

FIG. 1A

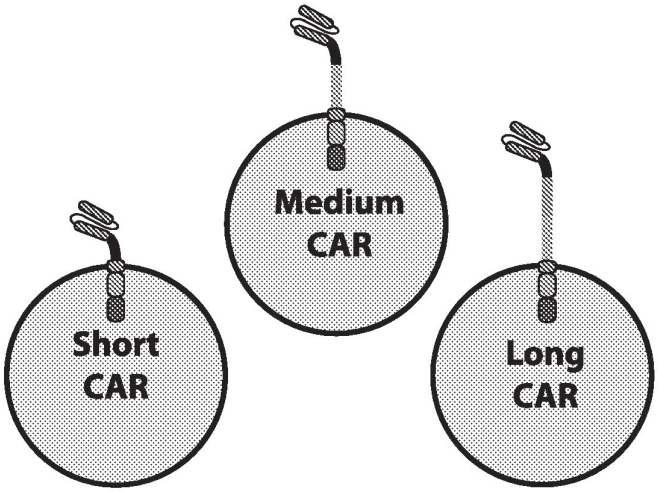


FIG. 1B

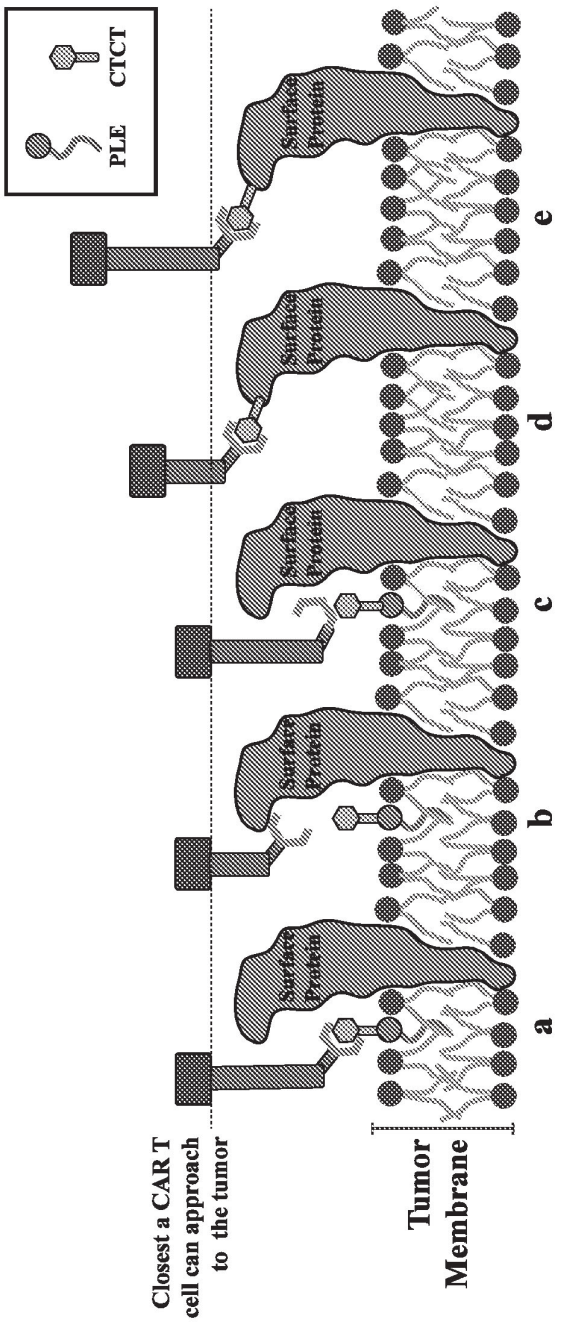

PLE
CTCT
Surface Protein
Surface Protein
Surface Protein
Surface Protein
Surface Protein
a
b
c
d
e
Tumor Membrane
Closest a CAR T cell can approach to the tumor

FIG. 2

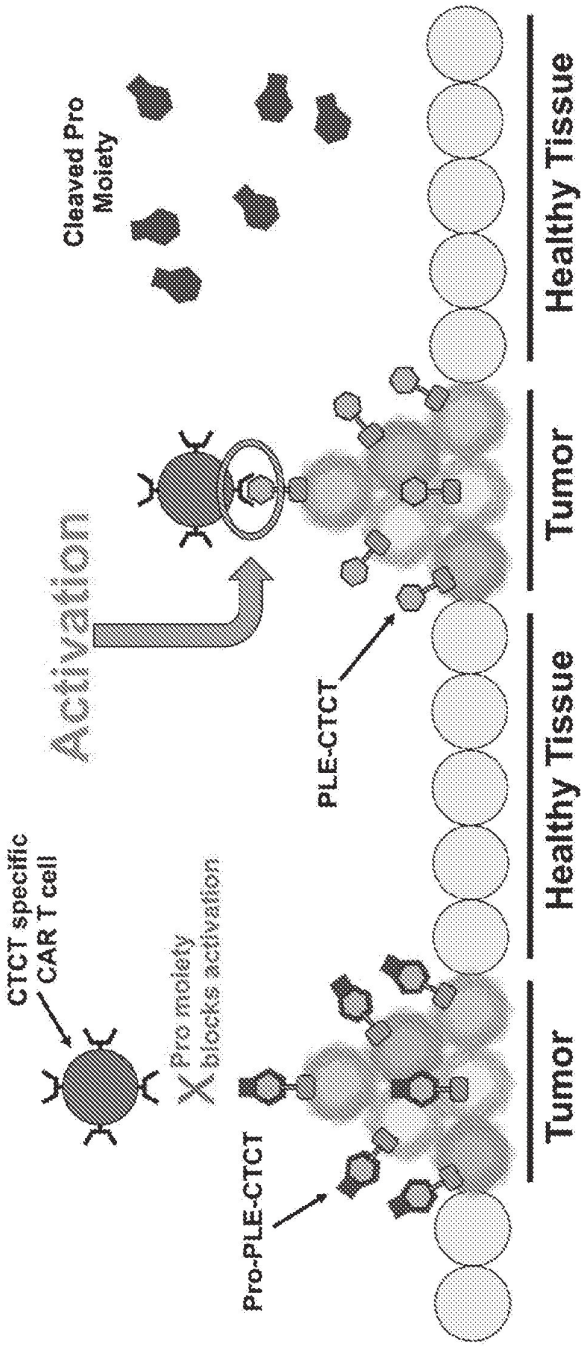

CTCT specific
CAR T cell
Pro moiety
blocks activation
Activation
Cleaved Pro
Moiety
Pro-PLE-CTCT
PLE-CTCT
Tumor
Healthy Tissue
Tumor
Healthy Tissue

FIG. 3

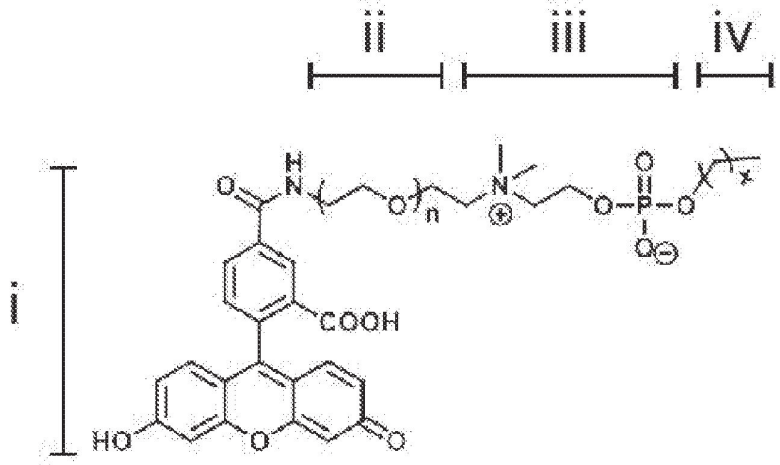


FIG. 4A

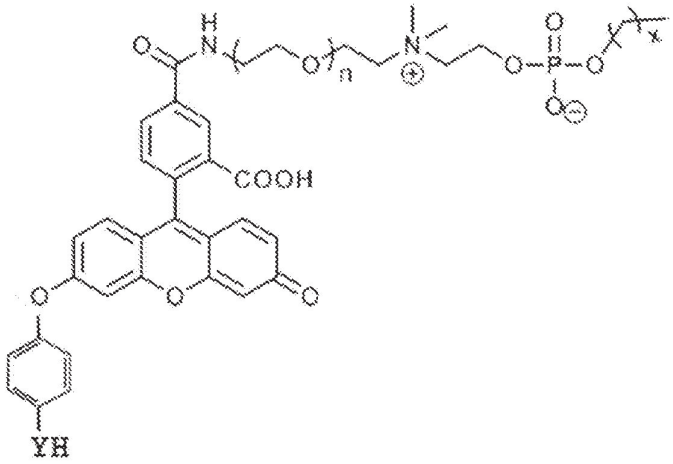


FIG. 4B

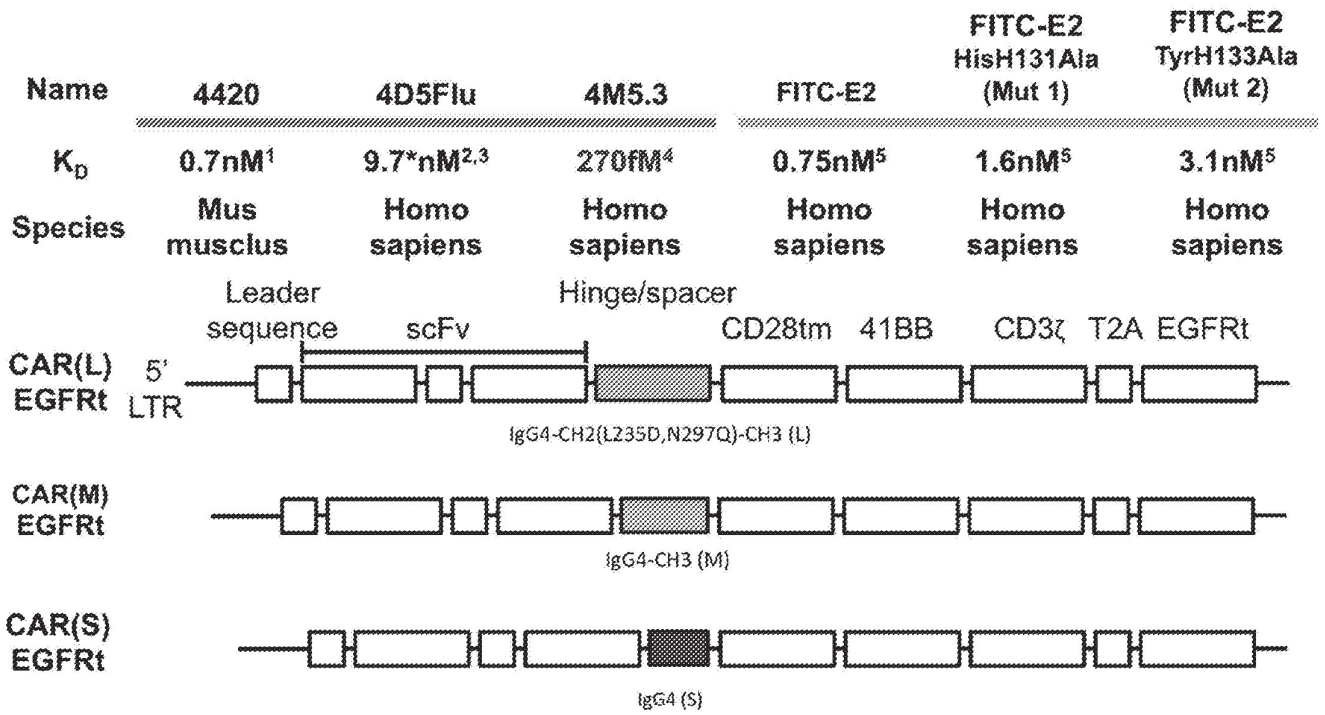

Name
4420
4D5Flu
4M5.3
FITC-E2
FITC-E2 HisH131Ala (Mut 1)
FITC-E2 TyrH133Ala (Mut 2)
K_D
0.7nM[1]
9.7*nM[2,3]
270fM[4]
0.75nM[5]
1.6nM[5]
3.1nM[5]
Species
Mus musclus
Homo sapiens
Homo sapiens
Homo sapiens
Homo sapiens
Homo sapiens
Leader sequence
scFv
Hinge/spacer
CD28tm
41BB
CD3ζ
T2A
EGFRt
CAR(L) EGFRt
5' LTR
IgG4-CH2(L235D,N297Q)-CH3 (L)
CAR(M) EGFRt
IgG4-CH3 (M)
CAR(S) EGFRt
IgG4 (S)

FIG. 5

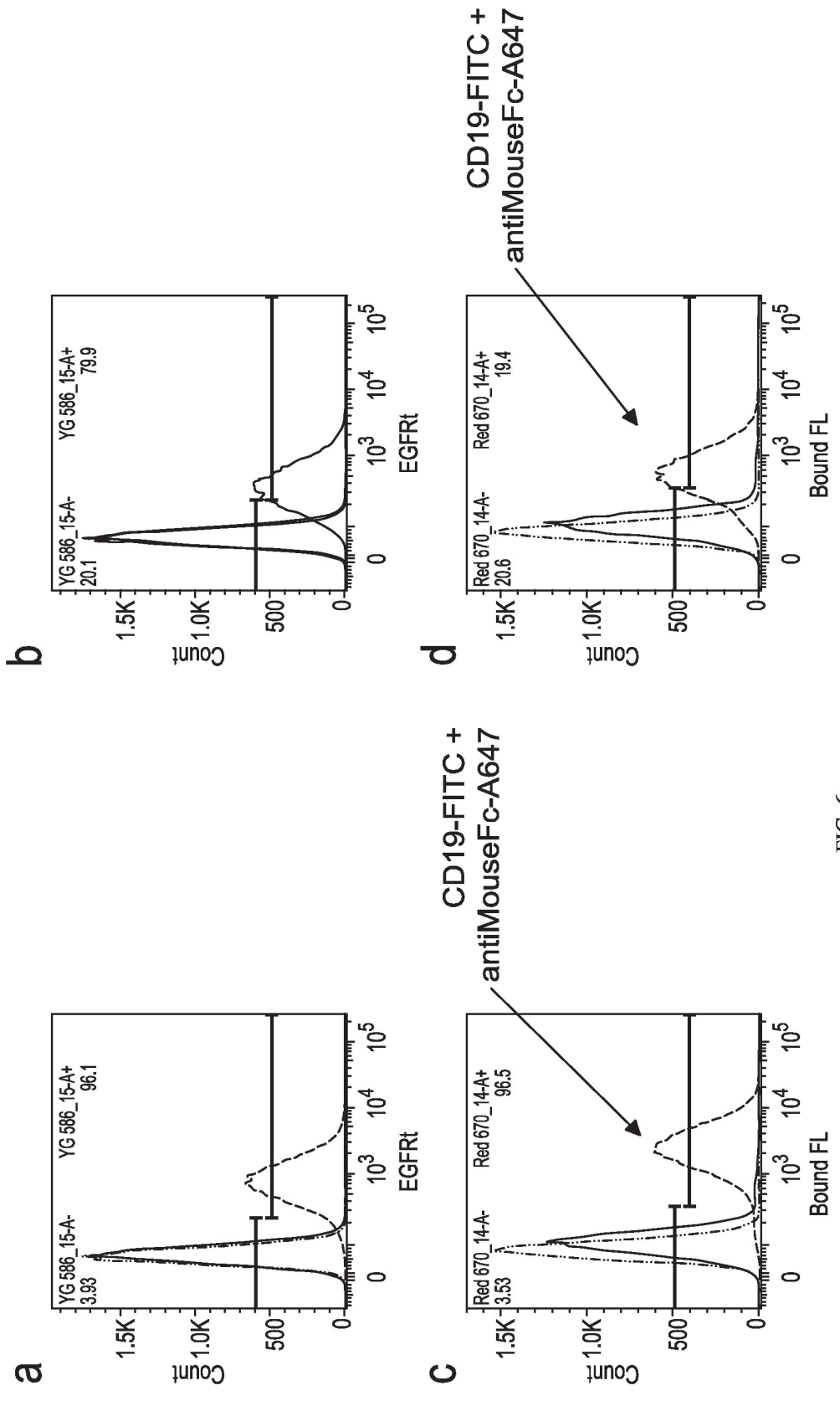

a
YG 586_15-A-
3.93
YG 586_15-A+
96.1
Count
EGFRt
b
YG 586_15-A-
20.1
YG 586_15-A+
79.9
Count
EGFRt
c
CD19-FITC +
antiMouseFc-A647
Red 670_14-A-
3.53
Red 670_14-A+
96.5
Count
Bound FL
d
CD19-FITC +
antiMouseFc-A647
Red 670_14-A-
20.6
Red 670_14-A+
19.4
Count
Bound FL

FIG. 6

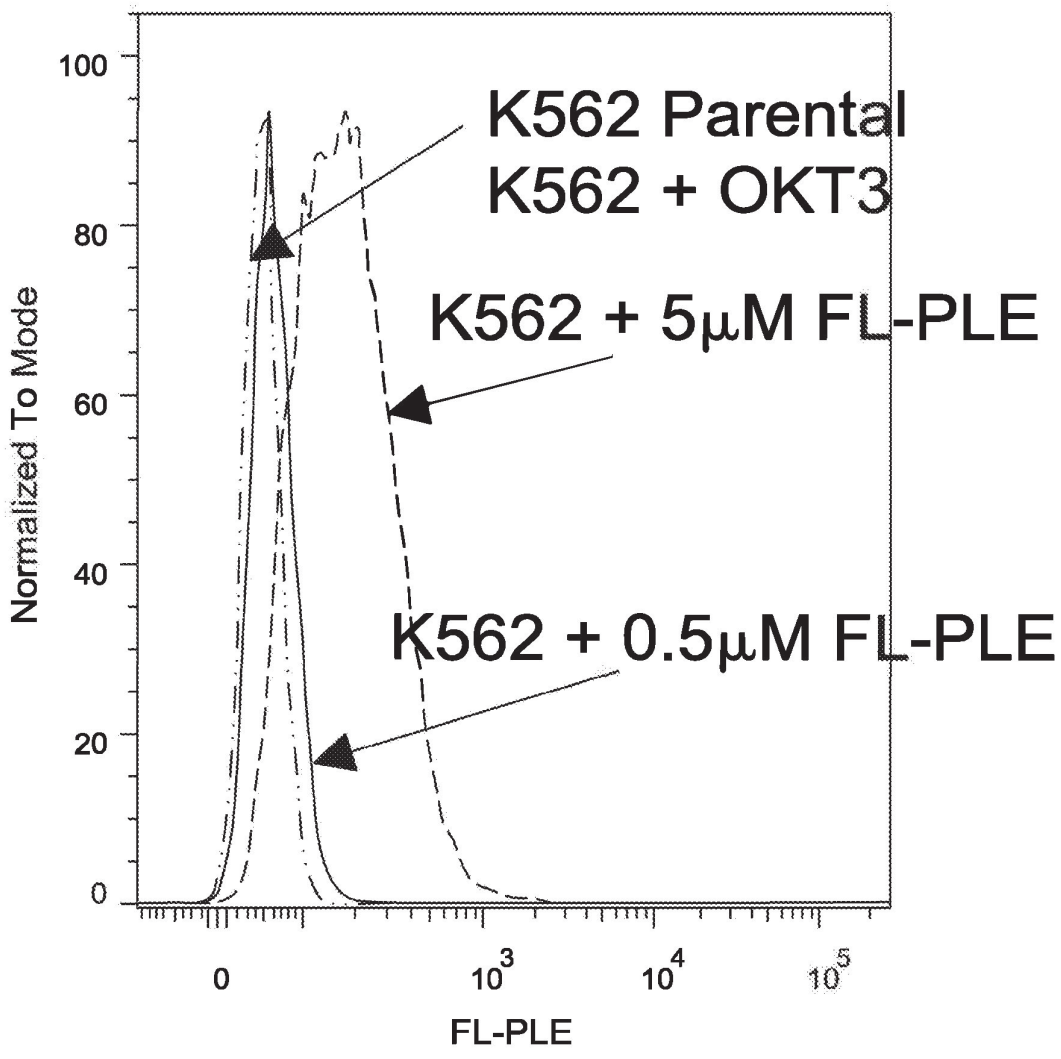

K562 Parental
K562 + OKT3
K562 + 5μM FL-PLE
K562 + 0.5μM FL-PLE
Normalized To Mode
100
80
60
40
20
0
0
10^3
10^4
10^5
FL-PLE

FIG. 7A

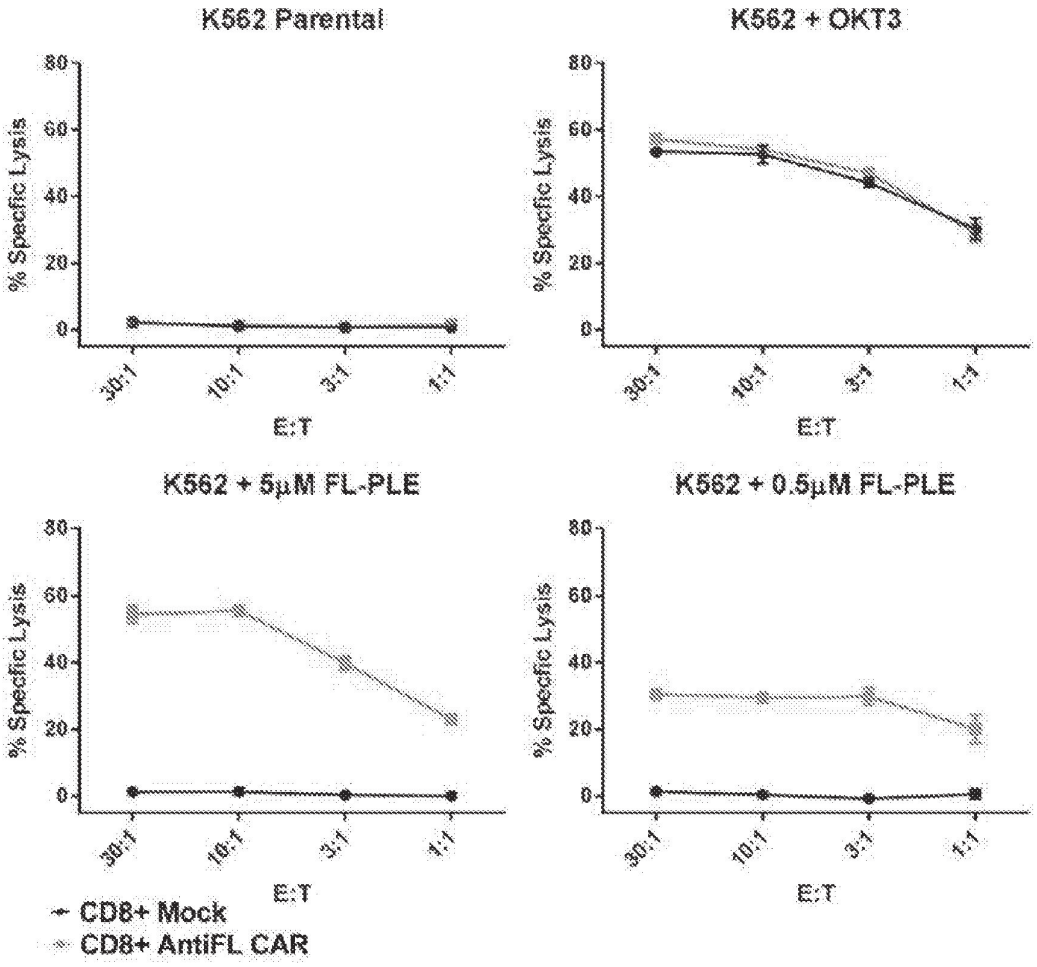

K562 Parental
K562 + OKT3
K562 + 5μM FL-PLE
K562 + 0.5μM FL-PLE
% Specific Lysis
E:T
30:1
10:1
3:1
1:1
0
20
40
60
80
CD8+ Mock
CD8+ AntiFL CAR

FIG. 7B

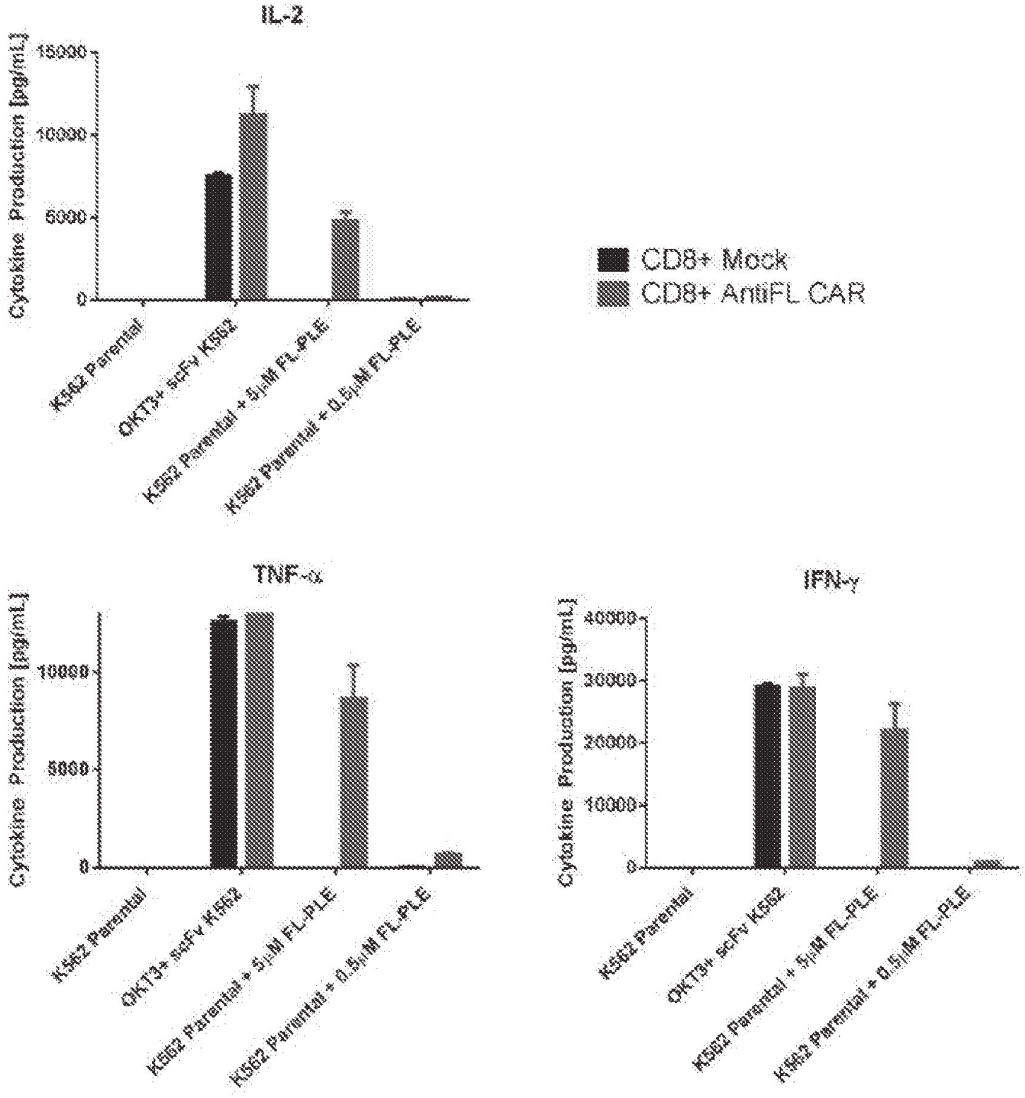

IL-2
Cytokine Production [pg/mL]
15000
10000
5000
0
K562 Parental
OKT3+ scFv K562
K562 Parental + 5μM FL-PLE
K562 Parental + 0.5μM FL-PLE
CD8+ Mock
CD8+ AntiFL CAR
TNF-α
Cytokine Production [pg/mL]
10000
5000
0
K562 Parental
OKT3+ scFv K562
K562 Parental + 5μM FL-PLE
K562 Parental + 0.5μM FL-PLE
IFN-γ
Cytokine Production [pg/mL]
40000
30000
20000
10000
0
K562 Parental
OKT3+ scFv K562
K562 Parental + 5μM FL-PLE
K562 Parental + 0.5μM FL-PLE

FIG. 7C

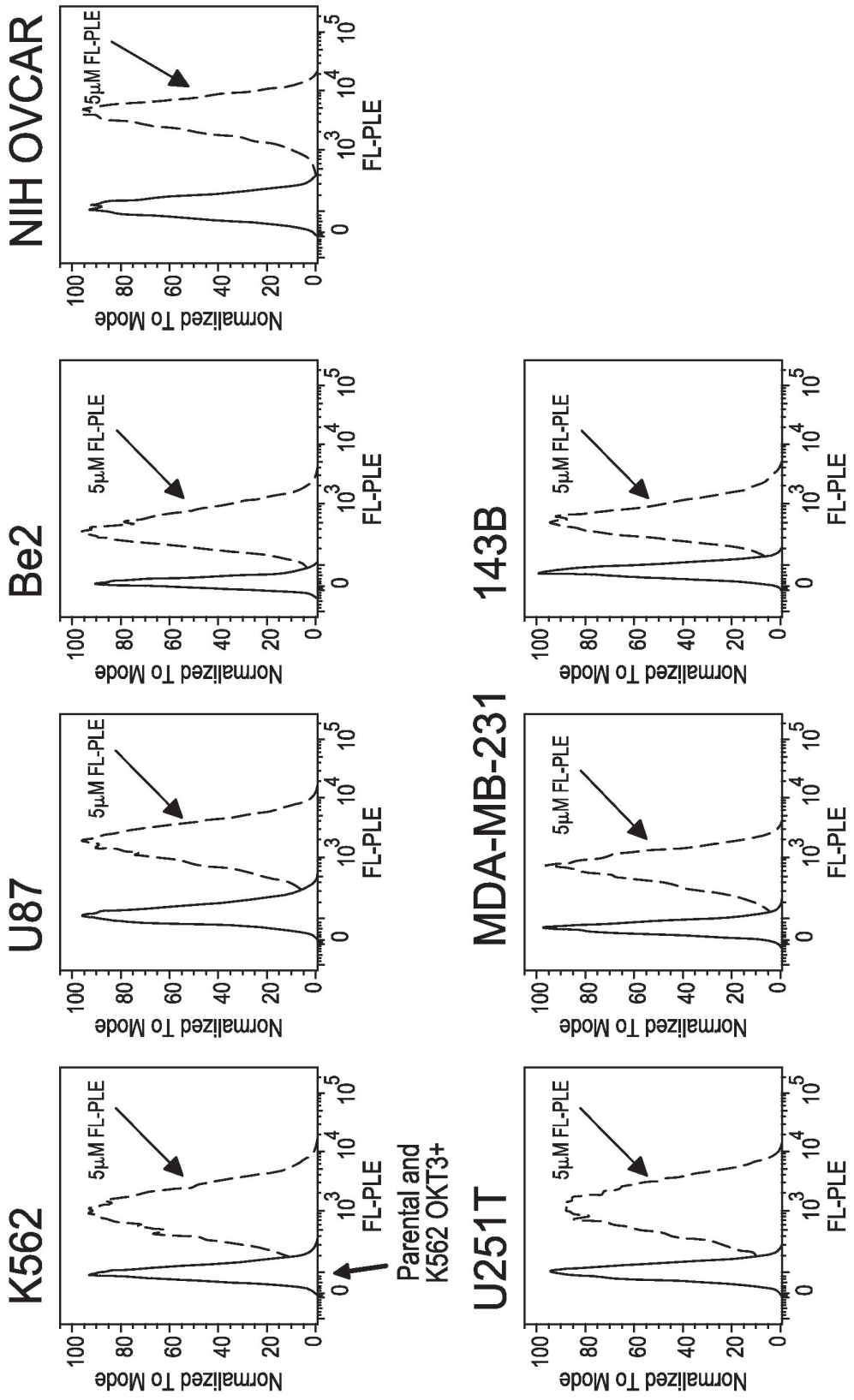

K562
U87
Be2
NIH OVCAR
U251T
MDA-MB-231
143B
5μM FL-PLE
FL-PLE
Normalized To Mode
Parental and
K562 OKT3+

FIG. 8A

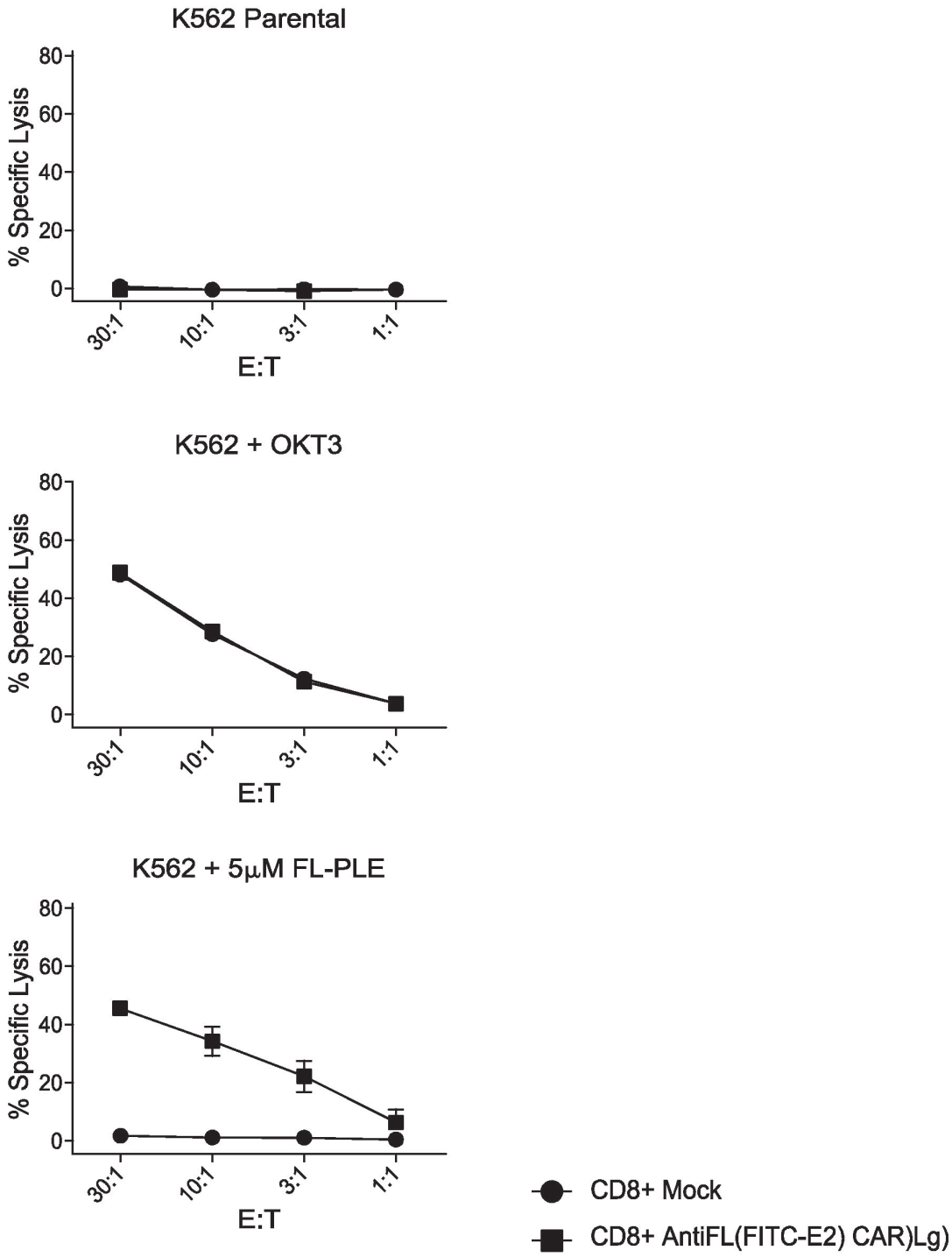

K562 Parental
% Specific Lysis
80
60
40
20
0
30:1
10:1
3:1
1:1
E:T
K562 + OKT3
% Specific Lysis
80
60
40
20
0
30:1
10:1
3:1
1:1
E:T
K562 + 5μM FL-PLE
% Specific Lysis
80
60
40
20
0
30:1
10:1
3:1
1:1
E:T
CD8+ Mock
CD8+ AntiFL(FITC-E2) CAR)Lg)

FIG. 8B

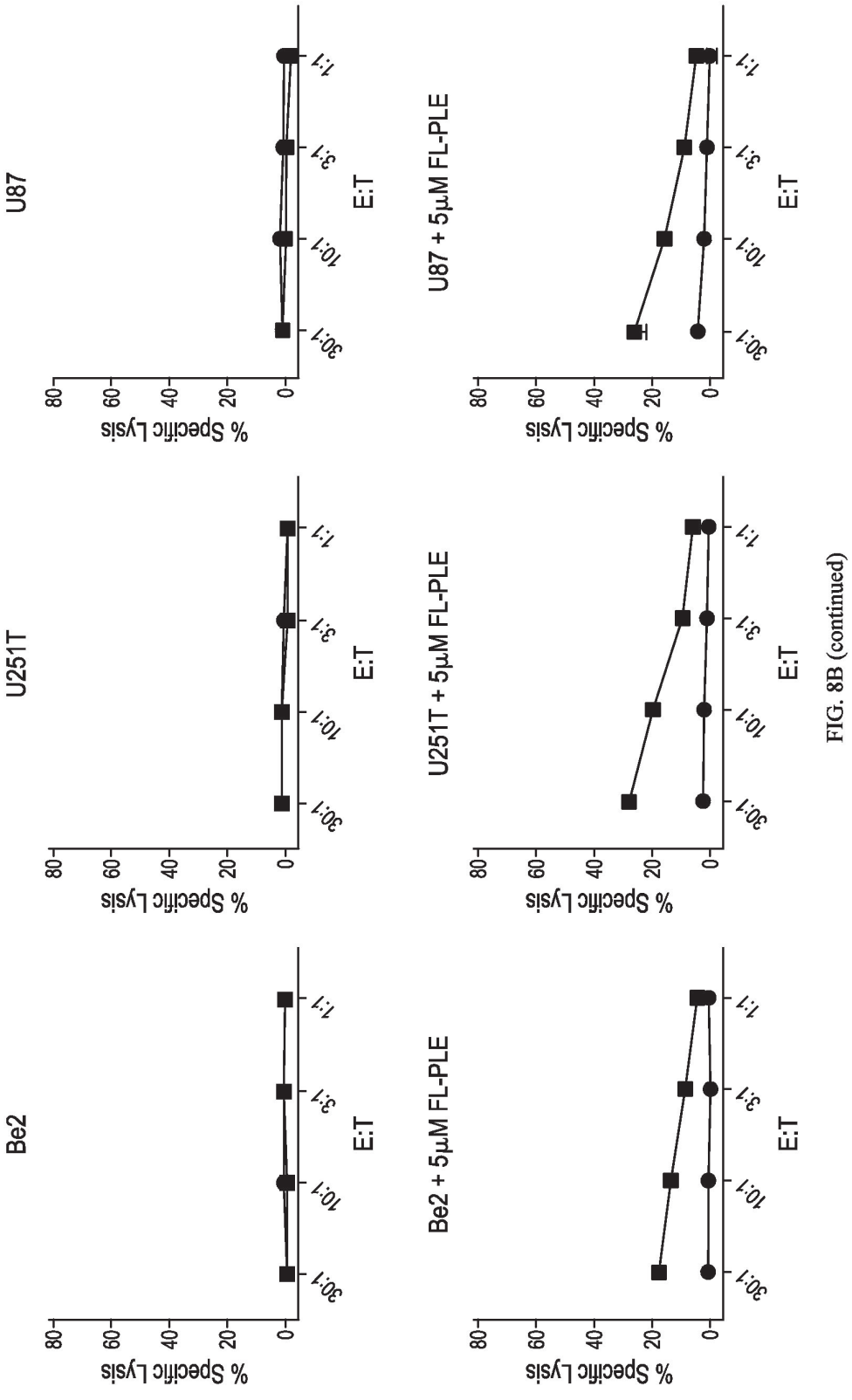


FIG. 8B (continued)

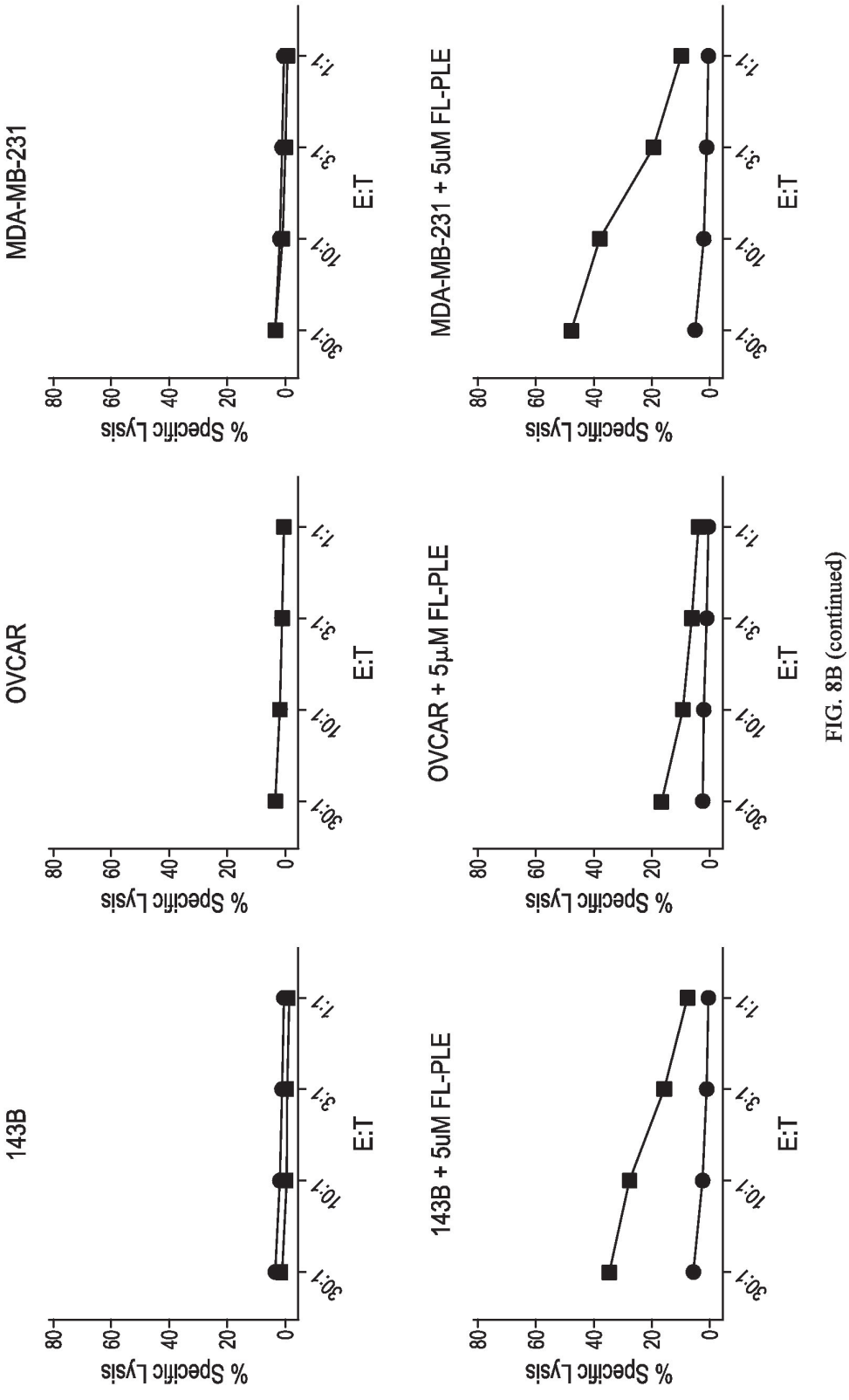


FIG. 8B (continued)

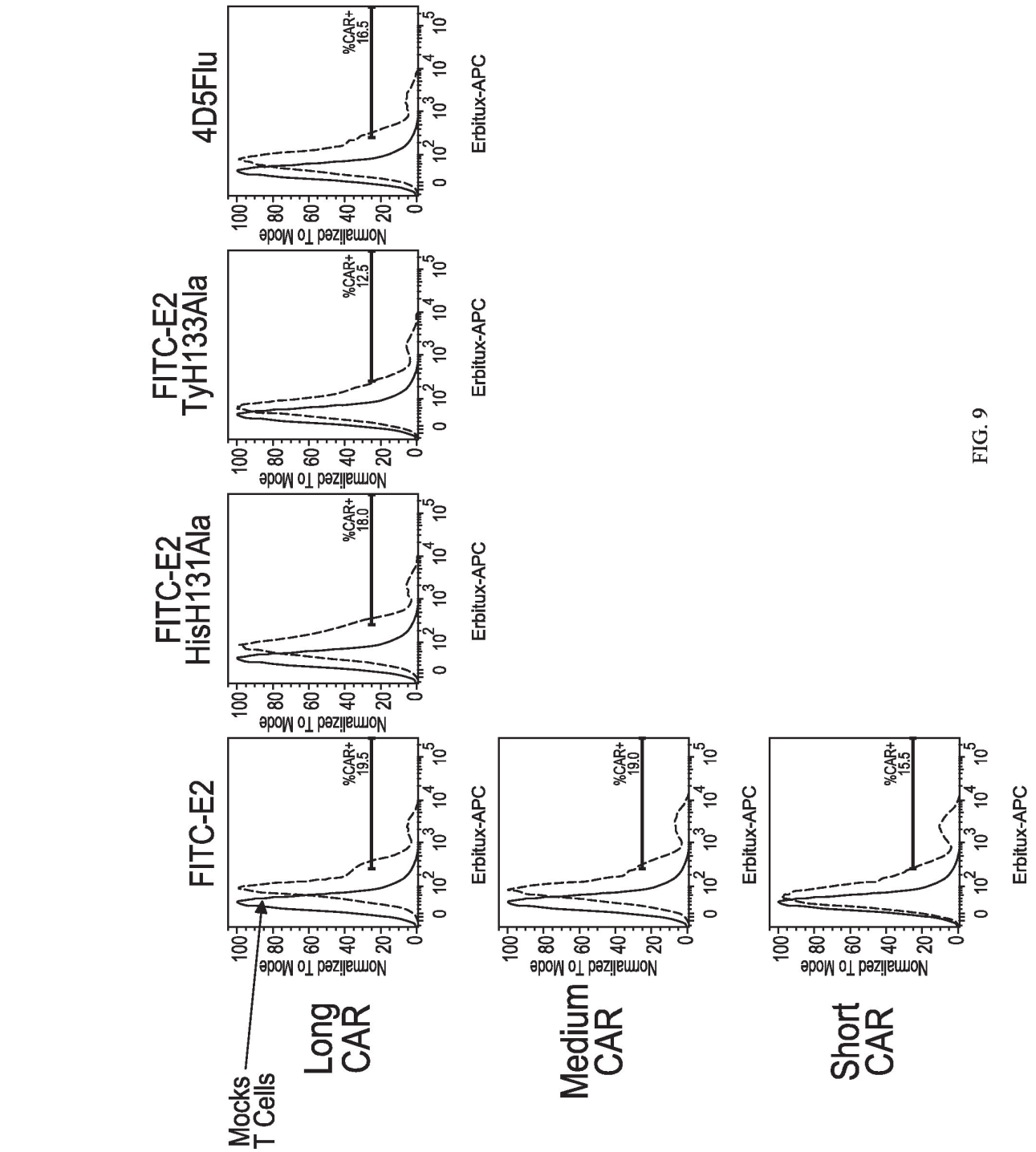

FITC-E2
FITC-E2 HisH131Ala
FITC-E2 TyH133Ala
4D5Flu
Mocks T Cells
Long CAR
Medium CAR
Short CAR
Normalized To Mode
Erbitux-APC
%CAR+ 19.5
%CAR+ 18.0
%CAR+ 12.5
%CAR+ 16.5
%CAR+ 19.0
%CAR+ 15.5

FIG. 9

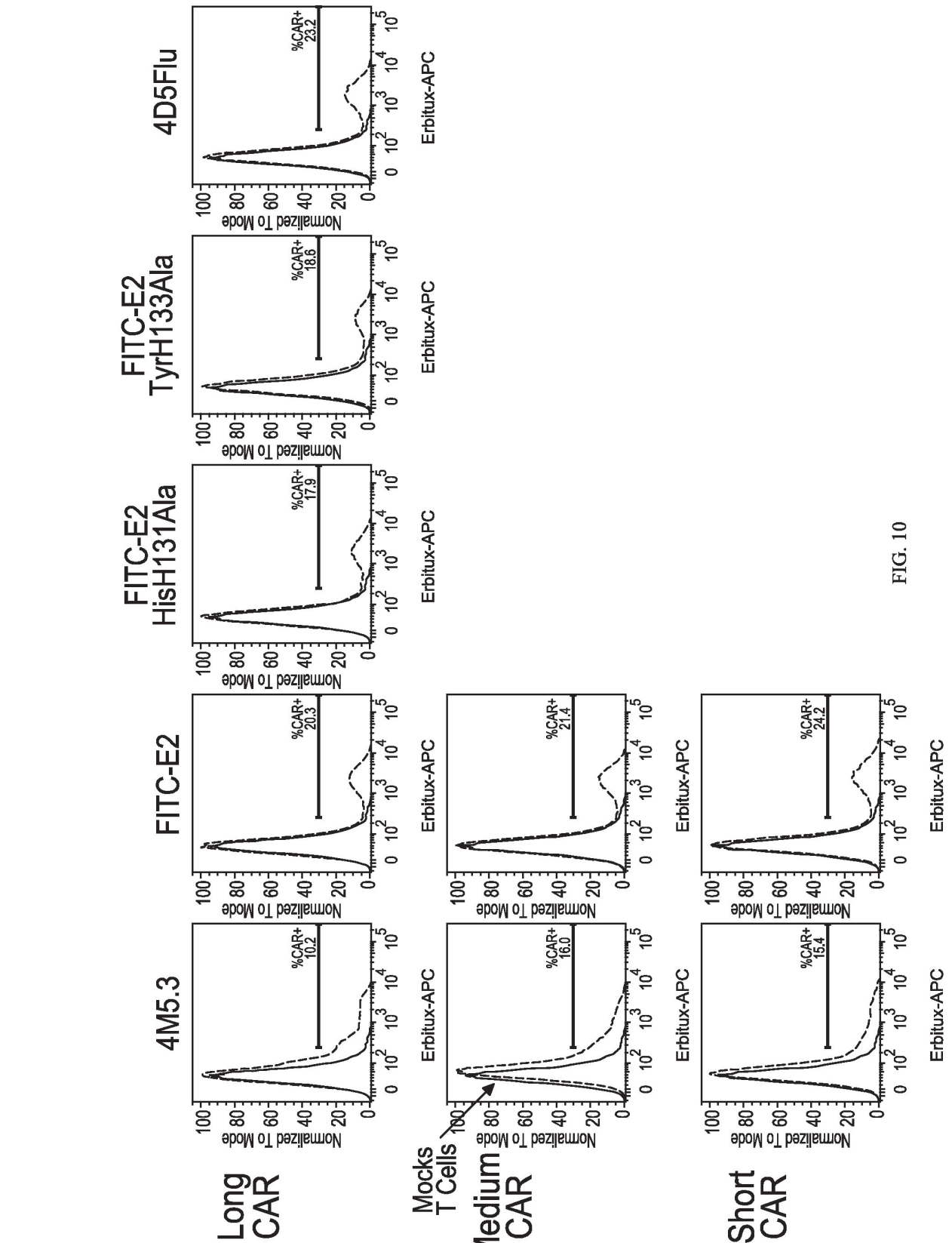

4M5.3
FITC-E2
FITC-E2 HisH131Ala
FITC-E2 TyrH133Ala
4D5Flu
Long CAR
Medium CAR
Short CAR
Mocks T Cells
%CAR+ 10.2
%CAR+ 20.3
%CAR+ 17.9
%CAR+ 18.6
%CAR+ 23.2
%CAR+ 16.0
%CAR+ 21.4
%CAR+ 15.4
%CAR+ 24.2
Normalized To Mode
Erbitux-APC

FIG. 10

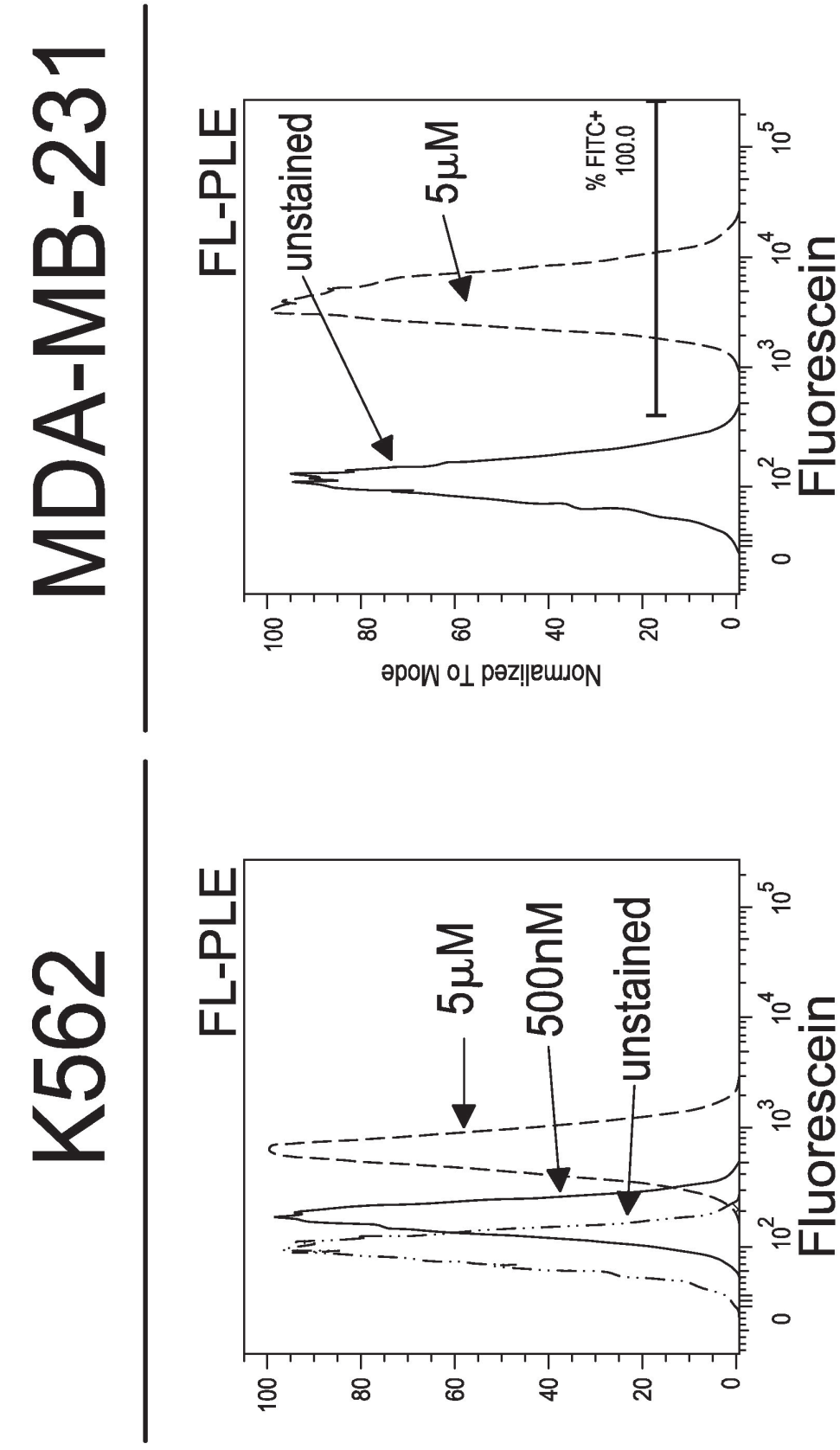

K562
FL-PLE
5µM
500nM
unstained
Fluorescein
MDA-MB-231
FL-PLE
unstained
5µM
% FITC+
100.0
Normalized To Mode
Fluorescein

FIG. 11

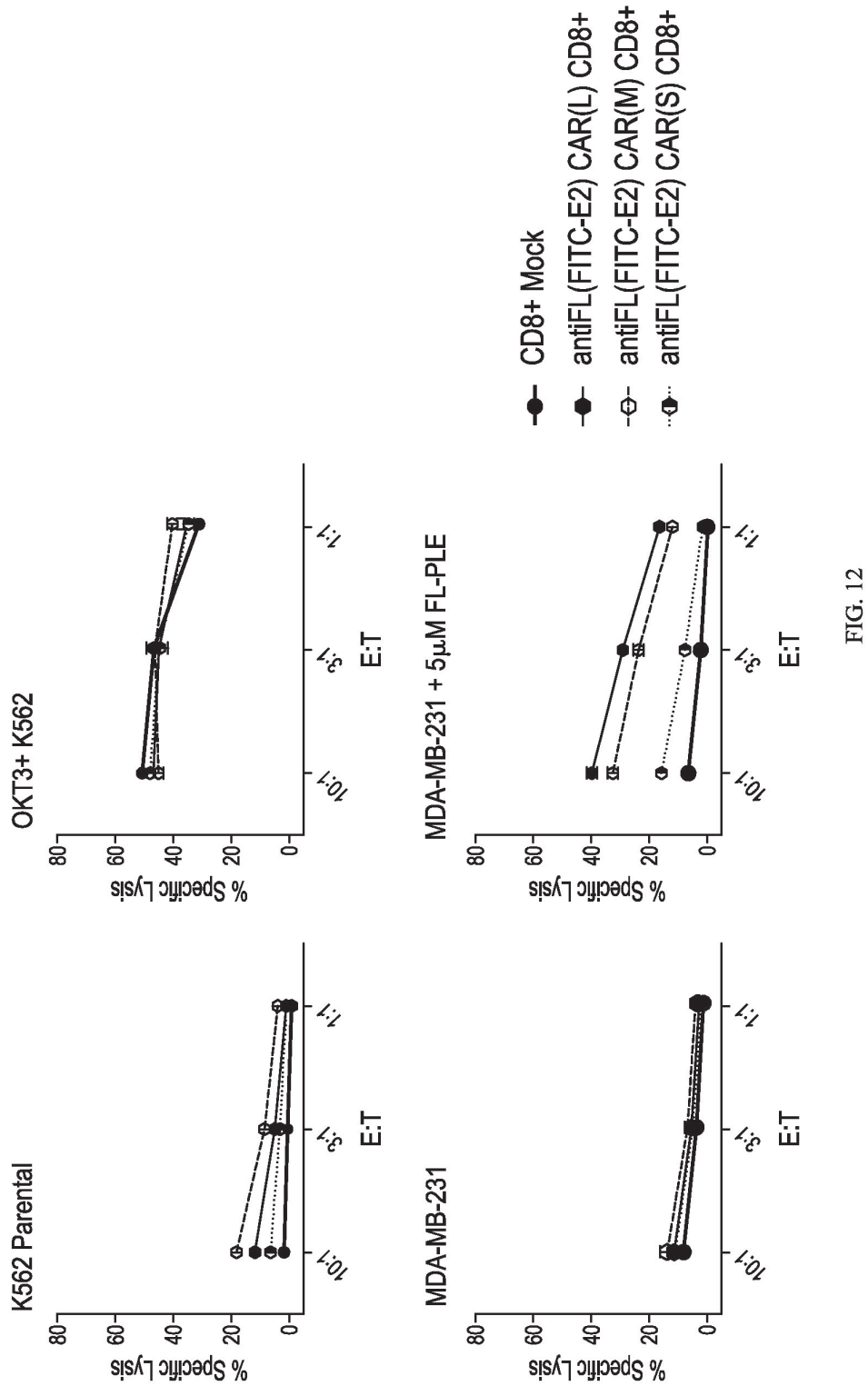

K562 Parental
OKT3+ K562
MDA-MB-231
MDA-MB-231 + 5µM FL-PLE
% Specific Lysis
0
20
40
60
80
10:1
3:1
1:1
E:T
CD8+ Mock
antiFL(FITC-E2) CAR(L) CD8+
antiFL(FITC-E2) CAR(M) CD8+
antiFL(FITC-E2) CAR(S) CD8+

FIG. 12

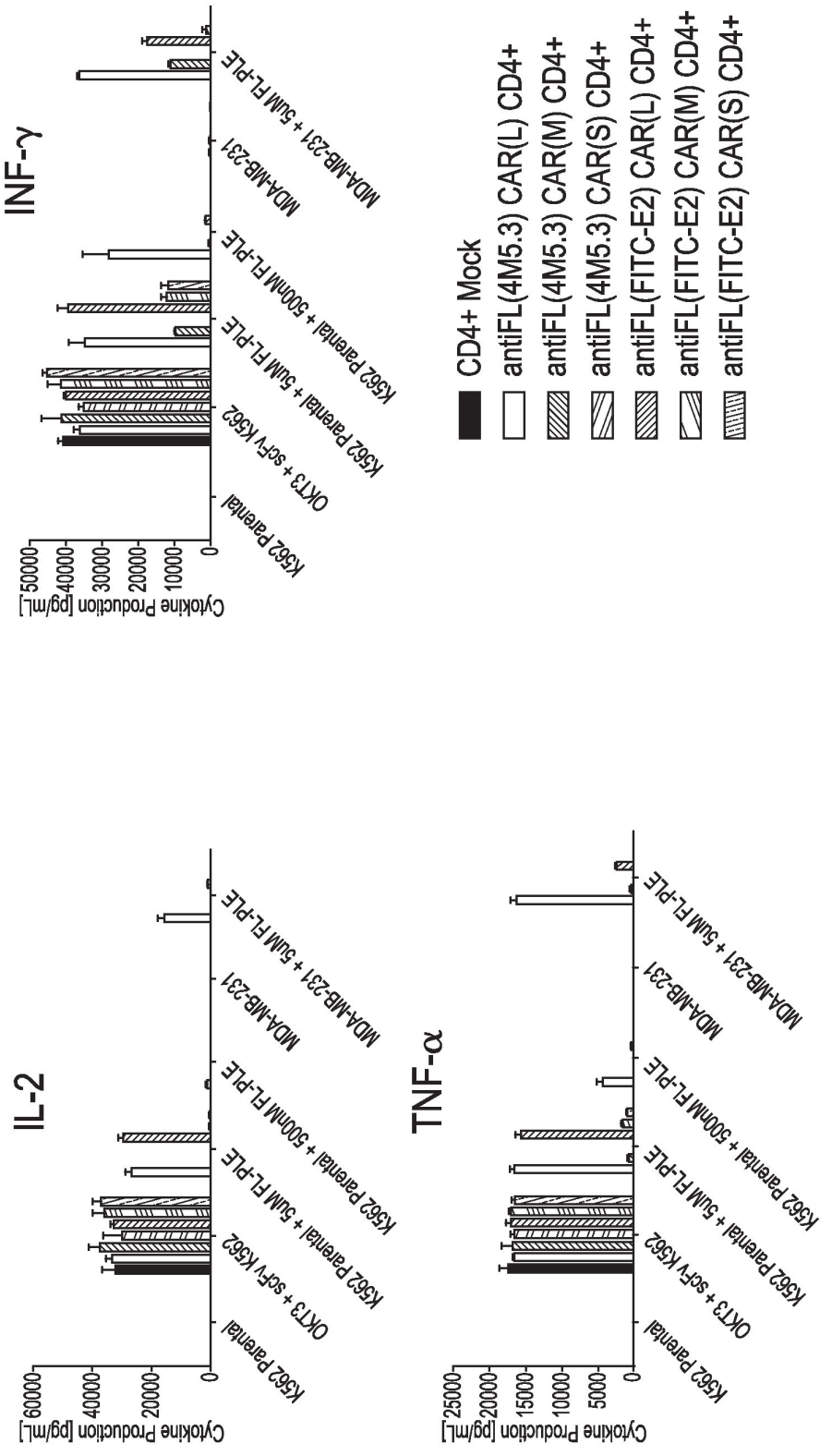

IL-2
Cytokine Production [pg/mL]
60000
40000
20000
0
K562 Parental
OKT3 + scFv K562
K562 Parental + 5uM FL-PLE
K562 Parental + 500nM FL-PLE
MDA-MB-231
MDA-MB-231 + 5uM FL-PLE
TNF-α
Cytokine Production [pg/mL]
25000
20000
15000
10000
5000
0
K562 Parental
OKT3 + scFv K562
K562 Parental + 5uM FL-PLE
K562 Parental + 500nM FL-PLE
MDA-MB-231
MDA-MB-231 + 5uM FL-PLE
INF-γ
Cytokine Production [pg/mL]
50000
40000
30000
20000
10000
0
K562 Parental
OKT3 + scFv K562
K562 Parental + 5uM FL-PLE
K562 Parental + 500nM FL-PLE
MDA-MB-231
MDA-MB-231 + 5uM FL-PLE
CD4+ Mock
antiFL(4M5.3) CAR(L) CD4+
antiFL(4M5.3) CAR(M) CD4+
antiFL(4M5.3) CAR(S) CD4+
antiFL(FITC-E2) CAR(L) CD4+
antiFL(FITC-E2) CAR(M) CD4+
antiFL(FITC-E2) CAR(S) CD4+

FIG. 13

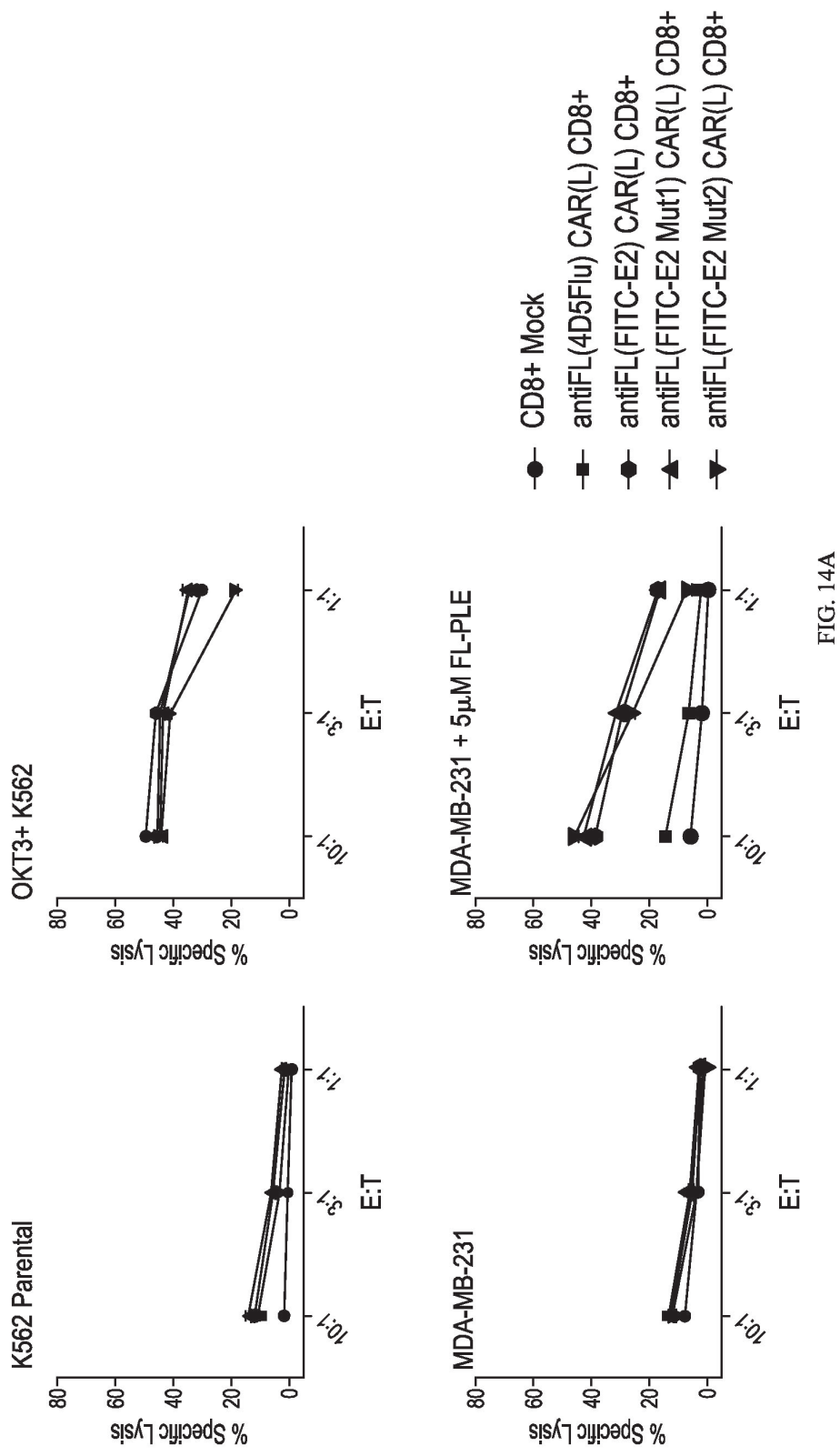

K562 Parental
OKT3+ K562
MDA-MB-231
MDA-MB-231 + 5µM FL-PLE
% Specific Lysis
0
20
40
60
80
10:1
3:1
1:1
E:T
CD8+ Mock
antiFL(4D5Flu) CAR(L) CD8+
antiFL(FITC-E2) CAR(L) CD8+
antiFL(FITC-E2 Mut1) CAR(L) CD8+
antiFL(FITC-E2 Mut2) CAR(L) CD8+

FIG. 14A

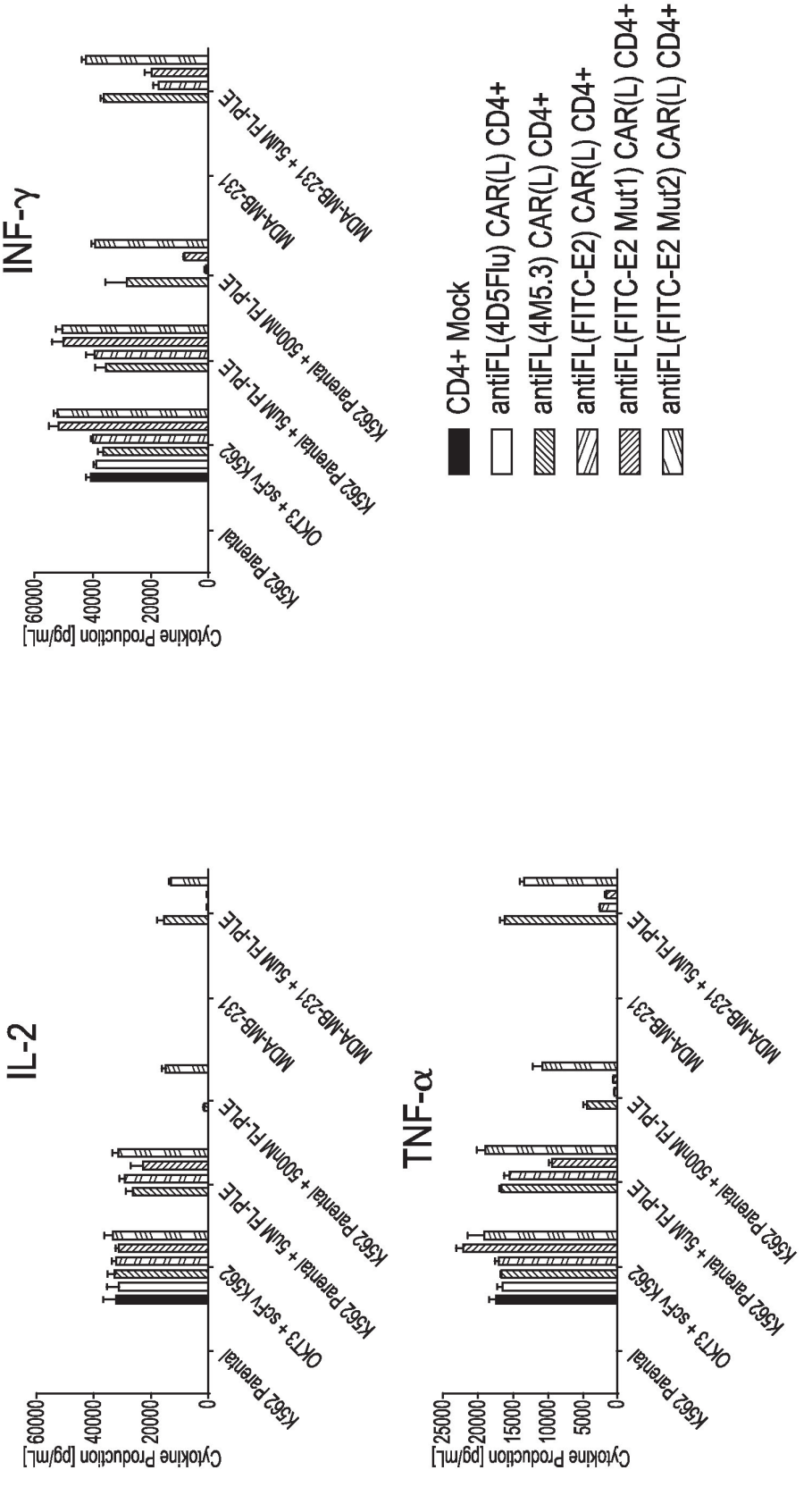

IL-2
INF-γ
TNF-α
Cytokine Production [pg/mL]
60000
40000
20000
0
25000
20000
15000
10000
5000
K562 Parental
OKT3 + scFv K562
K562 Parental + 5uM FL-PLE
K562 Parental + 500nM FL-PLE
MDA-MB-231
MDA-MB-231 + 5uM FL-PLE
CD4+ Mock
antiFL(4D5Flu) CAR(L) CD4+
antiFL(4M5.3) CAR(L) CD4+
antiFL(FITC-E2) CAR(L) CD4+
antiFL(FITC-E2 Mut1) CAR(L) CD4+
antiFL(FITC-E2 Mut2) CAR(L) CD4+

FIG. 14B

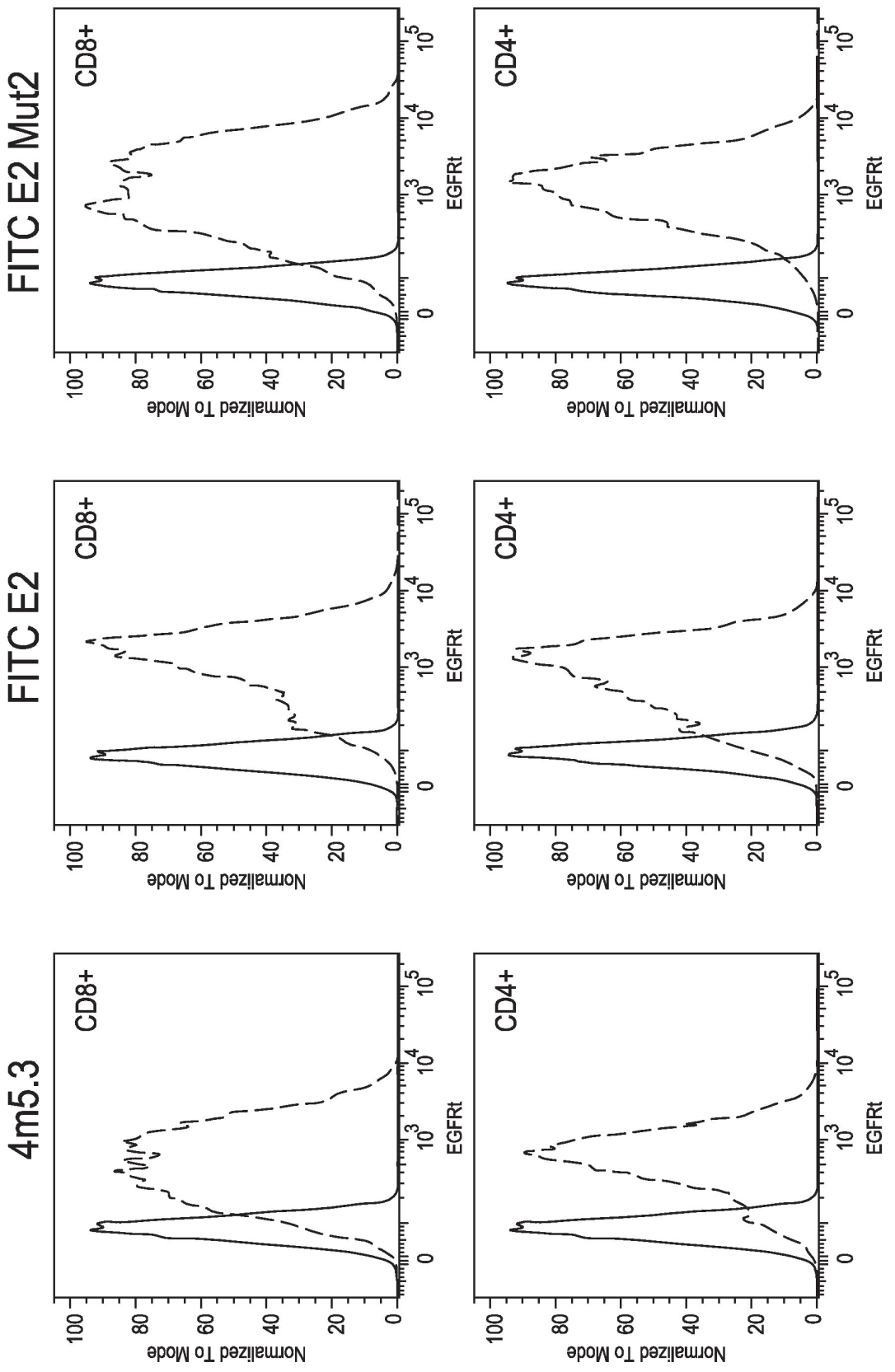

4m5.3
FITC E2
FITC E2 Mut2
CD8+
CD4+
Normalized To Mode
EGFRt
0
20
40
60
80
100
10^3
10^4
10^5

FIG. 15A

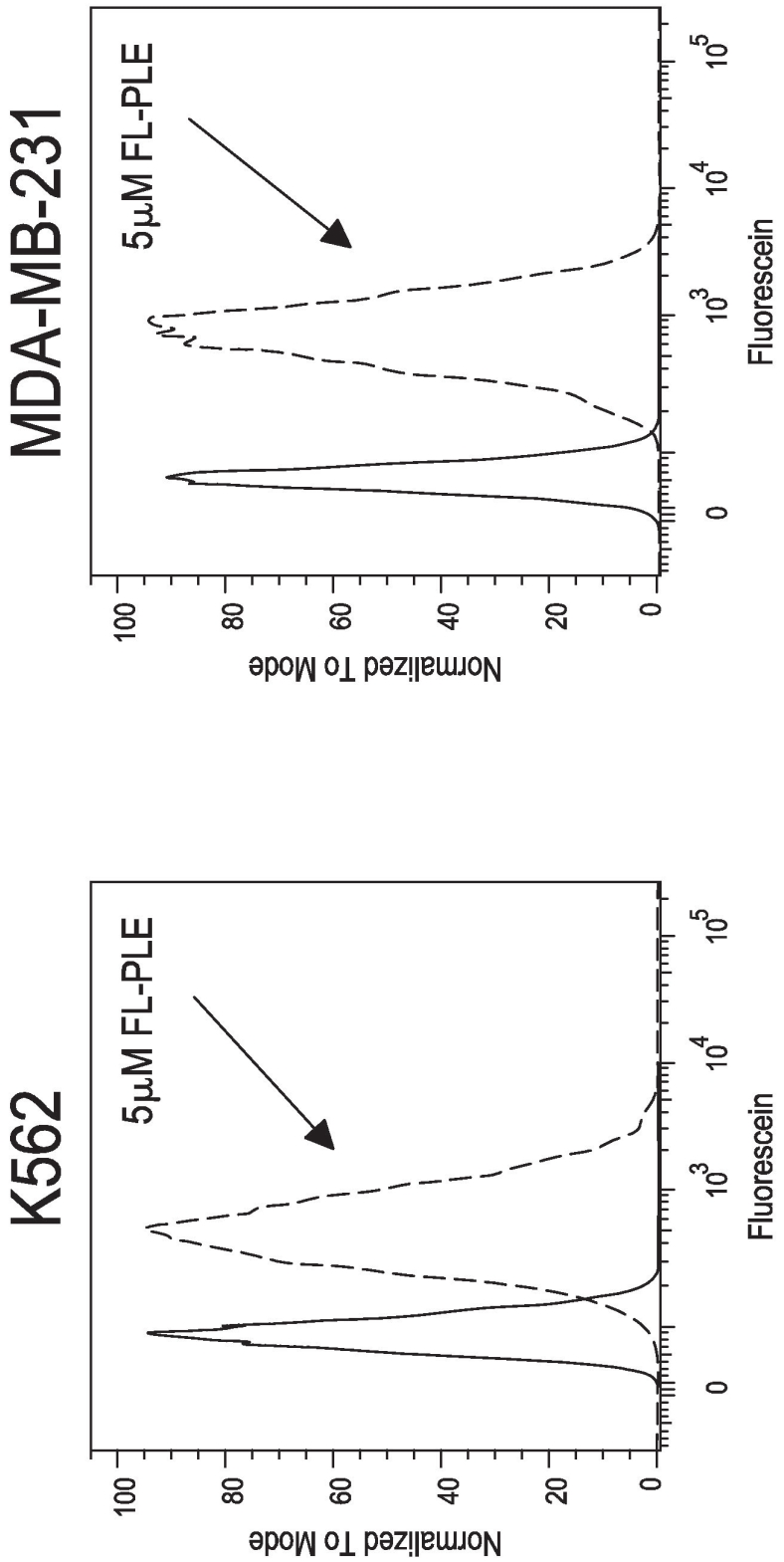

MDA-MB-231
5μM FL-PLE
Normalized To Mode
Fluorescein
0
20
40
60
80
100
0
10^3
10^4
10^5
K562
5μM FL-PLE
Normalized To Mode
Fluorescein

FIG. 15B

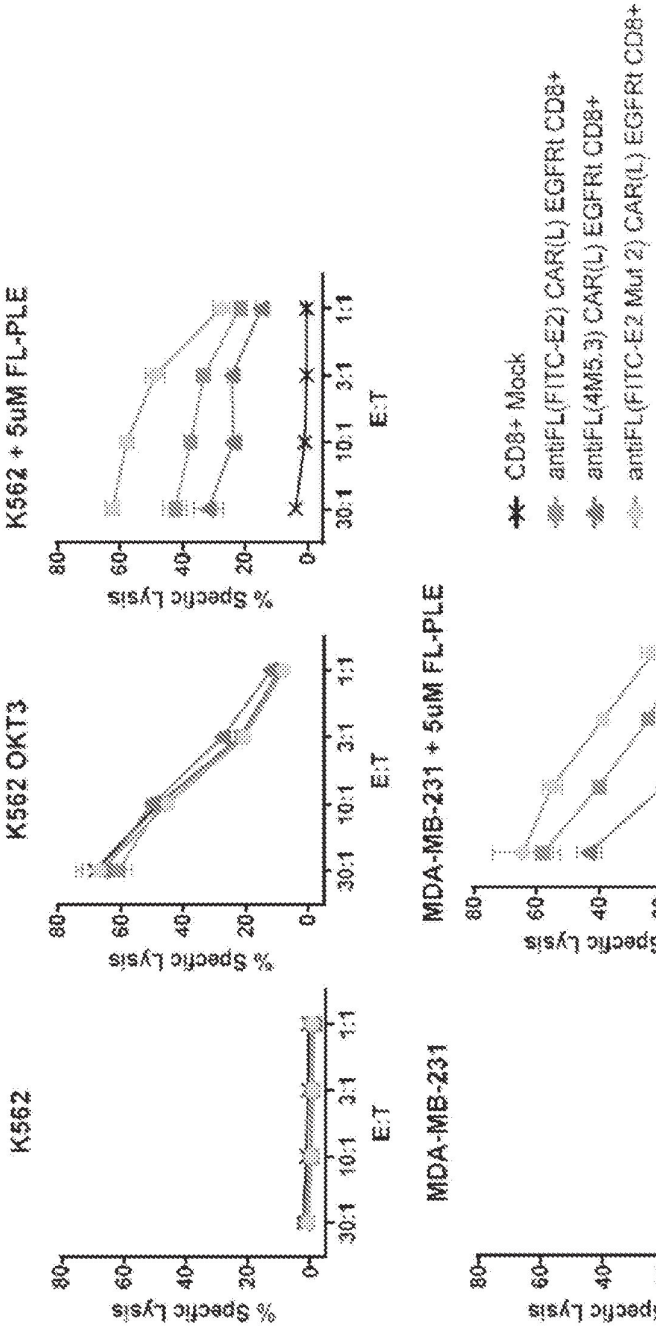

K562
K562 OKT3
K562 + 5uM FL-PLE
MDA-MB-231
MDA-MB-231 + 5uM FL-PLE
% Specific Lysis
0
20
40
60
80
30:1
10:1
3:1
1:1
E:T
CD8+ Mock
antiFL(FITC-E2) CAR(L) EGFRt CD8+
antiFL(4M5.3) CAR(L) EGFRt CD8+
antiFL(FITC-E2 Mut 2) CAR(L) EGFRt CD8+

FIG. 15C

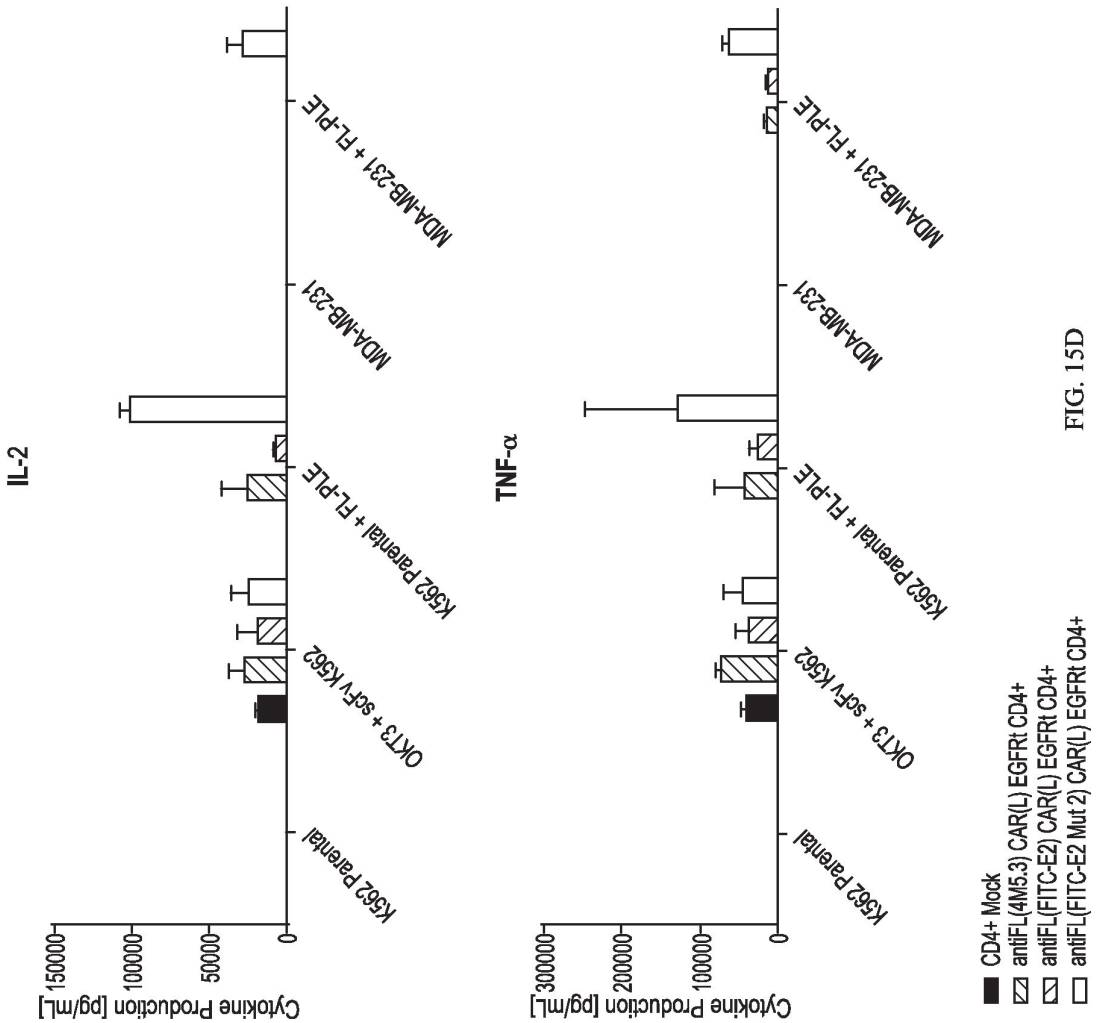

IL-2
Cytokine Production [pg/mL]
150000
100000
50000
0
K562 Parental
OKT3 + scFv K562
K562 Parental + FL-PLE
MDA-MB-231
MDA-MB-231 + FL-PLE
TNF-α
Cytokine Production [pg/mL]
300000
200000
100000
0
K562 Parental
OKT3 + scFv K562
K562 Parental + FL-PLE
MDA-MB-231
MDA-MB-231 + FL-PLE
CD4+ Mock
antiFL(4M5.3) CAR(L) EGFRt CD4+
antiFL(FITC-E2) CAR(L) EGFRt CD4+
antiFL(FITC-E2 Mut 2) CAR(L) EGFRt CD4+

FIG. 15D

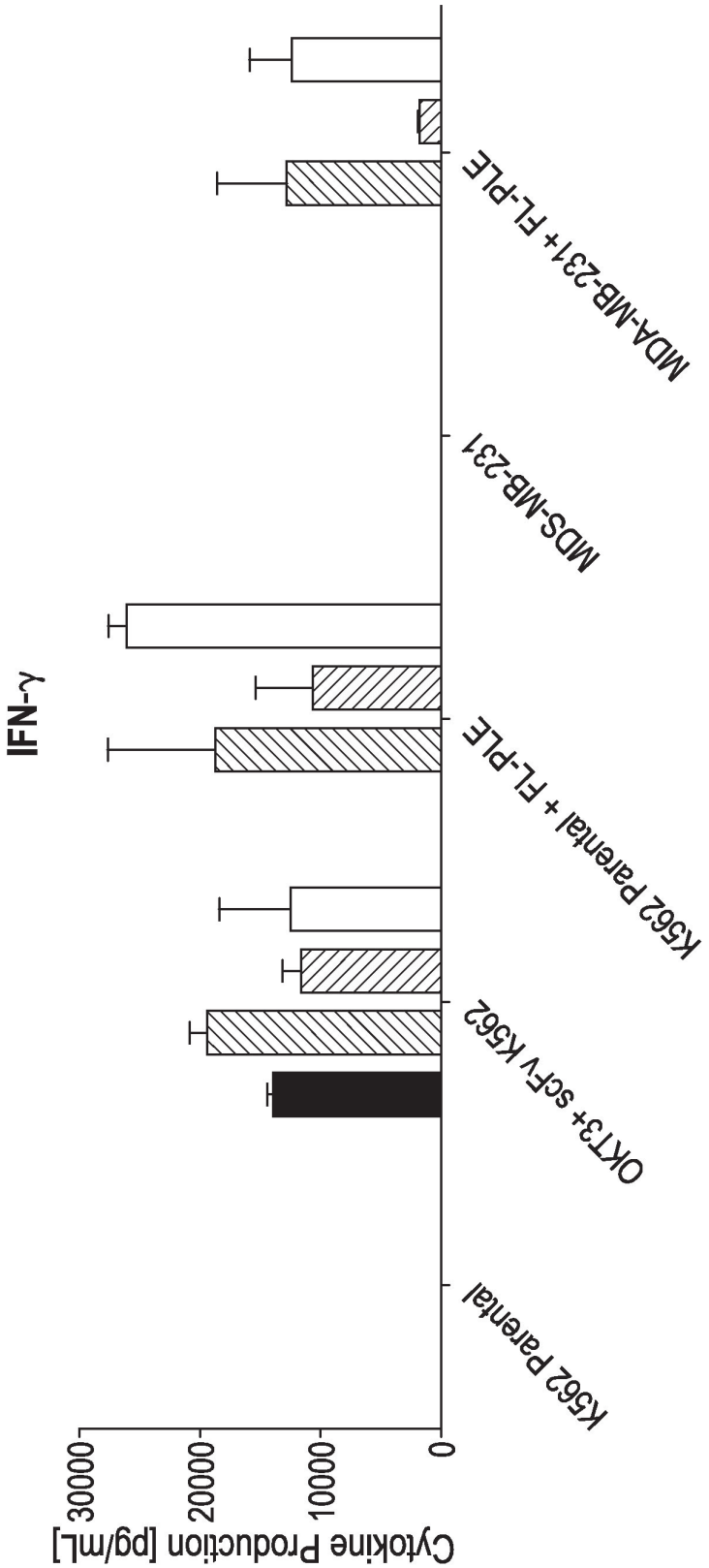


FIG. 15D (continued)

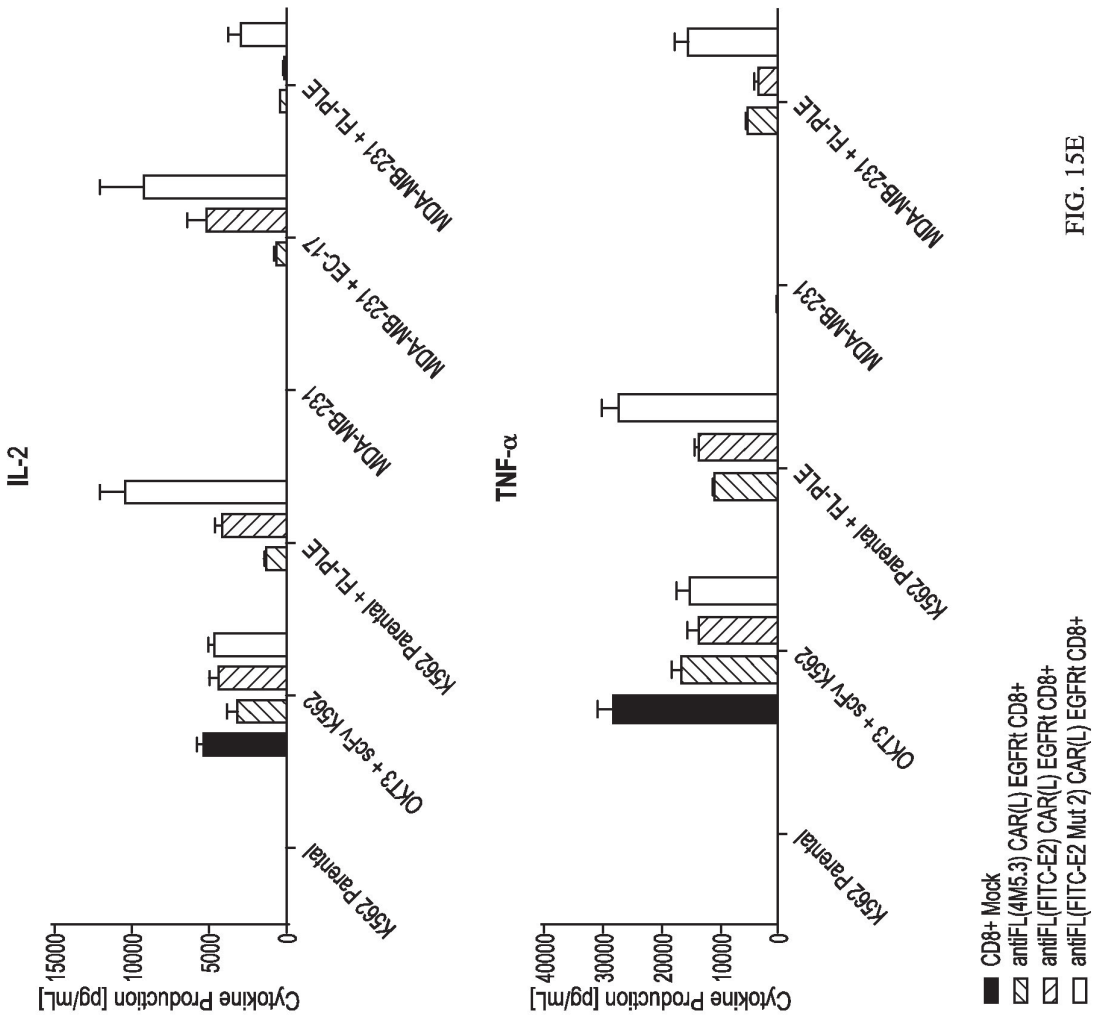

IL-2
Cytokine Production [pg/mL]
15000
10000
5000
0
K562 Parental
OKT3 + scFv K562
K562 Parental + FL-PLE
MDA-MB-231
MDA-MB-231 + EC-17
MDA-MB-231 + FL-PLE
TNF-α
Cytokine Production [pg/mL]
40000
30000
20000
10000
0
K562 Parental
OKT3 + scFv K562
K562 Parental + FL-PLE
MDA-MB-231
MDA-MB-231 + FL-PLE
CD8+ Mock
antiFL(4M5.3) CAR(L) EGFRt CD8+
antiFL(FITC-E2) CAR(L) EGFRt CD8+
antiFL(FITC-E2 Mut 2) CAR(L) EGFRt CD8+

FIG. 15E

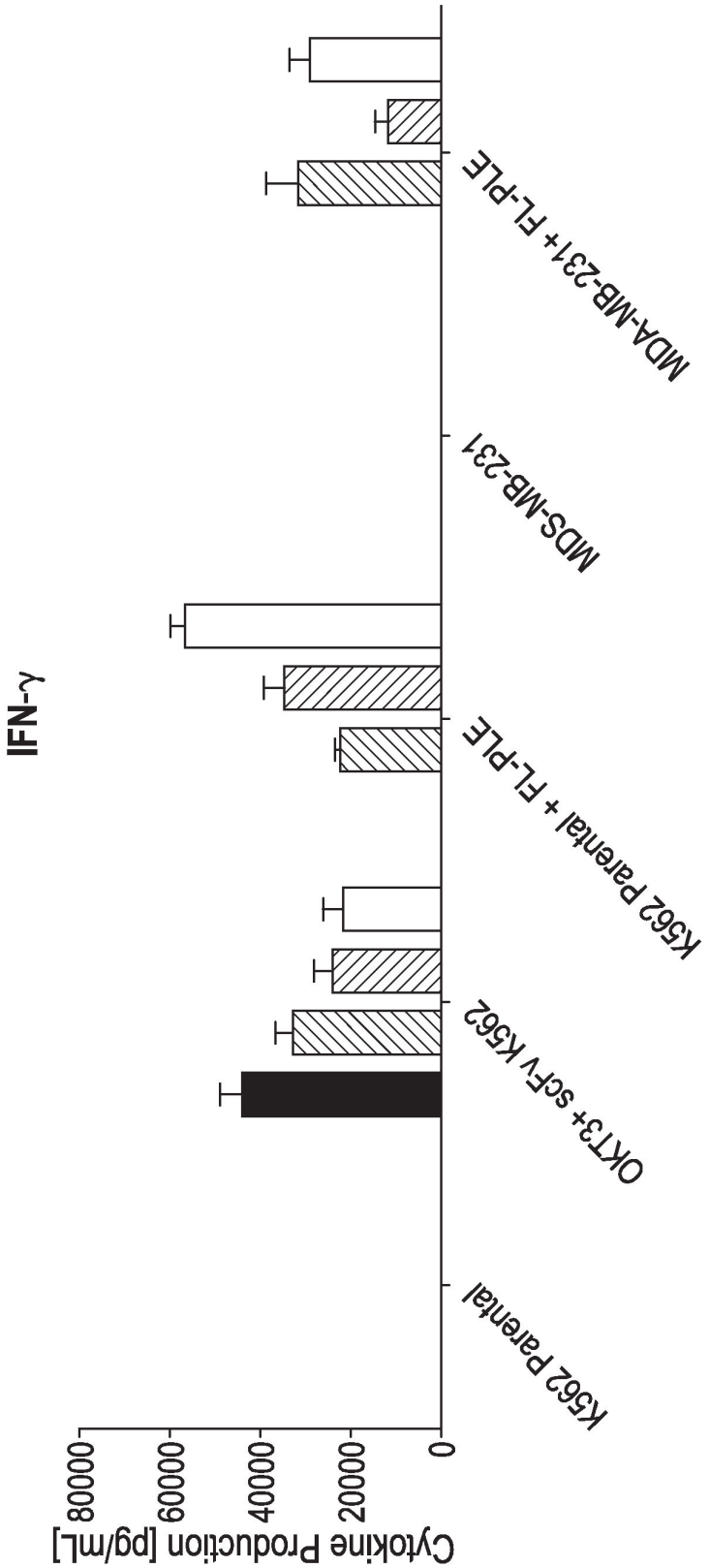


FIG. 15E (continued)

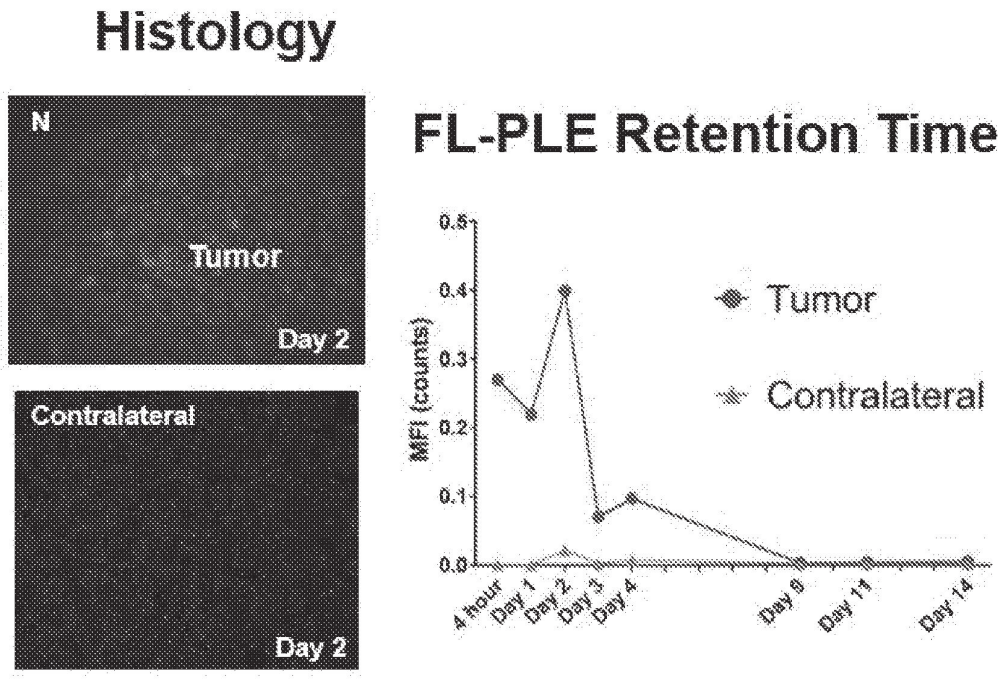

Histology
N
Tumor
Day 2
Contralateral
Day 2
FL-PLE Retention Time
MFI (counts)
0.5
0.4
0.3
0.2
0.1
0.0
Tumor
Contralateral
4 hour
Day 1
Day 2
Day 3
Day 4
Day 8
Day 11
Day 14

FIG. 16

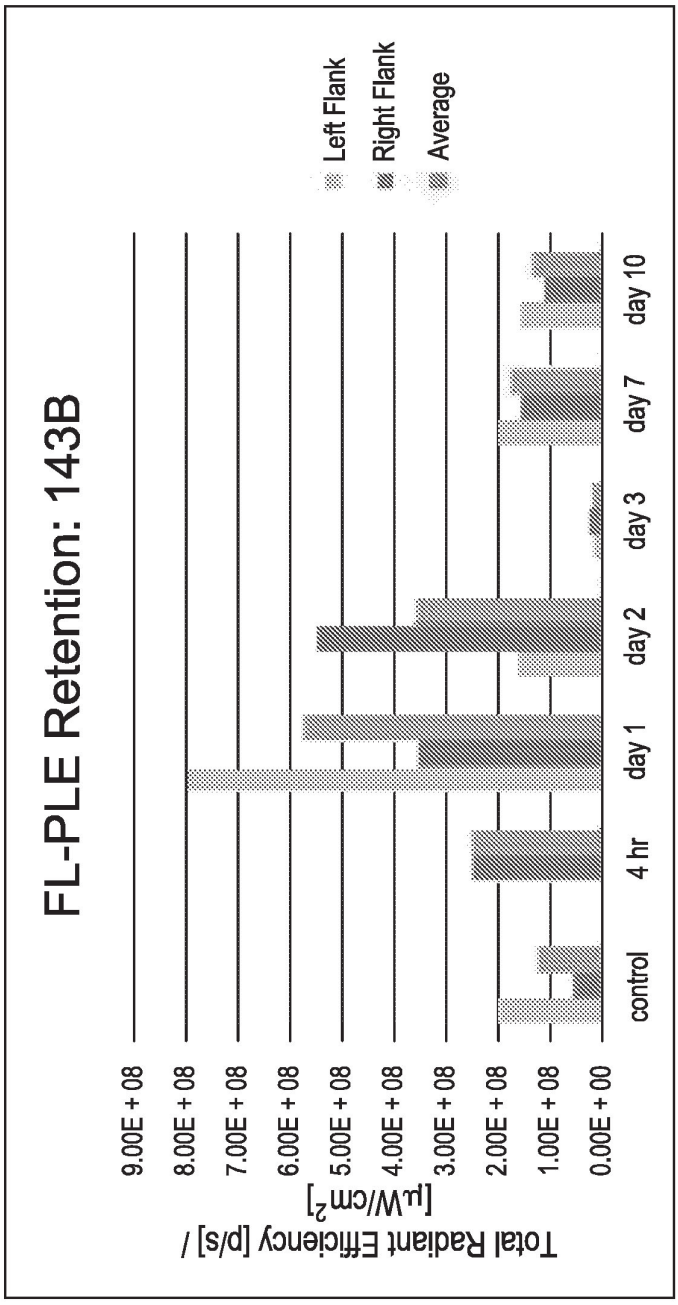

FL-PLE Retention: 143B
Total Radiant Efficiency [p/s] / [μW/cm²]
9.00E + 08
8.00E + 08
7.00E + 08
6.00E + 08
5.00E + 08
4.00E + 08
3.00E + 08
2.00E + 08
1.00E + 08
0.00E + 00
control
4 hr
day 1
day 2
day 3
day 7
day 10
Left Flank
Right Flank
Average

FIG. 17

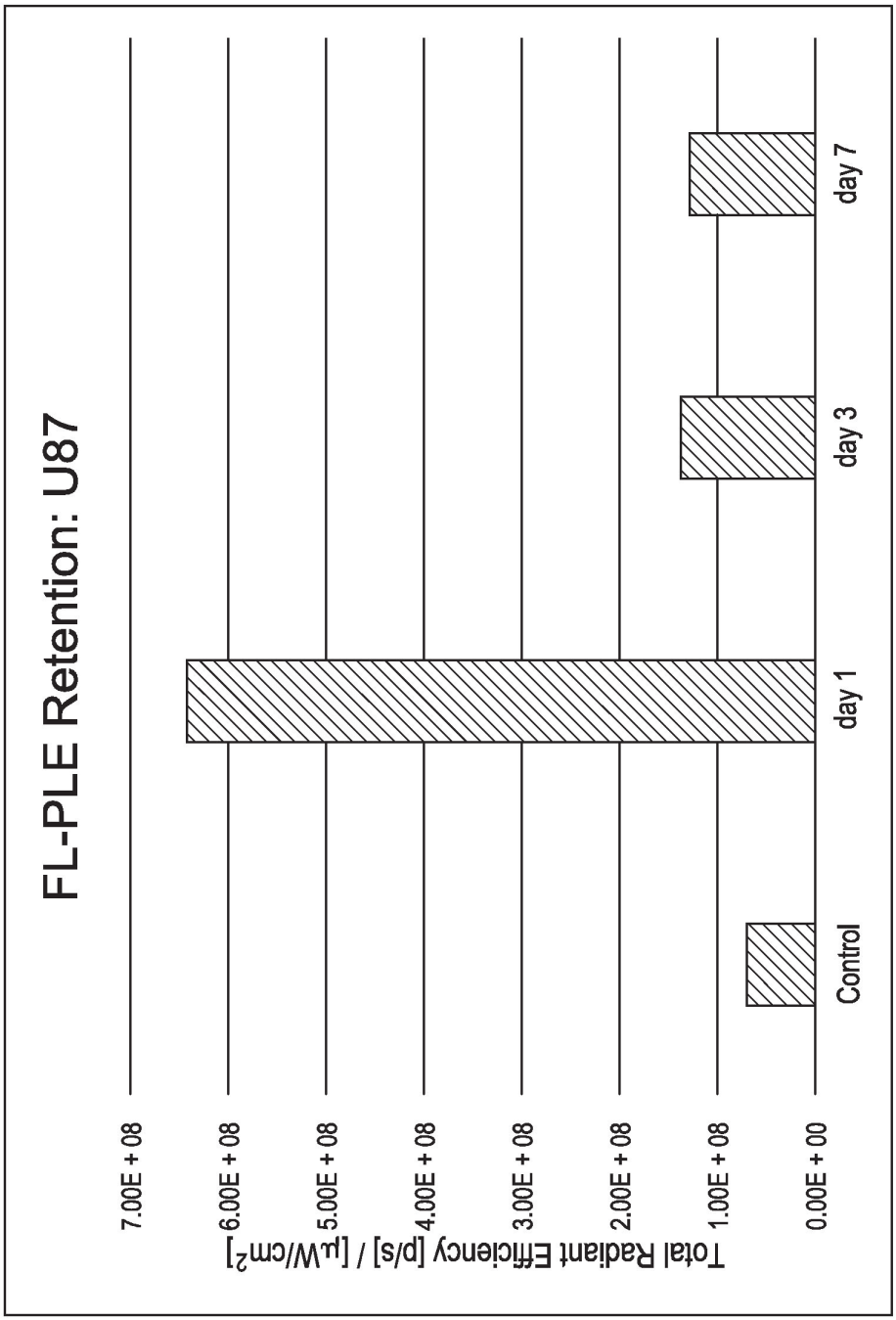


FIG. 17 (continued)

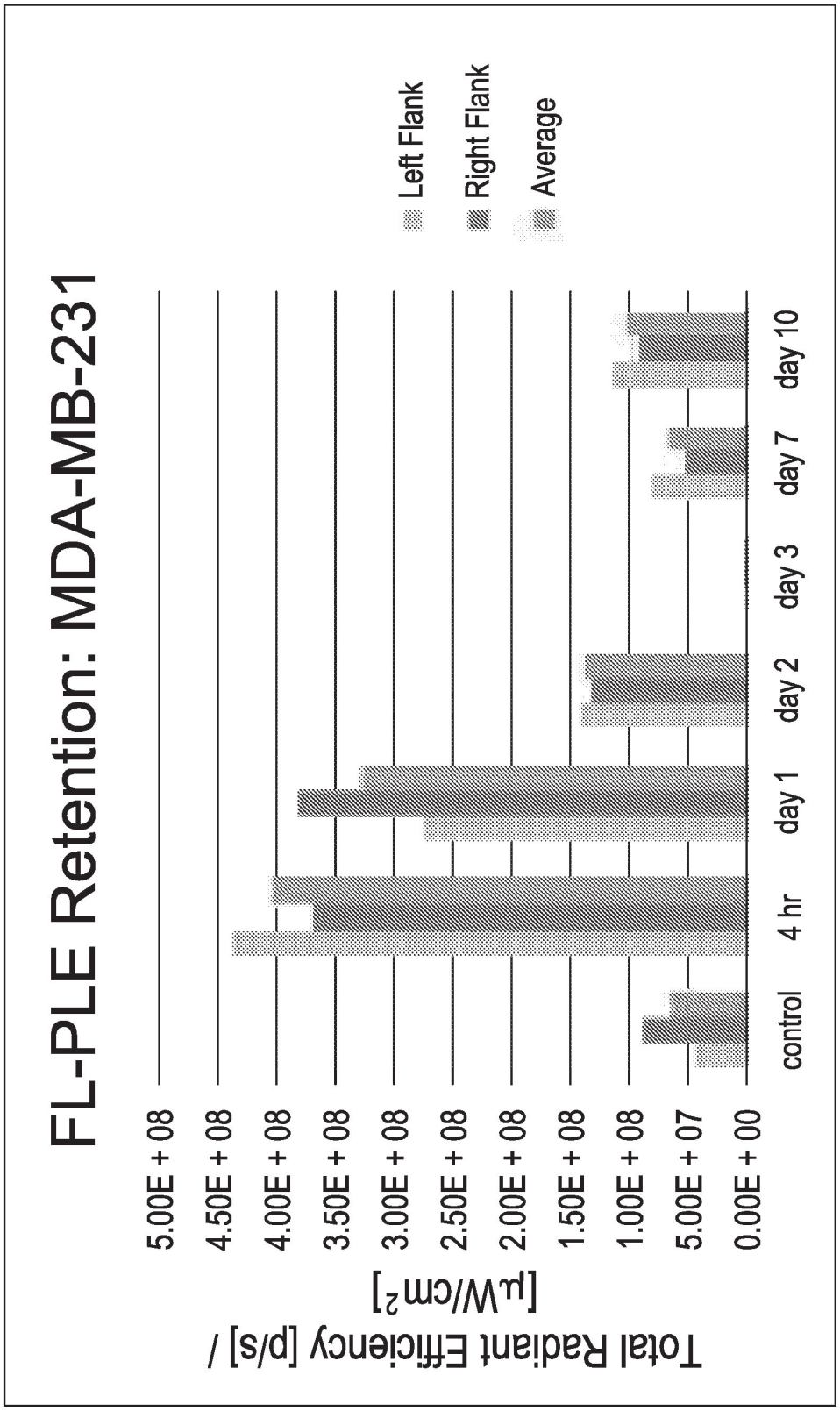


FIG. 17 (continued)

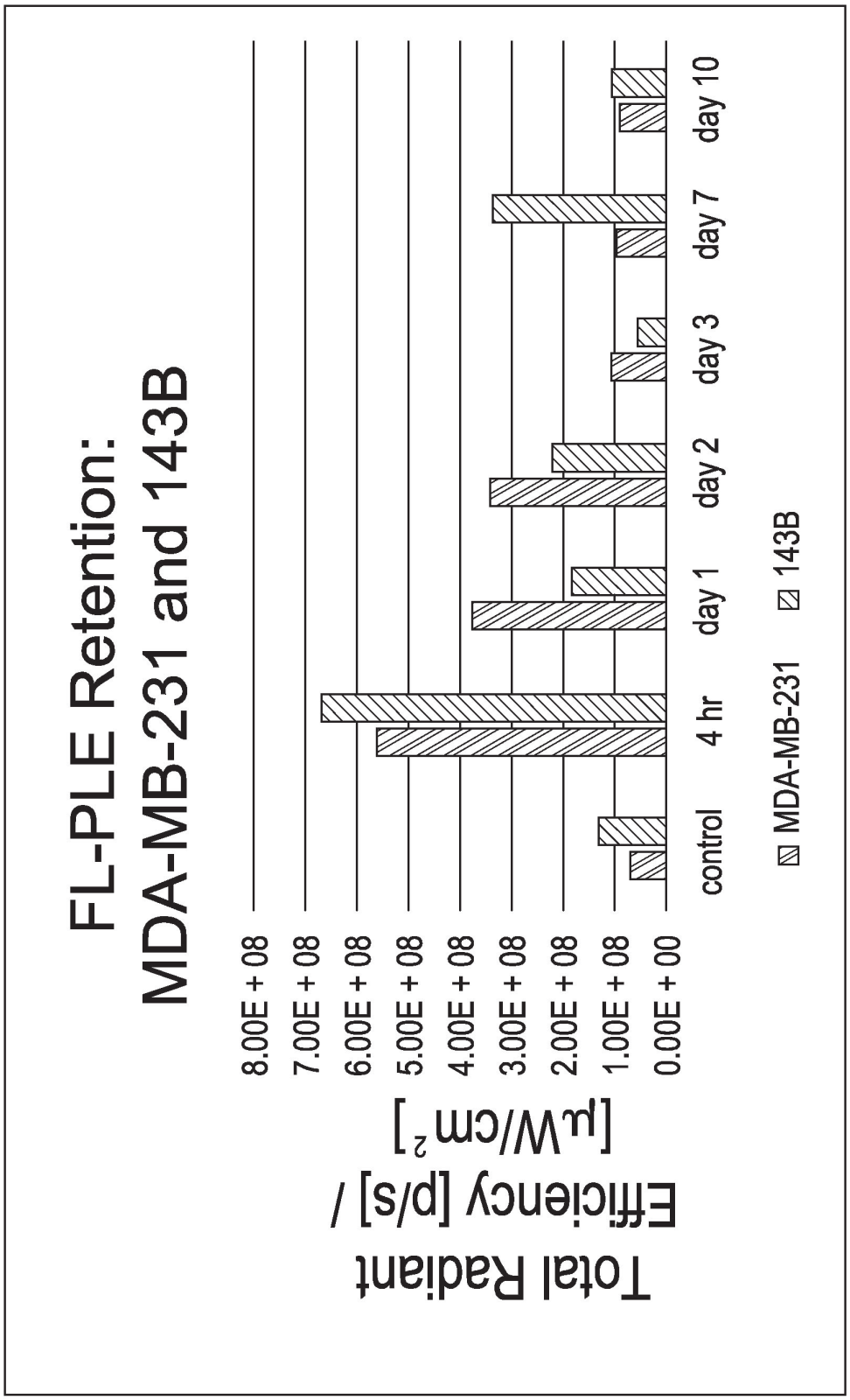


FIG. 17 (continued)

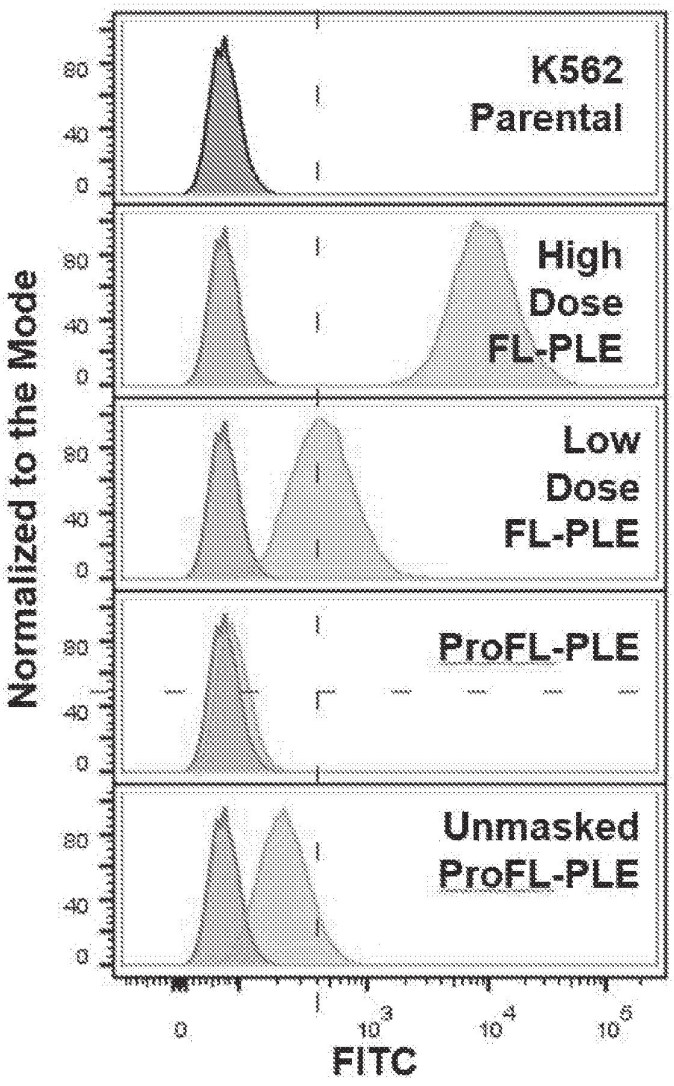

K562
Parental
High
Dose
FL-PLE
Low
Dose
FL-PLE
ProFL-PLE
Unmasked
ProFL-PLE
Normalized to the Mode
FITC
0
10^3
10^4
10^5

FIG. 18A

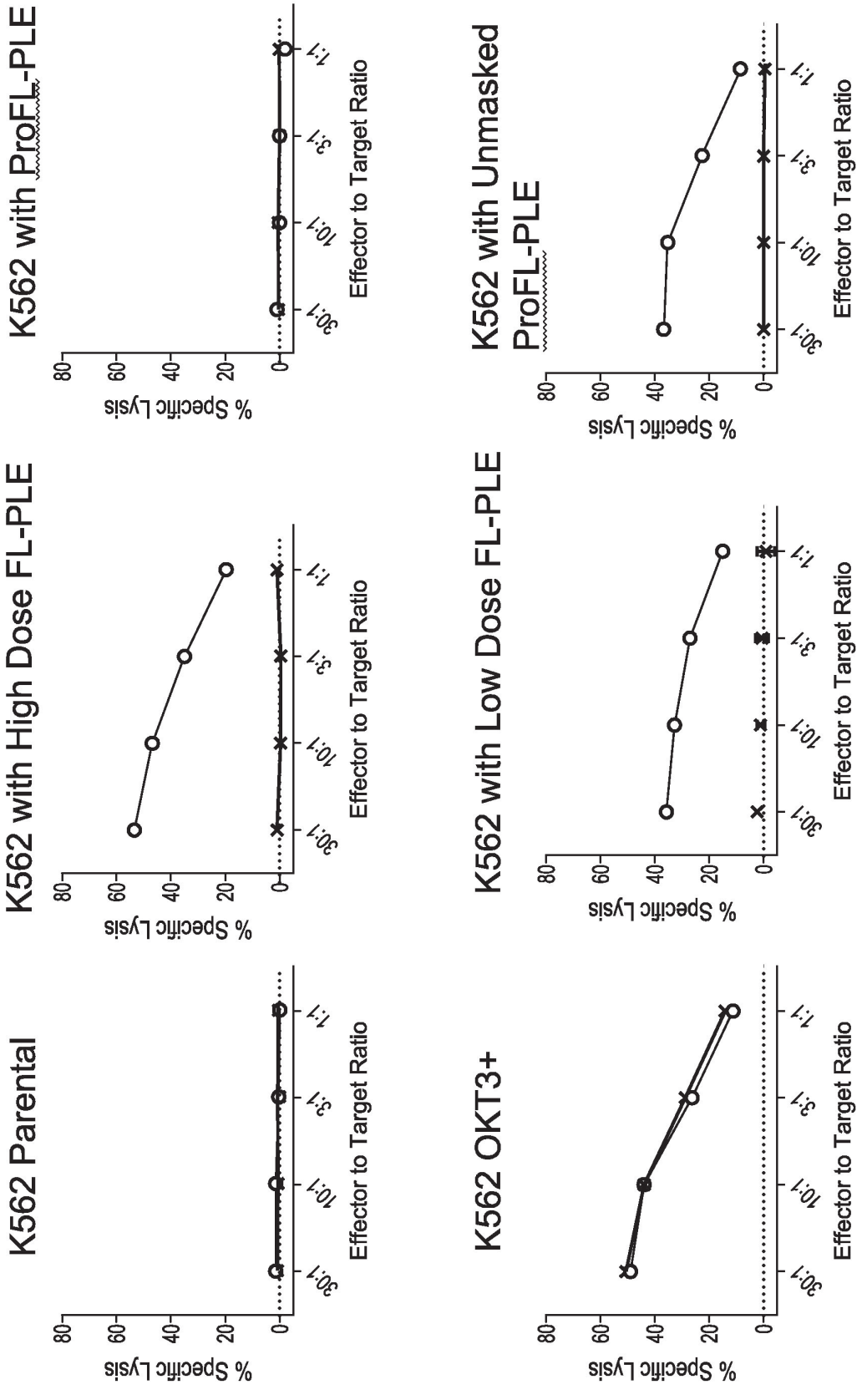

K562 Parental
K562 with High Dose FL-PLE
K562 with ProFL-PLE
K562 OKT3+
K562 with Low Dose FL-PLE
K562 with Unmasked ProFL-PLE
% Specific Lysis
0
20
40
60
80
Effector to Target Ratio
1:1
3:1
10:1
30:1

FIG. 18B

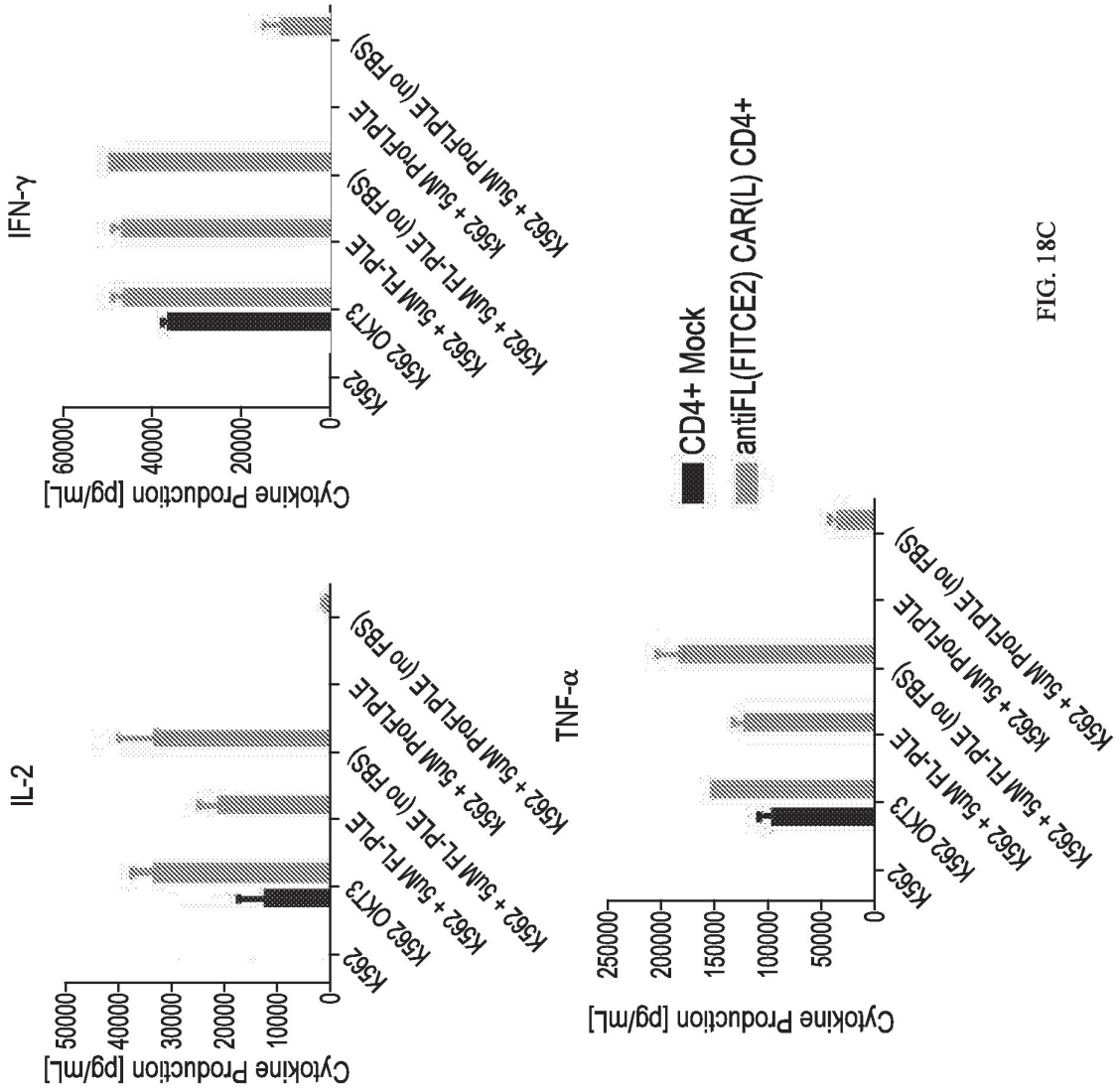

IL-2
IFN-γ
TNF-α
Cytokine Production [pg/mL]
CD4+ Mock
antiFL(FITCE2) CAR(L) CD4+
K562
K562 OKT3
K562 + 5uM FL-PLE
K562 + 5uM FL-PLE (no FBS)
K562 + 5uM ProFLPLE
K562 + 5uM ProFLPLE (no FBS)

FIG. 18C

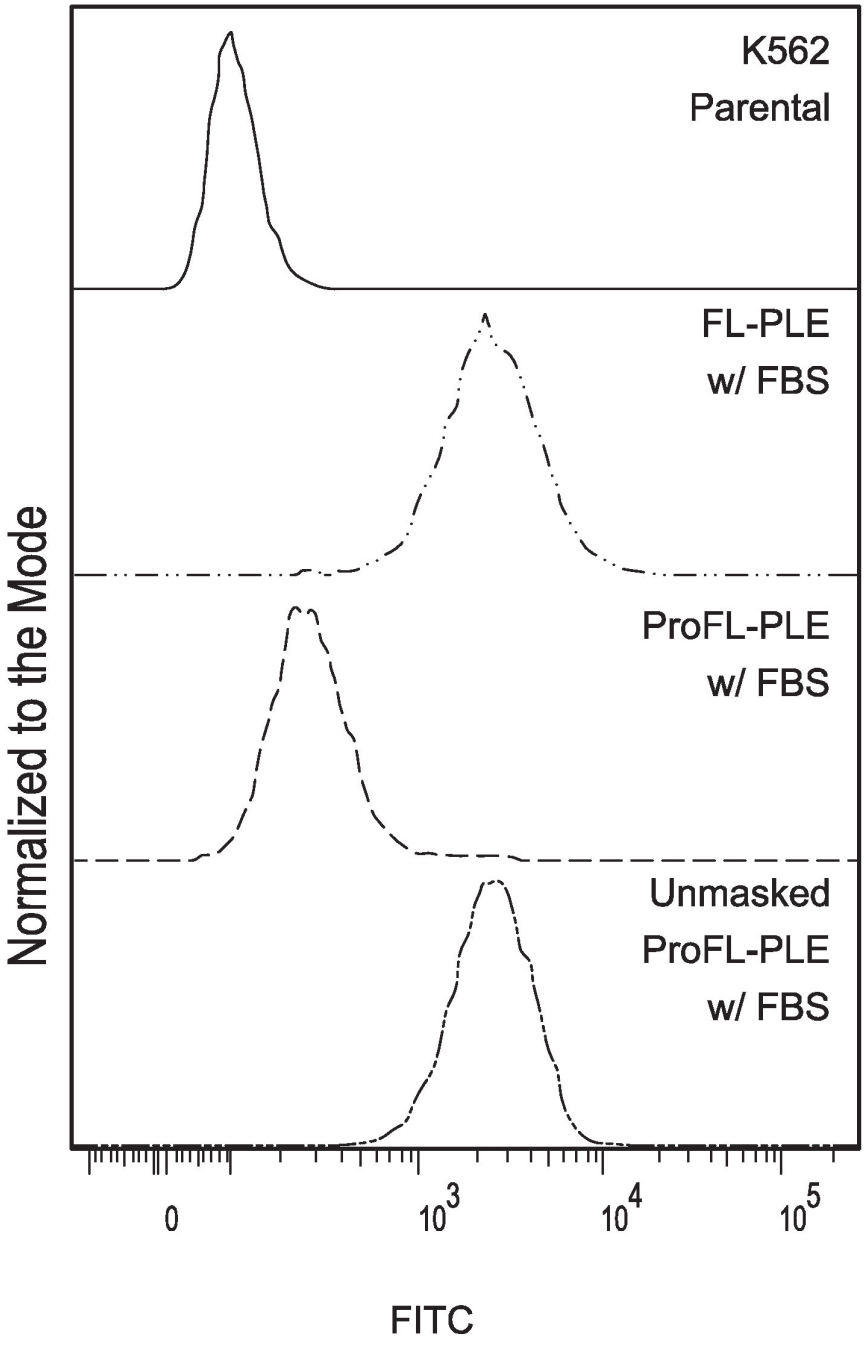

K562
Parental
FL-PLE
w/ FBS
ProFL-PLE
w/ FBS
Unmasked
ProFL-PLE
w/ FBS
Normalized to the Mode
0
10^3
10^4
10^5
FITC

FIG. 19A

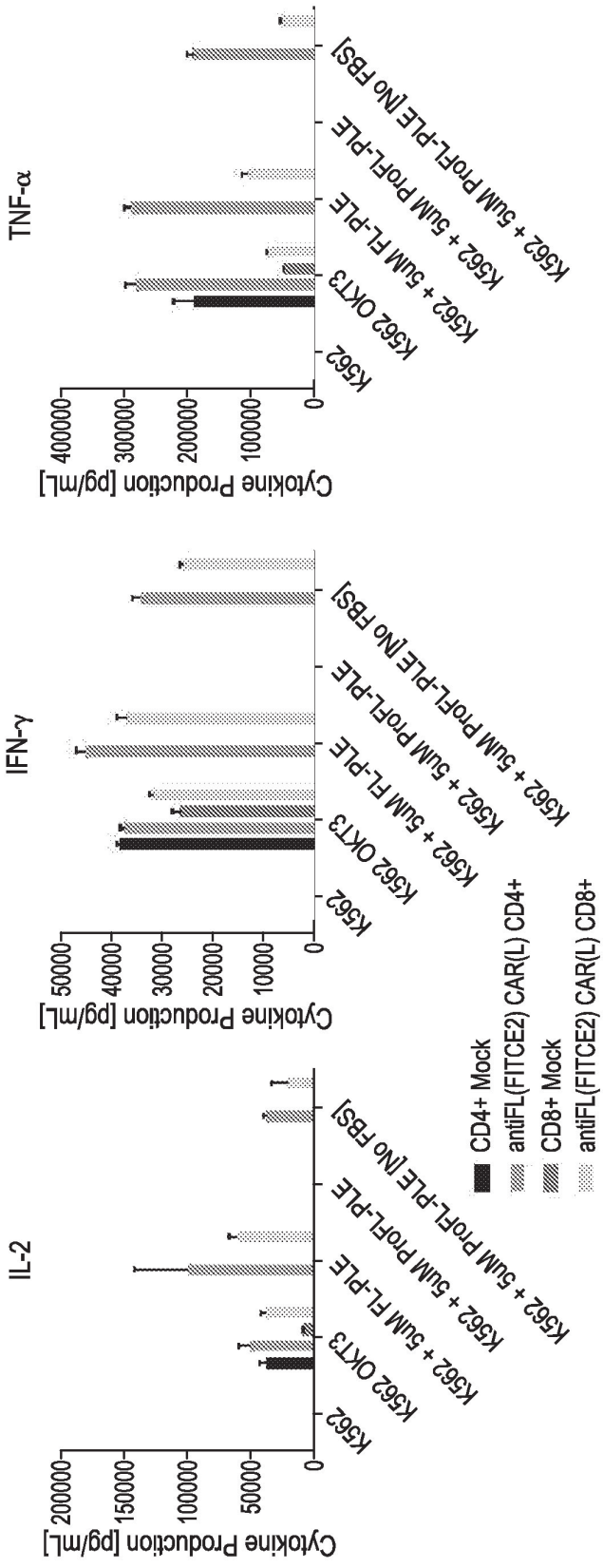

IL-2
IFN-γ
TNF-α
Cytokine Production [pg/mL]
200000
150000
100000
50000
0
50000
40000
30000
20000
10000
0
400000
300000
200000
100000
0
K562
K562 OKT3
K562 + 5uM FL-PLE
K562 + 5uM ProFL-PLE
K562 + 5uM ProFL-PLE [No FBS]
CD4+ Mock
antiFL(FITCE2) CAR(L) CD4+
CD8+ Mock
antiFL(FITCE2) CAR(L) CD8+

FIG. 19B

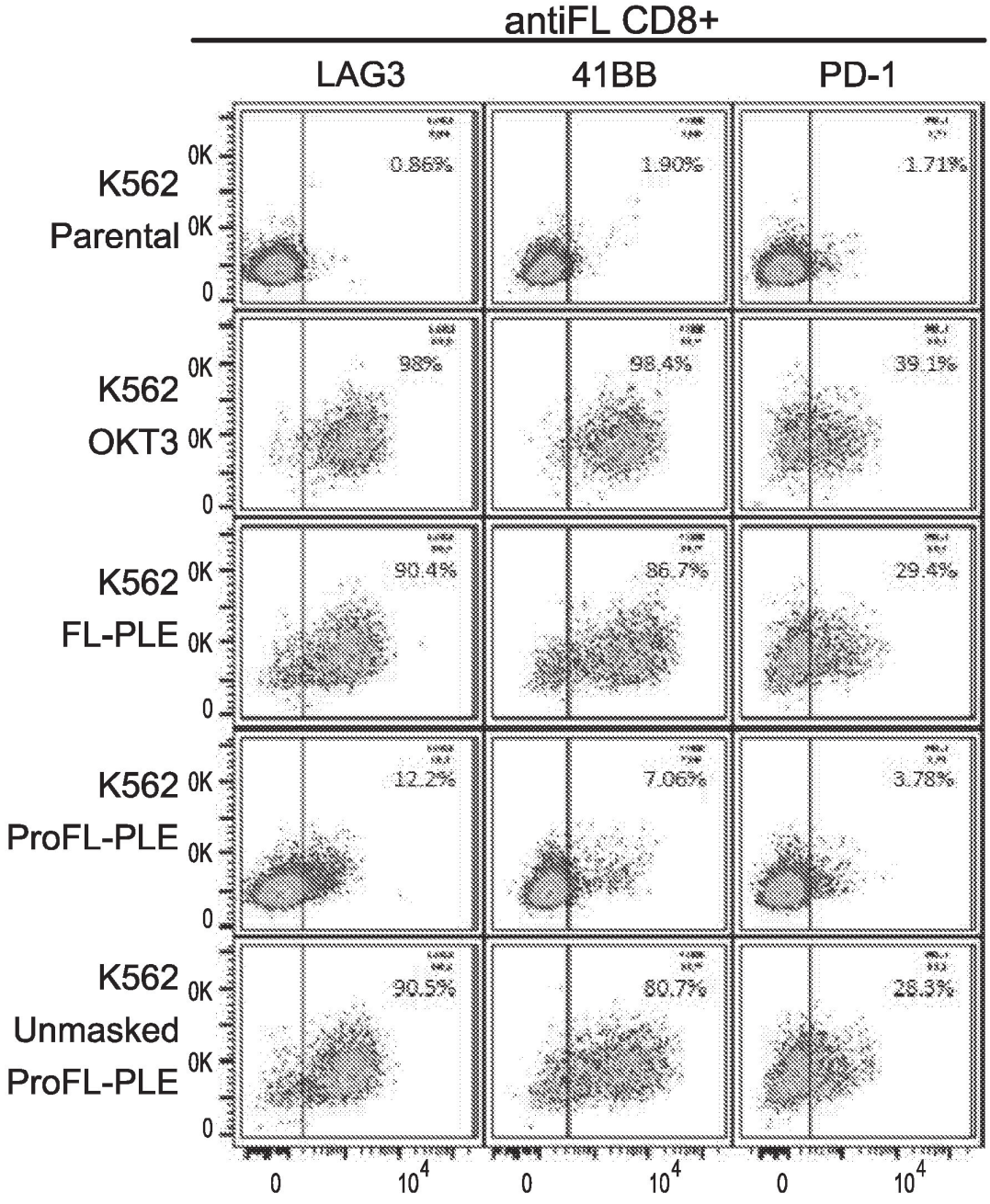

antiFL CD8+
LAG3
41BB
PD-1
K562 Parental
K562 OKT3
K562 FL-PLE
K562 ProFL-PLE
K562 Unmasked ProFL-PLE
0.86%
1.90%
1.71%
98%
98.4%
39.1%
90.4%
86.7%
29.4%
12.2%
7.06%
3.78%
90.5%
80.7%
28.3%
0K
0
10^4

FIG. 19C

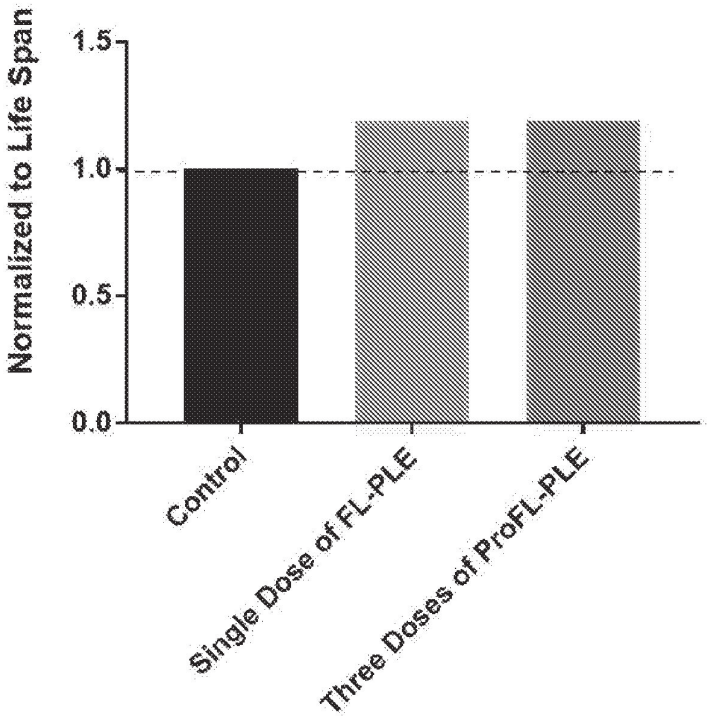
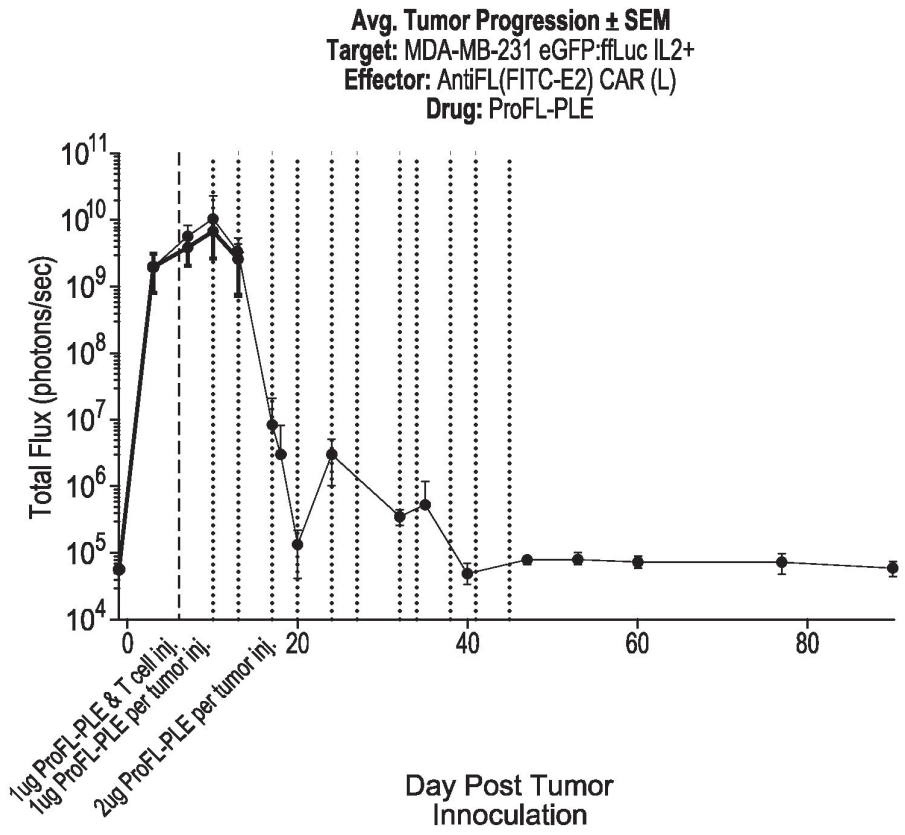
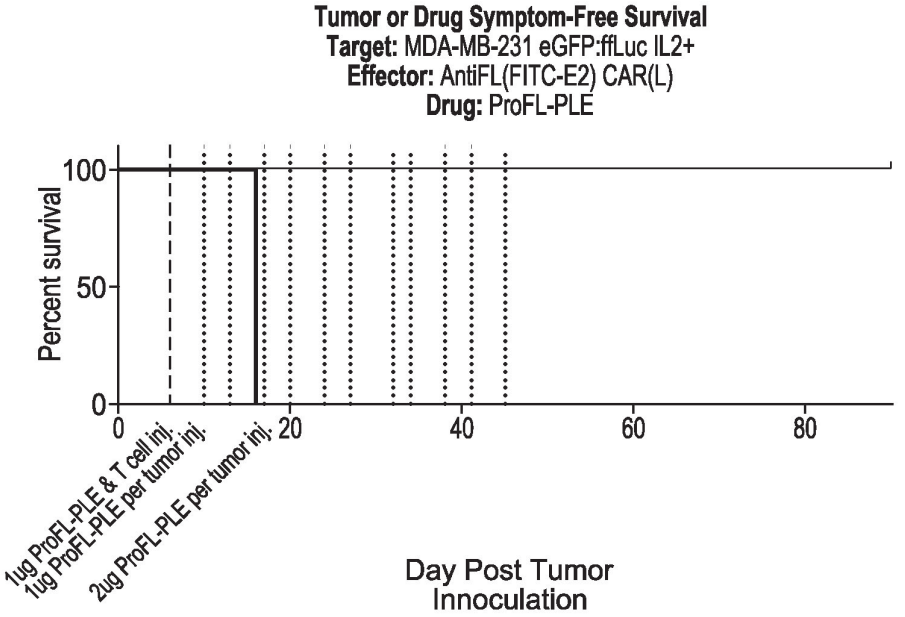

Normalized to Life Span
1.5
1.0
0.5
0.0
Control
Single Dose of FL-PLE
Three Doses of ProFL-PLE

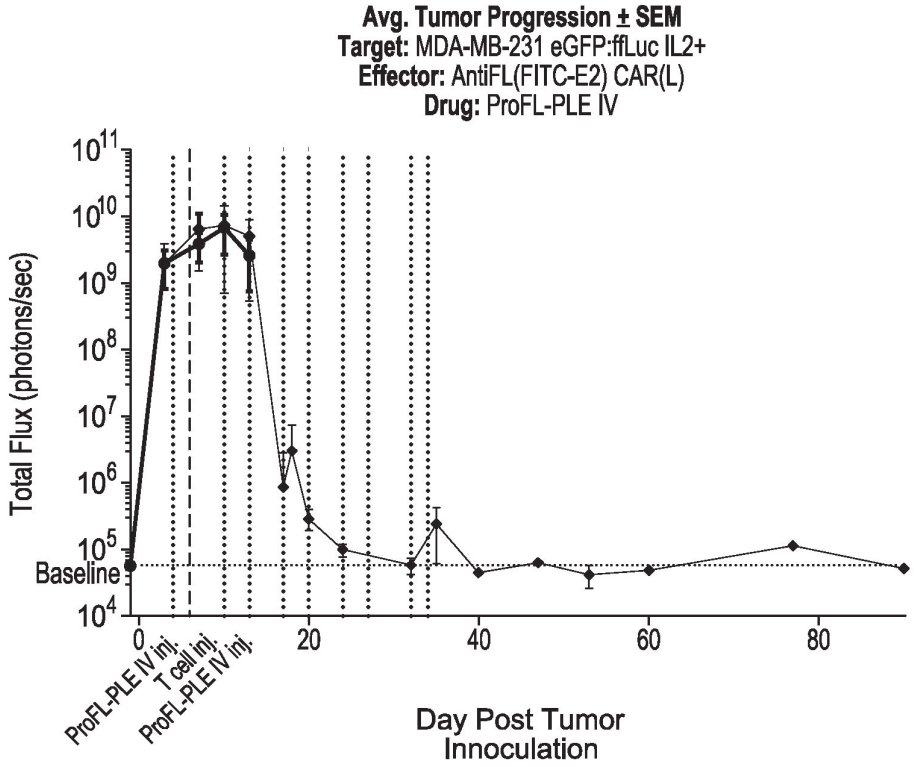
FIG. 20

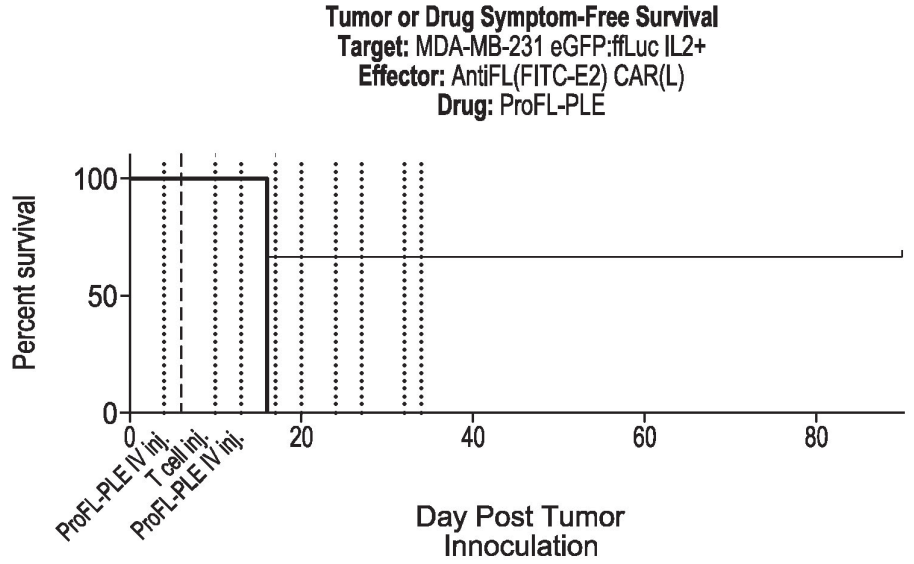




— No Drug
— 1ug or 2ug ProFL-PLE per tumor (intratumoral inj) (6 days post tumor) with redose

FIG. 21A





No Drug

0.75mg ProFL-PLE IV (4 days post tumor) with Redose

FIG 21B