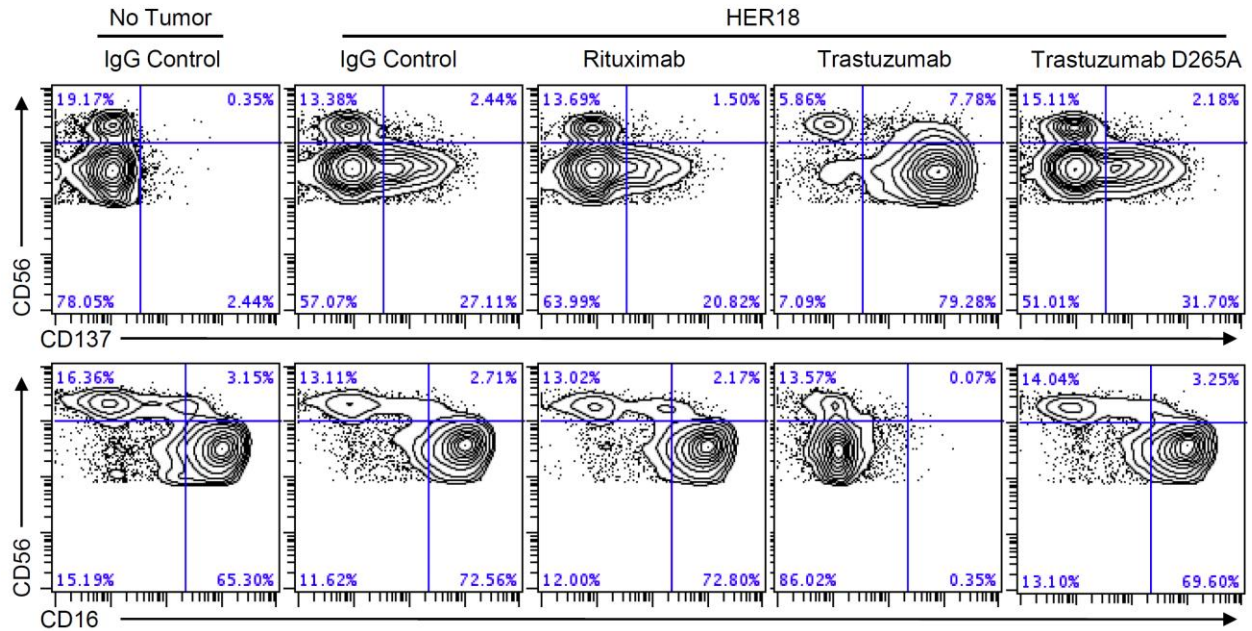
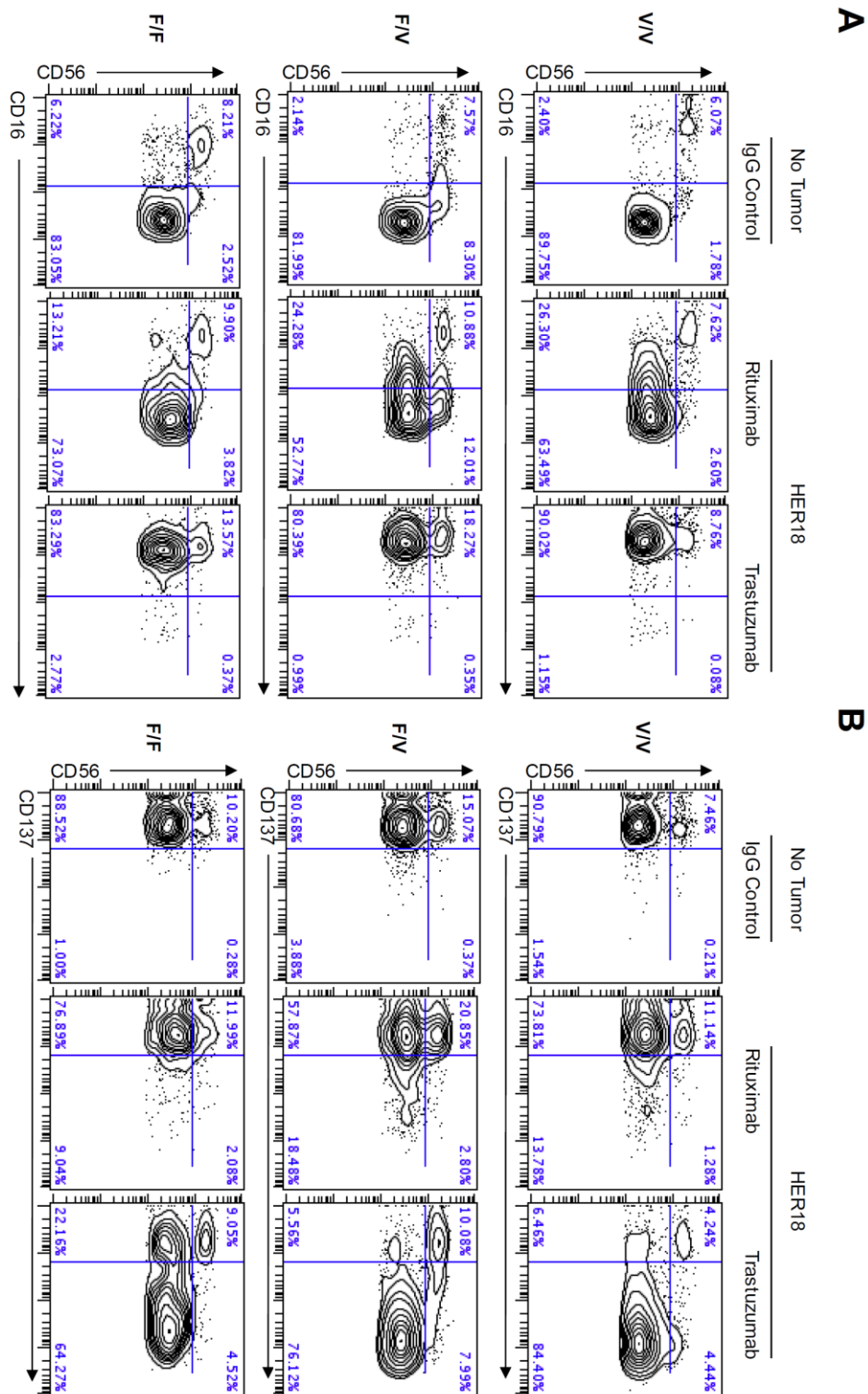


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Supplementary Figure 1

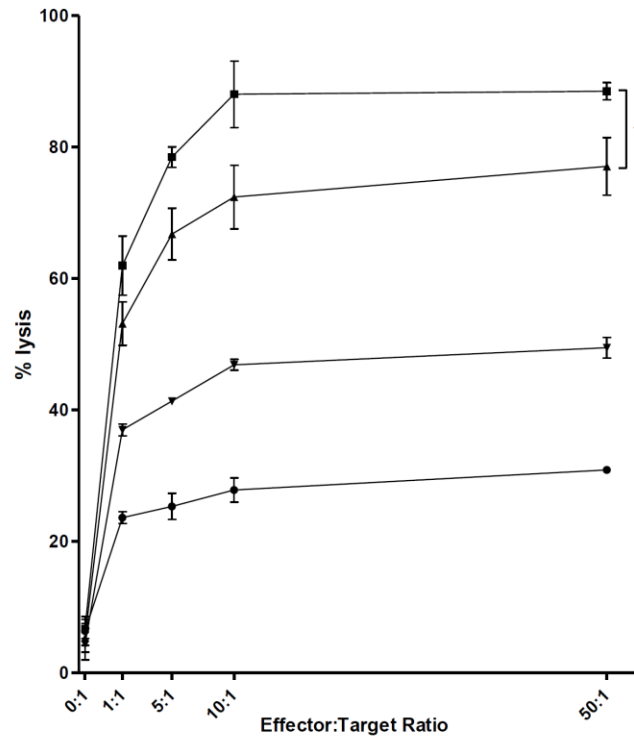
Trastuzumab induced CD137 upregulation requires Fc-FcγR binding on human NK cells following exposure to HER2-overexpressing tumor cells. Peripheral blood from a healthy donor was analyzed for CD137 expression on CD3⁺CD56⁺ NK cells after 24 hour culture with breast cancer cell line, HER18, or no tumor and IgG control, rituximab, trastuzumab, or trastuzumab D265A (D265A mutation prevents Fc-FcγR binding). CD137 and CD16 expression on NK cell subsets cells CD3⁺CD56^{bright} and CD3⁺CD56^{dim} from a healthy donor after 24 hour culture with IgG control alone, HER18 and IgG control, HER18 and rituximab, HER18 and trastuzumab, and HER18 and trastuzumab D265A is shown.



Supplementary Figure 2

Genetic polymorphisms with variable FcγRIIIa affinity impact degree of NK cell CD137 expression. Peripheral blood from healthy donors with FcγRIIIa-158 genotypes V/V (high affinity FcγR), V/F, and F/F (low affinity FcγR) was analyzed for CD137 expression on CD3⁺CD56⁺ NK cells after 24 hour culture with breast cancer cell line, HER18, or no tumor and IgG control, rituximab, or trastuzumab. (A) shows CD137 expression on NK cell subsets cells CD3⁺CD56^{bright} and CD3⁺CD56^{dim} from healthy donors after 24 hour culture with IgG control alone, HER18 and rituximab, and HER18 and trastuzumab. (B) shows CD16 expression on NK cell subsets cells CD3⁺CD56^{bright} and CD3⁺CD56^{dim} from healthy donors after 24 hour culture with IgG control alone, HER18 and rituximab, and HER18 and trastuzumab.

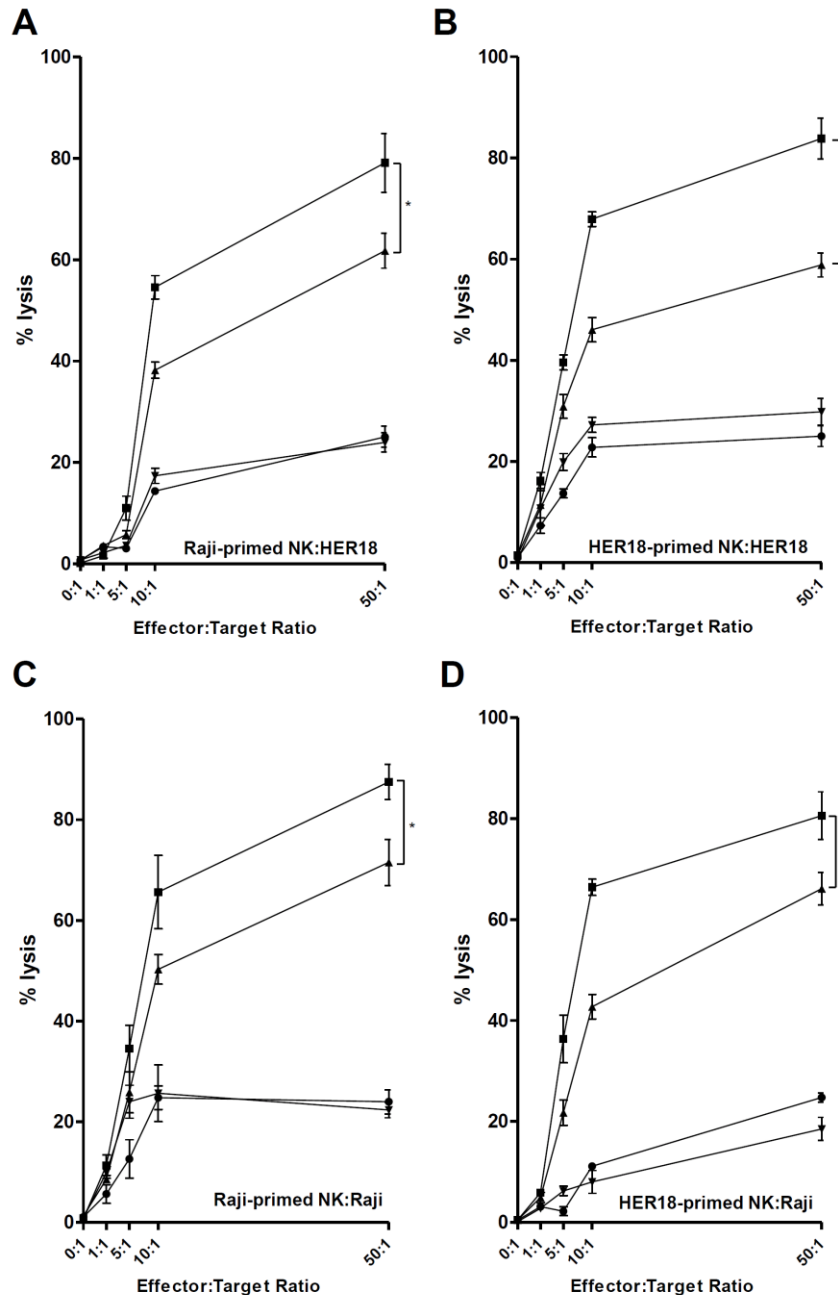
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Supplementary Figure 3

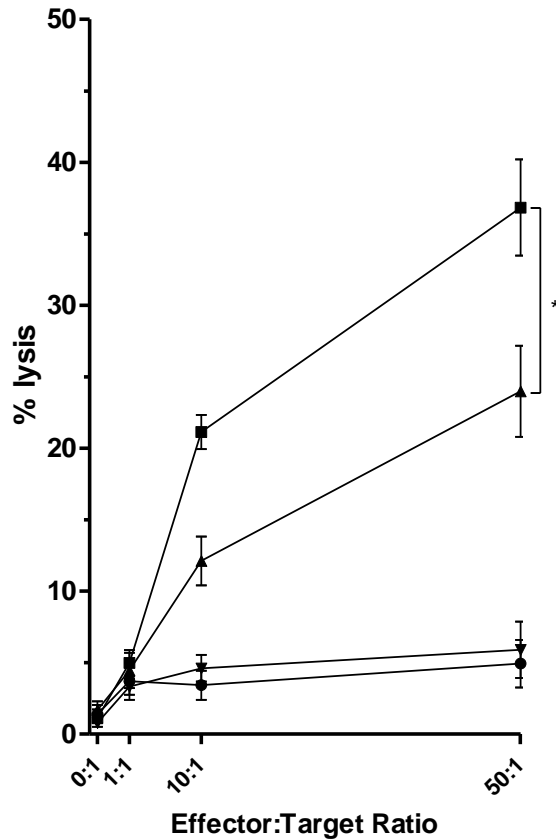
Trastuzumab-mediated cytotoxicity of activated, unpurified NK cells is augmented by anti-CD137 agonistic mAb. NK cells were isolated from healthy PBMCs and cultured for 24 hours together with trastuzumab (10 $\mu\text{g}/\text{mL}$) and irradiated (5,000 rads) breast cancer cells (HER18) at a ratio of 1:1. After 24 hours, the activated NK cells were washed and added to chromium-labeled breast cancer cells, HER18, for 4 additional hours in media alone, or with anti-CD137 mAb (BMS-663513, 10 $\mu\text{g}/\text{mL}$) alone, trastuzumab (10 $\mu\text{g}/\text{mL}$) alone, or trastuzumab plus anti-CD137 mAbs. Shown is percent lysis of target cells by chromium release at varying effector (activated NK cells):target cell ratios cultured with media alone(●), anti-CD137(▼), trastuzumab(▲), or trastuzumab and anti-CD137(■) antibodies (* $p=0.050$).

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Supplementary Figure 4

Enhanced mAb-mediated cytotoxicity of activated NK cells following anti-CD137 agonistic mAb is not restricted to the antibody-coated tumor used to induce NK cell expression of CD137. Healthy PBMCs were cultured for 24 hours together with trastuzumab (10 µg/mL) and irradiated (5,000 rads) HER2-expressing breast cancer cells (HER18, B and D) or with rituximab (10 µg/mL) and irradiated (5,000 rads) CD20⁺ lymphoma cells (Raji, A and C) at a ratio of 1:1. After 24 hours, NK cells were isolated by negative selection and assessed for purity (>90% purity as defined by CD3⁺CD56⁺ flow cytometry) and activation (>50% expression of CD137). Chromium-labeled breast cancer cell line, HER18 (A and B), or lymphoma cell line, Raji (C and D) were cultured for 4 hours with preactivated, purified NK cells in media alone, or with anti-CD137 mAb (BMS-663513, 10 µg/mL) alone, trastuzumab or rituximab (10 µg/mL) alone, or anti-CD137 plus either trastuzumab or rituximab mAbs (each 10 µg/mL). Shown is percent lysis of target cells by chromium release at varying effector (activated NK cells):target cell ratios cultured with media alone (●), anti-CD137 (▼), trastuzumab (▲), or trastuzumab and anti-CD137 (■) antibodies (A *p* = .046; B **p* = .006) or with media alone (●), anti-CD137 (▼), rituximab (▲), or rituximab and anti-CD137 (■) antibodies (C **p* = .048; D **p* = .049).



205 **Supplementary Figure 5**
 206 Anti-CD137 agonistic mAb increases trastuzumab-mediated NK cell cytotoxicity on
 207 trastuzumab-resistant tumor cells as assayed by chromium release. To evaluate NK cell
 208 cytolytic function, healthy PBMCs were cultured for 24 hours together with trastuzumab (10
 209 µg/mL) and irradiated (5,000 rads) breast cancer cells (HCC1659) at a ratio of 1:1. After 24
 210 hours, NK cells were isolated by negative selection and assessed for purity (>90% purity as
 211 defined by CD3⁺CD56⁺ flow cytometry) and activation (>50% expression of CD137). Chromium-
 212 labeled breast cancer cell line, HCC1569 were cultured for 4 hours with preactivated, purified
 213 NK cells in media alone, or with anti-CD137 mAb (BMS-663513, 10 µg/mL) alone, trastuzumab
 214 (10 µg/mL) alone, or trastuzumab plus anti-CD137 mAbs. Shown is percent lysis of target cells
 215 by chromium release at varying effector (activated NK cells):target cell ratios cultured with
 216 media alone(●), anti-CD137(▼), trastuzumab(▲), or trastuzumab and anti-CD137(■) antibodies
 217 (*p=.046).
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