T-cell receptor engineering of NK-cells to therapeutically target tumours and tumour immune evasion.

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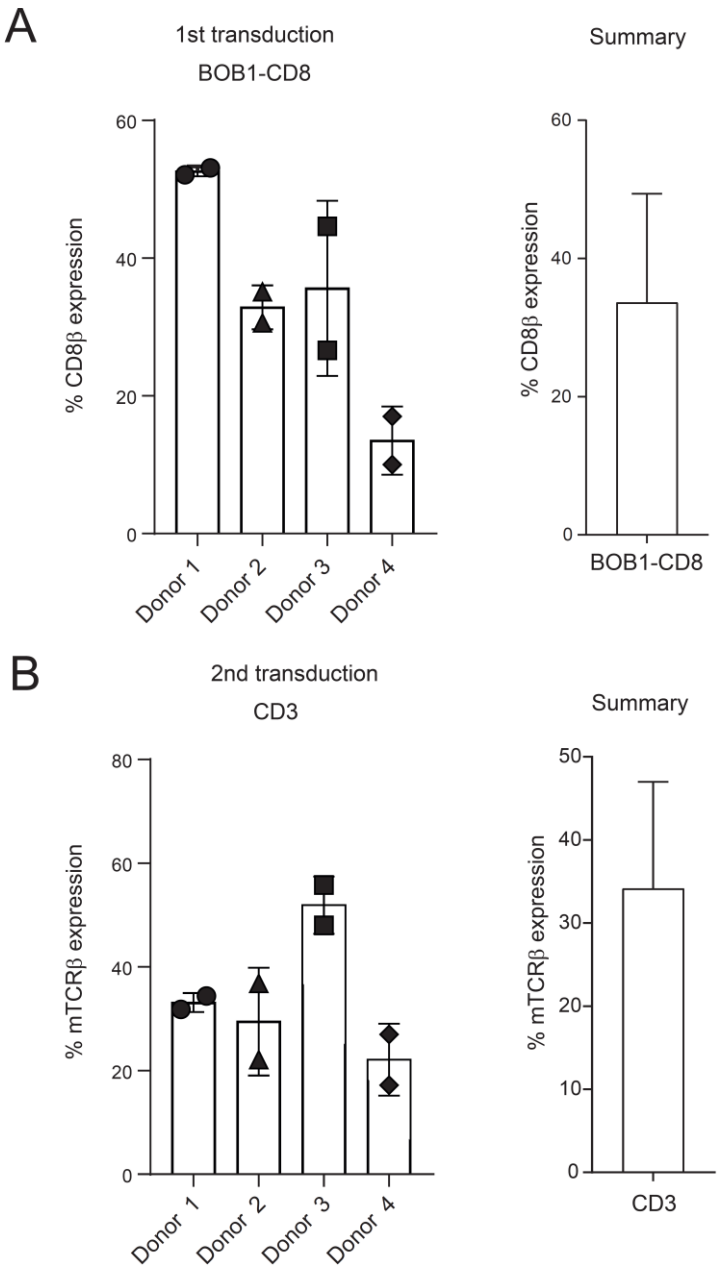
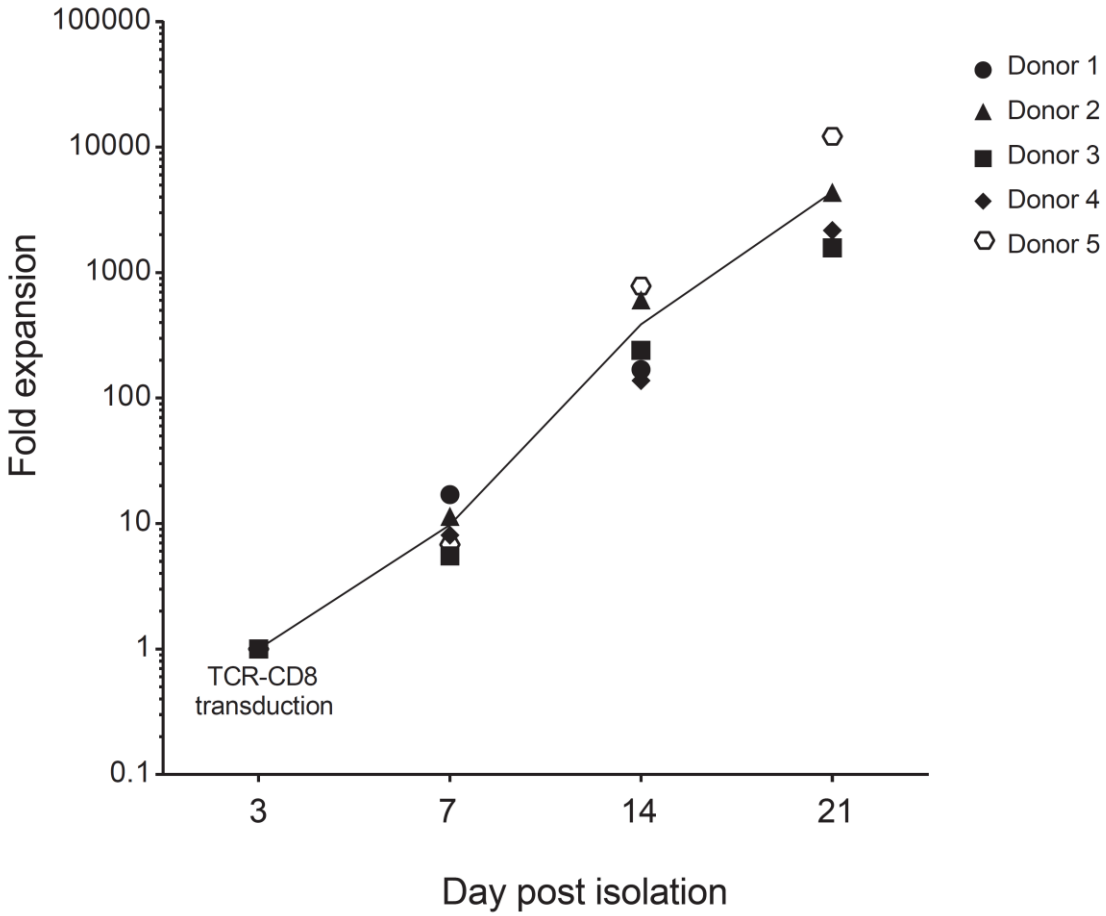
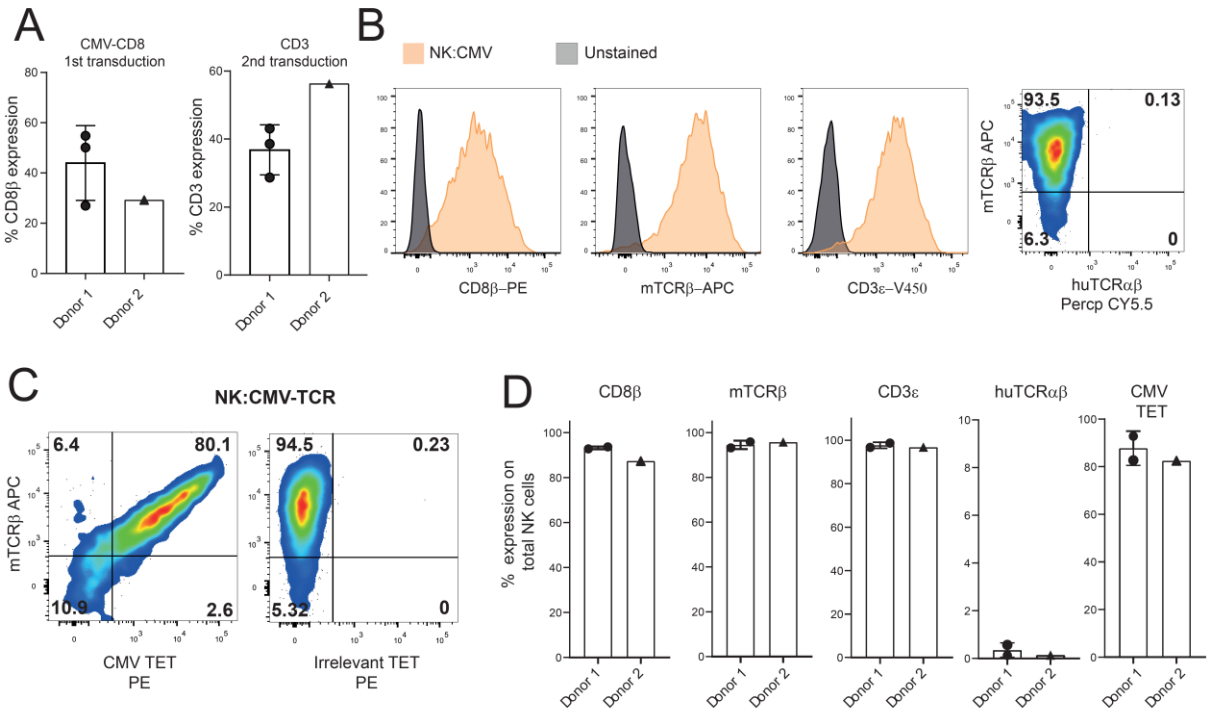
Supplemental data



Supplemental figure S1 Transduction efficiencies of NK-cells.
NK-cells were retrovirally transduced to express the murinised BOB1-TCR following a 2-step transduction protocol. 1st step was transduction with TCR-CD8 A) TCR-CD8 transduction efficiencies analysed on Day 7 by FACS for expression of CD8β. 2nd transduction was with the 4 invariant chains of CD3εδγζ signaling complex B) CD3 transduction efficiencies analyzed on Day 14 by FACS for expression of mTCRβ. Each symbol represents an individual donor and error bars depict mead and SD.

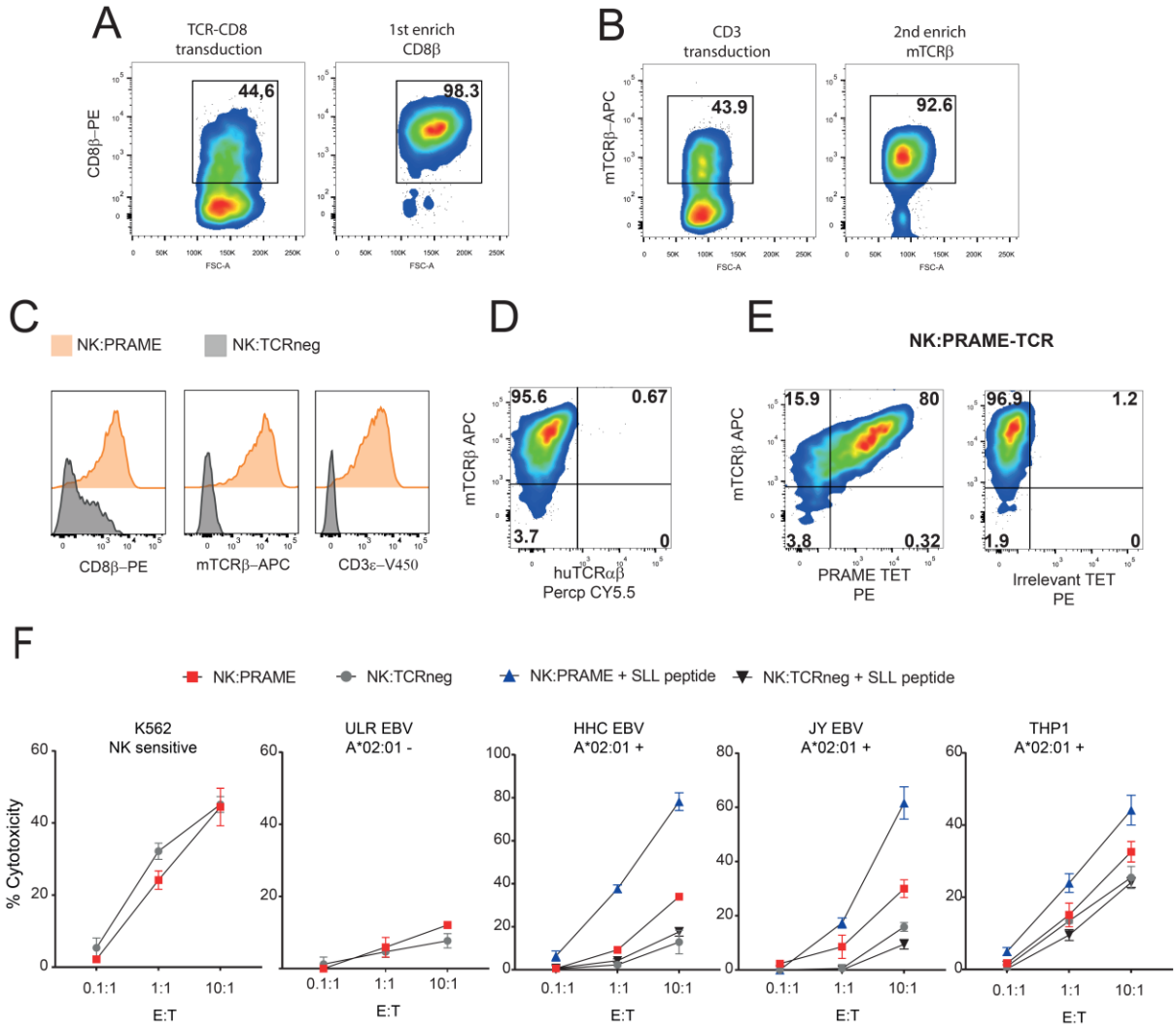


Supplemental figure S2 Expansion kinetics of NK:BOB1 cell products.
NK-cells were transduced on Day 3 with TCR-CD8 gene constructs encoding the BOB1-specific TCR. At each time point cell number was determined by counting total viable cells present in culture. Each symbol represents a NK:BOB1 cell product from different donors and the solid line represents the mean expansion.



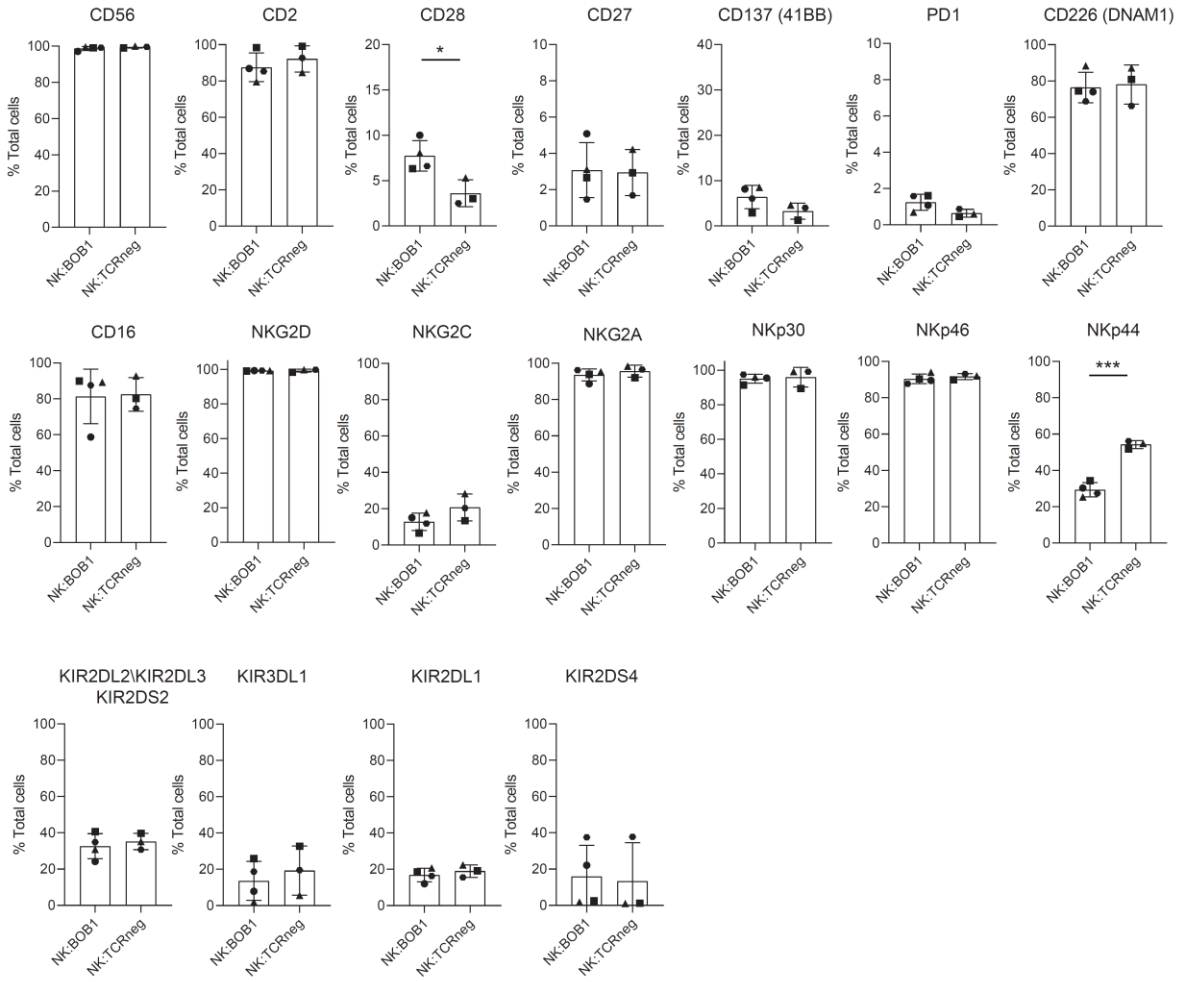
Supplemental figure S3 Expression of CMV-specific TCR in NK-cells following two-step transduction protocol.

NK-cells were transduced on Day 3 with TCR-CD8 encoding the HLA-A*02:01 restricted, CMV-specifc TCR and with the 4 invariant chains of CD3 on Day 9. A) Transduction efficiencies of the 1st TCR-CD8 transduction and the 2nd CD3 transduction. B) Representative histograms of CD8β, mTCRβ, CD3ε and human TCRαβ expression in a final NK-TCR cell product expressing CMV-specific TCR (NK:CMV) on Day 21. Representative FACS plot of NK:CMV stained for mTCRβ and human(hu) TCRαβ (T-cells) expression. C) Representative FACS plot of NK:CMV stained with CMV-specific or an irrelevant pMHC tetramer on day 21 post isolation . D) Summary of expression frequencies of CD8β, mTCRβ, CD3ε, huTCRαβ expression and CMV-specific tetramer binding frequencies on Day 21 of NK:CMV cell products. Symbols represent a different donor and error bars depict mean and standard deviation.

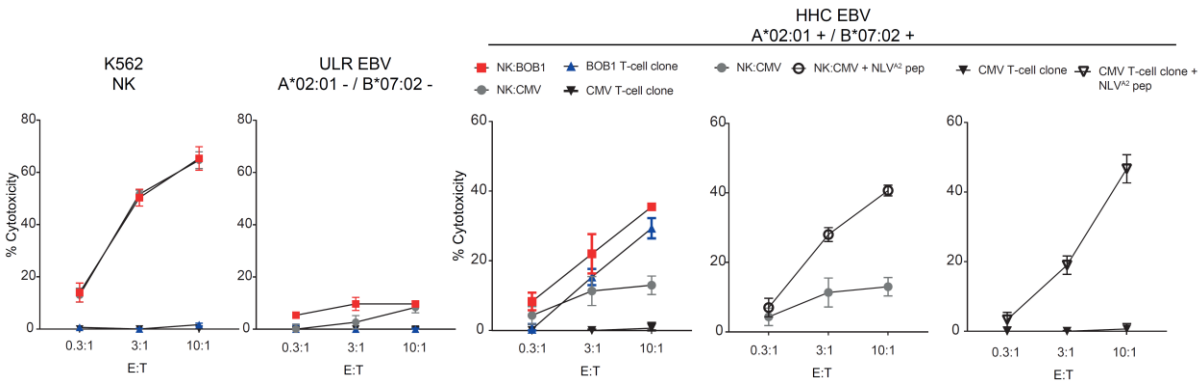


Supplemental figure S4 Expression of PRAME-specific TCR on NK-cells permits PRAME-specific cytotoxic responses.

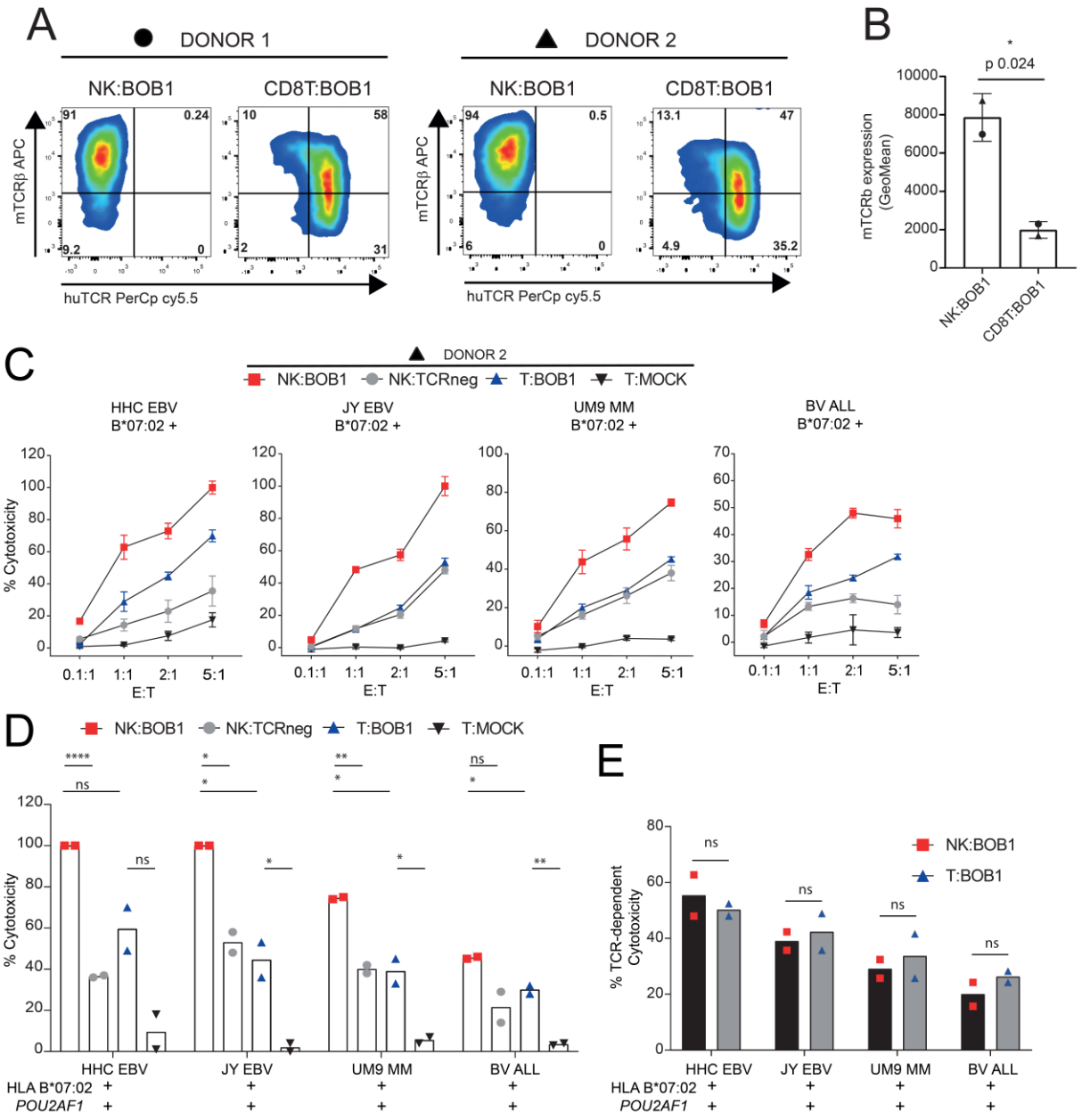
NK-cells derived from donor 3 were transduced to express PRAME-specific TCR targeting targeting preferentially-expressed-antigen In melanoma (PRAME) restricted to peptide SLLQHLIGL presented in HLA-A*02:01 (NK:PRAME). A) Transduction and enrichment efficiencies and of PRAME-CD8 and B) CD3 viral constructs measured by FACS by CD8β expression or CD3ε expression respectively on total NK-cells. C) Histograms of CD8β, murine TCRβ (mTCRβ) and CD3ε expression on NK:PRAME final cell product. D) Dotplot of human TCRαβ (T-cells) verses mTCRβ (transduced) expression on NK:PRAME final cell product E) FACS analysis for PRAME-specific\Irrelevant tetramer binding of NK:PRAME. F) Cytotoxicity data of NK:PRAME and MOCK transduced NK cells against K562 (NK sensitive) and low PRAME-expressing target cell lines. HLA-A*02:01+ cell lines were loaded with 1µM SLLQHLIGL peptide. Error bars represent mean and SD of technical triplicates



Supplemental figure S5 Phenotype of NK-TCR cell products on Day 21.
NK-cells were transduced to express the BOB1-specific TCR (NK:BOB1) and in parallel NK-cells not expressing TCR (NK:TCRneg) were similarly expanded without transduction. On day 21, without further stimulation, NK-cells were harvested from culture and stained with monoclonal antibodies targeting NK activation and inhibitory receptors and co-stimulation molecules and subsequenctly analysed by FACS. NK-cells were 1st gated on CD56 expression and positive gates were determined using PBMCs. Each symbol represents a different donor and error bars depict mean and SD. Statistical test used was unpaired T-test.

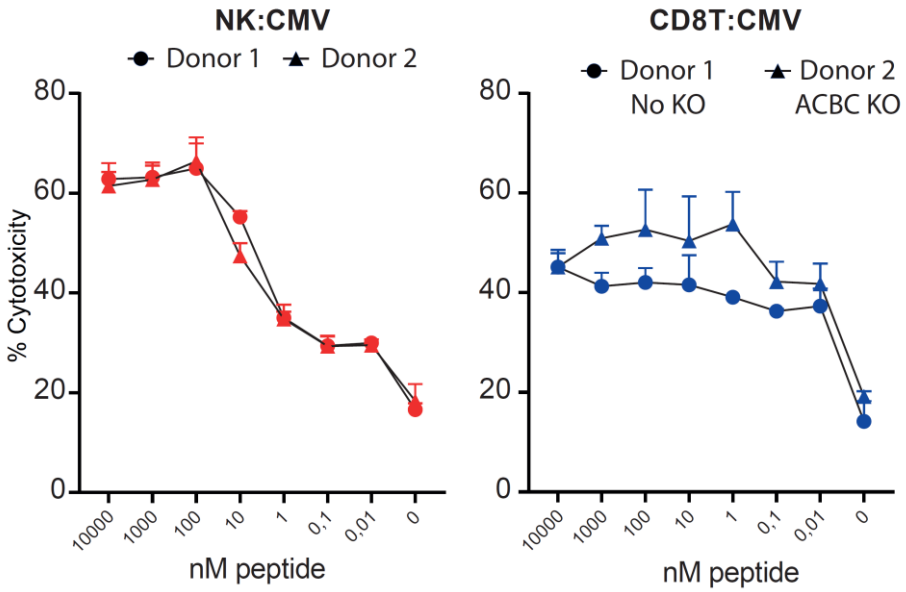


Supplemental figure S6 Antigen-specific cytotoxicity of NK:CMV cell products.
NK-cells were transduced to express the HLA-A*02:01 restricted CMV-specific TCR (NK:CMV). Cytotoxicity data of NK:CMV, NK:BOB1(as a control), CMV-specific T-cell clone and BOB-specific T-cell clone against K562 (NK sensitive), HLA-A*02:01- EBV-LCL and HLA-A*02:01 + EBV-LCL cell lines were loaded with 1µM NLVPMVATV CMV-derived peptide. Error bars represent mean and SD of technical triplicates

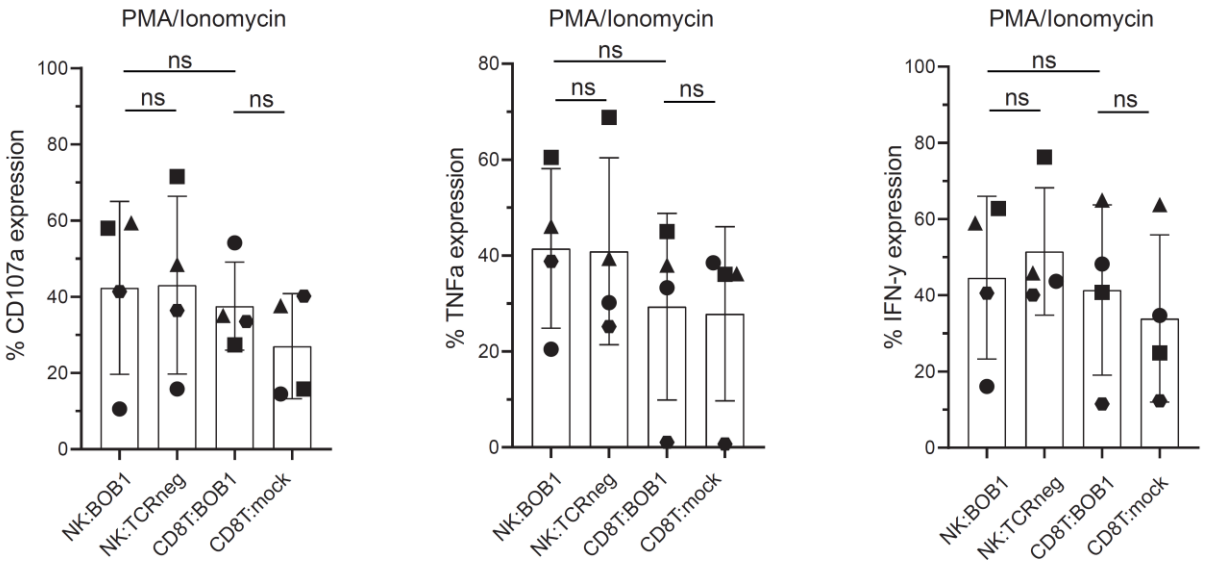


Supplemental figure S7 NK:BOB1 demonstrates increased antigen-specific cytotoxicity compared to CD8 T-cells expressing BOB1-TCR.

CD8 T-cells, without endogenous TCRαβ Knock out, and NK cells were isolated from the same healthy donor and transduced to express BOB1-specific TCR (CD8T:BOB1 and NK:BOB1). As negative control TCR negative NK-cells (NK:TCRneg) or CMV-specific TCR transduced CD8 T-cells (T-MOCK) were used. A) FACS analysis depicting human TCR (huTCR) expression (endogenous TCR) and murine TCR (mTCR) expression (tgTCR) from donor 1 and 2. B) Geometric Mean of mTCR expression in NK:BOB1 and CD8T:BOB1. Error bars represent SD. C) Representative data from donor 2 of BOB1-specific NK and CD8 T cell cytotoxicity at multiple effector:target (E:T) ratios. Error bars represent mean and standard error of technical triplicates. D) Combined Cytotoxicity data at 5:1 E:T ratio by NK and CD8 T cells derived from donor 1 and donor 2. E) % TCR-dependent killing calculated as the difference in killing at 5:1 E:T ratio between BOB1-TCR and negative controls. Combined data acquired from donor 1 and donor 2. *POU2AF1* encodes the BOB1 protein and expression was predetermined by qPCR. Statistical test used was unpaired T-test corrected for multiple comparisons using the Holm-Sidak method.

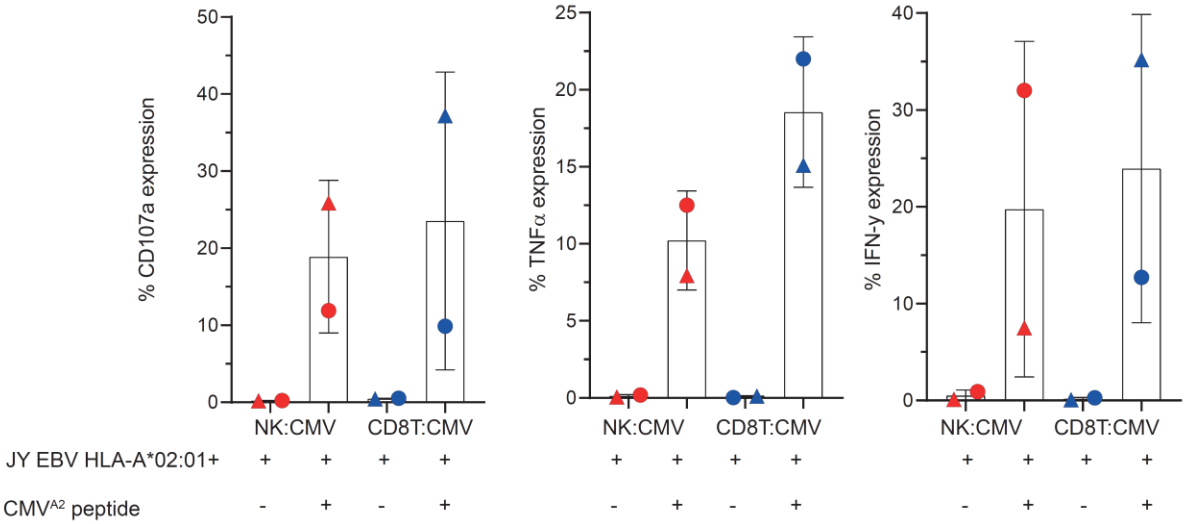


Supplemental figure S8 NK:CMV and CD8T:CMV cytotoxicity against exogenously loaded antigen. CD8 T-cells and NK cells were isolated from the same healthy donor and transduced to express HLA-A*02:01 restricted, CMV-specific TCR (NK:CMV) and (CD8T:CMV). For CD8T:CMV, donor 1 expressed its endogenous TCRαβ (NO KO) whereas donor 2 had been CRISPR/Cas9 edited to remove endogenous TCRαβ expression (ACBC KO). HLA-A*02:01+ EBV-LCL was exogenously loaded with NLVPMVATV CMV-derived peptide at different concentrations and subsequently co-cultured with effector cells at a 10:1 E:T ratio for 6 hours Cr release assay.



Supplemental figure S9 PMA/Ionomycin stimulation elicits cytokine production and degranulation from cell products.

CD8T expressing BOB1-TCR or CMV-TCR (D10 post stimulation) and NK-cells expressing BOB1-TCR or TCR negative NK-cells (Day 7 post stimulation) were stimulated overnight with PMA/Ionomycin in the presence of Brefeldin-A and anti-CD107a. After 12-14 hours incubation, cells were stained for inflammatory cytokines TNFα and IFN-γ and assessed by FACS. Depicted is the combined data of cell products derived from different donors for A) CD107a, B)TNFα and C)IFN-γ expression. Live NK-cells were previously gated on CD56 expression and Live CD8T were gated on CD8 expression. Positive cells were gated according to unstimulated. Each symbol represents a different donor and error bars represent mean and SD of biological replicates. Statistical test used was unpaired T-test.



Supplemental figure S10 NK:CMV induces antigen-specific cytokine production and degranulation from cell products.

CD8T expressing HLA-A*02:01 restricted CMV-TCR (D10 post stimulation) and NK-cells expressing CMV-TCR (Day 7 post stimulation) were stimulated overnight with HLA-A*02:01+ EBV-LCL in the presence of Brefeldin-A and anti-CD107a. EBV-LCL was loaded with and without 1µM NLVPMVATV CMV-derived peptide. After 12-14 hours incubation, cells were stained for inflammatory cytokines TNFα and IFN-γ and assessed by FACS. Depicted is the combined data of cell products derived from different donors for A) CD107a, B)TNFα and C)IFN-γ expression. Live NK-cells were previously gated on CD56 expression and Live CD8T were gated on CD8 expression. Positive cells were gated according to unstimulated. Each symbol represents a different donor and error bars represent mean and SD of biological replicates..