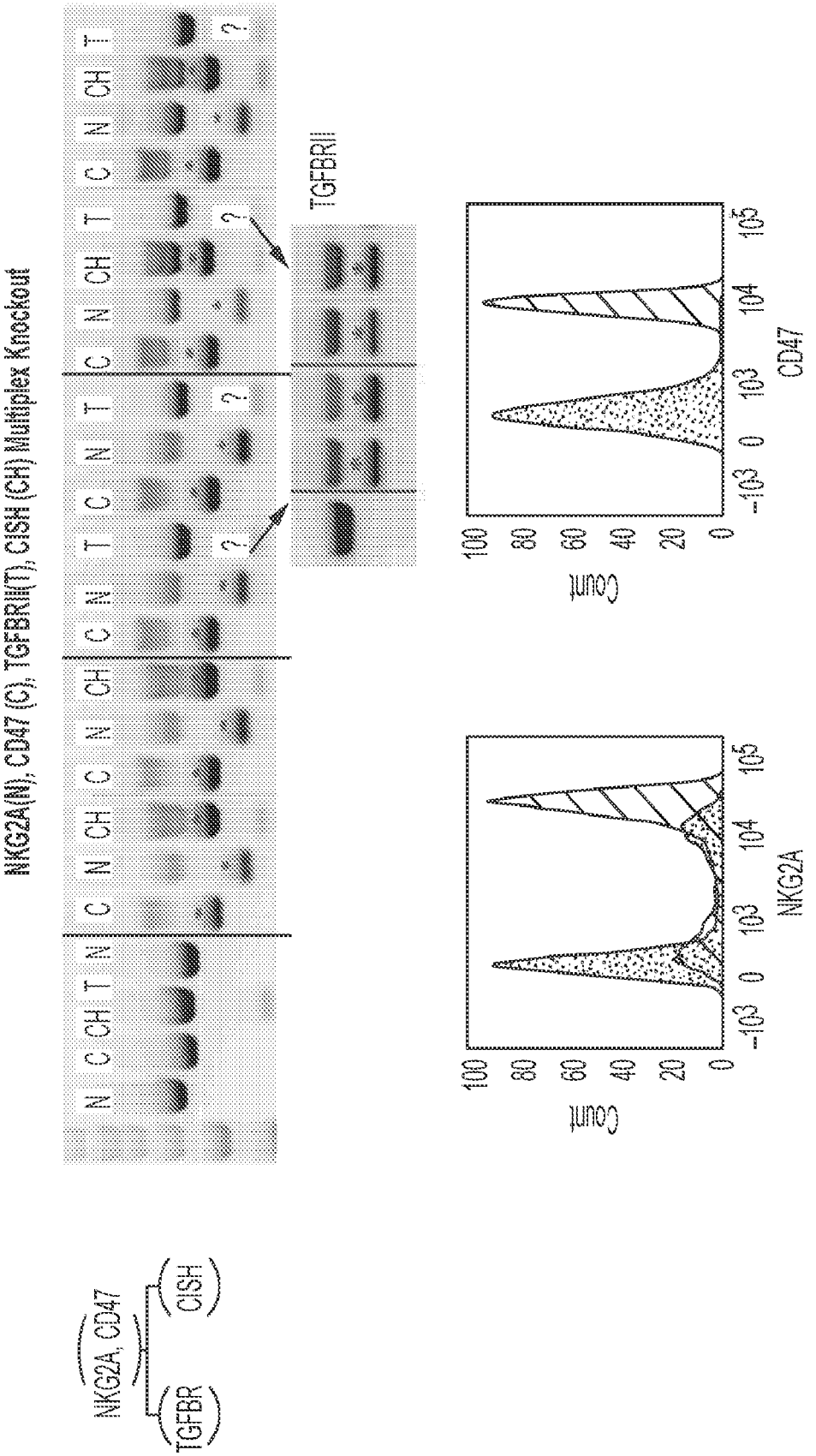

NKG2A(N), CD47 (C), TGFBRII(T), CISH (CH) Multiplex Knockout
N C CH T N C N CH C N CH C N T C N T C N CH T C N CH T
TGFBRII
?
NKG2A, CD47
TGFBR
CISH
Count
100
80
60
40
20
0
-10^3
0
10^3
10^4
10^5
NKG2A
CD47

FIG. 1

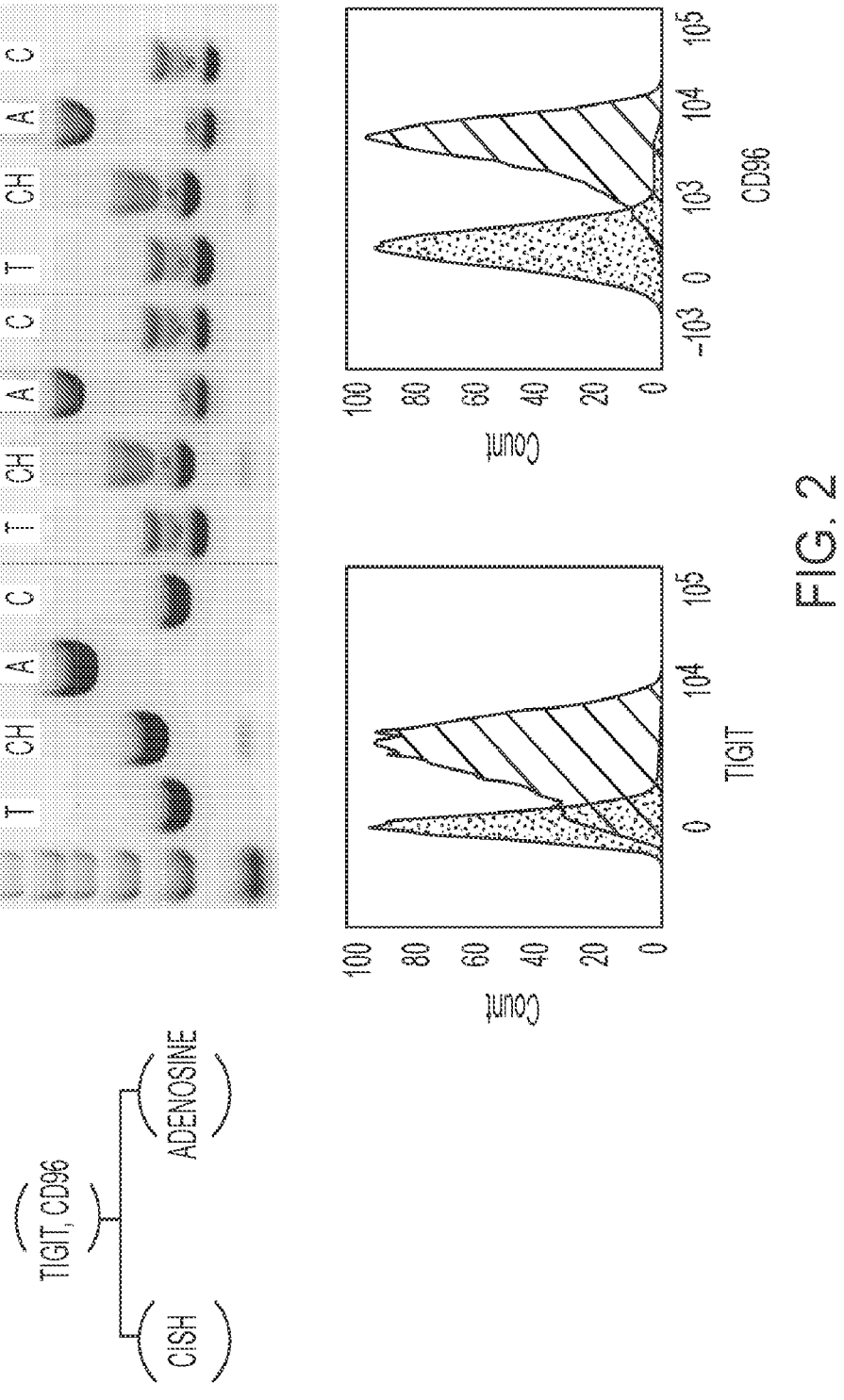

TIGIT (T), CD96 (C), CISH (CH), ADENOSINE (A) Multiplex Knockout
T CH A C T CH A C T CH A C
TIGIT, CD96
CISH
ADENOSINE
Count
100
80
60
40
20
0
0
104
105
TIGIT
Count
100
80
60
40
20
0
-103
0
103
104
105
CD96

FIG. 2

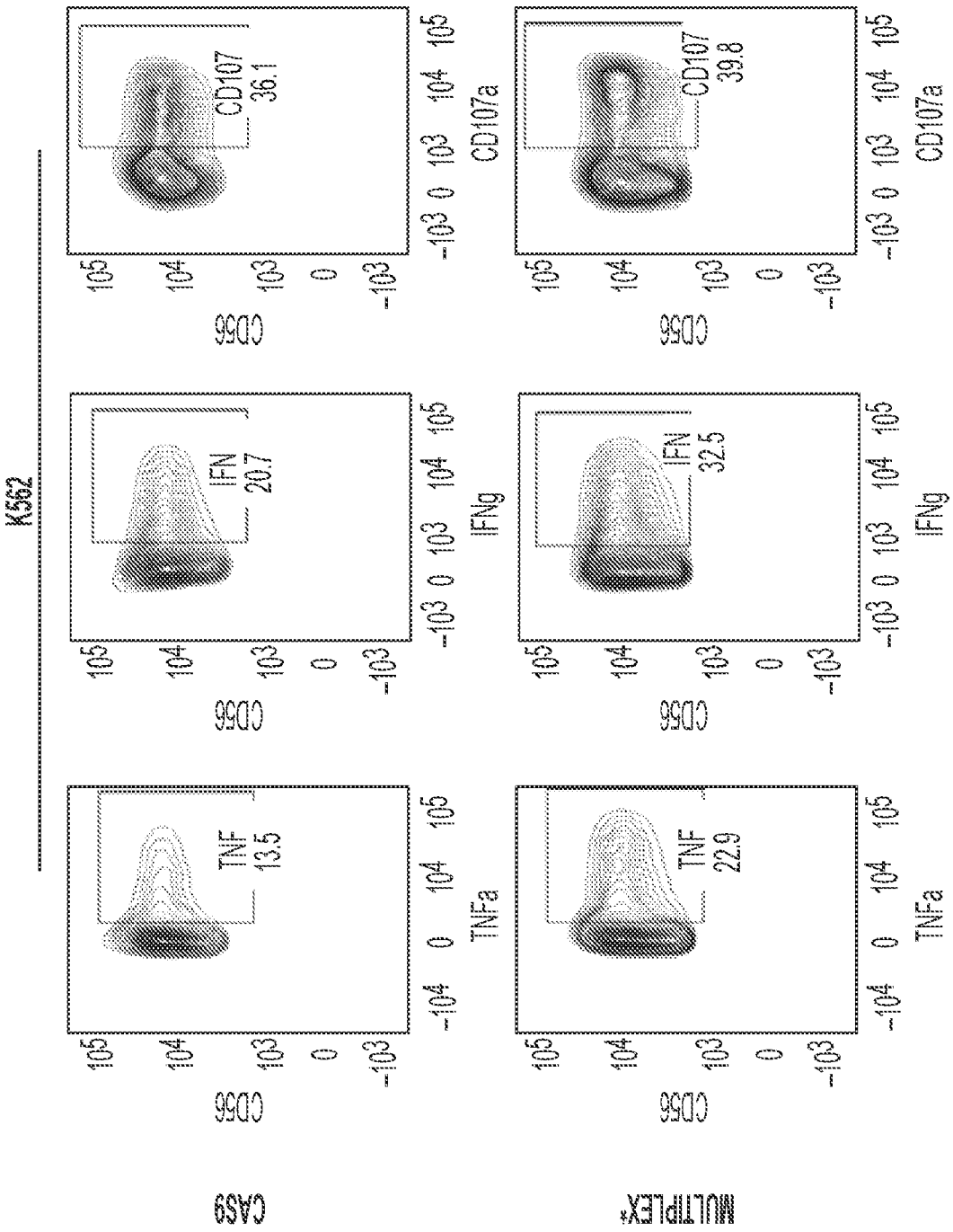

K562
CAS9
MULTIPLEX*
CD56
TNF
13.5
TNFa
IFN
20.7
IFNg
CD107
36.1
CD107a
TNF
22.9
IFN
32.5
CD107
39.8

FIG. 3

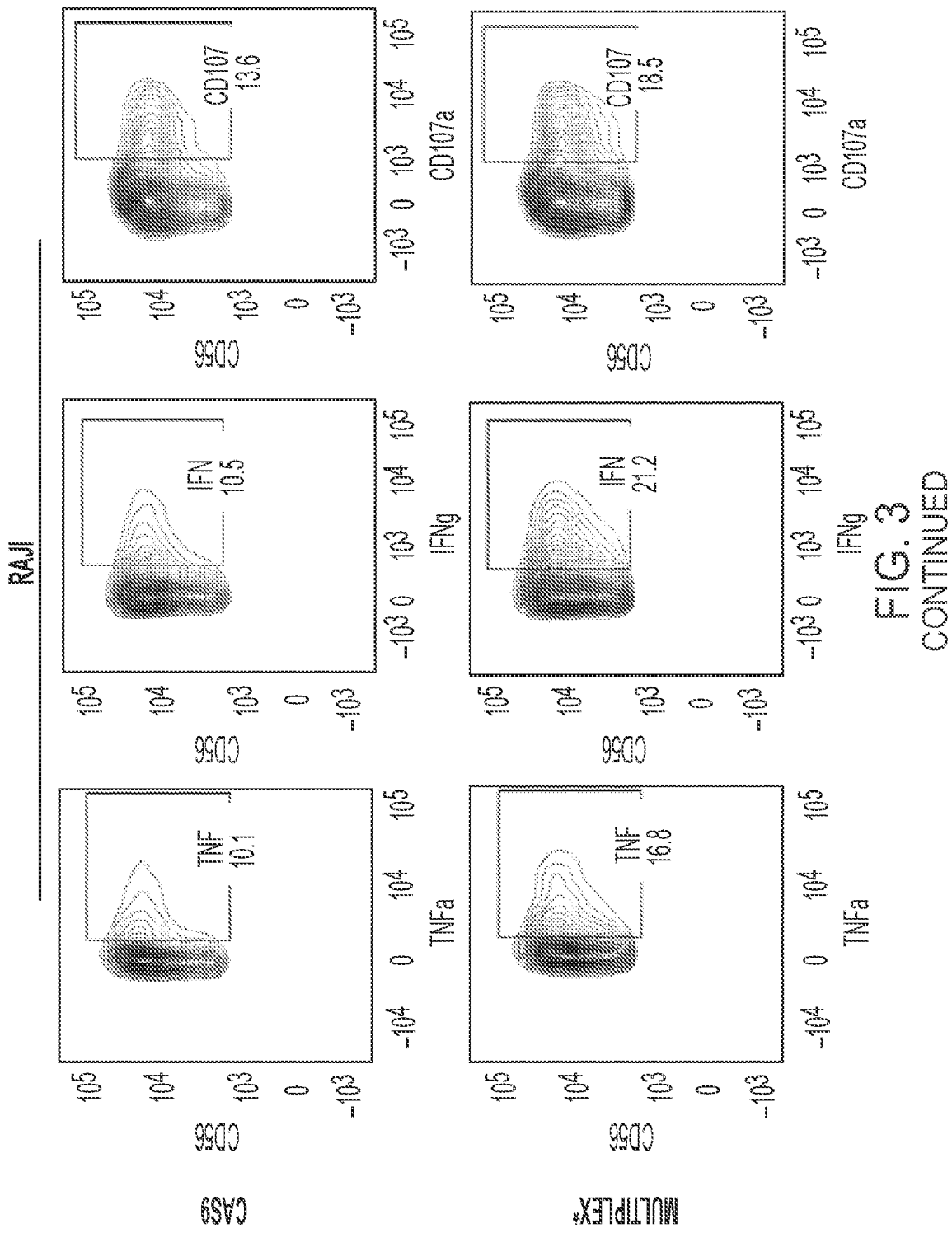


FIG. 3
CONTINUED

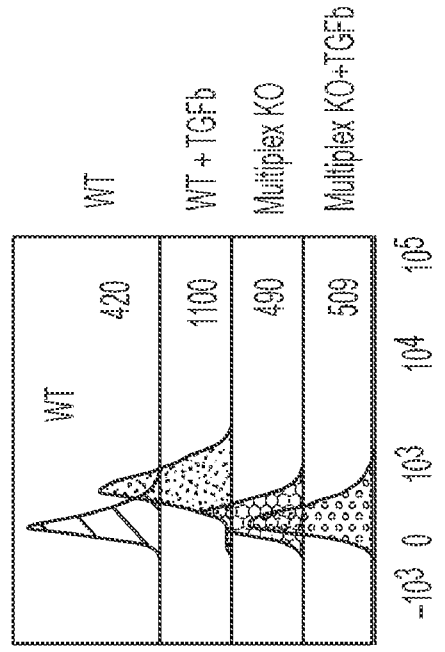

WT
WT + TGFb
Multiplex KO
Multiplex KO+TGFb
WT
420
1100
490
509
-10^3
0
10^3
10^4
10^5
pSMAD2/3

FIG. 4B

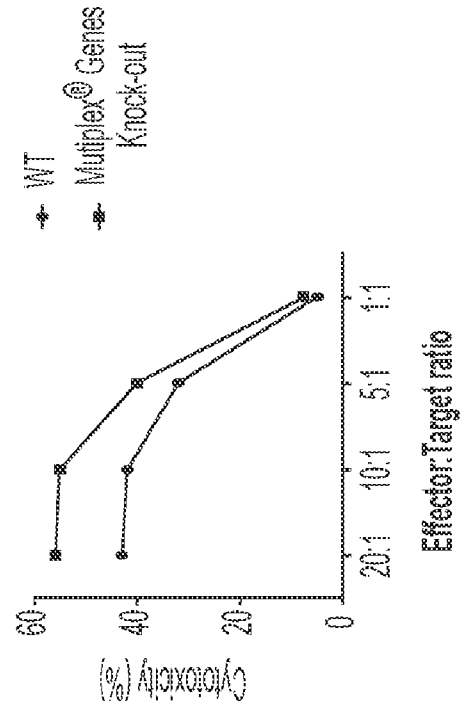

WT
Multiplex® Genes Knock-out
60
40
20
0
Cytotoxicity (%)
20:1
10:1
5:1
1:1
Effector:Target ratio

FIG. 4A

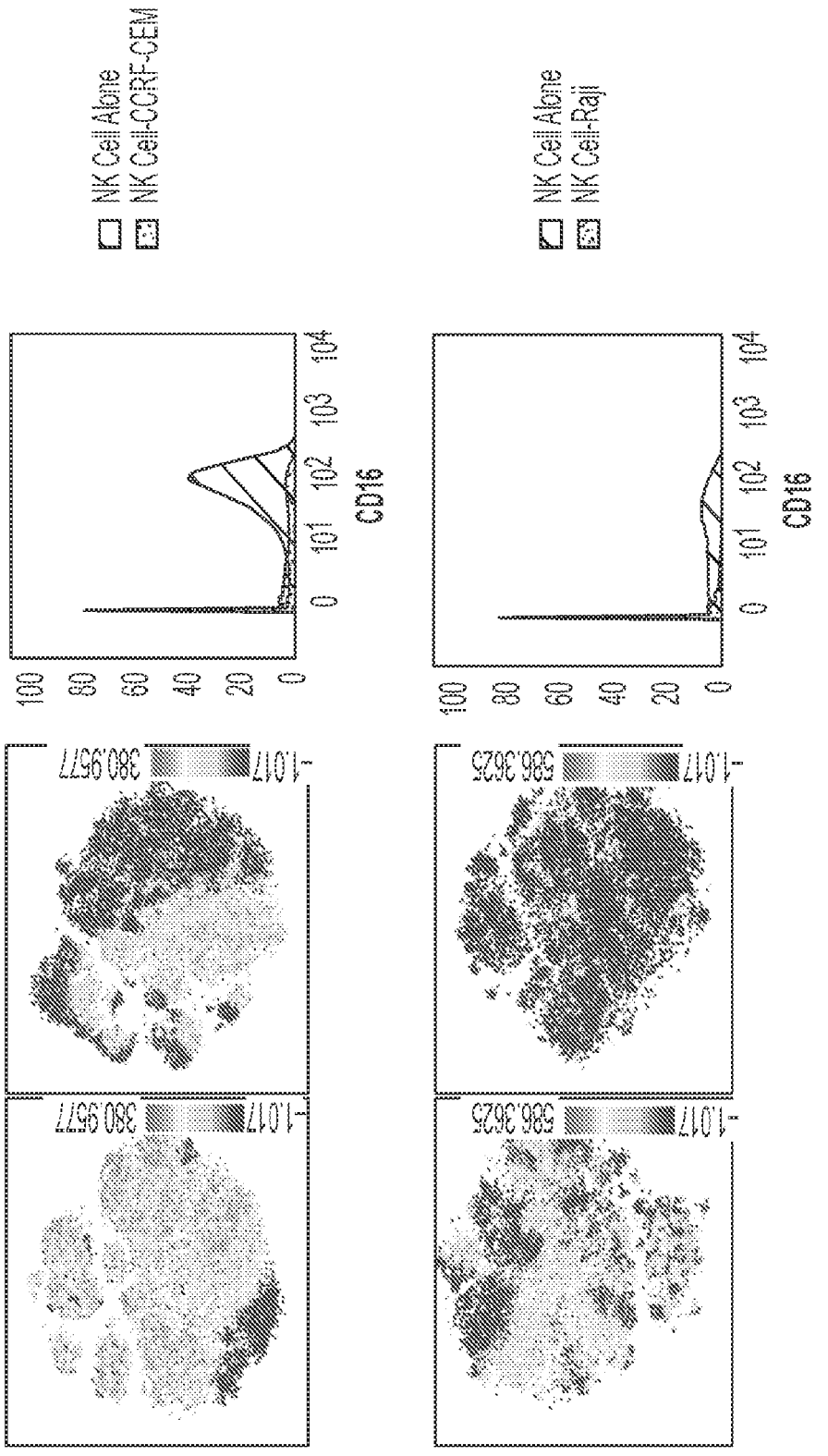

NK Cell Alone
NK Cell-CCRF-CEM
NK Cell Alone
NK Cell-Raji
CD16
CD16
100
80
60
40
20
0
0
10^1
10^2
10^3
10^4
-1.017
380.9577
586.3625

FIG. 5

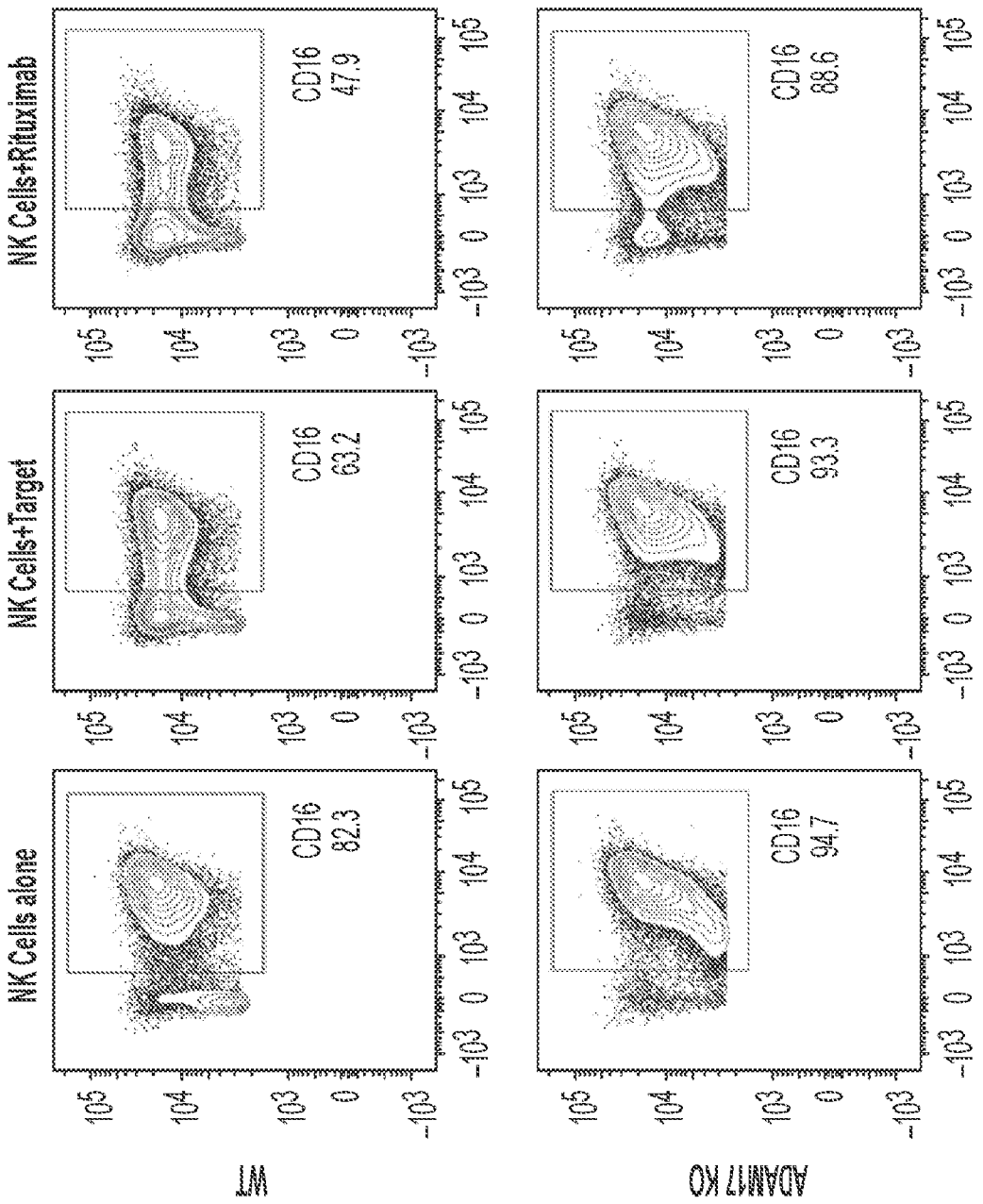

NK Cells alone
NK Cells+Target
NK Cells+Rituximab
WT
ADAM17 KO
CD16
82.3
CD16
63.2
CD16
47.9
CD16
94.7
CD16
93.3
CD16
88.6
105
104
103
0
-103
-103
0
103
104
105

FIG. 6

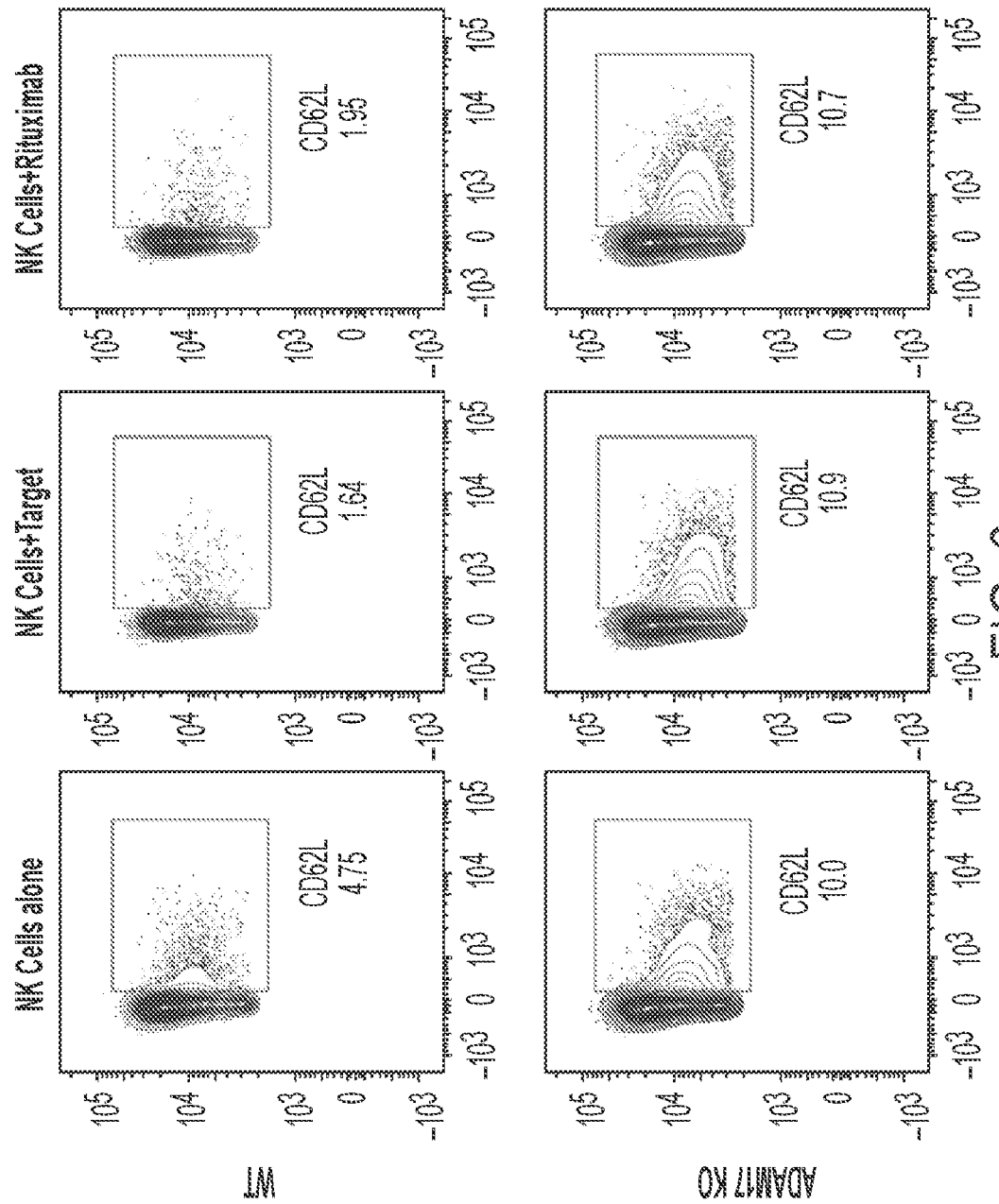


FIG. 6
CONTINUED

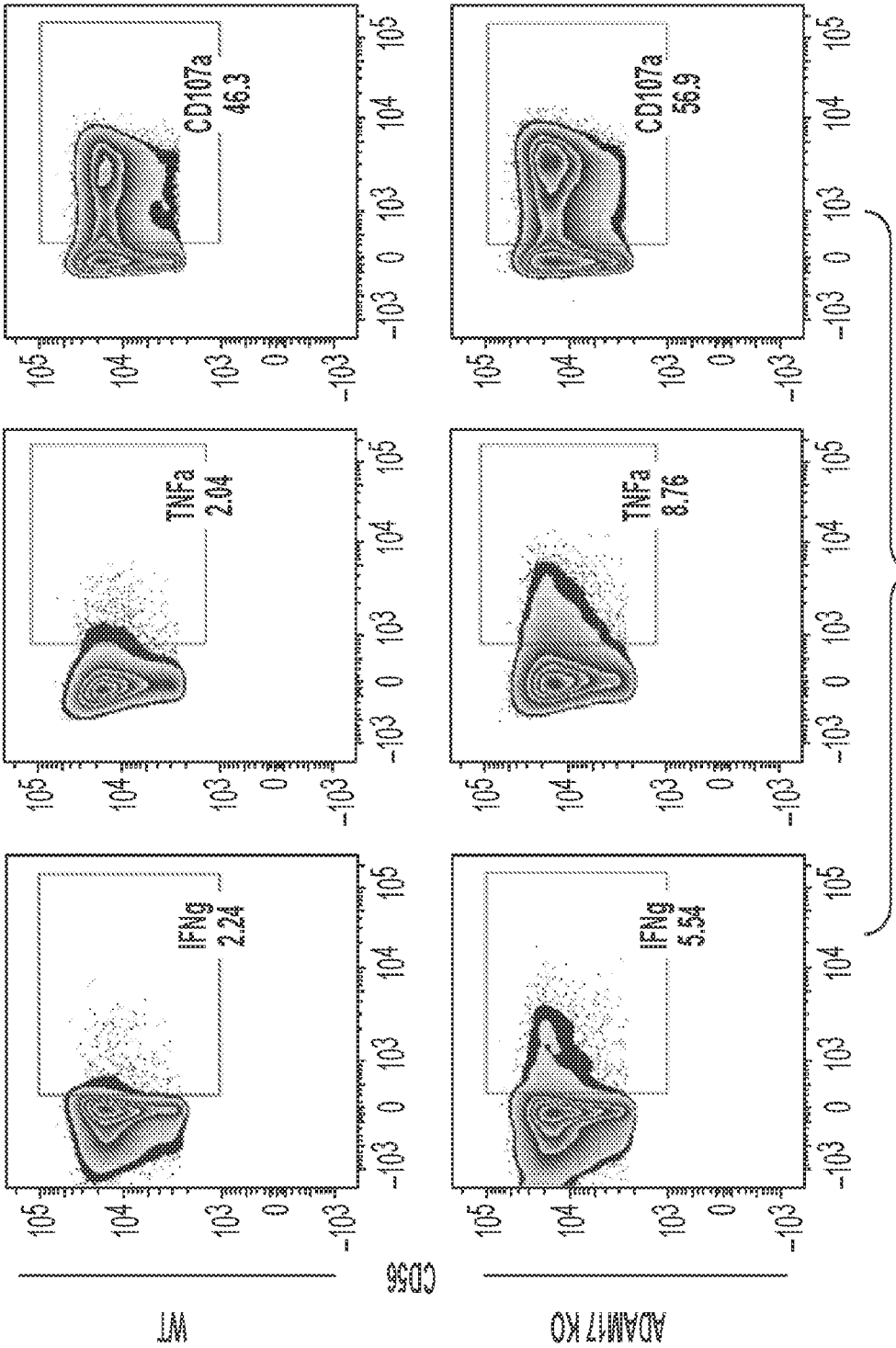

WT
ADAM17 KO
CD35
IFNg
2.24
TNFa
2.04
CD107a
46.3
IFNg
5.54
TNFa
8.76
CD107a
56.9
ADCC

FIG. 7

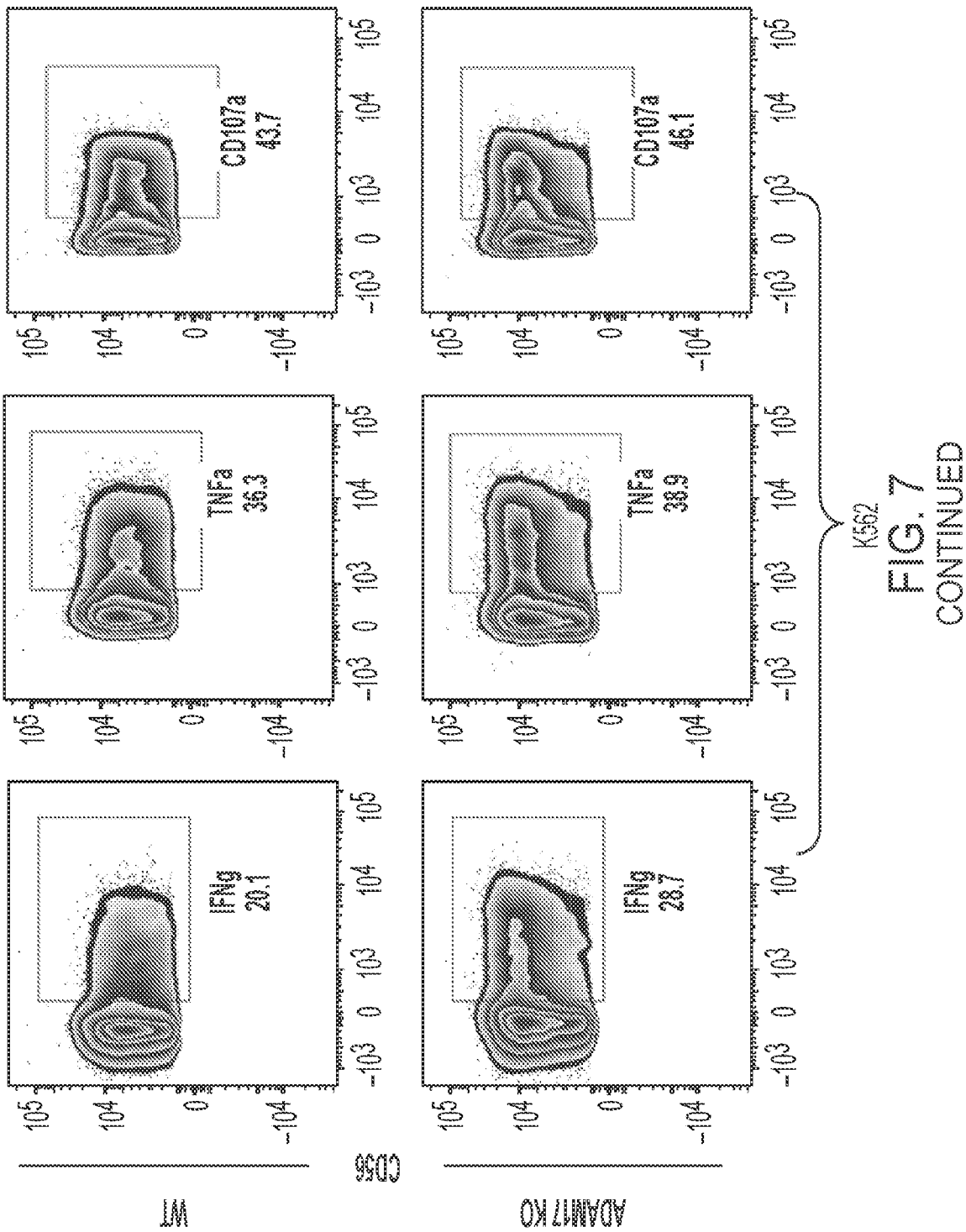


FIG. 7
CONTINUED

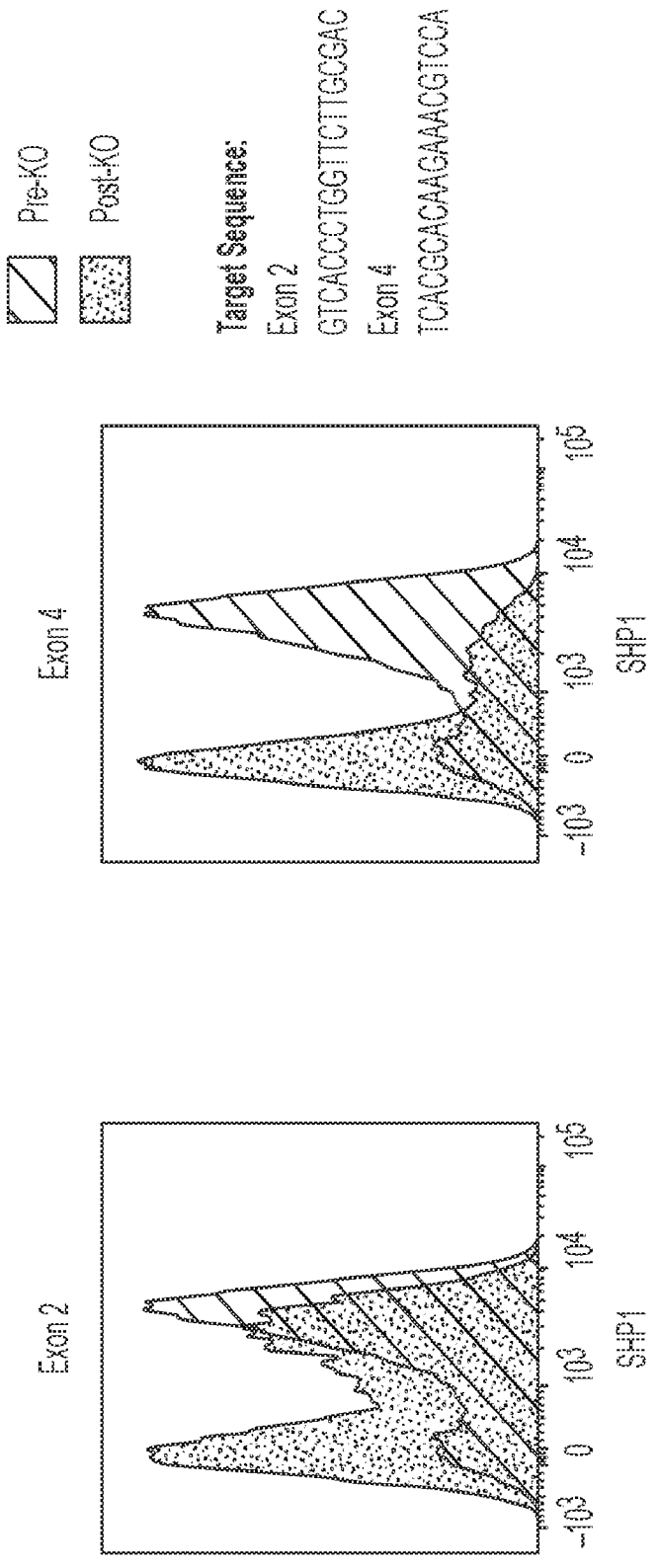

Pre-KO
Post-KO
Target Sequence:
Exon 2
GTCACCCTGGTTCTTGCGAC
Exon 4
TCACGCACAAGAAACGTCCA
Exon 4
-103
0
103
104
105
SHP1
Exon 2
-103
0
103
104
105
SHP1

FIG. 8

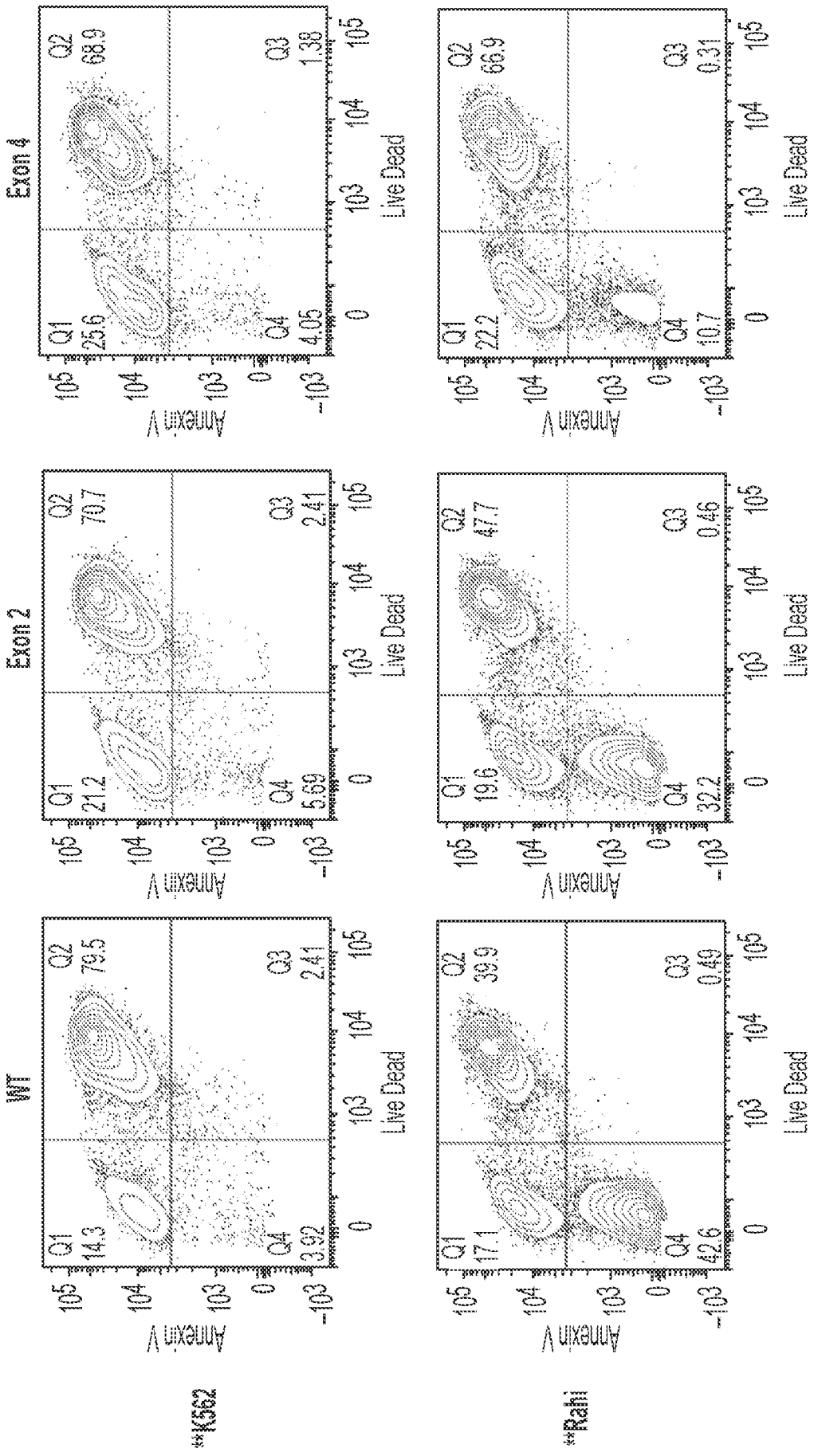

WT
Exon 2
Exon 4
**K562
**Rahi
Annexin V
Live Dead
Q1
Q2
Q3
Q4
14.3
79.5
2.41
3.92
21.2
70.7
2.41
5.69
25.6
68.9
1.38
4.05
19.6
47.7
0.46
32.2
17.1
38.9
0.49
42.6
22.2
66.9
0.31
10.7

FIG. 9

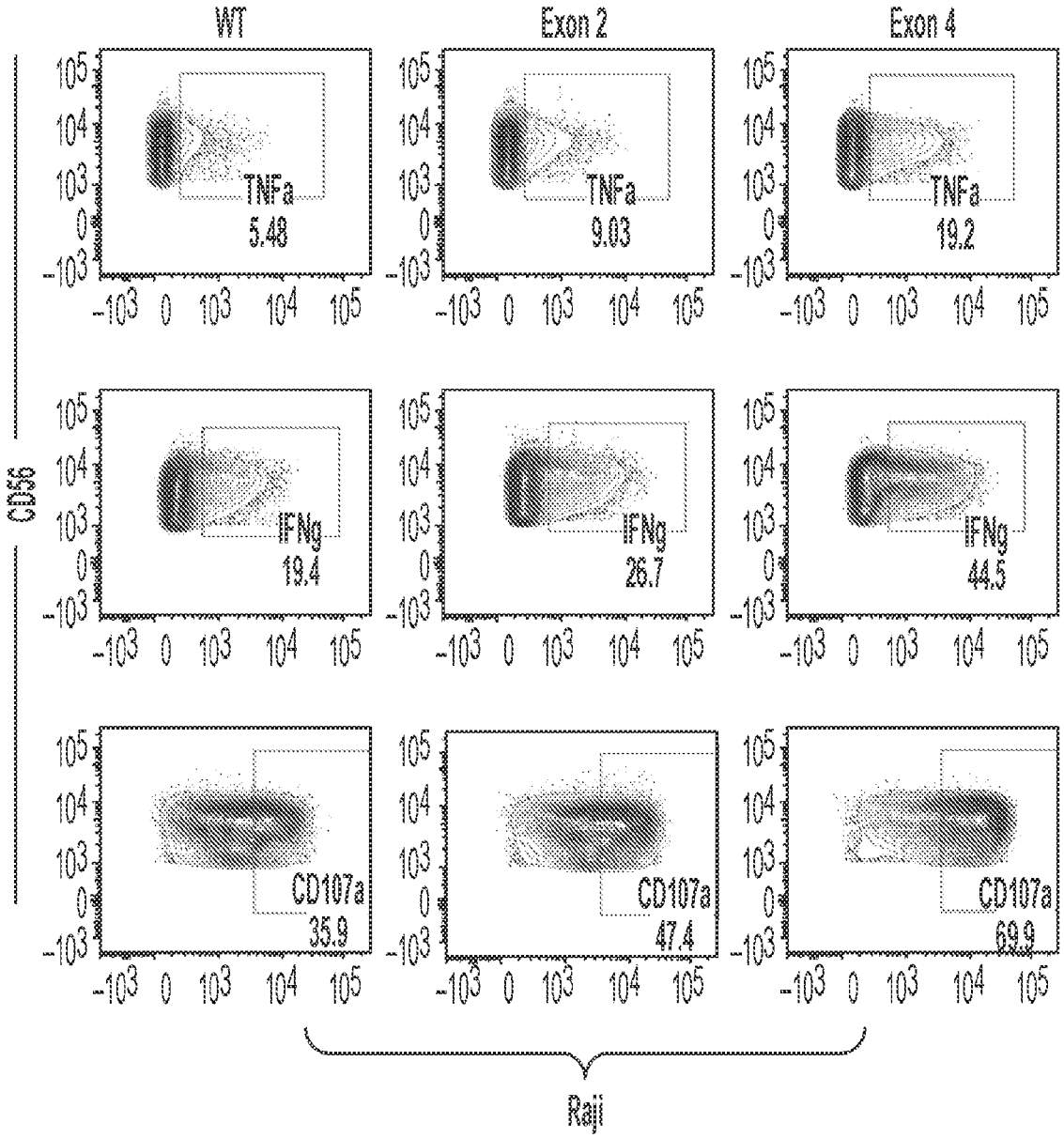

WT
Exon 2
Exon 4
CD56
TNFa
5.48
TNFa
9.03
TNFa
19.2
IFNg
19.4
IFNg
26.7
IFNg
44.5
CD107a
35.9
CD107a
47.4
CD107a
69.9
Raji

FIG. 10A

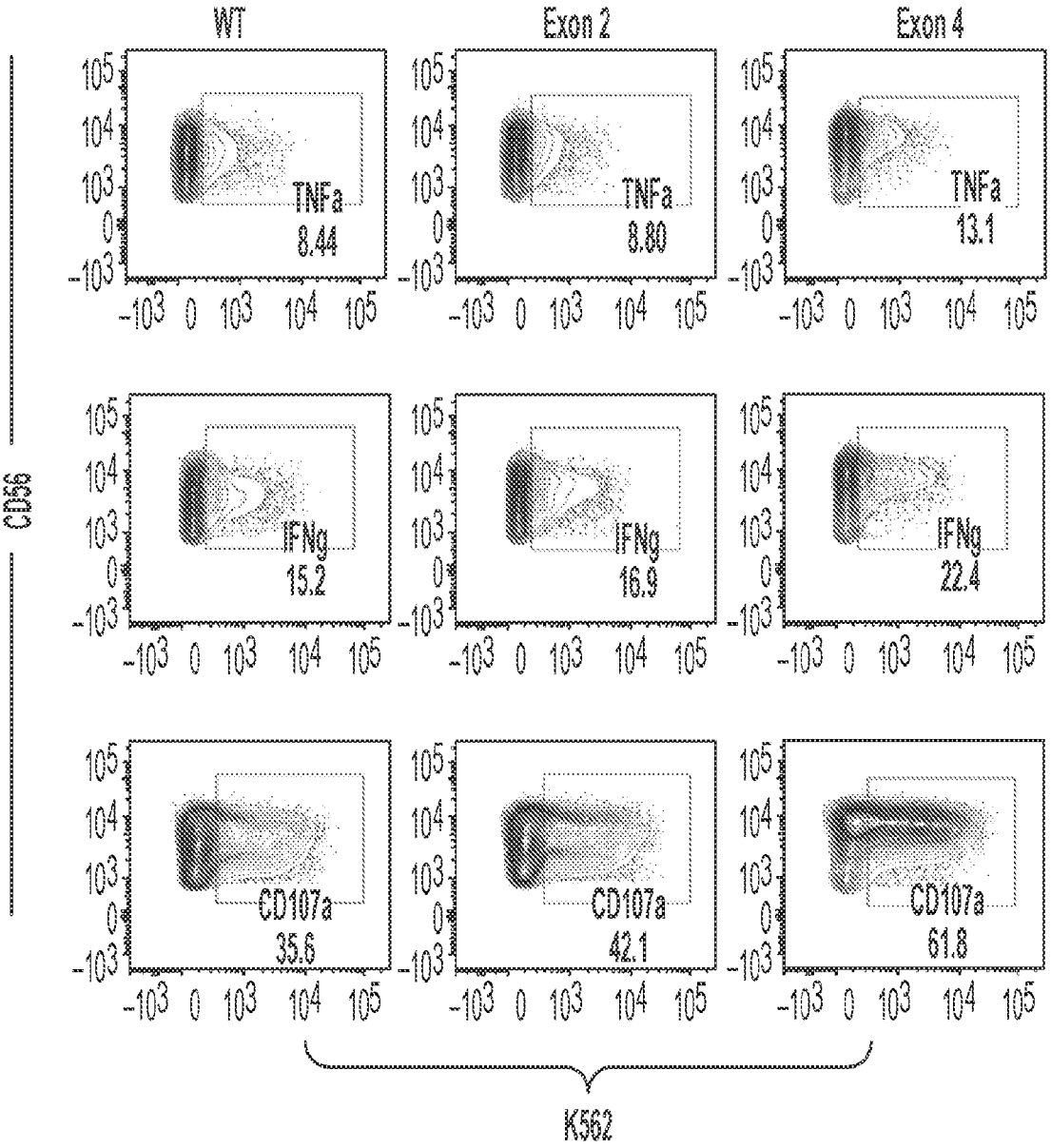


FIG. 10A
CONTINUED

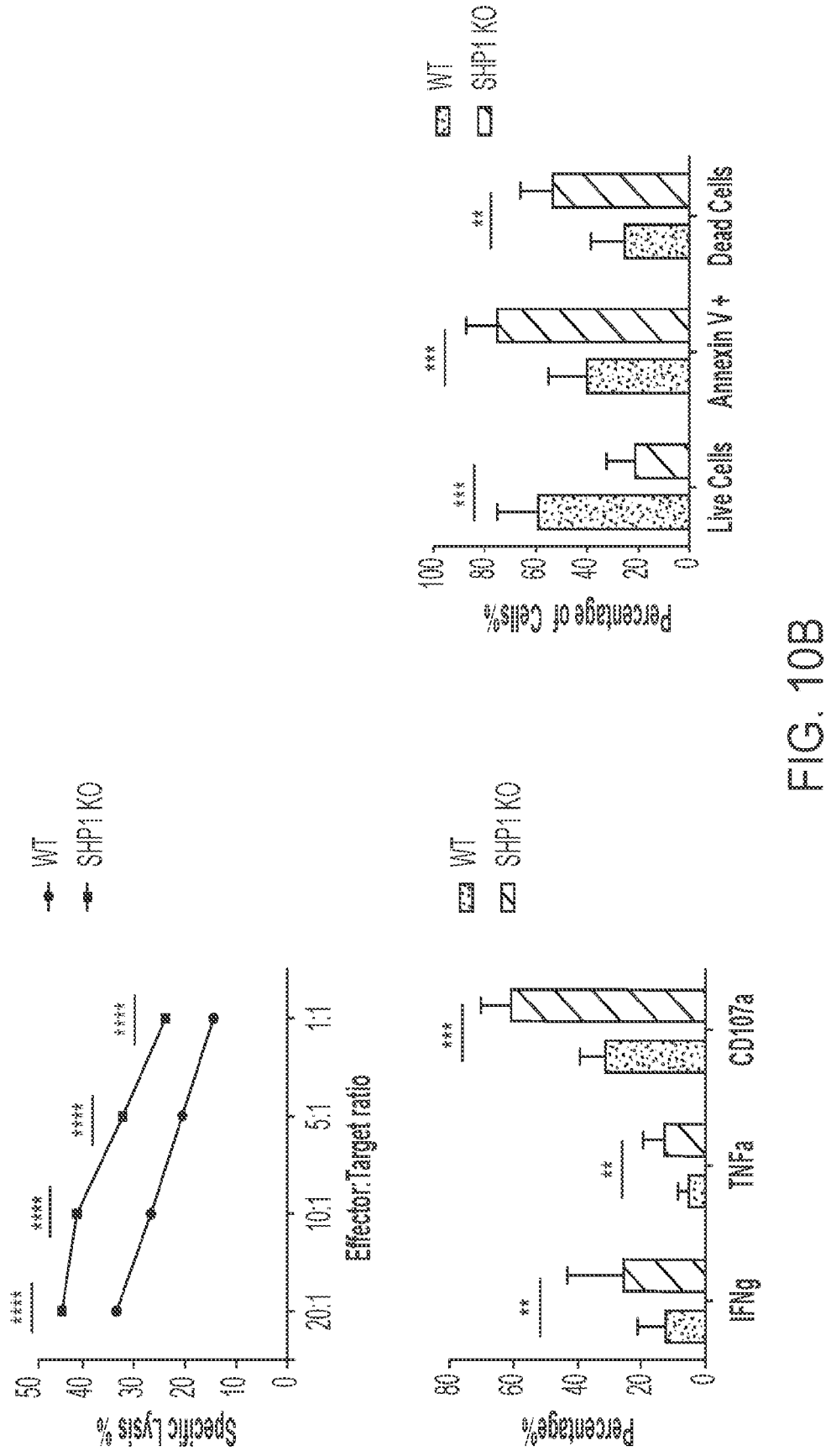

WT
SHP1 KO
Specific Lysis %
Effector:Target ratio
20:1
10:1
5:1
1:1
****
WT
SHP1 KO
Percentage%
IFNg
TNFa
CD107a
Percentage of Cells%
Live Cells
Annexin V+
Dead Cells

FIG. 10B

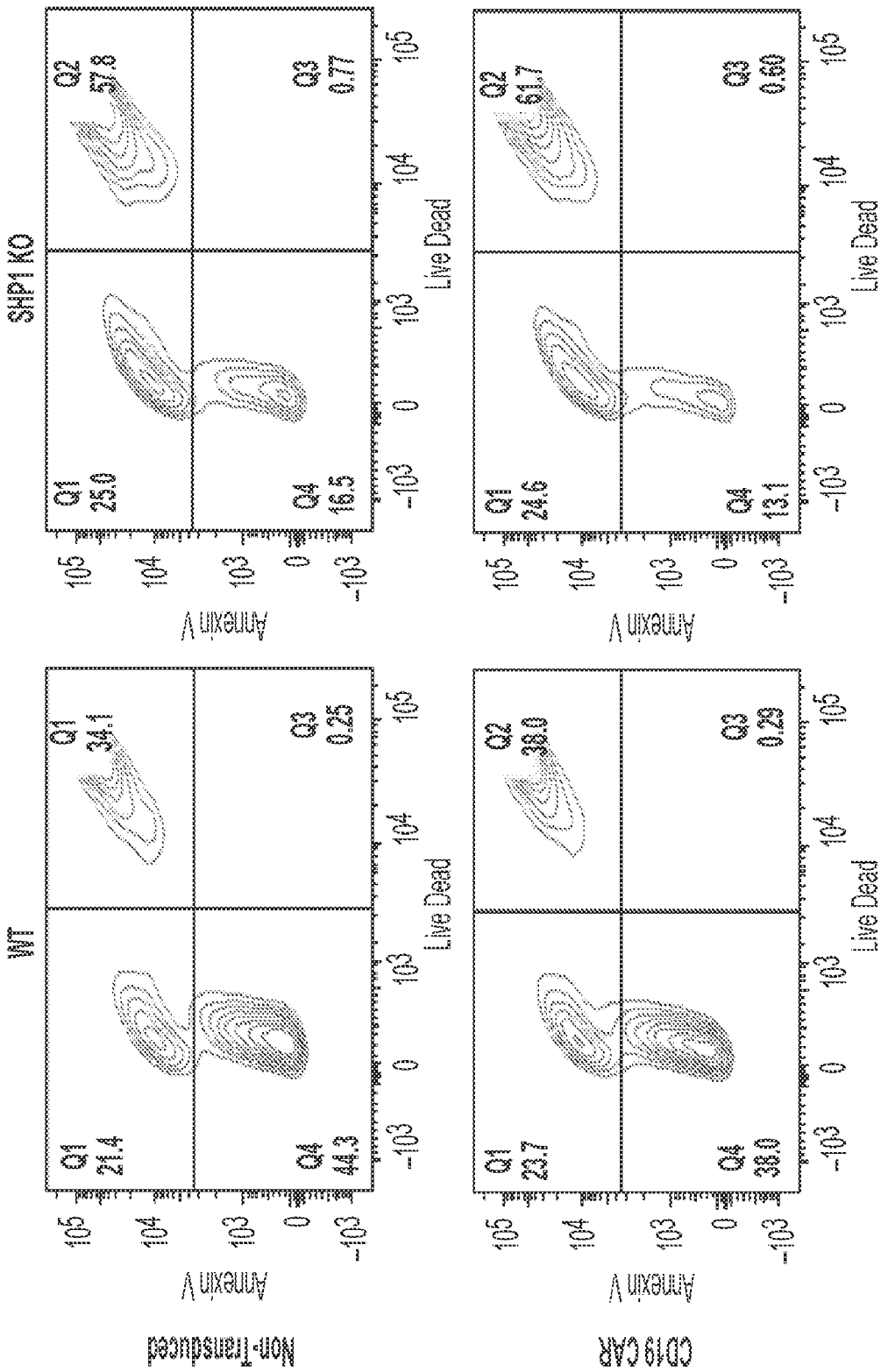

Non-Transduced
CD19 CAR
WT
SHP1 KO
Annexin V
Live Dead
Q1
21.4
Q4
44.3
Q3
0.25
Q1
34.1
Q1
23.7
Q4
38.0
Q2
38.0
Q3
0.29
Q1
25.0
Q4
16.5
Q2
57.8
Q3
0.77
Q1
24.6
Q4
13.1
Q2
61.7
Q3
0.60

FIG. 11

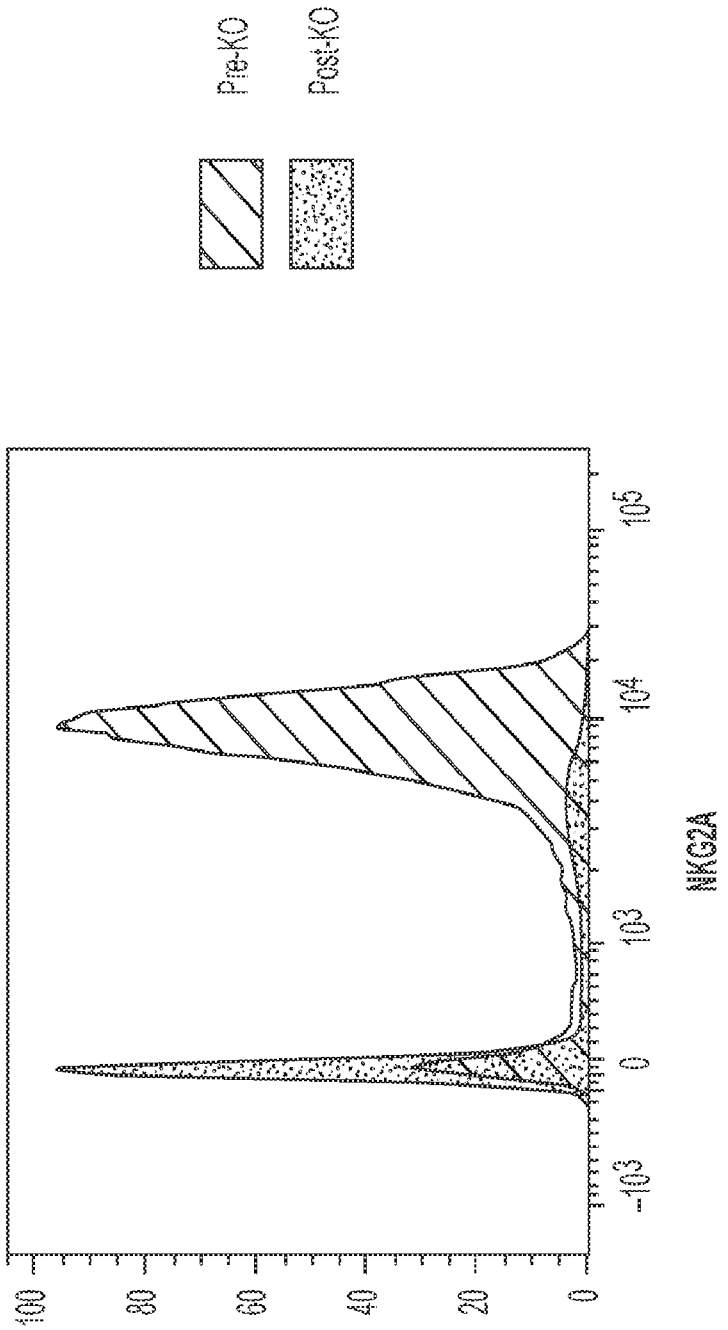


FIG. 12A

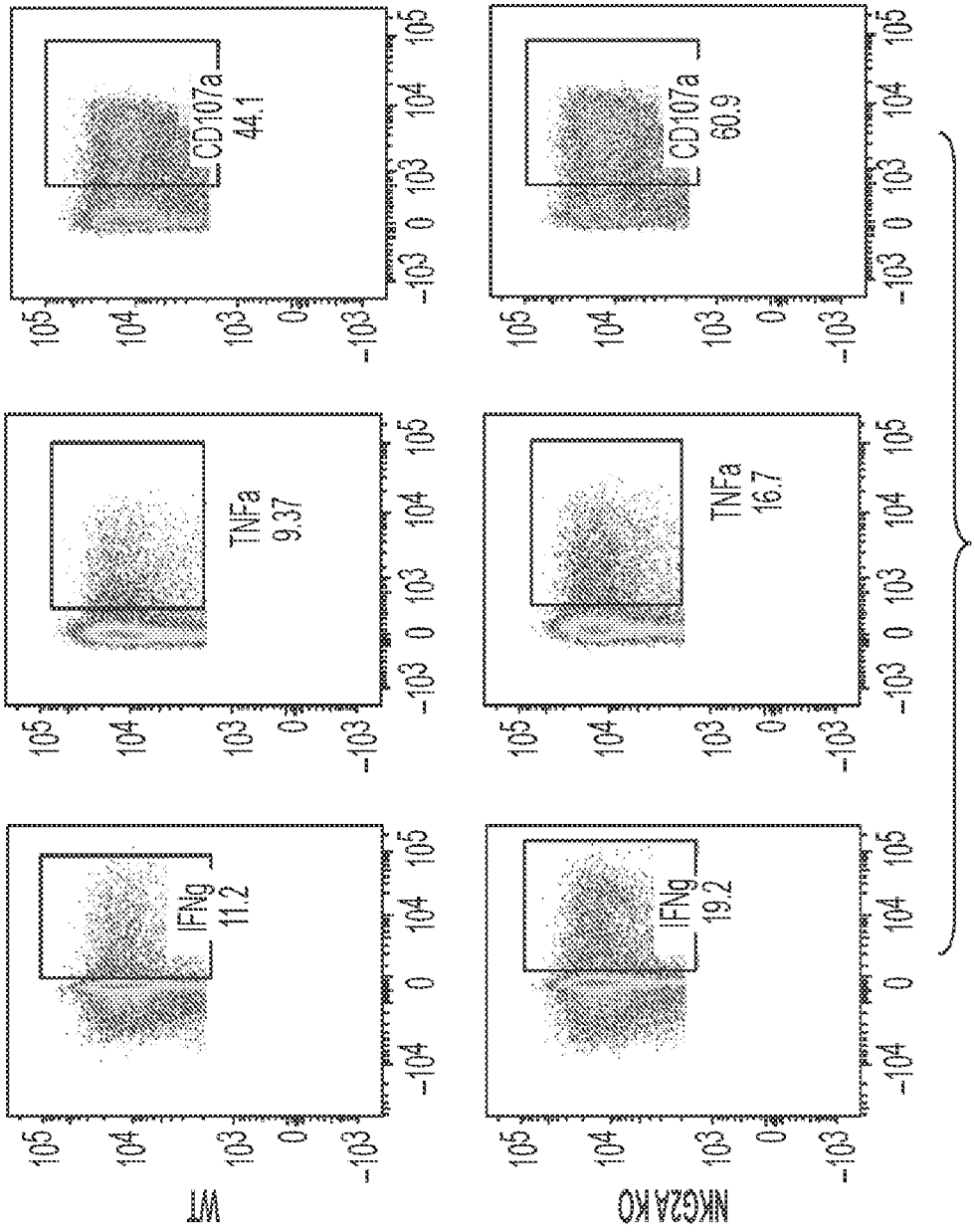

WT
NKG2AKO
IFNg
11.2
TNFa
9.37
CD107a
44.1
IFNg
19.2
TNFa
16.7
CD107a
60.9
Donor 1

FIG. 12B

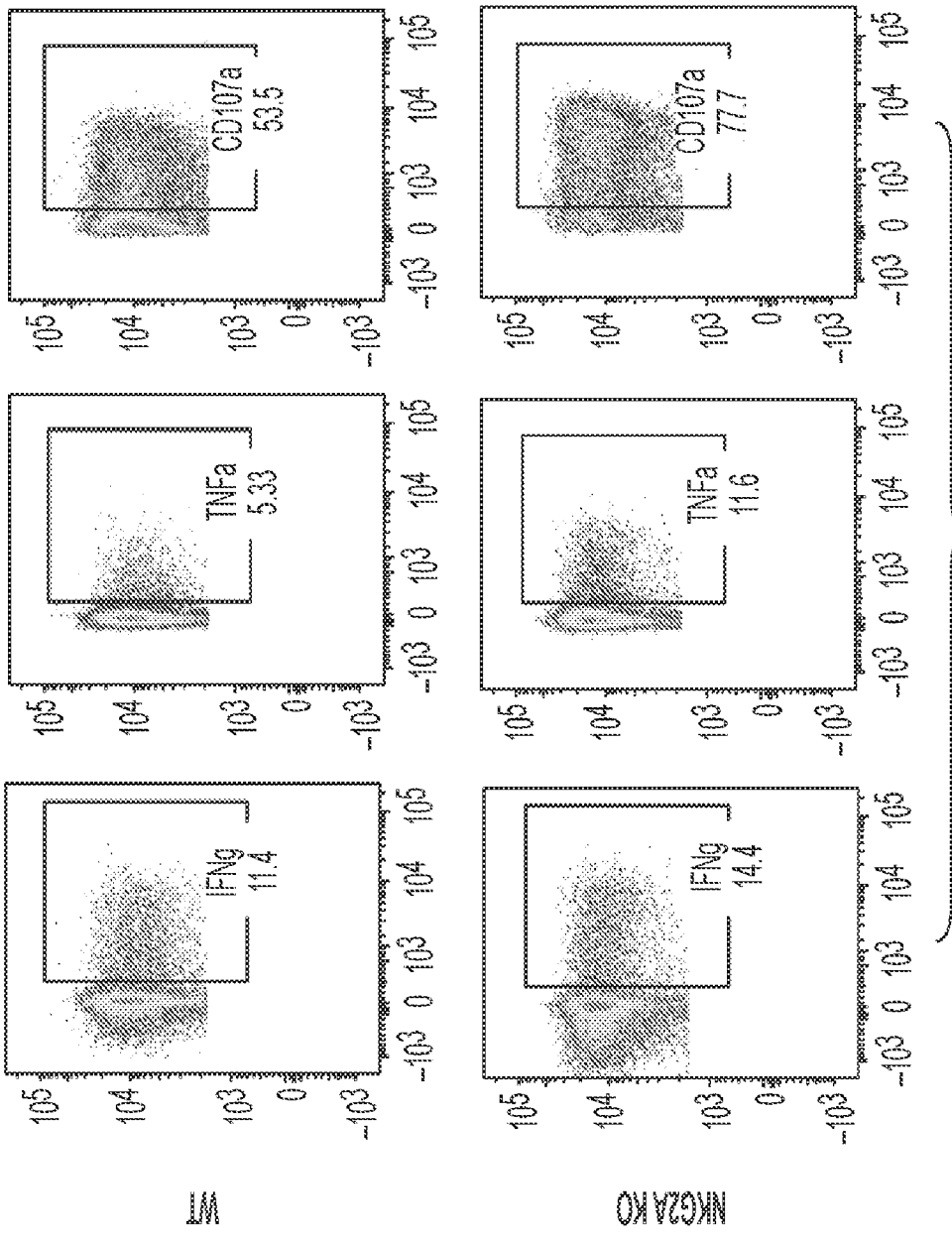


FIG. 12B
CONTINUED

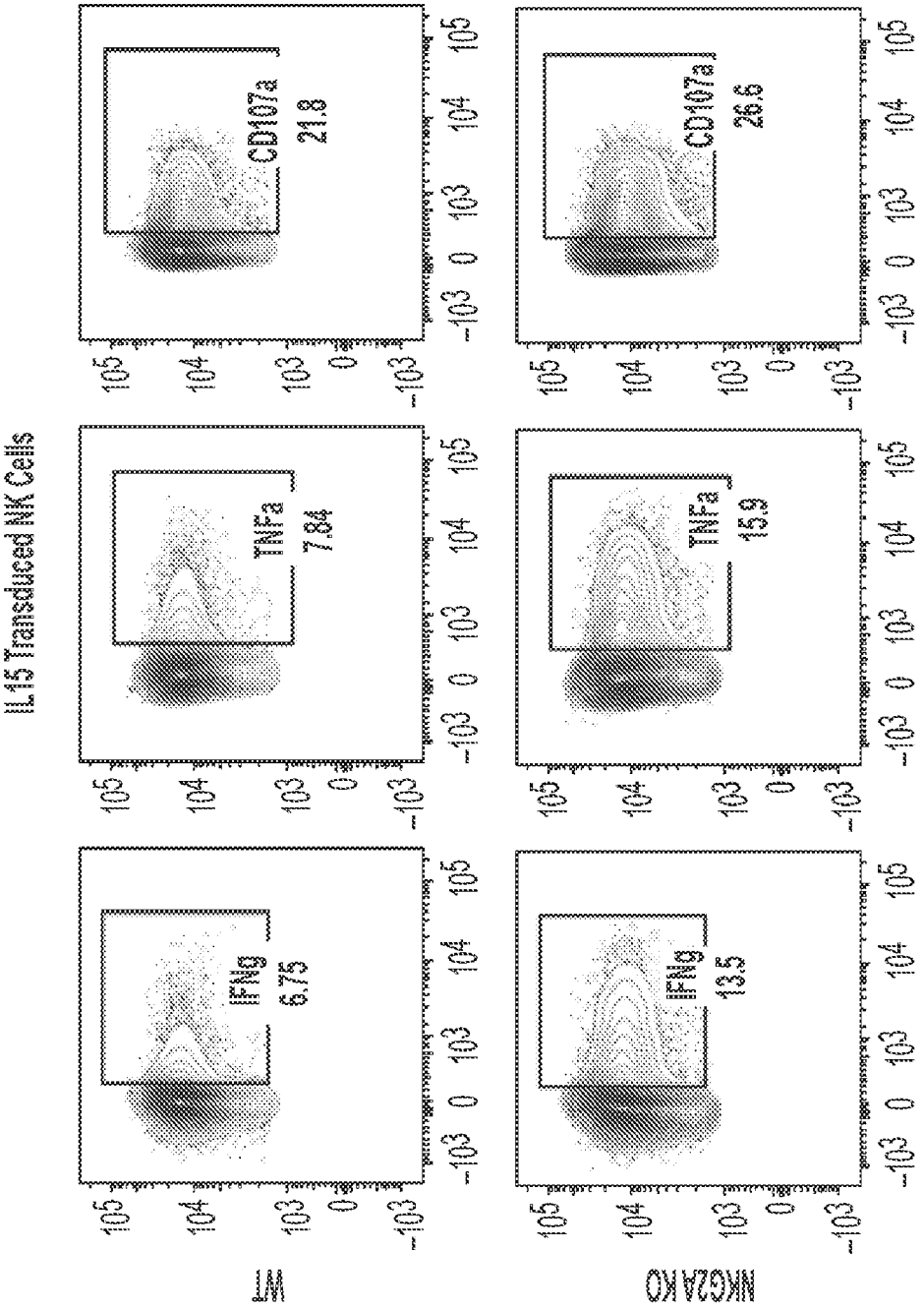


FIG. 12C
CONTINUED

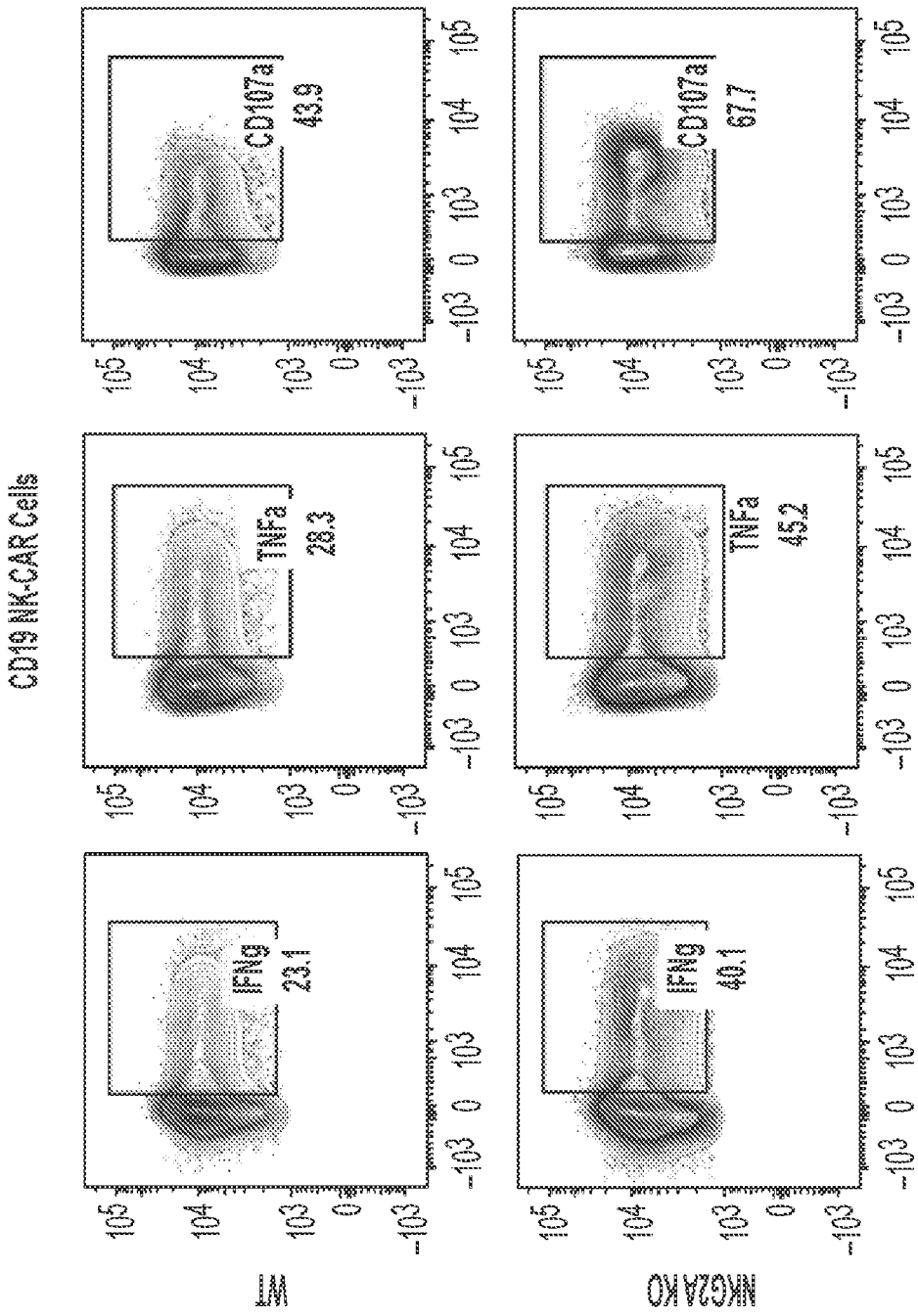


FIG. 12C
CONTINUED

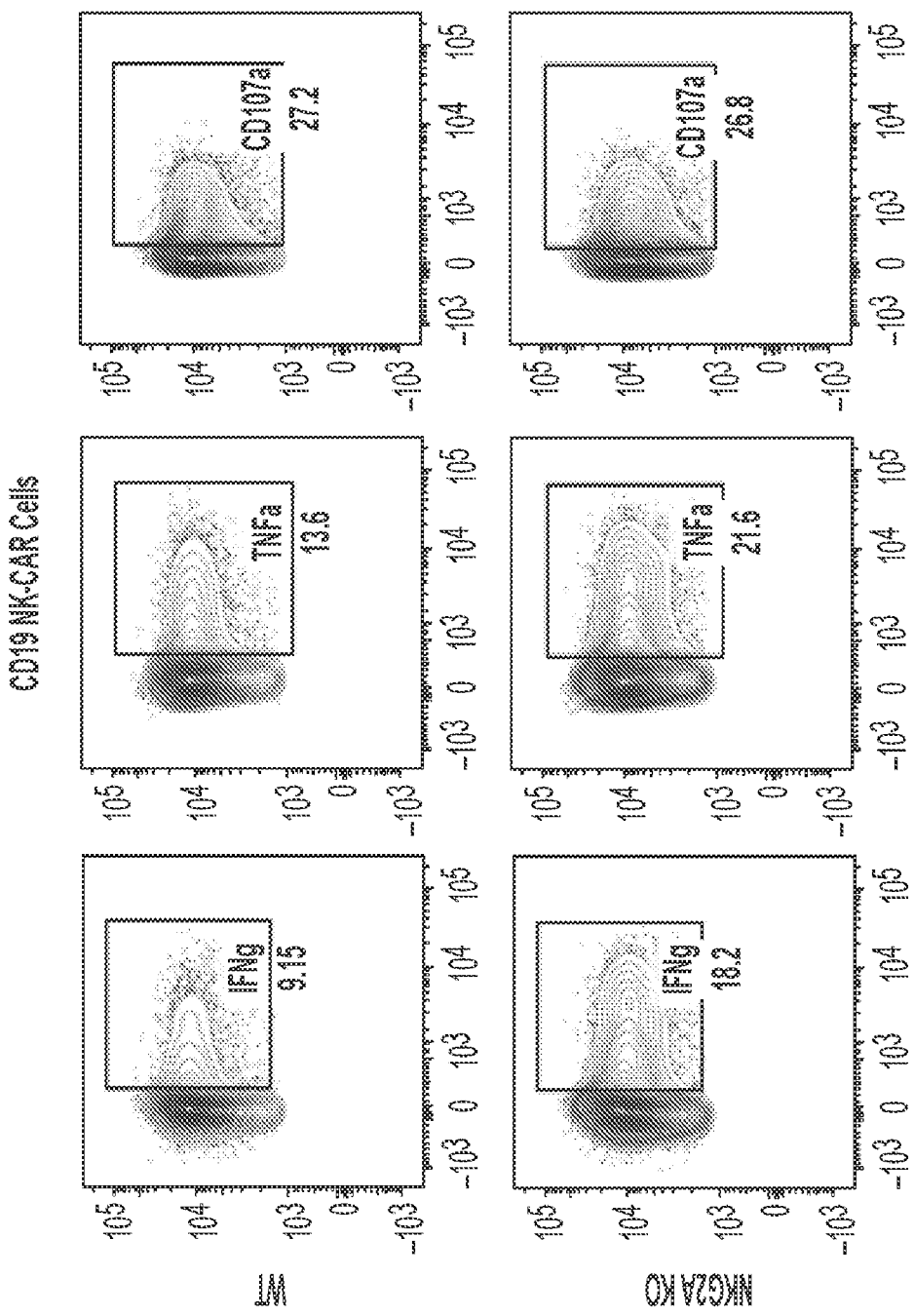


FIG. 12C
CONTINUED

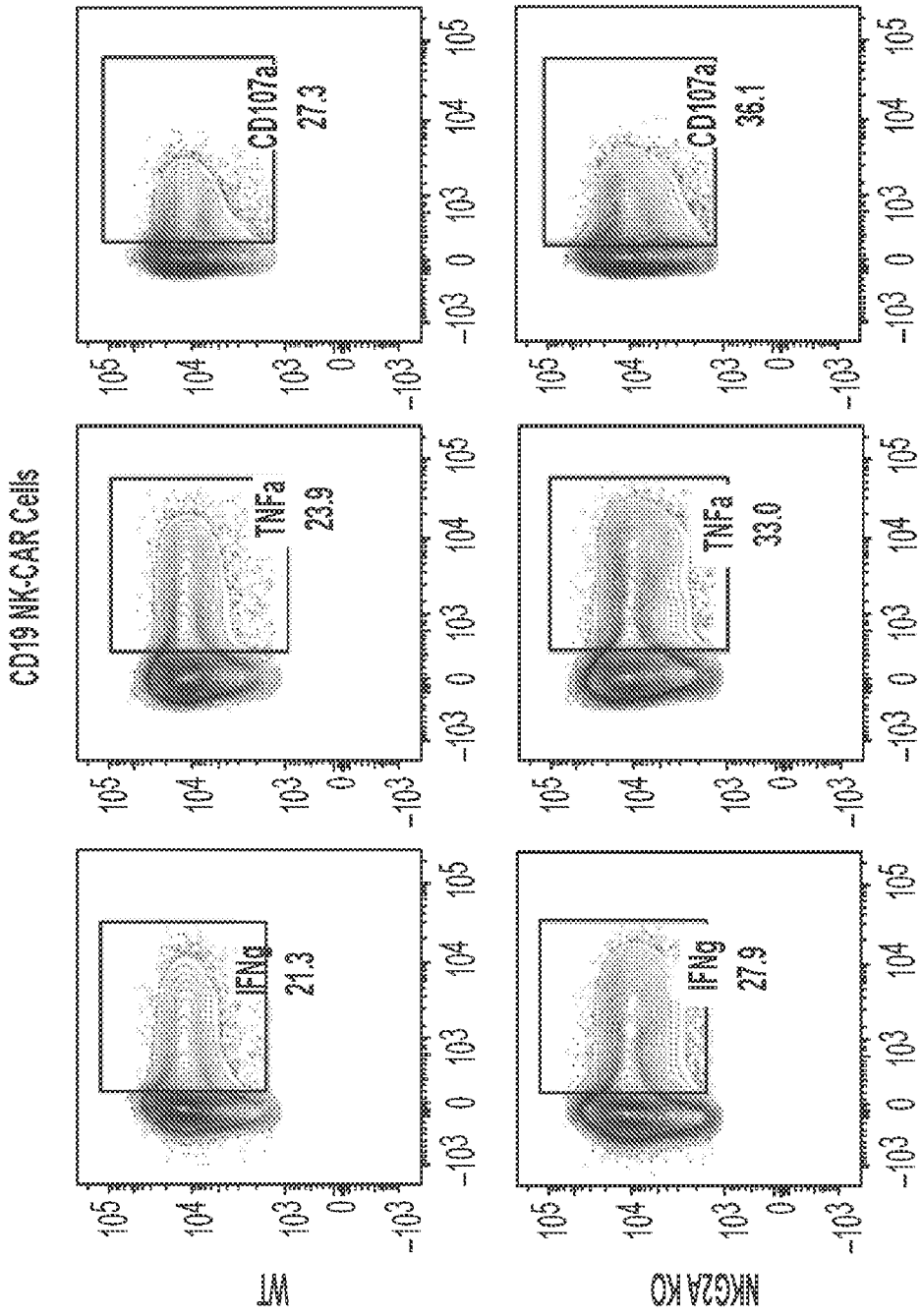
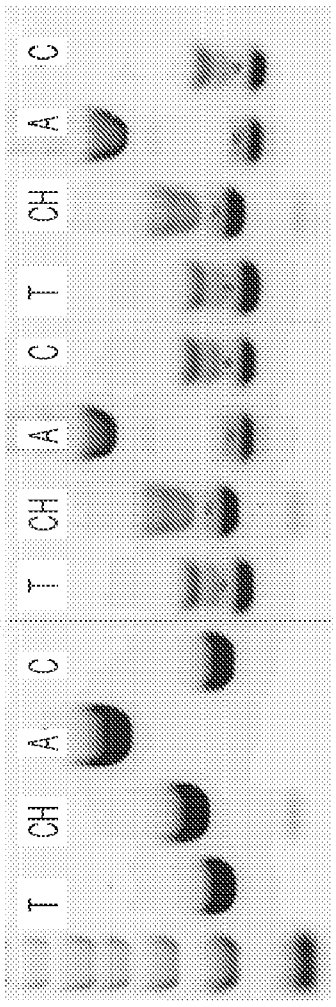
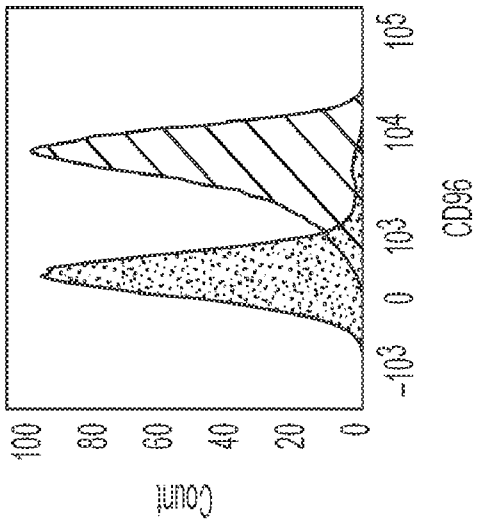
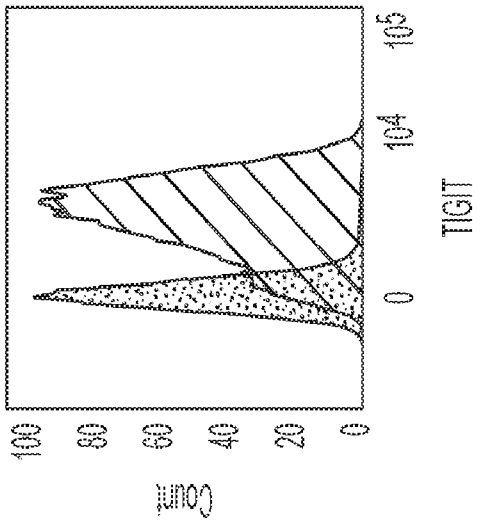


FIG. 12C
CONTINUED


T
CH
A
C
T
CH
A
C
T
CH
A
C


Count
100
80
60
40
20
0
-10^3
0
10^3
10^4
10^5
CD96


Count
100
80
60
40
20
0
0
10^4
10^5
TIGIT

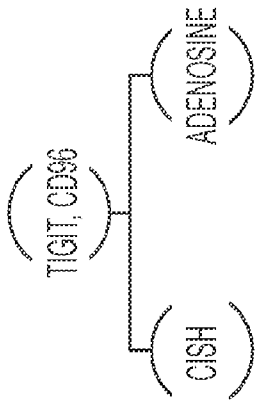

TIGIT, CD96
CISH
ADENOSINE

FIG. 13

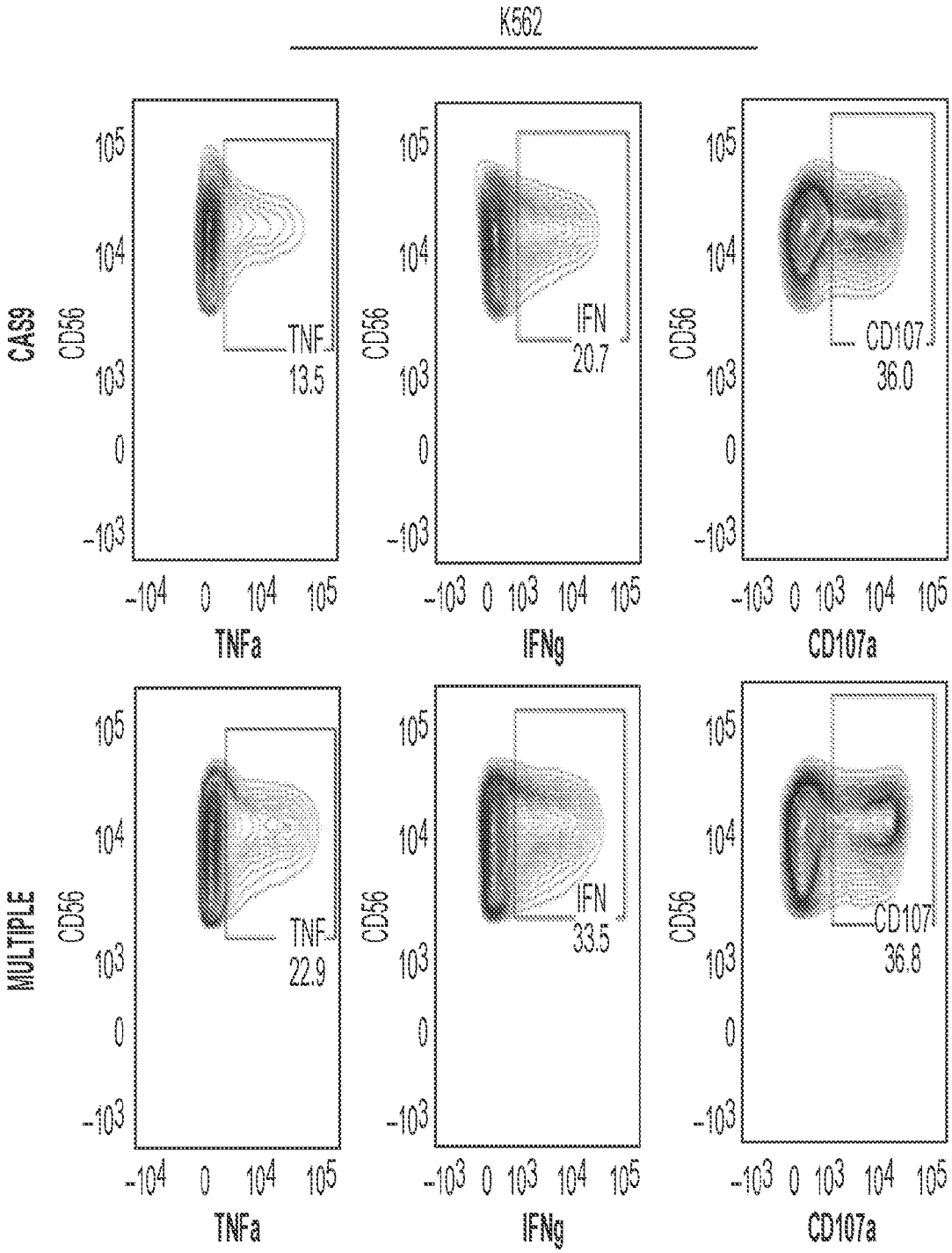

K562
CAS9
MULTIPLE
CD56
TNFa
IFNg
CD107a
TNF
13.5
IFN
20.7
CD107
36.0
TNF
22.9
IFN
33.5
CD107
36.8

FIG. 14

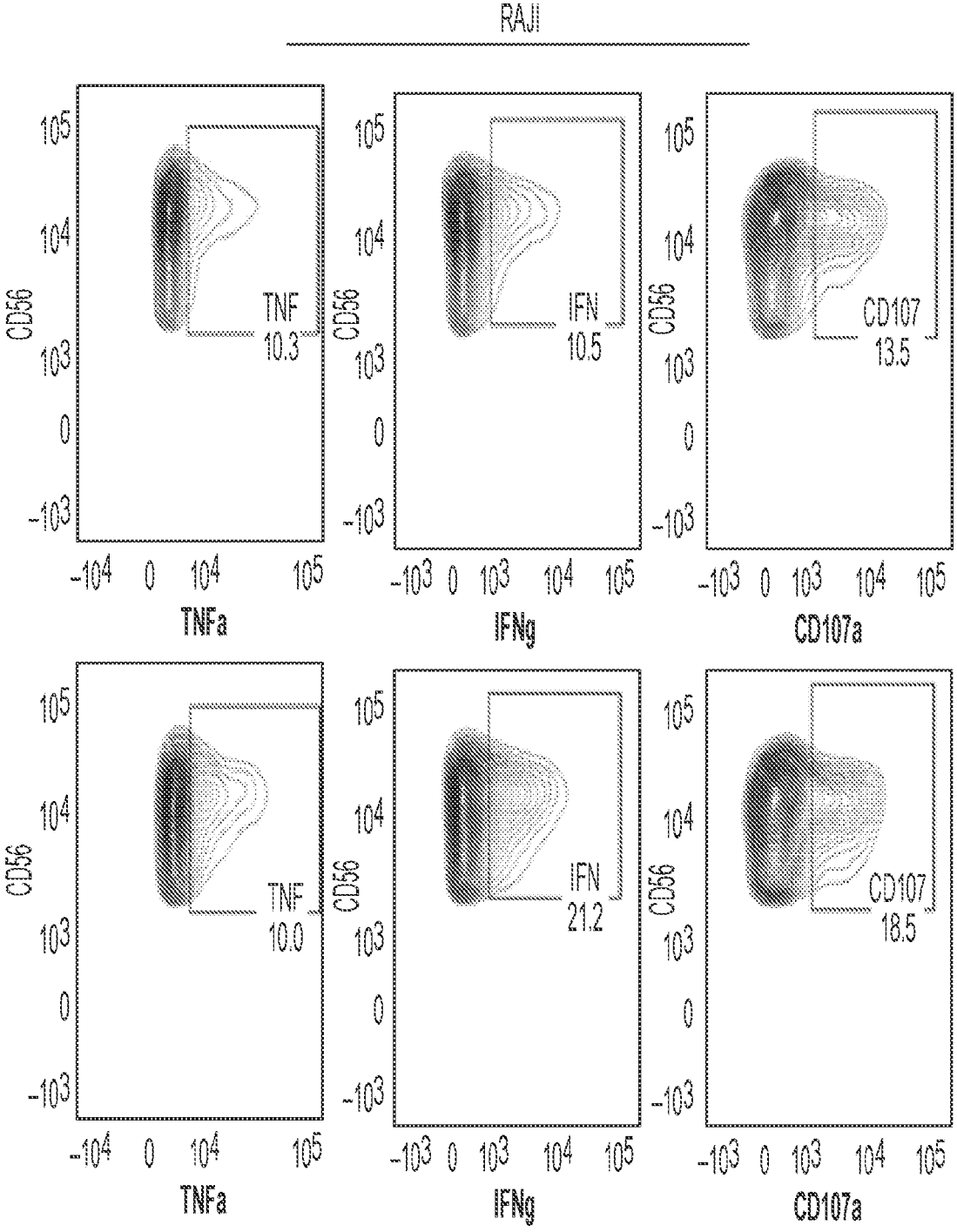


FIG. 14
CONTINUED

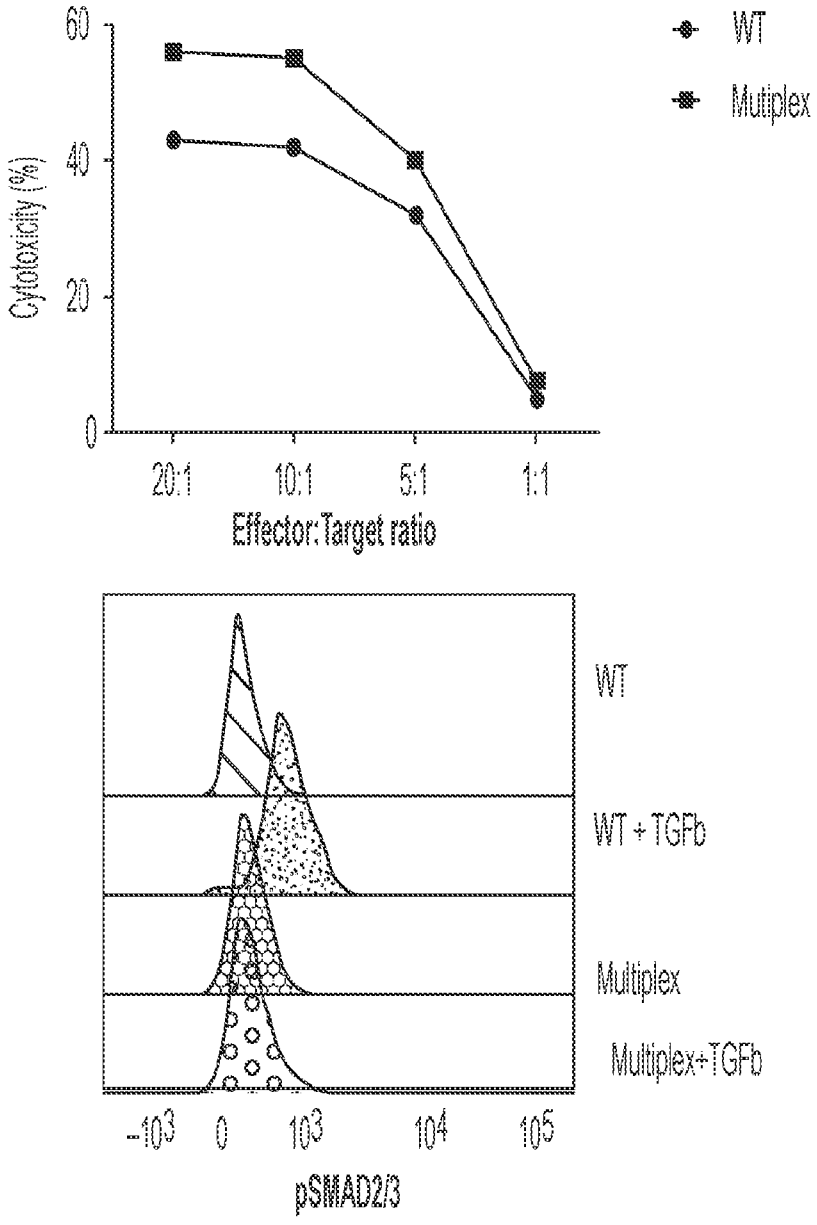


FIG. 14
CONTINUED

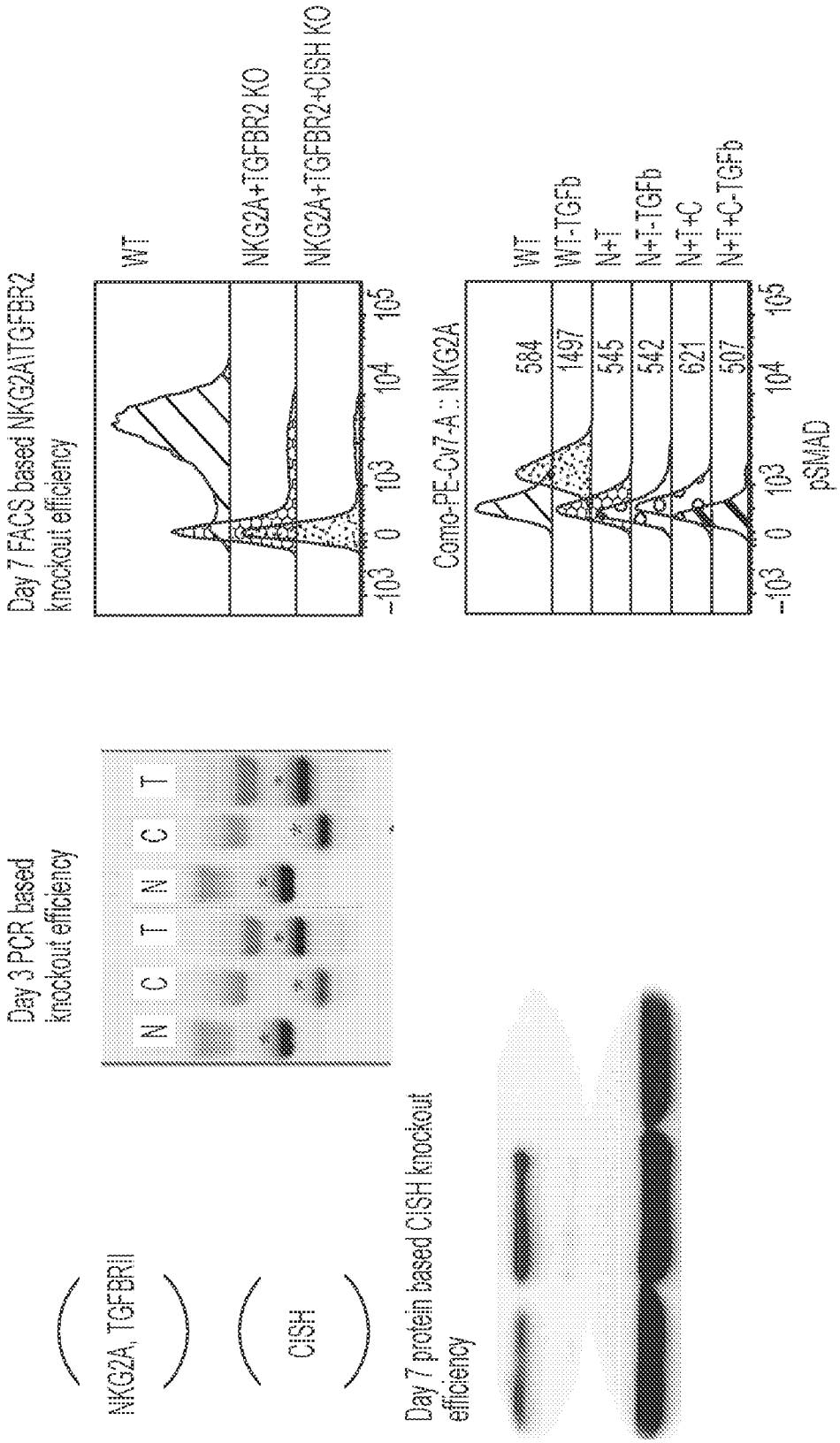

Day 7 FACS based NKG2A/TGFBR2 knockout efficiency
WT
NKG2A+TGFBR2 KO
NKG2A+TGFBR2+CISH KO
-10^3 0 10^3 10^4 10^5
Como-PE-Cy7-A :: NKG2A
584
1497
545
542
621
507
WT
WT-TGFb
N+T
N+T-TGFb
N+T+C
N+T+C-TGFb
pSMAD
Day 3 PCR based knockout efficiency
N C T N C T
NKG2A, TGFBRII
CISH
Day 7 protein based CISH knockout efficiency

FIG. 15

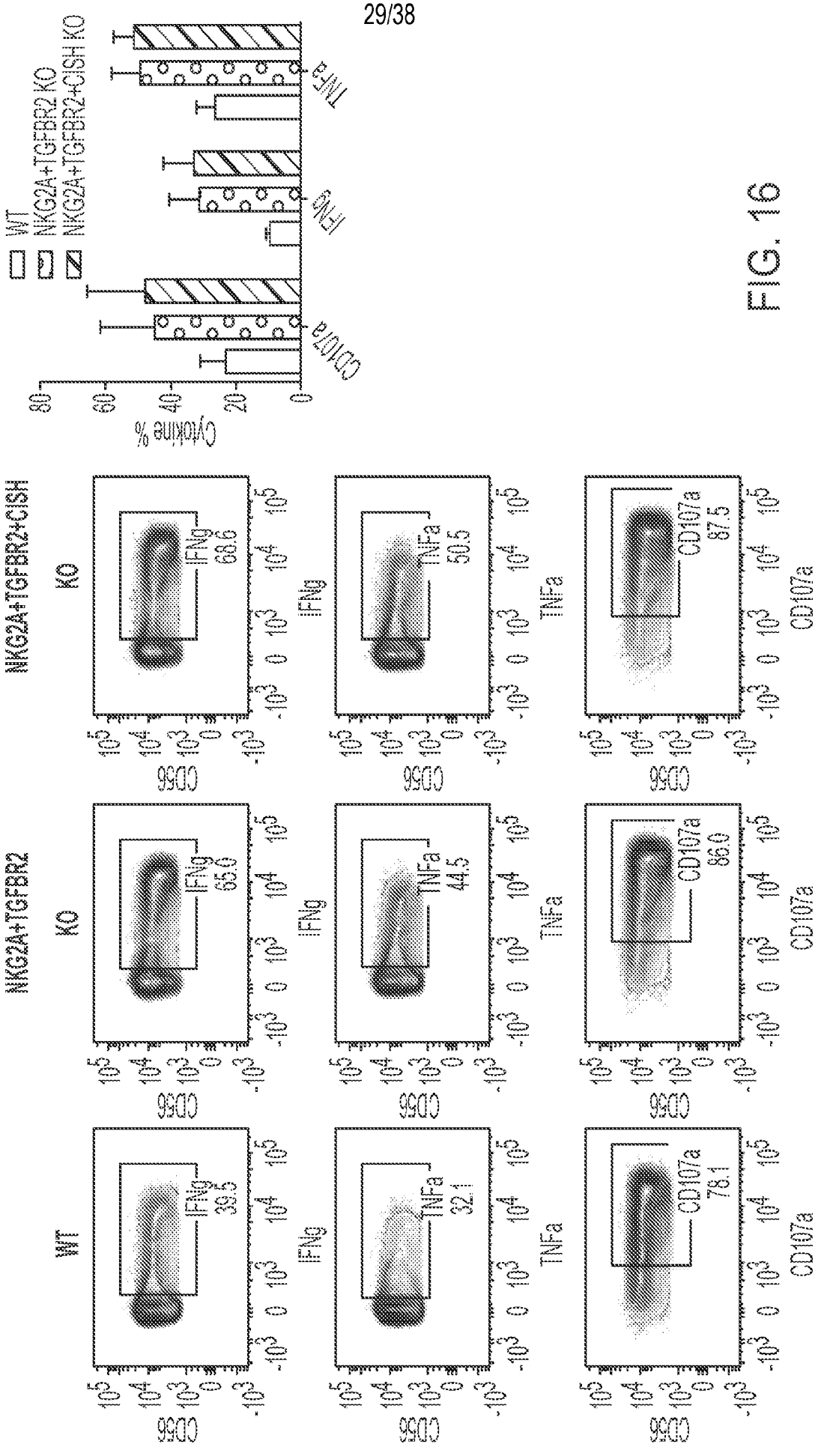

WT
NKG2A+TGFBR2 KO
NKG2A+TGFBR2+CISH KO
Cytokine %
0
20
40
60
80
CD107a
IFNg
TNFa
NKG2A+TGFBR2+CISH
KO
NKG2A+TGFBR2
KO
WT
CD56
IFNg
TNFa
CD107a
39.5
32.1
78.1
65.6
44.5
86.0
68.6
50.5
87.5

FIG. 16

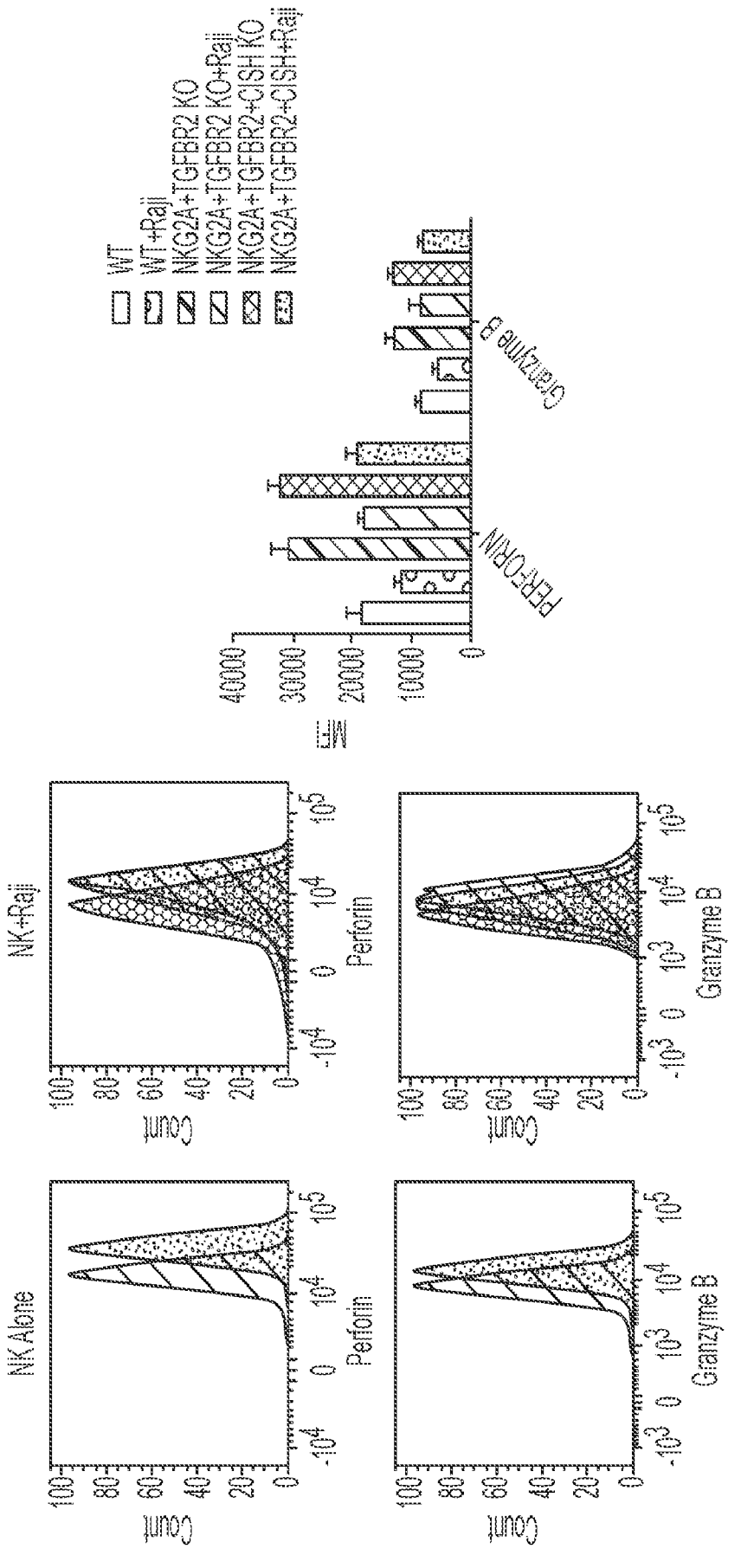


FIG. 16
CONTINUED

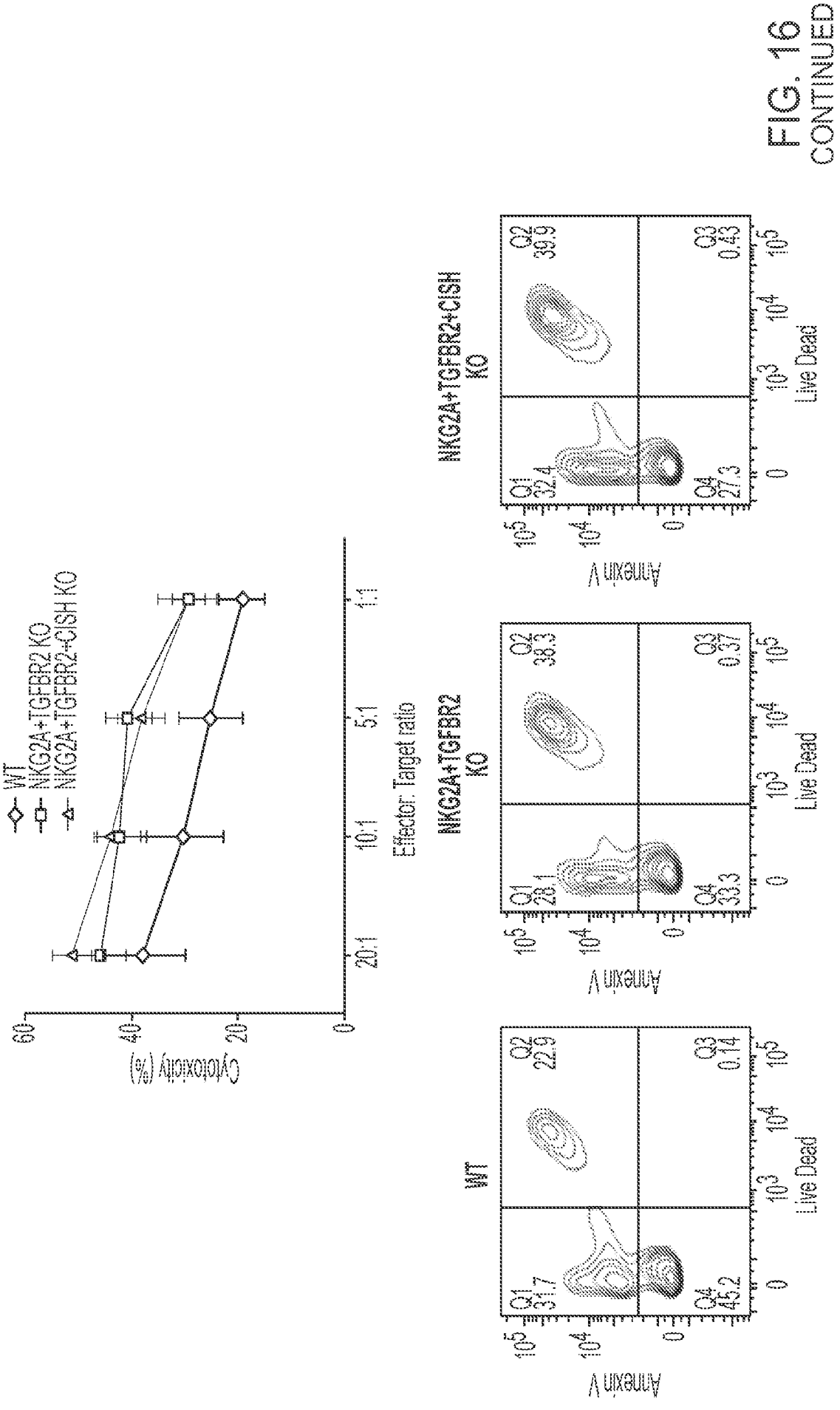


FIG. 16
CONTINUED

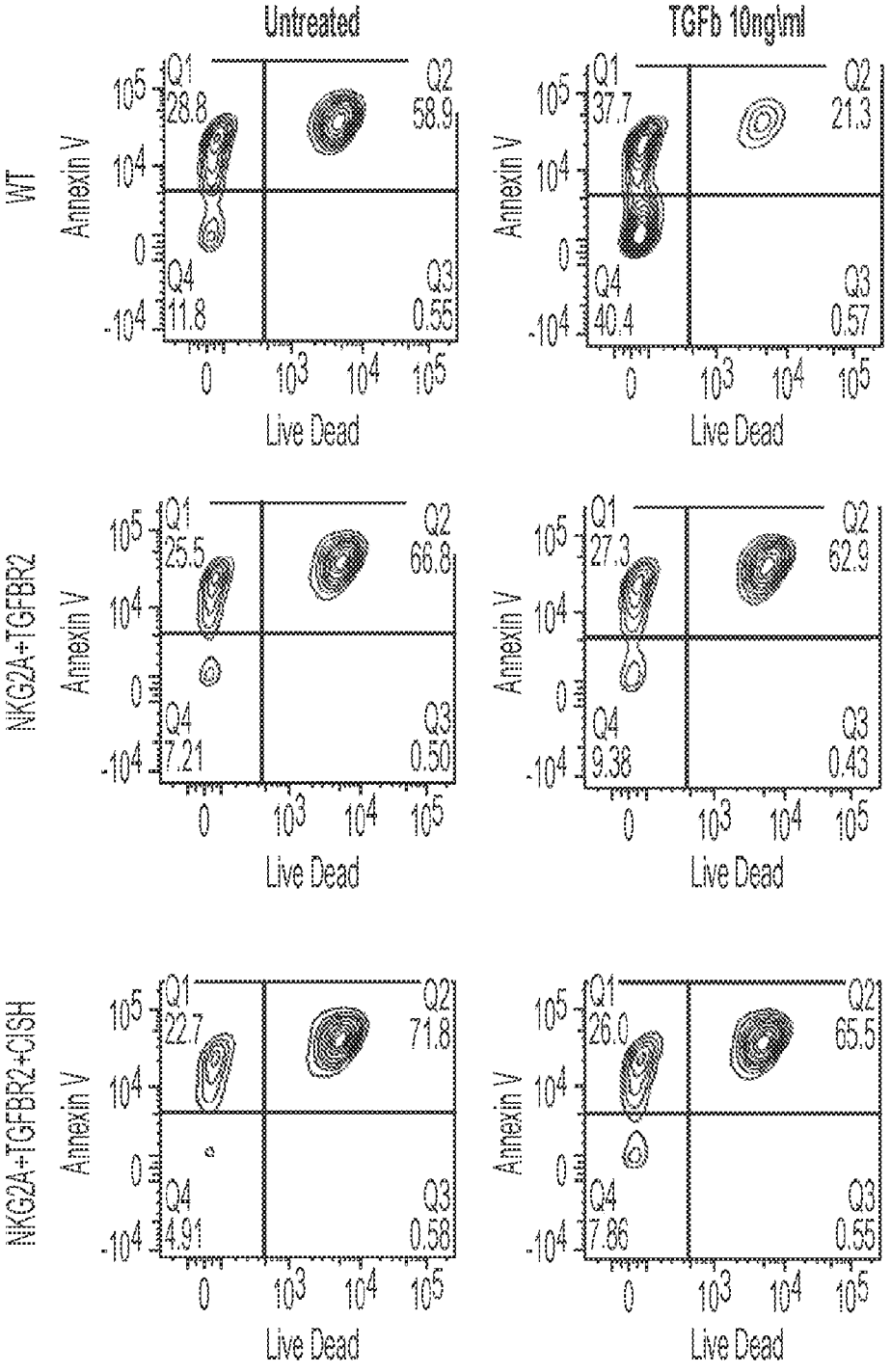
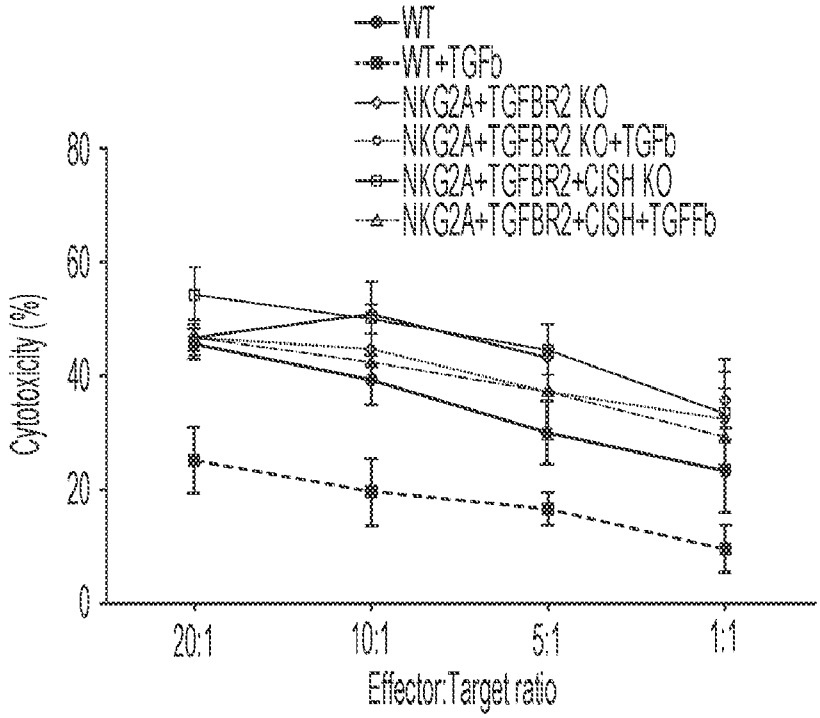

Untreated
TGFb 10ng/ml
WT
NKG2A+TGFBR2
NKG2A+TGFBR2+CISH
Annexin V
Live Dead
Q1
Q2
Q3
Q4
28.8
58.9
11.8
0.55
37.7
21.3
40.4
0.57
25.5
66.8
7.21
0.50
27.3
62.9
9.38
0.43
22.7
71.8
4.91
0.58
26.0
65.5
7.86
0.55

FIG. 17



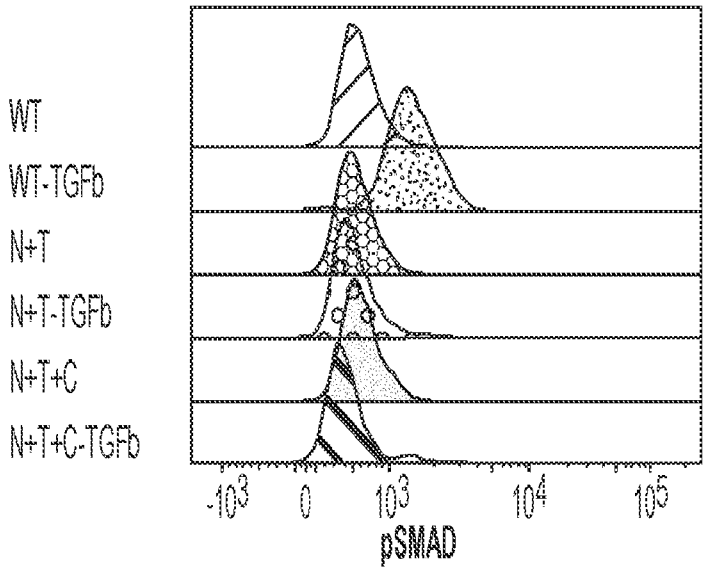


FIG. 17
CONTINUED

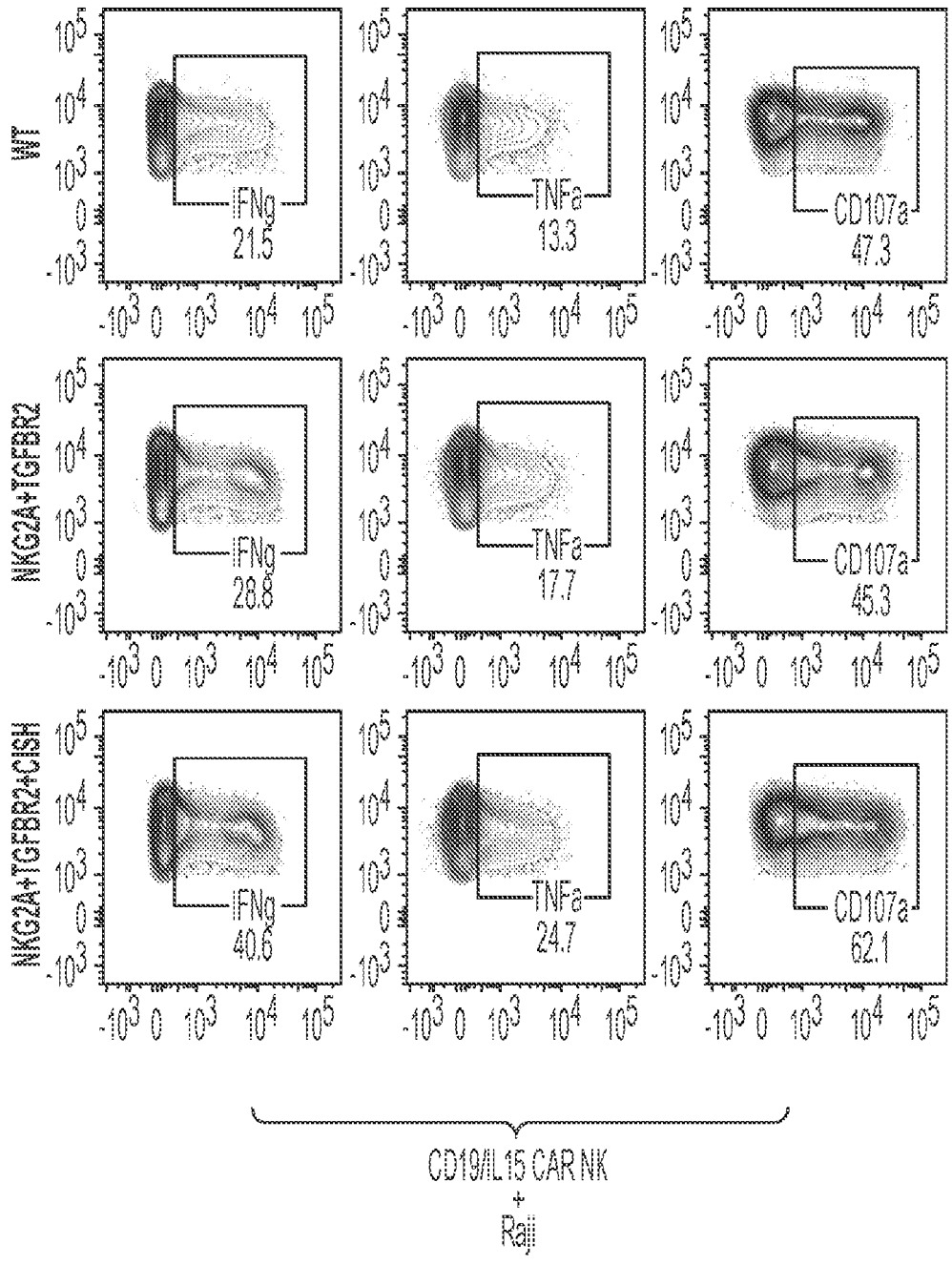

WT
NKG2A+TGFBR2
NKG2A+TGFBR2+CISH
IFNg
21.5
TNFa
13.3
CD107a
47.3
IFNg
28.8
TNFa
17.7
CD107a
45.3
IFNg
40.6
TNFa
24.7
CD107a
62.1
CD19/IL15 CAR NK
+
Raji

FIG. 18

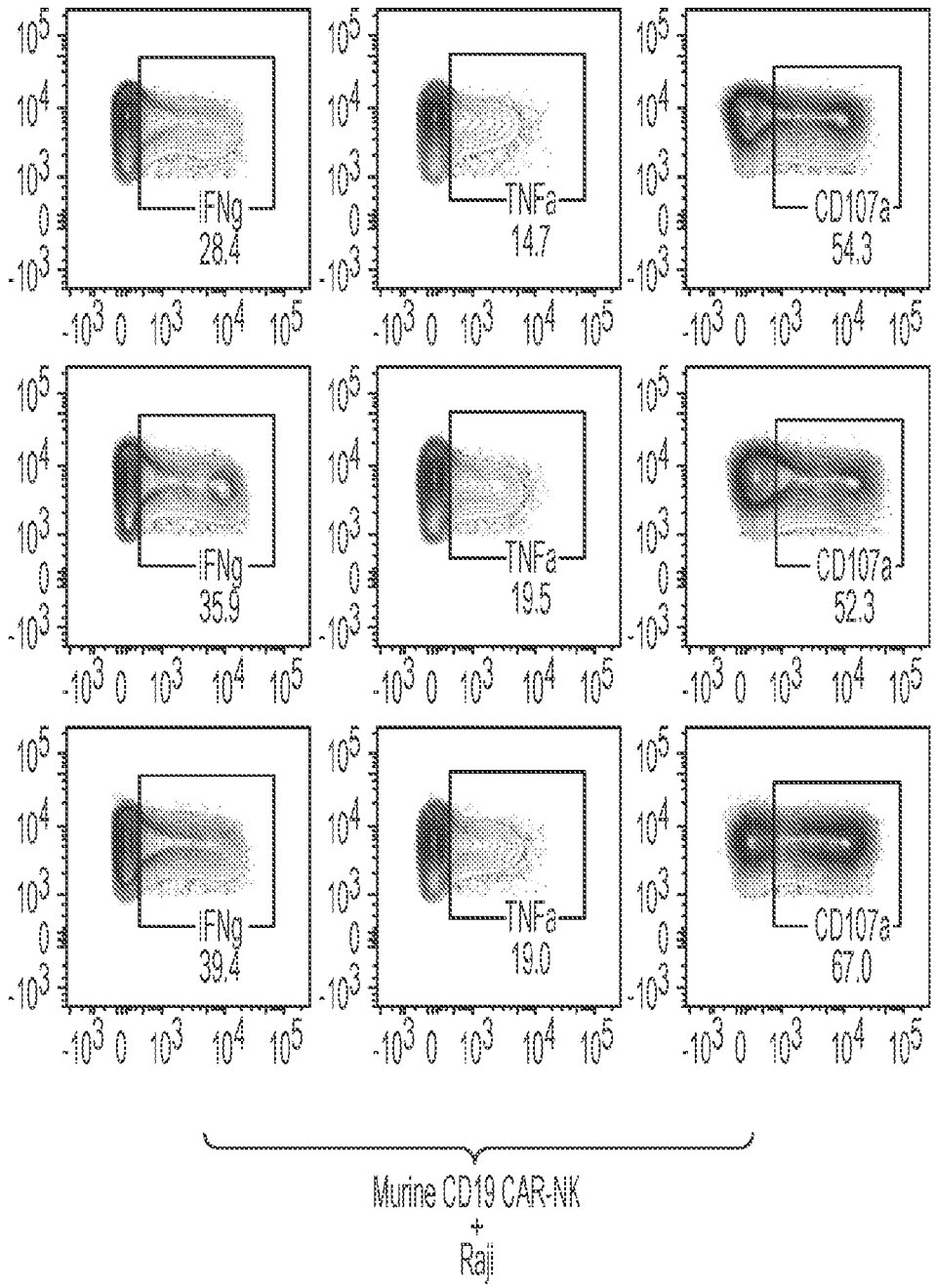


FIG. 18
CONTINUED

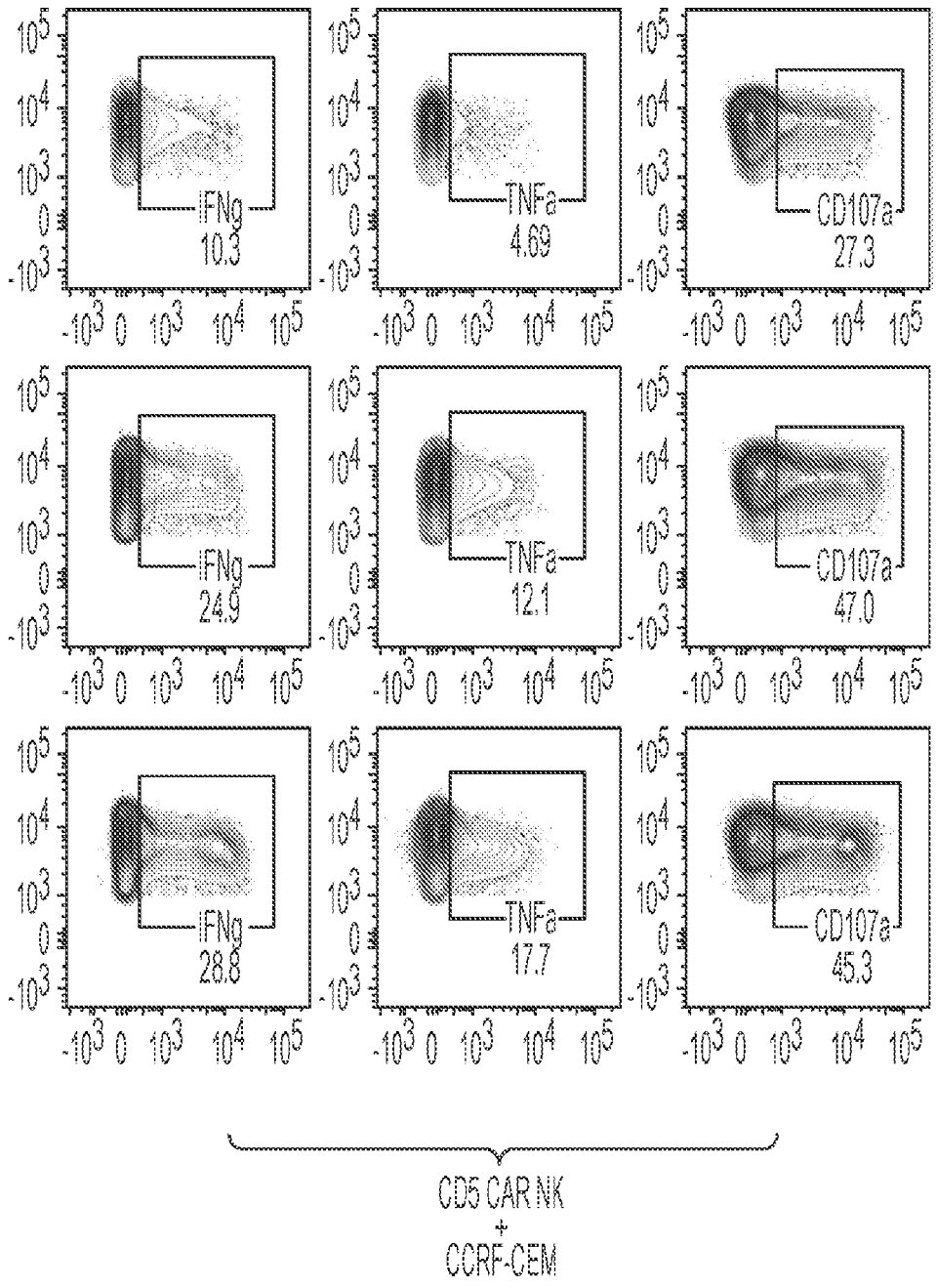


FIG. 18
CONTINUED

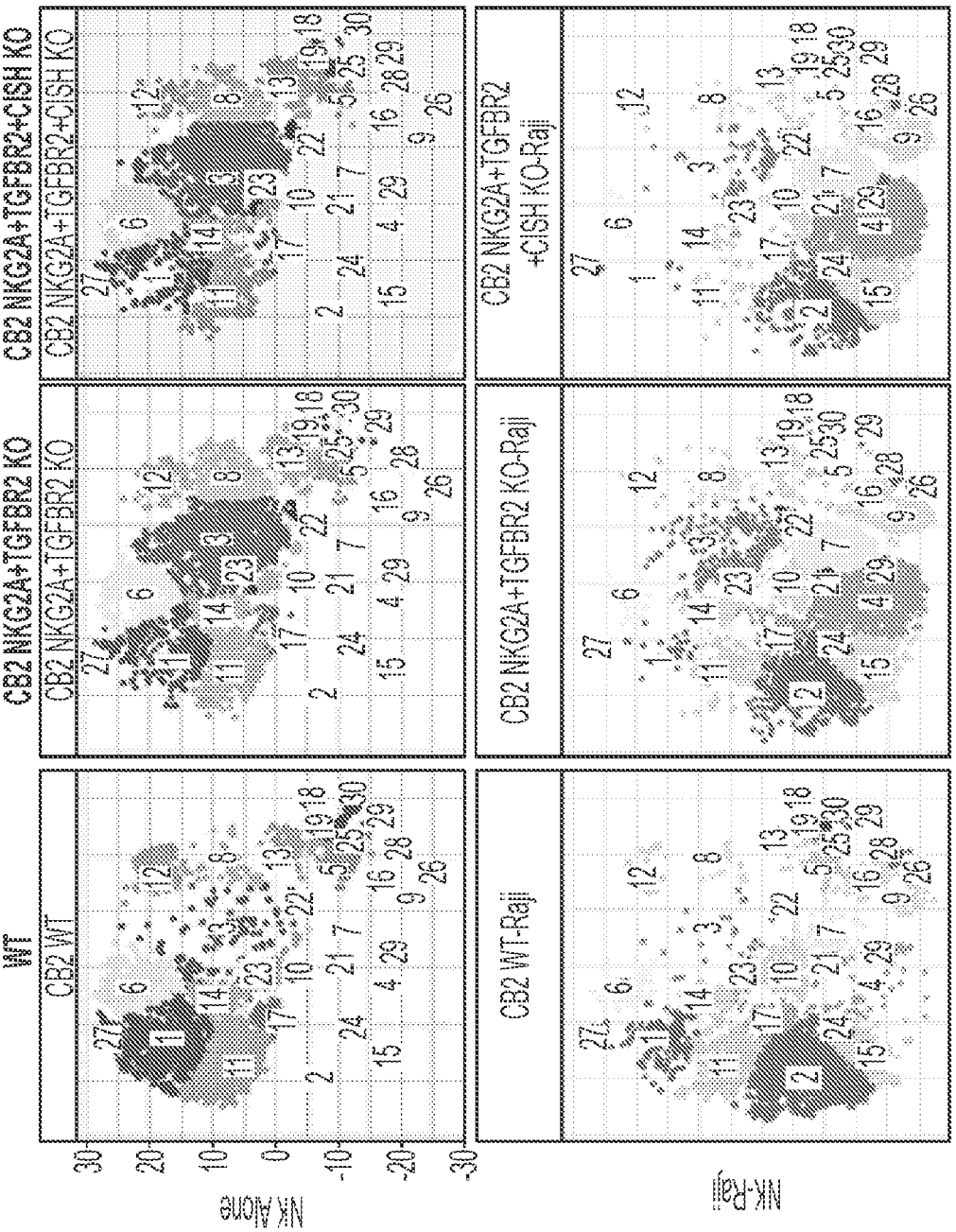

WT
CB2 NKG2A+TGFBR2 KO
CB2 NKG2A+TGFBR2+CISH KO
CB2 WT
CB2 NKG2A+TGFBR2 KO
CB2 NKG2A+TGFBR2+CISH KO
CB2 WT-Raji
CB2 NKG2A+TGFBR2 KO-Raji
CB2 NKG2A+TGFBR2 +CISH KO-Raji
NK Alone
NK-Raji
30
20
10
0
-10
-20
-30

FIG. 19

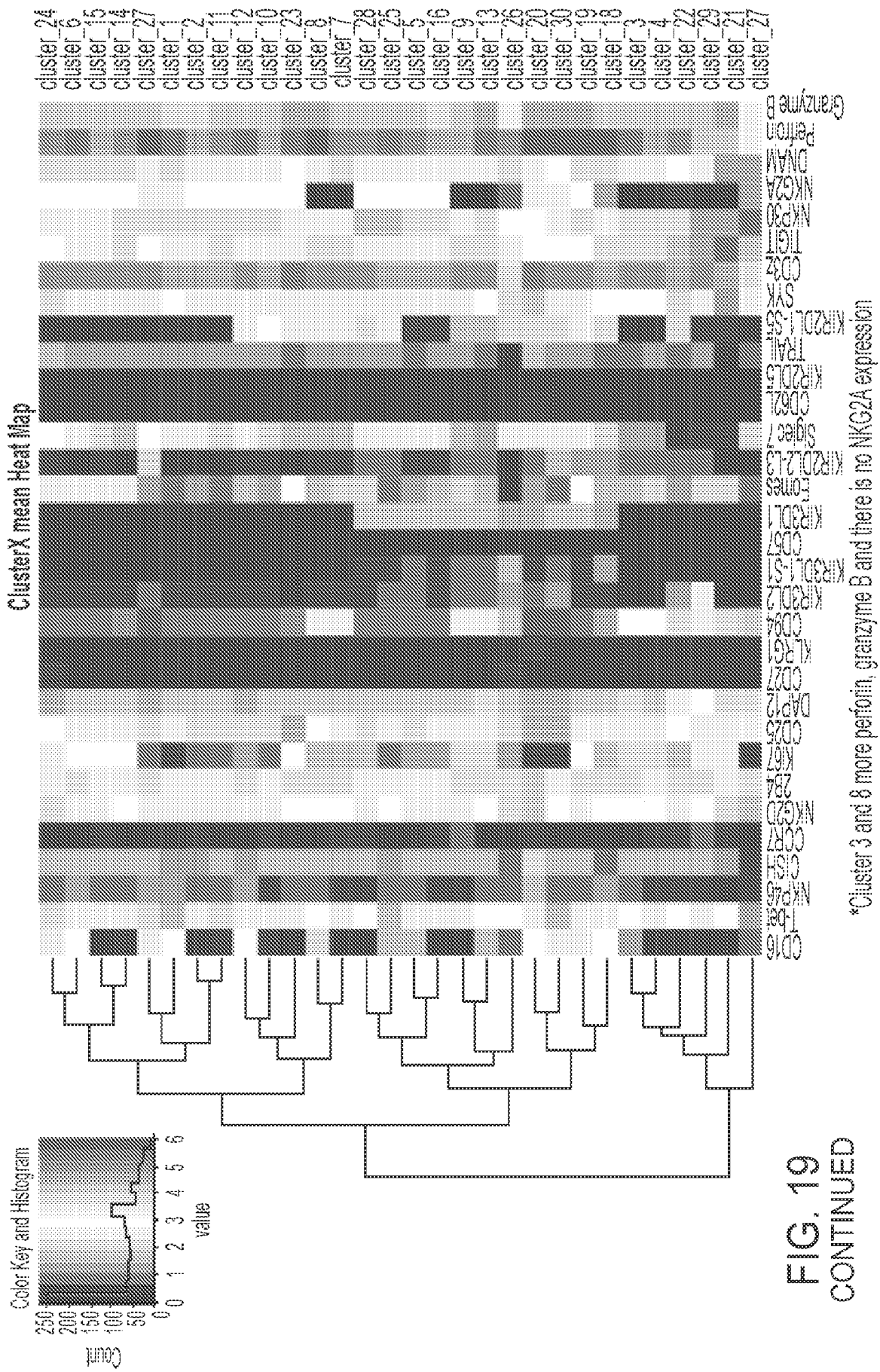


FIG. 19
CONTINUED