

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

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editas MEDICINE

# Disclosure

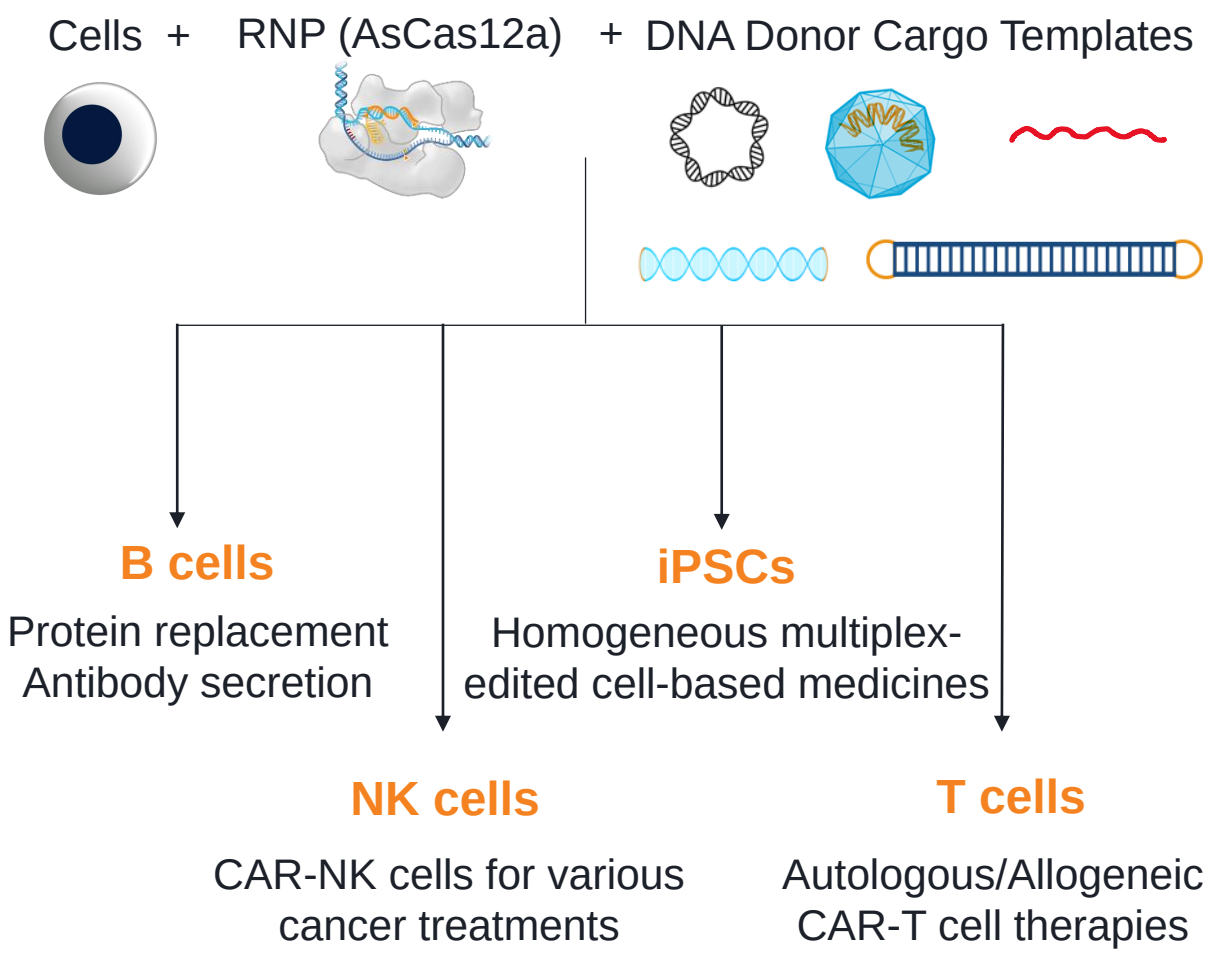
I am an employee and shareholder of Editas Medicine

# Our Goal was to Develop an Editing Technology That Could Fundamentally Improve the Generation of Cell-Based Medicines

SLEEK™ GENE EDITING

**SeLection by Essential-gene Exon Knock-In**

- Enables >95% knock-in efficiency
- High-level, tunable cargo expression
- Homogeneous editing
- Efficient multicistronic cargos
- Simplifies iPSC clone selection process
- Robust, lineage-independent, expression of functional cargo in iPSCs




CAR: chimeric antigen receptor; Cas: CRISPR-associated protein; iNK: IPSC-derived natural killer cell; iPSC: induced pluripotent stem cell; NK: natural killer; RNP: ribonucleoprotein; TME: tumor microenvironment.

# The Specificity Case for Selecting AsCas12a Over SpCas9

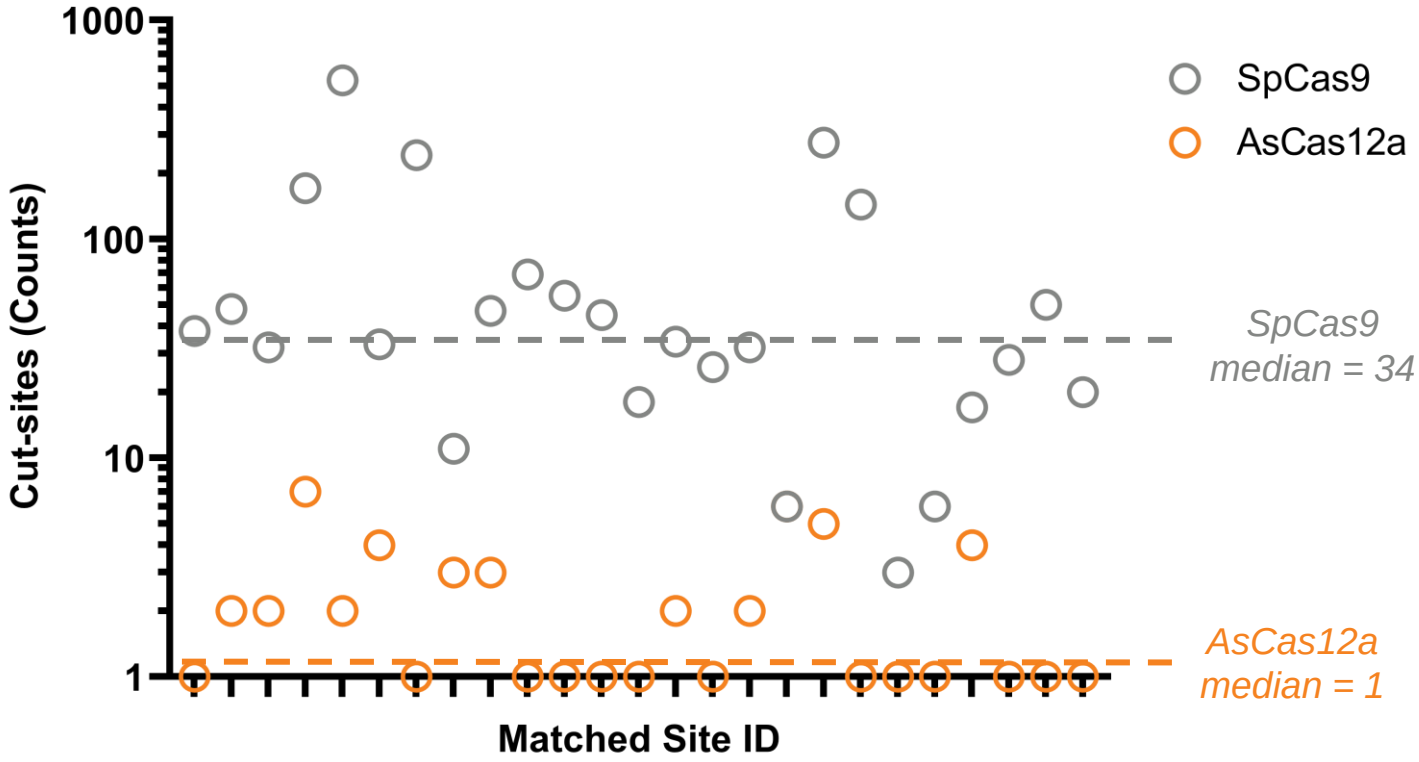
## Experimental Design to Assess Specificity

- Assessed using Digenome-Seq—a fully reconstituted cutting and WGS detection assay
- Assayed 25 randomly selected “matched sites” in the genome, and run at saturating RNP concentration and time

**Matched Target Site** (20-Ns):
**TTTV**NNNNNNNNNNNNNNNNNNNN**NGG**

**AsCas12a PAM** **SpCas9 PAM**

## Digenome-Seq with 25 Matched Genomic Sites




*Gotta et al. Cold Spring Harbor 2019*

**AsCas12a is 10-100x More Specific Than SpCas9**

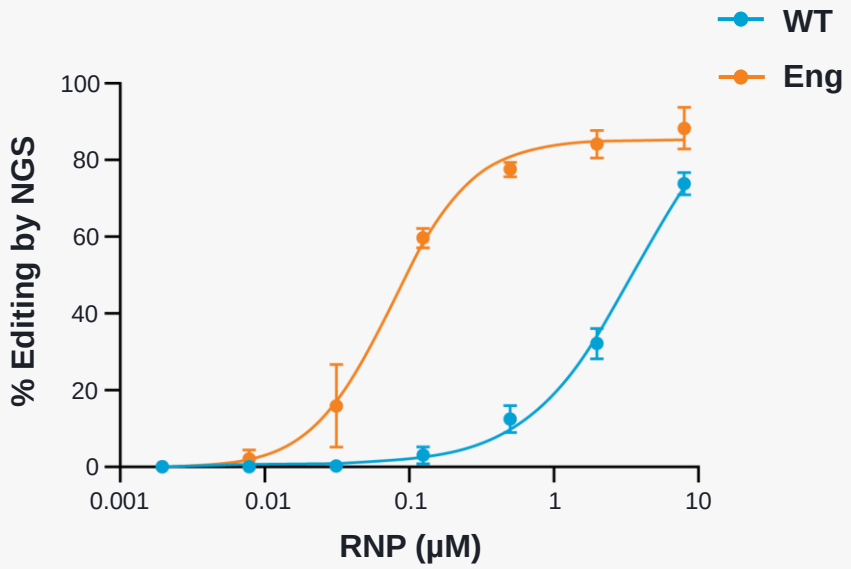
RNP: ribonucleoprotein; PAM: protospacer adjacent motif; Cas: CRISPR-associated protein; WGS: whole genome sequencing.

For more on Cas12a superior specificity to SpCas9, see: Kim et al. *Nat Biotech* 2016; Kleinstiver et al. *Nat Biotech* 2016; Kim et al. *Nat Methods* 2017; Strohkendl et al. *Mol Cell* 2018; Zhang et al. *Nat Comms* 2021.

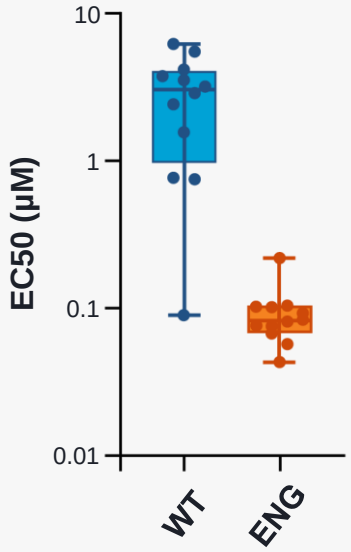
# Engineered AsCas12a Shows Robust Efficiency and Potency

*T cells*

## AsCas12a Engineering Results in a 2-Log Potency Improvement




## All Guides Screened are Highly Active with Eng. AsCas12a




**Engineered AsCas12a PAM Variants are Also Available, Further Expanding Target Space**

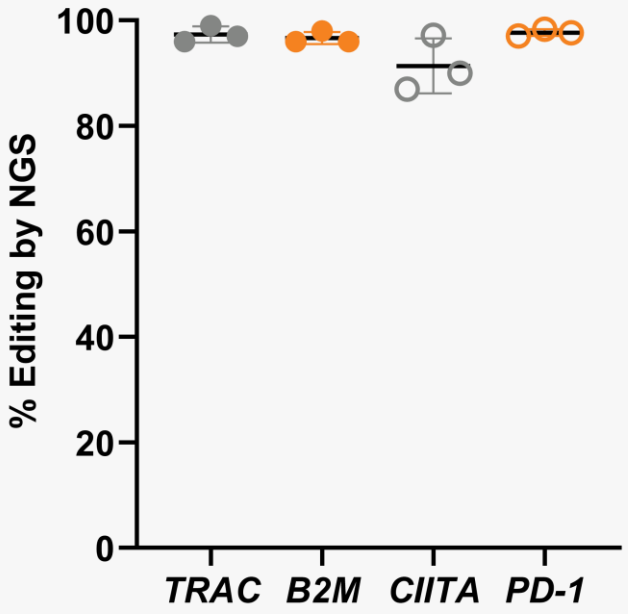
Cas: CRISPR-associated protein; Eng: engineered; NGS: next generation sequencing; RNP: ribonucleoprotein; WT: wildtype.

Zhang, Zuris et al. *Nat Commun.* 2021

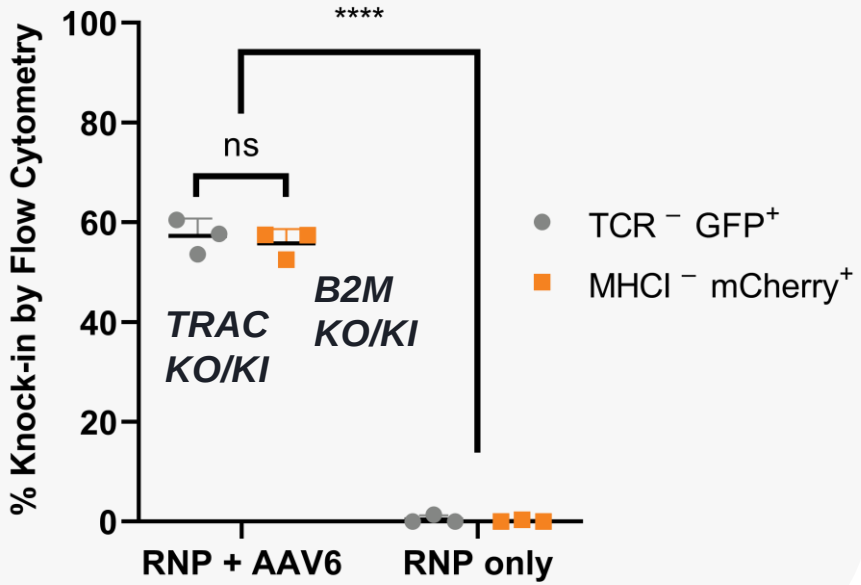
# Despite Major Progress, Efficient Knock-in Remains a Challenge

***T cells***

## Robust Multiplexed Gene KO

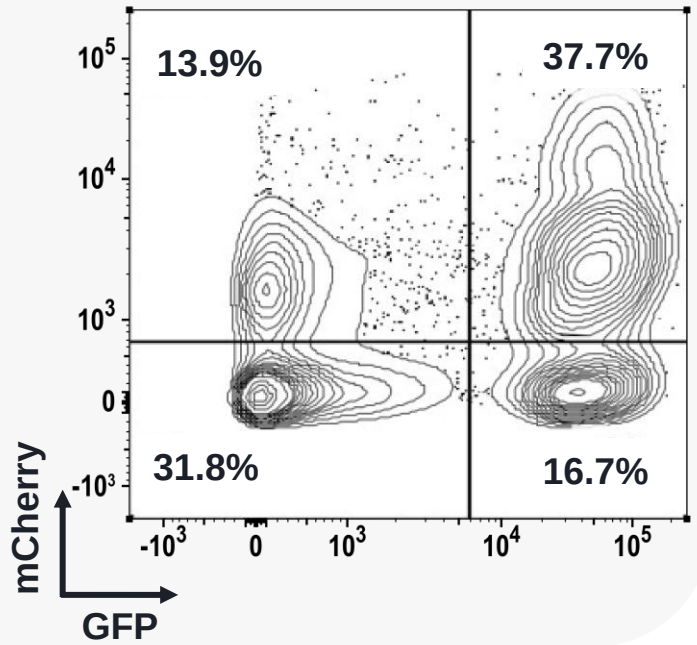



## Transgene Knock-in with AAV6




## Dual Knock-in with AAV6

***TRAC KO / GFP KI and B2M KO / mCherry KI***




**Impressive KO Results Near 100%, Single Knock-in ~60%, Double Knock-in ~40%**

AAV: adeno-associated virus type 6; B2M; beta-2 macroglobulin; CIITA: class II major histocompatibility complex transactivator; GFP: green fluorescent protein; KI: knock-in; KO: knock-out; NGS: next generation sequencing; MHC: major histocompatibility complex; PD: programed cell death protein; RNP: ribonucleoprotein; TRAC: T-cell receptor α constant

# What if We Could Overcome This Knock-in Challenge?

**SLEEK: SeLection by Essential-gene Exon Knock-in**

## Desired Capability

- Selection for knock-in over indel edits
- High-level constitutive expression of cargo(s)

## Key Criteria

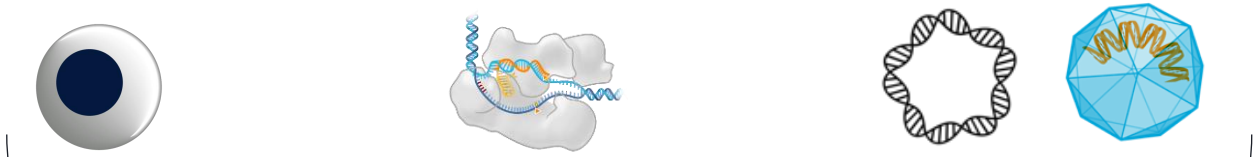
- Indels are lethal
- Editing (NHEJ) rates must be high
- High-level constitutive promoter

**Editing Overview**

Cells of interest

RNP (Engineered AsCas12a)

DNA Donor Template Example: Plasmid or AAV6



Electroporate and Recover for ~7 Days

**Editing Outcomes**

**Unedited**

Normal essential protein
*No effect on cell health*

Positive Selection

**Indel (NHEJ)**

Inactivates essential protein
*Lethal to cells*

Negative Selection

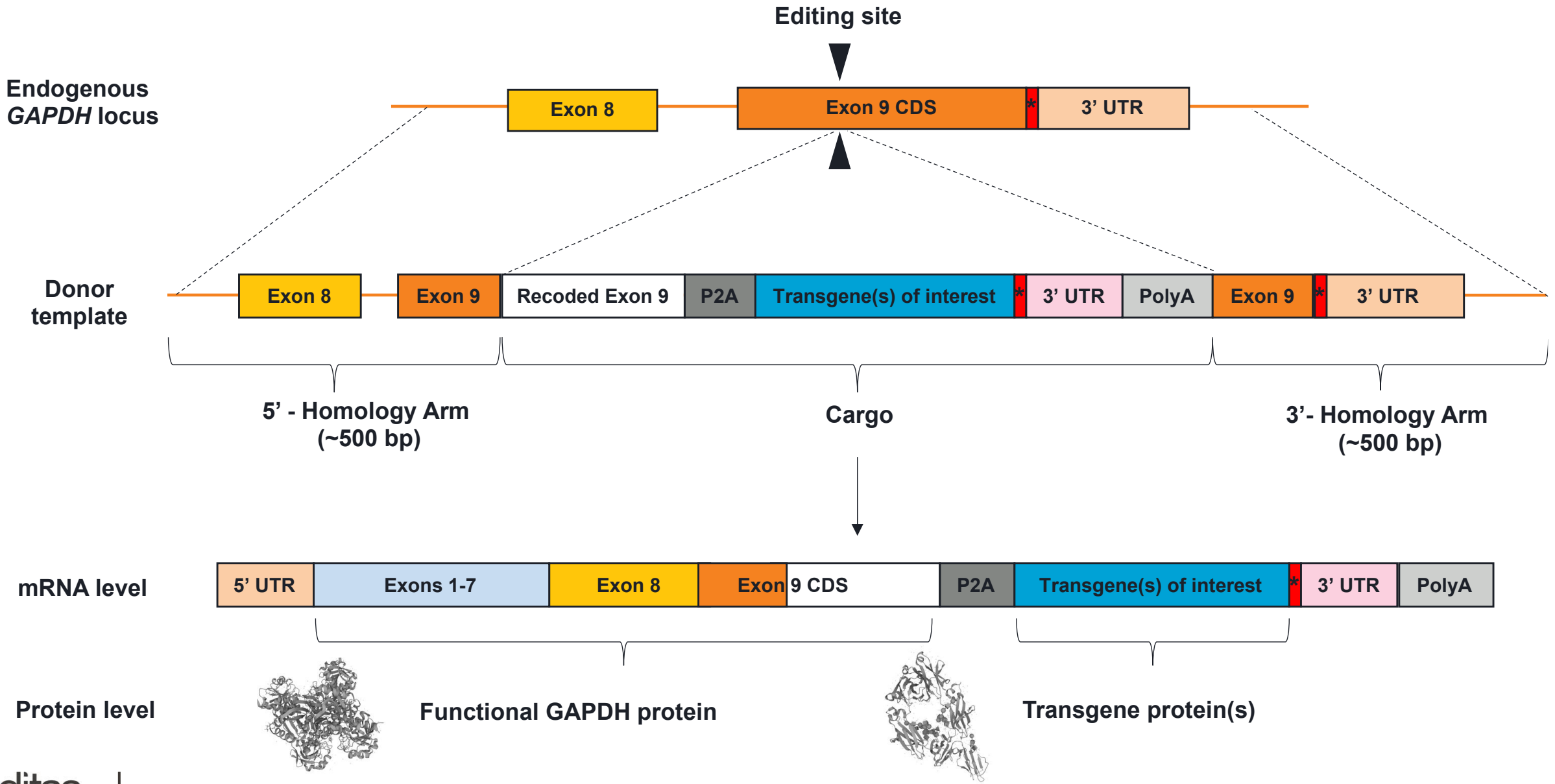
**Repair (HDR)**

Normal essential protein
*No effect on cell health*

Positive Selection

Cas: CRISPR-associated protein; HDR: homology directed recombination; RNP: ribonucleoprotein; NHEJ: non-homologous end joining.

# Molecular Design of SLEEK Knock-in Construct

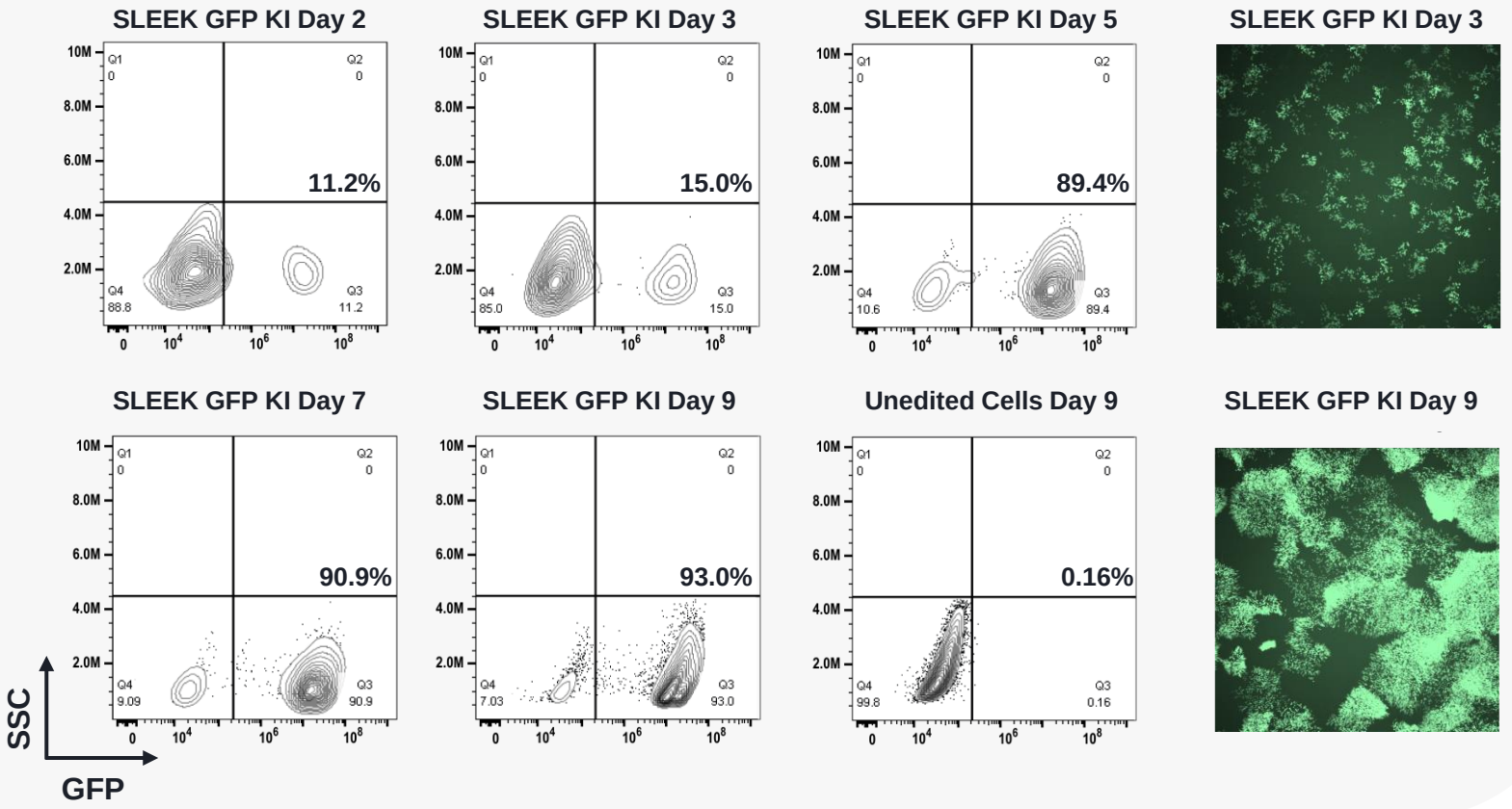



CDS: coding DNA sequence; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; mRNA: messenger RNA; P2A: peptide 2A; UTR: untranslated region.

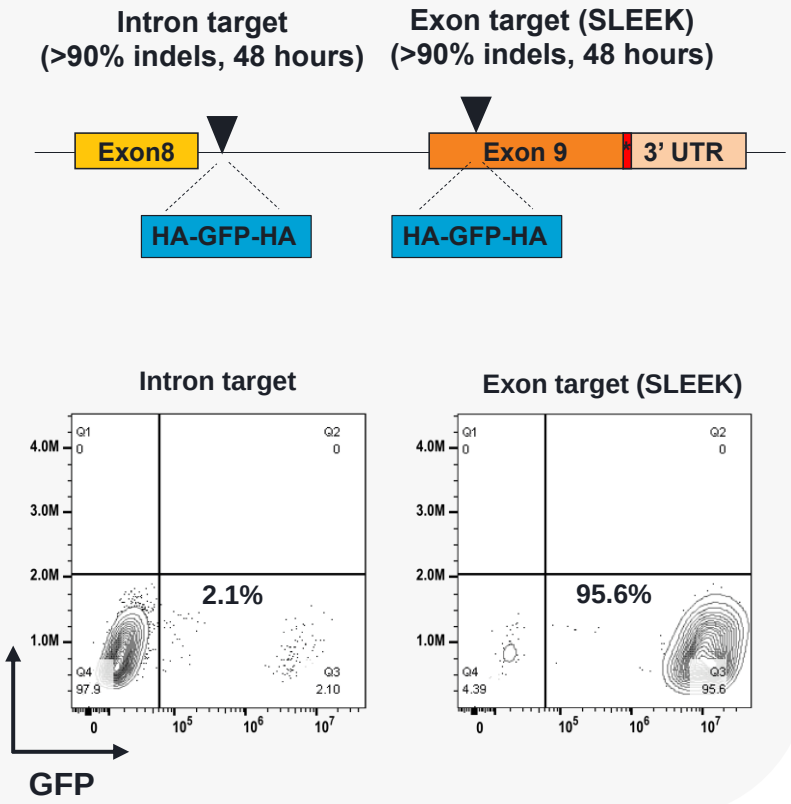
# Reduction to Practice of SLEEK Technology

*iPSCs*

## Selection of Knock-in Edit Over KO Edit




## Validation of SLEEK Hypothesis

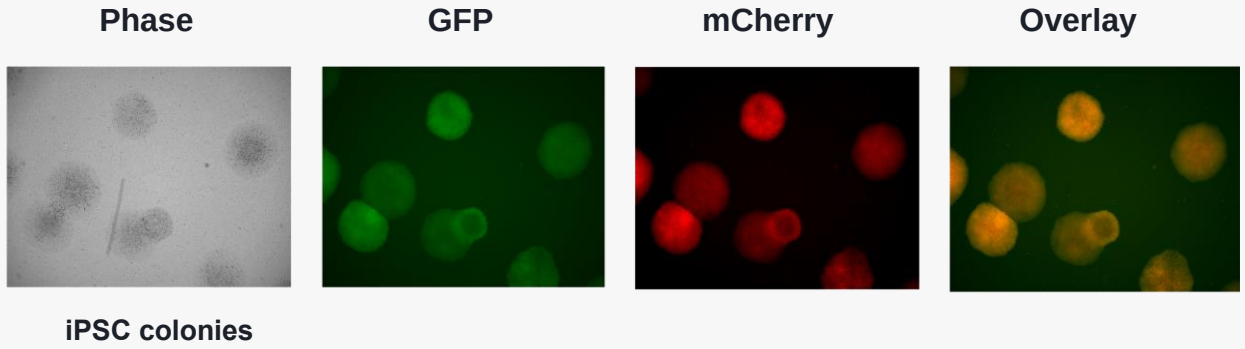



HA: homology arm; GFP: green fluorescent protein; iPSC: induced pluripotent stem cell; KI: knock-in; KO: knock-out; SSC: side scatter; UTR: untranslated region.

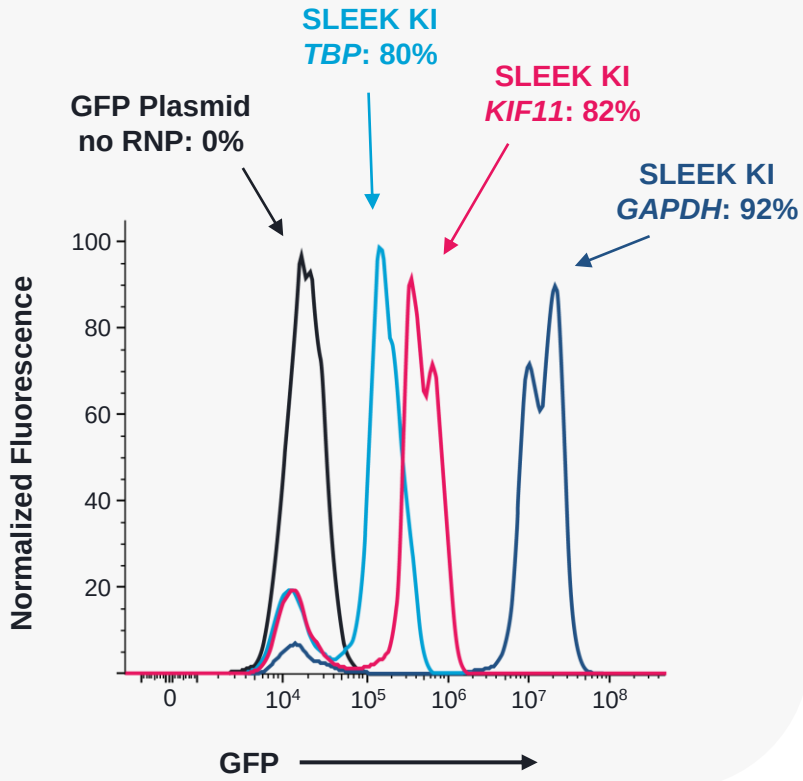
# Multicistronic Knock-in and Tunable Expression With SLEEK

*iPSCs*

## Multicistronic KI of GFP and mCherry Transgene Cargos


## Tunable Cargo Expression by KI at Different Genes



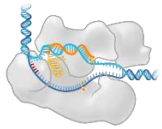
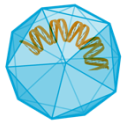

CDS: coding DNA sequence; GFP: green fluorescent protein; KIF11: kinesin family member 11; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; KI: knock-in; P2A: peptide 2A; RNP: ribonucleoprotein; TBP: TATA binding protein; UTR: untranslated region.

# SLEEK is More Efficient and Potent Than *TRAC* KI Gold Standard

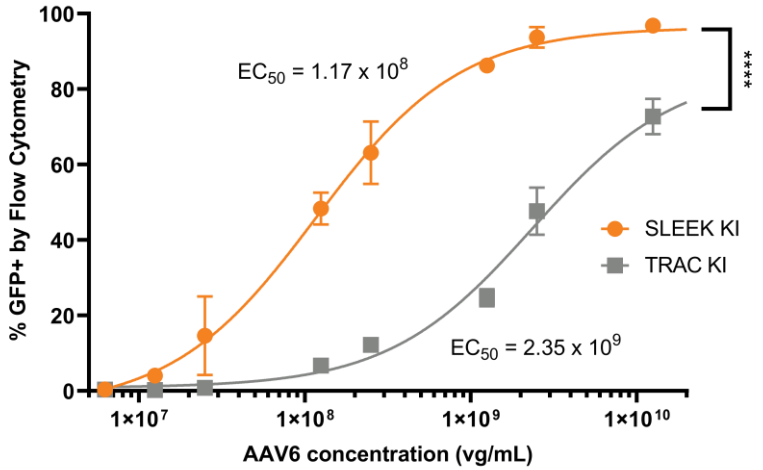
**T cells**

## Experimental Schematic

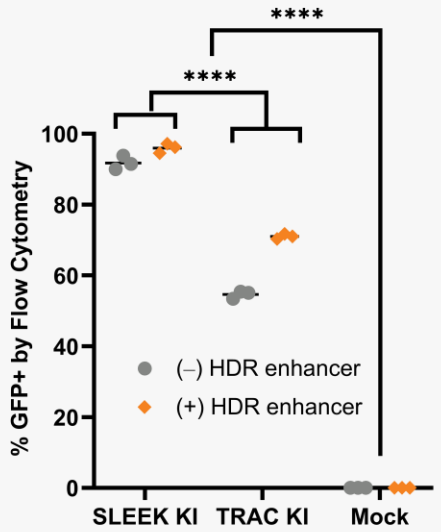
T cells + TRAC or SLEEK RNPs + AAV6 GFP (TRAC or SLEEK)

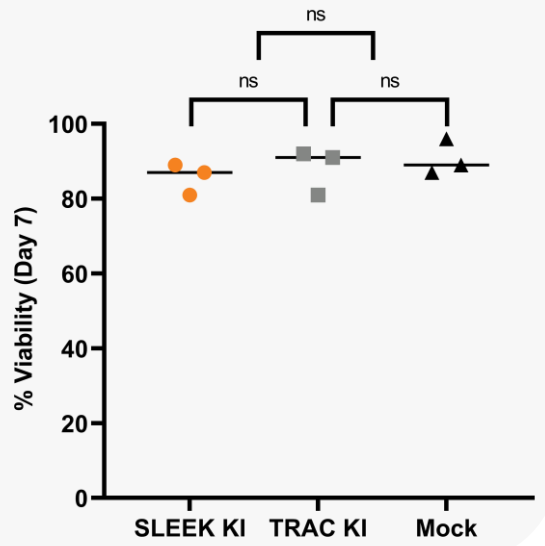
→ KI Efficiency
Viability
Expansion



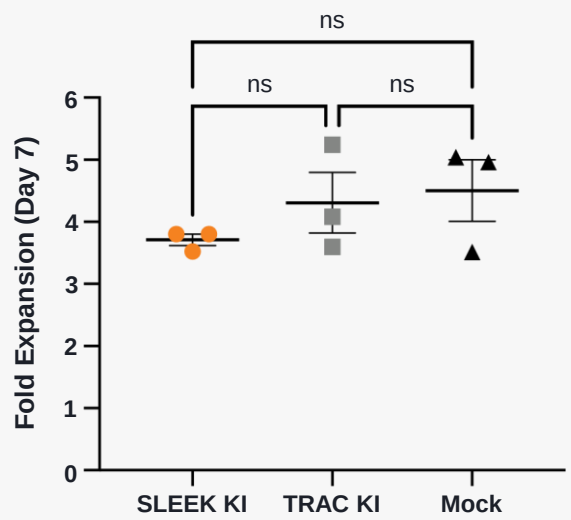

### KI Efficiency




### Viability



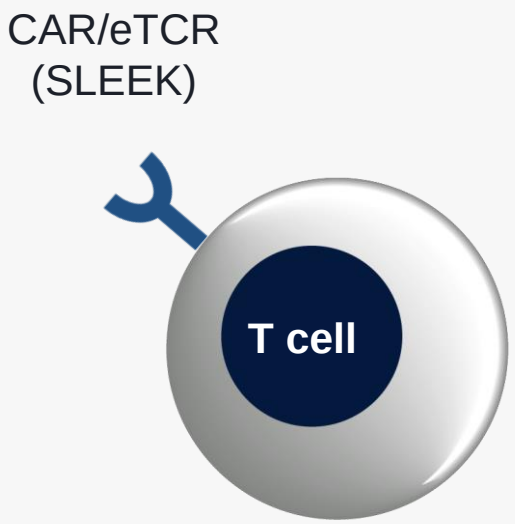

### Expansion



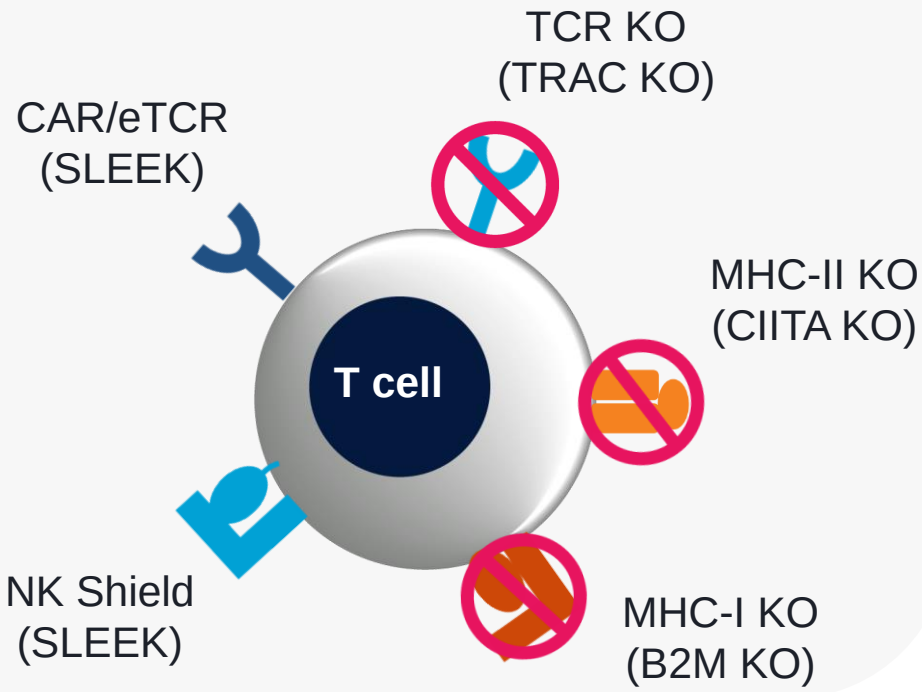

AAV6: adeno-associated virus type 6; GFP: green fluorescent protein; HDR: homology-directed repair; KI: knock-in; ns: not significant; RNP: ribonucleoprotein; TRAC: T-cell receptor α constant.

# Many Opportunities to Use SLEEK to Generate Highly Homogeneous Engineered T Cell and NK Cell Medicines

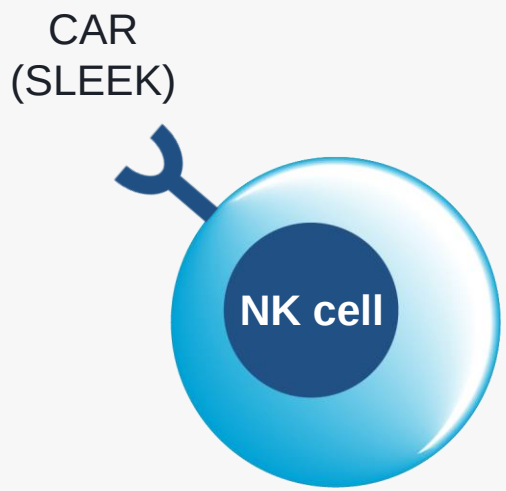
## Autologous Engineered T cell




## Allogeneic Healthy Donor Engineered T Cell




## Allogeneic Healthy Donor Engineered NK Cell

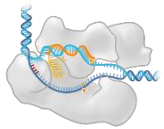


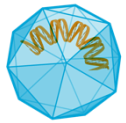

B2M: beta-2 macroglobulin; CAR: chimeric antigen receptors; CIITA: class II major histocompatibility complex transactivator; KI: knock-in; KO: knock-out; MHC: major histocompatibility complex; NK: natural killer; TCR: T-cell receptor.

# SLEEK KI of a CD19 CAR in T Cells Leads to Robust Tumor Killing

**Experimental Schematic**

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



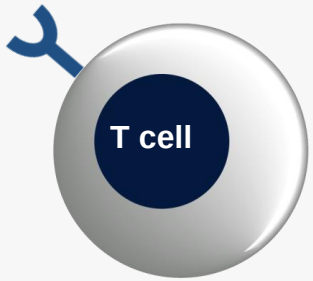


Flow Cytometry
Tumor Killing Assay

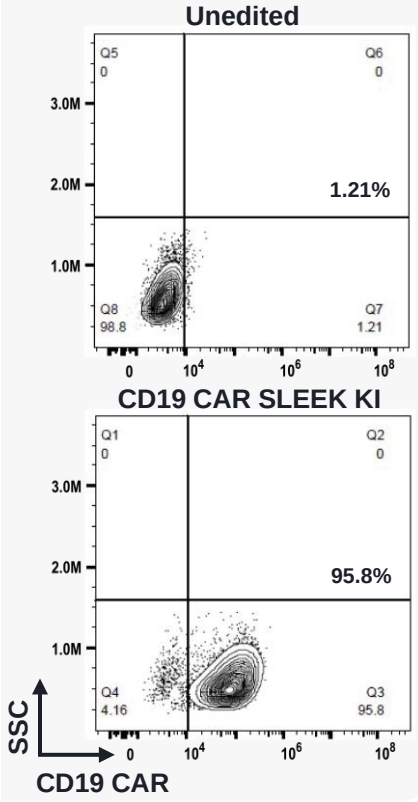
***T cells***

## Allogeneic Healthy Donor Applications

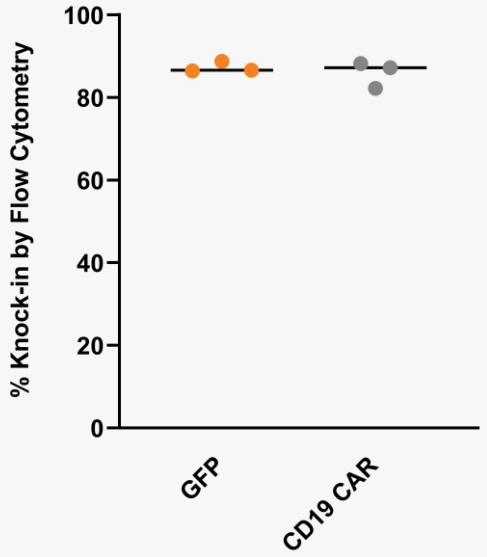
CAR/eTCR (SLEEK)



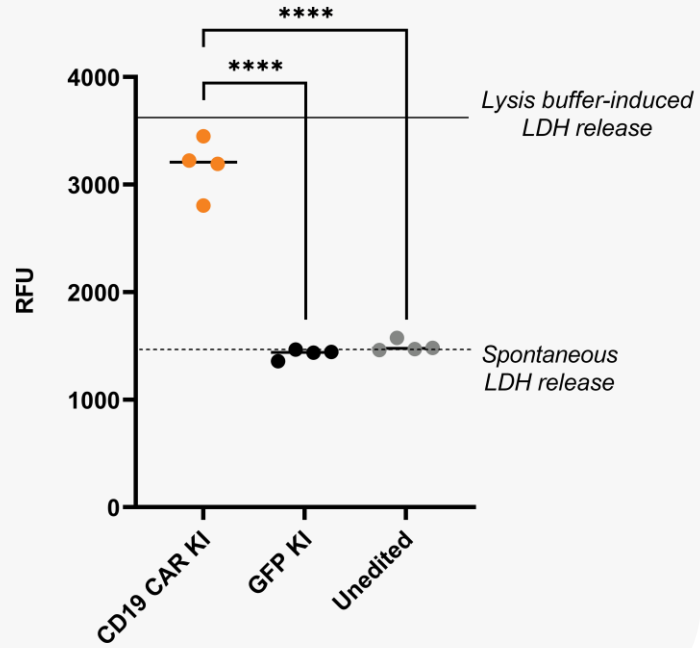

## Validation of KI




## KI Efficiency




## Tumor Killing



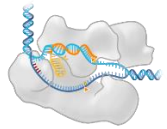

AAV6: adeno-associated virus type 6; Cas: CRISPR-associated protein; CAR: chimeric antigen receptors; CD: cluster of differentiation; GFP; green fluorescent protein; LDH: lactate dehydrogenase; KI: knock-in; NK: natural killer; TCR:T-cell receptor; TRAC: T-cell receptor α constant; RNP: ribonucleoprotein; RFU: relative fluorescence units; SSC: side scatter.

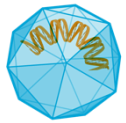
# SLEEK Enables the Generation of Multi-Edited Cell-Based Medicines

**T cells**

**Experimental Schematic**

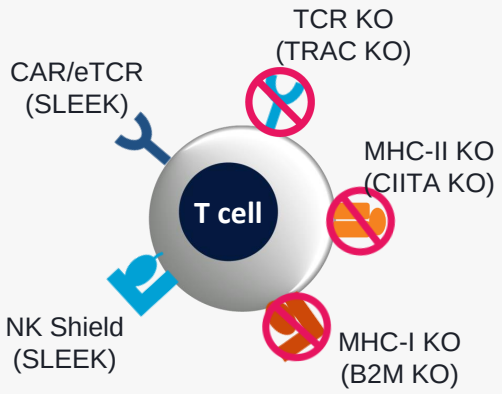
T cells + TRAC, B2M, CIITA and SLEEK RNPs + AAV6 (CD19 CAR)





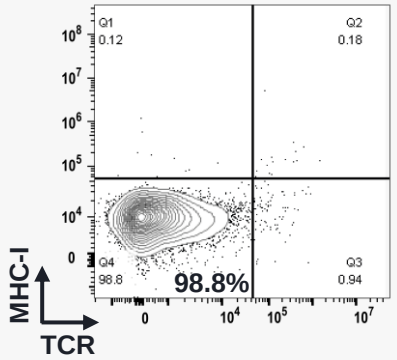
→ Flow Cytometry
Tumor Killing Assay

## Allogeneic Healthy Donor Applications

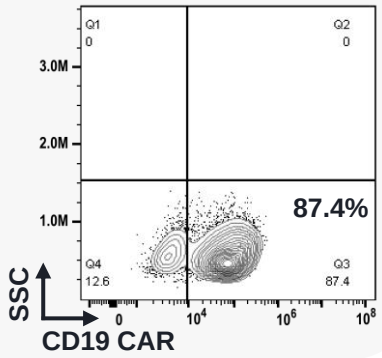



## Validation of KO/KI

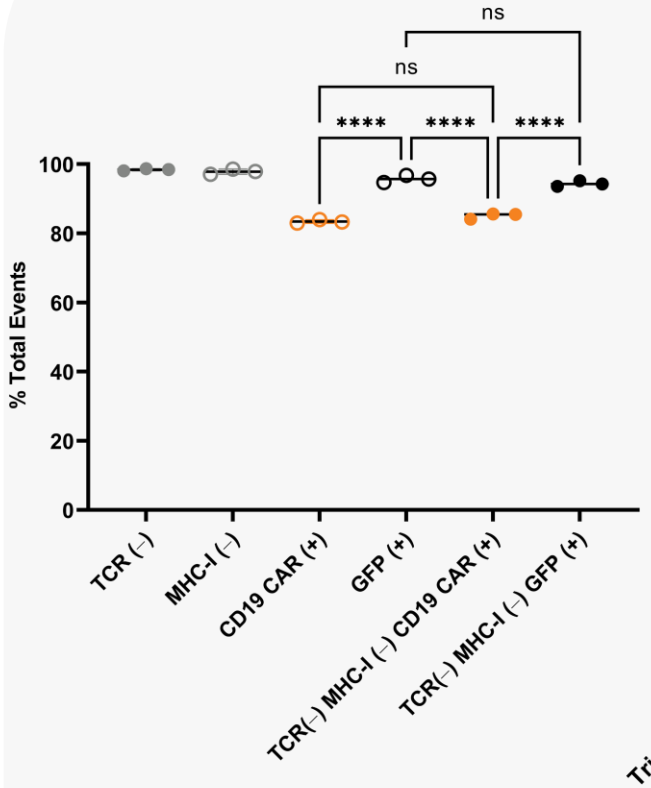
MHC-I and TCR KO



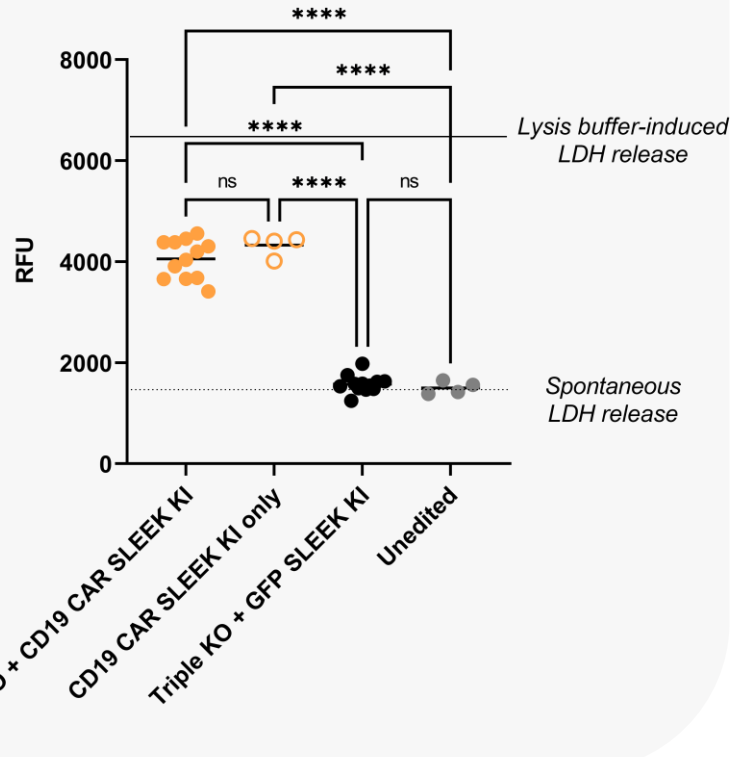

CD19 CAR SLEEK KI




## KI Efficiency




## Tumor Killing



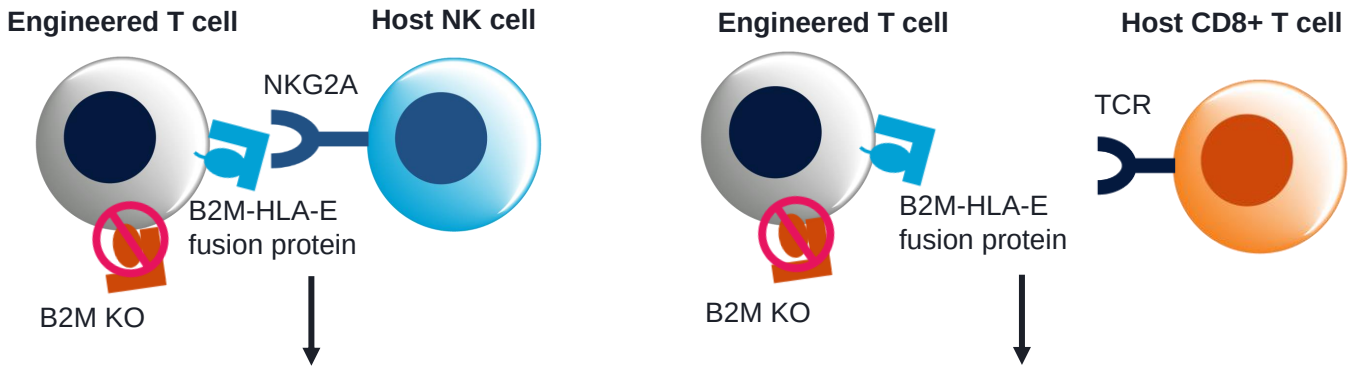

AAV6: adeno-associated virus type 6; B2M; beta-2 macroglobulin; CAR: chimeric antigen receptors; CIITA: class II major histocompatibility complex transactivator; CD: cluster of differentiation; GFP; green fluorescent protein; KI: knock-in; KO: knock-out; MHC: major histocompatibility complex; NK: natural killer; SSC: side scatter; TRAC: T-cell receptor α constant; TCR: T-cell receptor.

# Inhibition of Host NK Response by SLEEK KI of an NK Shield

*T cells*

## Proposed Solution for Engineering of an Allogenic T Cell:

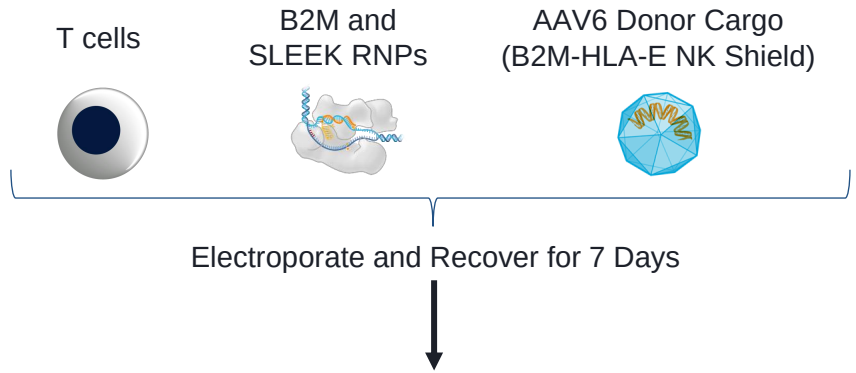
**CD8+ T Cell Shield (B2M KO) + SLEEK KI of NK Shield (B2M-HLA-E Fusion)**

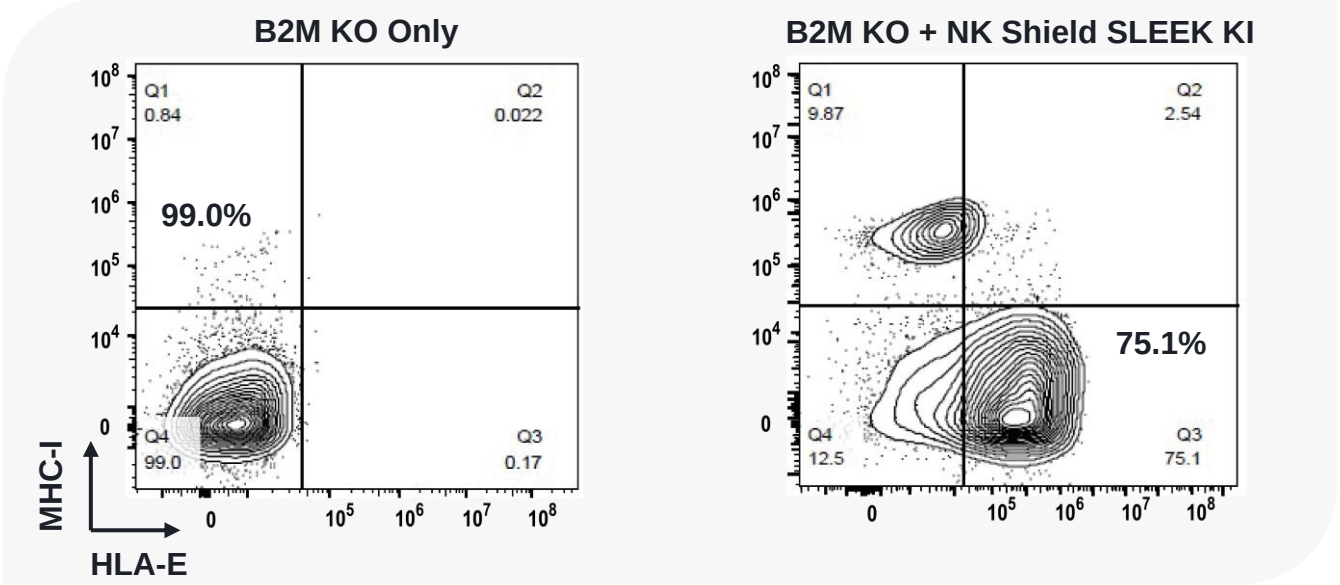


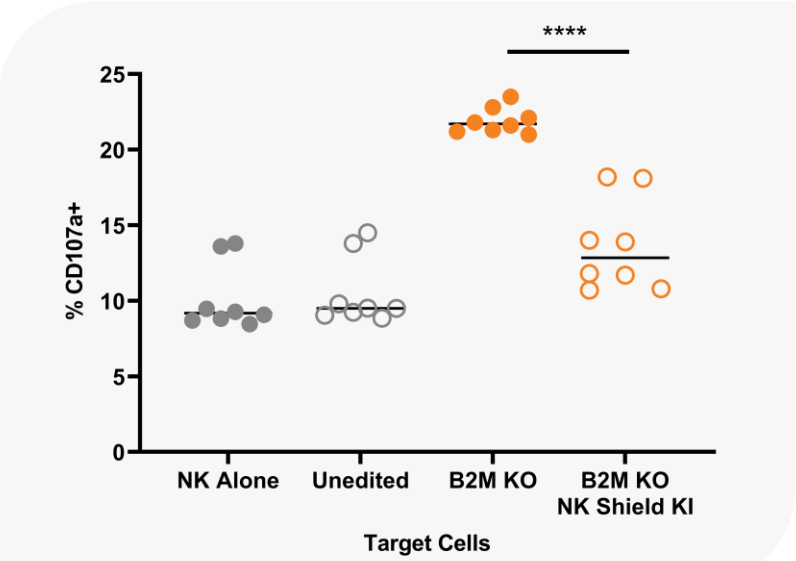

**Inhibition of host NK cell response as NKG2A should recognize HLA-E (+) engineered T cell as "self"**

**Host T cell should avoid recognition of conserved HLA-E (MHC-I) as "non-self"**

**Allogeneic T Cell Generation and Degranulation Assay Schematic**



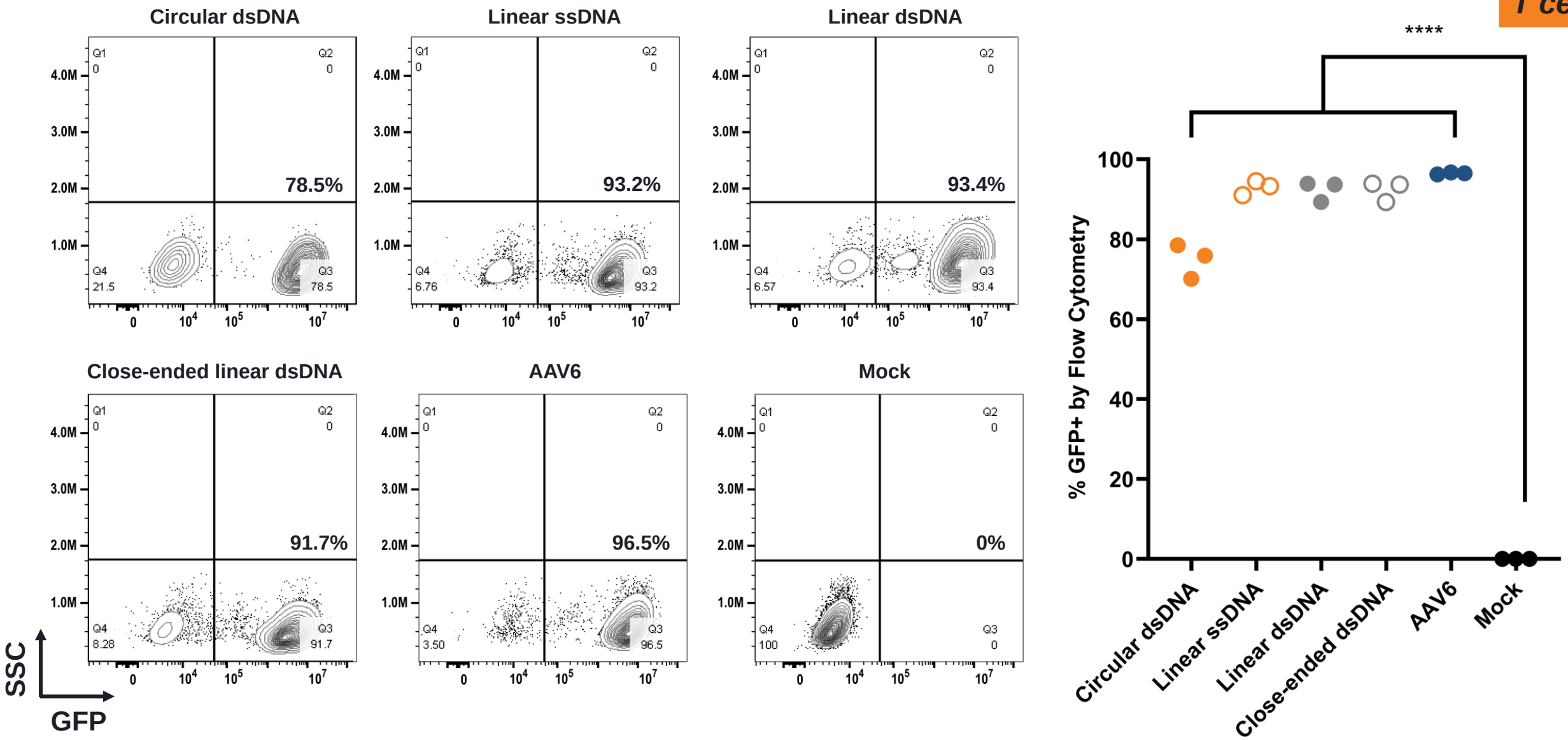




AAV6: Adeno-associated virus type 6; B2M; beta-2 macroglobulin; CD: cluster of differentiation; HLA-E: human leukocyte antigen E; KI: knock-in; KO: knock-out; MHC: major histocompatibility complex; NK: natural killer; NKG2A: NK group 2 member A; RNP: ribonucleoprotein; TCR: T-cell receptor.

# SLEEK is Similarly Efficient With Non-Viral DNA Templates

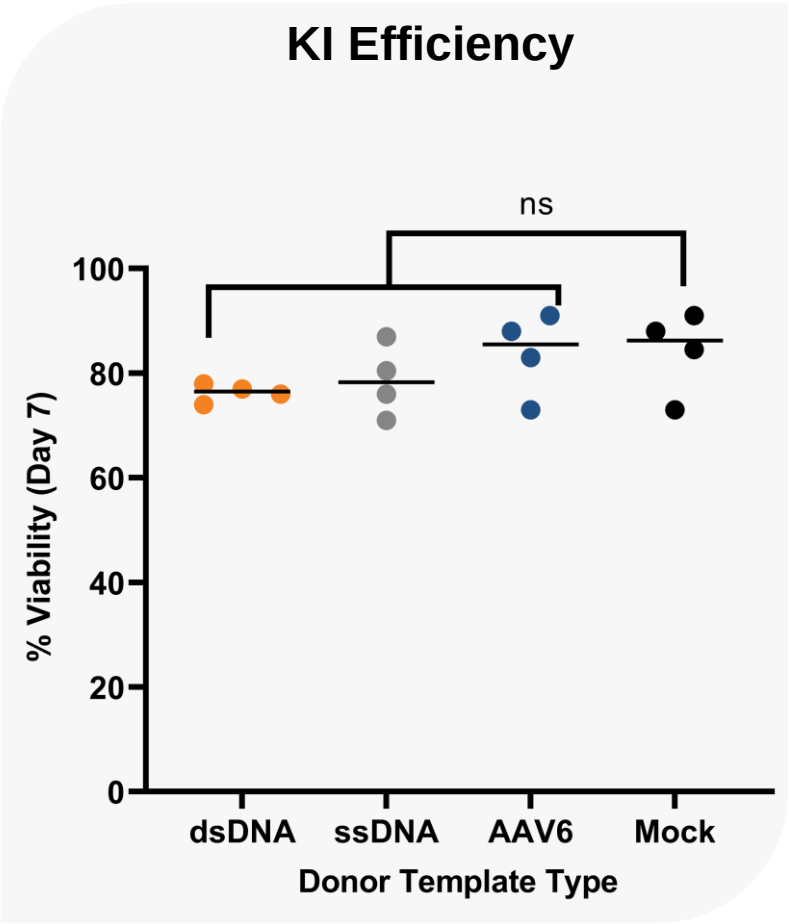
**T cells**

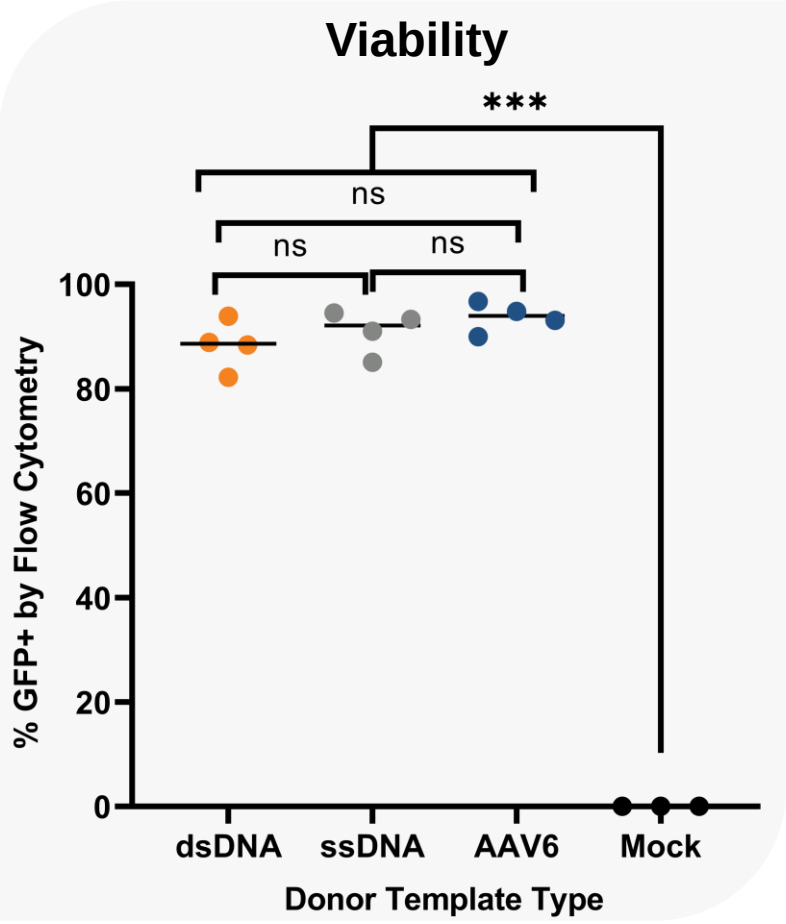


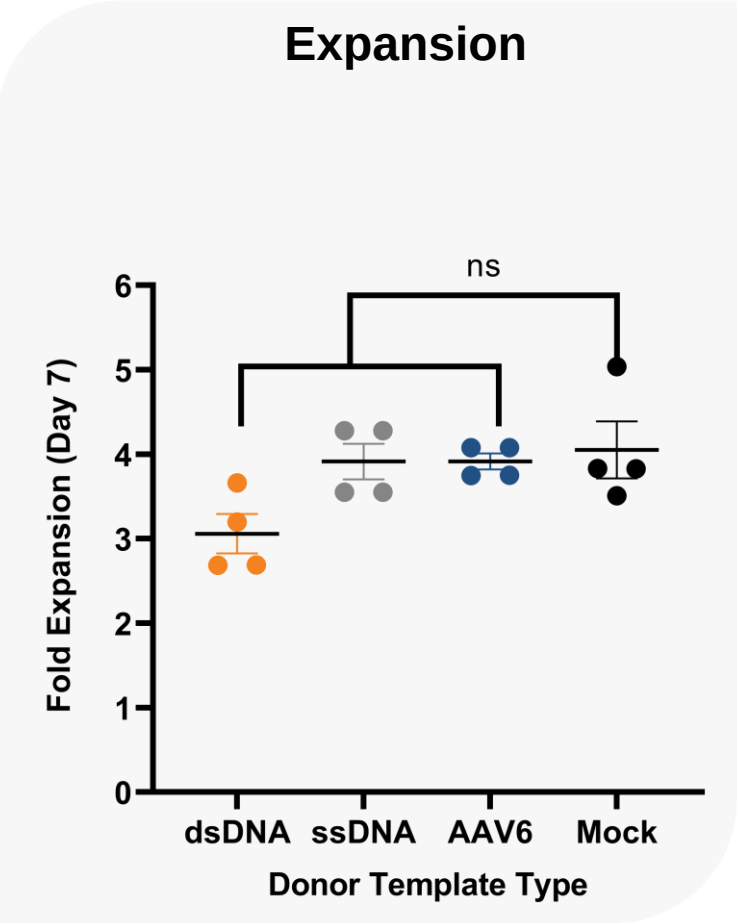

AAV6: adeno-associated virus type 6; dsDNA: double stranded deoxyribonucleic acid; GFP: green fluorescent protein; SSC: side scatter; ssDNA: single stranded deoxyribonucleic acid.

# Negligible Effect on Viability or Expansion with Non-Viral Templates

***T cells***



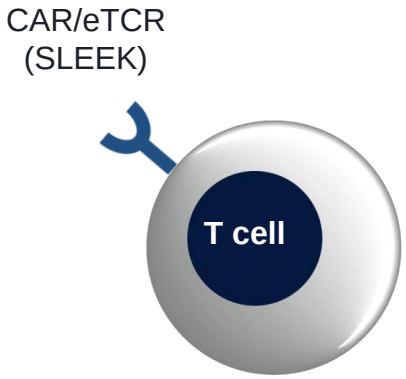

## Non-Viral DNA Templates Offer Greater Cargo Sizes Than AAV6 and Faster Manufacturing Times

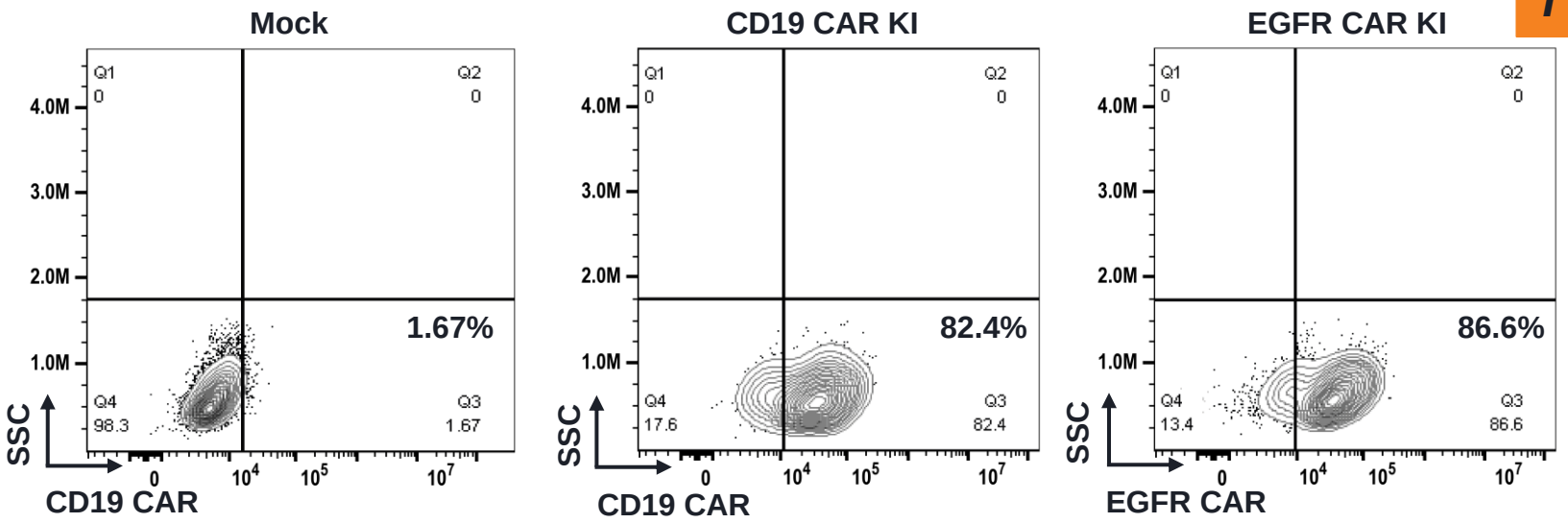
AAV6: adeno-associated virus type 6; dsDNA: double stranded deoxyribonucleic acid; GFP: green fluorescent protein; KI: knock-in; ssDNA: single stranded deoxyribonucleic acid.

# SLEEK KI of Functional Cargos With Non-Viral DNA Templates

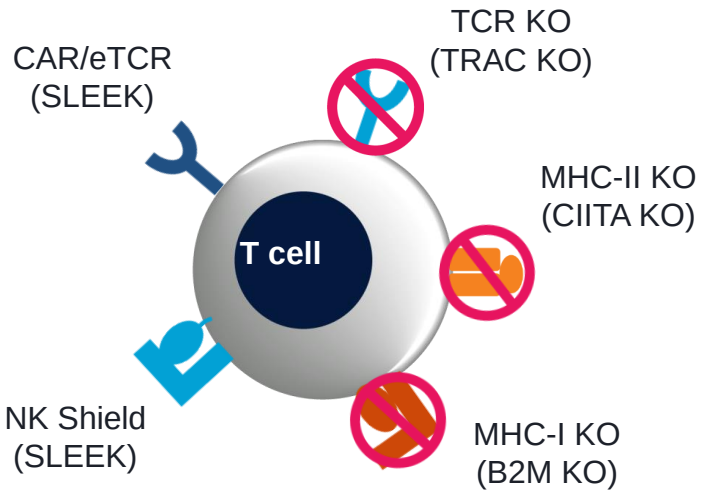
**T cells**

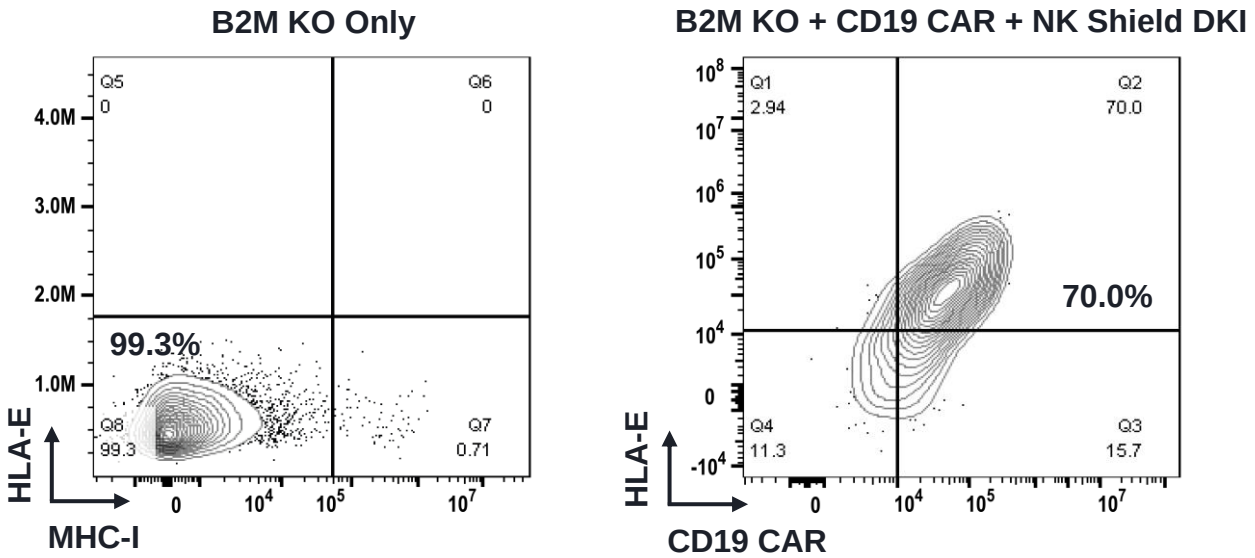
## Autologous



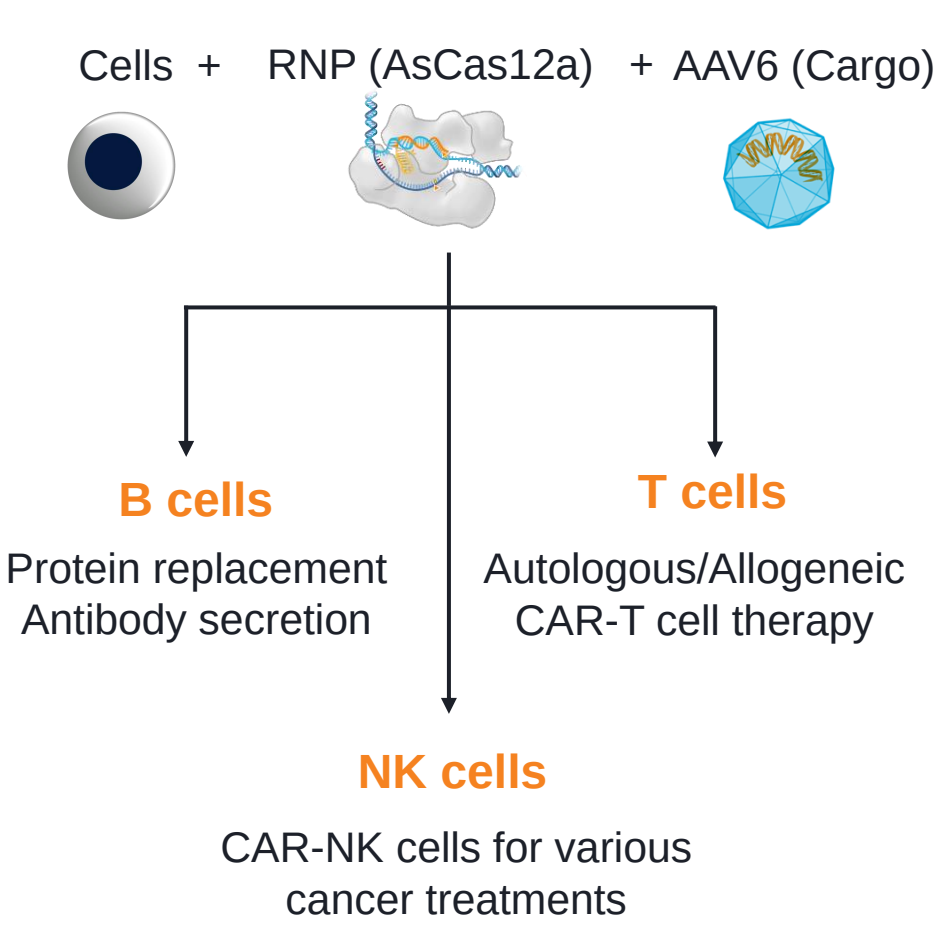

## Allogeneic

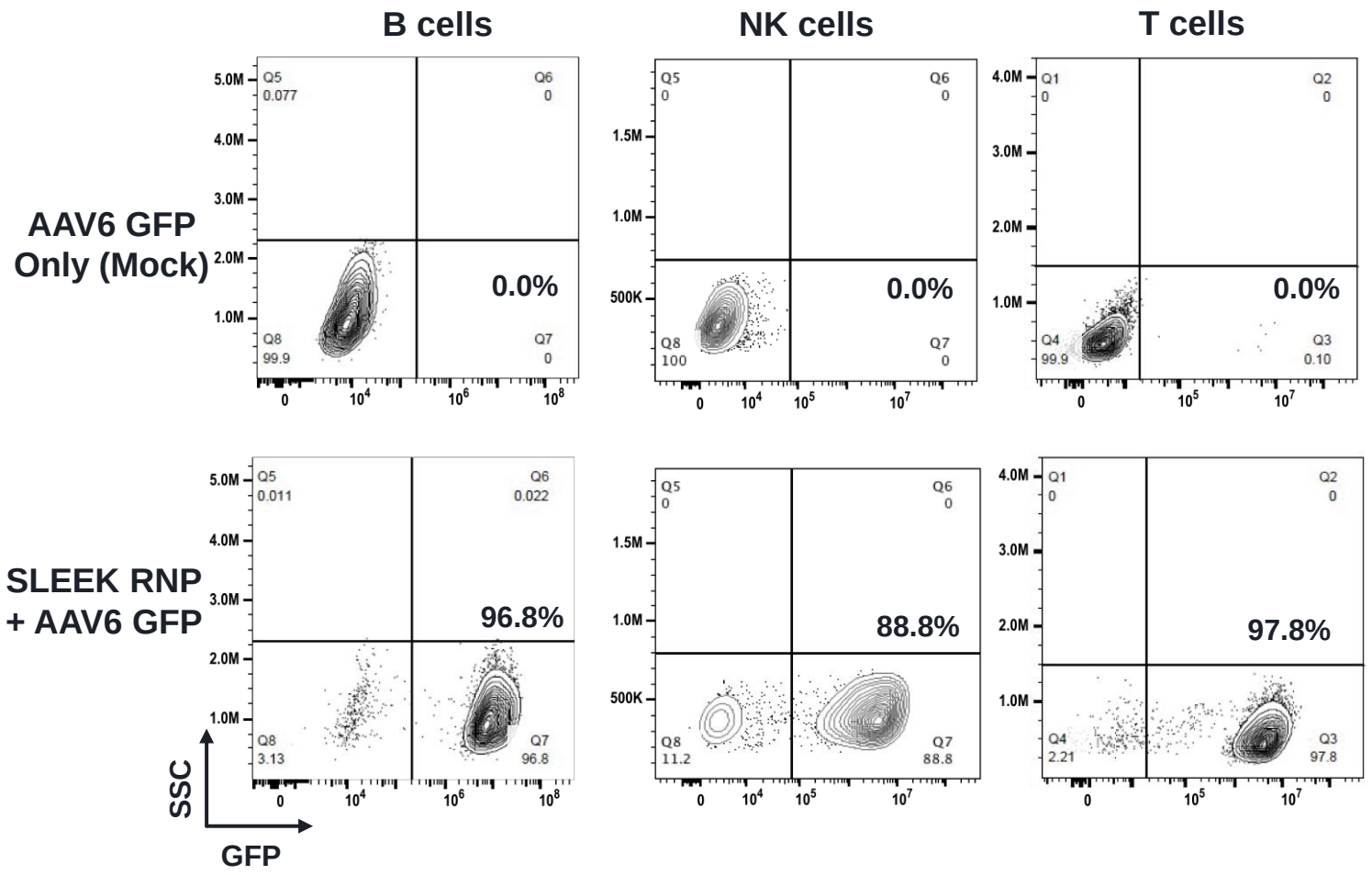



B2M; beta-2 macroglobulin; CAR: chimeric antigen receptors; CD: cluster of differentiation; DKI: double knock-in; EGFR: epidermal growth factor receptor; HLA-E: human leukocyte antigen E; GFP; green fluorescent protein; KI: knock-in; KO: knock-out; MHC: major histocompatibility complex; NK: natural killer; SSC: side scatter; TCR: T-cell receptor.

# SLEEK Achieves Best-in-Class Knock-in Rates Across Cell Types




## SLEEK KI Efficiency Approaches 100% Which Improves Product Purity of Edited Cell Medicines

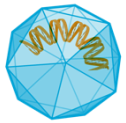
AAV6: adeno-associated virus type 6; Cas: CRISPR-associated protein; CAR: chimeric antigen receptors; GFP; green fluorescent protein; KI: knock-in; NK: natural killer; RNP: ribonucleoprotein; SSC: side scatter.

# SLEEK KI of a CD19 CAR in NK Cells Leads to Robust Tumor Killing

**NK cells**

**Experimental Schematic**

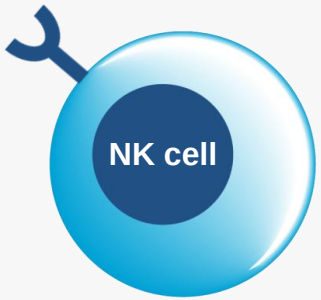
NK cells + SLEEK RNP + AAV6 (CD19 CAR)



Flow Cytometry
Tumor Killing Assay

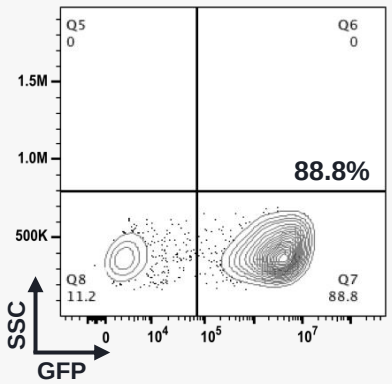
## Allogeneic Healthy Donor Applications

CAR (SLEEK)

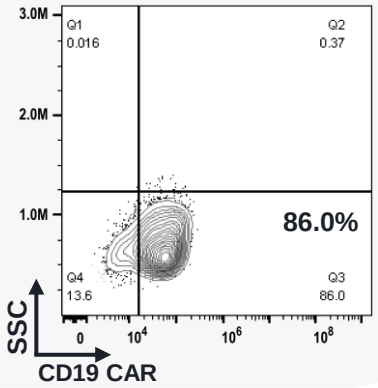



## Validation of KI

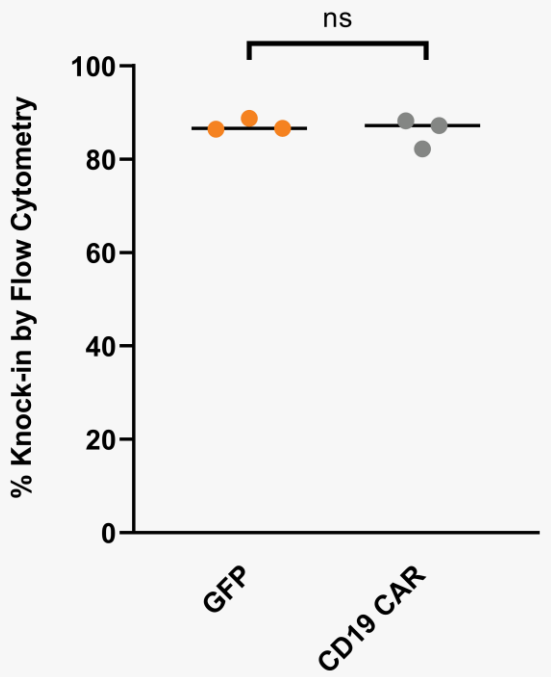
GFP SLEEK KI



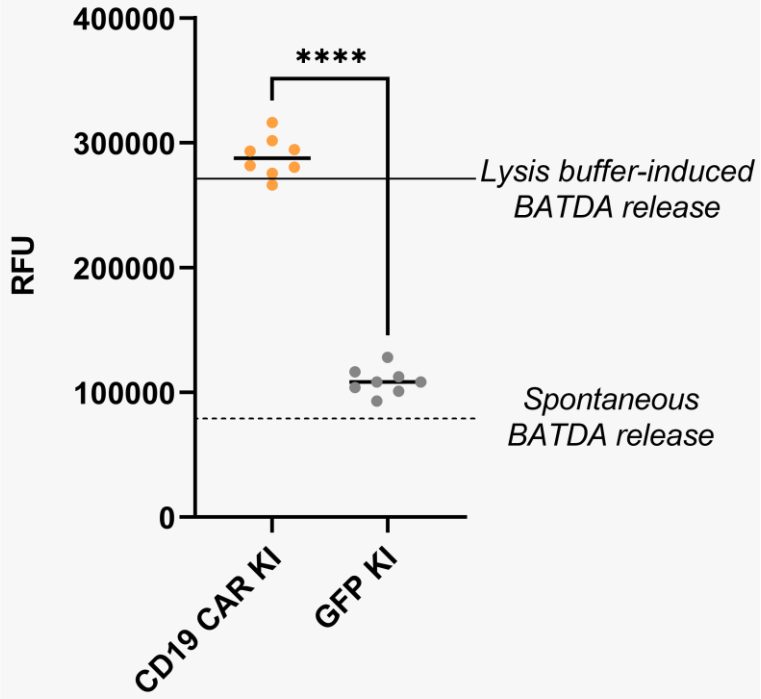

CD19 CAR SLEEK KI




## KI Efficiency



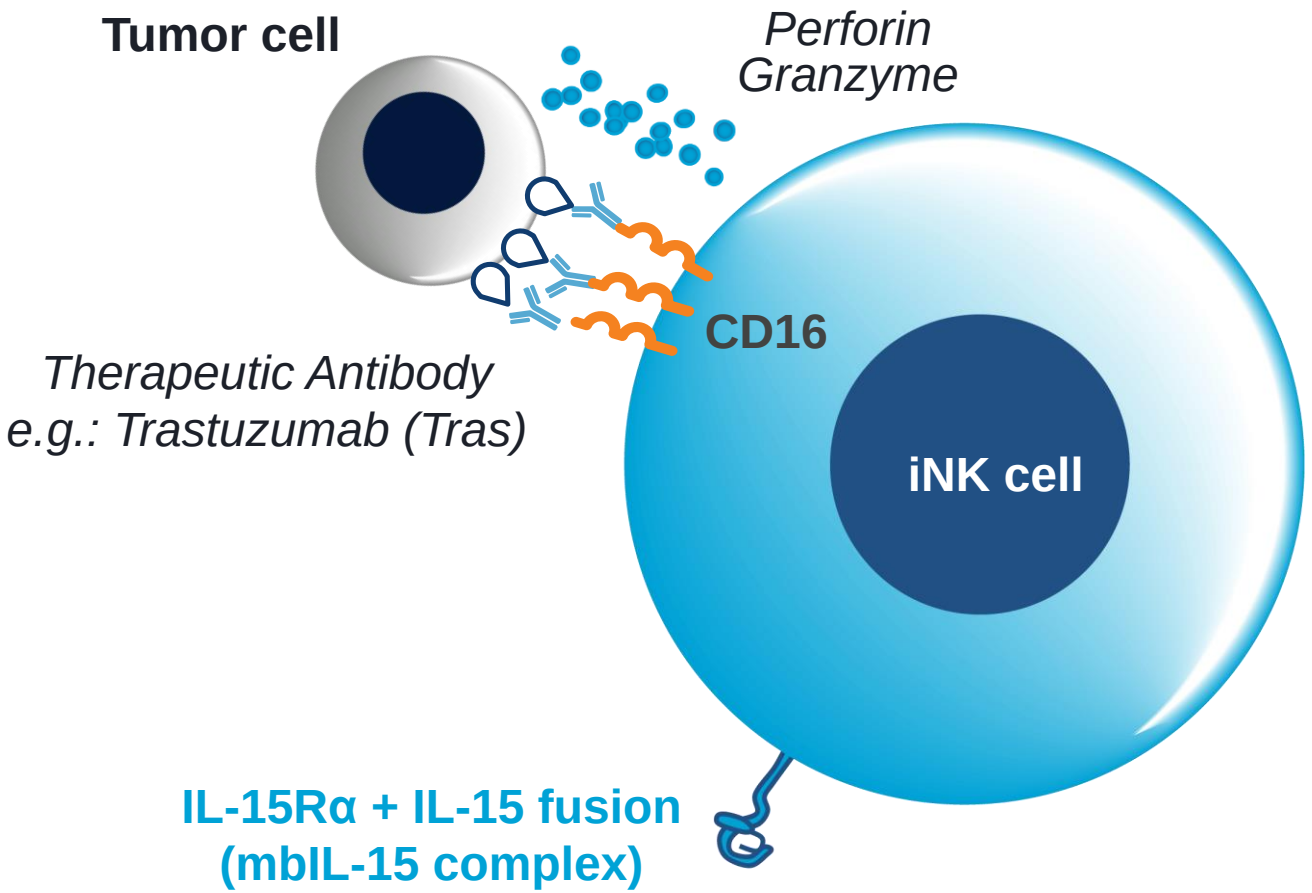

## Tumor Killing




AAV6: adeno-associated virus type 6; Cas: CRISPR-associated protein; CAR: chimeric antigen receptors; CD: cluster of differentiation; GFP; green fluorescent protein; KI: knock-in; NK: natural killer; RFU: relative fluorescence units; RNP: ribonucleoprotein; SSC: side scatter.

# Engineering an iNK Cell With Enhanced Functions Using SLEEK

***iNK cells***




**Enhance Antibody-Dependent Cellular Cytotoxicity (ADCC)**

Constitutive overexpression of **CD16** through **CD16** knock-in

**Enhance survival and/or expansion of iNK cells**

Increased **IL-15** signaling through knock-in of **mbIL-15** complex

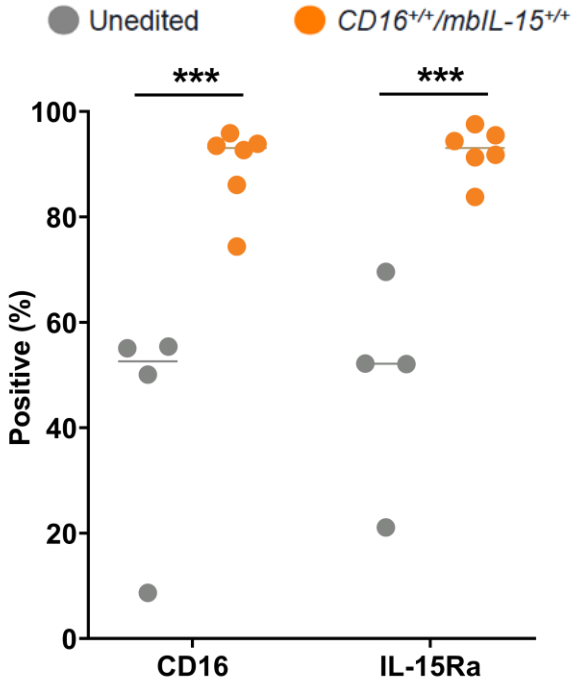
**Generation of an Edited iNK Cell Through SLEEK Double Knock-in of CD16 and mbIL-15 (SLEEK DKI)**

ADCC: Antibody-Dependent cellular cytotoxicity; CD: cluster of differentiation; iNK: IPSC-derived natural killer cell; IL-15Rα: interleukin-15 receptor α; mbiL-15: membrane-bound IL-15; NK: natural killer.

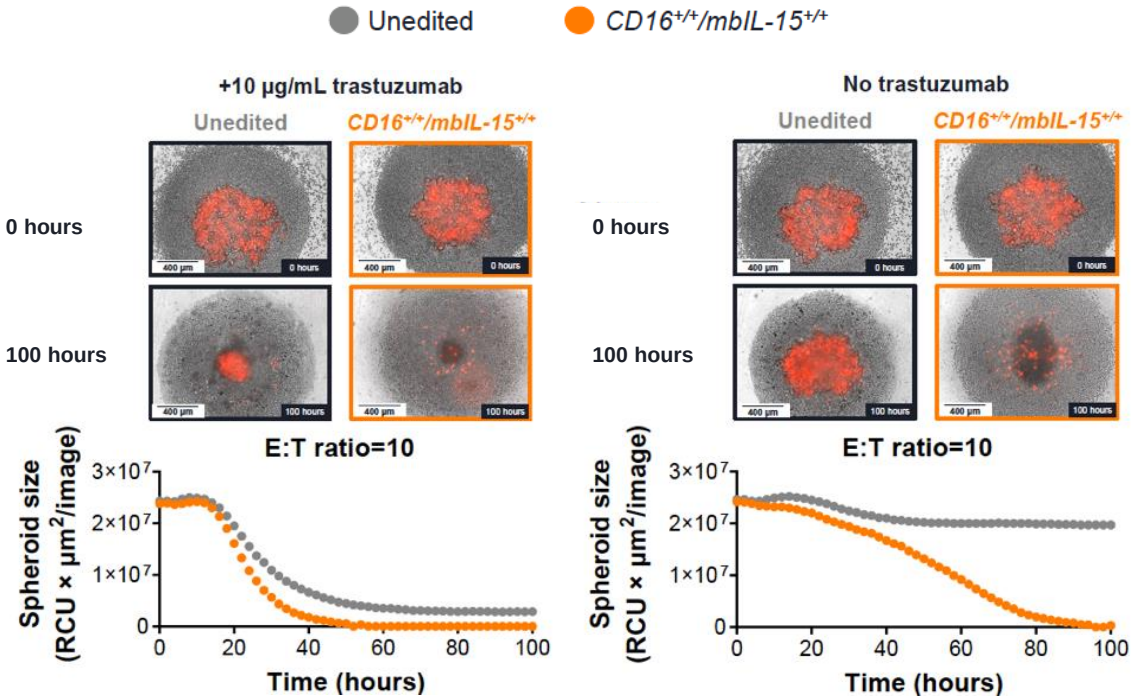
# SLEEK Knock-in iNKs Show Enhanced ADCC and Persistence

*iNK cells*

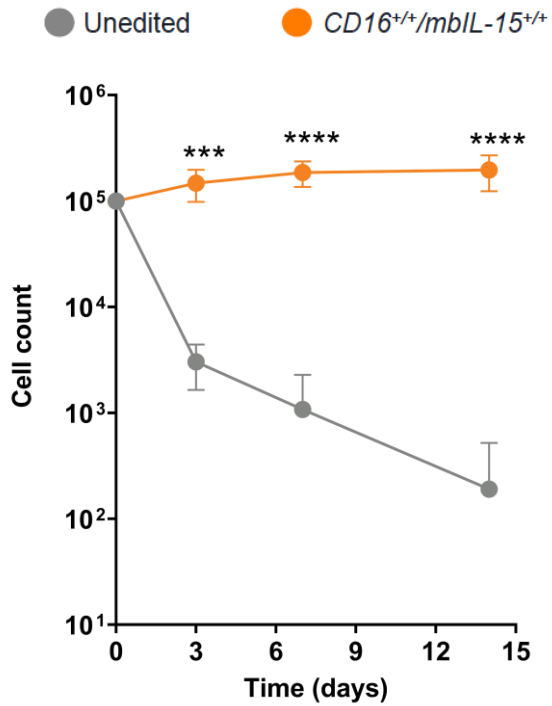
## CD16 and mbIL-15 Expression in SLEEK DKI iNKs




## Greater Tumor Reduction with SLEEK DKI iNKs




## Increased Persistence in SLEEK DKI iNKs




**Increased ADCC and Persistence Attributed to Robust Expression of CD16 and mbIL-15 Cargos by SLEEK**

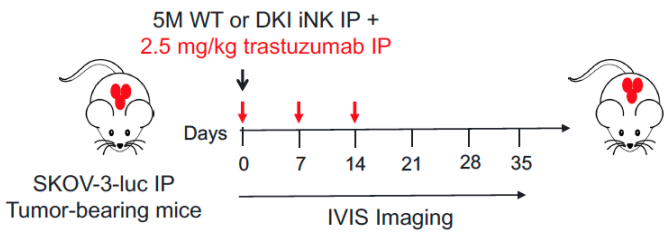
ADCC: antibody-dependent cellular cytotoxicity; CD: cluster of differentiation; DKI: double knock-in; E: effector; iNK: ; IL-15Rα: interleukin-15 receptor α; mbiL-15: membrane-bound IL-15; IPSC-derived natural killer cell; RCU: red calibration unit; T: target; WT: wild-type.

Pattali et al. SITC, 2021.

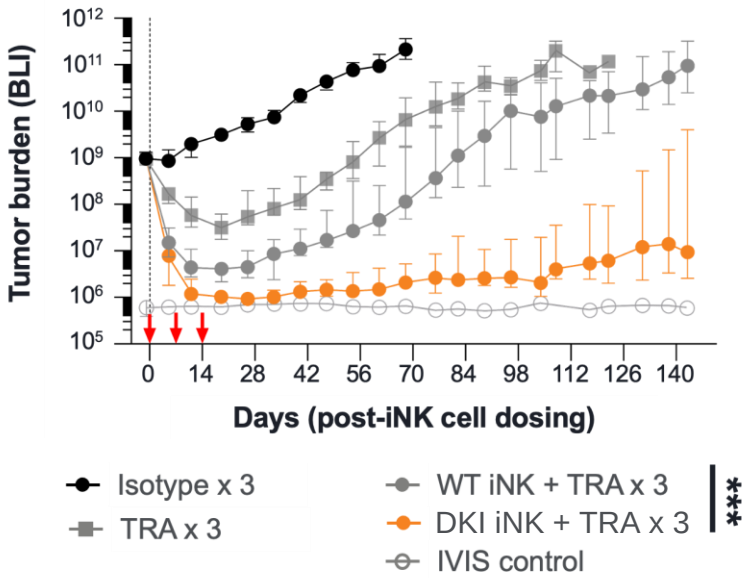
# SLEEK DKI iNKs Administered in Combination With Trastuzumab Induced Significant to Complete Tumor Clearance in Mice

*iNK cells*

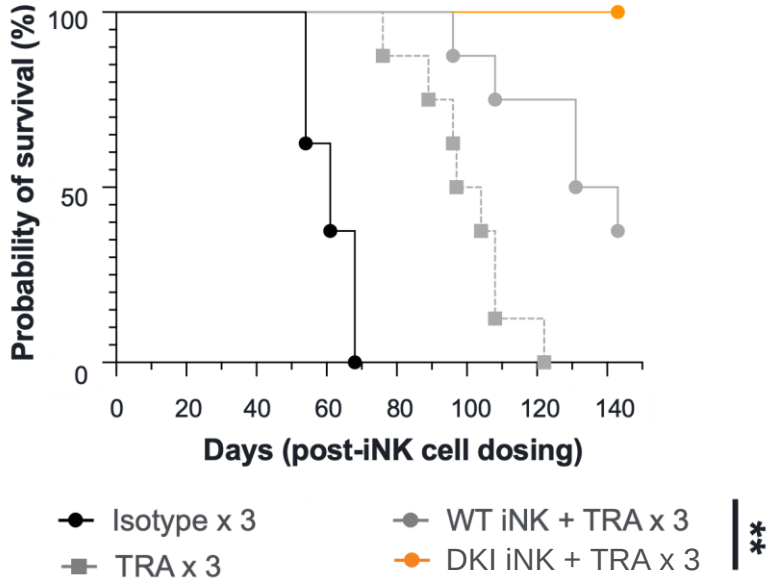
**Overview of in vivo study to assess SLEEK DKI iNK**




**During the course of the 144-day experiment, 6/8 DKI iNK-treated mice had no detectable tumor**




**On day 144, 100% of DKI iNK-treated mice were alive, compared with <50% of WT iNK-treated mice**



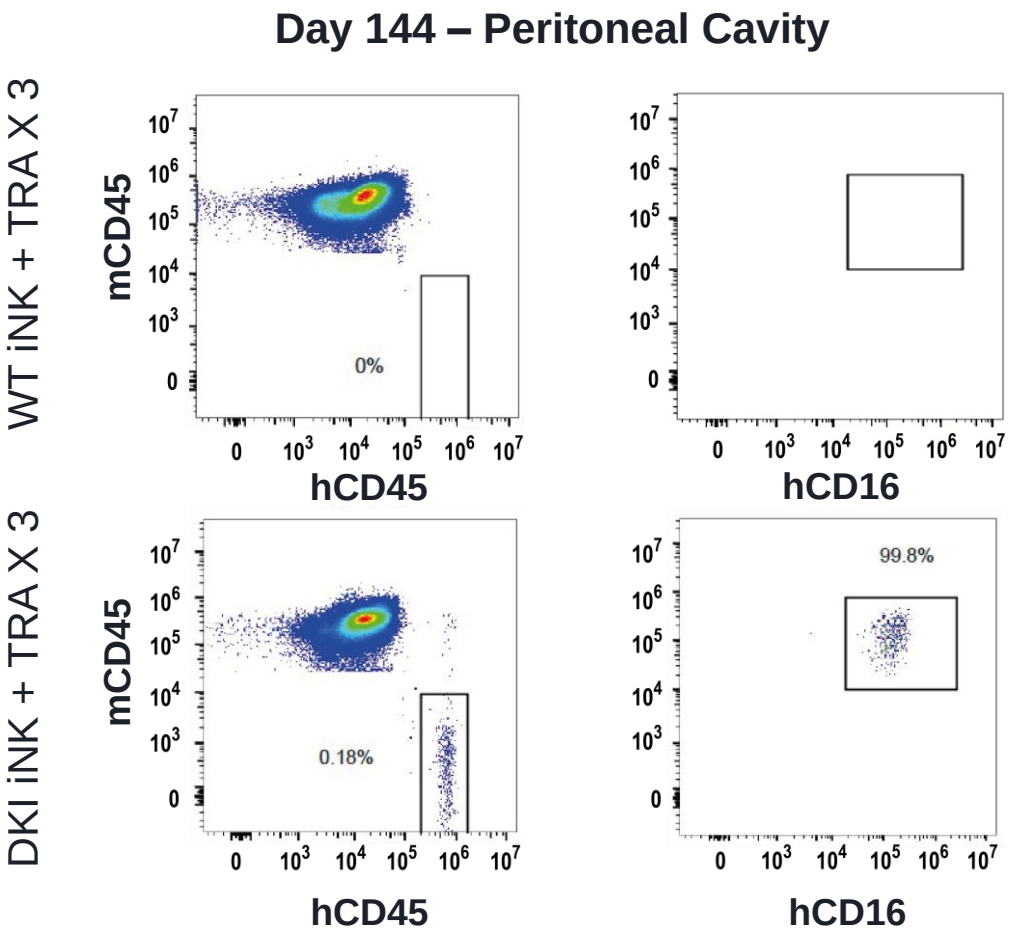

## Strong Tumor Clearance Attributed to Robust Expression of CD16 Cargo by SLEEK

DKI: double knock-in; iNK: IPSC-derived natural killer cell; IP: intraperitoneal; Iso: isotype; IVIS: in vivo imaging system; NK: natural killer; SKOV-3-luc: luciferase-expressing SKOV-3 cell line; TRA: trastuzumab; WT: wild-type.

Gerew et al. ASH, 2021; Allen, Khan et al. AACR, 2022.

# SLEEK DKI iNKs Show Prolonged *In Vivo* Persistence Beyond 144 Days

***iNK cells***




- SLEEK iNKs continued to express high levels of CD16 up to Day 144 post-dosing
- No exogenous cytokine support needed

**Impressive Persistence in iNKs From Robust Expression of mbIL-15 Cargo by SLEEK**

CD: cluster of differentiation; iNK: IPSC-derived natural killer cell; hCD: human CD; mCD: mouse CD; NK: natural killer; TRA: trastuzumab; WT: wild-type.

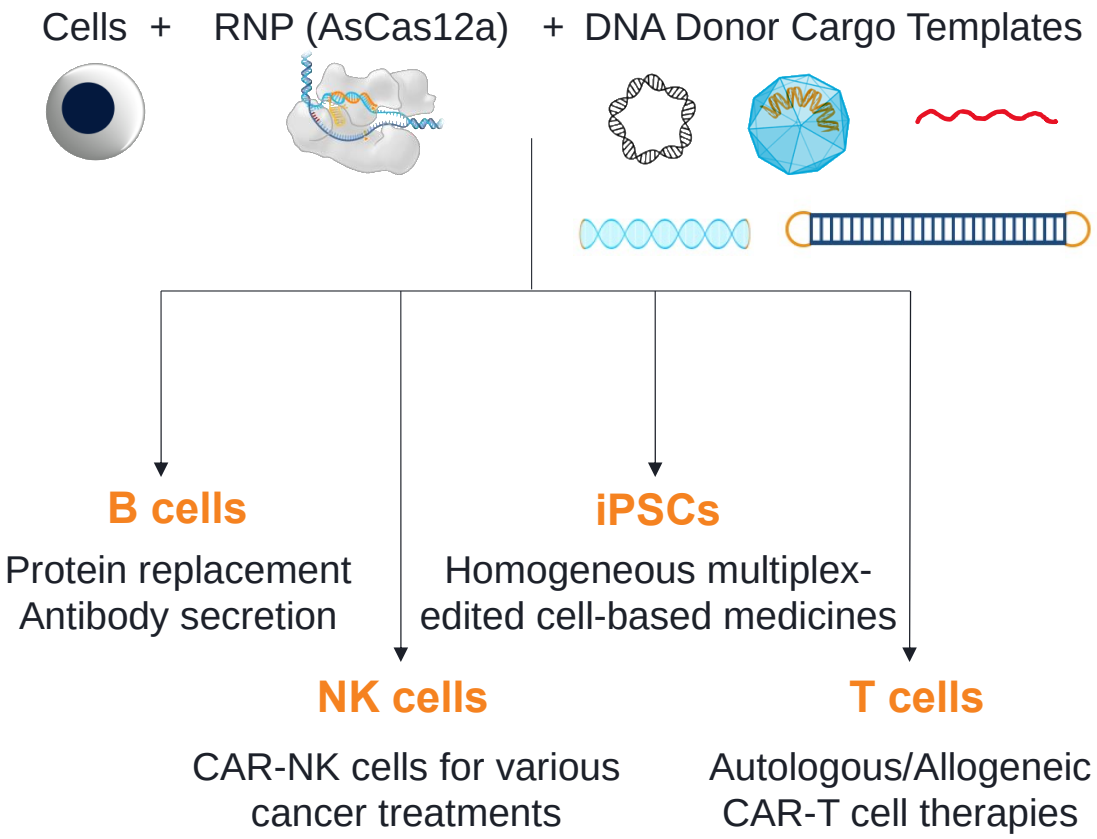
Allen, Khan et al. AACR, 2022.

# Our Goal was to Develop an Editing Technology that Could Fundamentally Improve the Generation of Cell-Based Medicines

SLEEK™ GENE EDITING

**S**e**L**ection by **E**ssential-gene **E**xon **K**nock-In

- Enables >95% knock-in efficiency
- High-level, tunable cargo expression
- Homogeneous editing
- Efficient multicistronic cargos
- Simplifies iPSC clone selection process
- Robust, lineage-independent, expression of functional cargo in iPSCs




**We Believe SLEEK Fundamentally Improves the Generation and Clinical Potential of Cell-based Medicines**

CAR: chimeric antigen receptor; Cas: CRISPR-associated protein; iNK: IPSC-derived natural killer cell; iPSC: induced pluripotent stem cell; NK: natural killer; RNP: ribonucleoprotein; TME: tumor microenvironment.