

FATE NK-100: A novel NK cell-mediated cancer therapy

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EXECUTIVE SUMMARY

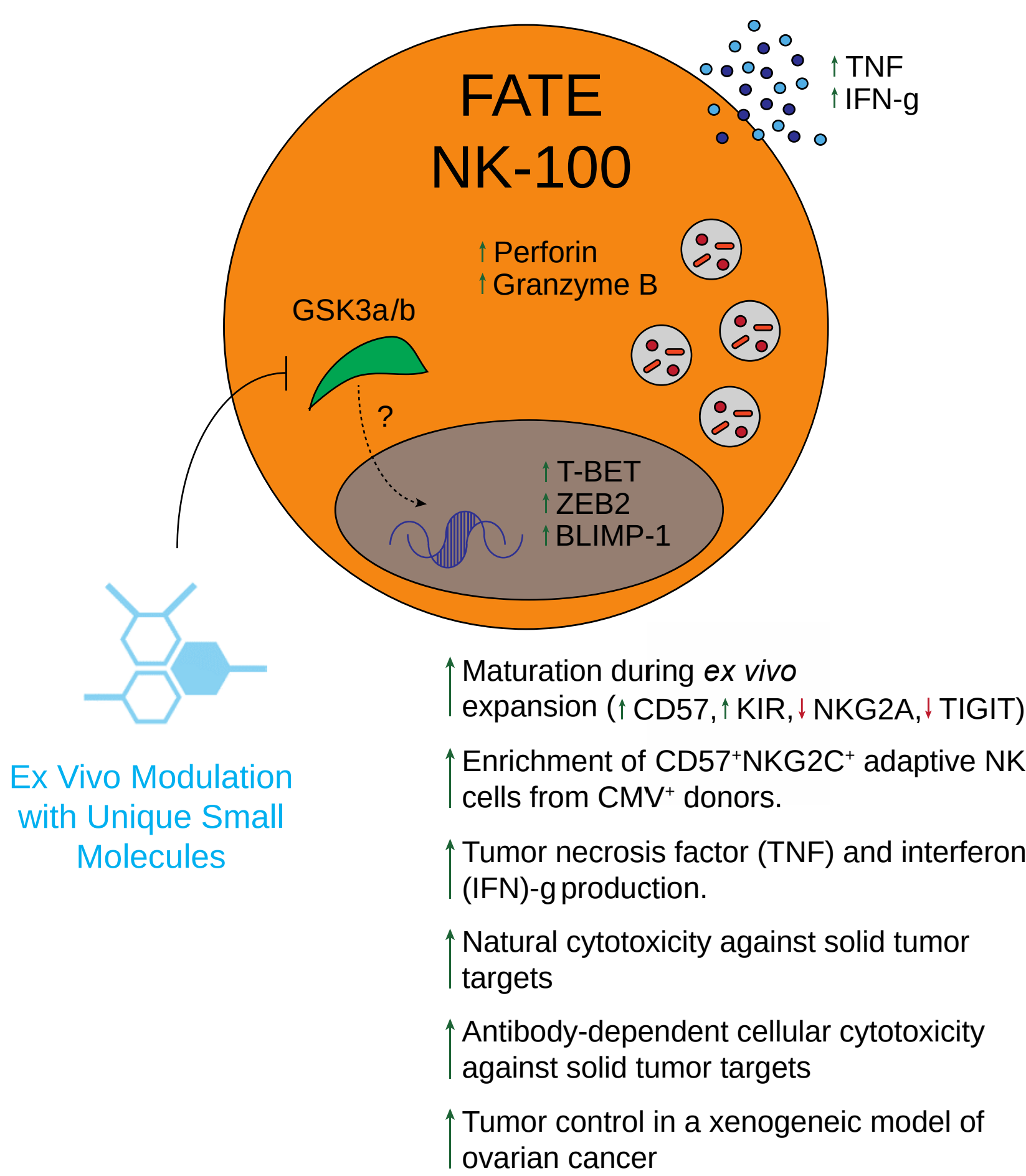
Here, we demonstrate that addition of a small molecule inhibitor of glycogen synthase kinase (GSK) 3 to peripheral blood NK cells expanded ex vivo with interleukin (IL)-15 markedly enhances CD57⁺ acquisition and NK cell maturation.

GSK3 inhibition leads to increased expression of several transcription factors associated with late-stage NK cell maturation including T-BET, ZEB2 and BLIMP-1 without affecting cell viability or proliferation.

NK cells expanded with the GSK3 inhibitor exhibit significantly higher TNF and IFN- γ production in response to K562 myeloid leukemia cells and higher natural cytotoxicity and ADCC against A549 lung carcinoma cells, SKOV-3 ovarian adenocarcinoma cells and PANC-1 epitheloid carcinoma cells. These NK cells also demonstrate stronger and more consistent tumor control after adoptive transfer using a xenogeneic model of ovarian cancer.

Our novel ex vivo NK cell expansion protocol (FATE NK-100) has been successfully scaled-up under cGMP conditions, and a first-in-human clinical trial will open in 2017.

GRAPHICAL ABSTRACT



RESULTS

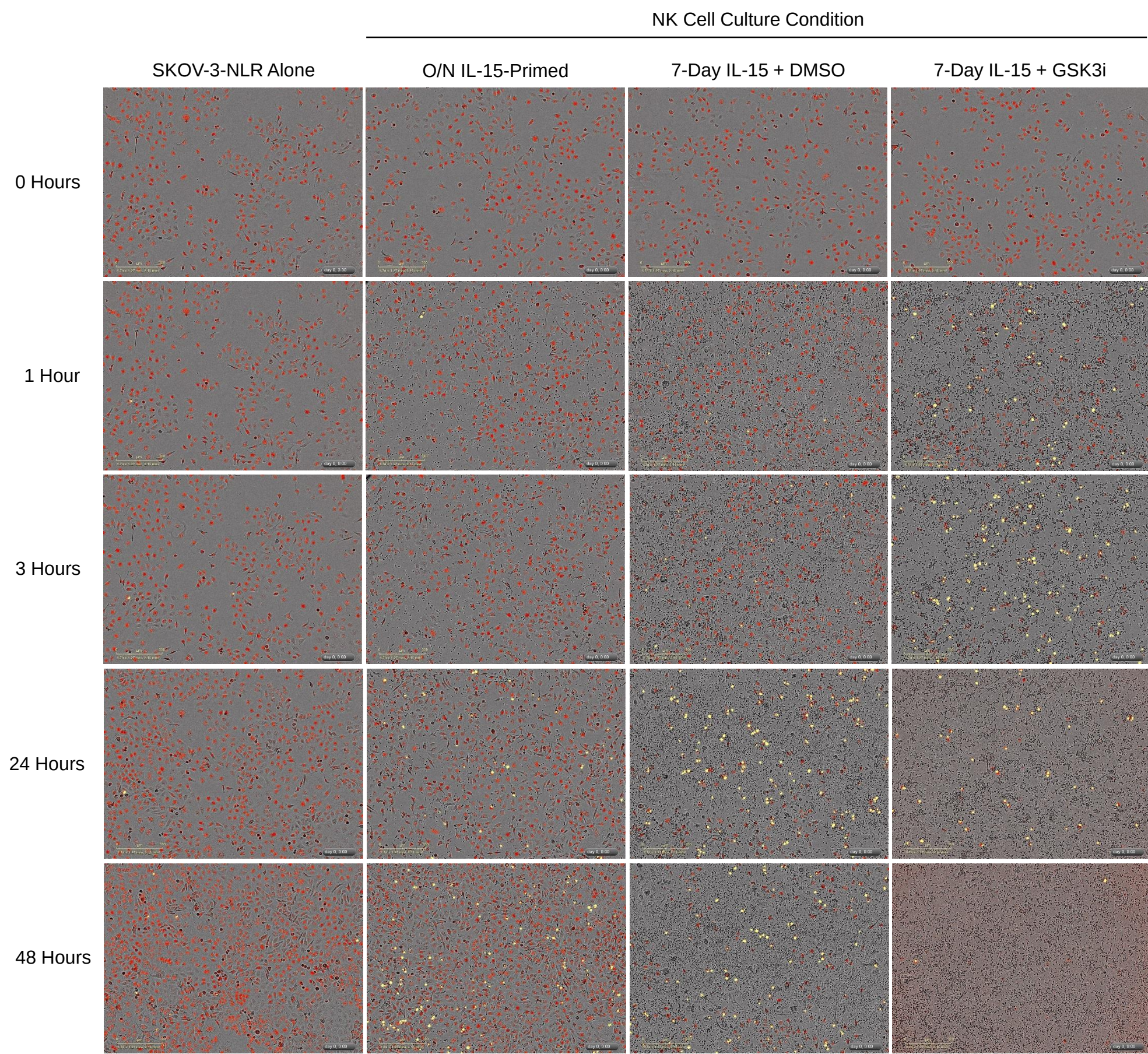


Figure 5. NK cells expanded with GSK3i exhibit rapid killing kinetics of SKOV-3 ovarian adenocarcinoma cells. CD3/19-depleted PBMCs from CMV seropositive donors were cultured overnight with 10 ng/ml IL-15, for 7 days with 10 ng/ml IL-15 and DMSO or for 7 days with 10 ng/ml IL-15 and GSK3i. NK cells from these 3 conditions were incubated with SKOV-3 cells expressing NuLight Red fluorescent protein that were labeled with Caspase-3/7 Green Detection Reagent at a 3:1 ratio. An IncuCyte Imaging System was used to measure caspase activation over 48 hours. The images shown are representative of 3 independent experiments. In this assay, SKOV-3 targets can be visualized by their red nuclei. The appearance of yellow indicates caspase 3/7 activity within SKOV-3 cells upon NK cell-mediated lysis.

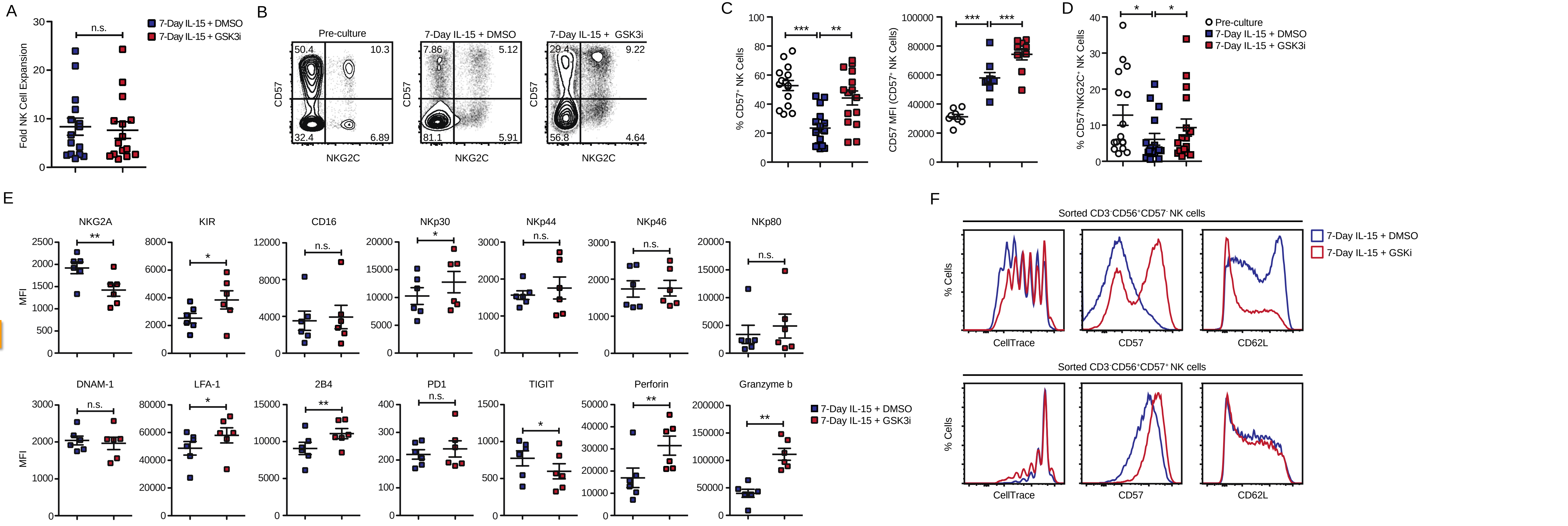


Figure 1. Addition of GSK3i to NK cells expanded with autologous monocytes enriches for NK cells with a mature phenotype. CD3/19-depleted PBMCs from CMV seropositive donors were cultured for 7 days with 10 ng/ml IL-15 and either DMSO or GSK3i. (A) Shown is fold NK cell expansion after 7 days in each culture condition relative to day 0 ($n = 15$). (B) Representative FACS scatter plots of CD57 and NKG2C expression on gated CD3⁺CD56⁺ NK cells before and after culture. (C) Cumulative ($n = 15$) data showing the percentages (left panel) of CD57⁺ NK cells and the mean fluorescence intensity of CD57 gated on CD57⁺ NK cells (right panel) within the total CD3⁺CD56⁺ NK cell population before and after culture. Results are from 5 independent experiments. (D) Cumulative ($n = 15$) data showing the percentages of adaptive CD57⁺NKG2C⁺ NK cells before and after culture. (E) Cumulative ($n = 6$) data showing the relative mean fluorescence intensity for each indicated receptor or cytotoxic granule component on gated CD3⁺CD56⁺ NK cells from CD3⁺CD56⁺ NK cells cultured relative to DMSO controls. (F) CD3⁺CD56⁺CD57⁺ NK cells and CD3⁺CD56⁺CD57⁺ NK cells were sorted from donor PBMCs ($n = 4$) by FACS, labeled with CellTrace dye and cultured for 7 days at a 2:1 ratio with sorted, autologous CD14⁺ monocytes with 10 ng/ml IL-15 and either DMSO or 5 μ M CHIR99021. Shown are representative FACS histogram plots of CellTrace, CD57 and CD62L from each sorted population after culture. All cumulative data is shown as mean $\pm$ SEM, and paired Student's t tests were used for statistical analyses. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

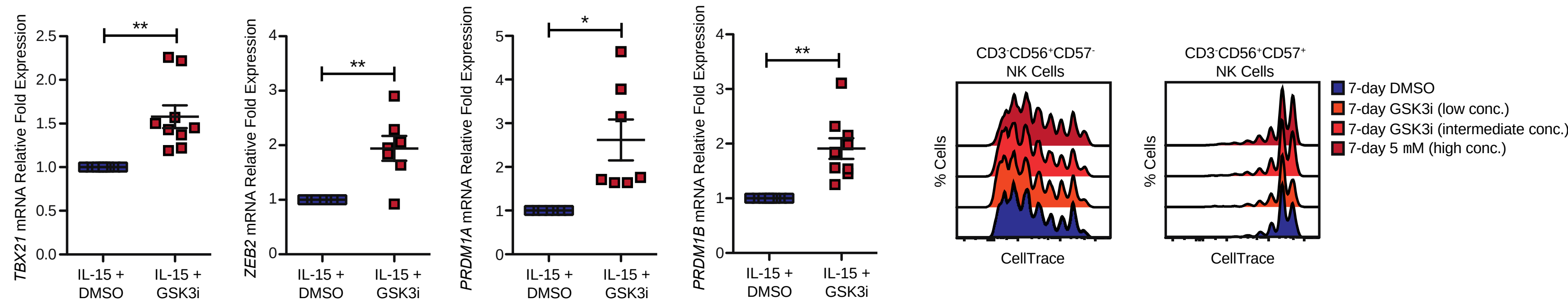


Figure 2. NK cells expanded with GSK3i exhibit elevated expression of transcription factors associated with late-stage NK cell maturation. CD3/19-depleted PBMCs from CMV seropositive donors were cultured for 7 days with 10 ng/ml IL-15 and either DMSO or GSK3i ($n = 9$). CD3⁺CD56⁺ NK cells were then isolated from each culture condition and analyzed by qRT-PCR for expression of TBX21, ZEB2, PRDM14, PRDM18 and EOMES mRNA. Shown are cumulative data of fold expression values of cells cultured with GSK3i relative to cells cultured with DMSO. All data are normalized against ACTB. Results are from 2 independent experiments. Data is shown as mean $\pm$ SEM, and paired Student's t tests were used for statistical analyses. * $p \leq 0.05$, ** $p \leq 0.01$.

Figure 3. Inhibition of GSK3 does not affect NK cell proliferation. CD3⁺CD56⁺CD57⁺ NK cells were sorted from PBMCs, labeled with CellTrace proliferation dye, and cultured for 7 days with 10 ng/ml IL-15 and either DMSO or increasing concentrations of GSK3i. Shown are representative FACS histogram plots of CellTrace proliferation dye dilution in gated CD3⁺CD56⁺CD57⁺ (left panel) and CD3⁺CD56⁺CD57⁺ NK cells (right panel).

Figure 4. Inhibition of GSK3 enhances NK cell production of TNF and IFN- γ in response to K562 myeloid leukemia cells. CD3/19-depleted PBMCs from CMV seropositive donors were cultured overnight with 10 ng/ml IL-15, for 7 days with 10 ng/ml IL-15 and DMSO or for 7 days with 10 ng/ml IL-15 and GSK3i. NK cells from these 3 conditions were incubated with K562 cells at a 1:1 ratio, and intracellular FACS was used to measure the percentage of CD3⁺CD56⁺ NK cells positive for TNF (left panel) or IFN- γ (right panel) ($n = 7$). Results are from 4 independent experiments and are shown as mean $\pm$ SEM. Paired Student's t tests were used for statistical analyses. * $p \leq 0.05$, ** $p \leq 0.01$.

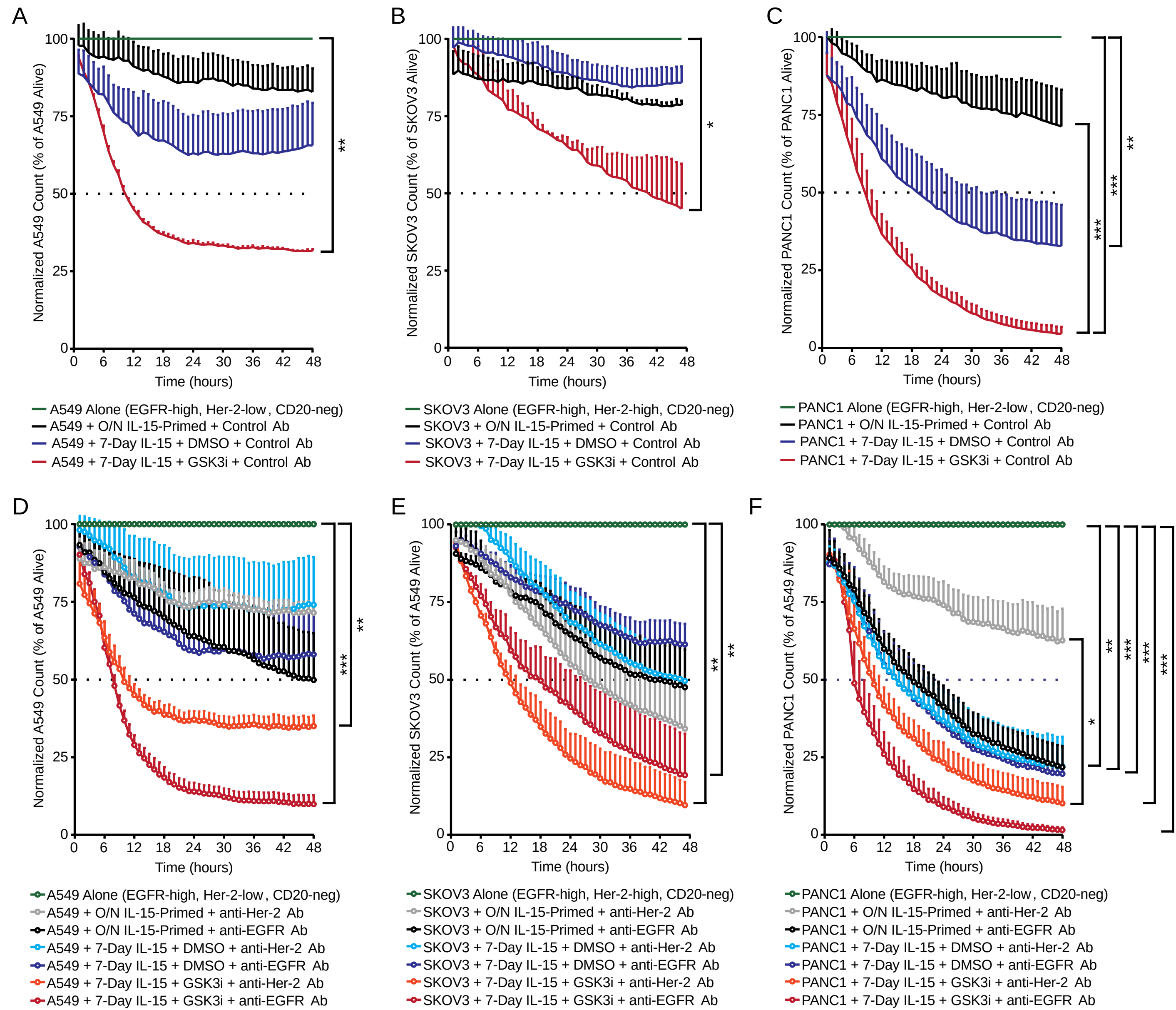


Figure 6. Inhibition of GSK3 enhances NK cell natural cytotoxicity and ADCC function against solid tumor cell lines in vitro. CD3/19-depleted PBMCs from CMV seropositive donors were cultured overnight with 10 ng/ml IL-15, for 7 days with 10 ng/ml IL-15 and DMSO or for 7 days with 10 ng/ml IL-15 and GSK3i. NK cells from these 3 conditions were incubated with (A) A549 cells, (B) SKOV-3 cells and (C) PANC-1 cells expressing NuLight Red fluorescent protein at a 3:1 ratio with the addition of a control anti-CD20 antibody. Parallel assays with NK cells ($n = 3$) cultured in the conditions above were performed with (D) A549 cells, (E) SKOV-3 cells and (F) PANC-1 cells expressing NuLight Red fluorescent protein at a 3:1 ratio with the addition of either anti-HER2 or anti-EGFR antibodies. An IncuCyte Imaging System was used to measure target cell killing over 48 hours. Results are from 3 independent experiments and are shown as mean $\pm$ SEM. Two-way ANOVA with Tukey's multiple comparisons tests were used to determine significance. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

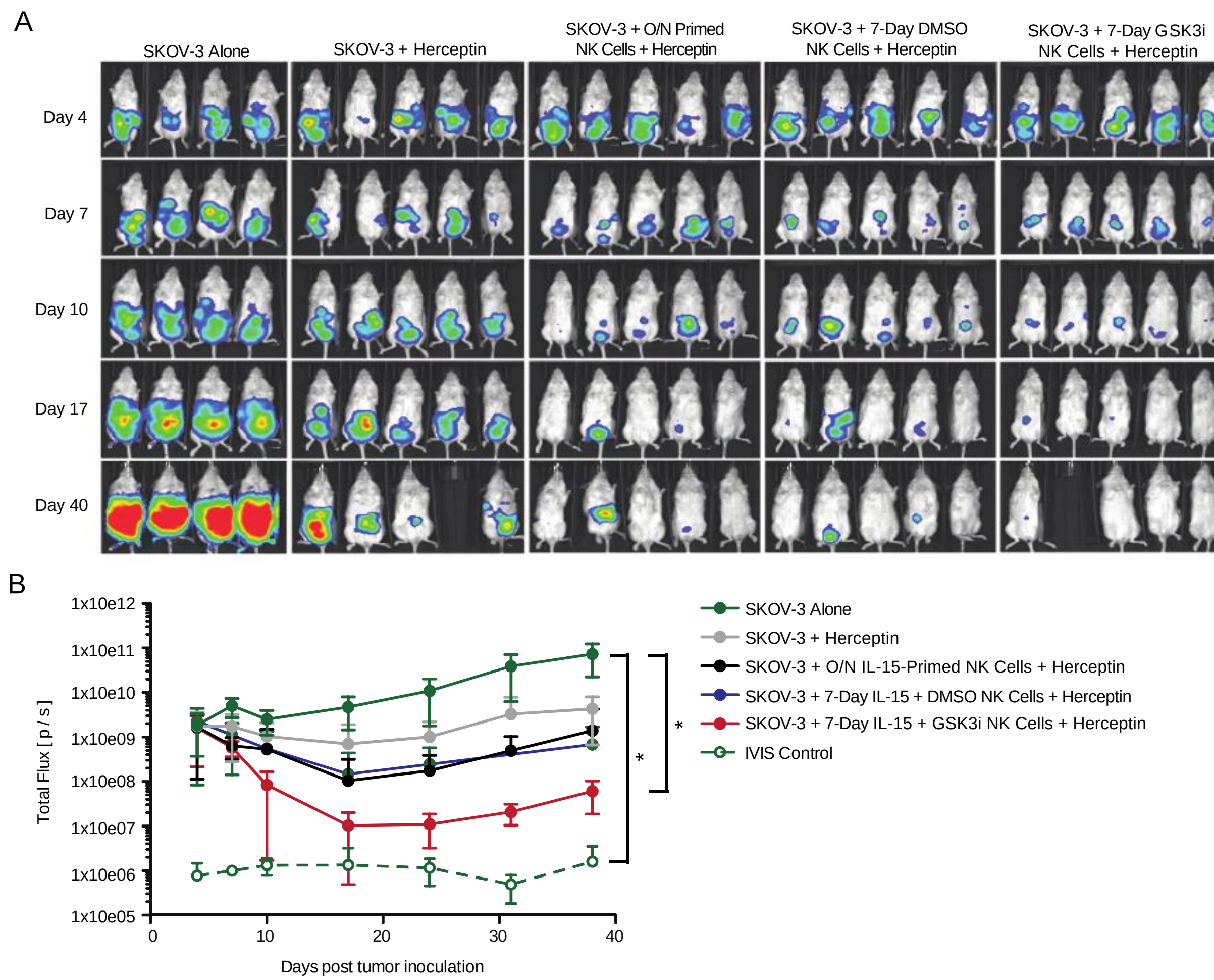


Figure 7. Inhibition of GSK3 enhances NK cell anti-tumor function in vivo. Mice with established SKOV-3 tumors were left untreated ($n = 17$), treated with Herceptin alone ($n = 20$), treated with overnight IL-15-primed NK cells plus Herceptin ($n = 15$), treated with NK cells cultured for 7 days with 10 ng/ml IL-15 and DMSO plus Herceptin ($n = 10$) or treated with NK cells cultured for 7 days with 10 ng/ml IL-15 and GSK3i plus Herceptin ($n = 16$). All donors were CMV seropositive, and cultures were performed using CD3/19-depleted PBMCs. Tumor progression was monitored weekly by IVIS imaging over 40 days. (A) Bioluminescence images of mice from each group at the indicated intervals post-adoptive NK cell transfer from a representative experiment are shown. (B) Cumulative bioluminescence values were plotted over time representing all mice in each experiment. Results are from 4 independent experiments, and data is shown as mean $\pm$ SEM. One-way ANOVA with Bonferroni's Multiple Comparisons Tests were used to determine significance. * $p \leq 0.05$.