Directed Evolution of AAV9 Peptide Display Libraries Identifies a Family of Cross-Species Variants with Enhanced Brain Tropism in Non-Human Primates and Mice Following Systemic Administration

Tyler Moyer, Scientist II, Novel Capsid Discovery – Voyager Therapeutics

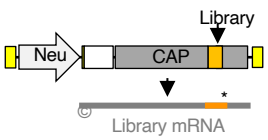
ASGCT 2022- Novel AAV Capsids for the Brain, Eye, and Kidney

May 19, 2022

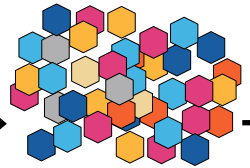
Disclosure: Tyler Moyer is a full-time employee of Voyager Therapeutics.

The TRACER Platform - RNA Enrichment Analysis for Multiplexed Capsid Fitness

TRACER library

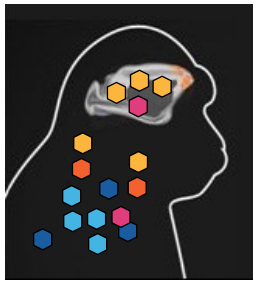


Starting Library
Capsid mRNA driven by neu-specific promoter



I.V.

In vivo Selection



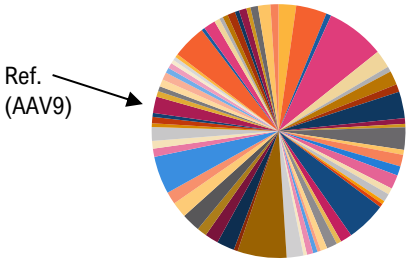
1 mo

RNA Recovery (Neuron-restricted)

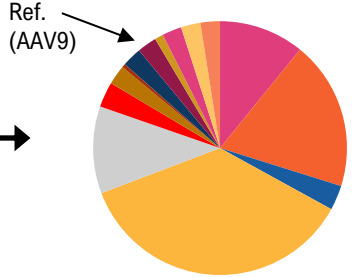
RT-PCR

Amplicon deep sequencing

Before Selection (Virus)



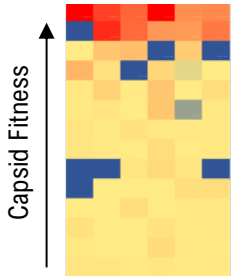
After Selection (RNA)



E=Enrichment score of output viral RNA over Input Viral DNA (R = rel. abundance)

$$Ex = \frac{Rx_{OutputRNA}}{Rx_{InputVirus}}$$

$$nFx = \frac{Ex}{E_{ref}}$$



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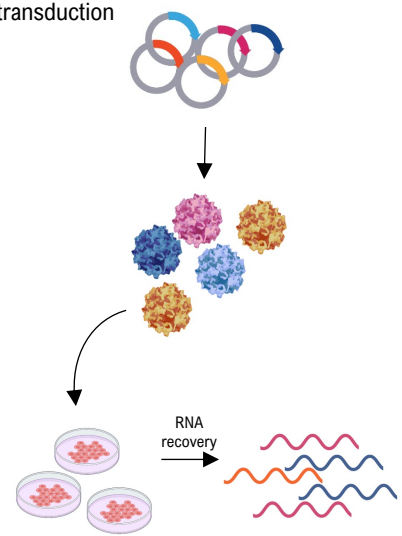
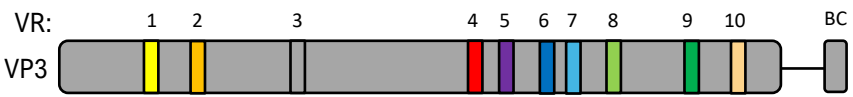
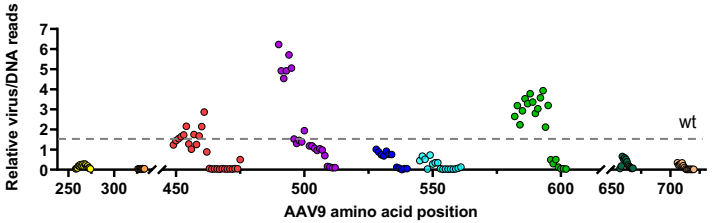
AAV9 ‘Hotspot’ Library Reveals Favorable Positions Within the 3-fold Axis for High Library Viability

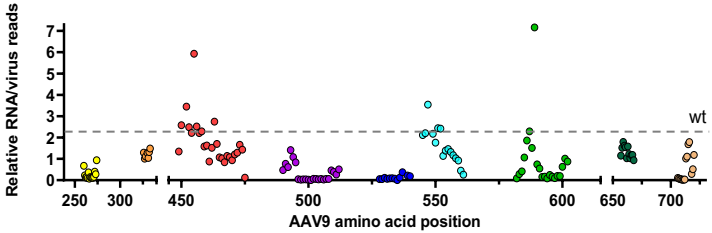
AAV9 Hotspot Library:

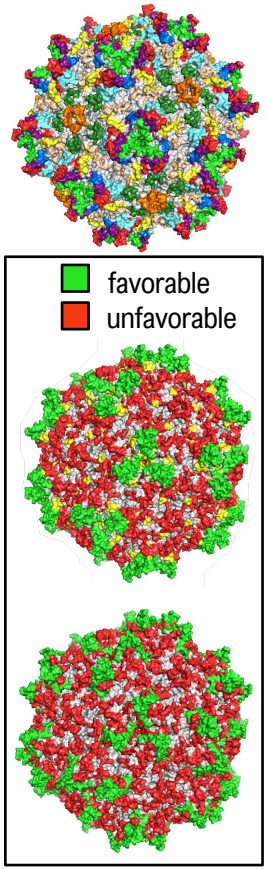
- Small-scale library with random 6mer peptide insertions at each position with each VR. 153 total positions surveyed.
- Library was barcoded based on insertion position, not sequence identity.
- NGS was used to assess favorable positions for capsid production as well as transduction



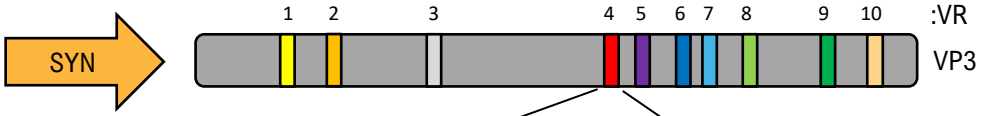






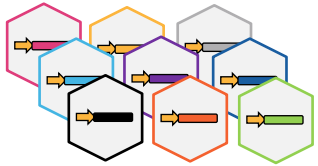


AAV9 VR-IV Peptide Insertion Scan Library Design and Dosing Strategy



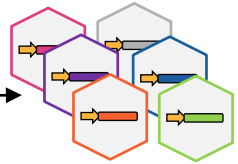
GXXXXXXSGQNQQT
GSXXXXXXGQNQQT
GSGXXXXXXQNQQT
GSGQXXXXXXNQQT
GSGQNXXXXXXQQT
GSGQNQXXXXXXQT
GSGQNQQXXXXXXT
GSGQNQQTXXXXXX

P_0 Virus library



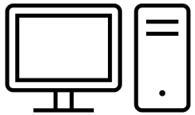
P_1 Macaque Brain

RNA recovery

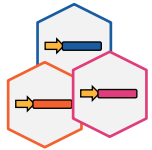


P_2 Macaque Brain

RNA Recovery and NGS



Synthetic Library (~1500 capsids)



P_3 Macaque Brain

P_3 C57Bl/6 Brain
(n = 3)

P_3 BALB/c Brain
(n = 3)

*All dosings were n=2 NHPs; intravenous; 28 days in-life; 2-3E13 VG/kg

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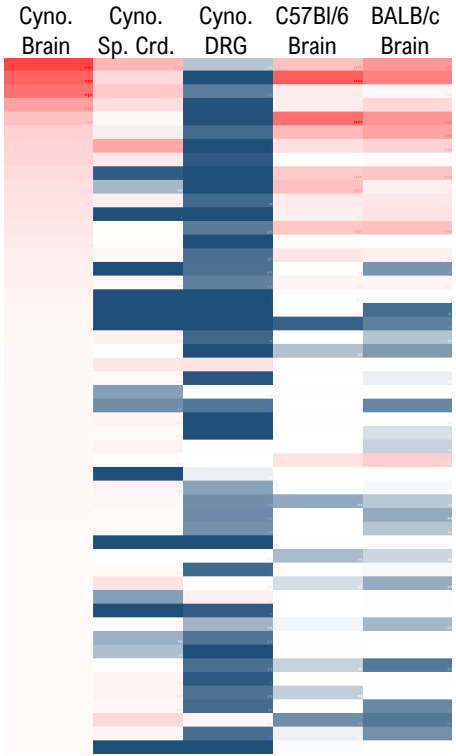
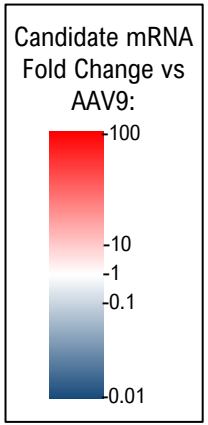
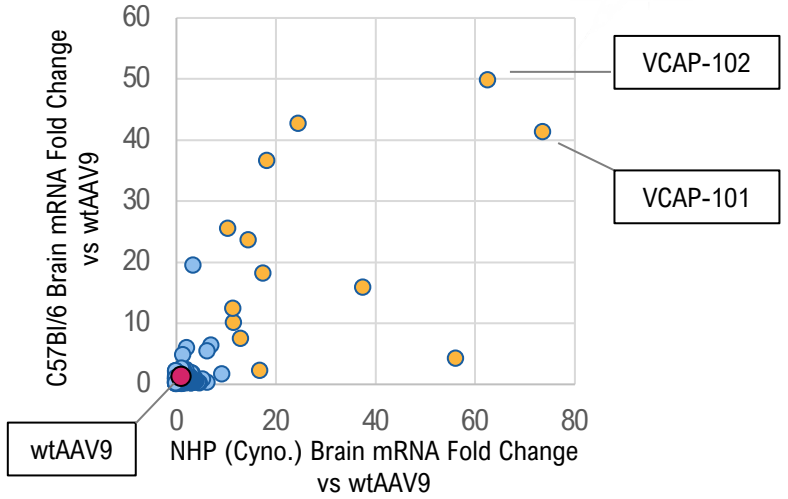
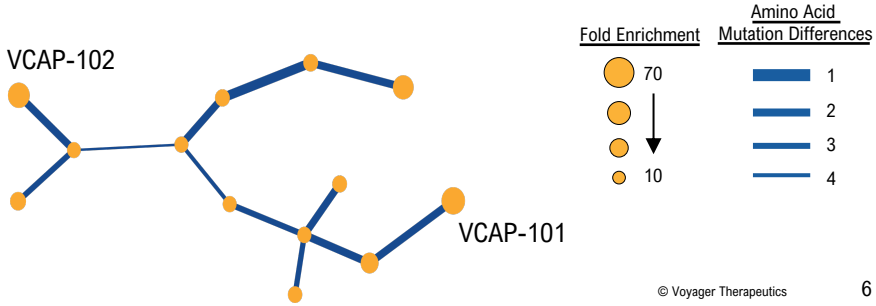
VCAP-101 and VCAP-102 Demonstrate Increased CNS Tropism in Both Macaques and Rodents







Network Clustering of Hits with FC/wtAAV9 > 10:



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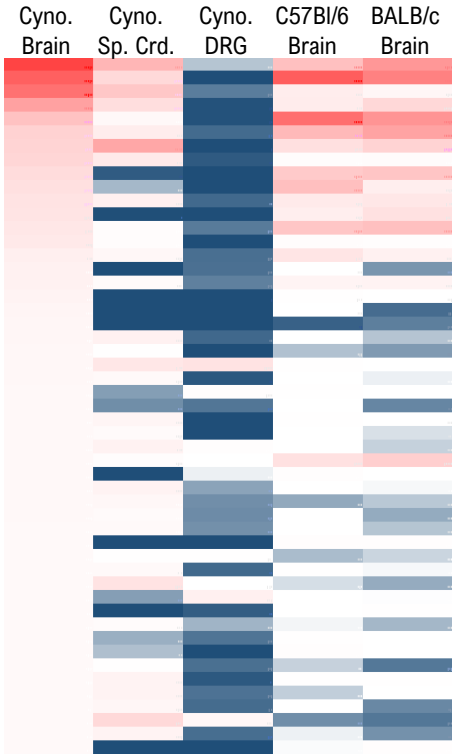
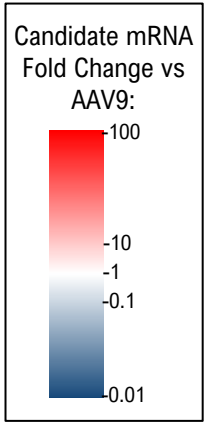
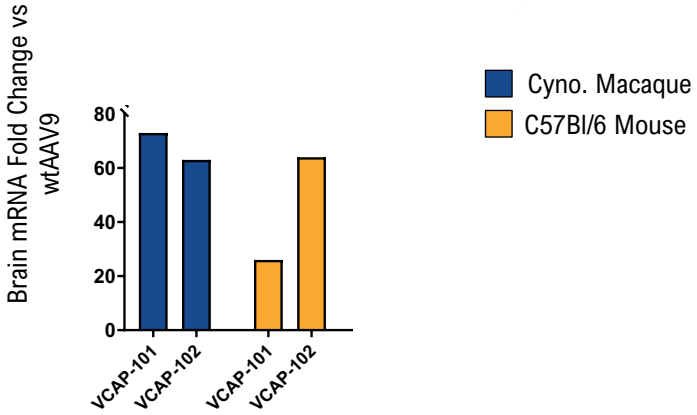
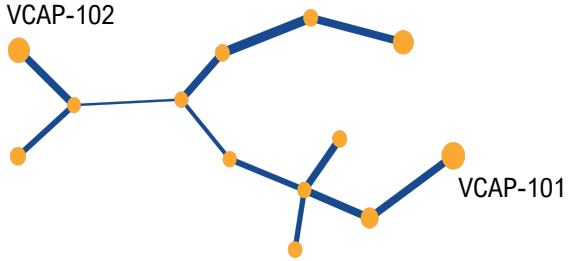
VCAP-101 and VCAP-102 Demonstrate Increased CNS Tropism in Both Macaques and Rodents







Network Clustering of Hits with FC/wtAAV9 > 10:



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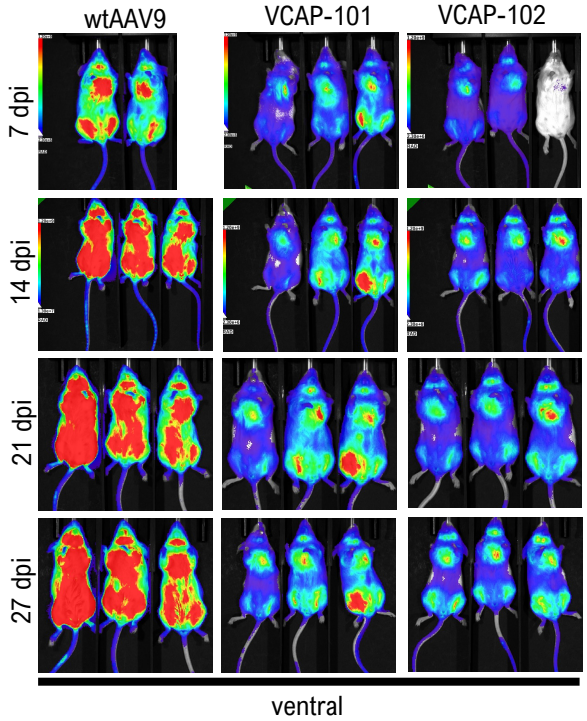
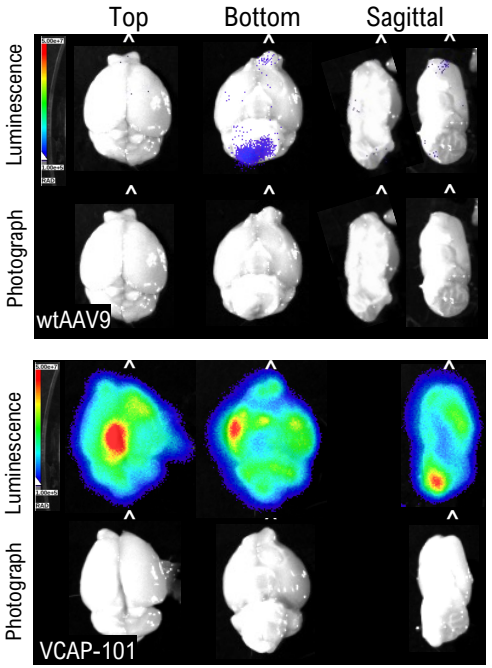
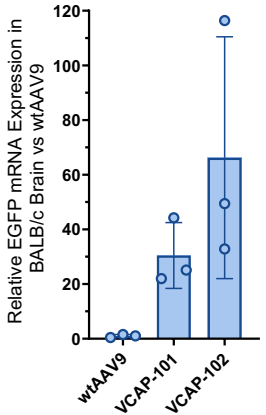
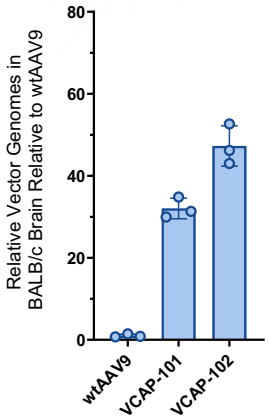
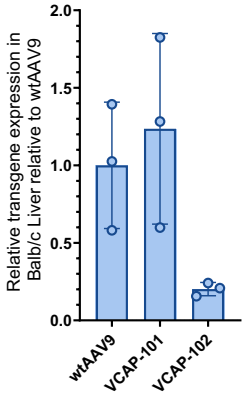
Validation Efforts Confirm Increased CNS Tropism of VCAP-101 and VCAP-102 in Mice

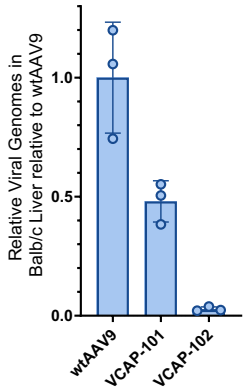












- IV dosing; 28 days in life
- Transgene: -ssCBA-Luc2-T2A-EGFP; 2.5E13 VG/kg

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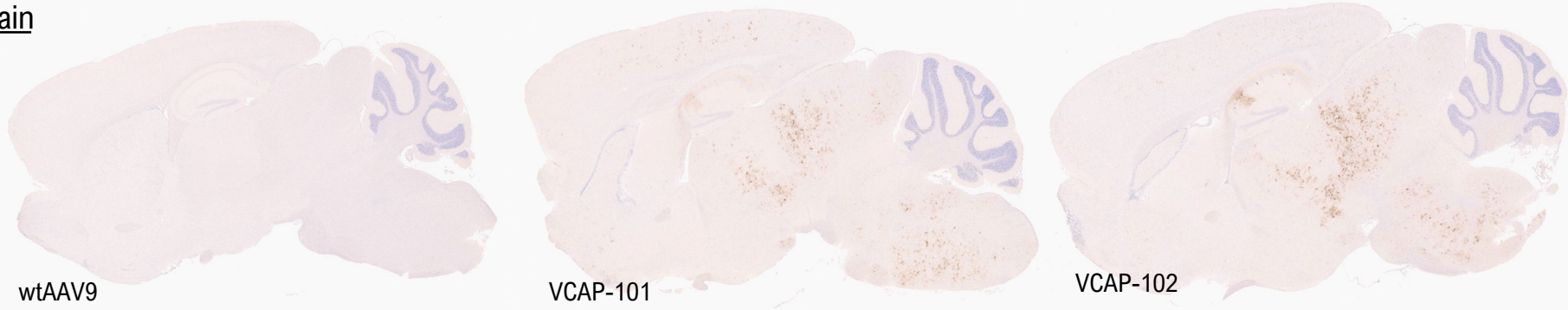
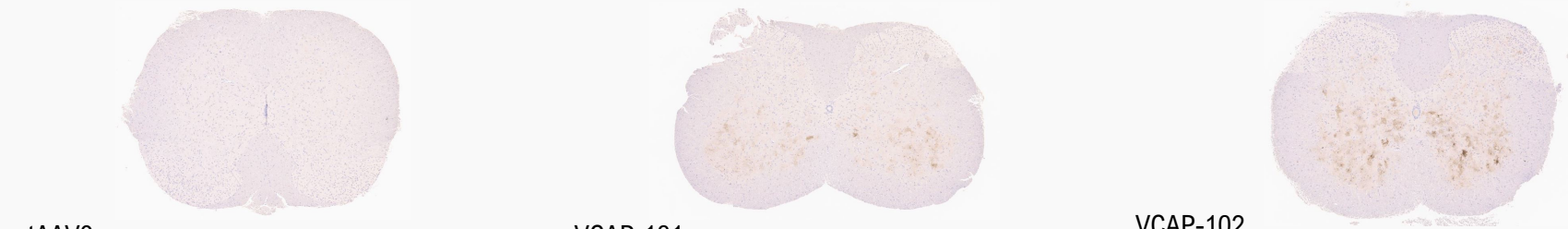
VCAP-101 and VCAP-102 Display a Unique Glial Tropism in Mouse Brain and Spinal Cord

Brain



wtAAV9 VCAP-101 VCAP-102

Spinal Cord



wtAAV9 VCAP-101 VCAP-102 Anti-GFP

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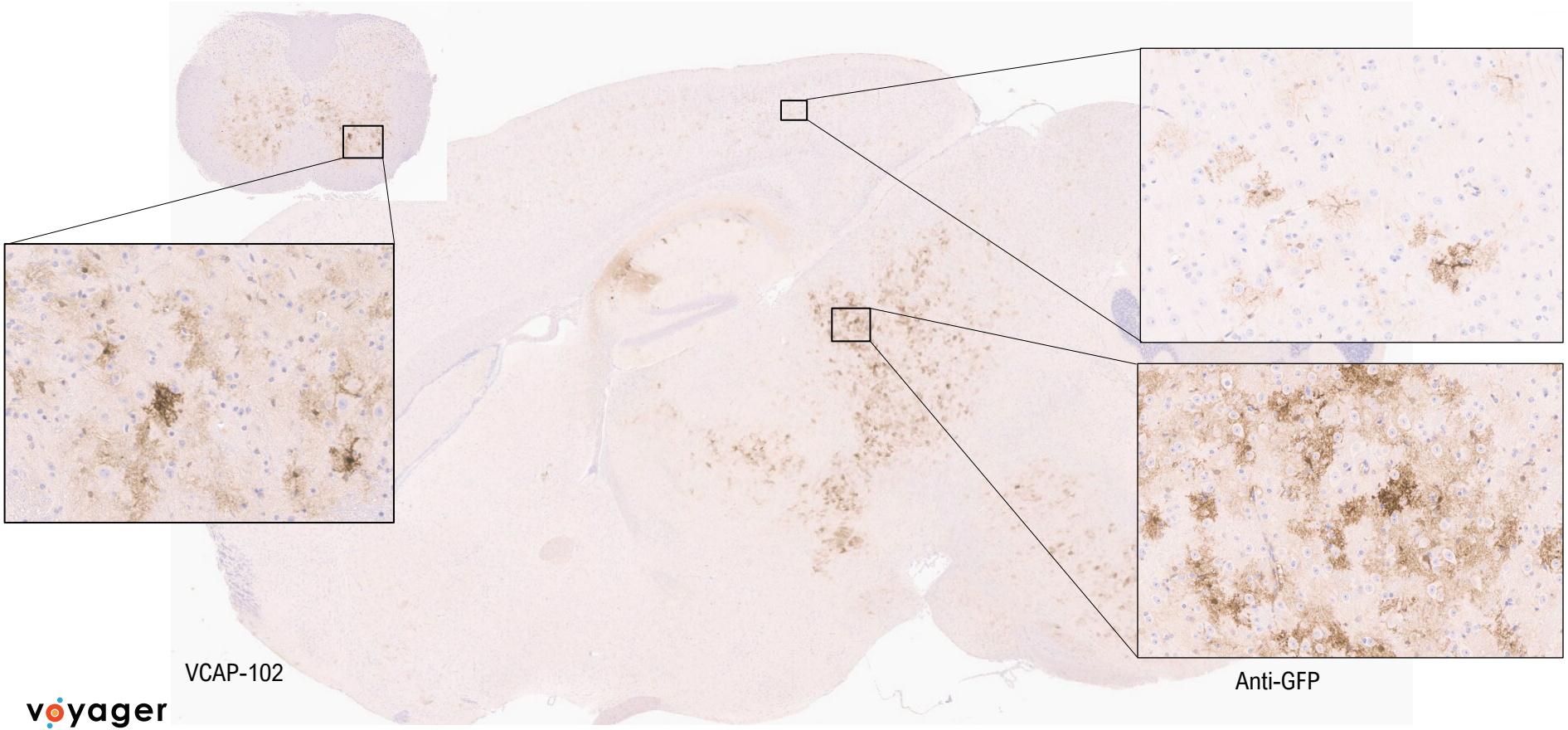
VCAP-101 and VCAP-102 Display a Unique Glial Tropism in Mouse Brain and Spinal Cord



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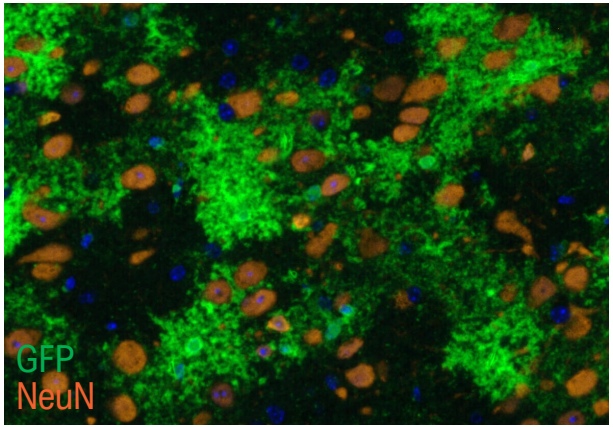
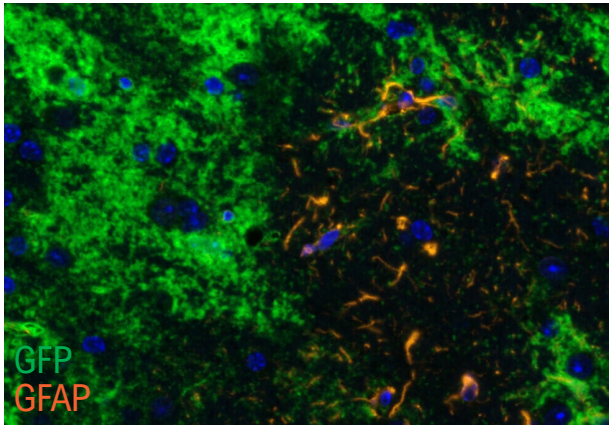
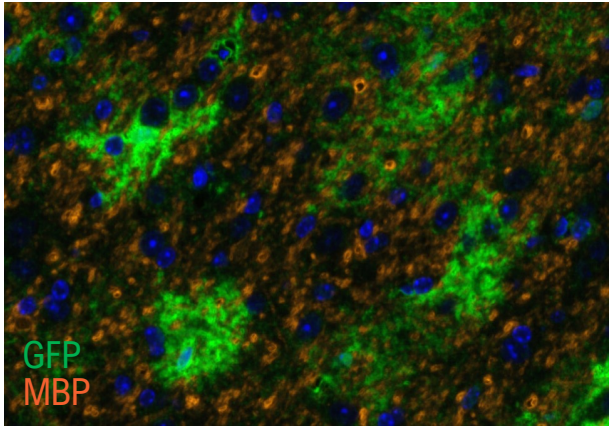
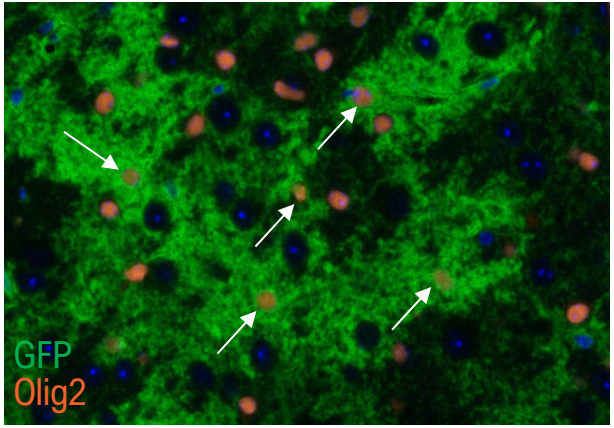
Co-stainings Suggest That VCAP-102 Transduces a Population of Olig2+ Glial Cells









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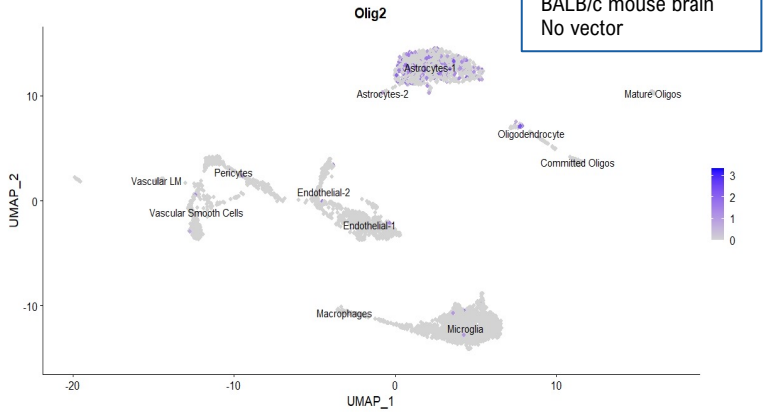
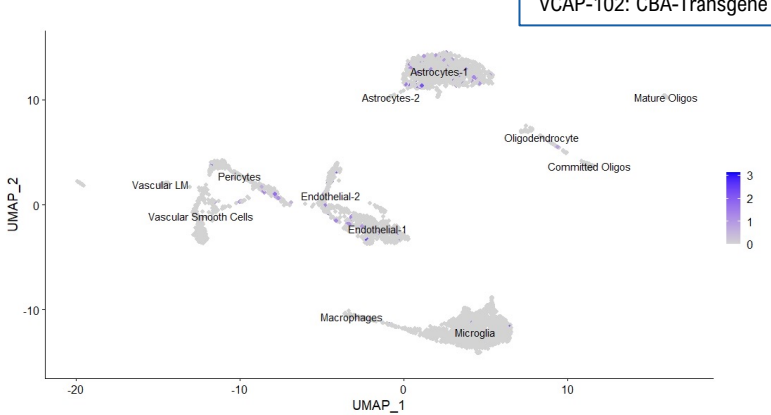
Single Cell RNA-seq Analysis Suggests that VCAP-102 Transduces Astrocytes and Cells of the Blood Brain Barrier in Mice

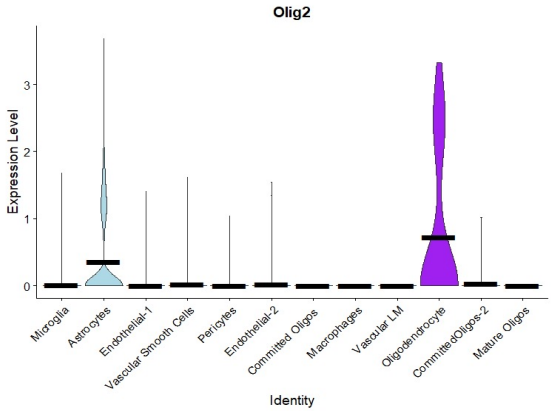
BALB/c mouse brain
No vector

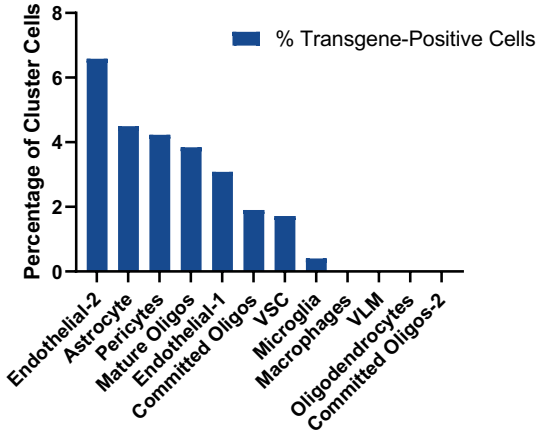


VCAP-102: CBA-Transgene



Dan Laks
Abstract 63





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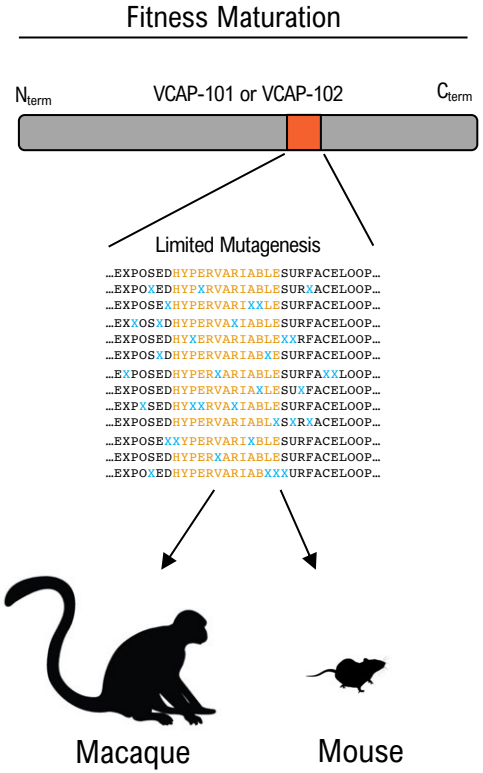
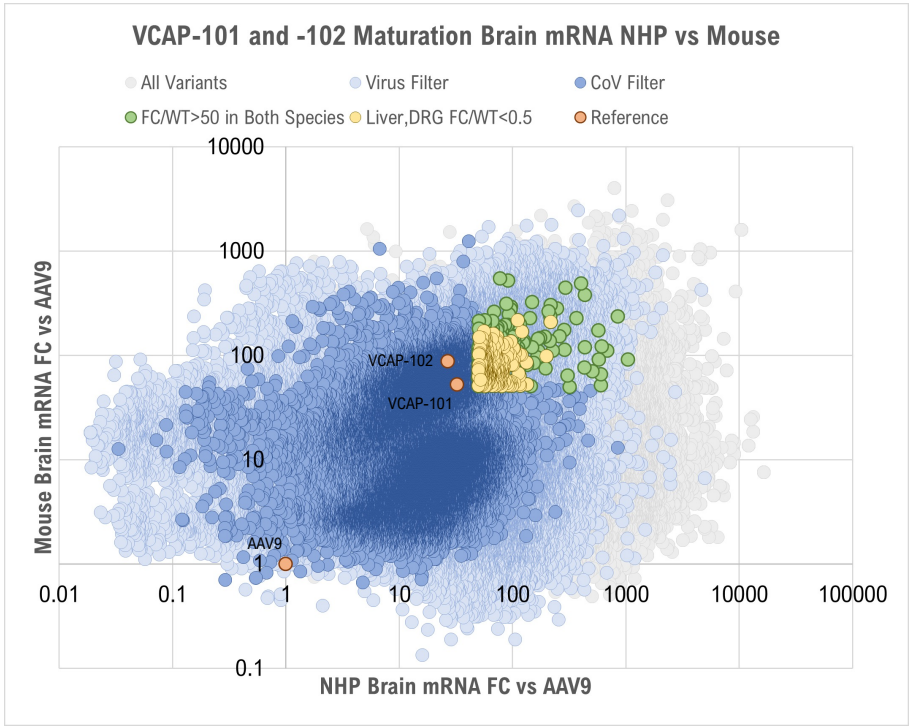
Fine-Tuning of VCAP-101 and VCAP-102 Generates Variants with Increased CNS Tropism Across Species





Total Candidates:	**680,000**
Virus Input Filter:	**184,000**
Coefficient of Variation Filter:	**64,000**
Brain FC/WT > 50 in Both Species:	**486**
FC/WT < 0.5 in Liver and DRG:	**225**

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Acknowledgements

Capsid Discovery Team
- Mathieu Nonnenmacher
- Damien Maura
- Matthew Child
- Roop Kaur
- Kristin Graham
- Brett Hoffman
- Tatiana Knox
- Jing Lin
- Amy Ren
- Gordon Ta
- Jiangyu Li
- Wei Wang
- Jiachen Liu

In vivo team
- Ambreen Sayed-Zahid
- Mike Grannan

Histology
- Katherine Tyson
- Anupriya Kulkarni
- Jessenia Laguna-Torres
- Nilesh Pande

Single Cell RNA-Seq
- Dan Laks
- Sam Hasson

Histology Outsourcing
- Charlotte Chung

NHP Study Coordination
- Mike Hefferan
- Andrew Cameron

NGS Data Analysis
- Katie D'Aco – Diamond Age

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QUESTIONS?
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tmoyer@vygr.com