FIG. 1A

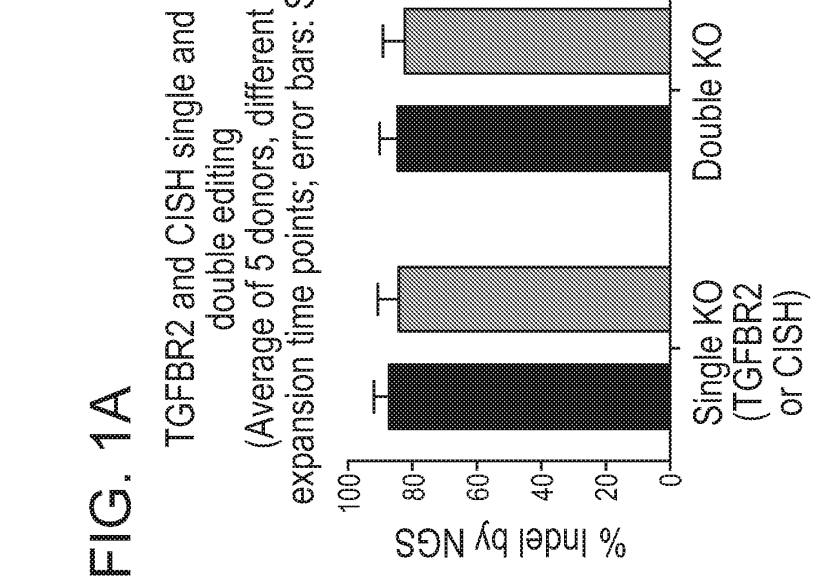
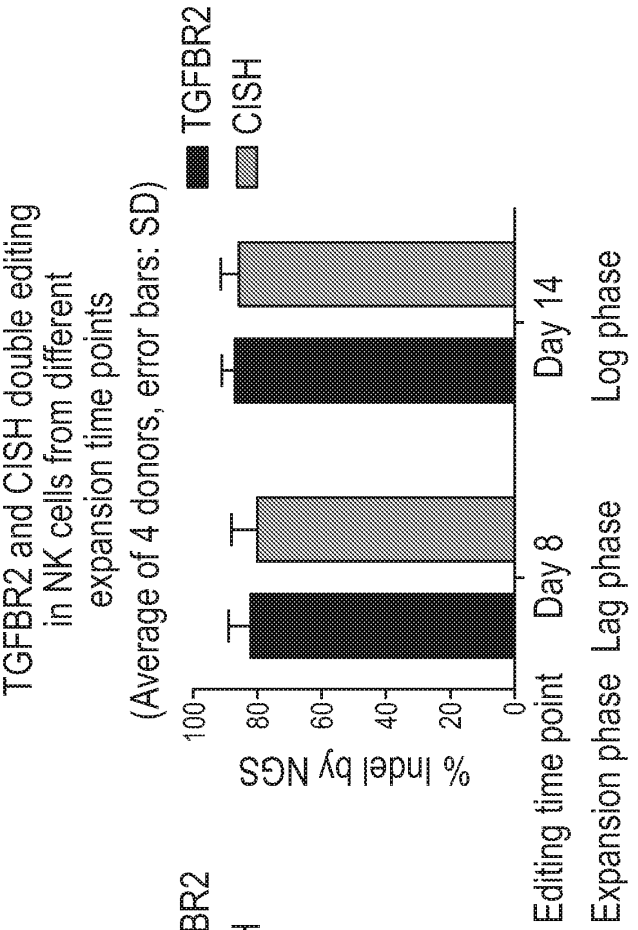

TGFBR2 and CISH single and double editing
(Average of 5 donors, different expansion time points; error bars: SD)
TGFBR2
CISH
% Indel by NGS
100
80
60
40
20
0
Single KO (TGFBR2 or CISH)
Double KO

FIG. 1B


TGFBR2 and CISH double editing in NK cells from different expansion time points
(Average of 4 donors, error bars: SD)
TGFBR2
CISH
% Indel by NGS
100
80
60
40
20
0
Editing time point
Day 8
Day 14
Expansion phase
Lag phase
Log phase

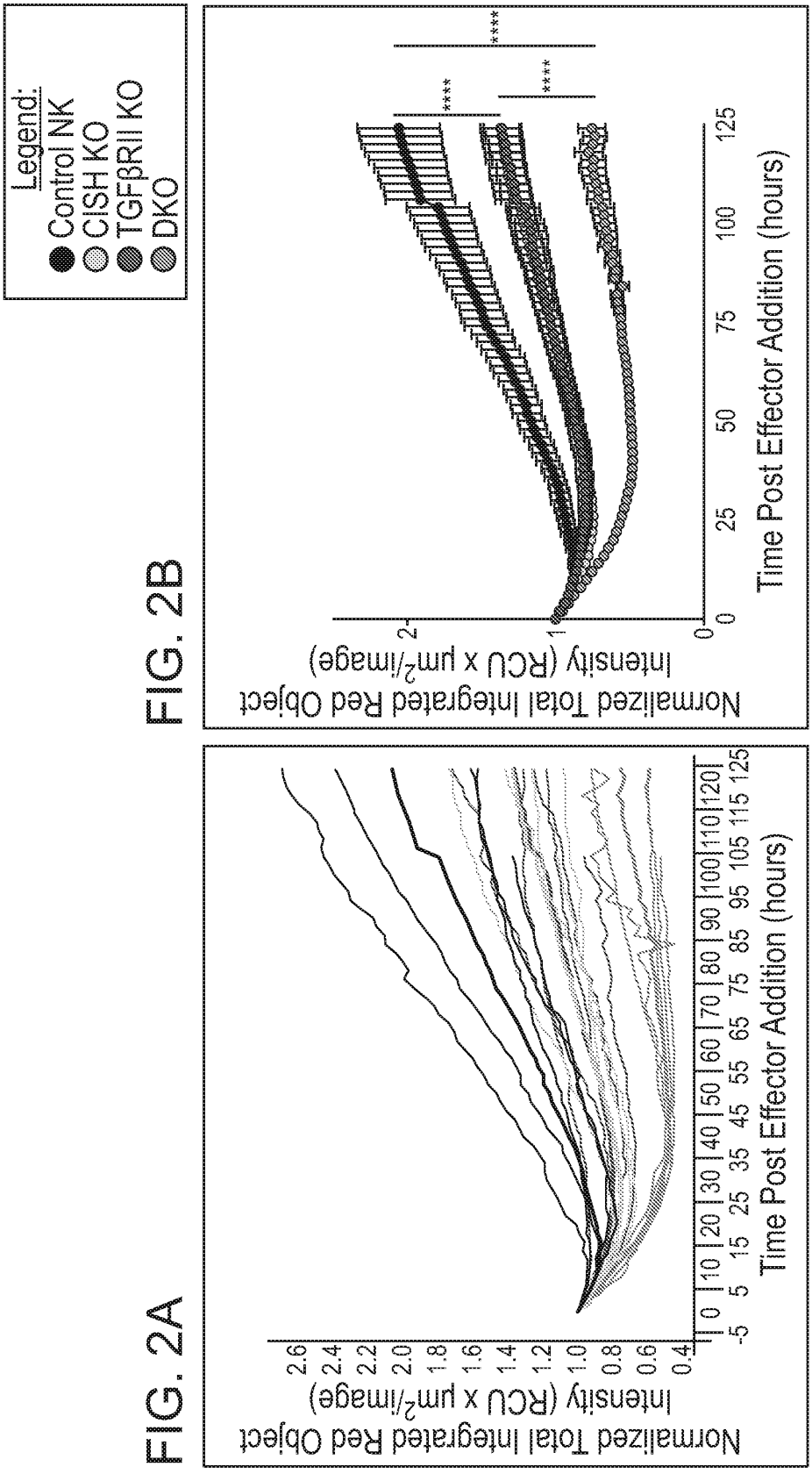
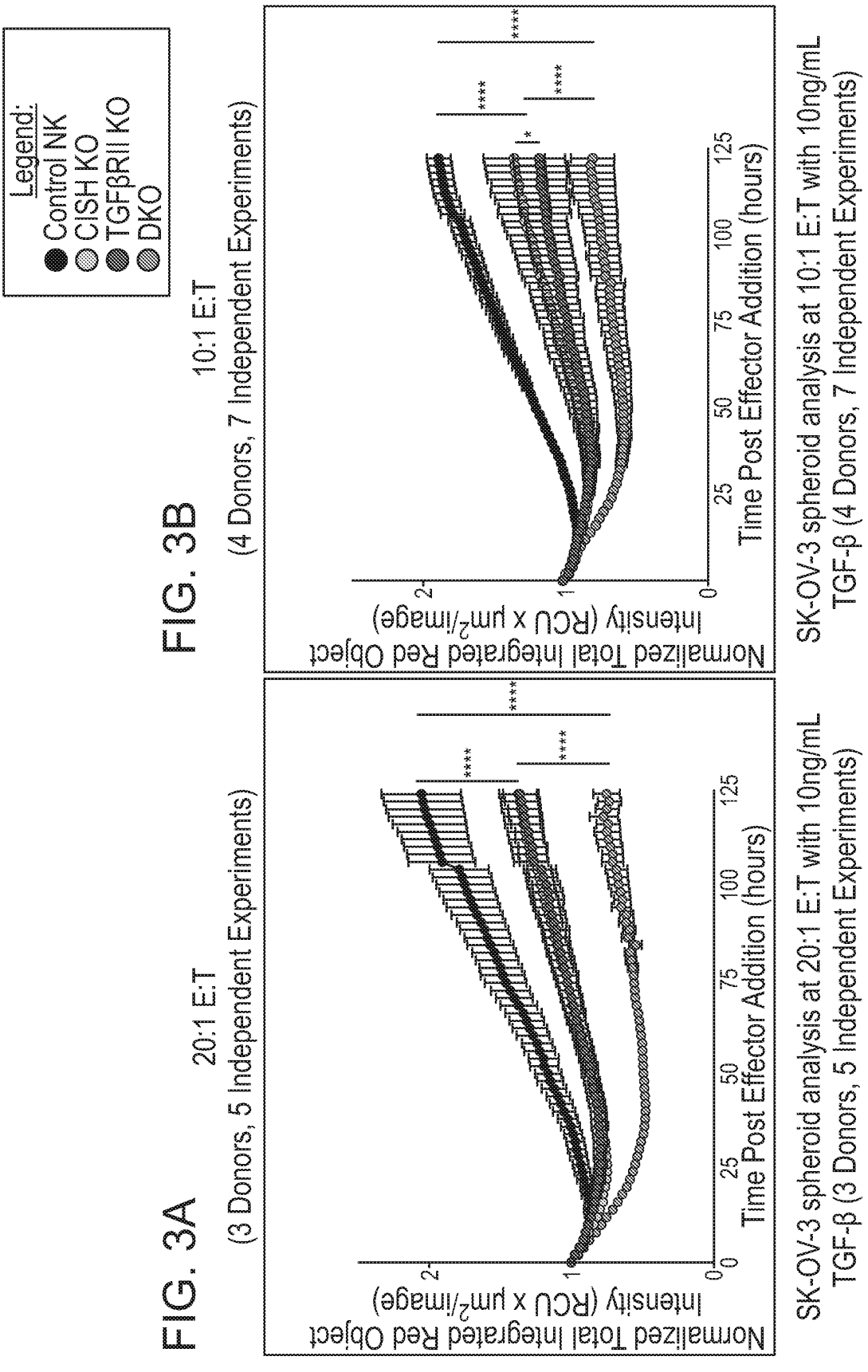
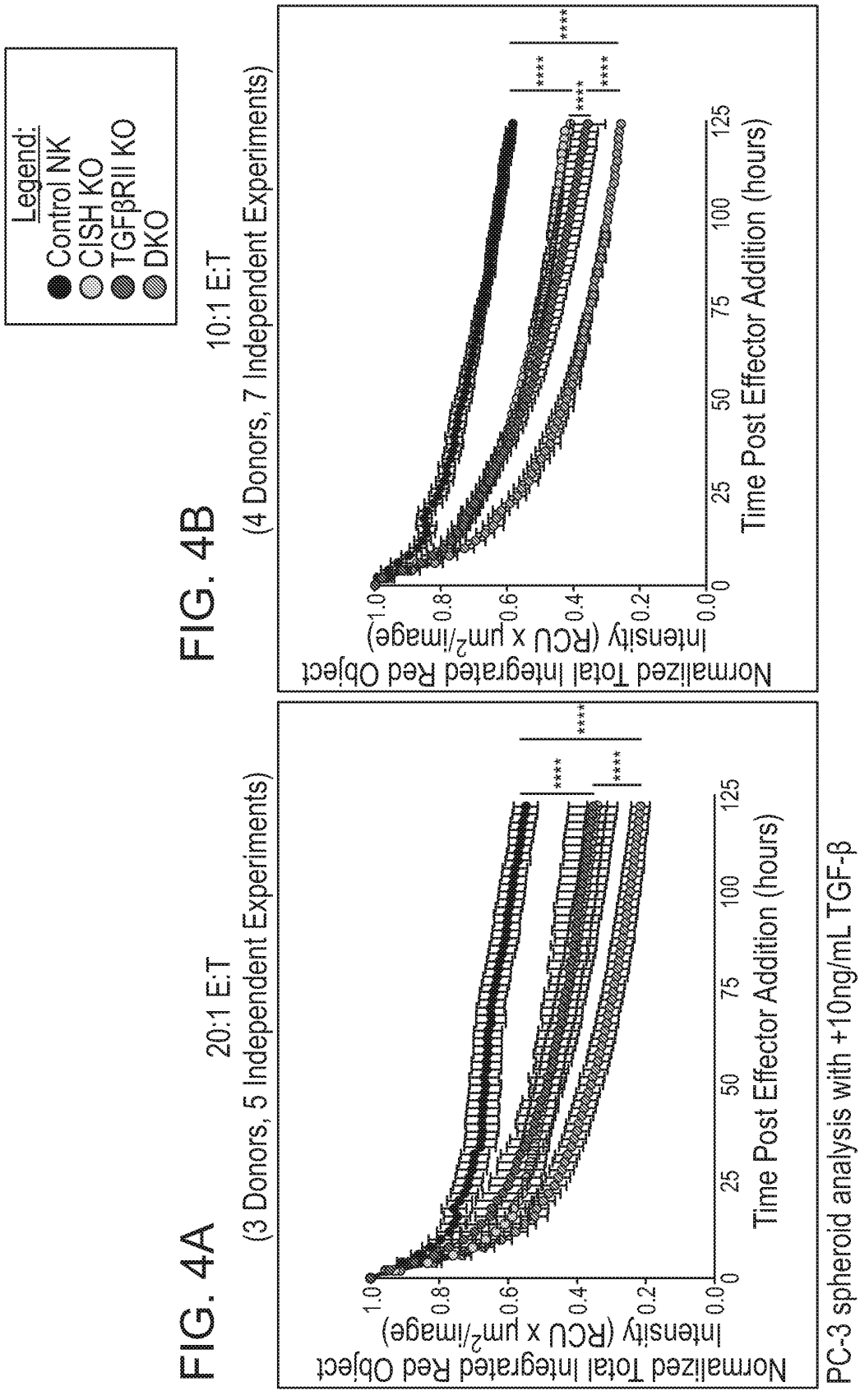

Legend:
Control NK
CISH KO
TGFβRII KO
DKO
Normalized Total Integrated Red Object Intensity (RCU x μm²/image)
Time Post Effector Addition (hours)
****
****
****
0
25
50
75
100
125
1
2
Normalized Total Integrated Red Object Intensity (RCU x μm²/image)
Time Post Effector Addition (hours)
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
2.6
-5
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125

FIG. 2B

FIG. 2A


Legend:
Control NK
CISH KO
TGFβRII KO
DKO
FIG. 3A
20:1 E:T
(3 Donors, 5 Independent Experiments)
FIG. 3B
10:1 E:T
(4 Donors, 7 Independent Experiments)
Normalized Total Integrated Red Object Intensity (RCU x μm²/Image)
Time Post Effector Addition (hours)
0
25
50
75
100
125
1
2
*
****
SK-OV-3 spheroid analysis at 20:1 E:T with 10ng/mL TGF-β (3 Donors, 5 Independent Experiments)
SK-OV-3 spheroid analysis at 10:1 E:T with 10ng/mL TGF-β (4 Donors, 7 Independent Experiments)


Legend:
Control NK
CISH KO
TGFβRII KO
DKO
FIG. 4A
20:1 E:T
(3 Donors, 5 Independent Experiments)
FIG. 4B
10:1 E:T
(4 Donors, 7 Independent Experiments)
Normalized Total Integrated Red Object Intensity (RCU x µm²/image)
Time Post Effector Addition (hours)
0.0
0.2
0.4
0.6
0.8
1.0
0
25
50
75
100
125
****
PC-3 spheroid analysis with +10ng/mL TGF-β

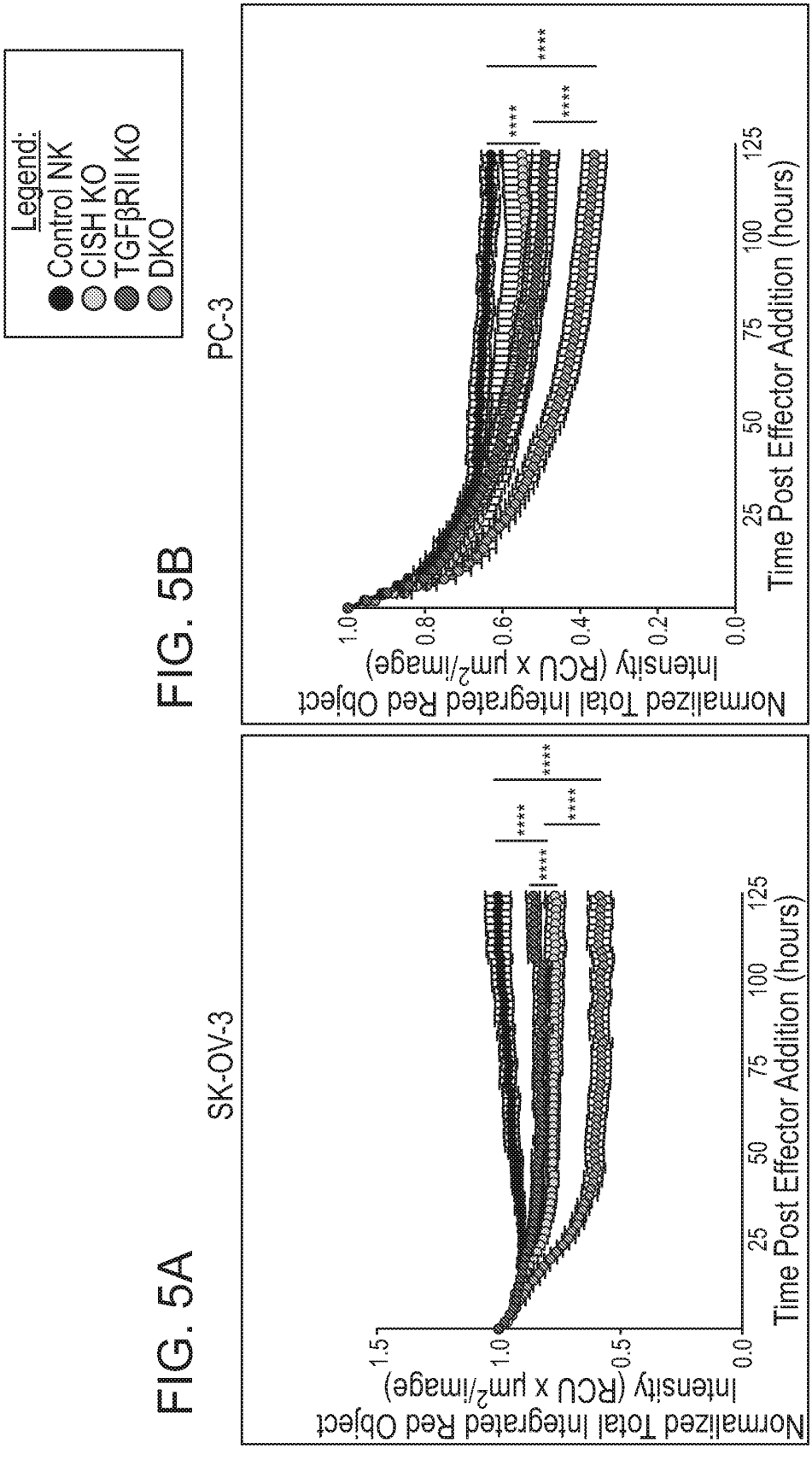


FIG. 5A

FIG. 5B

Spheroid analysis at 10:1 E:T (no exogenous cytokine)
(4 Donors, 7 Independent Experiments)

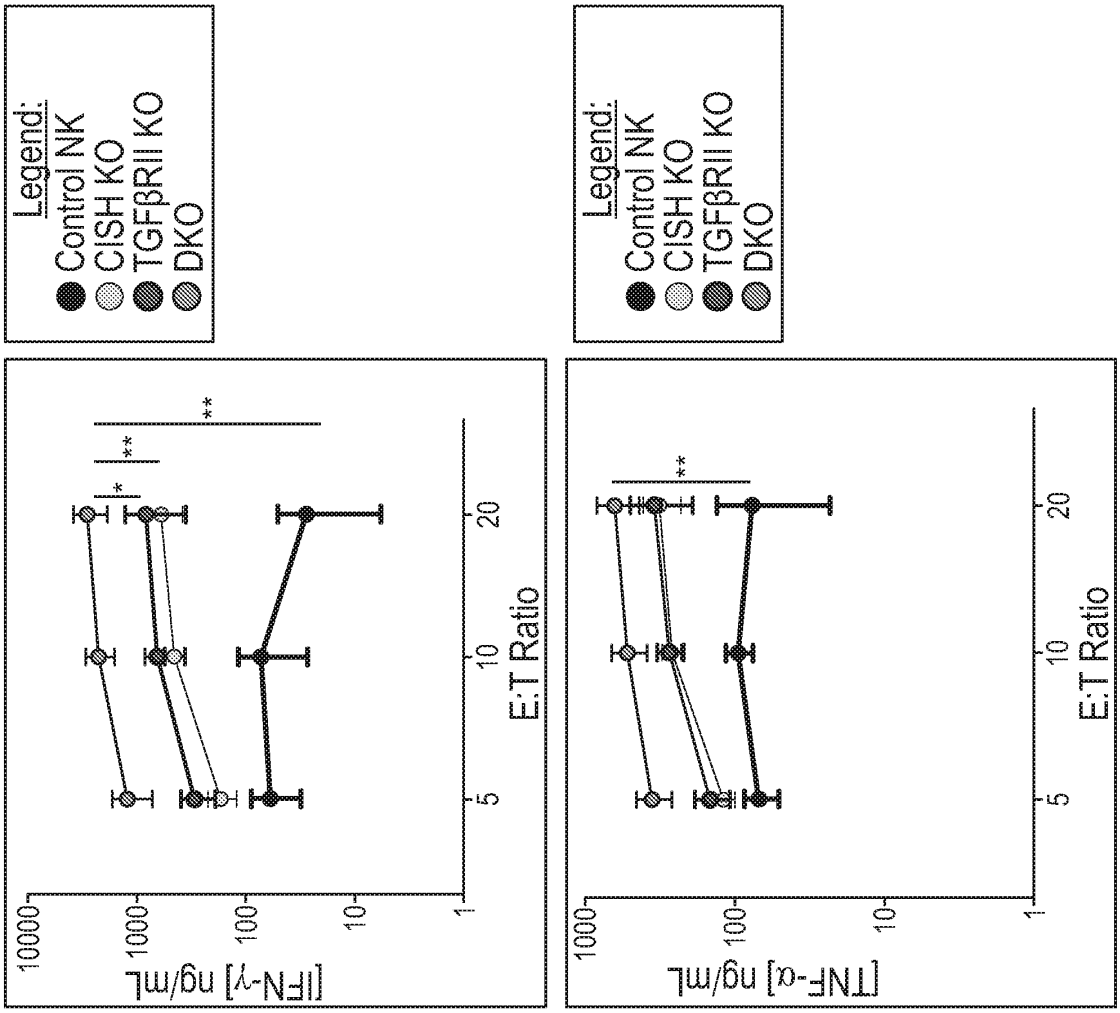
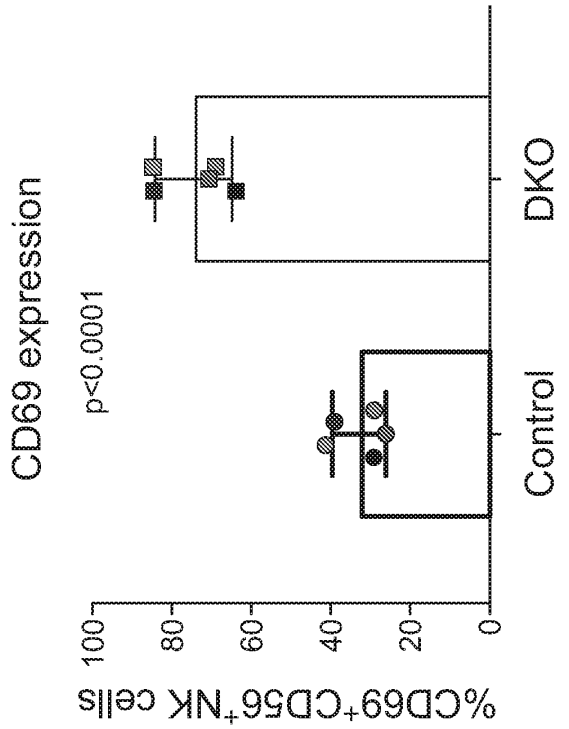

Legend:
Control NK
CISH KO
TGFβRII KO
DKO
[IFN-γ] ng/mL
10000
1000
100
10
1
1
5
10
20
E:T Ratio
*
**
**

FIG. 6A

Legend:
Control NK
CISH KO
TGFβRII KO
DKO
[TNF-α] ng/mL
1000
100
10
1
1
5
10
20
E:T Ratio
**

FIG. 6B


CD69 expression
p<0.0001
%CD69+CD56+NK cells
100
80
60
40
20
0
Control
DKO

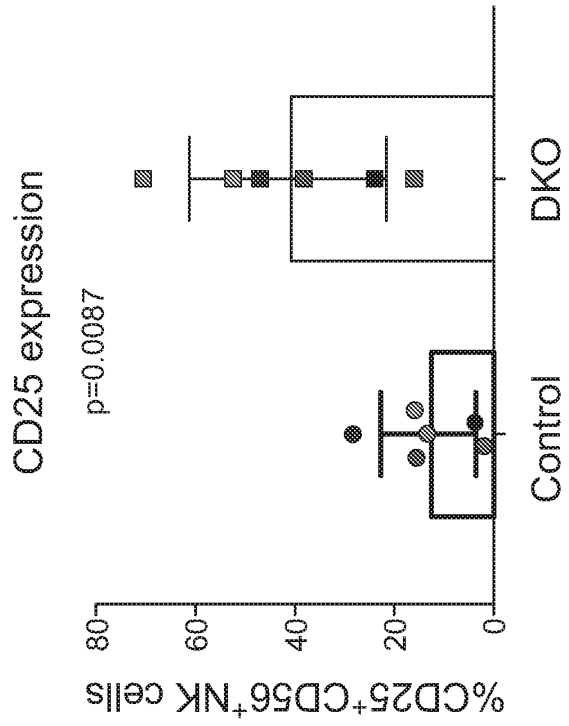

CD25 expression
p=0.0087
%CD25+CD56+NK cells
80
60
40
20
0
Control
DKO

FIG. 6C

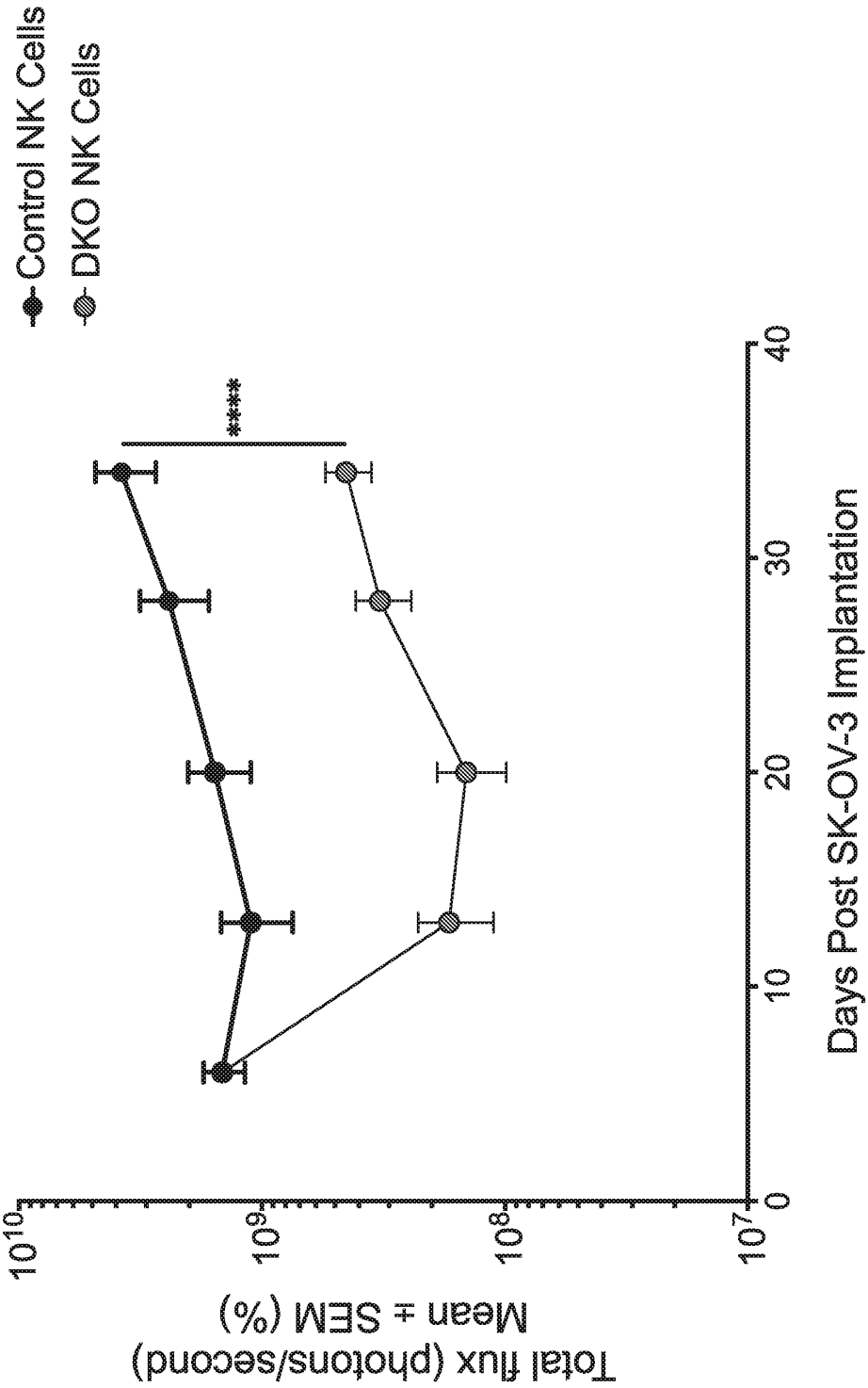

Control NK Cells
DKO NK Cells
****
Total flux (photons/second)
Mean ± SEM (%)
10^{10}
10^{9}
10^{8}
10^{7}
0
10
20
30
40
Days Post SK-OV-3 Implantation

FIG. 6D

FIG. 7A

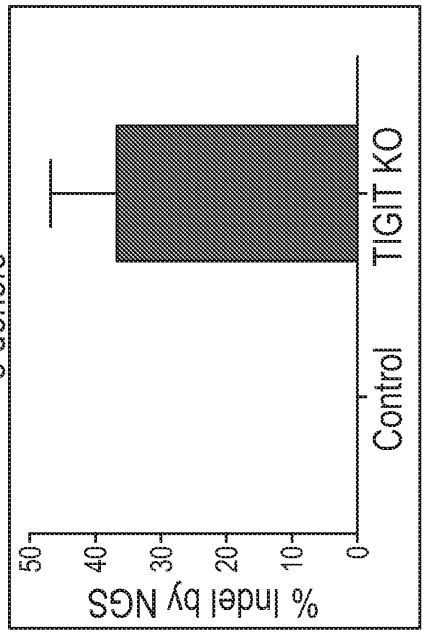


FIG. 7B

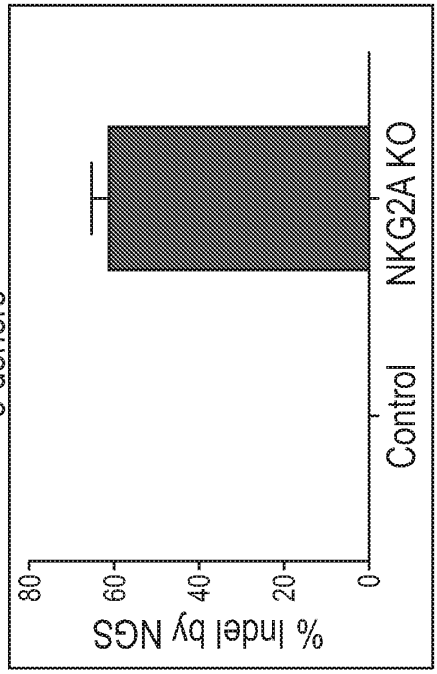


FIG. 7C

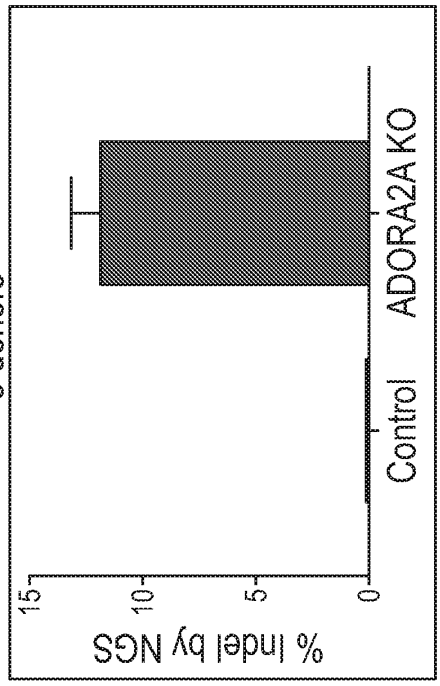


FIG. 8A

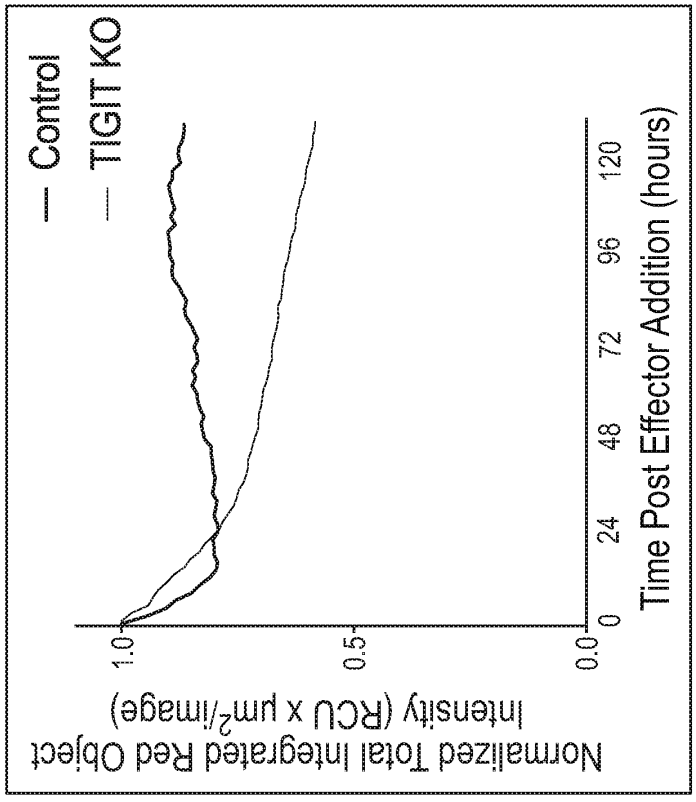

Normalized Total Integrated Red Object Intensity (RCU x μm²/image)
1.0
0.5
0.0
0
24
48
72
96
120
Time Post Effector Addition (hours)
— Control
— TIGIT KO

FIG. 8B

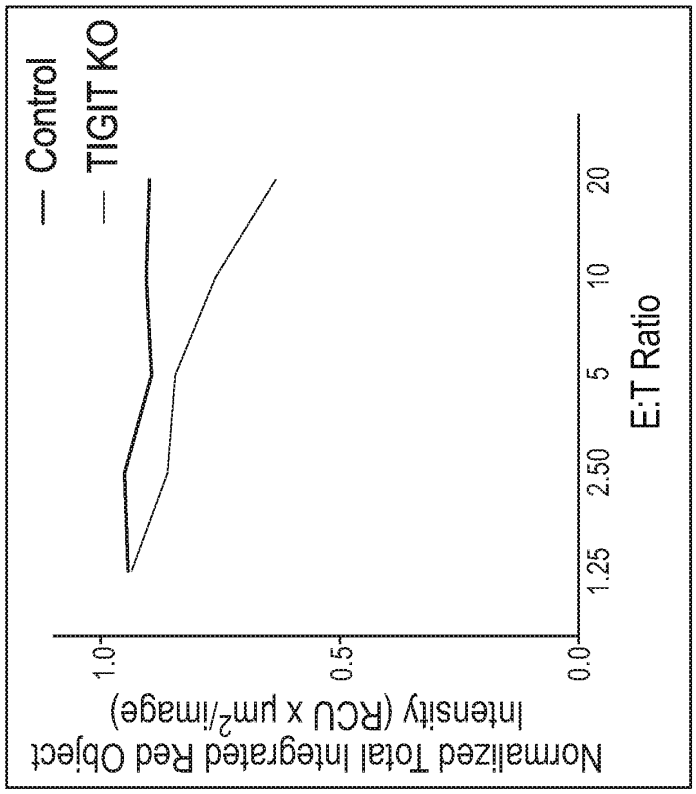

Normalized Total Integrated Red Object Intensity (RCU x μm²/image)
1.0
0.5
0.0
1.25
2.50
5
10
20
E:T Ratio
— Control
— TIGIT KO

FIG. 9A

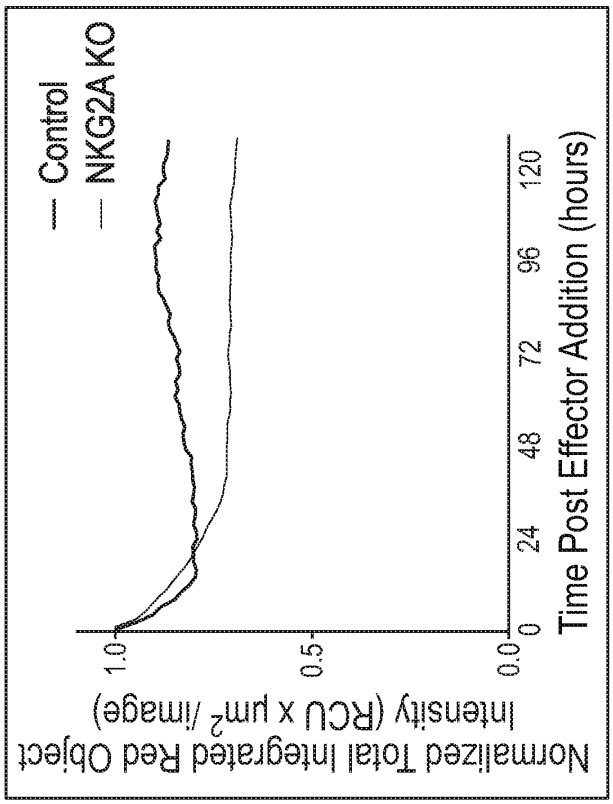

Control
NKG2A KO
Normalized Total Integrated Red Object Intensity (RCU x μm² /image)
1.0
0.5
0.0
0
24
48
72
96
120
Time Post Effector Addition (hours)

FIG. 9B

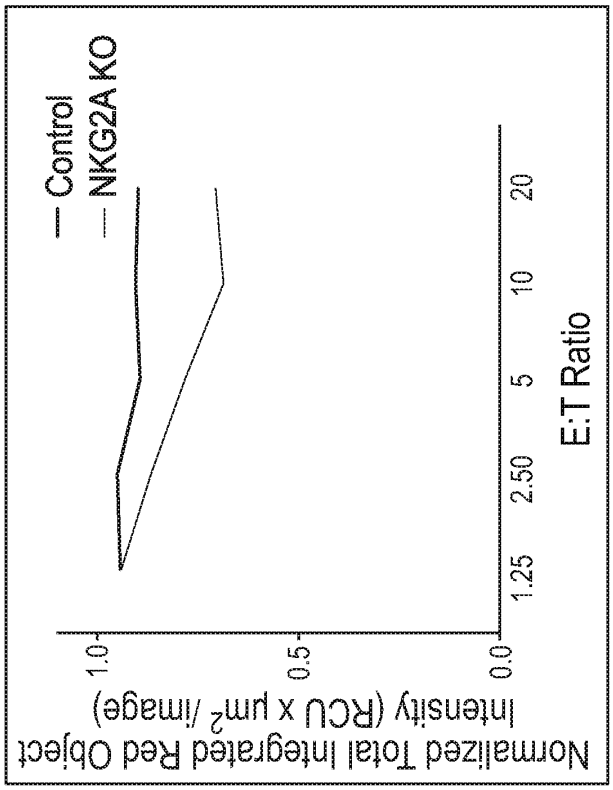

Control
NKG2A KO
Normalized Total Integrated Red Object Intensity (RCU x μm² /image)
1.0
0.5
0.0
1.25
2.50
5
10
20
E:T Ratio

FIG. 10A

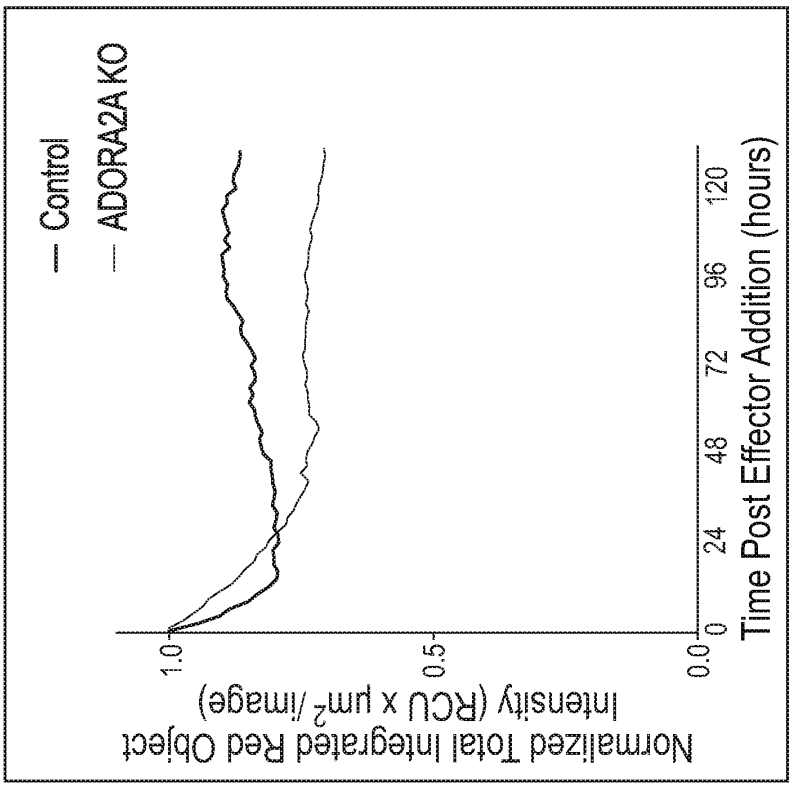


FIG. 10B

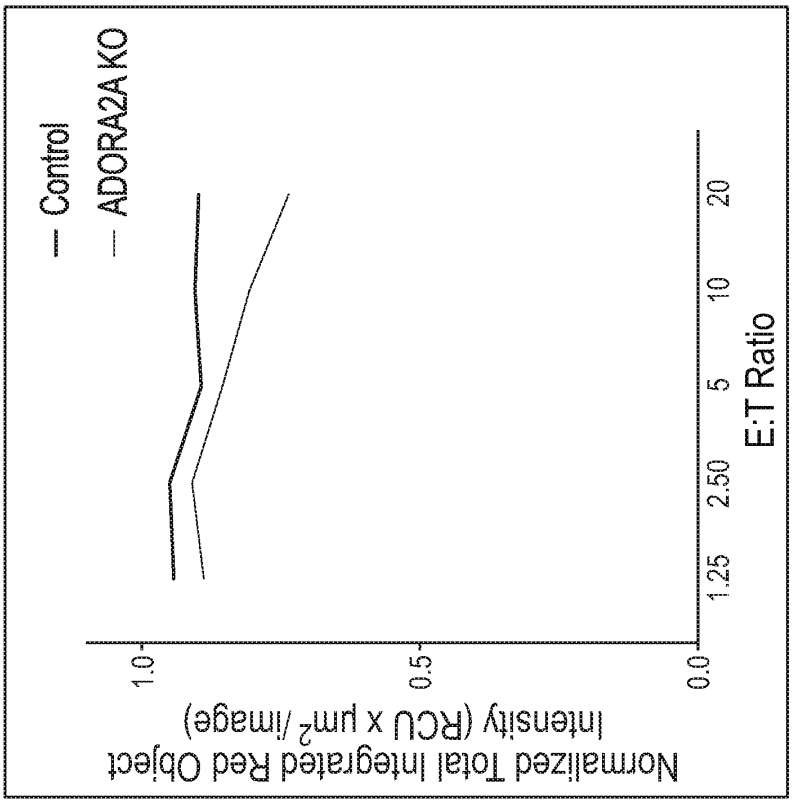


FIG. 11

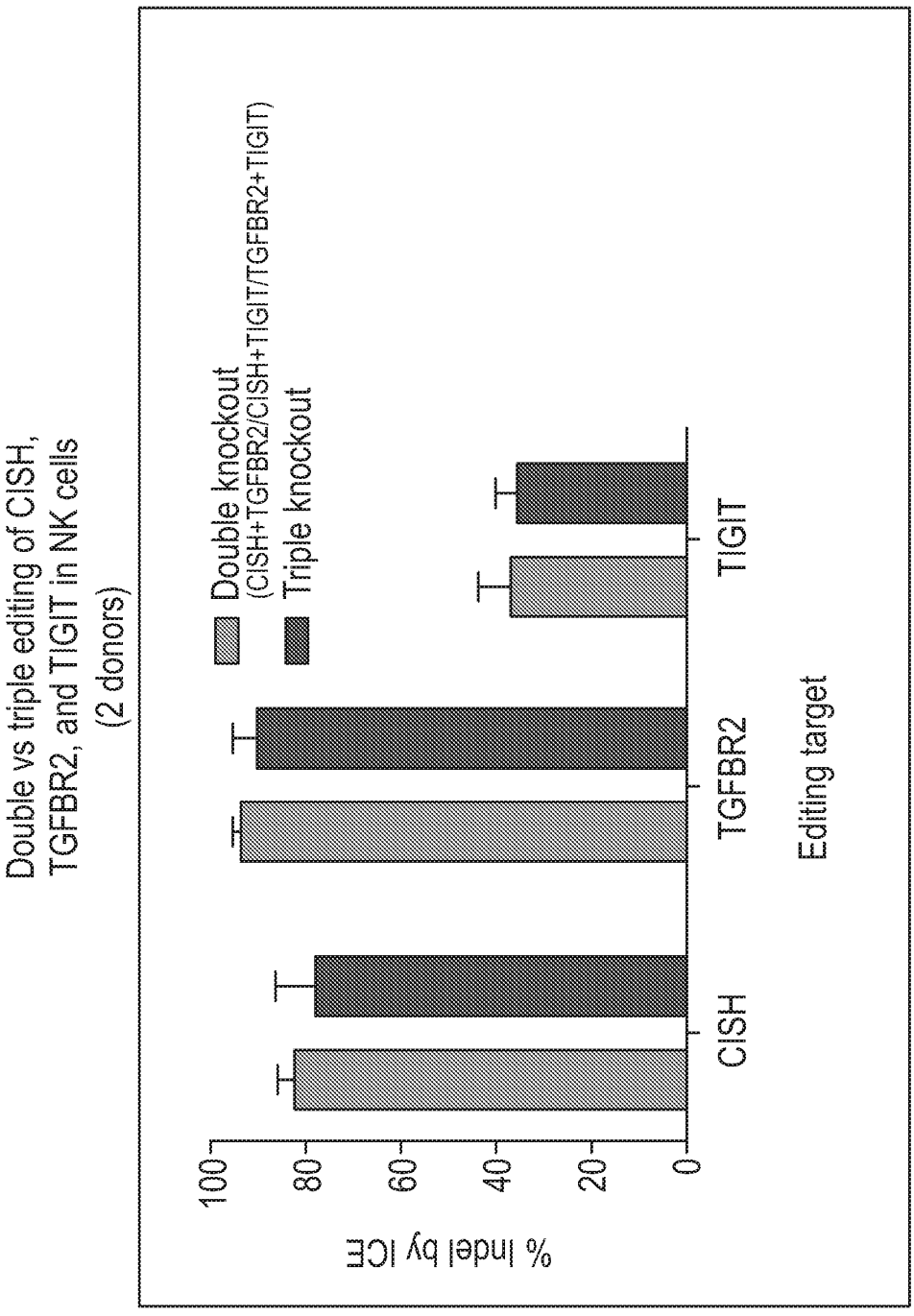

Double vs triple editing of CISH, TGFBR2, and TIGIT in NK cells
(2 donors)
Double knockout
(CISH+TGFBR2/CISH+TIGIT/TGFBR2+TIGIT)
Triple knockout
% Indel by ICE
100
80
60
40
20
0
CISH
TGFBR2
TIGIT
Editing target

FIG. 12A

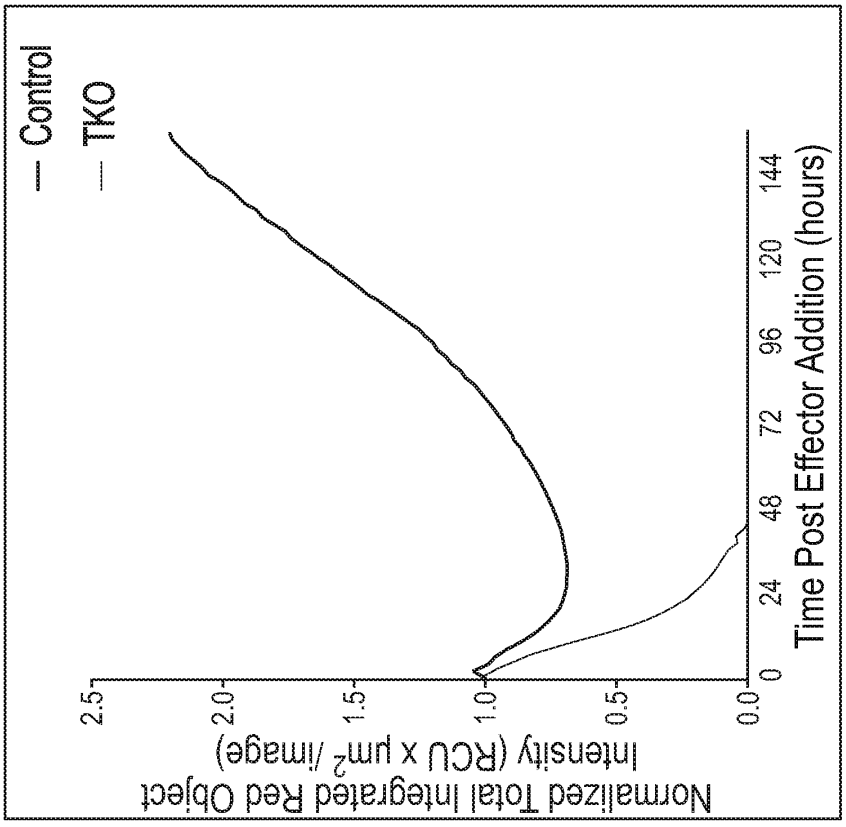


FIG. 12B

