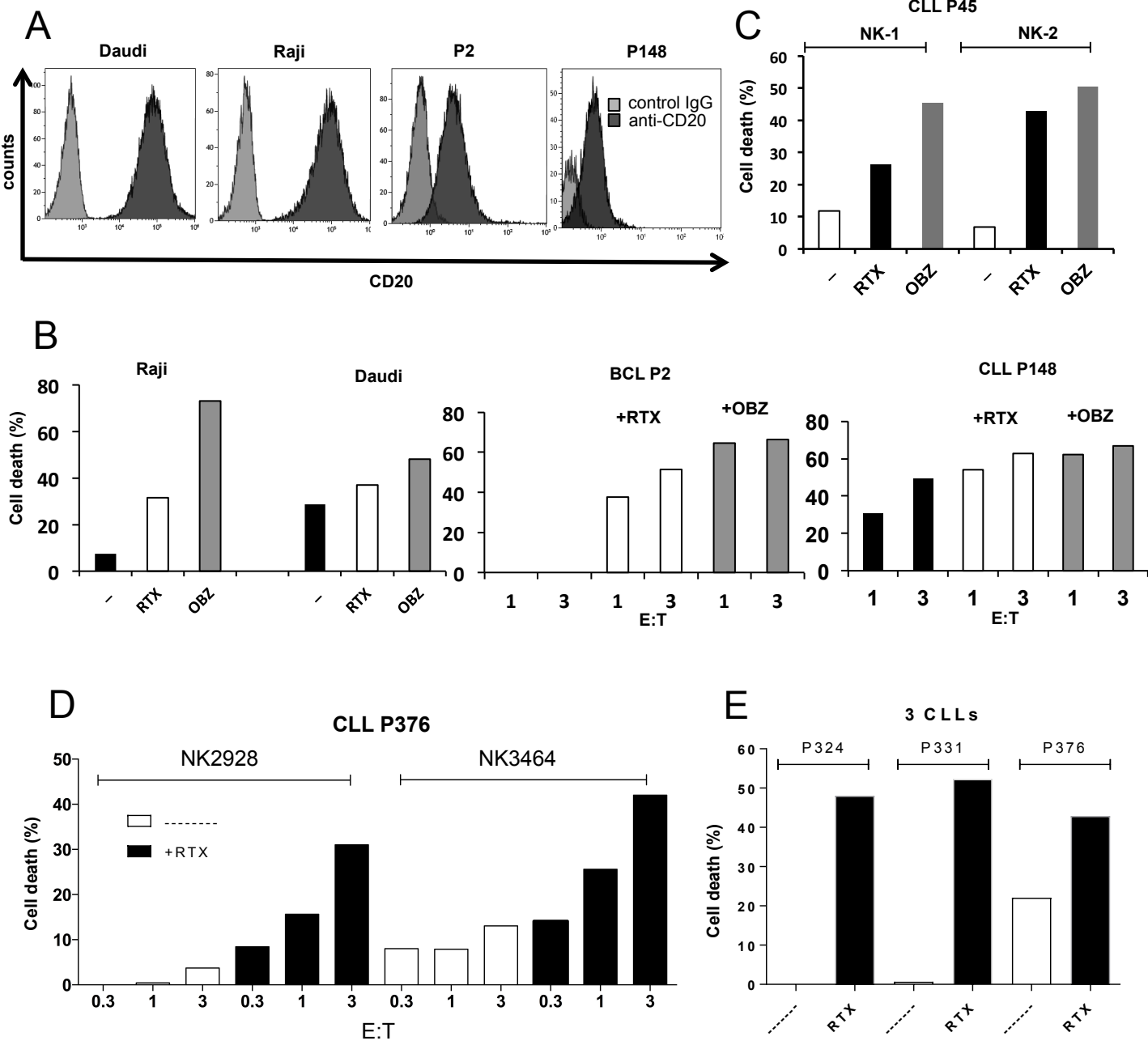
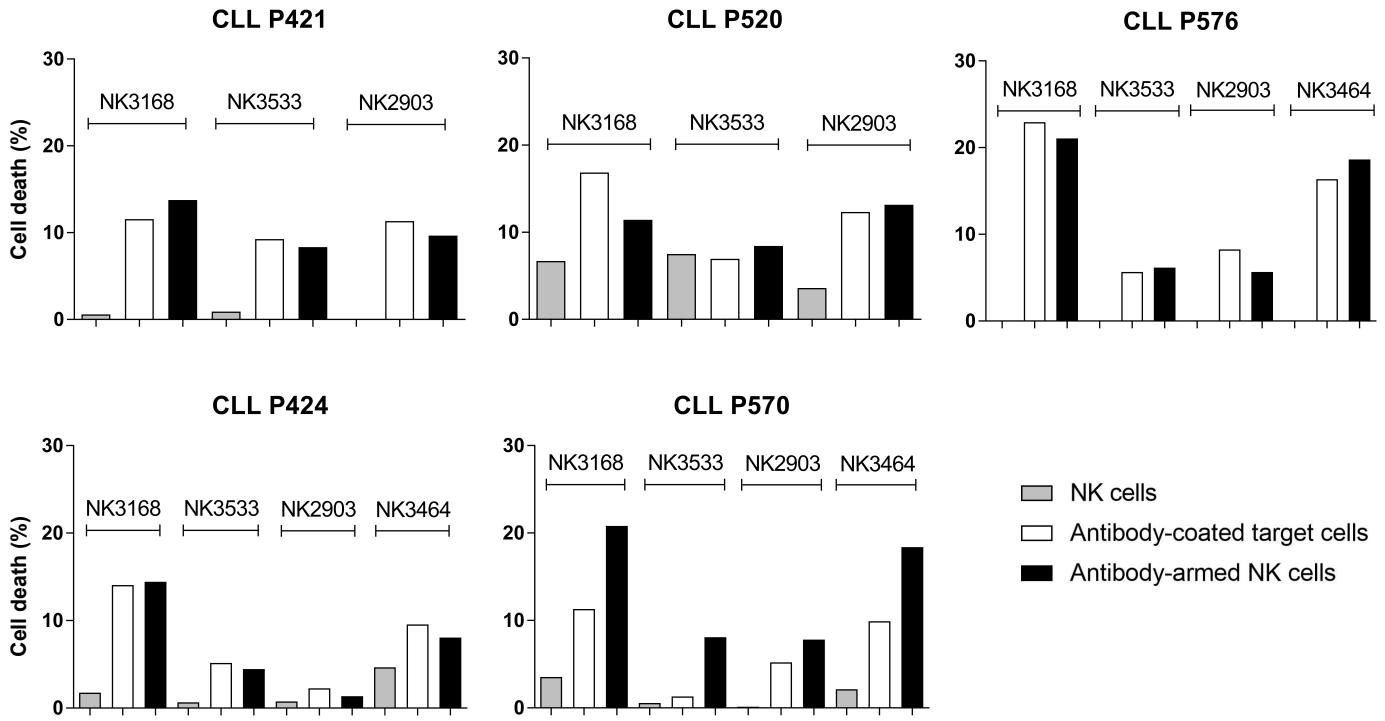


Supplemental Figure 1. Comparison of IL-2- and costimulation-drive expansions. A) Daudi cells were incubated overnight with costimulation- (circles) or IL-2-activated (triangles) NK cells from three different donors (2903,3464,2928), in presence (black symbols) or absence (white symbols) of RTX (10 μ g/ml), at different E:T ratios. Cell death was analyzed by 7-AAD staining. B) UCB NK cells were expanded for 10 days with IL-2 or with costimulation and the expression of the depicted markers was analyzed. The upper right graph showed CD16 expression after activation by costimulation and this expression after freezing/thawing the cells. The mean fluorescence intensity (MFI) depicted in the graphs correspond to the positive population. Significance was determined by paired t-test between both stimulations, * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$.

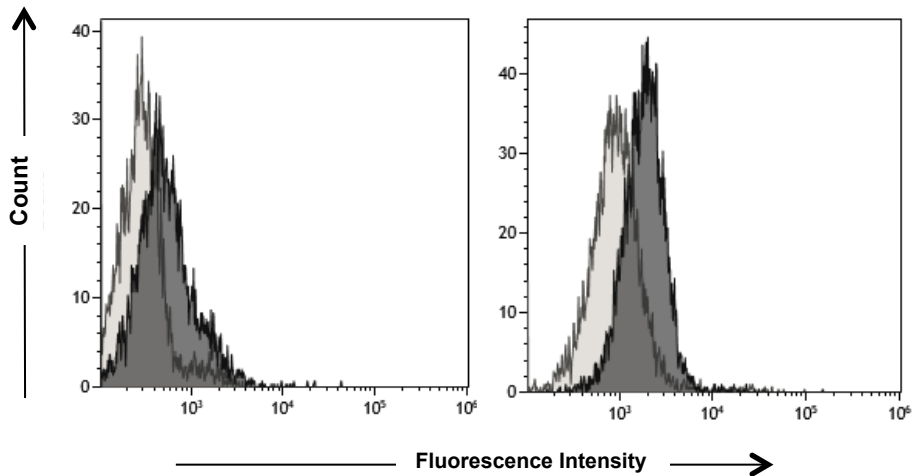


Supplemental Figure 2. e-NK mediate ADCC with anti-CD20 mAbs: improved effects with the afucosylated OBZ (GA101). A) Expression of CD20 antigen in the different cell lines and patient samples used in this figure. B) PBMCs from CLL patient 45 were incubated for 1h with 10 μ g/ml of the corresponding antibodies and overnight with e-NK from donors 3001 and 3002 at 3:1 E:T ratio. C) CD20 positive cell lines (Raji and Daudi) and two different patient samples (B-cell lymphoma (P2) and B-CLL (P148) were pre-incubated for 1h in presence or absence of RTX or OBZ antibodies (10 μ g/ml) and then cultured overnight with e-NK at 1:1 (Daudi and Raji) or 1:1 and 3:1 E:T ratio. Cell death was analyzed by 7AAD staining D) PBMCs from CLL patient 376 were incubated for 1h with 10 μ g/ml RTX (black bars) or not (white bars). e-NK from donors 2928 and 3464 were added overnight at different E:T ratio. Cell death was analyzed by 7-ADD staining. E) e-NK cells from donor 2903 were used in presence (black bars) or absence (white bars) of RTX at 3:1 E:T against CLL samples of three different patients (324, 331, 376).

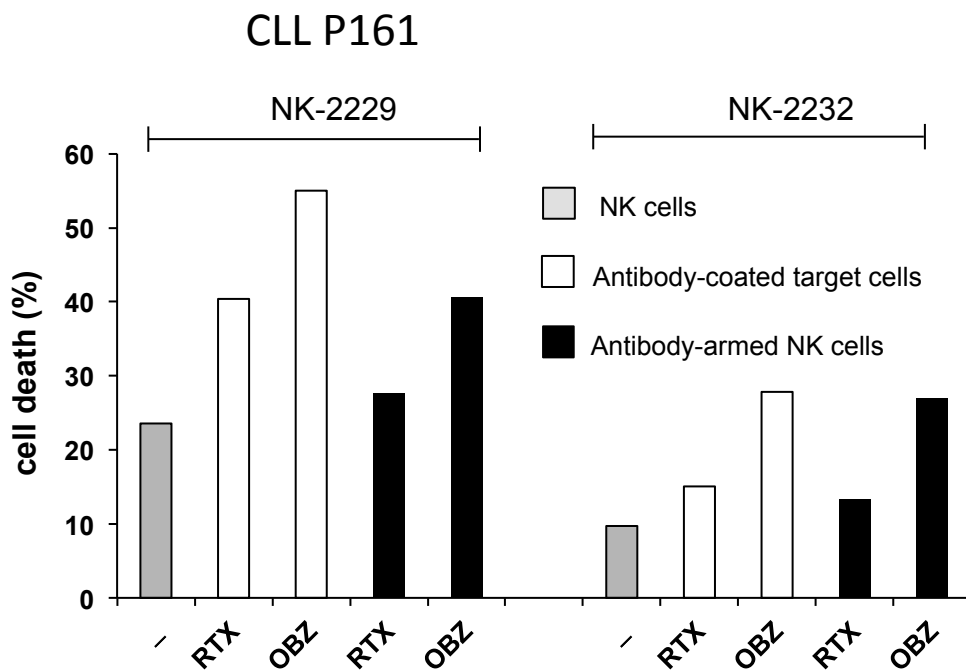
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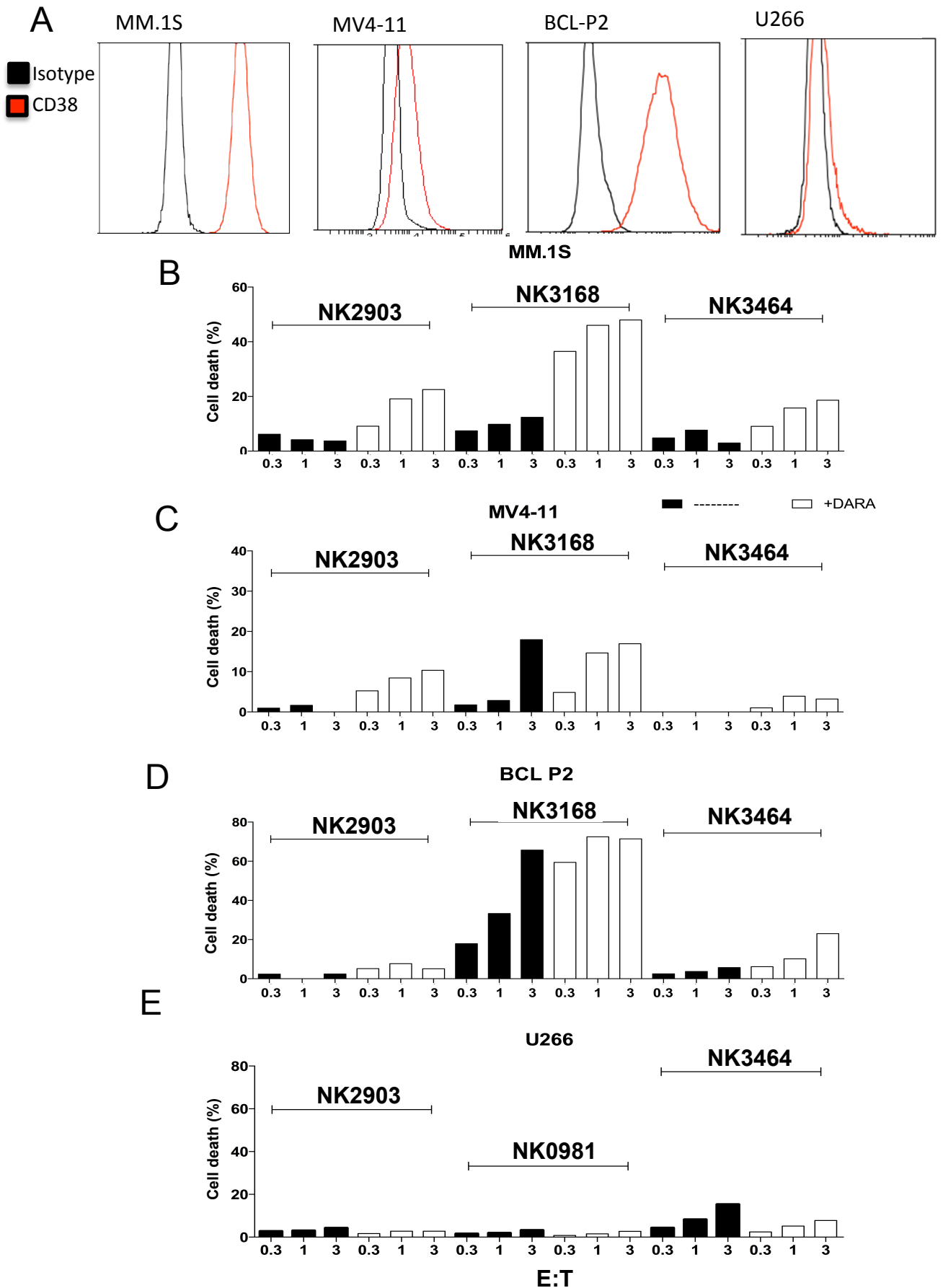
B



Supplemental Figure 3. Anti-CD20-armed e-NK show ADCC activity. A) PBMCs from 5 CLL samples were incubated for 1h with 10 μ g/ml of RTX and overnight with 3 or 4 donor e-NK cells at 3:1 E:T ratio (antibody-coated target cells condition). Alternatively, e-NK cells were incubated for 1 h with 10 μ g/ml of RTX before incubating them overnight with target cells (antibody-armed NK cell condition). Target B-CLL cell death was analyzed by 7-AAD staining. **B)** e-NK derived from 2 donors were incubated for 1 h with RTX, washed and incubated with a goat F(ab')₂ anti human IgG (H+L). As a control cells were only stained with the a goat F(ab')₂ anti human IgG (light grey).

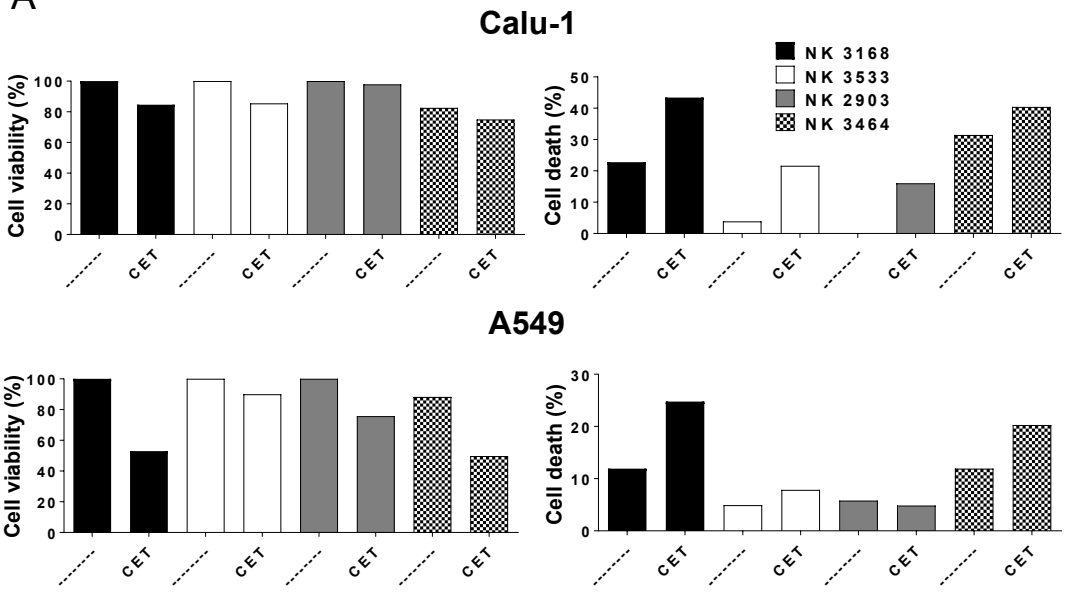


Supplemental Figure 4. Anti-CD20-armed e-NK cells enhance NK cell activity. PBMCs from a CLL patient (p161) were incubated for 1h with 10 μ g/ml of RTX (RIT) or OBZ (GA101) and overnight with 2 donor e-NK cells at 3:1 E:T ratio (antibody-coated condition). Alternatively, e-NK cells were incubated for 1 h with 10 μ g/ml of the anti-CD20 before incubating them overnight with target cells (antibody-armed condition). Cell death was analyzed by 7-AAD staining.

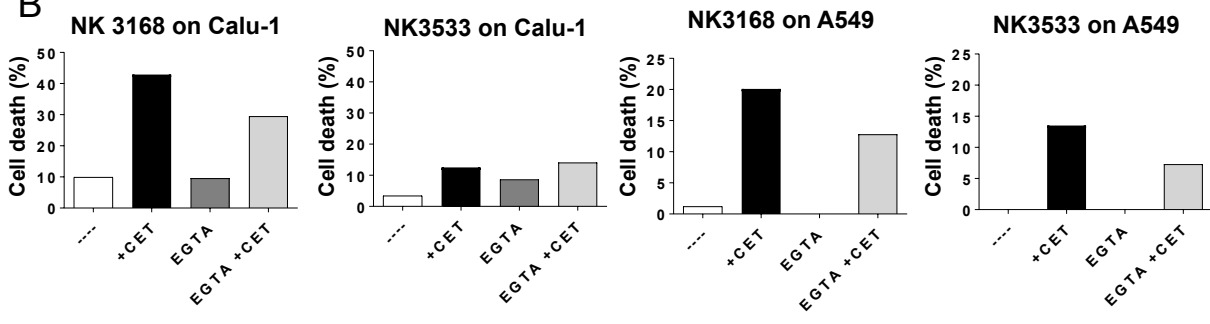


Supplemental Figure 5. e-NK produce ADCC with daratumumab. A) CD38 expression in different cell lines. B-D) Different e-NK cell productions from different donors were tested against the CD38⁺ cell lines MM.1S, MV4-11 and primary tumor cells from a BCL patient (P2), or against the CD38⁻ cell line U266. In the white bars target cells were pre-incubated for 1 h with 5 μ g/ml daratumumab. Cell death was analyzed by 7-AAD staining.

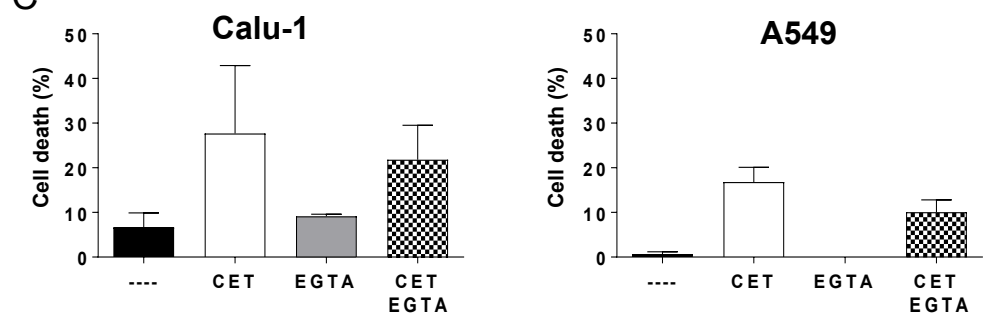
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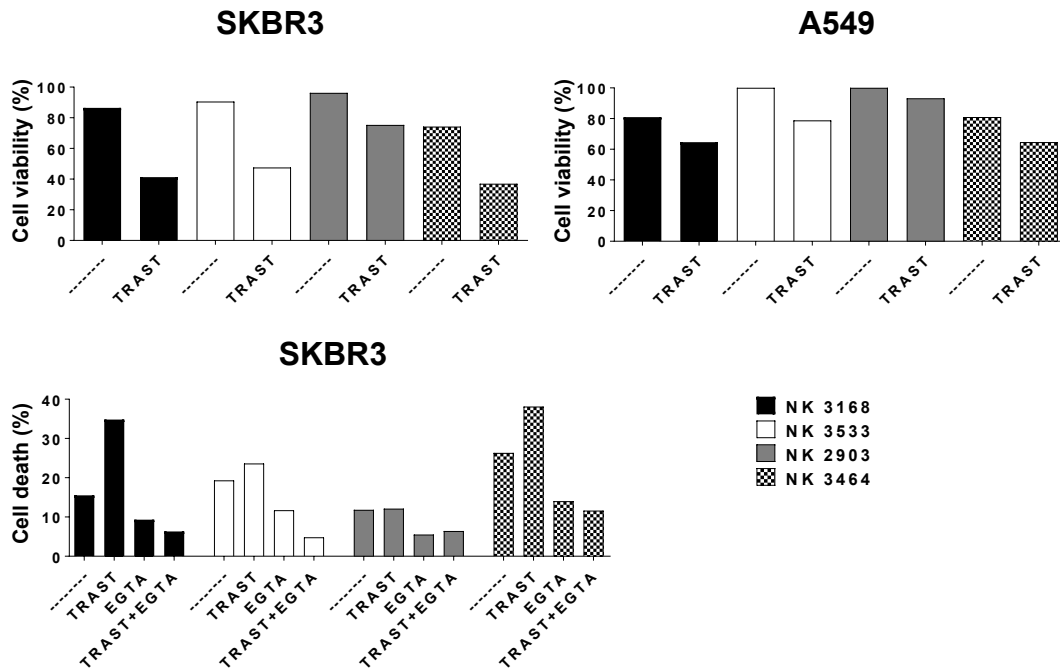


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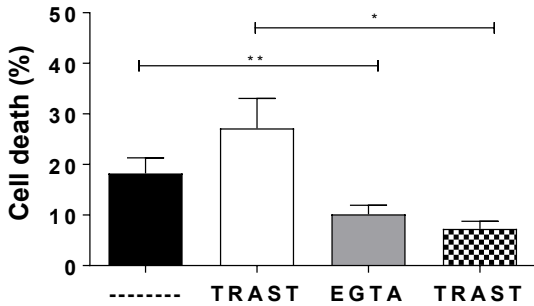


Supplementary Figure 6. e- NK perform ADCC with cetuximab and trastuzumab against EGFR and HER2 positive cell lines, respectively. A) Calu-1 and A549 cells were incubated with 5 µg/ml cetuximab for 1 h and overnight with e-NK cells from 4 different donors at 3:1 E:T ratio. Subsequently, we measured cell viability (MTT assay) and cell death (annexin-V FITC/propidium iodide (PI)). B) Cells were treated with 1 mM EGTA during the cytotoxic assay. C) Statistical analysis of 2 e-NK productions on CALU-1 (left) and A549 (right) cells after cetuximab and/or EGTA treatment.

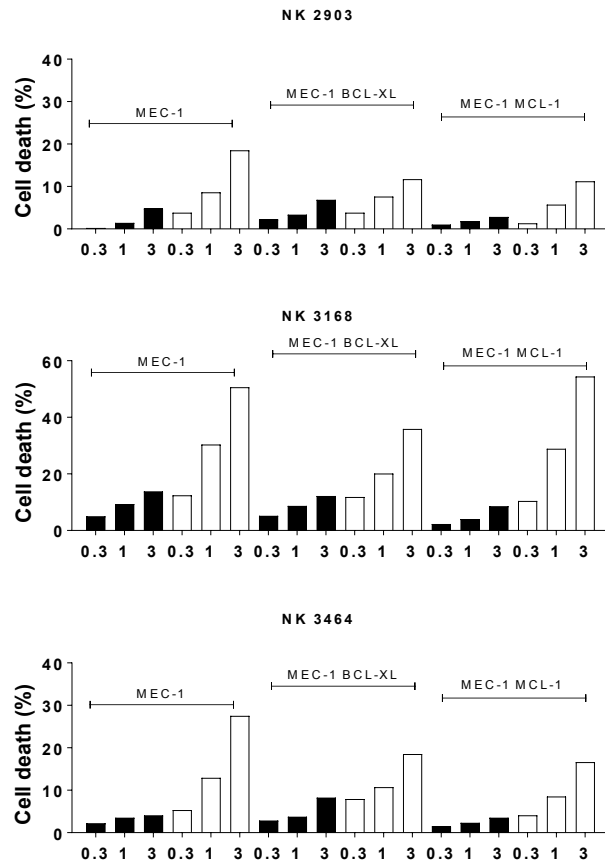
A



B



Supplementary Figure 7. e- NK perform ADCC with cetuximab and trastuzumab against EGFR and HER2 positive cell lines, respectively. A) Upper panels: SK-BR-3 and A549 cells were incubated with 5 μ g/ml trastuzumab for 1 h and overnight with e-NK cells from 4 different donors at 3:1 E:T ratio. Subsequently, we measured cell viability (MTT assay). Lower panel: SK-BR-3 cells were treated with 1 mM EGTA during the cytotoxic assay and cell death analyzed (annexin-V/7-AAD). B) Statistical analysis of paired t-test one-tailed e-NK productions on SK-BR-3 cells after trastuzumab and/or EGTA treatment.



Supplemental Figure 8. e-NK cell-induced ADCC overcome anti-apoptotic mechanisms of drug resistance. CD20⁺ MEC-1 cells overexpressing BCL-X_L and MCL1 were incubated in presence (white graphs) or absence (black graphs) of RTX (10 µg/ml). After 1 hour, e-NK cells from 3 different donors were added overnight at different E:T ratio. Cell death was analyzed by 7AAD/annexin-V labeling.