

Induced Pluripotent Stem Cell Derived NK Cells Genetically Modified to Express NKG2C/DAP12 Mediate Potent Function When Targeted through an NKG2C/IL15/CD33 TriSpecific Killer Engager (TriKE)

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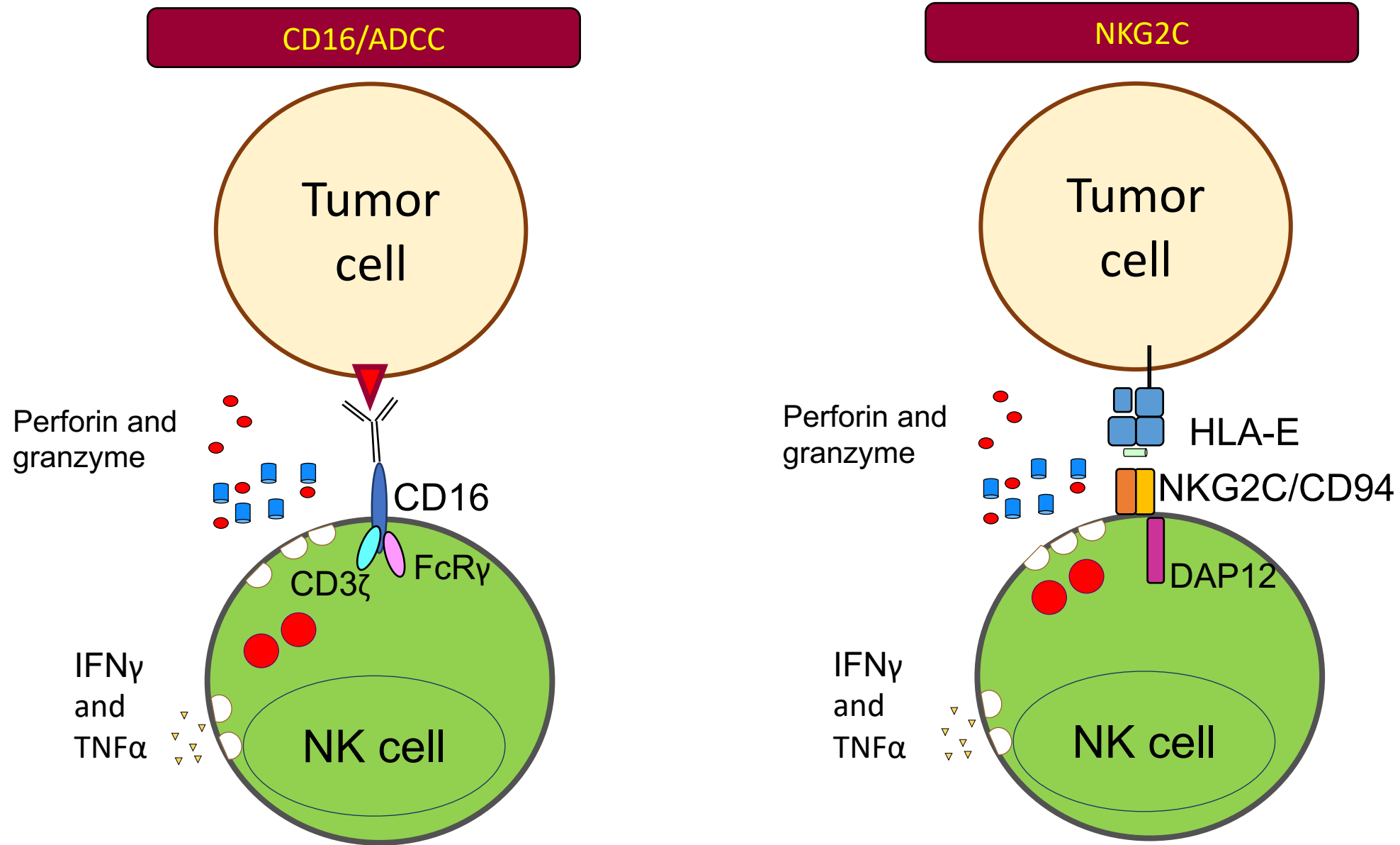
NK Cell Immunotherapy

- Natural killer cells recognize tumors and virally infected cells
- They mediate cytotoxicity and produce cytokines
- Lymphodepleting chemotherapy and haploidentical NK cell adoptive transfer have been used successfully to treat patients with refractory AML with CR rates of 30-50%
- New strategies are needed to
 - Further improve the rate and duration of CR
 - Make NK cell products immediately available in a cost-effective off-the-shelf manner
 - Enhance NK cell activity and persistence by engineering and to combine with strategies that promote antigen specificity

Barriers to current NK cell therapy

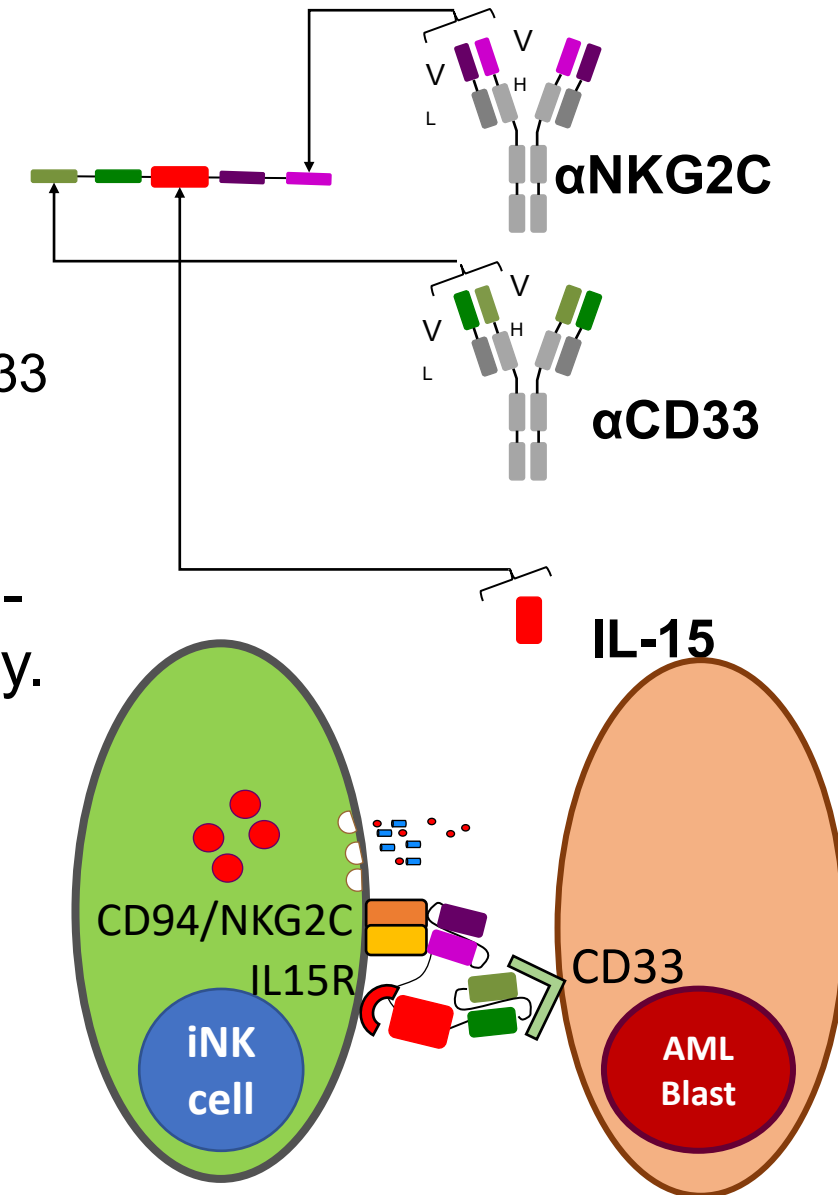
- Variability within the population makes selection of NK donors difficult and inconsistent
- Gene editing peripheral blood NK cells is difficult and inconsistent making the production of gene modified NK products impractical
- The “one donor-one product” paradigm and the associated complex GMP requirements limit peripheral blood donor NK cell adoptive transfer to specialized clinical centers
- Unlike T cells, NK cells are not antigen-specific, precluding the ability to target and expand NK cells upon engagement with tumor associated antigen

NK cells mediate anti-tumor efficacy through their Fc receptor (CD16) and NKG2C

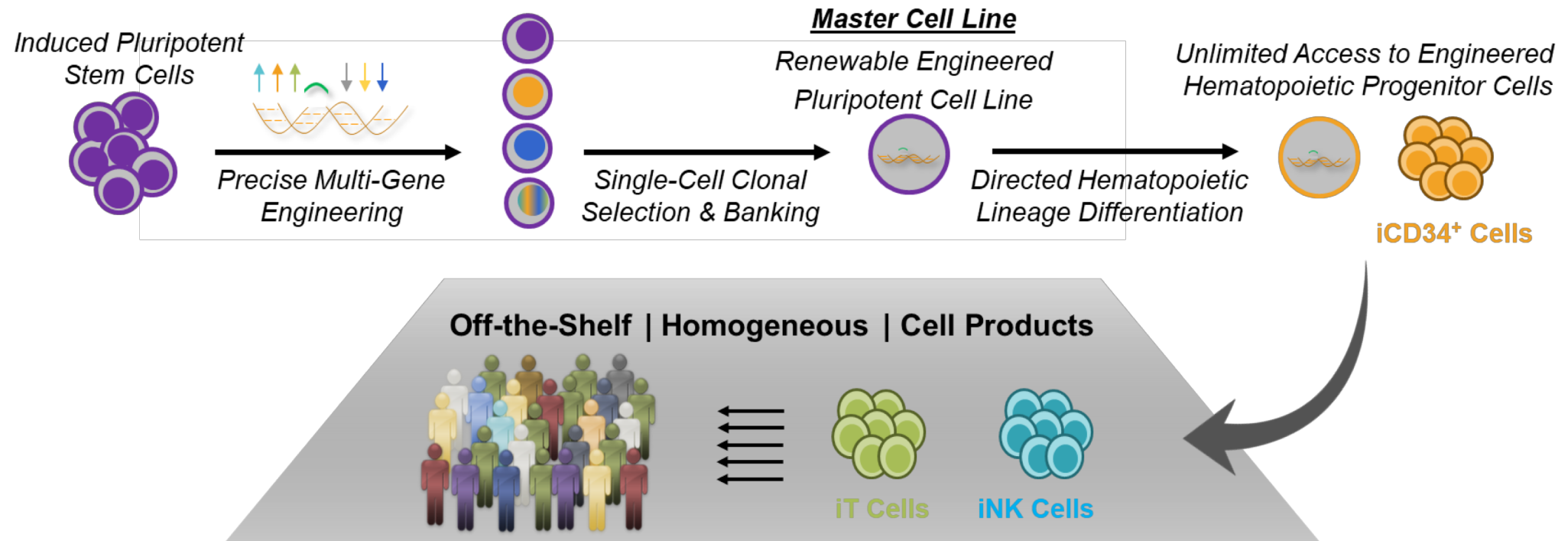


Developing an effective combinatorial off-the-shelf therapy

- We hypothesized that:
 - NK cells activated by signaling through NKG2C are effective killers of AML and other cancer types.
 - NK specificity could be enhanced by using Trispecific Killer Engagers (TriKE) designed to bind NKG2C on NK cells and CD33 on myeloid tumors with an IL-15 linker to enhance activation, proliferation and survival.
- We partnered with Fate Therapeutics to create an off-the-shelf iPSC-derived NK (iNK) cell adoptive immunotherapy.
- We genetically engineered iNK to express NKG2C ± its adaptor DAP12 be given with NKG2C1533 TriKE for preclinical testing.



Off-the-shelf hematopoietic cell products derived from single cell engineered master pluripotent cell lines



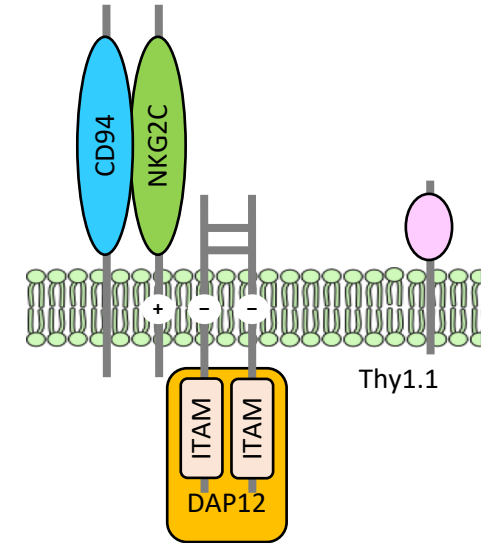
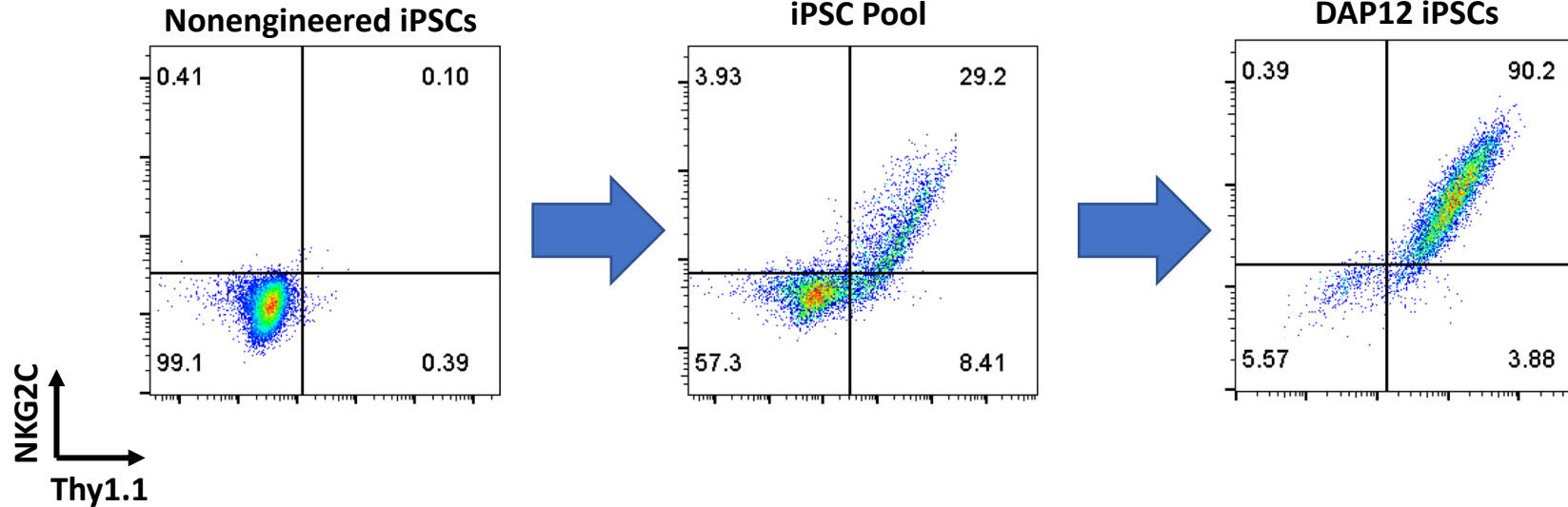
Does not require patient-sourced cells Off-the-shelf production of cells

Consistent and reliable product forms Unprecedented scalability Cost-effective

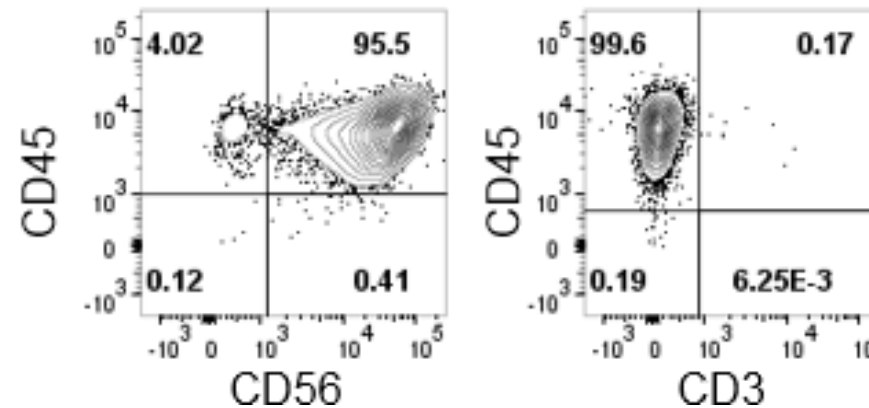
Addresses Critical Limitations of Patient-Sourced Cellular Therapies

Engineering and differentiation of iPSCs containing NKG2C/DAP12

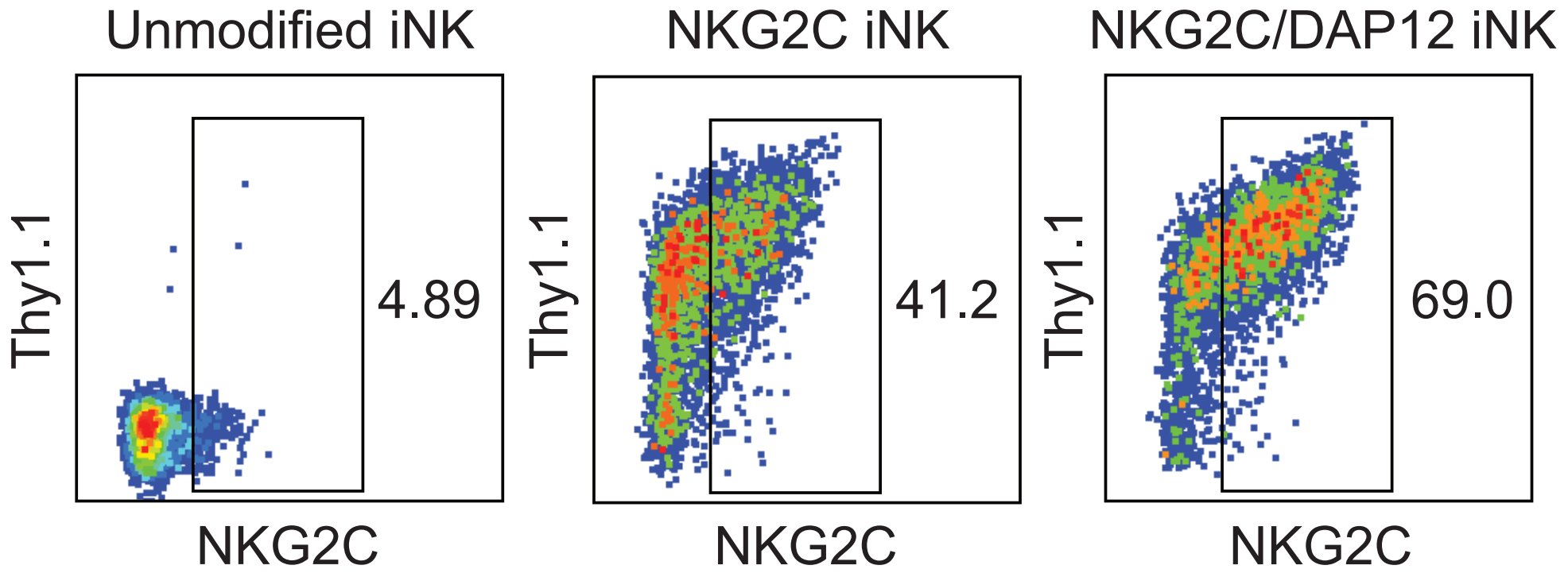
iPSC Engineering and Enrichment



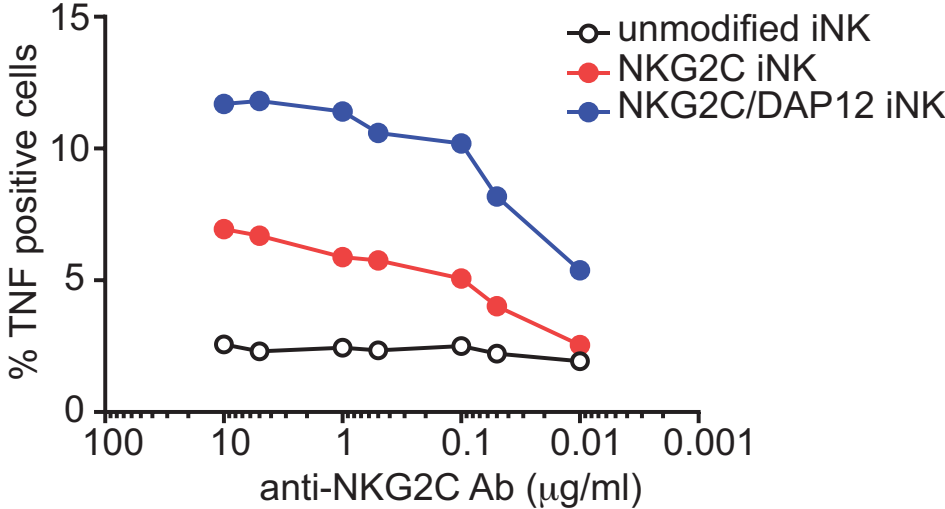
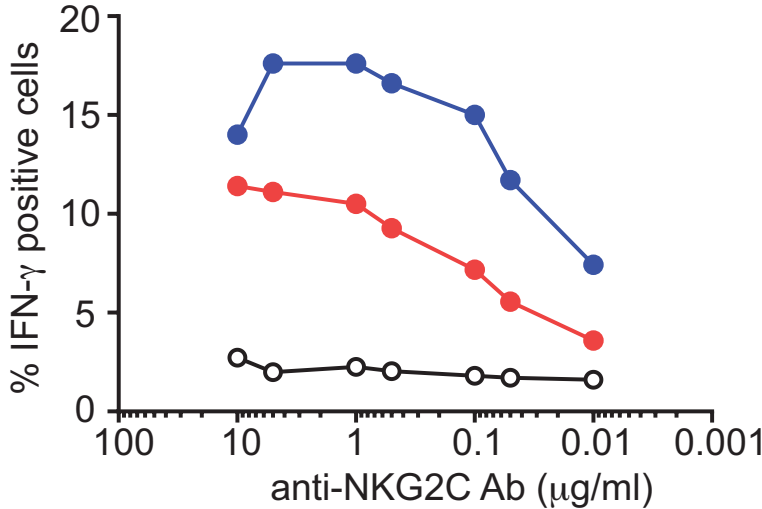
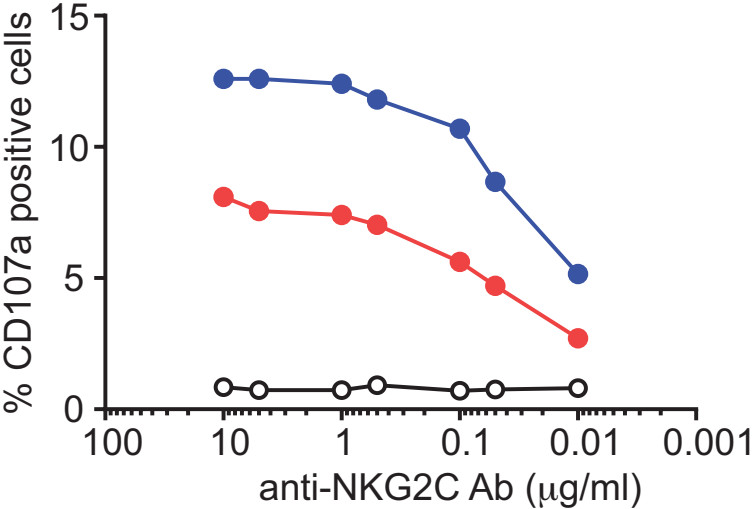
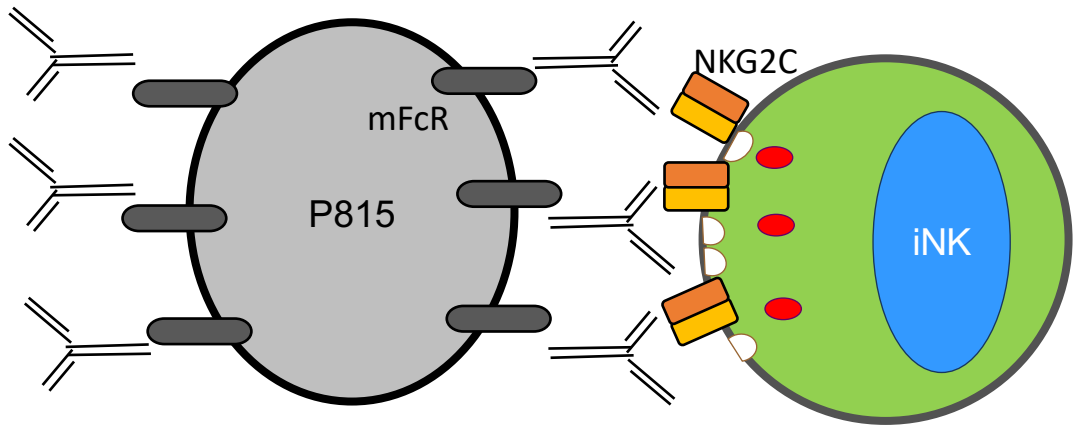
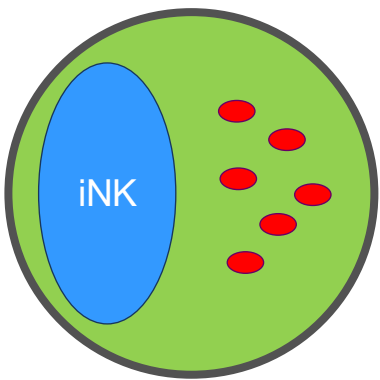
iNK cell Differentiation Profile



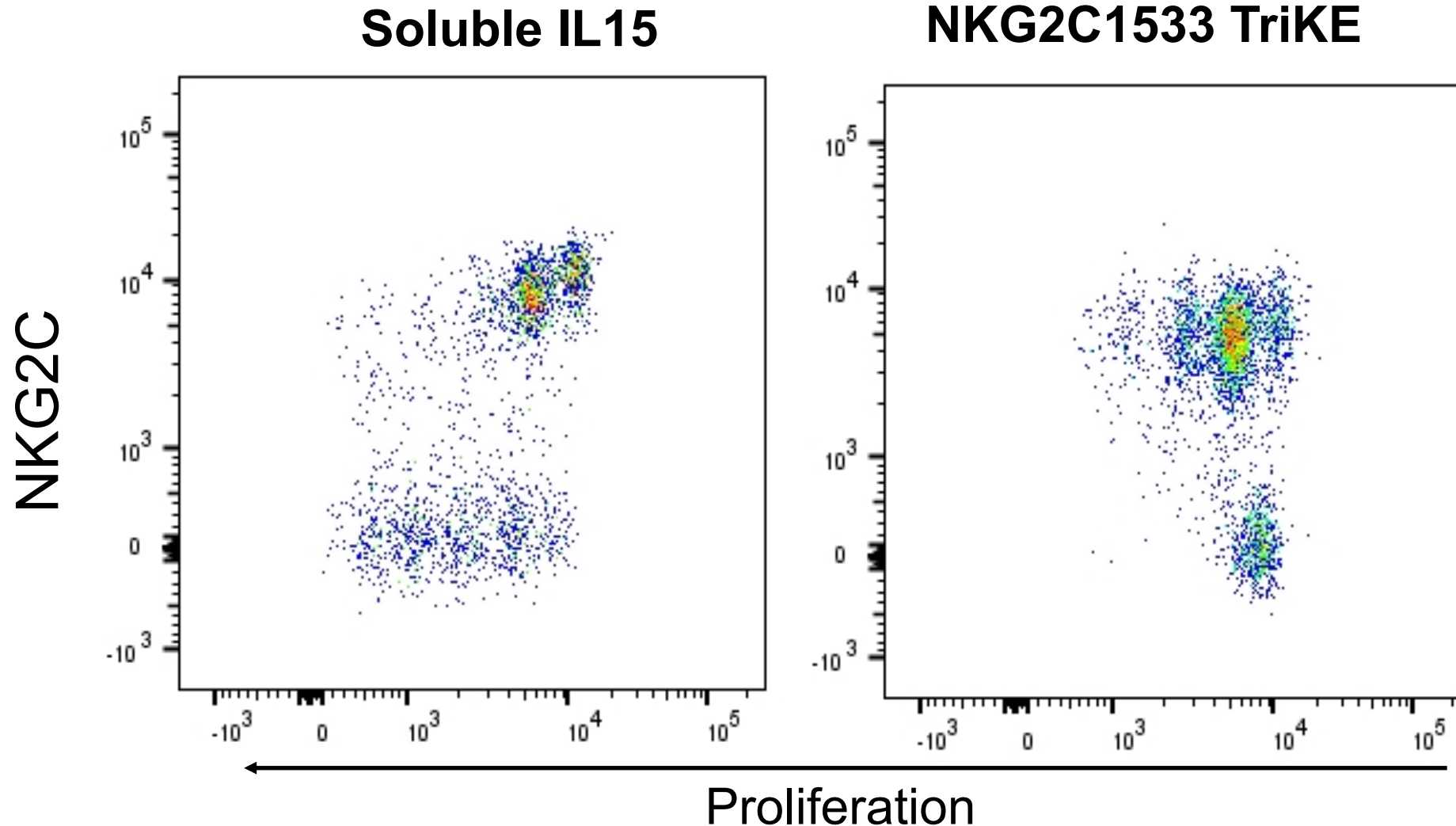
High surface expression of NKG2C on NKG2C/DAP12-transduced iNKs



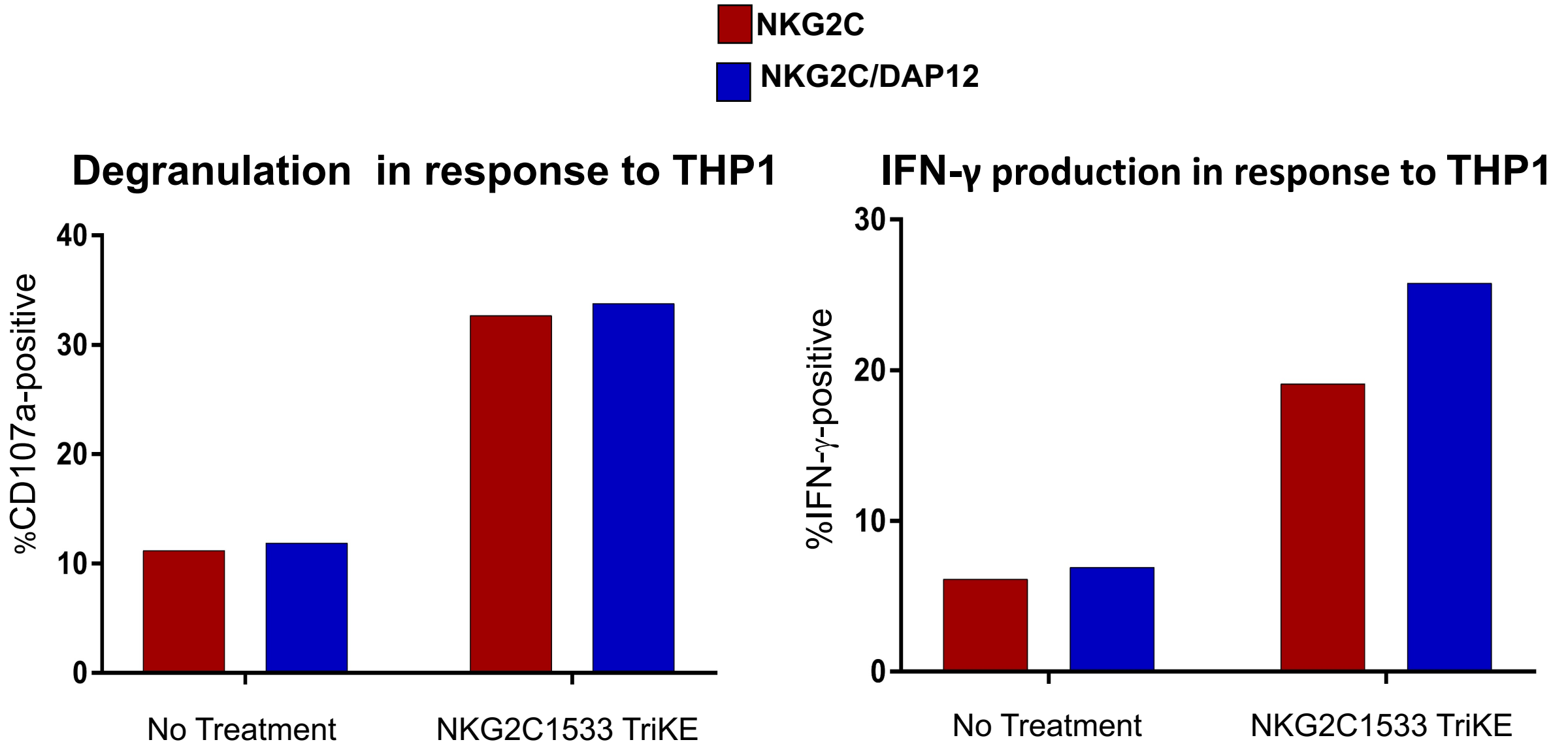
NKG2C/DAP12 iNK cells are highly functional in reverse ADCC assays



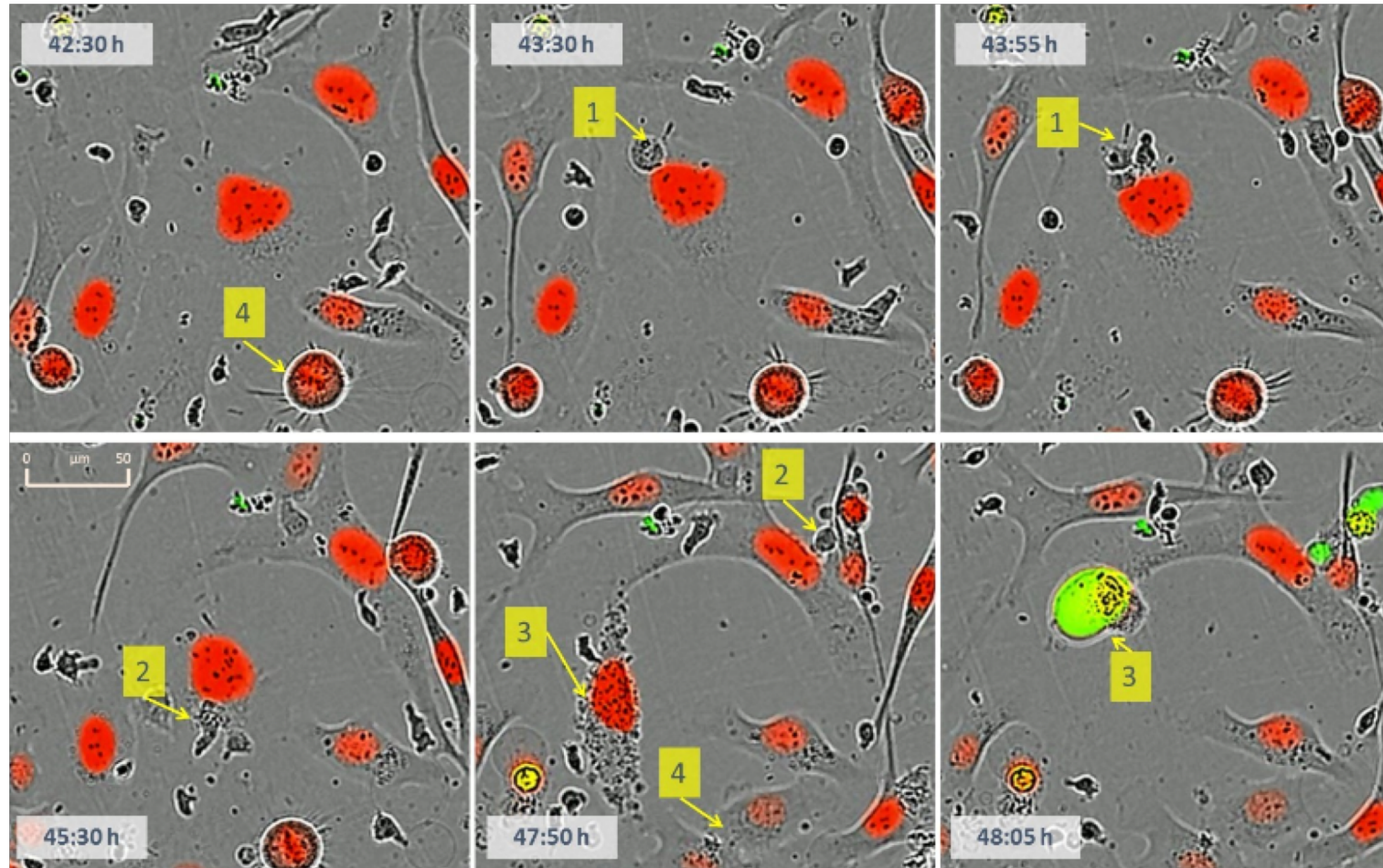
PB NKG2C⁺ NK cells proliferate selectively after NKG2C1533 TriKE but not IL-15 exposure



Degranulation and IFN γ production by NKG2C/DAP12 iNKs exposed to AML are enhanced by NKG2C1533 TriKE



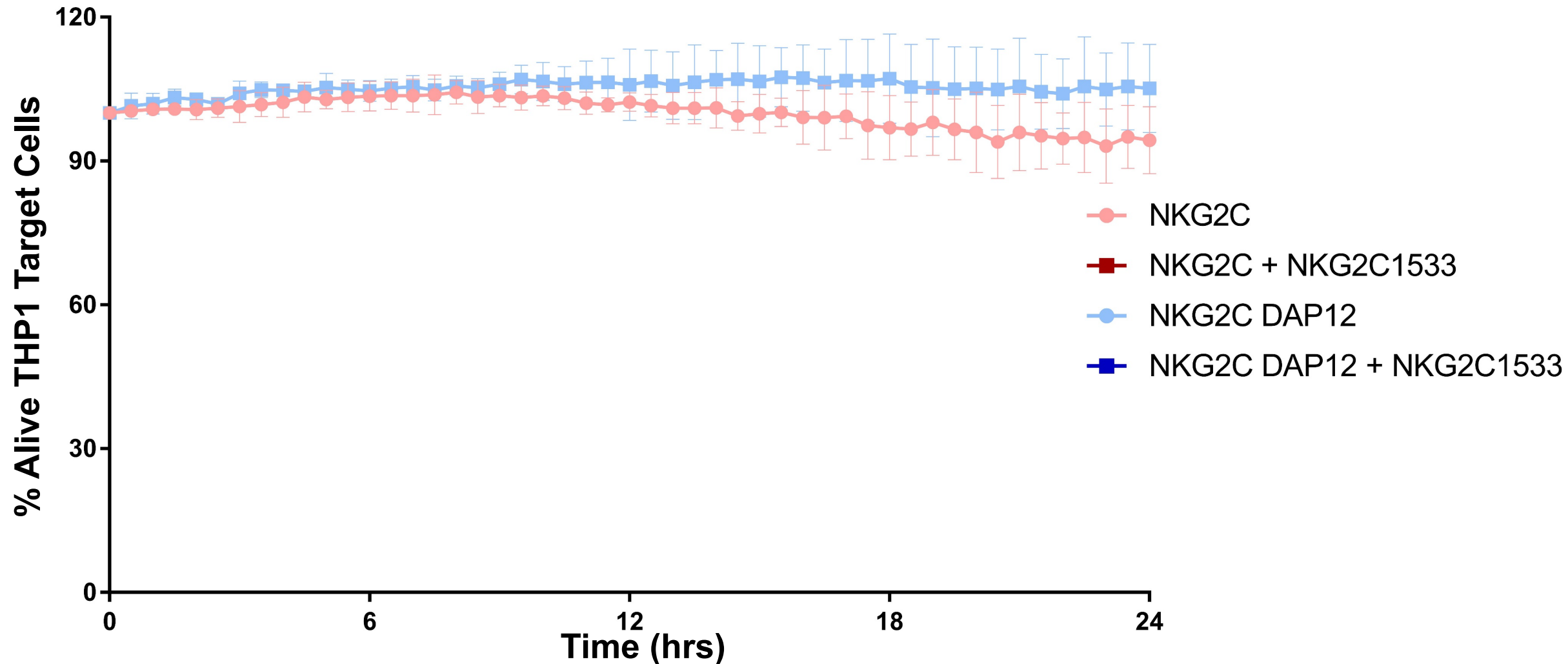
IncuCyte imaging of NK cells killing targets



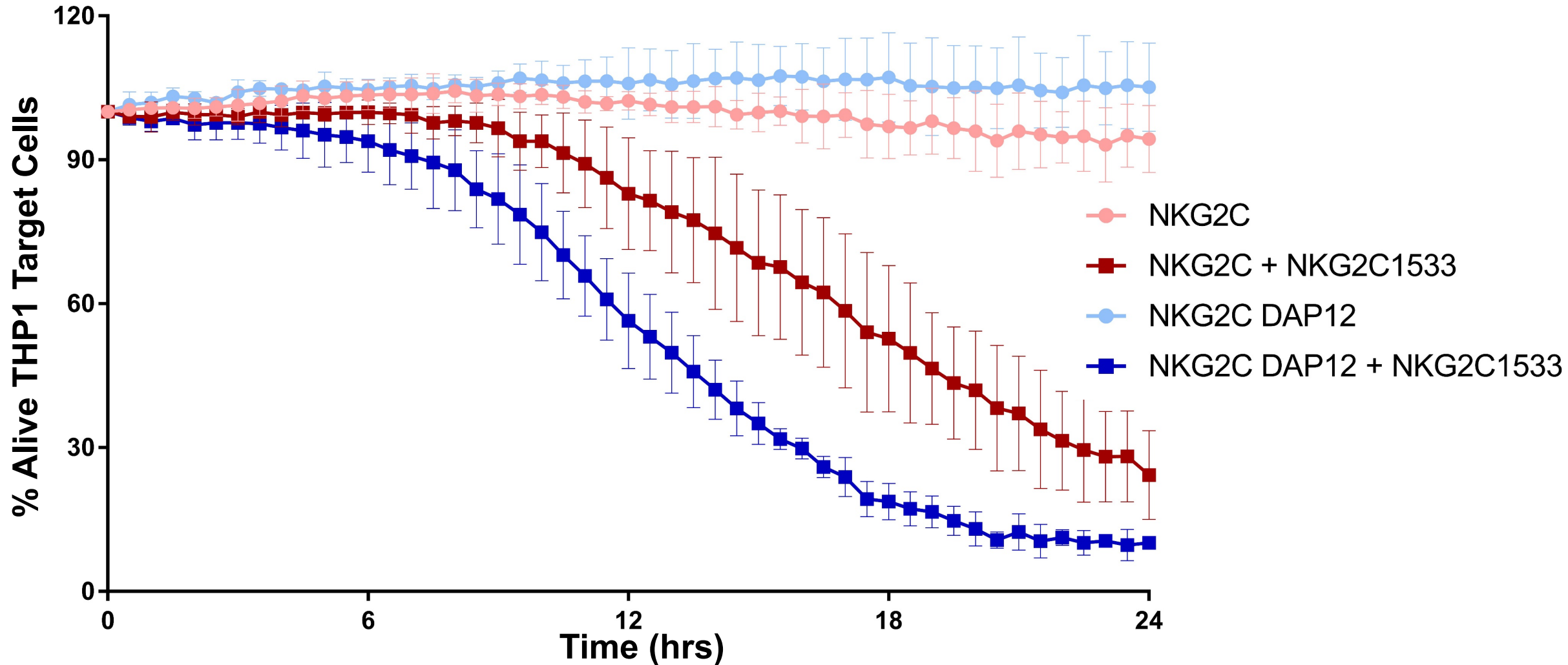
Red = tumor

Green = caspase activity (dying cell)

NKG2C-DAP12 iNKs and NKG2C1533 TriKE synergize to effectively eliminate THP1 (AML) cells



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Conclusions

- We have developed a renewable off-the-shelf population of purified NKG2C/DAP12 iNK cells through genetic modification of iPSCs followed by directed differentiation to NK cells.
- The engineered iPSCs differentiate efficiently, expand robustly in culture and are fully functional.
- iNK engineered to express NKG2C with its adaptor DAP12 have enhanced function and expansion.
- TriKEs designed to engage through NKG2C are more NK specific than CD16 TriKE, as neutrophils and monocytes are also CD16⁺.
- Xenogeneic experiments testing NKG2C/DAP12 iNK and NKG2C1533 TriKE against established myeloid tumors are ongoing.

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