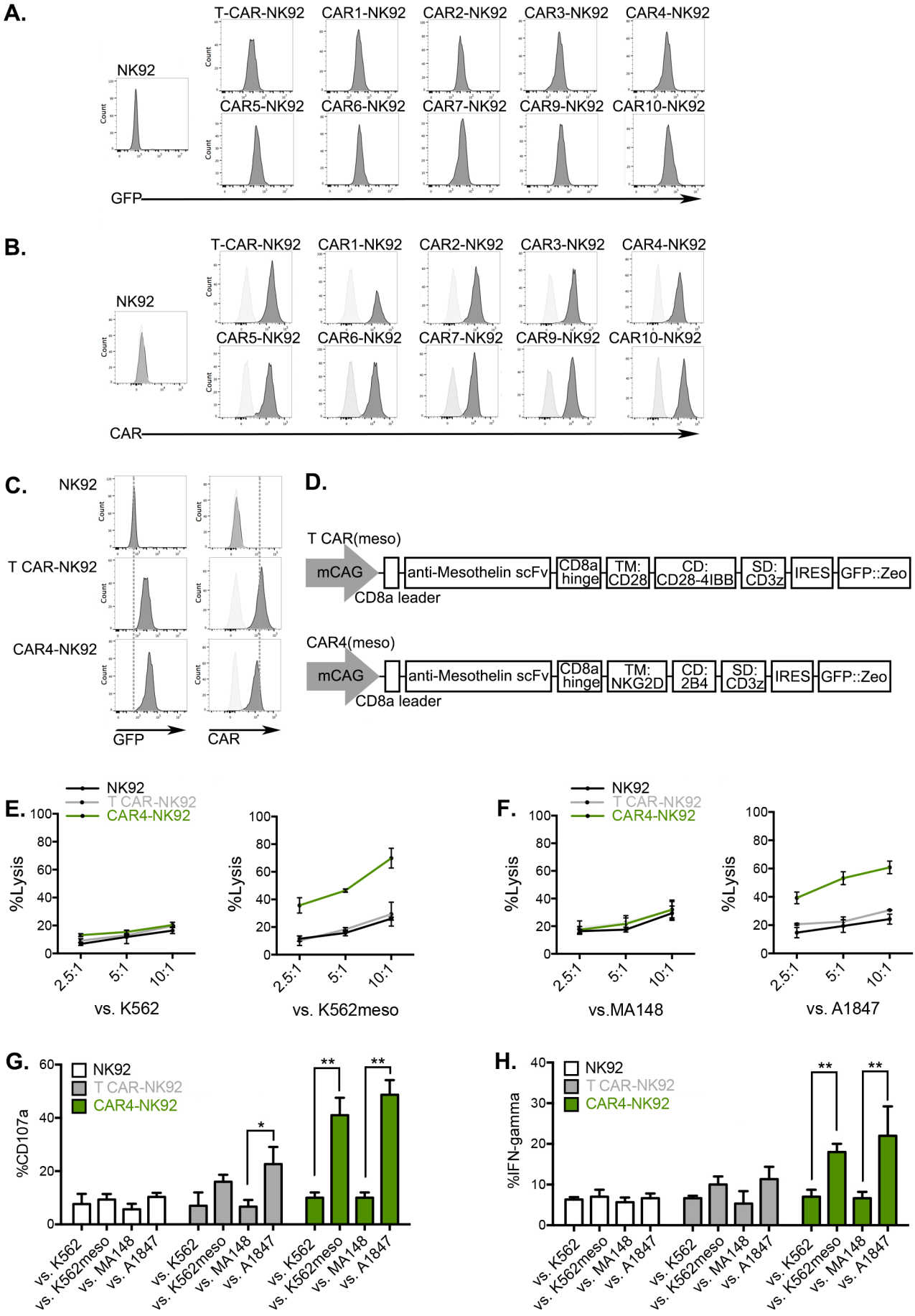

A.
NK92
T-CAR-NK92
CAR1-NK92
CAR2-NK92
CAR3-NK92
CAR4-NK92
CAR5-NK92
CAR6-NK92
CAR7-NK92
CAR9-NK92
CAR10-NK92
Count
GFP
B.
NK92
T-CAR-NK92
CAR1-NK92
CAR2-NK92
CAR3-NK92
CAR4-NK92
CAR5-NK92
CAR6-NK92
CAR7-NK92
CAR9-NK92
CAR10-NK92
Count
CAR
C.
NK92
T CAR-NK92
CAR4-NK92
Count
GFP
CAR
D.
T CAR(meso)
mCAG
CD8a leader
anti-Mesothelin scFv
CD8a hinge
TM: CD28
CD: CD28-4IBB
SD: CD3z
IRES
GFP::Zeo
CAR4(meso)
mCAG
CD8a leader
anti-Mesothelin scFv
CD8a hinge
TM: NKG2D
CD: 2B4
SD: CD3z
IRES
GFP::Zeo
E.
NK92
T CAR-NK92
CAR4-NK92
%Lysis
2.5:1
5:1
10:1
vs. K562
vs. K562meso
F.
NK92
T CAR-NK92
CAR4-NK92
%Lysis
2.5:1
5:1
10:1
vs.MA148
vs. A1847
G.
NK92
T CAR-NK92
CAR4-NK92
%CD107a
*
**
**
vs. K562
vs. K562meso
vs. MA148
vs. A1847
H.
NK92
T CAR-NK92
CAR4-NK92
%IFN-gamma
**
**
vs. K562
vs. K562meso
vs. MA148
vs. A1847

Supplemental Figure S1. Expression, cytotoxicity and NK cell activation response in CAR-expressing NK92 cells. Related to Table 1 and Figure 1

(A and B) Expression GFP (A) and the surface expression of CAR (B) was measured in NK92 cells by flow cytometry to demonstrate similar levels of surface CAR expression by the different constructs.

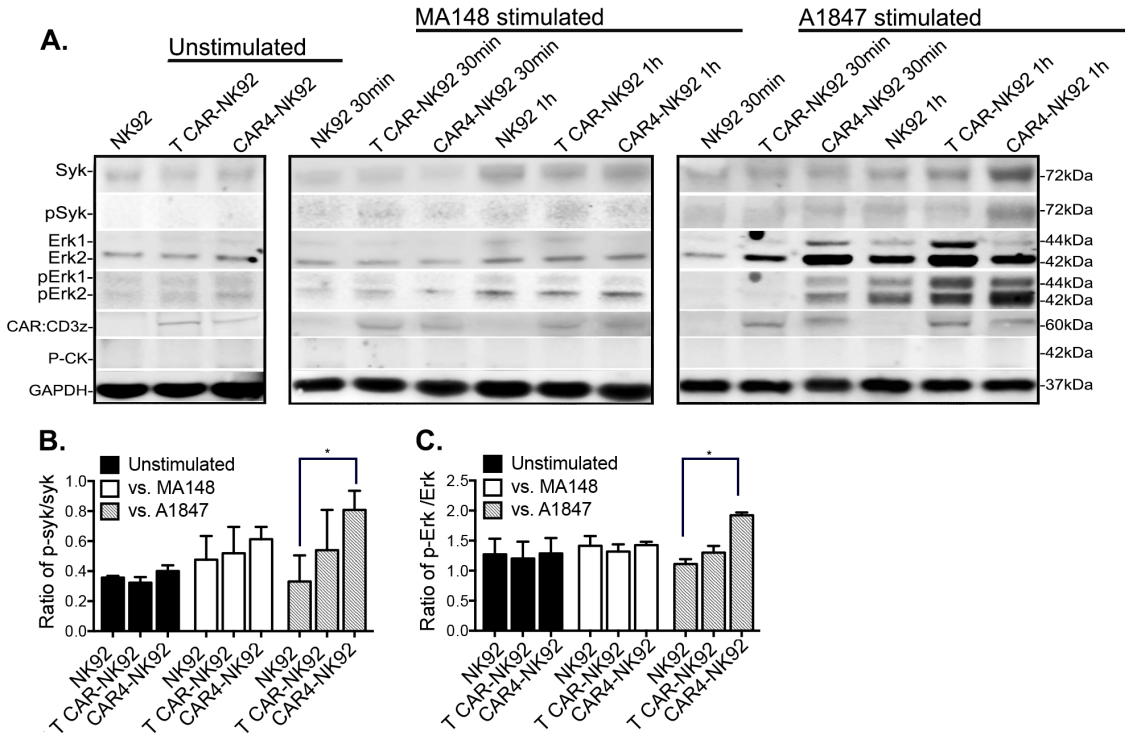
(C) Expression transcriptional marker GFP and the surface expression of CARs in NK92 cells, T-CAR transfected NK92 cells and CAR4 transfected NK92 cells assessed by flow cytometry.

(D) Schematic representation of the transposon vector encoding CARs such as T-CAR and CAR4. The selection marker was linked with IRES region. CARs are driven by mCAG promoter.

(E and F) Effector cell of NK92 and CAR expressing NK92 co-cultured with (C) europium-loaded leukemia targets cell K562 ($meso^{neg}$), K562meso ($meso^{high}$) for 2 hours, and (D) europium-loaded ovarian cancer target cells of MA148 ($meso^{low}$) and A1847 ($meso^{high}$) for 3 hours as different effector to target ratios.

(G and H) Surface staining of (E) CD107a expression and intracellular staining of (F) IFN-γ production was analyzed in anti-CD56 labeled NK effector cells of NK92 and CAR expressing NK 92 after 3-hour co-cultured with target cells. Data were normalized to the percentage of media-treated effector cells, shown as mean±S.D,

Statistic: two tailed Student t-test, * $P<0.05$, ** $P<0.01$.

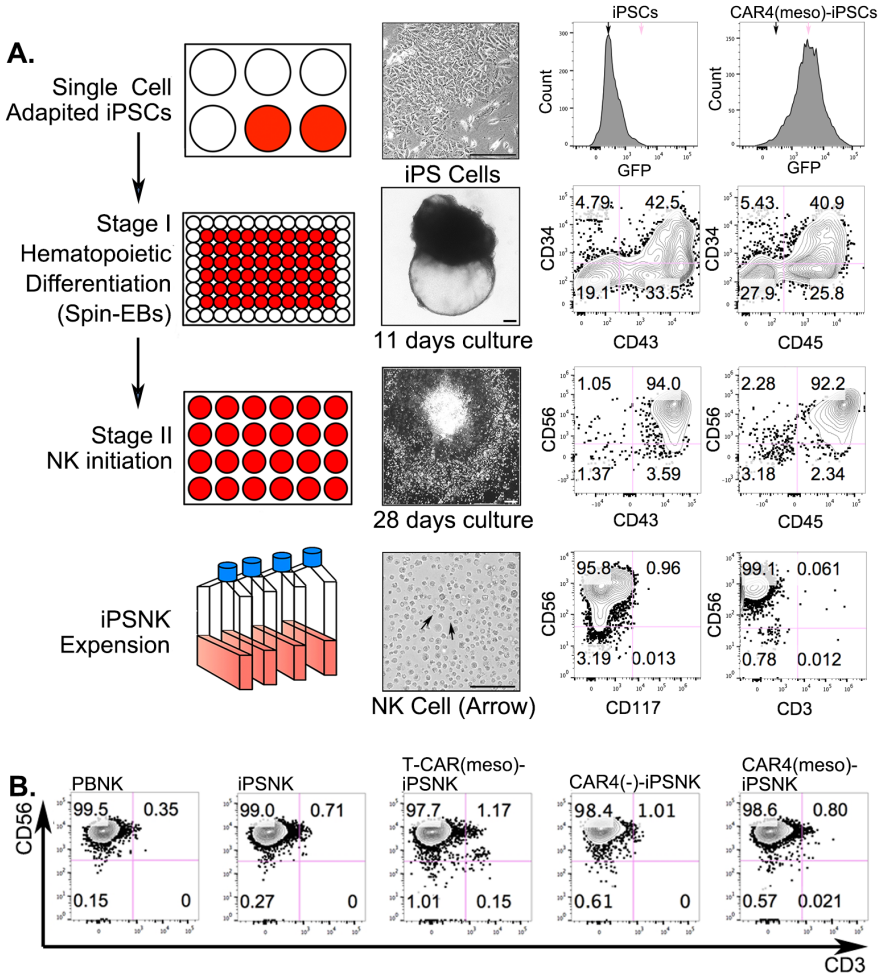


Supplemental Figure S2. Activation of NK cell intracellular signaling pathways in CAR expressing NK92 cells, Related to Figure 1 and Figure 2

(A) Total and phosphot-protein analysis of Syk, Erk1/2 in cell lysate of NK92 cells, T-CAR-NK92 cells, and CAR4-NK92 cells by immunoblots in different condition. Left: unstimulated; middle: meso^{low} MA148 cell stimulated for 30 min and1 hours; right: meso^{high} A1847 cell stimulated for 30 min and 1 hours. Anti-CD3ζ antibody was used to determine CAR expression in estimated molecular size, epithelial marker P-CK and GAPDH were used as loading controls.

(B and C) Measurement of phosphorylated level over total level in NK effector cells co-cultured with target cells were performed in protein of (B) Syk and (C) Erk. Data were normalized to the values of endogenous GAPDH as mean and data was presented as mean ± S.D.

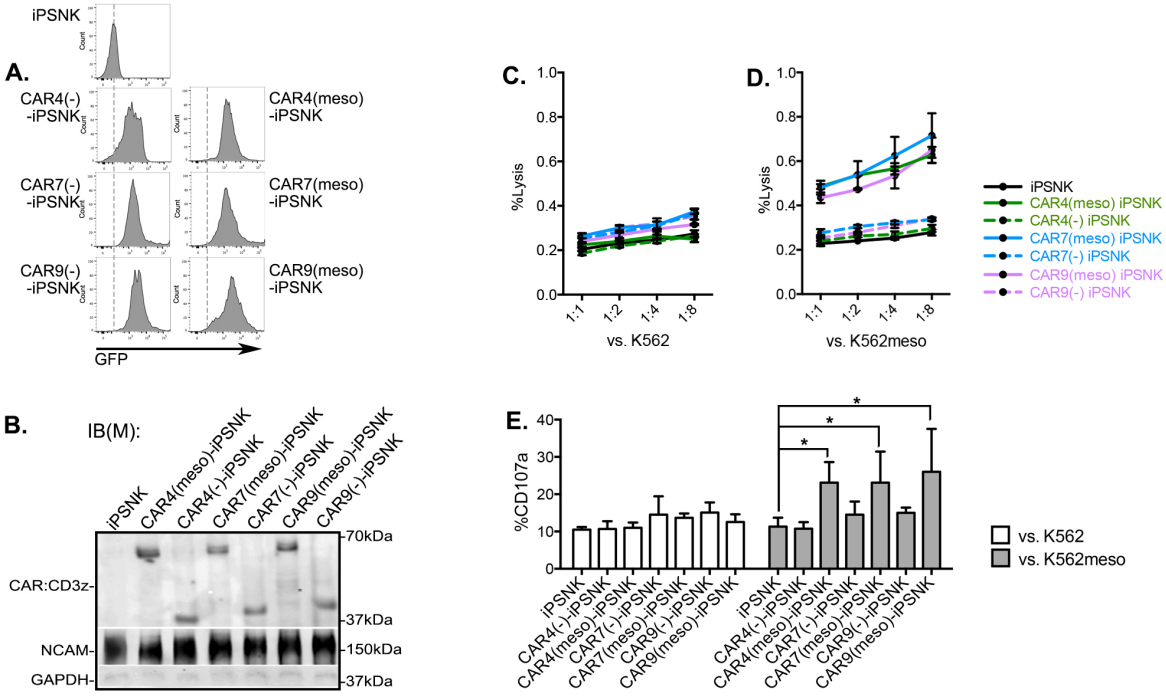
Statistic: two tailed student t-test, * P<0.05.



Supplemental Figure S3. Derivation of NK cells from CAR-expressing iPSCs. Schematic for the derivation of iPSC-derived NK cells, Related to Figure 3

(A) CAR transfected iPSCs were dissociated and plated in spin-EB conditions for 11 days. After 11 days in spin EB culture, cells were evaluated for hematopoietic progenitor cells by flow cytometry for $CD34^{+}/CD43^{+}$ and $CD34^{+}/CD45^{+}$. Then, spin-EBs were transferred to conditions supporting NK cell development for 4 weeks in NK cell differentiation culture. Suspension cells were collected and analyzed by flow cytometry for expression of CD56, CD43, and CD45. NK cells were then expanded via artificial antigen presenting cell (aAPC) + IL2 co-culture. Mature NK cells were analyzed by flow cytometry for expression of CD56, CD117, and CD3. Each flow plot is representative of at least five independent experiments. EB: embryonic body.

(B) Flow cytometric analysis of surface markers of CD56 and CD3 in aAPC-expanded PB-NK cells, iPSC-NK cells, T CAR-iPSC-NK cells, CAR4(-)-iPSC-NK cells, and CAR4(meso)-iPSC-NK cells.



Supplementary Figure 4. CAR mediated anti-tumor activities in iPSC-NK cells, Related to Table 1 and Figure 3.

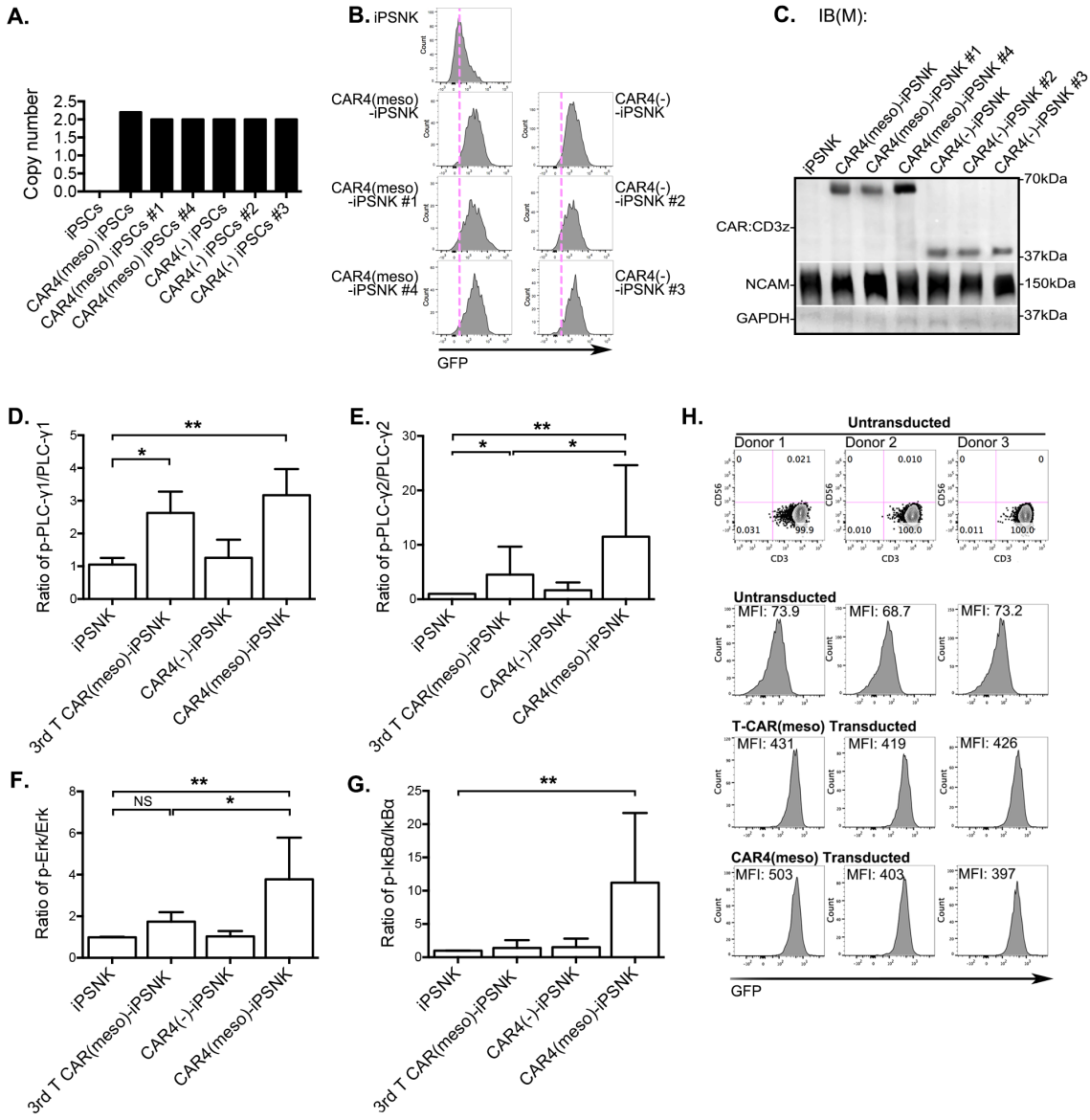
(A) Expression transcriptional marker GFP in NK cell derived from CAR(meso) transfected iPSCs assessed by flow cytometry.

(B) Membrane protein analysis in cell lysate of NK cell derived from CAR transfected iPSCs by Immunoblots. Anti-CD3ζ antibody was used to determine CAR expression in estimated molecular size, NCAM and GAPDH were used as loading controls.

(C and D) Effector cells of iPSC-NK cells and CAR(meso) expressing iPSC-NK cells were co-cultured with (C) europium-loaded meso^{neg} target cells of K562, and (D) europium-loaded meso^{high} target cells of K562meso for 2 hours as different effector to target ratios.

(E) Surface staining of CD107a expression is determined in anti-CD56 labeled NK effector cells such as iPSC-NK and CAR(meso) expressing iPSC-NK after 3-hour co-cultured with leukemia target cells of K562 and K562meo. Data were normalized to the percentage of media-treated effector cells, shown as mean ± S.D.

Statistic: two tailed student t-test, * P<0.01.



Supplementary Figure S5. Expression of CAR-iPSC-NK cells, Related to Figure 3.

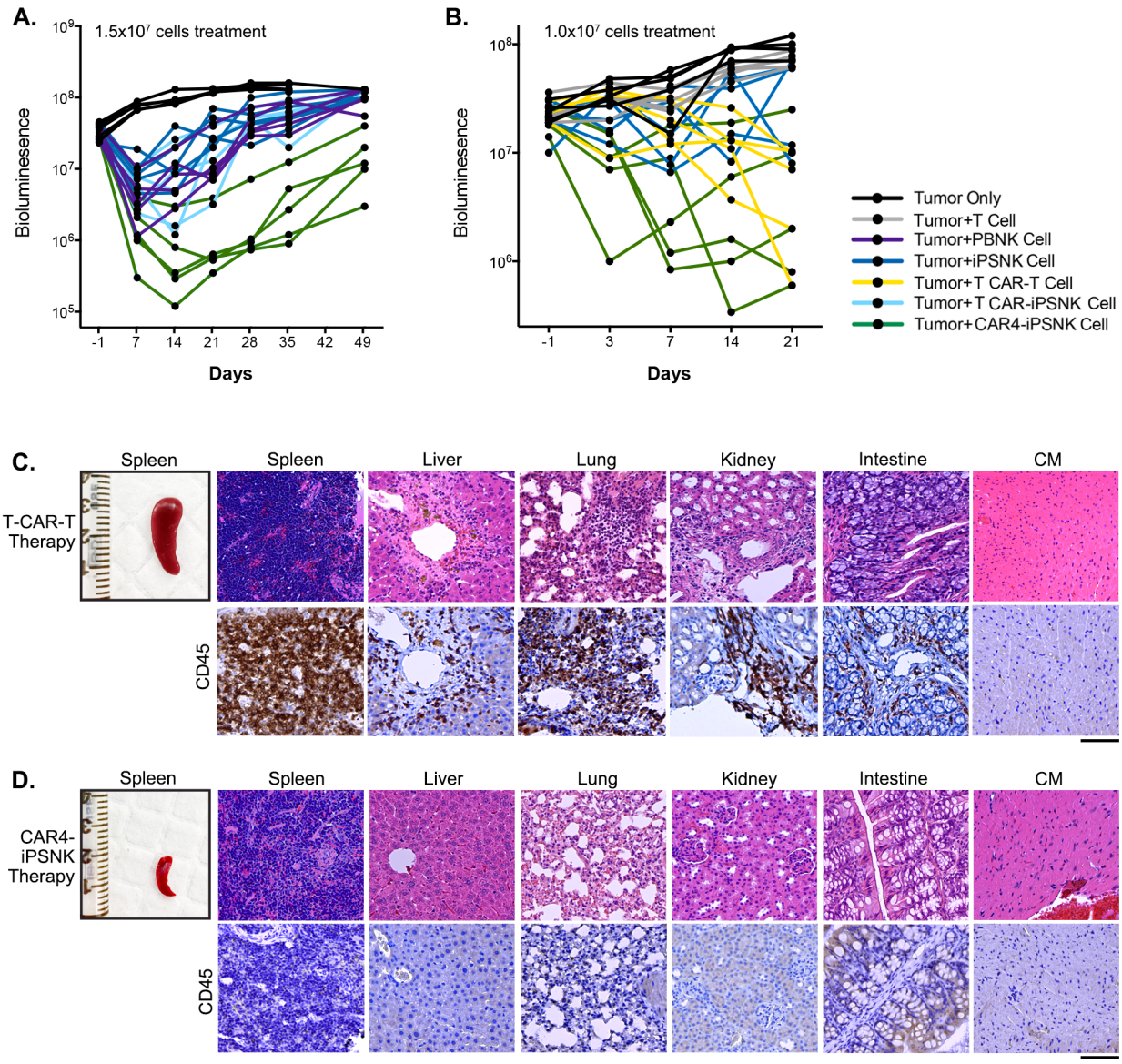
(A) Reprogramming copy number of monoclone of CAR4(meso)-iPSC #1, CAR4(meso)-iPSC #4, CAR4(-)-iPSC #2, CAR4(-)-iPSC #3 assessed by qPCR.

(B) Expression transcriptional marker GFP in NK cell derived from monoclone of CAR-iPSCs assessed by flow cytometry.

(C) Membrane protein analysis in cell lysate of NK cell derived from monoclone of CAR-iPSCs by Immunoblots. Anti-CD3ζ antibody was used to determine CAR expression in estimated molecular size, NCAM and GAPDH are used as loading controls.

(D-G) Measurement of phosphorylated level over total level in NK effector cells co-cultured with meso^{high} A1847 target cells were performed in protein of (D) PLC-γ1,15min; (E) PLC-γ2, 15min, (F) Erk1/2, 30min; and (G) IκBα, 30min. Data were normalized to the values of endogenous GAPDH as mean and presented as mean ± S.D. Statistic: Two-tailed one-way ANOVA, * P<0.05, ** P<0.01, NS: not significant.

(H) Flow cytometric analysis of surface markers of CD56 and CD3 in primary T cell from 3 donors (upper), and expression transcriptional marker GFP in CAR transduced T cells assessed (lower).



Supplementary Figure S6. Anti-tumor activities and toxicity of CAR expressing immune effectors in vivo, Related to Figure 4 and Figure 5.

(A) Individual meso^{high} A1847 ovarian tumor burden plotted from treated mice by giving one dose of 1.5X10^7 NK cell populations such as PB-NK cells, iPSC-NK cells, T-CAR(meso) iPSC-NK cells, CAR4(meso) iPSC-NK cells with tumor bearing only as control.

(B) Individual meso^{high} A1847 ovarian tumor burden plotted from treated mice by giving one dose of 1.0X10^7 immune effector cell populations such as T cells, T-CAR(meso) T cells, iPSC-NK cells, and CAR4(meso) iPSC-NK cells with tumor bearing only as control. BLI was used to monitor tumor burden weekly and BLI data was plotted.

(C and D) T-CAR-T cells treated mice (C) and NK-CAR4-iPSC-NK cells treated mice (D) were subjected to necropsy and histopathological analysis in spleen, liver, lung, kidney, intestine, and cardiac muscle (CM). CD45-IHC staining was performed to observe the lymphocyte infiltration in organs. Scare bar is shown as 100μm.