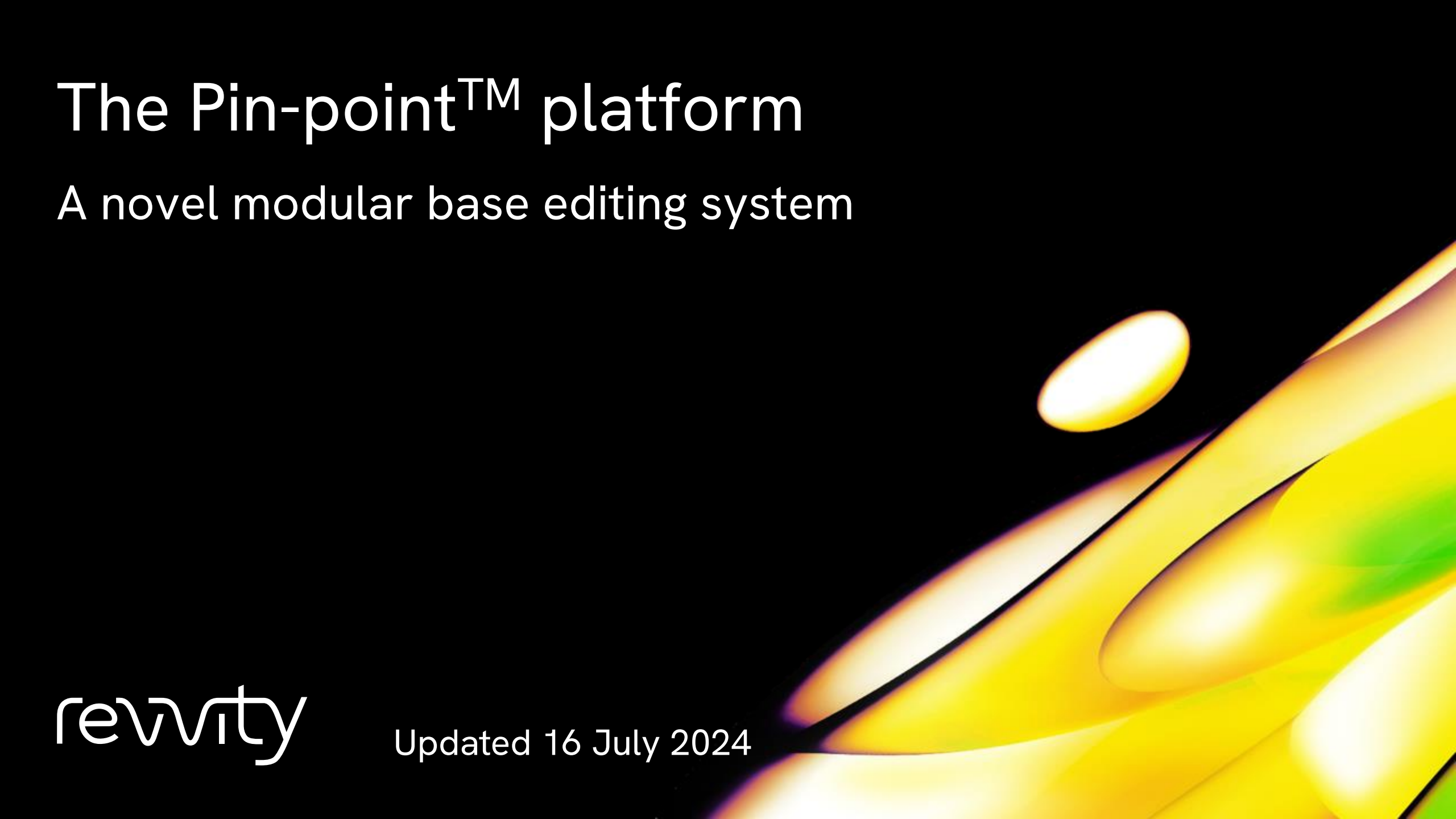
The Pin-point™ platform

A novel modular base editing system



revvity

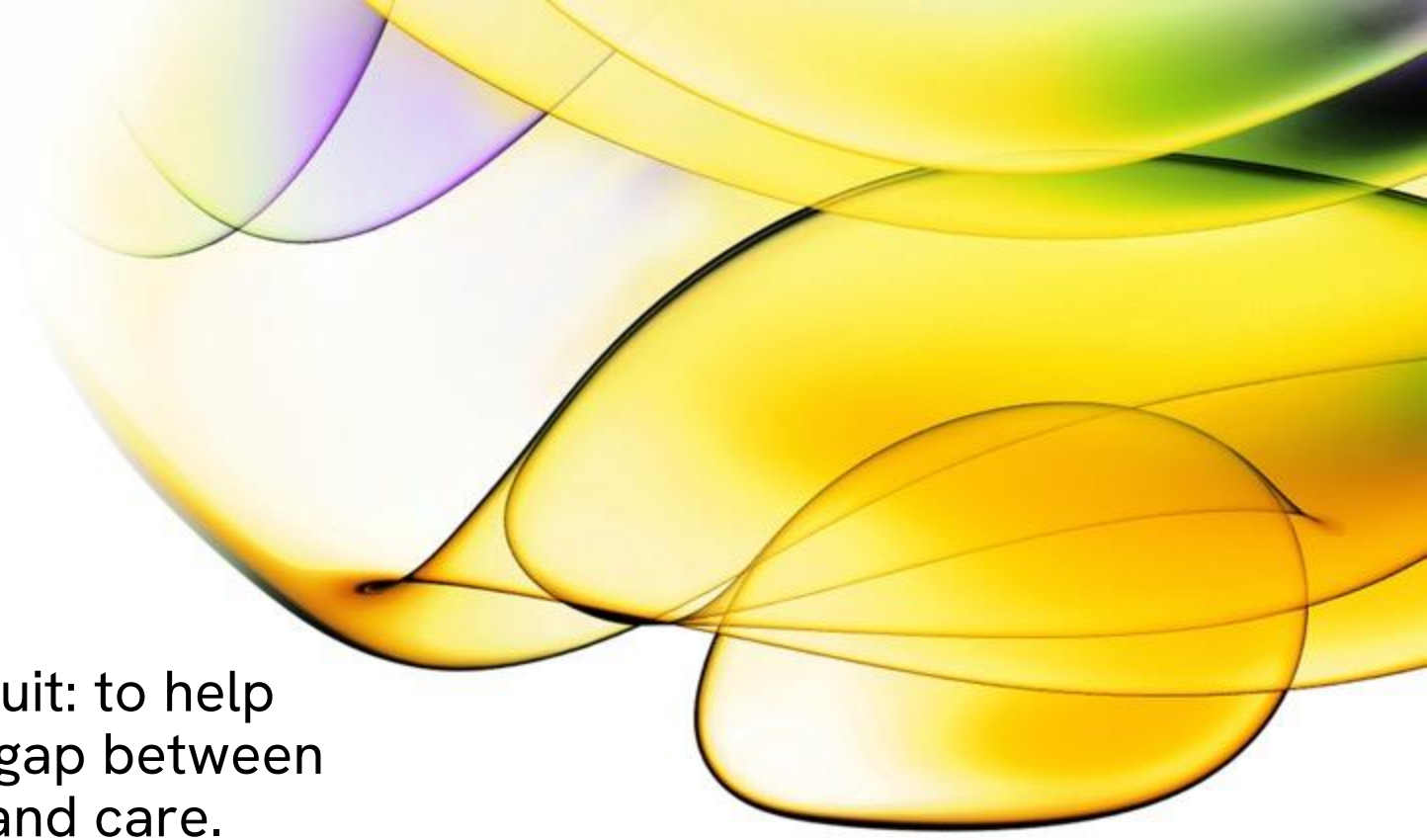
Updated 16 July 2024

Outline

1. Introduction
2. Data Overview
3. Summary

Supplemental Sections

1. Knockout explained
2. T cell datapack
3. iPSC datapack
4. HSPC datapack
5. Complex Engineering
6. Flexible Modularity
7. Screening – Arrayed & Pooled

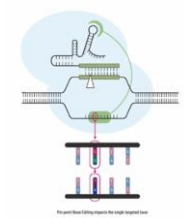


Revvity is born of a single-minded pursuit: to help improve human health by bridging the gap between science and people through precision and care.

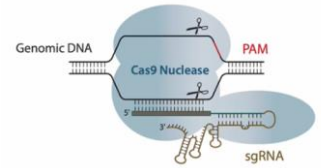
We innovate and collaborate to empower our partners to see science in unexpected ways that deliver breakthrough results.

Revvity's Cell & Gene Therapy Research Portfolio

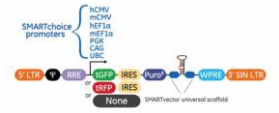
GENE EDITING & MODULATION



Pin-point™ base editing platform

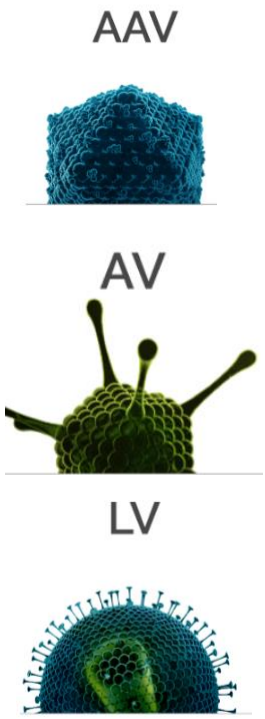


CRISPR-Cas9



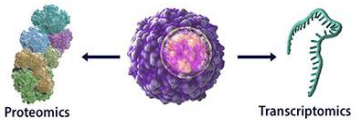
Dharmacon™ RNAi shRNA and siRNA

VIRAL VECTORS



LentiBOOST®

CELL ANALYSIS



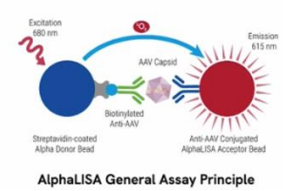
Total-Seq

Cell selection & single cell proteogenomics

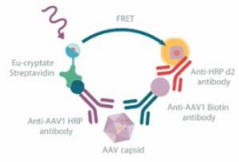
CELL COUNTING

Cell viability, potency & yield

DISCOVERY & QC



AlphaLISA General Assay Principle



HTRF General Assay Principle

LabChip AAV empty/full

Cell and AAV characterization and QC

return to outline

Targeted knock-in

Programmable

Dividing, non-dividing,
& primary cells

Efficient

Flexible

Predictable

Precise

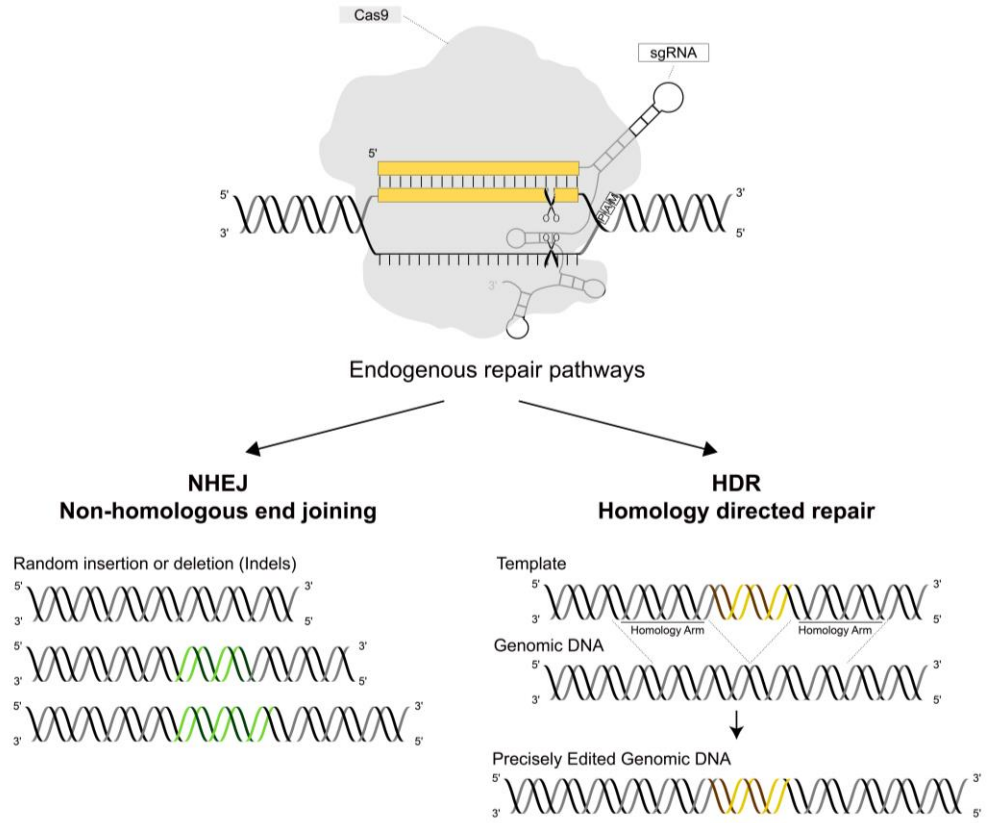
Modular

Multiplexing

Safe

Avoid DSBs

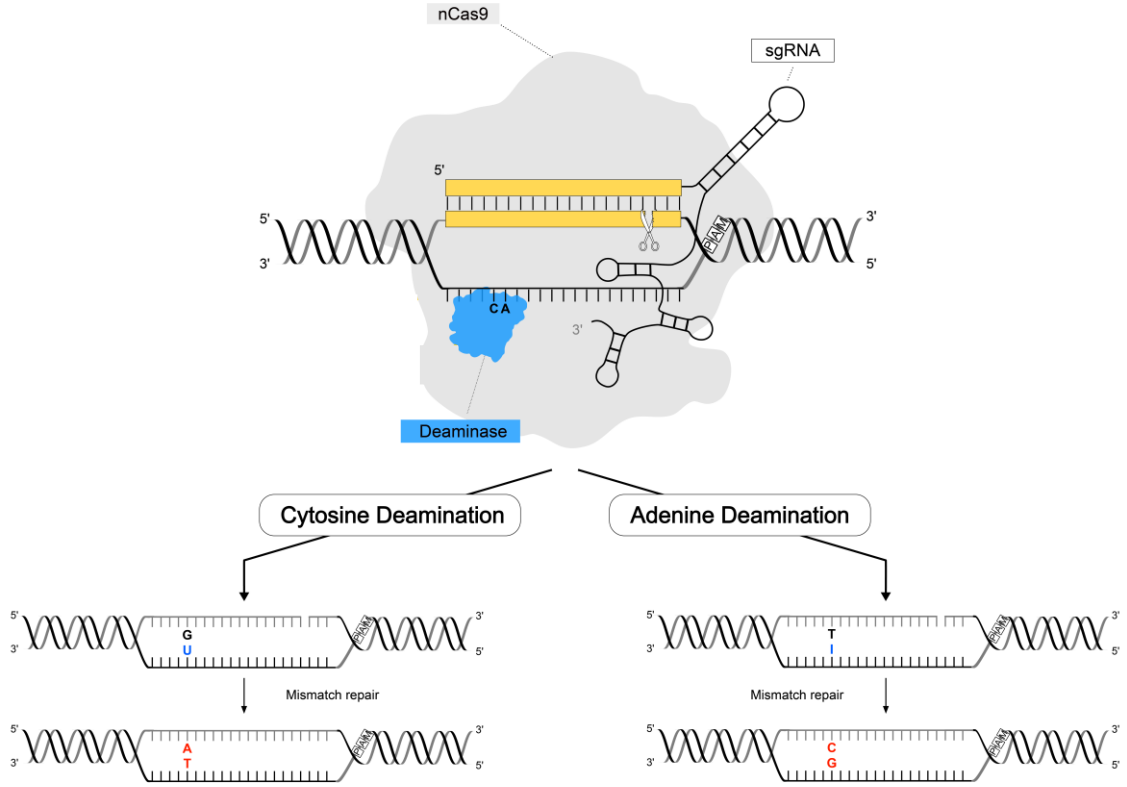
CRISPR gene editing



GENE DISRUPTION BY A DSDNA BREAK

- Indel formation to disrupt gene sequence
- complex population of indels

Base editing



GENE MODIFICATION BY POINT MUTATIONS

- Creation of stop codons or splice site disruption for knockout
- Introduction of single base conversion

Competitive advantages of base editing

1st generation Cas enzymes
Gene disruption by a dsDNA break

2nd generation base editing
Gene modification by point mutation

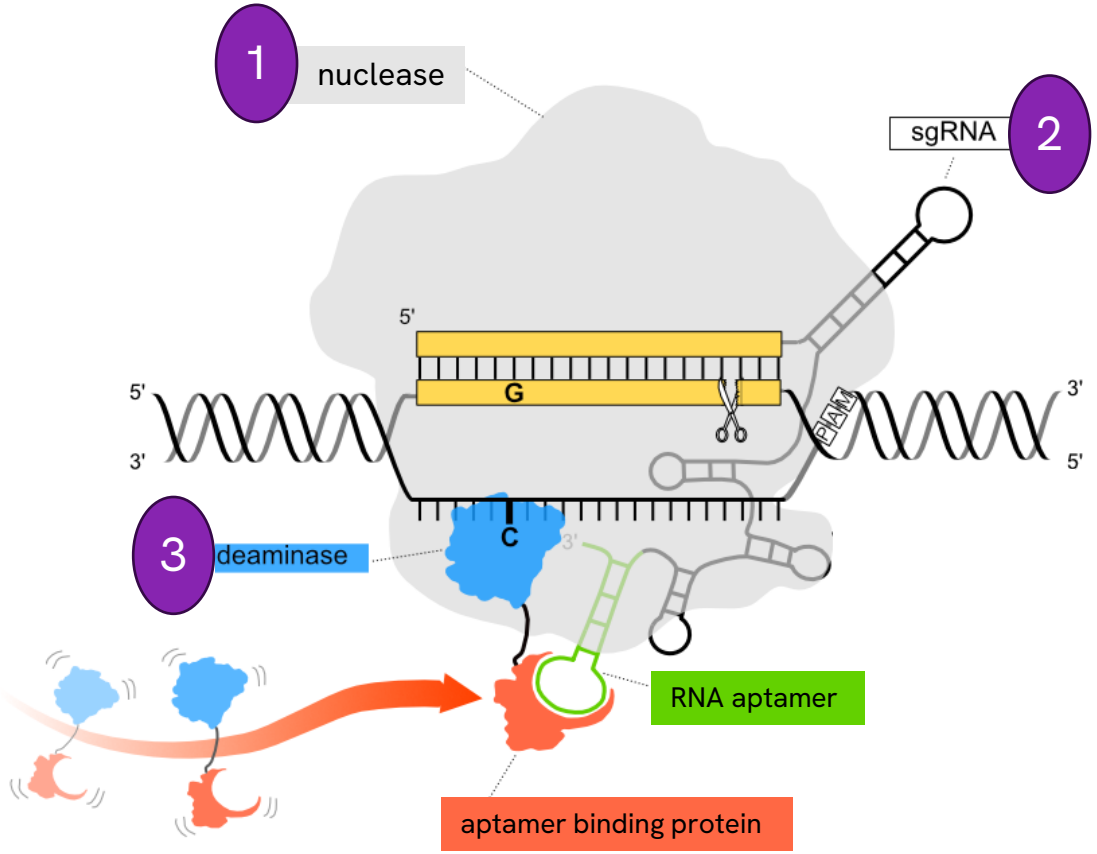
- ✓ *Avoidance of double strand DNA breaks for **reduced cytotoxicity** and **high viability***
- ✓ ***Permanent change** to the DNA*
- ✓ *Flexible creation of stop codons or splice site disruption for **knockout** or introduction of **single base conversion***
- ✓ ***Predictable**, **precise**, and **efficient** single or multi-gene editing*
- ✓ *Components are easy to **design**, **synthesize**, and **deliver***

New generation Pin-point™ base editing platform

- ☑ **Simultaneous knock-in and knockout** in a single reaction
- ☑ Nuclease and deaminase **flexible**
- ☑ **Modular control** over target and editing window to specifically reach your gene of interest
- ☑ A single **novel, patented aptamer-recruited base editing platform** for your therapeutic development

What is the Pin-point™ platform?

Based on a patented aptamer-recruited base editing arrangement



3 component system

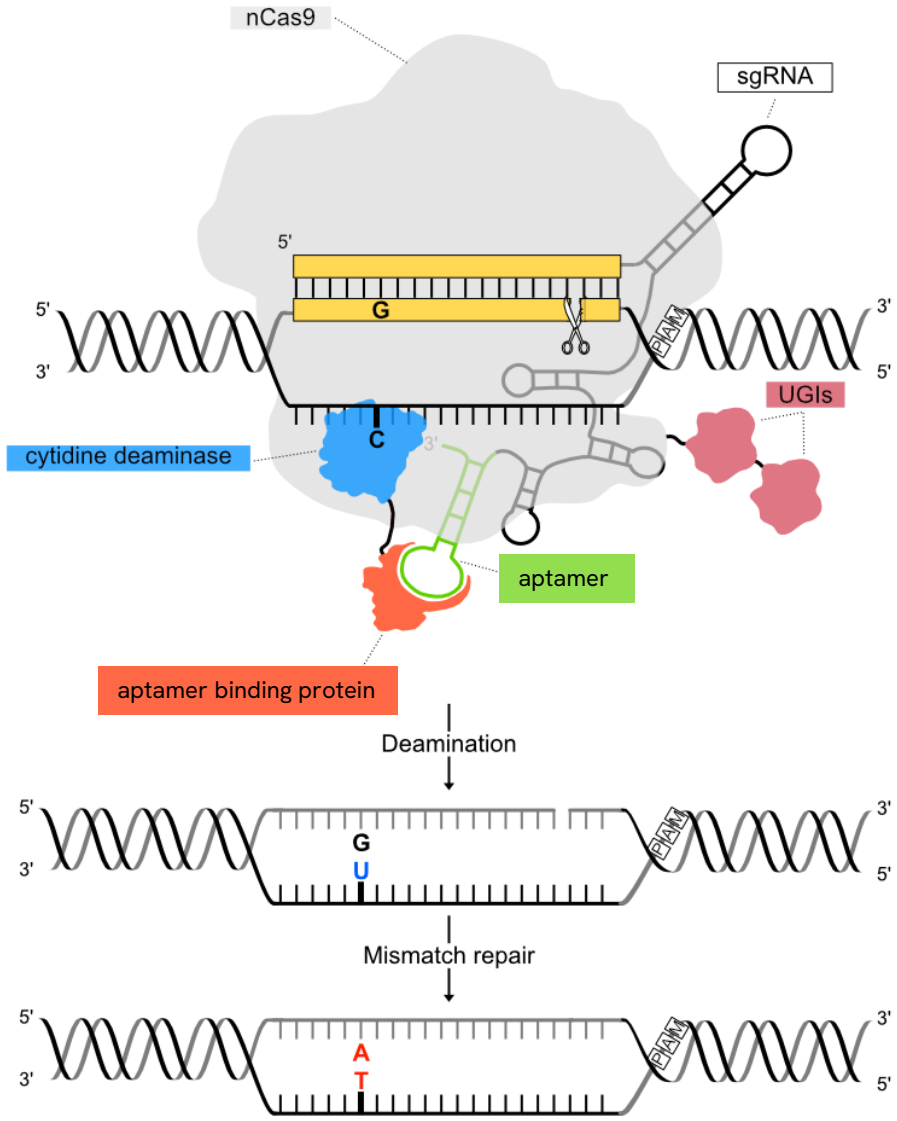
1. RNA-guided enzyme
2. Guide RNA with aptamer
3. Deaminase and recruitment protein

Demonstrated advantages

- ☑ Multiplex gene editing including knock-in and knockout with high efficiency and safety
- ☑ Validated performance in T cells, iPSCs, and HSPCs
- ☑ Mix-and-match for target specificity and efficiency

*Schematic depicts nCas9 configuration

Base editing terminology



"Base editing window"

5′	20bp spacer																			3′
N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	G	G
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	PAM	

nCas9/rat APOBEC is most likely to edit C's in positions 4-7

"Bystander editing" is any editing other than the target base of interest

"Off-target editing" is any editing other than at the locus that is targeted

The Pin-point™ base editing platform is accelerating therapeutic development research

Molecular Therapy
Original Article

An aptamer-mediated base editing platform for simultaneous knockin and multiple gene knockout for allogeneic CAR-T cells generation

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Gene editing technologies hold promise for enabling the next generation of adoptive cellular therapies. In conventional gene editing platforms that rely on nuclease activity, such as clustered regularly interspaced short palindromic repeats CRISPR-associated protein 9 (CRISPR-Cas9), allow efficient introduction of genetic modifications; however, these modifications occur via the generation of DNA double-strand breaks (DSBs) and can lead to unwanted genomic alterations and genotoxicity. Here, we apply a novel modular RNA aptamer-mediated Pin-point base editing platform to simultaneously introduce multiple gene knockouts and site-specific integration of a transgene in human primary T cells. We demonstrate high editing efficiency and purity at all target sites and significantly reduced frequency of chromosomal translocations compared with the conventional CRISPR-Cas9 system. Site-specific knockin of a chimeric antigen receptor and multiplex gene knockout are achieved within a single intervention and without the requirement for additional sequence-targeting components. The ability to perform complex genome editing efficiently and precisely highlights the potential of the Pin-point platform for application in a range of advanced cell therapies.

INTRODUCTION

Gene editing technologies have entered the clinic and show significant potential for advancing next-generation therapies, particularly in the development of more efficient chimeric antigen receptor (CAR)-T cell therapies to address hematological malignancies.[1–3] To overcome the logistical and infrastructure-related challenges and product variability barriers of the autologous cell therapy paradigm, recent focus has shifted to realizing the potential of allogeneic cell therapies. The manufacture of allogeneic cell products requires multiple edits to prevent both graft-versus-host disease and immune rejection by the host, which would otherwise limit efficacy, persistence, and safety of the cell product. To expand the scope of these innovative off-the-shelf therapies to solid tumors, further edits will also be required to ensure therapeutic cells retain their efficacy in the refractory and heterogeneous tumor microenvironment.[4] These factors, together with the need to provide new functions to the cells to make effective and safe therapies that offer wider patient accessibility and therapy deployment, ultimately demand increasingly refined genome editing strategies.

Gene editing technologies such as zinc-finger nucleases, transcription activator-like effector nucleases and CRISPR-Cas9 have all been employed to successfully perform targeted editing at genomic loci for effective knockout and knockin applications. However, the generation of double-strand breaks (DSBs) inherent to their mechanism of conferring a DNA edit can lead to chromosomal loss or structural variation.[5–11] The occurrence of chromosomal aberrations is enhanced in the context of multi-gene editing as more concurrent DSBs are generated, and the extent of this damage is expanded if DNA breaks also occur at off-target sites. Although many structural aberrations in a cell may not be viable, it has been reported that

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GEN Biotechnology

REVIEW

Genome Editing of Pluripotent Stem Cells for Adoptive and Regenerative Cell Therapies

Robert Blassberg*

Abstract

The explosion of interest in CRISPR-Cas systems over recent years has delivered rapid advances in precision genome engineering technologies that hold enormous potential for application across the biotechnology sector. Within the cell therapy space, adoptive immunotherapies continue to lead the translation of increasingly sophisticated genetic engineering strategies toward clinical application. Although early focus was placed on engineering primary immune cells collected from patient blood, the democratization of these therapeutics will require the development of off-the-shelf allogeneic cell therapy products that

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DOI: 10.1089/crispr.2020.0035

The CRISPR Journal

RESEARCH ARTICLE

Development and Characterization of a Modular CRISPR and RNA Aptamer Mediated Base Editing System

Juan Carlos Collantes,[1] Victor M. Tan,[1] Huiting Xu,[1] Melany Ruiz-Urigüen,[1] Amer Alasadi,[1] Jingjing Guo,[1] Hanlin Tao,[1] Chi Su,[1] Katarzyna M. Tyc,[2] Tommaso Selmi,[3] John J. Lambourne,[4] Jennifer A. Harbottle,[4] Jesse Stombaugh,[5] Jinchuan Xing,[2] Ceri M. Wiggins,[1] and Shengkan Jin[1,*]

Abstract

Conventional CRISPR approaches for precision genome editing rely on the introduction of DN
breaks (DSB) and activation of homology-directed repair (HDR), which is inherently genotoxic
somatic cells. The development of base editing (BE) systems that edit a target base without req
of DSB or HDR offers an alternative. Here, we describe a novel BE system called Pin-point™ th
base-modifying enzyme through an RNA aptamer within the gRNA molecule. Pin-point is capa
modifying base pairs in the human genome with precision and low on-target indel formation.
potentially be applied for correcting pathogenic mutations, installing premature stop codons
genes, and introducing other types of genetic changes for basic research and therapeutic de

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News Details

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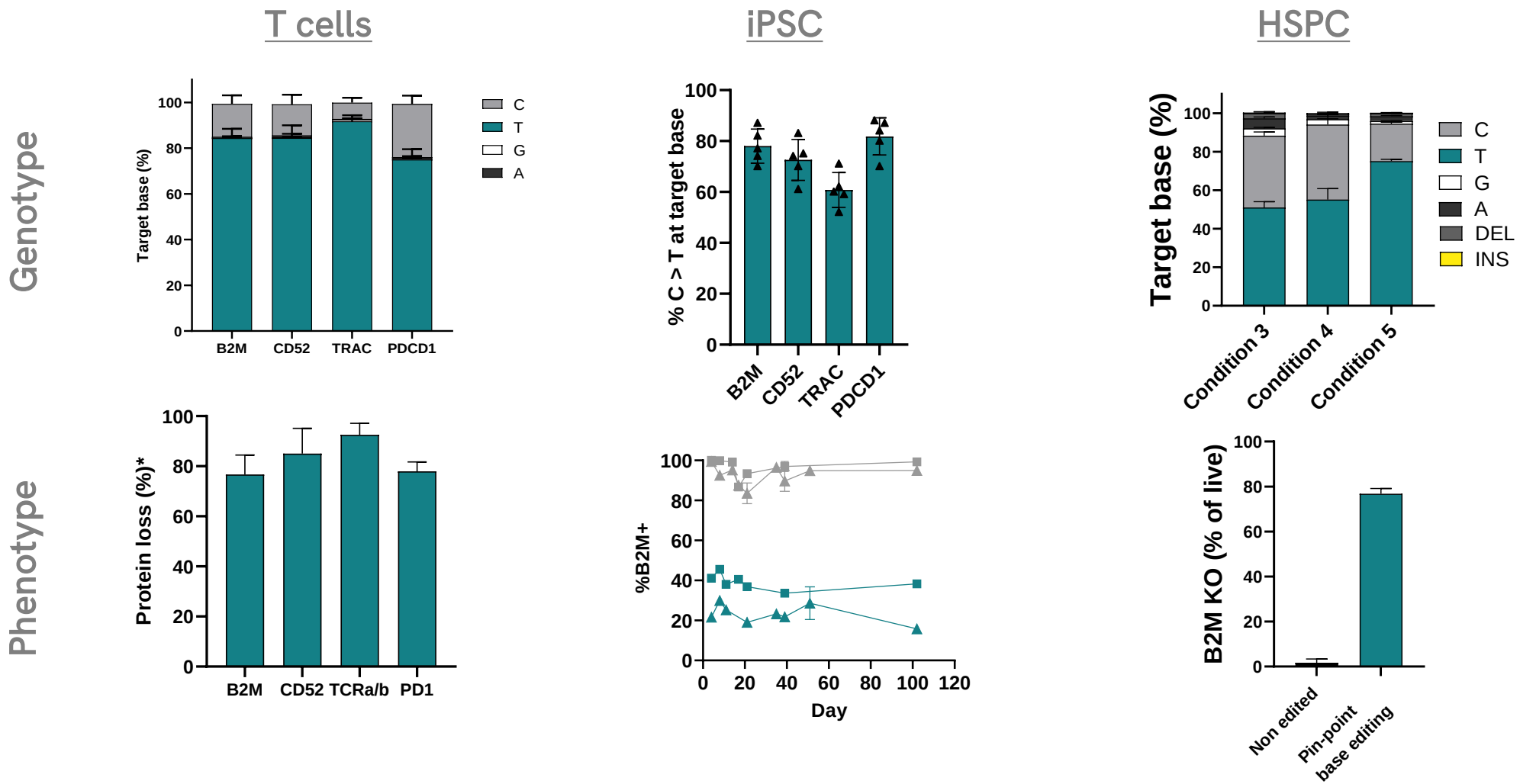
Revvity Announces New License Agreement for Next-Generation Base Editing Technology

05/18/2023

https://www.sciencedirect.com/science/article/pii/S1525001624004234
https://doi.org/10.1089/genbio.2021.0010
https://www.biorxiv.org/content/10.1101/2023.06.20.545315v1
Press release

return to outline

Highly efficient and precise single and multiplex editing in T cells, iPSCs, and HSPCs



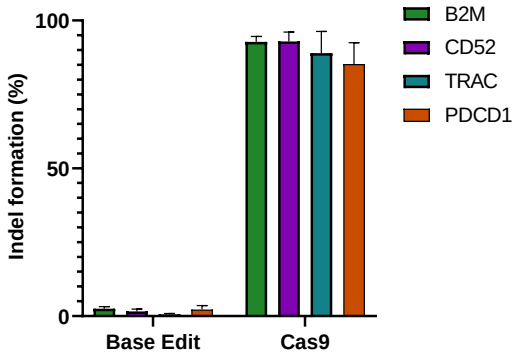
The Pin-point™ base editing platform is highly efficient in multiple cell types

*Normalized to controls

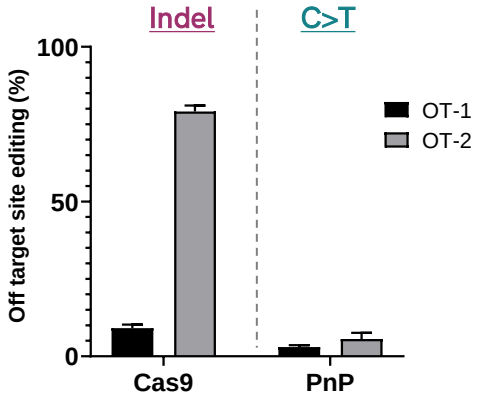
Jump to clinical enrichment of T cells return to outline

Strong safety profile compared to CRISPR/Cas9 editing

Reduced indel formation (T cells)

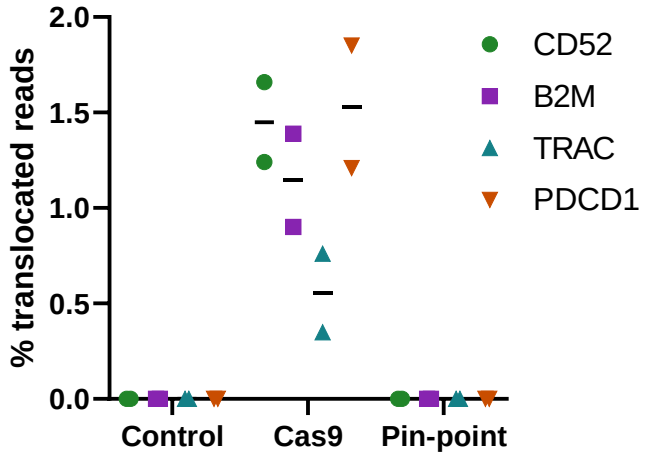


Negligible off-target edits (T cells)

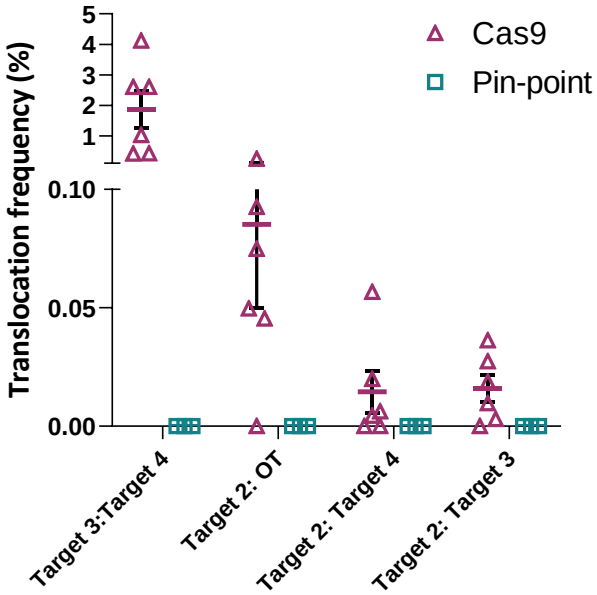


No detected translocations with the Pin-point platform

T cells



iPSC



The Pin-point™ base editing platform is precise and avoids translocations

*Normalized to controls

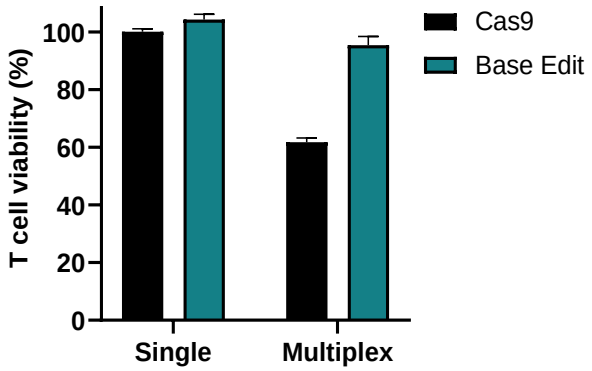
Additional off-target analysis: https://www.sciencedirect.com/science/article/pii/S1525001624004234

return to outline

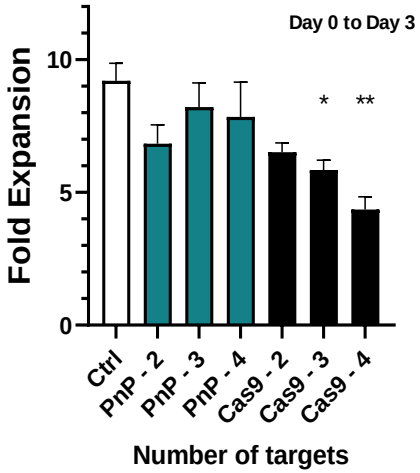
High viability and cell health, even when editing multiple targets

T cells

Cell viability maintained in multiplex

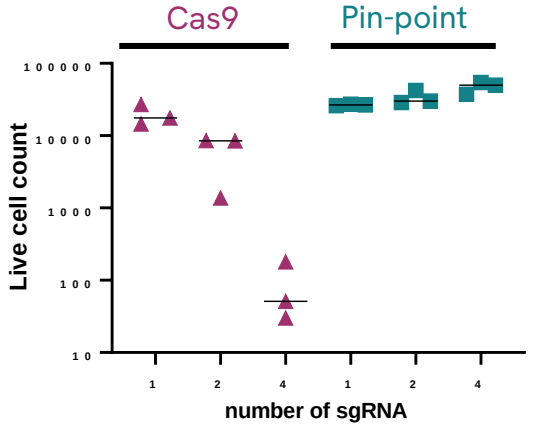


Rate of cell expansion unaffected

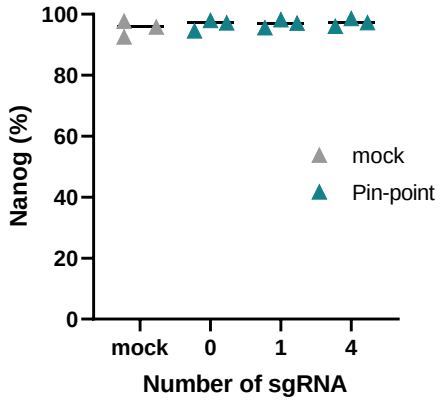


iPSCs

High viability in multiplex



Cells retain pluripotency

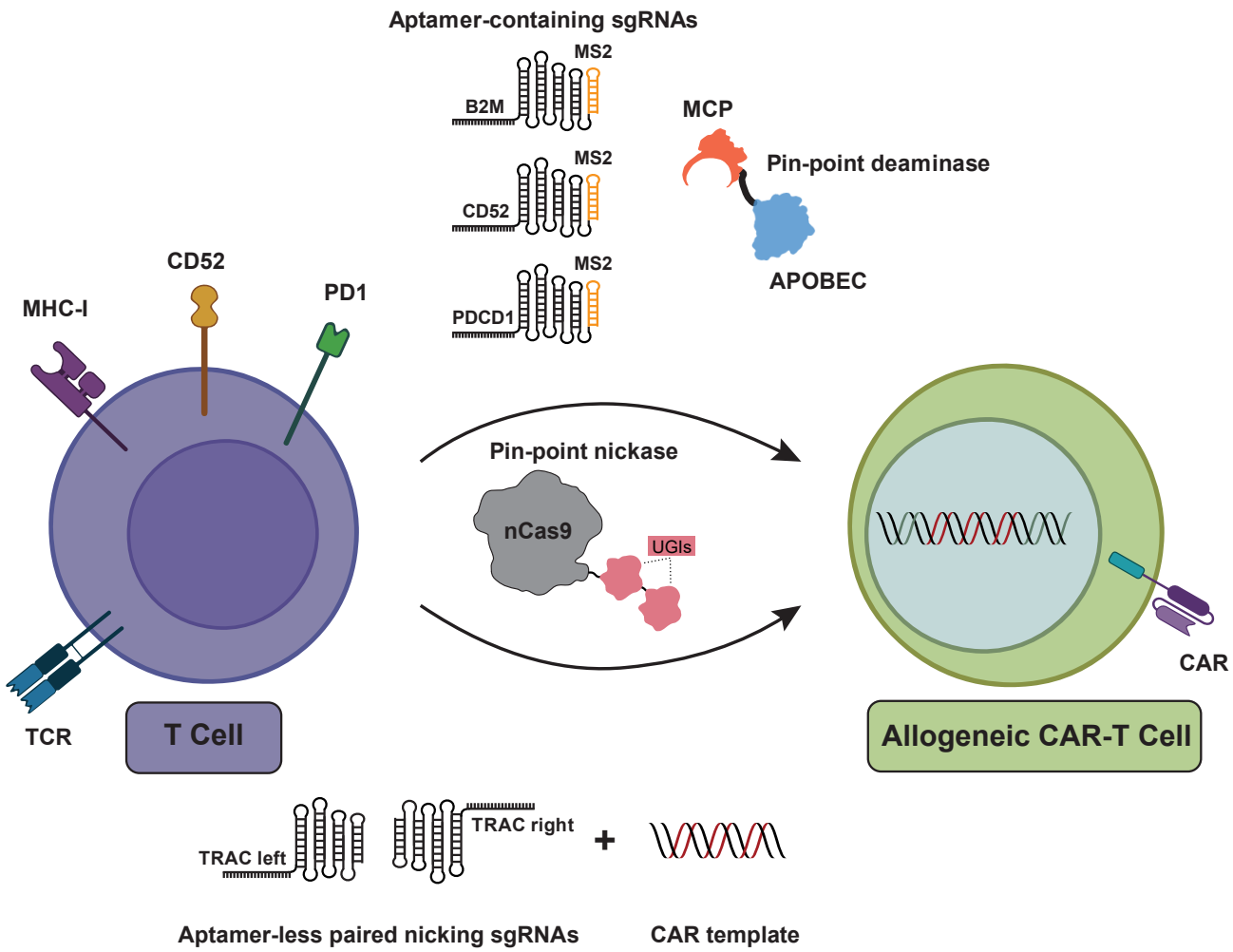


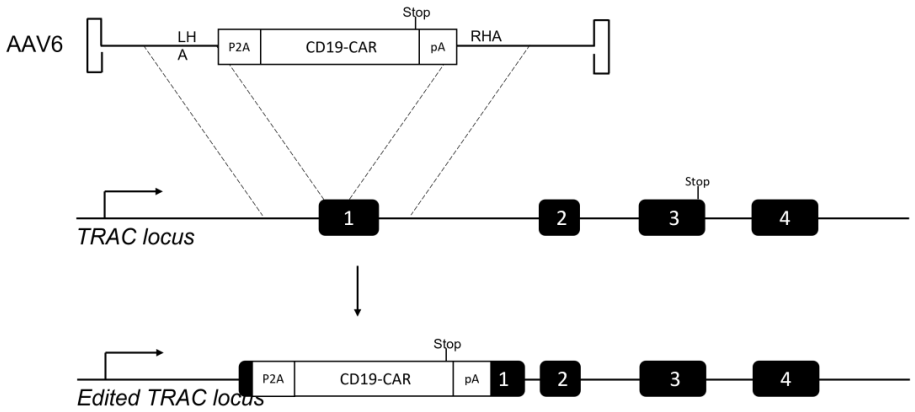
The Pin-point™ base editing platform is gentle on sensitive cell types

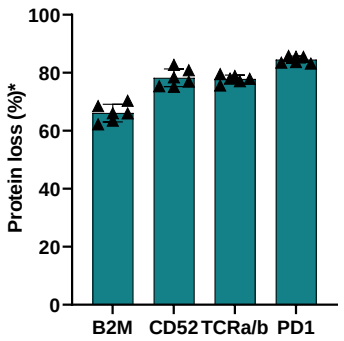
*Normalized to controls

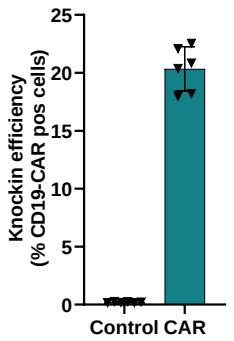
return to outline

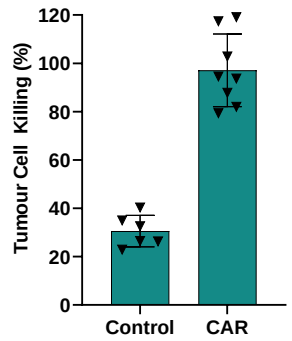
A solution for complex engineering: One-step simultaneous knock-in and knockout in T cells







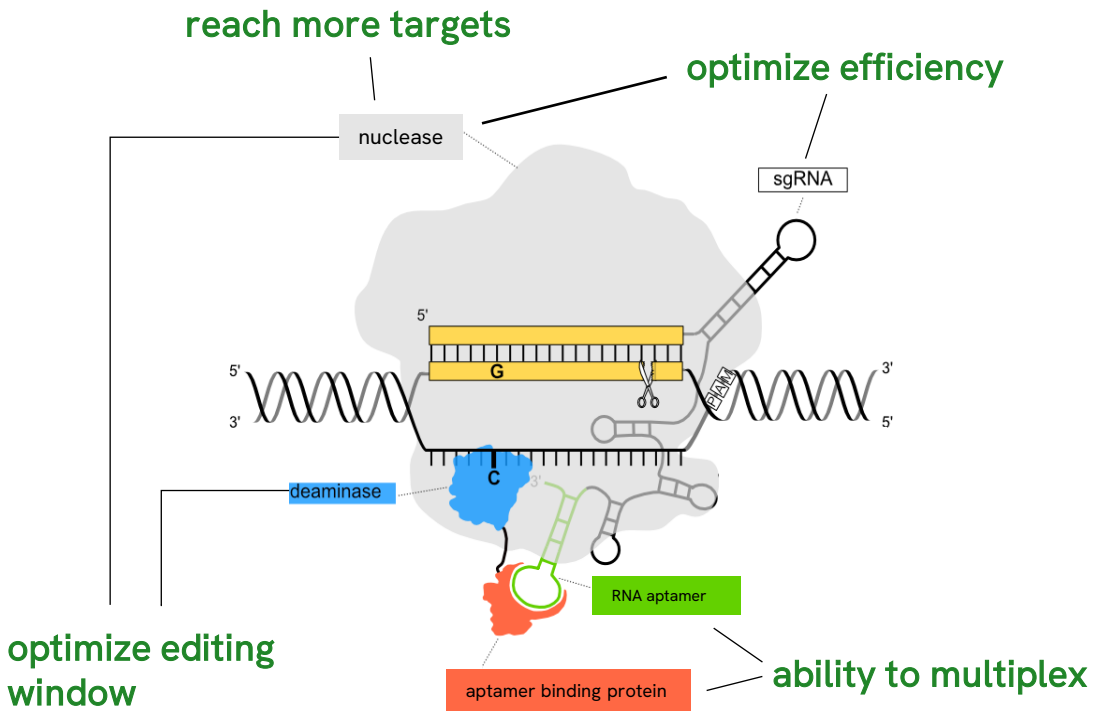




Efficient and accurate for concurrent transgene insertion and multiplex base editing

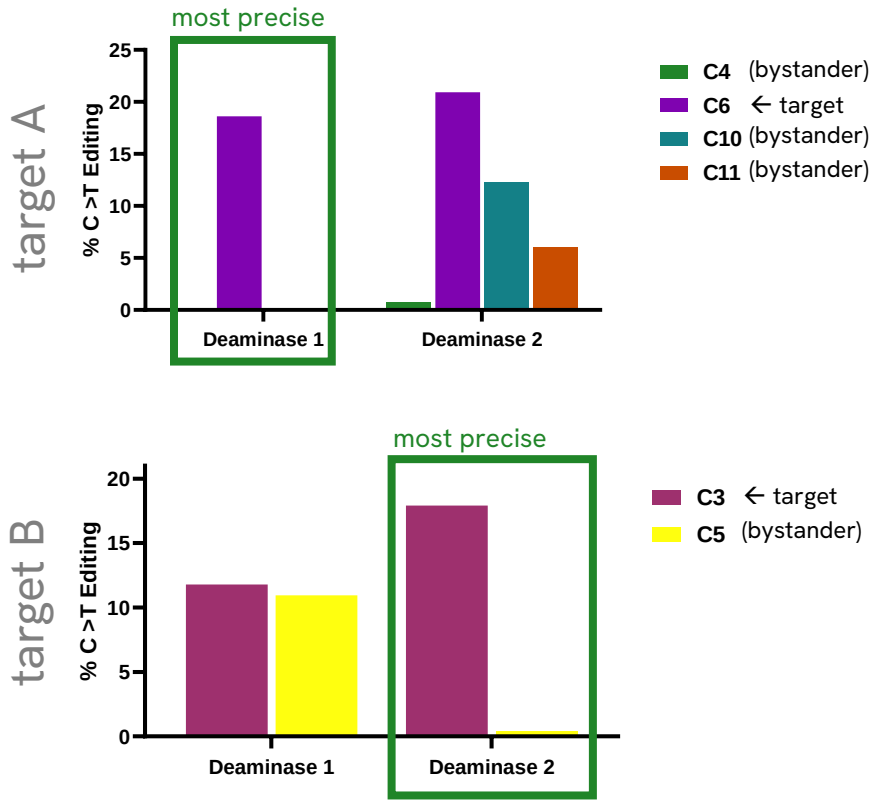
Choose components for locus-specific optimization

Increased potential to correct more pathogenic SNVs that are not reachable with existing published systems*



Schematic depicts nCas9 configuration

Example of optimization of the editing window by selecting the best guide RNA and deaminase pairs

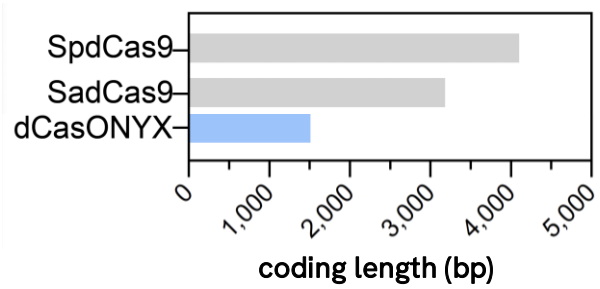


The modular Pin-point™ platform can be customized to combine optimal components for a wide range of base editing applications

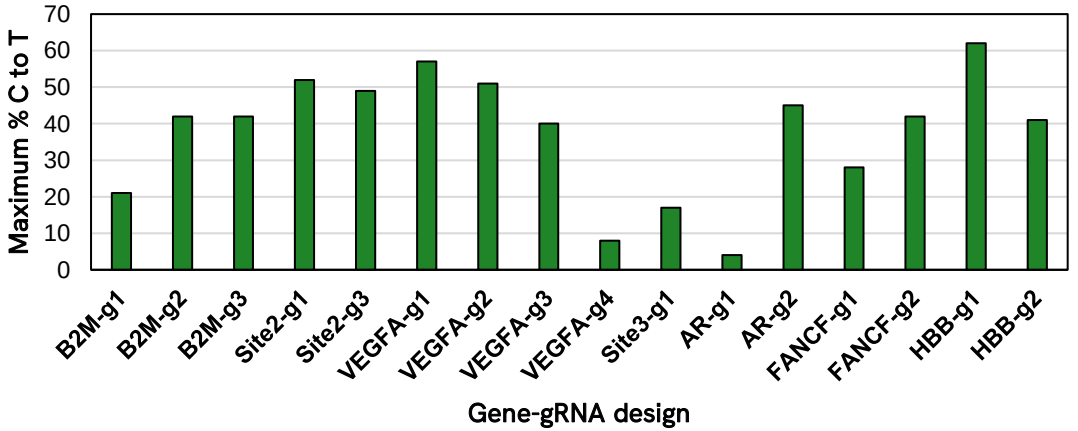
*Lavrov et al. *BMC Med Genomics* 2020

The Pin-point™ platform configured with Epic Bio's ultracompact Type V effector protein, dCasONYX

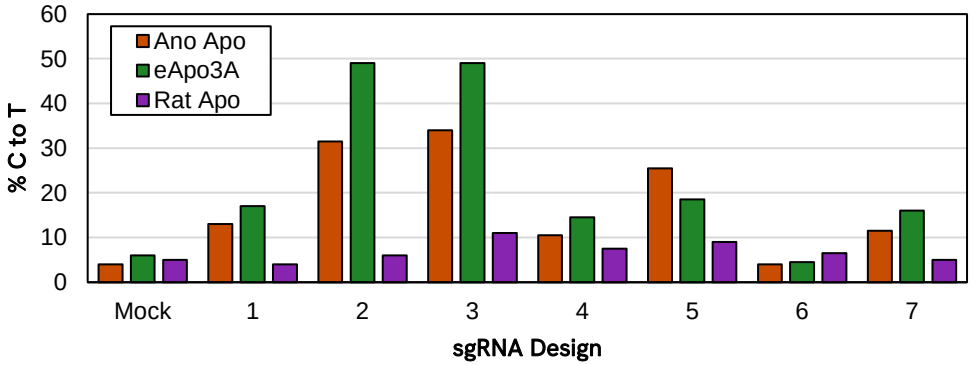
Ultra-compact engineered Cas12f variant dCasONYX is under 1.5 kb



Robust targeting capability



Efficient editing with multiple deaminases when optimal gRNA scaffold is used



Additional benefits of dCasONYX

- ✓ **Rapidly advancing to the clinic:** Epic Bio's asset EPI-321 for the potential cure for FSHD
- ✓ **Superior off-target profile:** described in Xin et al. Nature Communications[1]
- ✓ **Fully deactivated nuclease:** base editing without the risk of cutting the DNA
- ✓ **Low immunogenicity:** no prior exposure to dCasONYX in 10 human T cell donors, while 80% of human population have prior exposure to Cas9[2]
- ✓ **Small size:** Coding length less than 1.5 kb ideal for AAV packaging

A dCasONYX Pin-point configuration is one potential alternate to Cas9 for therapeutic applications

1 - doi: 10.1038/s41467-022-33346-1
2 - doi: 10.1038/s41591-018-0326-x

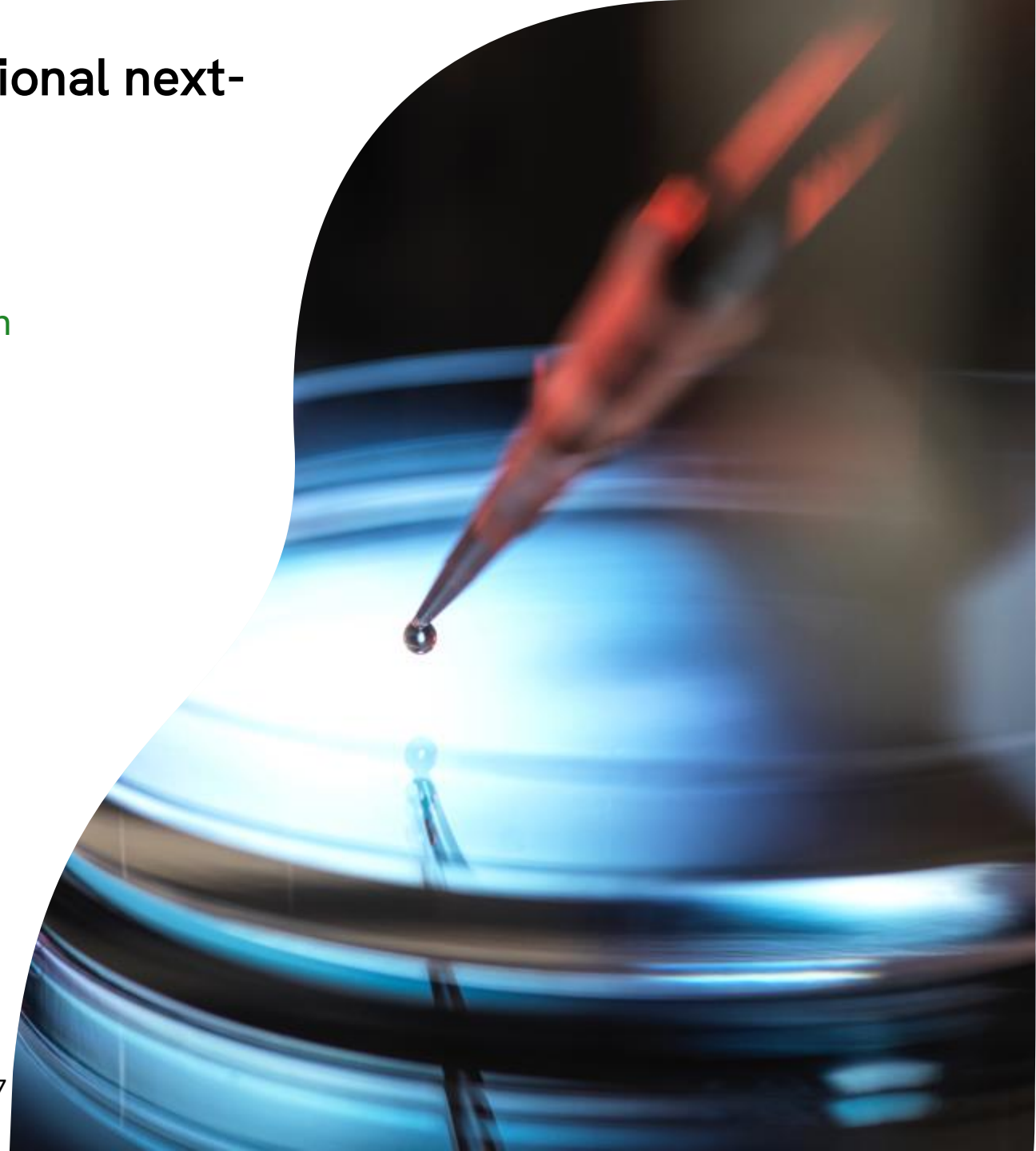
jump to base editing terminology return to outline

The Pin-point™ platform is a transformational next-generation gene editing technology

Highly effective editing platform, even for **complex edits**

Versatile technology modular and capable of generating locus-specific effects for novel therapies

Improved safety compared to standard CRISPR-Cas9 systems



Access the Pin-point™ base editing platform

Licensing

Licenses for therapeutic development

Comprehensive support

Collaboration opportunities

Research Reagents

Synthetic off-the-shelf reagents

Validated controls

Custom guide RNAs

Services

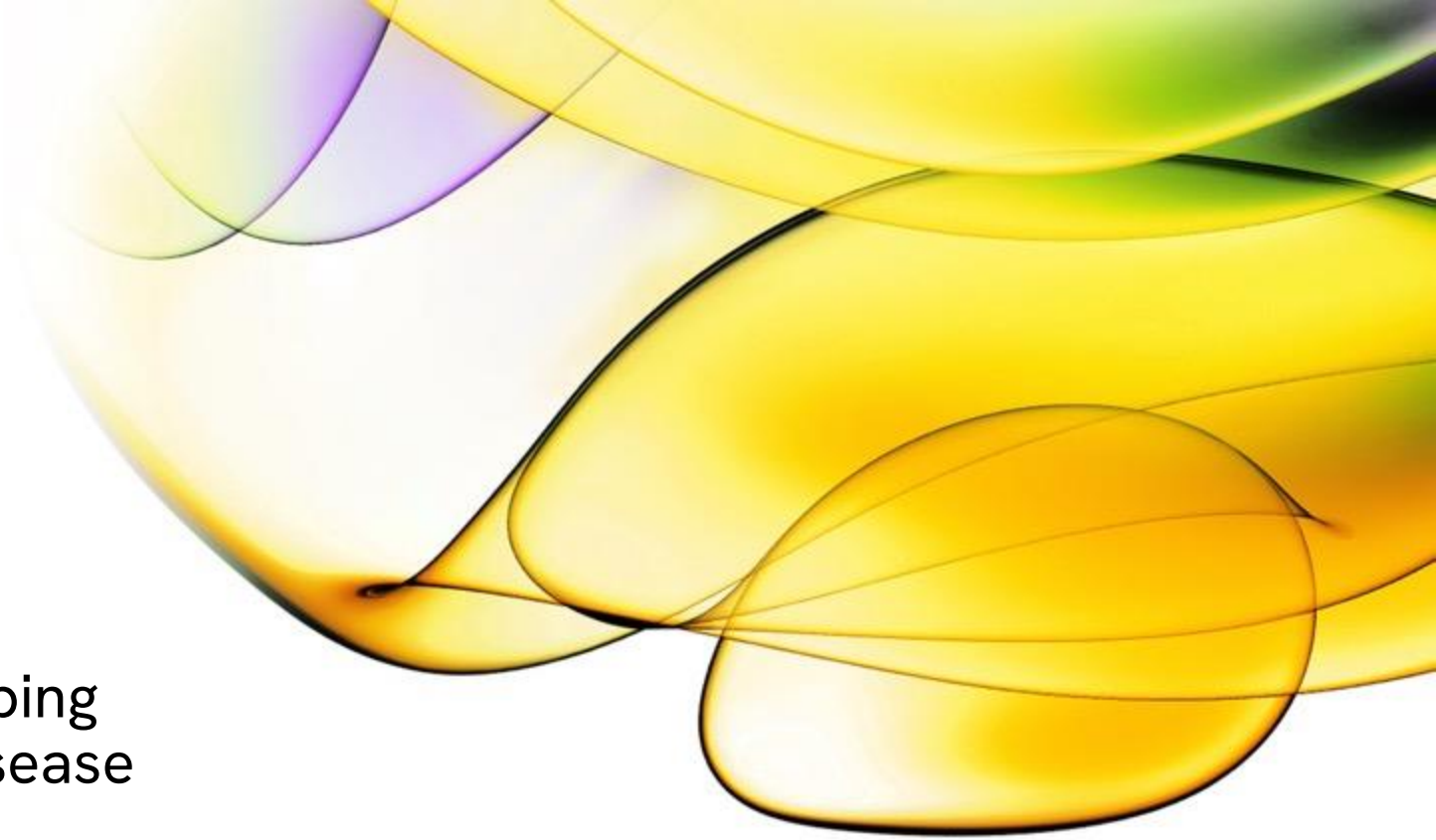
Tiled pooled screening

Functional genomics

Cell models

https://horizondiscovery.com/en/gene-editing/pin-point-base-editing-platform

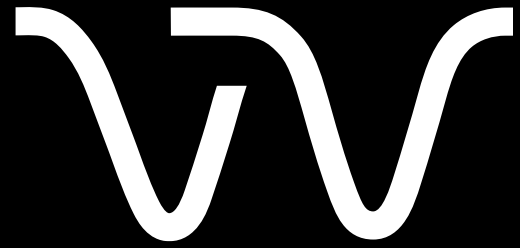
BaseEditing@HorizonDiscovery.com



We are a visionary partner in developing technologies and solutions across disease research pathways.

Here for a healthier humankind.

return to outline

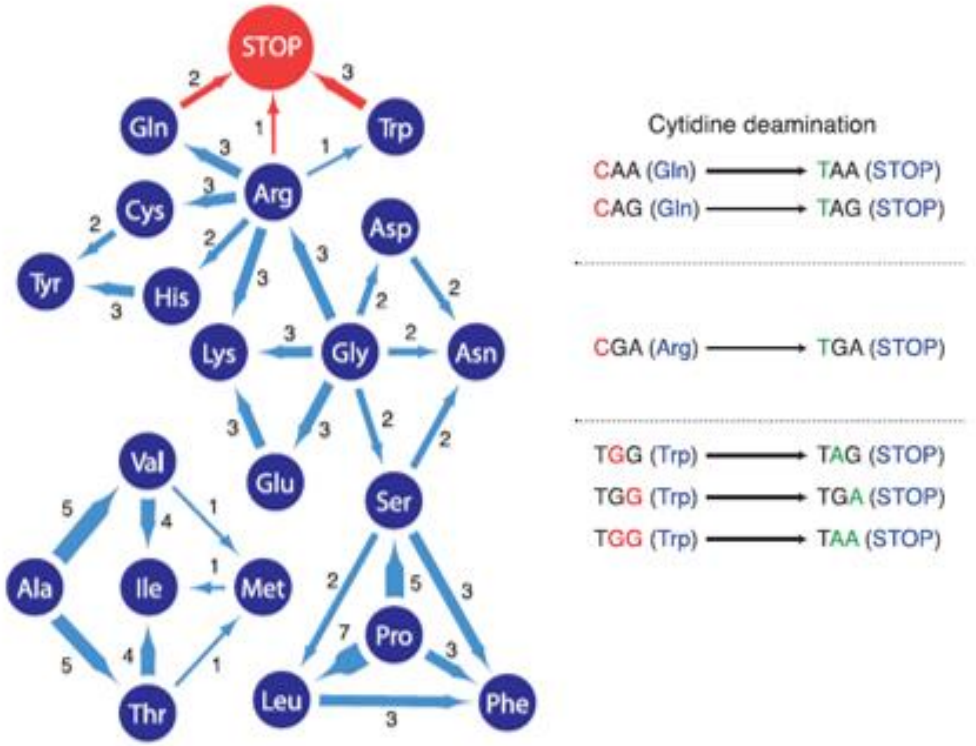


Revvity.com

Supplemental slides

How can base editing be used to create gene knockout?

Premature Stop Codons



Cytidine deamination

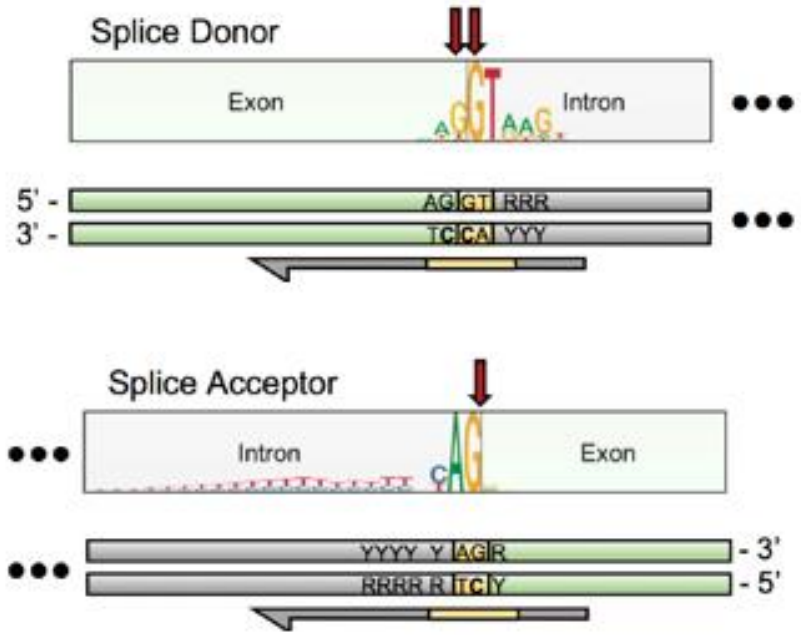
CAA (Gln) → TAA (STOP)
CAG (Gln) → TAG (STOP)

CGA (Arg) → TGA (STOP)

TGG (Trp) → TAG (STOP)
TGG (Trp) → TGA (STOP)
TGG (Trp) → TAA (STOP)

Billon et al. 2017. doi.org/10.1016/j.molcel.2017.08.008

Splice Sites Disruption

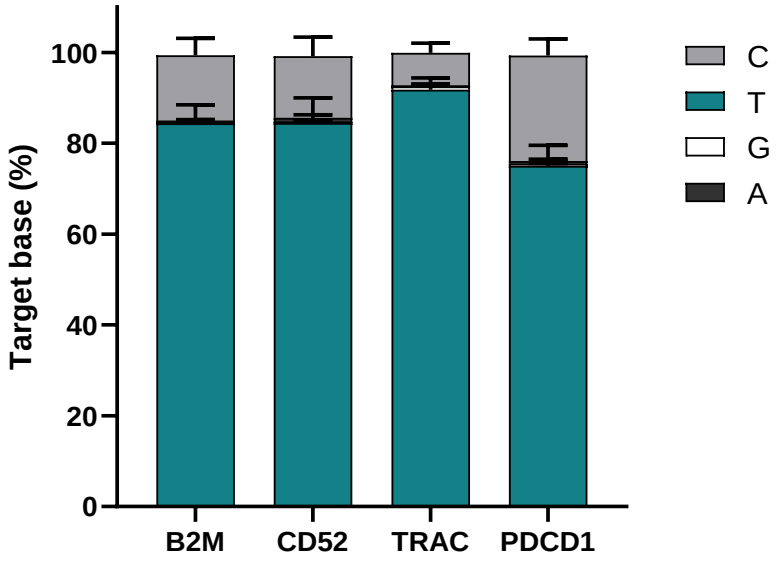


Webber et al 2019. doi.org/10.1038/s41467-019-13007-6

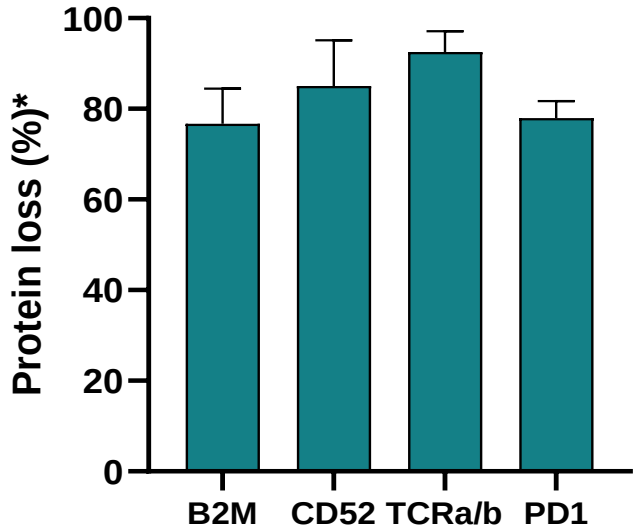
Validated performance in primary T cells

Highly efficient and precise multiplex T cell editing

>75% editing in each target base without enrichment

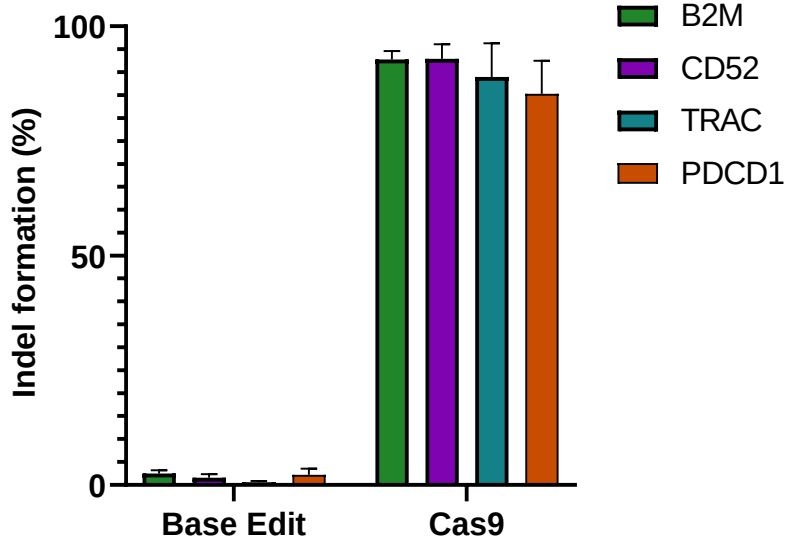


~50% of all 4 proteins knocked out (no enrichment)



*Normalized to controls

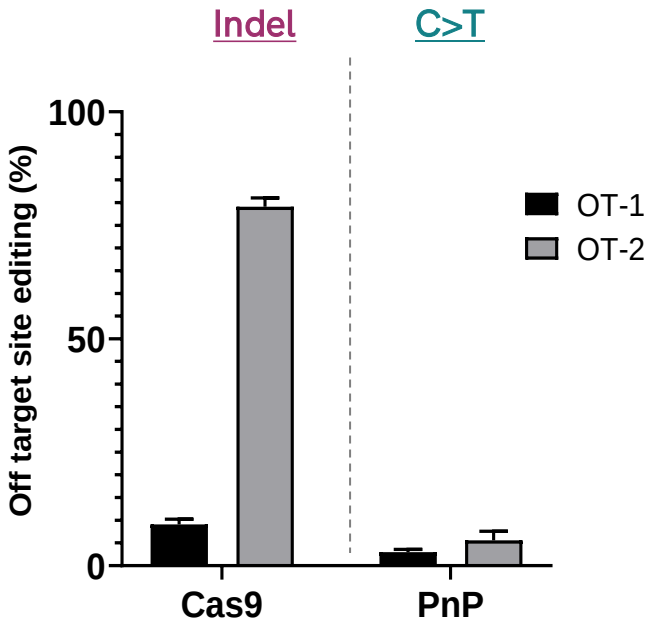
No indel formation



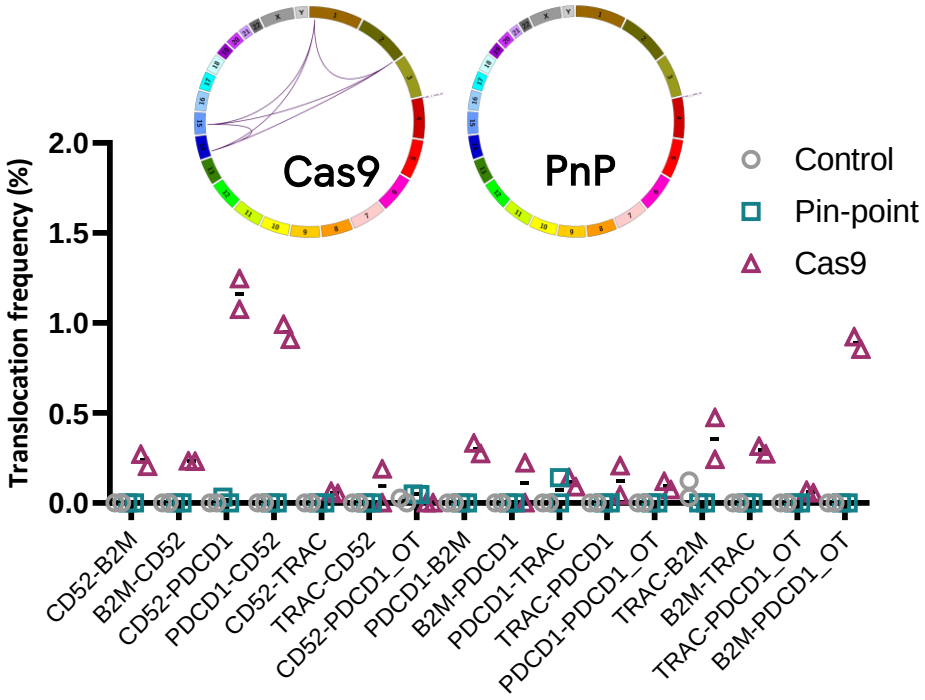
Pin-point™ base editing system is highly efficient and avoids potentially catastrophic DNA damage

Strong safety profile in T cells

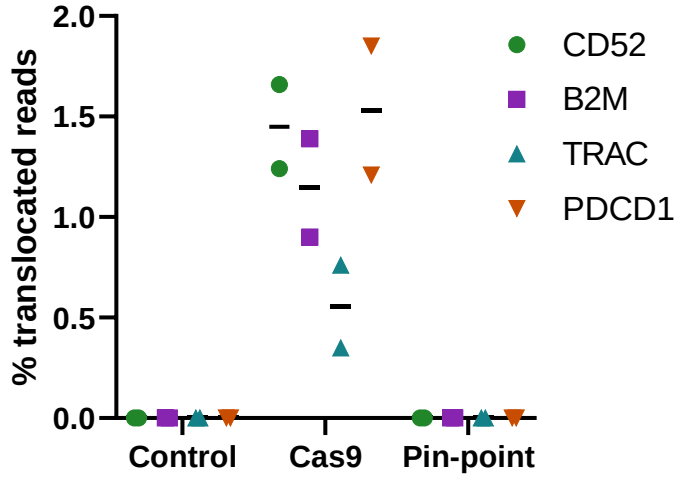
Negligible off-target edits



No detected translocations: targets and known off-target sites



No detected translocations: fusions



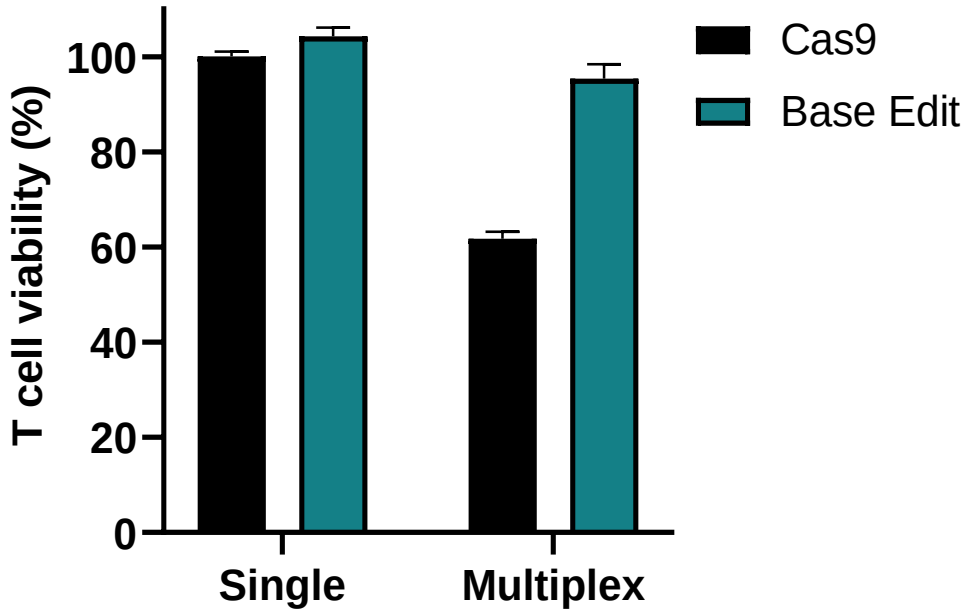
A cleaner and safer approach to multiplex gene editing in T cells

Additional off-target analysis: https://www.sciencedirect.com/science/article/pii/S1525001624004234

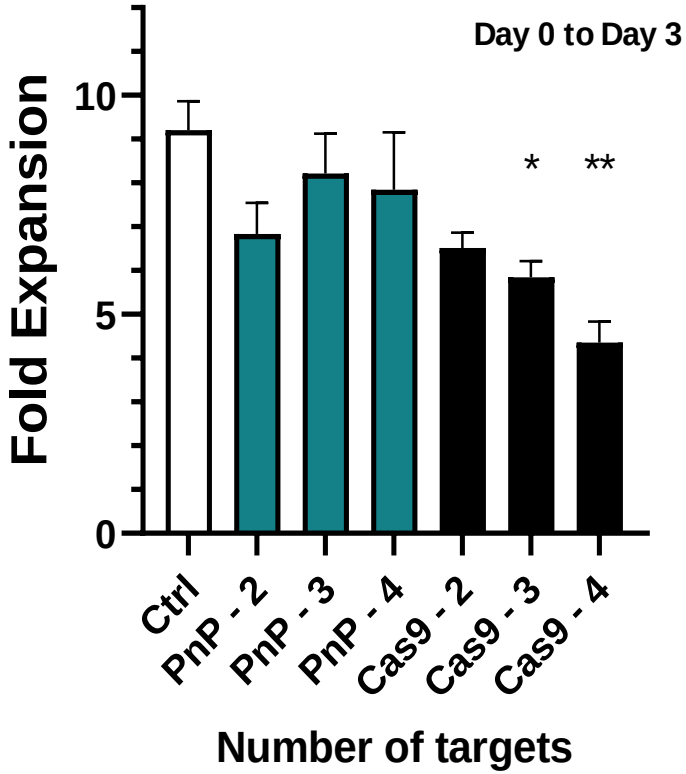
return to outline

No impact on T cell health

Cell viability maintained



Rate of cell expansion unaffected

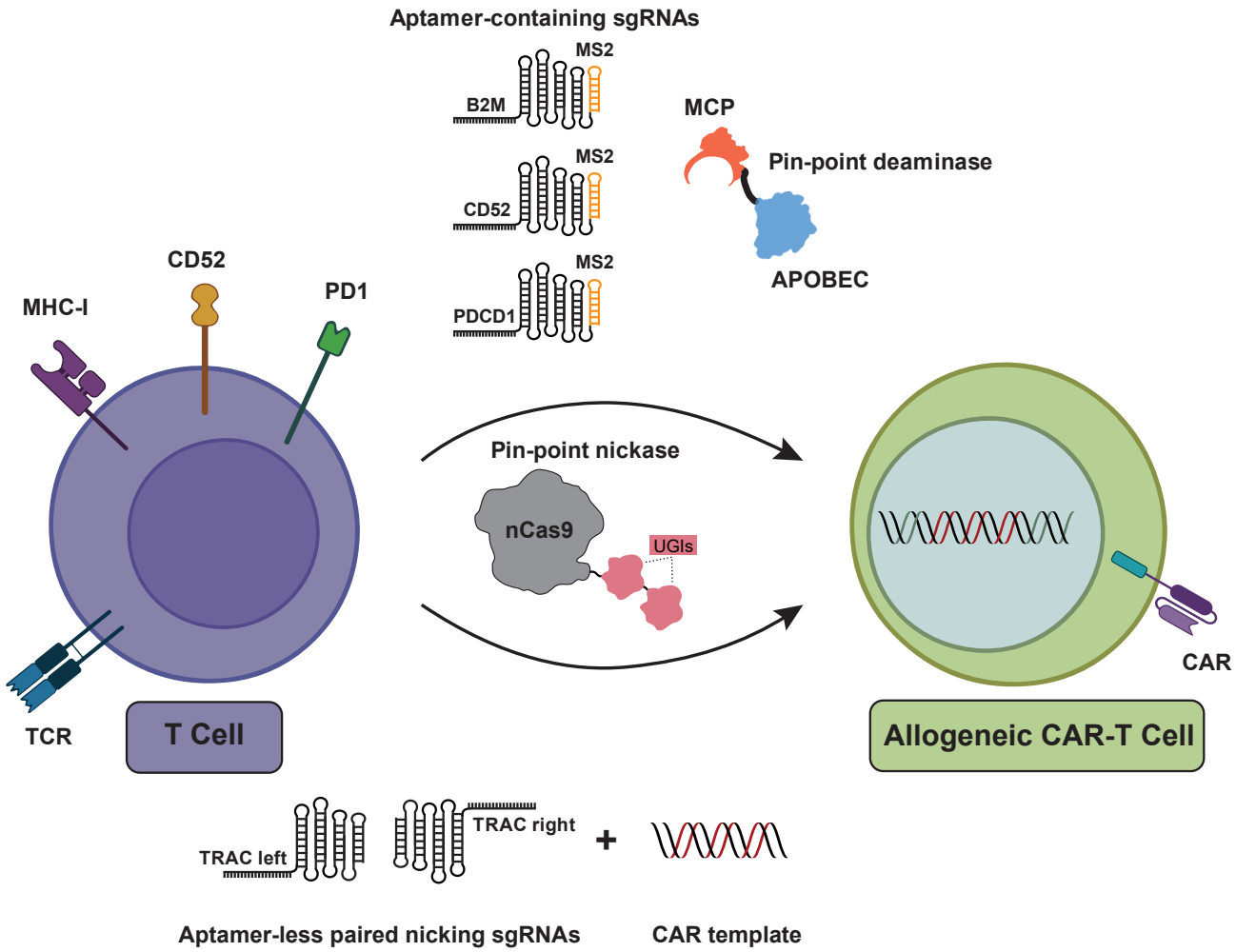


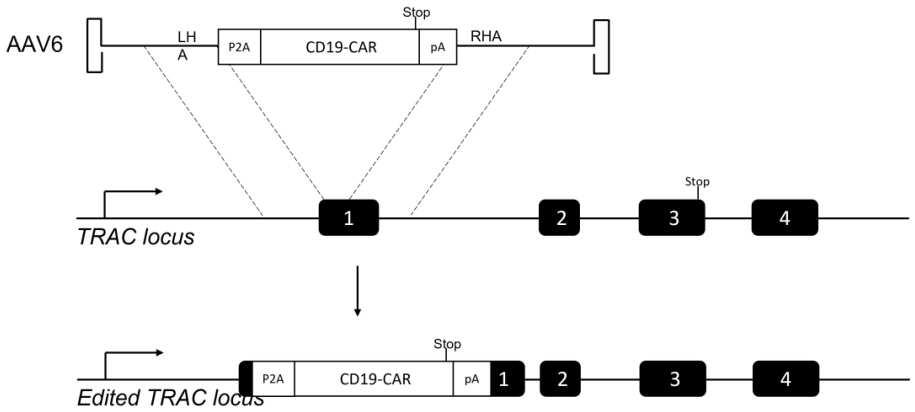
High multiplexing does not compromise cellular health or yield

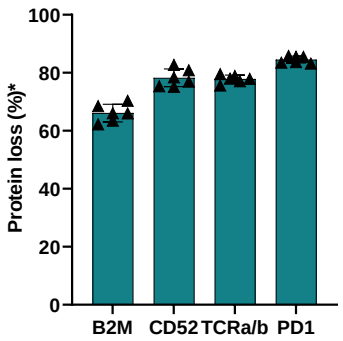
Biological replicates = 3 | Data normalized to controls

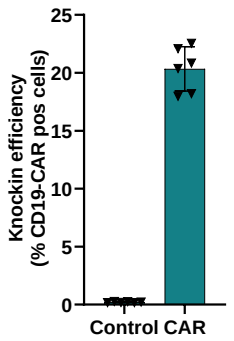
return to outline

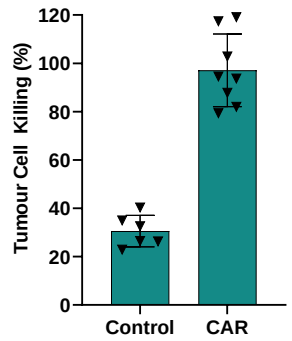
A solution for complex engineering: One-step simultaneous knock-in and knockout in T cells











Efficient and accurate for concurrent transgene insertion and multiplex base editing

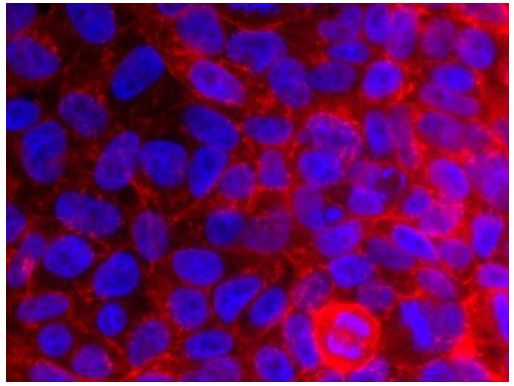
Also demonstrated in iPSCs, data available in supplemental slides

https://www.sciencedirect.com/science/article/pii/S1525001624004234

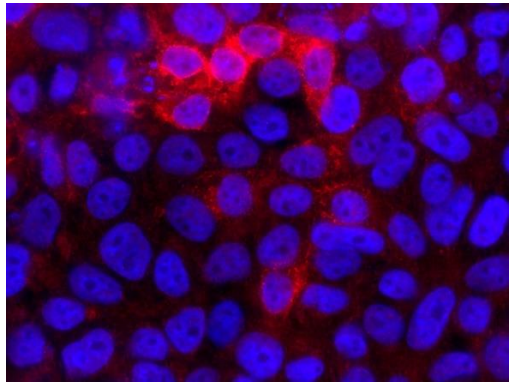
Validated performance in iPSCs

Base editing with a Pin-point™ platform in iPSCs

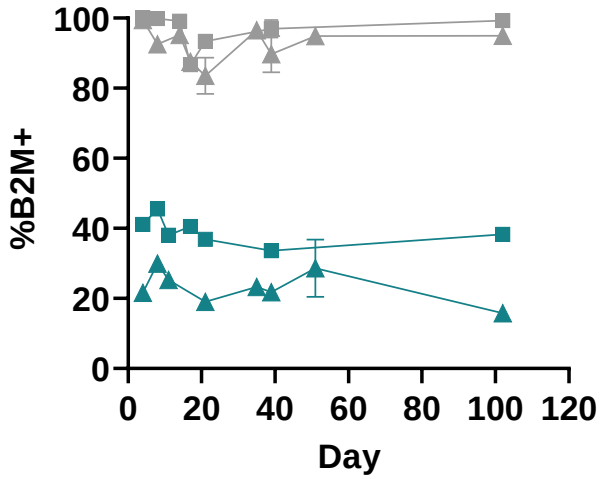
Mock

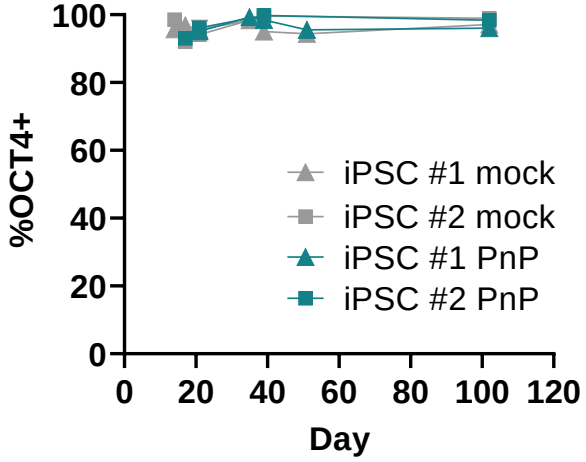


Pin-point BE

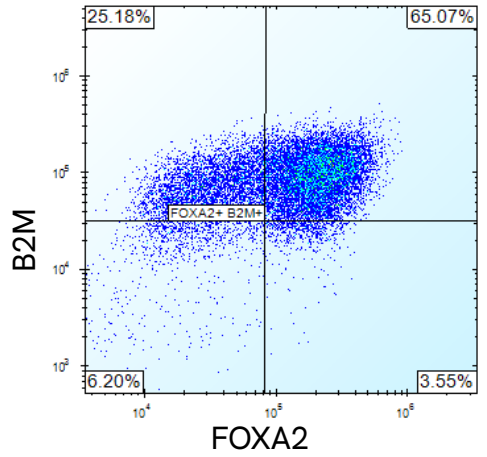


DAPI (nucleus) B2M

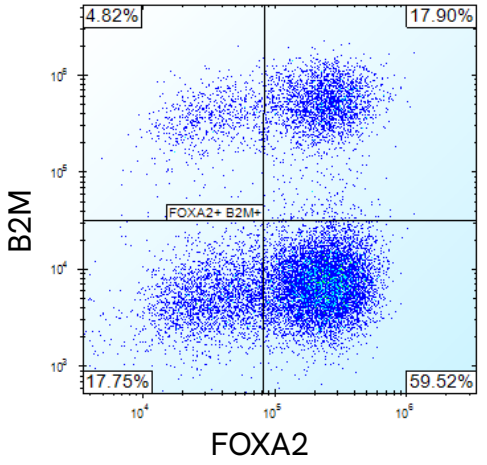




Mock



Pin-point BE

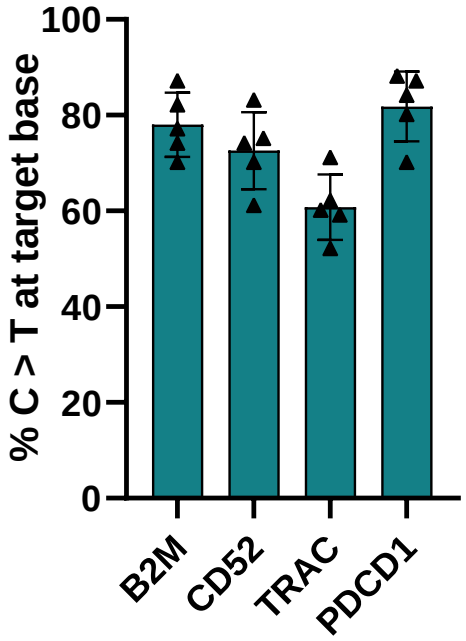


Edited iPSCs are stable with no growth defects when cultured up to 100 days and retain differentiation potential

return to outline

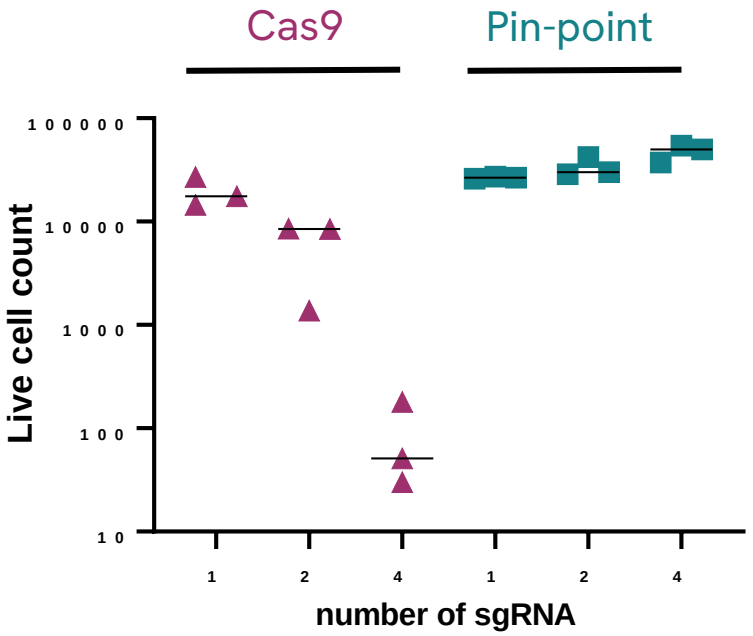
Multi-gene editing in iPSCs

Effective multiplex base editing



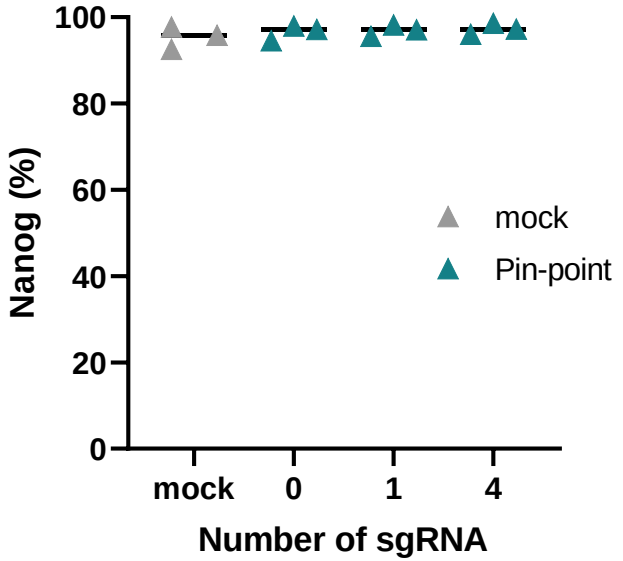
High base editing efficiency at target loci in a multiplex setting

Edited cells are viable



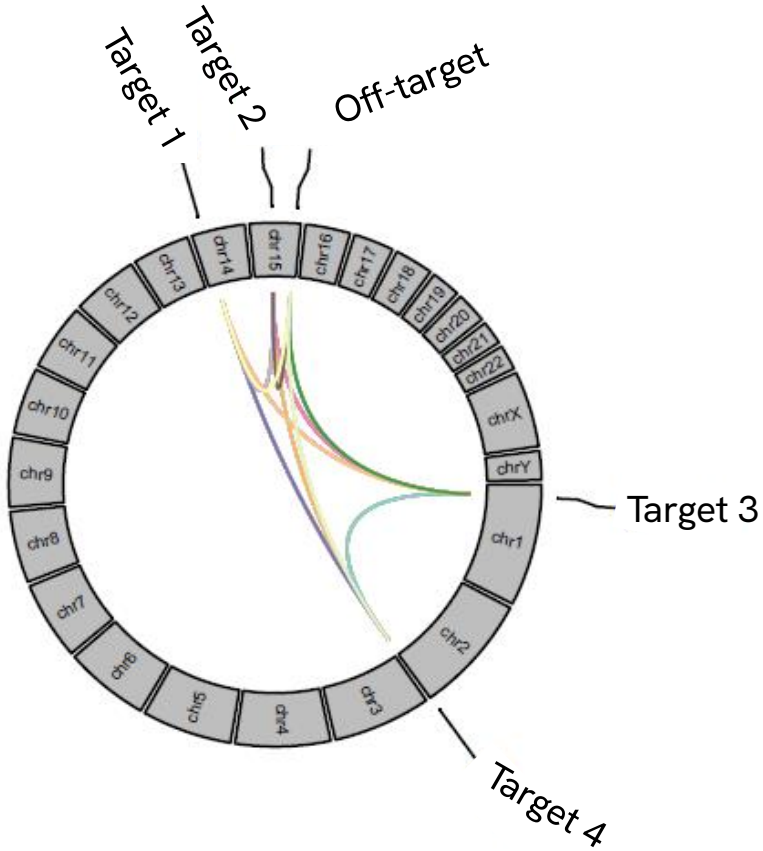
High survival of multi-edited iPSCs with a Pin-point™ system

Edited cells retain their pluripotency



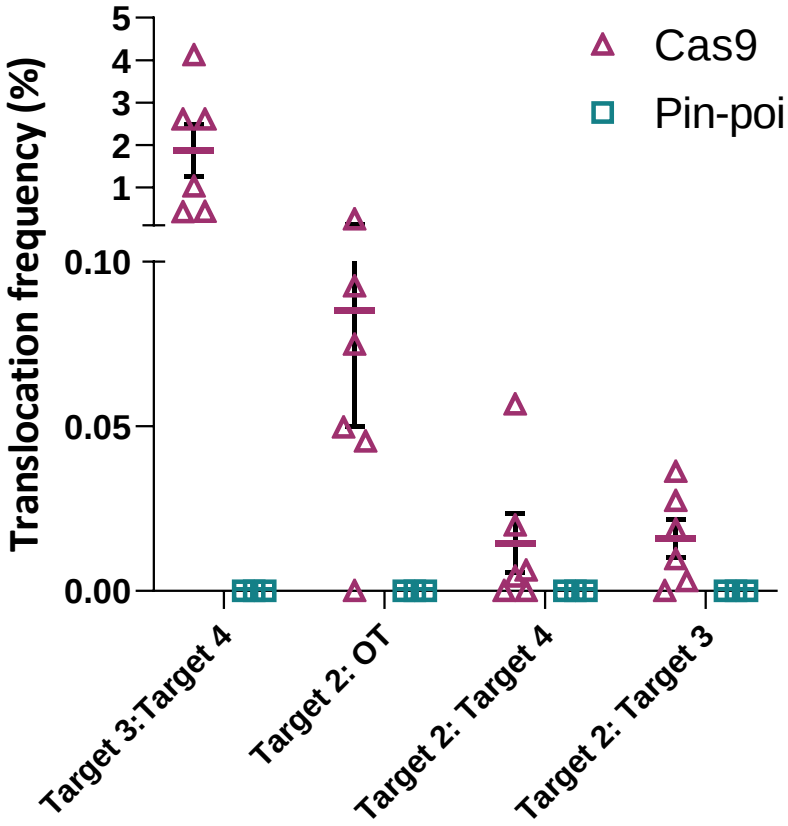
Pluripotency is retained in iPSCs edited with a Pin-point system

Strong safety profile in iPSCs



in-silico predicted translocations previously validated in T cells

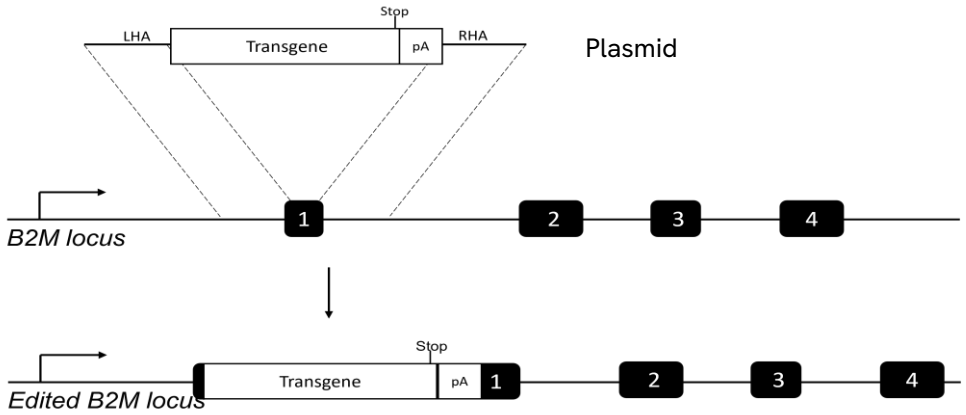
Undetectable translocations after multiplex base editing with a Pin-point™ system



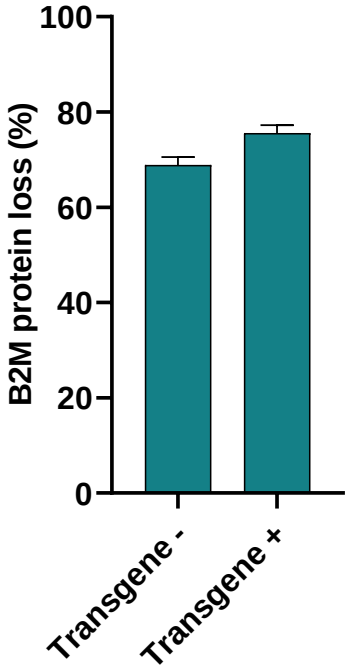
A cleaner and safer approach to multiplex gene editing in iPSCs

Demonstrated simultaneous knock-in and multiple knockout in iPSCs

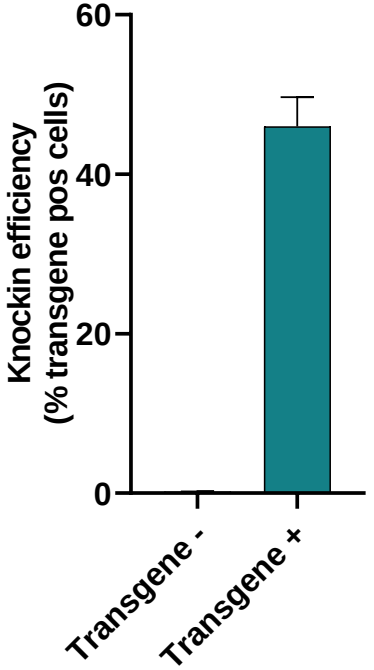
Base editing
Knockout CIITA
&
Insertion of a transgene
in B2M (promoter-less GFP)



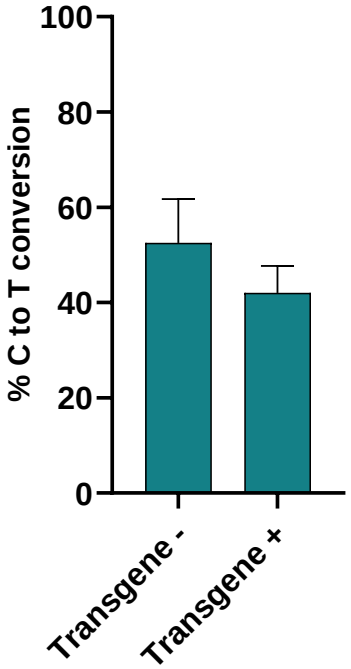
High level of B2M knockout



High level of transgene knock-in



… and base editing (CIITA KO)



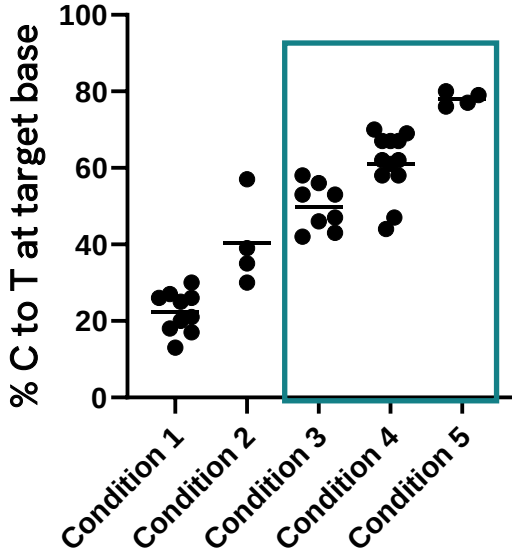
The Pin-point™ platform enables one-step simultaneous knock-in and multiple knockout in iPSCs

Validated performance in HSPCs

Highly efficient base editing in HSPCs

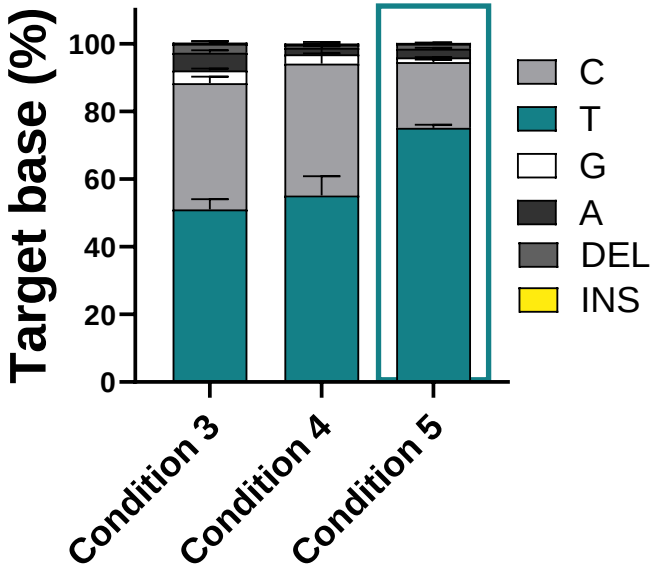
High level of editing achieved with optimised conditions

Sanger sequencing

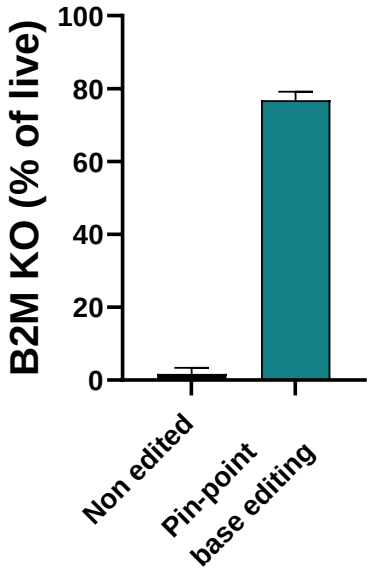


High level of editing and purity achieved at the target site

NGS



High level of B2M phenotypic knock-out



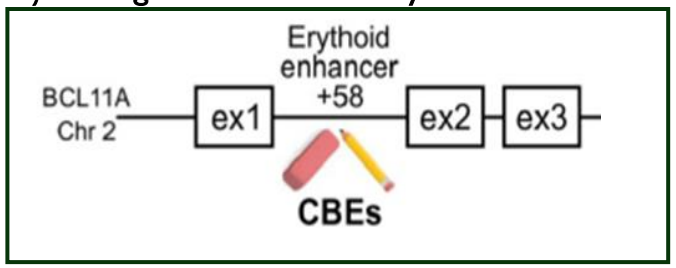
Optimized application of the Pin-point™ platform achieves high levels of editing in HSPCs with high purity of C to T conversion

Therapeutic editing of HSPCs with the Pin-point™ platform

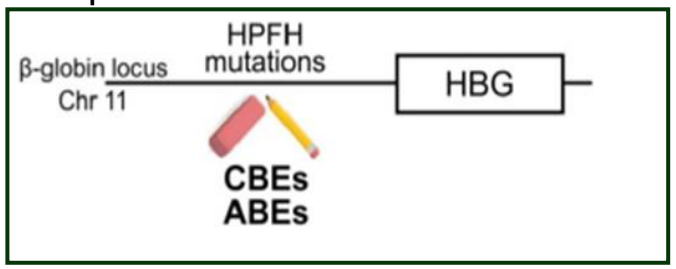
https://doi.org/10.3389/fgeed.2021.618406

Reactivating Fetal Haemoglobin

1) Editing of the BCL11A erythroid enhancer



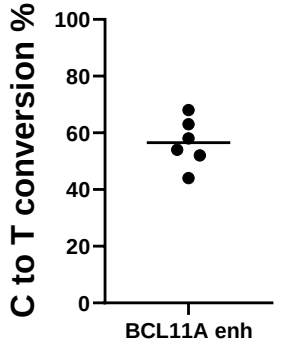
2) Editing of the BCL11A binding site in the HBG promoter

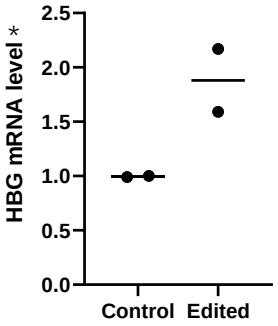


Editing Efficiency

GATA1 binding site

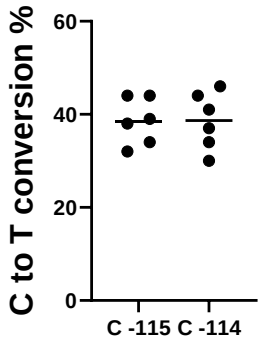
Base	T	T	T	A	T	C	A	C	A	G	G	C	T	C	C	A	G	G	A	A
G	0	0	0	0	0	4	0	0	6	-	-	0	0	0	0	4	-	-	0	5
A	0	0	0	-	0	0	-	0	-	0	0	0	0	0	0	-	0	0	-	-
T	-	-	-	0	-	68	0	5	0	0	0	0	-	5	0	0	0	0	0	0
C	0	0	0	0	0	-	0	-	0	0	0	-	6	-	-	0	0	0	0	0

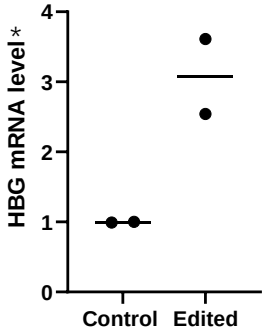




BCL11A binding site

Base	C	T	T	G	A	C	C	A	A	T	A	G	C	C	T	T	G	A	C	A
G	0	0	0	-	0	0	0	0	0	0	0	-	0	0	0	0	-	0	0	0
A	0	0	0	0	-	0	0	-	-	0	-	0	0	0	0	0	0	-	0	-
T	0	-	-	0	0	38	37	0	0	-	0	5	0	0	-	-	0	0	0	0
C	-	0	0	0	0	-	-	0	0	0	0	0	-	-	0	0	0	0	-	0





Induction of Fetal Haemoglobin

*qPCR data expressed as HBG/HBA and normalised on expression in control samples

The Pin-point base editing platform achieves therapeutic editing in HSPCs

return to outline

Uniquely capable of complex engineering

A solution for complex engineering

One-step simultaneous knock-in and multiple knockout in T cells

One transfection

Pin-point deaminase

Pin-point aptamer gRNA (3)

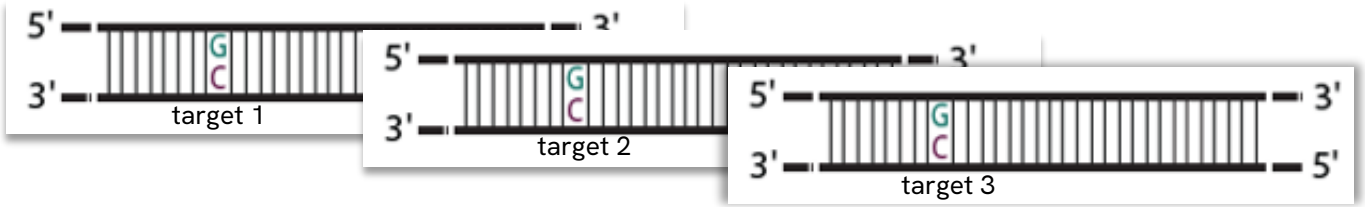
Nickase Cas

Nicking gRNAs (2)

Donor insert template

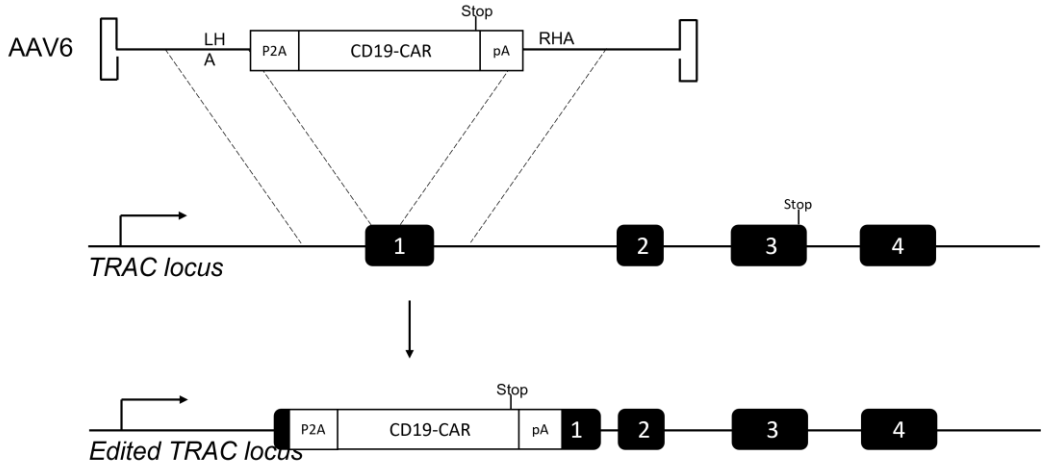
Base Editing with aptamer gRNAs

Knockout B2M, CD52, PDCD1



Insertion of a transgene by non-aptamer nicking gRNAs

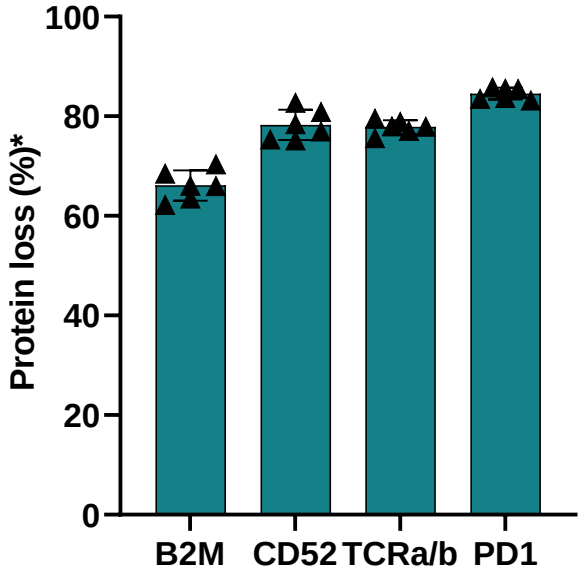
CAR in TRAC



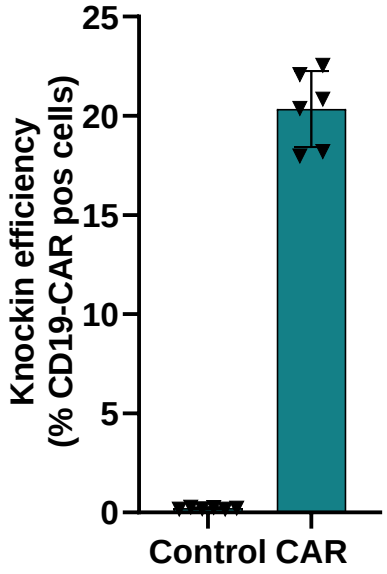
return to outline

Streamlined creation of CAR-T cells is enabled with the Pin-point™ platform

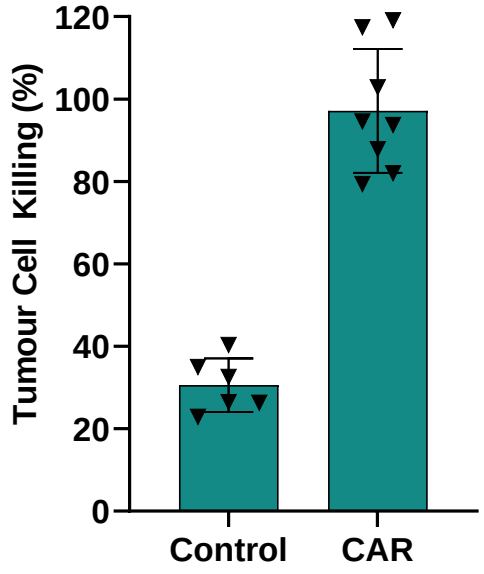
Multiple proteins are knocked out



... while enabling protein knock-in



... and weaponizing T cells against cancer cells



The Pin-point platform is efficient and accurate for concurrent transgene insertion and multiplex base editing

Biological replicates = 2 *Normalized to controls

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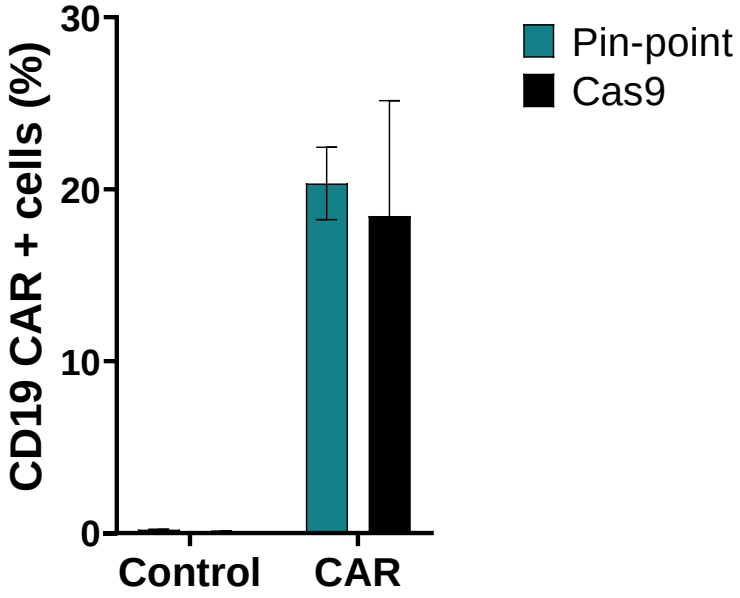
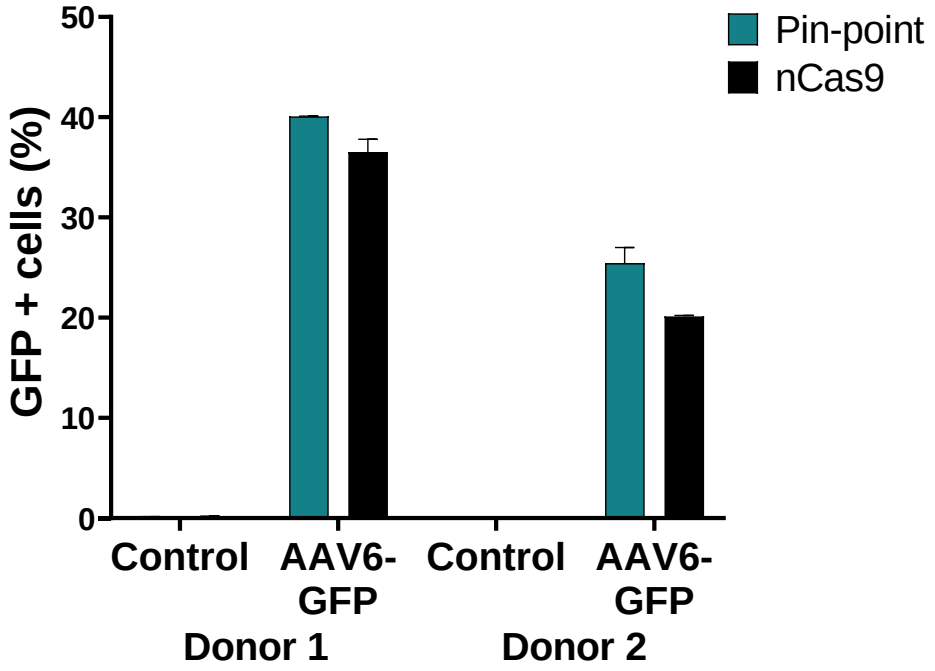
No loss of efficiency in payload deliveries

Equivalent to dsDNA knock-in



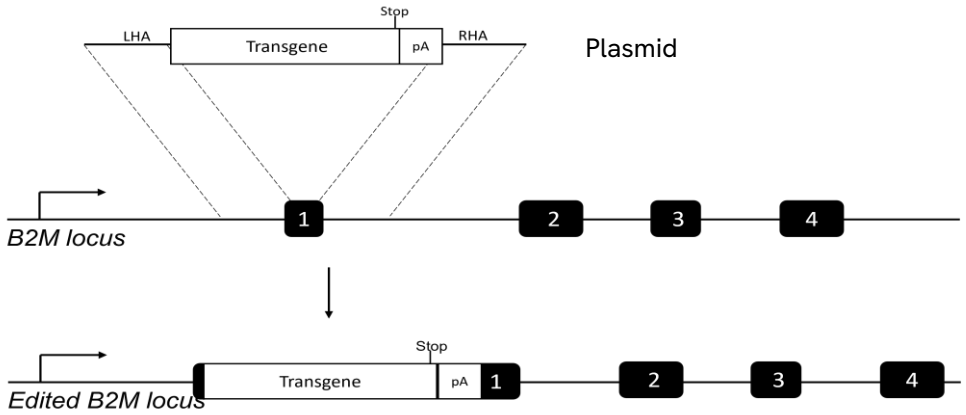
Presence of modular deaminase has no impact on knock-in



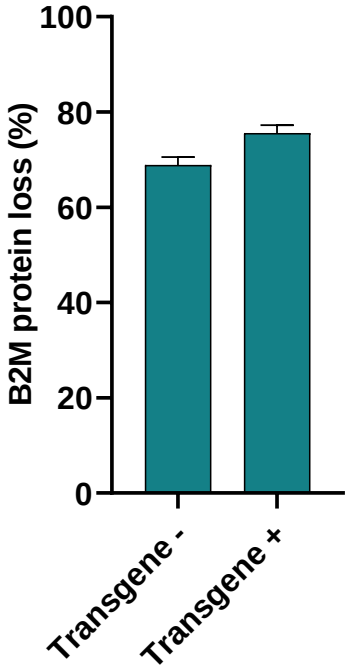
The Pin-point™ platform can deliver payloads equivalently to standard Cas9 or nCas9 knock-in strategies

Demonstrated simultaneous knock-in and multiple knockout in iPSCs

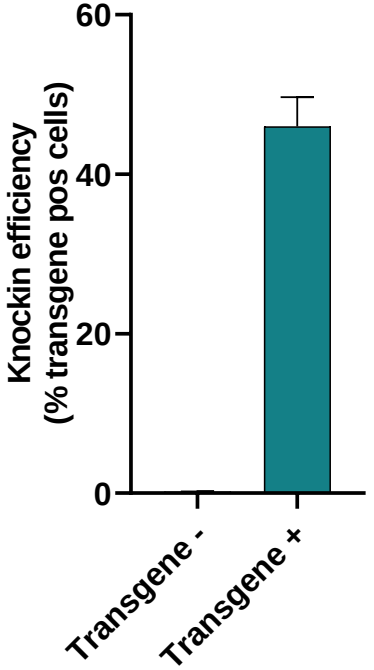
Base editing
Knockout CIITA
&
Insertion of a transgene
in B2M (promoter-less GFP)



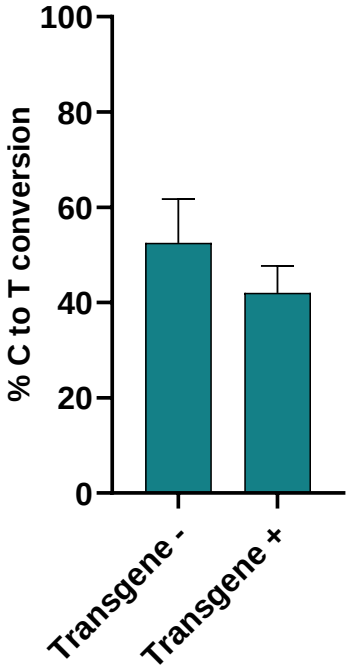
High level of B2M knockout



High level of transgene knock-in



… and base editing (CIITA KO)

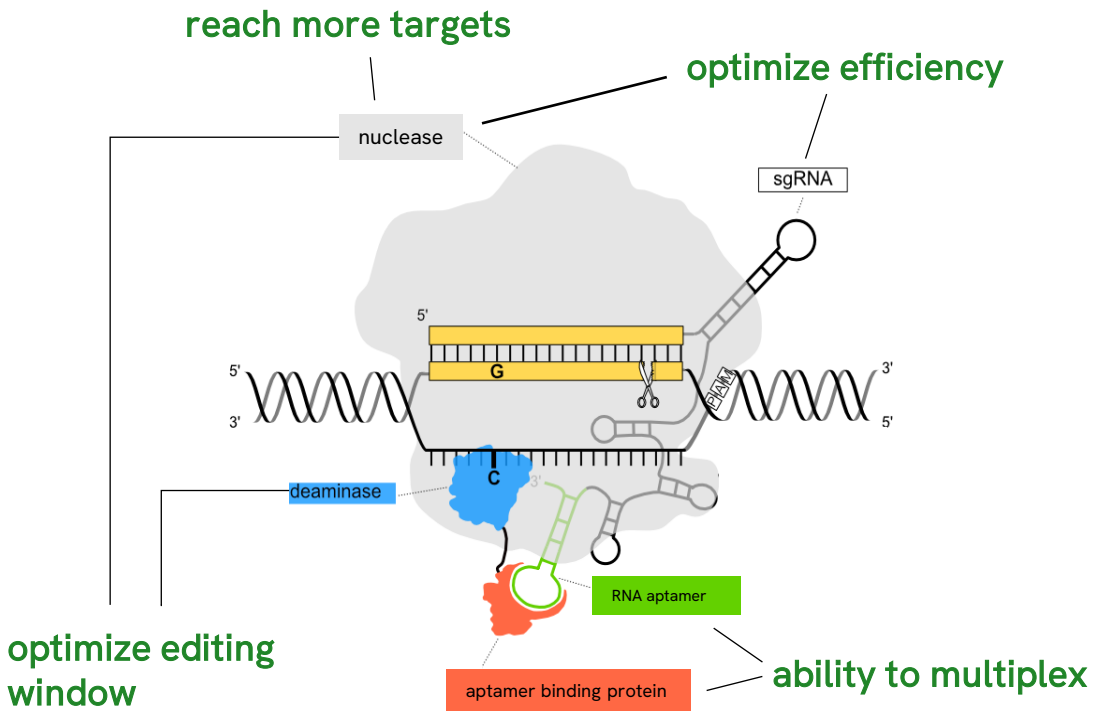


The Pin-point™ platform enables one-step simultaneous knock-in and multiple knockout in iPSCs

Flexibility for target optimization

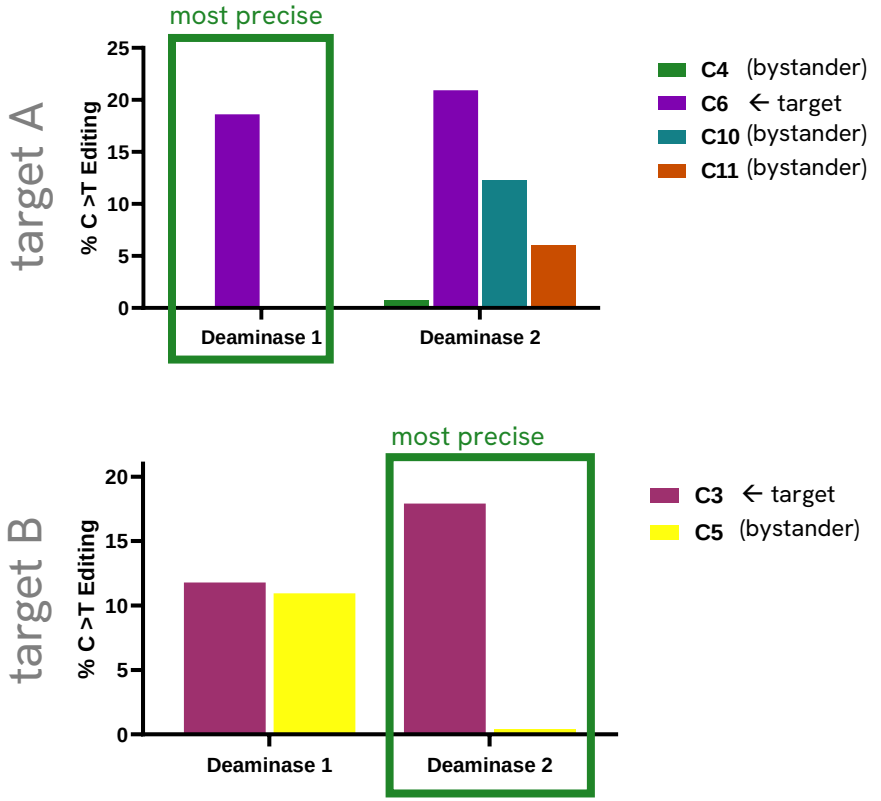
Choose components for locus-specific optimization

Increased potential to correct more pathogenic SNVs that are not reachable with existing published systems*



Schematic depicts nCas9 configuration

Example of optimization of the editing window by selecting the best guide RNA and deaminase pairs



The modular Pin-point™ platform can be customized to combine optimal components for a wide range of base editing applications

*Lavrov et al. *BMC Med Genomics* 2020

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A benefit of modularity of the Pin-point™ platform

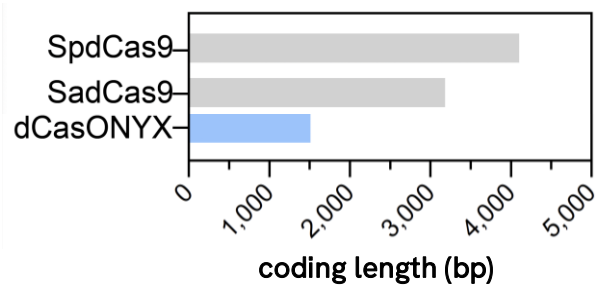
Demonstrated compatibility with numerous nucleases

	Type II			Type V						
	A	B	C	D	E	F	G	H	I	J
Enzyme activity	nickase	nickase	nickase	deactivated	deactivated	deactivated	deactivated	deactivated	deactivated	deactivated
Demonstrated nuclease activity in mammalian cells	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Demonstrated with the Pin-point system	✓	✓	In progress	✓	In progress	✓	✓	✓	✓	In progress
sgRNA optimized	✓	In progress		In progress		✓	✓	✓	✓	
Enzyme optimized	✓					✓				
Confirmed at multiple targets (2+)	✓	In progress				✓	✓	✓	✓	
Demonstrated in multiple cell types (2+)	✓	In progress				✓	In progress	✓		
Demonstrated with multiple deaminases (2+)	✓						In progress	✓	✓	

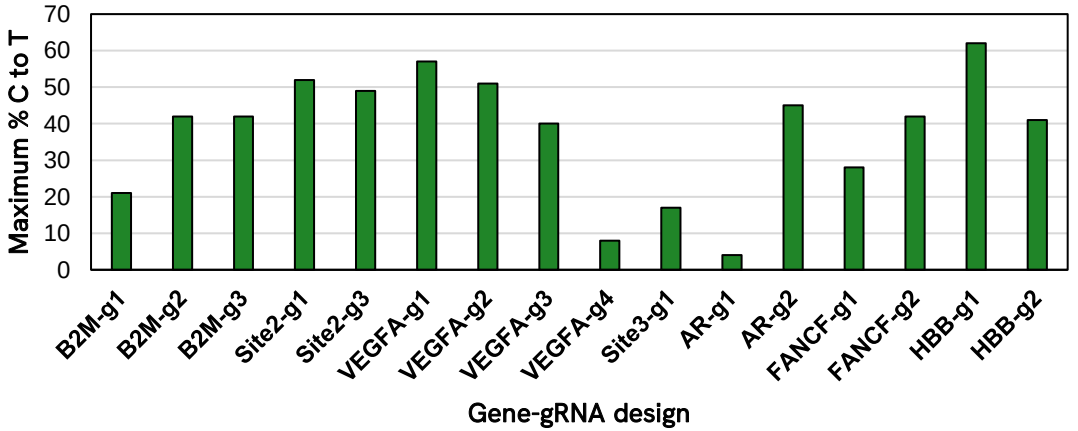
The Pin-point platform enables utilization of a variety of RNA-guided nucleases, which can be further optimized for editing efficiency

The Pin-point™ platform configured with Epic Bio's ultracompact Type V effector protein, dCasONYX

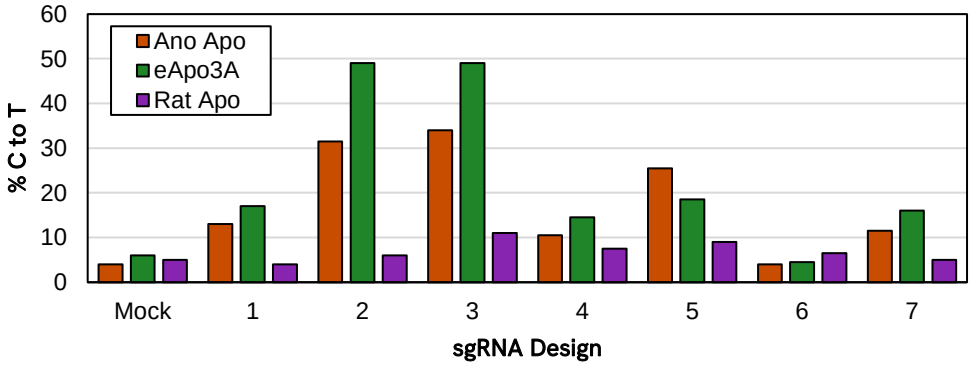
Ultra-compact engineered Cas12f variant dCasONYX is under 1.5 kb



Robust targeting capability



Efficient editing with multiple deaminases when optimal gRNA scaffold is used



Additional benefits of dCasONYX

- ✓ **Rapidly advancing to the clinic:** Epic Bio's asset EPI-321 for the potential cure for FSHD
- ✓ **Superior off-target profile:** described in Xin et al. Nature Communications[1]
- ✓ **Fully deactivated nuclease:** base editing without the risk of cutting the DNA
- ✓ **Low immunogenicity:** no prior exposure to dCasONYX in 10 human T cell donors, while 80% of human population have prior exposure to Cas9[2]
- ✓ **Small size:** Coding length less than 1.5 kb ideal for AAV packaging

A dCasONYX Pin-point configuration is one potential alternate to Cas9 for therapeutic applications

1 - doi: 10.1038/s41467-022-33346-1
2 - doi: 10.1038/s41591-018-0326-x

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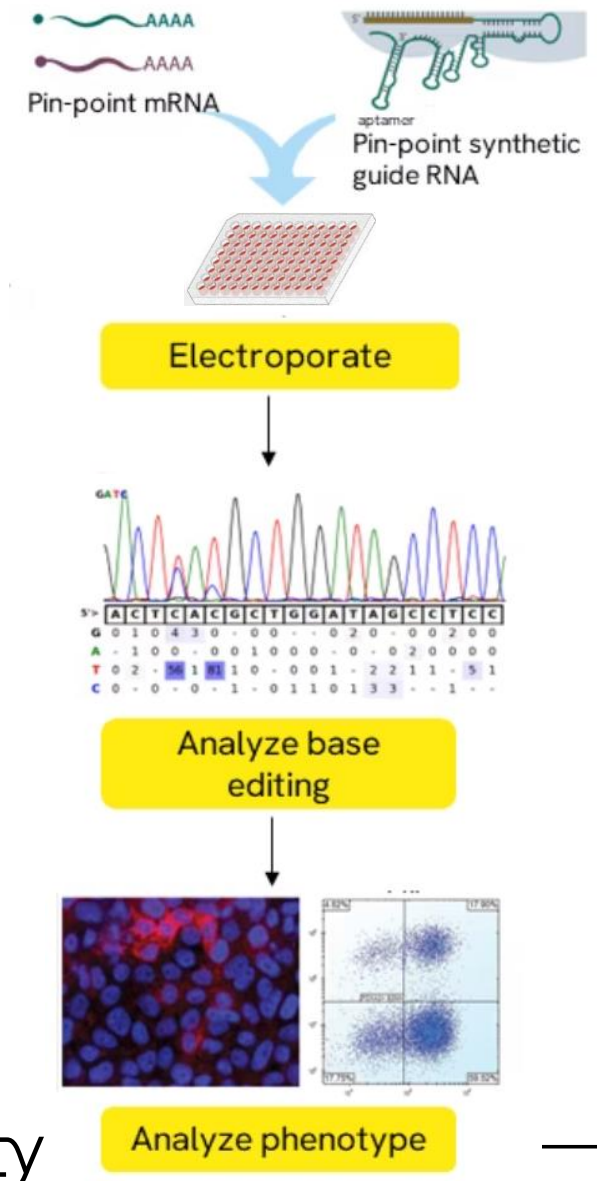
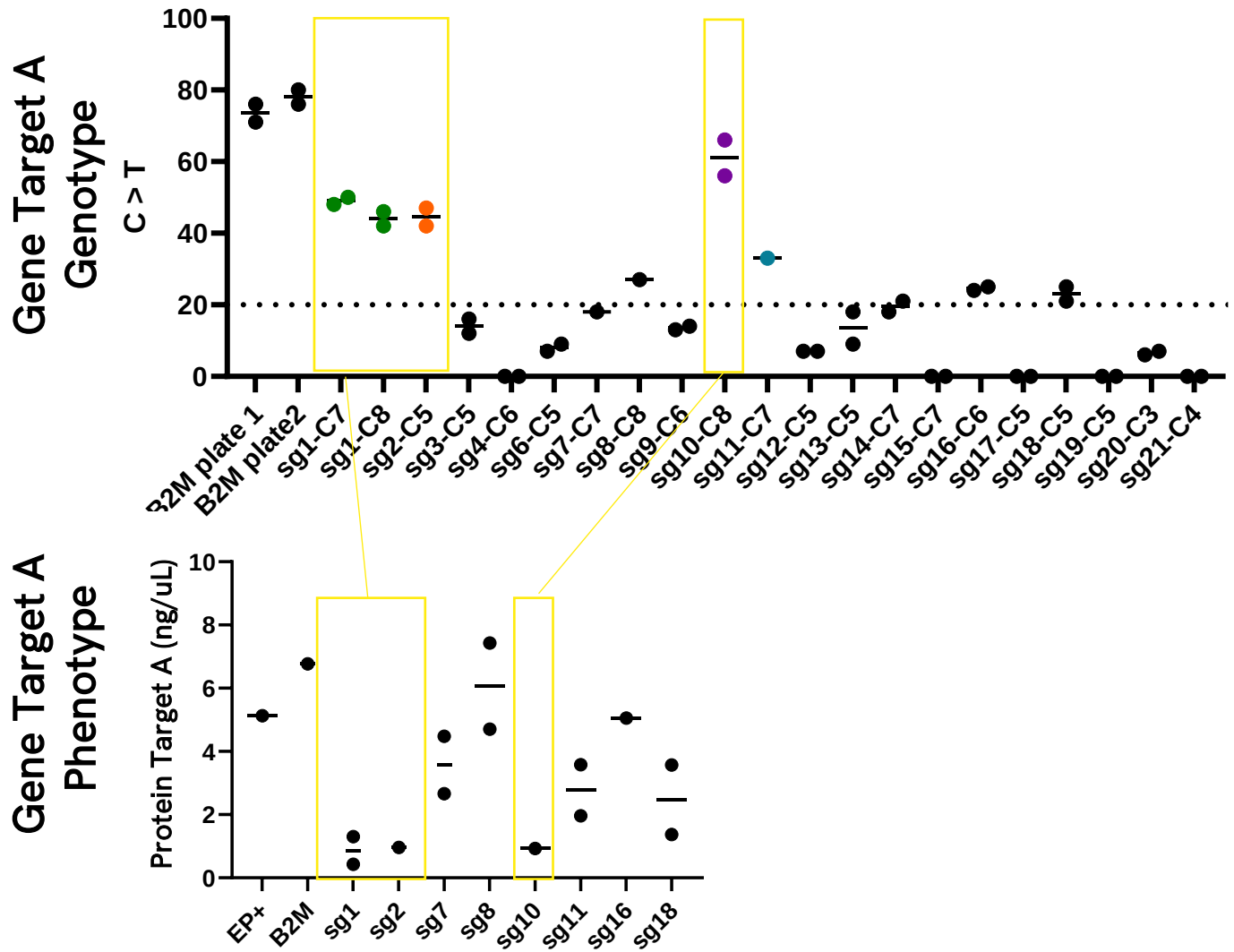
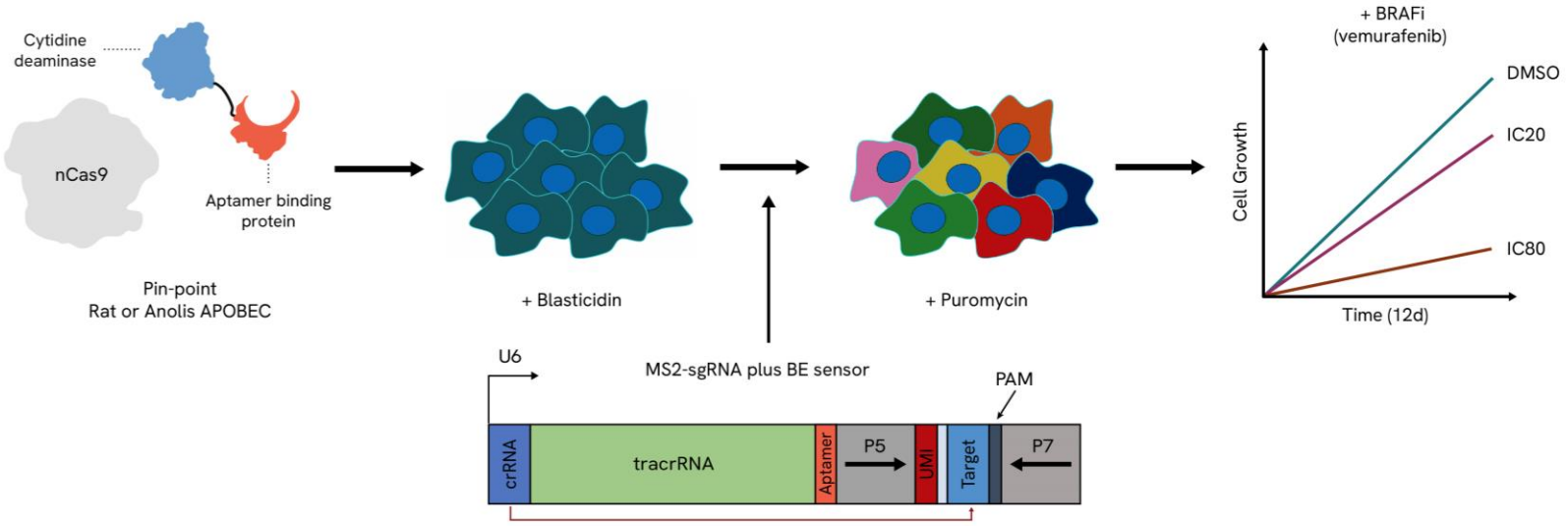
Synthetic reagents for arrayed screening applications



Confirm results of genotype with phenotype



Pooled tiled base editing screening services

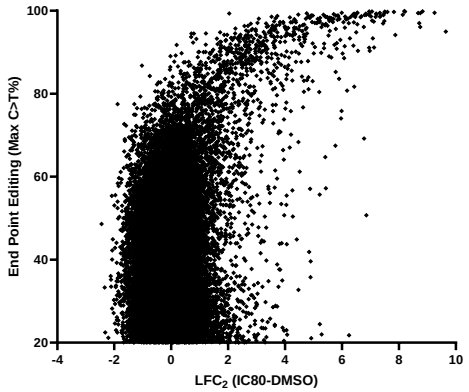


Modify up to every PAM-accessible cytidine in the gene of interest to gain unparalleled understanding of the genotype-phenotype relationship.

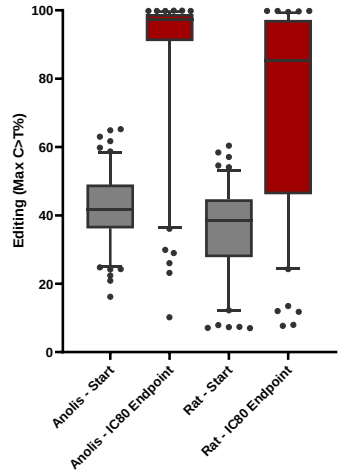
- **Target splice sites, introduce premature stop codons, and introduce all possible missense mutations** to recapitulate and then go way beyond CRISPRko and CRISPRi screens.
- Include **loss of function** and **gain of function** mutations in a single screen to elucidate possible mechanisms of drug sensitivity and resistance, or protein function.
- **Utilize different deaminases** to maximize the editing window and evaluate more of the genetic sequence.
- Data analysis to generate hit lists and potentially **identify putative causative mutations**.

Pooled tiled base editing screening services | example BRAFi resistance screen

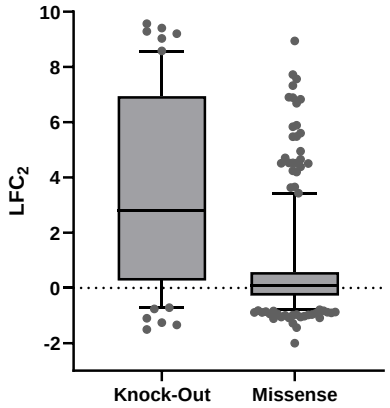
Enrichment of guides that confer resistance to the drug correlate with increased levels of editing



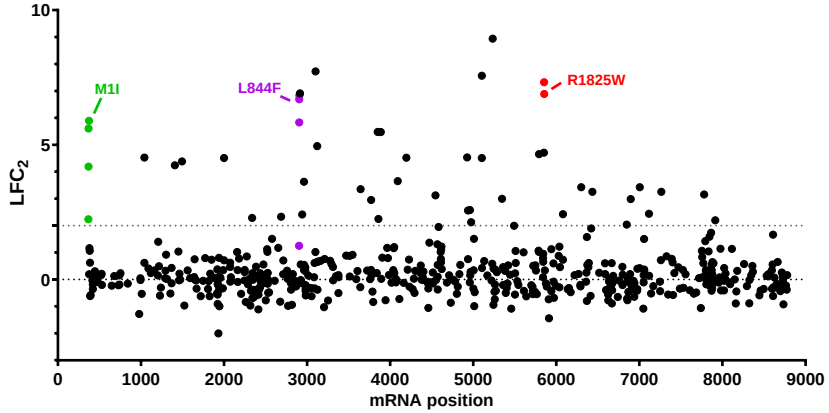
Strong enrichment for edited genomes



NF1 knockout by targeting either the mRNA slice sites or introducing nonsense mutations are more likely to confer drug resistance



resistance hits show no hotspot regions in the NF1 gene, agrees with clinical observations. Multiple guides introducing the same resistance conferring mutation are shown to demonstrate similar screen phenotypes.



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