CHROMA MEDICINE

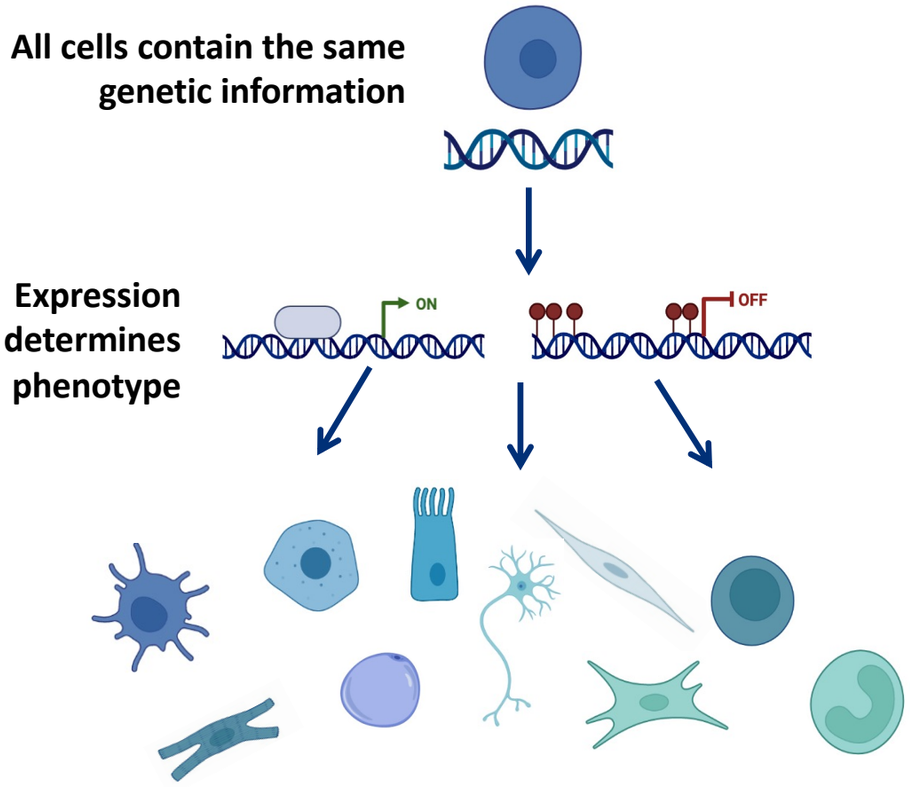
Therapeutic Development of Epigenetic Editors

Morgan Maeder, PhD

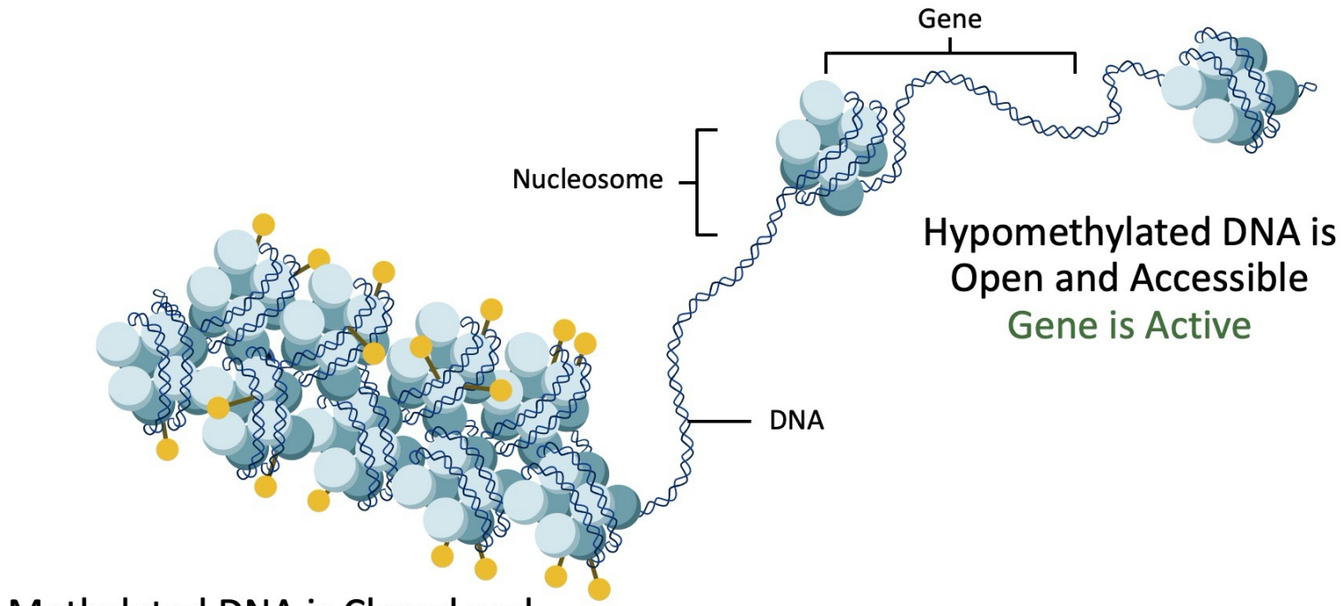
Senior Director, Payload Sciences

Epigenetics regulates gene expression to determine cell identity and function

Regulation of gene expression determines cell identity and function



Chromatin packaging and epigenetics regulates DNA transcription



Hypomethylated DNA is Open and Accessible
Gene is Active

Methylated DNA is Closed and Inaccessible
Gene is Inactive

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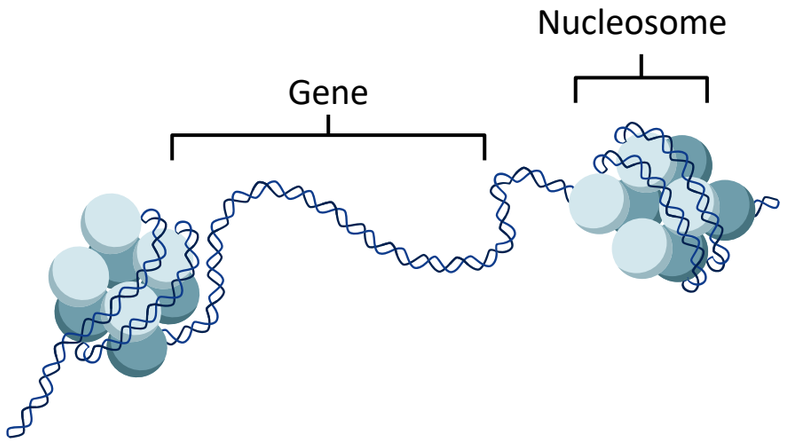
Epigenetic editing leverages the endogenous system to precisely control gene expression

Durable change in phenotype without a change in genotype



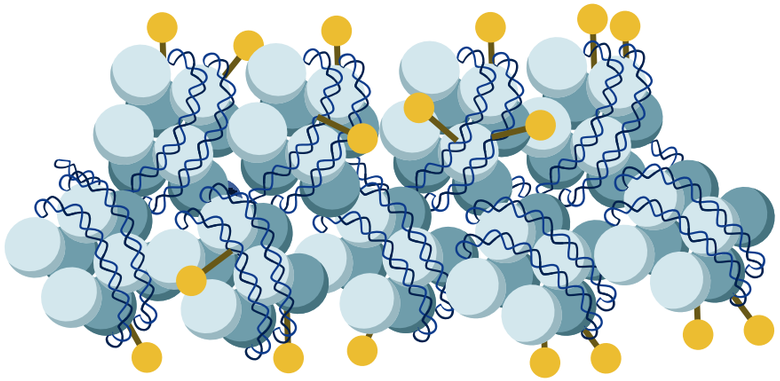
Gene is Active
DNA is Open and Accessible

Epigenetic Repressor
Methylates Targets

Transient Application

Epigenetic Activator
Demethylates Targets

Gene is Inactive
DNA is Closed and Inaccessible



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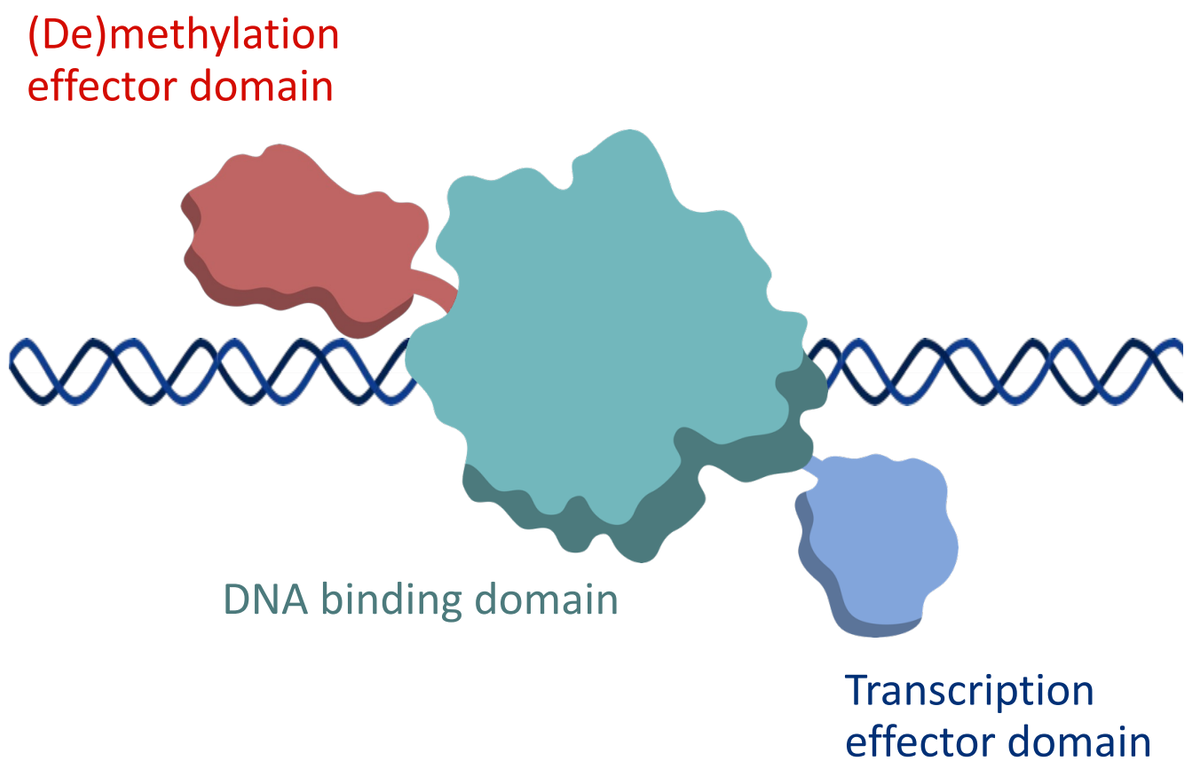
Chroma’s epigenetic editors are designed to be modular and versatile

Chroma’s Epigenetic Editors



- **DNA binding domain** precisely localizes effector domains to target sequence
- **Transcription effector domain** transiently represses or activates target gene
- **Methylation / Demethylation effector domain** durably silences / activates target gene

Nuñez JK, Chen J, Pommier GC, et al. Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. *Cell*. 2021;184(9):2503-2519.e17.
Amabile A, Migliara A, Capasso P, et al. Inheritable silencing of endogenous genes by hit-and-run targeted epigenetic editing. *Cell*. 2016;167(1):219-232.e14.

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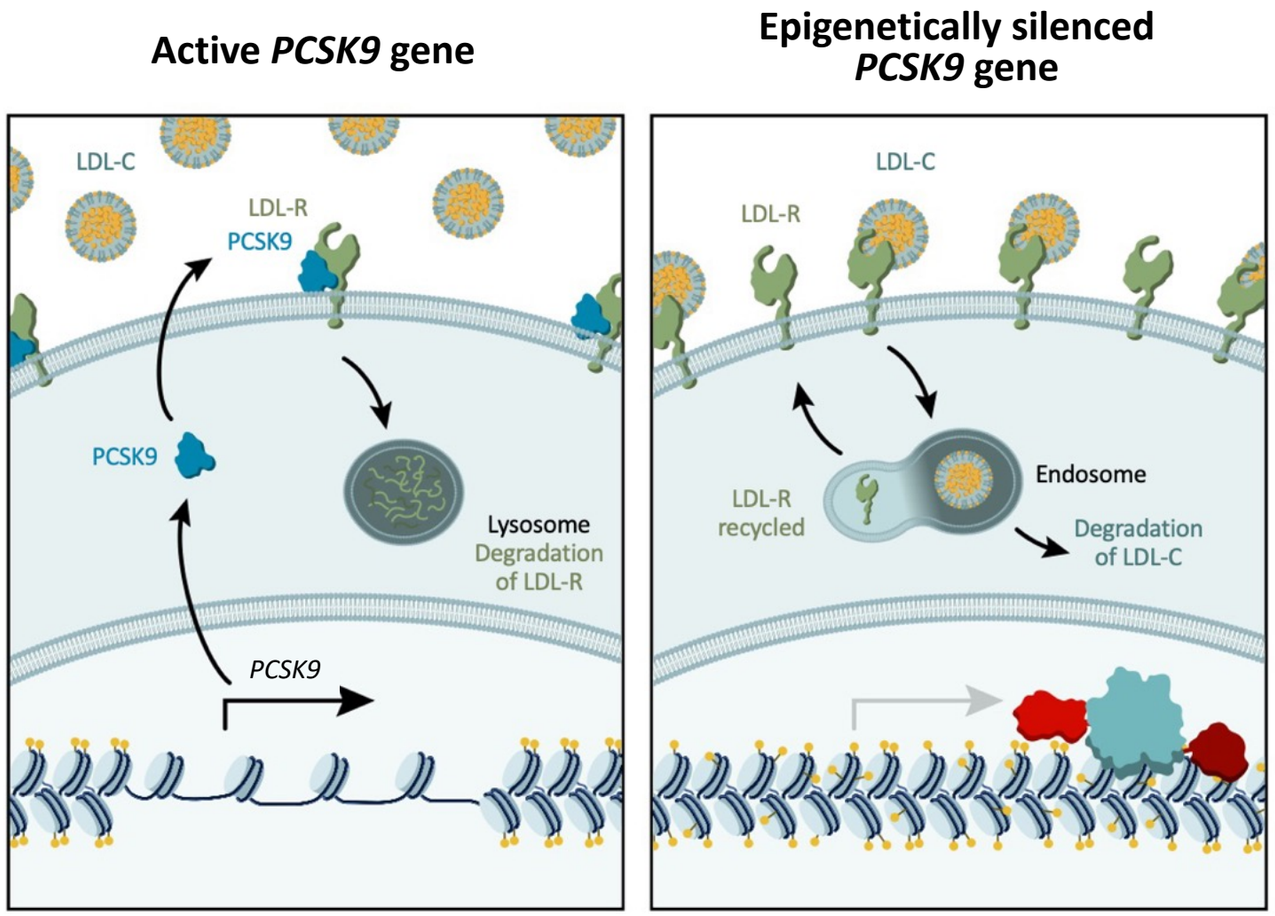
Epigenetic editor targeting PCSK9 to lower LDL-C



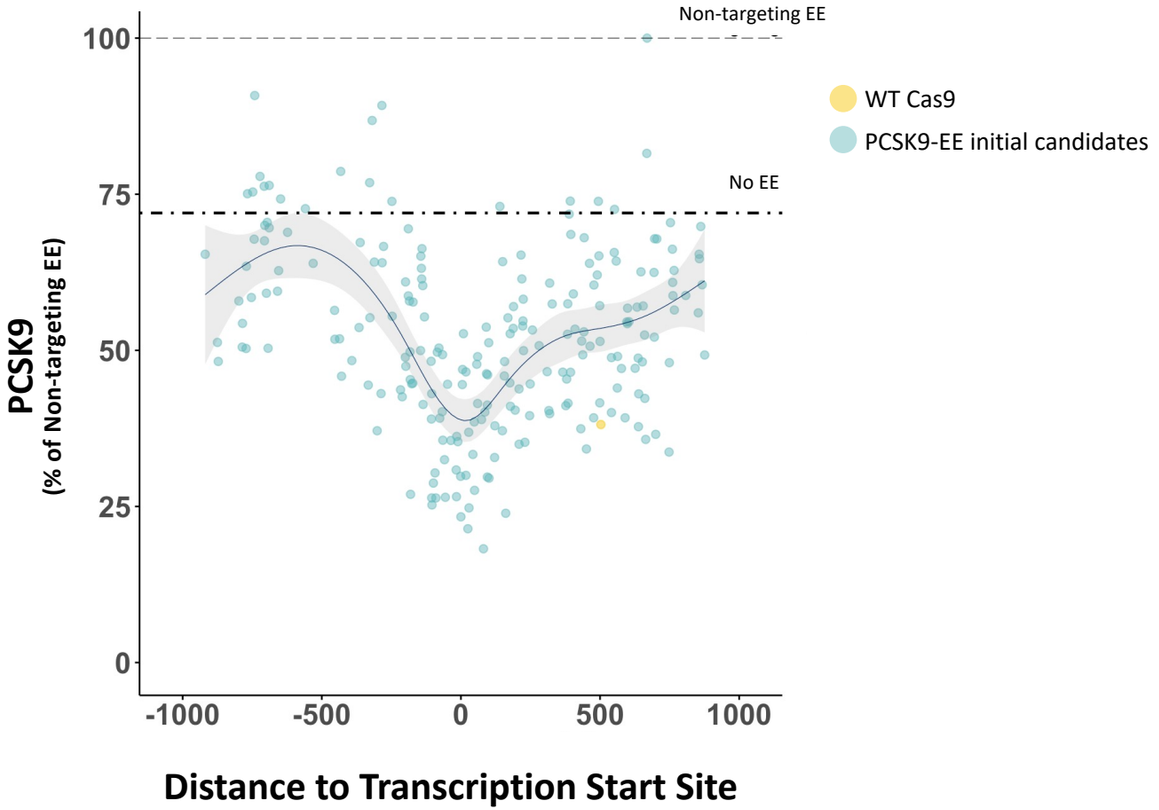
Epigenetic editor is designed to:

- Silence *PCSK9* through DNA methylation
- Prevent degradation of LDL-R, which increases removal of LDL-C from blood
- Not cause undesirable genomic consequences from cutting or nicking the DNA
- Be durable for lifetime of the patient

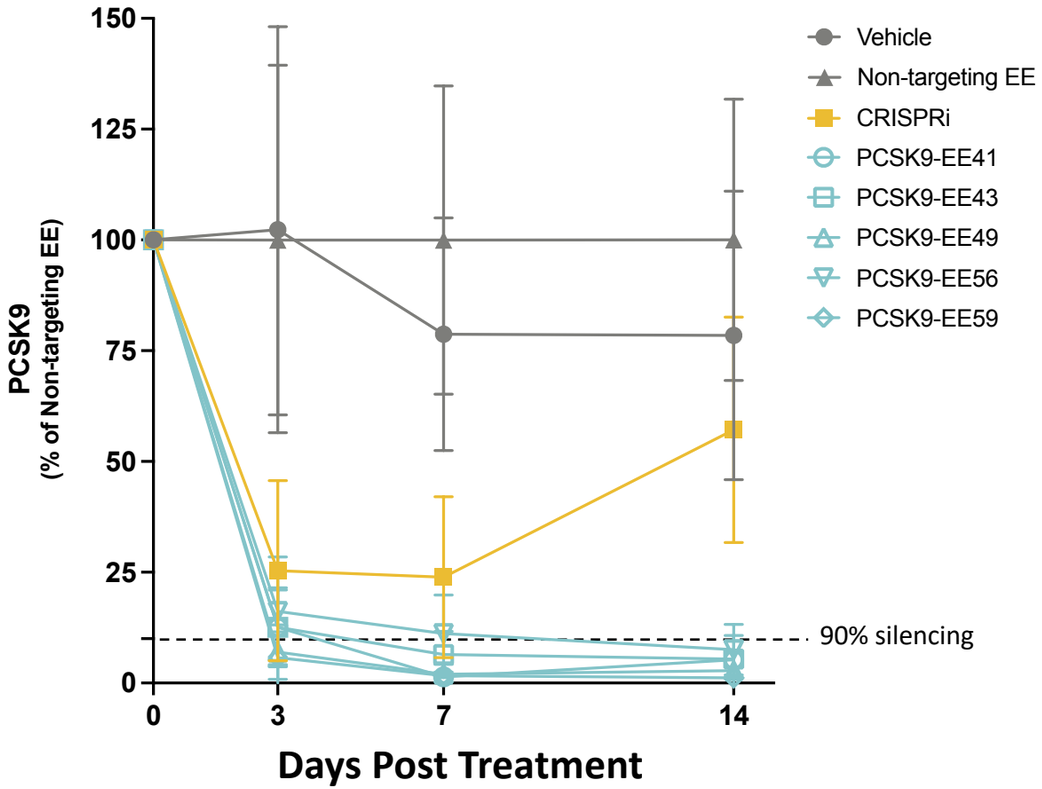
LDL-C = low-density lipoprotein cholesterol; LDL-R = low-density lipoprotein receptor; PCSK9 = proprotein convertase subtilisin/kexin type 9.

PCSK9-EE screen identified hits with robust activity in primary human hepatocytes (PHH)

PCSK9-EE Screen in Immortalized Liver Cells



PCSK9-EE Hit Confirmation in PHH



Tremblay F, Kelly K, Shah S, A single administration of an epigenetic editor targeting human *PCSK9* robustly and durably lowers cholesterol in vivo, AHA 2023
EE = epigenetic editor; PHH = primary human hepatocyte; WT = wild type; error bars represent standard deviation.

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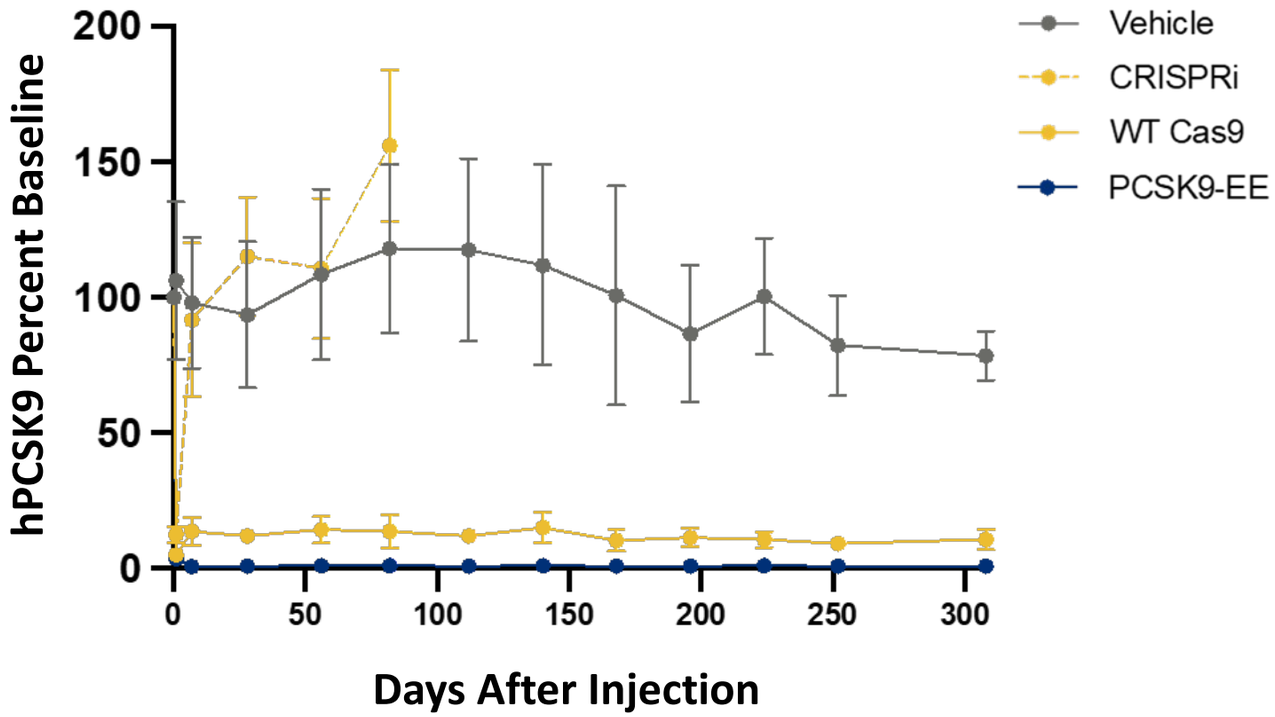
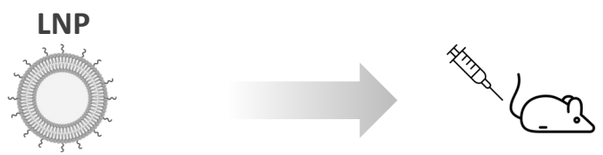
Near-complete *PCSK9* silencing achieved in transgenic mice

- Transgenic mouse containing the human *PCSK9* locus
- Tested optimized single construct epigenetic editor
- **>98% silencing maintained for over 300 days post single IV injection**





Experiment

- hPCSK9 Tg mouse
- Single IV administration
- PCSK9 analysis by ELISA

Tremblay F, Kelly K, Shah S, A single administration of an epigenetic editor targeting human *PCSK9* robustly and durably lowers cholesterol in vivo, AHA 2023

EE = epigenetic editor; ELISA = enzyme-linked immunosorbent assay; IV = intravenous; hPCSK9 = human proprotein convertase subtilisin/kexin type 9; LNP = lipid nanoparticles; Tg = transgenic; WT = wild type; error bars represent standard deviation.

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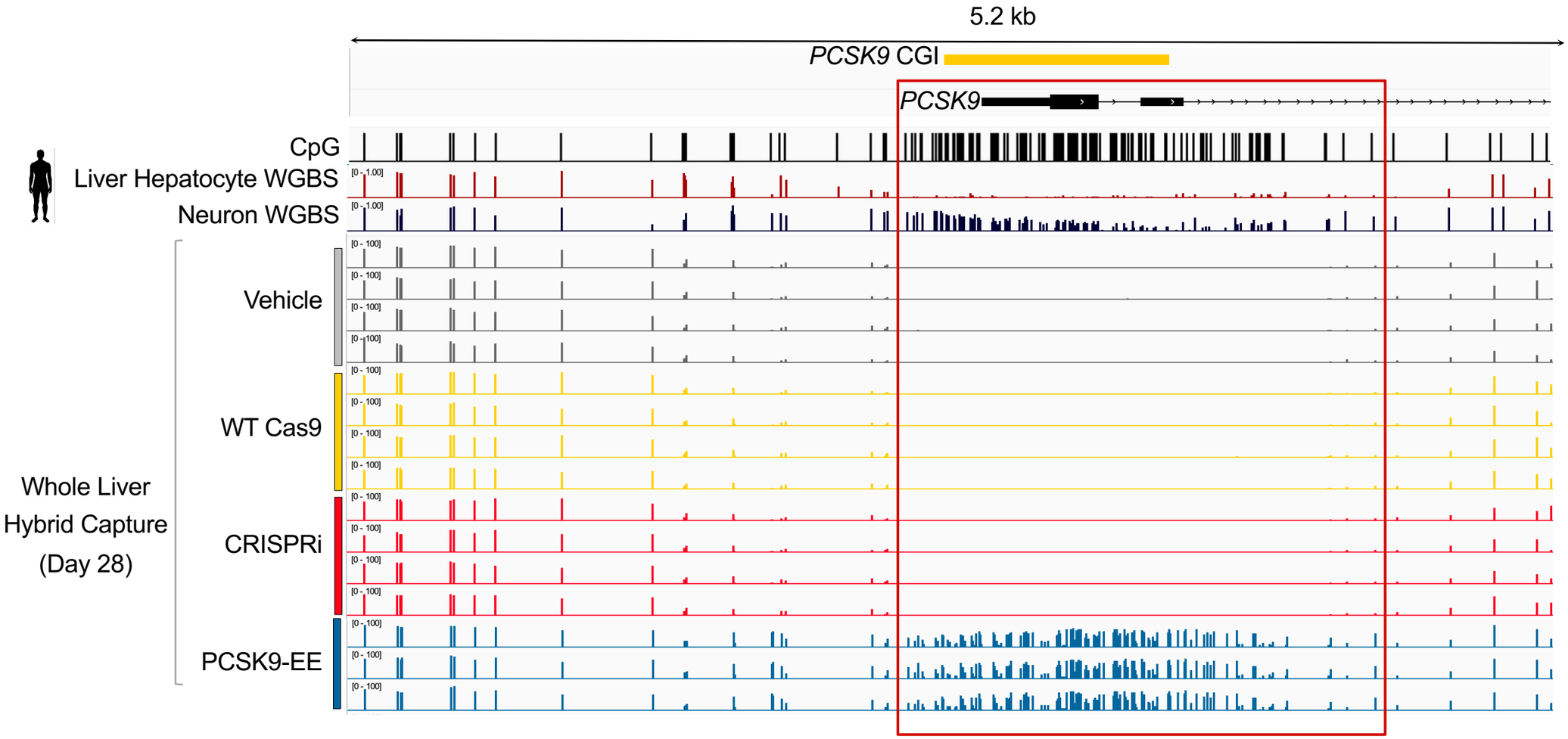
PCSK9-EE induced durable, targeted CpG methylation at the human *PCSK9* locus in vivo



Tremblay F, Kelly K, Shah S, A single administration of an epigenetic editor targeting human *PCSK9* robustly and durably lowers cholesterol in vivo, AHA 2023
EE = epigenetic editor; WT = wild type.

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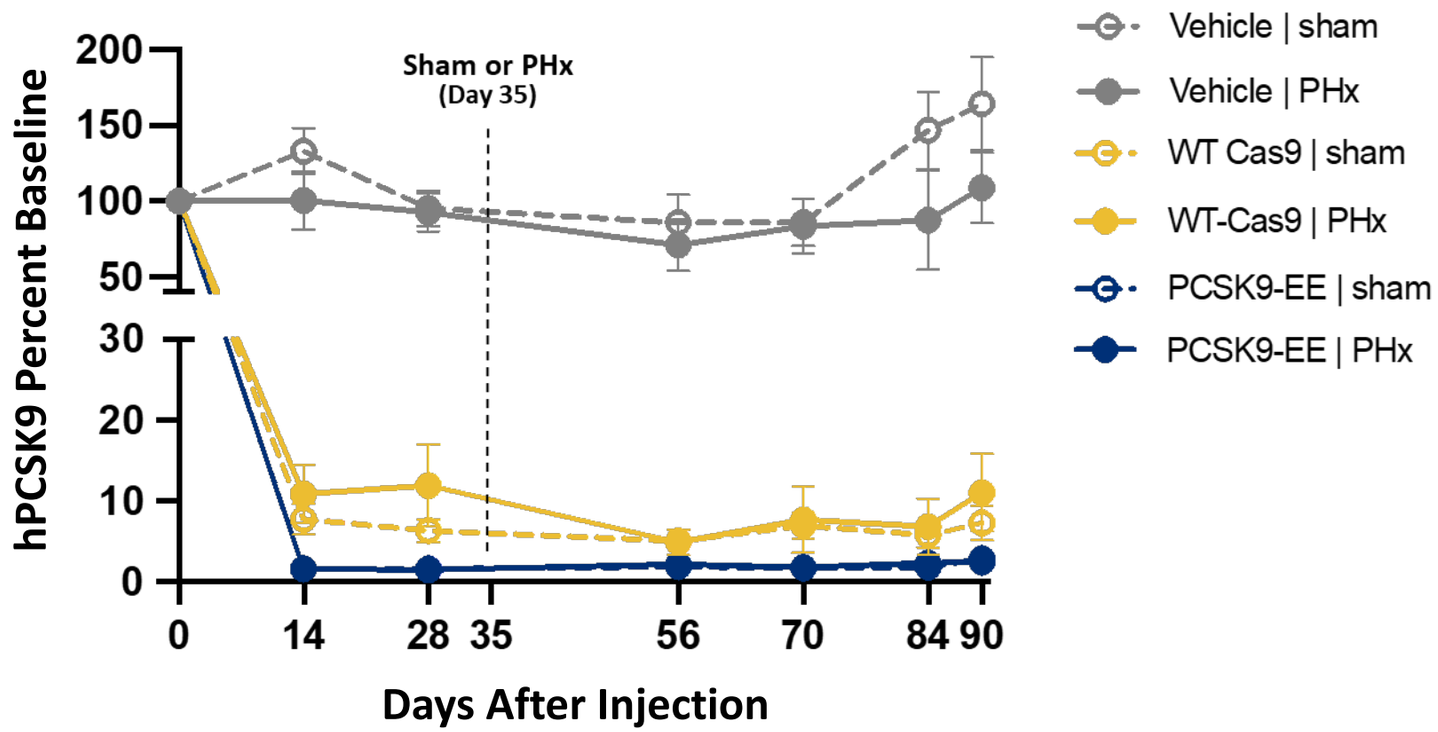
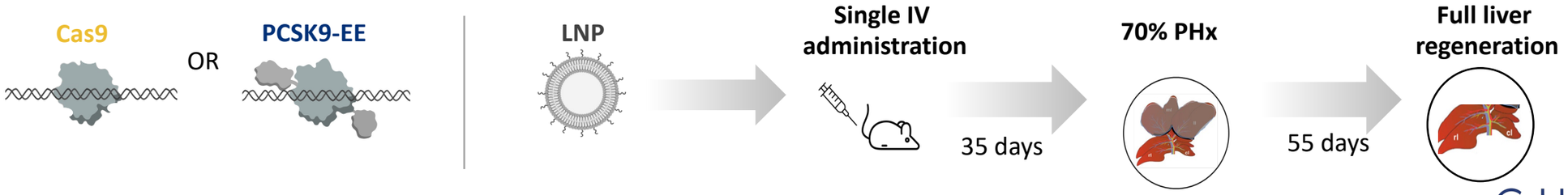
PCSK9 silencing was maintained through liver regeneration

- 70% partial hepatectomy (PHx) is a gold standard surgical model to induce liver regeneration in rodents
- **Single administration of PCSK9-EE demonstrated durable PCSK9 silencing pre- and post- partial hepatectomy**





Tremblay F, Kelly K, Shah S, A single administration of an epigenetic editor targeting human *PCSK9* robustly and durably lowers cholesterol in vivo, AHA 2023

EE = epigenetic editor; IV = intravenous; hPCSK9 = human proprotein convertase subtilisin/kexin type 9; LNP = lipid nanoparticles; PHx = partial hepatectomy; WT = wild type; error bars represent standard deviation.

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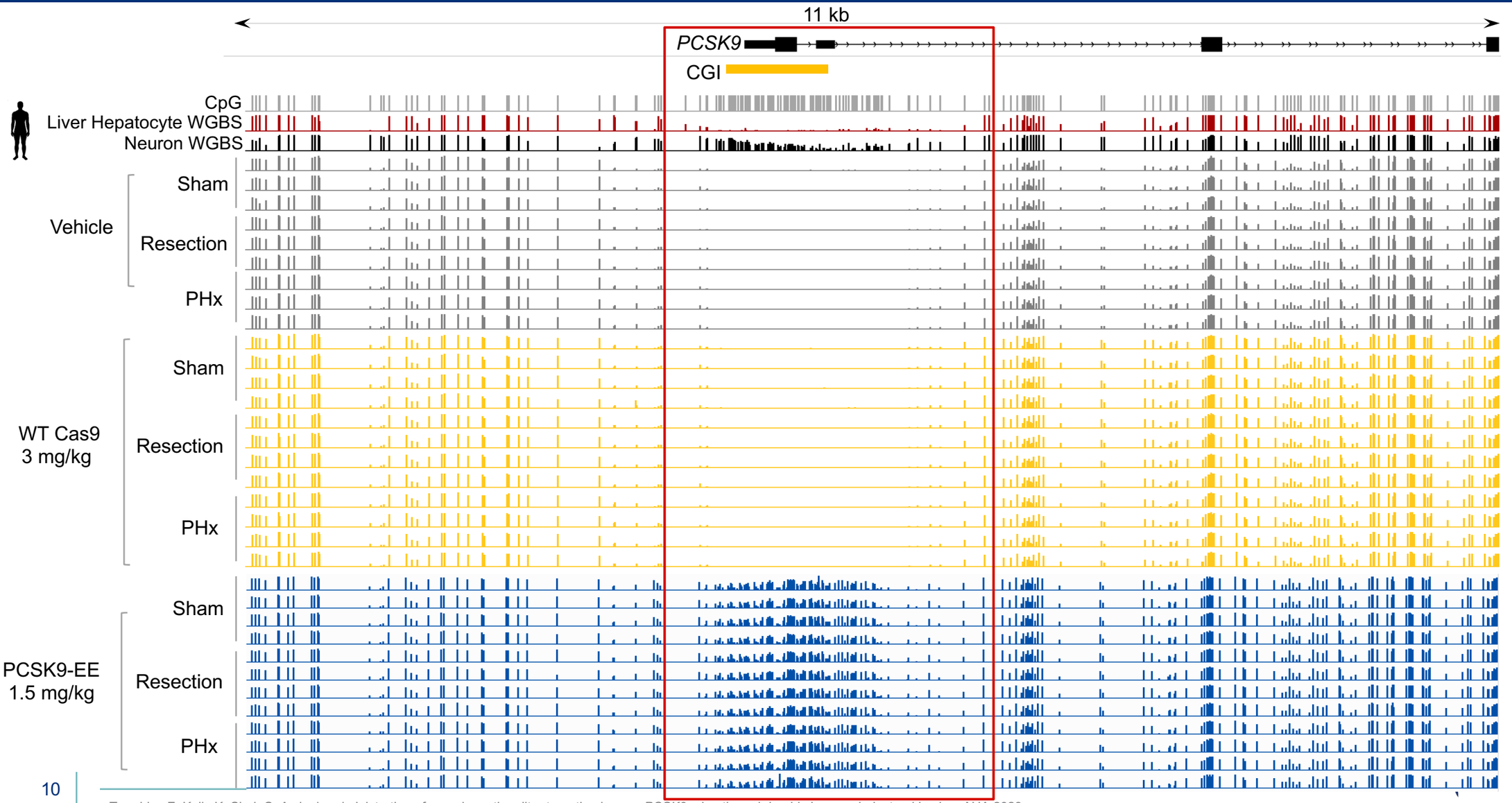
PCSK9-EEs demonstrated durable DNA methylation at *PCSK9* locus pre- and post-partial hepatectomy (PHx)

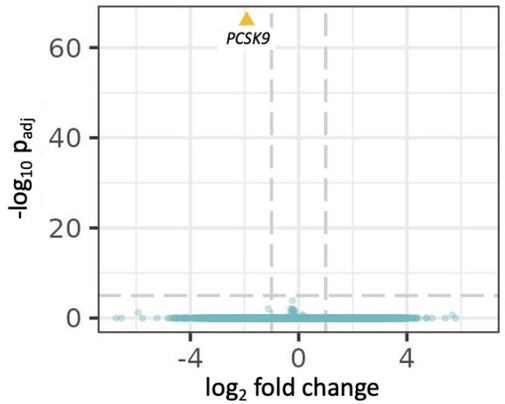


Tremblay F, Kelly K, Shah S, A single administration of an epigenetic editor targeting human *PCSK9* robustly and durably lowers cholesterol in vivo, AHA 2023

EE = epigenetic editor; WT = wild type

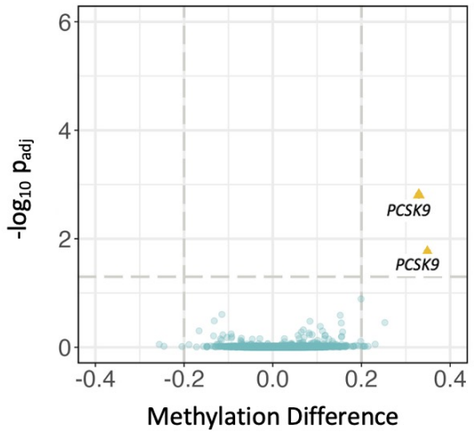
PCSK9-EEs can be highly specific with no off-target changes in expression or methylation in primary human hepatocytes

RNA Expression



Differentially Expressed Gene: PCSK9

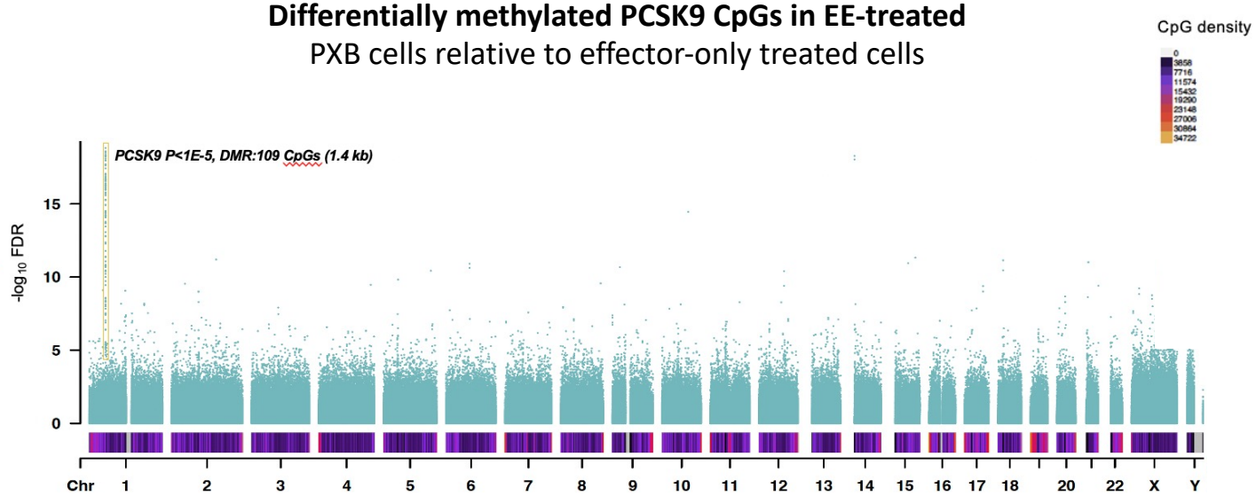
Methylation at CpG-enriched sites



Differentially Methylated Region: PCSK9

Genome-wide Methylation

Differentially methylated PCSK9 CpGs in EE-treated
PXB cells relative to effector-only treated cells



- **PCSK9-EEs can be highly specific**
- **No off-target changes in gene expression** with epigenetic repressor in primary human hepatocytes as measured by RNA-seq
- **No off-target changes in methylation** with epigenetic repressor in primary human hepatocytes as measured by Illumina Methylation Array and whole genome bisulfite sequencing

Tremblay F, Kelly K, Shah S, Development of a human PCSK9-targeting epigenetic editor with durable, near-complete in vivo silencing, ASGCT 2023;
Tremblay F, Kelly K, Shah S, A single administration of an epigenetic editor targeting human PCSK9 robustly and durably lowers cholesterol in vivo, AHA 2023

EE = epigenetic editor; PHH = primary human hepatocytes.

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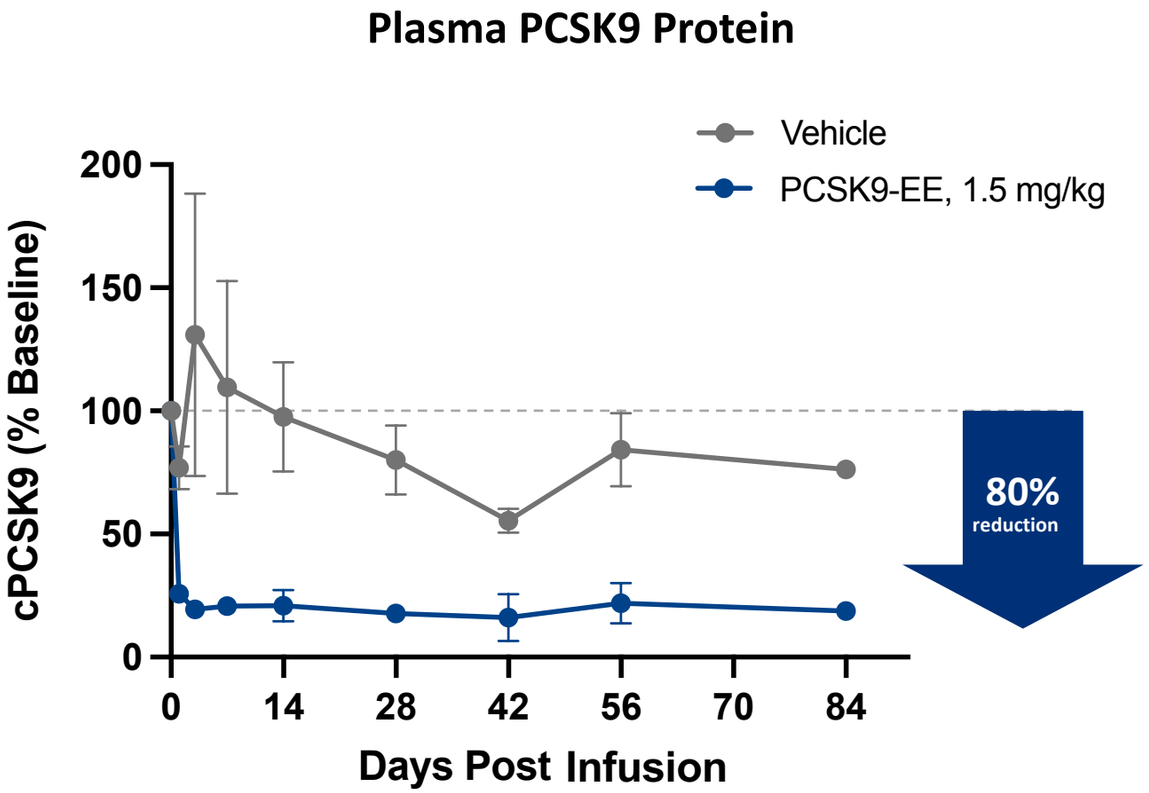
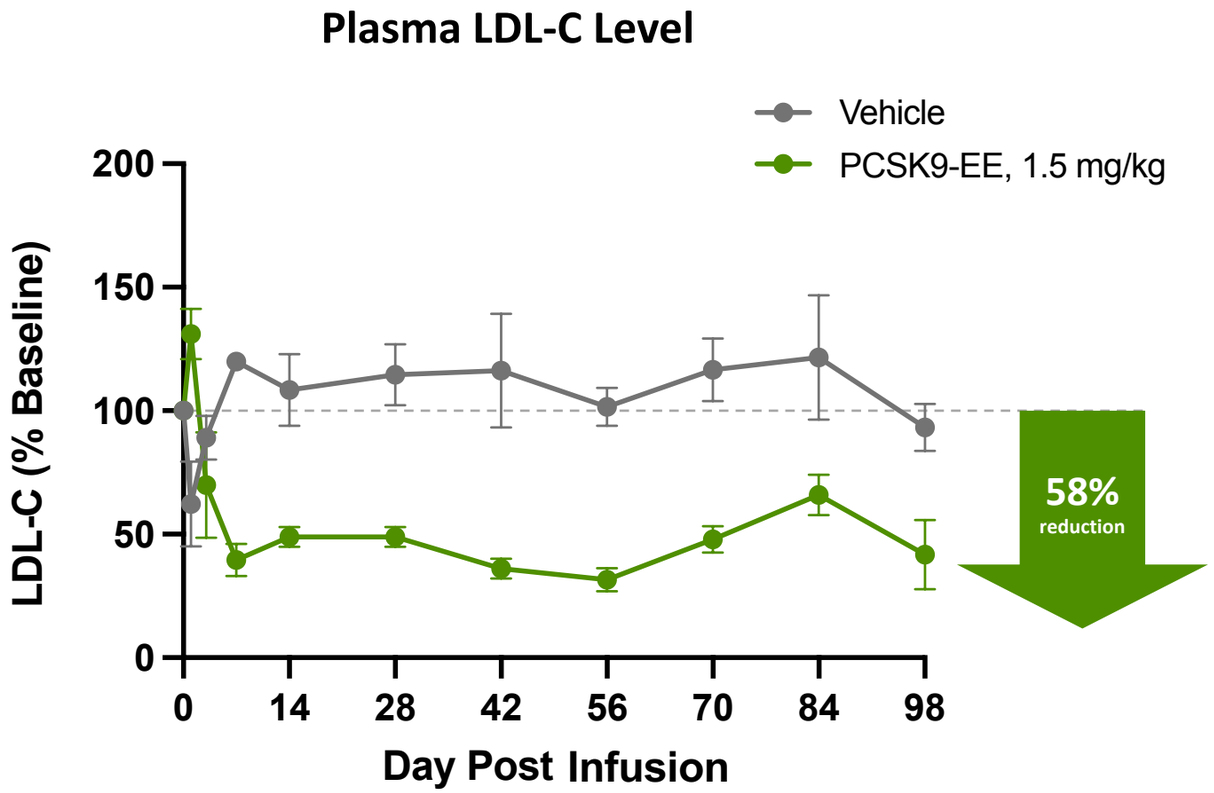
In NHPs, PCSK9-EE achieved 80% reduction in PCSK9 and 58% in LDL-C with durability out to 3 months





Tremblay F, Kelly K, Shah S, A single administration of an epigenetic editor targeting human *PCSK9* robustly and durably lowers cholesterol in vivo, AHA 2023

cPCSK9 = cyno proprotein convertase subtilisin/kexin type 9; EE = epigenetic editor; IV = intravenous; LDL-C = low-density lipoprotein cholesterol; LNP = lipid nanoparticles; NHP = non-human primates; WT = wild type; error bars represent standard deviation.

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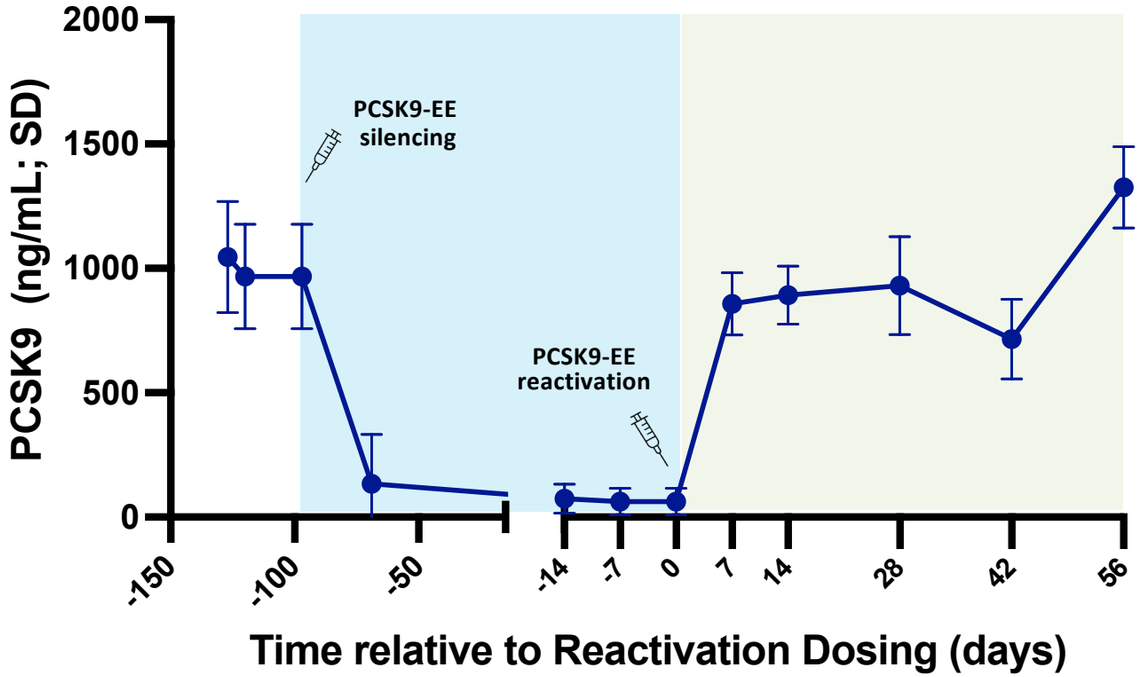
Achieved early proof-of-concept for reactivation of PCSK9 in mice

- Transgenic mouse containing the human *PCSK9* locus
- Single administration of epigenetic editor to silence PCSK9 was given 100 days prior to reactivation
- **Single administration of epigenetic activator restored PCSK9 expression by day 7 and was durable to day 56**



Tremblay F, Kelly K, Shah S, A single administration of an epigenetic editor targeting human *PCSK9* robustly and durably lowers cholesterol in vivo, AHA 2023
EE = epigenetic editor; hPCSK9 = human proprotein convertase subtilisin/kexin type 9; IV = intravenous; LNP = lipid nanoparticles; error bars represent standard deviation.

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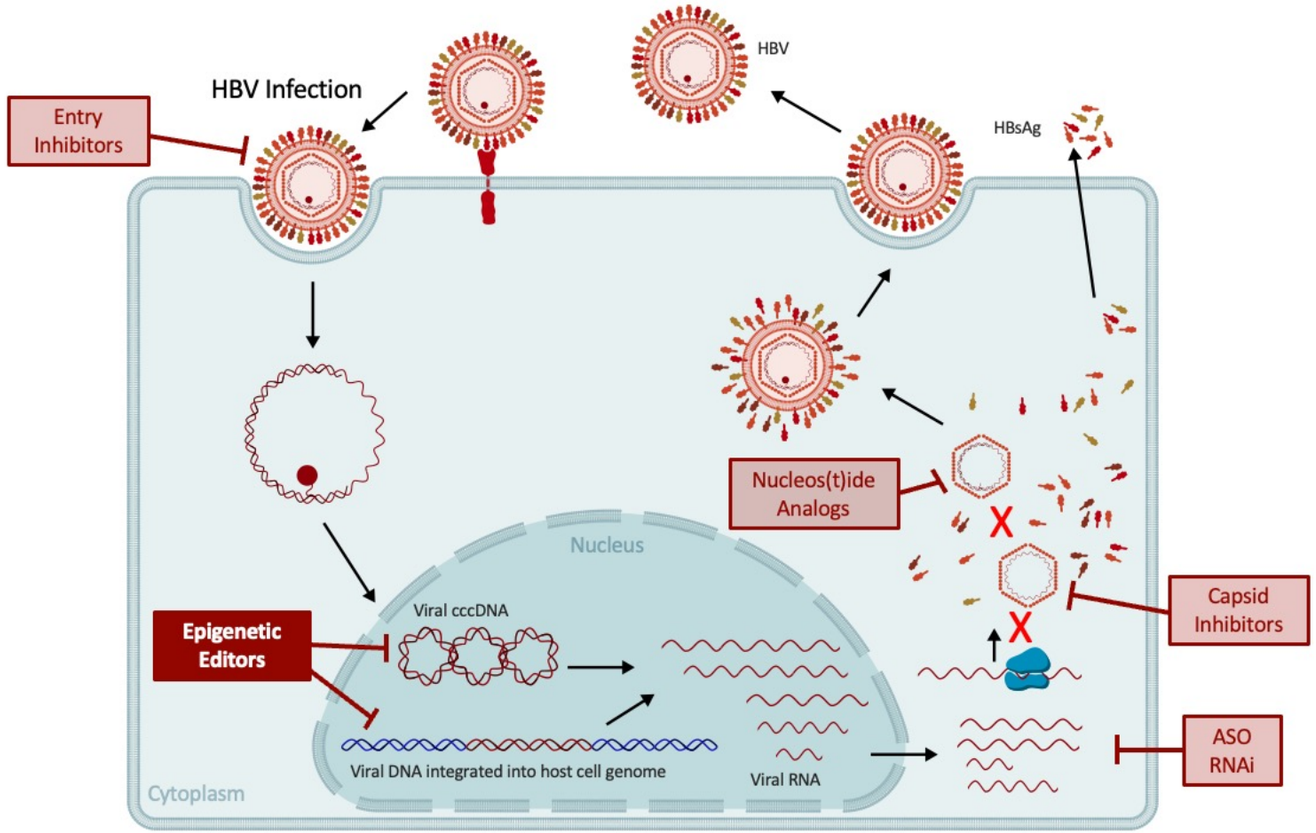
HBV epigenetic editors target both cccDNA and intDNA



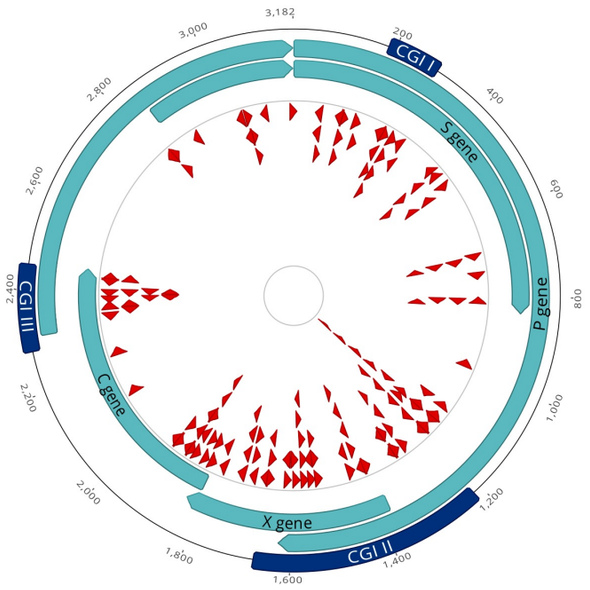
Epigenetic editors are designed to:

- Target both sources of pathogenesis, cccDNA and intDNA, in infected cells
- Prevent production of all viral transcripts and proteins
- Avoid introducing risk of increasing viral integrations
- Be durable for lifetime of the patient

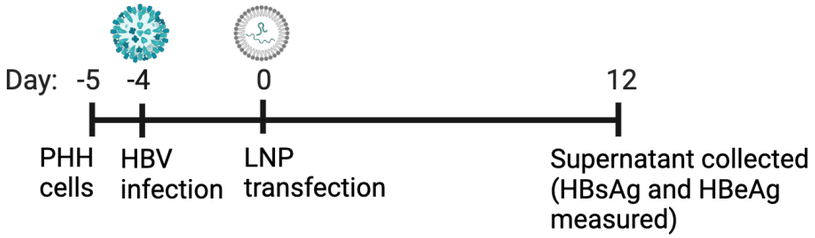
ASO = antisense oligonucleotides; cccDNA = covalently closed circular DNA; HBV = hepatitis B virus; HBsAg = hepatitis B surface antigen; intDNA = integrated DNA; RNAi = RNA interference.

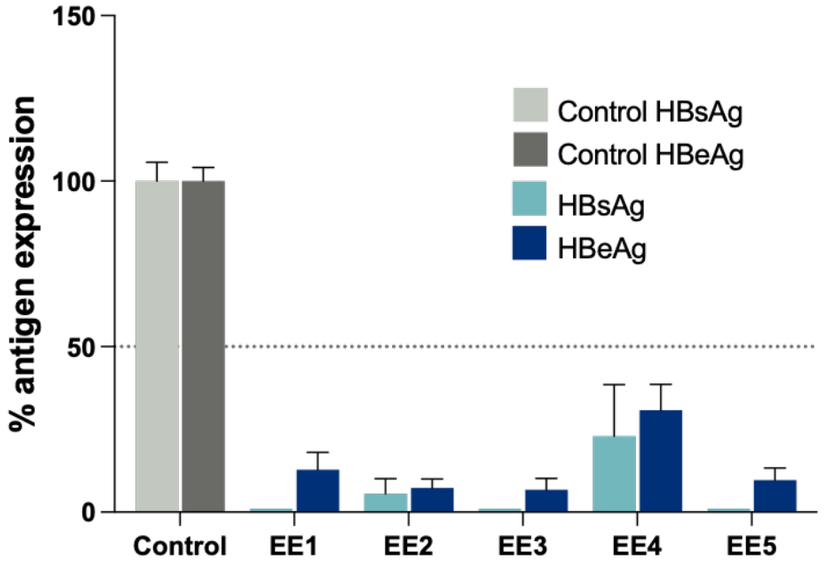
HBV-EE screen identified hits with robust activity in primary human hepatocytes (PHH)

Epigenetic editors were designed to cover the entire HBV genome and are conserved for most common genotypes of HBV

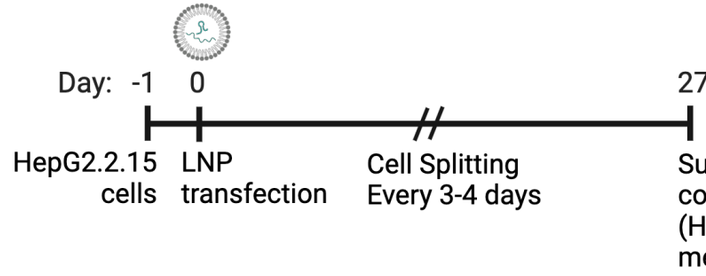


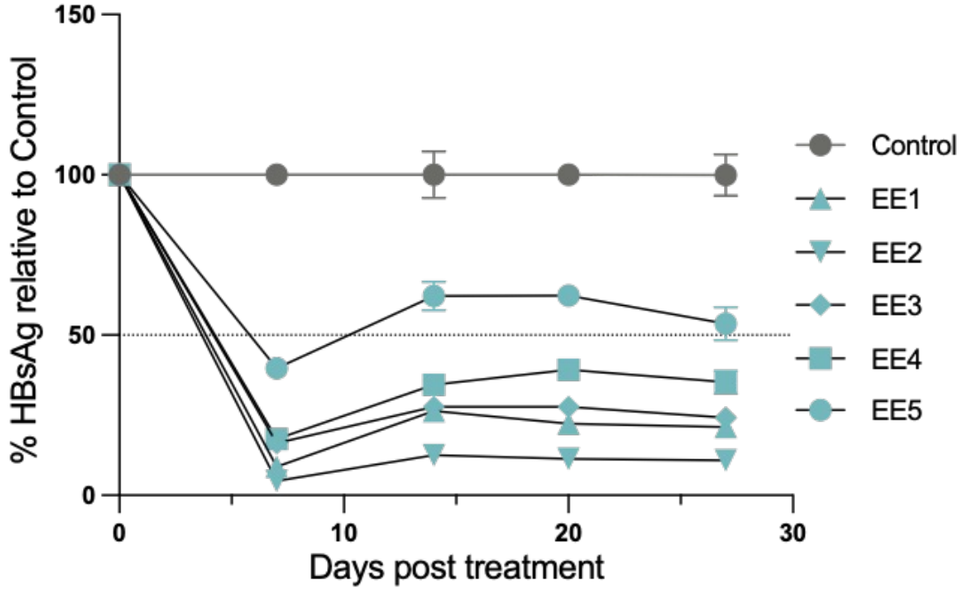
Strong reduction of viral markers in HBV infected primary human hepatocytes





Epigenetic repression of HBsAg is maintained in vitro for nearly a month in dividing cells containing integrated HBV





Anglero-Rodriguez Y, Abubucker S, Xiong J et al Development of HBV-targeting epigenetic repressors with deep, durable in vivo silencing of viral markers. International HBV Meeting 2023
EE= epigenetic editor; HBsAg = hepatitis B surface antigen; LNP = lipid nanoparticle.

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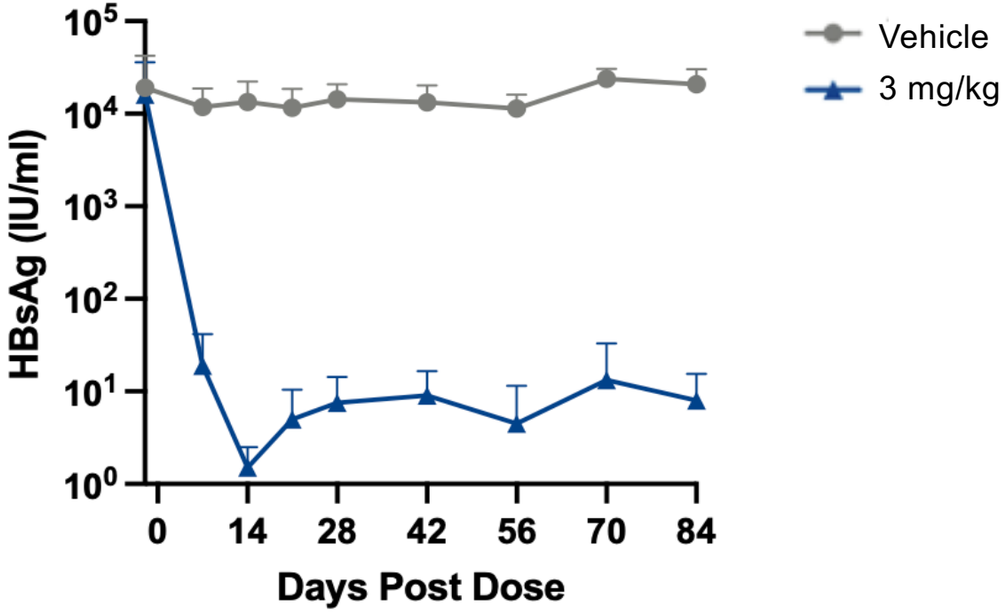
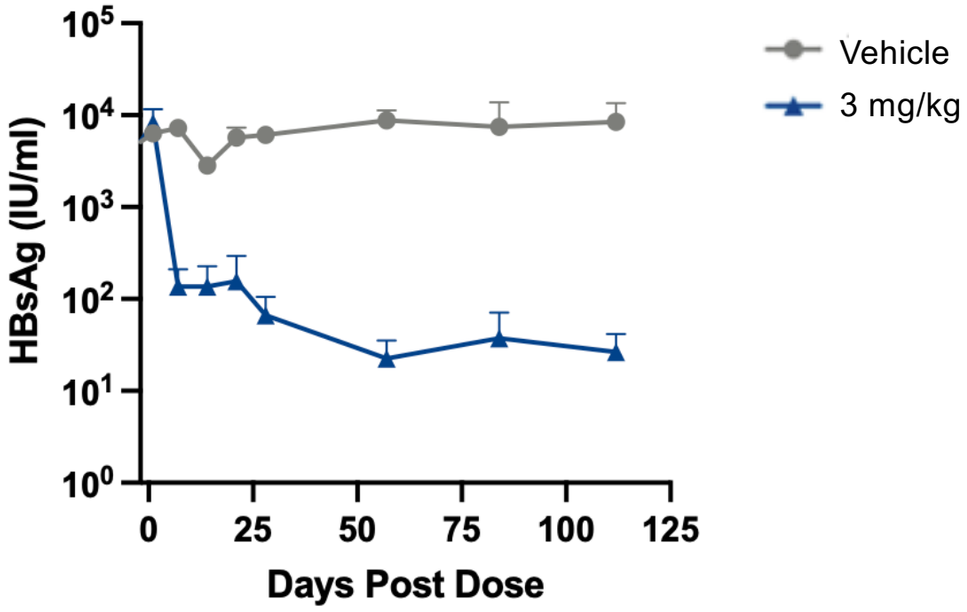
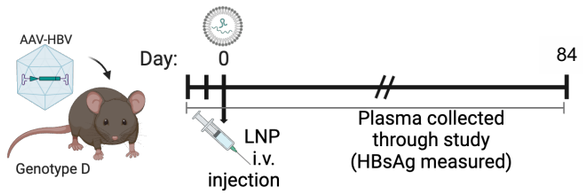
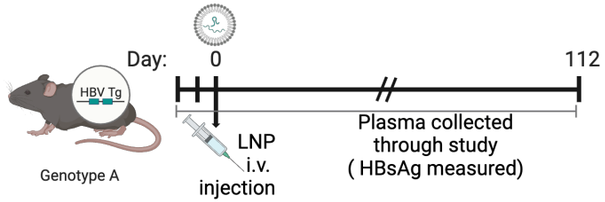
HBV epigenetic editors deeply and durably reduced HBsAg in both AAV8-HBV and Tg-HBV mice









AAV = adeno-associated virus; HBsAg = hepatitis B surface antigen; IV = intravenous; LNP = lipid nanoparticles; Tg = transgenic.

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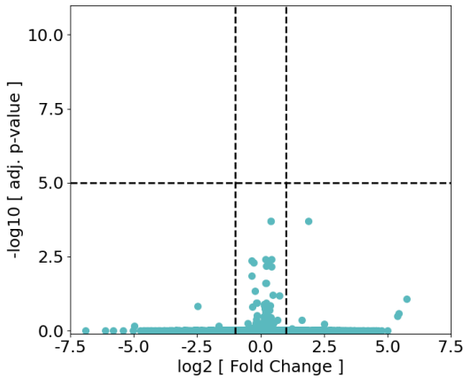
HBV epigenetic editors were highly specific, with no off-target changes in gene expression or methylation in PHH

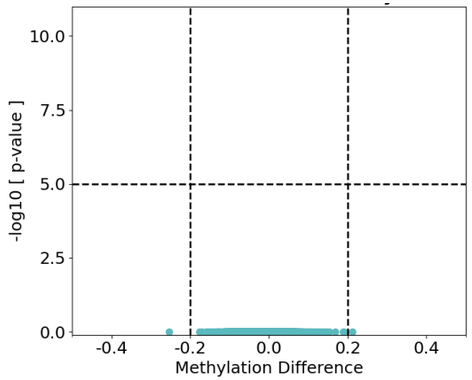
RNA Expression



No off-target changes in gene expression with HBV epigenetic editor in primary human hepatocytes as measured by RNA-seq

# of Differentially Expressed Genes: 0

Methylation at CpG-enriched sites

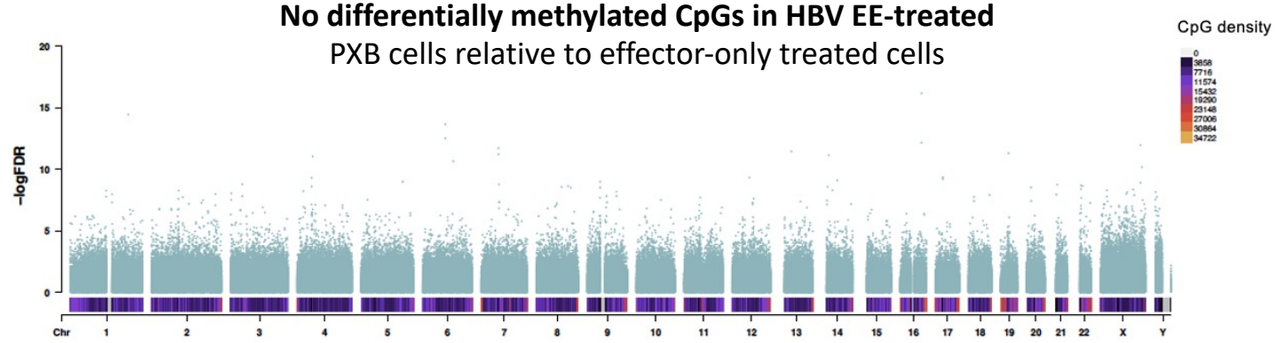


No off-target changes in methylation with HBV epigenetic editor in primary human hepatocytes as measured by methylation array

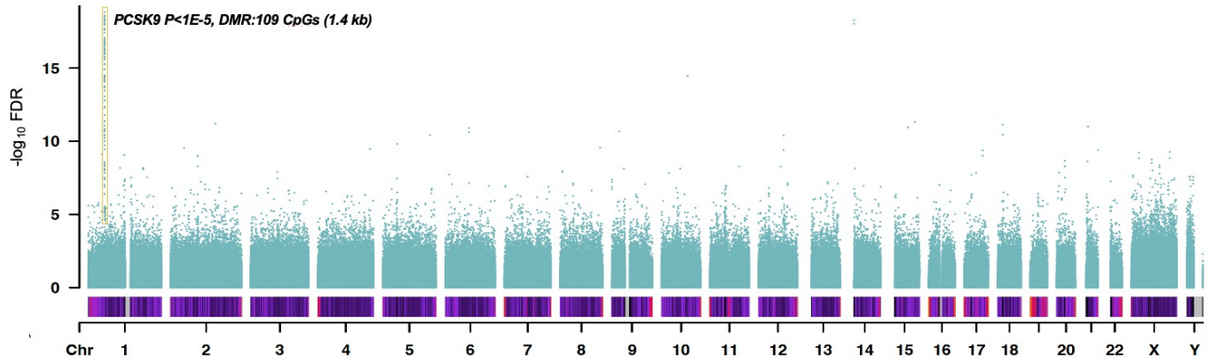
# of Differentially Methylated Regions: 0

Genome-wide Methylation

No differentially methylated CpGs in HBV EE-treated PXB cells relative to effector-only treated cells



PCSK9-EE positive control



EE = epigenetic editor; PHH = primary human hepatocytes.

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Advantages of epigenetic editing for highly engineered cell therapies

Epigenetic gene regulation is designed to be highly efficient, specific, and durable without any cuts, nicks, or changes to the underlying DNA

1 **Potential to enable multiplexing without genotoxic consequences**

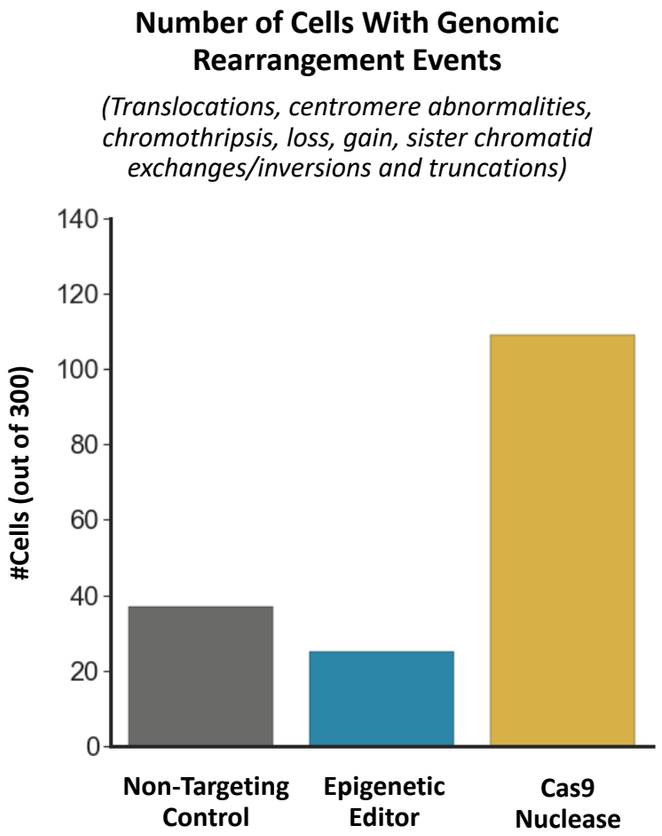
- Simultaneous silencing of a large number of targets without introducing DNA damage

2 **Potential to streamline manufacturing**

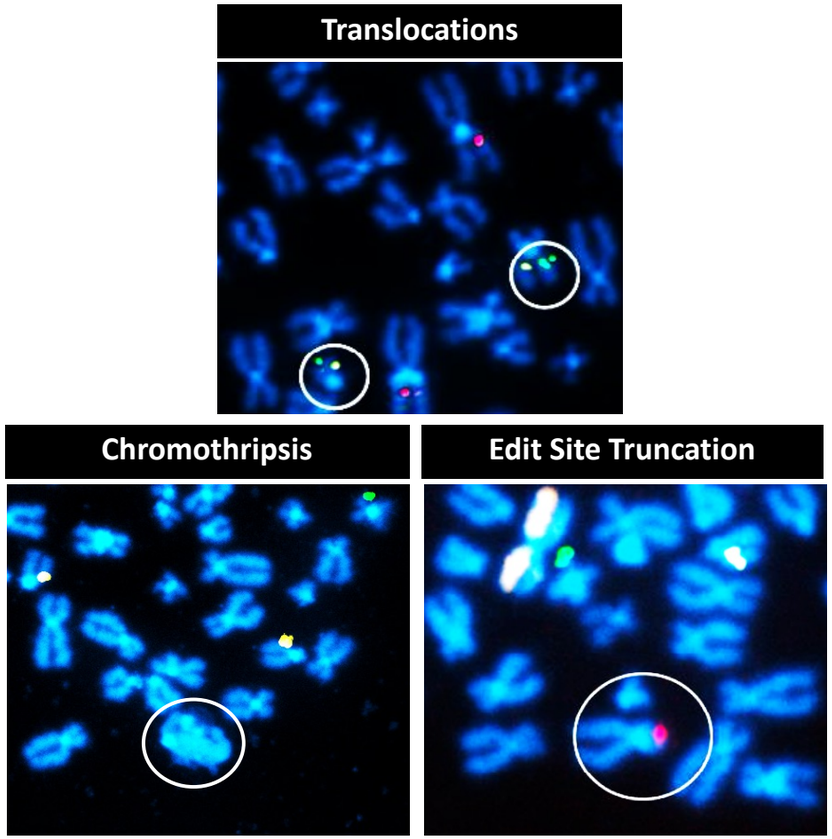
- Accomplish a high number of multiplex edits in a single step; eliminates need for sequential administration required with nuclease editing
- No requirement for in-depth characterization of edited T cells for translocations and chromosomal rearrangements

Multiplexing with epigenetic editors did not induce translocations or genomic rearrangement events

Triple epigenetically silenced cells were compared to triple Cas9 knockout T cells



Gross chromosomal abnormalities evident in Cas9 nuclease-treated cells



Abubucker S, Collins M, Hildebrand E, Multiplexed editing without chromosomal rearrangements using epigenetic editors, ASGCT 2023

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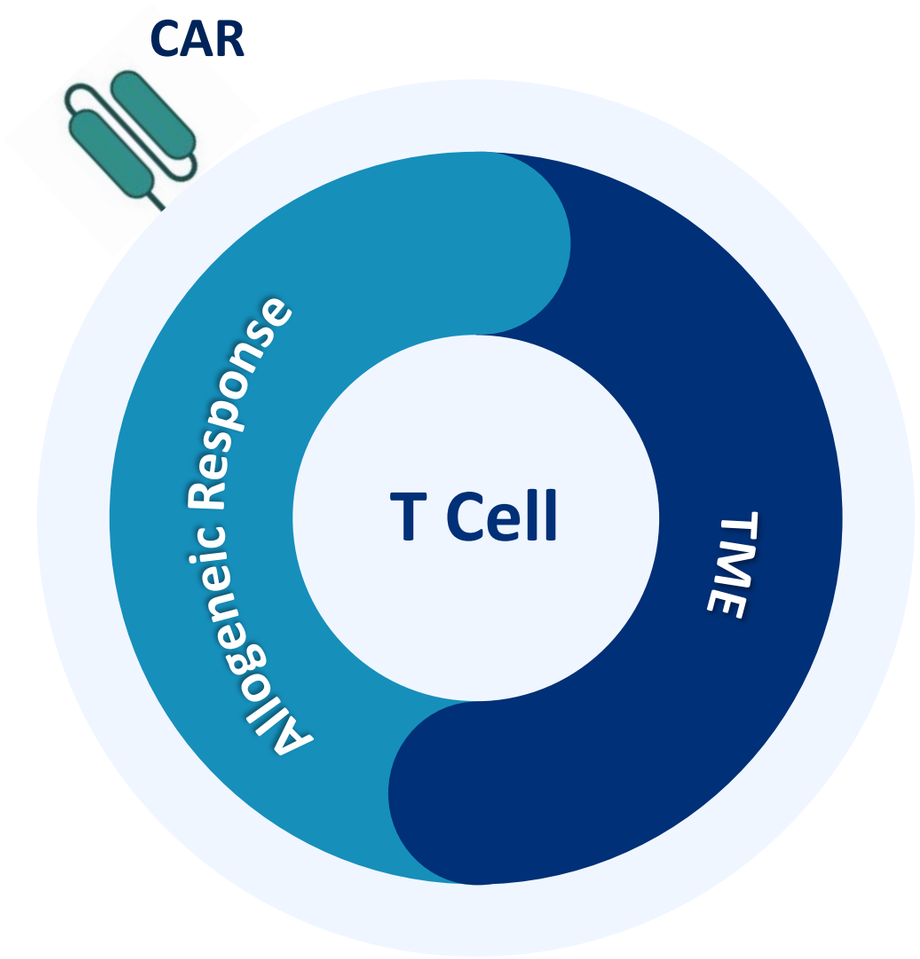
Modular cassette approach is designed to maximize cell therapy flexibility and functionality



Allogeneic Response

- Enables “off-the shelf” CAR T therapy that maintains potency and durability to treat a greater number of patients

Targets:

B2M	RFXAP	CD3D, CD3G	HLA-E
CD8+ T resistance	CD4+ T resistance	Eliminate GvHD	NK resistance

Tumor Microenvironment

- Overcomes immunosuppressive tumor environment to create a more persistent and efficacious CAR T

Targets:

TGFβR2	ADORA2A
Evade TGFβ	Reduce immunosuppression

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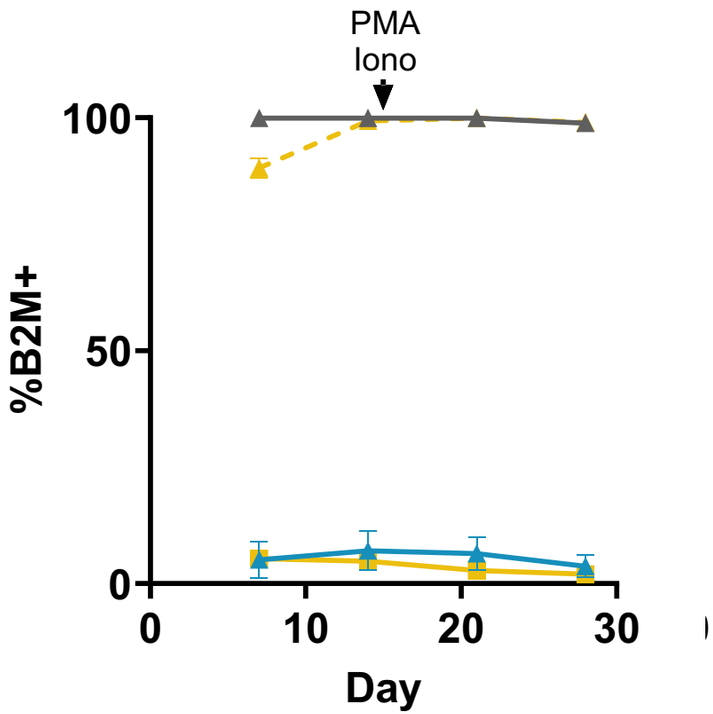
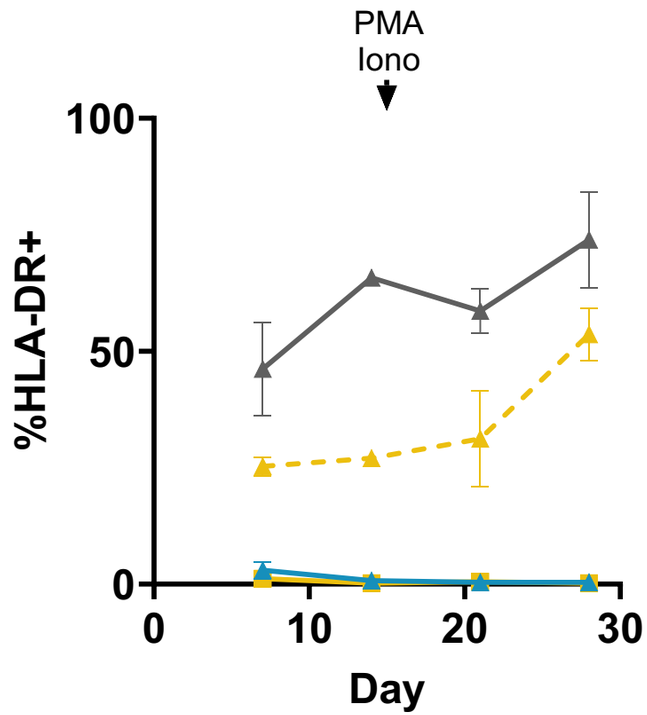
Efficient and durable multiplex silencing of three allogeneic targets

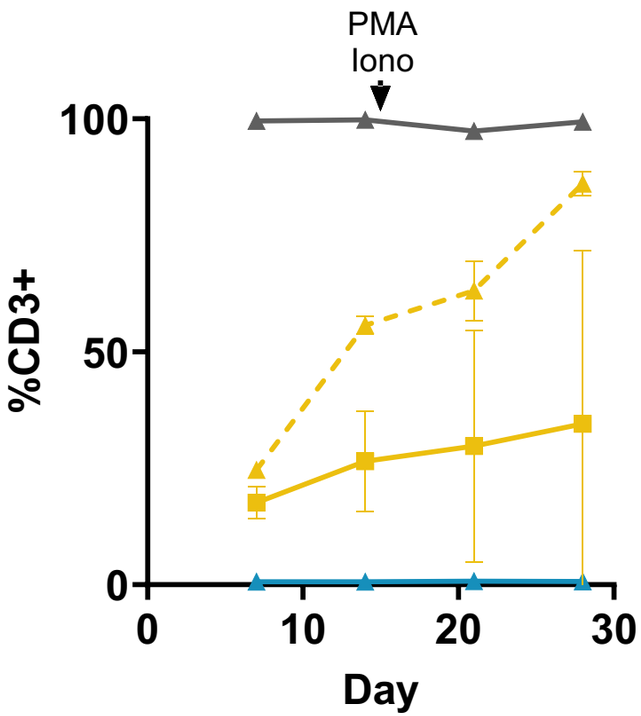
B2M

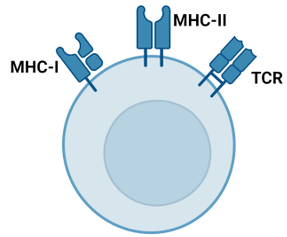


RFXAP



CD3D, CD3G





EE (No guide)
Cas9
CRISPRi
EE

Mean ± SD; N=3 biological replicates from one donor; representative of results obtained with two donors

Schafer J, Trombley J, Hallisey B, Durable multiplex epigenetic editing for generation of allogeneic CAR T without chromosomal rearrangements, ASH 2023
EE = epigenetic editor; MHC = major histocompatibility complex; TCR = T cell response.

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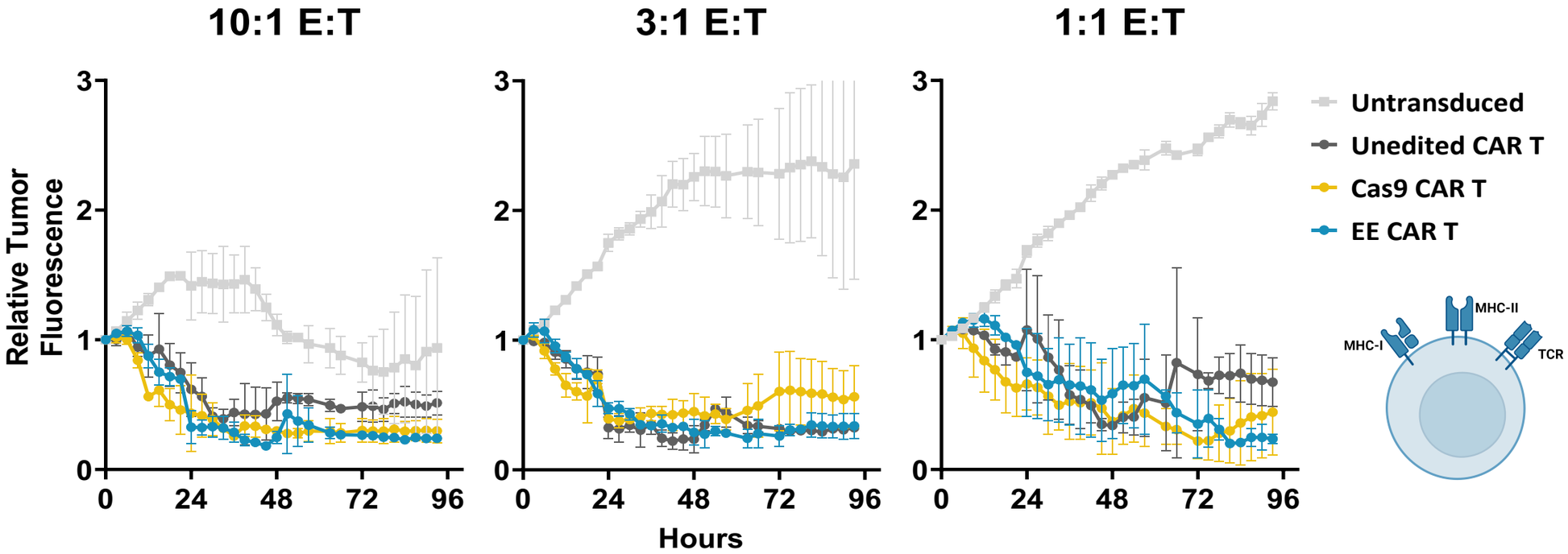
Multiplex epigenetically-edited CAR T have comparable cytotoxic function to unedited CAR T in vitro



Mean ± SD; N=2 technical replicates from one donor

- Multiplex epigenetically-edited CAR T kill tumor cells as effectively as unedited CAR T and demonstrates comparable tumor killing vs. triple Cas9 knock out CAR T for the same three targets
- Additional functional data demonstrates no GvHD response by, or alloresponse to, our epigenetically silenced T cells

Schafer J, Trombley J, Hallisey B, Durable multiplex epigenetic editing for generation of allogeneic CAR T without chromosomal rearrangements, ASH 2023
E:T = effector: target; GvHD = graft-versus-host disease; MHC = major histocompatibility complex; TCR = T cell response.

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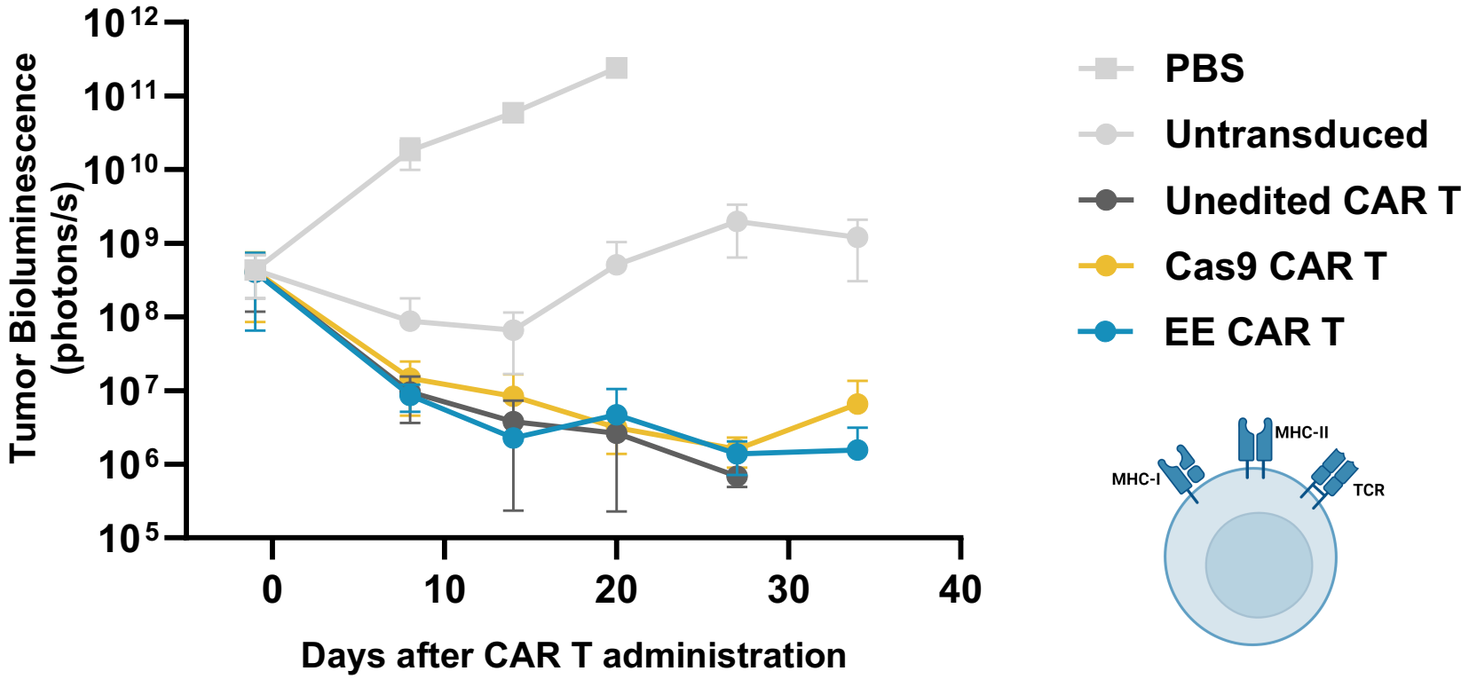
Multiplex epigenetically-edited CAR T have comparable cytotoxic function to unedited CAR T in vivo

- In vivo control of tumor in an NSG MM.1S model is equivalent for multiplex allogeneic EE CAR T and unedited CAR T
- GvHD response observed in unedited CAR T-treated animals required early euthanization
- Prolonged GvHD-free survival in EE CAR T- and Cas9 CAR T-treated animals



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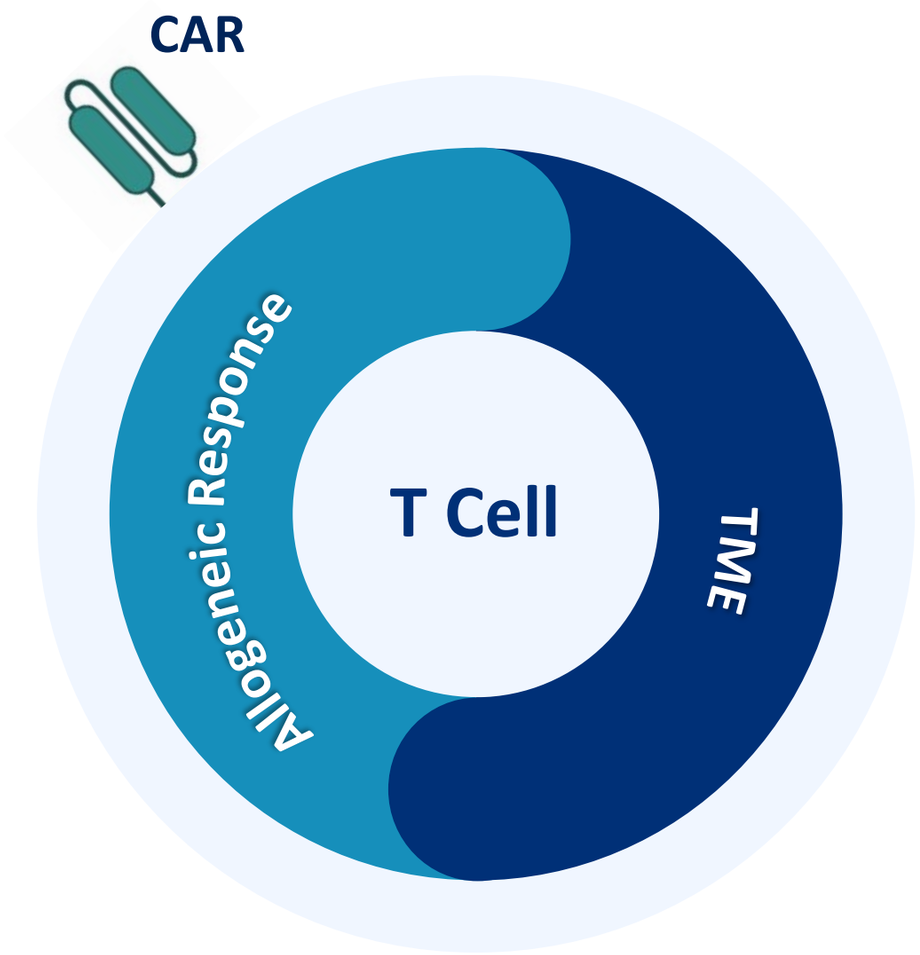
Modular cassette approach is designed to maximize cell therapy flexibility and functionality



Allogeneic Response

- Enables “off-the shelf” CAR T therapy that maintains potency and durability to treat a greater number of patients

Targets:

B2M	RFXAP	CD3D, CD3G	HLA-E
CD8+ T resistance	CD4+ T resistance	Eliminate GvHD	NK resistance

Tumor Microenvironment

- Overcomes immunosuppressive tumor environment to create a more persistent and efficacious CAR T

Targets:

TGFβR2	ADORA2A
Evade TGFβ	Reduce immunosuppression

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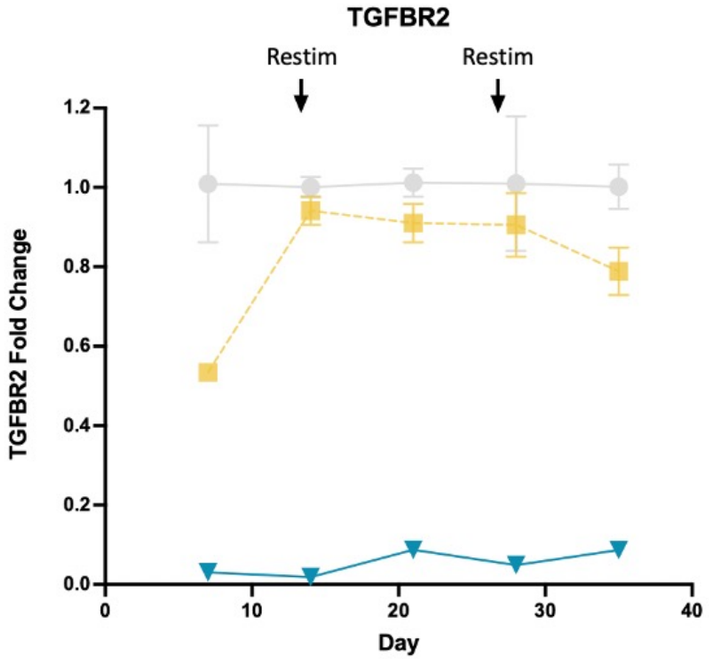
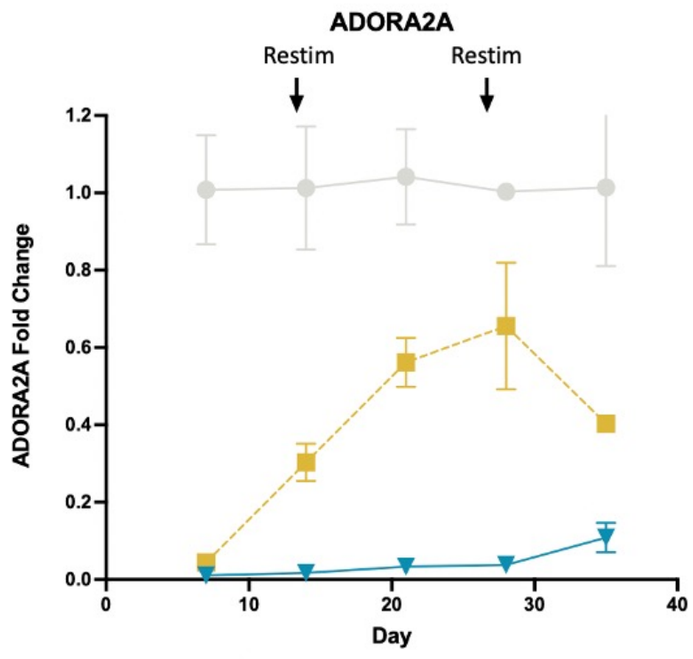
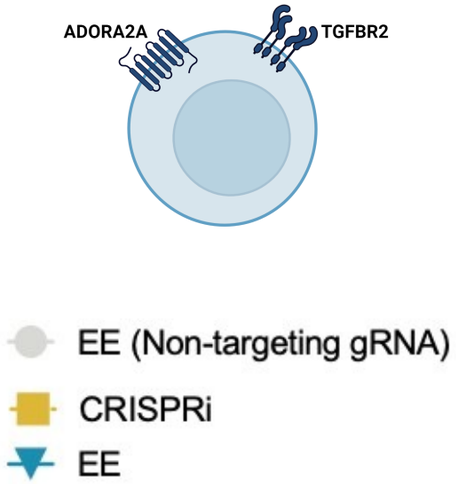
Efficient and durable multiplex silencing of TME resistance targets

TGFβR2



ADORA2A





- Efficient and durable multiplex silencing of two TME-resistance targets, ADORA2A and TGFBR2, was achieved in primary human T cells

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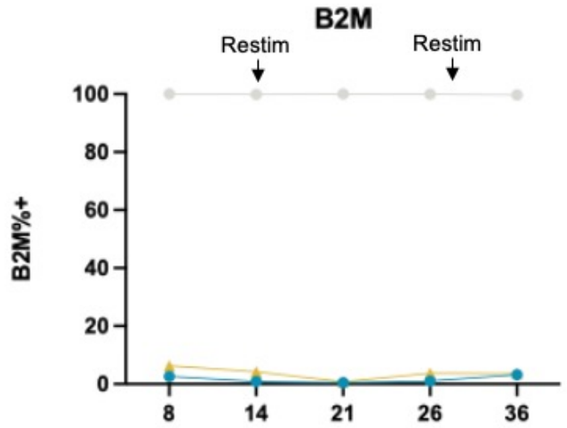
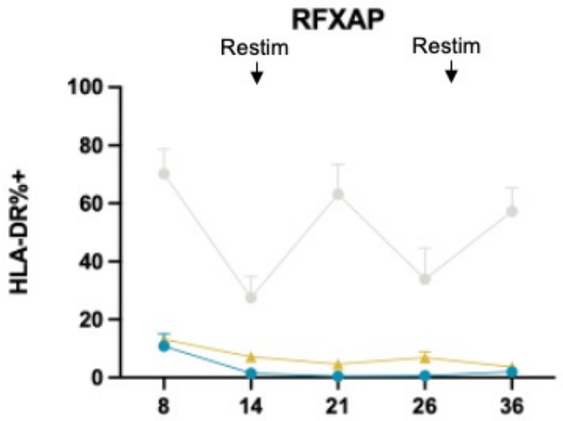
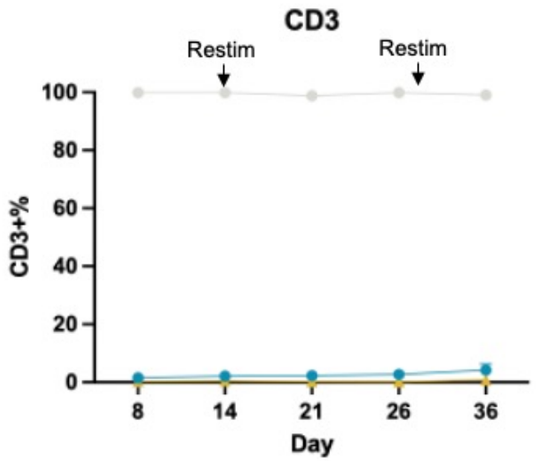
Allo/TME five-target multiplexing is both highly efficient and durable

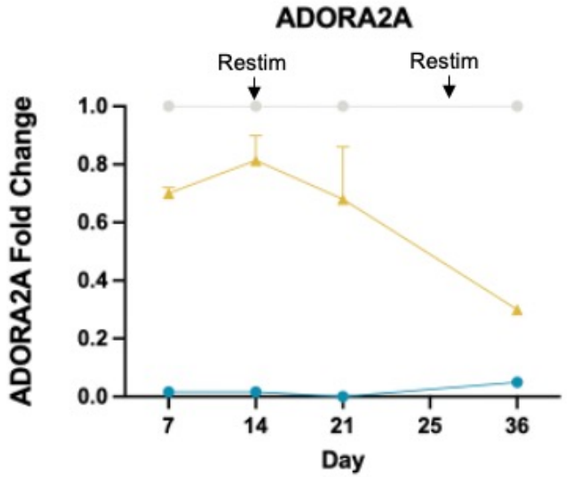
Allo

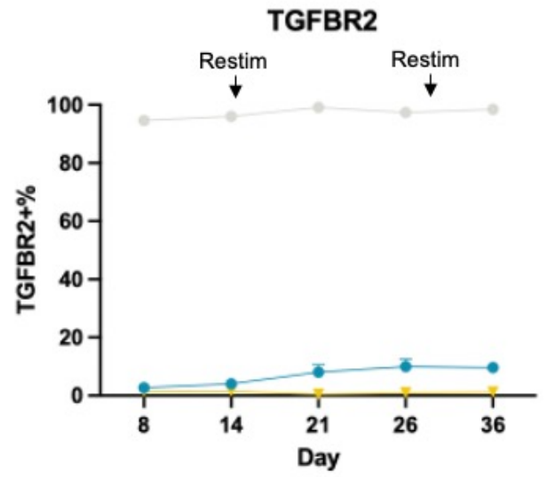
TME

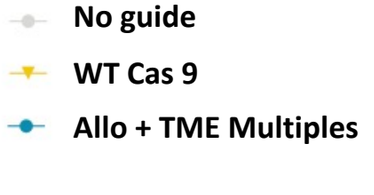


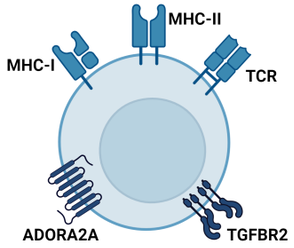












- Efficient 5-target silencing out to 35 days shows minimal impact of multiplexing on overall silencing efficiency through two PMA/ionomycin restimulations on Days 14 and 28

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Acknowledgements

Thank you to the entire Chroma team, our collaborators, and partners!

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