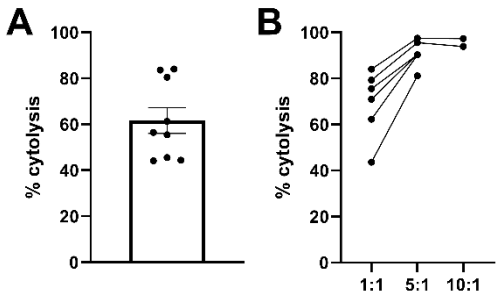
iScience, Volume *26*

Supplemental information

Early TRAIL-engagement elicits potent multimodal targeting of melanoma by CD34^{+} progenitor cell-derived NK cells

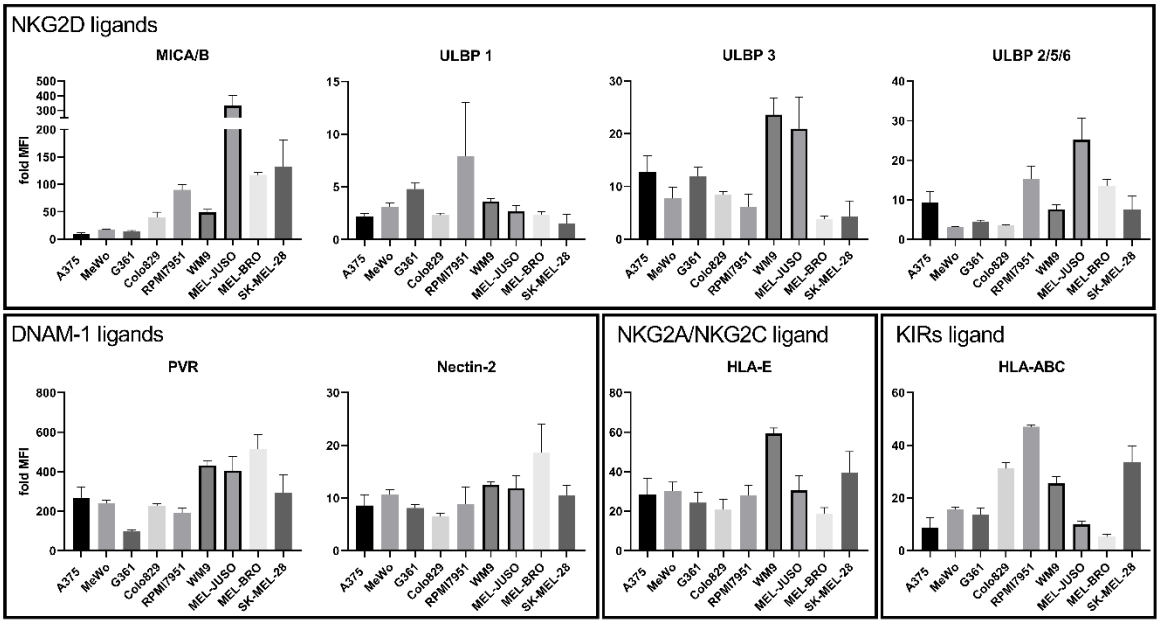
Amanda A. van Vliet, Ella Peters, Denise Vodegel, Daniëlle Steenmans, Monica Raimo, Susan Gibbs, Tanja D. de Gruijl, Adil D. Duru, Jan Spanholtz, and Anna-Maria Georgoudaki

SUPPLEMENTARY FIGURES

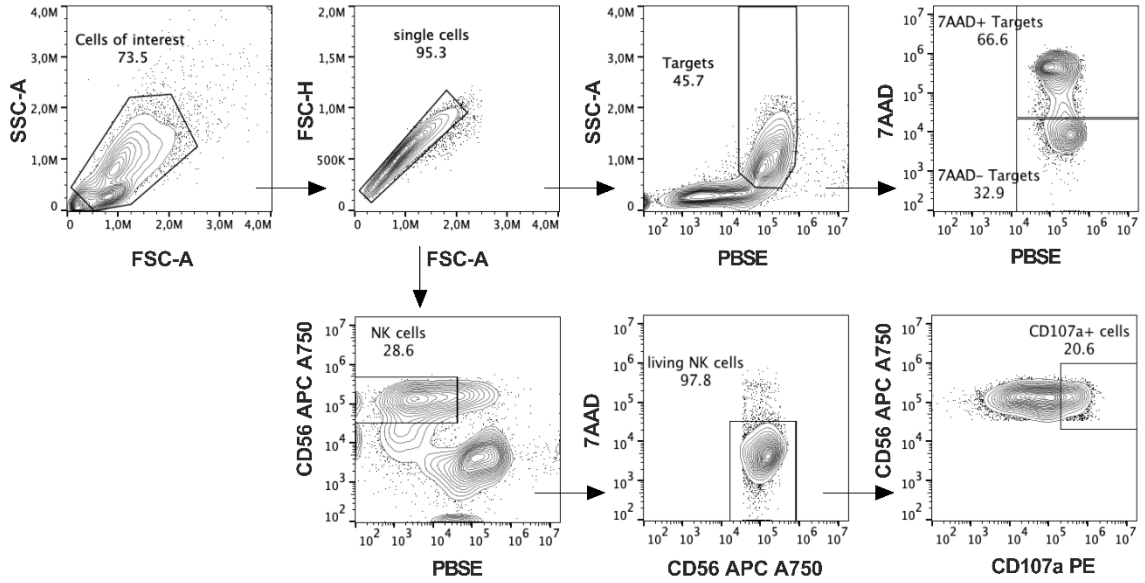


Supplementary figure 1. Flowcytometry-based analysis of NK cytotoxicity against K562 target cell line, related to figure 1. (**A**) The 9 selected UCB donors were co-cultured with K562 which were pre-stained with PBSE, for 20h in an E:T ratio of 1:1. Data are shown as mean ± SEM/ (**B**) 6 additional UCB donors were co-cultured with K562 which were pre-stained with PBSE, for 20h in an E:T ratio of 1:1, 5:1 and 10:1. Percentage of cytolysis was determined as follows: %cytolysis = 100-((count living K562 in co-culture/ count living K562 cells in ctrl)*100).

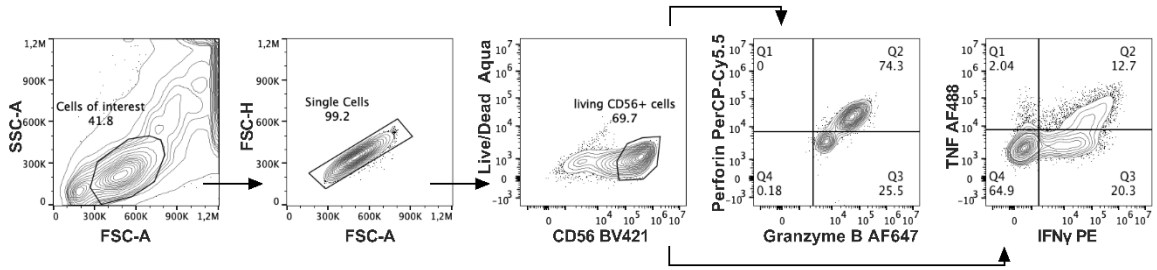
cell line	tissue of origin
A375	primary melanoma
MeWo	lymph node metastasis
G361	primary melanoma
Colo829	primary melanoma
RPMI7951	lymph node metastasis
WM9	lymph node metastasis
MEL-JUSO	primary melanoma
MEL-BRO	primary melanoma
SK-MEL-28	primary melanoma



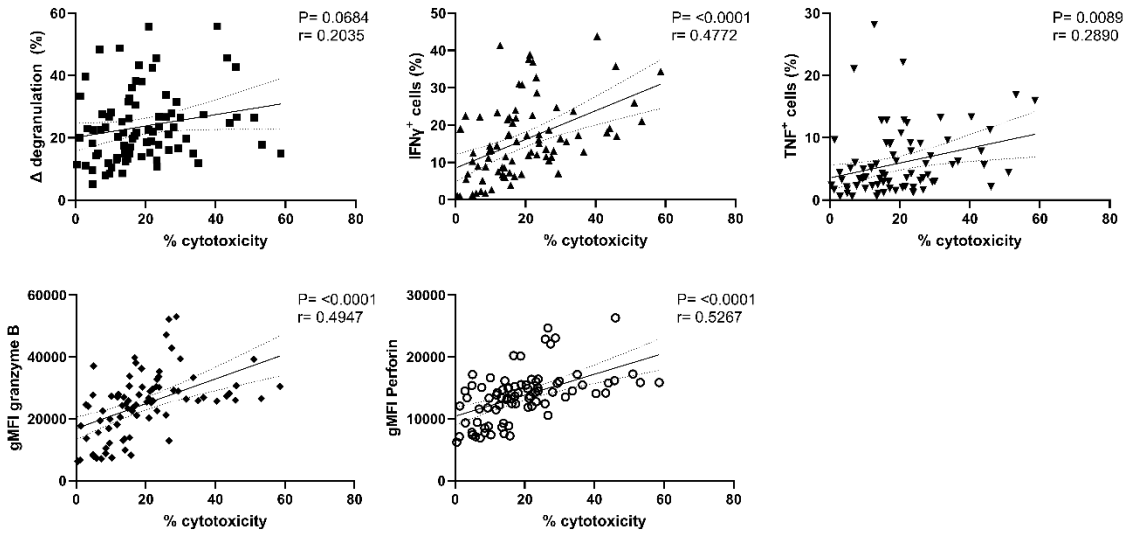
Supplementary figure 2. Tissue of origin and surface ligand expression of melanoma cell lines, related to figure 1. The following antibodies were used: anti-MICA/B PE (6D4), anti-HLA-ABC BV510 (W6/32) from Biolegend, anti-ULBP- 2,5,6 PE (165903), anti-ULBP-1 PE (170818), anti-ULBP-3 PE (166510) from R&D systems and anti-nectin-2 PE (R2.525), anti-PVR PE (REA519), anti-HLA-E APC (3D12) from Miltenyi. All antibodies were used at a dilution of 1:11. Relative expression was calculated as geometric MFI (gMFI) of the stained samples divided by the gMFI of the unstained sample (n=3 from independent experiments).



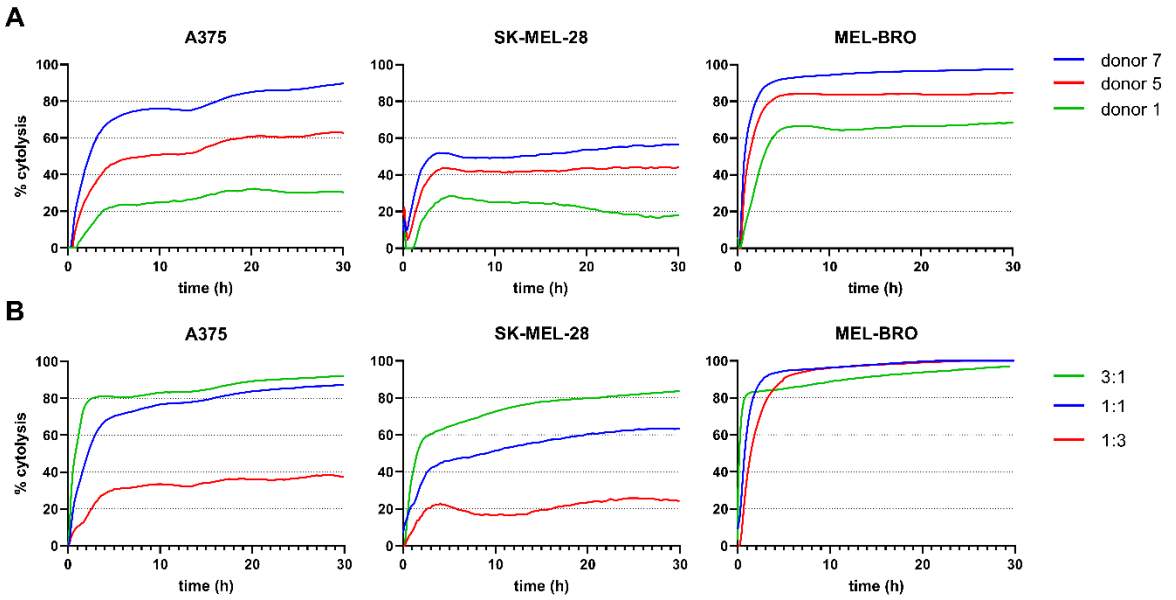
Supplementary figure 3. Gating strategy for cytotoxicity and degranulation, related to figure 1. Debris was excluded in the FSC/SSC gate followed by gating on single cells using FSC-A/FSC-H. From this gate, target cells were gated by PBSE+ and NK cells were gated by CD56+/PBSE-. Viability of targets was determined by $PBSE^+$ $7AAD^-$ or $7AAD^+$ cells using target only condition as control. Degranulation of NK cells was determined by gating first on living NK cells using $7AAD^-/CD56^+$ gating followed by $CD56^+/CD107a^+$ gating using NK only condition as control.



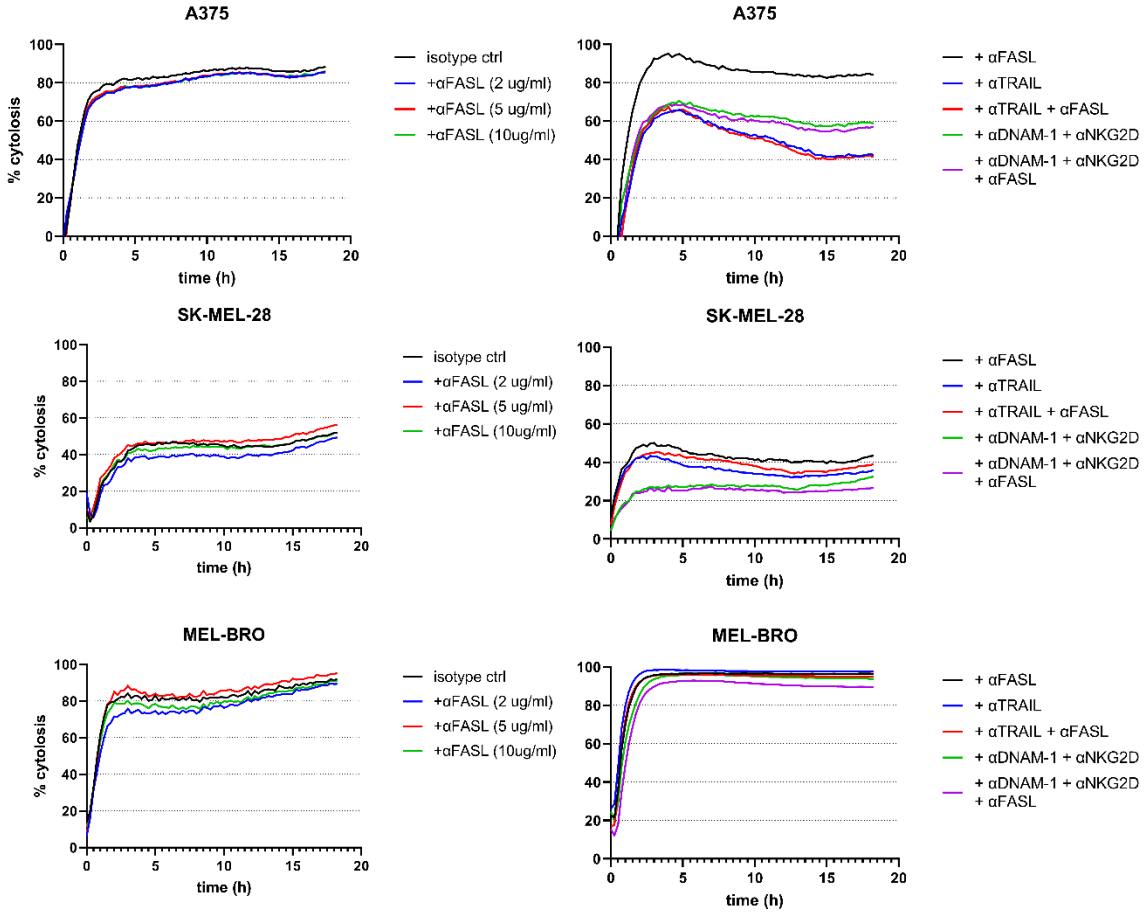
Supplementary figure 4. Gating strategy for intracellular levels of granzyme B, perforin, TNF and IFNγ, related to figure 3. Cells of interest were gated in the FSC/SSC plot followed by gating on single cells using FSC-A/FSC-H. NK cells were selected based on $CD56^+$ and live/$dead^-$ gating. From the living NK cells, perforin/granzyme B or TNF/IFNγ levels could be determined. Gates of perforin/Granzyme B and IFNγ and TNF are based on isotype control.



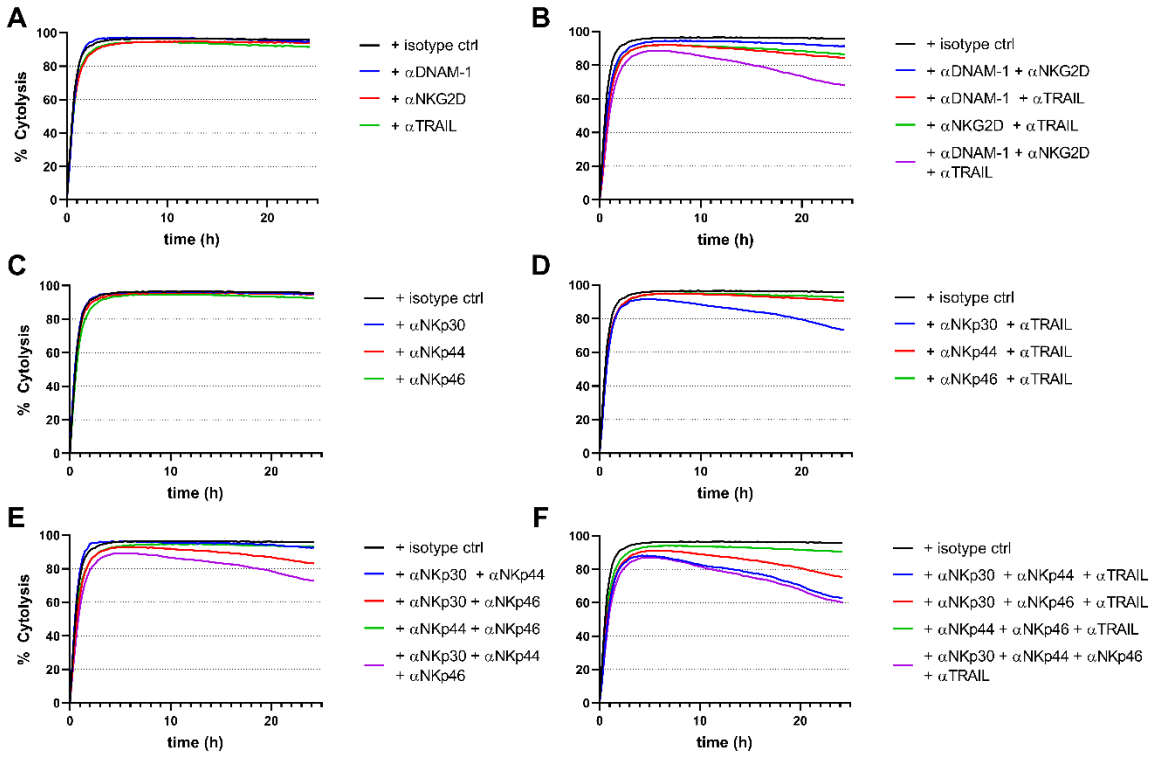
Supplementary figure 5. Correlation analysis of cytotoxicity vs. percentage of degranulation, IFNγ$^+$ cells, TNF$^+$ cells or of granzyme B or perforin levels (gMFI), related to figure 3. Data of all 9 cell lines and all 9 UCB donors are combined which results in n=81. Pearson r value and p value are depicted in the graphs.



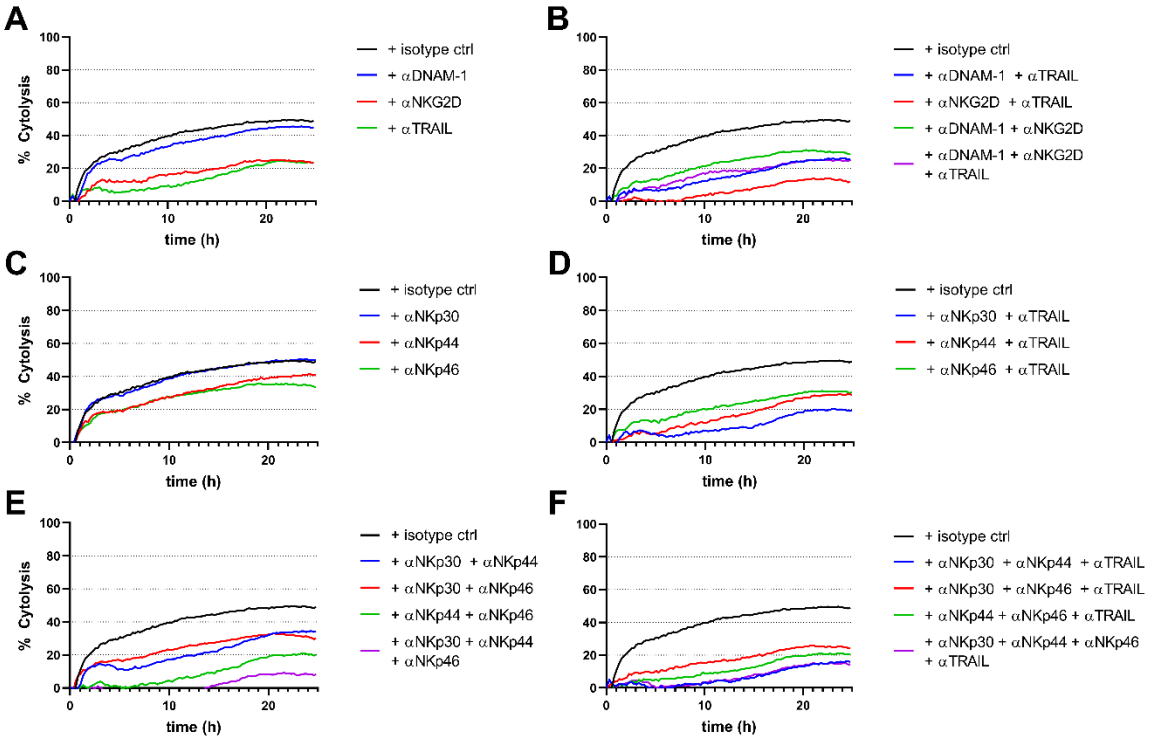
Supplementary figure 6. Cytolysis graph based on impedance, related to figure 5. (**A**) UCB donors #1, #5 and #7 were co-cultured with A375, SK-MEL-28 or MEL-BRO for 30h in a 1:1 E:T ratio. (**B**) UCB donor #8 was seeded at 3 different E:T ratios with A375, SK-MEL-28 or MEL-BRO for 30h.



Supplementary figure 7. Effect on cytolysis of A375, SK-MEL-28 and MELBRO upon blocking FASL or combinations with FASL and TRAIL/NKG2D/DNAM-1, related to figure 5. NK cells were pre-incubated with different combinations of blocking mAbs and seeded in co-culture with A375, SK-MEL-28 or MEL-BRO in a 1:1 E:T ratio for 24h.



Supplementary figure 8. Effect on cytolysis of MEL-BRO upon blocking various receptors, related to figure 5. NK cells were pre-incubated with different combinations of blocking mAbs and seeded in co-culture with MEL-BRO in a 1:1 E:T ratio for 24h. Representative graphs of MEL-BRO cytolysis in co-culture with donor #7 are shown representing the mean of technical triplicates after blocking (**A**) DNAM-1, NKG2D, or TRAIL, (**B**) combination of DNAM-1, NKG2D and TRAIL, (**C**) NKp30, NKp44 or NKp46, (**D**) combination of NKp30, NKp44 or NKp46 with TRAIL, (**E**) combination of NKp30, NKp44 and NKp46, (**F**) combination of two out of NKp30, NKp44 or NKp46 with TRAIL or all four combined. All blocking mAbs were used at 10 µg/ml. Concentration of isotype ctrl Ab is matched to the concentration of four added blocking mAbs.



Supplementary figure 9. Effect on cytolysis of SK-MEL-28 after blocking various receptors, related to figure 5. NK cells were pre-incubated with different combinations of blocking mAbs and seeded in co-culture with SK-MEL-28 in a 1:1 E:T ratio for 24h. Representative graphs of MEL-BRO cytolysis in co-culture with donor #7 are shown representing the mean of technical triplicates after blocking (**A**) DNAM-1, NKG2D, or TRAIL, (**B**) combination of DNAM-1, NKG2D and TRAIL, (**C**) NKp30, NKp44 or NKp46, (**D**) combination of NKp30, NKp44 or NKp46 with TRAIL, (**E**) combination of NKp30, NKp44 and NKp46, (**F**) combination of two out of NKp30, NKp44 or NKp46 with TRAIL or all four combined. All blocking mAbs were used at 10 µg/ml. Concentration of isotype ctrl Ab is matched to the concentration of four added blocking mAbs.