A Method for Highly Efficient Knock-in and Expression of Transgene Cargos for Next-Generation Cell-based Medicines

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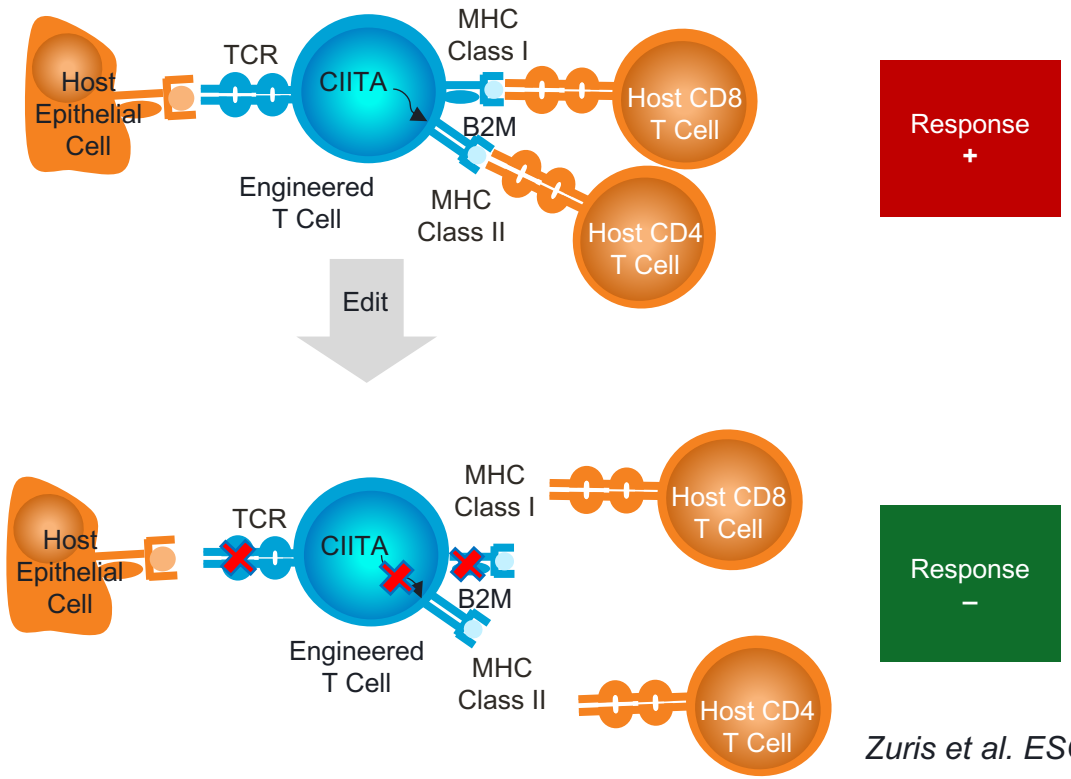
Disclosure

I am an employee and shareholder of Editas Medicine

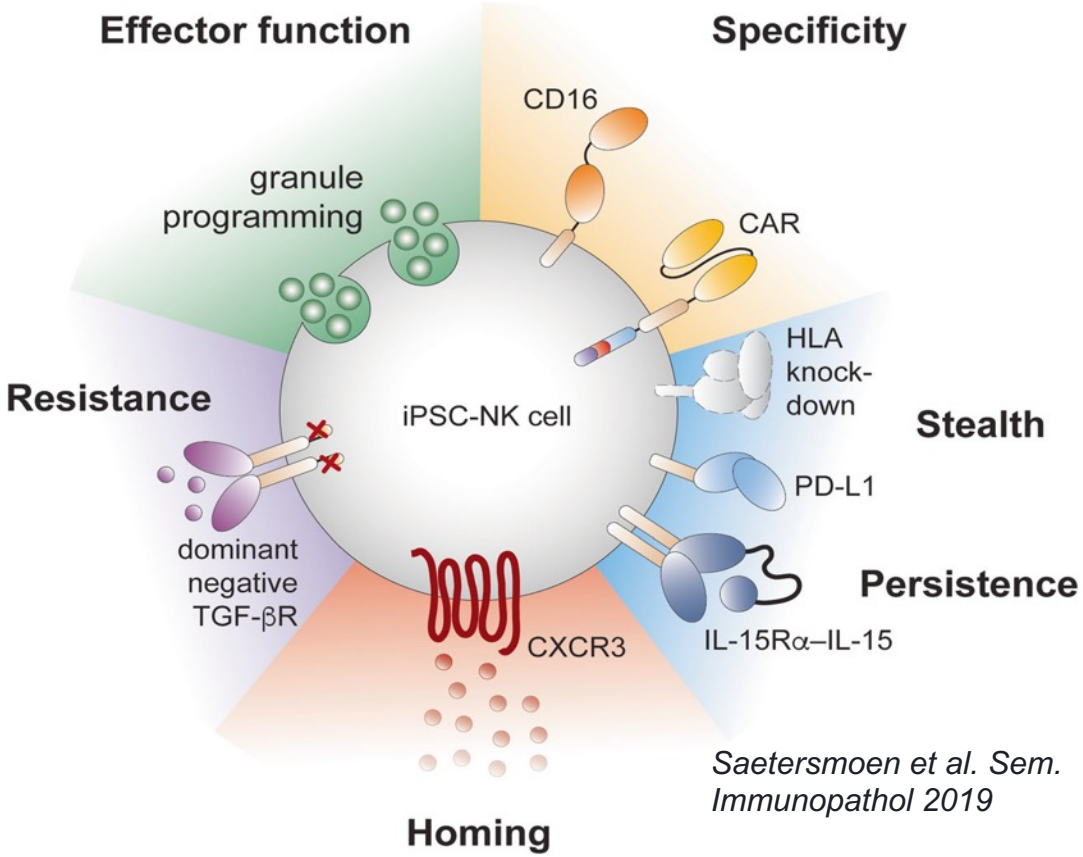
Multiplexed Gene Editing Should Generate the Best Cell Therapies

Proposed Allogeneic T cell Edits



Zuris et al. ESGCT 2018

Proposed iNK Edits



Saetersmoen et al. Sem. Immunopathol 2019

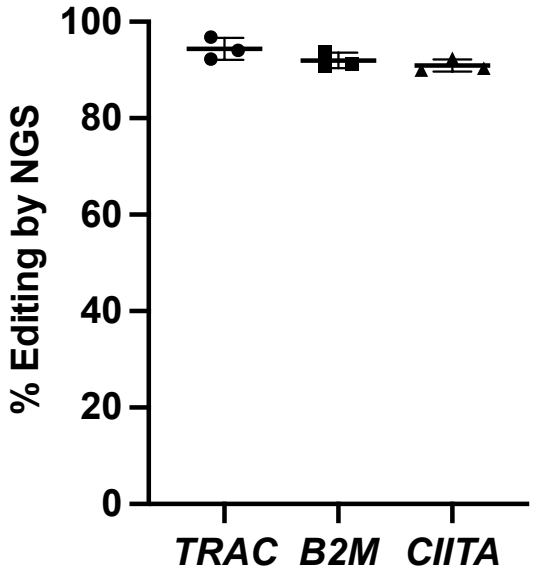
The high activity and specificity of our engineered AsCas12a enables highly multiplexed editing

For more on engineered AsCas12a, see Zhang, Zuris et al. Nat Commun 2021

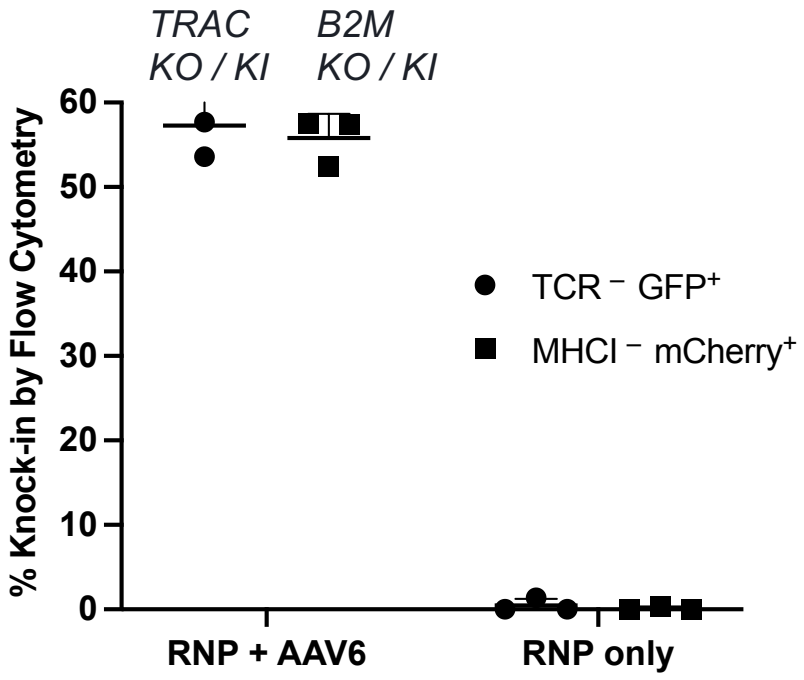
Despite Major Progress Efficient Knock-in Continues to be a Challenge

T cells

Robust multiplexed editing

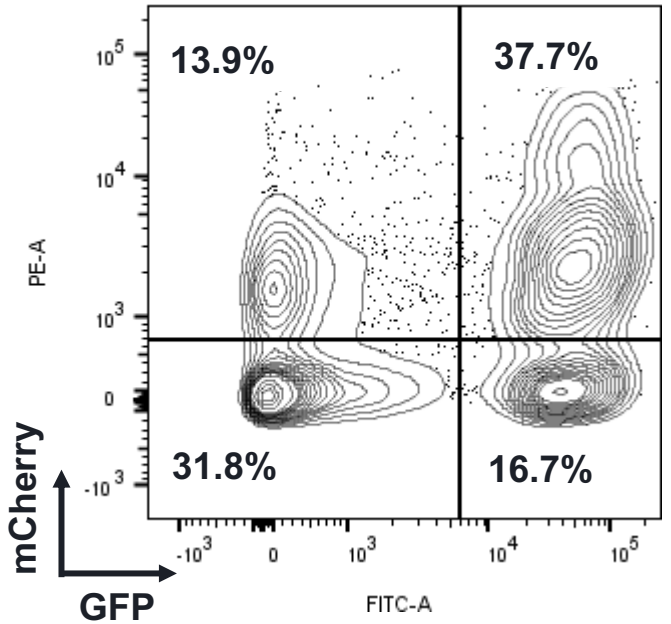


Transgene Knock-in with AAV6



Dual Knock-in with AAV6

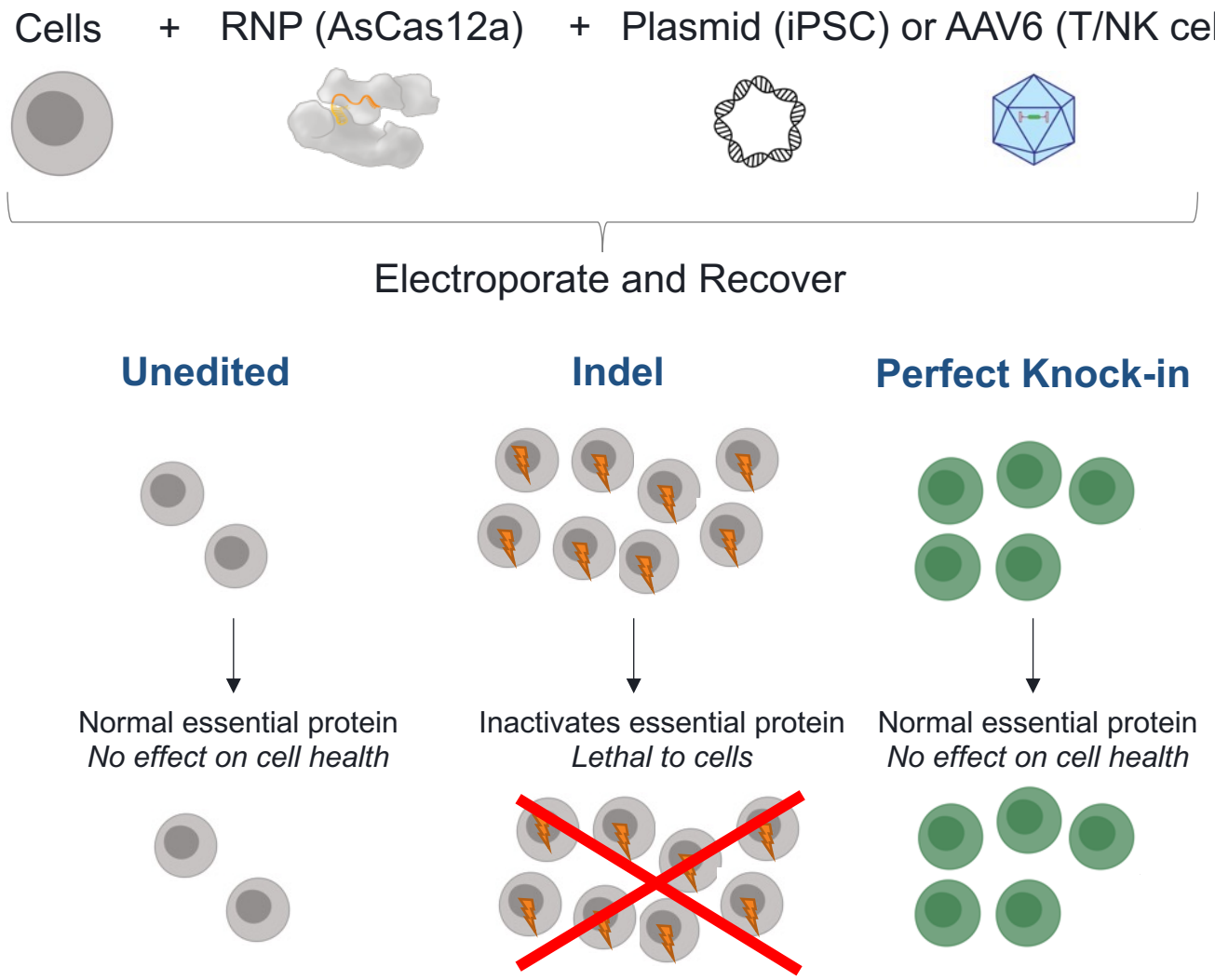
TRAC KO / GFP KI and
B2M KO / mCherry KI



Zhang, Zuris et al. Nat Commun 2021

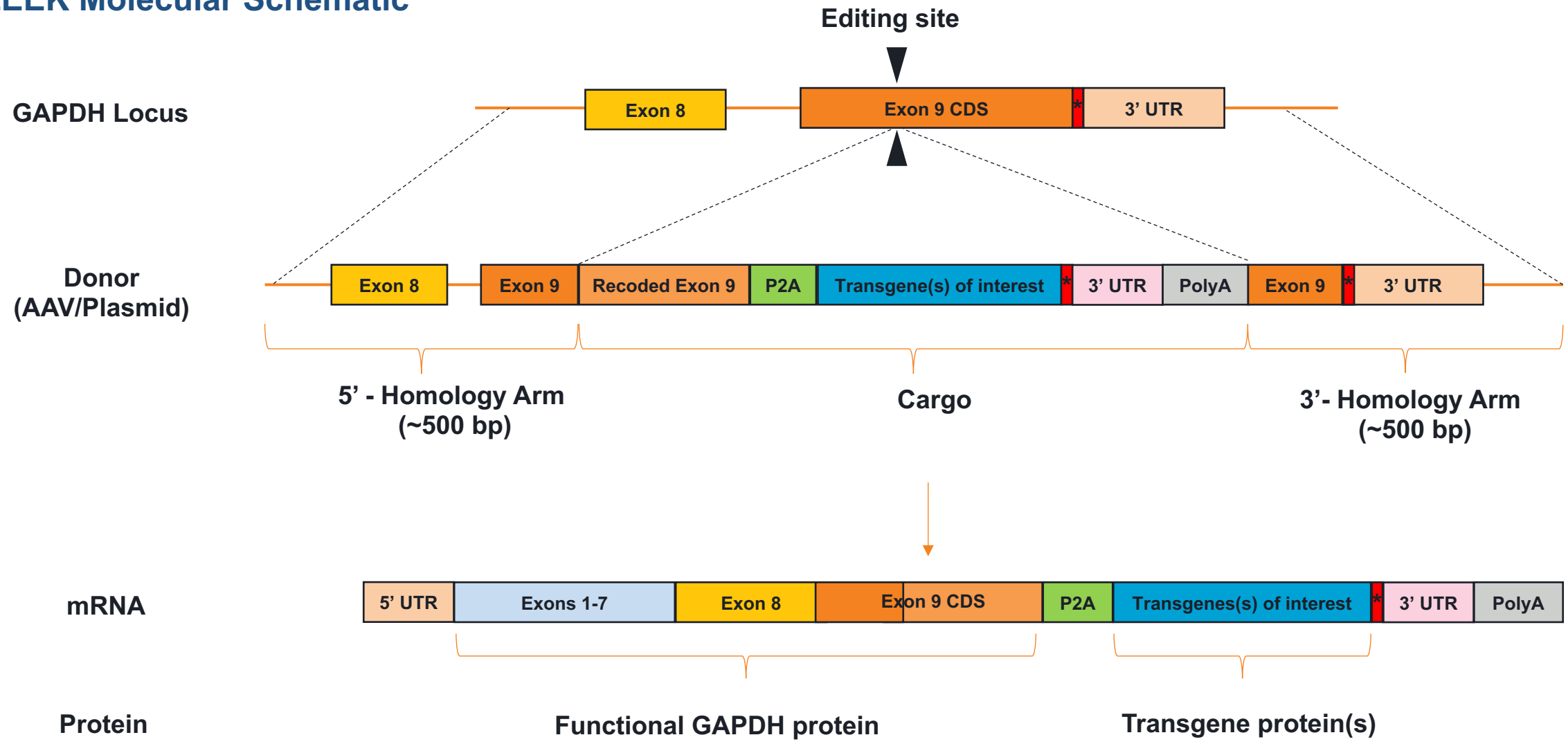
What if We Could Overcome this Knock-in Challenge?

- Desired Capability:
  - Selection for knock-in over indel edits
  - High-level constitutive expression
- Key Criteria
  - High-level constitutive gene expression at the locus must be critical for cell survival
  - Editing (NHEJ) rates must be high and this can be achieved with our engineered AsCas12a
  - Indels lead to disruption in protein function so that indel types are lethal without repair (HDR)



The Method is Called SLEEK: SeLection by Essential-gene Exon Knock-in

SLEEK Molecular Schematic

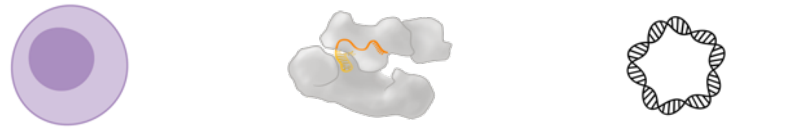


SLEEK Achieved >95% Knock-in and Enables Tunable Cargo Expression

iPSCs

SLEEK Reduced to Practice

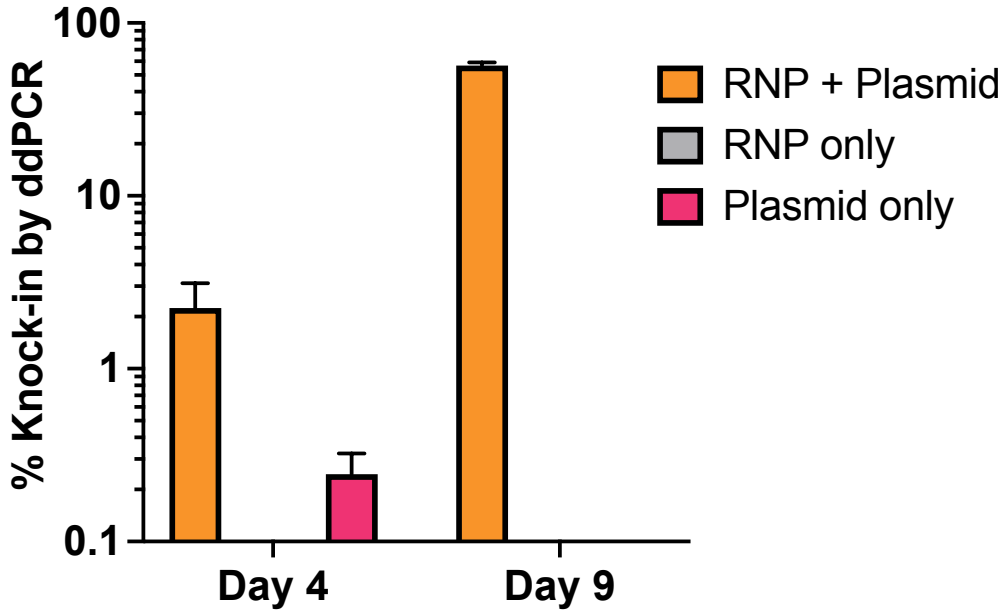
iPSCs + SLEEK RNP + Plasmid (iPSC)



Electroporate and Recover for 4 – 9 days

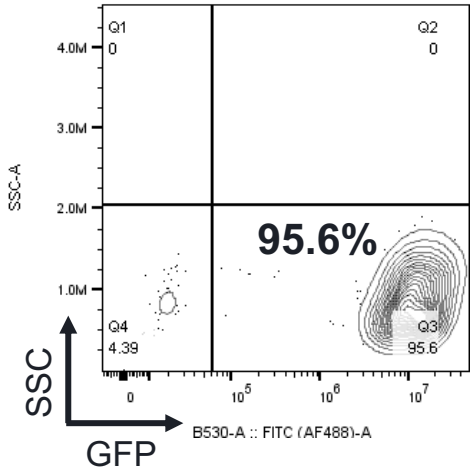
Flow Cytometry
ddPCR

SLEEK Knock-in Time Course

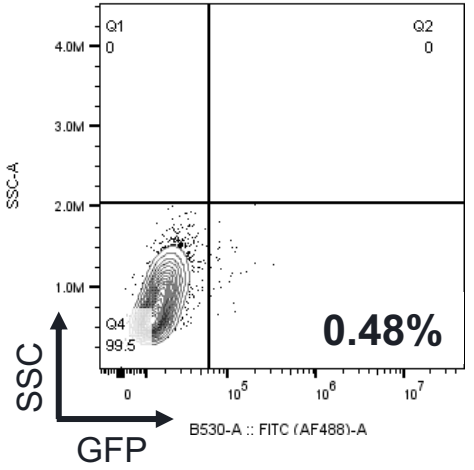


Tunable Cargo Expression with SLEEK

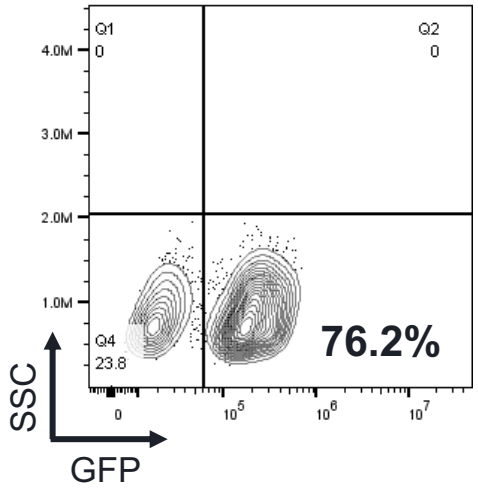
GAPDH RNP + plasmid



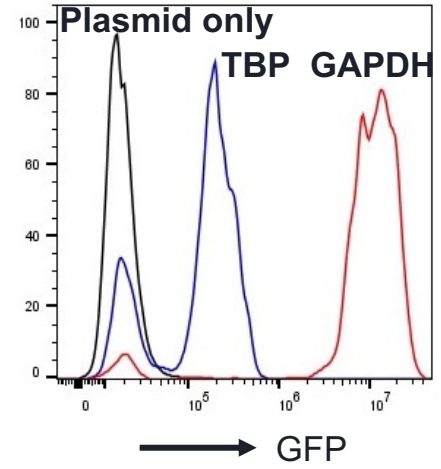
Plasmid only



TBP RNP + plasmid



KI at different genes



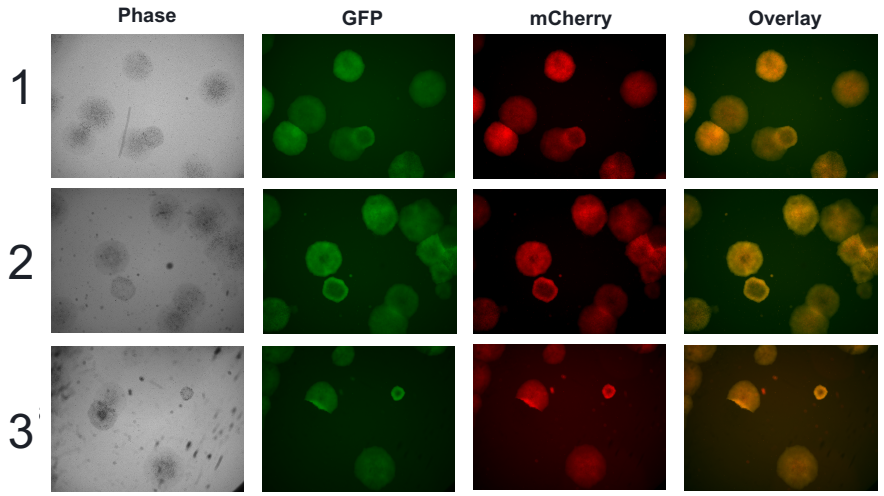
Efficient Multicistronic Knock-in Simplifies iPSC Clone Selection Process

iPSCs

Robust Knock-in and Expression of Multicistronic Cargos

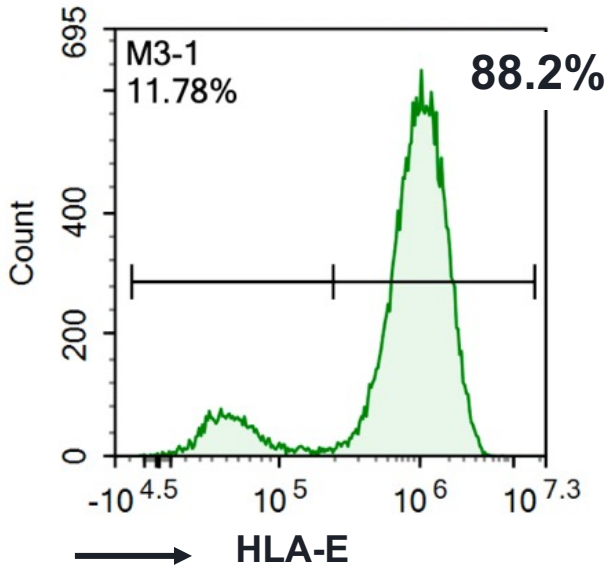
Cargo: HA-GFP-P2A-mCherry-HA

iPSC clonal colonies



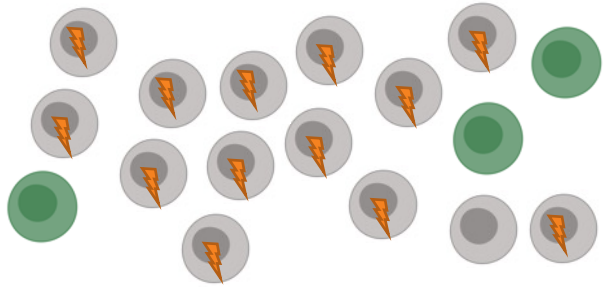
Robust Knock-in and Expression of Key Functional Cargo in iPSCs

HLA-E Allo Shield

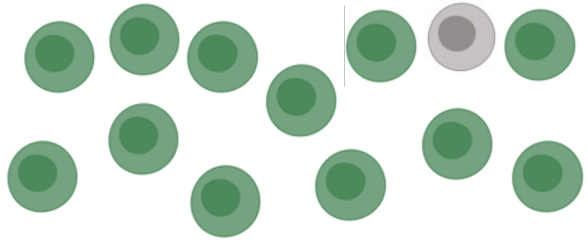


SLEEK Simplifies the iPSC Clone Selection Process

Current Low KI Methods

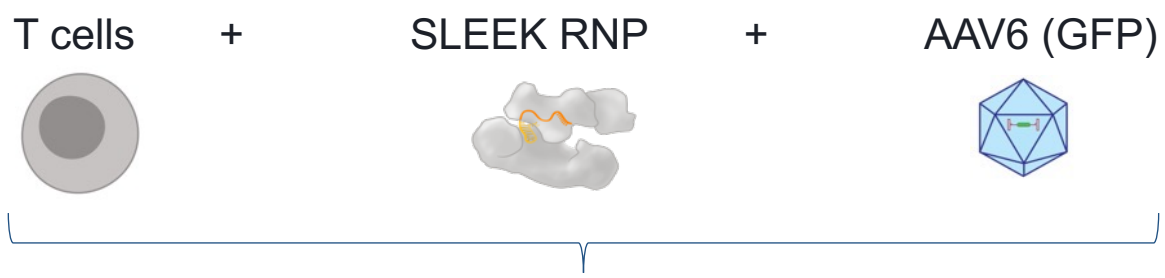


SLEEK KI Method



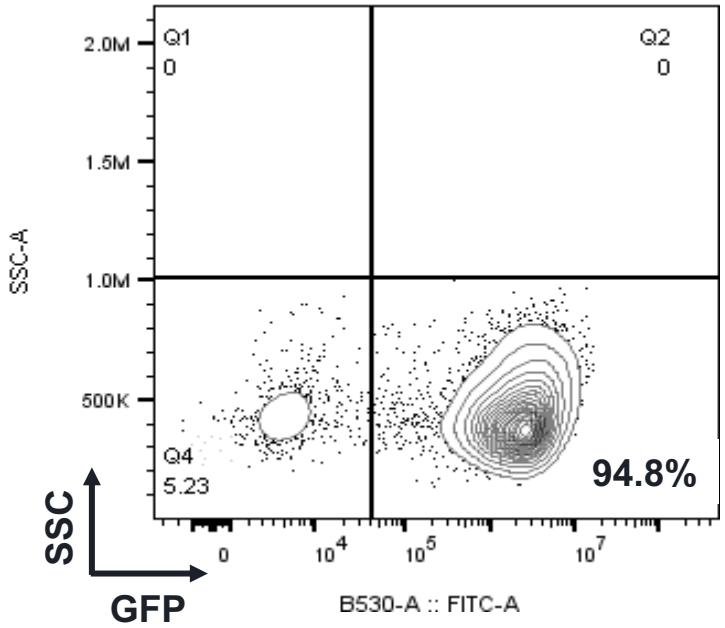
SLEEK Shows Superior Knock-in to Current State-of-the-Art Methods

T cells



Flow Cytometry
Cell viability and expansion

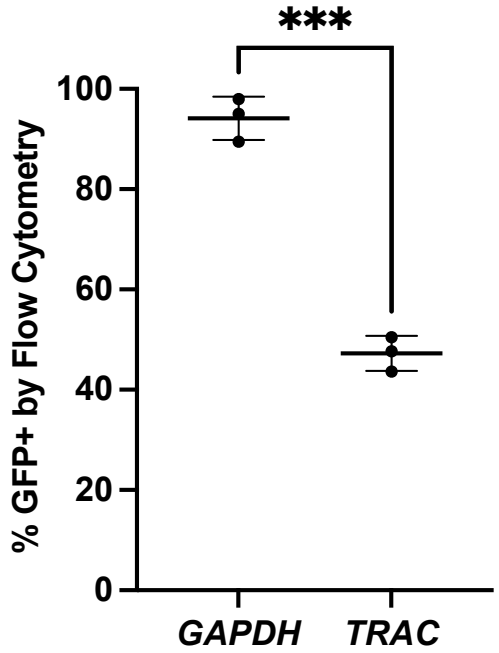
SLEEK Efficiency in T cells



Expansion and Viability Data

	Mock	RNP + AAV6
% Viability day 0	94	90.5
% Viability day 7	93.5	86.5
Fold-change in expansion day 7	2.4	2.1

Comparison to *TRAC* Knock-in

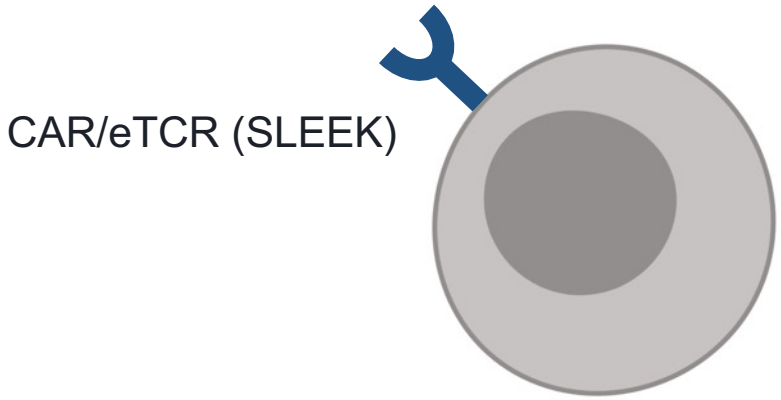


N = 3 biological replicates
p < 0.001, Unpaired t-test

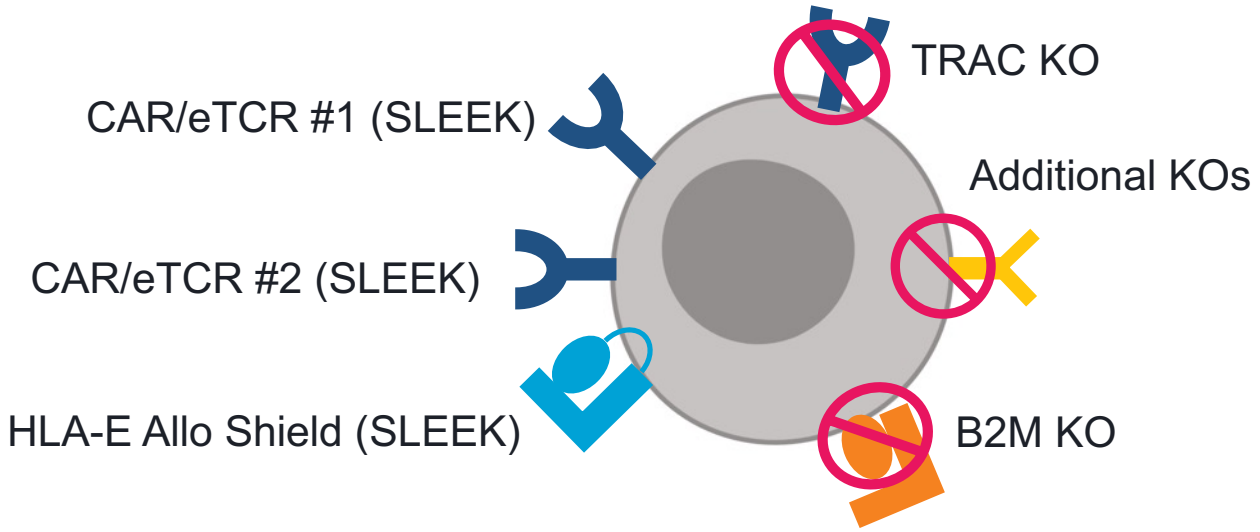
SLEEK Enables Engineered T cells with High Overall Homogeneity

T cells

Autologous Applications



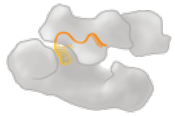
Allogeneic Healthy Donor Applications



Demonstration of TRAC Edit and SLEEK Knock-in of CD19 CAR

T cells

T cells + TRAC and SLEEK RNPs + AAV6 (CD19 CAR)

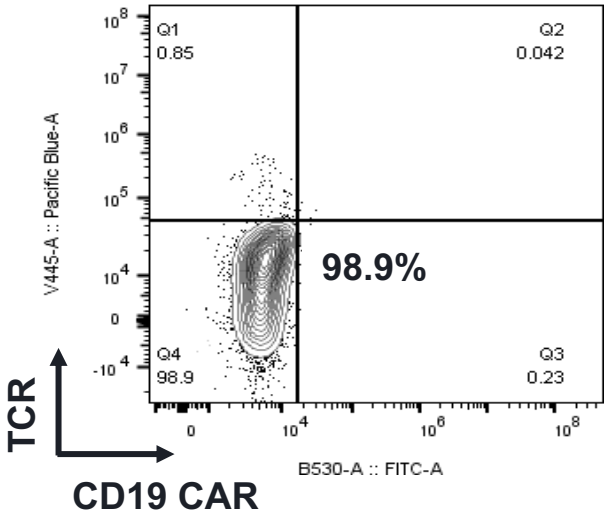


Electroporate and Recover for 4 – 7 days

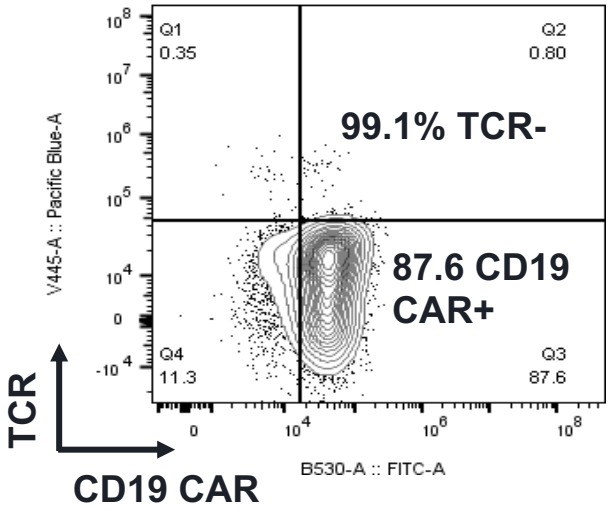
→ Flow Cytometry
Tumor Killing Assay

Near-Homogeneous Editing Outcomes Achieved with SLEEK

TRAC KO only

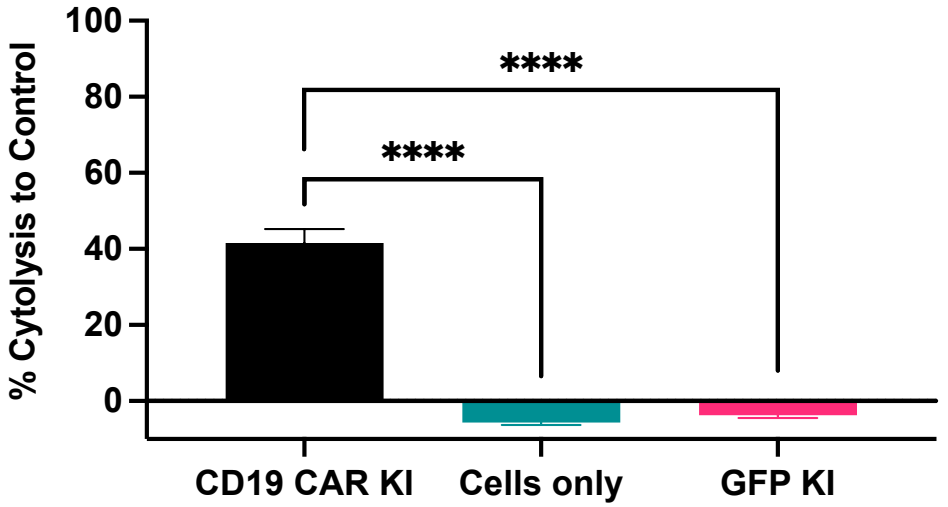


TRAC KO + CD19 CAR KI



CD19 CAR SLEEK KI Enables Potent Tumor Killing

Killing of CD19+ Raji Cells



LDH assay after 24 hours, N = 4, 1 biological replicates in 4 technical replicates, +/- SEM, one-way ANOVA, ****, p < 0.0001

Knock-in of Allo Shield to Protect Against Host NK and T Cell Responses

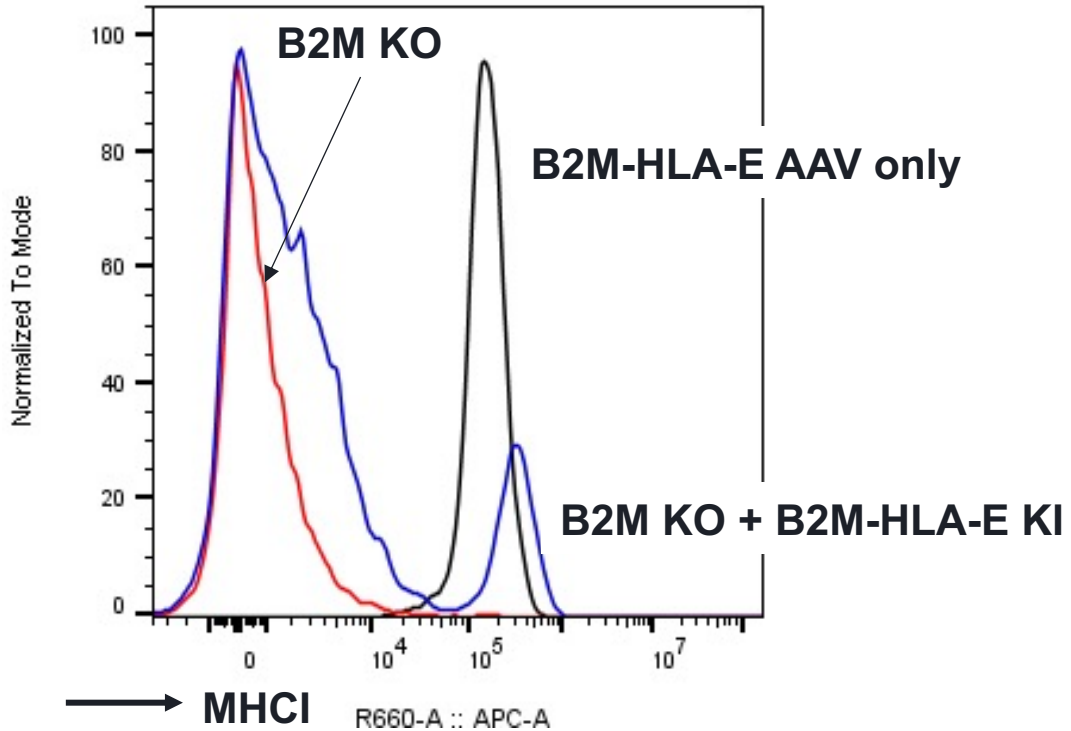
T cells

T cells + B2M and SLEEK RNPs + AAV6 (B2M-HLA-E Allo Shield)

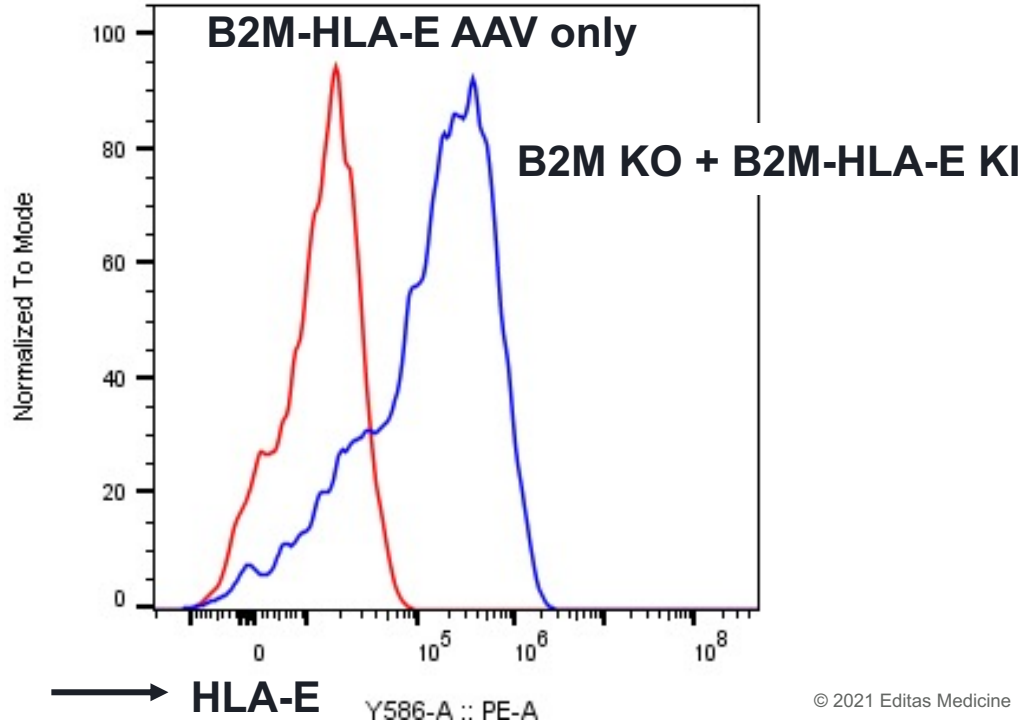
Electroporate and Recover for 4 – 7 days

→ Flow Cytometer
ddPCR
NGS

Effect of B2M KO on Overall MHCI Surface Expression



SLEEK KI Shows Increased Expression of HLA-E

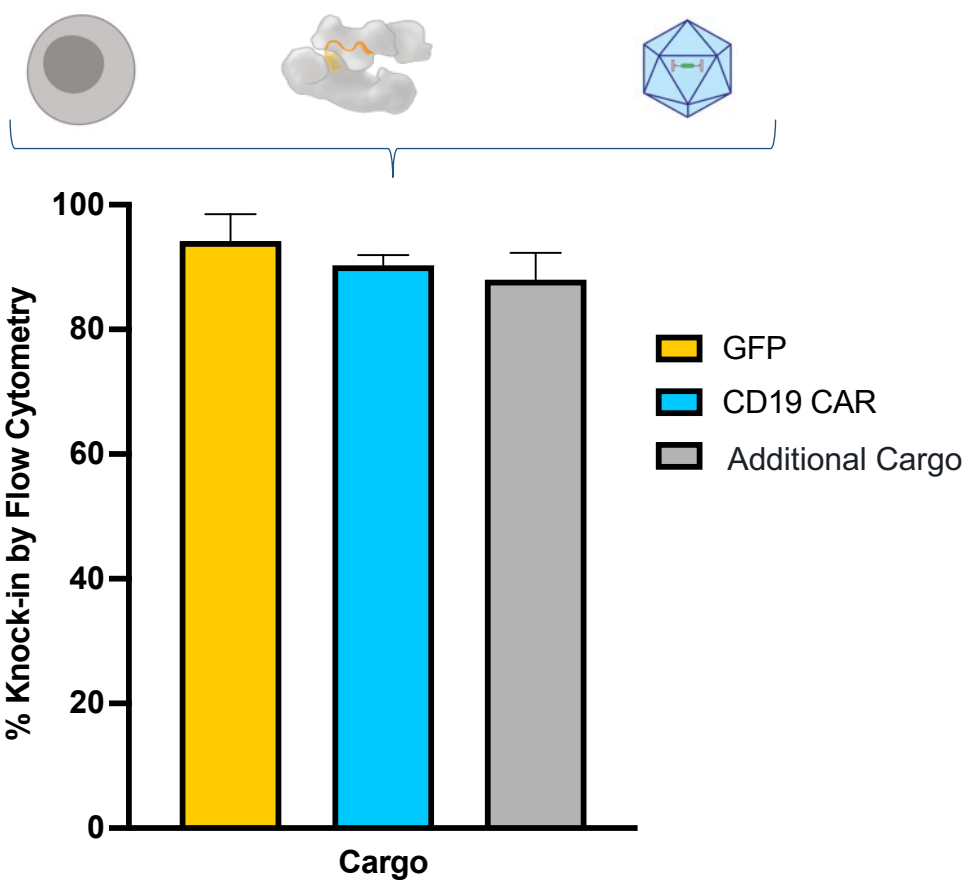


SLEEK Enables the Generation of Near-Homogeneous Edited T Cells

T cells

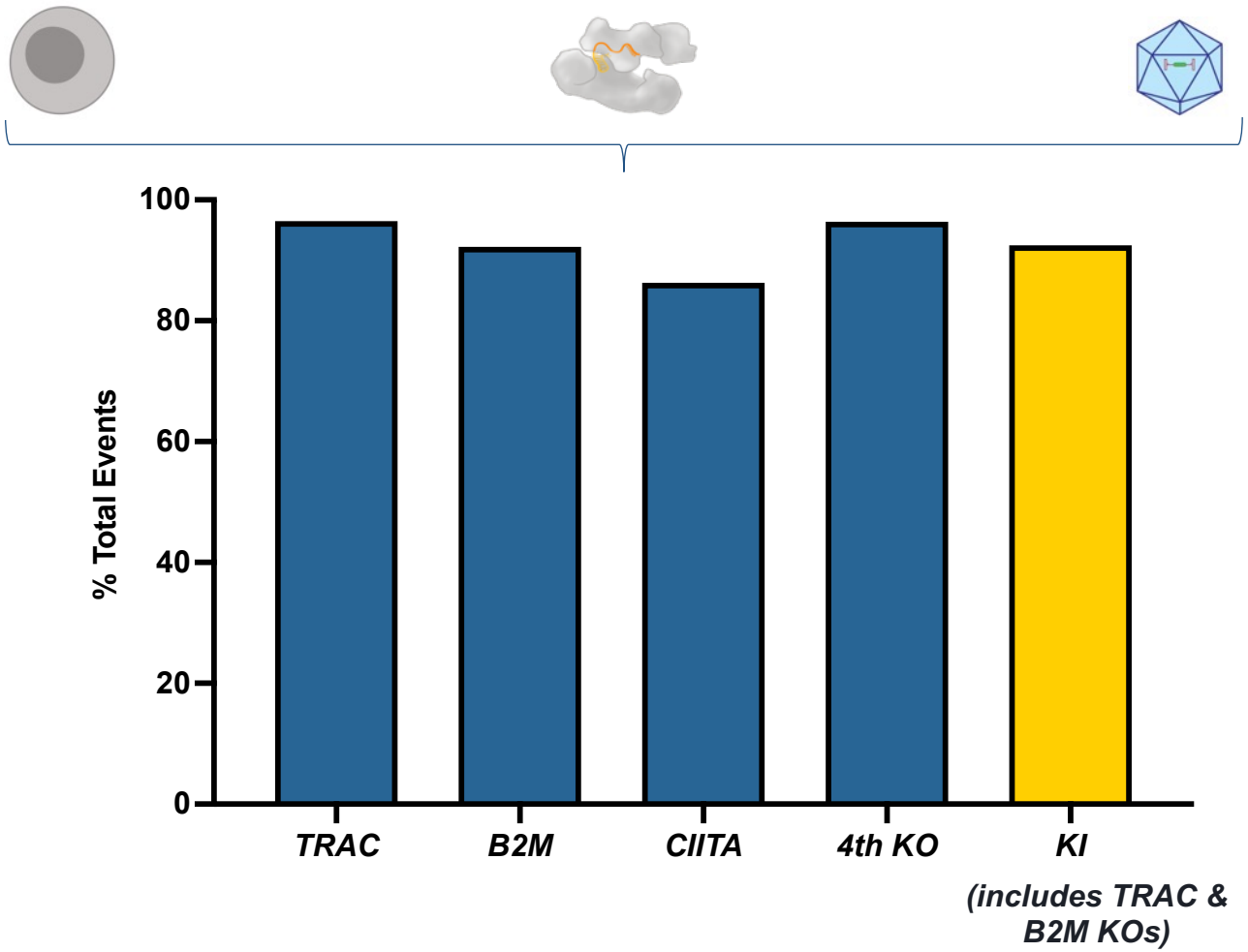
Autologous T cell Applications: Efficient SLEEK KI of Different Cargos

T cells + SLEEK RNP + AAV6 (Cargo)



Allogeneic Healthy Donor T cell Applications: Demonstration of Multiplex KO + SLEEK KI

T cells + TRAC, B2M, CIITA, 4th KO + SLEEK RNPs + AAV6 (Cargo)



Demonstration of CD19 CAR Knock-in in NK cells with SLEEK

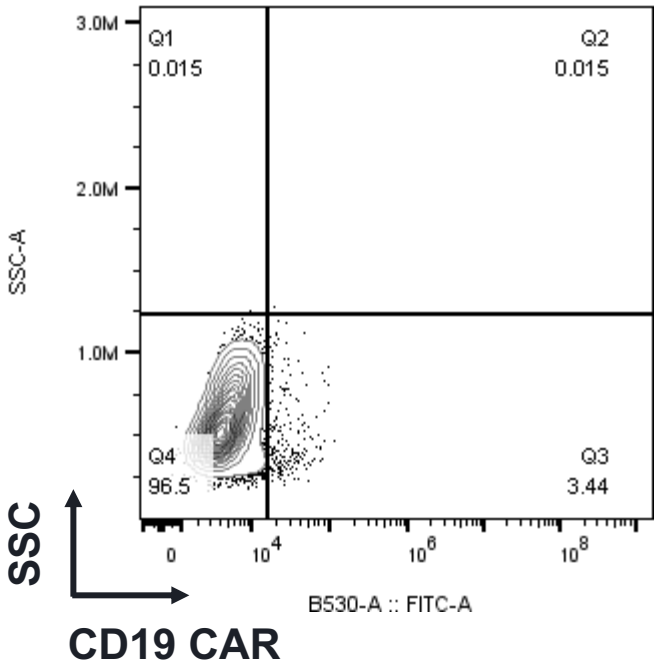
NK cells

NK cells + SLEEK RNP + AAV6 (CD19 CAR)

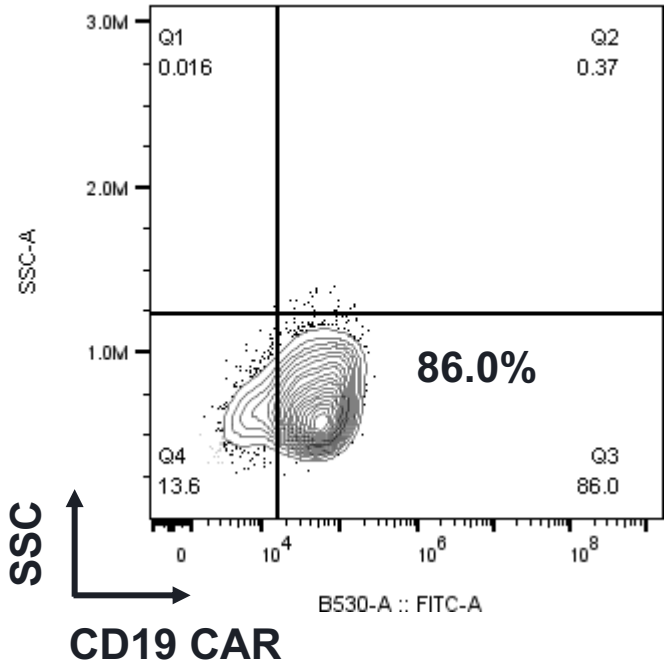
Electroporate and Recover for 4 – 7 days

Flow Cytometry
Tumor Killing Assay

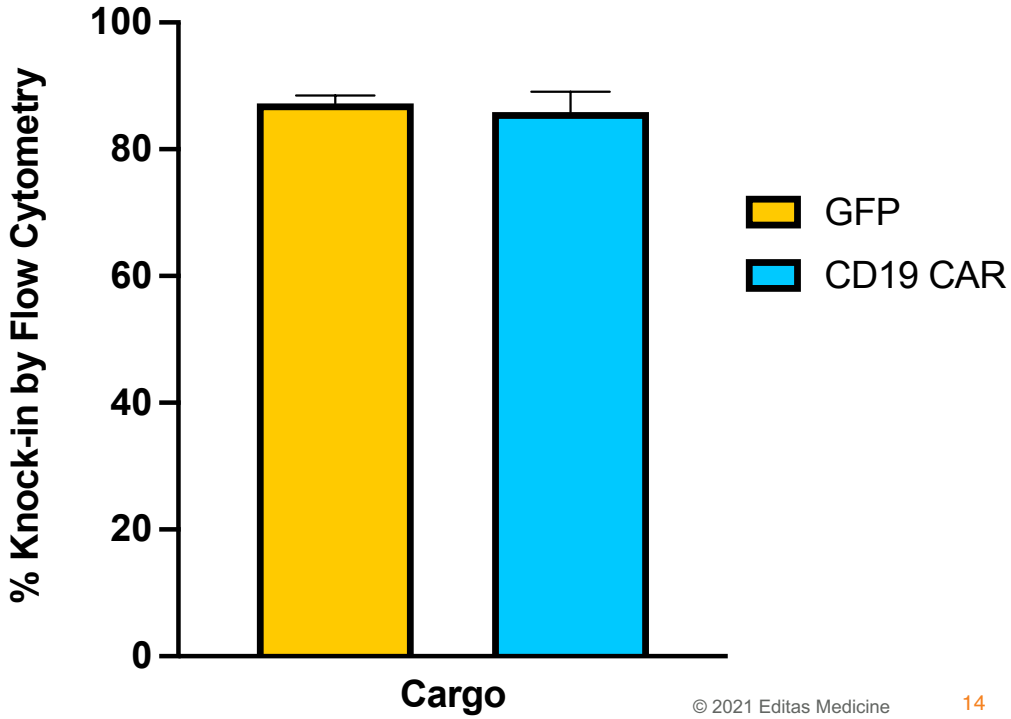
CD19 CAR AAV only



CD19 CAR AAV + SLEEK RNP



SLEEK KI in NK Cells

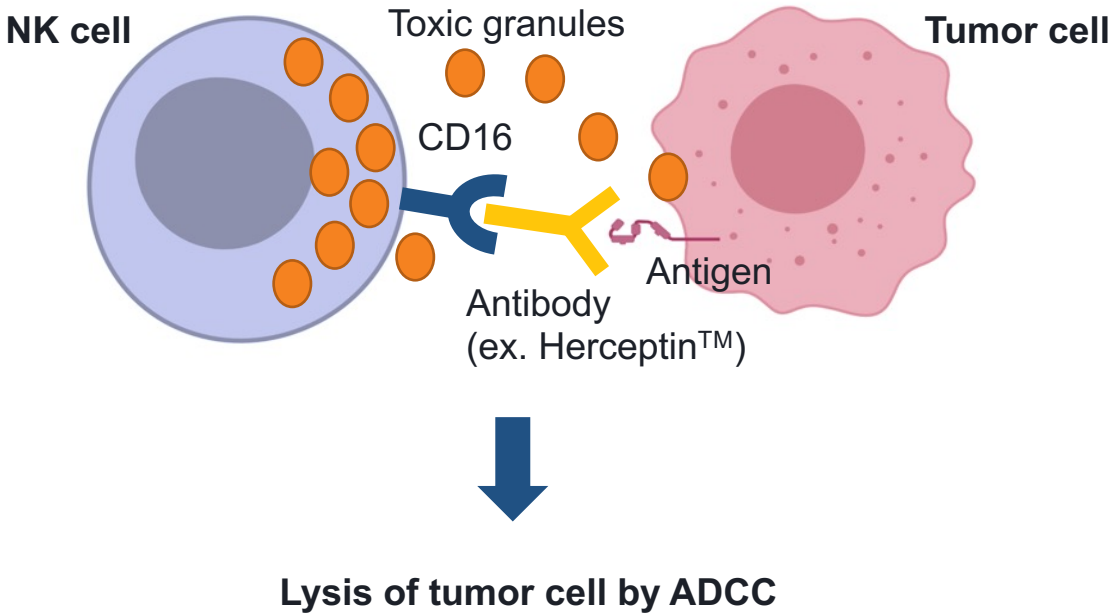


SLEEK Method to Ensure Robust Surface Expression of CD16 in iNKs

iNK cells

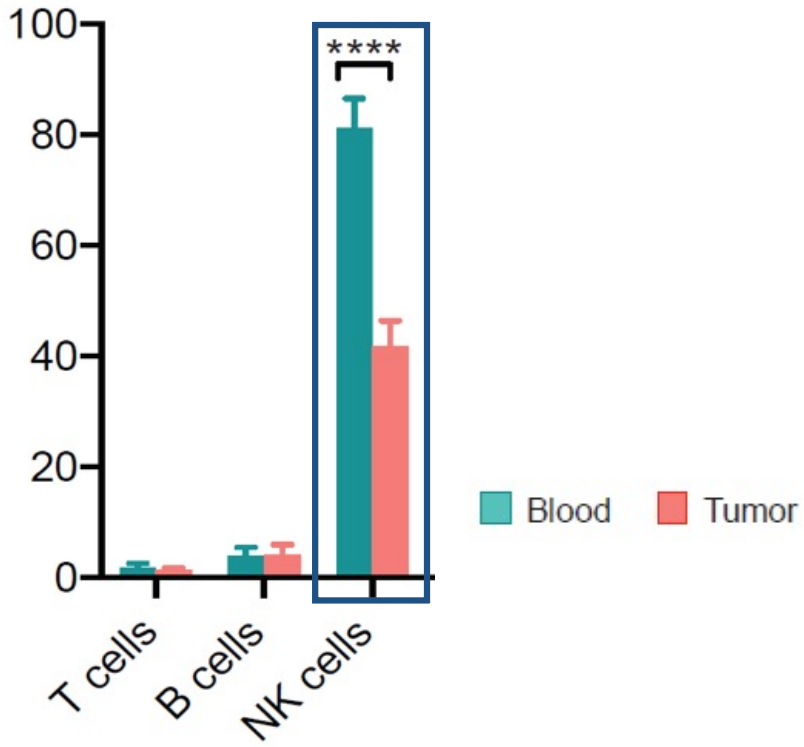
Importance of CD16 for NK Tumor Killing

Antibody-dependent cellular cytotoxicity (ADCC)



Lysis of tumor cell by ADCC

Loss of CD16 Expression on NK cells in Tumors

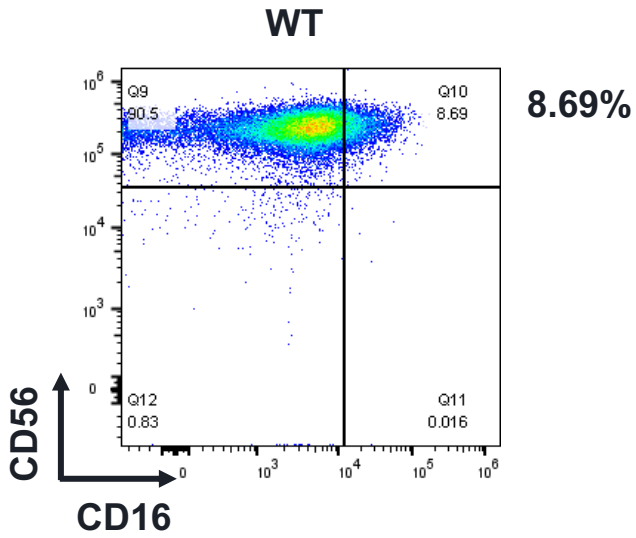


Vargas et al. Cancer Cell 2018

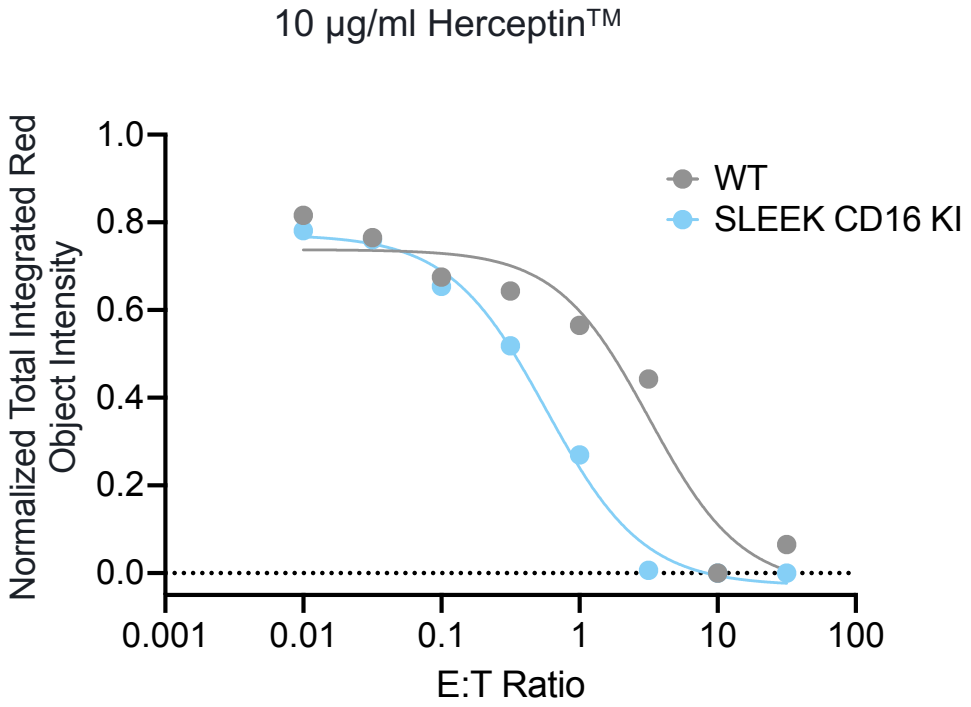
Robust Constitutive Expression of CD16 in iNKs Led to Improved ADCC

iNK cells

Robust Expression of CD16 in knock-in iNKs



CD16 KI iNK Cells Show Improved ADCC



Cell Line	IC50
WT	3.0
SLEEK CD16 KI	0.5

Summary of SLEEK Technology

KNOCK-IN IS DRIVEN BY RESTORATION OF EXON	▪ Exon repair through HDR is required for survival after editing at an essential gene ▪ Cells with disruptive indels in the essential gene are selected against ▪ SLEEK is enabled by an efficient and specific engineered AsCas12a nuclease
HIGH LEVEL EXPRESSION OF MULTIPLE CARGOS	▪ GAPDH knock-in offers highly robust cargo expression ▪ P2A and other multicistronic elements can be used to expressed multiple proteins ▪ No exogenous promoter needed with SLEEK
SLEEK KNOCK-IN EFFICIENCY IS HIGHEST IN THE FIELD	▪ Knock-in rates of 90% shown with AAV6 in iPSCs, T cells and NK cells ▪ SLEEK CD16 KI iNKs maintained high CD16 surface expression during ADCC ▪ Currently focused on leveraging this powerful technology across many programs

We're Hiring

ABOUT EDITAS MEDICINE

Pioneering the Possible

Editas Medicine is a leading genome editing company focused on translating the power and potential of the CRISPR/Cas9 and CRISPR/Cpf1 (also known as Cas12a) genome editing systems into a robust pipeline of medicines for people living with serious diseases around the world. Editas Medicine aims to discover, develop, manufacture, and commercialize transformative, durable, and precise genomic medicines for a broad class of diseases.

Our corporate headquarters is located in Cambridge, MA, and we have a significant and growing site in Boulder, CO.

We are pioneering the possibilities of genomic medicines through gene editing. Jump down to learn more about our work and the people who make it possible:

The Editas Medicine Mission

Our mission is to translate the power and potential of genome editing into a broad class of gene edited medicines that transform lives of people living with serious diseases.

Come work on SLEEK and several other amazing technologies that we are leveraging to help bring medicines to patients!

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