



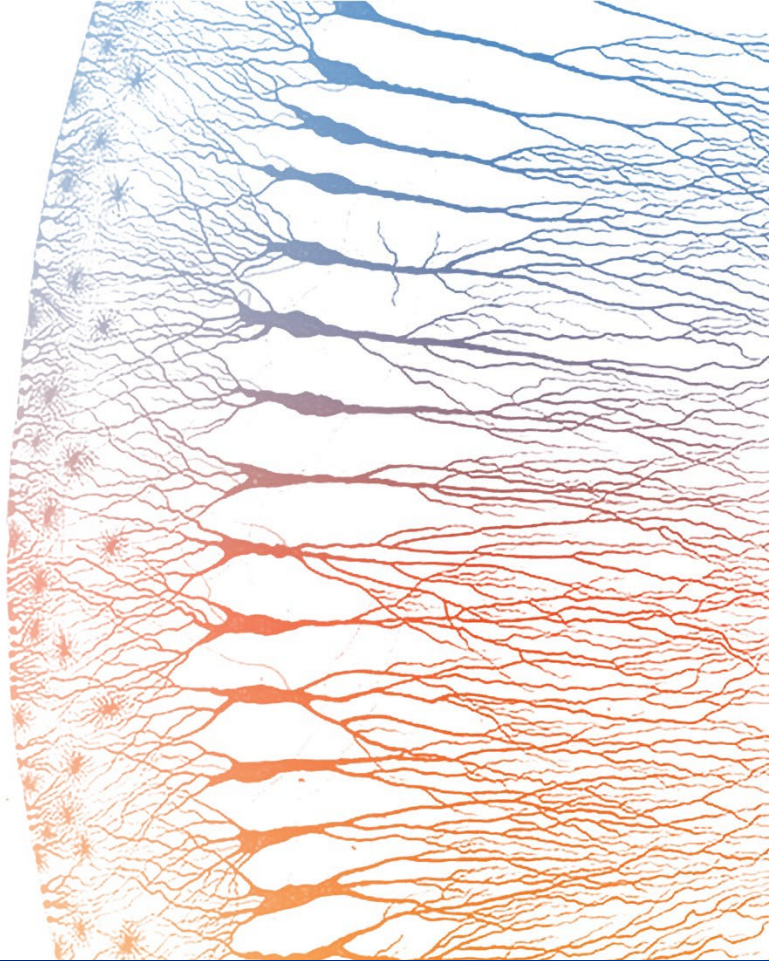
# Continued directed evolution of VCAP-101 and VCAP-102 identifies second generation capsids with increased brain tropism in non-human primates and mice (#119)

Tyler Moyer, Ph.D  
Senior Scientist I, Novel Capsid Discovery

ASGCT 27<sup>th</sup> Annual Meeting

Breaking Barriers to the CNS via AAV Capsid Engineering

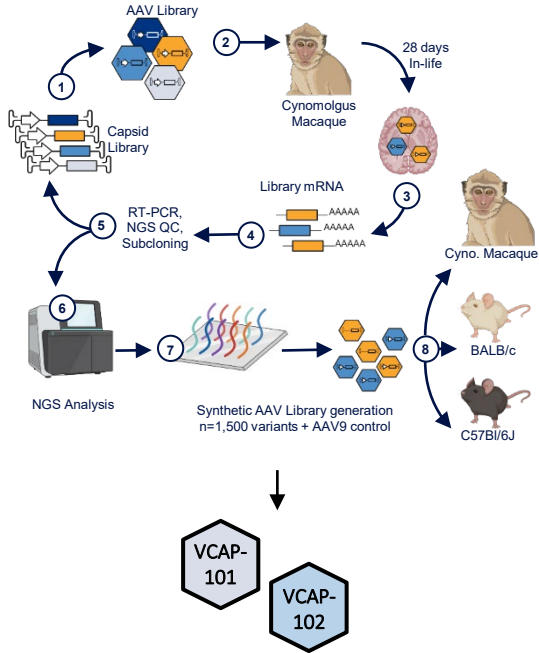
Wednesday May 8, 2024



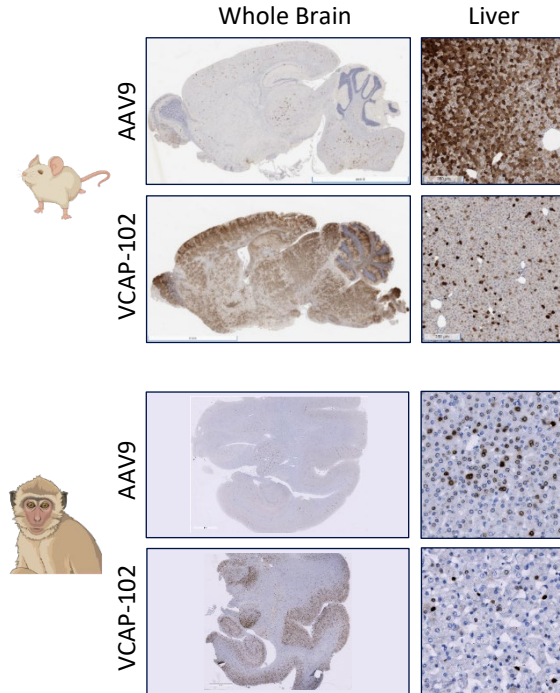
Disclosure: Tyler Moyer is a full-time employee of Voyager Therapeutics.

# VCAP-101 and VCAP-102 Demonstrate Increased CNS-Tropism Across Species

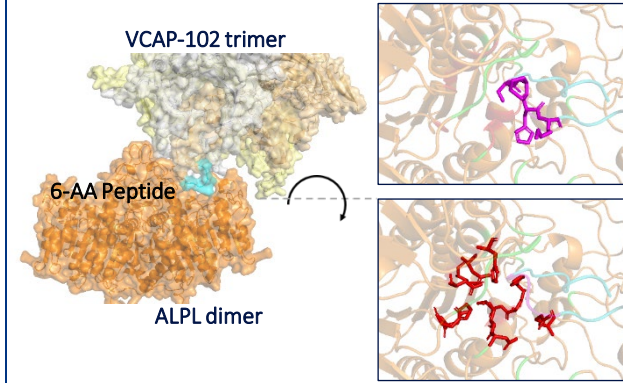
AAV9-based TRACER™ screen identifies VCAP-101 and VCAP-102 as cross-species, BBB-penetrant capsids



VCAP-102 demonstrates increased CNS-tropism and liver detargeting



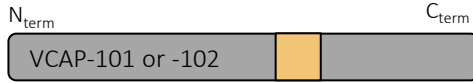
VCAP-102 crosses the BBB through direct and specific interaction with Tissue Non-Specific Alkaline Phosphatase (ALPL)



Identification and characterization of a highly conserved cell surface receptor utilized by engineered BBB-penetrant AAV capsids with enhanced brain tropism in non-human primates and mice (#975).  
Thursday, May 9, 2024, 12:00 p.m. ET

# Gen2 Candidates were Selected for Increased CNS Tropism and Liver Detargeting

## Fitness Maturation



Mutagenesis

...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...  
...EXPOSEDHYPERVARIABLESURFACELOOP...



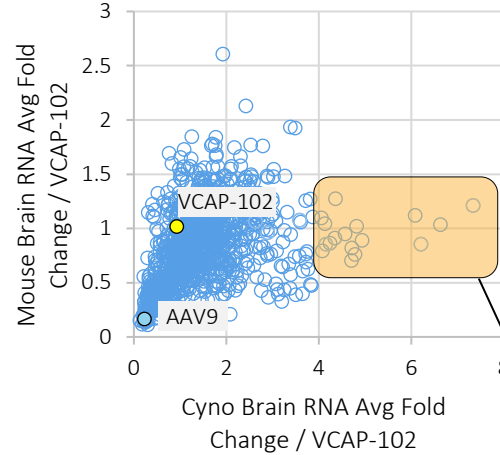
Macaque



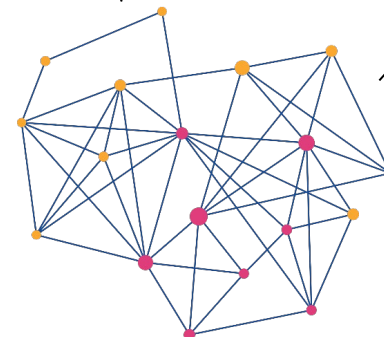
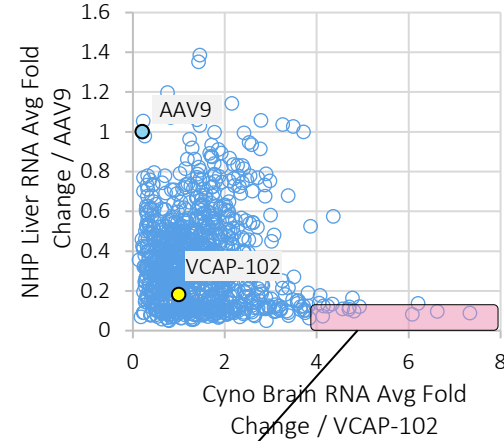
Mouse

## Gen2 Candidates Selection

### Brain Transduction



### Liver Detargeting



# Multiplexed Capsid Evaluation in NHP – Multi-Tag Approach

AAV9-Tg-Tag1

Capsid A-Tg-Tag2

Capsid B-Tg-Tag3

Capsid C-Tg-Tag4

Capsid D-Tg-Tag5

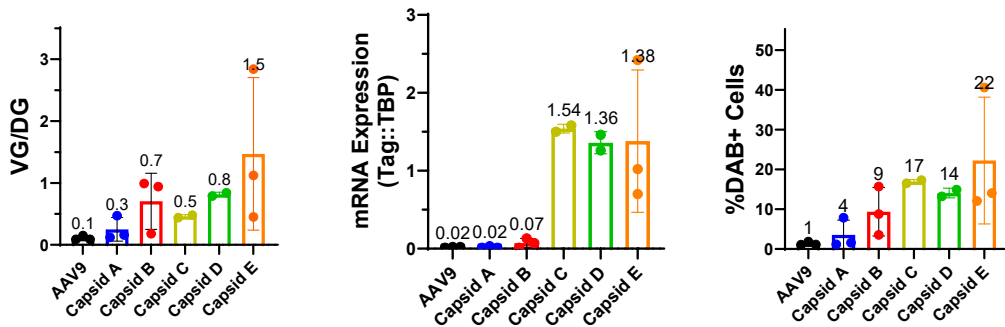
Capsid E-Tg-Tag6



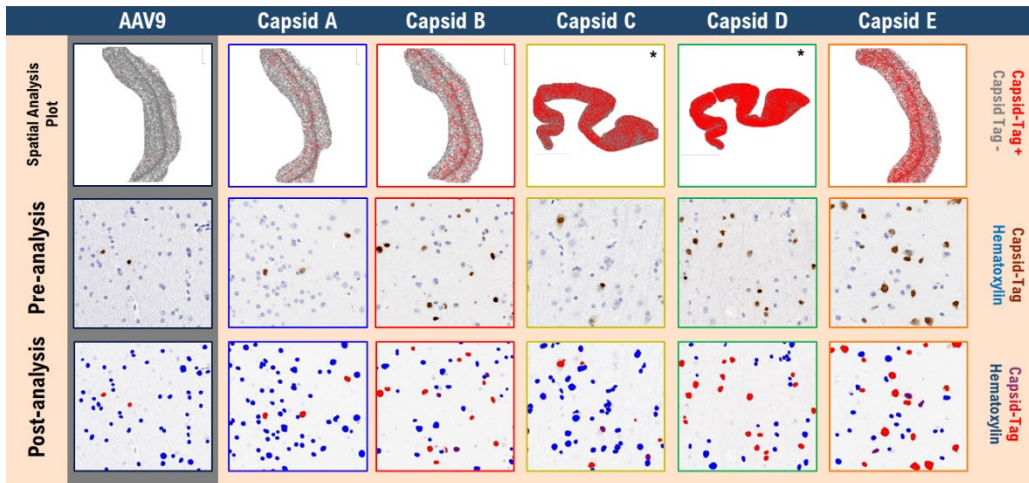
3 x  
Cynomolgus  
macaques

Each capsid dosed at 4E12 vg/kg

High-resolution quantitative analysis of multiple AAV capsids in rodent and primate models using multiplexed reporter protein tagging platform. (#511). Wednesday, May 8, 2024, 12:00 p.m.



Motor  
Cortex



\*Some images depict pre-motor cortex

# Multiplexed Capsid Evaluation in NHP – Multi-Tag Approach

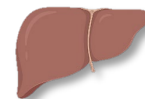
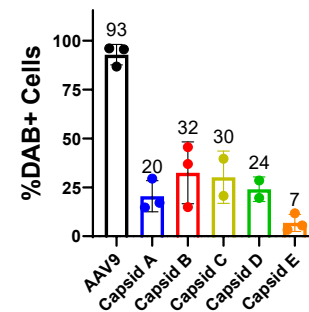
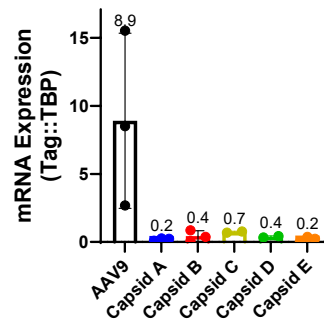
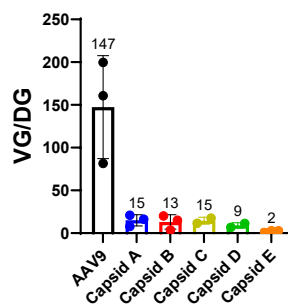
- AAV9-Tg-Tag1
- Capsid A-Tg-Tag2
- Capsid B-Tg-Tag3
- Capsid C-Tg-Tag4
- Capsid D-Tg-Tag5
- Capsid E-Tg-Tag6



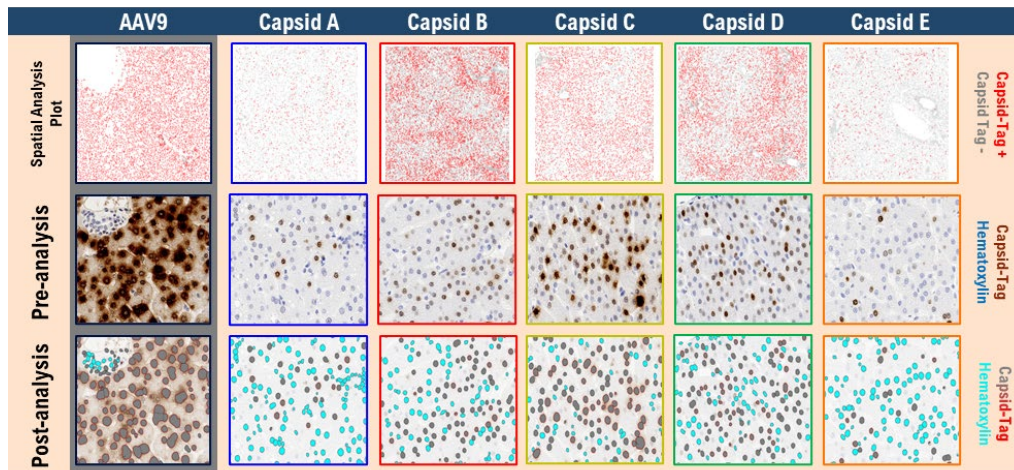
3 x  
Cynomolgus  
macaques

Each capsid dosed at 4E12 vg/kg

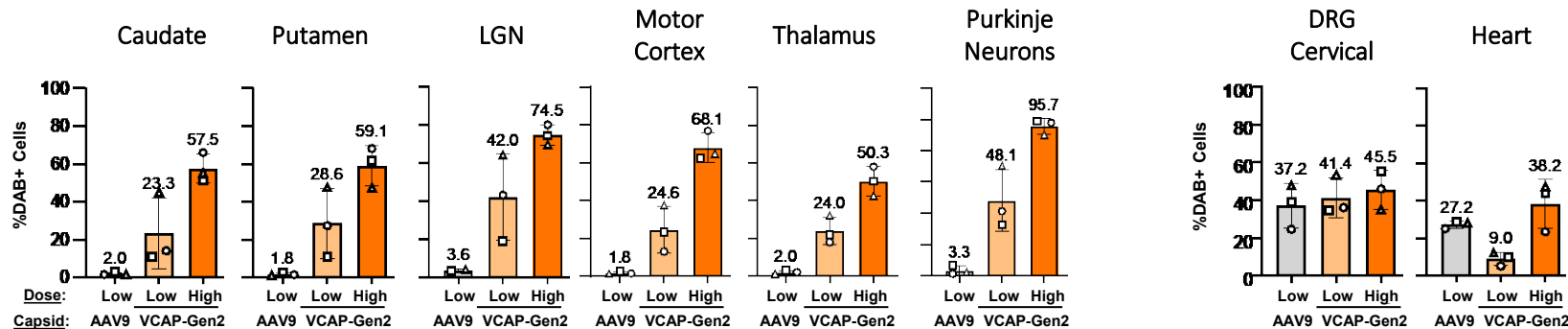
High-resolution quantitative analysis of multiple AAV capsids in rodent and primate models using multiplexed reporter protein tagging platform. (#511). Wednesday, May 8, 2024, 12:00 p.m.



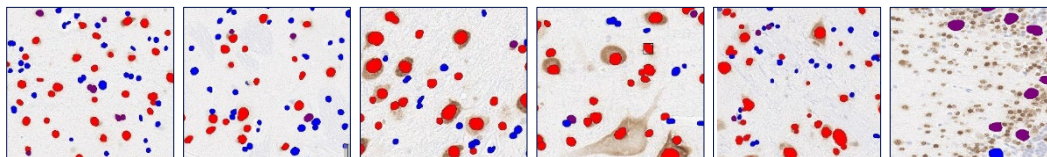
Liver



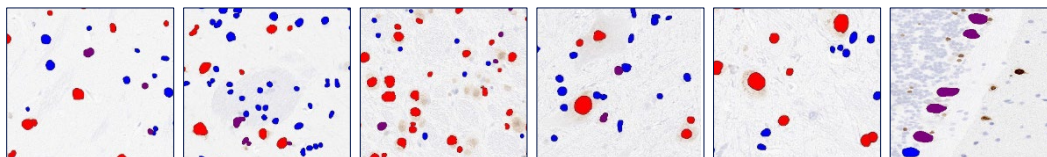
# VCAP-Gen2 Transduces Cells Across Diverse Brain Regions



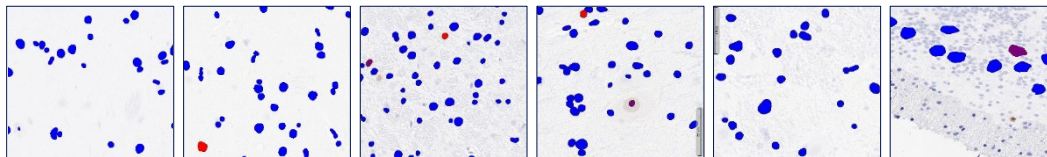
High Dose  
Single Capsid,  
VCAP-Gen2  
3E13 vg/kg



Low Dose  
Multi-Tag,  
VCAP-Gen2  
4E12 vg/kg



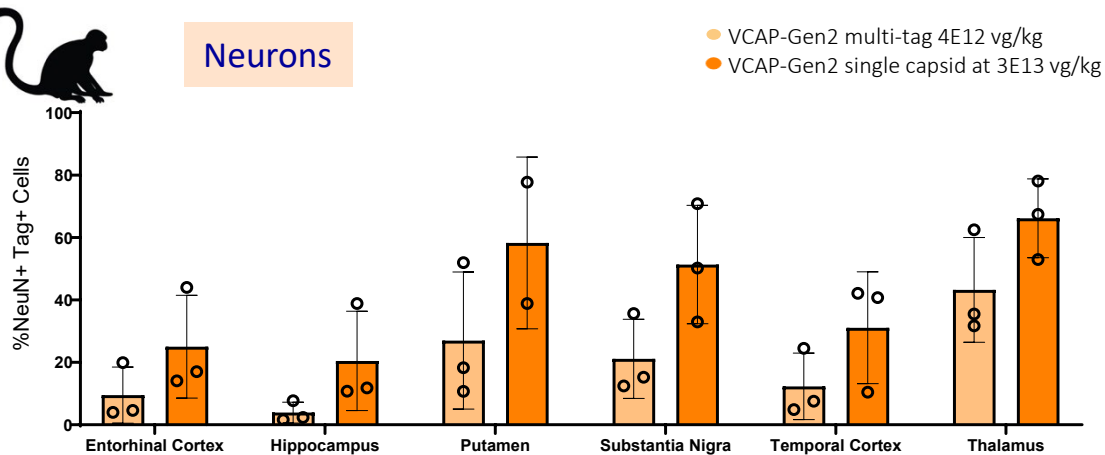
Low Dose  
Multi-Tag,  
AAV9  
4E12 vg/kg



- Transgene-Positive Nuclei
- Transgene-Negative Nuclei

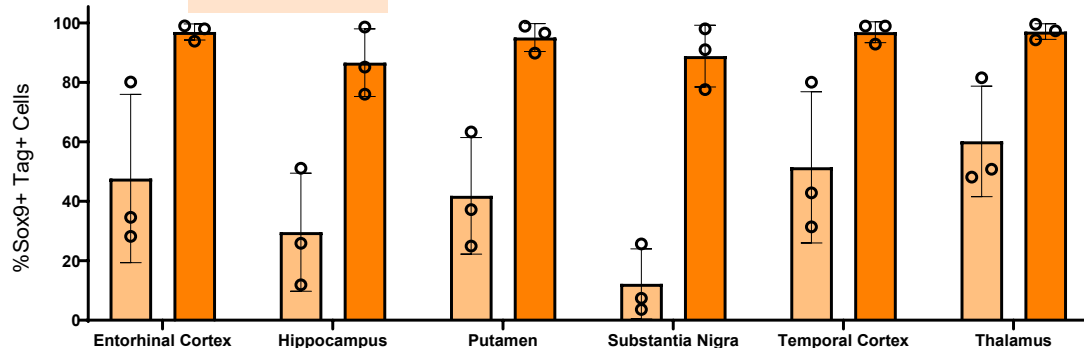
# VCAP-Gen2 Transduces Both Neurons and Astrocytes

## Neurons



Brain region	% NeuN <sup>+</sup> tag <sup>+</sup> of total NeuN <sup>+</sup> neurons		
	AAV9 multi-tag	VCAP-Gen2 Multi tag	VCAP-Gen2 single capsid
Entorhinal Cortex	0	10	25
Hippocampus	0	4	21
Putamen	0	27	58
Substantia Nigra	2	21	51
Temporal Cortex	0	12	31
Thalamus	2	43	66

## Astrocytes



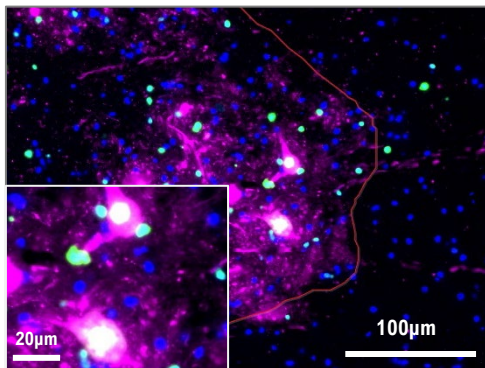
Brain region	% Sox9 <sup>+</sup> tag <sup>+</sup> of total Sox9 <sup>+</sup> neurons		
	AAV9 multi-tag	VCAP-Gen2 Multi tag	VCAP-Gen2 single capsid
Entorhinal Cortex	0	48	97
Hippocampus	0	30	87
Putamen	0	42	95
Substantia Nigra	2	12	89
Temporal Cortex	0	51	97
Thalamus	2	60	97



# VY9323 Demonstrates Upwards of 80% Knockdown of SOD1 mRNA in Spinal Cord Motor Neurons

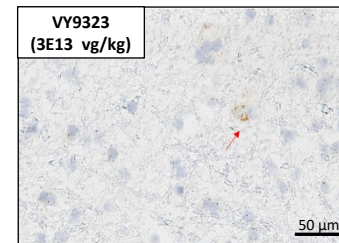
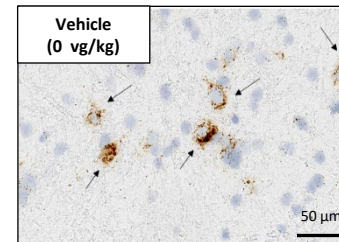
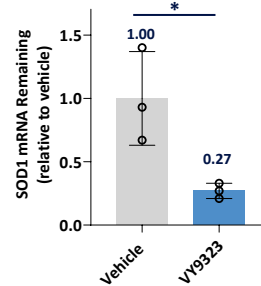


Cervical Spinal Cord  
anti-HA + anti-ChAT

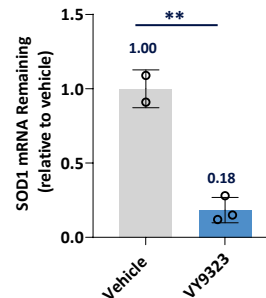


	% ChAT+ tag+ of total ChAT+ gray matter		
Spinal Cord region	AAV9 multi-tag (4E12 VG/kg)	VCAP-Gen2 multi-tag (4E12 VG/kg)	VCAP-Gen2 single capsid (3E13 VG/kg)
Cervical (C2)	17	65	84
Thoracic (T8)	34	39	86
Lumbar (L2)	20	57	94

Cervical Laser Captured Motor Neurons  
SOD1 mRNA

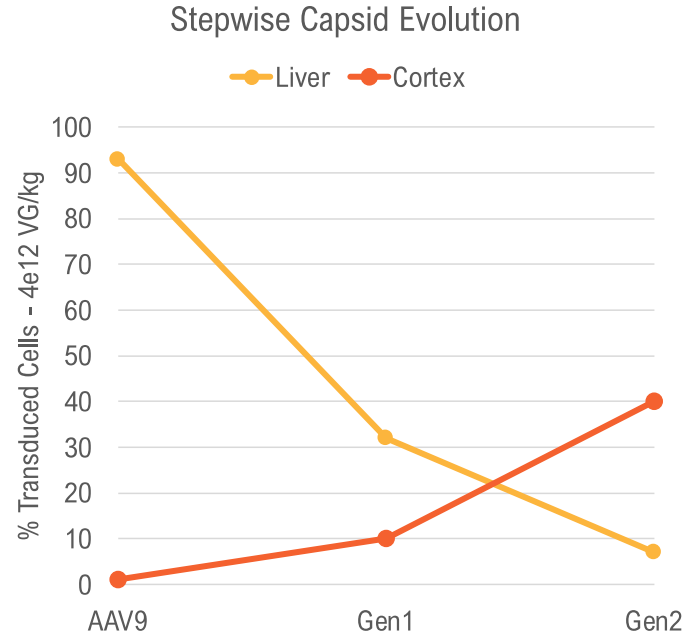
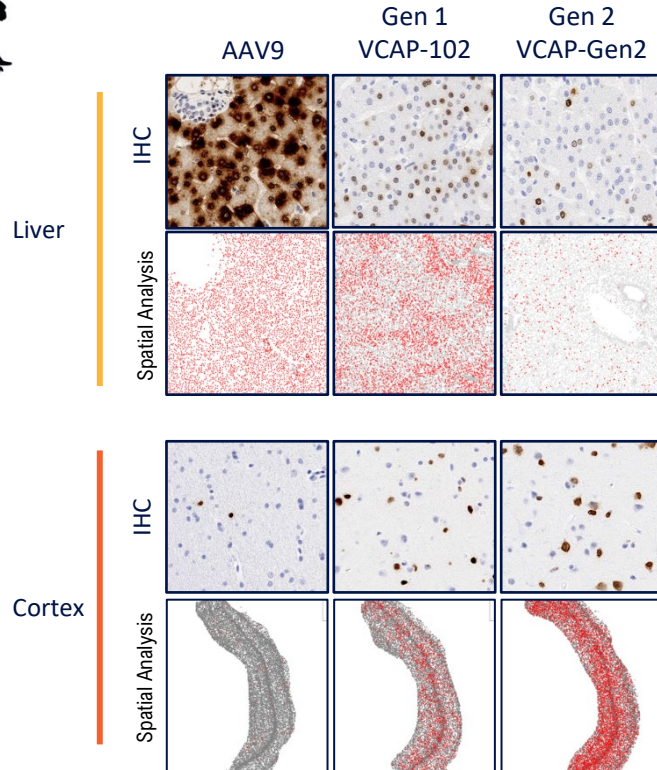


Lumbar Laser Captured Motor Neurons  
SOD1 mRNA

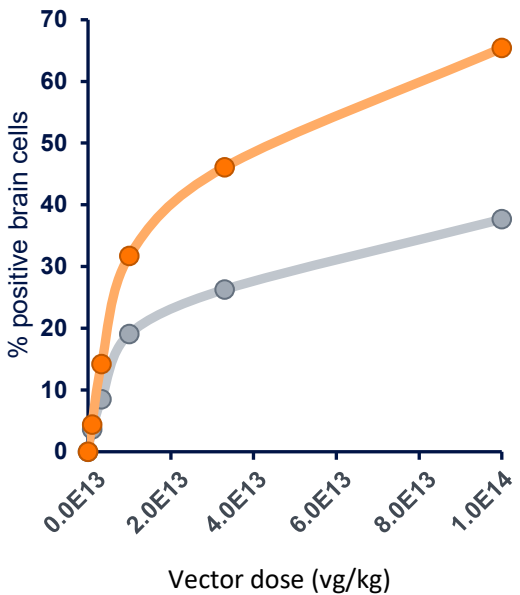
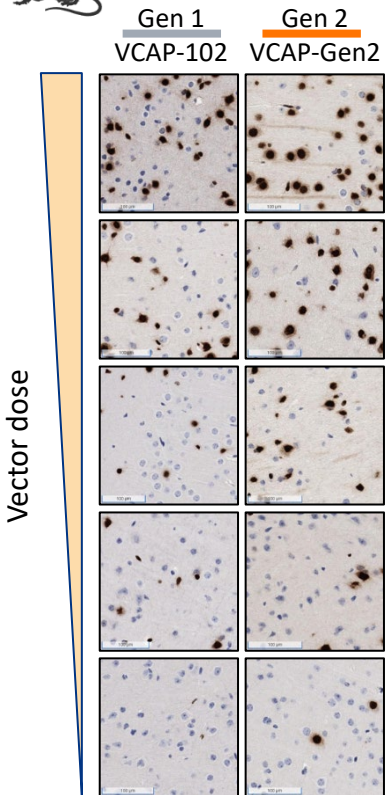


Intravenous delivery of AAV gene therapy for the treatment of SOD1-ALS provides broad SOD1 lowering in NHP. (#1647).  
Friday, May 10, 2024,  
12:00 p.m.

# Iterative Capsid Evolution : Increased Brain Tropism and Liver Detargeting

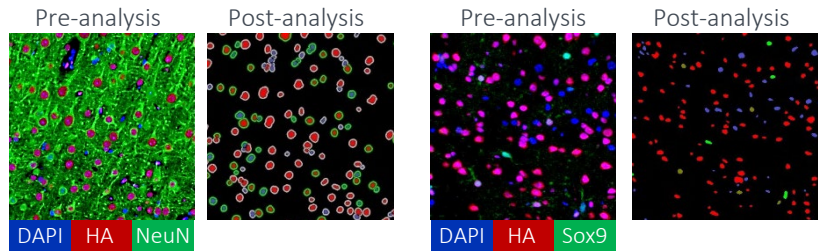


# VCAP-Gen2 Targets Neurons and Astrocytes in Mice



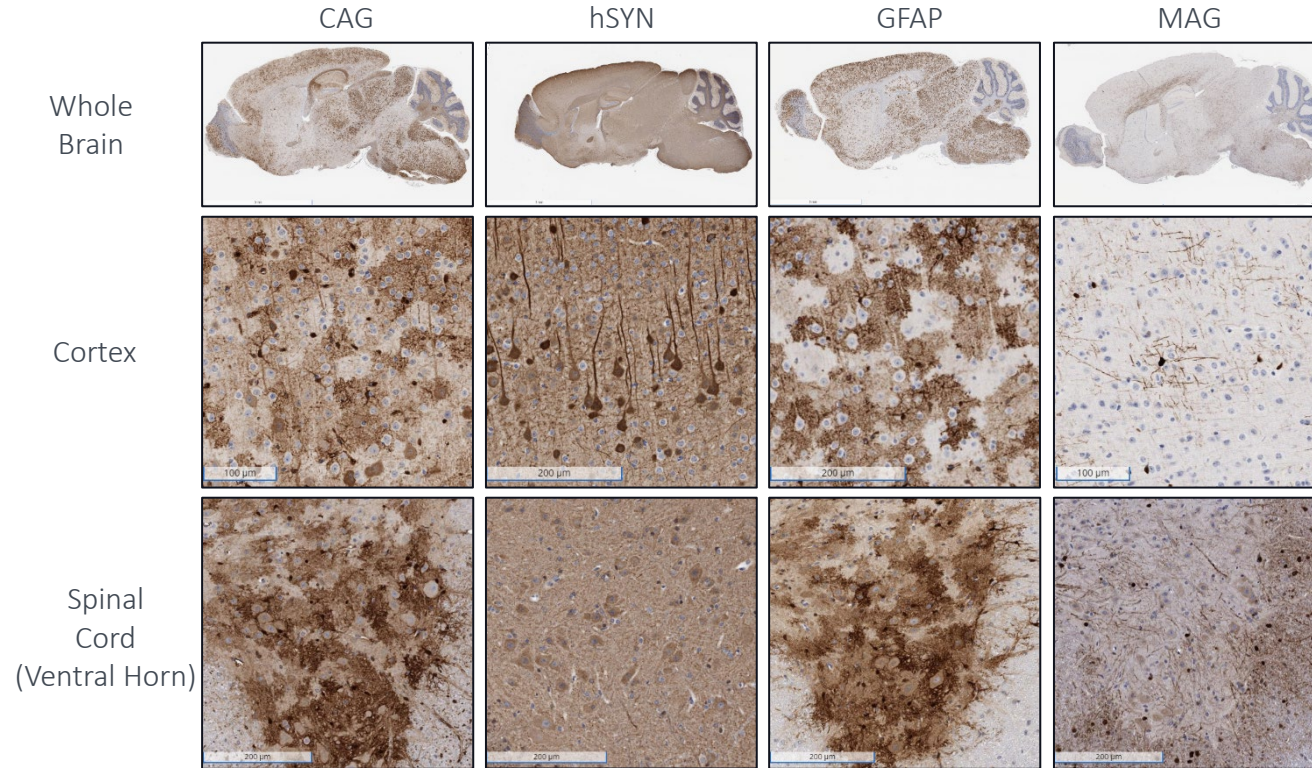
ss-CAG-transgene-HA at a range of doses (see right); tail vein injection; 28 days in-life; 6-8 week old female Balb/c

VCAP-Gen2 cellular expression of ssCAG-Transgene-HA in mouse cortex across a range of doses



Evaluation of cross-species expression across four species and cellular tropism of VCAP-102, an engineered blood-brain barrier-penetrating AAV derived capsid from TRACER Platform screens ([#1452](#)). Friday, May 10, 2024, 12:00 p.m.

# VCAP-Gen2 Expression in Mice Can Be Optimized Using Cell-Type Specific Promoters



Dosings were at 1E13 VG/kg of sc-ZsGreen-HA under the control of varying promoters; tail vein injection; 28 days in-life; 6-8 week old female Balb/c

- VCAP-Gen2 is member of a family of second generation TRACER™-derived capsids with increased CNS tropism relative to AAV9 in primates and mice.
- This family of capsids utilizes the highly conserved receptor ALPL to cross the blood-brain barrier.
- Up to 75% of cells were transduced across diverse brain regions in primates with VCAP-Gen2 at 3E13 vg/kg, including both neurons and astrocytes.
- VCAP-Gen2 is detargeted from the primate liver relative to AAV9 (approximately 45X less expression in NHPs).
- Cell-type specific expression of VCAP-Gen2 can be optimized through the use of varying CNS-promoters.

## Capsid Discovery Team

- Mathieu Nonnenmacher
- Brett Hoffman
- Kristin Graham
- Matthew Child
- Roop Kaur
- Zach Thorpe
- All other team members

## NGS Data Analysis

- Jiachen Liu
- Xiaoqin Ren

## In vivo/Neuroscience team

- Mike Grannan
- Meg Dalrymple

## NHP Study/Data Coordination

- Johnny Yao
- Alex Kutchin
- Amy Johnson
- Chanchal Sadhu
- Arpana Khatri

## Histology Team

- Nilesh Pande
- Hamza Khalid
- Anupriya Kulkarni
- Alexa Tsolias
- Amy Bruce
- Rong Zhao
- Wenya Yang
- Chiso Egbuchulam
- Madeline Barnes

## Bioanalytics Team

- Jeff Thompson
- Joe Clement
- Alexis Bloedel
- Rebecca Spellman
- Camila Arce
- Joydeep Ghosh

## Vector Production Team

- Kyle Grant
- Jayashree Natasan
- Ruohong Zhou
- Timothy Fiore
- Dhiral Shah
- Alex Kubiak
- Dillon Kavanagh
- Jack Studer
- Jordan O'Brien
- Usman Hameedi

Questions?

