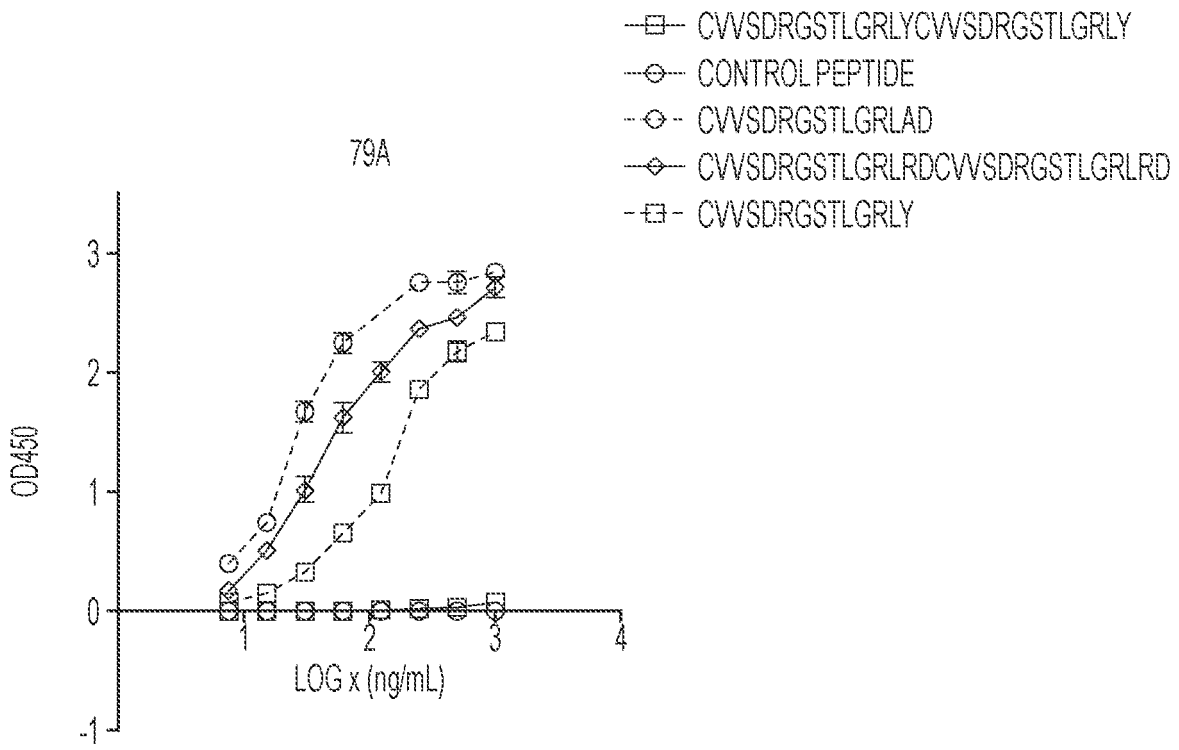

CVVSDRGSTLGRLYCVVSDRGSTLGRLY
CONTROL PEPTIDE
CVVSDRGSTLGRLAD
CVVSDRGSTLGRLRDCVVSDRGSTLGRLRD
CVVSDRGSTLGRLY
79A
OD450
3
2
1
0
-1
1
2
3
4
LOG x (ng/mL)

FIG. 1A

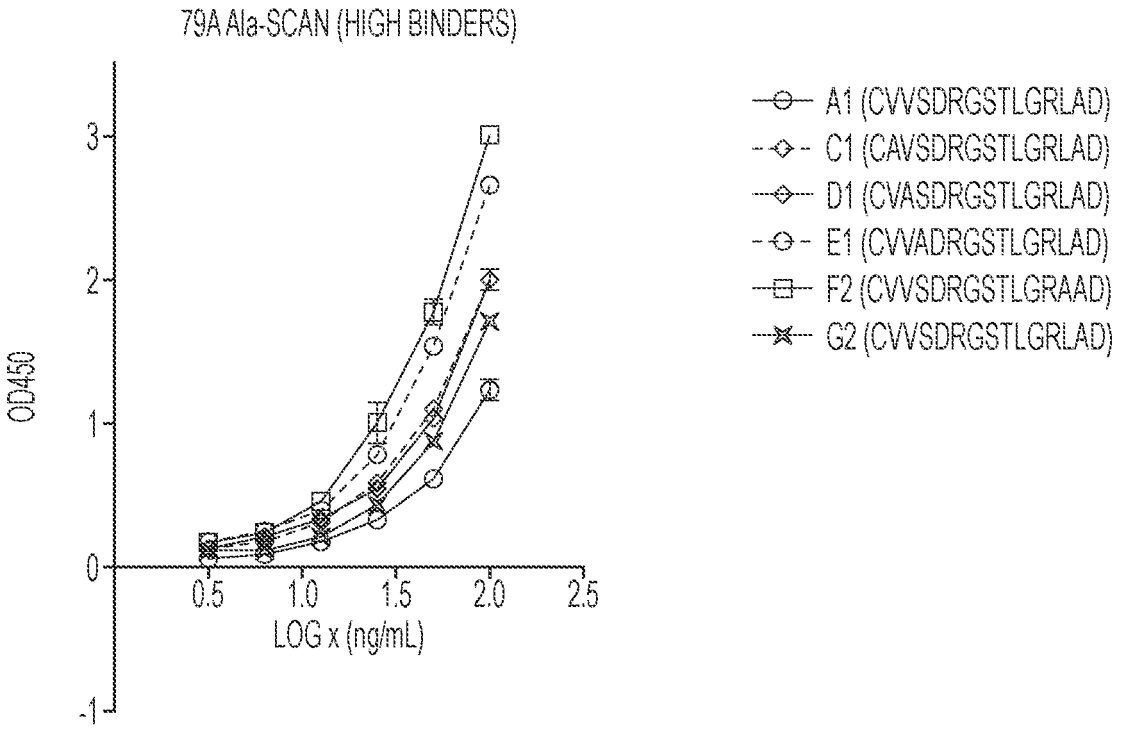

79A Ala-SCAN (HIGH BINDERS)
OD450
3
2
1
0
-1
0.5
1.0
1.5
2.0
2.5
LOG x (ng/mL)
A1 (CVVSDRGSTLGRLAD)
C1 (CAVSDRGSTLGRLAD)
D1 (CVASDRGSTLGRLAD)
E1 (CVVADRGSTLGRLAD)
F2 (CVVSDRGSTLGRAAD)
G2 (CVVSDRGSTLGRLAD)

FIG. 1B

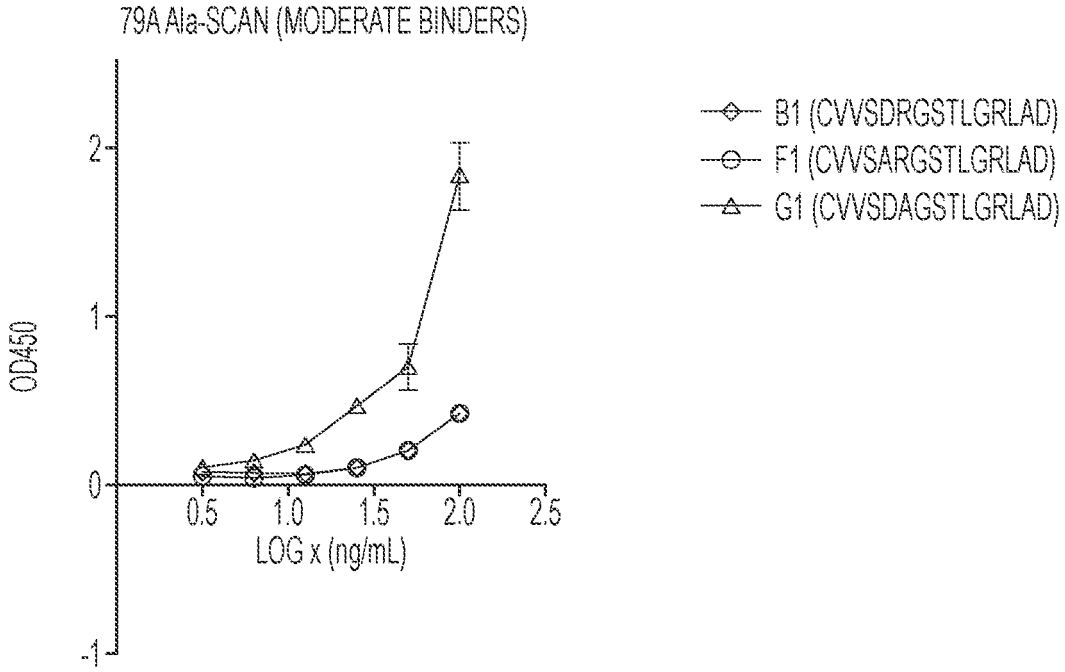

79A Ala-SCAN (MODERATE BINDERS)
OD450
2
1
0
-1
0.5
1.0
1.5
2.0
2.5
LOG x (ng/mL)
B1 (CVVSDRGSTLGRLAD)
F1 (CVVSARGSTLGRLAD)
G1 (CVVSDAGSTLGRLAD)

FIG. 1C

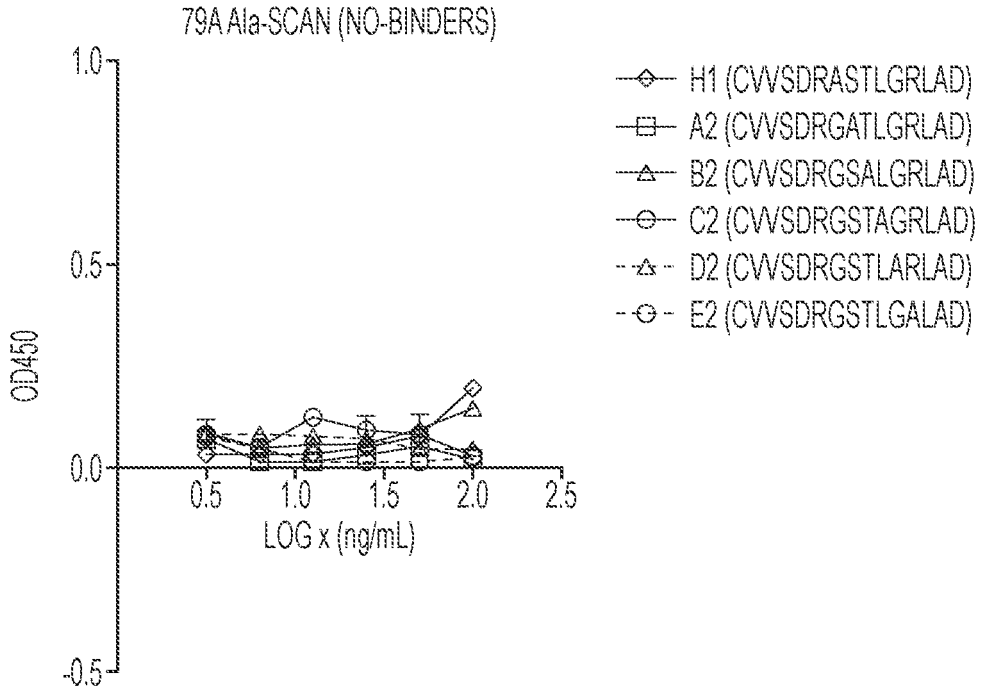

79A Ala-SCAN (NO-BINDERS)
1.0
0.5
0.0
-0.5
OD450
0.5
1.0
1.5
2.0
2.5
LOG x (ng/mL)
H1 (CWSDRASTLGRLAD)
A2 (CWSDRGATLGRLAD)
B2 (CWSDRGSALGRLAD)
C2 (CWSDRGSTAGRLAD)
D2 (CWSDRGSTLARLAD)
E2 (CWSDRGSTLGALAD)

FIG. 1D

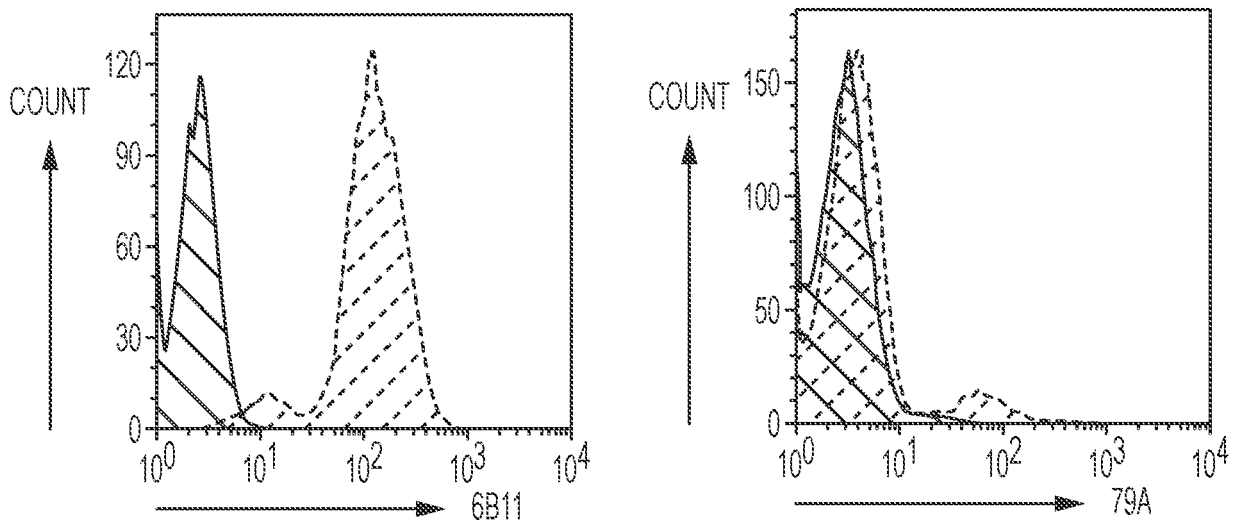

COUNT
120
90
60
30
0
10^0
10^1
10^2
10^3
10^4
6B11
COUNT
150
100
50
0
10^0
10^1
10^2
10^3
10^4
79A

FIG. 1E(i)

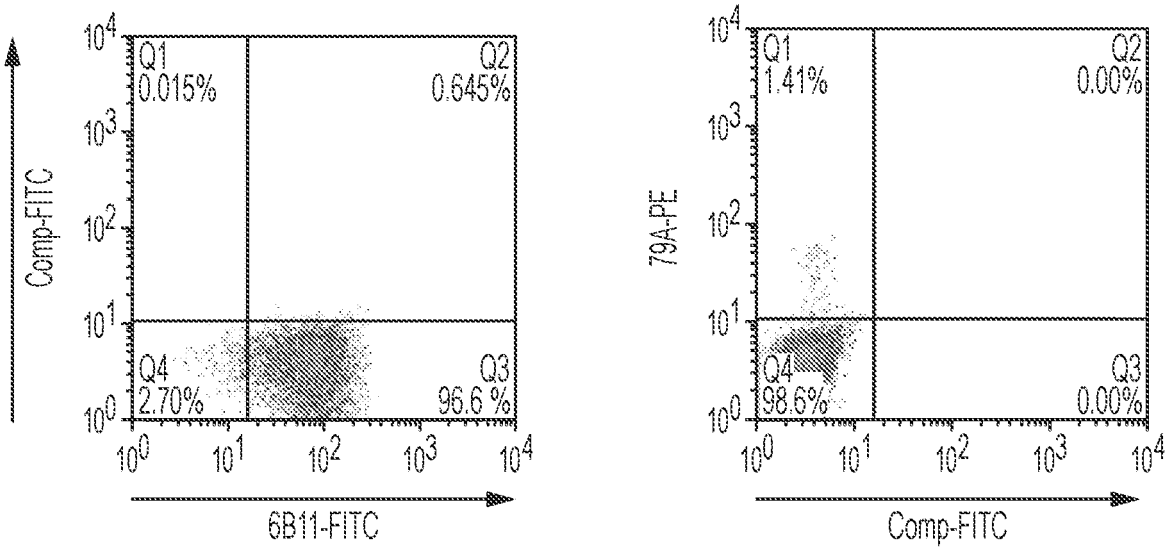

Comp-FITC
Q1
0.015%
Q2
0.645%
Q4
2.70%
Q3
96.6 %
6B11-FITC
79A-PE
Q1
1.41%
Q2
0.00%
Q4
98.6%
Q3
0.00%
Comp-FITC

FIG. 1E(ii)

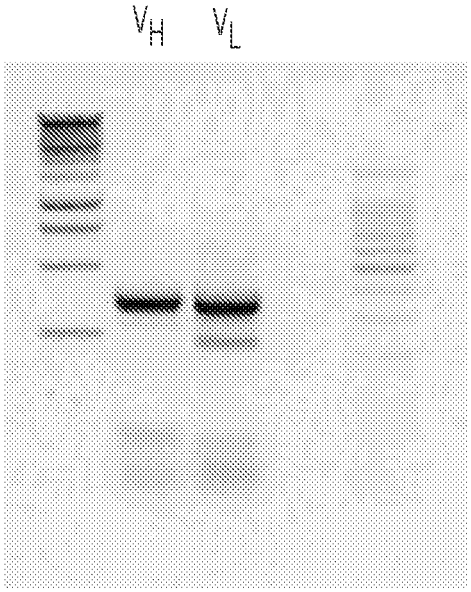
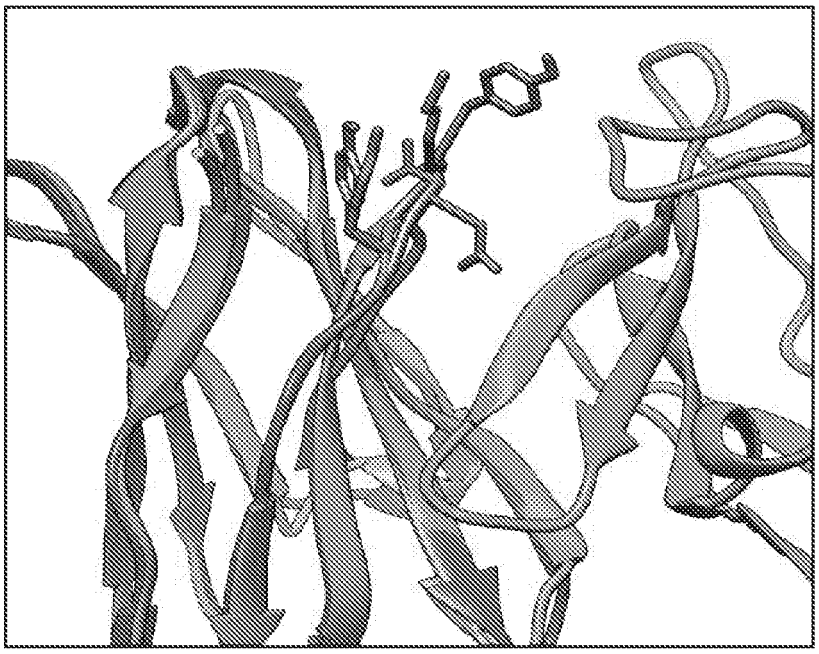
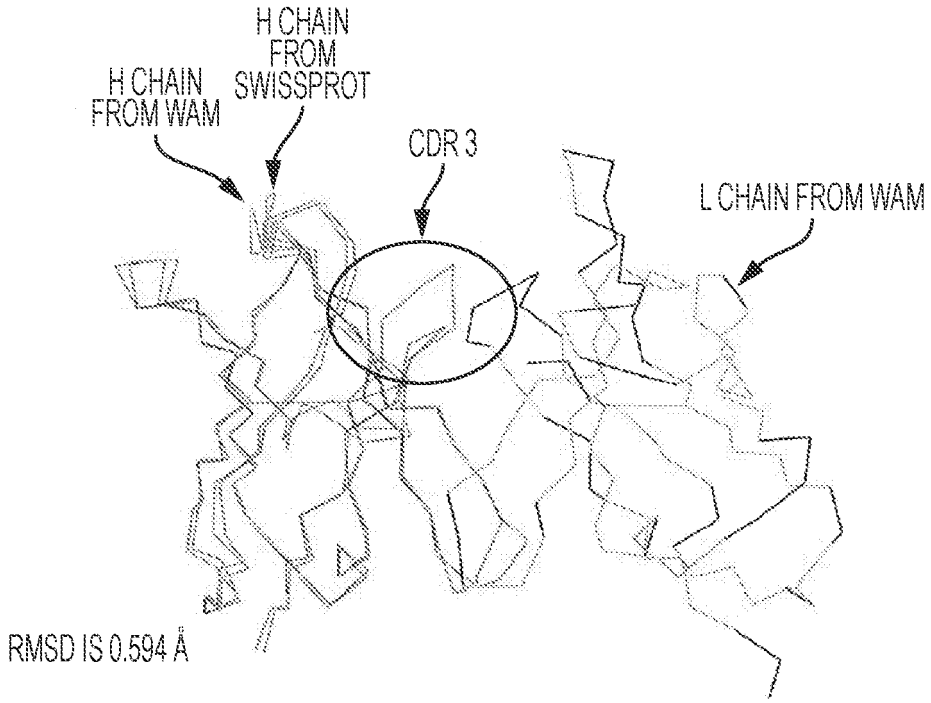


FIG. 2



FIGS. 3A


H CHAIN FROM WAM
H CHAIN FROM SWISSPROT
CDR 3
L CHAIN FROM WAM
RMSD IS 0.594 Å

FIGS. 3B

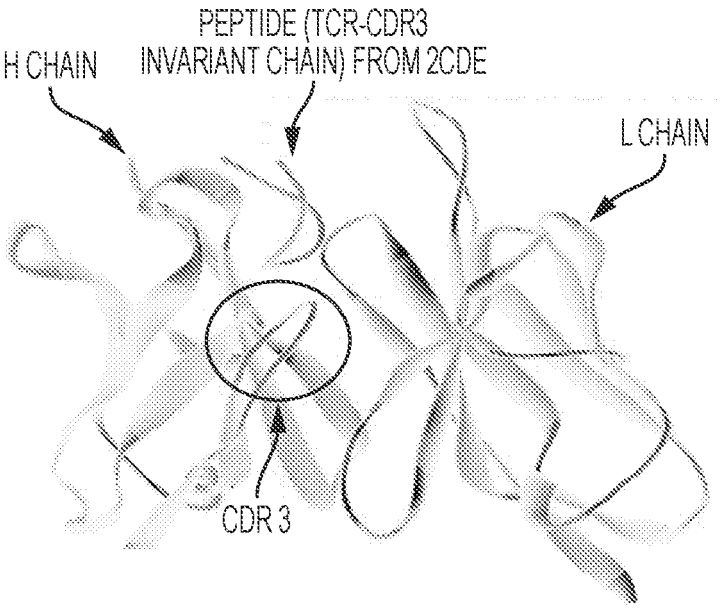

PEPTIDE (TCR-CDR3
INVARIANT CHAIN) FROM 2CDE
H CHAIN
L CHAIN
CDR 3

FIG. 3C

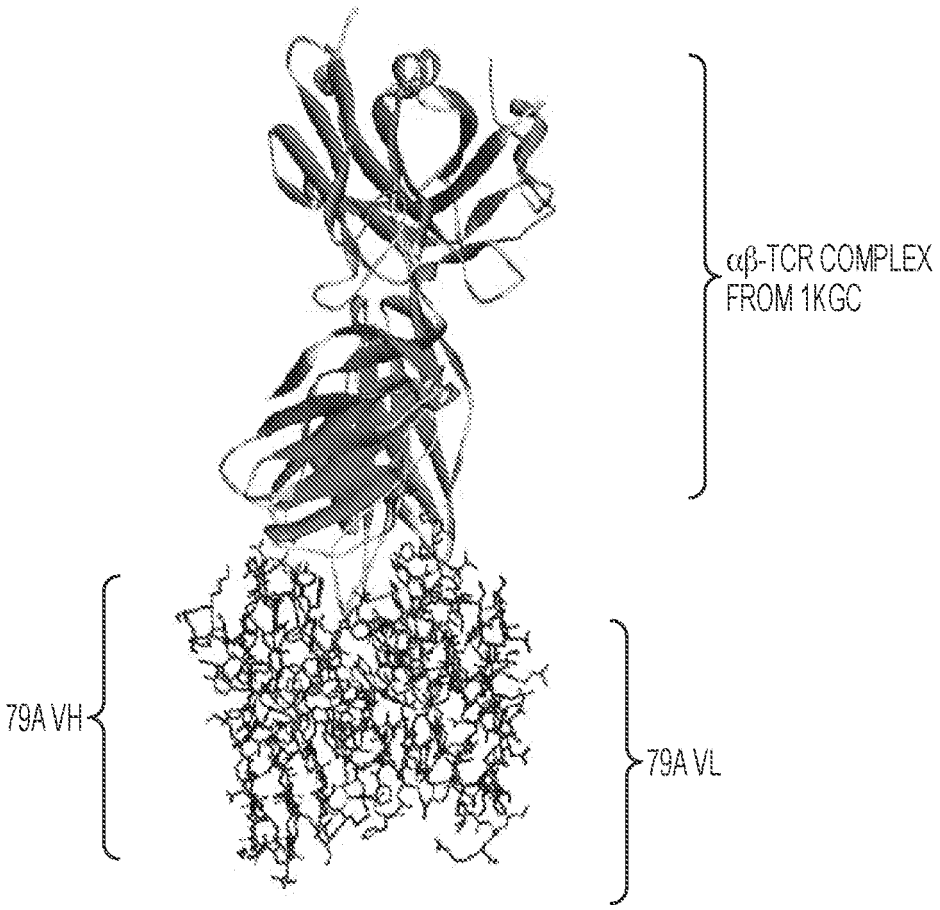


FIG. 3D

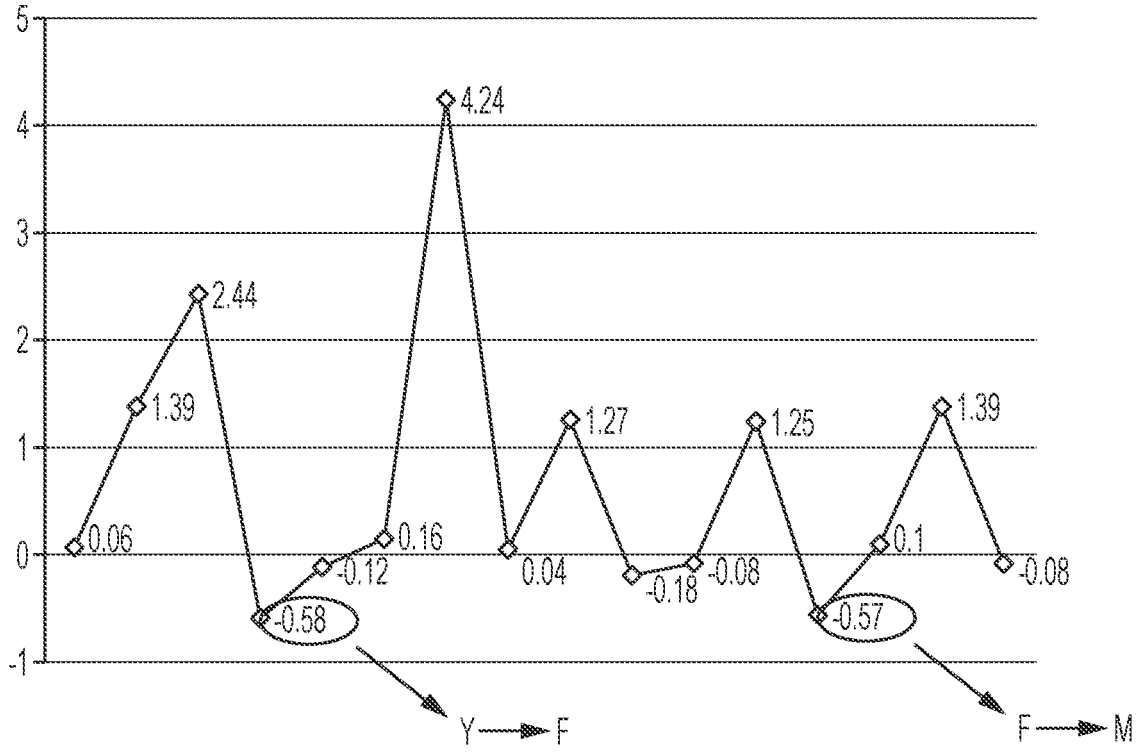

5
4
3
2
1
0
-1
0.06
1.39
2.44
-0.58
-0.12
0.16
4.24
0.04
1.27
-0.18
-0.08
1.25
-0.57
0.1
1.39
-0.08
Y
F
F
M

FIG. 4A

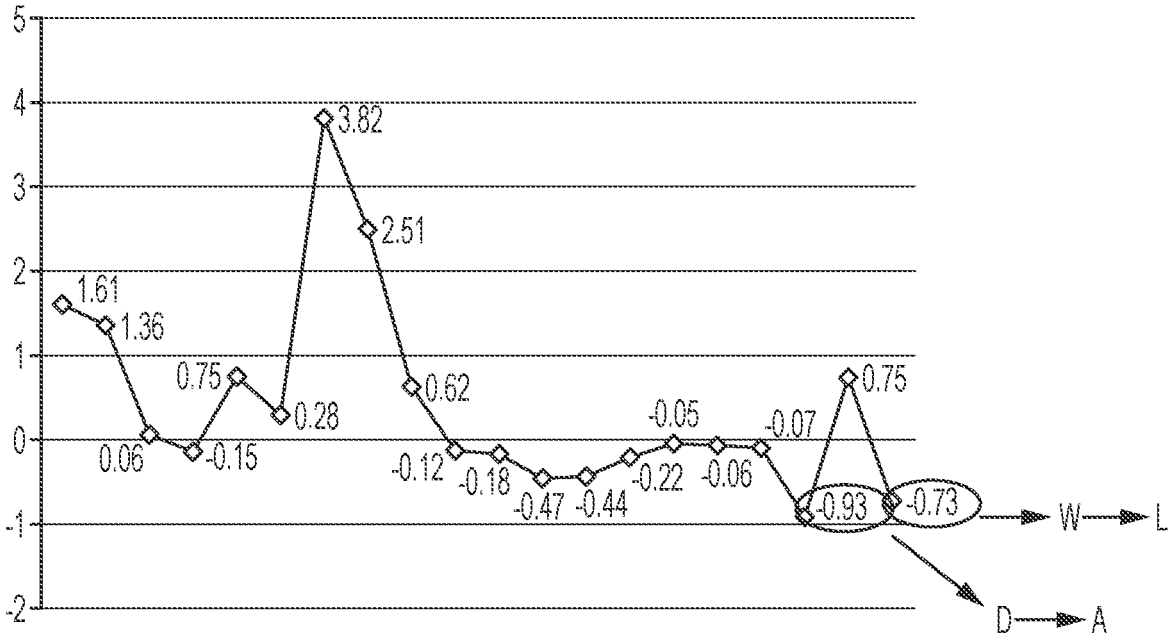

5
4
3
2
1
0
-1
-2
1.61
1.36
0.06
0.75
-0.15
0.28
3.82
2.51
0.62
-0.12
-0.18
-0.47
-0.44
-0.22
-0.05
-0.06
-0.07
-0.93
0.75
-0.73
W
L
D
A

FIG. 4B

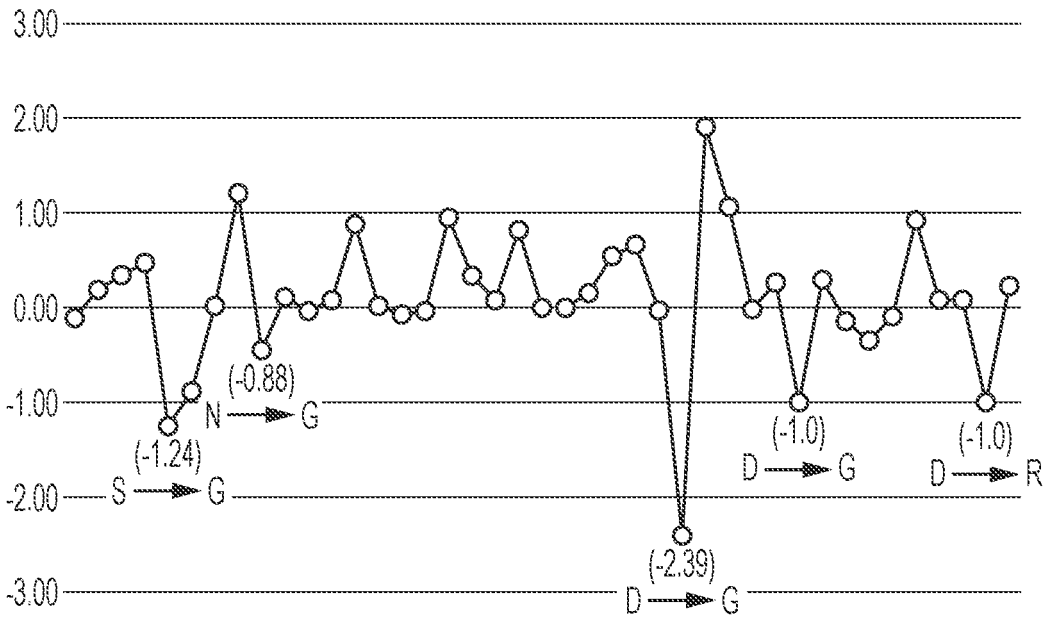


FIG. 4C

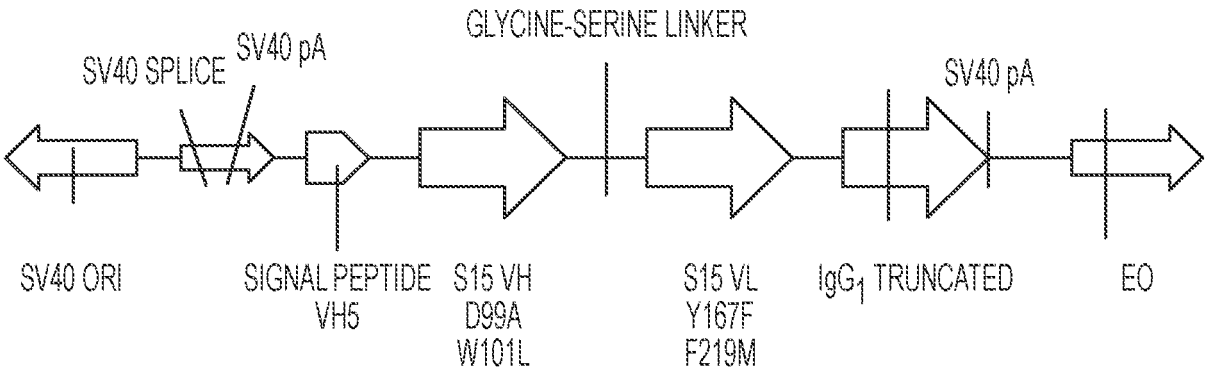

GLYCINE-SERINE LINKER
SV40 SPLICE
SV40 pA
SV40 pA
SV40 ORI
SIGNAL PEPTIDE VH5
S15 VH D99A W101L
S15 VL Y167F F219M
IgG1 TRUNCATED
EO

FIG. 5A(i)

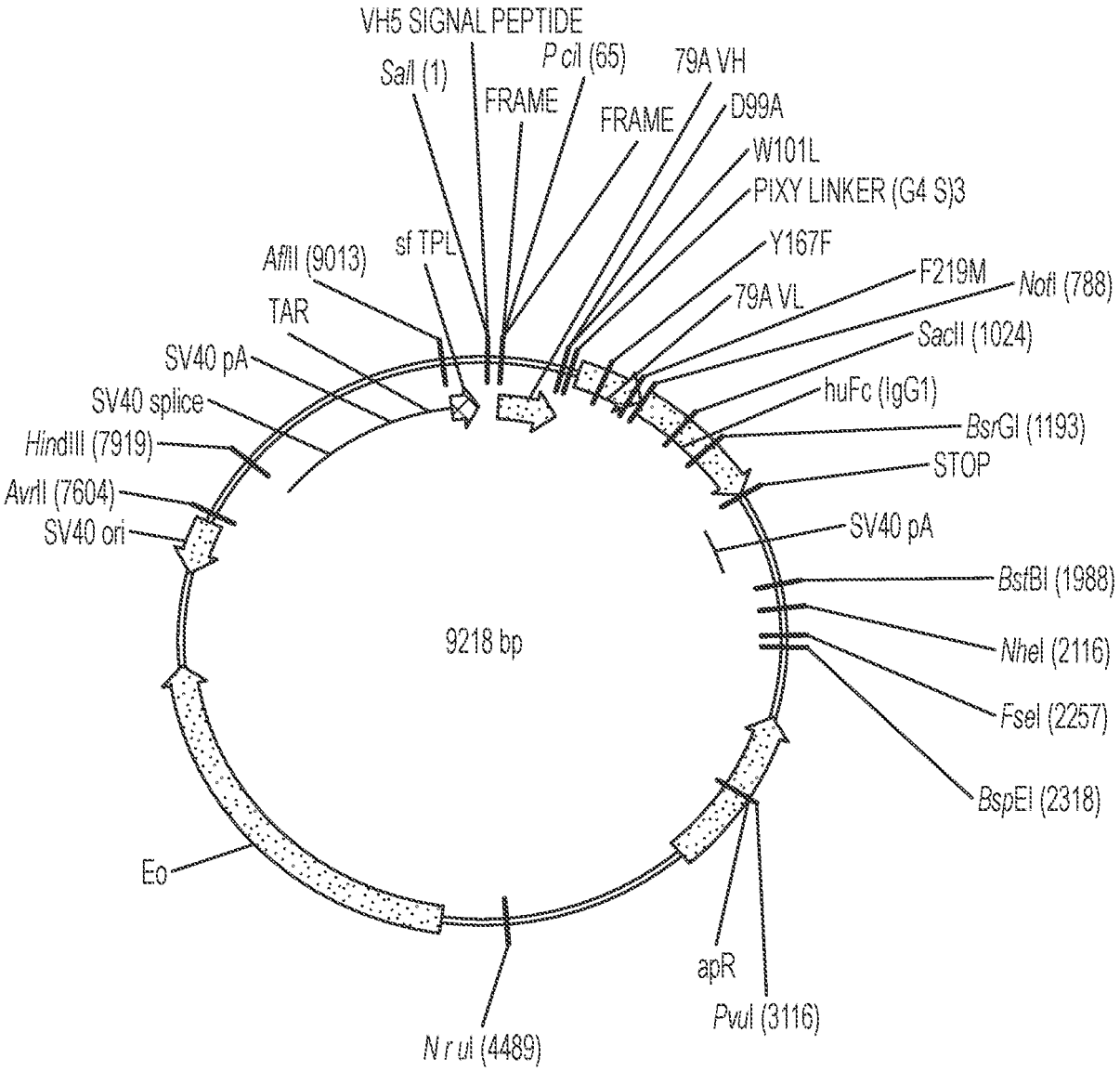

VH5 SIGNAL PEPTIDE
SalI (1)
PciI (65)
79A VH
FRAME
FRAME
D99A
W101L
PIXY LINKER (G4 S)3
Y167F
sf TPL
AflII (9013)
F219M
NotI (788)
79A VL
TAR
SacII (1024)
SV40 pA
huFc (IgG1)
SV40 splice
BsrGI (1193)
HindIII (7919)
STOP
AvrII (7604)
SV40 pA
SV40 ori
BsfBI (1988)
9218 bp
NheI (2116)
FseI (2257)
BspEI (2318)
Eo
apR
PvuI (3116)
NruI (4489)

FIG. 5A(ii)

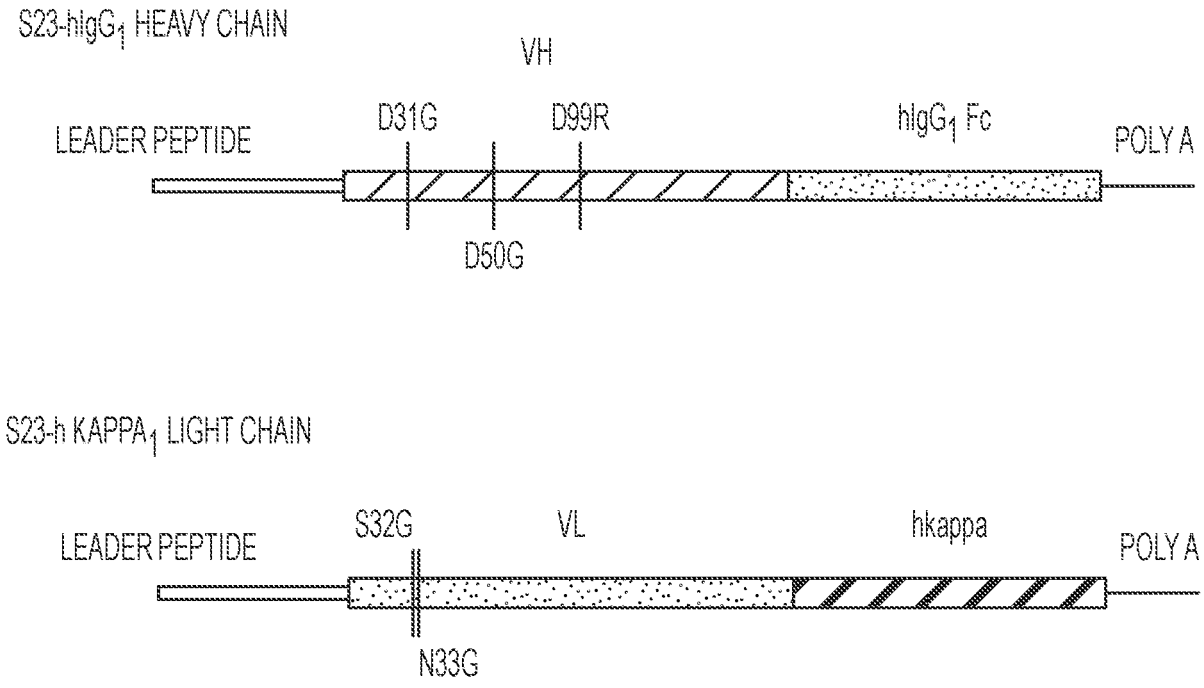
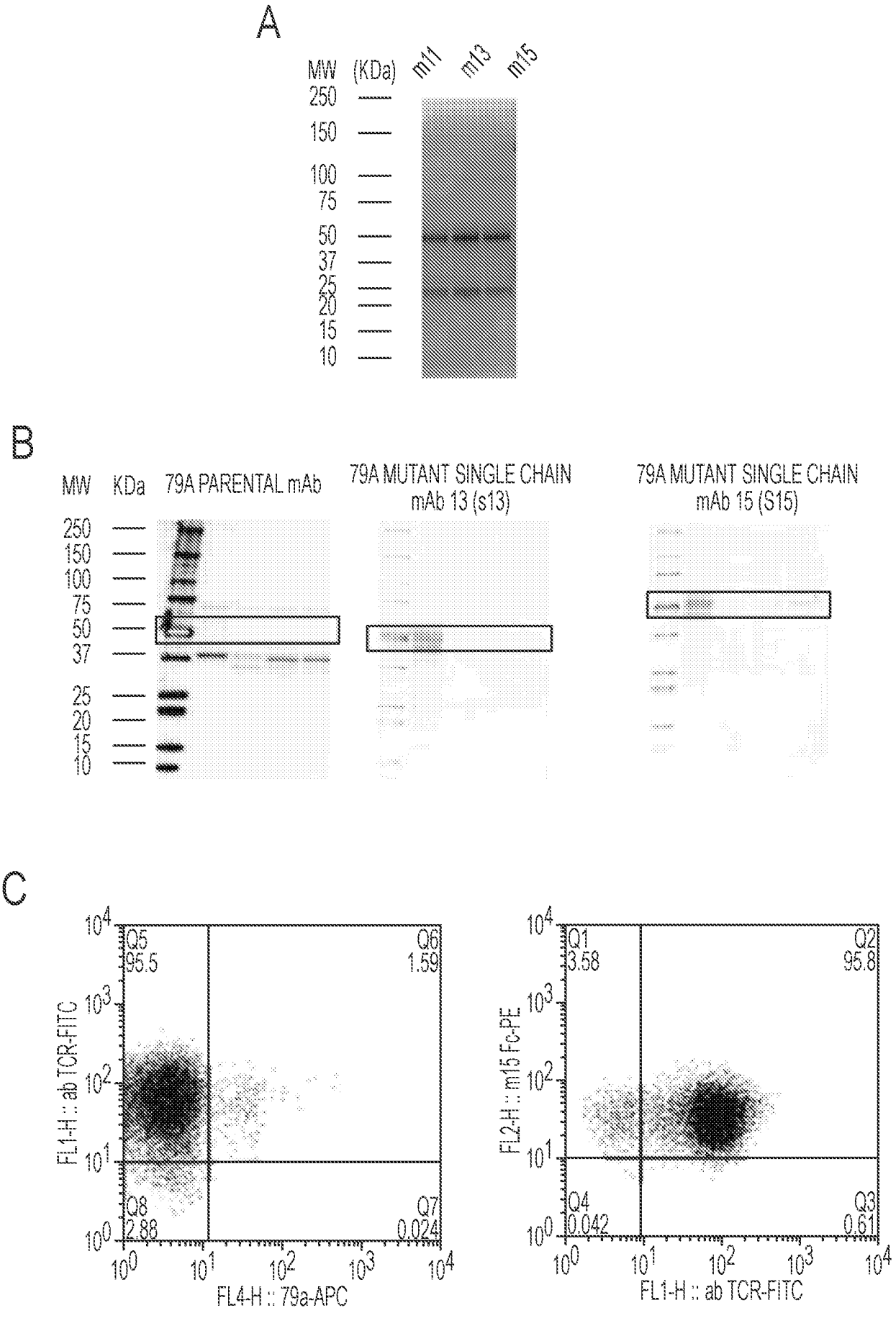

S23-hIgG1 HEAVY CHAIN
VH
LEADER PEPTIDE
D31G
D99R
hIgG1 Fc
POLY A
D50G
S23-h KAPPA1 LIGHT CHAIN
LEADER PEPTIDE
S32G
VL
hkappa
POLY A
N33G

FIG. 5B


A
MW (KDa)
m11
m13
m15
250
150
100
75
50
37
25
20
15
10
B
MW
KDa
79A PARENTAL mAb
79A MUTANT SINGLE CHAIN mAb 13 (s13)
79A MUTANT SINGLE CHAIN mAb 15 (S15)
C
FL1-H :: ab TCR-FITC
FL4-H :: 79a-APC
Q5 95.5
Q6 1.59
Q8 2.88
Q7 0.024
FL2-H :: m15 Fc-PE
FL1-H :: ab TCR-FITC
Q1 3.58
Q2 95.8
Q4 0.042
Q3 0.61

FIGS. 6A-C

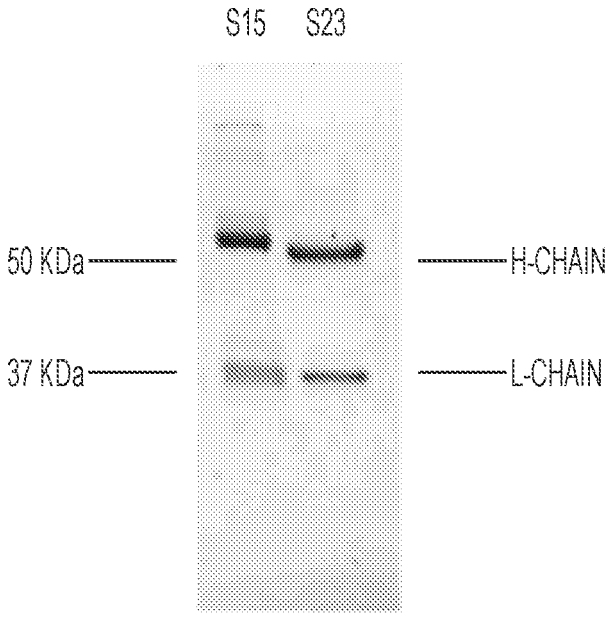


FIG. 6D

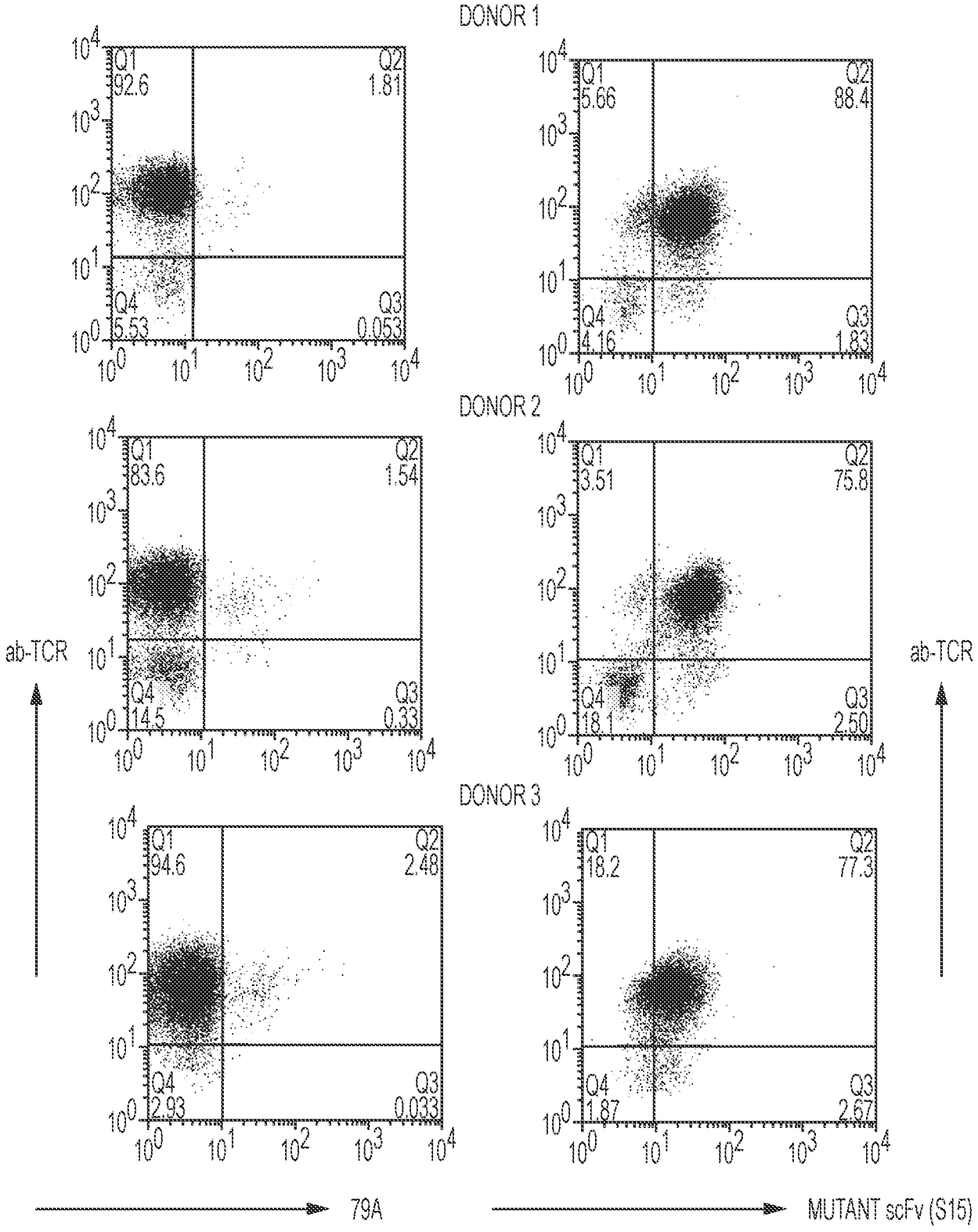

DONOR 1
Q1 92.6
Q2 1.81
Q4 5.53
Q3 0.053
Q1 5.66
Q2 88.4
Q4 4.16
Q3 1.83
DONOR 2
Q1 83.6
Q2 1.54
Q4 14.5
Q3 0.33
Q1 3.51
Q2 75.8
Q4 18.1
Q3 2.50
DONOR 3
Q1 94.6
Q2 2.48
Q4 2.93
Q3 0.033
Q1 18.2
Q2 77.3
Q4 1.87
Q3 2.67
ab-TCR
ab-TCR
79A
MUTANT scFv (S15)

FIG. 6E

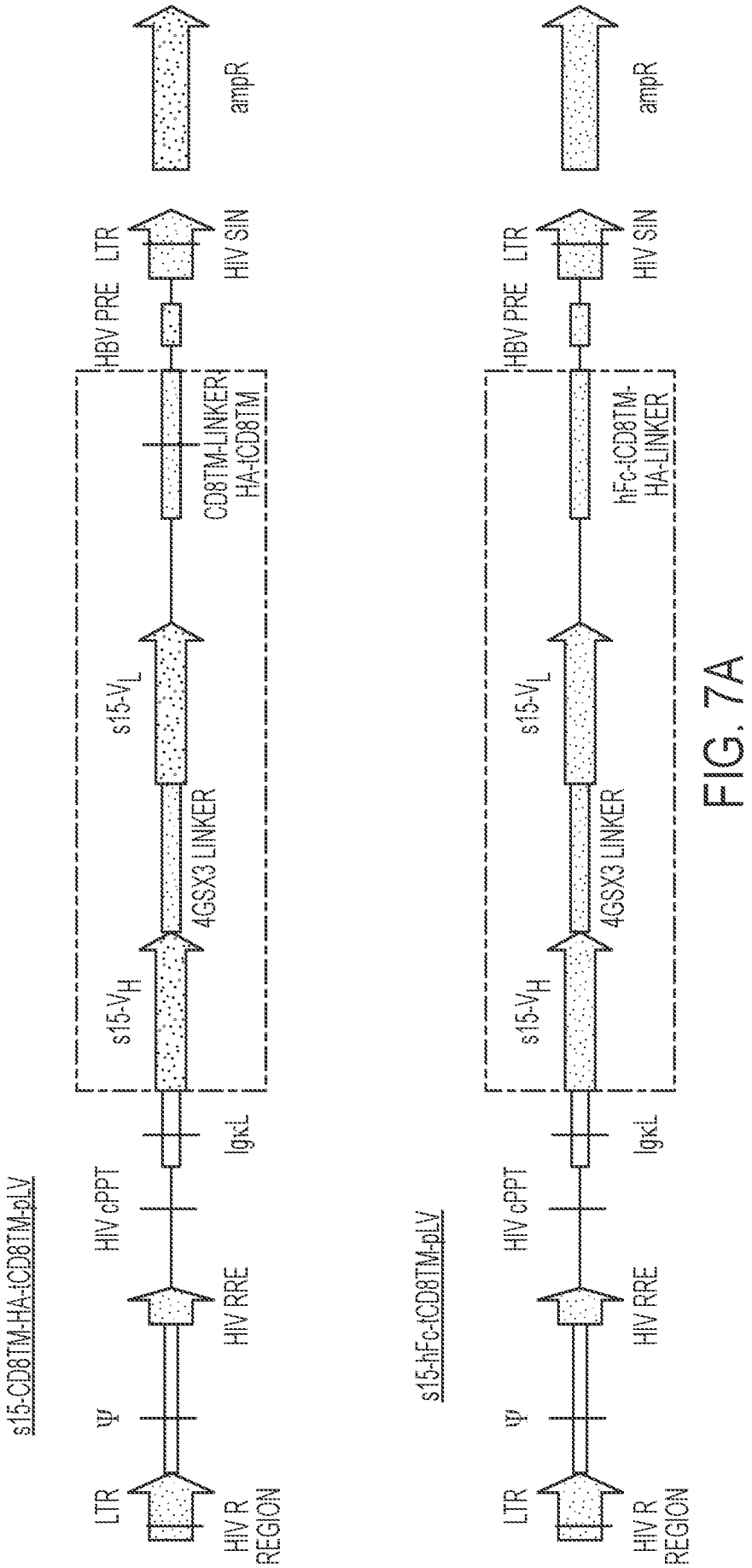

s15-CD8TM-HA-tCD8TM-pLV
LTR
HIV R REGION
Ψ
HIV RRE
HIV cPPT
IgκL
s15-V_H
4GSX3 LINKER
s15-V_L
CD8TM-LINKER HA-tCD8TM
HBV PRE
LTR
HIV SIN
ampR
s15-hFc-tCD8TM-pLV
LTR
HIV R REGION
Ψ
HIV RRE
HIV cPPT
IgκL
s15-V_H
4GSX3 LINKER
s15-V_L
hFc-tCD8TM-HA-LINKER
HBV PRE
LTR
HIV SIN
ampR

FIG. 7A

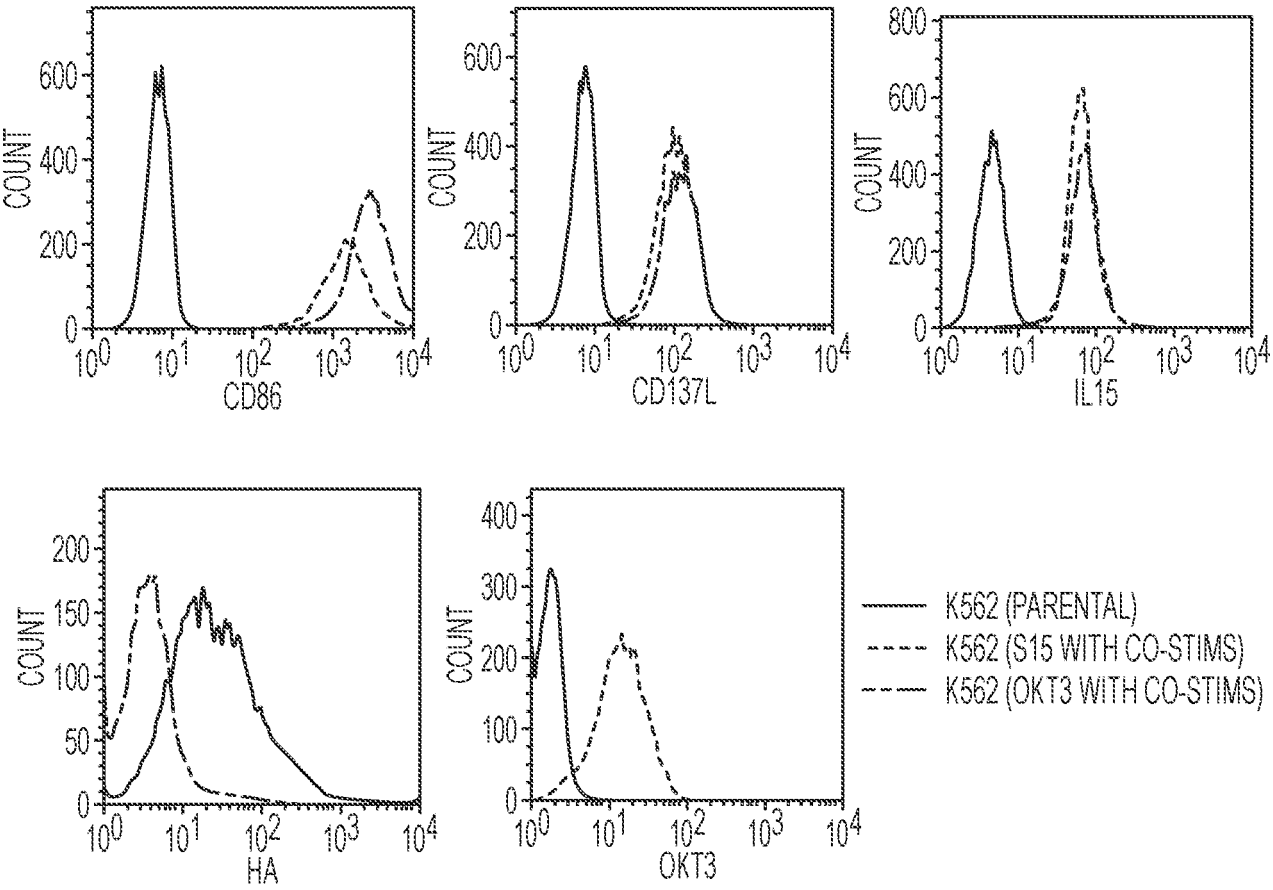

COUNT
CD86
CD137L
IL15
HA
OKT3
K562 (PARENTAL)
K562 (S15 WITH CO-STIMS)
K562 (OKT3 WITH CO-STIMS)

FIG. 7B

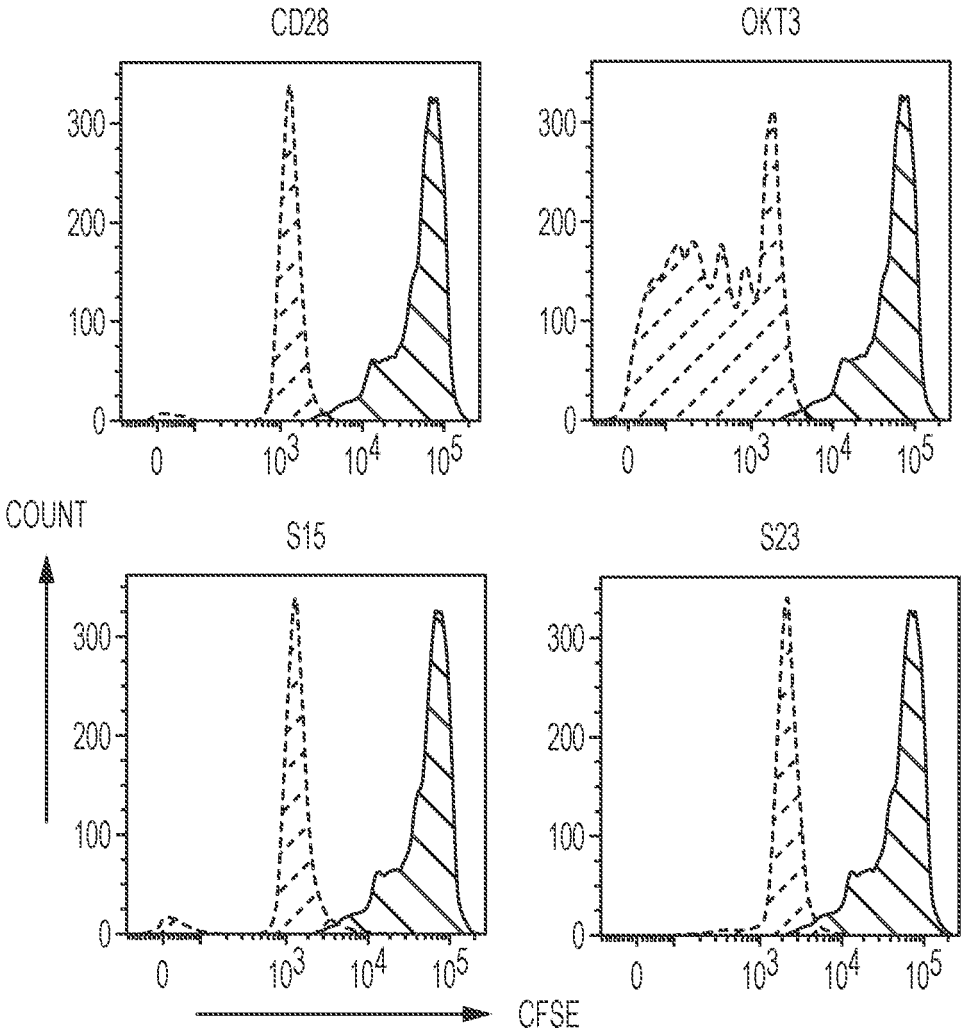


FIG. 7C

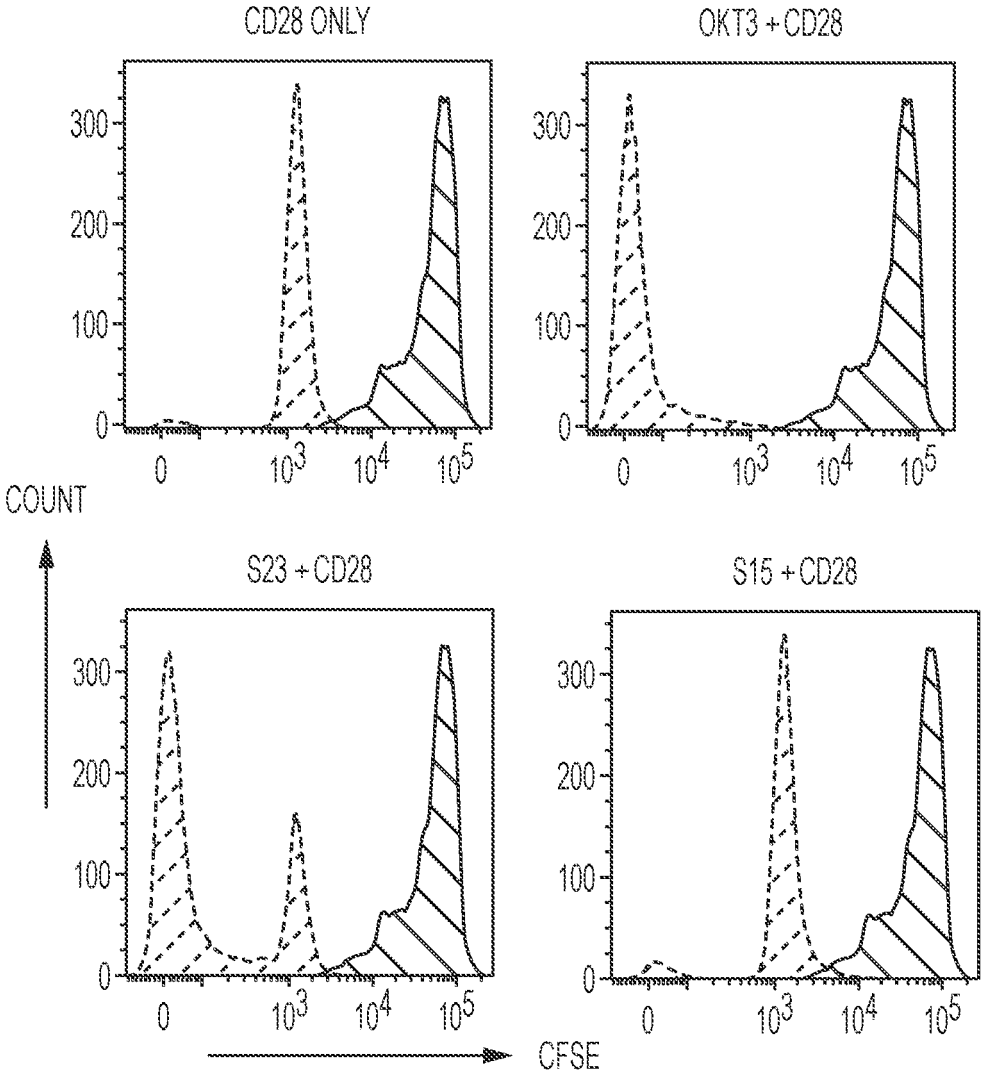

CD28 ONLY
OKT3 + CD28
S23 + CD28
S15 + CD28
COUNT
CFSE
300
200
100
0
0
10^3
10^4
10^5

FIG. 7D

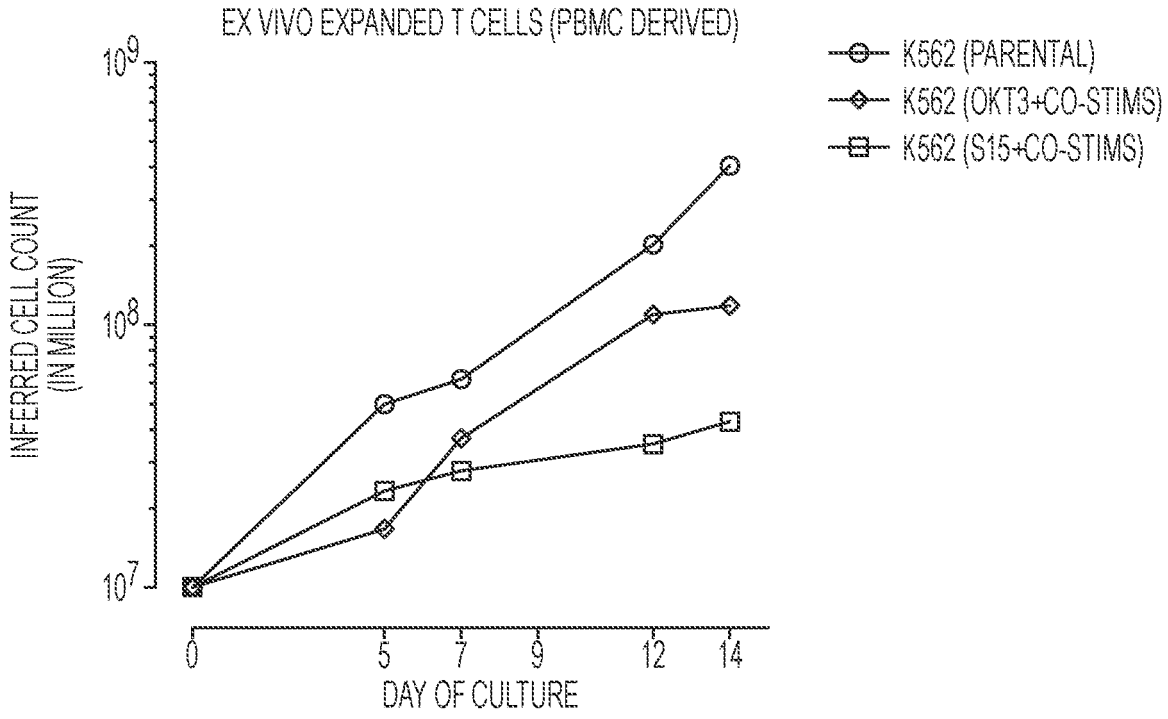

EX VIVO EXPANDED T CELLS (PBMC DERIVED)
INFERRED CELL COUNT (IN MILLION)
10^9
10^8
10^7
0
5
7
9
12
14
DAY OF CULTURE
K562 (PARENTAL)
K562 (OKT3+CO-STIMS)
K562 (S15+CO-STIMS)

FIG. 7E

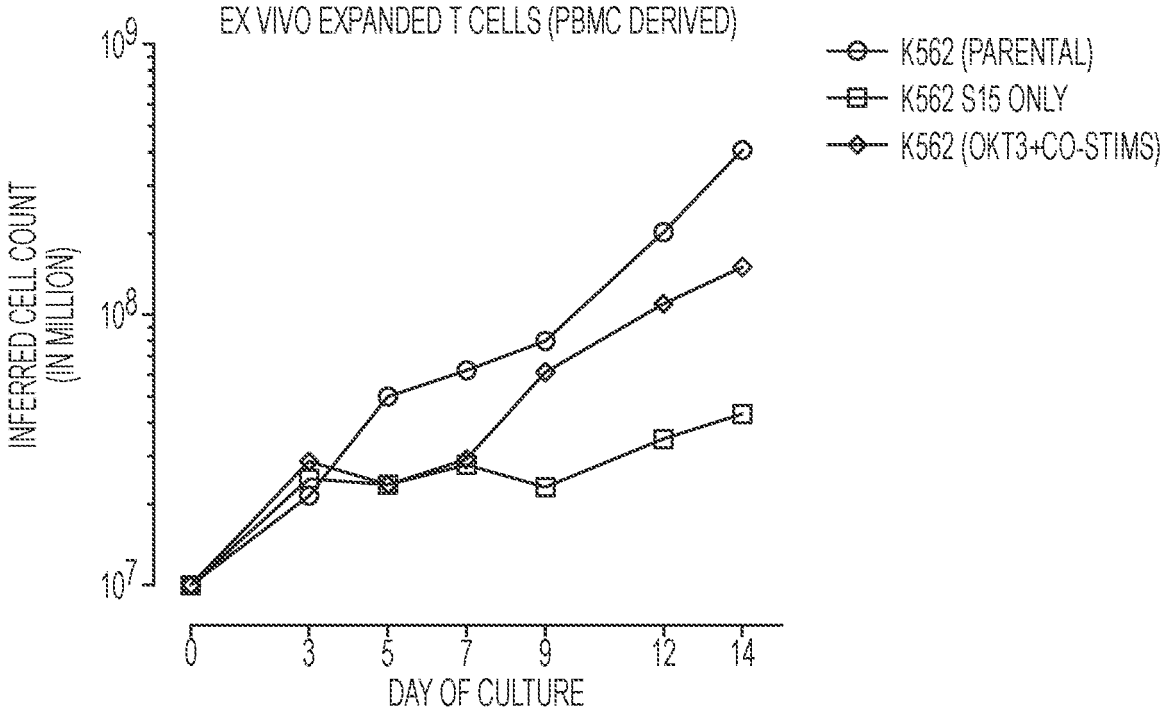

EX VIVO EXPANDED T CELLS (PBMC DERIVED)
INFERRED CELL COUNT (IN MILLION)
10^9
10^8
10^7
0
3
5
7
9
12
14
DAY OF CULTURE
K562 (PARENTAL)
K562 S15 ONLY
K562 (OKT3+CO-STIMS)

FIG. 7F

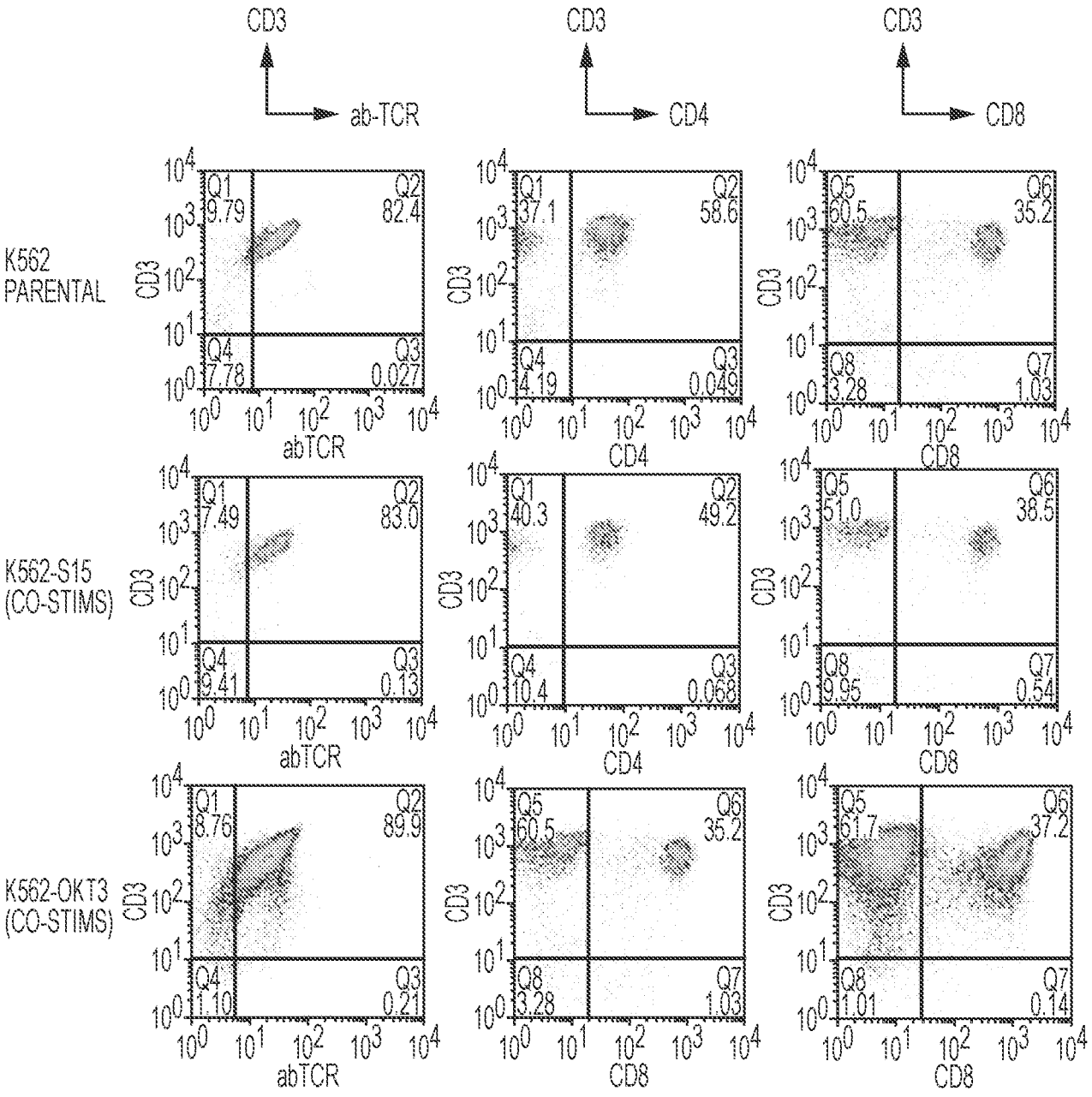

CD3
ab-TCR
CD4
CD8
K562
PARENTAL
K562-S15
(CO-STIMS)
K562-OKT3
(CO-STIMS)
abTCR
Q1 9.79
Q2 82.4
Q4 7.78
Q3 0.027
Q1 37.1
Q2 58.6
Q4 4.19
Q3 0.049
Q5 60.5
Q6 35.2
Q8 3.28
Q7 1.03
Q1 7.49
Q2 83.0
Q4 9.41
Q3 0.13
Q1 40.3
Q2 49.2
Q4 10.4
Q3 0.068
Q5 51.0
Q6 38.5
Q8 9.95
Q7 0.54
Q1 8.76
Q2 89.9
Q4 1.10
Q3 0.21
Q5 60.5
Q6 35.2
Q8 3.28
Q7 1.03
Q5 61.7
Q6 37.2
Q8 1.01
Q7 0.14

FIG. 7G

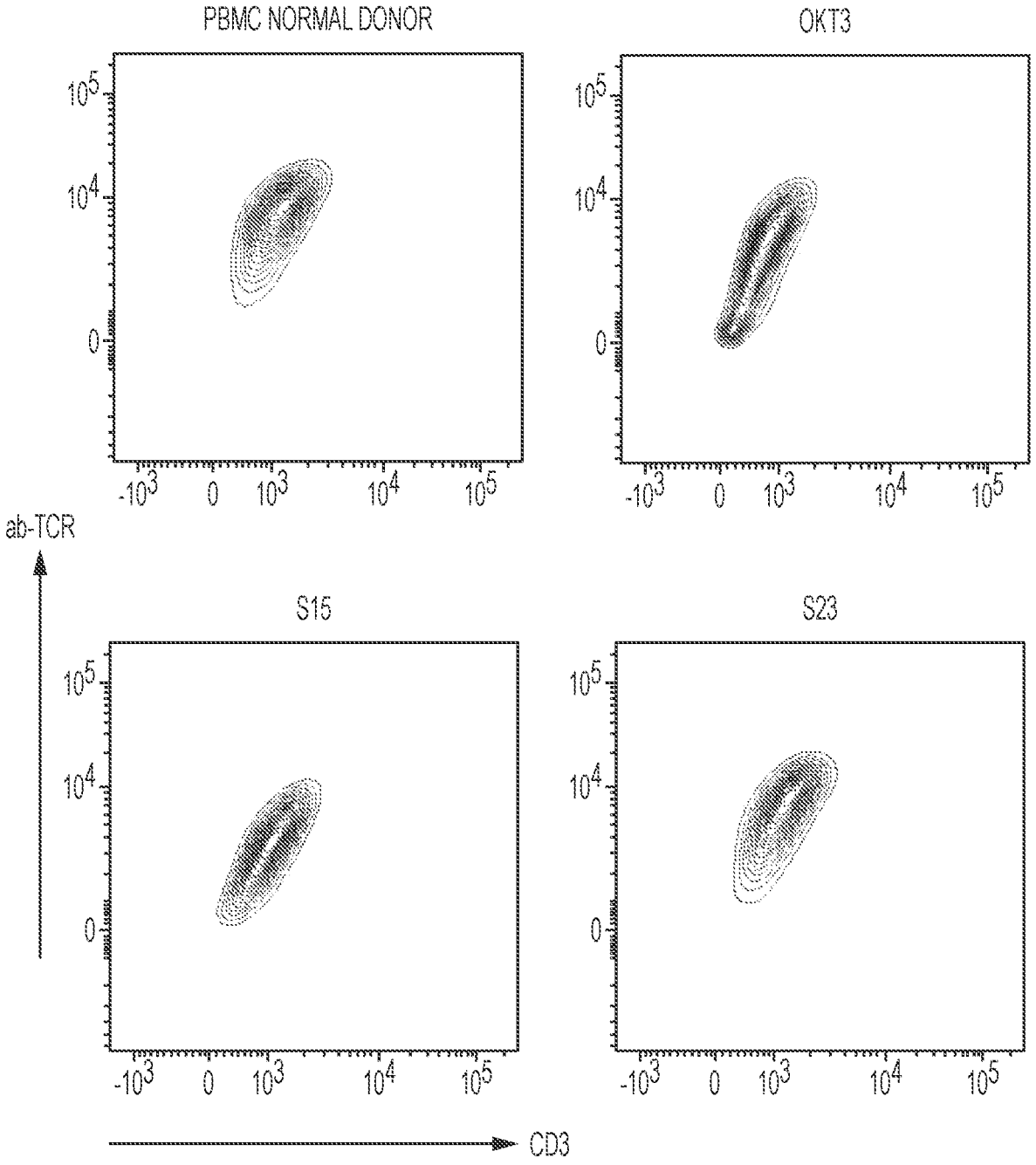

PBMC NORMAL DONOR
OKT3
S15
S23
ab-TCR
CD3
10^5
10^4
0
-10^3
0
10^3
10^4
10^5

FIG. 7H

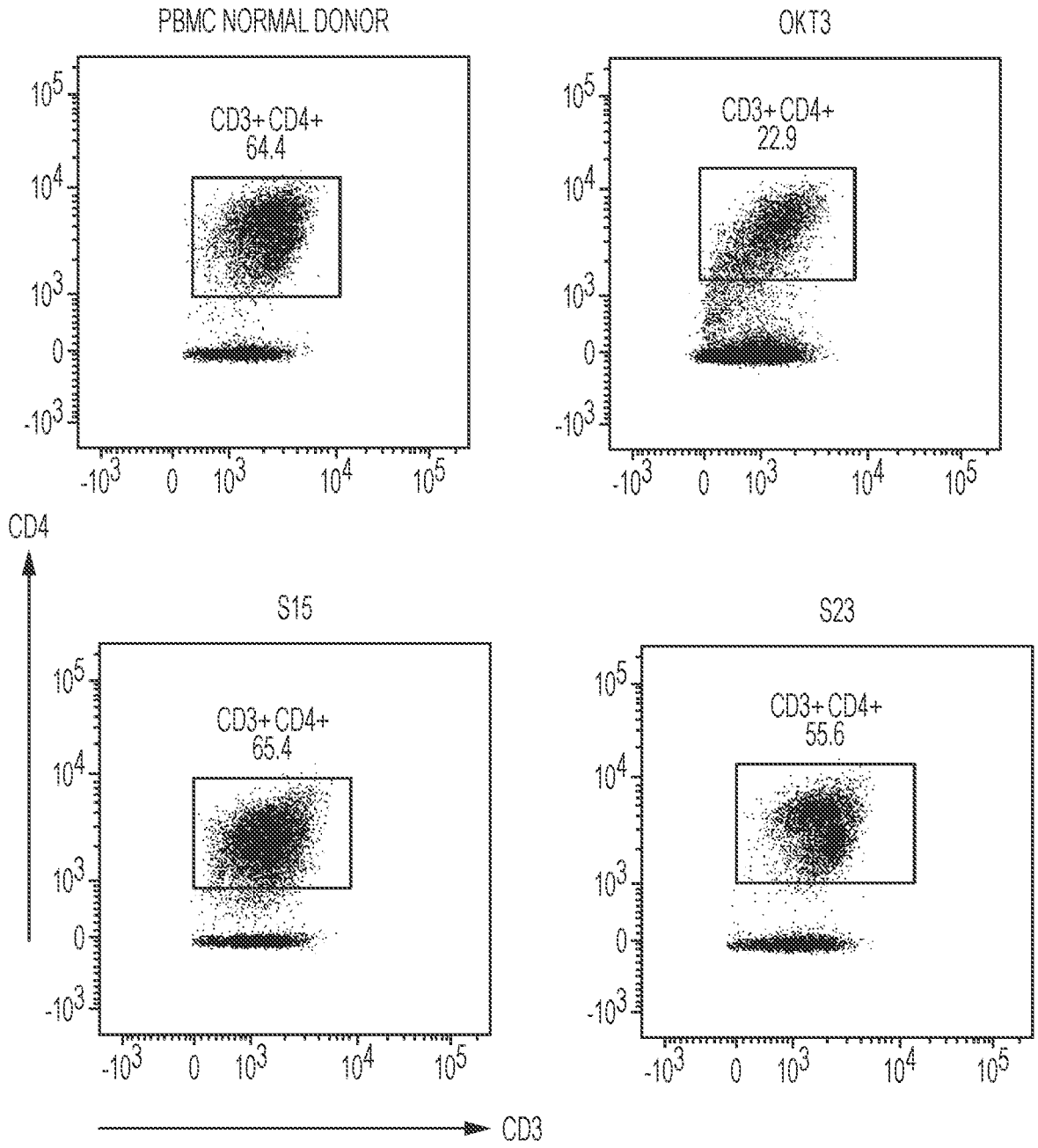

PBMC NORMAL DONOR
CD3+CD4+
64.4
OKT3
CD3+CD4+
22.9
S15
CD3+CD4+
65.4
S23
CD3+CD4+
55.6
CD4
CD3
10^5
10^4
10^3
0
-10^3

FIG. 7I

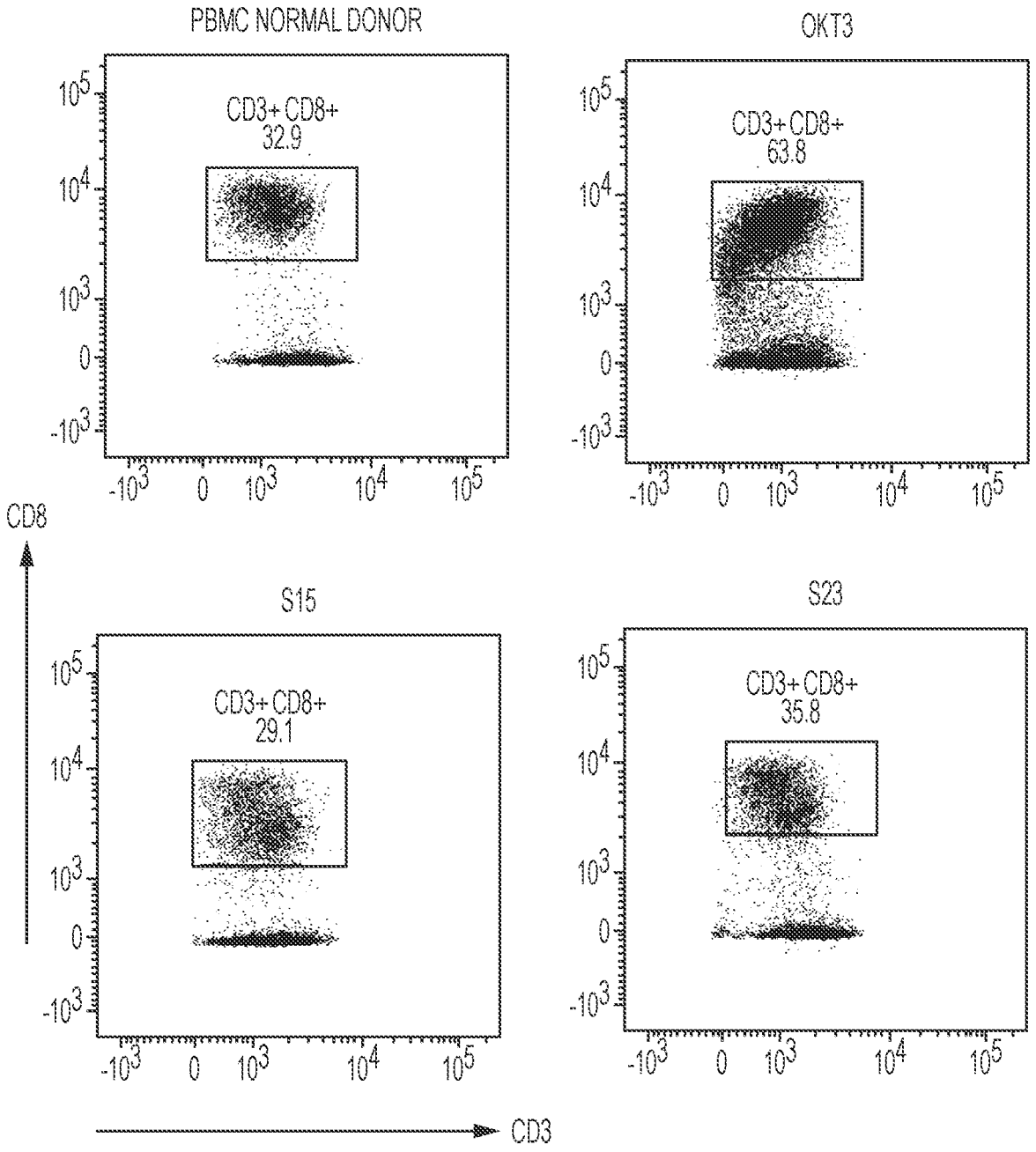

PBMC NORMAL DONOR
CD3+ CD8+
32.9
OKT3
CD3+ CD8+
63.8
S15
CD3+ CD8+
29.1
S23
CD3+ CD8+
35.8
CD8
CD3
10^5
10^4
10^3
0
-10^3

FIG. 7J

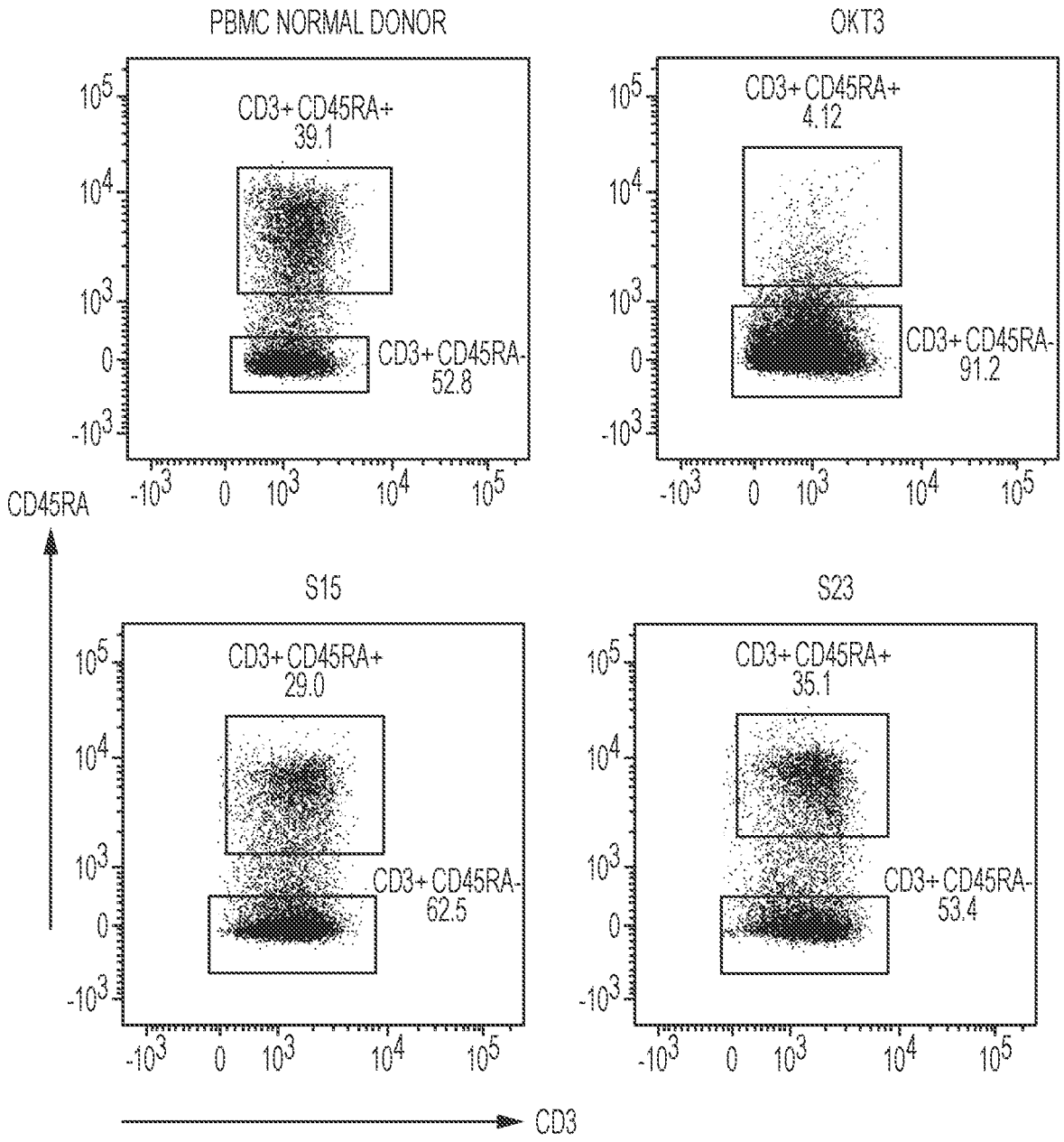

PBMC NORMAL DONOR
CD3+CD45RA+
39.1
CD3+CD45RA-
52.8
OKT3
CD3+CD45RA+
4.12
CD3+CD45RA-
91.2
S15
CD3+CD45RA+
29.0
CD3+CD45RA-
62.5
S23
CD3+CD45RA+
35.1
CD3+CD45RA-
53.4
CD45RA
CD3
10^5
10^4
10^3
0
-10^3

FIG. 7K

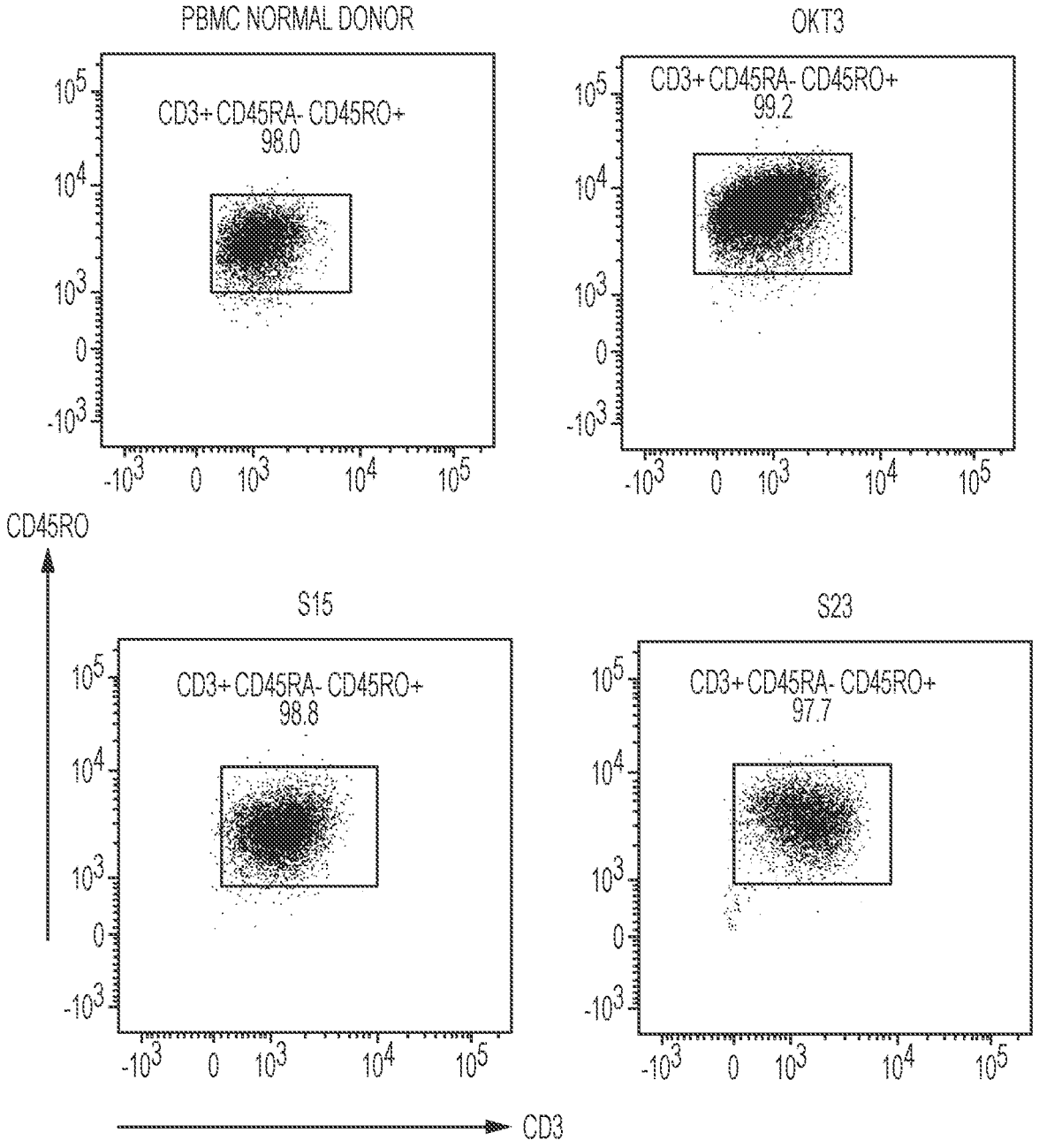

PBMC NORMAL DONOR
CD3+CD45RA-CD45RO+
98.0
OKT3
CD3+CD45RA-CD45RO+
99.2
CD45RO
S15
CD3+CD45RA-CD45RO+
98.8
S23
CD3+CD45RA-CD45RO+
97.7
CD3

FIG. 7L

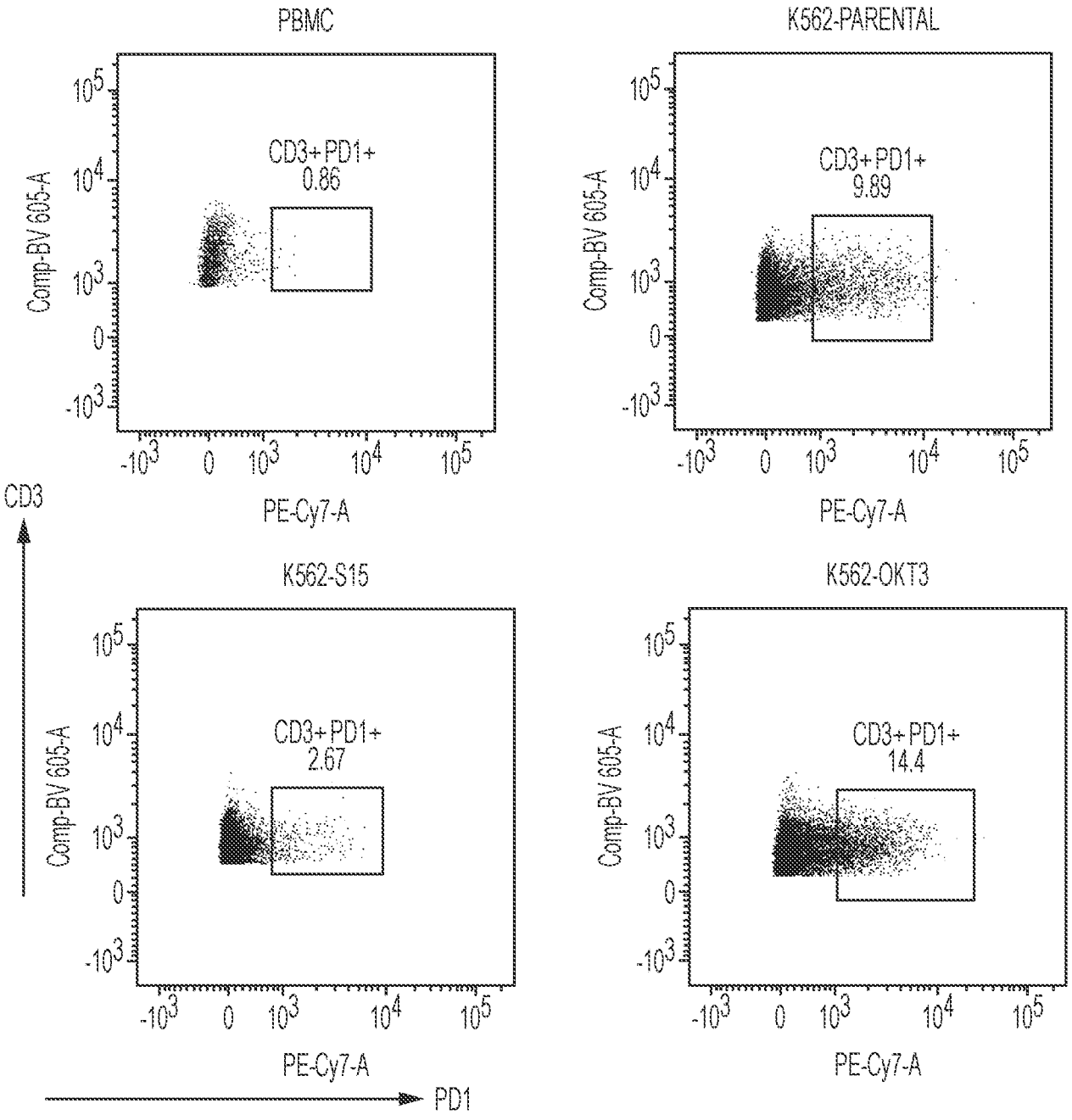

PBMC
CD3+PD1+
0.86
K562-PARENTAL
CD3+PD1+
9.89
K562-S15
CD3+PD1+
2.67
K562-OKT3
CD3+PD1+
14.4
Comp-BV 605-A
PE-Cy7-A
CD3
PD1

FIG. 7M

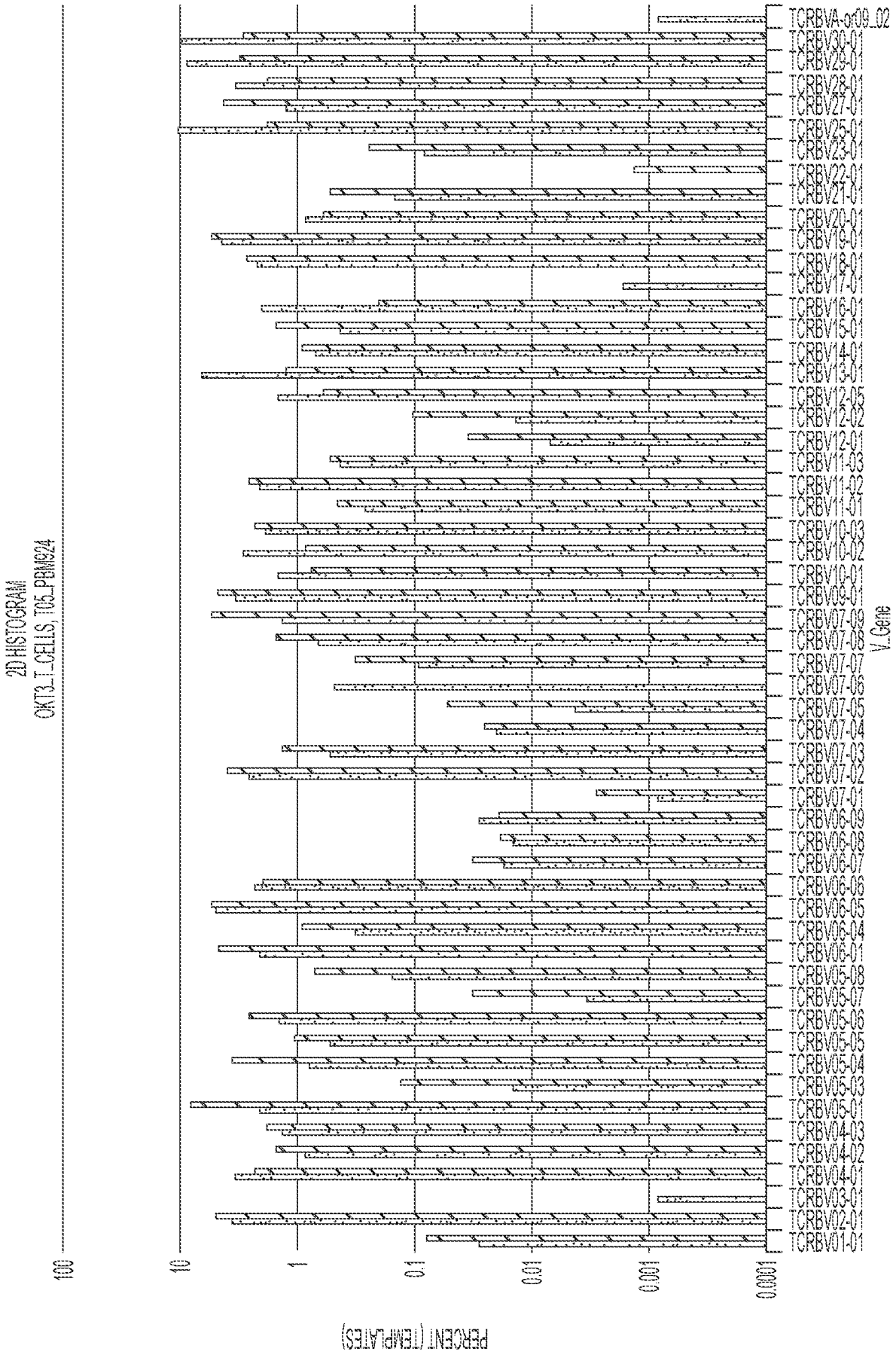

2D HISTOGRAM
OKT3_T_CELLS, T05_PBM924
PERCENT (TEMPLATES)
100
10
1
0.1
0.01
0.001
0.0001
TCRBVA-or09_02
TCRBV30-01
TCRBV29-01
TCRBV28-01
TCRBV27-01
TCRBV25-01
TCRBV23-01
TCRBV22-01
TCRBV21-01
TCRBV20-01
TCRBV19-01
TCRBV18-01
TCRBV17-01
TCRBV16-01
TCRBV15-01
TCRBV14-01
TCRBV13-01
TCRBV12-05
TCRBV12-02
TCRBV12-01
TCRBV11-03
TCRBV11-02
TCRBV11-01
TCRBV10-03
TCRBV10-02
TCRBV10-01
TCRBV09-01
TCRBV07-09
TCRBV07-08
TCRBV07-07
TCRBV07-06
TCRBV07-05
TCRBV07-04
TCRBV07-03
TCRBV07-02
TCRBV07-01
TCRBV06-09
TCRBV06-08
TCRBV06-07
TCRBV06-06
TCRBV06-05
TCRBV06-04
TCRBV06-01
TCRBV05-08
TCRBV05-07
TCRBV05-06
TCRBV05-05
TCRBV05-04
TCRBV05-03
TCRBV05-01
TCRBV04-03
TCRBV04-02
TCRBV04-01
TCRBV03-01
TCRBV02-01
TCRBV01-01
V_Gene
T05_PBM924
OKT3_T_CELLS

FIG. 8A

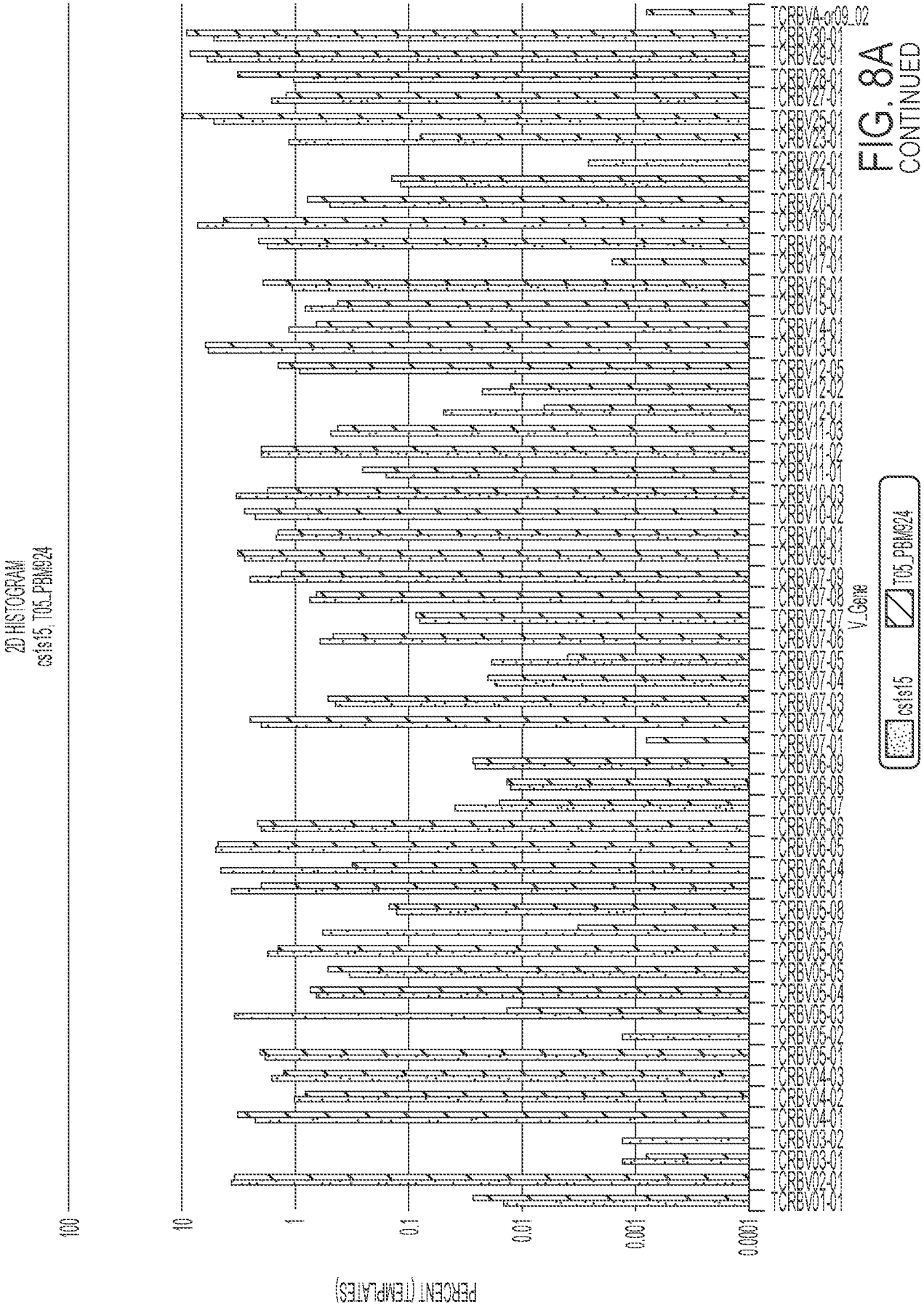


FIG. 8A
CONTINUED

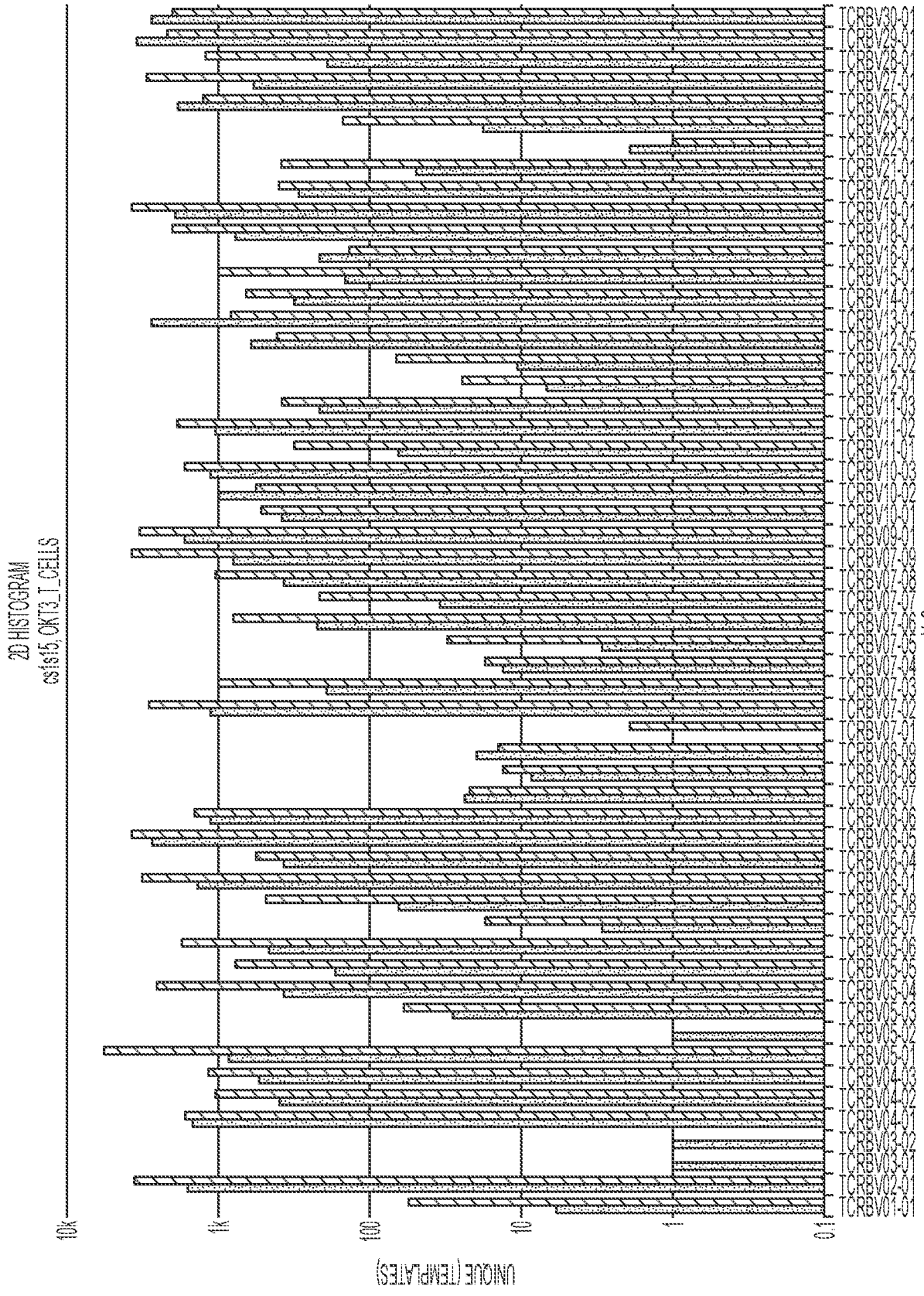

2D HISTOGRAM
cs1s15_OKT3_T_CELLS
10k
1k
100
10
1
0.1
UNIQUE (TEMPLATES)
V_Gene
cs1s15
OKT3_T_CELLS

FIG. 8B

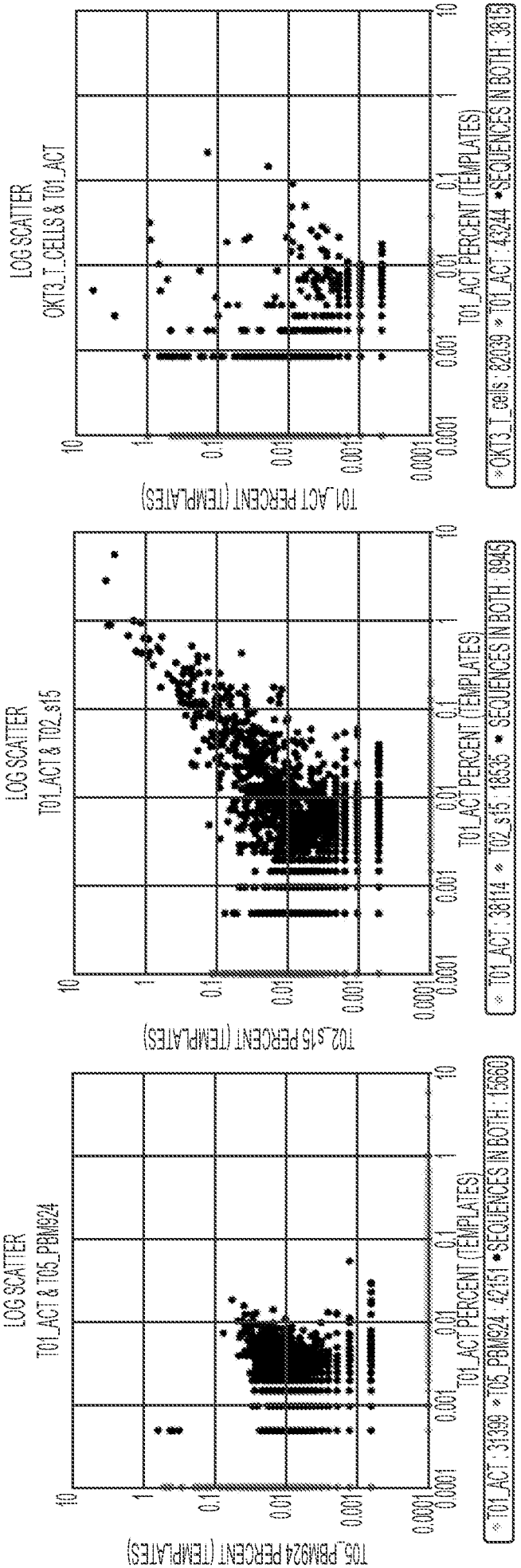

LOG SCATTER
OKT3_T_CELLS & T01_ACT
T01_ACT PERCENT (TEMPLATES)
*OKT3_T_cells: 82039 * T01_ACT: 42244 *SEQUENCES IN BOTH: 3813
LOG SCATTER
T01_ACT & T02_s15
T02_s15 PERCENT (TEMPLATES)
T01_ACT PERCENT (TEMPLATES)
* T01_ACT: 38114 * T02_s15: 18535 * SEQUENCES IN BOTH: 8945
LOG SCATTER
T01_ACT & T05_PBMI924
T05_PBMI924 PERCENT (TEMPLATES)
T01_ACT PERCENT (TEMPLATES)
* T01_ACT: 31399 * T05_PBMI924: 42151 * SEQUENCES IN BOTH: 15660

FIG. 8C

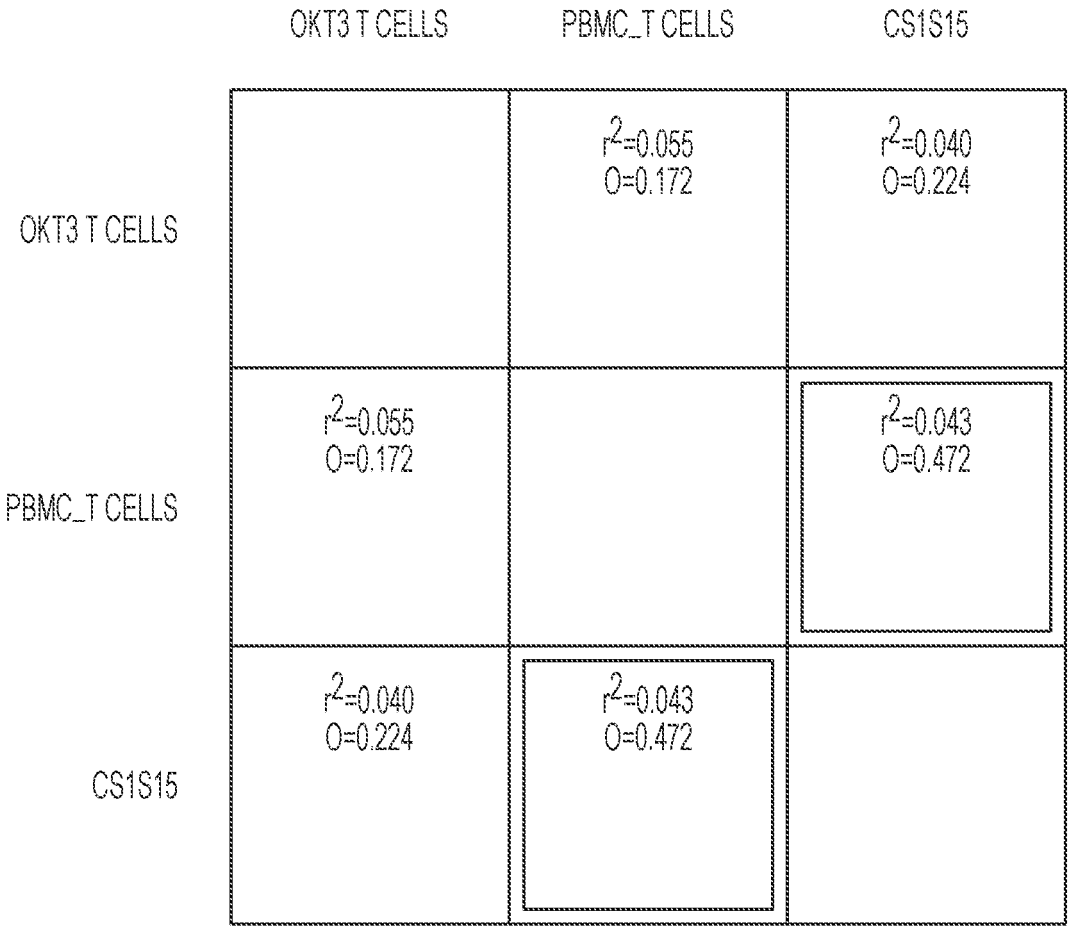

OKT3 T CELLS
PBMC_T CELLS
CS1S15
OKT3 T CELLS
PBMC_T CELLS
CS1S15
r2=0.055
O=0.172
r2=0.040
O=0.224
r2=0.055
O=0.172
r2=0.043
O=0.472
r2=0.040
O=0.224
r2=0.043
O=0.472

FIG. 8D

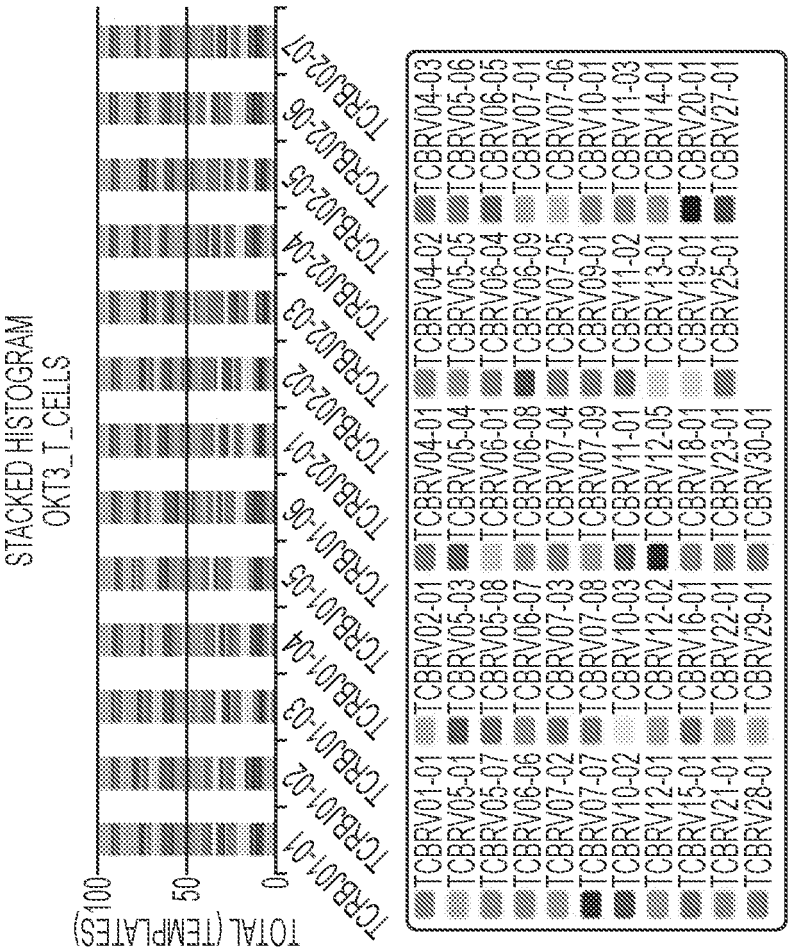
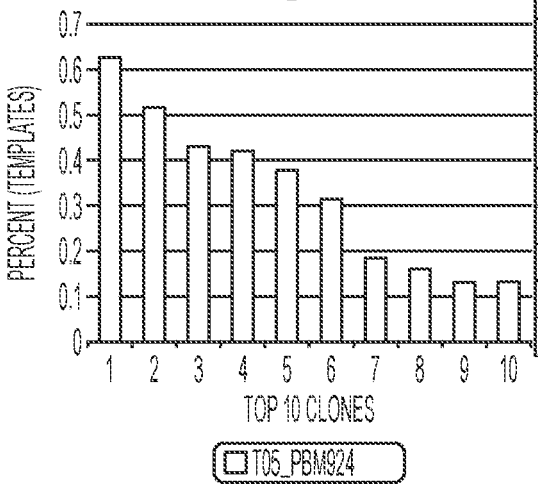

STACKED HISTOGRAM
OKT3_T_CELLS
TOTAL (TEMPLATES)
0
50
100
TCRBJ01-01
TCRBJ01-02
TCRBJ01-03
TCRBJ01-04
TCRBJ01-05
TCRBJ01-06
TCRBJ02-01
TCRBJ02-02
TCRBJ02-03
TCRBJ02-04
TCRBJ02-05
TCRBJ02-06
TCRBJ02-07
TCBRV01-01
TCBRV02-01
TCBRV04-01
TCBRV04-02
TCBRV04-03
TCBRV05-01
TCBRV05-02
TCBRV05-03
TCBRV05-04
TCBRV05-05
TCBRV05-06
TCBRV05-07
TCBRV05-08
TCBRV06-01
TCBRV06-04
TCBRV06-05
TCBRV06-06
TCBRV06-07
TCBRV06-08
TCBRV06-09
TCBRV07-01
TCBRV07-02
TCBRV07-03
TCBRV07-04
TCBRV07-05
TCBRV07-06
TCBRV07-07
TCBRV07-08
TCBRV07-09
TCBRV09-01
TCBRV10-01
TCBRV10-02
TCBRV10-03
TCBRV11-01
TCBRV11-02
TCBRV11-03
TCBRV12-01
TCBRV12-02
TCBRV12-05
TCBRV13-01
TCBRV14-01
TCBRV15-01
TCBRV16-01
TCBRV18-01
TCBRV19-01
TCBRV20-01
TCBRV21-01
TCBRV22-01
TCBRV23-01
TCBRV25-01
TCBRV27-01
TCBRV28-01
TCBRV29-01
TCBRV30-01

FIG. 9

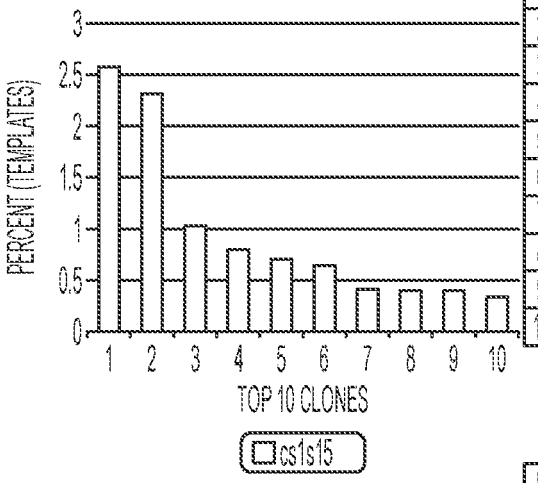
CLONAL ABUNDANCE (HEALTHY DONOR PBMC)



PBMC-T CELLS

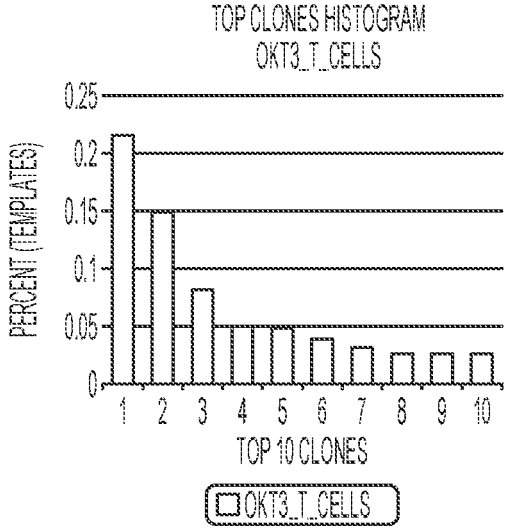
	AMINO ACID	V	J	PERCENTAGE (%)
1	CASSSLSANSPLHF	TCRBV28-01*01	TCRBJ01-06*01	0.63
2	CASSPGHTGELFF	TCRBV28-01*01	TCRBJ02-02*01	0.52
3	CASSIDREDNSPLHF	TCRBV28-01*01	TCRBJ01-06*01	0.43
4	CASSVSGGSNTGELFF	TCRBV25-01*01	TCRBJ02-02*01	0.42
5	CASSLAGGGTNEKLFF	TCRBV05-06*01	TCRBJ01-04*01	0.38
6	CASSEFKQGNTEAFF	TCRBV25-01*01	TCRBJ01-01*01	0.318
7	CASSNHPGPFNEQYF	TCRBV05-05*01	TCRBJ02-07*01	0.187
8	CASSQDPGGVETQYF	TCRBV04-03*01	TCRBJ02-05*01	0.162
9	CASSHVASTDTQYF	TCRBV04-02*01	TCRBJ02-03*01	0.132
10	CASTTGGRTEAFF	TCRBV06-06	TCRBJ01-01*01	0.132

CLONAL ABUNDANCE (K562-S15) AND K562-OKT3 SAMPLES



K562-S15 CO-STIM-T CELLS

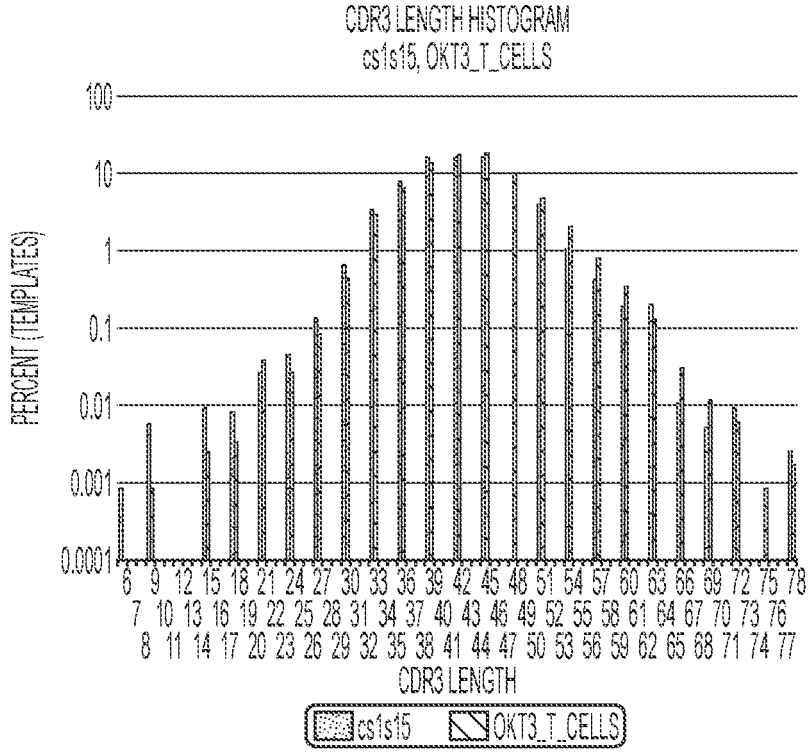
	AMINO ACID	V	J	PERCENTAGE (%)
1	CASSWYREVIDGYTF	TCRBV19-01	TCRBJ01-02*01	2.58
2	CARSFRAGRDGTF	TCRBV05-03	TCRBJ01-02*01	2.32
3	CAGSGGASYNEQFF	TCRBV10-03*01	TCRBJ02-01*01	1.04
4	CASSLNWDSGFF	TCRBV13-01*01	TCRBJ02-01*01	0.81
5	CASSQWDYNSPLHF	TCRBV23-01*01	TCRBJ01-06*01	0.71
6	CASSDSGTHYGYTF	TCRBV02-01*01	TCRBJ01-02*01	0.64
7	CASSQDRQGTDTF	TCRBV14-01*01	TCRBJ01-02*01	0.42
8	CASSGLACSGANVLTF	TCRBV06-04	TCRBJ02-06*01	0.4
9	CASSLWAGDRVKNIQYF	TCRBV05-07*01	TCRBJ02-04*01	0.395
10	CASTREGQQNTEAFF	TCRBV28-01*01	TCRBJ01-01*01	0.34



K562-(OKT3-CO-STIM) T CELLS

	AMINO ACIDS	V	J	PERCENTAGE (%)
1	CASSSLAGLLNYGYTF	TCRBV07-09	TCRBJ01-02*01	0.21
2	CASTSRQGVNTEAFF	TCRBV19-01	TCRBJ01-01*01	0.14
3	CASSLTSGGDNEQFF	TCRBV06-05*01	TCRBJ02-01*01	0.08
4	CAGSGGASYNEQFF	TCRBV10-03*01	TCRBJ02-01*01	0.05
5	CASSPARNTEAFF	TCRBV04-03*01	TCRBJ01-01*01	0.04
6	CATSPLAGEEQFF	TCRBV15-01*01	TCRBJ02-01*01	0.038
7	CARSFRAGRDGTF	TCRBV05-03	TCRBJ01-02*01	0.03
8	CASSQWTGPPYEQYF	TCRBV05-06*01	TCRBJ02-07*01	0.026
9	CASRLGSGGGNYGYTF	TCRBV27-01*01	TCRBJ01-02*01	0.026
10	CASSSAGSNQPQHF	TCRBV10-02*01	TCRBJ01-05*01	0.34

FIG. 10


CDR3 LENGTH HISTOGRAM
cs1s15, OKT3_T_CELLS
PERCENT (TEMPLATES)
100
10
1
0.1
0.01
0.001
0.0001
6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78
7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76
8 11 14 17 20 23 26 29 32 35 38 41 44 47 50 53 56 59 62 65 68 71 74 77
CDR3 LENGTH
cs1s15
OKT3_T_CELLS

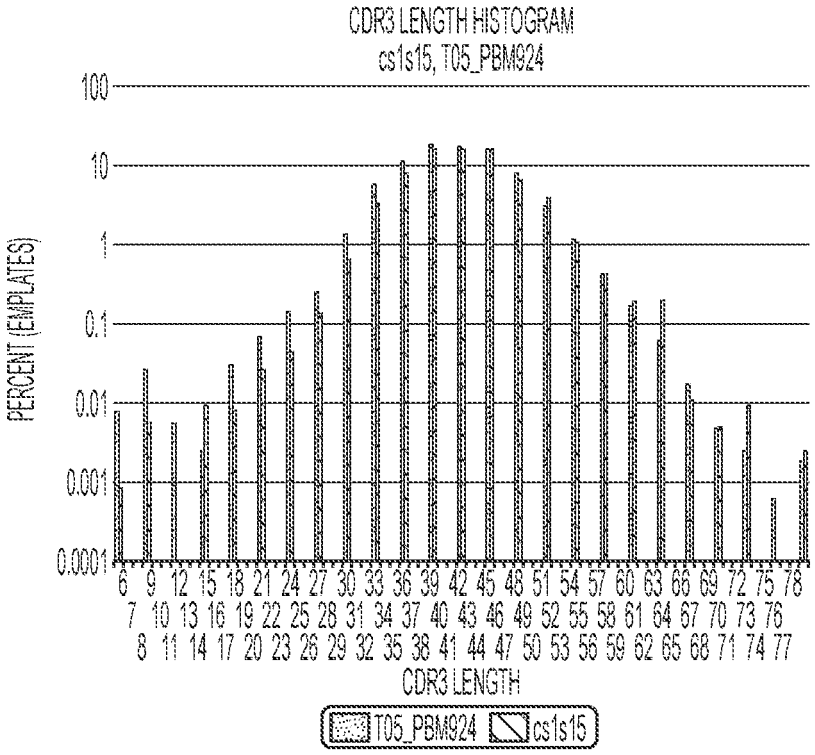

CDR3 LENGTH HISTOGRAM
cs1s15, T05_PBM924
PERCENT (EMPLATES)
100
10
1
0.1
0.01
0.001
0.0001
6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60 63 66 69 72 75 78
7 10 13 16 19 22 25 28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76
8 11 14 17 20 23 26 29 32 35 38 41 44 47 50 53 56 59 62 65 68 71 74 77
CDR3 LENGTH
T05_PBM924
cs1s15

FIG. 11