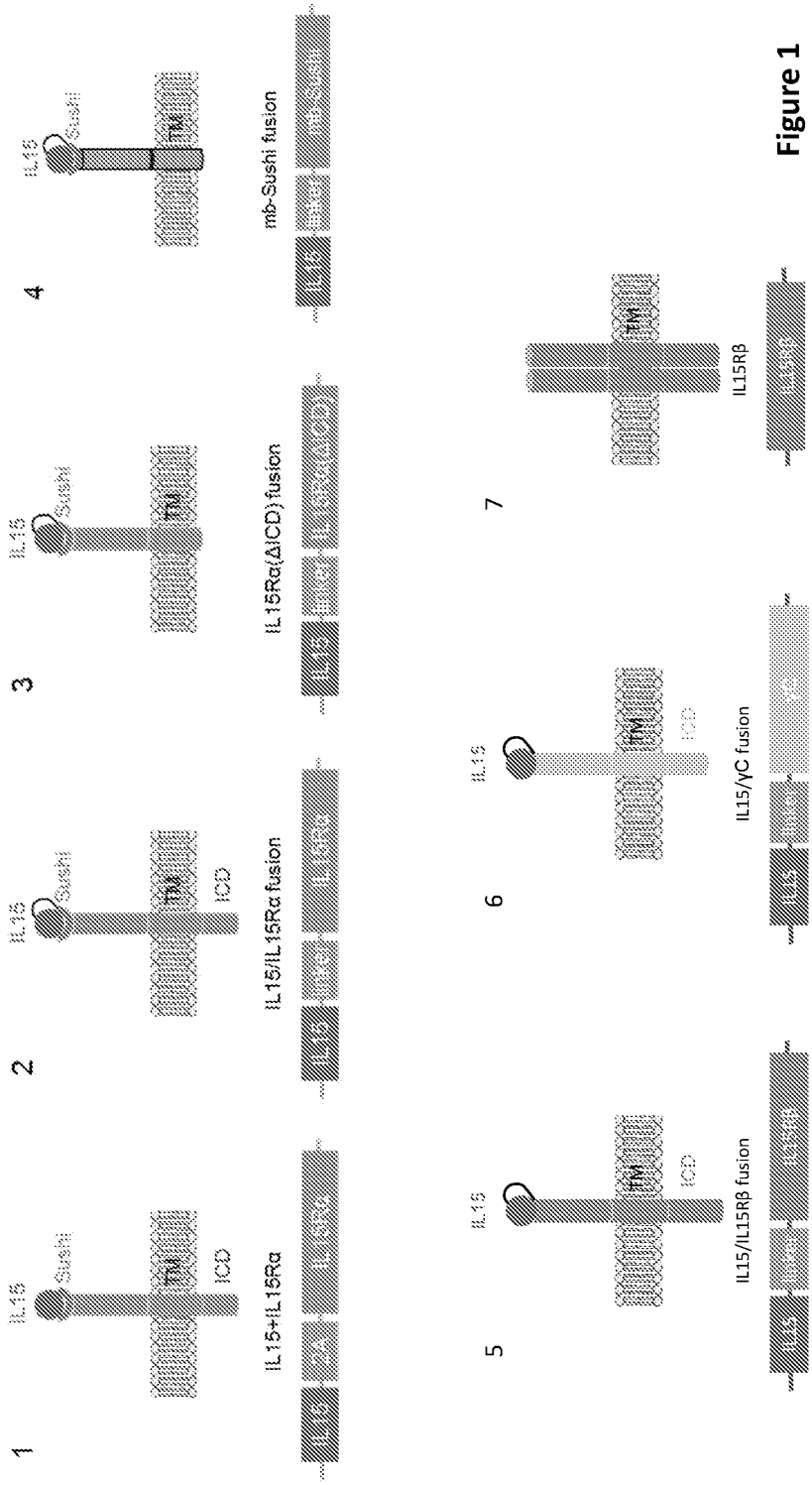

1
IL15
Sushi
TM
ICD
IL15+IL15Rα
2
IL15
Sushi
TM
ICD
IL15/IL15Rα fusion
3
IL15
Sushi
TM
IL15Rα(ΔICD) fusion
4
IL15
Sushi
TM
mb-Sushi fusion
5
IL15
TM
ICD
IL15/IL15Rβ fusion
6
IL15
TM
ICD
IL15/γC fusion
7
TM
IL15Rβ

Figure 1

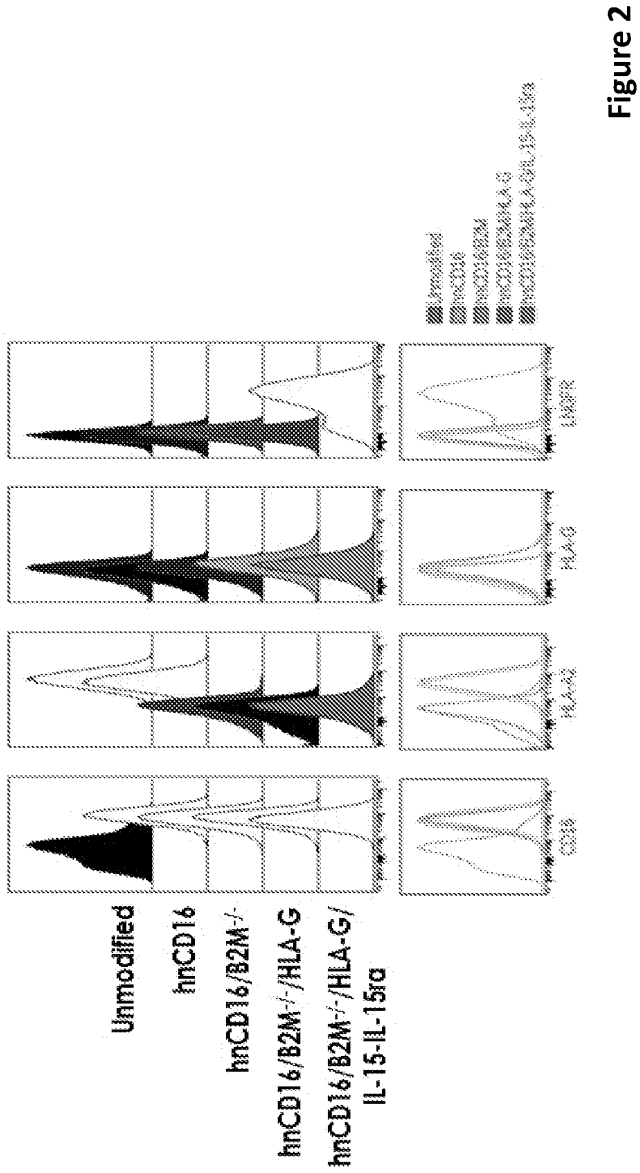

Unmodified
hnCD16
hnCD16/B2M-/-
hnCD16/B2M-/-/HLA-G
hnCD16/B2M-/-/HLA-G/
IL-15-IL-15rα
CD16
HLA-A2
HLA-G
LNGFR
Unmodified
hnCD16
hnCD16/B2M
hnCD16/B2M/HLA-G
hnCD16/B2M/HLA-G/IL-15-IL-15rα

Figure 2

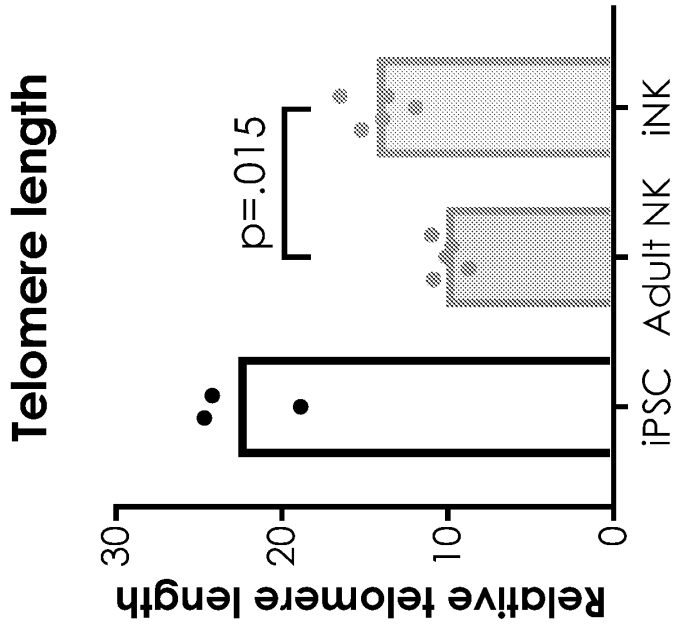

Telomere length
Relative telomere length
30
20
10
0
p=.015
iPSC
Adult NK
iNK

Figure 3

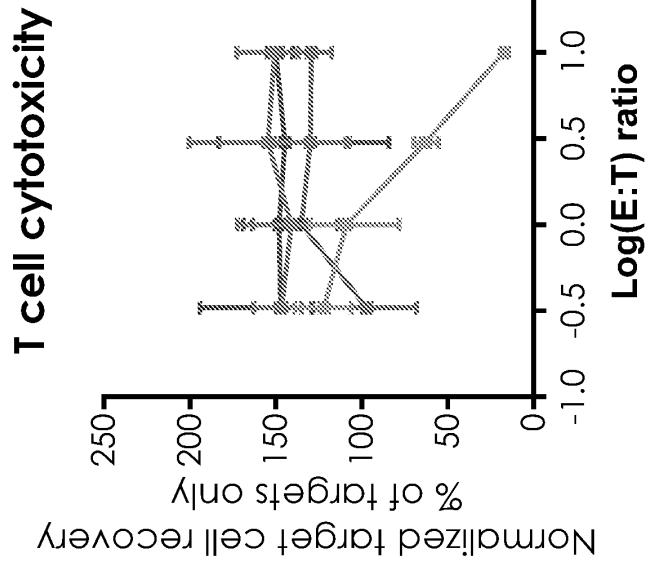

T cell cytotoxicity
Normalized target cell recovery
% of targets only
250
200
150
100
50
0
-1.0
-0.5
0.0
0.5
1.0
1.5
Log(E:T) ratio
hnCD16/B2M-/-
hnCD16/B2M-/-HLA-G
hnCD16/B2M-/-HLA-G/
IL-15-IL-15ra
hnCD16

Figure 4

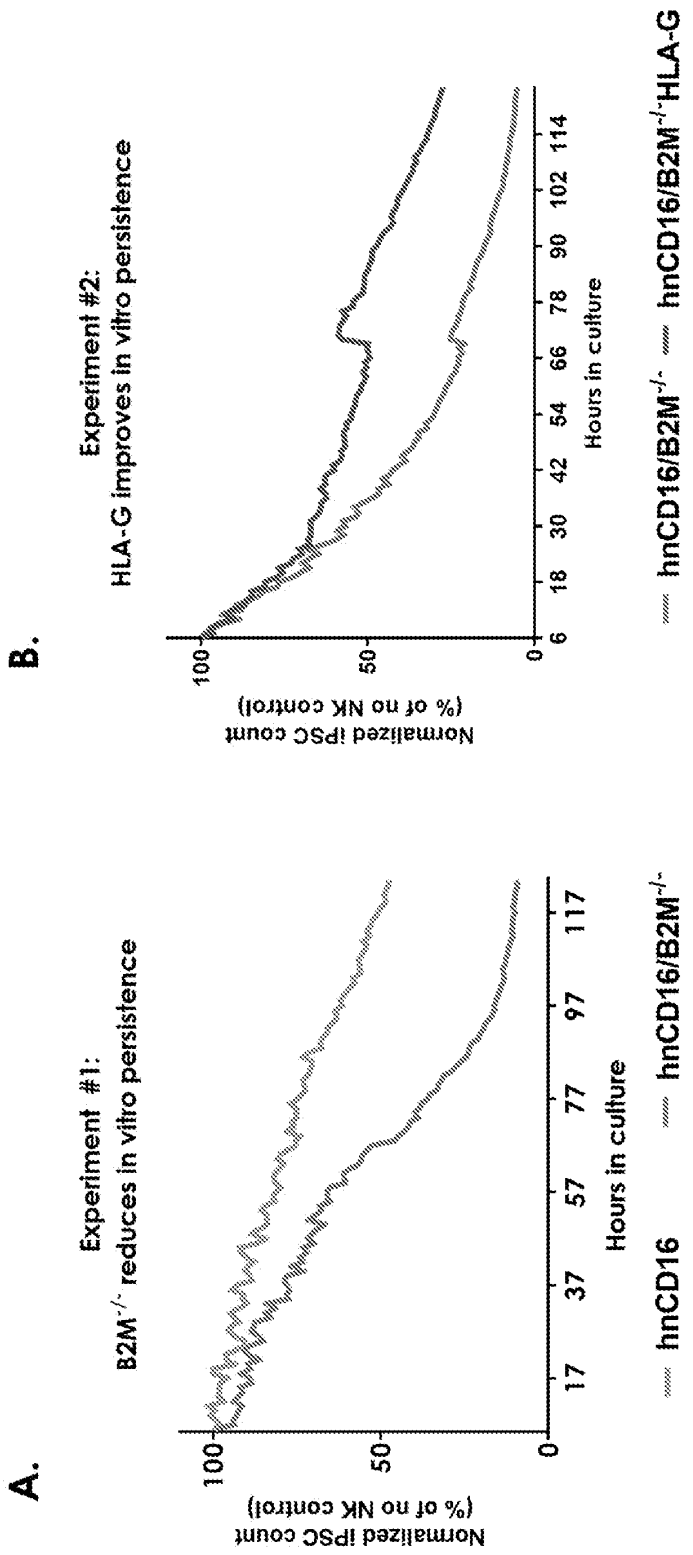

A.
Experiment #1:
B2M-/- reduces in vitro persistence
Normalized iPSC count
(% of no NK control)
100
50
0
17
37
57
77
97
117
Hours in culture
hnCD16
hnCD16/B2M-/-
B.
Experiment #2:
HLA-G improves in vitro persistence
Normalized iPSC count
(% of no NK control)
100
50
0
6
18
30
42
54
66
78
90
102
114
Hours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

Figure 5

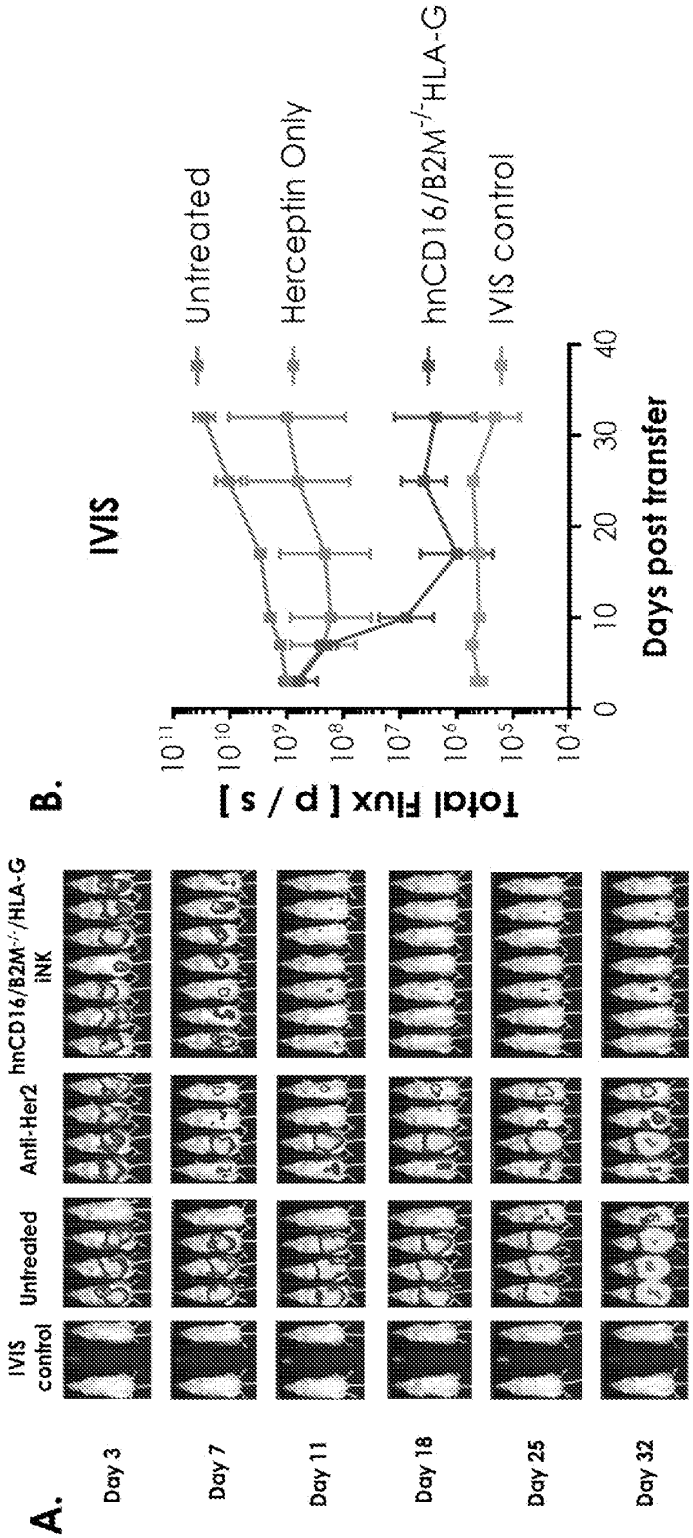

A.
Day 3
Day 7
Day 11
Day 18
Day 25
Day 32
IVIS control
Untreated
Anti-Her2
hnCD16/B2M-/-/HLA-G iNK
B.
IVIS
Total Flux [p / s]
Days post transfer
Untreated
Herceptin Only
hnCD16/B2M-/-HLA-G
IVIS control

Figure 6

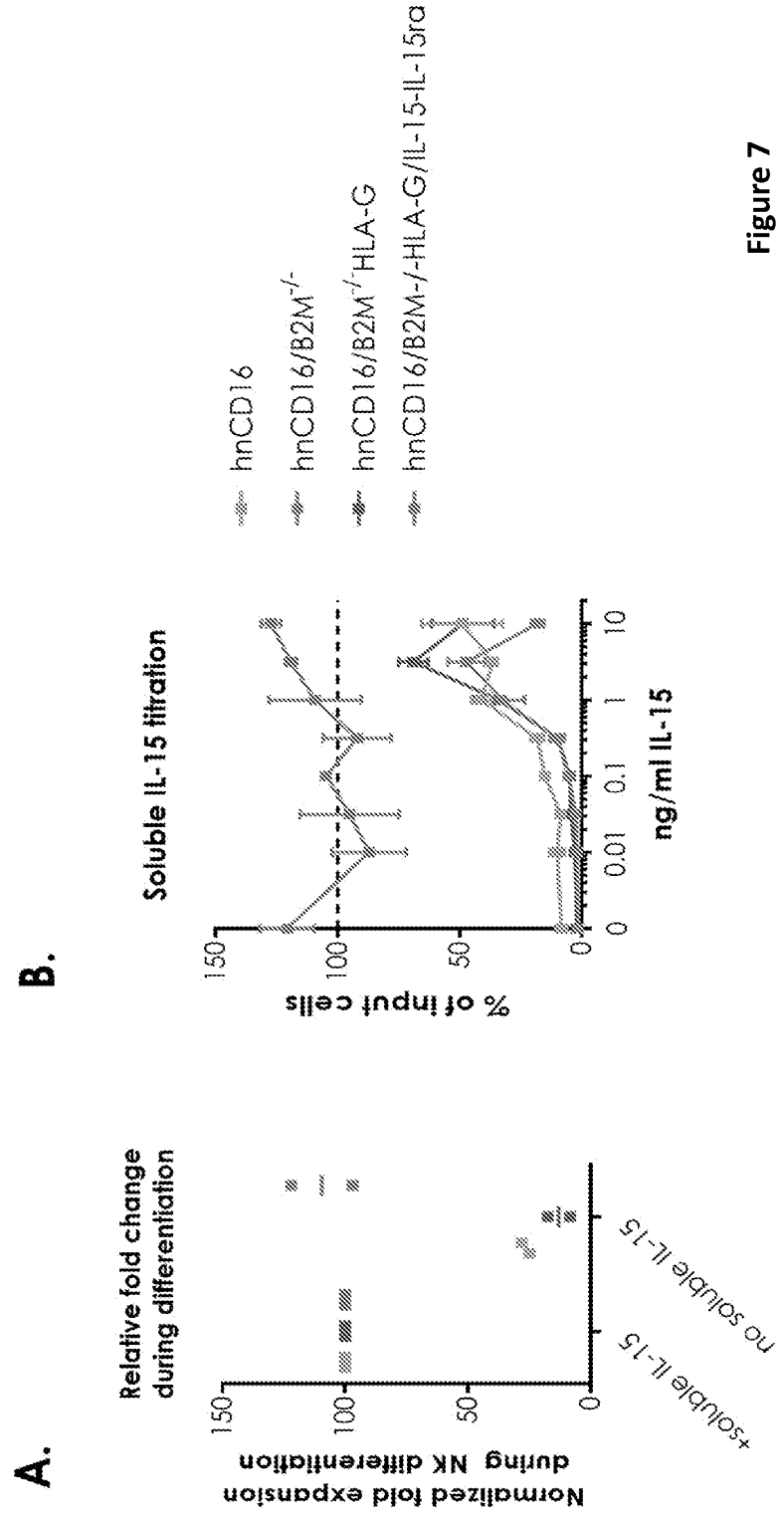

A.
Relative fold change during differentiation
Normalized fold expansion during NK differentiation
150
100
50
0
+soluble IL-15
no soluble IL-15
B.
Soluble IL-15 titration
% of input cells
150
100
50
0
0
0.01
0.1
1
10
ng/ml IL-15
hnCD16
hnCD16/B2M-/-
hnCD16/B2M-/-HLA-G
hnCD16/B2M-/-HLA-G/IL-15-IL-15ra

Figure 7

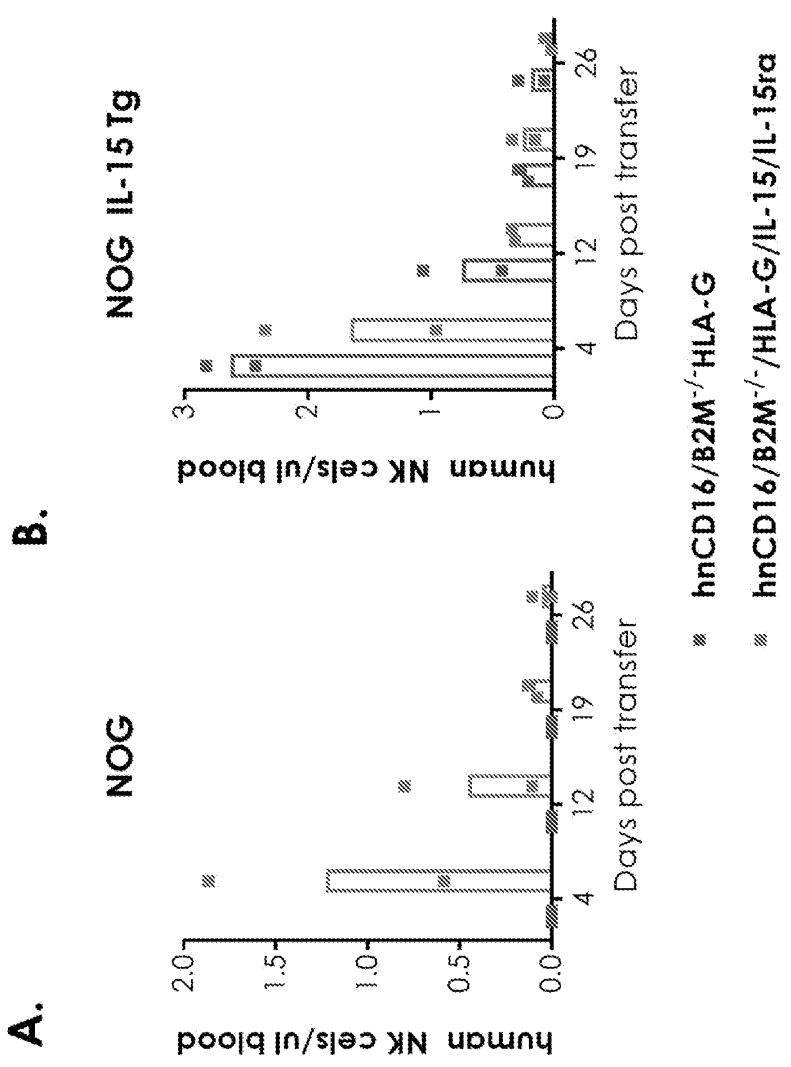

A.
NOG
human NK cels/ul blood
2.0
1.5
1.0
0.5
0.0
4
12
19
26
Days post transfer
B.
NOG IL-15 Tg
human NK cels/ul blood
3
2
1
0
4
12
19
26
Days post transfer
hnCD16/B2M-/-HLA-G
hnCD16/B2M-/-/HLA-G/IL-15/IL-15ra

Figure 8

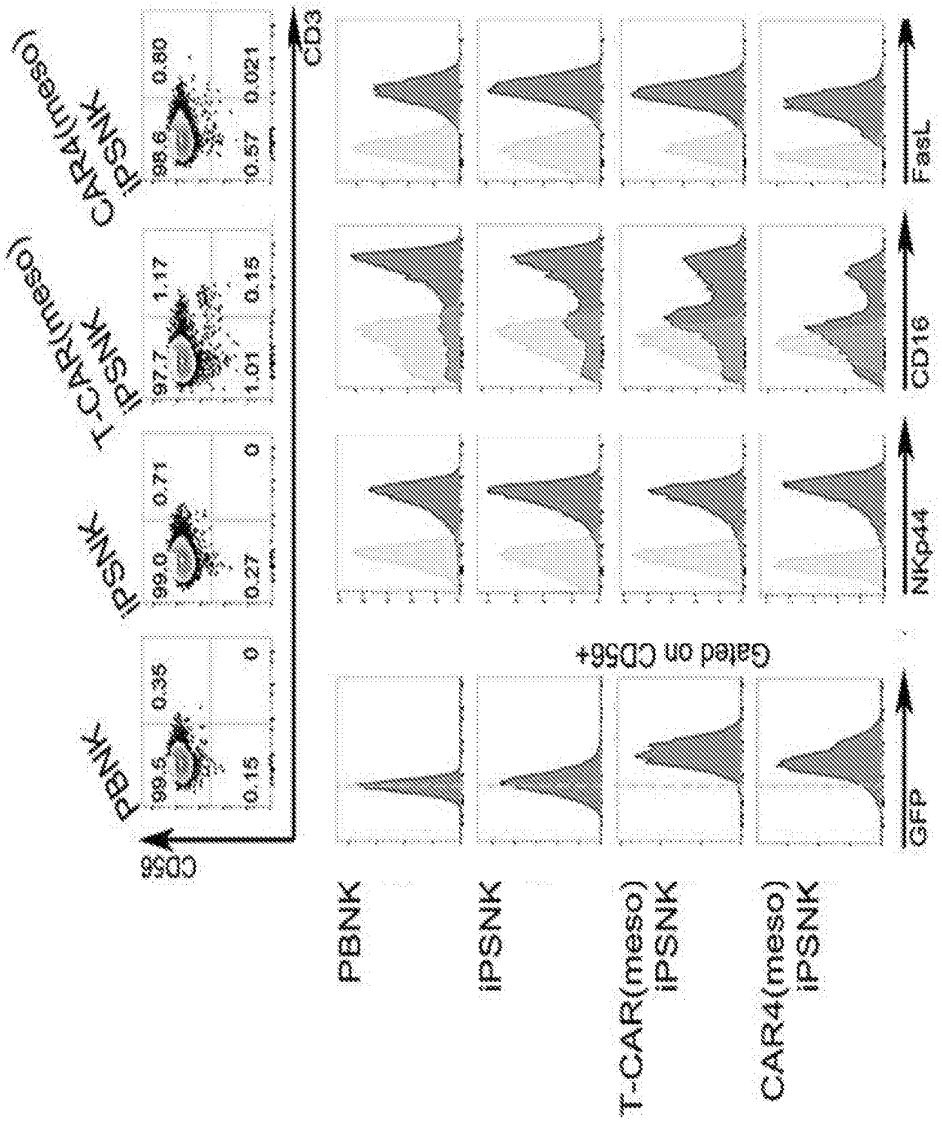

PBNK
iPSNK
T-CAR(meso) iPSNK
CAR4(meso) iPSNK
CD56
CD3
99.5
0.35
0.15
0
99.0
0.71
0.27
0
97.7
1.17
1.01
0.15
99.6
0.80
0.57
0.021
Gated on CD56+
GFP
NKp44
CD16
FasL

Figure 9

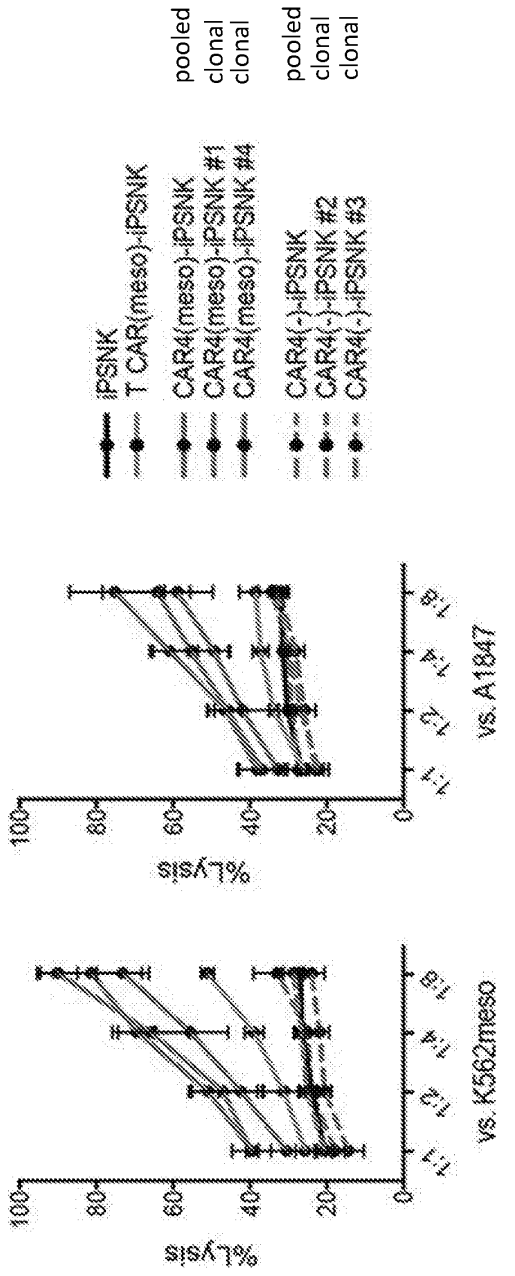

iPSNK
T CAR(meso)-iPSNK
CAR4(meso)-iPSNK
CAR4(meso)-iPSNK #1
CAR4(meso)-iPSNK #4
CAR4(-)-iPSNK
CAR4(-)-iPSNK #2
CAR4(-)-iPSNK #3
pooled
clonal
clonal
pooled
clonal
clonal
%Lysis
vs. K562meso
vs. A1847
A
B

Figure 10

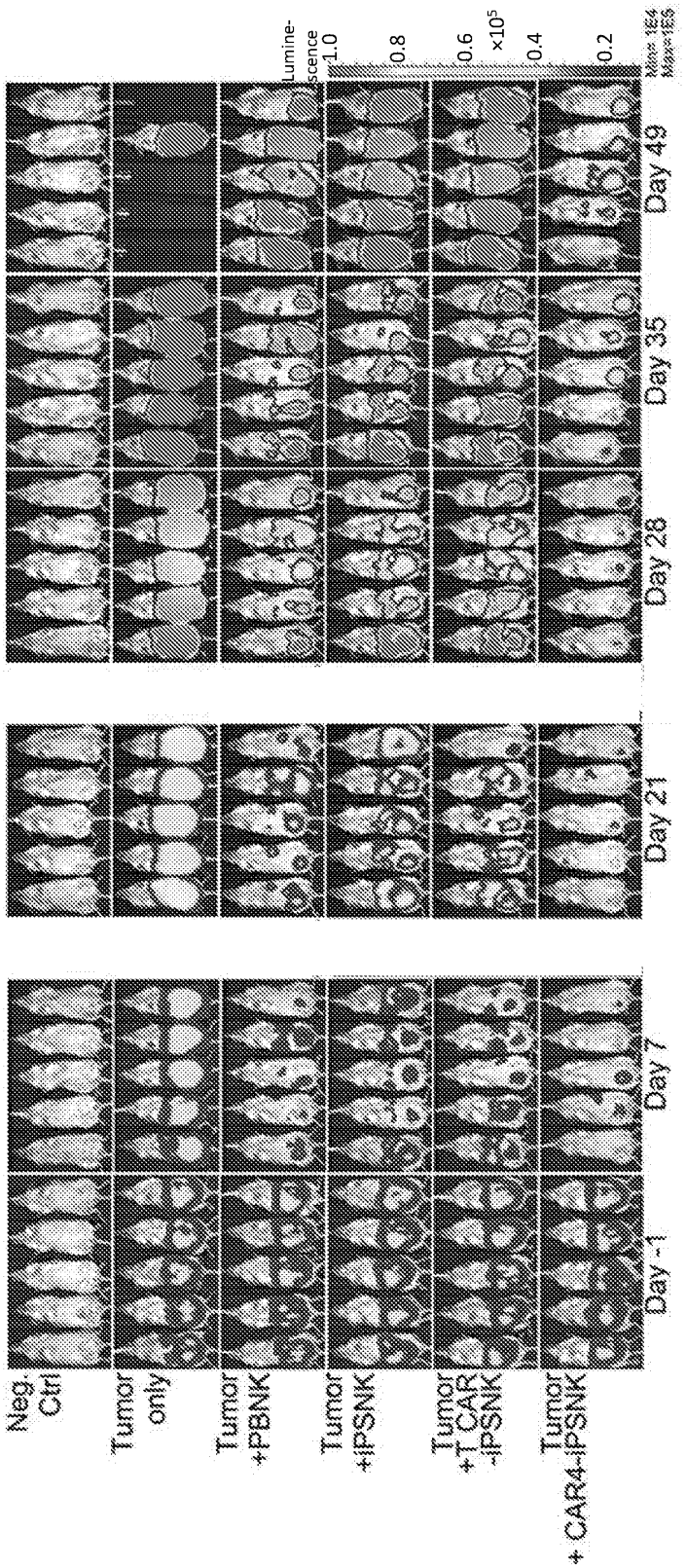

Lumine-
scence
1.0
0.8
0.6
×10^5
0.4
0.2
Min= 1E4
Max=1E5
Day 49
Day 35
Day 28
Day 21
Day 7
Day -1
Neg. Ctrl
Tumor only
Tumor +PBNK
Tumor +iPSNK
Tumor +TCAR -iPSNK
Tumor + CAR4-iPSNK

Figure 11

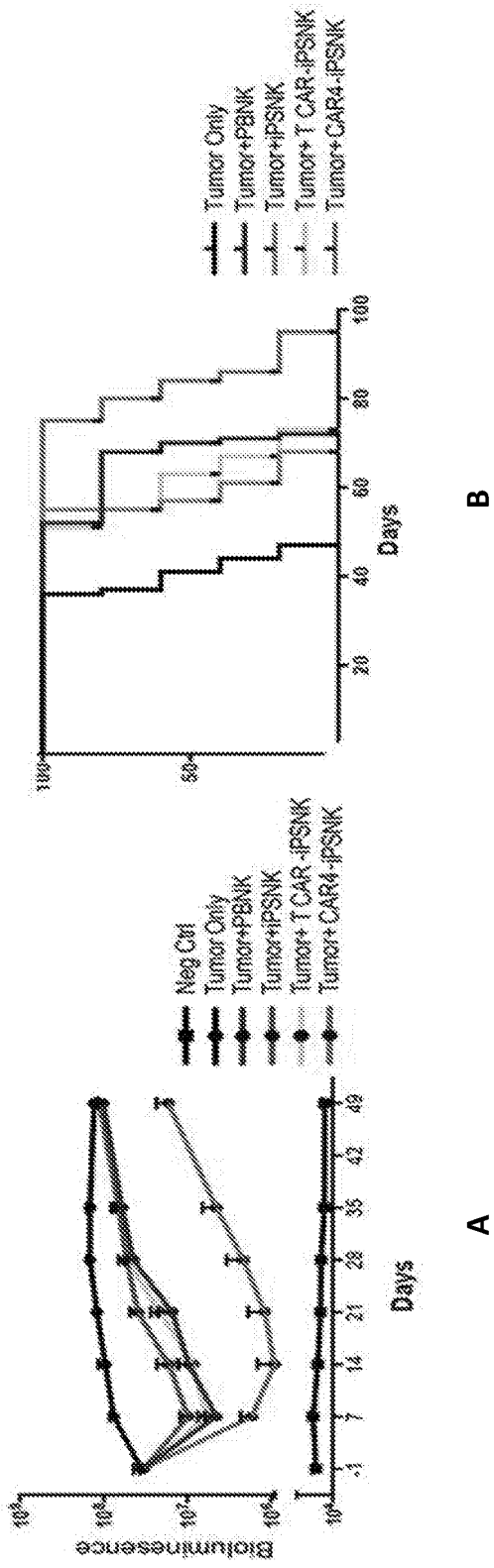

Neg Ctrl
Tumor Only
Tumor+PBNK
Tumor+iPSNK
Tumor+ T CAR-iPSNK
Tumor+CAR4-iPSNK
Bioluminescence
Days
A
Tumor Only
Tumor+PBNK
Tumor+iPSNK
Tumor+ T CAR-iPSNK
Tumor+CAR4-iPSNK
Days
B

Figure 12

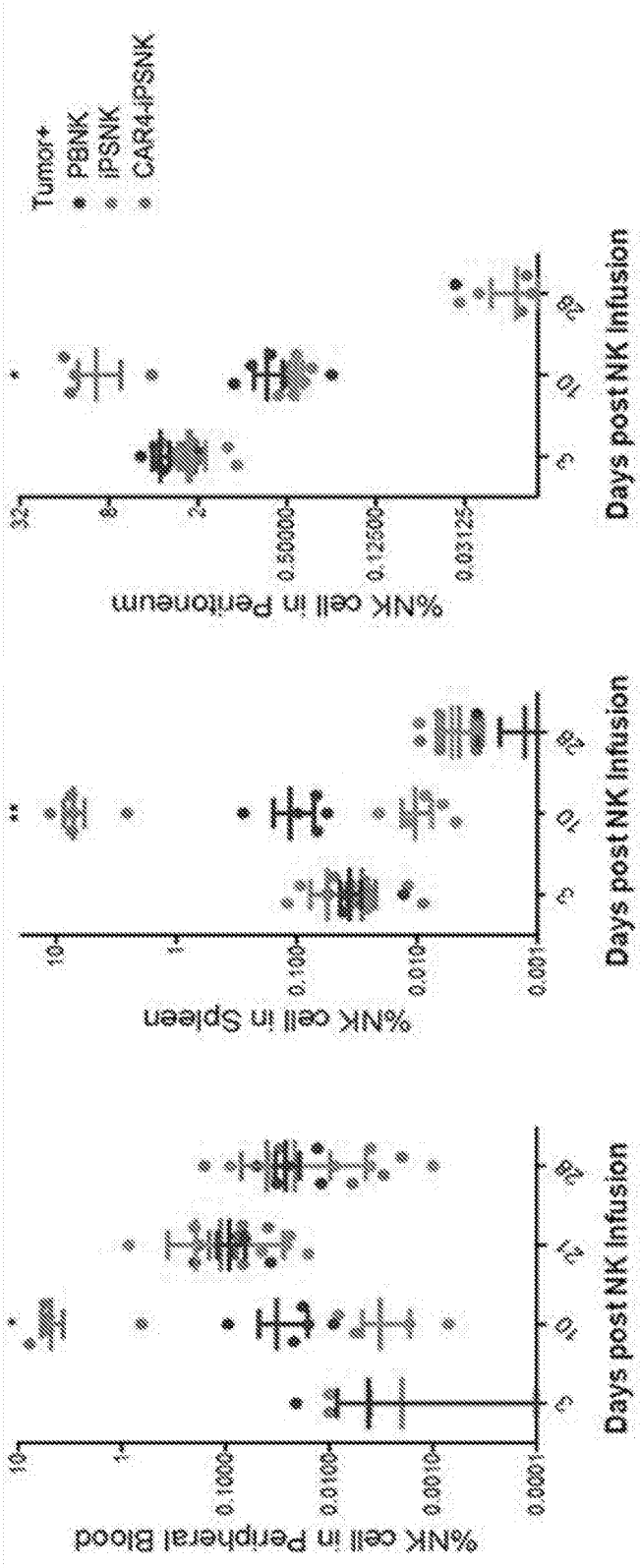

Tumor+
PBNK
IPSNK
CAR4-IPSNK
%NK cell in Peripheral Blood
%NK cell in Spleen
%NK cell in Peritoneum
Days post NK Infusion
A
B
C

Figure 13

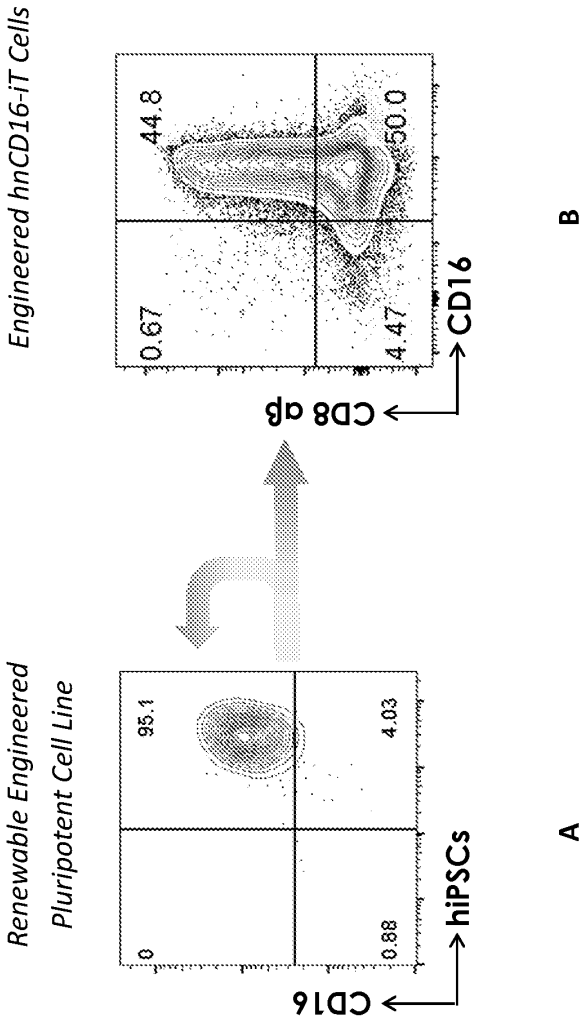
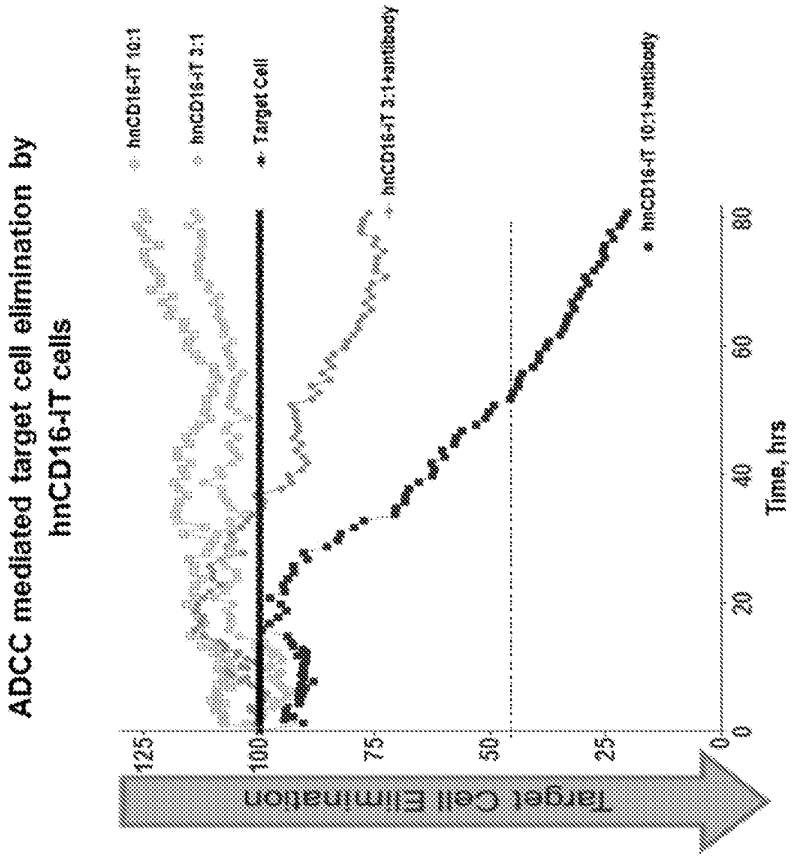

Renewable Engineered Pluripotent Cell Line
hiPSCs
CD16
95.1
4.03
0
0.88
A
Engineered hnCD16-iT Cells
CD16
CD8 αβ
44.8
50.0
0.67
4.47
B

Figure 14

Figure 15


ADCC mediated target cell elimination by hnCD16-iT cells
hnCD16-iT 10:1
hnCD16-iT 3:1
Target Cell
hnCD16-iT 2:1+antibody
hnCD16-iT 10:1+antibody
Target Cell Elimination
125
100
75
50
25
0
0
20
40
60
80
Time, hrs

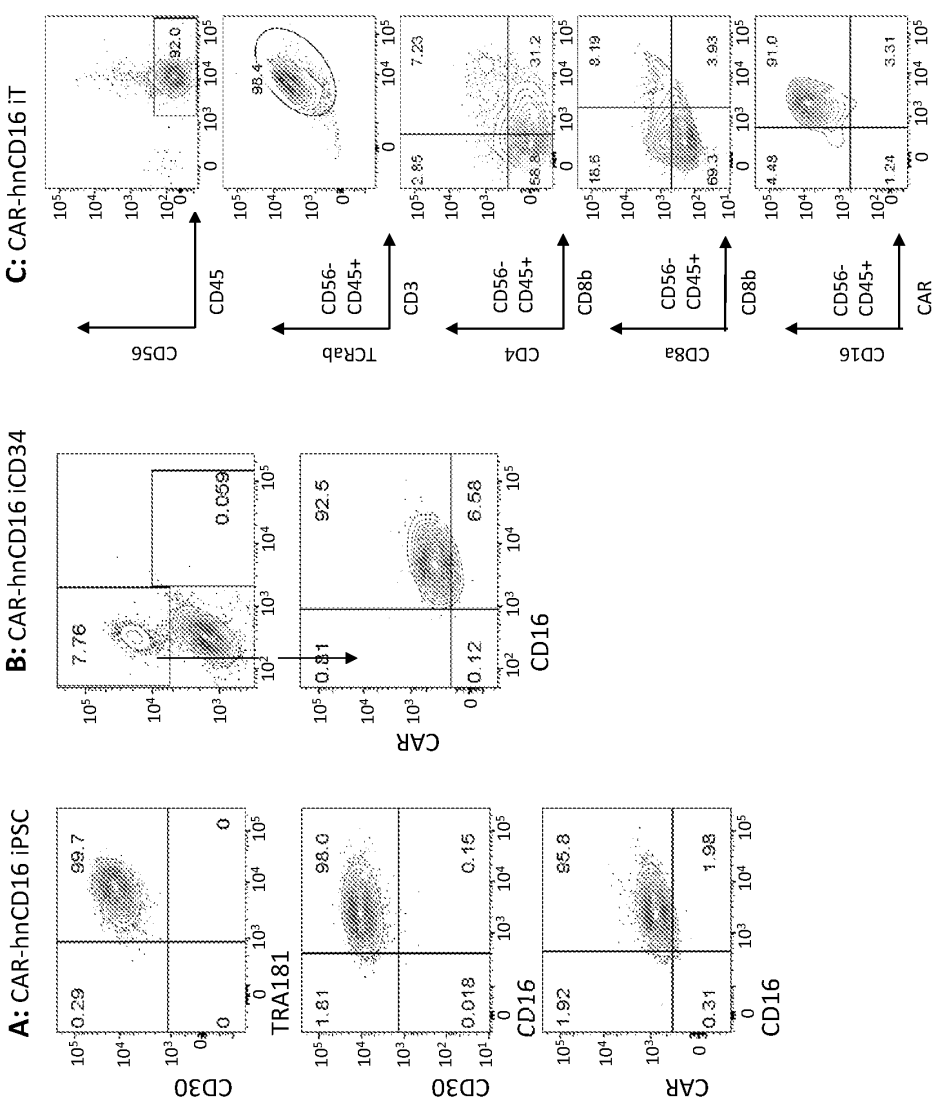

A: CAR-hnCD16 iPSC
CD30
TRA181
CD30
CD16
CAR
CD16
B: CAR-hnCD16 iCD34
CAR
CD16
C: CAR-hnCD16 iT
CD56
CD45
TCRab
CD56-CD45+
CD3
CD4
CD56-CD45+
CD8b
CD8a
CD56-CD45+
CD8b
CD16
CD56-CD45+
CAR

Figure 16

Figure 17

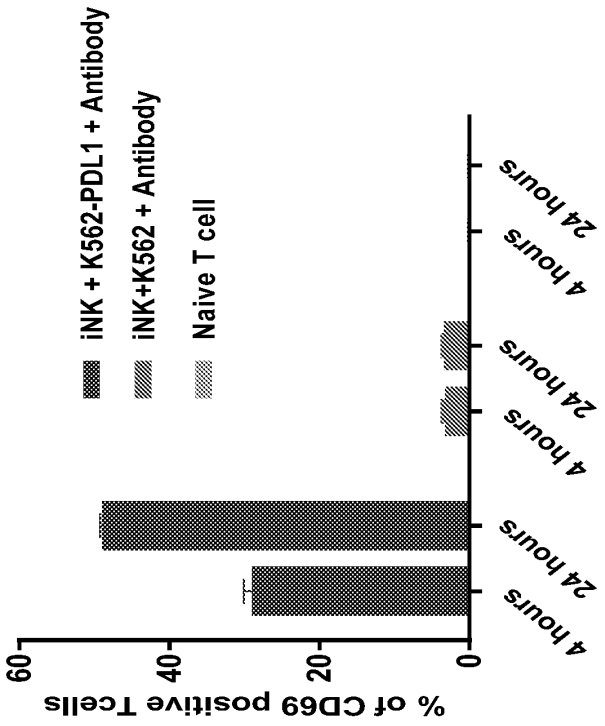
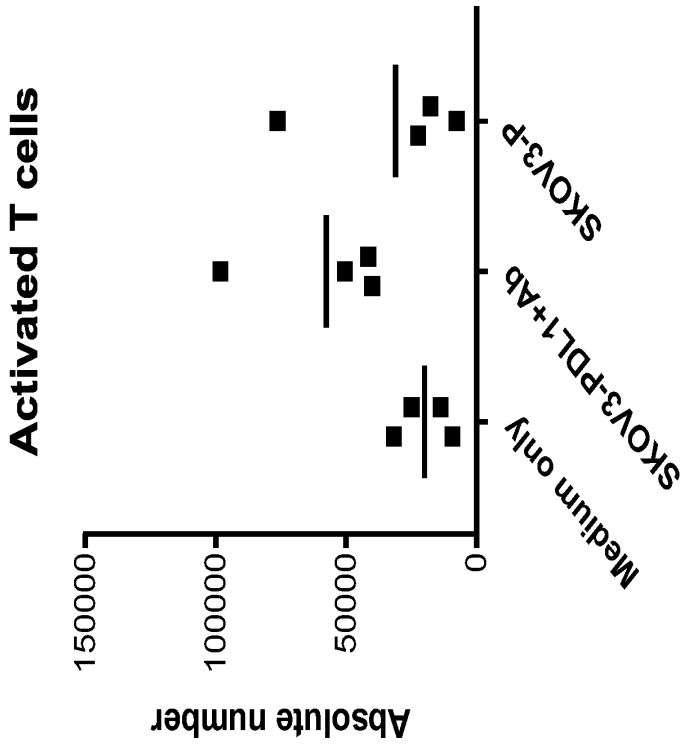

iNK + K562-PDL1 + Antibody
iNK+K562 + Antibody
Naive T cell
% of CD69 positive Tcells
0
20
40
60
4 hours
24 hours
4 hours
24 hours
4 hours
24 hours

Figure 18


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

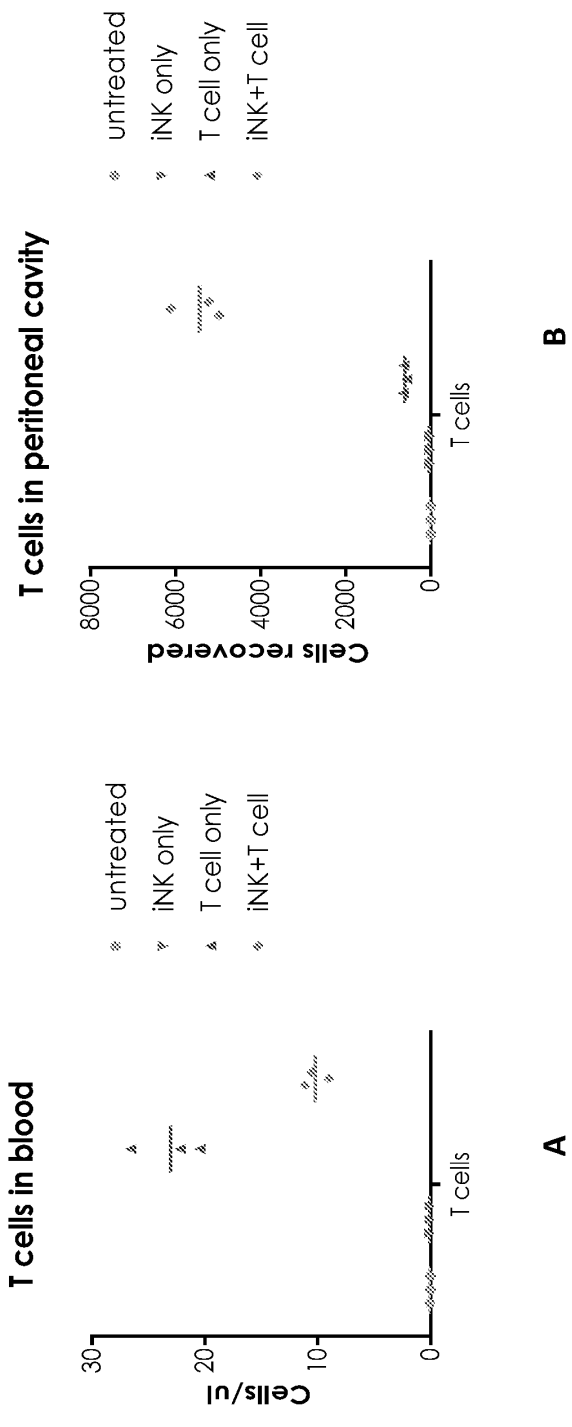
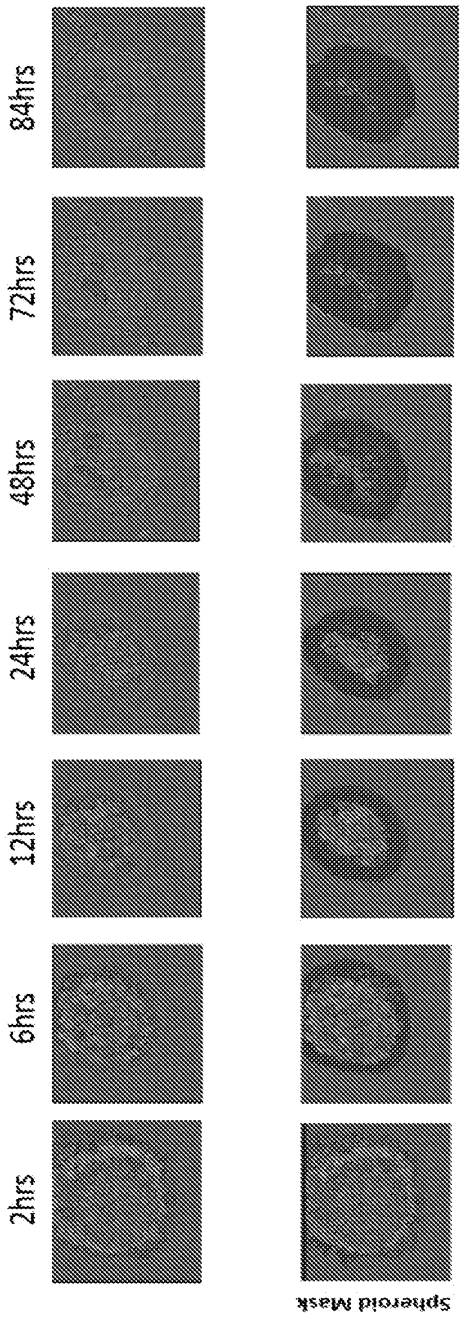

T cells in blood
Cells/ul
30
20
10
0
T cells
untreated
iNK only
T cell only
iNK+T cell
A
T cells in peritoneal cavity
Cells recovered
8000
6000
4000
2000
0
T cells
untreated
iNK only
T cell only
iNK+T cell
B

Figure 19

Figure 20



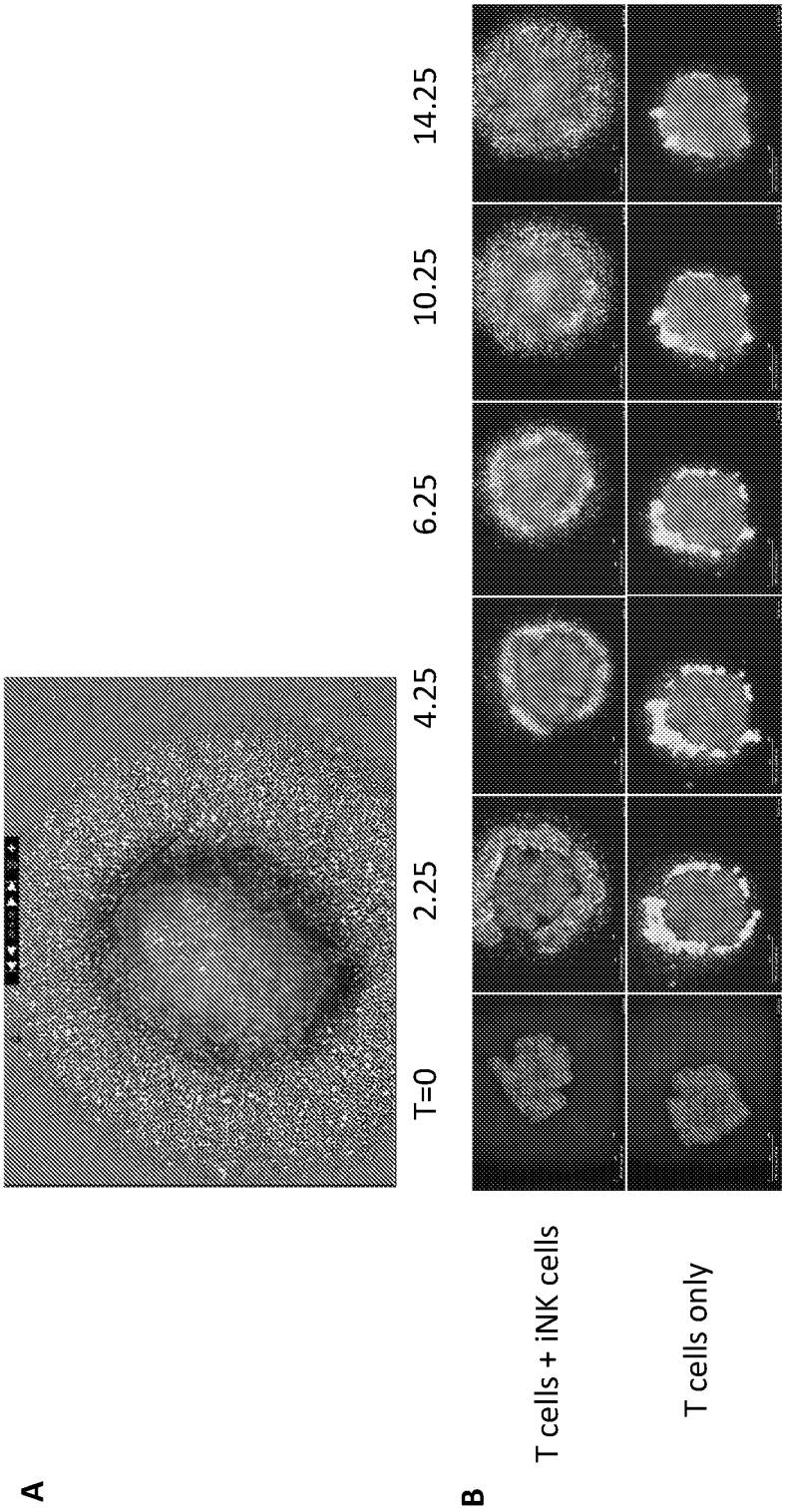


Figure 21

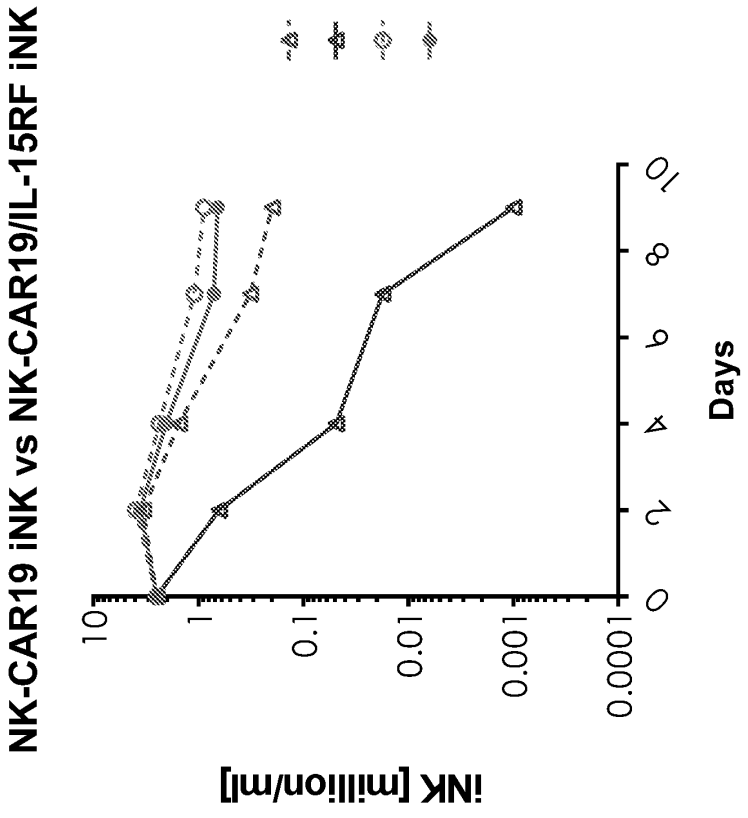

NK-CAR19 iNK vs NK-CAR19/IL-15RF iNK
NK-CAR19
NK-CAR19 w/o IL-2
NK-CAR19 IL-15RF
NK-CAR19 IL-15RF w/o IL-2
iNK [million/ml]
10
1
0.1
0.01
0.001
0.0001
0
1
4
6
8
10
Days

Figure 22

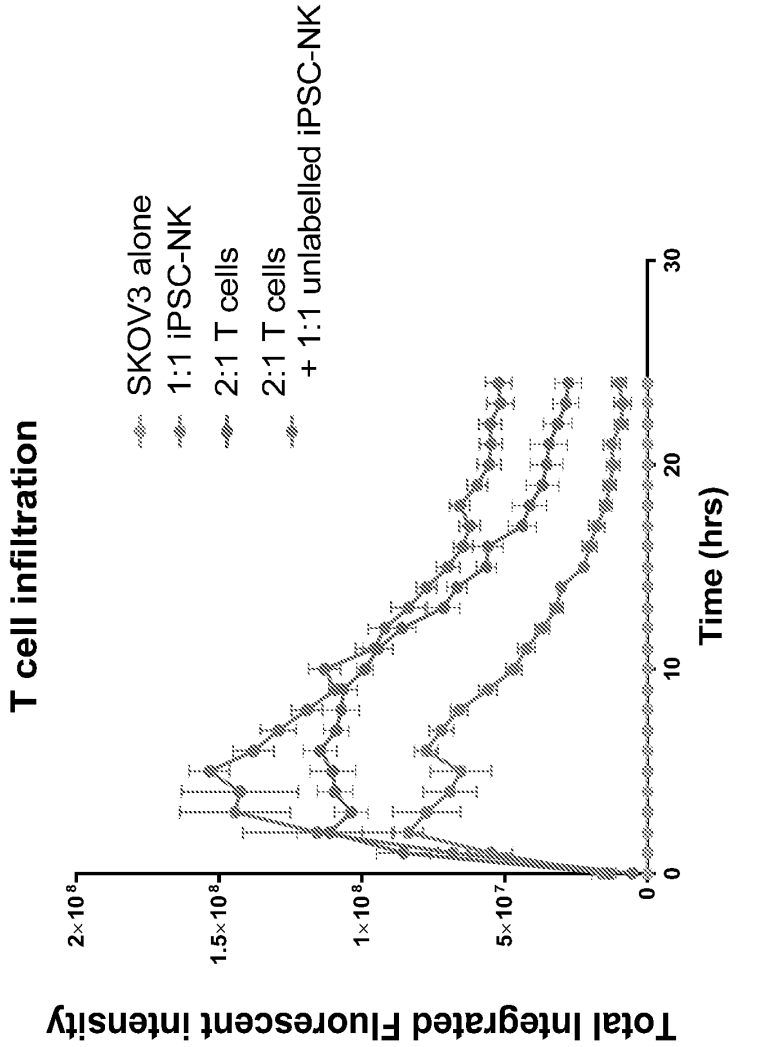

T cell infiltration
SKOV3 alone
1:1 iPSC-NK
2:1 T cells
2:1 T cells
+ 1:1 unlabelled iPSC-NK
Total Integrated Fluorescent Intensity
2×10^8
1.5×10^8
1×10^8
5×10^7
0
0
10
20
30
Time (hrs)

Figure 23

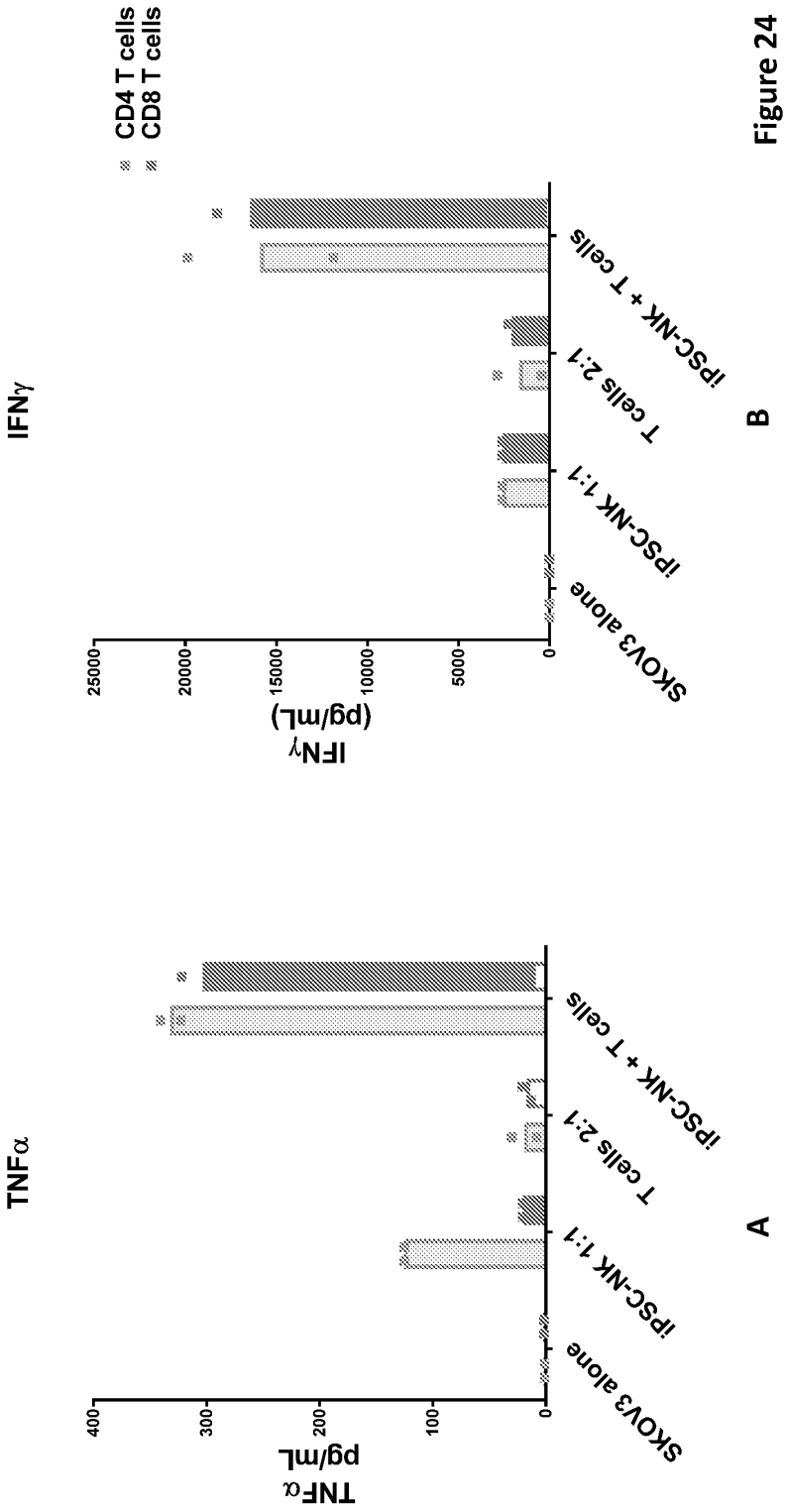

CD4 T cells
CD8 T cells
IFNγ
IFNγ (pg/mL)
25000
20000
15000
10000
5000
0
SKOV3 alone
iPSC-NK 1:1
T cells 2:1
iPSC-NK + T cells
B
TNFα
TNFα pg/mL
400
300
200
100
0
SKOV3 alone
iPSC-NK 1:1
T cells 2:1
iPSC-NK + T cells
A

Figure 24

Figure 25

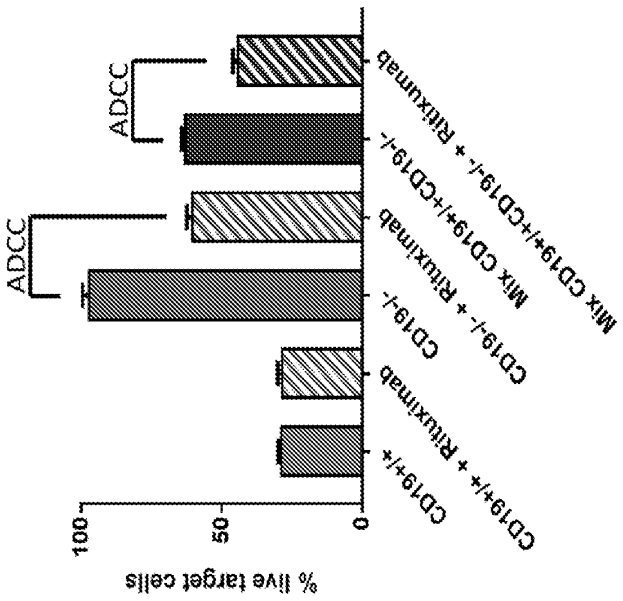
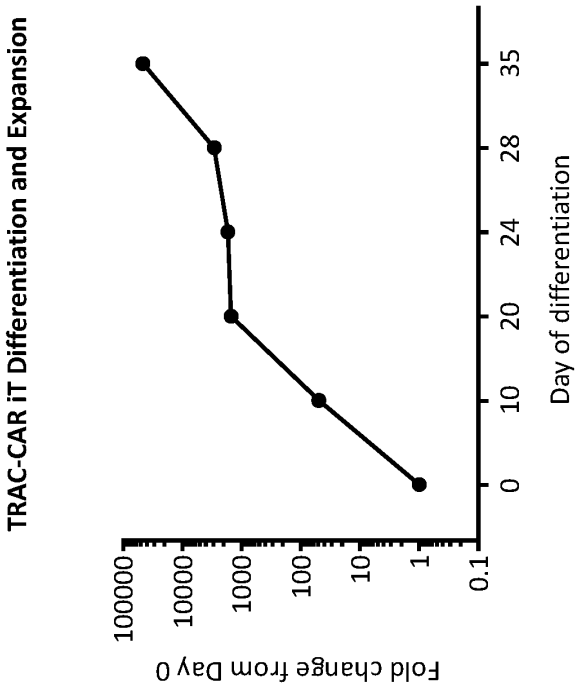

% live target cells
100
50
0
CD19+/+
CD19+/+ + Rituximab
CD19-/-
CD19-/- + Rituximab
Mix CD19+/+CD19-/-
Mix CD19+/+CD19-/- + Rituximab
ADCC
ADCC

Figure 26


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

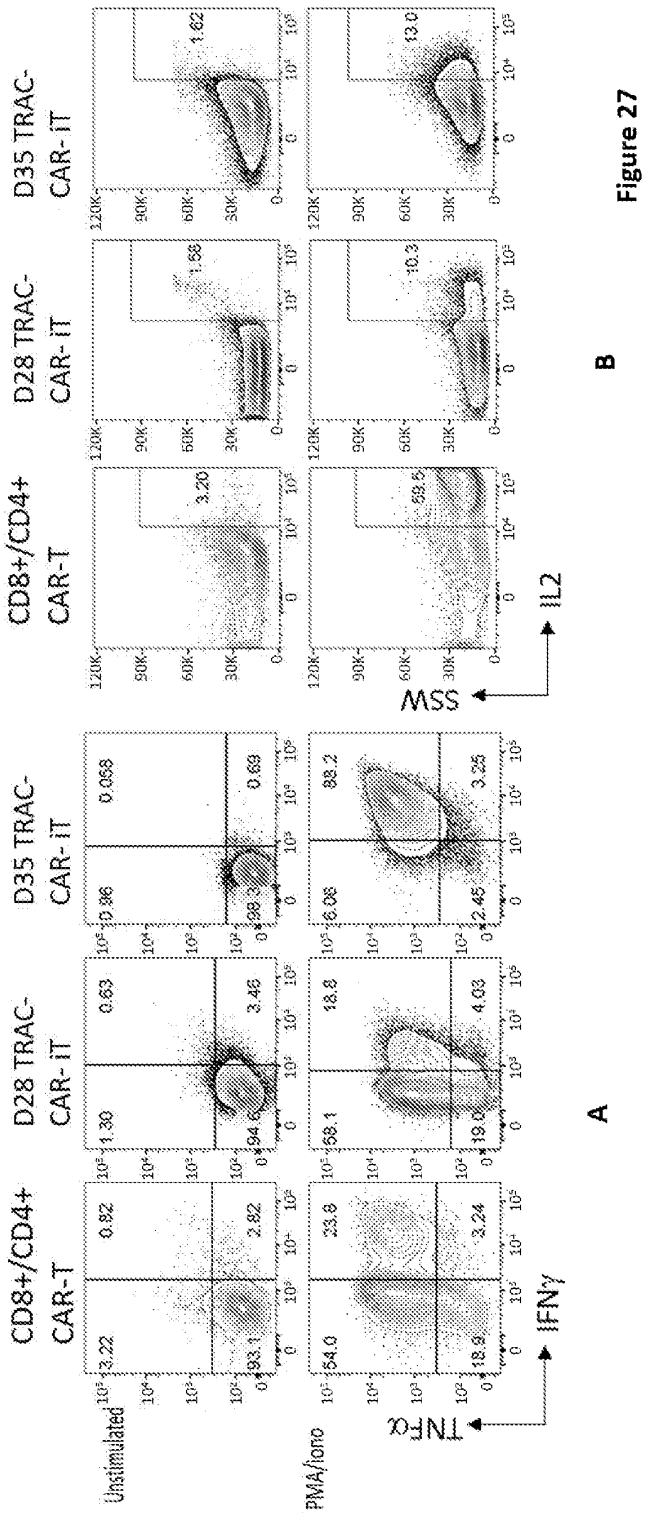
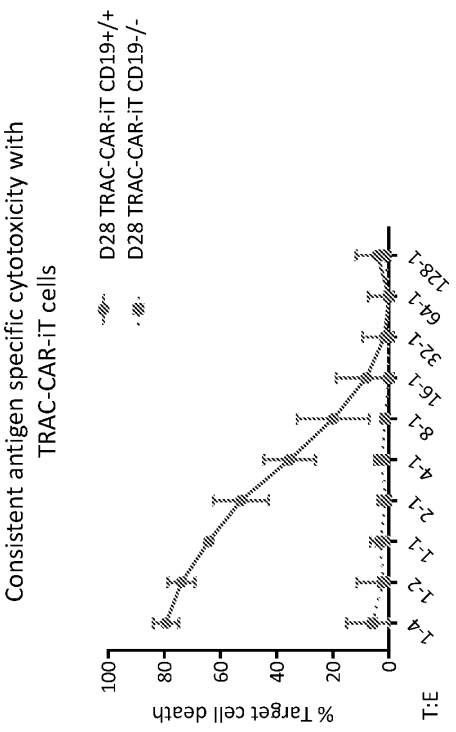

CD8+/CD4+ CAR-T
D28 TRAC-CAR-iT
D35 TRAC-CAR-iT
Unstimulated
PMA/Iono
TNFα
IFNγ
A
SSW
IL2
B

Figure 27


Consistent antigen specific cytotoxicity with TRAC-CAR-iT cells
D28 TRAC-CAR-iT CD19+/+
D28 TRAC-CAR-iT CD19-/-
% 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
B

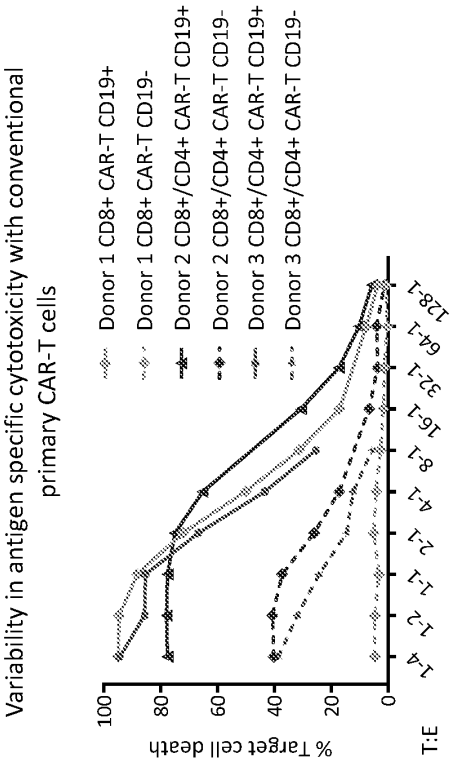

Variability in antigen specific cytotoxicity with conventional primary CAR-T cells
Donor 1 CD8+ CAR-T CD19+
Donor 1 CD8+ CAR-T CD19-
Donor 2 CD8+/CD4+ CAR-T CD19+
Donor 2 CD8+/CD4+ CAR-T CD19-
Donor 3 CD8+/CD4+ CAR-T CD19+
Donor 3 CD8+/CD4+ CAR-T CD19-
% Target cell death
100
80
60
40
20
0
T:E
1:4
1:2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

Figure 28

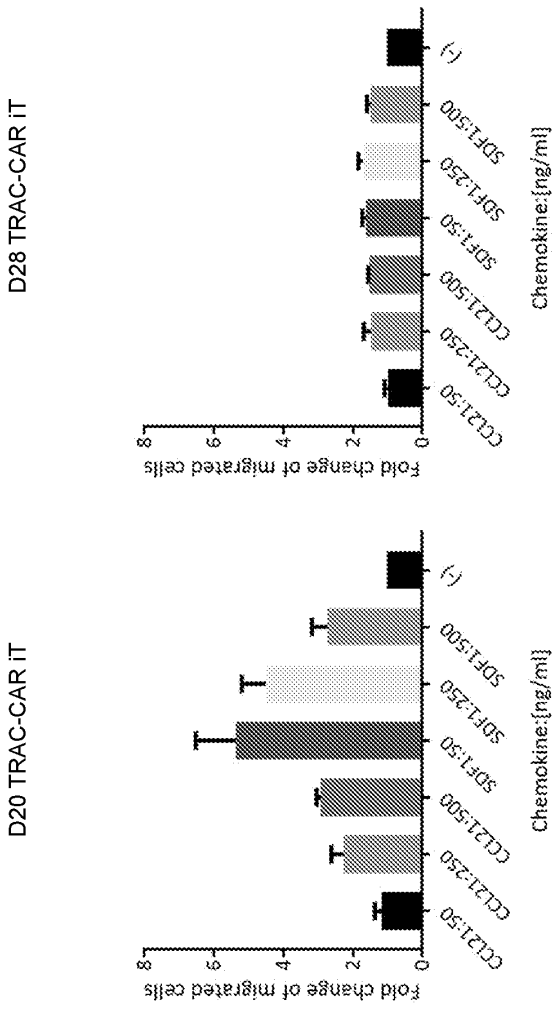

D20 TRAC-CAR iT
D28 TRAC-CAR iT
Fold change of migrated cells
0
2
4
6
8
CCL21:50
CCL21:250
CCL21:500
SDF1:50
SDF1:250
SDF1:500
(-)
Chemokine:[ng/ml]
A
B

Figure 29

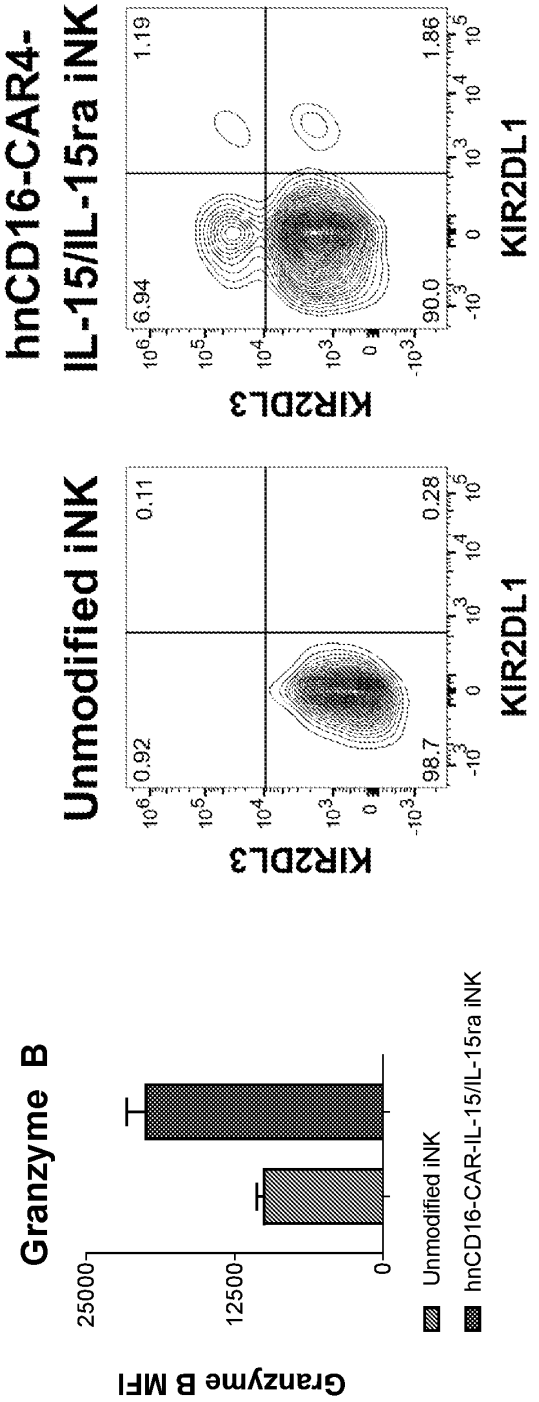

Granzyme B
Granzyme B MFI
25000
12500
0
Unmodified iNK
hnCD16-CAR-IL-15/IL-15ra iNK
A
Unmodified iNK
KIR2DL3
KIR2DL1
0.92
0.11
98.7
0.28
hnCD16-CAR4-IL-15/IL-15ra iNK
6.94
1.19
90.0
1.86
B

Figure 30

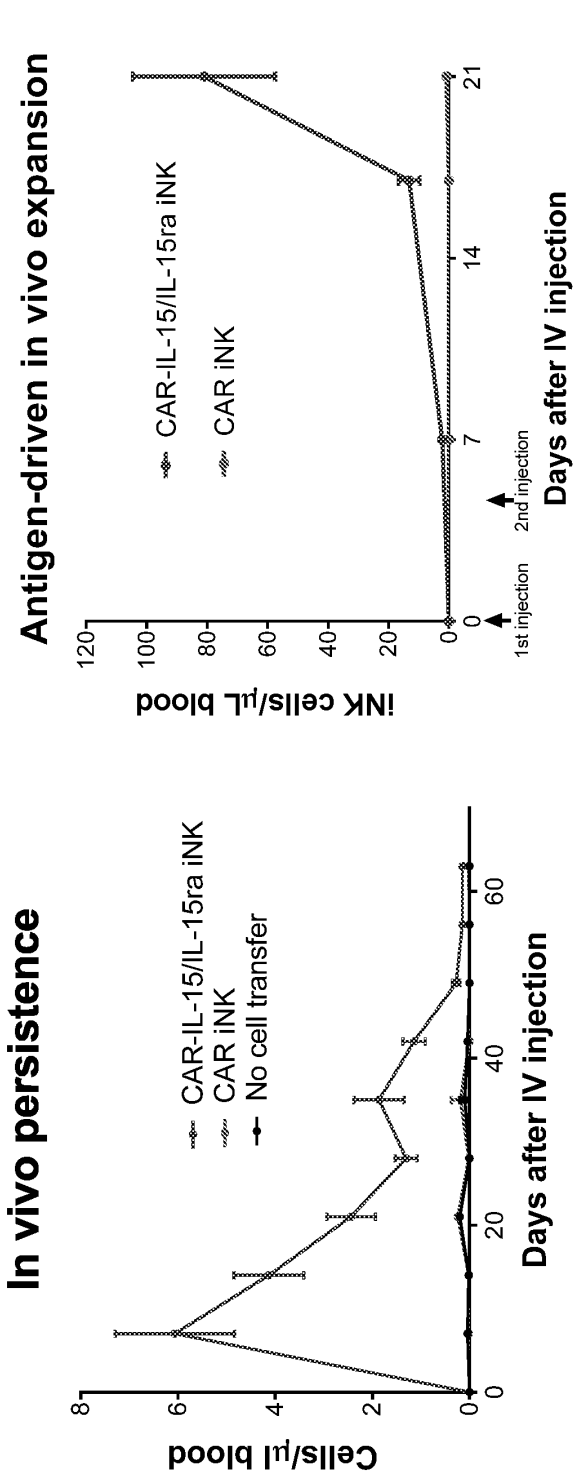
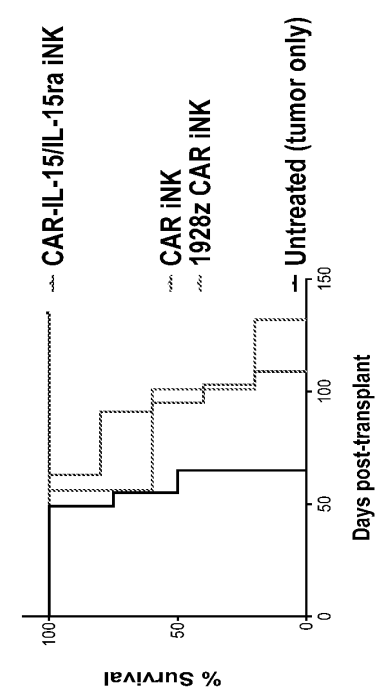
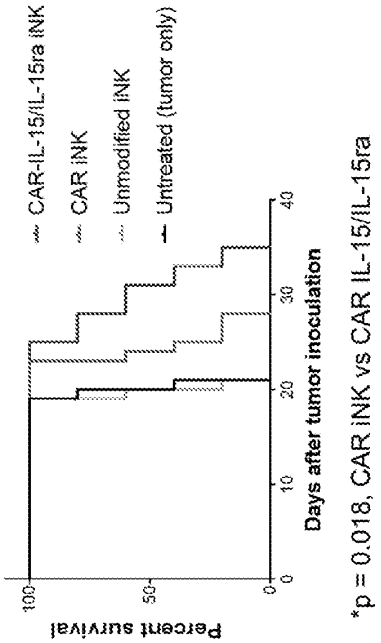

In vivo persistence
Cells/μl blood
0
2
4
6
8
0
20
40
60
Days after IV injection
CAR-IL-15/IL-15ra iNK
CAR iNK
No cell transfer
A
Antigen-driven in vivo expansion
iNK cells/μL blood
0
20
40
60
80
100
120
0
7
14
21
Days after IV injection
1st injection
2nd injection
CAR-IL-15/IL-15ra iNK
CAR iNK
B

Figure 31



B



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

A

Figure 32

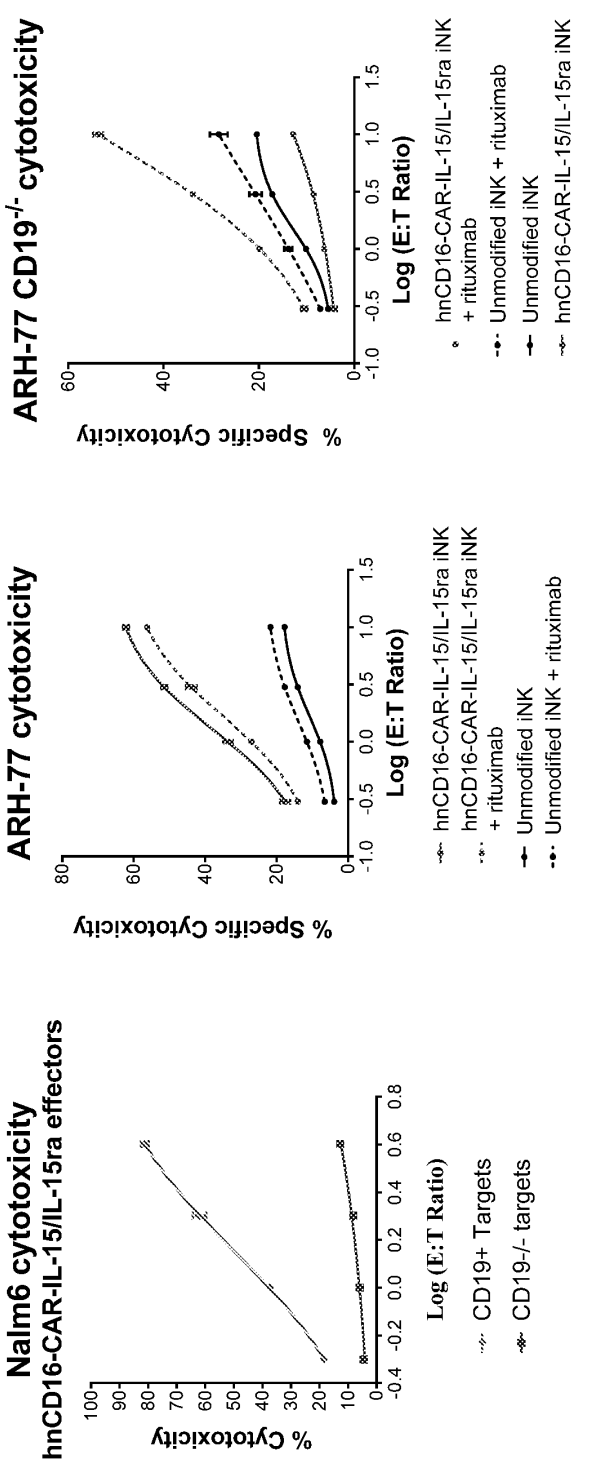

Nalm6 cytotoxicity
hnCD16-CAR-IL-15/IL-15ra effectors
% Cytotoxicity
Log (E:T Ratio)
CD19+ Targets
CD19-/- targets
A
ARH-77 cytotoxicity
% Specific Cytotoxicity
Log (E:T Ratio)
hnCD16-CAR-IL-15/IL-15ra iNK
hnCD16-CAR-IL-15/IL-15ra iNK + rituximab
Unmodified iNK
Unmodified iNK + rituximab
B
ARH-77 CD19-/- cytotoxicity
% Specific Cytotoxicity
Log (E:T Ratio)
hnCD16-CAR-IL-15/IL-15ra iNK + rituximab
Unmodified iNK + rituximab
Unmodified iNK
hnCD16-CAR-IL-15/IL-15ra iNK
C

Figure 33

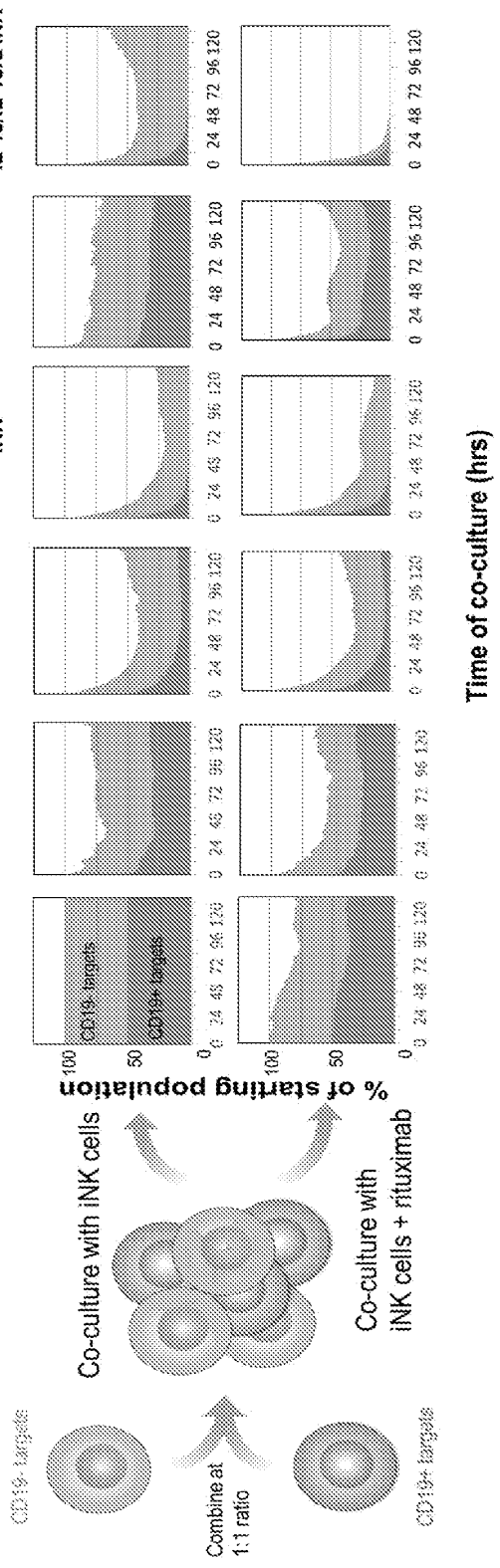

No effectors
Unmodified iNK
CAR4 iNK
CAR4-IL-15/IL-15ra iNK
hnCD16 iNK
hnCD16-CAR4-IL-15/IL-15ra iNK
CD19- targets
CD19+ targets
% of starting population
Time of co-culture (hrs)
Co-culture with iNK cells
Co-culture with iNK cells + rituximab
Combine at 1:1 ratio
CD19- targets
CD19+ targets

Figure 34

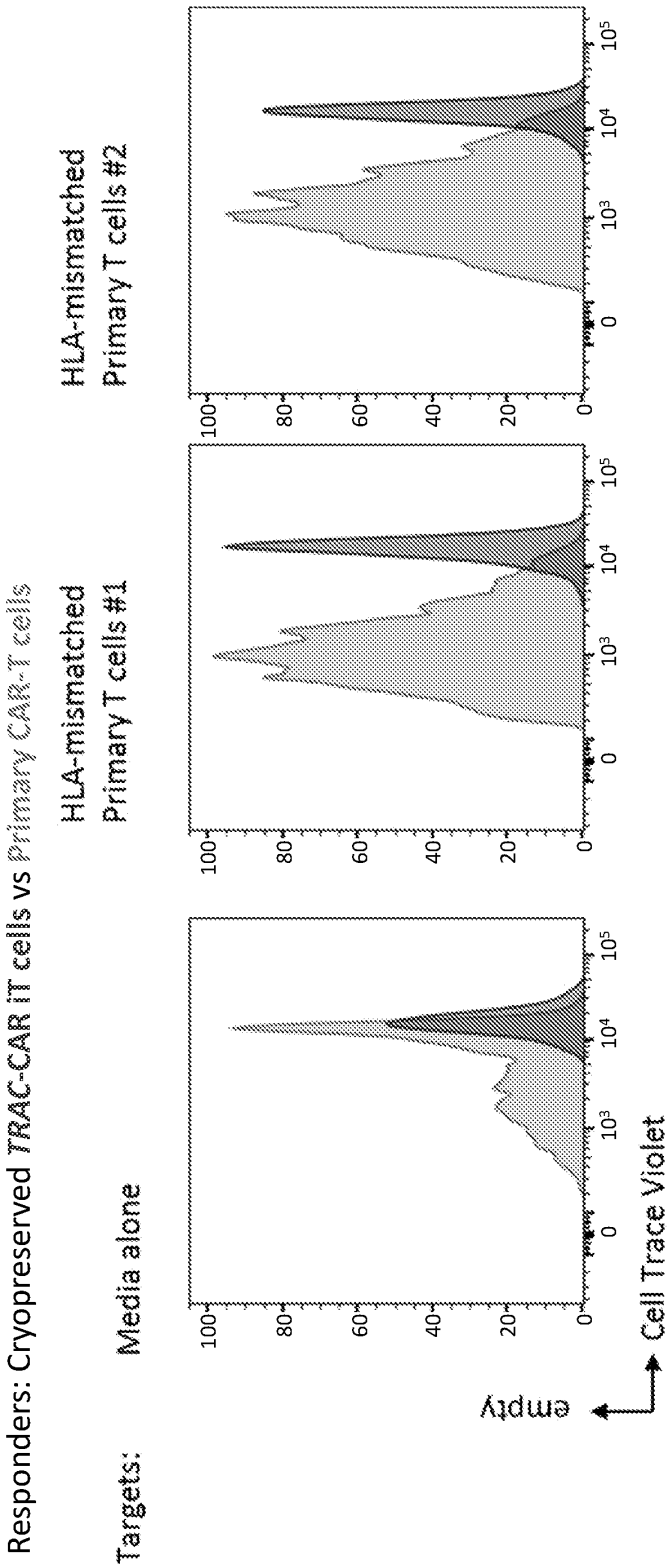

Responders: Cryopreserved TRAC-CAR iT cells vs Primary CAR-T cells
Targets:
Media alone
HLA-mismatched Primary T cells #1
HLA-mismatched Primary T cells #2
empty
Cell Trace Violet
0
20
40
60
80
100
10^3
10^4
10^5

Figure 35

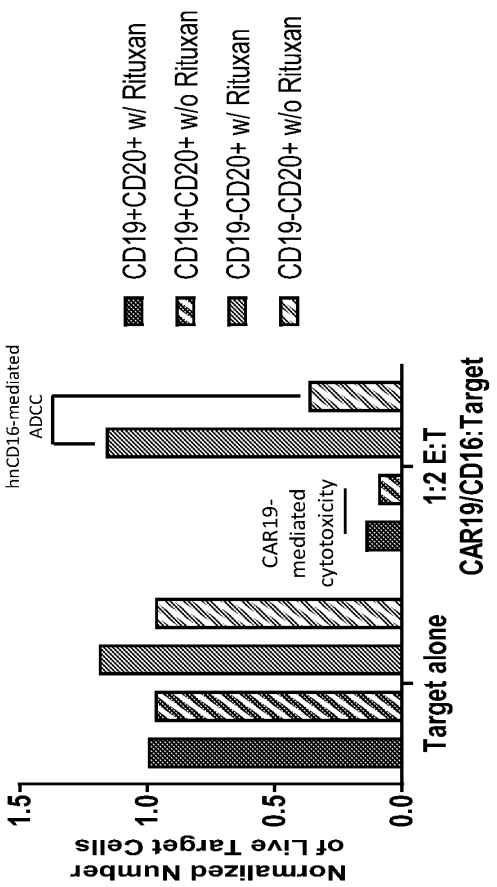

CD19+CD20+ w/ Rituxan
CD19+CD20+ w/o Rituxan
CD19-CD20+ w/ Rituxan
CD19-CD20+ w/o Rituxan
hnCD16-mediated ADCC
CAR19-mediated cytotoxicity
Normalized Number of Live Target Cells
1.5
1.0
0.5
0.0
Target alone
1:2 E:T CAR19/CD16:Target

Figure 36