Sustained Brain-wide Reduction of Prion via Zinc Finger Repressors in Mice and Nonhuman Primates as a Potential One-Time Treatment for Prion Disease

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Some illustrations were created with BioRender.com

Disclosure

I am a full-time employee of Sangamo Therapeutics

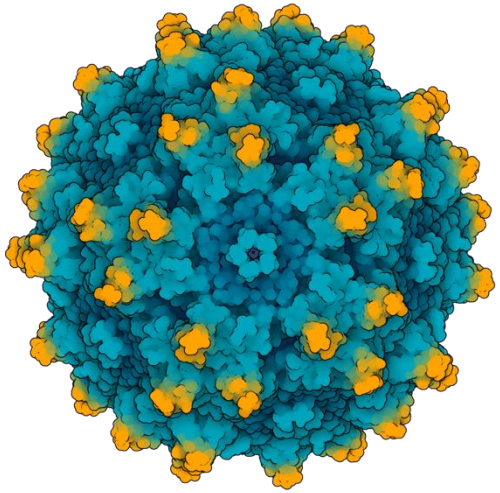
Sangamo pairs epigenetic regulation and capsid delivery capabilities to create genomic medicines for neurological diseases

Zinc finger epigenetic regulators

- Most abundant DNA-binding proteins in human genome*
- Tune gene expression up, down, or off
- Cell-type specific
- No permanent genome or epigenome changes

Blood brain barrier (BBB) penetrant capsids

- Intravenous (IV) delivery
- One-time dosing
- ~700x increase in brain expression**
- ~100x de-targeted from liver**



** >700 human transcription factors that regulate the epigenome contain ZF domains*
*** Results for STAC-BBB compared to AAV9*

Sangamo THERAPEUTICS

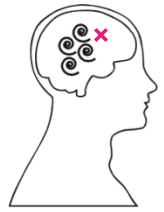
Prion disease: Rapidly progressing and fatal with no effective treatments

- Rapidly progressing disease, can strike at any age
- Pronounced brainwide neurodegeneration
- Cognitive, psychiatric and motor symptoms
- Median survival is 5 months
- No disease-modifying therapies

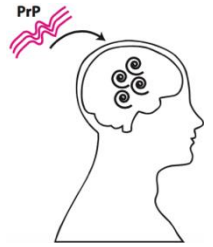
~1,300 new cases each year
in the US and Europe *

3 forms account for
nearly all cases

85%
Sporadic



15%
Genetic



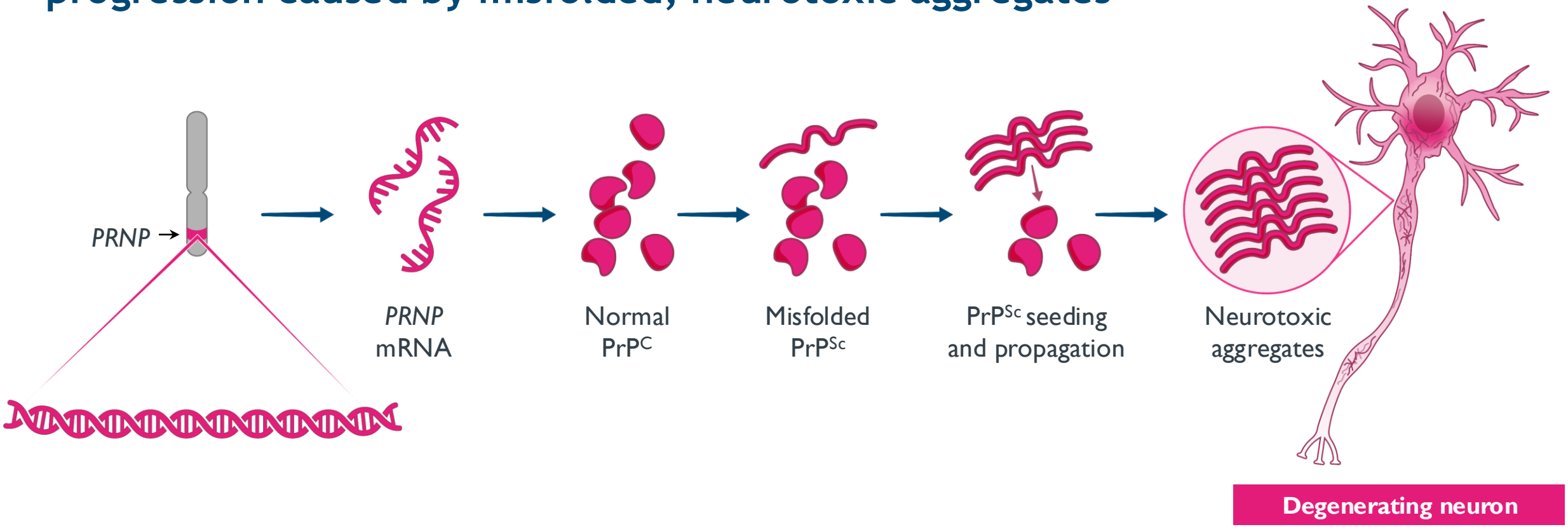
<1%
Transmitted

Exceptionally high unmet medical need and rapid clinical POC for our genomic medicine platforms

** US (per CDC) and Europe (https://www.eurocjd.ed.ac.uk/)*
Mead et al., 2019; Maddox et al., 2020; Corbie et al., 2022; Hermann et al., 2022

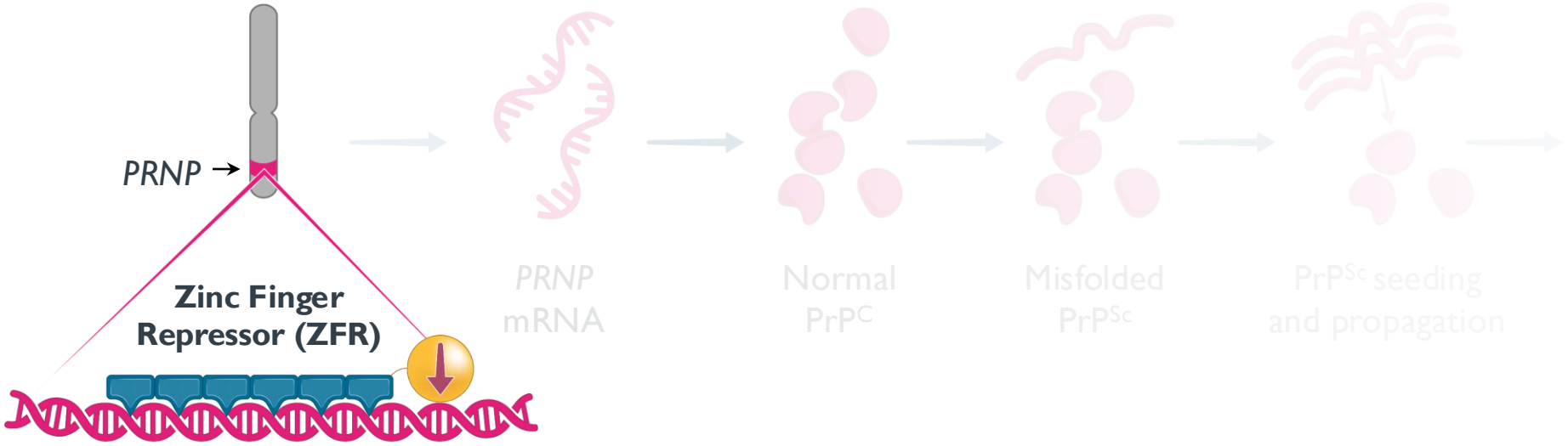
Sangamo THERAPEUTICS

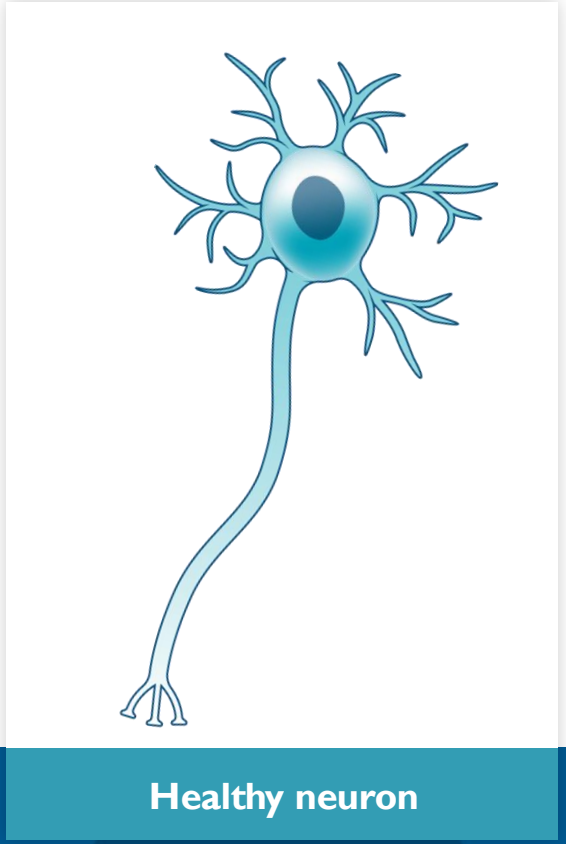
Lowering prion protein (PrP) can slow and prevent disease progression caused by misfolded, neurotoxic aggregates



Prusiner 1982; Bueler et al., 1992, 1993, 1994; Sailer et al., 1994; Brandner et al., 1996; Mallucci et al., 2003; Richt et al., 2007; Yu et al., 2009; Benestad et al., 2012; Raymond et al., 2019; Salvesen et al., 2020; Minikel et al., 2020; Vallabh et al., 2020; Lakkaraju et al., 2022; Gentile et al., 2024; An et al., 2025

Lowering prion protein (PrP) can slow and prevent disease progression caused by misfolded, neurotoxic aggregates





Healthy neuron

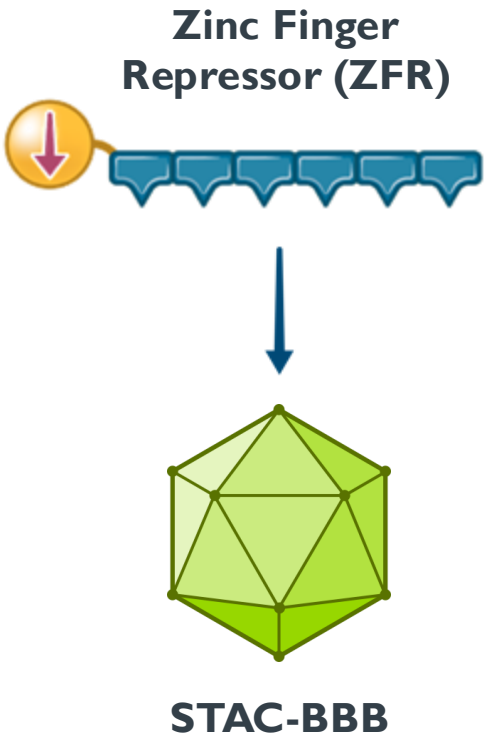
Excellent fit for a ZFR approach that blocks PrP expression at the DNA level

- Prion knock-out (KO) animals do not get disease
- Prion KO is well tolerated (including in mice, rats, goats, sheep, cows)
- Neuronal PrP removal is sufficient to prevent disease
- Pre-symptomatic PrP lowering in mice can substantially extend survival

Prusiner 1982; Bueler et al., 1992, 1993, 1994; Sailer et al., 1994; Brandner et al., 1996; Mallucci et al., 2003; Richt et al., 2007; Yu et al., 2009; Benestad et al., 2012; Raymond et al., 2019; Salvesen et al., 2020; Minikel et al., 2020; Vallabh et al., 2020; Lakkaraju et al., 2022; Gentile et al., 2024; An et al., 2025

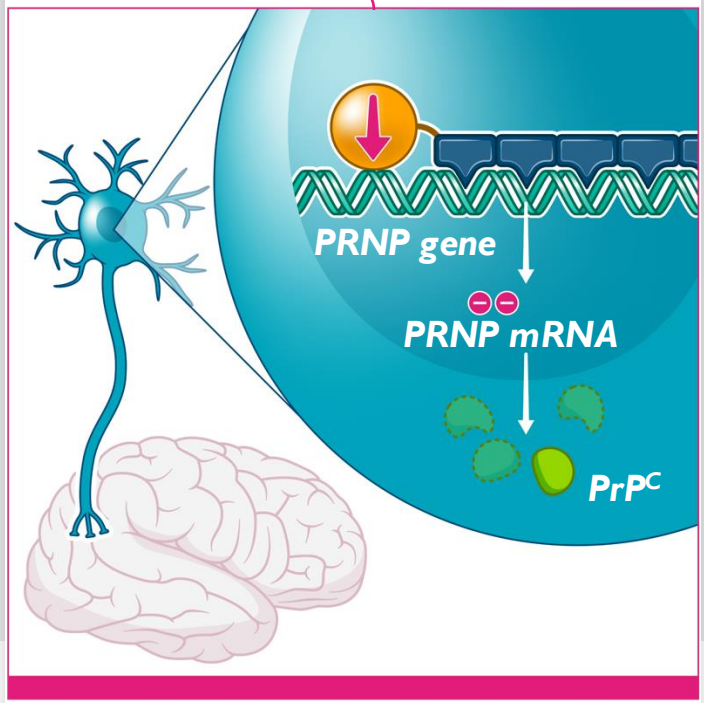
ST-506: A one-time IV-administered zinc finger repressor targeting the prion gene delivered by STAC-BBB

Neuron-targeted ZFR packaged into STAC-BBB AAV capsid



One-time IV administration

Stable PrP reduction in neurons in the brain



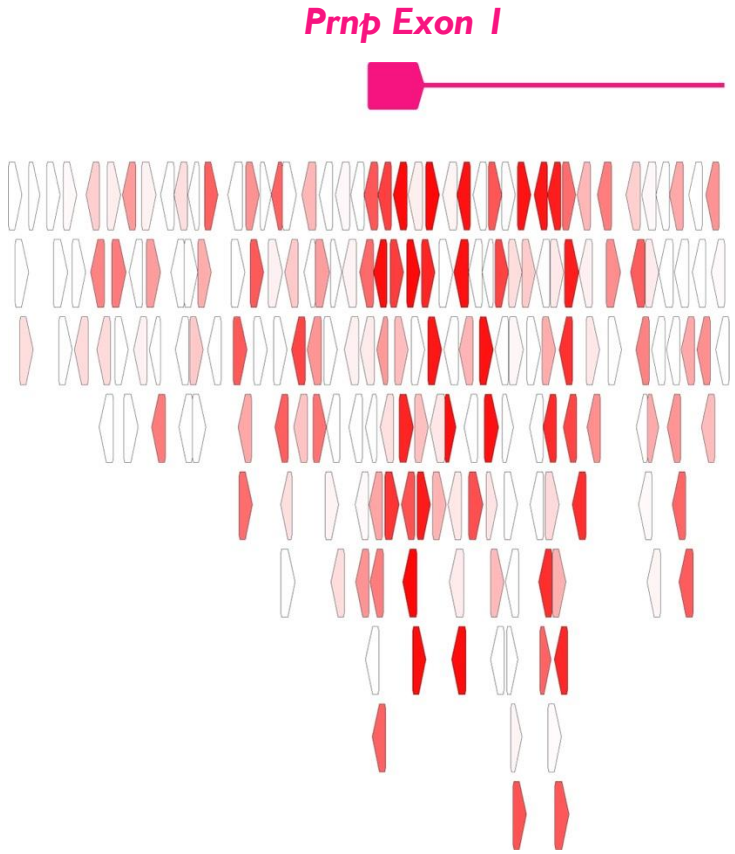
One ZFR design set yielded potent ZFRs targeting *Prnp* with no detectable off targets

>90% *Prnp* repression in screening cells

192 ZFRs

N2a cells
mRNA delivery

Prnp mRNA
0 100

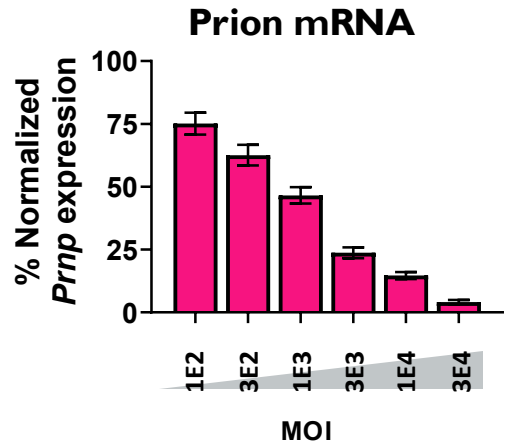


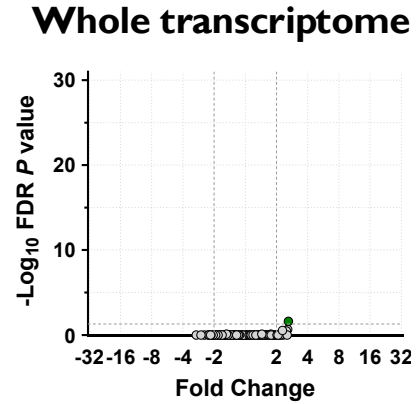
>90% *Prnp* repression in primary neurons

mZFR1

Primary mouse cortical neurons

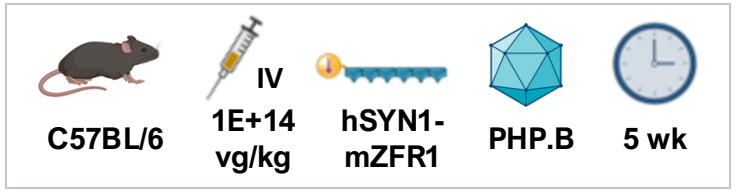
AAV6-hSYN1-mZFR1



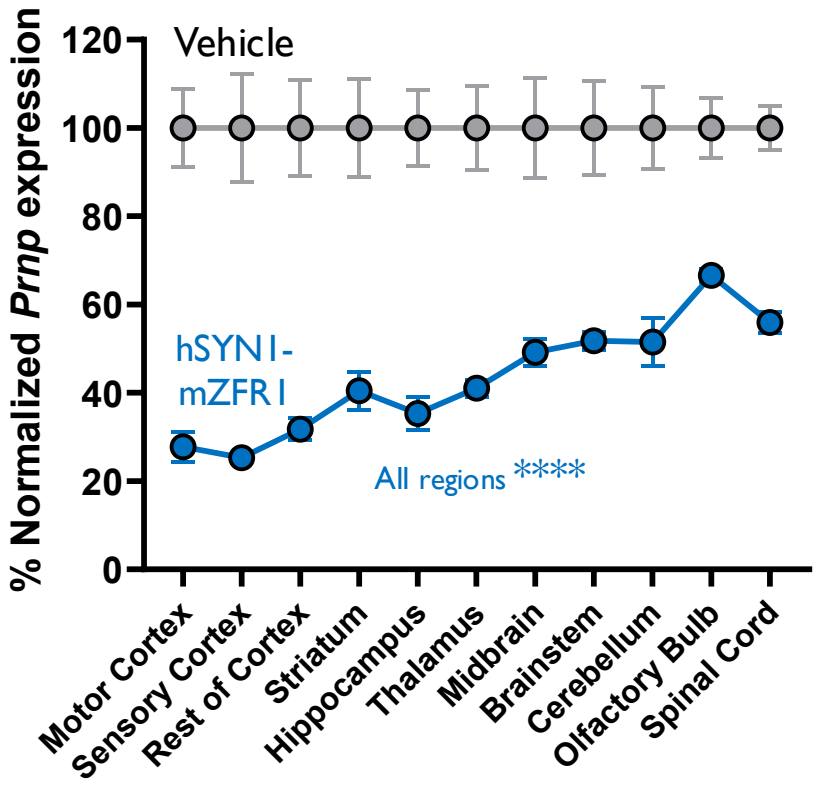


Prnp RT-qPCR data normalized to mean of Atp5b, Eif4a2 and scaled to control. Left: 1000 ng ZFR mRNA dose shown. Right: 1E+2 – 3E+4 MOIs shown.
Off-target analysis conducted using Clariom S gene arrays. N=5-6 biological replicates at 3E+3 MOI. P-values shown as False Discovery Rate (FDR).

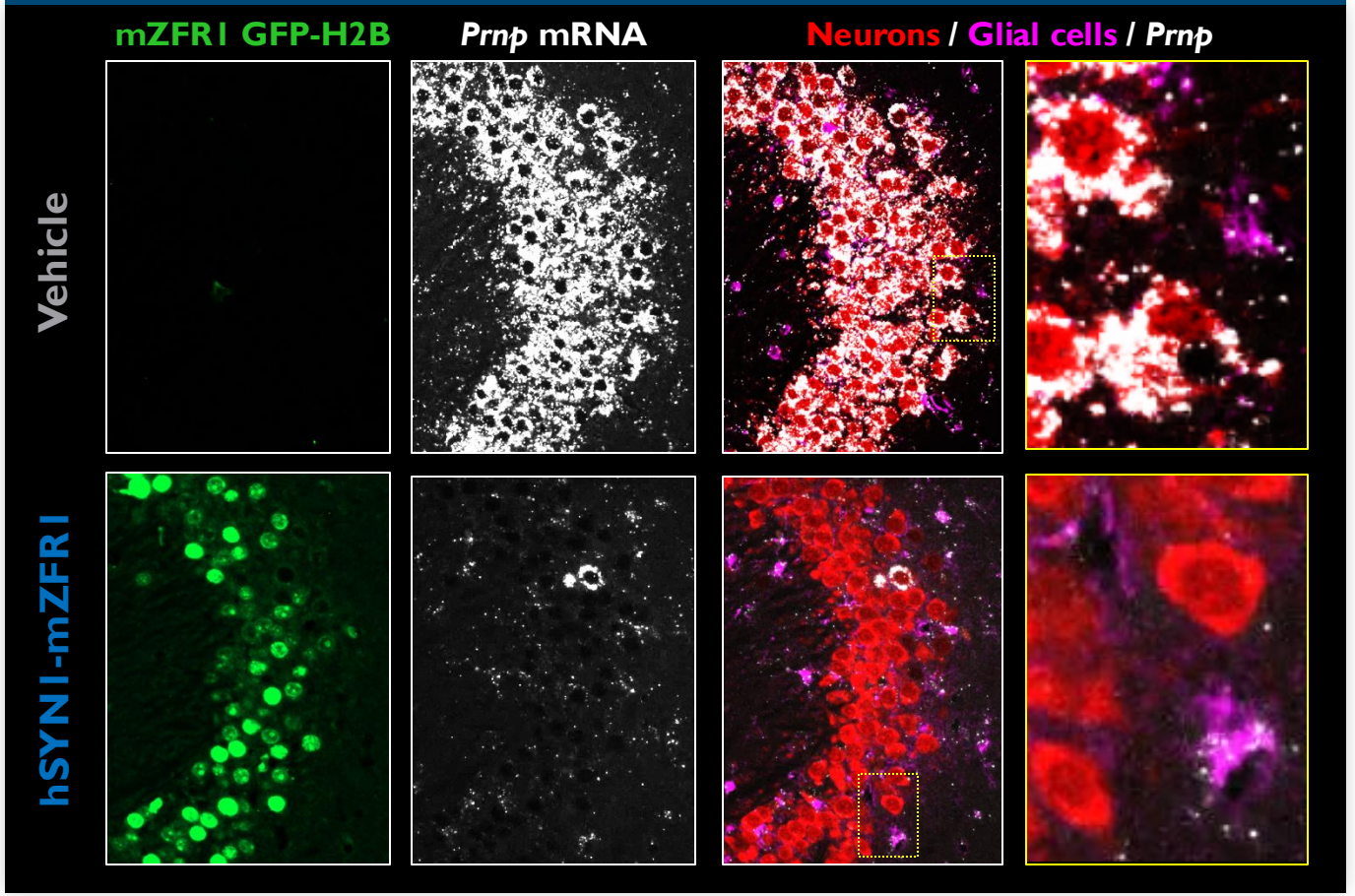
ZFRs mediate brainwide repression of *Prnp* mRNA specifically in neurons



Brainwide >40-80% *Prnp* repression



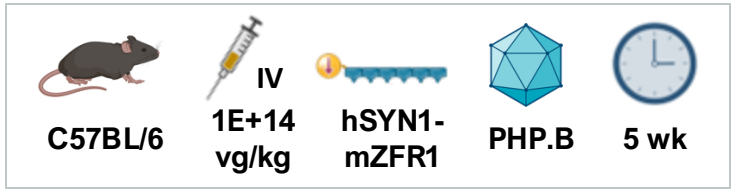
Potent, neuron-restricted *Prnp* repression



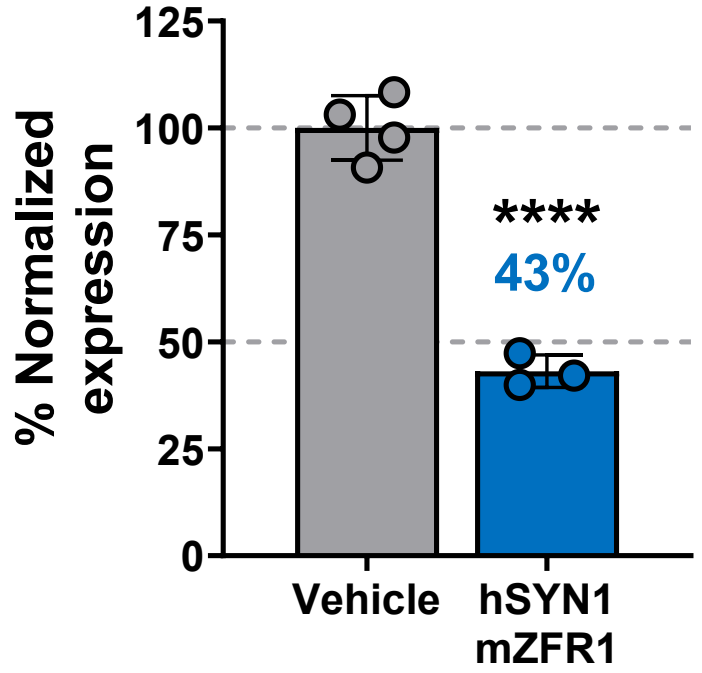
*N=7-8 mice per group. Mean ± SD; Two-way ANOVA; Sidak's multiple comparisons against to vehicle group. **** P < 0.0001. PHP.B developed by Deverman et al 2016.*
Prnp RT-qPCR data normalized to mean of Atp5b, Eif4a2, Gapdh and scaled to the Vehicle group mean.
Multiplexed IHC for neurons (NeuN), glial cells (S100b) and GFP with RNAscope for Prnp. Representative images shown for hippocampal CA3 region.

Sangamo THERAPEUTICS

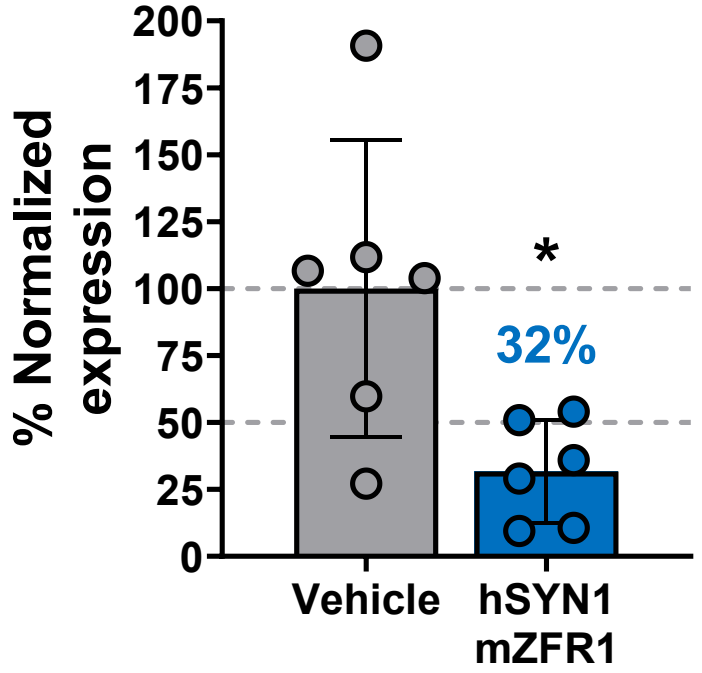
ZFRs mediate whole brain and CSF prion protein reduction in mice



>50% PrP knockdown in mouse brain

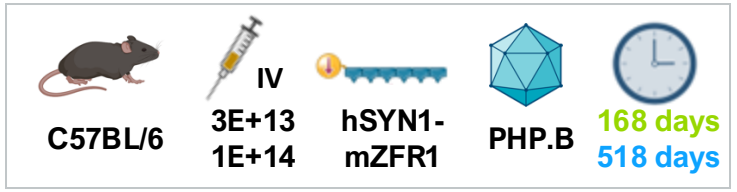


>60% PrP knockdown in CSF

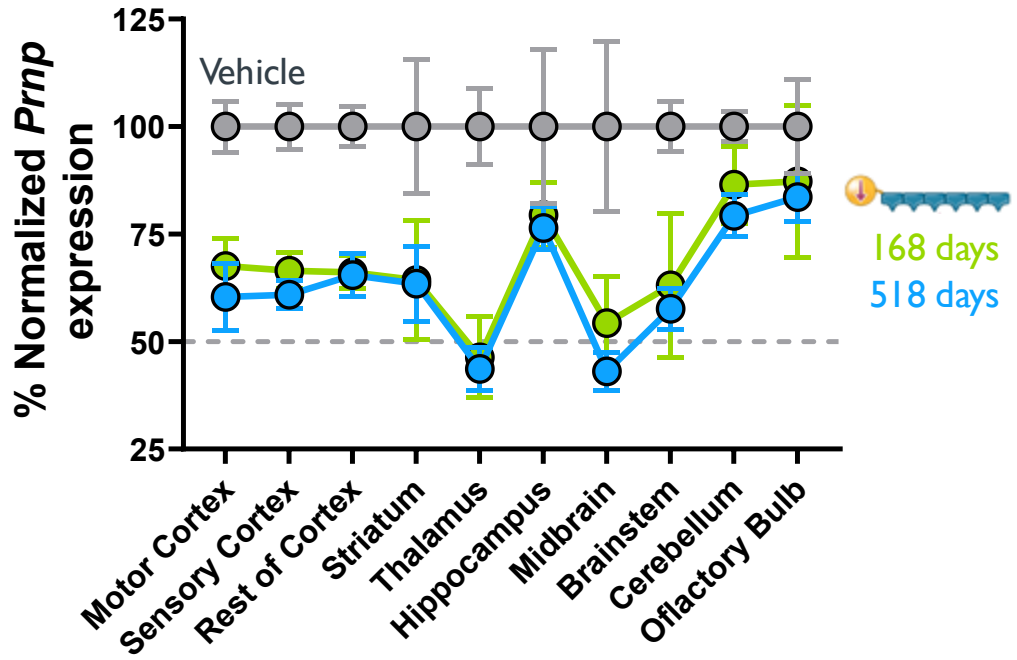


*Brain PrP ELISA: N=3-4 mice per group. Mean ± SD; Unpaired t test. **** P < 0.0001.*
*Cerebrospinal fluid (CSF) PrP ELISA: N=6 mice per group. Mean ± 95%CI; Mann Whitney test. * P < 0.05.*

Prion mRNA and protein reduction sustained for at least 17 months across the mouse brain

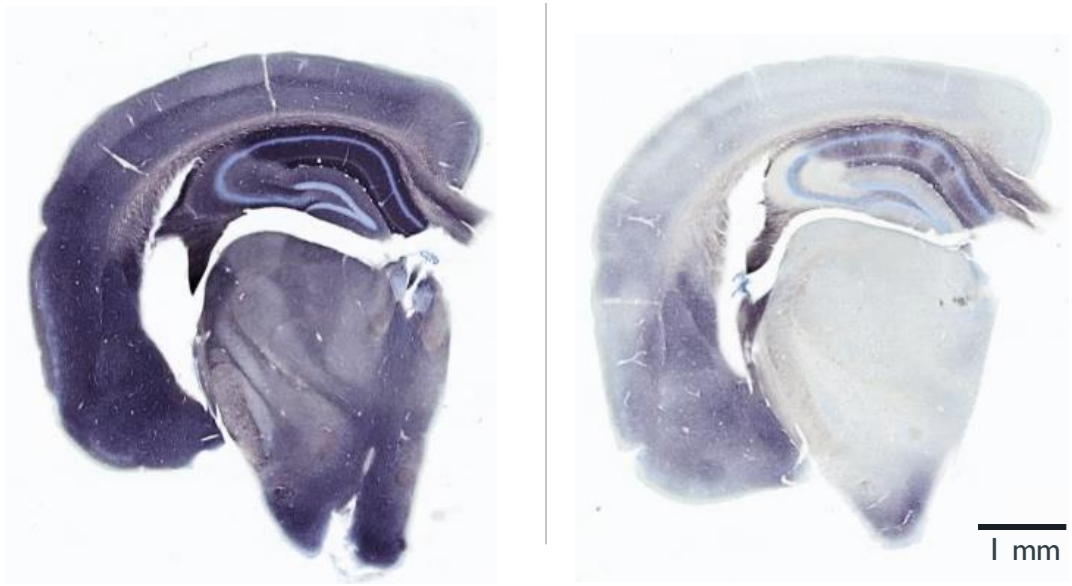


Sustained brainwide *Prnp* mRNA repression



3E+13 vg/kg dose shown for both time points; this was the only dose evaluated at 518 days for RT-qPCR.

Sustained brainwide PrP knockdown at 518 days

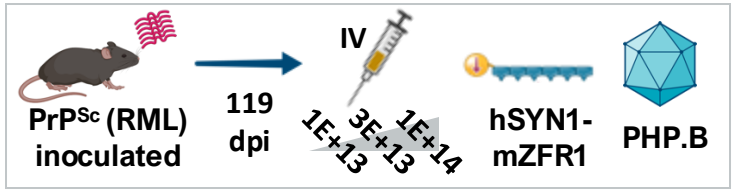


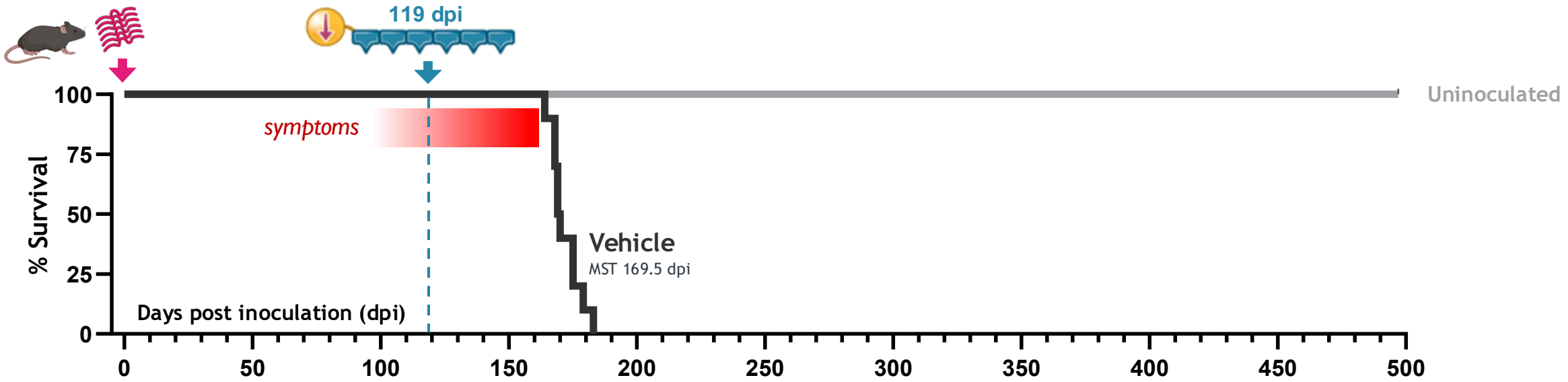
1E+14 vg/kg dose shown.

Persistent ZFR activity suggests the potential for lifelong PrP suppression in neurons

Prnp mRNA: N=10 mice per group. Mean ± SD; Two-way ANOVA with Dunnett's multiple comparisons. P < 0.0001 for all regions except P < 0.01 for cerebellum and P < 0.05 for olfactory bulb.
PrP IHC: N=5 mice per group. Anti-PrP (DAB, black). Nissl staining (blue). IHC data were only collected at the 518 day timepoint.

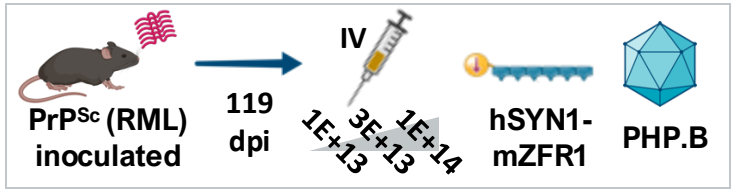
ZFRs mediate a profound survival extension in prion disease mice treated after symptom onset

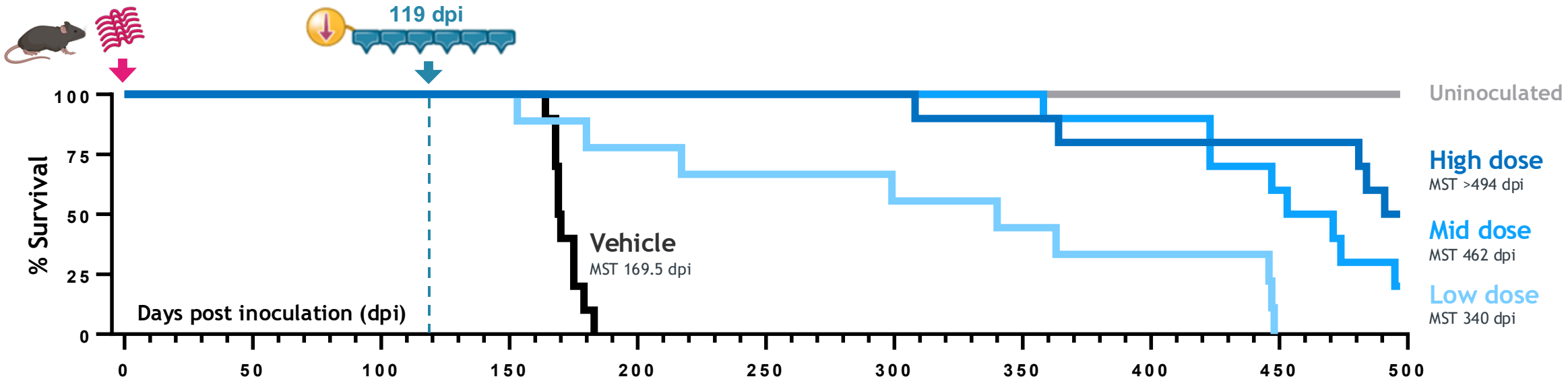


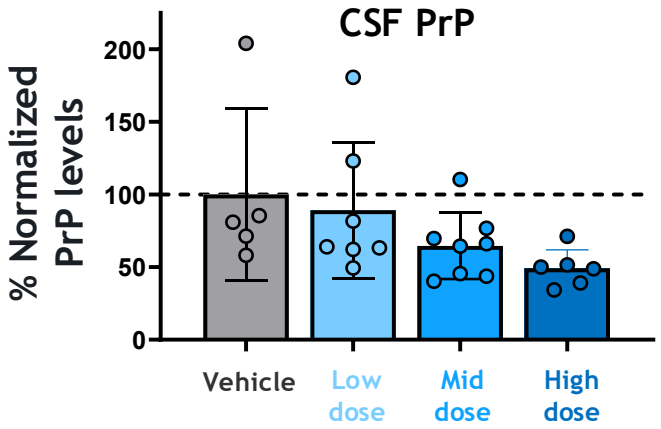


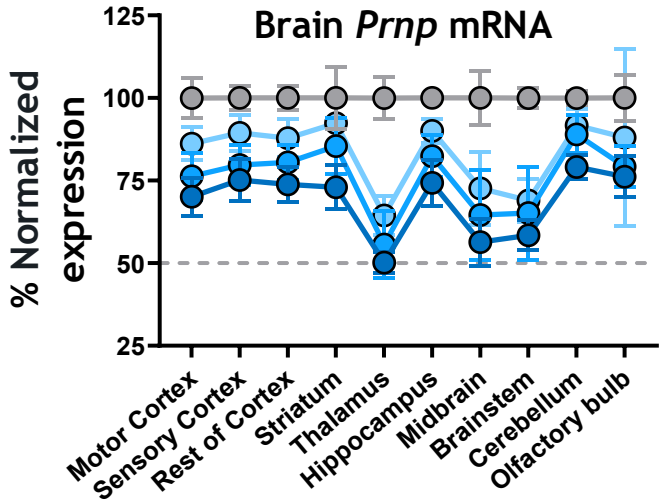
Mice inoculated with RML PrP^{Sc} at 0 dpi received hSYN1-mZFR1 or vehicle (n = 10 per group) at 119 dpi. Survival and body weight were monitored to 497 dpi, the predetermined end of study. Uninoculated mice received vehicle at -21 dpi (n = 5). For Prnp/PrP analyses, uninoculated mice received vehicle or hSYN1-mZFR1 at -21 and 119 dpi, respectively. Tissues were collected at 301 dpi.

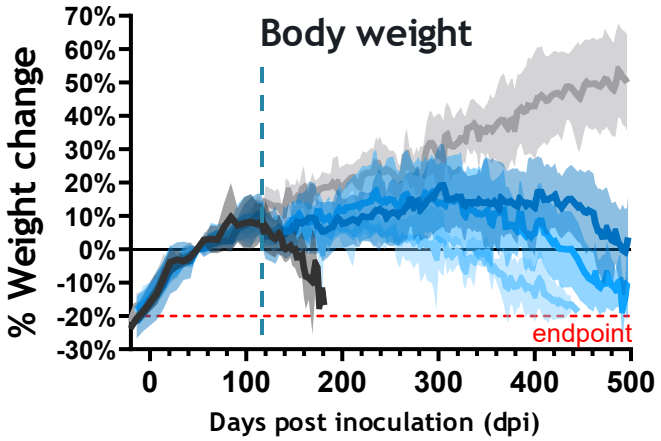
ZFRs mediate a profound survival extension in prion disease mice treated after symptom onset











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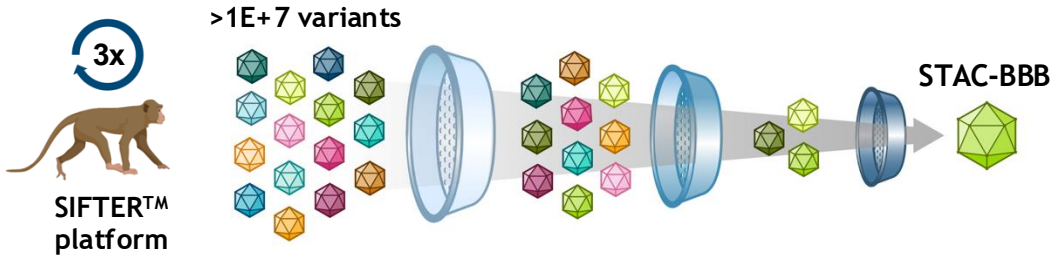
Our discovery of STAC-BBB potentially enables translation of the ZFR approach to humans

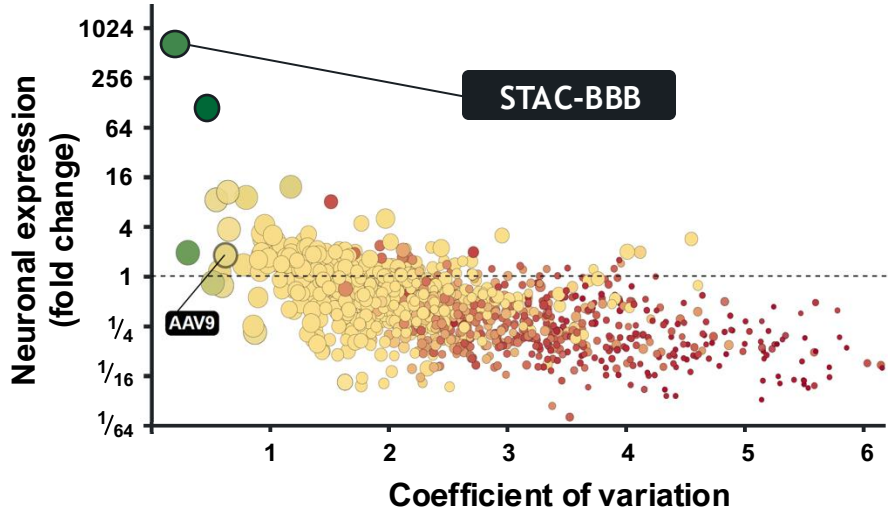
Mouse POC studies

PHP.B
Works exclusively in mice

Brain delivery for NHPs and humans

STAC-BBB
Engineered for human therapeutics





PHP.B (Deverman et al 2016)

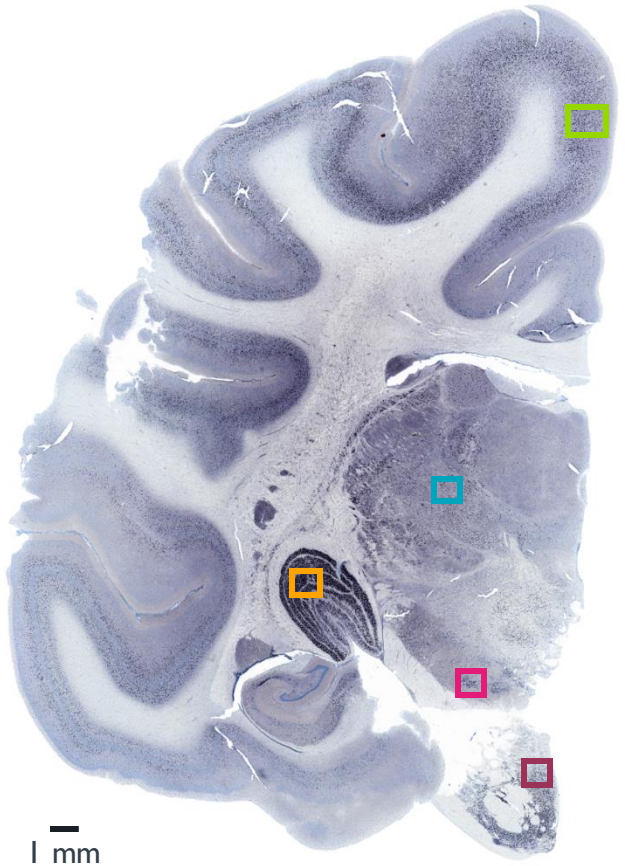
Sangamo's lead blood-brain-barrier penetrant capsid STAC-BBB (Tiffany et al 2024)

Sangamo THERAPEUTICS

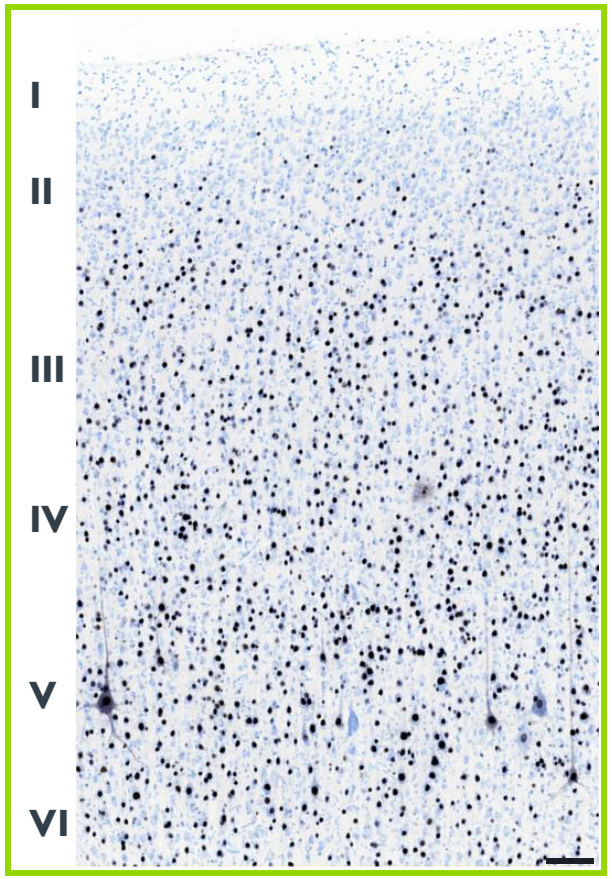
STAC-BBB drives widespread and robust expression throughout the nonhuman primate brain

STAC-BBB

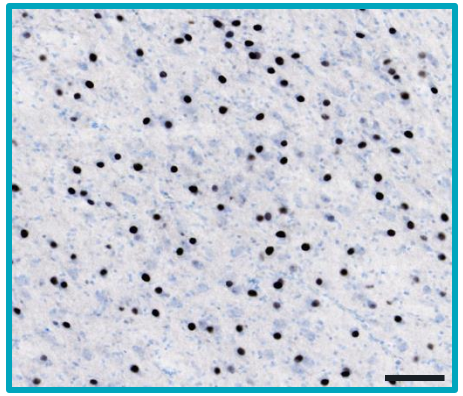
STAC-BBB
(Nuclear-localized GFP)



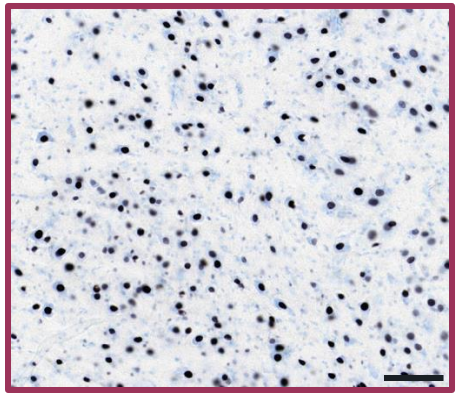
Motor Cortex



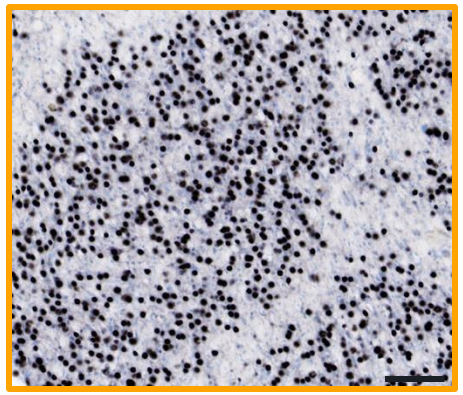
Thalamus



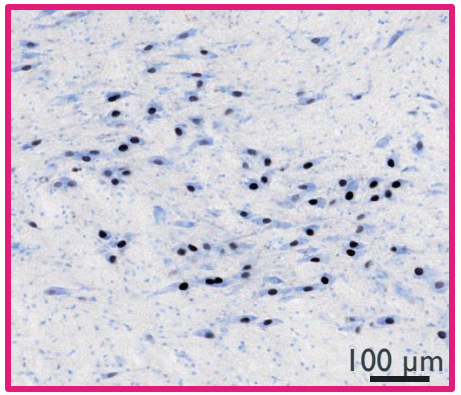
Pons



Lateral Geniculate Nucleus

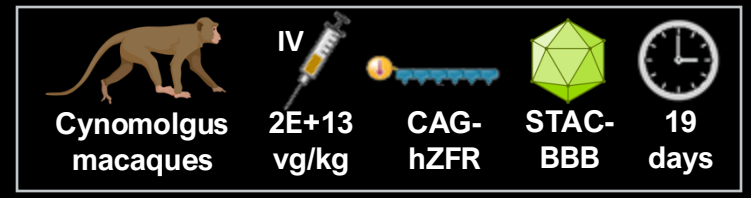


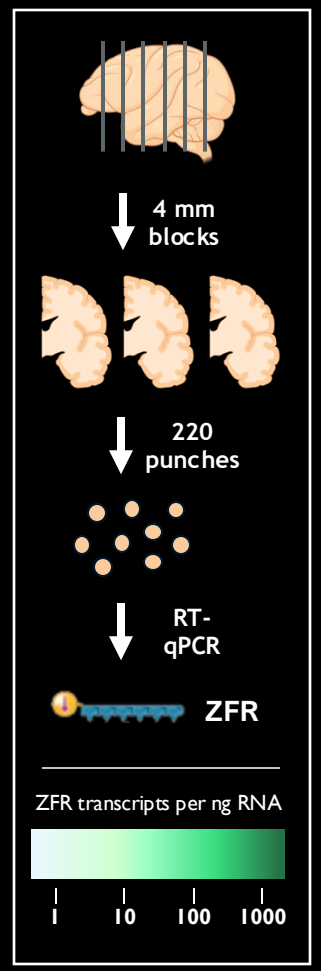
Substantia Nigra

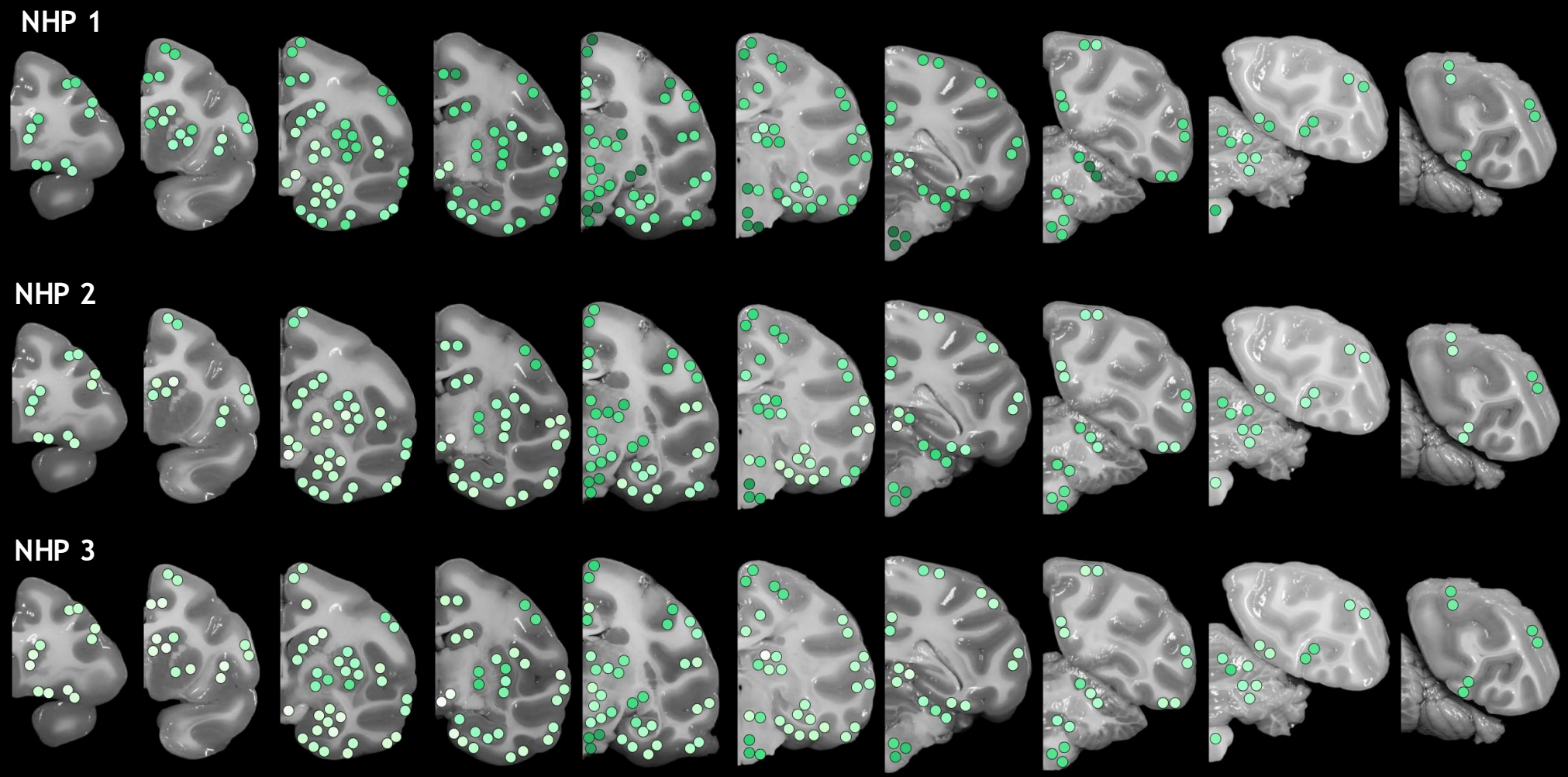


2e13 vg/kg STAC-BBB, 19 days post administration
Antibody labeling for green florescent protein (black), STAC-BBB transduced cells; Nissl staining (light blue), all nuclei

STAC-BBB mediates widespread ZFR expression throughout the nonhuman primate neuraxis







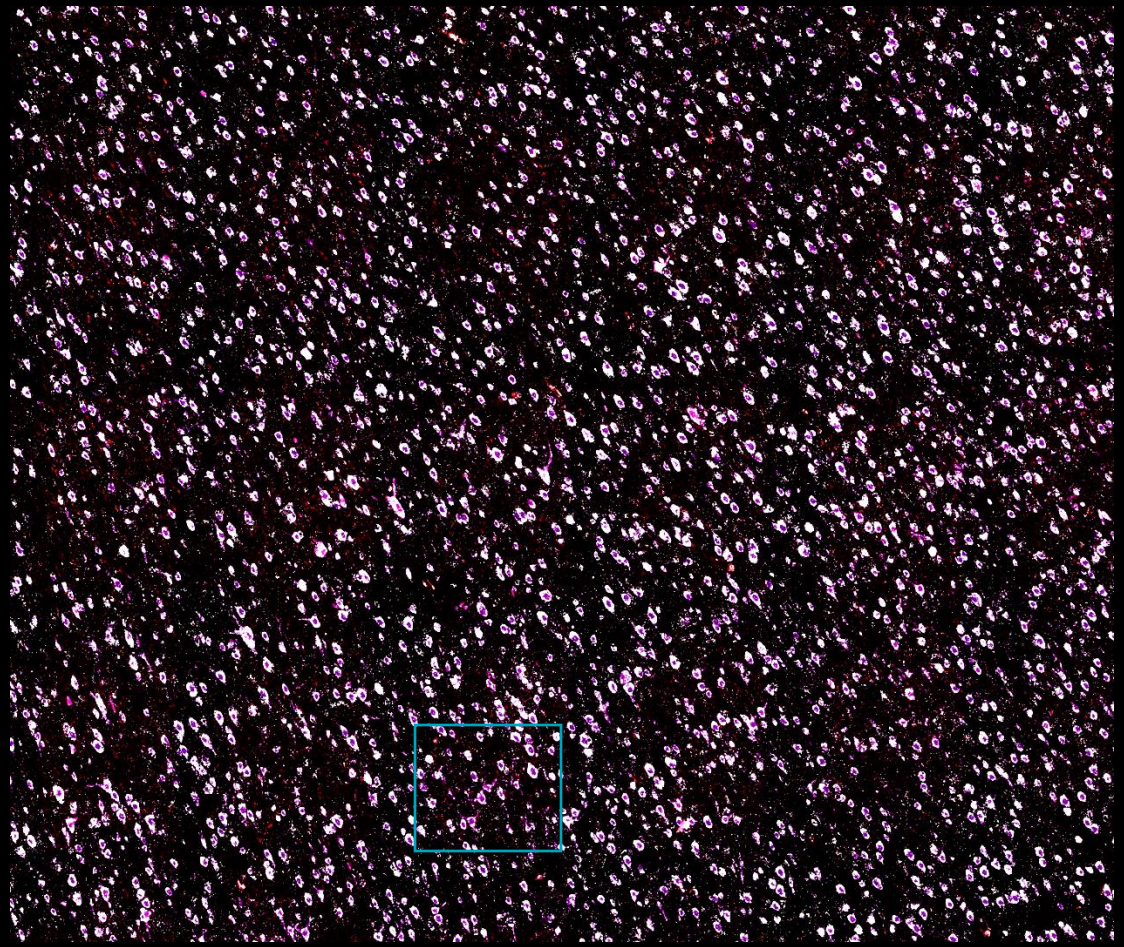
2e13 vg/kg dose, 19 days post administration, RT-qPCR bulk analysis for ZFR transgene expression.

STAC-BBB mediates widespread neuronal ZFR expression and prion repression in the cortex

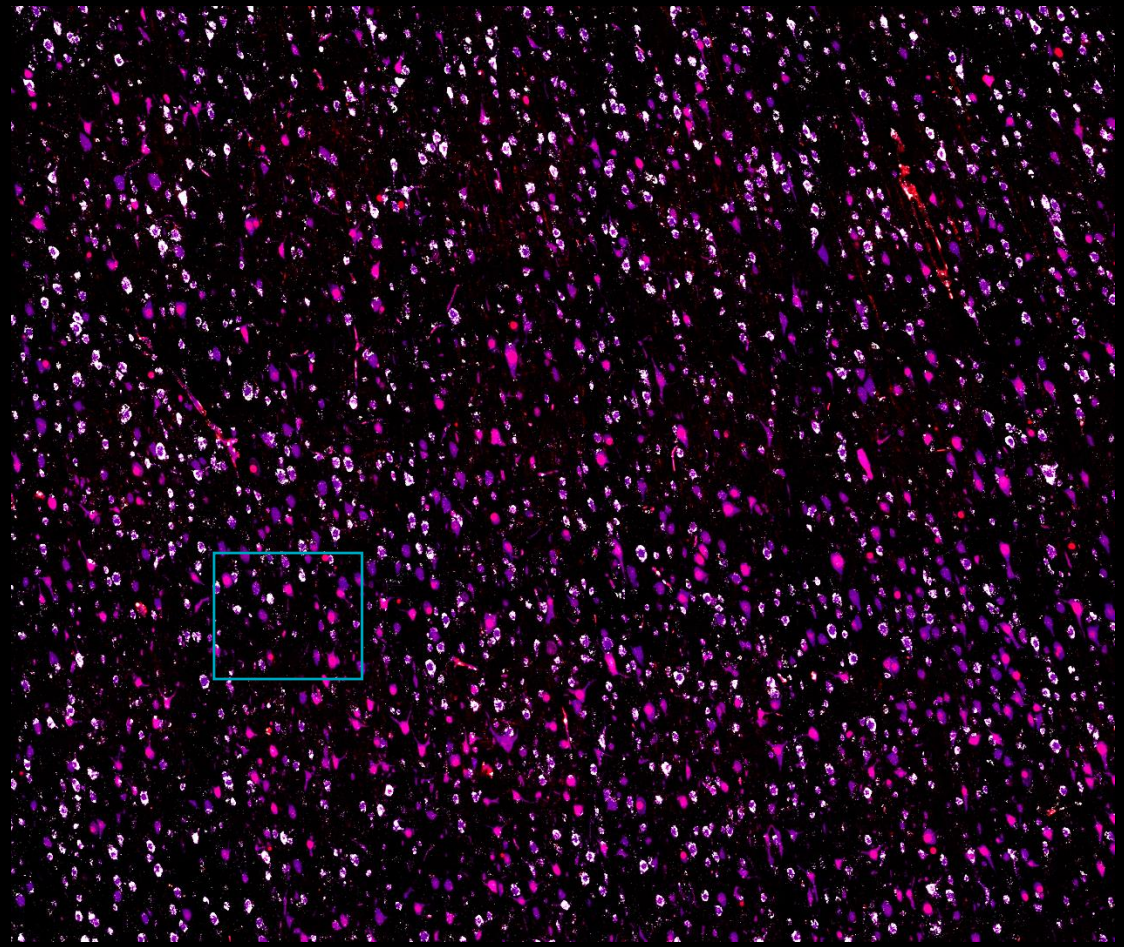
Neurons (NeuN) GFP
PRNP mRNA

STAC-BBB

Vehicle Control



STAC-BBB



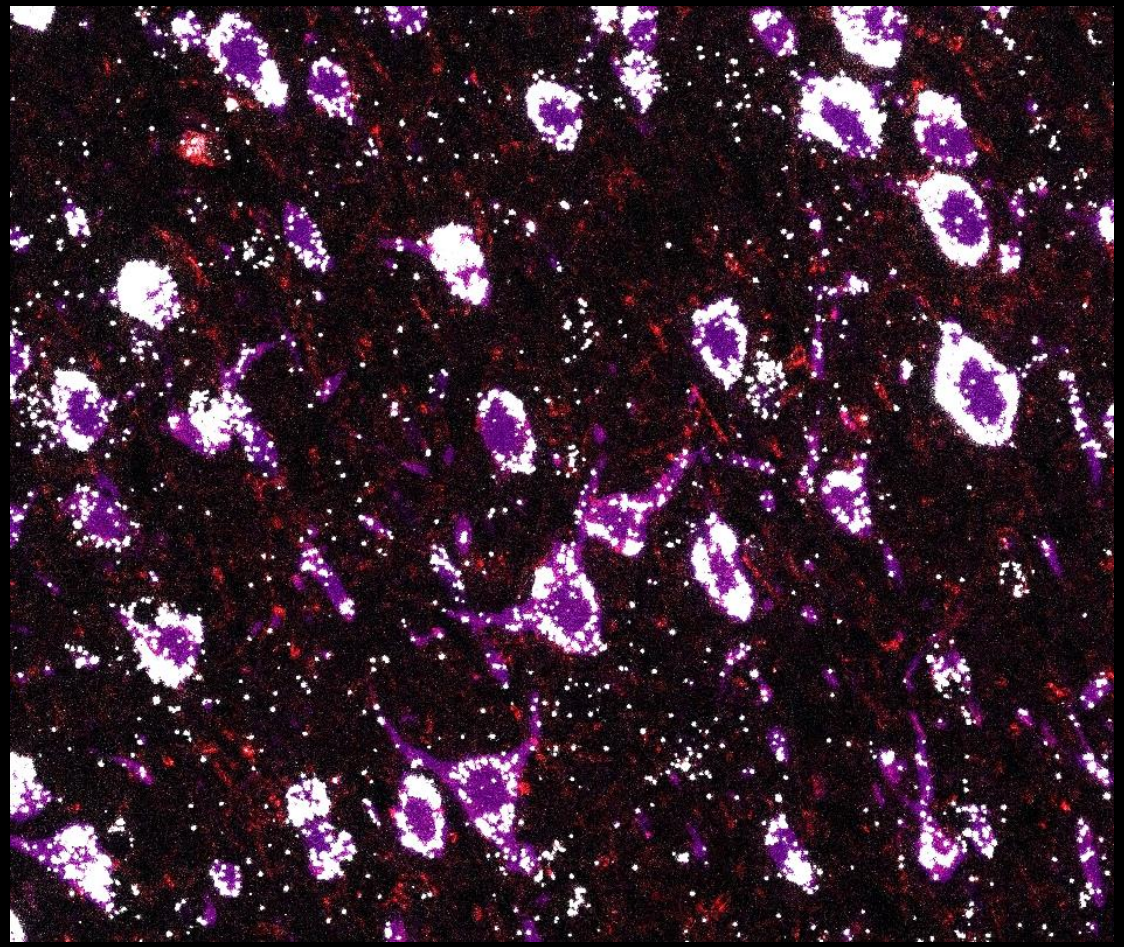
Multiplexed RNAscope ISH / IHC assay for NeuN, GFP, PRNP mRNA, and ZFR mRNA. Precentral gyrus shown.
2e13 vg/kg dose, 19 days post administration

STAC-BBB mediates widespread neuronal ZFR expression and prion repression in the cortex

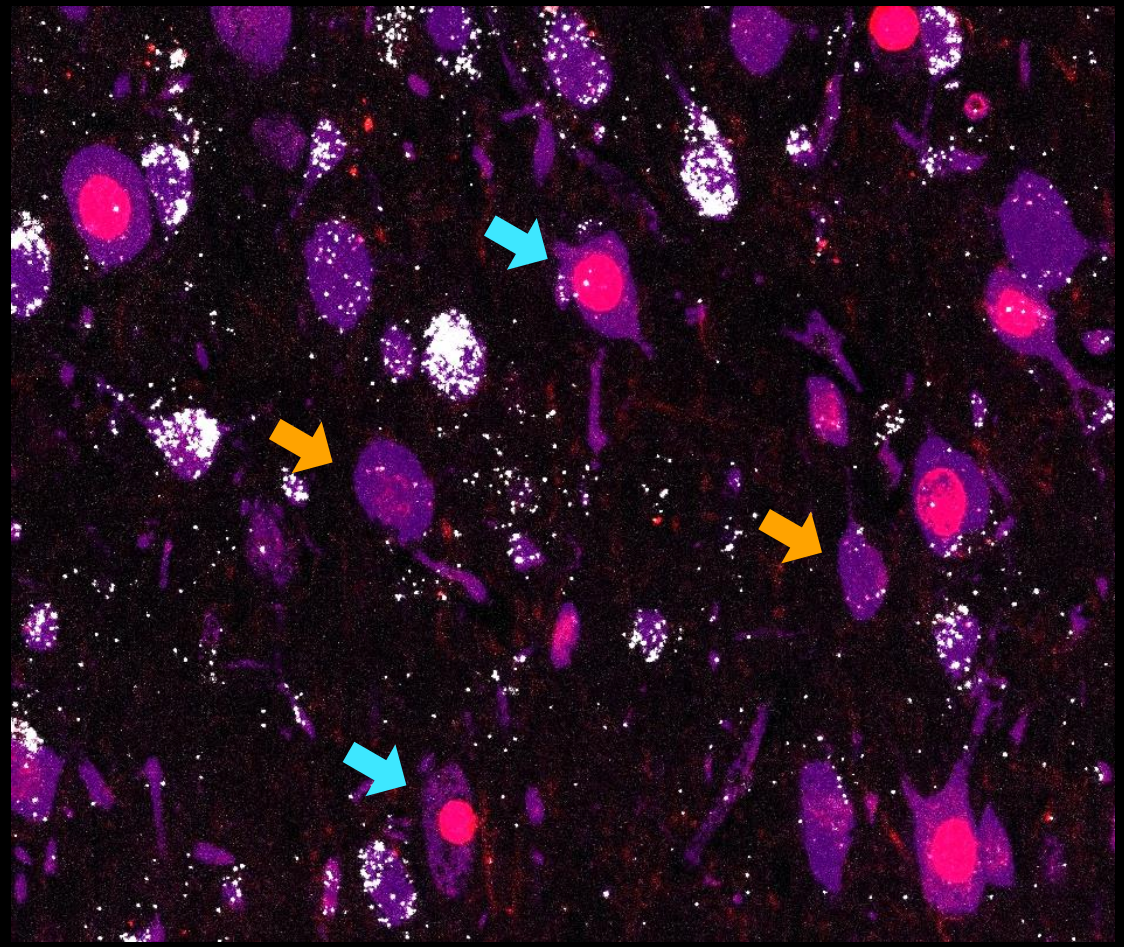
Neurons (NeuN) GFP
PRNP mRNA

STAC-BBB

Vehicle Control



STAC-BBB



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

PRNP repressed
ZFR detected

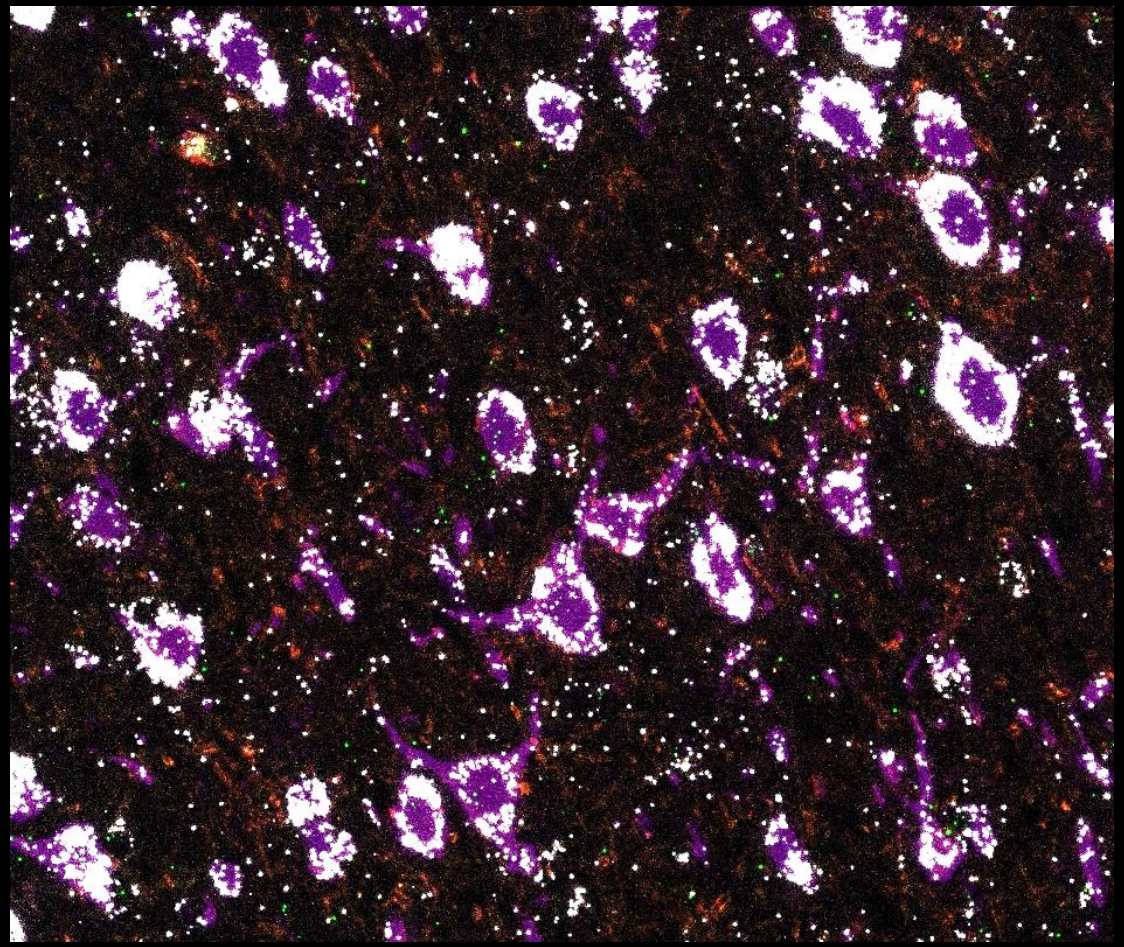
PRNP repressed
ZFR low or not detected

STAC-BBB mediates widespread neuronal ZFR expression and prion repression in the cortex

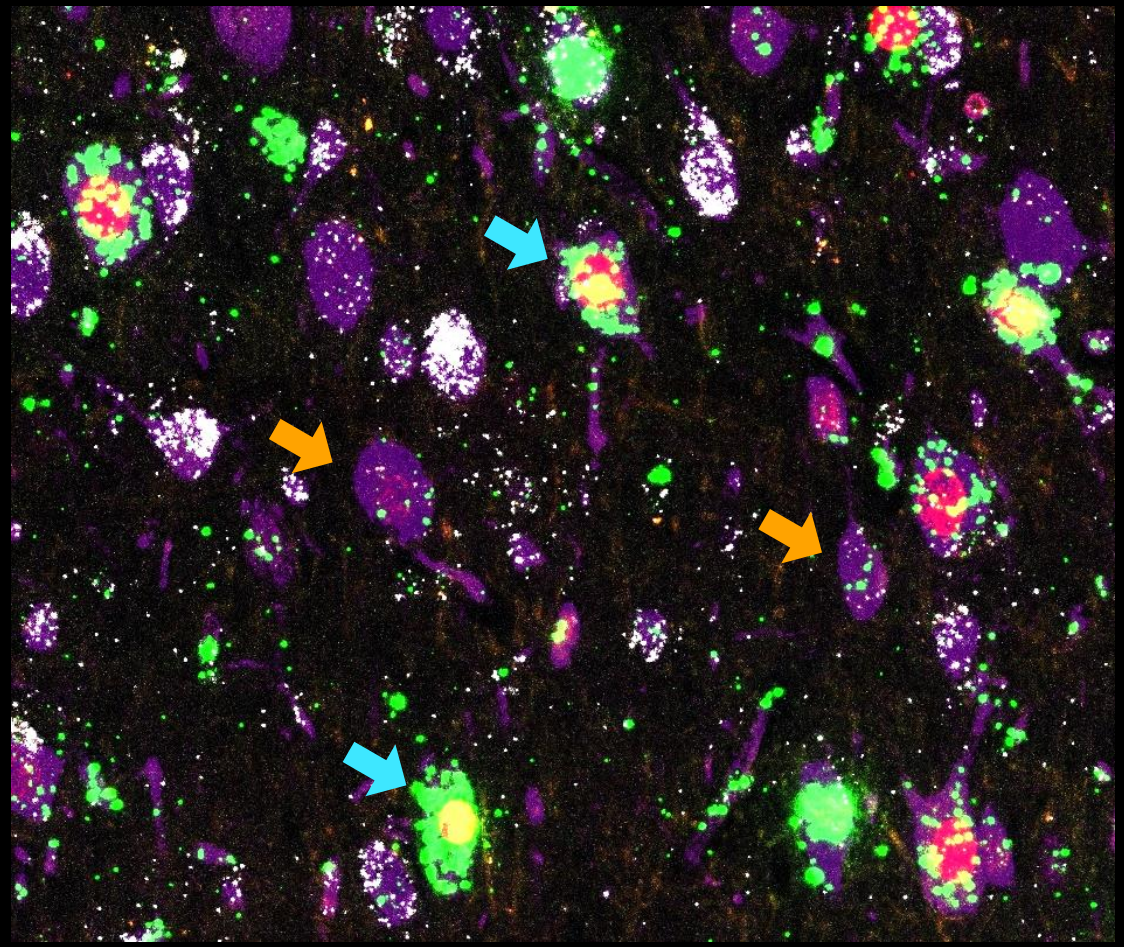
Neurons (NeuN) GFP
PRNP mRNA ZFR mRNA

STAC-BBB

Vehicle Control



STAC-BBB



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

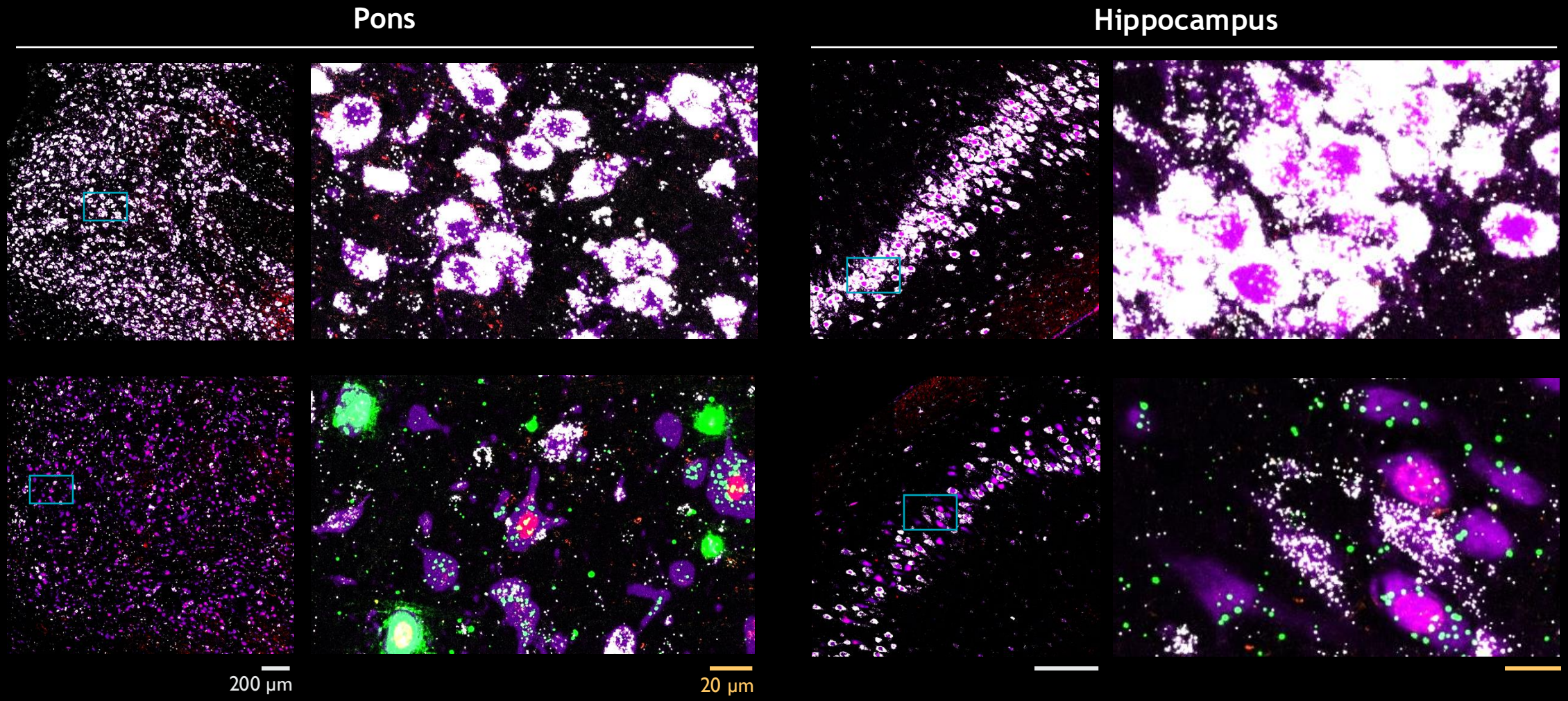
PRNP repressed ZFR detected

PRNP repressed ZFR low or not detected

STAC-BBB mediates widespread neuronal ZFR expression and prion repression in deeper regions

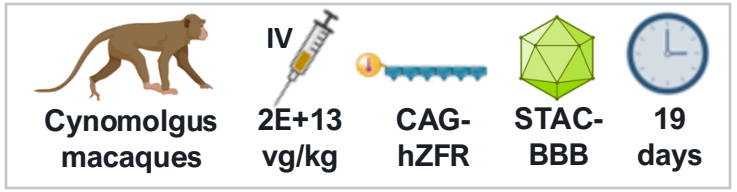
Neurons (NeuN) GFP
PRNP mRNA ZFR mRNA

STAC-BBB

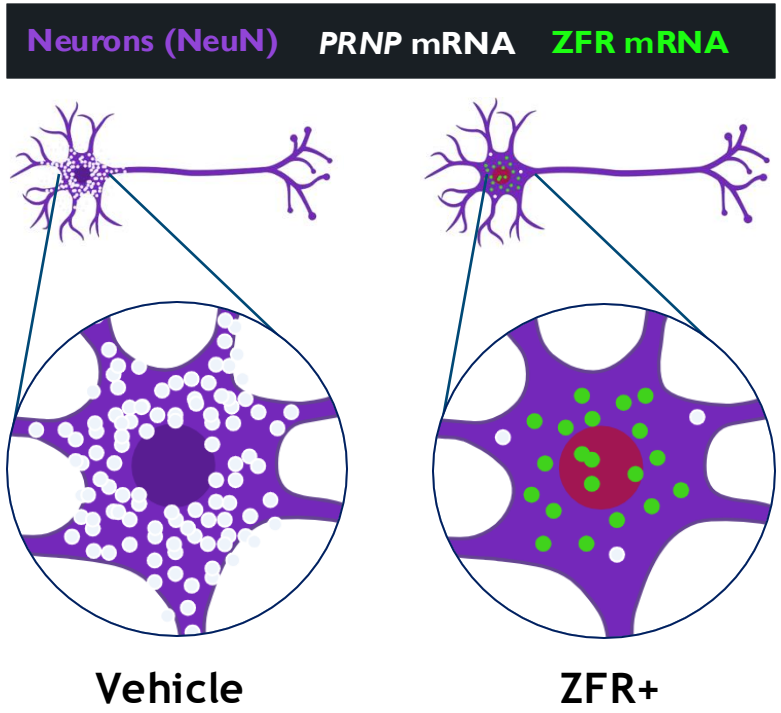


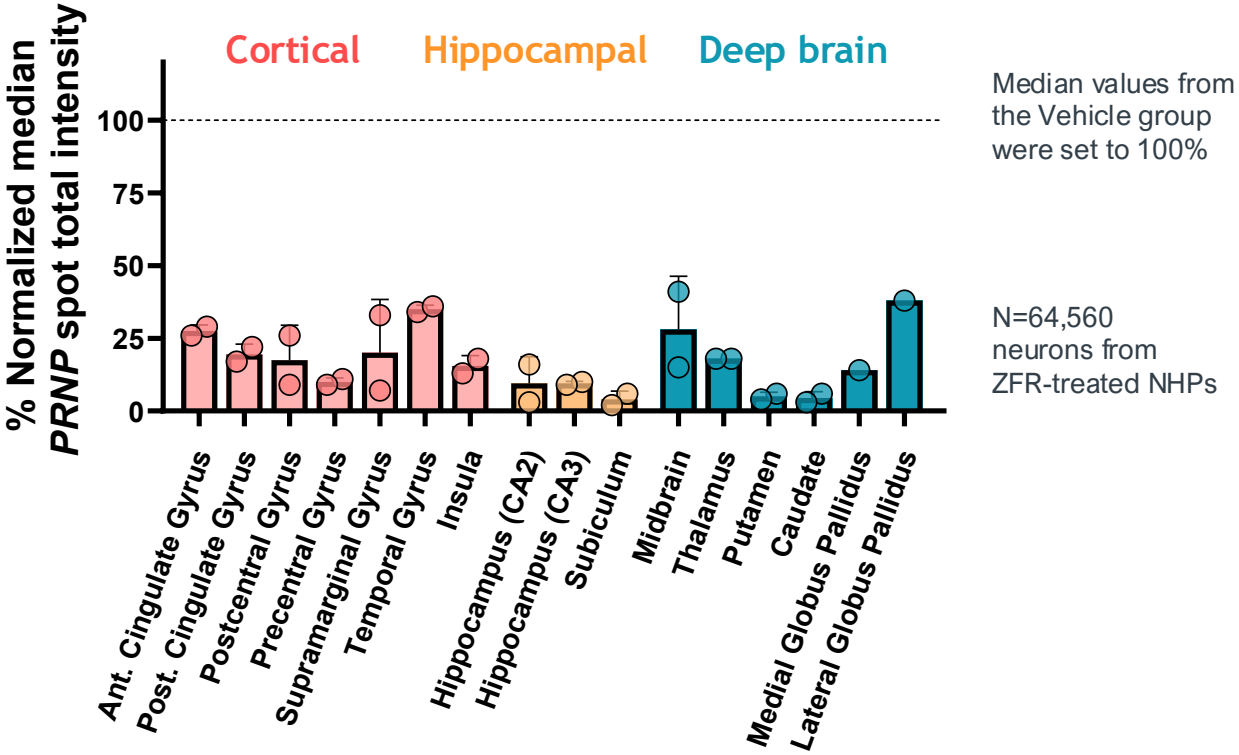
Multiplexed RNAscope ISH / IHC assay for NeuN, GFP, PRNP mRNA, and ZFR mRNA
2e13 vg/kg dose, 19 days post administration

Single-cell quantification revealed potent repression in neurons throughout the brain



~65-98% median *PRNP* transcript reduction with individual neurons across 16 brain regions





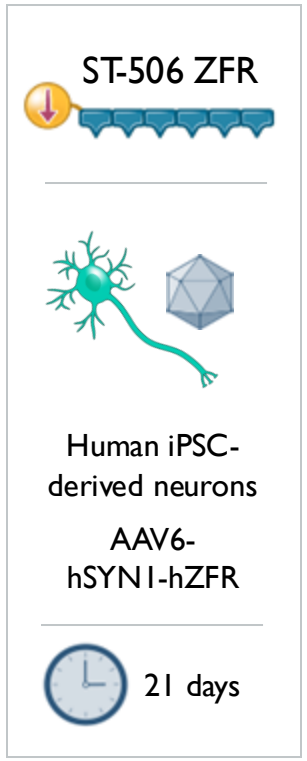
Median values from the Vehicle group were set to 100%

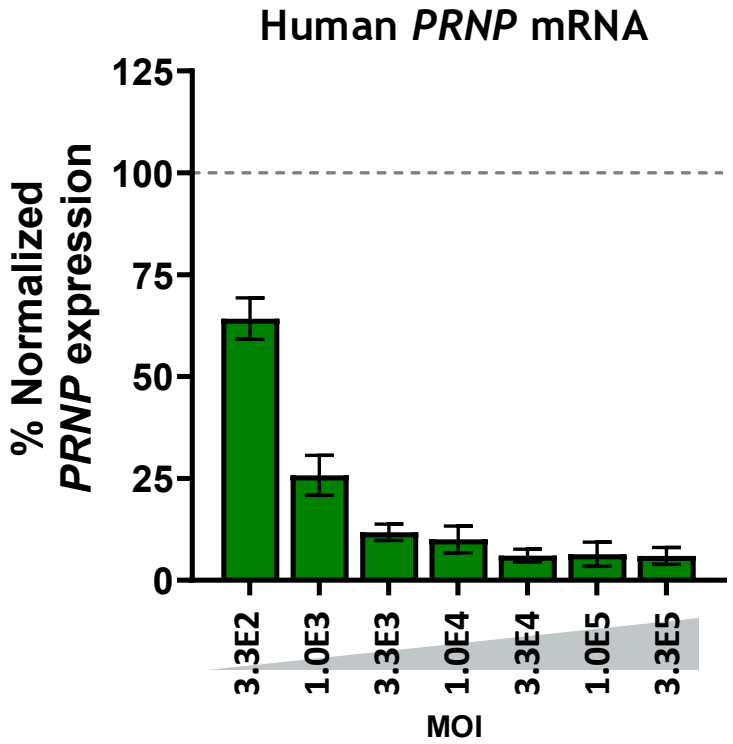
N=64,560 neurons from ZFR-treated NHPs

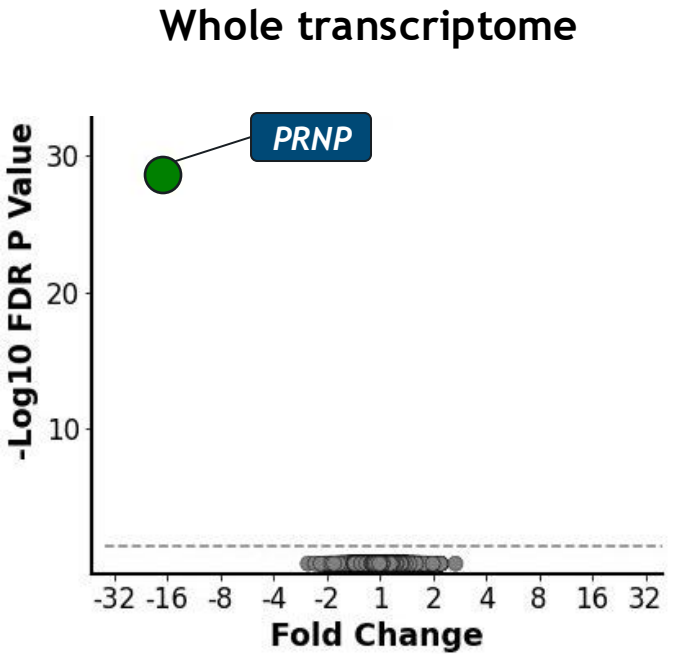
Multiplexed RNAscope ISH / IHC assay for NeuN, GFP, PRNP mRNA, and ZFR mRNA.
Median spot total intensity was calculated as described in Chou et al., 2025. Individual values represent the median for one NHP. N=2 animals.
N=59,947 NeuN+ cells were analyzed for vehicle; N=64,560 NeuN+ cells were analyzed for the ZFR-treated controls.

The ST-506 clinical lead ZFR mediates potent prion repression in human neurons with no detectable off targets

>90% *PRNP* repression in human iPSC neurons with no detectable off-targets

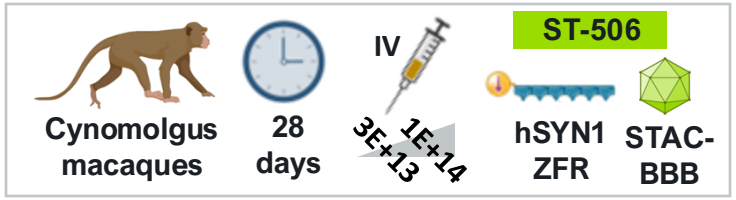


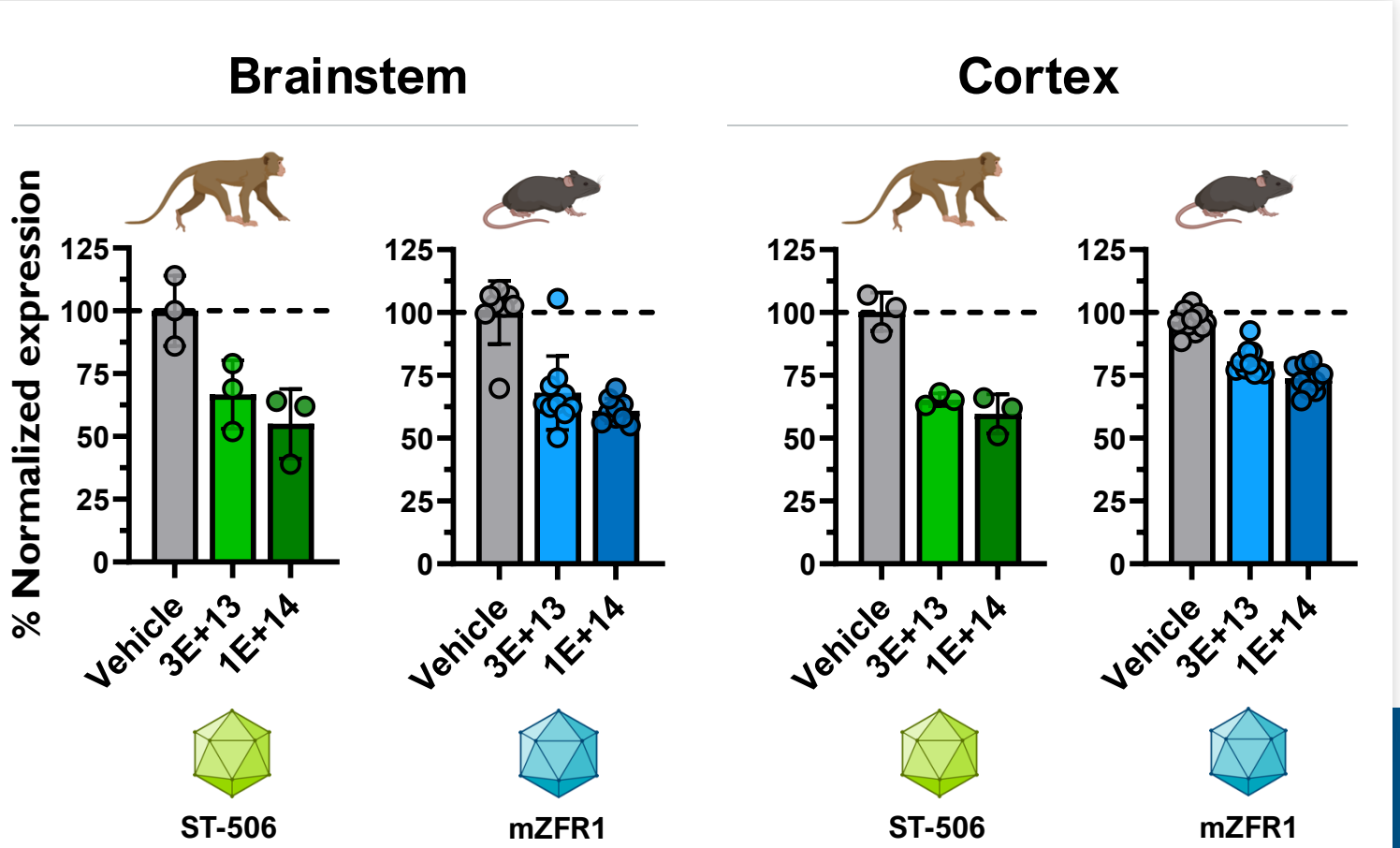


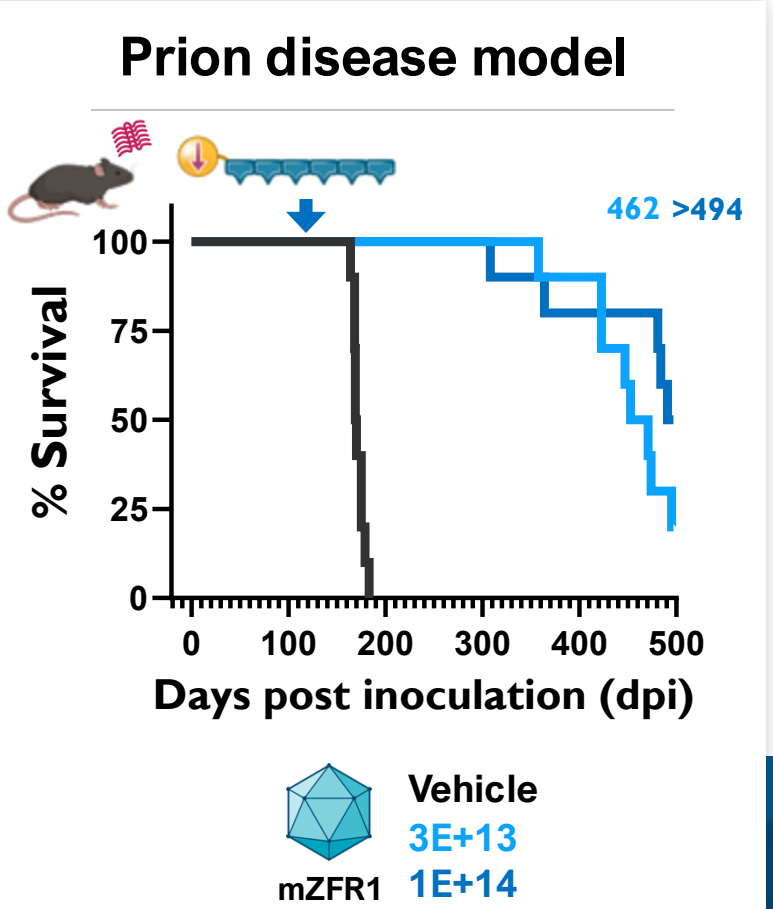


Prnp RT-qPCR data normalized to mean of Atp5b, Eif4a2 and scaled to control levels. Left: 3E+2 – 3E+5 MOIs.
Off-target analysis conducted using Clariom S gene arrays. N=6 biological replicates at 3E+4 MOI. P-values shown as False Discovery Rate (FDR).

ST-506 mediates prion repression that matches or exceeds levels associated with profound survival extension in mice







ST-506 was safe at both dose levels, with no adverse safety findings in any tissue

3E+13, 1E+14 vg/kg STAC-BBB, 28 days post-administration in cynomolgus macaques. N=3 per group. Mean values from multiple punches from pons and middle frontal gyrus shown.
3E+13, 1E+14 vg/kg PHP.B, 168 days post administration to ~7 mo old C57BL/6 mice. N=7-10 per group. Brainstem and cortex shown.

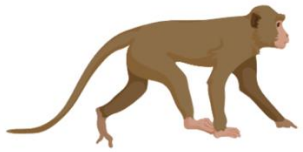
Understanding the translational potential of STAC-BBB

Mouse POC

PHP.B

Works exclusively in mice

NHP safety and pharmacology



STAC-BBB

High performance in macaques

Delivery to the human CNS

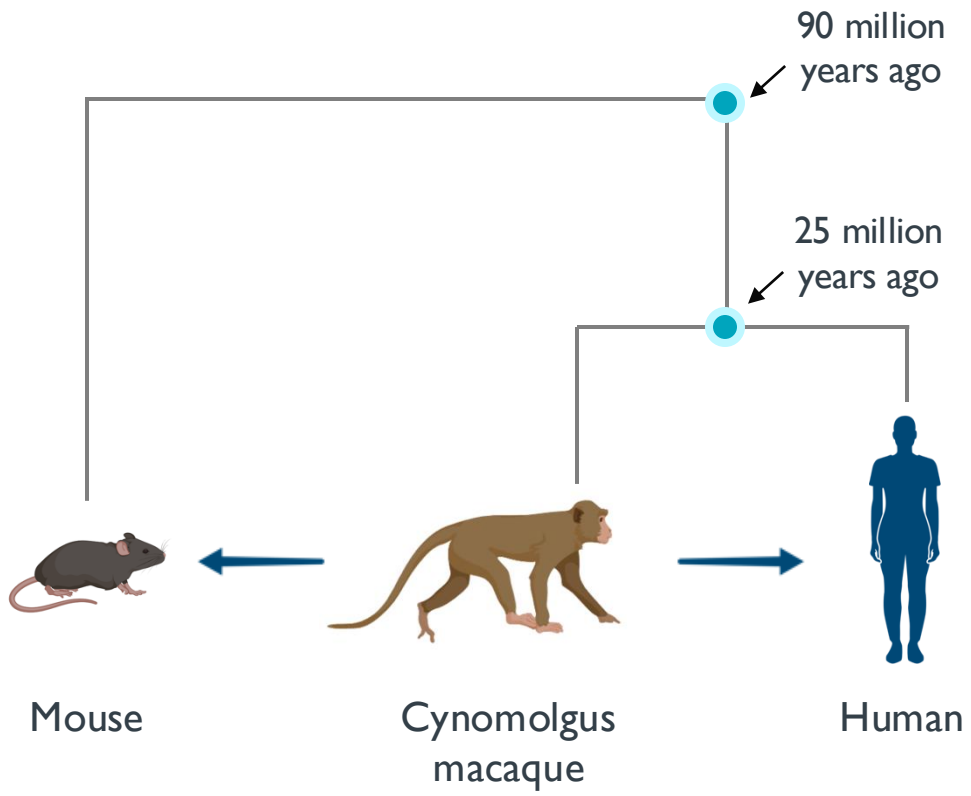
STAC-BBB

To be tested in our ST-506 clinical study

PHP.B (Deverman et al 2016)

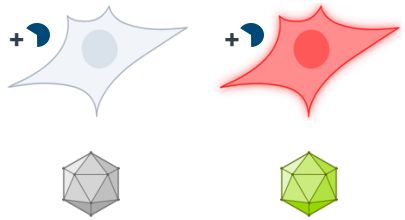
Cross-species performance and utilization of a conserved receptor support the translational potential of STAC-BBB in humans

Cross-species performance

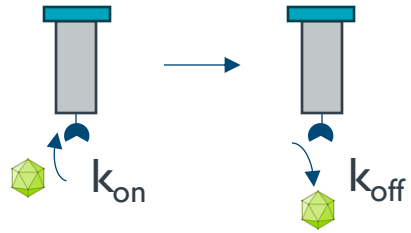


Receptor characterization

Overexpression studies

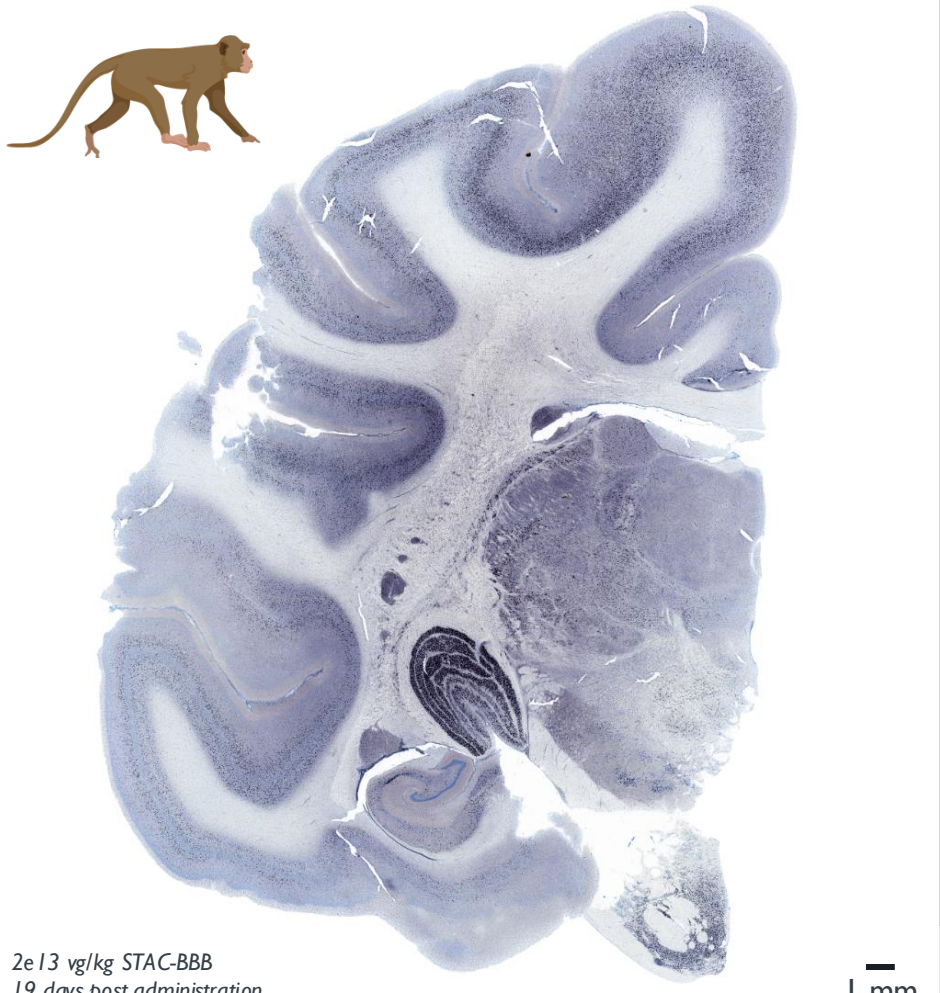


Biophysical measurements



STAC-BBB mediates brainwide delivery in nonhuman primates and mice

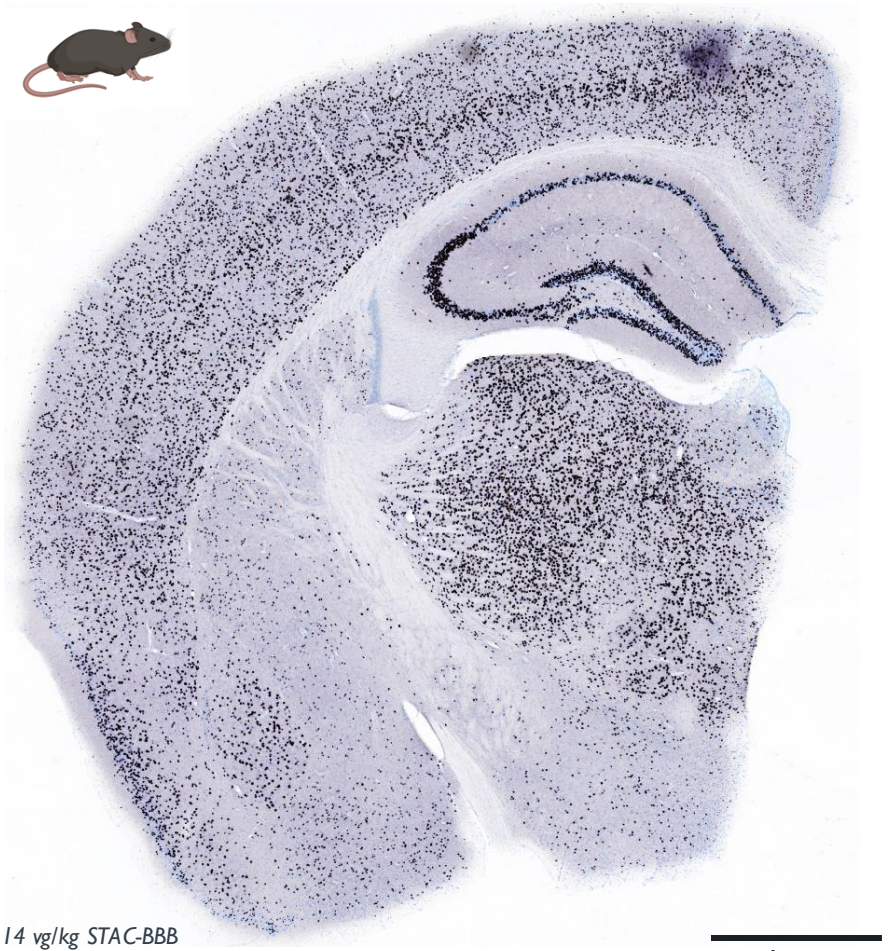
Nonhuman Primate



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

1 mm

Mouse



1e14 vg/kg STAC-BBB
28 days post administration

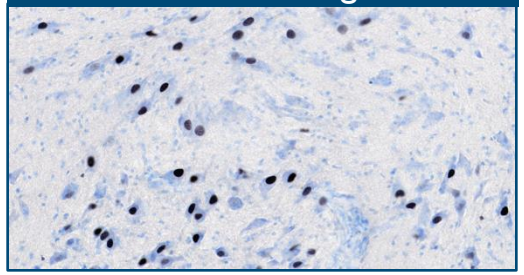
1 mm

Light blue, Nissl staining for cell nuclei. Black, IHC labeling for nuclear-localized GFP-H2B reporter.

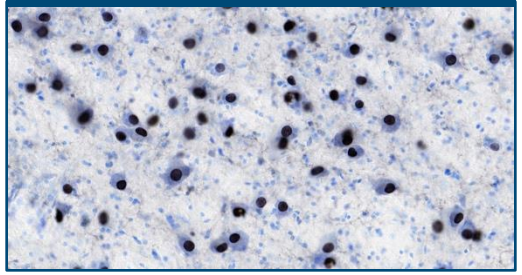
STAC-BBB mediates brainwide delivery in nonhuman primates and mice

STAC-BBB

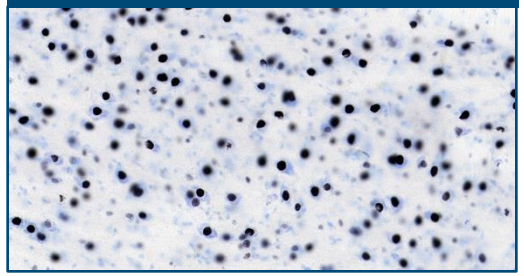
Substantia nigra



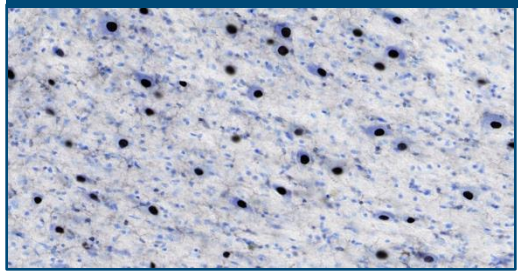
Thalamus



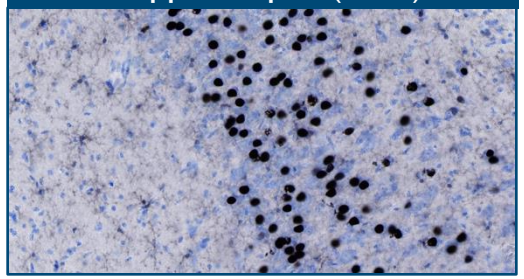
Pons



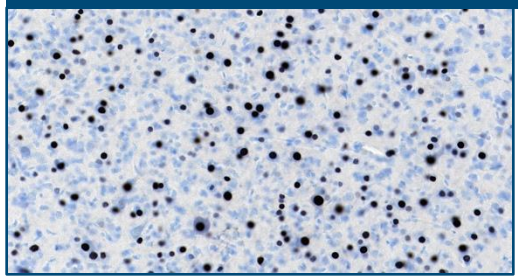
Dentate nucleus



Hippocampus (CA2)



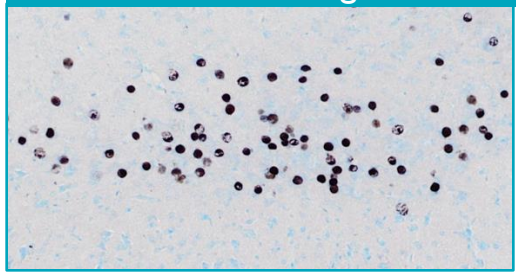
Motor cortex



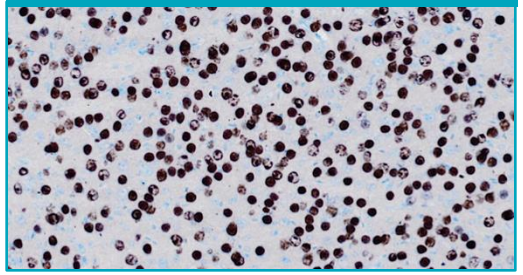
2e13 vg/kg STAC-BBB, 19 days post administration

STAC-BBB

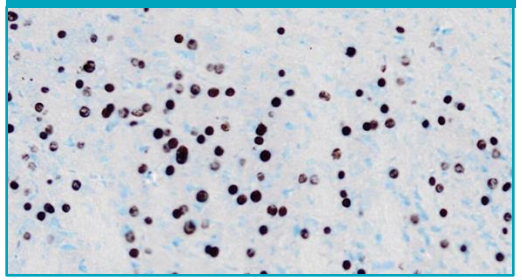
Substantia nigra



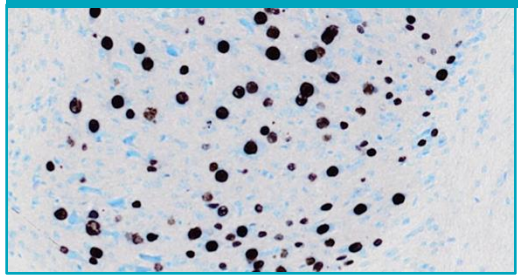
Thalamus



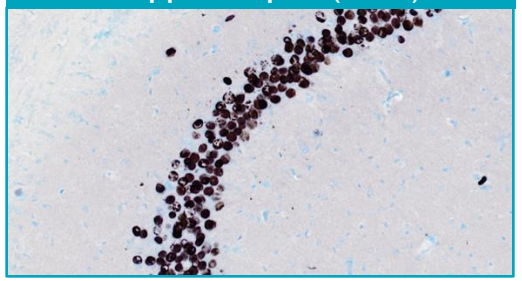
Pons



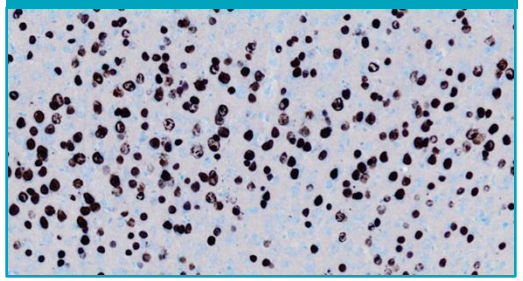
Dentate nucleus



Hippocampus (CA2)



Motor cortex

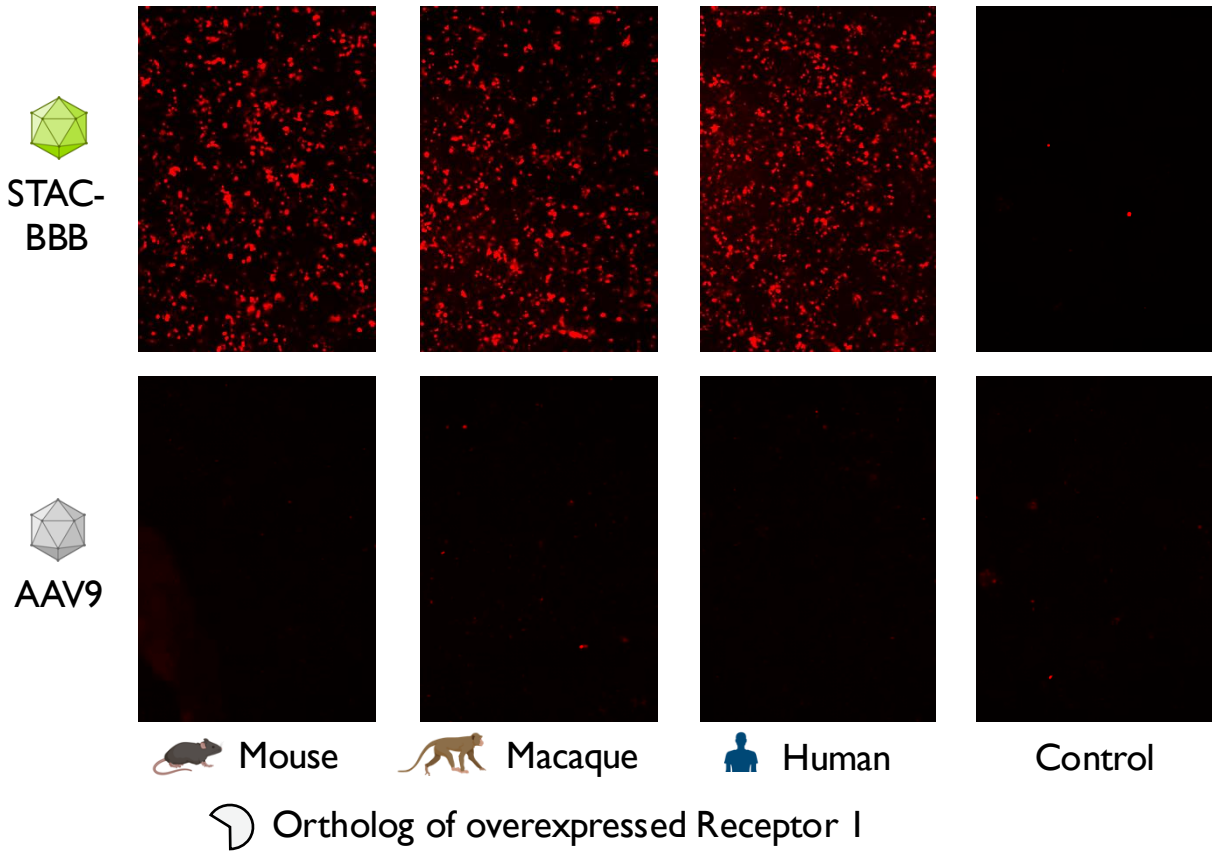


1e14 vg/kg STAC-BBB, 28 days post administration to C57BL/6 mice.

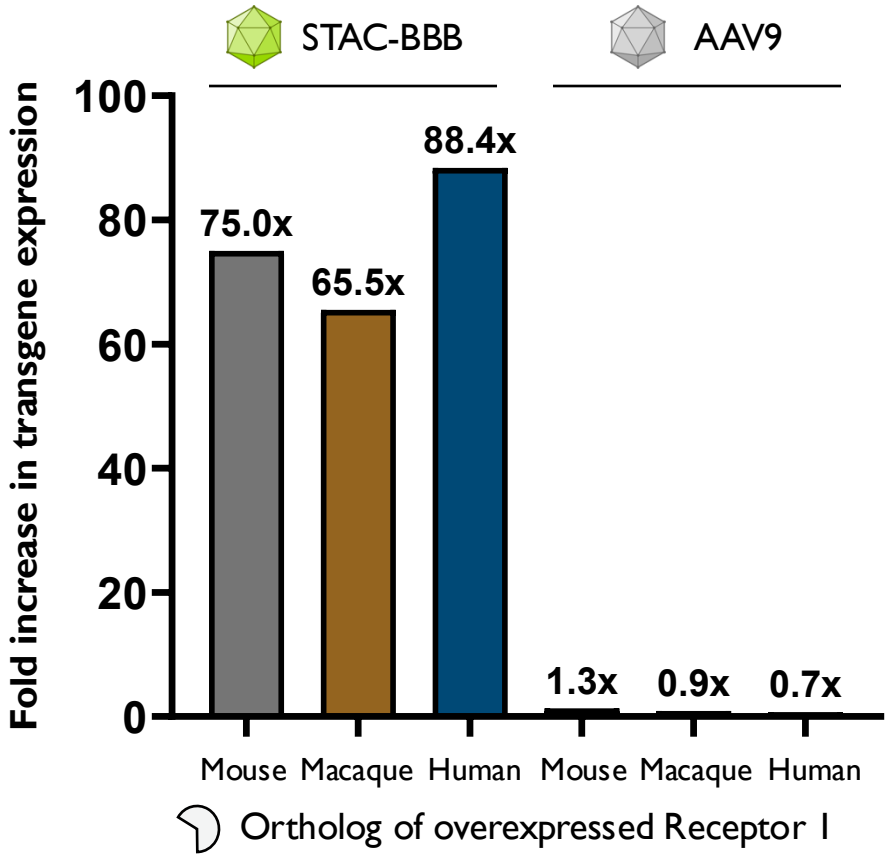
Sangamo THERAPEUTICS

Overexpression of mouse, macaque, and human Receptor 1 confers enhanced transduction for STAC-BBB

All tested Receptor 1 orthologs mediate improved transduction for STAC-BBB but not AAV9

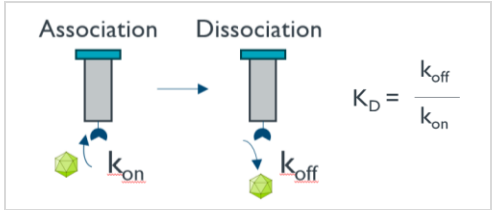


66-88x increase in transgene expression for STAC-BBB but not AAV9

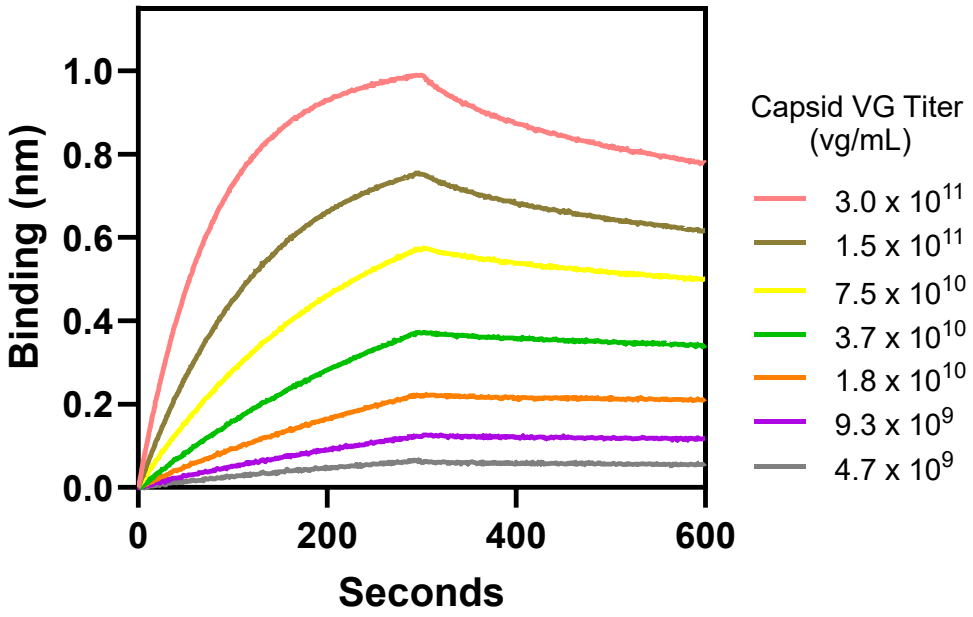


Expression of an mCherry reporter was evaluated MOIs ranging from 1E+3 to 1E+5 in HEK293 cells. Relative normalized transgene expression was assessed by RT-qPCR. A nonlinear regression model was used to interpolate values for each capsid-construct condition. These values were then scaled to a GFP transfection control value for each capsid.

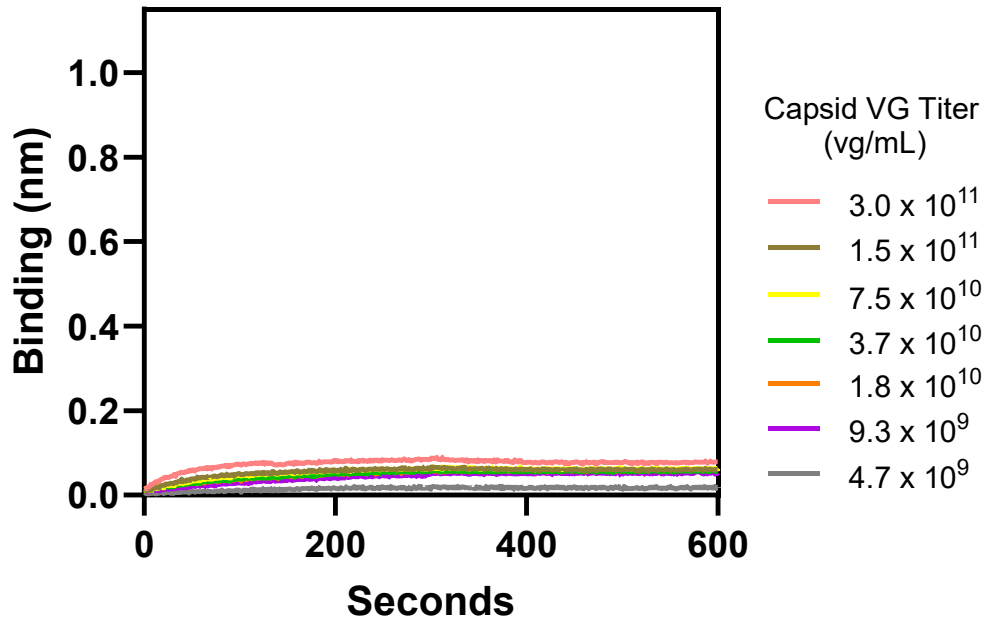
STAC-BBB binds with high affinity to human Receptor 1



STAC-BBB + Human Receptor 1



AAV9 + Human Receptor 1



STAC-BBB binds human receptor 1 with a dissociation constant in the low picomolar range. The parental serotype AAV9 exhibits no binding.

Human Receptor 1 was immobilized on Octet BLI sensors. Binding to STAC-BBB or AAV9 was measured across a range of capsid concentrations.

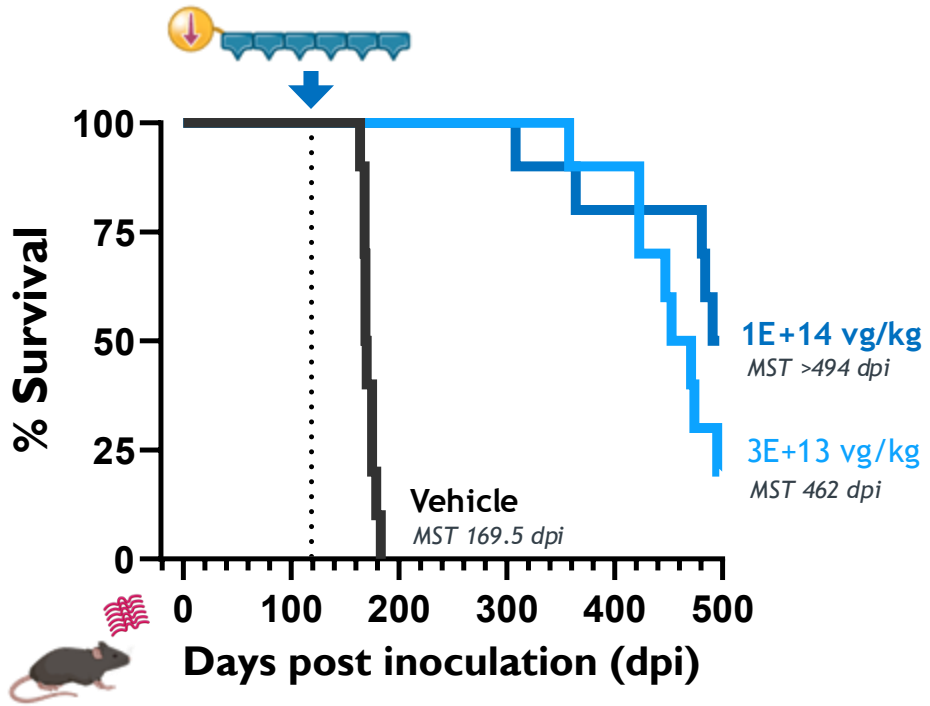
ST-506 shows great promise for the potential treatment of prion disease

- Profound survival extension in prion disease model even when dosed post-symptomatically
- Sustained prion repression for at least 17 months
- Brainwide delivery and repression in NHPs and mice
- High potential for human translation
- Anticipate start of the ST-506 clinical study in 2026

ZF epigenetic regulators and STAC-BBB could transform the treatment of many other neurological diseases

Chou et al, 2025

Zinc Finger Repressors mediate widespread PRNP lowering in the nonhuman primate brain and profoundly extend survival in prion disease mice



MST, median survival time

Please join us for additional Sangamo abstracts

May 13
Tuesday

Recombinant Adeno-Associated Virus (rAAV) Production in Spodoptera Frugiperda (Sf9) Cells: **Viral Cathepsin Mediated Capsid Cleavage and Mitigation Strategies**
Leah Benedict, **#987**
Poster presentation

A **Protein-Guided Modular Integrase** (MINT) Platform Enables Compact Therapeutic Payloads and Efficient Targeted Integration in T Cells
Jeff Miller, **#648**
Poster presentation

May 14
Wednesday

Sustained **Brain-wide Reduction of Prion via Zinc Finger Repressors** in Mice and Nonhuman Primates as a Potential One-Time Treatment for Prion Disease
Bryan Zeitler, **#2**
11:30 AM, Hall F

The Impact of Empty Capsids on AAV Manufacturing and Strategies for Enhancing Yield, **Purity, and Stability in the Production** of a Novel Blood-Brain Barrier Penetrant AAV Capsid
Taeho Kim, **#1464**
Poster presentation

May 15
Thursday

Assessment of **Adeno-Associated Virus (AAV) Purity** by Capillary Electrophoresis-Based Western
Julyana Acevedo, **#1814**
Poster presentation

Characterization of **receptor-targeted blood-brain barrier penetrant** AAV capsids
David Ojala, **#1896**
Poster presentation

Fitness maturation of **STAC-BBB yields second-generation capsid variants** with enhanced delivery to the central nervous system
Matt Tiffany, **#1909**
Poster presentation

May 16
Saturday

AAV-mediated Delivery of an Engineered Zinc Finger Leads to Selective and Potent **Repression of Nav1.7 in Human** Sensory Neurons and Nonhuman Primates DRG Nociceptors Following Intrathecal Injection
Mohammad Samie, **#369**
8:45 AM, New Orleans Theater B

Preclinical Development of an AAV-delivered Zinc Finger Transcriptional Repressor Targeting the Prion Gene as a **Novel Epigenetic Gene Therapy for Prion Disease**
Toufan Parman, **#389**
8:45 AM, Room 288-290

Sangamo THERAPEUTICS

- AAV Engineering and Production for the Central Nervous System
- Neurology Epigenetic Regulation
- Next-Generation Genome Engineering

Thank You

Sangamo Therapeutics, Fall 2024

Sangamo	All current and former colleagues
Evotec SE	Giulia Cisbani, Finn Peters, Tim Fieblinger, Chiara Melis
Broad Institute	Sonia Vallabh, Eric Vallabh Minikel, Meredith Mortberg, and the entire Vallabh-Minikel lab

Bryan Zeitler, PhD

Senior Director, Gene Regulation Neurology Research

bzeitler@sangamo.com