



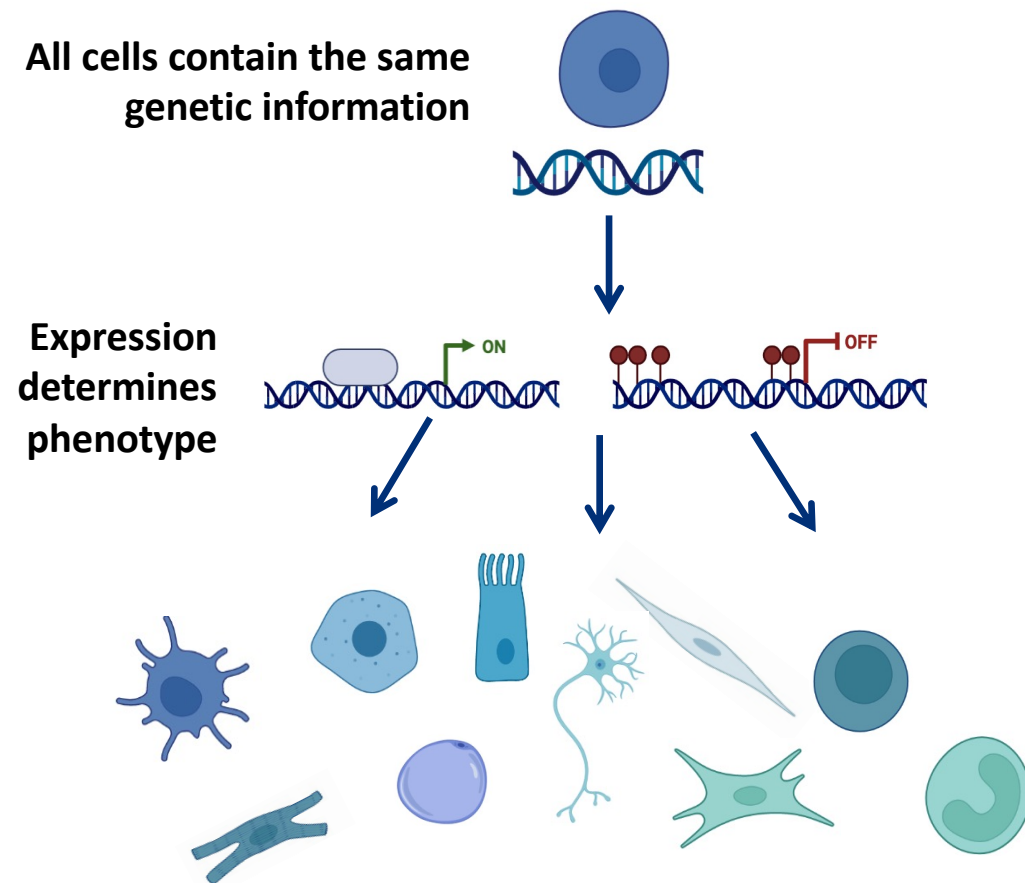
Development of a human PCSK9-targeting epigenetic editor with durable, near-complete in vivo silencing

Aron Jaffe, SVP, Head of Research

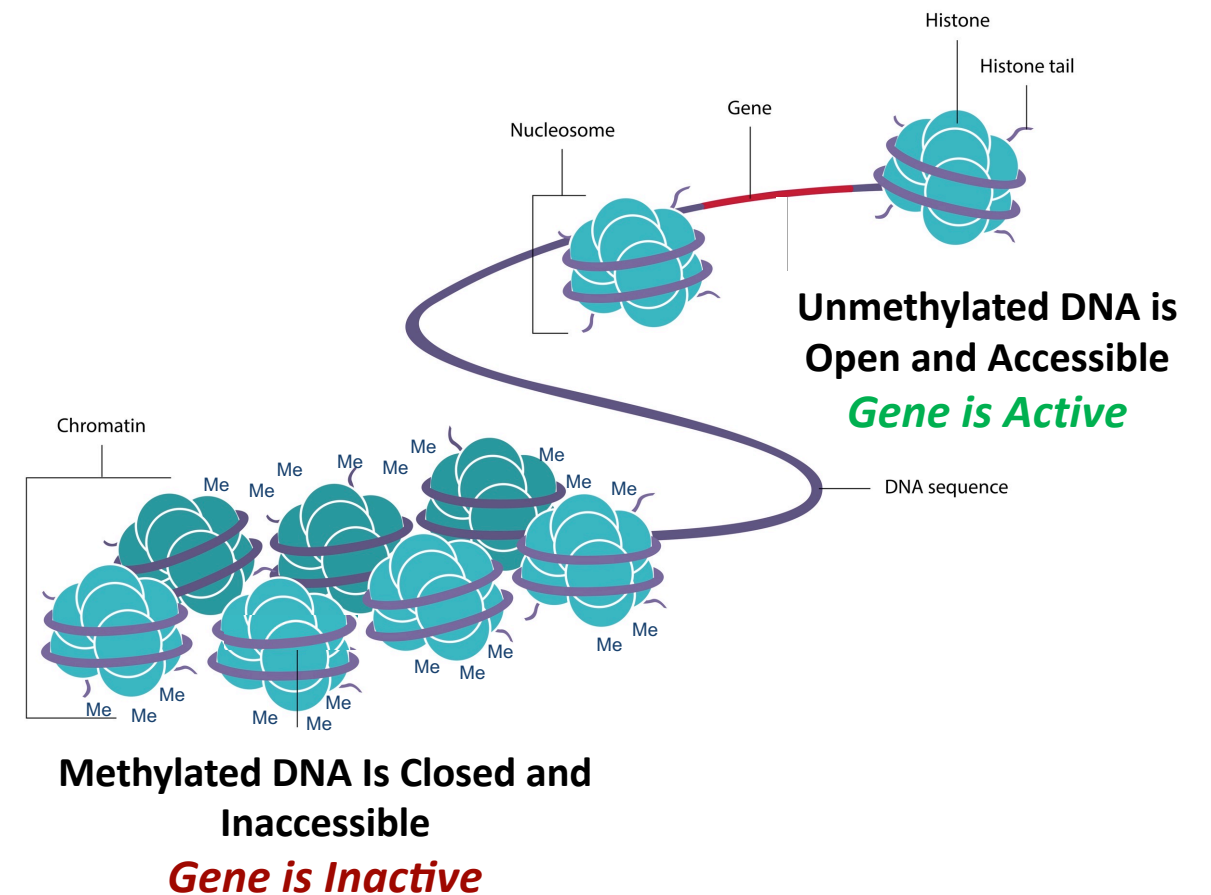


Epigenetics regulates gene expression to determine cell identity and function

Regulation of gene expression determines cell identity and function

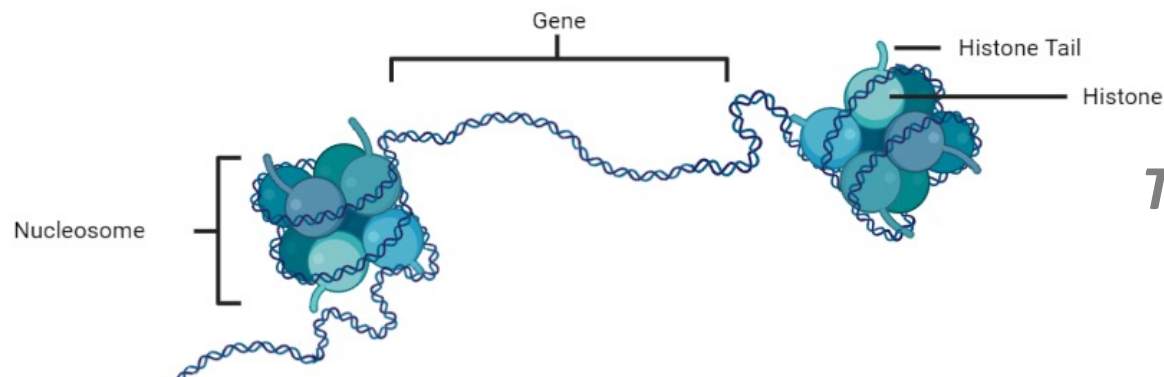


Chromatin packaging and epigenetics regulates DNA transcription



Epigenetic editing leverages the cell's 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

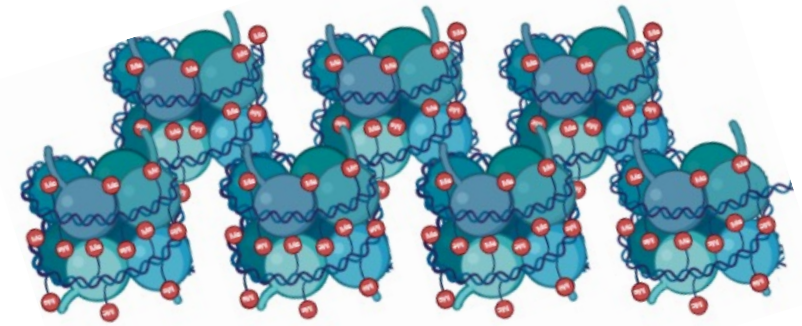


Transient Application



Epigenetic Activator
Demethylates Targets

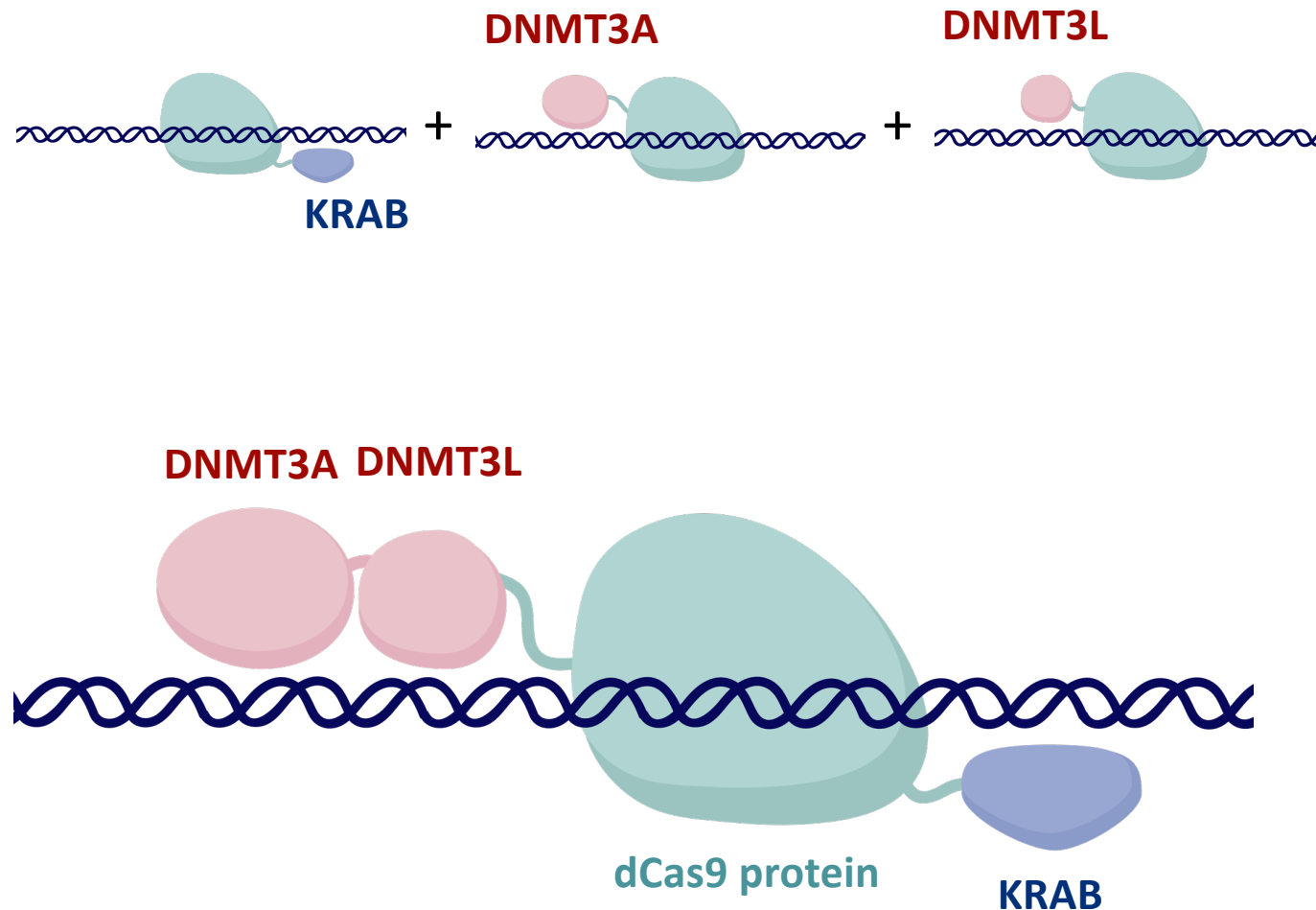
Gene is Inactive
DNA is Closed and Inaccessible



Chroma's epigenetic editors are single fusion proteins with three functional domains

Triple ETR

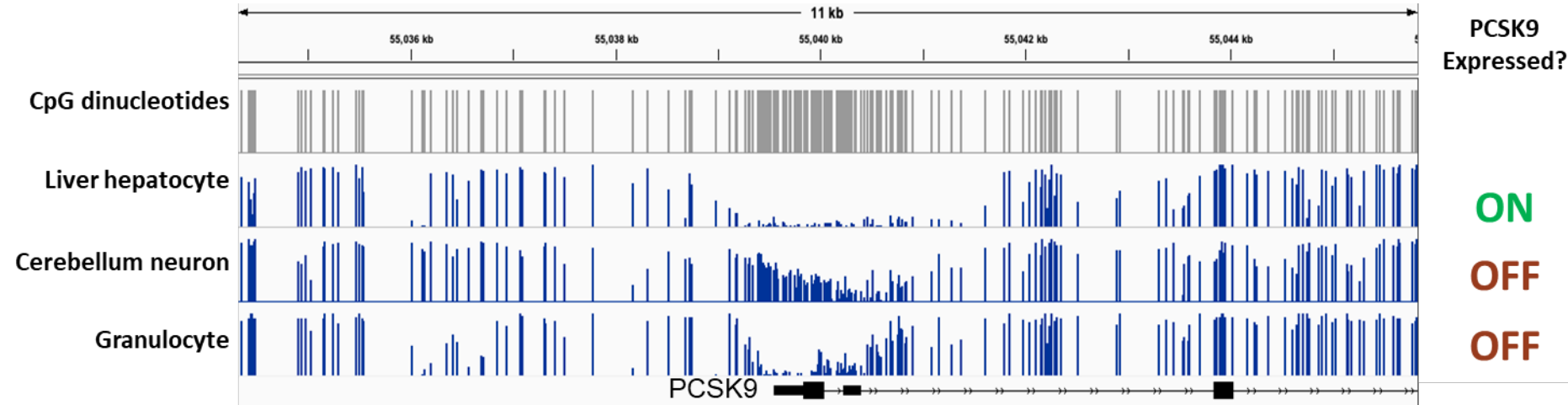
Amabile et al., 2016



Chroma Epigenetic Repressor

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

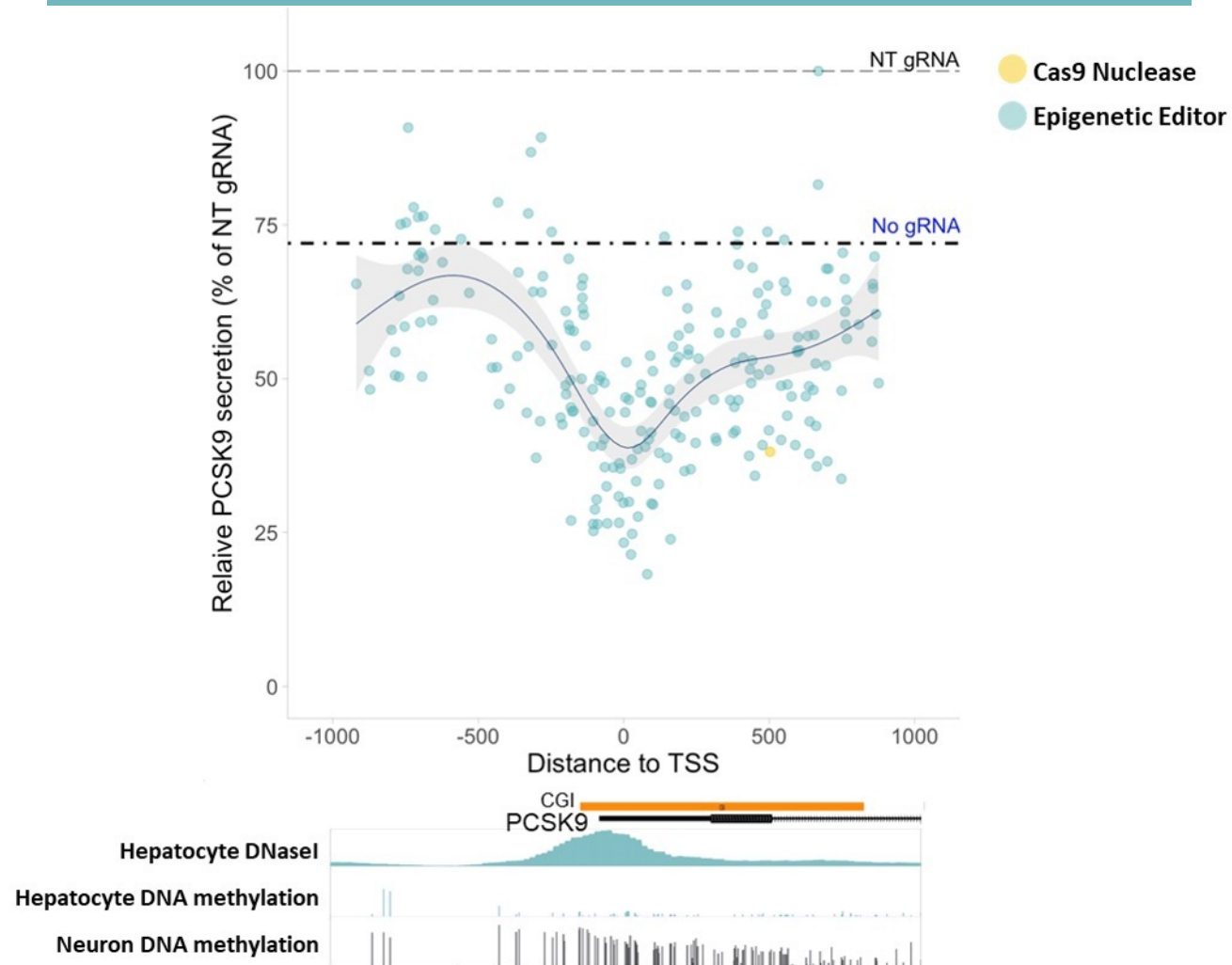
CpG methylation spanning the TSS governs PCSK9 expression across cell types



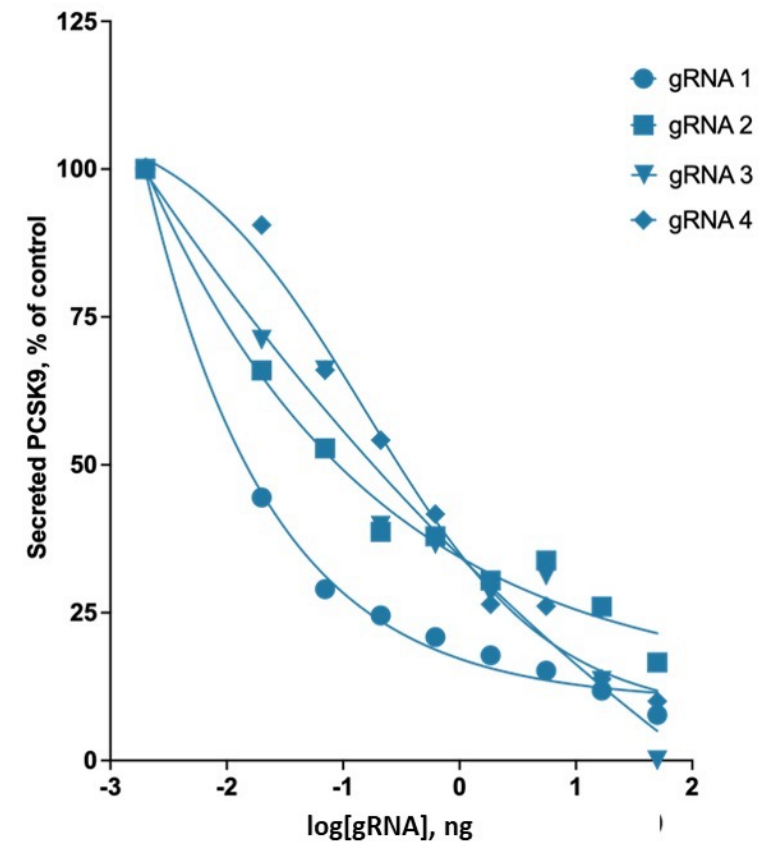
- WGBS analysis of 205 methylomes from 39 different cell types from 137 donors
- Patterns are very consistent across individuals within a cell type (>99.5% identical), and across cell types (>95% identical)
- Focal patterns around promoters and enhancers are the sites of differences across cell types and determine gene expression
- PCSK9 methylation spanning the TSS is inversely correlated with expression

Epigenetic editor screen identified multiple potent PCSK9 gRNAs

PCSK9 gRNA Screen



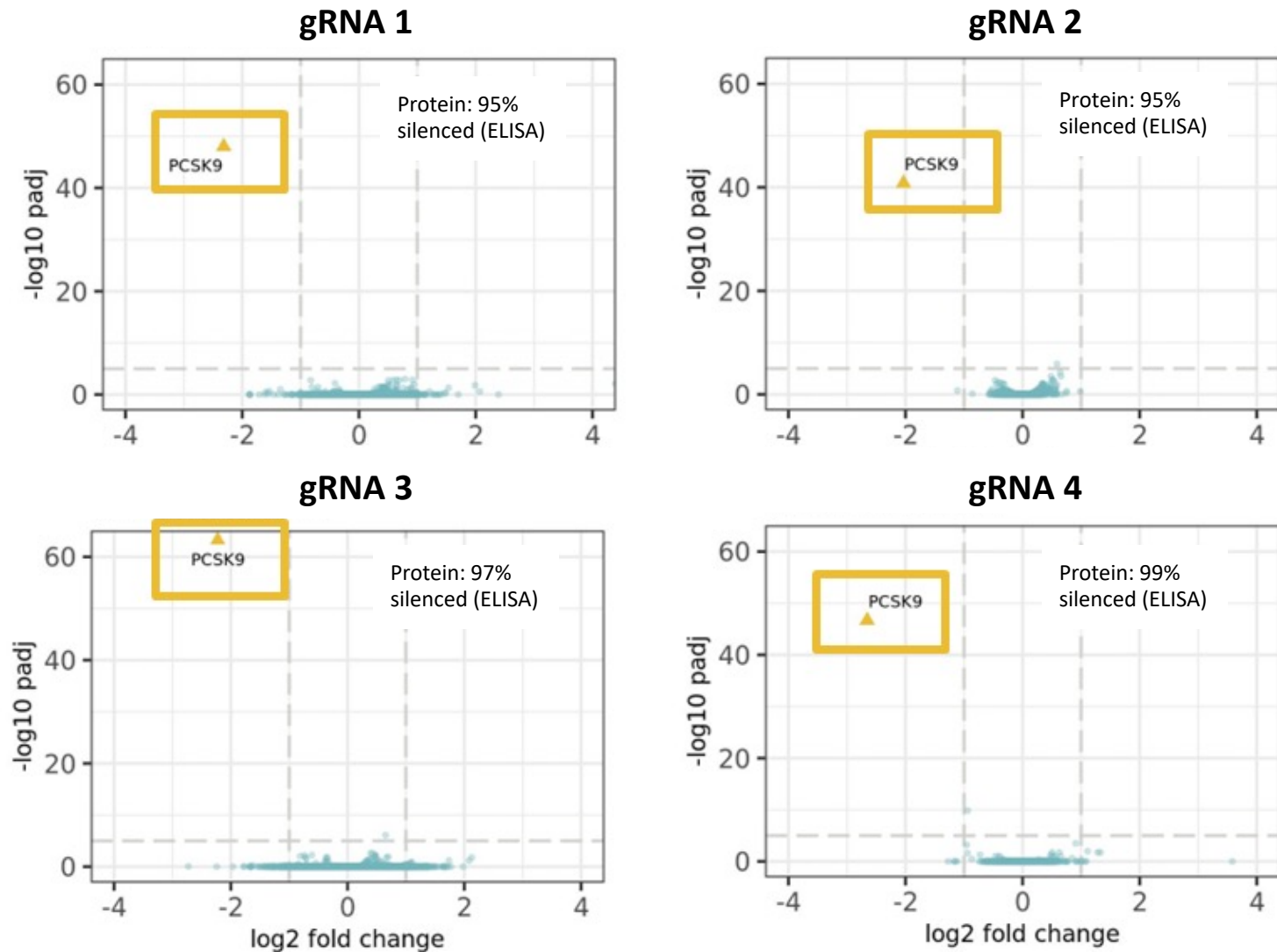
Hit Confirmation: Dose Response



Epigenetic silencing of PCSK9 is highly specific with no off-target changes in expression or methylation

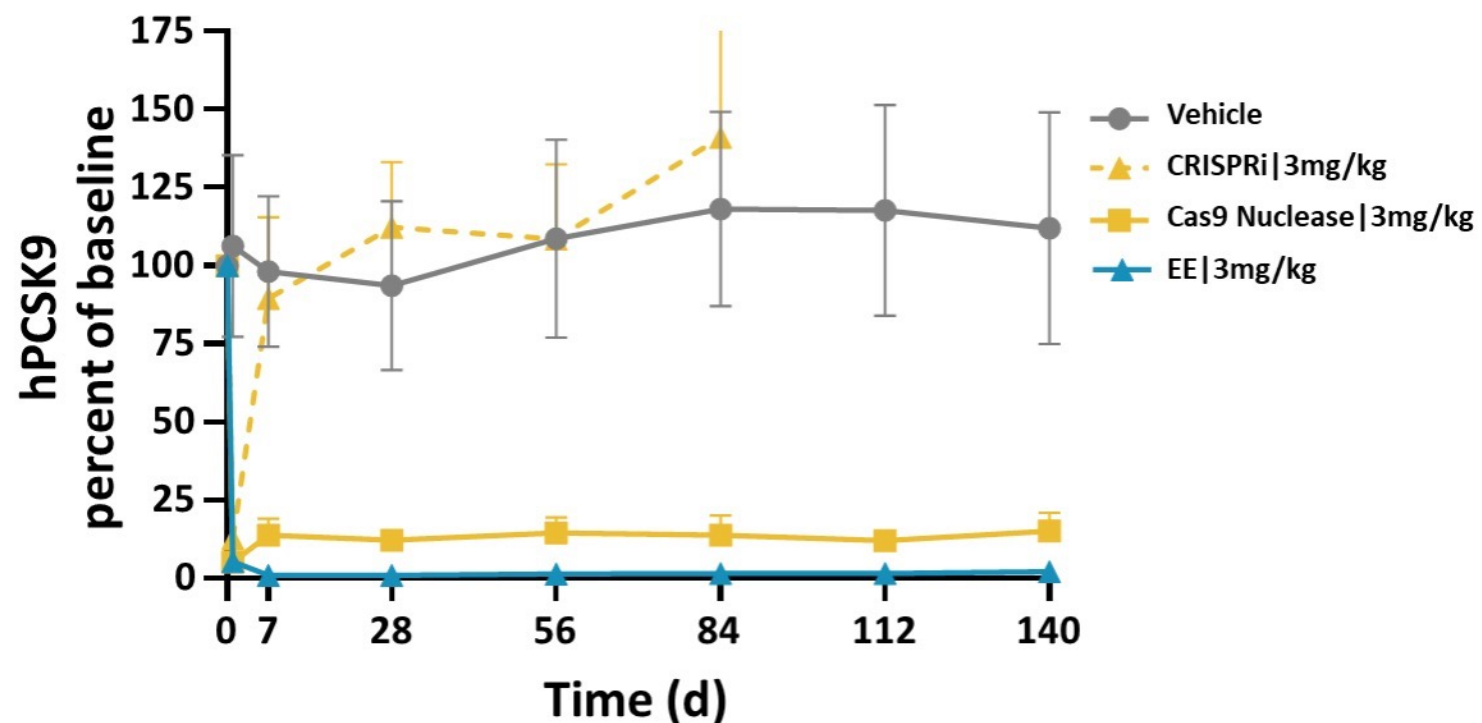
- Lead gRNAs demonstrated high specificity with epigenetic repressor in primary human hepatocytes *in vitro*
 - ✓ No off-target changes in expression (RNA-seq)
 - ✓ No off-target methylation (Illumina Methylation Array, not shown)

Epigenetic Repressor Specificity Analysis: Expression of Targeted vs. Control



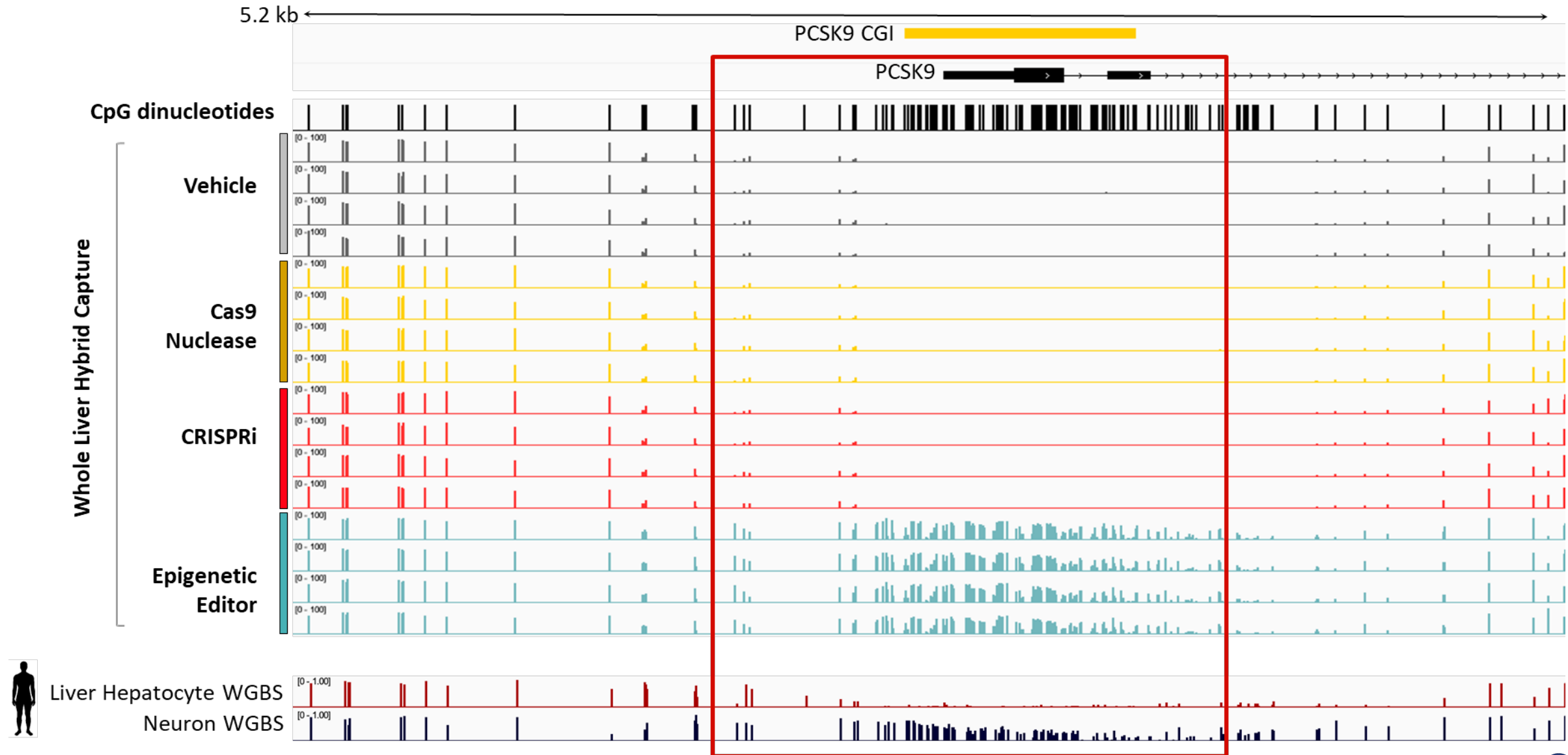
Chroma's epigenetic editors are highly efficacious and durable in vivo

- Transgenic mouse containing human PCSK9 locus
- Controls:
 - Cas9 nuclease: durable, 90% reduction of PCSK9
 - CRISPRi: transient reduction of PCSK9
- **Epigenetic editor achieves 99% silencing, durable at least 5 months post-dose**



Chroma's epigenetic editor induces stable, targeted CpG methylation at the human PCSK9 TSS in vivo

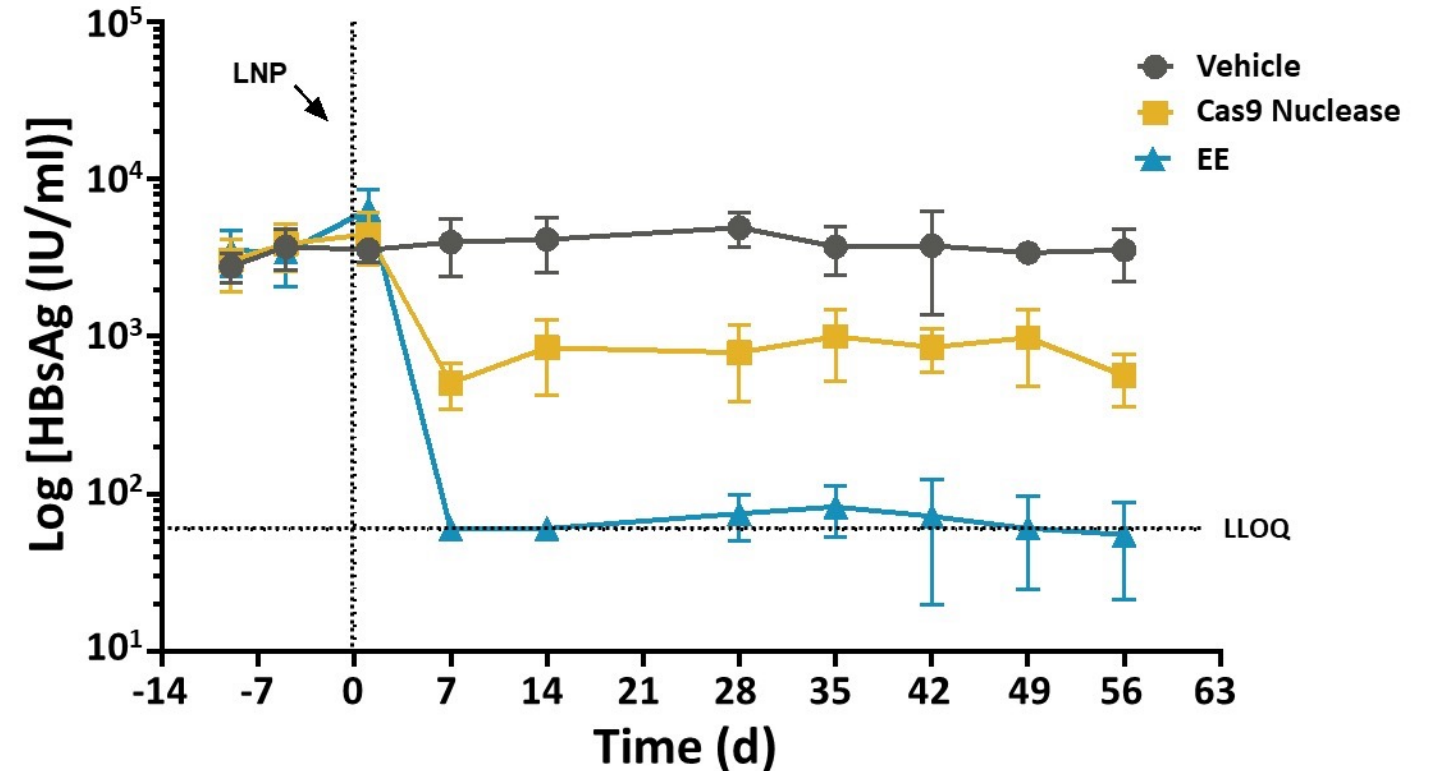
Methylation of PCSK9 locus at Day 28



Chroma's epigenetic editors are easily reprogrammable

Robust and durable reduction of HBV markers in Tg-HBV mouse model

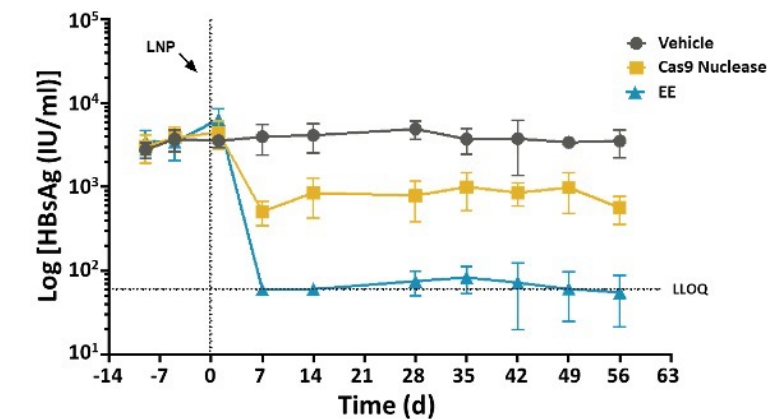
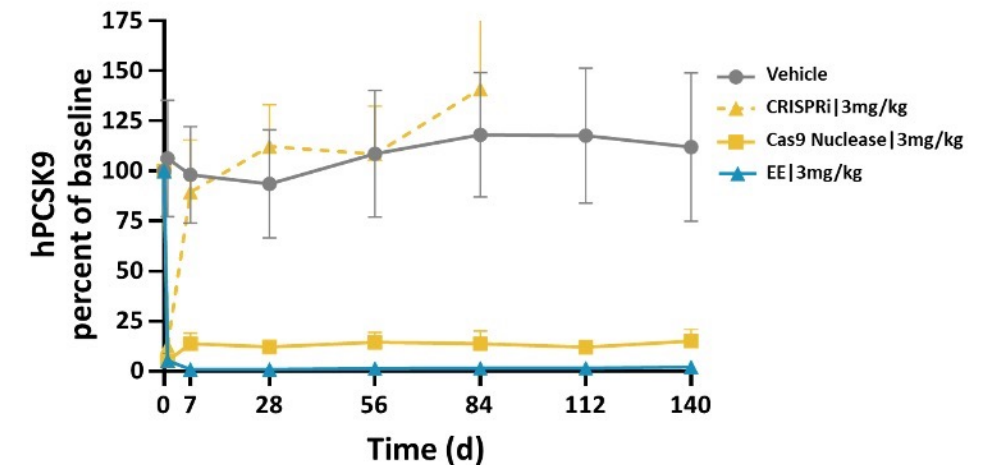
- Editor retargeted to HBV by changing gRNA sequence
- Transgenic mouse containing the HBV virus
- Effective and durable reduction of HBsAg (below LLOQ)
- Similar reduction seen in circulating viral HBV DNA



Summary

- Epigenetic editing leverages an endogenous mechanism for regulating gene expression
- Chroma's epigenetic editors robustly and durably silence multiple targets in vivo
 - Prototype epigenetic editor drove robust silencing of mPCSK9, durable through partial hepatectomy (Cappellutti et al., ASGCT 2023)
 - Chroma's PCSK9 epigenetic editor highly specific
 - PCSK9 epigenetic editor achieved durable, 99% silencing in vivo
 - HBV-targeted epigenetic editor achieved durable silencing of HBsAg in vivo below the LLOQ

Chroma epigenetic editor robustly and durably silences multiple targets in vivo



Acknowledgements

Thank you to the entire Chroma team and our partners!



CHROMA
MEDICINE

