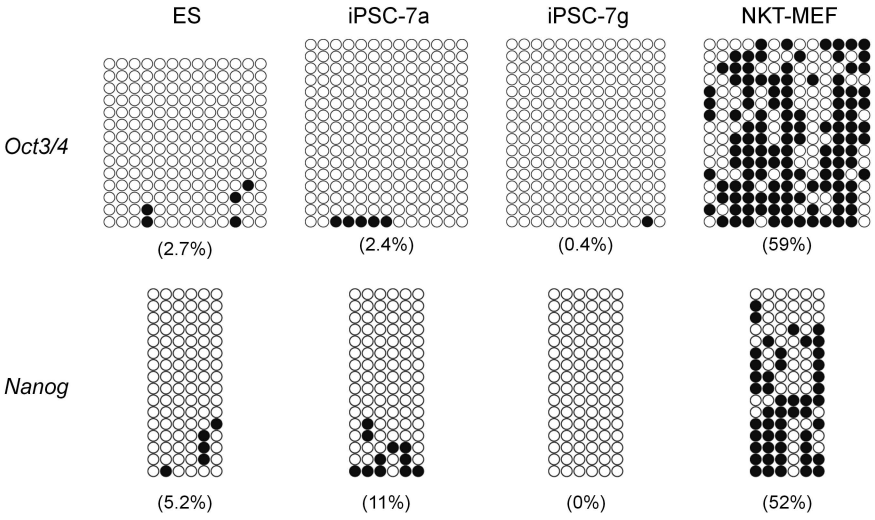
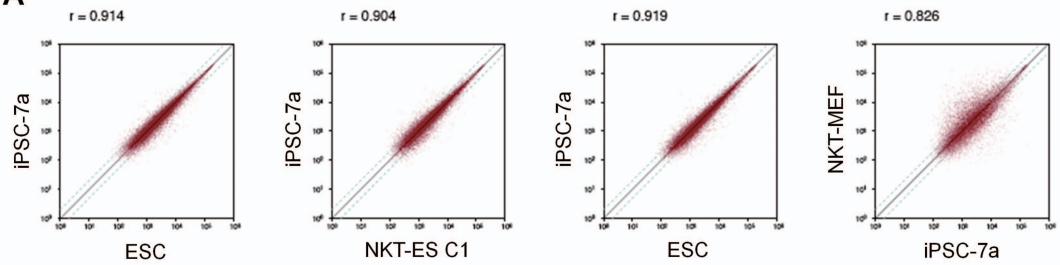


Supplemental Figure 1

DNA methylation status at ESC-specific gene loci in the iPSC-7a and iPSC-7g cells. Bisulfite DNA sequence analysis was performed in the *Oct3/4 and Nanog* loci for ESCs, iPSC-7a, iPSC-7g and NKT-MEF. Open circles represent unmethylated CpG sites and closed circles methylated. The percentage of methylated CpG is indicated in parentheses. Note the extensive demethylation in the iPSC-7a and iPSC-7g cells, similar to the level in ESCs, but not to those in NKT-MEF.

A



B

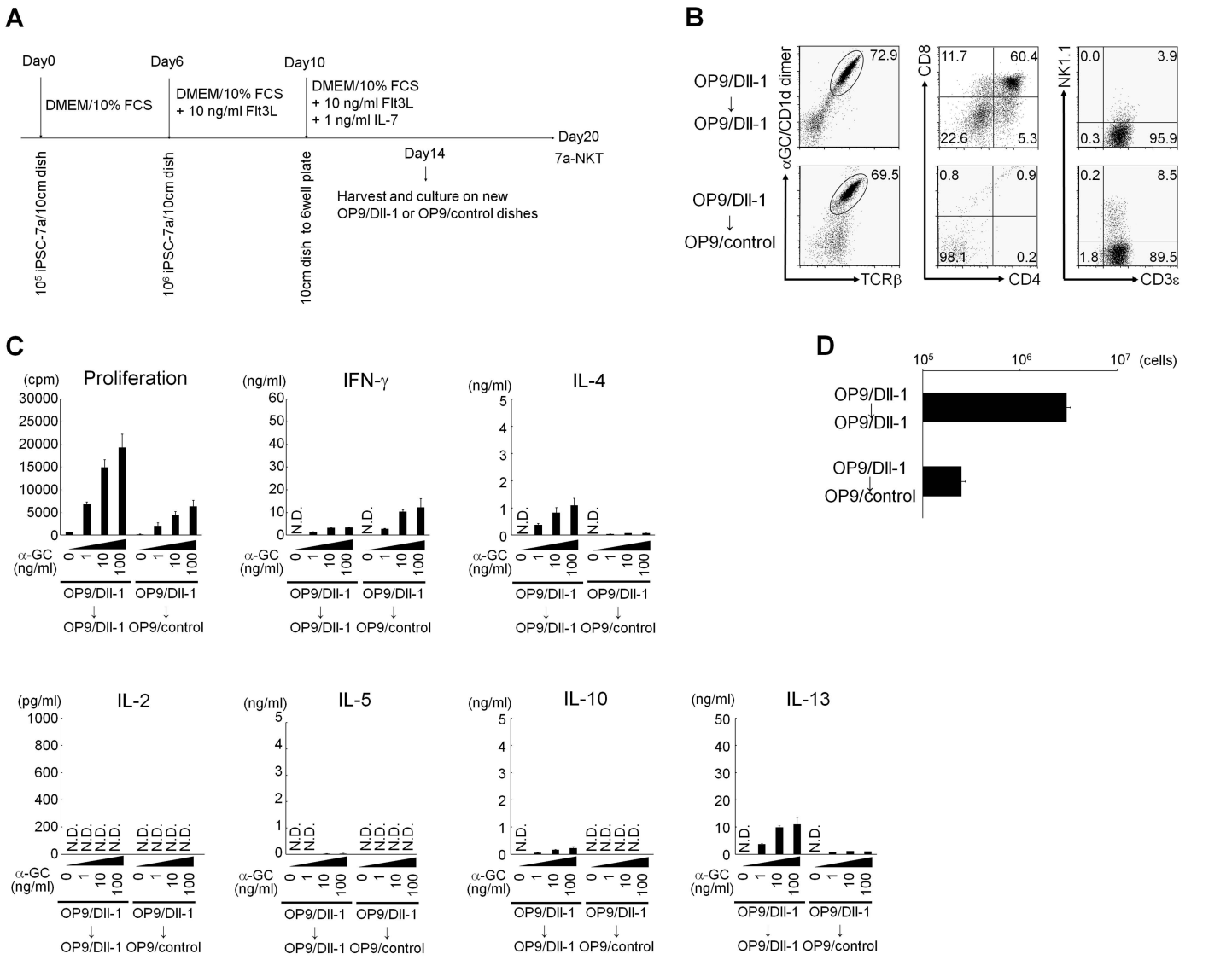
	NKT-MEF	NKT-ES D-1	NKT-ES C-1	iPSC-7a	iPSC-7g	ESC
ESC	0.837	0.917	0.919	0.914	0.925	1.000
iPSC-7g	0.831	0.909	0.911	0.925	1.000	
iPSC-7a	0.826	0.901	0.904	1.000		
NKT-ES C-1	0.808	0.928	1.000			
NKT-ES D-1	0.808	1.000				
NKT-MEF	1.000					

C

	splenic NKT2	splenic NKT1	7g-NKT	7a-NKT	iPSC-7g	iPSC-7a
iPSC-7a	0.684	0.712	0.734	0.765	0.925	1.000
iPSC-7g	0.684	0.715	0.738	0.769	1.000	
7a-NKT	0.803	0.855	0.905	1.000		
7g-NKT	0.798	0.862	1.000			
splenic NKT1	0.834	1.000				
splenic NKT2	1.000					

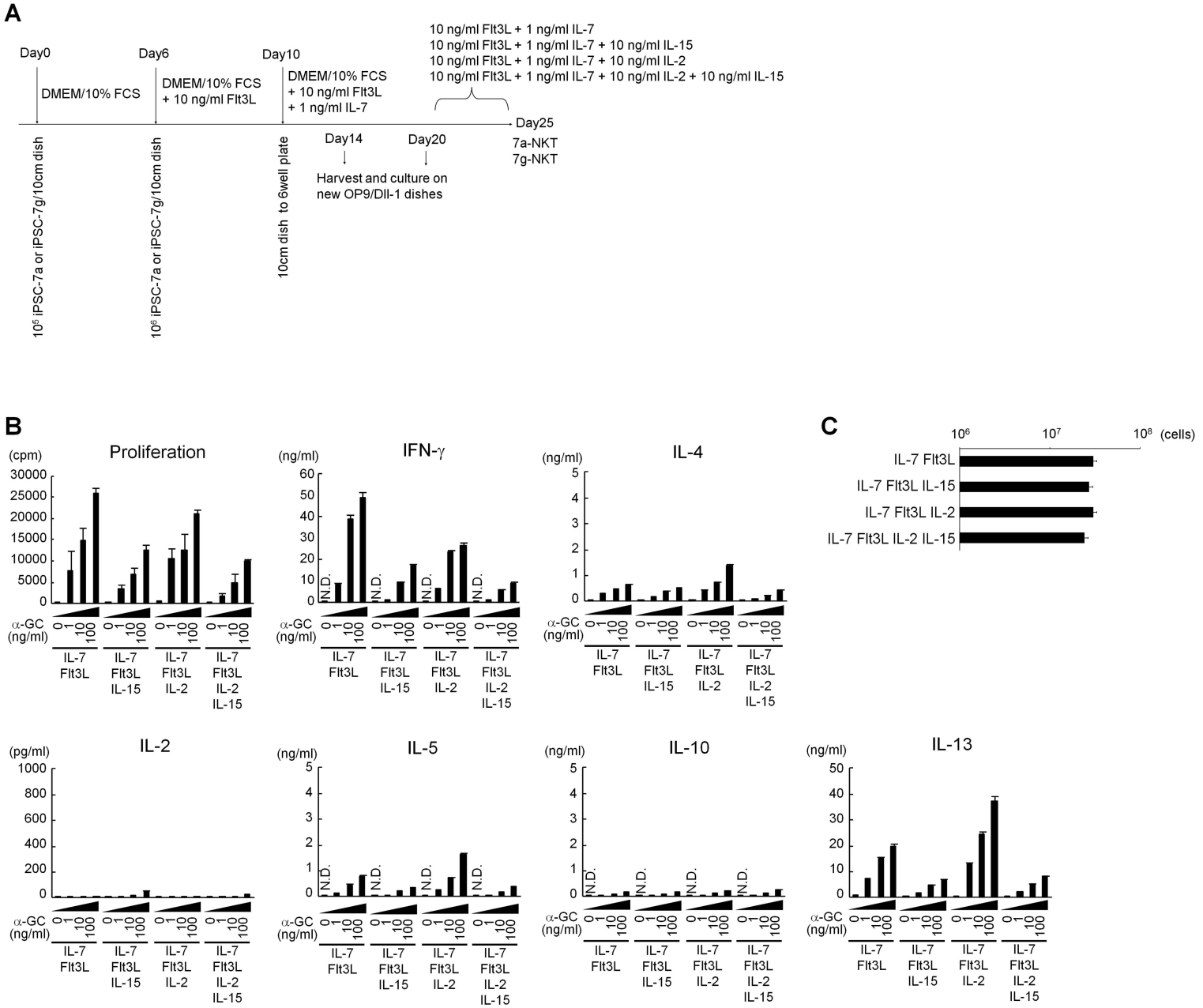
Supplemental Figure 2

Global gene expression profiles in the iPSC-7a and iPSC-7g cells. (**A**) Global gene expression of the iPSCs-7a cells compared to ESCs, NKT-ES C-1 or NKT-MEF by scatter plot analysis. The correlation coefficient for each comparison is indicated above each panel. (**B** and **C**) Tree view representation of clustering analysis among the iPSC-7a, iPSC-7g, NKT-ES C-1, NKT-ES D-1 and NKT-MEF (**B**), and splenic NKT (1 and 2), 7a-NKT, 7g-NKT, iPSC-7a, and iPSC-7g cells, (**C**). The values represent coefficient between indicated panels. r^2 >0.9 in red, 0.8< r^2 <0.9 in orange, and r^2 <0.8 in blue.



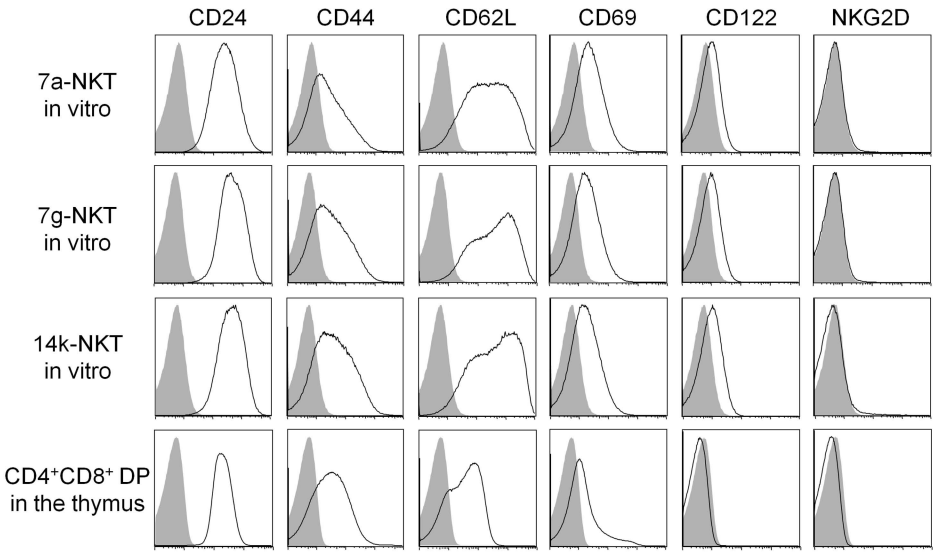
Supplemental Figure 3

A protocol for generation of NKT cells from iPSCs in the 20-day culture used for generation from ESCs *in vitro*. For the comparison with the 25-day culture system described in Figure 2 and Figure 4, experiments using iPSCs under the same protocols were carried out as described for ESCs (13). (**A**) The iPSC-7a cells were co-cultured with OP9/Dll-1 for 14 days in the presence of Flt3L (Day6-10), Flt3L and IL-7 (Day10-14). Cells were harvested on the Day14 and further co-cultured on new OP9/Dll-1 or OP9/control dishes. On day 20, iPSC-derived NKT cells (7a-NKT and 7g-NKT) were used for further analysis. (**B**) The expression of cell surface markers on iPSC-derived NKT cells (7a-NKT). The 7a-NKT cells were gated as the α-GalCer/CD1d dimer$^+$ TCRβ$^+$ population and further analyzed for the expression of the indicated markers, NK1.1 vs. CD3ε and CD4 vs. CD8. (**C**) Proliferative response and cytokine production capacity of 7a-NKT cells upon stimulation with α-GalCer. The 7a-NKT cells generated in (**A**) (10^6/ml) were assayed for their function (proliferation and cytokine production) after stimulation with DCs (10^5/ml) in the presence of the indicated dose of α-GalCer (0, 1, 10, 100 ng/ml). N.D.: not detected. The mean ± standard deviation of triplicate wells is shown. One representative of three experiments is shown. (**D**) Total cell numbers of iPSC-derived NKT cells (7a-NKT) generated after culturing 10^5 iPSC-7a.



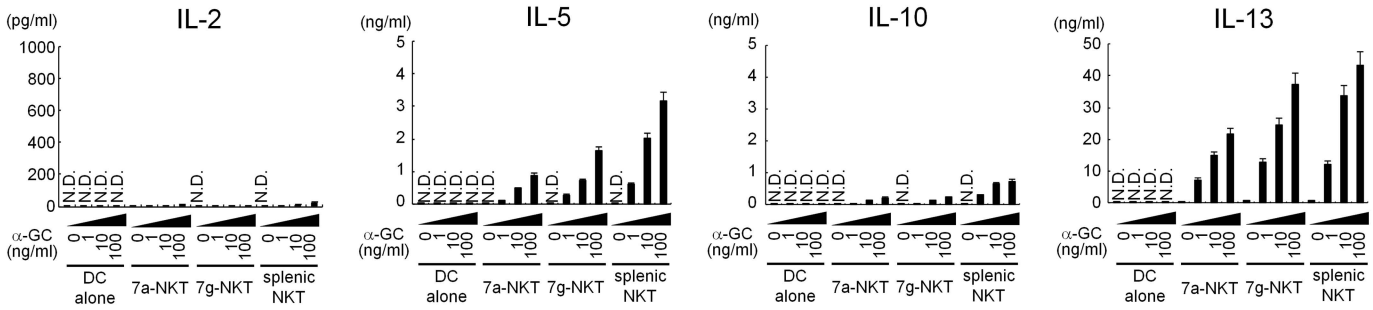
Supplemental Figure 4

A protocol for generation of NKT cells from iPSCs by culturing 25 days *in vitro*. (**A**) The iPSC-7a or iPSC-7g cells were co-cultured with OP9/Dll-1 for 25 days in the presence of Flt3L (Day6-10), Flt3L and IL-7 (Day10-20), combination of Flt3L, IL-7, IL-2 and IL-15 (Day20-25). Cells were harvested every 4 or 5 days and further co-cultured on new OP9/Dll-1 dishes. On day 25, iPSC-derived NKT cells (7a-NKT and 7g-NKT) were used for further analysis. (**B**) Proliferative response and cytokine production capacity of 7a-NKT cells upon stimulation with α-GalCer. The 7a-NKT cells generated in various cytokine combinations in (**A**) (10^6/ml) were assayed for their function (proliferation and cytokine production) after stimulation with DCs (10^5/ml) in the presence of the indicated dose of α-GalCer (0, 1, 10, 100 ng/ml). N.D.: not detected. The mean ± standard deviation of triplicate wells is shown. One representative of three experiments is shown. (**C**) Total cell numbers of iPSC-derived NKT cells (7a-NKT) generated after culturing 10^5 iPSC-7a.



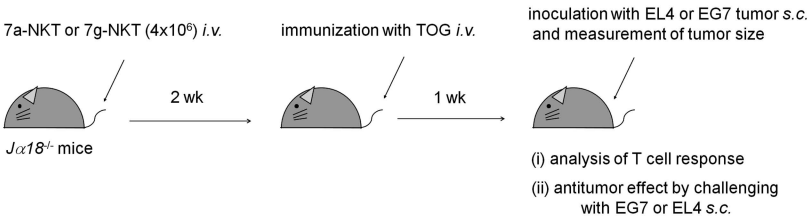
Supplemental Figure 5

The expression of cell surface markers on the iPSC-derived 7a-NKT, 7g-NKT and 14k-NKT cells generated in the 25-day culture system in vitro. Gated fractions in Figure 2A (7a-NKT and 7g-NKT) and Figure 4F (14k-NKT) were further analyzed for expression of the indicated markers and compared with those of $CD4^+CD8^+$ (Double positive; DP) thymocytes. Shadowed profiles indicated isotype-matched control staining.



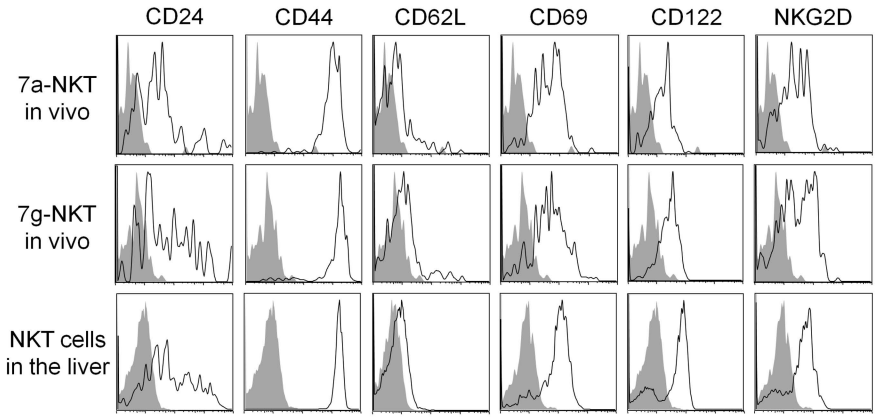
Supplemental Figure 6

Cytokine production of iPSC-derived NKT cells upon stimulation with α-GalCer. The 7a-NKT, 7g-NKT or WT spleen NKT cells (10^6/ml) were assayed for their cytokine production with DCs (10^5/ml) in the presence of the indicated dose of α-GalCer (0, 1, 10, 100 ng/ml). N.D.: not detected. The mean of triplicate wells ±one standard deviation is shown. One representative of three experiments is shown.



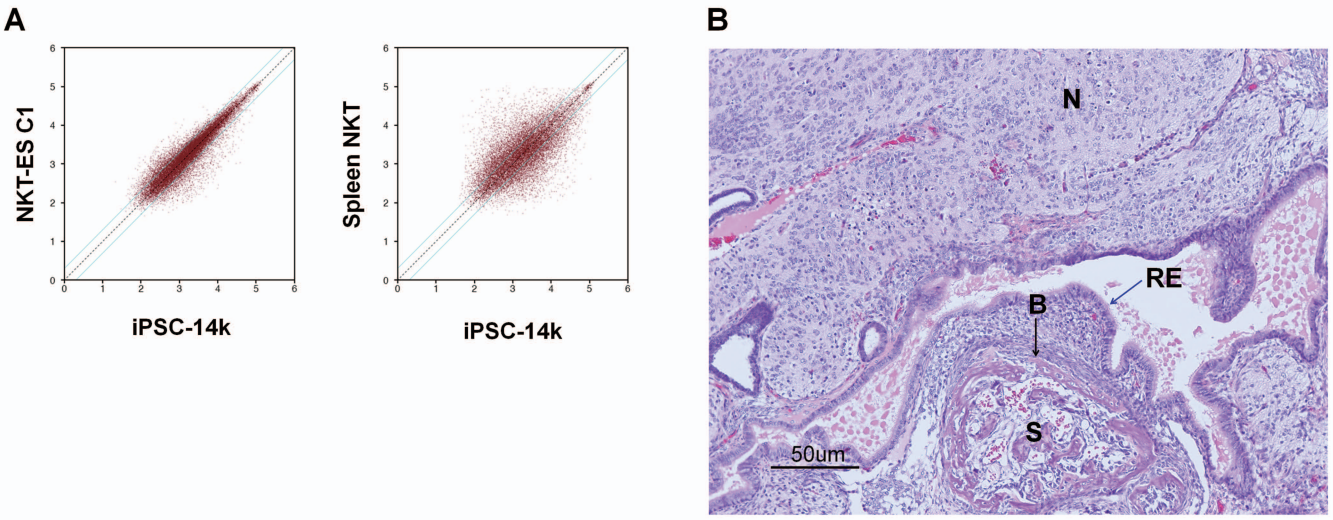
Supplemental Figure 7

Schematic representation of the experimental procedure to measure adjuvant activity of the iPSC-derived 7a-NKT and 7g-NKT cells. The OVA-specific CD8T cell response in WT mice or the 7a-NKT- or 7g-NKT-transferred *Jα18*$^{-/-}$ mice that had been immunized with or without TOG was evaluated. In brief, two weeks after cell transfer, *Jα18*$^{-/-}$ mice that had been transferred were primed with a combination of $2x10^7$ *TAP*$^{-/-}$ OVA-loaded spleen cells and 2 μg α-GalCer (TOG) i.v. One week after TOG immunization, the production of IFN-γ in response to re-stimulation by OVA peptide and the anti-tumor effect after challenge with EG7 or EL4 cells were analyzed.



Supplemental Figure 8

The expression of cell surface markers on the iPSC-derived 7a-NKT and 7g-NKT cells after transferred *in vivo*. The 7a-NKT and 7g-NKT cells (4 x 10^6 cells /mouse) generated in the 25-day culture system *in vitro* were transferred into $J\alpha18^{-/-}$ mice. Two weeks later, gated fractions of the liver MNCs in $J\alpha18^{-/-}$ mice in Figure 3A were further analyzed for expression of the indicated markers and compared with those of NKT cells in the liver MNCs of C57BL/6 mice. Shadowed profiles indicated isotype-matched control staining.



Supplemental Figure 9

Global gene expression profile of the iPSC-14k cells, and their capacity to form teratomas in nude mice. (**A**) Global gene expression of the iPSC-14k cells compared to NKT-ES C-1 and splenic NKT cells by scatter plot analysis. (**B**) Histology of teratomas derived from the iPSC-14k cells shows inclusion of immature nervous tissue (N), tall columnar respiratory epithelium (RE), and mature bone tissue (B) containing marrow stroma (S). Stratified squamous epithelium, bundles of striate muscles and neuroepithelial tubules were often found (not shown). Teratomas formed in the subcutaneous regions of nude mice were dissected out 4 weeks after inoculation of the iPSC-14k cells ($1x10^6$).