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Supplemental information

Expanded human NK cells armed with CAR uncouple potent anti-tumor activity from off-tumor toxicity against solid tumors

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Figure S1

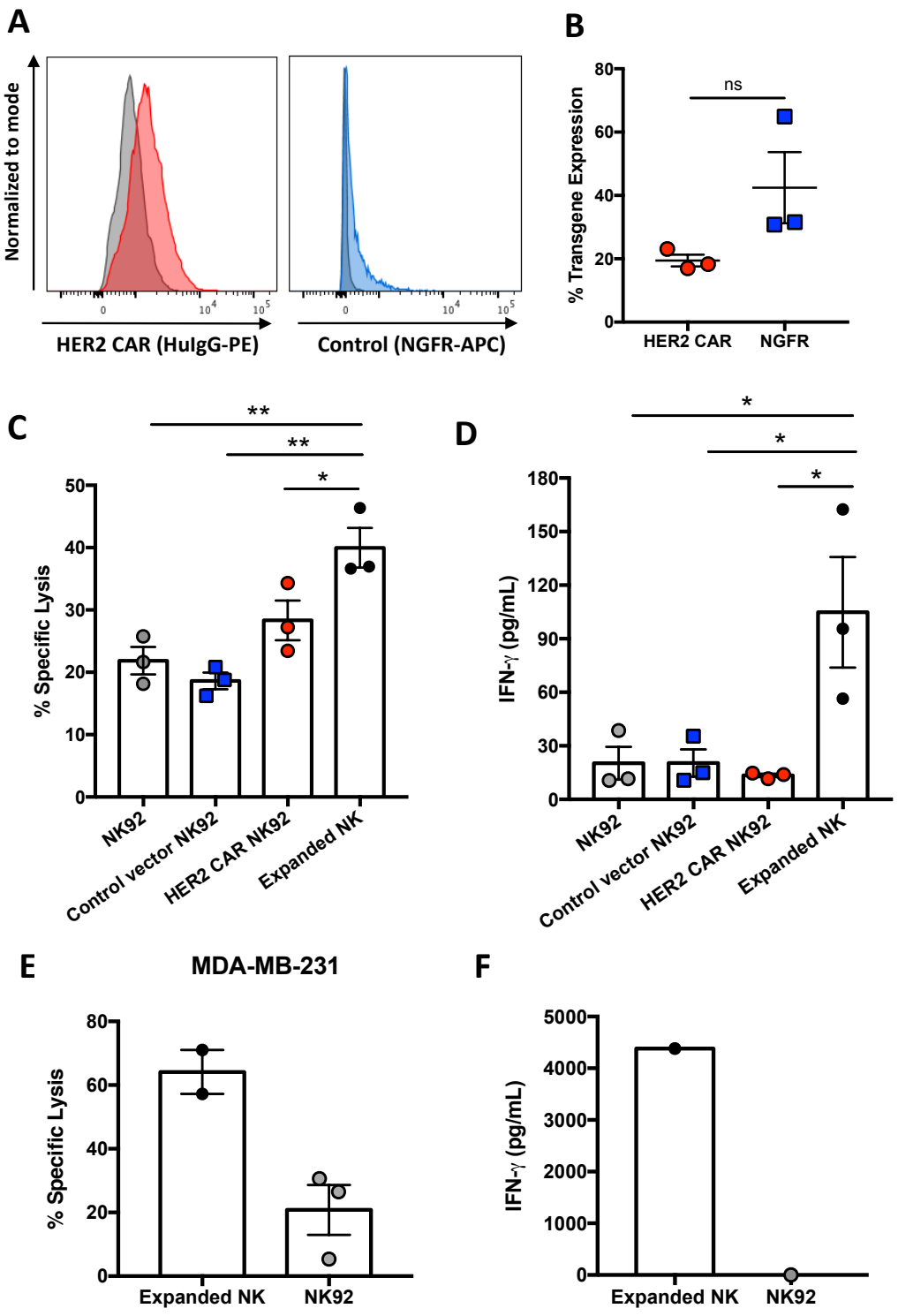

Figure S1. Unmodified expanded NK cells have higher anti-tumor function than the human NK-92 cell line, Related to Figure 1. (A) Representative histograms of HER2 CAR (red) and NGFR (blue) transgene expression in human NK-92 cells compared to non-transduced control (grey). (B) HER2 CAR

and NGFR (control vector) expression on NK-92 cells was measured by flow cytometry on the day of functional assays. (C) Cytotoxicity of parental NK-92 cells, control vector transduced-, HER2 CAR-NK-92 cells against SKBR3 target cells at a 1:1 E:T ratio was determined by flow cytometry and compared to expanded NK cells. (D,F) Cell-free supernatants were collected after incubation with target cells and IFN-γ release was measured by ELISA. (E) Expanded NK cell and NK-92 cytotoxicity against MDA-MB-231breast cancer target cells was measured by flow cytometry at a 5:1 E:T ratio. Data represent mean ± SEM from one to three biological replicates per condition. *$p < 0.05$, **$p < 0.01$ (B, two-sided t test; C and D, one-way ANOVA with Dunnett's post hoc tests). ns, no significant difference.

Figure S2

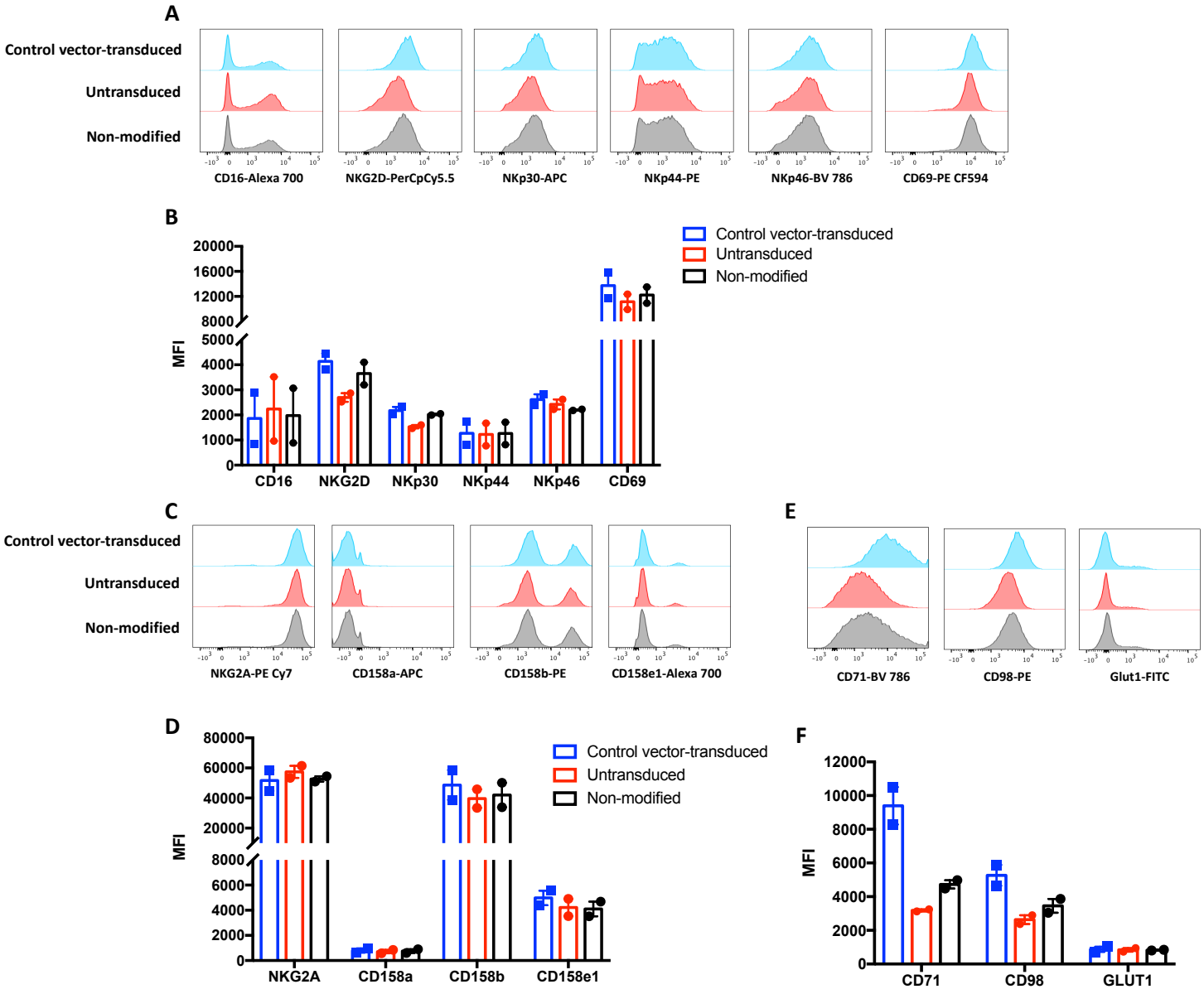

Figure S2. Expression of activation, inhibitory and nutrient receptors on lentiviral transduced expanded NK cells, Related to Figure 1. (A) Representative histograms of activation receptor expression and (B) quantified median fluorescence intensity (MFI) of activation receptor expression in control vector transduced-NK cells, untransduced NK cells, and non-modified controls. (C) Representative histograms of inhibitory receptor expression and (D) quantified MFI of inhibitory receptor expression in control vector transduced-NK cells, untransduced NK cells, and non-modified controls. (E) Representative histograms of nutrient receptor expression and (F) quantified MFI on nutrient receptor expression in control vector transduced-NK cells, untransduced NK cells, and non-modified controls. Data represent mean ± SEM from two donors per condition.

Figure S3

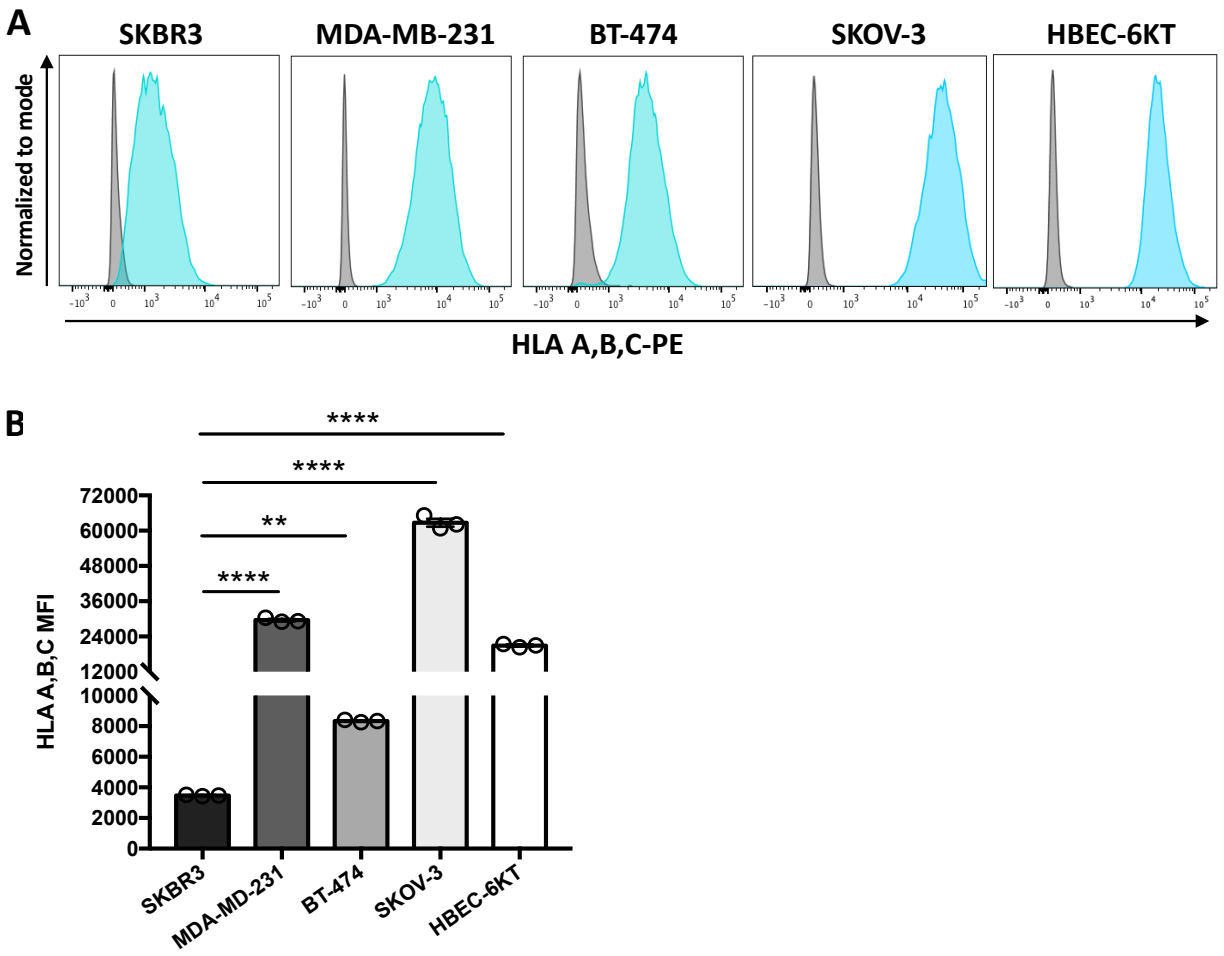

Figure S3. MHC Class I expression various target cell lines used in the study, Related to Figure 2 and Figure 4. (A) Level of MHC Class 1 expression and (B) quantified MFI of MHC class 1 expression on SKBR3 breast cancer, triple-negative MDA-MB-231breast cancer, BT-474 breast cancer, SK-OV-3 ovarian cancer, and healthy epithelial HBEC-6KT cells measured by flow cytometry using a fluorescent conjugated antibody against HLA-A,B,C. Data represent mean ± SEM from three replicates per condition. **p < 0.01, ****p < 0.0001 (B, one-way ANOVA with Dunnet's post hoc tests).

Figure S4

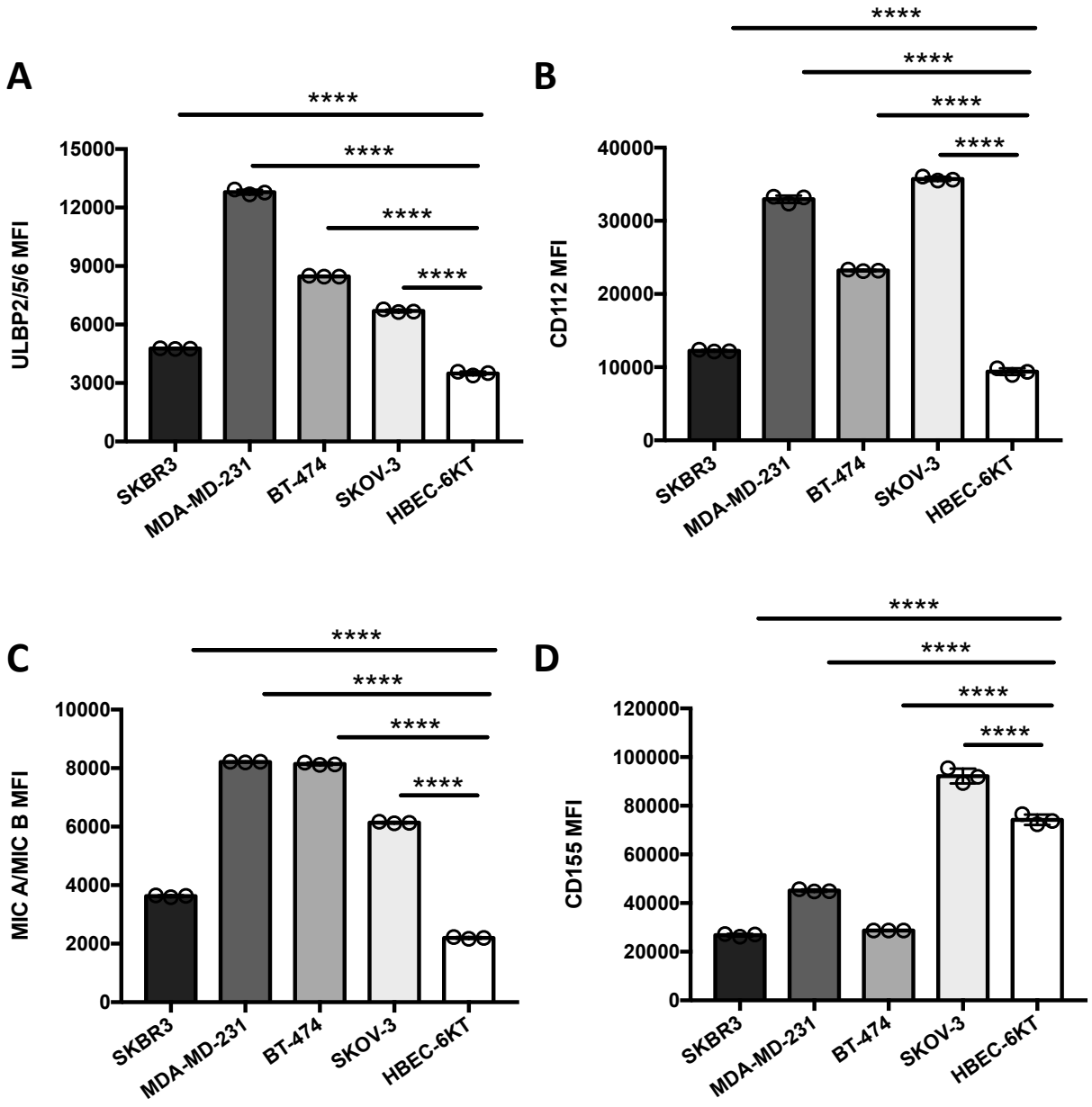

Figure S4. Stress-induced ligand expression on various target cell lines used in the study, Related to Figure 4. Expression of stress-induced ligands on SKBR3, MDA-MB-231, BT-474, SK-OV-3 and HBEC-6KT target cells were measured by flow cytometry. (A) Quantified MFI of ULBP2/5/6 ligand expression. (B) Quantified MFI of CD112 expression. (C) Quantified MFI of MIC A/MIC B ligand expression. (D) Quantified MFI of CD155 expression. Data represent mean ± SEM from three replicates per condition. ****p <0.0001 (A-D, one-way ANOVA with Dunnett's post hoc tests).

Figure S5

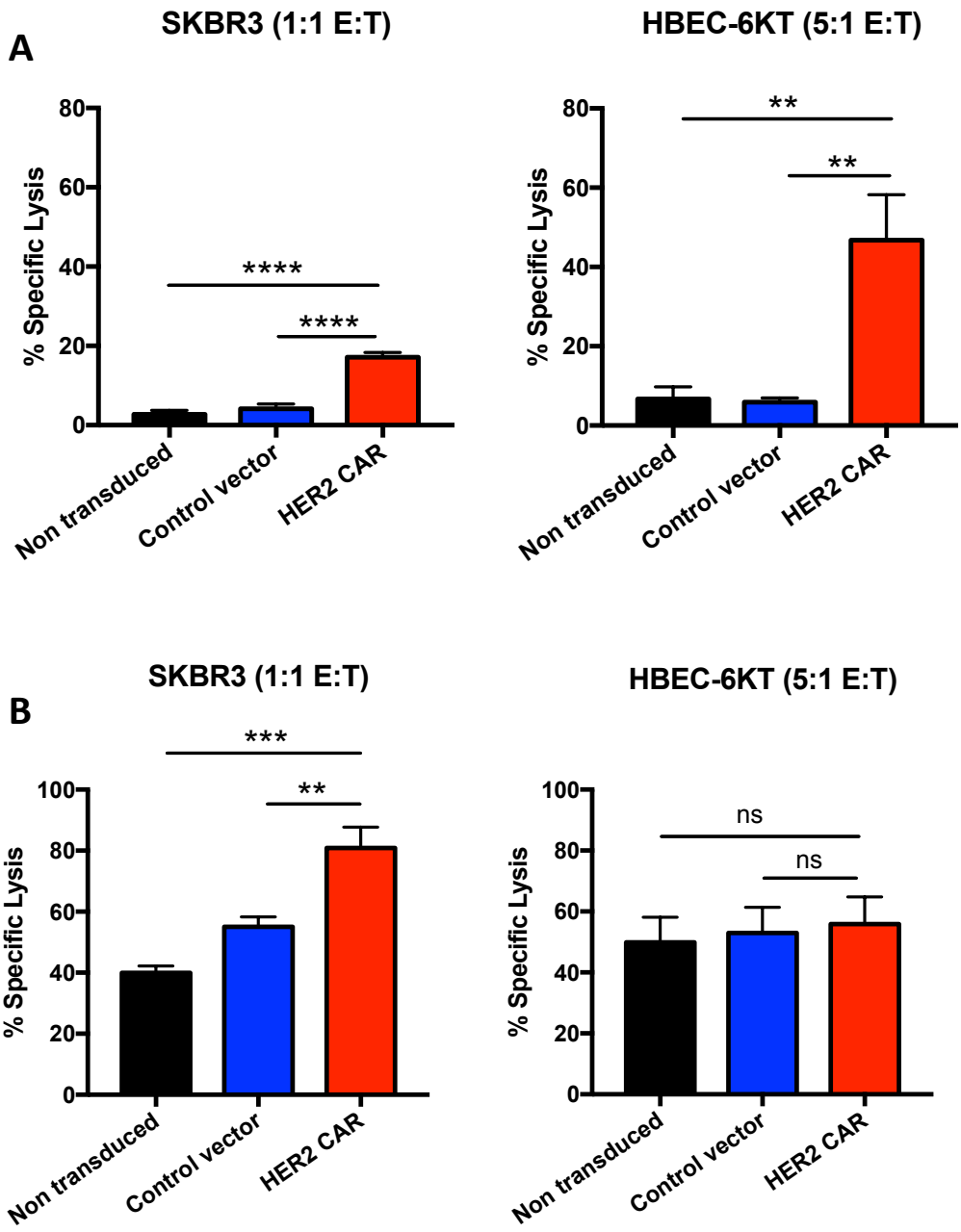

Figure S5. Cell-mediated cytotoxicity of HER2 CAR-T cells and HER2 CAR-NK cells against SKBR3 tumor or HBEC-6KT cells, Related to Figure 4. (A) Percent specific lysis of HER2 CAR-T cells against SKBR3 HER2-positive tumor cells at 1:1 E:T ratio or HBEC-6KT cells at a 5:1 E:T ratio. (B) Percent specific lysis of HER2 CAR-NK cells against SKBR3 HER2-positive tumor cells at 1:1 E:T ratio or HBEC-6KT cells at a 5:1 E:T ratio. Data represent mean ± SEM from four donors per condition. **p < 0.01, ***p < 0.001, ****p < 0.0001 (A and B, one-way ANOVA with Tukey's post hoc tests). ns, no significant difference.

Figure S6

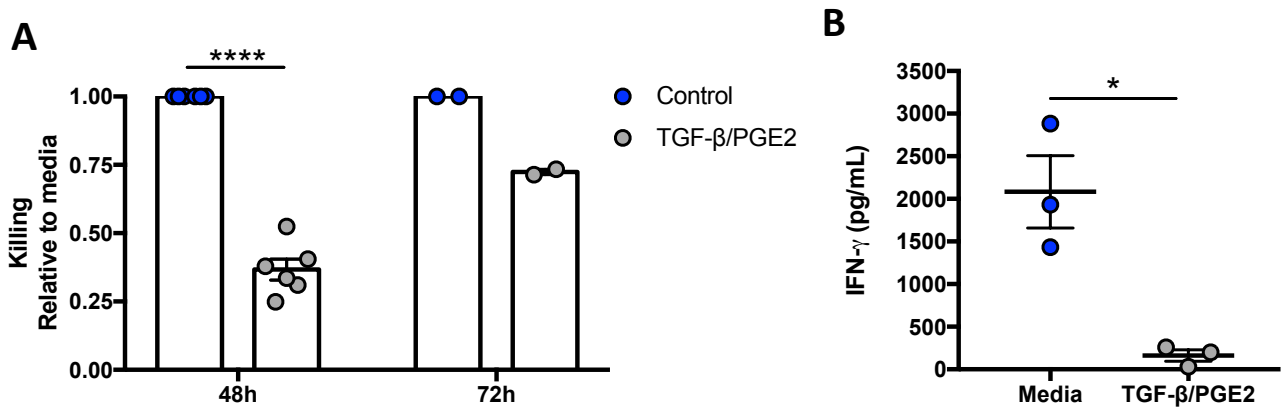

Figure S6. The immunosuppressive factors TGF-β and PGE2 suppress the anti-tumor functions of expanded NK cells, Related to Figure 5. Expanded NK cells were co-cultured in the presence of TGF-β (5 ng/mL) and PGE2 (10 μg/mL) or media only control for 48h or 72h. (A) Cell-mediated cytotoxicity against SKBR3 cells was assessed by flow cytometry. Relative change in specific lysis was calculated for each donor compared to the media only control. (B) IFN-γ production was measured by ELISA. Data represent mean ± SEM from two to six biological replicates per condition. *p < 0.05, ****p < 0.0001 (A, two-way ANOVA with Sidak's post hoc tests; B, two-sided t test).

Figure S7

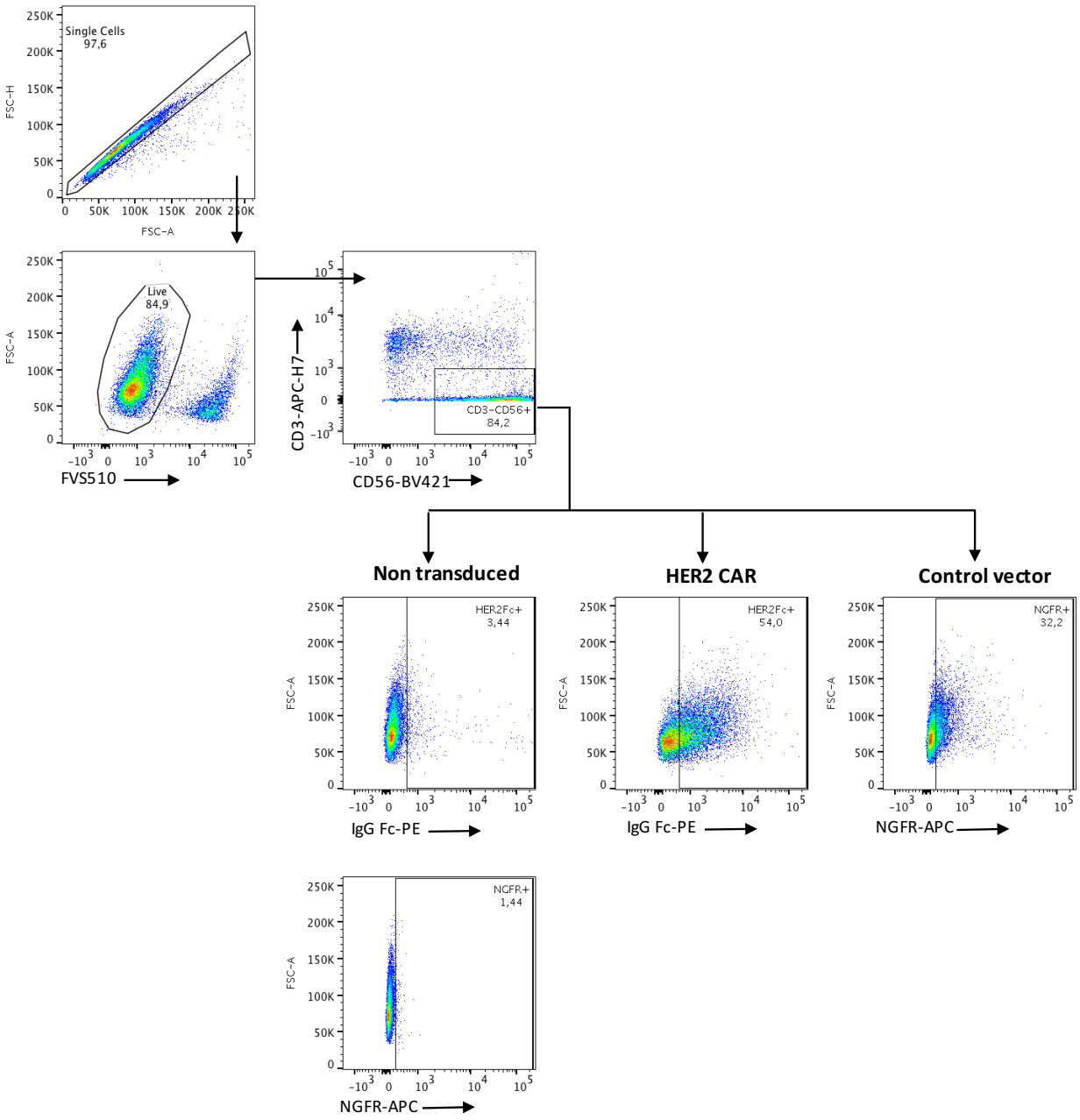

Figure S7. Gating strategy used for flow cytometry analysis of HER2 CAR and NGFR expression on human NK cells, Related to STAR Methods. Singlets are discriminated (FSC-H v. FSC-A) and live cells are gated (FVS510-negative). Non-transduced, HER2 CAR- and control vector transduced-expanded NK cells are assessed for purity by CD3-CD56+ gating, and then assessed for HER2 CAR or NGFR expression.

Figure S8

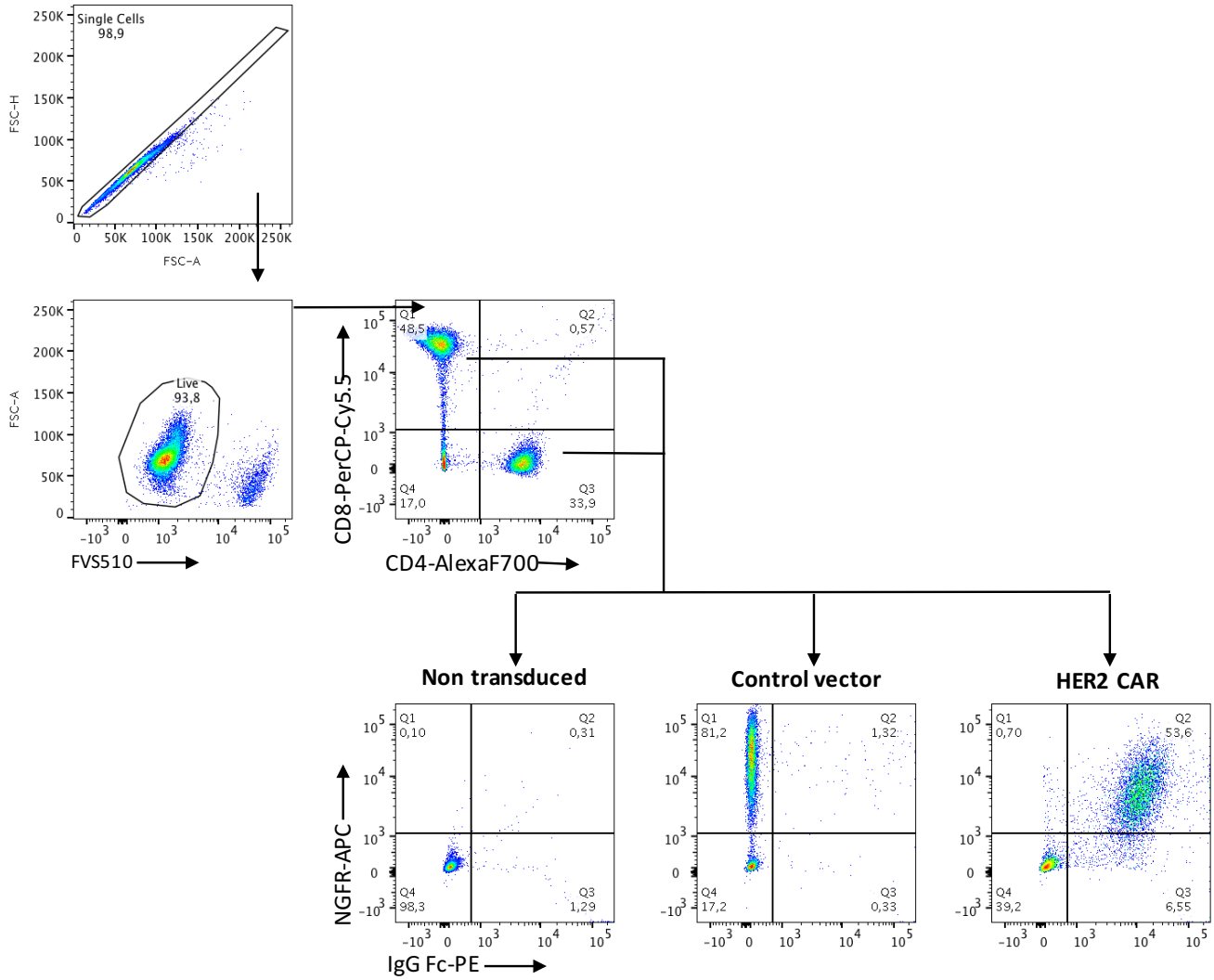

Figure S8. Gating strategy used for flow cytometry analysis of HER2 CAR and NGFR expression on human T cells, Related to STAR Methods. Singlets are discriminated (FSC-H v. FSC-A) and live cells are gated (FVS510-negative). Non-transduced, HER2 CAR- and control vector transduced-T cells were gated on CD4+ or CD8+ T cells, and then subsequently assessed for HER2 CAR or NGFR expression.