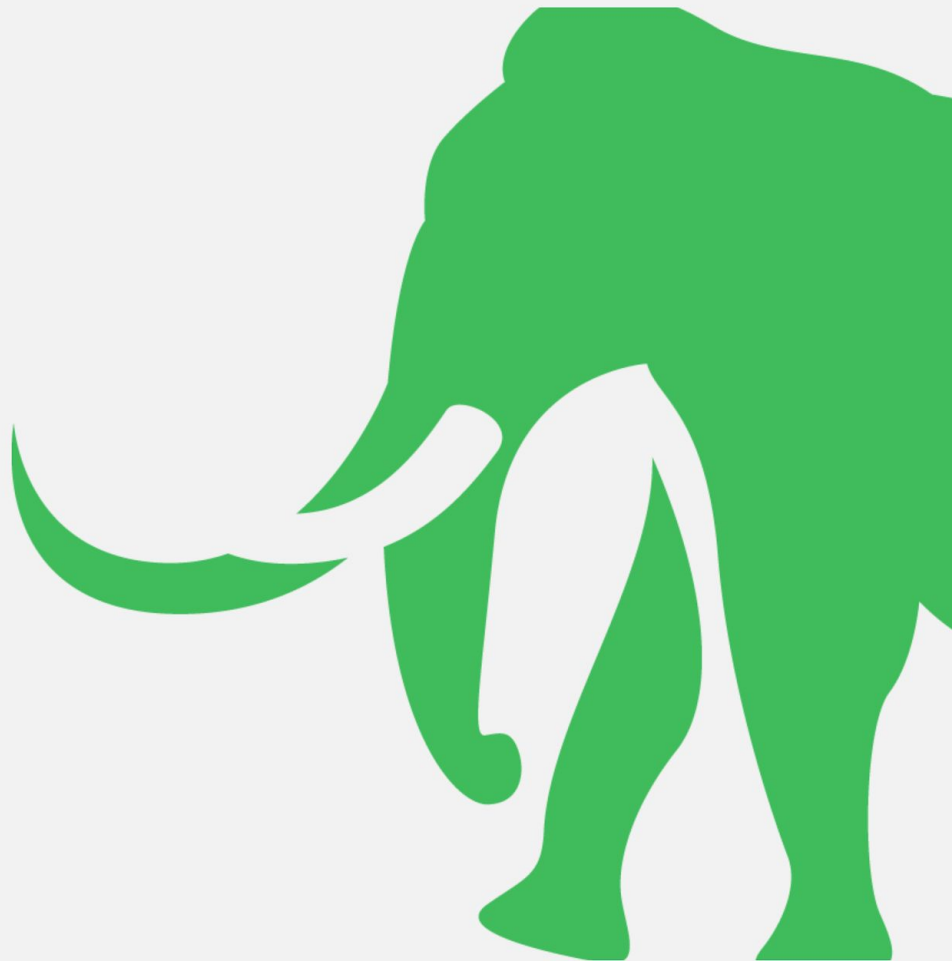


MammothBiosciences

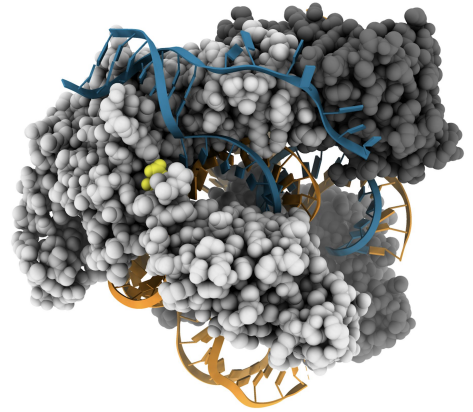
Developing *in vivo* Therapeutics with Ultracompact CRISPR Systems

Janice Chen, PhD
Co-Founder & CTO
Keystone Symposia
Precision Genome Engineering
January 25, 2024





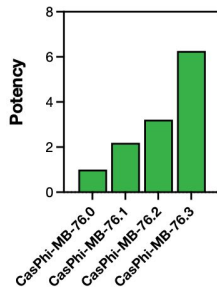
Mammoth aims to usher in a new era of gene editing by overcoming delivery limitations with our **Ultracompact CRISPR** systems



Key highlights

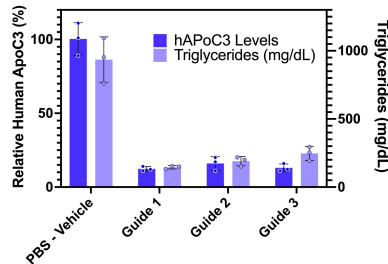
1

Potent ultracompact nucleases developed through protein discovery and engineering



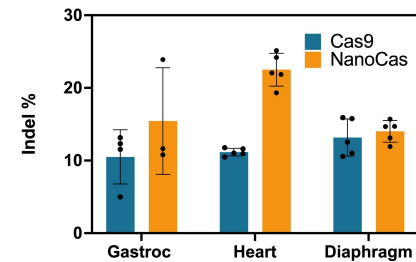
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Liver program shows robust triglyceride reduction *in vivo*

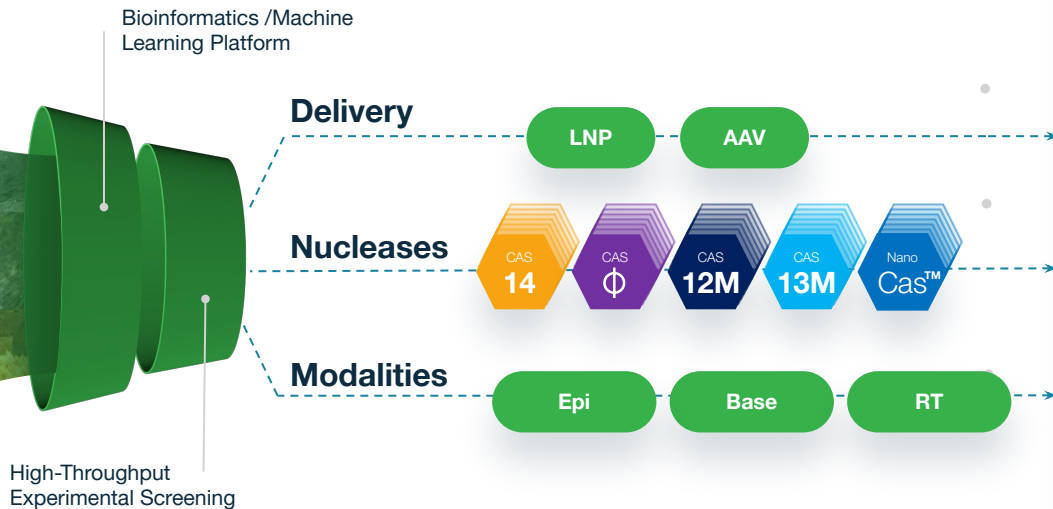


3

Single AAV injection of NanoCas shows high levels of gene editing in muscle



We leverage **discovery** and **engineering** to advance all components of gene editing



34 Billion

Proteins

Including exclusive data sources and proprietary analysis approaches

Biomanufacturing

Agriculture

Diagnostics

Therapeutics

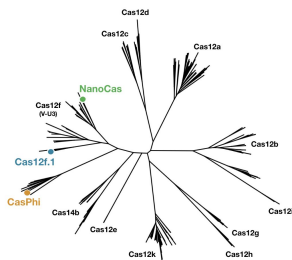
Research Tools

Other

Our fundamental innovation on CRISPR cargo unlocks the potential for gene editing

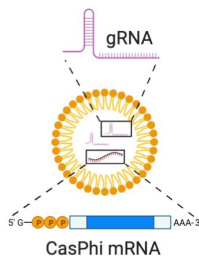
1

**Establishing the
broadest portfolio of
ultracompact CRISPR
systems**



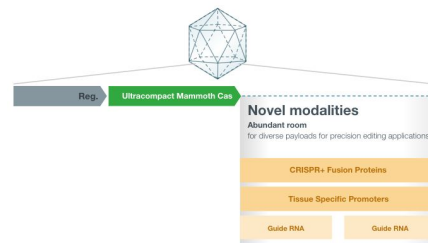
2

**De-risking novel
CRISPR cargo with
validated delivery
technologies**

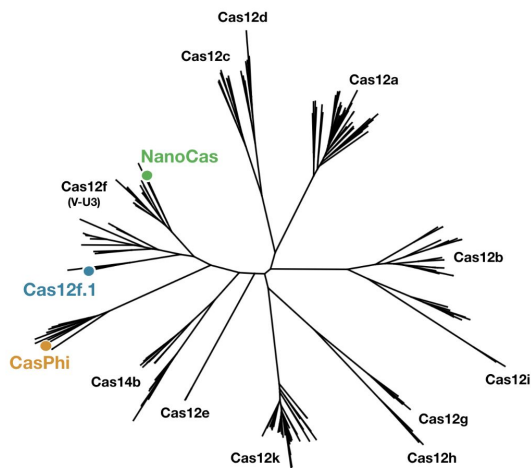


3

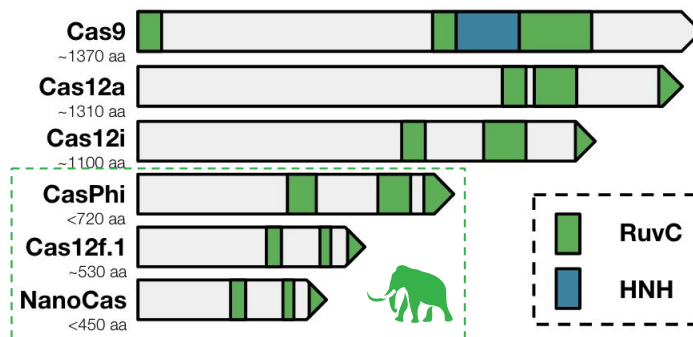
**Unlocking *in vivo*
extrahepatic targeting
with new editing
modalities**



Mammoth's Ultracompact CRISPR systems (<750aa) offer unique advantages



Mammoth's systems are significantly smaller than legacy systems



Advantages include:

- Small size
- Allele-specificity
- Fidelity
- Targeting range
- Stability
- Short gRNA
- Delivery compatibility

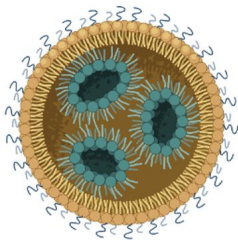
Zetche & Gootenberg *et al.*, Cell (2015)
Burstein, Harrington & Strutt *et al.*, Nature (2016)
Yan & Hunnewell *et al.*, Science (2019)
Harrington & Burstein *et al.*, Science (2018)
Pausch & Al-Shayeb *et al.*, Science (2020)

Our Ultracompact systems are compatible with any delivery modality, beginning with validated technologies



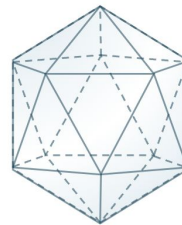
Clinically Validated Delivery Technologies

LNP



- **Smaller mRNAs allow for more efficient packaging with LNP**
- De-risk ultracompact nucleases in commercially attractive target with unmet patient need and validated biology

AAV



- **Ultracompact systems enable all-in-one single AAV delivery**
- Combine de-risked ultracompact nucleases with new editing modalities in commercially attractive targets with very high unmet patient needs

Novel Delivery

Non-viral, non-LNP
(e.g., retargeted LNPs,
exosomes, VLPs)

Our strategy involves sequential de-risking to address diseases of high unmet need beyond the liver

Leveraging our breadth of modalities to develop the right therapeutic



Established component

Next-gen component

Liver



Safety

Efficacy

Modality

Delivery

Muscle



Safety

Efficacy

Modality

Delivery

CNS



Safety

Efficacy

Modality

Delivery

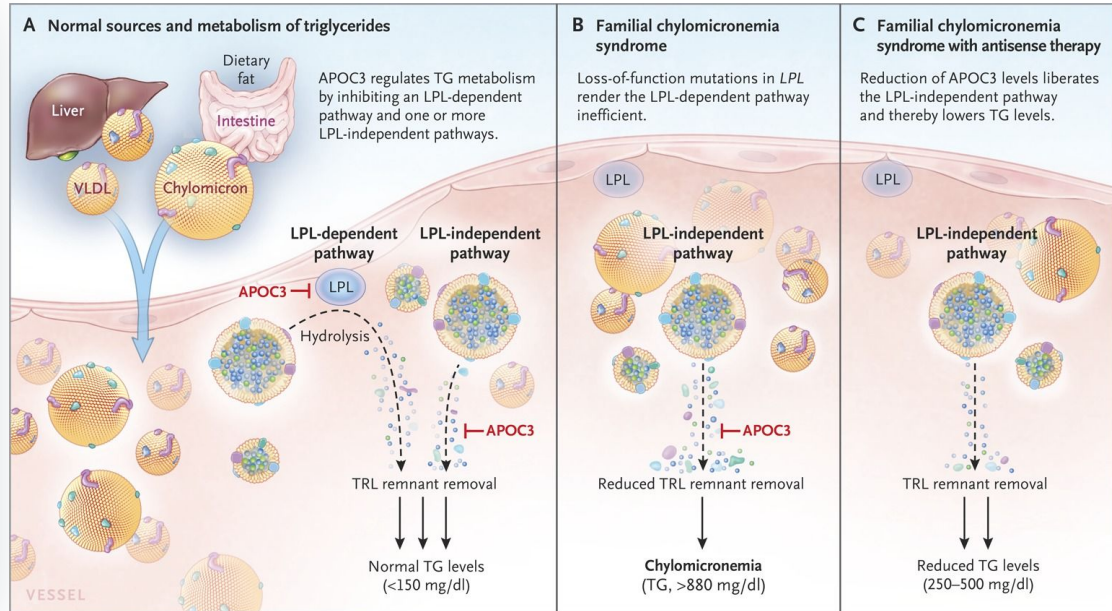
Familial Chylomicronemia Syndrome (FCS) is a life-threatening disease characterized by severely elevated triglycerides (TG)



Gene editing may offer a durable, “one and done” lifelong cure by lowering TG levels

Familial Chylomicronemia Syndrome

- Severely elevated triglycerides (>880 mg/dL and often over 2,000 mg/dL); currently no FDA-approved treatments.
- Loss-of-function in genes responsible for LPL-dependent triglyceride clearance (LPL most commonly, APOC2, APOA5, LMF1).
- Multiple systemic manifestations: recurrent abdominal pain; acute pancreatitis; neurocognitive problems; type 2 diabetes mellitus; eruptive xanthomas.
- Estimated 3000-5000 patients worldwide.
- Mainly managed by adhering to an extremely low fat diet of <10% daily caloric intake

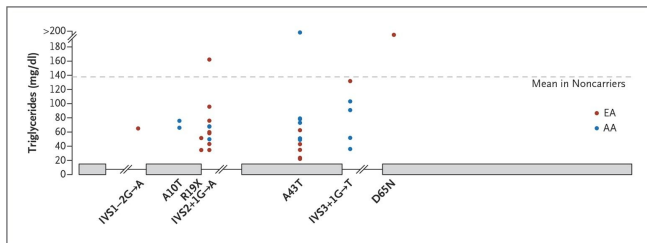


Human genetics and clinical data suggest that APOC3 knockout is a potentially safe and curative therapeutic approach

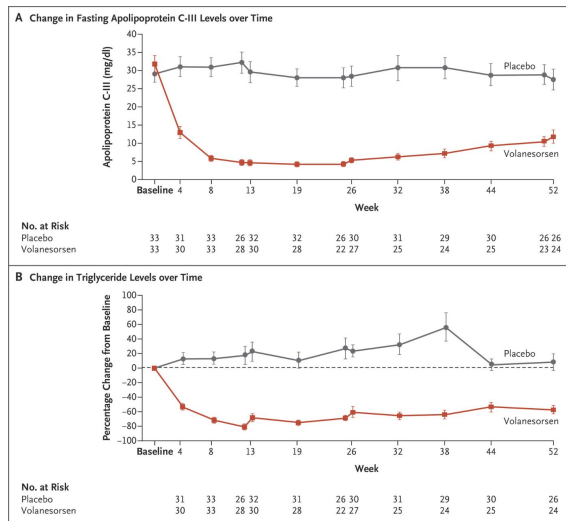


Other knockdown approaches demonstrate MOA and clinical efficacy but are not curative

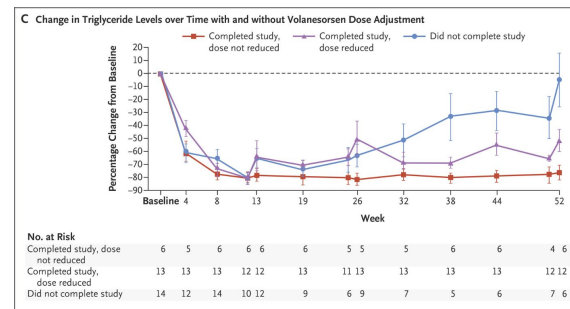
Naturally-occurring loss of function mutations in APOC3 are safe and associated with lower TG levels



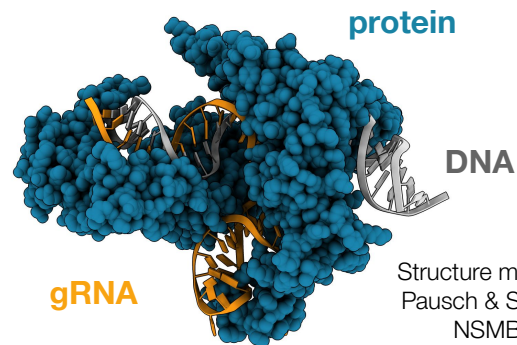
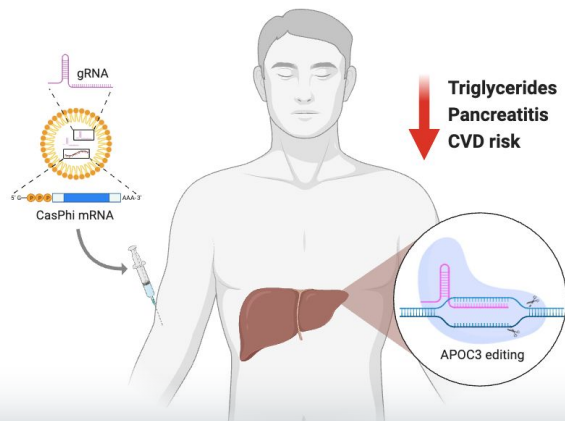
APOC3 knockdown by weekly ASO treatment demonstrated decreased TG levels in patients with FCS



TG levels returned to baseline in FCS patients that did not complete the study



MB-111 is a single-dose, *in vivo* gene editing therapy to treat FCS by targeting APOC3



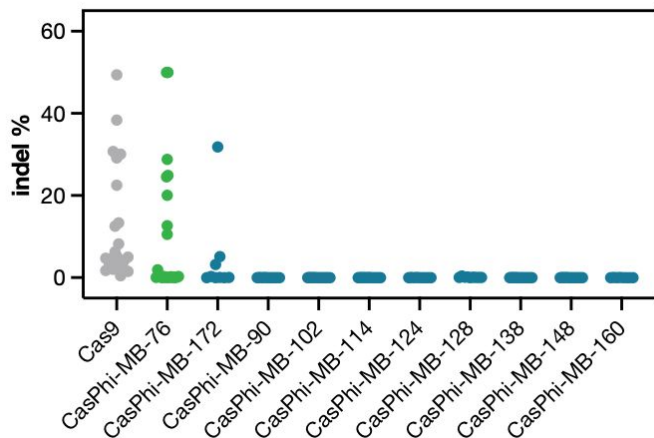
Developing a permanent cure for FCS

- Nuclease mRNA and gRNA encapsulated in lipid nanoparticles for targeted liver delivery
- Facilitates indel formation and genetic ablation of APOC3
- APOC3 knockdown results in permanent triglyceride lowering and amelioration of FCS symptoms

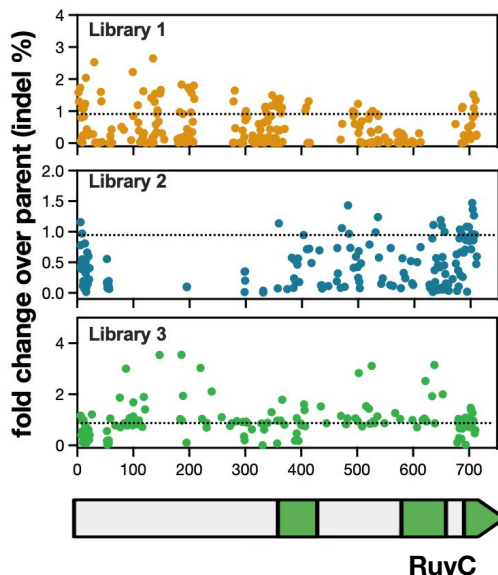
CasPhi	
Protein length	<720 aa
gRNA length	~40 nt
Single Guide?	yes
Protein structure	monomer
PAM	NTTN

Engineered CasPhi variants shows improved activity *in vitro*

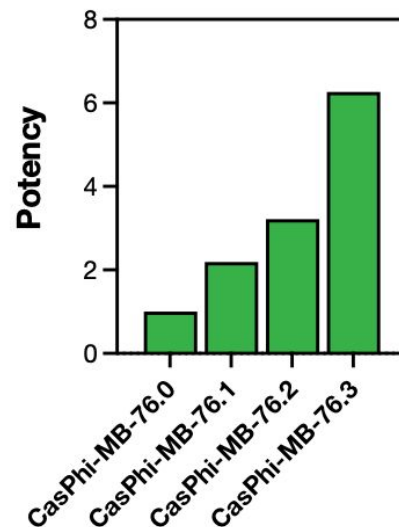
Activity of natural variants



Semi-rational HTP scanning mutagenesis

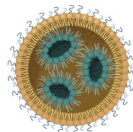


in vitro potency improvement of consolidated variants



Engineered CasPhi variants shows improved activity *in vivo*

LNP/mRNA/guide Formulation & QC



Design and Production of LNP

- CasPhi mRNA
- gRNA
- LNP lipids

IV Administration in WT mice



IV Administration

- Single Administration
- 1-week long study
- 2 mg/kg

Liver Tissue Sampling



Tissue Harvest

- Liver

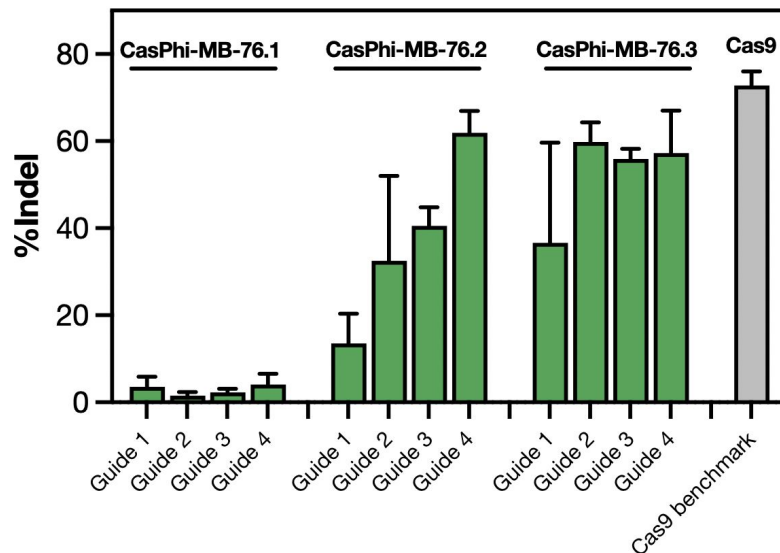
NGS Analysis



Indel Analysis

- Genomic DNA extraction
- Amplicon Sequencing
- INDEL Analysis

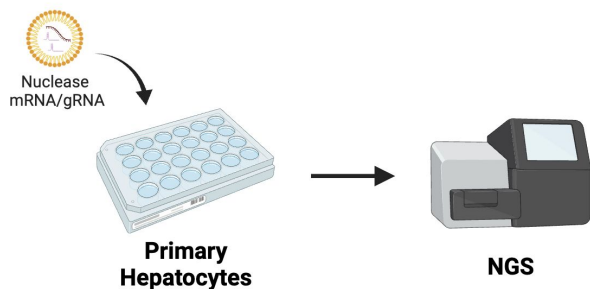
Engineering cycles



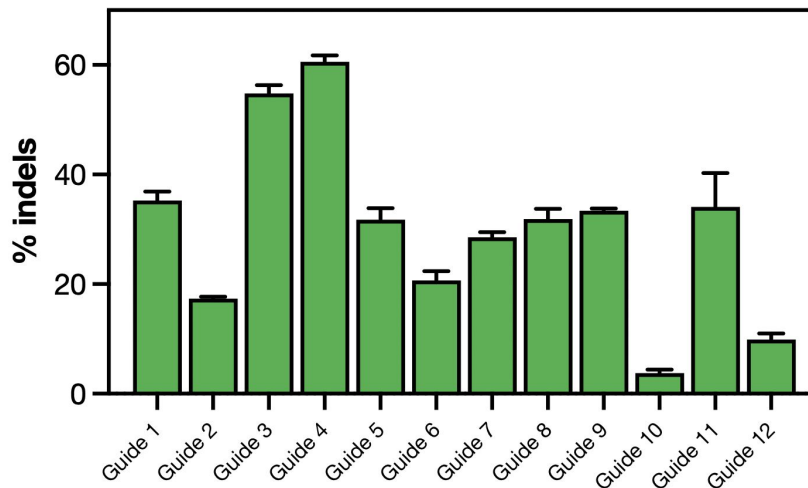
APOC3 guide screening in human primary cells yields multiple candidate gRNAs



mRNA/gRNA transfection of primary human hepatocytes



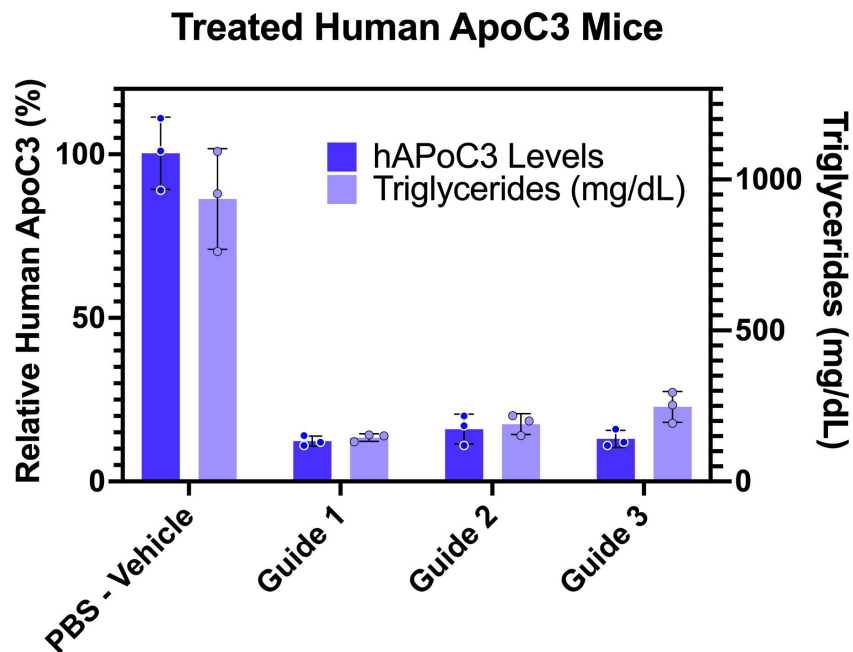
In vitro guide screen



In vivo editing of APOC3 reduces protein and triglyceride levels by ~80%



Humanized transgenic mice harbor human APOC3 gene and exhibit severely elevated triglycerides



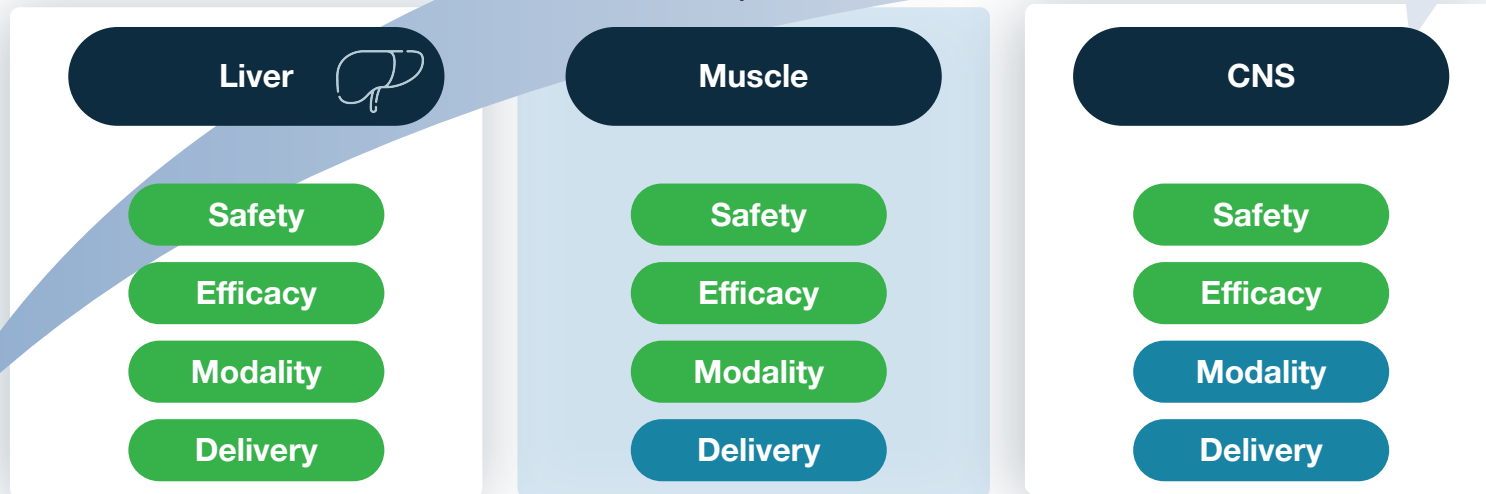
Our strategy involves sequential de-risking to address diseases of high unmet need beyond the liver

Leveraging our breadth of modalities to develop the right therapeutic



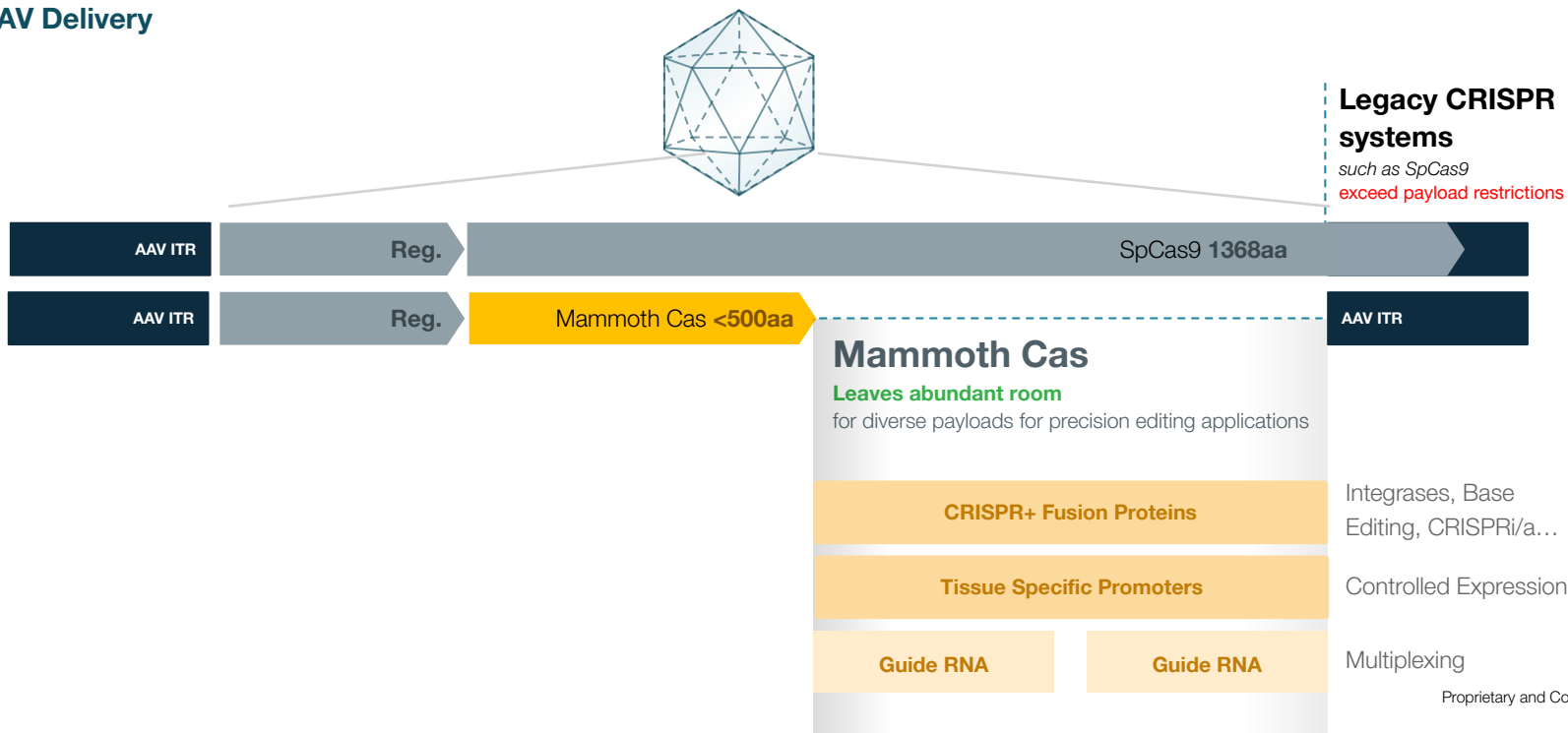
Established component

Next-gen component



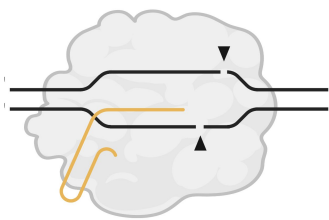
Mammoth CRISPR Systems Enable All-In-One AAV Delivery To Unlock Targets Beyond the Liver

AAV Delivery



Unlocking extrahepatic delivery with Ultracompact nucleases

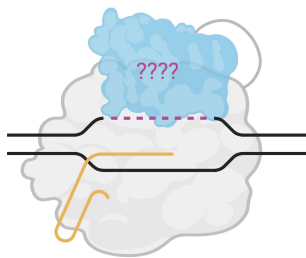
Mammoth's smallest nucleases leave room for CRISPR+ modalities in AAV



Ultracompact Nuclease

CRISPR 1.0

Double Stranded Breaks



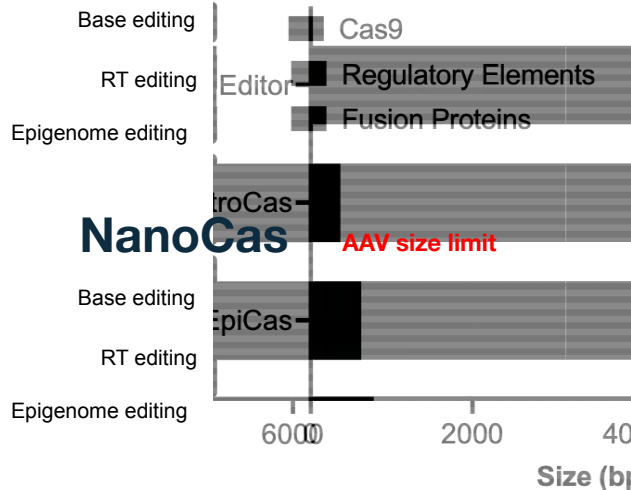
CRISPR+

CRISPR 2.0

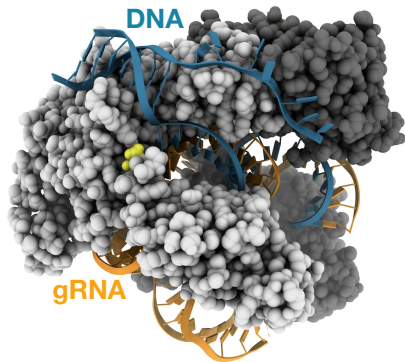
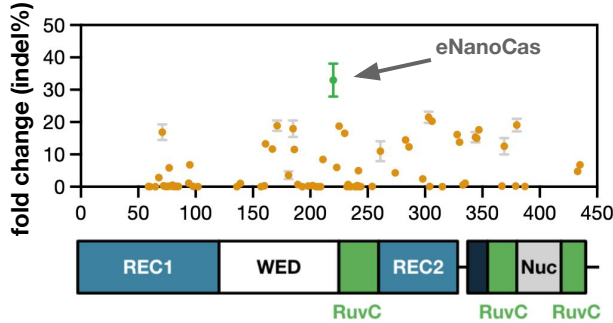
Beyond Double
Stranded Breaks

SaCas9

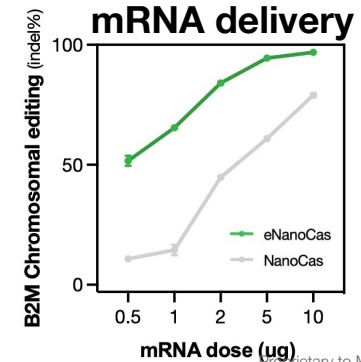
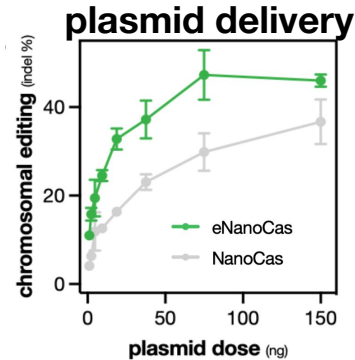
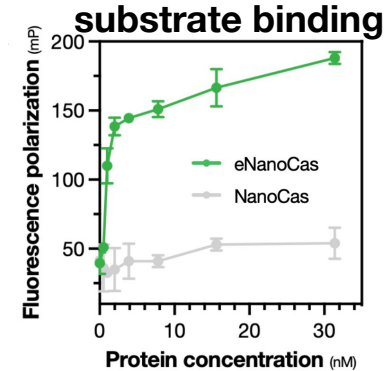
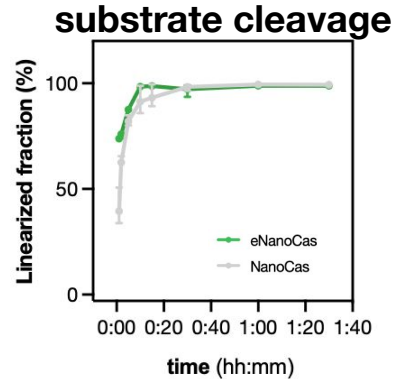
AAV size limit



NanoCas variants show increased potency over several rounds of protein engineering



Structure from: Takeda *et al.*, Mol Cell (2021)

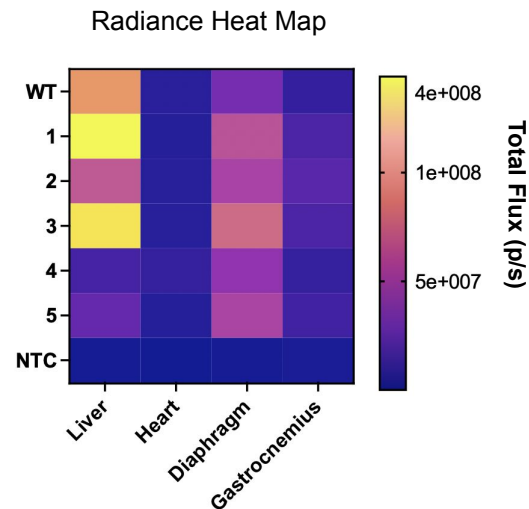
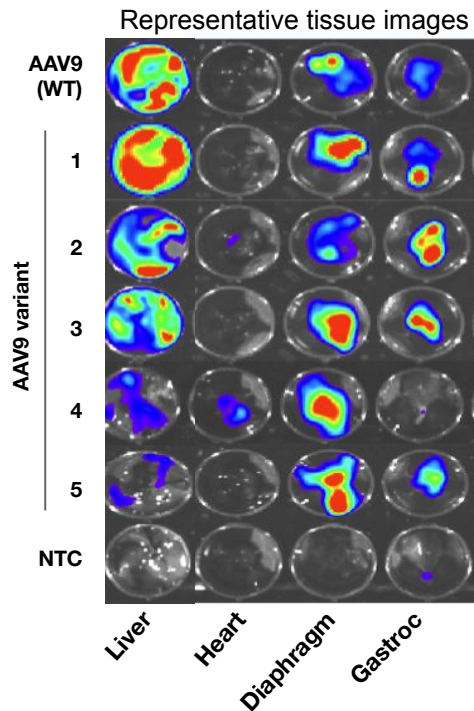
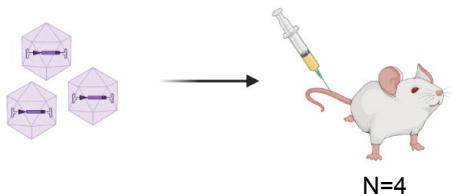


AAV9 serotype variants result in improved transgene muscle delivery compared to Wild Type AAV9



AAV-NanoLuc reporter
Intravenously

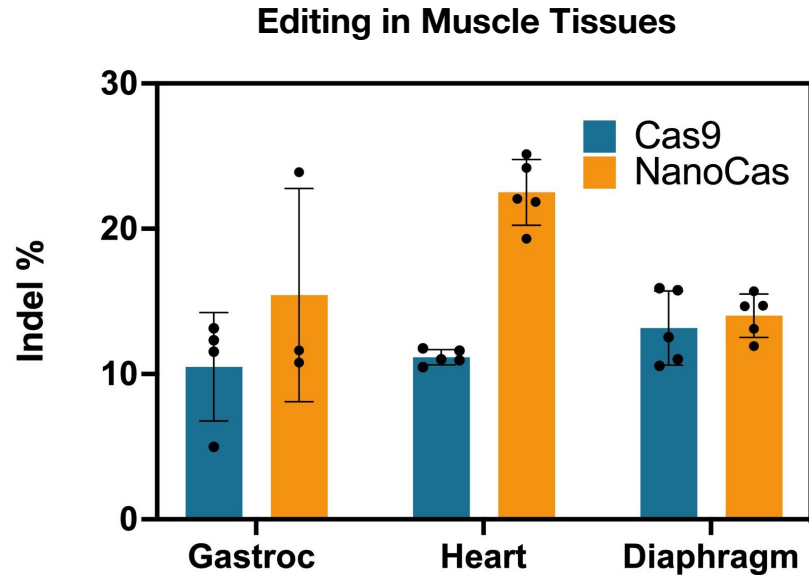
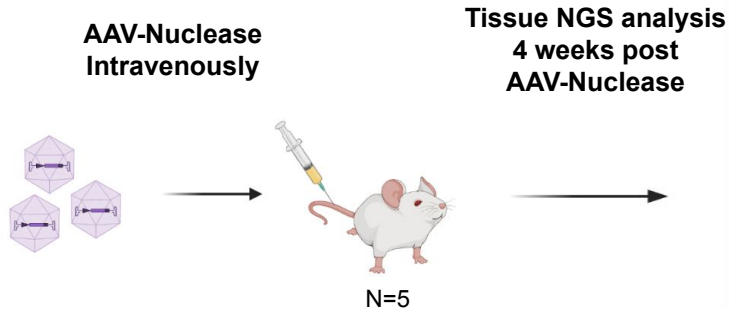
Ex vivo tissue imaging
4 weeks post
AAV-NanoLuc



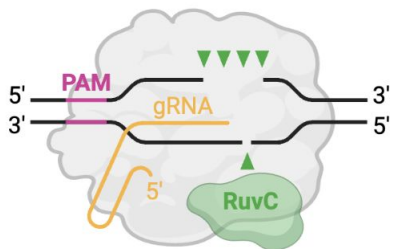
First *in vivo* demonstration of gene editing in muscle with NanoCas delivered by AAV administration



AAV9-based vector encoding NanoCas induces high levels of gene editing in muscle

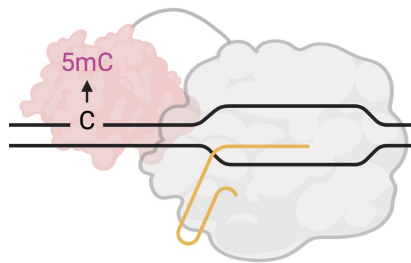


Ultracompact systems enable new CRISPR+ modalities that can address a broad range of genetic diseases



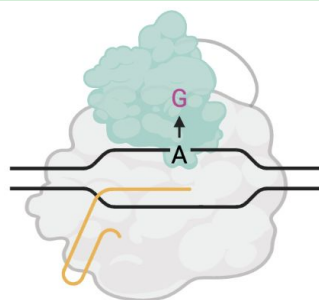
Nuclease editing

Ultracompact versions of CRISPR that make double stranded breaks to inactivate genes



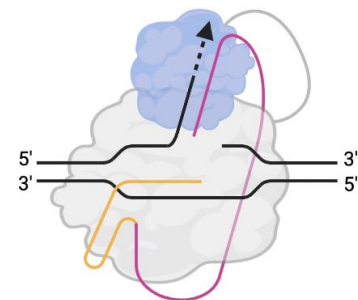
Epigenetic editing

A platform technology that uses epigenetic modifications to silence genes



Base editing

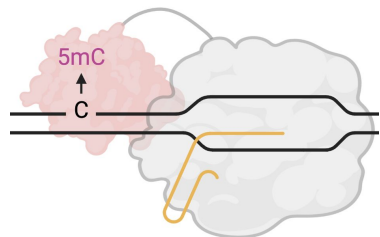
A platform technology that makes a single-letter change to convert A to G or C to T



RT editing

A platform technology that makes versatile RNA-encoded insertions, deletions or substitutions

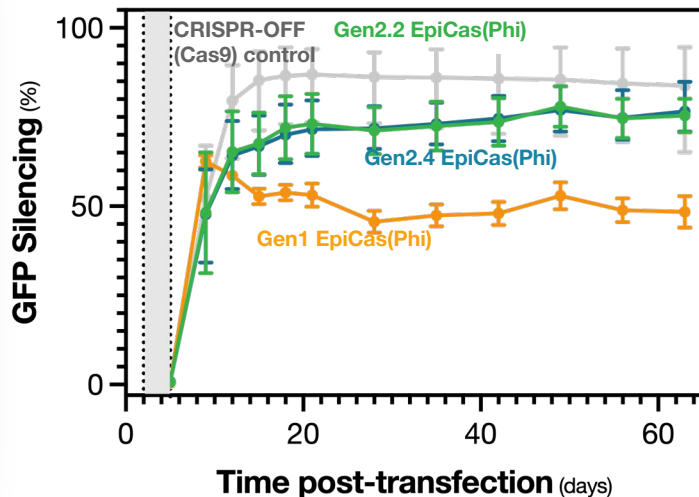
EpiCas is a robust epigenetic silencing platform that leverages ultracompact systems and fits within a single AAV



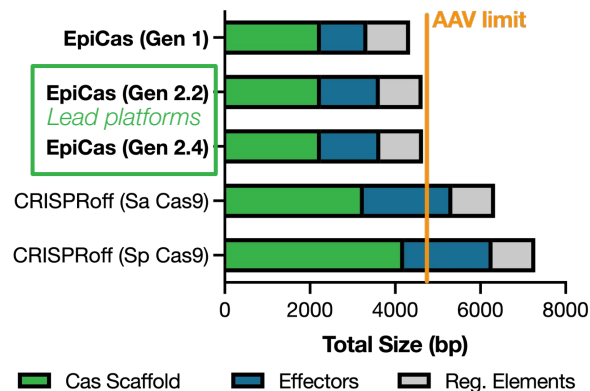
Epigenetic editing

A platform technology that uses epigenetic modifications to silence genes

Durable silencing in HEK293T



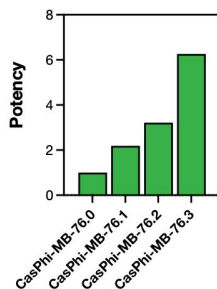
Epigenetic editor fusion sizes



Key takeaways

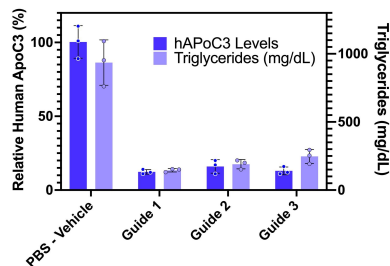
1

Potent ultracompact nucleases developed through protein discovery and engineering



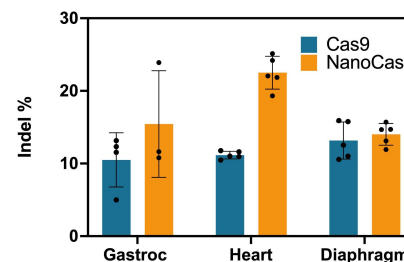
2

Liver program shows robust triglyceride reduction *in vivo*



3

Single AAV injection of NanoCas shows high levels of gene editing in muscle





Thanks to the entire
Mammoth Biosciences team!

Keith Abe
Sean Coakley
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Jennifer Mathes
Robert Lamboy
Mark DeWitt
Siming Xu
Dennis Ho
Alyssa Sancio
David Yuan
Sierra Rybarczyk
Susan Providenza
Sophia Yunanda
Aaron DeLoughery
Shravanti Suresh
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Jackie Laurel
Kelsey Docouto
Tony Gao
Ben Rauch
Ray Hickey
Lucas Harrington