Fig. S1. Comparison of the proportion of NK cells in PBMCs derived from healthy adult donors and lung cancer patients. (A) Flow cytometric analysis of PBMCs derived from healthy adult volunteers and lung cancer patients. Peripheral blood samples were obtained from healthy adult volunteers (HD-A and HD-B) and lung cancer patients (LC-A and LC-B) using heparin-containing tubes and PBMCs were purified by gradient centrifugation. PBMCs were stained with PE-conjugated anti-CD56 mAb and FITC-conjugated anti-CD3 mAb and analyzed using a FACSCalibur flow cytometer. (B) Comparison of the proportion of CD3−CD56+ cells in PBMCs derived from healthy adult volunteers and lung cancer patients. The proportion of NK cells in PBMCs derived from 25 lung cancer patients (LC) was compared with that from 21 healthy adult volunteers (HD): 19.2 ± 10.5% (mean ± SD) for LC and 17.3 ± 6.8% for HD. A p value between LC and HD is shown.
Fig. S2. Time course of cell surface marker expression on NK cells stimulated with IL-2 and IL-2/IL-18. The expression of cell surface markers on peripheral blood CD3- cells expanded with IL-2 in the absence or presence of IL-18 were analyzed on the basis of mean fluorescence intensity on days 5, 7 and 10. CD80, CD86, HLA-DR and HLA-DQ typically expressed on antigen-presenting cells were markedly induced on IL-2/IL-18-stimulated NK cells. The experiments were performed two times and representative results are shown.
Fig. S3. Effect of IL-18 on the expression of cell surface markers on NK cells stimulated with IL-2. Peripheral blood samples were obtained from a healthy adult volunteer using heparin-containing tubes and PBMCs were purified by gradient centrifugation. After CD3+ cells were removed using anti-CD3 mAb-coated beads and a magnet holder, CD3- cells were stimulated with IL-2 (100 U/mL) or IL-2 (100 U/mL) plus IL-18 (100 ng/mL) at 37°C with 5% CO2. The expression of cell surface markers were detected through flow cytometry using a fluorescein isothiocyanate (FITC)-conjugated anti-CD56 mAb and phycoerythrin (PE)-conjugated anti-NKG2D, DNAM-1, Fas-L, TRAIL and CD16 mAbs: filled histograms, stimulated with IL-2/IL-18; open histograms, stimulated with IL-2. The proportions of cell surface marker-positive cells in CD56+ cells after expansion are indicated.
Fig. S4. Effect of IL-18 on the expression of cell surface markers on CD3− cells stimulated with IL-2. Peripheral blood was obtained from a healthy adult volunteer using heparin-containing tubes and PBMCs were purified by gradient centrifugation. After CD3+ cells were removed using anti-CD3 mAb-coated beads and a magnet holder, CD3− cells were stimulated with IL-2 (100 U/mL) plus IL-18 (100 ng/mL) for 10 days at 37°C with 5% CO2. The expression of cell surface markers were detected through flow cytometry using fluorescein isothiocyanate (FITC)-conjugated anti-CD56 mAb and phycoerythrin (PE)-conjugated anti-CD80, CD86, HLA-DR, HLA-DQ or CD14 mAbs, and the expression of cell surface markers on CD56+ cells was analyzed: filled histograms, indicated antibodies; open histograms, control.
Fig. S5. Comparison of NK cells induced with various stimulants. (A) Stimulation of NK cells with interleukins. Peripheral blood CD3\(^{-}\) cells were stimulated with IL-2, IL-2/IL-18, IL-2/IL-21 or IL-15 and the cell images were monitored under a microscope on day 7. (B) Expansion of NK cells with interleukins. Before and after stimulation of peripheral blood CD3\(^{-}\) cells with IL-2, IL-2/IL-18, IL-2/IL-21 or IL-15, the number of cells was counted. (C) Phenotypic analysis of interleukin-induced NK cells. After 10 days of stimulation with interleukins, cell surface markers were analyzed using a FACSCalibur flow cytometer. Mean fluorescence intensity is indicated in the panels.
**Fig. S6. Interaction between NK cells and γδ T cells.** Human γδ T cells in green and IL-2/IL-18-induced NK cells in red were mixed and the resulting cell clusters were observed under a confocal microscope. For preparation of γδ T cells, PBMCs were obtained from a healthy adult volunteer. Heparinized PBMCs (10 mL) were diluted with 10 mL of PBS and loaded on 20 mL of Ficoll-Paque™ PLUS (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) in a 50 mL conical tube. After centrifugation at 600 x g for 30 min at room temperature, a lymphocyte layer was removed in a 50 mL conical tube containing 35 mL of PBS. After being washed two times with PBS, the lymphocytes were resuspended in 6 mL of Yssel’s medium [1] supplemented with 10% heat-inactivated human AB serum. The cell suspension was stimulated with PTA [2], a nitrogen-containing bisphosphonate prodrug, and incubated with IL-2 for 10 days. The purity of the resulting γδ T cells was more than 99%. For preparation of IL-2/IL-18-induced NK cells, PBMCs were purified as described above and resuspended in 80 µL of PBS/2% BSA/2mM EDTA. CD3- cells were purified and IL-2/IL-18-induced NK cells were prepared as described in “Materials and Methods”. γδ T cells (4 x10^6 cells) in 4 mL of phenol red-free RPMI1640 medium supplemented with 10% FCS were treated with 5 µM CellTracker™Green CMFDA (Life Technologies Corp., Carlsbad, CA) for 15 min at 37°C. After centrifugation at 600 x g for 5 min at 4°C, the cells were washed three times with 5 ml of the medium and resuspended in 4 mL of the medium. IL-2/IL-18-induced cells were similarly stained with 5 µM CellTracker™Red CMTPX and resuspended in 4 mL of the medium. The stained γδ T cells and IL-2/IL-18-induced NK cells were mixed and placed in 35-mm glass-base dishes (Glass 27φ, Asahi Glass Co., Ltd., Chiyoda-ku, Tokyo, Japan). After incubation for 4 h at 37°C, the cells were observed under an LSM 710 microscope (Carl Zeiss AG, Oberkochen, Baden-Württemberg Germany) and the images were analyzed using a Zen software. Left panel: γδ T cells in green; center panel: IL-2/IL-18-induced NK cells in red; Right panel: a merged image.


Fig. S7. Flow cytometric analysis of cell surface markers expressed on tumor cells. (A) Expression of EGFR on the surface of lung carcinoma and renal cell carcinoma cells. PC-9 lung carcinoma cells and ACHN and VMRC-RCW renal cell carcinoma cells were grown in RPMI1640 media at 37°C with 5% CO₂ overnight. The tumor cells were harvested and the expression of EGFR was analyzed through flow cytometry using a biotinylated anti-EGFR mAb and green fluorescence protein-conjugated biotin-binding proteins. Mean fluorescence intensity is indicated. (B) Expression of CD20 on the surface of Burkitt’s lymphoma cells. Raji and RAMOS-RAI Burkitt’s lymphoma cells were examined for the expression of CD20 as in (A). The flow cytometric profiles of EGFR and CD20 expression are displayed as filled histograms with solid contours and those of unstained controls as open histograms with dotted contours. Mean fluorescence intensity is indicated.