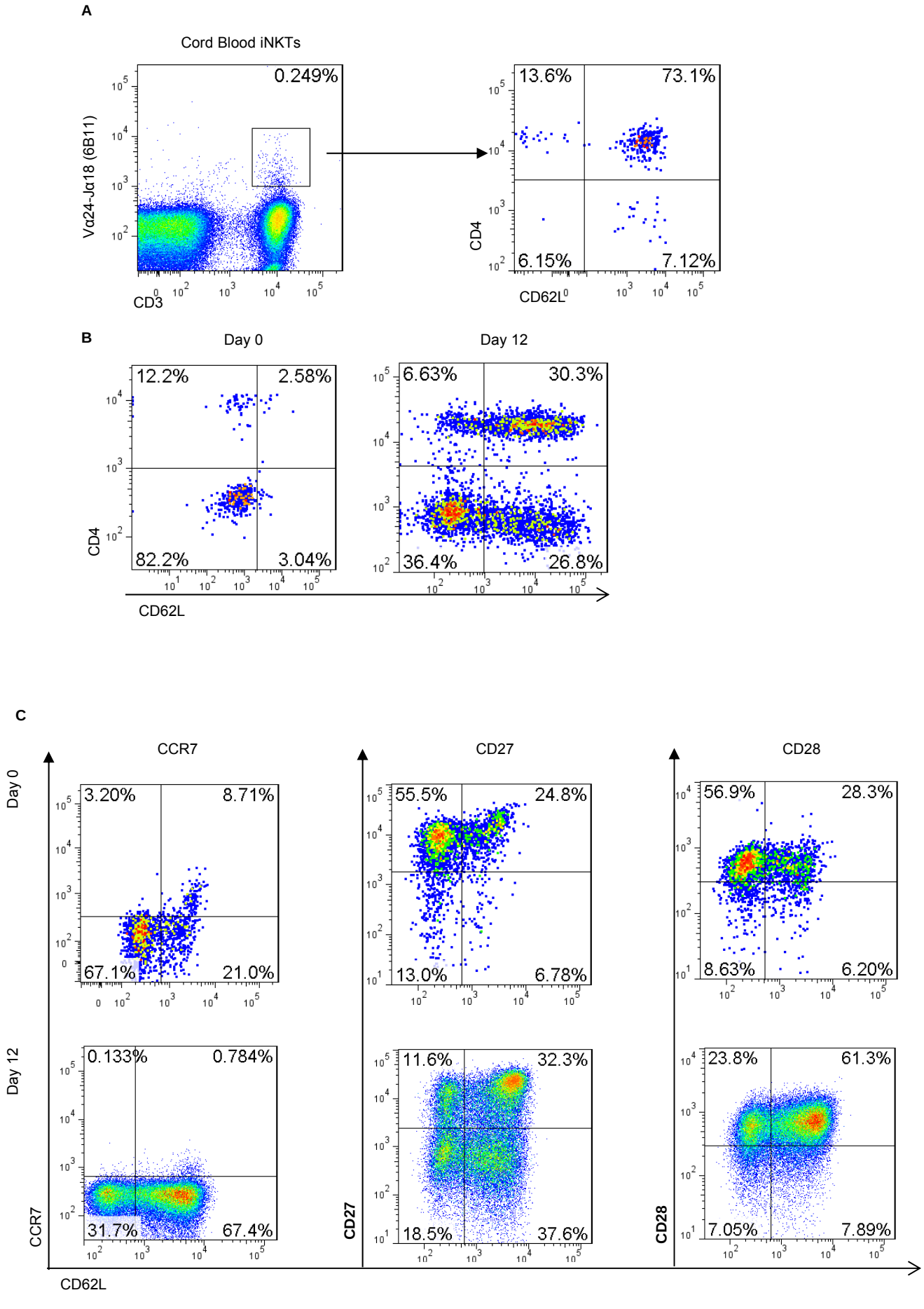
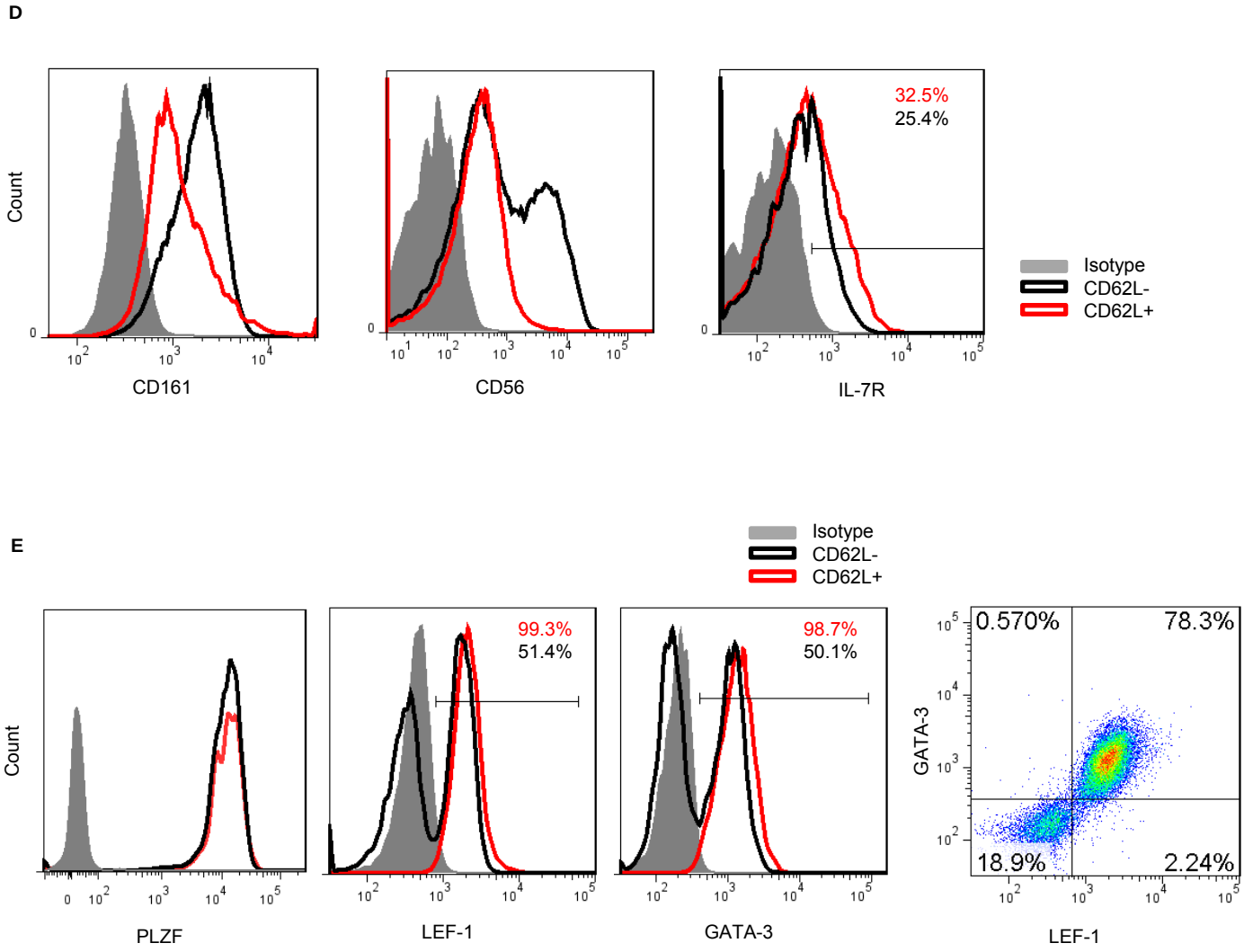
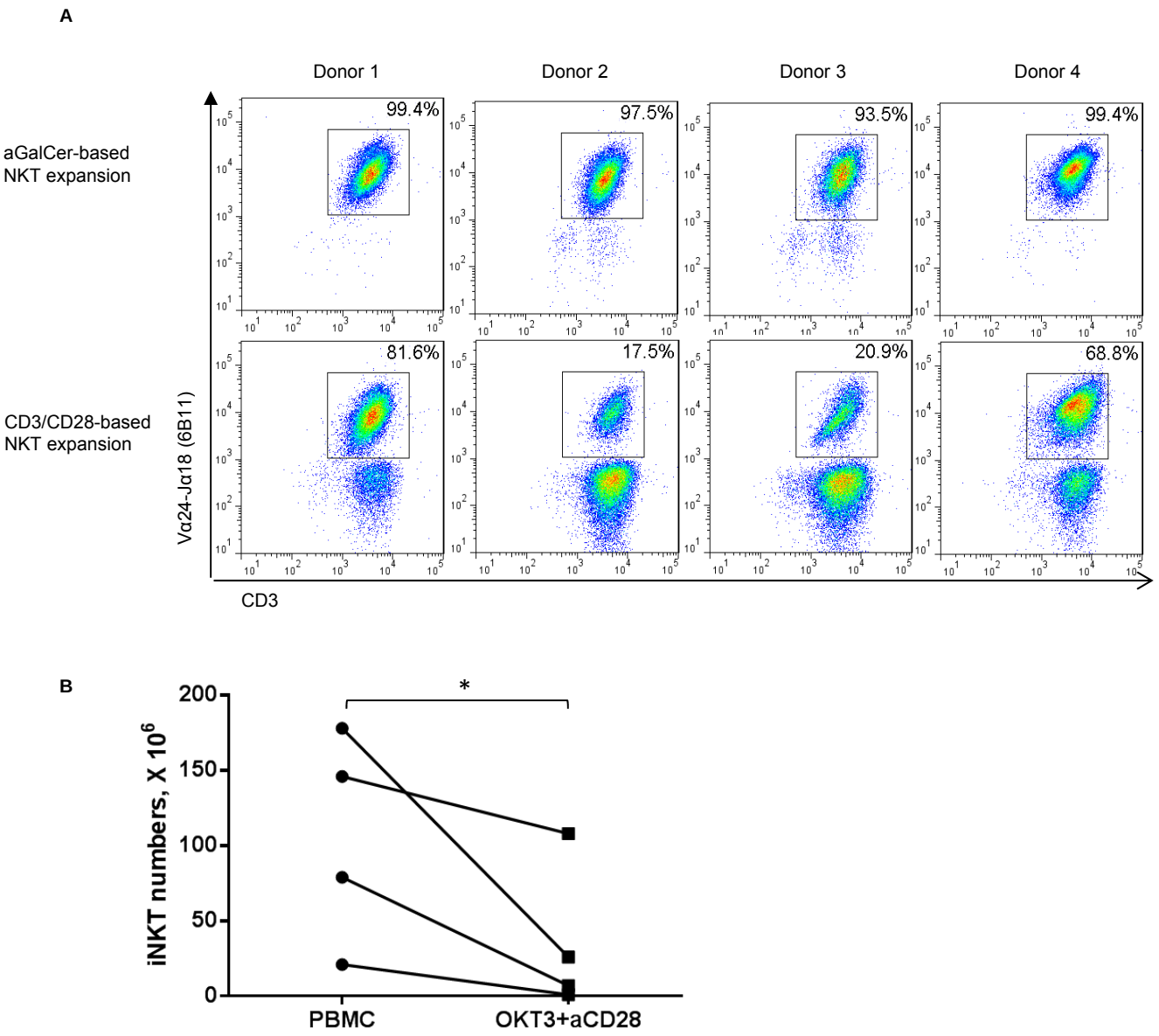
Figure S1


A
Cord Blood iNKTs
0.249%
Vα24-Jα18 (6B11)
CD3
13.6%
73.1%
6.15%
7.12%
CD4
CD62L
B
Day 0
Day 12
12.2%
2.58%
82.2%
3.04%
6.63%
30.3%
36.4%
26.8%
CD4
CD62L
C
CCR7
CD27
CD28
Day 0
Day 12
3.20%
8.71%
67.1%
21.0%
55.5%
24.8%
13.0%
6.78%
56.9%
28.3%
8.63%
6.20%
0.133%
0.784%
31.7%
67.4%
11.6%
32.3%
18.5%
37.6%
23.8%
61.3%
7.05%
7.89%
CCR7
CD27
CD28
CD62L



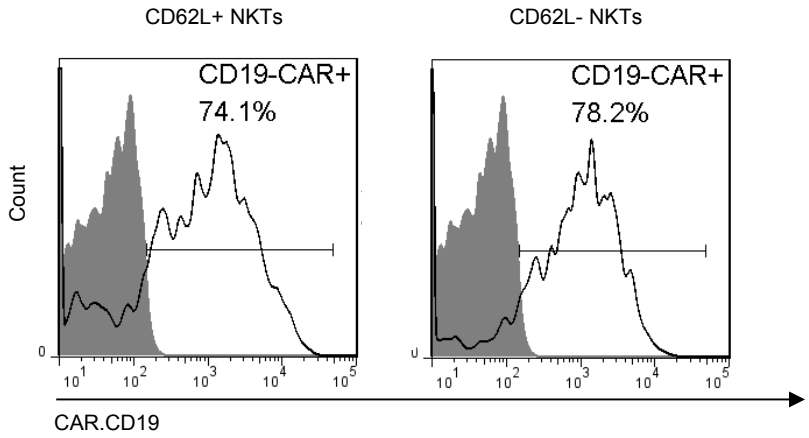
Supplemental Figure 1. **Phenotypic analysis of freshly isolated and *in vitro* expanded NKTs.** **(A)** CD4 and CD62L expression was examined by FACS in primary NKTs (gating on CD3+Vα24-Jα18+ subset) in freshly isolated cord blood mononuclear cells (CBMC). Plots are from a representative of 5 CBMC donors. **(B)** CD4 and CD62L expression was examined in primary NKTs after gating as in A before (day 0) and 12 days after stimulation with αGalCer and in vitro expansion. Plots are from a representative of 10 PBMC donors. **(C)** Expression of CCR7, CD27 and CD28 in relationship to CD62L expression in primary NKTs after gating as in A before (day 0) and 12 days after stimulation with αGalCer and *in vitro* expansion. Plots are from a representative of 6 PBMC donors. **(D**) Expression of CD161, CD56 and IL7Rα in relationship to CD62L expression on day 12 after stimulation with αGalCer and in vitro expansion. Plots are from a representative of 3 PBMC donors. **(E)** Expression of PLZF, LEF1, and GATA3 in relationship to CD62L expression and co-expression of LEF1 and GATA3 was analyzed using intracellular flow cytometry on day 12 after stimulation with αGalCer and in vitro expansion. Plots are from a representative of 4 PBMC donors.



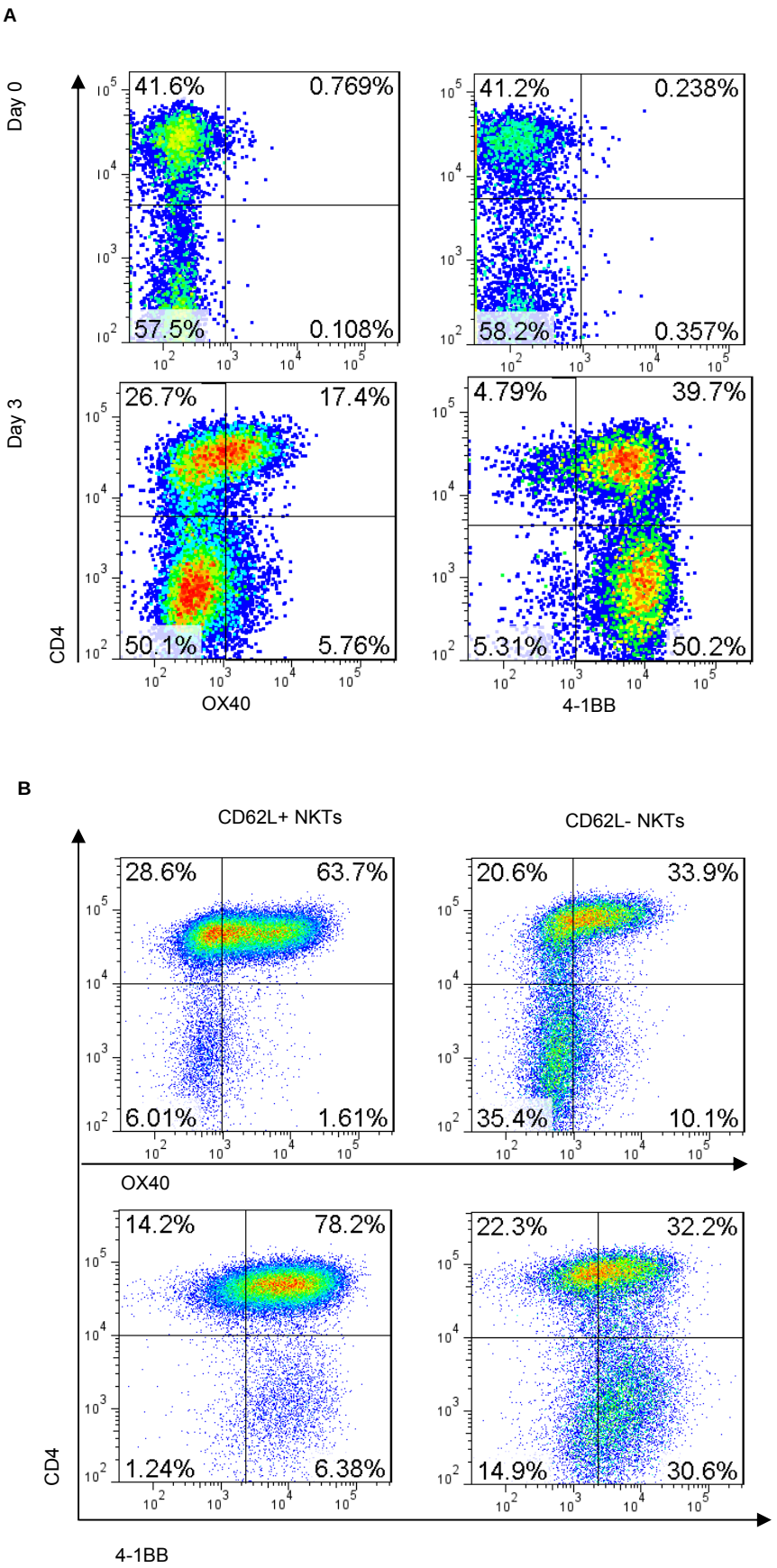
Supplemental Figure 2. **The comparison of NKT-cell purity and absolute numbers after expansion with CD3/CD28 agonistic mAbs vs. aGalCer-pulsed irradiated PBMC.** NKTs were isolated from four PBMCs. Half of them were stimulated using autologous irradiated PBMC pulsed with aGalCer, another half were stimulated with CD3/CD28 mAb-coated plate. In both cases, cells were propagated in culture with the IL-2 (200 U/ml) added every other day. At day 12, cultures were analuzed for: **(A)** NKT-cell purity was determined by flow cytometry as percent cells expressing CD3 and iTCRα. **(B)** NKT-cell absolute cell count was performed using trypan blue exclusion assay in triplicates. * $P < 0.05$, data were analyzed after Log(2) transformation using paired t test.

A

B

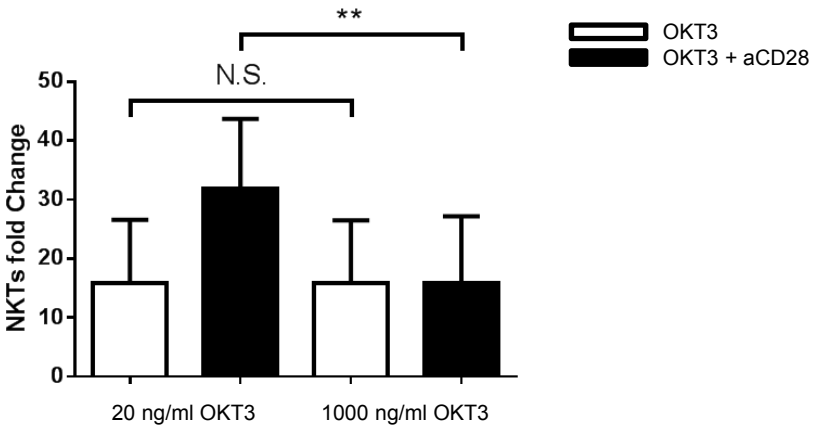


Supplemental Figure 3. **NKT-cell transduction with CAR.CD19**. **(A)** Schematic presentation of CAR.CD19 construct. **(B)** NKTs were re-stimulated with autologous PBMC (irradiated with 40 Gy). On day 3 after re-stimulation, 24 well, non-tissue culture plates were coated with retronectin and after washing inoculated with 1 ml of retroviral supernatant containing CAR.CD19. The viral supernatant was then removed and NKTs were added to the wells in complete media and 200 U/ml rhIL-2. NKTs were then magnetically sorted to CD62L+ and CD62L- subsets and CAR.CD19 surface expression was analyzed with 2D3 mAb staining by FACS 12 days after transduction. Shown are FACS plots from a representative of 3 independent experiments.

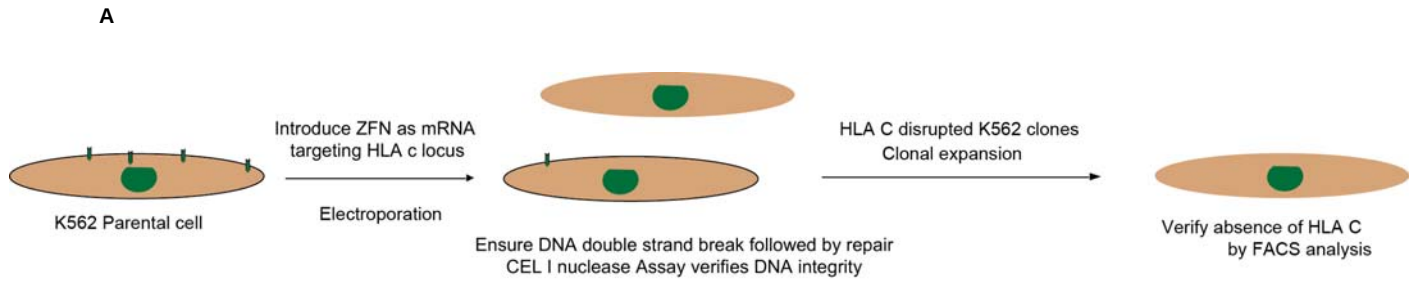


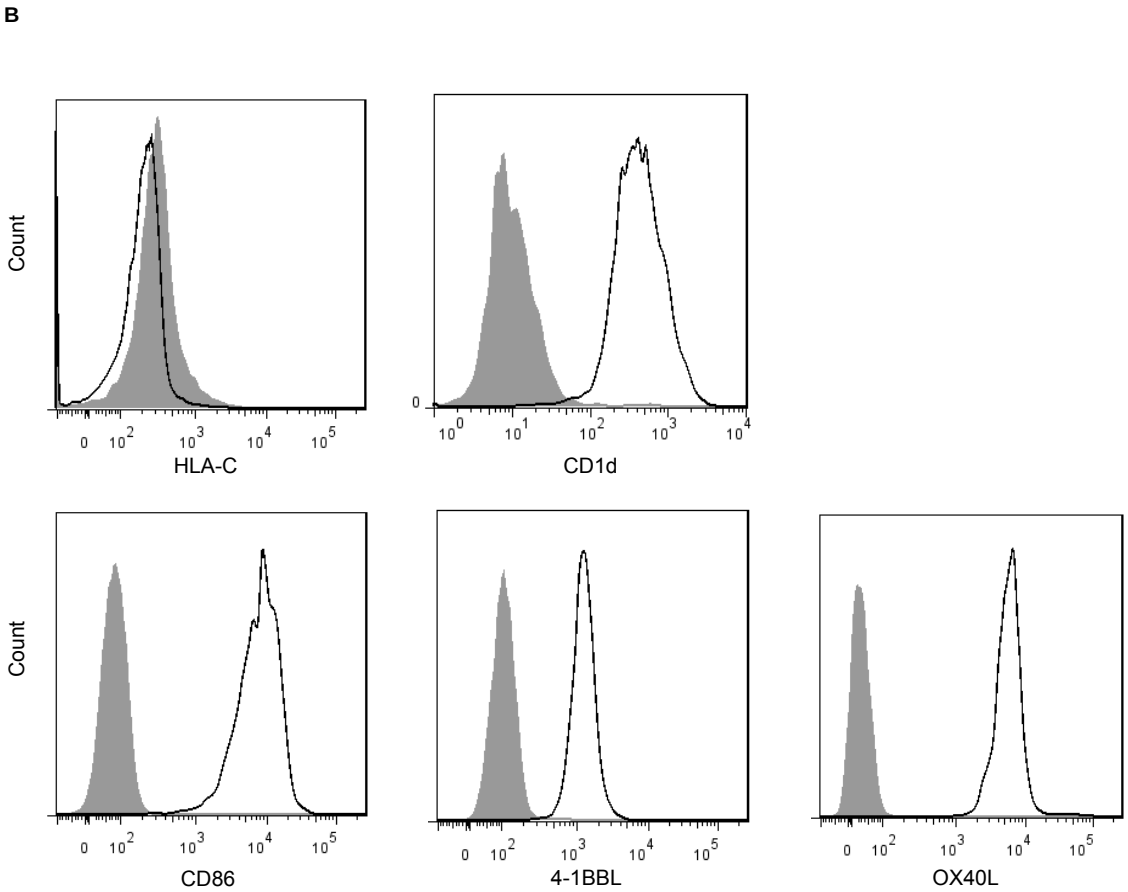
Supplemental Figure 4. Expression of co-stimulatory receptors on resting and activated NKTs. (A) FACS analysis of OX40 and 4-1BB expression in relation to CD4 on resting NKTs (day 12 after primary stimulation) and 3 days after re-stimulation with αGalCer. Plots are from a representative of 6 PBMC donors. **(B)** Magnetically sorted CD62L+ and CD62L- NKTs were analyzed for OX40 and 4-1BB expression in relation to CD4 3 days after NKT cell re-stimulation with αGalCer. Plots are from a representative of 4 PBMC donors.

Figure S5

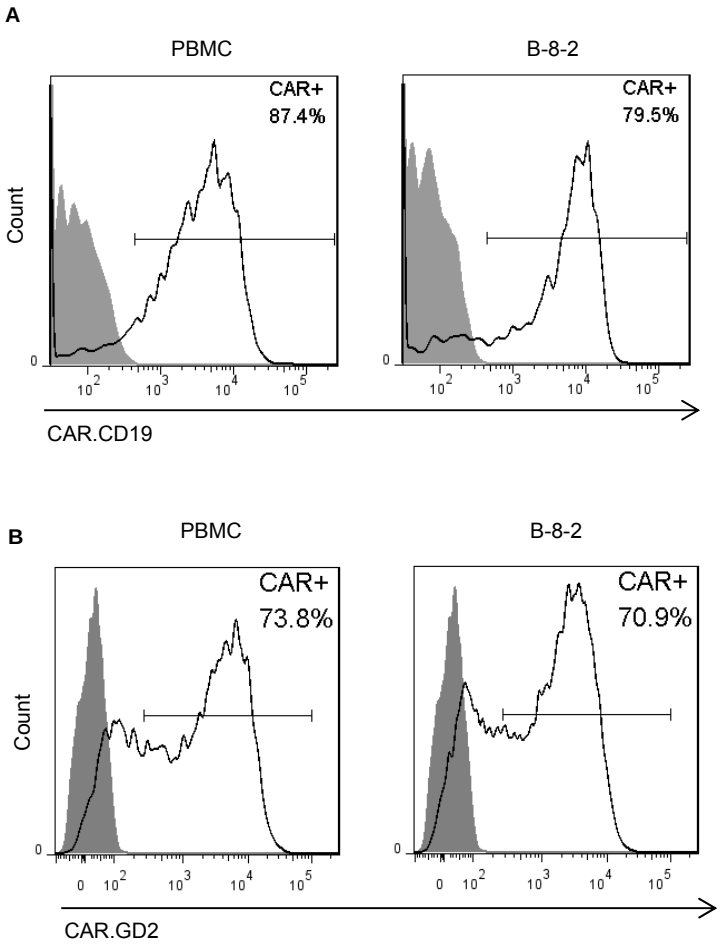


Supplemental Figure 5. **The comparison of NKT-cell expansion using low and high concentrations of plate-bound OKT3 mAb.** In vitro expanded quiescent NKTs were stimulated with anti-CD3 OKT3 mAb at 20 ng/ml or 1 μg/ml alone or with 500 ng/ml anti-CD28 CD28.2 mAb. Cells were propagated in culture with the IL-2 (200 U/ml) added every other day. At day 12, NKT-cell absolute cell count was performed using trypan blue exclusion assay in triplicate and divided to the input number at day 0. Data are M ± SD, N = 4. ** P = 0.01, paired t test.

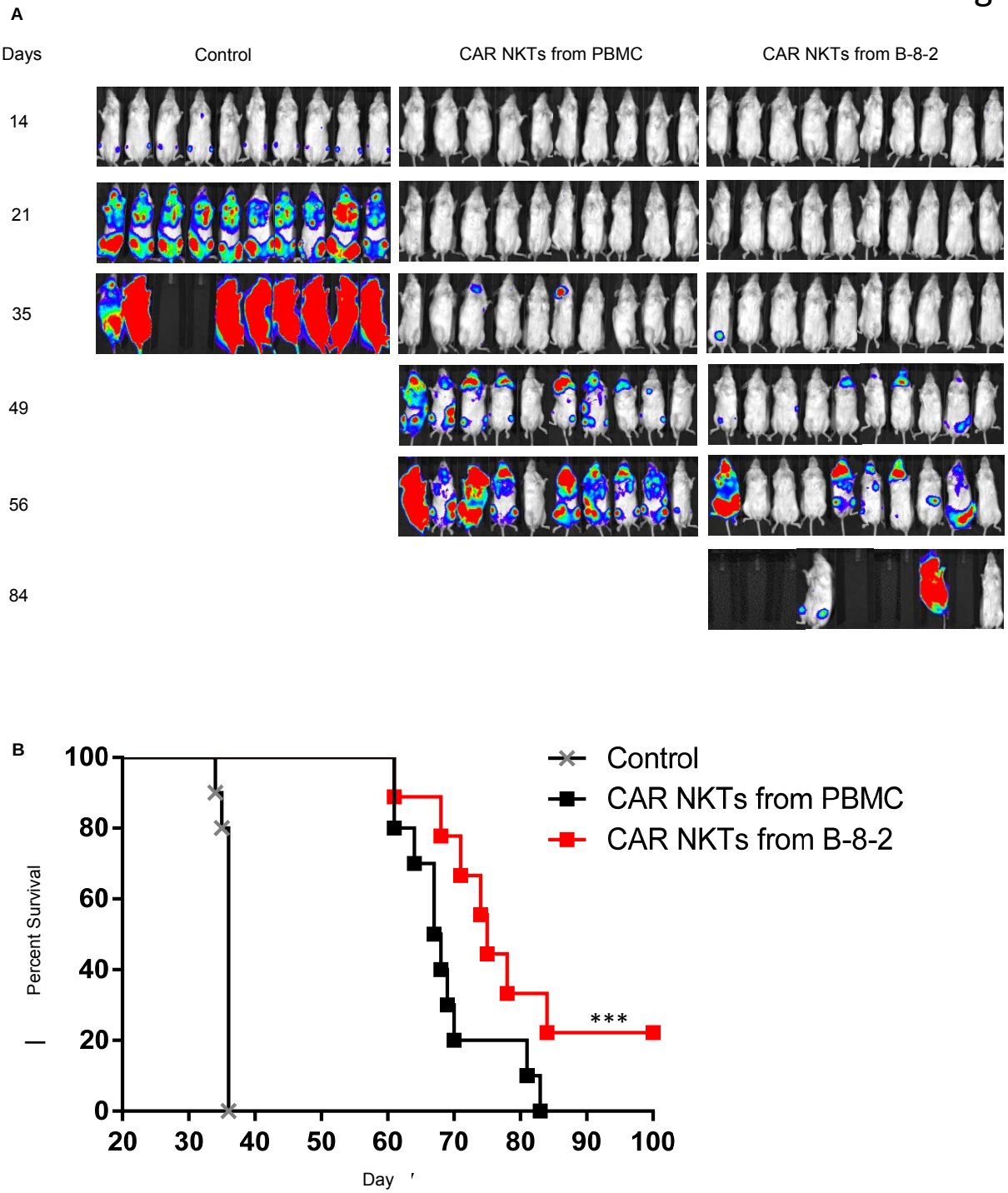




Supplemental Figure 6. Generation of aAPC clones. (A) Schematic presentation of the process of HLA-C gene deletion from K562 cell genome using an HLA-C-specific zinc-finger nuclease (ZFN). **(B)** Immunophenotype of B-8-2 aAPC clone as determined by FACS.



Supplemental Figure 7. Transduction of NKTs expanded with PBMC or B-8-2 with CAR. Gene transduction with (**A**) CAR.CD19 or (**B**) CAR.GD2 was performed as described in S. Figure 3 and "Methods" section. Shown are FACS plots from a representative of 2 independent experiments.



Supplemental Figure 8. aAPC-expanded CAR-NKTs have superior anti-tumor activity in Daudi B-cell lymphoma model. Mice received i.v. injection of 2 X 10^5 luciferase-transduced Daudi cells (day 0) followed by (day 4) i.v. injection of 10^7 NT or CAR.CD19 NKTs with IL-2 (1000 U/mouse). Tumor growth was monitored using bioluminescent imaging once per week. Data were analyzed by the Kaplan-Meier method (10 mice per group). The differences in survival were then compared using the Log-rank test.

Supplemental Table 1. Differential gene expression in CD62L+ and CD62L- NKT cells

GENE NAME	FOLD CHANGE	P VALUE
CD62L	118.32	0.0001
LEF1	5.46	0.0089
S1PR1	4.69	0.0024
ICAM4	3.67	0.0015
IL7R	2.85	0.0006
AIRE	3.31	0.0173
IL21R	2.19	0.0016
CCR8	2.78	0.0050
CD8B	2.57	0.0175
NCAM1(CD56)	-6.44	0.0014
CCL3	-4.89	0.0002
PDCD1(PD-1)	-4.78	0.0207
KLRB1(CD161)	-3.49	0.0001
LAG-3	-2.92	0.0027
CD244	-2.92	0.0025
IRAK2	-2.76	0.0001
IKZF3	-2.71	0.0038
KLRC1	-2.64	0.0026
CXCR6	-2.5	0.0028
CCR1	-2.37	0.0097
HAVCR2(TIM-3)	-1.82	0.0011

On day 12 after primary stimulation, NKTs were magnetically sorted into CD62L+ and CD62L- subsets followed by RNA isolation and gene expression analysis using Human Immunology Panel v.2 and nCounter Analysis System. The table shows the log2 fold changes (CD62+/CD62L-) of genes with p-values less than 0.02 and average fold change greater than 1.8. Data were generated from 6 NKT-cell donors (12 paired samples), paired t-test.