

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

CSHL Genome Engineering: CRISPR Frontiers 2022

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Editas Medicine

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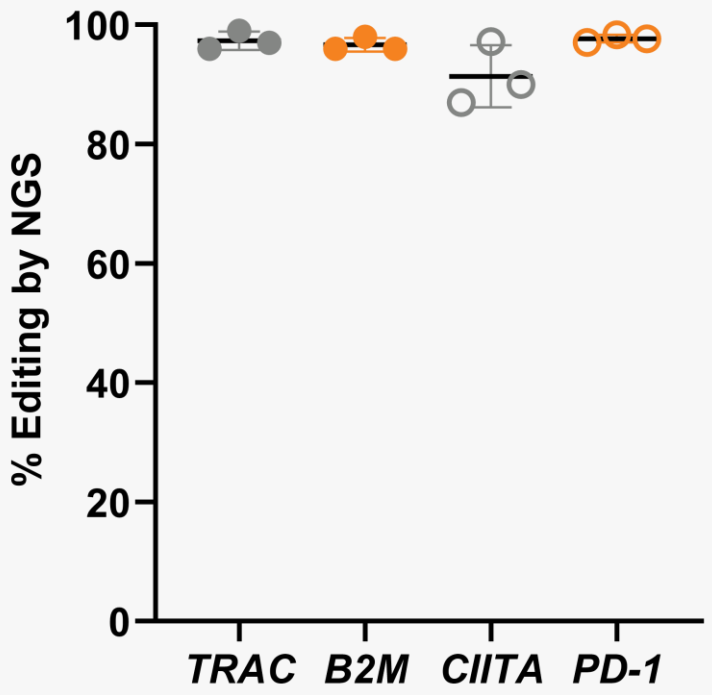
Disclosure

I am an employee and shareholder of Editas Medicine

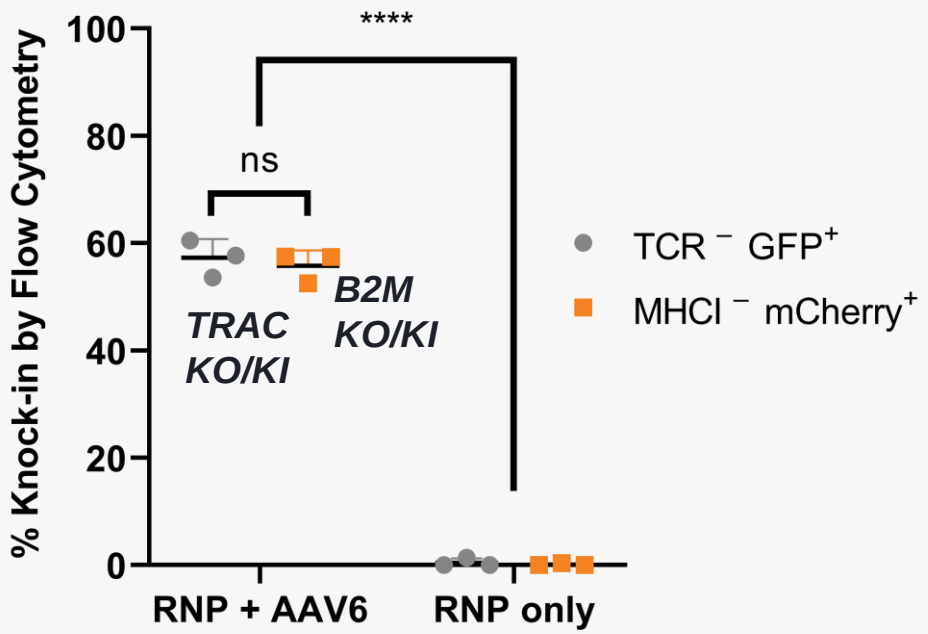
Efficient Knock-in Remains a Challenge in the Cell Therapy Field

T cells

Robust Multiplexed Gene KO



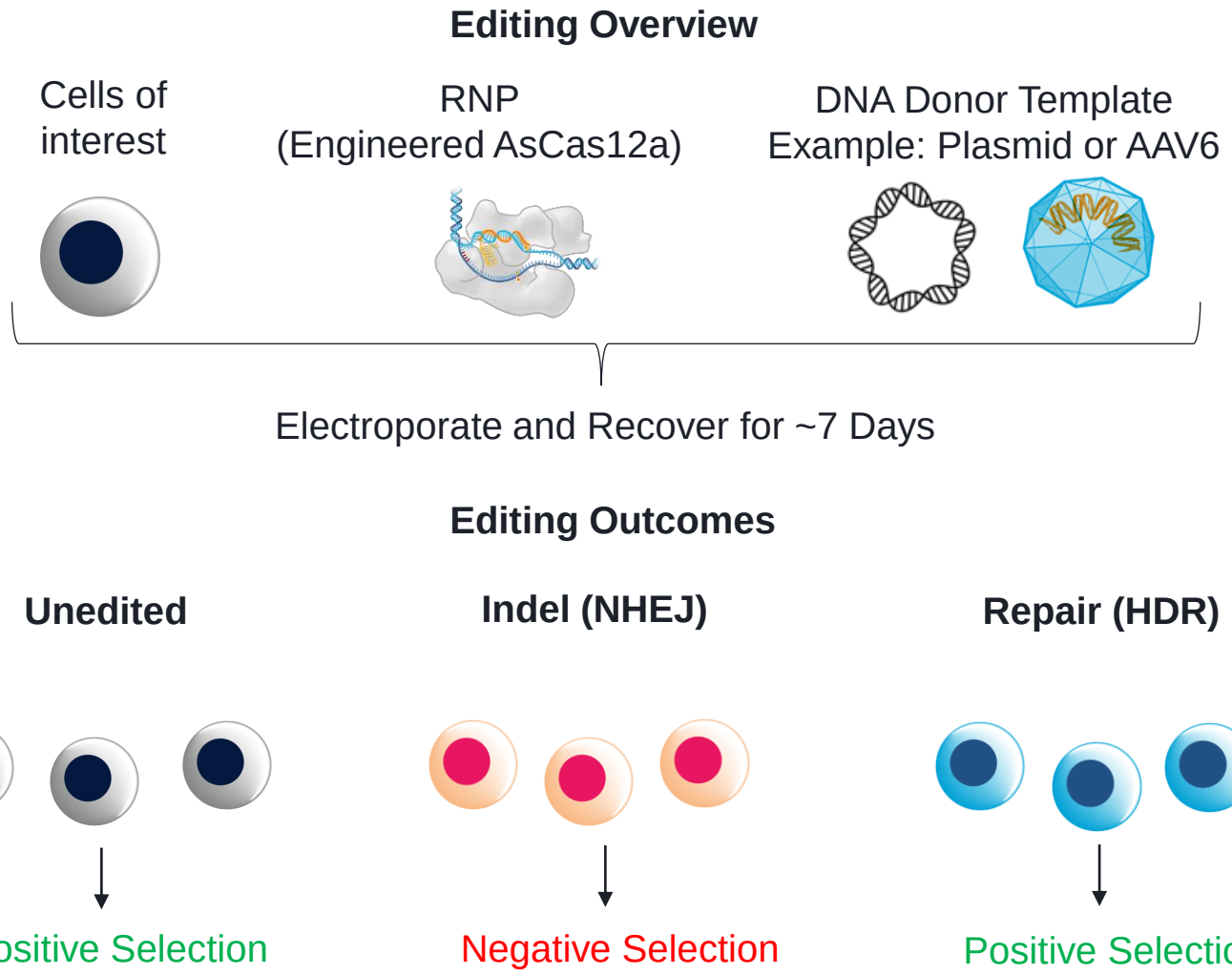
Transgene Knock-in with AAV6



AAV6: 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

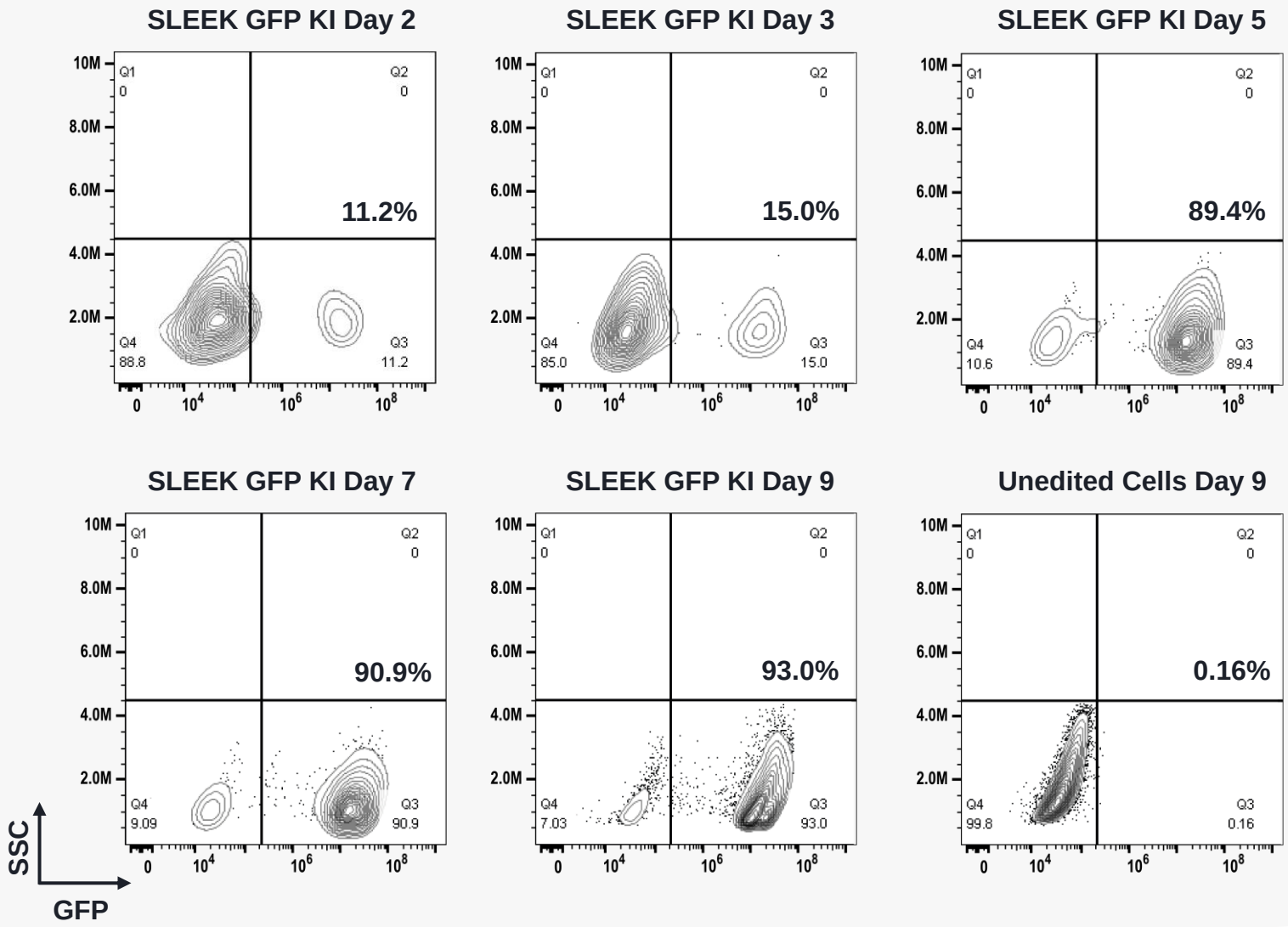


Cas: CRISPR-associated protein; HDR: homology directed recombination; RNP: ribonucleoprotein; NHEJ: non-homologous end joining.

Reduction to Practice of SLEEK Technology

iPSCs

Selection of Knock-in Edit Over KO Edit

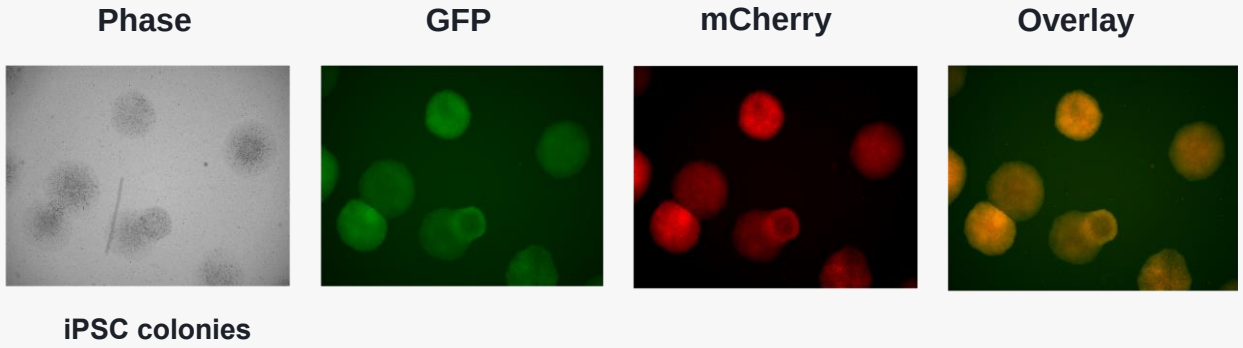


HA: homology arm; GFP: green fluorescent protein; iPSC: induced pluripotent stem cell; KI: knock-in; KO: knock-out; SSC: side scatter; UTR: untranslated region.

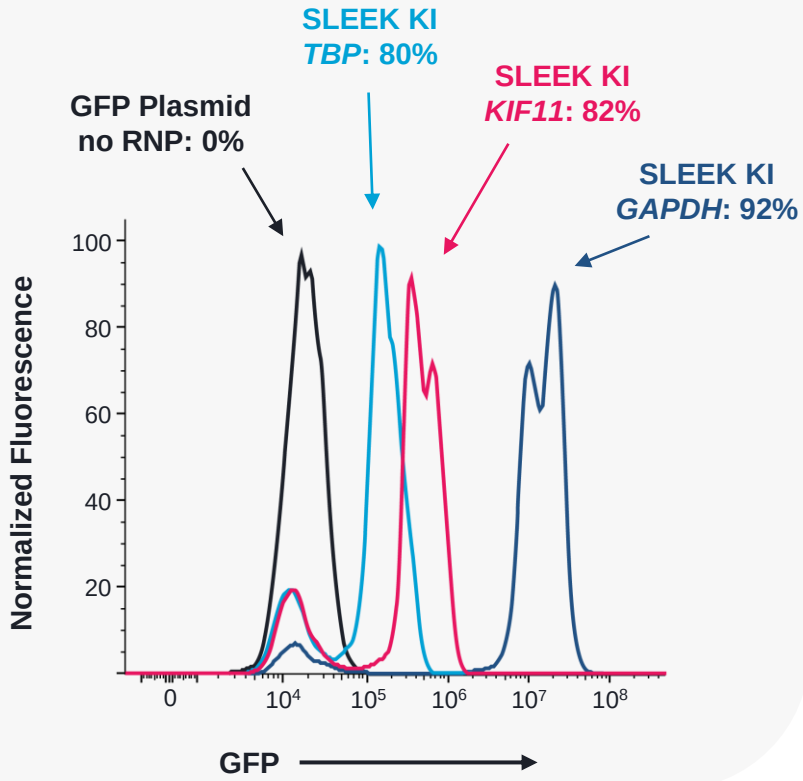
Multicistronic Knock-in and Tunable Expression With SLEEK

iPSCs

Multicistronic KI of GFP and mCherry Transgene Cargos



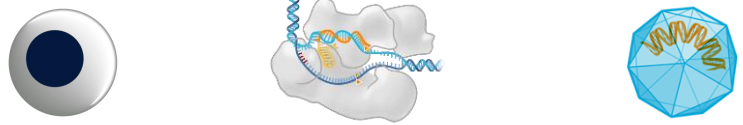
Tunable Cargo Expression by KI at Different Genes



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

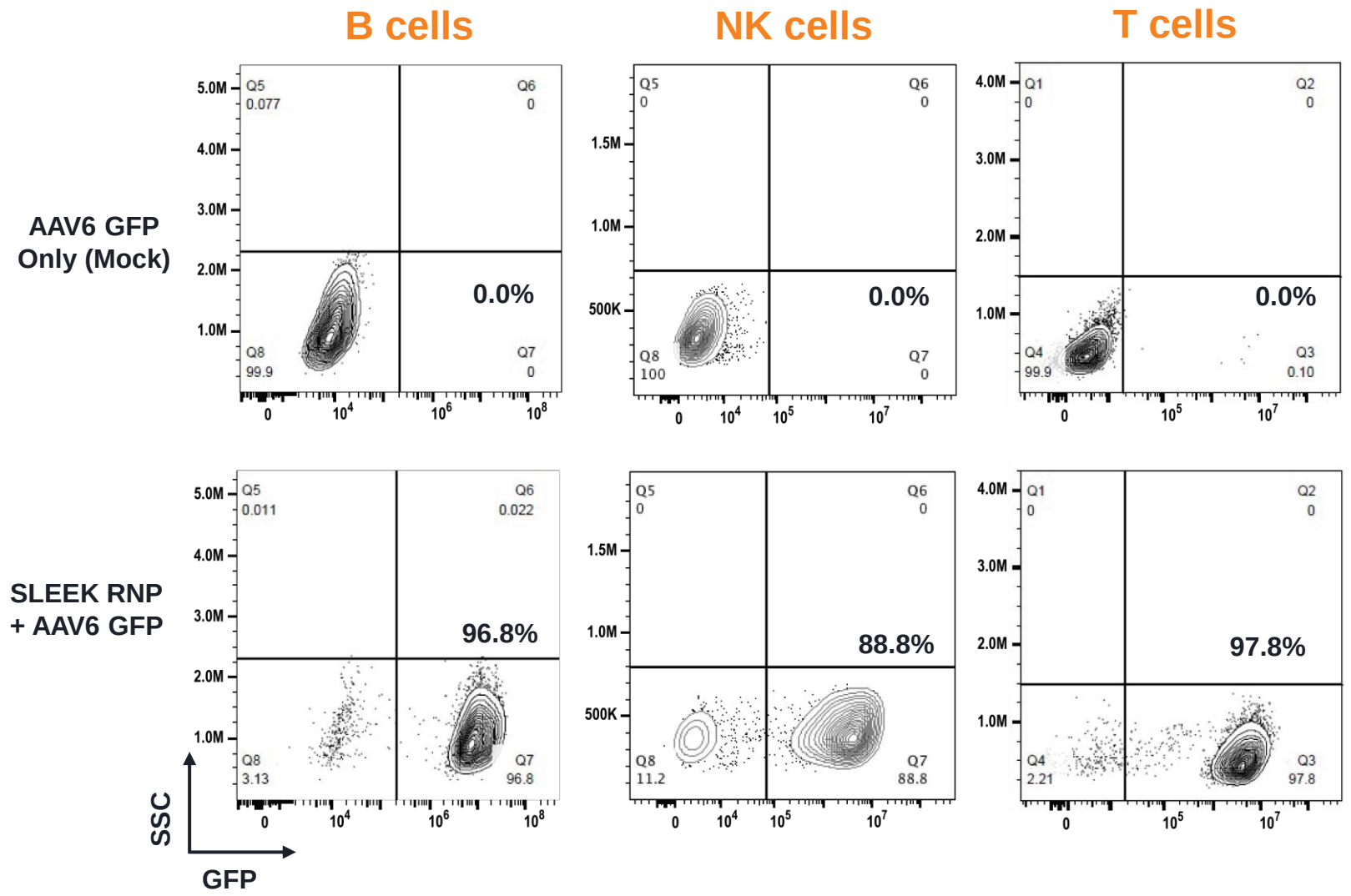
Cells + RNP (AsCas12a) + AAV6 (Cargo)



B cells

T cells

NK cells

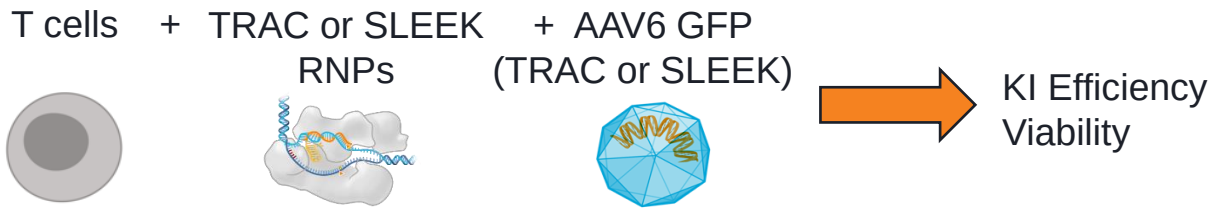


AAV6: adeno-associated virus type 6; Cas: CRISPR-associated protein; CAR: chimeric antigen receptors; GFP; green fluorescent protein; KI: knock-in; NK: natural killer; RNP: ribonucleoprotein; SSC: side scatter.

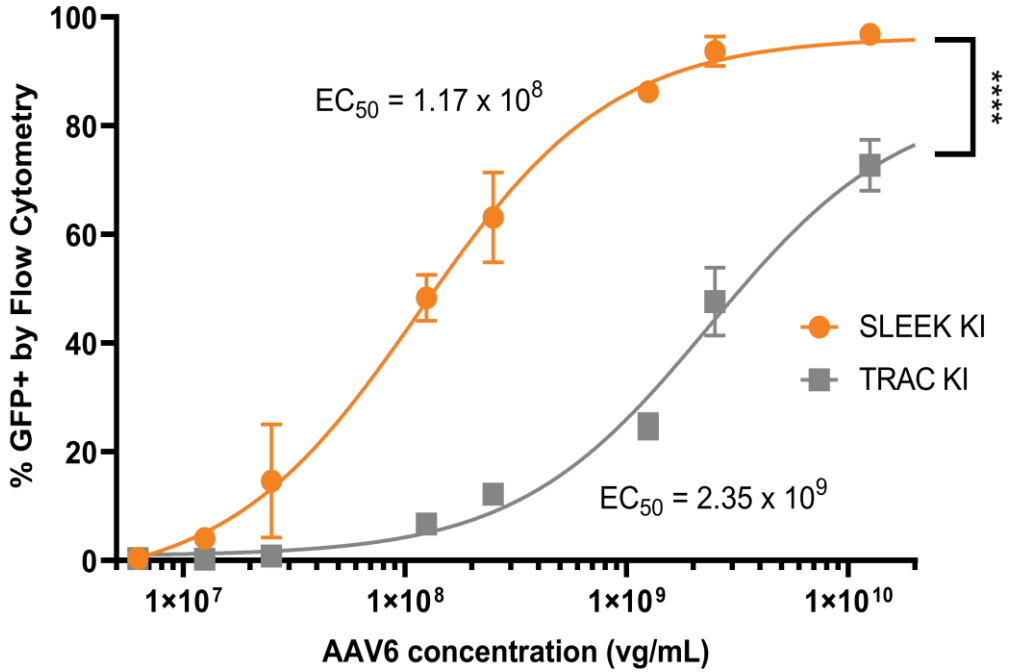
SLEEK is More Efficient and Potent Than *TRAC* KI Gold Standard

T cells

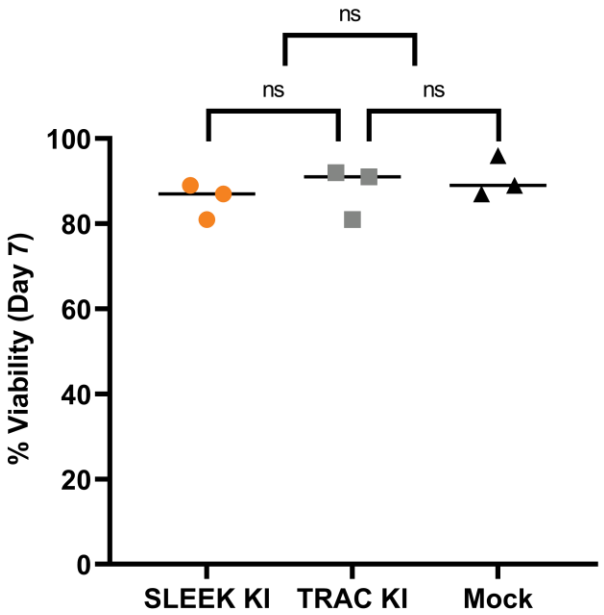
Experimental Schematic



Superior Efficiency and Potency to *TRAC* KI



Similar Day 7 Viability

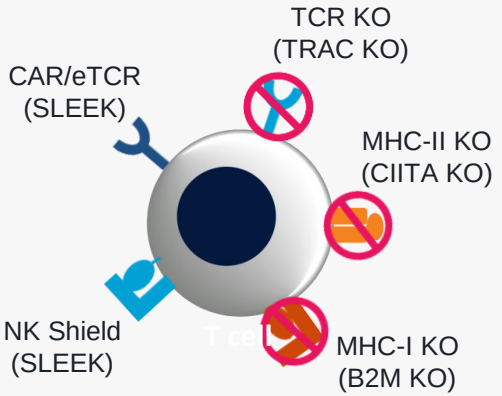


AAV6: adeno-associated virus type 6; GFP: green fluorescent protein; HDR: homology-directed repair; KI: knock-in; ns: not significant; RNP: ribonucleoprotein; TRAC: T-cell receptor α constant.

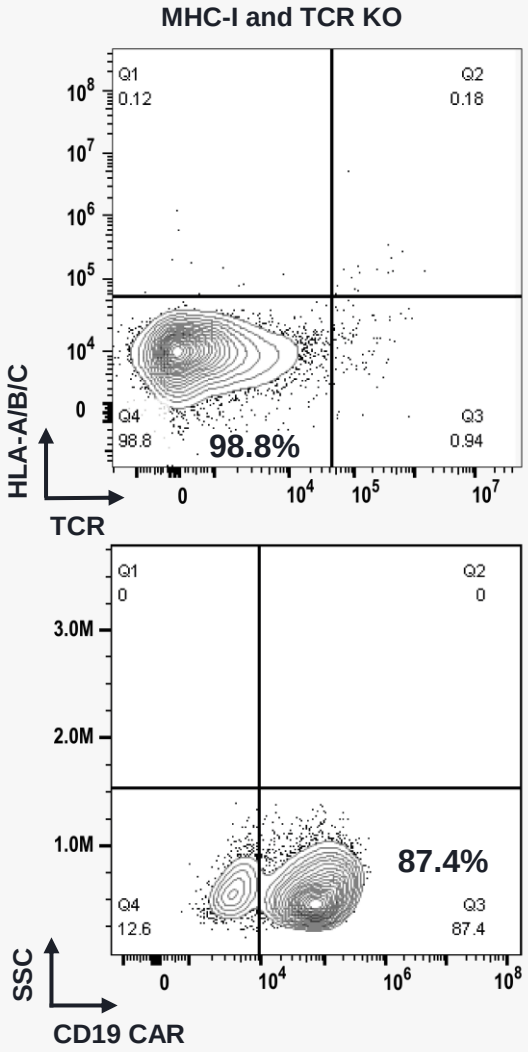
SLEEK Enables the Generation of Multi-Edited Cell-Based Medicines

T cells

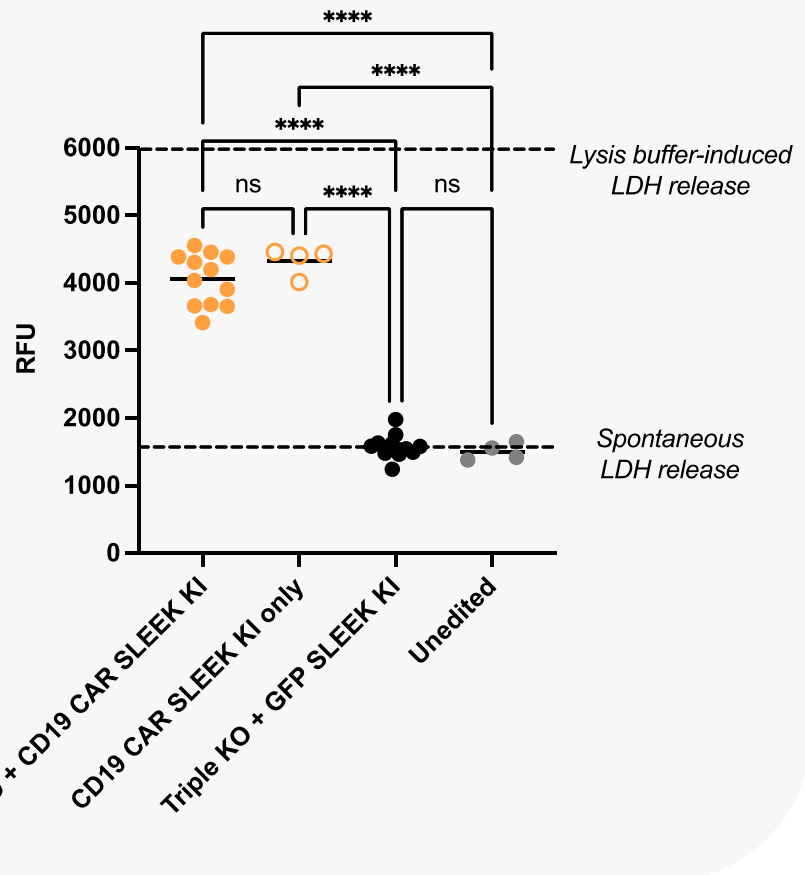
Allogeneic Healthy Donor Applications



Validation of KO/KI



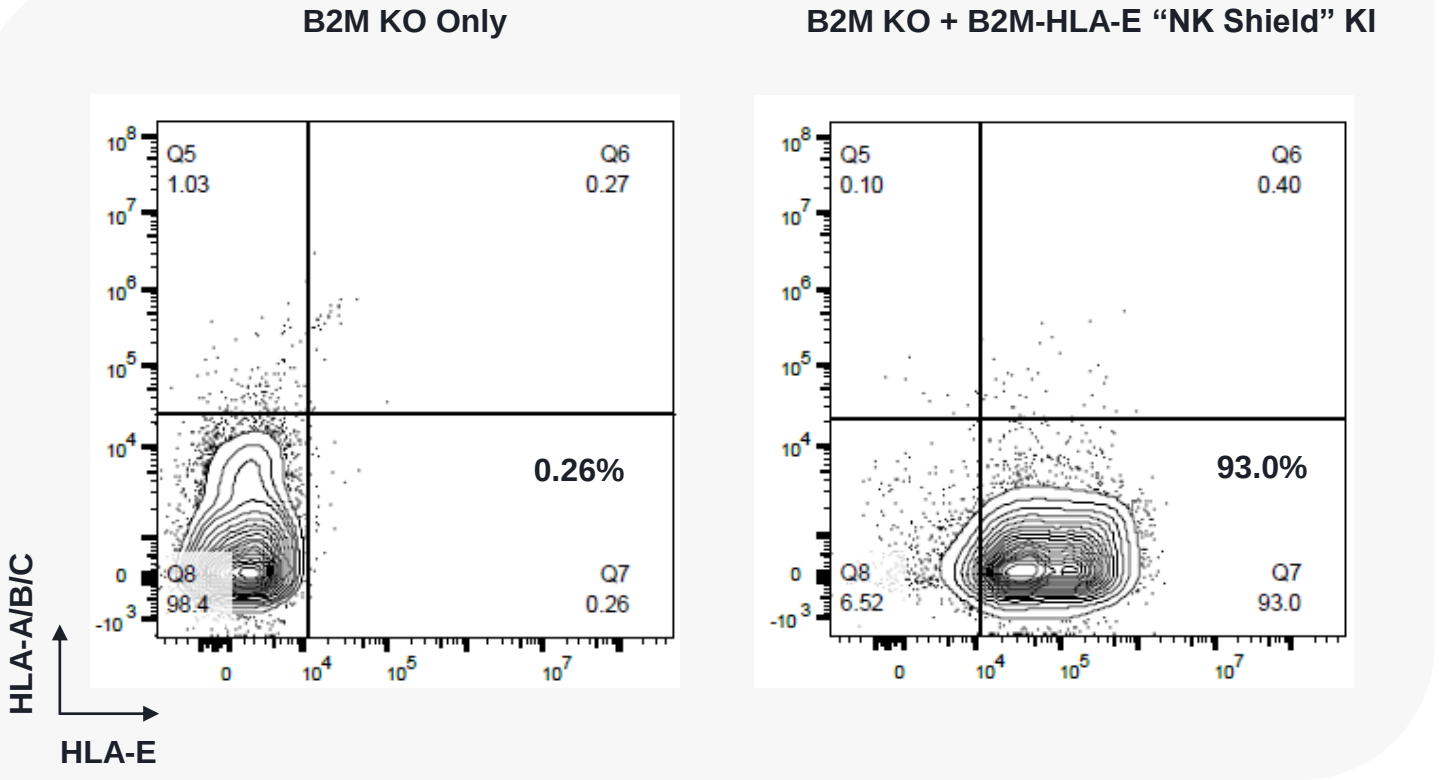
Tumor Killing

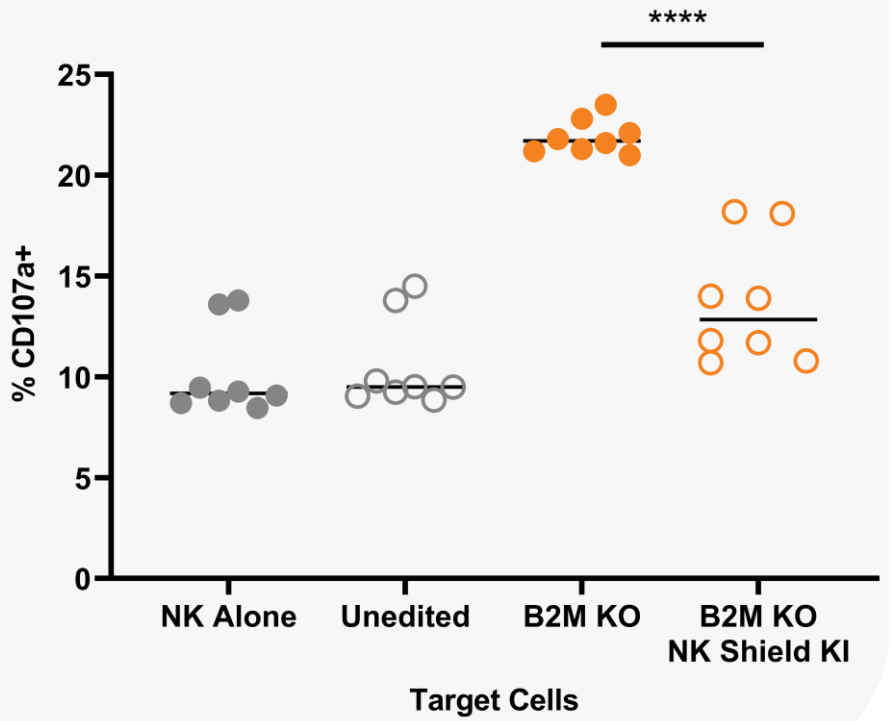


AAV6: adeno-associated virus type 6; B2M; beta-2 macroglobulin; CAR: chimeric antigen receptors; CIITA: class II major histocompatibility complex transactivator; CD: cluster of differentiation; GFP; green fluorescent protein; KI: knock-in; KO: knock-out; MHC: major histocompatibility complex; NK: natural 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

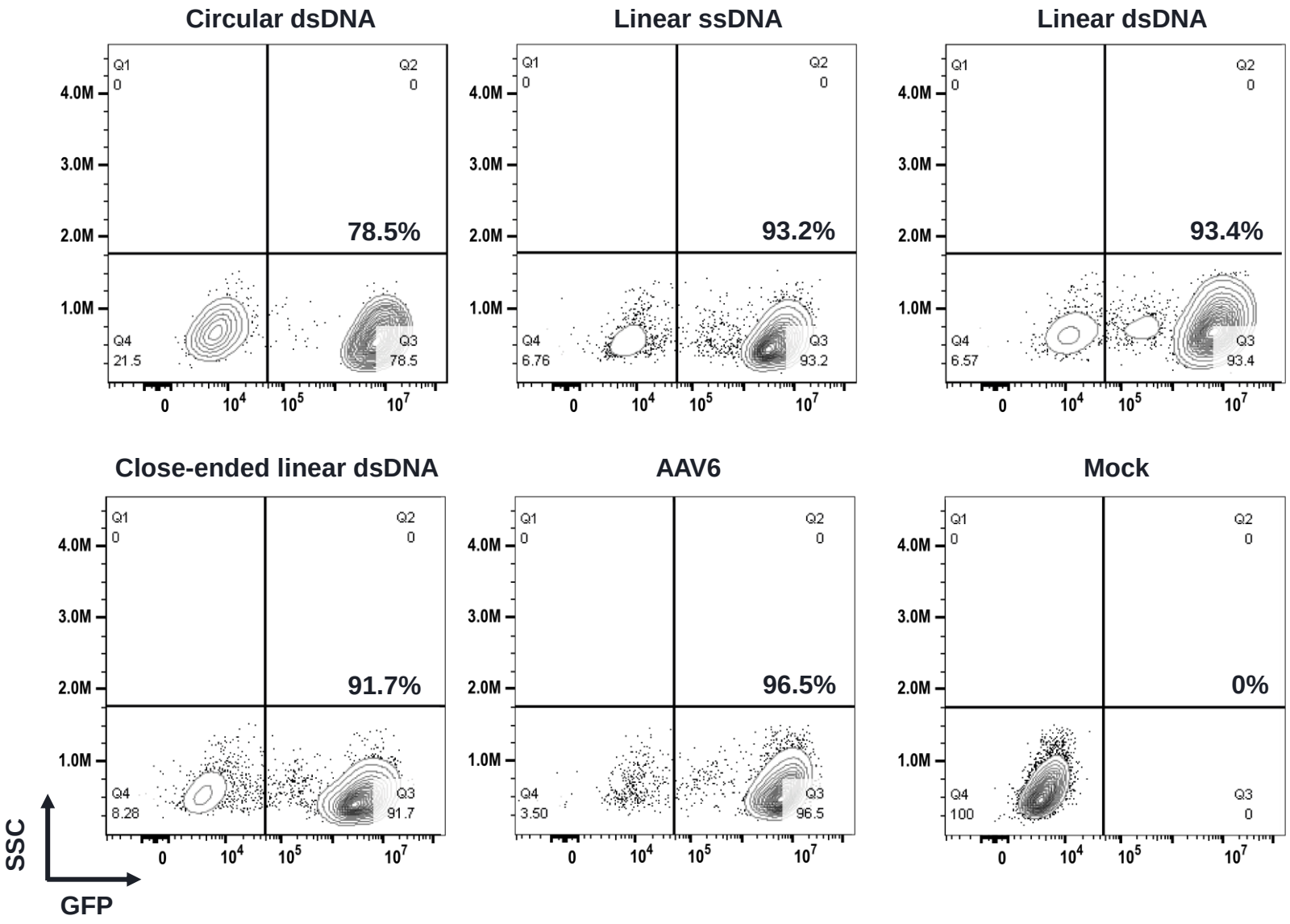


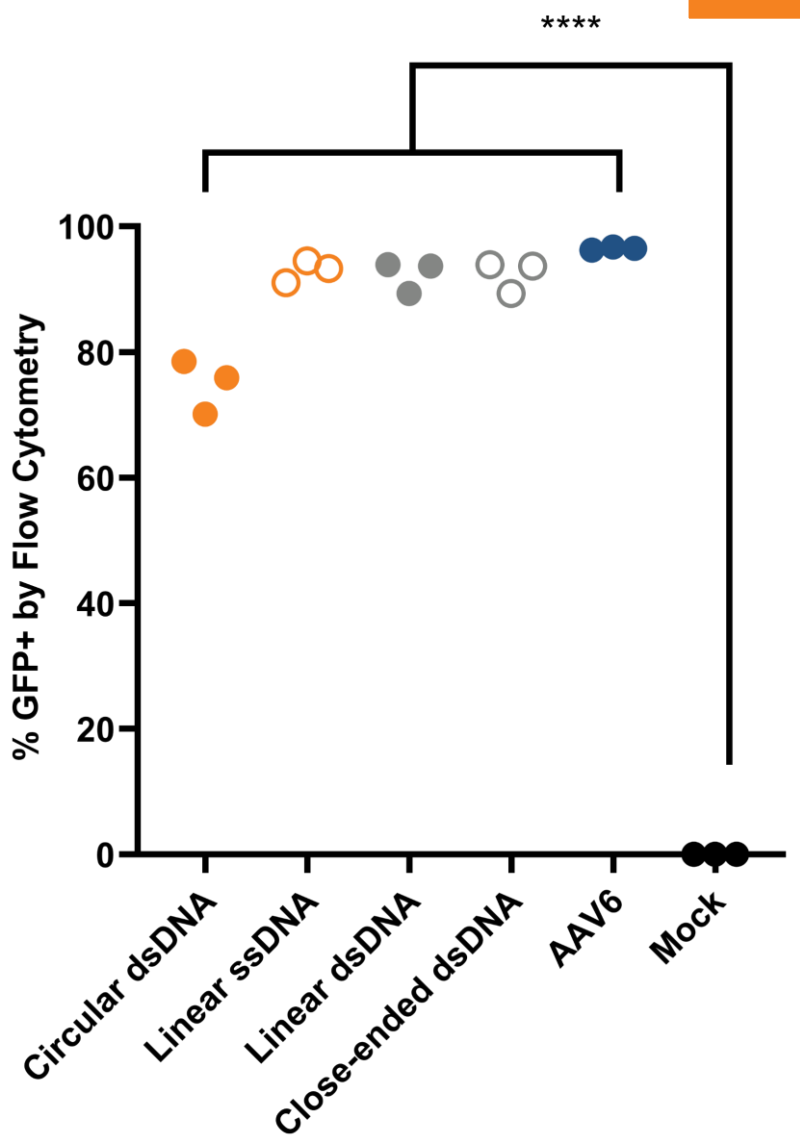


AAV6: Adeno-associated virus type 6; B2M; beta-2 macroglobulin; CD: cluster of differentiation; HLA-E: human leukocyte antigen E; KI: knock-in; KO: knock-out; MHC: major histocompatibility complex; NK: natural killer; NKG2A: NK group 2 member A; RNP: ribonucleoprotein; TCR: T-cell receptor.

SLEEK is Similarly Efficient With Non-Viral DNA Templates

T cells

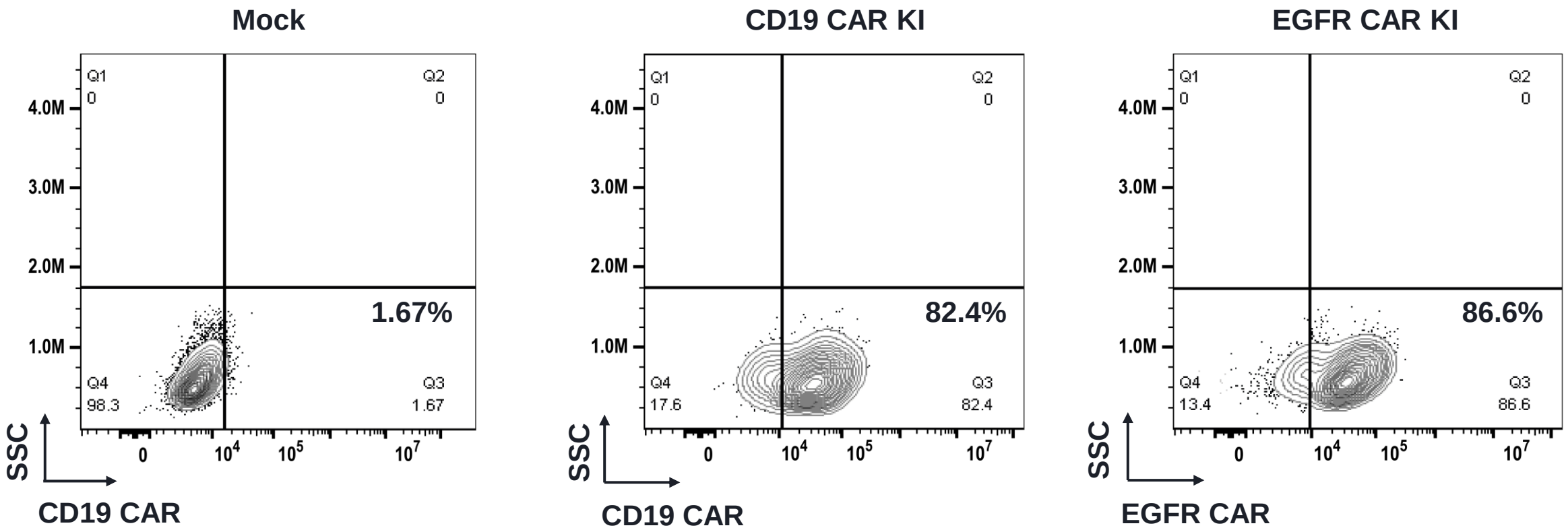




AAV6: adeno-associated virus type 6; dsDNA: double stranded deoxyribonucleic acid; GFP: green fluorescent protein; SSC: side scatter; ssDNA: single stranded deoxyribonucleic acid.

SLEEK KI of Relevant Cargos With Non-Viral DNA Templates

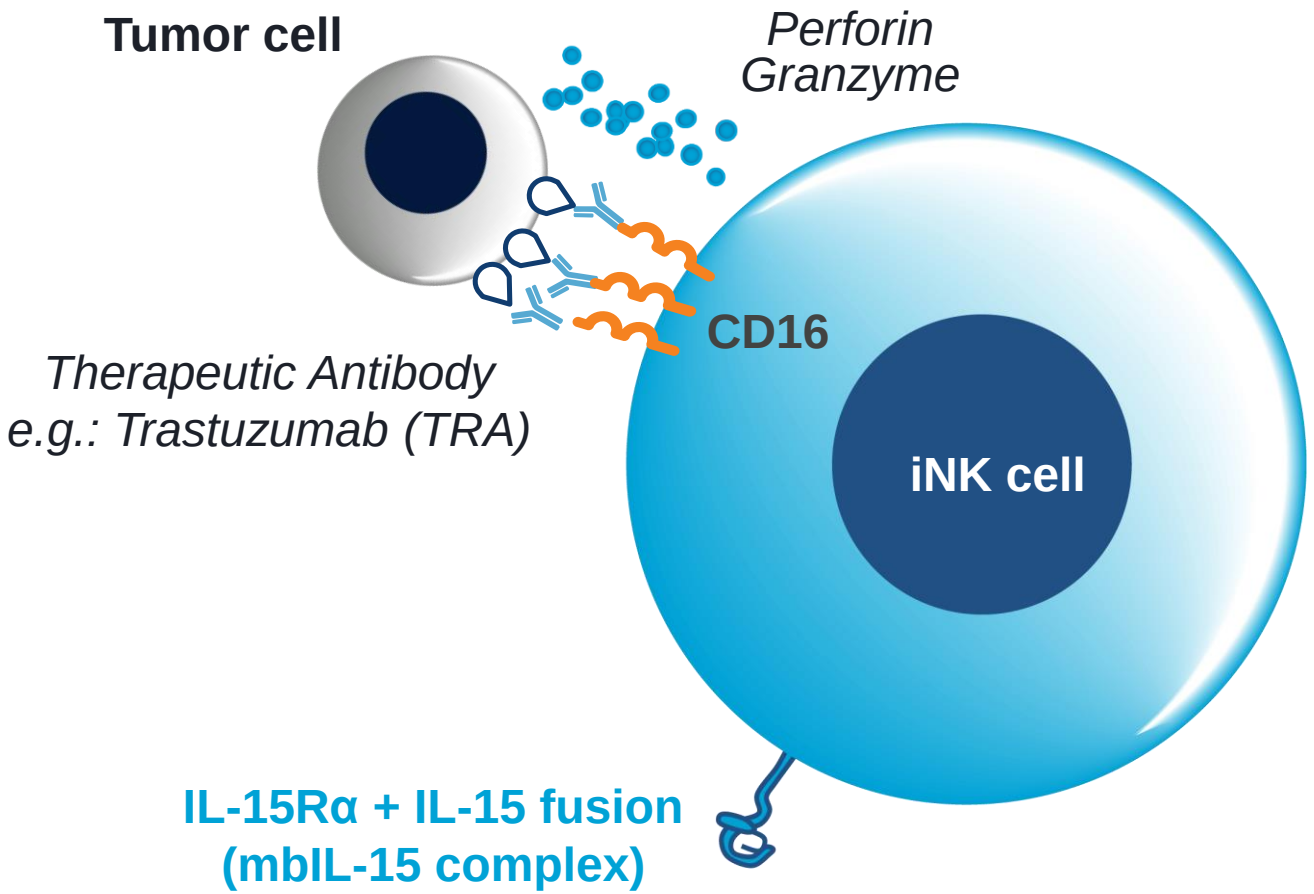
T cells



CAR: chimeric antigen receptors; CD: cluster of differentiation; EGFR: epidermal growth factor receptor; KI: knock-in; KO: knock-out; 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

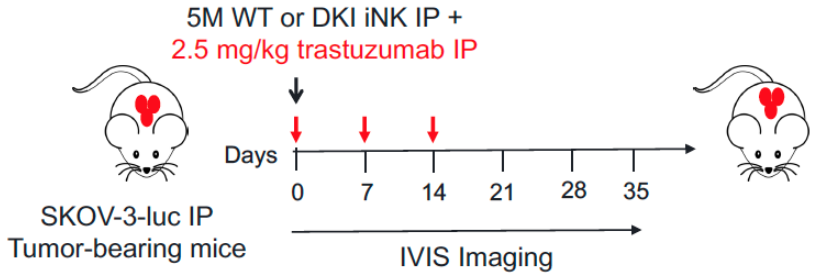
Generation of an Edited iNK Cell Through SLEEK Double Knock-in of CD16 and mbIL-15 (SLEEK DKI)

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.

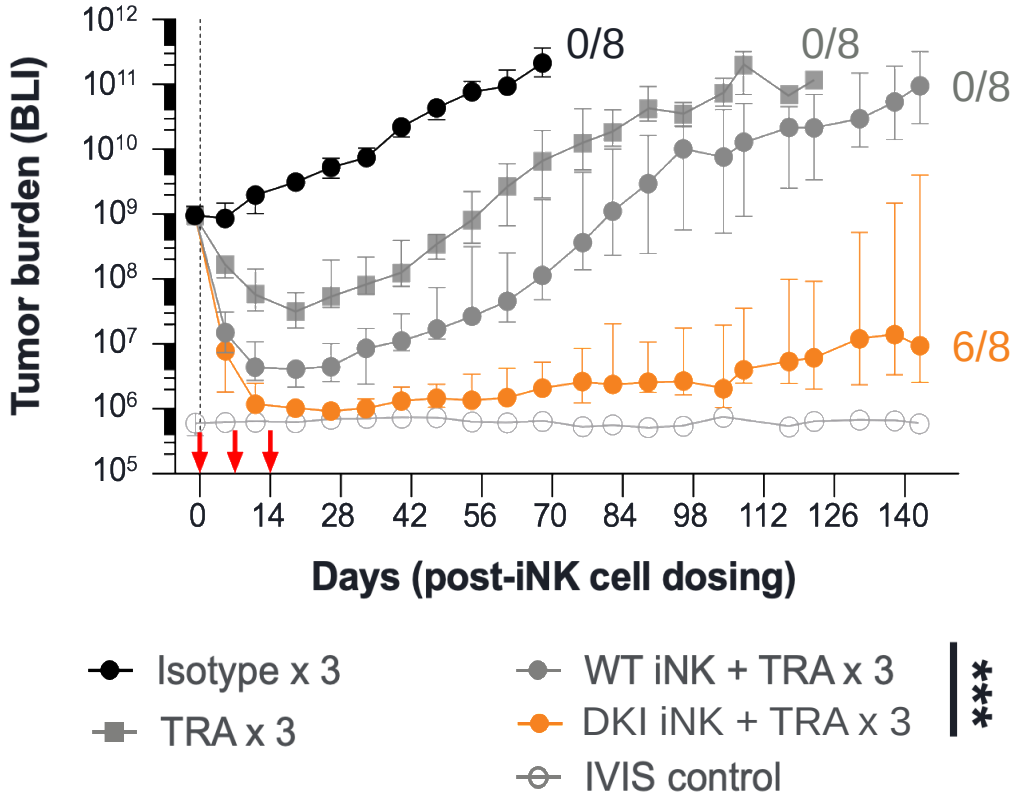
SLEEK DKI iNKs Administered in Combination with Trastuzumab Induced Significant to Complete Tumor Clearance in Mice

iNK cells

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



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



Strong Tumor Clearance Attributed to Robust Expression of Our Cargos 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.

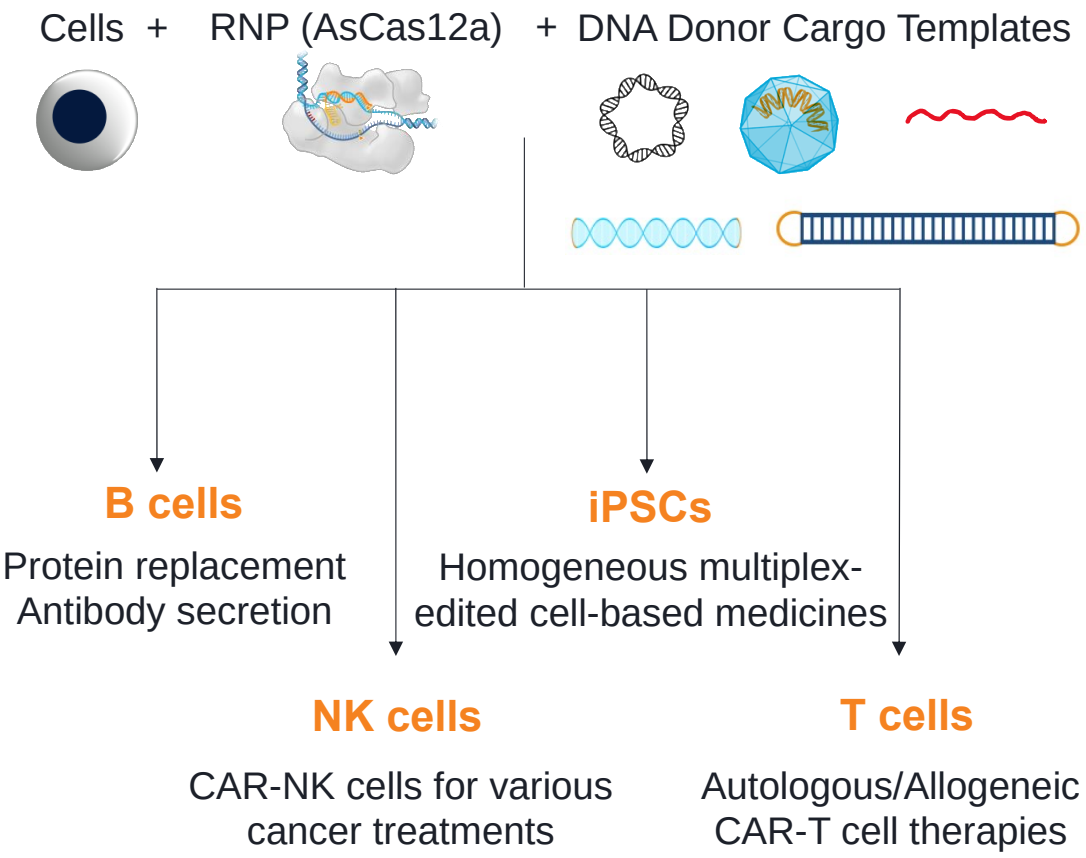
Gerew et al. ASH, 2021; Allen, Khan et al. AACR, 2022.

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

SLEEK™ GENE EDITING

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

- Enables >95% knock-in efficiency
- High-level, tunable cargo expression
- Achieves near-homogeneous editing
- Efficient knock-in of multicistronic cargos
- Works equivalently well with both viral and non-viral DNA cargo formats
- Robust, lineage-independent, expression of functional cargo in iPSCs to generate iNK cells with unprecedented persistence *in vivo*



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

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