CAPSIDA BIOTHERAPEUTICS

Directed Evolution of AAV9 Libraries in Non-Human Primates Identifies a Capsid Family with Enhanced Central Nervous System Tropism and Liver De-Targeting Following Systemic Delivery

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Principal Scientist

Disclosure

- Xiaojing Shi is a full-time employee of Capsida Biotherapeutics.

Capsida Biotherapeutics

Foundation in capsid engineering with focus on building a new class of targeted, non-invasive gene therapies

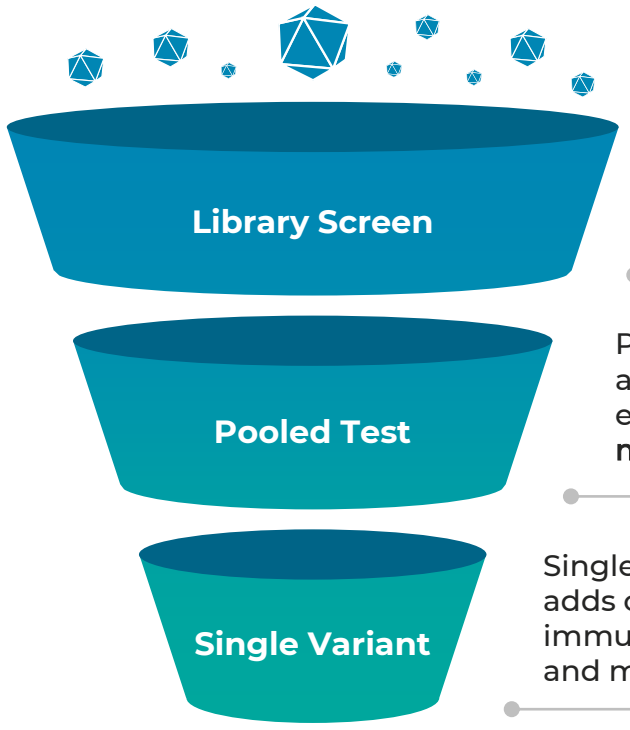
Pipeline of wholly owned and partnered programs in rare and more common Neurological and Ophthalmology diseases

Fully integrated capabilities: capsid engineering, cargo optimization, discovery, preclinical research, process development, manufacturing, and clinical development

NHP Driven Targeted Gene Therapy Engineering Platform

High-throughput Automated Process Identifies Capsids that Target Desired Tissues and Cell Types While De-targeting Undesired Tissues

Evolving Capsids to Meet **Target Capsid Profile**



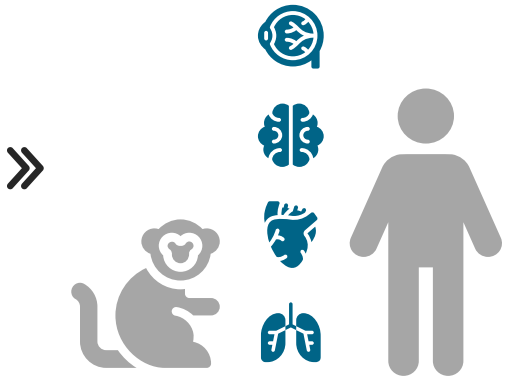
Billions of variants screened via Next Gen sequencing for enriched DNA and RNA

Pools with tens of variants adds assessment of protein expression and preliminary **manufacturability**

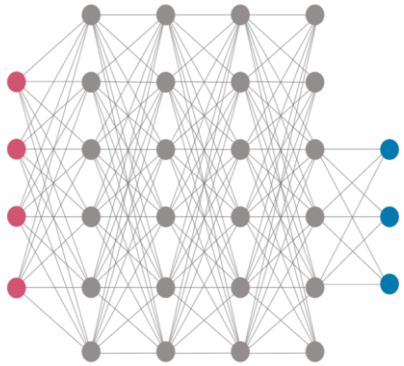
Single variant characterization adds cell-type distribution, immunogenicity / toxicity profile, and manufacturing scalability

IND-enabling

Variant Optimization and Re-Diversification

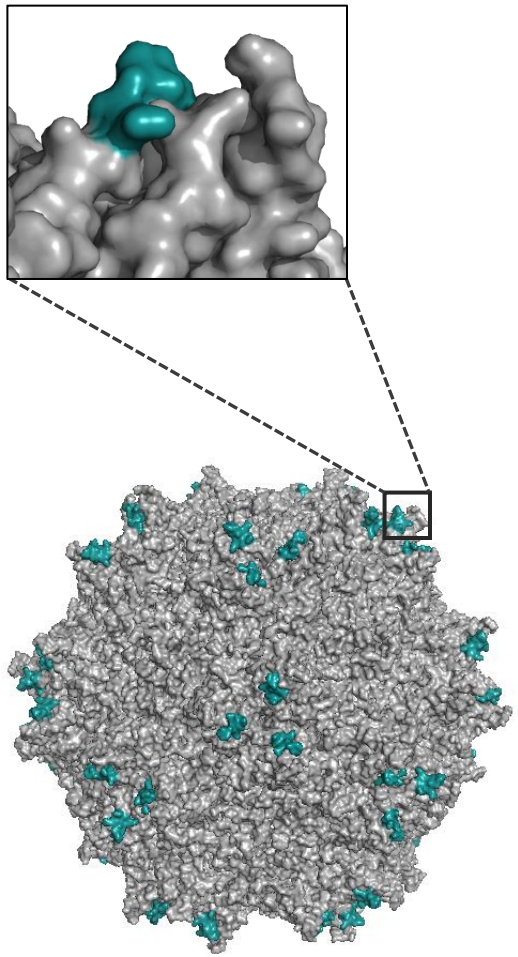


Automated Biologically Driven NHP Screening Combined with Analysis of Murine and Human Cells In Vitro



Sequence and Structure based analysis

Library Design and Screening Strategy



Initial Library Screening

Peptide Insertion
VR-VIII Loop

XXXXXXXX

2 Rounds of Screening

IV

Multiple Families
of Lead Variants

Variant Optimization

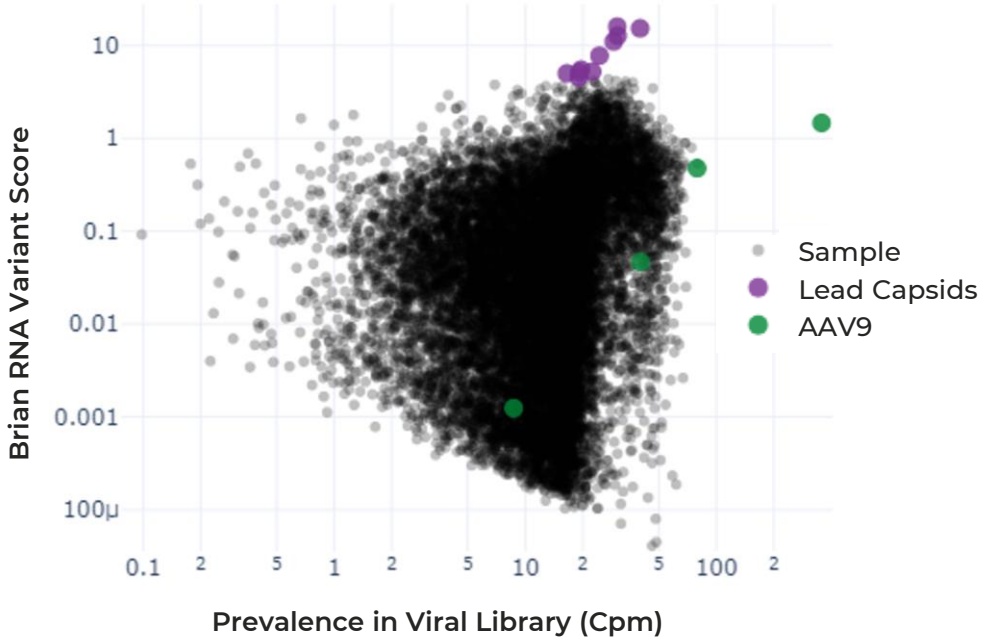
Multi-positional Mutagenesis

2 Rounds of Screening

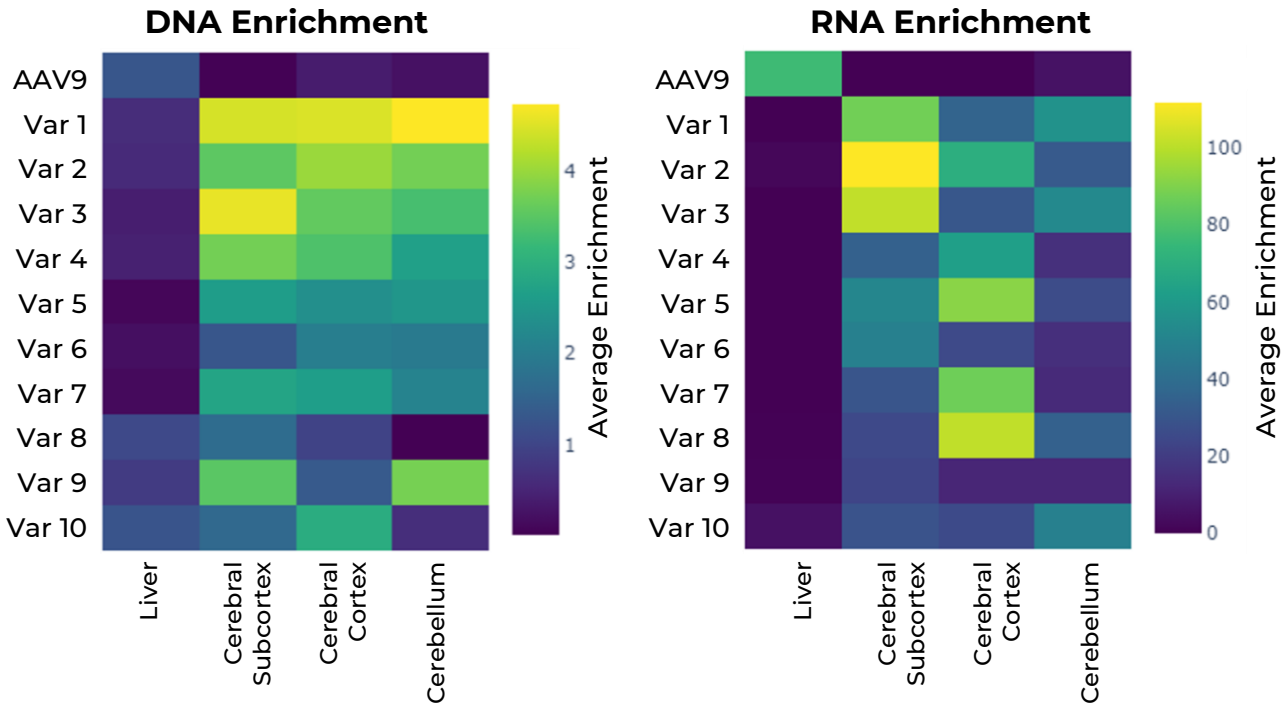
IV

Gen5 Capsid Family was Identified to be Enriched in Brain and De-target Liver

Brain Variant Score



Variant Enrichment



Variant score considers enrichment values and consistency in performance across codon replicates, tissue replicates and animals

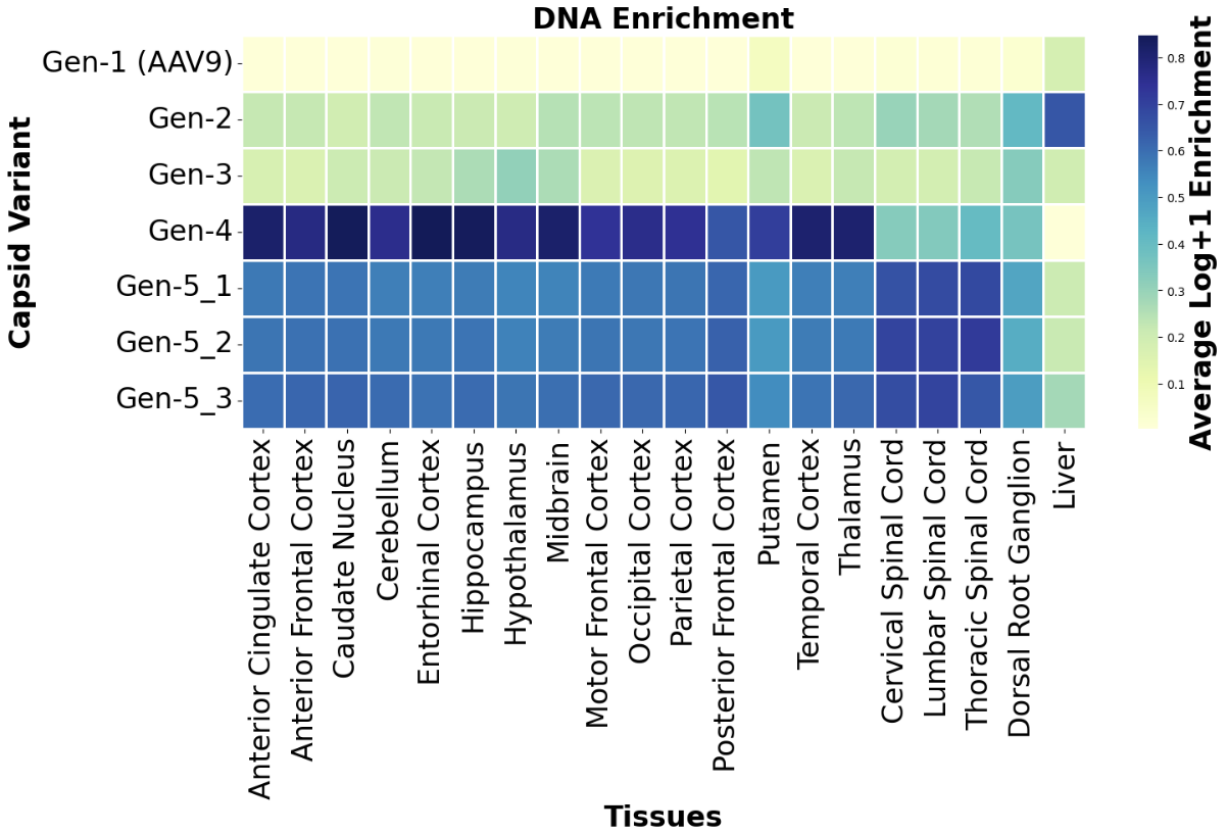
Reference capsid (AAV9) was titrated at multiple doses in the library

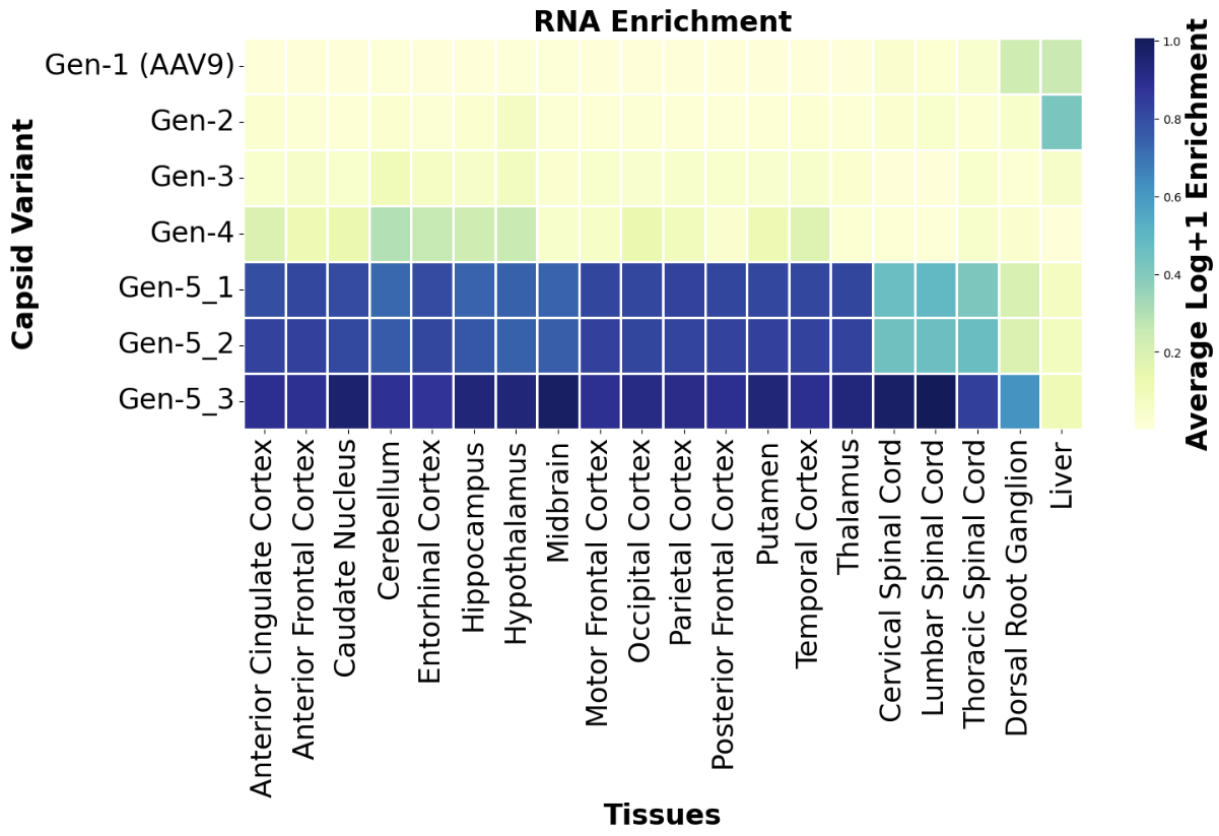
Each box represents the average of 3 codon replicates for a variant and the average across four animals

Enrichment (Tissue Cpm / Viral Cpm)

Gen5 Capsids Demonstrate Improved RNA Expression Across CNS Regions

Platform and Process Improvements Led to Continuous Advancements of Variants Across Generations



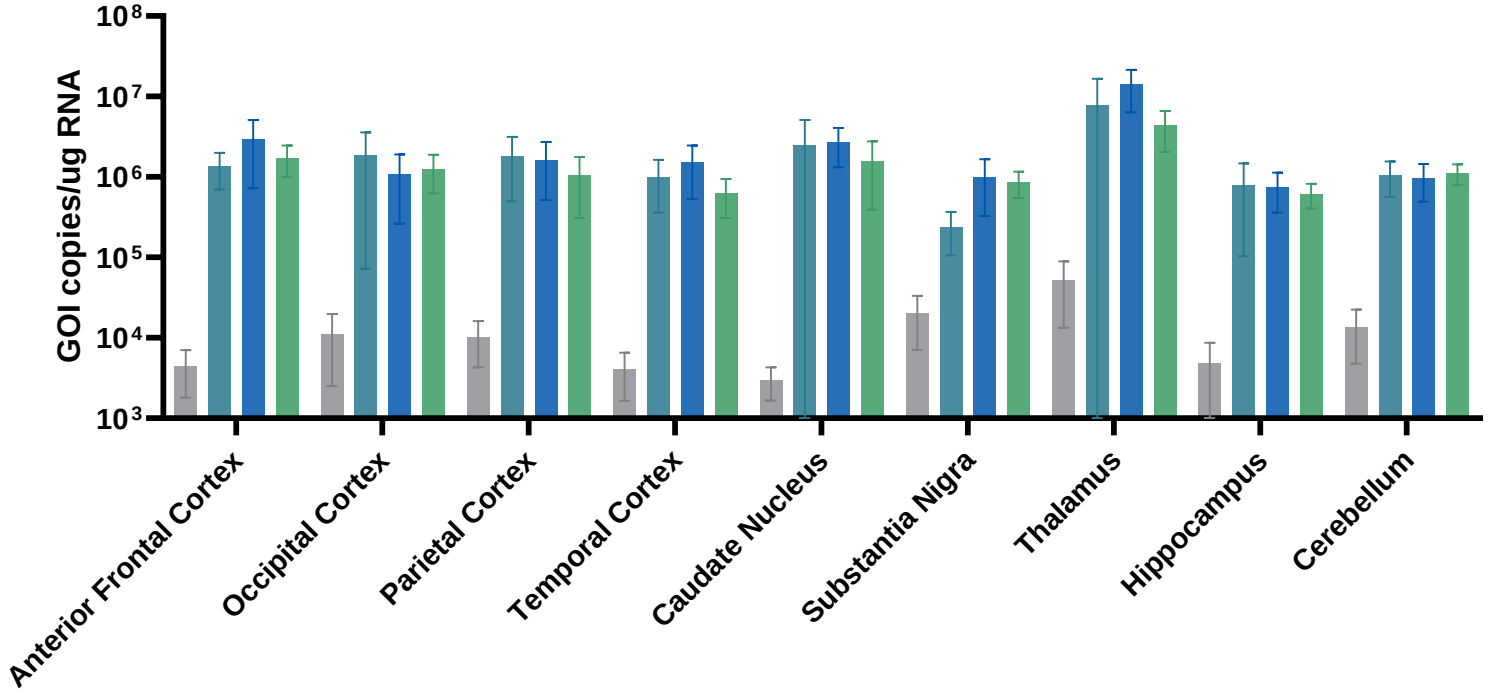


- Each box represents the average of 3 barcodes for a variant and the average across four animals
- Log10 +1 Enrichment (Tissue Cpm / Viral Cpm)

Gen 5 Capsids Yield Breakthrough Transduction Across The CNS And Significant Liver De-Targeting vs WT AAV9

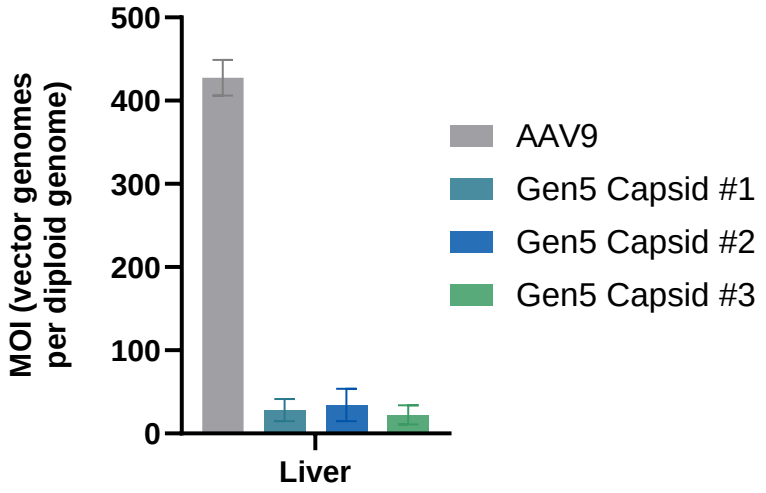
Broad CNS Transduction

Up to ~300x increase in CNS Expression



Liver De-targeting

Up to ~16x decrease in Liver De-targeting



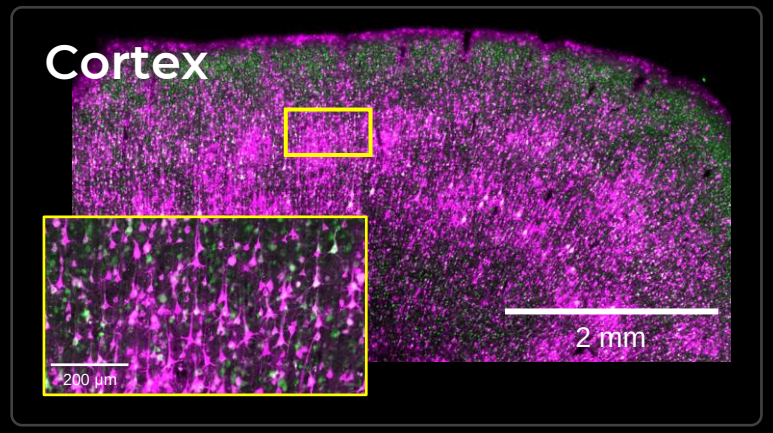
Cargo: HA-GOI | **Dose:** 2.5E13 vg/kg

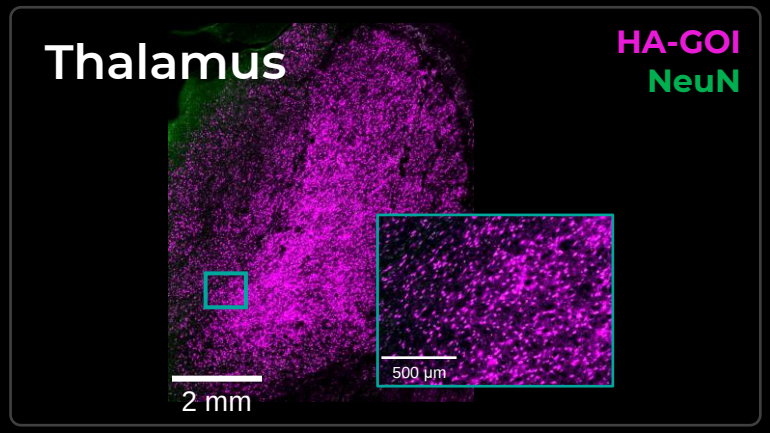
Species/Age: N = 3 cynomolgus macaques/group, 36-42mo

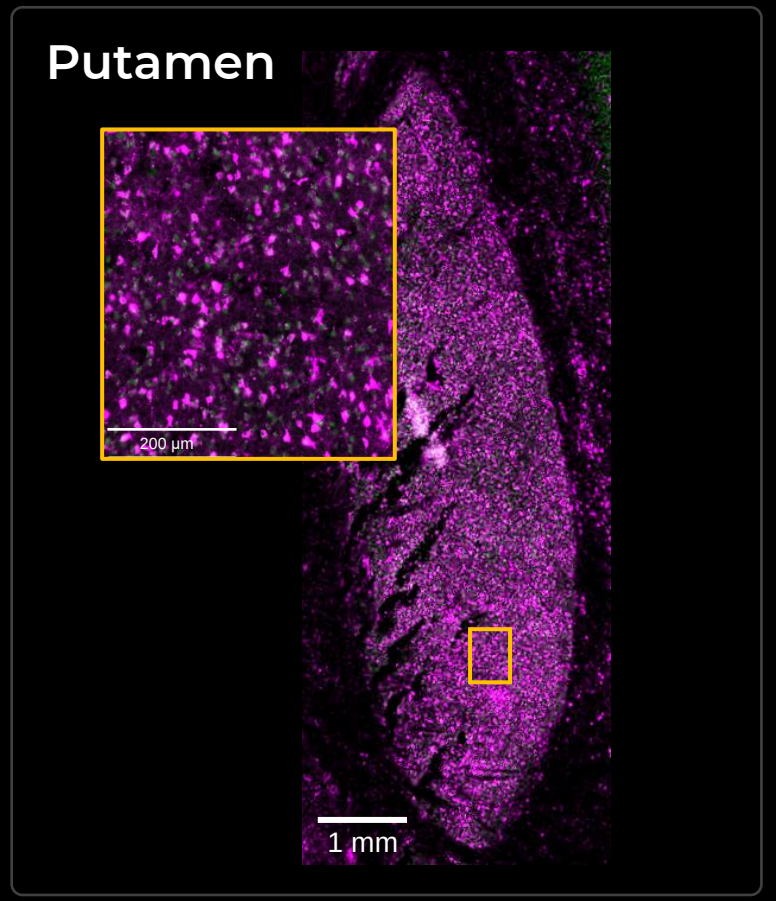
Gen 5 capsids are **well tolerated** with no clinical pathology or immunogenicity findings

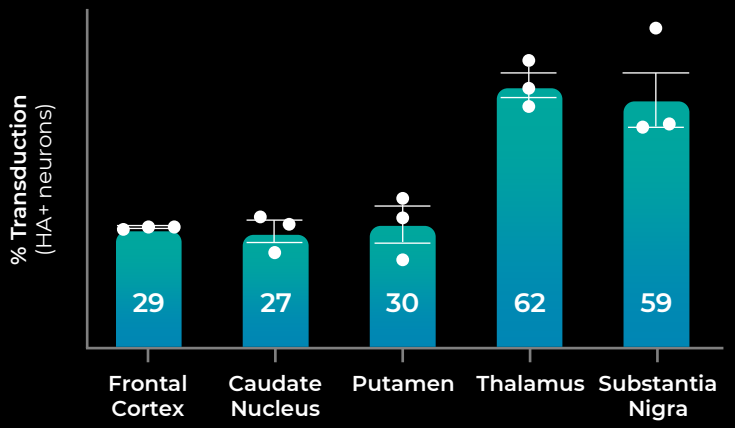
Unremarkable histopathology across the body, including **liver** and **DRGs**

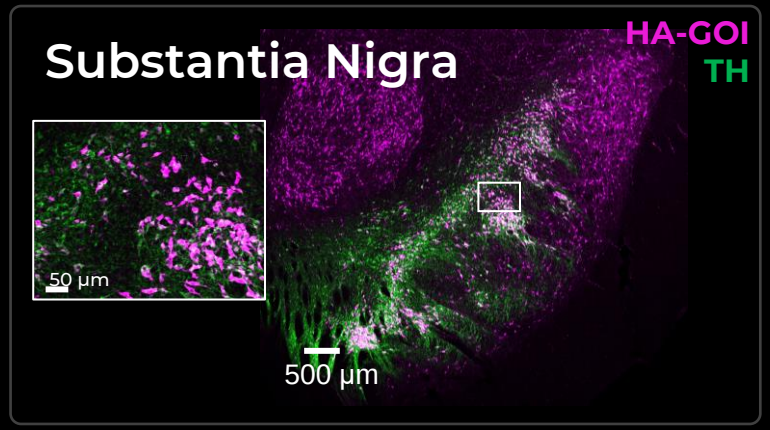
Gen5 Capsid Achieves Widespread Protein Expression Across the Brain Following IV Delivery in NHPs







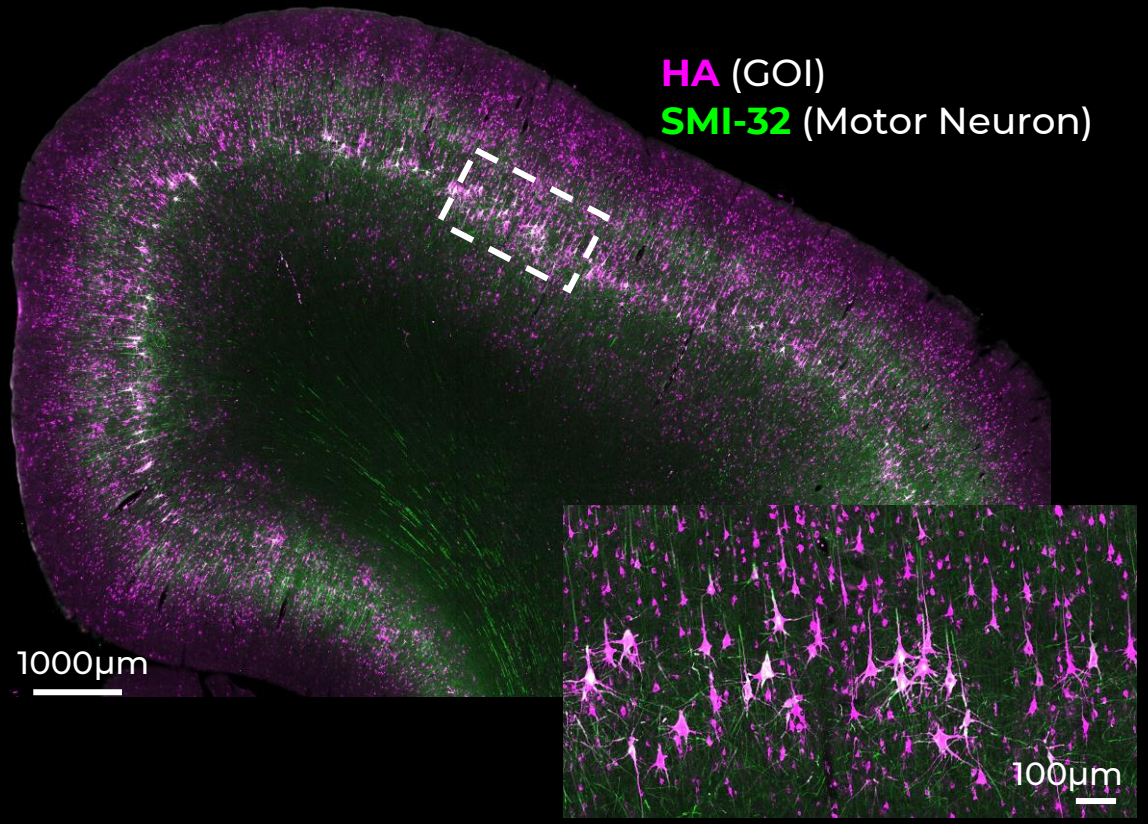




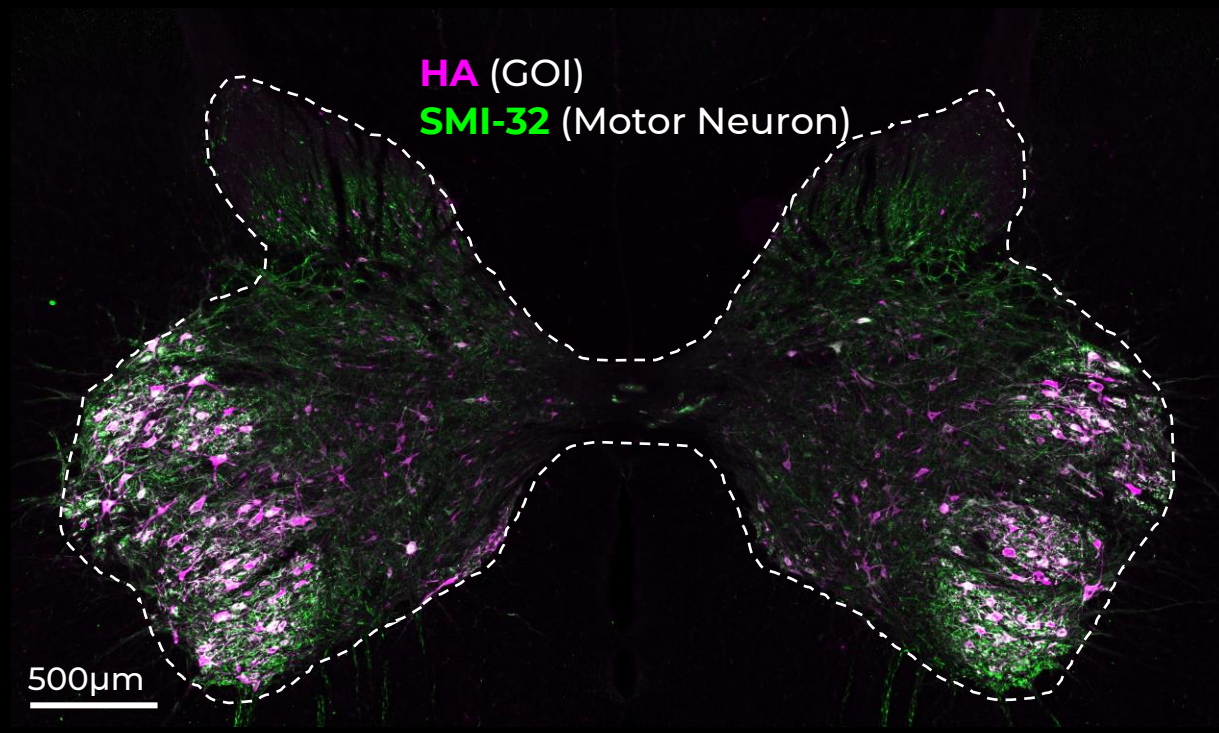
Capsid: Gen 5; **Dose**: 1.25E13 vg/kg
Cargo: HA-GOI; **In-life:** 6 weeks
Species/Age: N = 3 cynomolgus macaques, ~42mo

Gen5 Capsid Results in Extensive Transduction of NHP Motor Neurons

Motor Cortex



Spinal Cord



85%-95% Motor Neuron Transduced

Cargo: HA-GOI, **Dose:** 1.25E13 vg/kg, **In-life:** 6 weeks, **Species:** Cynomolgus macaques, **Age:** ~42mo

Summary

Demonstrated **success in engineering next-generation AAV capsids** for specific, widespread CNS expression via Capsida's NHP-driven targeted gene therapy platform

Breakthrough CNS transduction and **effective liver de-targeting** achieved with Gen 5 capsids in NHPs following systemic administration

Up to 70% neuron transduction (over 85% motor neurons) achieved at low doses with Gen 5 capsids

Advancement of wholly owned and partnered CNS-targeted gene therapy programs enabled by Gen 5 capsids, including treatments for genetic epilepsy (STXBP1 mutations) and Parkinson's Disease (GBA mutations)

Efficient **production of Gen 5 capsids at yields comparable to wild type AAV9**, enabled by Capsida's suspension manufacturing platform

Acknowledgements

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Marsela Jorgolli
Fan Wu
Jeremy Patrick
Matthew Frias

Vector Production

Pasha Tchourilov
Sara Jabalameli
Jordyn Wheeler
Kyle Douglas
Vernon Benedicto
Gordon Gibson

Other Capsida Presentations

Oral Presentations

- AAV Gene Therapy Corrects Neurological Phenotypes with Clinically Relevant Doses in a Mouse Model of STXBP1-Related Development and Epileptic Encephalopathy; **Abs # 38 – Wu Chen (BCM) – Tues May 7, 3:00-3:15 PM**
- Systemic AAV Gene Therapy with CNS-Targeted Engineered Capsids Achieves Significant GCase Activity Increases in the Primate Brain to Support the Potential Treatment of GBA-PD; **Abs # 274 – Nicholas Flytzanis – Fri May 10, 3:00 - 3:15 PM**

Poster Presentations

- CAP-002: Systemic AAV Gene Therapy with Next Generation Capsida for Treatment of STXBP1 Encephalopathy; **Abs # 504 – Allison Knoll – Wed, May 8**
- Directed Evolution of AAV2 Libraries Yields Capsids with Improved Performance in the Central Nervous System and Cross-species Translatability; **Abs # 992 – Sean Gross – Thurs, May 9**
- Alternative Plasmid Designs Including Two Plasmid Transfection Systems for Improved Production and Packaging of Engineered AAV Capsids; **Abs # 530 – Lysa-Anne Volpe – Wed, May 8**
- Characterization of engineered AAV capsids from different HEK293 cell culture fractions, crude lysate versus cell pellet material; **Abs # 529 – Heidy Morales – Wed, May 8**
- Separation of Empty and Full Engineered Adeno-Associated Virus Capsids Using a Weak Anion Exchanger; **Abs # 1038 – Varun Gejji – Thurs, May 9**

CAPSIDA BIOTHERAPEUTICS

Our Pipeline is Making the Impossible Possible

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