An Engineered AsCas12a Nuclease Facilitates the Rapid Generation of Therapeutic Cell Medicines

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Disclosure

- I am an employee and shareholder of Editas Medicine

Overview of Presentation

GENERATION OF A HIGHLY ACTIVE ASCAS12A VARIANT CALLED ASCAS12A ULTRA	▪ Collaboration with IDT to try and engineer a highly active AsCas12a variant ▪ Assessment of AsCas12a editing across targets and cell types ▪ Assessment of how AsCas12a Ultra specificity compared to WT nuclease
ASCAS12A ULTRA COMPARISON TO ENCAS12A	▪ Comparison of AsCas12a editing efficiency and potency to enCas12a and HiFi variant across targets and cell types ▪ Demonstration of the effects of combining mutations between AsCas12a Ultra and the enCas12a engineered nucleases
APPLICATIONS OF ASCAS12A ULTRA IN CELL MEDICINES	▪ Demonstration of multiplexed editing in T cells ▪ Demonstration of highly efficient single and dual knock-in in T cells ▪ Demonstration of functional knock-out and knock-in in NK cells and potential applications for these edits in oncology

This Work was Part of a Collaboration with Integrated DNA Technologies

DIRECTED EVOLUTION OF ENGINEERED ASCAS12A AND TESTING IN CANCER CELL LINES

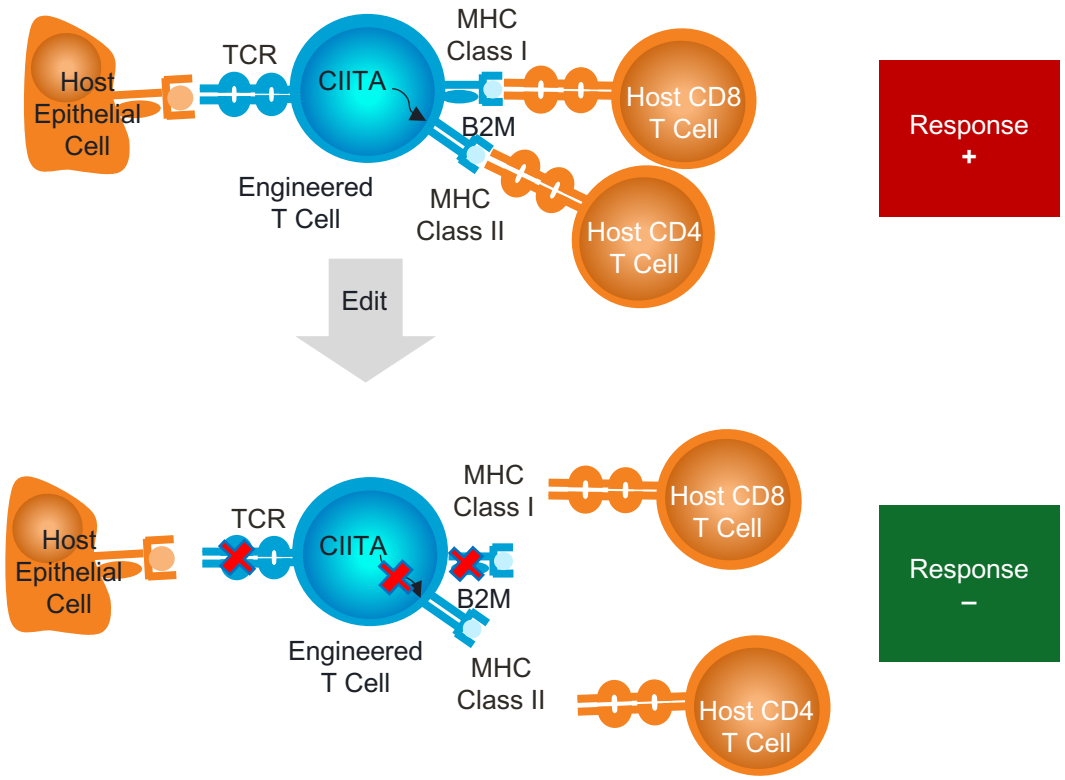
- Liyang Zhang
- Chris Vakulskas

OPTIMIZATION OF CLINICALLY RELEVANT KNOCK OUT AND KNOCK-IN EDITS IN PRIMARY CELLS

- Ramya Viswanathan
- Jasmine Edelstein
- Swarali Lele
- Sean Scott
- Kevin Wasko
- Steven Sexton
- Chris Borges

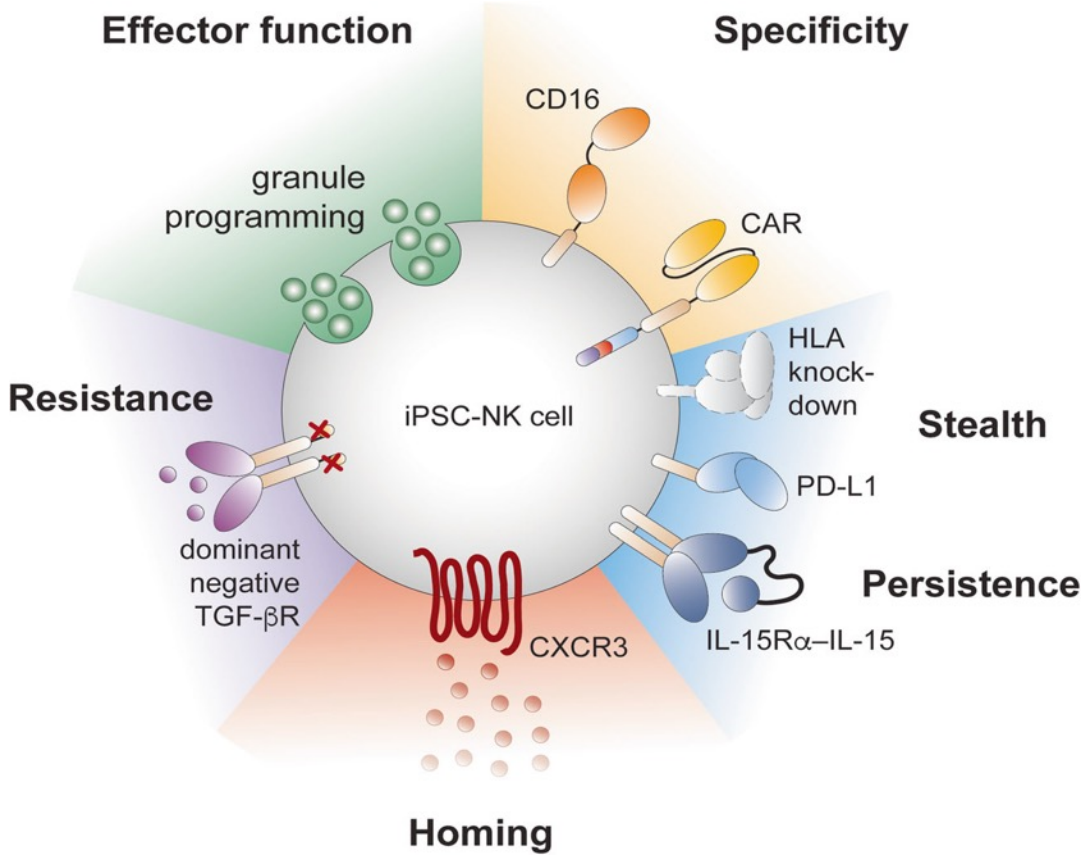
Multiplexed Gene Editing is Key for the Next Generation of Cell Medicines

Proposed Allogeneic T cell Edits



Zuris et al. ESGCT 2018

Proposed iNK Edits



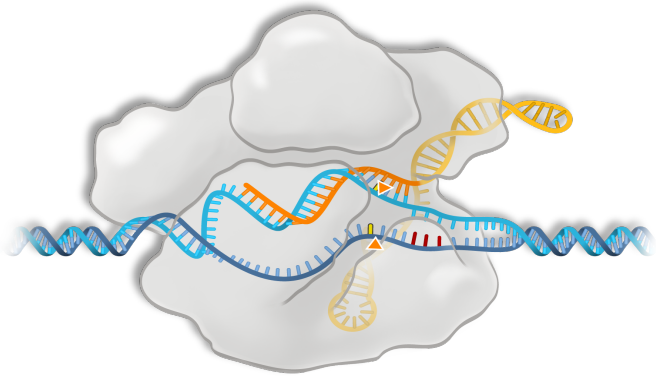
Saetersmoen et al. Seminars in Immunopathology 2019

The ideal nuclease must be highly efficient, highly specific, and be able to achieve efficient knock-in

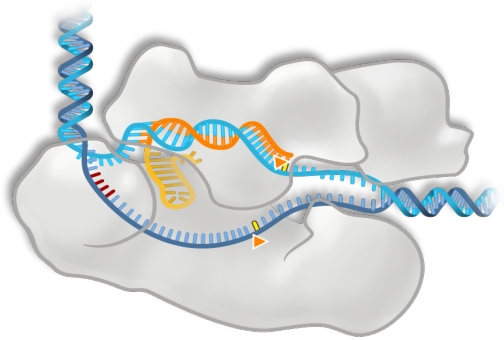
There are Several CRISPR Nuclease Options for the Potential Generation of Engineered Cell Medicines

Editas general suite of CRISPR nucleases

Variant	PAM	Frequency (bp)
SpCas9	NGG	1 in 8
SaCas9	NNGRRT	1 in 32
SaCas9 KKH	NNNRRT	1 in 8
AsCas12a	TTTV	1 in 43
AsCas12a RR	TYCV/CCCC	1 in 18
AsCas12a RVR	TATV	1 in 43



Cas9



Cas12a

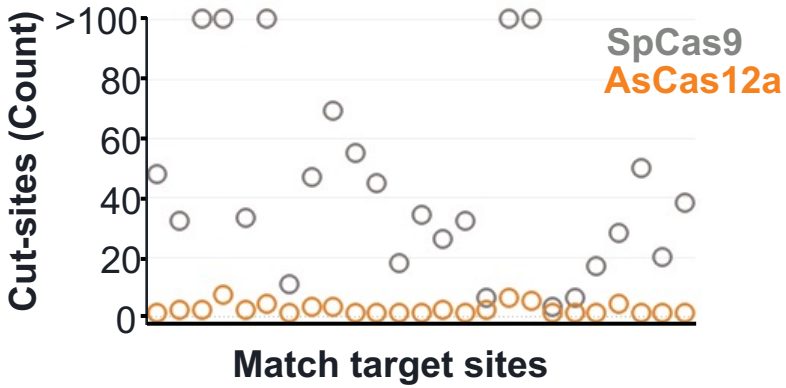
Takeaway:

PAM sites are generally not limiting for general multi-gene knock-out and large transgene knock-in applications

The AsCas12a Nuclease has Higher Intrinsic Specificity and Higher Sequence Fidelity in the Shorter Chemically Synthesized Guide RNA

Specificity:

Matched Target Site (20-Ns):
TTTVNNNNNNNNNNNNNNNNNNNNNGG



Gotta et al. CSHL 2019

Takeaway:

AsCas12a is more specific across matched sites in the genome in contrast to SpCas9

Guide RNA synthesis:

Chemical synthesis of gRNAs occurs in the 3' → 5' direction and purity and yield of the entire gRNA sequence drops with increasing length

Cas12a gRNAs (~40mer) are much shorter than SpCas9 gRNAs (~100mer)

Cas9 guide is **most sensitive** to mismatches at 5' end which is the location of **lowest sequence fidelity** as this is where synthesis ends

Cas12a guide is **most sensitive** to mismatches at 3' end which is the location of **highest sequence fidelity** as this is where synthesis starts

Lack of sequence fidelity will lead to unanticipated off-target editing due to errors in RNA sequence targeting the protospacer region

Takeaway:

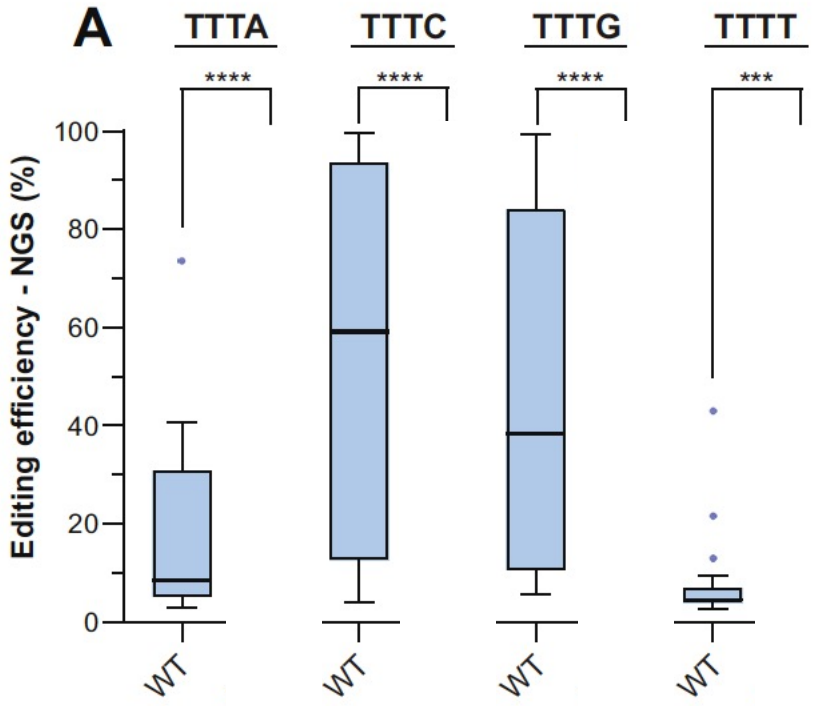
AsCas12a synthetic gRNAs have reduced risk of off-target editing that results from synthesis errors

References on Cas12a specificity:
Kim et al. Nat Biotech 2016, Kleinstiver et al. Nat Biotech 2016, Strohkendl et al. Mol Cell 2018, Swarts et al. Biochem Soc Trans 2019

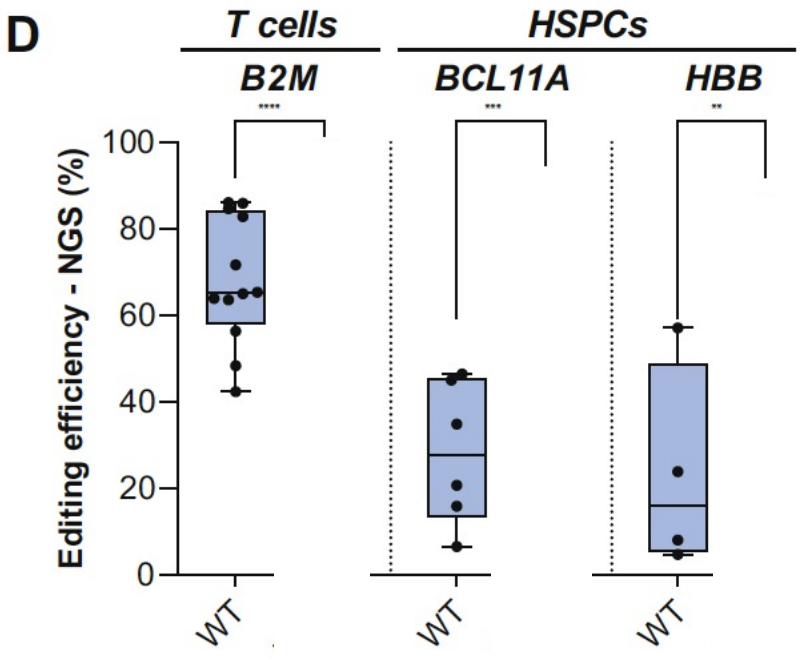
Given the Clear Specificity Advantages of AsCas12a Why has it Not Been Widely Adopted in *Ex Vivo* Applications in Research and the Clinic?

Assessment of WT AsCas12a editing and HDR efficiency

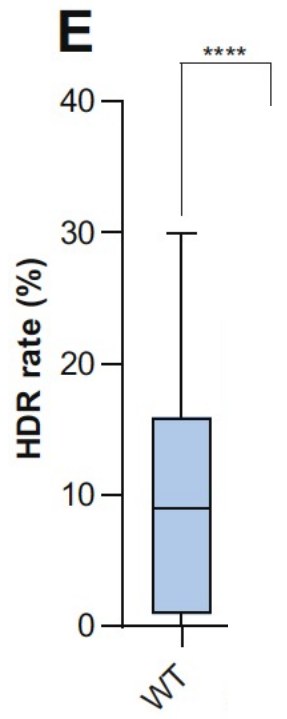
Cancer cell lines



Primary cells



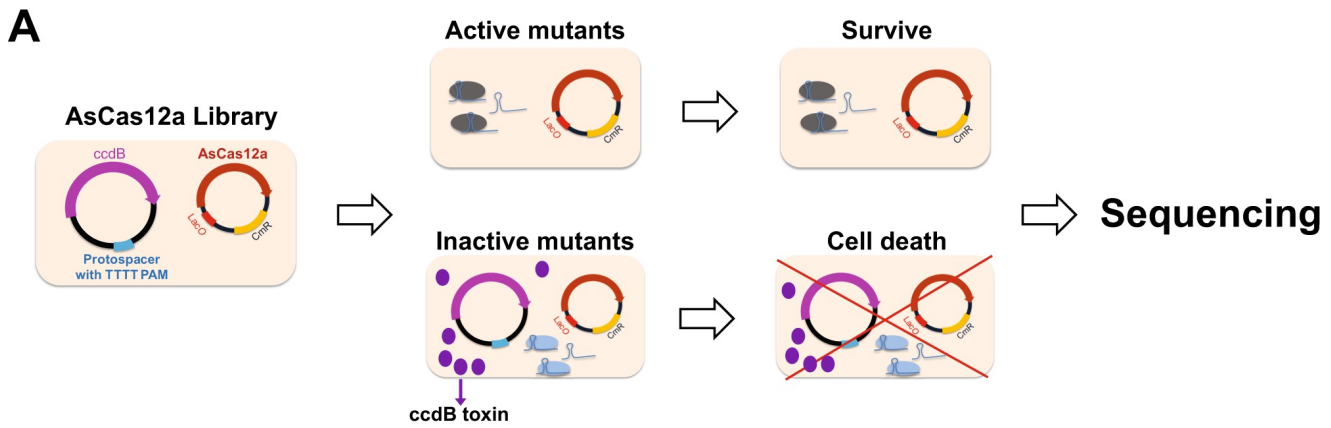
HDR in cancer cell lines

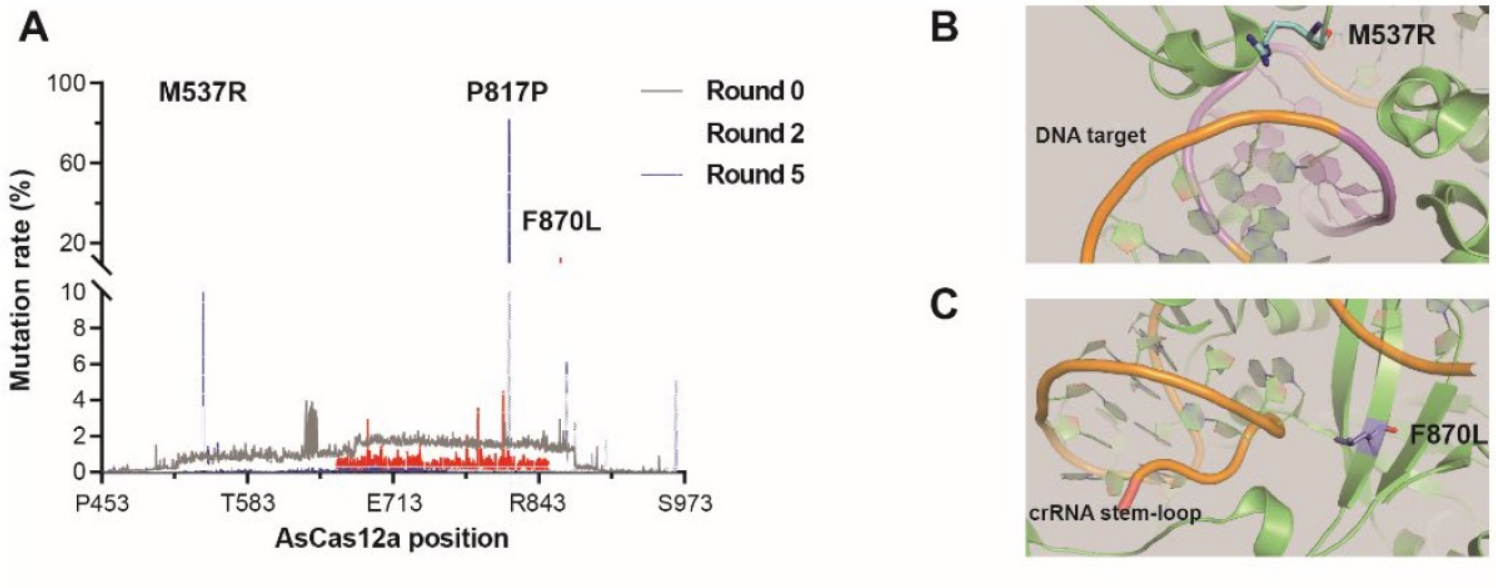


WT AsCas12a is generally inefficient for both gene KO and KI across most targets and cell types

Zhang et al. Nature Communications 2021

Bacterial Selection Experiment Performed by IDT Team Revealed a Mutant AsCas12a Protein with Potentially Higher Activity

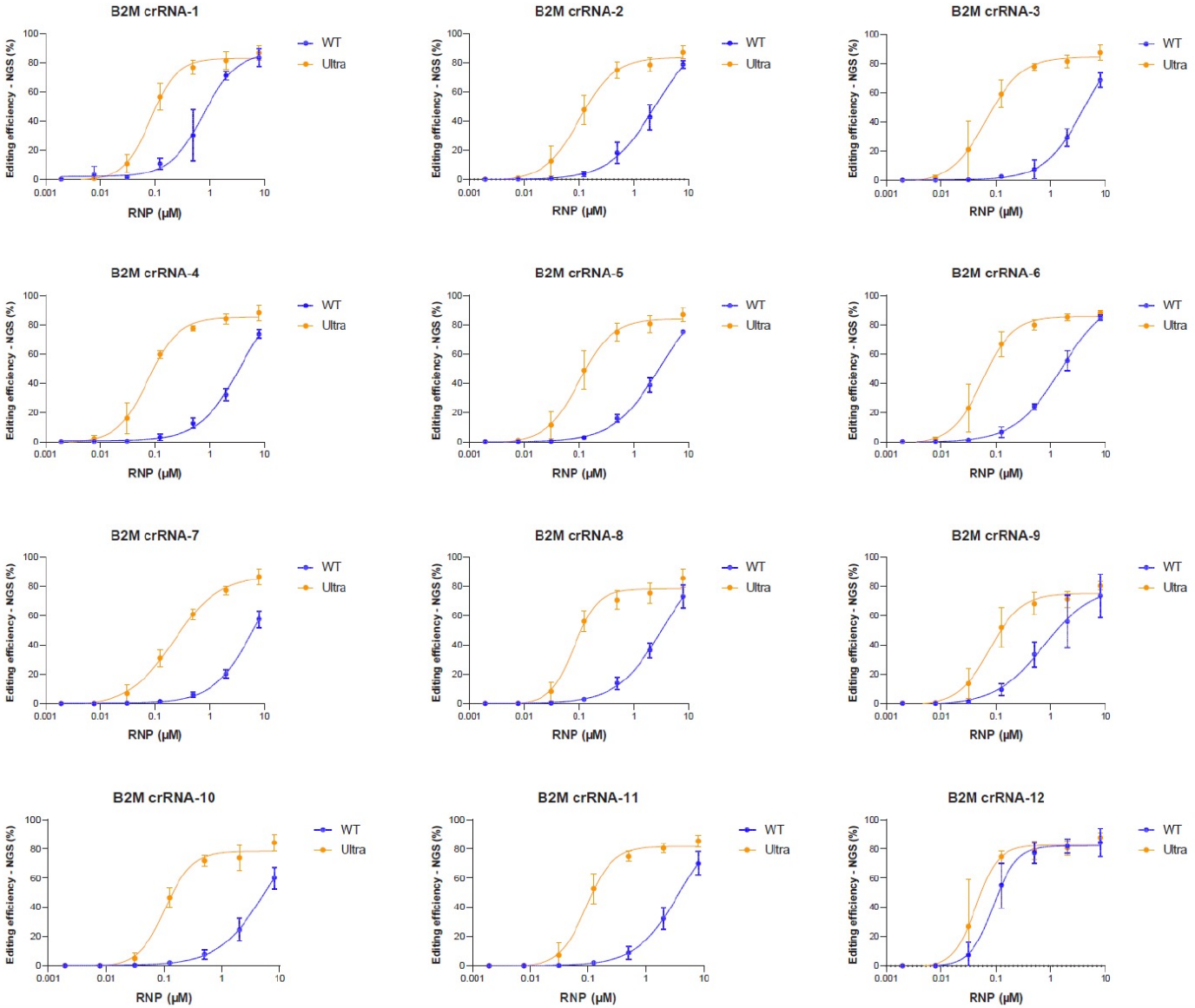




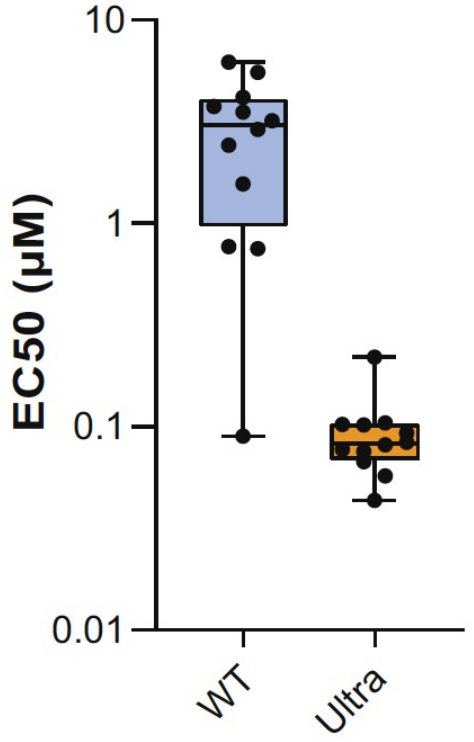
M537R and F870L point mutations were combined and taken forward to make AsCas12a “Ultra”

Zhang et al. Nature Communications 2021

AsCas12a Ultra is 40-fold More Potent On Average Compared to WT AsCas12a Across All Potential Targets at the *B2M* Locus in T cells



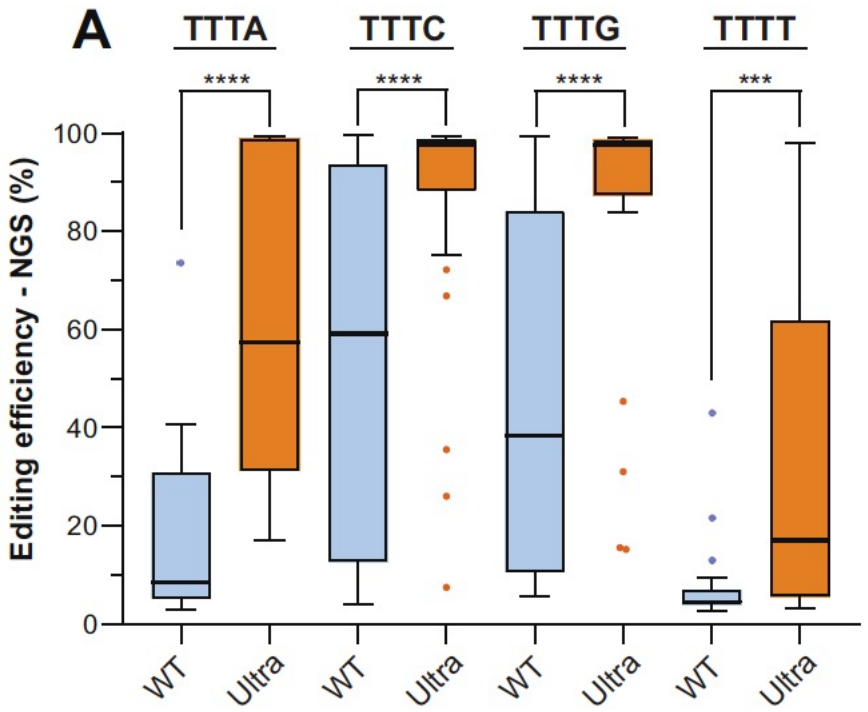
40-fold increase in EC50



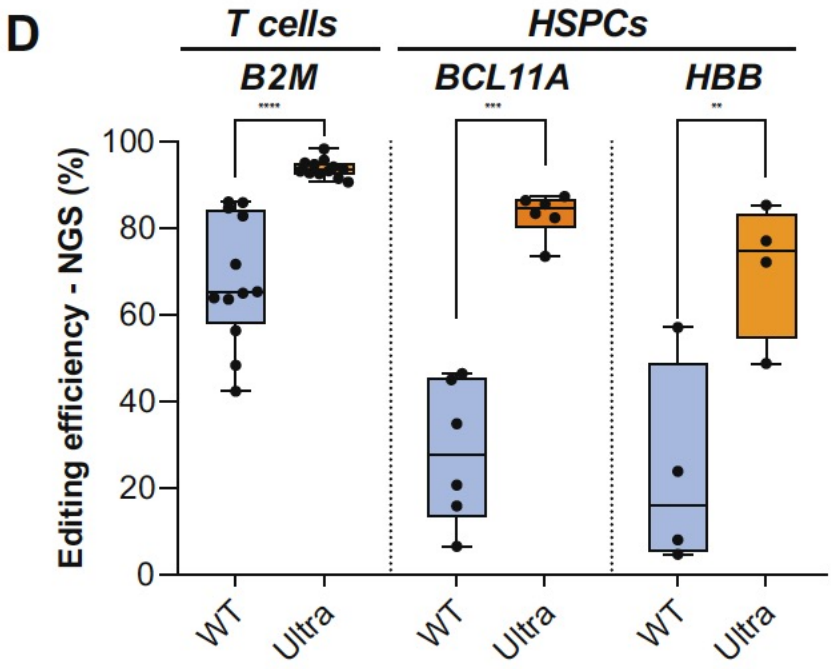
AsCas12a Ultra Shows Highly Efficient Editing Across All Guides Tested Across Different Loci and Across All Tested Cell Types

Assessment of WT AsCas12a editing and HDR efficiency

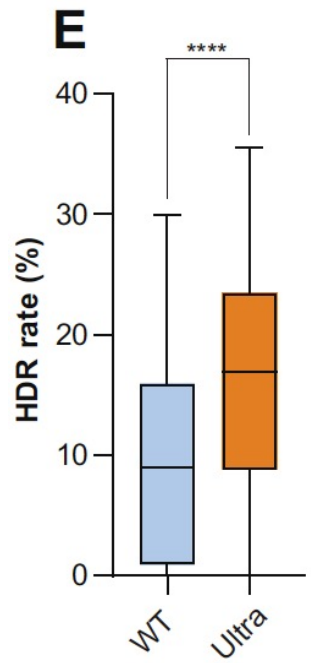
Cancer cell lines



Primary cells

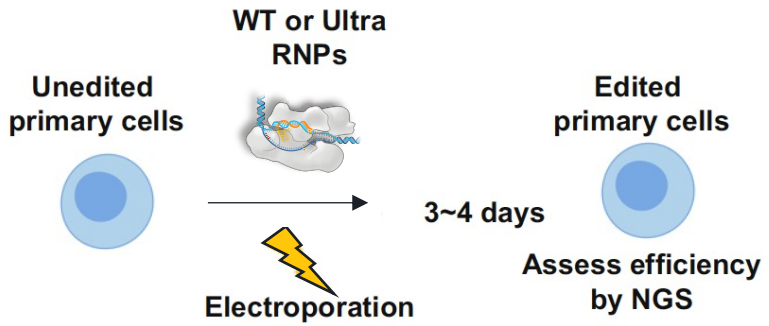


HDR in cancer cell lines

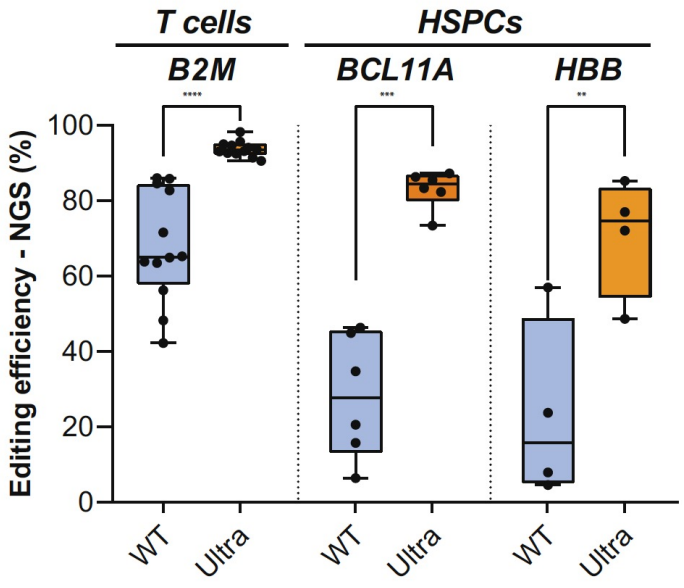


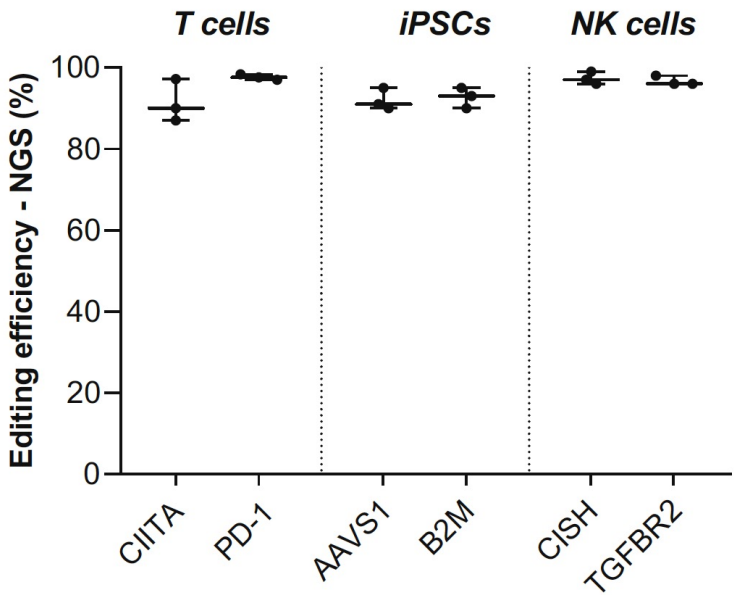
WT
Ultra

AsCas12a Ultra Enables the Generation of Engineered Cell Medicines



Cell Type	Target Locus (# of crRNA)			
T cells	TRAC (1)	B2M (12)	CIITA (1)	PD-1 (1)
HSCs	BCL11A (5)	HBB (4)		
NK cells	CISH (1)	TGFBR2 (1)		
iPSCs	AAVS1 (1)	B2M (1)		





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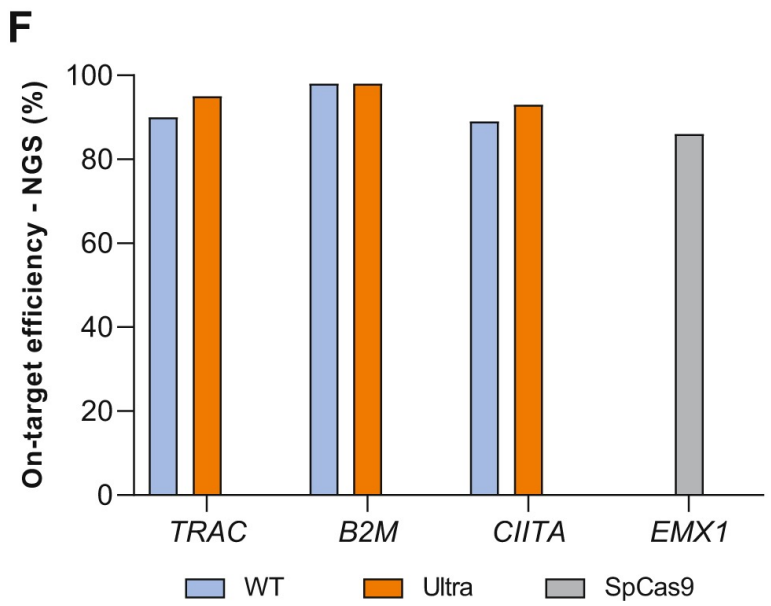
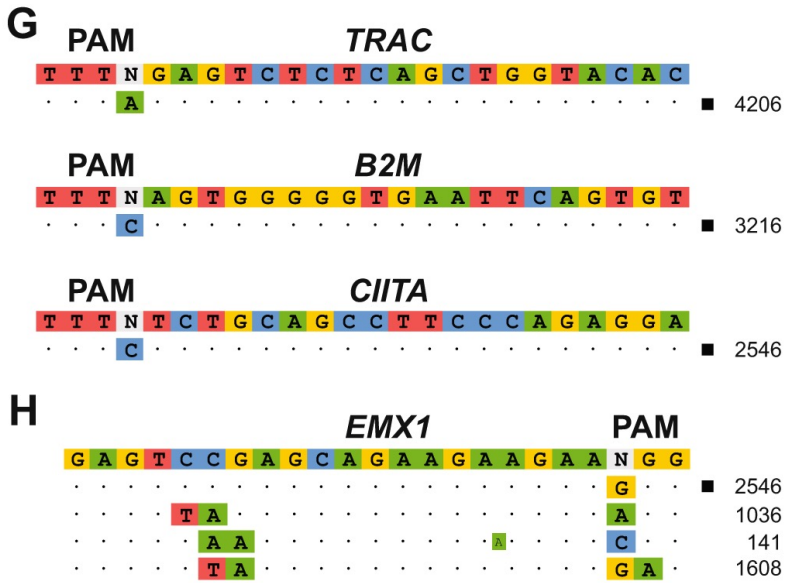
Specificity is Retained with AsCas12a Ultra

On-target Editing in GUIDE-Seq Experiment



No Off-targets Detected by GUIDE-Seq



We continually see this high specificity across current or potential future clinical targets

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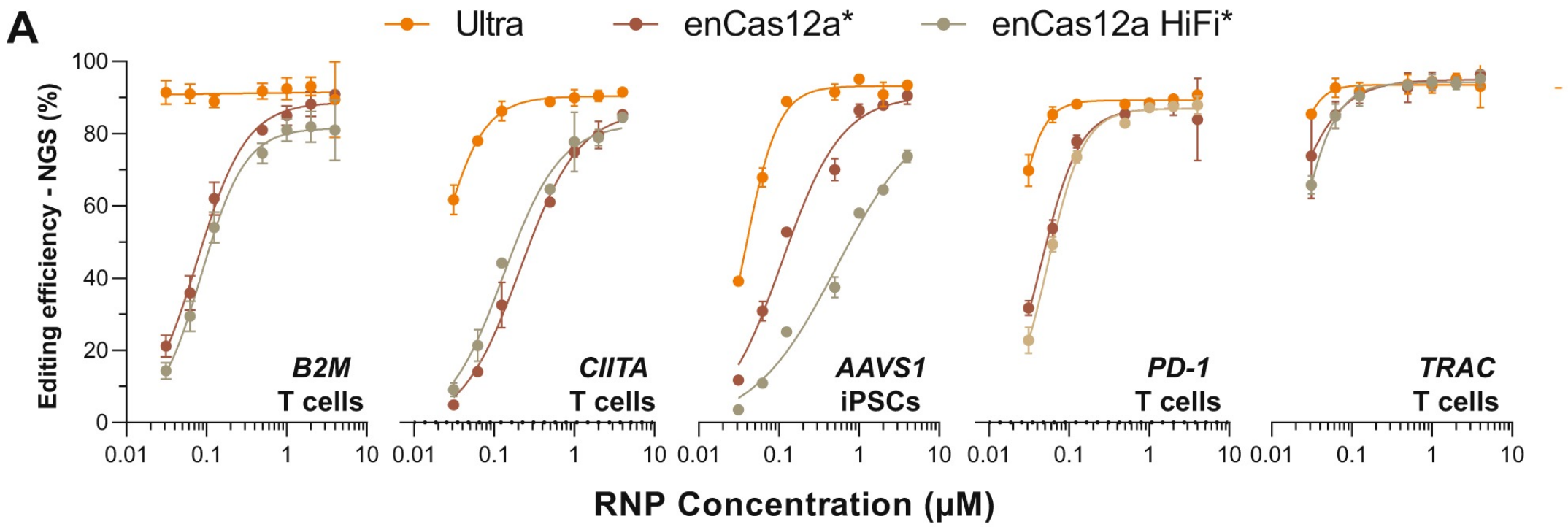
AsCas12a Ultra is More Potent than enCas12a and HiFi Variant

enCas12a and enCas12a HiFi shown to have higher activity and expanded PAM targeting range to WT AsCas12a as reported in *Kleinstiver et al. Nat Biotech 2019*



Key Takeaway: for maximal editing efficiency AsCas12a Ultra should be nuclease of choice

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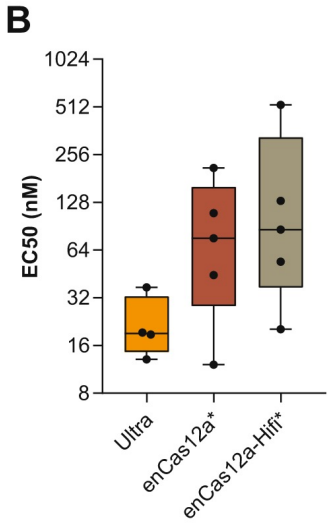
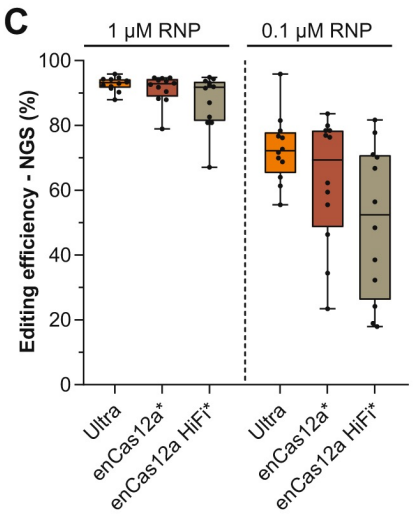
AsCas12a Ultra has Superior Potency Across Key Primary Cell Types

Potency across a wide range of targets

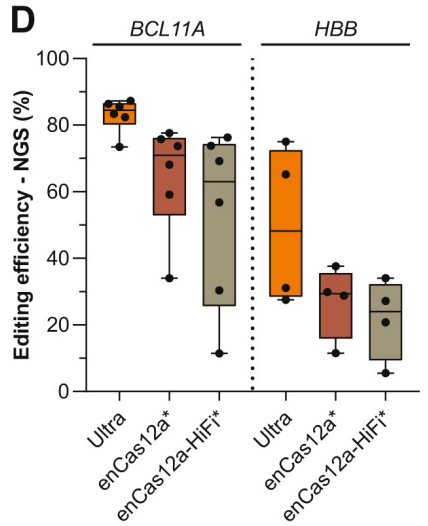


AsCas12a Ultra is 4-fold more active than enCas12a

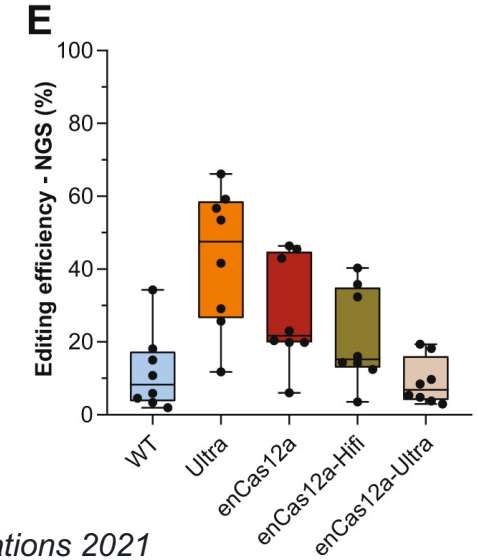
Case example in T cells

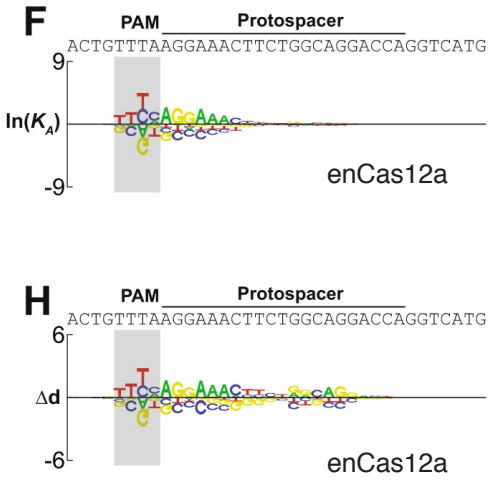


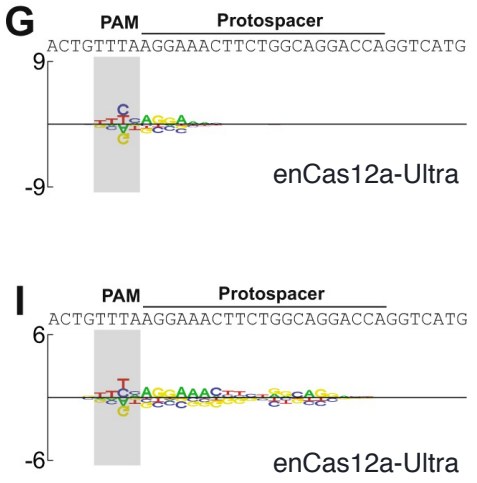
Case examples in HSPCs



Tested at suboptimal concentration of RNP to better observe potency differences







Sequence specificities of enCas12a and enCas12a-Ultra are systematically lower than AsCas12a Ultra

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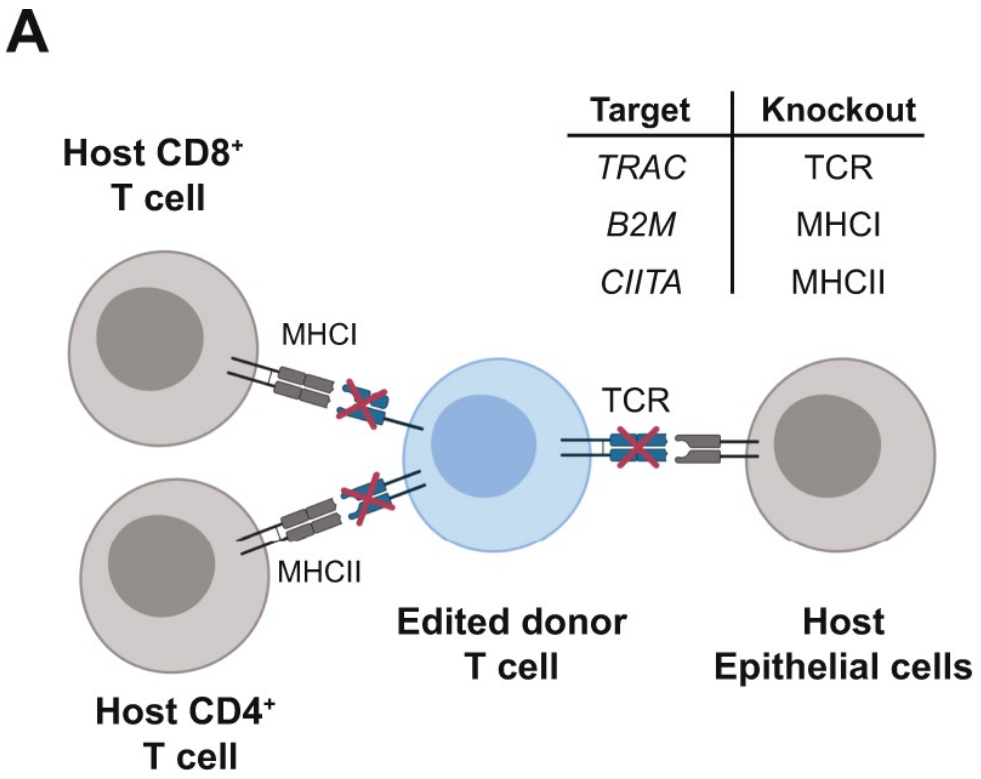
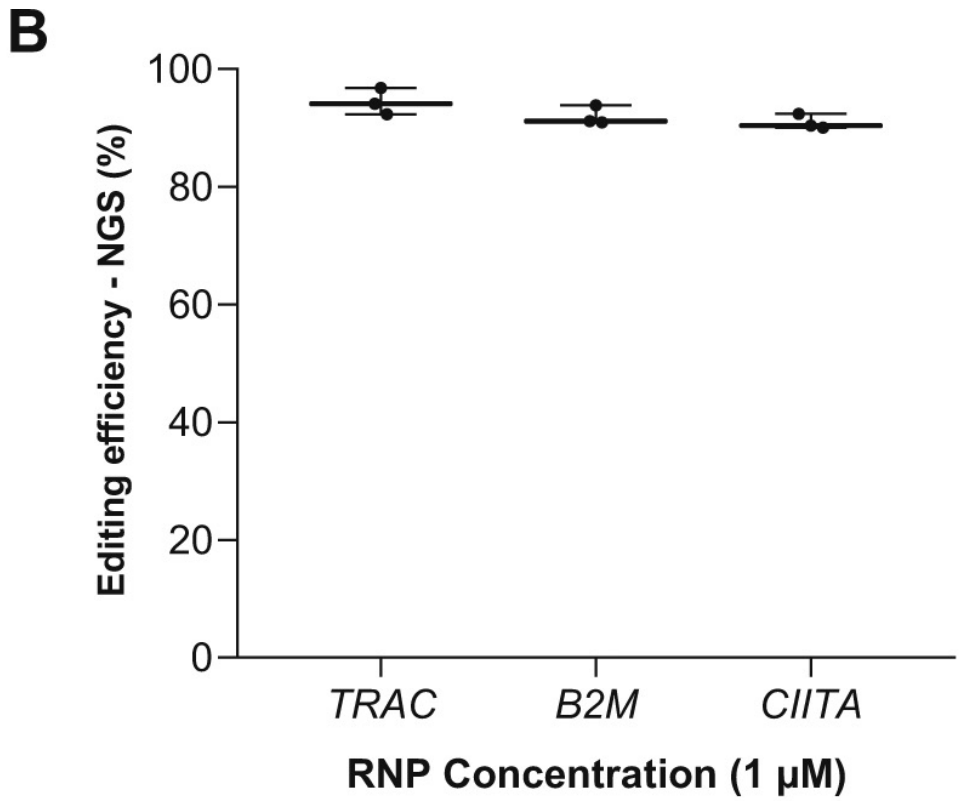
AsCas12a Ultra Allows for Highly Efficient Multiplexed Editing in T cells





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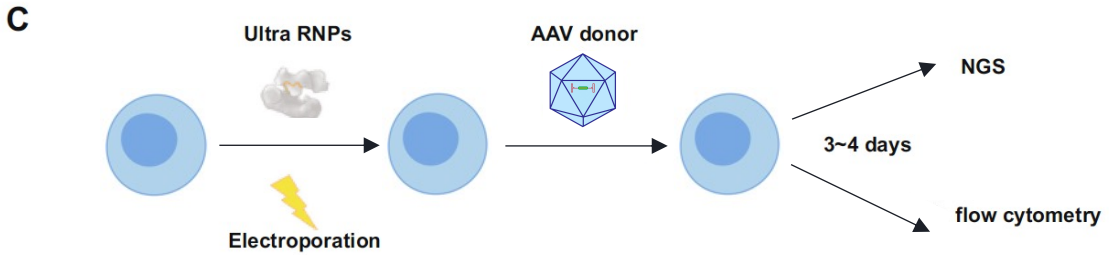
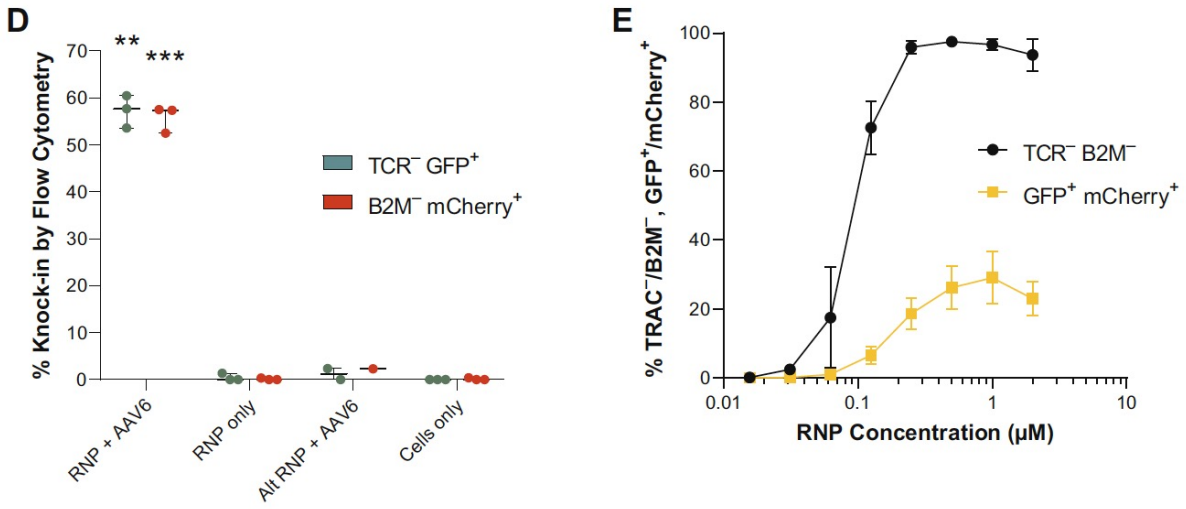
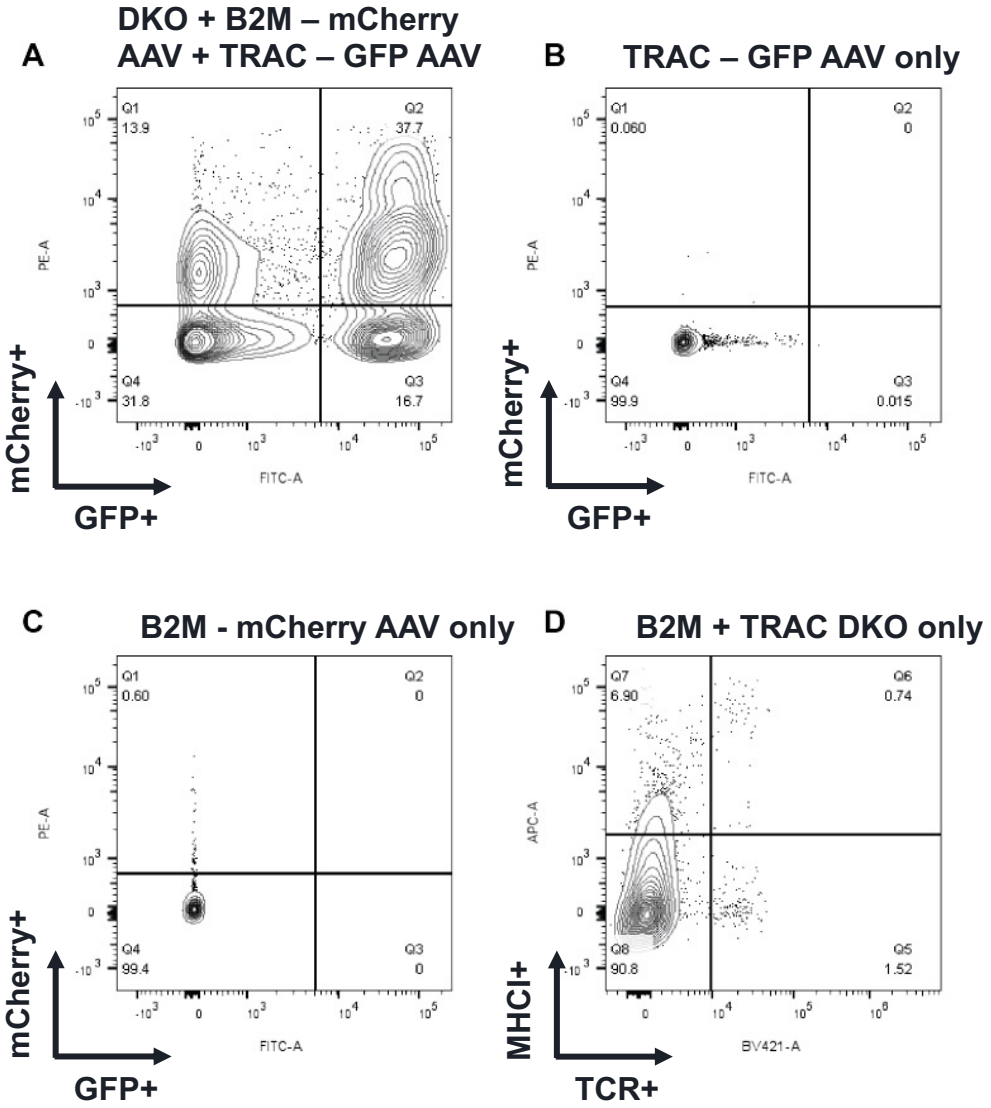
AsCas12a Ultra Allows for Highly Efficient Single and Multigene Knock-in







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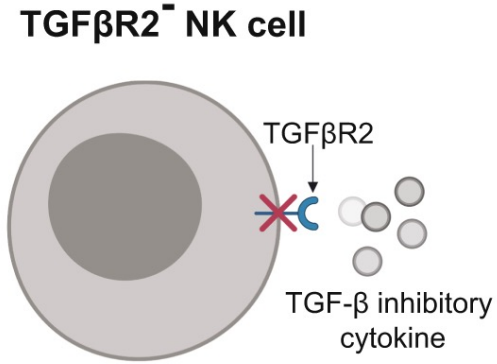
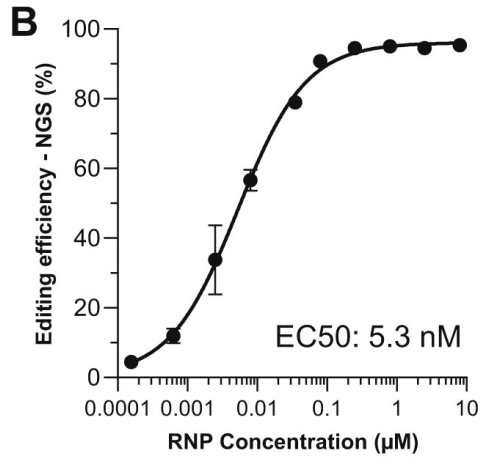
AsCas12a Ultra Generates Highly Efficient KO of a Tumor Suppressive Cytokine Receptor Which Leads to Enhanced NK Cell Killing Function

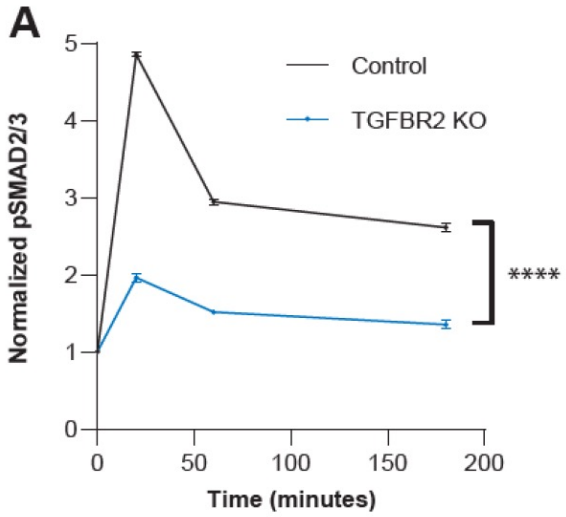
TGFBR2 KO to inhibit suppression of NK cells by TGF-β



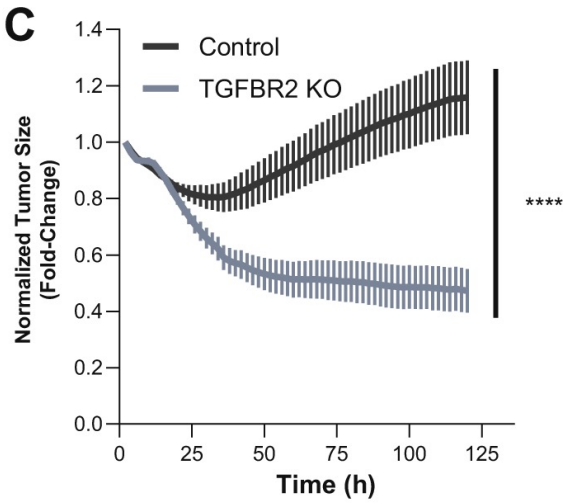
TGFBR2 editing is highly efficiency with AsCas12a Ultra



Functional assay shows decreased SMAD phosphorylation due to TGF-β/TGFBR2 pathway signaling inhibition



Increased tumor killing by TGFBR2 KO NK cells in spheroid assay in presence of TGF-β immunosuppressive cytokine



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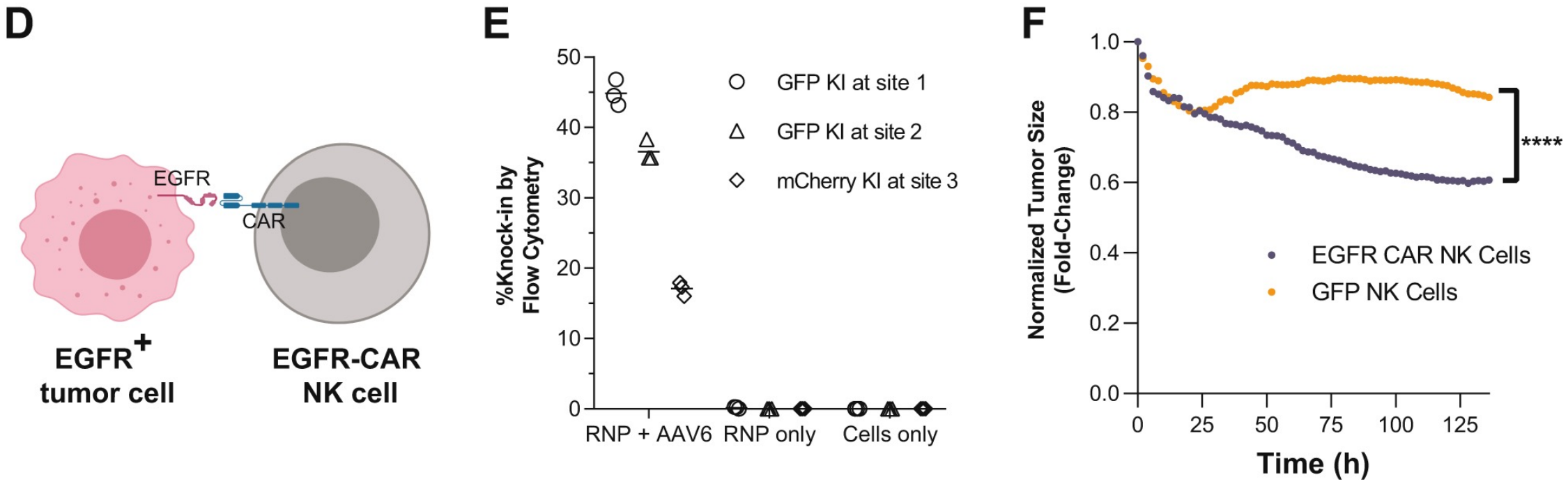
AsCas12a Ultra Enables Efficient Knock-in of Many Transgenes Including an EGFR CAR Which Leads to Increased NK Cell Killing of Tumors Cells



Key takeaway: AsCas12a can be leveraged for AAV6-mediated knock-in of a CAR in NK cells

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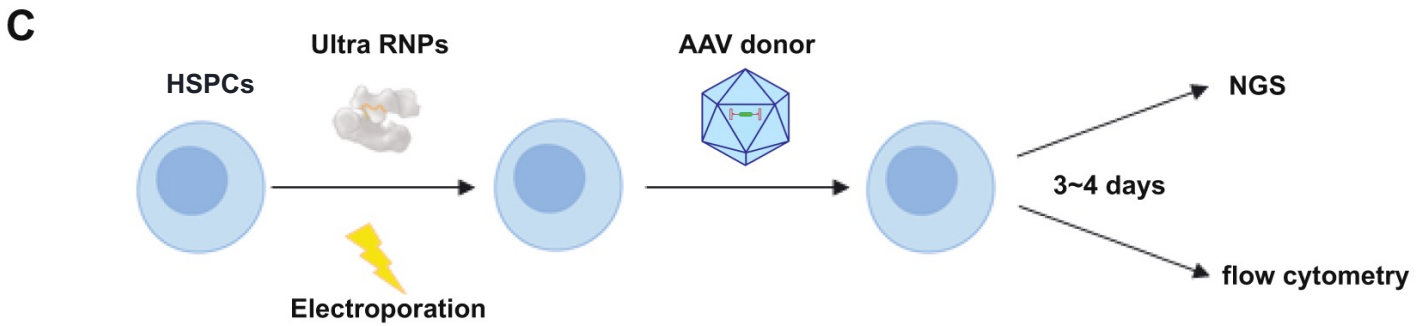
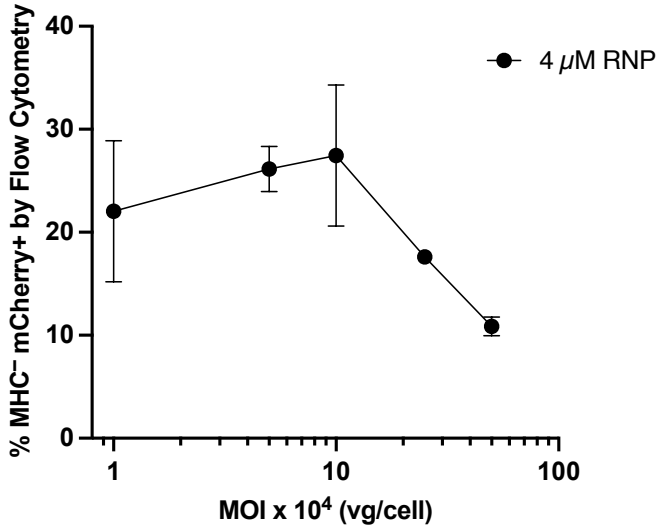
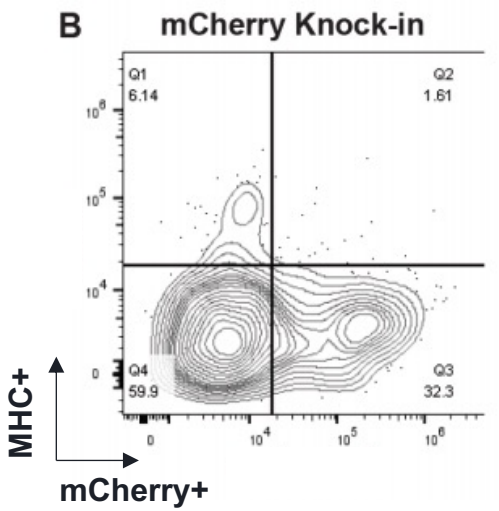
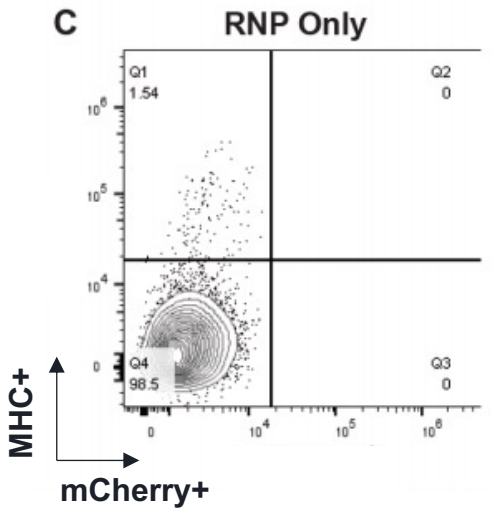
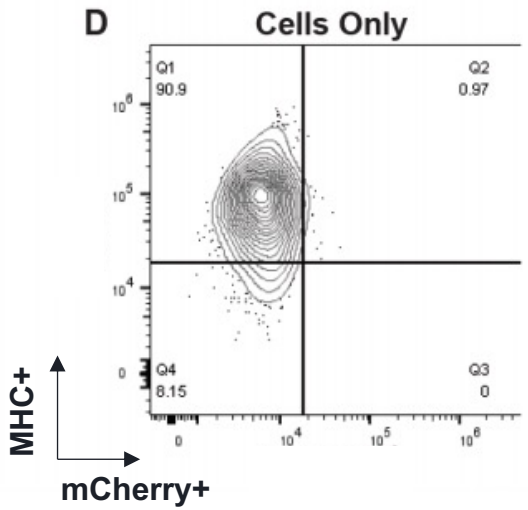
AsCas12a Ultra Enables Efficient Knock-in Using AAV6 in HSPCs







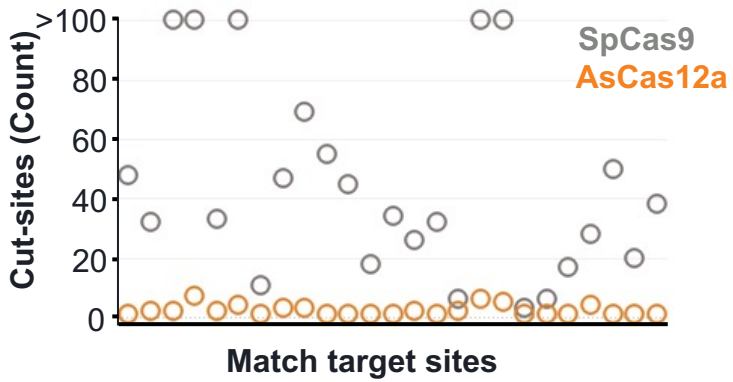




Zhang et al. Nature Communications 2021

AsCas12a Ultra Mutations Incorporated into an Engineered AsCas12a at Editas which is Being Used in Our Sickle Cell Program: EDIT-301

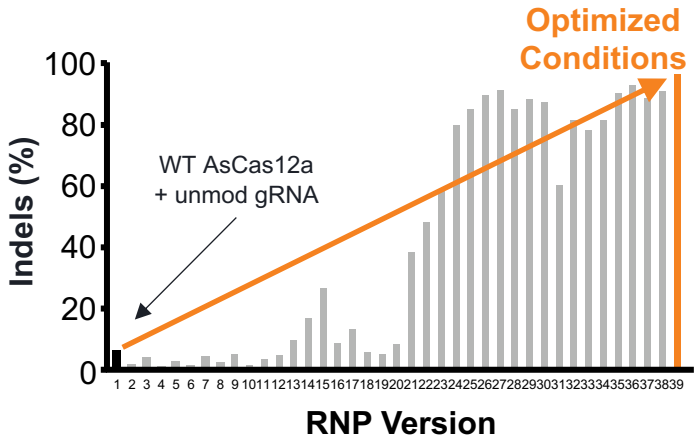
Editing Specificity



AsCas12a is Highly Specific primarily due to its DNA target engagement mechanism that is distinct from SpCas9

Gotta et al. Cold Spring Harbor 2019

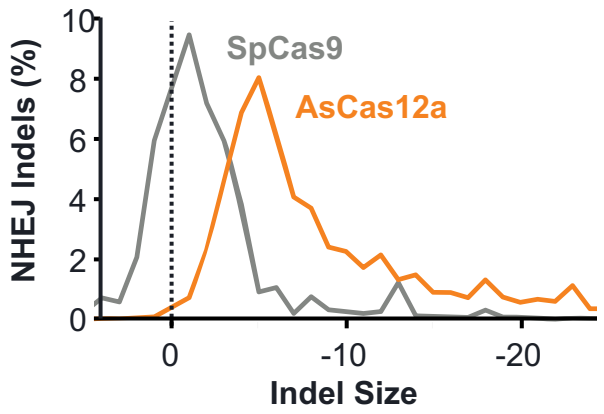
Editing Efficiency



Editas engineered AsCas12a RNP demonstrates **high editing efficiency** in CD34+ cells

De Dreuzy et al, ASH 2019

Indel Profile



AsCas12a staggered cut generate larger deletions than SpCas9, **leading to higher HbF induction** at the HBG locus

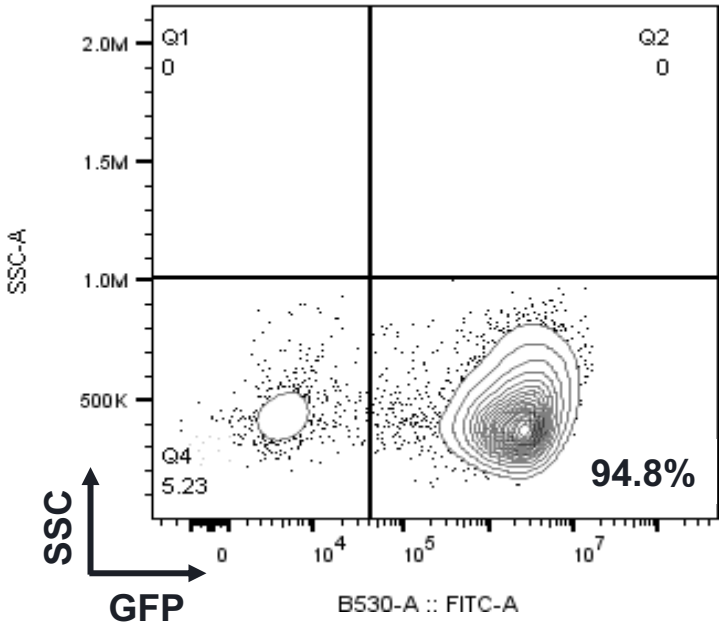
De Dreuzy et al, ASH 2019
De Dreuzy et al, ASGCT 2018

Engineered AsCas12a high potency and specificity led to incorporation into EDIT-301, now in the clinic

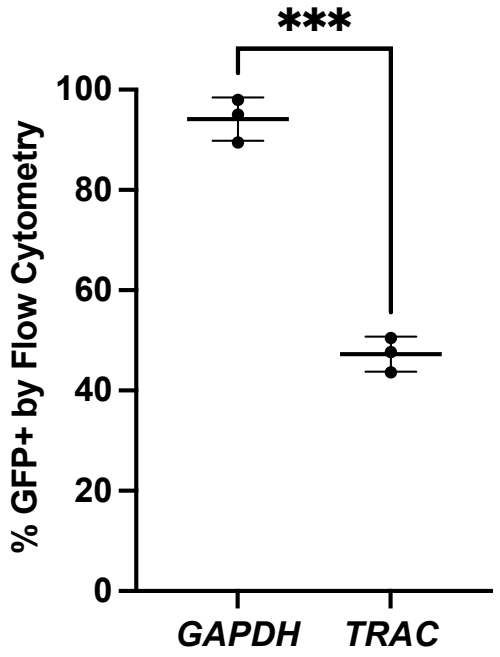
New HDR Technology Leverages Engineered AsCas12a

SLEEK = SeLection by Essential-gene Exon Knock-in

SLEEK Efficiency in T cells

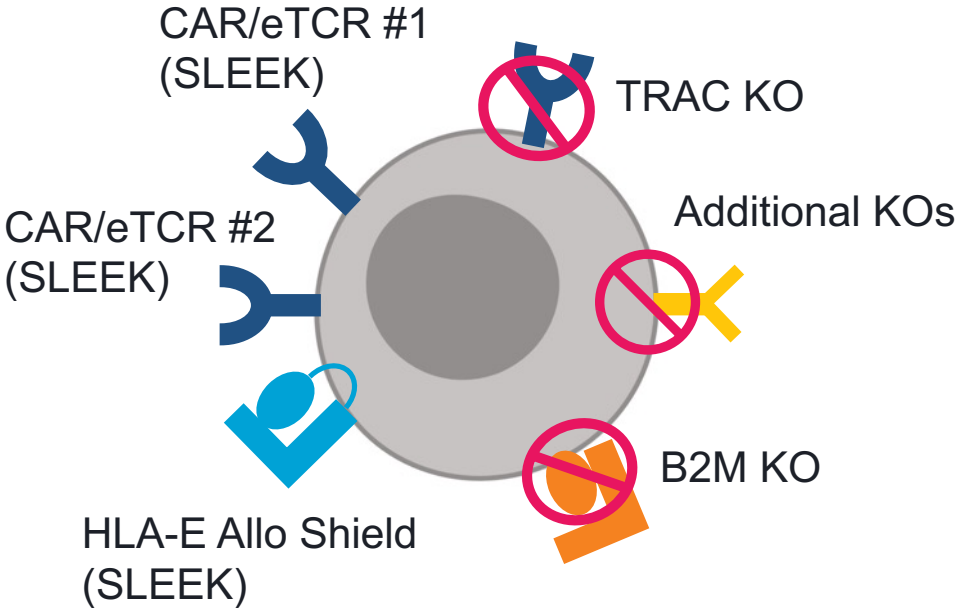


Comparison to *TRAC* Knock-in



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

Allogeneic Healthy Donor Applications



Presented at Cold Spring Harbor Laboratory's Genome Engineering: CRISPR Frontiers on August 20th, 2021

https://www.editasmedicine.com/gene-editing-pipeline/

Summary

- In collaboration with IDT, we generated and tested a highly active variant of AsCas12a across many different clinically relevant targets and cell types

- This highly active AsCas12a variant:
  - Achieves high editing rates with all cell types tested and is uniformly active across on-target sites
  - Retains the high specificity of the WT AsCas12a nuclease
  - Shows superior potency to enCas12a across all tested sites and cell types
  - Demonstrates high rates editing in T cells, NK cells, HSCs, and iPSCs
  - Demonstrated highly efficient multiplexed editing, as well as single and dual site-specific knock-ins
  - High knock-in efficiency achieved with this nuclease and our proprietary SLEEK technology

For More Exciting Information on Editas's CRISPR Platform Please See Our Other Presentations Taking Place at TIDES USA 2021

- Oral presentations:
  - **IND-enabling Small-Scale Guide RNA Production Under GMP for CRISPR Based Cell Therapies** – Keith Jarvis
  - **Characterization of gRNAs and Ribonucleoproteins for CRISPR Applications** – Steve Wolk
- Posters:
  - **Understanding CRISPR RNA Secondary Structure Impact on The Ribonucleotide Protein (RNP) Behavior by SEC-PAGE** – Pranjali Ghude
  - **Characterization of CRISPR RNPs by Ion Exchange Chromatography** – Jean-Noel Lemercier

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:

Come Work on Our Engineered AsCas12a and Our Many Other Amazing Technologies and Bring CRISPR Medicines to Patients

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.

https://www.editasmedicine.com/careers/

https://www.linkedin.com/in/john-zuris-5a5b2537/