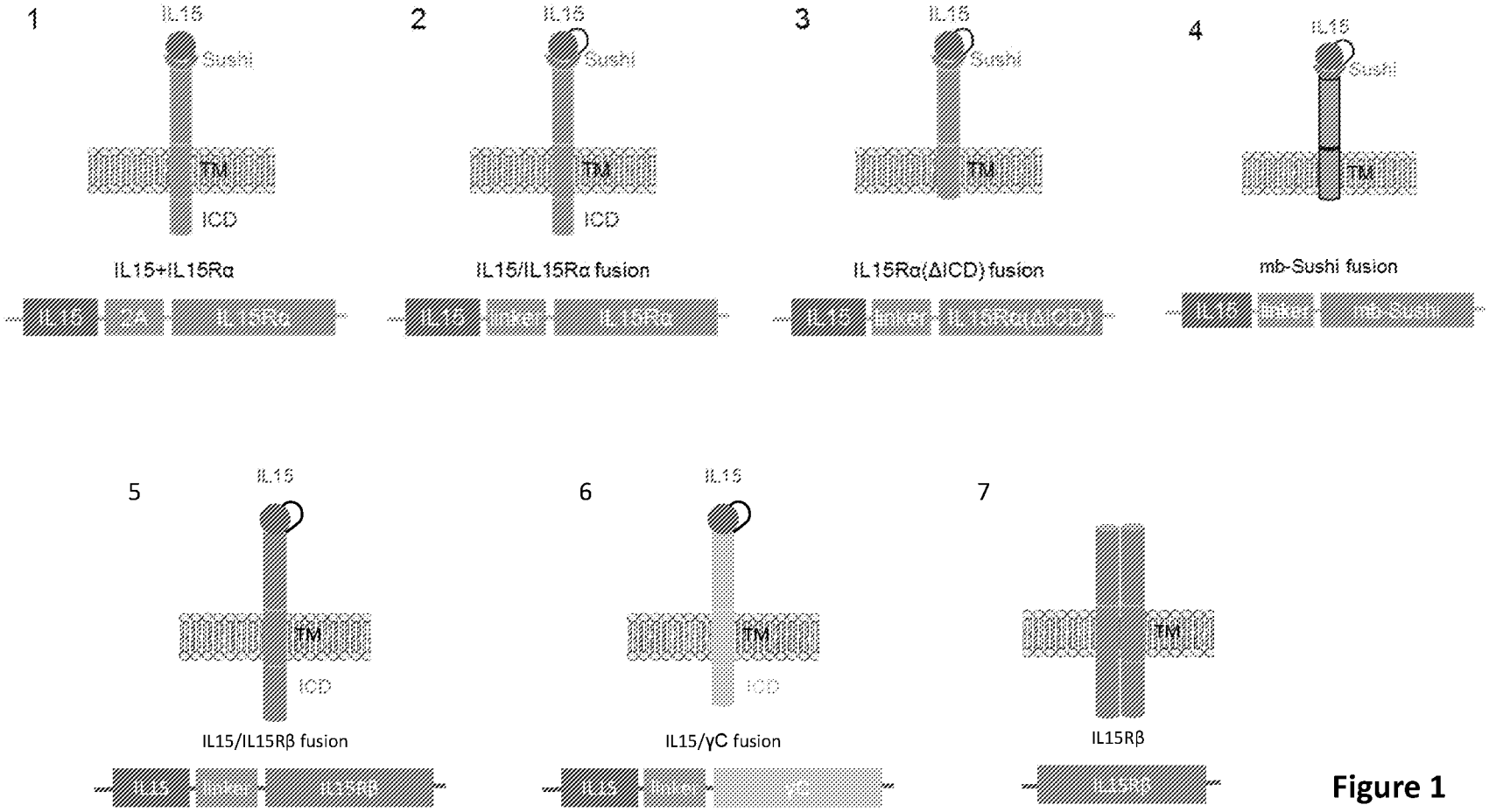

1
IL15
Sushi
TM
ICD
IL15+IL15Rα
2
IL15
Sushi
TM
ICD
IL15/IL15Rα fusion
3
IL15
Sushi
TM
IL15Rα(ΔICD) fusion
4
IL15
Sushi
TM
mb-Sushi fusion
5
IL15
TM
ICD
IL15/IL15Rβ fusion
6
IL15
TM
ICD
IL15/γC fusion
7
TM
IL15Rβ

Figure 1

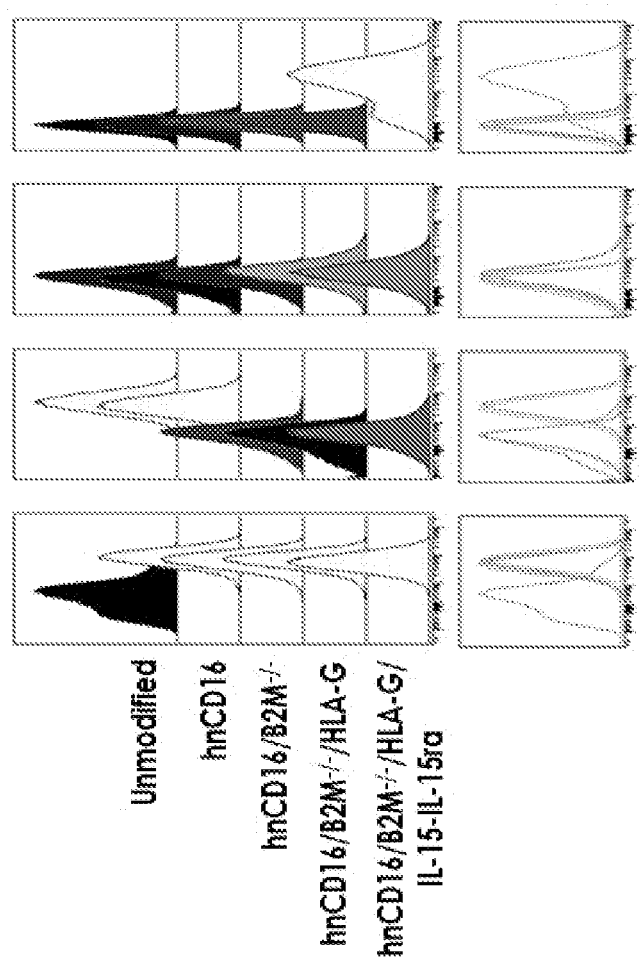

Unmodified
hnCD16
hnCD16/B2M-/-
hnCD16/B2M-/-/HLA-G
hnCD16/B2M-/-/HLA-G/
IL-15-IL-15rα
CD16
HLA-A2
HLA-G
LMIR
Unmodified
hnCD16
hnCD16/B2M
hnCD16/B2M/HLA-G
hnCD16/B2M/HLA-G/IL-15-IL-15rα

Figure 2

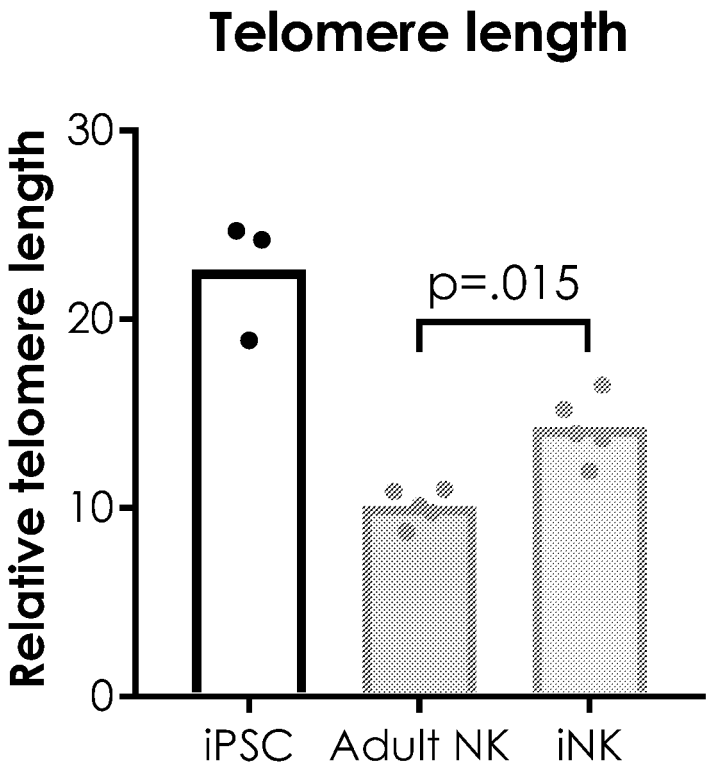

Telomere length
Relative telomere length
30
20
10
0
p=.015
iPSC
Adult NK
iNK

Figure 3

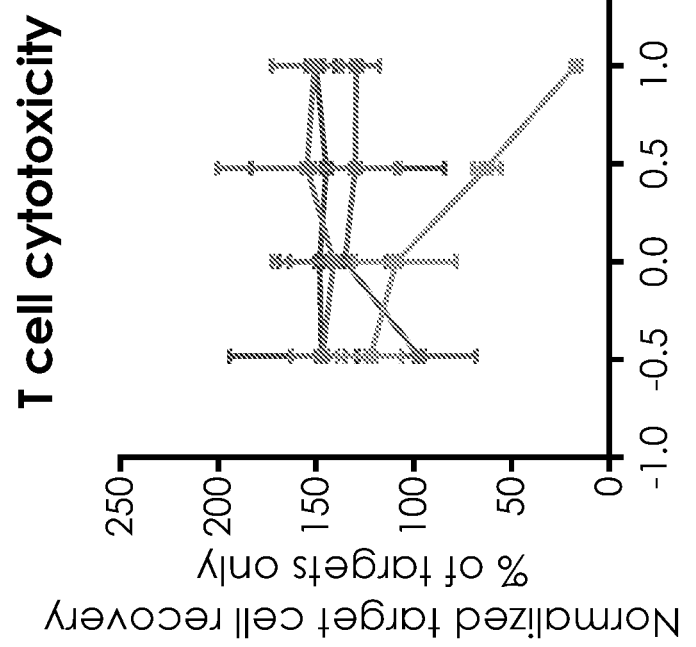

T cell cytotoxicity
Normalized target cell recovery
% of targets only
250
200
150
100
50
0
-1.0
-0.5
0.0
0.5
1.0
1.5
Log(E:T) ratio
hnCD16/B2M-/-
hnCD16/B2M-/-HLA-G
hnCD16/B2M-/-HLA-G/
IL-15-IL-15ra
hnCD16

Figure 4

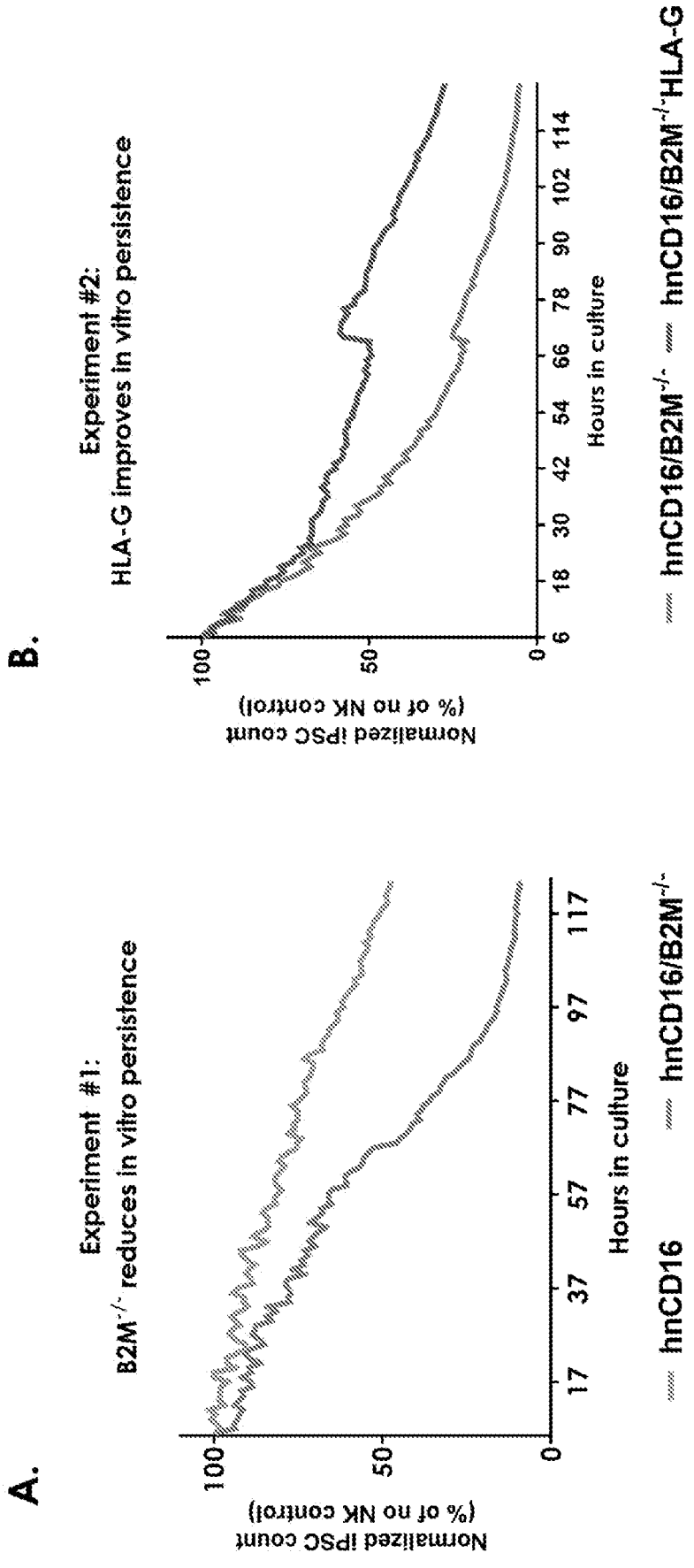

A.
Experiment #1:
B2M-/- reduces in vitro persistence
Normalized iPSC count
(% of no NK control)
100
50
0
17
37
57
77
97
117
Hours in culture
hnCD16
hnCD16/B2M-/-
B.
Experiment #2:
HLA-G improves in vitro persistence
Normalized iPSC count
(% of no NK control)
100
50
0
6
18
30
42
54
66
78
90
102
114
Hours in culture
hnCD16/B2M-/-
hnCD16/B2M-/-HLA-G

Figure 5

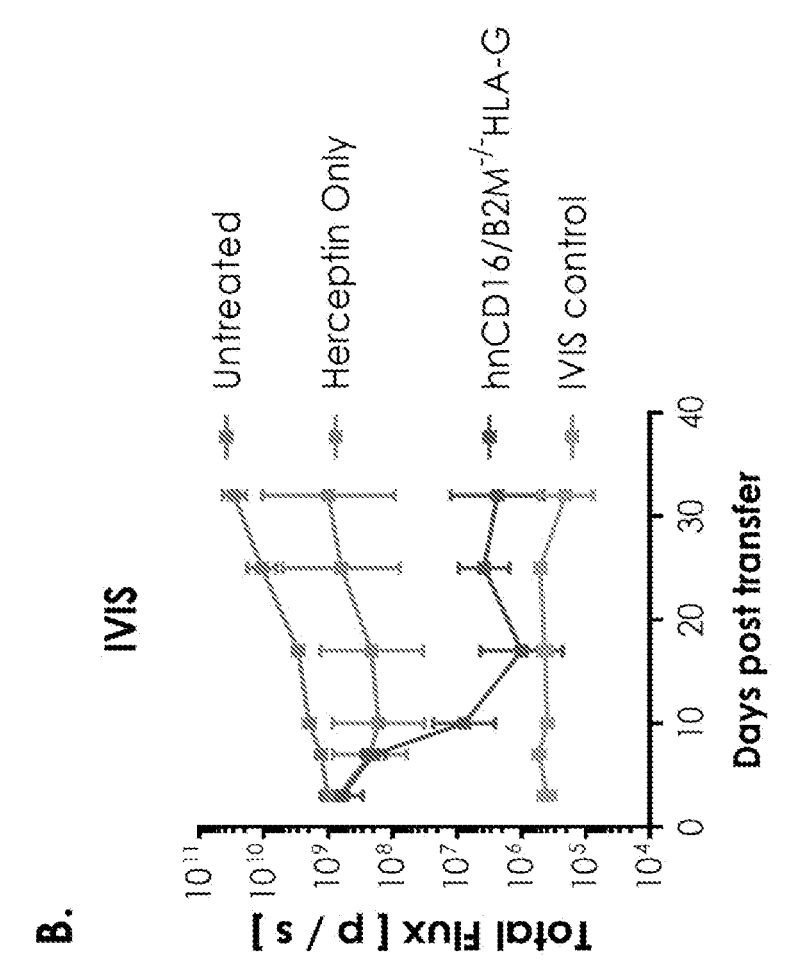

A.
IVIS control
Untreated
Anti-Her2
hnCD16/B2M-/-/HLA-G iNK
Day 3
Day 7
Day 11
Day 18
Day 25
Day 32
B.
IVIS
Total Flux [p / s]
Days post transfer
Untreated
Herceptin Only
hnCD16/B2M-/-HLA-G
IVIS control

Figure 6

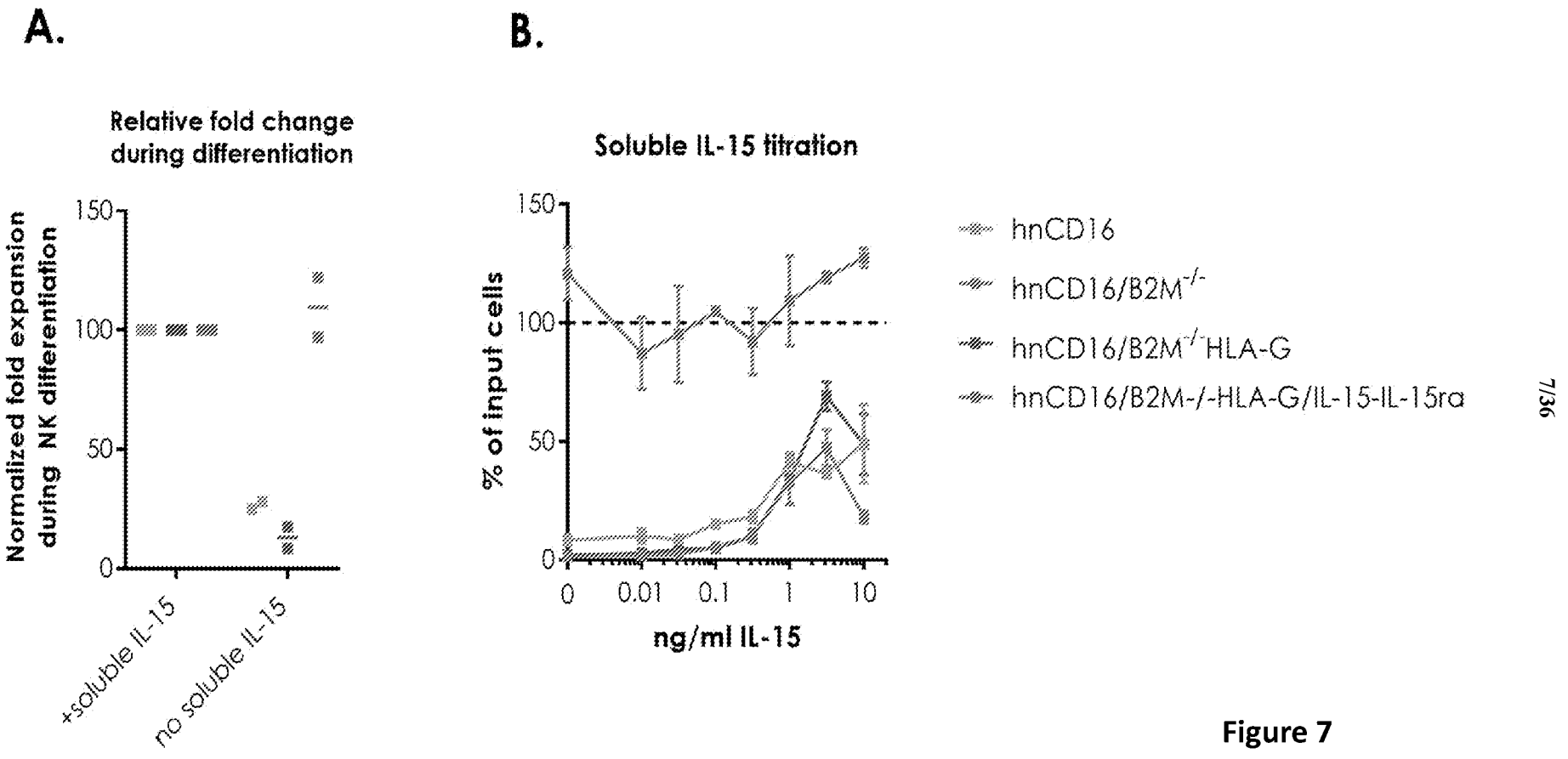

A.
Relative fold change during differentiation
Normalized fold expansion during NK differentiation
150
100
50
0
+soluble IL-15
no soluble IL-15
B.
Soluble IL-15 titration
% of input cells
150
100
50
0
0
0.01
0.1
1
10
ng/ml IL-15
hnCD16
hnCD16/B2M-/-
hnCD16/B2M-/-HLA-G
hnCD16/B2M-/-HLA-G/IL-15-IL-15ra

Figure 7

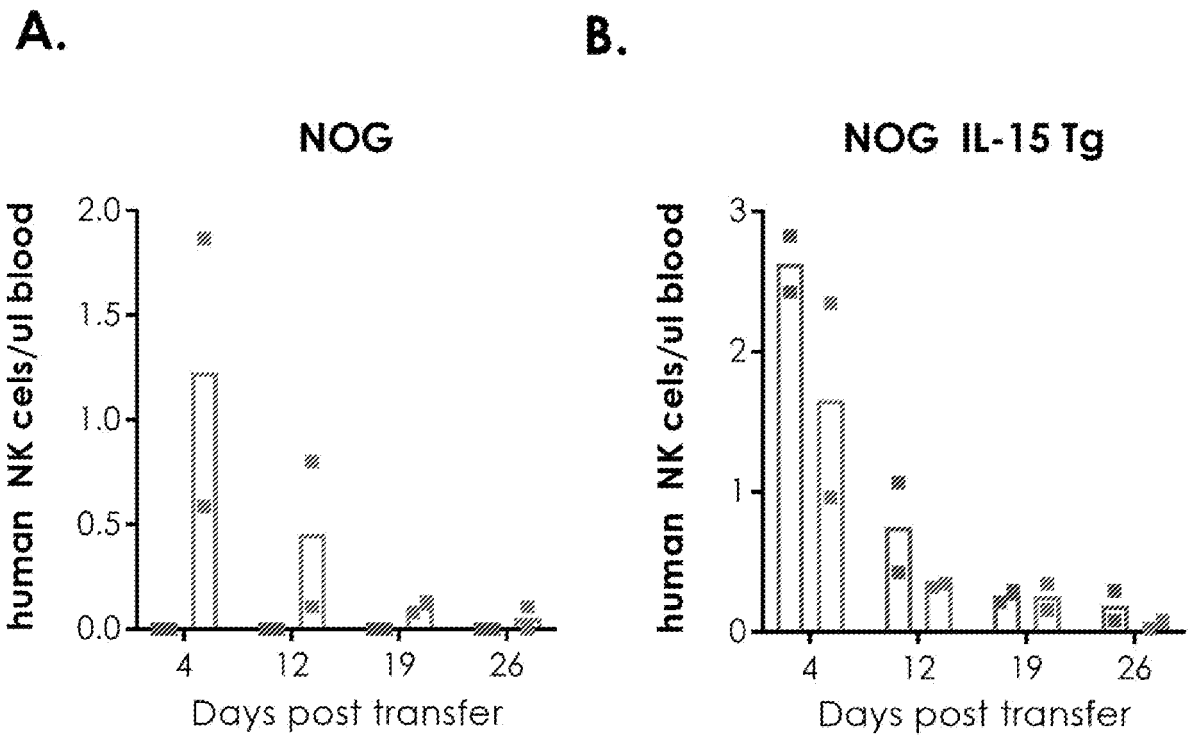

A.
B.
NOG
NOG IL-15 Tg
human NK cels/ul blood
2.0
1.5
1.0
0.5
0.0
3
2
1
0
4
12
19
26
Days post transfer
hnCD16/B2M-/-HLA-G
hnCD16/B2M-/-/HLA-G/IL-15/IL-15ra

Figure 8

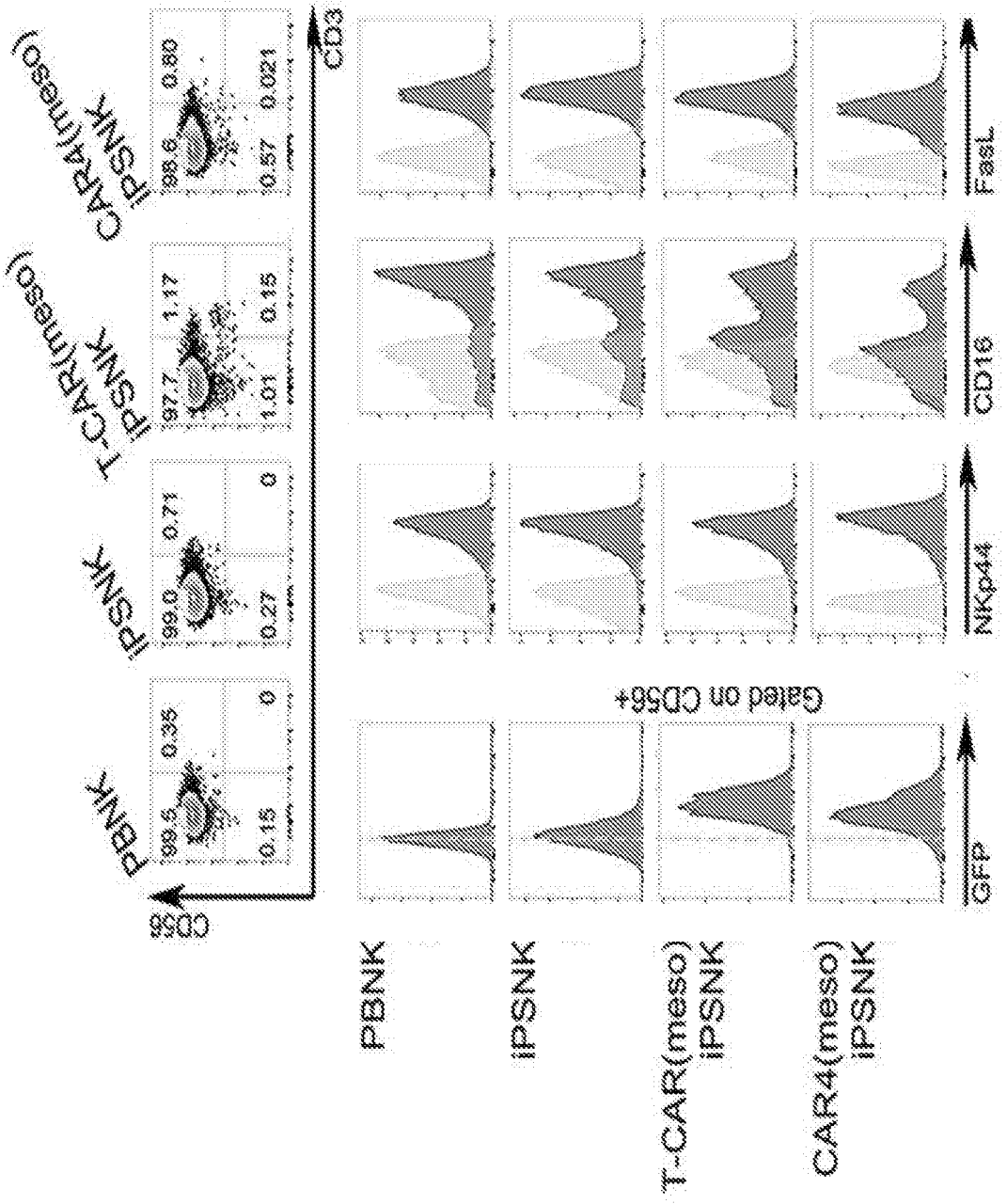
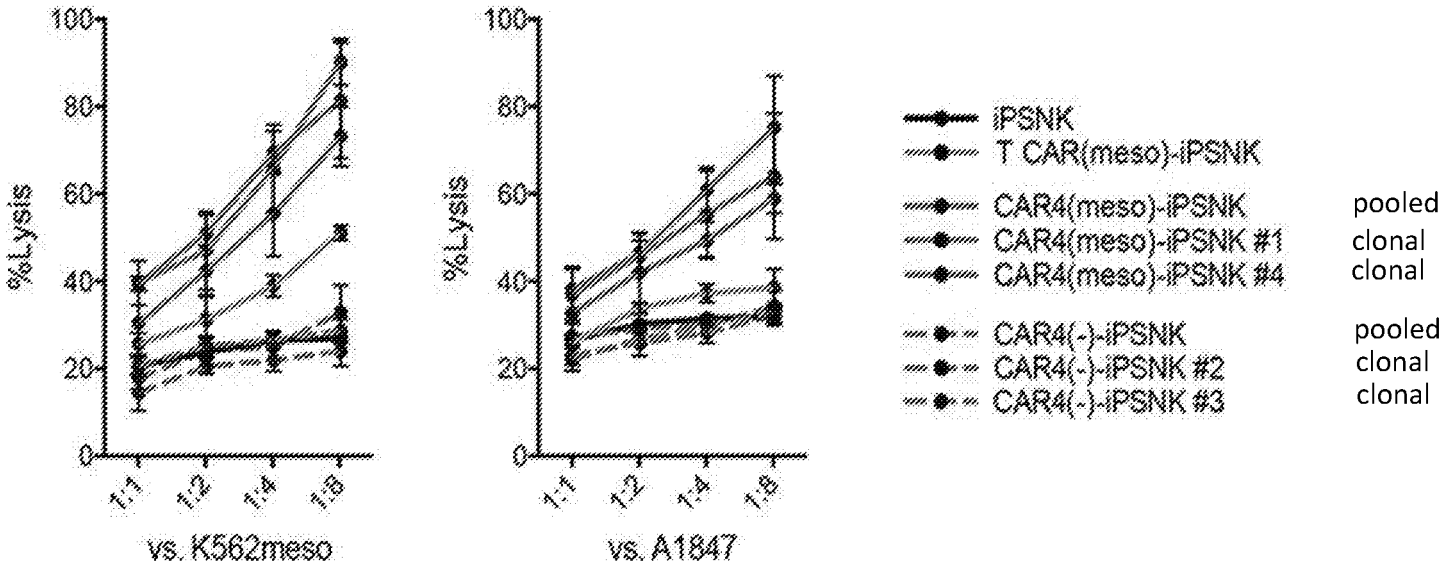

PBNK
iPSNK
T-CAR(meso) iPSNK
CAR4(meso) iPSNK
CD56
CD3
99.5
0.35
0.15
0
99.0
0.71
0.27
0
97.7
1.17
1.01
0.15
98.6
0.80
0.57
0.021
Gated on CD56+
PBNK
iPSNK
T-CAR(meso) iPSNK
CAR4(meso) iPSNK
GFP
NKp44
CD16
FasL

Figure 9


100
80
60
40
20
0
%Lysis
1:1
1:2
1:4
1:8
vs. K562meso
vs. A1847
iPSNK
T CAR(meso)-iPSNK
CAR4(meso)-iPSNK
CAR4(meso)-iPSNK #1
CAR4(meso)-iPSNK #4
CAR4(-)-iPSNK
CAR4(-)-iPSNK #2
CAR4(-)-iPSNK #3
pooled
clonal
clonal
pooled
clonal
clonal

A **B**

Figure 10

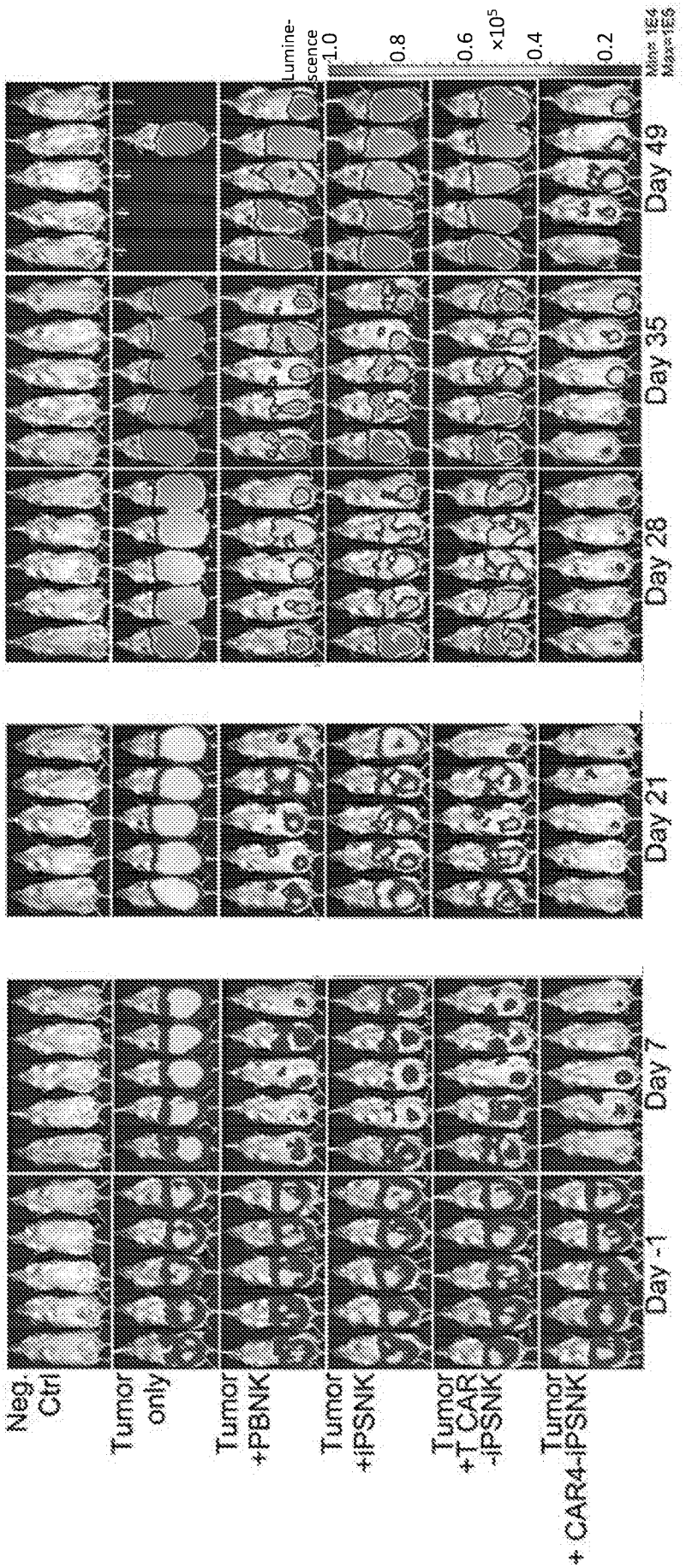

Neg. Ctrl
Tumor only
Tumor +PBNK
Tumor +iPSNK
Tumor +T CAR -iPSNK
Tumor + CAR4-iPSNK
Day -1
Day 7
Day 21
Day 28
Day 35
Day 49
Lumine-scence
1.0
0.8
0.6
×10^5
0.4
0.2
Min=1E4
Max=1E5

Figure 11

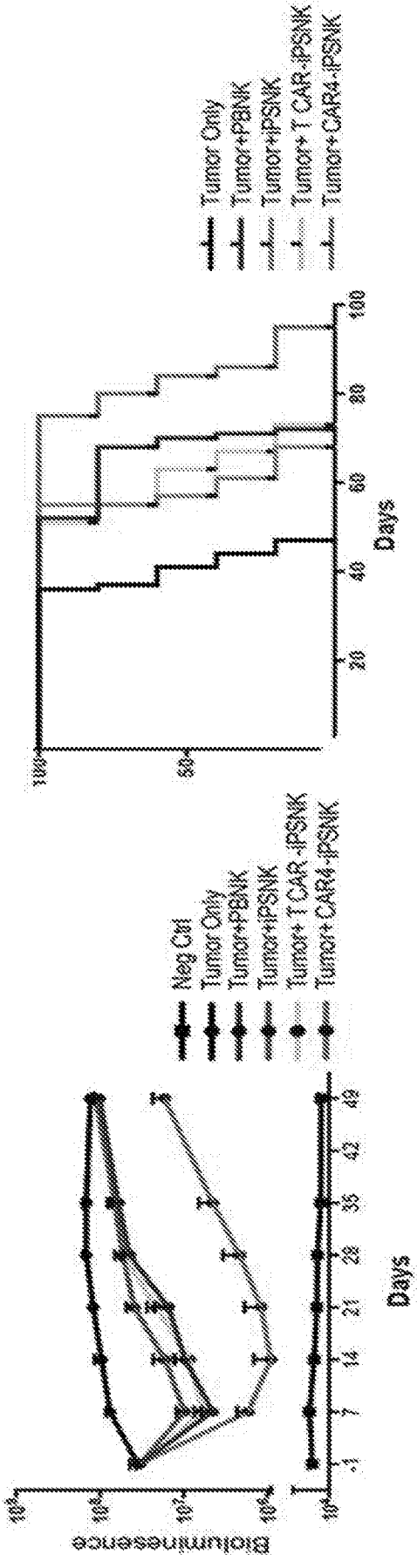

Bioluminescence
Days
Neg Ctrl
Tumor Only
Tumor+PBNK
Tumor+iPSNK
Tumor+ T CAR -iPSNK
Tumor+ CAR4 -iPSNK
A
Days
Tumor Only
Tumor+PBNK
Tumor+iPSNK
Tumor+ T CAR -iPSNK
Tumor+ CAR4 -iPSNK
B

Figure 12

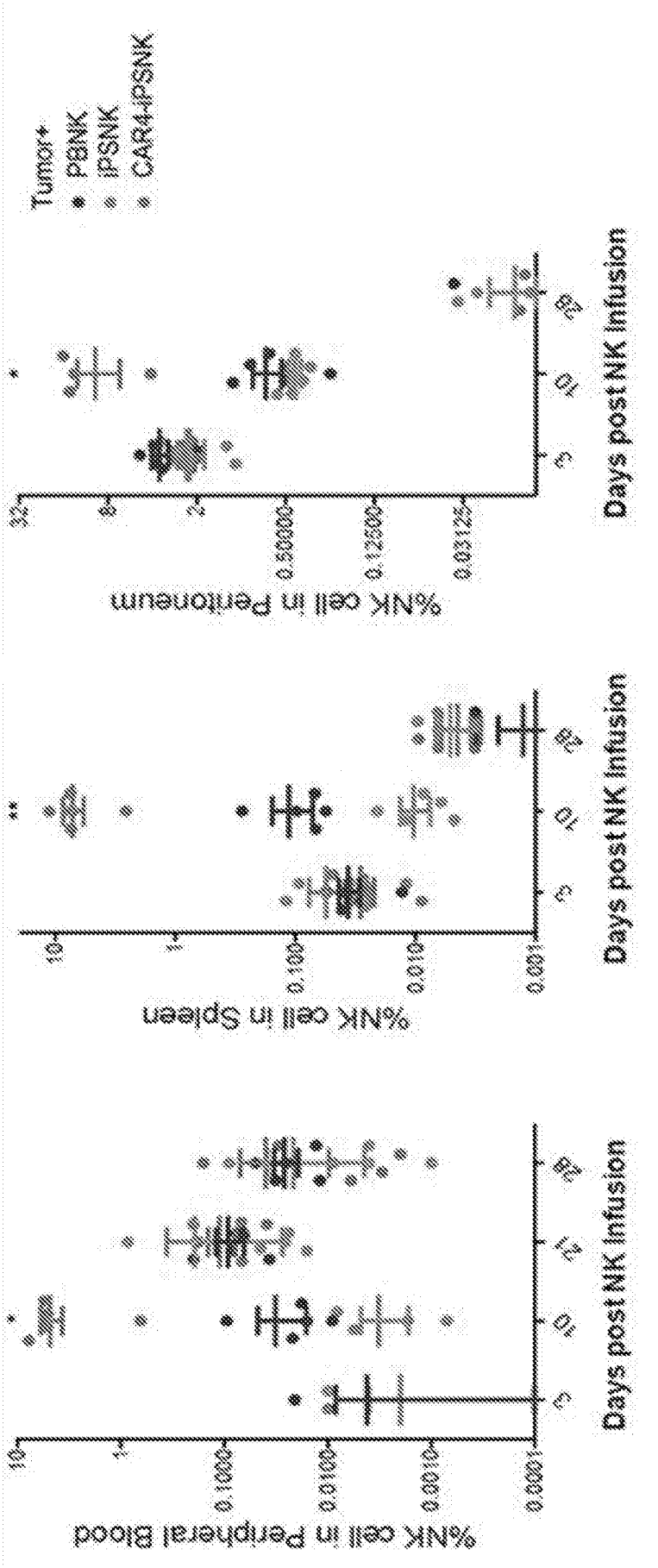

%NK cell in Peripheral Blood
10
1
0.1000
0.0100
0.0010
0.0001
Days post NK Infusion
0
7
10
28
A
%NK cell in Spleen
10
1
0.100
0.010
0.001
Days post NK Infusion
0
7
10
28
B
%NK cell in Peritoneum
32
8
2
0.50000
0.12500
0.03125
Days post NK Infusion
0
5
10
28
C
Tumor+
PBNK
IPSNK
CAR4-IPSNK

Figure 13

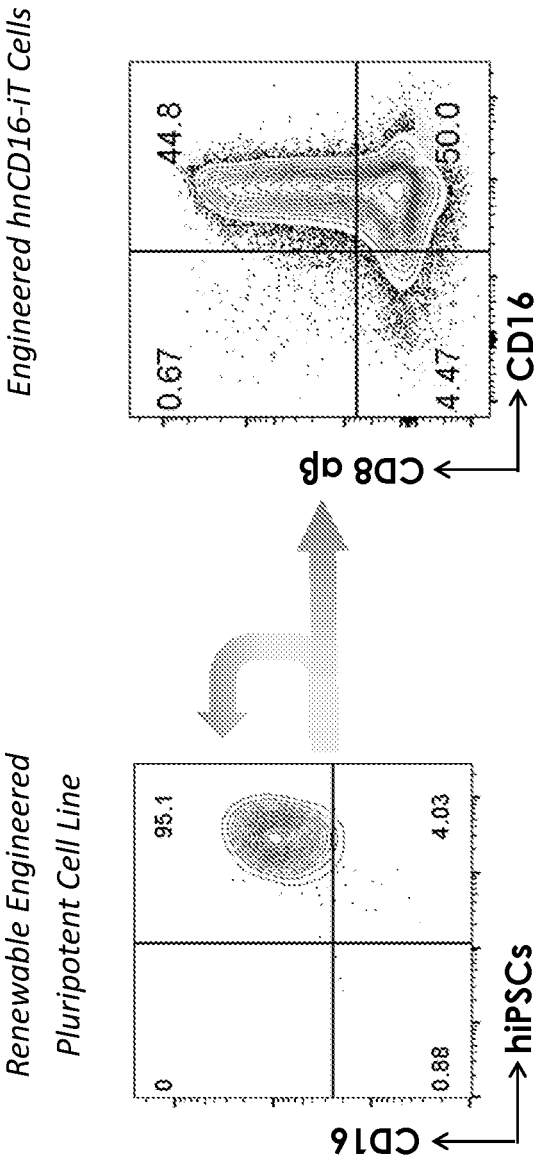

Renewable Engineered
Pluripotent Cell Line
CD16
hiPSCs
0
95.1
0.88
4.03
A
Engineered hnCD16-iT Cells
CD8 αβ
CD16
0.67
44.8
4.47
50.0
B

Figure 14

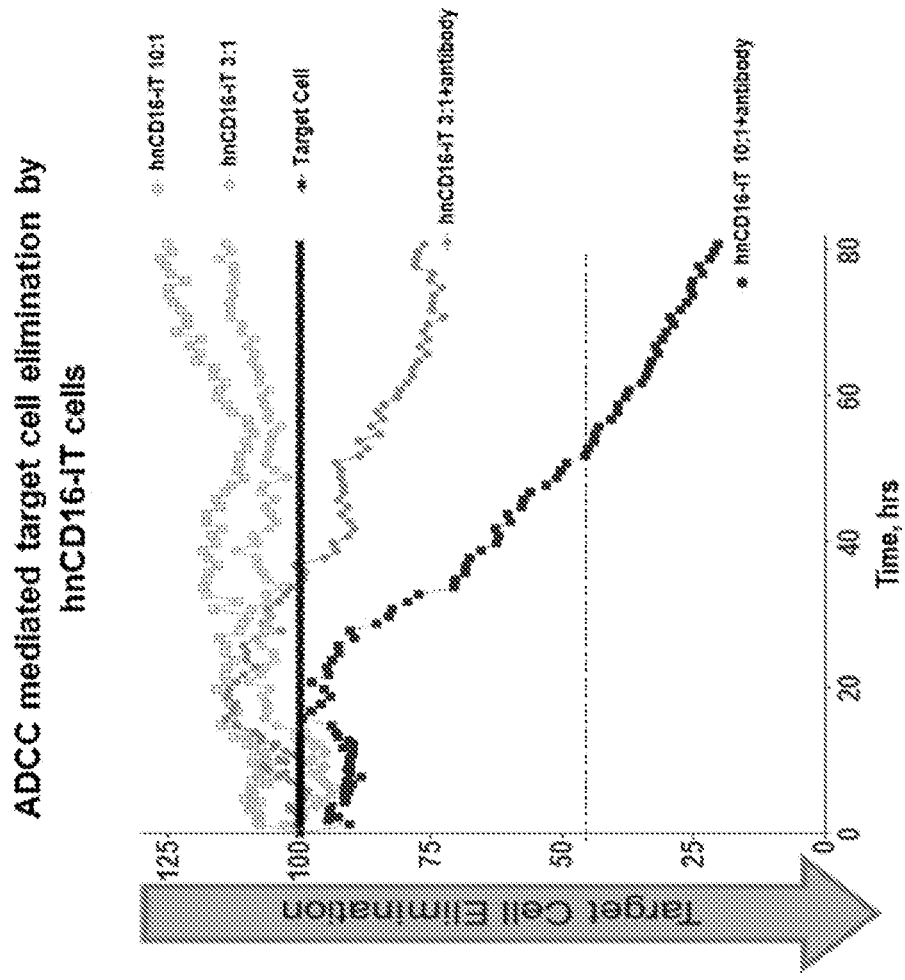

ADCC mediated target cell elimination by hnCD16-iT cells
hnCD16-iT 10:1
hnCD16-iT 3:1
Target Cell
hnCD16-iT 3:1+antibody
hnCD16-iT 10:1+antibody
Target Cell Elimination
125
100
75
50
25
0
0
20
40
60
80
Time, hrs

Figure 15

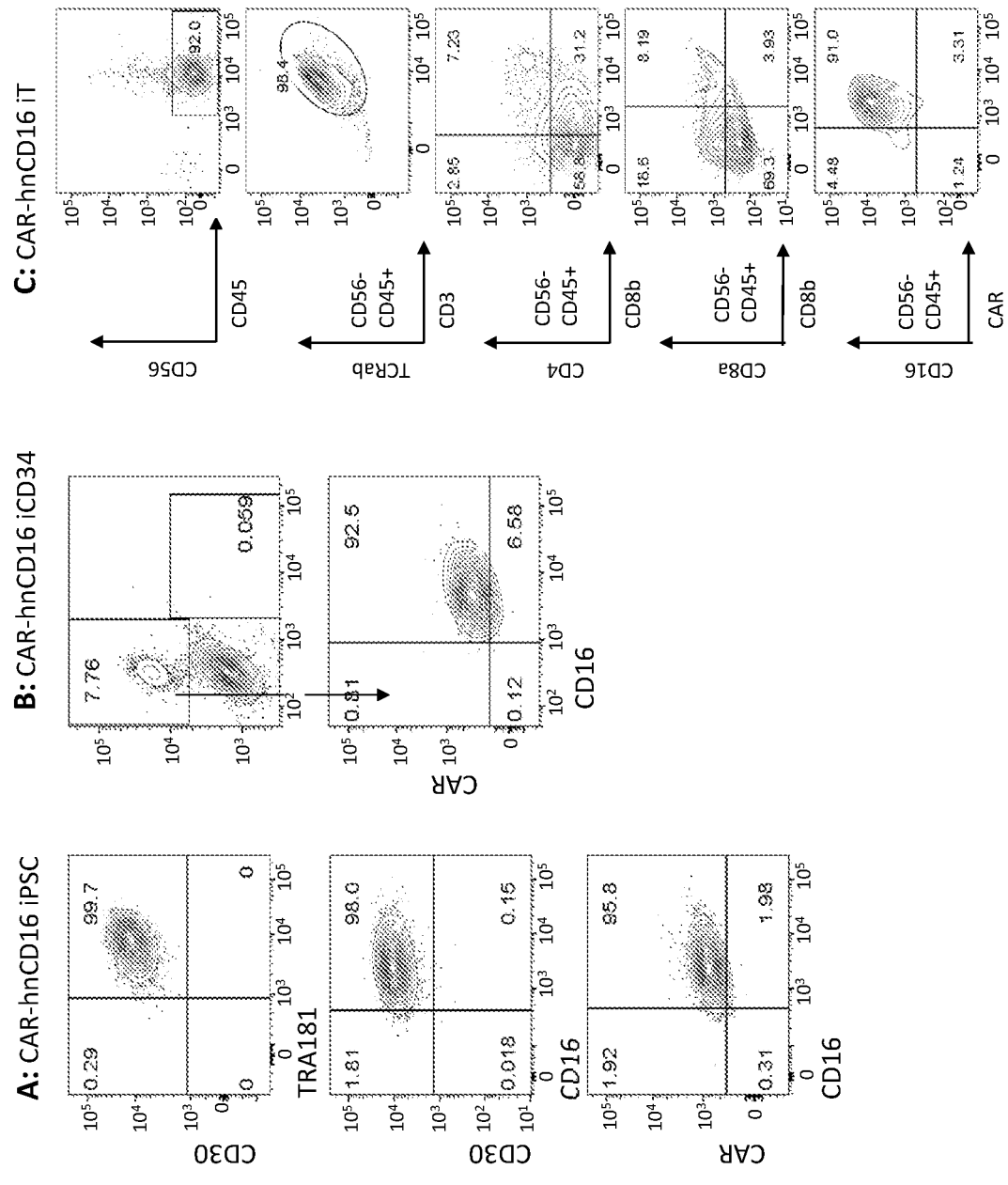

A: CAR-hnCD16 iPSC
CD30
TRA181
CD30
CD16
CAR
CD16
B: CAR-hnCD16 iCD34
CAR
CD16
C: CAR-hnCD16 iT
CD56
CD45
TCRab
CD3
CD4
CD8b
CD8a
CD8b
CD16
CAR

Figure 16

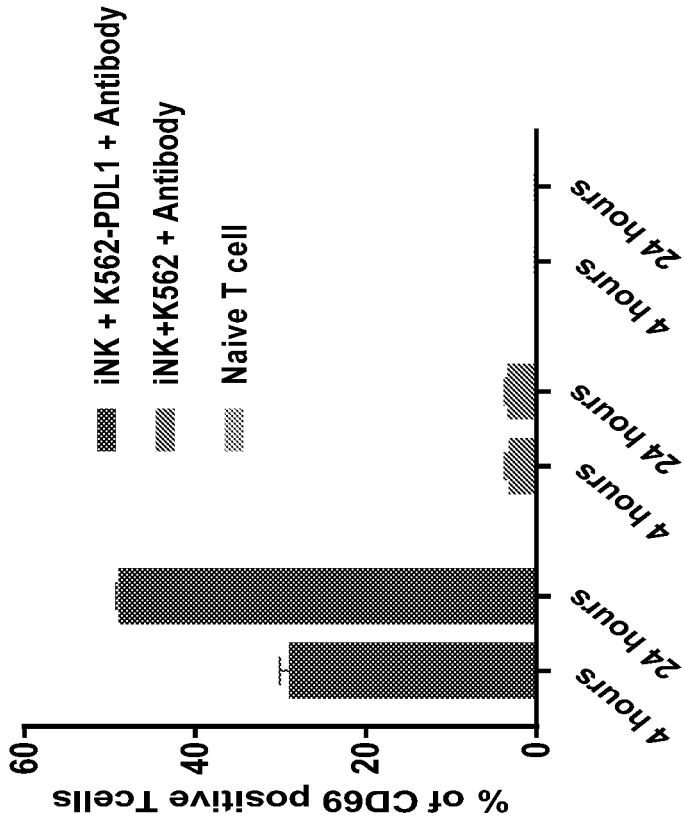

iNK + K562-PDL1 + Antibody
iNK+K562 + Antibody
Naive T cell
% of CD69 positive Tcells
0
20
40
60
4 hours
24 hours
4 hours
24 hours
4 hours
24 hours

Figure 17

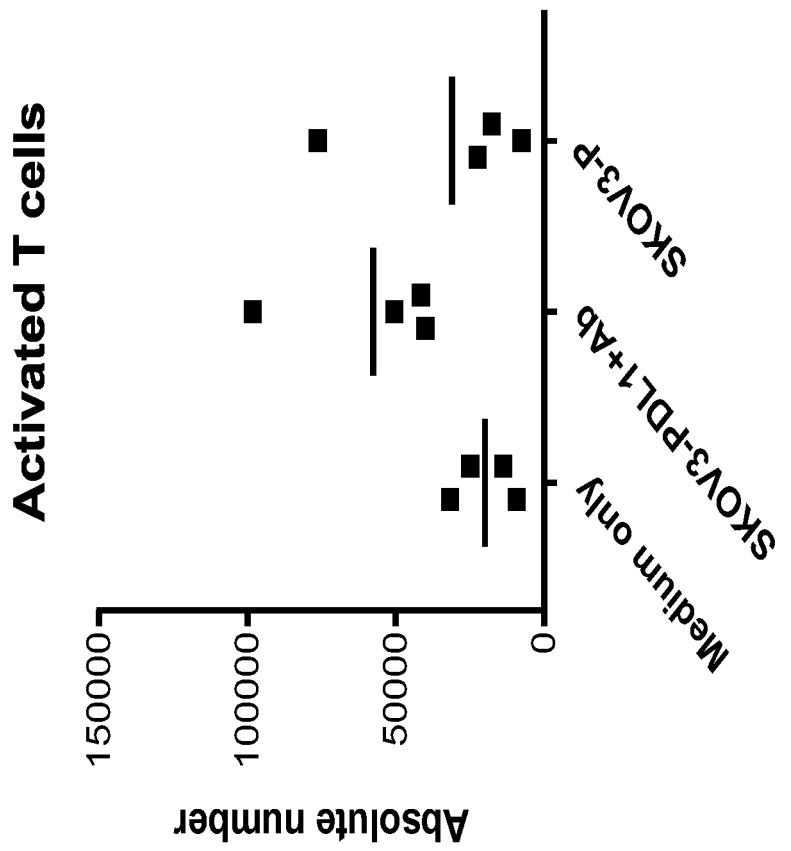
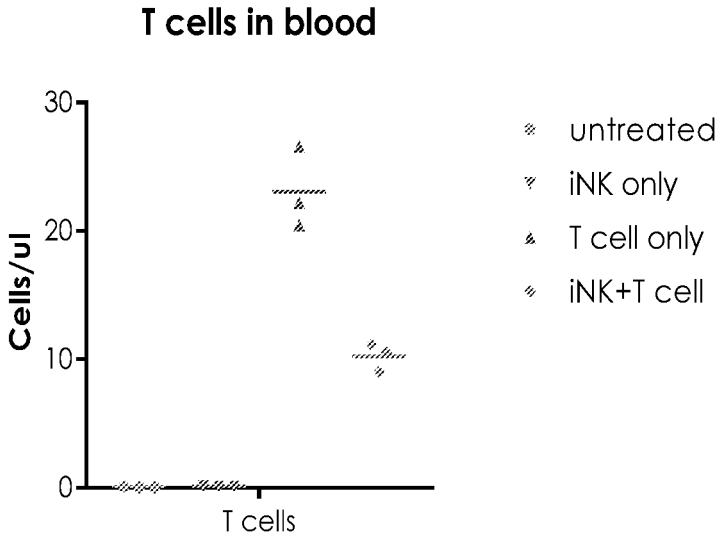
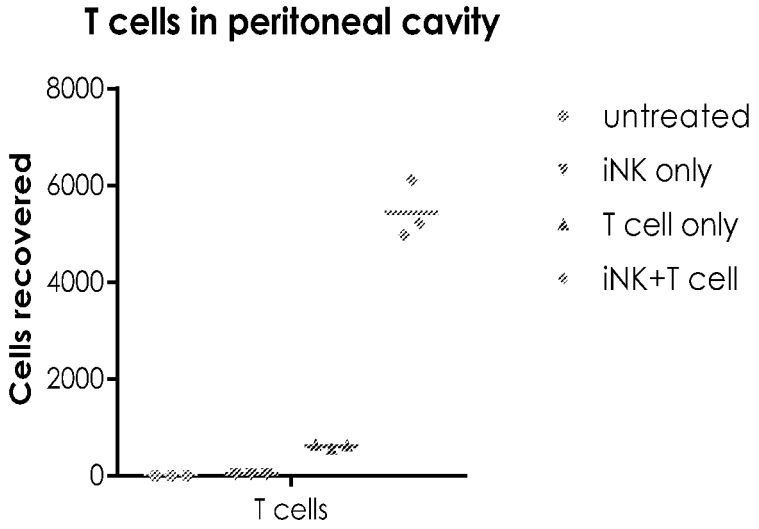

Activated T cells
Absolute number
150000
100000
50000
0
Medium only
SKOV3-PDL1+Ab
SKOV3-P

Figure 18


T cells in blood
Cells/ul
30
20
10
0
T cells
untreated
iNK only
T cell only
iNK+T cell

A


T cells in peritoneal cavity
Cells recovered
8000
6000
4000
2000
0
T cells
untreated
iNK only
T cell only
iNK+T cell

B

Figure 19

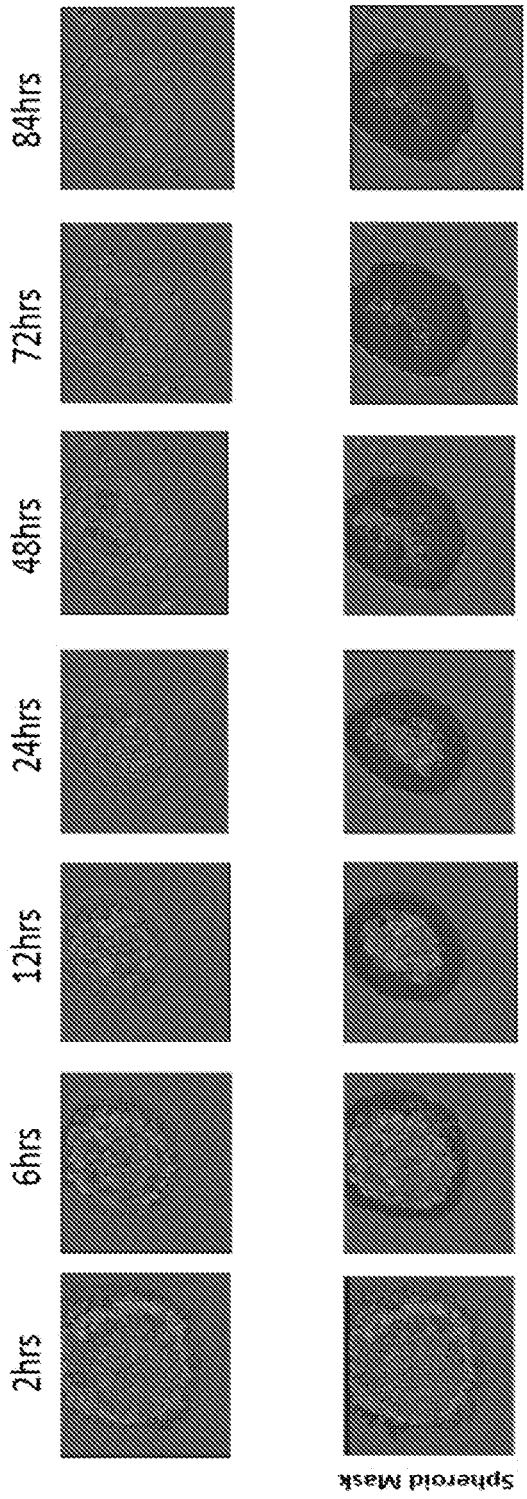
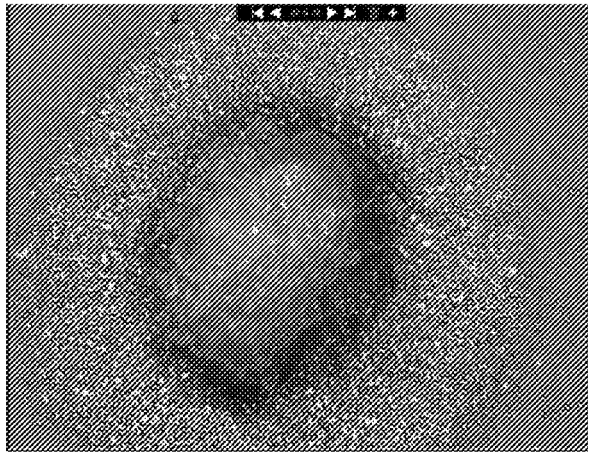


Figure 20

A



T=0 2.25 4.25 6.25 10.25 14.25

B

T cells + iNK cells

T cells only

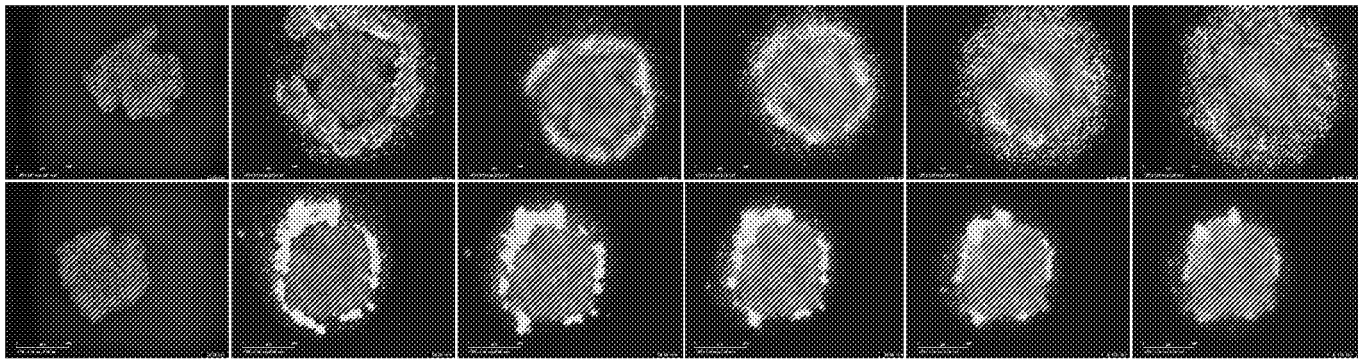


Figure 21

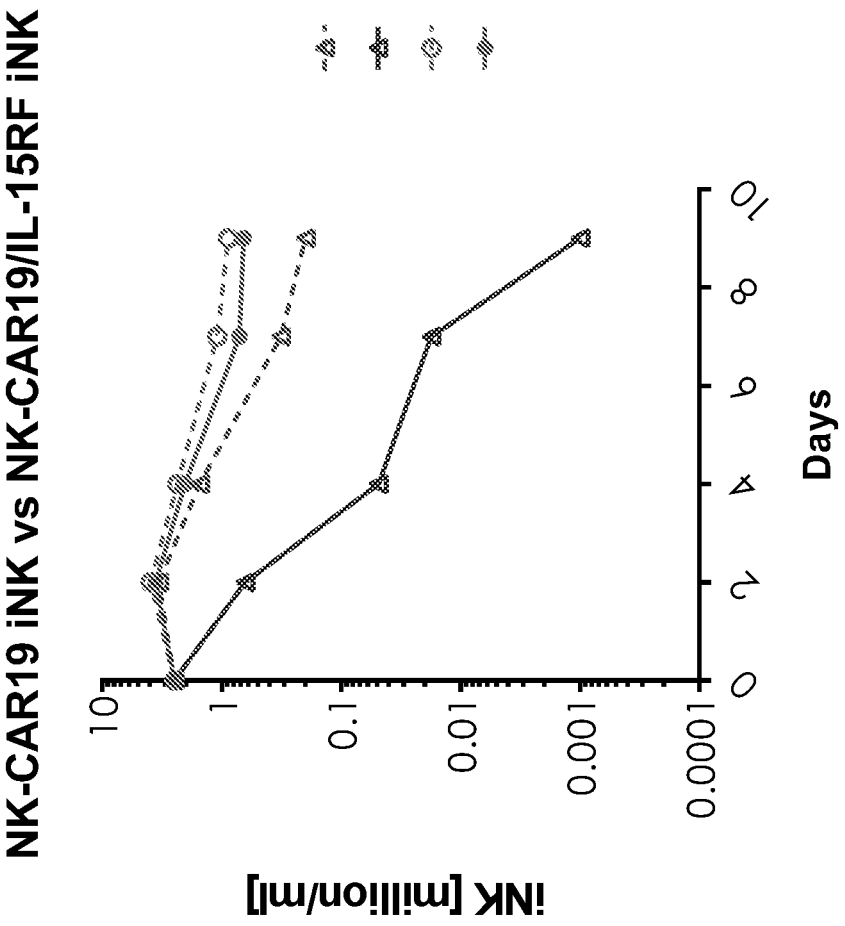

NK-CAR19 iNK vs NK-CAR19/IL-15RF iNK
NK-CAR19
NK-CAR19 w/o IL-2
NK-CAR19 IL-15RF
NK-CAR19 IL-15RF w/o IL-2
iNK [million/ml]
10
1
0.1
0.01
0.001
0.0001
0
2
4
6
8
10
Days

Figure 22

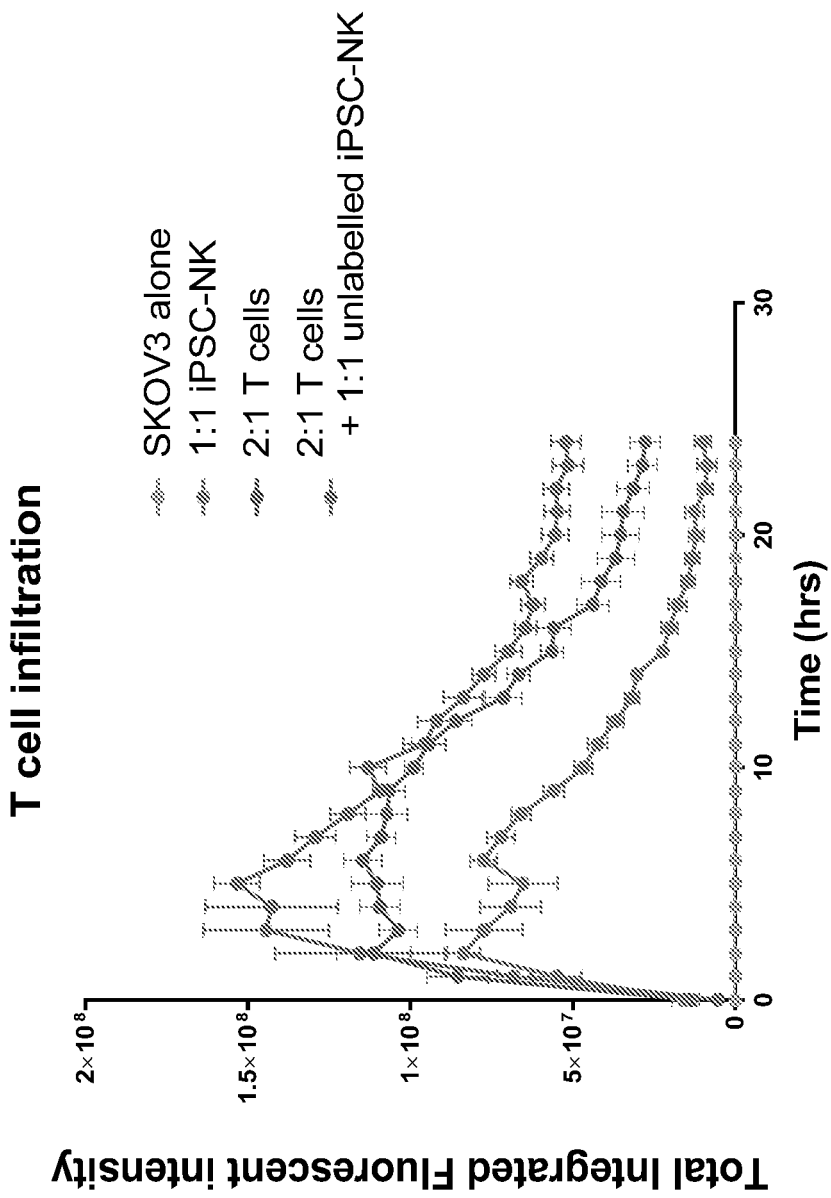

T cell infiltration
SKOV3 alone
1:1 iPSC-NK
2:1 T cells
2:1 T cells
+ 1:1 unlabelled iPSC-NK
Total Integrated Fluorescent intensity
2×10^8
1.5×10^8
1×10^8
5×10^7
0
0
10
20
30
Time (hrs)

Figure 23

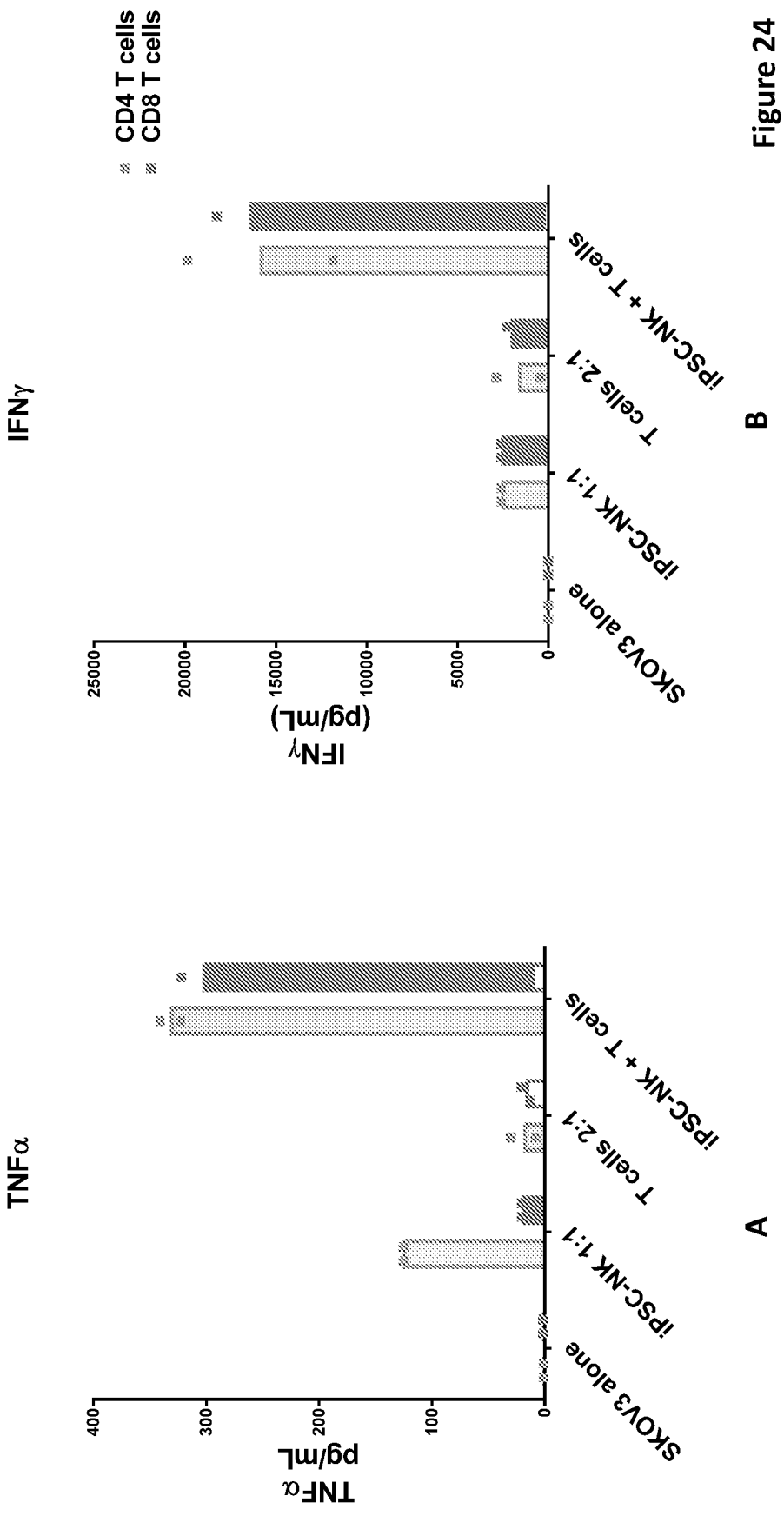

TNFα
TNFα pg/mL
400
300
200
100
0
SKOV3 alone
iPSC-NK 1:1
T cells 2:1
iPSC-NK + T cells
A
IFNγ
CD4 T cells
CD8 T cells
IFNγ (pg/mL)
25000
20000
15000
10000
5000
0
SKOV3 alone
iPSC-NK 1:1
T cells 2:1
iPSC-NK + T cells
B

Figure 24

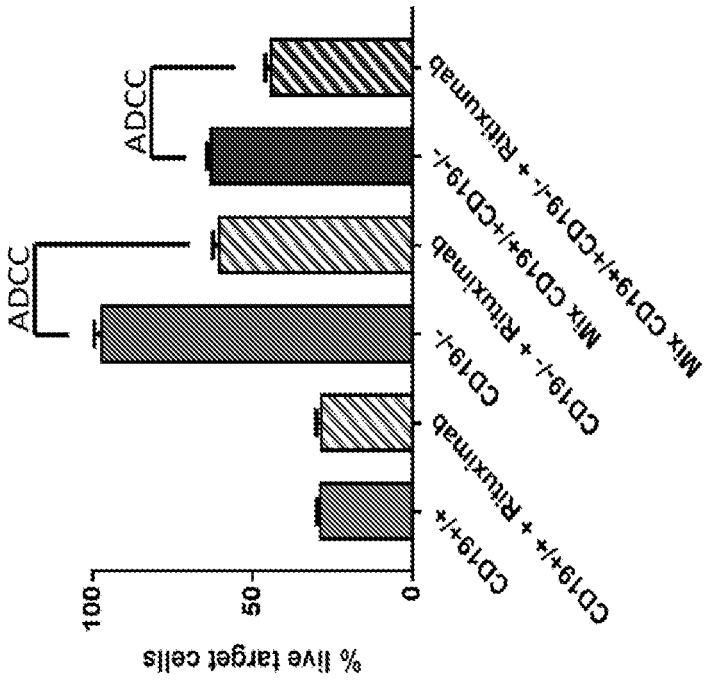

% live target cells
100
50
0
ADCC
ADCC
CD19+/+
CD19+/+ + Rituximab
CD19-/-
CD19-/- + Rituximab
Mix CD19+/+CD19-/-
Mix CD19+/+CD19-/- + Rituximab

Figure 25

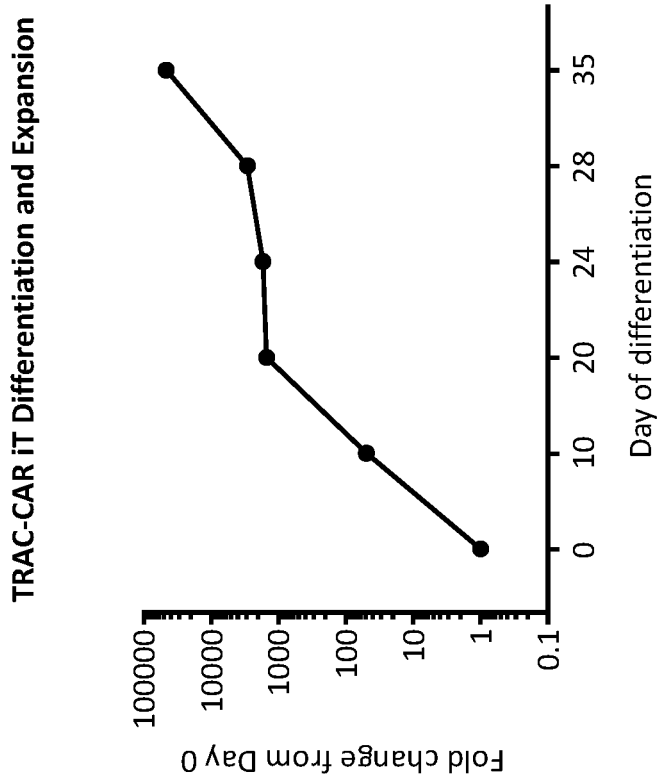

TRAC-CAR iT Differentiation and Expansion
Fold change from Day 0
100000
10000
1000
100
10
1
0.1
0
10
20
24
28
35
Day of differentiation

Figure 26

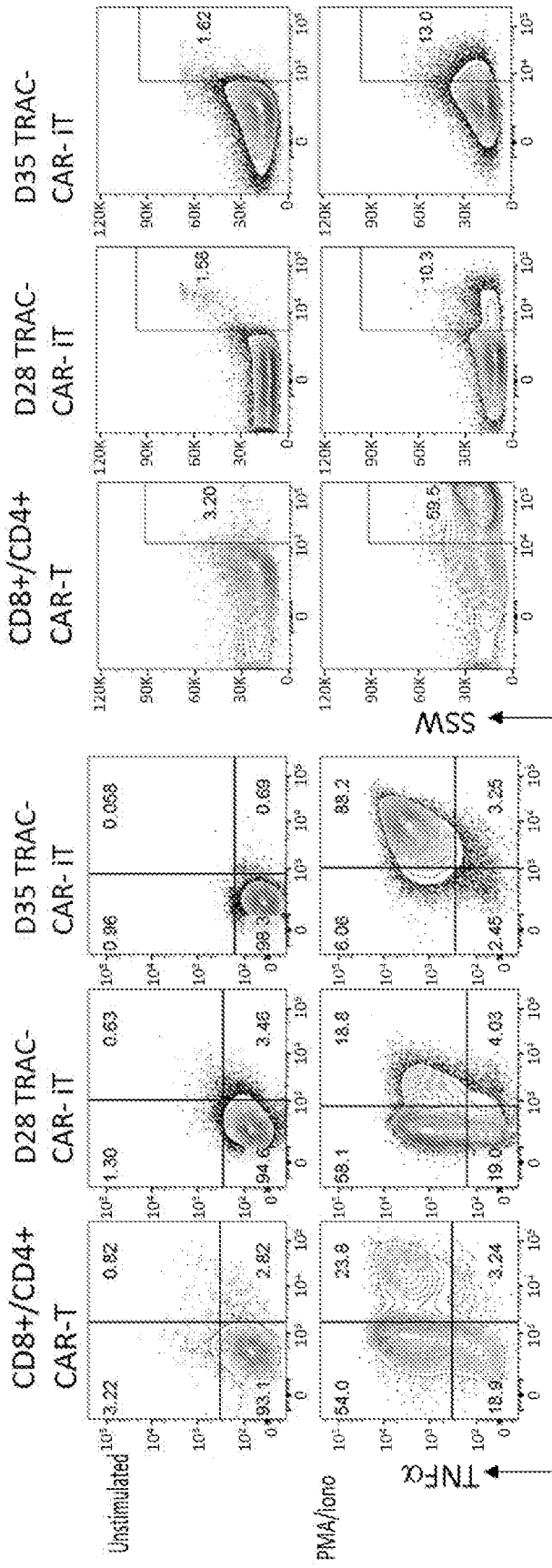
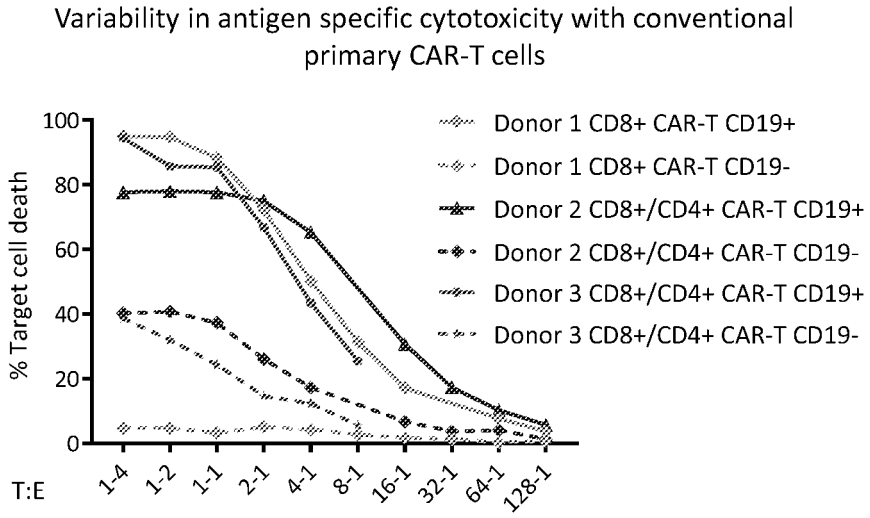
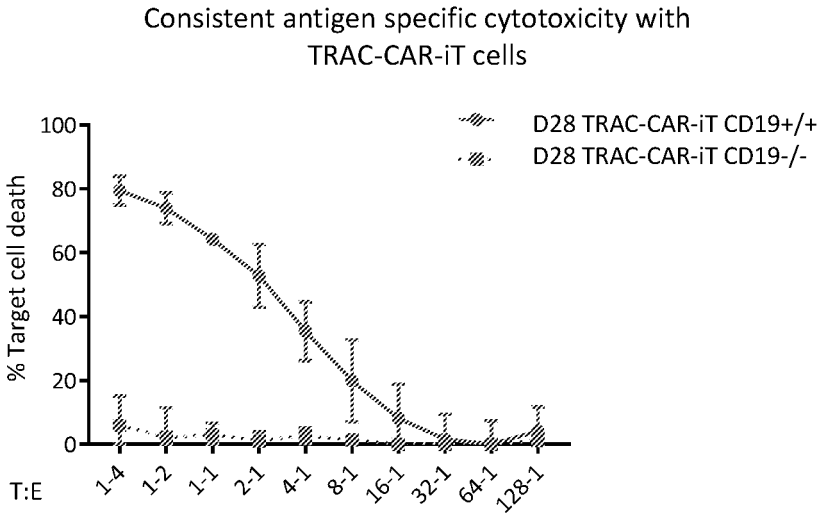

CD8+/CD4+ CAR-T
D28 TRAC-CAR- iT
D35 TRAC-CAR- iT
Unstimulated
PMA/Iono
TNFα
IFNγ
A
SSW
IL2
B

Figure 27


Variability in antigen specific cytotoxicity with conventional primary CAR-T cells
% Target cell death
100
80
60
40
20
0
T:E
1-4
1-2
1-1
2-1
4-1
8-1
16-1
32-1
64-1
128-1
Donor 1 CD8+ CAR-T CD19+
Donor 1 CD8+ CAR-T CD19-
Donor 2 CD8+/CD4+ CAR-T CD19+
Donor 2 CD8+/CD4+ CAR-T CD19-
Donor 3 CD8+/CD4+ CAR-T CD19+
Donor 3 CD8+/CD4+ CAR-T CD19-

A


Consistent antigen specific cytotoxicity with TRAC-CAR-iT cells
% Target cell death
100
80
60
40
20
0
T:E
1-4
1-2
1-1
2-1
4-1
8-1
16-1
32-1
64-1
128-1
D28 TRAC-CAR-iT CD19+/+
D28 TRAC-CAR-iT CD19-/-

B

Figure 28

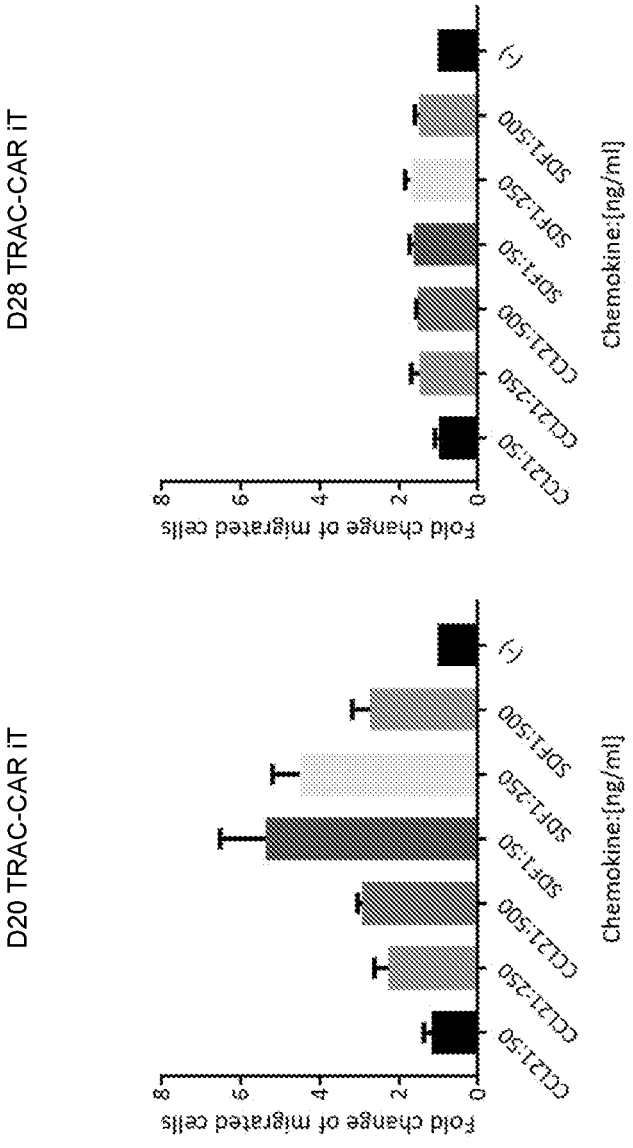

D20 TRAC-CAR iT
D28 TRAC-CAR iT
Fold change of migrated cells
0
2
4
6
8
CCL21:50
CCL21:250
CCL21:500
SDF1:50
SDF1:250
SDF1:500
(-)
Chemokine:[ng/ml]
A
B

Figure 29

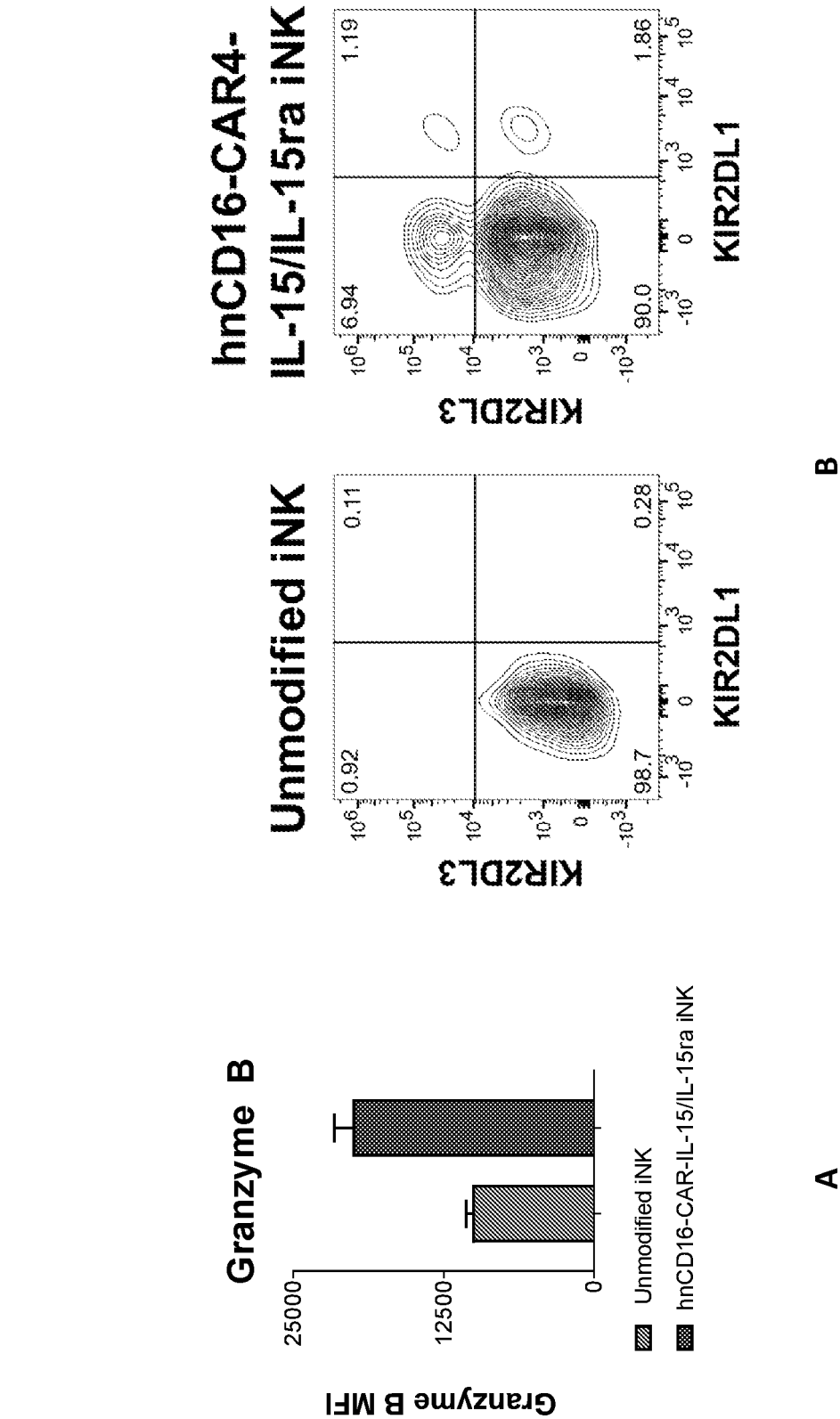
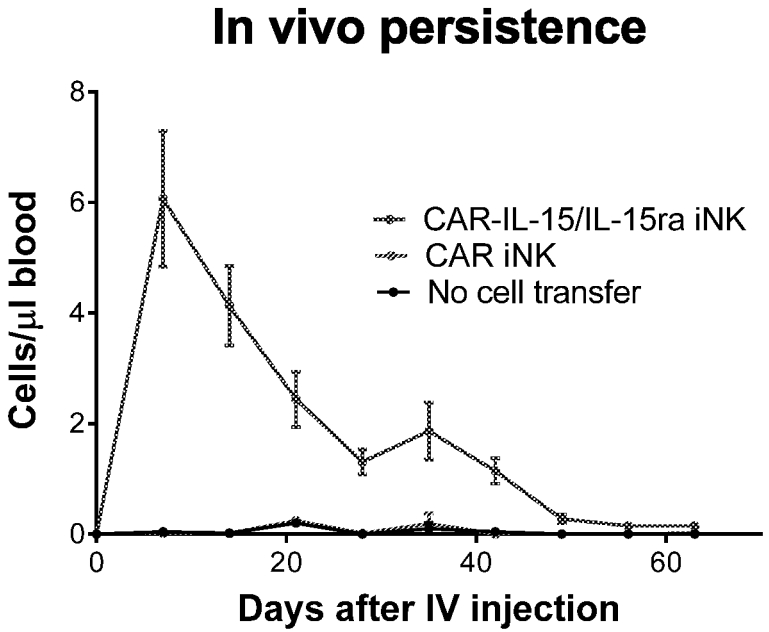
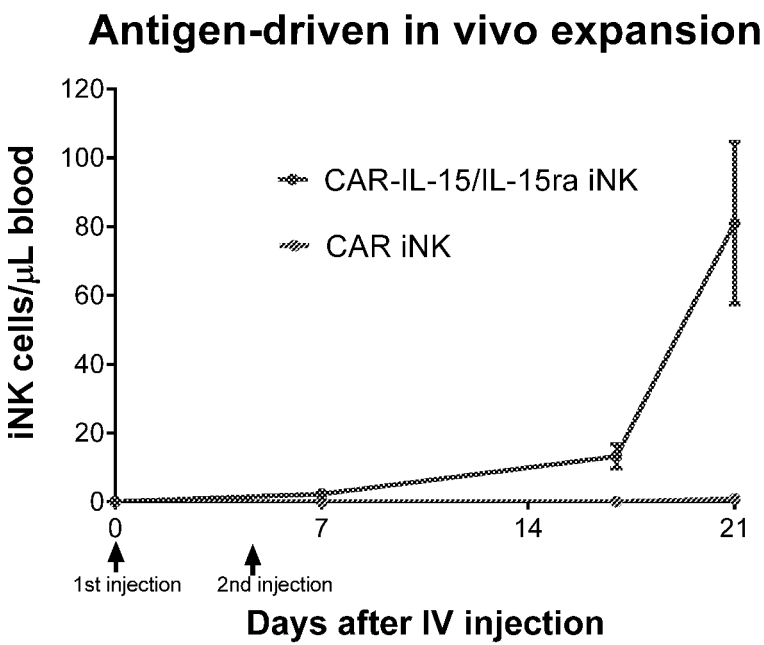

Granzyme B
Granzyme B MFI
25000
12500
0
Unmodified iNK
hnCD16-CAR-IL-15/IL-15ra iNK
A
Unmodified iNK
KIR2DL3
KIR2DL1
0.92
0.11
98.7
0.28
hnCD16-CAR4-IL-15/IL-15ra iNK
KIR2DL3
KIR2DL1
6.94
1.19
90.0
1.86
B

Figure 30

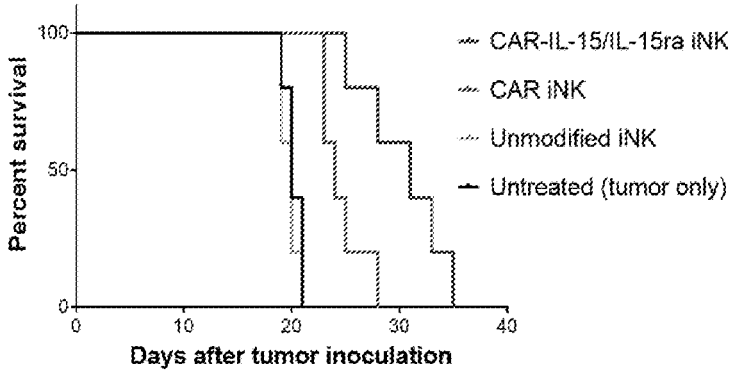

In vivo persistence
Cells/μl blood
8
6
4
2
0
0
20
40
60
Days after IV injection
CAR-IL-15/IL-15ra iNK
CAR iNK
No cell transfer

A


Antigen-driven in vivo expansion
iNK cells/μL blood
120
100
80
60
40
20
0
0
7
14
21
1st injection
2nd injection
Days after IV injection
CAR-IL-15/IL-15ra iNK
CAR iNK

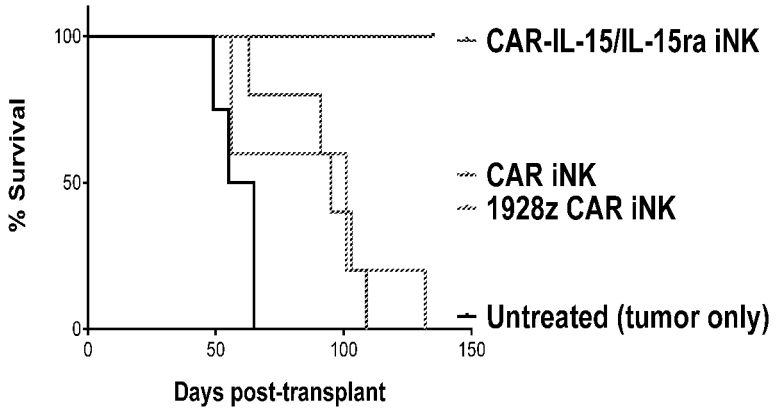
B

Figure 31



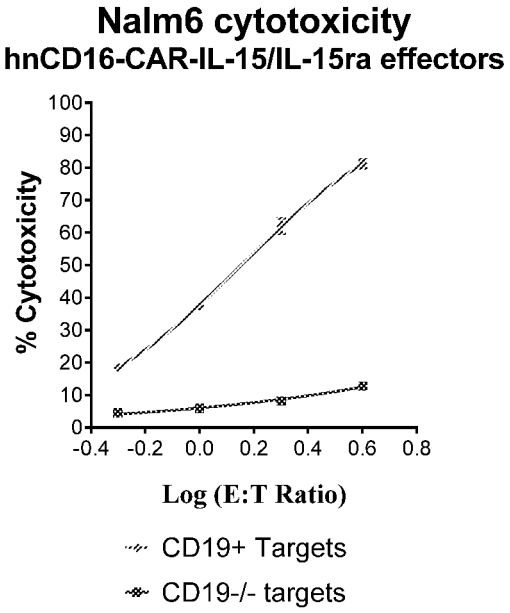
*p = 0.018, CAR iNK vs CAR IL-15/IL-15ra

A

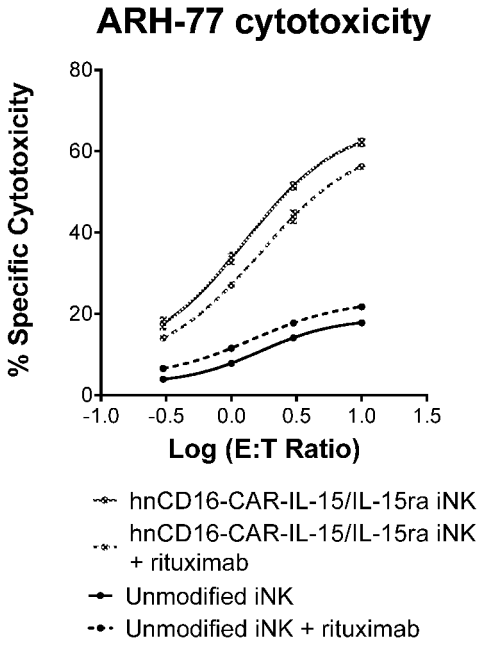


B

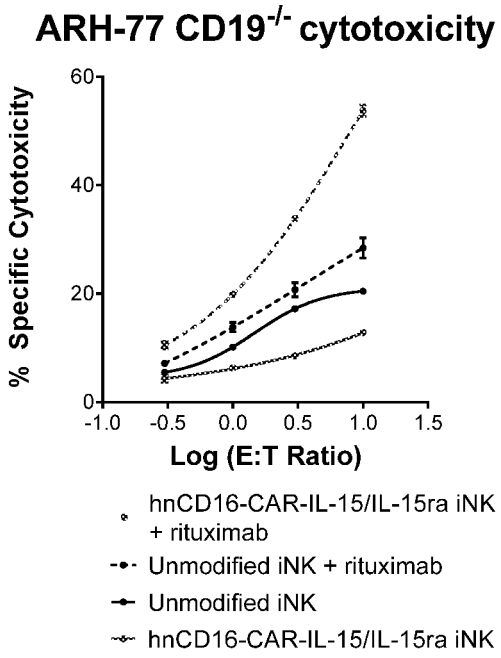
Figure 32



A



B



C

Figure 33

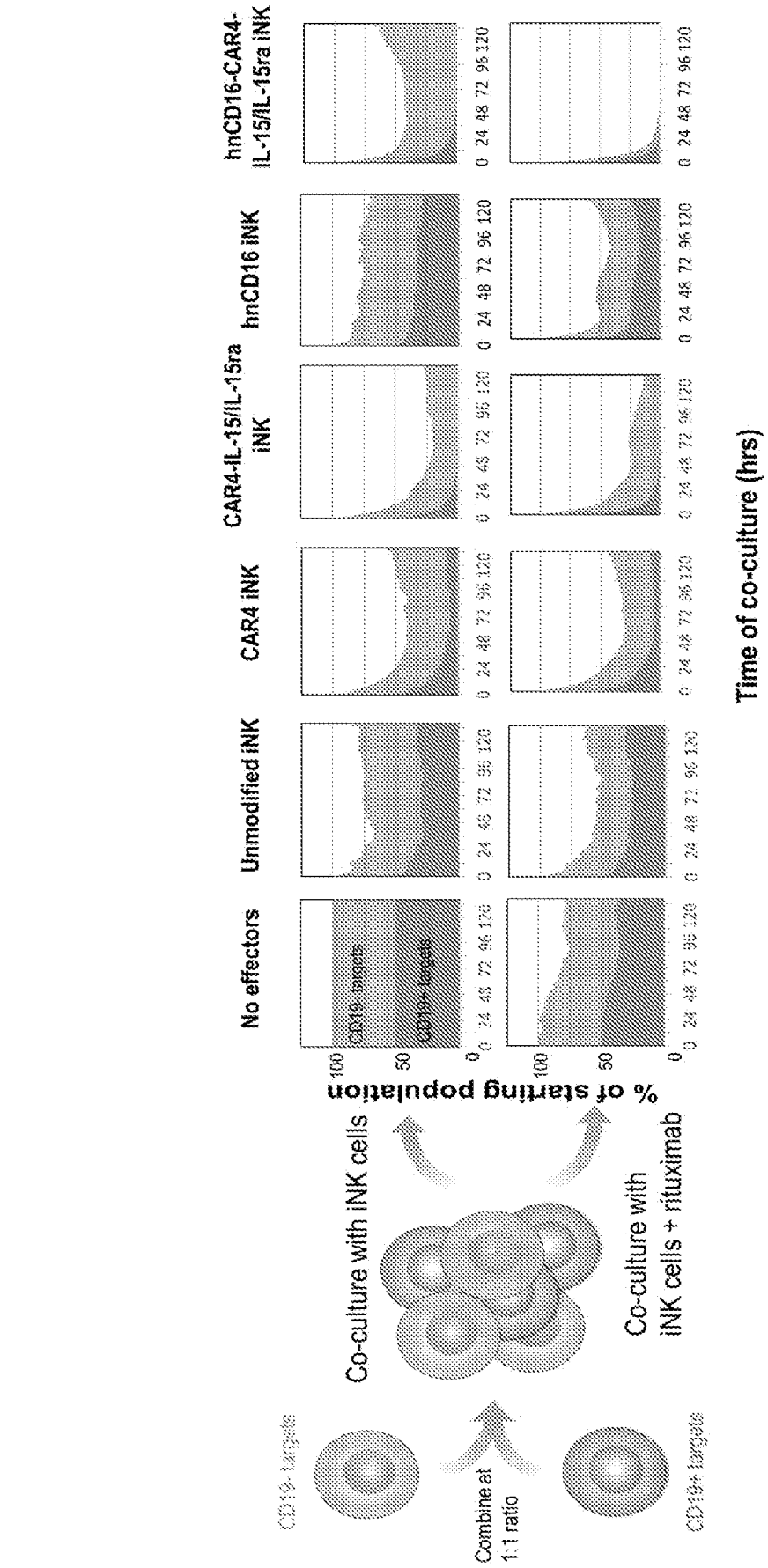

CD19- targets
Combine at 1:1 ratio
CD19+ targets
Co-culture with iNK cells
Co-culture with iNK cells + rituximab
% of starting population
CD19- targets
CD19+ targets
No effectors
Unmodified iNK
CAR4 iNK
CAR4-IL-15/IL-15ra iNK
hnCD16 iNK
hnCD16-CAR4-IL-15/IL-15ra iNK
0 24 48 72 96 120
Time of co-culture (hrs)

Figure 34

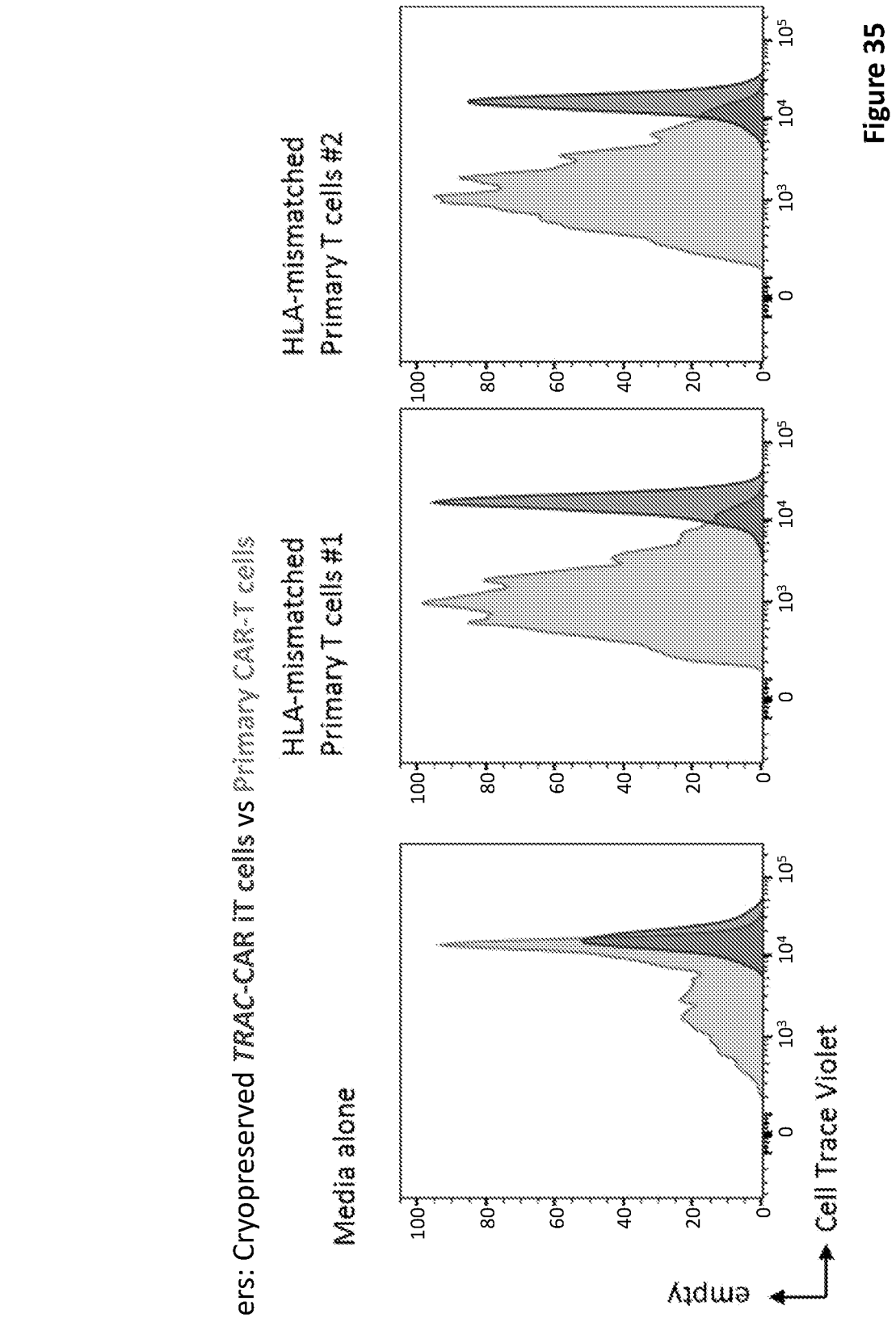

Responders: Cryopreserved TRAC-CAR iT cells vs Primary CAR-T cells
Targets:
Media alone
HLA-mismatched Primary T cells #1
HLA-mismatched Primary T cells #2
empty
Cell Trace Violet
0
20
40
60
80
100
10^3
10^4
10^5

Figure 35

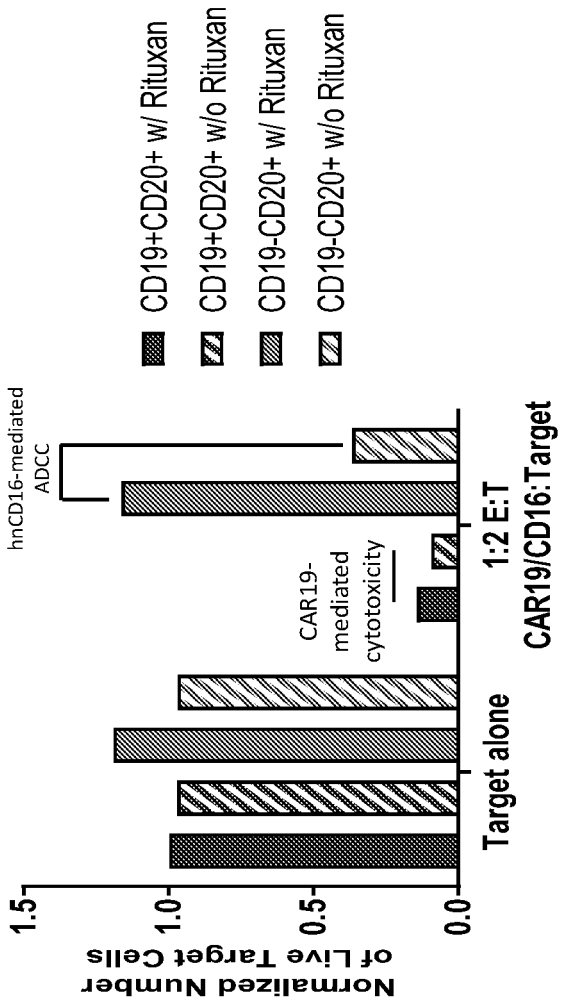

CD19+CD20+ w/ Rituxan
CD19+CD20+ w/o Rituxan
CD19-CD20+ w/ Rituxan
CD19-CD20+ w/o Rituxan
hnCD16-mediated ADCC
CAR19-mediated cytotoxicity
Normalized Number of Live Target Cells
1.5
1.0
0.5
0.0
Target alone
1:2 E:T CAR19/CD16:Target

Figure 36