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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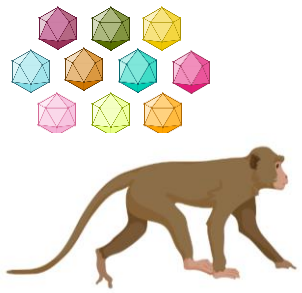
Disclosure

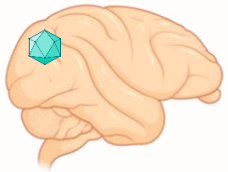
I am a full-time employee of Sangamo Therapeutics

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

AAV library administered to cyno macaques

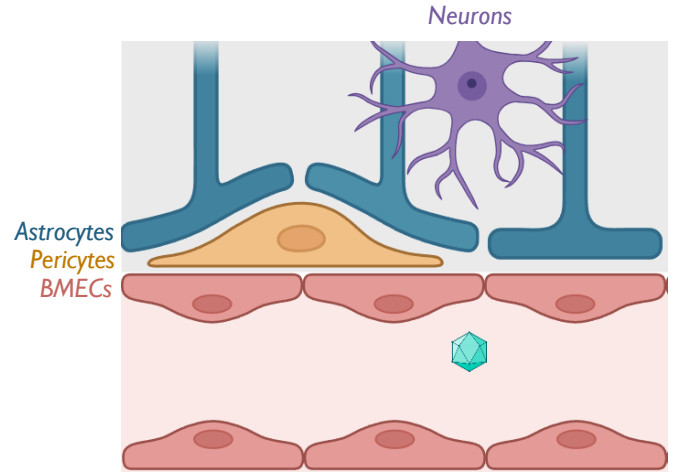


Capsids reach brain tissue

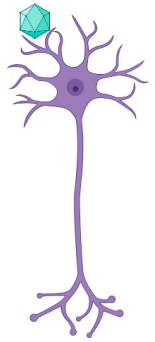


Traditional methods stop here

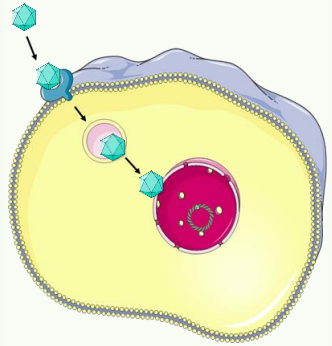
Overcome biological barriers



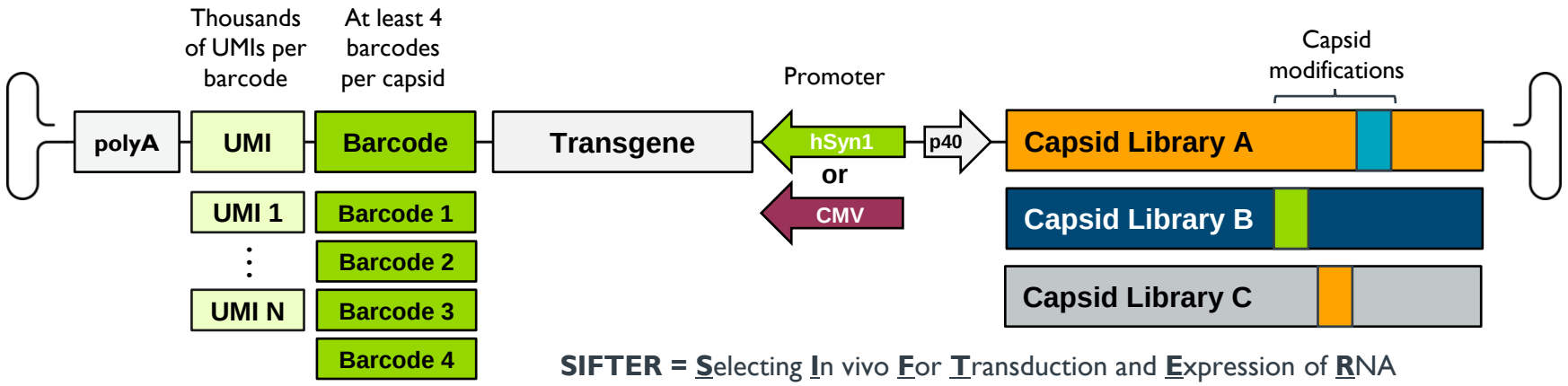
Transduce neurons



Express transgene in neurons

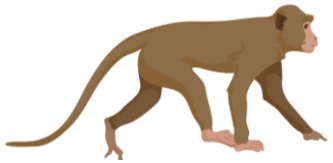
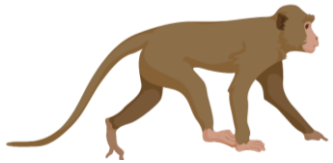
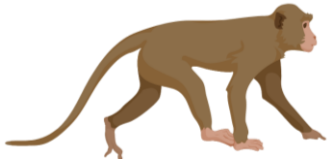
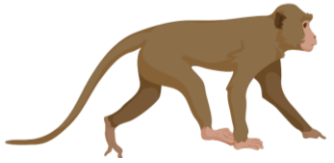


Delivery goal



SIFTER = Selecting In vivo For Transduction and Expression of RNA

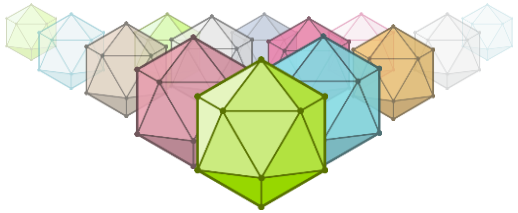
Multiple library screening rounds were conducted to identify STAC-BBB

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

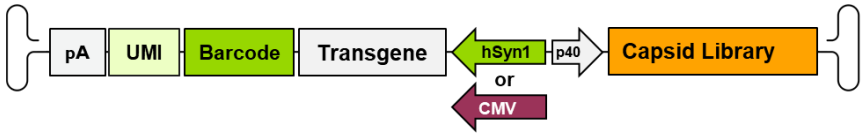
Objective: Determine relative performance of 1,260 capsid variants and select lead variant for individual evaluation

Capsid



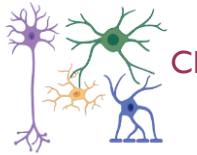
1,260 Capsid Variant Library

Cargo



Multiplexed promoters

hSyn1 promoter (Neurons only)



CMV promoter (All cells)

Dose & Duration

5E13 vg/kg

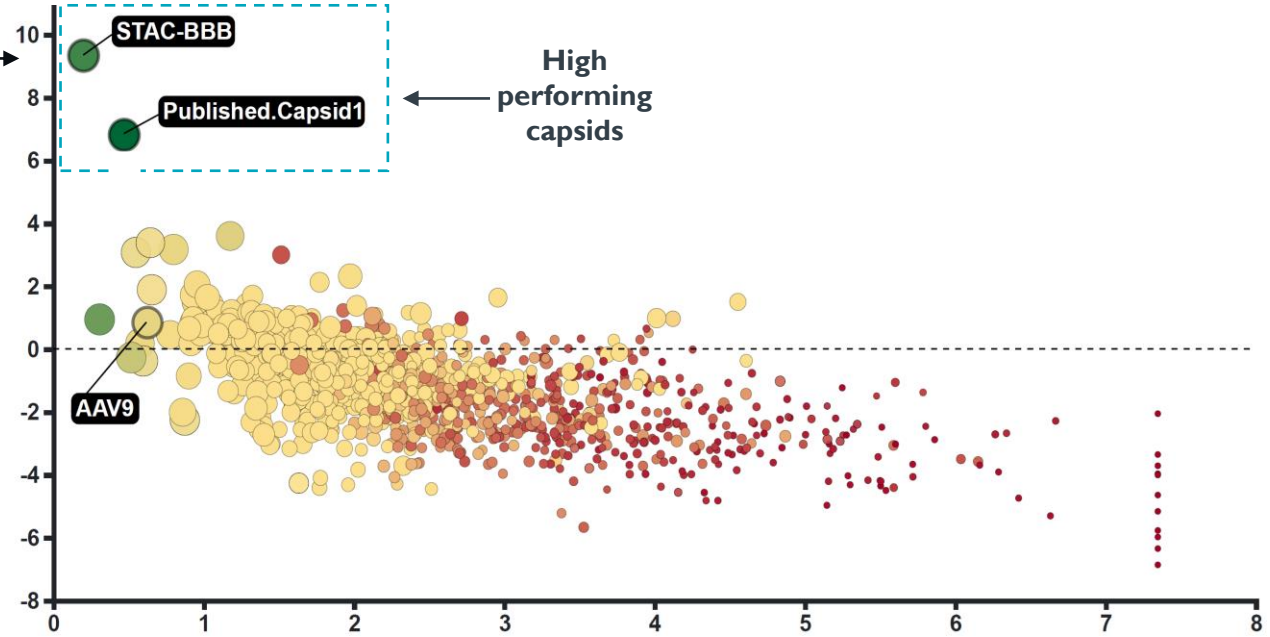
3-week study

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

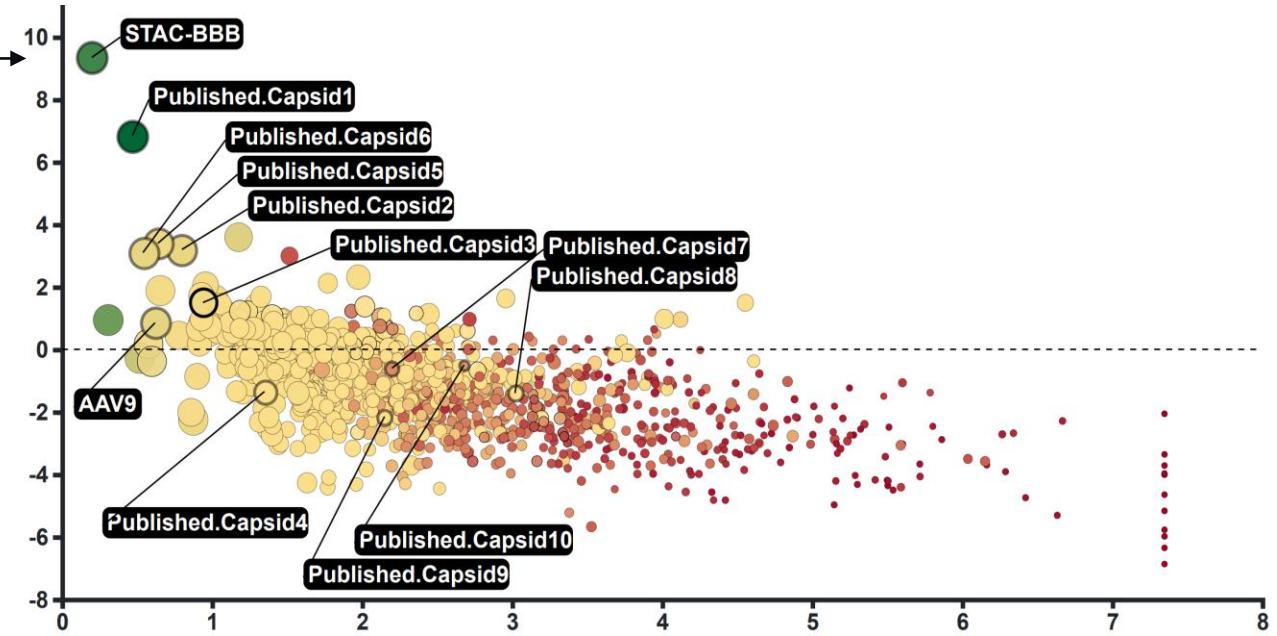
Unique Molecular Identifier count (Color): Informs number of unique AAV transduction events **Darker green is better**

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 →

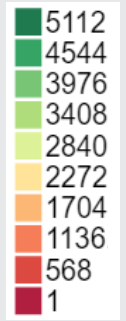


Log_2 Fold Change in Neuronal mRNA Expression (Y-axis): Enrichment score relative to the administered library **Larger value is better**

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**

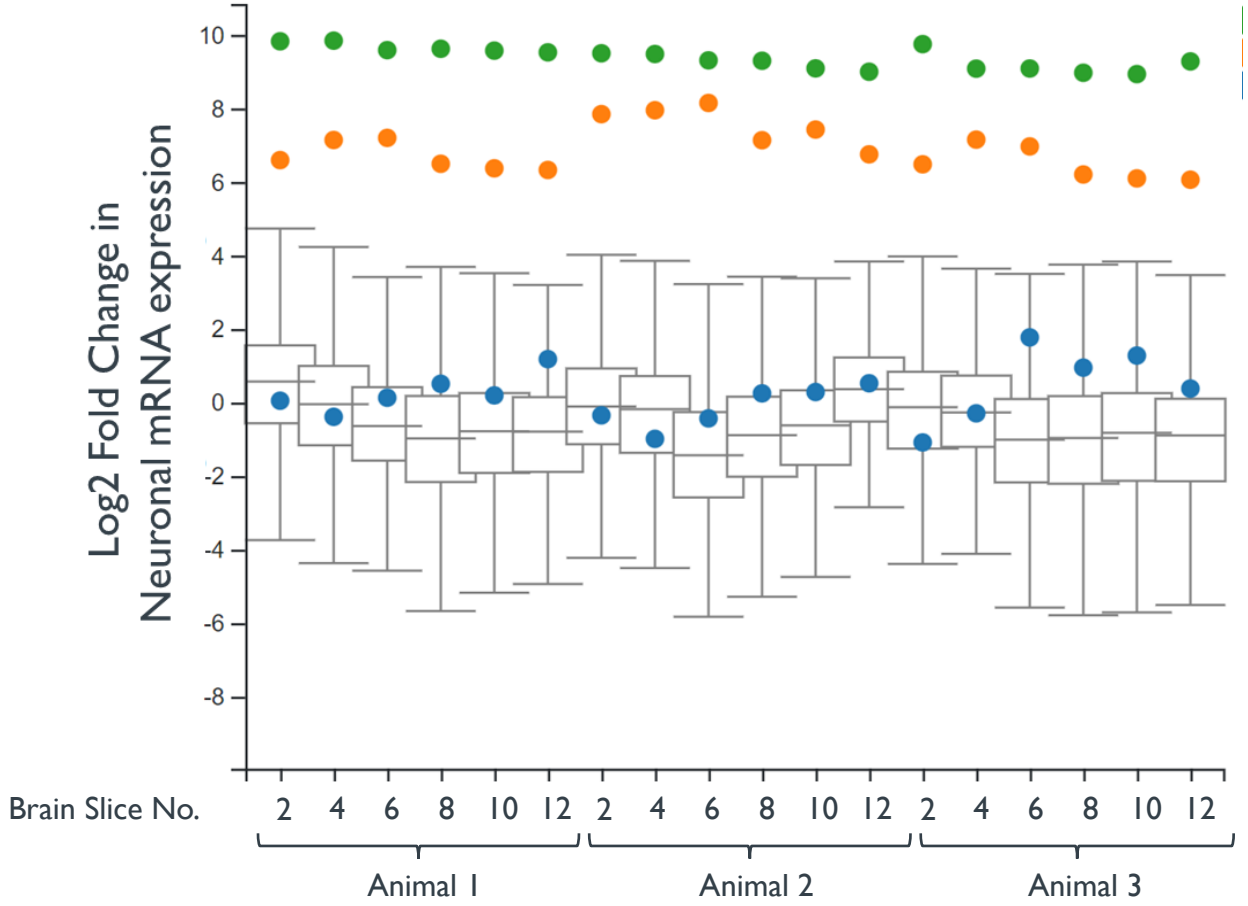


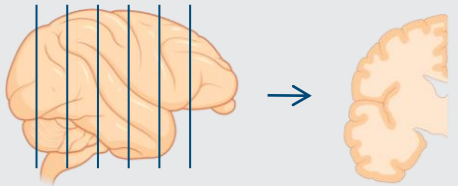
Unique Molecular Identifier count (Color): Informs number of unique AAV transduction events **Darker green is better**

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





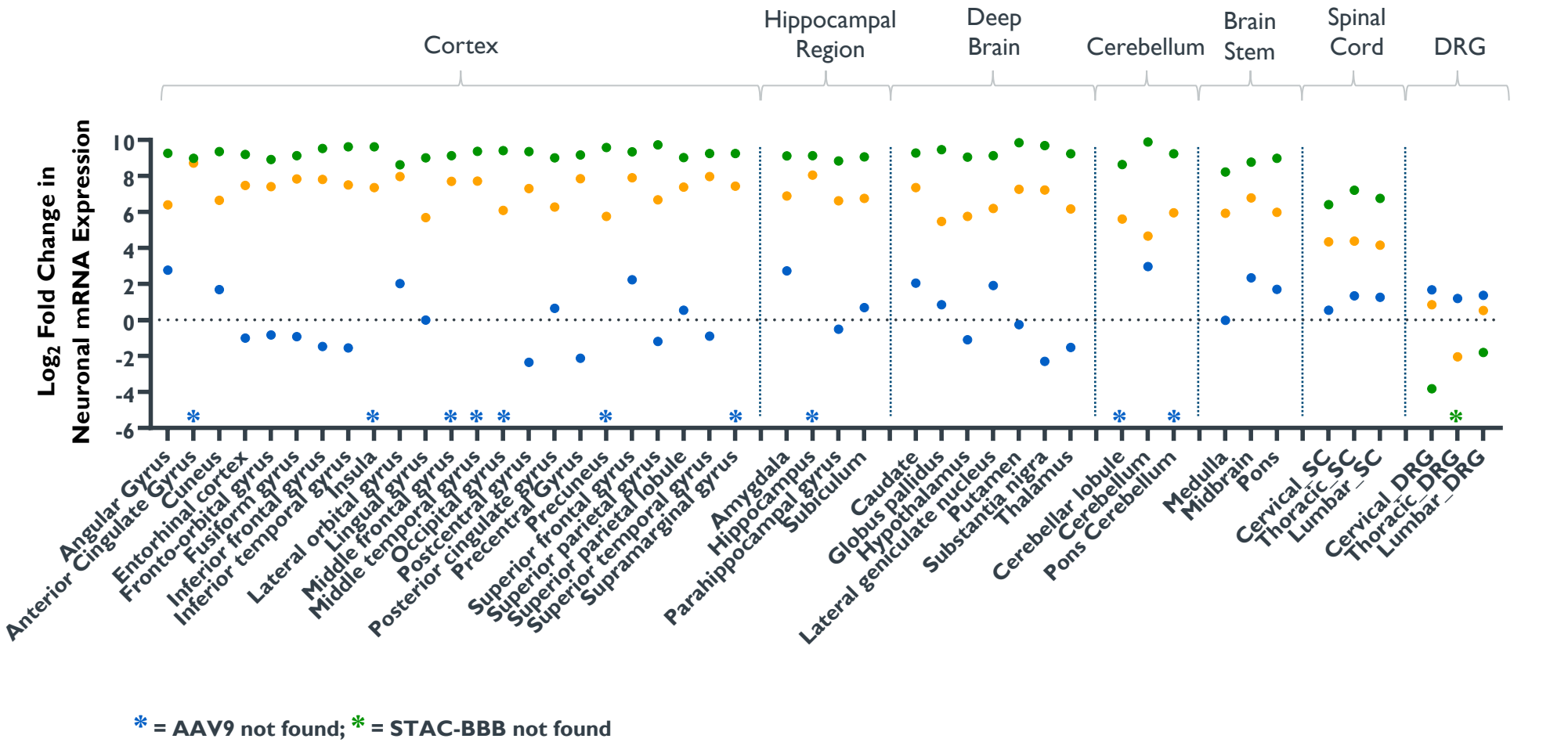
Assessment across brain slices and animals

- STAC-BBB
- Published capsid
- AAV9

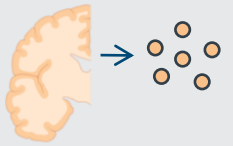
hSyn I neuron specific promoter

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

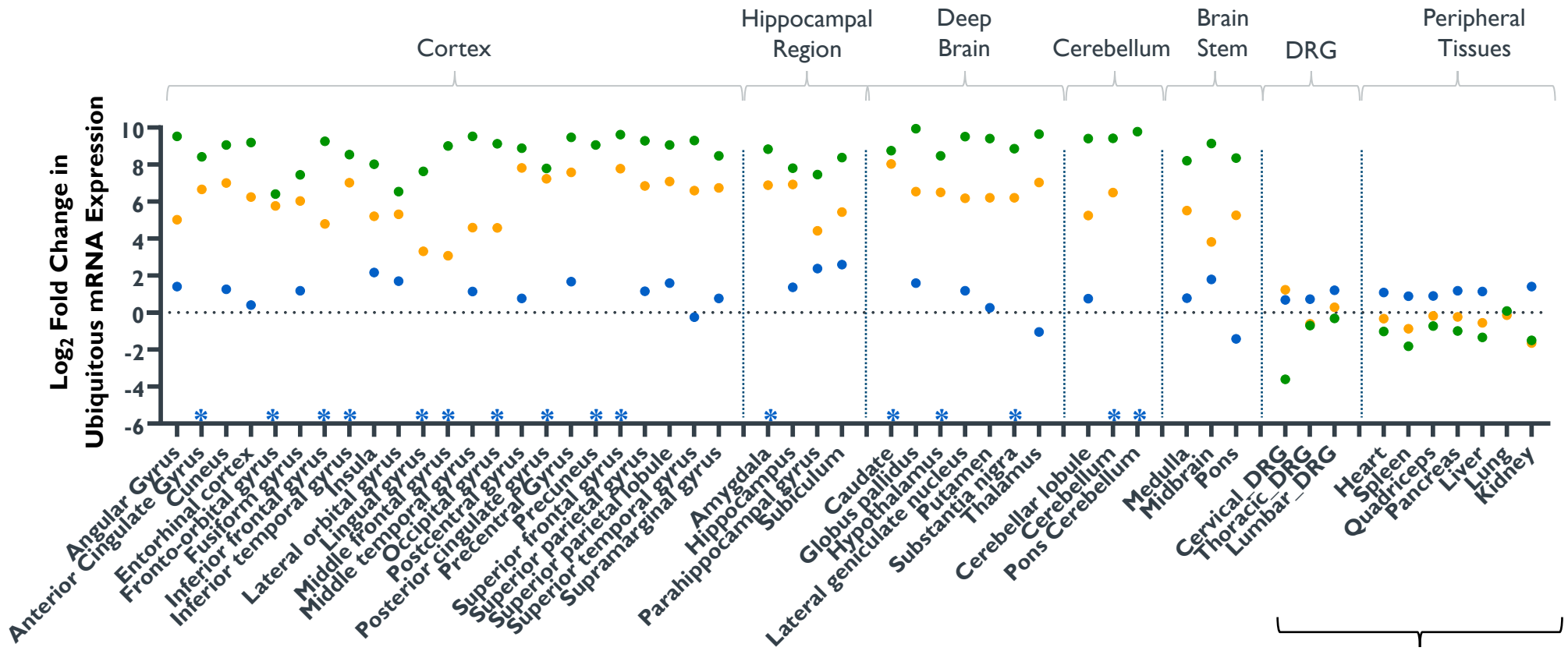
- STAC-BBB
- Published capsid
- AAV9

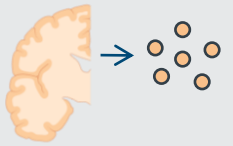
hSyn I neuron specific promoter

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

Sangamo THERAPEUTICS

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

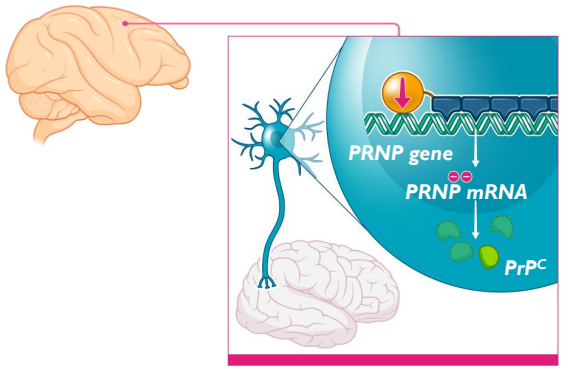
CAG – ubiquitous promoter

Nuclear localized EGFP

2A peptide

Prion ZFR

Therapeutic Approach



Dose & Duration

2E13 vg/kg

3-week study

Species

Cynomolgus Macaque
N=3

Endpoints

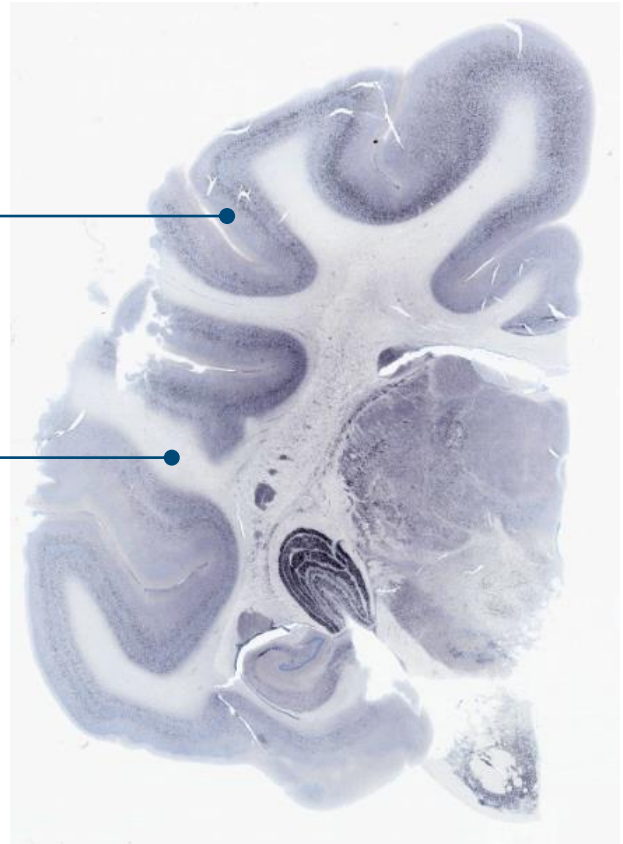
- Immunohistochemistry (GFP) images
- RNAscope images (ZFR/Prion)
- Molecular analyses

STAC-BBB drives widespread and robust expression throughout the brain

Grey matter (cell bodies)

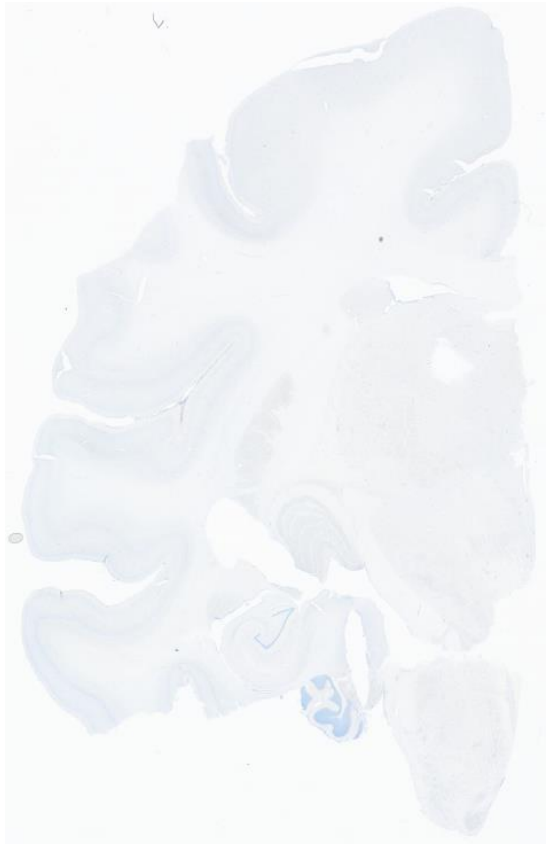
White matter (nerve fibers)

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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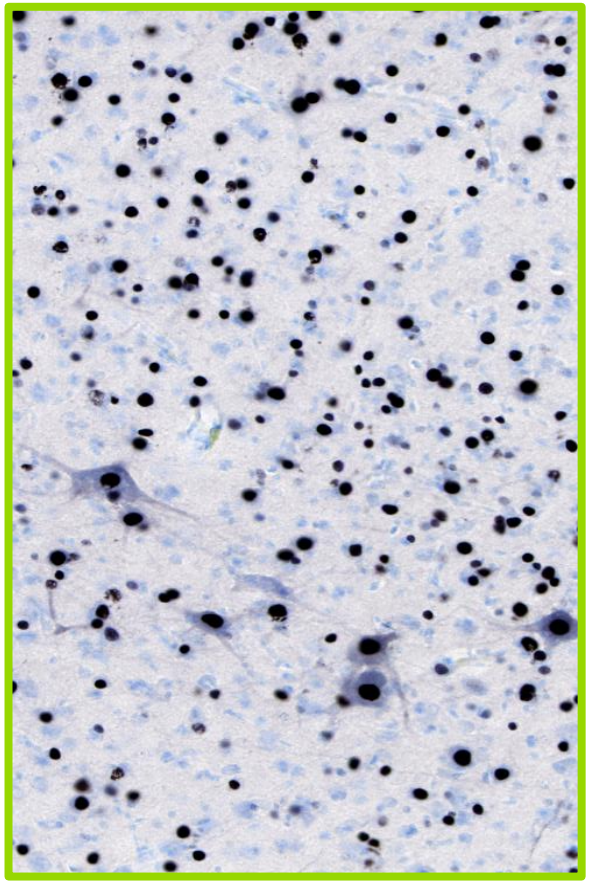
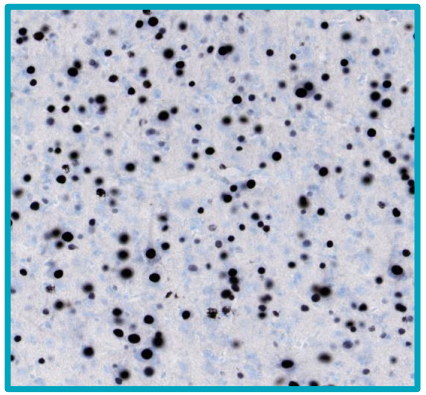
STAC-BBB shows widespread neuronal transduction across all cortical regions

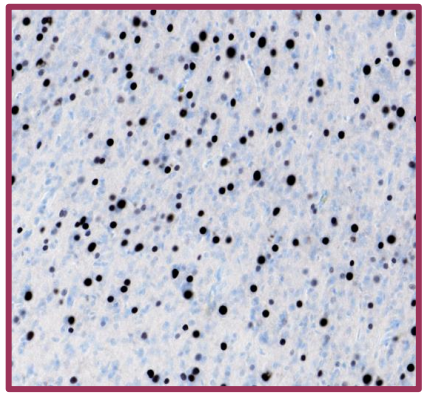
Precentral Gyrus (Motor Cortex)



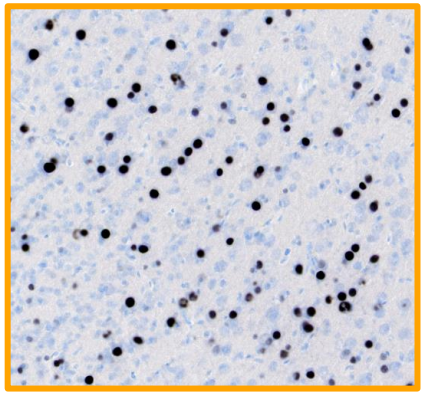
Postcentral Gyrus



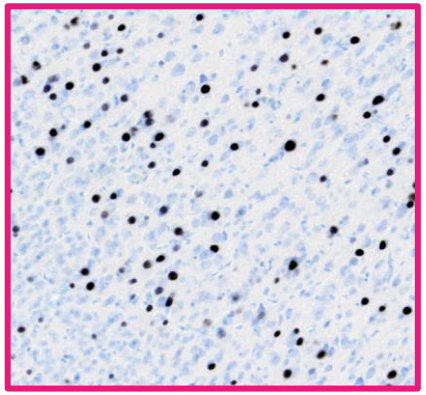
Superior Temporal Gyrus



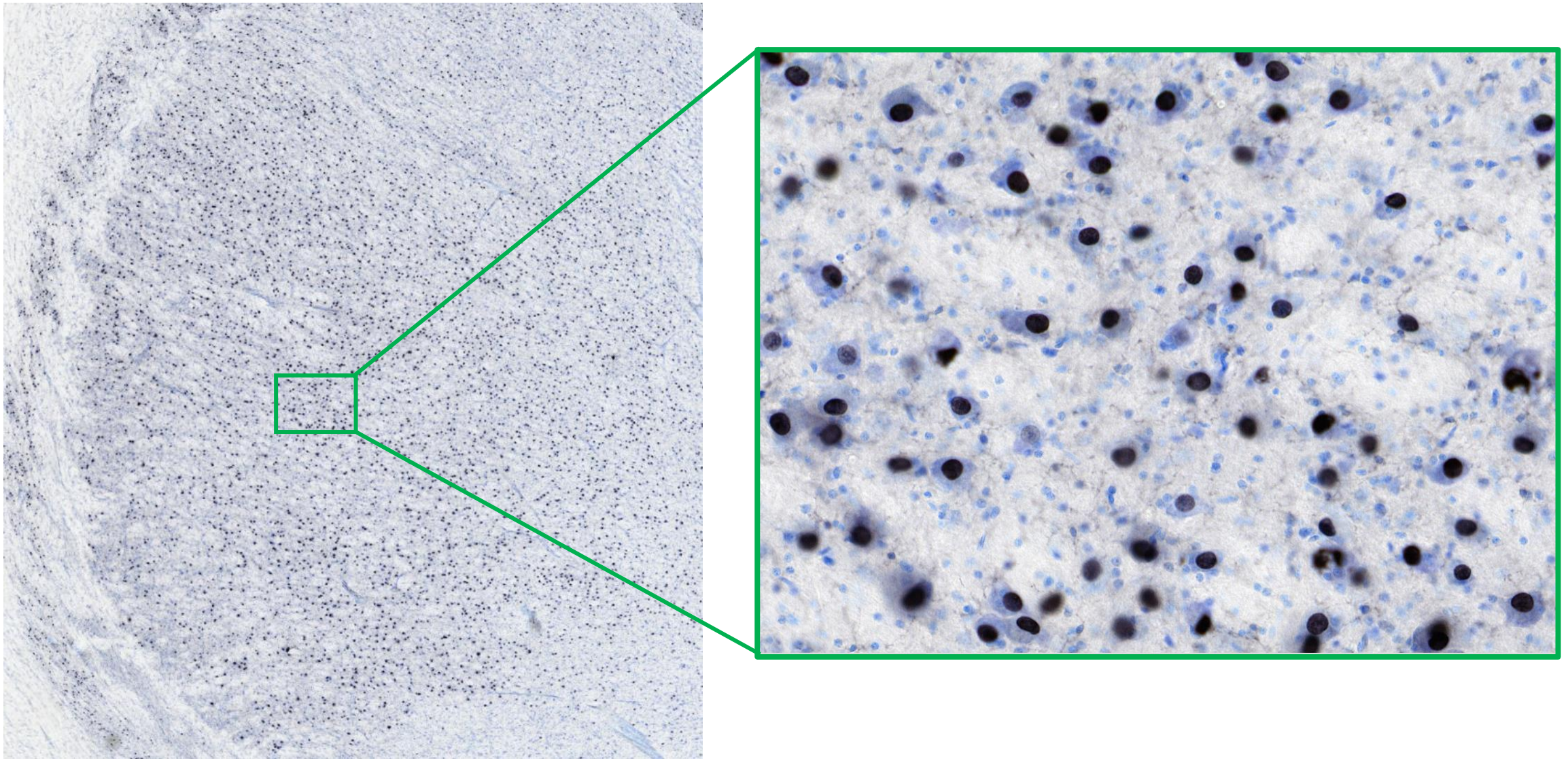
Middle Temporal Gyrus



Inferior Temporal Gyrus



STAC-BBB mediates widespread neuronal transduction in the thalamus

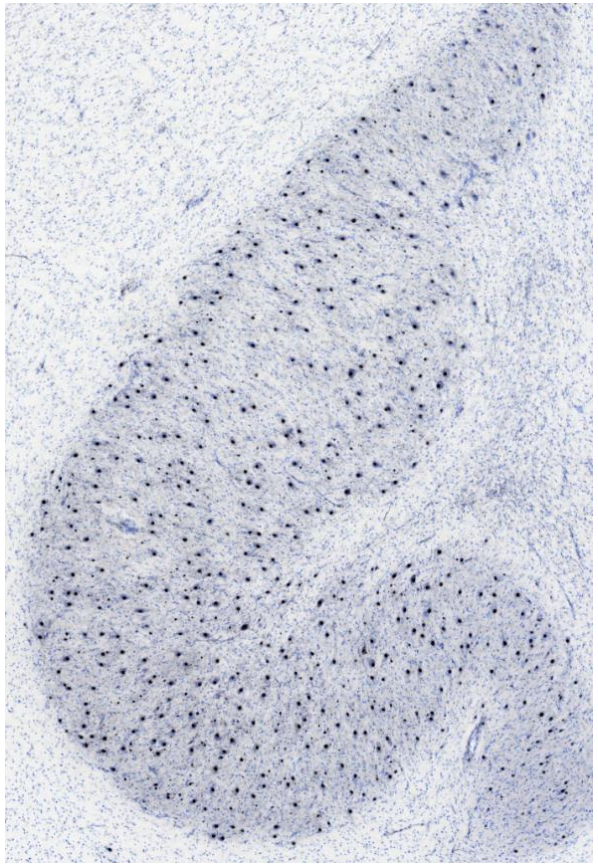


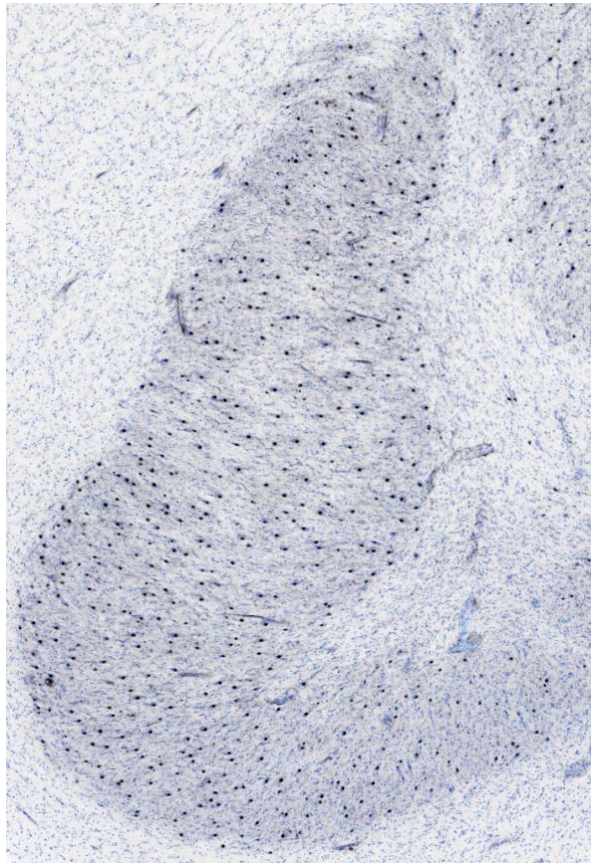
2e13 vg/kg STAC-BBB, 19 days post administration

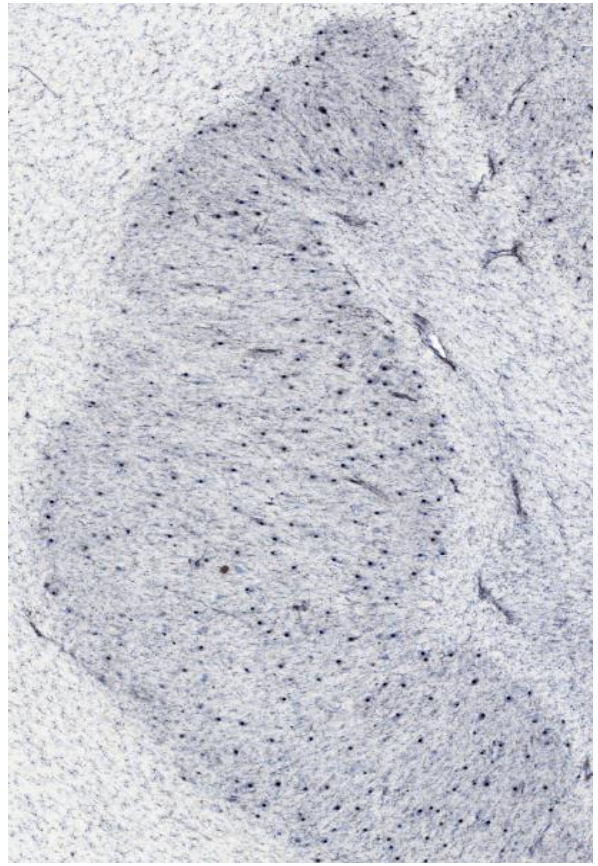
STAC-BBB transduction is consistent across all animals

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

NHP 1	NHP 2	NHP 3







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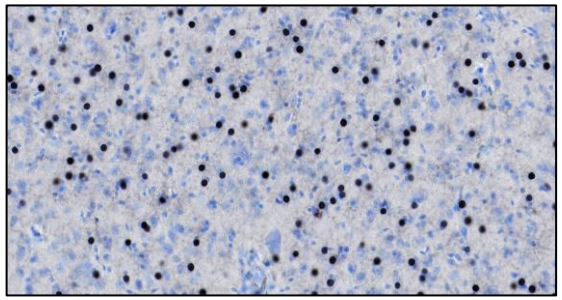
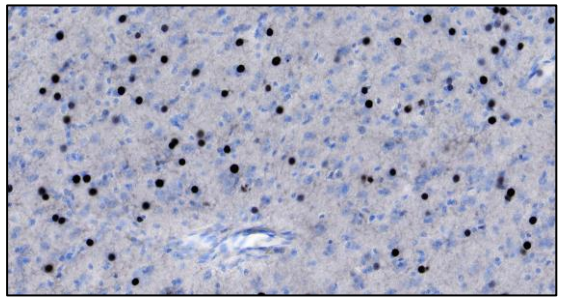
2e13 vg/kg STAC-BBB, 19 days post administration

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

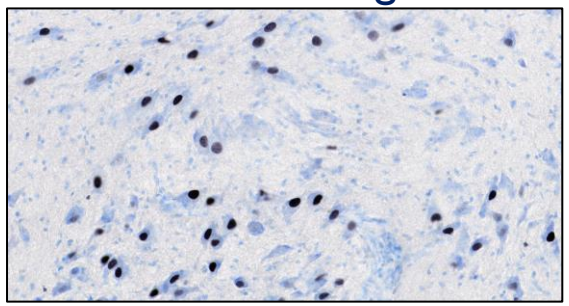
Putamen



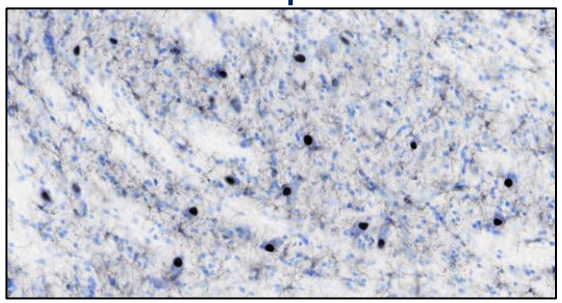
Caudate



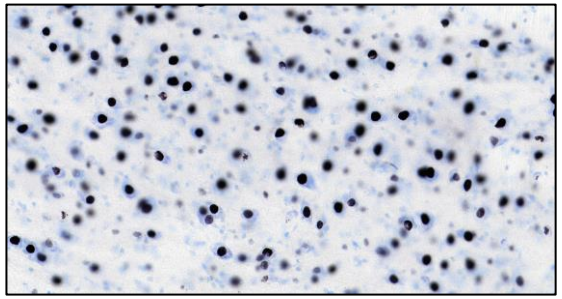
Substantia nigra



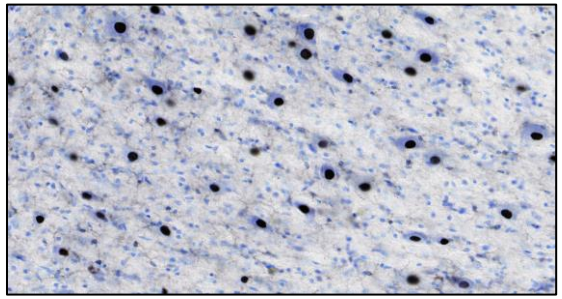
Globus pallidus



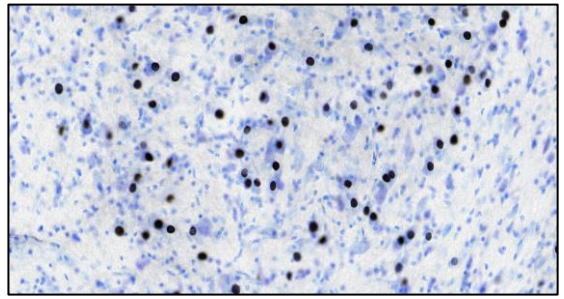
Pons



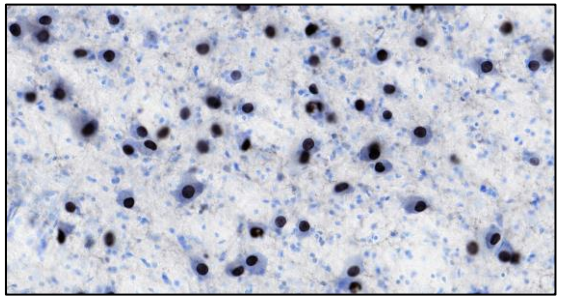
Dentate nucleus



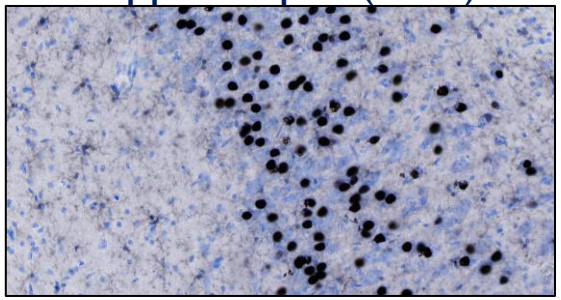
Cuneate nucleus



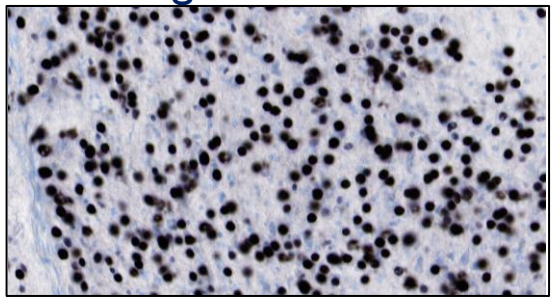
Thalamus



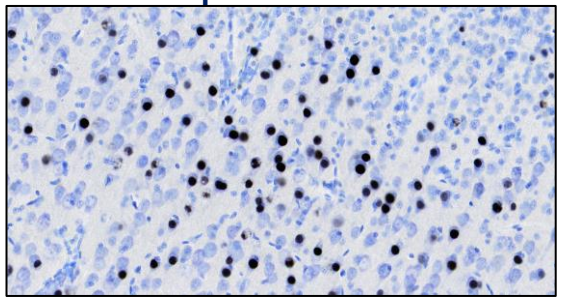
Hippocampus (CA2)



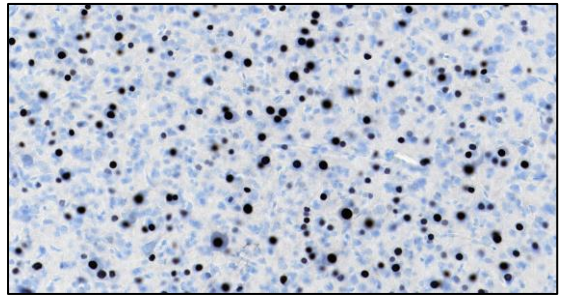
Lateral geniculate nucleus



Temporal cortex



Motor cortex

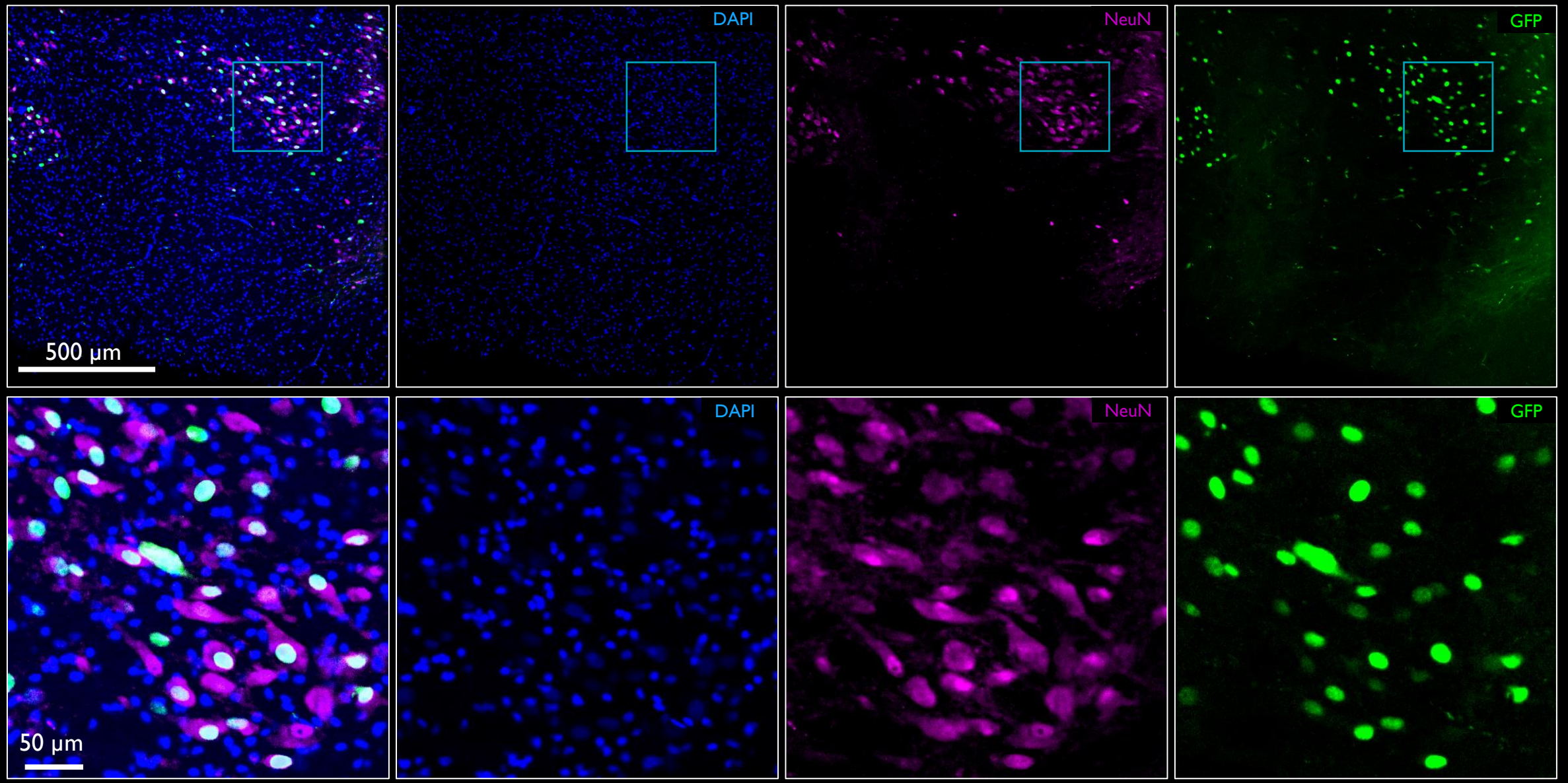


Sangamo THERAPEUTICS

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

STAC-BBB transduces neurons in the substantia nigra

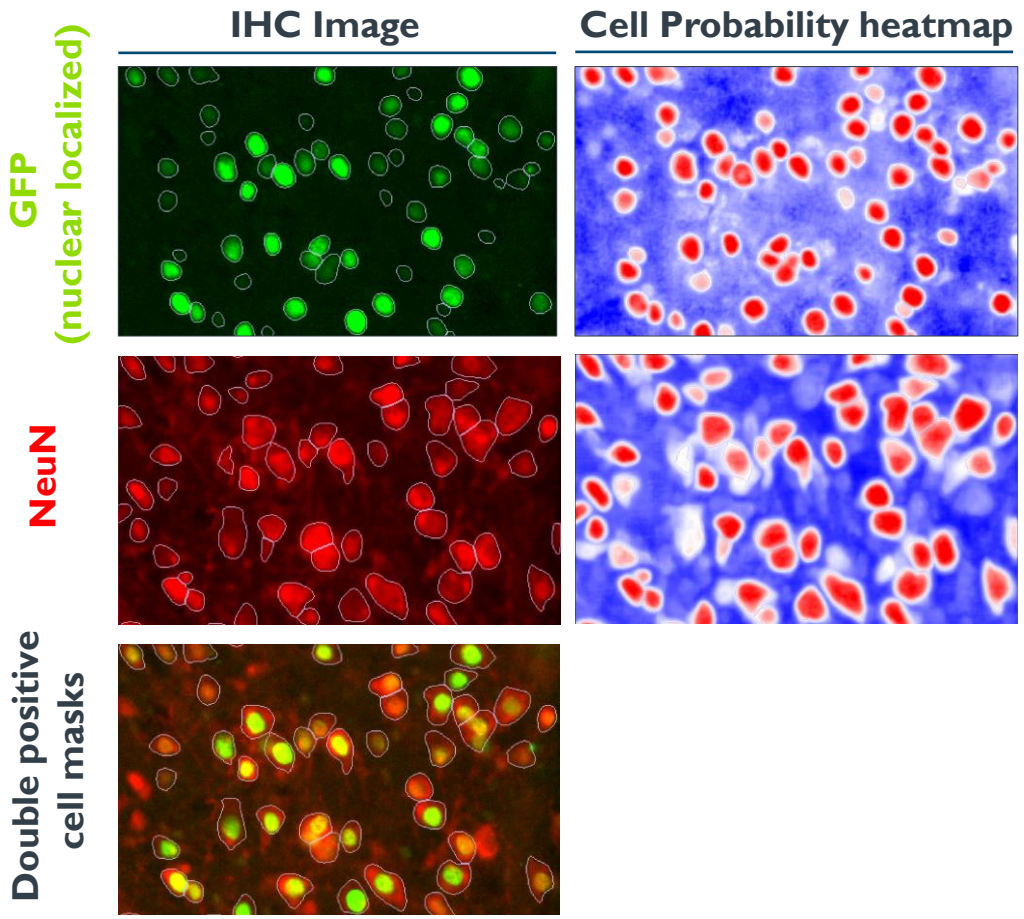
DAPI
NeuN
GFP



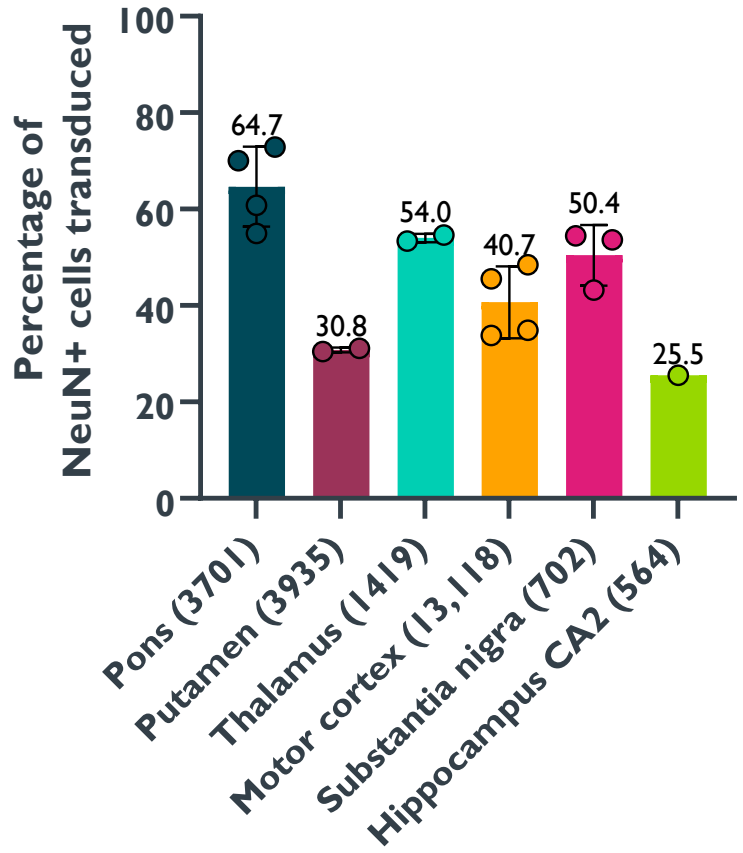
CAG Promoter, 2e13 vg/kg STAC-BBB, 19 days post administration

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

Deep-learning based cell segmentation



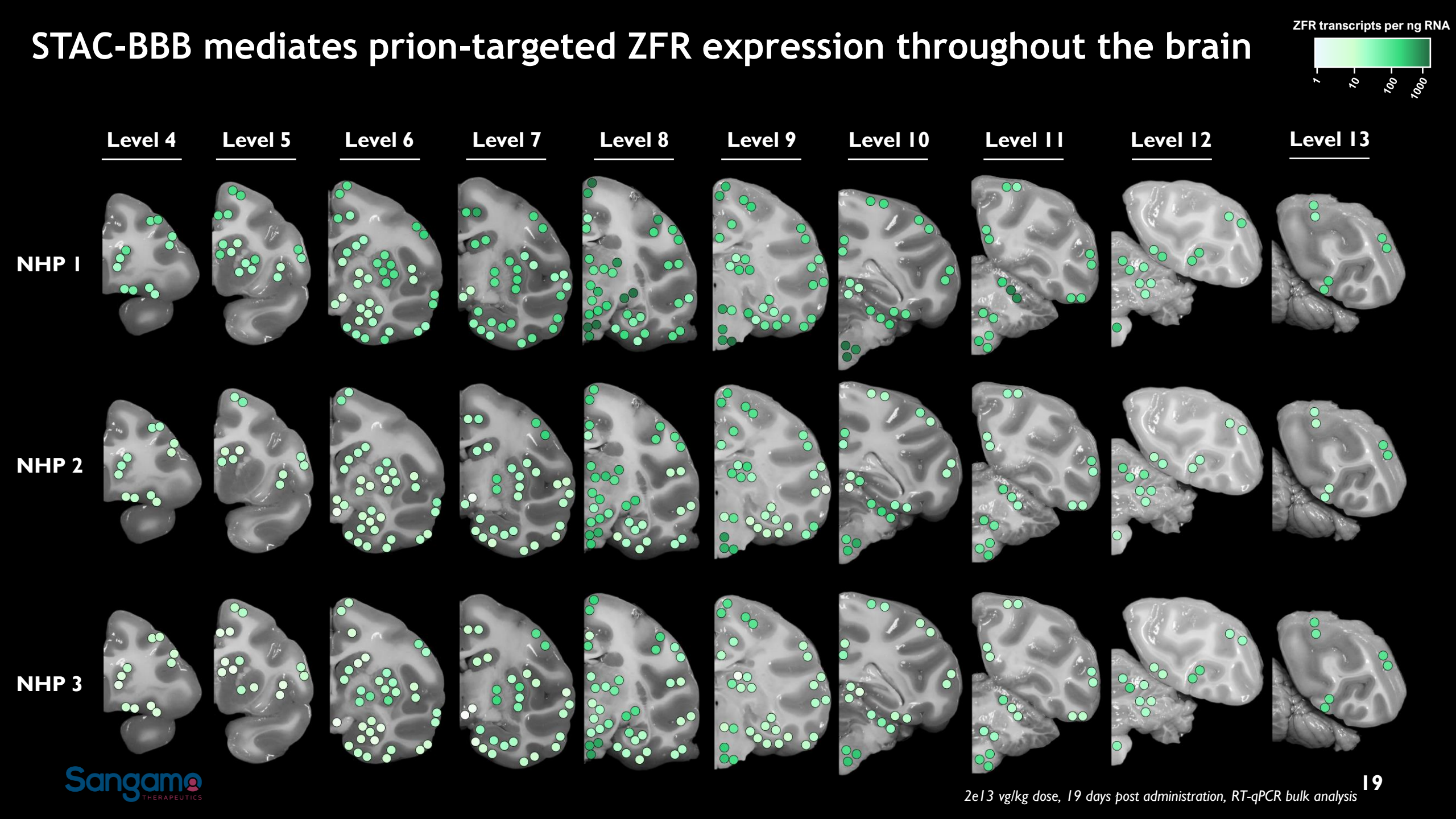
STAC-BBB NeuN+ cell transduction



Number of NeuN+ cells counted per structure is in parenthesis

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

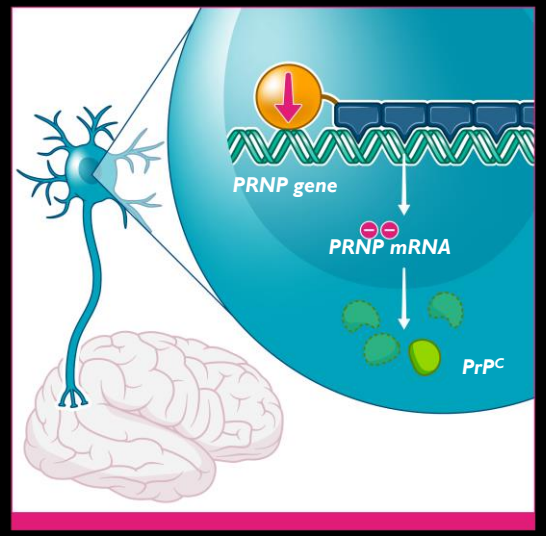
STAC-BBB mediates prion-targeted ZFR expression throughout the brain



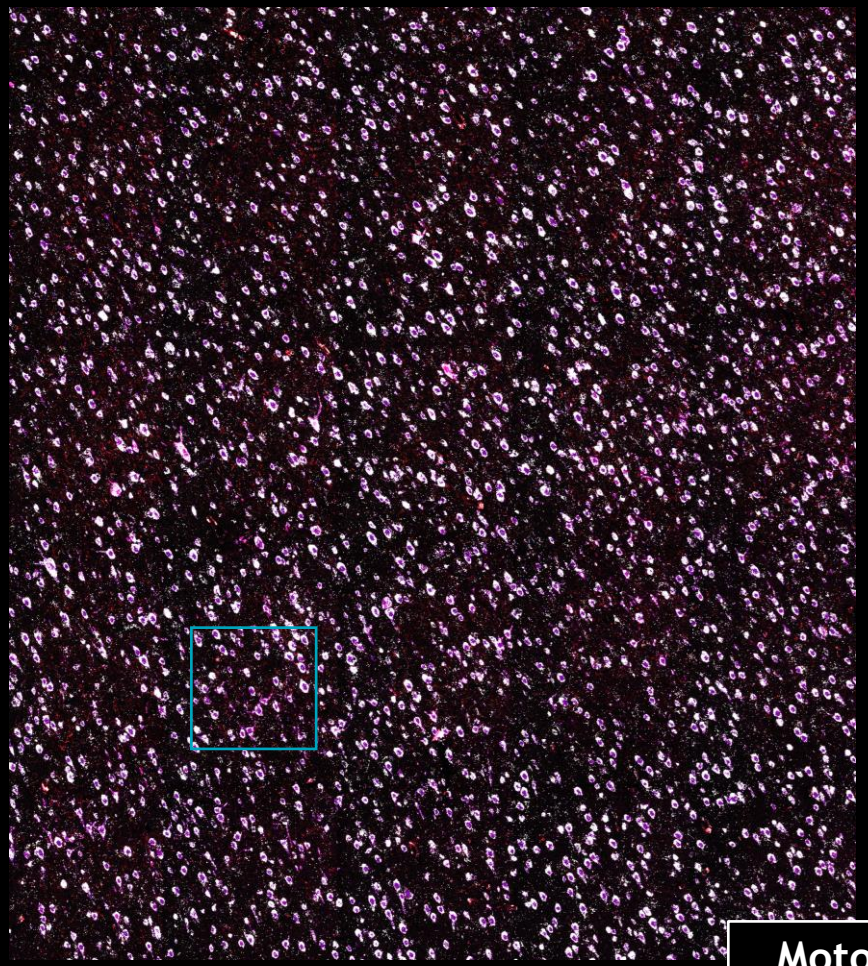
2e13 vg/kg dose, 19 days post administration, RT-qPCR bulk analysis

STAC-BBB mediates ZFR expression and Prion repression in neurons

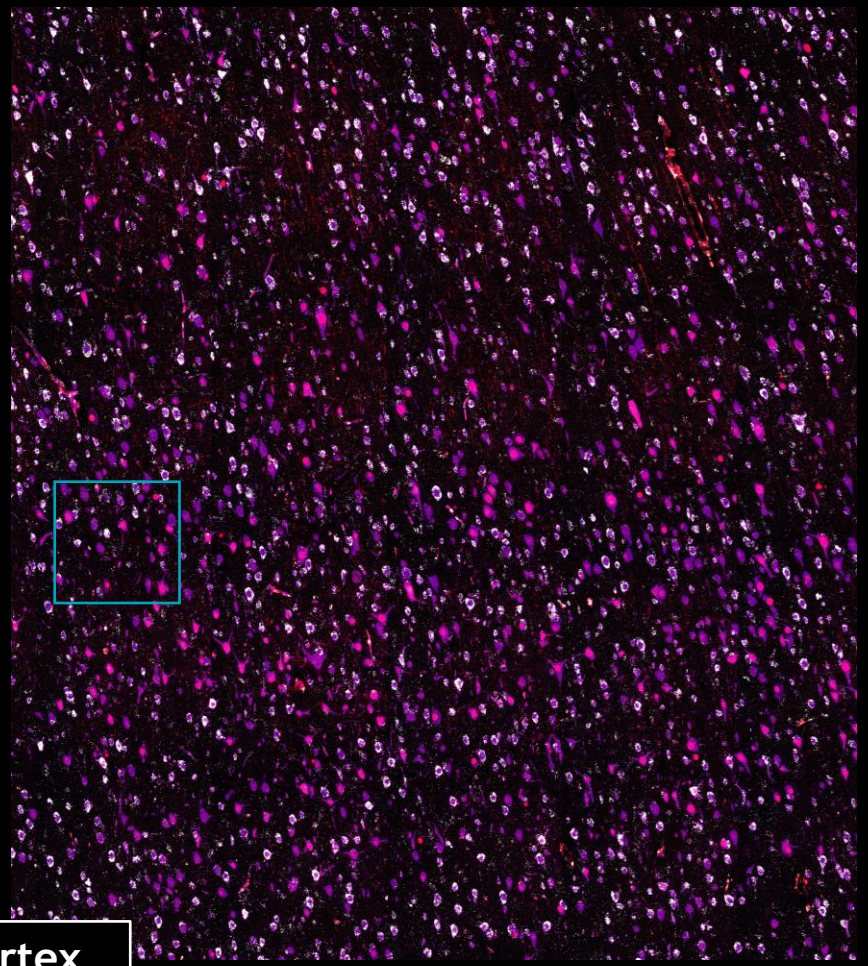
GFP
Neurons (NeuN)
Prion mRNA



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

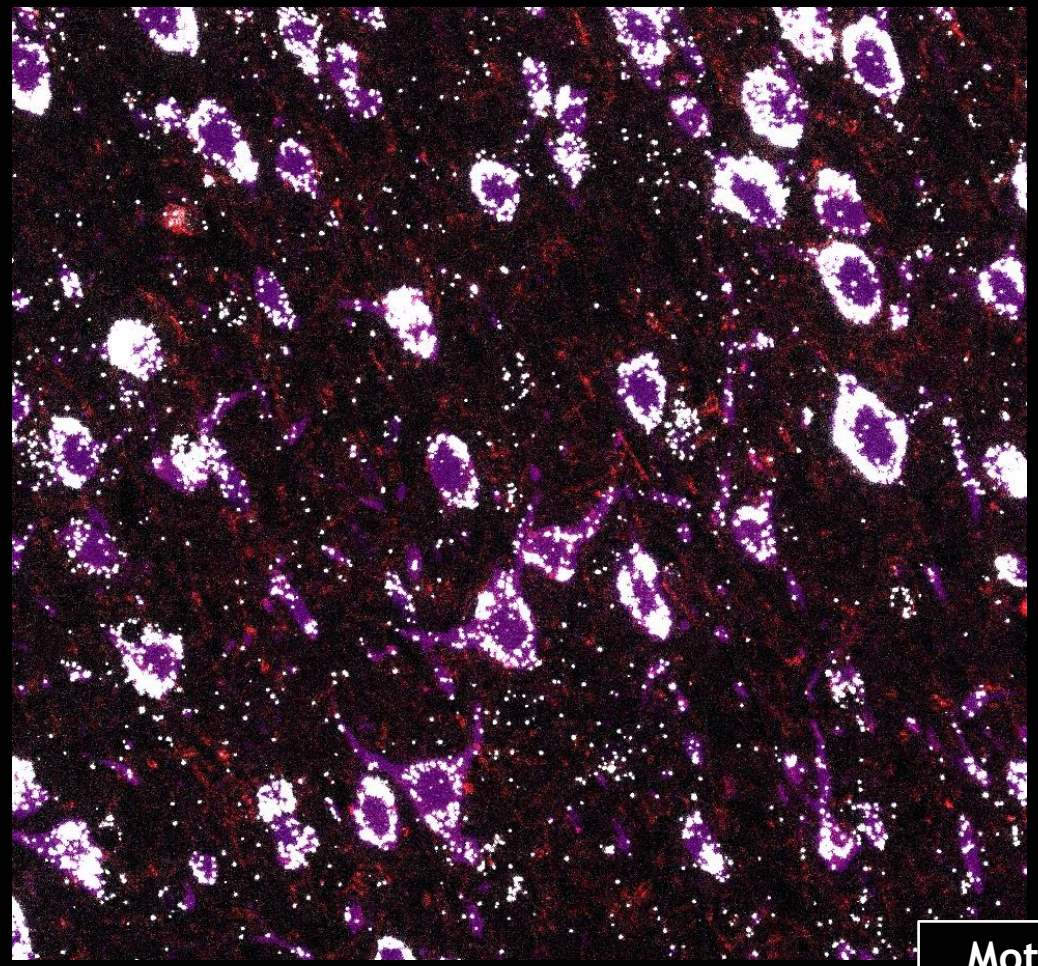
STAC-BBB mediates ZFR expression and Prion repression in neurons

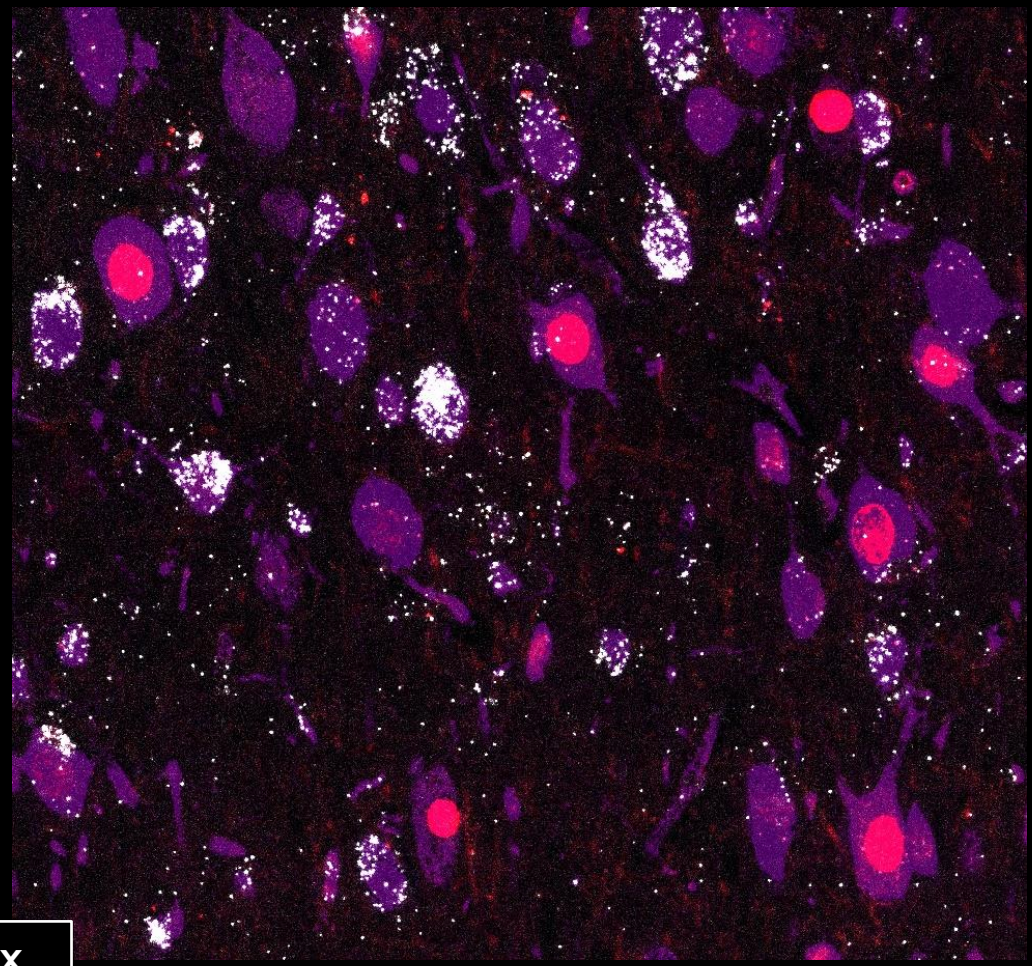
GFP
Neurons (NeuN)
Prion mRNA

Vehicle Control

STAC-BBB

Motor cortex





Motor cortex

Multiplexed RNAscope ISH / IHC assay for NeuN, GFP, PRNP mRNA, and ZFR mRNA
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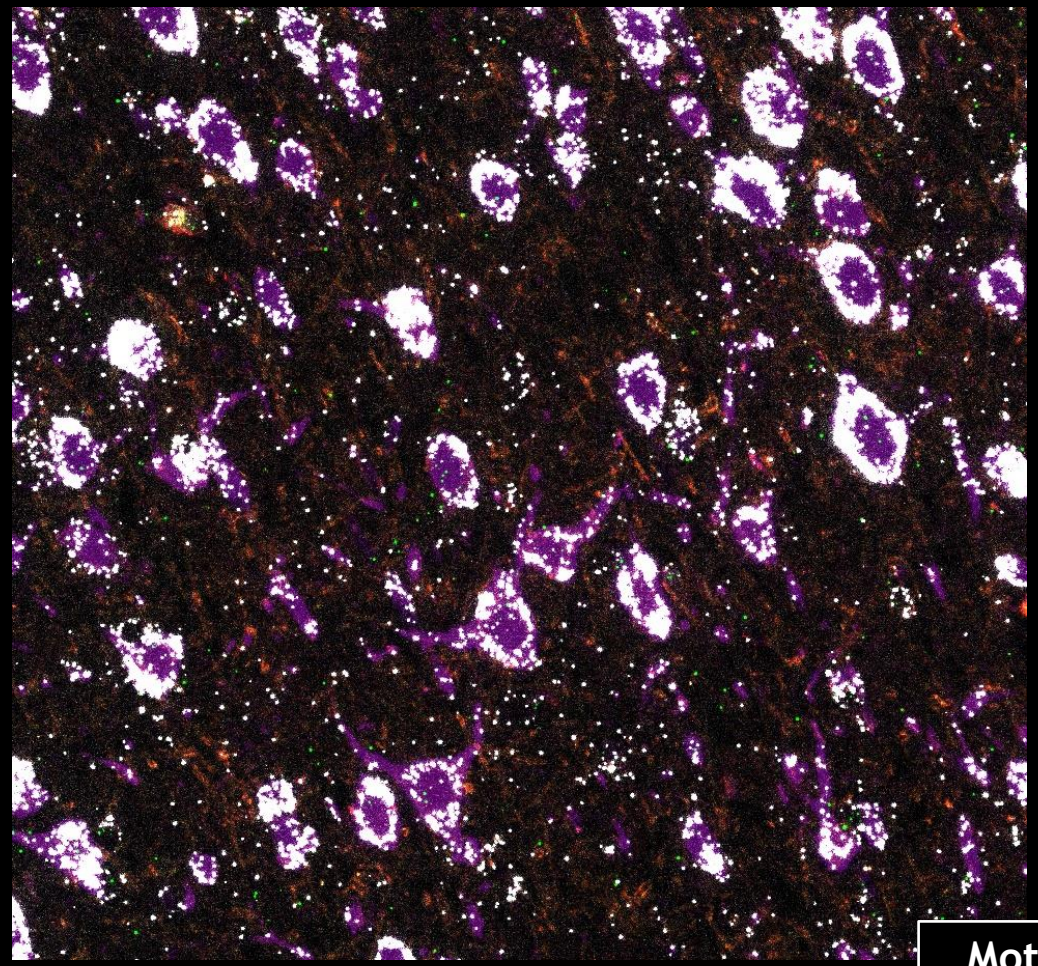
STAC-BBB mediates ZFR expression and Prion repression in neurons

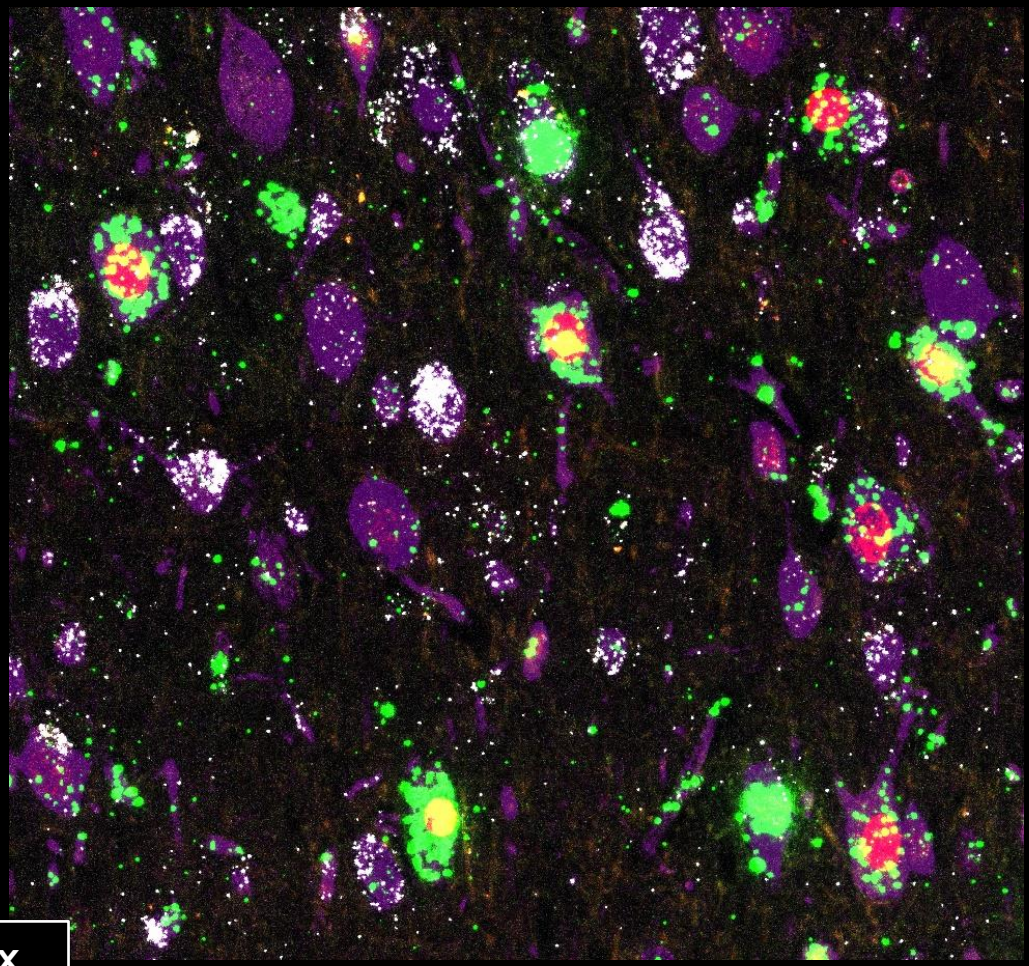
GFP
Neurons (NeuN)
Prion mRNA
ZFR mRNA

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

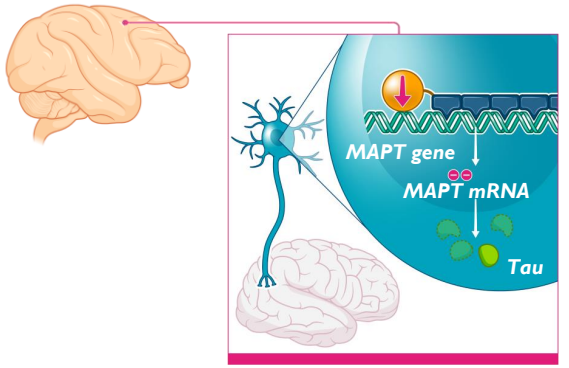
STAC-BBB

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

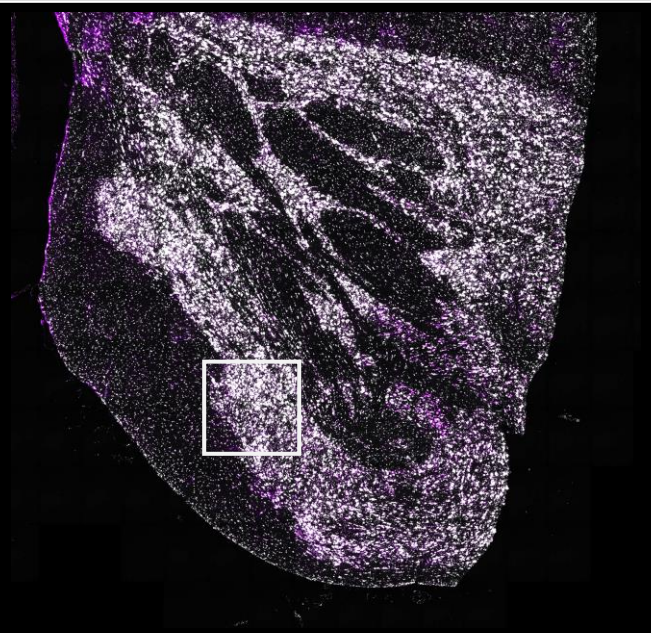
Endpoints

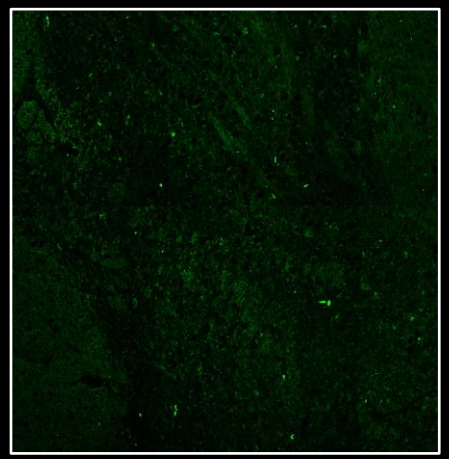
- RNAscope images
- Molecular analyses

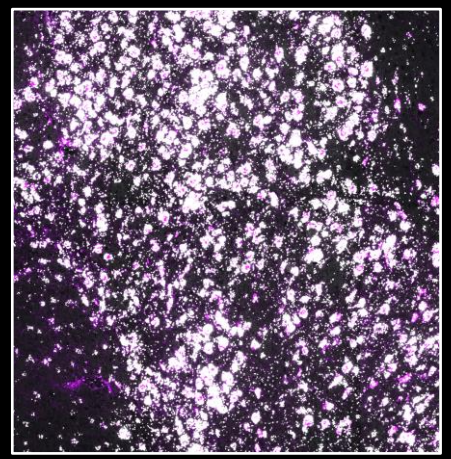
STAC-BBB mediates robust repression of neuronal tau in the pons

ZFR mRNA
Neurons (NeuN)
Tau mRNA (MAPT)

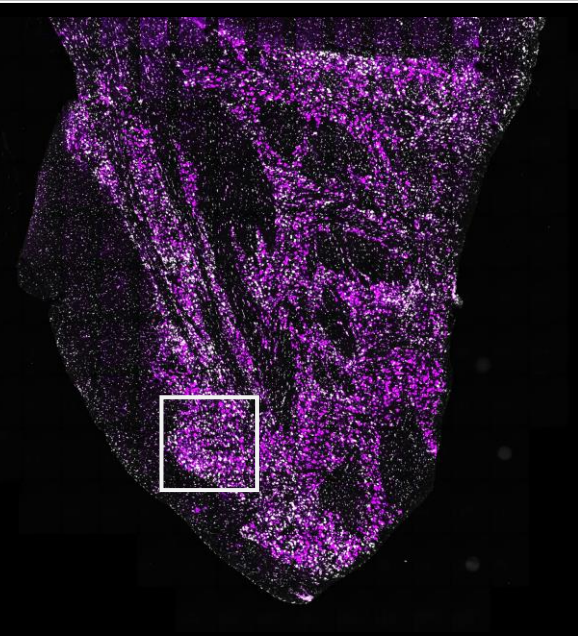
Vehicle Control

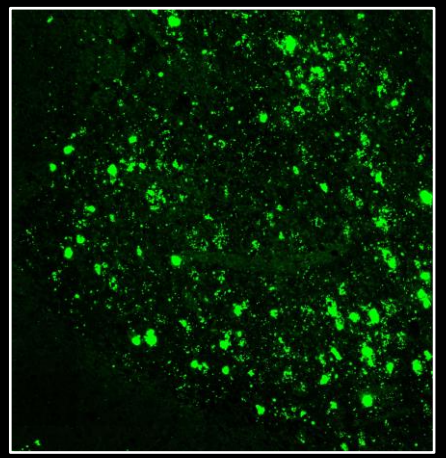


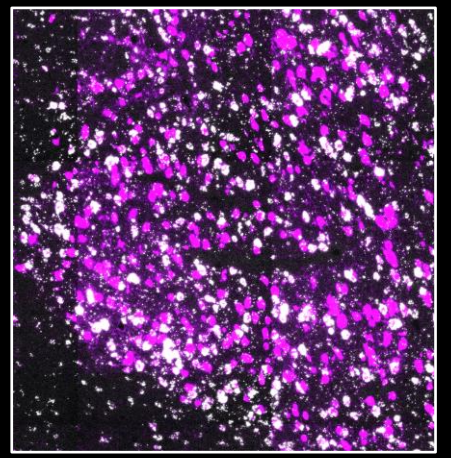




STAC-BBB







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

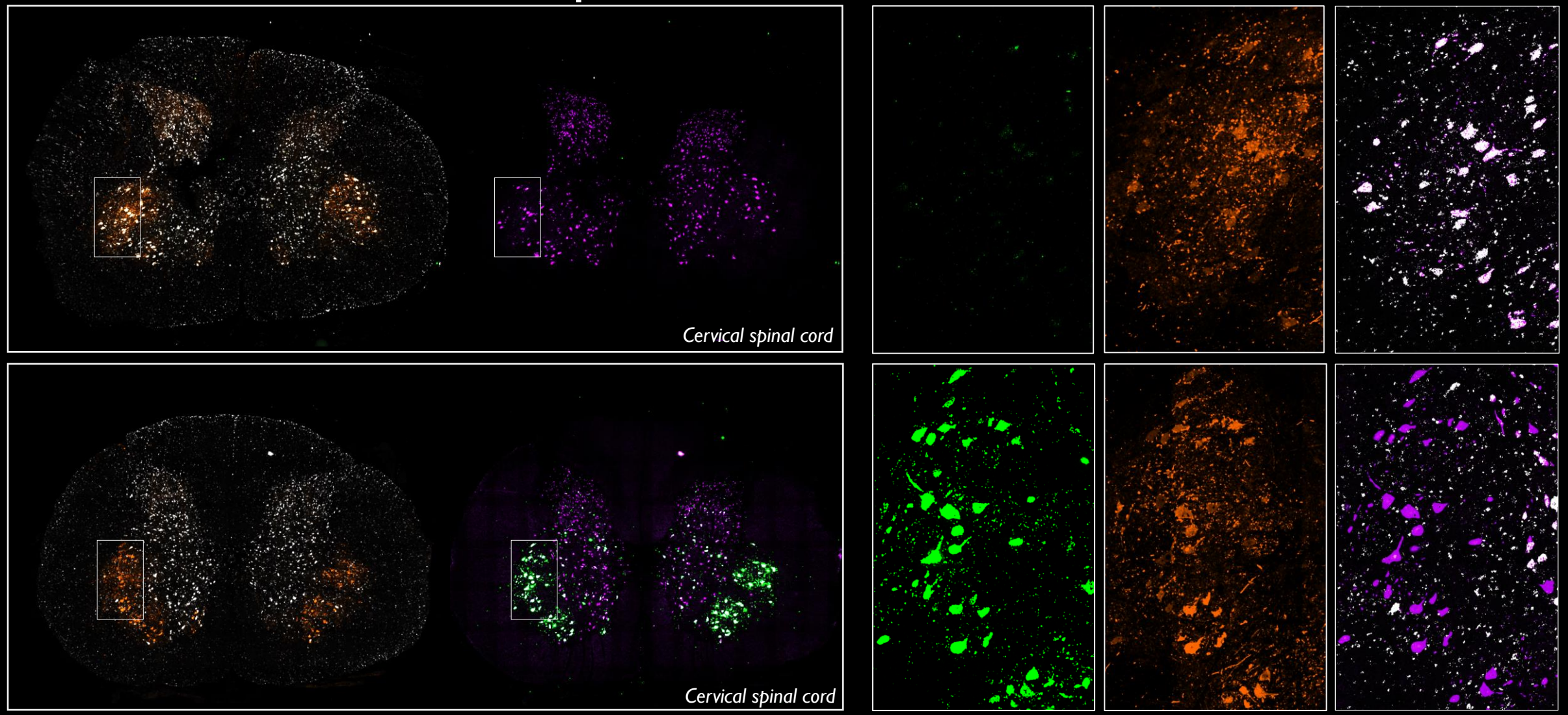
Sangamo THERAPEUTICS

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

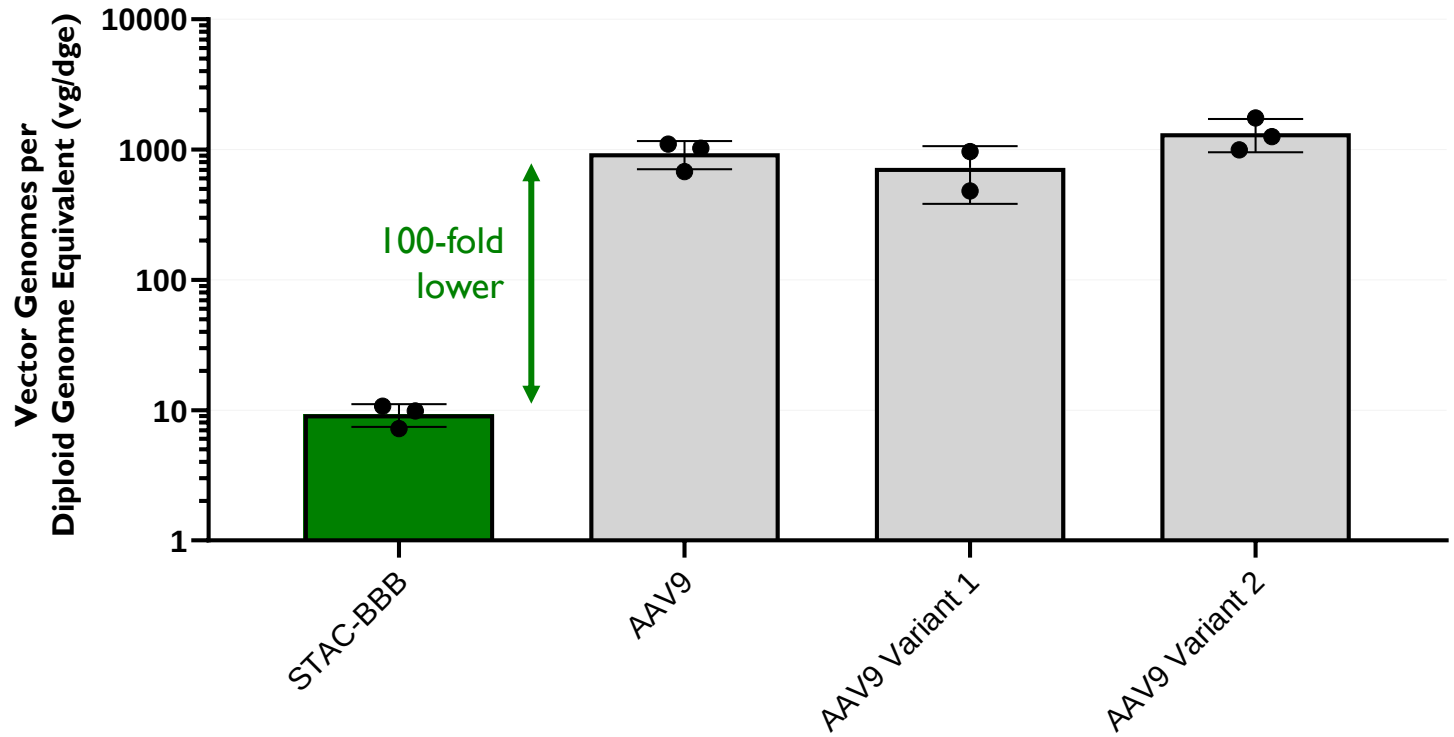


Percentage of ChAT+NeuN+ motor neurons transduced in the ventral horn: Cervical 95%, Thoracic 84%, Lumbar 98%

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

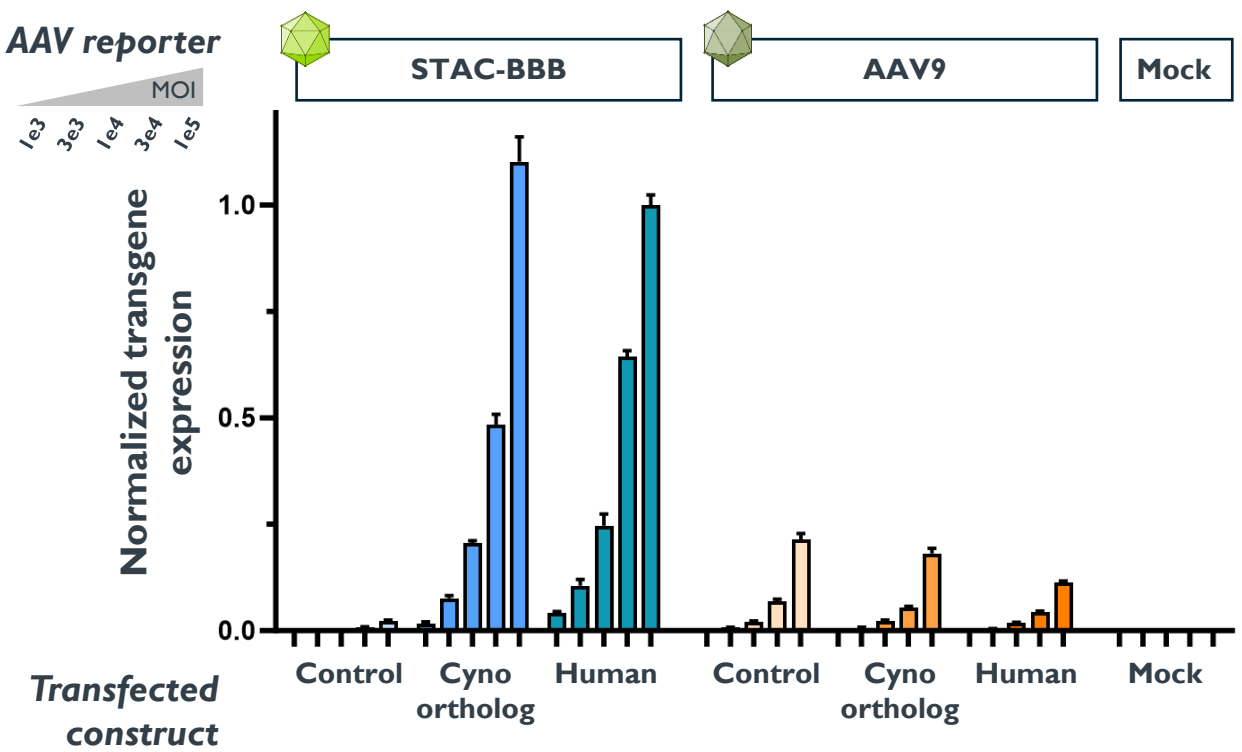
High liver exposure after intravenous administration is a limitation of conventional AAV serotypes including AAV9

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

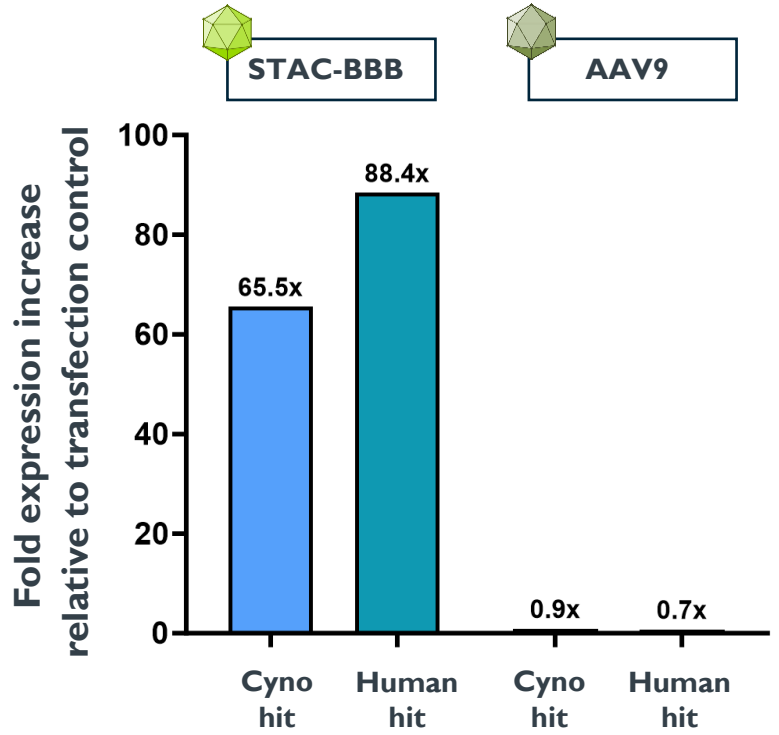
Overexpression of putative receptor confers a gain-of-function for STAC-BBB transduction *in vitro*

Transgene expression – RT-qPCR



HEK293 cells
Transgene expression evaluated 48 hr post transduction

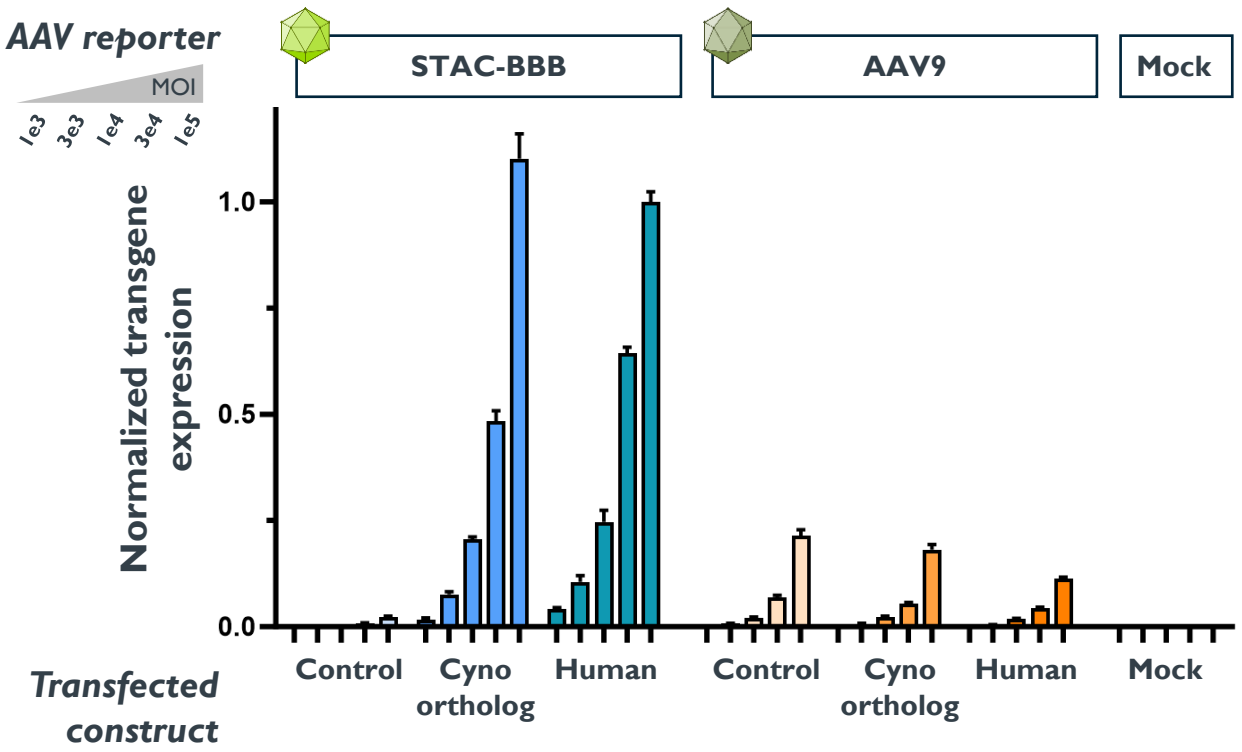
Fold increase relative to control



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.

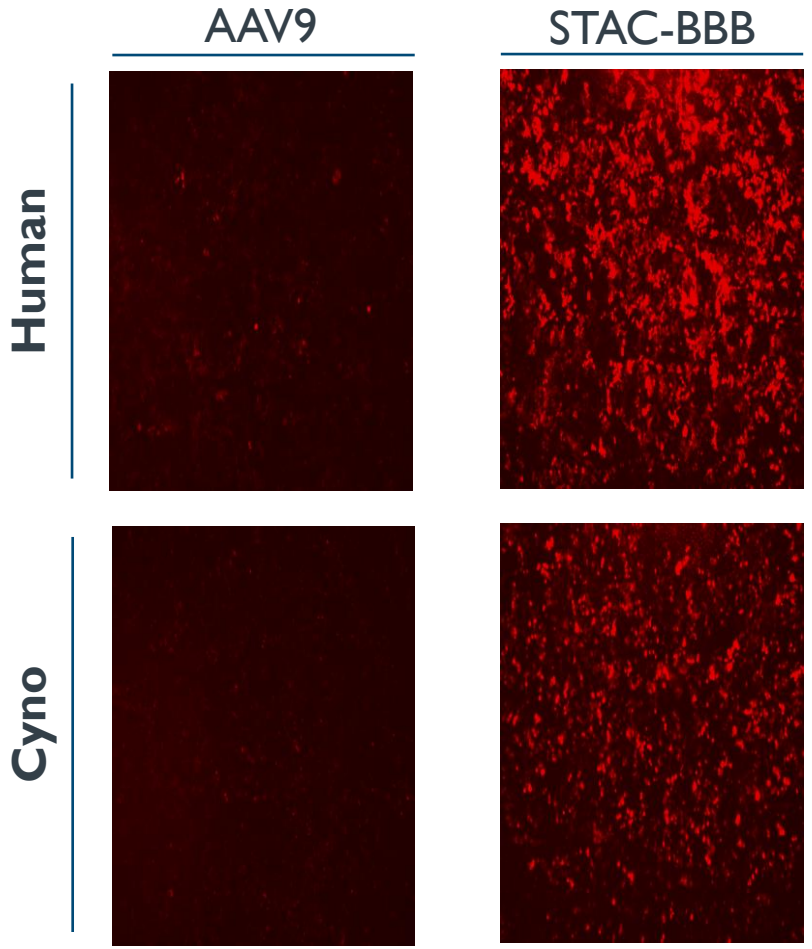
Overexpression of putative receptor confers a gain-of-function for STAC-BBB transduction *in vitro*

Transgene expression – RT-qPCR

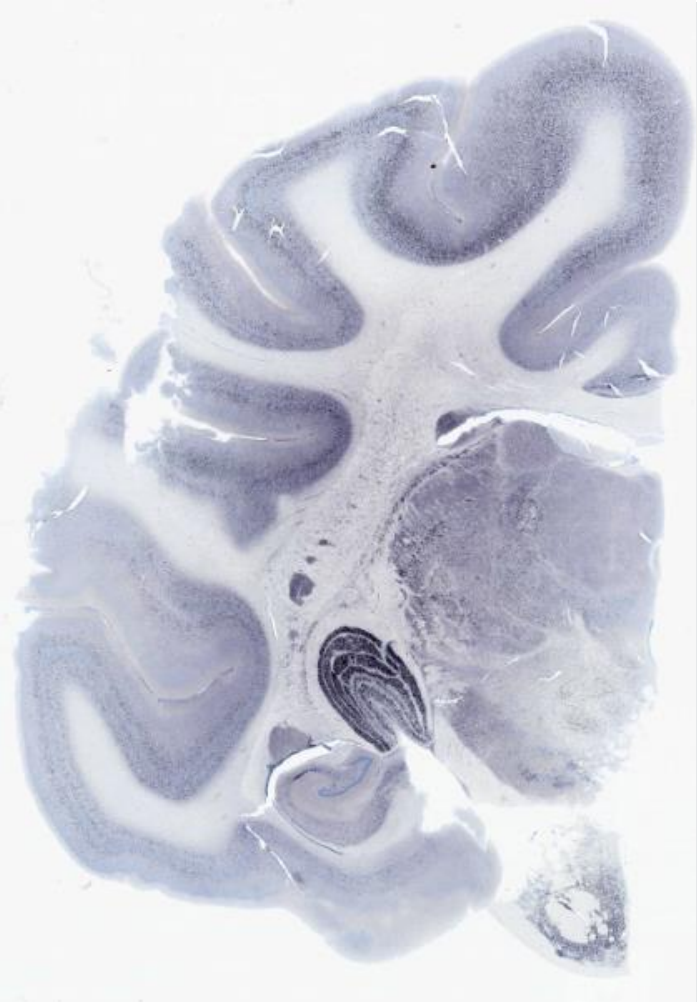


HEK293 cells
Transgene expression evaluated 48 hr post transduction

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**