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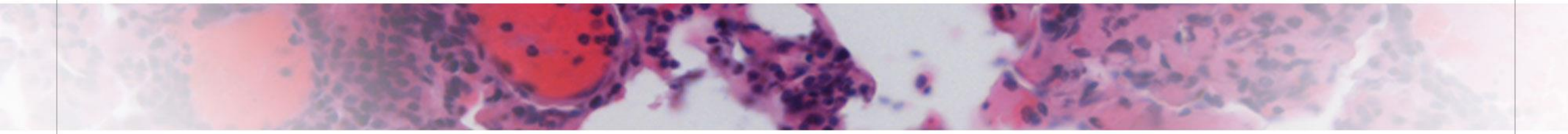
Abstract #3257



Generation of Natural Killer Cells with Enhanced Function from a CRISPR/Cas12a-Edited Induced Pluripotent Stem Cell Line

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Disclosures

- Employees and shareholders of Editas Medicine:
  J-I.M., M.S.C., A.T.B., S.S., K.W., J.M.N., L.P.A., K.K.W., C.M.B., R.A.M., G.G.W.
- Previous employment with bluebird bio:
  R.A.M.

Editing of *CISH* and *TGFBR2* is hypothesized to enhance effector function and anti-tumor activity of iPSC-derived NK cells

Advantages of NK cells

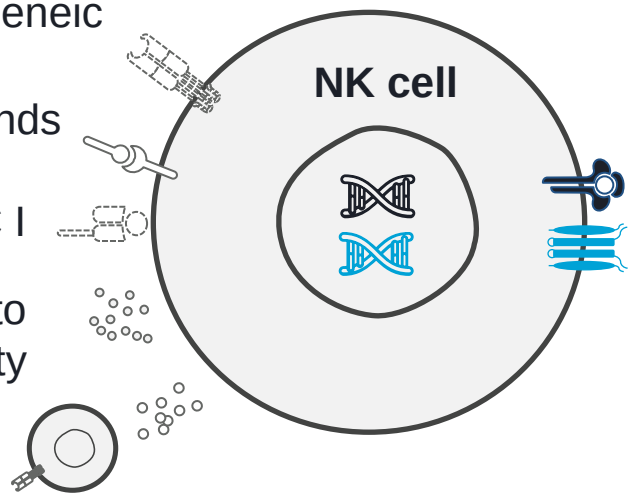
Allogeneic

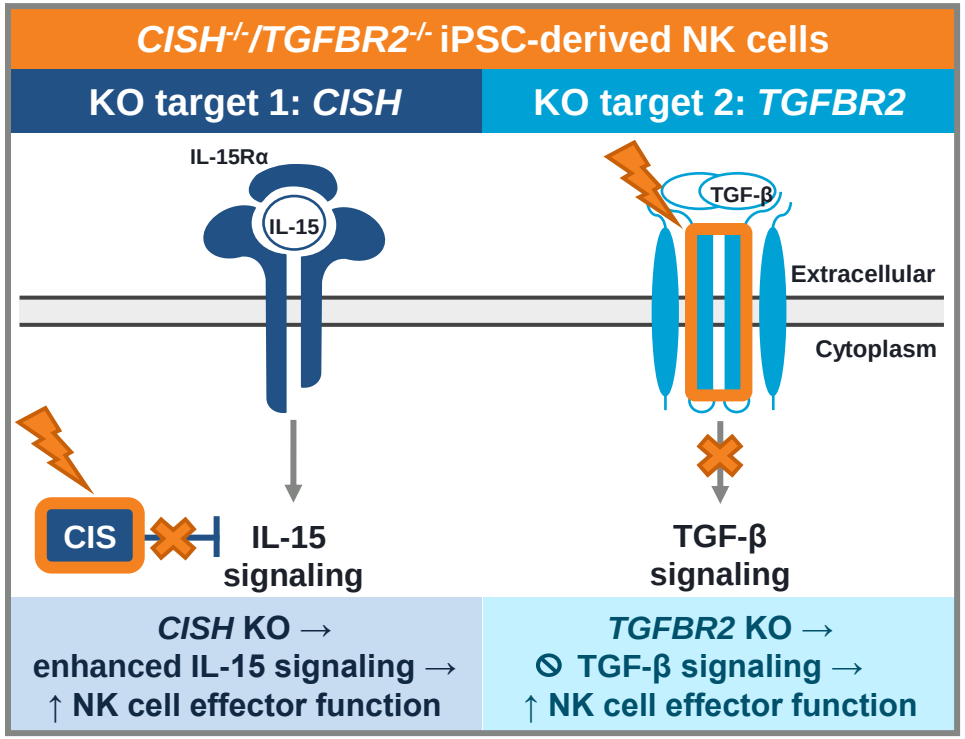
Recognize broad array of tumor ligands

Recognize malignant cells that lack MHC I

Rapid degranulation leads to robust tumor cytotoxicity

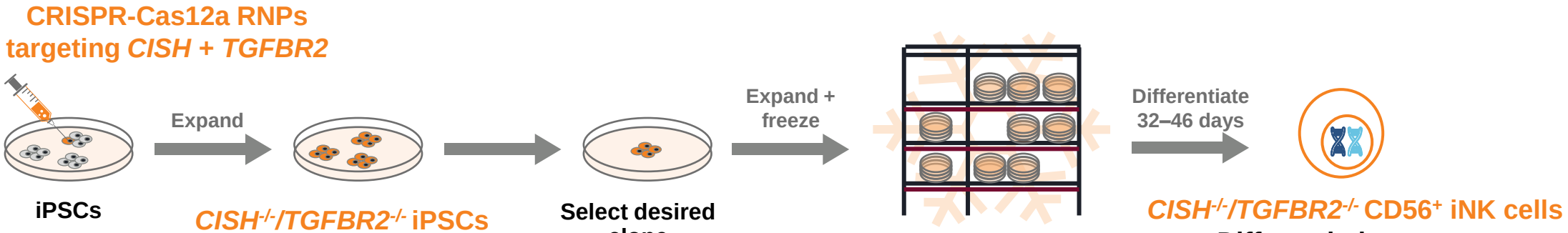
Recruit and engage cells of the adaptive immune system to enhance tumor cytotoxicity





CISH: cytokine-inducible SH2-containing protein gene; IL: interleukin; iPSC: induced pluripotent stem cell;
KO: knock out; MHC: major histocompatibility complex; NK: natural killer; TGF-β: tumor growth factor beta; TGFBR2: TGF-β receptor II gene

Development of a CRISPR-Cas12a-edited iPSC platform for the generation of enhanced CD56^{+} iNK cells



- Editing efficacy (next-generation sequencing)
- Pluripotency & differentiation potential (IHC)

Differentiation assays

- Flow cytometry
- Embryoid bodies

Molecular and functional analyses

- pFLOW
- Cytokine production
- Spheroid killing assay (SK-OV-3)
- Serial killing assay

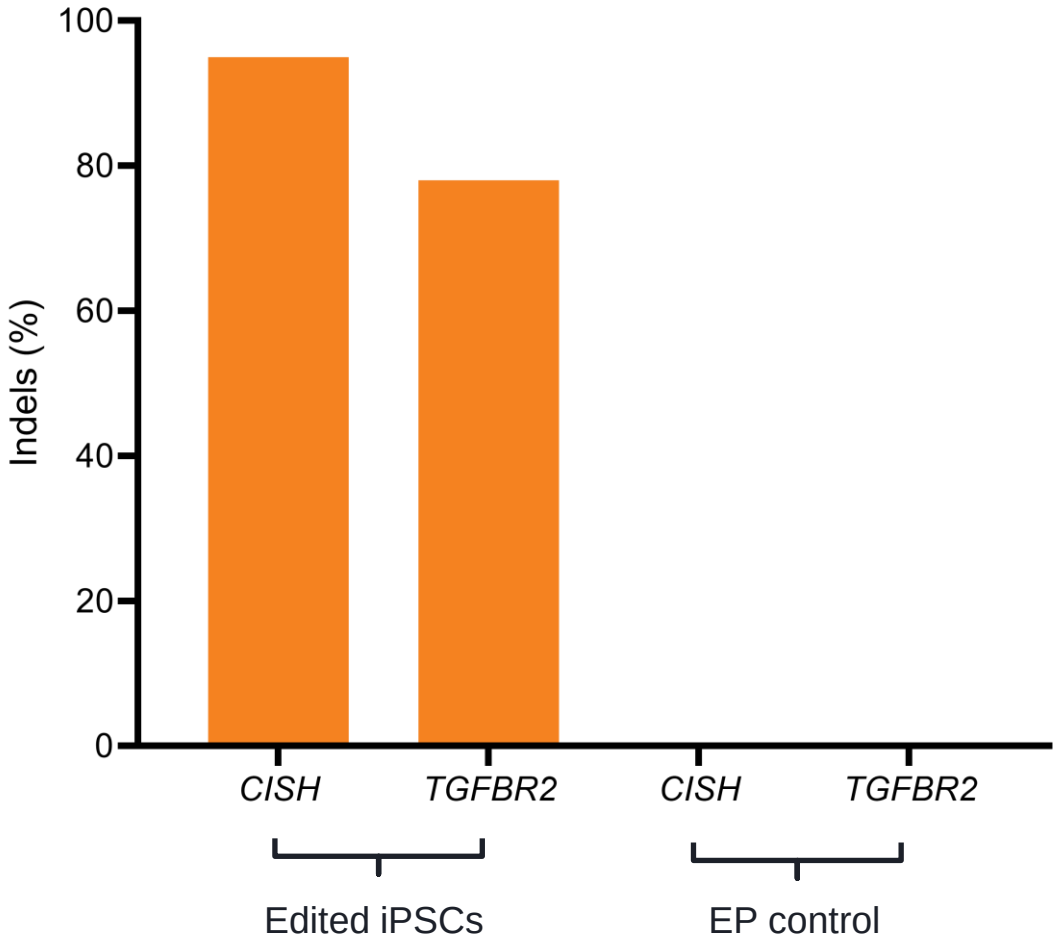
Objective:

To evaluate the enhanced effector function of CRISPR-Cas12a ***CISH$^{-/-}$/TGFBR2$^{-/-}$*** **human iPSC-derived NK cells** *in vitro*

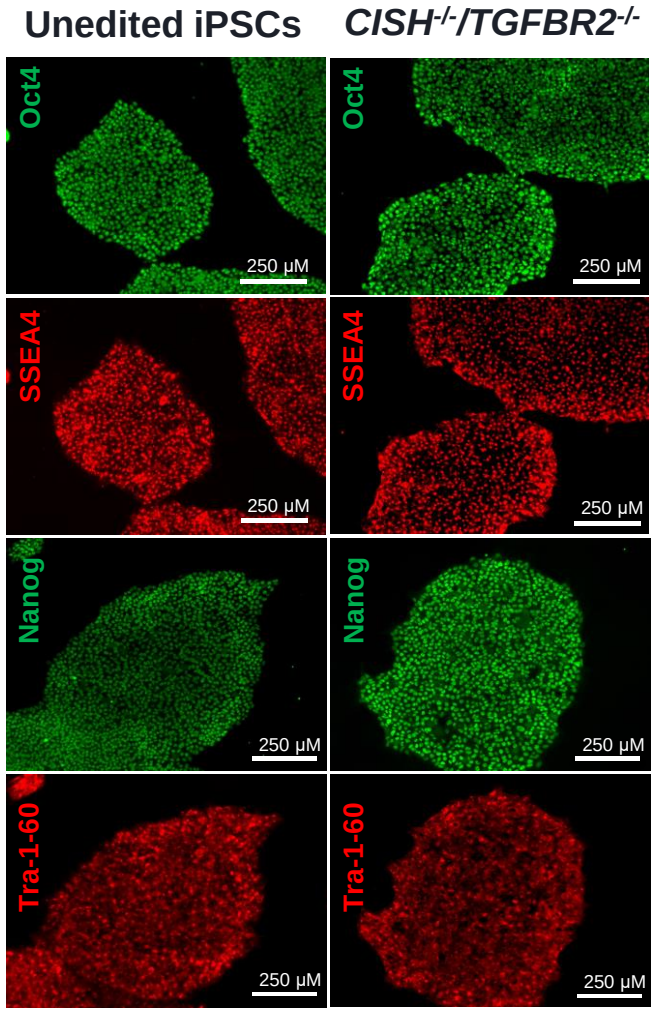
CRISPR-Cas12a: clustered regularly interspaced short palindromic repeats-Caspase 12a;
IHC: immunohistochemistry; iNK: natural killer cell derived from induced pluripotent stem cells; pFLOW: phosphorylating flow cytometry; RNP: ribonucleic protein

Successful generation of *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ iPSCs that retain normal stemness marker expression

Robust multiplex editing



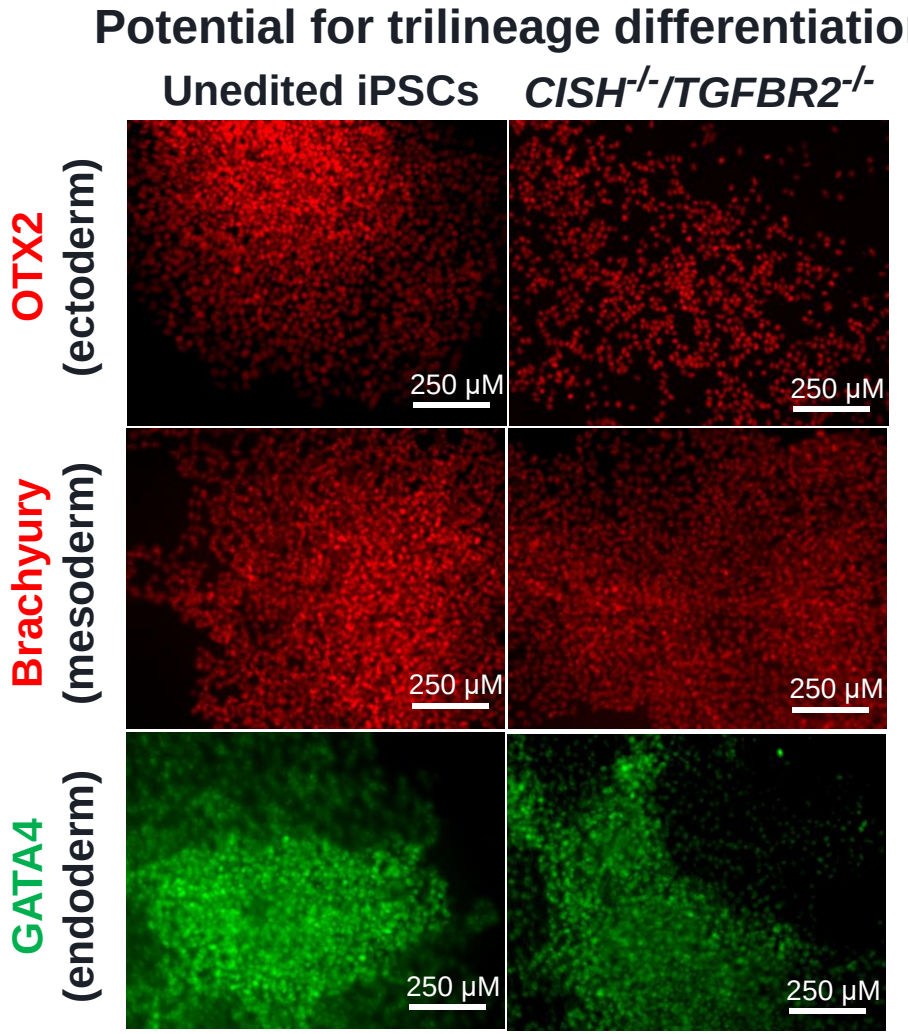
Normal stemness



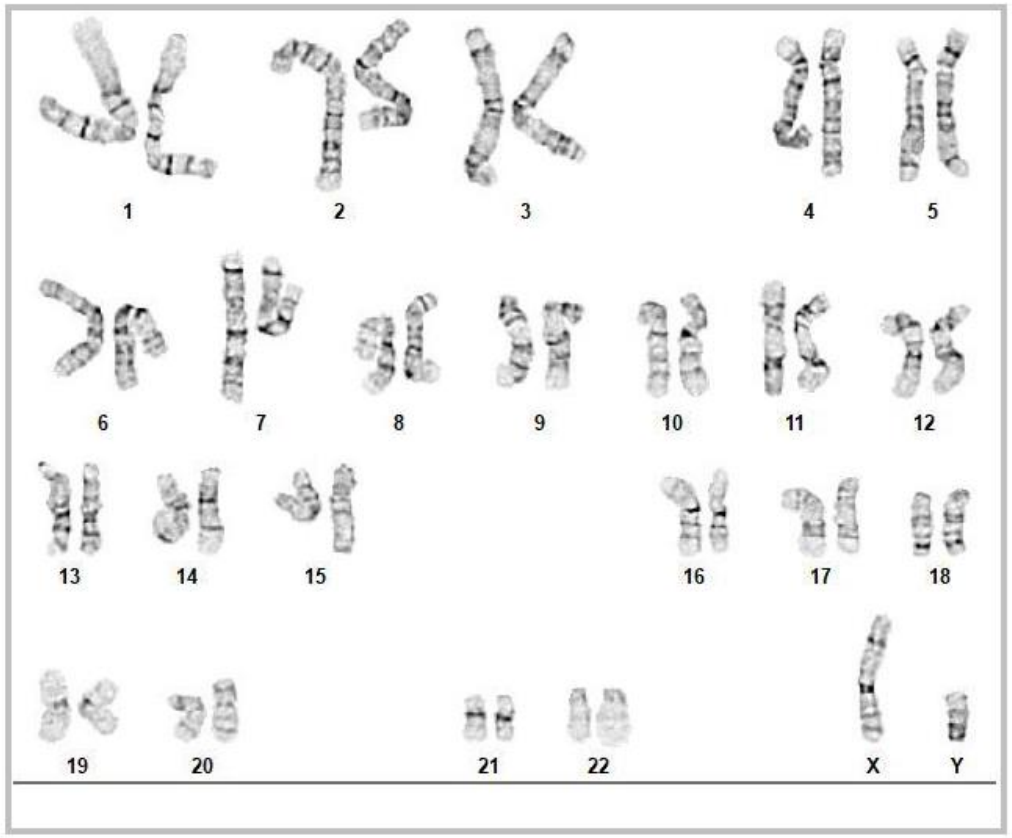
EP: electroporation

CISH$^{-/-}$/*TGFBR2*$^{-/-}$ iPSCs retain their potential for trilineage differentiation and have normal karyotypes

Potential for trilineage differentiation

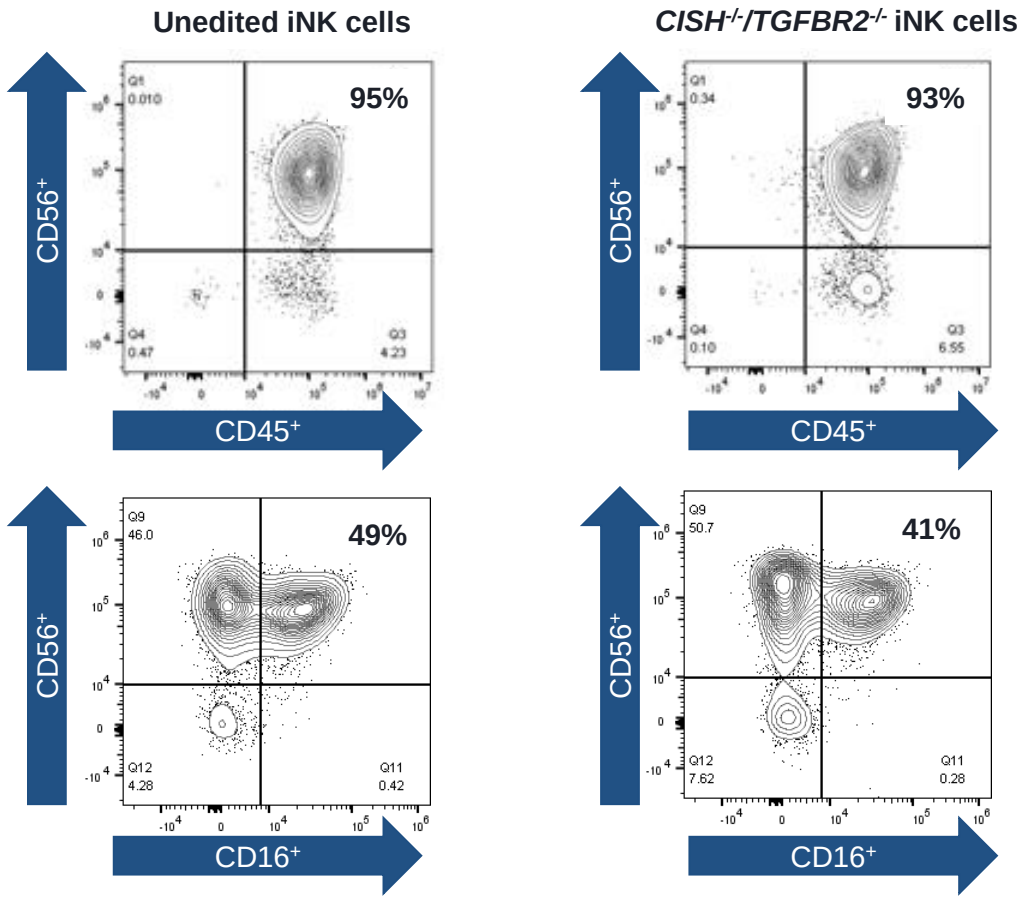


Normal karyotypes with no translocation between the cut sites



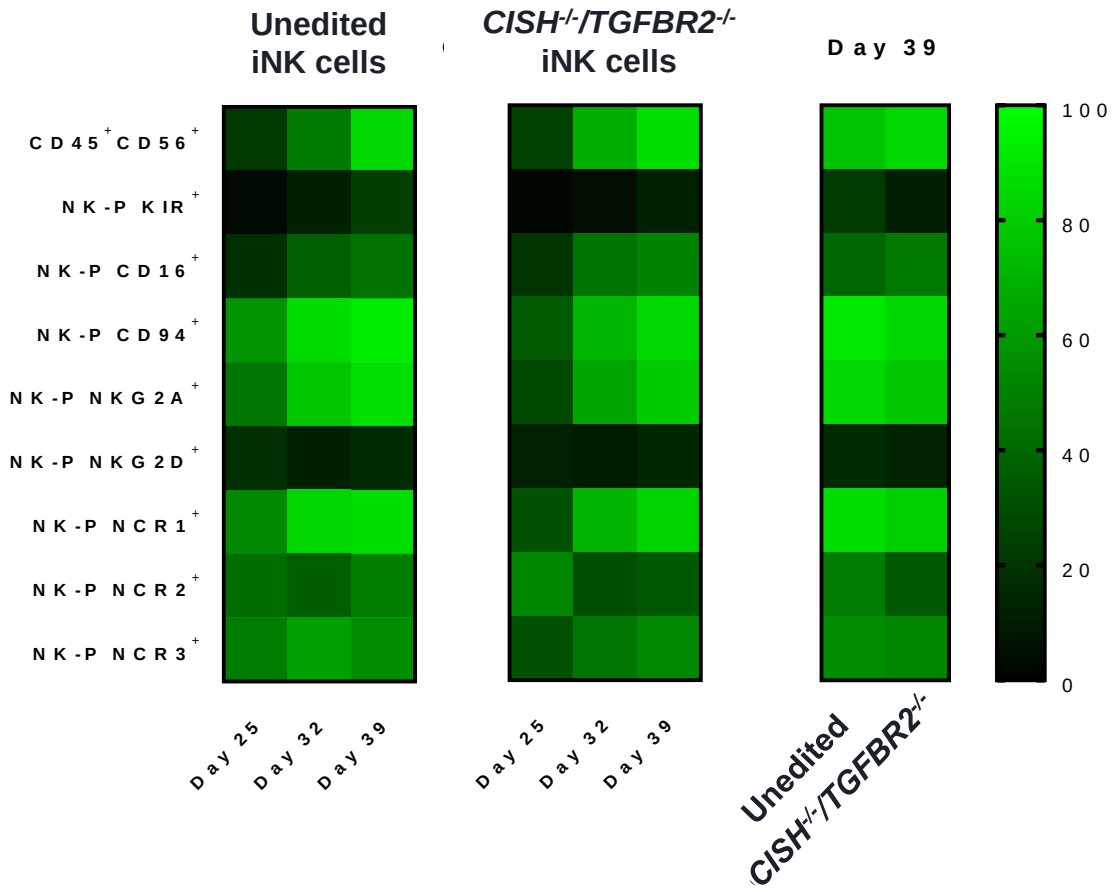
CISH$^{-/-}$/*TGFBR2*$^{-/-}$ iPSCs successfully differentiated into CD56^{+} iNK cells with canonical NK cell markers

Identification of distinct iNK cell populations by flow cytometry



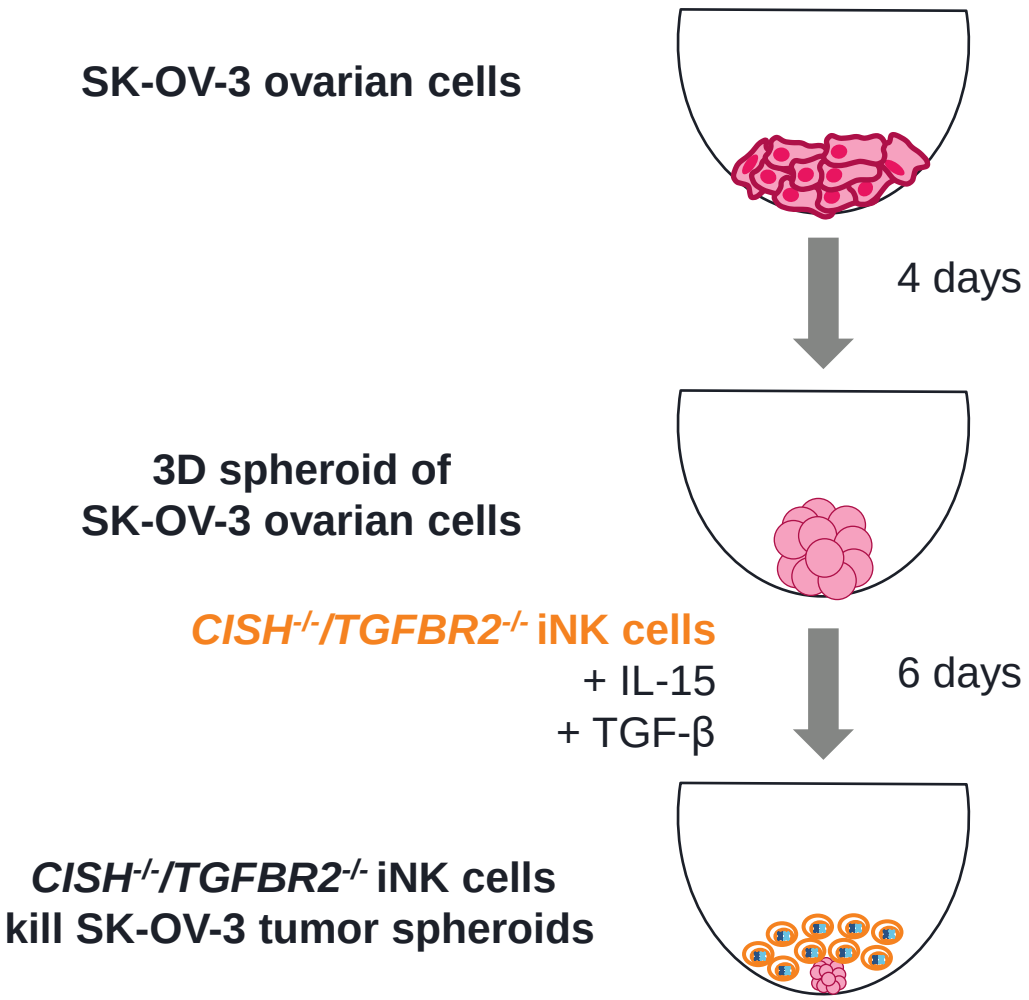
Representative plots from a single experiment

Edited iNK cells expressed NK cell markers, similar to unedited controls

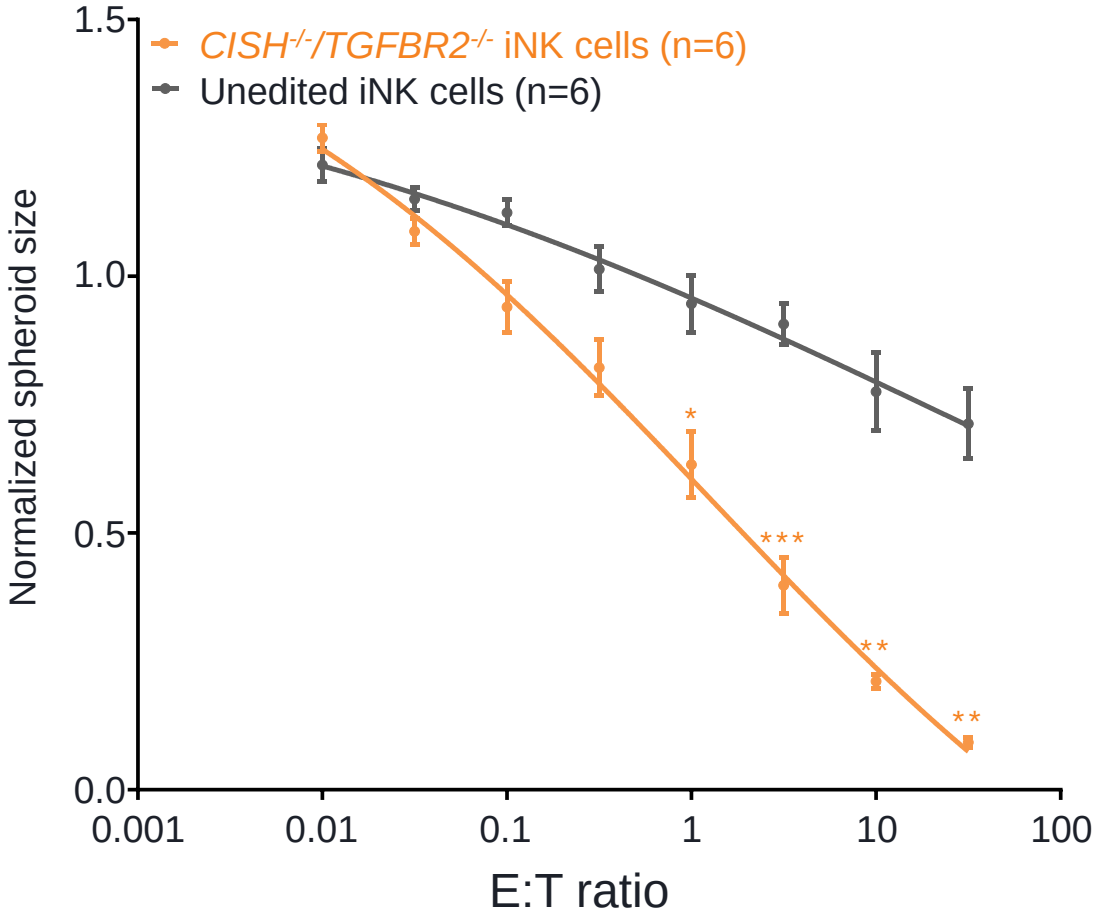


Average values from >5 differentiations

CISH$^{-/-}$*/TGFBR2*$^{-/-}$ iNK cells demonstrated enhanced anti-tumor activity against SK-OV-3 ovarian tumor spheroids



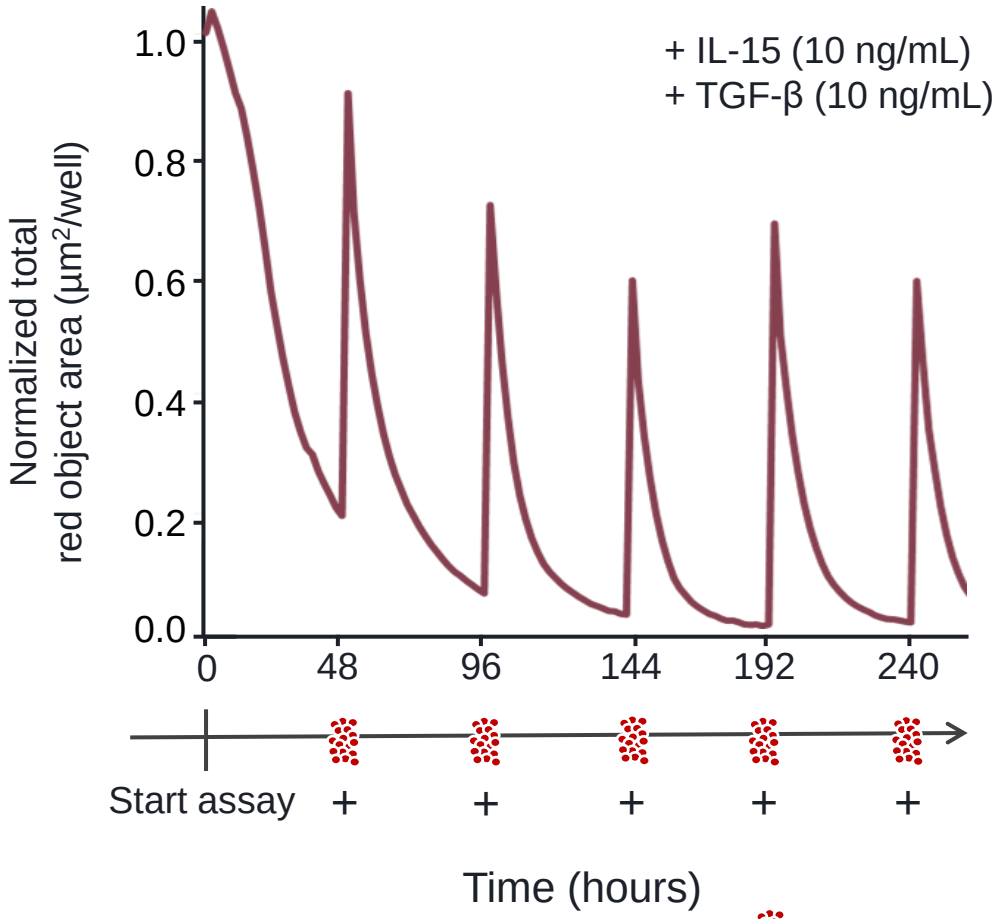
Greater reduction of SK-OV-3 spheroid size



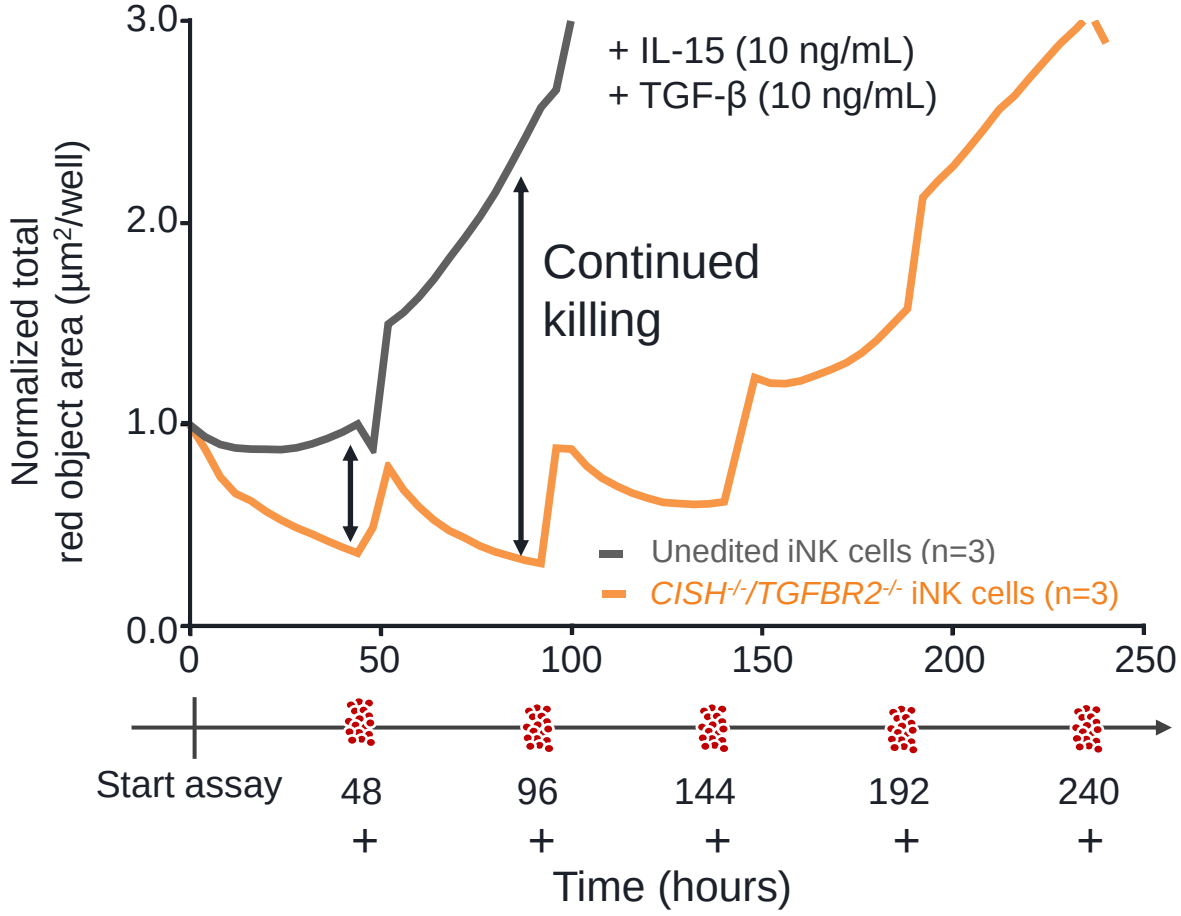
*p<0.05; **p<0.01; ***p<0.001 vs unedited iNK cells (two-way ANOVA, Sidak's multiple comparisons test)

CISH$^{-/-}$/*TGFBR2*$^{-/-}$ iNK cells demonstrated enhanced sustained serial killing of a B cell leukemia cell line (Nalm6)

Serial killing assay challenges NK cells with new Nalm6 cells every 48 hours for up to 12 days



Enhanced sustained serial killing of Nalm6 cells



= Tumor cell addition (5×10^6 cells)

Conclusions

Established a **robust, consistent, and highly effective CRISPR-Cas12a editing platform in iPSCs** that may be generalized to other targets

Unedited and *CISH*$^{-/-}$/*TGFBR2*$^{-/-}$ iPSCs differentiated into **iNK cells with canonical NK cell markers**

***CISH*$^{-/-}$*/TGFBR2*$^{-/-}$ iNK cells demonstrated enhanced anti-tumor activity** against tumor cell lines derived from both solid and hematological malignancies

This demonstrates the utility of this iPSC platform to create **multiple, off-the-shelf edited cell therapy medicines** for future application to a broad range of oncology indications

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