

Single Intravenous Treatment with Zinc Finger Repressor Leads to Brain-wide Reduction of Prion in Nonhuman Primates and Significantly Prolongs Survival in the RML Mouse Model

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¹ Sangamo Therapeutics Inc., ² Broad Institute of MIT and Harvard, ³ Evotec SE.

Prion Conference, October 23, 2024

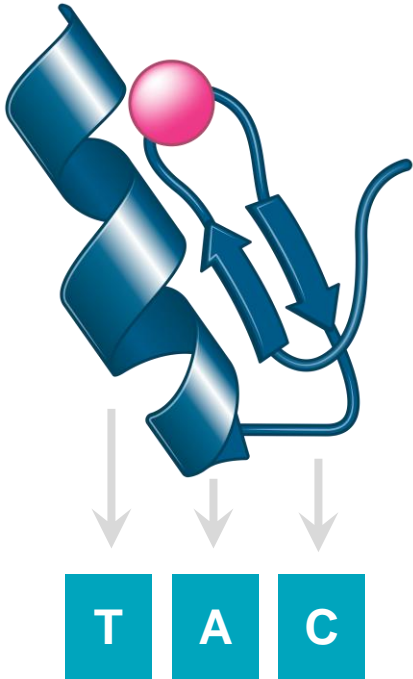
— Disclosure

I am a full-time employee of Sangamo Therapeutics

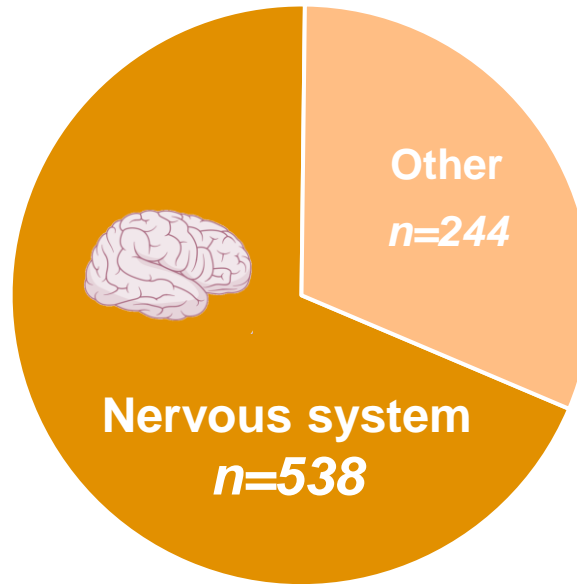
— Outline of this talk

1. Background of zinc finger repressor (ZFR) technology
2. Potential therapeutic approach for prion disease
3. Overview of compelling data supporting this approach
 - Potency and specificity of ZFR
 - ZFR efficacy in RML prion disease mouse model
 - AAV-ZFR delivery and prion repression in non-human primates

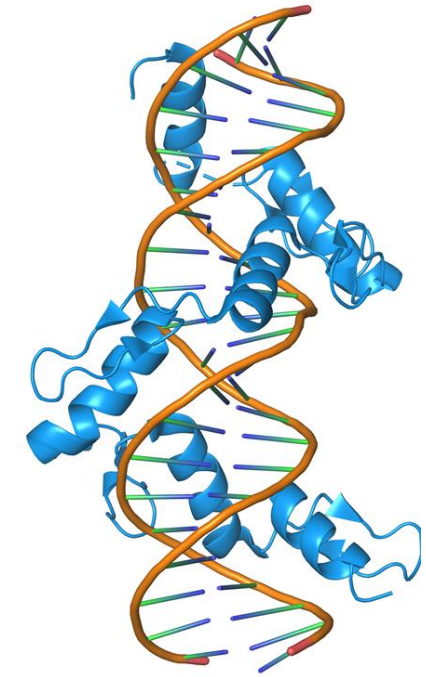
Zinc fingers are nature's solution for highly specific DNA binding



Zinc Fingers are **natural proteins** that bind DNA sequences with high specificity



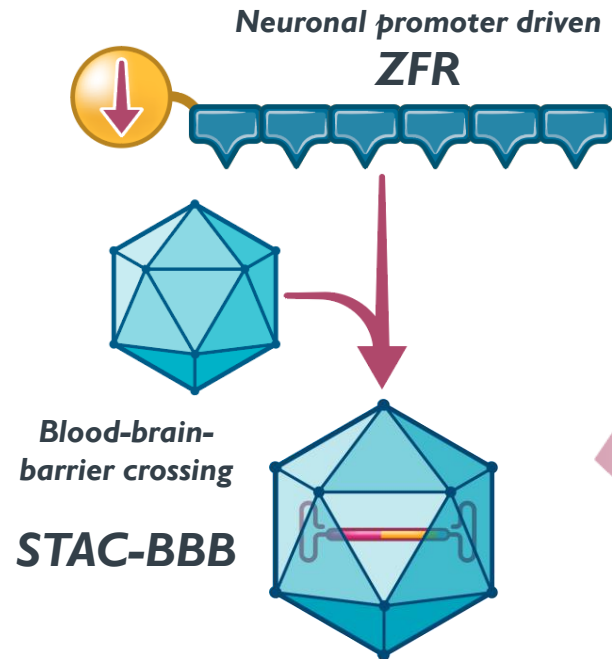
At least **782 human genes** encode for Zinc Finger Proteins




Most natural Zinc Finger Proteins function to **regulate the epigenetic state** of other genes

One-time IV administration of a ZFR for prion disease

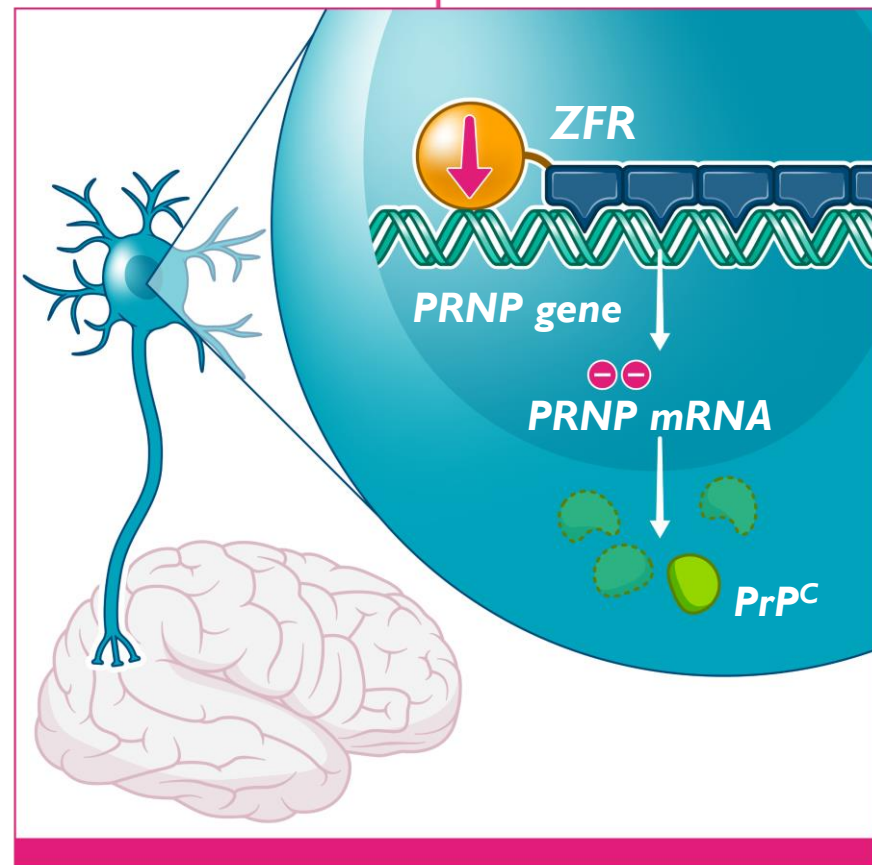
ZFR cassette packaged into AAV vector



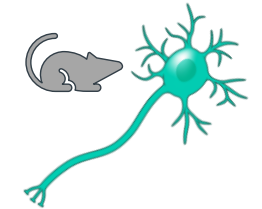
One-time IV administration 



Stable PrP^C reduction in neurons in the brain



Potent ZFR repression of mouse *Prnp* with high specificity *in vitro*



Mouse primary cortical neurons

+



AAV6 + hSYN1-ZFR

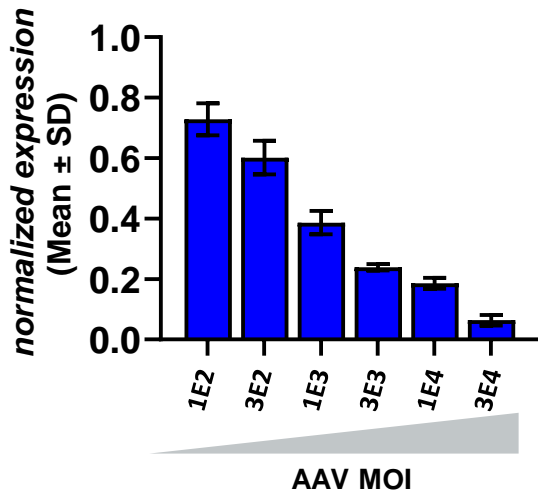
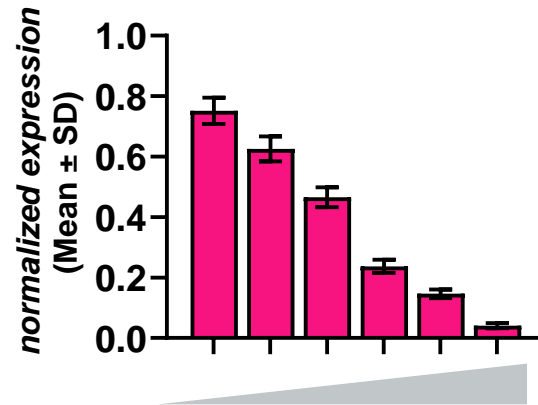
7 days ↓

Readouts:
Prnp mRNA,
Transcriptomics

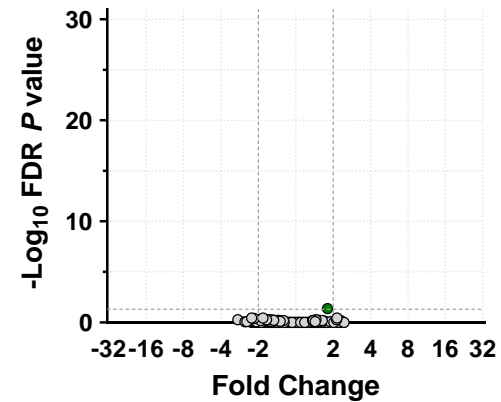
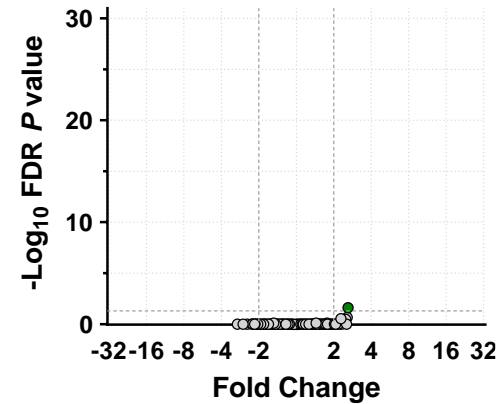
mZFR 1

mZFR 2

Prnp mRNA



Whole transcriptome



- 384 ZFRs were designed against the mouse *Prnp* locus and screened for on- and off-target activity
- Candidates mZFR1 and mZFR2 were selected due to potent *Prnp* mRNA repression in primary mouse neurons *in vitro*
- Whole transcriptome profiling of >20,000 transcripts revealed no off-target genes were significantly downregulated, demonstrating the ZFRs had excellent specificity
- These lead mouse ZFRs were advanced to *in vivo* evaluation

- FDR p-val < 0.05; Downregulated
- FDR p-val < 0.05; Upregulated
- FDR p-val > 0.05; Not Significant

Potent *in vivo* reduction of *Prnp* mRNA across the mouse brain



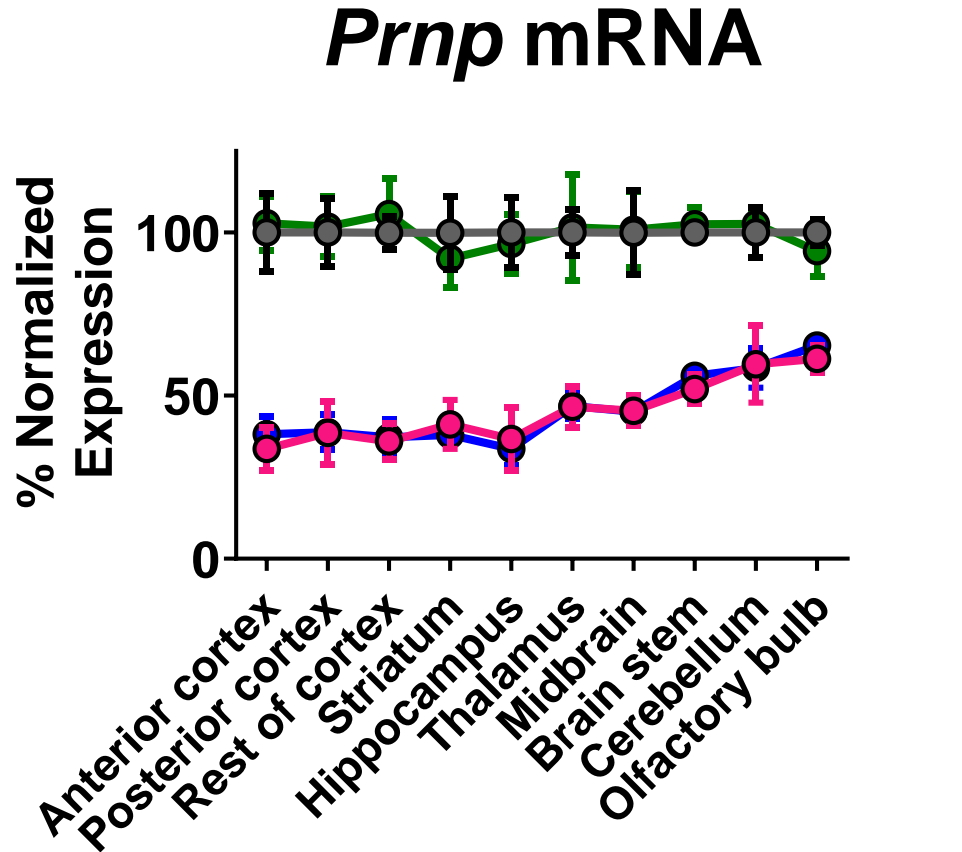
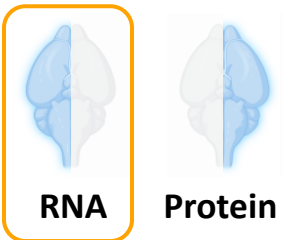
C57BL/6
adult mice

+



AAV PHP.B +
hSYN1-ZFR
1e14 vg/kg

5 wk

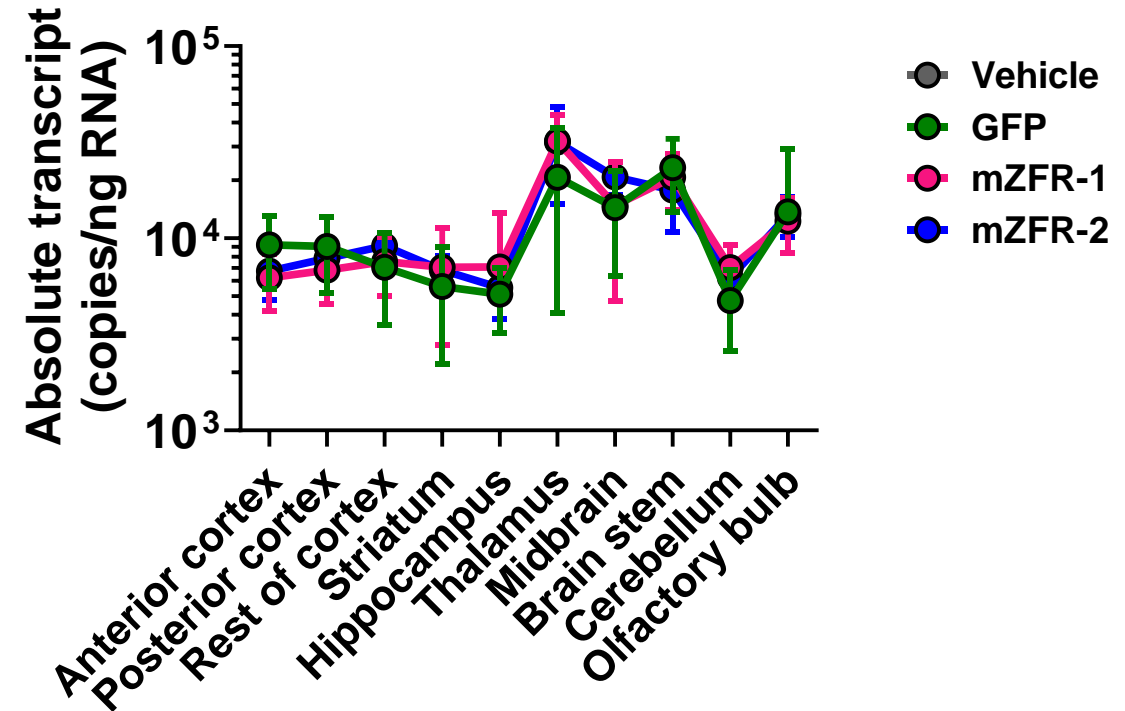


mPrnp RT-qPCR data normalized to mean of *Atp5b*, *Eif4a2*, *Gapdh*

N=4-8 mice per group. Mean ± SD; Two-way ANOVA; Dunnett's

Every region **** comparisons against GFP group
Every region ****

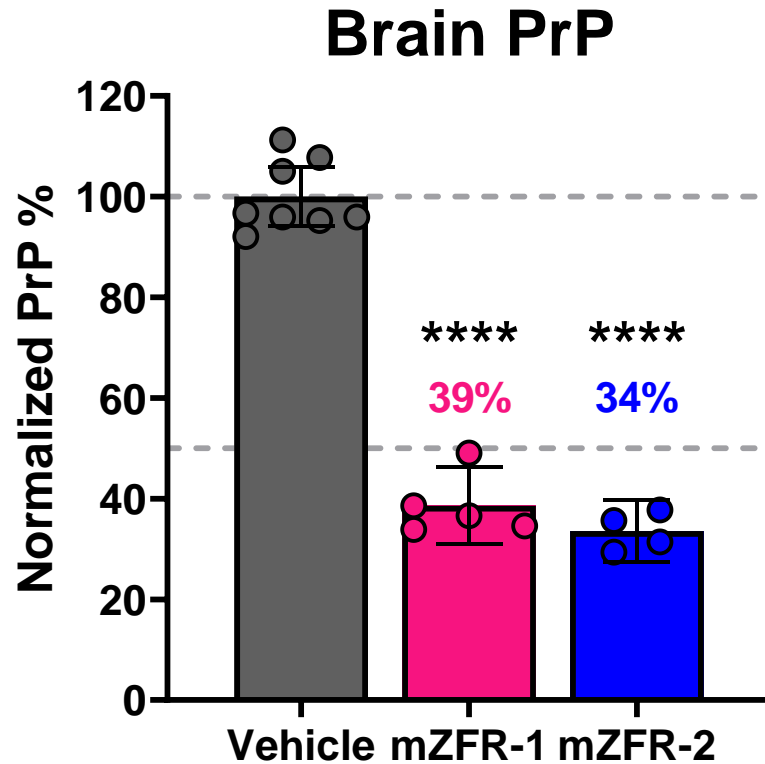
Transgene expression



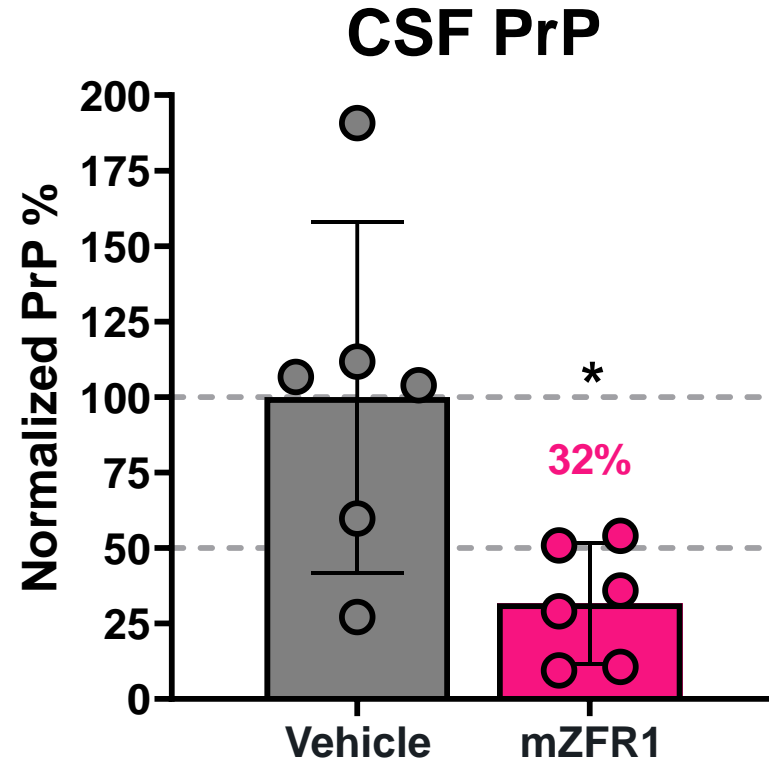
N=4-8 mice per group. Mean ± SD; Two-way ANOVA; Dunnett's comparisons against GFP group

Thalamus* ; all other regions ns
Thalamus** ; all other regions ns

Potent reduction of PrP protein in the brain and CSF of mice

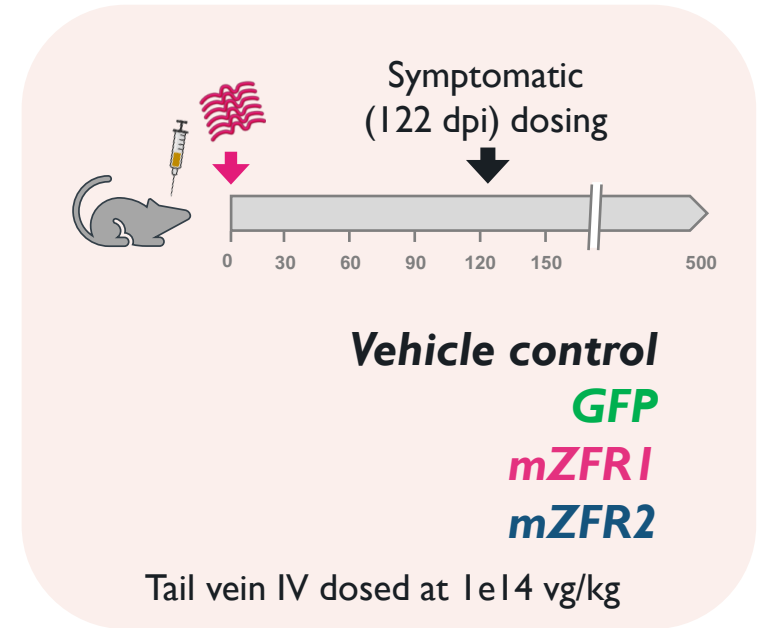
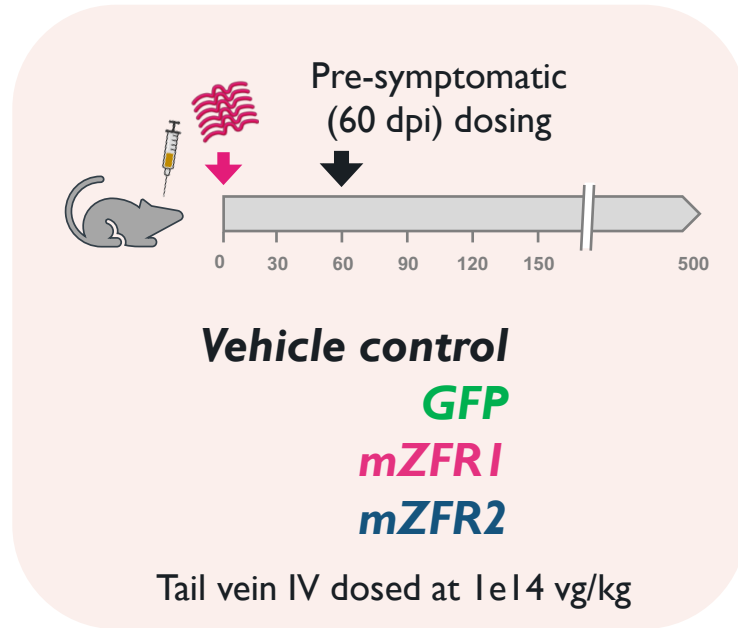
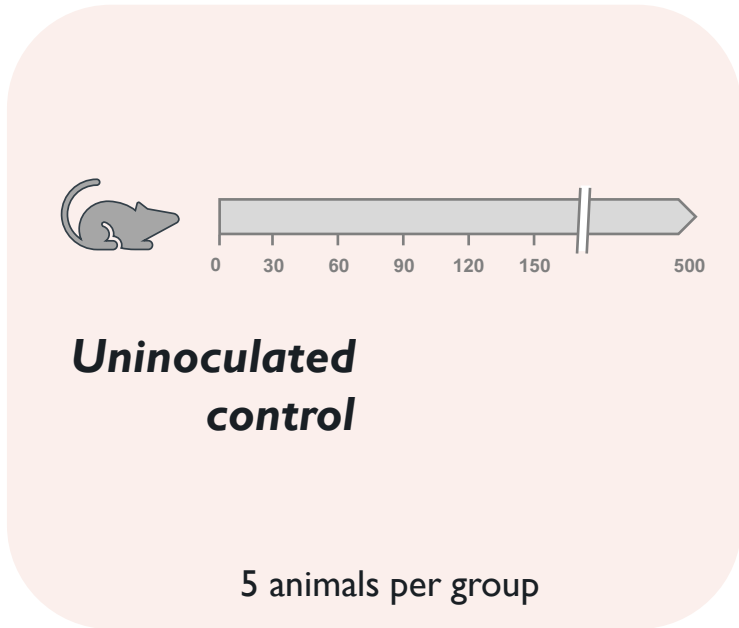
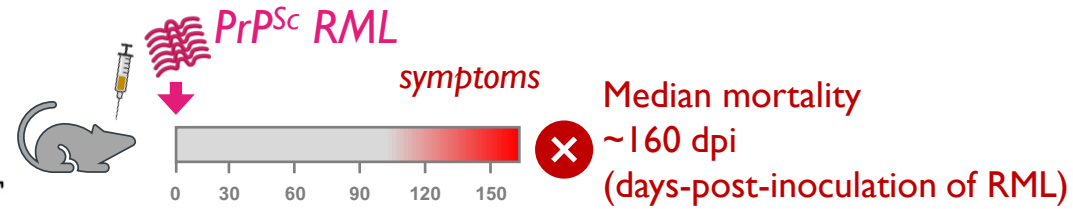
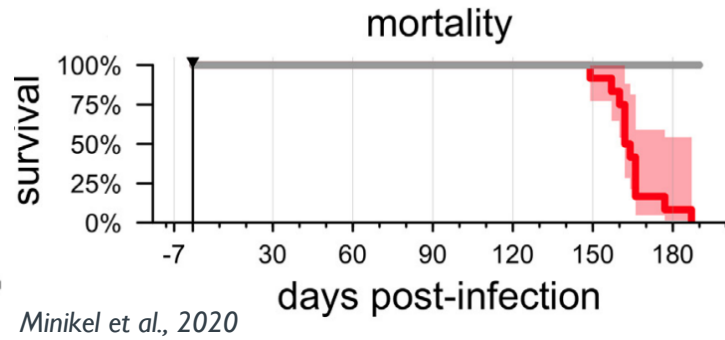
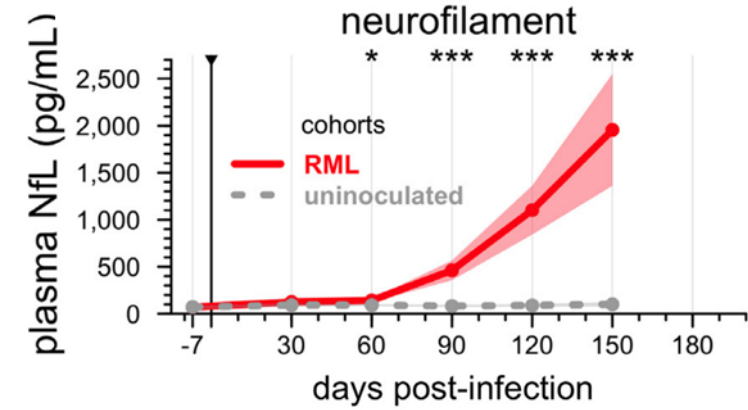


N=4-8 mice per group. Mean ± 95%CI; One-way ANOVA; Dunnett's post test; comparisons against Vehicle group



N=6 mice per group. Mean ± 95%CI; Mann Whitney test comparisons against Vehicle group

Efficacy study #1: evaluate survival of RML-inoculated mice with a single ZFR dose at pre-symptomatic and symptomatic

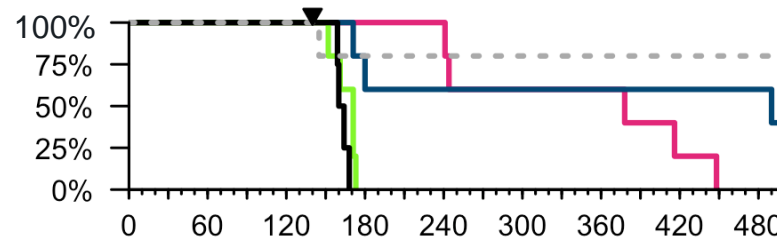
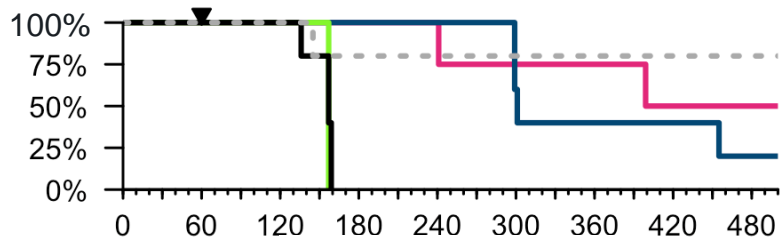


mZFRs extend survival, slow NfL increase in plasma, and improve weight gain of RML-inoculated mice treated at pre-symptomatic and symptomatic

60dpi (Pre-symptomatic)

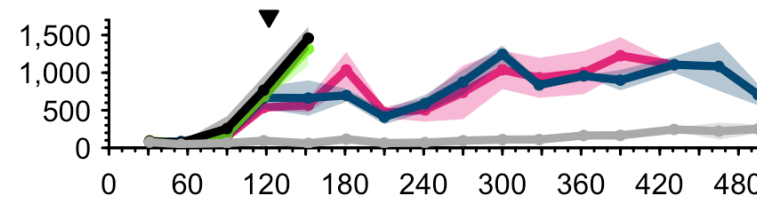
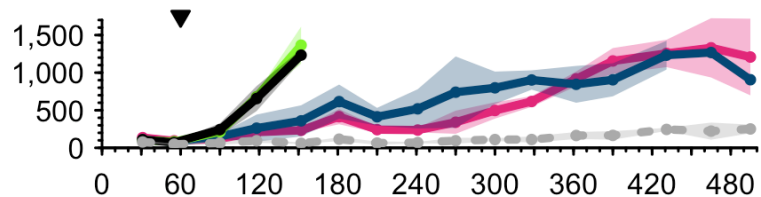
122dpi (Symptomatic)

Survival

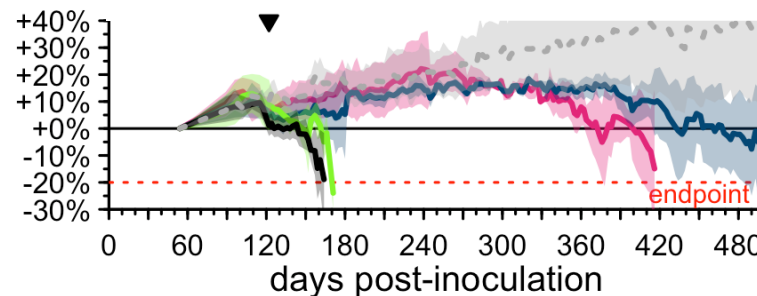
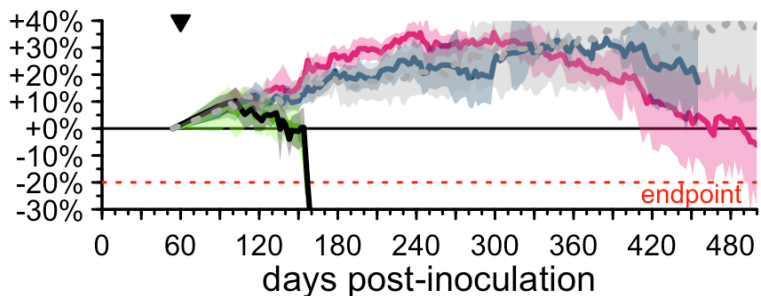


uninoculated
Vehicle
GFP
mZFR1
mZFR2

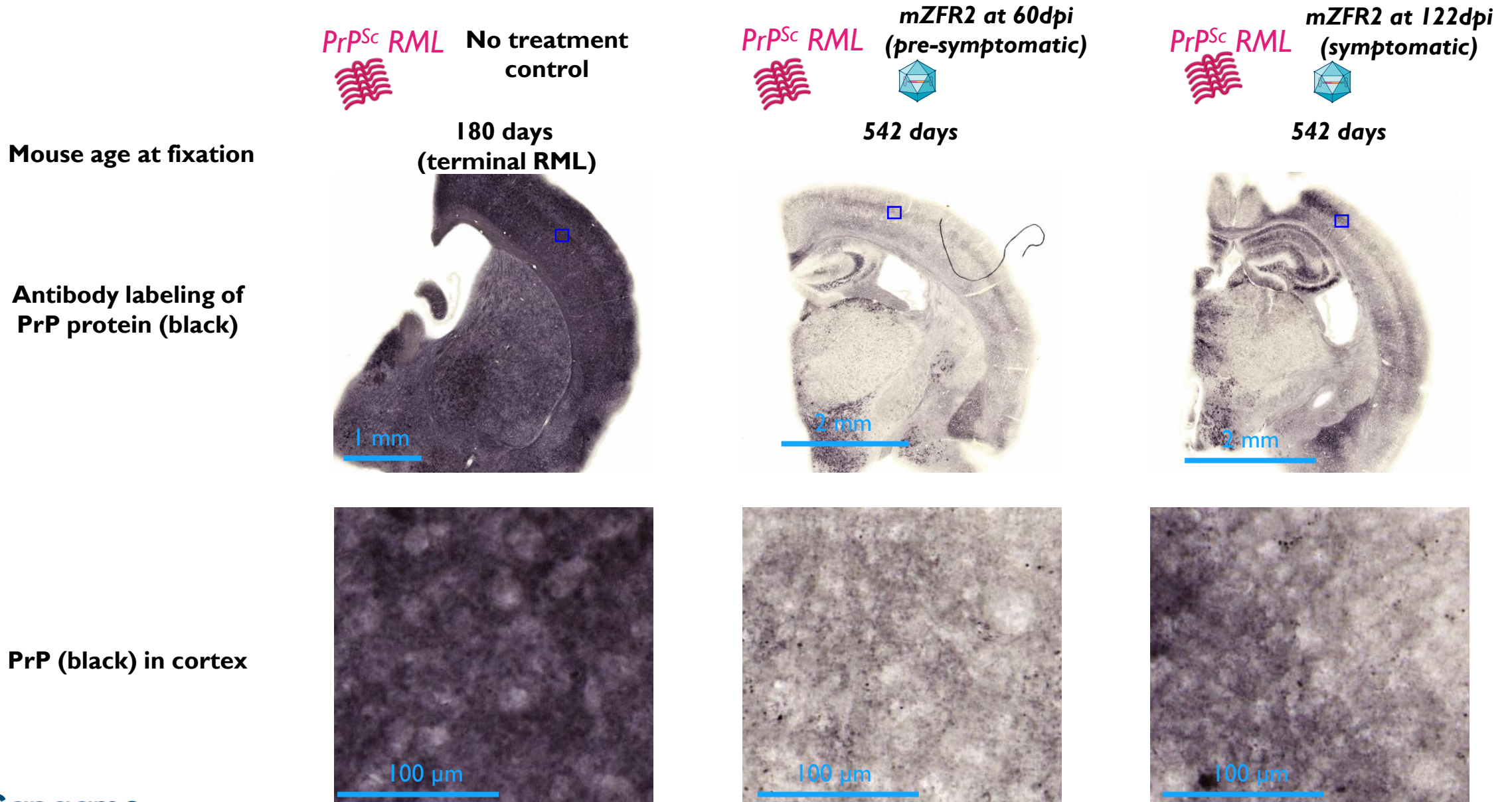
Plasma NfL (pg/mL)



Body weight Trajectory (% change)

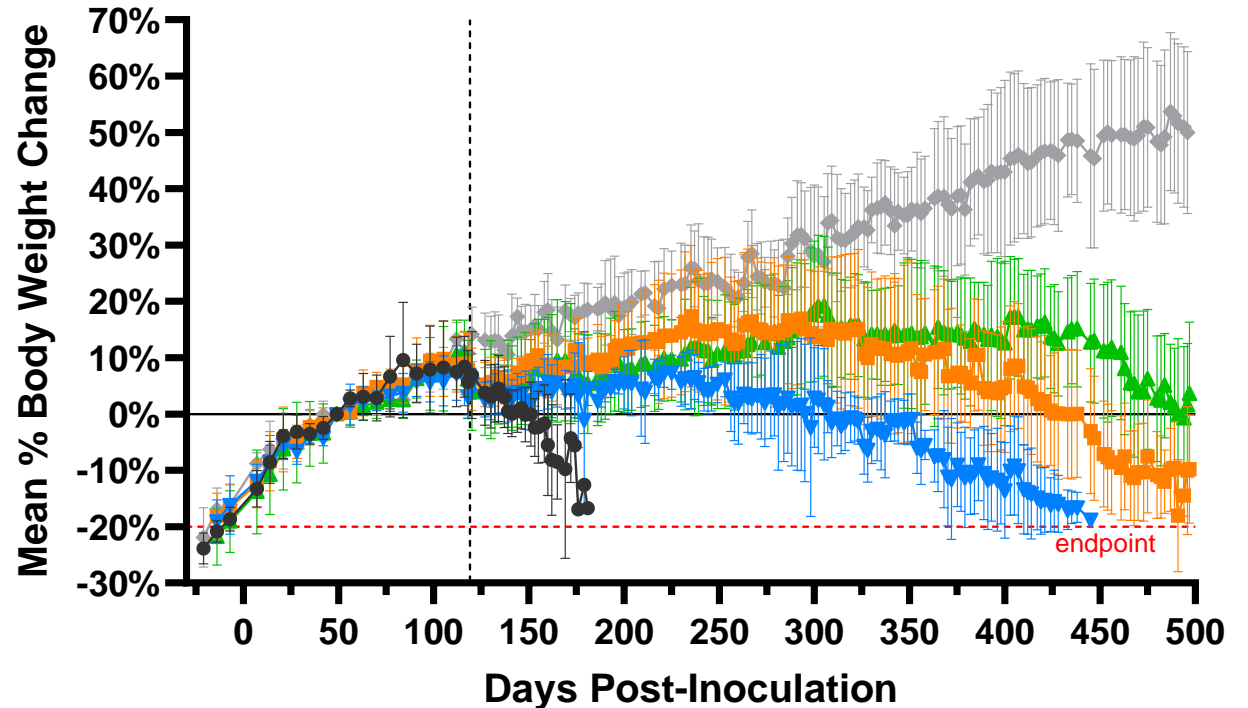
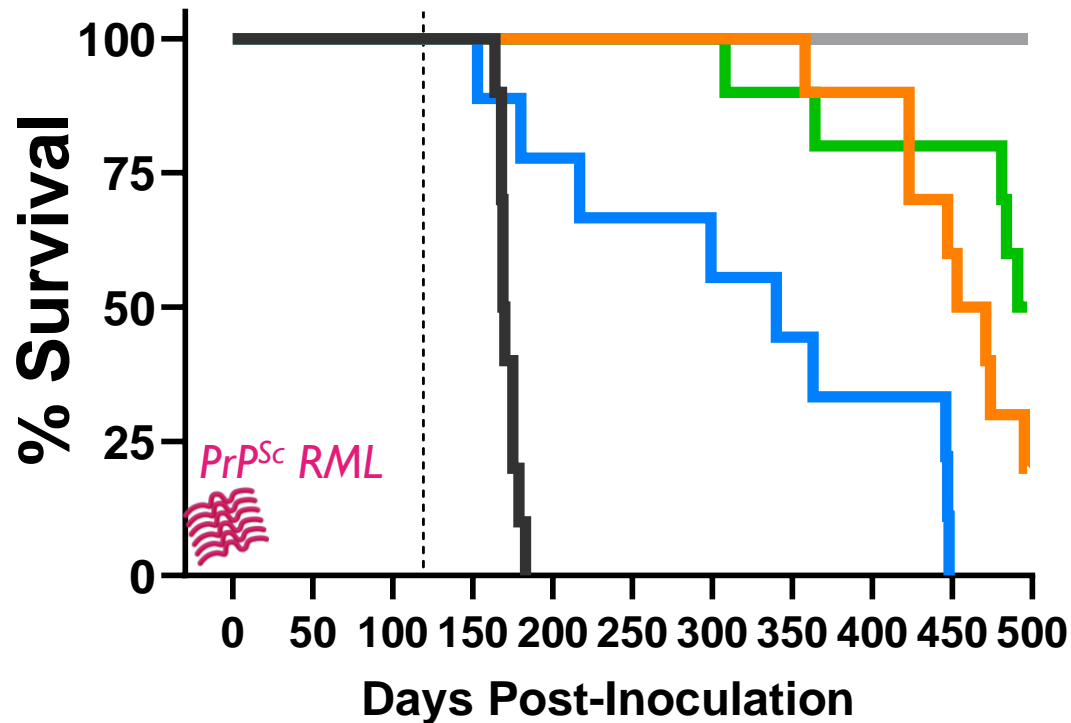


mZFRs significantly reduced PrP^{Sc} protein in RML-inoculated mice



Efficacy study#2: mZFR, at a wide dosing range, successfully extended survival and improved body weight of RML-inoculated mice

	Uninoculated mice with Vehicle (-21 dpi)	
	Vehicle (-21 dpi)	170 days
Median survival	1E13 vg/kg (119dpi)	340 days
	3E13 vg/kg (119dpi)	462 days
	1E14 vg/kg (119dpi)	491 days



Prnp mRNA and PrP protein reduction level correlates with survival extension in a dose-dependent manner

Wildtype



mZFR1 (180 days ZFR exposure)

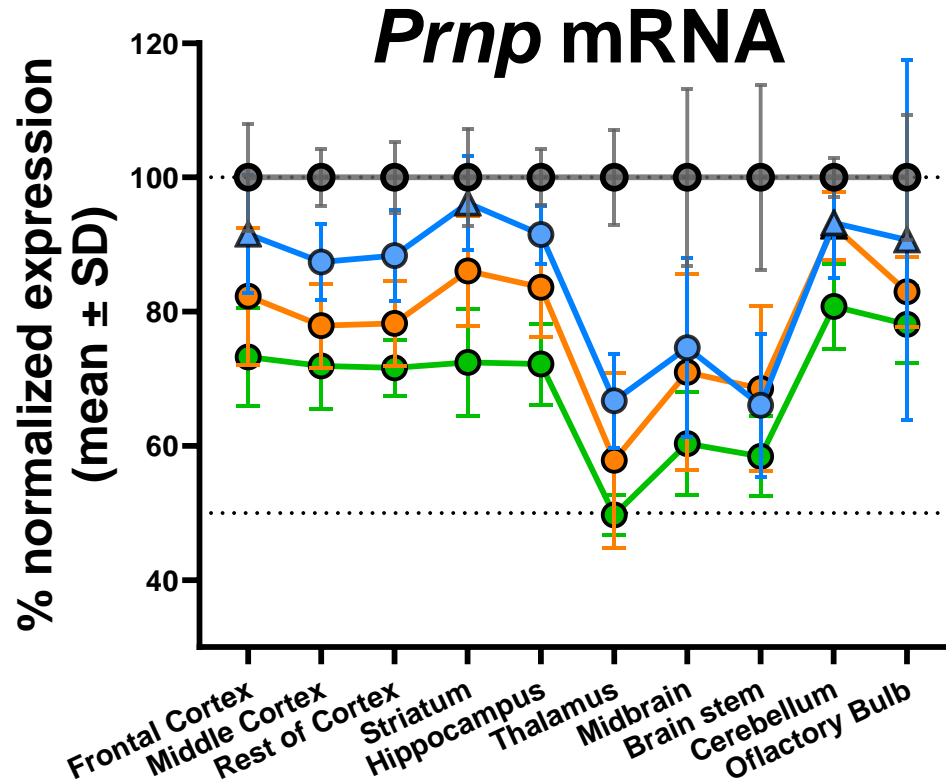


Vehicle

1E13 vg/kg

3E13 vg/kg

1E14 vg/kg

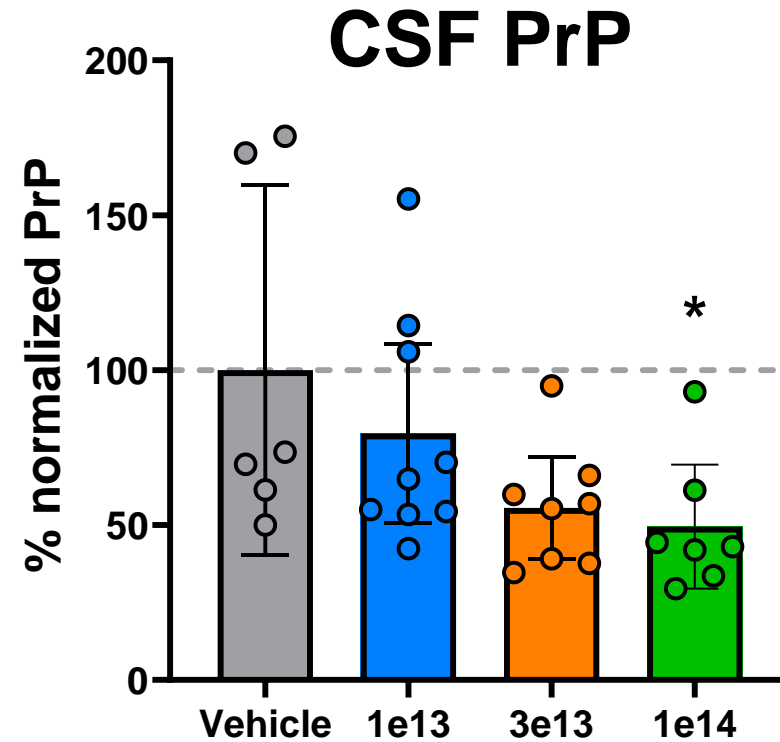


N=8-9 mice per group. Mean ± SD; Two-way ANOVA; Dunnett's test comparisons against Vehicle group

1E13 vg/kg : ns in ▲ regions; *~** for other regions

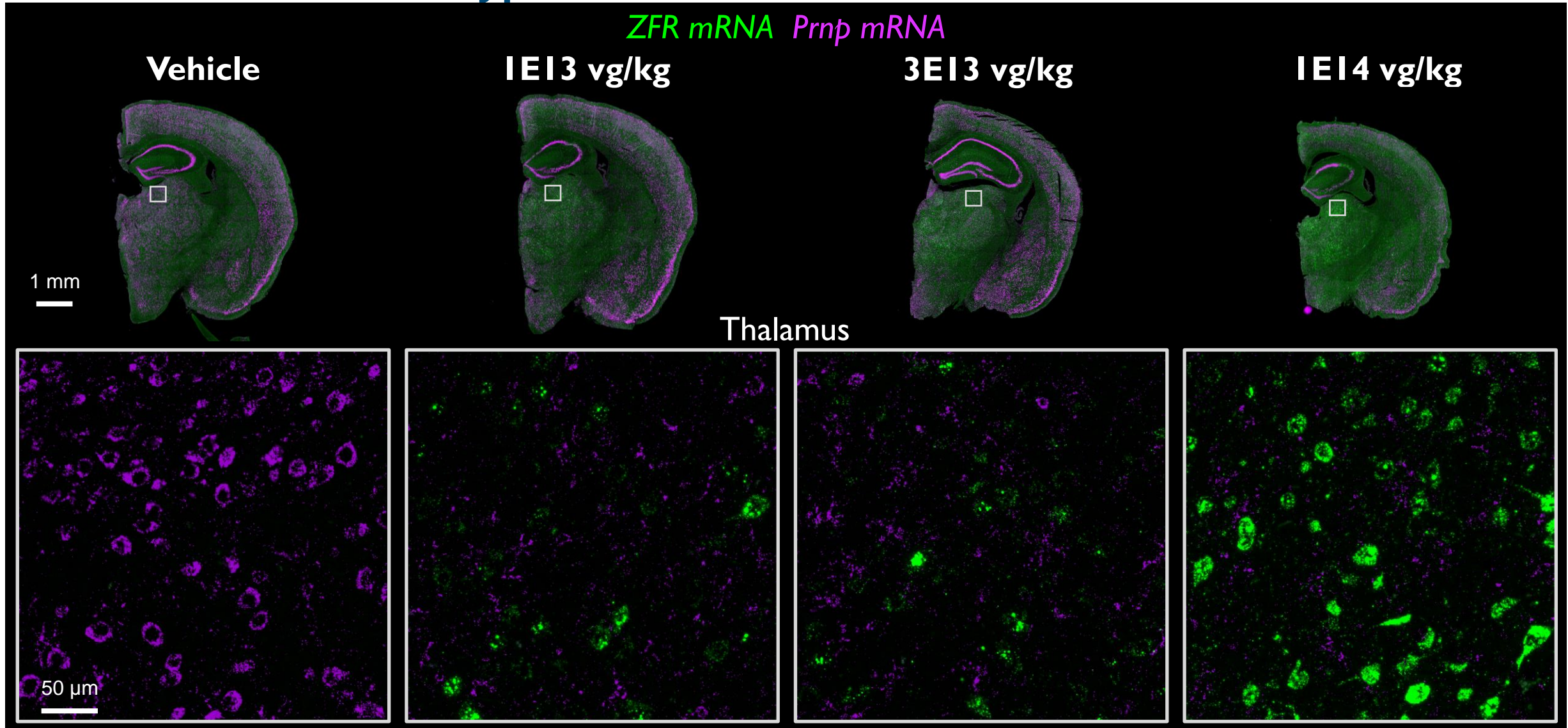
3E13 vg/kg : ***~**** for every regions except cerebellum (ns)

1E14 vg/kg : *** for every regions

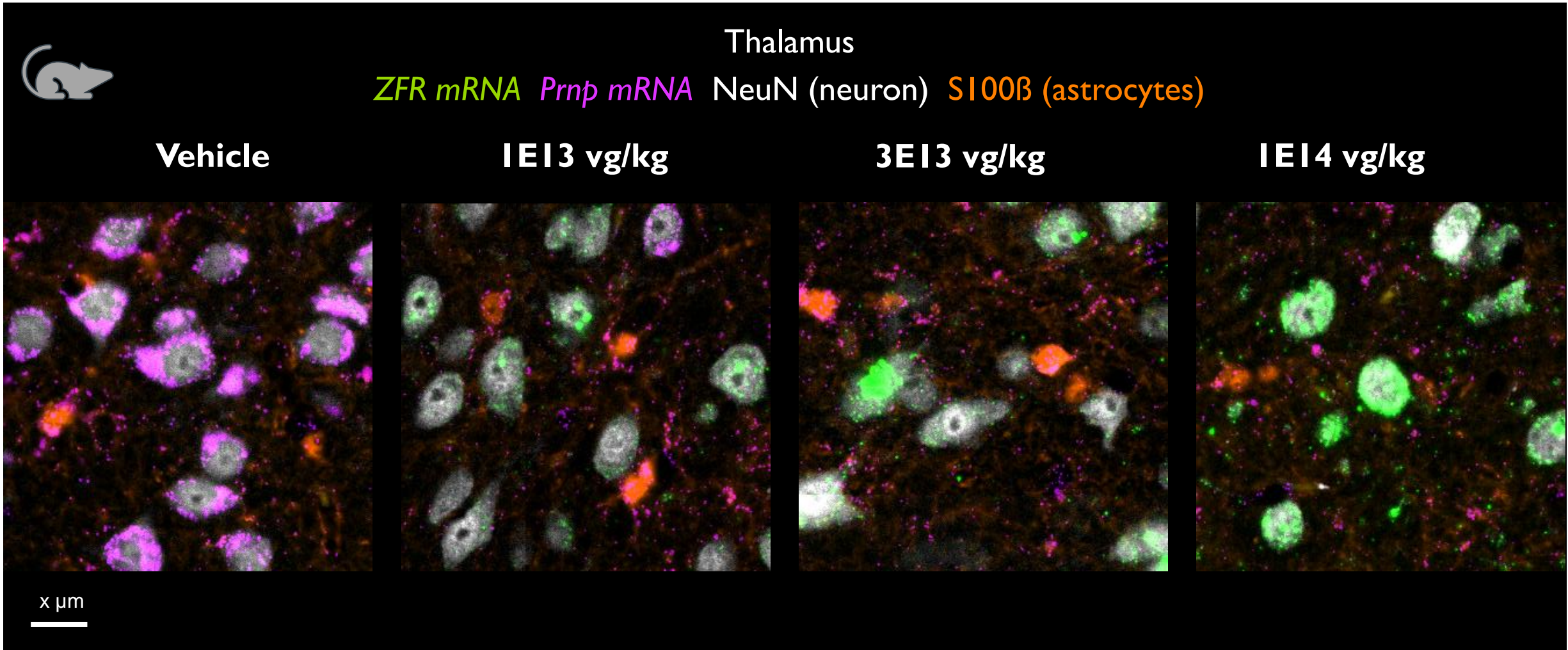


N=6-9 mice per group. Mean ± 95%CI; Kruskal-Wallis, Dunnett's test comparisons against Vehicle group

Dose-dependent ZFR expression and *Prnp* repression specifically in neurons of wild type mice



AAV-ZFR dose titrated % transduction and ZFR expression but not the *Prnp* repression in a single neuron received ZFR



 180 days exposure to mZFR1

PrP lowering with ZFR as a therapeutic treatment



- ✓ **Potent, rapid ZFR-mediated *Prnp* mRNA and PrP protein repression** in neurons throughout the brain **significantly extend survival** in RML-mice
- ✓ **Clear dose response** for both ZFR expression and target gene repression throughout the brain at both tissue and single cell level

Evaluation of STAC-BBB capsid with *PRNP*-targeting ZFR cargo

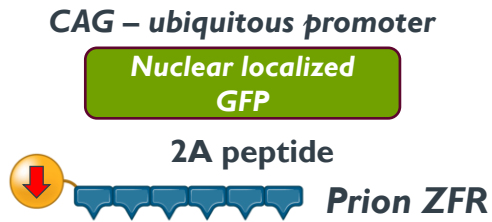
Objective: Assess STAC-BBB biodistribution in CNS and peripheral tissues

Capsid

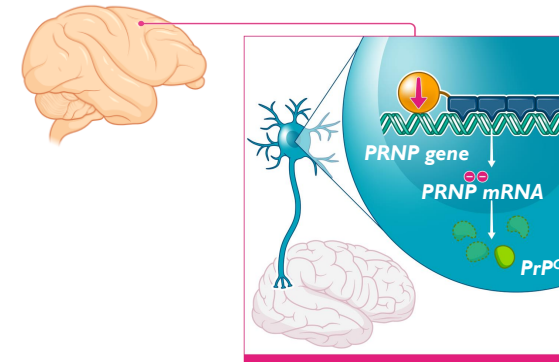


STAC-BBB

Cargo



Therapeutic Approach



Dose & Duration



2E13 vg/kg



3-week study

Species



Cynomolgus Macaque
N=3

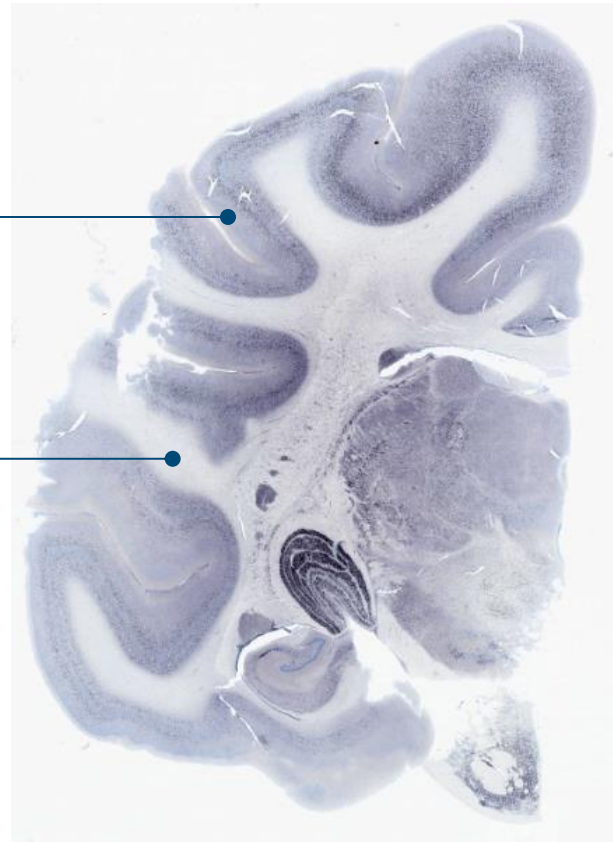
Endpoints

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

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

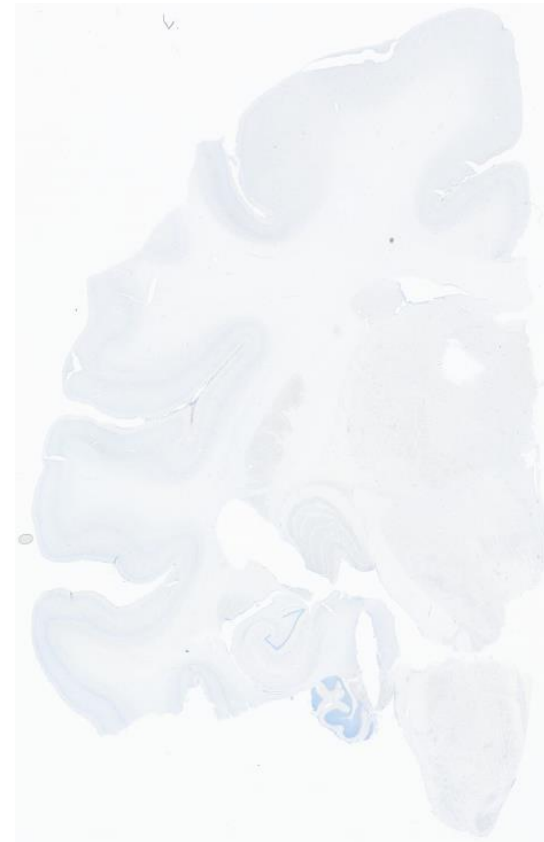


STAC-BBB
(Nuclear-localized GFP)



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

Negative control
(no AAV treatment) – No signal



Grey matter
(cell bodies)

White matter
(nerve fibers)

Nissl staining (light blue):

All cell nuclei

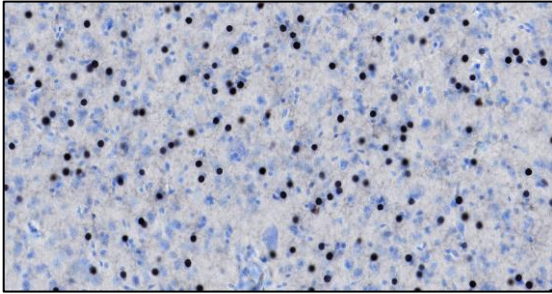
*Antibody labeling
for green fluorescent protein
(GFP) expression (black):*

**Cells transduced
with STAC-BBB**

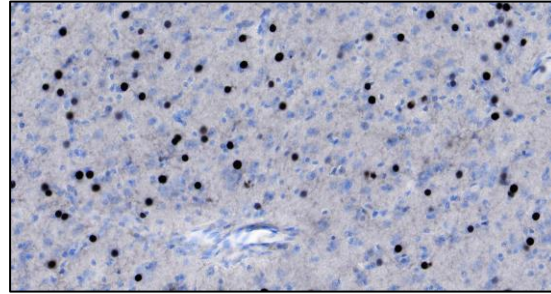
STAC-BBB mediates widespread NHP brain transduction



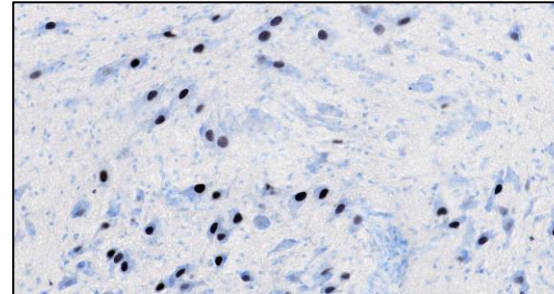
Putamen



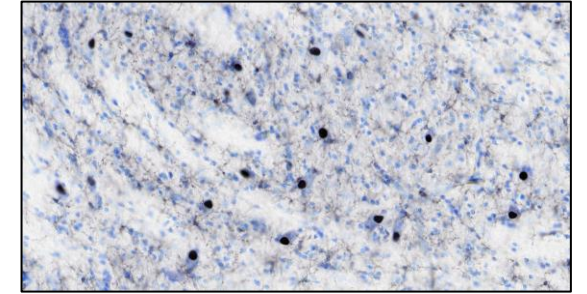
Caudate



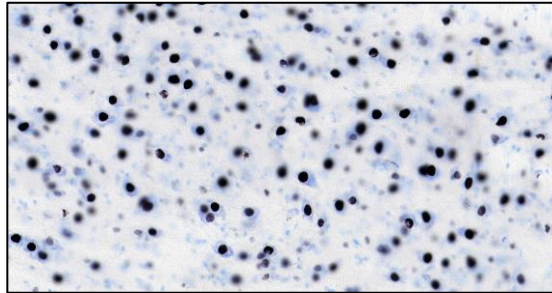
Substantia nigra



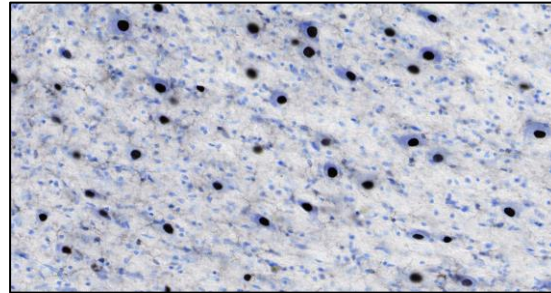
Globus pallidus



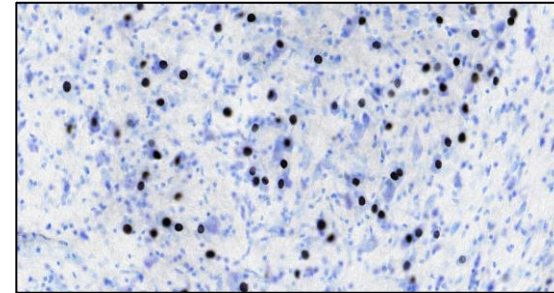
Pons



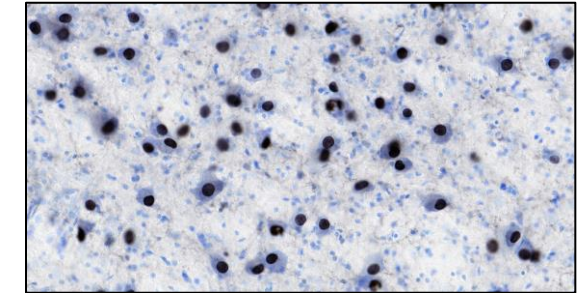
Dentate nucleus



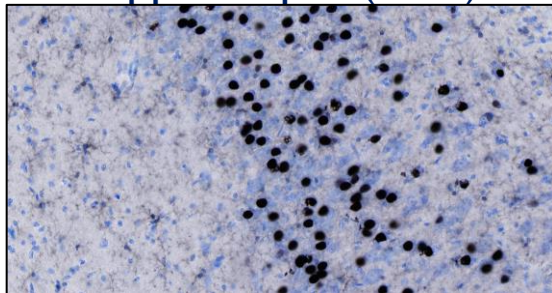
Cuneate nucleus



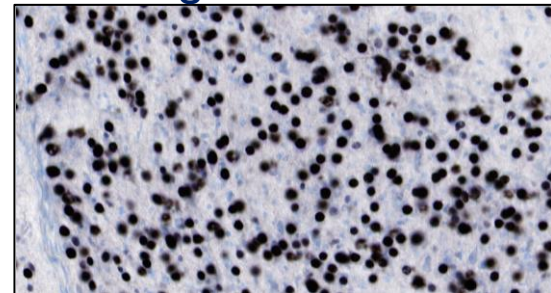
Thalamus



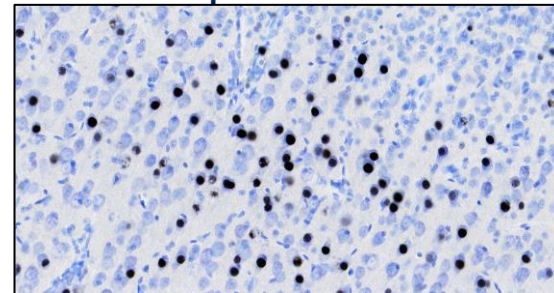
Hippocampus (CA2)



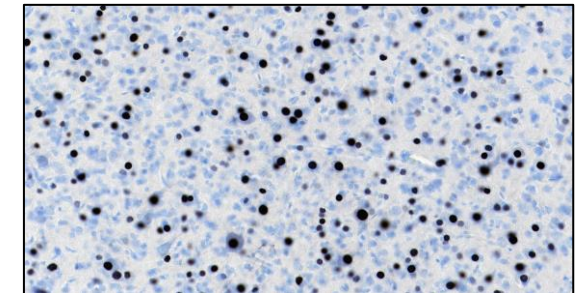
Lateral geniculate nucleus



Temporal cortex



Motor cortex

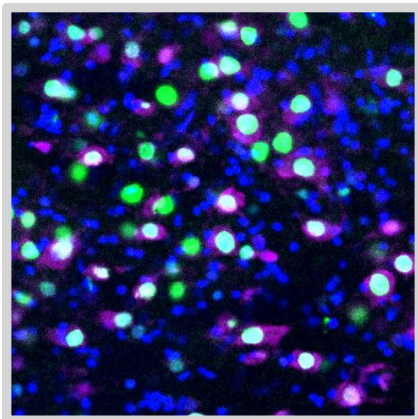


STAC-BBB widely transduced neurons across the NHP brain

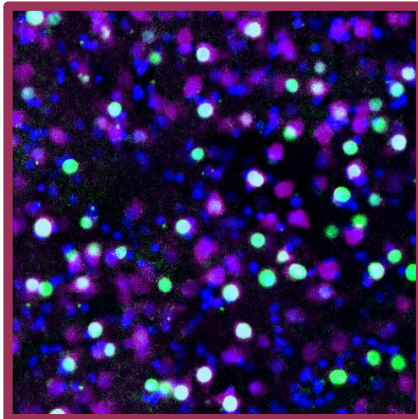


DAPI NeuN GFP

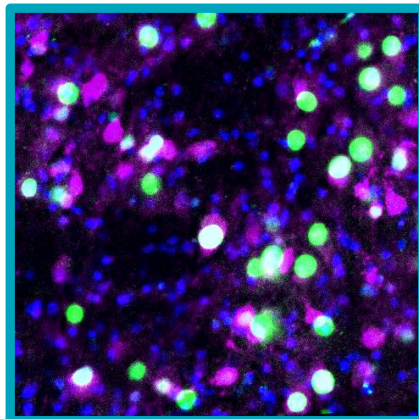
Pons



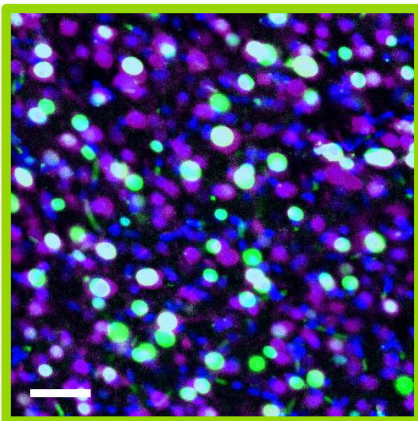
Putamen



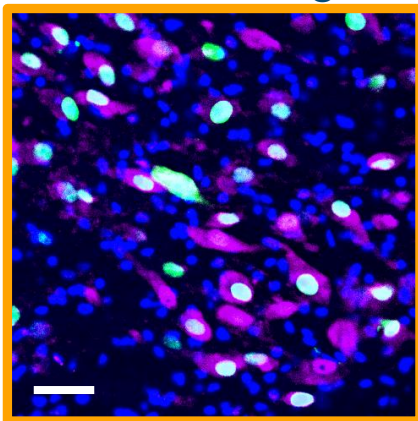
Thalamus



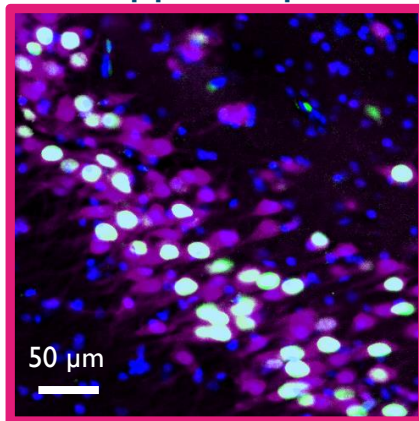
Motor Cortex



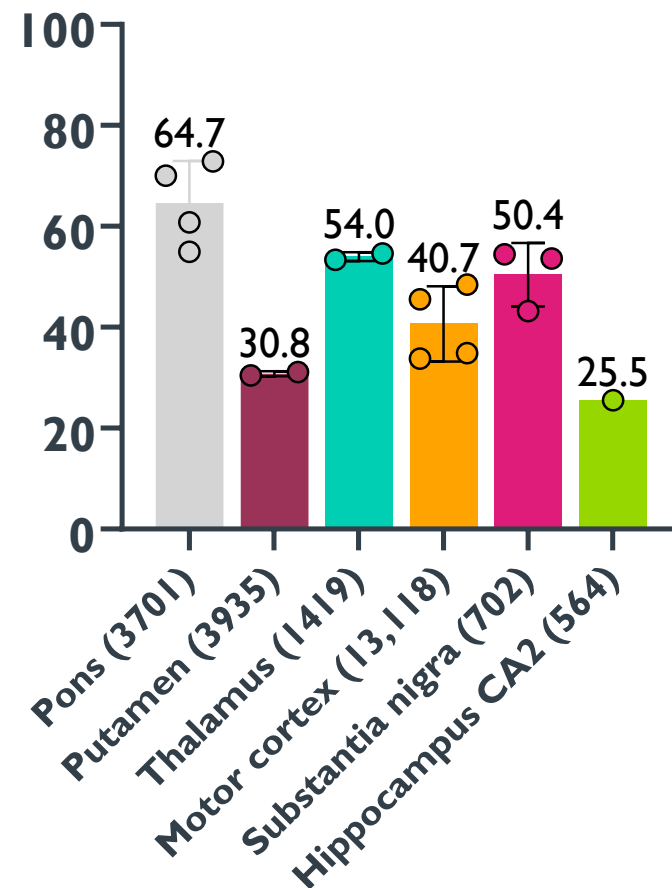
Substantia Nigra



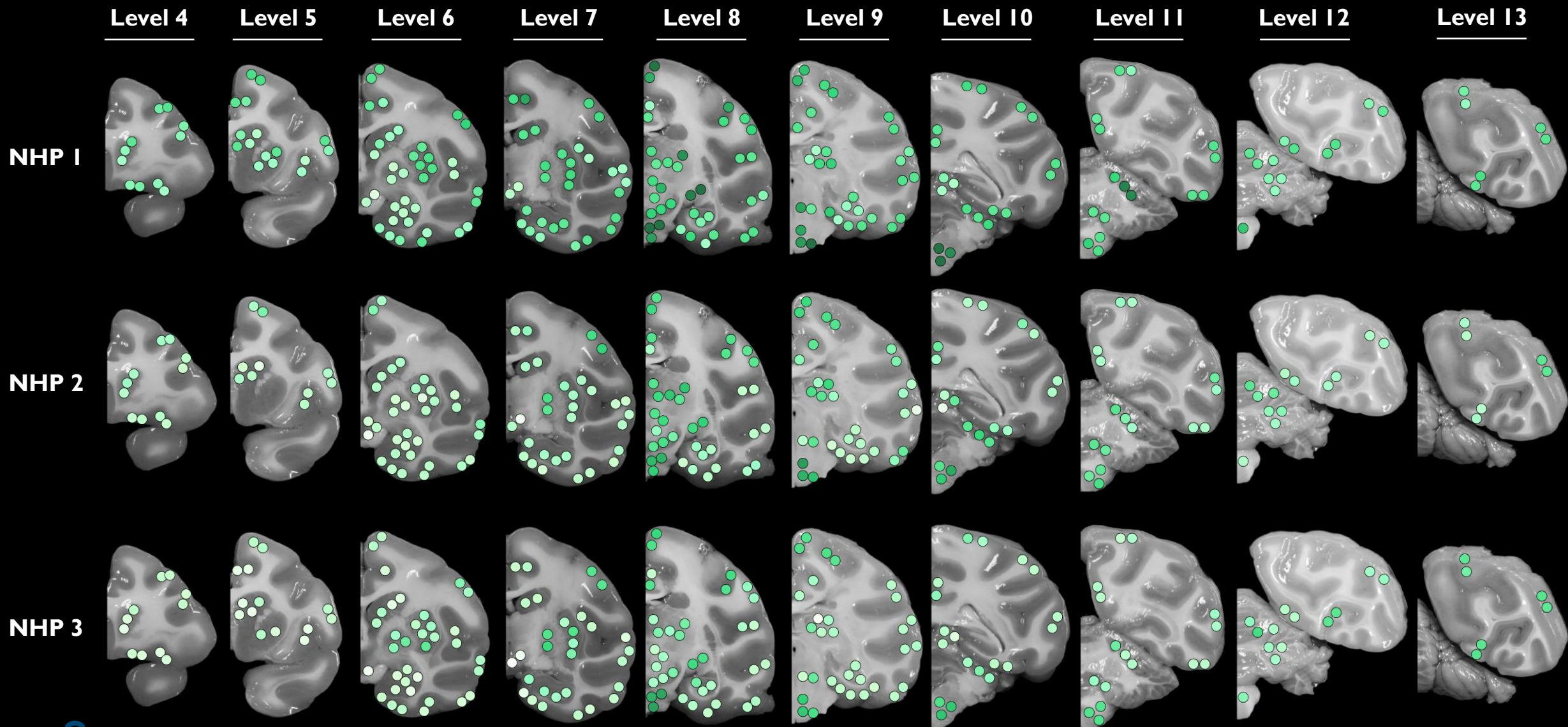
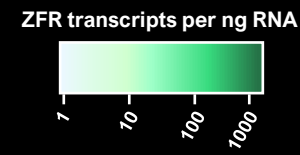
Hippocampus



Percentage of NeuN+ cells transduced



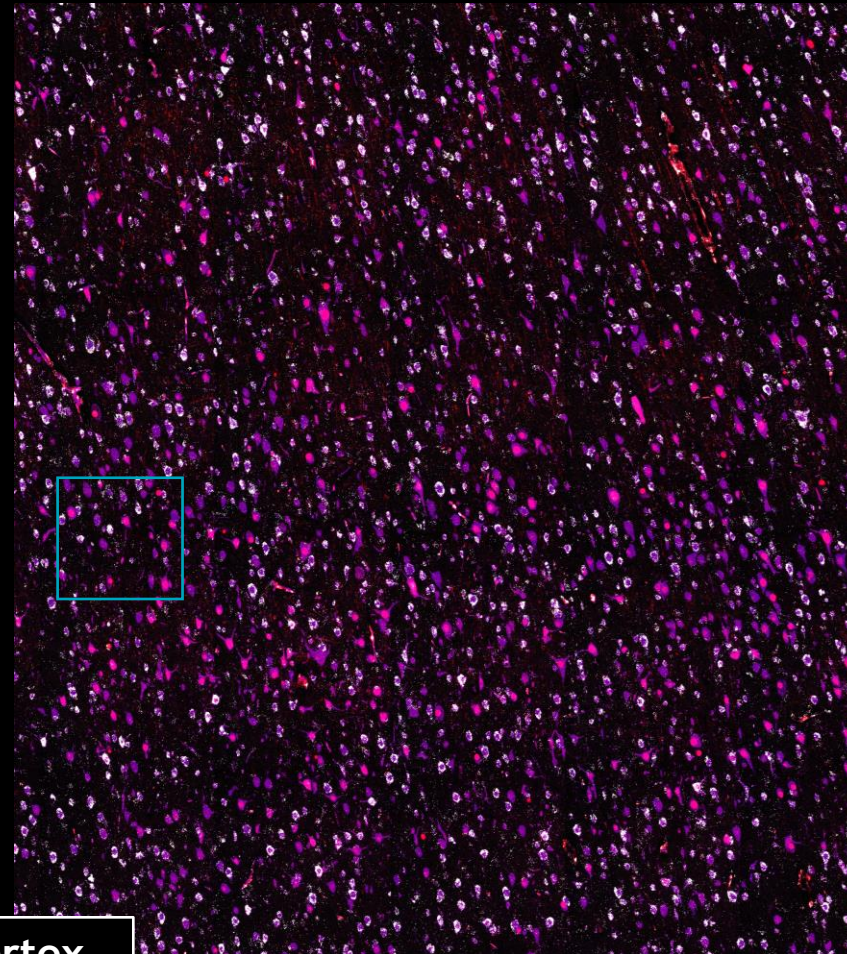
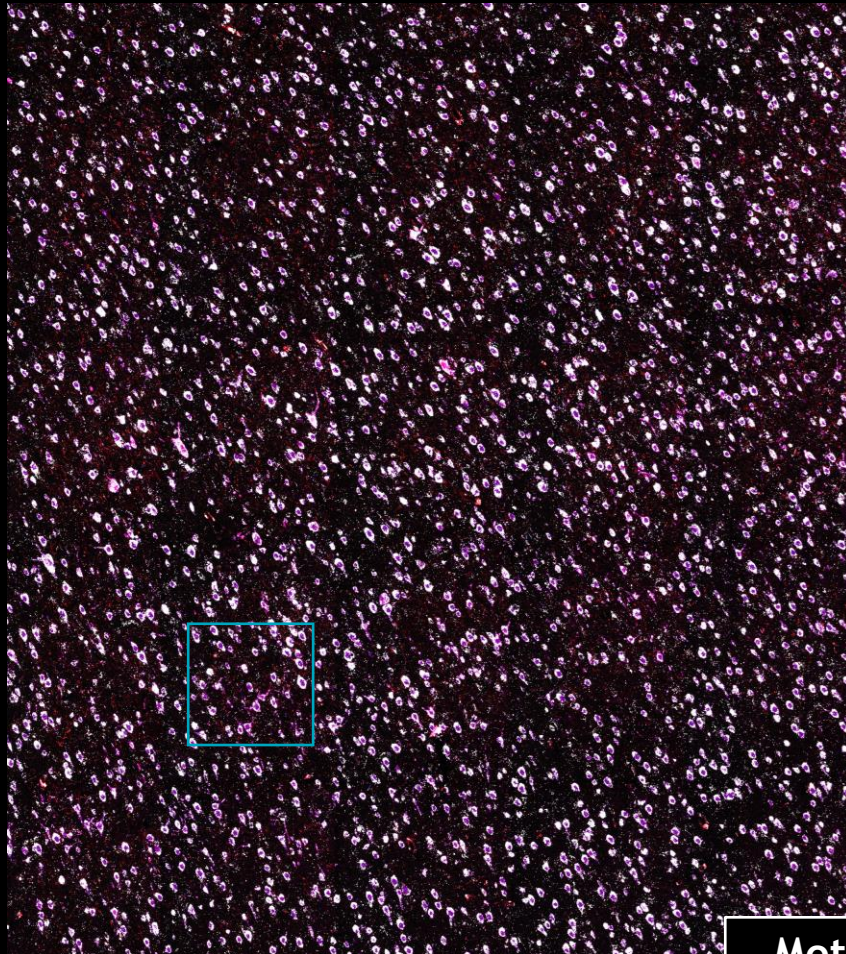
STAC-BBB mediates prion-targeted ZFR expression throughout the entire brain in all animals tested



STAC-BBB mediates ZFR expression and Prion repression in neurons

Vehicle Control

STAC-BBB



GFP
Neurons (NeuN)
Prion mRNA

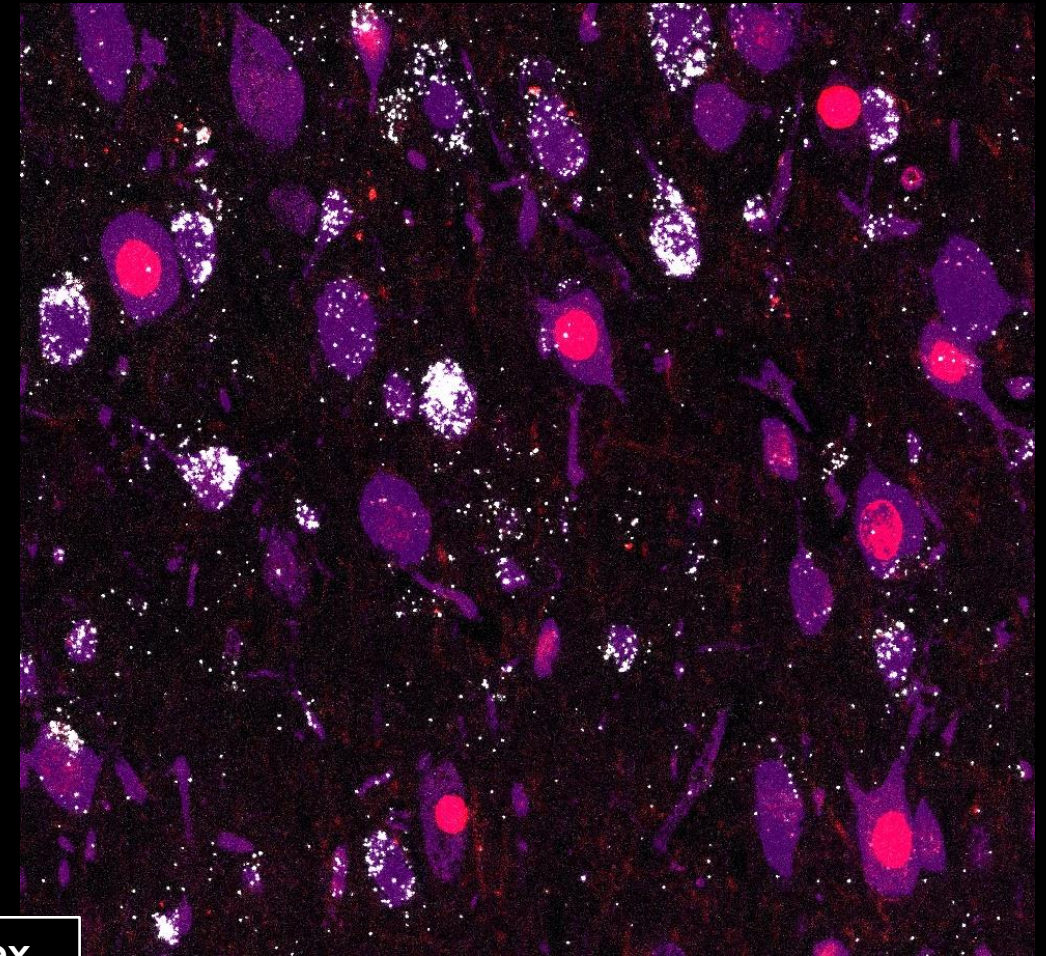
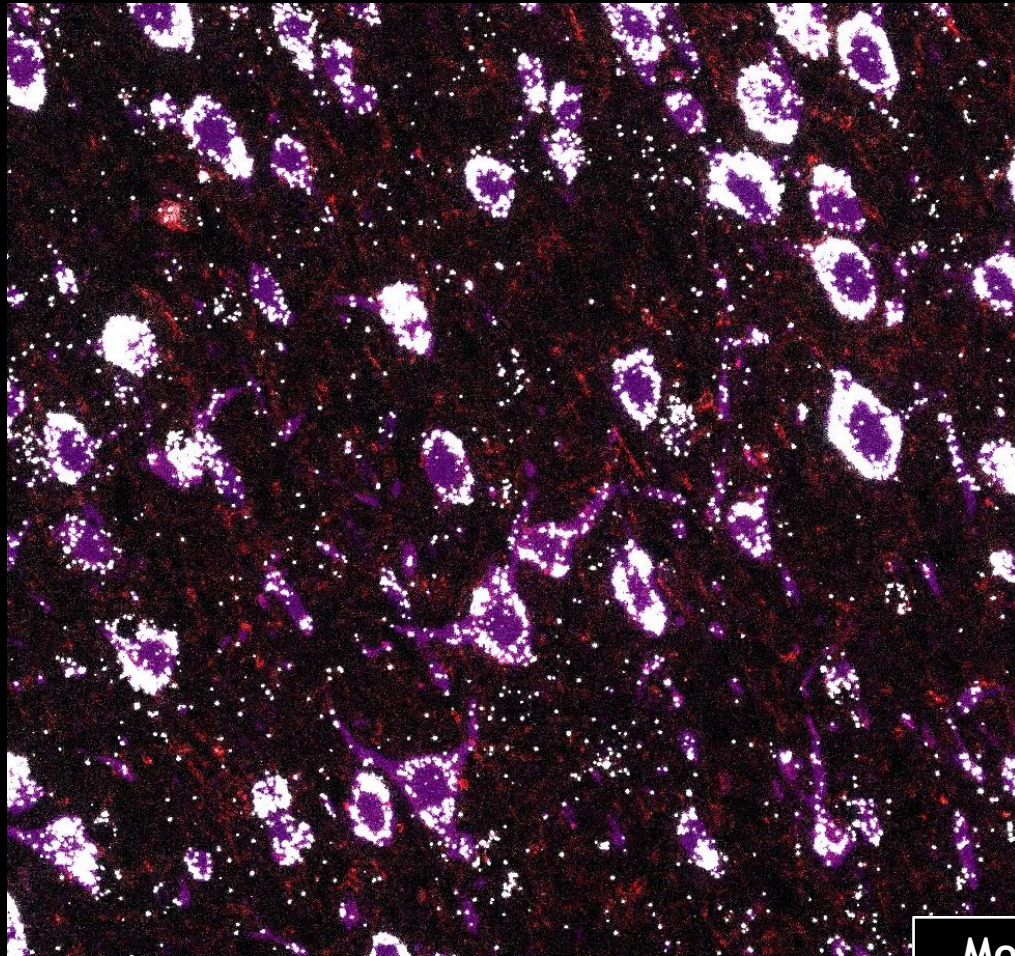
Motor cortex

STAC-BBB mediates ZFR expression and Prion repression in neurons

GFP
Neurons (NeuN)
Prion mRNA

Vehicle Control

STAC-BBB



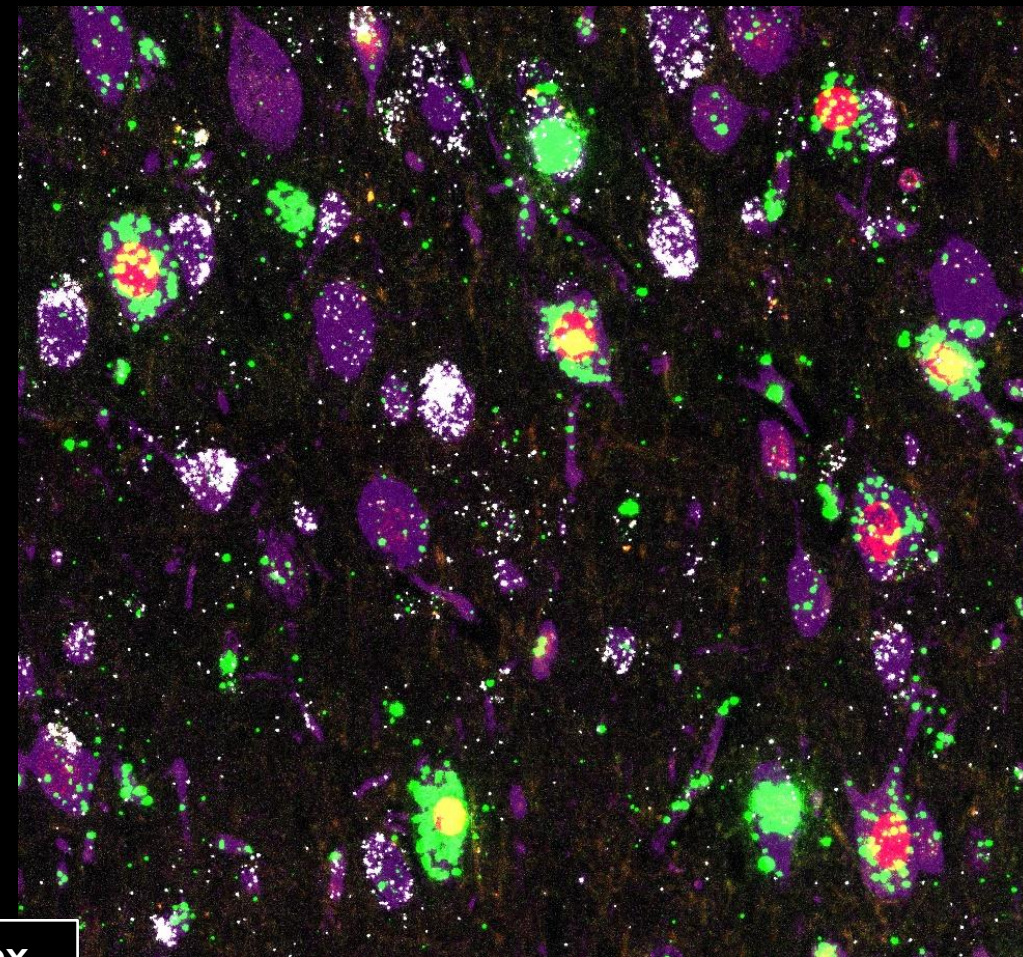
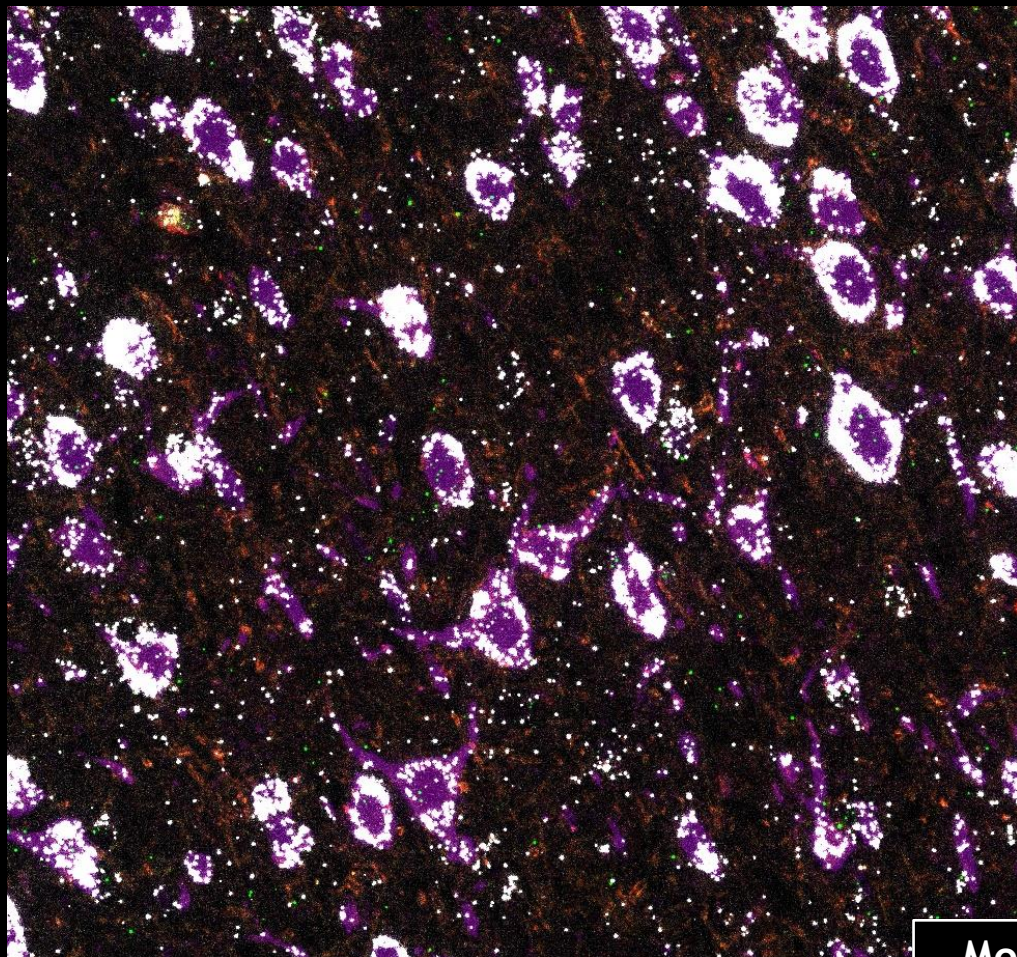
Motor cortex

STAC-BBB mediates ZFR expression and Prion repression in neurons

GFP
Neurons (NeuN)
Prion mRNA
ZFR mRNA

Vehicle Control

STAC-BBB

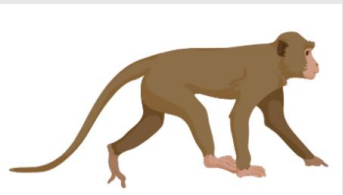


Motor cortex

Sangamo is progressing development of this one-time IV administered ZFR therapy for prion disease



- ✓ **Potent, rapid ZFR-mediated *Prnp* mRNA and PrP protein repression** in neurons throughout the brain **significantly extend survival** in RML-mice
- ✓ **Clear dose response** for both ZFR expression and target gene repression throughout the brain at both tissue and single cell level



- ✓ Novel STAC-BBB AAV capsid demonstrated robust **blood-brain barrier crossing** and **widespread neuronal transduction** throughout the adult nonhuman primate brain.
- ✓ **STAC-BBB-ZFR transduced neurons showed lower *PRNP* mRNA expression** in nonhuman primate brain.

Acknowledgements

Sangamo Team

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Alaric Falcon	Madeleine Jensen
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Adrian Woolfson
Ben Deverman
Melanie Hermann



Thank You