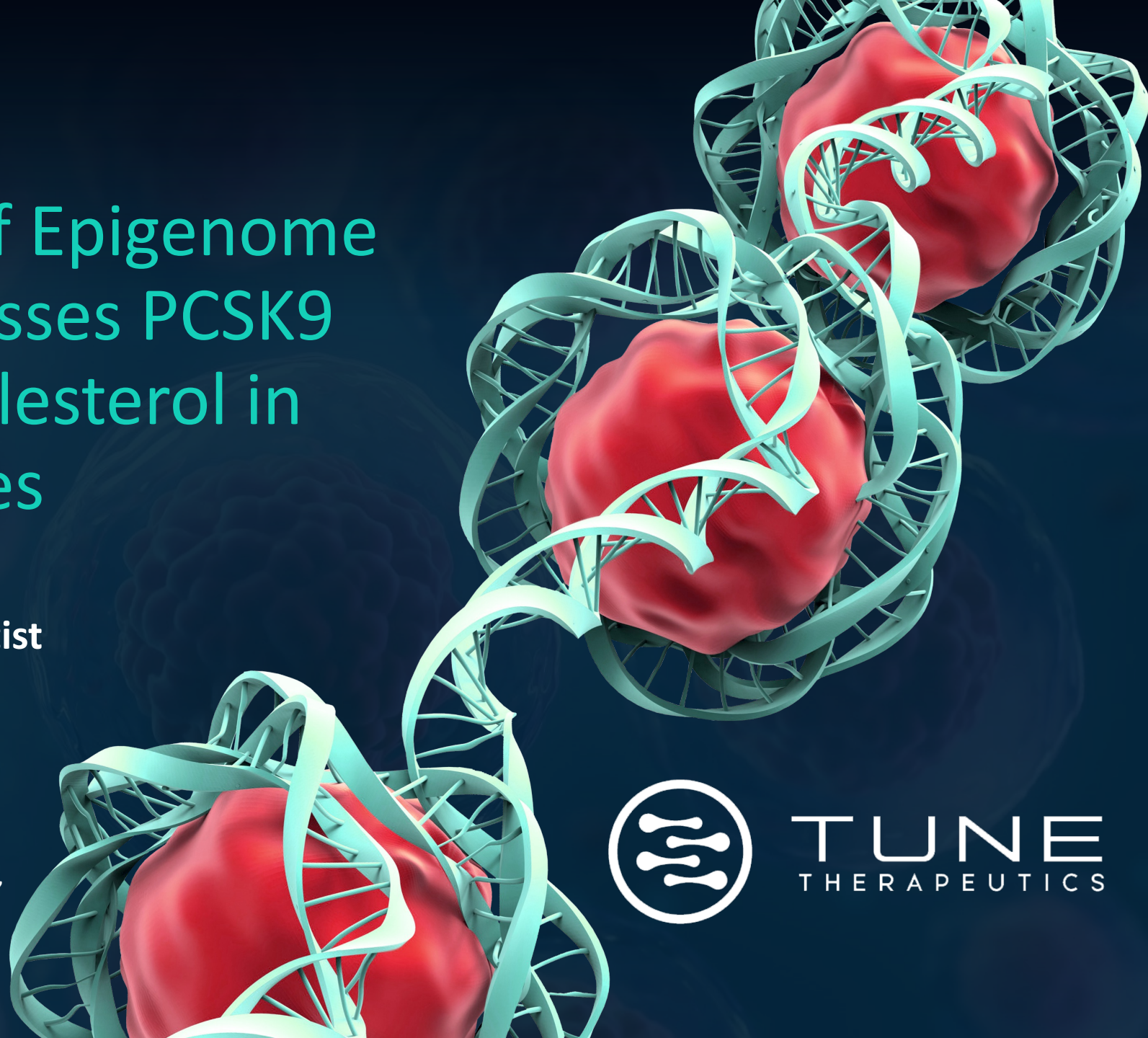


Transient Delivery of Epigenome Editors Stably Represses PCSK9 and Lowers LDL Cholesterol in Non-Human Primates

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ASGCT | May 19, 2023

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Disclosures



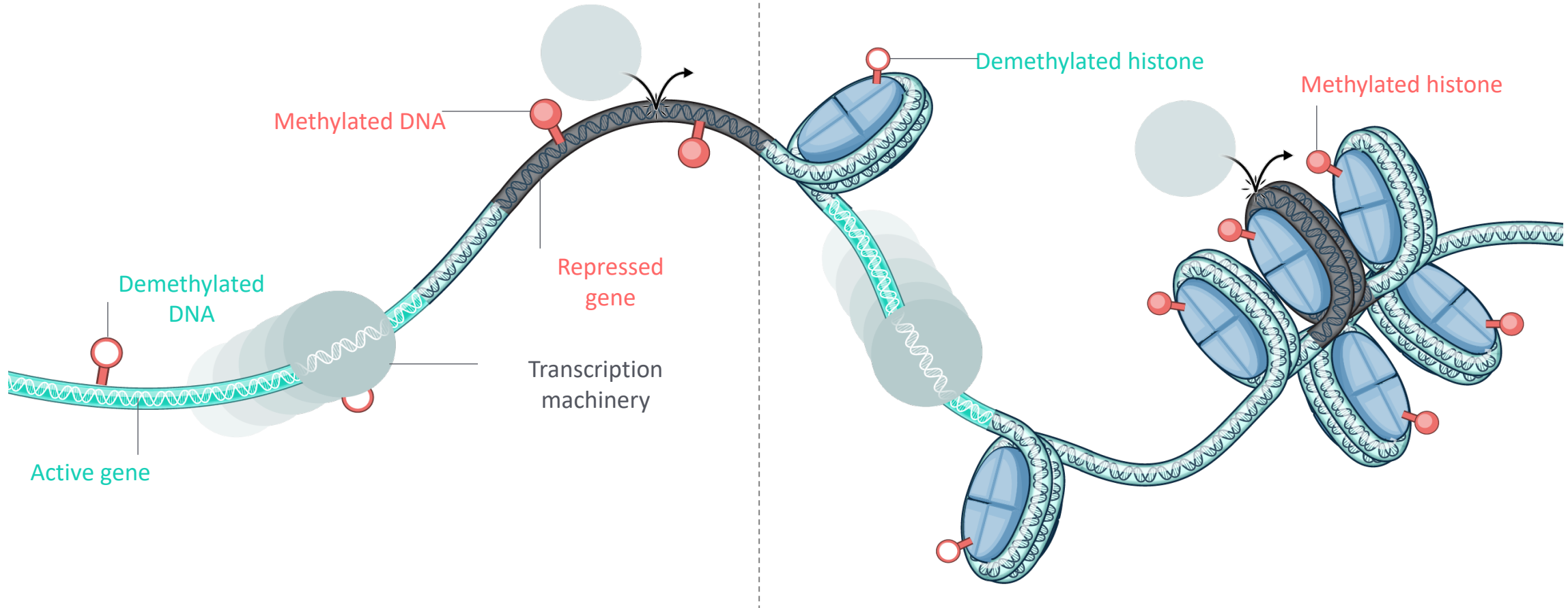
- I am an employee of Tune Therapeutics.

The epigenome controls levels of gene expression

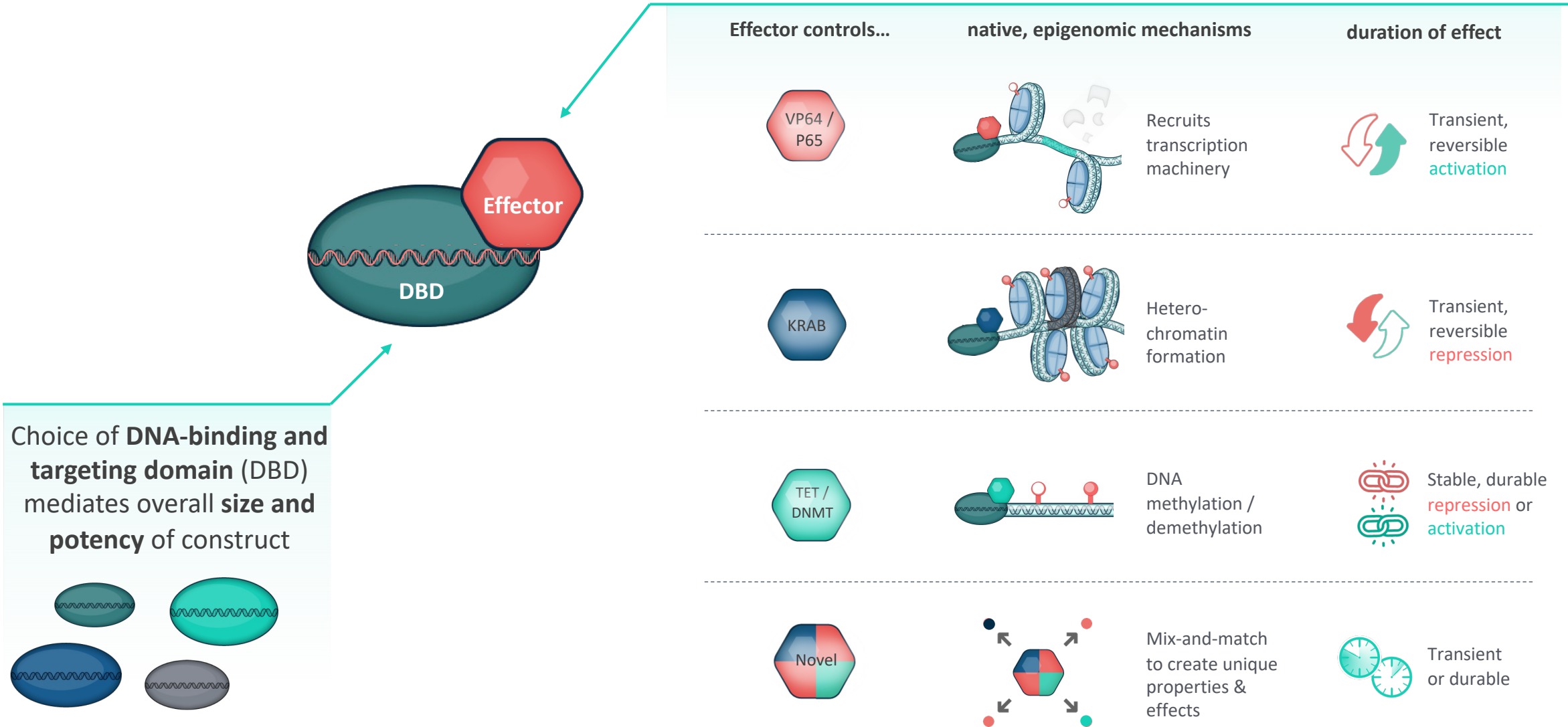
Mechanisms either **increase** or **restrict** the ability of transcription factors to read DNA

DNA modification (**methylation** or **demethylation**) alters binding site recognition by transcription factors

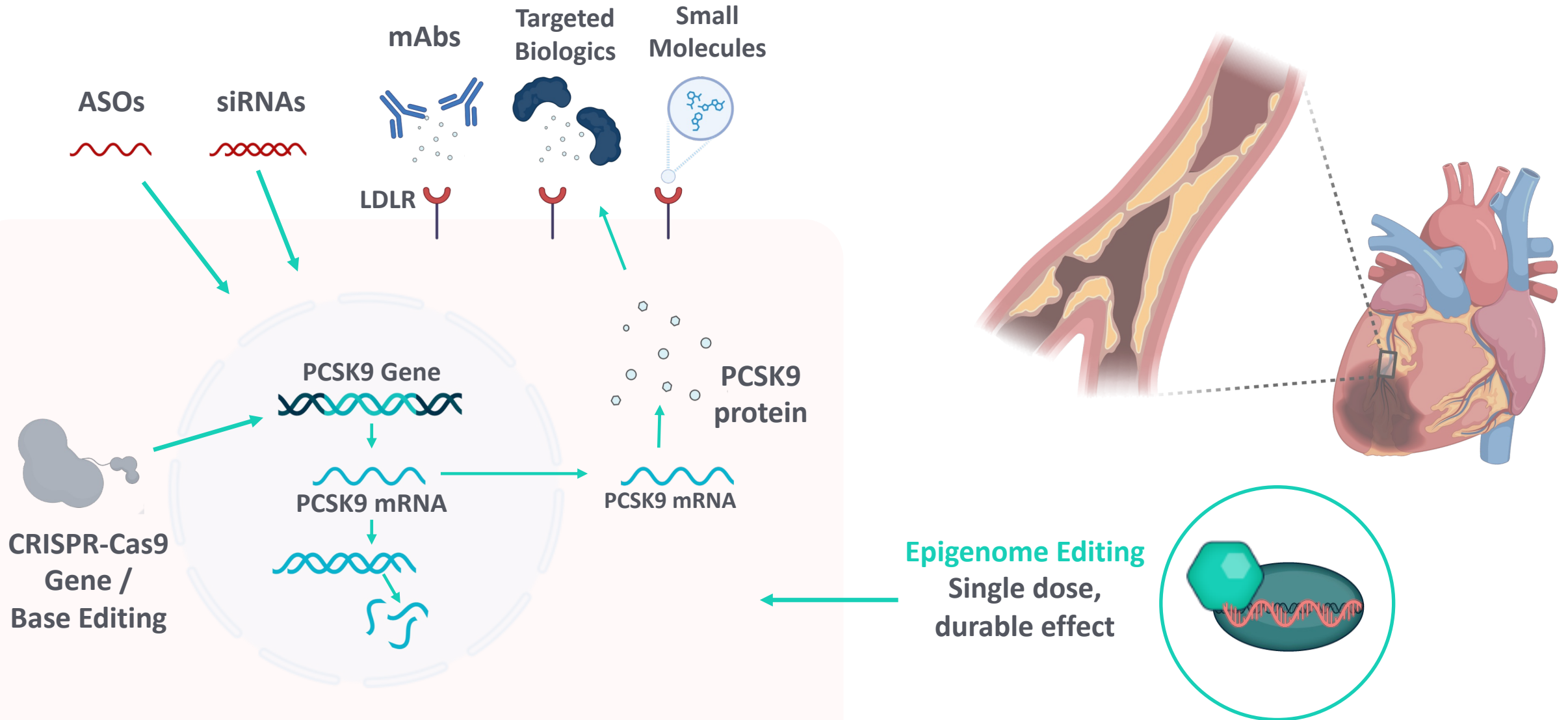
Histone modification alters DNA shape, and can act synergistically with DNA modification to regulate expression



Anatomy of the modular TEMPO tuning platform



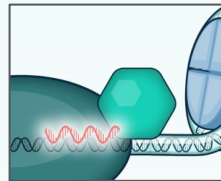
PCSK9: a well-defined target for therapeutic regulation



Single dose of epi-editor targeting PCSK9 to durably reduce LDL-C

In vivo delivery with LNP

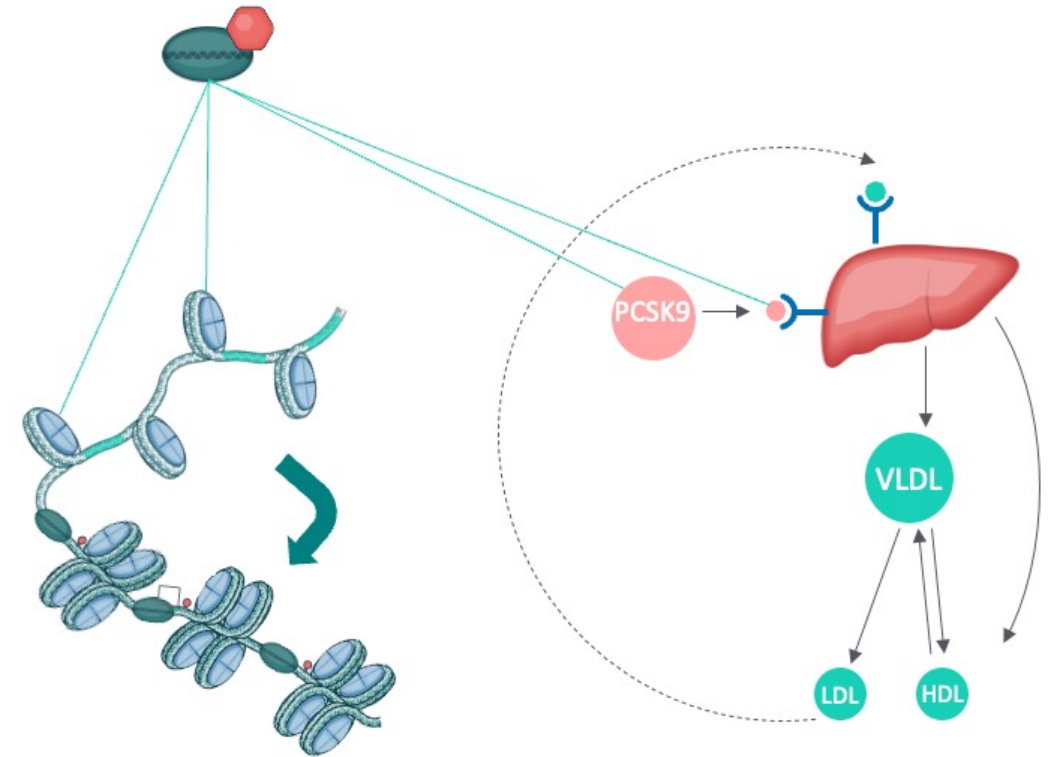
TEMPO drives
epigenetic
change



Tuned cell

Lipid nanoparticles (LNP)
make ideal transient
delivery vehicles for liver
targets with lipid
accumulation

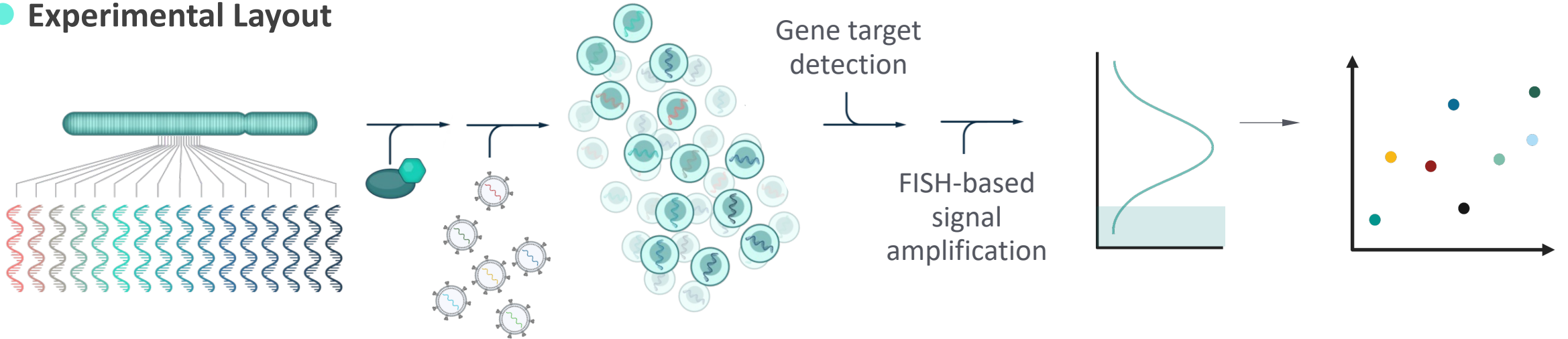
Epigenetic repression of PCSK9 as a
therapeutic strategy for LDL-C reduction



FlowFISH screen to identify PCSK9 gRNAs

Saturating PCSK9 gRNA screen to identify top repressors

Experimental Layout



1 Designed a gRNA library of ~1500 gRNAs saturating the PCSK9 promoter and potential cis-regulatory regions

2 Cells expressing epi-editor transfected with library of gRNAs

3 Sort for low PCSK9 expression

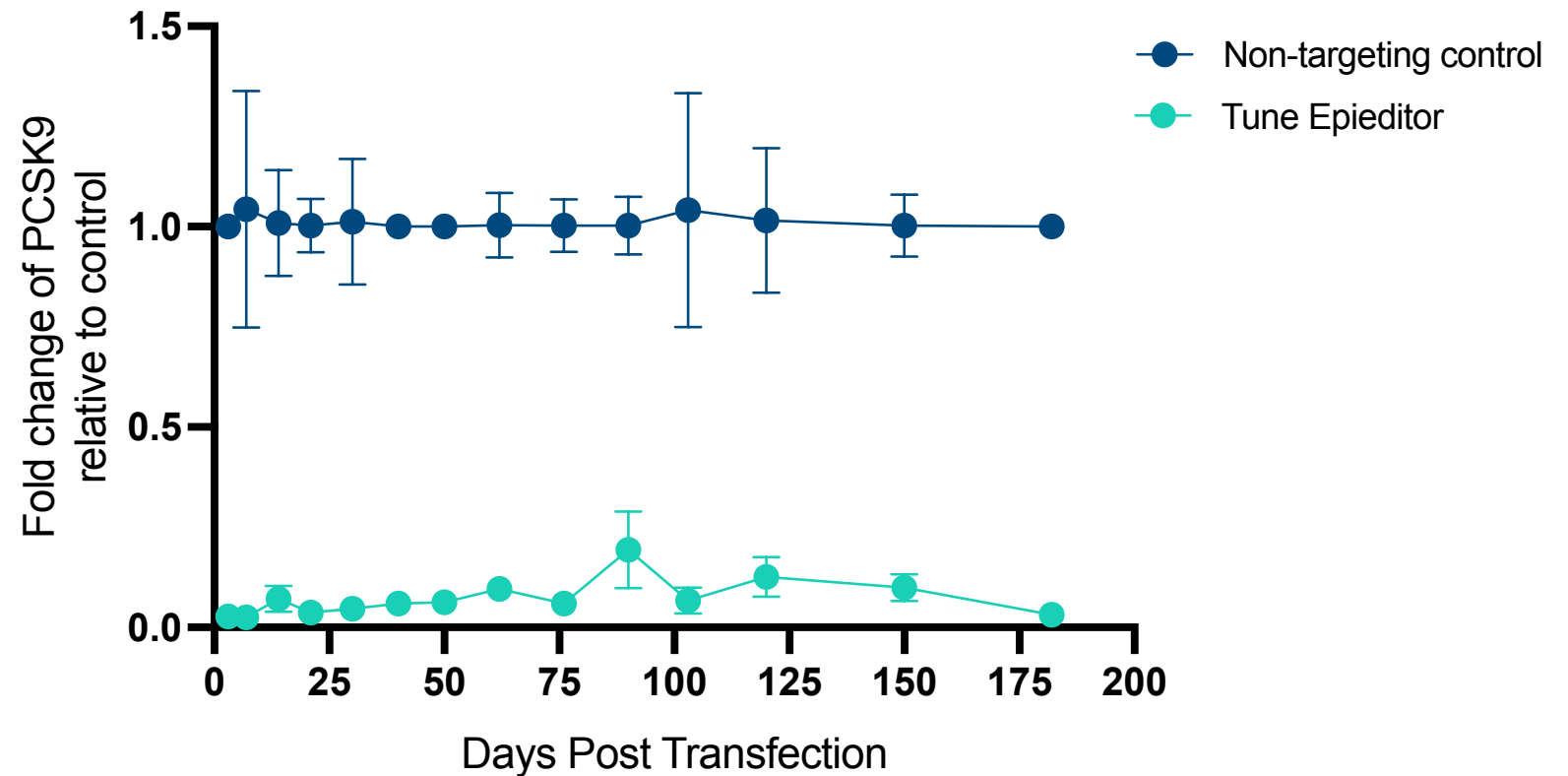
4 Sequencing-based counting of gRNA frequencies

Heritable PCSK9 repression *in vitro* 6 months post-transfection in dividing cells

Experimental Layout

- Single transfection of Tune targeting or non-targeting epi-editor mRNA in Huh7 cells doubling time of 24 hours
- PCSK9 mRNA repression assessed 3 days post transfection and out to 6 months

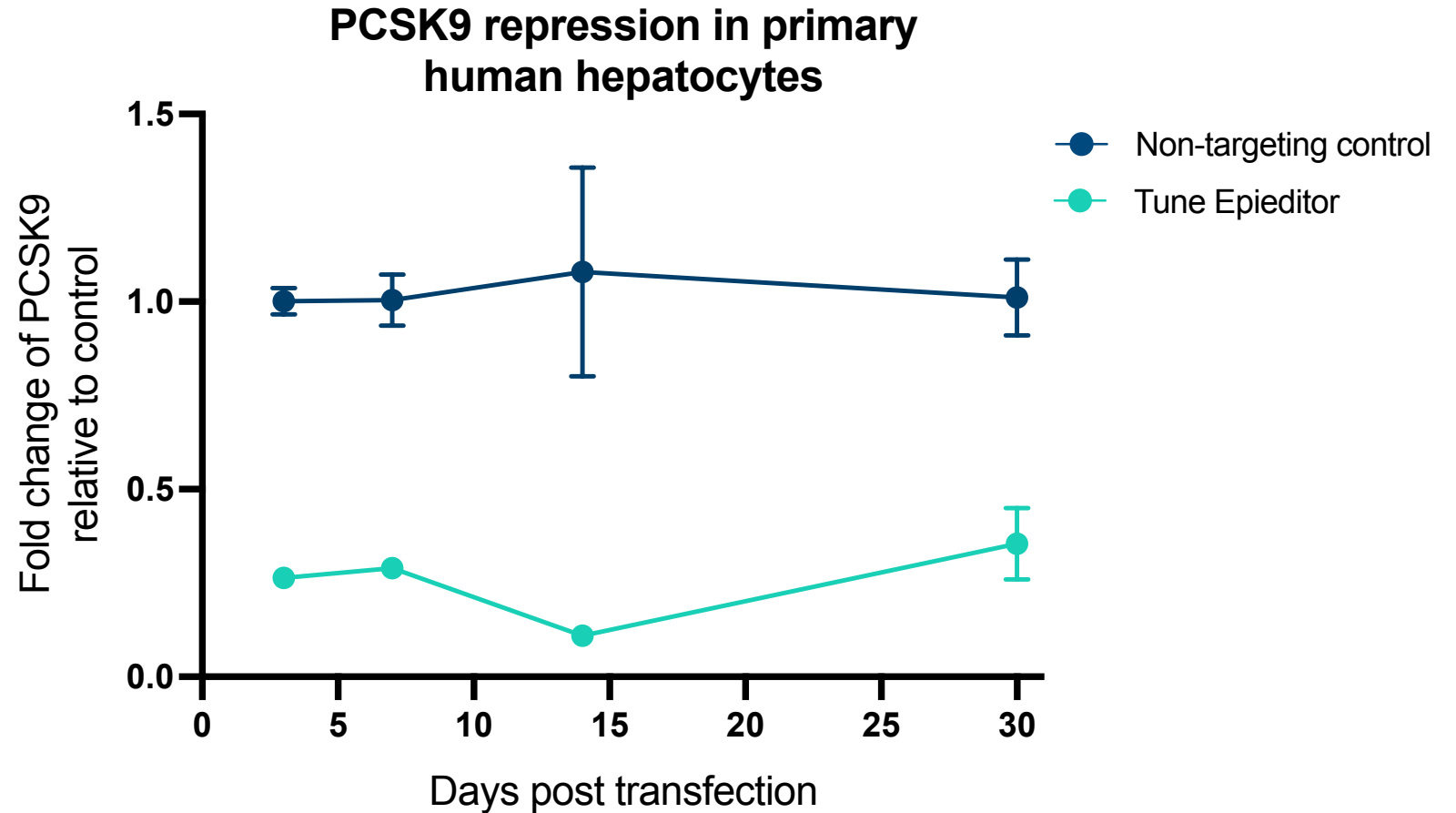
PCSK9 repression in Huh7 cells after single transfection



Sustained PCSK9 repression in primary human hepatocytes

Experimental Layout

- Single transfection of Tune targeting or non-targeting epi-editor in non-dividing primary human hepatocytes
- PCSK9 mRNA repression assessed out to 30 days – maximum lifespan of cells in vitro

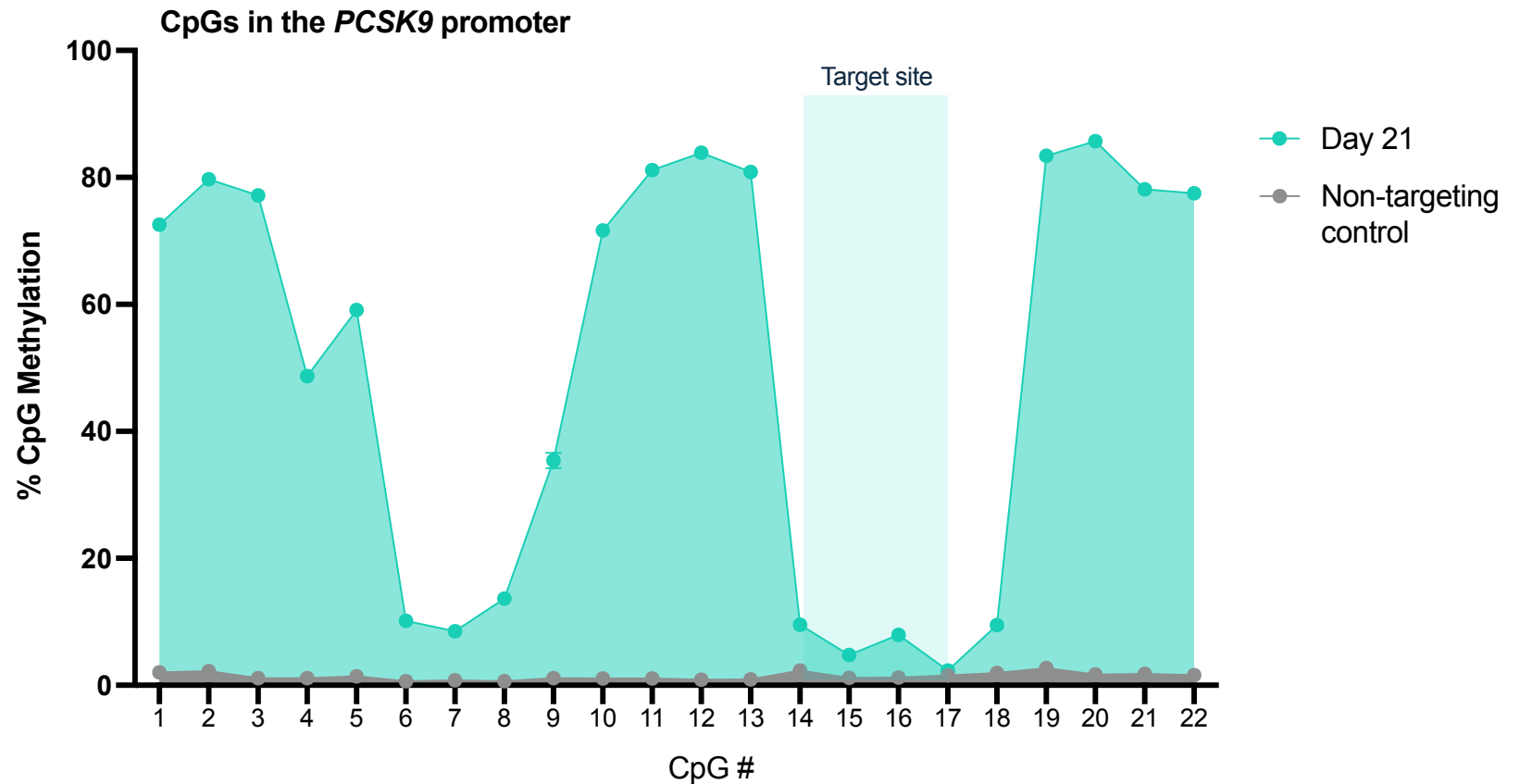


Sustained methylation signature at PCSK9 promoter

Bisulfite sequencing around PCSK9 promoter at early and late timepoints in Huh7 cells

Experimental Layout

- Single transfection of Tune targeting or non-targeting epi-editor mRNA in Huh7 cells
- Bisulfite-sequencing of ~250 bp region surrounding the gRNA target site capturing 22 CpGs in the CpG island

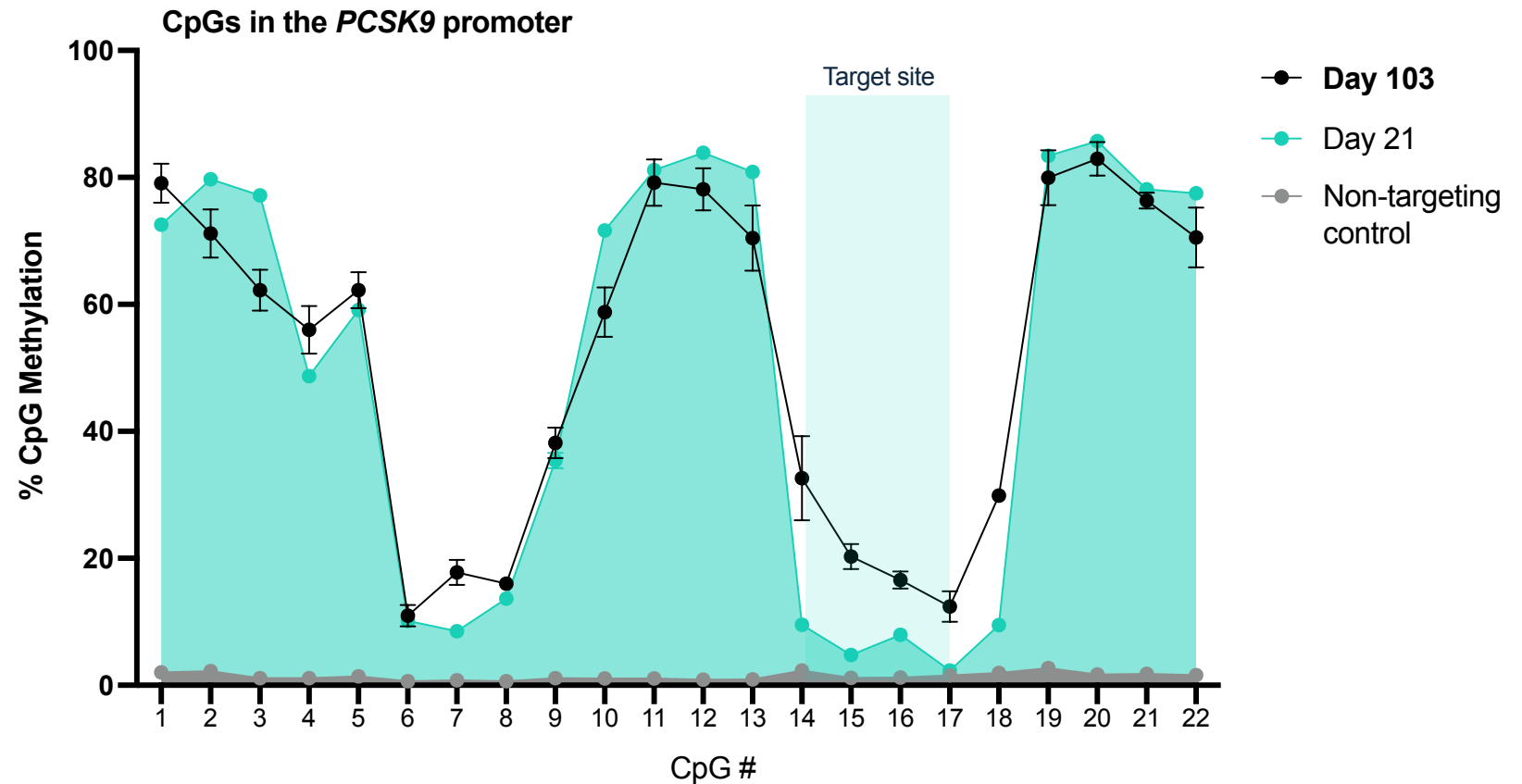


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Experimental Layout

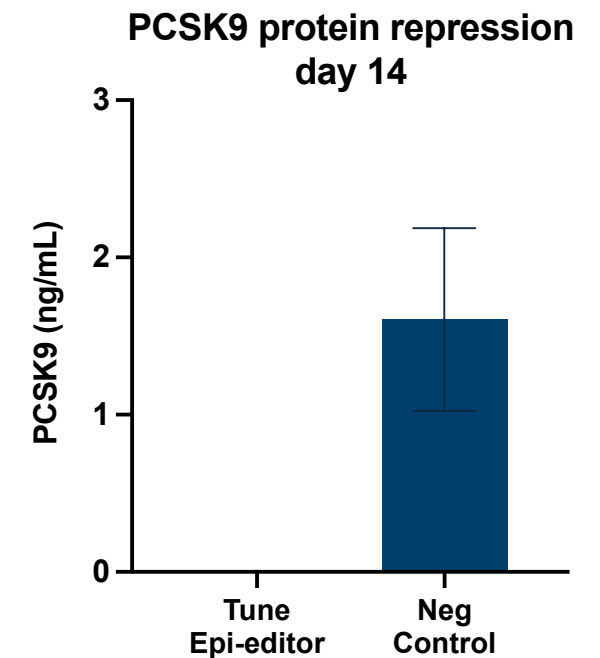
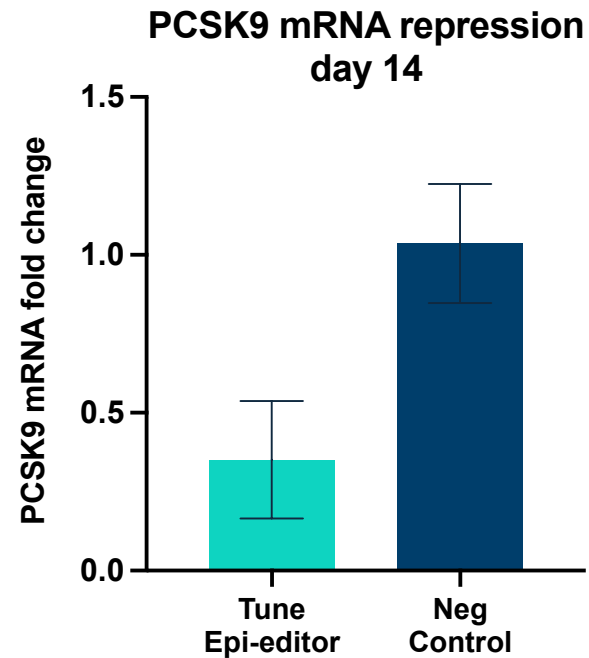
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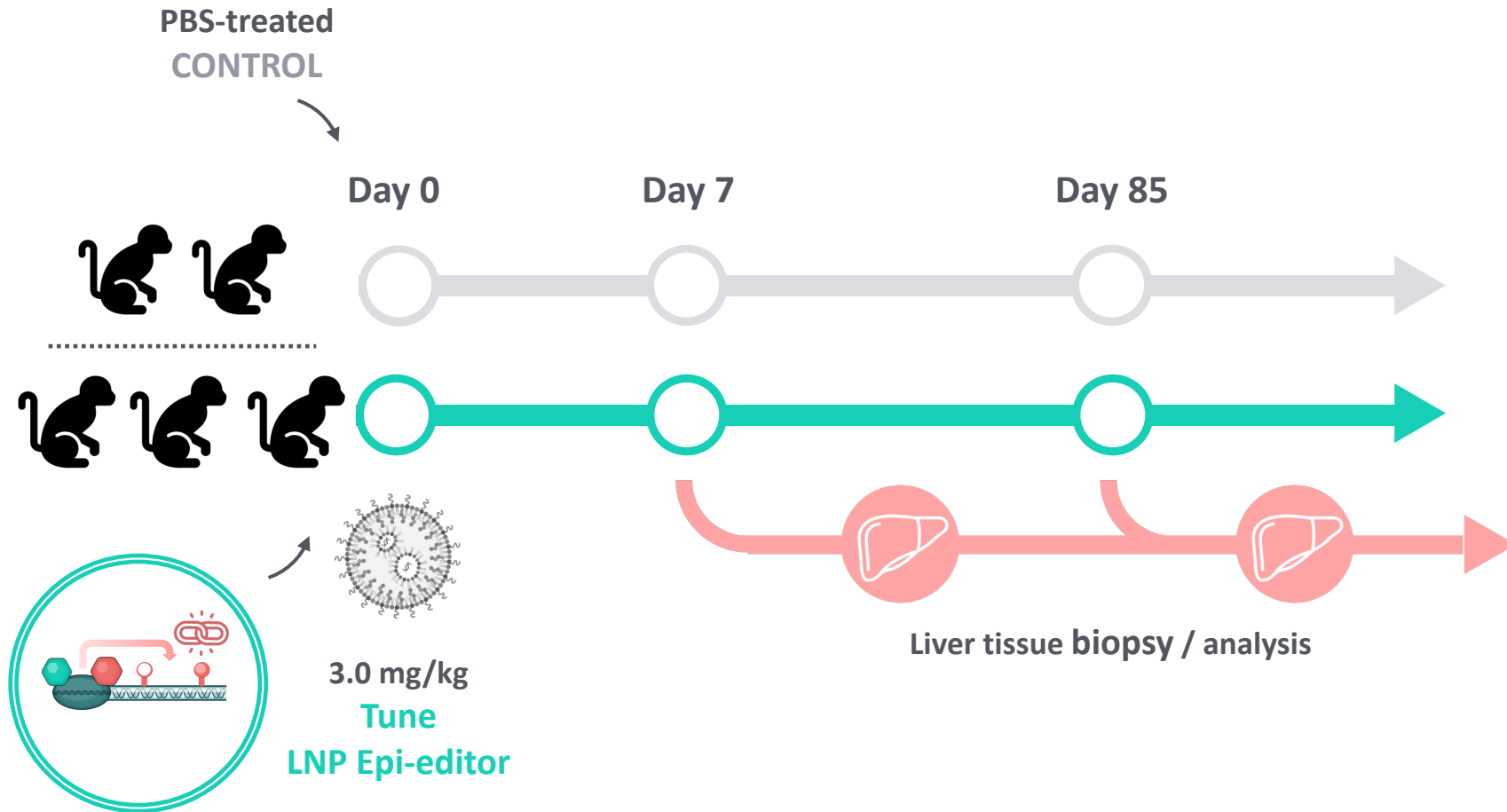
Sustained PCSK9 repression in primary NHP hepatocytes

Experimental Layout

- Single transfection of Tune targeting or non-targeting epi-editor in non-dividing primary NHP hepatocytes
- PCSK9 mRNA and protein repression assessed out to 14 days – maximum lifespan of cells in vitro



NHP PCSK9-targeting study outline



Primary Endpoints

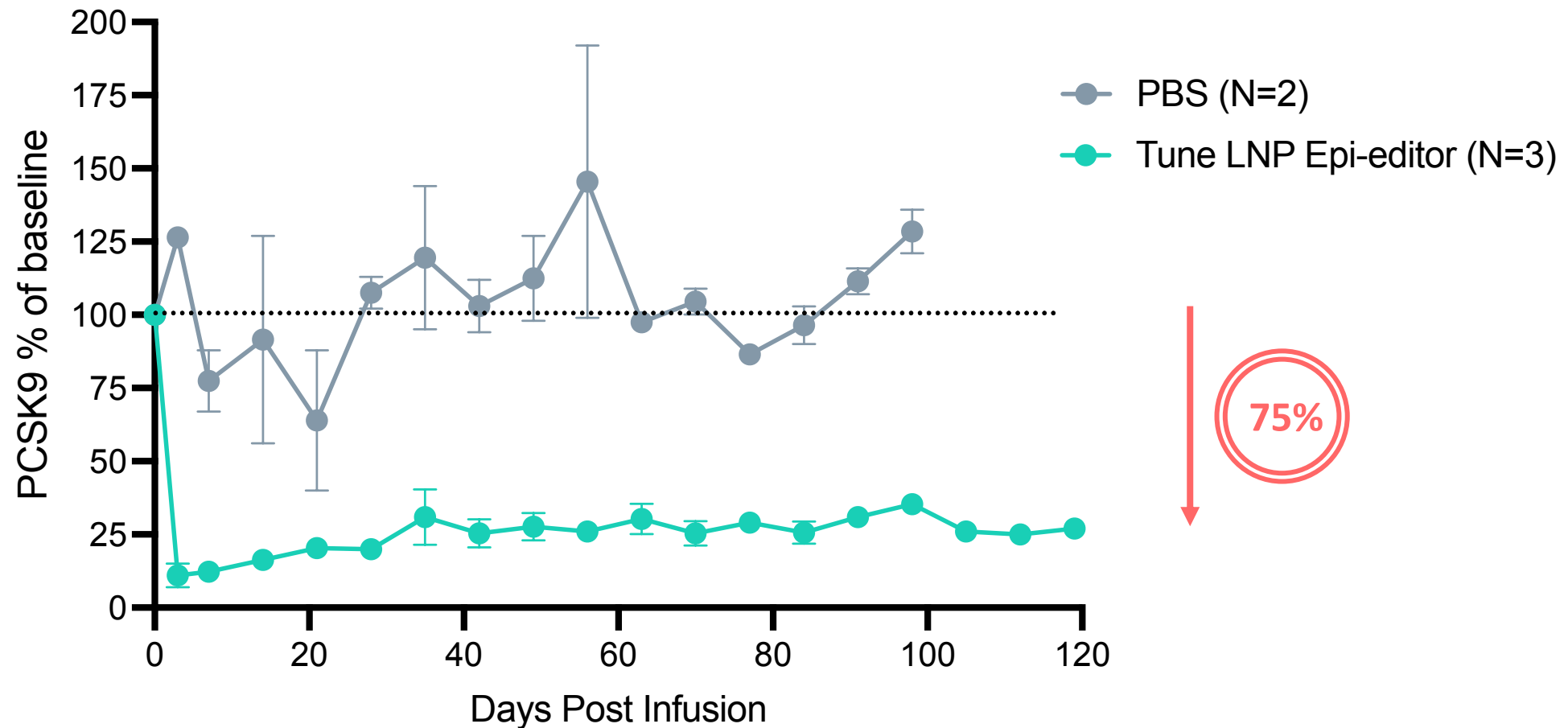
Blood

- PCSK9 protein
- LDL-C level
- Liver enzymes

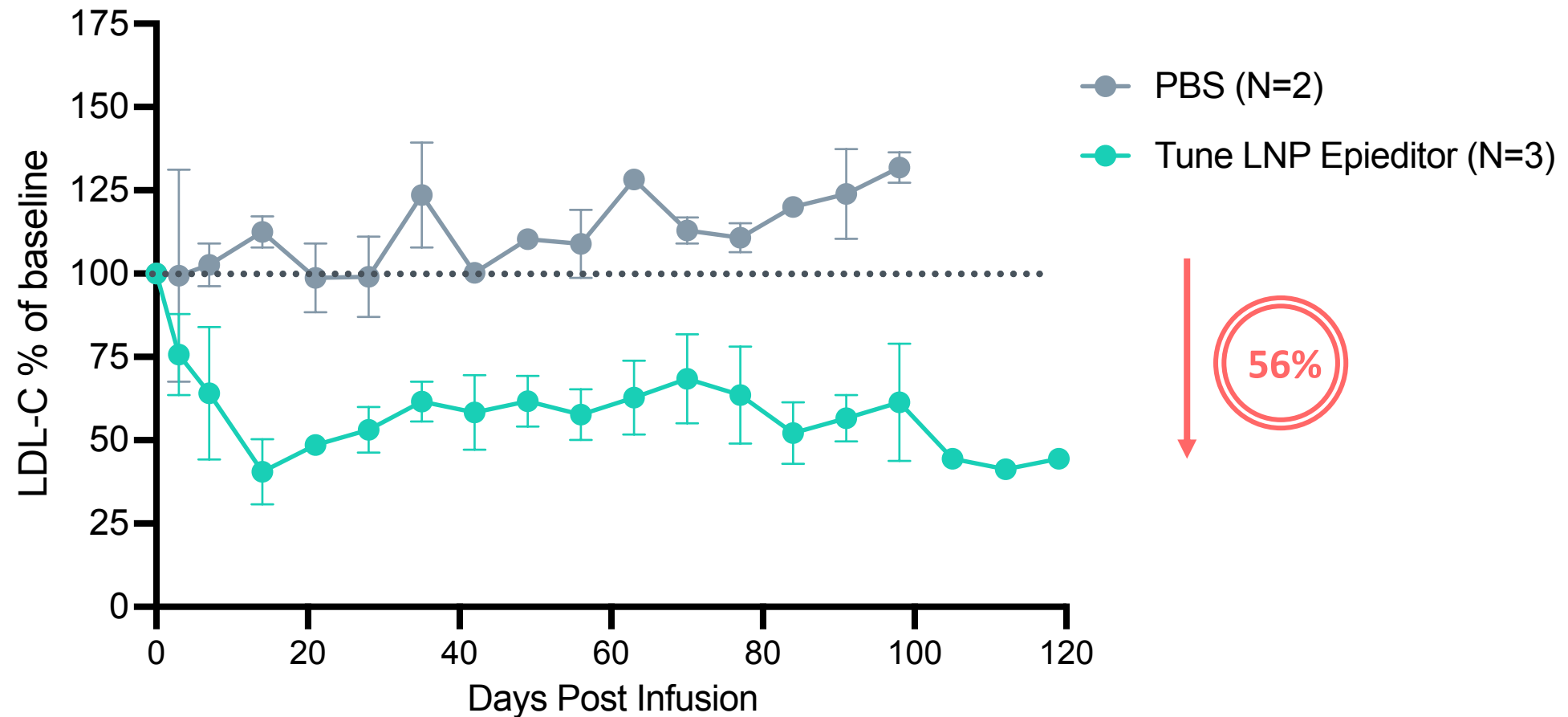
Liver Biopsies

- Methylation analysis

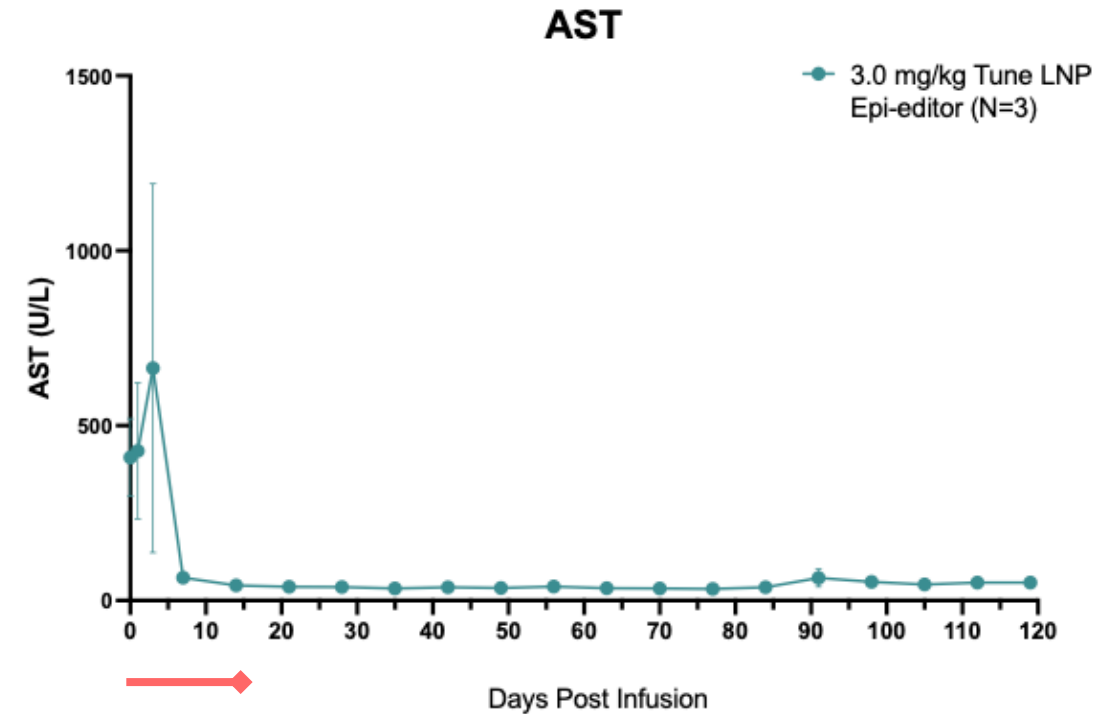
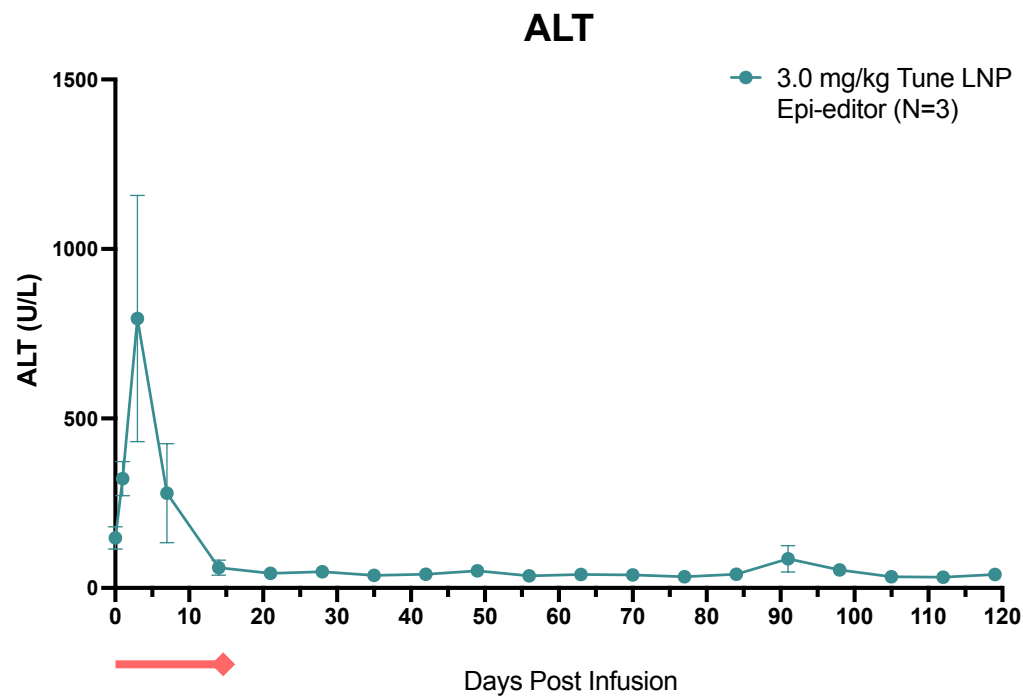
Blood PCSK9 protein: 75% reduction 120 days after single LNP epi-editor administration



Blood **LDL-C** protein: 56% reduction 120 days after single LNP epi-editor administration

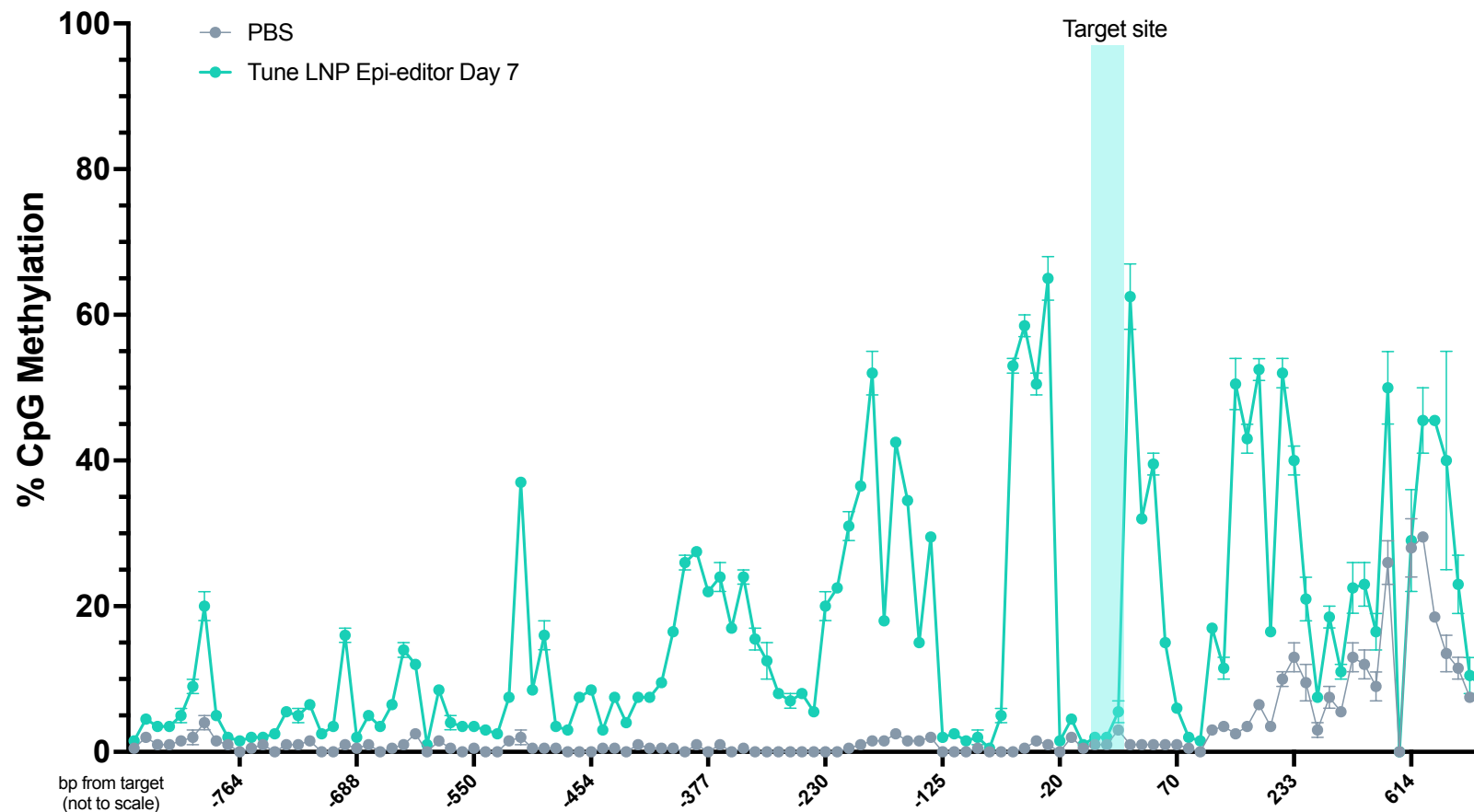


Transient increase in ALT/AST resolves after two weeks



Methylation signature of PCSK9 7 days post LNP

Liver Biopsies - Tune LNP treated NHPs



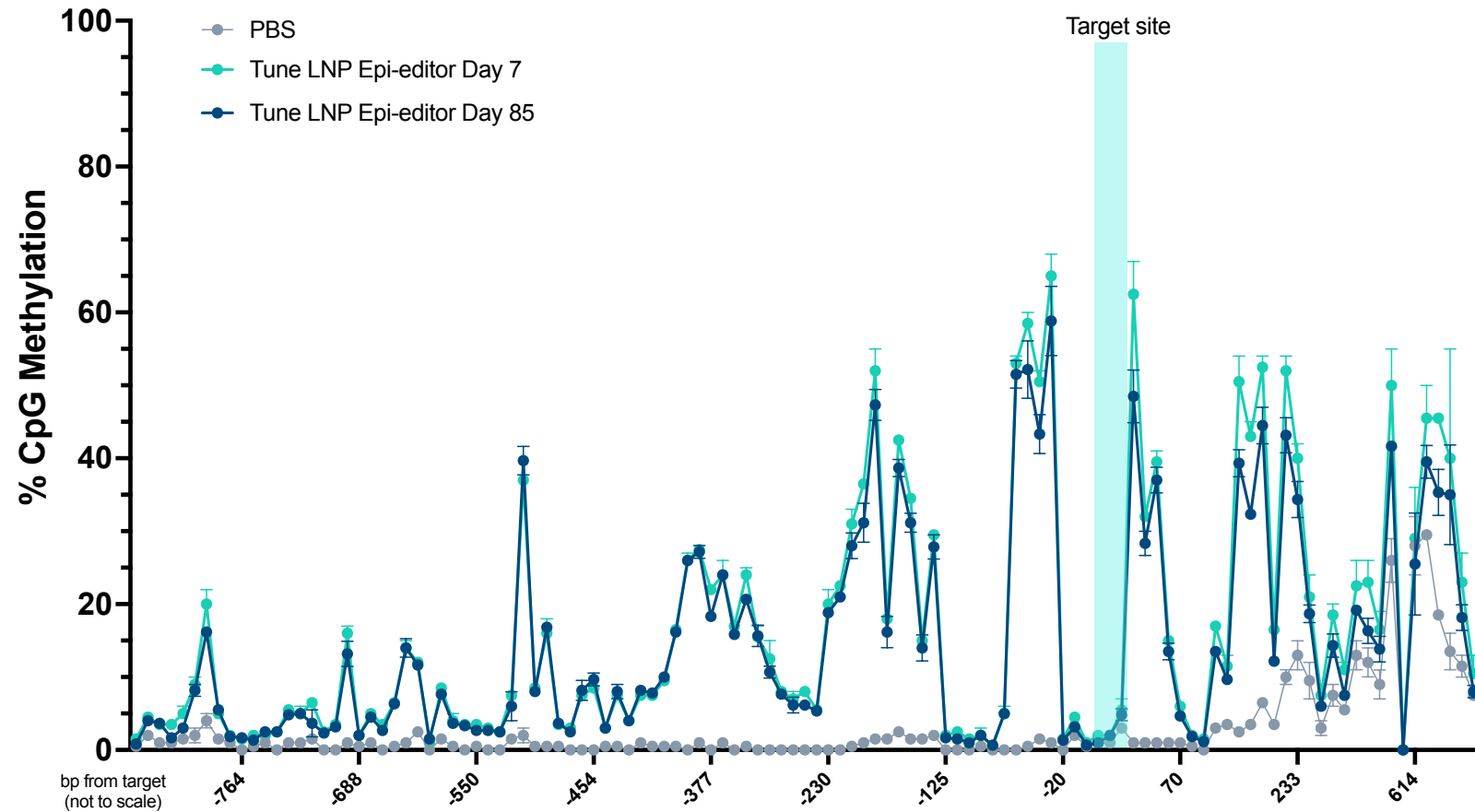
Experimental Layout

- Day 7 FNA biopsies collected
- DNA processed for Methyl-seq with panel designed around PCSK9 locus

Methylation of PCSK9 is highly stable over 85 days



Liver Biopsies - Tune LNP treated NHPs

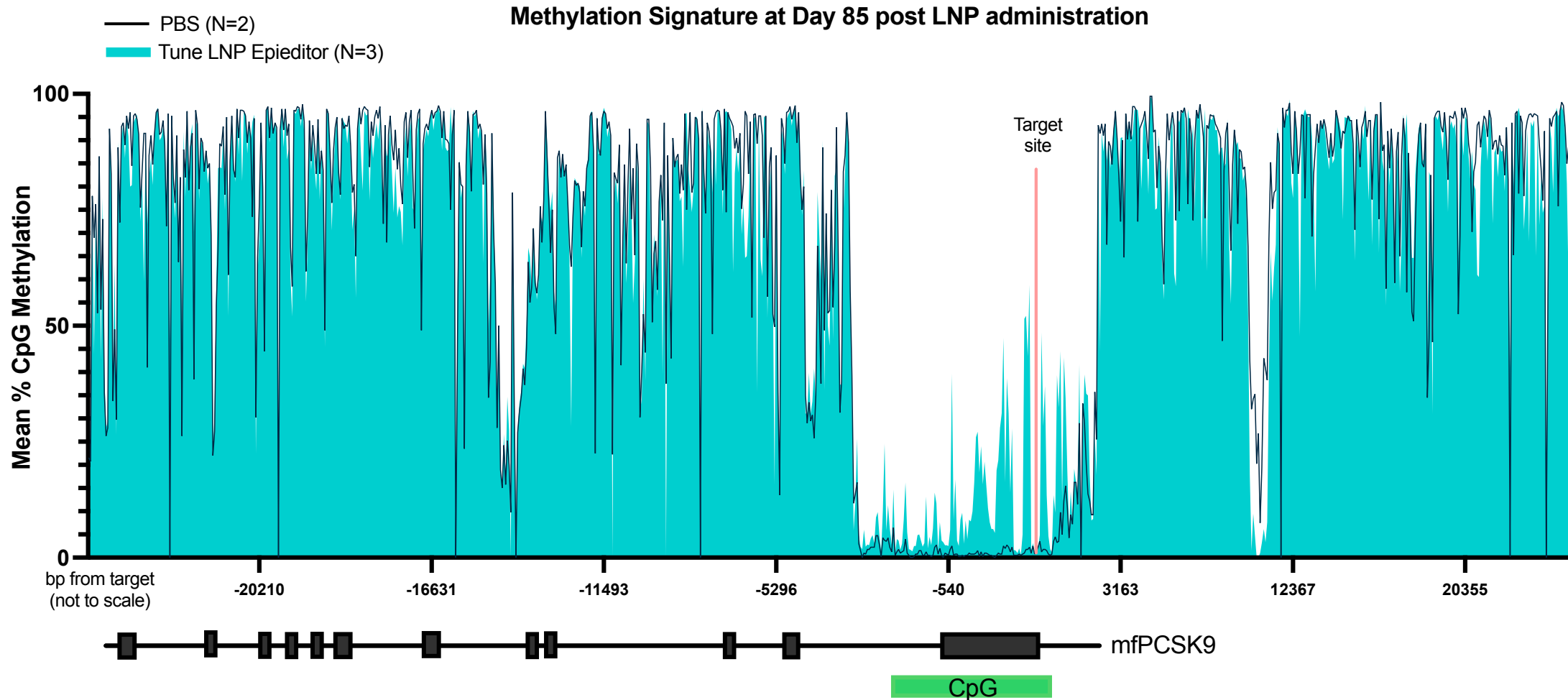


Experimental Layout

- Day 85 FNA biopsies collected
- DNA processed for Methyl-seq with panel designed around PCSK9 locus

Methylation signature across 50kb PCSK9 panel

Methylation spread limited to 2kb region around target site



Conclusions

- Tune Epi-editor demonstrated efficacy and durability of PCSK9 repression in dividing hepatoma cell line out to 6 months
- Single dose of LNP with Tune Epi-editor resulted in stable repression of PCSK9 and LDL-C out to 4 months in NHPs
- Confirmed stable epigenetic remodeling around target locus in vitro and in vivo



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THANK YOU