## The Pin-point<sup>™</sup> platform

A novel modular base editing system



Updated 9 June 2023

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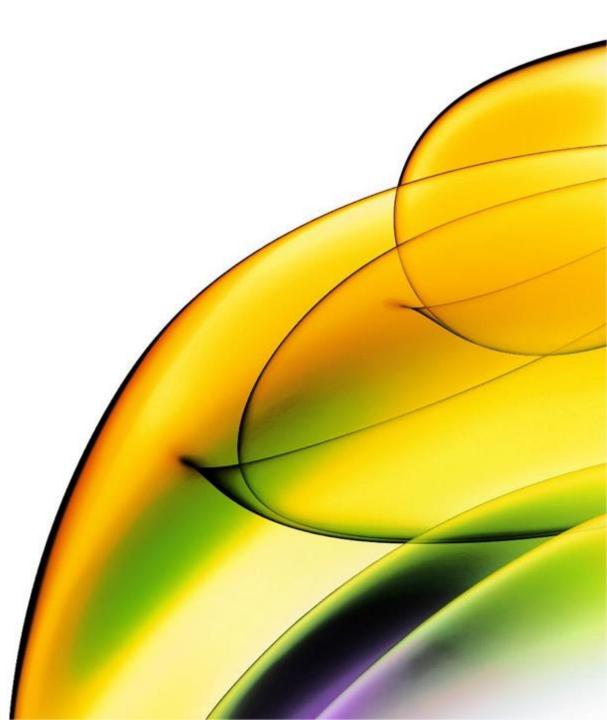
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## The Pin-point<sup>™</sup> platform

Accelerating therapeutic development and reach into the clinic

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

Robert Blassberg\*

REVIEW

(Fig. 1).2.

The explosion of interest in CRISPR-Cas systems over rece engineering technologies that hold enormous potential for therapy space, adoptive immunotherapies continue to lear neering strategies toward clinical application. Although ea collected from patient blood, the democratization of the allogeneic cell therapy products that can be manufacture substrate for such products, and many of the challenges adoptive cell therapies are common with those holding b cusses how genome editing can overcome these challer generation technologies in the development of novel or

D luripotent stem cells (PSCs) hold the potential to generate

embryos generated for the purpose of in vitro fertilization were the first PSCs to raise hopes for clinical application in

cell therapies.<sup>1</sup> The paradigm was shifted in the mid 2000s

by the pioneering work of Kazutoshi Takahashi and Shinya Yamanaka who demonstrated that PSCs can be "induced"

from adult cells by reactivating embryonic gene expression

programs with defined cocktails of transcription factors

Such induced PSCs (IPSCs) can be derived either from pro spective patients or adult donors, overcoming the practical and ethical considerations associated with the clinical transla

tion of ESCs. Like ESCs, IPSCs can be stably propagated in cul ture, are amenable to transgenesis, gene editing, and clonal sion, and can be banked for future use, circumve

any cell type found in the body, presenting vast oppor tunities for the development of cell based therapeutics Embryonic stem cells (ESCs) derived from surplus early stage The CRISPR Journal Volume 4, Number 1, 2021 © Mary Ann Liebert, Inc DOI: 10.1089/crispr.2020.0035

CRISPR Journal

#### RESEARCH ARTICLE

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

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

#### Abstrac

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

#### Introduction

eral technical challenges inherent to the genetic manipulation of differentiated cells in ex vivo culture or intact tissues. Advan ces in the fields of developmental biology and bioengineeri have refined capabilities to control the differentiation of iPSCs Horizon Discourse a PerkinUmer Company, Cambridge, United Ringdom orrespondence to: Robert Blassberg, Horizon Discovery, a PerkinDimer Compa

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homology-directed repair (HDR), which is low in nondi- is to engineer the guide RNA (gRNA) component of the editing without the need for HDR.

DSBs or HDR activity, and has been successfully applied Gene editing technologies mediated by CRISPR systems to models from animals to crops, 8.9 highlighting its versaprovide powerful tools for biotechnology and biomedical tility and biotechnological and biomedical potential. A research in general.1-3 However, conventional CRISPR variation to direct fusion of a deaminase to dCas9/ technologies rely on the generation of DNA double- nCas9 has been published, where an effector deaminase strand break (DSBs) at target sites, which may have is recruited to dCas9/nCas9 through the use of proteinoncogenic liability. Moreover, for a precision repair, con-protein interaction domains, ventional CRISPR technologies requires the activation of An alternative to fusing an effector deaminase to Cas9

viding somatic cells.45 Thus, there is a need for the de- CRISPR-Cas9 complex as an anchor for recruitment. In velopment of new gene editing systems that limit the this approach, the gRNA is engineered to include an introduction of DNA DSBs and facilitate precise gene RNA aptamer, which interacts with its cognate ligand fused to an effector protein. This mode of recruitment To overcome the limitations associated with the re- has been utilized for transcriptional regulation,<sup>11,12</sup> the laquirement of DSB and HDR posed by conventional beling of genomic loci,<sup>13,14</sup> and targeted hypermuta-CRISPR-Cas systems, an elegant gene editing method tion.<sup>13,16</sup> The separation of the DNA recognition module called base editing (BE) was developed.<sup>6</sup> BE harnesses from the effector module and the use of RNA aptamer the DNA targeting ability of a nuclease defective Cas9 for effector recruitment has potential advantages, such as (dCas9 or nCas9), combined with the DNA editing activ- allowing convenient reconfiguration of the system by the ity of the cytidine deaminases APOBEC-1.6 By directly mix and match of individual components and simultaneous fusing the deaminase effector to the nuclease deficient recruitment of different effectors to different target sites. Cas9 protein, these tools introduce targeted point muta-Here, we report the engineering and optimization of an tions in genomic DNA<sup>6</sup> or RNA<sup>7</sup> without requiring RNA aptamer-mediated BE system named Pin-point™

Department of Pharmacology, Robert Wood Johnson Medical School, Piscataway, New Jensey, USA; <sup>2</sup>Department of Genetics, Rutgers, The State University of New Jensey-iscataway, New Jersey, USA; and <sup>3</sup>Horizon Discovery, Cambridge, United Kingdom.

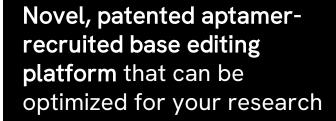
\*Address correspondence to: Shengkan Jin, PhD, Department of Pharmacology, Robert Wood Johnson Medical School, 675 Hoes Lane West, Piscataway, NJ 08854-5635, USA Email: victor i

www.ncbi.nlm.nih.gov/pmc/articles/PMC7898459/pdf/cris pr.2020.0035.pdf

### Why choose the Pin-point<sup>™</sup> system?







Modular, tunable system allows you to reach your targets of interest

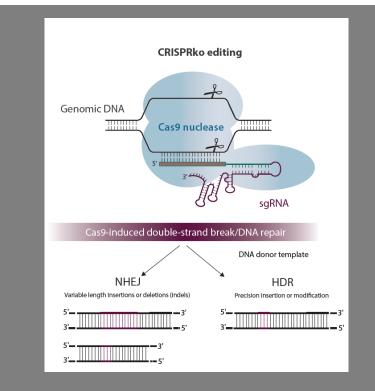


**Exemplary safety profile** with no unintended impact on cell viability or functionality

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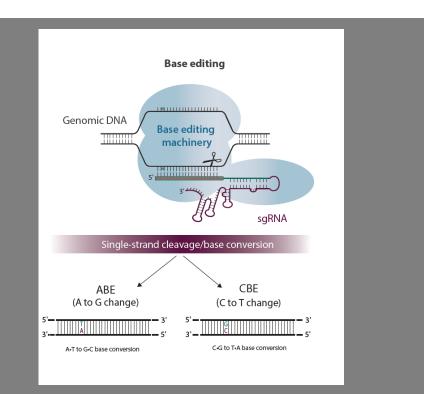
### **CRISPR** gene editing

### **Base editing**



#### GENE DISRUPTION BY A DSDNA BREAK

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



#### GENE MODIFICATION BY POINT MUTATIONS

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

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## The gene editing evolution is now

#### **1<sup>st</sup> generation Cas enzymes** Gene disruption by a dsDNA break

#### 2<sup>nd</sup> generation base editing

Gene modification by point mutation

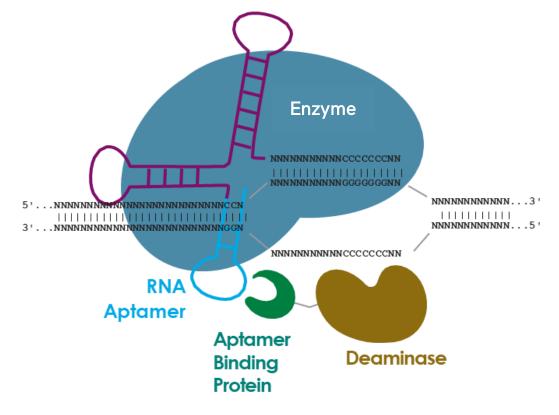
- creation of stop codons or splice site disruption for knockout
- not reliant on dsDNA break
- introduction of single base conversion

## New generation Pin-point™ base editing system

- Predictable, precise and efficient single and multi-gene editing
- Simultaneous knock-in and knockout in a single reaction
- Modular control over target and editing window to specifically reach your gene of interest

#### What is the Pin-point<sup>™</sup> system?

Novel | patented | aptamer-recruited base editing platform



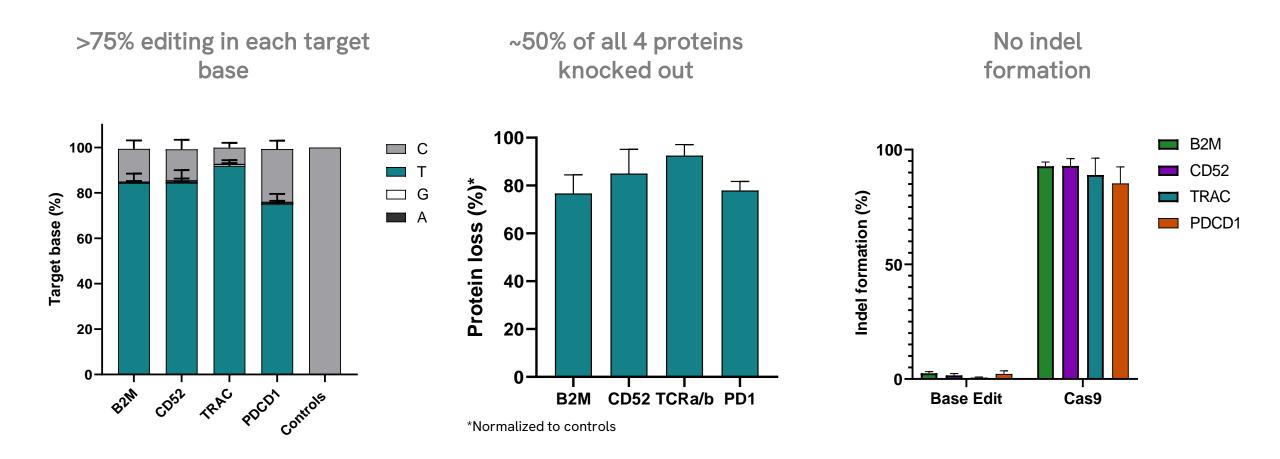
#### 3 component system

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

#### **Advantages**

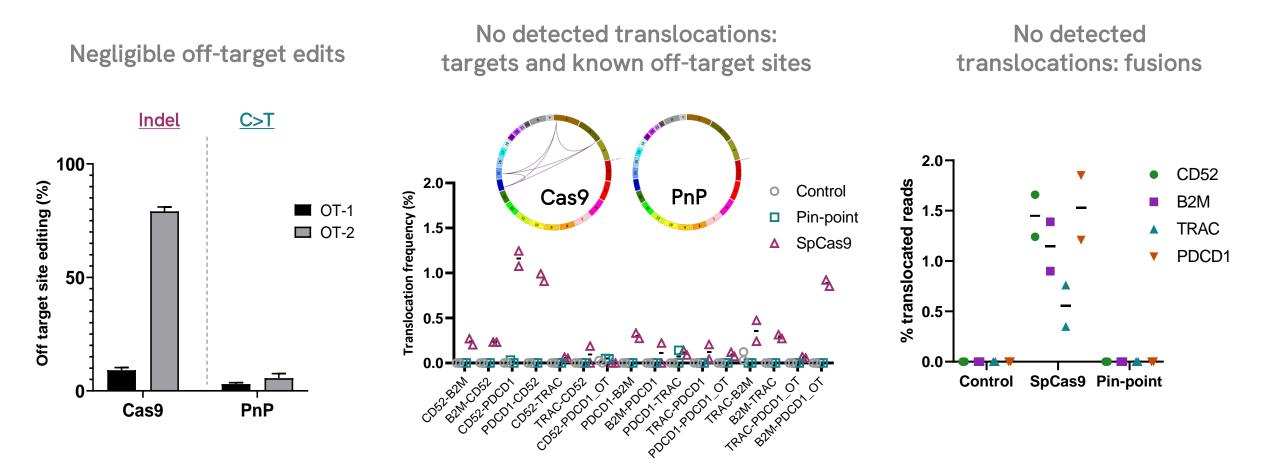
- Multiplex gene editing including knock-in and knockout with high efficiency and safety and no impact on cellular health
- Validated performance in T cells and iPSCs
- Completely mix-and-match for target specificity and efficiency

### Highly efficient and precise multiplex T cell editing



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

#### Strong safety profile in T cells



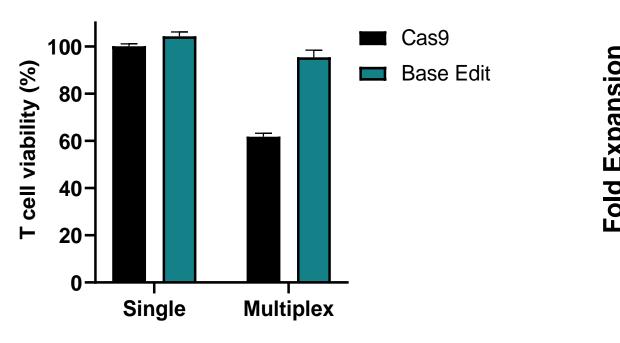
A cleaner and safer approach to multiplex gene editing in T cells



#### No impact on T cell health

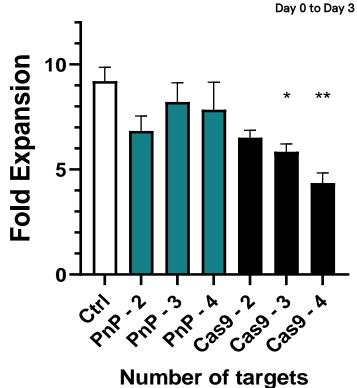
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Cell viability maintained



High multiplexing does not compromise cellular health or yield

#### Rate of cell expansion unaffected



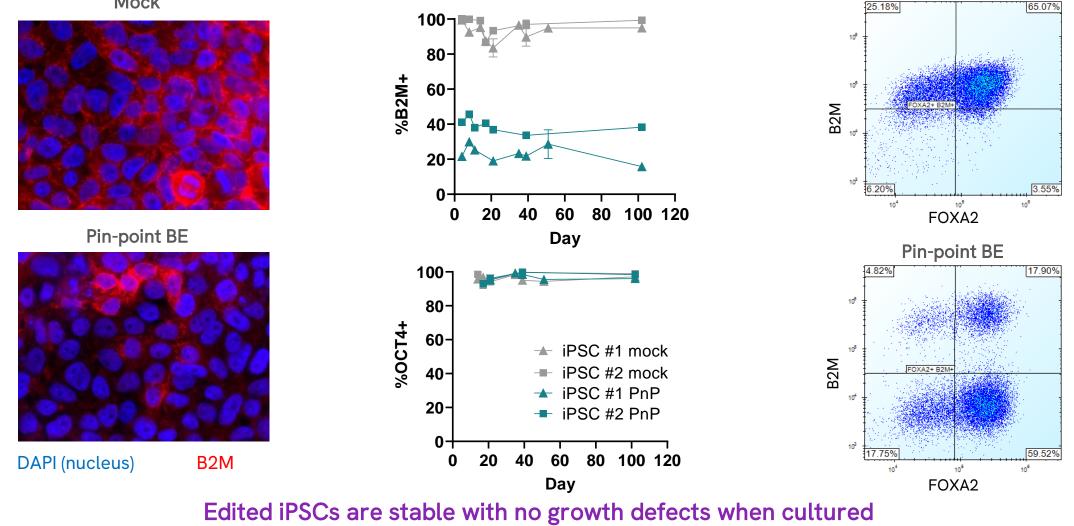
Biological replicates = 3 | Data normalized to controls

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#### Base editing with a Pin-point<sup>™</sup> system in iPSCs

Mock

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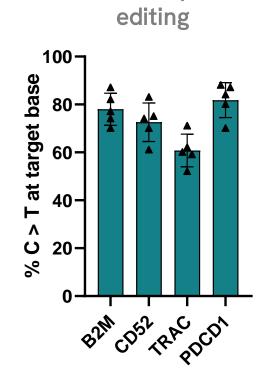
Mock

**-** 12 V

up to 100 days and retain differentiation potential

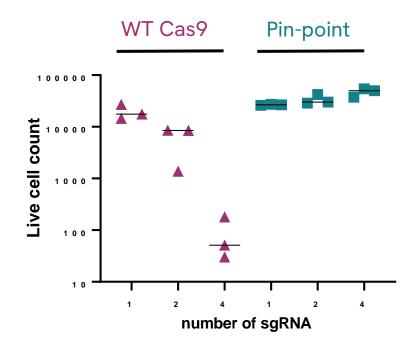
#### Multi-gene editing in iPSCs

Effective multiplex base

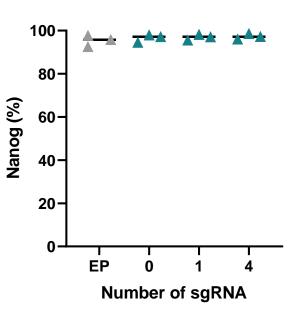


High base editing efficiency at target loci in a multiplex setting

Edited cells are viable



High survival of multi-edited iPSCs with a Pin-point system Edited cells retain their pluripotency



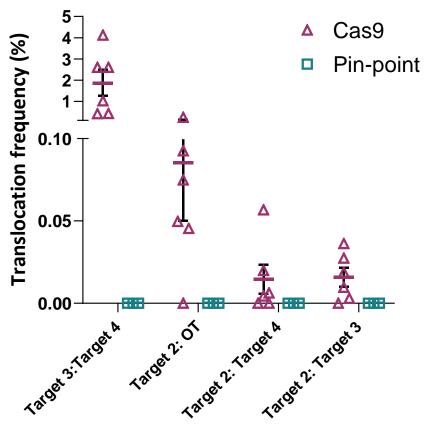
Pluripotency is retained in iPSCs edited with a Pin-point system

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#### Strong safety profile in iPSCs

Target 2 Targett Off-target 5 **4** Translocation frequency (%) 3 2 **0.10** T chr9 0.05 Target 3 0.00 Target 4 in-silico predicted translocations previously validated in T cells

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

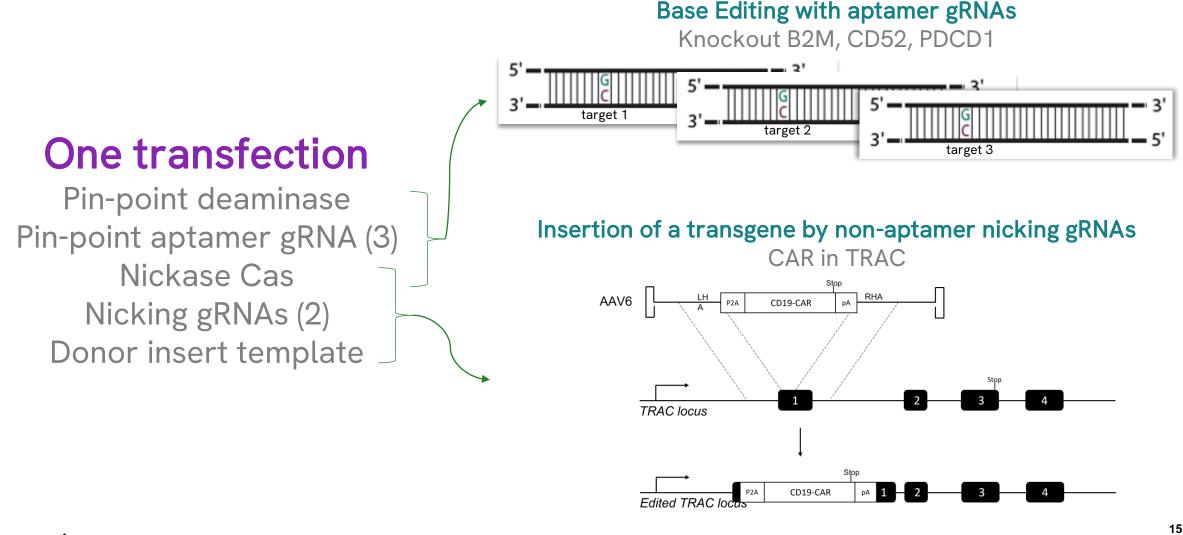


A cleaner and safer approach to multiplex gene editing in iPSCs

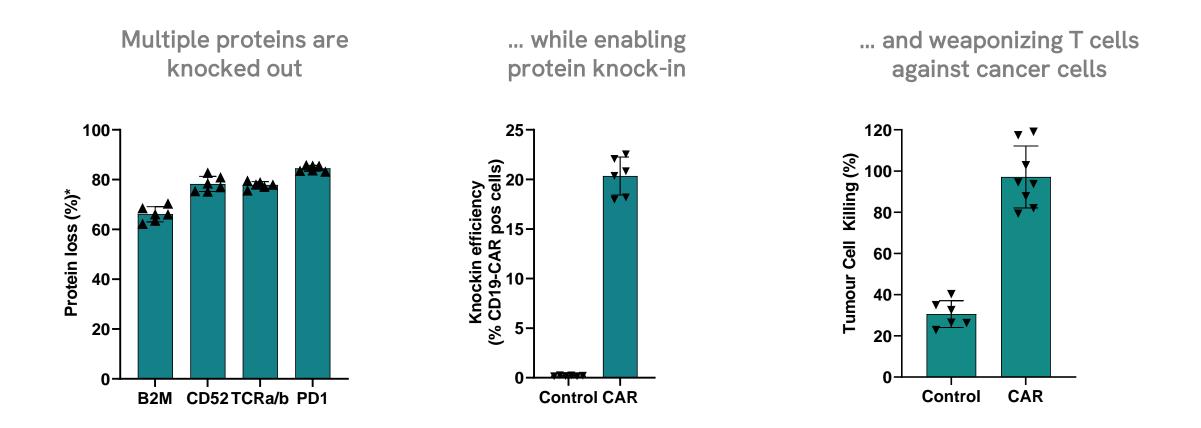


## A solution for complex engineering

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



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

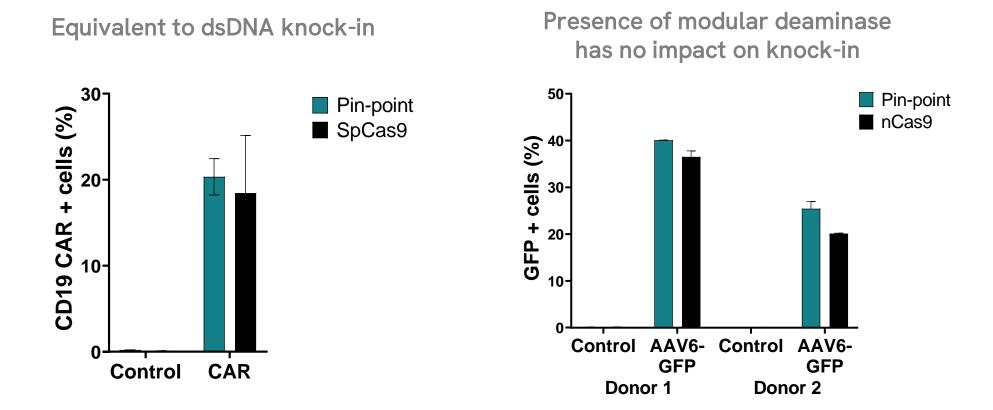


## The Pin-point platform is efficient and accurate for concurrent transgene insertion and multiplex base editing



\*Normalized to controls

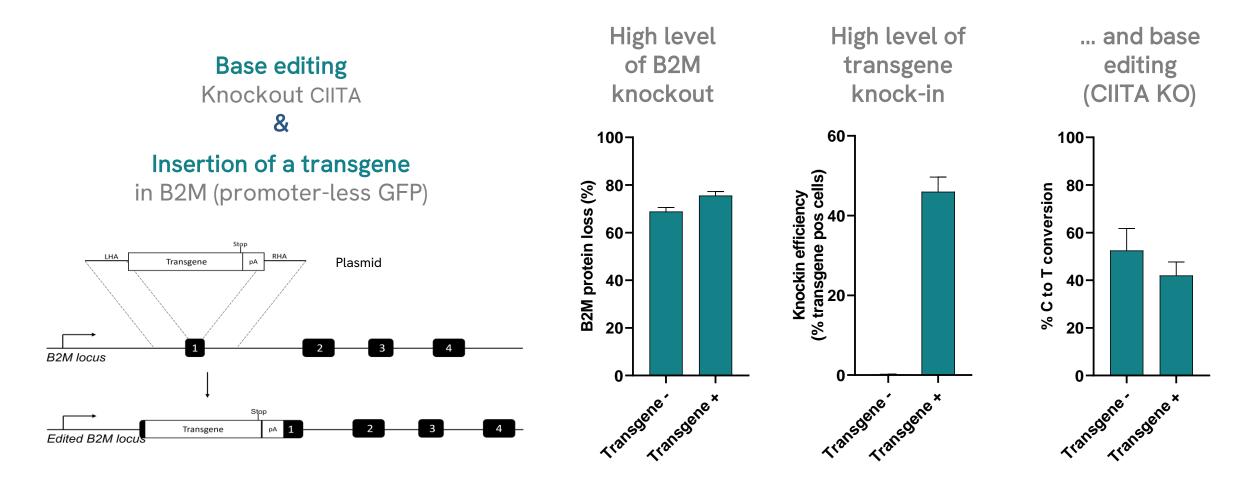
#### No loss of efficiency in payload deliveries



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



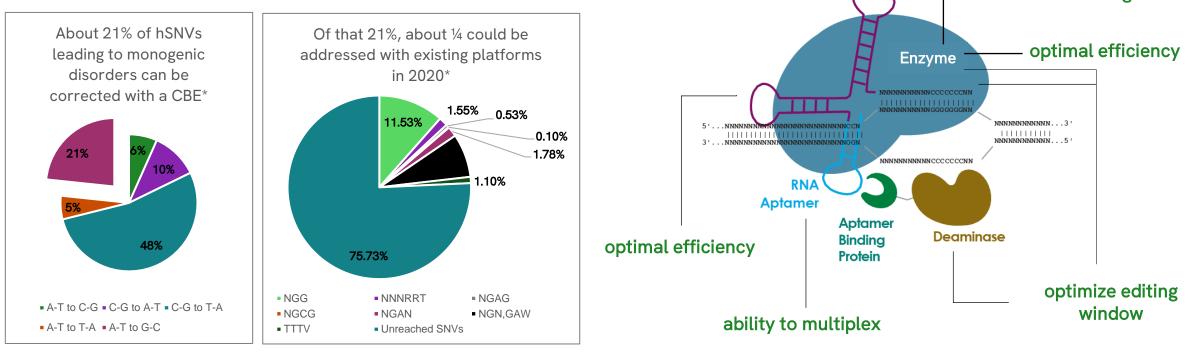
## Demonstrated simultaneous knock-in and multiple knockout in iPSCs



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

#### Choose components for locus-specific optimization

#### Most pathogenic SNVs with potential CBE correction are not reachable with published systems\*



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



reach more targets

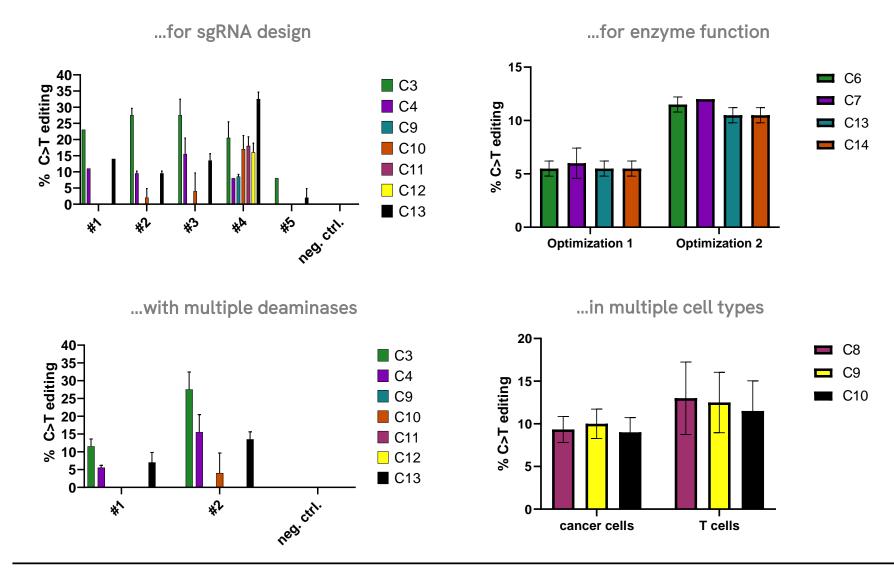
### A benefit of modularity of the Pin-point<sup>™</sup> platform Demonstrated compatibility with numerous nucleases

	Туре II			Туре V						
	Α	В	С	D	Е	F	G	Н	I	J
Enzyme activity	nickase	nickase	nickase	deactivated	deactivated	deactivated	deactivated	deactivated	deactivated	deactivated
Demonstrated activity in mammalian cells	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
Demonstrated with the Pin-point system	$\checkmark$	$\checkmark$	In progress	$\checkmark$	ln progress	$\checkmark$	ln progress	$\checkmark$	$\checkmark$	In progress
sgRNA optimized	$\checkmark$	In progress		In progress		$\checkmark$		$\checkmark$	$\checkmark$	
Enzyme optimized	$\checkmark$					$\checkmark$				
Confirmed at multiple targets (2+)	$\checkmark$	In progress				$\checkmark$		$\checkmark$	$\checkmark$	
Demonstrated in multiple cell types (2+)	$\checkmark$	In progress				$\checkmark$		$\checkmark$		
Demonstrated with multiple deaminases (2+)	$\checkmark$							$\checkmark$	$\checkmark$	

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

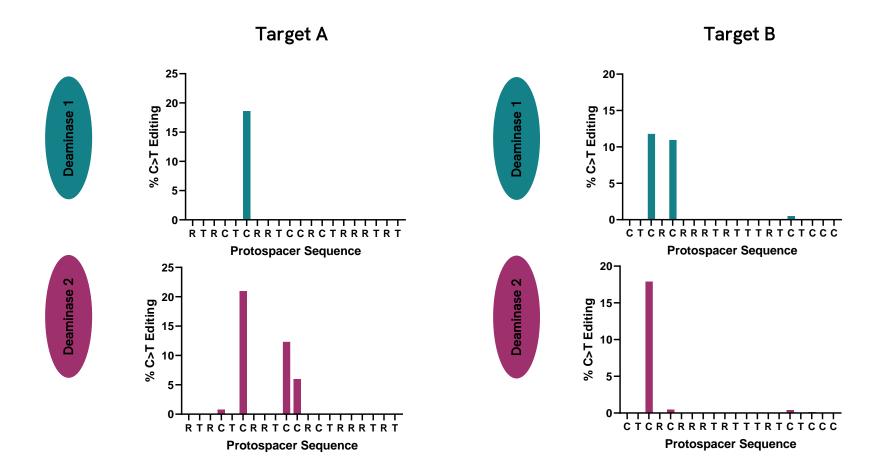
#### Optimization using the modularity of the Pin-point platform

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% C>T editing examples shown are in non-Cas9 alternative configurations of the Pin-point platform

#### Optimize synthetic guide RNAs and deaminase pairings



The Pin-point platform enables optimization of the editing window by selecting the best guide RNA and deaminase pairs



The Pin-point<sup>™</sup> system is a transformational nextgeneration gene editing technology



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



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

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Improved safety compared to standard CRISPR-Cas9 systems



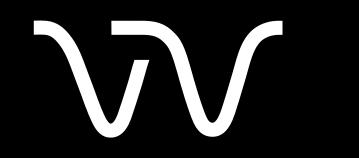


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24



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