

A 3D molecular model of the NanoCas system. It features a large, blue, textured protein structure in the center, surrounded by several yellow, textured protein subunits. A green DNA double helix is visible, intertwined with the protein structures. The background is a gradient from light gray to dark gray.

AAV Delivered NanoCas CRISPR System Edits Muscle in Non-Human Primates

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3D render of the NanoCas system [Mammoth Biosciences]

Out of the many challenges scientists face in bringing genomic medicine to patients, delivery is near the top of the list. Due to these barriers in getting the genetic payload to the correct target cells, current methods have been restricted to ex vivo approaches or in vivo liver editing. In addition, first-generation CRISPR systems (Cas9 and Cas12a) are too large for efficient in vivo delivery via a single adeno-associated viral (AAV) vector.

Now, new preclinical research presents the discovery and engineering of NanoCas—an ultracompact CRISPR nuclease capable of extending CRISPR's reach in vivo beyond liver targets. NanoCas is the first ultracompact CRISPR system capable of efficient extrahepatic editing when delivered systemically using a single AAV vector.

This work is published on *bioRxiv* in a preprint titled, “**Single-AAV CRISPR editing of skeletal muscle in non-human primates with NanoCas, an ultracompact nuclease.**”

NanoCas is a novel Cas enzyme (approximately one-third the size of Cas9) that can be accommodated within a single AAV vector while leaving room for additional payloads such as regulatory elements, guide RNAs, or non-double strand break editing machinery. This could be utilized for techniques such as reverse transcriptase editing, base editing, and epigenetic editing.

The research group at Mammoth Biosciences—a Brisbane, CA-based company—screened 176 ultracompact CRISPR systems found in metagenomic data and applied protein engineering approaches to enhance the editing efficiency of NanoCas. The optimized NanoCas, the authors say, “exhibits potent editing capabilities across various cell systems and tissues in vivo when administered via adeno-associated viral (AAV) vectors.”

When targeting the PCSK9 gene in mouse liver in vivo, NanoCas showed saturating editing efficiencies of approximately 60%, on par with that of SaCas9, which is about three-fold larger in size. Both CRISPR systems reduced serum PCSK9 protein to undetectable levels.

NanoCas also demonstrated 10% to 40% editing of the dystrophin gene across the quadricep, calf, and heart muscle in a humanized mouse model of Duchenne Muscular Dystrophy (DMD), when delivered via a single AAV vector.

Lastly, NanoCas achieved in vivo editing efficiencies of up to 30% when targeting dystrophin in the skeletal muscle of cynomolgus macaques. NanoCas also showed 15% editing across the heart, compared to 10% with SaCas9. And analysis of liver tissue showed minimal off-target editing.

“Potent editing of extrahepatic tissues in vivo has been a roadblock for the gene editing field,” said Trevor Martin, PhD, co-founder and CEO of Mammoth Biosciences. “NanoCas’ compact size makes it compatible with a wide range of gene editing modalities—including base editing, reverse transcriptase editing, and epigenetic modification—while still allowing for delivery using a single AAV vector. This study is a major step toward enabling any edit to be made in any cell in vivo, thereby dramatically increasing the number of patients who could benefit from genetic medicines and delivering on the full promise of CRISPR.”