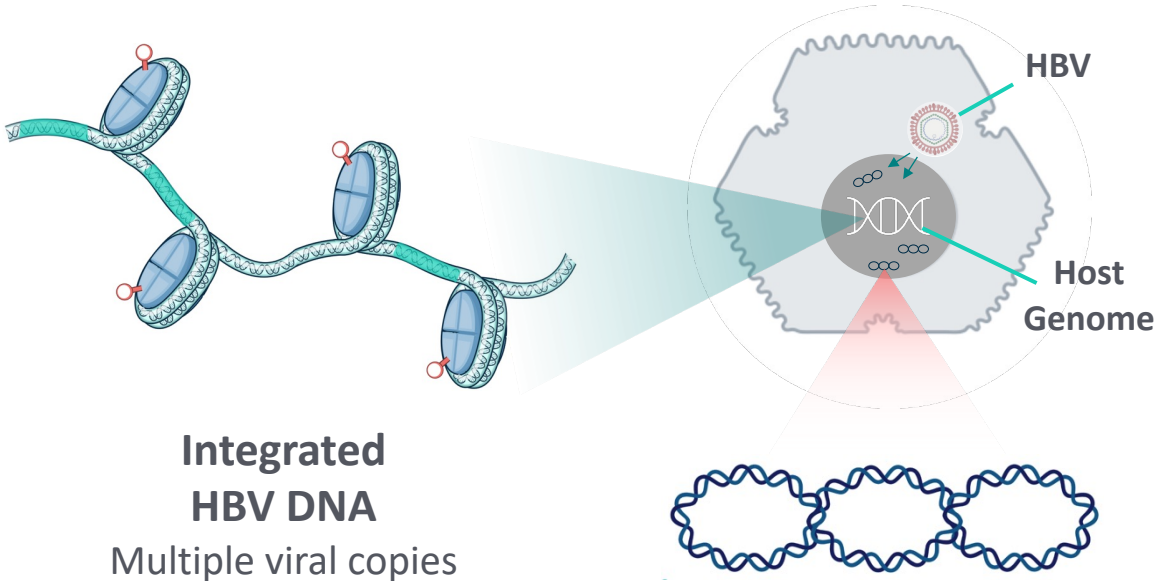
# Epigenetic editing for the treatment of HBV

## Tune Therapeutics

Brian Cosgrove, PhD

Hep-DART, December 5th, 2023

# The persistent challenge of Chronic Hepatitis B infection




**Integrated HBV DNA**
Multiple viral copies integrate into host genome, driving HBs antigen burden

**HBV cccDNA**
Source of viral replication and latent infection

**< 5%**
**of patients**
reach functional cure[1] with SOC

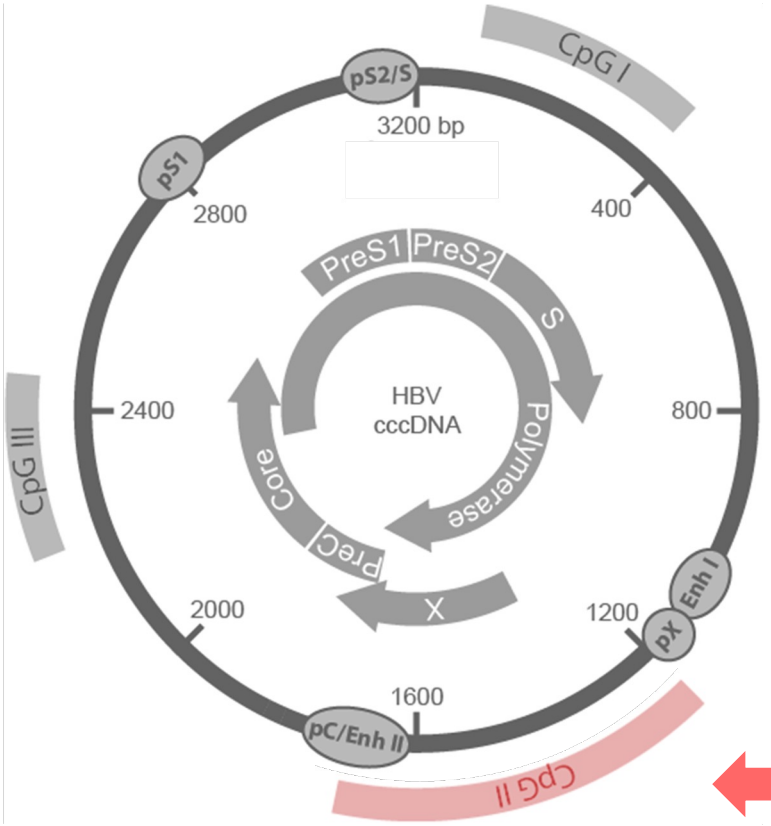
**Challenges of current and pipeline therapies include:**

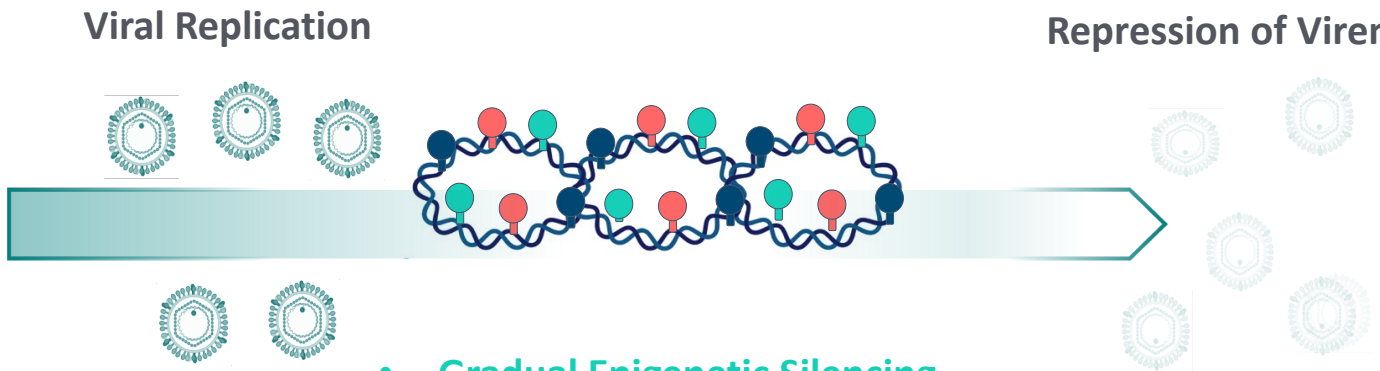
- Lack of cccDNA targeting
- Off-target safety
- Low response rates
- Low durability off-treatment
- Requirement for immune reactivation

**Clearance or Inactivation of cccDNA**
is widely regarded as crucial for curing chronic HBV[2,3]

[1] *EASL HBV Guidelines, 2017*
[2] *Nassal et al., Gut (2015)*
[3] *Lucifora & Protxer, J. Hepatol. (2015)*

# Hepatitis B viral replication is controlled by epigenetics




- **Gradual Epigenetic Silencing in Some Patients**[1-3]
- **IFNα Functions Partially Through Epigenetic Mechanisms**[4]

**Key Epigenetic Regions:**

The HBV genome is rich in CpG islands, with the **methylation of CpG island II** correlating with HBeAg status and reduced HBsAg expression in patients.[5]

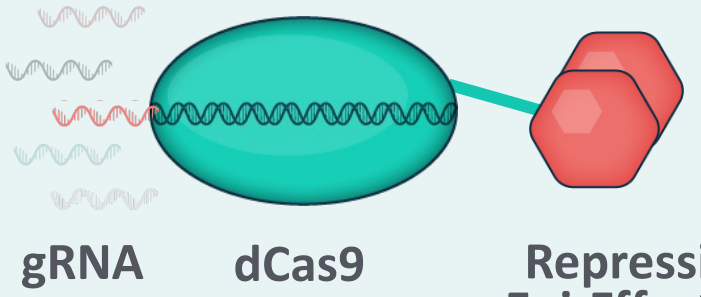
*Figure Adapted From:*

*Dandri et al. Sem Immunopathology (2020)*

*[1] Suslov et al., J Hepatology (2021) [2] Pollicino et al., Gastroenterology (2006) [3] Lebosse et al., Scientific Reports (2020)*

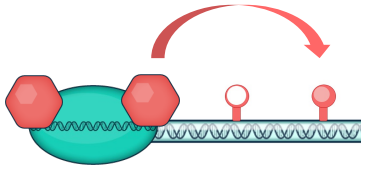
*[4] Belloni et al., J Clin Invest (2012) [5] Vivekanandan et al., Clin Infect Dis (2008)*

# Precise epigenetic silencing using the TEMPO platform

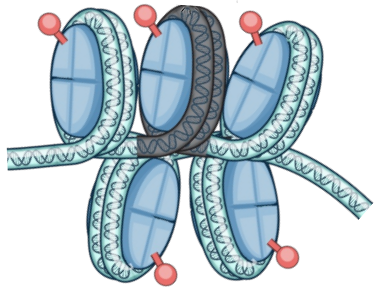



**TEMPO** directs...

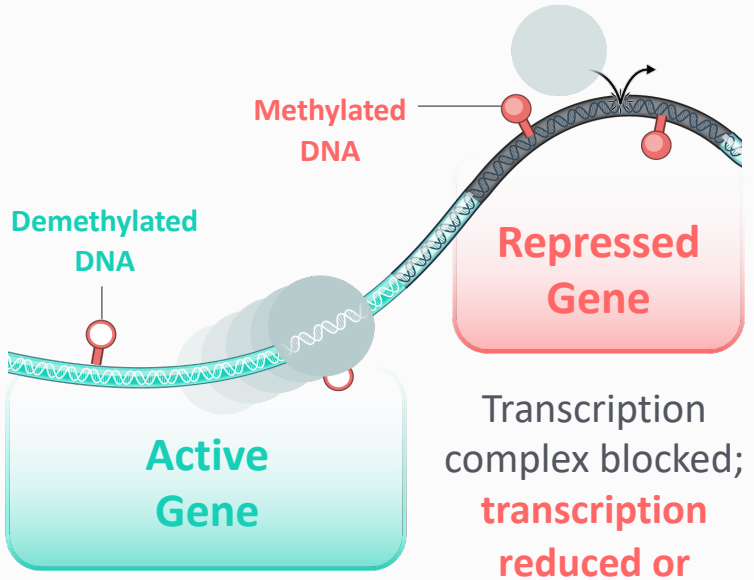
**targeted control of gene expression via epigenetic modulation**
(without cutting or nicking of DNA)



Targeted DNA methylation



Heterochromatin formation

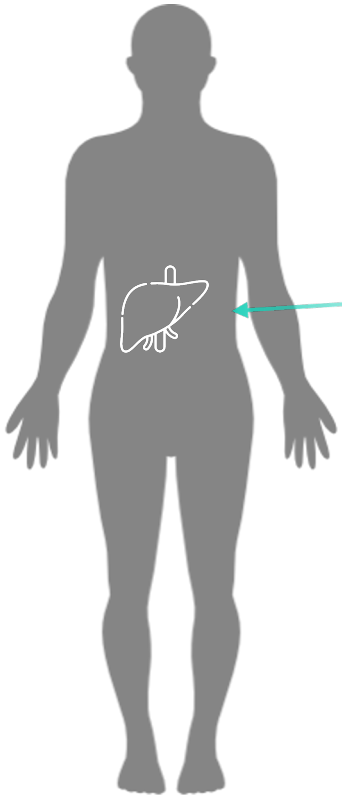


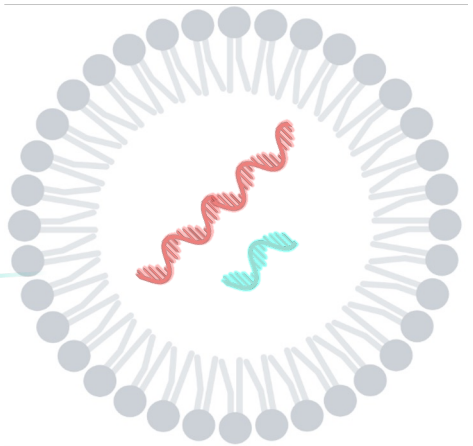

Transcription complex binds and initiates; **transcription active**

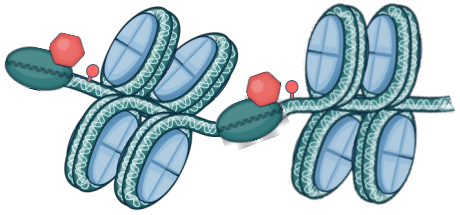
Transcription complex blocked; **transcription reduced or silenced**

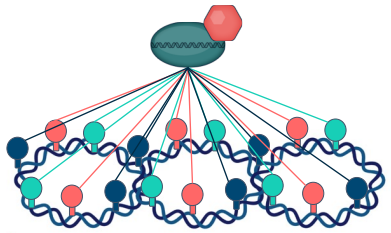
# Epi-silencing strategy targets integrated HBV *and* cccDNA

## In vivo delivery with LNP







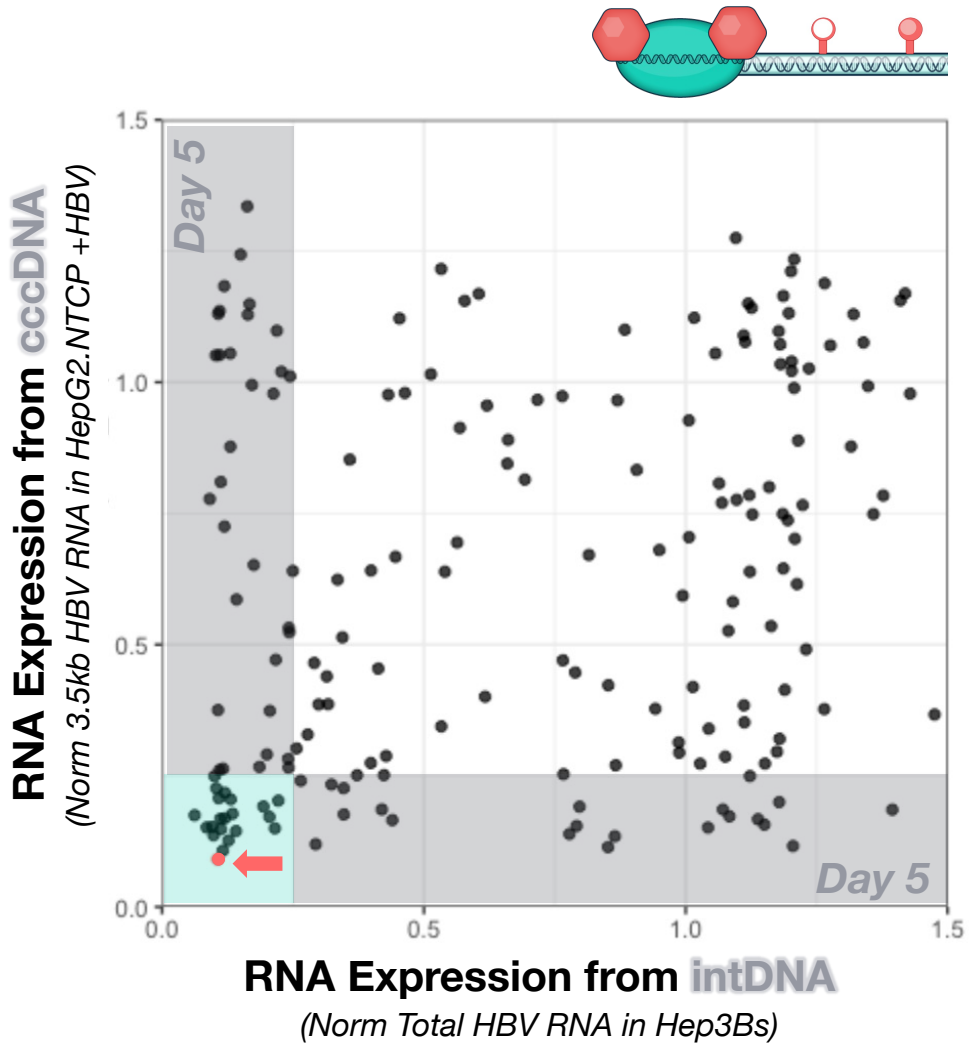


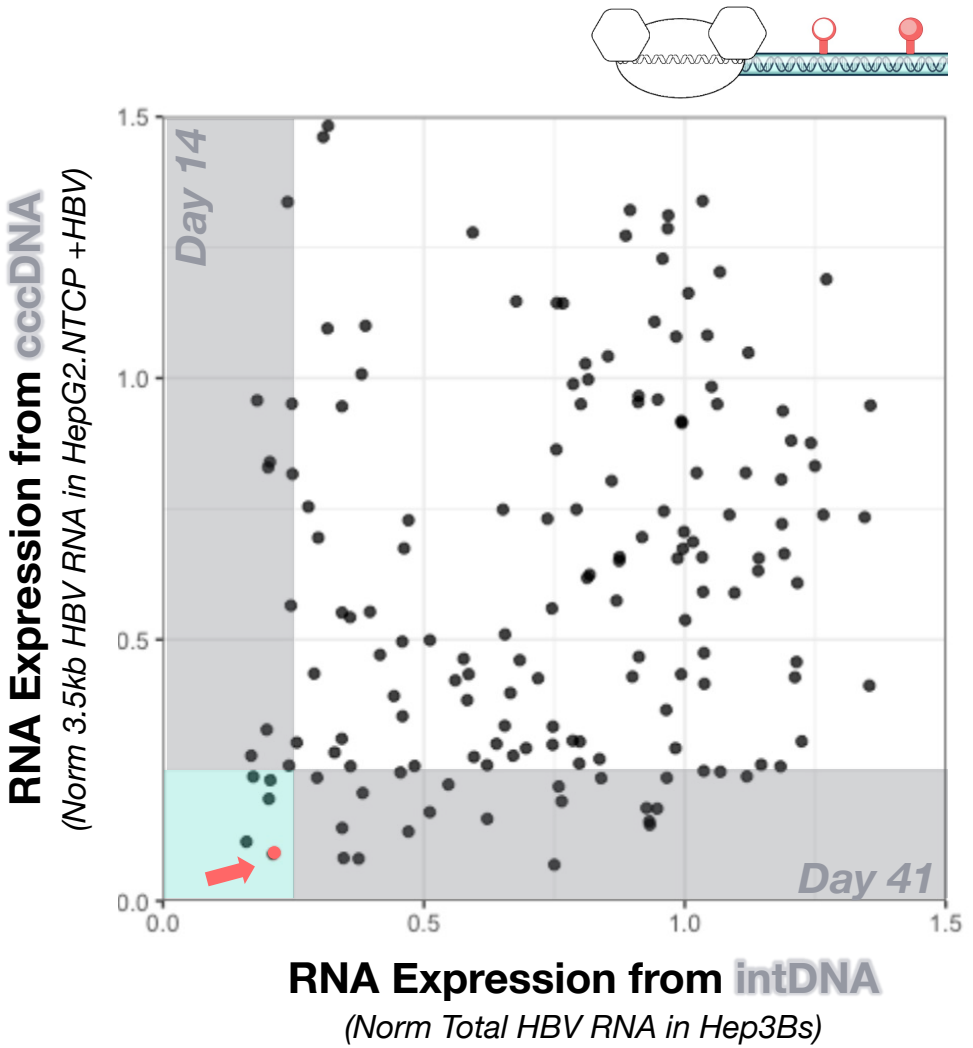
**Lipid nanoparticles (LNP)**
Ideal transient delivery vehicles for liver targets. Payload includes:

- **mRNA-encoded epi-silencer protein**
- **gRNA targeting HBV**

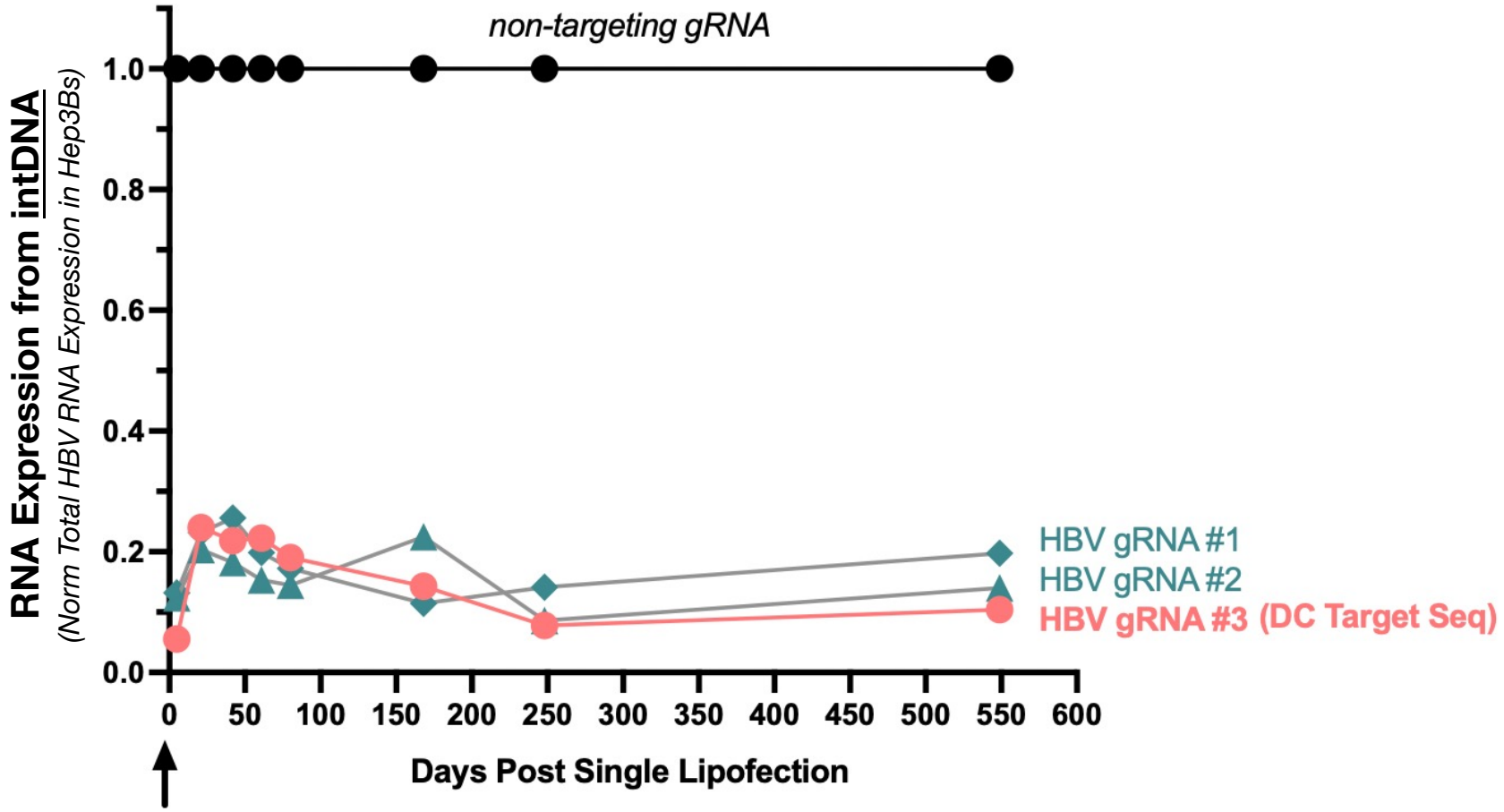
- **Transient delivery of epi-editor,** with durable effects
- **Direct & specific silencing of HBV DNA** at both cccDNA and intDNA
- **Durable repression without immune system engagement**

# Screening reveals gRNA targets active across both cccDNA *and* intDNA HBV contexts



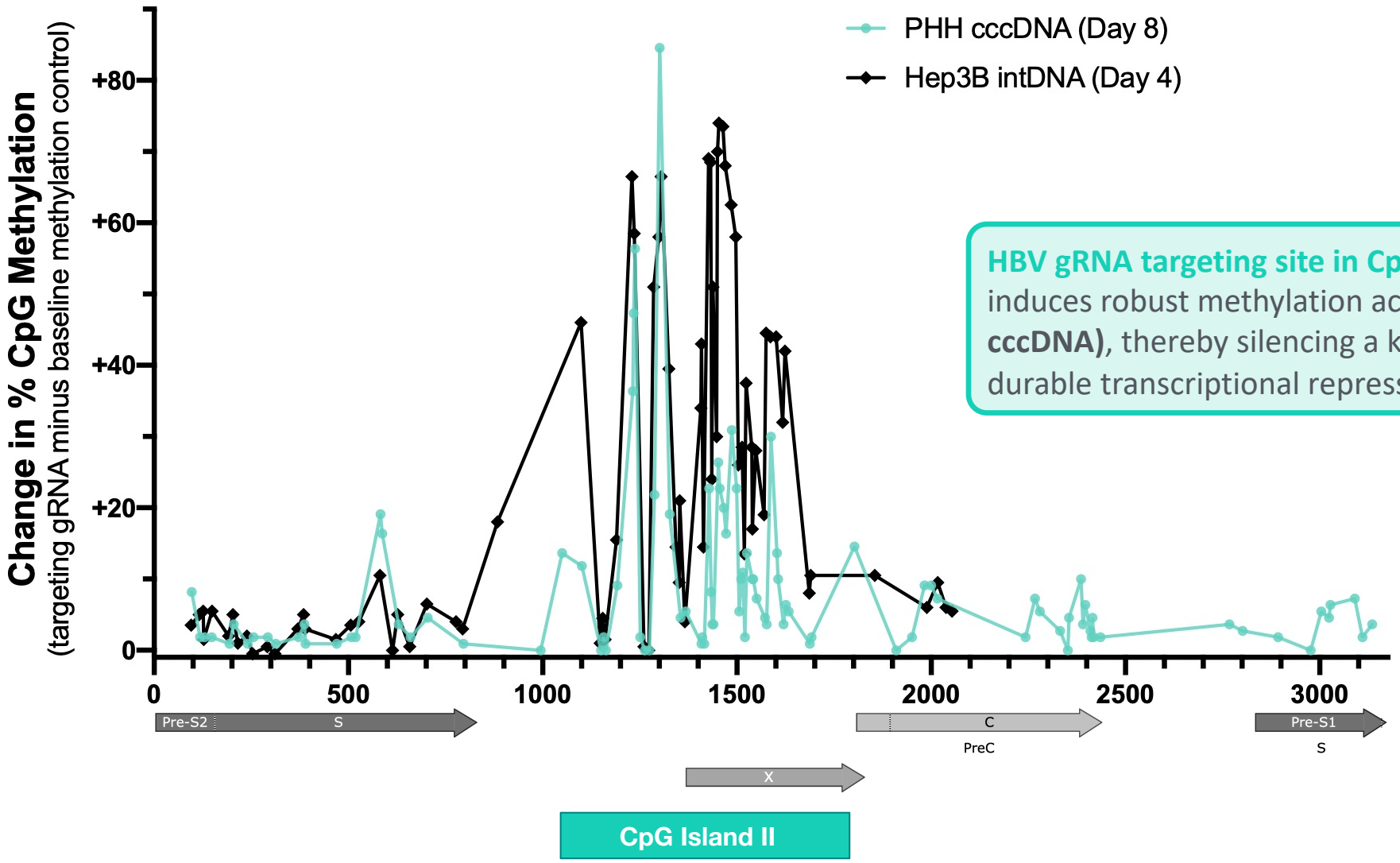



# Epi-silencing achieves durable repression of intDNA following a single transient lipofection




Multiple gRNAs maintain therapeutically-viable repression levels from integrated HBV DNA after **550 days** and **275+ cell divisions**

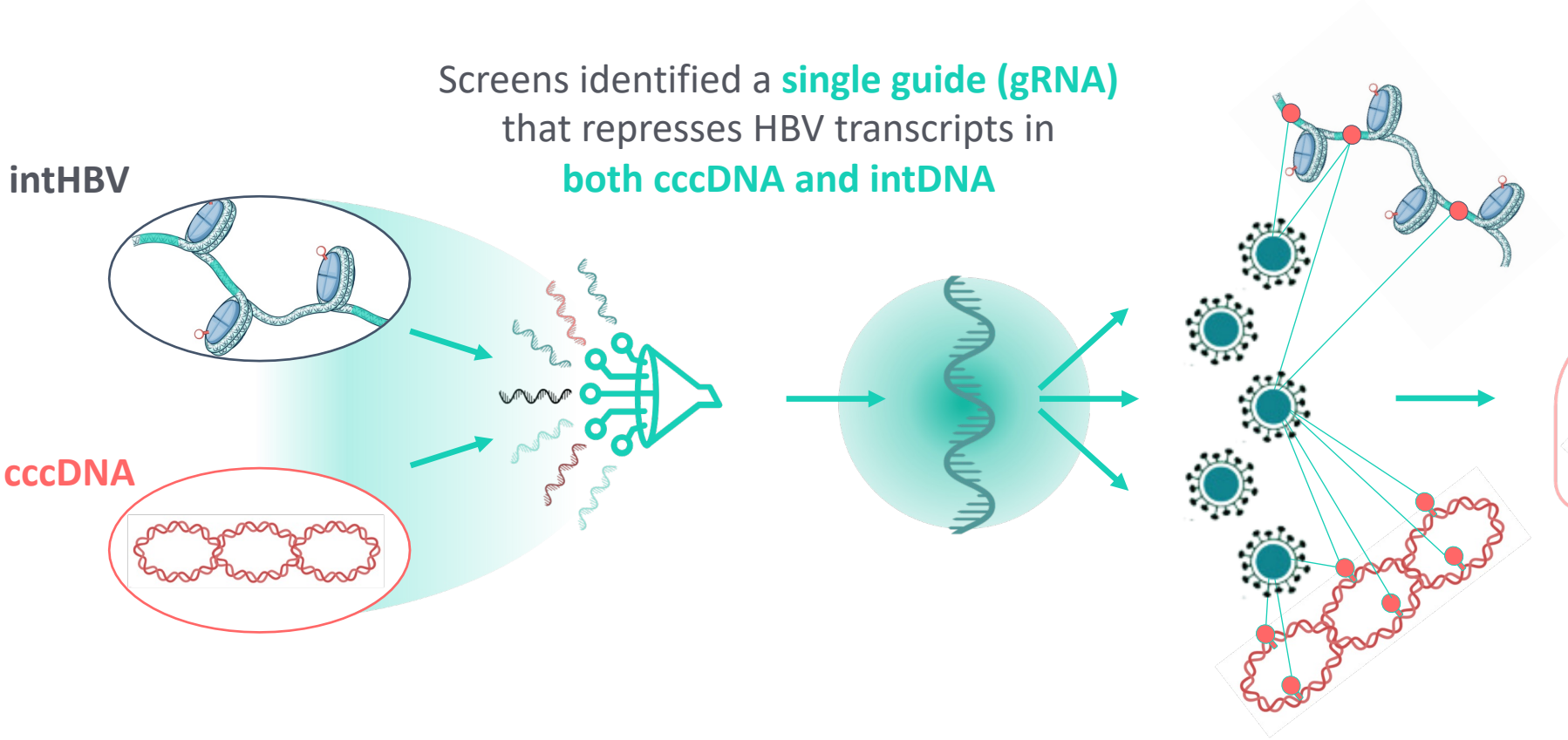
# Similar patterns of methylation deposited across both cccDNA and intDNA contexts



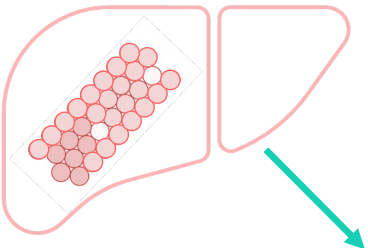

**HBV gRNA targeting site in CpG Island II**
induces robust methylation across **CpG Island II (in both intDNA and cccDNA)**, thereby silencing a key regulatory region and and enabling durable transcriptional repression across the entire viral genome

# Screening and selection of TUNE-401 discovery candidate for treatment of Chronic Hepatitis B

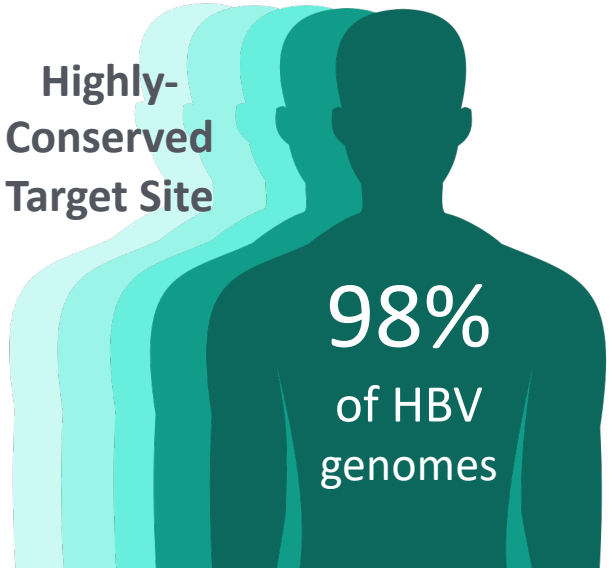
Screens identified a **single guide (gRNA)** that represses HBV transcripts in **both cccDNA and intDNA**




**Homogenous effect** across depots/cells and increased viral repression



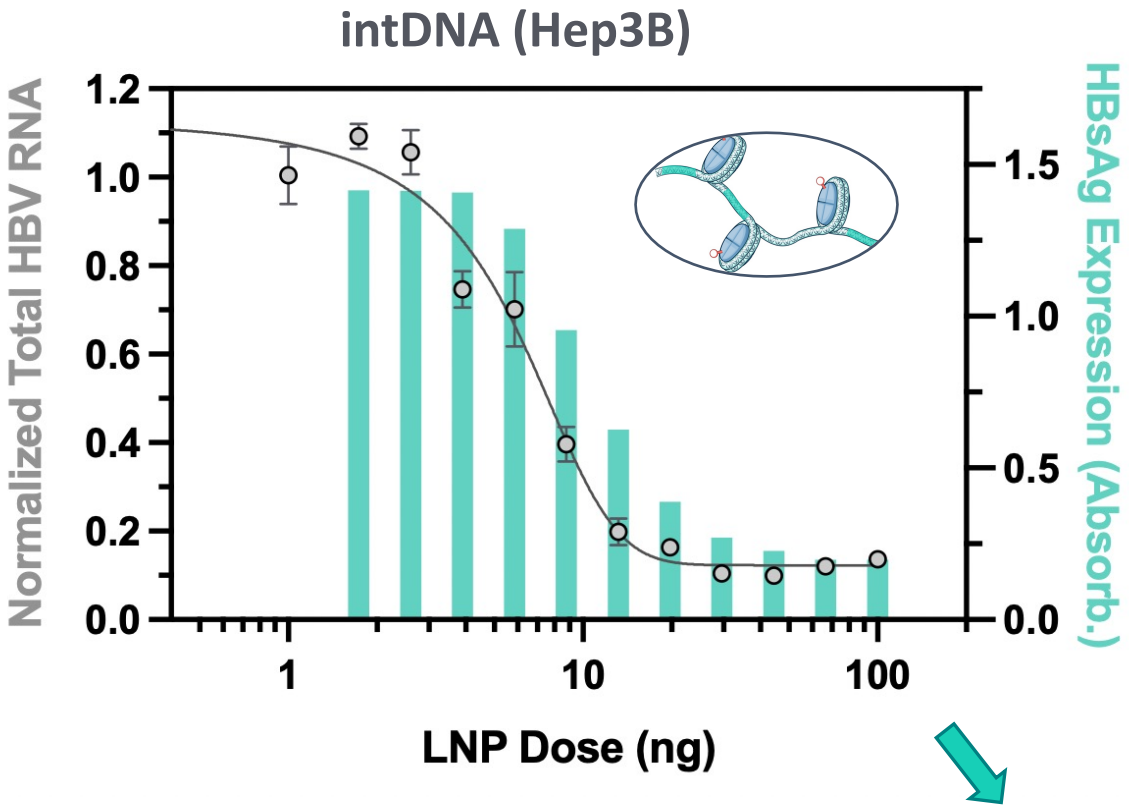
**Highly-Conserved Target Site**

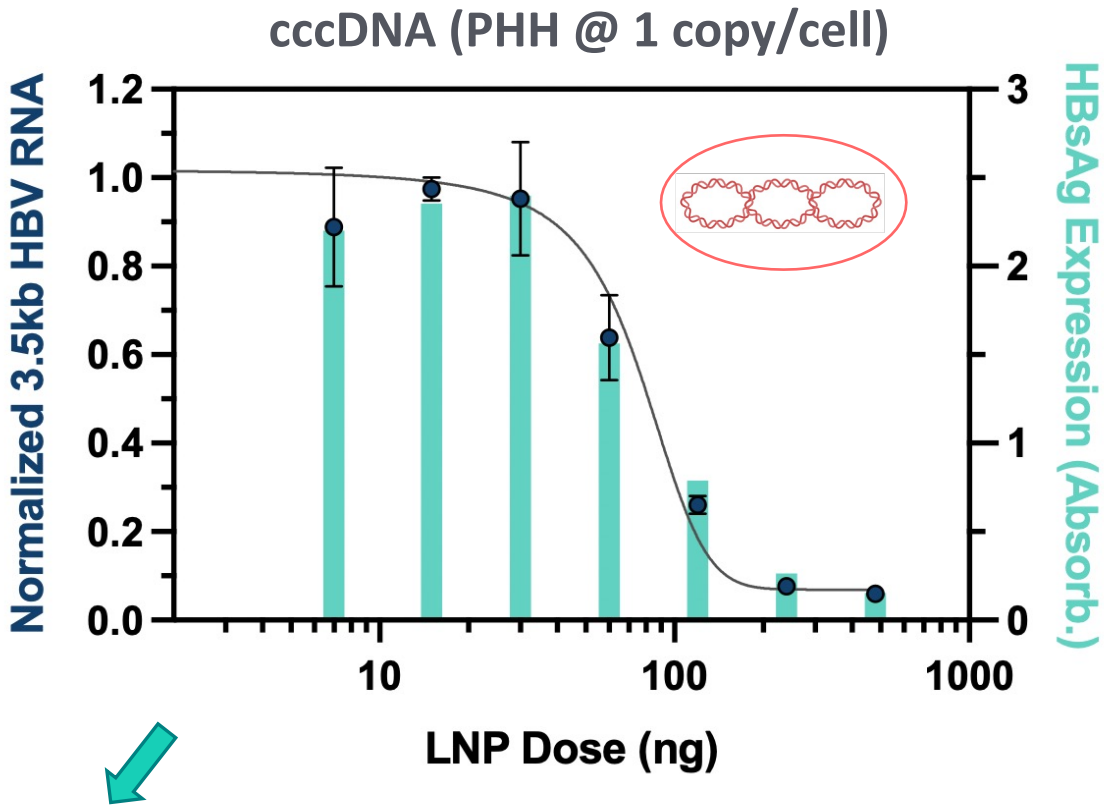



**Multi-system screening approach** cross-references both integrated HBV transcripts and viral cccDNA depots in cell lines and PHH

Using a **single gRNA** ensures **more robust LNP loading and delivery**

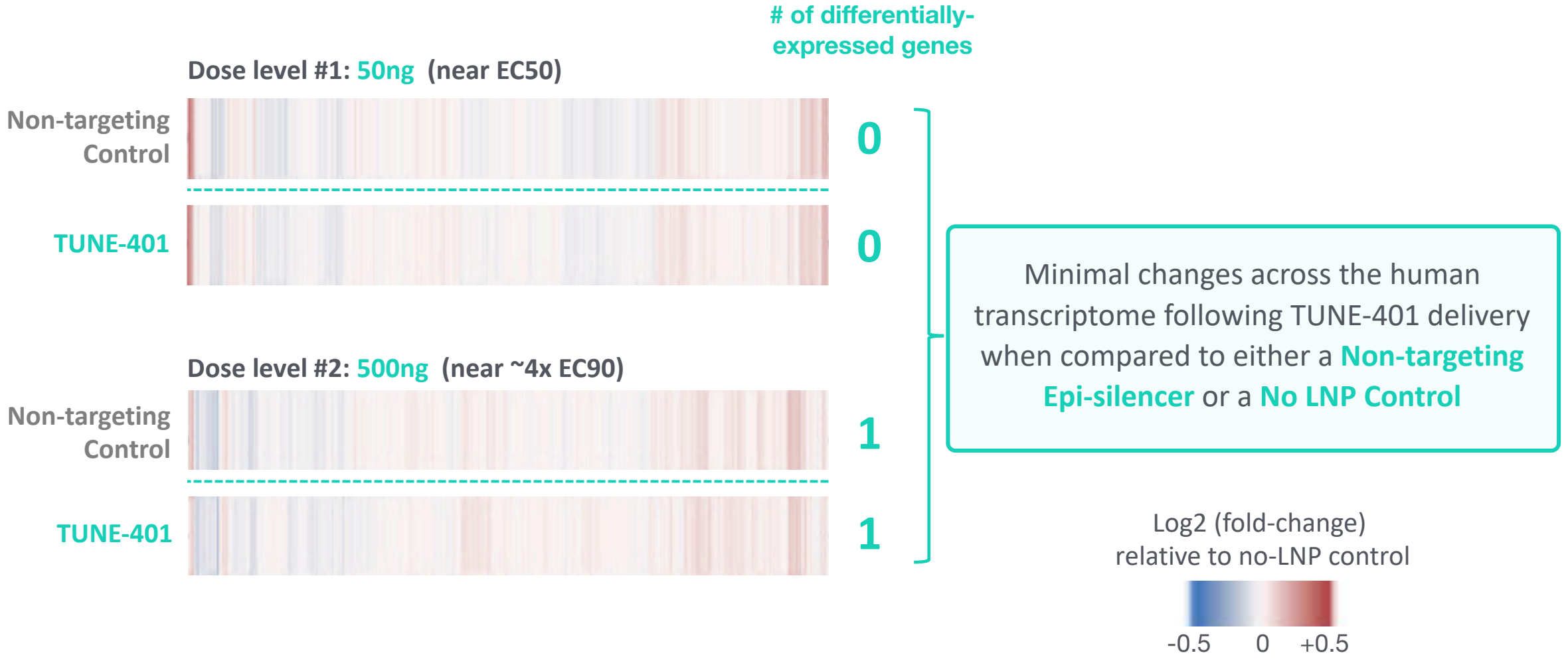
# Near-complete repression of transcription from HBV DNA sources with *in vitro* LNP delivery of TUNE-401




**~90-95% peak repression** seen across both **intDNA *and* cccDNA**, which represents **near-complete repression** when adjusted for max delivery efficiency per cell type

# RNA-Seq across the human transcriptome in uninfected PHH shows TUNE-401 is highly specific to HBV targets



# *In vitro* efficacy of TUNE-401 supports strong ability to precisely silence the HBV genome

**Depth of effect**

***90-95% repression*** *across both intDNA and cccDNA*

**Durability**

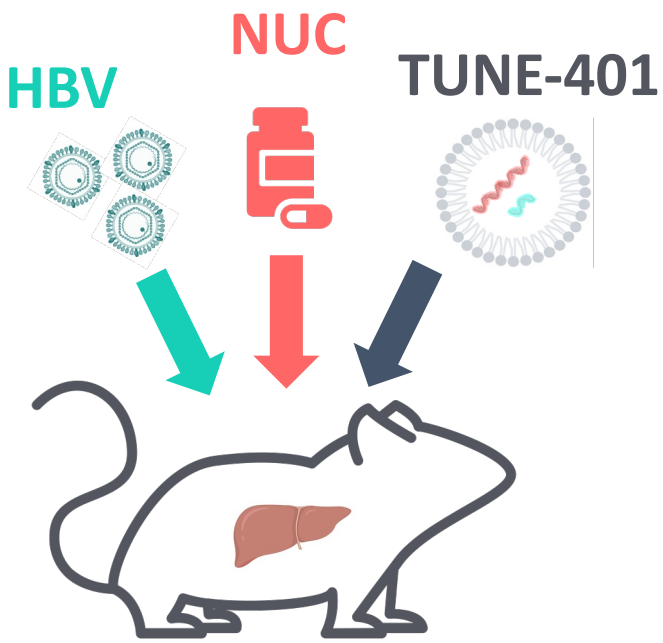
***> 1.5 years/275 doublings*** *for rapidly-cycling cell-types*

**Specificity**

***Highly precise modulation*** *of the HBV genome*

# Modeling cccDNA epi-silencing in a human chimeric liver mouse with HBV infection

Important to examine native cccDNA in human hepatocyte background, as **surrogate models of cccDNA might not have similar epigenetic regulation**

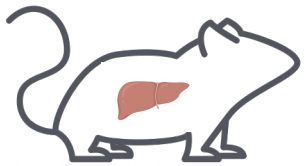



FRG Human Chimeric Liver Mouse + HBV

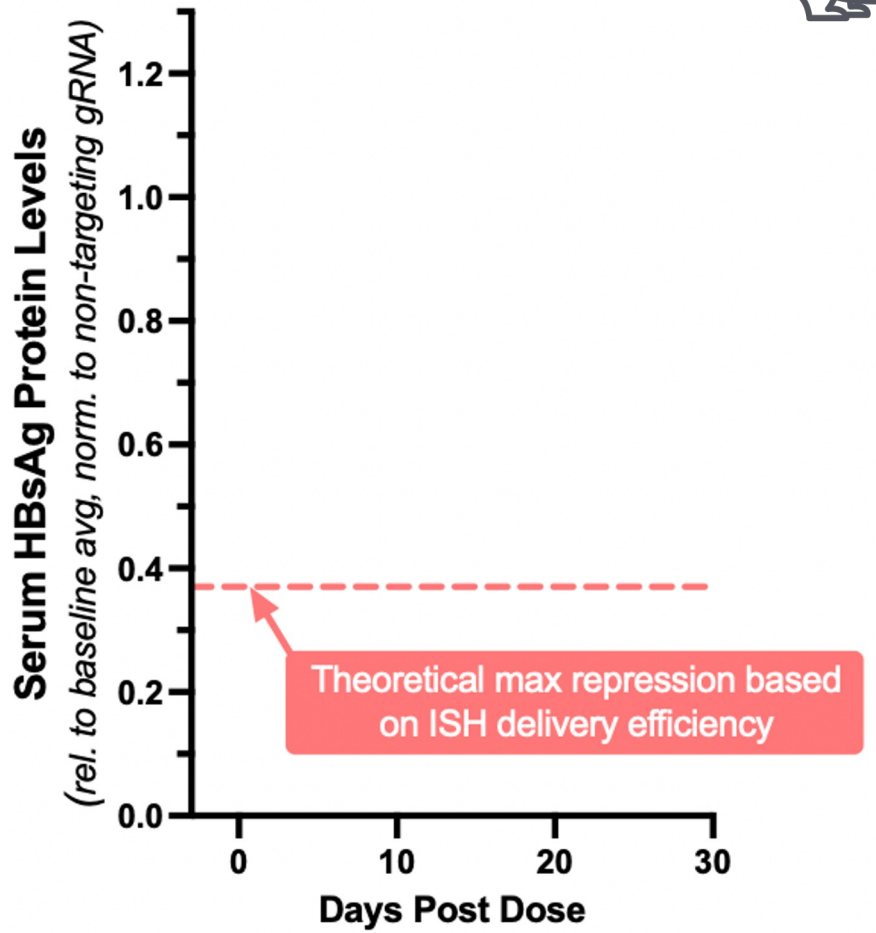
## FRG Chimeric Liver Mouse Study


- **~90% huPHH engraftment, 97% infection**
- **cccDNA @ 2.9 copies/cell** (via T5exo method)
- **HBsAg @ 76k IU/mL**

# Durable and strong silencing of cccDNA following TUNE-401 delivery in HBV-infected chimeric liver mice



LNP formulation in FRG + HBV context had delivery to 63% of Human Hepatocytes on average (via TUNE-401 mRNA ISH)



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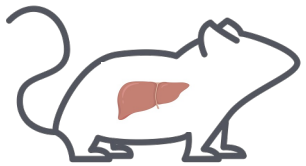
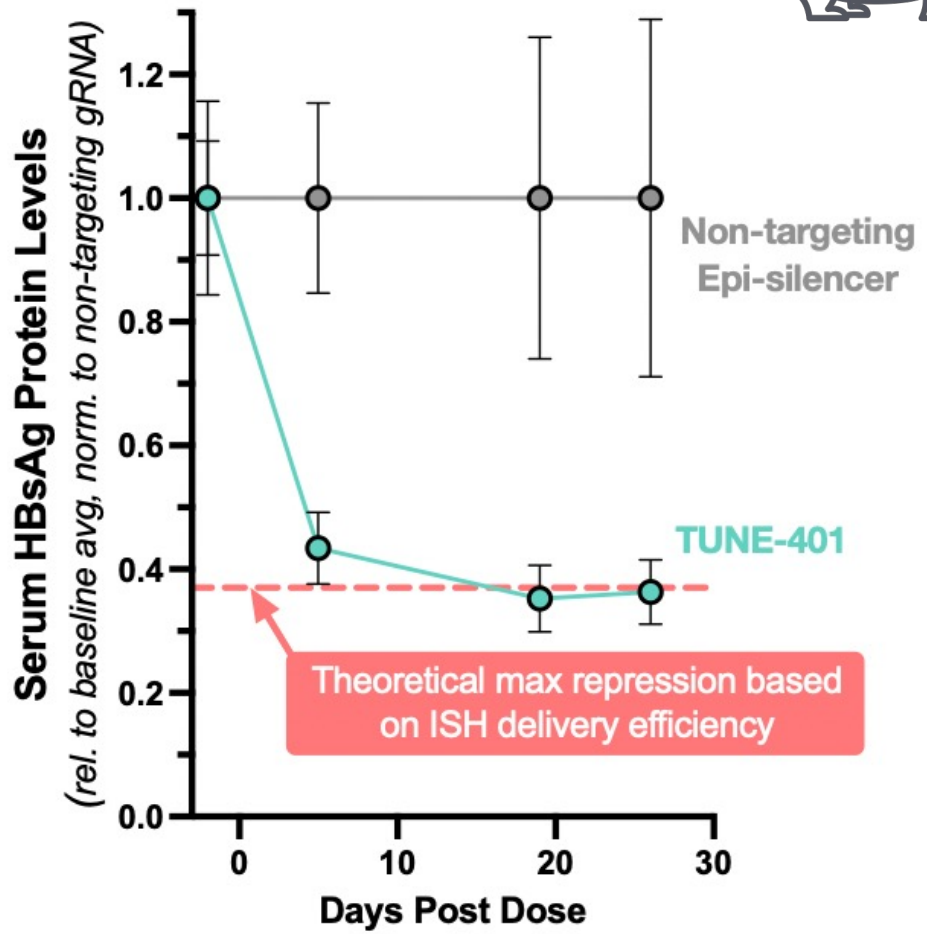


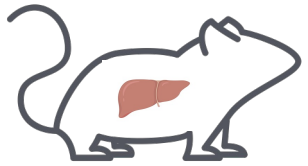
LNP formulation in FRG + HBV context had delivery to 63% of Human Hepatocytes on average (via TUNE-401 mRNA ISH)

Epi-silencer delivery results in **durable repression of cccDNA** (out to max lifespan of model) and data suggests **near-complete repression** in hepatocytes that received LNP



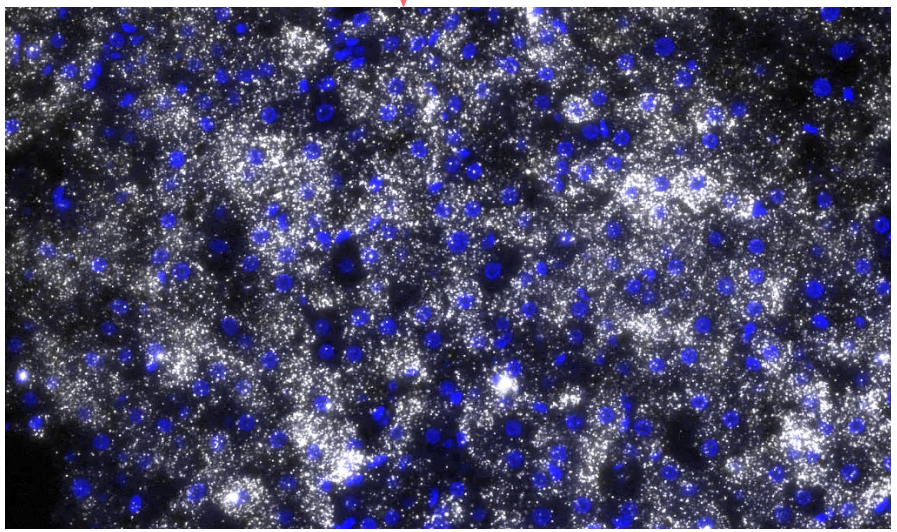

**2 mg/kg LNP dose, N=6 mice/group, Error bars +/- SEM.**

# RNA FISH data confirms near-complete HBV repression in hepatocytes from sub-regions with LNP delivery

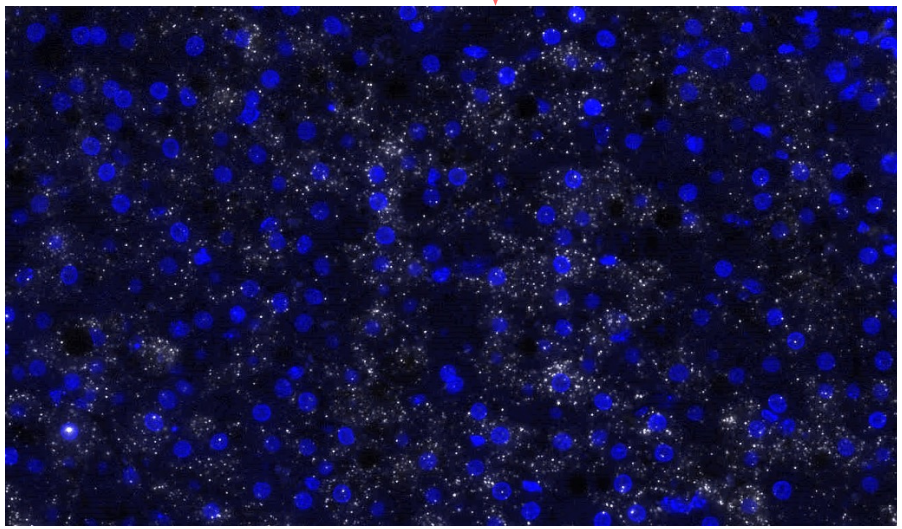


**Analyze hepatocytes in liver sub-regions with confirmed LNP delivery**

**HBV RNA** **Nuclei (any species)**

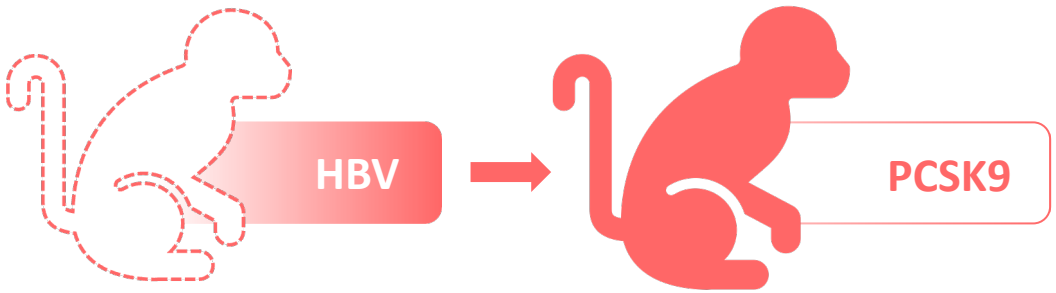


**Non-targeting Epi-silencer**



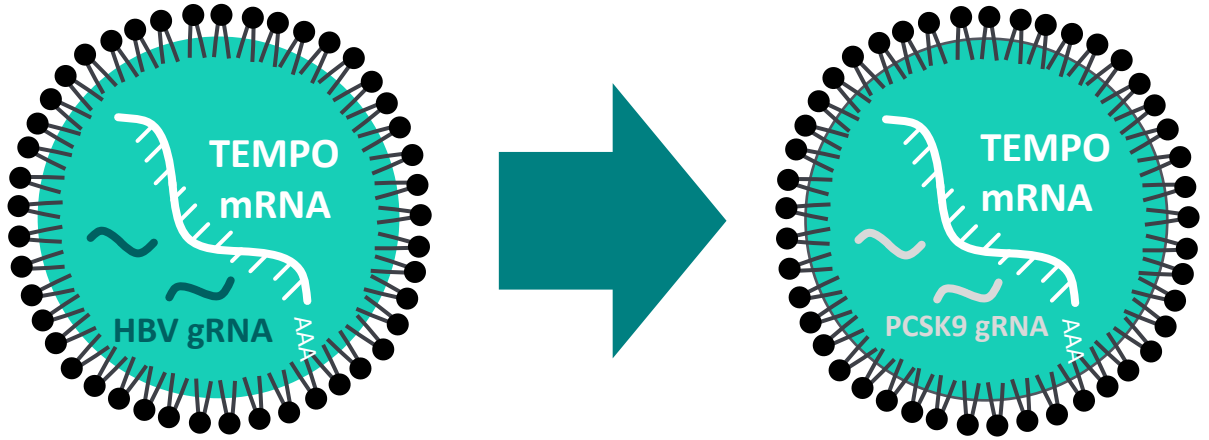
**TUNE-401**

# NHP Repression of PCSK9 a proxy for Integrated HBV Epi-silencing and for evaluation of safety/efficacy




No large animal model available

Surrogate liver target in established NHP model




**TUNE-401**
Lipid Nanoparticle (LNP)

**PCSK9 Surrogate**
Lipid Nanoparticle (LNP)

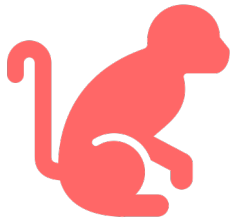
## Same mode of epigenetic action for repressing a gene in host chromatin

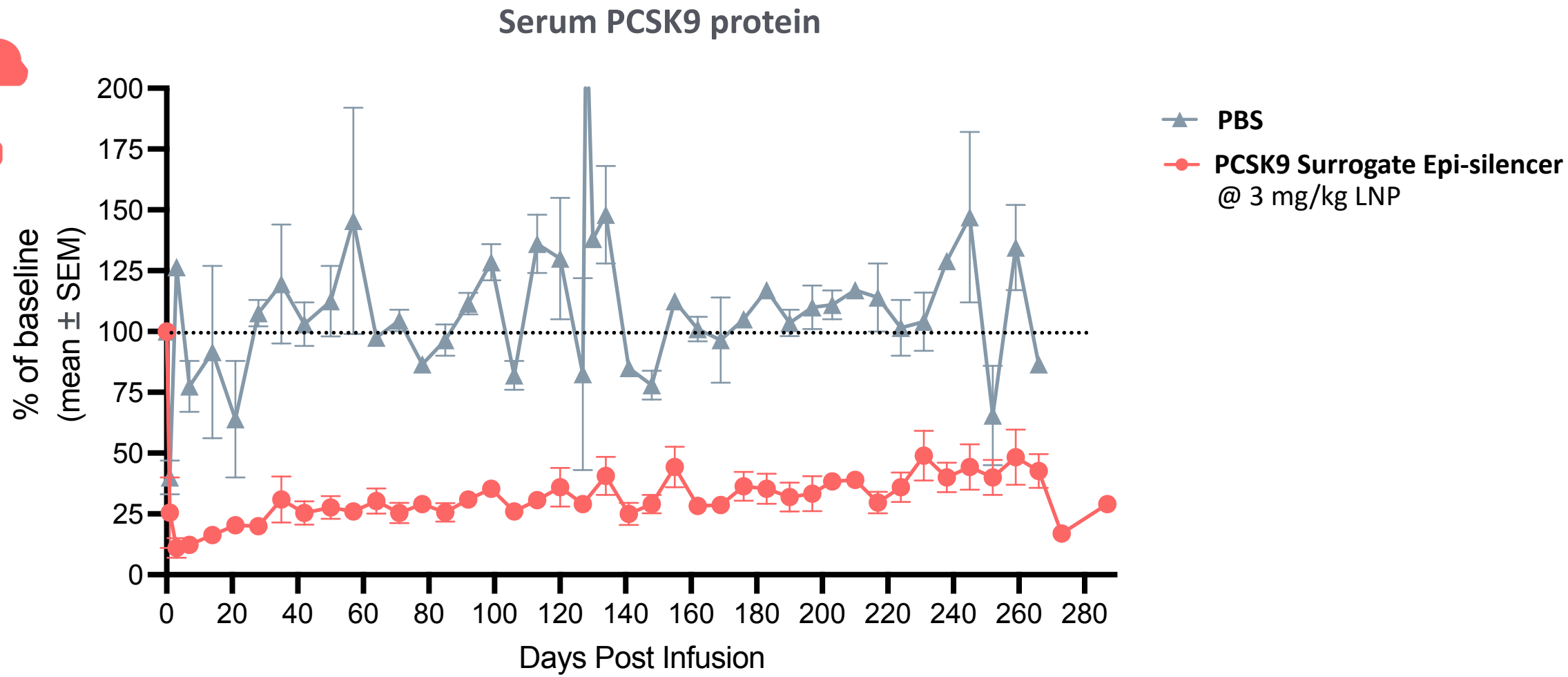
- Methylation of CpG islands leads to repression of transcription initiation

## Same delivery vehicle & payload

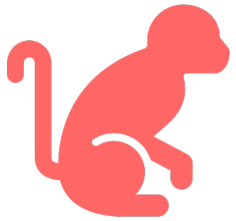
- Similar LNP and epi-silencer protein as HBV context, just a different gRNA dictating a PCSK9 target

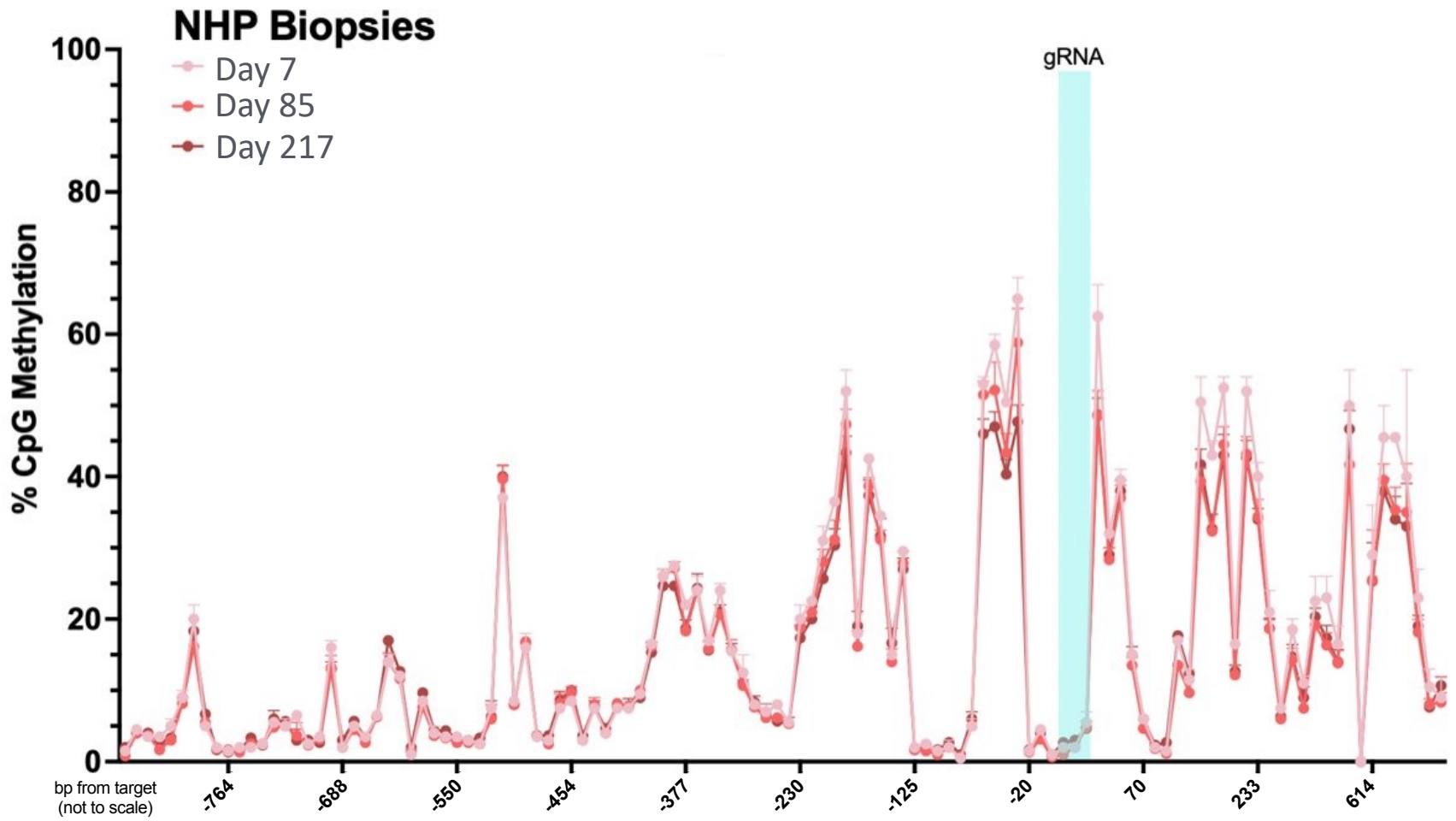
# Strong repression of PCSK9 proxy gene in NHPs at 9+ months post single LNP infusion





# PCSK9 locus methylation levels are highly stable in NHPs at 9+ months post single LNP infusion





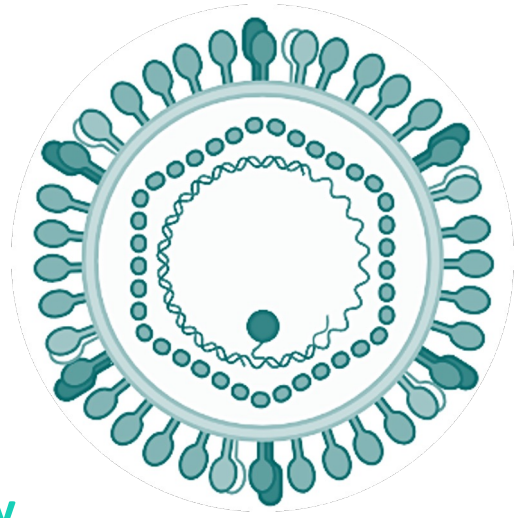
# Tune's epi-silencing approach paves the way towards a functional cure for HBV

## In Vitro Efficacy

Deep repression of both integrated DNA *and* cccDNA

## Durability

Repression held for over 275 mitotic events



## Specificity

RNA-seq demonstrates minimal off target expression in PHH

## Large Animal Safety

Comparable safety profile in NHP to other LNP-delivered therapeutics at efficacious doses.

## In Vivo Efficacy

Durable repression of cccDNA (in FRG chimeric mice) and proxy genes in host chromatin context (in NHP)

TUNE THERAPEUTICS

# THANK YOU

LIPID NANOPARTICLE TECHNOLOGY PROVIDED BY ACUITAS THERAPEUTICS