# 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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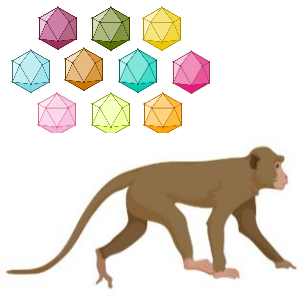
ASGCT 27th Annual Meeting – May 8, 2024

# Disclosure

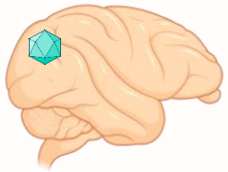
I am a full-time employee of Sangamo Therapeutics

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

AAV library administered to cyno macaques

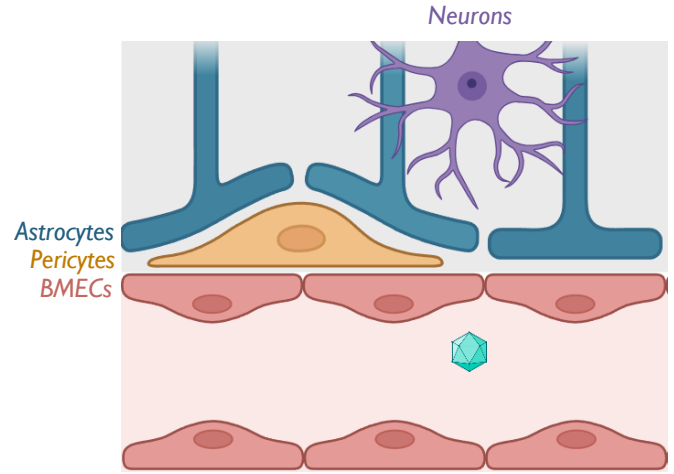


Capsids reach brain tissue

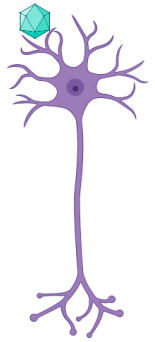


Traditional methods stop here

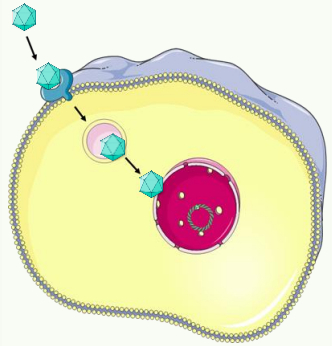
Overcome biological barriers



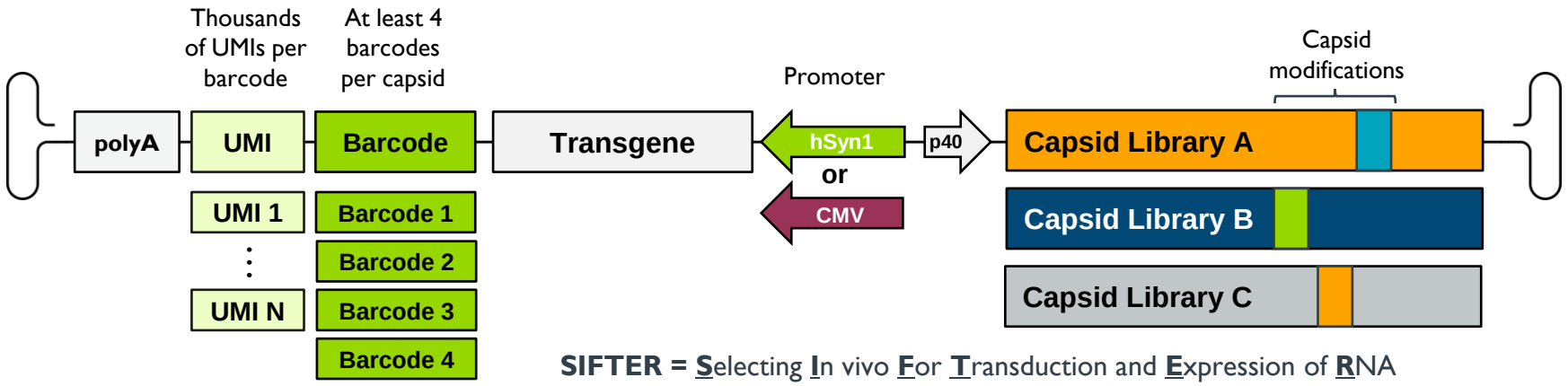

Transduce neurons



Express transgene in neurons

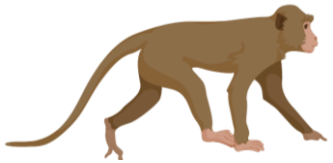
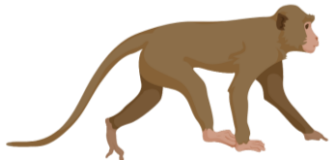
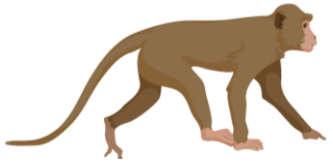
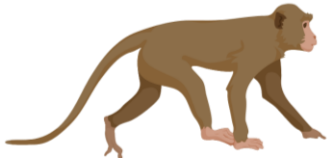


Delivery goal




**SIFTER = Selecting In vivo For Transduction and Expression of RNA**

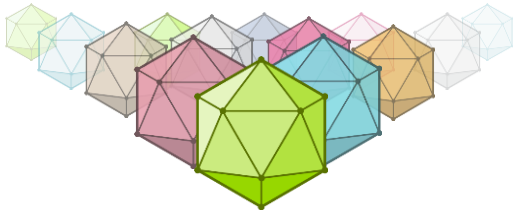
# Multiple library screening rounds were conducted to identify STAC-BBB

| Round 1 | Round 2 | Round 3 | Individual evaluation |
|---|---|---|---|
|  |  |  |  |
| **100 million** capsids screened. | **60,000** capsids with sequence replicates. Includes controls with known performance. | **1,260** capsids with at least 4 unique sequence replicates. Includes controls with known performance. | **Single best** capsid variant (STAC-BBB) with reporter gene or therapeutically-relevant ZF cargo. |

# Study design for Round 3 SIFTER library selection

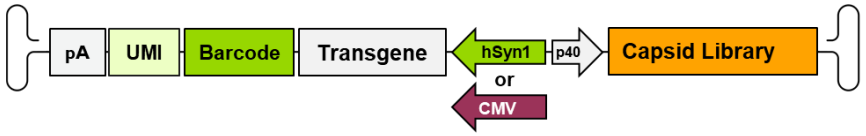
**Objective: Determine relative performance of 1,260 capsid variants and select lead variant for individual evaluation**

## Capsid



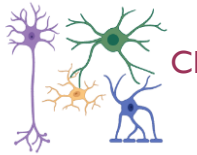
**1,260 Capsid Variant Library**

## Cargo




***Multiplexed promoters***

hSyn1 promoter (Neurons only)





CMV promoter (All cells)

## Dose & Duration

**5E13 vg/kg**

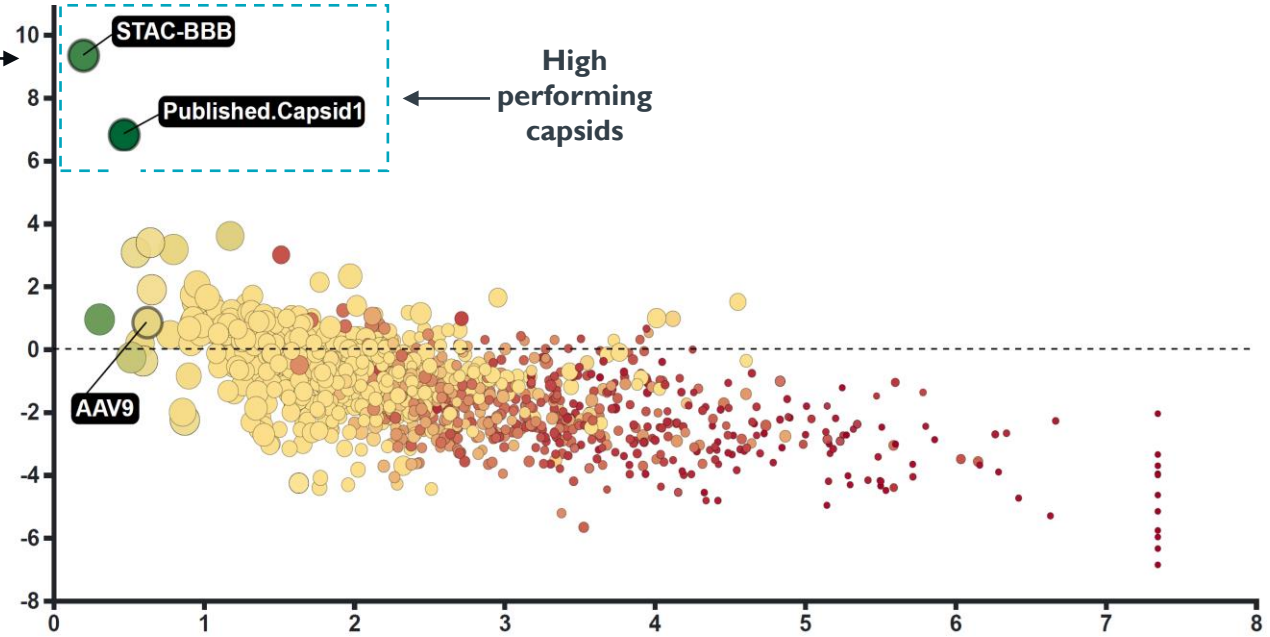
**3-week study**

## Species

***Cynomolgus Macaque***
**N=3 animals**

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

644-fold enrichment in brain →




← High performing capsids

**$Log_2$ Fold Change in Neuronal mRNA Expression (Y-axis)**: Enrichment score relative to the administered library **Larger value is better**

**Coefficient of Variation (X-axis)**: Variation in performance across tissue samples that were evaluated **Smaller value is better**

**Whole Brain Assessment**

hSyn1 neuron specific promoter

5112
4544
3976
3408
2840
2272
1704
1136
568
1

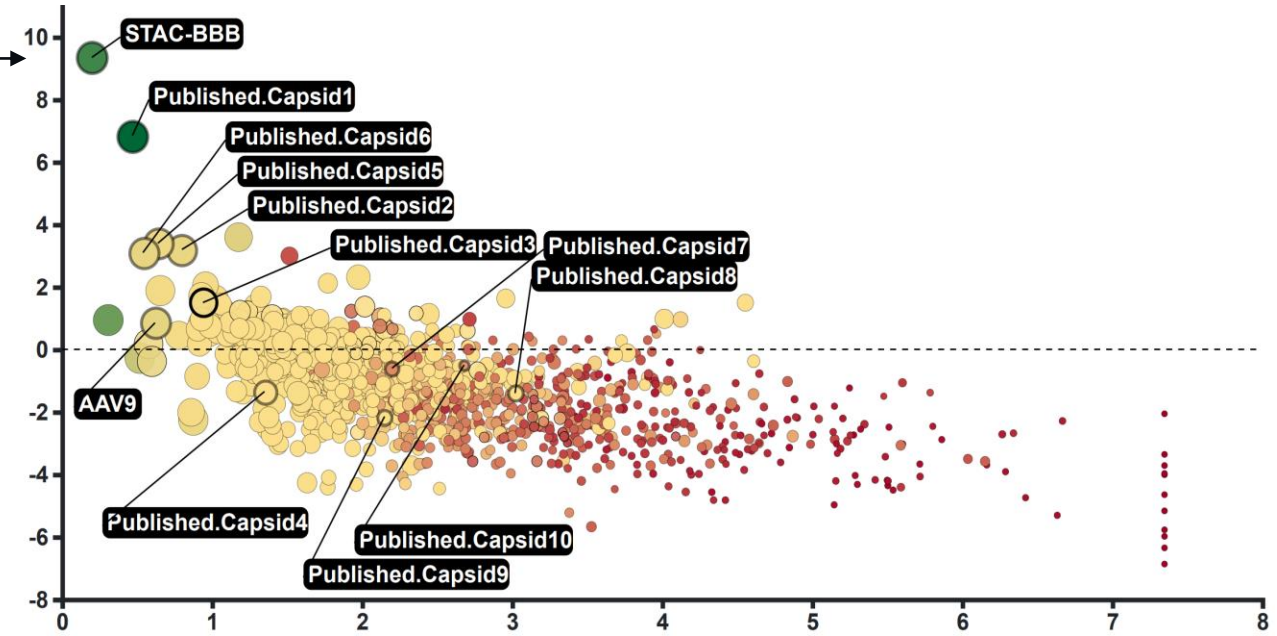
**Unique Molecular Identifier count (Color)**: Informs number of unique AAV transduction events **Darker green is better**

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

*Neuronal RNA expression (3-week study, hSyn1)*
*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

644-fold enrichment in brain →

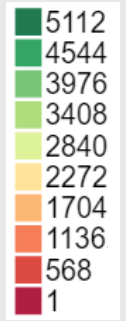



**$Log_2$ Fold Change in Neuronal mRNA Expression (Y-axis)**: Enrichment score relative to the administered library **Larger value is better**

**Whole Brain Assessment**

hSyn1 neuron specific promoter

**Coefficient of Variation (X-axis)**: Variation in performance across tissue samples that were evaluated **Smaller value is better**

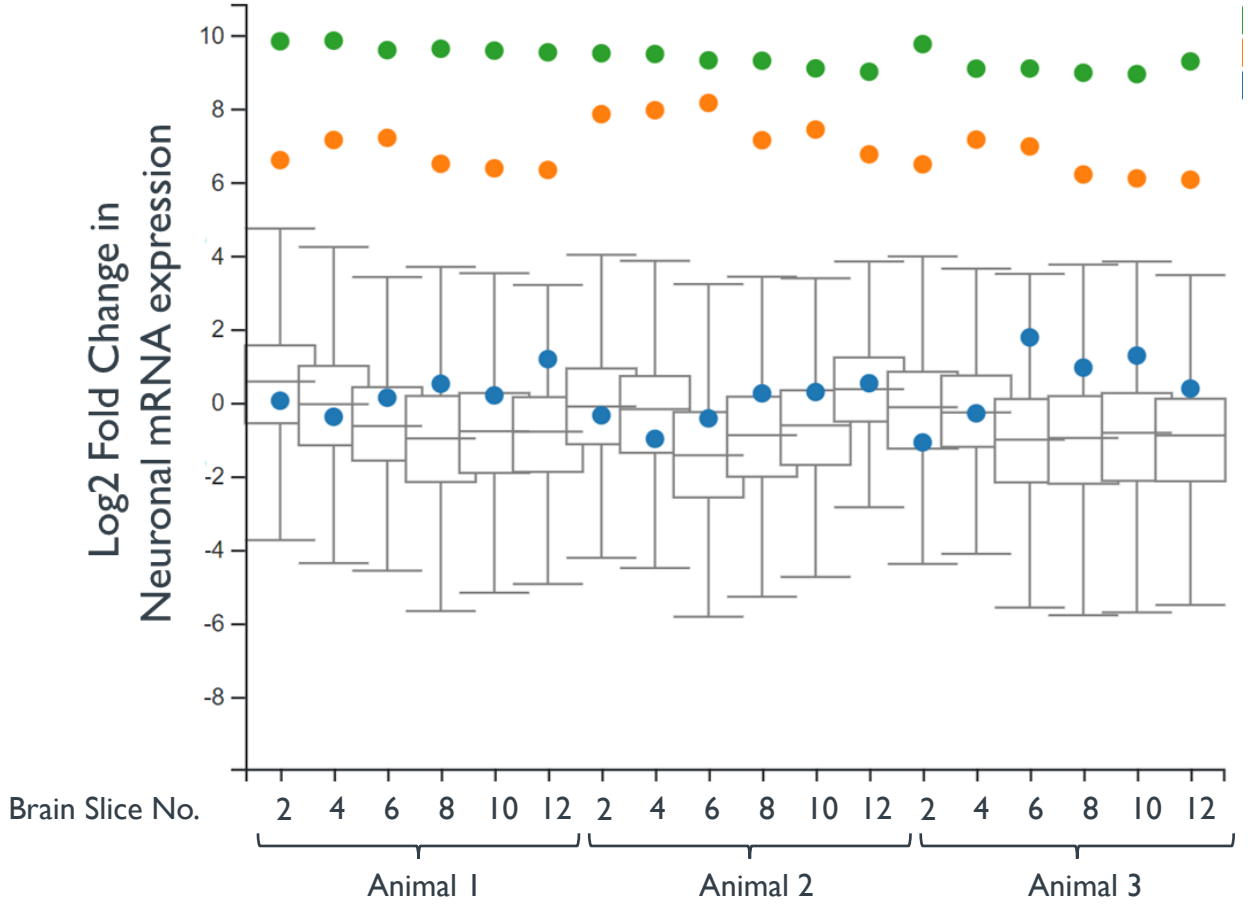


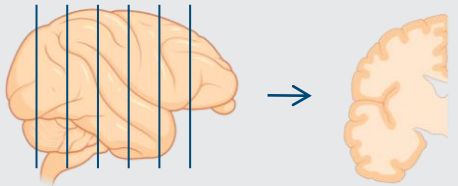

**Unique Molecular Identifier count (Color)**: Informs number of unique AAV transduction events **Darker green is better**

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

*Neuronal RNA expression (3-week study, hSyn1)*
*Data averaged from all three animals*

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



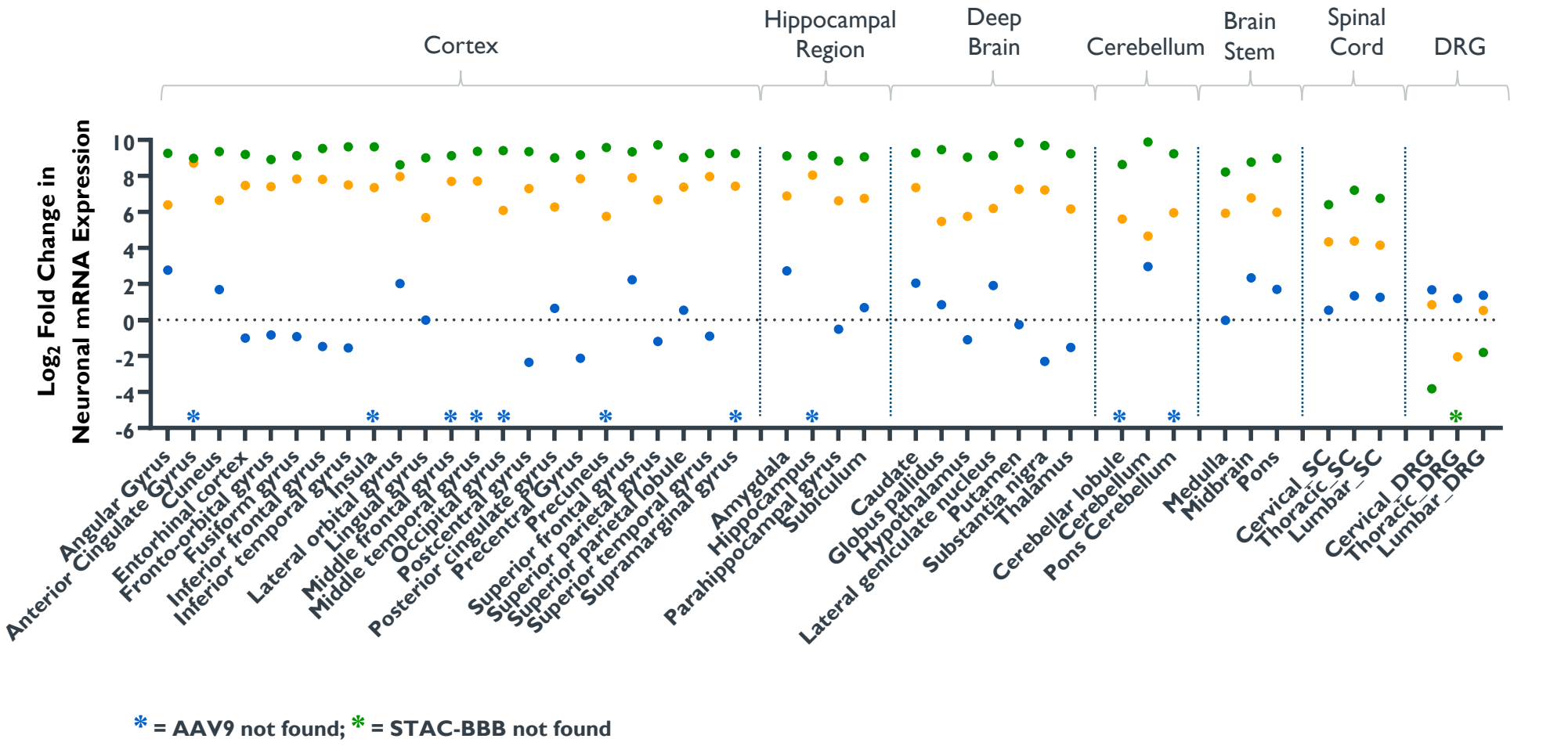
**Assessment across brain slices and animals**

- STAC-BBB
- Published capsid
- AAV9

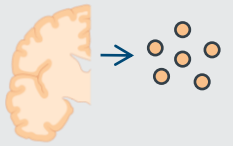
hSyn I neuron specific promoter

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

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




* = AAV9 not found; * = STAC-BBB not found



**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*

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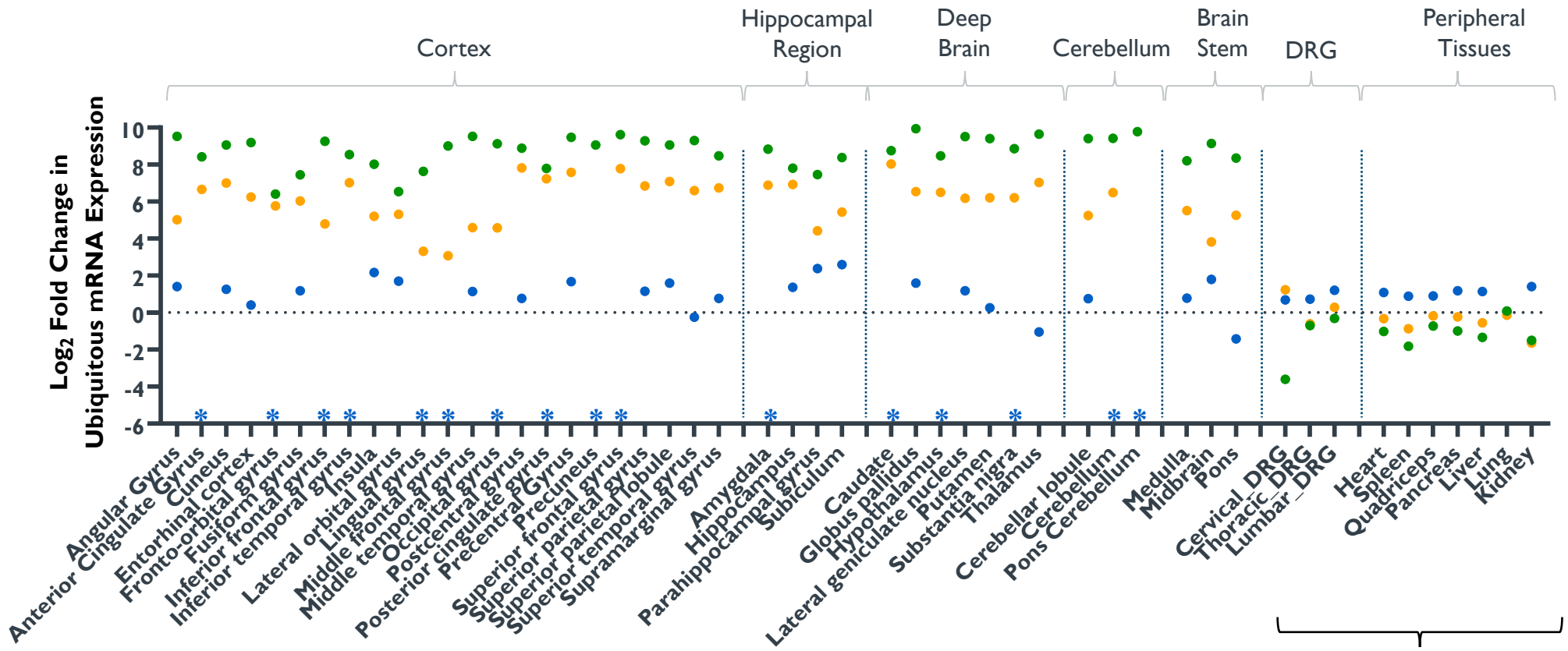
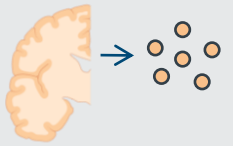
# STAC-BBB mediates higher ubiquitous mRNA expression in all CNS regions and is detargeted from DRG and peripheral tissues



150 brain punches analyzed

- STAC-BBB
- Published capsid
- AAV9

CMV ubiquitous promoter (All cells)

*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

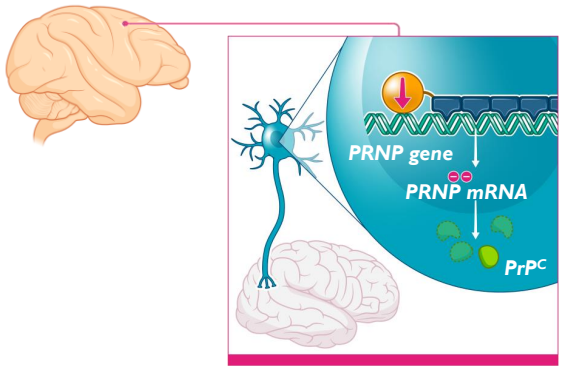
*CAG – ubiquitous promoter*

*Nuclear localized EGFP*

2A peptide

*Prion ZFR*

## Therapeutic Approach




## Dose & Duration

2E13 vg/kg

3-week study

## Species

*Cynomolgus Macaque*
N=3

## Endpoints

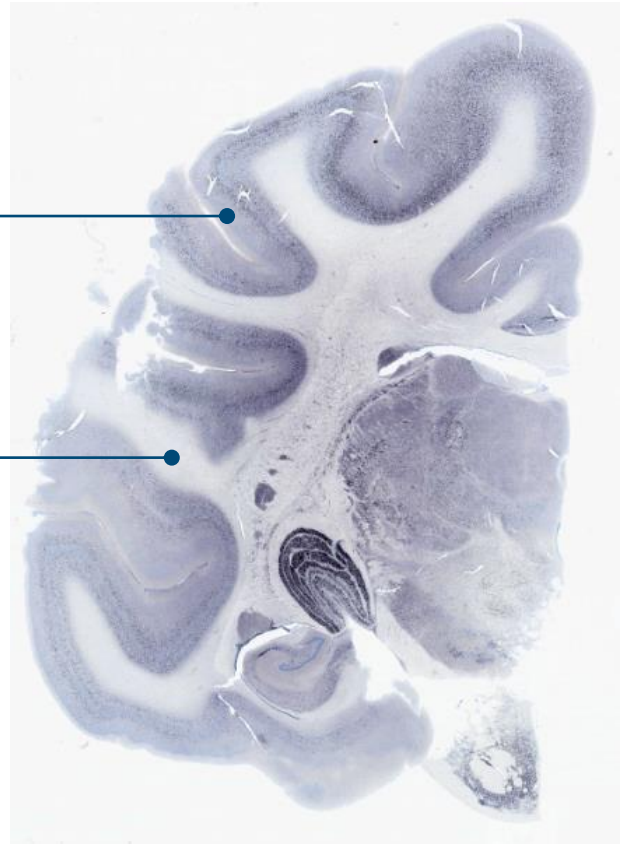
- Immunohistochemistry (GFP) images
- RNAscope images (ZFR/Prion)
- Molecular analyses

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

**Grey matter (cell bodies)**

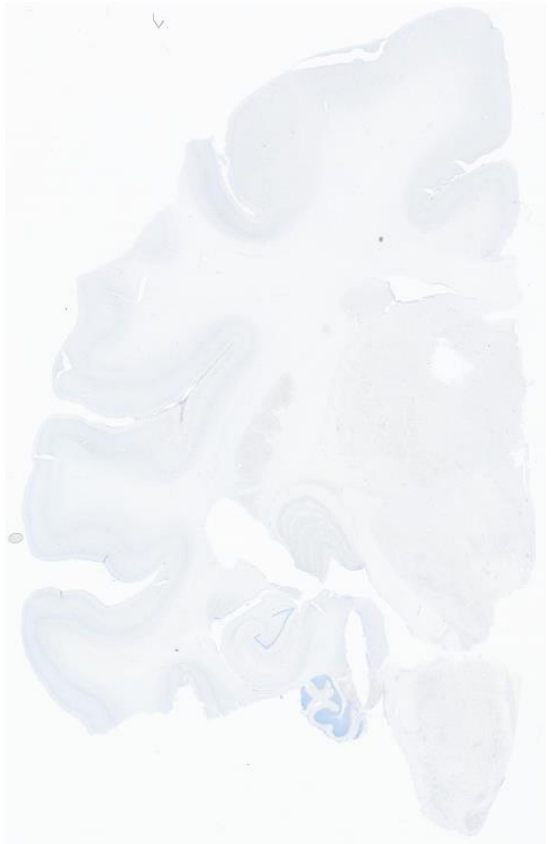
**White matter (nerve fibers)**

**STAC-BBB**
(Nuclear-localized GFP)



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

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



*Nissl staining (light blue):*

**All cell nuclei**

*Antibody labeling for green florescent protein (GFP) expression (black):*

**Cells transduced with STAC-BBB**

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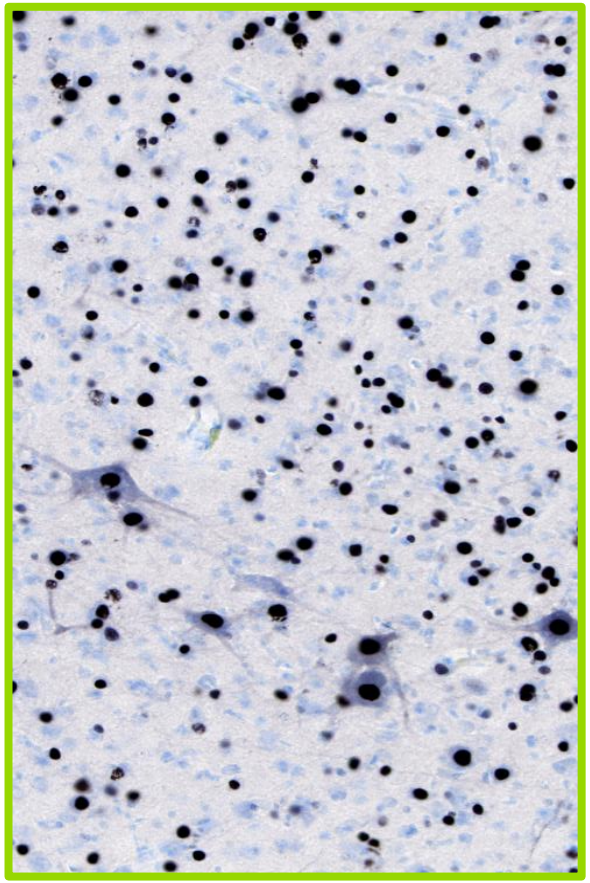
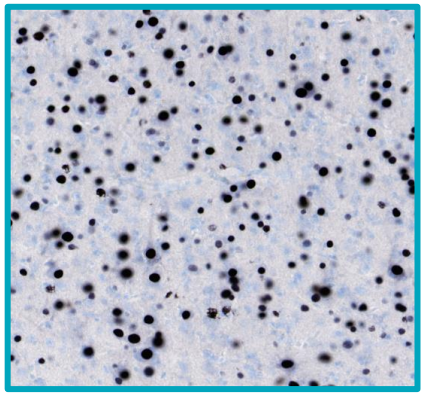
# STAC-BBB shows widespread neuronal transduction across all cortical regions

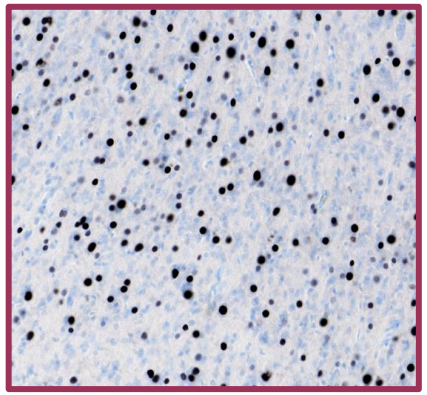
Precentral Gyrus (Motor Cortex)



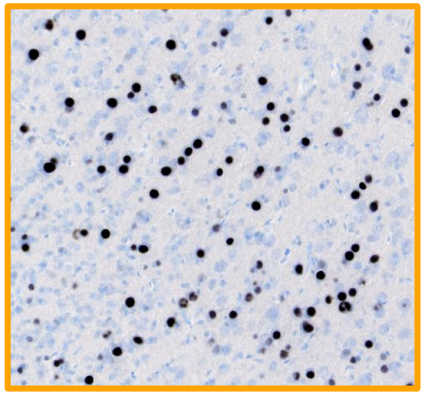
Postcentral Gyrus



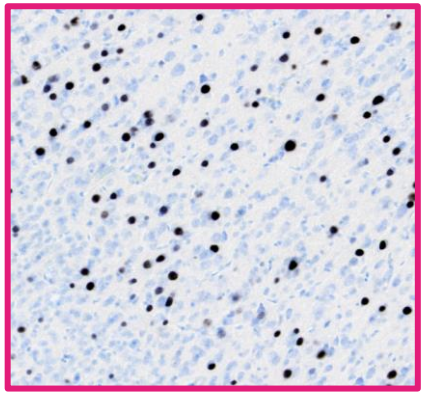
Superior Temporal Gyrus



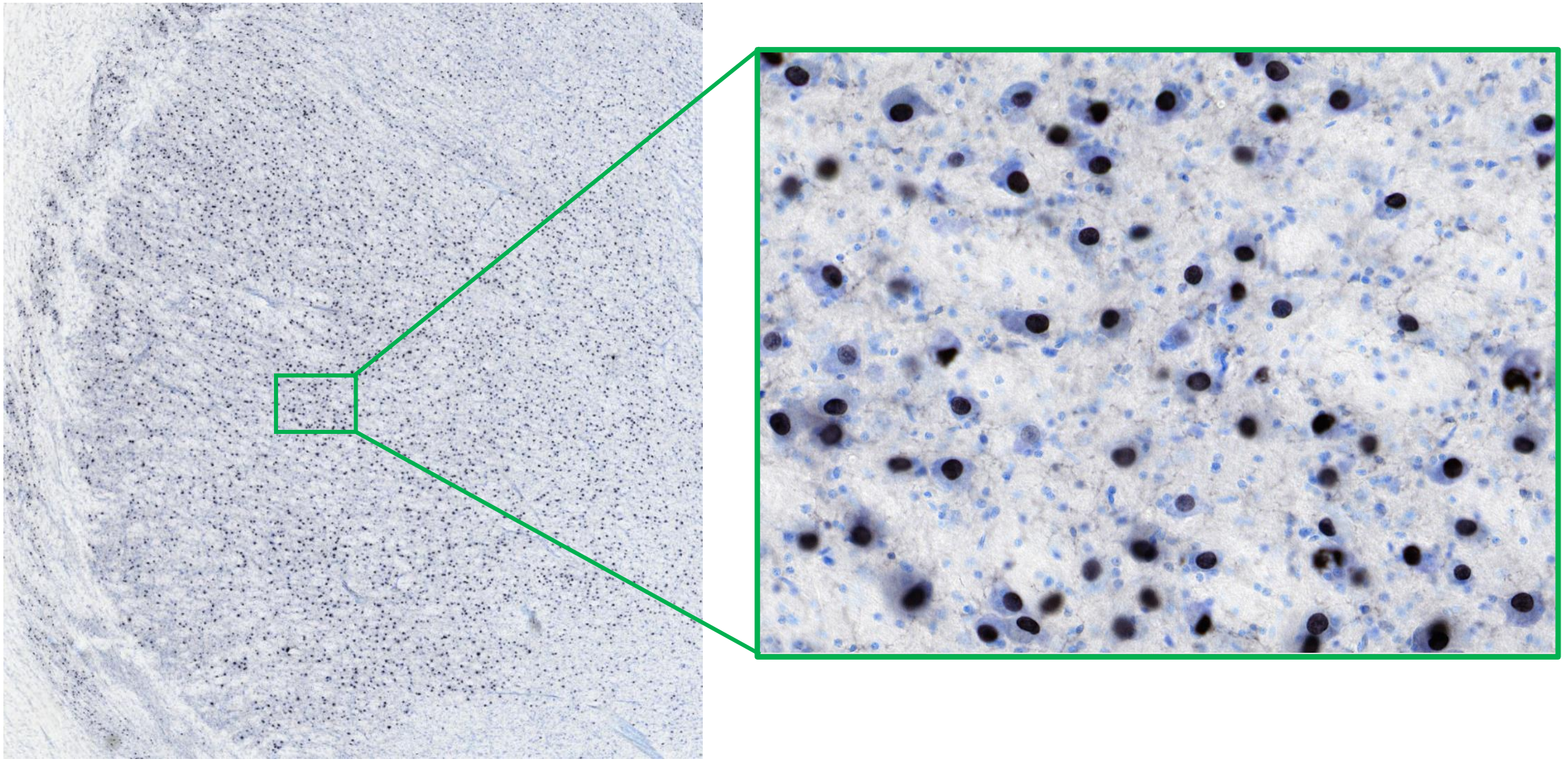
Middle Temporal Gyrus



Inferior Temporal Gyrus



# STAC-BBB mediates widespread neuronal transduction in the thalamus

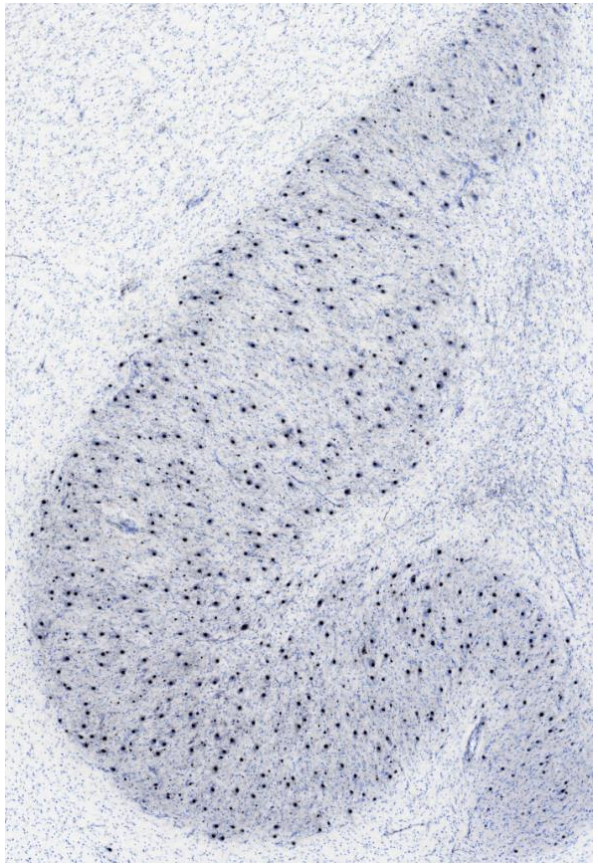


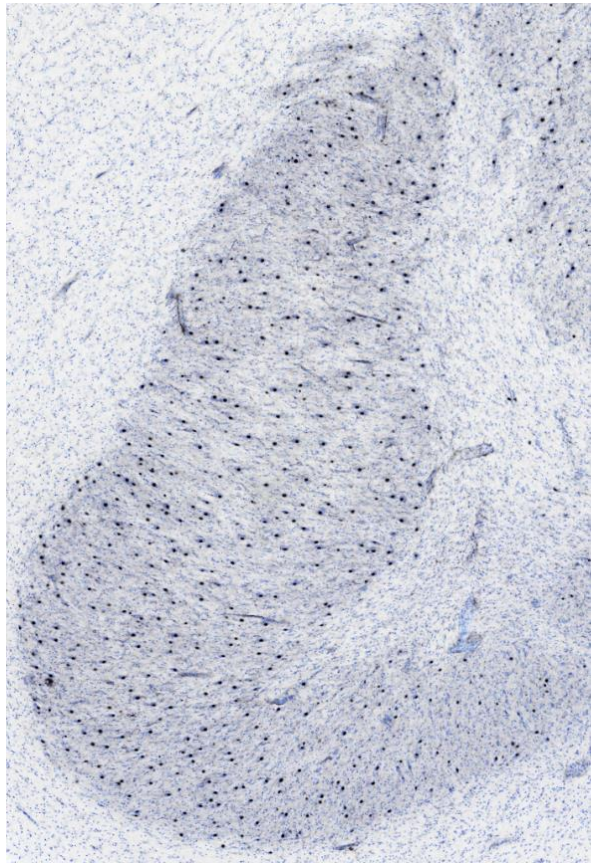
*2e13 vg/kg STAC-BBB, 19 days post administration*

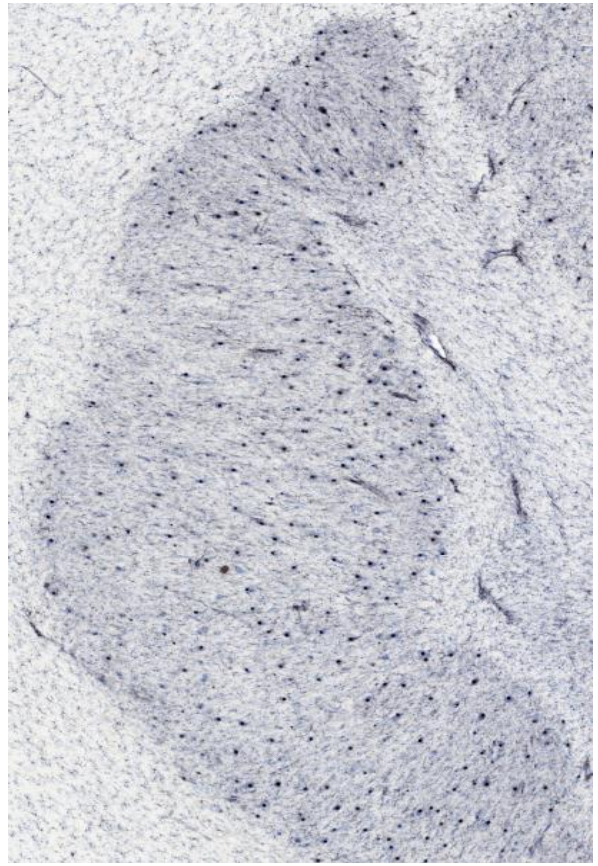
# STAC-BBB transduction is consistent across all animals

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

| NHP 1 | NHP 2 | NHP 3 |
|---|---|---|







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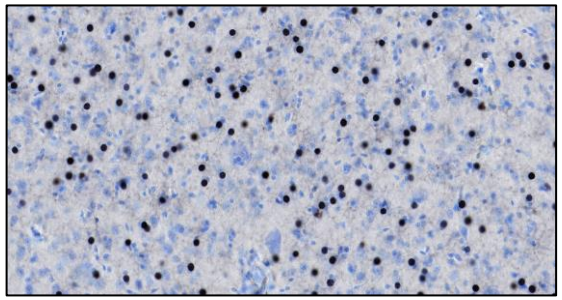
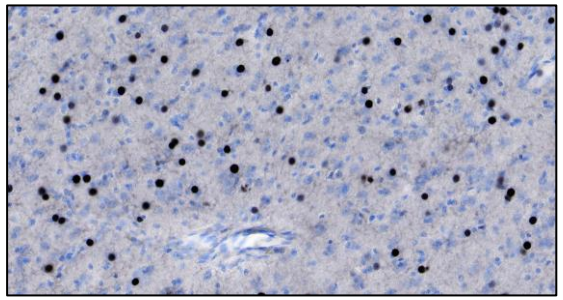
*2e13 vg/kg STAC-BBB, 19 days post administration*

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

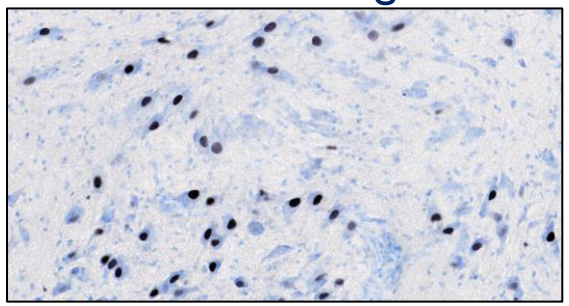
Putamen



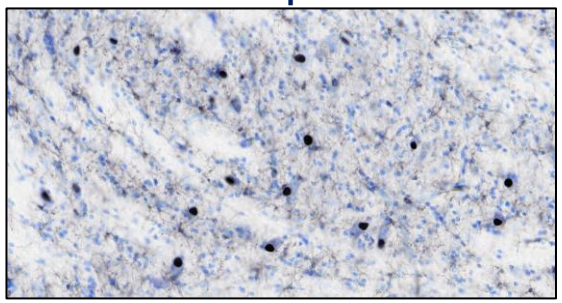
Caudate



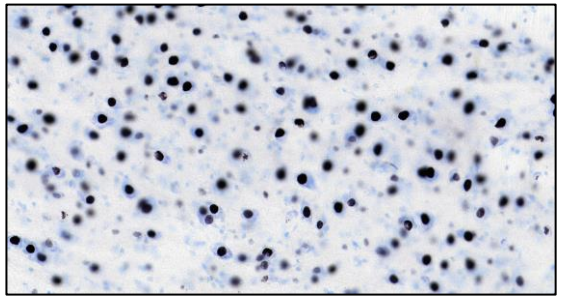
Substantia nigra



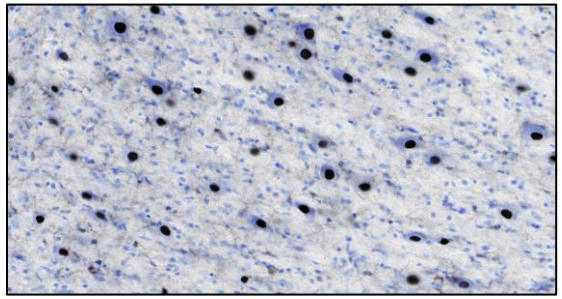
Globus pallidus



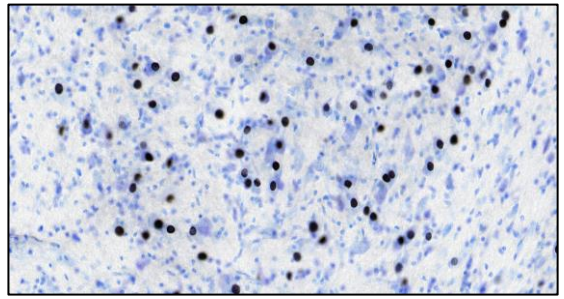
Pons



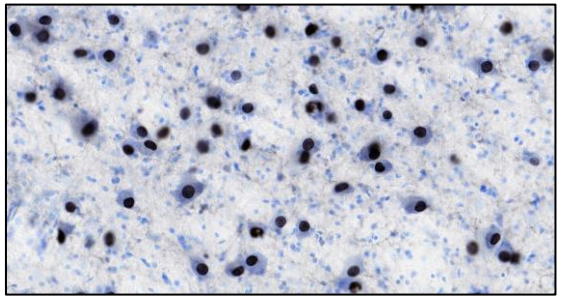
Dentate nucleus



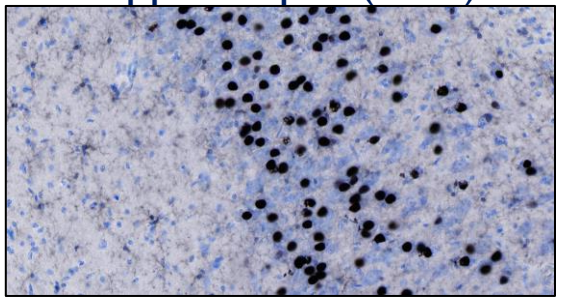
Cuneate nucleus



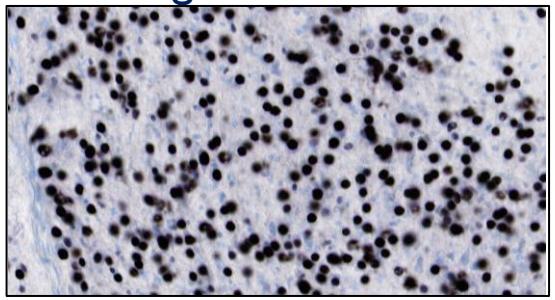
Thalamus



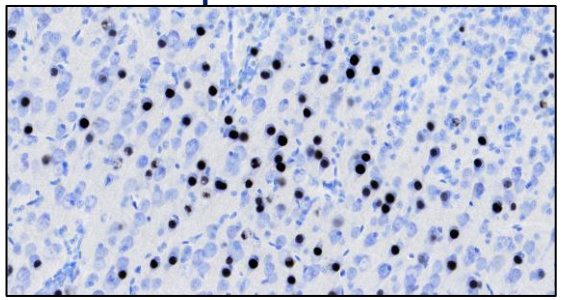
Hippocampus (CA2)



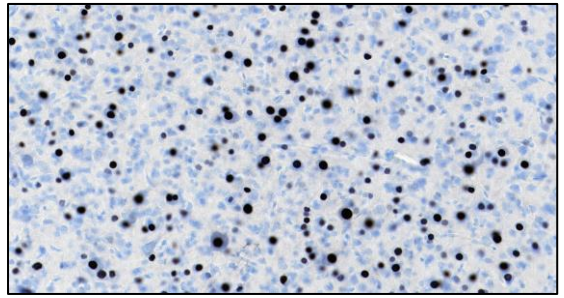
Lateral geniculate nucleus



Temporal cortex



Motor cortex

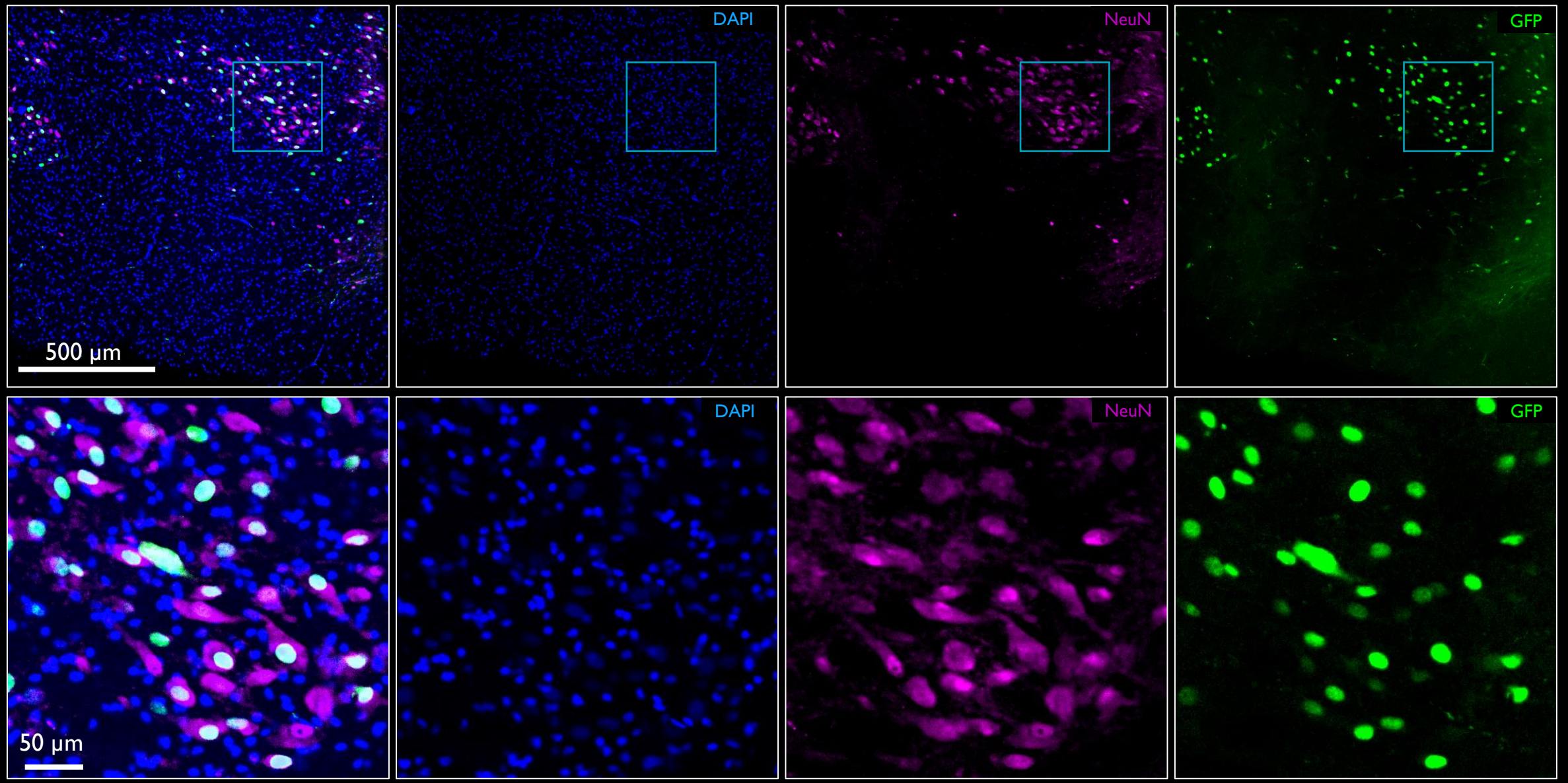


Sangamo THERAPEUTICS

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

# STAC-BBB transduces neurons in the substantia nigra

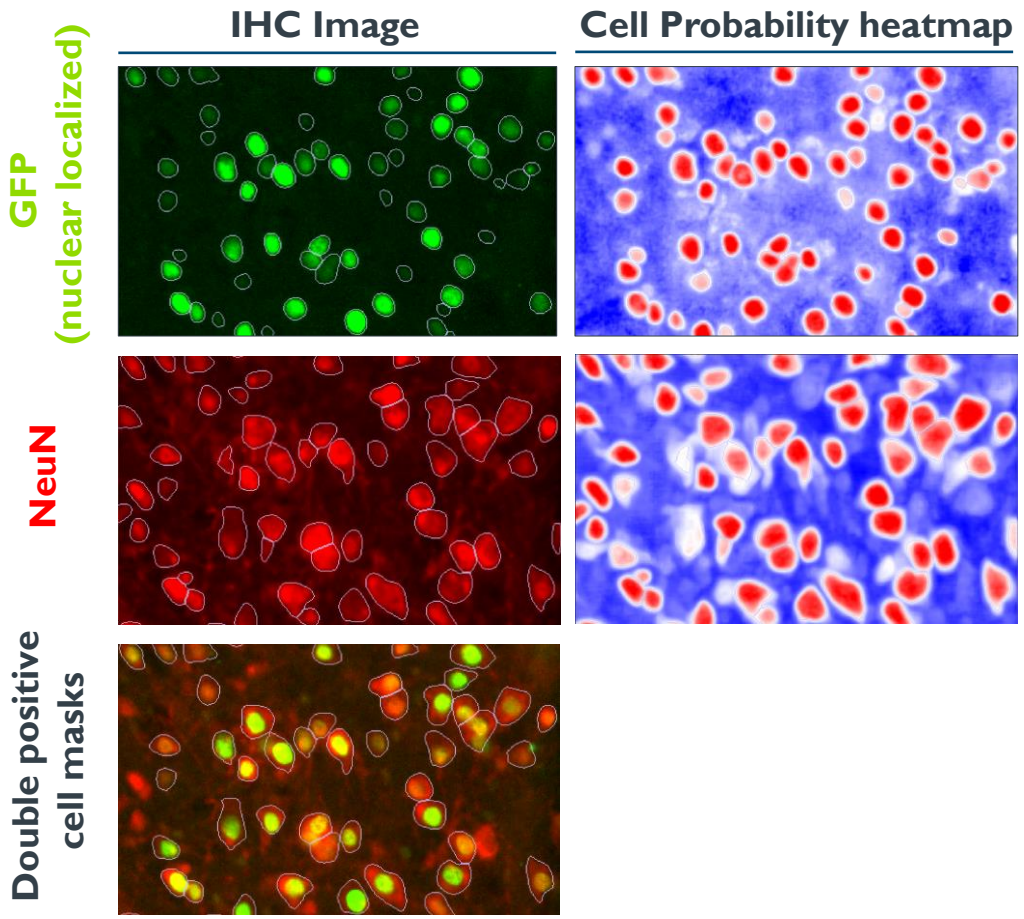
DAPI
NeuN
GFP



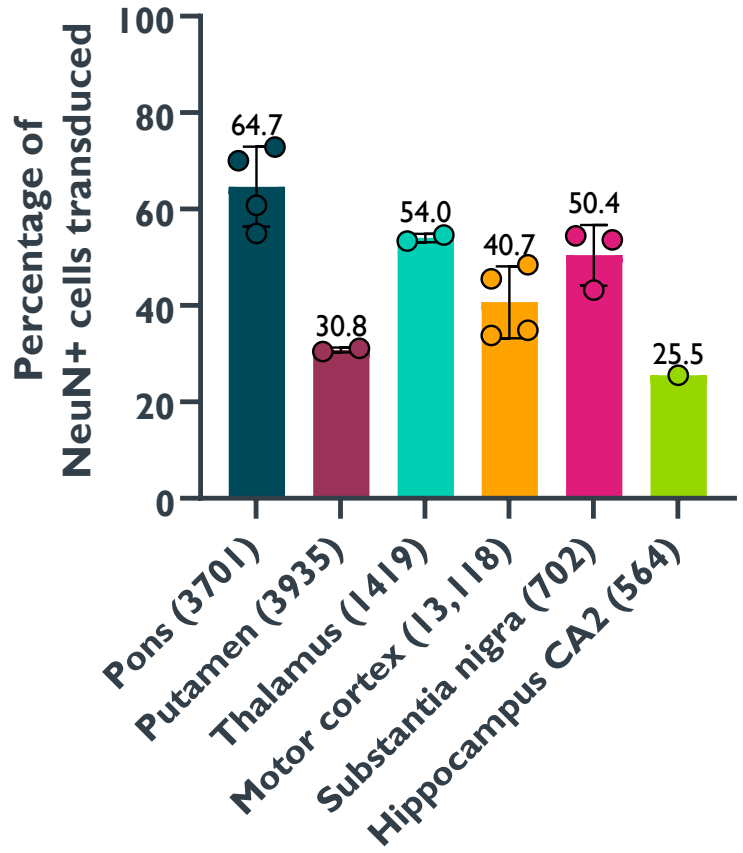

*CAG Promoter, 2e13 vg/kg STAC-BBB, 19 days post administration*

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

## Deep-learning based cell segmentation



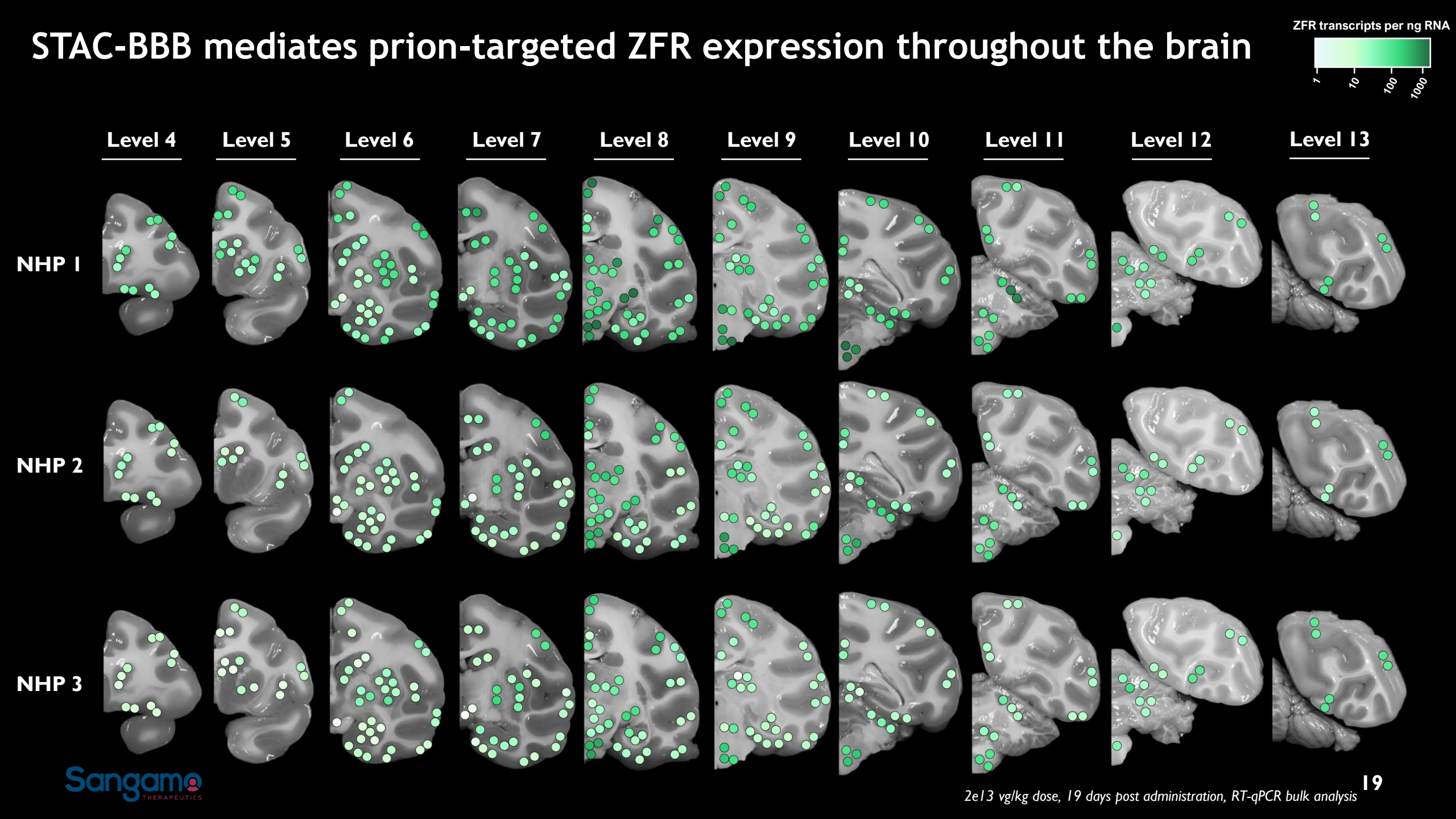

## STAC-BBB NeuN+ cell transduction




*Number of NeuN+ cells counted per structure is in parenthesis*

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

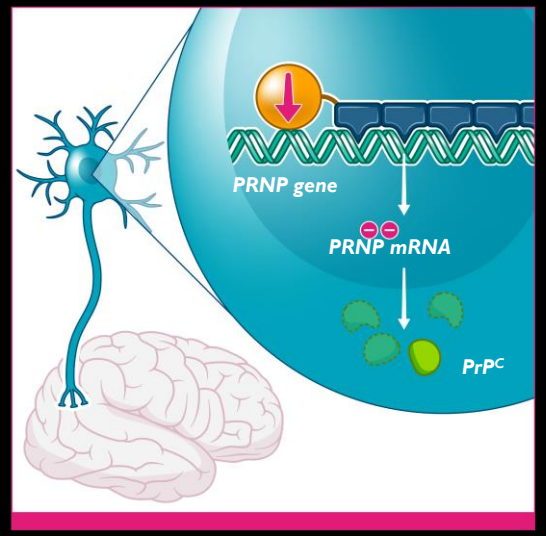
# STAC-BBB mediates prion-targeted ZFR expression throughout the brain



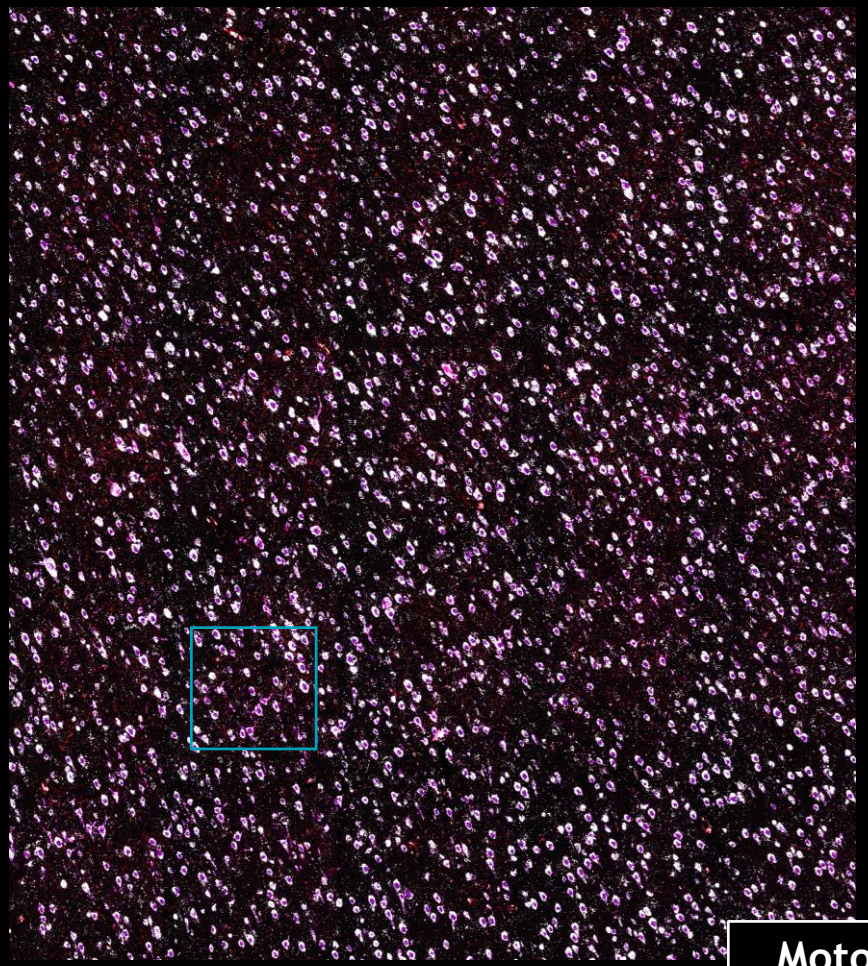

*2e13 vg/kg dose, 19 days post administration, RT-qPCR bulk analysis*

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

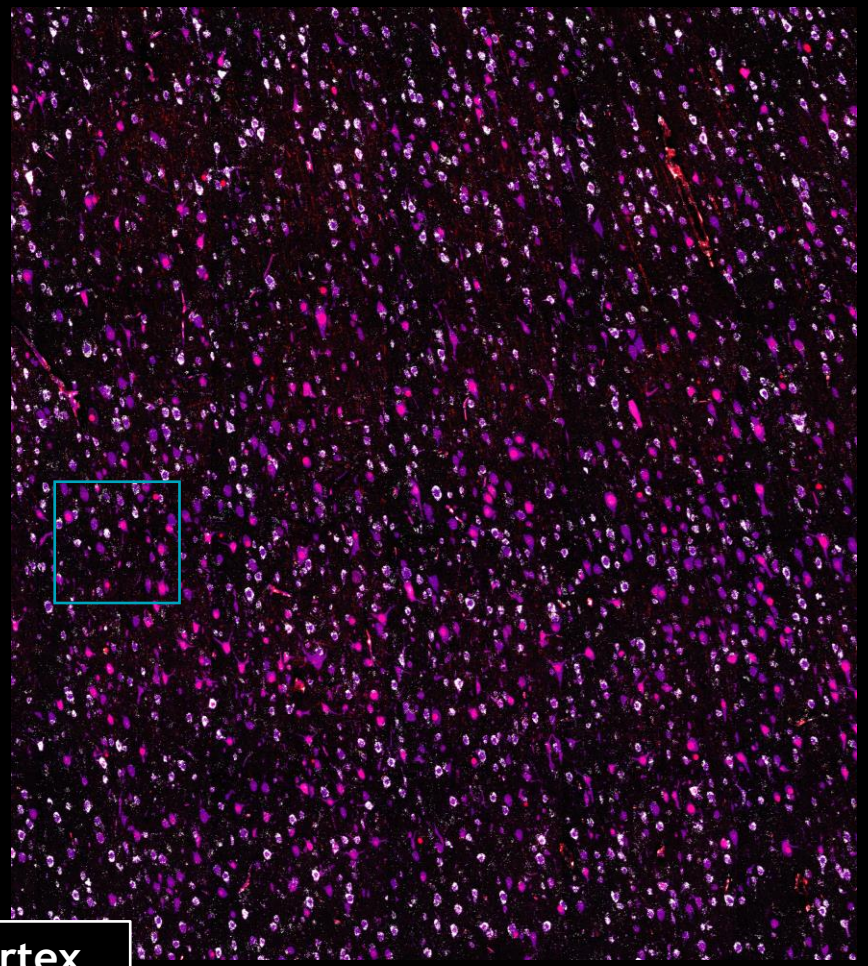
GFP
Neurons (NeuN)
Prion mRNA




Vehicle Control



STAC-BBB



Motor cortex

*Multiplexed RNAscope ISH / IHC assay for NeuN, GFP, PRNP mRNA, and ZFR mRNA*
*2e13 vg/kg dose, 19 days post administration*

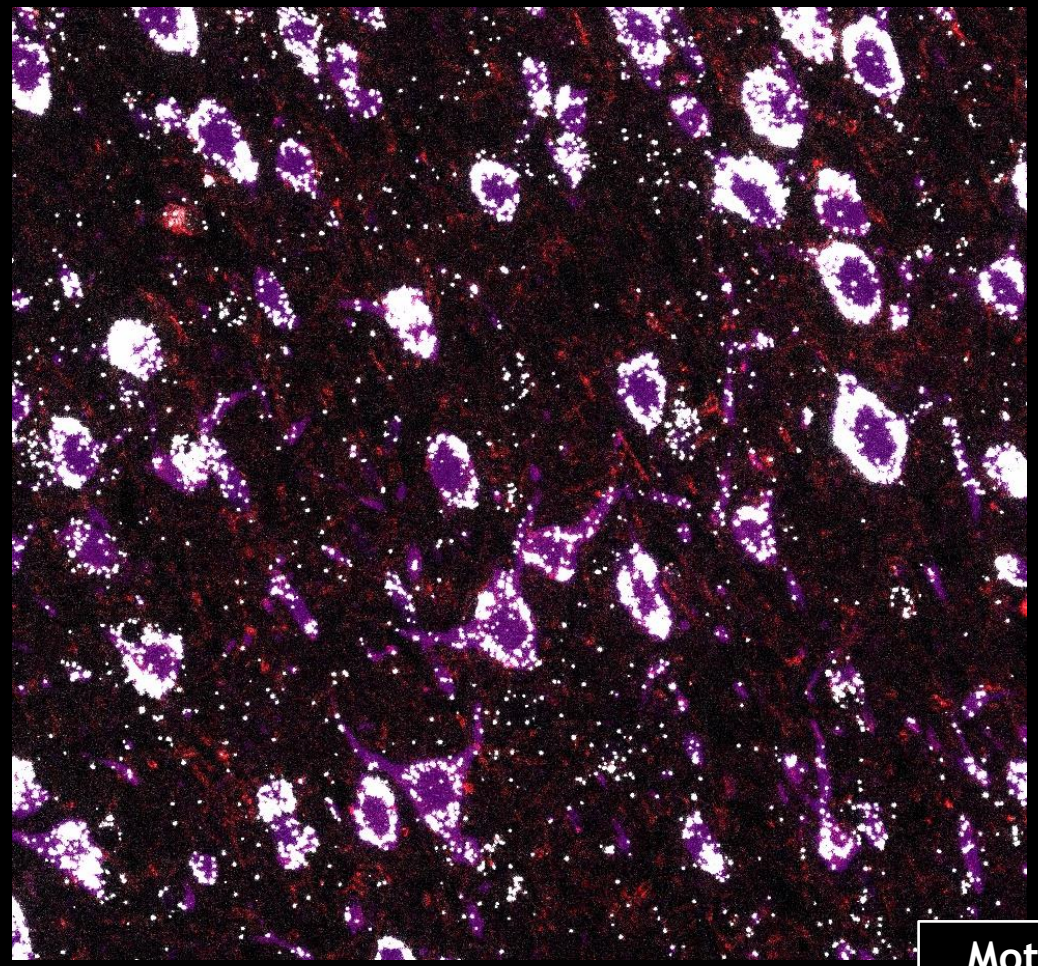
# STAC-BBB mediates ZFR expression and Prion repression in neurons

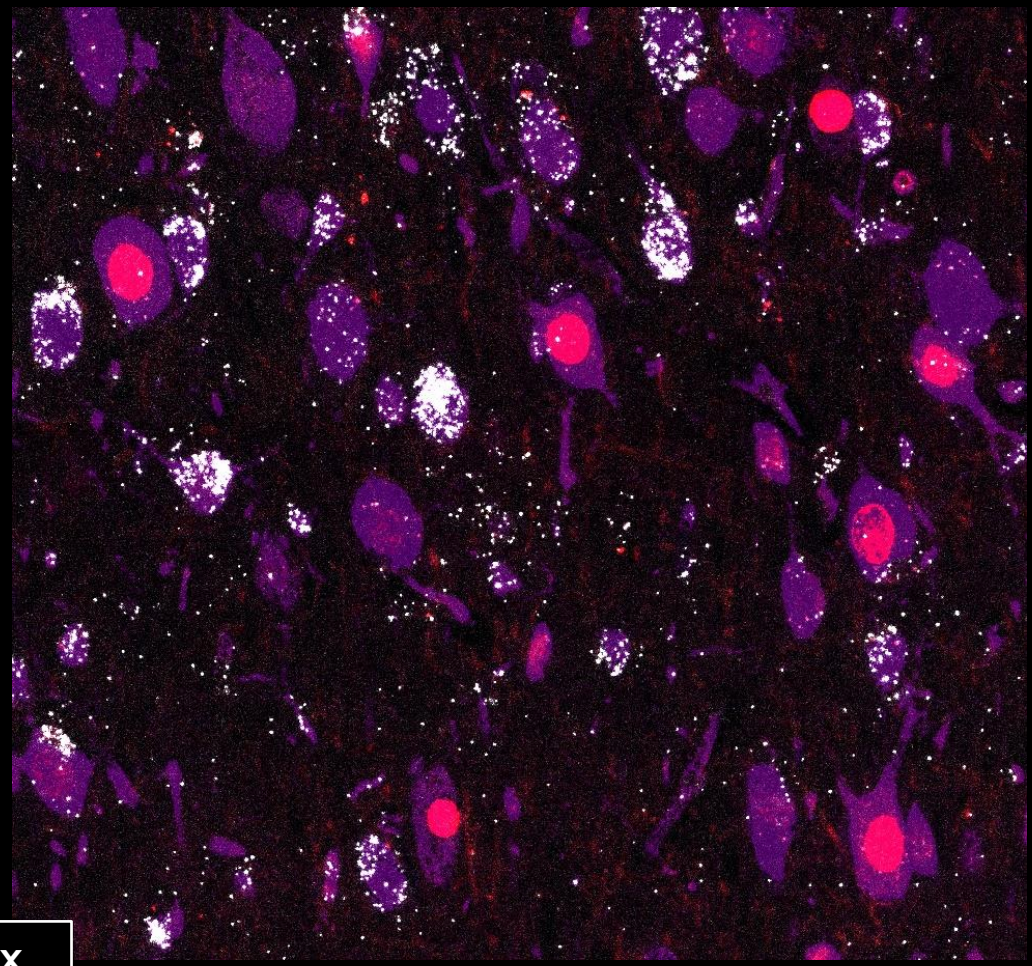
GFP
Neurons (NeuN)
Prion mRNA

Vehicle Control

STAC-BBB

Motor cortex





Motor cortex

*Multiplexed RNAscope ISH / IHC assay for NeuN, GFP, PRNP mRNA, and ZFR mRNA*
*2e13 vg/kg dose, 19 days post administration*

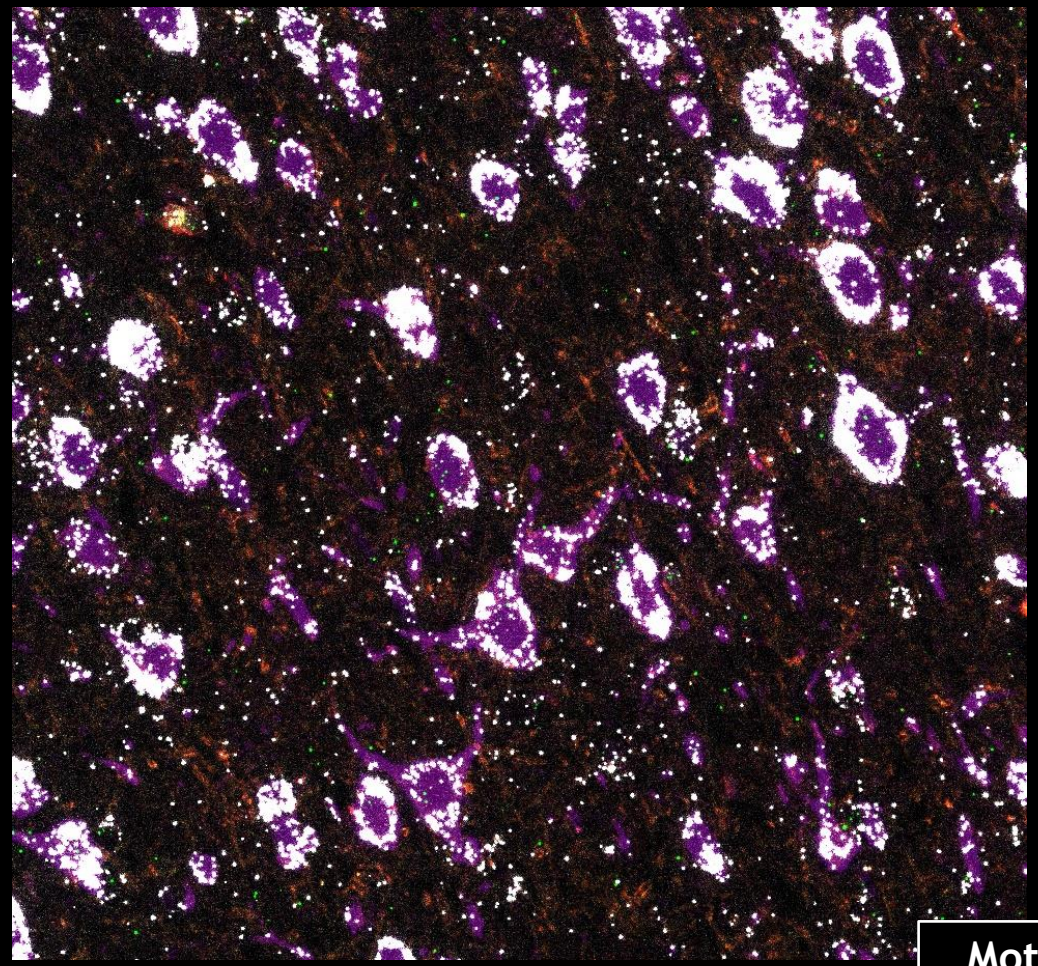
# STAC-BBB mediates ZFR expression and Prion repression in neurons

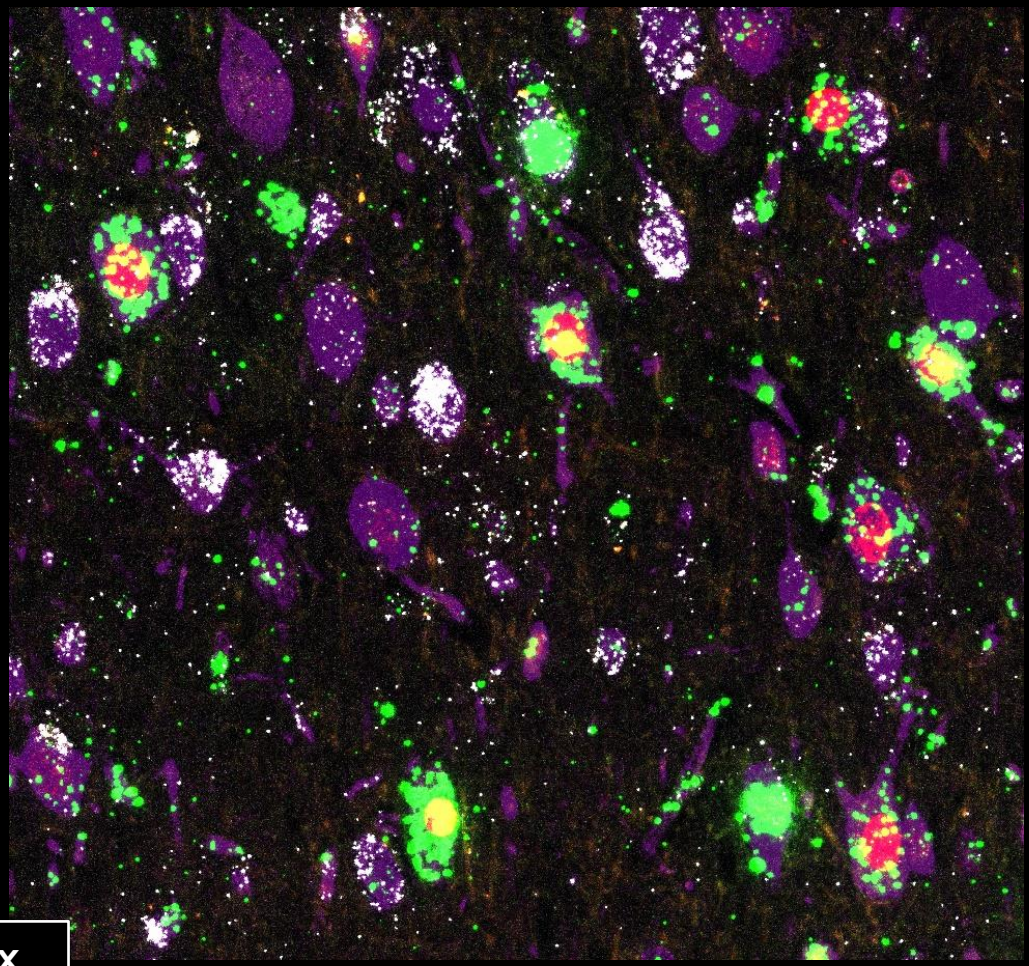
GFP
Neurons (NeuN)
Prion mRNA
ZFR mRNA

Vehicle Control

STAC-BBB

Motor cortex





Motor cortex

*Multiplexed RNAscope ISH / IHC assay for NeuN, GFP, PRNP mRNA, and ZFR mRNA*
*2e13 vg/kg dose, 19 days post administration*

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

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

## Capsid

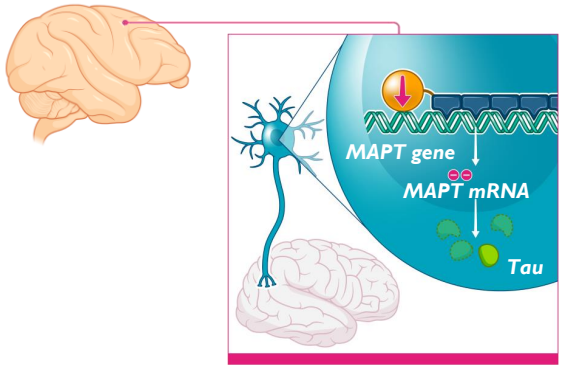
*STAC-BBB*

## Cargo

*hSyn1 – neuronal promoter*

*Tau ZFR*

## Therapeutic Approach




## Dose & Duration

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

4-week study

## Species

*Cynomolgus Macaque*
N=3 per group

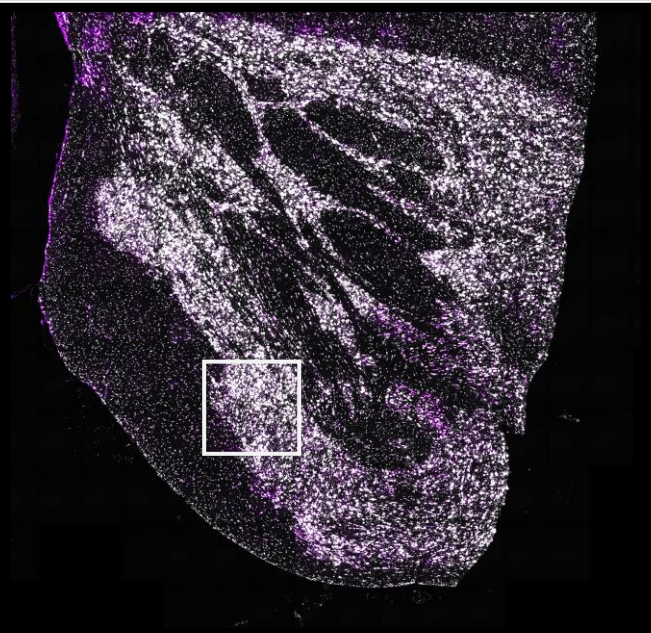
## Endpoints

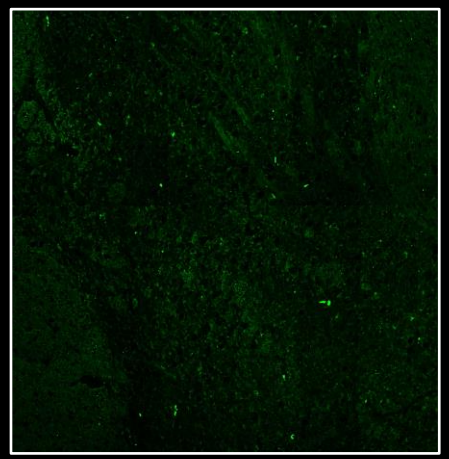
- RNAscope images
- Molecular analyses

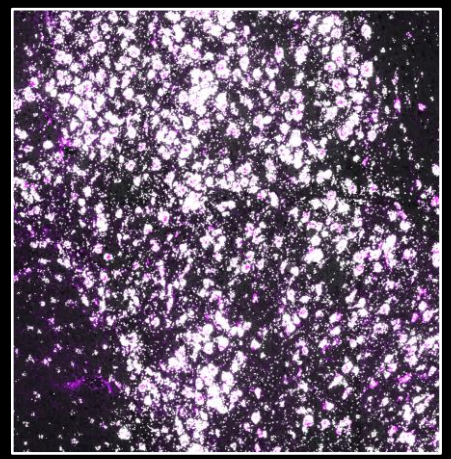
# STAC-BBB mediates robust repression of neuronal tau in the pons

ZFR mRNA
Neurons (NeuN)
Tau mRNA (MAPT)

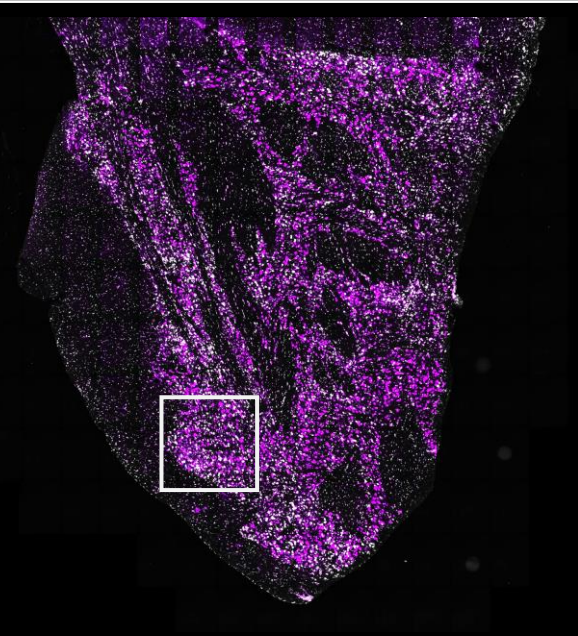
## Vehicle Control

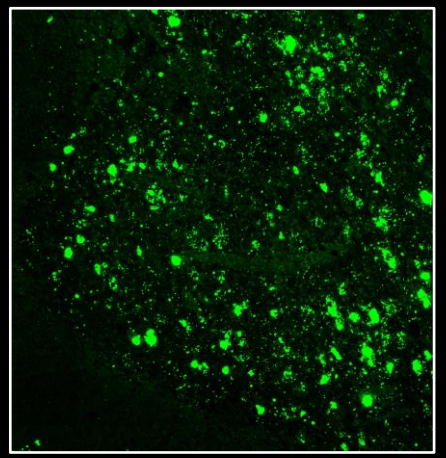


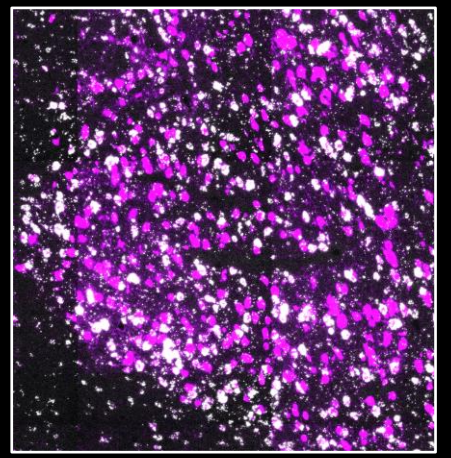




## STAC-BBB







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

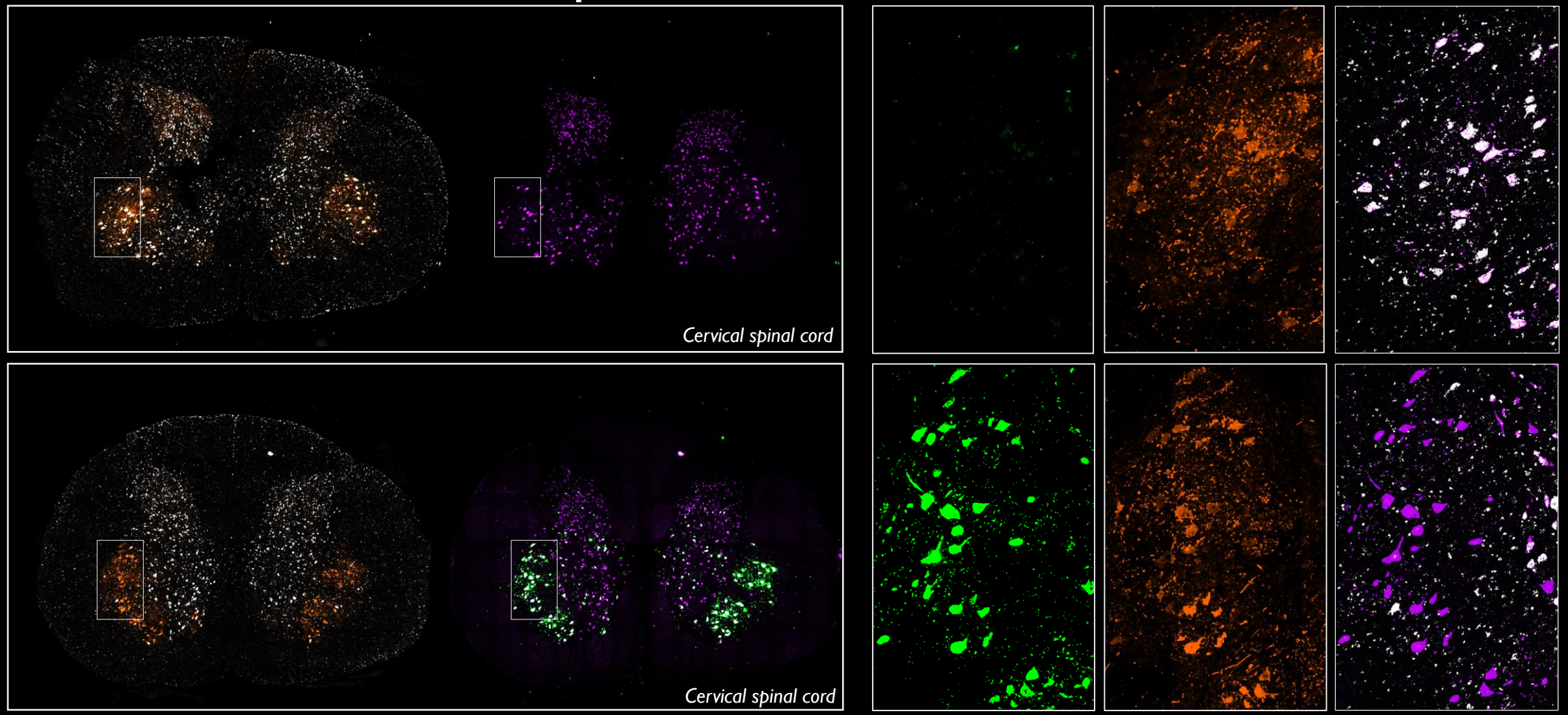
Sangamo THERAPEUTICS

# 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

STAC-BBB

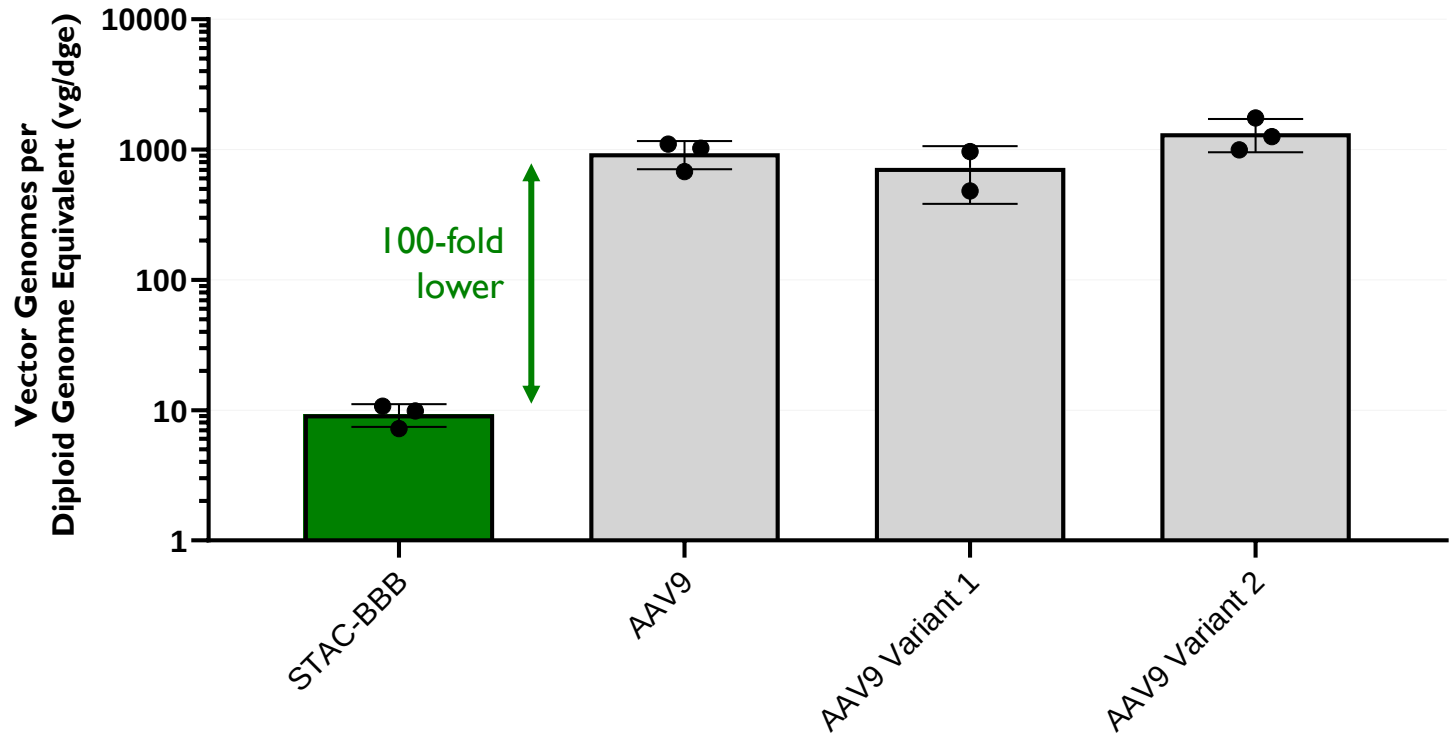



**Percentage of ChAT+NeuN+ motor neurons transduced in the ventral horn: Cervical 95%, Thoracic 84%, Lumbar 98%**

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

Sangamo THERAPEUTICS

# 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

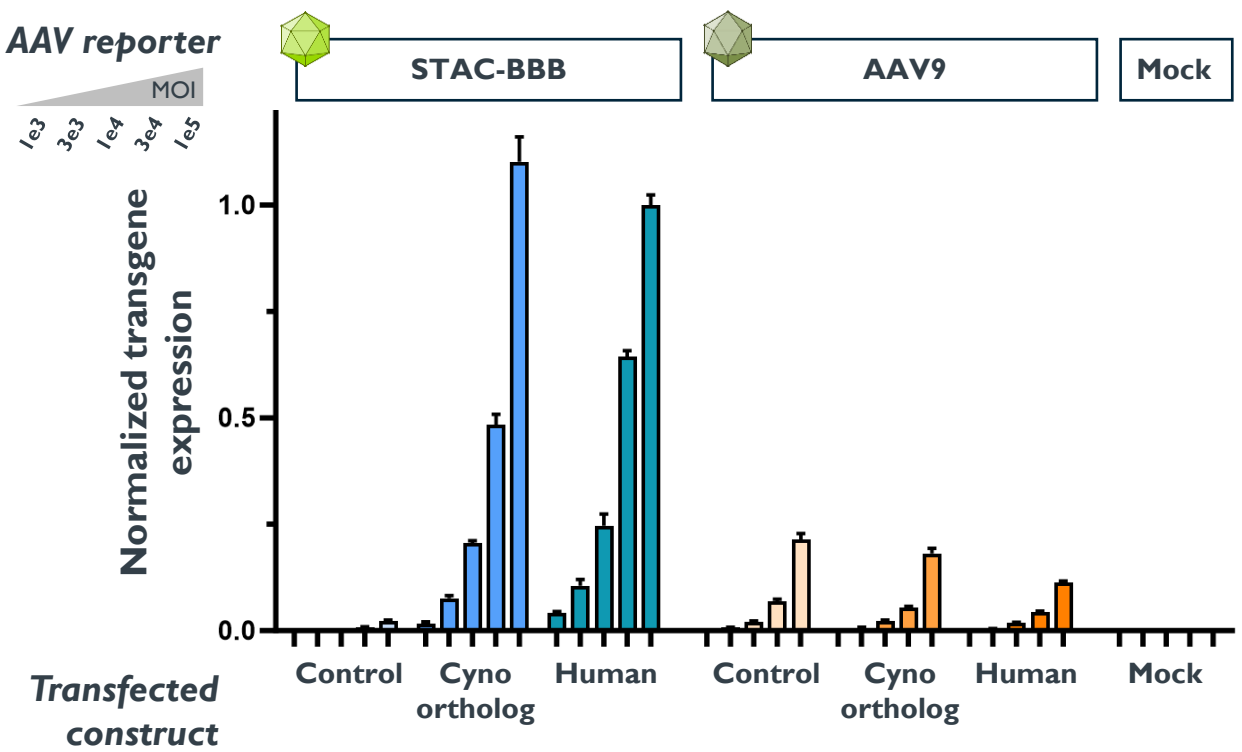
High liver exposure after intravenous administration is a limitation of conventional AAV serotypes including AAV9

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

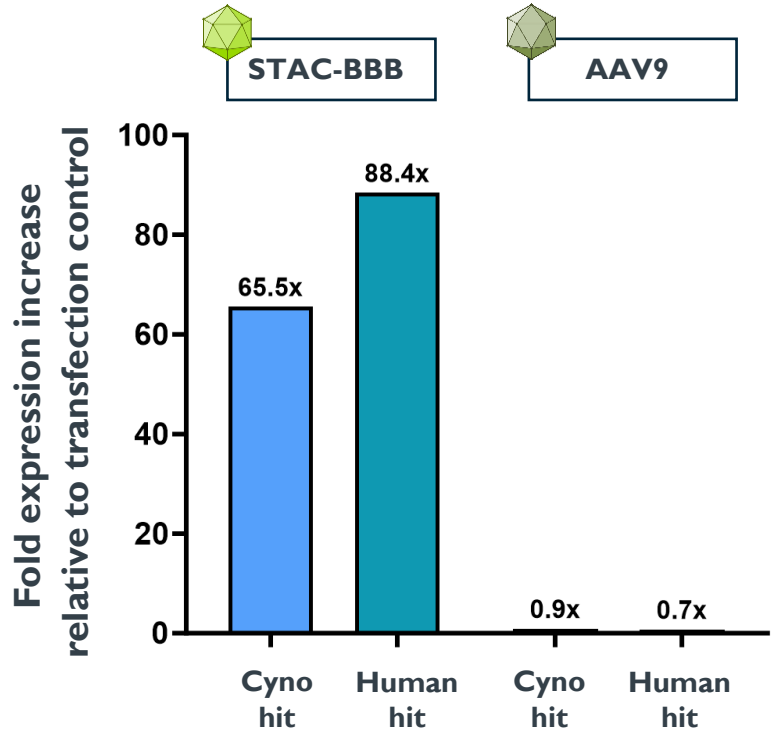
# Overexpression of putative receptor confers a gain-of-function for STAC-BBB transduction *in vitro*

## Transgene expression – RT-qPCR




*HEK293 cells*
*Transgene expression evaluated 48 hr post transduction*

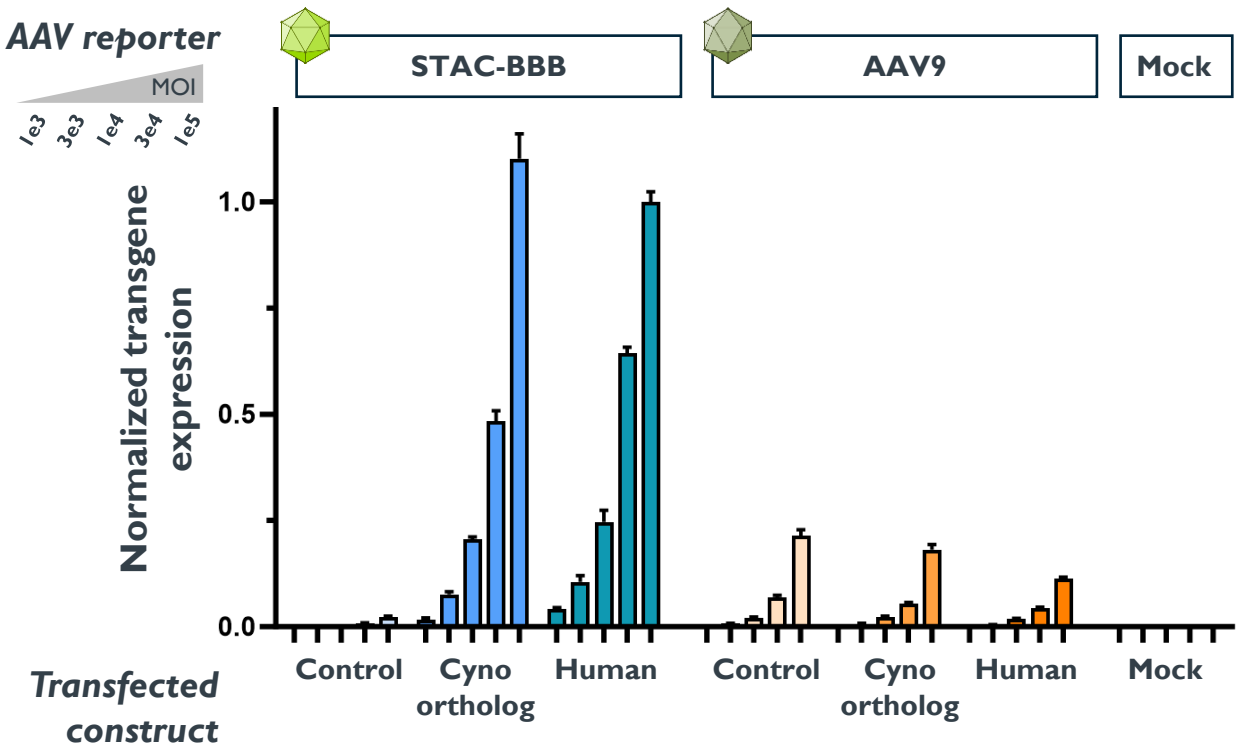
## Fold increase relative to control




*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.*

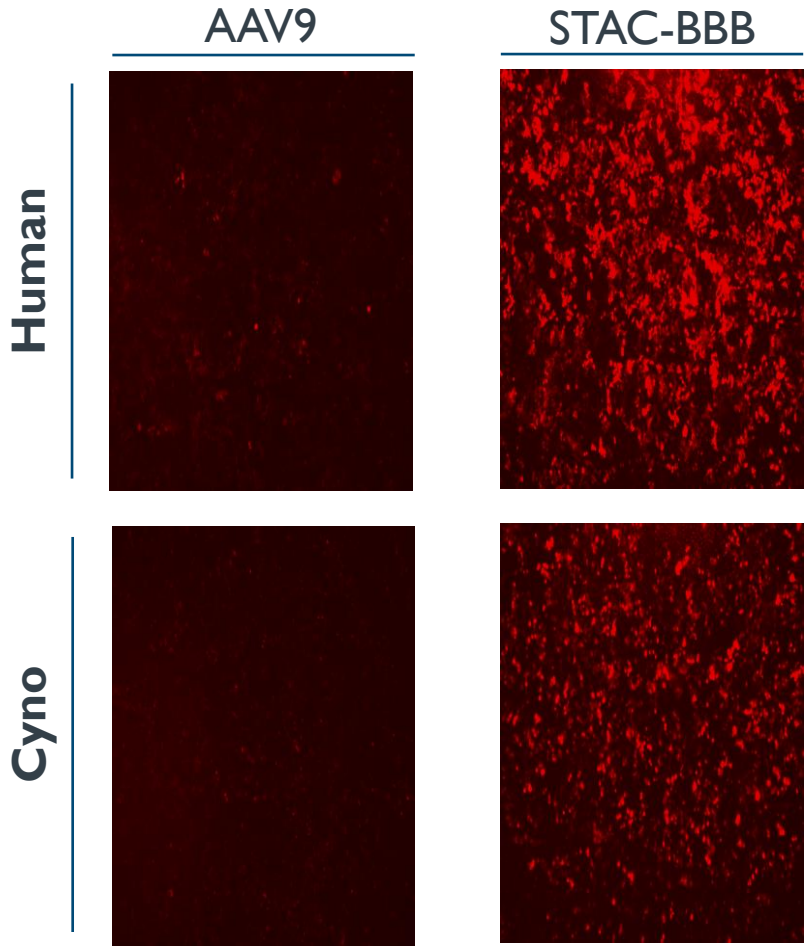
# Overexpression of putative receptor confers a gain-of-function for STAC-BBB transduction *in vitro*

## Transgene expression – RT-qPCR

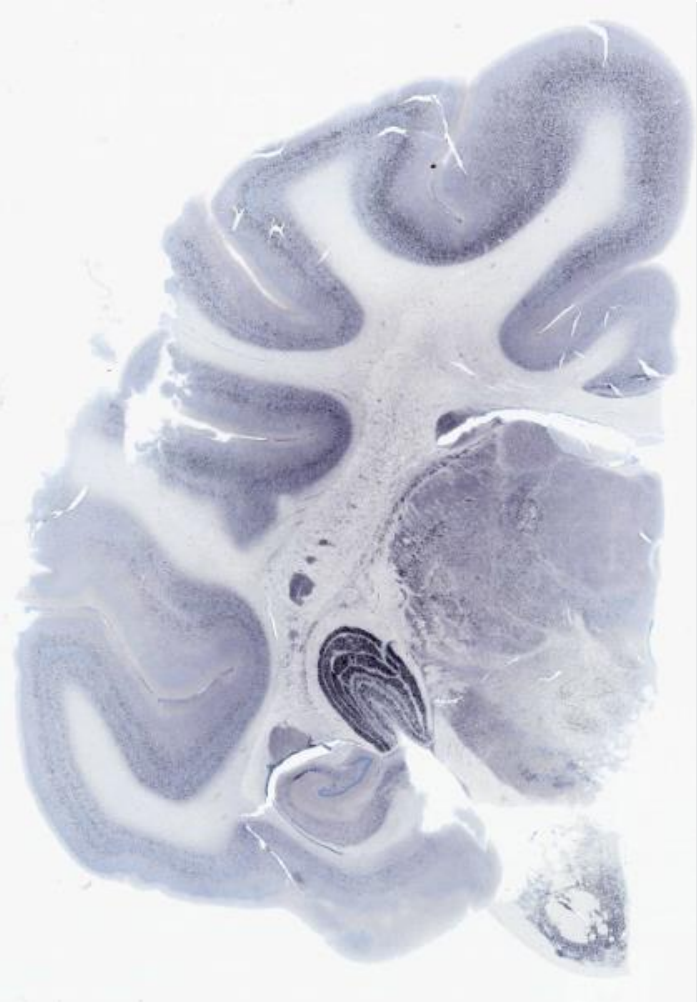



*HEK293 cells*
*Transgene expression evaluated 48 hr post transduction*

## 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 1e14 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**

# Acknowledgements


## Nonclinical Development: BBAS & Nonclinical Operations

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