

Renewable and genetically engineered natural killer cells for off-the-shelf adoptive cellular immunotherapy

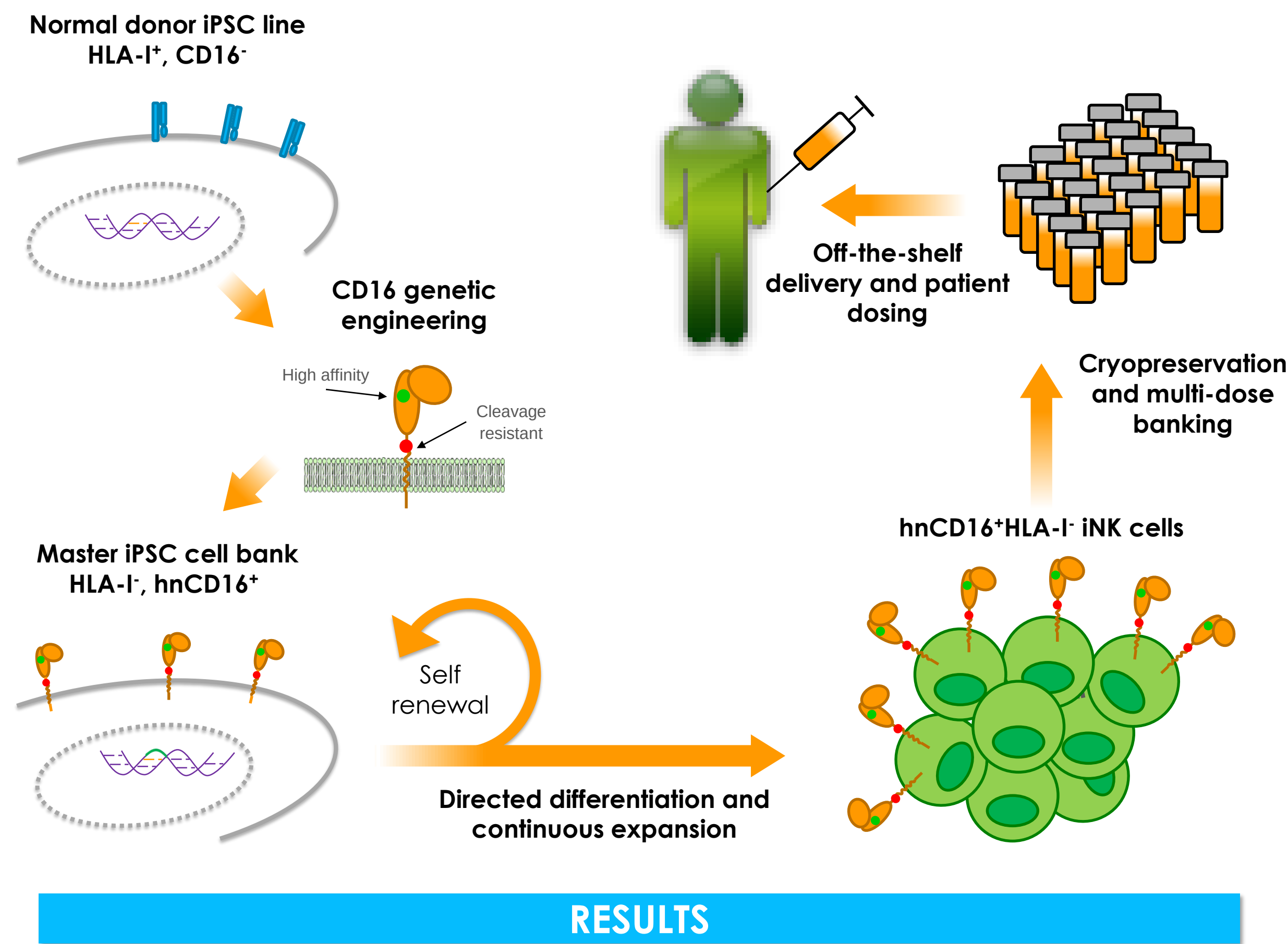
Ryan Bjordahl, Raedun Clarke, Svetlana Gaidarova, Brian Groff, Paul Rogers, Stacey Moreno, Ramzey Abujarour, Greg Bonello, Tom Tong Lee, Weijie Lan, Michelle Burrascano, Matthieu Bauer, Megan Robinson, Jeff Sasaki, Dave Robbins, Betsy Reznier, Stewart Abbot, Scott Wolchko, Daniel Shoemaker and Bahram Valamehr; Fate Therapeutics, San Diego, CA, USA Correspondence bob.valamehr@fatetherapeutics.com



EXECUTIVE SUMMARY

- Targeted genetic integration of **high affinity, non-cleavable CD16 (hnCD16)** promotes enhanced NK cell responsiveness to CD16 stimulation and antibody dependent cellular cytotoxicity (ADCC).
- NK cells differentiated from hnCD16 engineered iPSC (**hnCD16 - iNK**) are highly functional in vitro, with cytotoxicity and cytokine production that is comparable to peripheral blood NK cells.
- Production of hnCD16-iNK is highly scalable**, producing around 10^6 mature NK cells from a single iPSC.
- Genetic engineering of HLA molecules produces **reduced allogeneic rejection** in vitro of iPSC-derived NK cells across traditional histocompatibility barriers.
- hnCD16 iNK cells are highly functional in vitro, exhibiting potent cytokine production and **enhanced ADCC against multiple tumor targets**
- hnCD16 iNK cells are functional in vivo and mediate ADCC-dependent tumor regression in an ovarian cancer model**

GRAPHICAL ABSTRACT



RESULTS

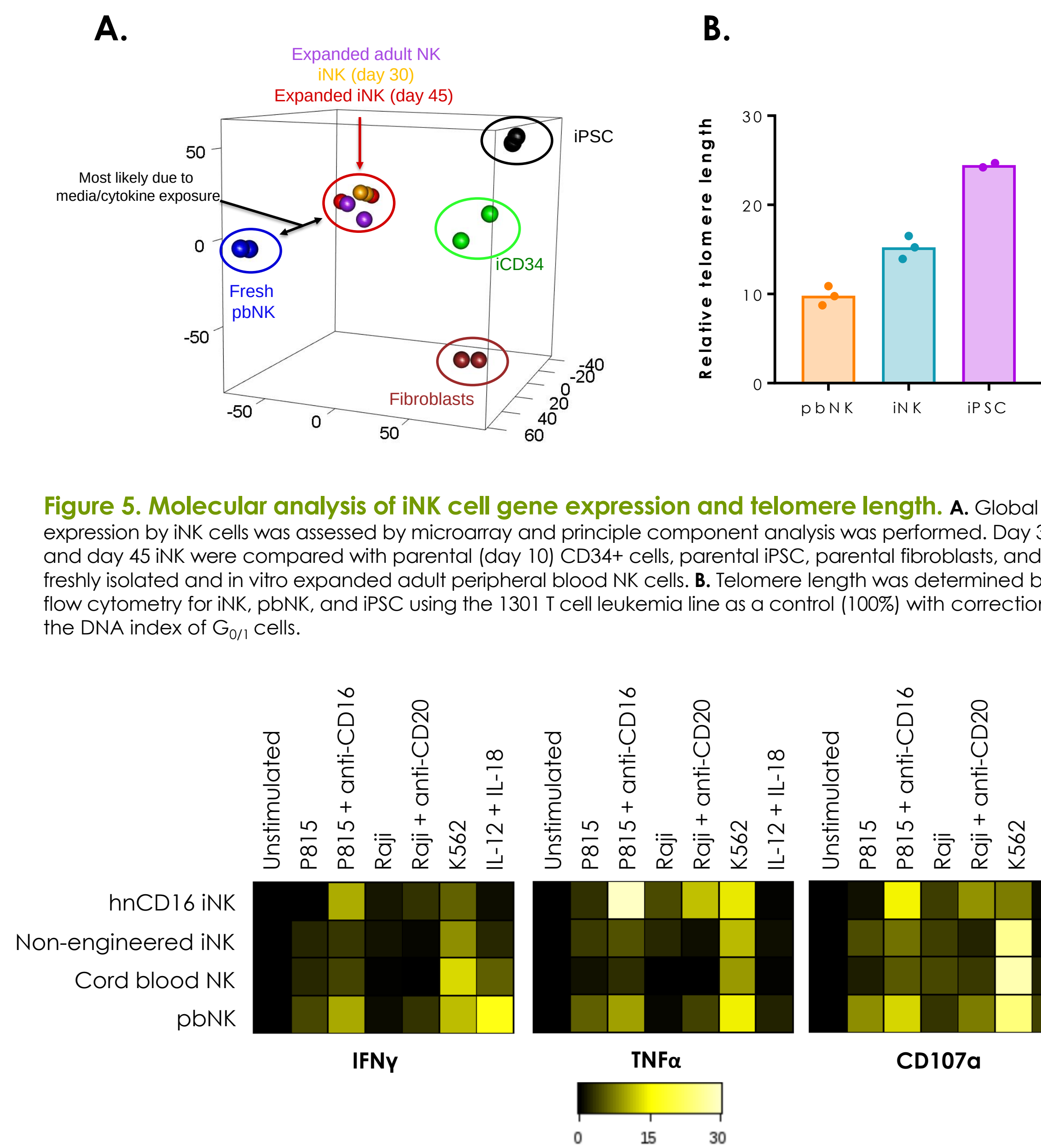


Figure 5. Molecular analysis of iNK cell gene expression and telomere length. **A.** Global gene expression by iNK cells was assessed by microarray and principle component analysis was performed. Day 30 iNK and day 45 iNK were compared with parental (day 10) CD34+ cells, parental iPSC, parental fibroblasts, and both freshly isolated and in vitro expanded adult peripheral blood NK cells. **B.** Telomere length was determined by flow cytometry for iNK, pbNK, and iPSC using the 1301 T cell leukemia line as a control (100%) with correction for the DNA index of $G_{0/1}$ cells. **C.** Heatmaps quantify the frequency of NK cells that are positive for IFN γ , TNF α , and CD107a and are scaled from 0 (black) to 30% (white), and are background subtracted such that unstimulated = 0. hnCD16 iNK are highly responsive to anti-CD16 stimulation.

RESULTS

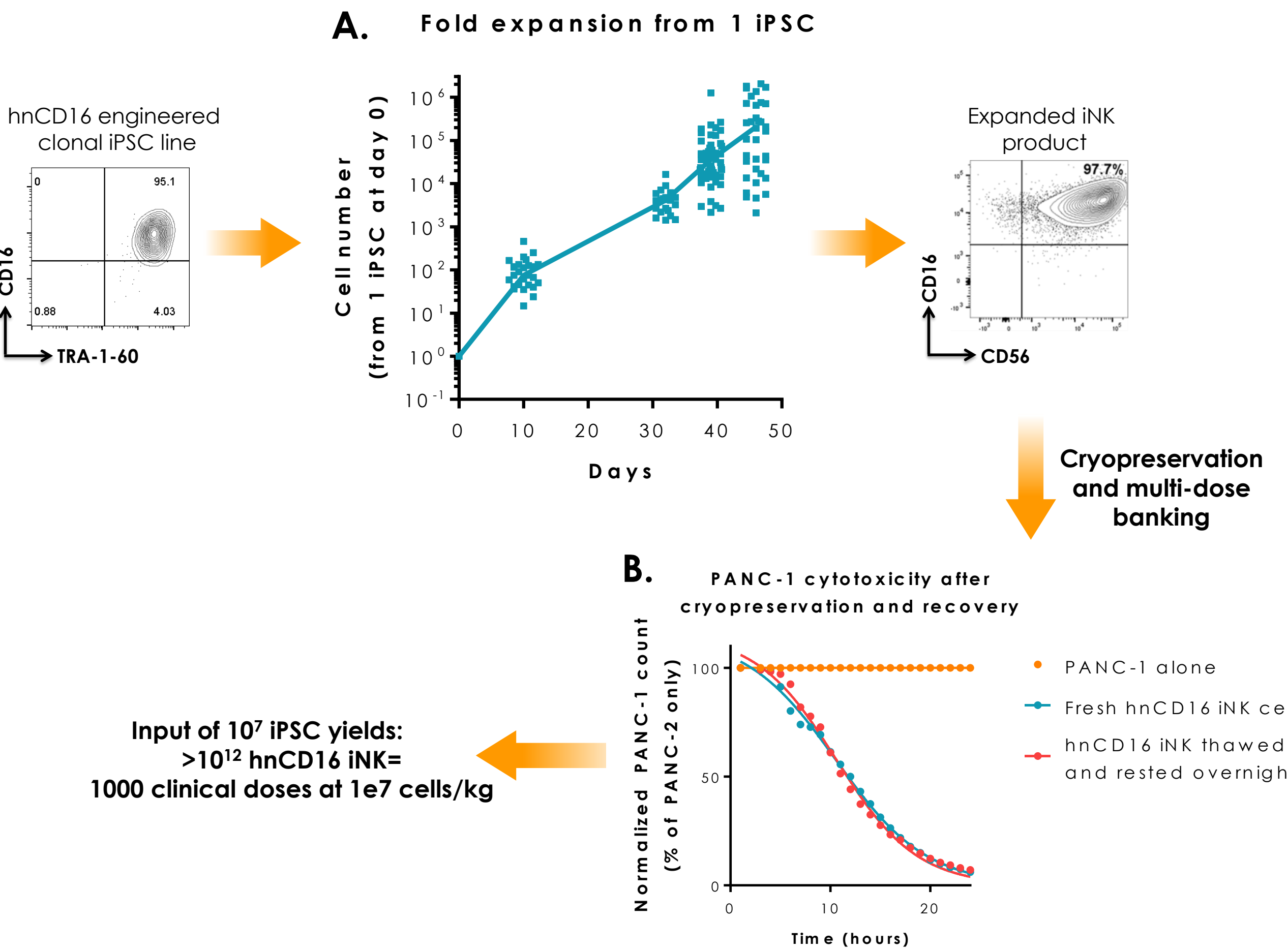


Figure 1. Robust centralized manufacturing platform for off the shelf, NK cell immunotherapy. **A.** Fold expansion from a single starting iPSC (day 0) through NK cell differentiation yields approximately 10^6 NK cells from a single iPSC (mean 7.5×10^5 , $n=39$). **B.** hnCD16 iNK cells retain function after cryopreservation and recovery. hnCD16 were cryopreserved and compared to cultured hnCD16 iNK (blue line) after thawing and resting overnight in IL-2 (red line).

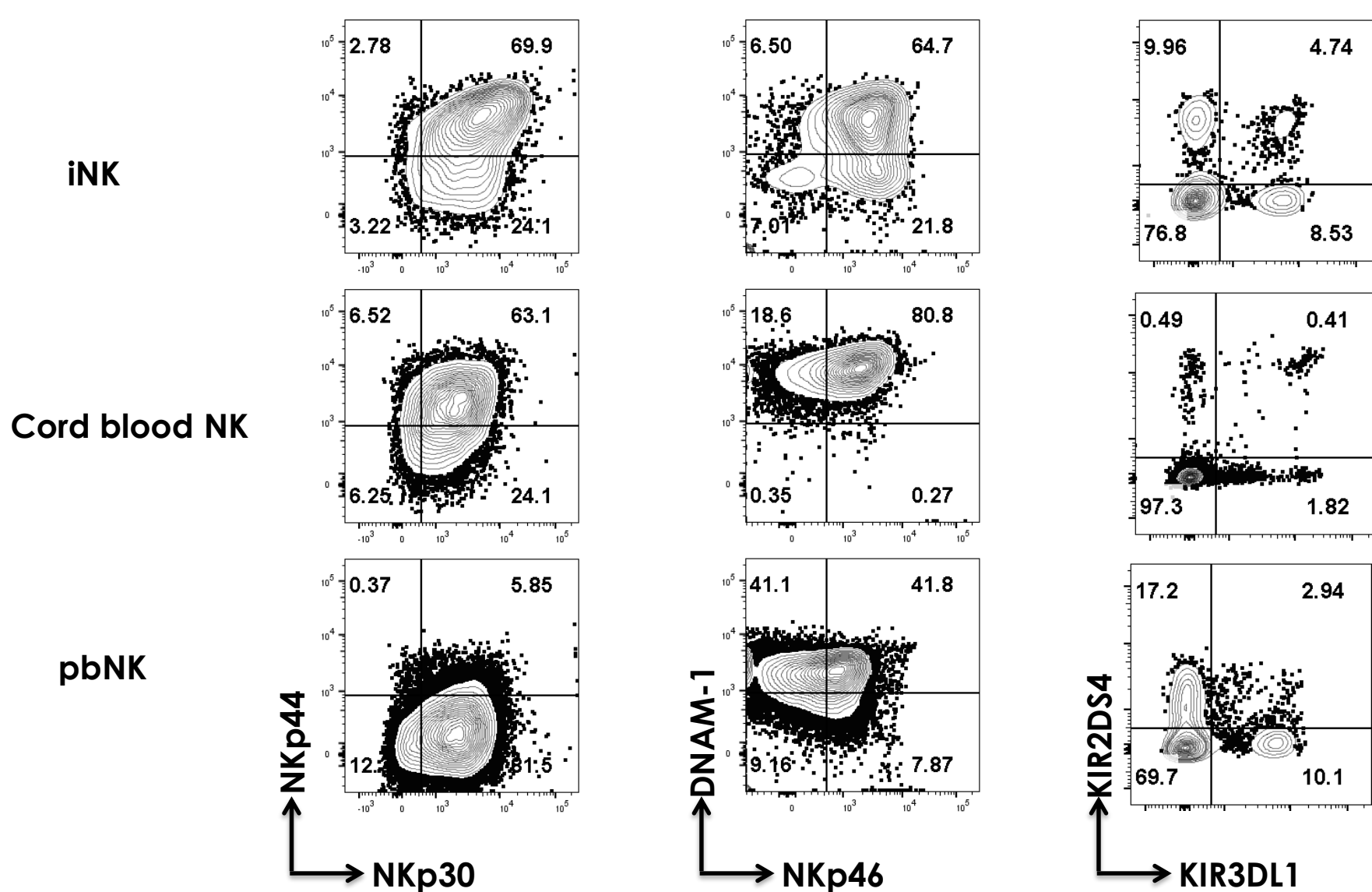


Figure 3. iPSC derived NK cells are phenotypically mature. iPSC-derived NK cells, NK cells differentiated in vitro from cord blood CD34+ cells, and adult peripheral blood NK cells were stained for a panel of NK cell receptors. Expression of each marker is shown by representative flow cytometry plots.

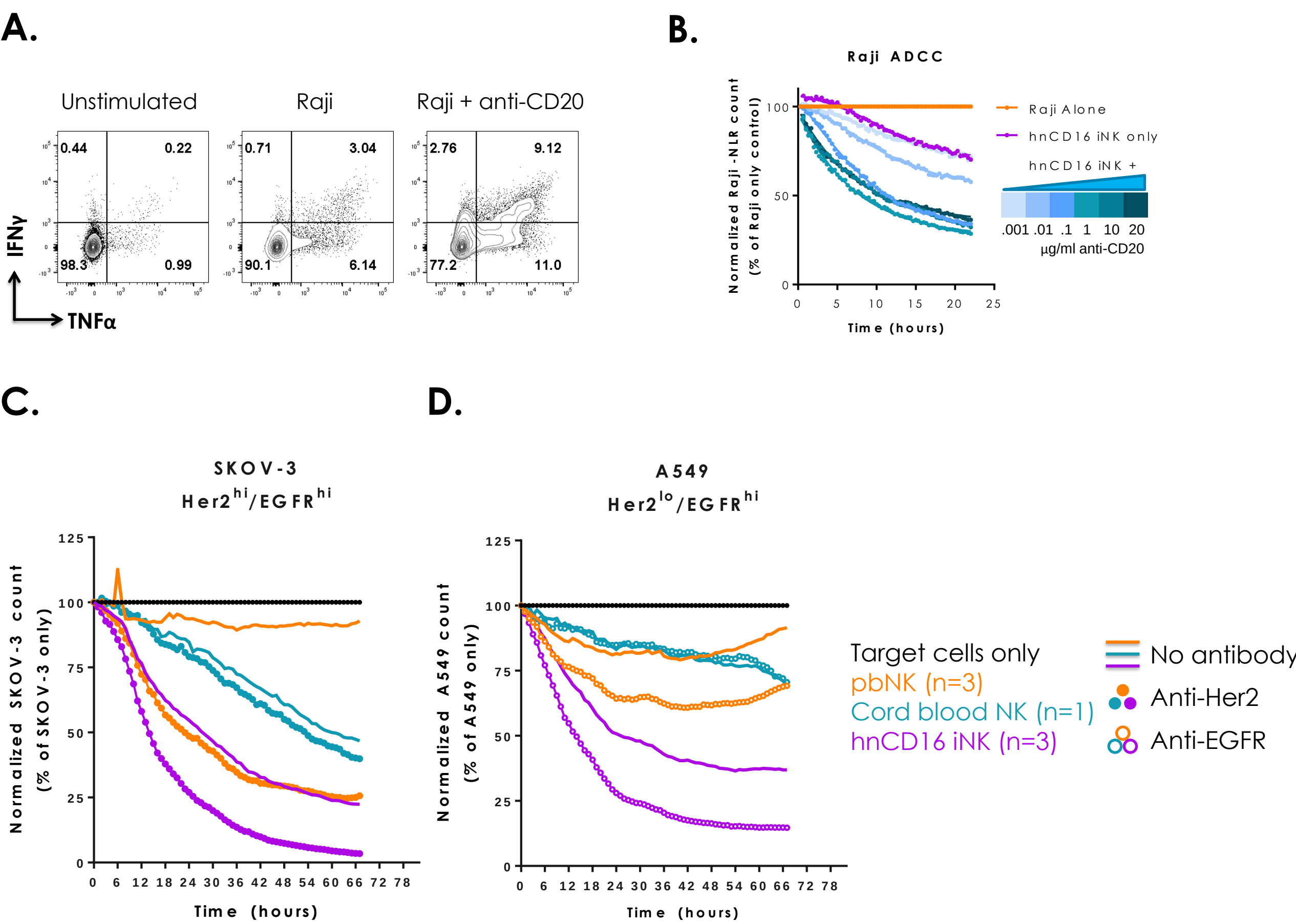


Figure 7. hnCD16-iNK cells have superior in vitro ADCC against multiple tumor types. **A.** hnCD16 iNK were left unstimulated or stimulated with a 1:1 ratio of Raji cells $\pm$ rituximab antibody and stained for TNF α and IFN γ 4 hours later. **B.** ADCC against Raji cells was determined using the Incucyte zoom imaging system. Anti-CD20 was titrated from .001 to 20 μ g/ml. **C & D.** hnCD16 cytotoxicity in vitro was measured directly against tumor cells (solid line), or with the addition of either **C.** Anti-Her2 (solid circles, SKOV-3) or **D.** anti-EGFR (open circles, A549). hnCD16-iNK cells (purple), were compared with both adult, peripheral blood NK cells (pbNK, orange), and in vitro differentiated cord blood NK cells (cord blood NK, blue). Incucyte imaging was performed, and the data are presented as the normalized frequency of target cells remaining, where target cells without NK effectors = 100%. Data represent mean, $n=3$.

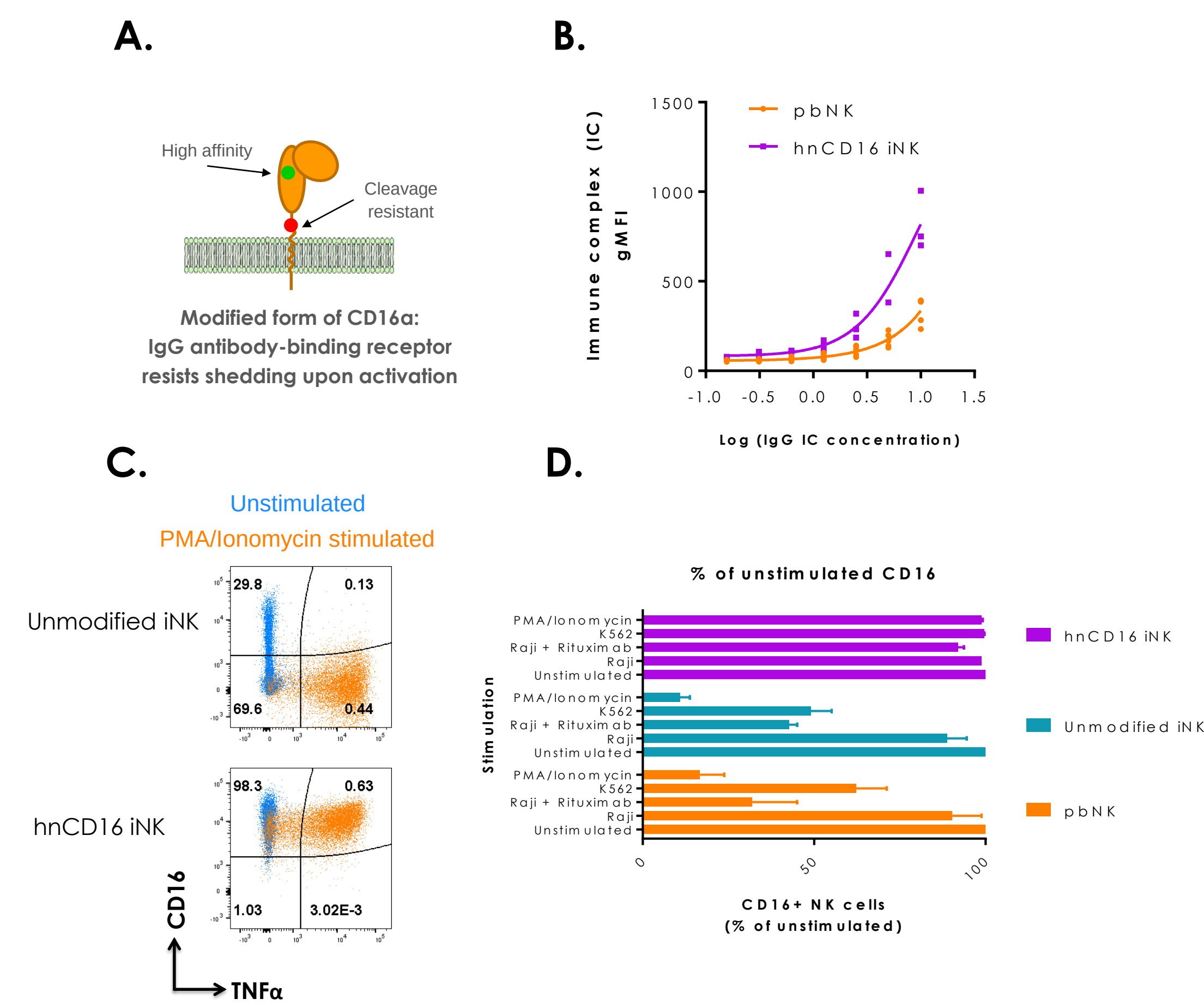


Figure 2. High affinity, non-cleavable CD16 (hnCD16) construct binds IgG immune complex with high affinity and resists activation induced cleavage. **A.** CD16 construct used to make hnCD16 iNK utilizes high affinity (F158V) and non-cleavable version of CD16. **B.** Binding of pre-formed immune complexes was tested for hnCD16 iNK (purple) or peripheral blood NK cells (orange). Staining determined for equal amounts of CD16 present on the cell surface. **C.** Unmodified iNK or hnCD16 iNK were left unstimulated (blue) or stimulated with PMA and ionomycin (orange). Activated iNK cells produce TNF α , but hnCD16 iNK resist CD16 cleavage. **D.** hnCD16 iNK, unmodified iNK, or peripheral blood NK were stimulated for 4 hours and CD16 shedding was determined by flow cytometry ($n=4-6$ per group).

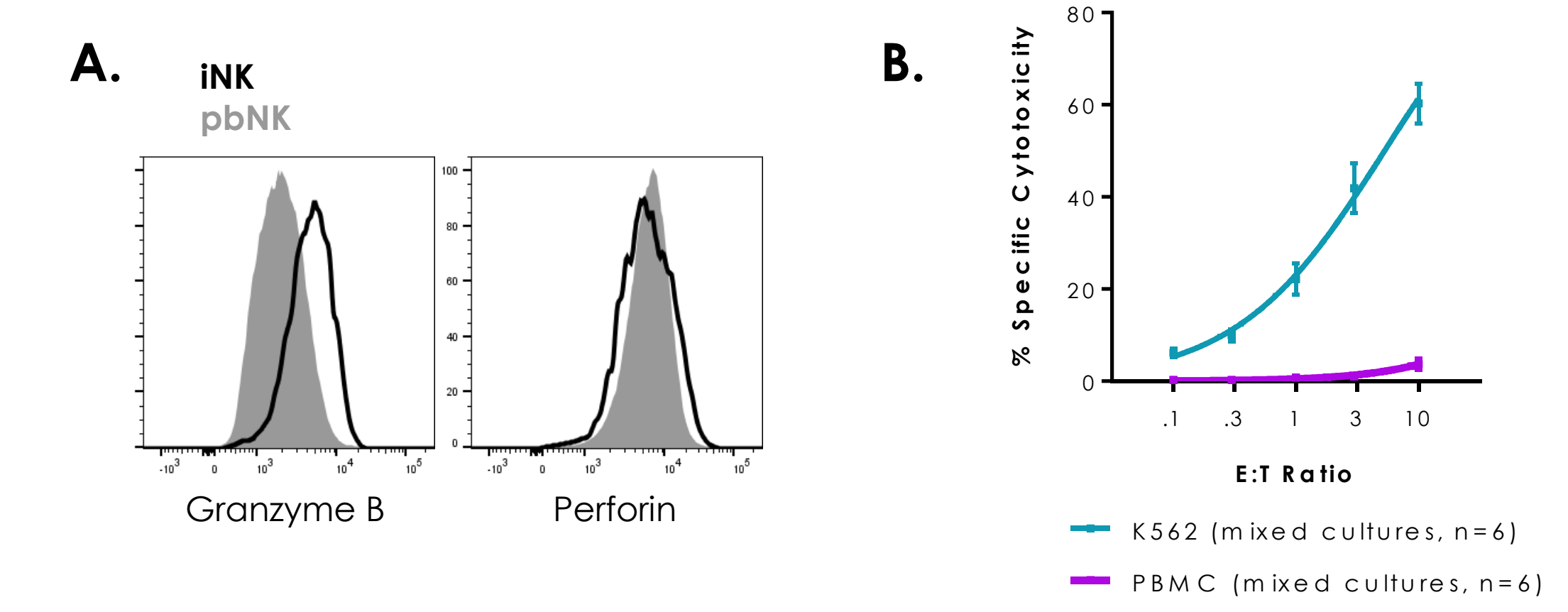


Figure 4. iNK cells express high levels of the cytolytic molecules perforin and granzyme B and specifically target HLA-null K562 targets while avoiding healthy PBMC. **A.** iPSC-derived NK cells (iNK) or peripheral blood NK cells (pbNK) were stained intracellularly for expression of granzyme B and perforin. **B.** hnCD16 iNK cells were used as effectors against a mixture of K562 and allogeneic PBMC target cells in a 4 hour in vitro cytotoxicity assay. Results are the mean $\pm$ SEM of 6 PMBC donors.

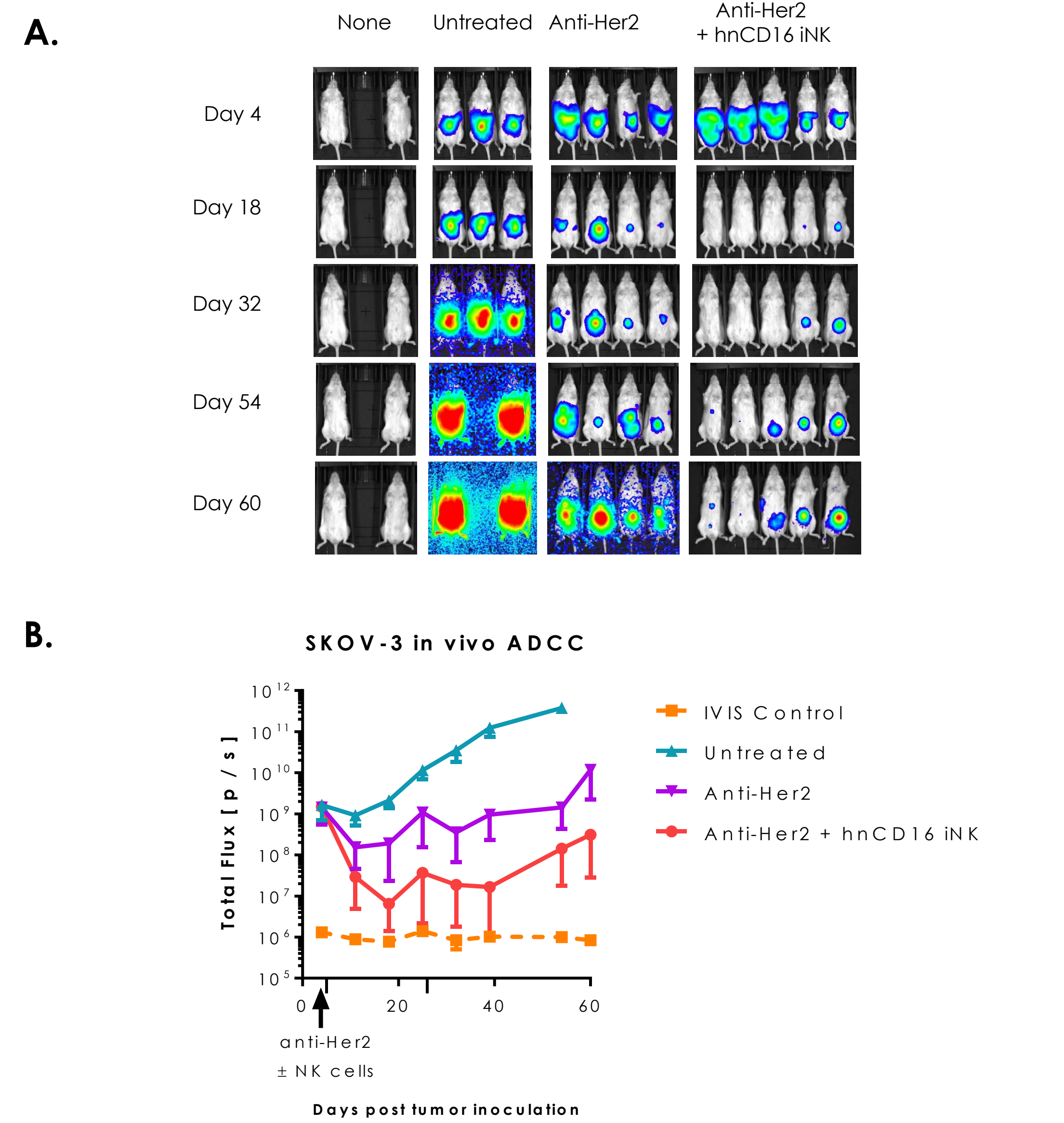


Figure 8. hnCD16-iNK effectively mediate ADCC in an in vivo ovarian cancer model. NSG mice were inoculated IP with 1×10^5 SKOV cells expressing the firefly luciferase gene, and tumor engraftment was assessed by IVIS imaging four days later. On day 5 after tumor transplant, mice were either left untreated or treated with 100 μ g anti-Her2 either alone or in combination with 5×10^6 hnCD16 iNK cells. NK cells were supported by twice-weekly injections of IL-2, and IVIS imaging was done weekly to track tumor load. **A.** IVIS images or **B.** the geometric mean - geometric SD for the mice shown in A.