Mammoth Biosciences

MB-111 · CasPhi Gene Editing Therapy

Off-Target Strategy for a Novel Small Nuclease

CASSS Cell & Gene Therapy · 2026

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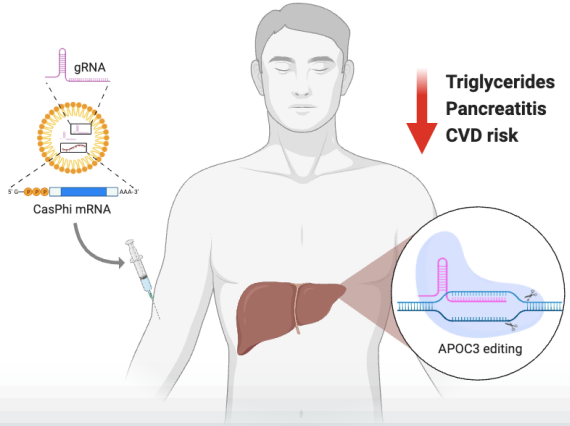
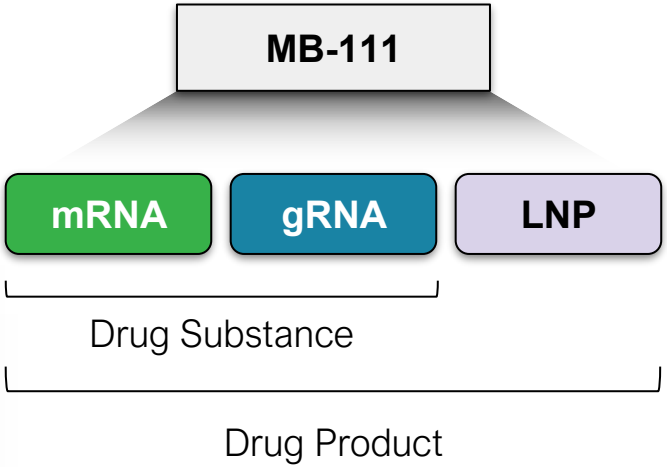
MB-111 is a single-dose, *in vivo* gene editing therapy to treat persistent chylomicronemia by targeting APOC3

Our goal is to develop a CRISPR-based permanent cure using engineered CasΦ

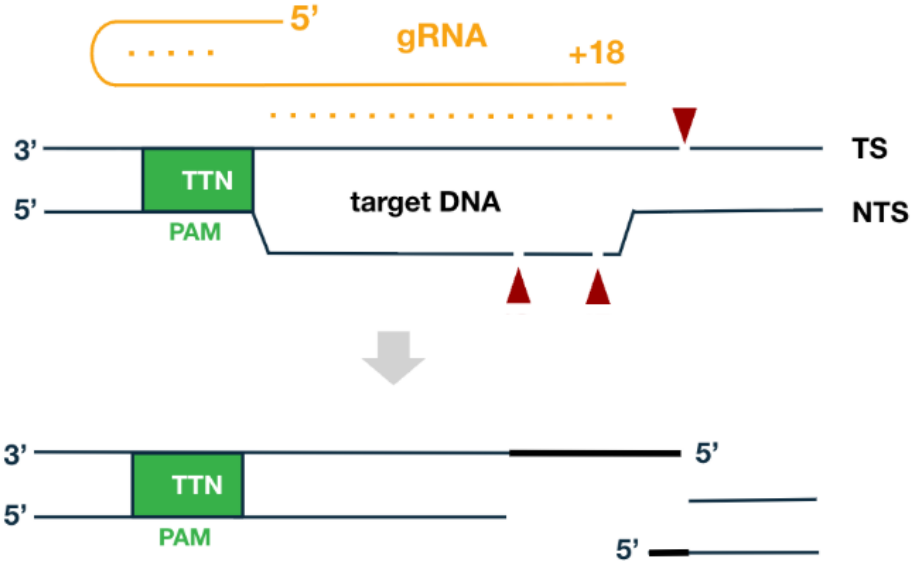




Developing a permanent treatment for hypertriglyceridemia

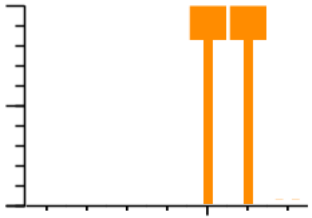
- **CRISPR-CasΦ** mRNA and gRNA encapsulated in lipid nanoparticles for targeted liver delivery
- Targeted DNA cutting leads to indel formation and **genetic ablation of APOC3**
- APOC3 knockdown results in **triglyceride lowering** and amelioration of persistent chylomicronemia symptoms based on clinically approved siRNA and ASOs

CasPhi is a compact, bacteriophage-derived nuclease with a restrictive PAM



PAM specificity

CasPhi: 5' TTN



Building confidence in CasPhi: guide, on-target, off-target

LNP-delivered CasPhi mRNA + guide RNA

Guide RNA

Optimized scaffold built for stability and target recognition

- **Architecture**
  - 65-mer with 3′ spacer
  - Chemical modifications

On-Target Characterization

Reproducible, dose-dependent editing in primary human hepatocytes

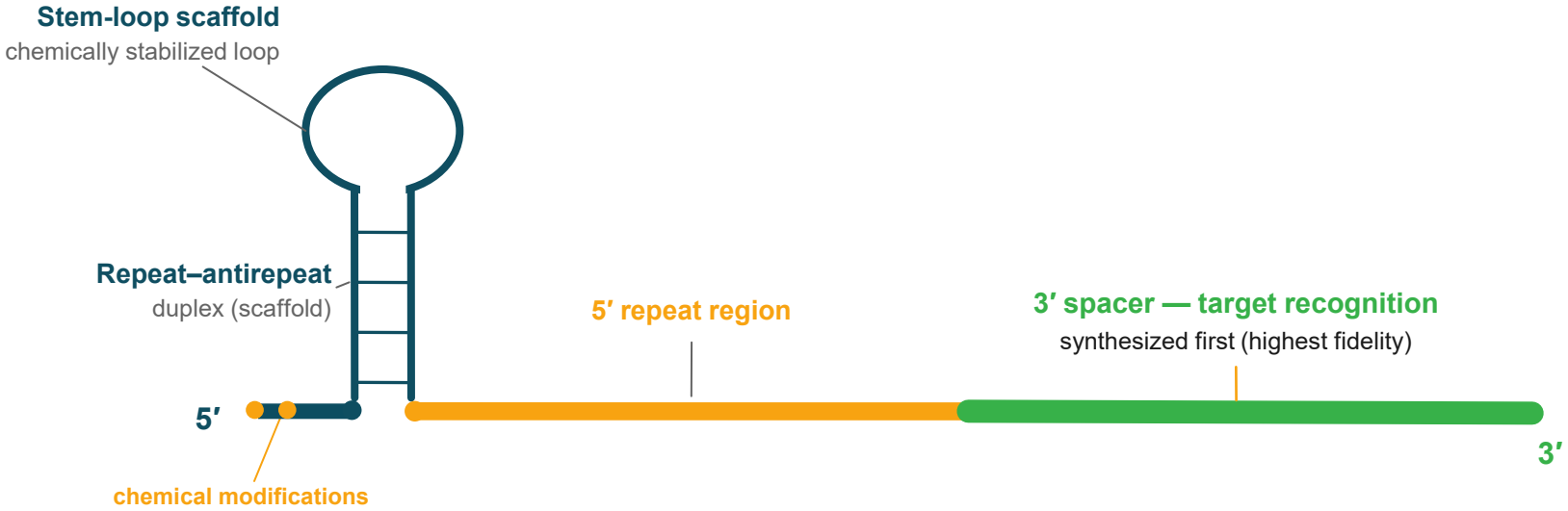
- **In vitro dose-response**
  - Primary human hepatocytes

Off-Target Characterization

No meaningful off-target activity across in silico, biochemical, and wet-lab assays

- **In silico nomination**
  - GRCh38 reference + variant-aware
- **Biochemical screen**
- **Wet-lab verification**
- **Structural integrity**

CasPhi Guide RNA Structure

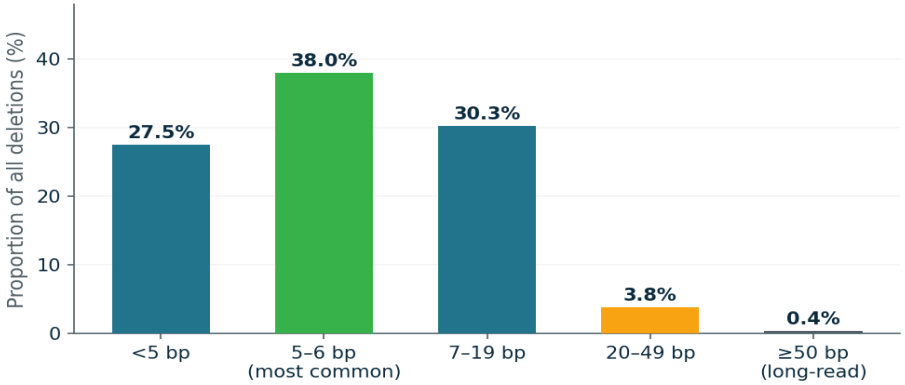


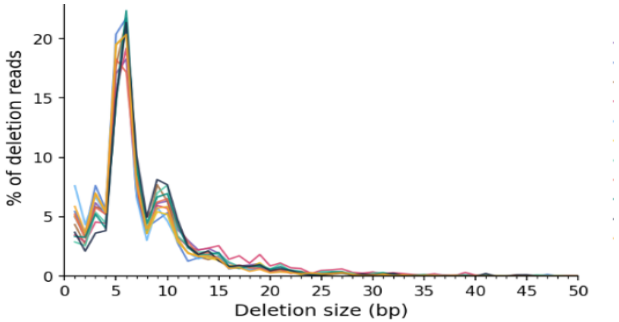
Why highlight the 3′ end of the spacer?

Solid-phase RNA synthesis proceeds **3′ → 5′**, so the 3′ spacer — the target-recognition region — is built first, before coupling errors and depurination accumulate.

Placing the critical targeting bases at the 3′ end keeps them in the highest-fidelity portion of the synthesis, giving lower error rates where on-target activity matters most.

On-target editing is precise: small deletions, consistent across 11 PHH donors





Editing profile

- 99.9% **of deletions <50 bp**
- 5–6 bp deletions most frequent (~38% at EC90)
- Large deletions (≥50 bp): **0.4%** at max dose (18x EC90)
- Consistent across 11 PHH donors
- Edits confined to APOC3 intron 1 splice junction
- No cis-regulatory elements disrupted

A novel nuclease requires a comprehensive off-target strategy

Three factors drove this approach

Novel nuclease

- CasPhi is less characterized than SpCas9
- No established regulatory precedent for off-target assessment

Modified cleavage mechanism

- Staggered 5' overhang cut → incompatible with standard SITE-Seq
- Required development of mSITE-Seq
- Verified vs Cas9/EMX1 control: 16/16 known OT sites recovered

FDA Guidance

- Orthogonal, multi-method nomination
- Variant-aware analysis for population genetic diversity
- Verification in most relevant model of target cell type (PHH) at supersaturating doses

Four-tier orthogonal strategy for comprehensive off-target characterization

Tier 1

In Silico Prediction
Cas-OFFinder
GRCh38 + DivRef
(variant-aware)

Tier 2

Biochemical Discovery
mSITE-Seq
(cell-free, CasPhi-compatible)

Tier 3

Targeted Verification
rhAmpSeq in PHH
(super-saturating followed by dose titration)

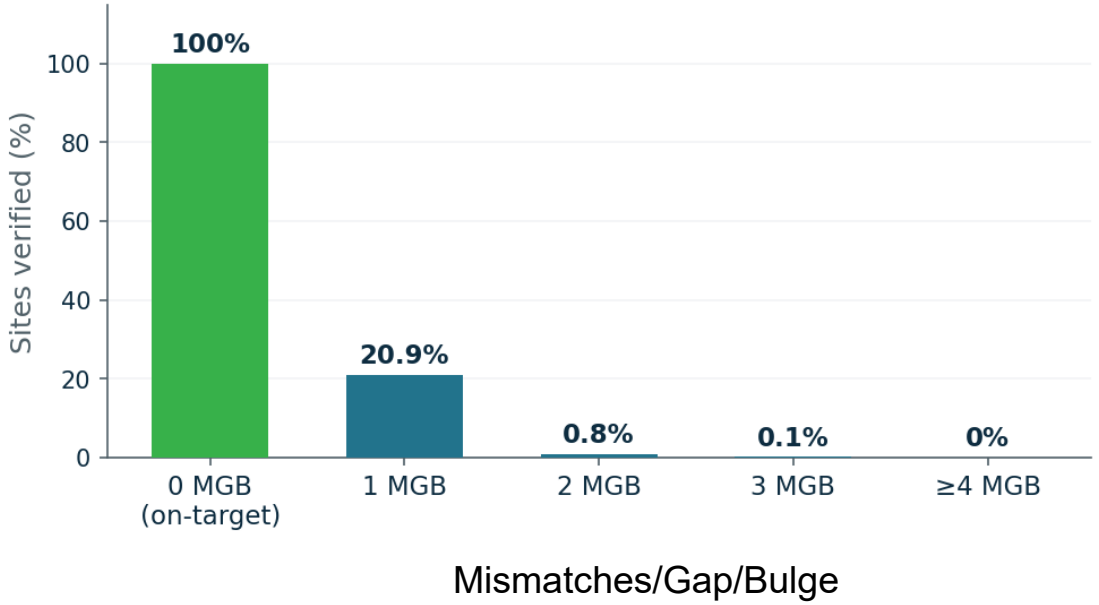
Tier 4

Impact Assessment
Genic context
Expression profile
Functional impact

317 in silico sites for verification

Verification rate drops sharply with increased mismatches

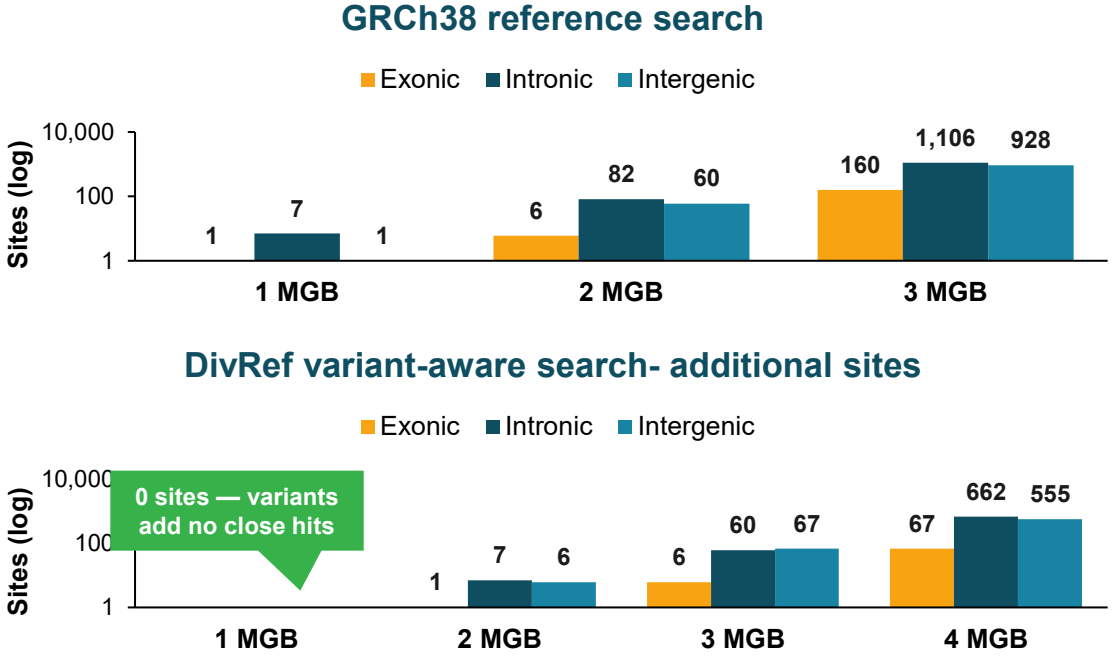
Verification rate drops sharply above 1 mismatch — empirically verified thresholds



Summary

- **On-target verified at 100%** — assay sensitivity confirmed at the intended cut site
- **1 mismatch sites verify at ~21%** — single mismatches are real risk that must be characterized
- **Sharp drop at ≥2 mismatch (<1%)** — CasPhi enforces a strict mismatch tolerance
- **Implication:** selecting guides without in silico predicted 1MM sites, focus wet lab characterization on any 1-2 MM off-target sites

In silico search covers reference genome and population variant diversity



Search parameters

- CasPhi 5′ TTN PAM (stringent)
- 17-nt spacer for GRCh38; 18-nt spacer for DivRef
- 5 ancestry groups (African/African American, American, EAS, SAS, NFE)

Biological relevance: Population variants introduce **zero new 1-MGB sites** — no close-mismatch hits emerge across 39M variants, so allelic diversity does not erode specificity at the highest-risk tier. Most off-targets remain in **intronic and intergenic** regions; exonic hits stay **<10 through 2 MGB** in both.

Four-tier orthogonal strategy for comprehensive off-target characterization

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GRCh38 + DivRef
(variant-aware)

317 in silico off-target sites

Tier 2

Biochemical Discovery
mSITE-Seq
(cell-free, CasPhi-compatible)

301 biochemical off-target sites nominated

Tier 3

Targeted Verification
rhAmpSeq in PHH
(super-saturating followed by dose titration)

Tier 4

Impact Assessment
Genic context
Expression profile
Functional impact

mSITE-Seq was developed for CasPhi's staggered cleavage

Standard SITE-Seq

Blunt-end adapter ligation- designed for Cas9

✗ CasPhi → no amplifiable library; high levels of ligation artifacts

Adapted →

mSITE-Seq (modified for CasPhi)

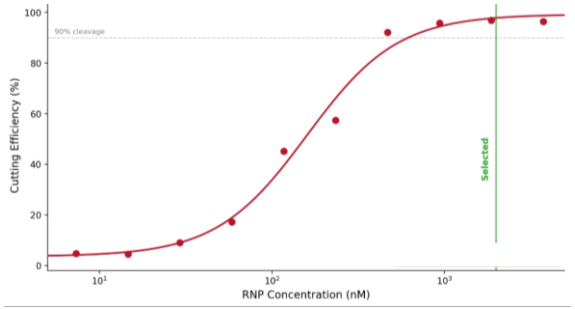
✓ Verification: Verified Cas9/EMX1 OT sites recovered by both protocols

✓ CasPhi → successful detection of off-target sites

Verified across:

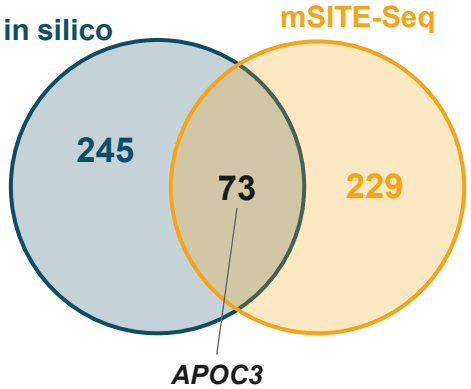
2 operators **3 gRNA lots**

CasPhi RNP Amplicon cleavage



Protein batch is tested for efficacy.
mSITEseq dose is >3X EC_{90} of the RNP.

301 biochemical off-target candidate sites nominated



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Targeted Verification
rhAmpSeq in PHH
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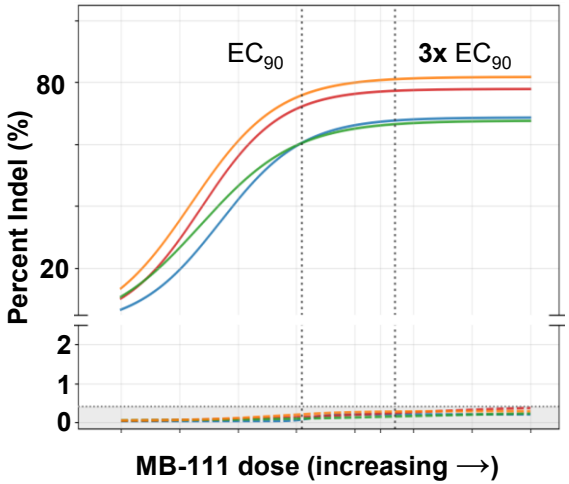
546 sites tested
0 verified
Editing > LoD
1 dose responsive
Editing < LoD

Tier 4

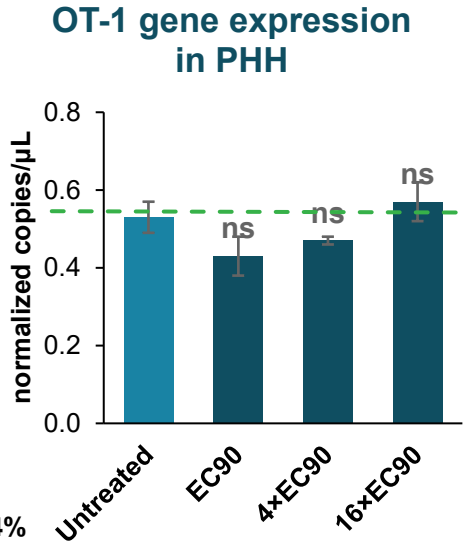
Impact Assessment
Genic context
Expression profile
Functional impact

OT-1: the single dose-responsive site is below LoD and de-risked

Intronic location; >250-fold on-target selectivity; no functional consequence



LOD= 0.4%



OT-1 risk assessment

- Location: **intronic** (non-coding)
- 2 mismatches/gaps/bulges from guide
- Editing rate: **0.1–0.4%** at 1.2–18.8× EC_{90}
- **>250-fold** on- vs off-target selectivity
- Gene: ubiquitous housekeeping function
- Gene expression: Similar to untreated in PHH (dPCR, p=NS)
- Redundant paralogs functionally compensate
- No disease phenotypes in databases (OMIM, ClinVar, DepMap)

Four-tier orthogonal strategy for comprehensive off-target characterization

Tier 1	Tier 2	Tier 3	Tier 4
In Silico Prediction Cas-OFFinder GRCh38 + DivRef (variant-aware)	**Biochemical Discovery** mSITE-Seq (cell-free, CasPhi-compatible)	**Targeted Verification** rhAmpSeq in PHH (super-saturating followed by dose titration)	**Impact Assessment** Genic context Expression profile Functional impact
317 in silico off-target sites	**301 biochemical off-target sites nominated**	**0 verified >0.4%** **1 dose responsive <0.4%**	**OT-1 de-risked**

Multi-modal structural integrity analysis reveals no treatment-associated events

Four orthogonal assays spanning chromosomal to nucleotide-level resolution

Assay	Resolution	Outcome
G-banding (karyotype)	Chromosome-level (Mb)	No treatment-related events
Optical Genome Mapping	Structural variants ≥500 bp	No novel treatment-associated SVs
Whole Genome Sequencing (250x)	Nucleotide-level	No treatment-related structural changes
rhAmpSeq translocation	APOC3 ↔ OT-1 junction	No significant junction reads

Conclusion

- No treatment-associated chromosomal rearrangements across all 4 assays
- Consistent in 3 PHH donors at doses > EC90

MB-111 demonstrates a highly specific genomic editing profile

On-Target Precision

- 99.9% of deletions <50 bp; 5–6 bp deletions most frequent
- Consistent across 11 PHH donors; confined to APOC3 intron 1 splice junction

In silico + Biochemical Nomination

- 548 candidates from GRCh38, DivRef, and mSITE-Seq
- variant-aware coverage

Targeted rhAmpSeq Verification

- 0 sites verified >0.4% at supersaturating dose
- OT-1: intronic, 0.1–0.4%, >250× on-target selectivity

Genomic Structural Integrity

- G-banding, OGM, WGS, and translocation-targeted rhAmpSeq
- No MB-111 associated structural variants in any assay

Acknowledgements

- Charles Barberi, Sarah Elsener, Ria Shah
- Clarissa Scholes, Julia Nussbacher, Katannya Kapeli
- Sara Ansaloni, Hui Liu
- Sean Chen, Ymer Bjornson, Lauren Uyesaka
- Jennifer Zeitler
- Raj Poudel

- OGM was conducted at Praxis
- G-banding conducted at Kromatid