hat include immune-inhibitory
ved suppressor cells (MDSCs)
ages (M2s). MDSCs and M2s
r the inhibitory receptor
K cells during their ex-vivo
tions being evaluated in
s are antibody-based and are
vailability and temporary
o overcome these limitations
sponse, we hypothesized that
R-NK cells would result in a
t in the anti-tumor activity of

ic neuroblastoma and sarcoma
igh surface expression of
tumor cells as well as intra-
the influence of TIGIT on CAR-
ssfully ablated TIGIT using
IT^{KO} primary human CAR-NK
okine secretion but not
ltures with MDSCs and
m TME culture system where
e first allowed to pre-establish
ntly-added TIGIT^{KO} CAR-NK
TIGIT^{WT} CAR-NK cells. Our
nhibition of CAR-NK cell
vel NK cell therapeutic
mmune suppression in the
f gene-edited CAR-NK cells to
n with solid tumors

e TIGIT-mediated suppression
tric TMEs.

Flow cytometry analysis of CD155 and CD112 expression in MDSCs and M2 from tumor samples of neuroblastoma patients (n=5)

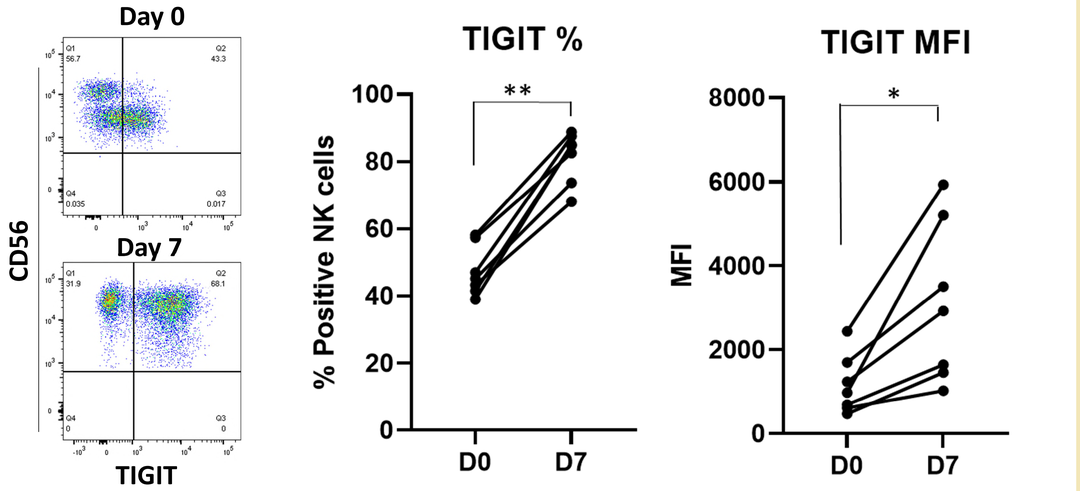


Figure 2:
Ex-vivo expanded NK Cells upregulate TIGIT expression.

Representative flow cytometry analysis of NK cells from human PBMCs before and after ex-vivo expansion using K562-41BBL-mbIL-15 feeder cells and exogenous IL-2 for 7 days (n=6). Paired t-test was used to calculate significance. *p<0.05 ,p<0.0005.**

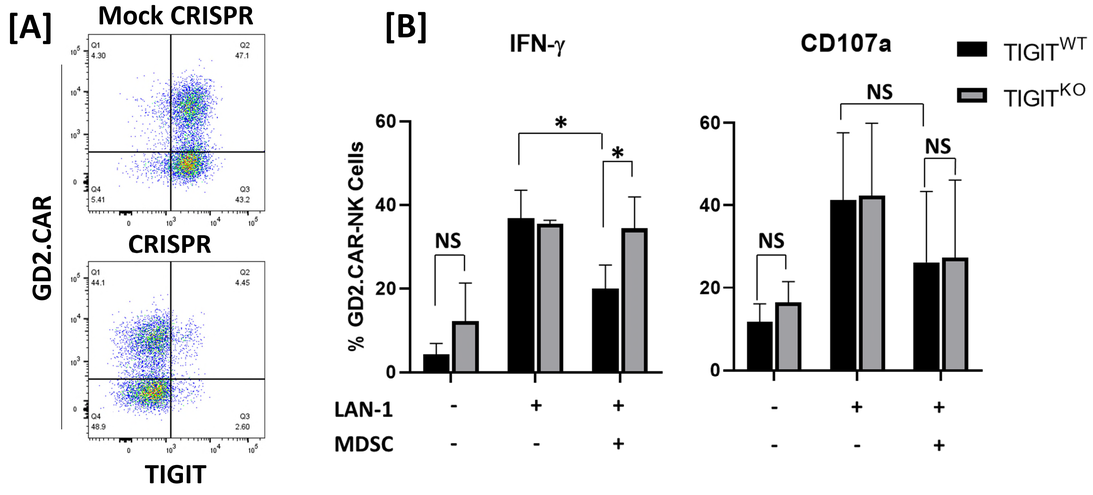
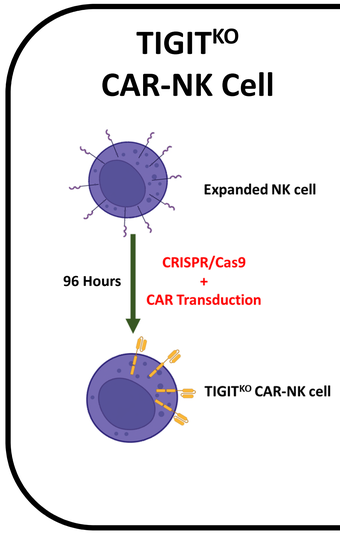


Figure 3:
TIGIT^{KO} GD2.CAR-NK cells exhibit increased IFN-γ release in short-term TME co-cultures

(A) Representative flow cytometry analysis of ex-vivo expanded GD2.CAR NK cells after sequential CRISPR and CAR transduction with exogenous IL-2 for 4 days (n=8). (B) TIGIT^{KO} and TIGIT^{WT} CAR-NK (5 x 10^4 cells) co-cultured with MDSCs (2 x 10^5 cells) and LAN-1 (4 x 10^5 cells) for 24 hours and CD107a and intracellular IFN-γ expression (n=3) were evaluated by flow cytometry. Students t-test was used to calculate significance. NS, not significant (p > 0.05); *p < 0.05.

macrophages were assesse
patients with neuroblastom
in NK cell TIGIT expression
a 7-day ex-vivo expansion
mbIL-15 cell line and exoge
TIGIT knockout (KO) GD2.CA
CRISPR/cas9 via electropor
followed immediately by
GD2.CAR. For short-term TM
NK cells were plated wi
neuroblastoma cell line LAN-
and CD107a and IFN-γ exp
cytometry. For long-term
monocytes and a GFP m
CHLA255 were plated on a 2
TIGIT^{KO} GD2.CAR-NK cell a
Tumor growth and CAR-
measured using GFP ex
respectively on an incucyte.
was determined via intracell



CONCLUSIONS

- **MDSCs and M2 mac ligands.**
- **TIGIT^{KO} maintains IF cells in a TME.**