

# Identification and Characterization of STAC-BBB, an Engineered AAV Capsid That Exhibits Widespread Transduction of the Central Nervous System in Cynomolgus Macaques

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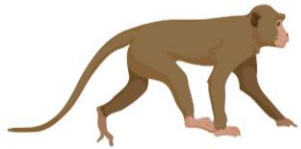
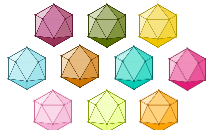
**Matthew Tiffany**, Stephanye Frias, Lori Andrews, Ankitha Nanjaraj, Russell Darst, Stephen Wist, Satria Sajuthi, Yuri Bendaña, Hung Tran, Sarah Mueller, Bryan Zeitler, Amy M. Pooler, David S. Ojala

## — Disclosure

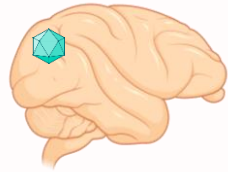
I am a full-time employee of Sangamo Therapeutics

# SIFTER platform leverages cell type specific measurement of capsid-mediated transgene expression

AAV library administered to cyno macaques



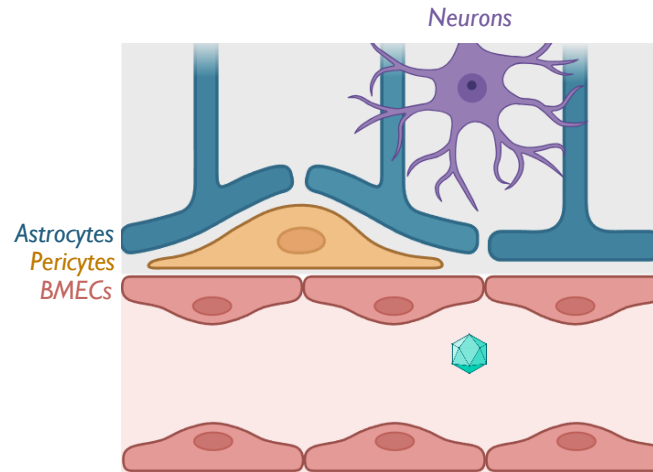
Capsids reach brain tissue



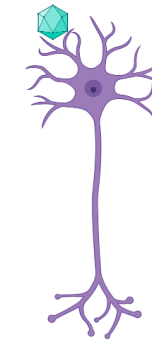
Traditional methods stop here



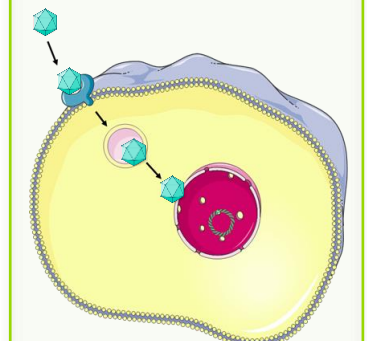
Overcome biological barriers



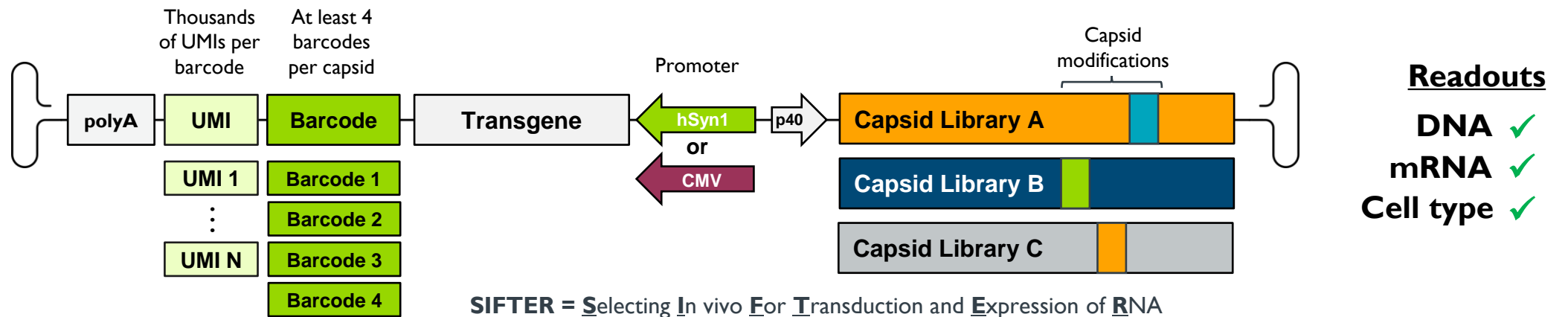
Transduce neurons



Express transgene in neurons



Delivery goal



# Multiple library screening rounds were conducted to identify STAC-BBB



✓

**Round 1**

**100 million**  
capsids screened.

✓

**Round 2**

**60,000** capsids with  
sequence replicates.  
Includes controls with  
known performance.

✓

**Round 3**

**1,260** capsids with at  
least 4 unique sequence  
replicates. Includes  
controls with known  
performance.

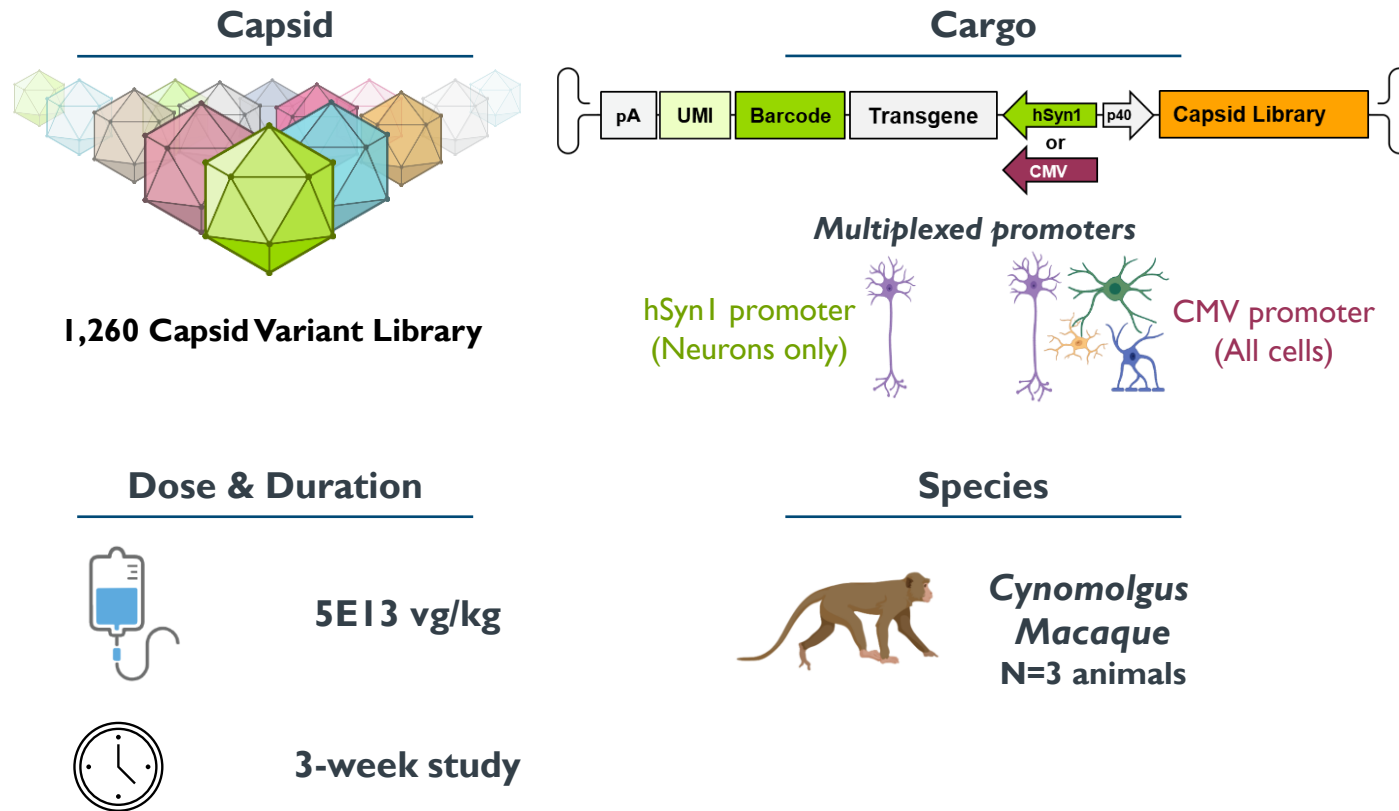
✓

**Individual evaluation**

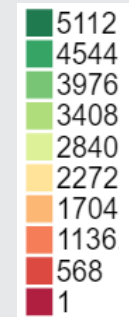
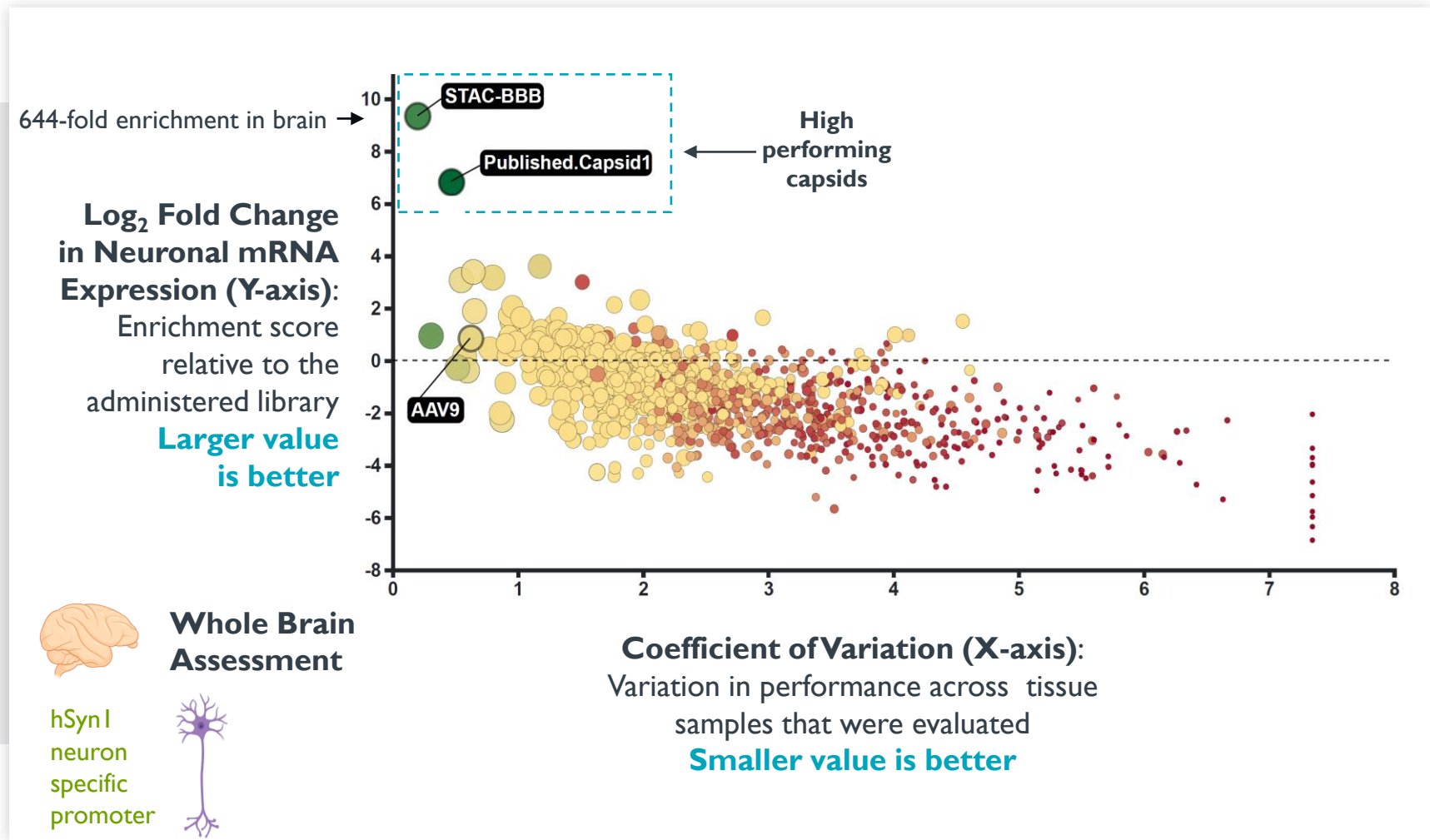
**Single best** capsid  
variant (STAC-BBB) with  
reporter gene or  
therapeutically-relevant  
ZF cargo.

# Study design for Round 3 SIFTER library selection

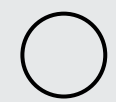
**Objective: Determine relative performance of 1,260 capsid variants and select lead variant for individual evaluation**



# STAC-BBB is the top performing capsid in the round 3 library for neuronal transduction



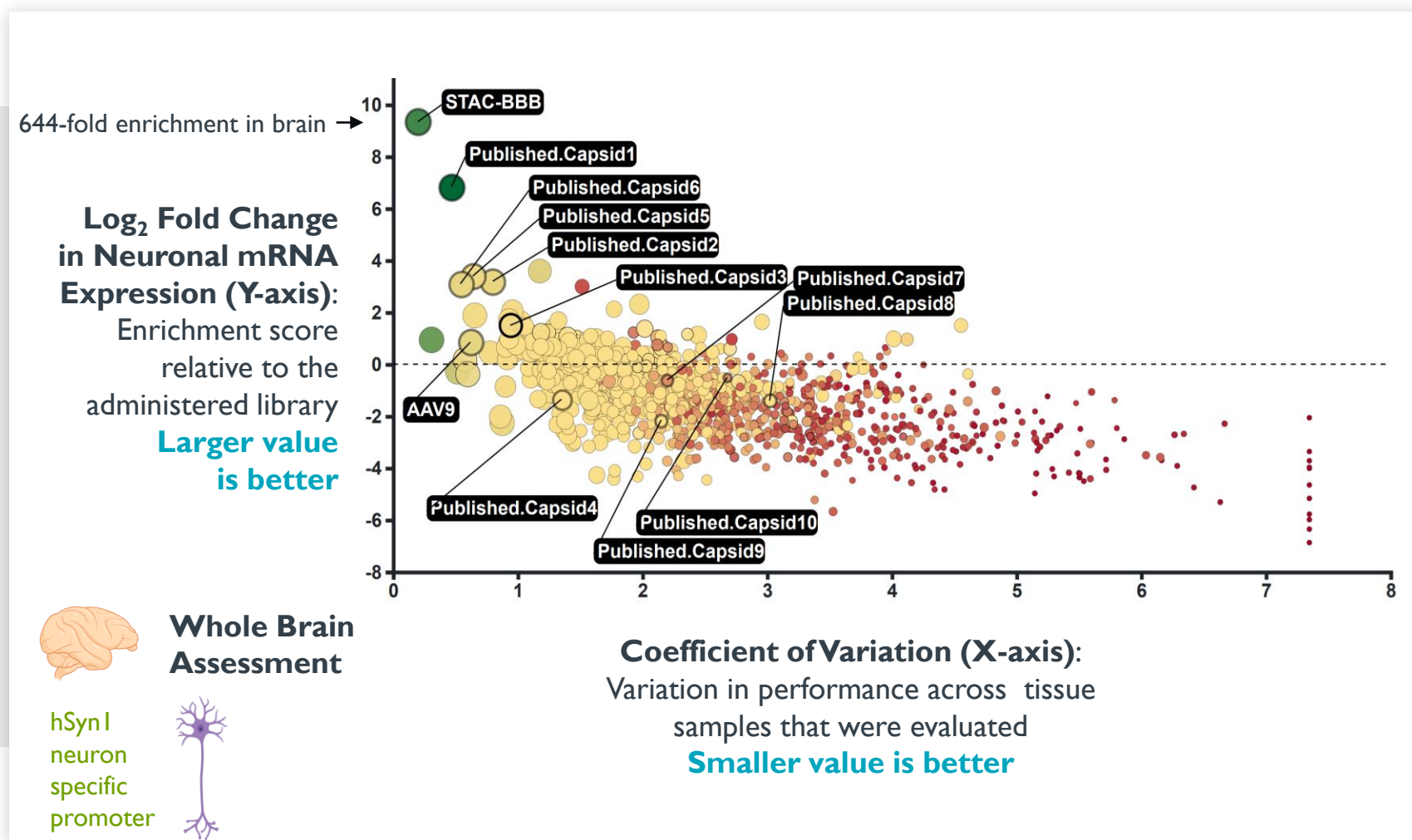
**Unique Molecular Identifier count (Color):**  
Informs number of unique AAV transduction events  
**Darker green is better**



**Fraction of replicates found (Bubble size):**  
Informs consistency of replicate recovery  
**Larger circle is better**

Neuronal RNA expression (3-week study, hSynI)  
Data averaged from all three animals  
STAC = Sangamo Therapeutics AAV Capsid

# STAC-BBB is the top performing capsid in the round 3 library for neuronal transduction



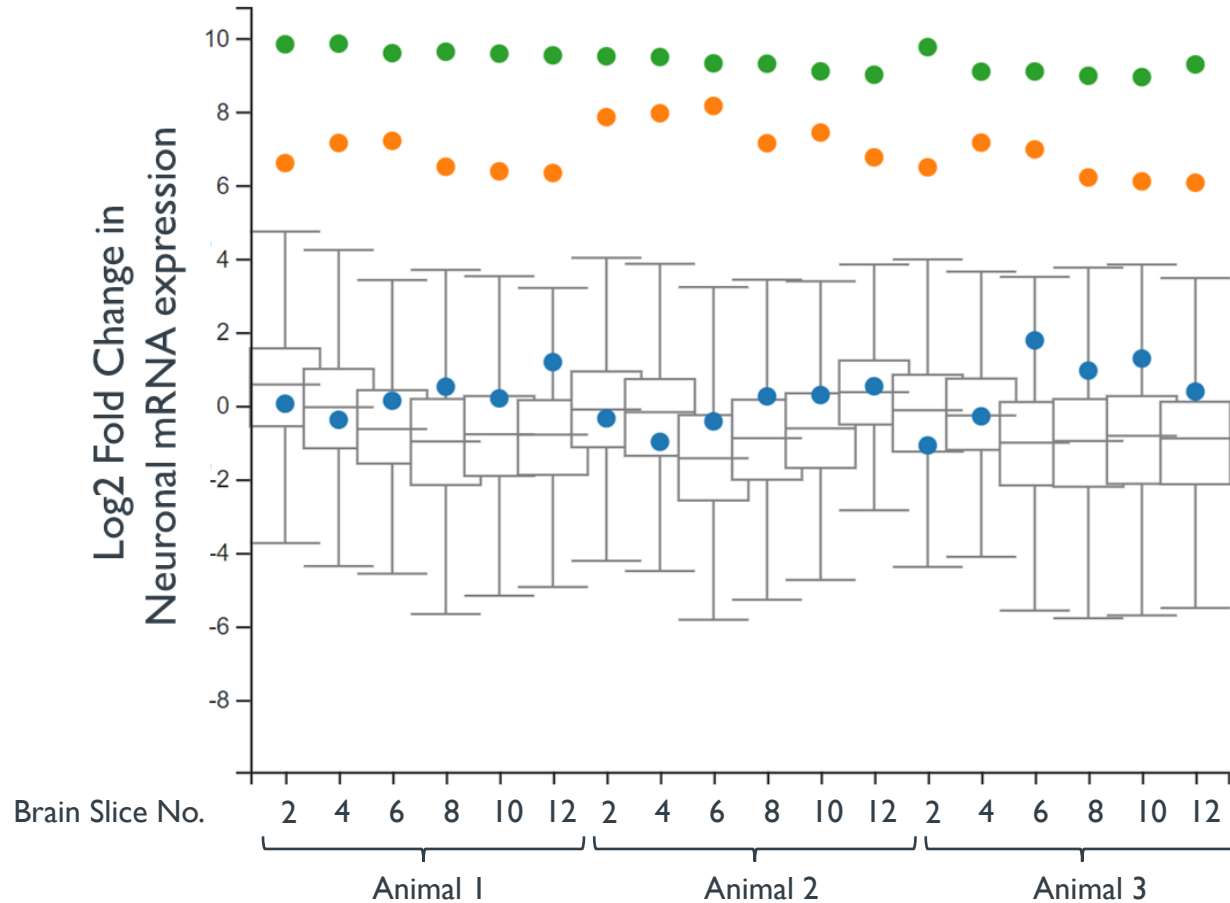
**Unique Molecular Identifier count (Color):**  
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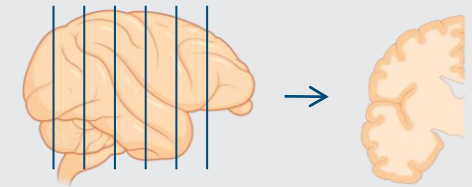
**Fraction of replicates found (Bubble size):**  
Informs consistency of replicate recovery  
**Larger circle is better**

Neuronal RNA expression (3-week study, hSynI)  
Data averaged from all three animals

# STAC-BBB exhibits 700-fold higher neuronal mRNA expression relative to AAV9



Neuronal RNA expression (3-week study, hSyn I)  
Box represents 25<sup>th</sup> – 75<sup>th</sup> percentile of library performance. Whiskers are 1.5x the interquartile range.



## Assessment across brain slices and animals

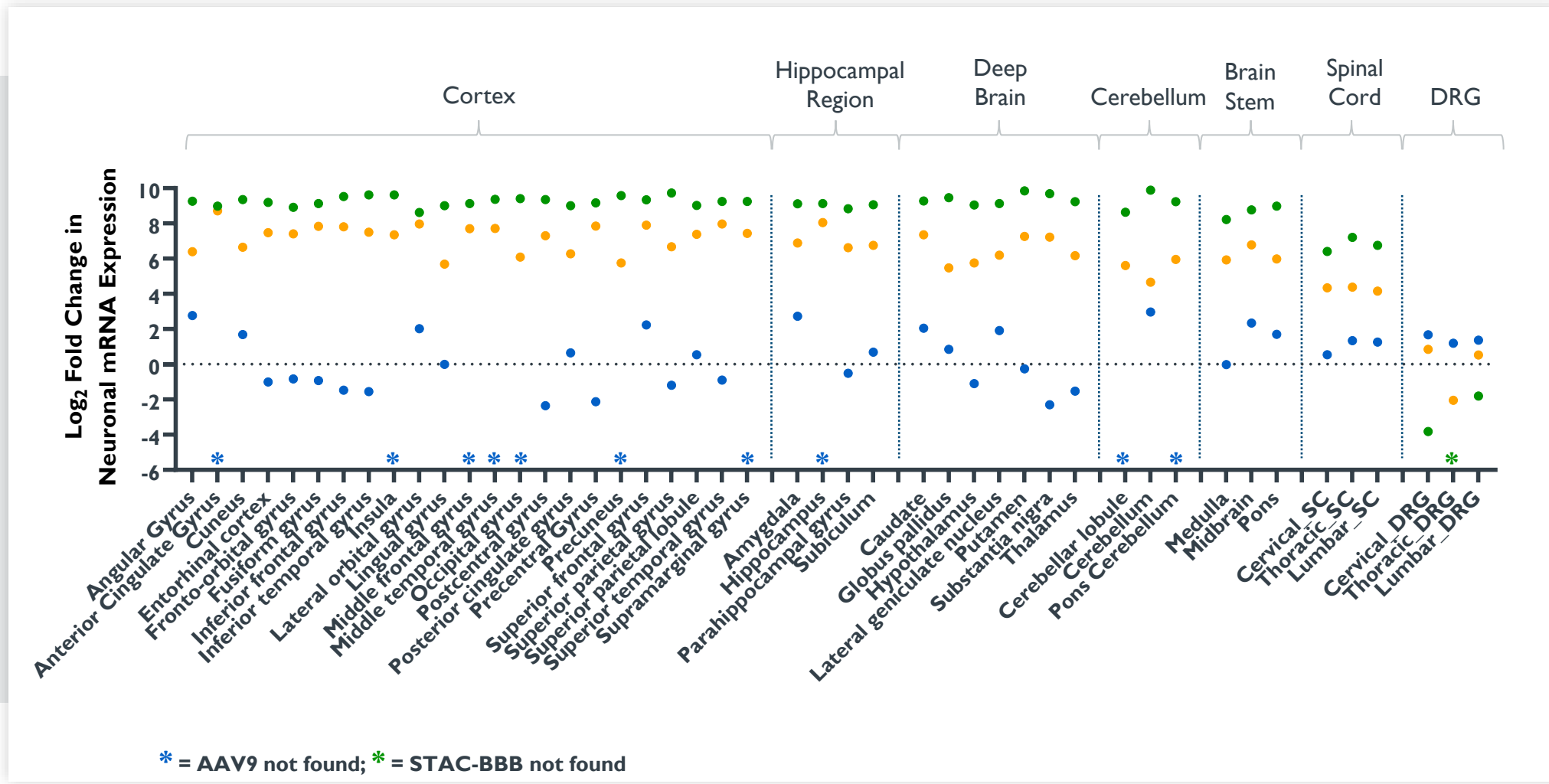
- STAC-BBB
- Published capsid
- AAV9

hSyn I  
neuron  
specific  
promoter





# STAC-BBB mediates higher neuronal mRNA expression in all CNS regions



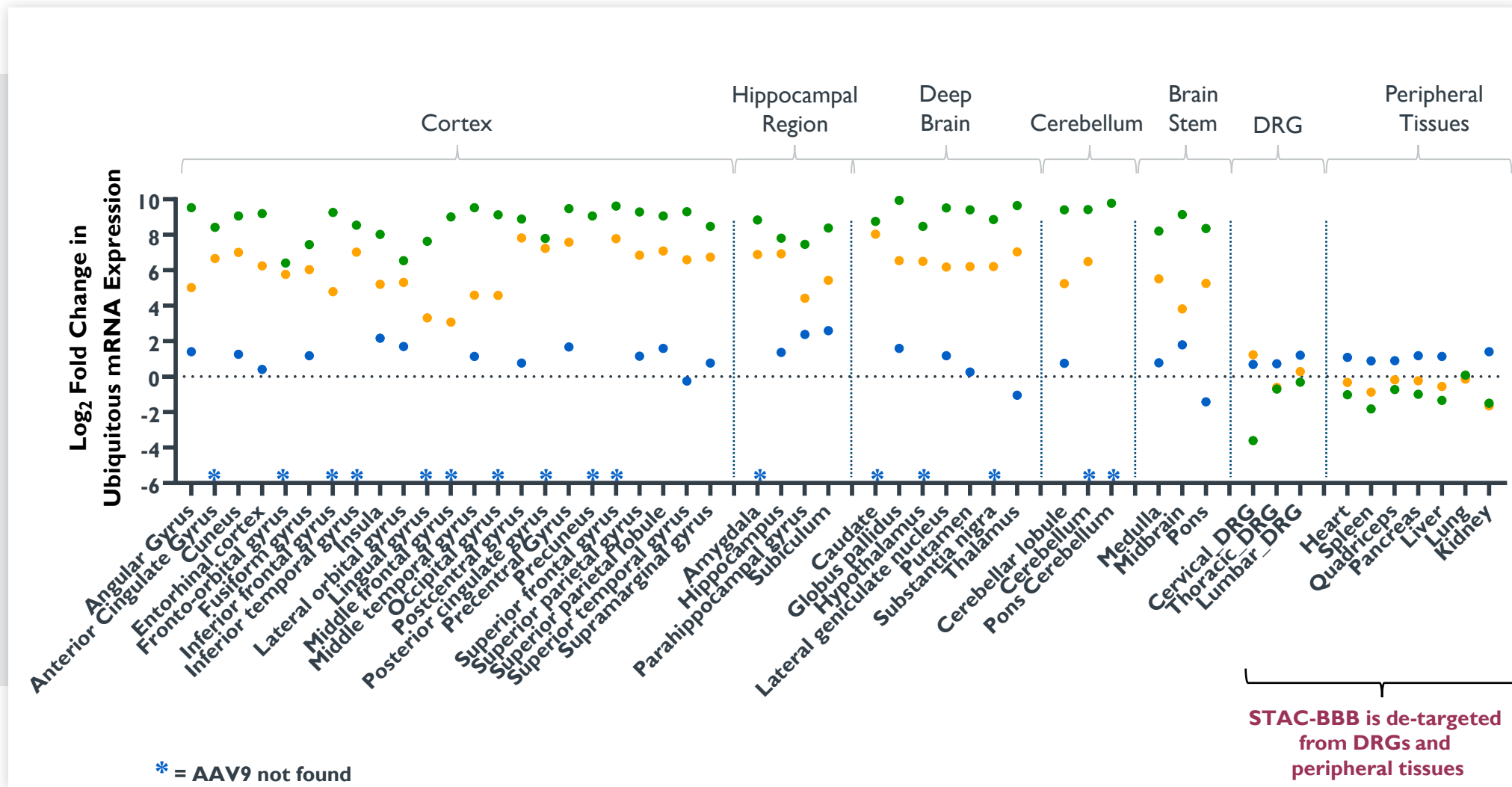
150 brain punches analyzed

- STAC-BBB
- Published capsid
- AAV9

hSyn I neuron specific promoter

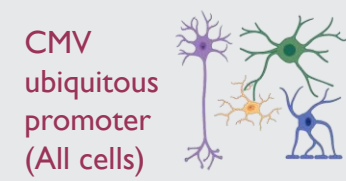
Neuronal RNA expression (3-week study, hSyn I)  
All three animals on study

# STAC-BBB mediates higher ubiquitous mRNA expression in all CNS regions and is detargeted from DRG and peripheral tissues



150 brain punches analyzed

- STAC-BBB
- Published capsid
- AAV9



**STAC-BBB is de-targeted from DRGs and peripheral tissues**

Ubiquitous RNA expression (3-week study, CMV)  
All three animals on study

# Individual evaluation of STAC-BBB capsid with zinc finger cargo

**Objective: Assess STAC-BBB biodistribution in CNS and peripheral tissues using a ubiquitous promoter.**

## Capsid



STAC-BBB

## Cargo

CAG – ubiquitous promoter

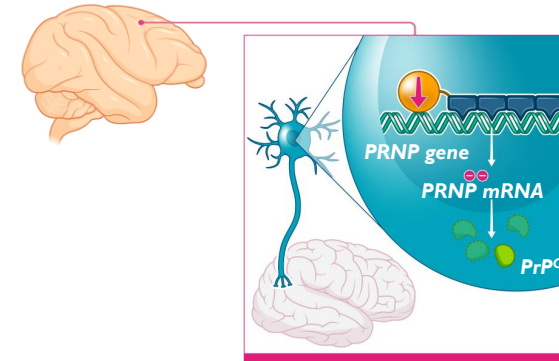
Nuclear localized  
EGFP

2A peptide



Prion ZFR

## Therapeutic Approach



## Dose & Duration



2E13 vg/kg



3-week study

## Species



*Cynomolgus  
Macaque*  
N=3

## Endpoints

- Immunohistochemistry (GFP) images
- RNAscope images (ZFR/Prion)
- Molecular analyses

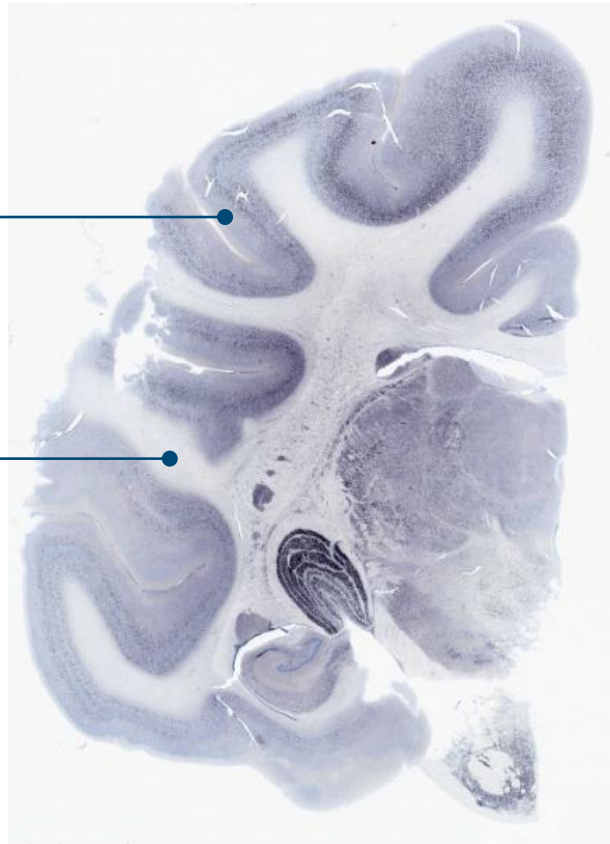
# STAC-BBB drives widespread and robust expression throughout the brain

## STAC-BBB (Nuclear-localized GFP)

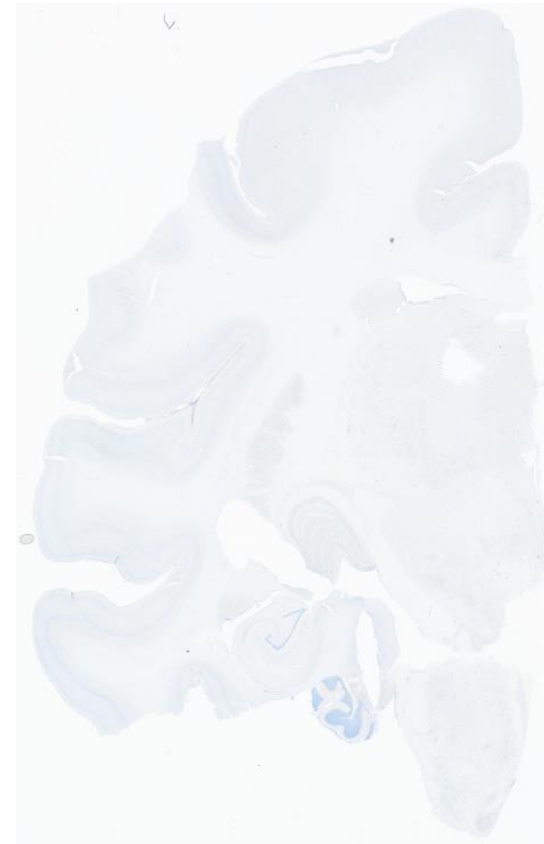
## Negative control (no AAV treatment) – No signal

Grey matter  
(cell bodies)

White matter  
(nerve fibers)



2e13 vg/kg STAC-BBB, 19 days post administration



Nissl staining (light blue):

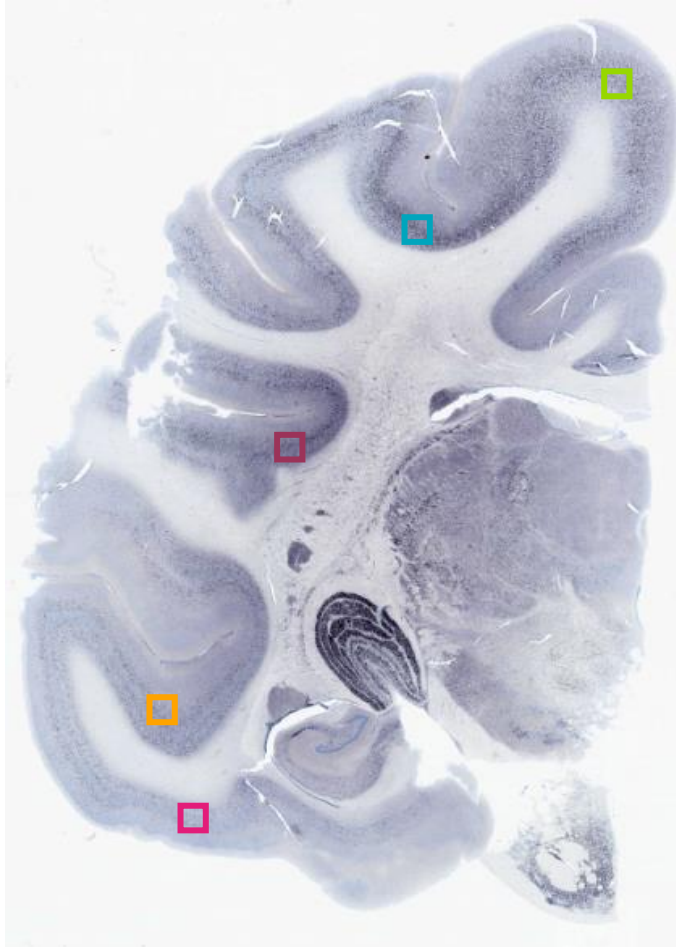
**All cell nuclei**

Antibody labeling  
for green fluorescent protein  
(GFP) expression (black):

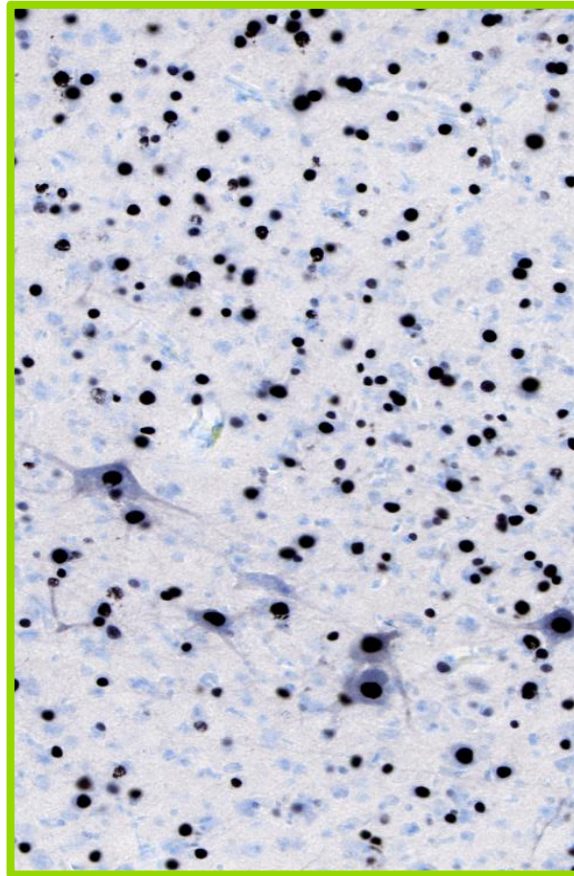
**Cells transduced  
with STAC-BBB**



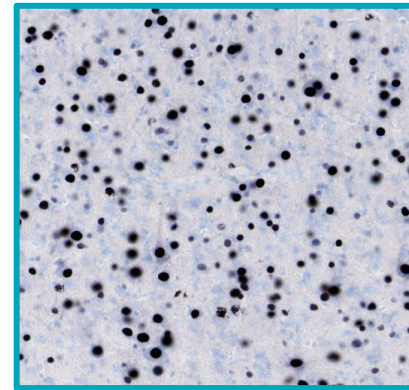
# STAC-BBB shows widespread neuronal transduction across all cortical regions



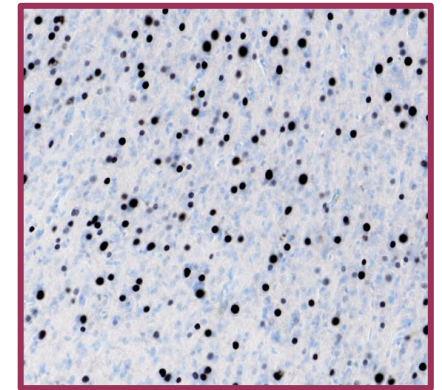
Precentral Gyrus (Motor Cortex)



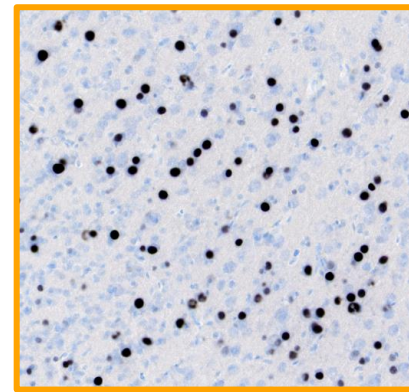
Postcentral Gyrus



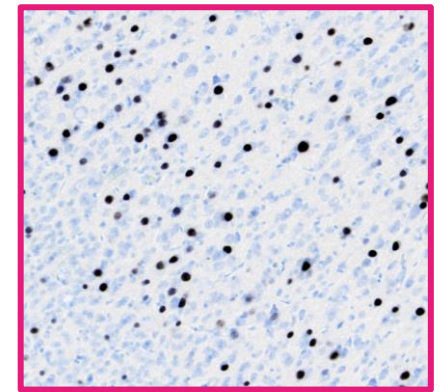
Superior Temporal Gyrus



Middle Temporal Gyrus

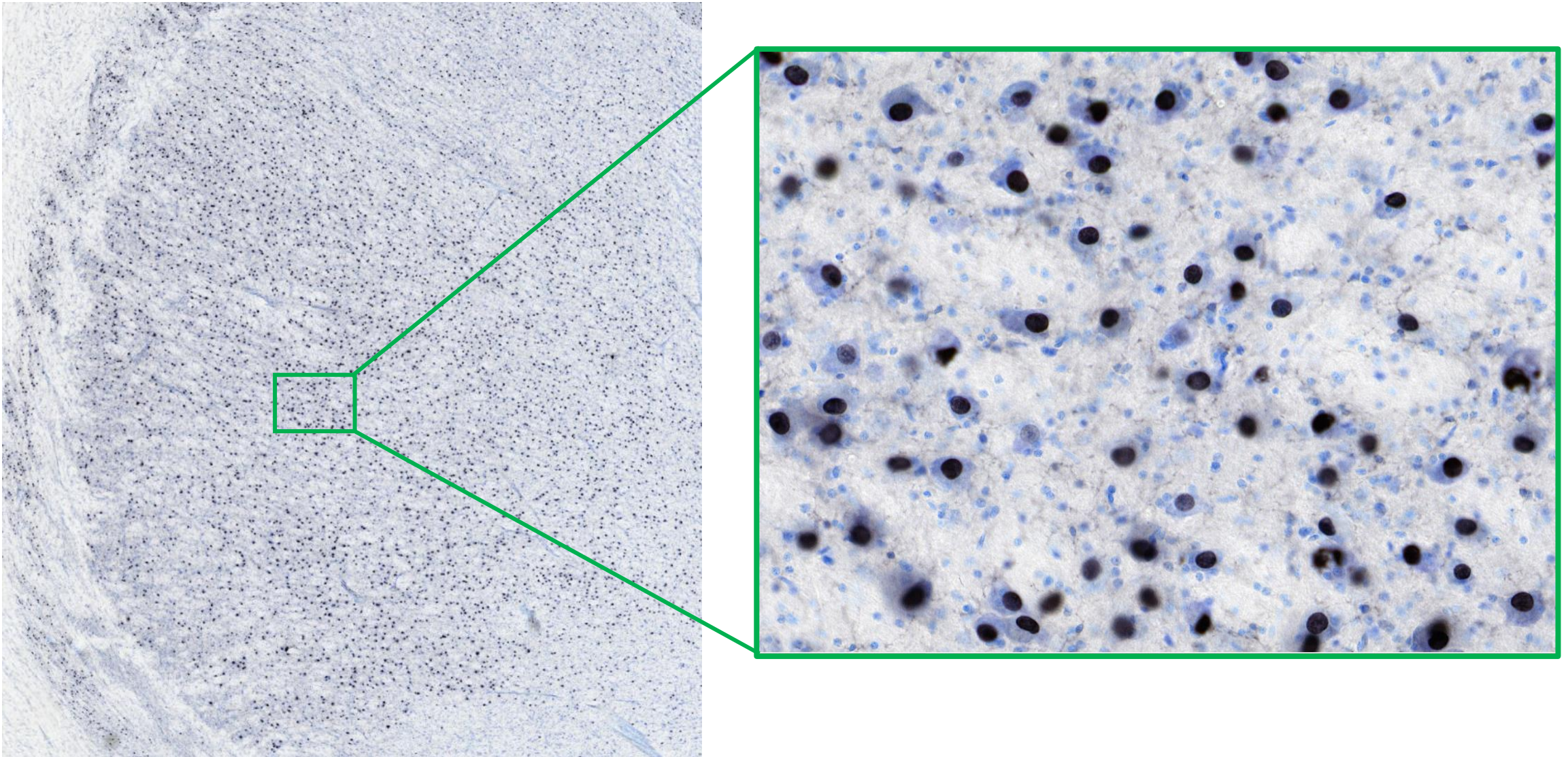


Inferior Temporal Gyrus





# STAC-BBB mediates widespread neuronal transduction in the thalamus

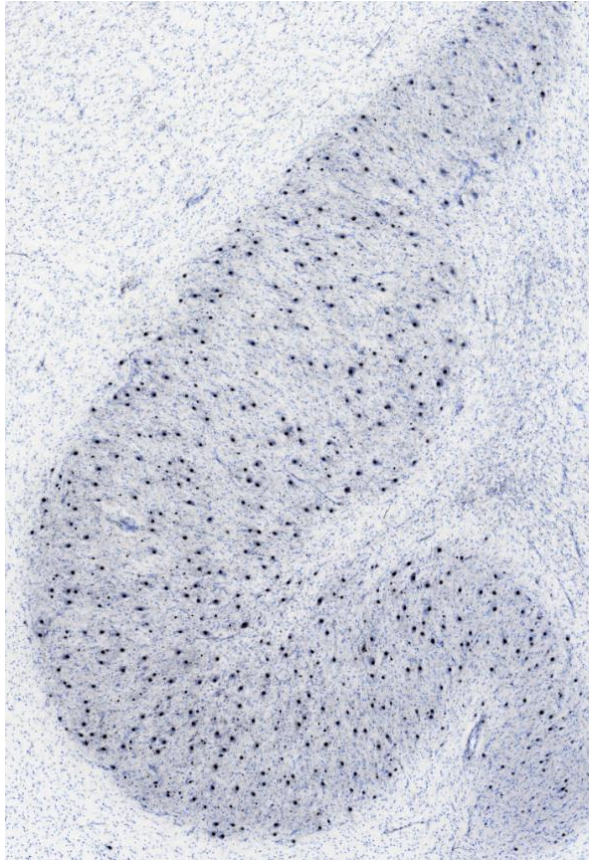




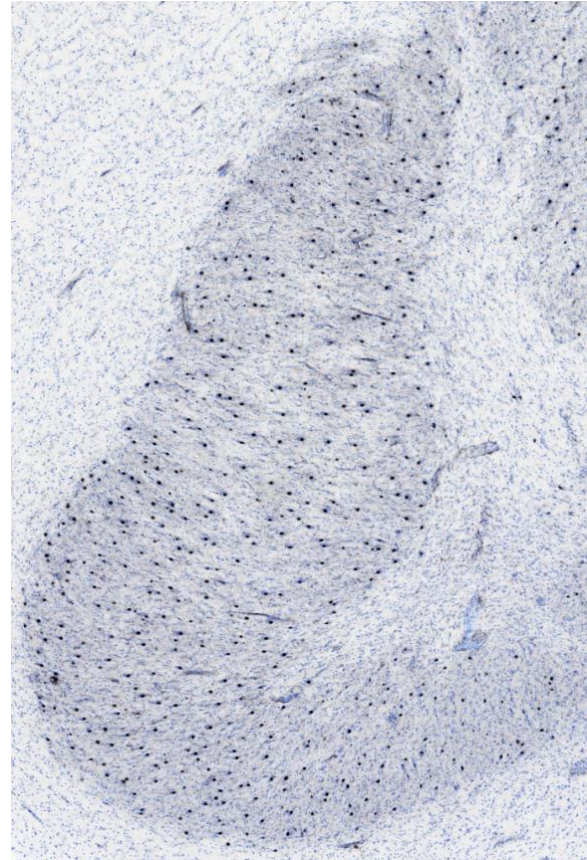
# STAC-BBB transduction is consistent across all animals

*Dentate nucleus - disease targets: Friedreich's ataxia, Spinocerebellar ataxias*

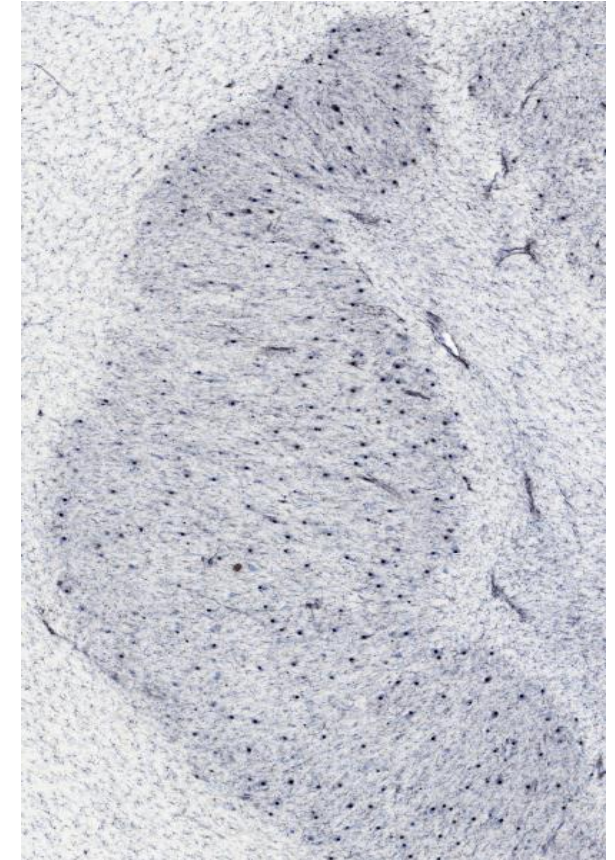
NHP 1



NHP 2



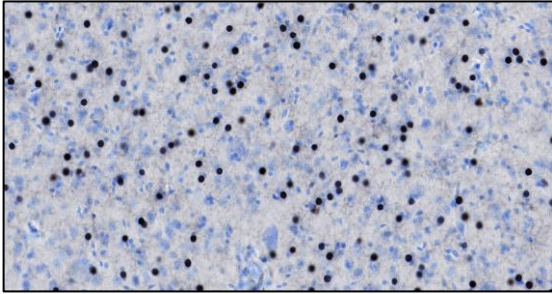
NHP 3



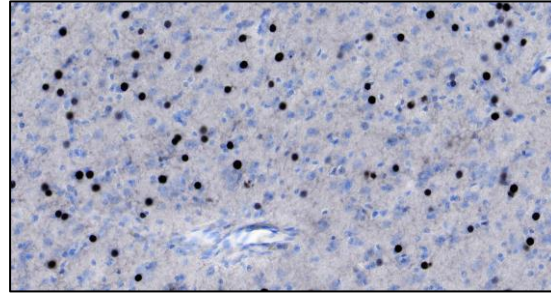


# STAC-BBB mediates widespread brain transduction at the 2e13 vg/kg dose

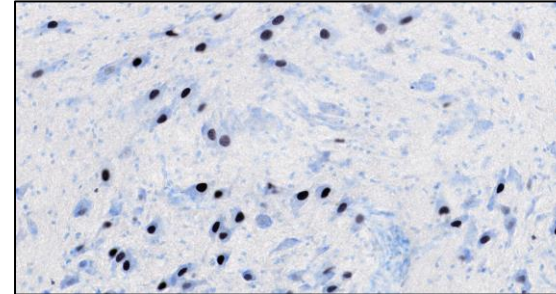
Putamen



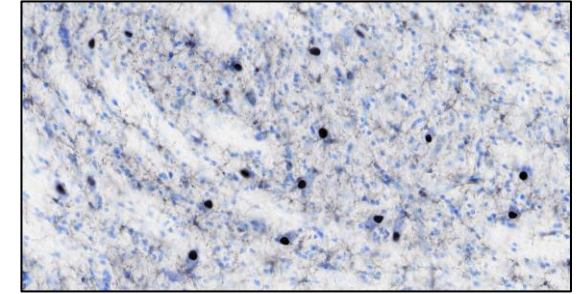
Caudate



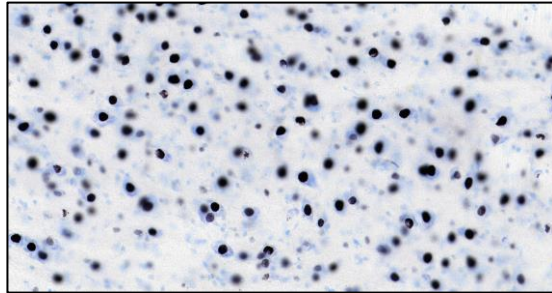
Substantia nigra



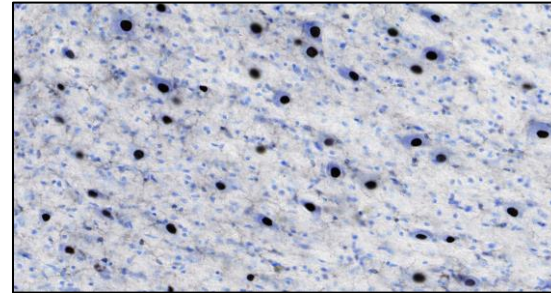
Globus pallidus



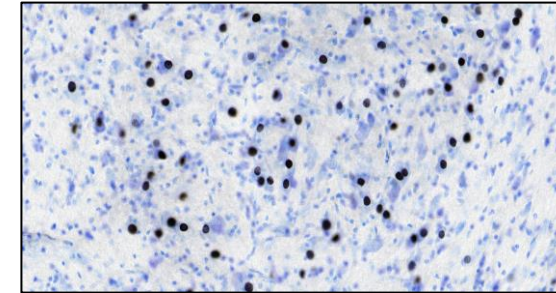
Pons



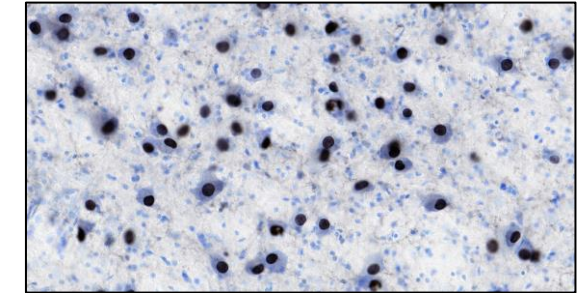
Dentate nucleus



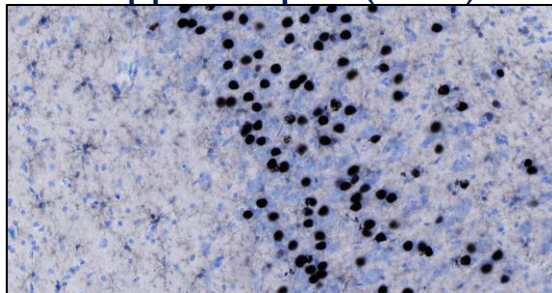
Cuneate nucleus



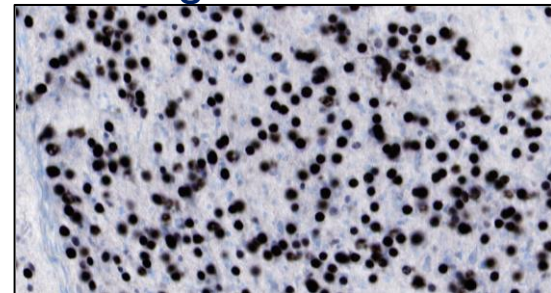
Thalamus



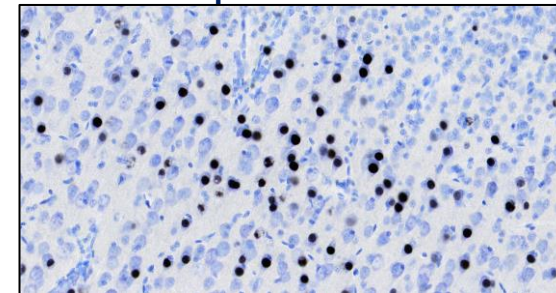
Hippocampus (CA2)



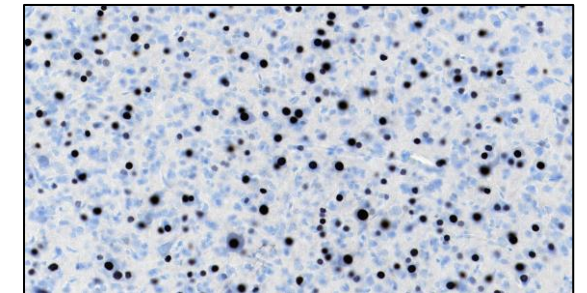
Lateral geniculate nucleus



Temporal cortex



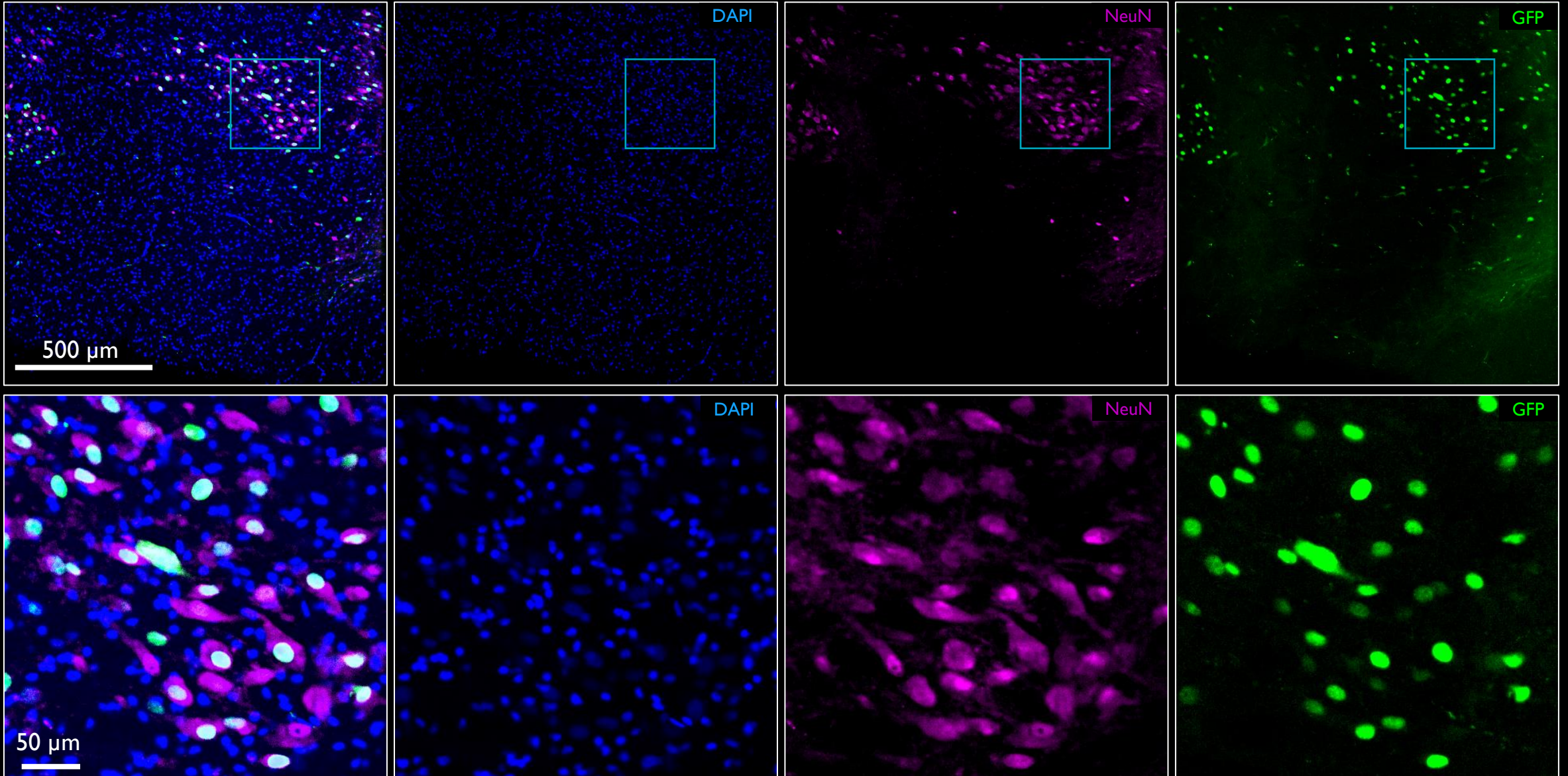
Motor cortex





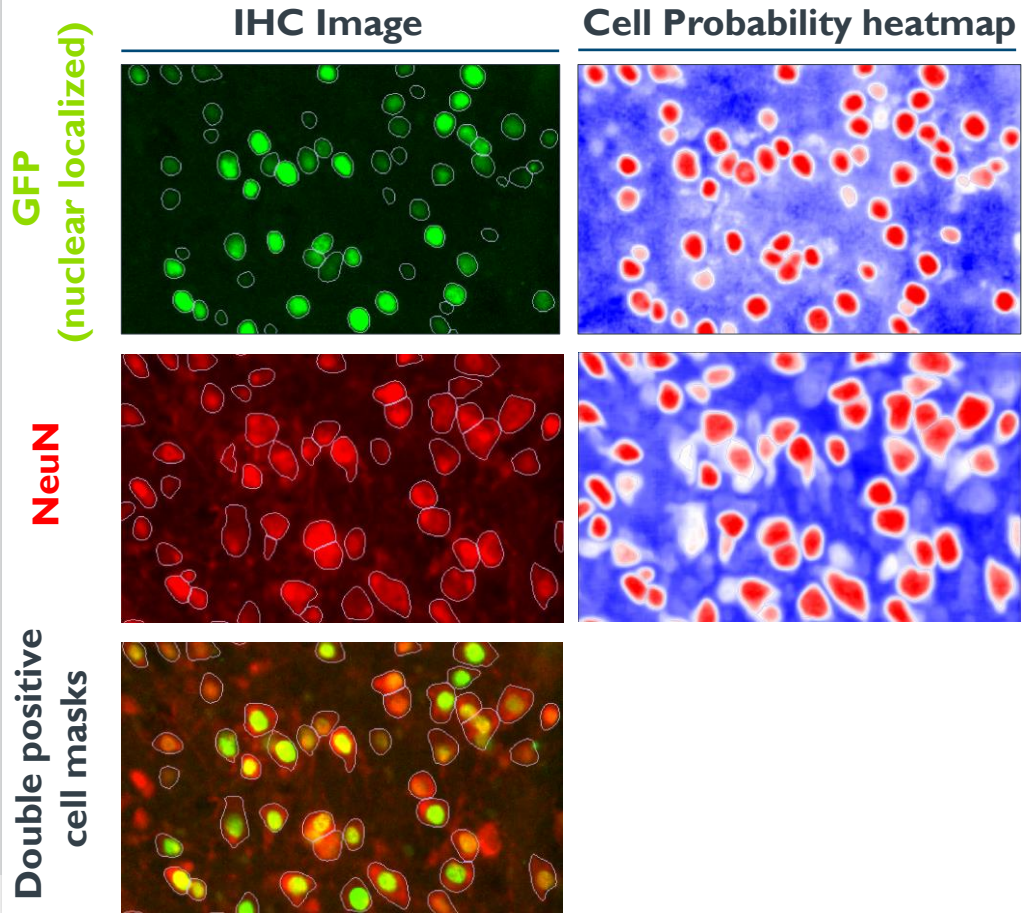
# STAC-BBB transduces neurons in the substantia nigra

DAPI  
NeuN  
GFP

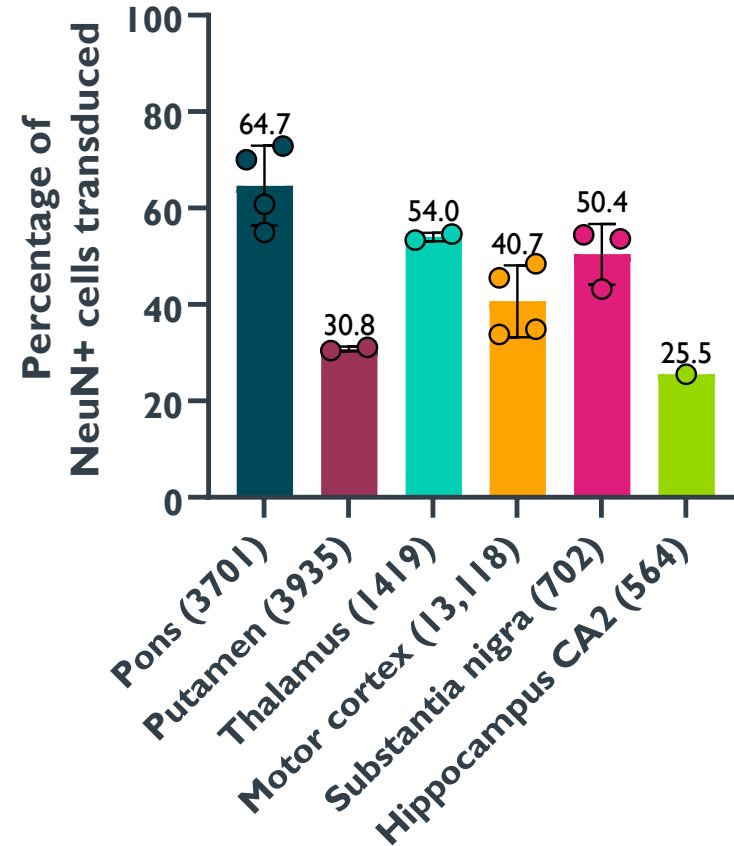


# STAC-BBB achieves high levels of NeuN+ cell transduction across the CNS

## Deep-learning based cell segmentation



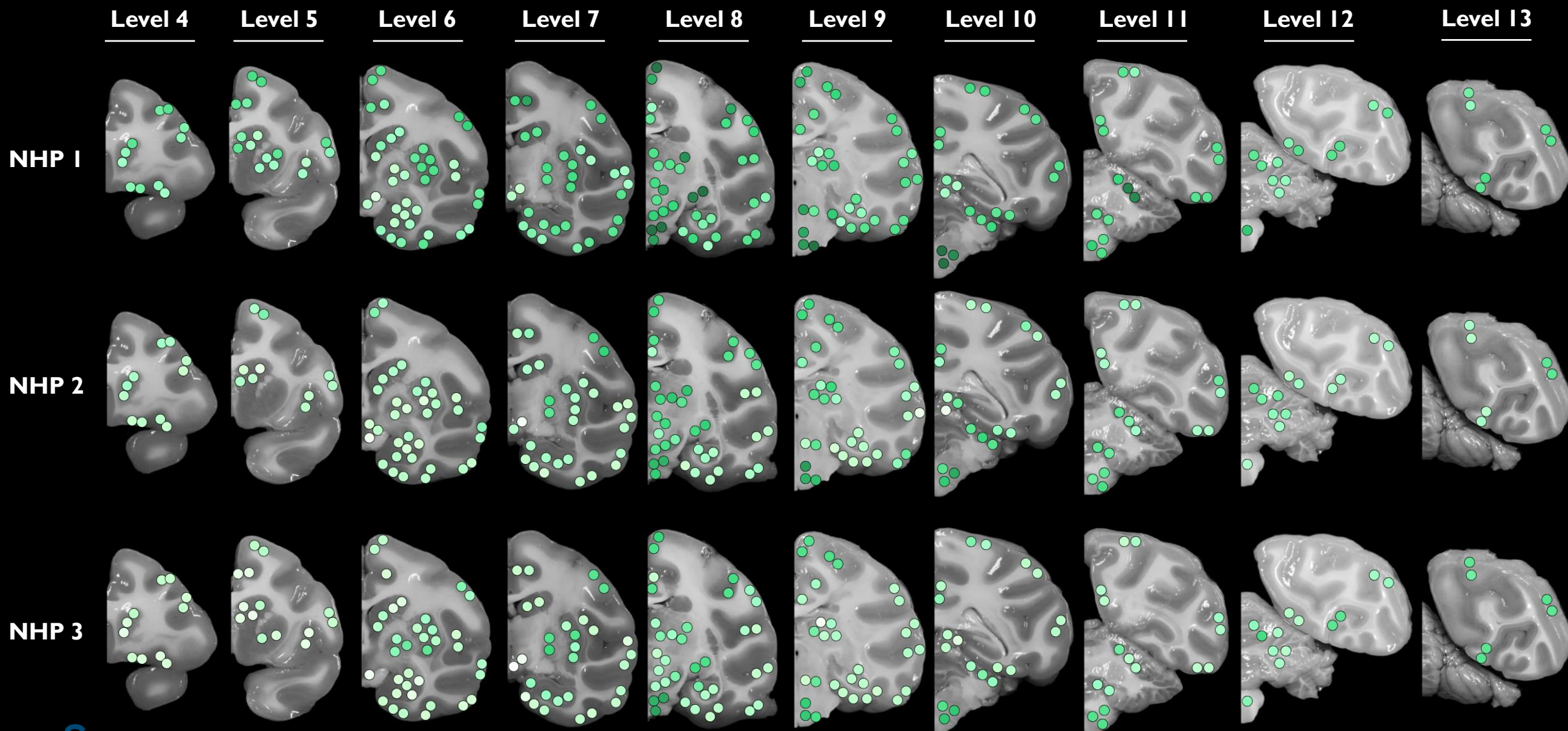
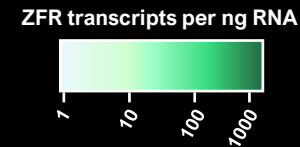
## STAC-BBB NeuN+ cell transduction



Number of NeuN+ cells counted per structure is in parenthesis



# STAC-BBB mediates prion-targeted ZFR expression throughout the brain



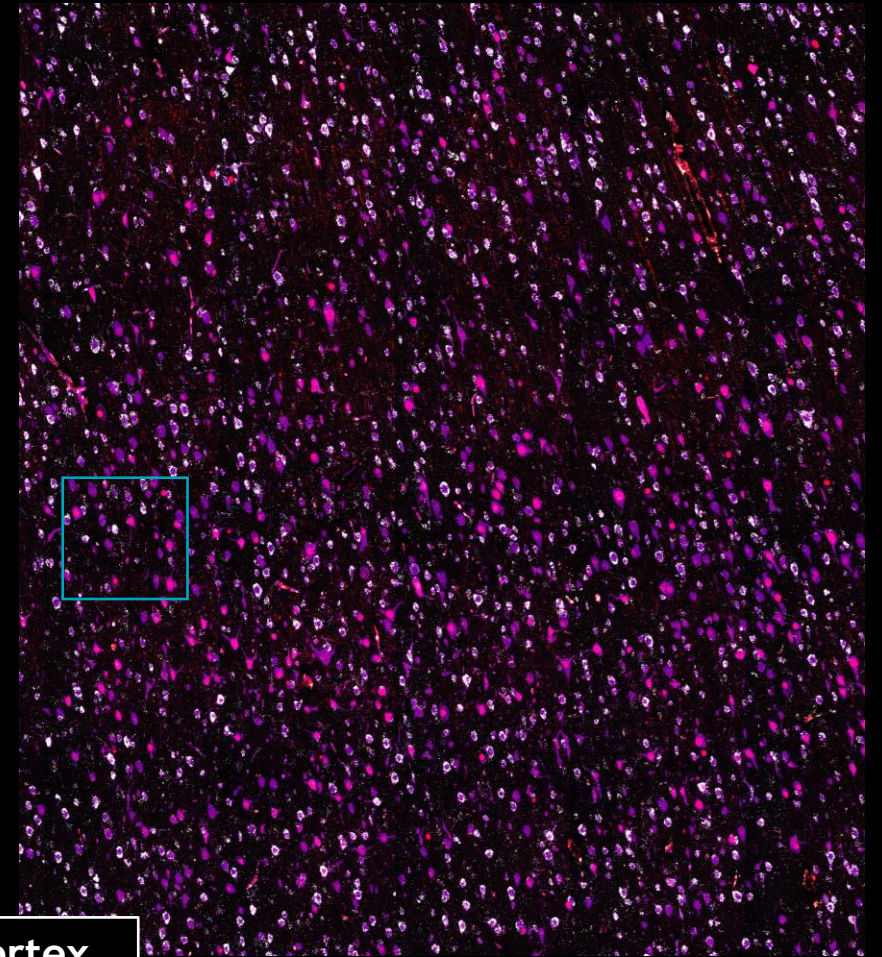
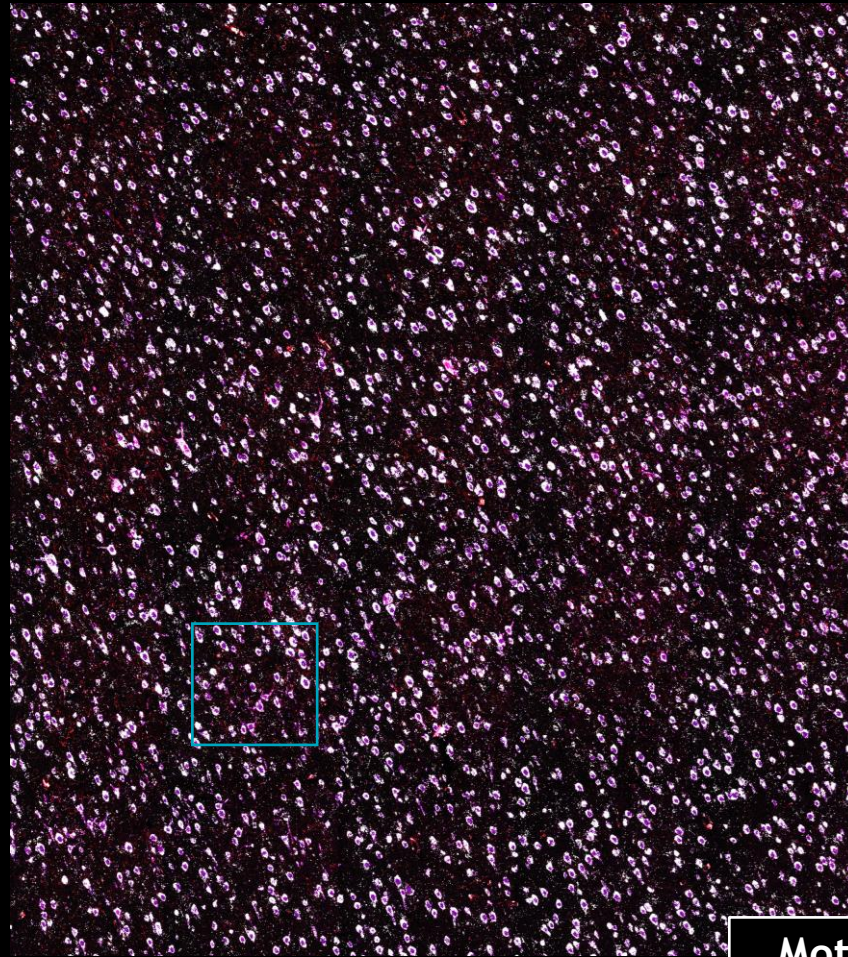
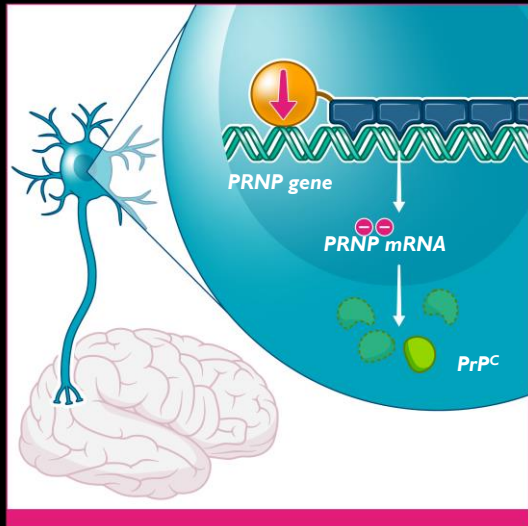


# STAC-BBB mediates ZFR expression and Prion repression in neurons

GFP  
Neurons (NeuN)  
Prion mRNA

Vehicle Control

STAC-BBB



Motor cortex



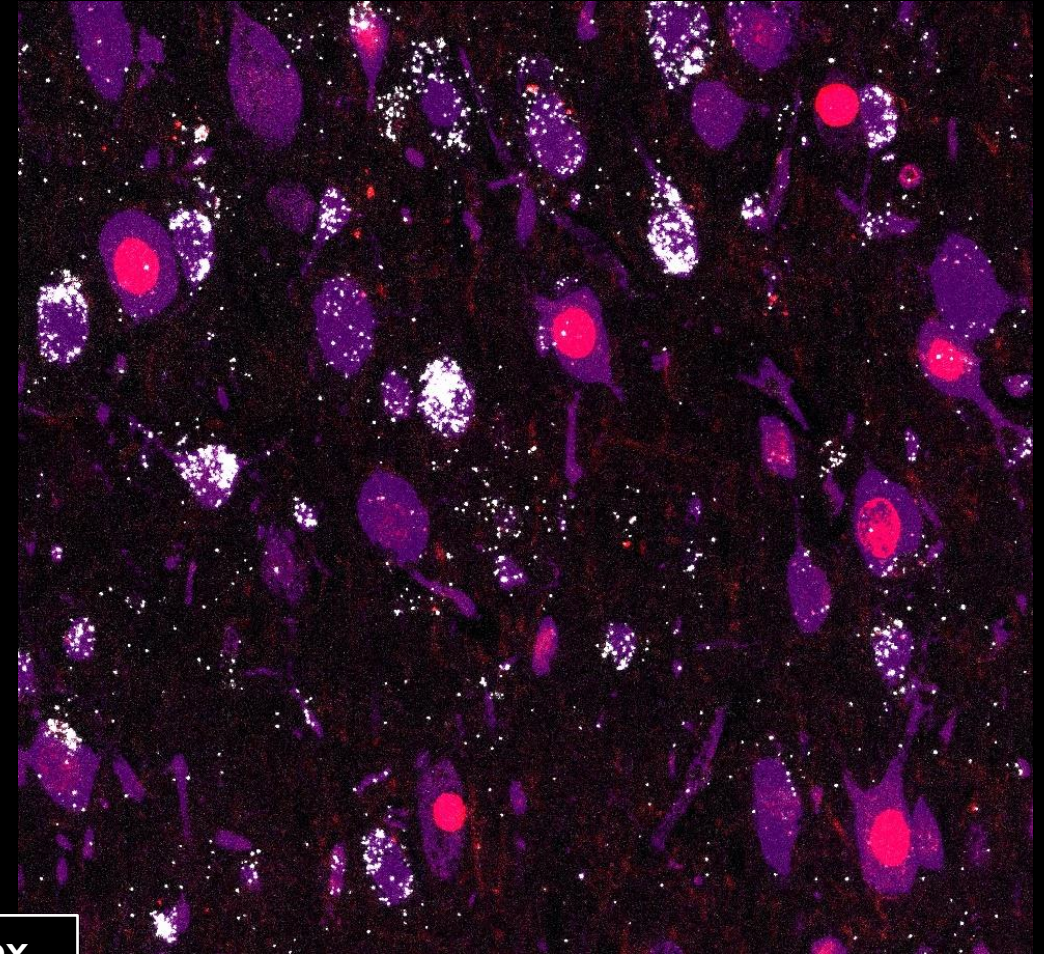
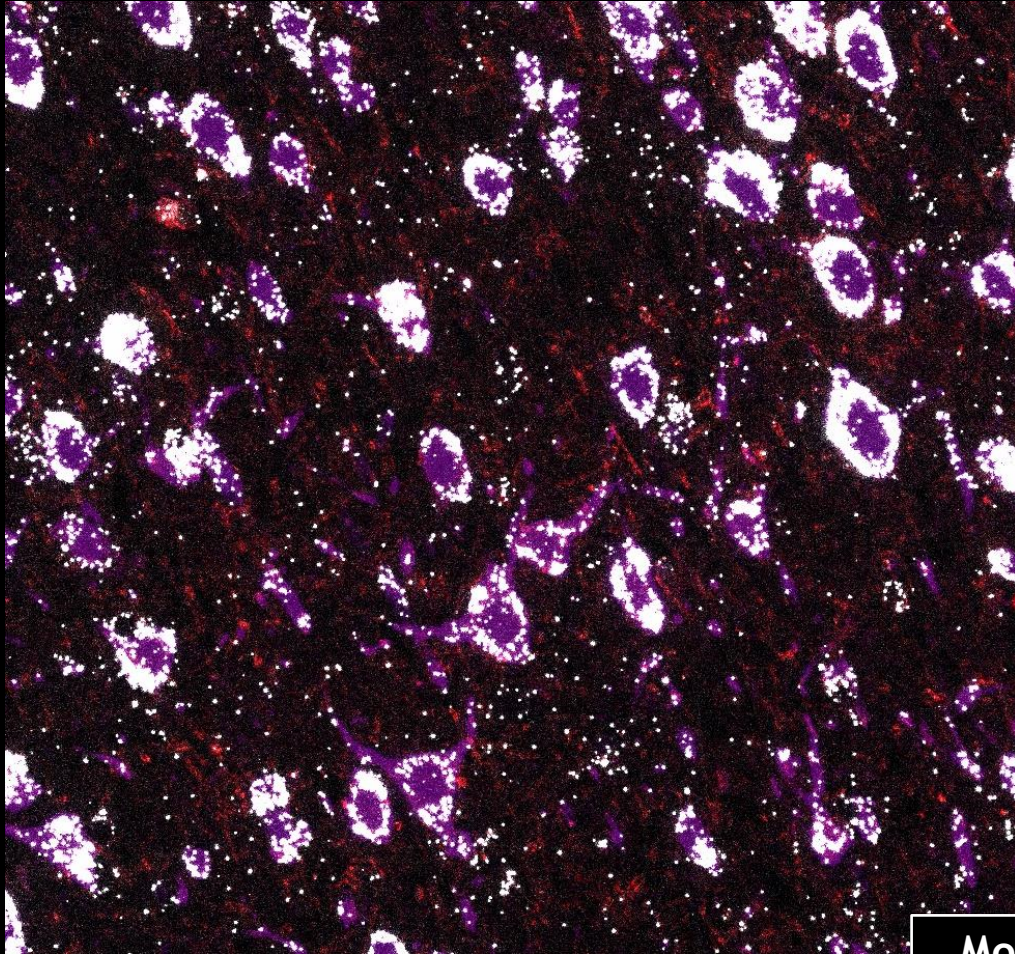
# STAC-BBB mediates ZFR expression and Prion repression in neurons

GFP  
Neurons (NeuN)  
Prion mRNA

Vehicle Control

STAC-BBB

Motor cortex



Motor cortex



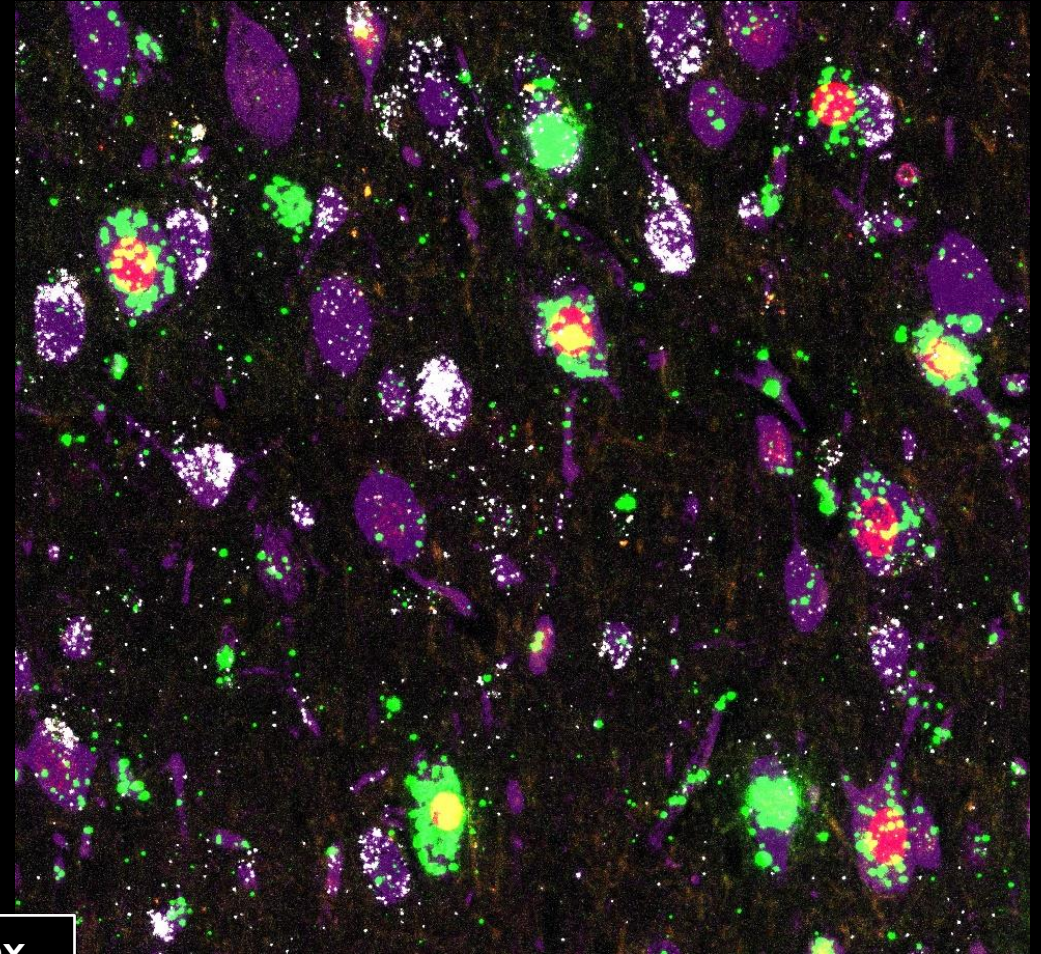
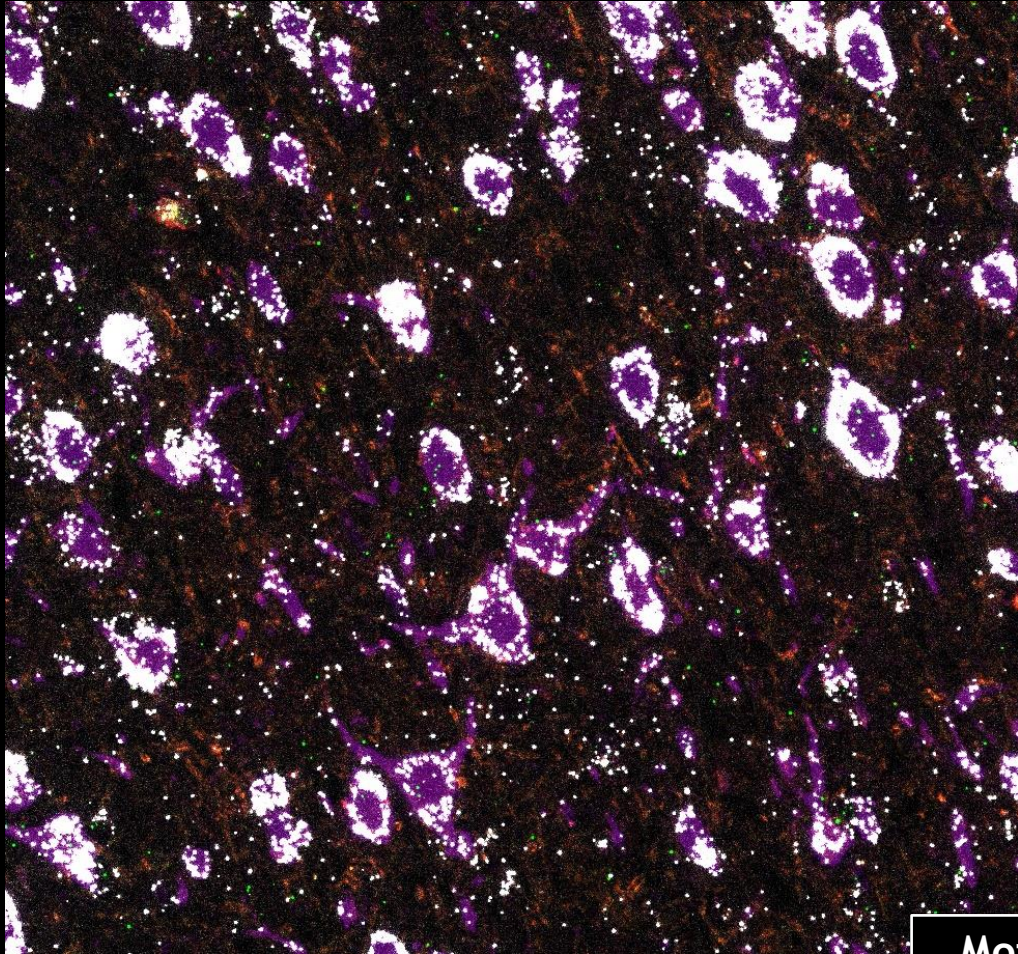
# STAC-BBB mediates ZFR expression and Prion repression in neurons

**GFP**  
**Neurons (NeuN)**  
**Prion mRNA**  
**ZFR mRNA**

Vehicle Control

STAC-BBB

Motor cortex



Motor cortex



# Individual evaluation of STAC-BBB capsid with zinc finger cargo

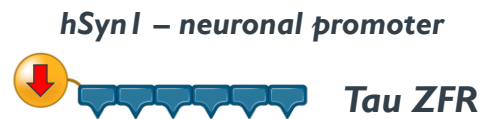
**Objective: Evaluate Tau clinical lead ZFR with STAC-BBB at multiple dose levels.**

## Capsid

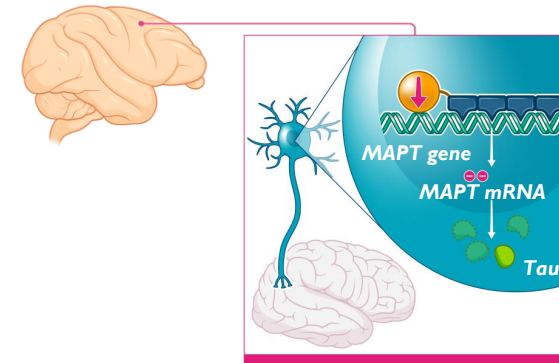


STAC-BBB

## Cargo



## Therapeutic Approach



## Dose & Duration



5E12 vg/kg  
2E13 vg/kg  
1E14 vg/kg



4-week study

## Species



*Cynomolgus*  
*Macaque*  
N=3 per group

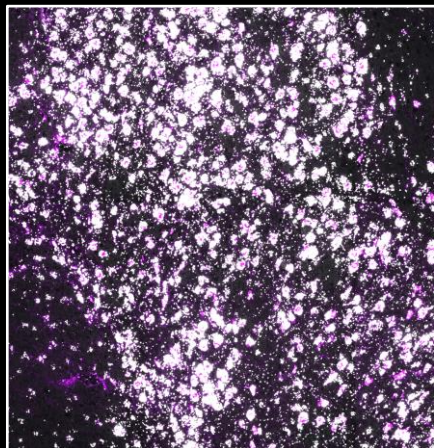
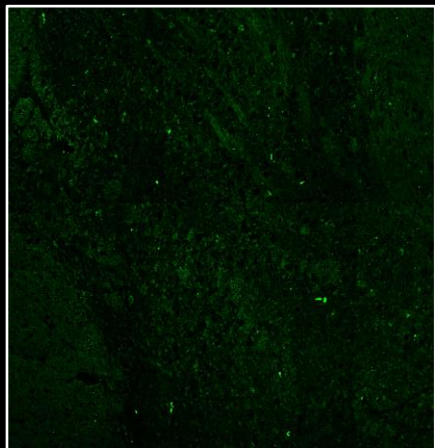
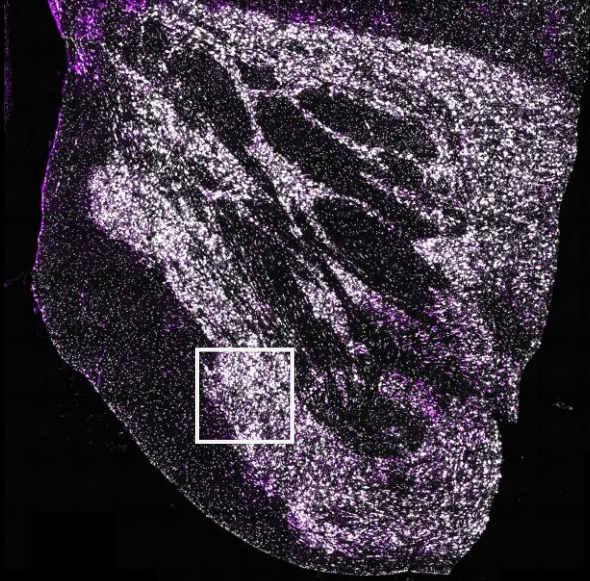
## Endpoints

- RNAscope images
- Molecular analyses

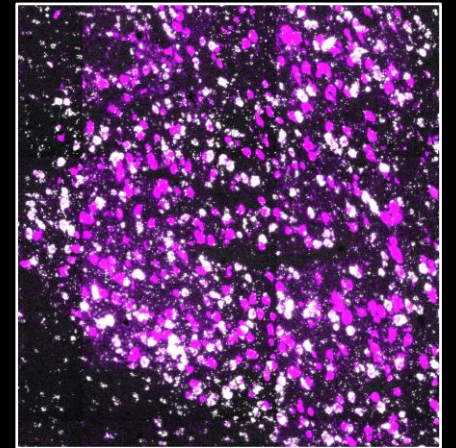
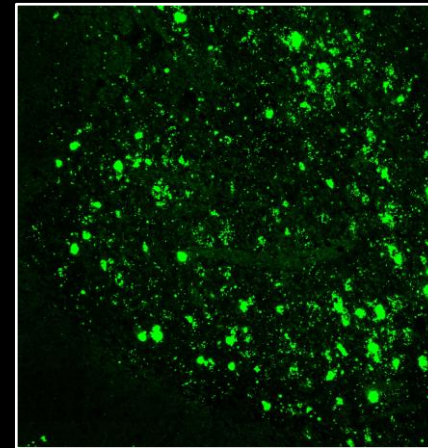
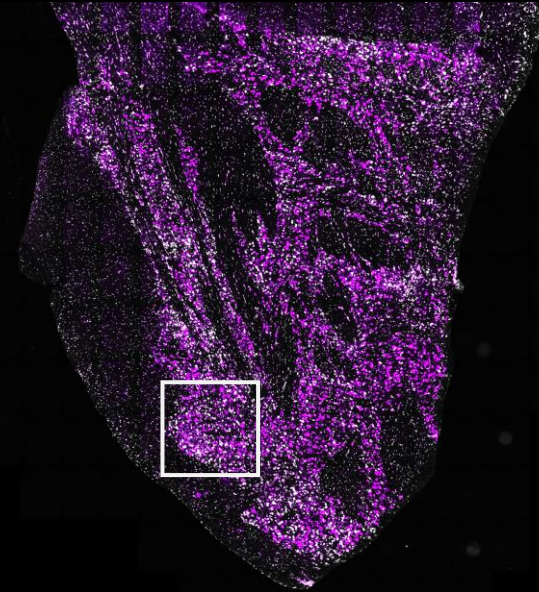
# STAC-BBB mediates robust repression of neuronal tau in the pons

ZFR mRNA  
Neurons (NeuN)  
Tau mRNA (MAPT)

Vehicle Control



STAC-BBB

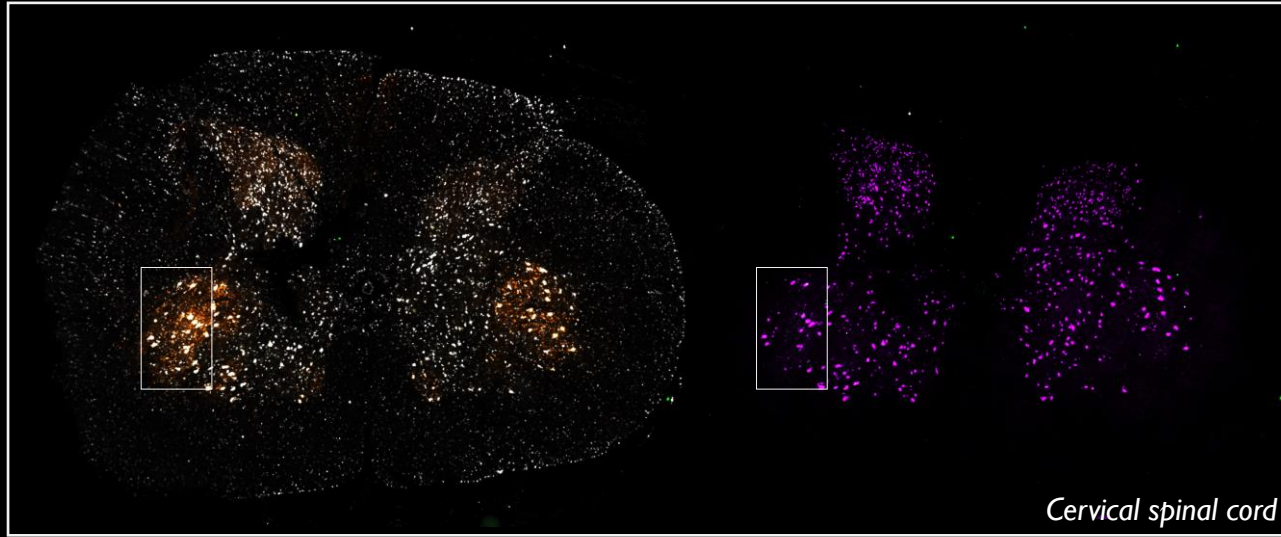




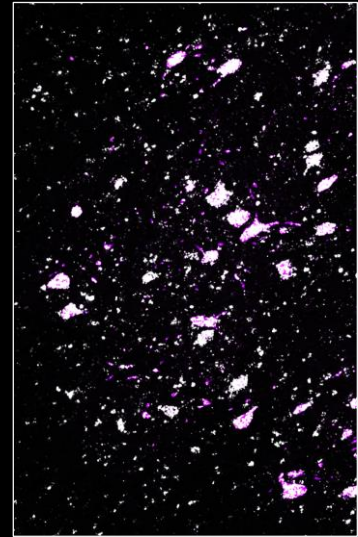
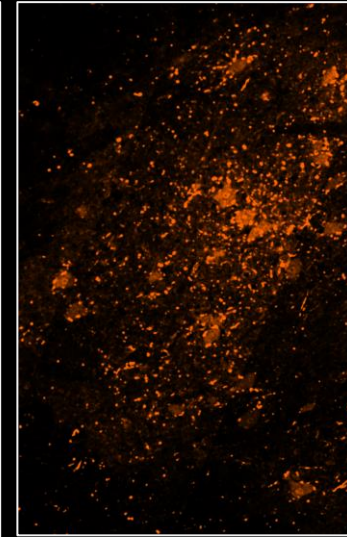
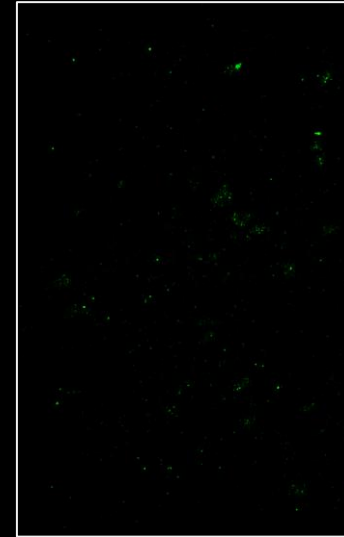
# STAC-BBB mediates ZFR expression and tau repression in ChAT+ motor neurons in the spinal cord

ZFR mRNA  
Tau mRNA  
Neurons (NeuN)  
ChAT+ neurons

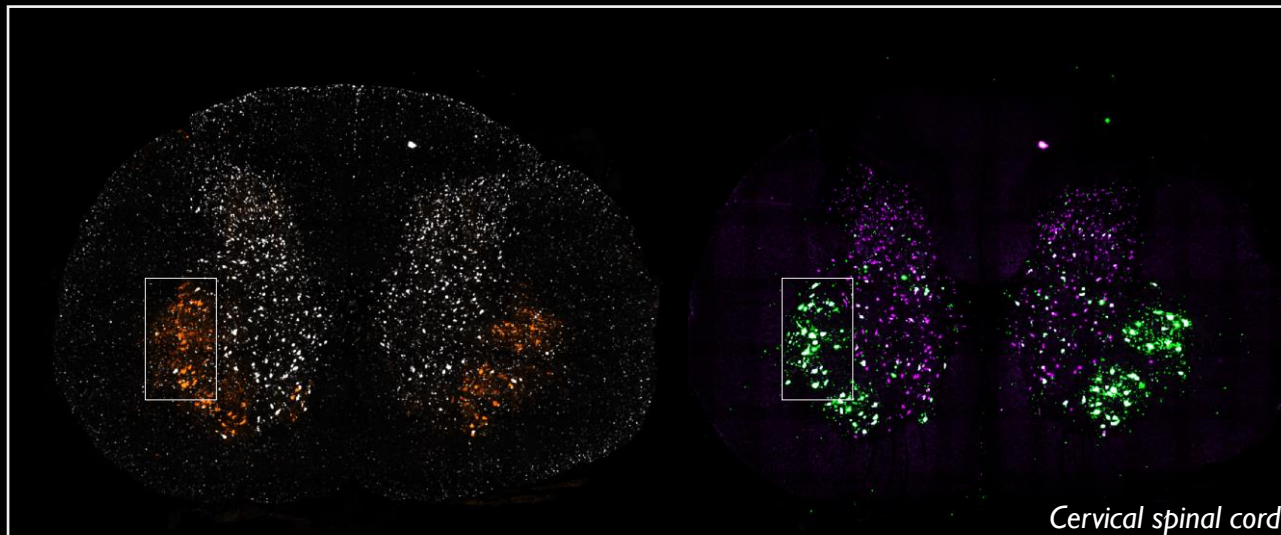
Vehicle Control



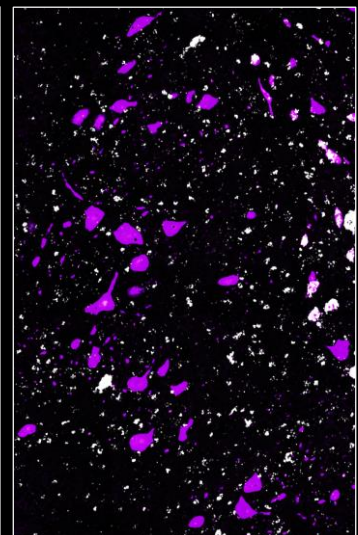
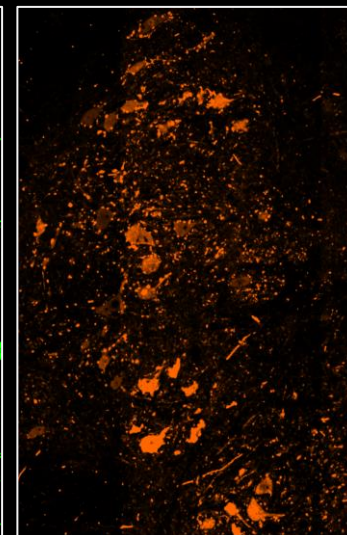
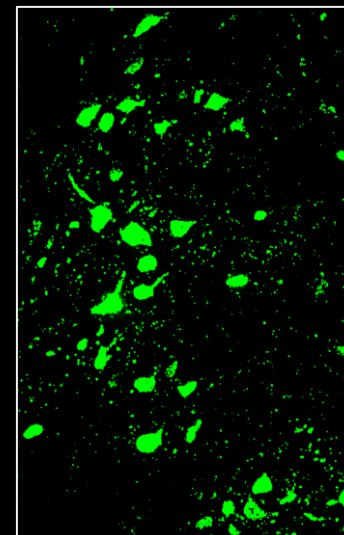
Cervical spinal cord



STAC-BBB



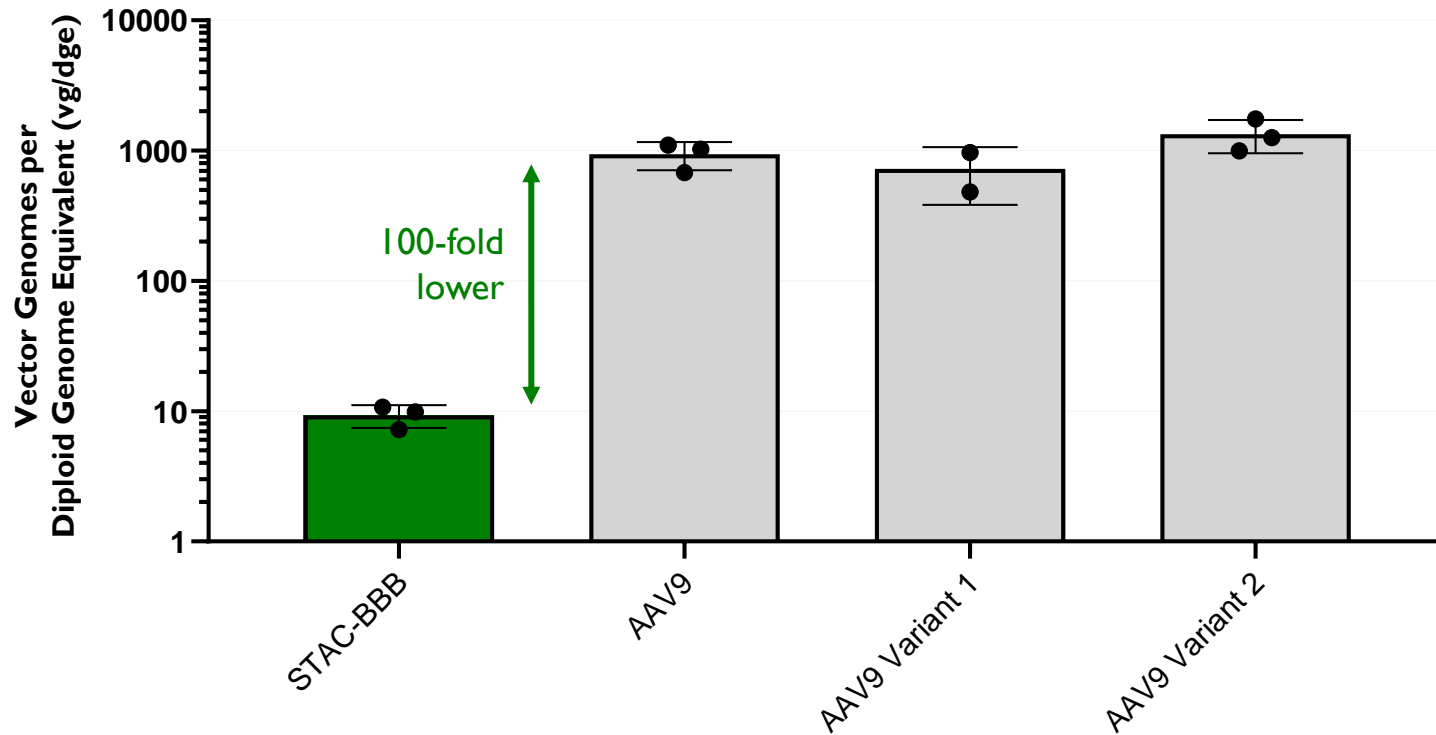
Cervical spinal cord



Percentage of ChAT+NeuN+ motor neurons transduced in the ventral horn:  
Cervical 95%, Thoracic 84%, Lumbar 98%

Multiplexed RNAscope ISH / IHC assay for NeuN, ChAT, MAPT mRNA, and ZFR mRNA  
1e14 vgl/kg dose, 28 days post administration

## STAC-BBB exhibits profound liver detargeting relative to AAV9



Comparison is relative to historical Sangamo studies, all data shown is for a 1e14 vg/kg dose

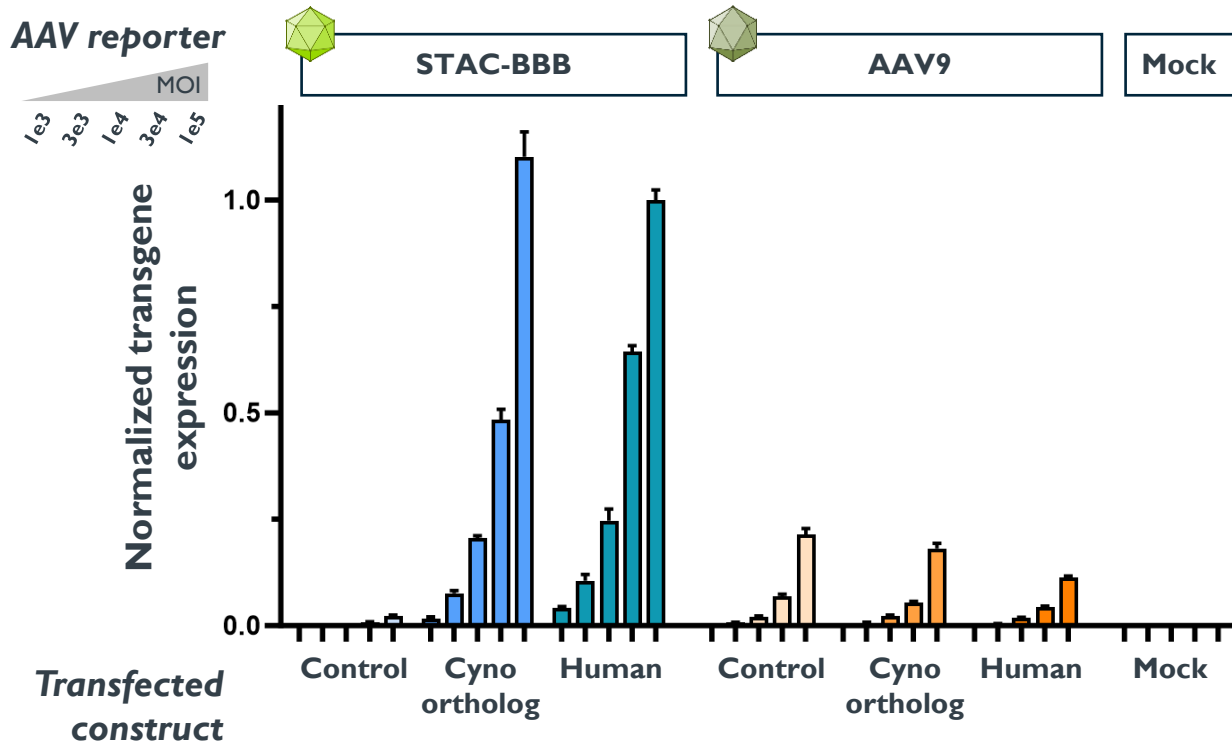
High liver exposure after intravenous administration is a limitation of conventional AAV serotypes including AAV9

STAC-BBB achieves efficient CNS delivery while maintaining low peripheral exposure in liver and dorsal root ganglia (DRG)

This is the ideal profile for a CNS-targeted capsid

# Overexpression of putative receptor confers a gain-of-function for STAC-BBB transduction *in vitro*

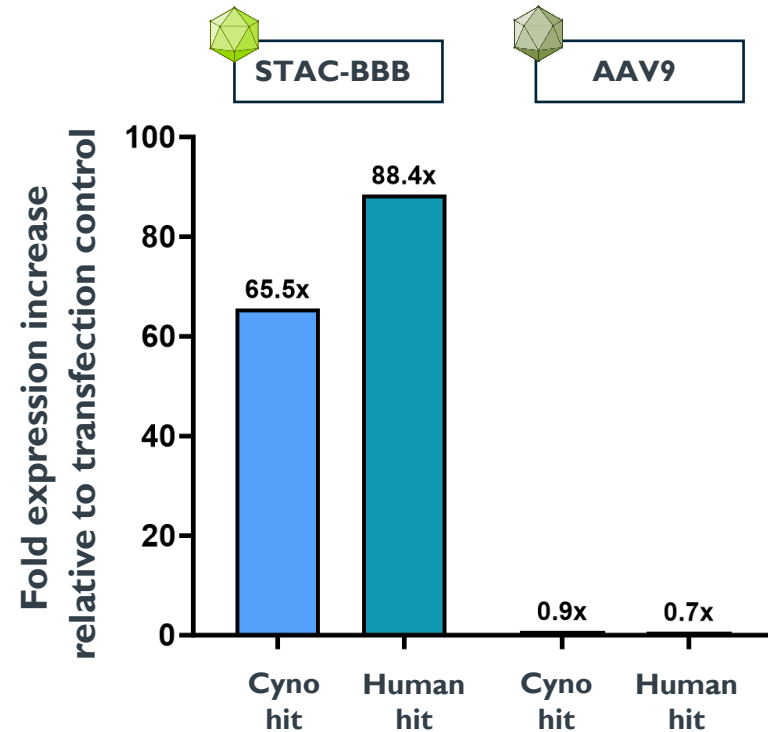
## Transgene expression – RT-qPCR



HEK293 cells

Transgene expression evaluated 48 hr post transduction

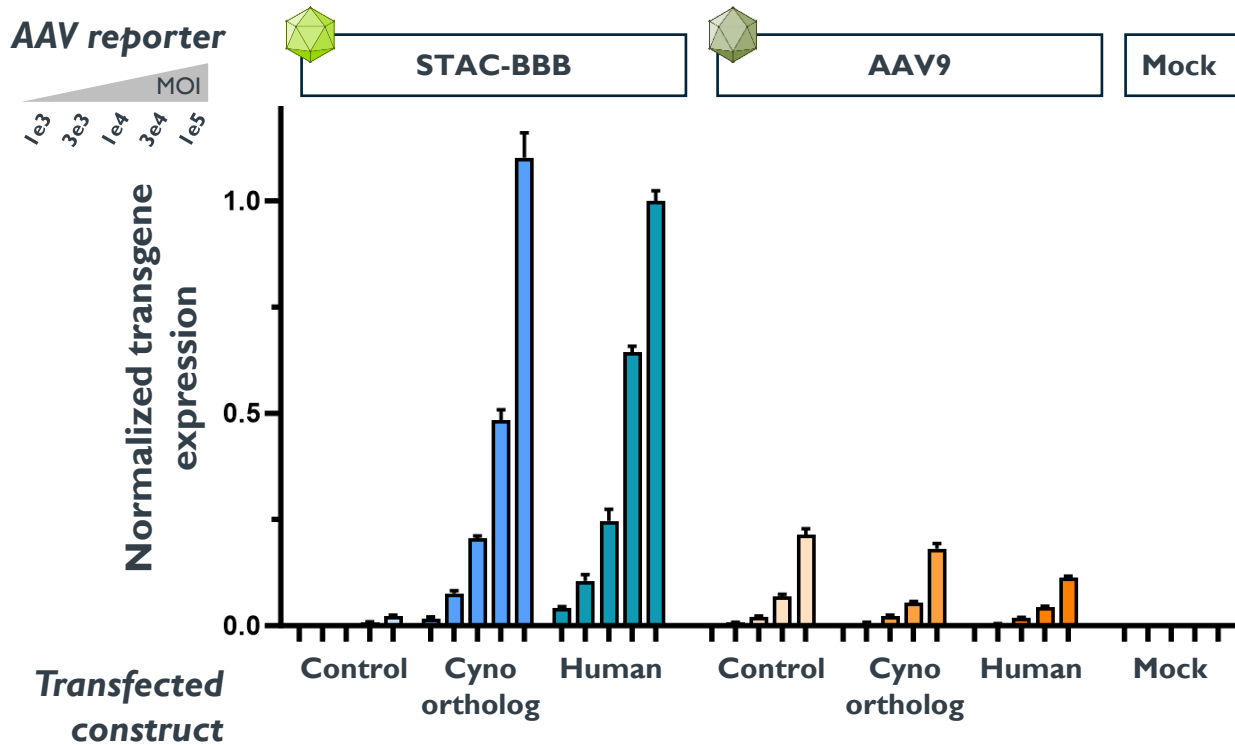
## Fold increase relative to control



A nonlinear regression model was used to interpolate relative transgene expression values for each capsid-construct condition. These values were then scaled to the transfection control value for each capsid.

# Overexpression of putative receptor confers a gain-of-function for STAC-BBB transduction *in vitro*

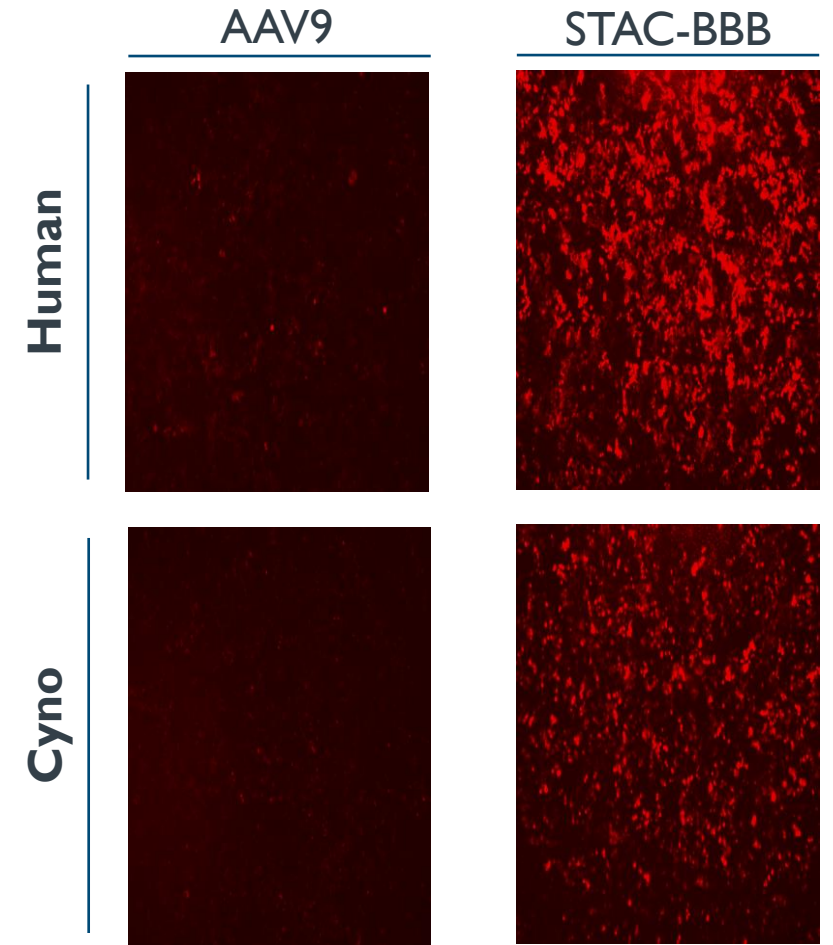
## Transgene expression – RT-qPCR



HEK293 cells

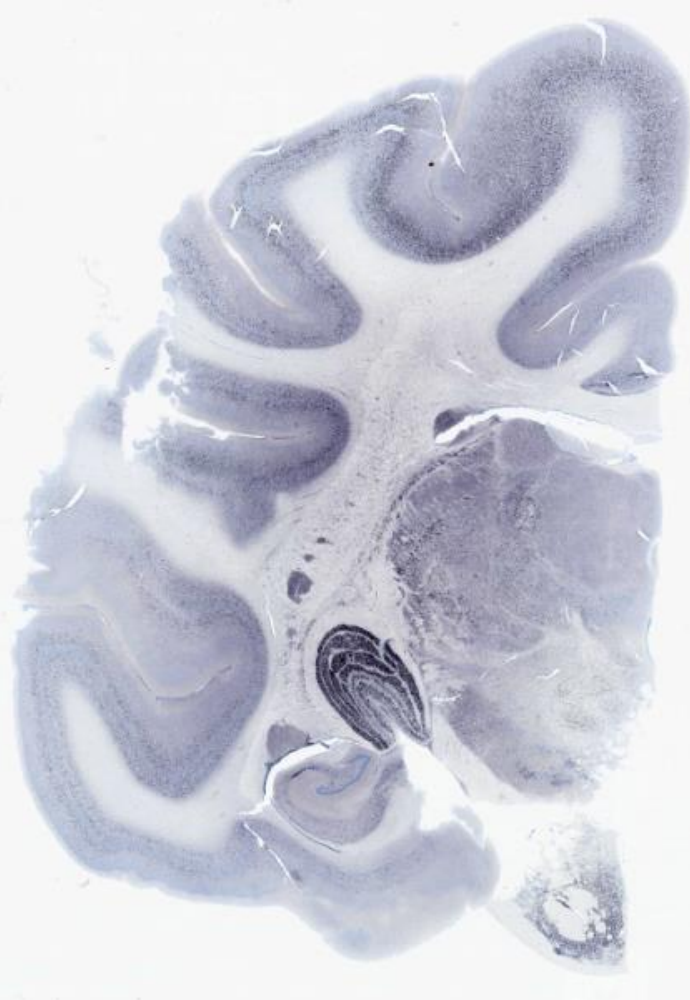
Transgene expression evaluated 48 hr post transduction

## Detection of fluorescent reporter





# STAC-BBB exhibits ideal characteristics of a blood-brain barrier penetrant capsid



- ✓ Robust blood-brain barrier crossing and **widespread transduction** throughout the brain
- ✓ **700-fold** enrichment compared to the benchmark AAV9
- ✓ Appears to **primarily target neurons** regardless of promoter
- ✓ Results are **consistent across individual animals and groups**
- ✓ **Clear dose response** for both ZF expression and repression of the disease target throughout the brain
- ✓ Vector genome biodistribution is enriched in the CNS and **de-targeted from the DRG and the liver**
- ✓ STAC-BBB was **well-tolerated** with no clinical findings related to test article and no histopathology findings in brain, spinal cord, and liver at doses up to  $1 \times 10^{14}$  vg/kg
- ✓ We have successfully scaled up STAC-BBB manufacturing to 50L

# Additional Sangamo abstracts

Related to STAC-BBB and  
Capsid Engineering

Neurology pipeline  
programs

Innovation and new  
cargo technologies

## Wednesday

- **Restoration of Normal Gene and Protein Expression in Mouse and Human Disease Models of [SCN2A](#) Haploinsufficiency Using Zinc Finger Activators**
  - Jenny Hodges, #636
- **Zinc Finger Mediated Repression and Replacement of MFN2 Leads to the Rescue of Cellular Disease Phenotype in [CMT2A](#) Patient-Derived Cells**
  - Mohammad Samie, #637
- **A Zinc Finger Activator Platform to Restore Normal Gene & Protein Expression in Cellular Models of [Dravet](#) Syndrome**
  - Jenny Hodges, #642
- **Optimal Drug Product Presentation and [Container Closure](#) Selection for [AAV-Based](#) Genomic Medicines**
  - Madhura Som, #547
- **Highly Specific Zinc Finger Proteins with Synthetic Target Sites Enable [Self-Regulated Expression](#) of Dosage-Sensitive Transgenes**
  - Gillian Houlihan, #722

## Thursday

- **Development of Blood-Brain Barrier Penetrant AAVs through [Receptor-Targeted Capsid Engineering](#)**
  - David Ojala, #985
- **Whole CNS Human [Tau](#) Knockdown for the Potential Treatment of Alzheimer's Disease and Other Tauopathies**
  - Bryan Zeitler, #1126
- **Process and Formulation [Development](#) for a Novel Blood-Brain Barrier Penetrant AAV Capsid**
  - Taeho Kim, #1052
- **[SNCA](#) Gene Repression Mediated by Zinc Finger Repressors (ZFRs) as a Therapeutic Approach for Parkinson's Disease**
  - Andrew Young, #1120
- **[UBE3A](#) Gene Activation Mediated by Zinc Finger Activators (ZFAs) as a Therapeutic Approach for Angelman Syndrome**
  - Andrew Young, #1121
- **Directed Evolution of [Bxb1](#) for the Development of [Modular Integrases](#) (MInts)**
  - Sebastian Arangundy-Franklin, #192, 4:00PM, Ballroom 3
- **Unraveling Impact of Manufacturing Process-Related [Stresses on AAV](#) Stability, Aggregation, and DNA Release**
  - Saba Ghazvini, #1032

## Friday

- **A Highly Potent [Engineered AAV Capsid](#), STAC-150, Enables High-Throughput AAV Production and Arrayed Epigenetic Regulator Screening Directly in Cultured Neurons**
  - Patrick Dunn, #351, 5:15PM, Room 339-342
- **Epigenetic Regulation of Human [Prion](#) Expression as a Potential One-Time Treatment for Prion Disease**
  - Victoria Chou, #1616
- **SOD1 Gene Repression Mediated by Zinc Finger Repressors (ZFRs) as a Therapeutic Approach for [SOD1-Mediated ALS](#)**
  - Andrew Young, #1597
- **PMP22 Gene Repression Mediated by Zinc Finger Repressors (ZFRs) as a Therapeutic Approach for [CMT1A](#)**
  - Andrew Young, #1600
- **[Shank3](#) Gene Activation Mediated by Zinc Finger Transcriptional Activators (ZFA) as a Therapeutic Approach for Phelan-McDermid Syndrome**
  - Andrew Young, #1605
- **Development of a Robust [Zinc Finger Activation](#) Platform for Treatment of Neurological Disorders**
  - Irene Tan, #1609
- **Site-directed integration of large DNA sequences into endogenous sites in the human genome using engineered [Modular Integrases](#) (MInts)**
  - Frieder Fauser, #1680