UCSF

PARKER INSTITUTE FOR CANCER IMMUNOTHERAPY

Synthetic Immunology

Harnessing the Tools of Synthetic Biology and Gene Editing to Engineer Next-Generation Immune Cell

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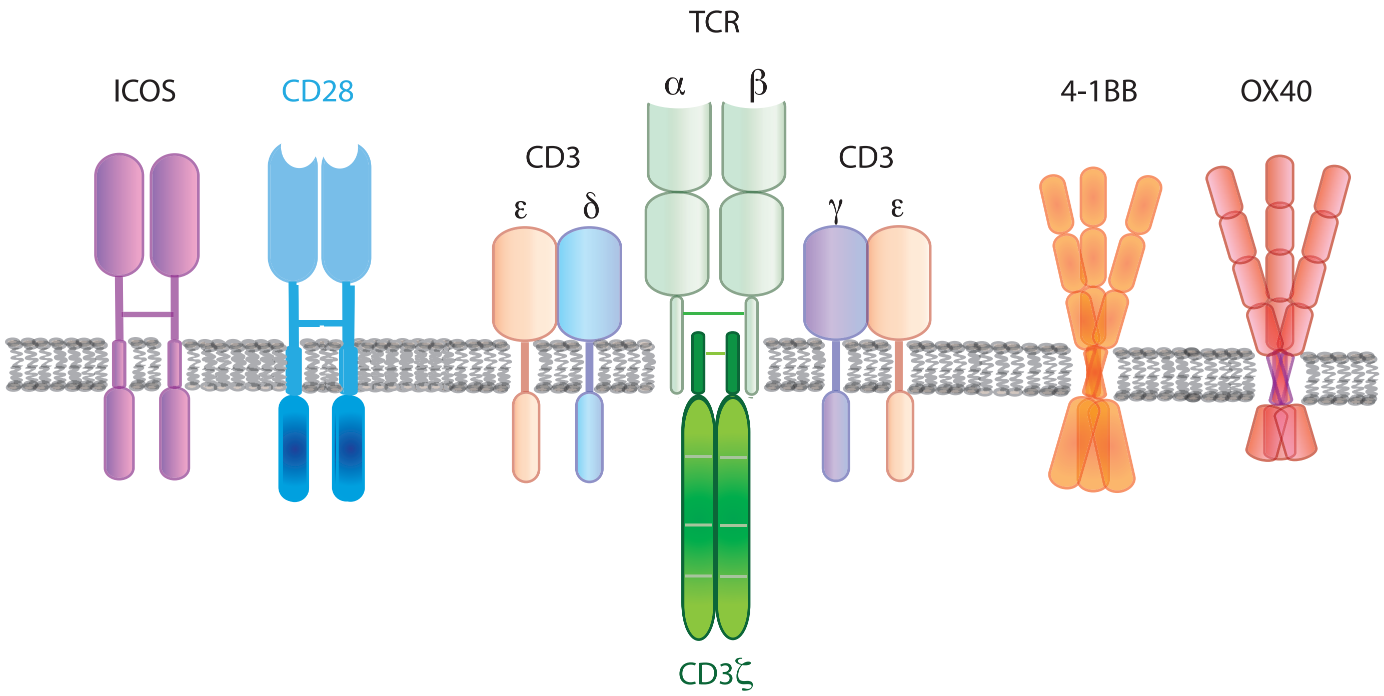
Parker Fellow
Immunology & Microbiology
UCSF

SITC - Houston
Jan 16th 2020

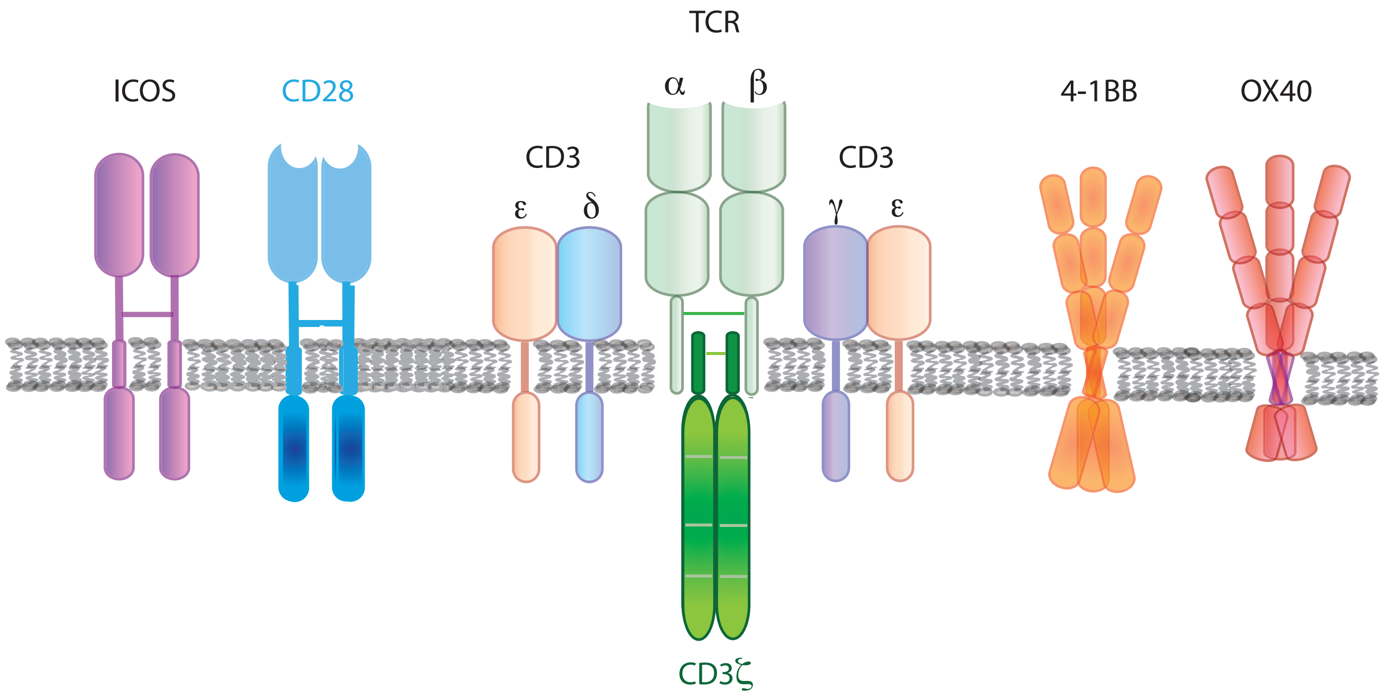
T Cell Receptor (TCR)

The TCR/CD3 complex and costimulatory constellation

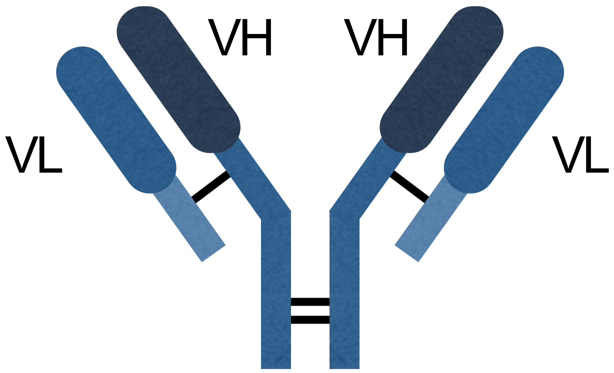


Chimeric Antigen Receptor (CAR)

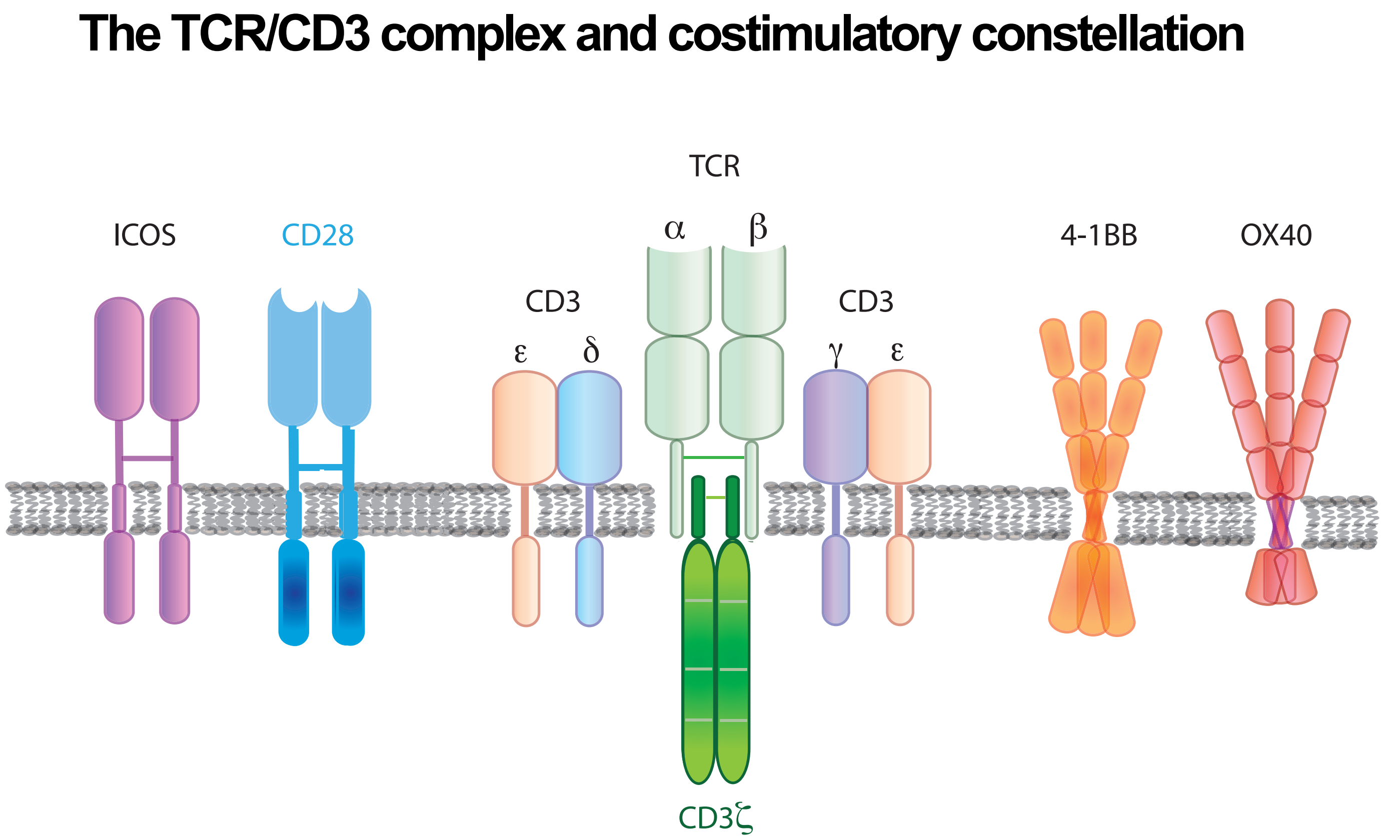
The TCR/CD3 complex and costimulatory constellation

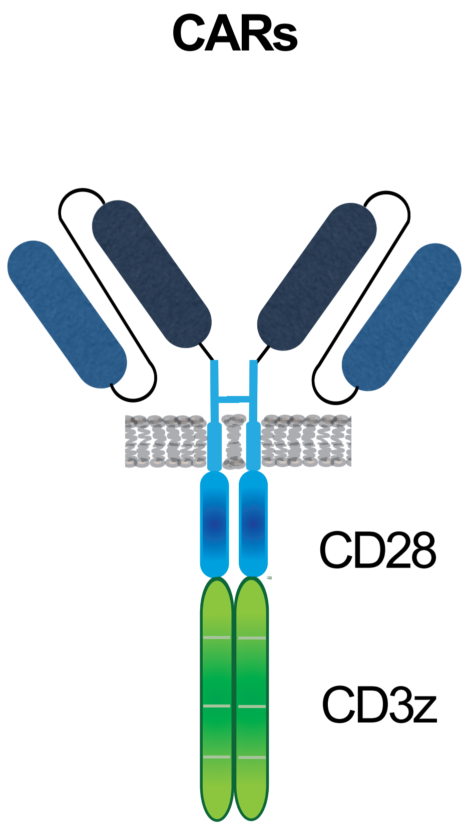


Monoclonal Antibody



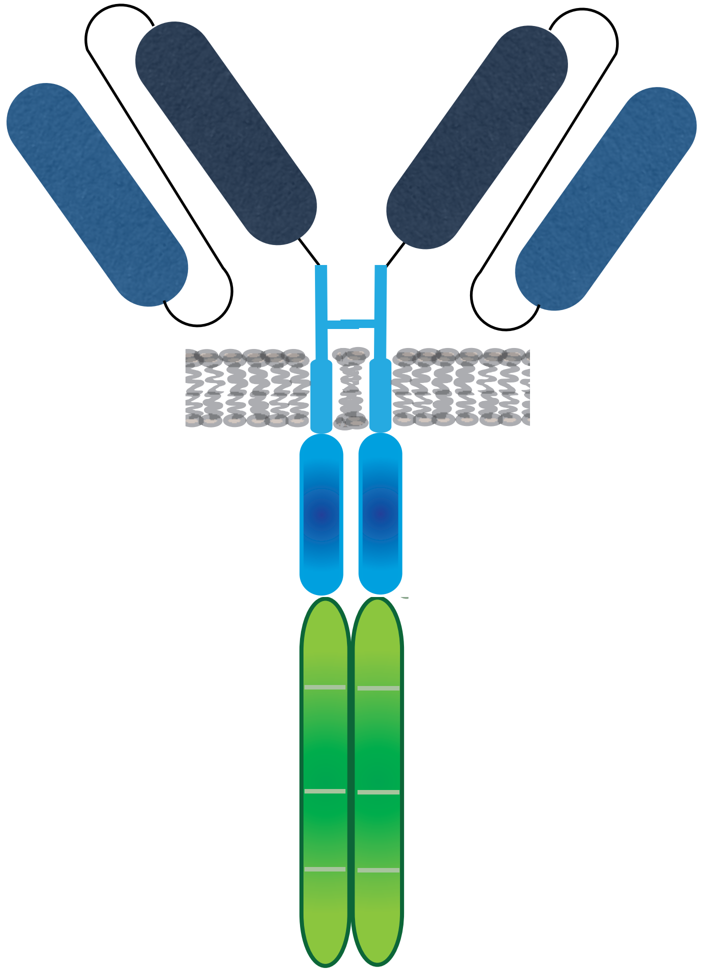
Chimeric Antigen Receptors (CAR)





Maher, Nat Biotech 2002
Sadelain, Riviere & Brentjens, Nat Rev Cancer, 2003
Sadelain, AACR Education Program, 2014

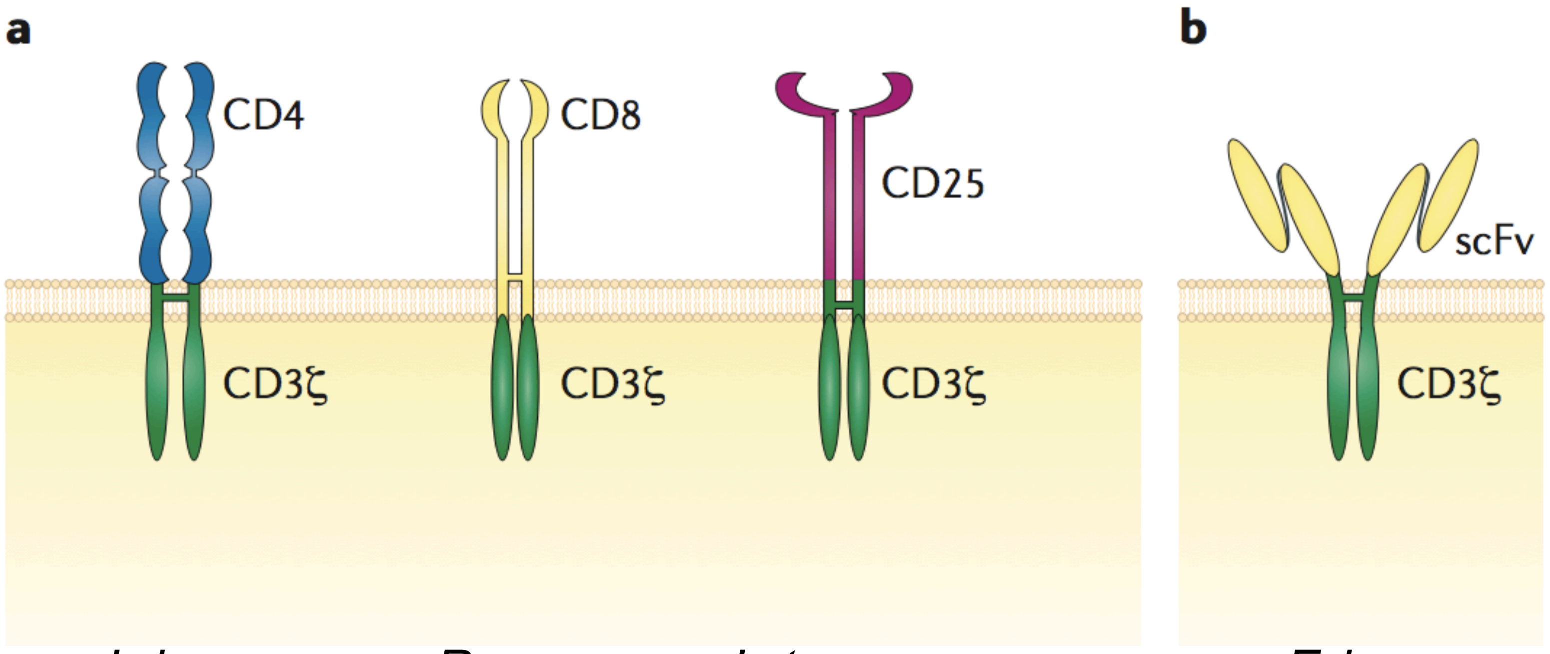
Chimeric Antigen Receptors (CAR)



- **Recognize cell surface antigen**
- **HLA independent**
- **T cell reprogramming**

Maher, Nat Biotech 2002
Sadelain, Riviere & Brentjens, Nat Rev Cancer, 2003
Sadelain, AACR Education Program, 2014

A historical perspective: early CAR designs



Irving, Cell, 1991

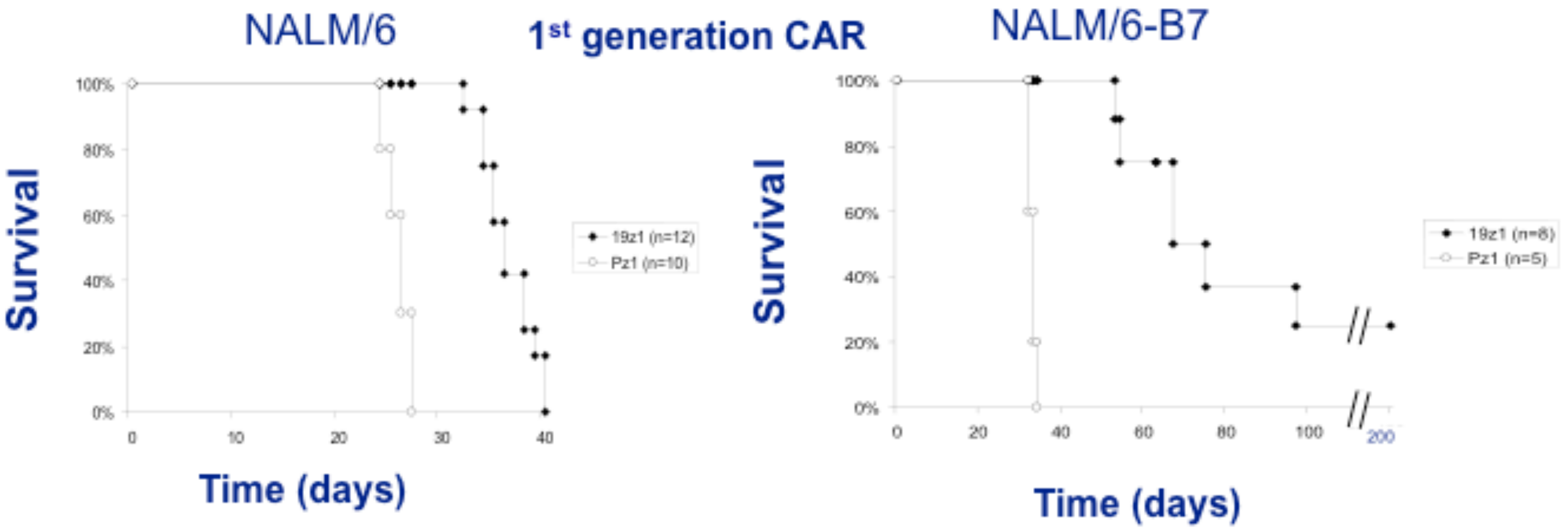
Romeo, Cell, 1991

Letourneur, PNAS, 1991

Eshar, PNAS, 1993

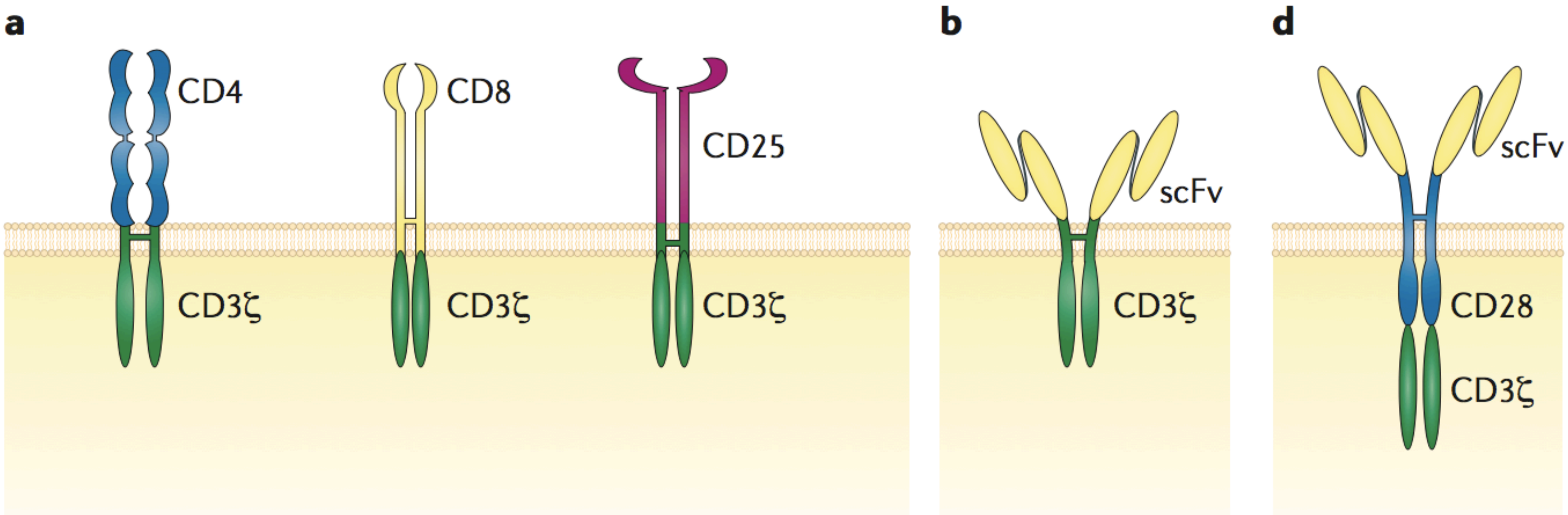
Adapted from Van der Stegen, Nat. Drug Discov 2015

CAR needs costimulation



Maher, Nat Biotech., 2002

A historical perspective: second gen CAR designs



Irving, Cell, 1991

Romeo, Cell, 1991

Letourneur, PNAS, 1991

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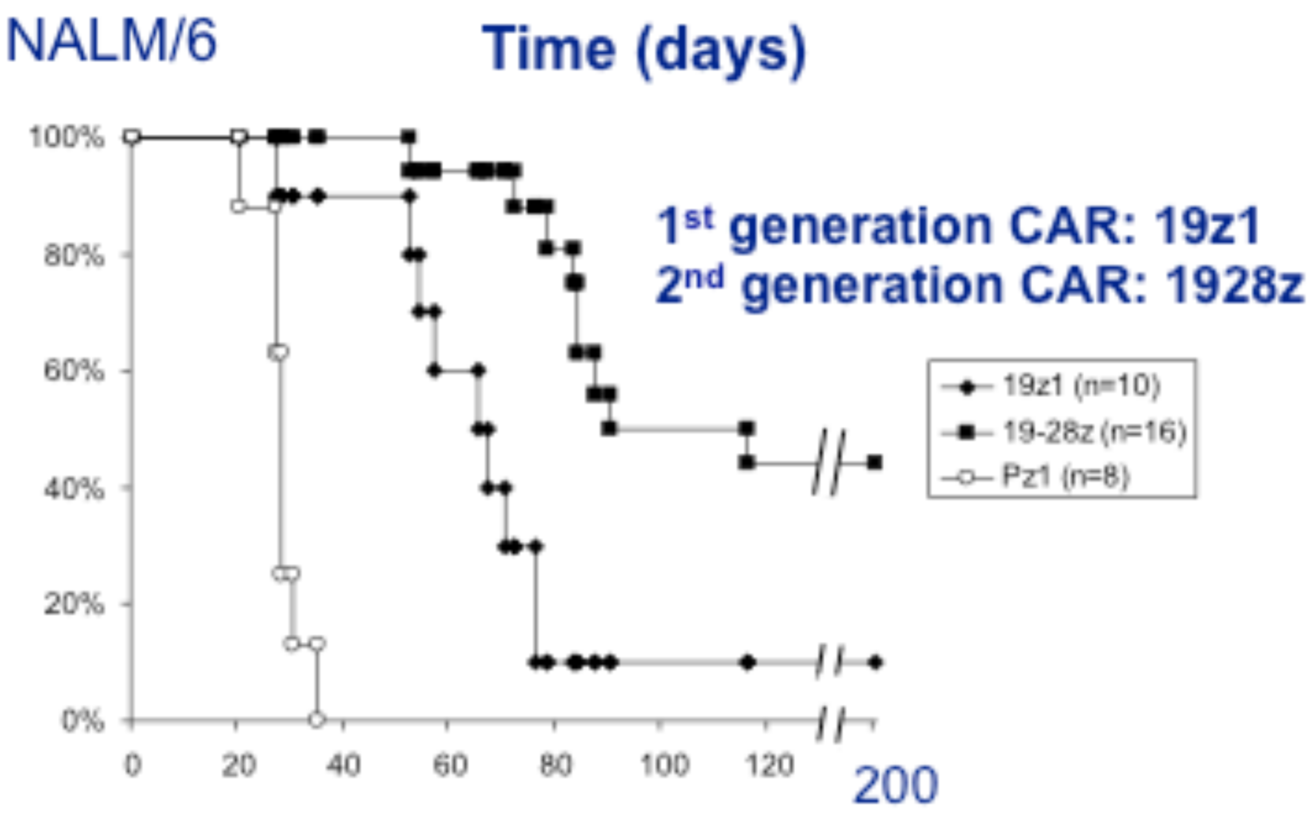
Maher, Nat Biotech., 2002

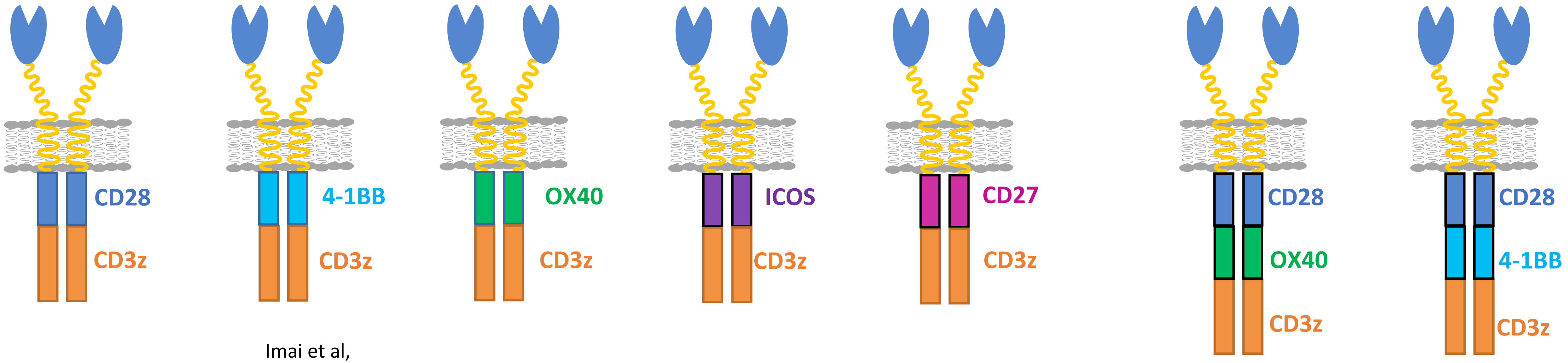
Adapted from Van der Stegen, Nat. Drug Discov 2015

CAR needs costimulation



Maher, Nat Biotech., 2002

2^{nd} and 3^{rd} gen CAR family



Maher et al,
Nat Biotech, 2002

Imai et al,
Leukemia, 2004;
Finney et al,
J Immunol, 2004

Finney et al,
J Immunol, 2004

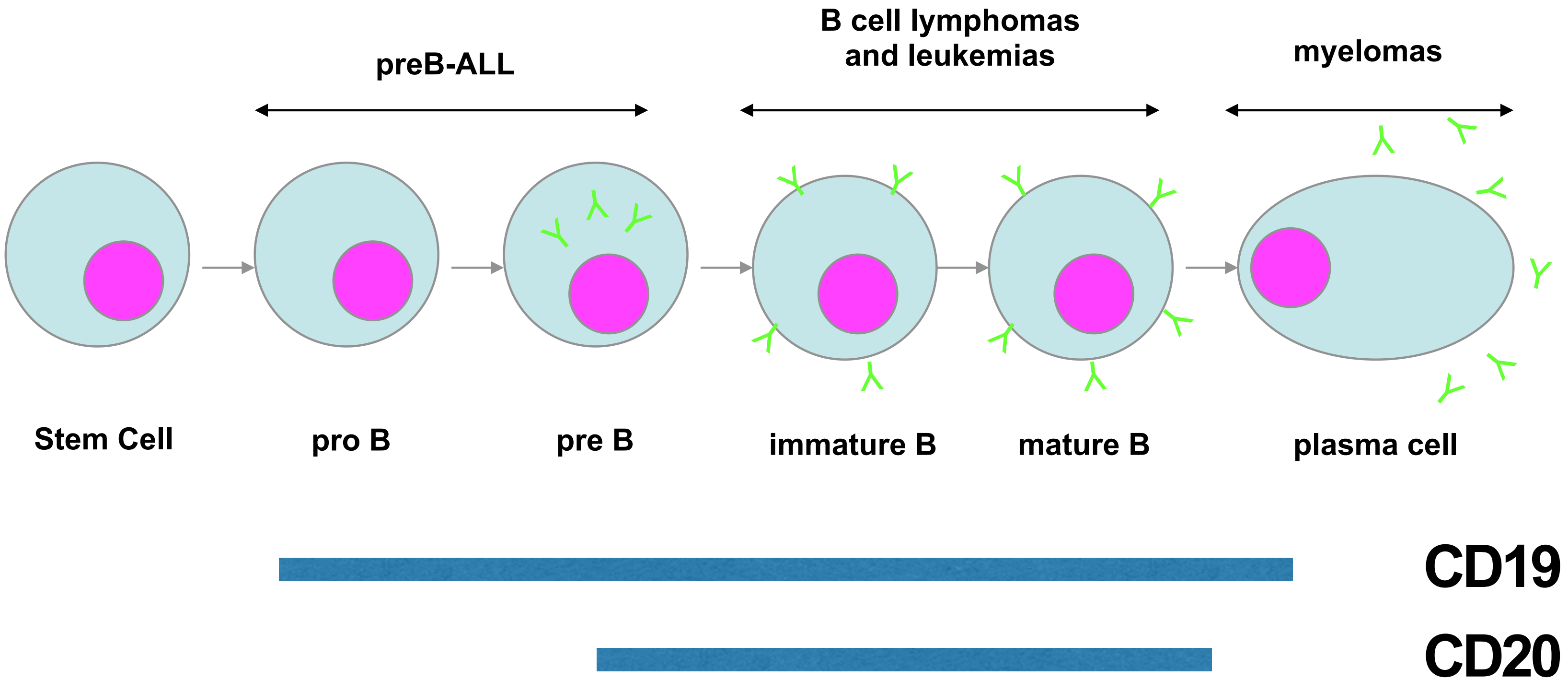
Finney et al,
J Immunol, 2004

Song et al,
Blood, 2012

Pule et al,
Mol Ther, 2005

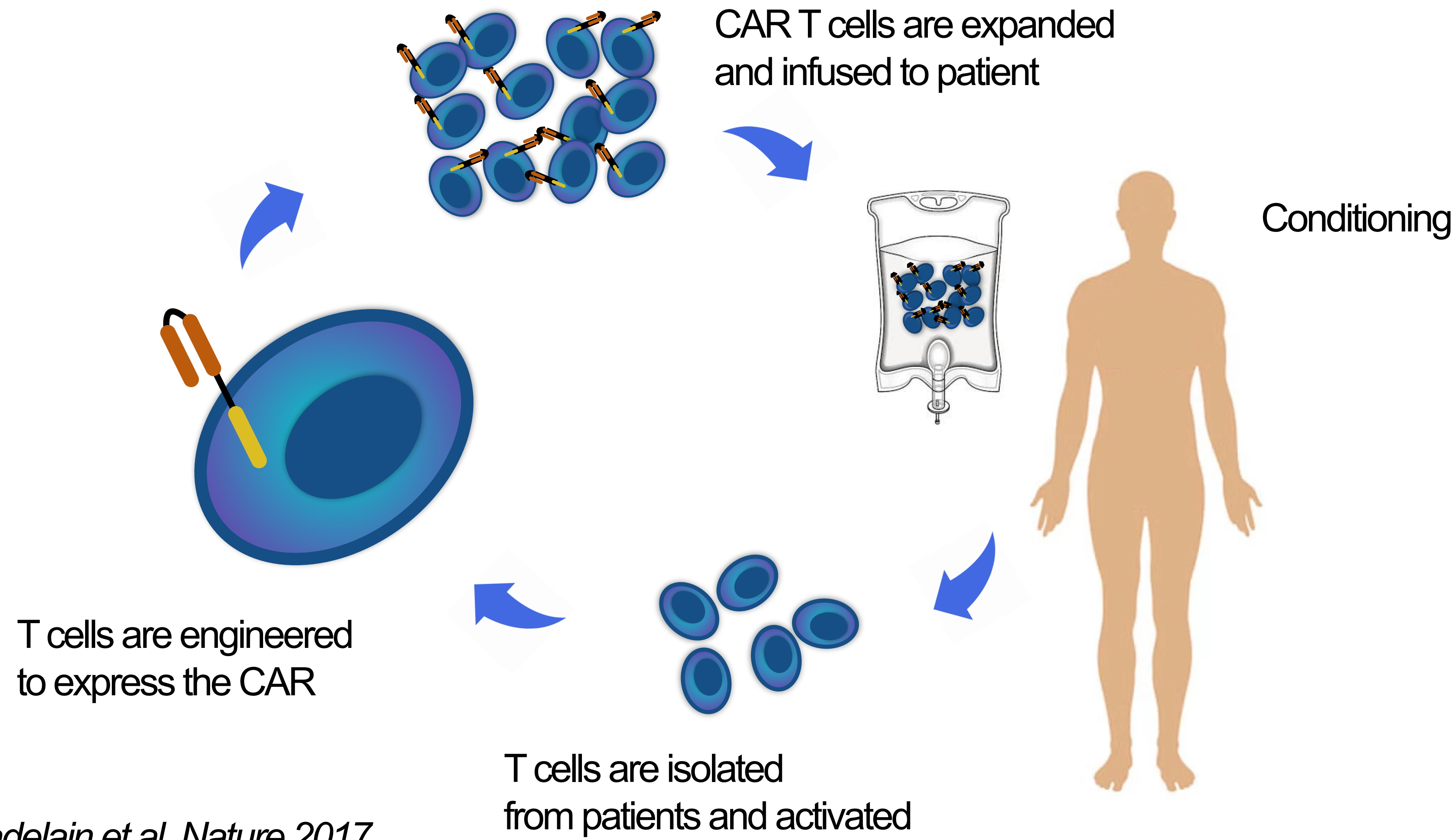
Pule et al,
Hum Gene Ther, 2007

Selecting CD19 as a target for CAR therapy



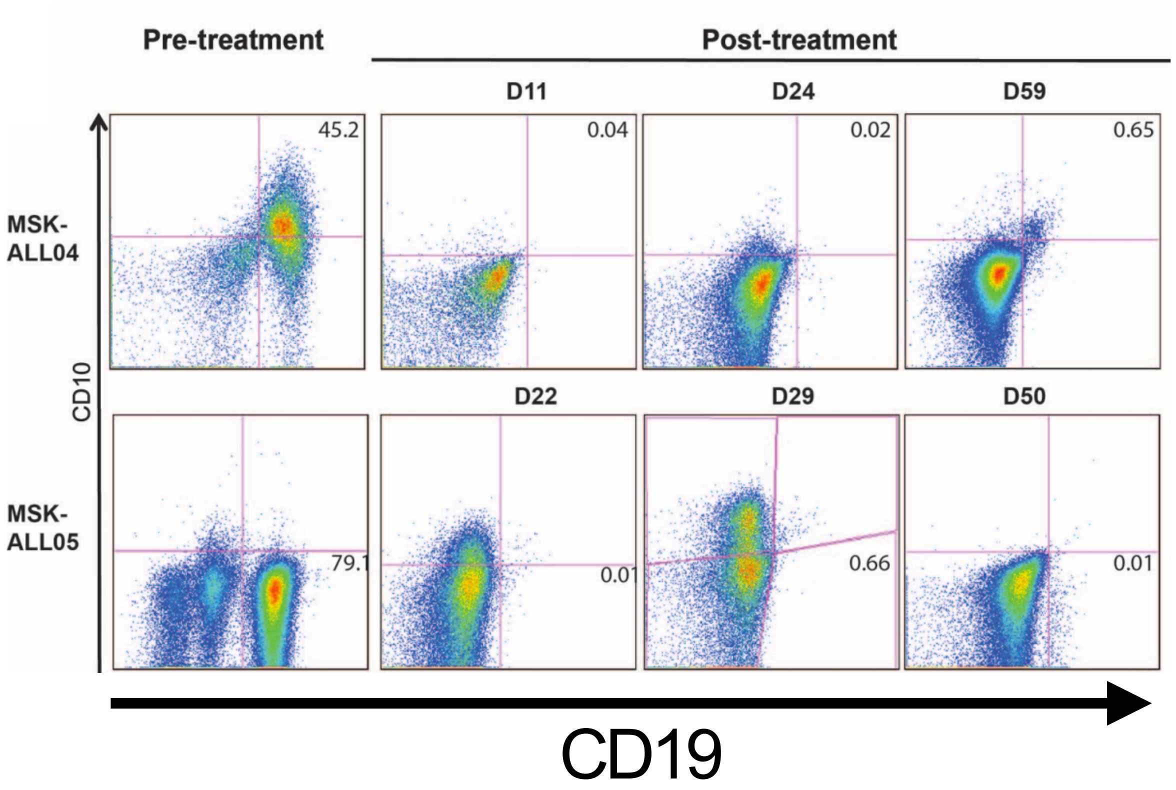
Cell surface CD19 and CD20 expression during B-cell development.
LeBien & Tedder, Blood, 2008.

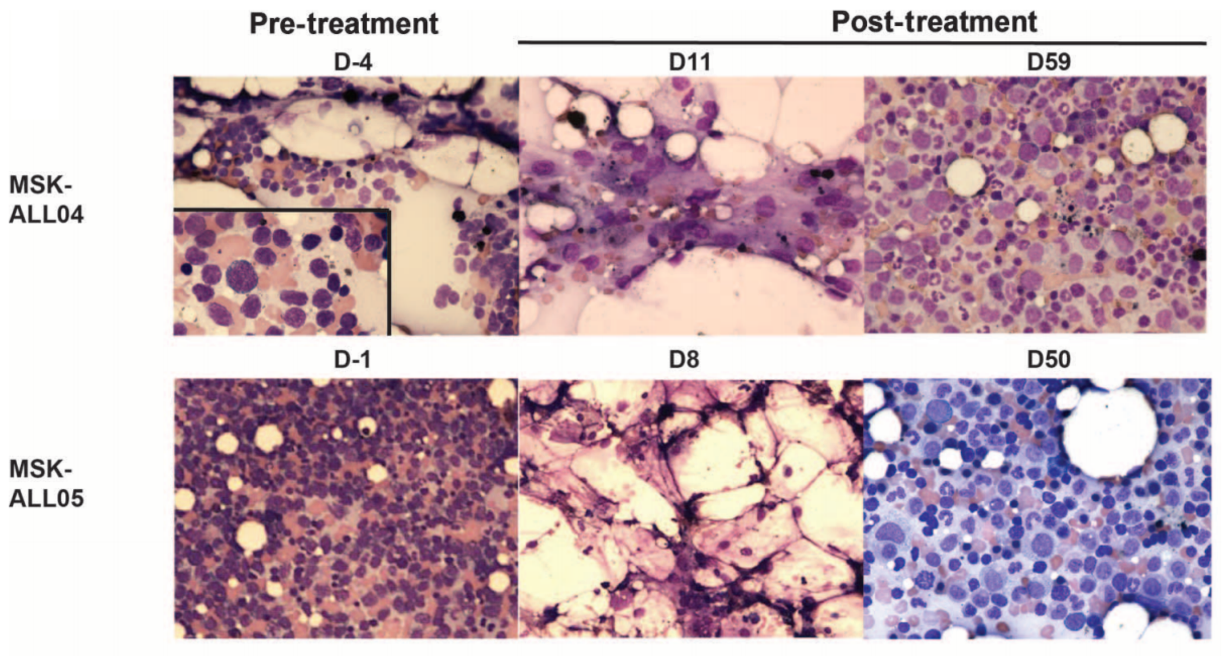
Chimeric Antigen Receptors (CARs)



Adapted from Sadelain et al. Nature 2017

Rapid Tumor elimination mediated by 1928z T cells in patient with refractory relapsed ALL





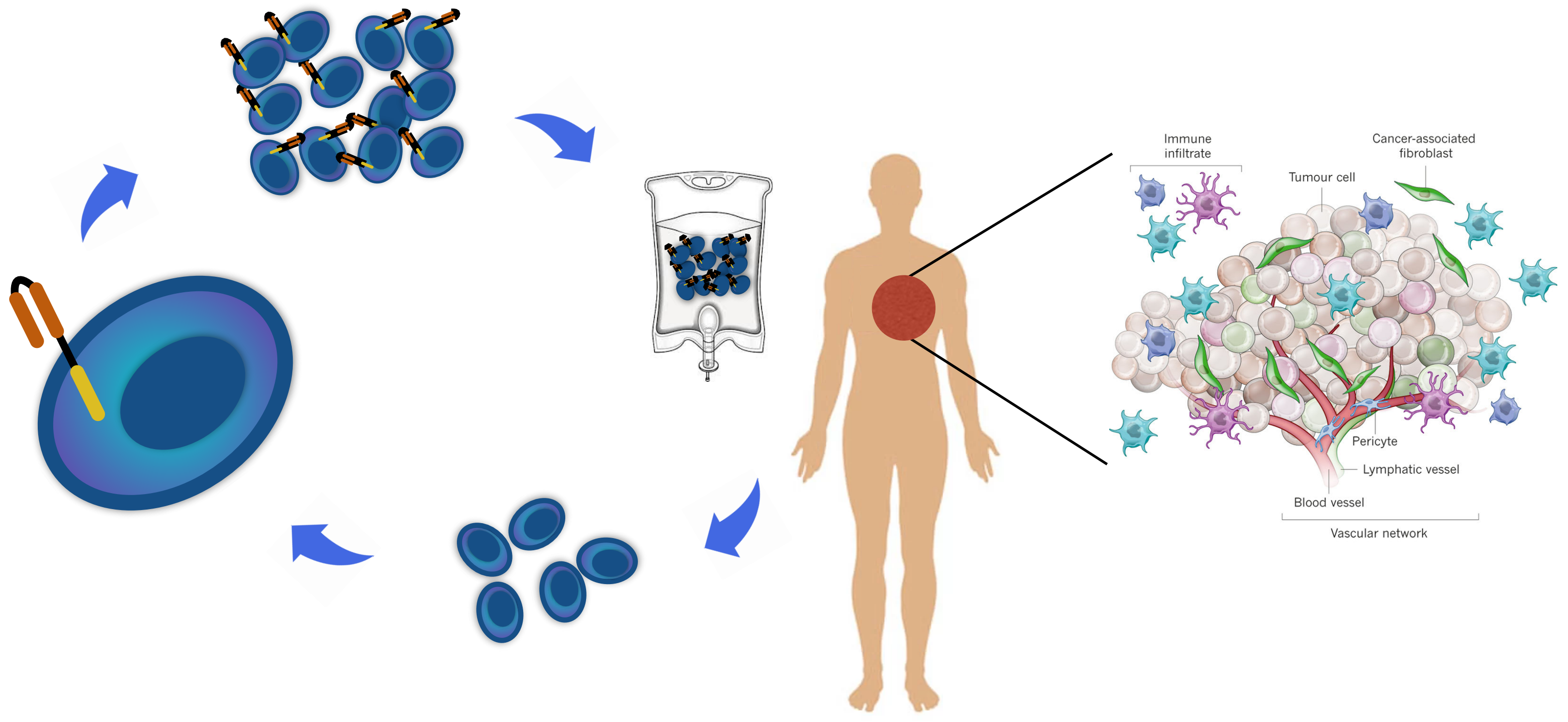
Brentjens, Davila, Riviere et al, Science Transl Med, 2013

CD19 targeting CAR for Relapsed, Chemo-refractory ALL

Center	Disease	CAR	Vector	Patients	CR rate
MSKCC Park, 2018	ALL (Ad.)	CD28	γRV	53	**83%**
Upenn Maud, 2018	ALL (Paed.)	4-1BB	LV	75	**81%**
NCI Lee, 2015	ALL (Paed.)	CD28	γRV	21	**68%**
FHCRC Turtle, 2016	ALL (Ad.)	4-1BB	LV	29	**93%**
UCL Qasim, 2015	ALL (Paed.)	4-1BB	LV	2	**100%**

Adapted from Sadelain et al. Nature 2017

Limits in CAR T cells



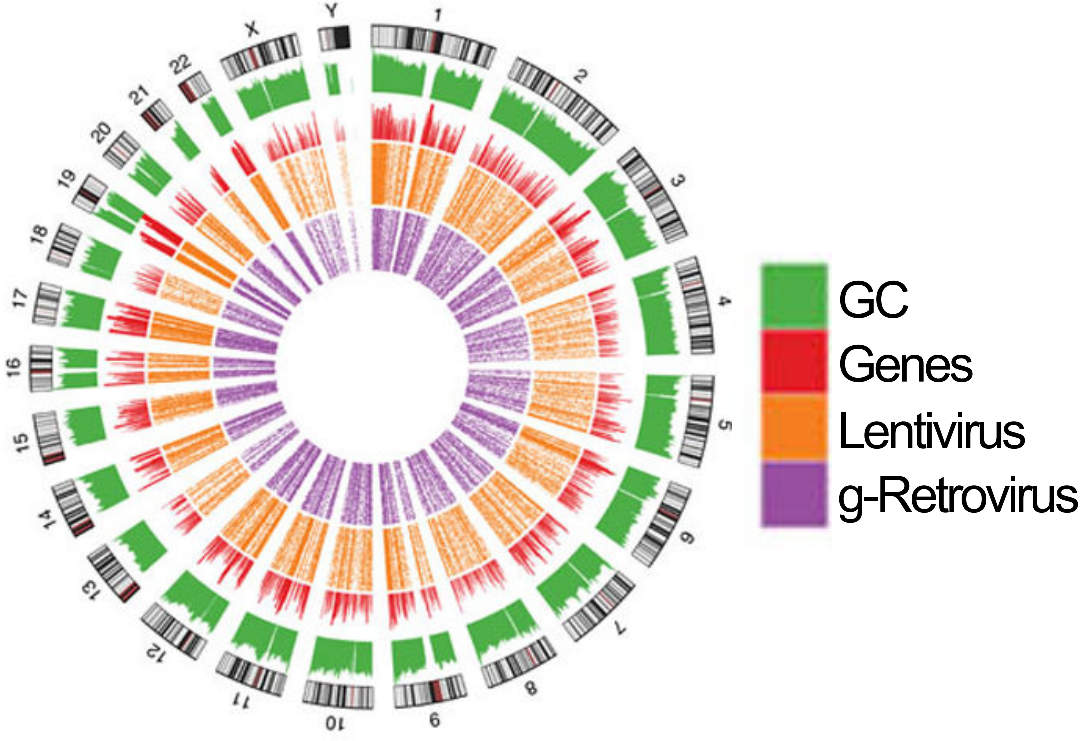
Relapse
- Low / negative antigen
- CAR not sensitive to low antigen
- Poor T cell persistence

Toxicities
- Cytokine Release Syndrome
- Cerebral Edema

Moderate activity in solid tumor
- Lack of ideal target
- Inefficient T cell homing
- T cell exhaustion/dysfunction

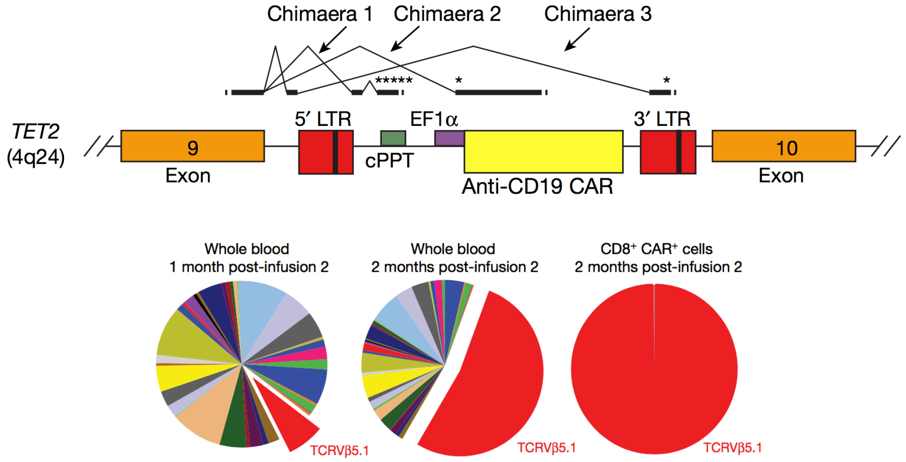
Manufacturing
- Cost
- Variability in the final product

Retroviral vectors: semi-random integration



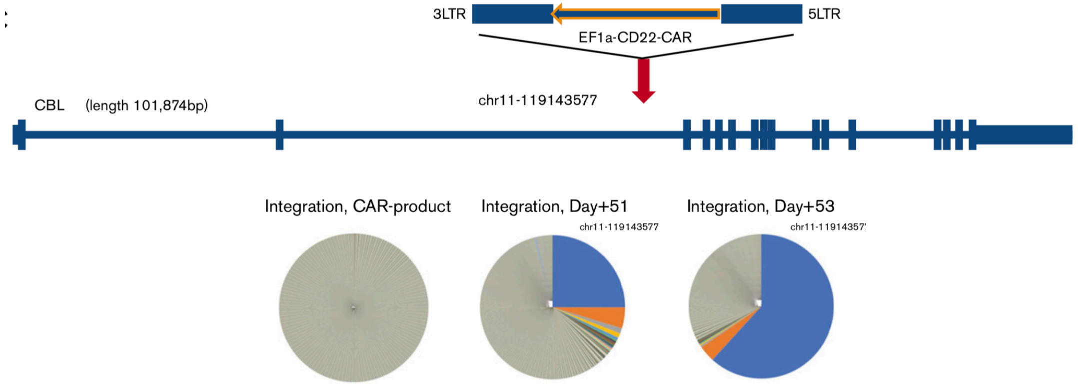
Craigie and Bushmann Microbiol. Spectr. 2014

TET2



Fraietta et al. Nature. 2018

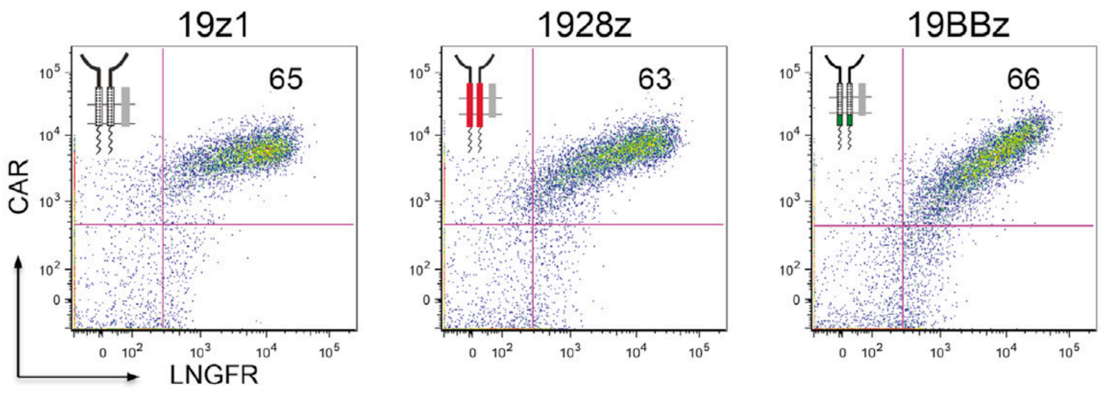
CBL



Shah et al. Blood. 2019

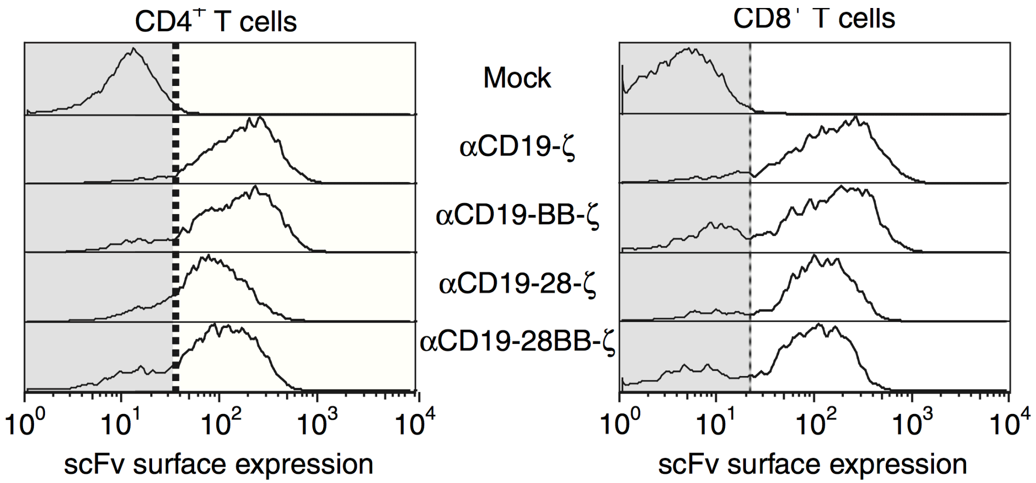
Retroviral vectors: variegated expression

g-Retrovirus



Zhao et al. Cancer cell 2015

Lentivirus



Milone et al. Molecular Therapy 2009

Ways to improve CAR T cells

Ways to improve CAR T cells

GOAL:

1. Control/Improve persistence
2. Prevent T cell exhaustion
3. Address tumor heterogeneity/target safety
4. Standardized manufacturing/reducing cost

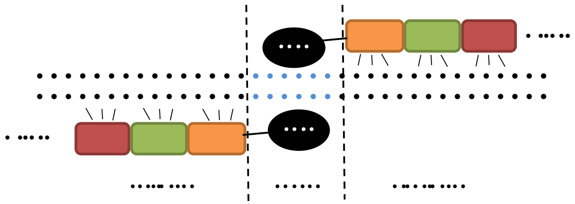
Tools:

1. Gene editing
2. CAR design
3. Logic gates
4. SynNotch

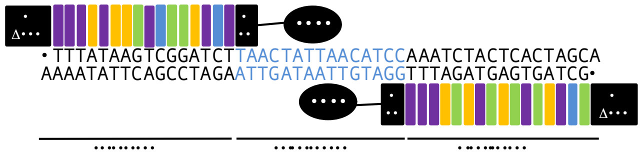
Gene edited CAR T cells

Genetic engineering

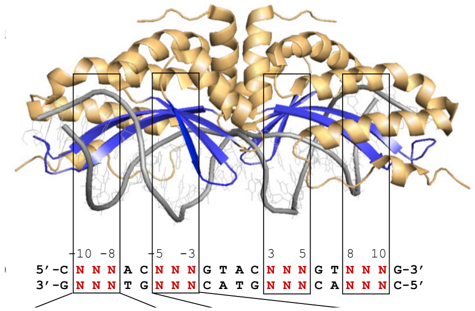
Zinc Finger Nuclease
(ZFN)



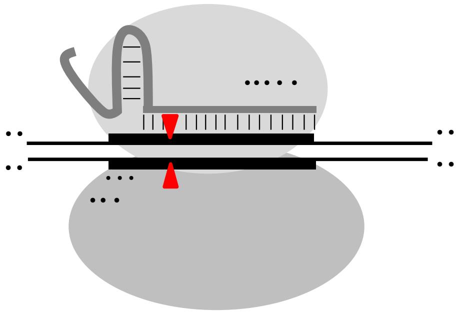
TAL Effector Nuclease
(TALEN)



Meganuclease



CRISPR/Cas9



Chandrasegaran, Carroll, Porteus, Stoddard, Dujon, Choulika, Belfort, Bonas, Bogdanove, Voytas, Joung, Doudna, Charpentier, Barrangou, Zhang, Church

Genome editing

Adapted form Joung et al. Nat biotech 2013

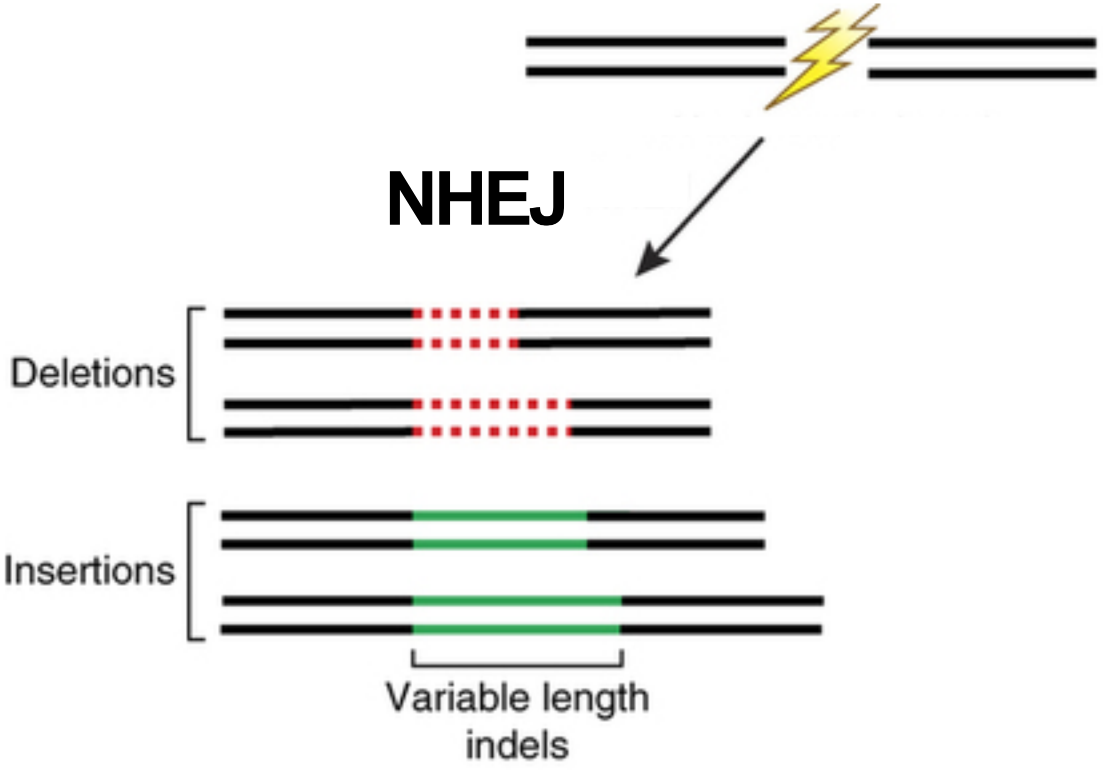
Nuclease-induced double-strand break

Genome editing

Adapted form Joung et al. Nat biotech 2013

Nuclease-induced double-strand break

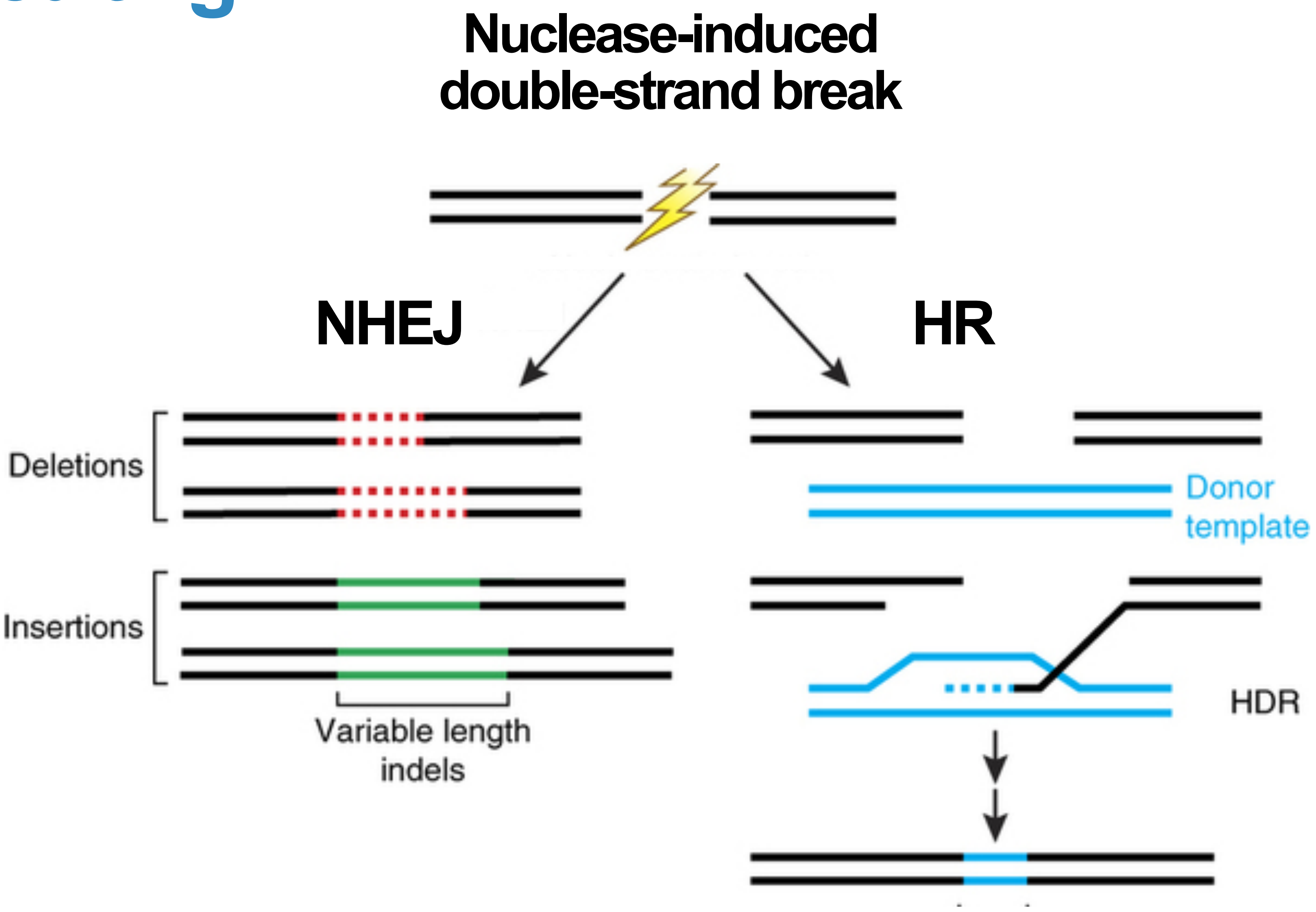
NHEJ



Gene disruption

Genome editing

Adapted form Joung et al. Nat biotech 2013



Gene disruption

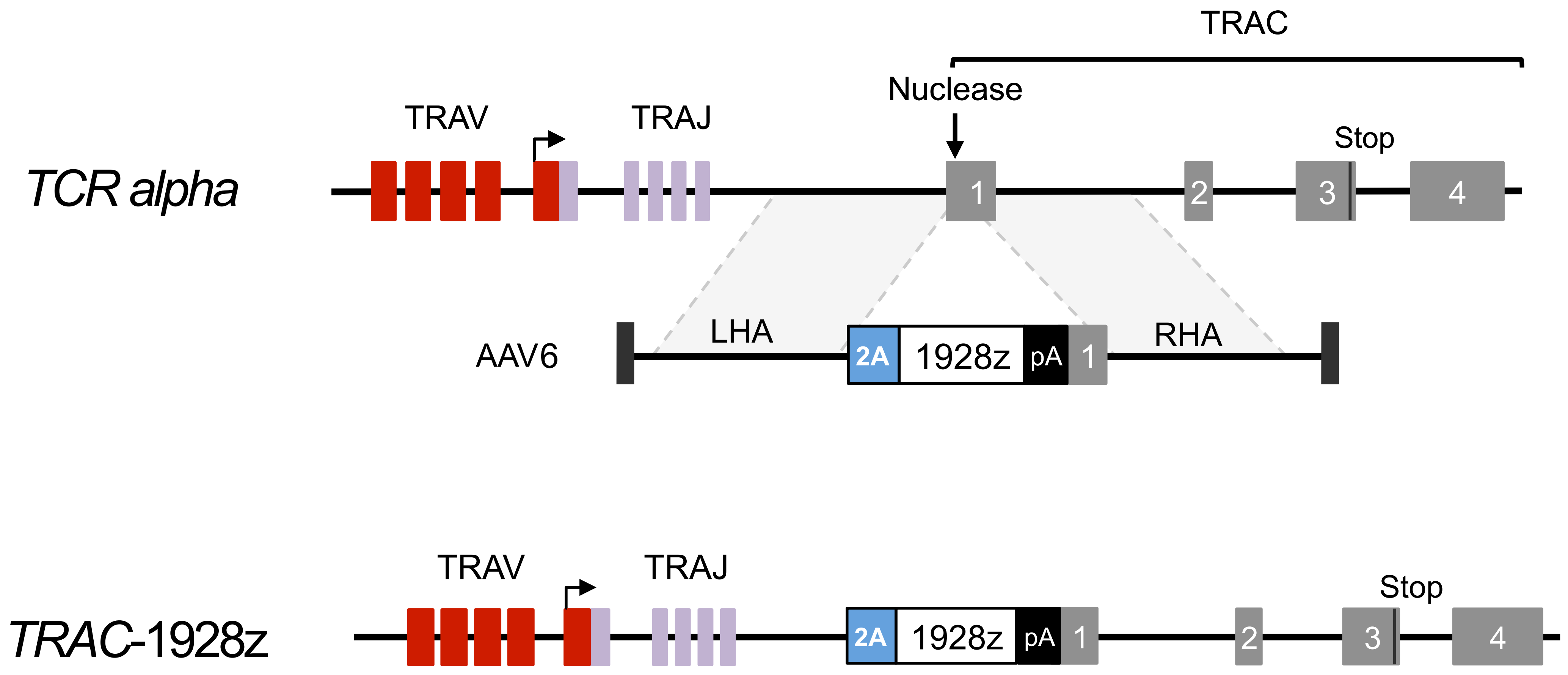
Gene tageting or correction

Editing CAR T cells

› Gene disruption

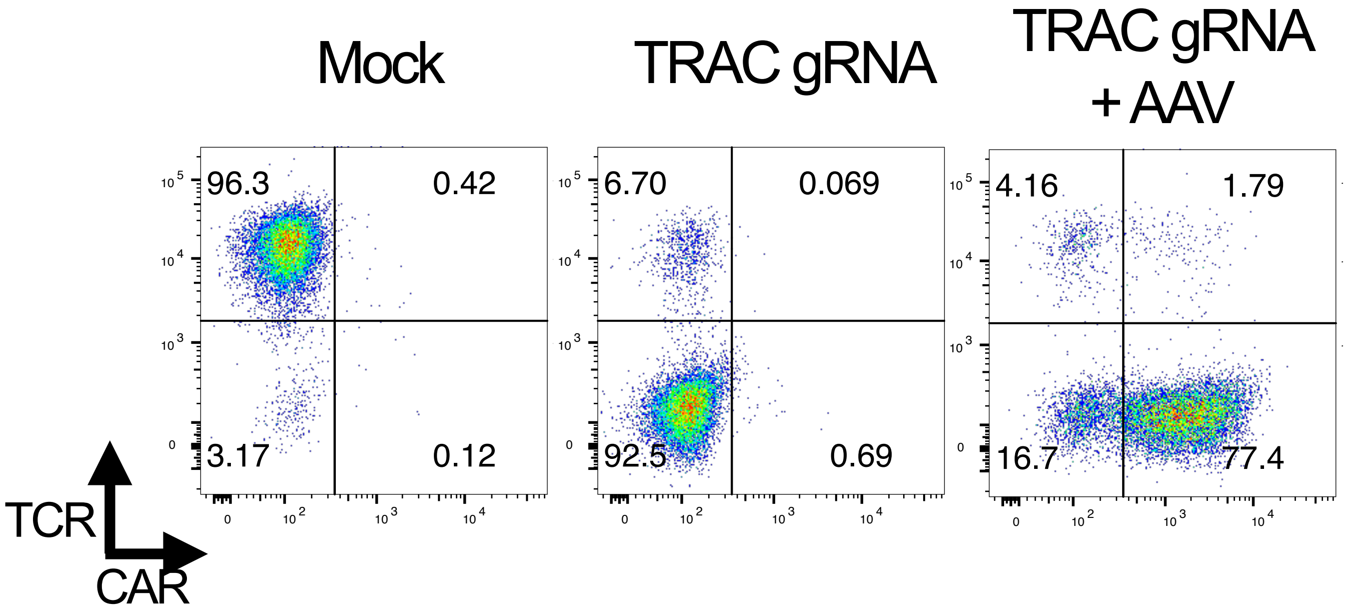
- Allogeneic:
  - TCR alpha/beta — *Torikai 2012, Berdien 2014, Poirot 2015*
  - B2M — *Ren 2017*
- Checkpoints: PD1 — *Su 2016, Rupp 2017*
- Cell death: Fas — *Ren 2017*
- Drug resistance:
  - CD52 (Alemtuzumab) — *Poirot 2015*
  - dCK (Clofarabine) — *Valton 2016*
- Exhaustion
  - NR4A — Chen 2019
  - TOX and TOX2 — Seo 2019

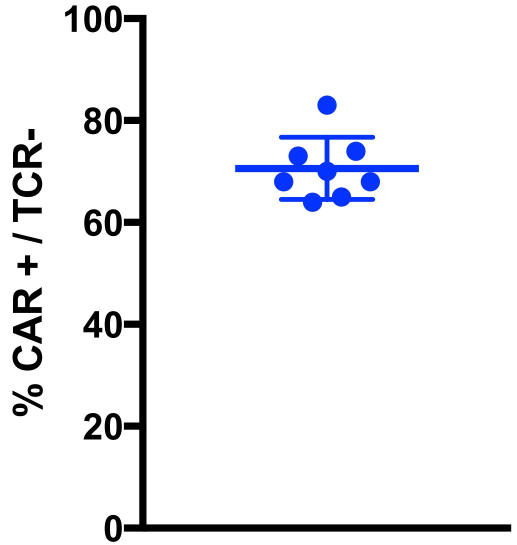
Targeting the CAR transgene



Eyquem, Mansilla-Soto et al., *Nature 2017*

Efficient CAR KI

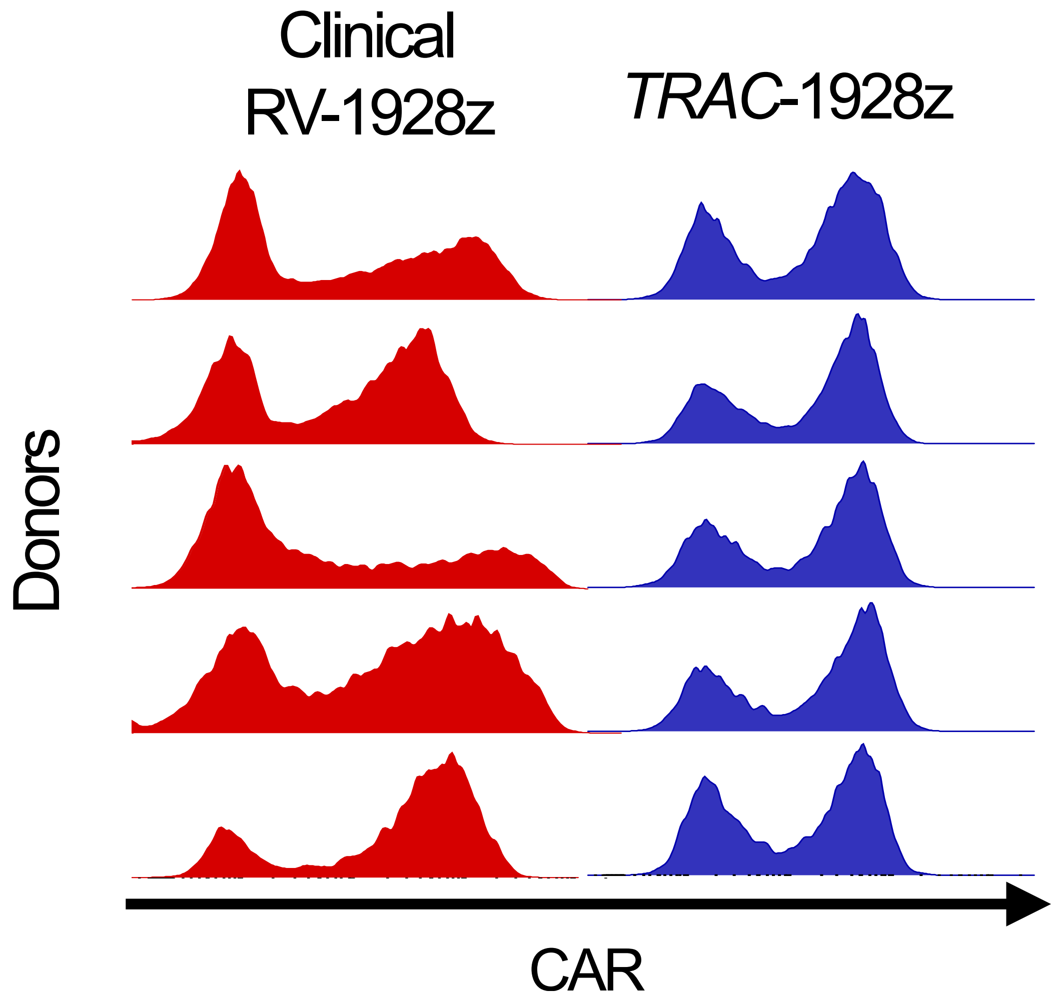


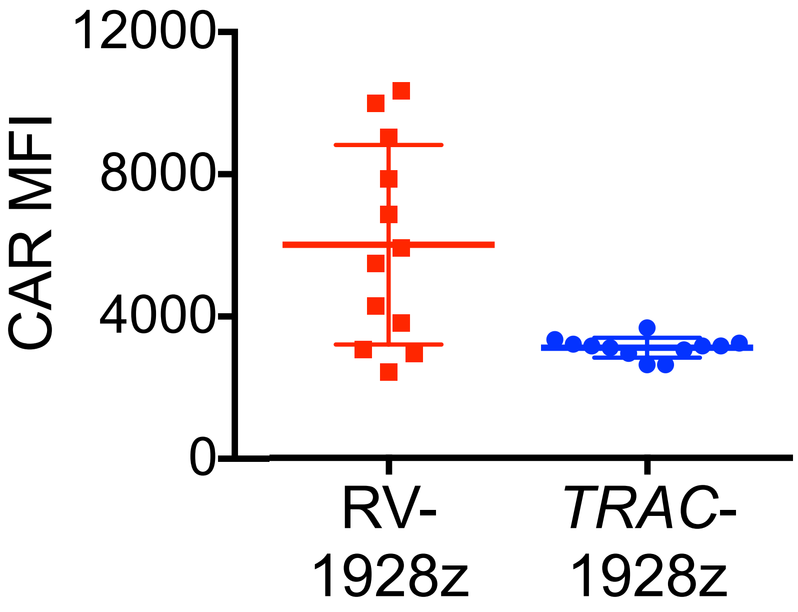


n = 8

Eyquem, Mansilla-Soto et al., *Nature 2017*

Homogeneous and Predictable CAR expression

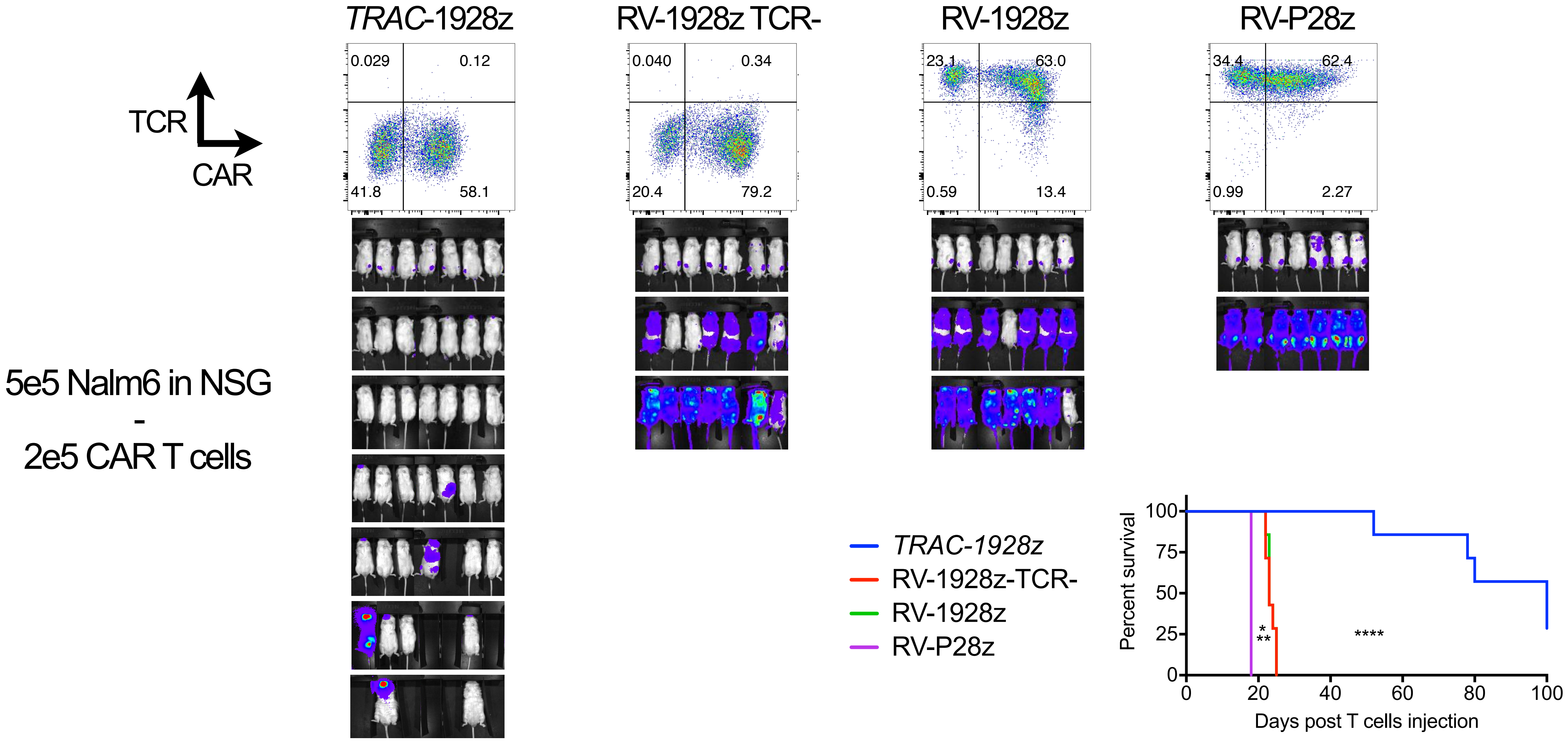




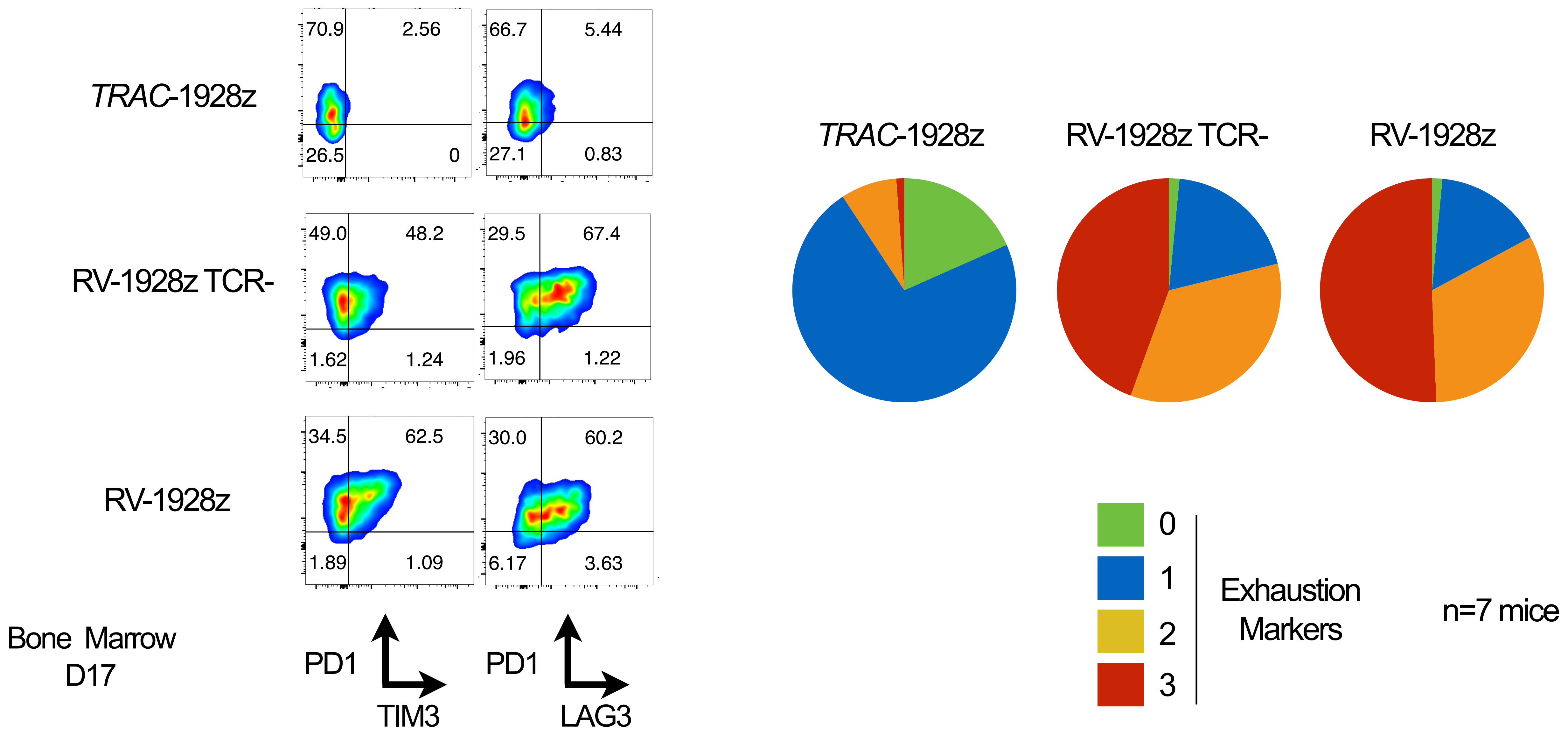
n=12

Eyquem, Mansilla-Soto et al., *Nature 2017*

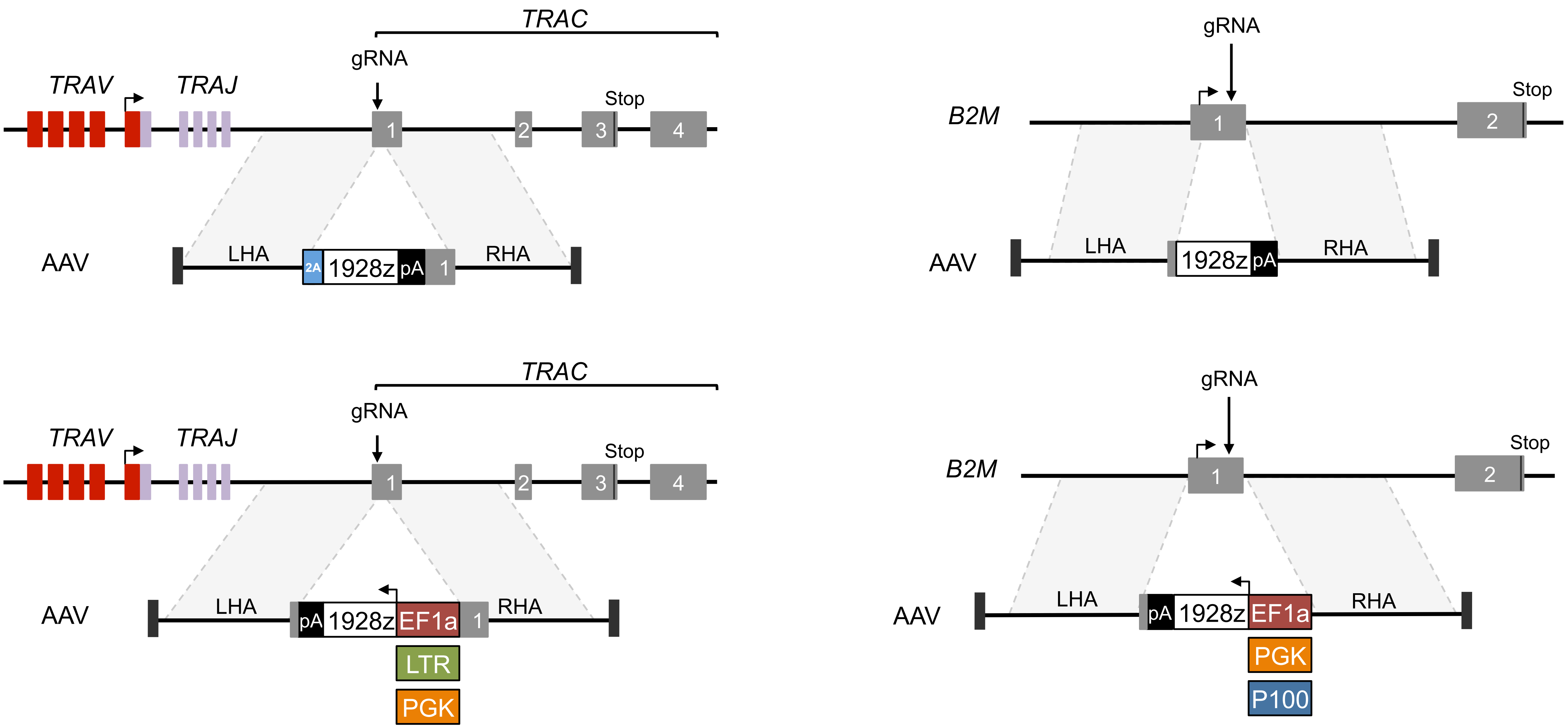
TRAC-CAR T cells display superior *in vivo* activity

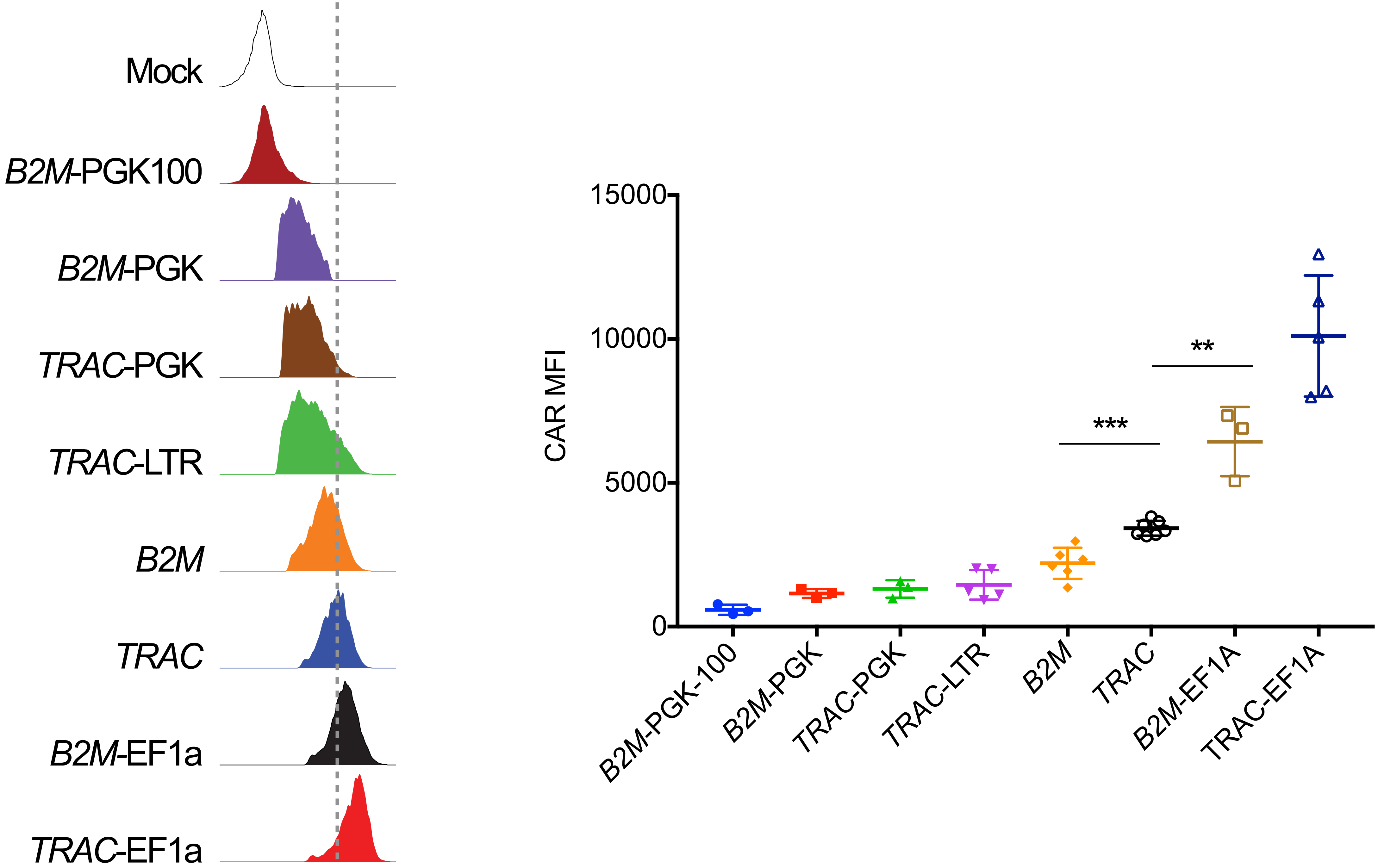


TRAC-CAR T cells are less exhausted



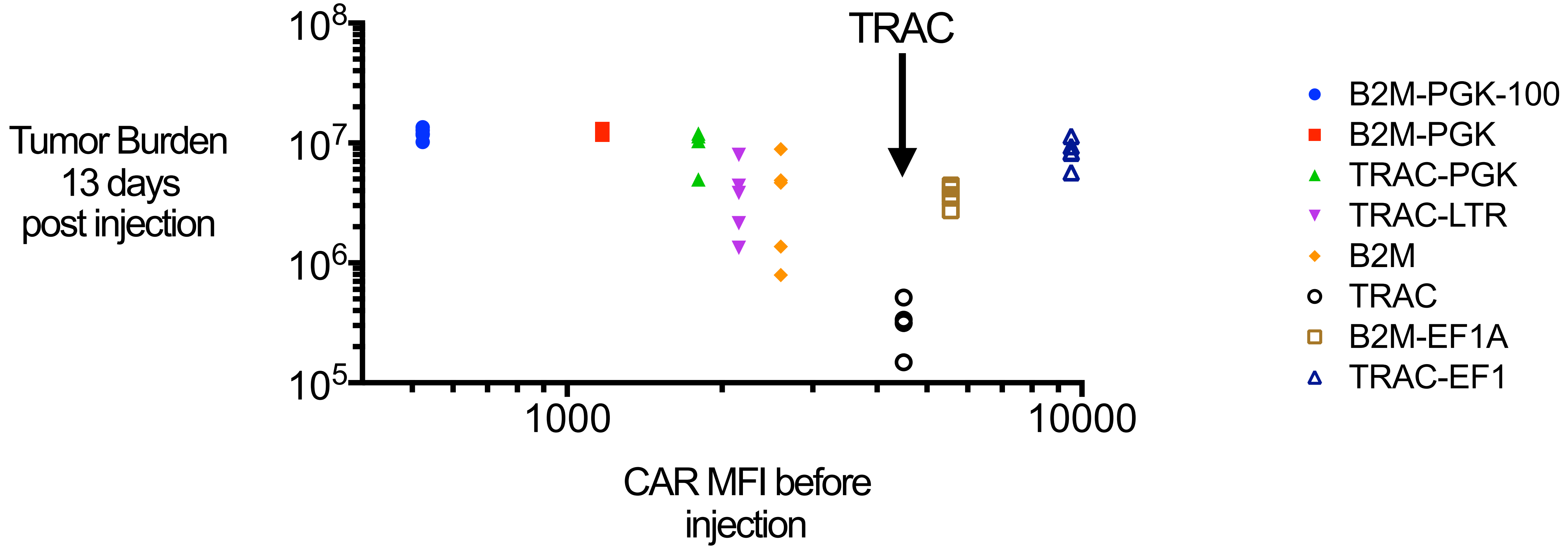
New Targeting constructs



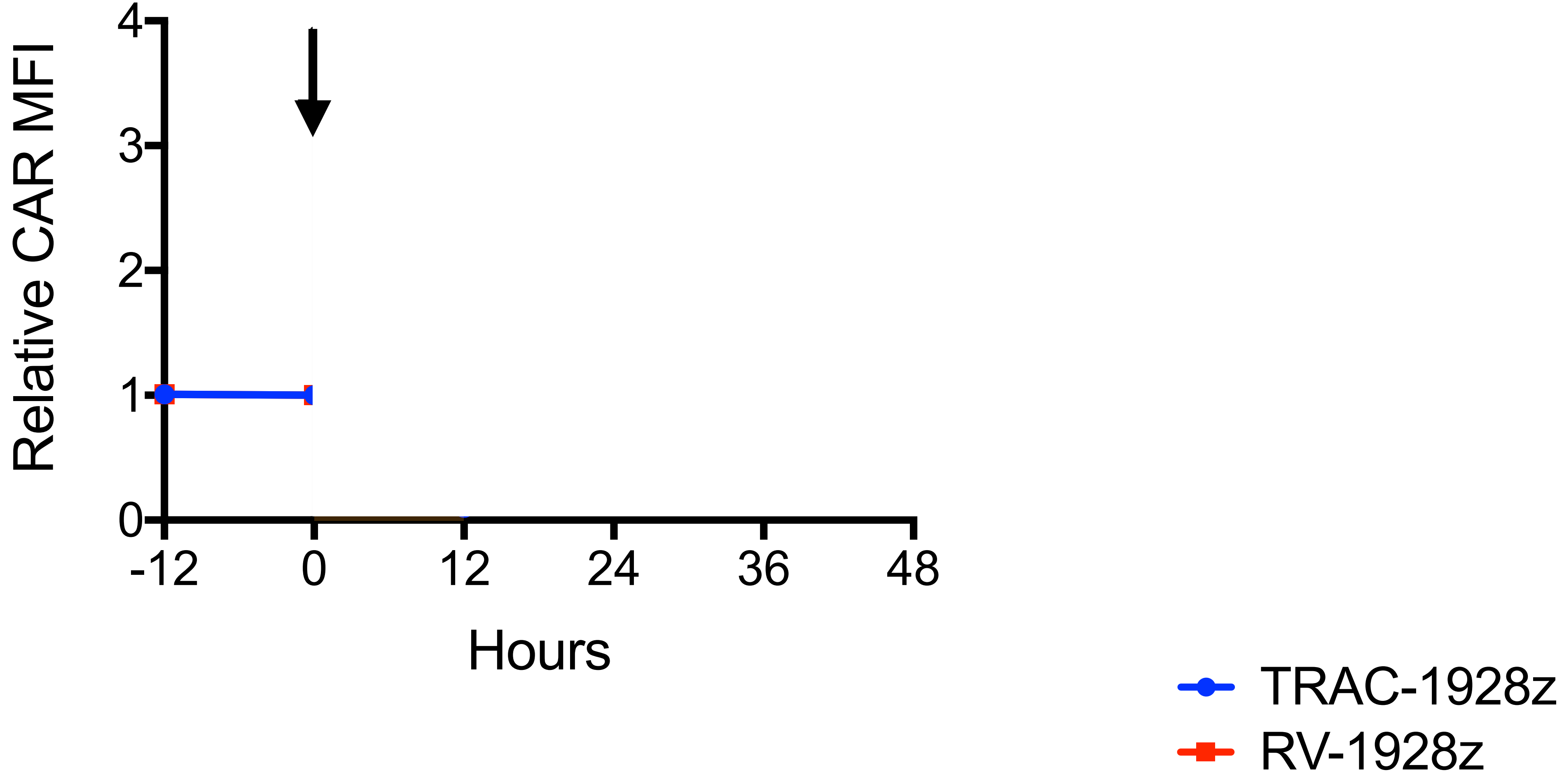

Mock
B2M-PGK100
B2M-PGK
TRAC-PGK
TRAC-LTR
B2M
TRAC
B2M-EF1a
TRAC-EF1a
CAR MFI
15000
10000
5000
0
***
**
B2M-PGK-100
B2M-PGK
TRAC-PGK
TRAC-LTR
B2M
TRAC
B2M-EF1A
TRAC-EF1A

TRAC-CAR T cells outperform other loci and promoters

5e5 Nalm6 in NSG
-
1e5 CAR T cells

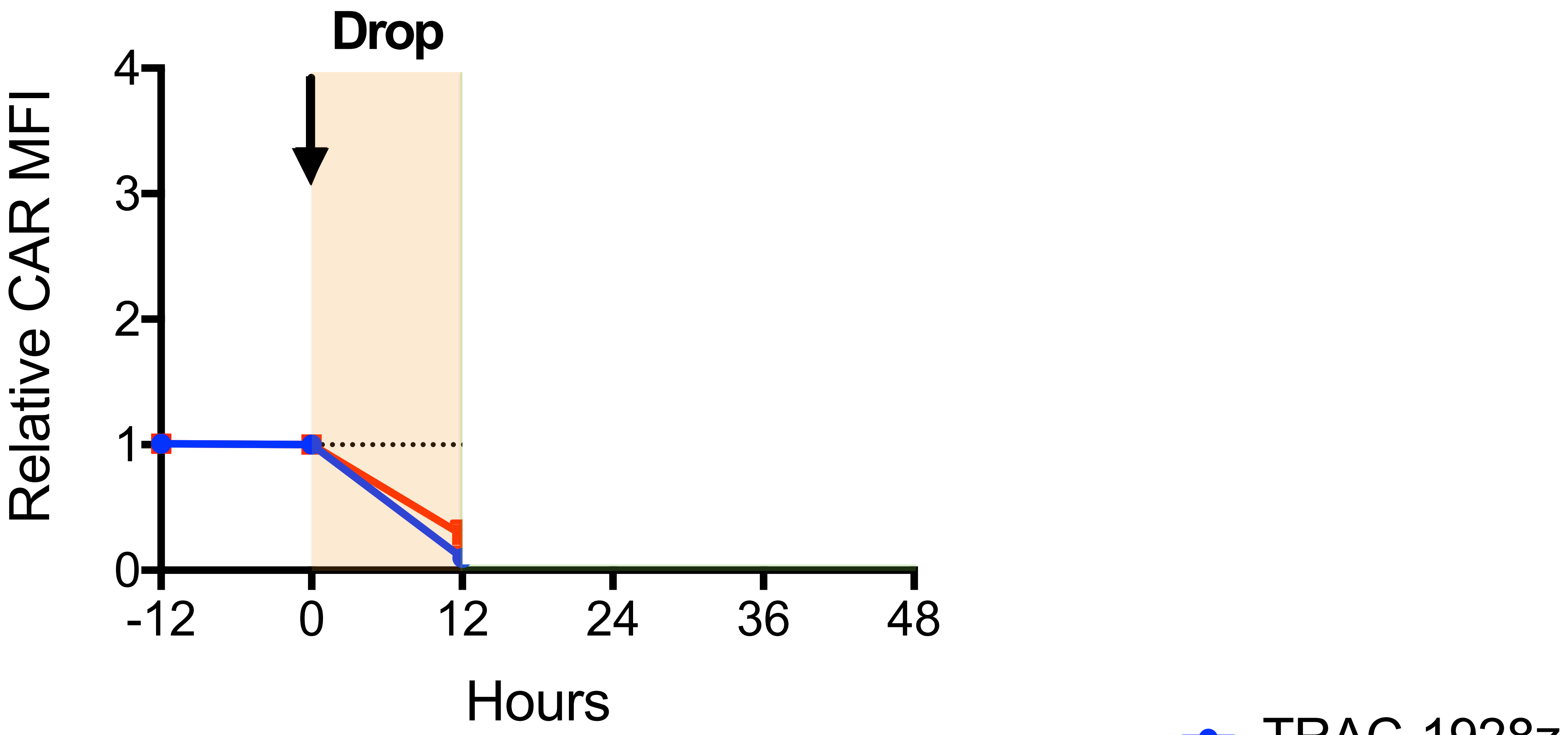


Model



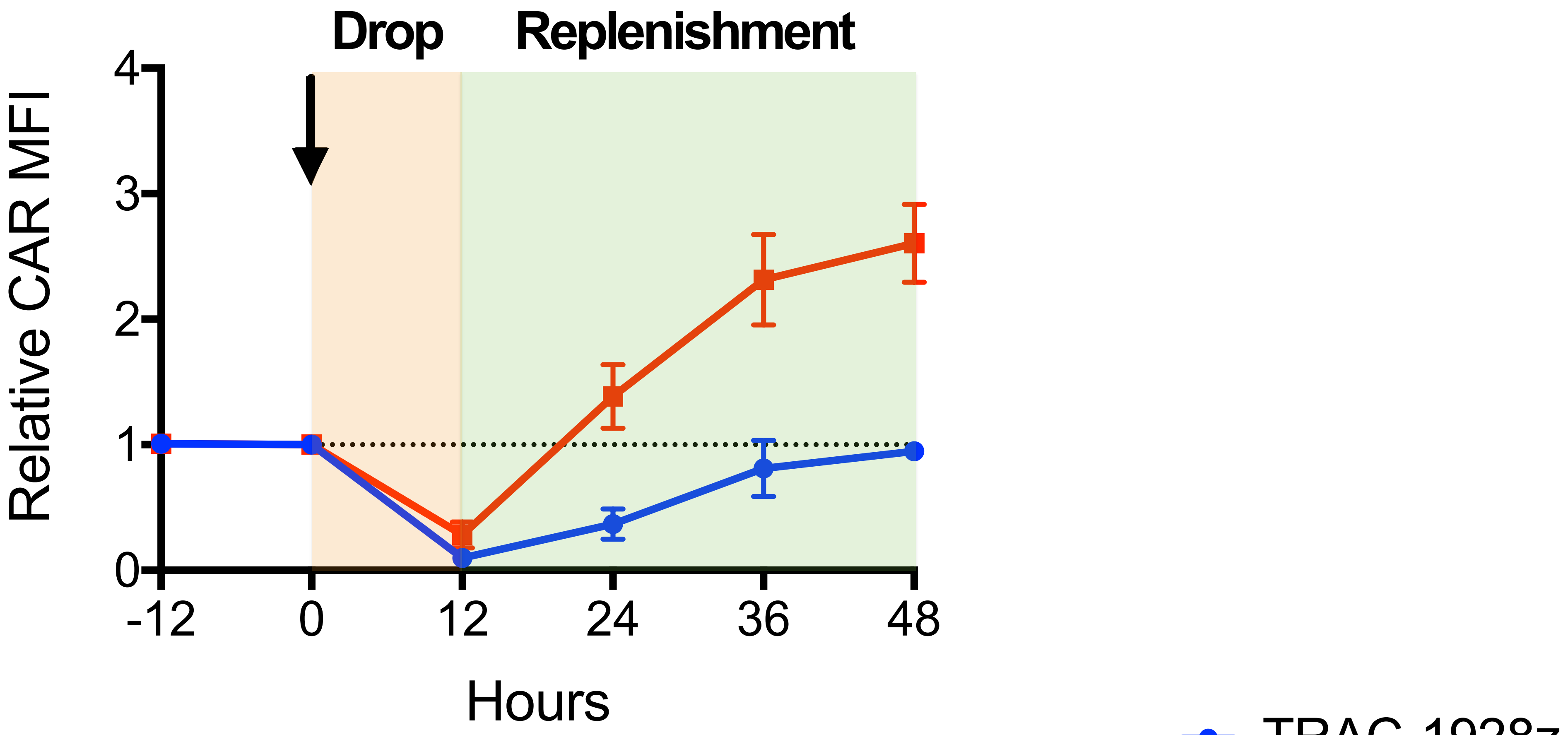
Relative CAR MFI
4
3
2
1
0
-12
0
12
24
36
48
Hours
TRAC-1928z
RV-1928z

Model



Antigen dependent

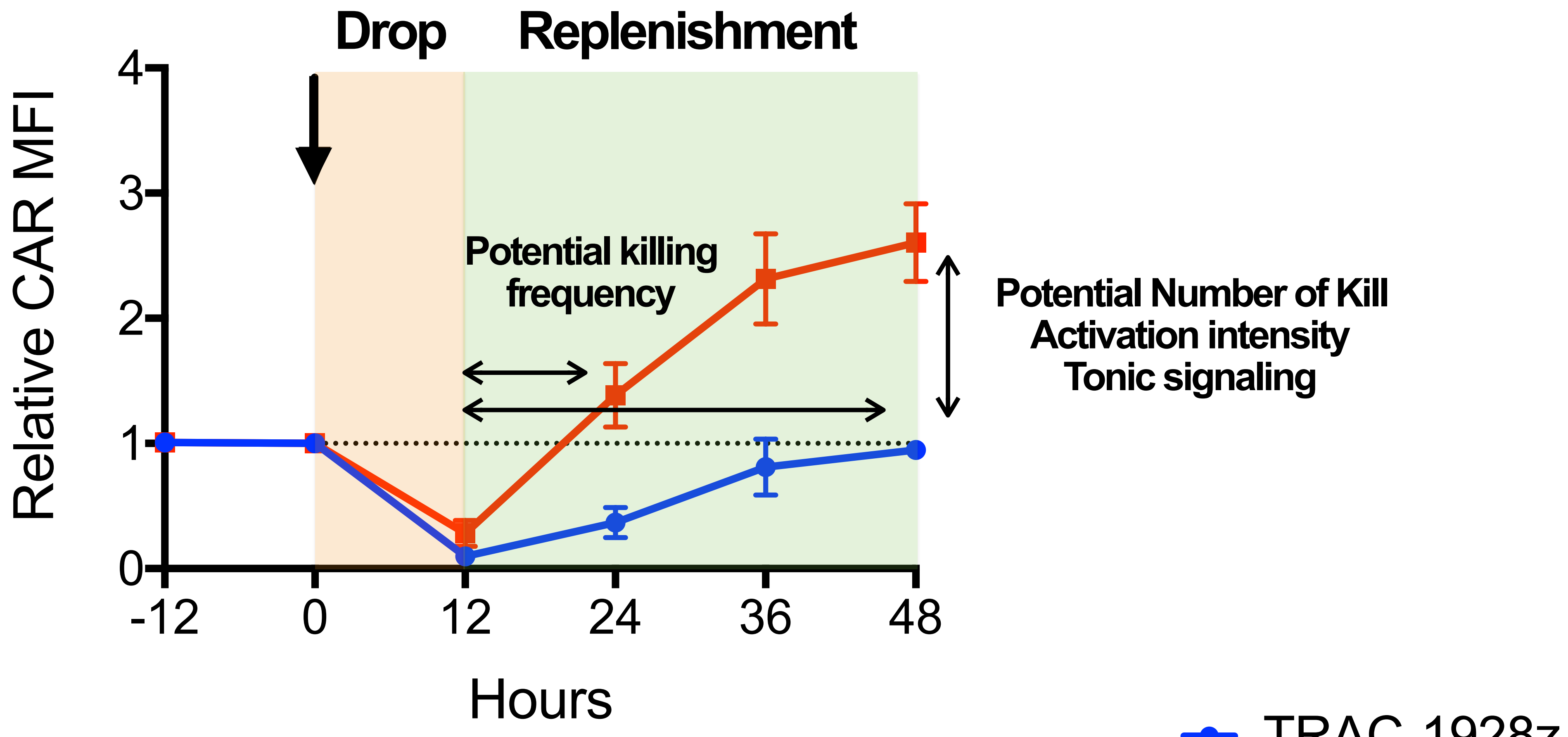
Model



Antigen dependent

Transcriptional

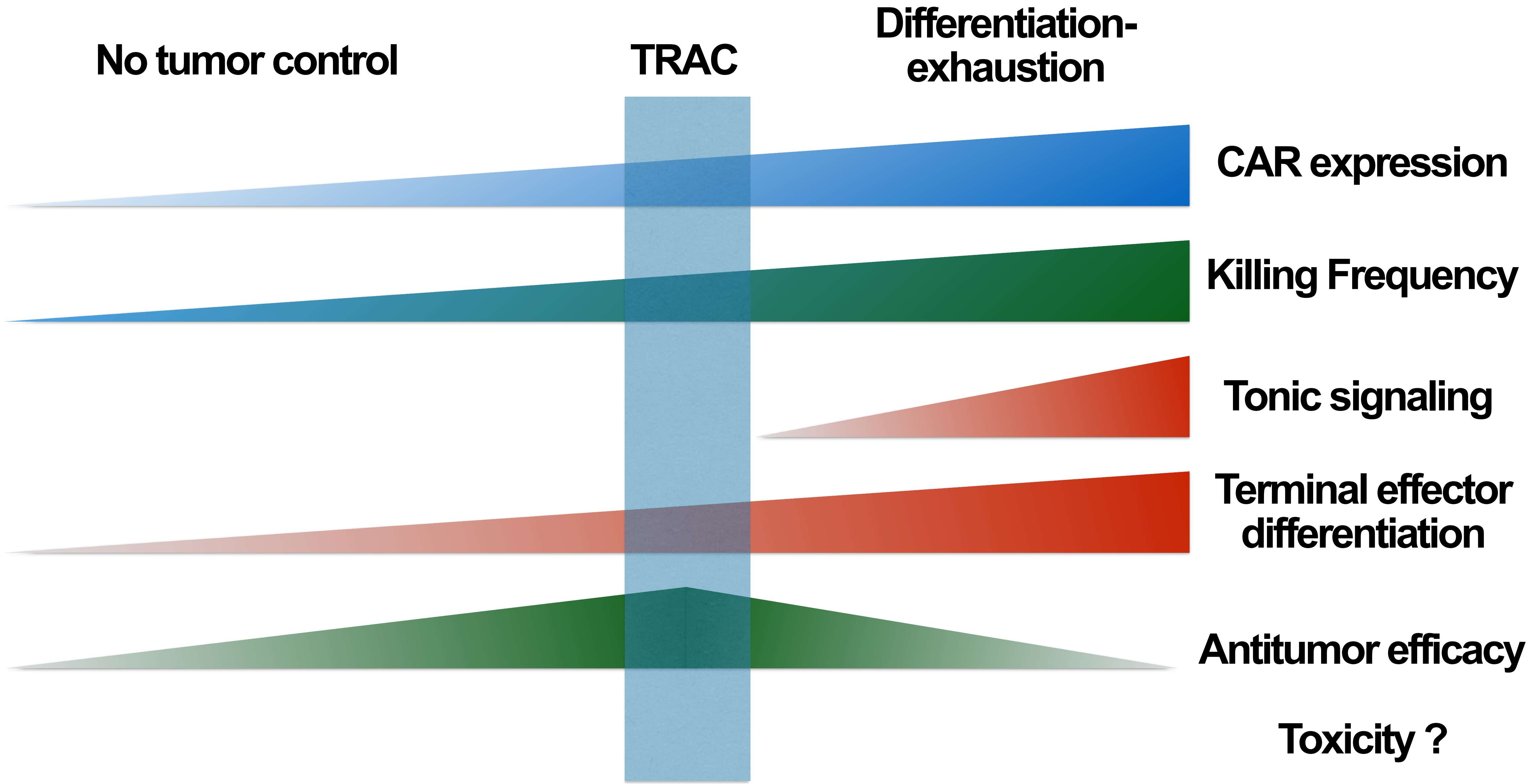
Model



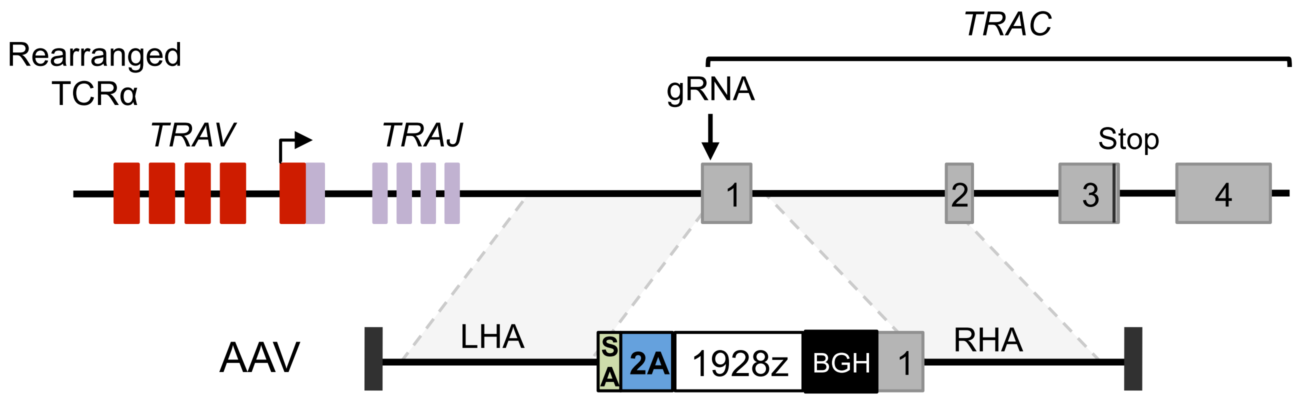
Antigen dependent

Transcriptional

Model - CAR expression / CAR T cells function

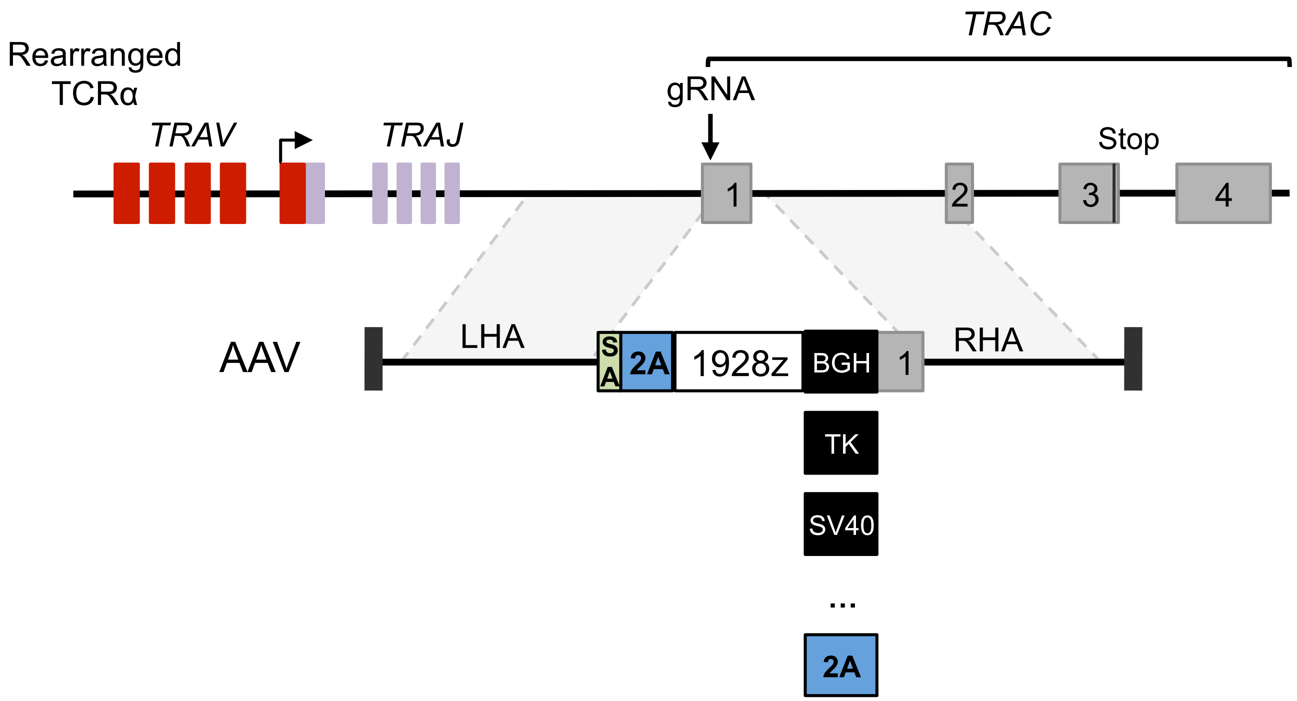


New TRAC-CAR cassettes



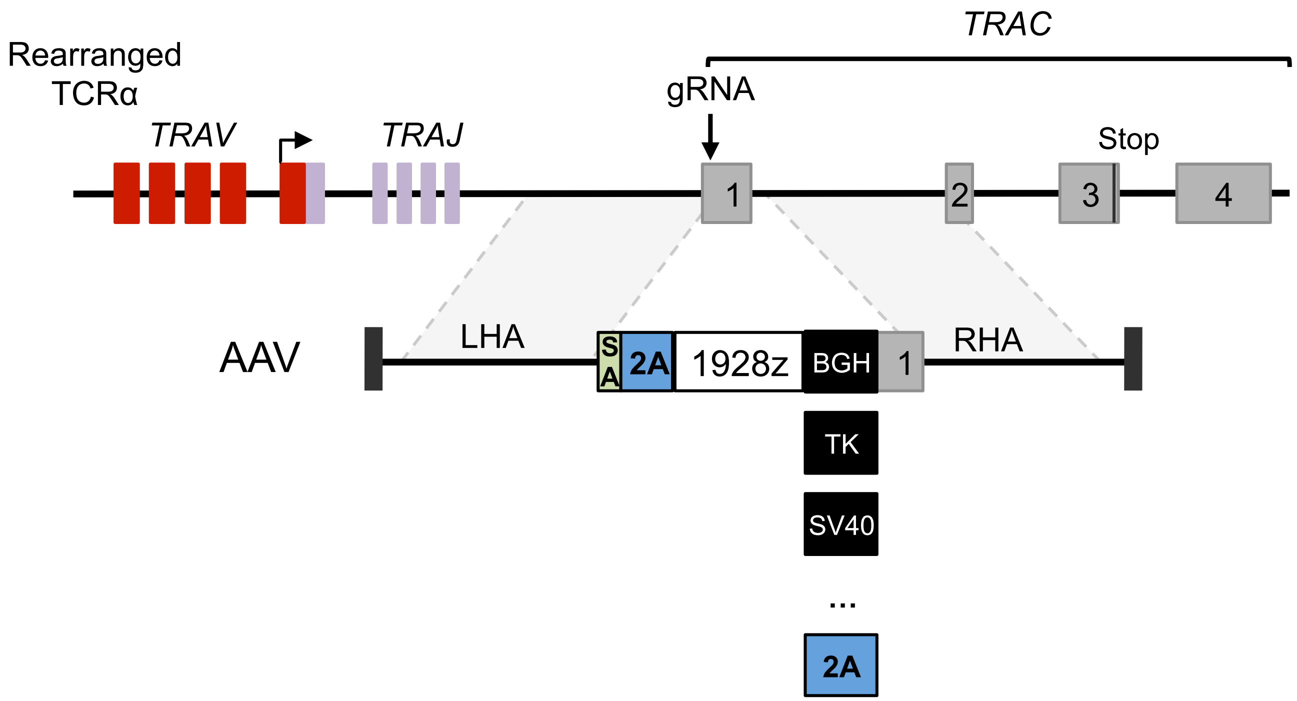
Eyquem et al., *Unpublished*

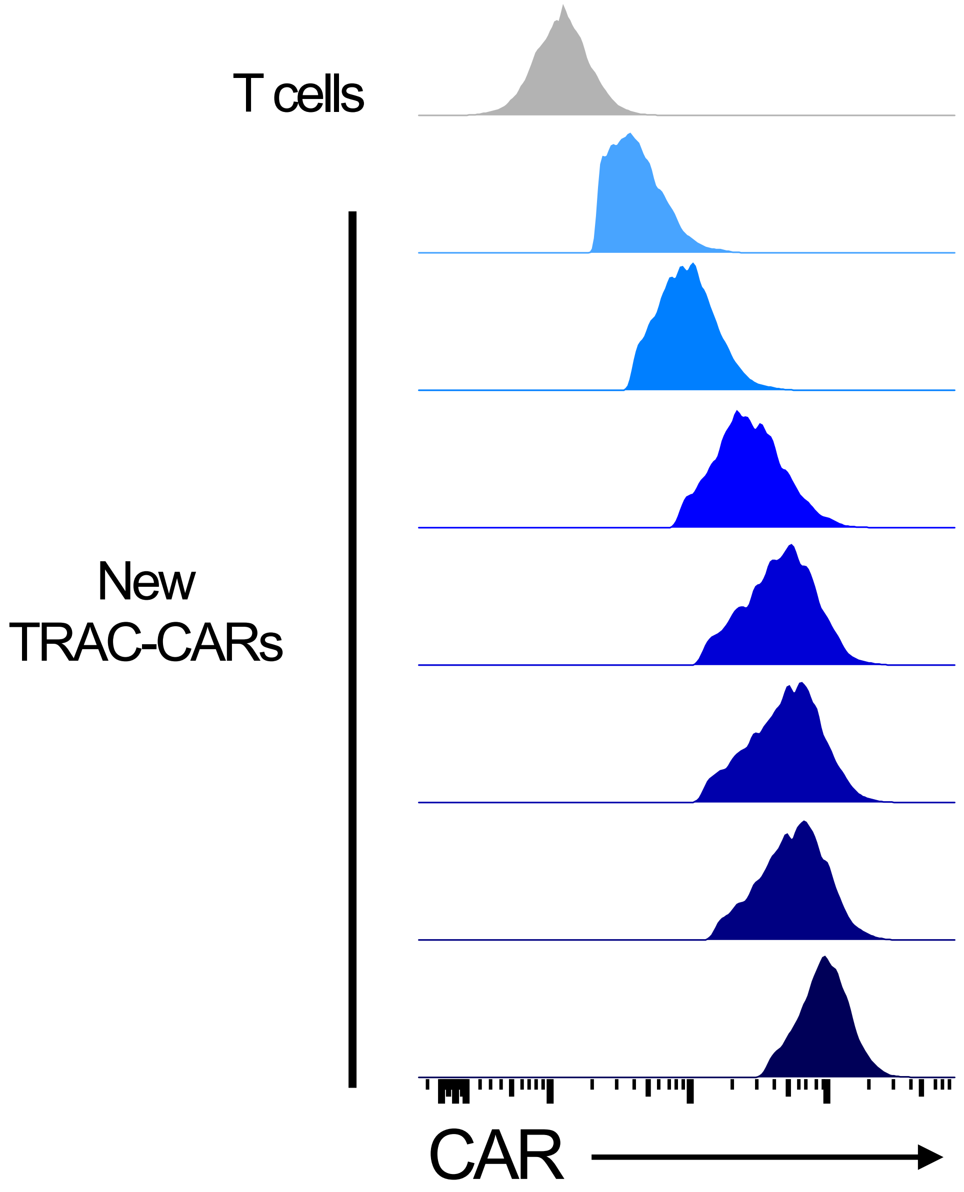
New TRAC-CAR cassettes



Eyquem et al., *Unpublished*

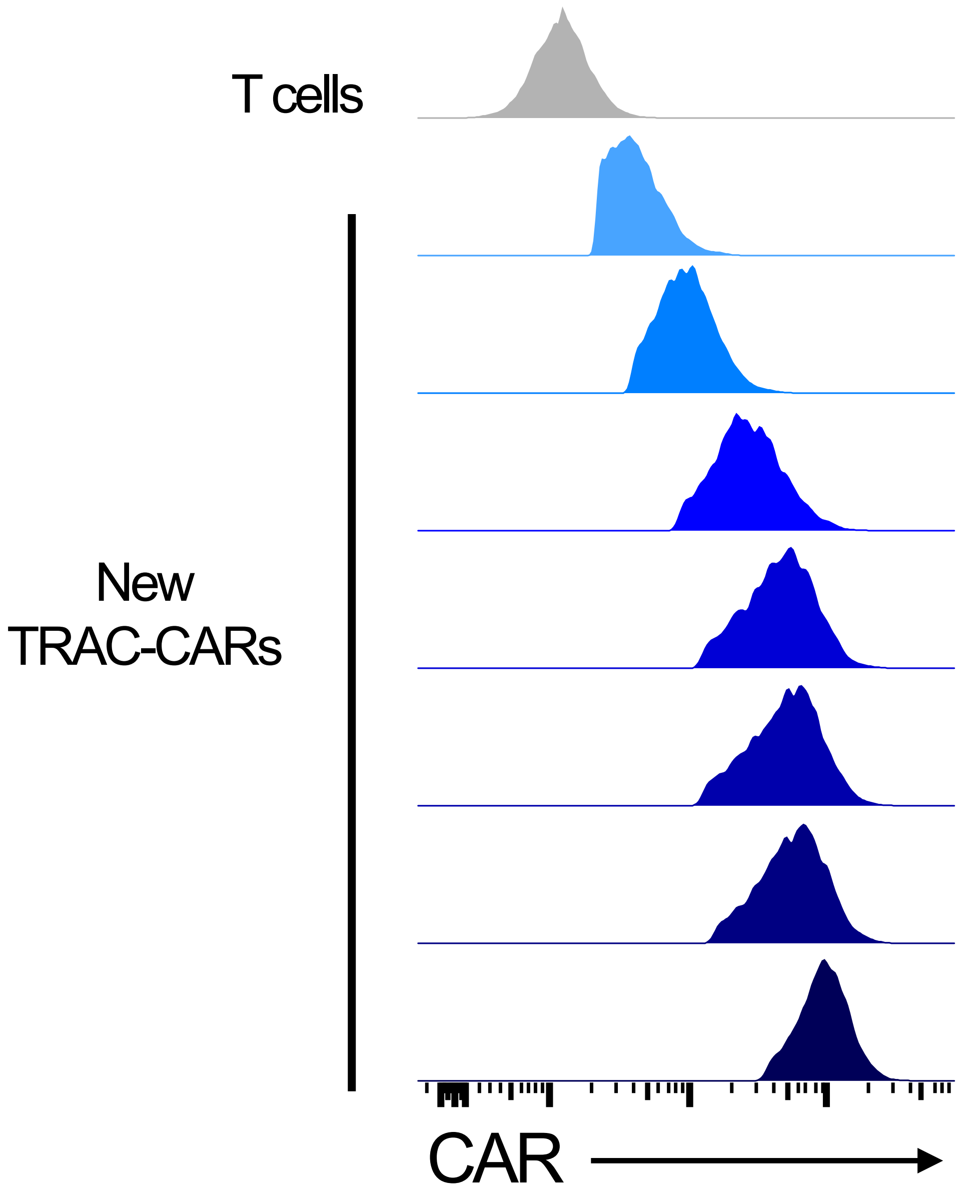
Different baseline level

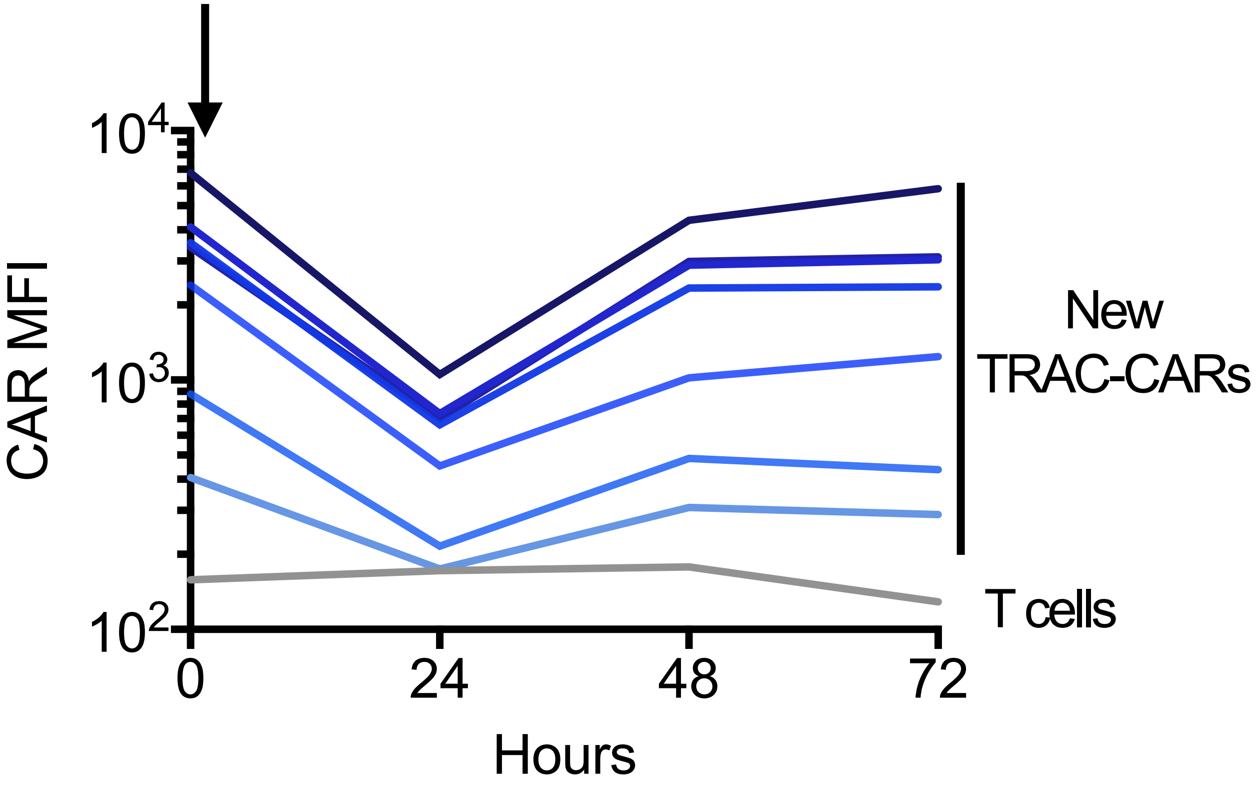




Eyquem et al., *Unpublished*

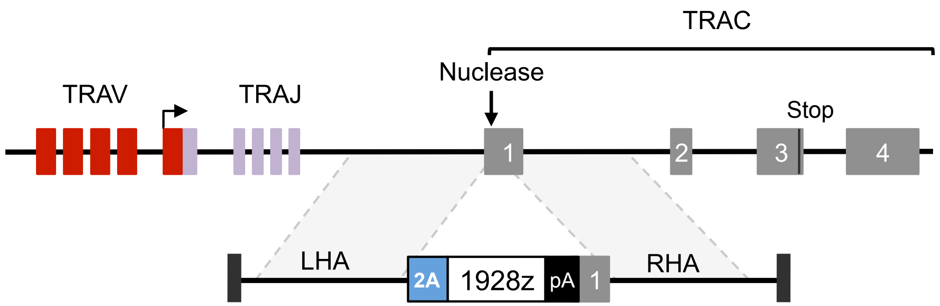
Different baseline level - similar regulation





Eyquem et al., *Unpublished*

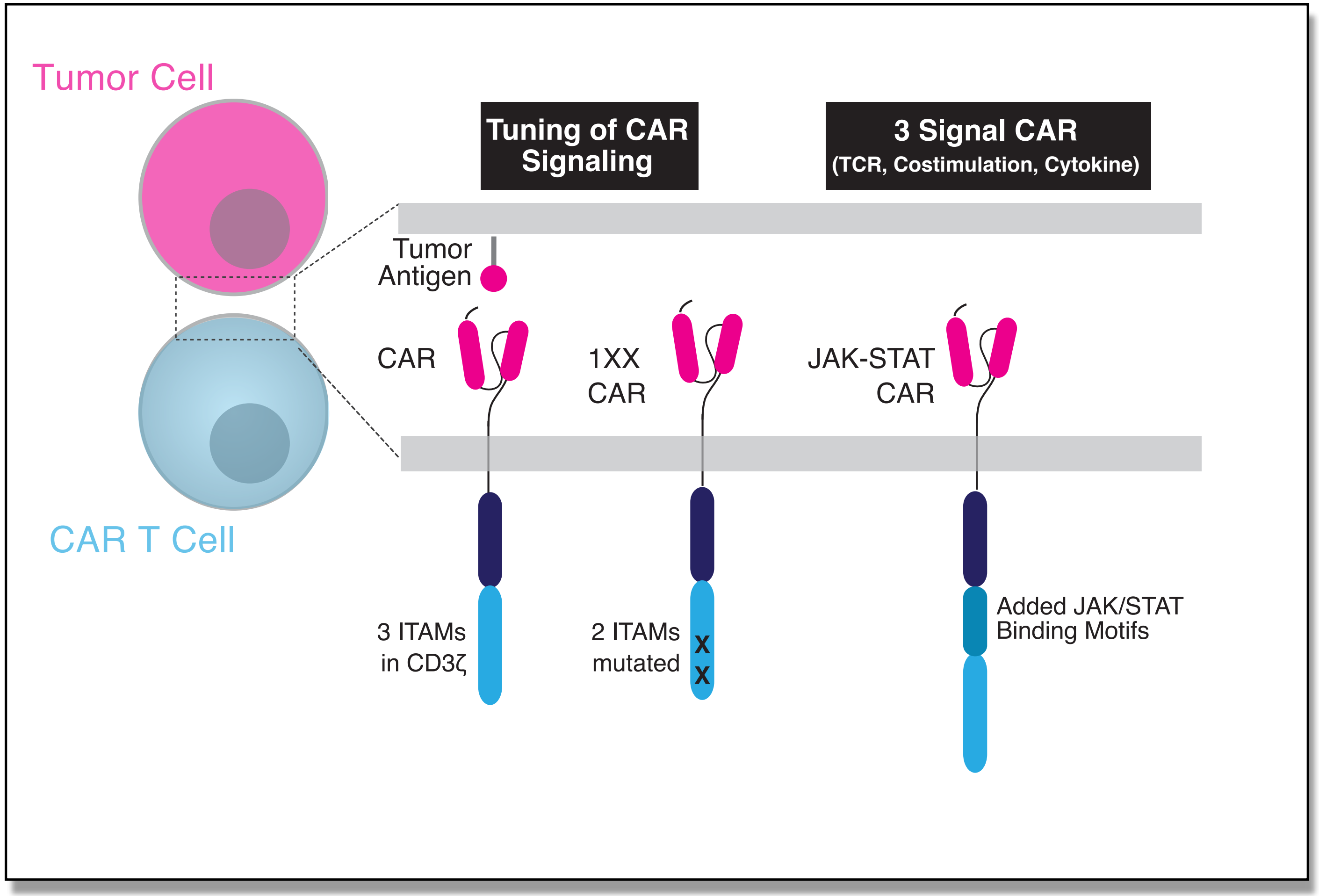
TRAC: An optimal locus for CARs and TCRs



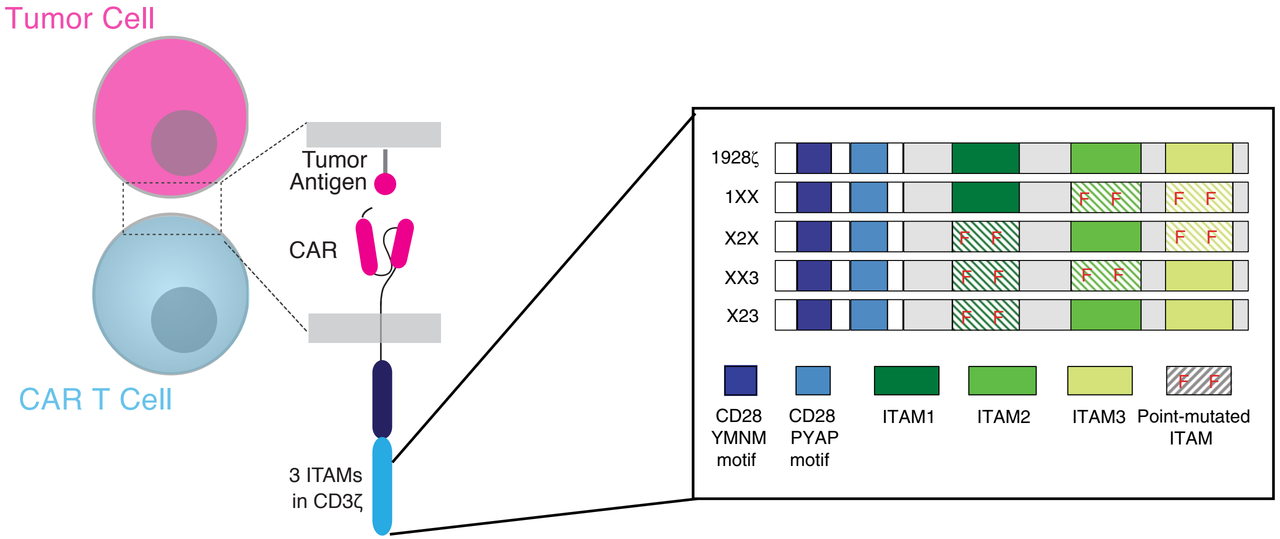
- **Safer:** targeted and promoter-less
- **Standardized:** Homogeneous, predictable expression
- **Controlled**: Improves therapeutic activity
- **Flexible:** cassette design, expression levels
- **Scalable**: Large clinical grade production on going
- **Adaptable** to every editing platform

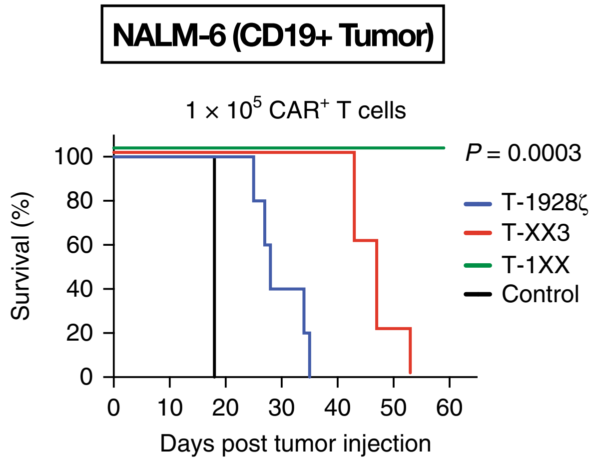
Next-generation CAR Designs

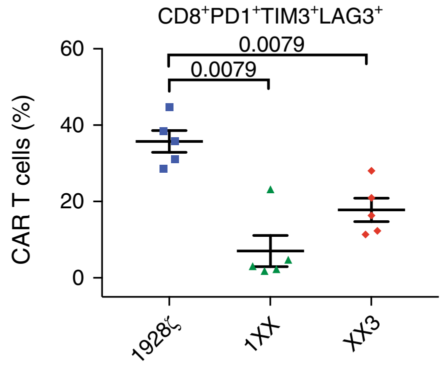
Next-Generation CARs with New Signaling Properties



Tuning CAR Signaling Through Signaling Motif Mutagenesis

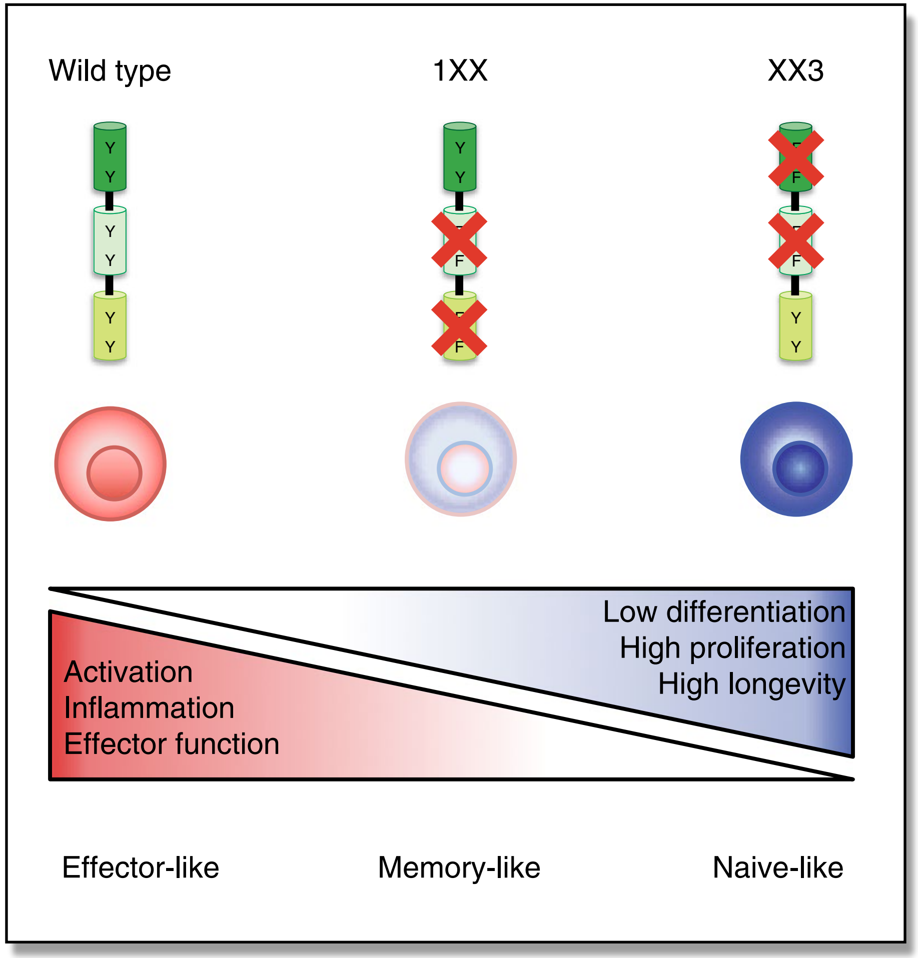




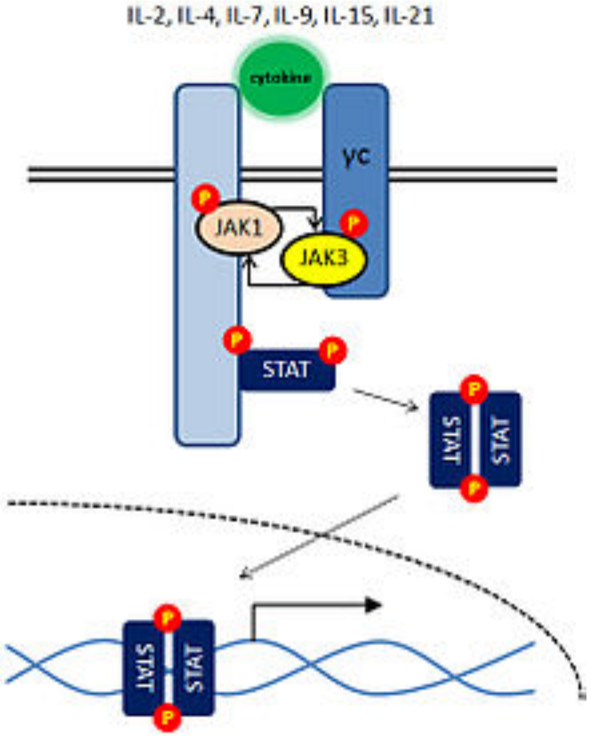


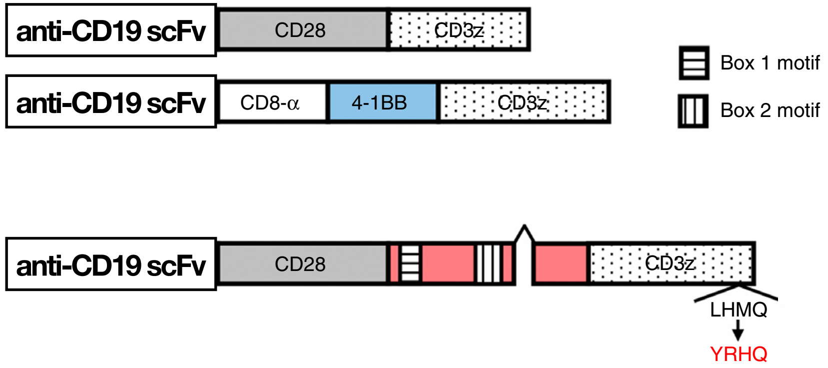
Feucht J et al. Nat Med. 2019

Balancing CAR Signaling Improve Therapeutic Efficacy



Adding New Signaling Capabilities to CARs

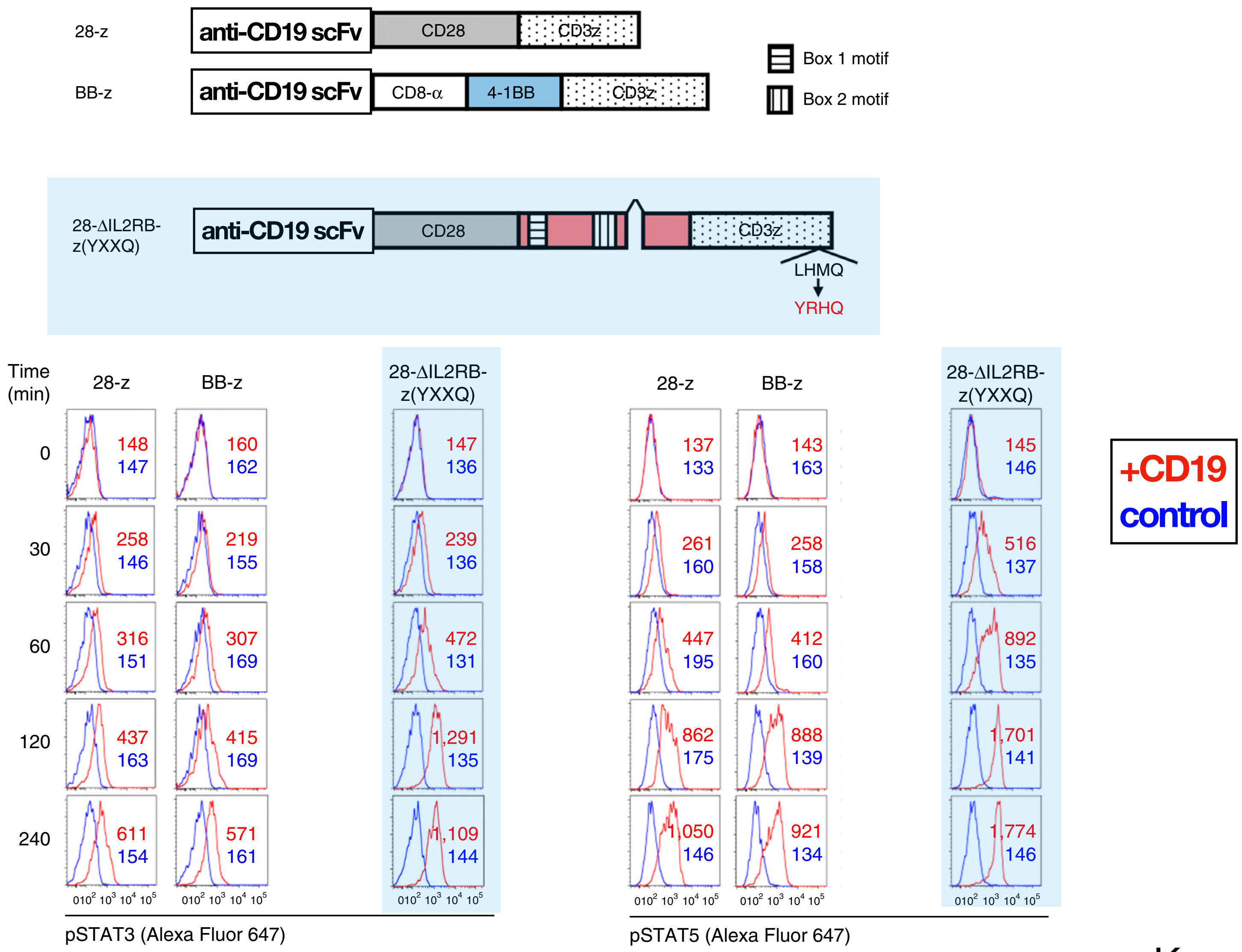




Enhanced proliferation and efficacy?

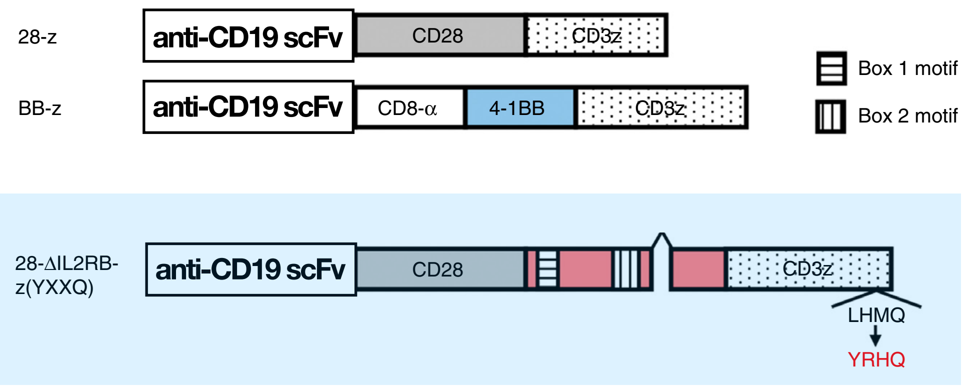
Kagoya Y et al. Nat Med. 2018

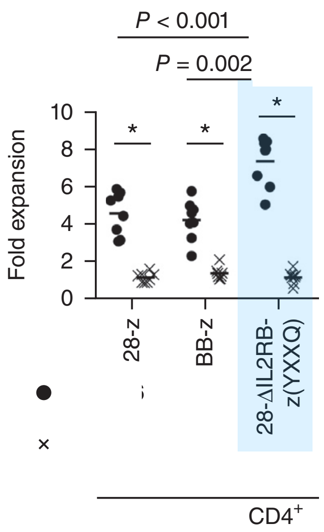
Adding New Signaling Capabilities to CARs

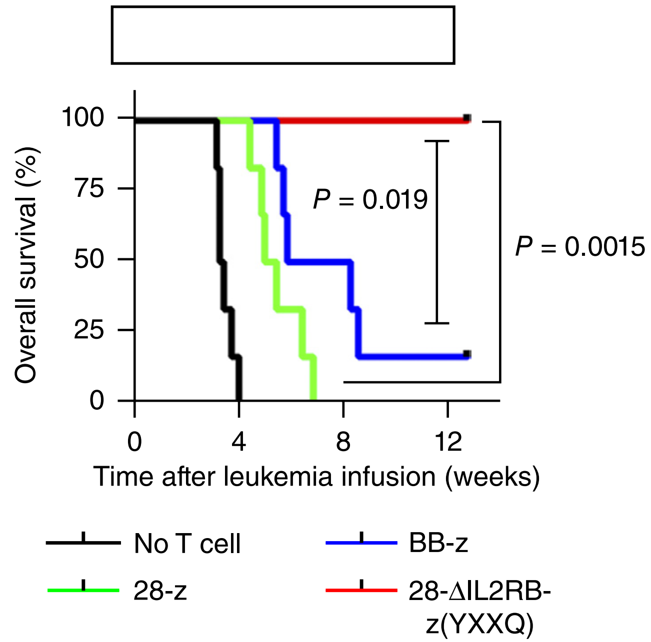


Kagoya Y et al. Nat Med. 2018

Adding New Signaling Capabilities to CARs







Kagoya Y et al. Nat Med. 2018

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

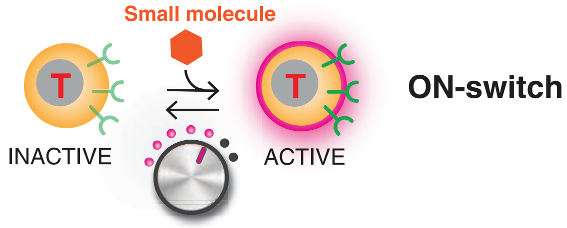
Logic Gating
- Multi-receptor systems
  - AND logic CARs
  - CAR/inhibitory CARs
  - synNotch/CAR circuits

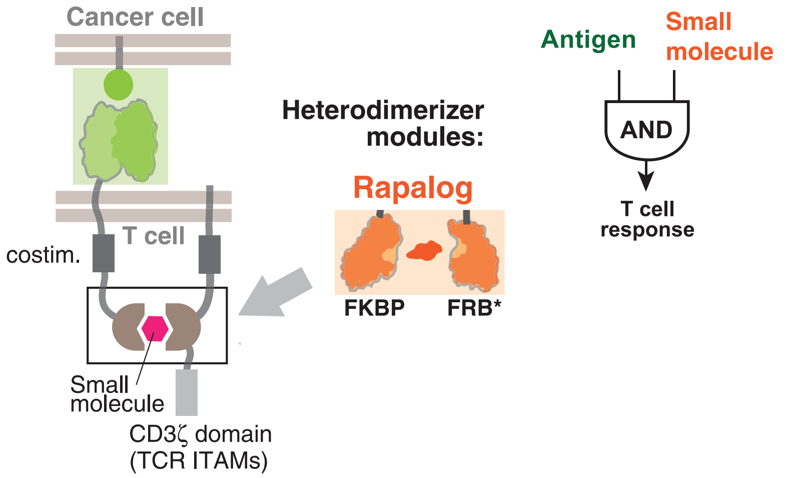
Enhancing & Sculpting T cell Activity
- cytokine/chemokine production
- customization of responses

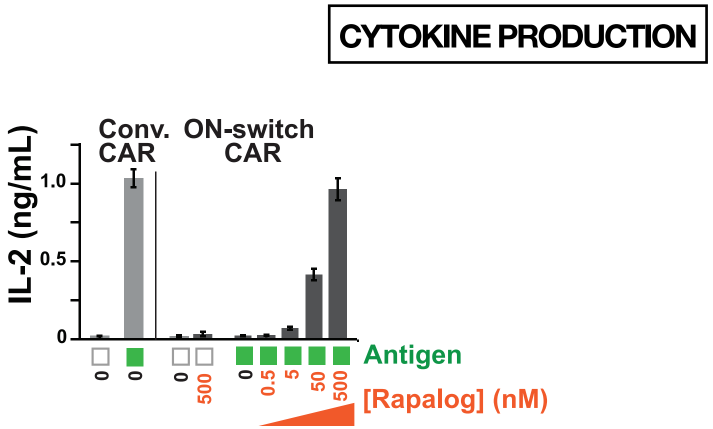
Drug Controlled CAR Activation

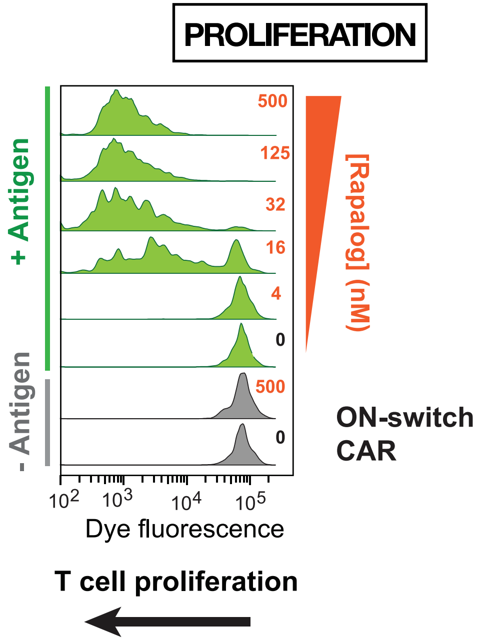
Remote Control of Adoptive T cell Therapies

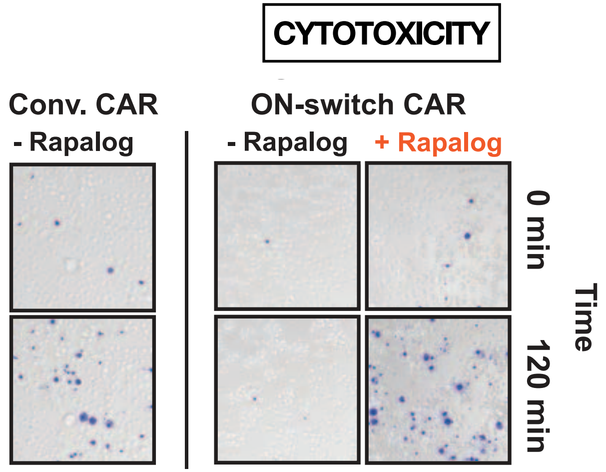
Conditional user-controlled switches





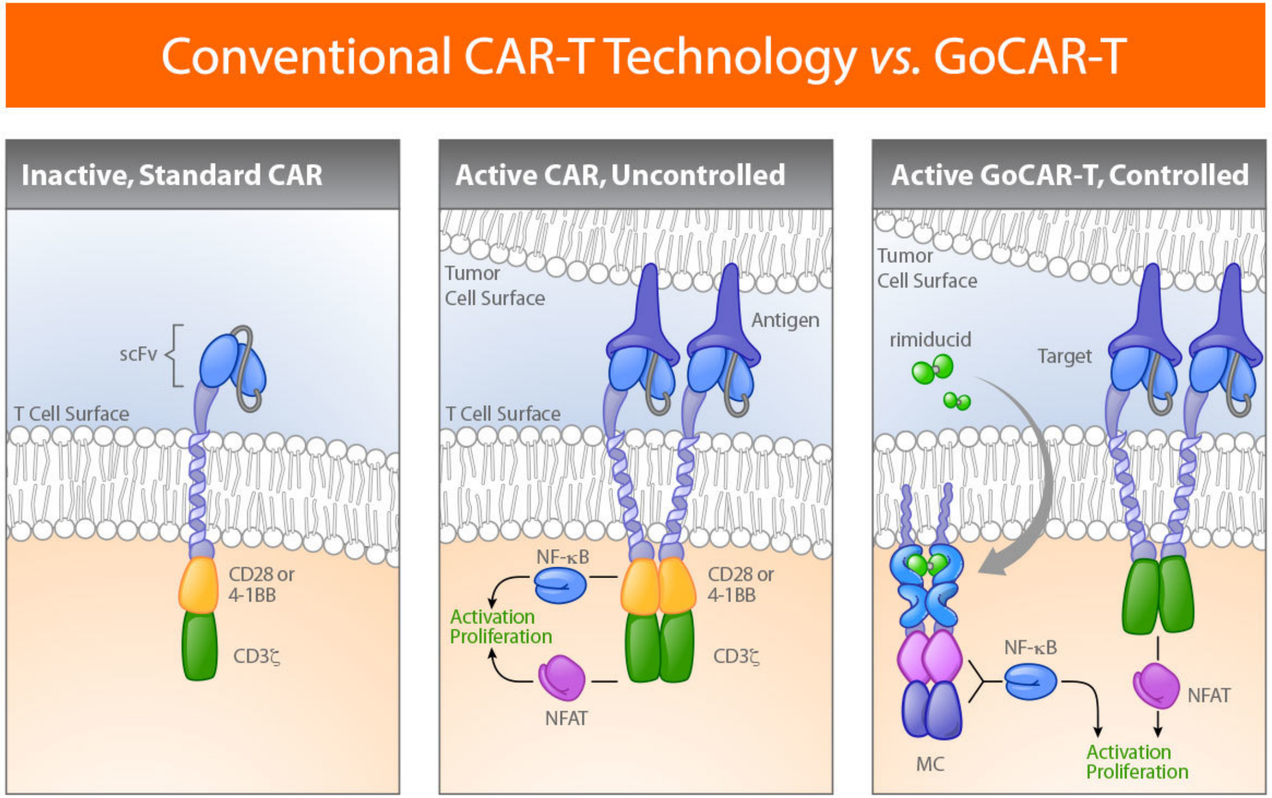


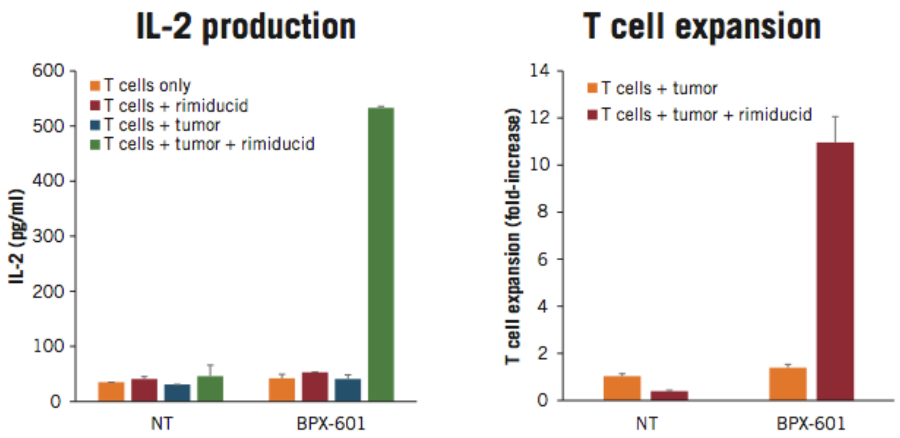


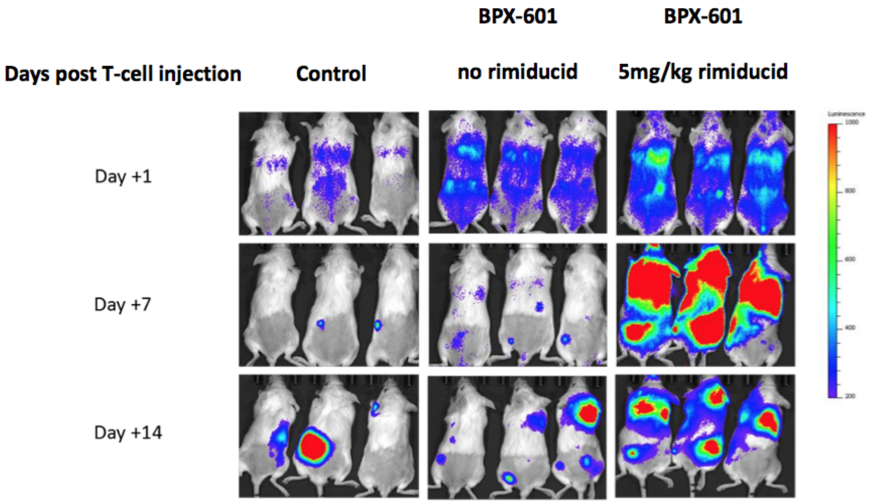


Wu C et al. *Science* 2015

Drug Controlled Costimulation
An Approach to Titrate Engineered T cell Effector Function

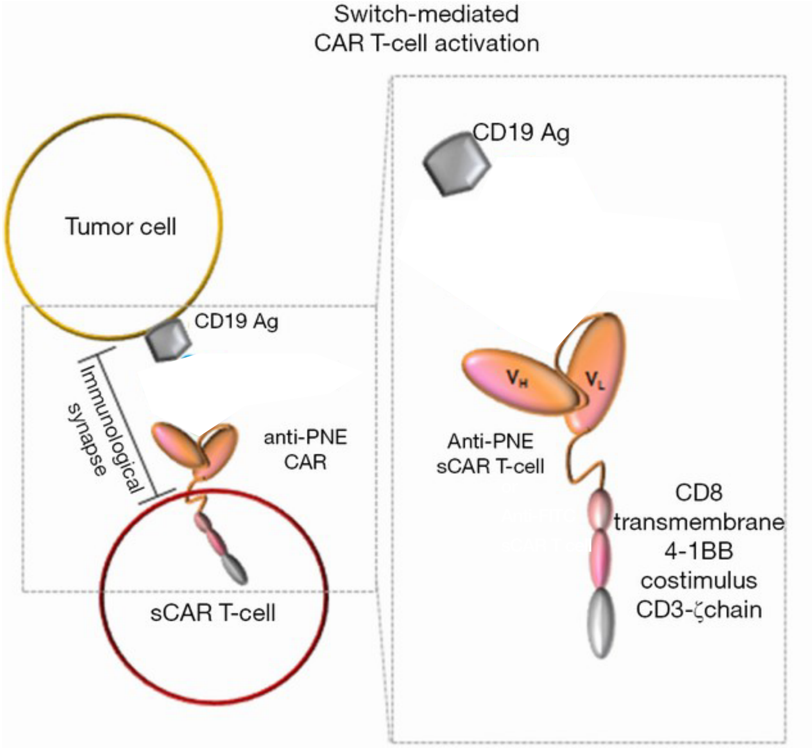


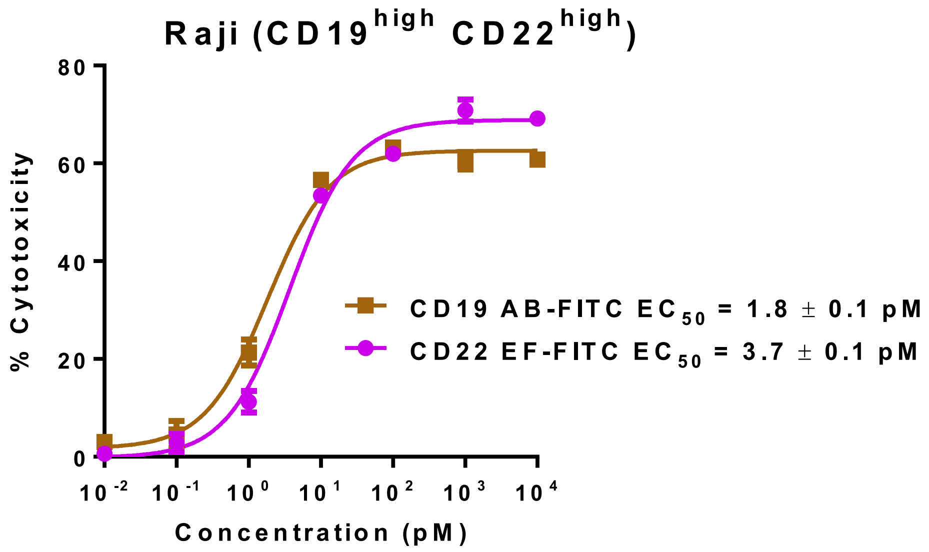




http://www.bellicum.com/technology/gocart/

Universal CAR T cells
Changing Antigen Specificity During Treatment





Ma JSY. PNAS. 2016

Universal CAR T cells
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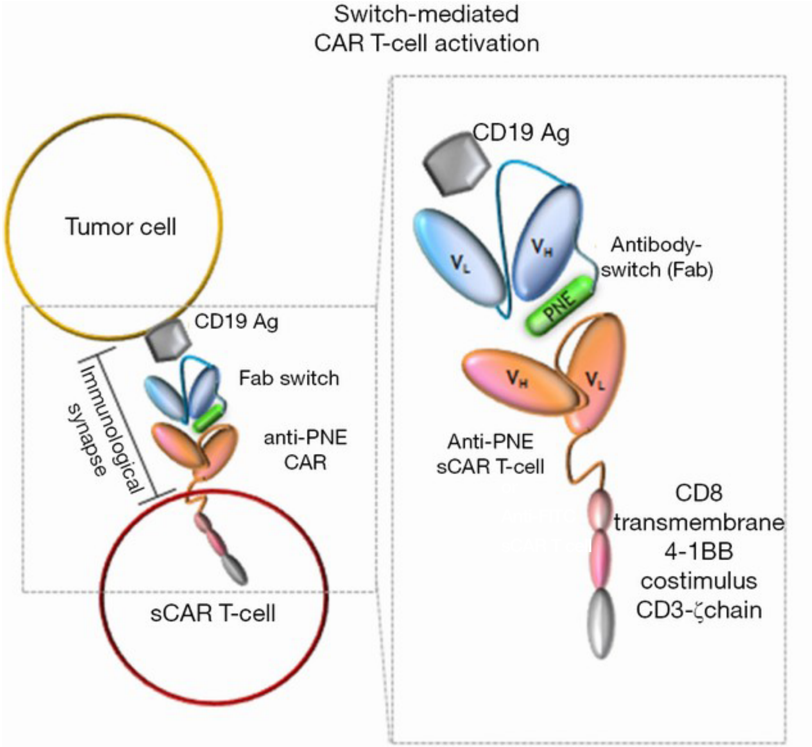
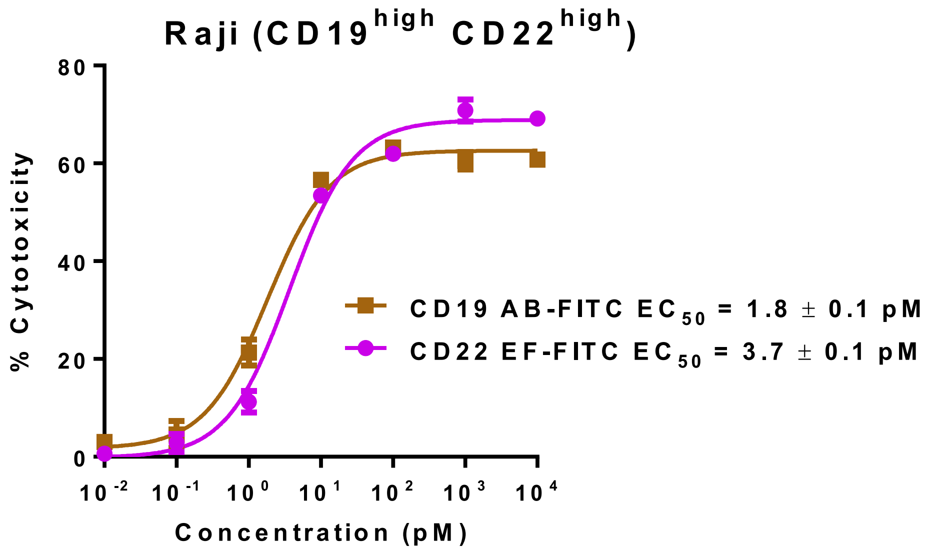
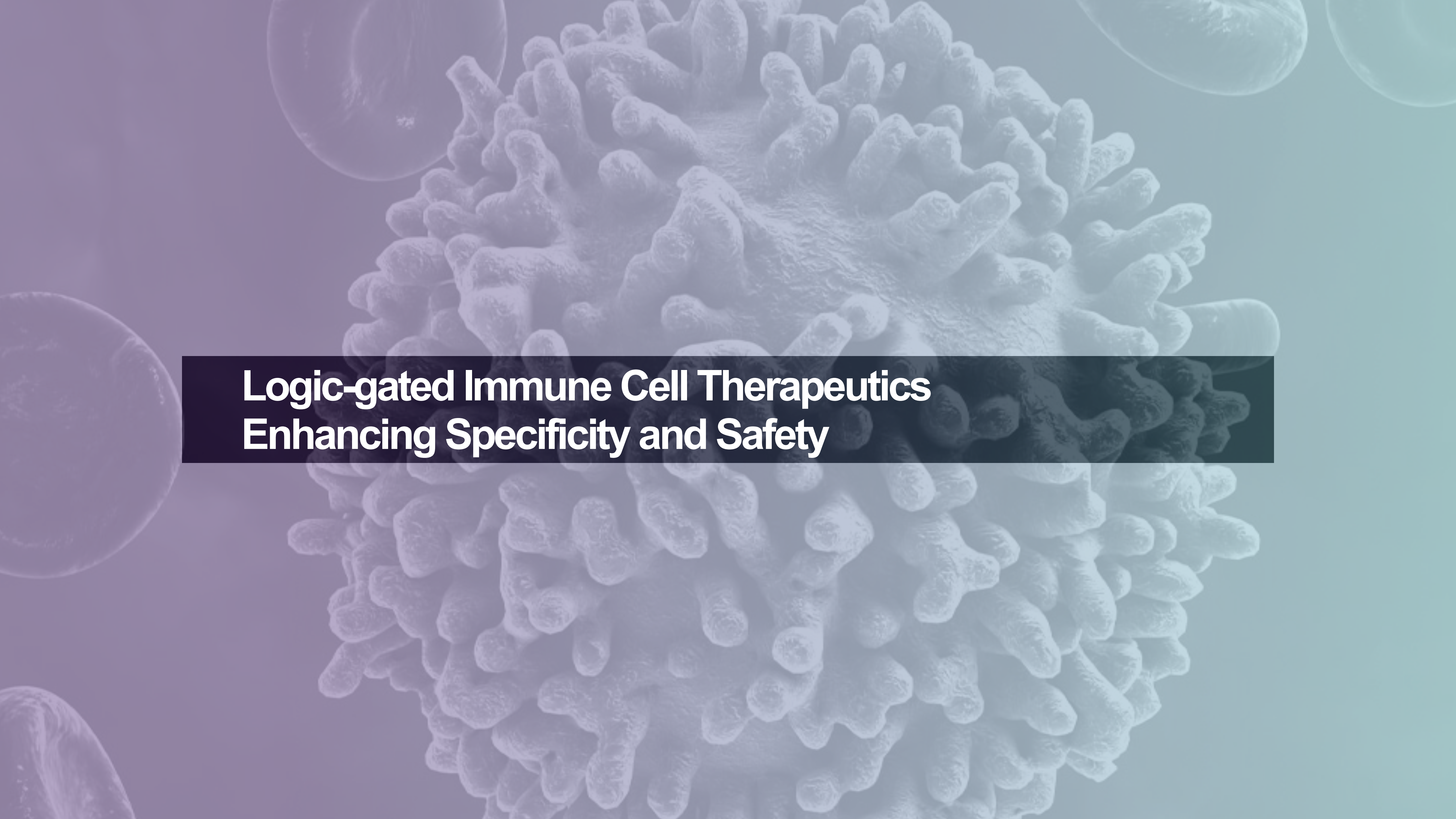


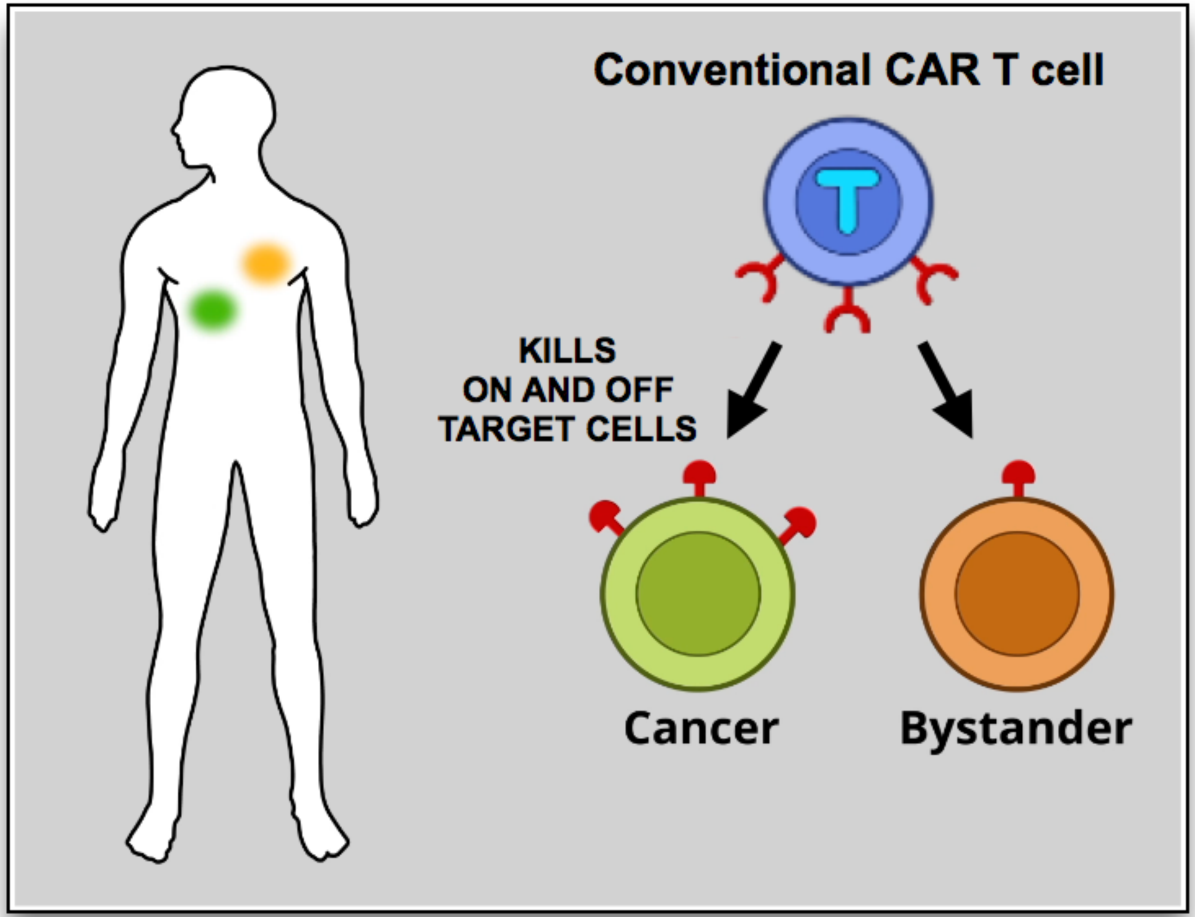


Ma JSY. PNAS. 2016

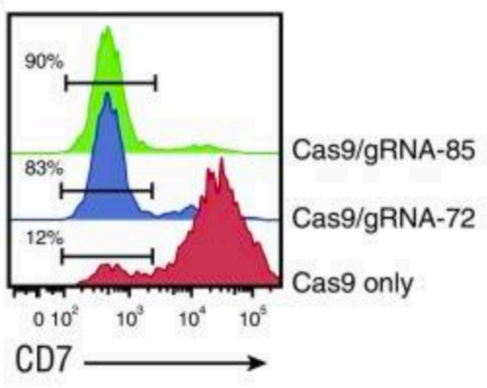


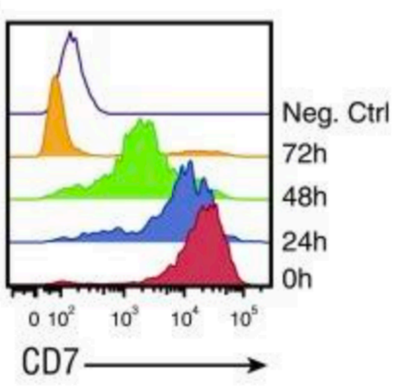
Logic-gated Immune Cell Therapeutics Enhancing Specificity and Safety

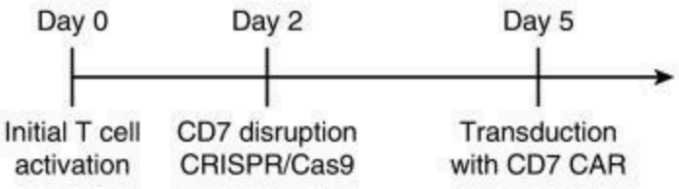
Redirecting the Specificity of T cells to Cancer
The Pitfalls of Single Antigen Targeting

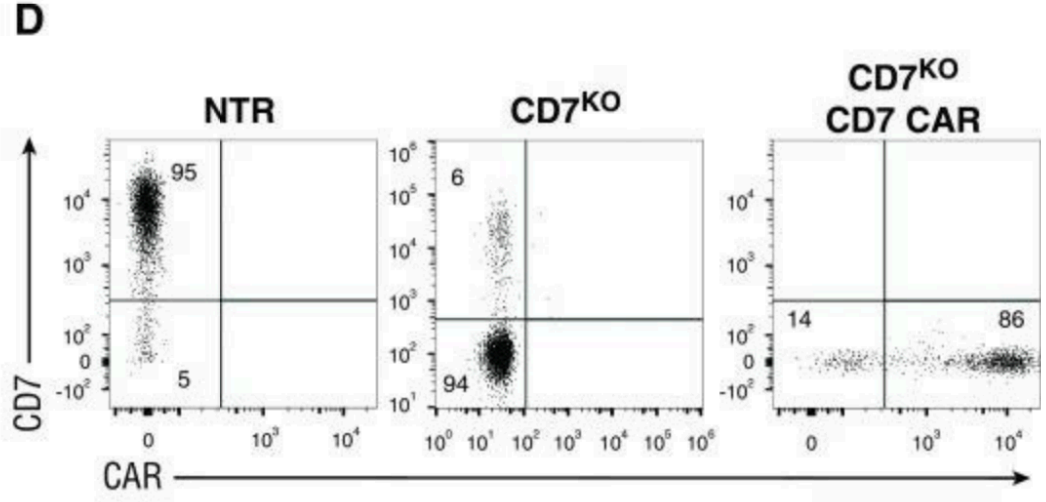


Limiting Fraticide Killing
Targeting T cell leukemia





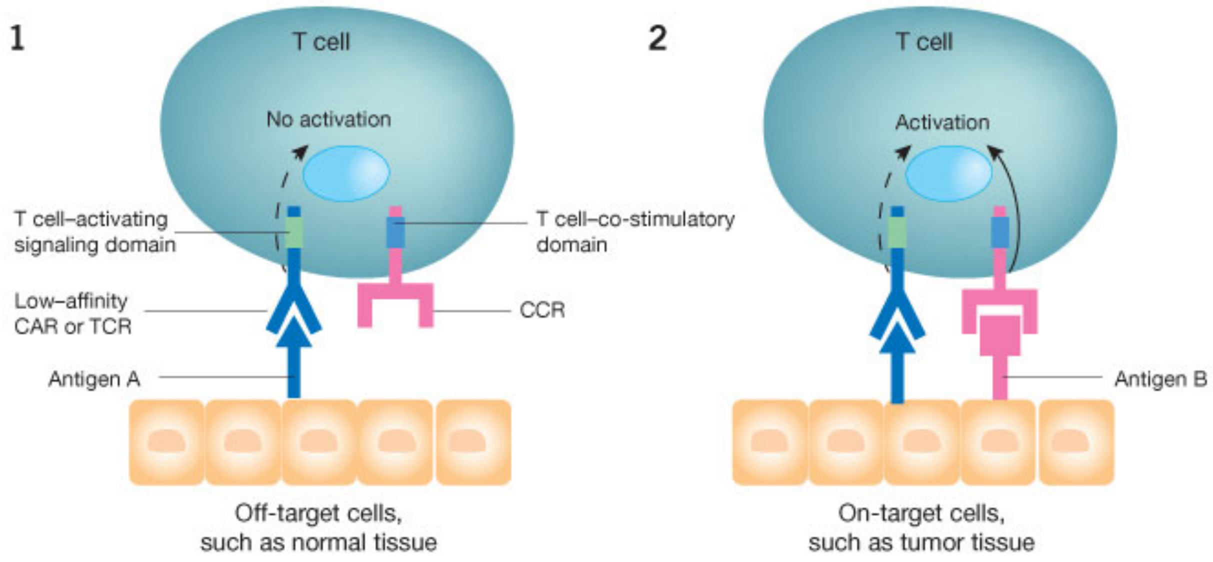


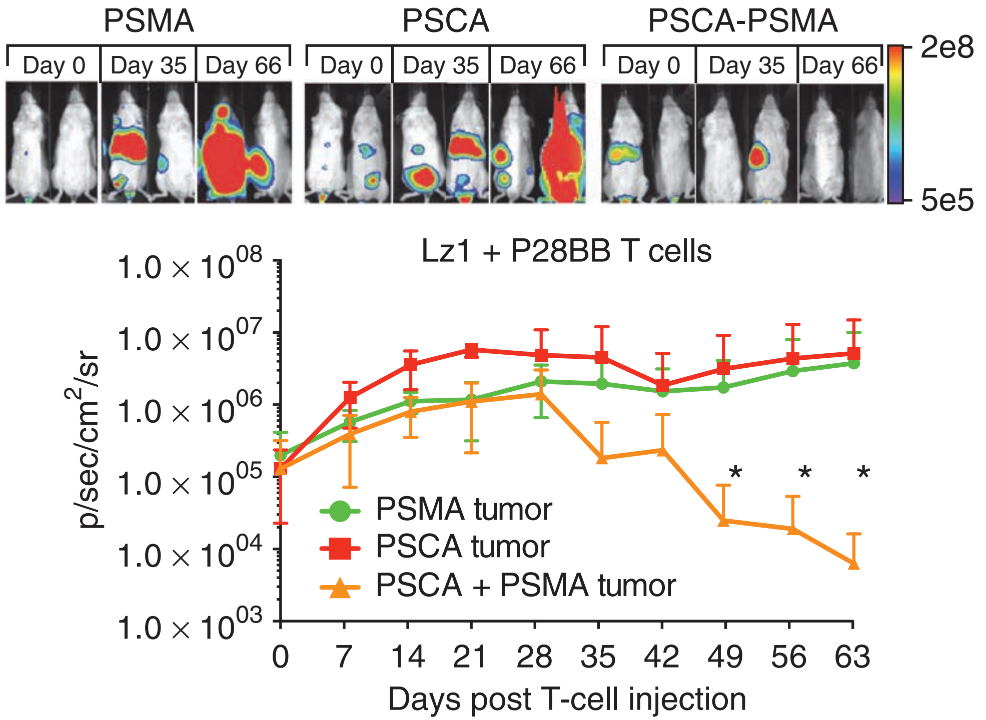


Gomez-Silva et. al Blood 2017

AND Gate CAR T cells

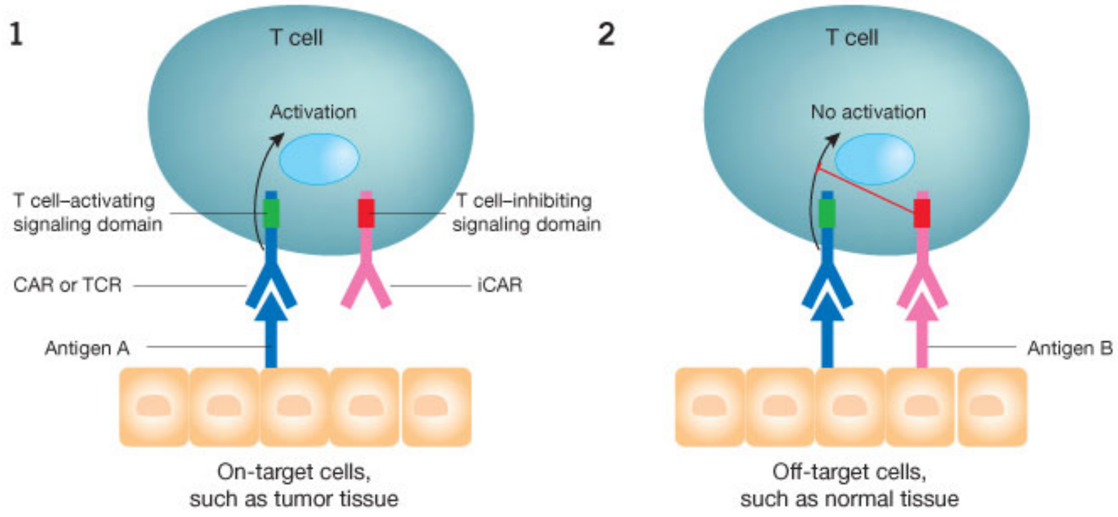
Separating Signal 1 (TCR) and Signal 2 (Costimulation)

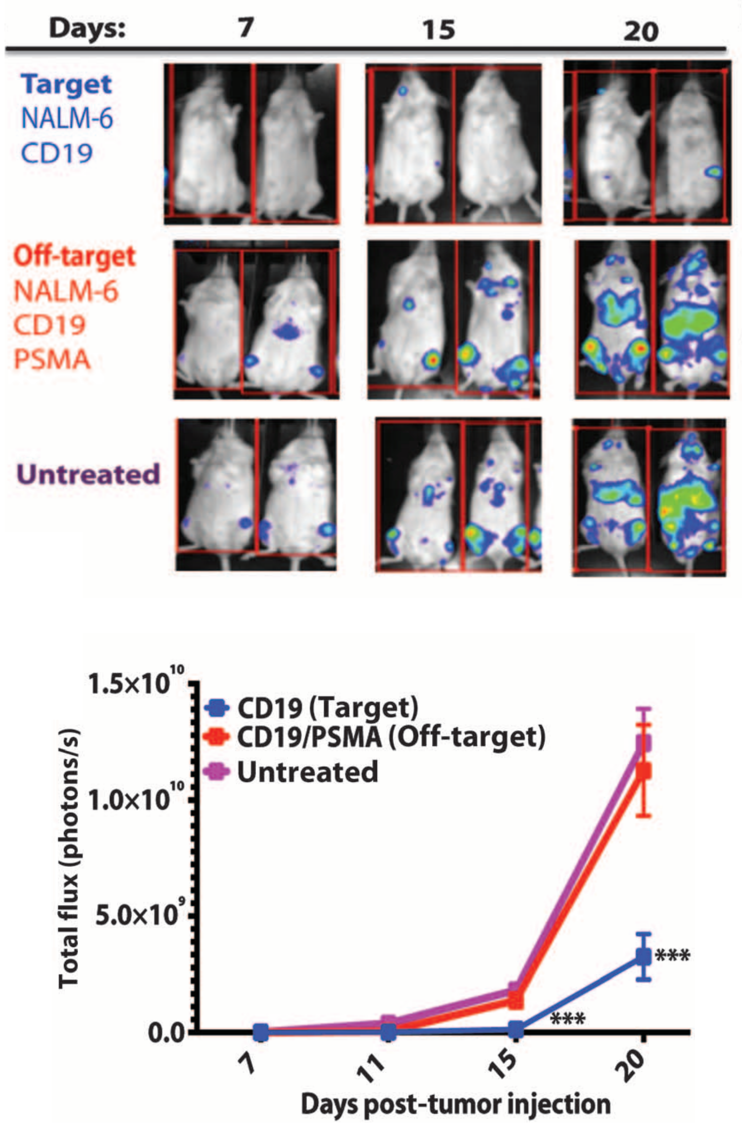




Kloss CC. Nat Biotech. 2013

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



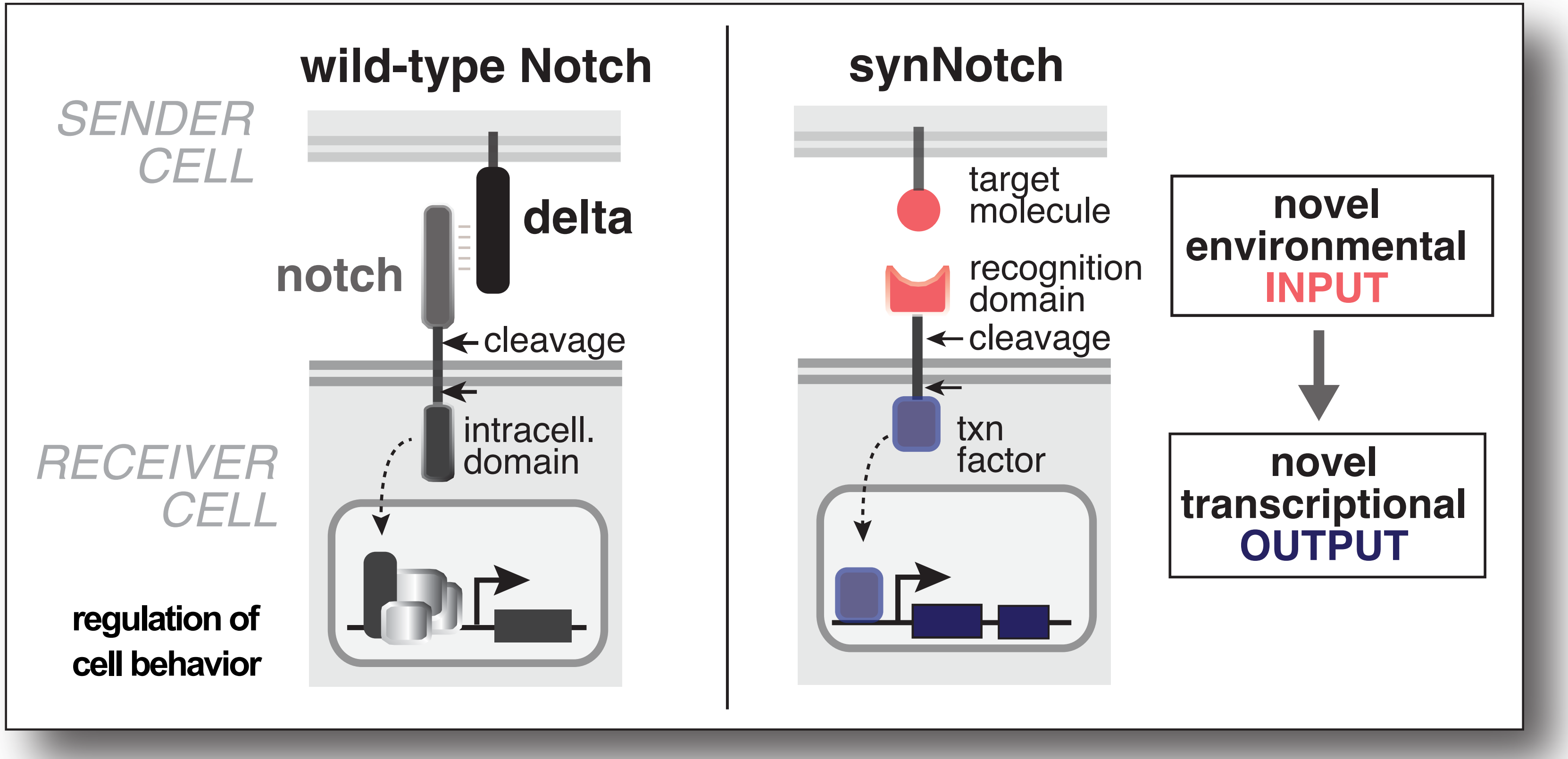


iCARS
use ICDs from T cell inhibitory receptors (e.g. CTLA4 and PD1)

Fedorov VD. Sci Trans Med. 2013

The Notch Receptor

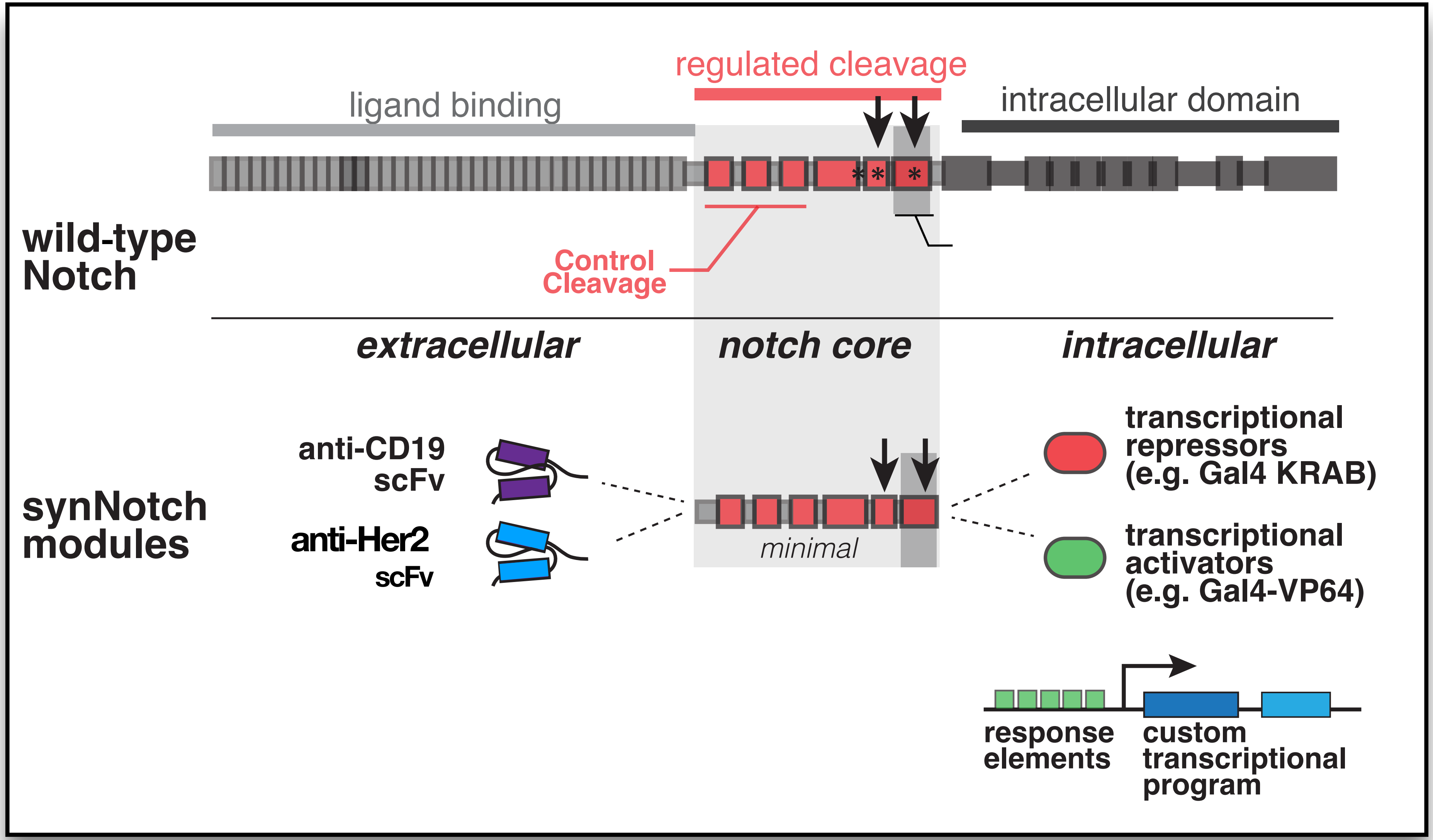
A Natural Environmental Sensor that Regulates Cells Through DIRECT Transcriptional Regulation



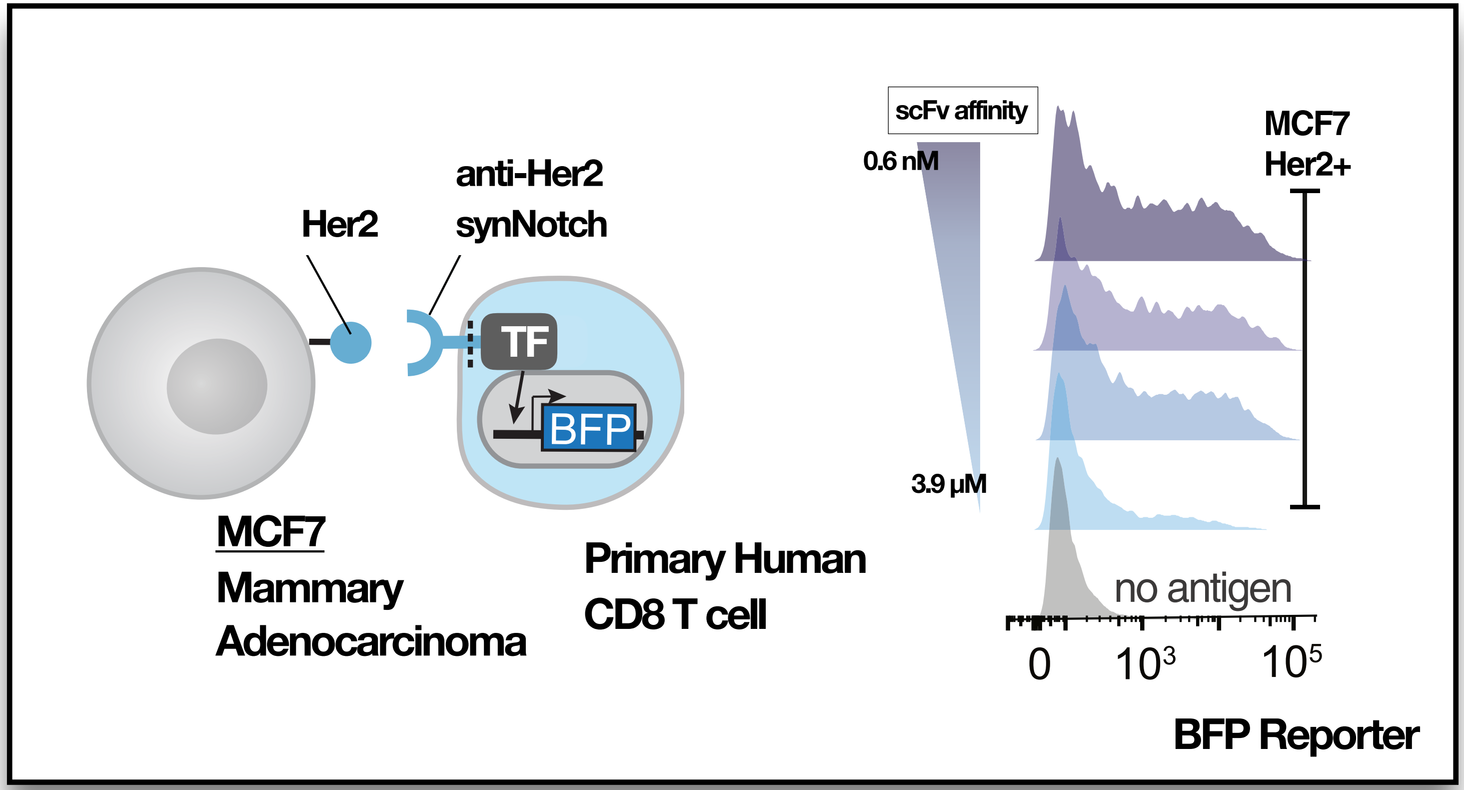
Roybal and Morsut et al. Cell. 2016

Synthetic Notch Receptors

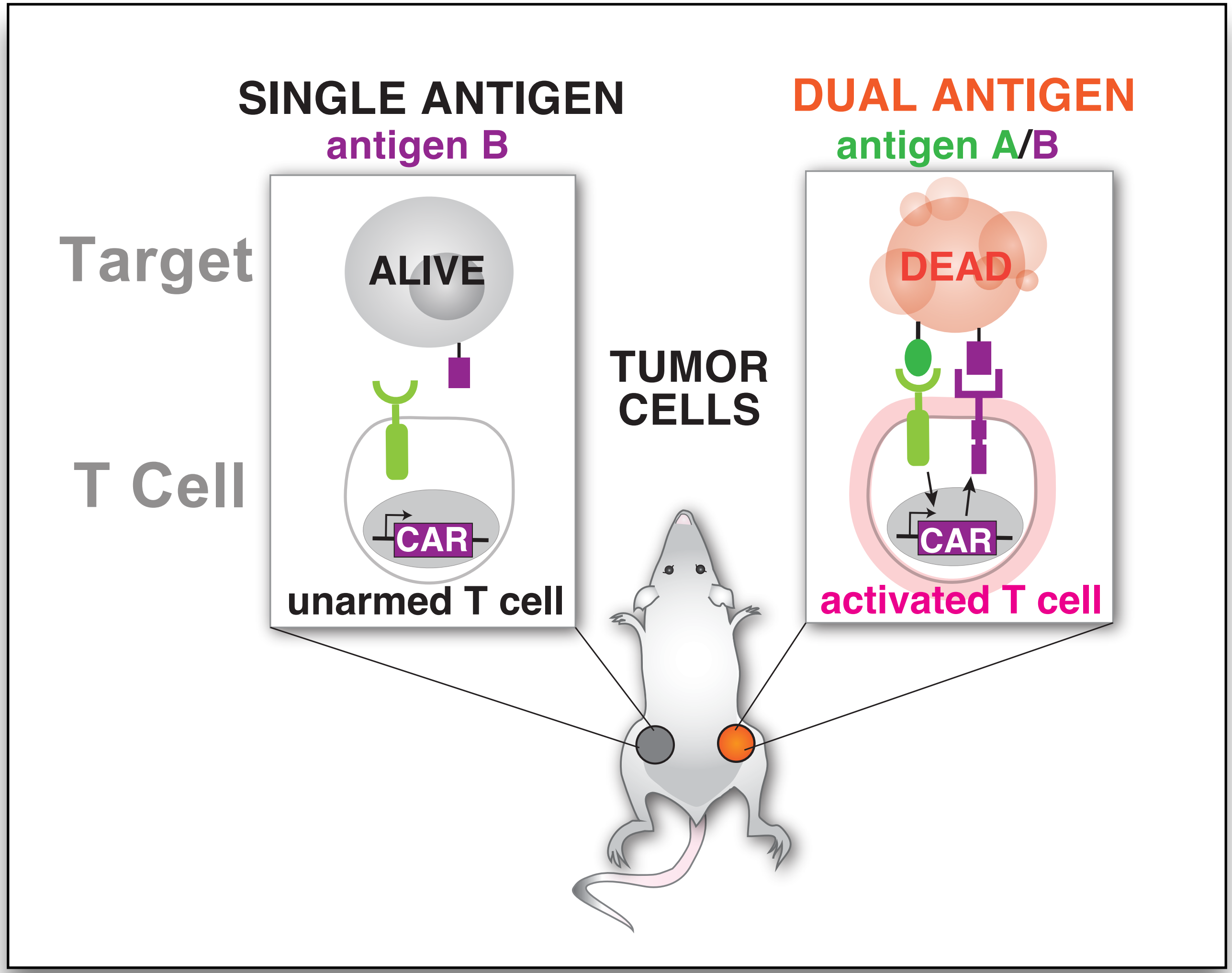
Customizable Cellular Sensing and Response Programs



SynNotch Receptors Drive Custom Transcriptional Circuits in Response to Tumor Antigens



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



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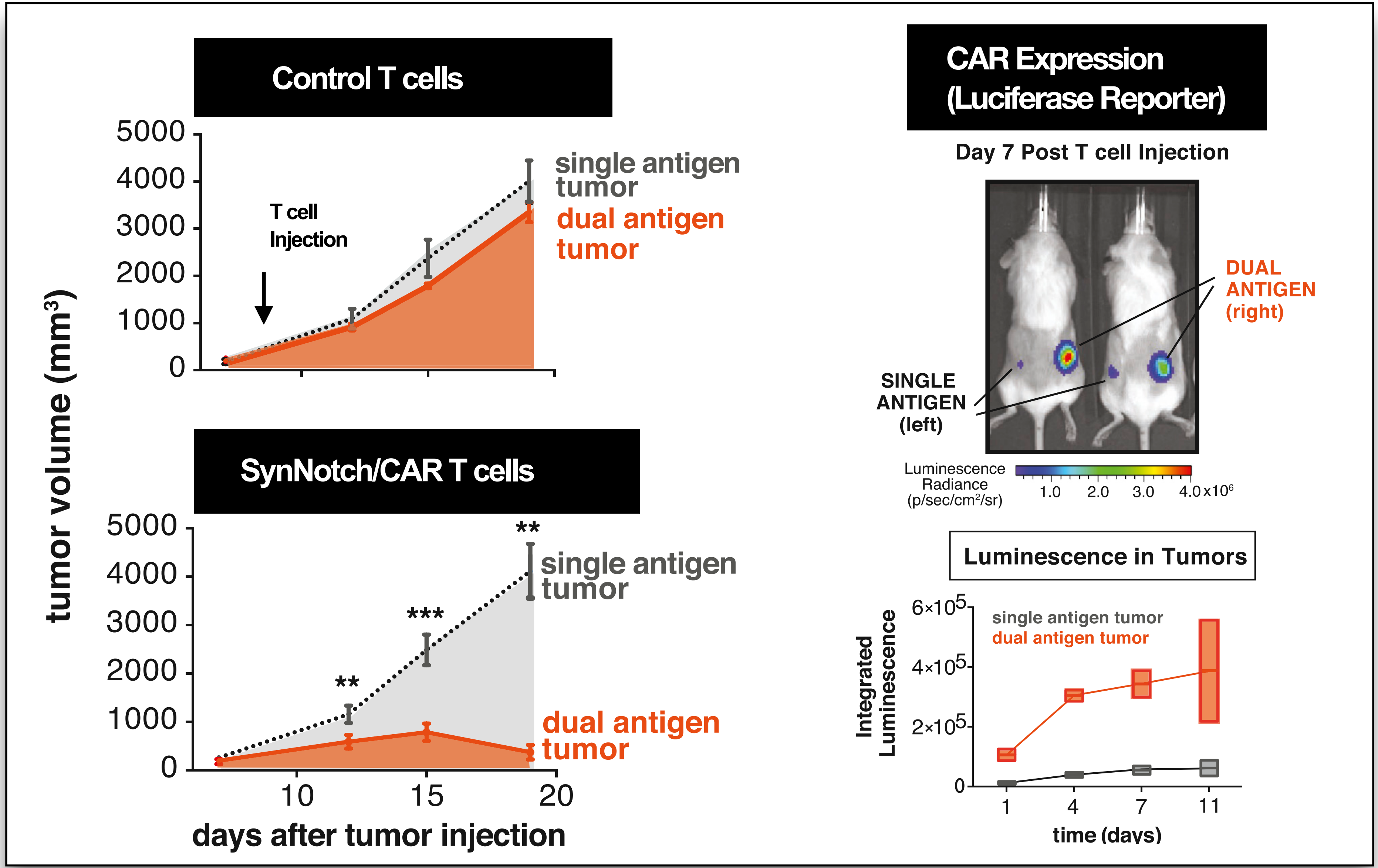
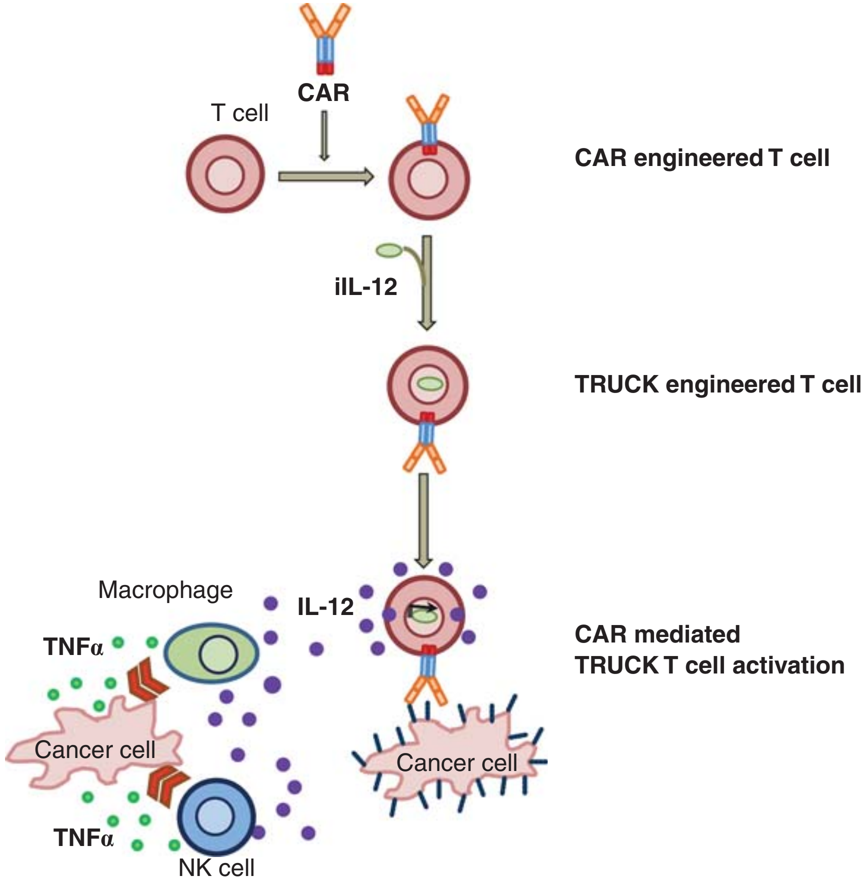
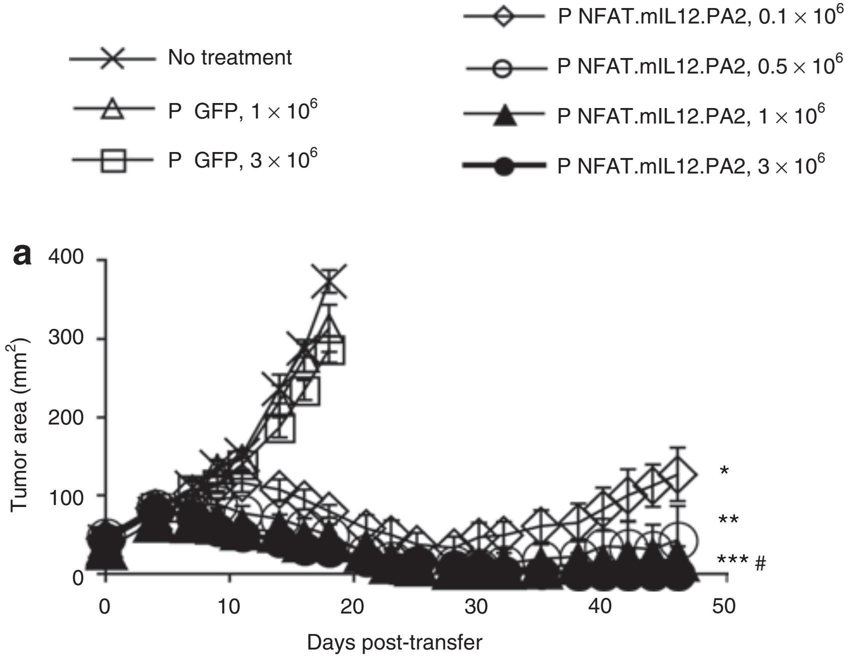


Enhancing and Sculpting the Immune Response

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

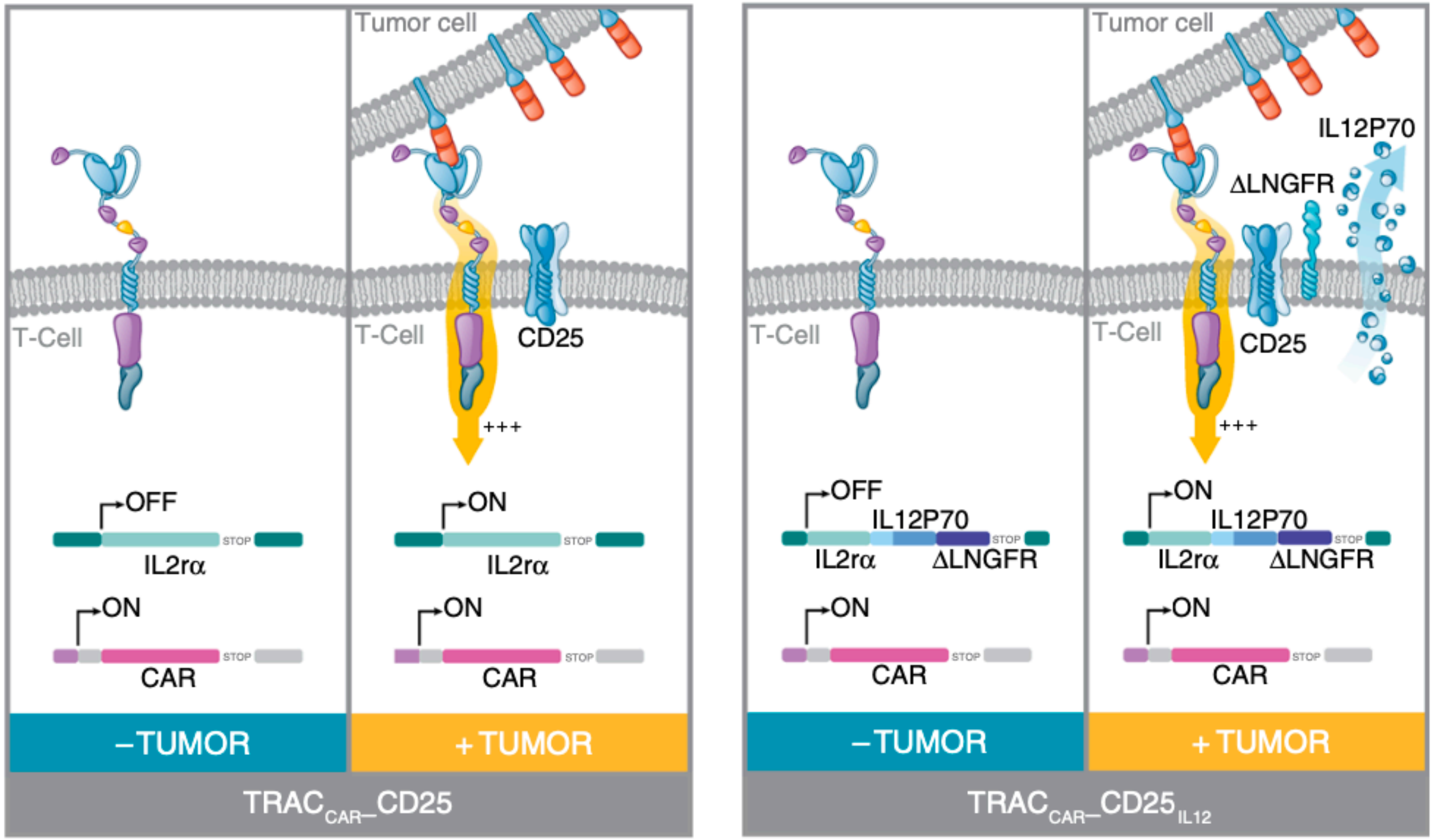




Chmielewski M. Expert Opin. Biol. Ther. 2015

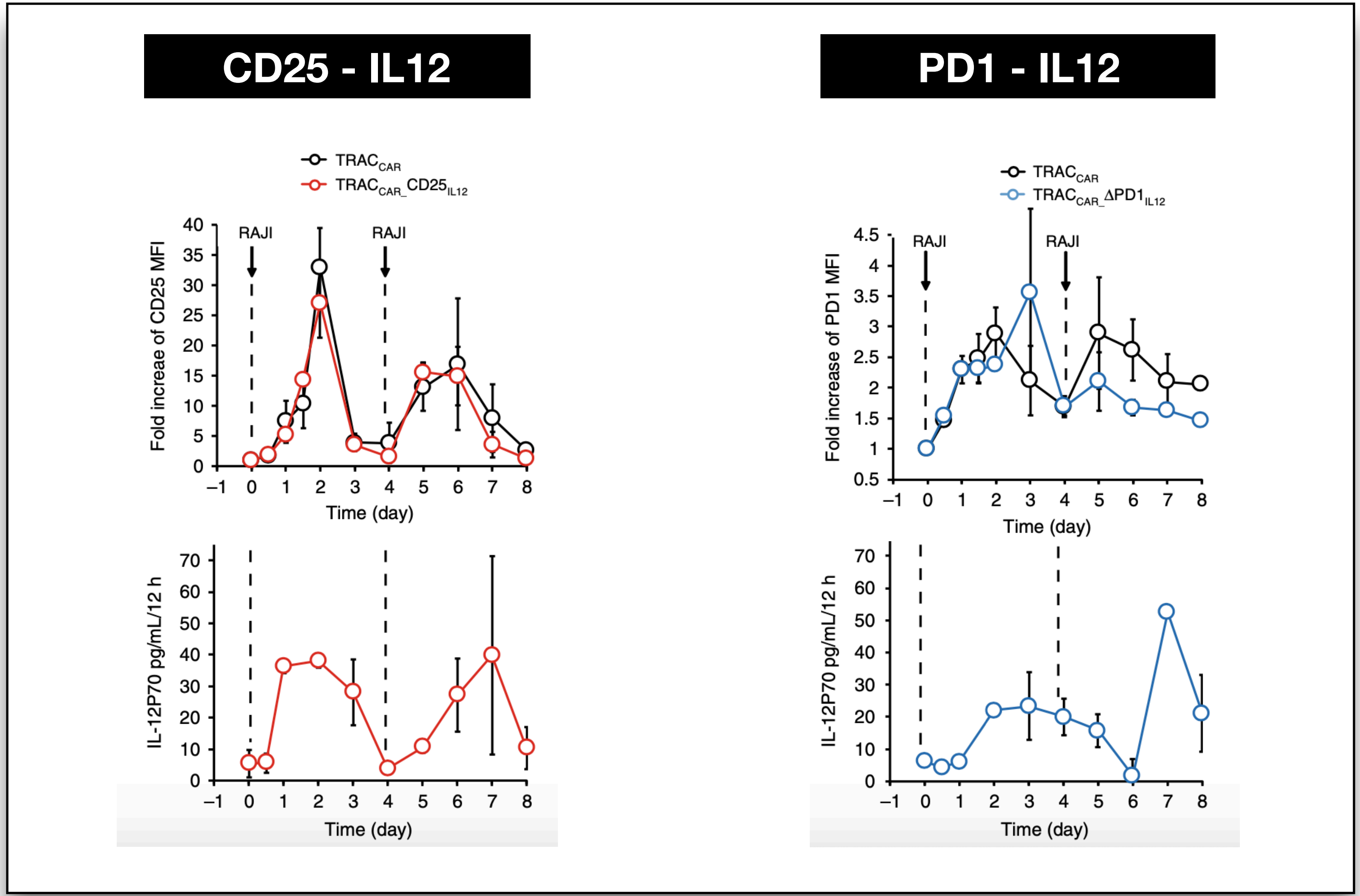
Zhang L. Molecular Therapy. 2011

Hijacking physiological transcriptional control to express therapeutic payload



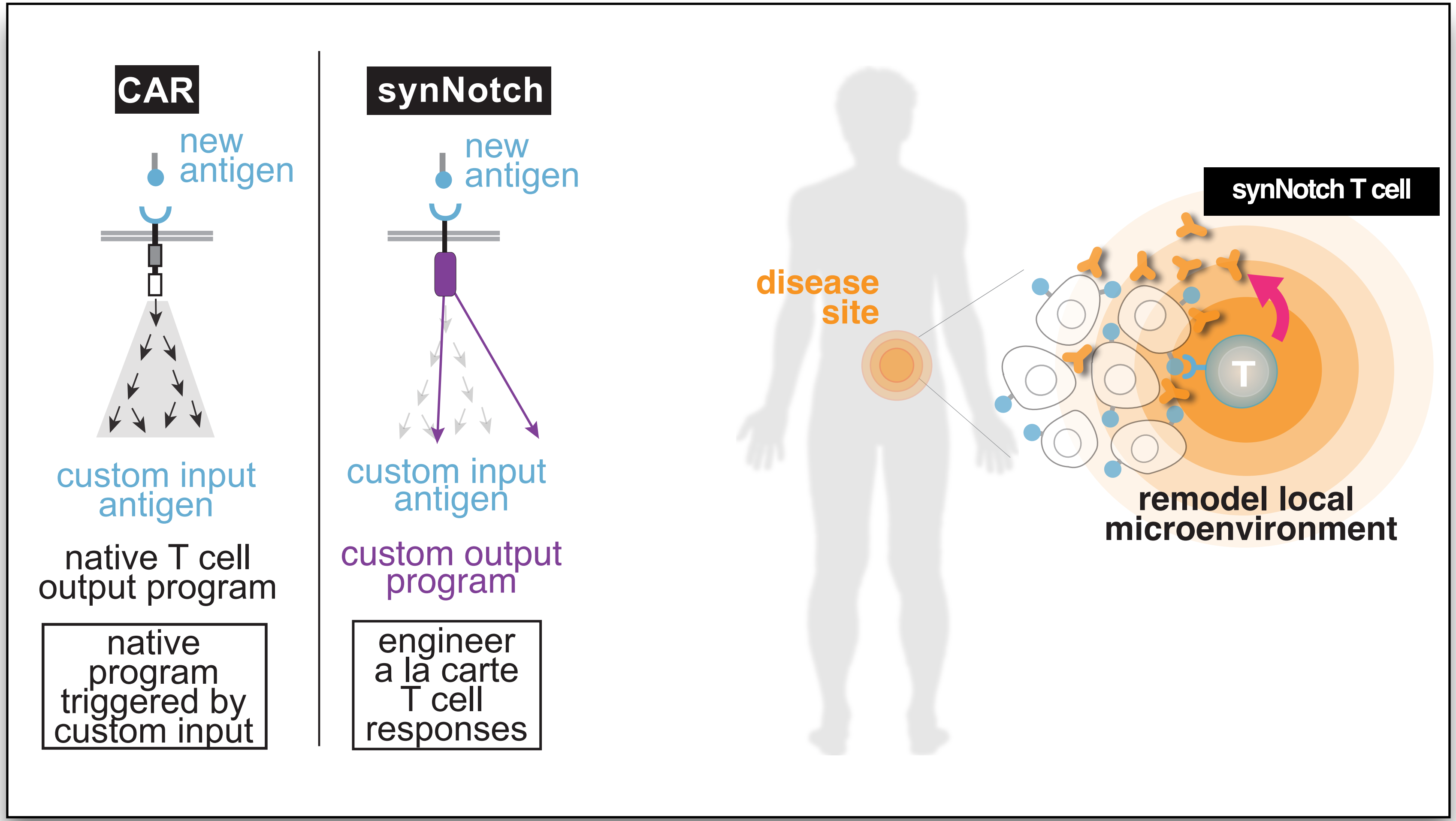
Sachdeva et al,. Nat Com 2019

Hijacking physiological transcriptional control to express therapeutic payload



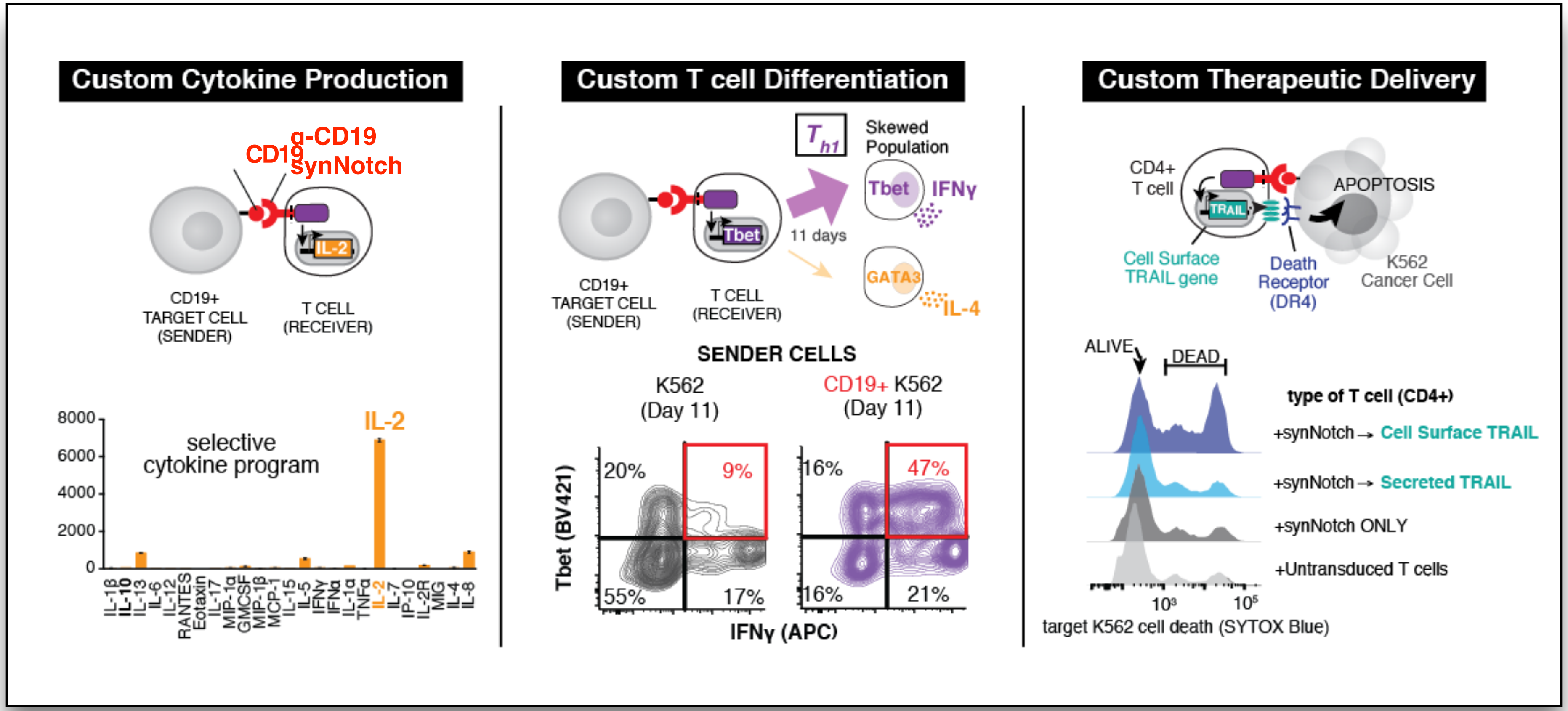
Sachdeva et al,. Nat Com 2019

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

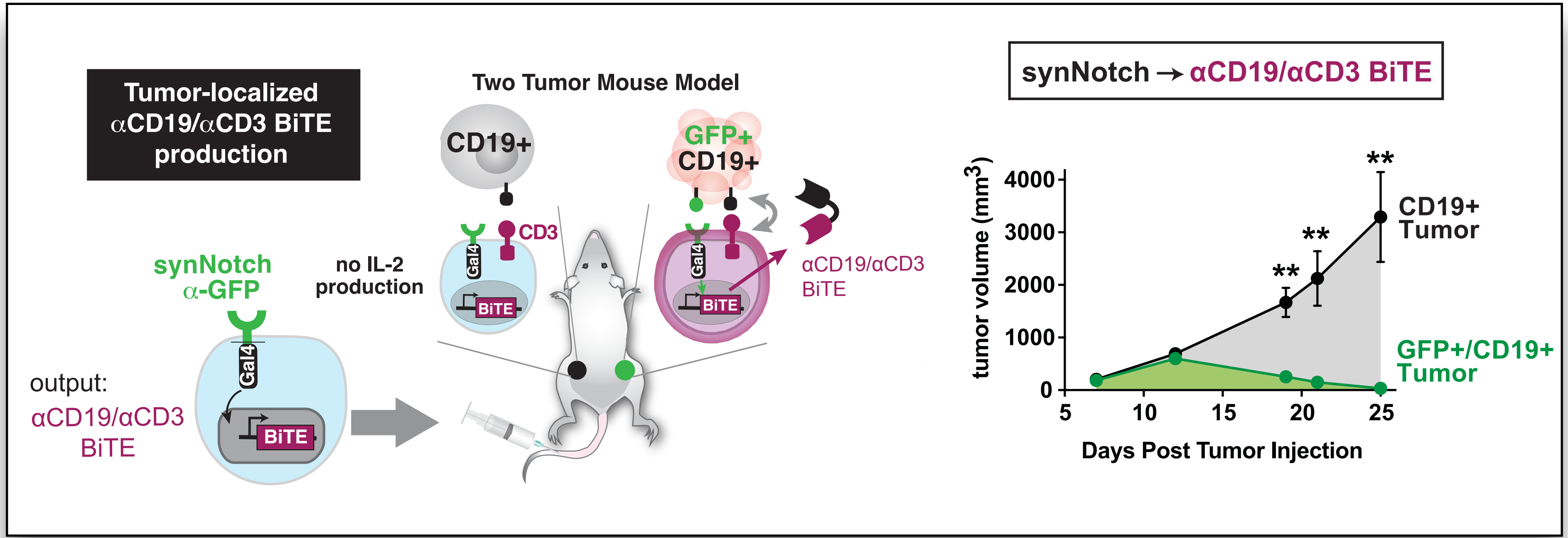


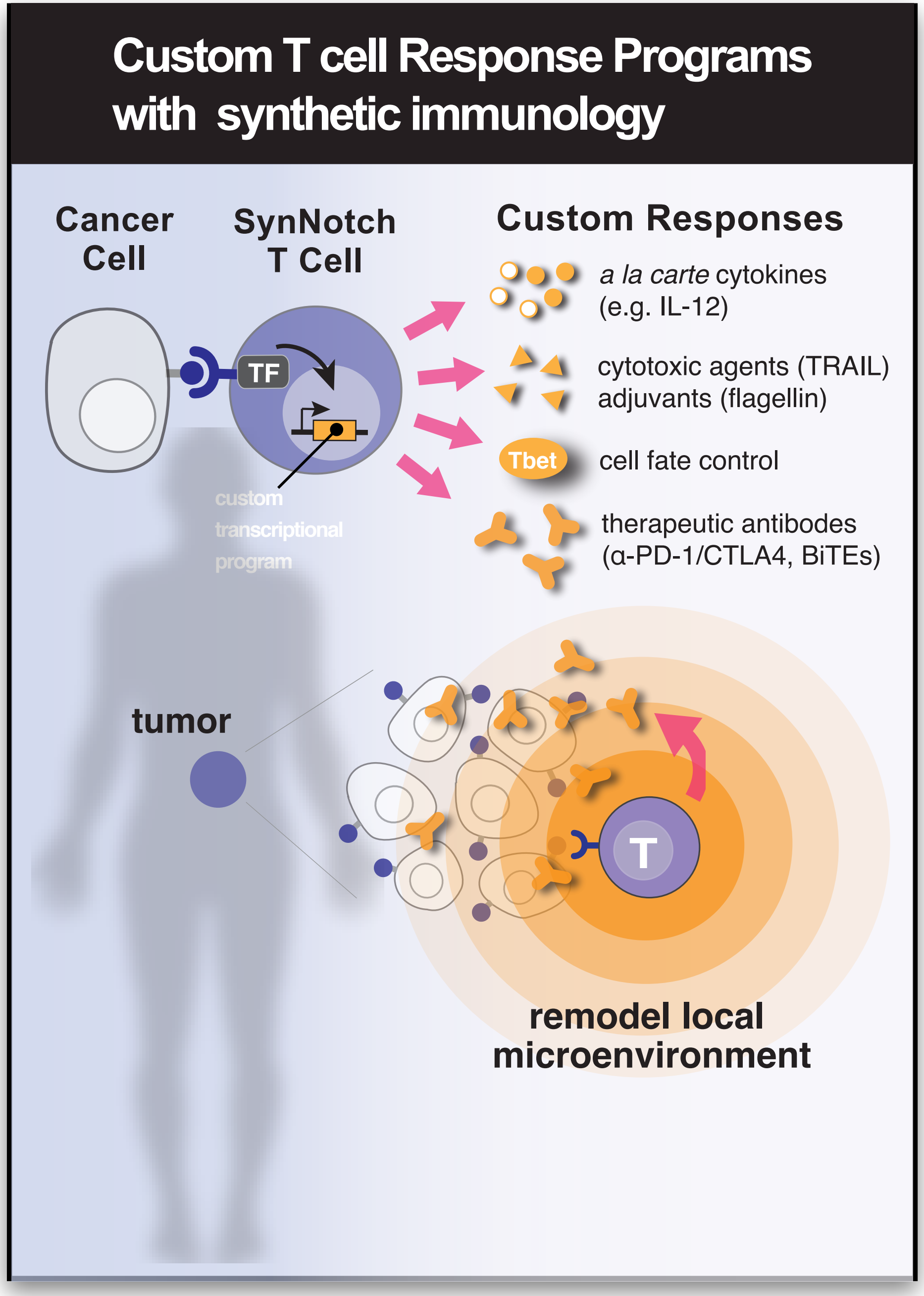
Roybal et al. Cell. 2016b

Customized T cell Responses with Synthetic Notch Receptors



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





Don't forget

- **Manufacturing**
- **Immunogenicity**
- **FDA**

Looking for postdocs

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Thank you!

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