

Bispecific γδ T-cell Engager Containing Heterodimeric BTN2A1 and BTN3A1 Promotes Targeted Activation of Vγ9Vδ2^{+} T cells

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3rd Gamma Delta T Therapy Summit
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SHATTUCK LABS

PIONEERING NOVEL BI-FUNCTIONAL FUSION PROTEINS
EXPANDING THE BOUNDARIES OF BIOLOGIC MEDICINES

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Outline

Overview of the role of butyrophilins (BTNs) in activation of γδT cells

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Introduction of Gamma Delta T Cell Engager (GADLEN™) Platform:
Leveraging heterodimeric BTNs to harness the cytolytic activity of γδT cells

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Proof of Concept: Functional characterization of heme-targeting GADLEN molecules

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Learnings from the characterization of GADLEN activity in Vγ9Vδ2 T cell-mediated target cell depletion

- Co-stimulation requirements
- Role of proliferation vs activation
- Serial killing potential by gamma/delta T cells in vivo

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Shattuck Labs (STTK) Overview

COMPANY

Clinical-stage biotech company developing next generation bifunctional fusion proteins

Two novel platforms for immune modulation:

- 400+ unique compounds to date
- High binding affinity/avidity to targets
- Rapid path from concept to compound to clinic

- **Founded in 2016**, first ARC asset to clinic in 2019
- **Listed on Nasdaq,** October 2020 (STTK)

CAPABILITIES

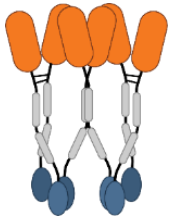
Experienced R&D, clinical and technical operations teams across offices in Durham, North Carolina and Austin, Texas

In-house cell line development, upstream and downstream process development, cell banking, formulation, analytical development and 200L scale manufacturing

100+ employees

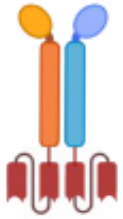
~$240M in cash as of March 31, 2022

PLATFORMS



ARC (Agonist-Redirected Checkpoint)

- Developed in 2016
- Two in clinic
  - SL-172154 (CD47 inhibitor + CD40 agonist)
  - SL-279252 (PD-1/L1 inhibitor + OX40 agonist)
- Multiple Phase 1 trials ongoing



GADLEN (Gamma Delta T Cell Engager)

- Developed in 2019
- Candidates for hematological and solid tumors
- Candidates for autoimmune disease
- Multiple in cell line development
- Manufacturing feasibility achieved
- Partnership opportunities to advance lead asset and other targets of interest

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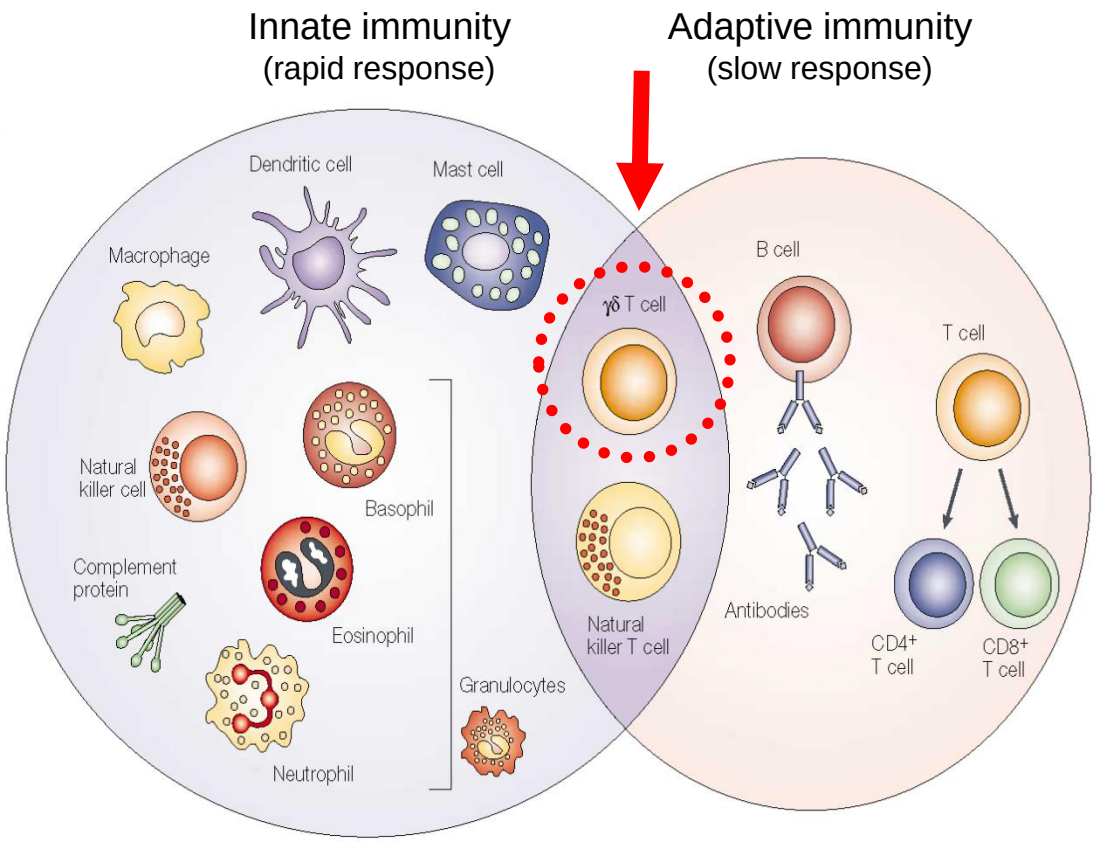
Targeting Gamma Delta ($\gamma\delta$) T Cells for Immunotherapy

At the Nexus of Innate and Adaptive Immunity

Host Immunity



Dranoff (2004) *Nature Reviews Cancer*

Key Attributes of $\gamma\delta$ T cells

- Shared innate and adaptive features
- Superior cytotoxic and serial killing potential (compared to conventional $\alpha\beta$T cells)
- ~1-5% of T cells in peripheral blood are Vγ9δ2
- Presence in tumors indicates strongest prognostic factor among all other immune cell types (Gentles, Nat Med 2015)
- MHC-independent antigen recognition

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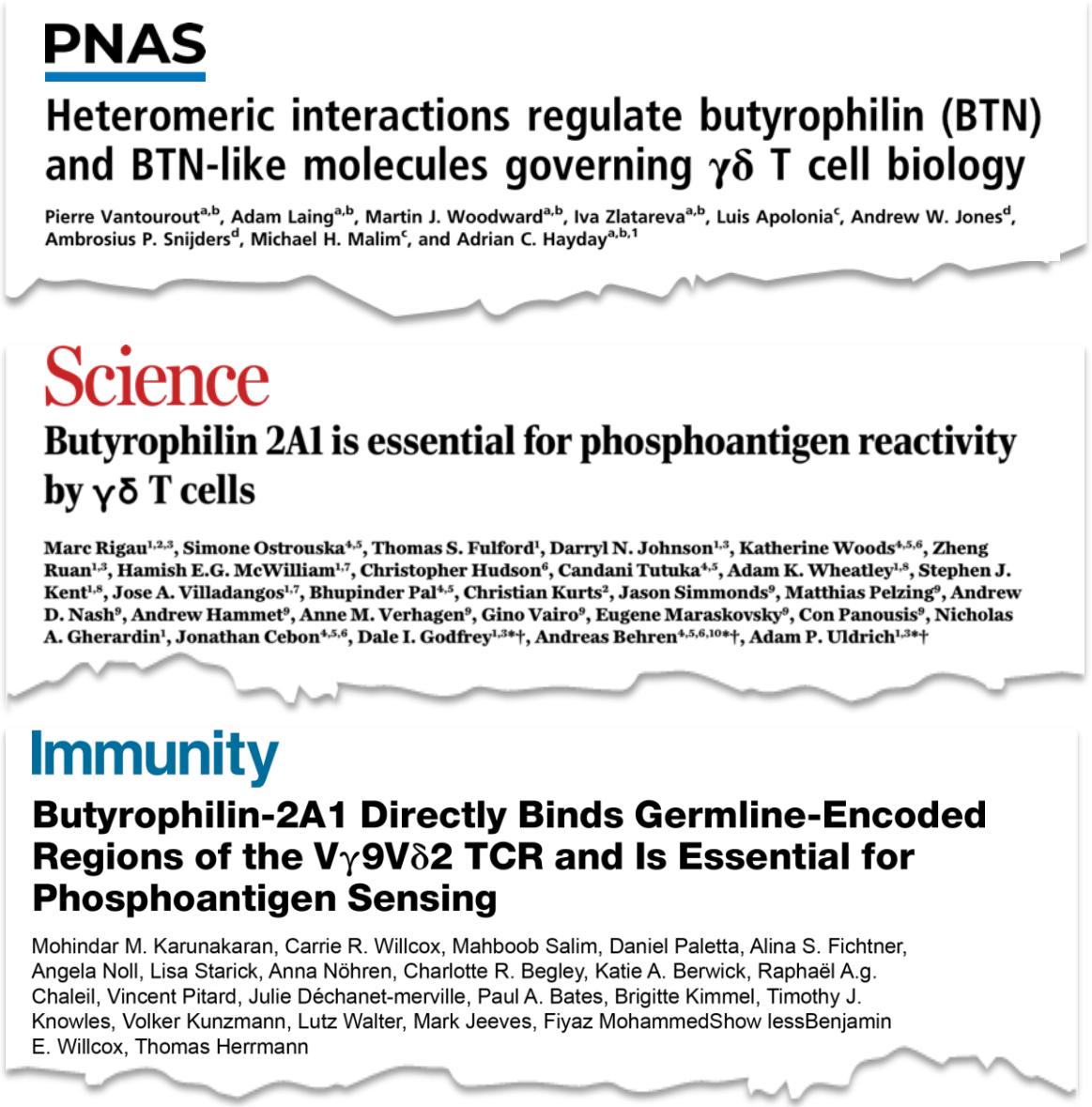
Role of Butyrophilin Proteins in $\gamma\delta$ T cell Function



PNAS

Heteromeric interactions regulate butyrophilin (BTN) and BTN-like molecules governing γδ T cell biology

Pierre Vantourout[a,b], Adam Laing[a,b], Martin J. Woodward[a,b], Iva Zlatareva[a,b], Luis Apolonia[c], Andrew W. Jones[d], Ambrosius P. Snijders[d], Michael H. Malim[c], and Adrian C. Hayday[a,b,1]

Science

Butyrophilin 2A1 is essential for phosphoantigen reactivity by γδ T cells

Marc Rigau[1,2,3], Simone Ostrouska[4,5], Thomas S. Fulford[1], Darryl N. Johnson[1,3], Katherine Woods[4,5,6], Zheng Ruan[1,3], Hamish E.G. McWilliam[1,7], Christopher Hudson[6], Candani Tutuka[4,5], Adam K. Wheatley[1,8], Stephen J. Kent[1,8], Jose A. Villadangos[1,7], Bhupinder Pal[4,5], Christian Kurts[2], Jason Simmonds[9], Matthias Pelzing[9], Andrew D. Nash[9], Andrew Hammet[9], Anne M. Verhagen[9], Gino Vairo[9], Eugene Maraskovsky[9], Con Panousis[9], Nicholas A. Gherardin[1], Jonathan Cebon[4,5,6], Dale I. Godfrey[1,3*†], Andreas Behren[4,5,6,10*†], Adam P. Uldrich[1,3*†]

Immunity

Butyrophilin-2A1 Directly Binds Germline-Encoded Regions of the Vγ9Vδ2 TCR and Is Essential for Phosphoantigen Sensing

Mohindar M. Karunakaran, Carrie R. Willcox, Mahboob Salim, Daniel Paletta, Alina S. Fichtner, Angela Noll, Lisa Starick, Anna Nöhren, Charlotte R. Begley, Katie A. Berwick, Raphaël A.g. Chaleil, Vincent Pitard, Julie Déchanet-merville, Paul A. Bates, Brigitte Kimmel, Timothy J. Knowles, Volker Kunzmann, Lutz Walter, Mark Jeeves, Fiyaz MohammedShow lessBenjamin E. Willcox, Thomas Herrmann

- The ligands responsible for activating $\gamma\delta$ T cells have long remained a mystery until recently
- A conserved role of heterodimeric butyrophilns (BTNs) in modulating function of multiple gdT cell subsets
- Heterodimerization of BTN2A1 and BTN3A1 are required for phosphoantigen induced activation of Vγ9δ2 T cells

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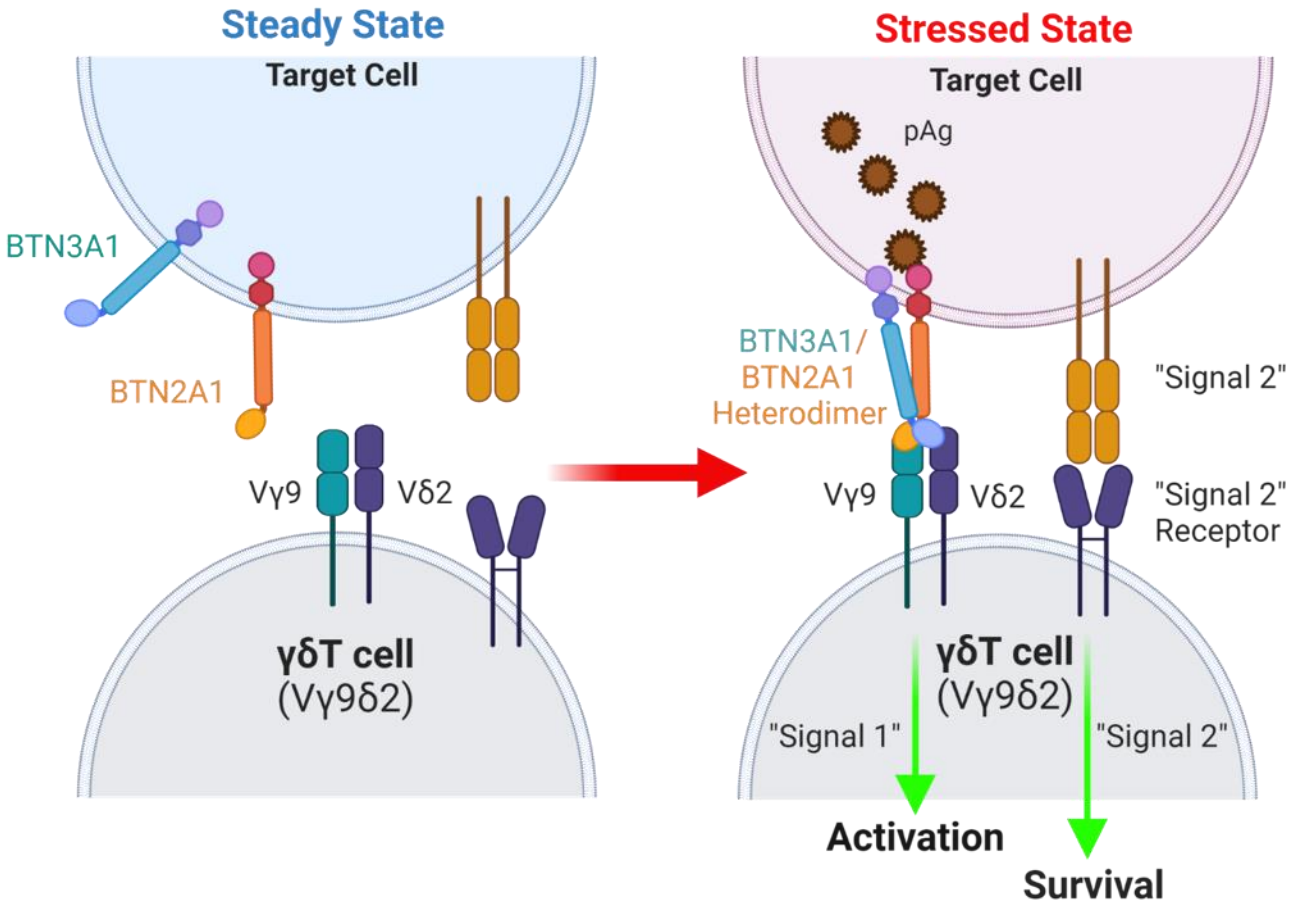
Basis for the GADLEN Design

Butyrophilins are Natural Ligands that Activate Vγ9δ2 T cells



Phosphoantigen (pAg) build up in cancer cells is sensed by Vγ9δ2 T cells via the heterodimerization of BTN2A1 and BTN3A1 on the cell surface.

Rigau, Science 2020
Karunakaran, Immunity 2020

The GADLEN Design:

- Activation of Vγ9δ2 T cells; main $\gamma\delta$ population in blood and many tumors
- Independent of phosphoantigen levels in cancer cells
- Independent of native butyrophilin expression
- Leverages signal 2/costim for $\gamma\delta$ T cell survival
- Directs cytotoxicity to antigen-expressing tumor cells

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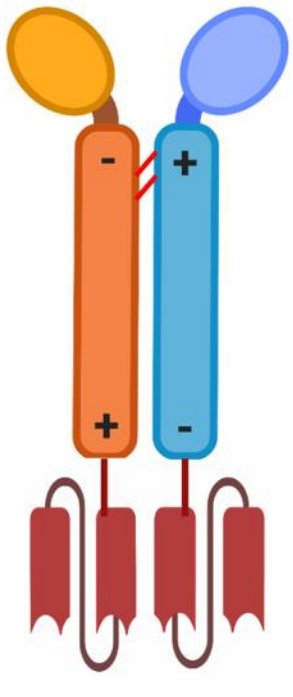
GADLEN: Butyrophilin Heterodimer-Based Engager Format

Using Antigen-Targeted scFv Domains to Tether BTN Heterodimers to the Surface of Tumor Cells

GADLEN construct includes an active BTN heterodimer



~150 kd

BTN2A1+3A1 heterodimer:
Stimulates $\gamma\delta$ T Cells

BTN2A1 and BTN3A1
Heterodimer specifically targets Vγ9δ2 T cells

Engineered Fc Domain
Facilitates Heterodimerization

Two Antibody Fragment Domains:
Tumor-Targeted

Format Enables:
- ✓ Dual tumor antigen targeting
- ✓ Biparatopic targeting
- ✓ Flexible, modular design

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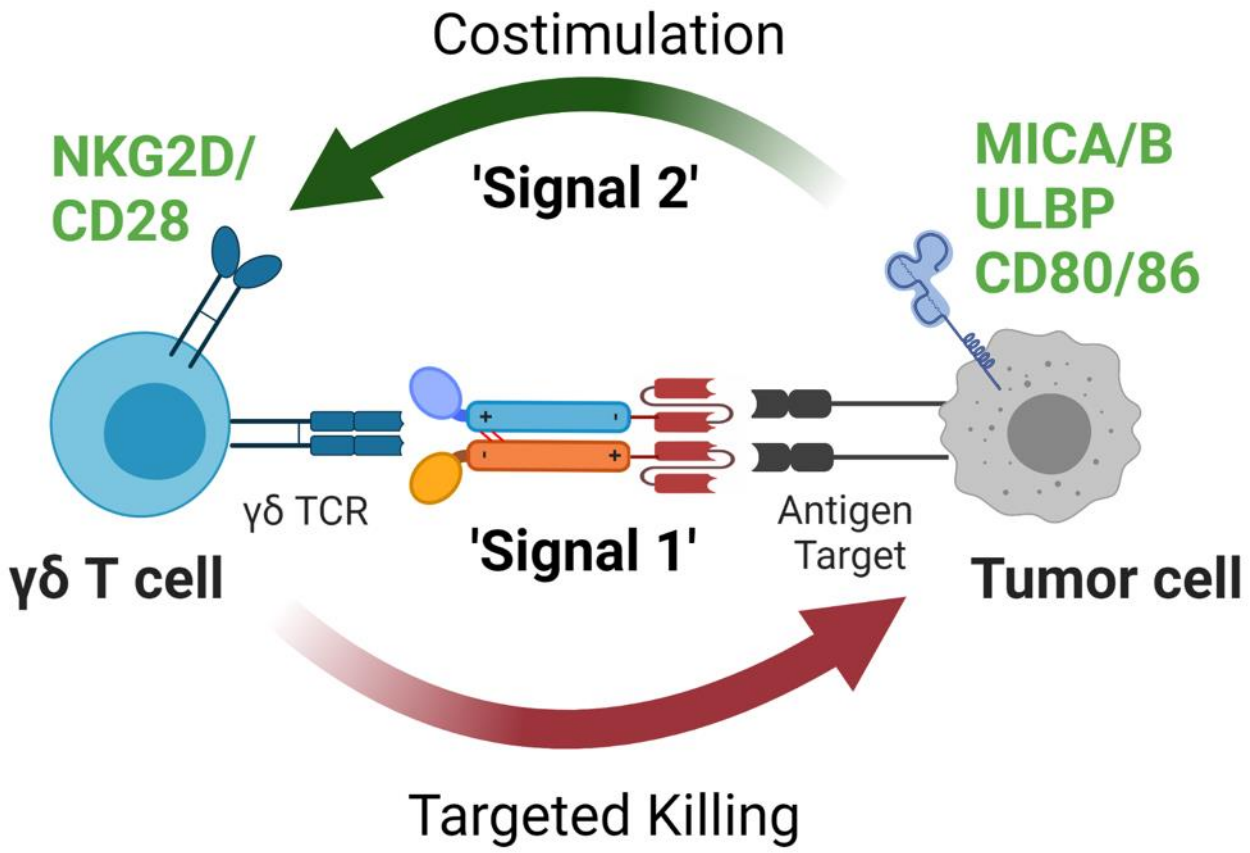
GADLEN: Mechanism of Action



➔ BTN heterodimer with costimulation (signal 2) leads to activation of Vγ9δ2 T Cells to promote killing of target cells

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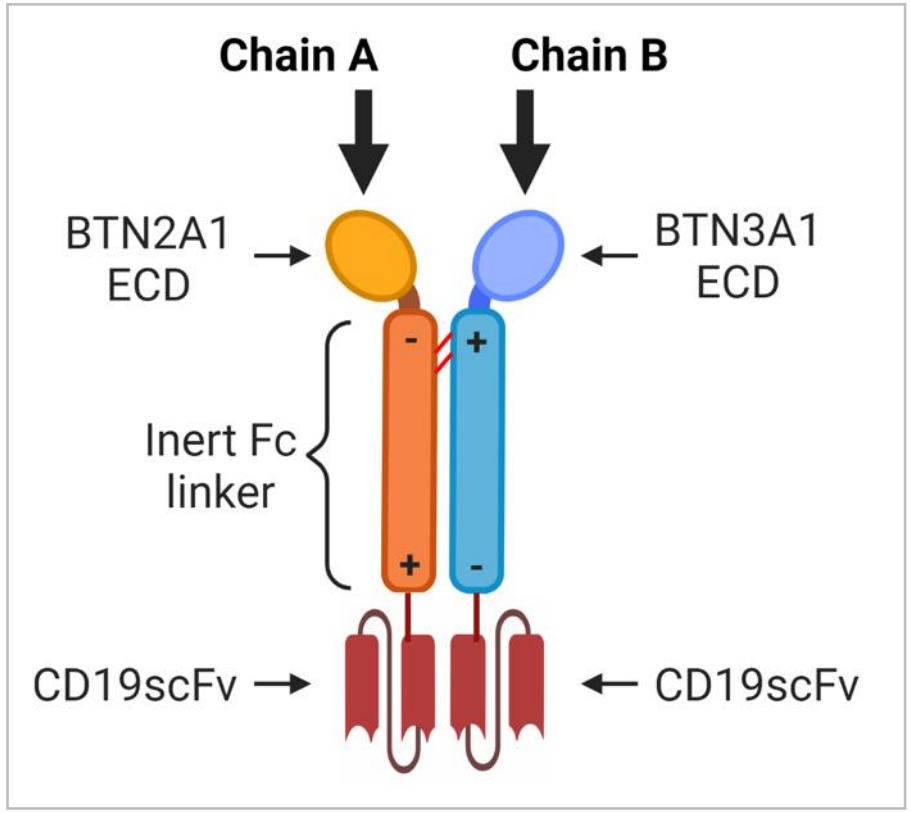
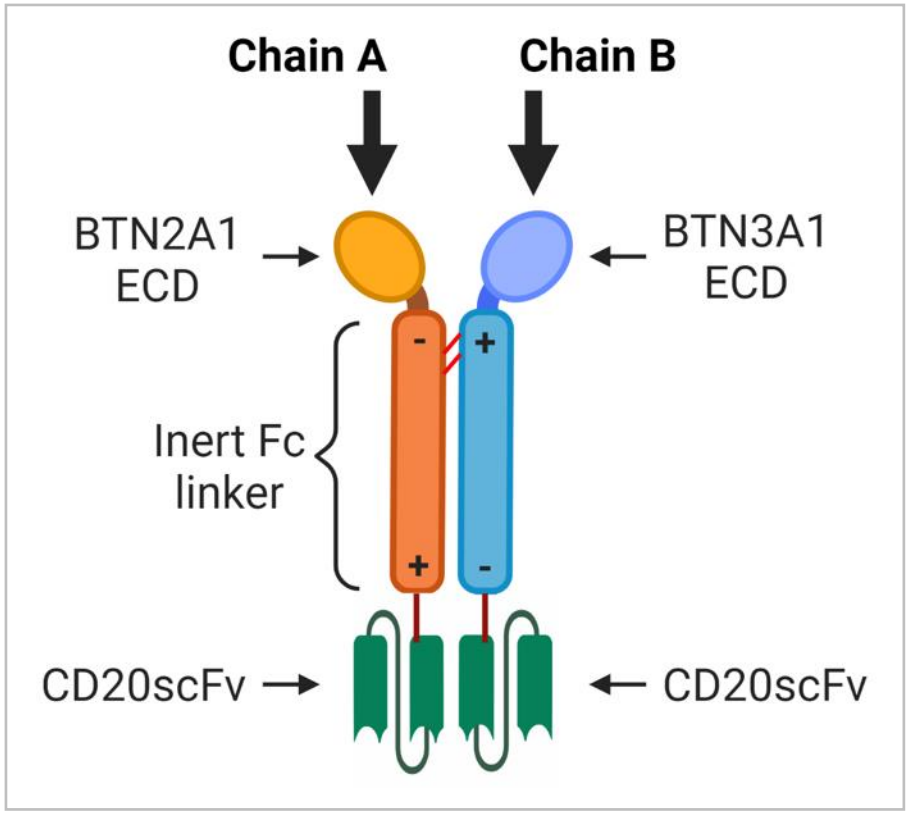
Characterization of Heme-Targeting GADLEN (Proof-of-Concept)





➔ CD19 and CD20 GADLEN molecules targeting CD19+/CD20+ lymphoma

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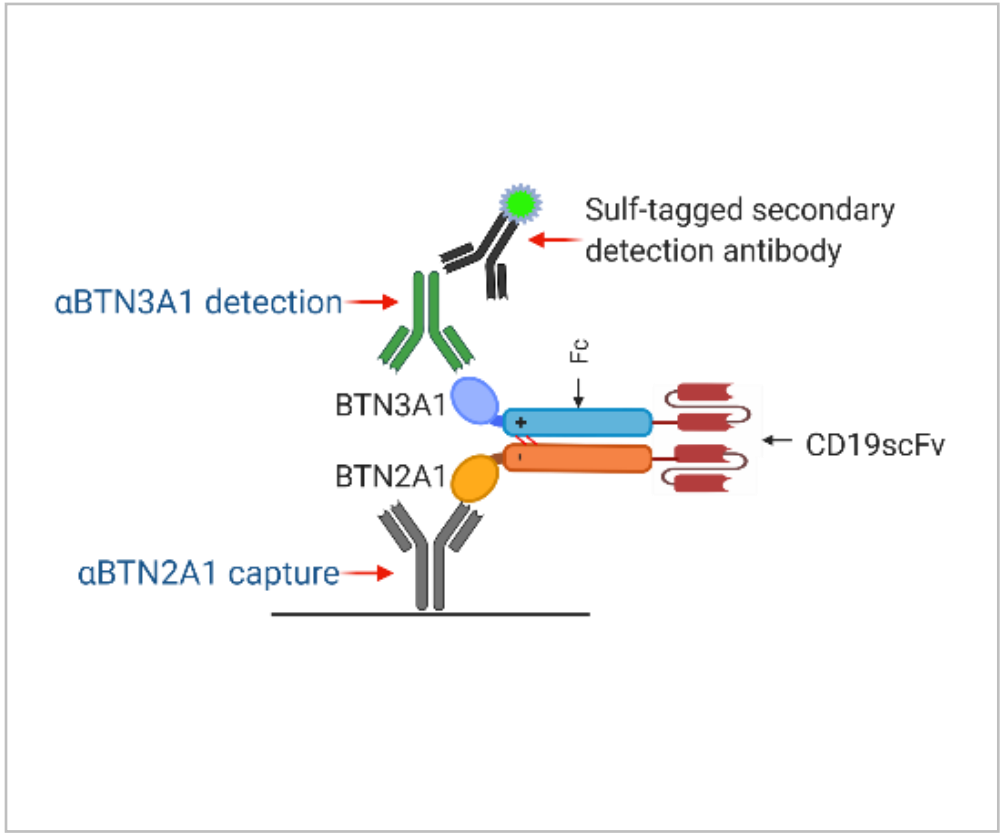
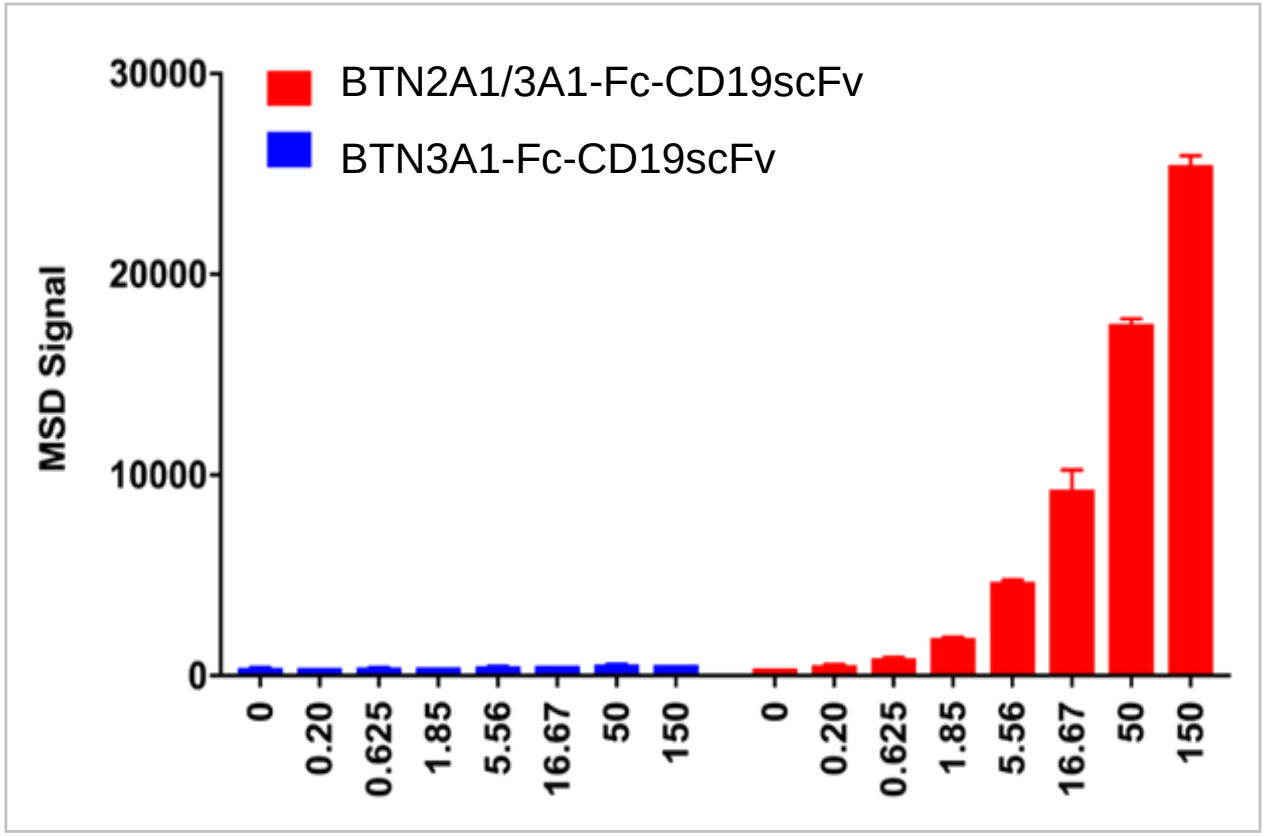
Presence of BTN2A1 and BTN3A1 Heterodimers in GADLEN Construct

Human BTN2A1/3A1-Fc-CD19scFv





→ Only GADLEN construct containing both BTN2A1 and BTN3A1 heterodimer, but not BTN3A1 homodimer, can be detected using dual-targeting MSD assay

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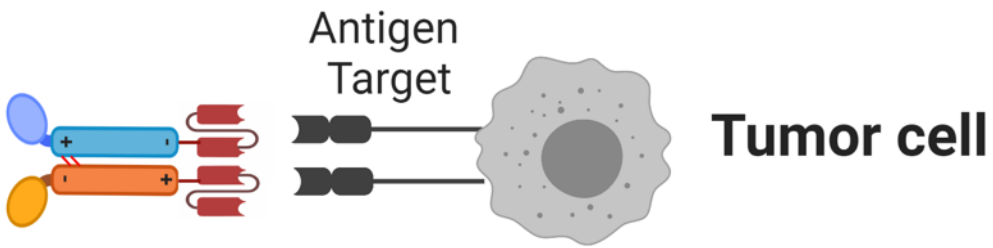
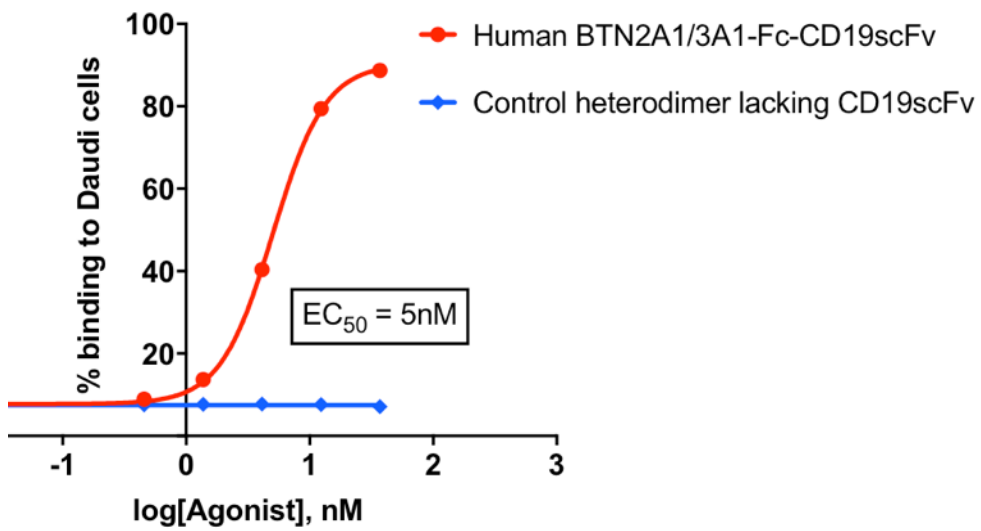
GADLEN Binds to γδT Cells and Targets Tumor Cells

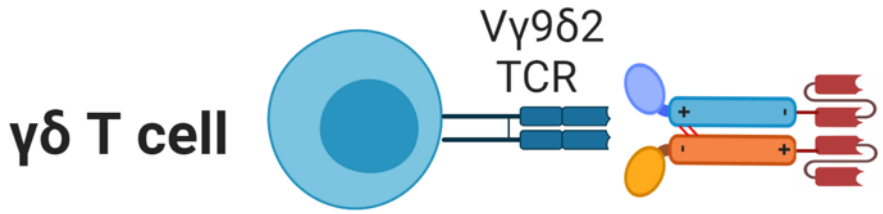
Human BTN2A1/3A1-Fc-CD19scFv

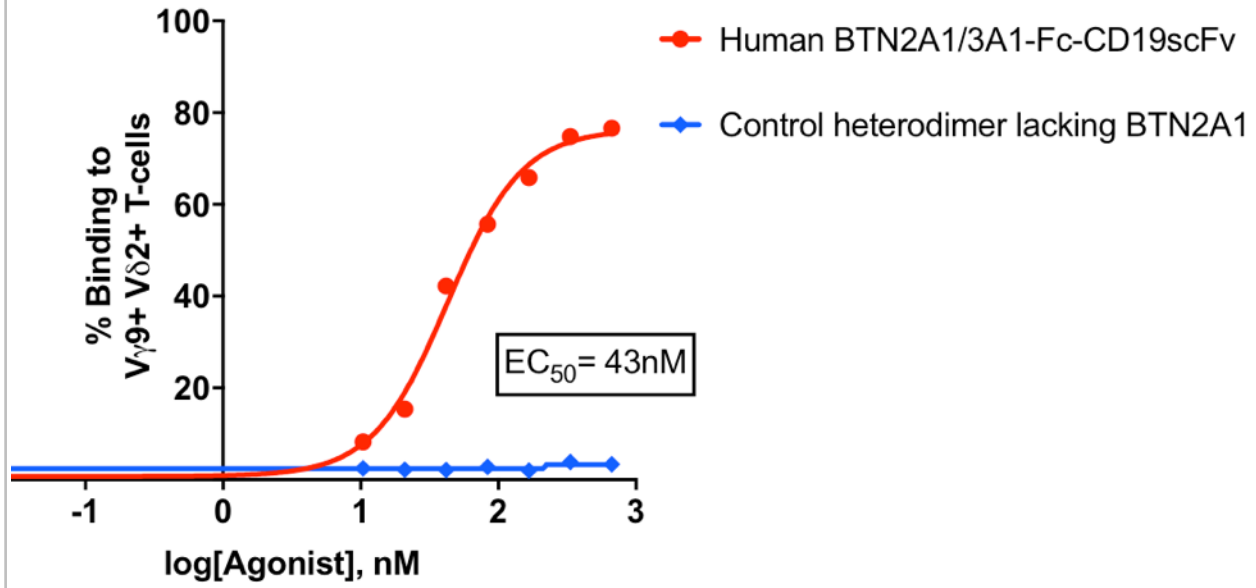
Binding to CD19+ Lymphoma Cells





Binding to γδT Cells





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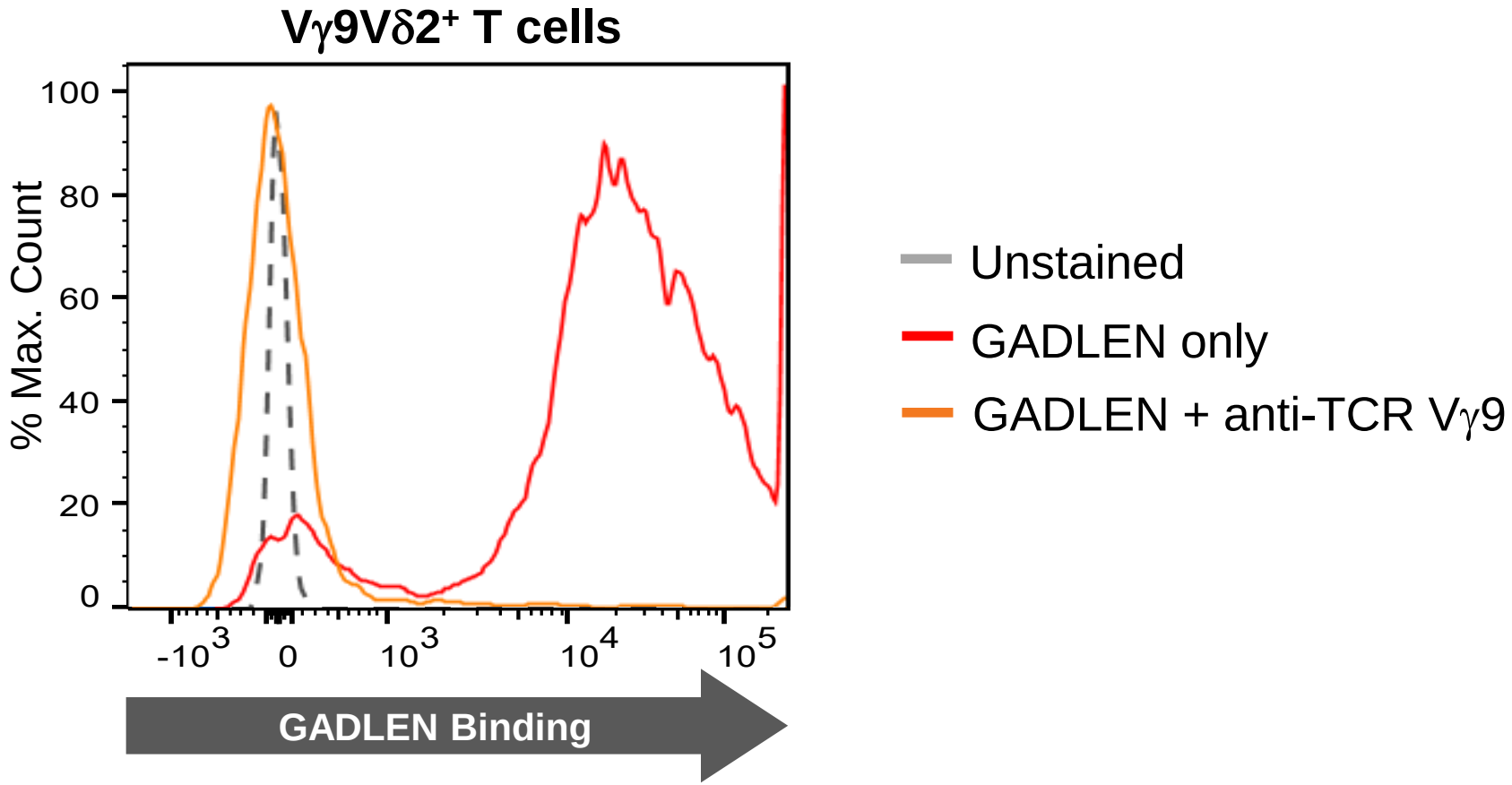
GADLEN Specifically Binds the Vγ9 Subunit of γδ T-Cell Receptor

Human BTN2A1/3A1-Fc-CD19scFv



➔ Co-incubation with anti-TCR Vg9 antibody blocked binding of GADLEN to Vg9Vd2+ T cells

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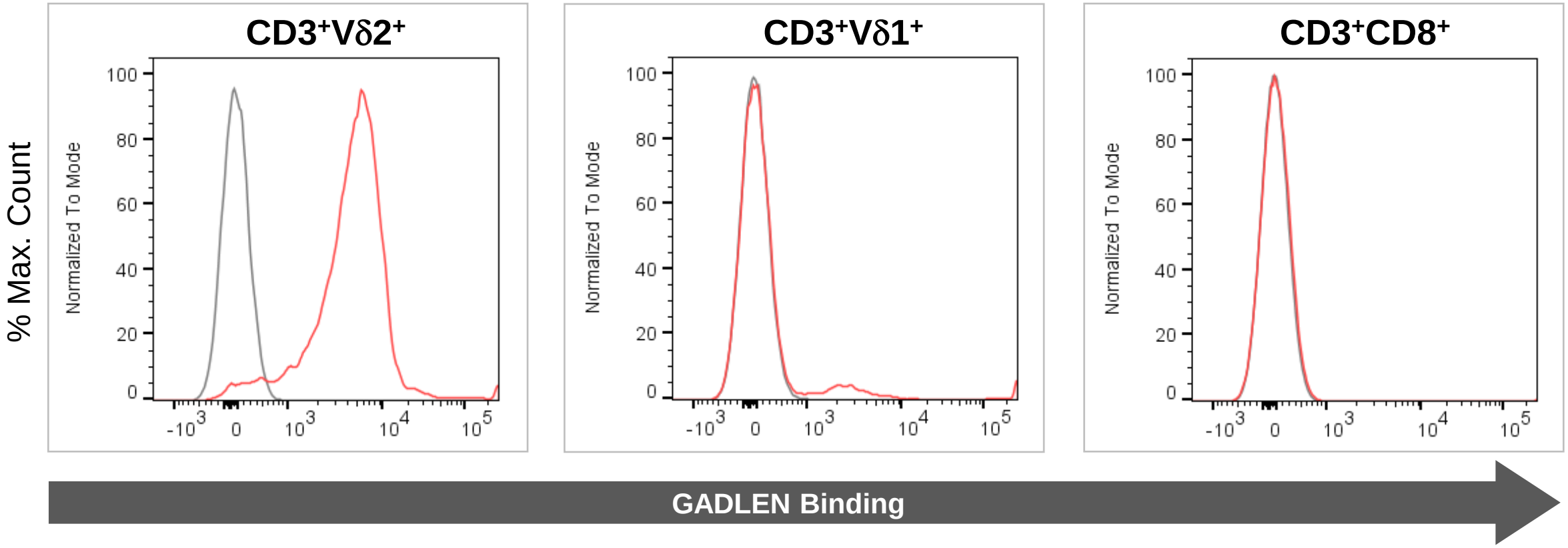
GADLEN Selectively Targets Vγ9Vδ2^{+} T Cells

Human BTN2A1/3A1-Fc-CD19scFv



➔ Cell binding analysis in PBMC: GADLEN specifically binds to Vd2+ T cells but not Vd1+ or other T cell subsets

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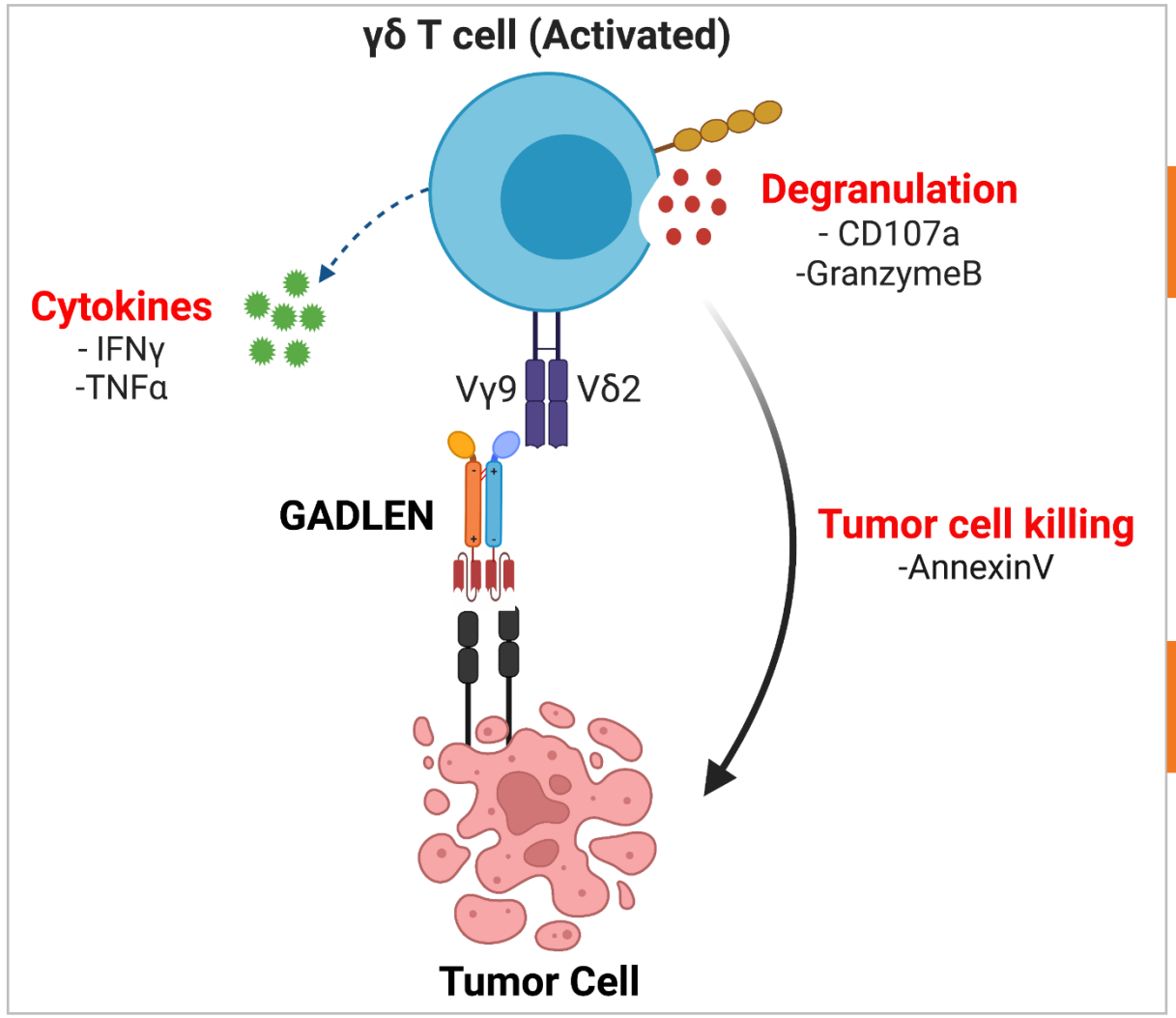
In Vitro Assays Used to Confirm GADLEN MOA

Human BTN2A1/3A1-Fc-CD19scFv



γδT cell Activation

Assessed in a tumor cell-free system

- CD107a expression by Flow Cytometry
- Cytokine production by Flow and ELISA

Tumor cell killing

Assessed in a co-culture system

- AnnexinV staining by Flow Cytometry
- Cytokine production by ELISA

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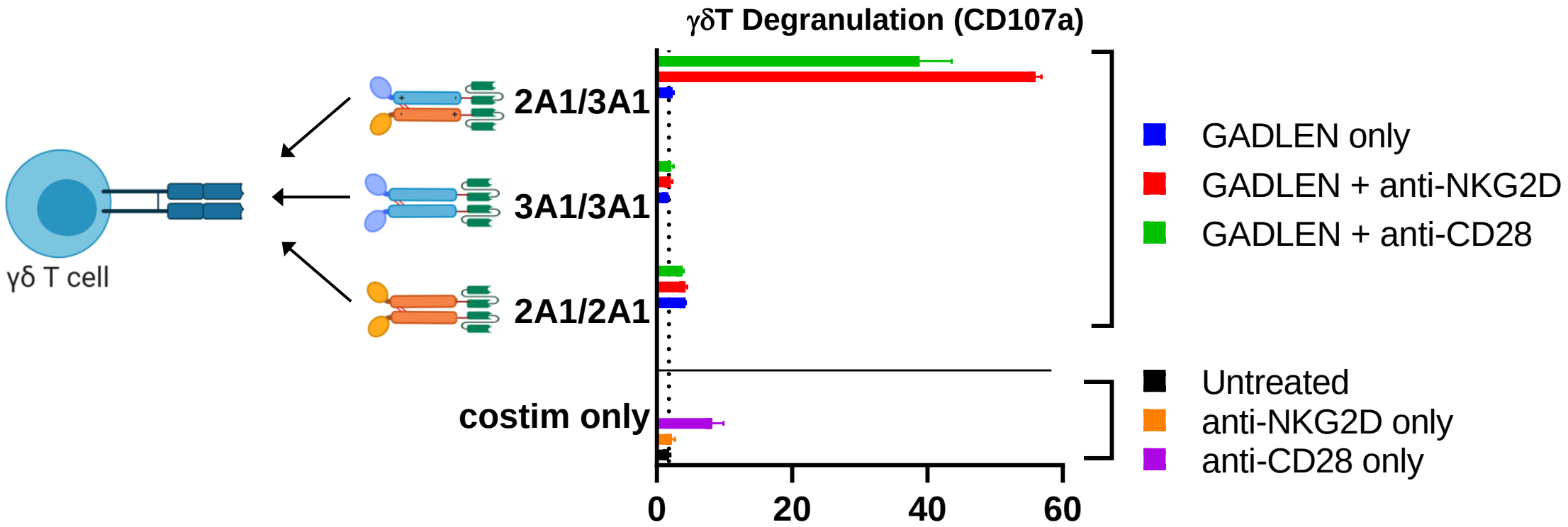
GADLEN Activates γδT Cells in the Presence of Costimulation

Human BTN2A1/3A1-Fc-CD19scFv



➔ Only GADLEN containing heterodimeric BTN2A1 and BTN3A1, but not homodimeric 2A1 and 3A1, promotes Vγ9Vδ2+ T degranulation

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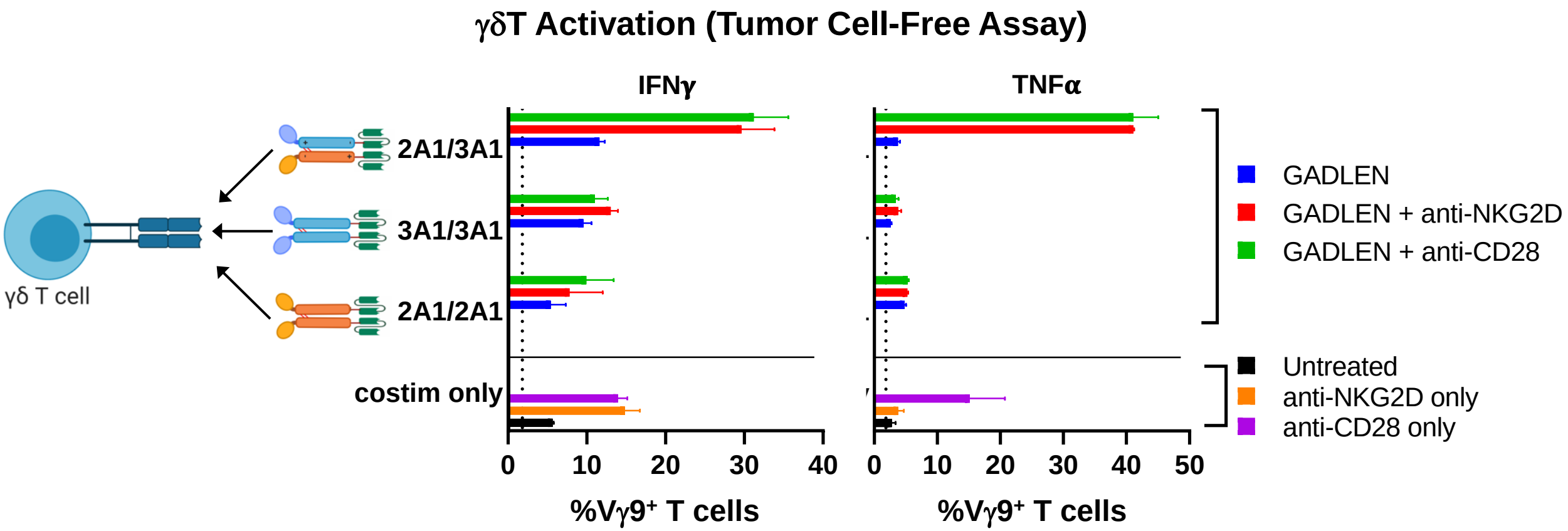
GADLEN Activates γδT Cells in the Presence of Costimulation

Human BTN2A1/3A1-Fc-CD19scFv



➔ Only GADLEN containing heterodimeric BTN2A1 and BTN3A1, but not homodimeric 2A1 and 3A1, promotes Vg9Vd2+ T degranulation

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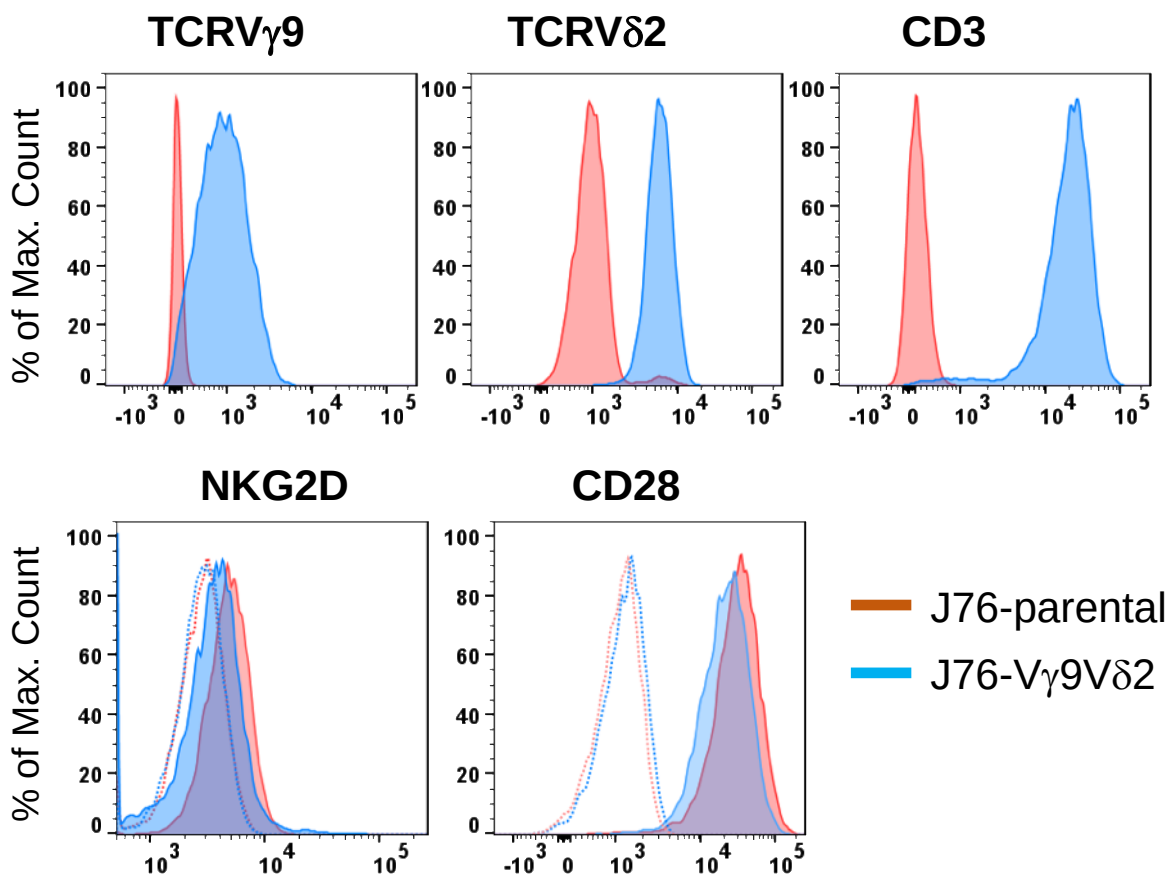
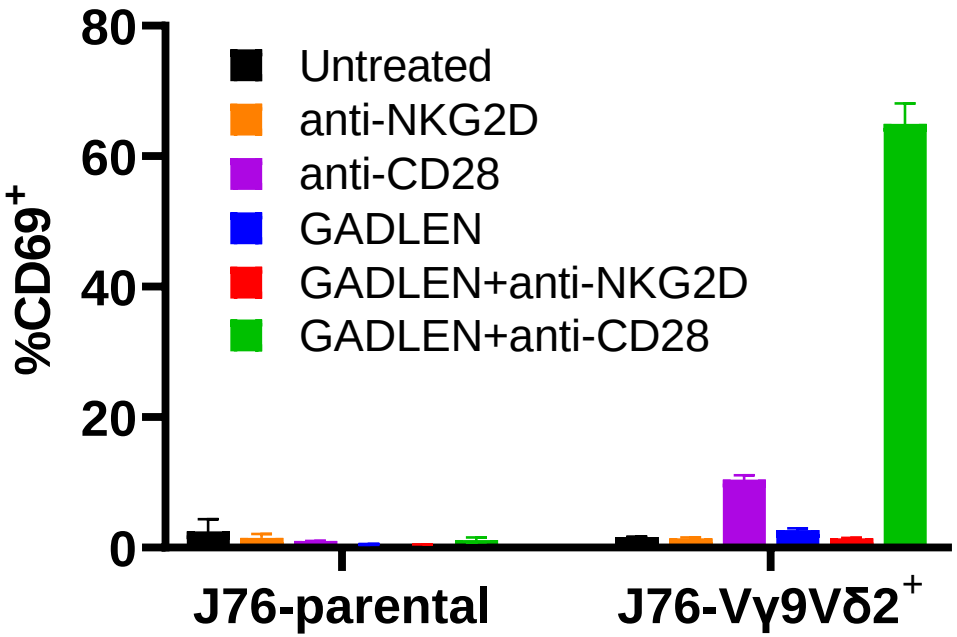
BTN Mediated ‘Signal 1’ and CD28 Mediated ‘Signal 2’ Determine Vγ9Vδ2 Activation

Human BTN2A1/3A1-Fc-CD19scFv

Jurkat 76 (TCR$^-$) engineered to express Vγ9Vδ2 TCR



Jurkat 76 Activation



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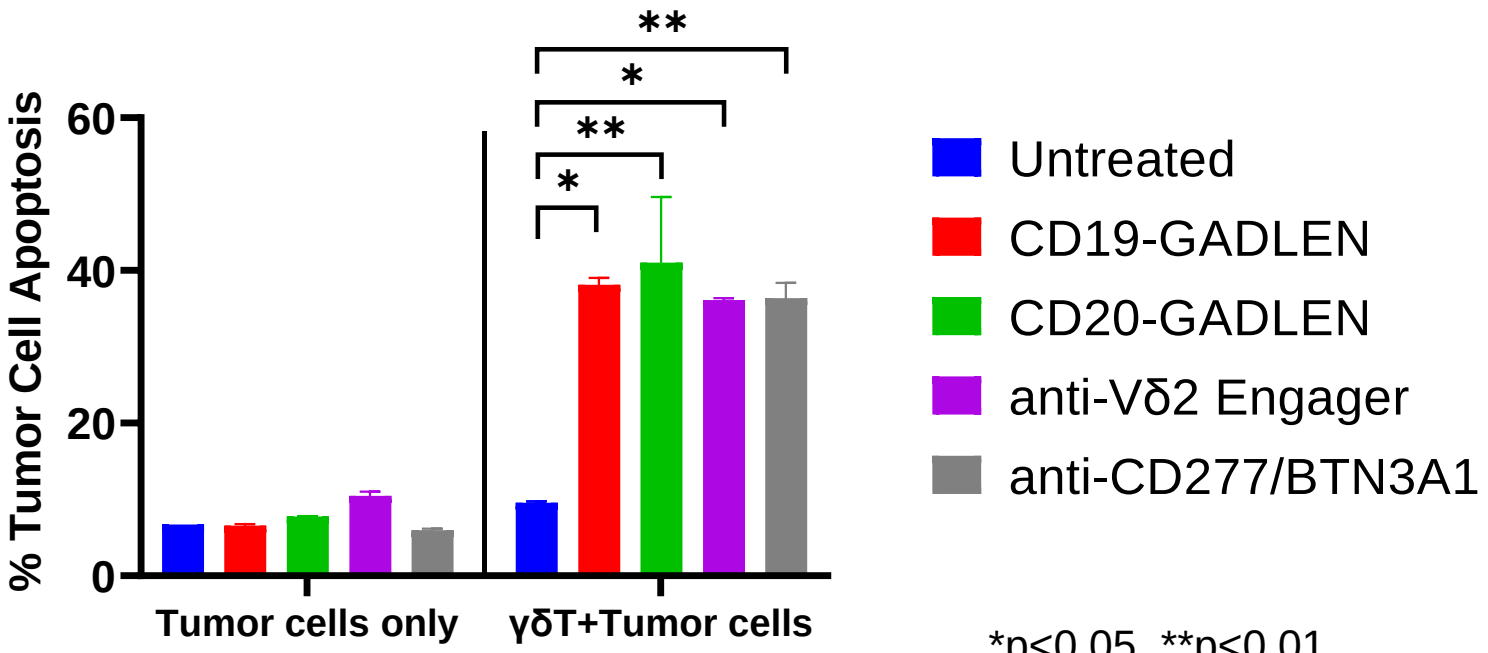
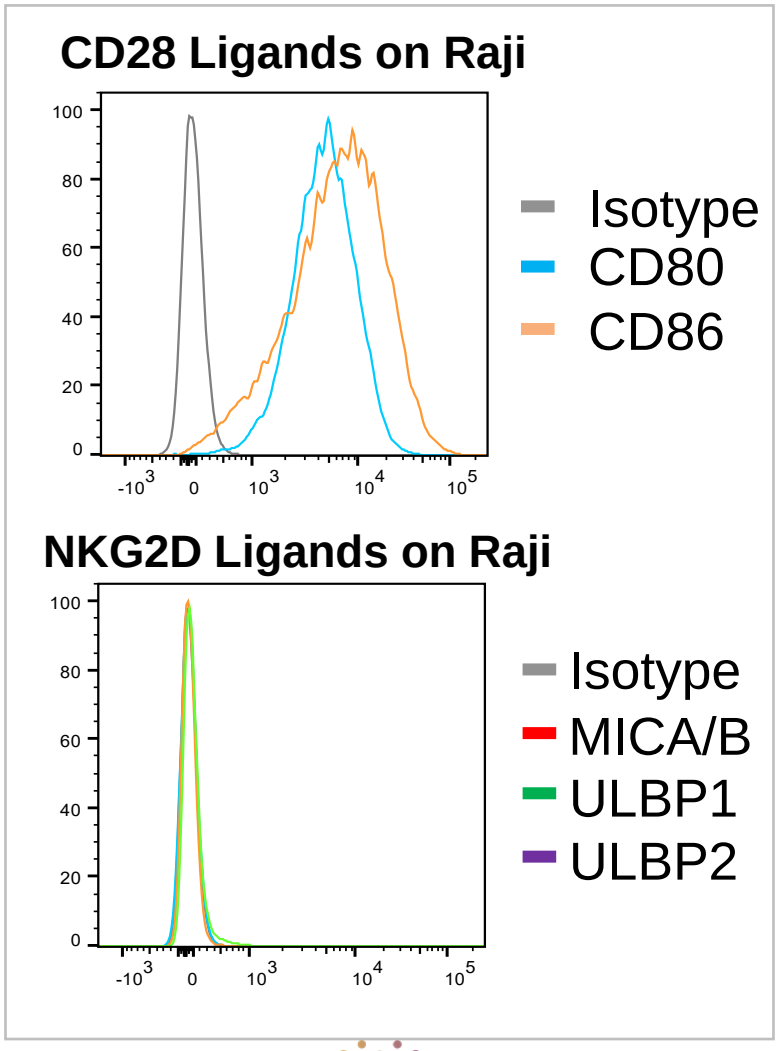
GADLEN Promotes Targeted Killing of Tumor Cells

Human BTN2A1/3A1-Fc-CD19scFv and BTN2A1/3A1-Fc-CD20scFv





- Single-agent activity in target cell killing
- Co-stimulatory signal is provided by tumor cells
- Tumor killing activity by GADLEN comparable to other γδT cell activating agents

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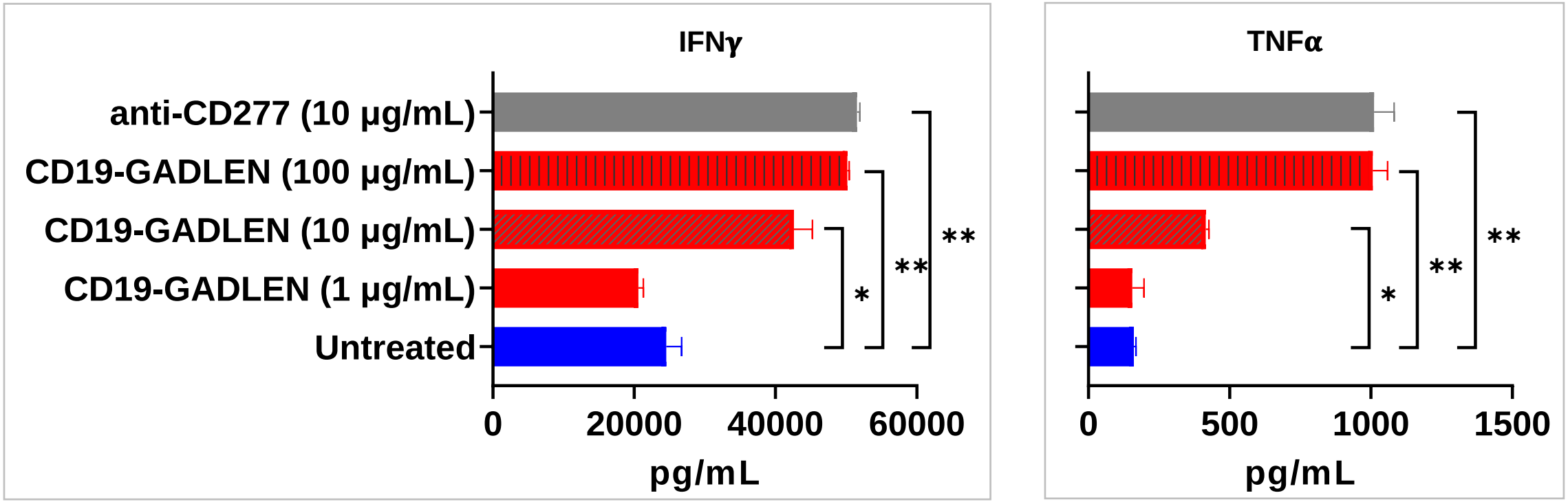
GADLEN-Activated Vγ9δ2 T Cells Secrete Key Pro-Inflammatory Cytokines

$\gamma\delta$T and Raji (CD19$^+$/CD20$^+$ Lymphoma) Co-Culture, 4hrs (E:T ratio = 1)



*p<0.05, **p<0.01

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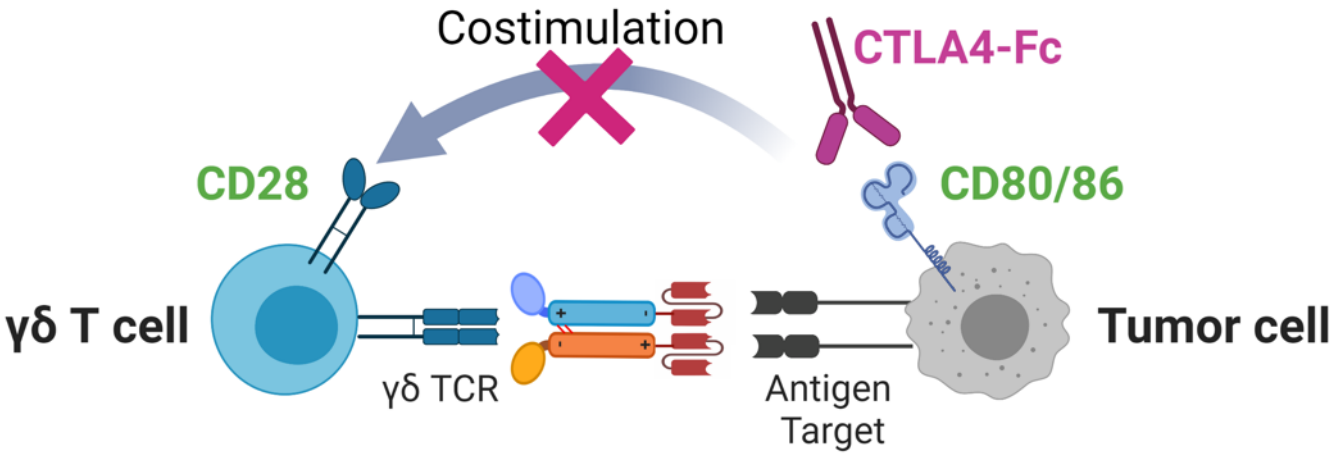
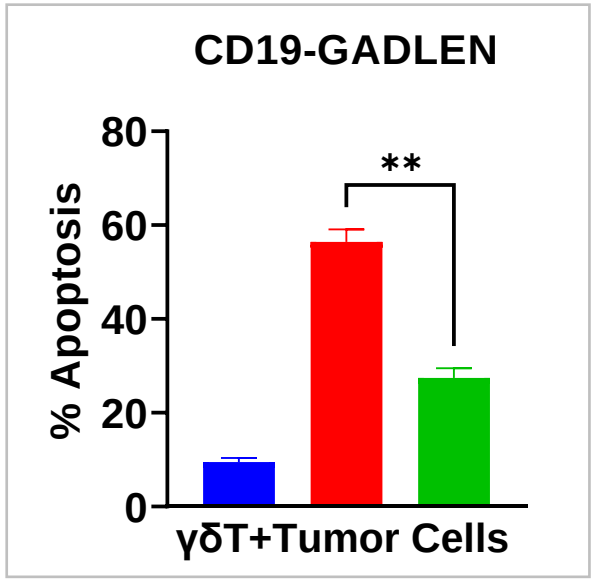
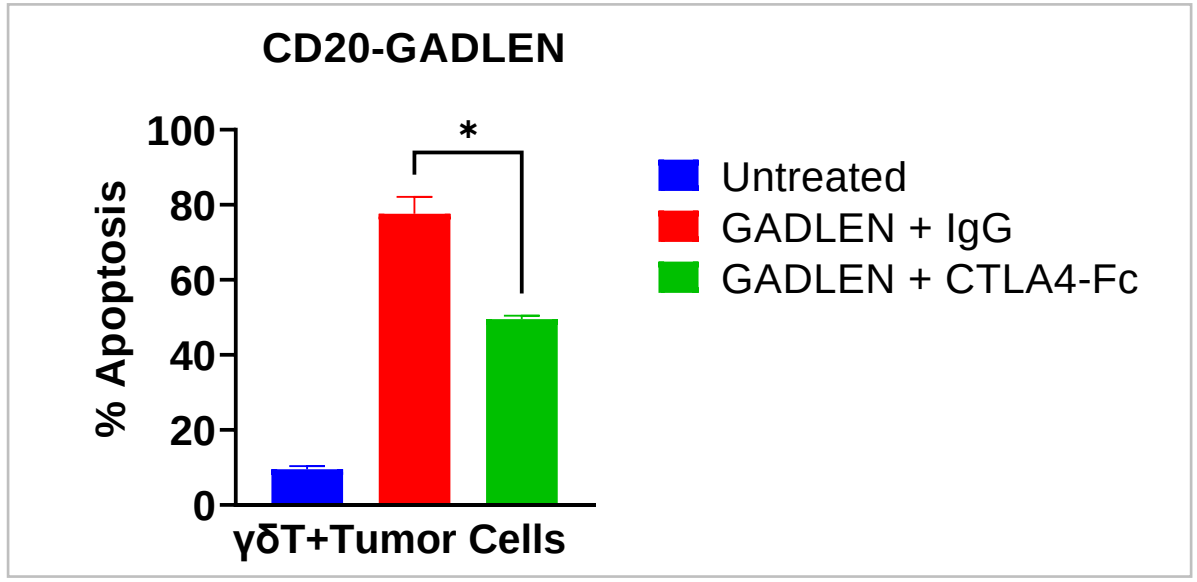
Blocking of Costimulatory Ligands on Tumor Cells Partially Attenuated GADLEN-Mediated γδT Cell Killing







Partial inhibition of tumor killing suggests the role of additional co-stimulatory pathways in mediating gdT cell cytotoxicity

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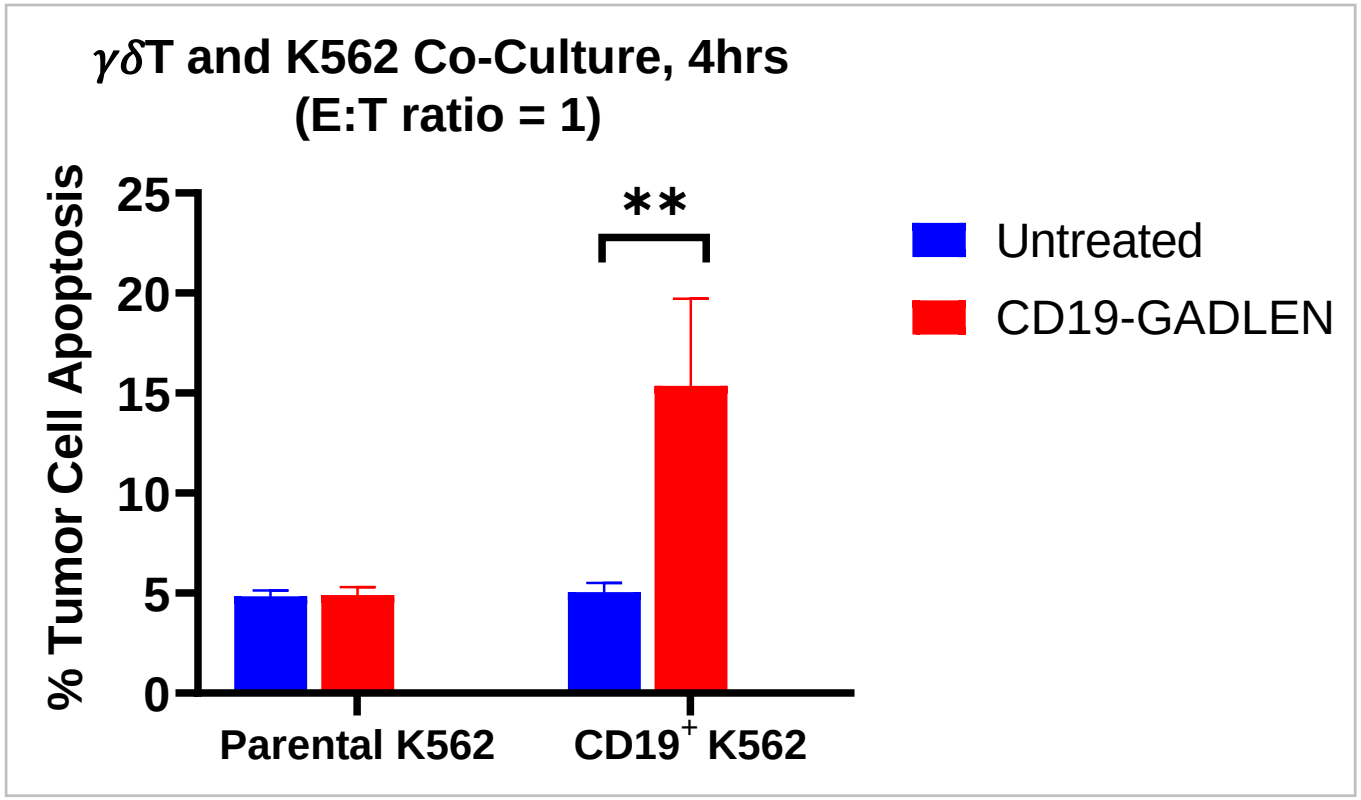
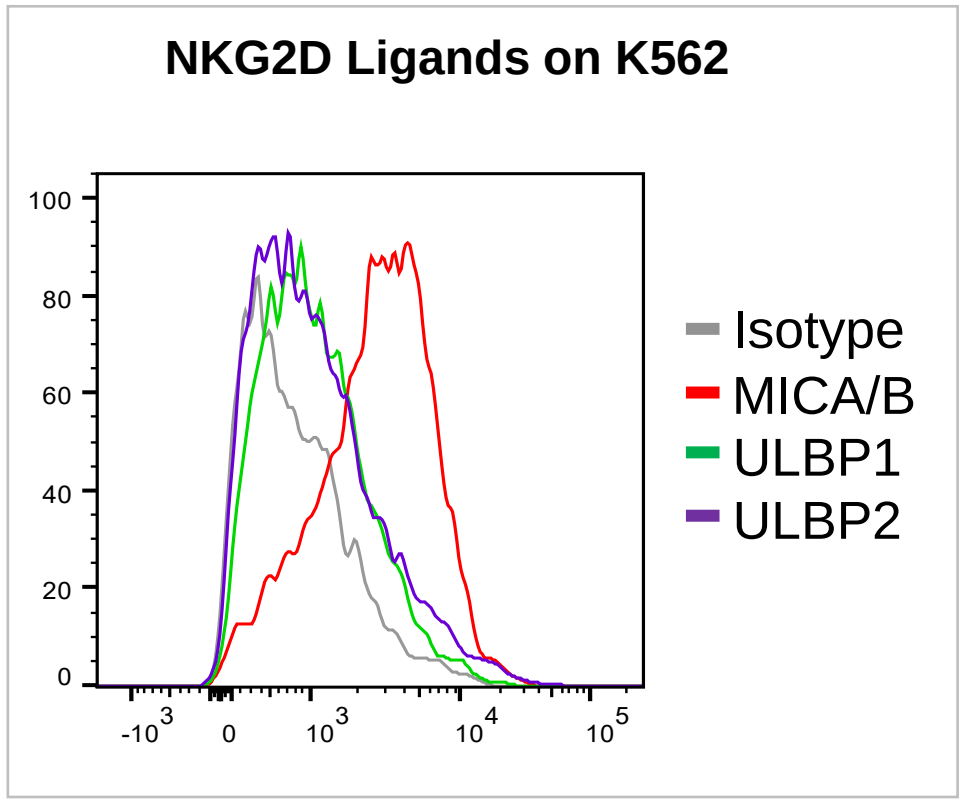
GADLEN Selectively Targets Tumor Cells Expressing Target Antigen

Human BTN2A1/3A1-Fc-CD19scFv





- K562 tumor cells were engineered to express CD19
- GADLEN directs targeted killing of tumor cells upon engagement with target antigen, and does not induce systemic activation of γδT cells

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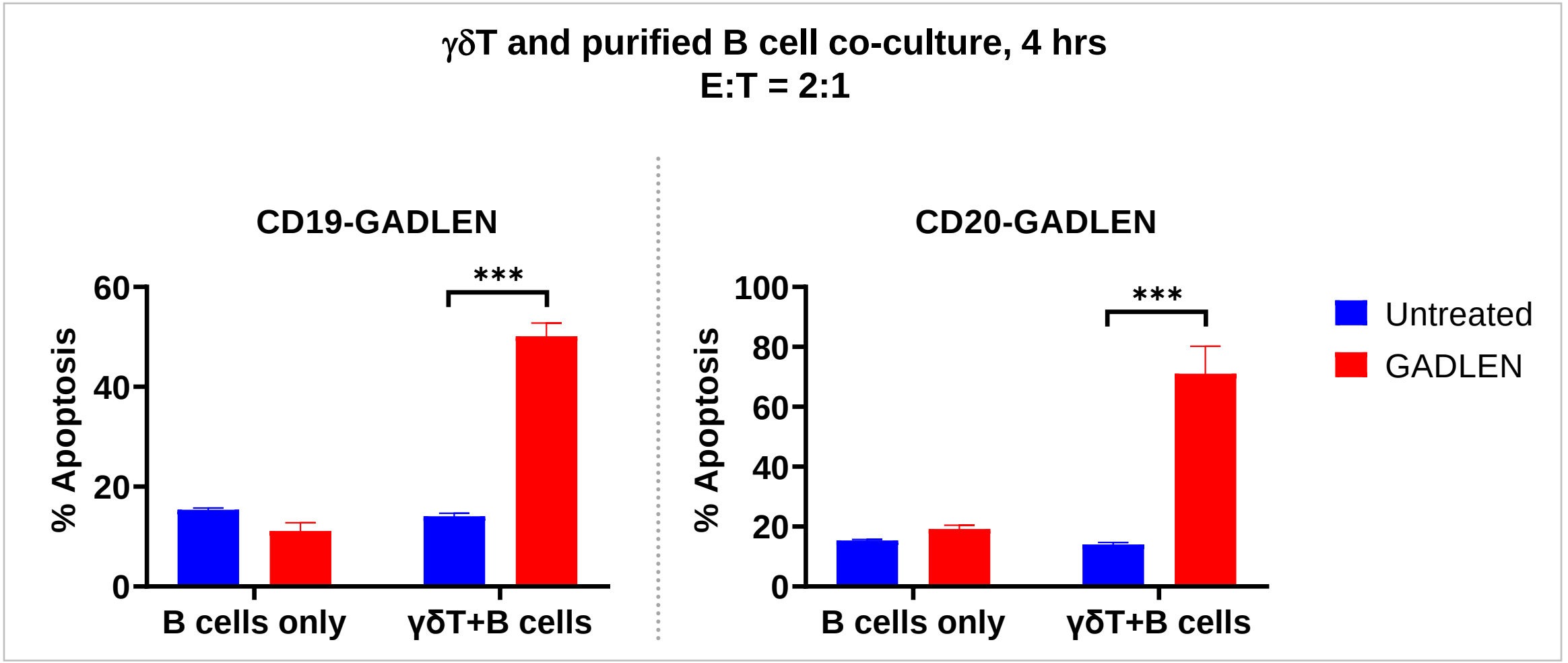
Expression of γδT Costimulatory Ligands (CD80 and CD86) Enables GADLEN Targeting of Human B Cells



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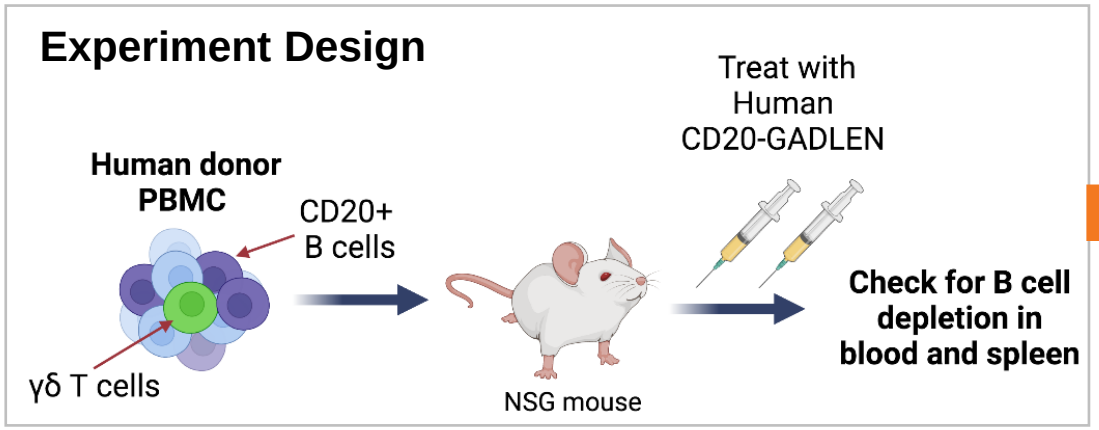
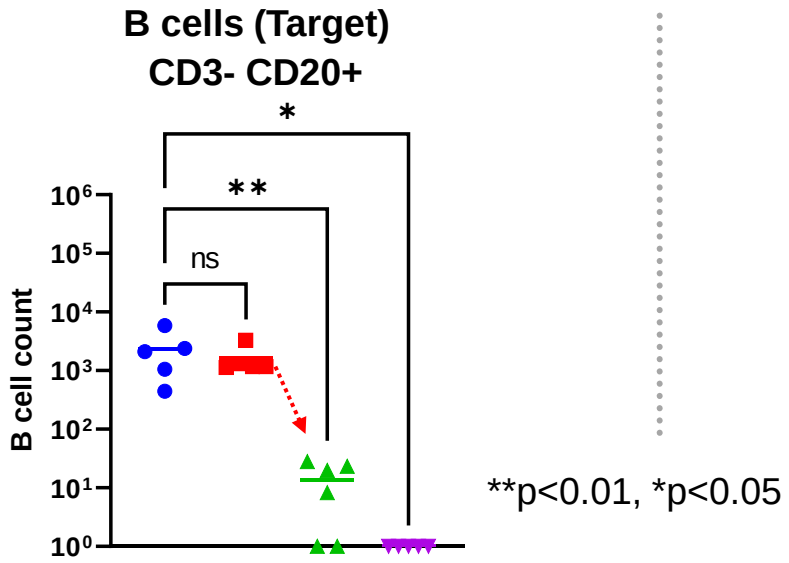
Selective *In Vivo* Depletion of Human Target Cells by CD20 GADLEN

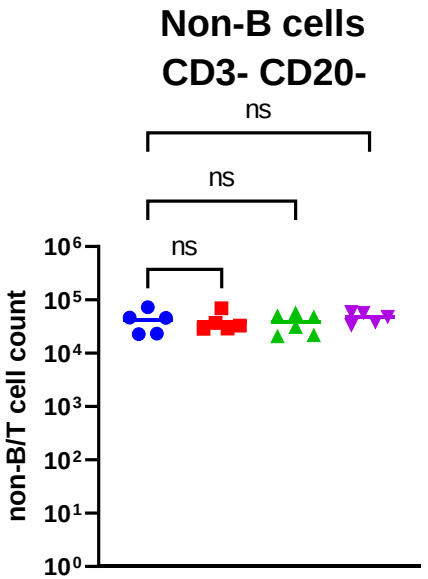
E:T Ratio = 1:2 (1 $\gamma\delta$ T cell per 2 B cells)



- CD20-GADLEN depleted >99% CD19+/20+ B cells *in vivo* (peripheral blood & spleen)
- Normal B cells express CD80 and CD86, which provide necessary costim signal (via CD28) for $\gamma\delta$ T cell targeting
- Absolute count for cells not expressing CD19/20 remains unchanged in spleen and blood
- CD33 targeted GADLEN included as specificity control



**p<0.01, *p<0.05



- Vehicle
- CD33GADLEN (200ug) - non-targeting control
- CD20GADLEN (50ug)
- CD20GADLEN (200ug)

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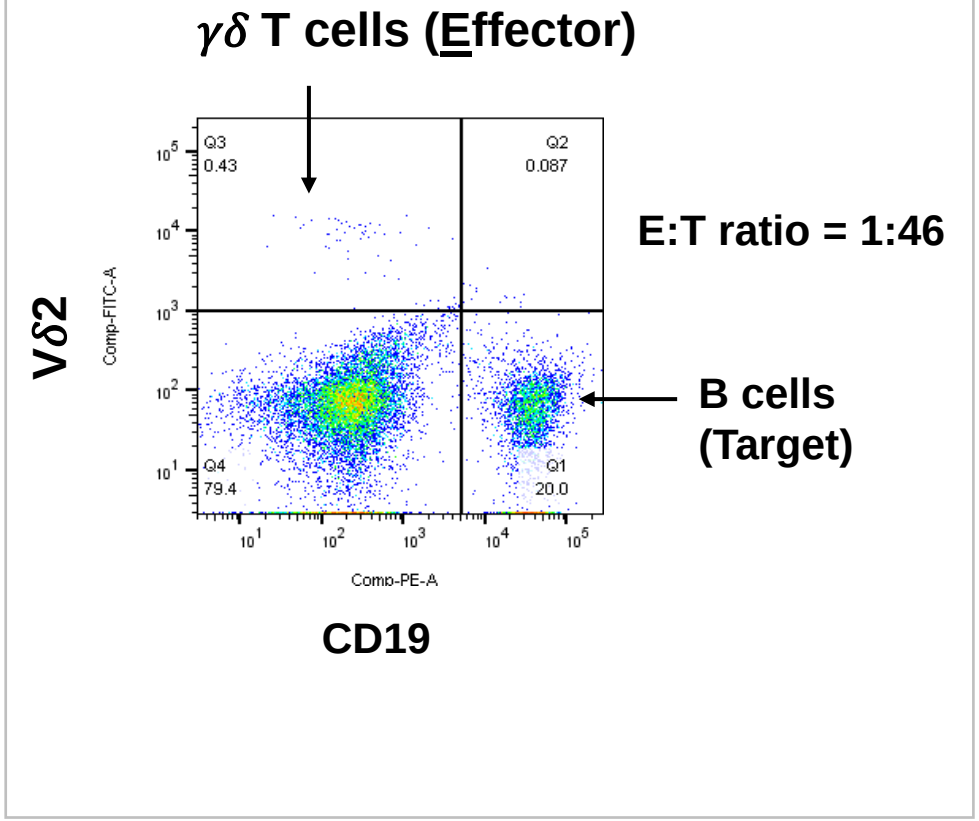
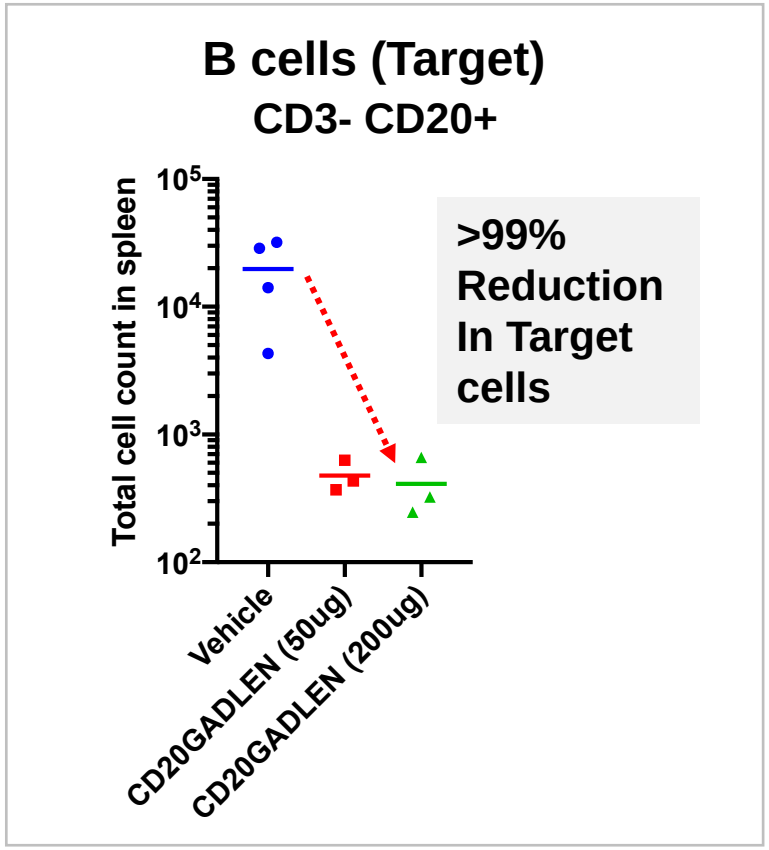
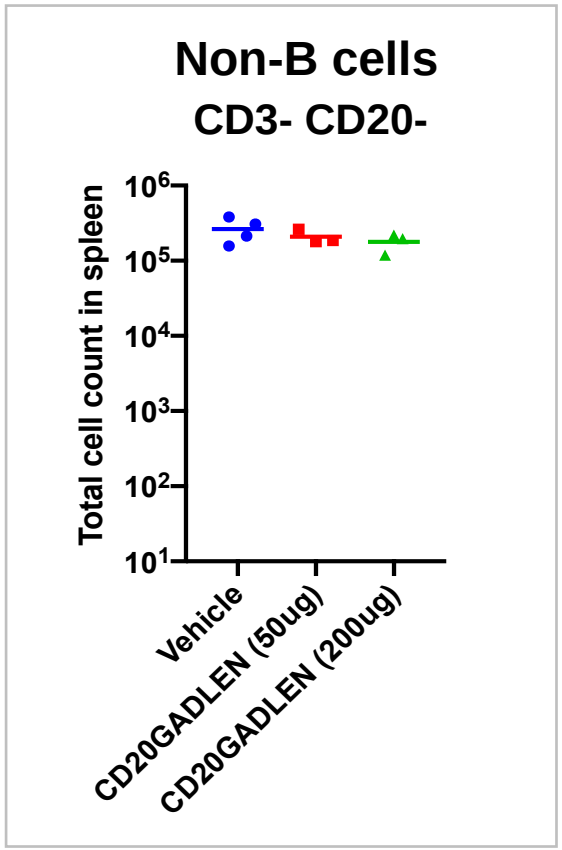
Efficient Depletion of Human Target Cells at Physiologic γδ Levels

E:T Ratio = 1:46 (approx. 1 γδ T cell per 50 B cells)







➔ Complete and specific B cell depletion in the presence of physiologically relevant frequencies of Vγ9δ2 T cells (less than 5% of total CD3+ T cells)

➔ Indicates rapid serial killing potential of Vγ9δ2 T cells

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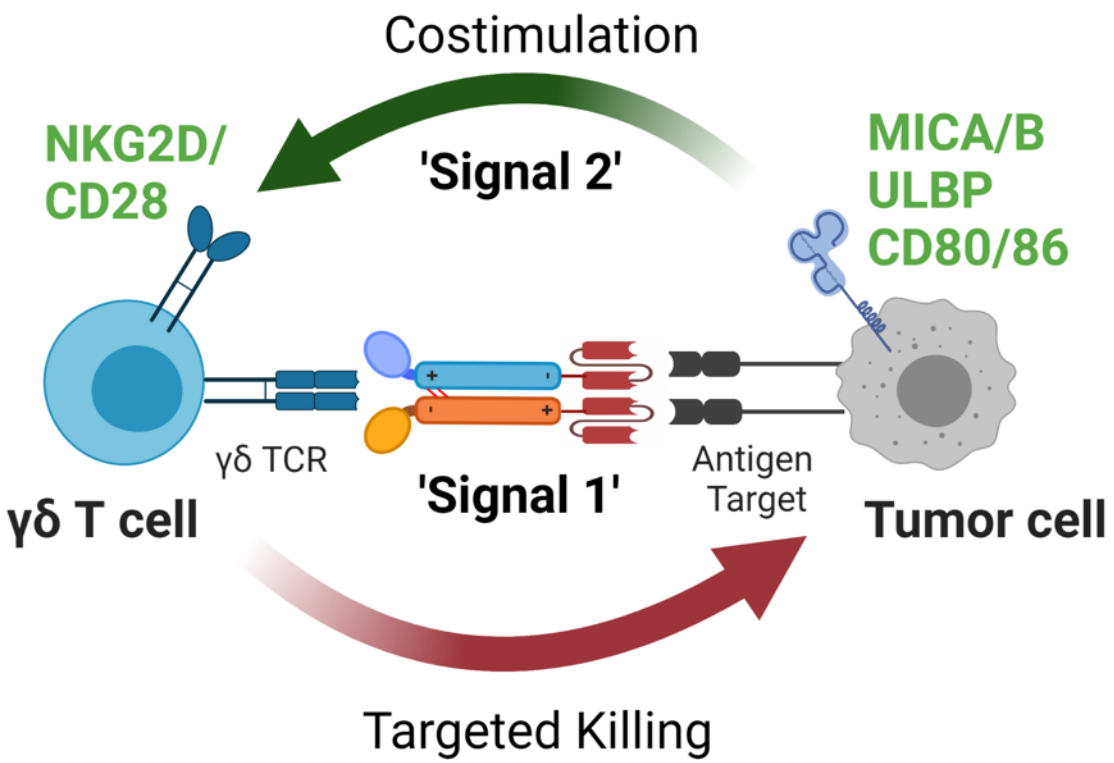
Summary of GADLEN Platform



- Activates $\gamma\delta$ T cells with on-target binding to antigen expressed on tumor cells
- Identified costimulatory requirement to promote BTN-mediated activation of $\gamma\delta$ T cells
- Selectively targets antigen-expressing tumor cells – without evidence of off-target cell killing
- Depletes >99% of target cells with E:T ratios of ~1:50, indicating serial killing at physiological levels of $\gamma\delta$ T cells
- Modular design enables single or dual scFv sequences for efficient tumor targeting
- Potential to target other $\gamma\delta$T subsets as role of additional BTN heterodimers are identified

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The GADLEN team

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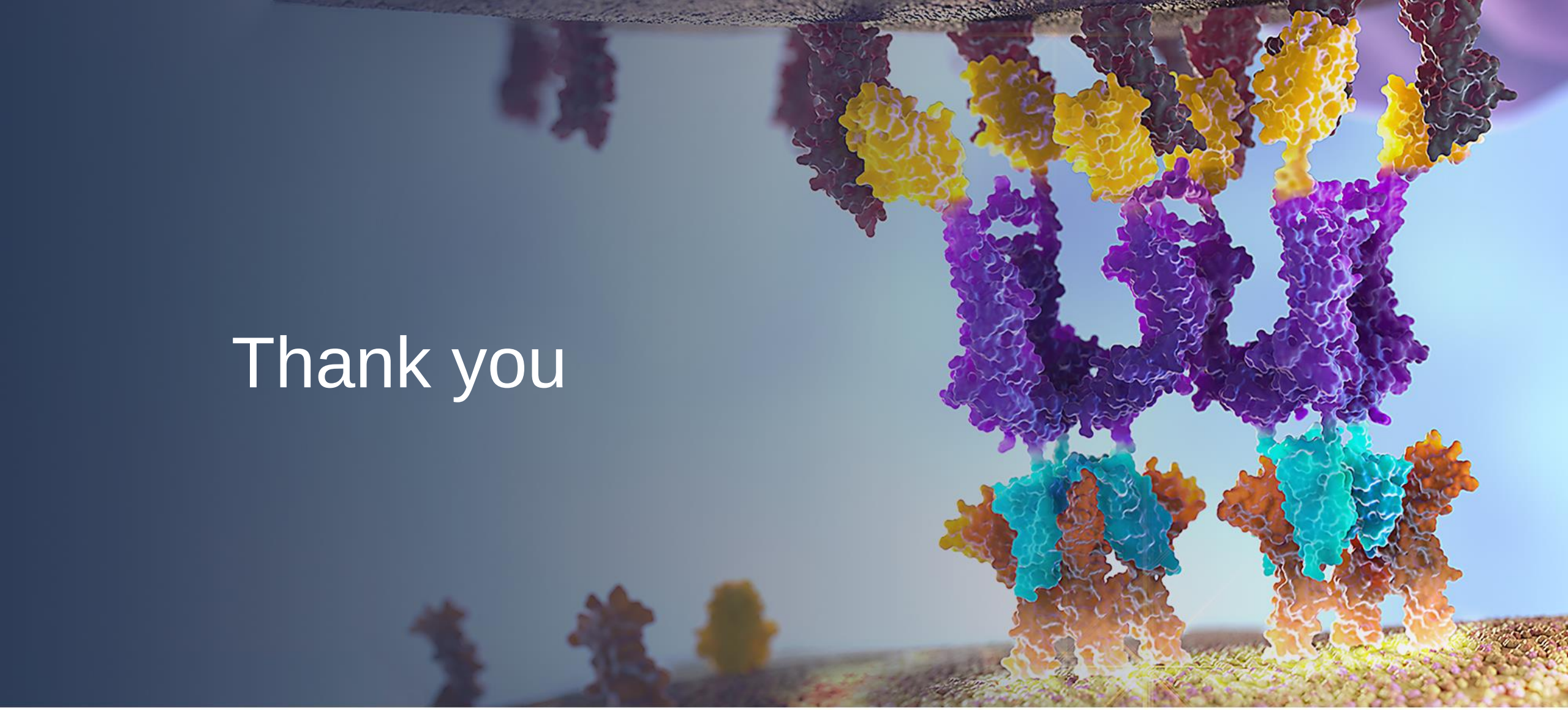

Thank you

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