

Supplementary Table S1. Association of disease stage with baseline NK-cell activity and ex vivo

Disease stage	Baseline NK cell activity (IFN- γ , pg/mL) (Mean $\pm$ SD)	Fold expansion of NK cells (Mean $\pm$ SD)
Stage I (n =7)	1514.98 $\pm$ 1022.42	131.47 $\pm$ 201.74
Stage II (n = 19)	1161.78 $\pm$ 1156.00	104.79 $\pm$ 131.89
Stage III-IV (n = 7)	895.69 $\pm$ 1155.83	121.45 $\pm$ 198.66
p value	0.593	0.925

NK-cell expansion capacity in patients with breast cancer

Supplementary Table S1 footnote:

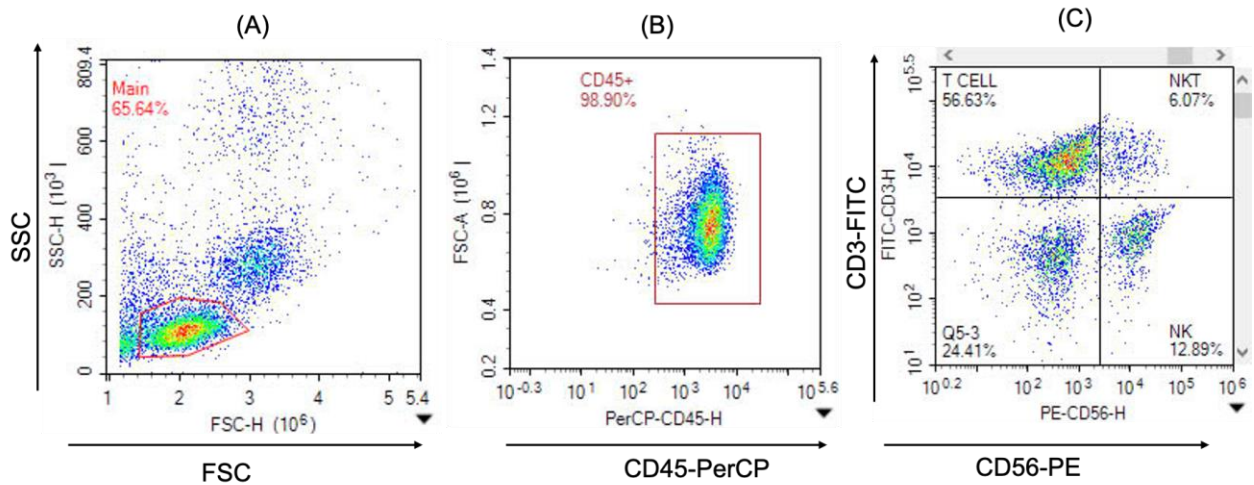
Values are presented as mean $\pm$ standard deviation (SD). Baseline NK-cell activity was assessed using the NKA-VUE whole-blood IFN- γ release assay and expressed as pg/mL. Fold expansion was calculated as the ratio of NK-cell number at day 14 to the initial NK-cell number at day 0. Comparisons among disease-stage groups were performed using one-way ANOVA.

Supplementary Table S2. Association of breast cancer molecular subtype with baseline NK-cell activity and ex vivo NK-cell expansion capacity

Disease stage	Baseline NK cell activity (IFN- γ , pg/mL) (Mean $\pm$ SD)	Fold expansion of NK cells (Mean $\pm$ SD)
Luminal A (n =8)	1091.38 $\pm$ 1189.95	85.23 $\pm$ 185.17
Luminal B HER2- (n = 7)	1673.79 $\pm$ 1213.26	77.11 $\pm$ 105.82
Luminal B HER2+ (n = 6)	1171.31 $\pm$ 1061.83	69.13 $\pm$ 33.39
HER2-enriched (n =8)	1334.81 $\pm$ 1158.52	235.17 $\pm$ 214.19
Triple-negative (TNBC) (n=3)	151.44 $\pm$ 202.68	63.52 $\pm$ 60.39
p-value	0.415	0.199

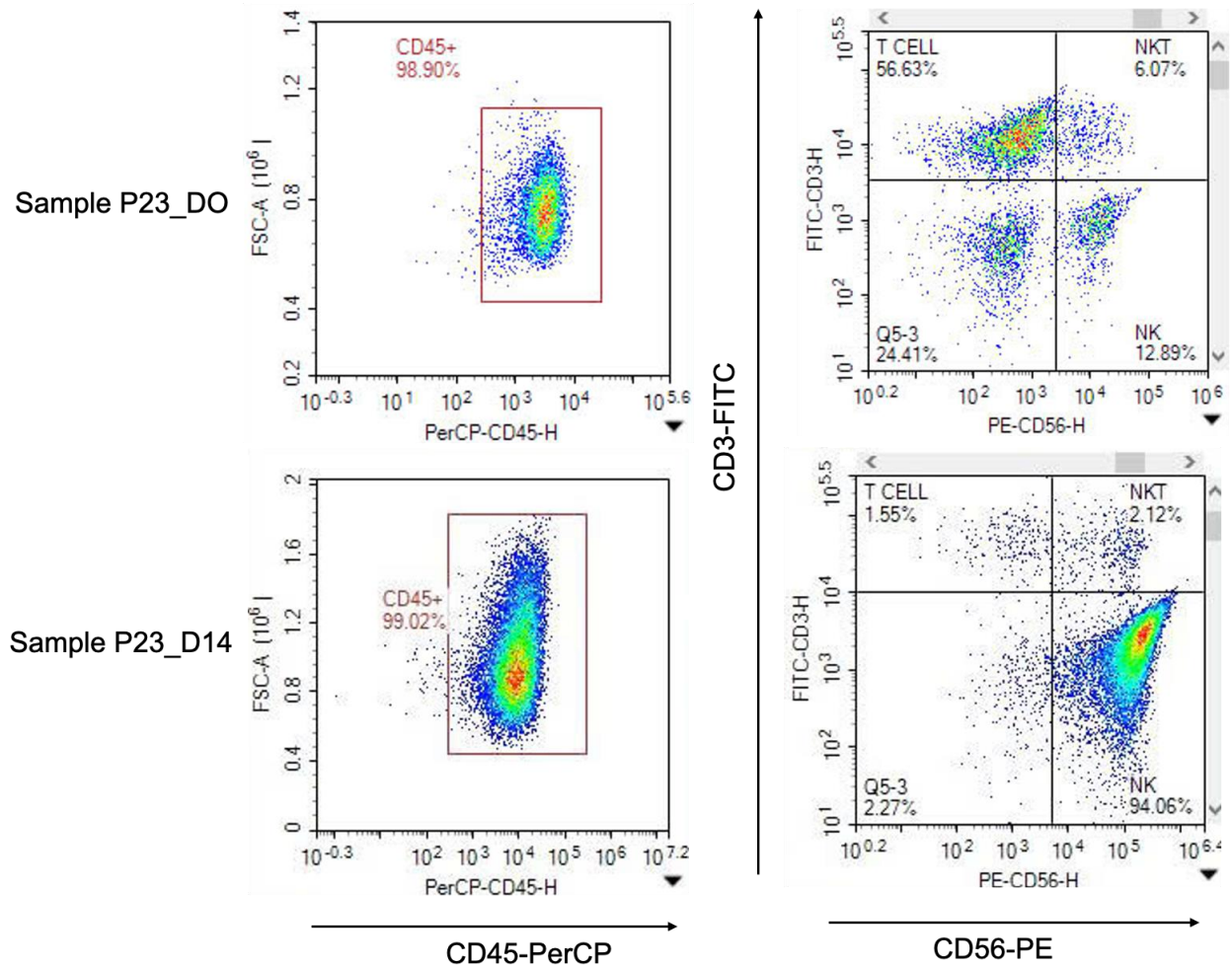
Supplementary Table S2 footnote:

Values are presented as mean $\pm$ standard deviation (SD). Baseline NK-cell activity was assessed using the NKA-VUE whole-blood IFN- γ release assay and expressed as interferon-gamma (IFN- γ) concentration (pg/mL). Fold expansion was calculated as the ratio of NK-cell number at day 14 to the initial NK-cell number at day 0. Breast cancer molecular subtypes were classified according to routine immunohistochemical assessment. Comparisons among molecular subtype groups were performed using one-way ANOVA. One patient with unavailable molecular subtype information was excluded from this analysis.



Supplementary Figure S1. Representative flow cytometric gating strategy for peripheral blood NK-cell identification.

(A) Lymphocyte gate established according to forward scatter (FSC) and side scatter (SSC) characteristics. (B) Selection of CD45⁺ leukocytes from the lymphocyte population. (C) Identification of NK cells as CD3⁻CD56⁺ lymphocytes. NK-cell frequency was expressed as the percentage of CD3⁻CD56⁺ cells among total peripheral blood lymphocytes.



Supplementary Figure S2. Representative flow cytometric assessment of NK-cell purity before and after ex vivo expansion.

Representative CD3 versus CD56 dot plots showing NK-cell frequency before culture (upper panels) and after 14 days of ex vivo expansion (lower panels). NK cells were defined as $CD3^-CD56^+$ lymphocytes. The expanded cell product demonstrated marked enrichment of the NK-cell population with concomitant depletion of $CD3^+$ T cells. Percentages indicate the proportion of cells within each gated population.