Synthetic Immunology

Harnessing the Tools of Synthetic Biology to Engineer Next-Generation Immune Cell Therapies

Kole T. Roybal, Ph.D.
Department of Microbiology & Immunology
University of California, San Francisco

ASCO-SITC Winter School
February 20th, 2019

PARKER INSTITUTE
for CANCER IMMUNOTHERAPY

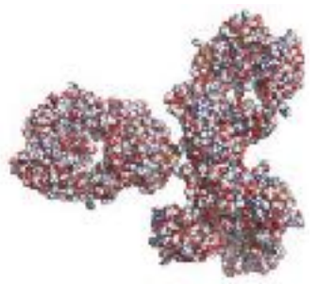
CHAN ZUCKERBERG BIOHUB

Cell-Based Therapeutics
The Next Pillar of Medicine

small molecules

chemical modifications

biologics



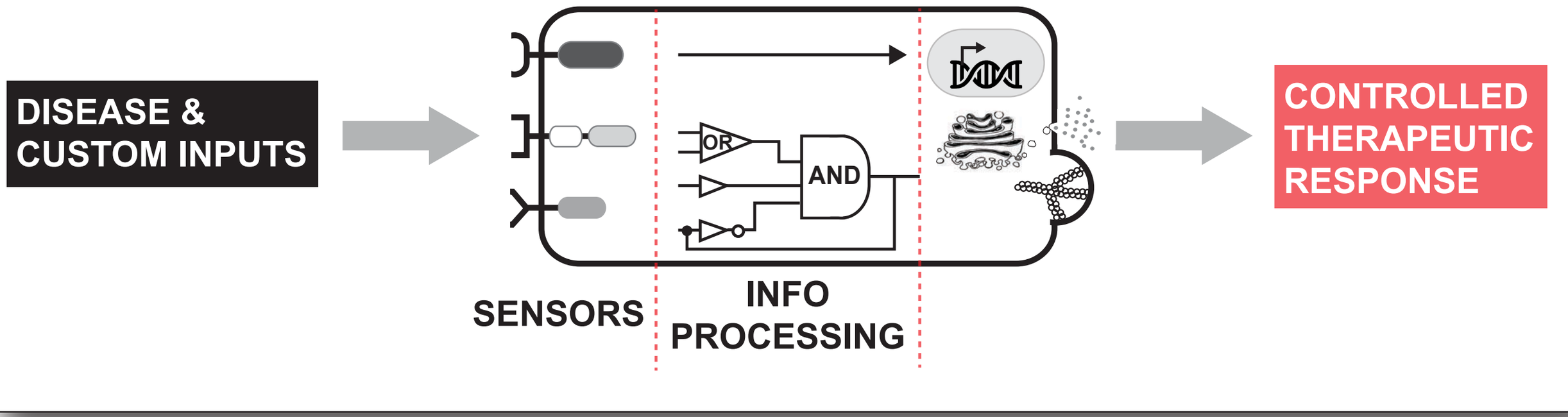
protein engineering

engineered cells

synthetic biology

Building New Sensing and Response Capabilities into Therapeutic Cells with Synthetic Biology

SMART THERAPEUTIC CELL

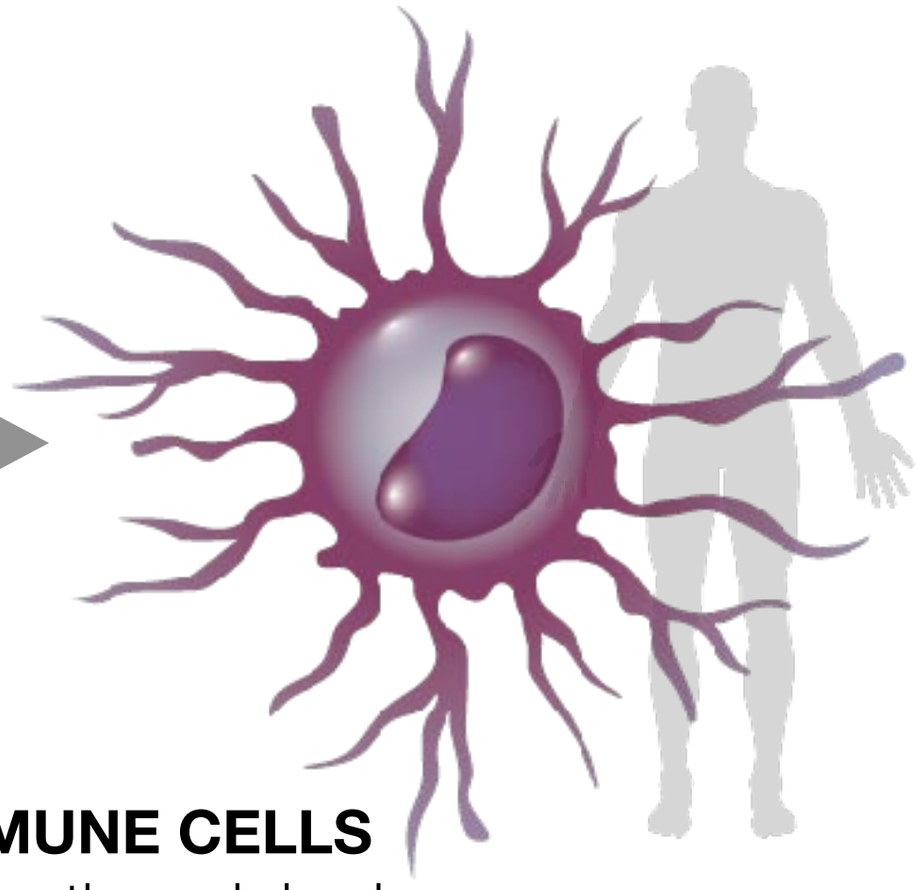


How can we engineer cells that:

1. Reliably discriminate disease from healthy tissue?
2. Dynamically regulate their therapeutic activity?
3. Tailor their therapeutic activity to the type of disease?
4. Drive a multi-pronged attack that is difficult to circumvent?

The Immune System as a Platform for Interfacing with Disease

SENSING
Infection
Injury
Tumor Cells

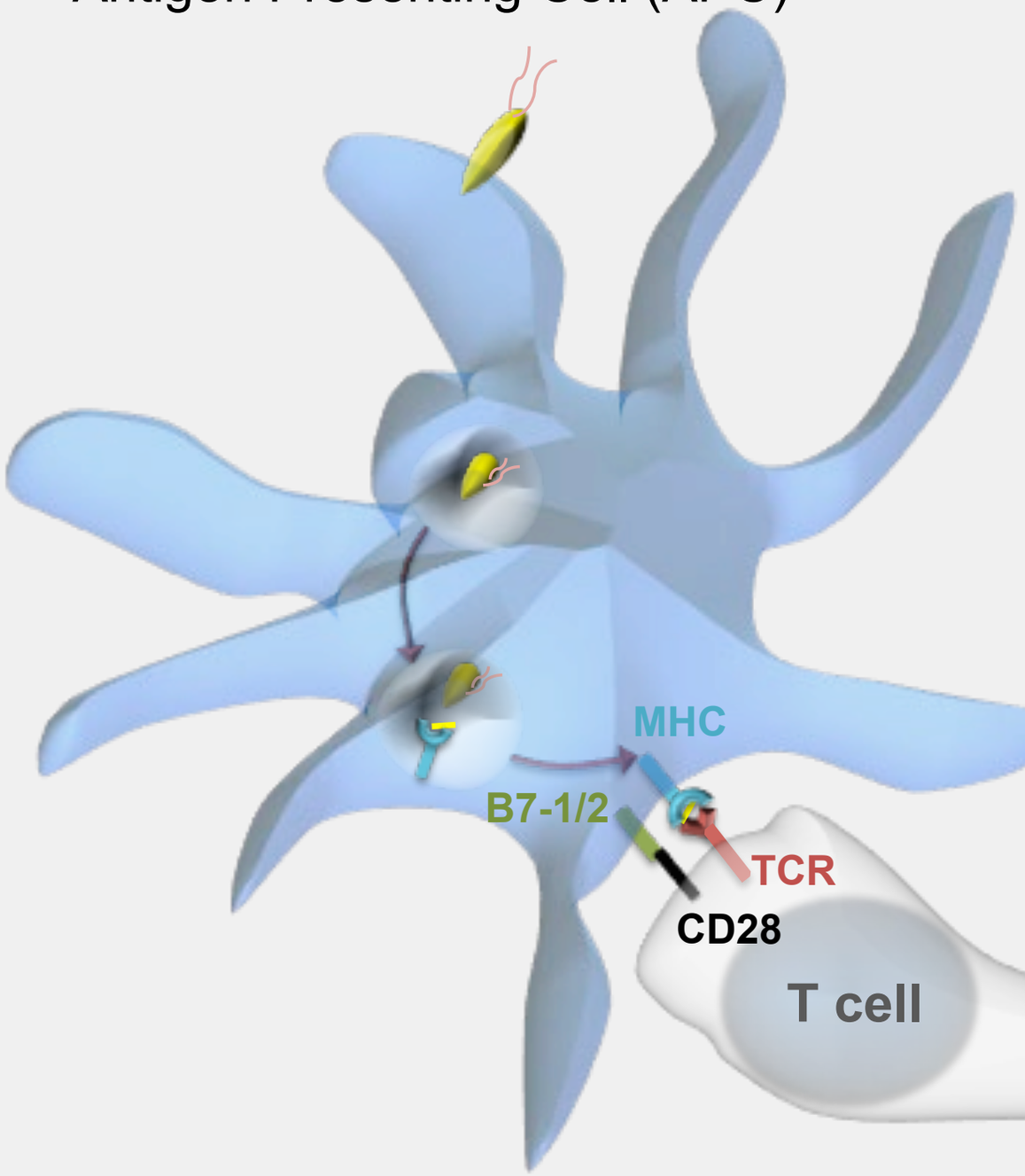


RESPONSE
Communicate
Eliminate disease
Coordinate repair

IMMUNE CELLS
Move through body
Monitor tissues
Conditionally activate
Display memory

T cell Activation Occurs Through a Cell-to-Cell Interaction

Dendritic cells/B cells/Macrophages
Antigen Presenting Cell (APC)



Targeted Release of Cytotoxic Granules (e.g. virally infected cells or tumor cells)

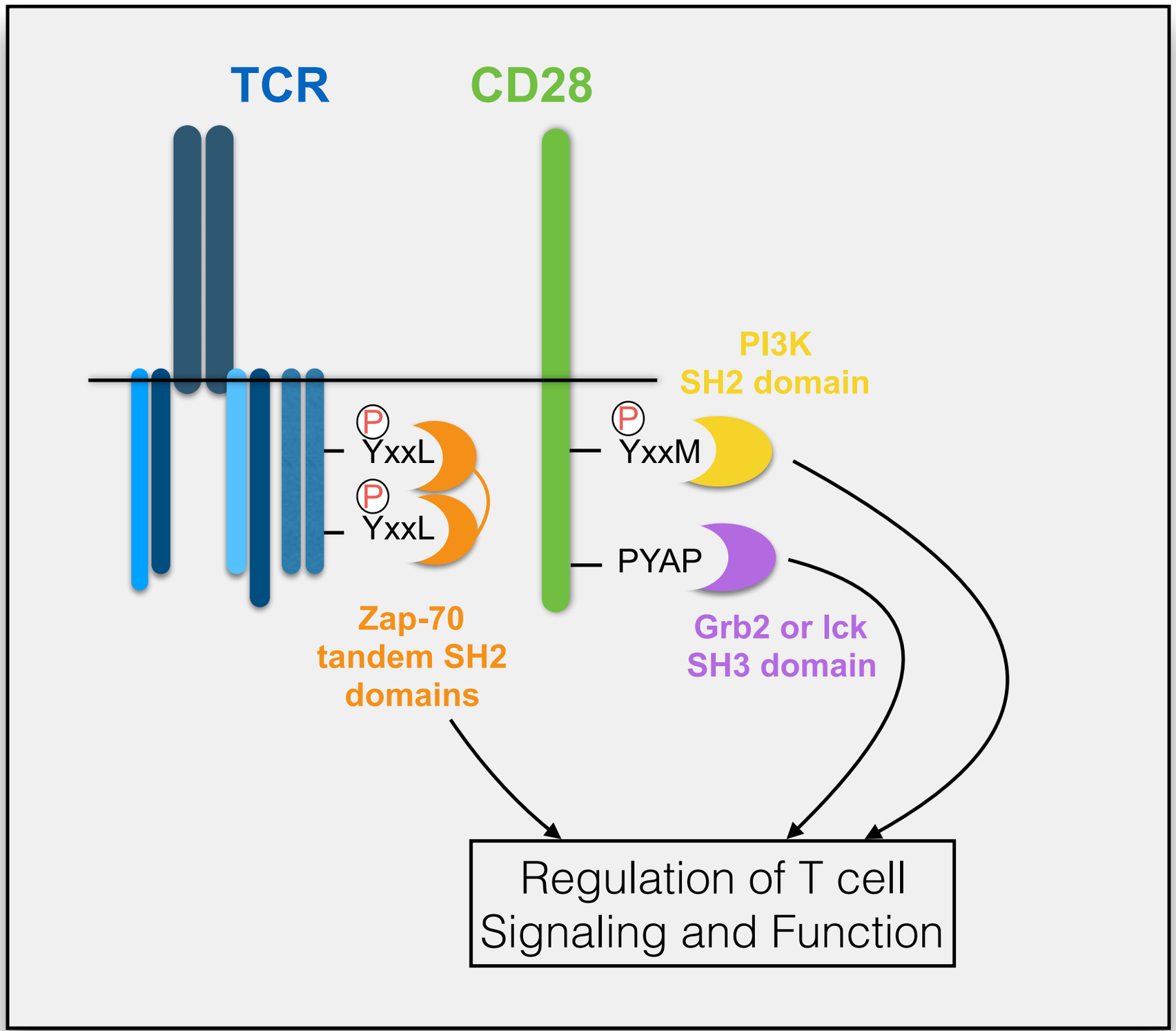
Cytokine Production

B cell Help for Antibody Production

Differentiation

Proliferation

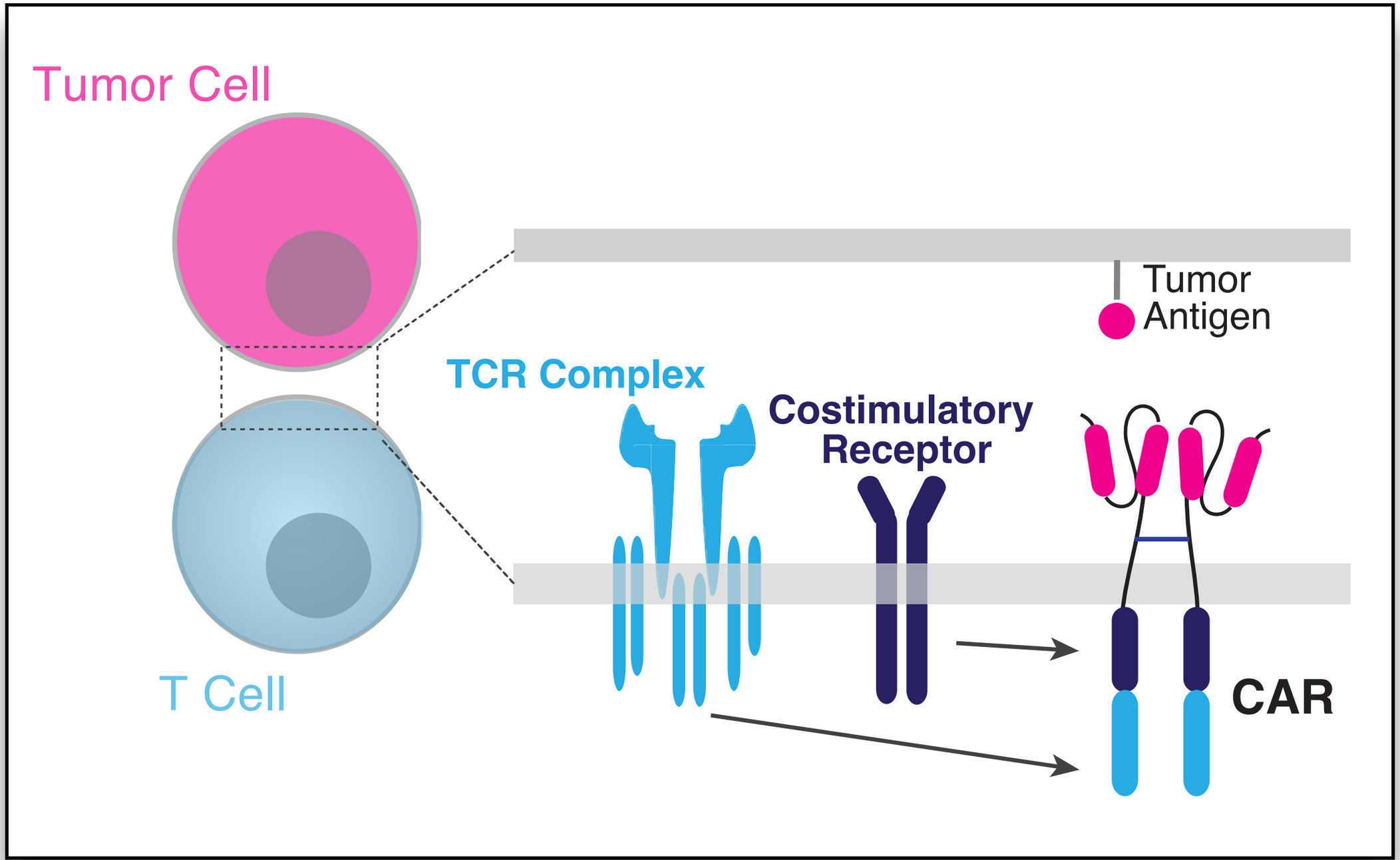
Linear Motifs Mediate T cell Receptor and Co-stimulatory Signaling



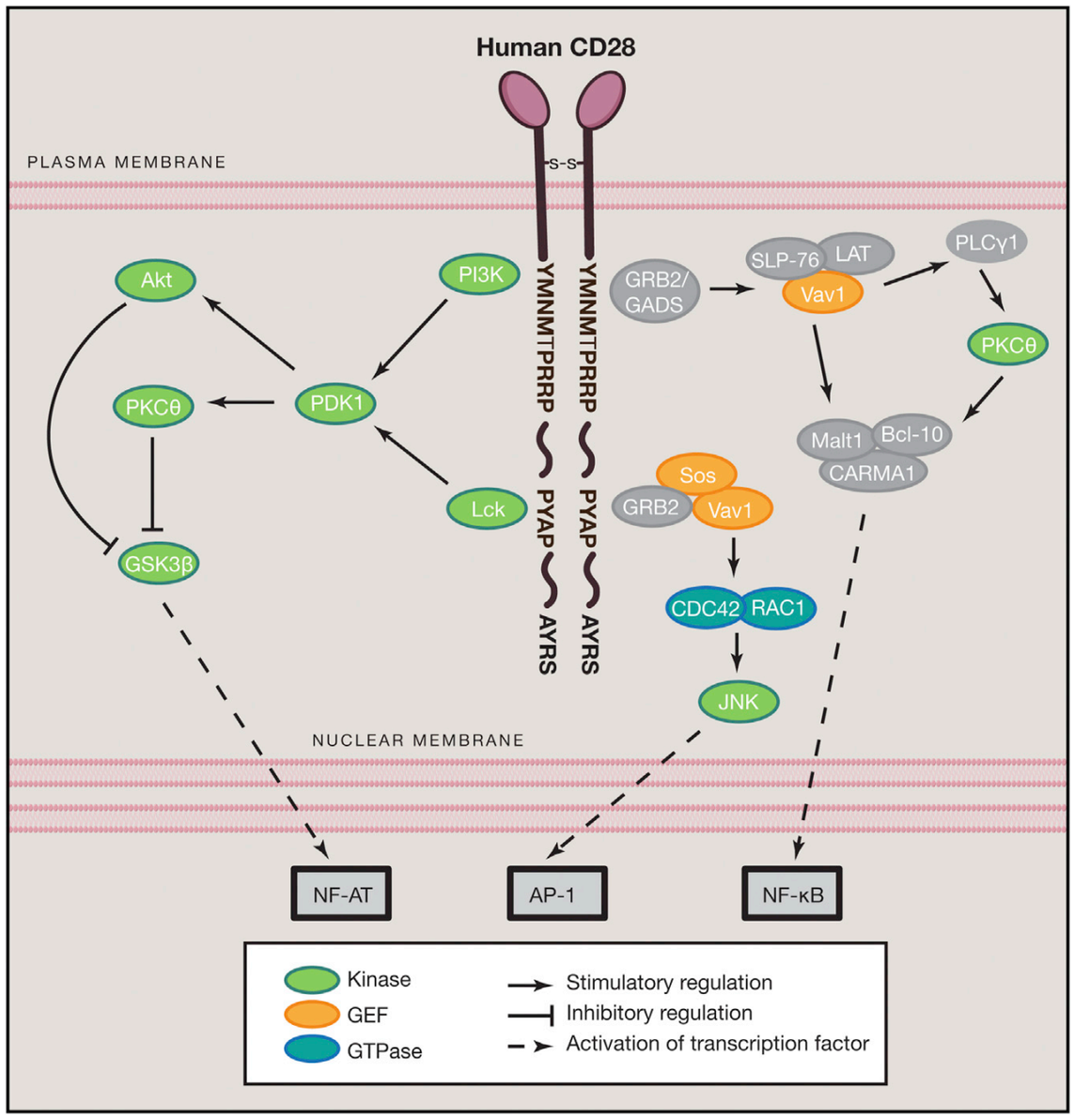
Chimeric Antigen Receptors

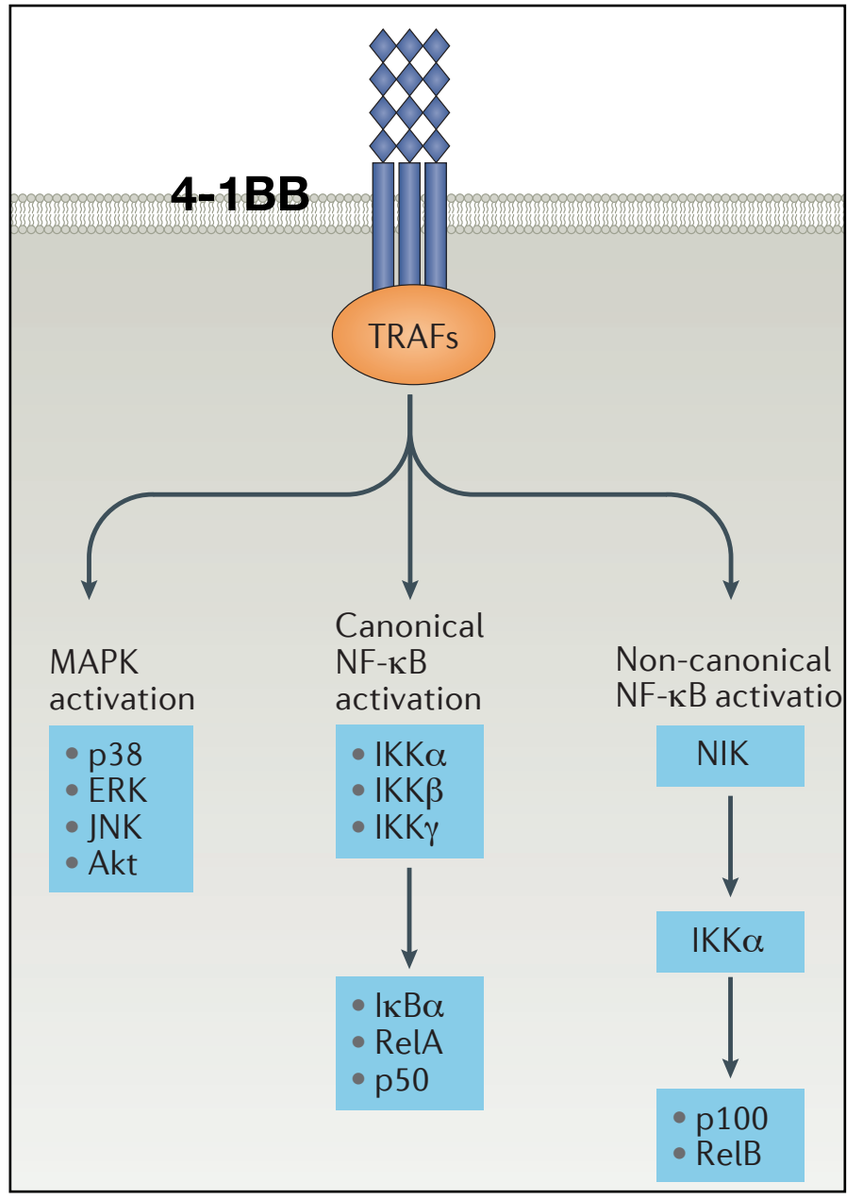
Synthetic Receptors for Retargeting T cells to Disease

Chimeric Antigen Receptor T cells (CARTs)



Adding Signal 2 (Co-stimulation) into CARs





Costimulation Enhances:

Proliferation

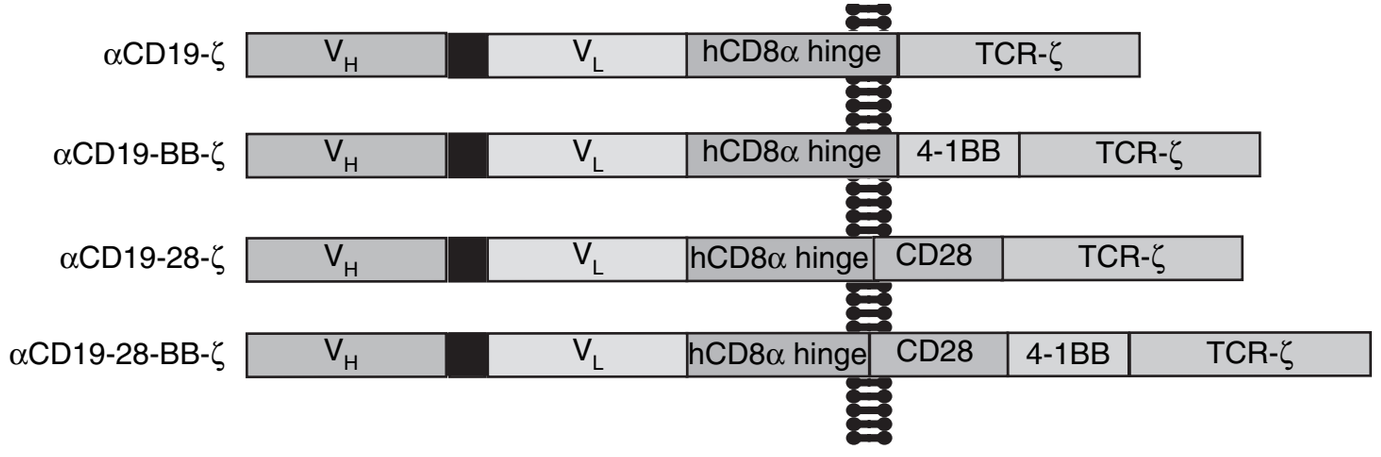
Changes Metabolism

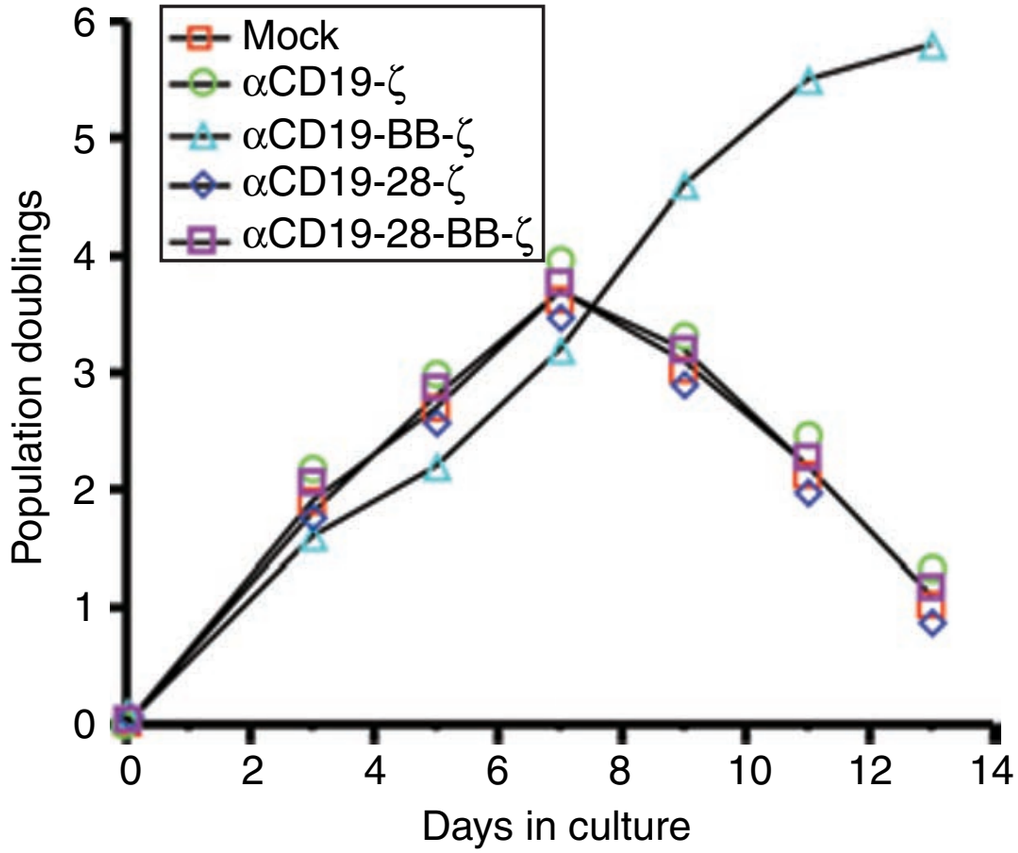
Alters Cytokine Production

Controls T cell longevity

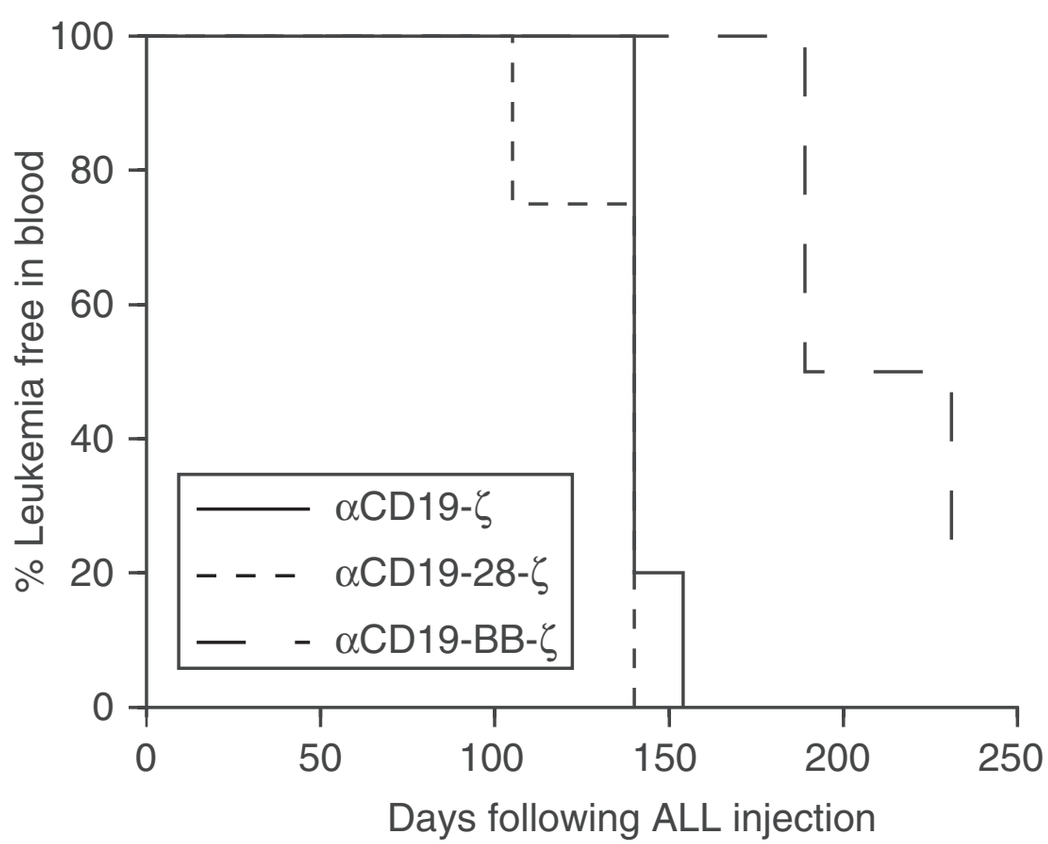
Esensten J. Immunity. 2016

Different Costimulatory Signals Can Affect the Engineered T cell Response to Cancer



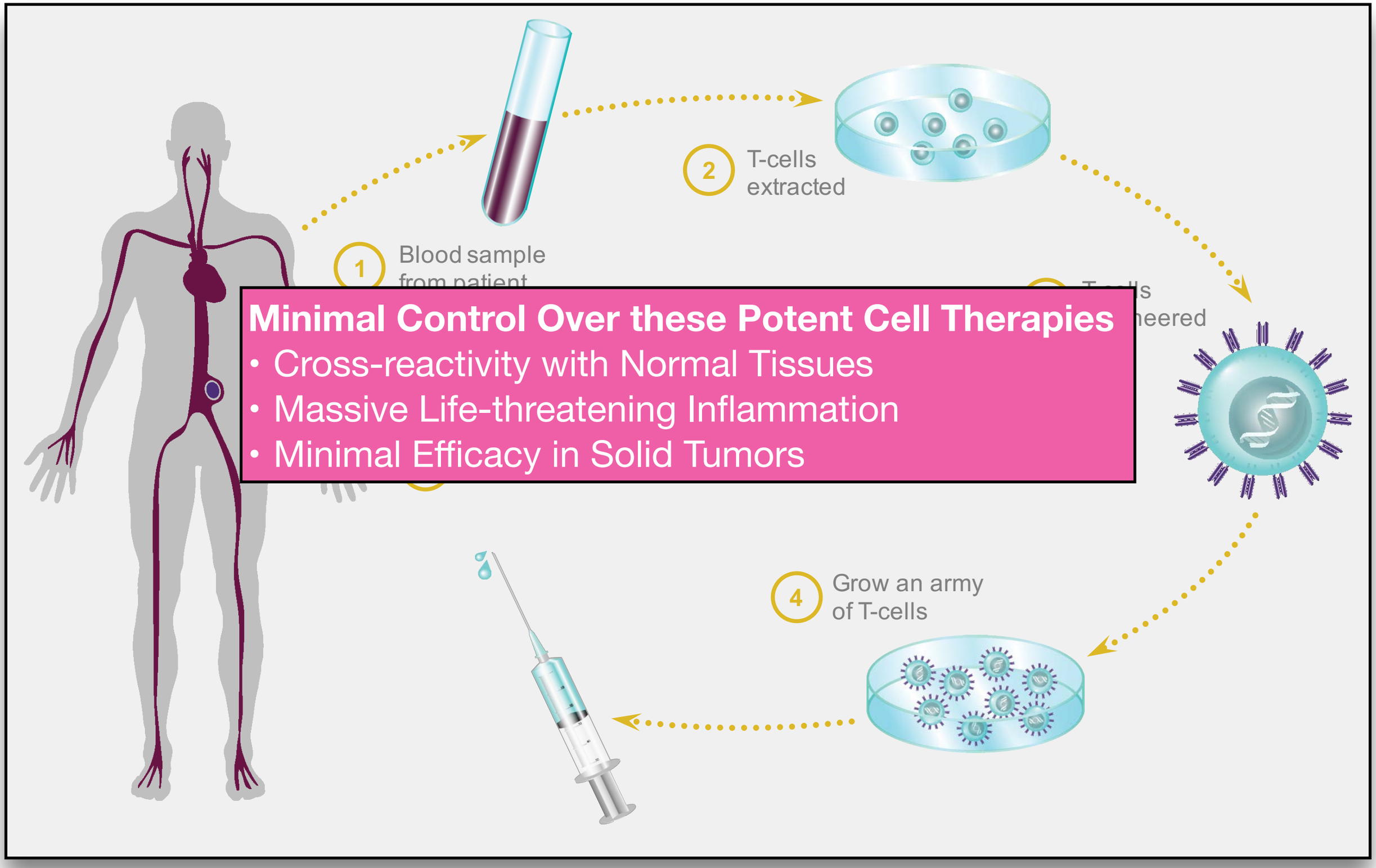


Efficacy in a Model of Acute Lymphoblastic Leukemia



Milone MC. Molecular Therapy. 2009

T cell Therapy Treatment and Pitfalls



Second Generation CAR T cell Trials in ALL

Table. Published Outcomes Using Second-Generation Anti-CD19 CART Cells

Study Group/ Reference	Signaling Domains Targeted	Lymphodepleting Agent(s)	Population	Response Rate	CRS Rate	Neurologic Toxicity Rate
Acute Lymphoblastic Leukemia (ALL)						
Penn/CHOP Maude et al[4]	CD3ζ, 4-1BB[a]	Varied	N = 30 pediatric and adult patients	CR: 90%	Total: 100% 27% severe	Total: 43% Encephalopathy, aphasia, seizures (1 patient)
MSKCC Davila et al[1]	CD3ζ, CD28	Cyclophosphamide	N = 16 adults	CR: 88%	43% severe	Grade 3/4: 25% Encephalopathy, seizures
NCI Lee et al[3]	CD3ζ, CD28	Fludarabine/ cyclophosphamide	N = 21 pediatric and adult patients	CR: 67% in intent-to-treat population	Total: 76% 28% severe	Total: 29% Hallucinations, dysphasia, encephalopathy
FHCRC Turtle et al[7]	CD3ζ, 4-1BB[a]	Cyclophosphamide and fludarabine/ cyclophosphamide	N = 29 adults	CR: 93%	Total: 83% 23% severe	Severe neurotoxicity: 50% TRM: 1 patient

Frey NV an Porter DL. 2016

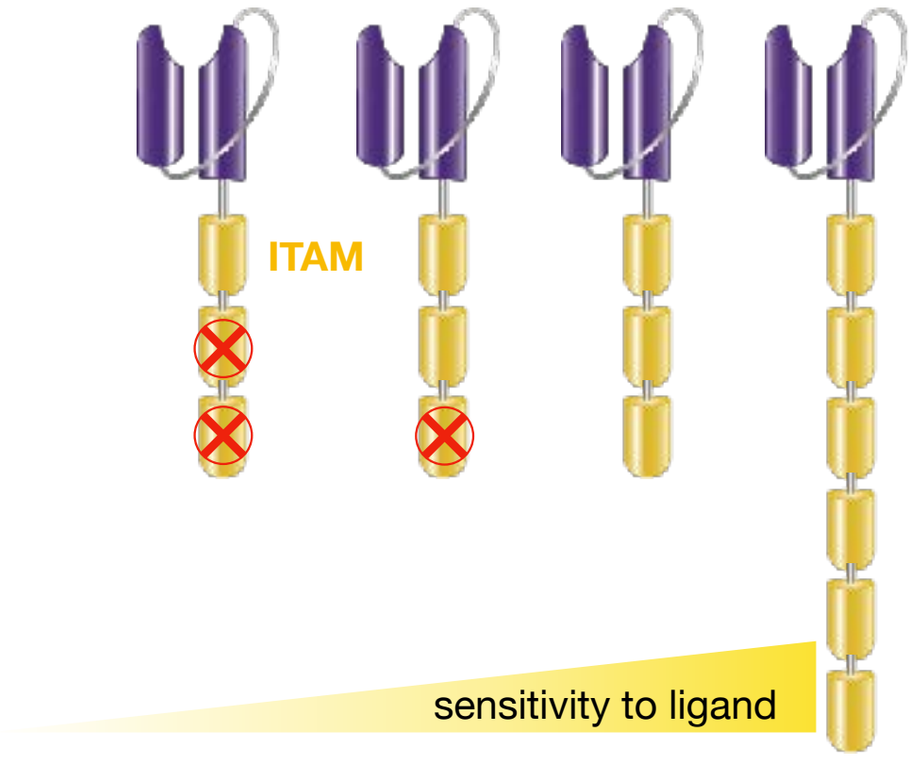
Next-generation CAR Designs

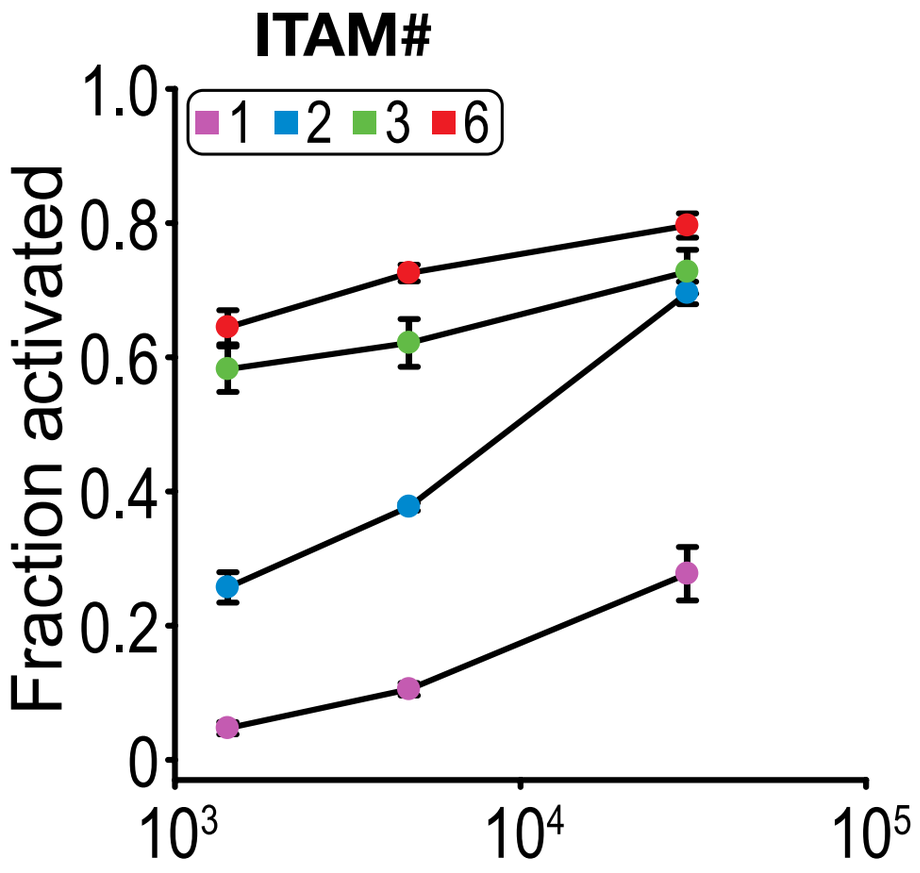
Can we improve efficacy for solid tumors?

GOAL:

1. Control/Improve persistence
2. Prevent T cell exhaustion
3. Optimize for particular T cell functions
4. Add non-natural capabilities
5. Improve sensitivity (activate at low antigen levels)
6. Reduce toxicity potential while maintaining efficacy

The Multiplicity of ITAMs Affects CAR Sensitivity





Immunoreceptor Tyrosine Activation Motif

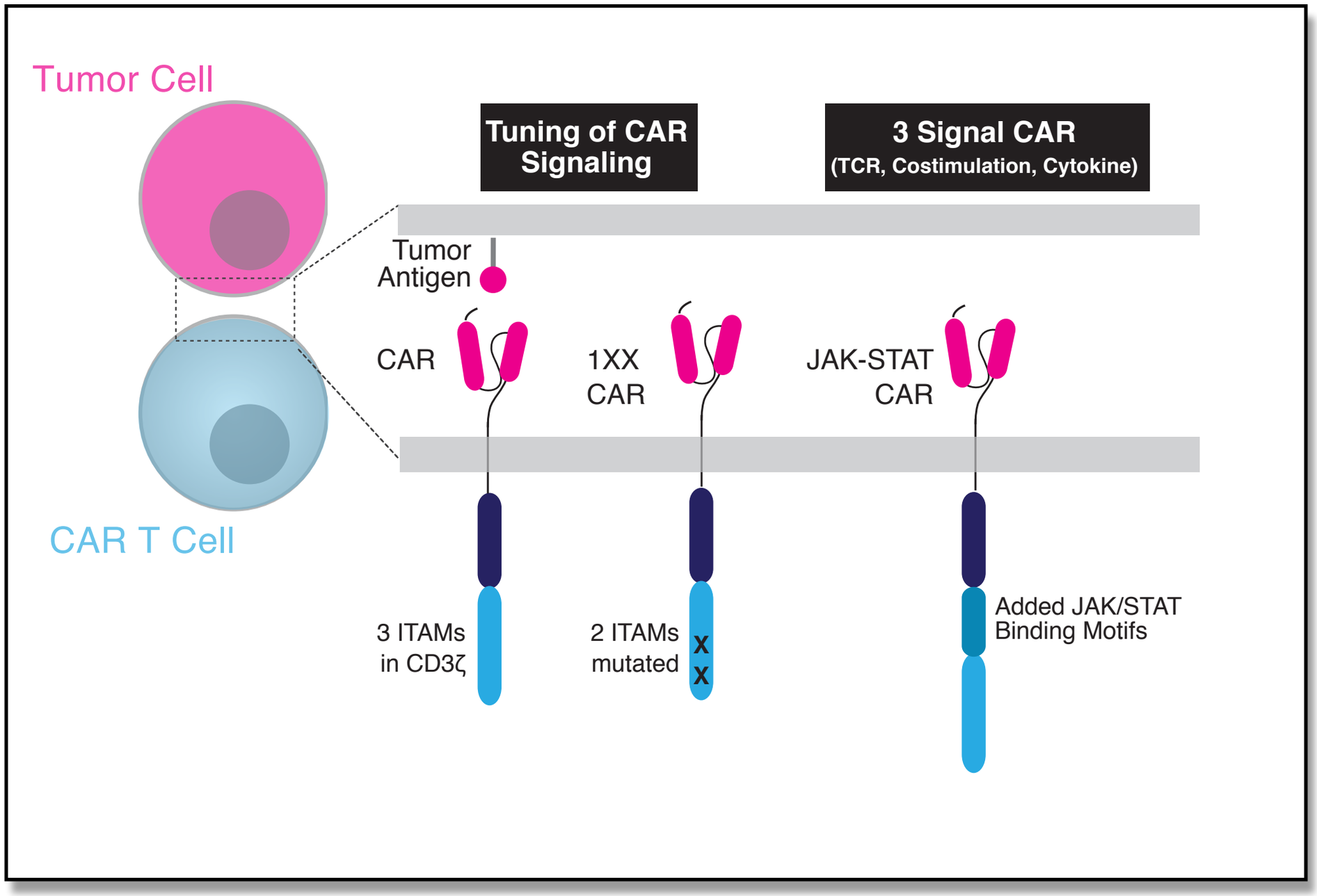
P P

YxxL/I $-x_{6\text{-}8}-$ YxxL/I

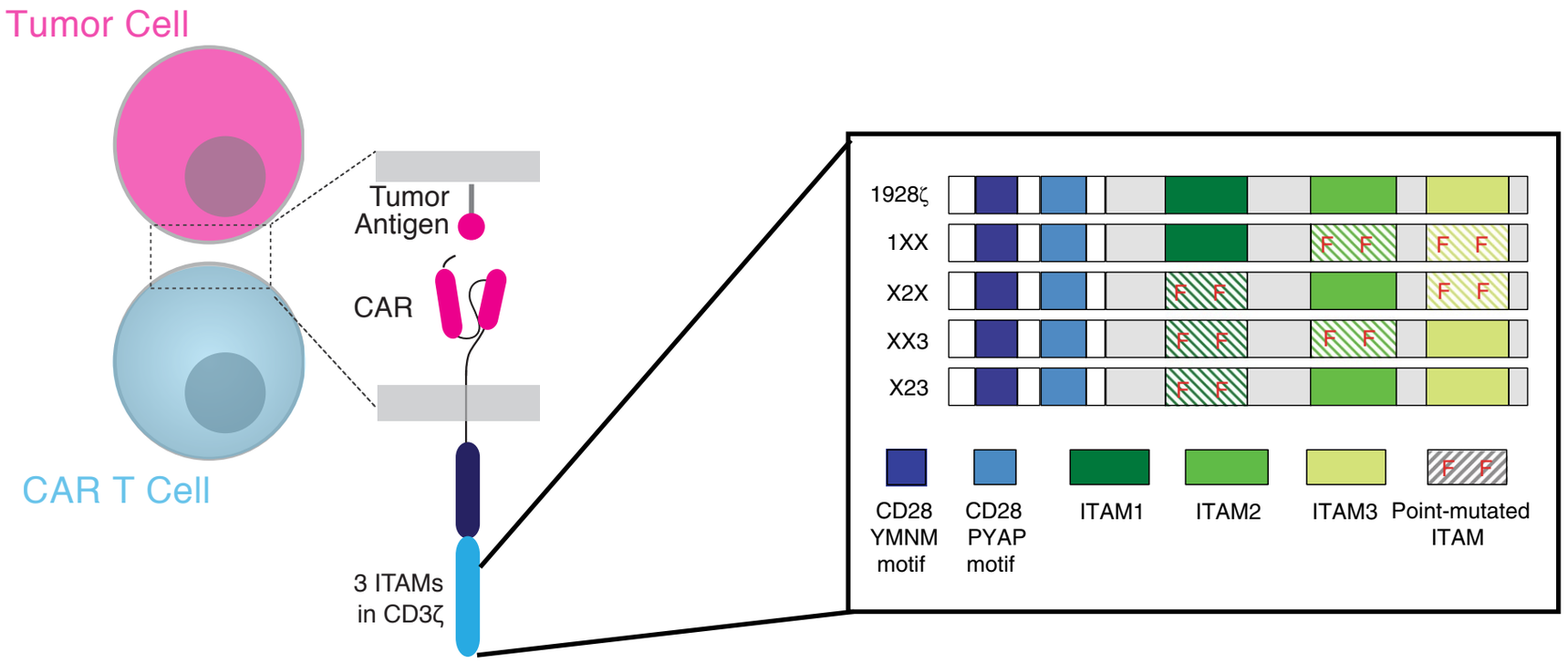
Human CD3 ITAM sequences	CD3γ	DQLYQPLK-DREDDQYSHL
	CD3δ	DQVYQPLR-DRDDAQYSHL
	CDε	NPDYEPIR-KGQRDLYSGL
	CD3ζ-1	NQLYNELN-LGRREEYDVL
	CD3ζ-2	EGLYNELQKDKMAEAYSEI
	CD3ζ-3	DGLYQGLS-TATKDTYDAL

James JR. Sci Signal. 2018

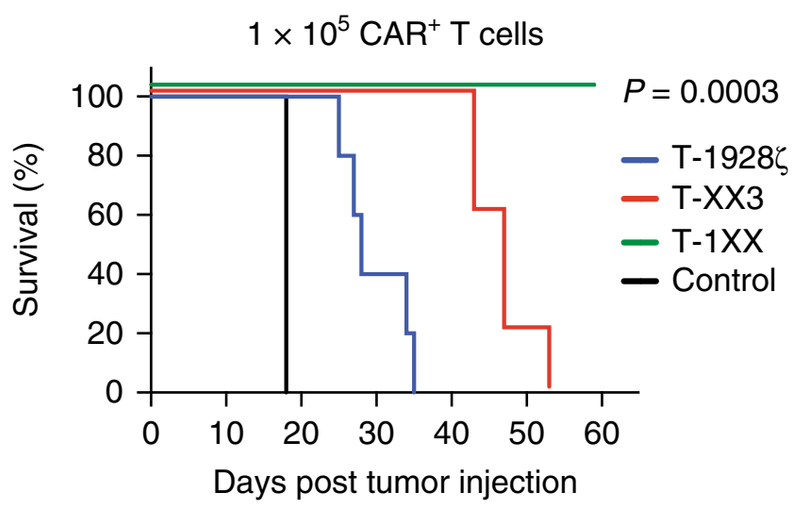
Next-Generation CARs with New Signaling Properties

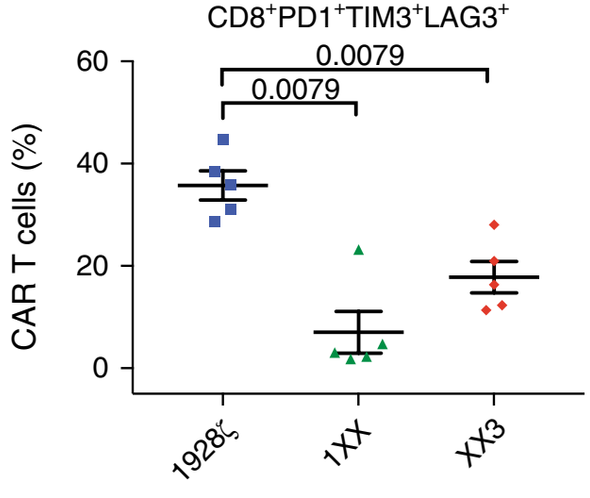


Tuning CAR Signaling Through Signaling Motif Mutagenesis



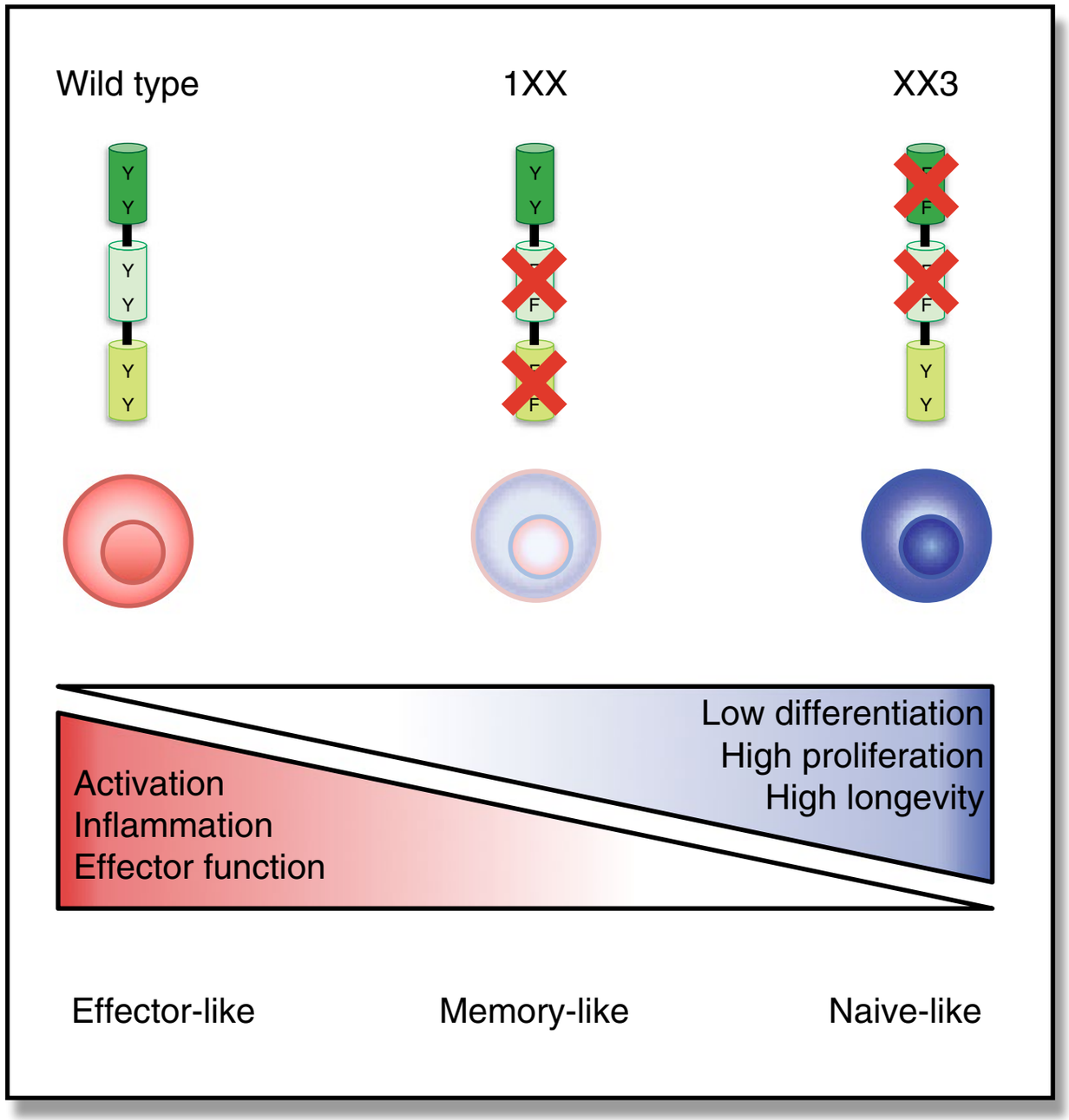
NALM-6 (CD19+ Tumor)





Feucht J et al. Nat Med. 2019

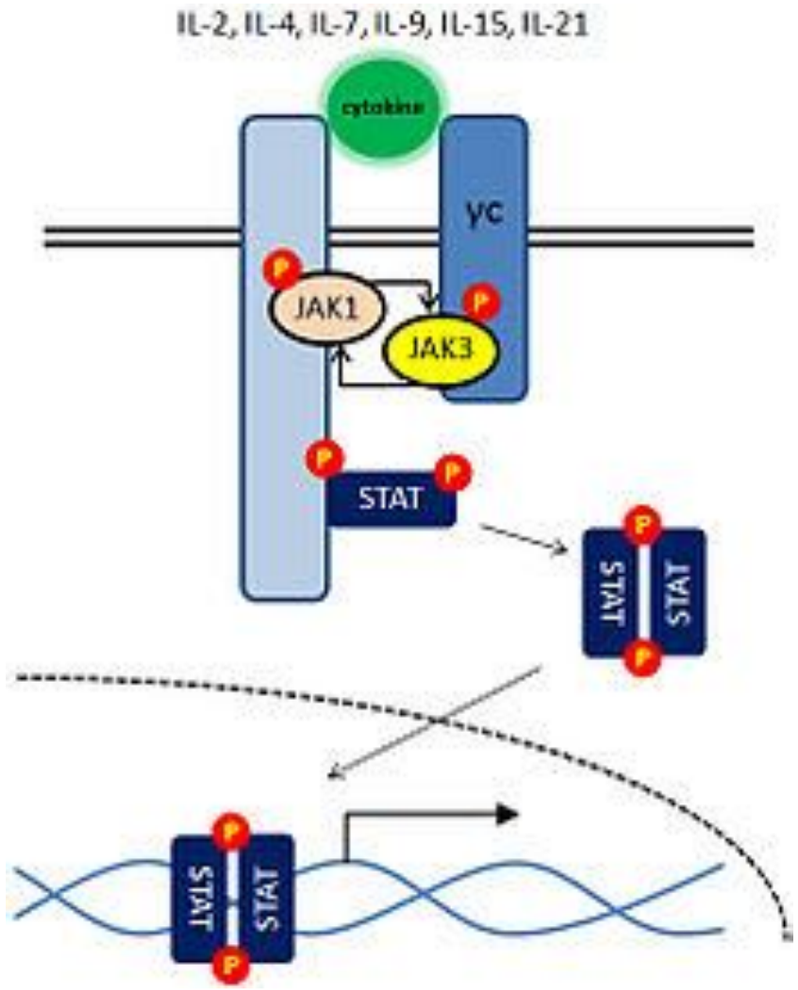
Balancing CAR Signaling Improve Therapeutic Efficacy

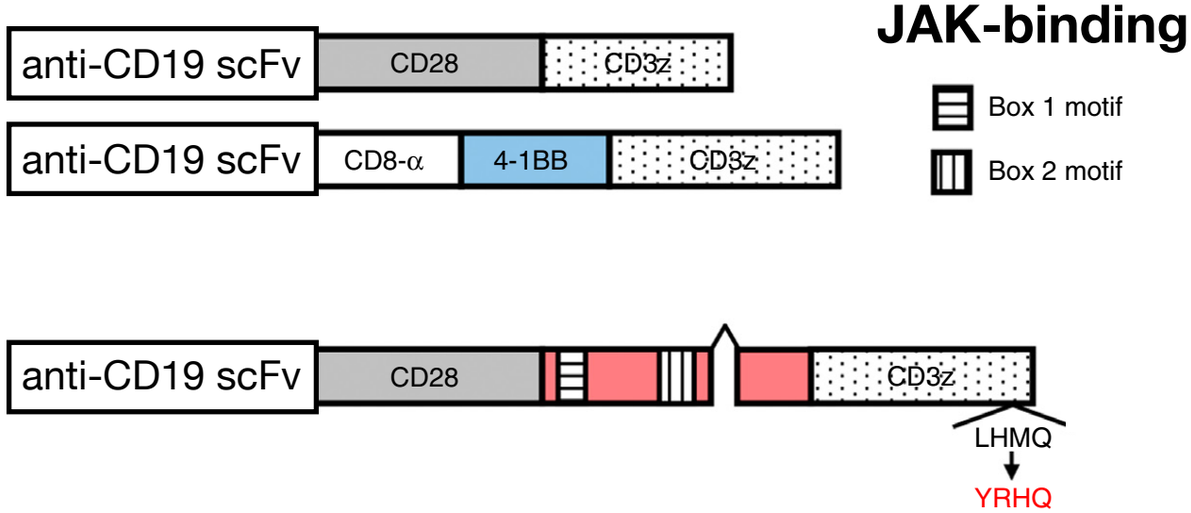


Feucht J et al. Nat Med. 2019

Adding New Signaling Capabilities to CARs

3 Signal CARs - TCR, Costimulation, and Cytokine



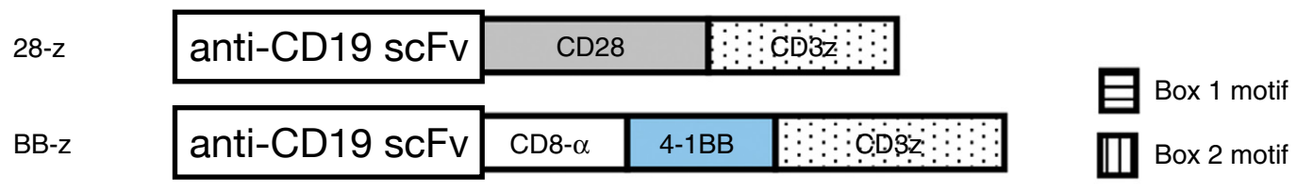


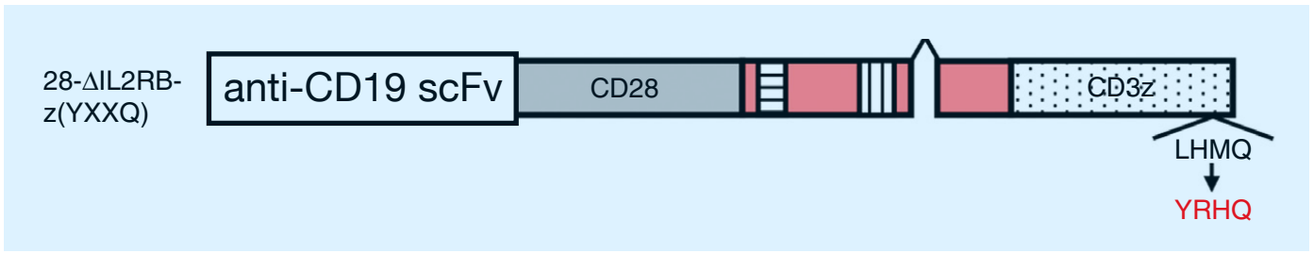
Enhanced proliferation and efficacy?

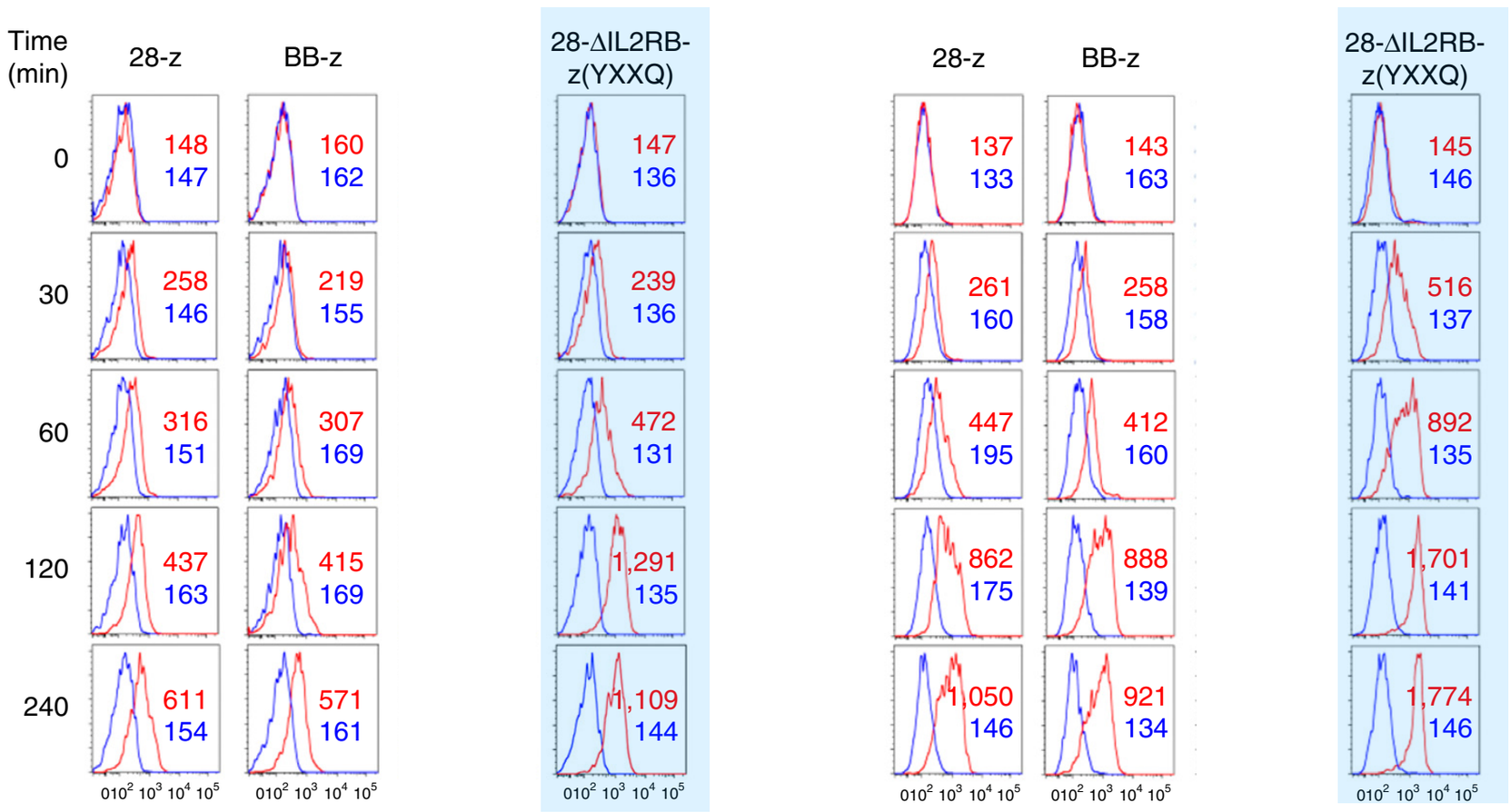
Kagoya Y et al. Nat Med. 2018

Adding New Signaling Capabilities to CARs

3 Signal CARs - TCR, Costimulation, and Cytokine





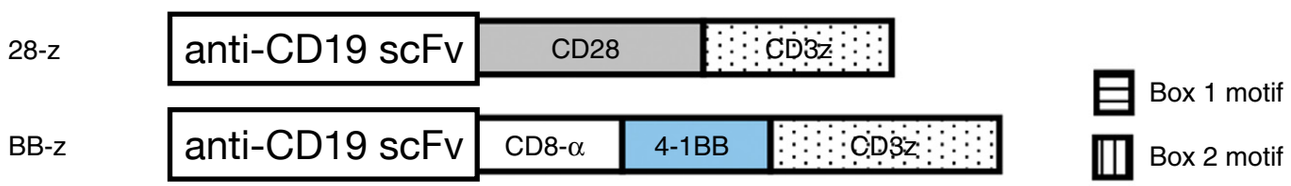


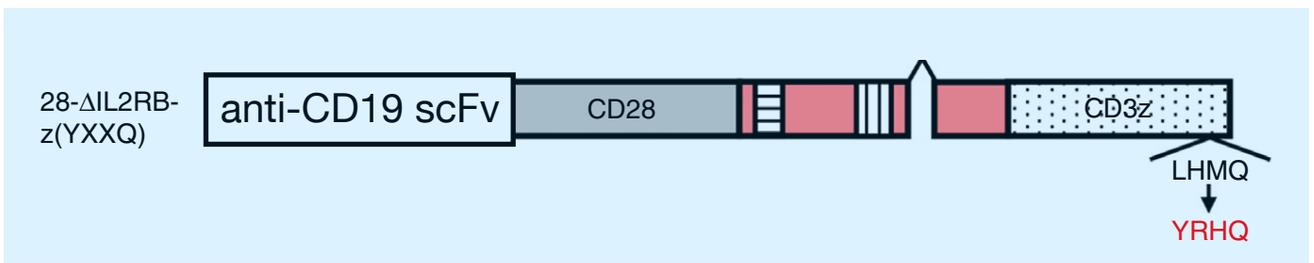
+CD19 control

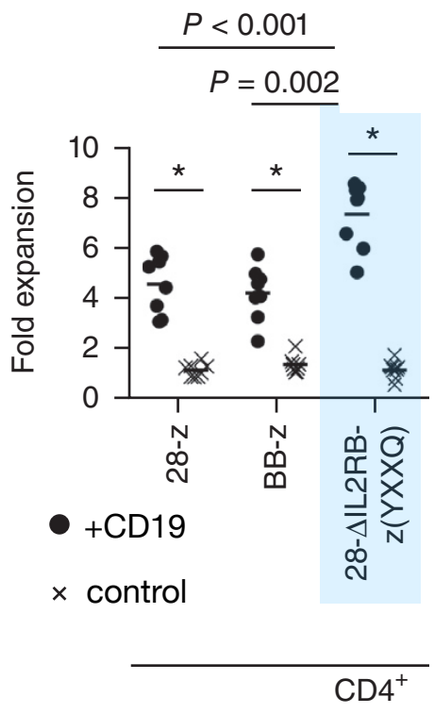
Kagoya Y et al. Nat Med. 2018

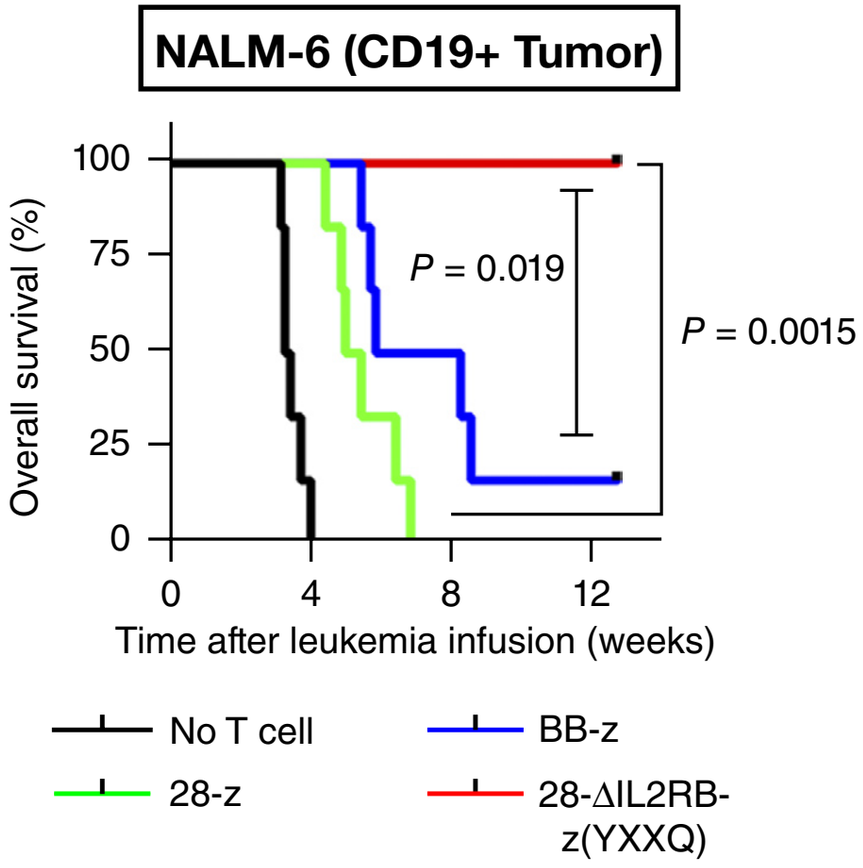
Adding New Signaling Capabilities to CARs

3 Signal CARs - TCR, Costimulation, and Cytokine





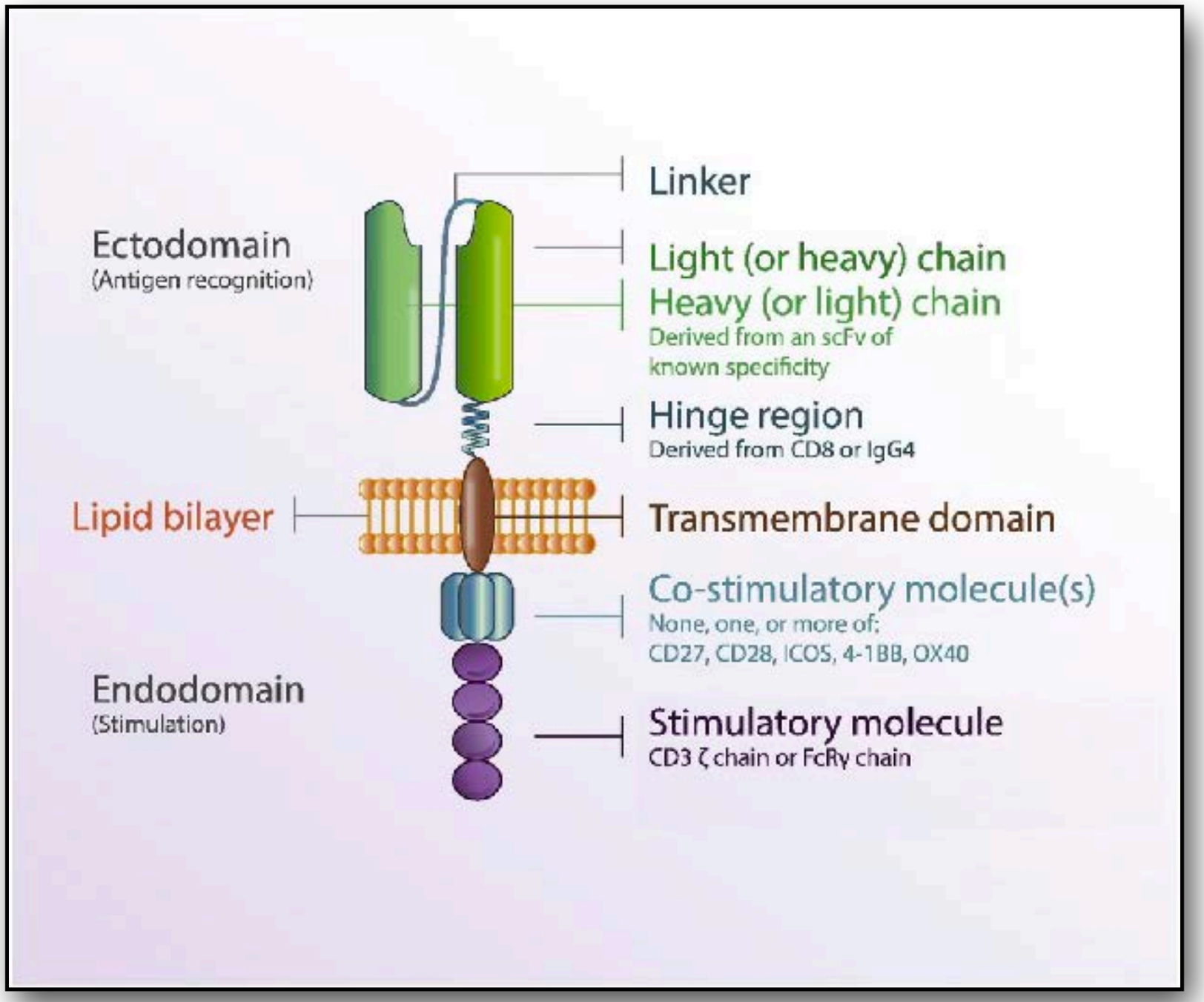




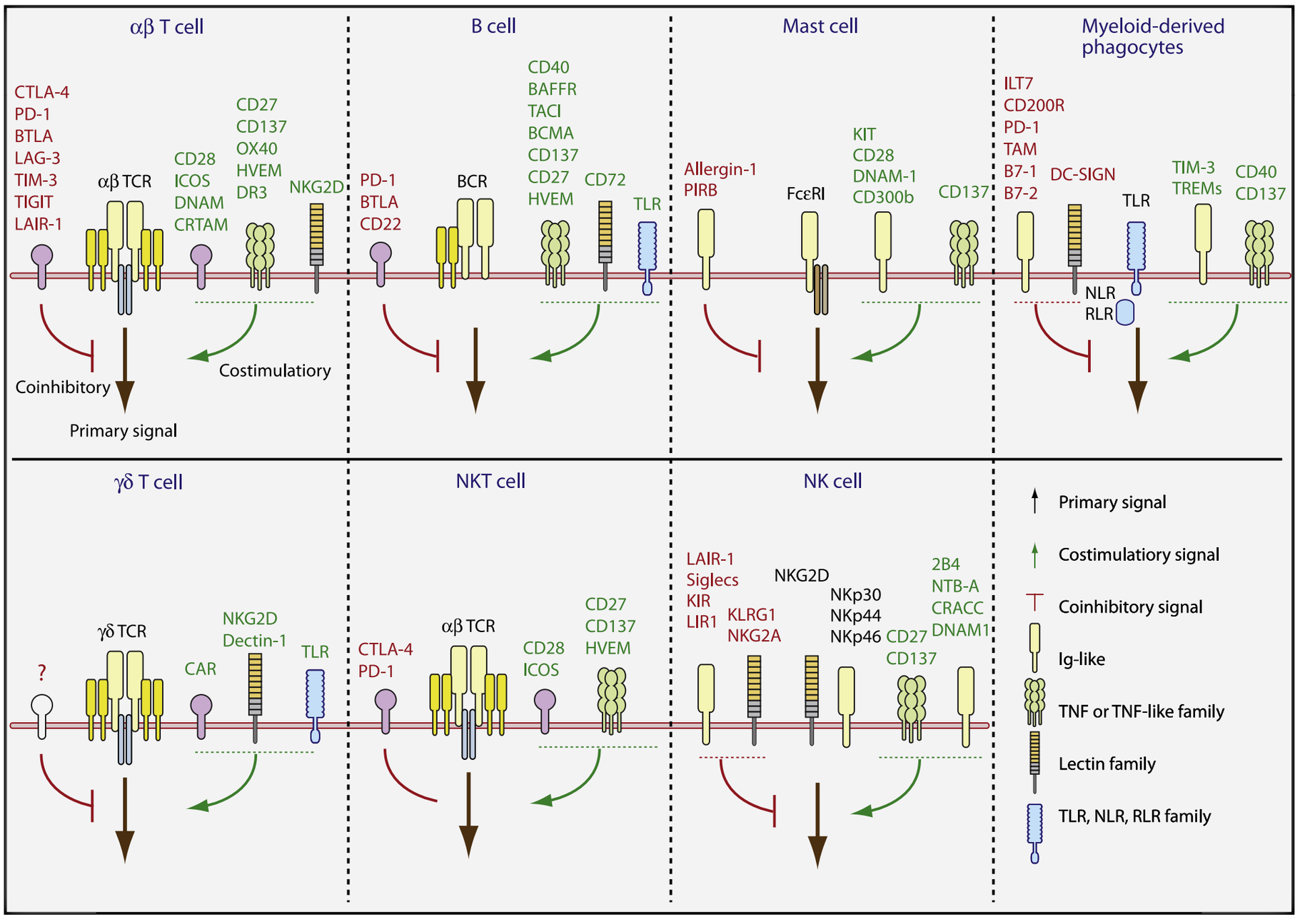
Kagoya Y et al. Nat Med. 2018

Exploring the Vast Landscape of CAR Design

General Domain Architecture of CARs



Coregulatory Receptors of the Immune System



Costimulators assoc. w/:

- PI3K
- Grb2
- Vav
- TRAF proteins
- Src family kinases
- Adaptor (e.g. LAT)
- PLCs
- STATs

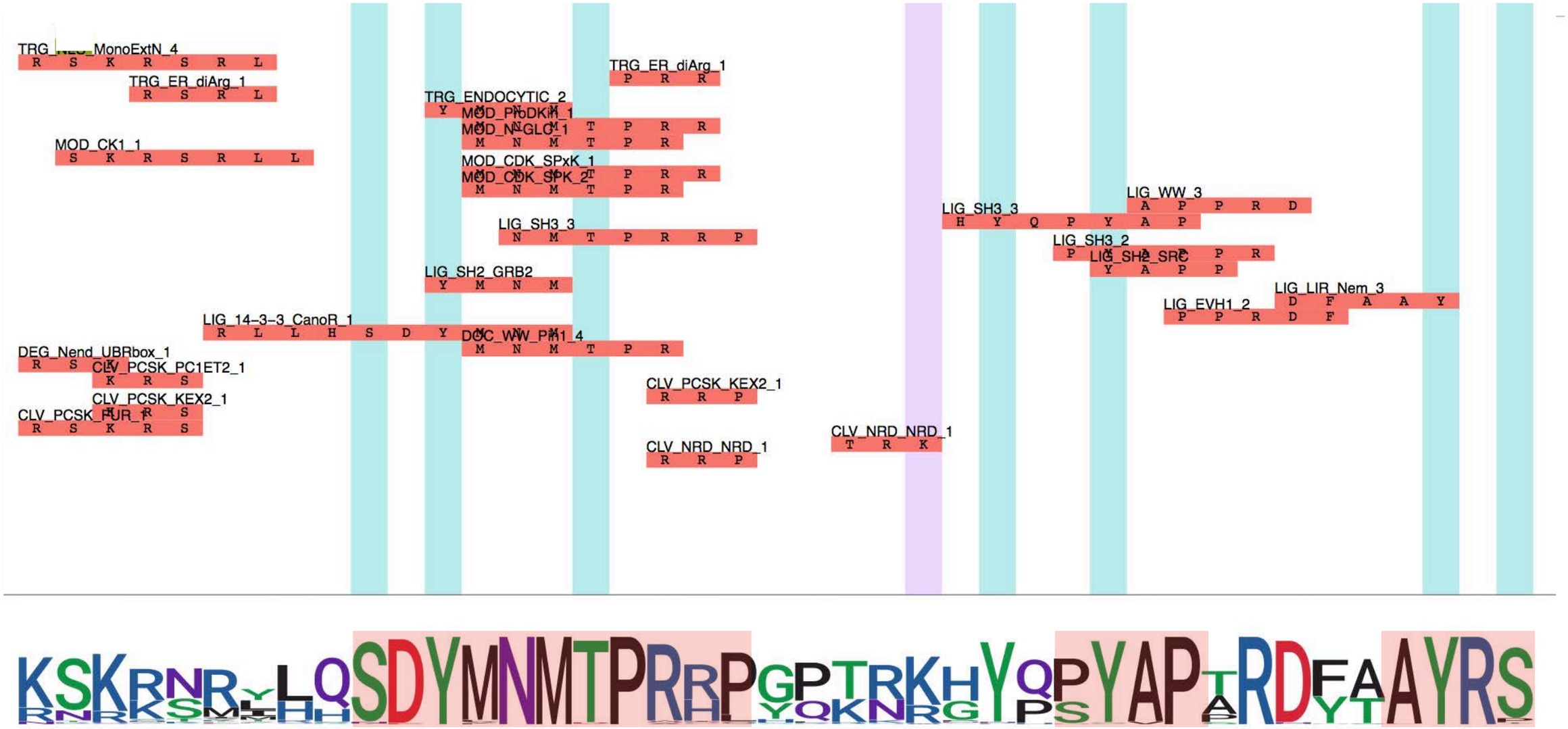
Coinhibitors assoc w/:

SHP1 and 2
Csk
EAT-2
Inhibitory Fc Receptors

Zhu Y. Immunity. 2011

Automated Cytoplasmic Domain Feature Annotation

CD28 cytoplasmic tail

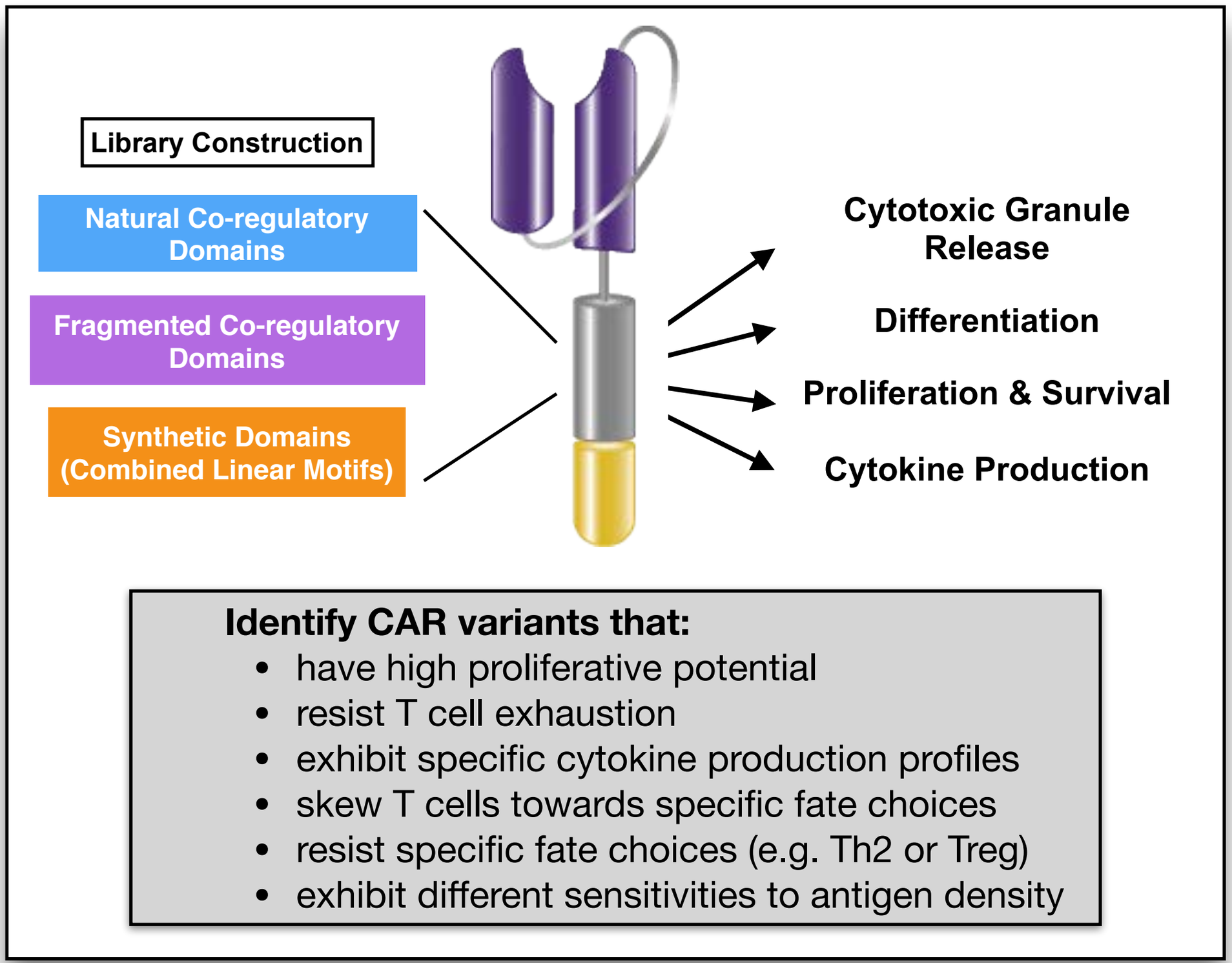


predicted linear motif

predicted phospho site

predicted ubiquitination site

Exploring the Vast Landscape of CAR Signaling



Identify CAR variants that:

- have high proliferative potential
- resist T cell exhaustion
- exhibit specific cytokine production profiles
- skew T cells towards specific fate choices
- resist specific fate choices (e.g. Th2 or Treg)
- exhibit different sensitivities to antigen density

Controlling Engineered T cell Activity and Specificity

NextGen T cell Therapies

<u>IMPROVING ENGINEERED T CELLS</u>

Controlling T cell Activity/Specificity
- Small molecule control
- Antigen switching

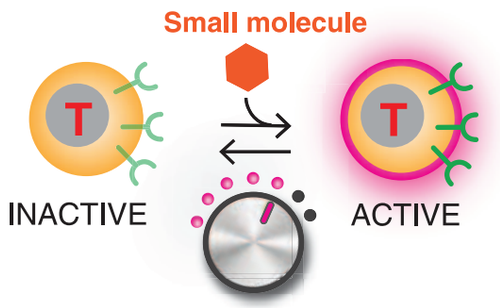
Logic Gating
- Multi-receptor systems
  - AND logic CARs
  - CAR/inhibitory CARs
  - synNotch/CAR circuits

Enhancing & Sculpting T cell Activity
- cytokine/chemokine production
- customization of responses

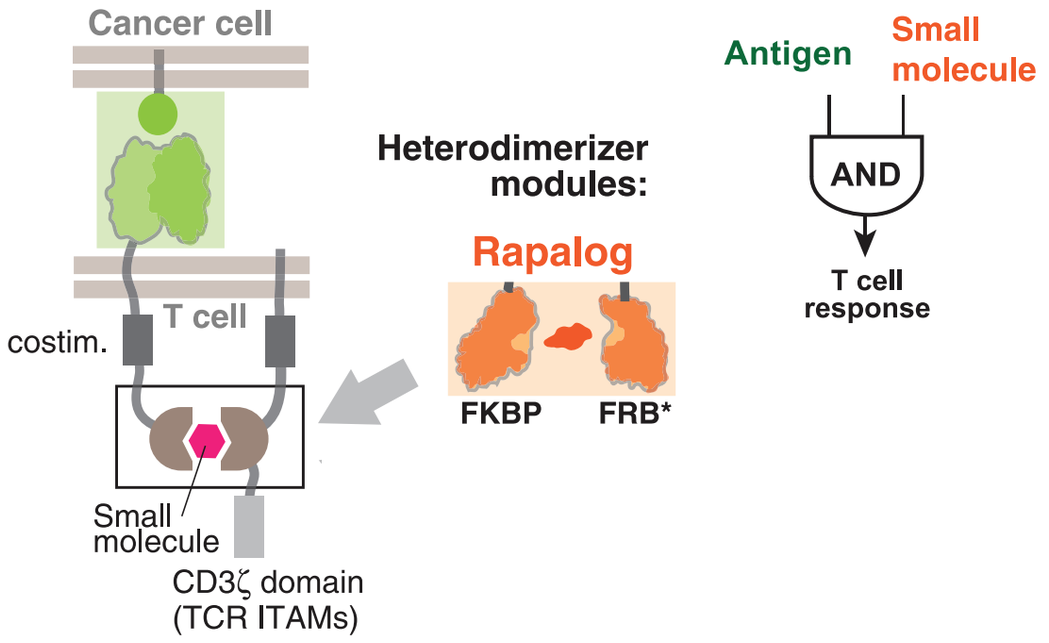
Drug Controlled CAR Activation

Remote Control of Adoptive T cell Therapies

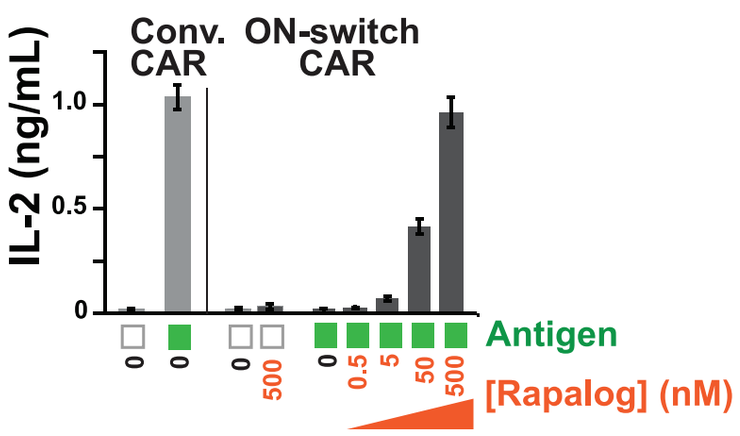
Conditional user-controlled switches



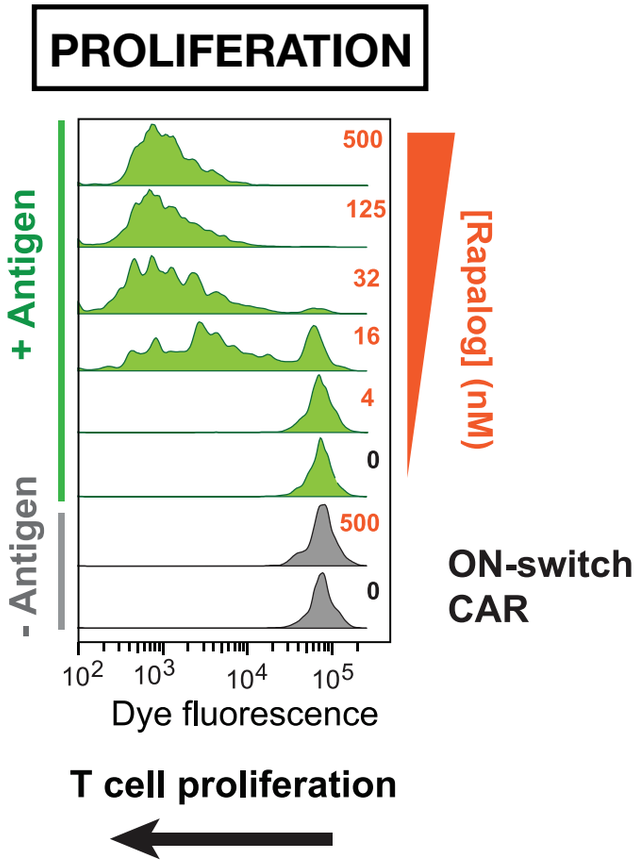
ON-switch



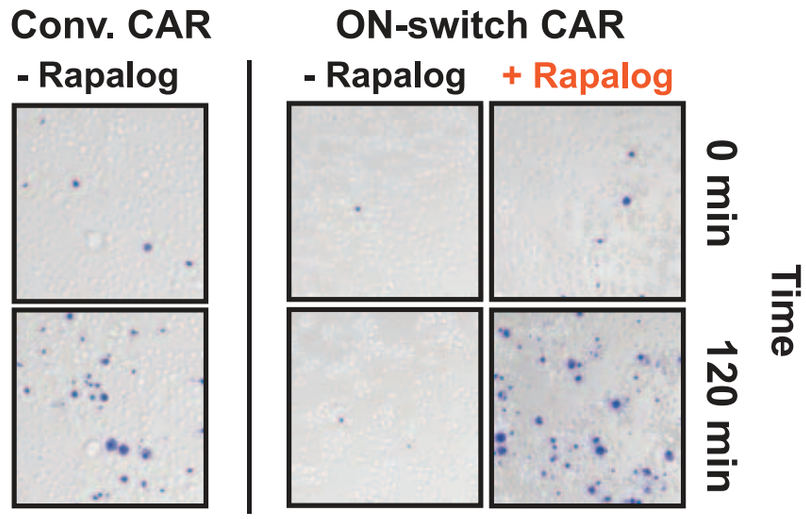
CYTOKINE PRODUCTION



PROLIFERATION



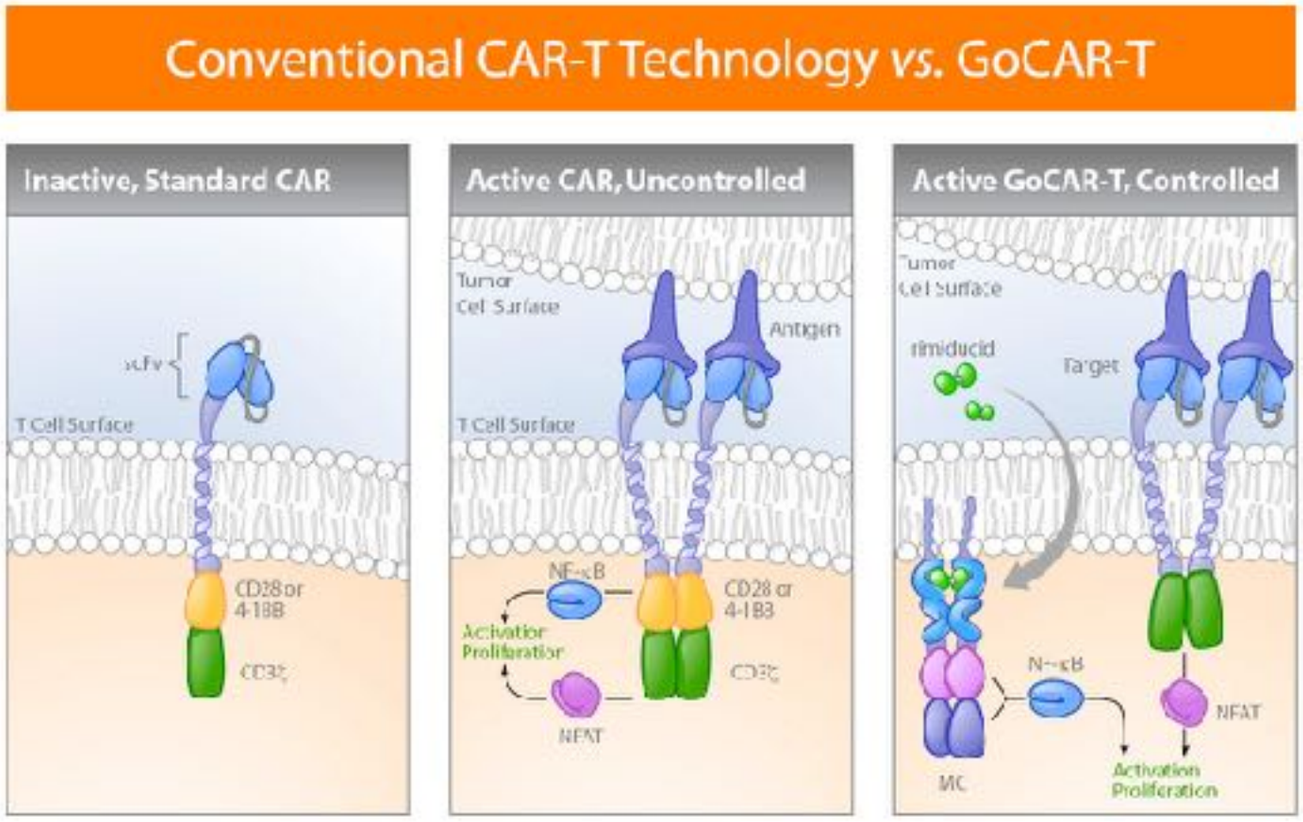
CYTOTOXICITY

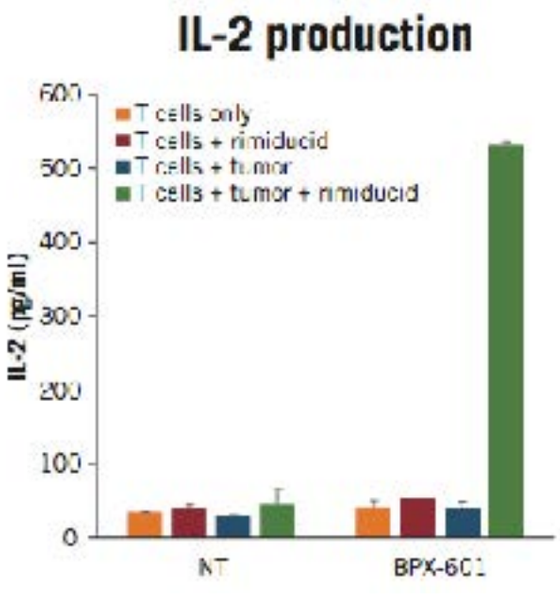


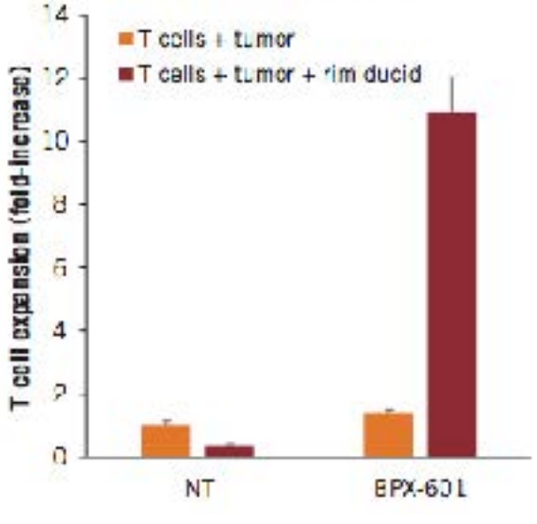
Wu C et al. *Science* 2015

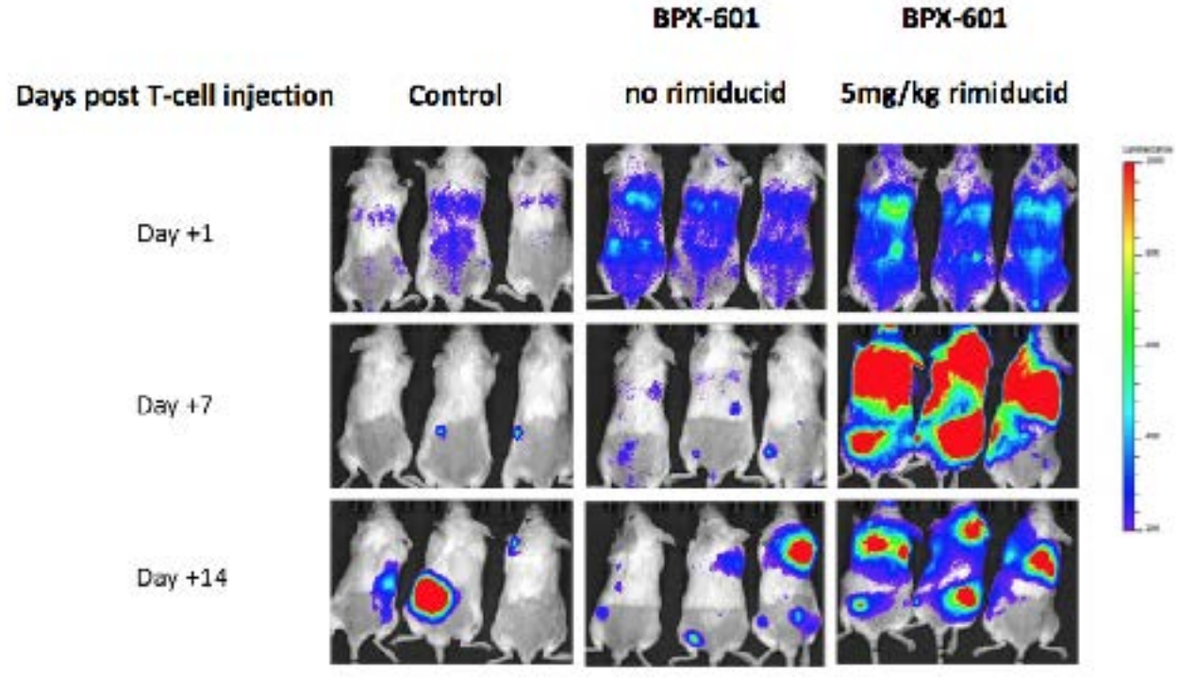
Drug Controlled Costimulation

An Approach to Titrate Engineered T cell Effector Function







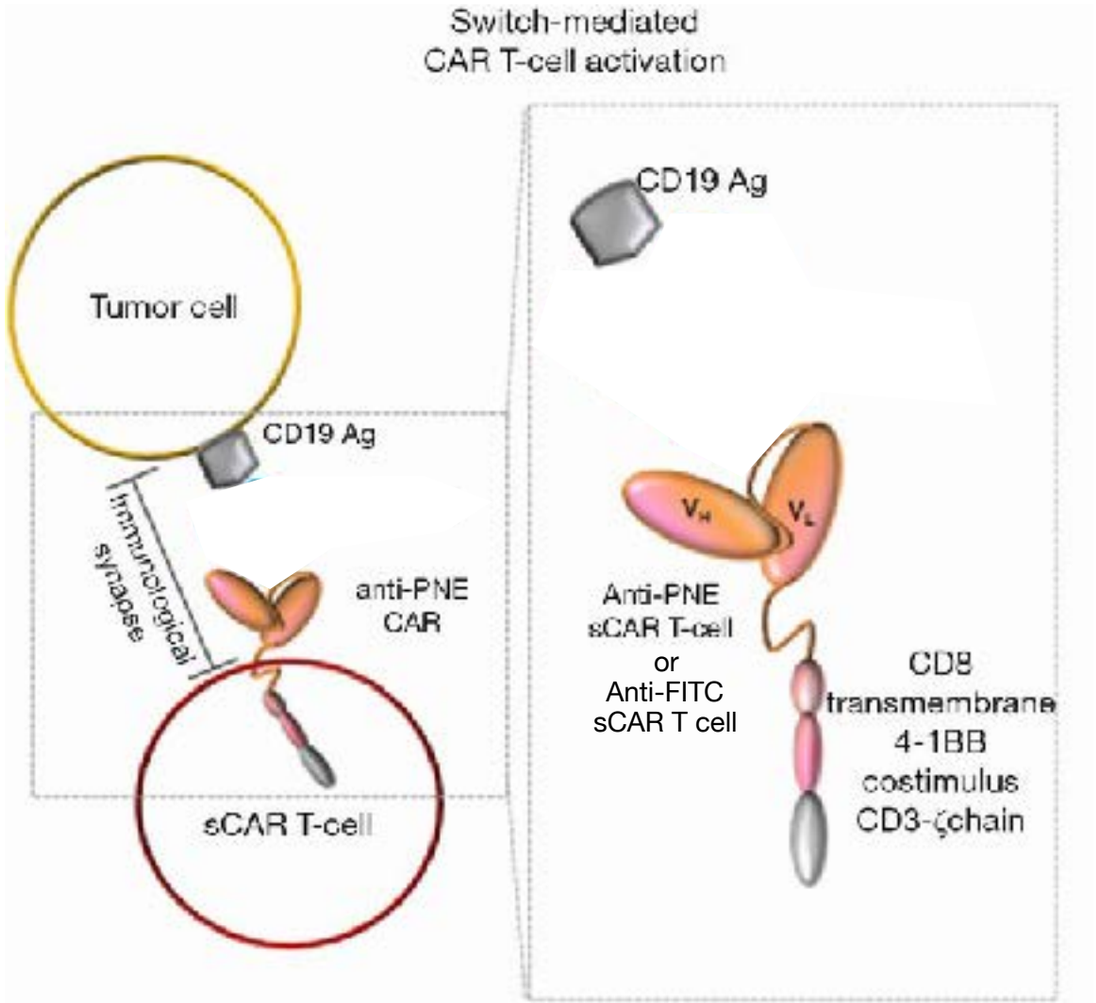


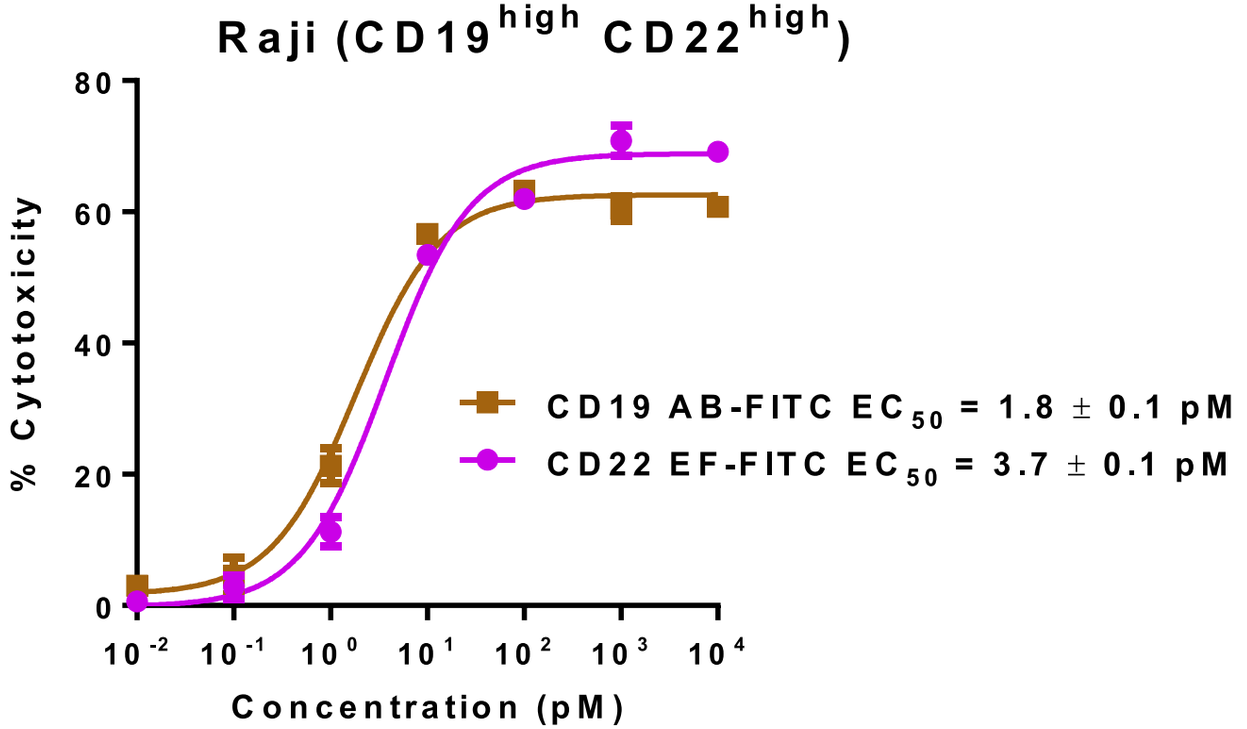
http://www.bellicum.com/technology/gocart/

T cell Proliferation

Universal CAR T cells

Changing Antigen Specificity During Treatment





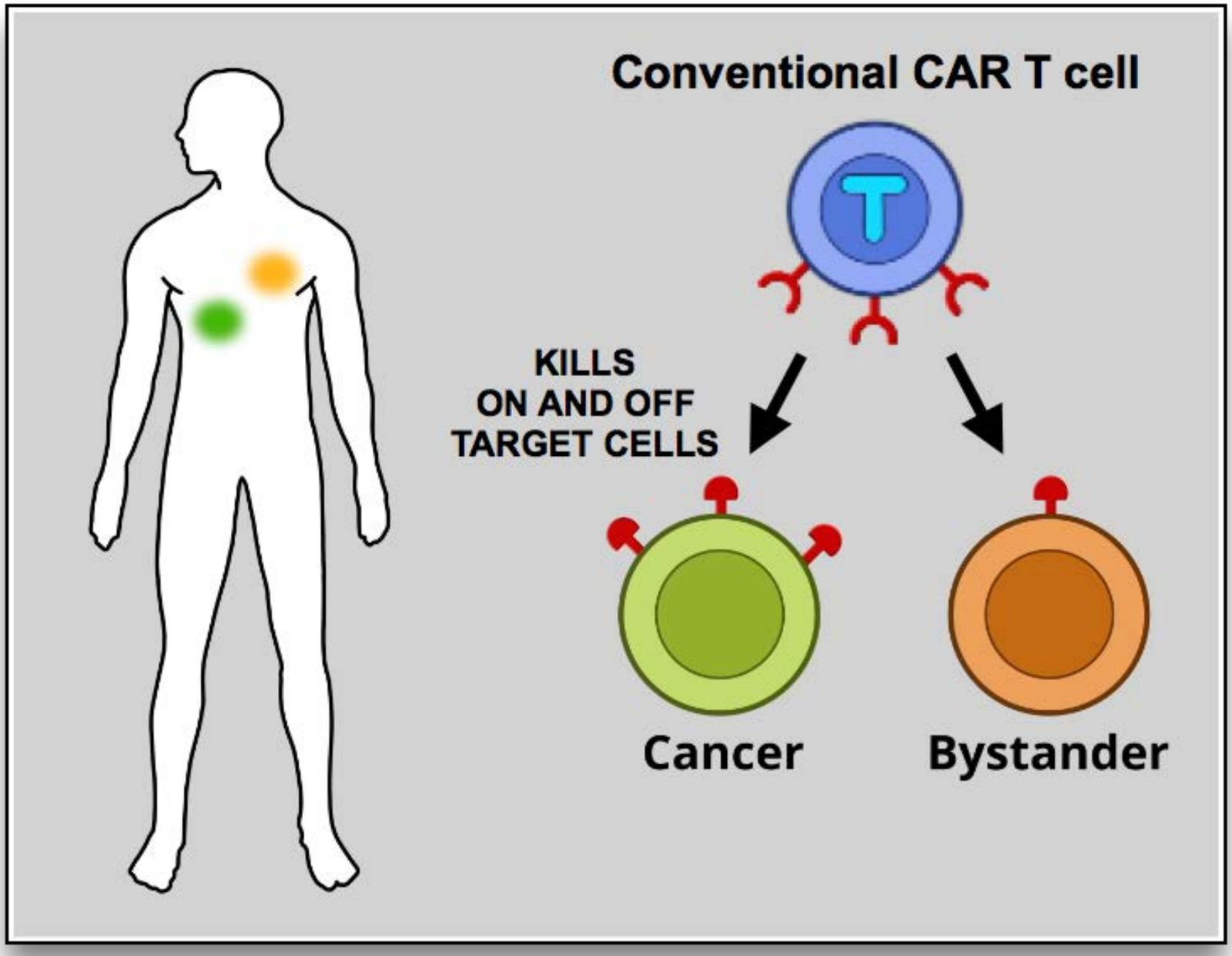
Ma JSY. PNAS. 2016

Logic-gated Immune Cell Therapeutics

Enhancing Specificity and Safety

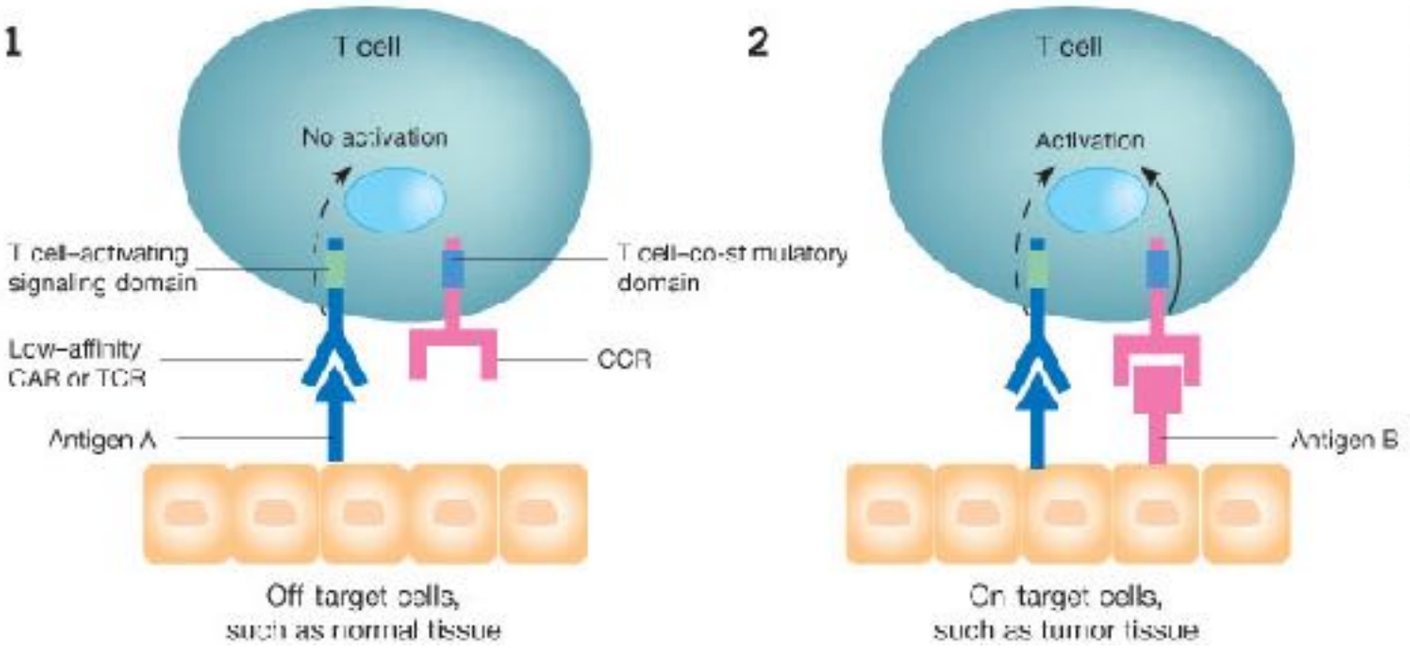
Redirecting the Specificity of T cells to Cancer

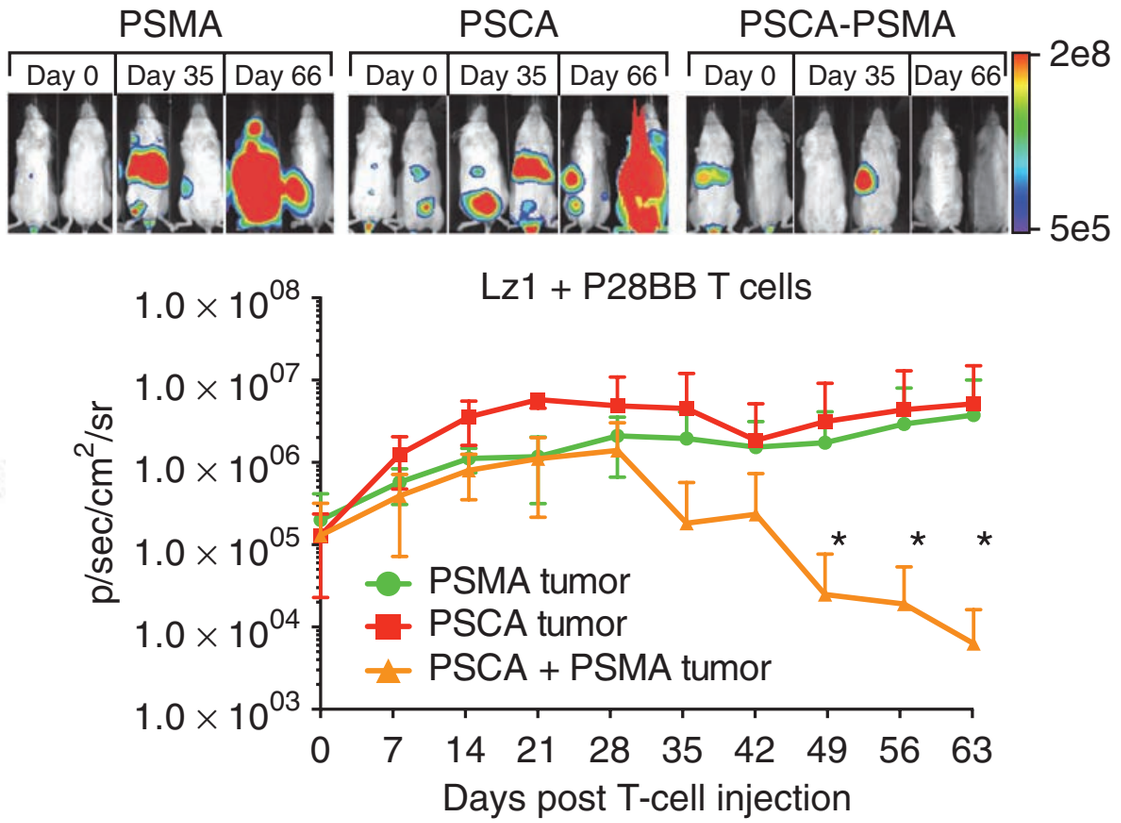
The Pitfalls of Single Antigen Targeting



AND Gate CAR T cells

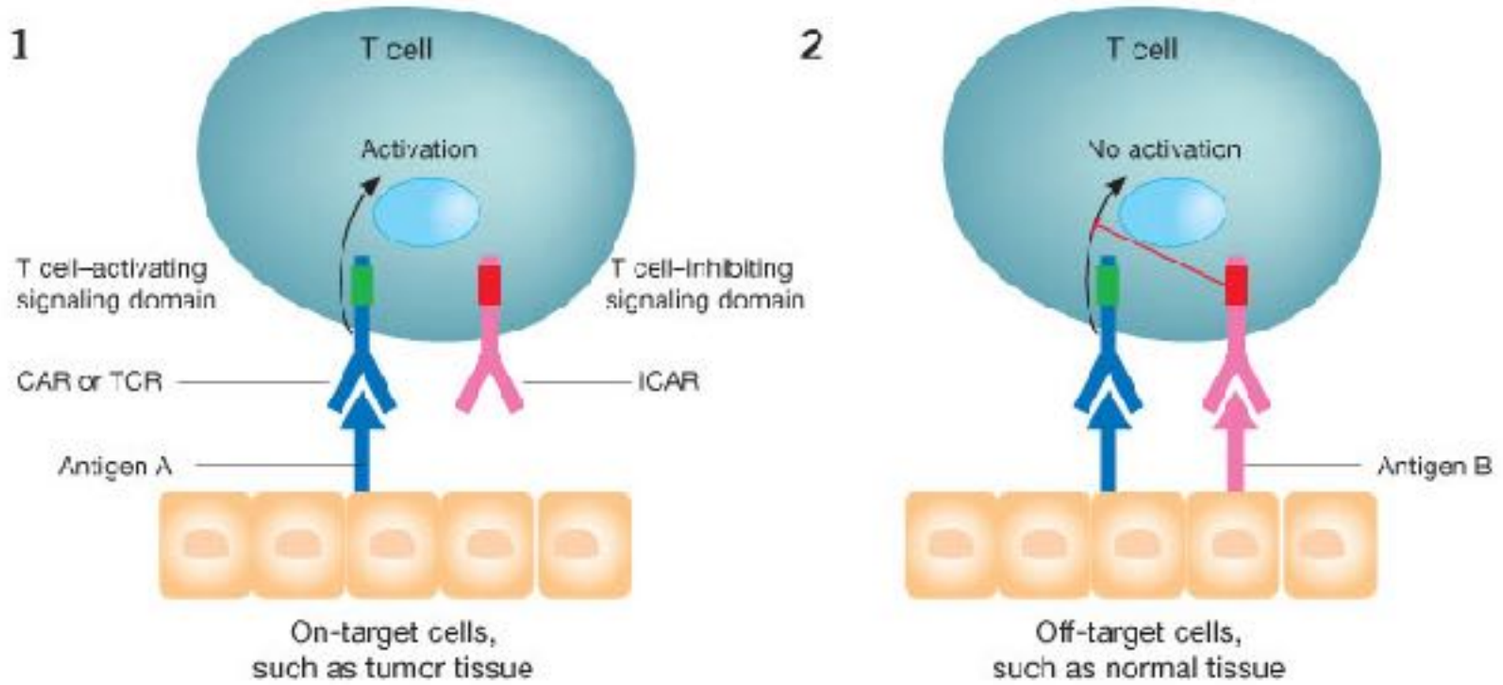
Separating Signal 1 (TCR) and Signal 2 (Costimulation)



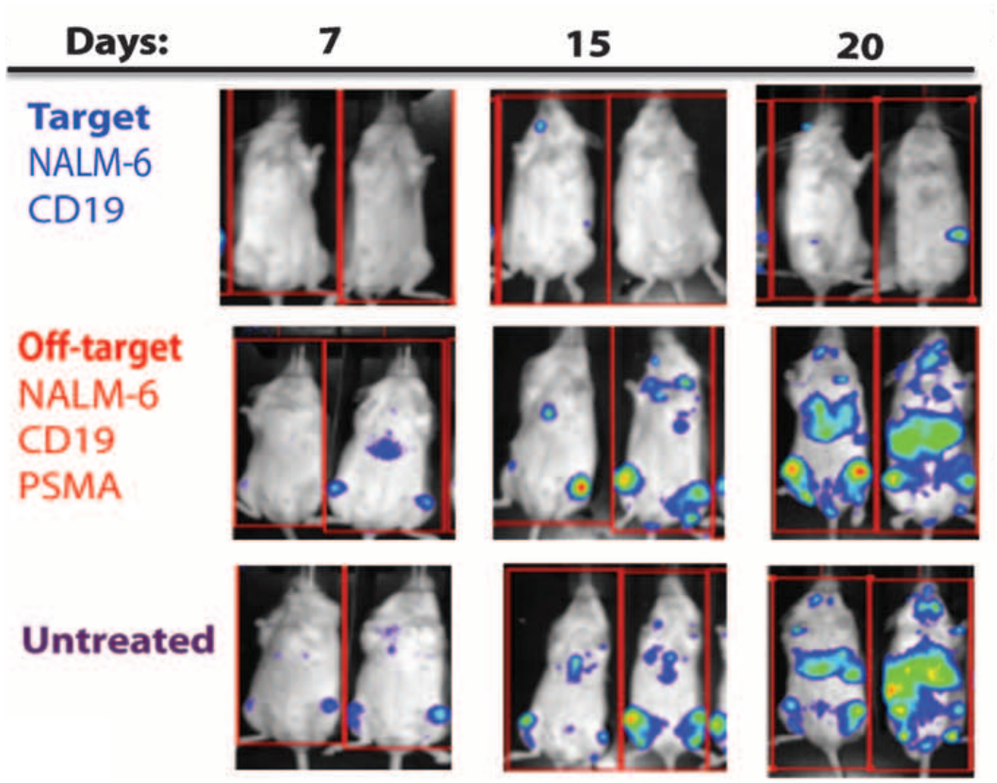


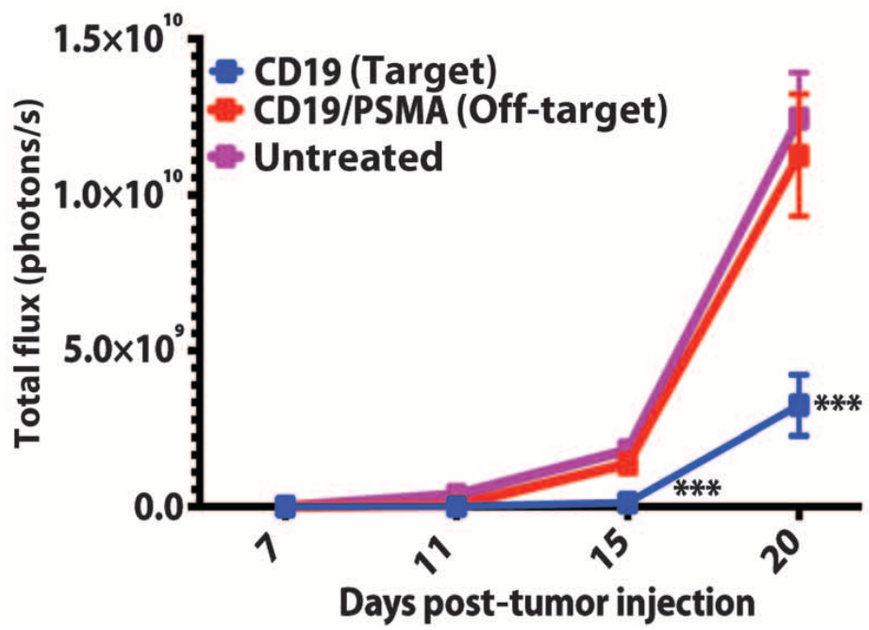
Kloss CC. Nat Biotech. 2013

NK cell-like Activation Paradigm for Engineered T cells with Inhibitory CARs (iCARs)



iCARS
use ICDs from T cell inhibitory receptors (e.g. CTLA4 and PD1)

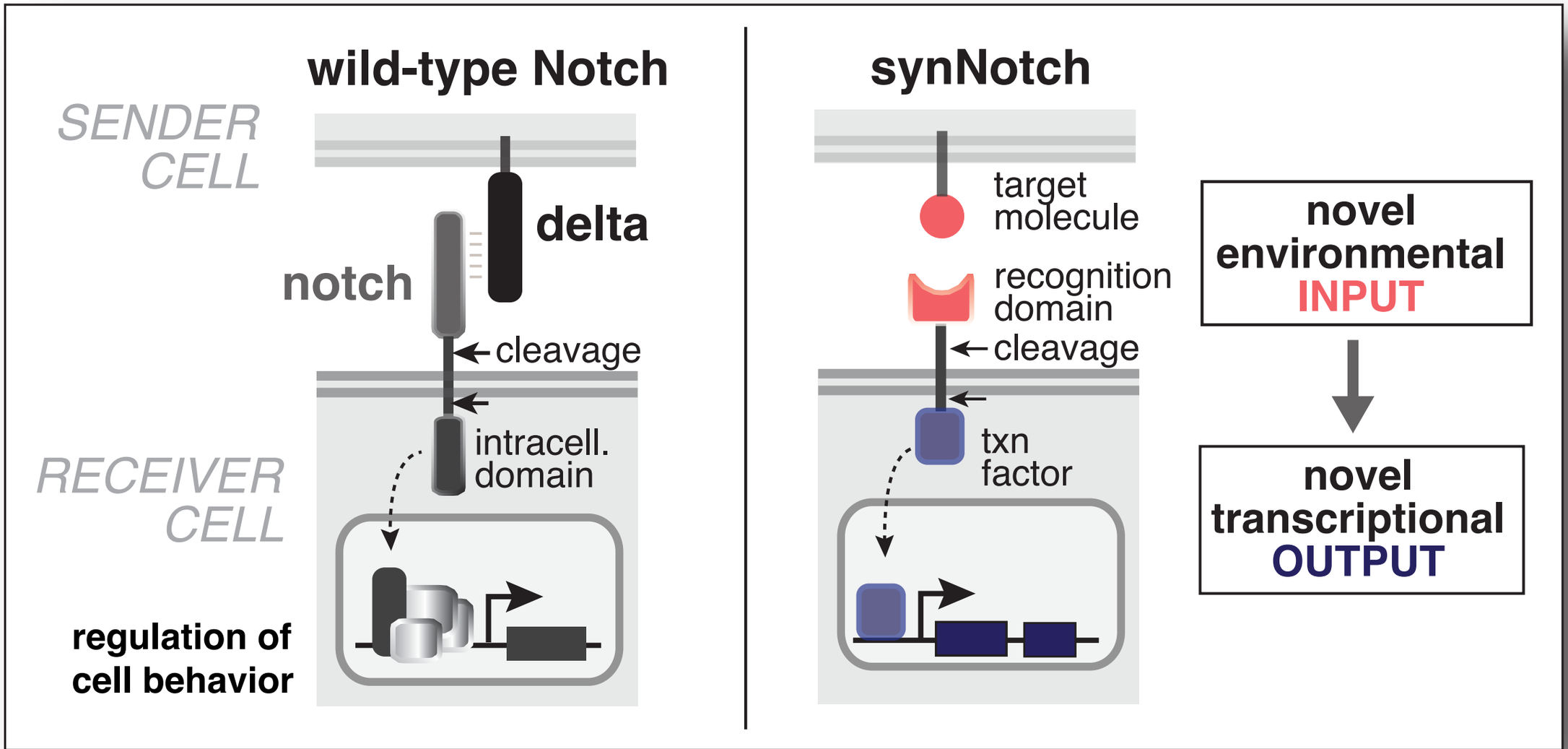




Fedorov VD. Sci Trans Med. 2013

The Notch Receptor

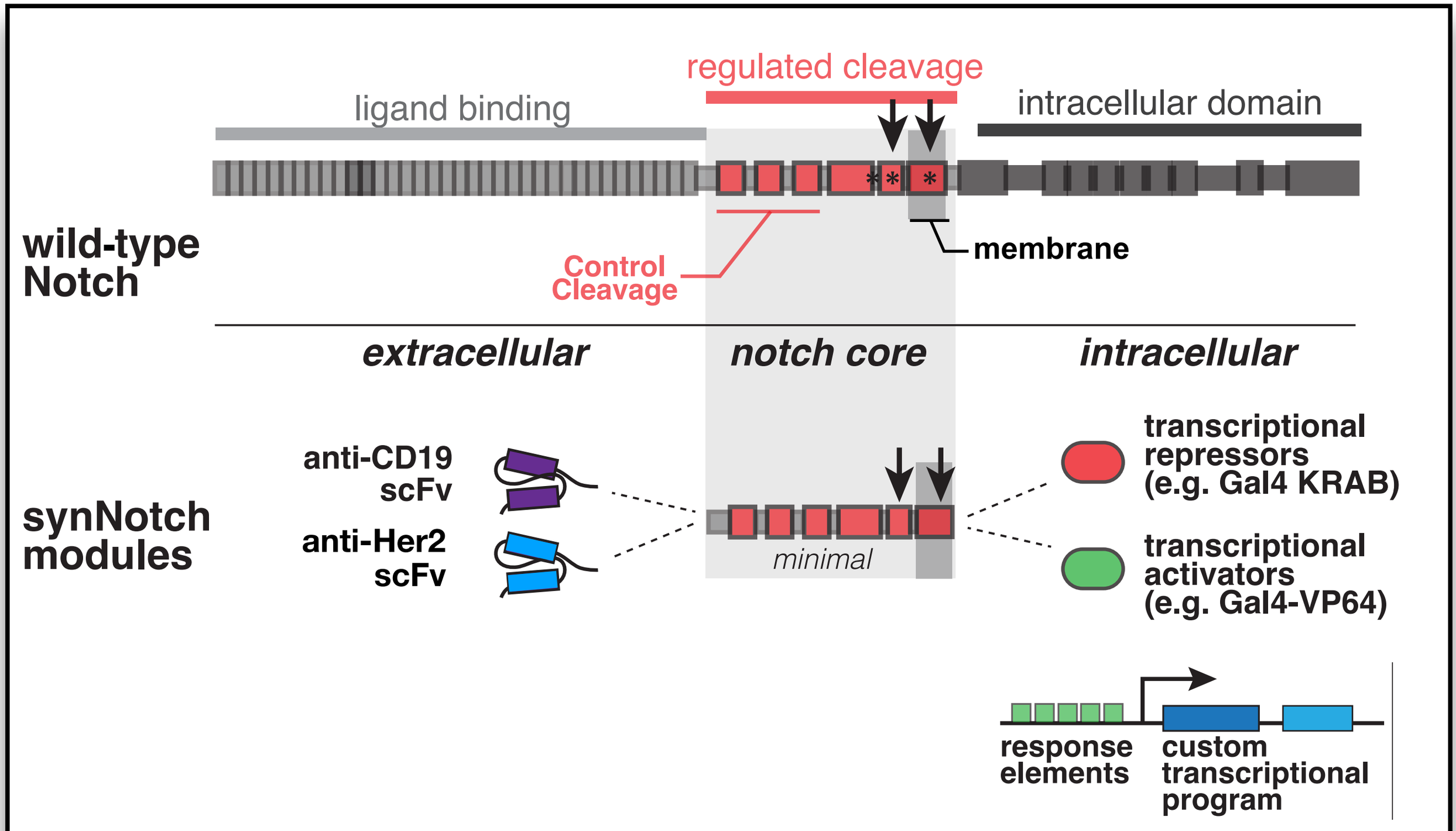
A Natural Environmental Sensor that Regulates Cells
Through DIRECT Transcriptional Regulation



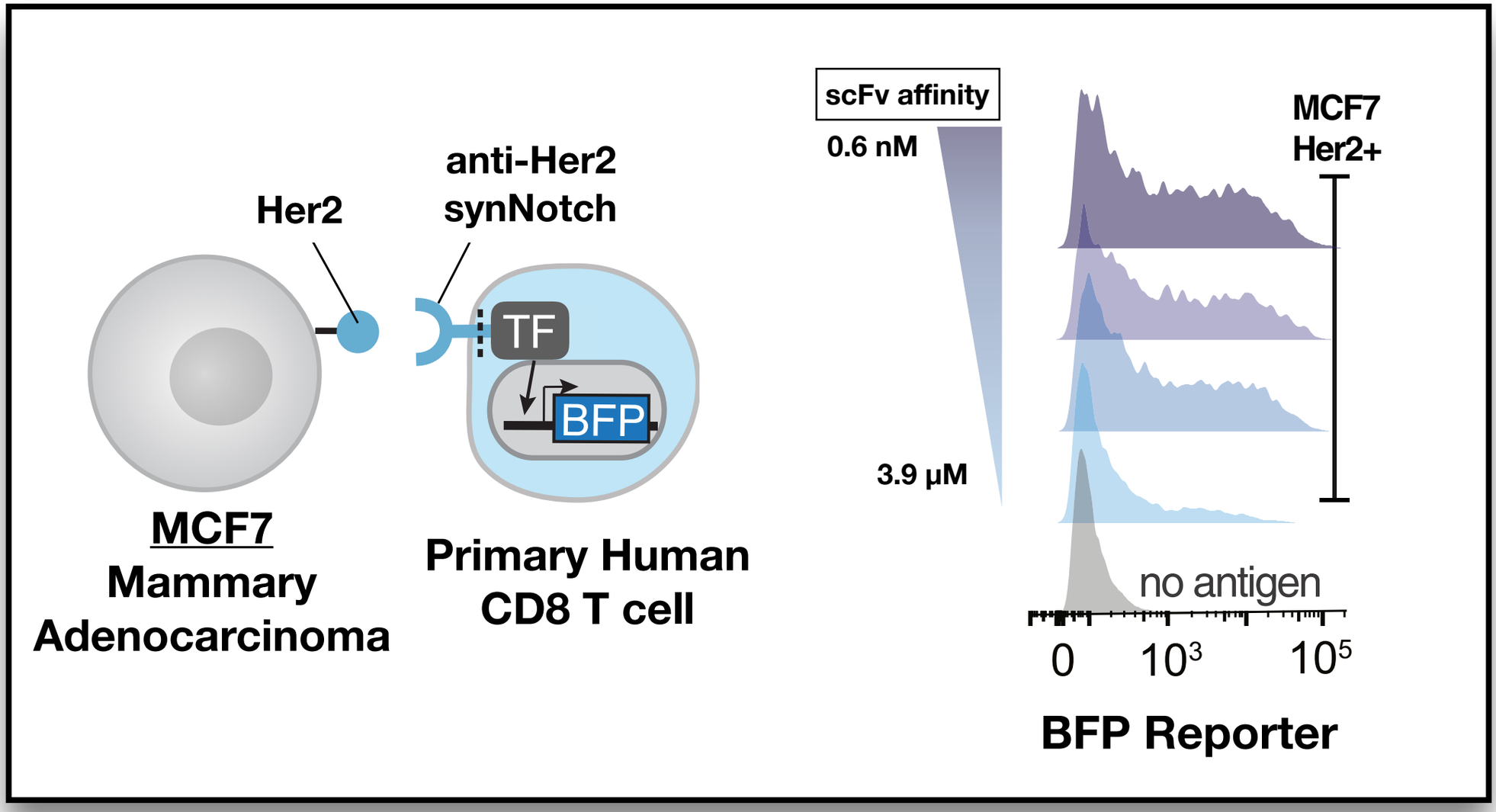
Roybal and Morsut et al. Cell. 2016

Synthetic Notch Receptors

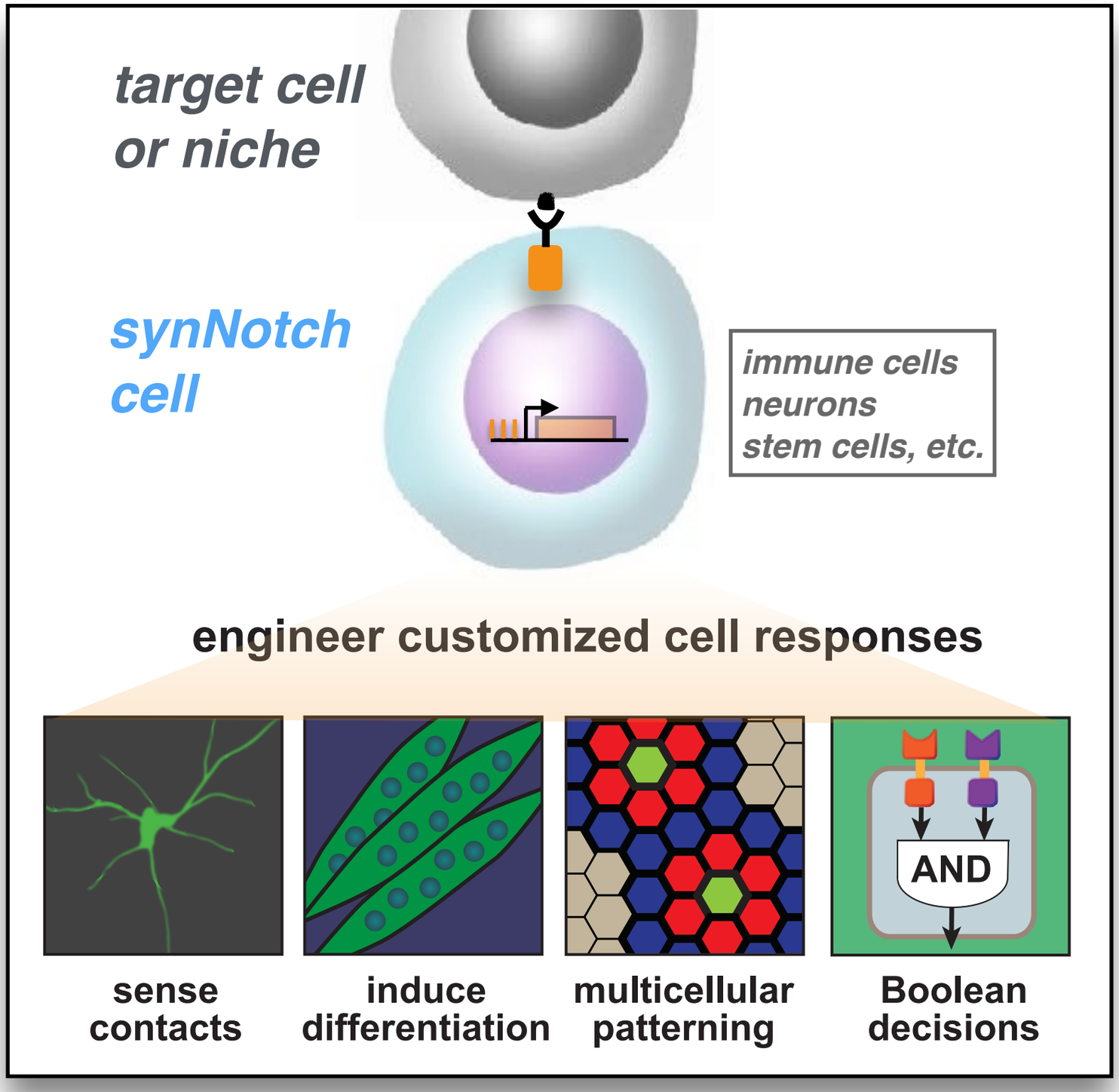
Customizable Cellular Sensing and Response Programs



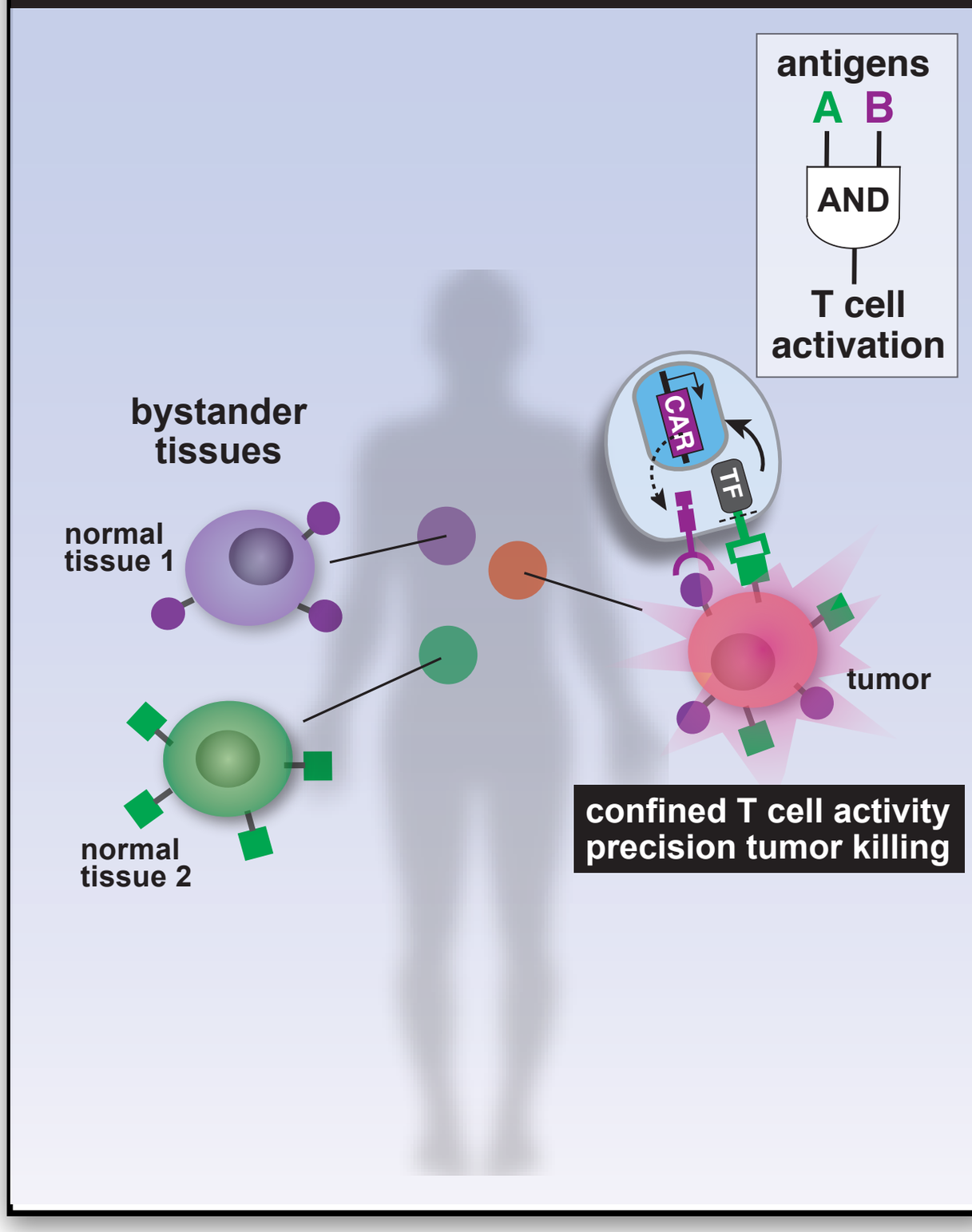
SynNotch Receptors Drive Custom Transcriptional Circuits in Response to Tumor Antigens



SynNotch Receptors are Versatile Regulators of Cellular Function in Response to Environmental Cues

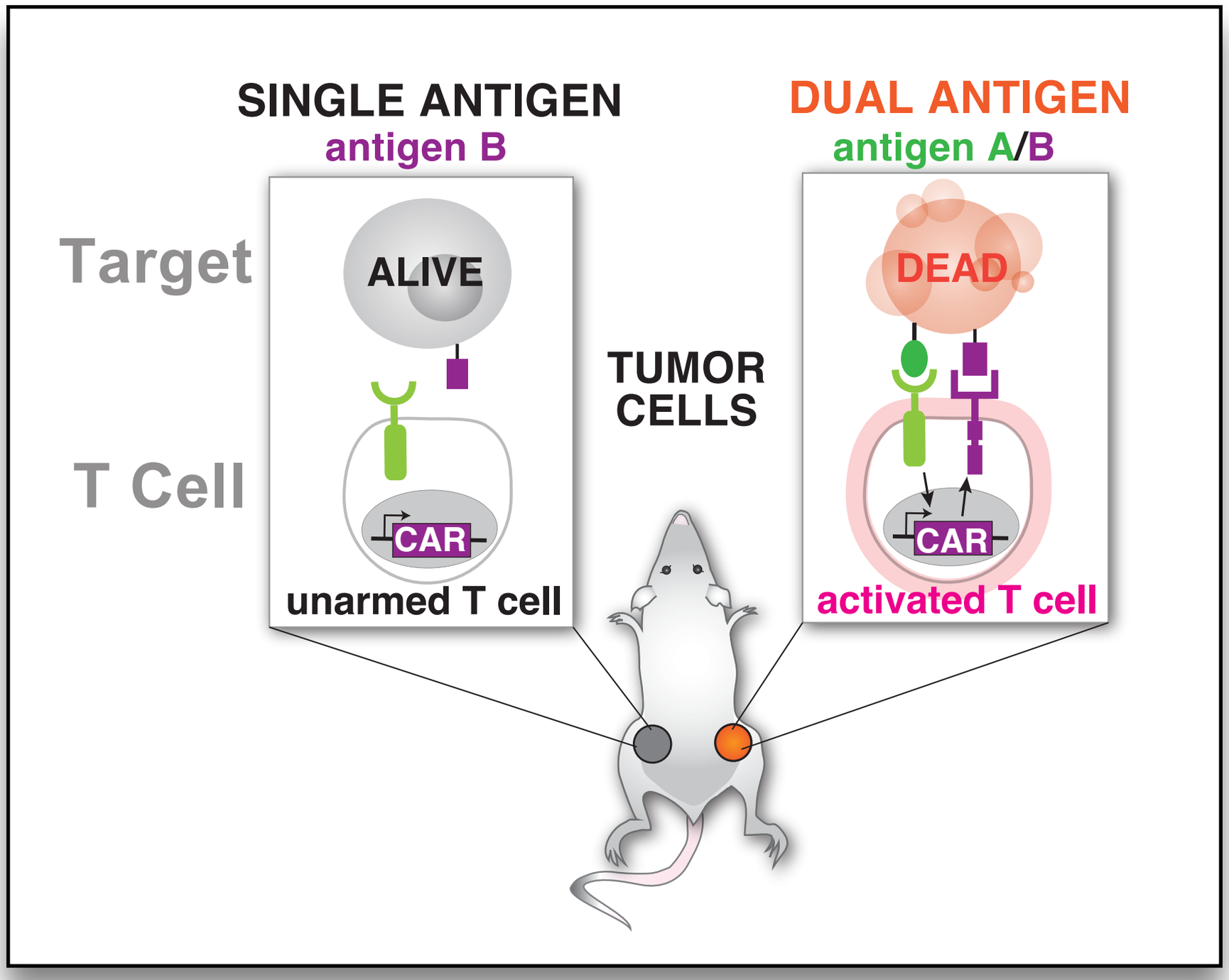


Precision Tumor Recognition with SynNotch:CAR Circuits



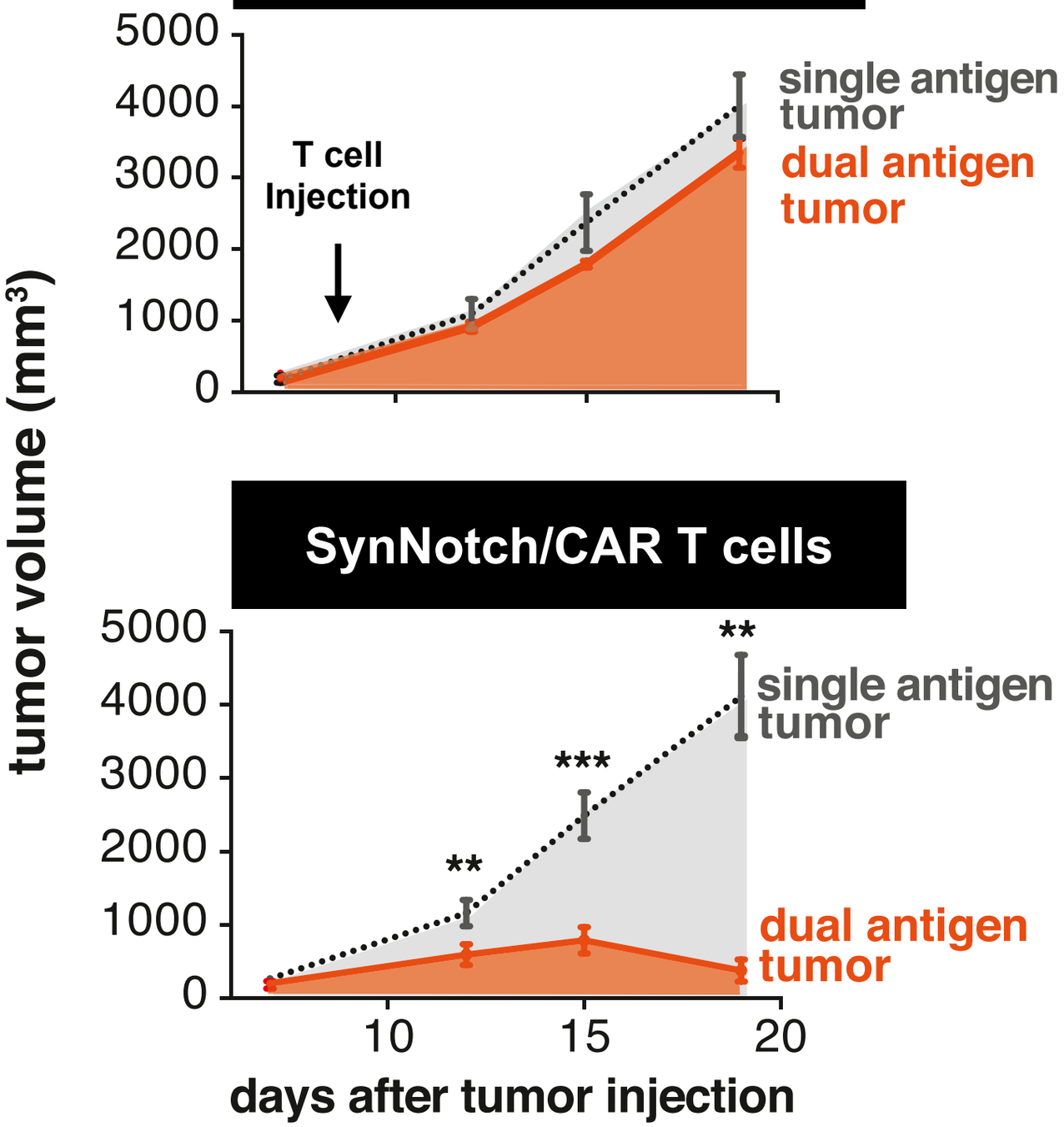
Roybal et al. Cell. 2016a

SynNotch/CAR T cells Exclusively Target Dual Antigen Tumors *In vivo*



SynNotch/CAR T cells Exclusively Target Dual Antigen Tumors *In vivo*

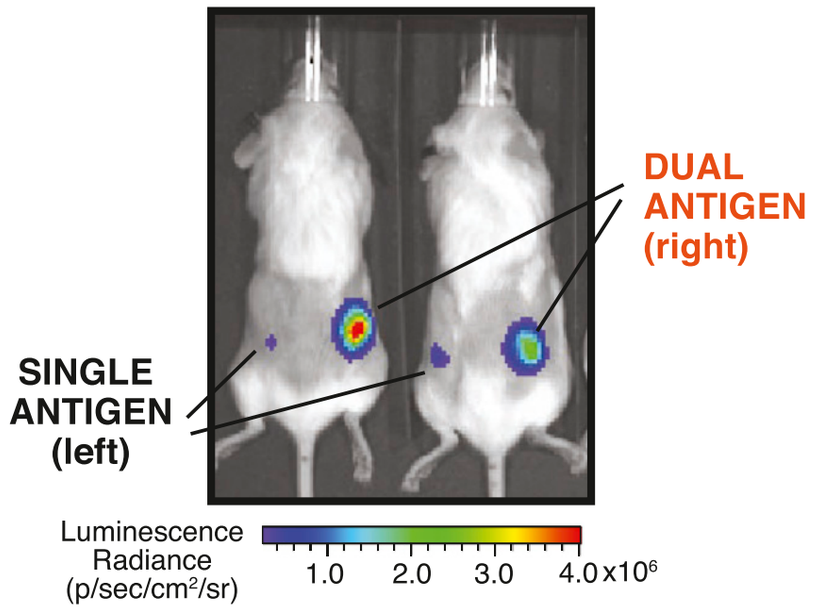
Control T cells



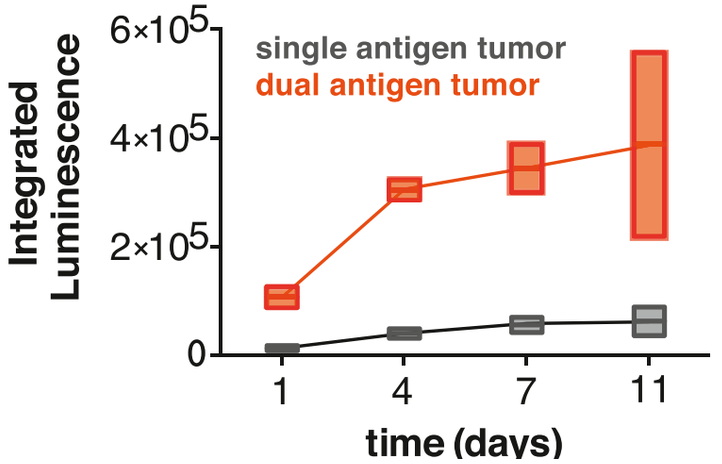
SynNotch/CAR T cells

CAR Expression (Luciferase Reporter)

Day 7 Post T cell Injection

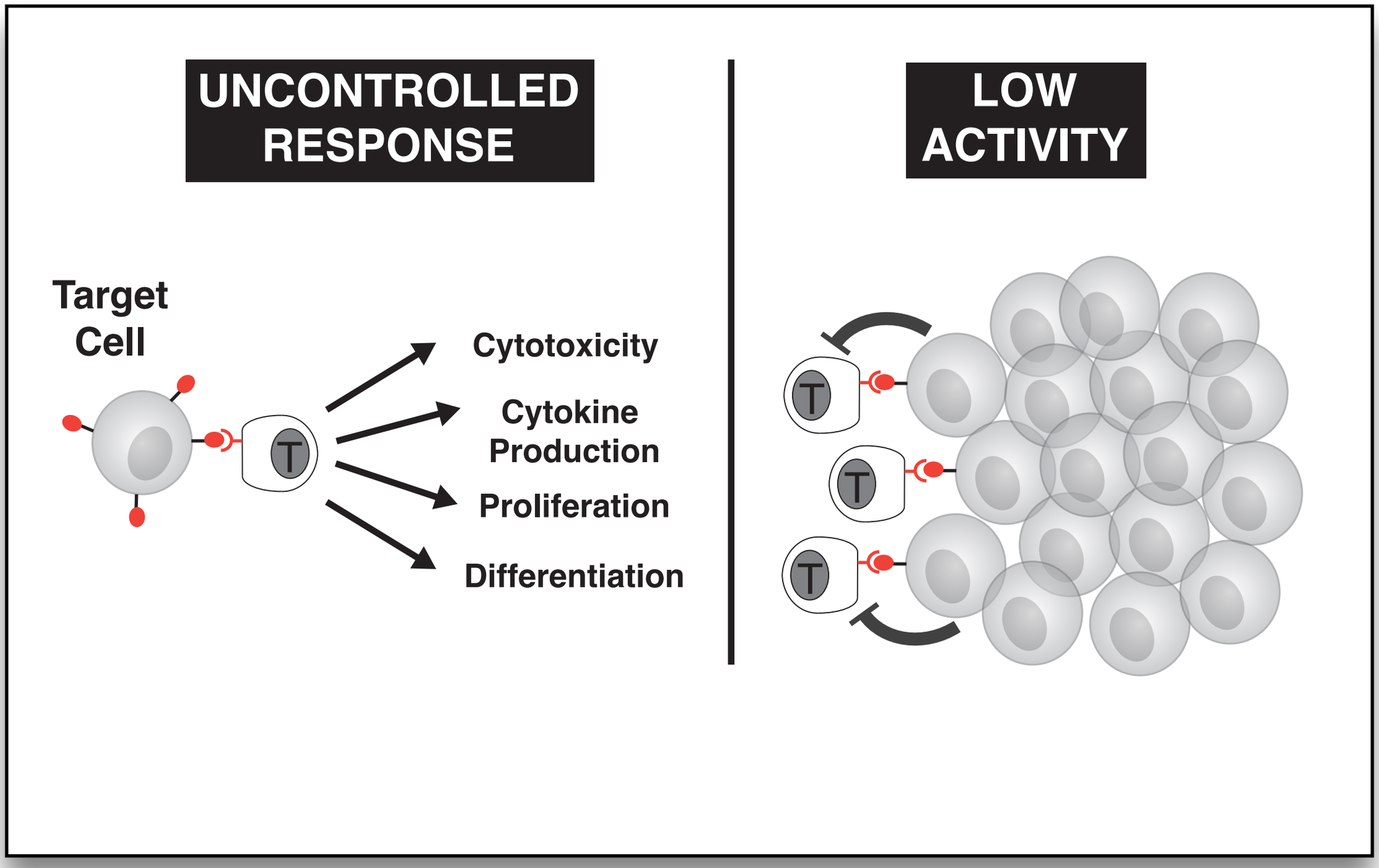


Luminescence in Tumors



Enhancing and Sculpting the Immune Response

Common Pitfalls of Cell-based Therapeutics

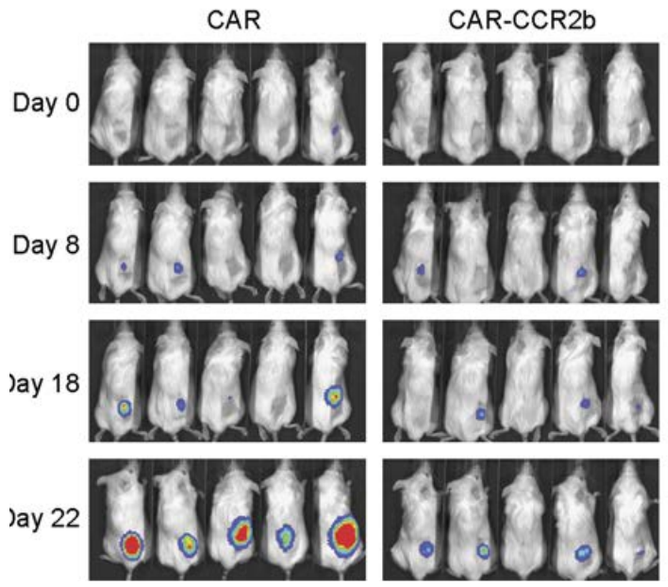


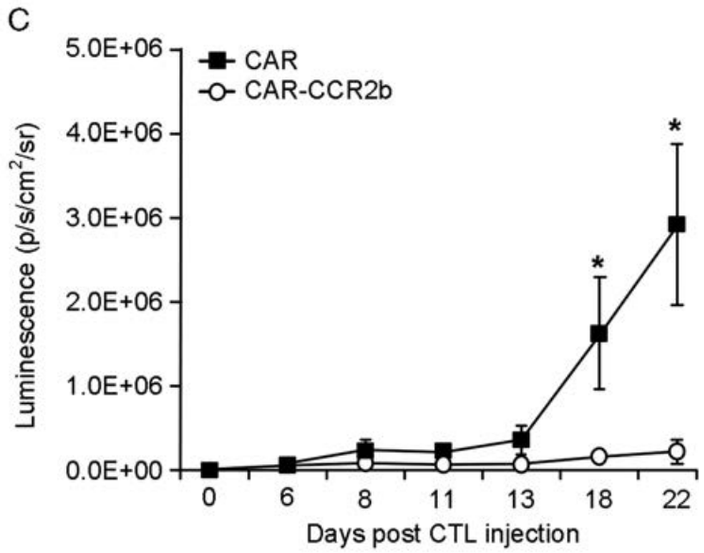
CAR T cells that Express Chemokine Receptors for Enhanced Trafficking to Tumors

TABLE 1. Neuroblastoma Cell Line Characteristics

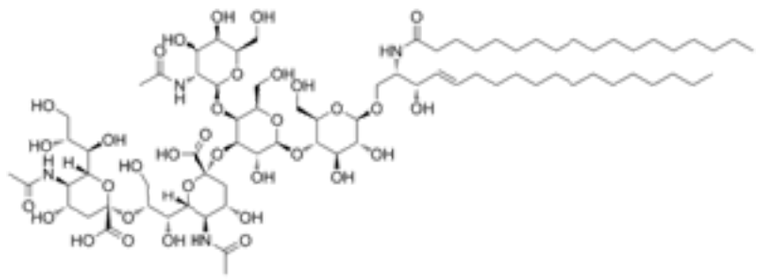
Cell Line	CCL2 (pg/mL)	GD2%/MFI	Myc-N Status
A, Established cell lines			
IMR-32	36 ± 3	79/75	Amplified
JF	40 ± 1	82/89.8	Amplified
LA-N-1	79 ± 5	85/97.3	Amplified
SK-N-AS	> 2000	47/7.5	Nonamplified
SK-N-SH	> 2000	6/0.1	Nonamplified
B, Primary cell lines			
P142	1393	60/29.2	Nonamplified
P175	457	39/4.9	Mixed
P246	1600	ND	ND
P247	932	34	ND
P275	5929	ND	Nonamplified
P283	1530	8/0.1	Nonamplified

MFI indicates mean fluorescence intensity; ND, not determined.



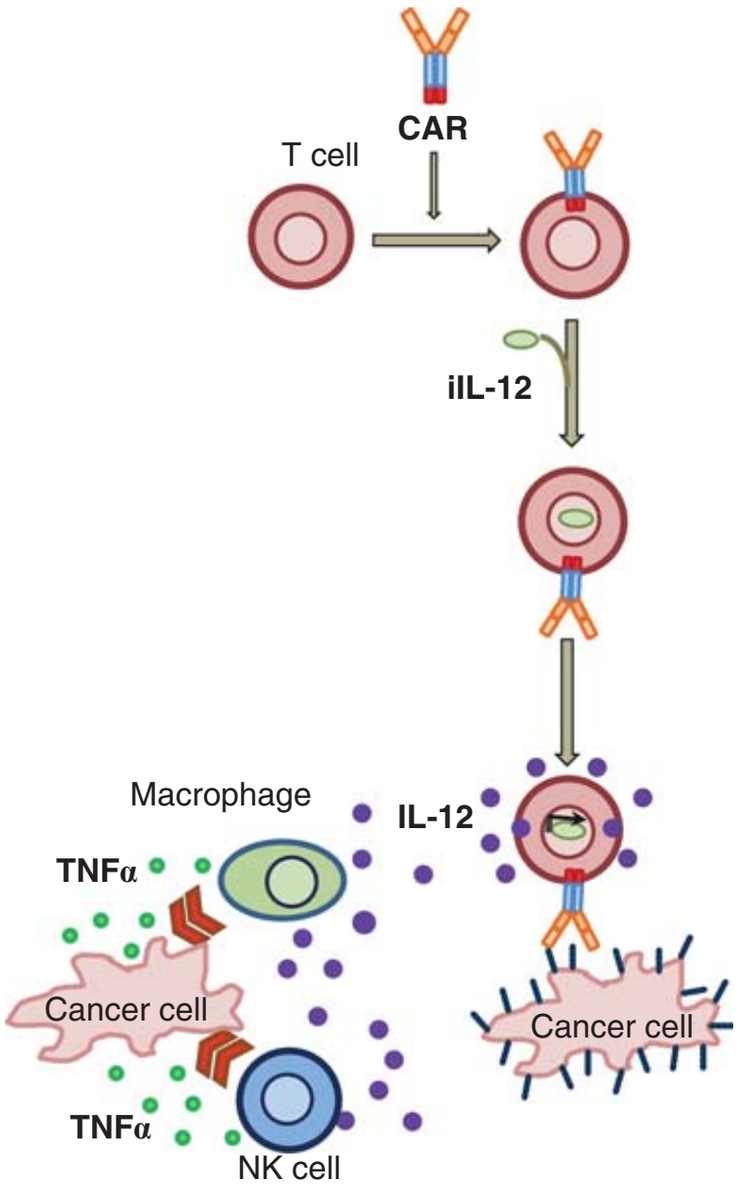


Ganglioside GD2 is found in overexpressed among pediatric and adult solid tumors, including neuroblastoma, glioma, retinoblastoma, Ewing's family of tumors, rhabdomyosarcoma, osteosarcoma, leiomyosarcoma, liposarcoma, fibrosarcoma, small cell lung cancer and melanoma.



Craddock JA. J. Immunotherapy. 2010

CAR T cells that Express Cytokines that Enhance Anti-tumor Immunity



CAR engineered T cell

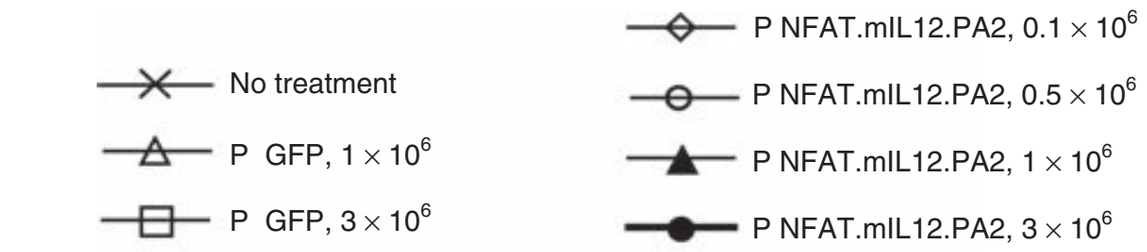
TRUCK engineered T cell

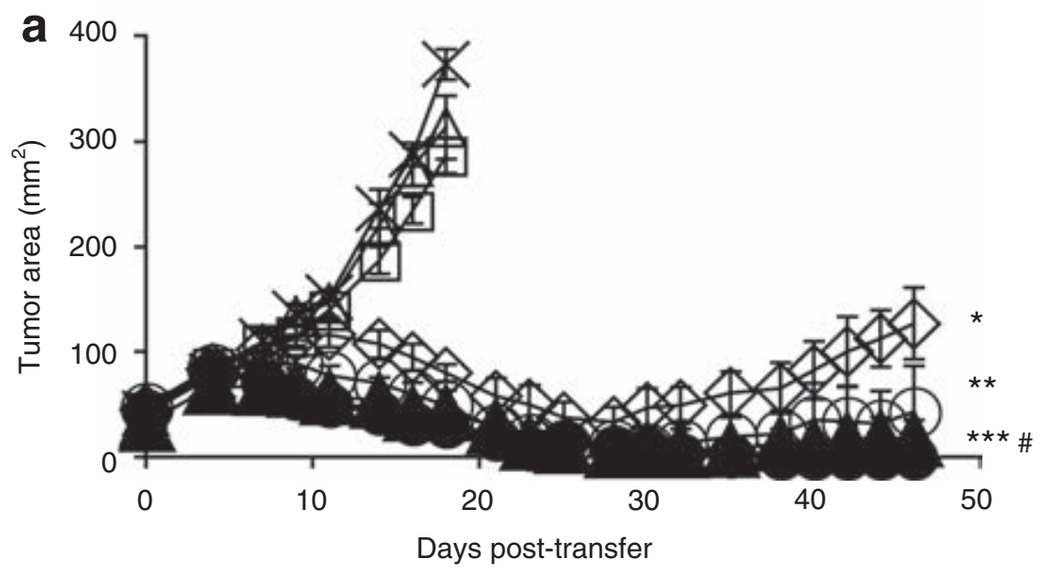
CAR mediated TRUCK T cell activation

Chmielewski M. Expert Opin. Biol. Ther. 2015

B16 Melanoma model
T cells engineered with:

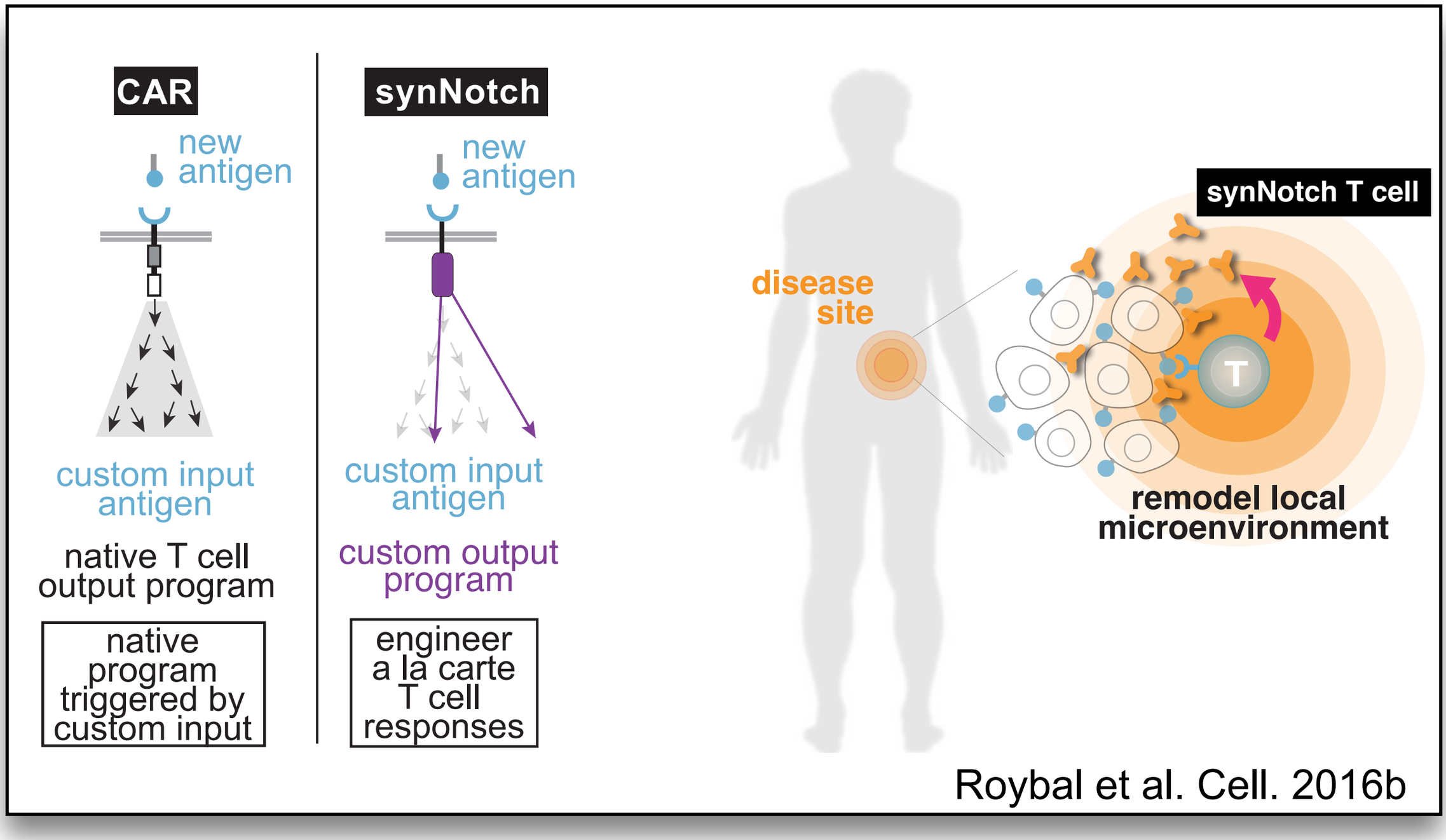
- MART 1 TCR (specific for melanoma)
- + NFAT Response Element controlled IL-12





Zhang L. Molecular Therapy. 2011

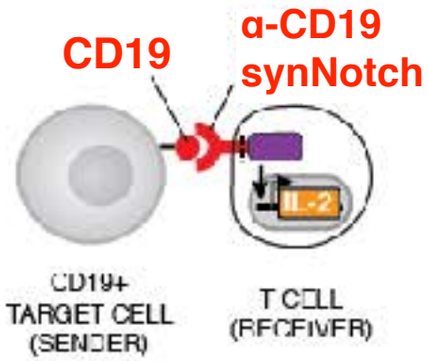
The Potential to Engineer Customized Therapeutic T cell Response Programs with SynNotch Receptors

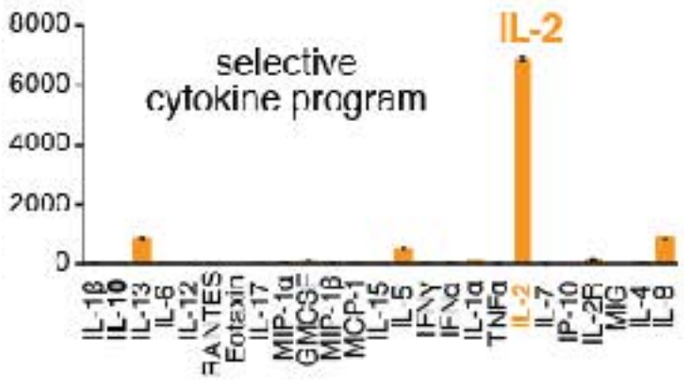


Roybal et al. Cell. 2016b

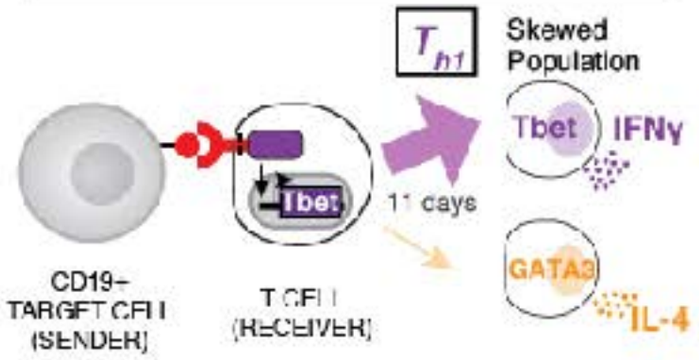
Customized T cell Responses with Synthetic Notch Receptors

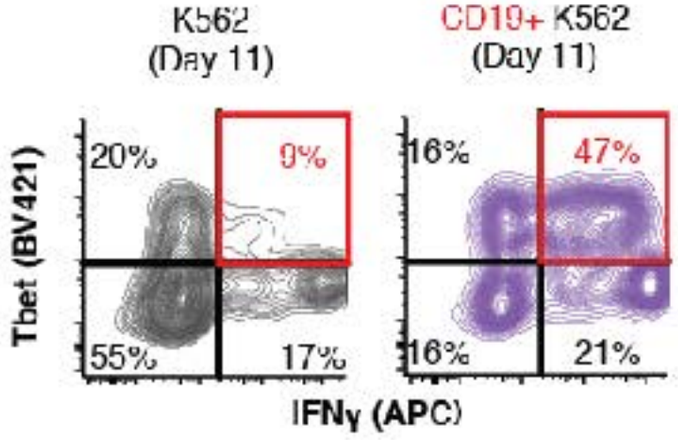
Custom Cytokine Production



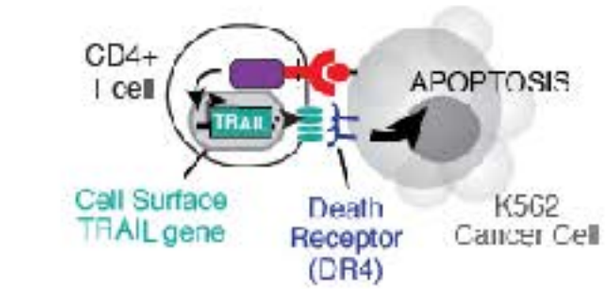


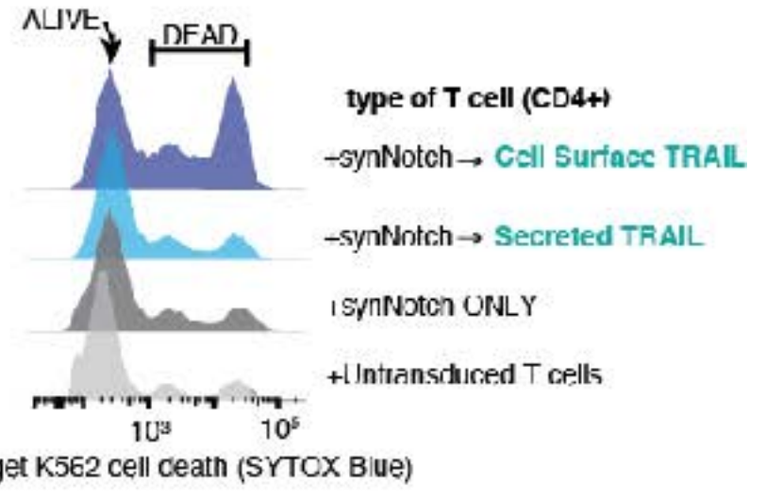
Custom T cell Differentiation



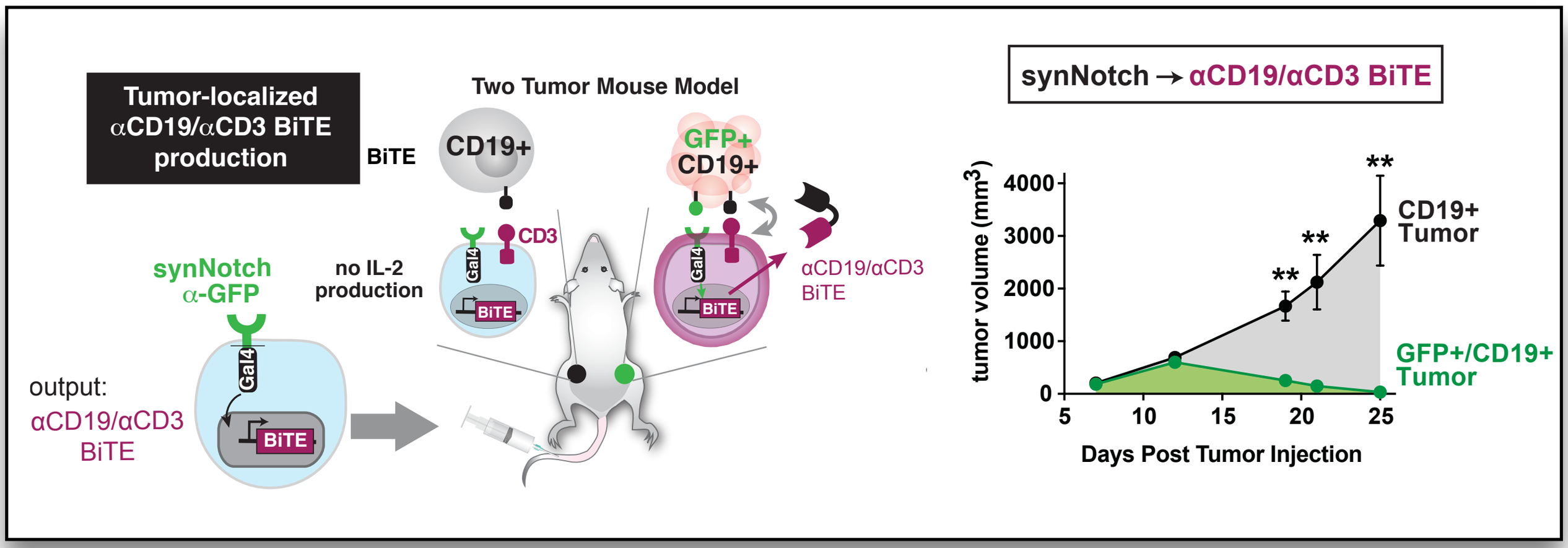


Custom Therapeutic Delivery

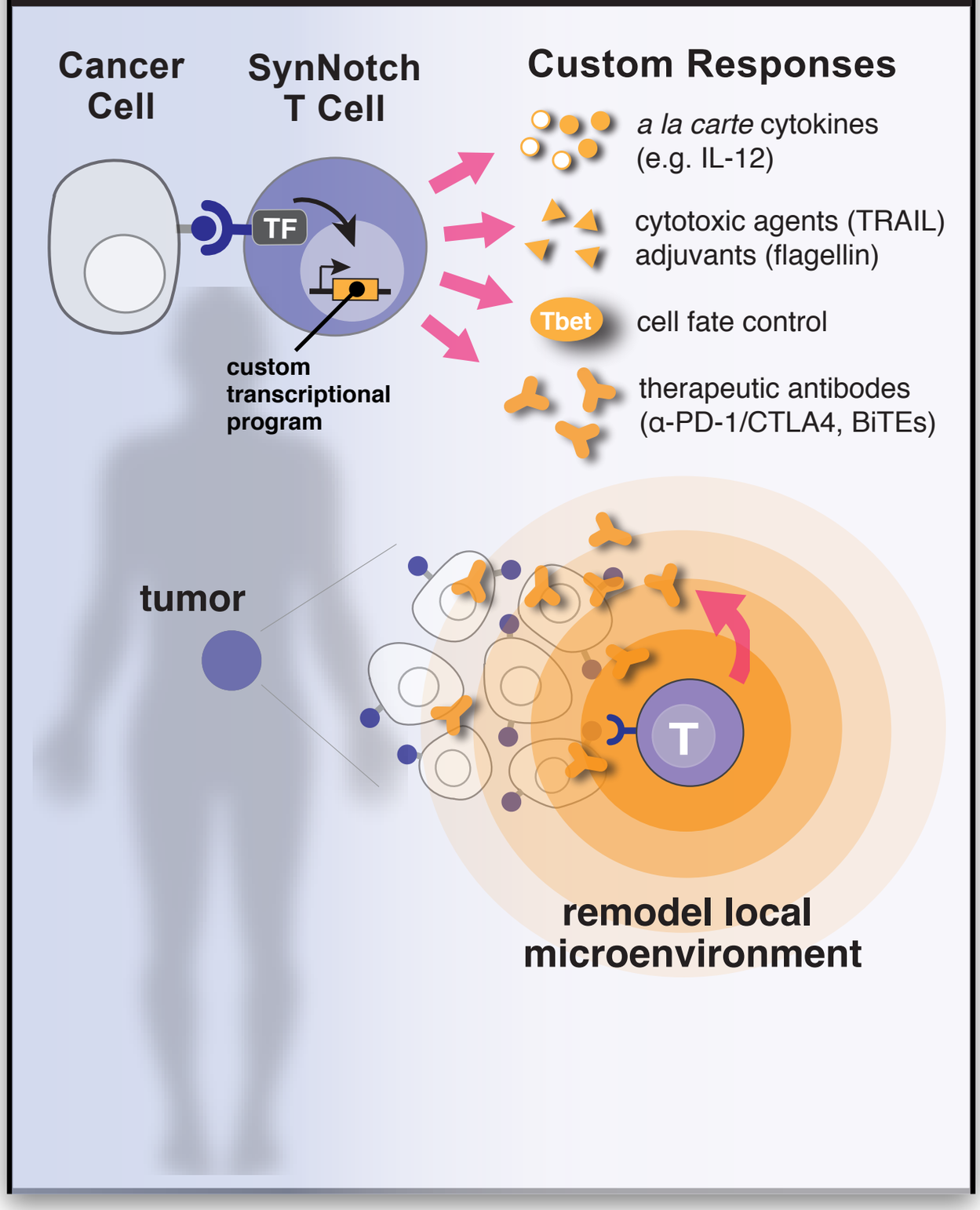




SynNotch Receptors Drive the Local Production of Therapeutic Antibodies *In vivo*

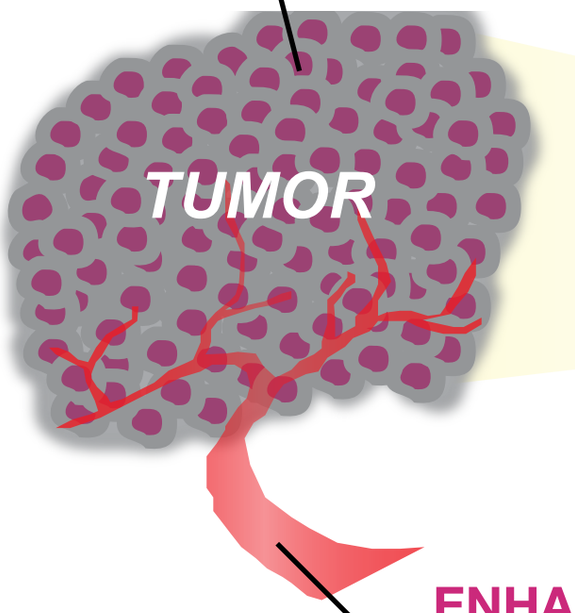


Custom T cell Response Programs with SynNotch Receptors



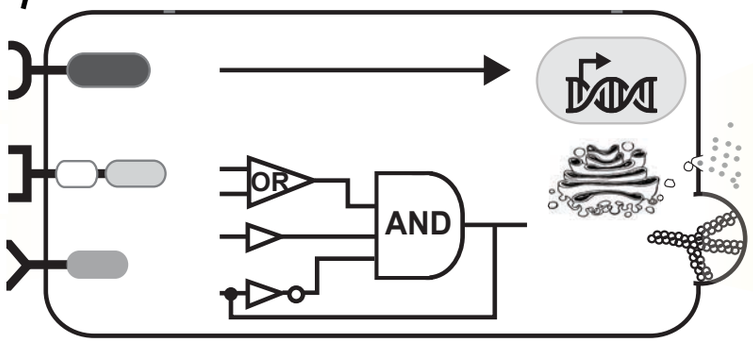
SYNTHETIC REGULATION OF IMMUNE CELL THERAPEUTICS

LOCAL MODULATION OF TUMOR MICROENVIRONMENT



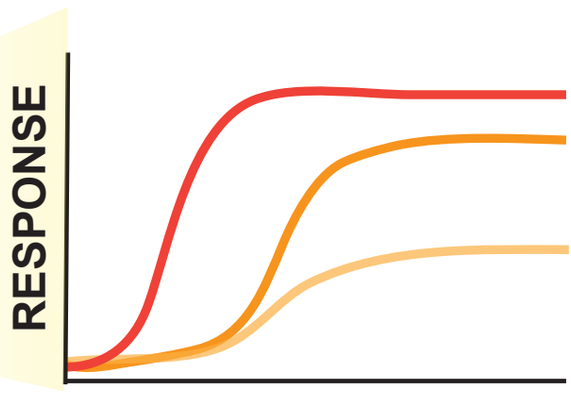
ENHANCE INFILTRATION & RETENTION

HIGH-THROUGHPUT RECEPTOR DESIGN



CELL AUTONOMOUS DECISION LOGIC (enhanced disease recognition)

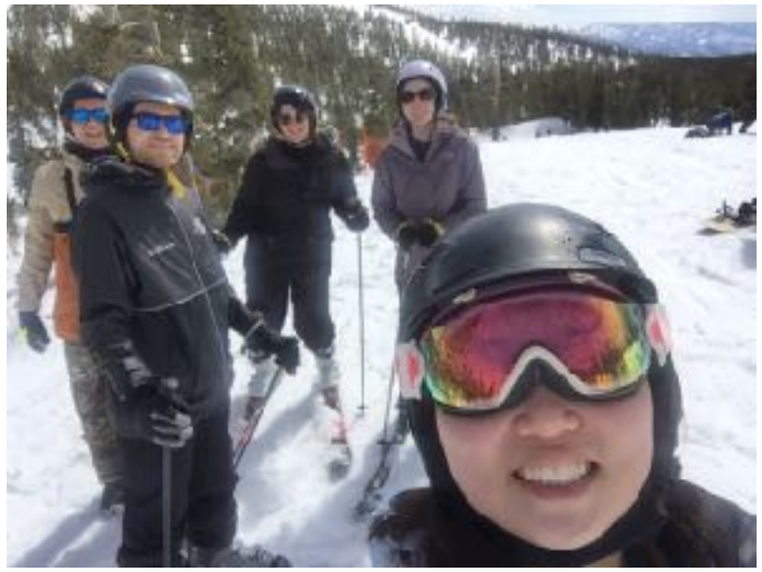
CONTROL THE DYNAMICS OF THERAPEUTIC RESPONSE PROGRAMS



UCSF

ROYBAL LAB

synthetic immunology



Axel Wittsten, PhD
Raymond Liu, PhD
Daniel Goodman, PhD
Casey Burnett
Camillia Azimi
Iowis Zhu
Kiavash Garakani
Garrett Montgomery
Emily Park
Kendall Kearns
Jaehoon Shin, MD, PhD

Collaborators:
Bin Liu, PhD - UCSF
Wendell Lim, PhD - UCSF
Hideho Okada, MD PhD - UCSF
Jeff Bluestone, PhD - UCSF
Qizhi Tang, PhD - UCSF
Lewis Lanier, PhD - UCSF
Andrei Goga, MD PhD - UCSF
Tudor Fulga, PhD - Oxford
Matthew Krummel, PhD - UCSF
James Rubenstein, MD PhD - UCSF

Funding:

PARKER INSTITUTE for CANCER IMMUNOTHERAPY

CHAN ZUCKERBERG BIOHUB

NIH DIRECTOR'S NEW INNOVATOR AWARD

NIH

CANCER RESEARCH UK GRAND CHALLENGE

Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation

UCSF Helen Diller Family Comprehensive Cancer Center

Emerson Collective

Multiple Myeloma Translational Initiative (MMTI)

UCSF Program for Breakthrough Biomedical Research (PBBR)