Figure S1. CD137 induction and temporal expression on NK cells following preactivation

Purified NK cells from healthy donors were analyzed for CD137 expression after 24 hour culture with media, rituximab, trastuzumab, lymphoma cell lines (Raji, Ramos, DHL-4, or OCI-Ly19) and rituximab (A). Purified NK cells from a healthy donor were analyzed for CD137 expression after 0, 4, 16, 24, 48 and 72 hour culture with Raji cell line and rituximab (B). For experiments shown in Figure 2C, peripheral blood mononuclear cells from a representative healthy donor were incubated with Raji, Ramos or DHL-4 and rituximab for 24 hours. Preactivated NK cells were then analyzed for CD137 expression (C) prior to performing the cytotoxicity assay.

Figure S2. Anti-CD137 agonistic mAb increases cytokine release and rituximab-mediated cytotoxicity of pre-activated NK cells

To evaluate NK cell interferon-γ secretion purified NK cells were isolated from healthy PBMCs and cultured for 24 hours together with rituximab (10 μg/mL) and irradiated (5,000 rads) lymphoma tumor cells (Raji) at a ratio of 1:1. After 24 hours, NK cells were isolated and assessed for purity (>90% purity as defined by $CD3^{-}CD56^{+}$ flow cytometry)(A-B). Preactivated, purified NK cells were then cultured for 4 hours in media alone, or with anti-CD137 mAb (BMS-663513, 10 μg/mL) alone, rituximab (10 μg/mL) alone, or rituximab plus anti-CD137 mAbs (both at 10 μg/mL) and supernatant was harvested and analyzed by ELISA for interferon-γ (A, **p*=.027). NK cell cytotoxicity on Raji tumor cells was analyzed in chromium release assay with and without prior NK cell preactivation (B). Preactivated, and non-preactivated, purified NK cells were incubated with chromium-labeled Raji for 4 hours. Percent lysis of target cells by chromium release at varying effector (preactivated NK cells depicted in continuous line, and non-preactivated NK cells depicted in dashed line):target (Raji) cell ratios cultured with media alone(♦), anti-CD137(▲), rituximab(●), or rituximab and anti-CD137(■) antibodies (**p*=.024).

Figure S3. Anti-CD137 agonistic mAb increases rituximab-mediated NK cell degranulation

NK cells isolated and purified from the peripheral blood of healthy donors were analyzed for degranulation by CD107a mobilization after 24 hour culture with media alone, CD20-positive lymphoma cell line (Raji, Ramos, or DHL-4), tumor and rituximab, tumor and anti-CD137 antibody, or tumor, rituximab, and anti-CD137 agonistic antibody (see Figure 2A). Representative flow cytometry plot of CD107a expression on NK cells after culture with Ramos.

FIGURE S1

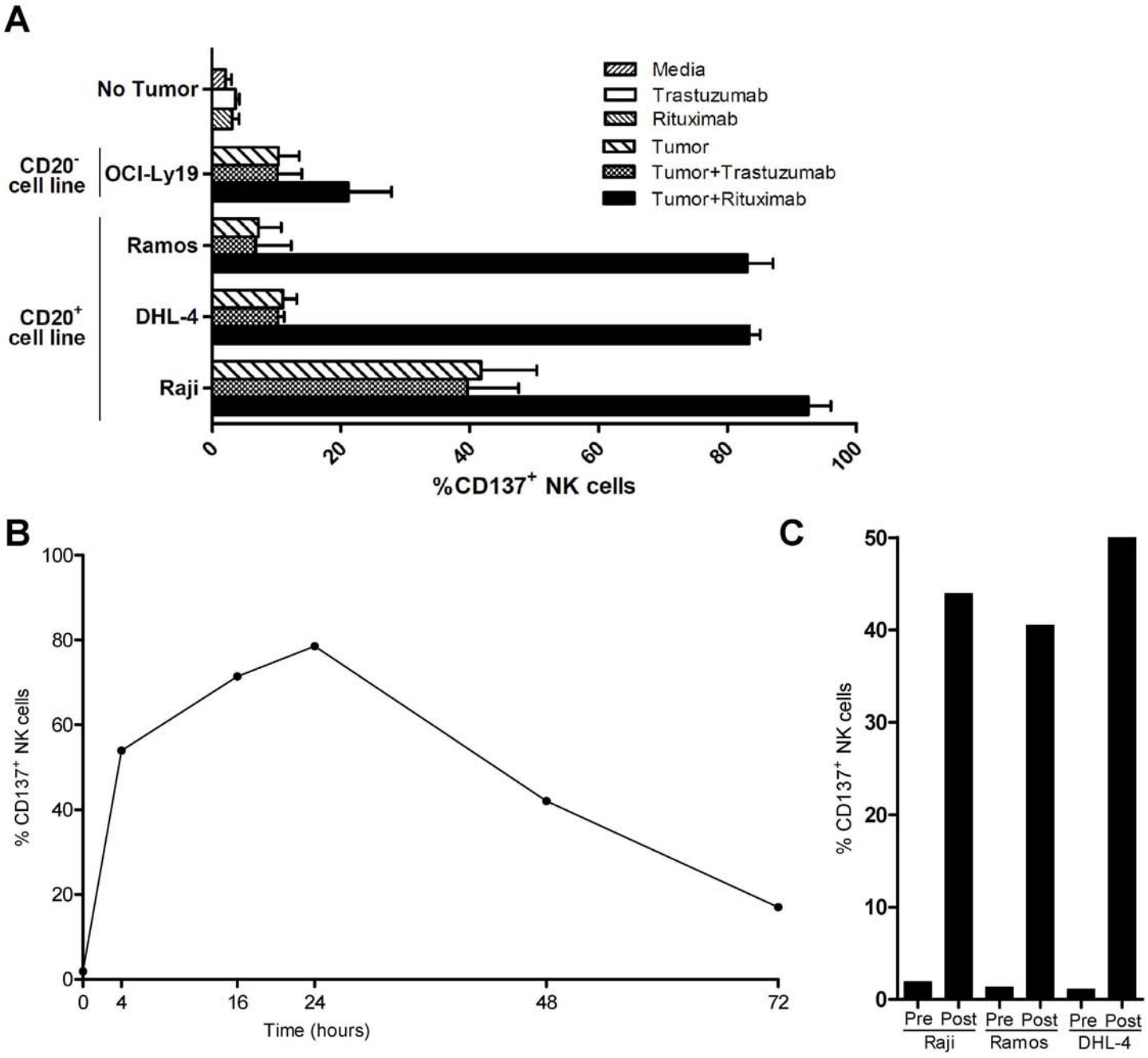
A
No Tumor
CD20- cell line
OCI-Ly19
CD20+ cell line
Ramos
DHL-4
Raji
Media
Trastuzumab
Rituximab
Tumor
Tumor+Trastuzumab
Tumor+Rituximab
0
20
40
60
80
100
%CD137+ NK cells
B
% CD137+ NK cells
0
20
40
60
80
100
0
4
16
24
48
72
Time (hours)
C
% CD137+ NK cells
0
10
20
30
40
50
Pre Post
Raji
Pre Post
Ramos
Pre Post
DHL-4

FIGURE S2

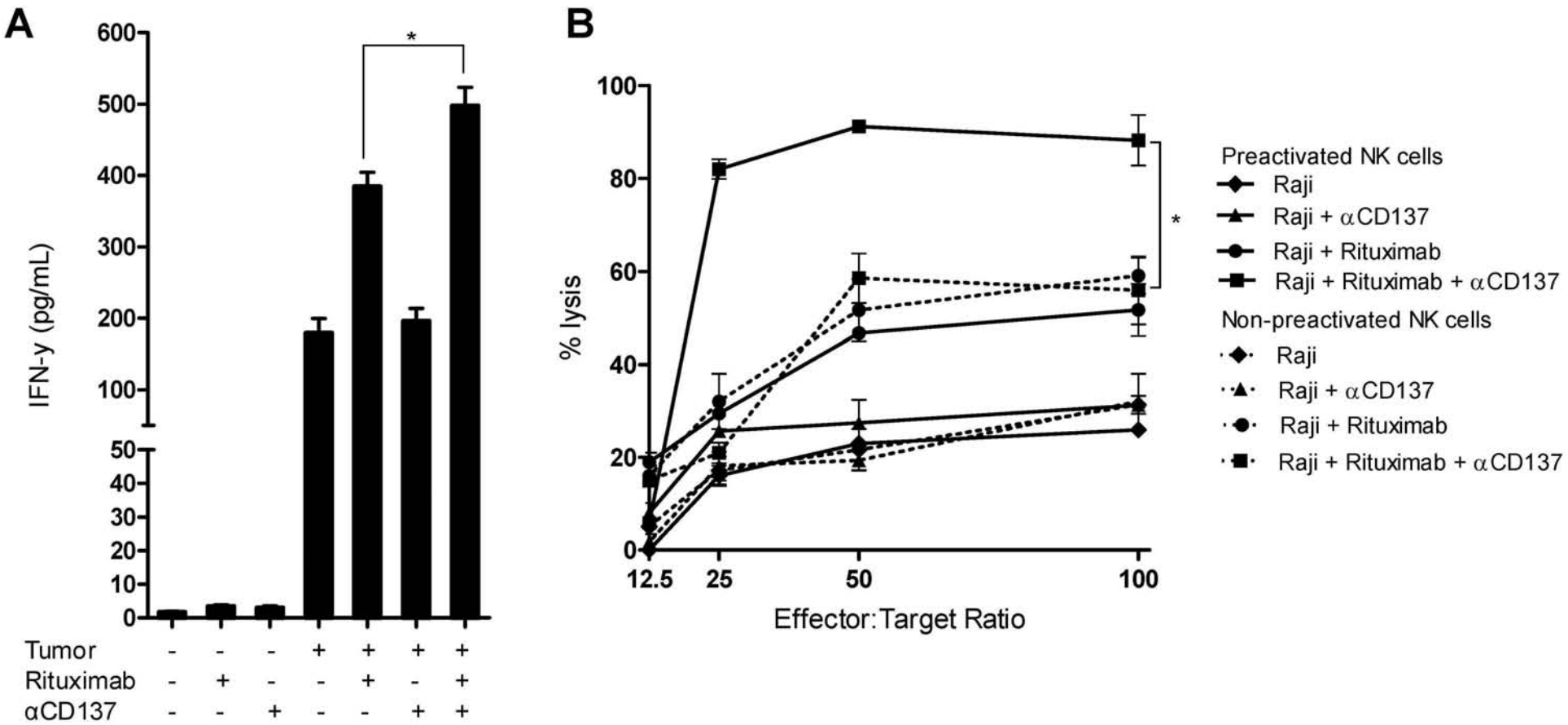
A
IFN-γ (pg/mL)
600
500
400
300
200
100
50
40
30
20
10
0
*
Tumor - - - + + + +
Rituximab - + - - + - +
αCD137 - - + - - + +
B
% lysis
100
80
60
40
20
0
12.5
25
50
100
Effector:Target Ratio
*
Preactivated NK cells
Raji
Raji + αCD137
Raji + Rituximab
Raji + Rituximab + αCD137
Non-preactivated NK cells
Raji
Raji + αCD137
Raji + Rituximab
Raji + Rituximab + αCD137

FIGURE S3