#AHA23

CHROMA MEDICINE

A SINGLE ADMINISTRATION OF AN EPIGENETIC EDITOR TARGETING HUMAN PCSK9 ROBUSTLY AND DURABLY LOWERS CHOLESTEROL IN VIVO

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American Heart Association.

Presenter Disclosures

Frederic Tremblay, PhD

FINANCIAL DISCLOSURE:
Employment by Chroma Medicine

American Heart Association.

Epigenetic editing – a ‘one and done’ approach to disrupt the current treatment paradigm for lowering LDL-C

The magnitude and cumulative duration of LDL-C lowering is key to reducing risk of atherosclerotic cardiovascular disease (ASCVD)

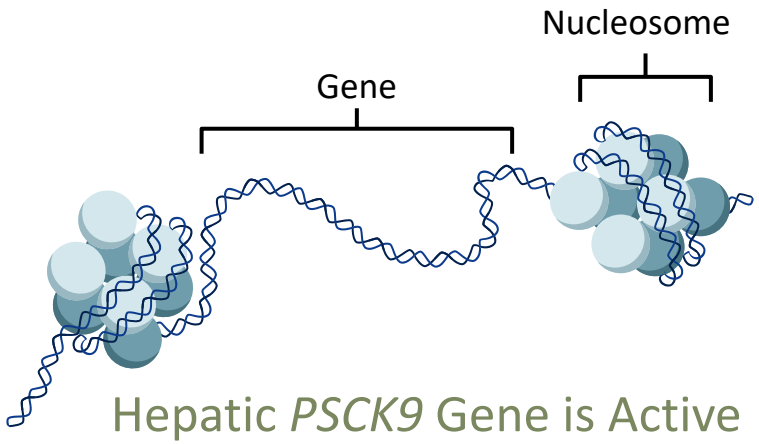
- Observational studies have shown that a large majority of ASCVD patients do not achieve their LDL-C target goal
- PCSK9 inhibitors have emerged as an effective class of LDL-lowering therapies but require life-long treatment

Epigenetic editing has the potential to provide a life-long lowering of LDL-C by silencing the *PCSK9* gene, thus providing durable and specific gene regulation without cutting or nicking the DNA

Gu J et al Lipid treatment status and goal attainment among patients with atherosclerotic cardiovascular disease in the United States: A 2019 Update *American Journal of Preventive Cardiology*, 2022.
Ray KK et al, EU-Wide Cross-Sectional Observational Study of Lipid-Modifying Therapy Use in Secondary and Primary Care: the DAVINCI study, *European Journal of Preventive Cardiology*, 2021.

Chroma's epigenetic editors can modulate *PCSK9* expression in hepatocytes

Durable change in phenotype without a change in genotype



Hepatic *PSCK9* Gene is Active

DNA is Open and Accessible

Epigenetic Repressor

Methylates Targets

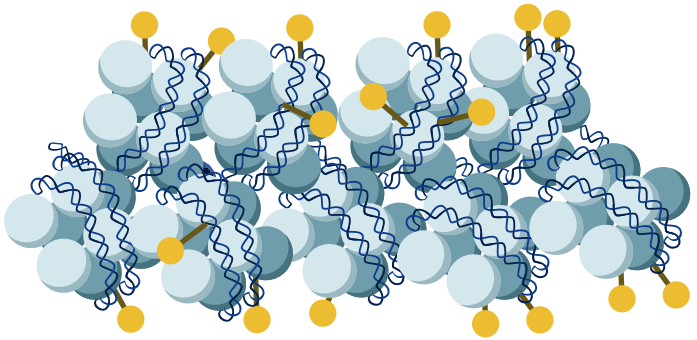
Transient application of Chroma's epigenetic editors

Epigenetic Activator

Demethylates Targets

Hepatic *PCSK9* Gene is Inactive

DNA is Closed and Inaccessible



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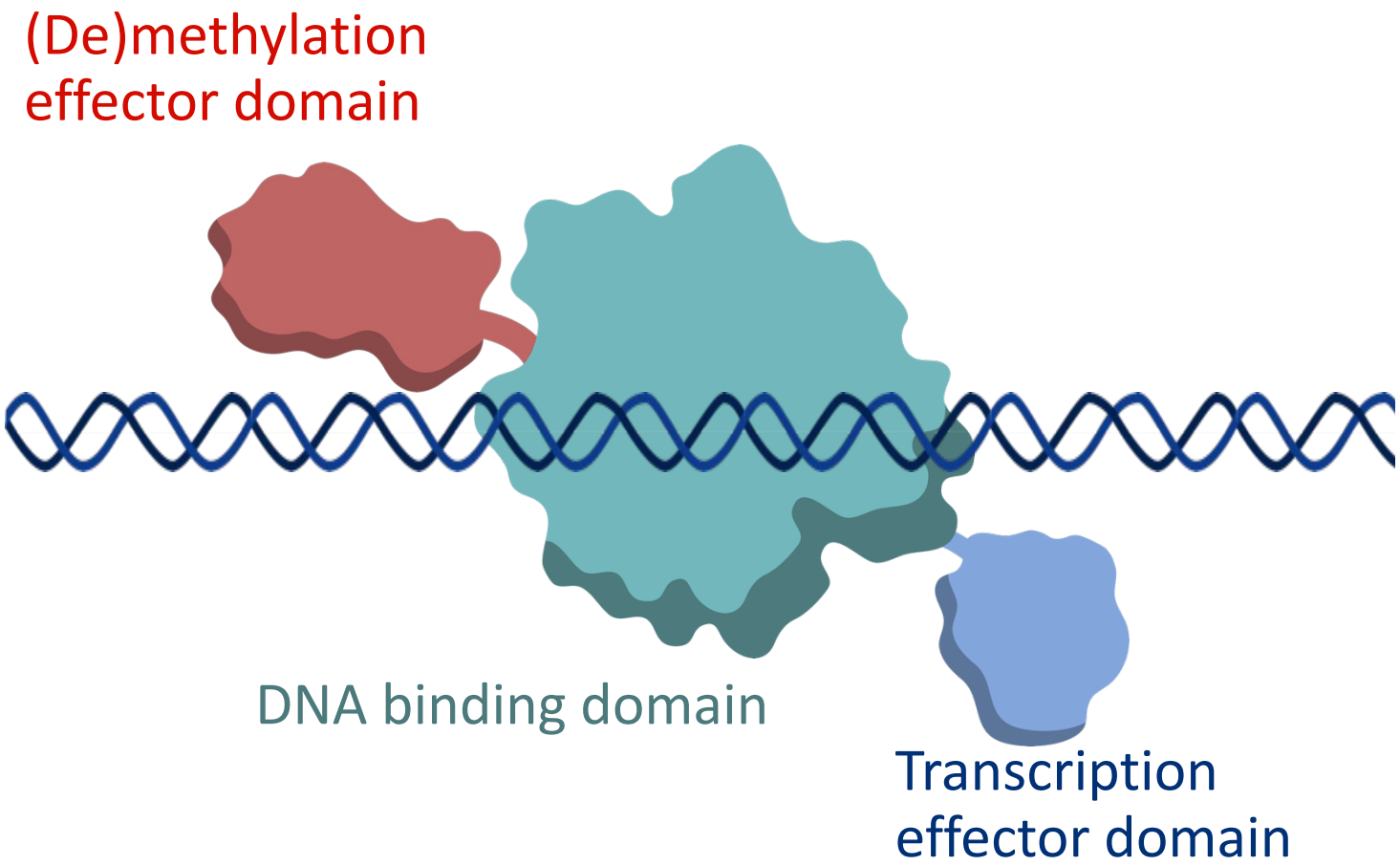
Chroma's epigenetic editors are modular and versatile

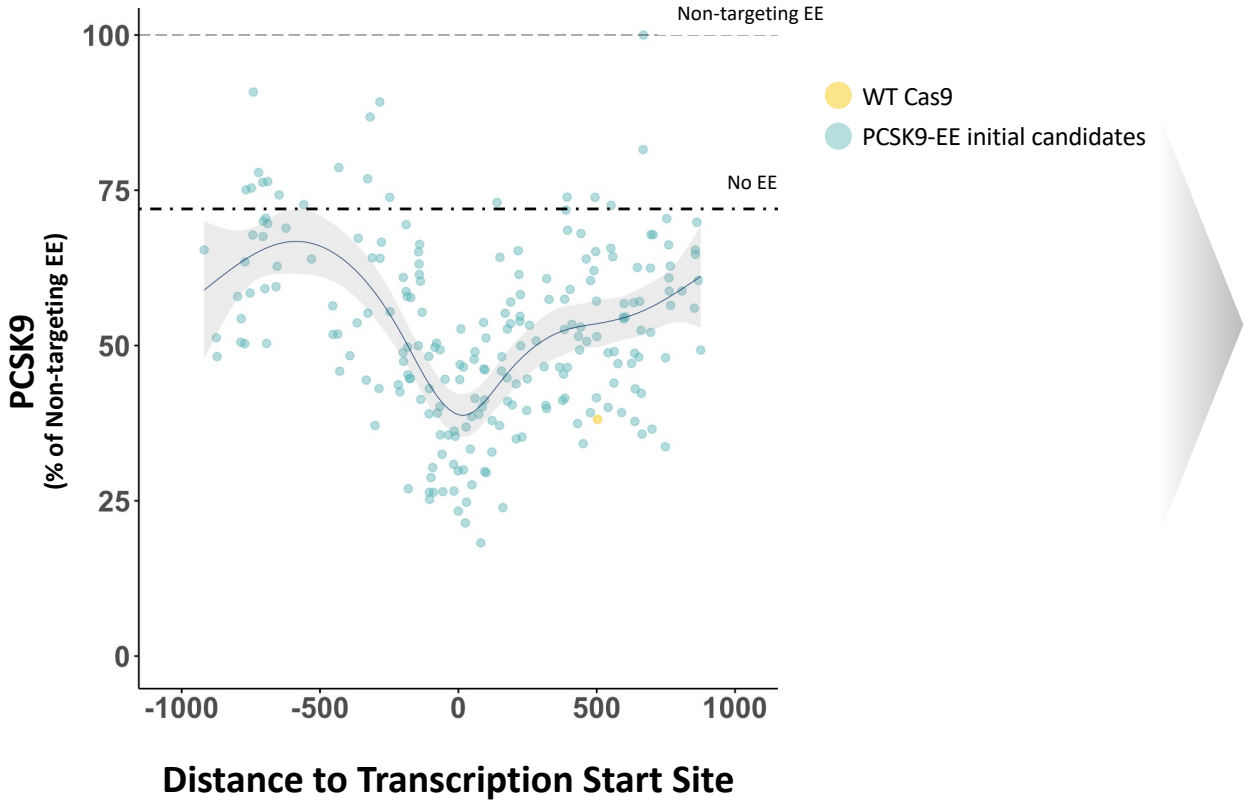
Chroma's Epigenetic Editors



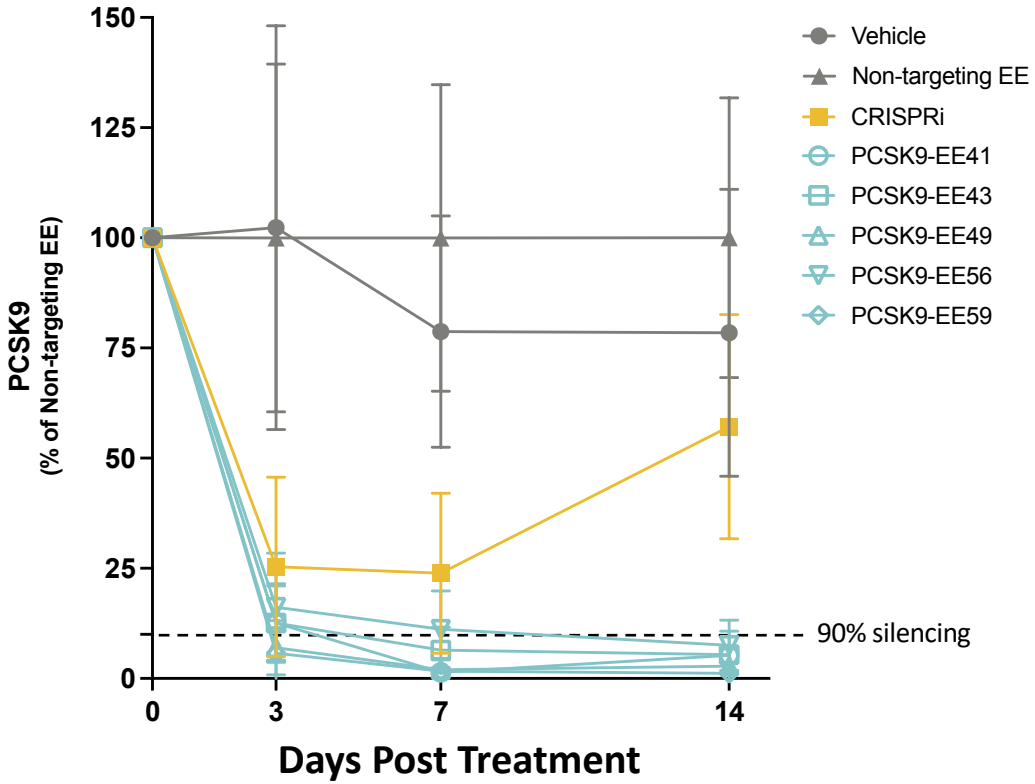
- **DNA binding domain** precisely localizes effector domains to target sequence
- **Transcription effector domain** transiently represses or activates target gene
- **Methylation / demethylation effector domain** durably silences / activates target gene

Robust: 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



EE = epigenetic editor; PHH = primary human hepatocyte; WT = wild type; error bars represent standard deviation

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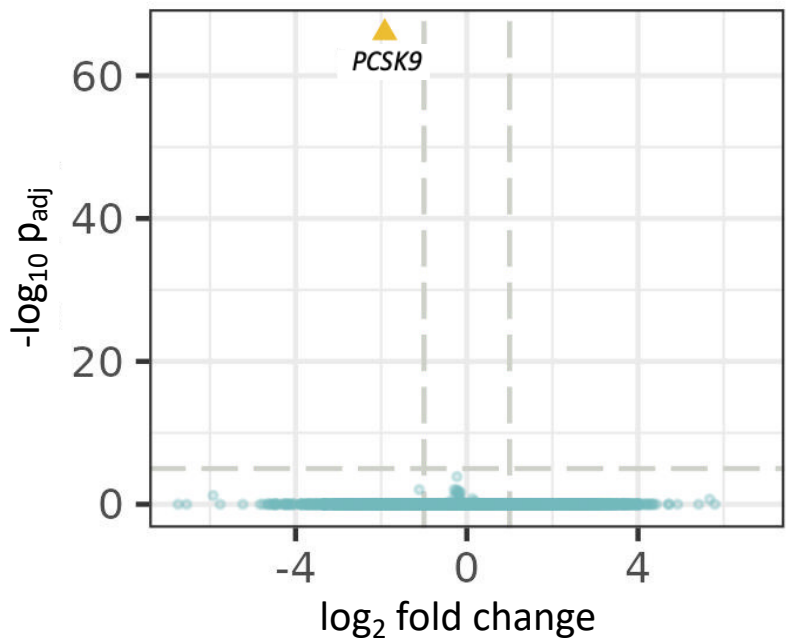
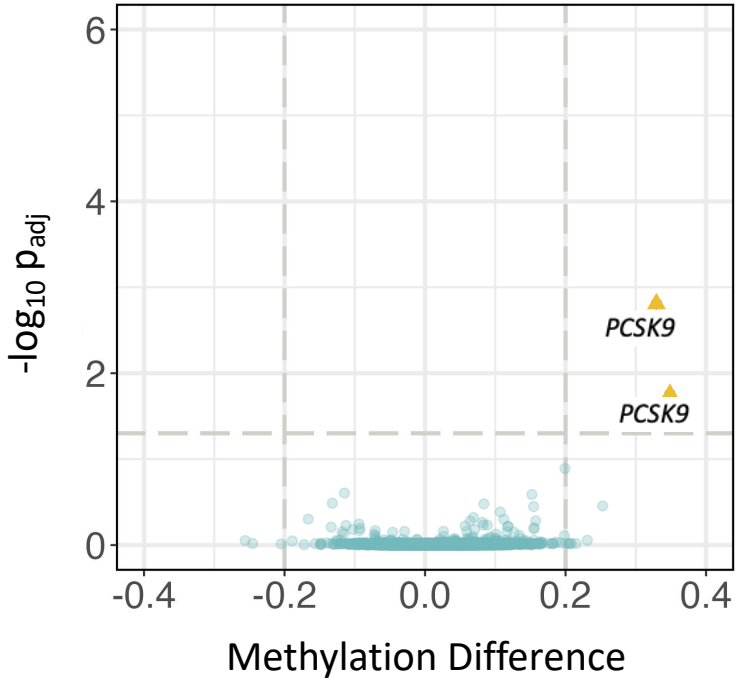
Specific: PCSK9-EEs can be highly specific with no off-target changes in expression or methylation in primary human hepatocytes

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



Only Differentially Expressed Gene: *PCSK9*

No off-target changes in methylation with epigenetic repressor in primary human hepatocytes as measured by Illumina Methylation Array



Only Differentially Methylated Regions: *PCSK9* Promoter

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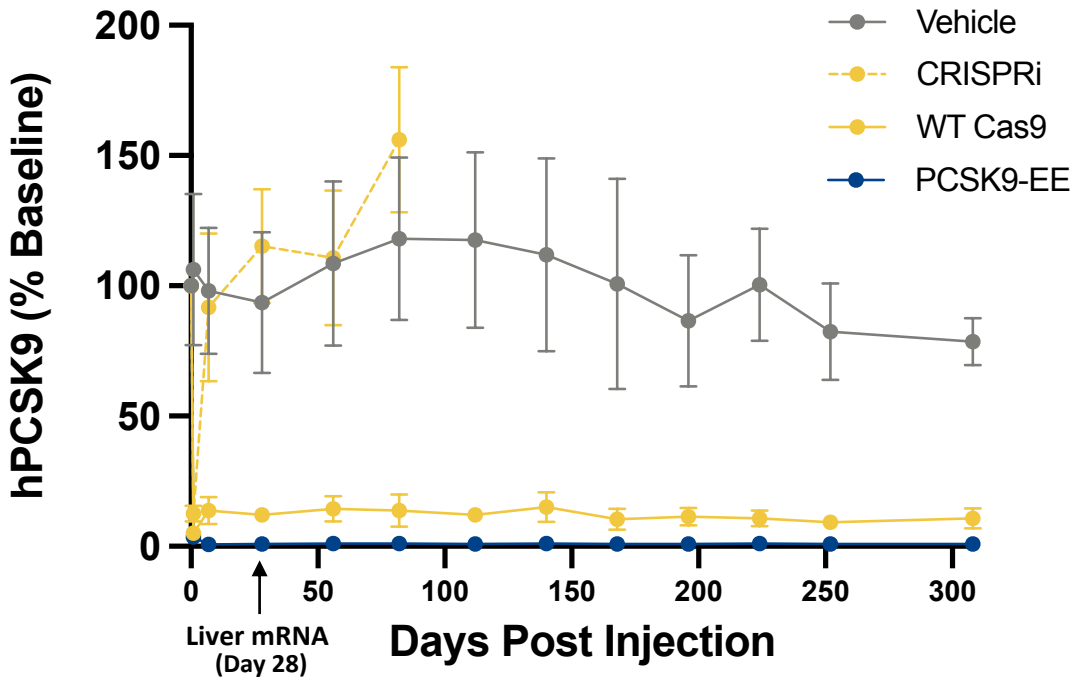
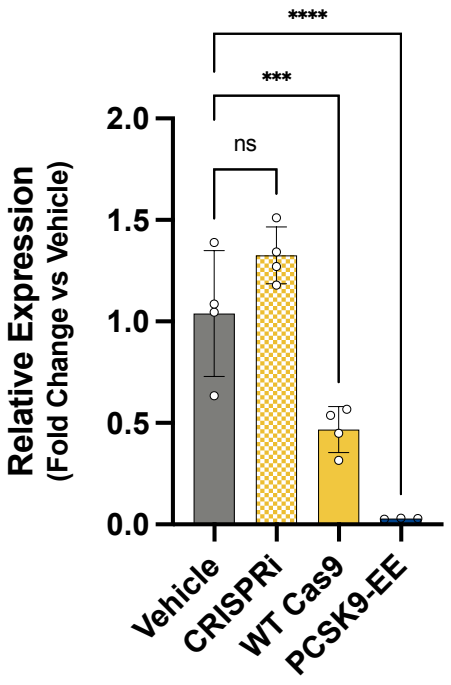
Efficient: In mice, PCSK9-EE achieved >98% PCSK9 silencing with durability out to 10 months

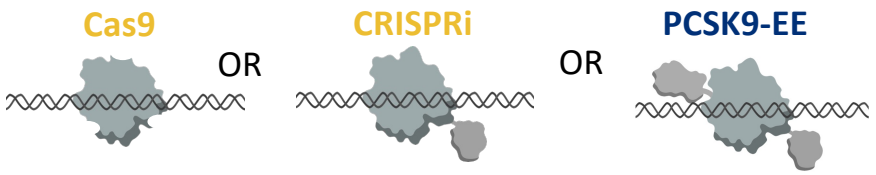
- Transgenic mouse containing the human *PCSK9* locus
- Tested optimized single construct epigenetic editor
- Near-complete silencing maintained for > 10 months post single IV injection
- Animals will be followed to confirm durability up to 1 year

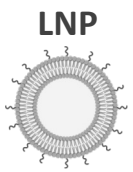
Plasma PCSK9 Protein



Liver *PCSK9* mRNA







Experiment

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

EE = epigenetic editor; 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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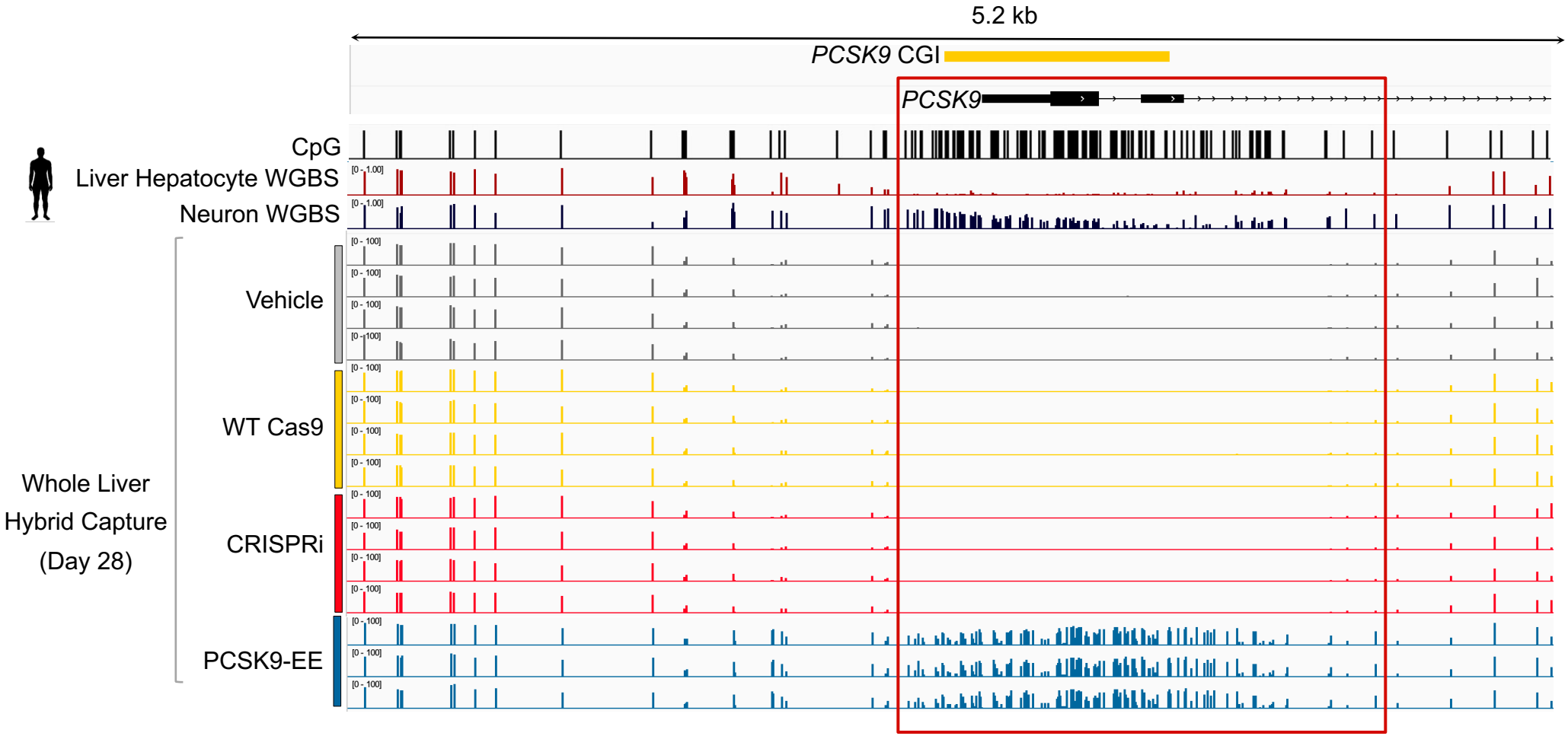
Efficient: PCSK9-EE induces stable, targeted CpG methylation at the human *PCSK9* locus in vivo



EE = epigenetic editor; WT = wild type

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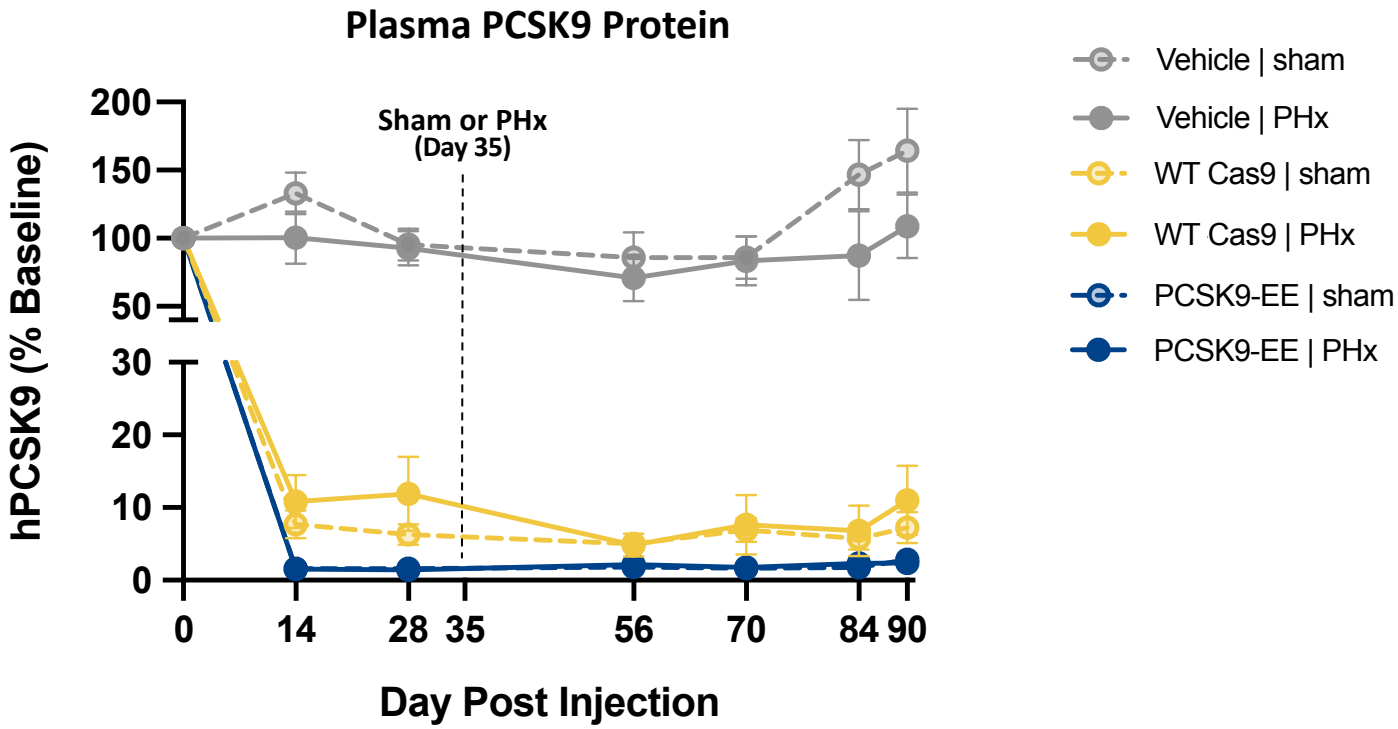
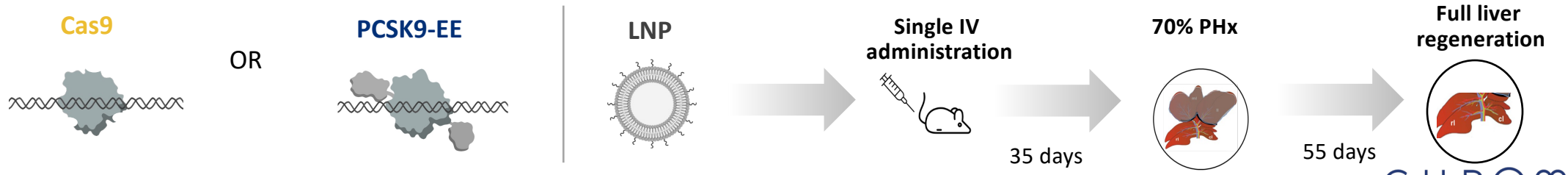
Durable: In mice, PCSK9-EE's effect is fully maintained after partial hepatectomy (PHx)





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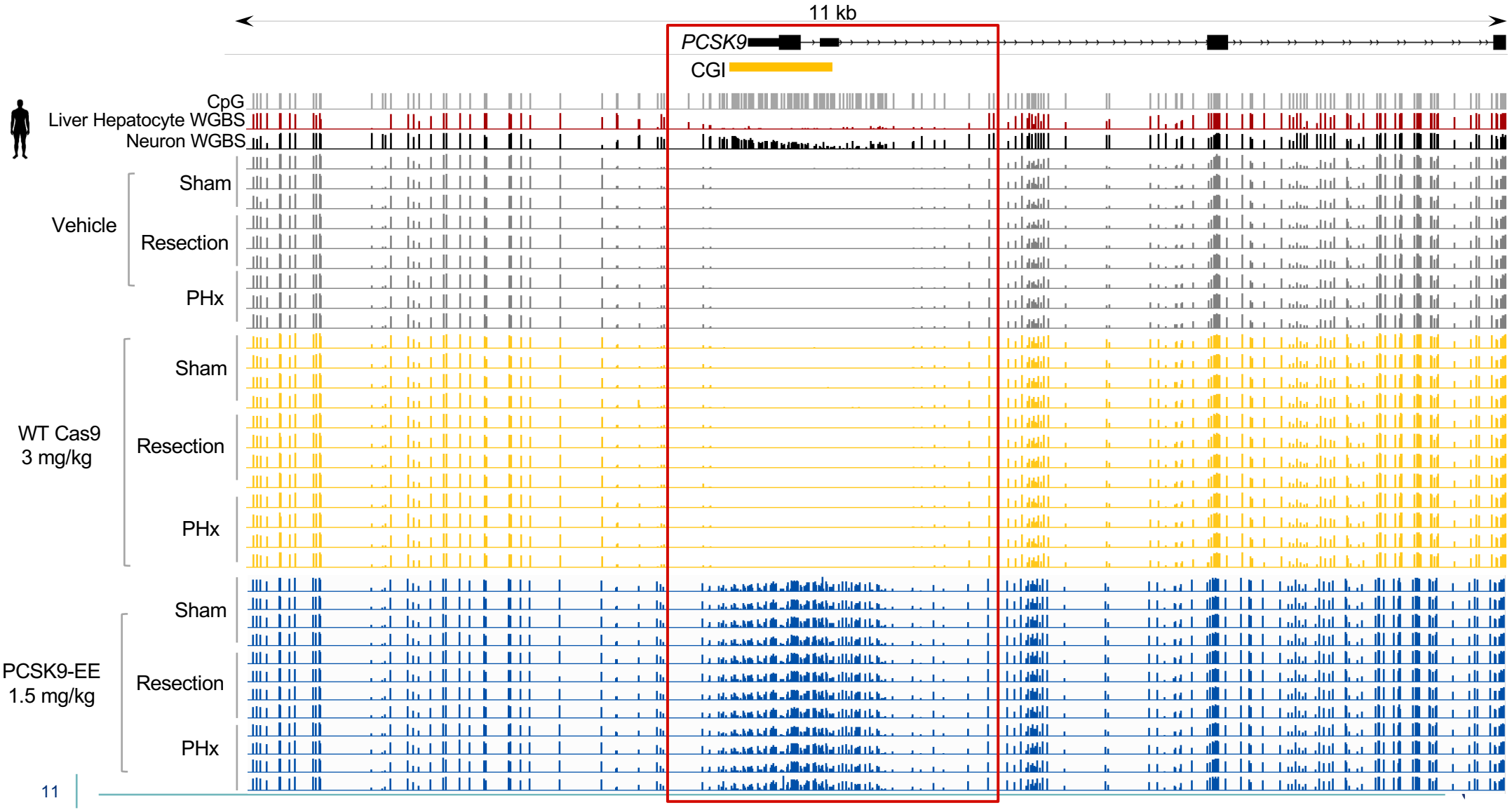
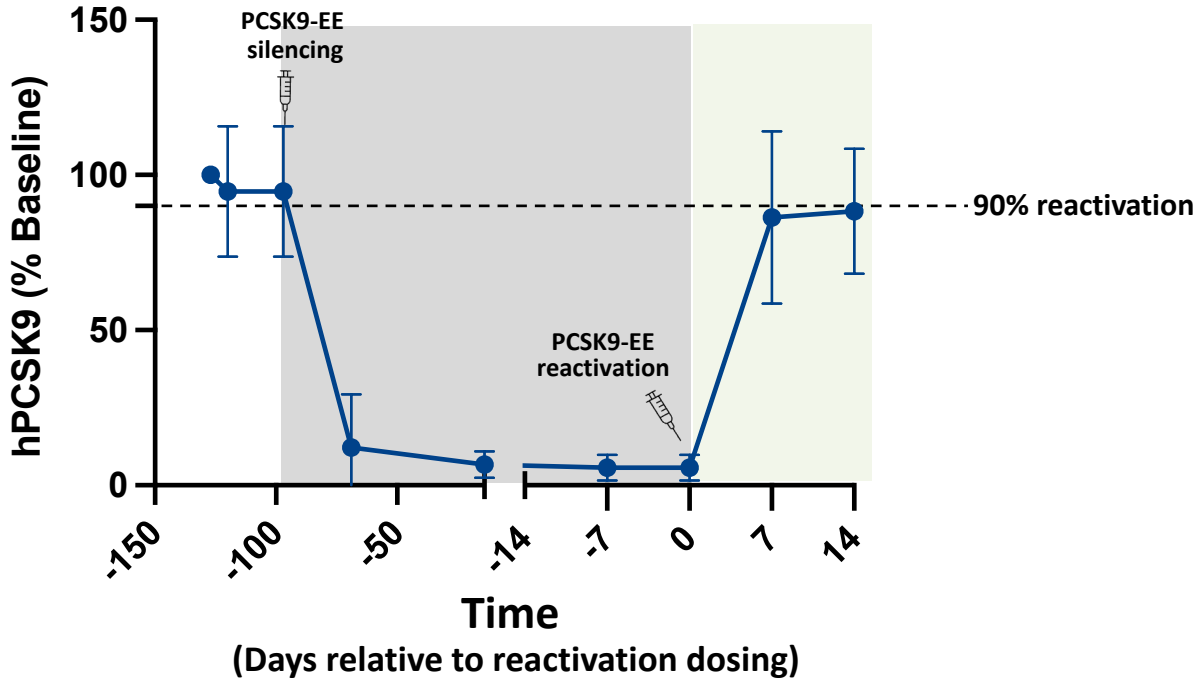
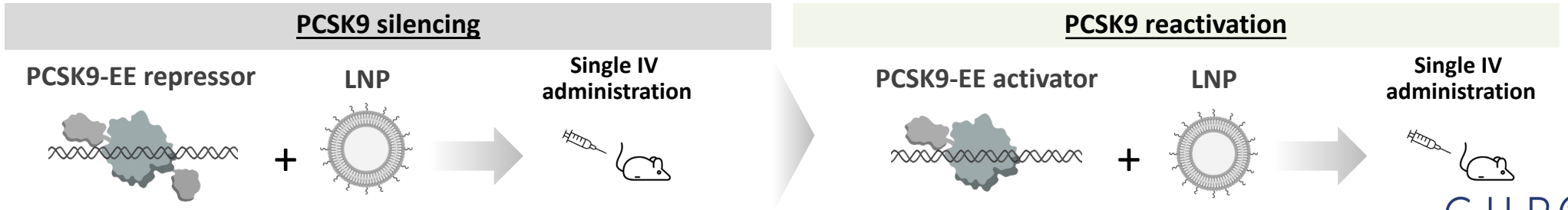
Durable: PCSK9-EEs demonstrate durable DNA methylation at *PCSK9* locus pre- and post-partial hepatectomy (PHx)



Reversible: In mice, PCSK9-EE's effect is reversed via targeted action of PCSK9-activator

- 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 at day 0**
- Animals will be followed to confirm durability





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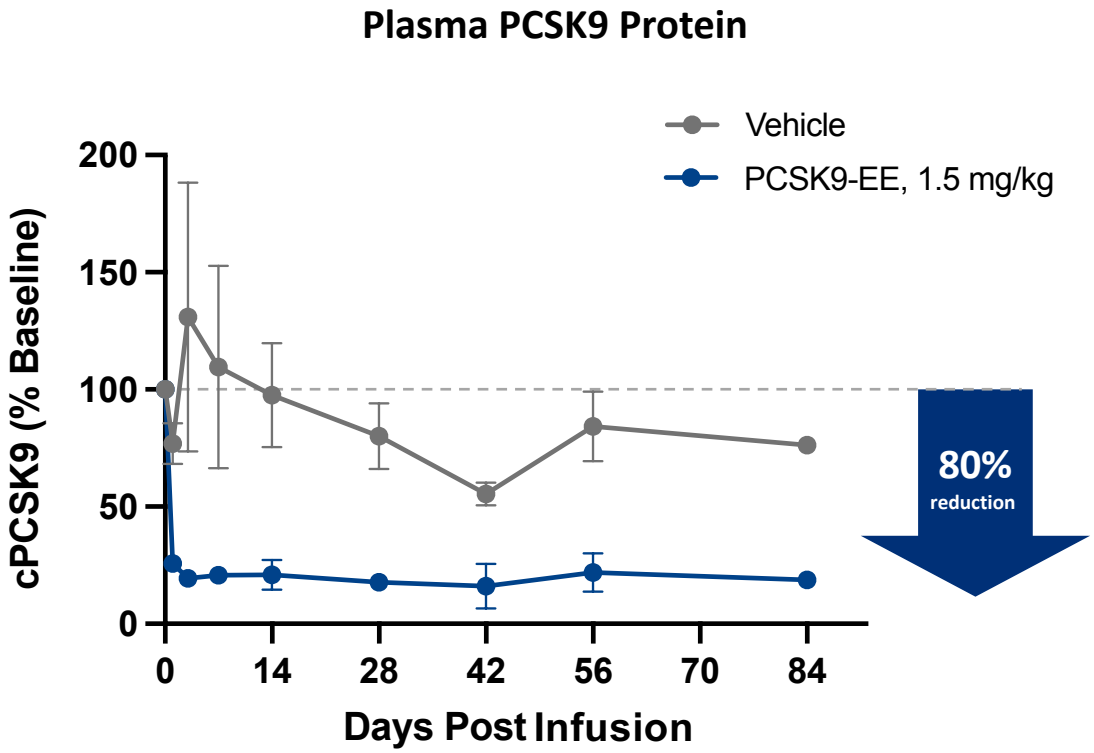
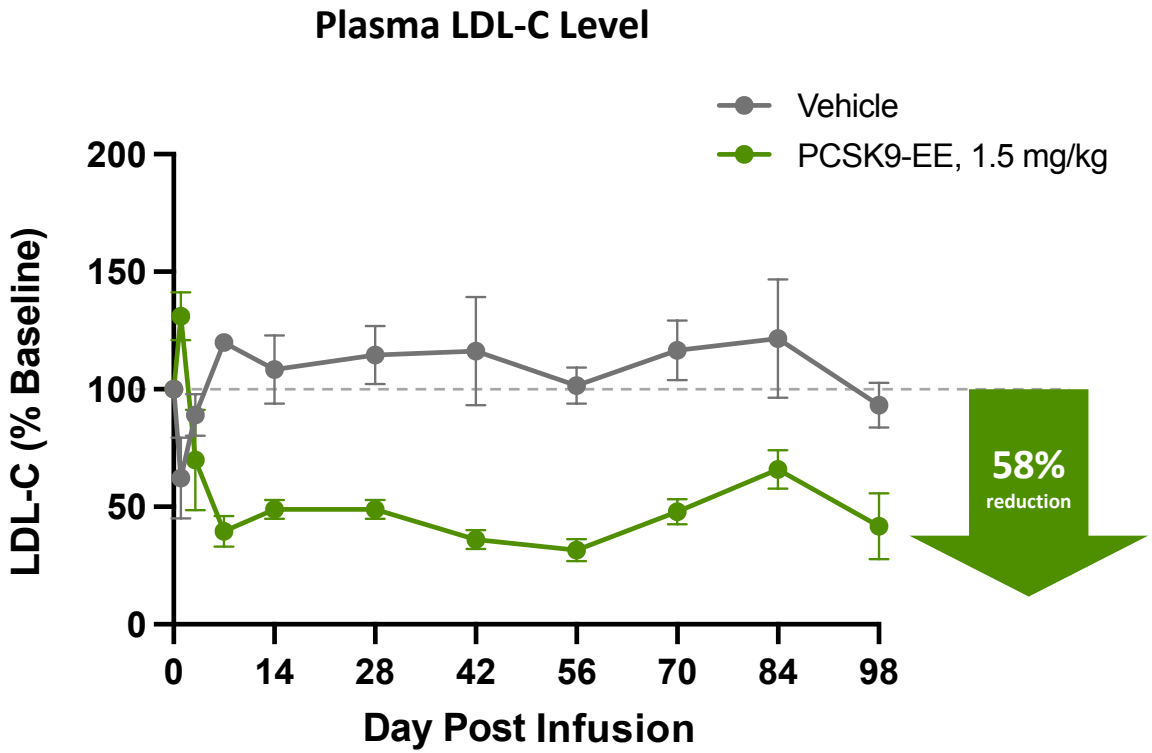
Translatable: In NHP, PCSK9-EE achieved 80% reduction in PCSK9 and 58% in LDL-C with durability out to 3 months





Experiment

- Cynomolgus monkey (*Macaca fascicularis*)
- Single IV infusion
- PCSK9 analysis by ELISA

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

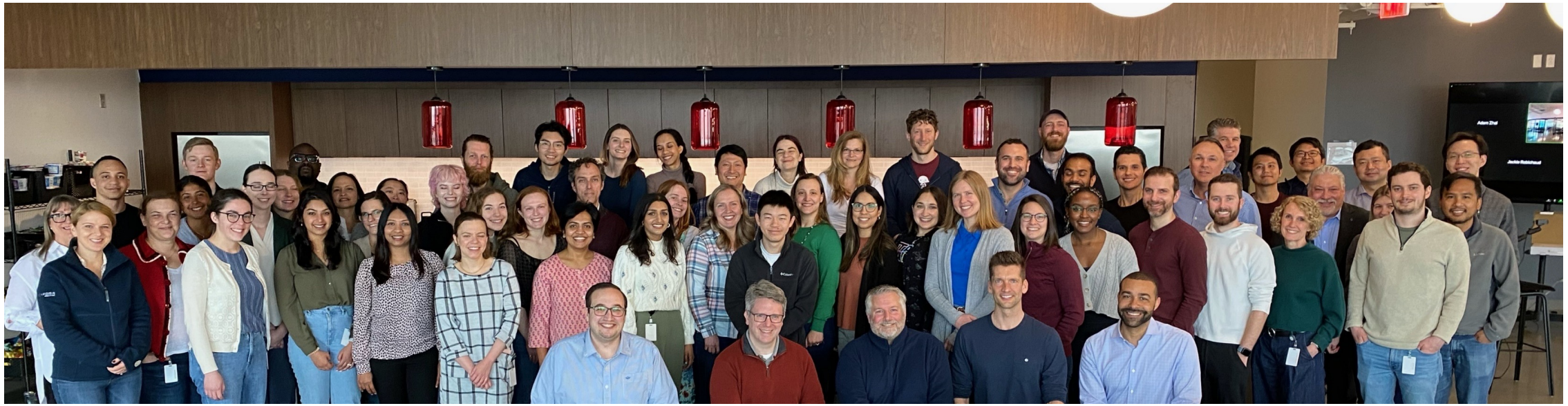
PCSK9-EE: a novel therapeutic approach for the specific, efficient and durable reduction of PCSK9

Chroma's PCSK9-EE:

- ✓ Leverages an endogenous mechanism for regulating *PCSK9* gene expression that does not rely on cutting or nicking the DNA
- ✓ Is highly specific with no off-target changes in gene expression or methylation
- ✓ Efficiently, reversibly, and durably suppresses PCSK9 in mice
- ✓ Translates to NHP with initial demonstration of efficient PCSK9 reduction and clinically meaningful reductions in LDL-C

Acknowledgements

Thank you to the entire Chroma team and our partner!



LNP technology provided by Acuitas Therapeutics, Inc.