CASEBIA THERAPEUTICS

Therapeutic Levels of FVIII Through CRISPR/Cas9-Mediated *In Vivo* Genome Editing in Hemophilia A Mice

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Abstract OC 40.2

Disclosures

All authors are employees of Casebia Therapeutics LLC

Introduction

Goal to Create a Curative Therapy for Hemophilia A

Constant expression of FVIII levels in the normal range (50-150%) providing continuous hemostasis

Recent successful progress with AAV based gene therapy, however challenges remain:

- Life-long persistence of episomal based vectors unproven
- Variable and unpredictable FVIII levels in each patient
- Development of neutralizing antibodies to AAV make re-dosing unlikely
- Concern about utility in pediatric patients due to vector dilution

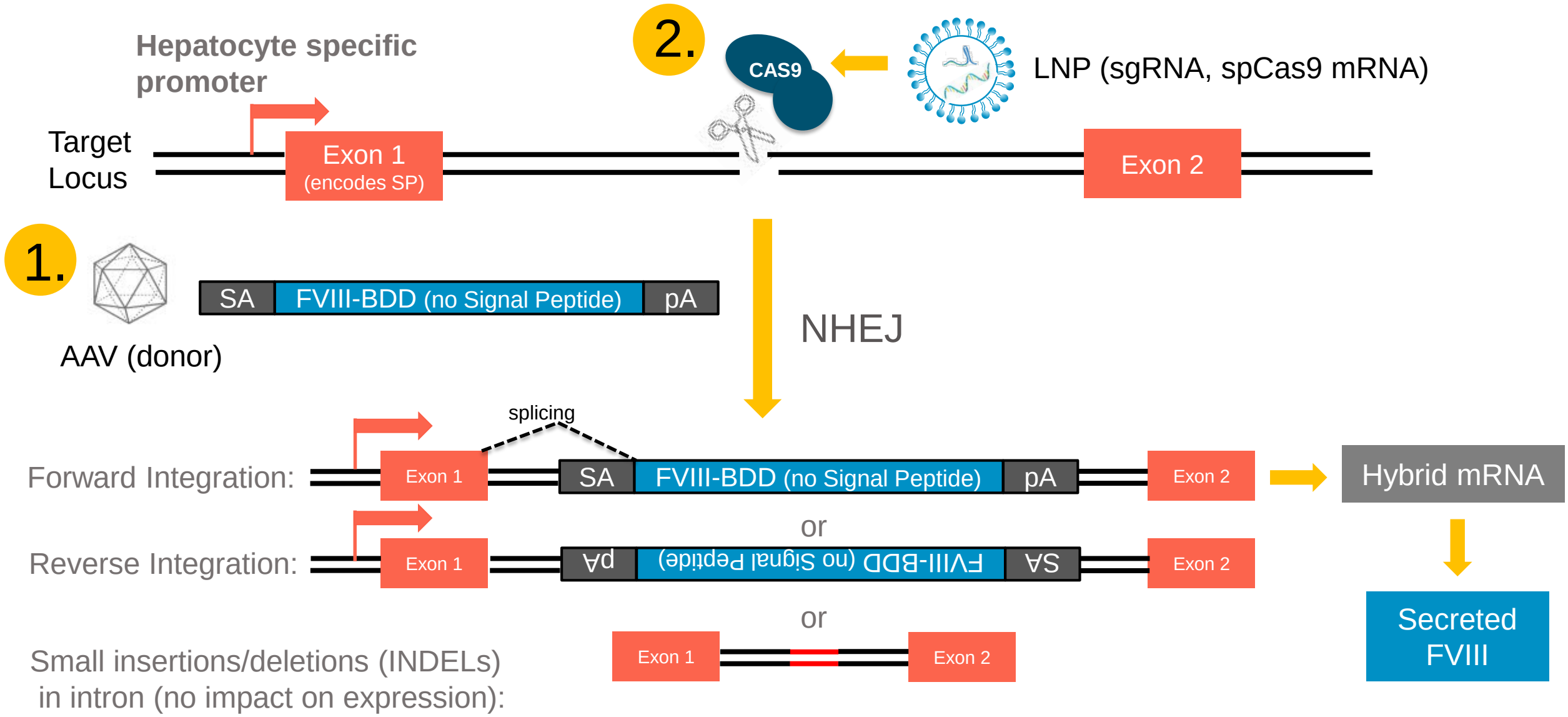
Casebia's CRISPR-based Approach

Expression from a FVIII gene integrated at a defined safe locus in the genome

Repeat dosing enables titration of FVIII to the normal range

Potentially a permanent curative approach, applicable in children and adults

Targeted Gene Insertion Mediated by CRISPR-Cas9

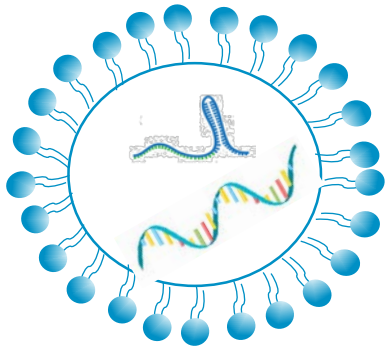


Dose Dependent Editing *in vivo* After Transient Non-Viral Delivery

Cationic lipid, neutral lipid, PEG-lipid
+ Single gRNA (chemically modified)
+ spCas9 mRNA (with NLS)

Self assembly

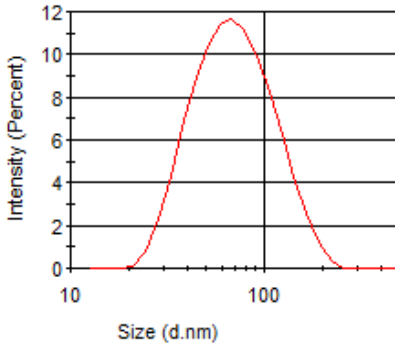
Precision NanoSystems flow cell



Lipid Nanoparticle (LNP)

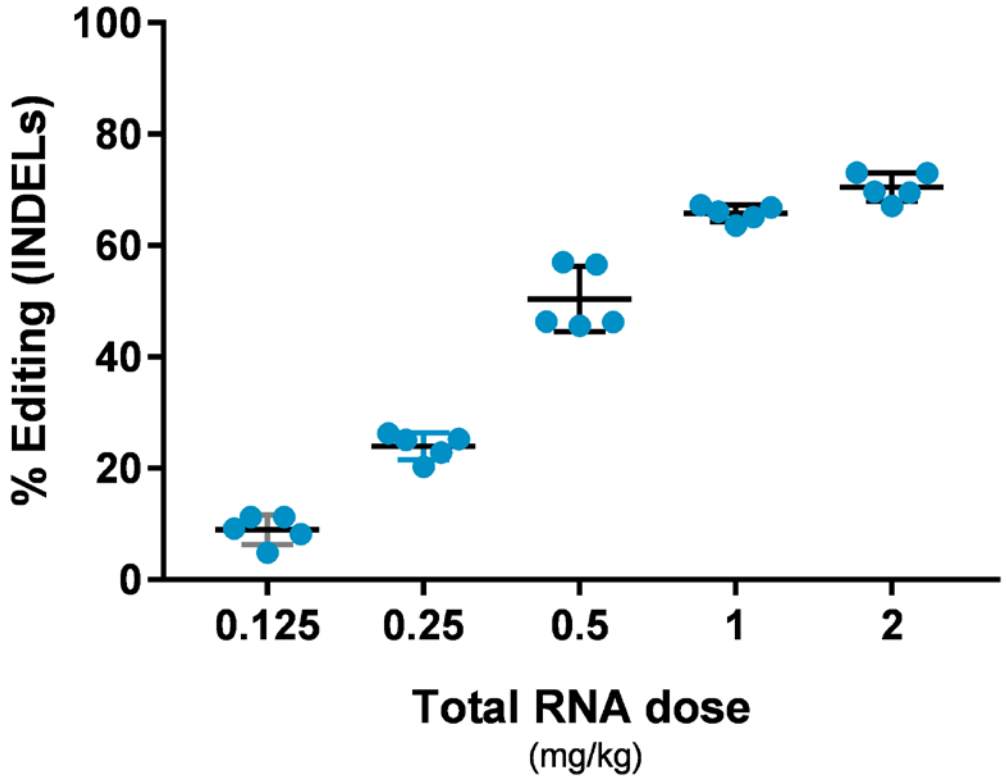
Size: 50 – 90 nm

Dynamic Light Scattering



Hemophilia A (HemA) Mice (FVIII knockout)

iv injection of LNP (sgRNA/spCas9 mRNA)
On-target editing in liver at day 7 (TIDE% analysis)



Dosed by total encapsulated RNA concentration (sgRNA + mRNA)

%: Tracking Indels by Decomposition (Brinkman, NAR, 2014)

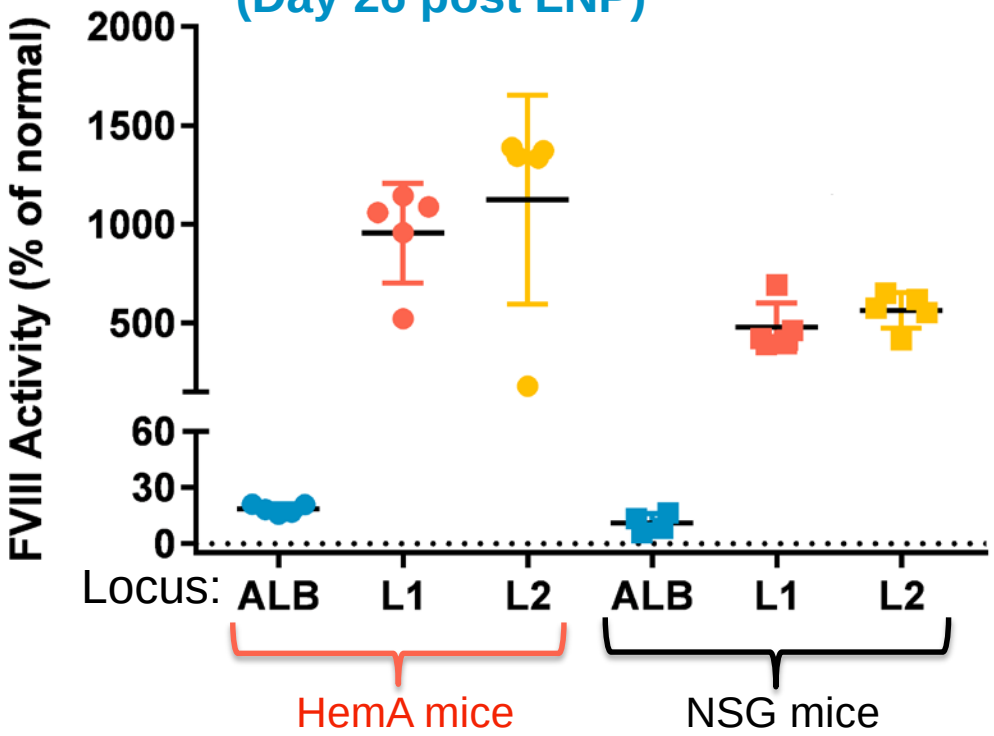
Improved FVIIII Expression From Integration at Novel Loci

HemA Mice
or **NSG Mice**

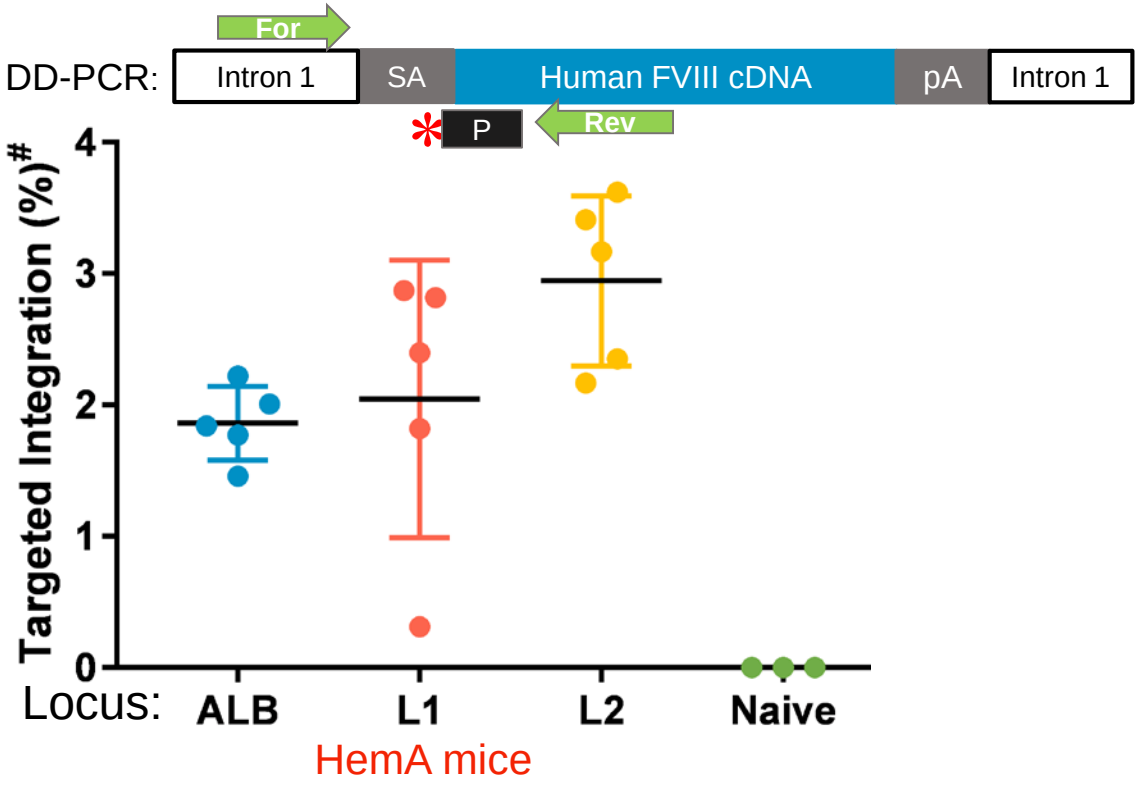
AAV8 virus — SA — Human FVIII-BDD cDNA — pA — 2e12 vg/kg

LNP — Cas9 mRNA
gRNA targeting one of **3 loci**

FVIII Activity by Chromogenic Assay# (Day 26 post LNP)



Integration of FVIII Gene at Target Site in the Liver



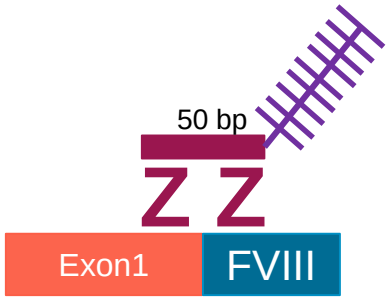
For NSG, Human FVIII specific capture followed by chromogenic assay
ALB: Serum albumin gene

copies per 100 haploid genomes (whole liver homogenized)

Distribution of Hybrid mRNA in Liver by Situ Hybridization

BaseScope™ Probe

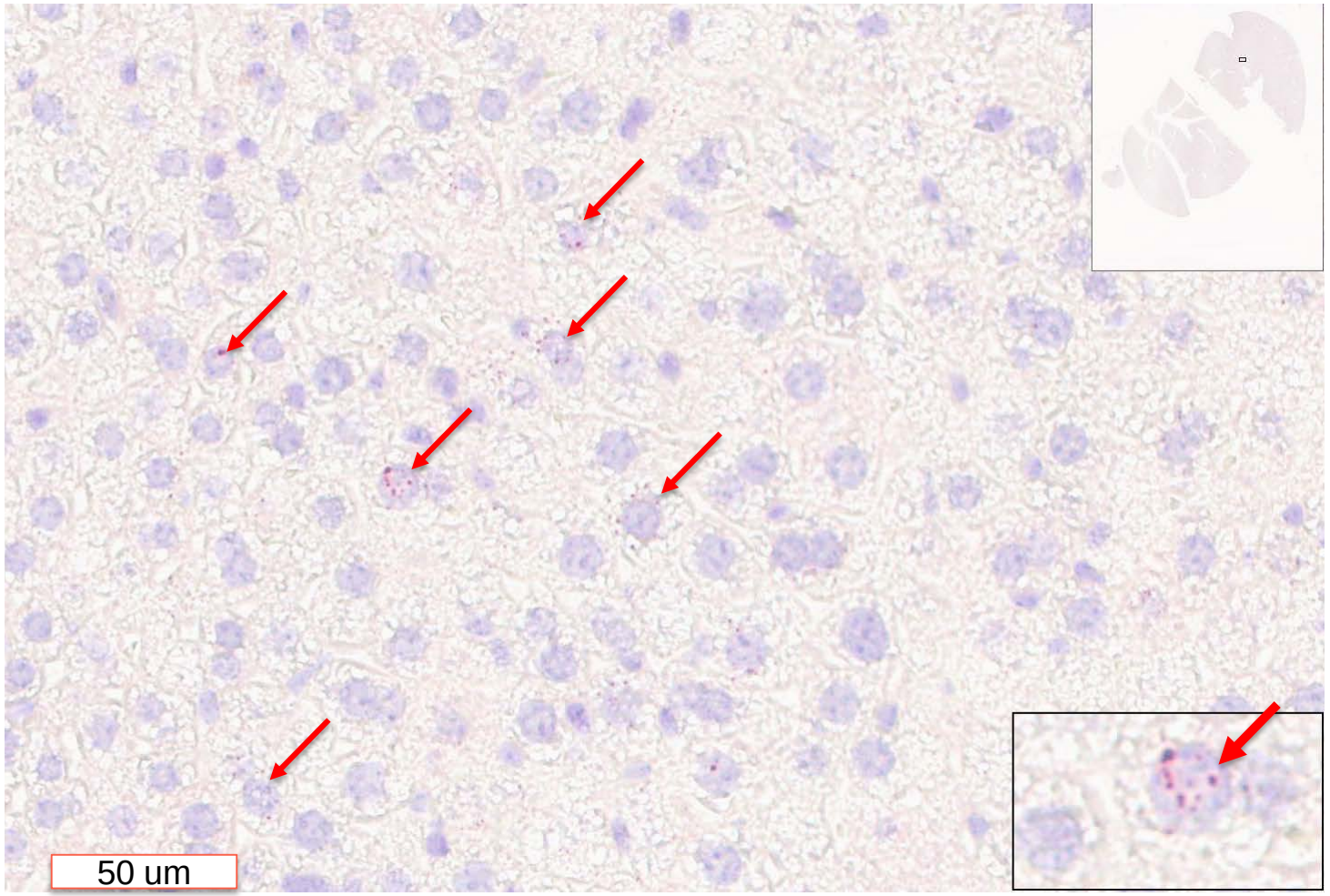
Detects exon1 of target gene fused to FVIII



- The hybrid mRNA predicted by the molecular approach is detected
- Frequency of expressing cells matches targeted integration measured by DD-PCR

Targeted to locus L2 (similar results for ALB and L1)

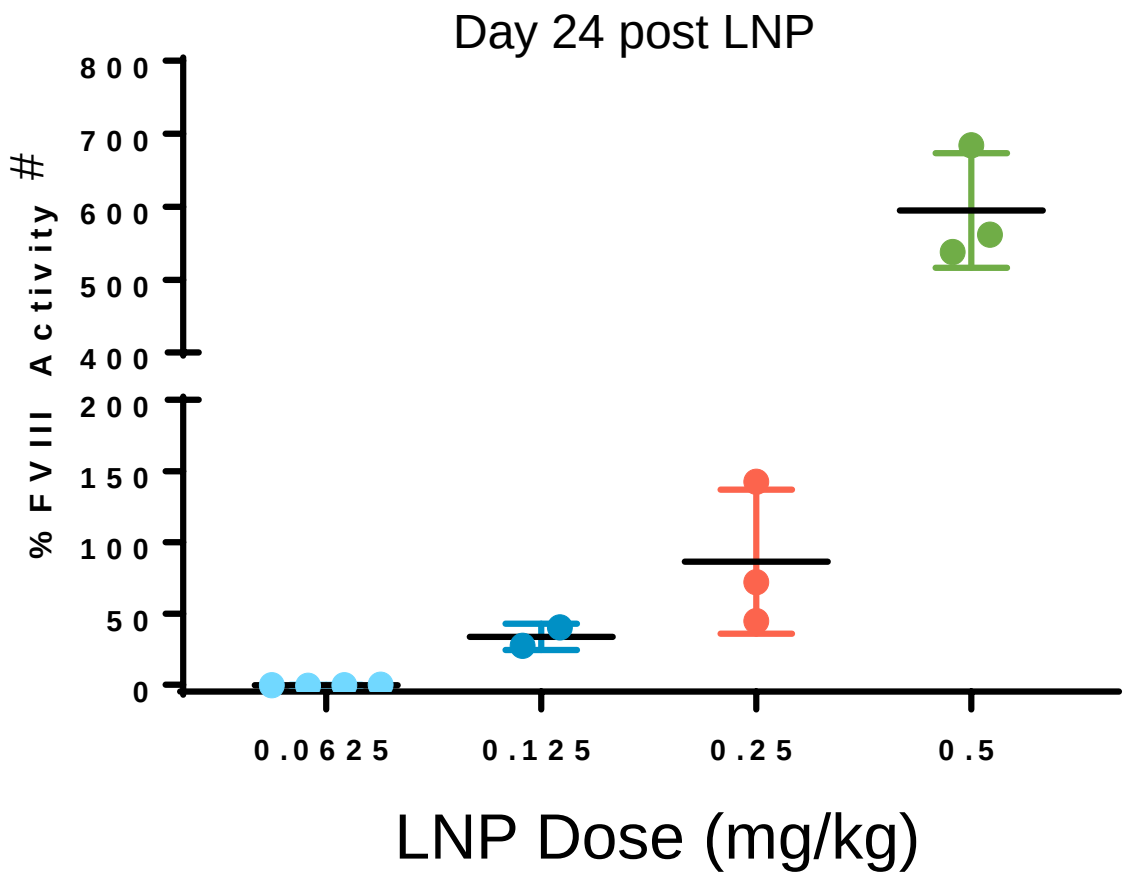
Estimate 2 – 4% of hepatocytes positive



Dose of LNP Modulates FVIII Levels

HemA Mice

AAV8-FVIII, 2e12 vg/kg
LNP: Cas9 mRNA, gRNA targeting L1



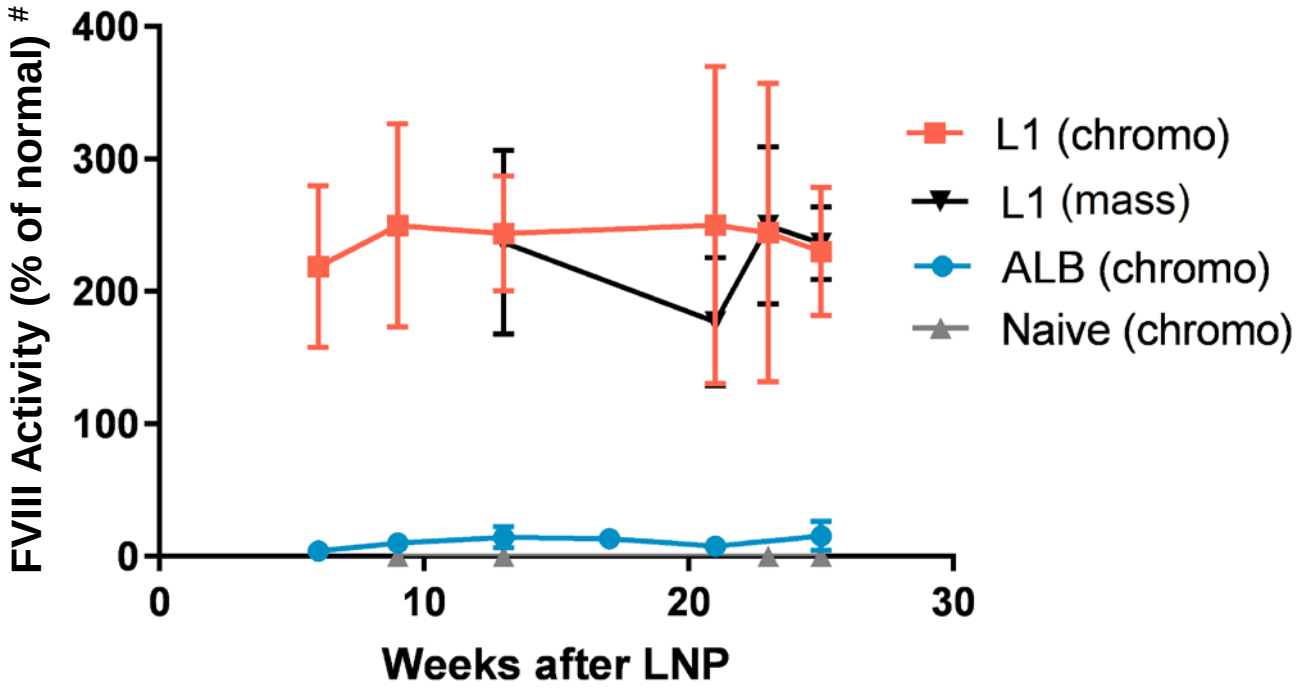
Chromogenic assay

Stable Expression of FVIII and Comparison to Promoter Driven AAV

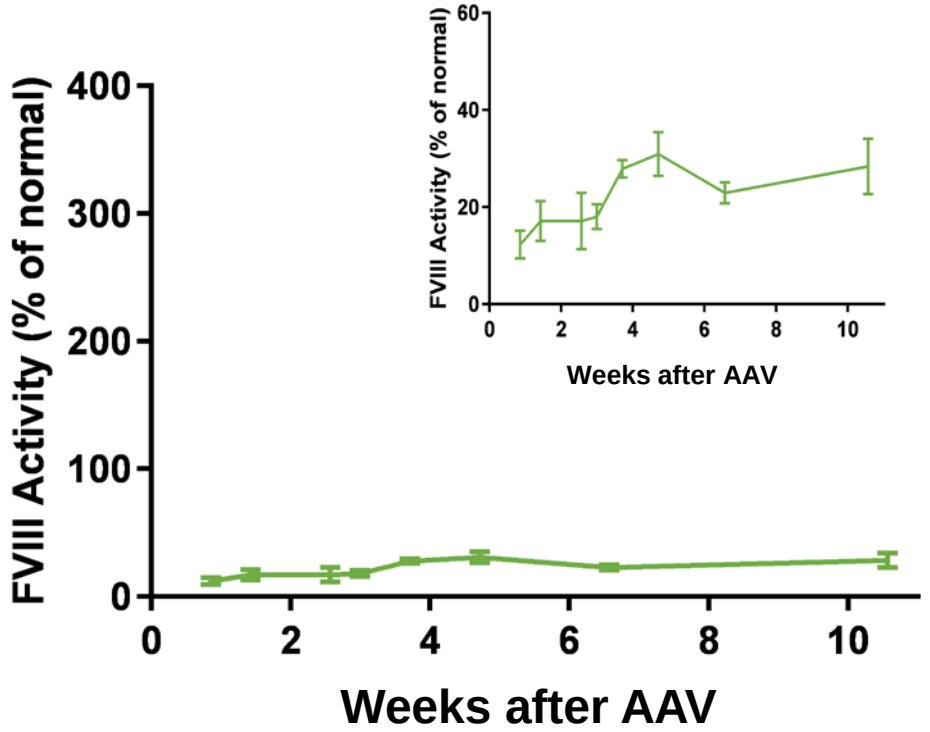
NSG Mice

AAV8-FVIII, 2e12 vg/kg

LNP: Cas9 mRNA, gRNA targeting ALB or L1



AAV8-HLP promoter-FVIII 2e12 vg/kg



8-fold higher expression than HLP promoter driven FVIII at same AAV dose
Stable expression through 6 months (when study was terminated)
Comparable FVIII activity by chromogenic and mass based assays

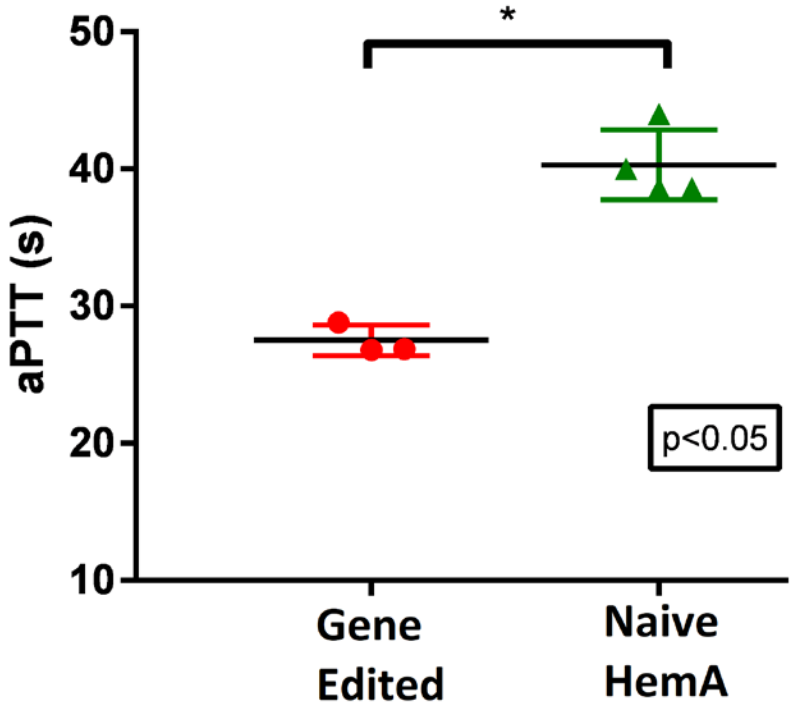
FVIII detected with human FVIII specific capture-Chromo assay or ELISA (mass-based) assay

FVIII Expressed by Gene Edited Mice is Functional

HemA Mice

AAV8-FVIII 2e12 vg/kg
Single dose of LNP #

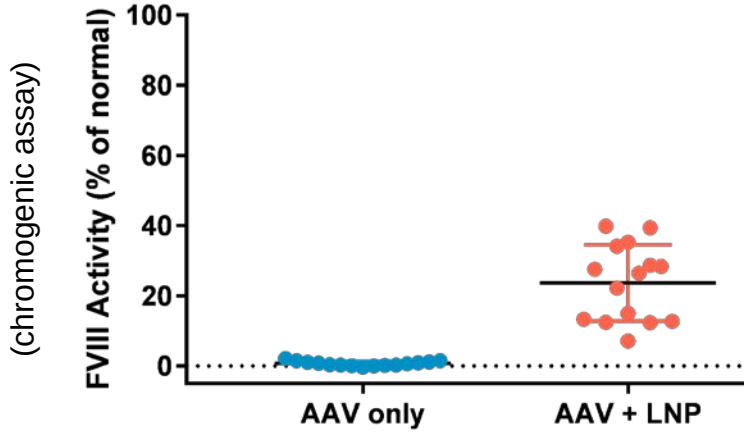
Day 10
aPTT

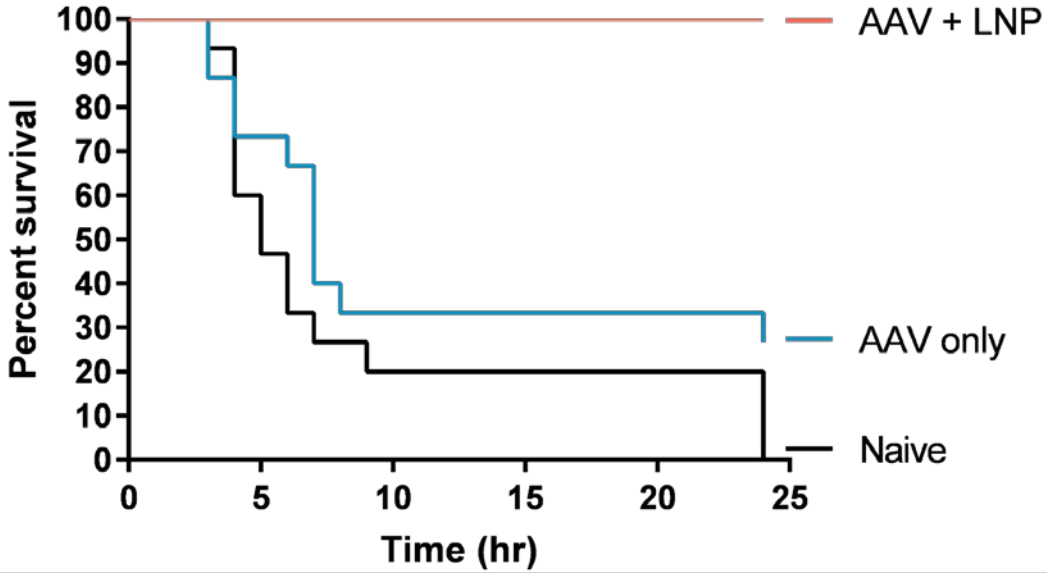


AAV8-FVIII 2e12 vg/kg
LNP# (low dose)

Day 5
FVIII
activity

Day 7
tail vein
transection



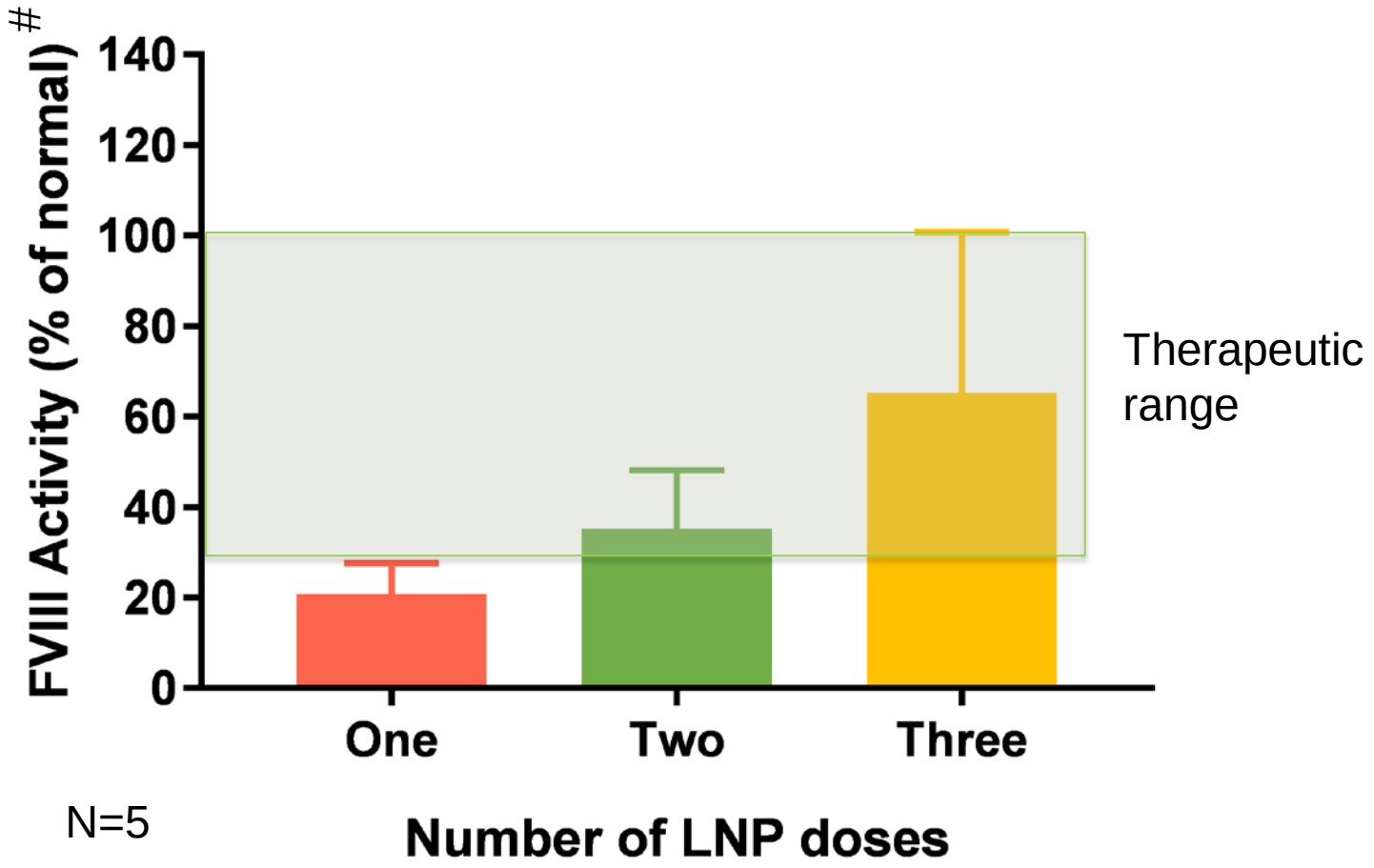


Cas9 mRNA, gRNA targeting L1

Repeat LNP Dosing Titrates FVIII Activity into Therapeutic Range

HemA Mice

AAV8-FVIII 2e12 vg/kg (single dose)
LNP: Cas9 mRNA, gRNA targeting L1, **3 doses 3 weeks apart**



Chromogenic assay

Summary and Conclusions

Therapeutic FVIII levels can be achieved by site-specific integration mediated by non-viral delivery of CRISPR-Cas9

Two target loci were identified that generate 40-fold higher FVIII than targeting to Albumin

FVIII is functional as assessed by chromogenic and aPTT assays and in a tail bleeding model

Stable expression expected as FVIII gene is integrated in the genome (6 months data in mice)

The level of FVIII can be controlled by LNP dose

Repeat dosing of LNP results in stepwise increase in FVIII expression illustrating the potential to titrate to optimal factor level

Further optimization and evaluation in higher species ongoing

Acknowledgments

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