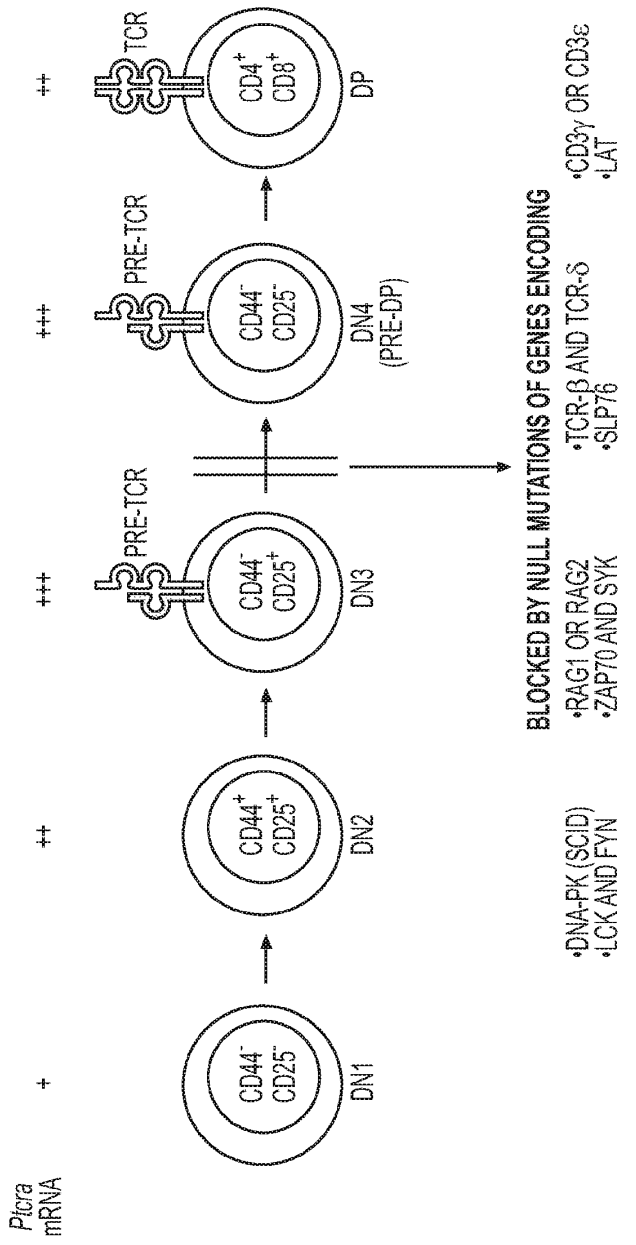
Ptcra mRNA
+
++
+++
+++
++
CD44- CD25- DN1
CD44+ CD25+ DN2
PRE-TCR
CD44- CD25+ DN3
PRE-TCR
CD44- CD25- DN4 (PRE-DP)
TCR
CD4+ CD8+ DP
BLOCKED BY NULL MUTATIONS OF GENES ENCODING
•DNA-PK (SCID)
•LCK AND FYN
•RAG1 OR RAG2
•ZAP70 AND SYK
•TCR-β AND TCR-δ
•SLP76
•CD3γ OR CD3ε
•LAT

FIG. 14

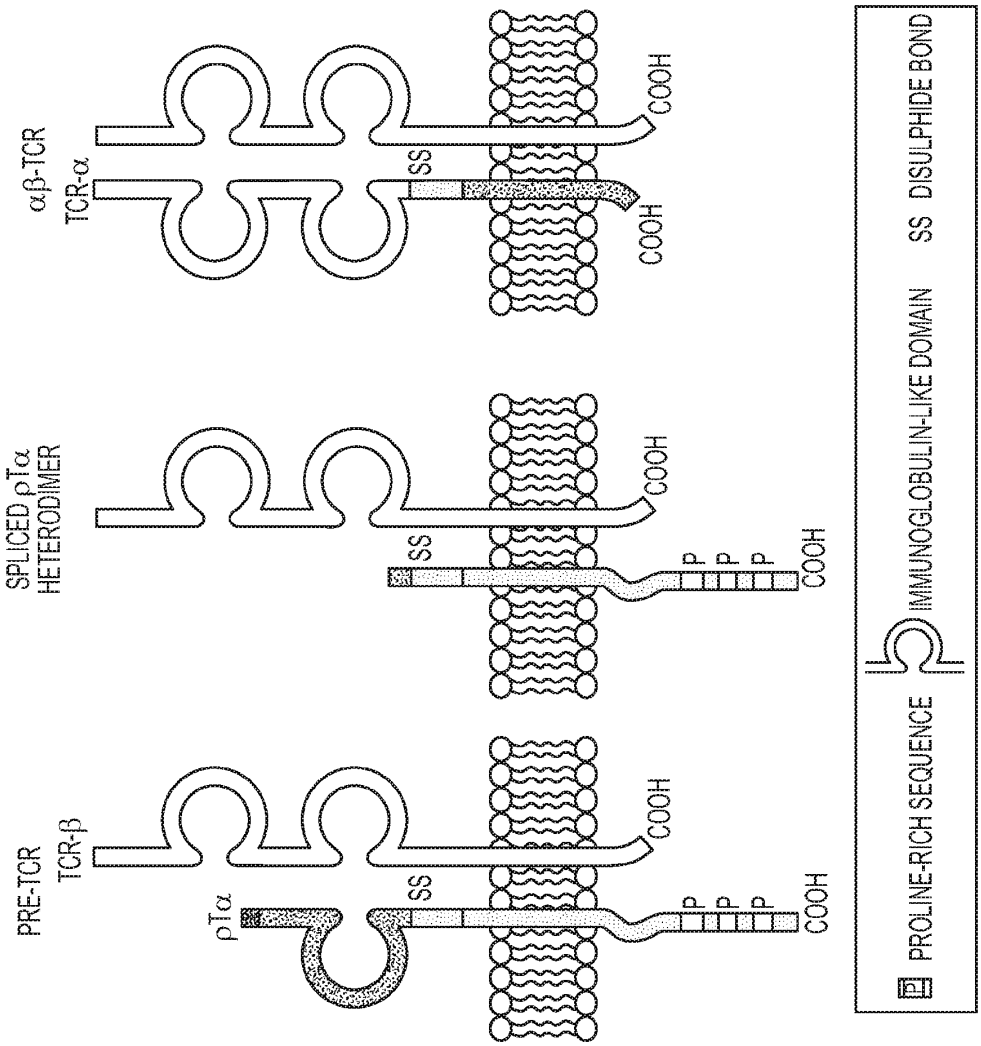
αβ-TCR
TCR-α
SS
COOH
COOH
SPLICED ρTα
HETERODIMER
SS
COOH
P
P
P
COOH
PRE-TCR
TCR-β
ρTα
SS
COOH
P
P
P
COOH
PROLINE-RICH SEQUENCE
IMMUNOGLOBULIN-LIKE DOMAIN
SS DISULPHIDE BOND

FIG. 15

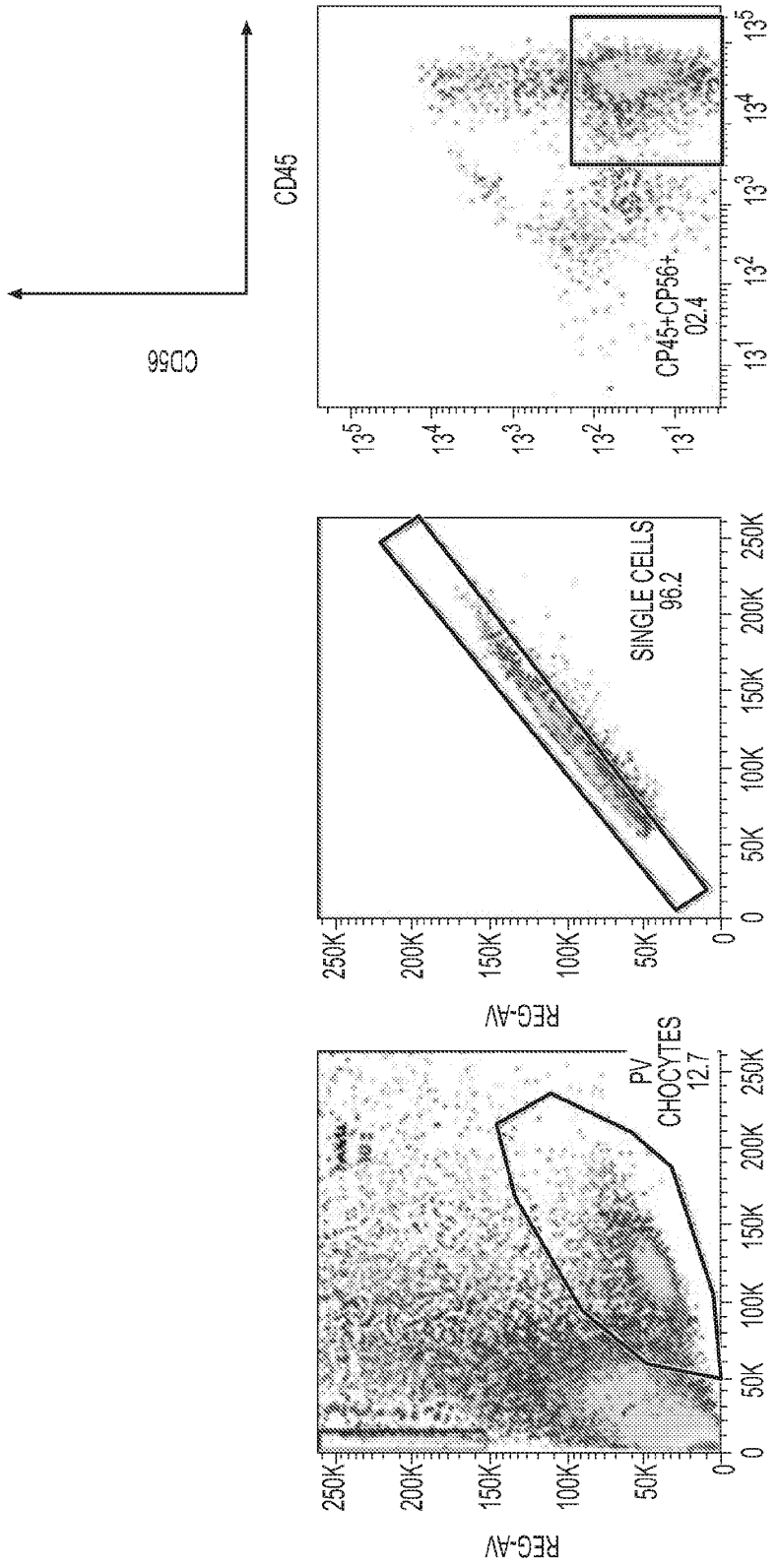
CD56
CD45
CP45+CP56+
02.4
SINGLE CELLS
96.2
PV
CHOCYTES
12.7
REG-AV
REG-AV

FIG. 16A

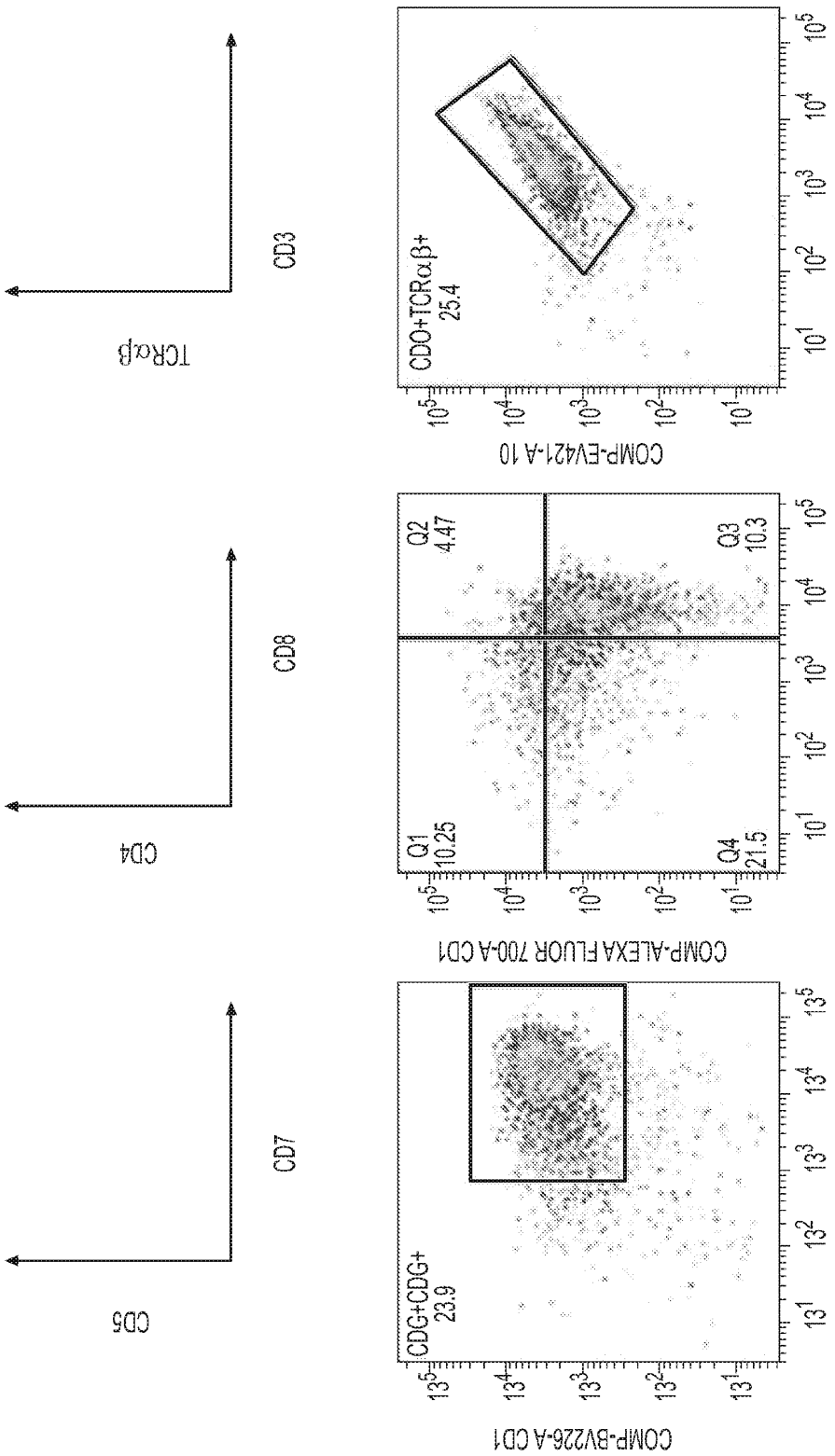

FIG. 16A
(CONT.-1)

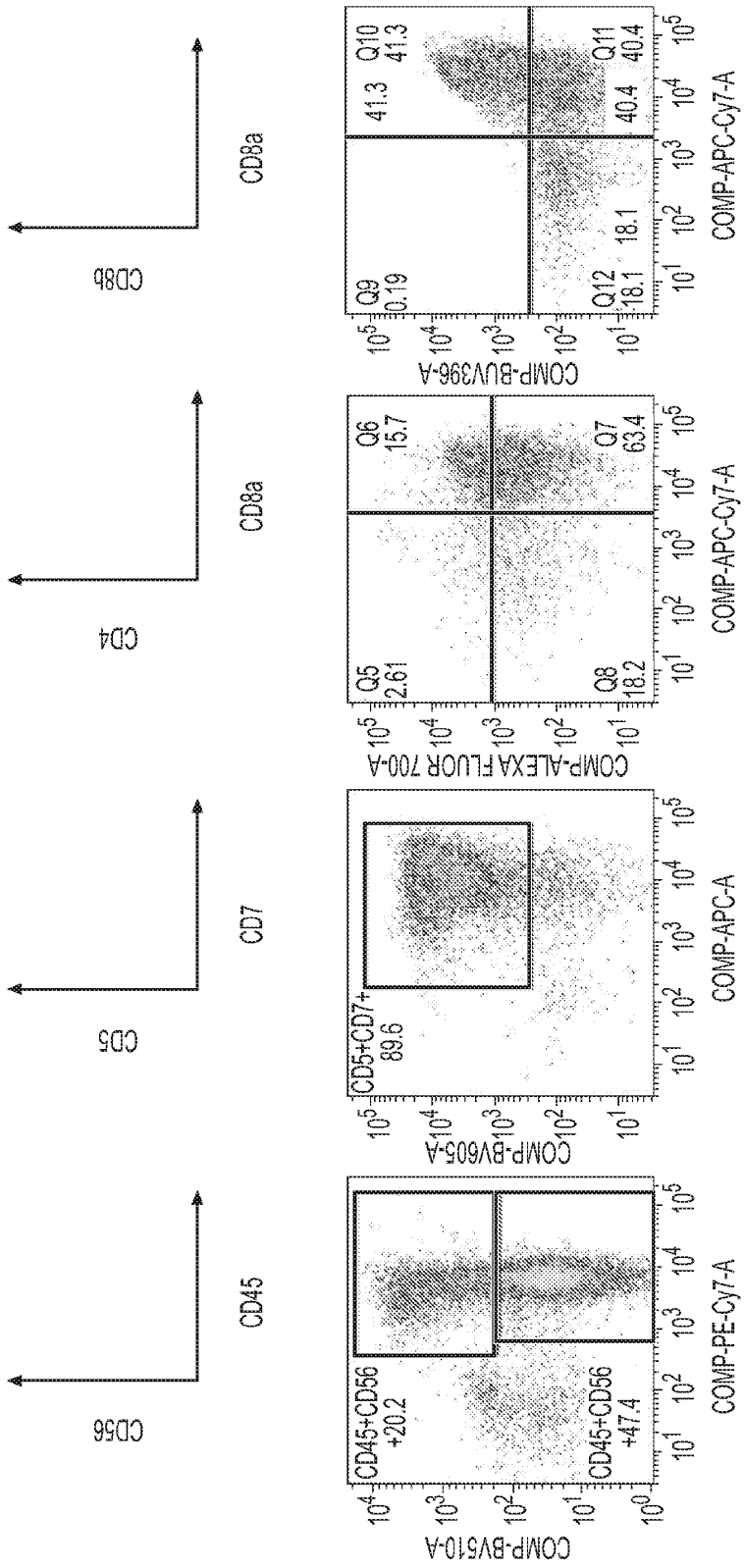
CD56
CD45
COMP-BV510-A
CD45+CD56
+20.2
CD45+CD56
+47.4
COMP-PE-Cy7-A
CD5
CD7
COMP-BV605-A
CD5+CD7+
89.6
COMP-APC-A
CD4
CD8a
COMP-ALEXA FLUOR 700-A
Q5
2.61
Q6
15.7
Q7
63.4
Q8
18.2
COMP-APC-Cy7-A
CD8b
CD8a
COMP-BUV396-A
Q9
0.19
Q10
41.3
Q11
40.4
Q12
18.1
41.3
40.4
18.1
COMP-APC-Cy7-A

FIG. 16B

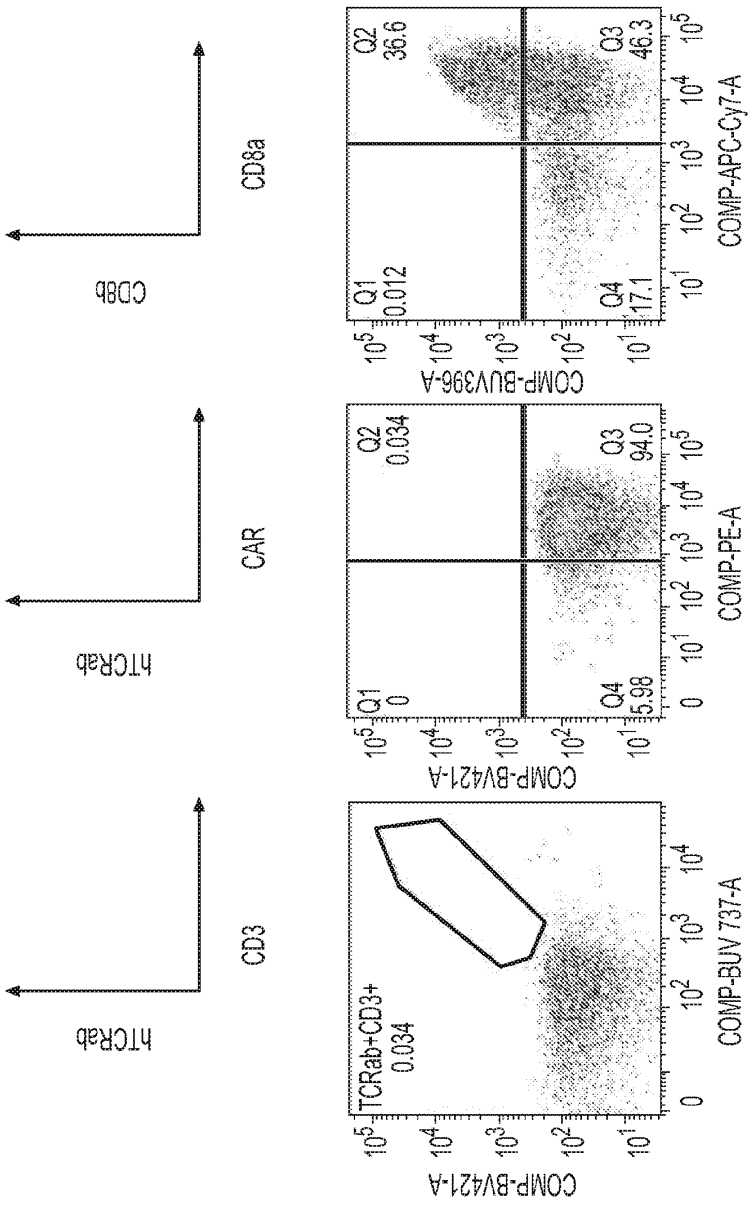

FIG. 16B
(CONT.-1)

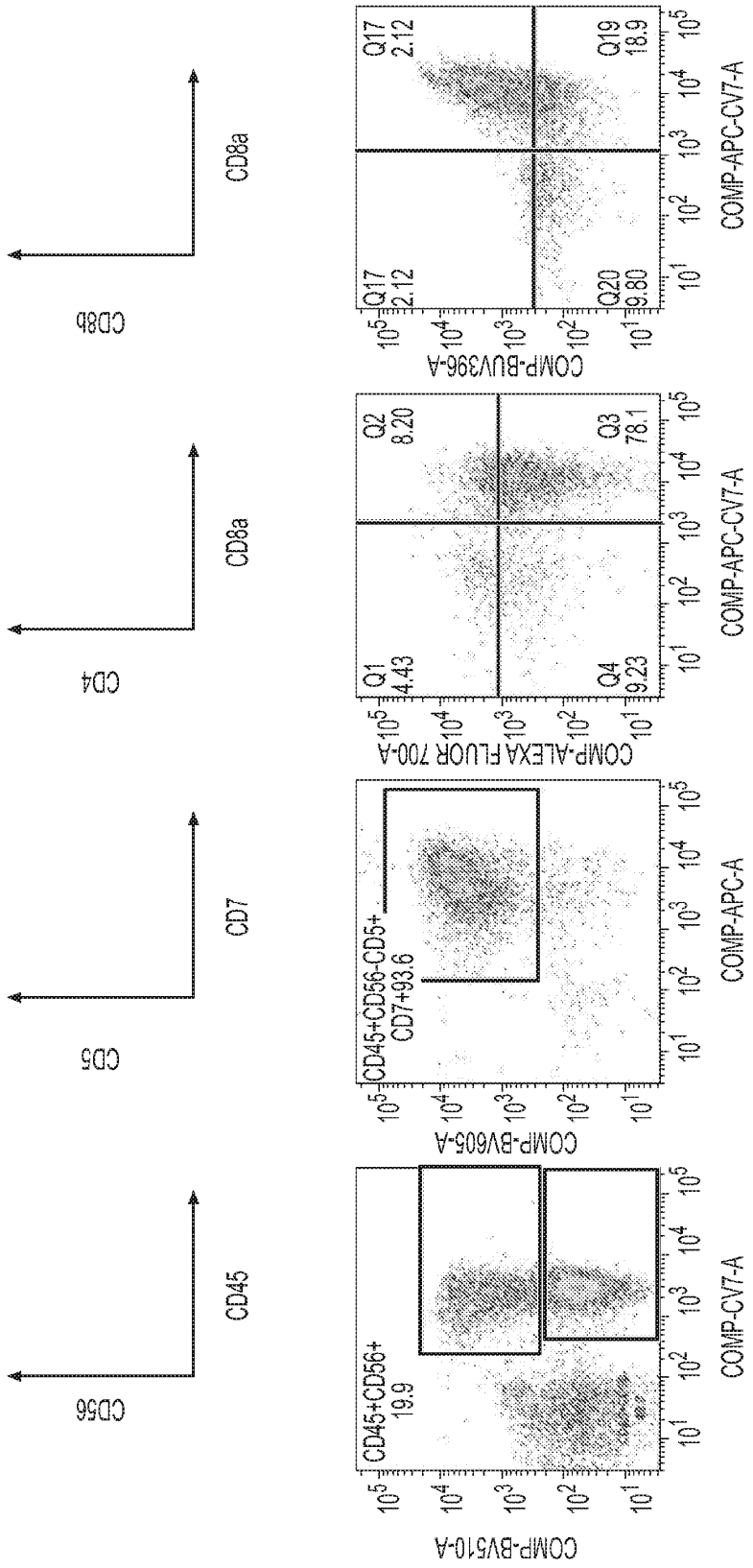
CD56
CD45
CD45+CD56+
19.9
COMP-BV510-A
COMP-CV7-A
CD5
CD7
CD45+CD56-CD5+
CD7+93.6
COMP-BV605-A
COMP-APC-A
CD4
CD8a
Q1
4.43
Q4
9.23
Q2
8.20
Q3
78.1
COMP-ALEXA FLUOR 700-A
COMP-APC-CV7-A
CD8b
CD8a
Q17
2.12
Q20
9.80
Q17
2.12
Q19
18.9
COMP-BUV396-A
COMP-APC-CV7-A

FIG. 16C

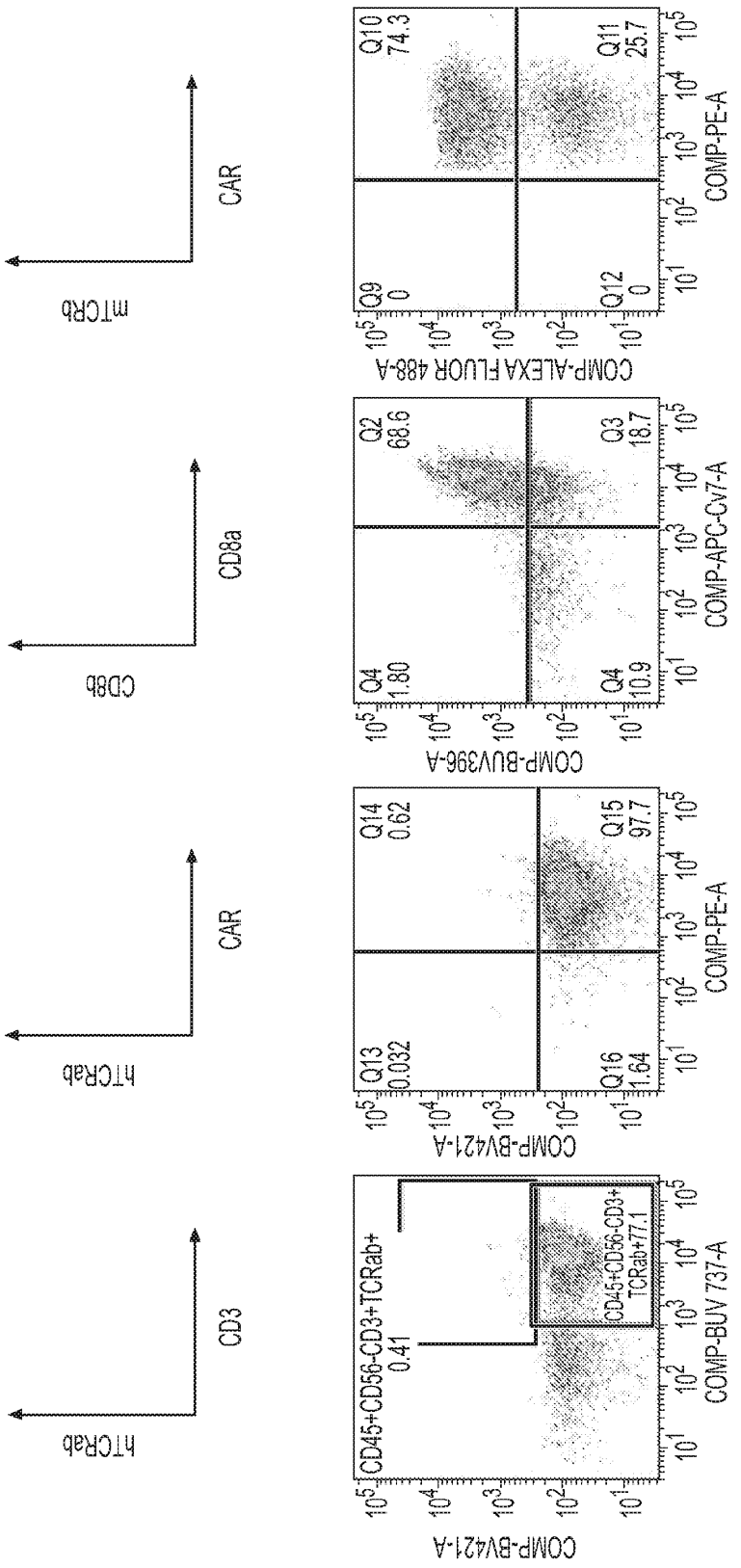

FIG. 16C
(CONT.-1)