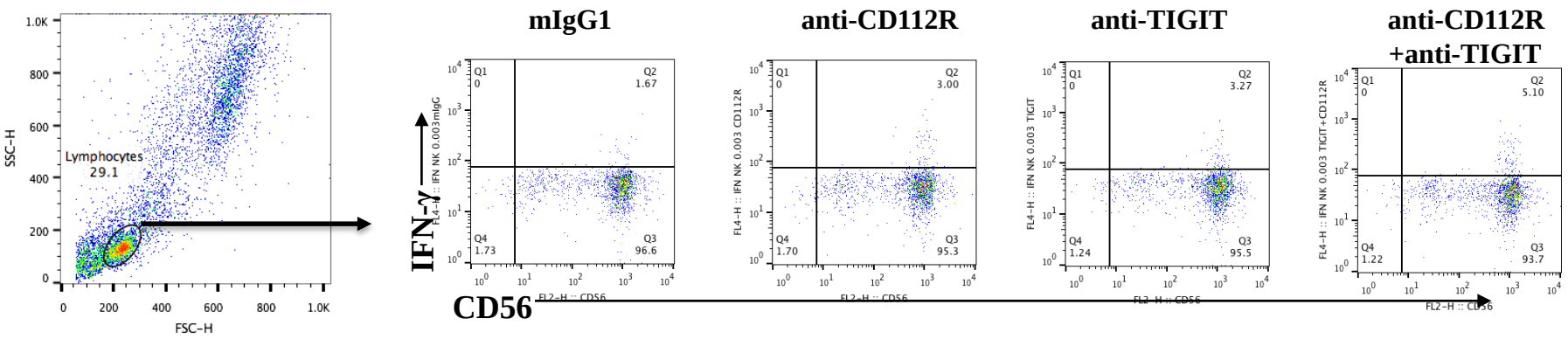
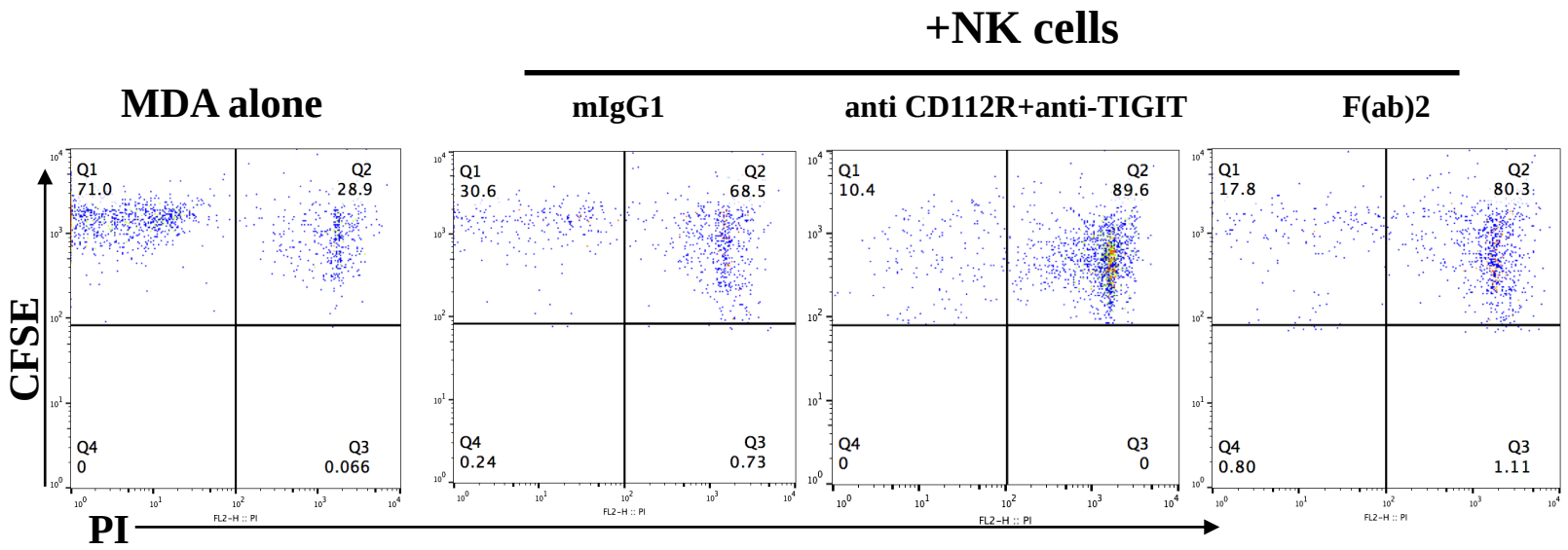
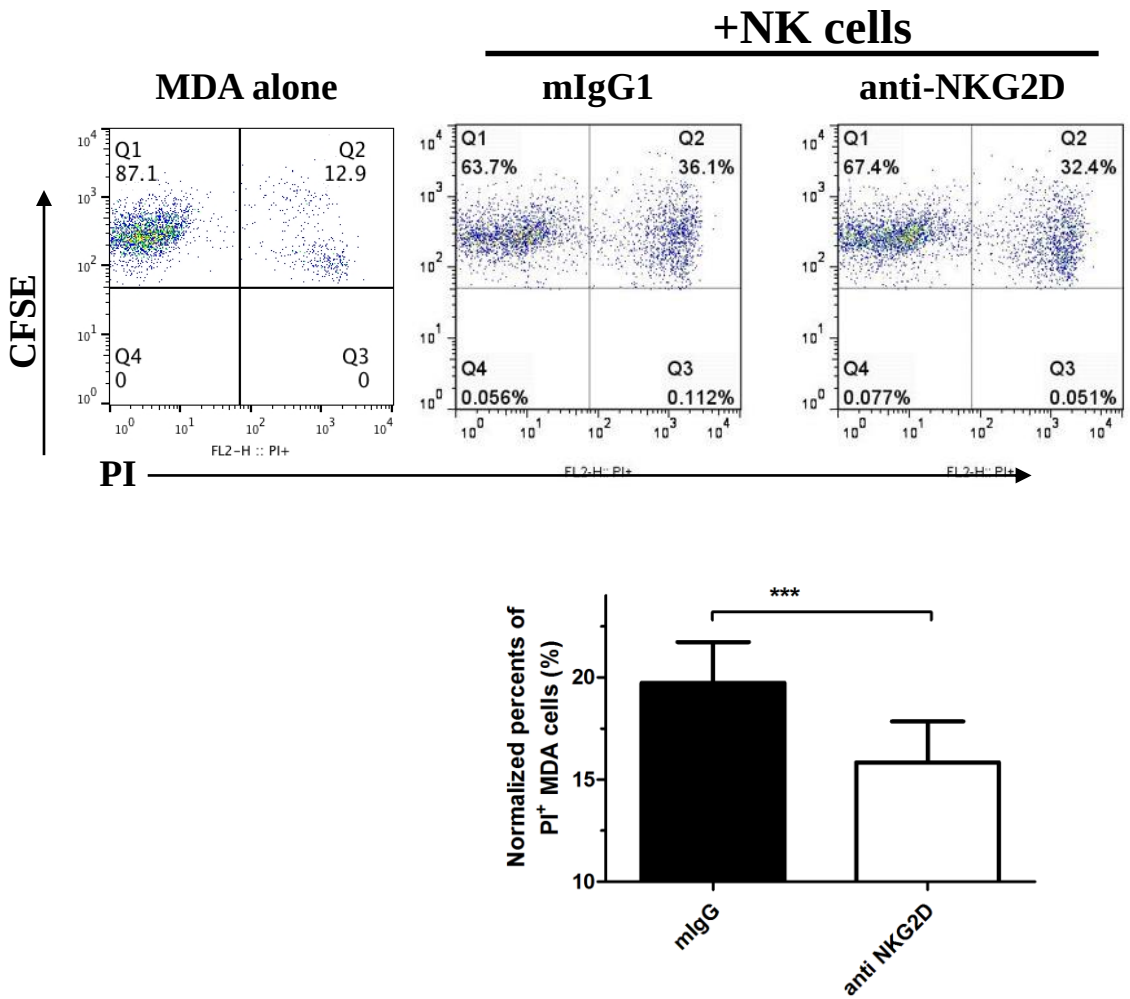


Supplemental Figure 1 Blockade of CD112R and TIGIT signals promotes IFN-γ production in human NK cells against breast cancer cells. Purified human NK cells were cultured with MDA tumor cells at a ratio of 2.5:1. Different neutralizing antibodies or isotype-matched mIgG1 control antibody were added from the beginning of culture as indicated. Harvested cells were stained for surface CD56 before intracellular staining of IFN-γ. Cells were gated on NK cell population based on morphology, and the percentages of IFN-γ-positive NK cells were determined via flow cytometry.



Supplemental Figure 2 Blockade of CD112R and TIGIT signals using F(ab)2 fragment promotes human NK cell cytotoxicity against breast cancer cells. CFSE-labeled MDA breast cancer cells were incubated with human NK cells overnight, with the presence of control, CD112R and TIGIT blocking antibodies, or F(ab)2 fragments generated from these two mAbs. The death of MDA cells (CFSE-positive) was revealed by PI staining. CFSE-labeled MDA cells cultured alone were used to reveal spontaneous death. Tumor killing was determined by the percentages of PI-positive tumor cells after subtracting natural death.



Supplemental Figure 3 Blockade of NKG2D receptor inhibits NK cell cytotoxicity against breast cancer cells. CFSE-labeled MDA breast cancer cells were incubated with human NK cells overnight, with the presence of control or NKG2D blocking mAb (Clone 149810, R&D systems). CFSE-labeled MDA cells cultured alone were used to reveal natural death. The death of MDA cells (CFSE-positive) was revealed by PI staining. Tumor killing was revealed by the percentages of PI-positive tumor cells after normalization.