

Large-Scale Expansion and Engineering of Human NK Cells Sourced from Peripheral Blood Versus Umbilical Cord Blood



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Introduction

Healthy adult peripheral blood natural killer (PBNK) cells are mature cytotoxic innate lymphocytes possessing an inherent capacity for tumor cell killing, making them attractive candidates for adoptive cell therapy. These NK cells are also amenable to chimeric antigen receptor (CAR) genomic engineering for enhanced functions. Moreover, NK cells possess an inherent capacity for off-the-shelf therapy since they are not known to cause graft-versus-host disease, unlike T cells. Approved CAR cell therapies are custom-made from each patient's own T cells, a process that can limit patient eligibility and contribute to product variability. In this study, we compare PBNK cells to umbilical cord blood NK (CBNK) cells to evaluate both as candidate starting materials for clinical and commercial supply of CAR NK cells and assess the ability of each to support large-scale production and manufacturing of CAR NK cells through multiple rounds of stimulation.

Methods

PBNK and CBNK cells were expanded using either a standard 14-day protocol and a single stimulation with Nkarta's NKSTIM cell line plus IL-2, or with 5 stimulations over 70 days [1]. IL-12 and IL-18 were added at the beginning and during the final stage of the 70-day expansion to drive memory-like NK cell differentiation (Figs. 1 and 2). We transduced NK cells to express CD19-targeted CAR and membrane-bound IL-15 following the first NKSTIM pulse. We measured cytotoxicity against 3 tumor cell lines by IncuCyte, and phenotyped cells to assess NK activation, exhaustion and expression of markers of differentiation, including CD57, NKG2A, NKG2C, and KIR (Fig. 3 and data not shown).

Results

Purified NK cells from 1 PBNK donor and 4 CBNK donors were successfully expanded and engineered to express high levels of CAR. The 70-day final product (FP) CBNK cells were CD57⁺KIR^{lo/-} and NKG2A⁺, consistent with a less differentiated phenotype, whereas the FP PBNK cells were educated, at more than 80% NKG2A⁺KIR⁺ (Fig. 4) [2]. CBNK cells expanded to approximately 11-million-fold, whereas PBNK cells surpassed 250-billion-fold expansion, without appearing to have reached a terminal expansion limit (Fig. 2). At the end of the study, 14-day process and FP PBNK cells were as potent or trended towards greater potency than CBNK cells against 3 different tumor targets (Fig. 5). Furthermore, FP PBNK cells were as or more potent than 14-day process PBNK cells, depending on the tumor target (Fig. 5). Lastly, educated and non-educated NK cells were FACS sorted, expanded, and then functionally tested at the subpopulation level. Whereas CAR-driven cytotoxicity was comparable for all NK cell subsets, natural cytotoxicity was markedly greater for the educated NK cell subgroups (Fig. 7). Intriguingly, greatest expansion was observed for KIR-educated NK cells (Fig. 7), which may explain the enrichment of KIR-educated NK cells over 70 days of culture.

Conclusion

We demonstrate healthy donor-derived PBNK cells can expand over 250 billion-fold while maintaining potency. These results show robust expansion capability of educated, potent NK cells and provide a rationale for the development of off-the-shelf CAR NK cell therapies using NK cells from donors selected to provide optimal product characteristics.

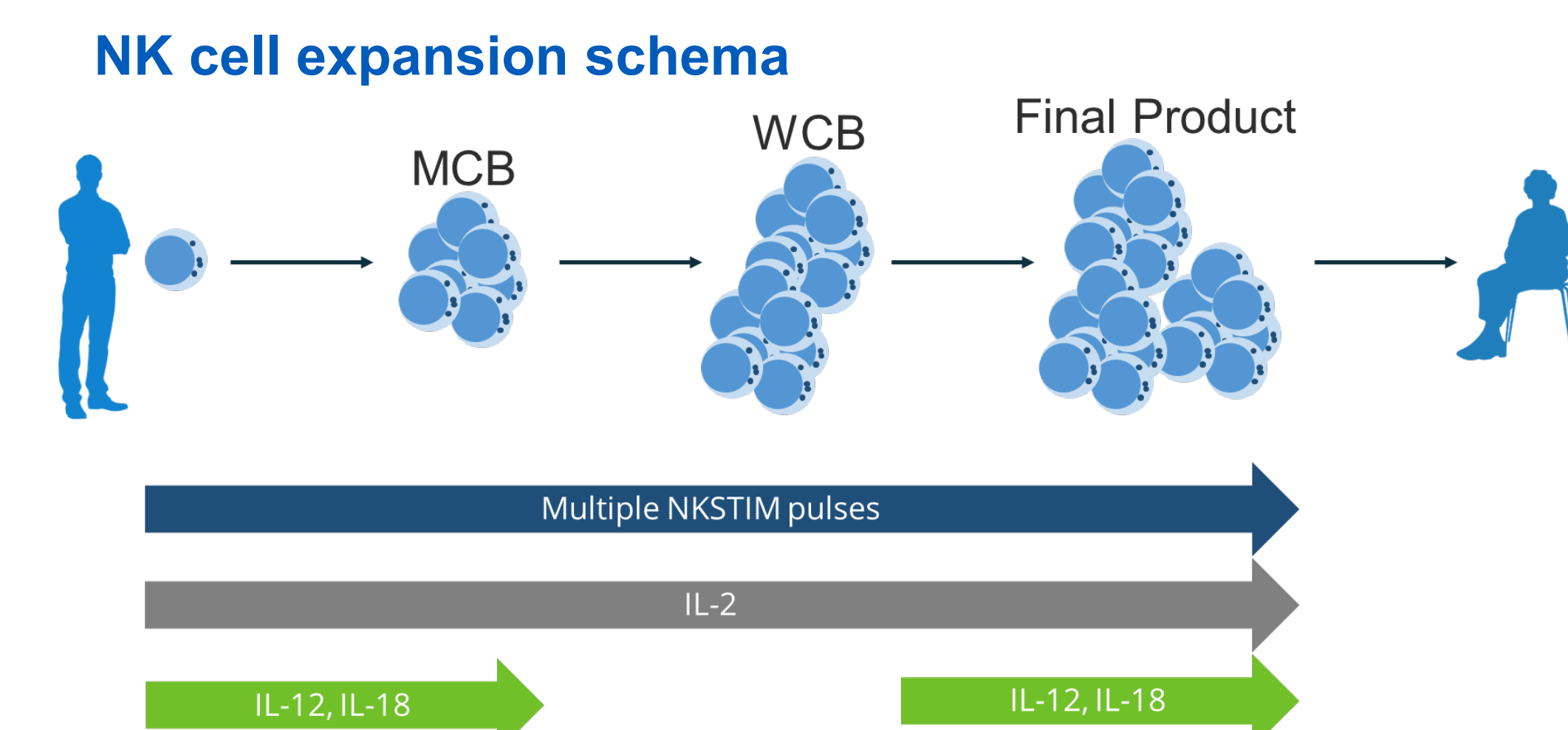


Figure 1. Schematic diagram of healthy donor-derived NK cell expansion for off-the-shelf use. Samples were cryopreserved at MCB (master cell bank), WCB (working cell bank) and FP (final product).

Substantial expansion of engineered peripheral blood NK cells can be achieved using multiple NKSTIM pulses

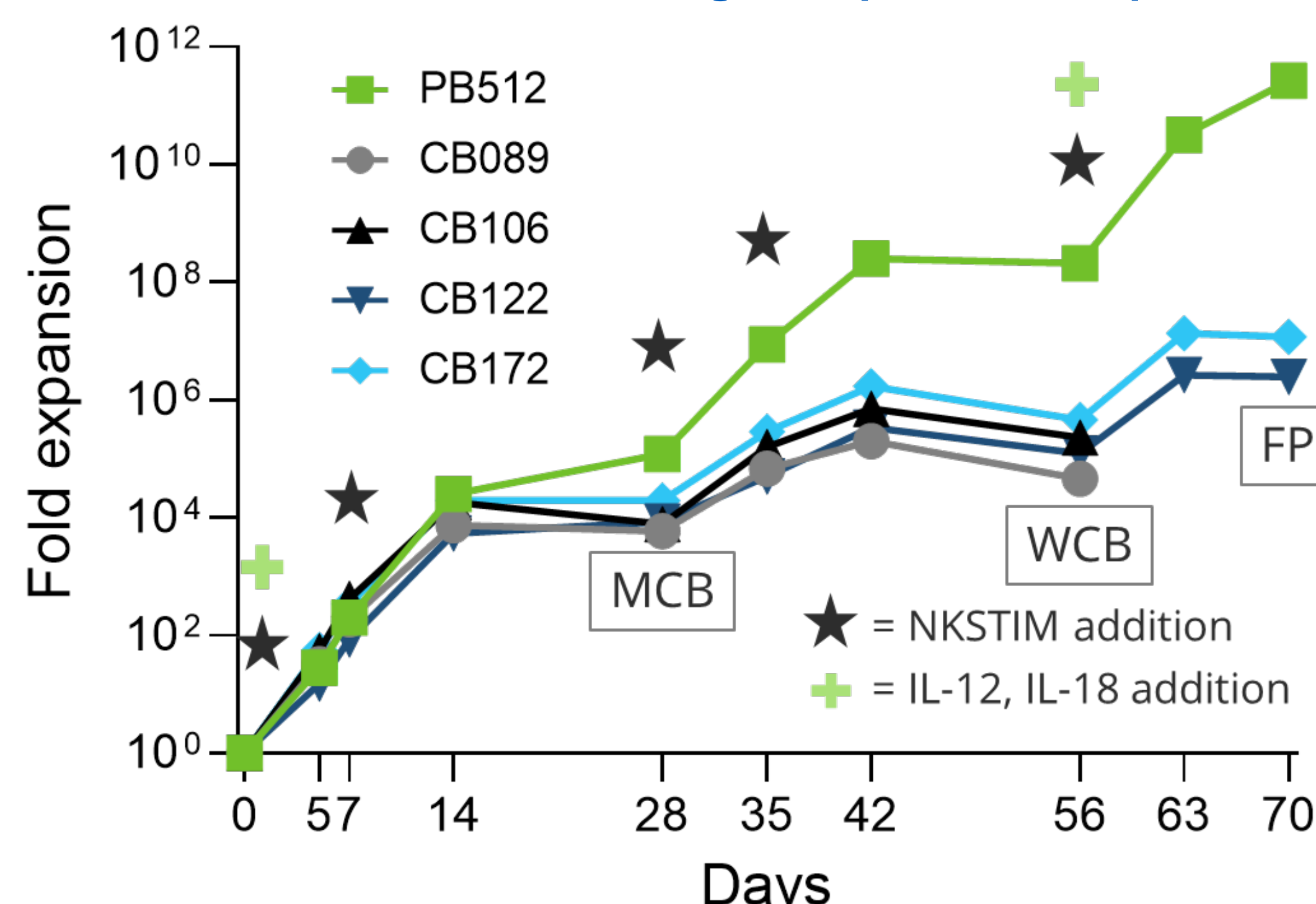


Figure 2. Greater than 250-billion-fold expansion was achieved for peripheral donor PB512. Expansion of NK cells from the three highest potency donors was characterized over the length of the study.

NK cells expanded from CMV seropositive donor PB512 are phenotypically consistent with adaptive NK cells

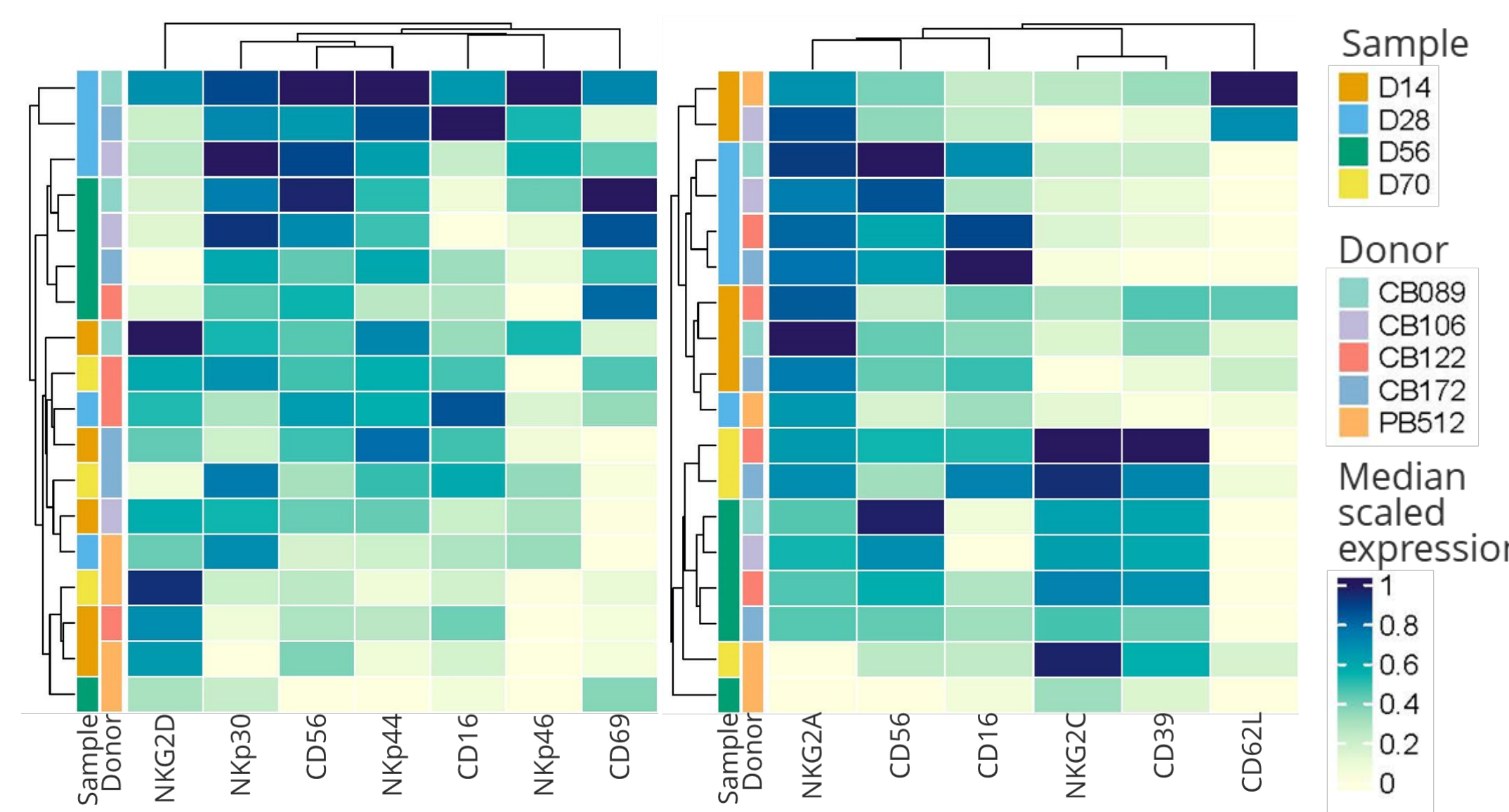


Figure 3. Longitudinal characterization of cell surface marker expression by flow cytometry and FlowSOM analysis. Adaptive NK cells, characterized as NKG2C⁺ NKG2A⁻ Nkp30⁻, are the major population in expanded PB512 NK cells [3]. D14, D28, D56 and D70 indicate day of expansion.

KIR-educated NK cells are enriched during expansion

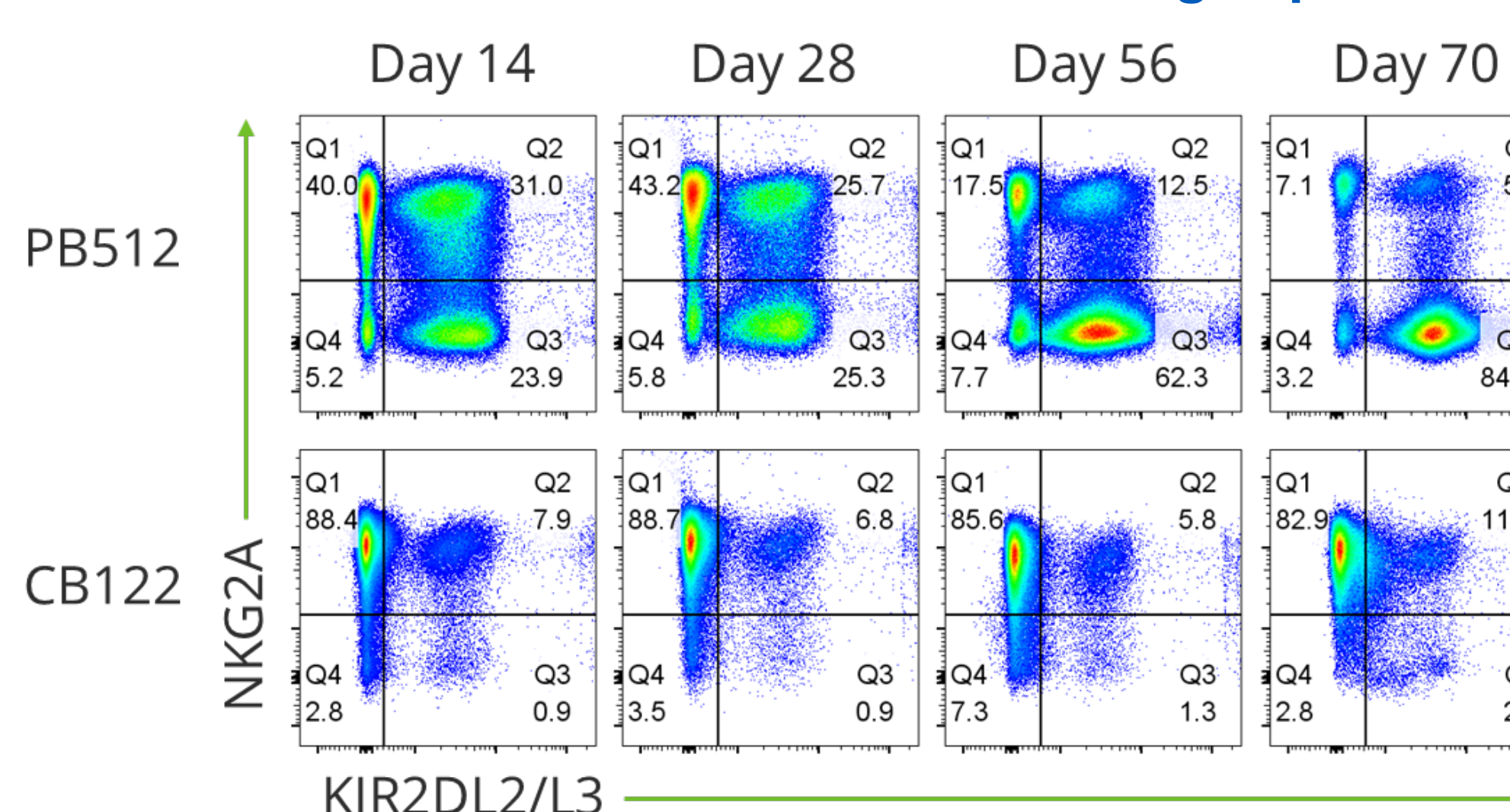


Figure 4. Enrichment of NKG2A⁺ KIR⁺ educated NK cells is observed for NK cells expanded from adult peripheral blood (PB512), but not from umbilical cord blood (CB122). Similar results were observed for CB172.

Potency of peripheral blood NK cells can be maintained after >250-billion-fold expansion

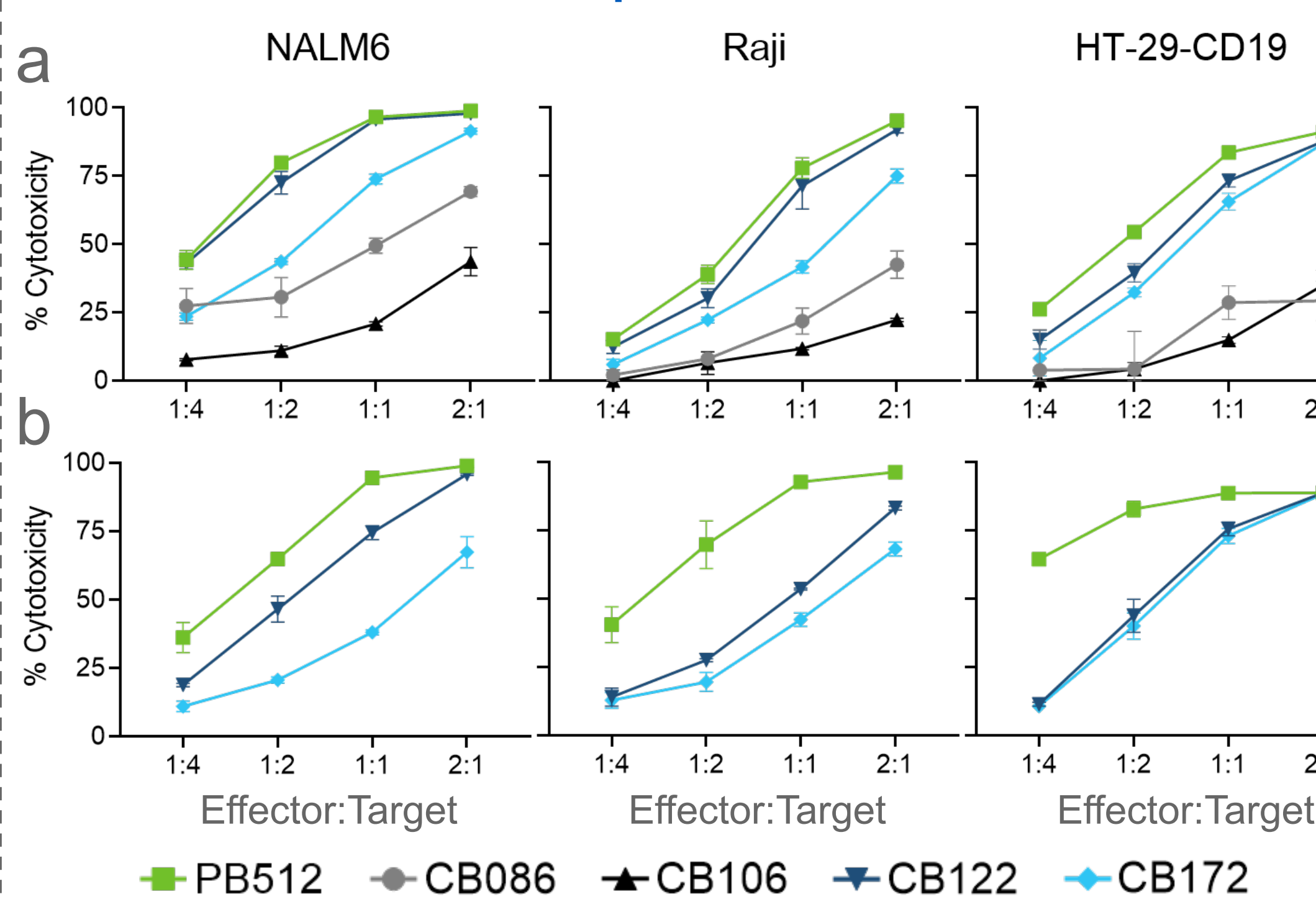


Figure 5. Extensively expanded healthy donor-derived peripheral blood NK cells are capable of killing multiple tumor cell types. (a) 14-day process NK cells and (b) final product NK cells were used in a cytotoxicity assay against NALM6 and Raji (B cell tumor cell lines that naturally express CD19) and HT-29-CD19 (a colorectal adenocarcinoma cell line that was transduced to ectopically express CD19). Percent cytotoxicity was calculated based on IncuCyte images collected at 72-hours after co-culture. % cytotoxicity = [(control - experiment) / control] x 100.

Chromosomal integrity is well-maintained in NK cells after >250-billion-fold expansion

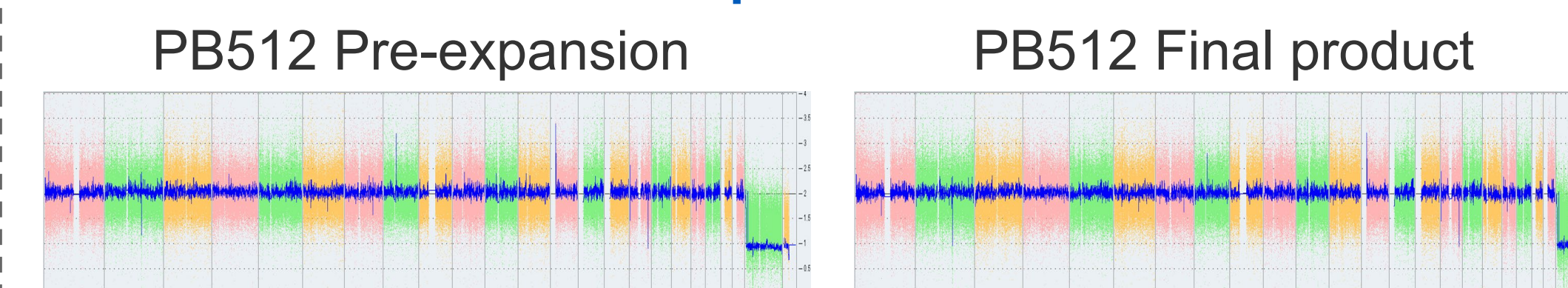


Figure 6. KaryoStat™ analysis shows no chromosomal aberrations in PB512 NK cells. 150k SNPs spread across the genome were analyzed and compared between donor matched pre- and post-expansion NK cells. X-axis indicates chromosome number and Y-axis indicates copy number.

KIR-educated NK cell subset has superior potential for expansion and natural cytotoxicity, but all subsets are capable of exceptional CAR-driven cytotoxicity

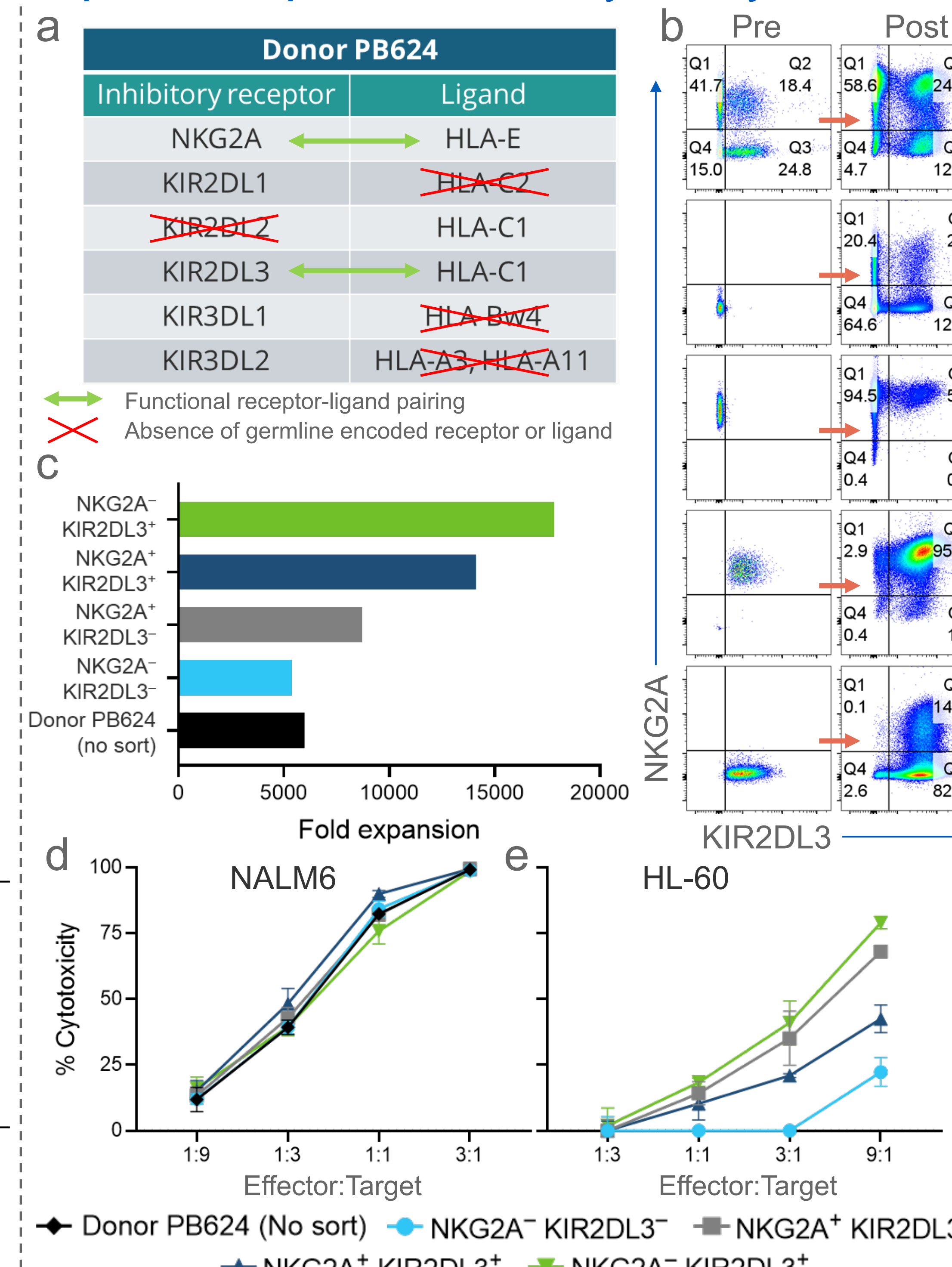


Figure 7. Expansion, phenotypic plasticity, and functional properties of isolated NK cell subpopulations. (a) Education of donor PB624 NK cells occurs through NKG2A and KIR2DL3 receptors. (b-e) Donor PB624 CD56^{dim} NK cells were sorted according to NKG2A, KIR2DL3, and CD57 expression, cultured for 14 days on NKSTIM plus IL-2, IL-12, and IL-18, +/- CD19 CAR transduction, and then reanalyzed for receptor expression or used in functional assays. CD57⁺ data are shown. Similar results were obtained for CD57⁻ cohort. (b) Comparison of NKG2A and KIR2DL3 expression on Pre- (left) and Post-expansion (right) NK cells. Top row represents control samples without prior sorting and bottom rows represent sorted samples. (c) Fold expansion results of sorted populations after 14 days of culture. (d) 4-hour cytotoxicity assay against NALM6 target cells using CD19 CAR transduced NK cells to assess CAR-driven cytotoxicity. (e) 4-hour cytotoxicity assay against HL-60 target cells using non-transduced NK cells to assess natural cytotoxicity. HL-60 cells are an acute promyelocytic leukemia cell line that do not express CD19.

References

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