

# **CRISPR-edited CAR-T cells**

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# **Disclosures**

**Patent – Transgenic T cell and Chimeric Antigen Receptor T cell compositions and related methods**

Licensed to Fate Therapeutics, Atara Biotherapeutics, and  
Takeda Pharmaceuticals

**Patent – Globin Gene Therapy for treating Hemoglobinopathies**

# $\gamma$ -Retrovirus vs lentivirus integration – site selection

## HIV-1 Integration in the Human Genome Favors Active Genes and Local Hotspots

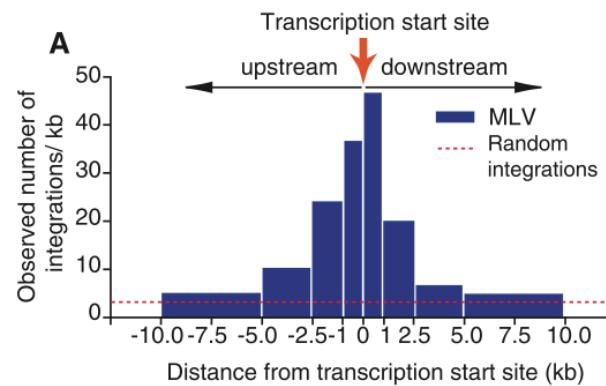
Astrid R.W. Schröder,<sup>1</sup> Paul Shinn,<sup>2</sup>  
Huaming Chen,<sup>2</sup> Charles Berry,<sup>3</sup> Joseph R. Eck,  
and Frederic Bushman<sup>1,4</sup>

Cell, 2002

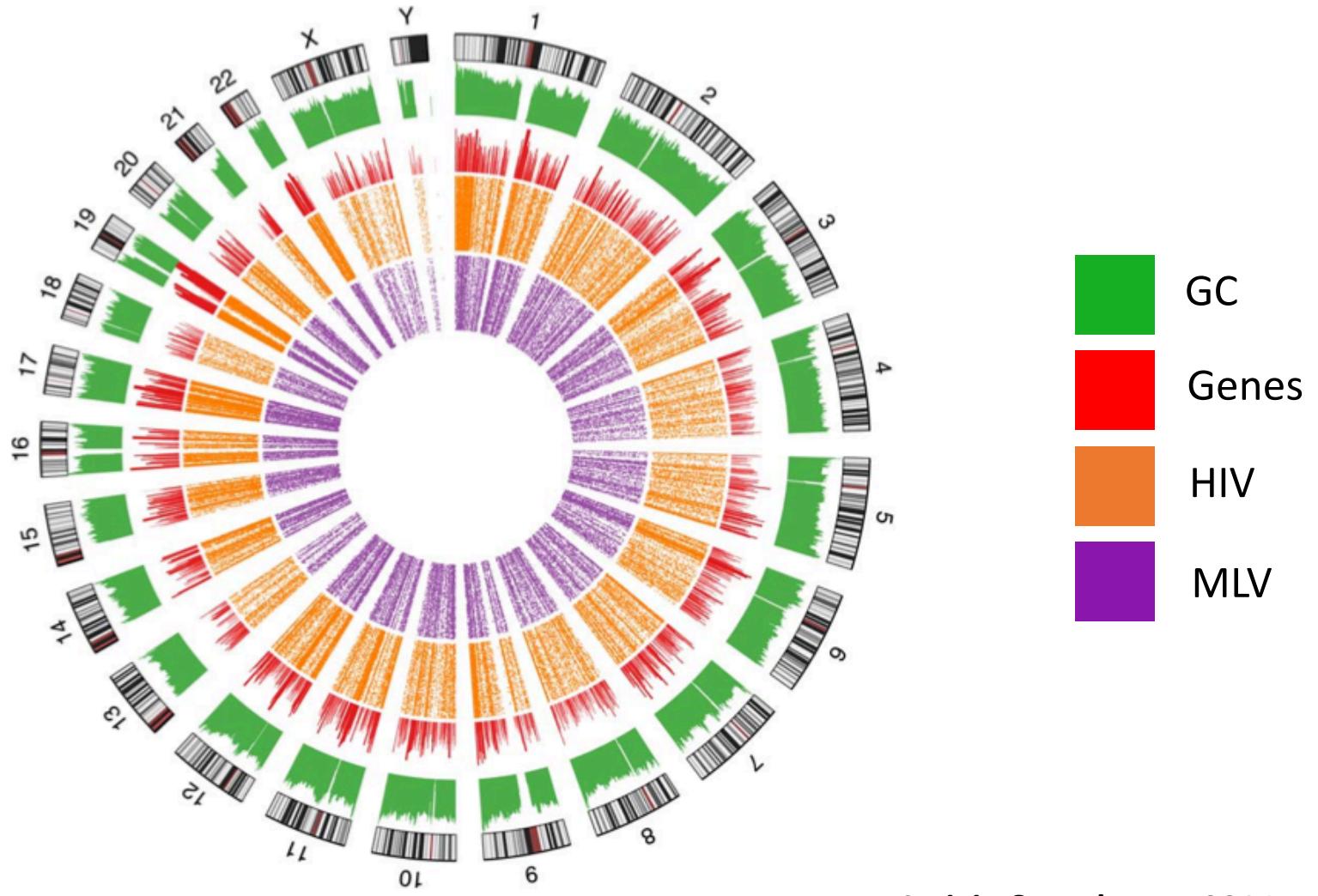
## Transcription Start Regions in the Human Genome Are Favored Targets for MLV Integration

Xiaolin Wu,<sup>1</sup> Yuan Li,<sup>2</sup> Bruce Crise,<sup>2</sup> Shawn M. Burgess<sup>1\*</sup>

Science, 2003

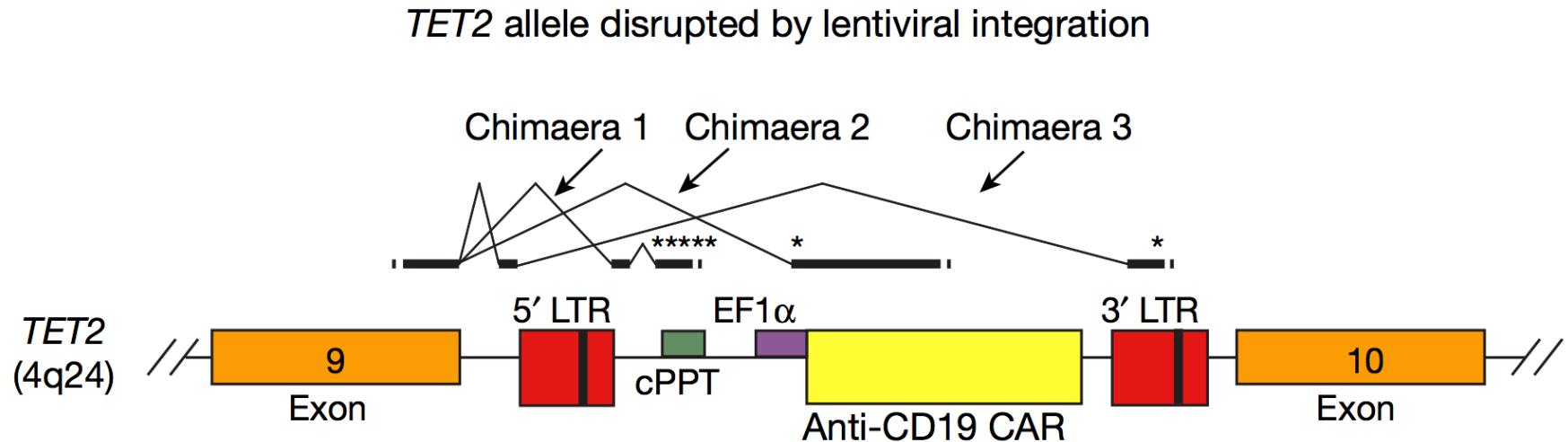


# $\gamma$ -Retroviral vector and lentivirus integration is semi-random in human T cells



Craigie & Bushman, 2014

# Clonal T cell expansion due to CAR lentiviral vector integration in the *TET2* gene

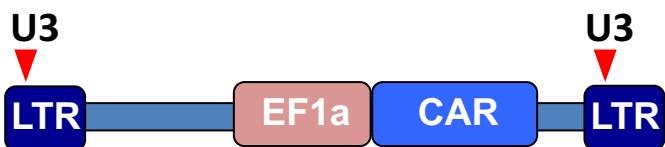
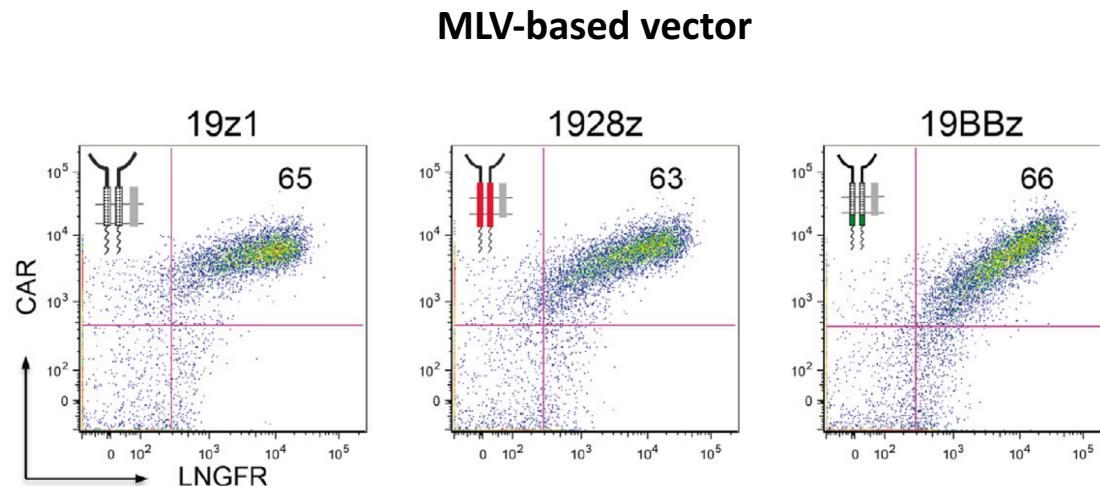


Fraietta et al., Nature 2018

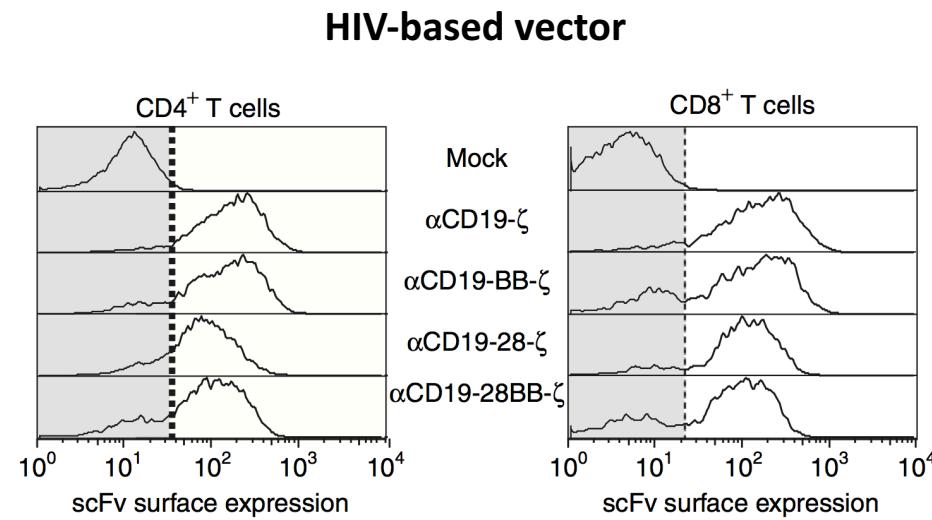
# $\gamma$ -Retroviral and lentiviral vector integration – expression variegation



Zhao et al., Cancer Cell , 2015



Milone et al., Mol. Therapy , 2009

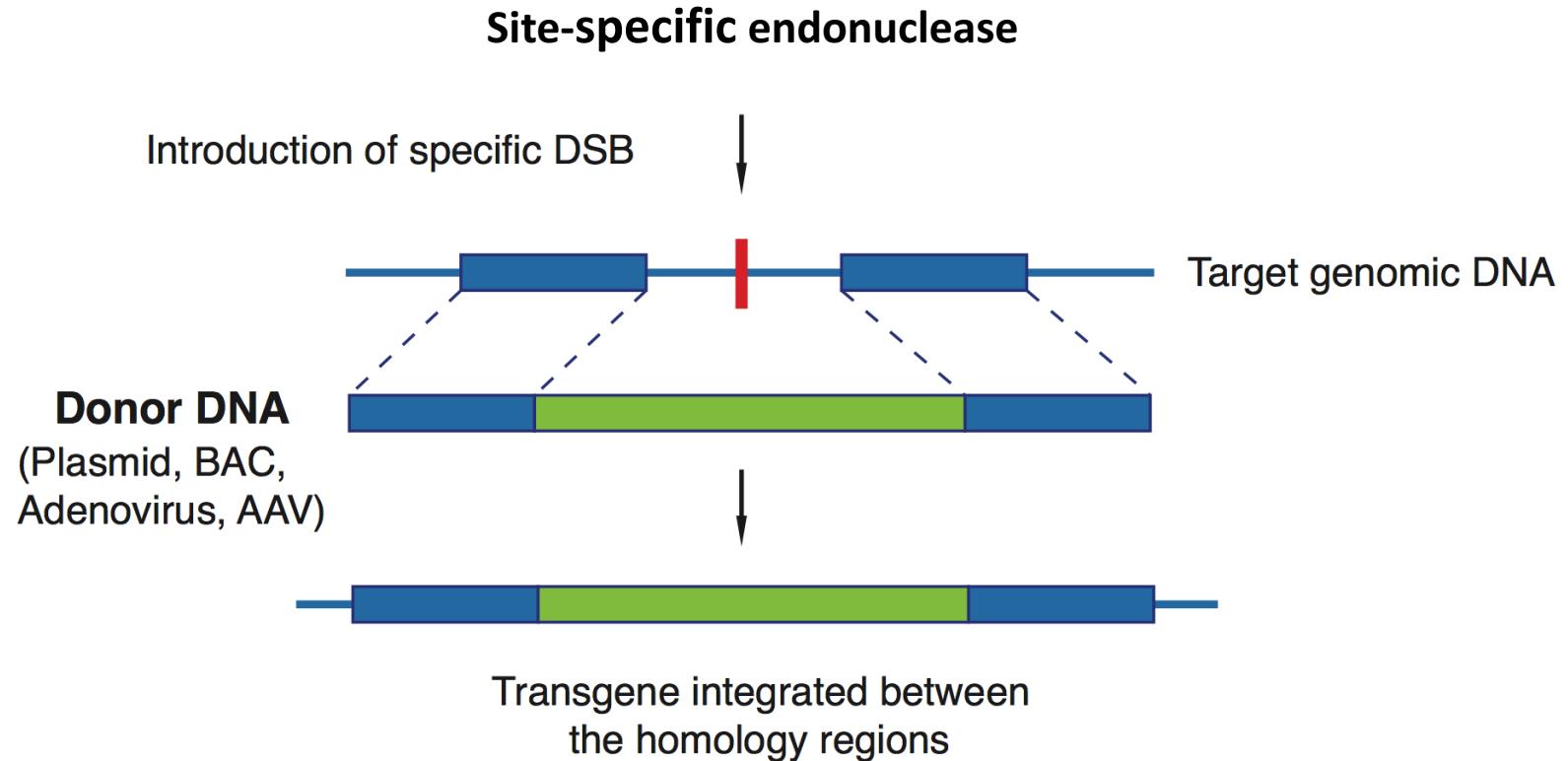


- Is there an optimal CAR expression level?
- Which locus can provide optimal expression level?
- Can an endogenous promoter drive optimal CAR expression level?

## Hypothesis

Targeting a CAR into a TCR locus provides homogenous  
and optimal CAR expression

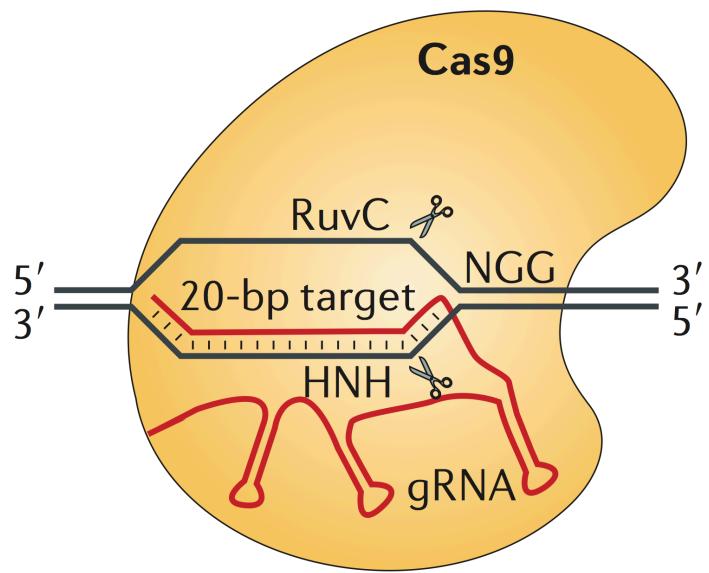
# Gene targeting in human T cells



Jasin and colleagues, 1996

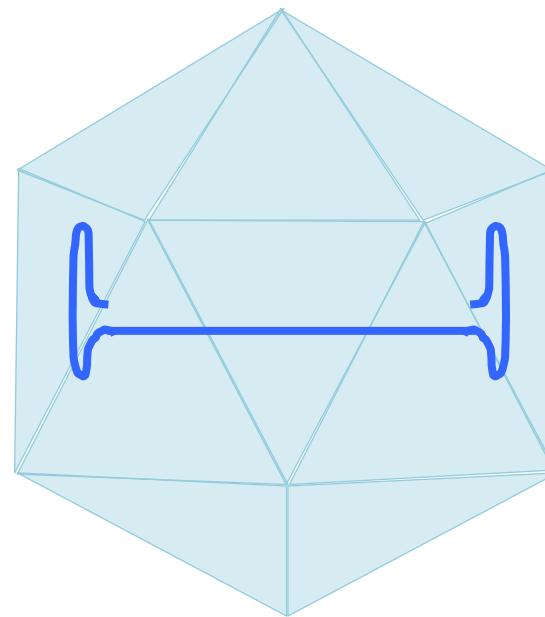
# Gene targeting in human T cells

CRISPR/Cas9



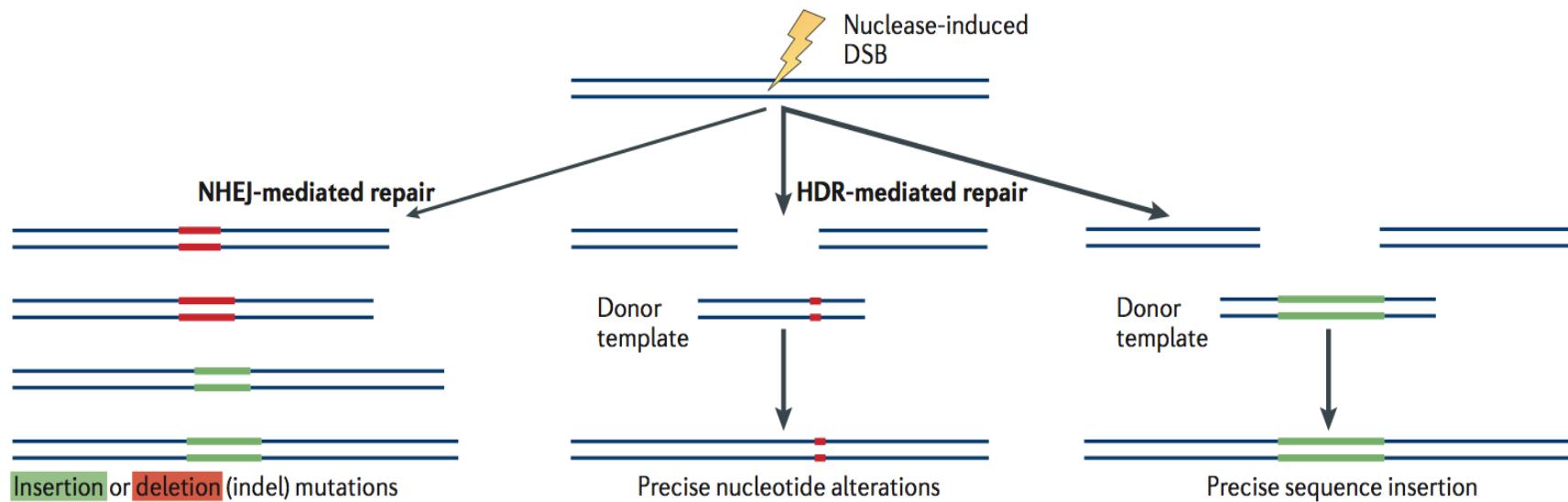
*Tsai and Joung, NRG 2016*

rAAV6

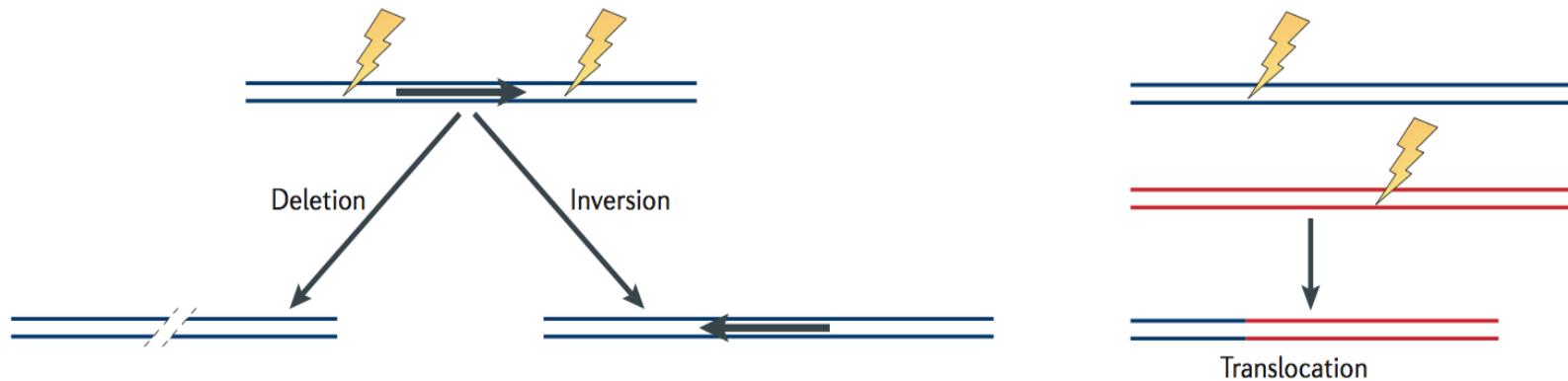


# DNA repair pathways in response to nuclease-induced DNA breaks

a



b

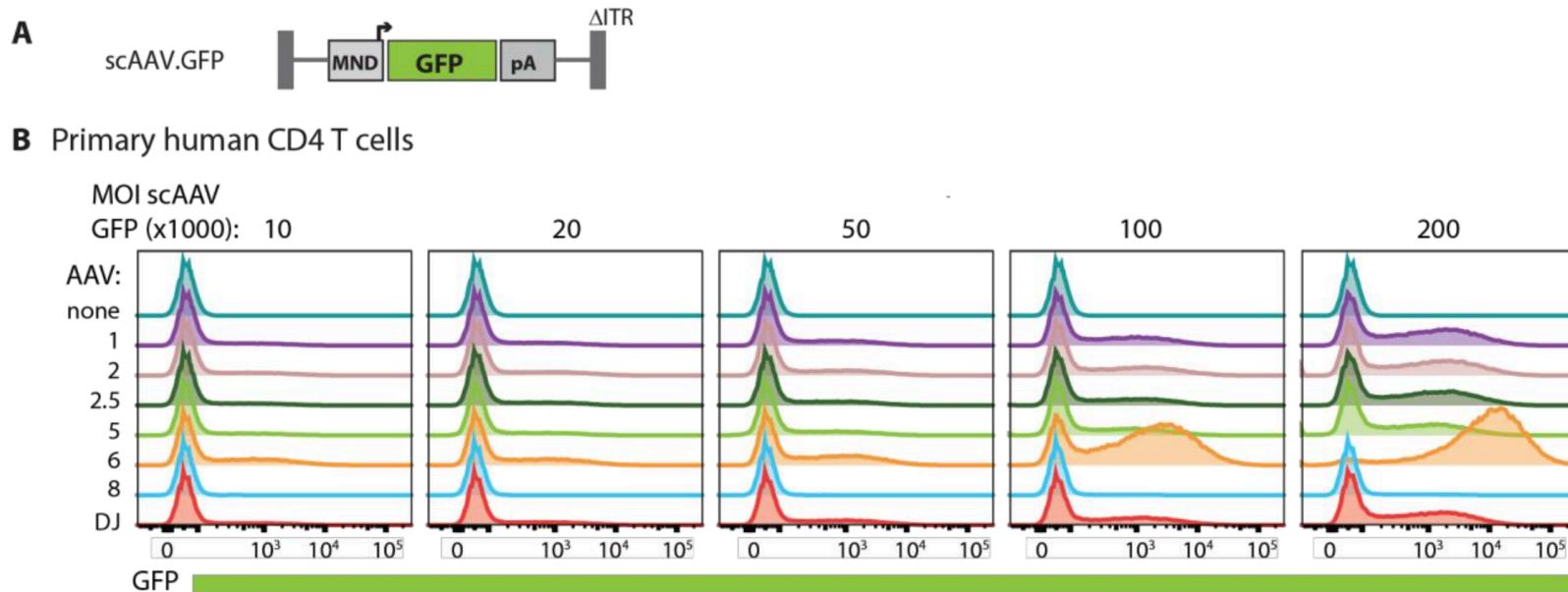


# Gene targeting in human T cells

## Efficient modification of CCR5 in primary human hematopoietic cells using a megaTAL nuclease and AAV donor template

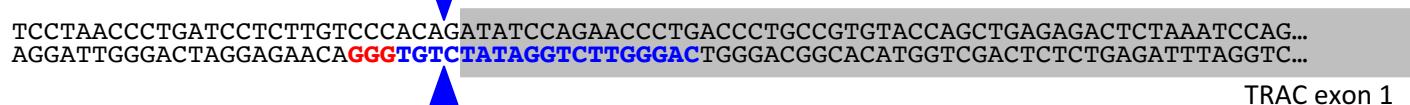
Blythe D. Sather,<sup>1\*</sup> Guillermo S. Romano Ibarra,<sup>1\*</sup> Karen Sommer,<sup>1</sup> Gabrielle Curinga,<sup>1</sup> Malika Hale,<sup>1</sup> Iram F. Khan,<sup>1</sup> Swati Singh,<sup>1</sup> Yumei Song,<sup>1</sup> Kamila Gwiazda,<sup>1</sup> Jaya Sahni,<sup>1</sup> Jordan Jarjour,<sup>2</sup> Alexander Astrakhan,<sup>2</sup> Thor A. Wagner,<sup>3,4</sup> Andrew M. Scharenberg,<sup>1,4,5†</sup> David J. Rawlings<sup>1,4,5†</sup>

Science Trans Med, 2015

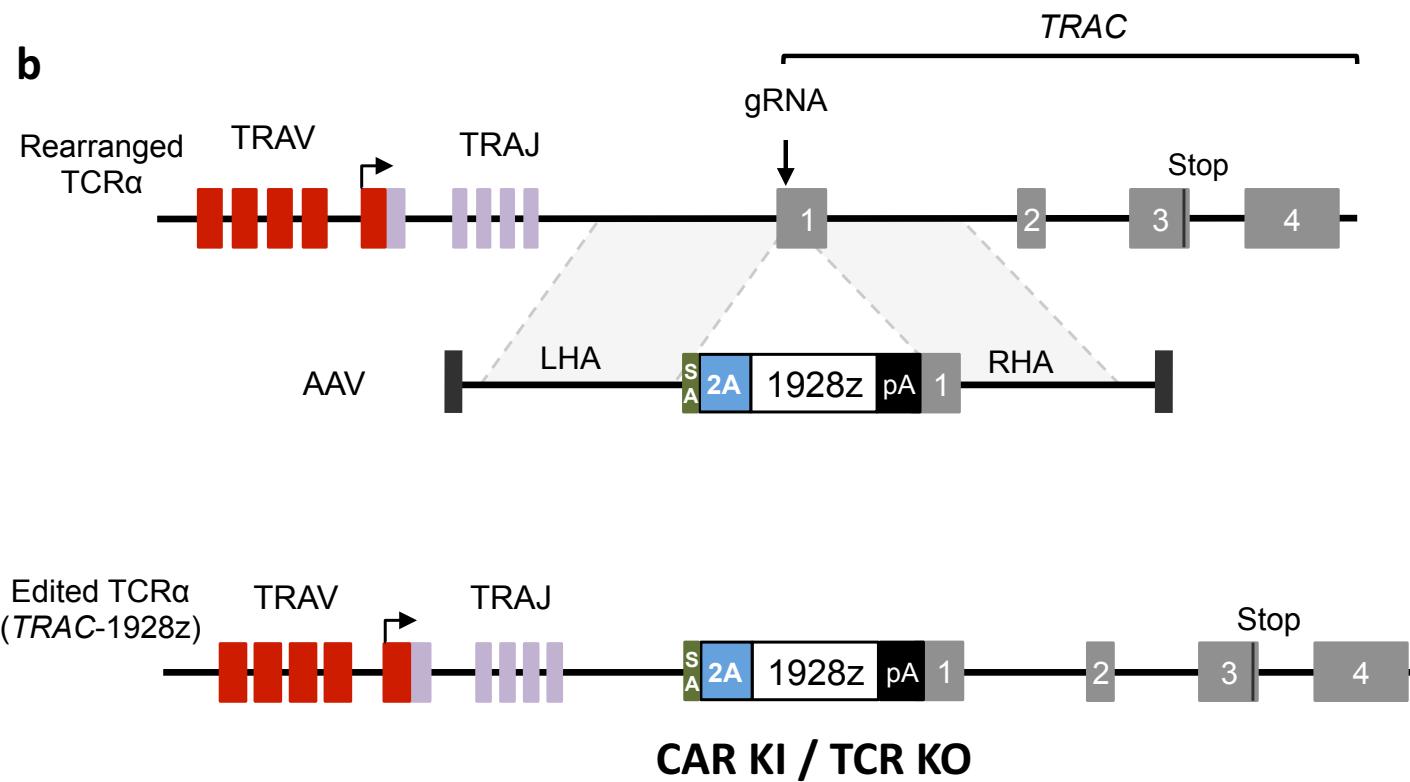


# CRISPR/Cas9-targeted integration into the *TRAC* locus

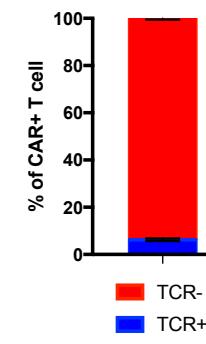
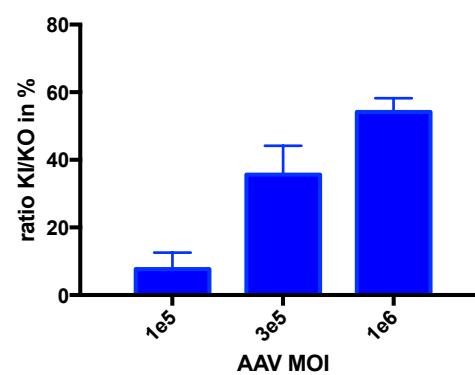
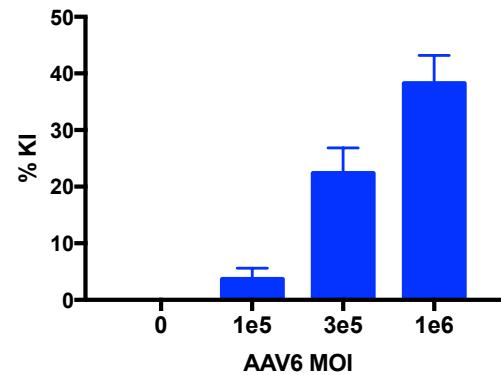
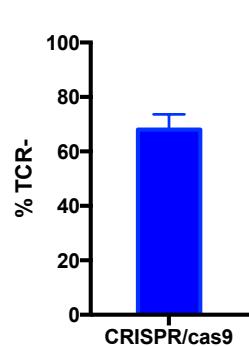
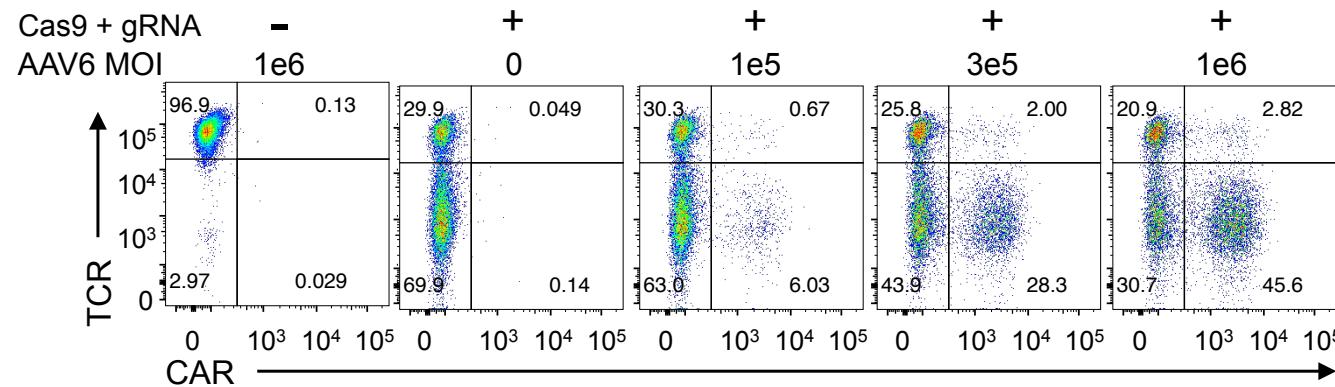
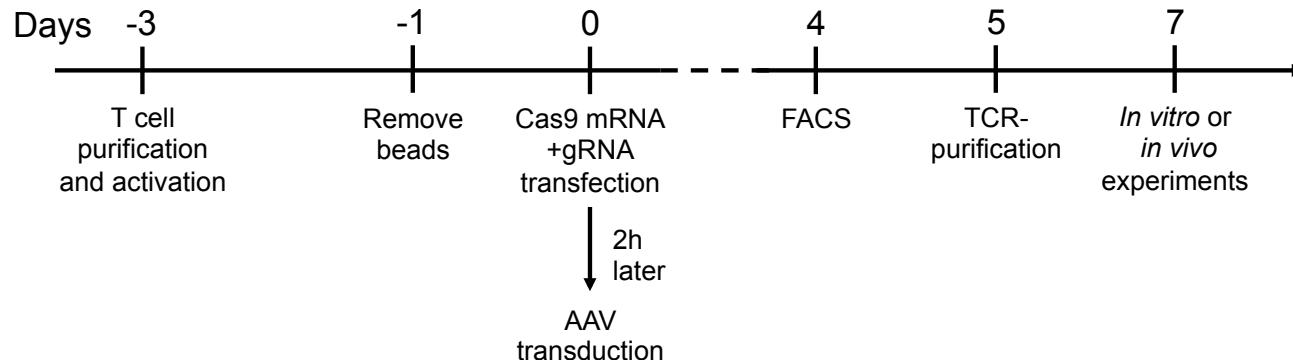
a



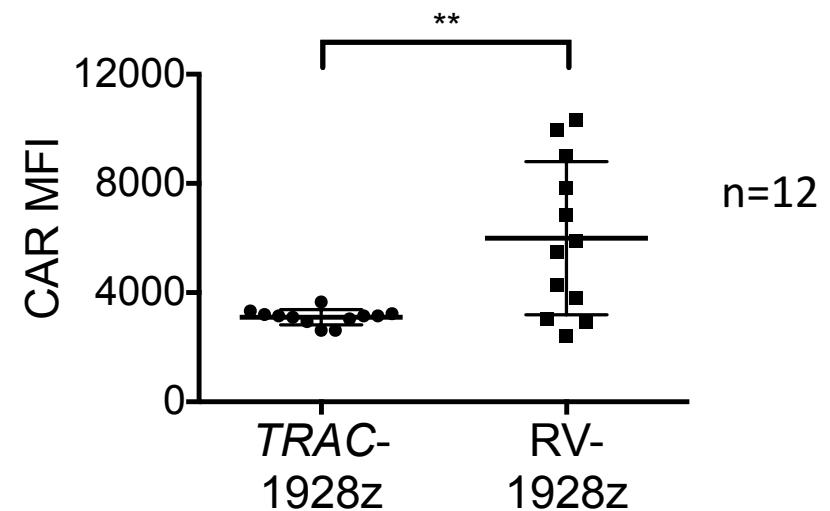
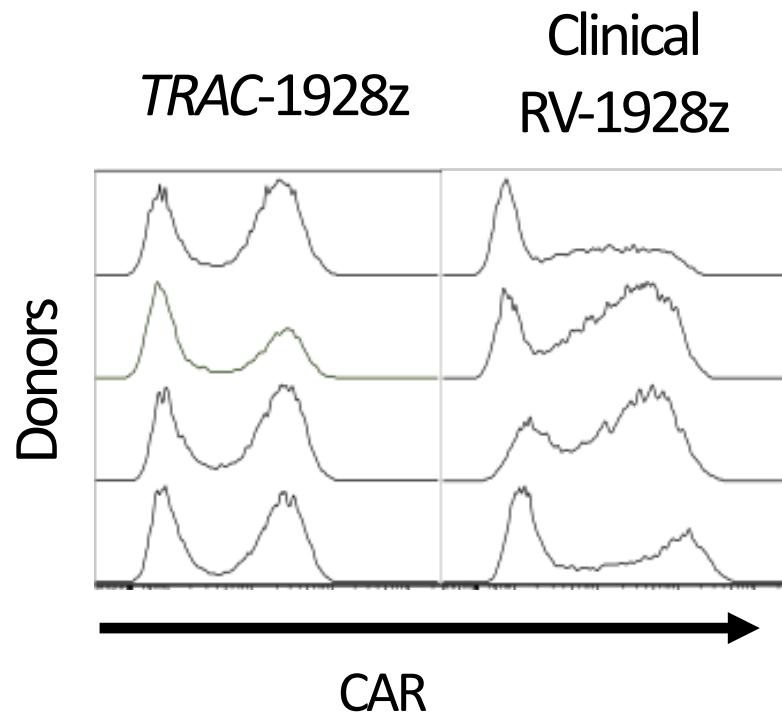
b



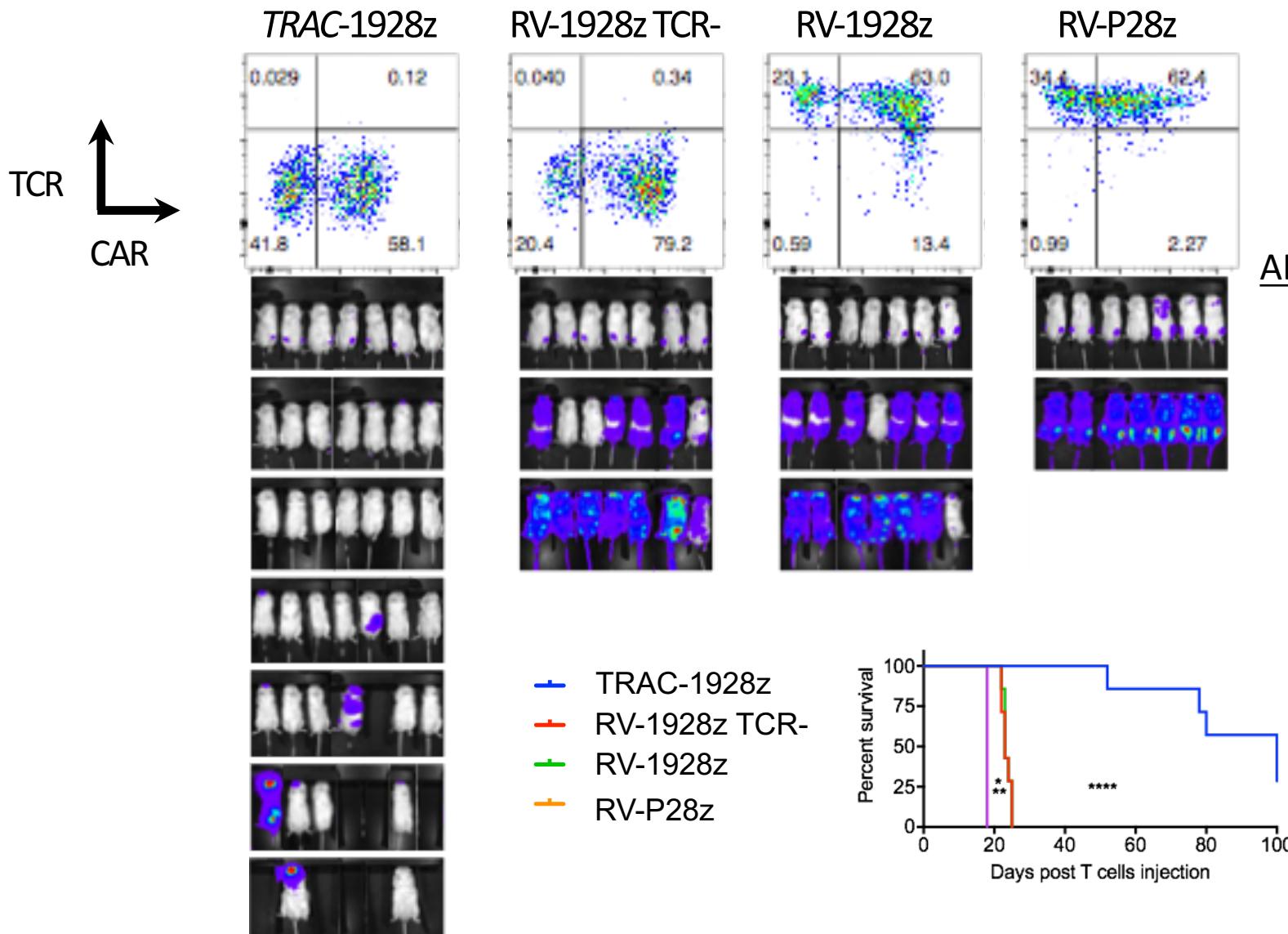
# CRISPR/Cas9-targeted integration into the TRAC locus results in efficient TCR knock-out/ CAR cDNA knock-in



## ***TRAC-CAR* T cells show homogenous CAR expression**

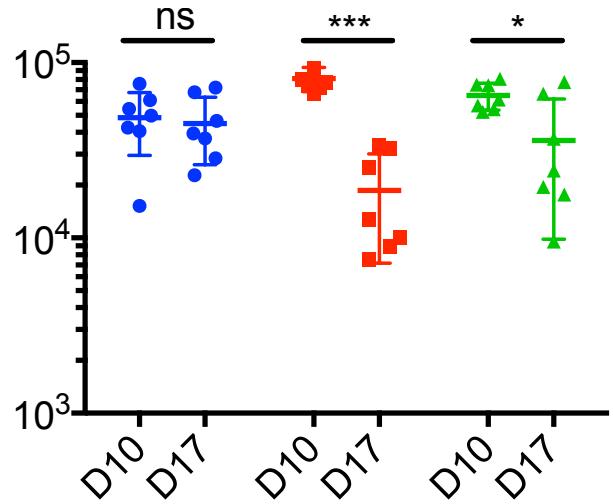


# *TRAC-CAR T cells display superior *in vivo* anti-tumor activity*

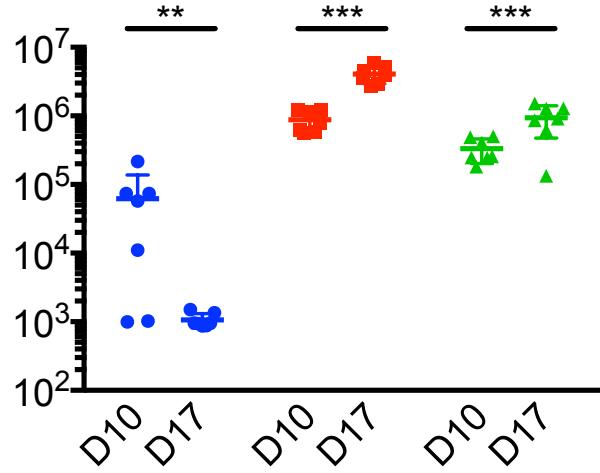


# *TRAC-CAR* T cells eradicates bone marrow tumor cells

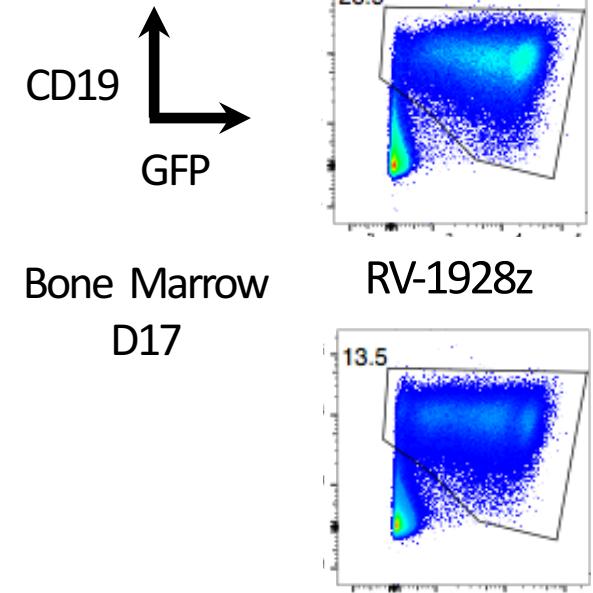
CAR T cells



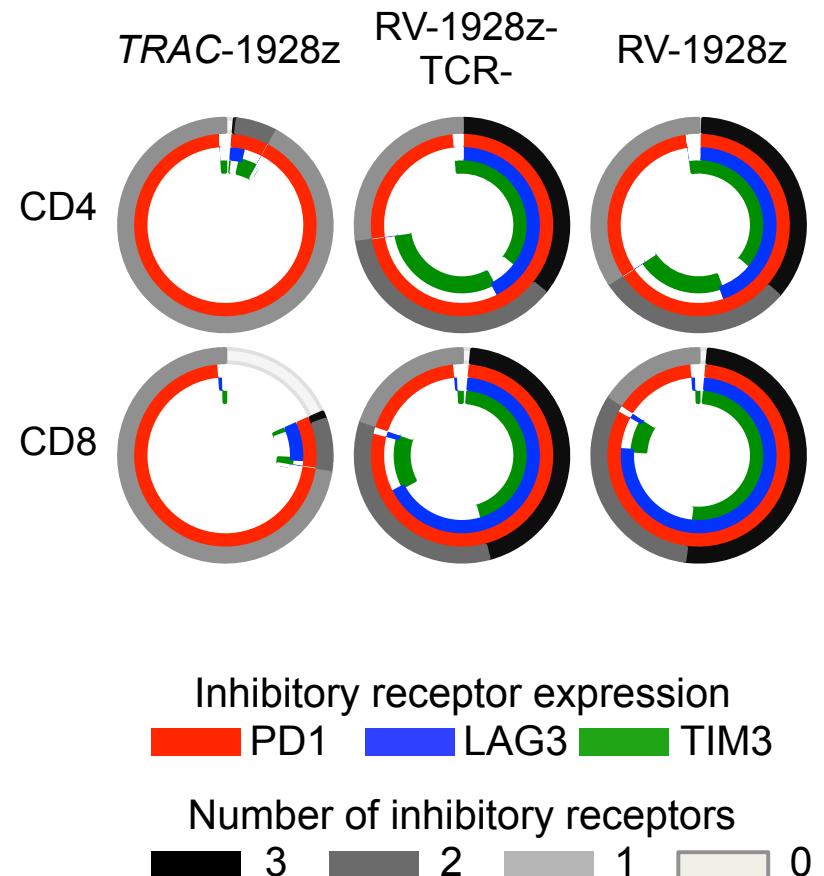
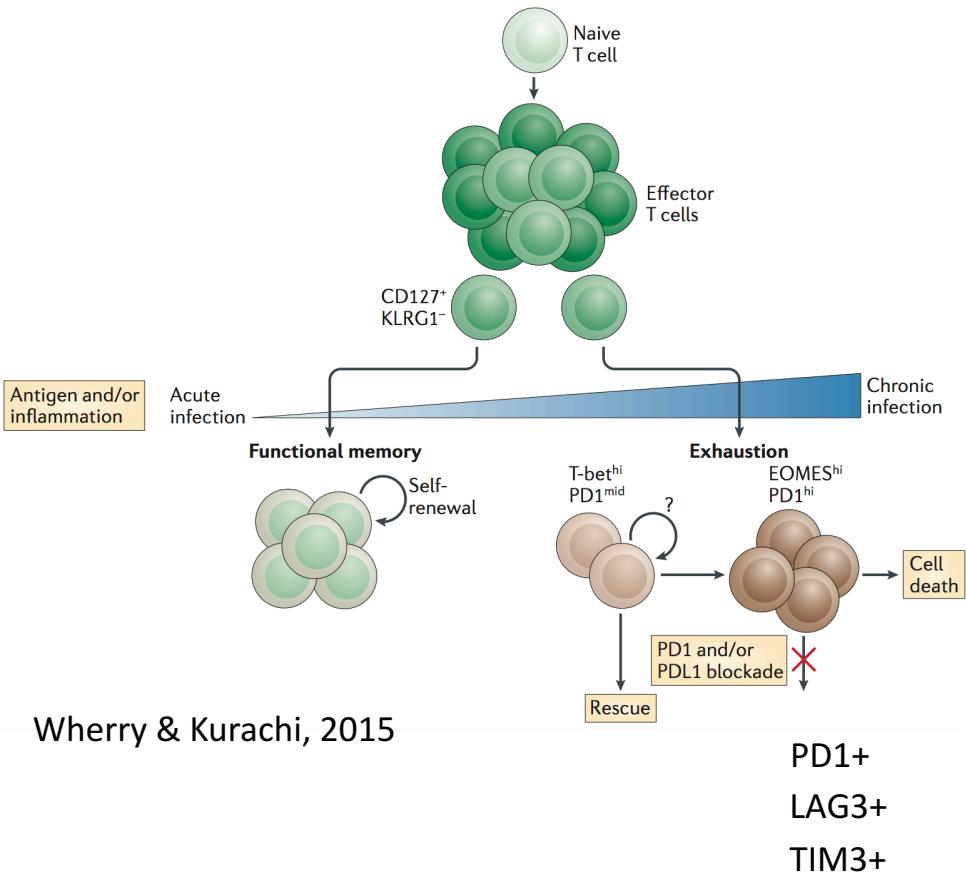
Tumor



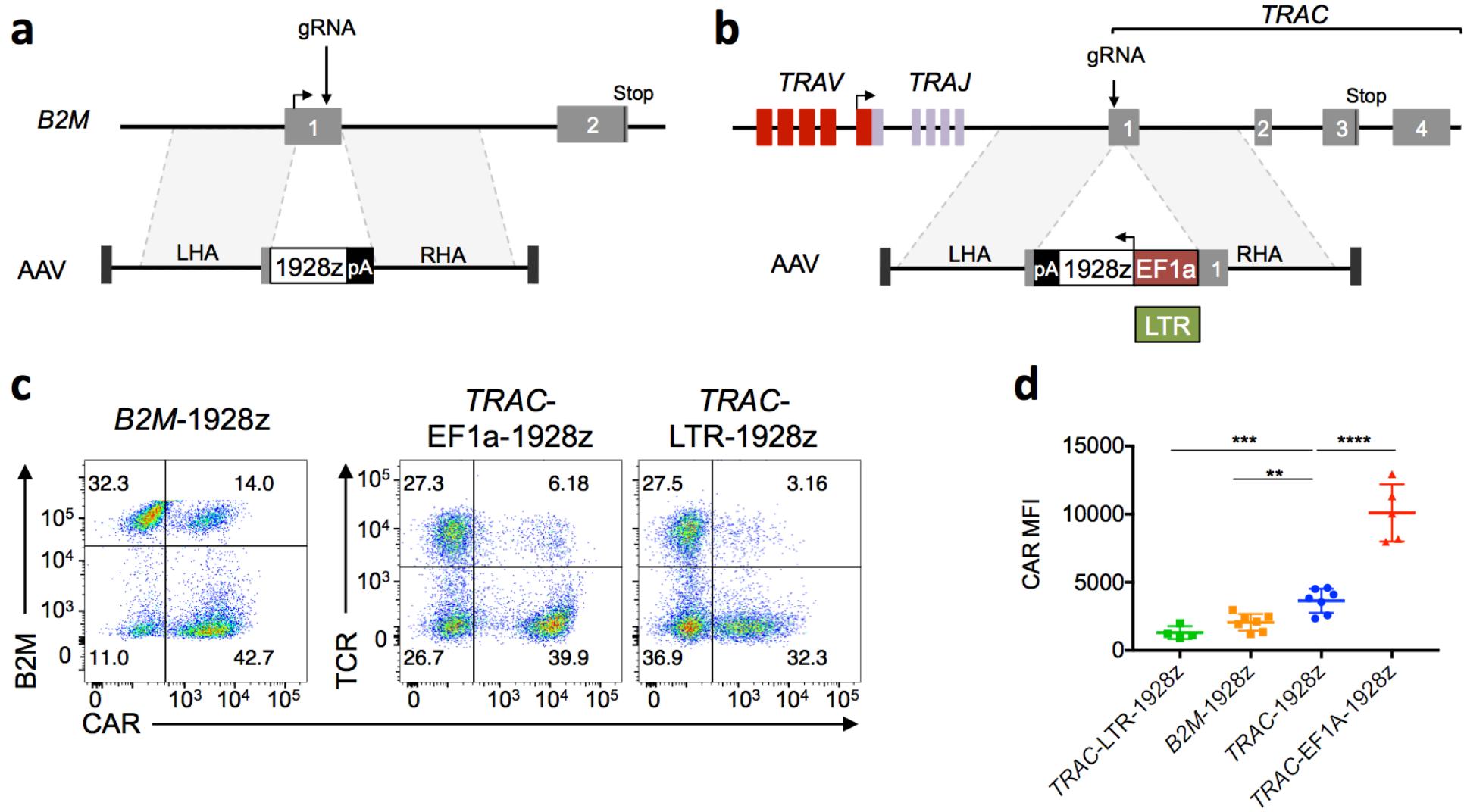
- *TRAC-1928z*
  - *RV-1928z-TCR-*
  - ▲ *RV-1928z*
- n=7 mice



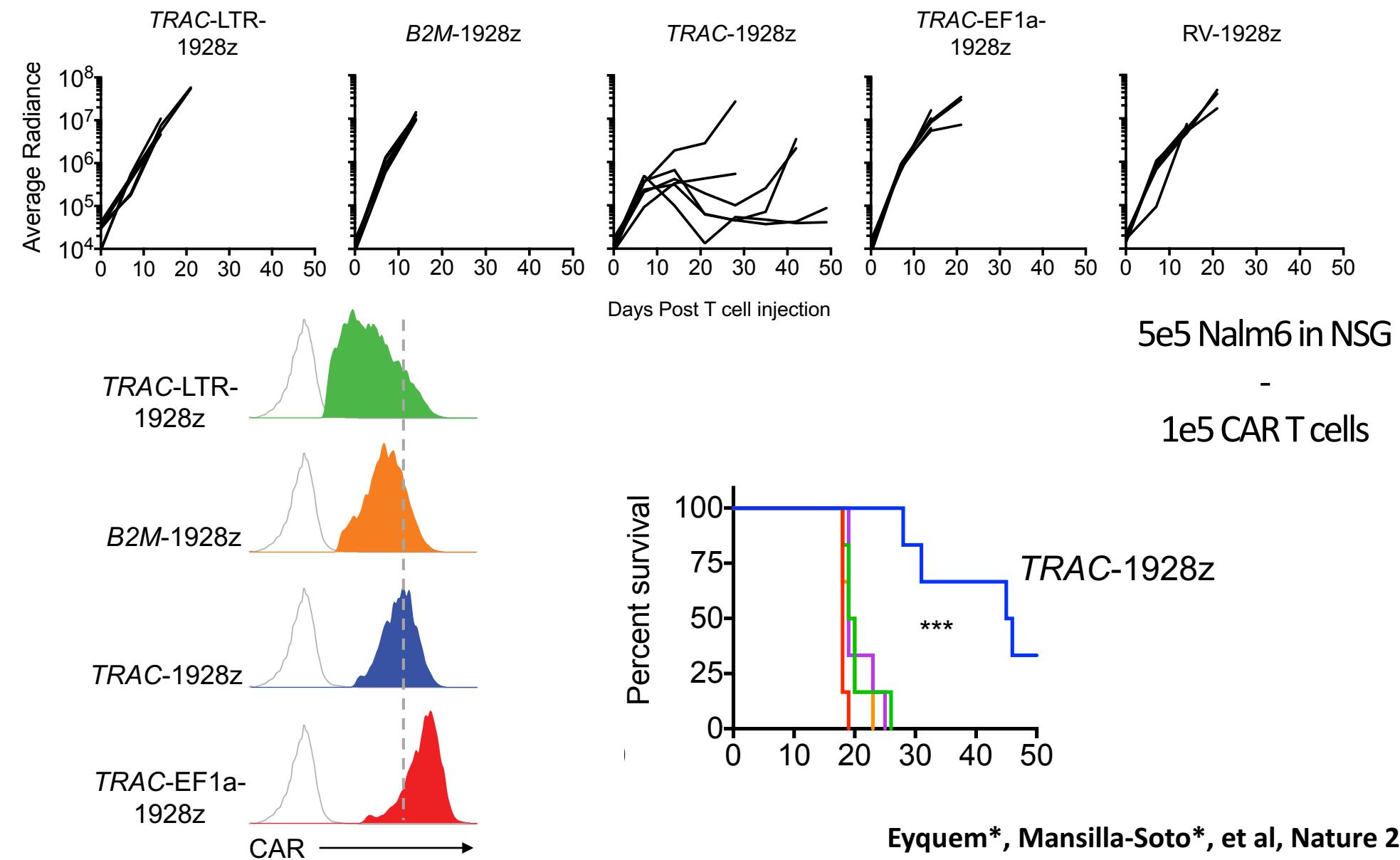
# TRAC-CAR prevents CAR T-cell exhaustion *in vivo*



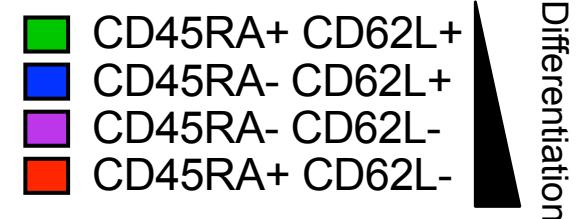
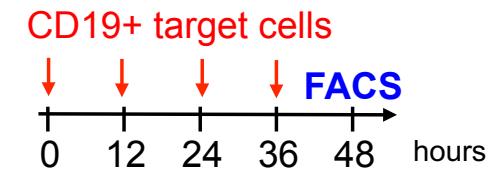
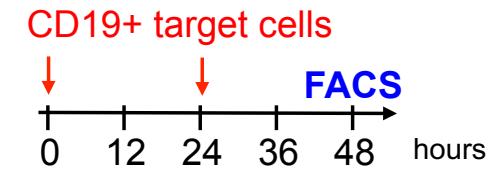
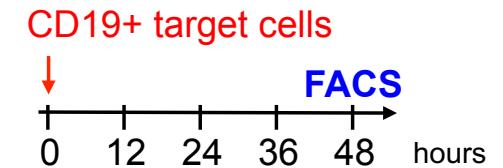
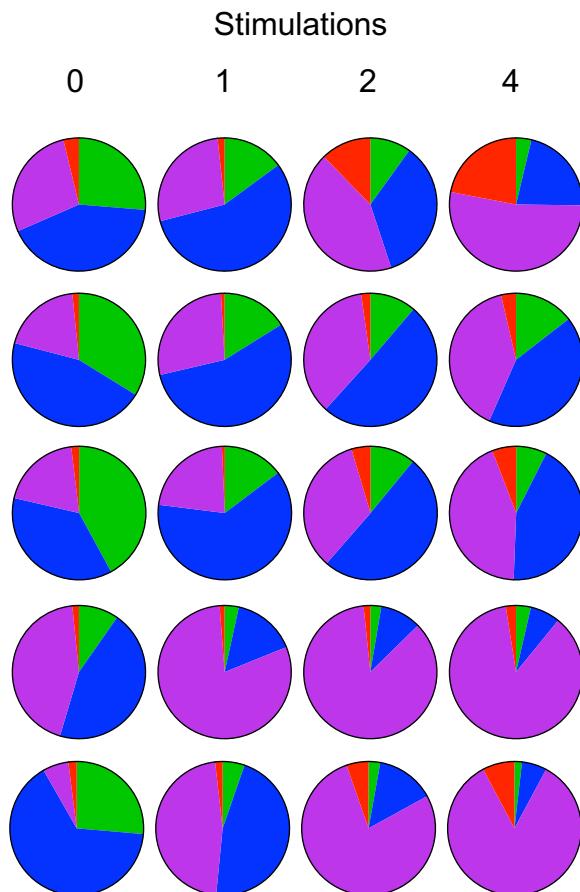
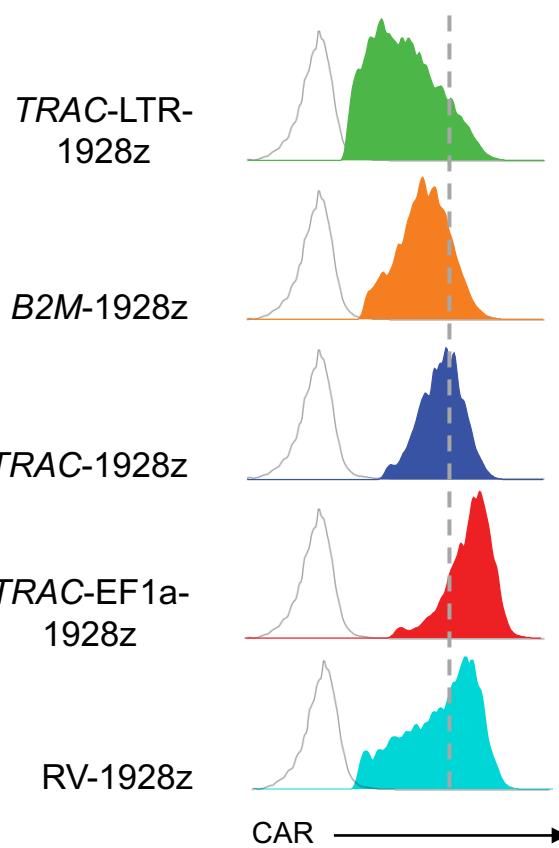
# Generation of CAR T cells with CRISPR/Cas9-targeted integration of CAR transgene at different loci



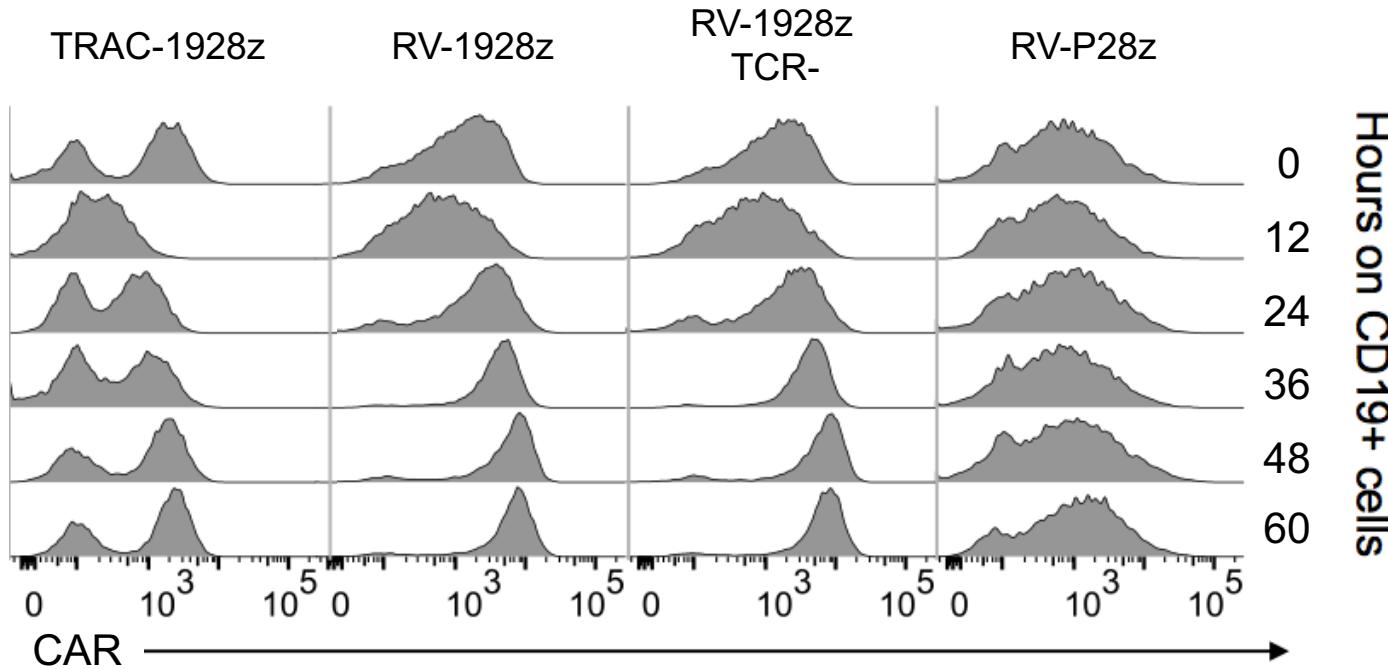
# *TRAC-CAR T cells outperform TRAC-EF1a-, TRAC-LTR-, and B2M-CAR T cells anti-tumor function *in vivo**



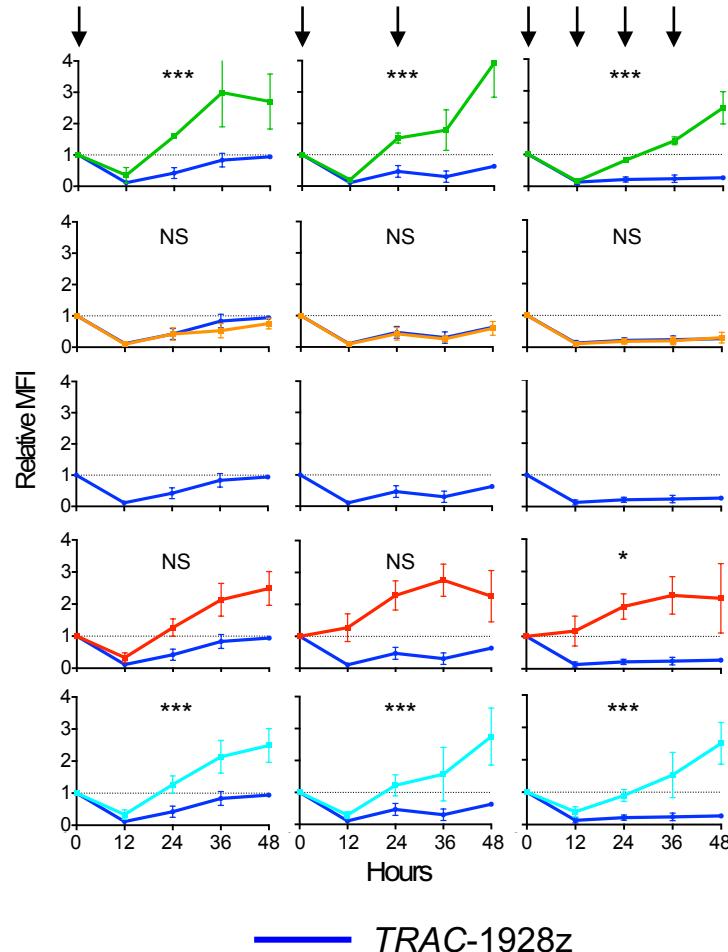
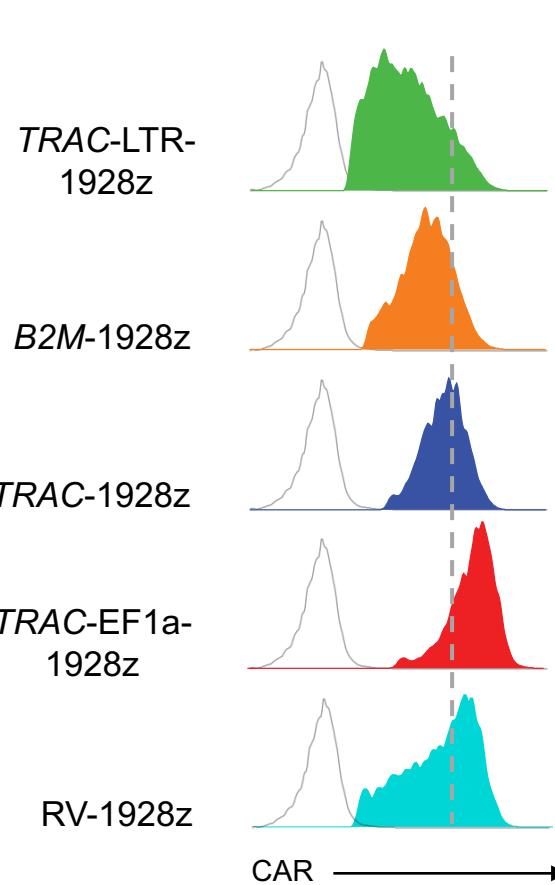
# CAR expression level is critical to prevent differentiation upon multiple stimulation



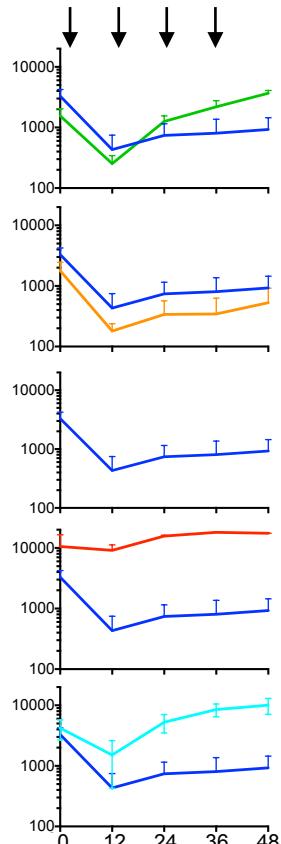
## CAR expression is down-regulated upon exposure to antigen



# Antigen-induced CAR expression follows different patterns at different loci and with different promoters



Absolute CAR MFI

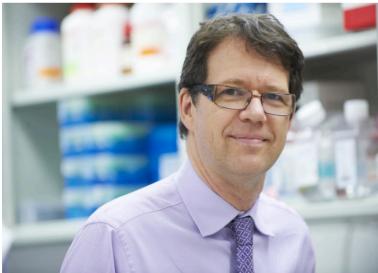


# Conclusions

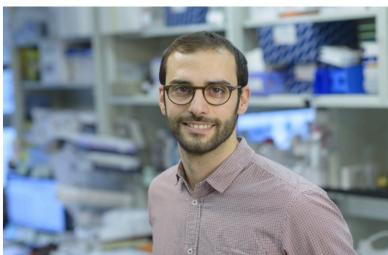
- *TRAC-CAR* T cells show enhanced *in vivo* anti-tumor activity compared to conventionally engineered CAR T cells
- The TCR alpha promoter provides highly homogeneous and optimal control of 19-28z CAR expression
- *TRAC-CAR* sustains functional persistence by averting rapid T cell differentiation and exhaustion
- CAR cell surface expression depends on both promoter strength and regulation
- Our findings on *TRAC-CAR* regulation highlight the value of precision engineering to advance T cell-based medicine
- **CAR-T cell manufacturing:**
  - Developed a clinical-scale T-cell CRISPR-editing step, where 85 millions TCR<sup>KO</sup> T cells are generated with a single electroporation
  - Simultaneous CAR knock-in and TCR knock-out enables allogeneic application

# Acknowledgments

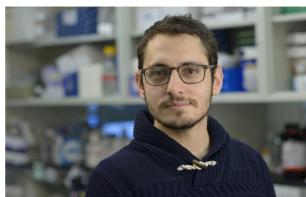
## Michel Sadelain lab Center for Cell Engineering



**Justin Eyquem**  
Research Fellow



**Theodoros Giavridis**  
Graduate Student



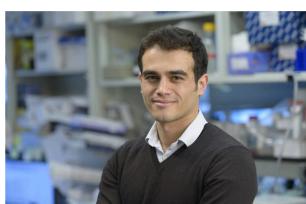
**Mohamad Hamieh**  
Research Fellow



**Sjoukje Van der Stegen**  
Research Fellow



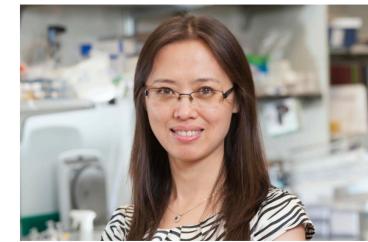
**Ashlesha Odak**  
Graduate Student



## Isabelle Rivière lab Center for Cell Engineering



**Xiuyan Wang**  
Assistant Director



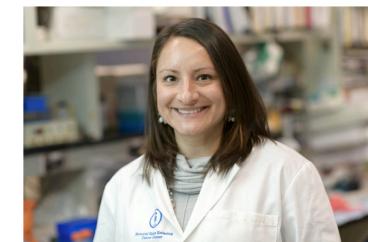
**Kristen Cunanan**



## Mithat Gönen lab Biostatistics Service



**Susan Zabierowski**



## Acknowledgments

- Lake Road Foundation
- Mr. William H. and Mrs. Alice Goodwin and the Commonwealth Foundation for Cancer Research
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- NYSTEM (New York State Stem Cell Science)
- NYSCF (New York Stem Cell Foundation)
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