

# ENGINEERED ALLOGENEIC CAR-NATURAL KILLER CELLS RESIST TUMOR MICROENVIRONMENT IMMUNO-SUPPRESSION BY EXPRESSION OF A TGFB2 DOMINANT NEGATIVE RECEPTOR

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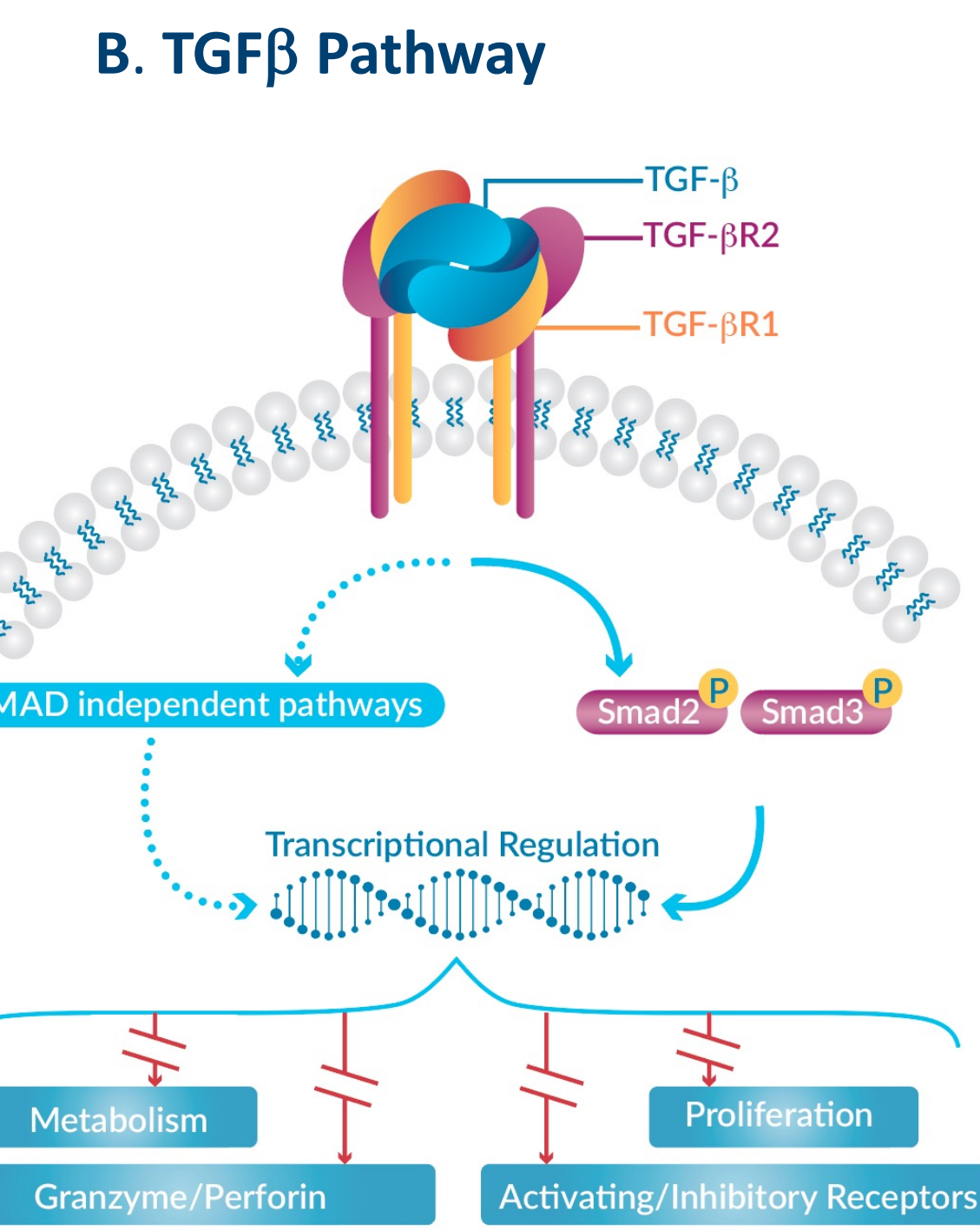
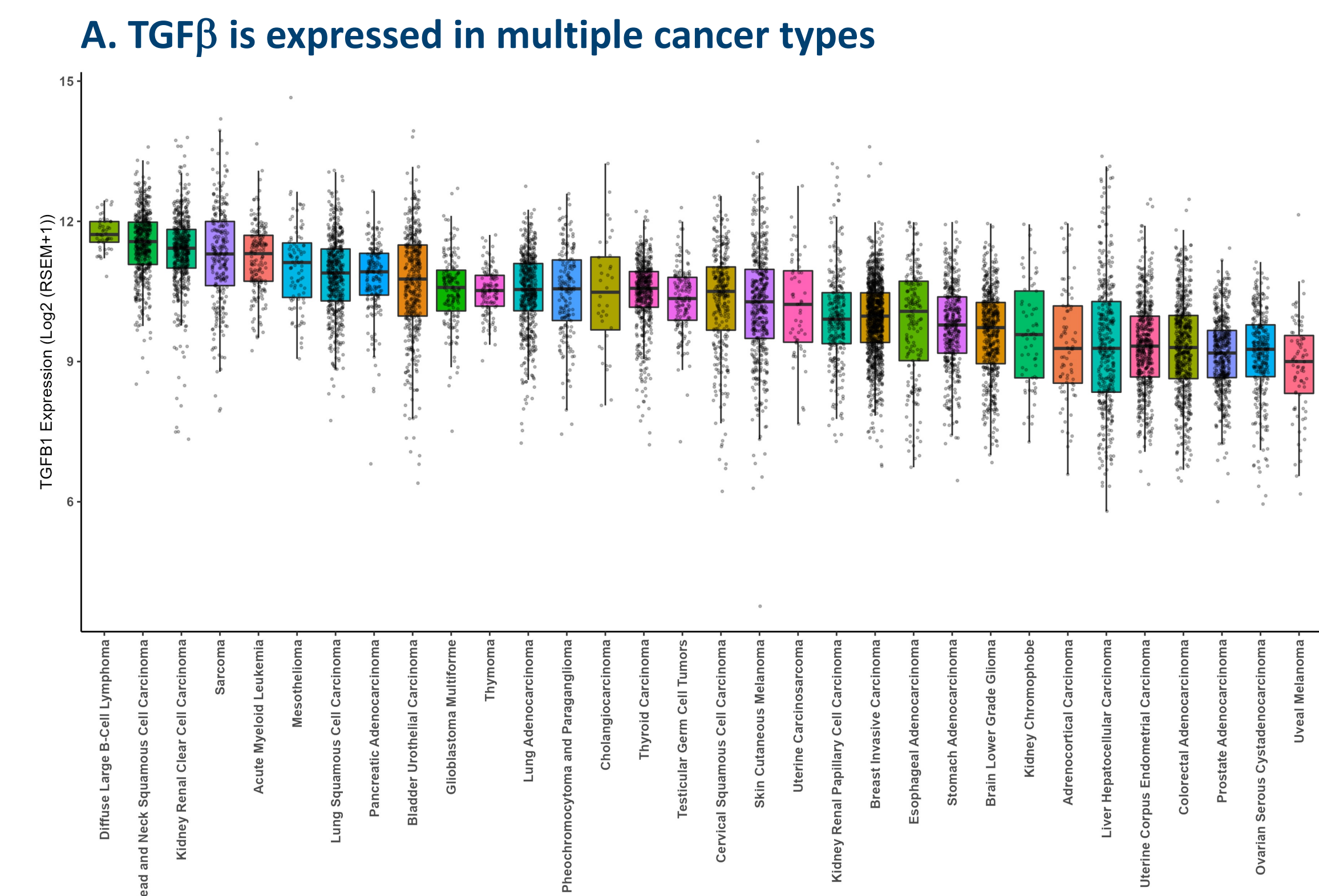
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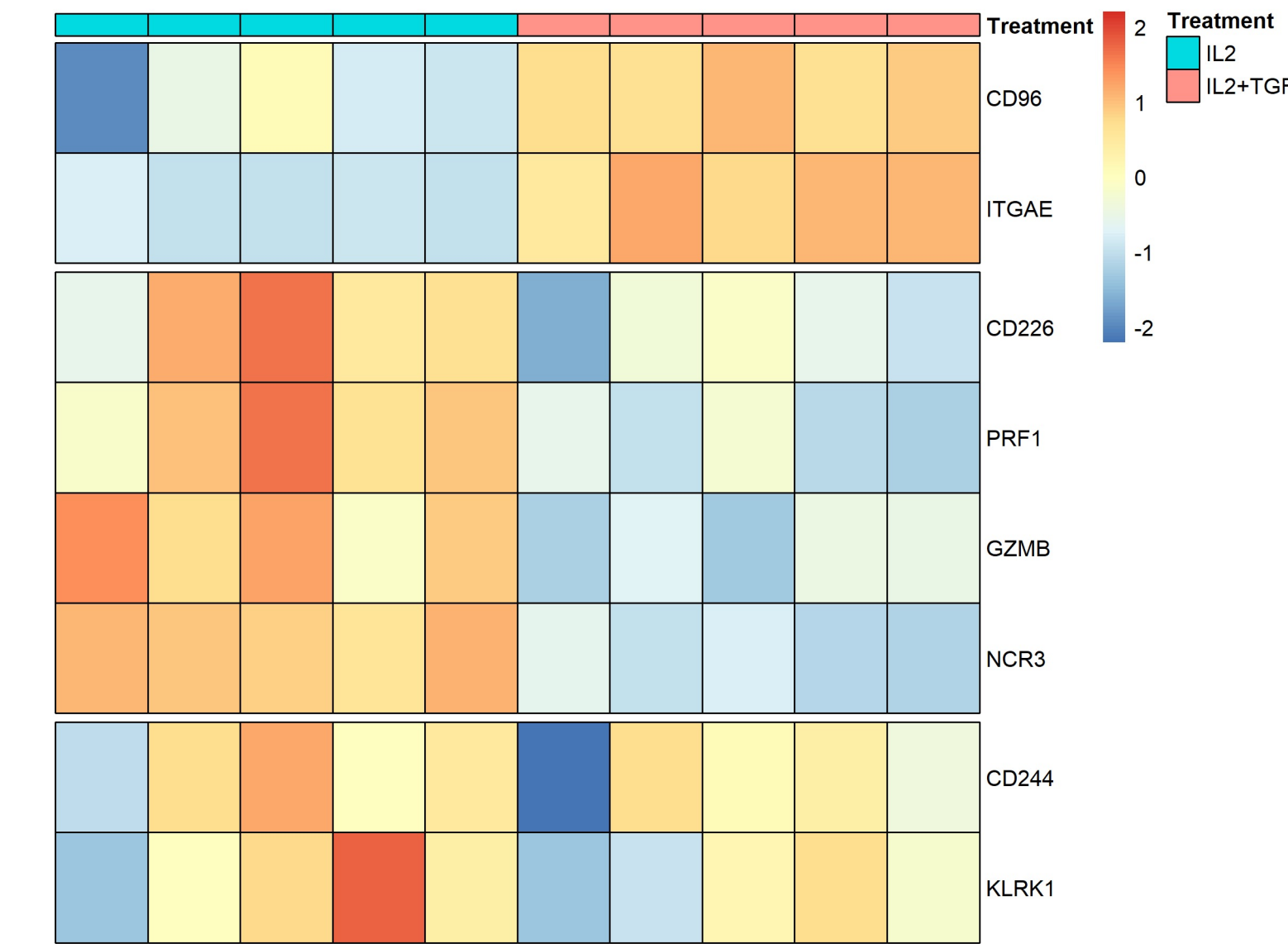
## INTRODUCTION

The deep and durable clinical responses seen with autologous chimeric antigen receptor (CAR) T cells in hematologic malignancies demonstrate the power and promise of adoptive immune cell therapy. However, clinical responses with CAR-T cells in solid tumors have been more limited. Furthermore, challenges with manufacturing of autologous cell therapy limit availability to patients. CAR-Natural Killer (NK) cell therapies offer new advantages, including their potential as allogeneic off-the-shelf products and their innate ability to kill tumor cells. However, clinical success in solid tumors will require overcoming the immunosuppressive tumor microenvironment (TME). TGFβ, a cytokine commonly expressed in the TME, limits the anti-tumor activities of NK cells through multiple mechanisms. **Here we developed a TGFB2 Dominant Negative Receptor (DNR) as a TME switch to protect the function of our engineered CAR-NK cell targeting HER2 for application in solid tumors.**

### 1 TGFβ is prevalent across cancer indications and perturbs expression of NK cell functional genes

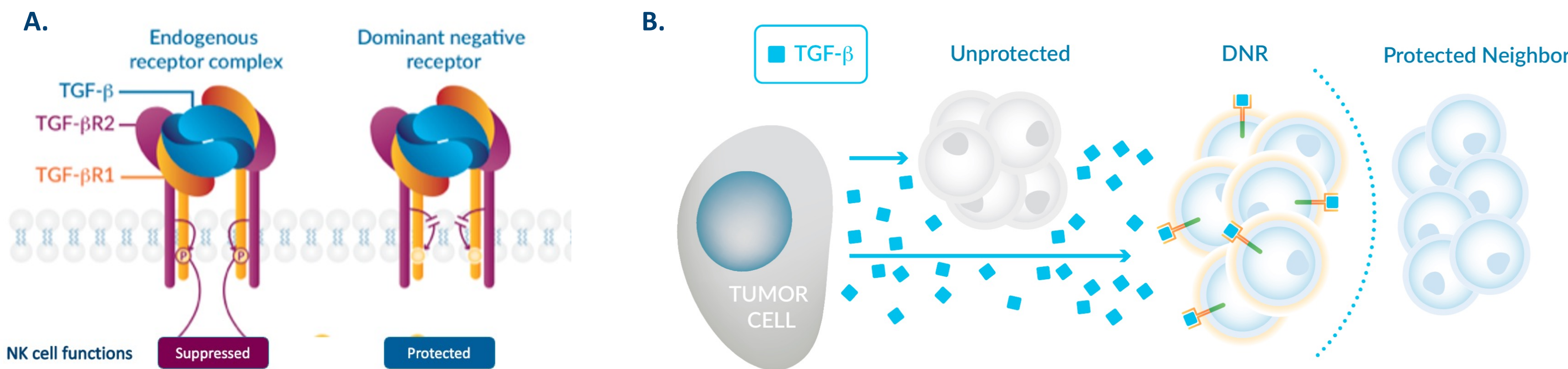


### C. TGFβ changes expression in multiple NK genes



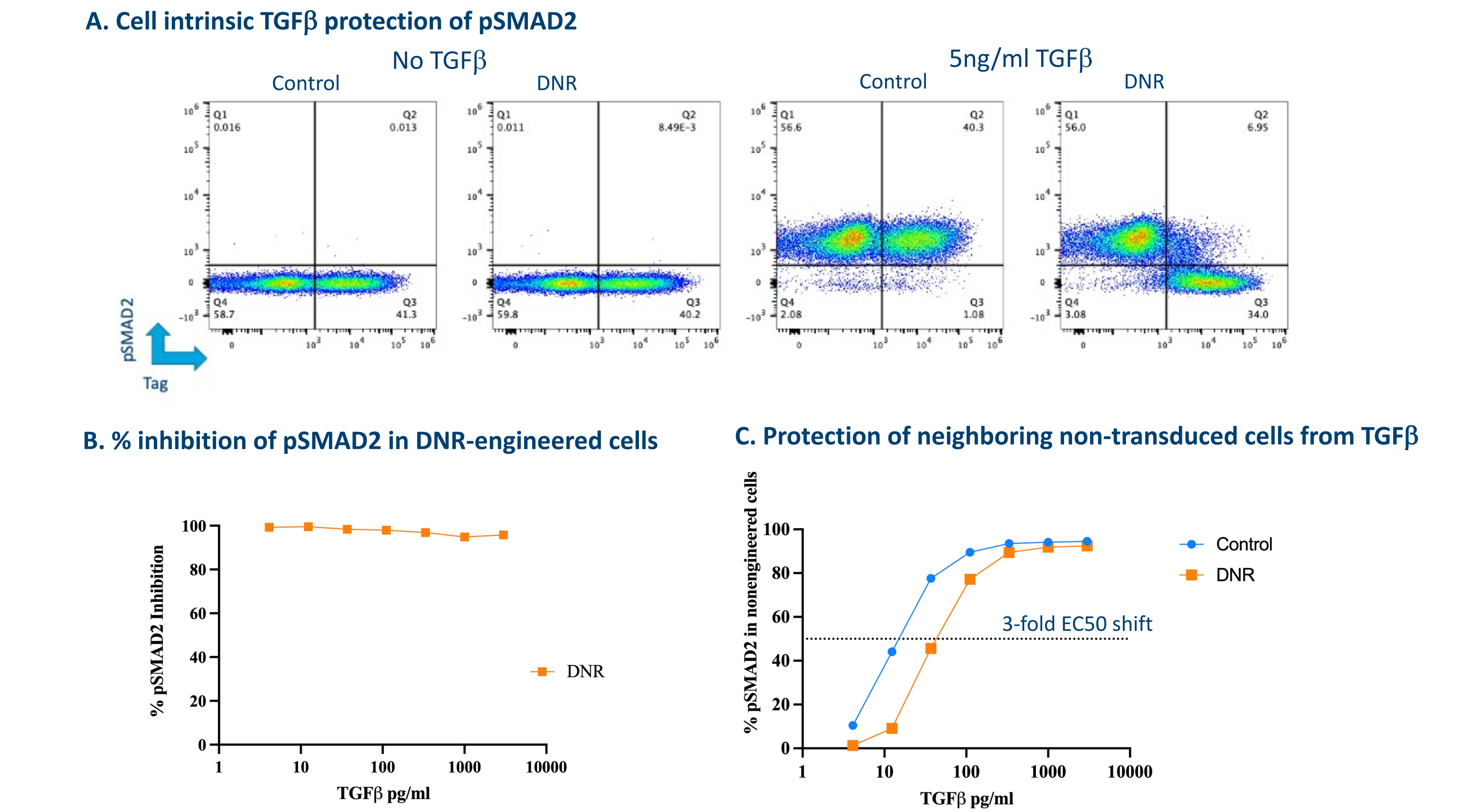
- A) TGFβ1 is highly expressed in multiple cancer indications. Data obtained from public TCGA dataset.  
B) TGFβ signaling pathway demonstrating how TGFβ can downregulate multiple parameters in NK cell function.  
C) Expanded NK cells were treated with or without 5 ng/ml TGFβ for 3 days, and then harvested for bulk RNA sequencing (Genewiz). Data was analyzed for various NK activating and inhibitory genes. N=5 donors tested.

### 2 TGFB2-DNR can protect against both intrinsic and extrinsic TGFβ suppression



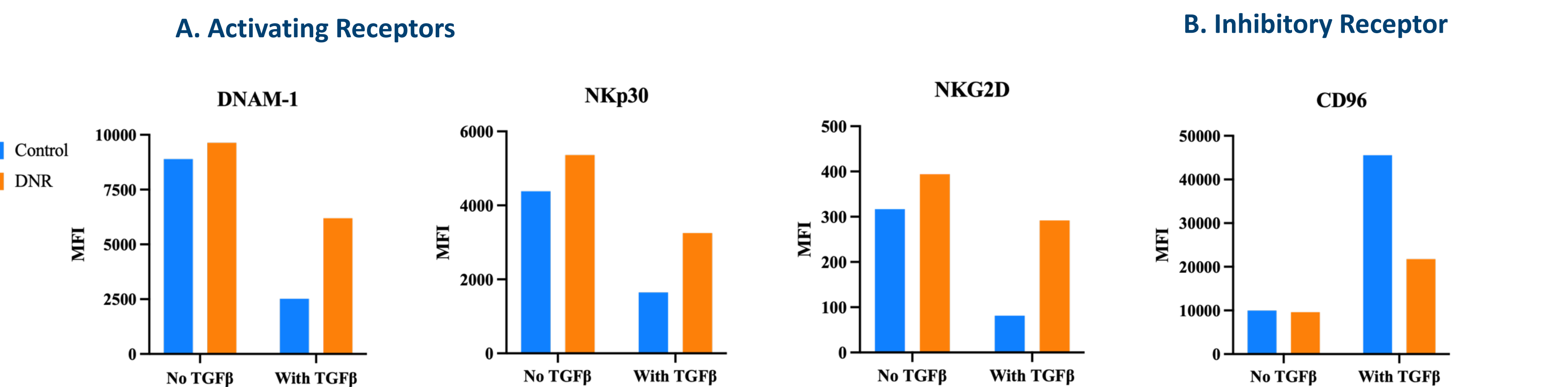
Schematics of A) the endogenous TGFβ complex vs dominant negative (with no kinase activity, based on the structure by Wieser et al. 1993) or B) how NK cells engineered with a DNR could prevent suppression of NK function, both intrinsically and for neighboring cells.

### 3 Engineered NK cells gain intrinsic DNR activity and provide extrinsic protection



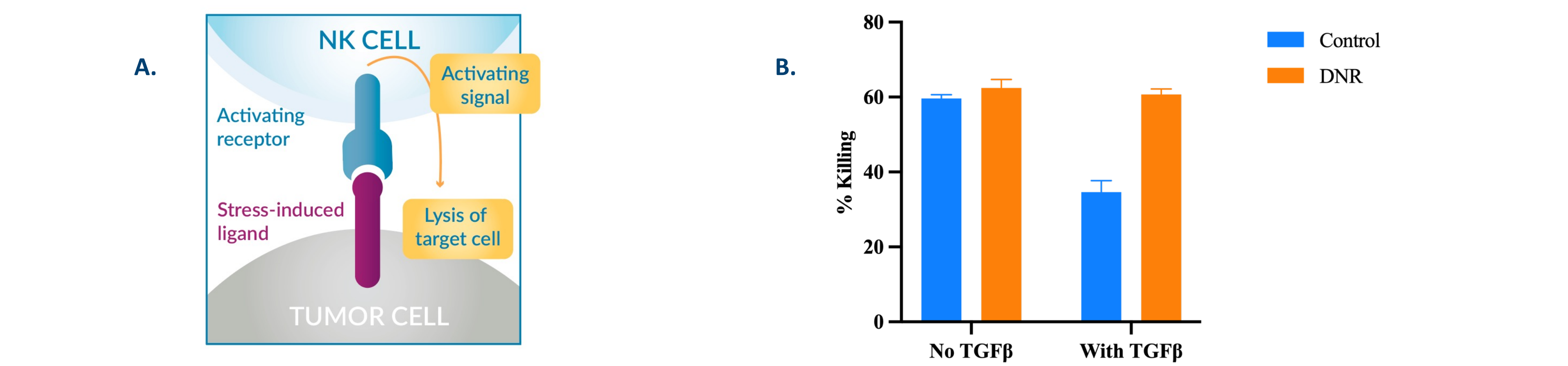
- A) DNR-engineered or control (truncated CD19)-engineered NK cells were treated with recombinant TGFβ for 1hr and then fixed, permeabilized, stained for phosphorylated SMAD2 (pSMAD2) and analyzed by flow cytometry. % of pSMAD2 positive cells was determined by gating on tagged engineered NK cells.  
B) % pSMAD2 inhibition of DNR (normalized to control) is plotted.  
C) Mock transduced NK cells were labeled with CellTrace™ Violet Dye and then mixed 1:1 with DNR-engineered or mock engineered NK cells. All cells were then treated with recombinant TGFβ for 1hr and then fixed, permeabilized, and stained for phosphorylated SMAD2 (pSMAD2) and analyzed by flow cytometry. % of pSMAD2 positive cells was determined by gating on non-engineered dye-labeled NK cells.

### 4 TGFB2-DNR protects NK cells from TGFβ mediated changes in receptor expression



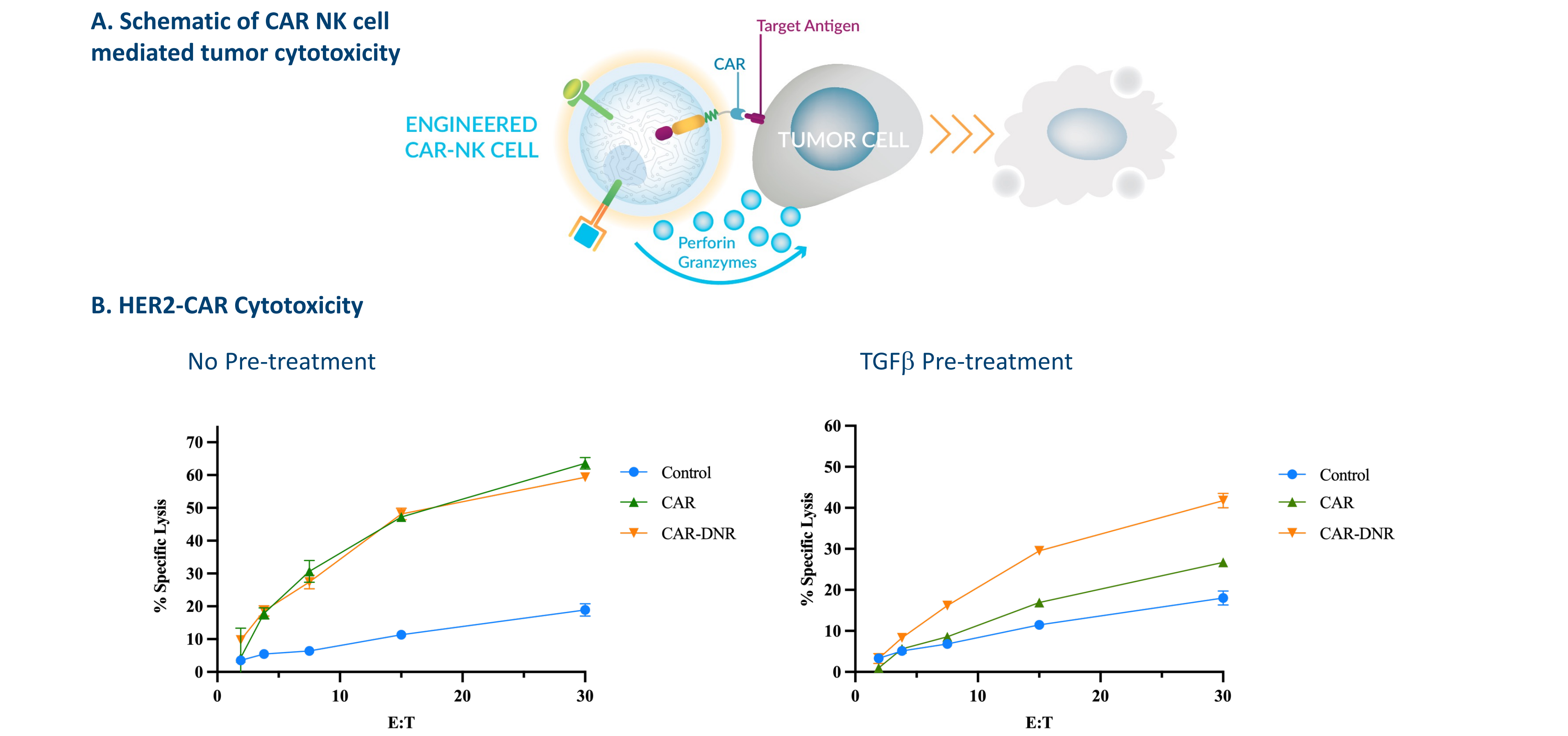
DNR-engineered or mock engineered were treated with or without 5 ng/ml TGFβ for 5 days. Cells were then stained for various surface markers and analyzed by flow cytometry by gating on the engineered population. A) Activating receptors, B) Inhibitory receptor. MFI = mean fluorescence intensity

### 5 TGFB2-DNR protects cells from TGFβ mediated inhibition of endogenous cytotoxicity



- A) Depiction of how NK cell activating receptors (such as those seen in Figure 4) engage endogenous NK cytotoxicity of tumor cells.  
B) DNR engineered or control (truncated CD19) engineered NK cells were pre-treated with 5 ng/ml TGFβ for 5 days, then co-cultured for 3 hours with K562-luciferase-tagged tumor cells (E:T 1:1) to evaluate innate NK mediated cell killing either in the presence or the absence of TGFβ.

### 6 TGFB2-DNR overcomes high TGFβ challenge to protect CAR dependent targeting of HER2+ tumors



- A) Demonstration of how a HER2-CAR engineered NK cell can cause tumor cell death by binding to target antigen.  
B) NK cells engineered with control, HER2-CAR, or HER2-CAR-DNR were treated for 5 days with or without 5ng/ml TGFβ. The cells were then tested for *in vitro* cytotoxicity activity by co-culturing with calcein labeled HER2 positive SK-OV-3 tumor cells for 4 hrs. % Specific lysis was determined by measuring amount of calcein released in the supernatant.

## CONCLUSIONS

Here we describe how a TGFB2 Dominant Negative Receptor can protect the function of engineered CAR-NK cells.

- TGFB2-DNR can reduce downstream TGFβ activity as shown by preventing >90% of SMAD2 phosphorylation even at supraphysiological doses of TGFβ (e.g., 5 ng/ml)
- TGFB2-DNR can also act as a TGFβ trap, thereby protecting neighboring NK cells from TGFβ signaling
- Engineered NK cells expressing the DNR are protected from decreases in NK activating receptor expression and from increases in NK inhibitory receptor expression
- Co-expression of DNR with a HER2-CAR construct restores CAR dependent cytotoxicity in the presence of TGFβ

The integration of TGFβ resistance provided by expression of the DNR with HER2-specific tumor targeting, achieved through co-expression of a HER2-CAR, provides an immediately achievable path towards novel engineered NK cell therapies with greater potential to treat solid tumors in cancer patients.