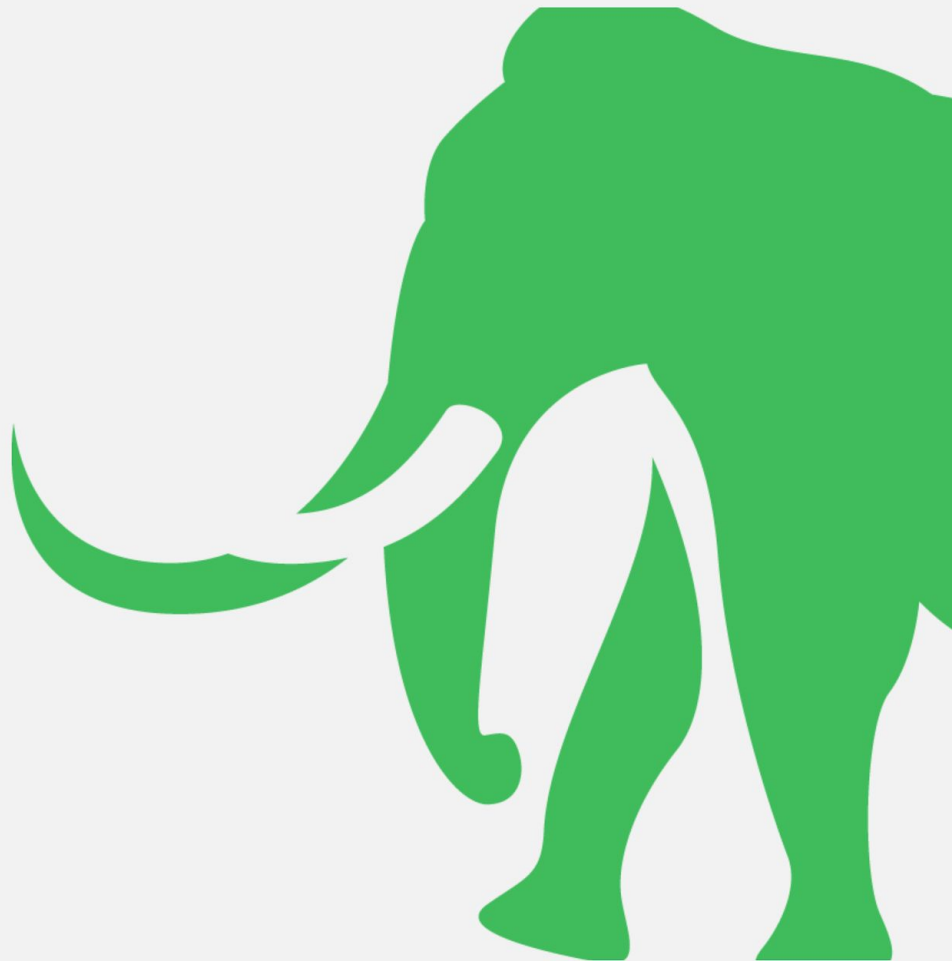


MammothBiosciences

Ultracompact CRISPR Systems to Overcome the Delivery Problem

October 14th, 2024

Lucas Harrington

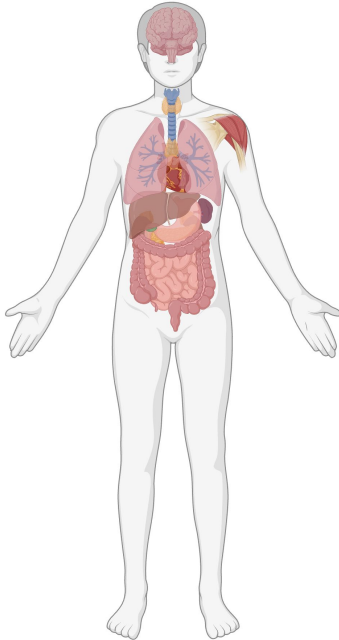


Ultracompact Systems are the Key to Expand Gene Editing

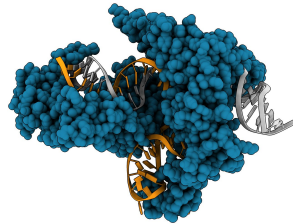
Advances in genetics and new editing technologies continue to be limited by delivery *in vivo*



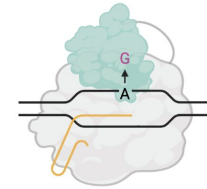
Diverse Genetic Disease
Beyond the Liver



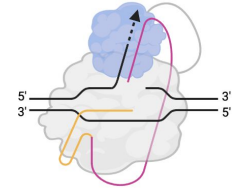
**Ultracompact
CRISPR Systems**



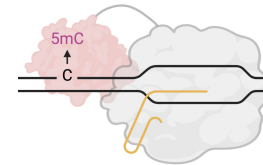
New Precise Methods for
Editing the Genome



Base Editing



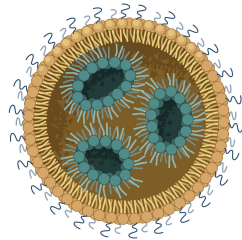
RT Editing



EpiEditing

Mammoth CRISPR Systems Enable All-In-One AAV Delivery To Unlock Targets Beyond the Liver

LNP Delivery

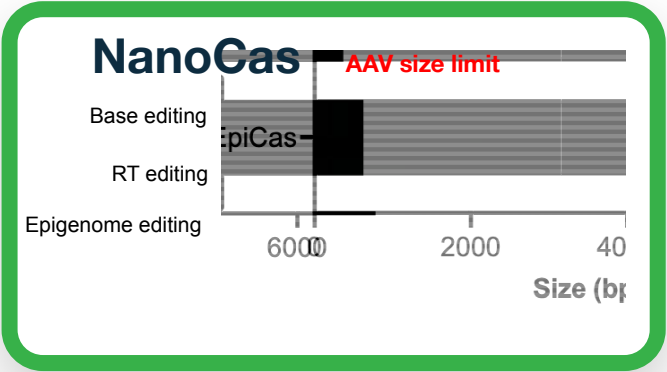


Smaller mRNAs allow for more efficient packaging and improved mRNA quality for delivery *in vivo*

AAV Delivery



Original CRISPR systems exceed payload restrictions



Mammoth Cas

Leaves abundant room for diverse payloads for precision editing applications

CRISPR+ Fusion Proteins

Tissue Specific Promoters

Guide RNA

Guide RNA

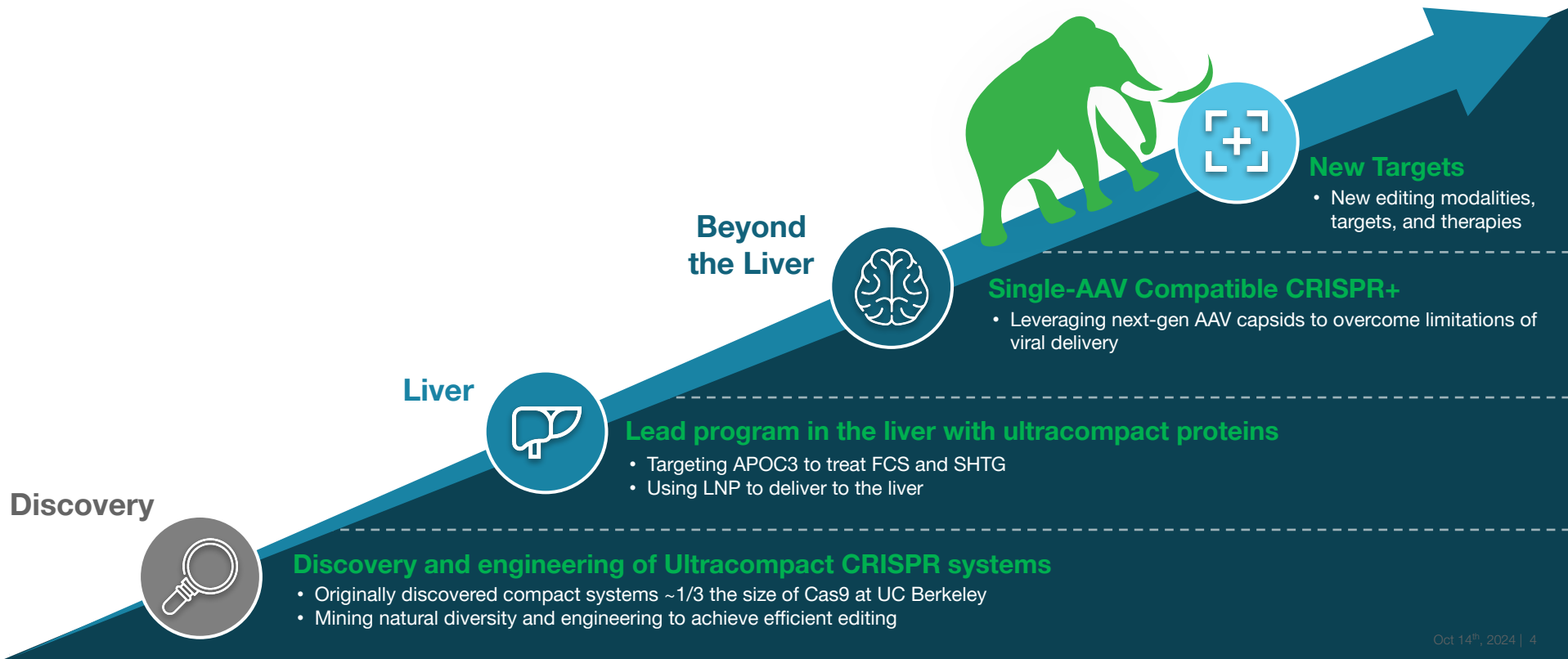
Integrases, Base Editing, CRISPRi/a...

Controlled Expression

Multiplexing

Roadmap for Developing Ultracompact Nucleases

Sequential derisking to expand gene editing applications



Mammoth Pipeline



Leveraging Ultracompact Systems to Unlock Tissues and Indications for *in vivo* Gene Editing

Target Tissue	Program	Indication	Editing Approach	Delivery	Partner	Research	Lead Optimization	IND Enabling	Clinical
Liver	MB-111	FCS + SHTG	Ultracompact Nuclease Knockout	LNP	Internal	<div><div></div></div>	<div><div></div></div>		
		Undisclosed	Ultracompact Nuclease Multiple Techniques	Multiple	Internal + Partner	<div><div></div></div>	<div><div></div></div>		
Neuromuscular		Undisclosed	Ultracompact Nuclease Multiple Techniques	AAV	Internal + Partner	<div><div></div></div>	<div><div></div></div>		
CNS		Undisclosed	Ultracompact Nuclease Multiple Techniques	AAV	Internal + Partner	<div><div></div></div>	<div><div></div></div>		

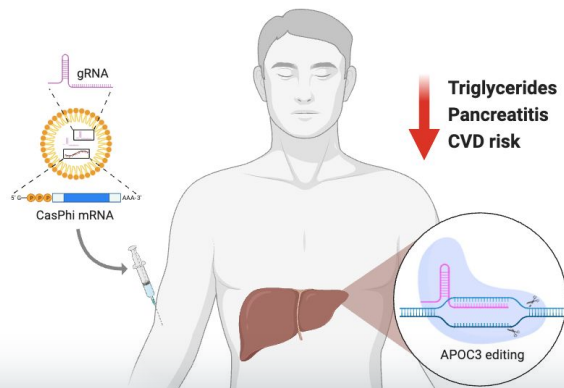
■ Proprietary ■ Proprietary and Partnered

Partners

REGENERON

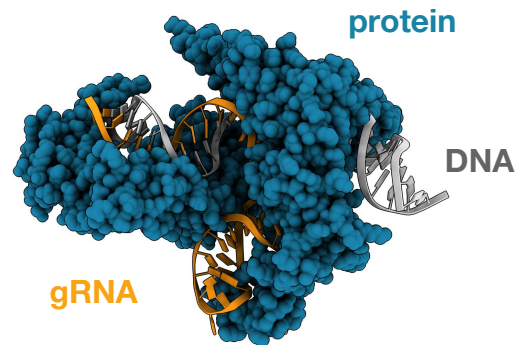


MB-111 is an *in vivo* Gene Editing Therapy to Treat Familial Chylomicronemia Syndrome (FCS) and SHTG by Targeting APOC3



We envision MB-111 as a durable cure for FCS and SHTG

- FCS and SHTG are characterized by accumulation of triglycerides in blood
- Estimated >2M patients worldwide for SHTG.
- Most severe forms of SHTG have TG >800 mg/dL and associated with acute pancreatitis.
- APOC3 encodes a small 74 aa lipoprotein that inhibits lipid catabolism, and silencing of APOC3 can speed up catabolism resulting in reduced triglycerides in serum
- MB-111 comprises of a **CasPhi nuclease mRNA** and **gRNA** encapsulated in a lipid nanoparticle targeting APOC3 in hepatocytes



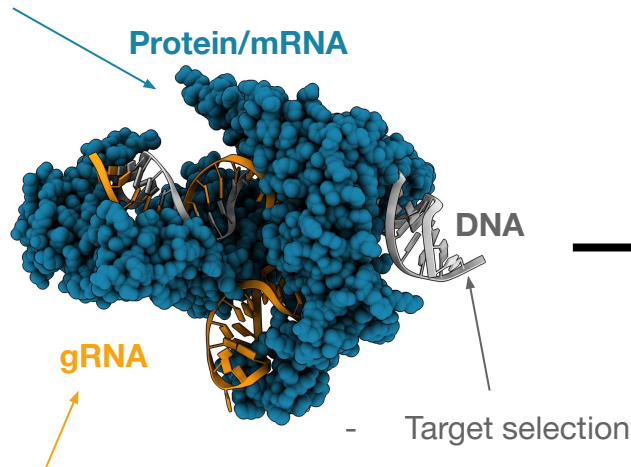
CasPhi	
Protein length	~720 aa
gRNA length	~40 nt
Single Guide?	yes
Protein structure	monomer
PAM	NTTN

Cas9 Equivalent Editing at Low Dose for hAPOC3 in Mice



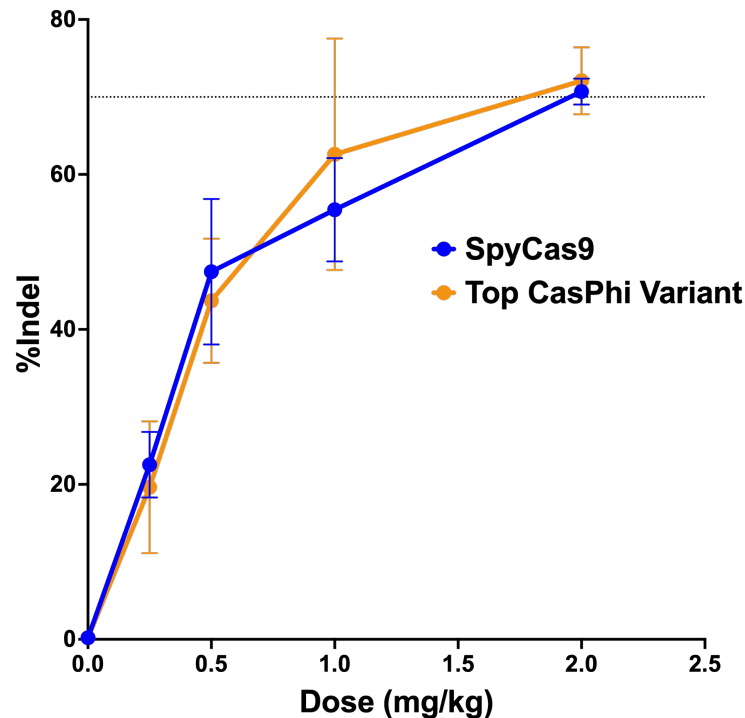
Putting all CasPhi improvements together achieves a major advance in MB-111 potency

- NLS optimization
- Protein engineering
- UTR
- ...



- Length optimization
- Chemical modifications
- Off target analysis
- ...

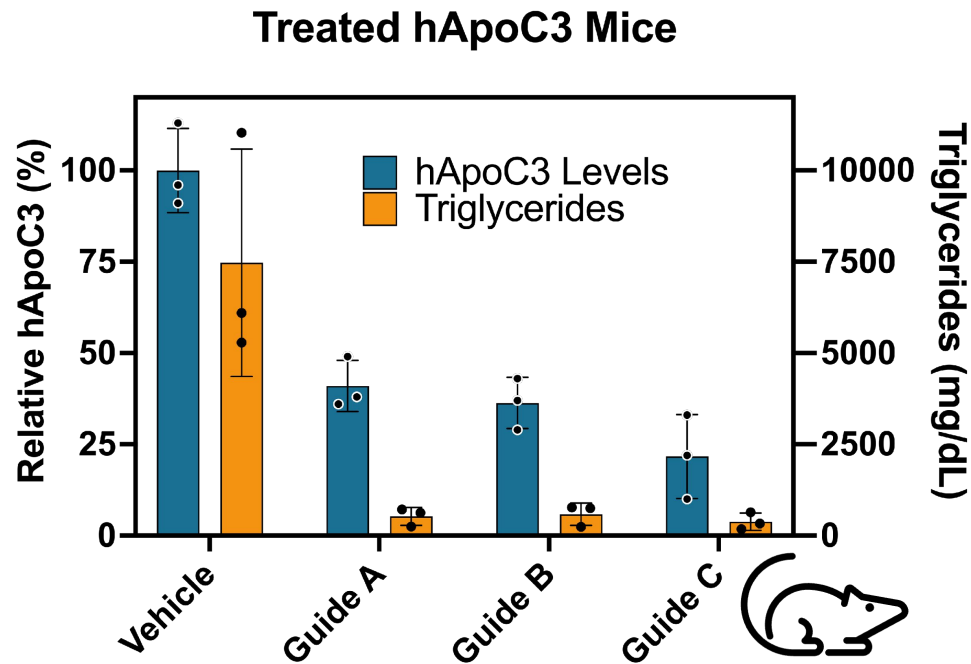
Top CasPhi variants — dose-response vs. Cas9



in vivo Editing of Hypertriglyceridemic hAPOC3 Mouse Reduces Triglycerides by up to 95%



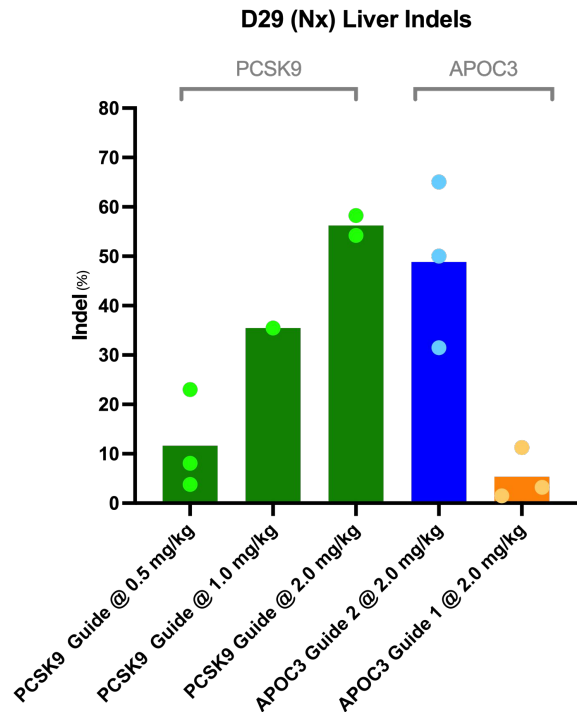
- Contains ~10 copies of human APOC3
- Hypertriglyceridemic mouse model
- Reduction in APOC3 protein by up to 80%
- Reduction in triglycerides by up to 95%
- LNP functions in hypertriglyceridemic conditions



Interim NHP Study for CasPhi and MB-111



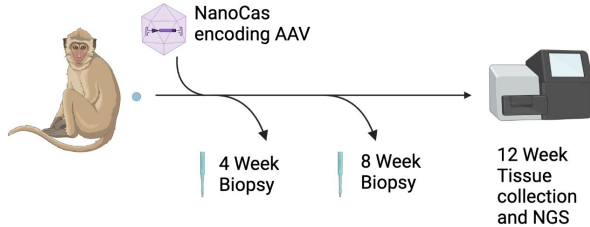
Large improvement seen in NHP editing efficiency with new protein variants



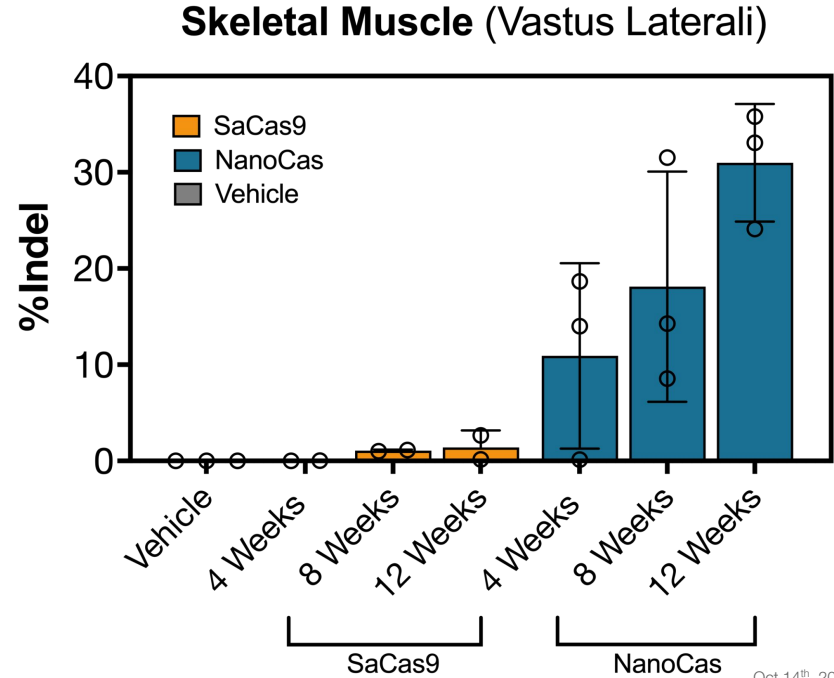
Grp	n=	Dose (mg/kg)	Cas mRNA-Guide	Liver Indels (Mean ± S.D.)
1	3	0.5	CasPhi - Guide 1 (PCSK9)	12% ± 10%
5	1	1.0		35%
2	2	2.0		56% ± 3%
3	3	2.0	CasPhi - Guide 3 (APOC3)	5% ± 5%
4	3	2.0	CasPhi - Guide 4 (APOC3)	49% ± 17%

First Test of Ultracompact Nucleases Delivered via AAV in NHPs

AAV-NanoCas shows robust editing in skeletal muscle that increases over time



- **Goal** Evaluate NanoCas AAV editing in NHPs
- **Results**
 - Robust editing with indels exceeding 30% in some muscle groups
 - NanoCas expression well tolerated in all animals with no adverse effects observed during 12-week study





Thanks to the Amazing Team at Mammoth!