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[1], Annemarie Ledeboer[1], Marina Falaleeva[1], Jing Hu[1], Madelena Nguyen[1], Shih-Wei Chou[1], Yonghua Pan[1], Kimberly Marlen[1], David Ojala[1], Matthew Tiffany[1], Daniel Chung[1], Mihika Jalan[1], Patrick Dunn[1], Lei Zhang[1], Jason Eshleman[1], Alaric Falcon[1], Carolyn Gasper[1], Meredith A Pokatayev[2], Michael Howard[2], Kenney Lenz[2], Kenia Guzman[2], Nikita Kamath[2], Alissa Coffey[2], Mary Lanier[3], Thanh-Thuy-Tran[3], Miranda Johnson[3], Cody Zurhellen[4], Yanmei Lu[1], Amy M Pooler[1], Eric Vallabh Minikel[2], Sonia M Vallabh[2], Bryan Zeitler[1], Kathleen Meyer[1]

[1] Sangamo Therapeutics Inc., [2] Broad Institute of MIT and Harvard, [3]SRI International, [4]Neuroscience Associates

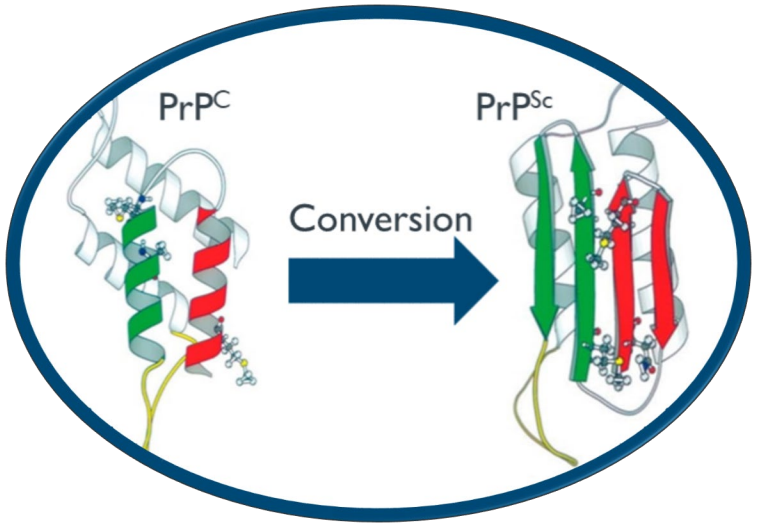
Some illustrations were created with BioRender.com

Presented at the ASGCT 28th Annual Meeting

Disclosure

I am a full-time employee of Sangamo Therapeutics

Prion disease



A rapid and fatal neurodegenerative disorder with high unmet medical need

Target: Entire brain - cortical and deep regions

Cause: Conversion of normal to misfolded prion protein

Prevalence: at least 1,300 new cases / year in US and Europe

Symptoms: Dementia, memory loss, movement disorder, cognitive decline → death

Life Expectancy: 5 months from diagnosis

Treatment: None

Given the lack of life-saving treatments there is an urgent need to develop novel therapeutics for the treatment of prion disease

Image modified from: Medd MM and Cao Q (2004) Perspectives on CRISPR Genome Editing to Prevent Prion Diseases in High-Risk Individuals. Biomedicines, 12(8): 1725; https://doi.org/10.3390/biomedicines12081725

Sangamo THERAPEUTICS

Sangamo utilizes zinc finger proteins with blood-brain barrier crossing AAV to advance a next-generation neurology genomic medicine

Potent Zinc Finger Cargo

Level of potency is precisely customizable to the indication being targeted

Versatility and Exquisite Specificity

We believe any gene in the genome is targetable for up- or down-regulation

All Human Derived

Potentially avoids issues with immunogenicity

Small Size. Easily Packaged.

Zinc fingers can be easily packaged into viral vectors

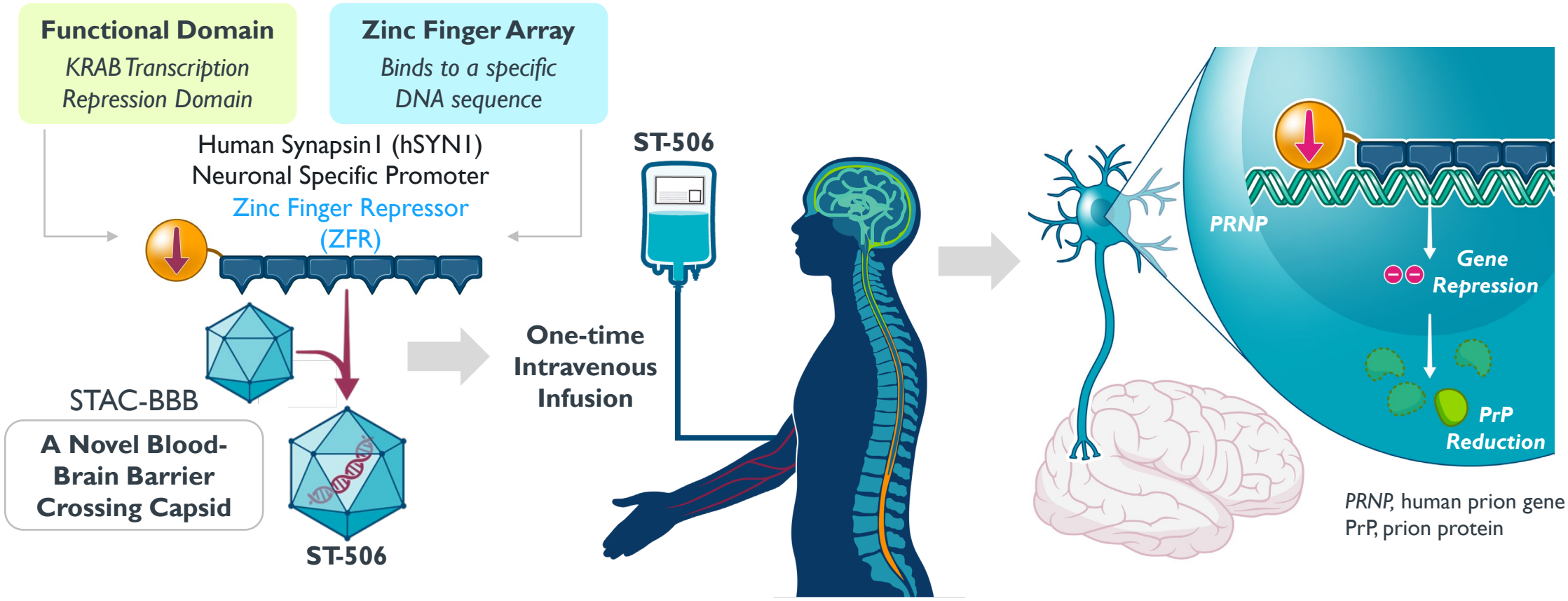
Powerful AAV Delivery Platform

Widespread zinc-finger 'cargo' delivery – via both intravenous AND intrathecal delivery

Industry Leading CNS Tropism

Robust penetration of the blood-brain barrier and widespread brain distribution in NHPs

STAC-BBB-delivered prion-lowering zinc finger protein as a potential therapy for prion disease



Nonclinical development strategy for ST-506: a potential therapy for prion disease

Surrogate ZFRs targeting mouse prion gene (*Prnp*, mZFRs) and surrogate capsid (PHP.B)

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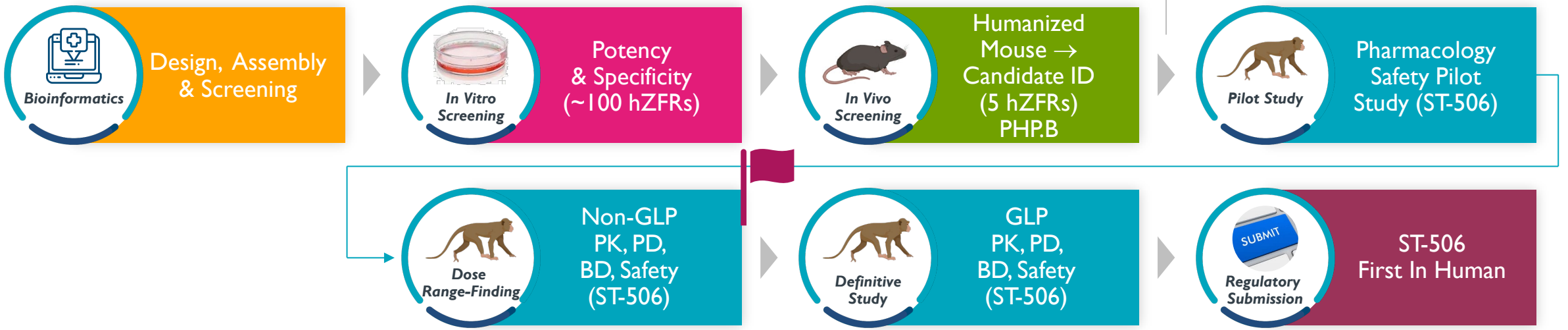
RML, Rocky Mountain Laboratory

ZFRs targeting human/nonhuman primate (NHP) *PRNP* (hZFRs)

Construct Optimization



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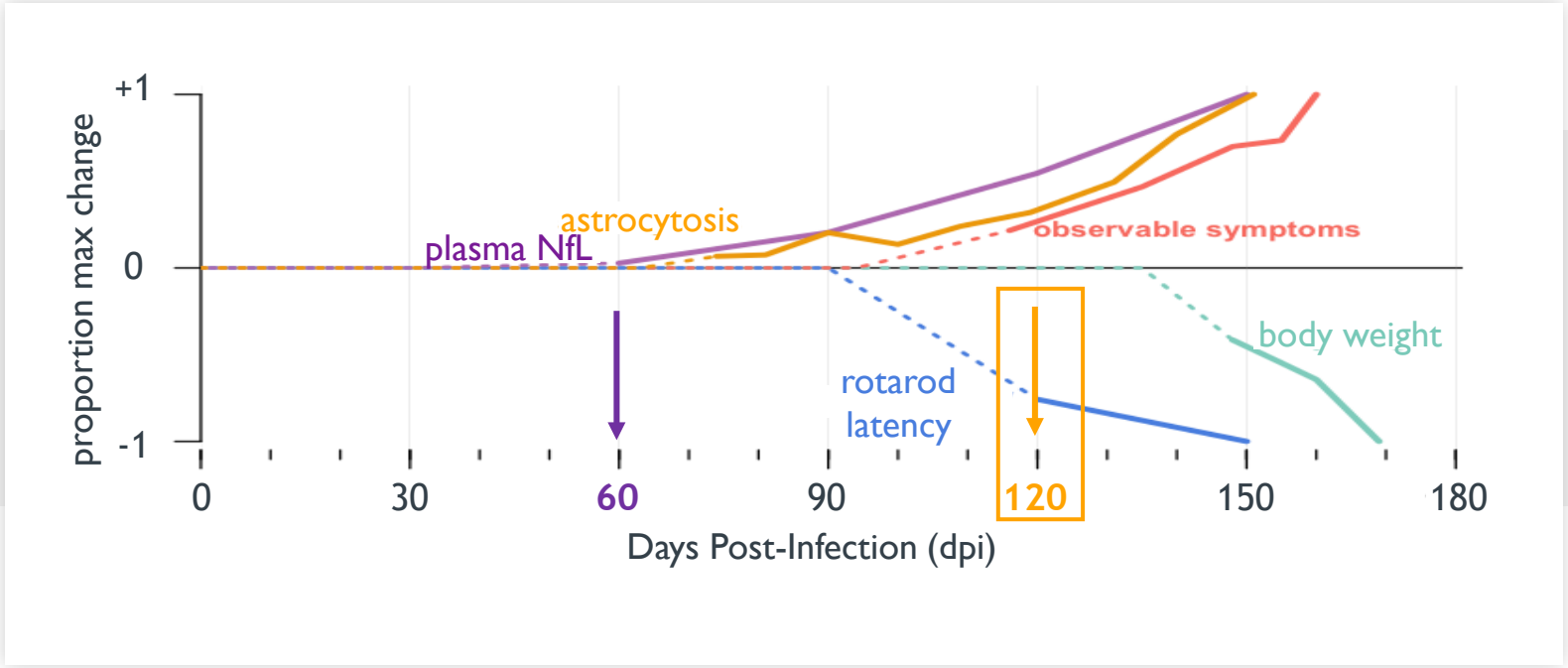
Rocky Mountain Laboratory (RML) inoculated mice are the gold standard model of prion disease



- Onset of molecular changes: 60 days post-inoculation (dpi)
- Onset of clinical symptoms: 120 dpi
- Median survival: ~160 dpi

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ZFRs are potent and selective repressors of gene expression

Surrogate mZFR1

Mouse Cortical neurons

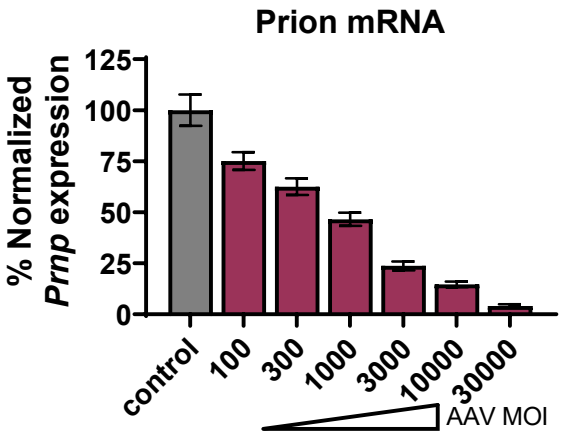
\+

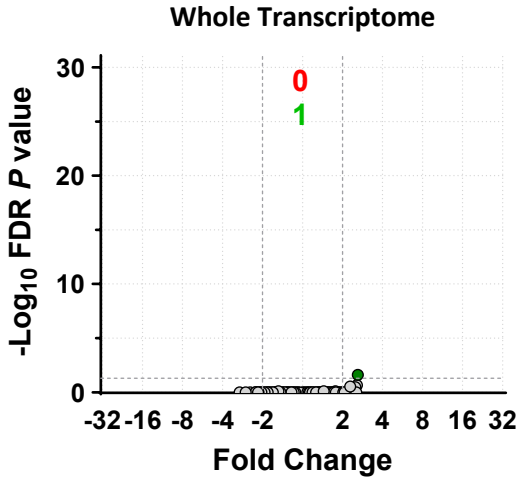
AAV6-hSYN1-mZFR1

7d

Prion expression,
Off-target assessment

Differential expression of 20,000 genes was evaluated





Clinical Candidate ZFR

iPSC-derived human neurons

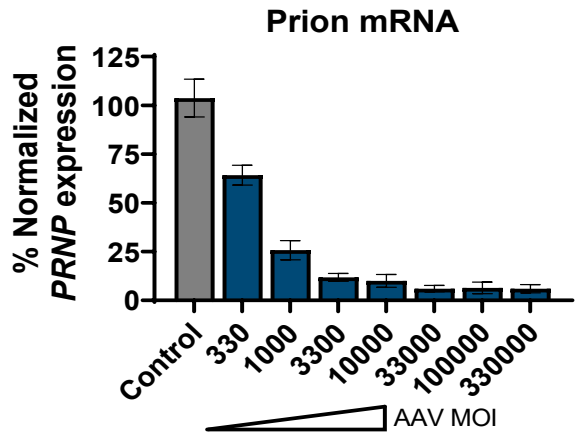
\+

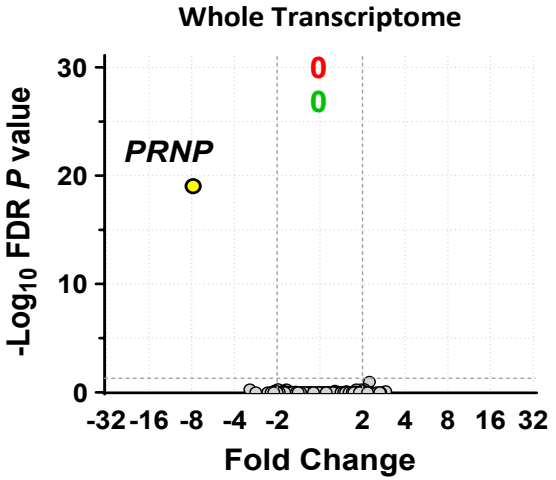
AAV6-Clinical Candidate ZFR

21d

Prion expression
Off-target assessment

Differential expression of 20,000 genes was evaluated





Genes down-regulated Genes up-regulated *Prion*

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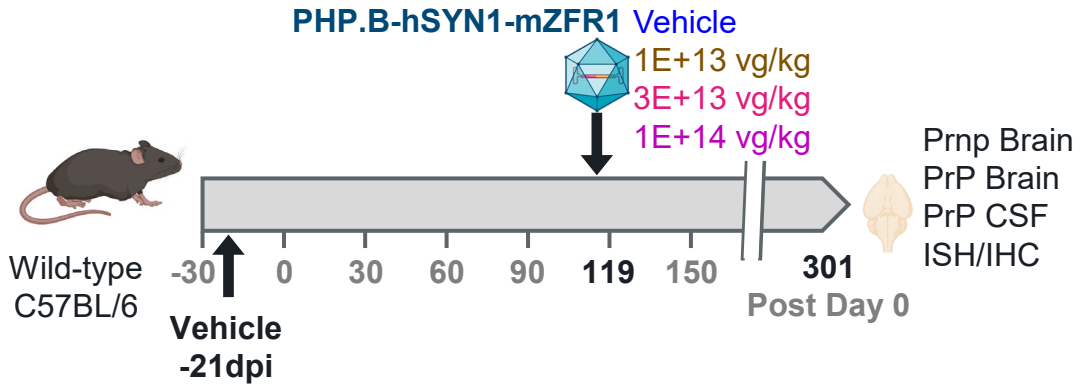
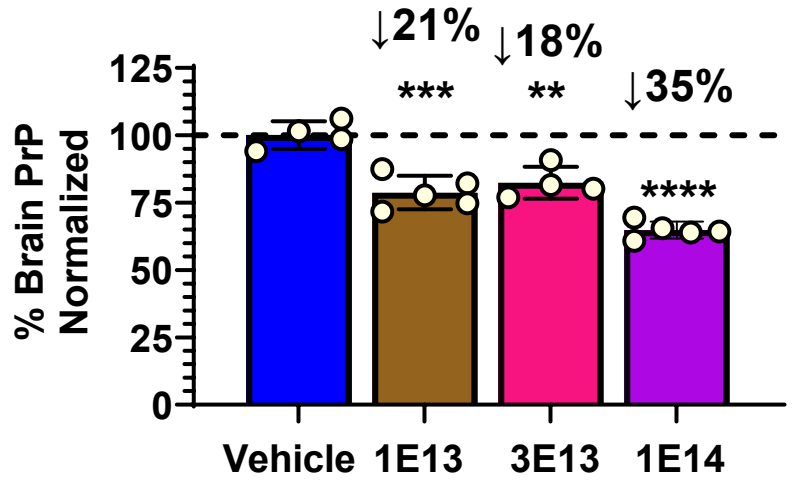
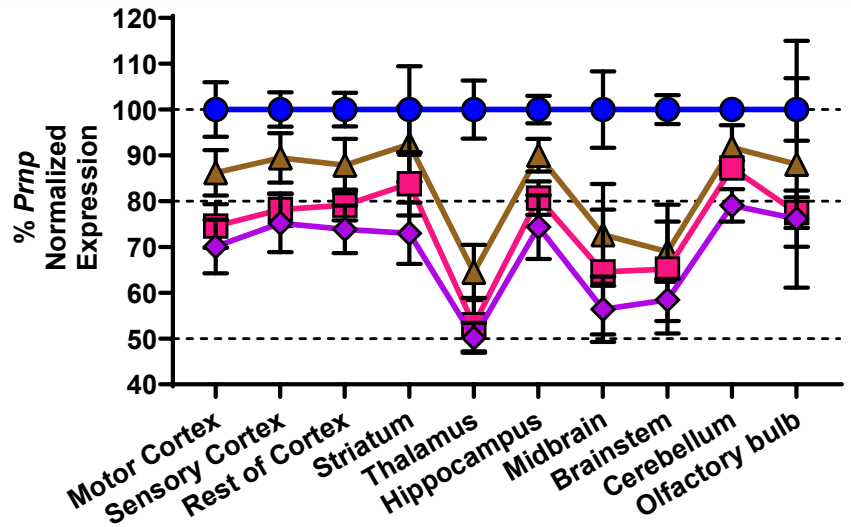
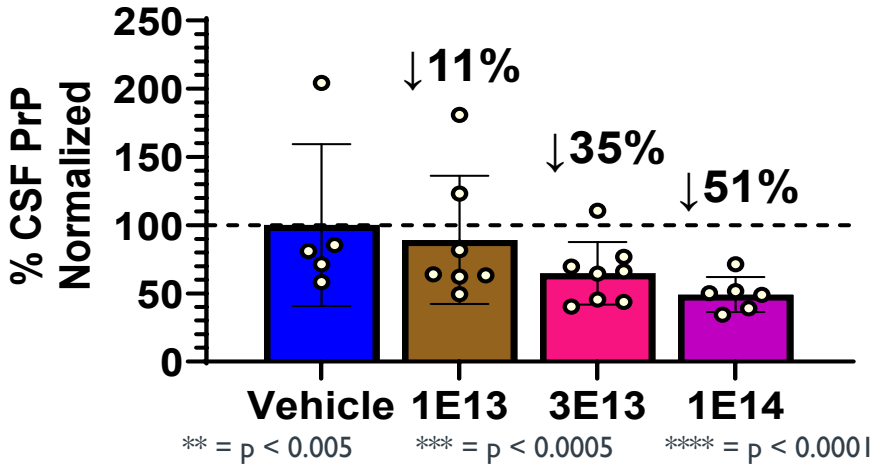
Well-tolerated surrogate ZFR dose-dependently represses prion gene and protein in wild-type mouse brain and CSF









Tolerability: No Mortality No Weight Loss No Abnormal Clinical Signs

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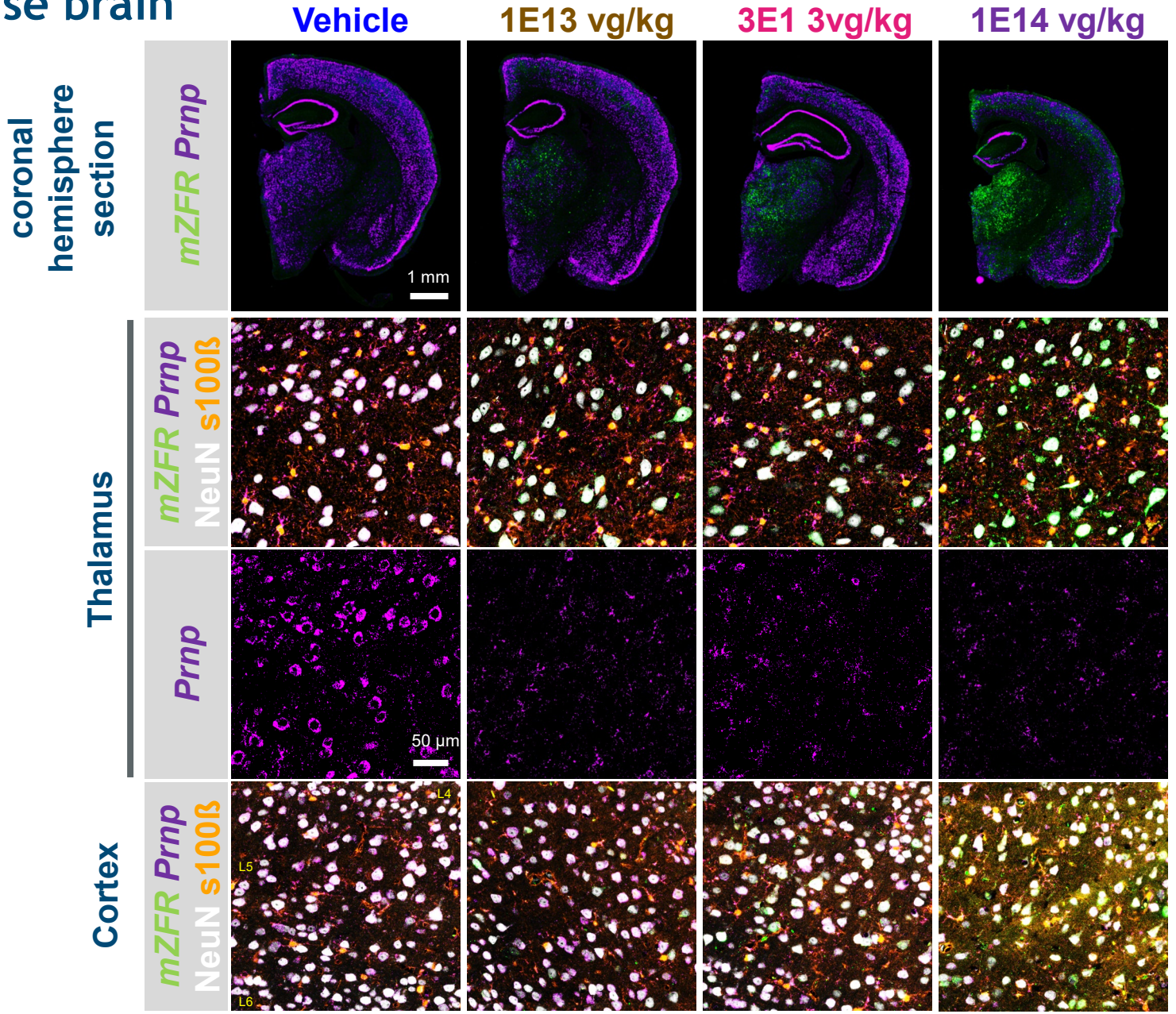
Surrogate ZFR dose-dependently represses *Prnp* expression in only neurons within wild-type mouse brain

PHP.B-hSYN1-mZFR1

Wild-type
C57BL/6



Prnp
mZFR
s100ß
Neun

Neuronal repression in both cortical and deep regions of the brain

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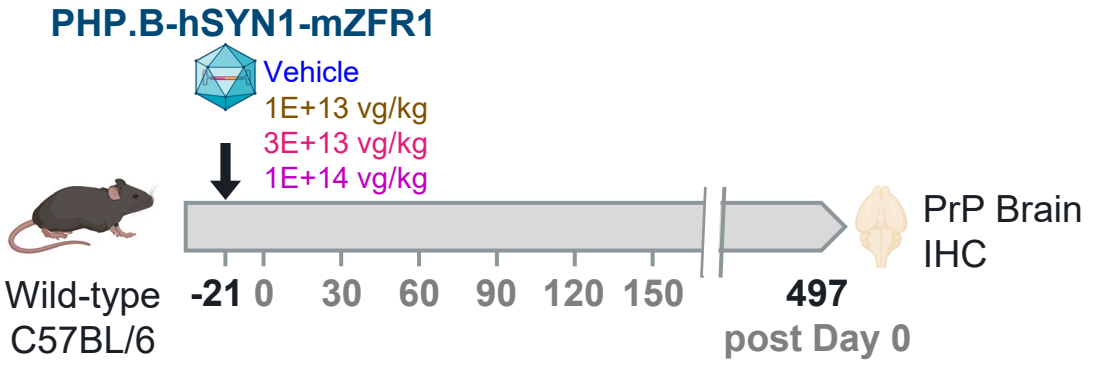
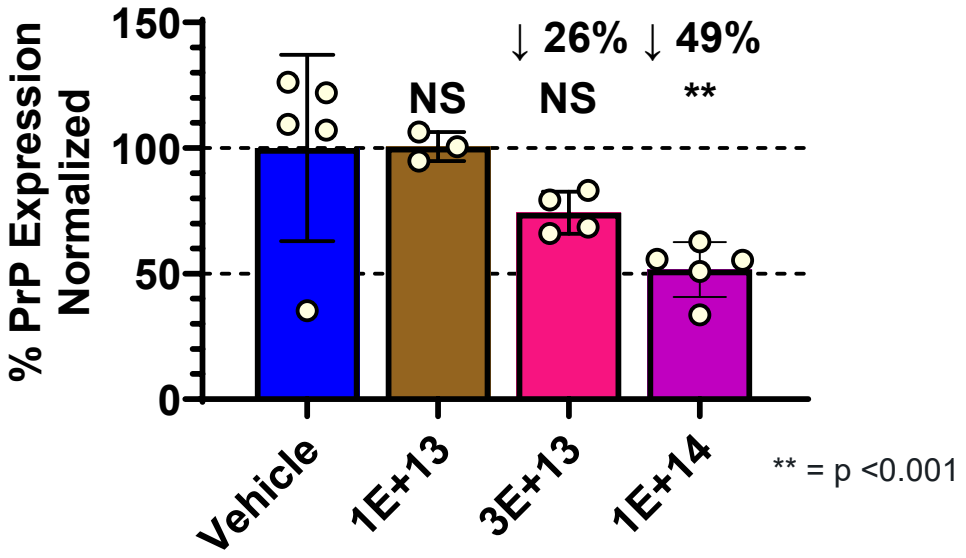
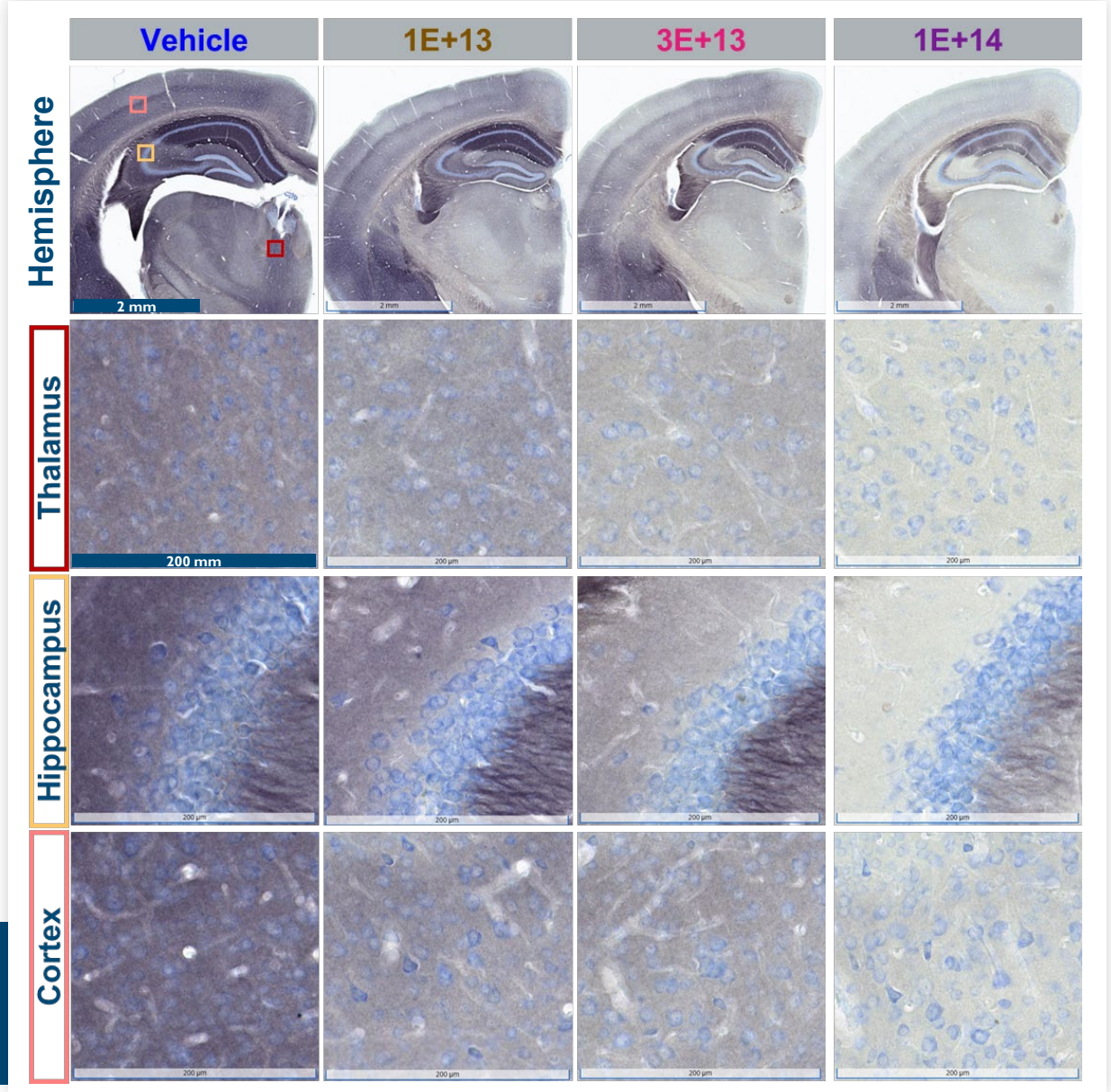
Surrogate ZFR-mediated PrP repression is durable in the mouse brain for at least ~17 months







ZFR-mediated prion protein repression was well-tolerated and lasted for the duration of the study: ~17 month longest ever reported

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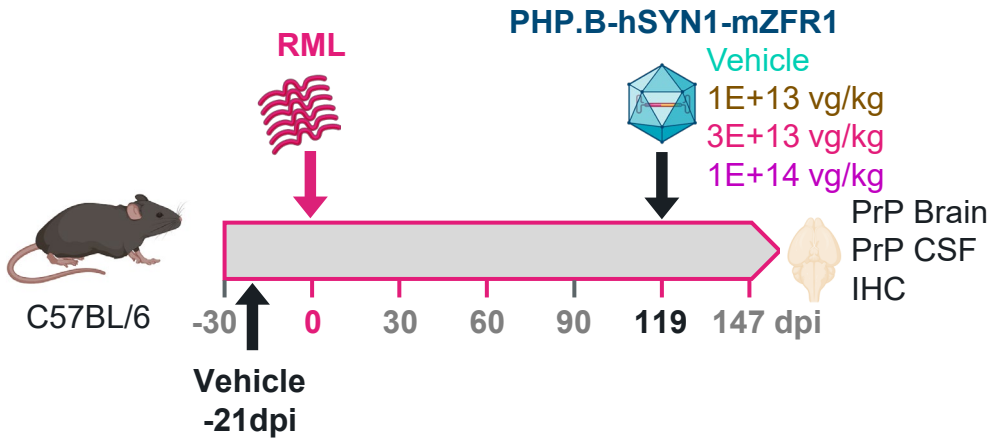
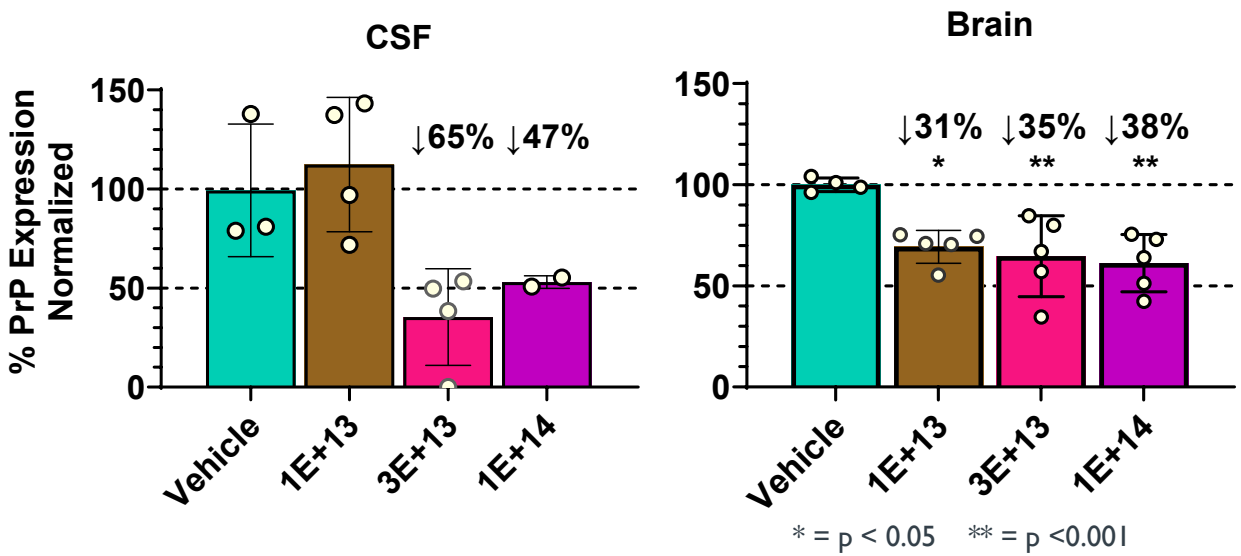
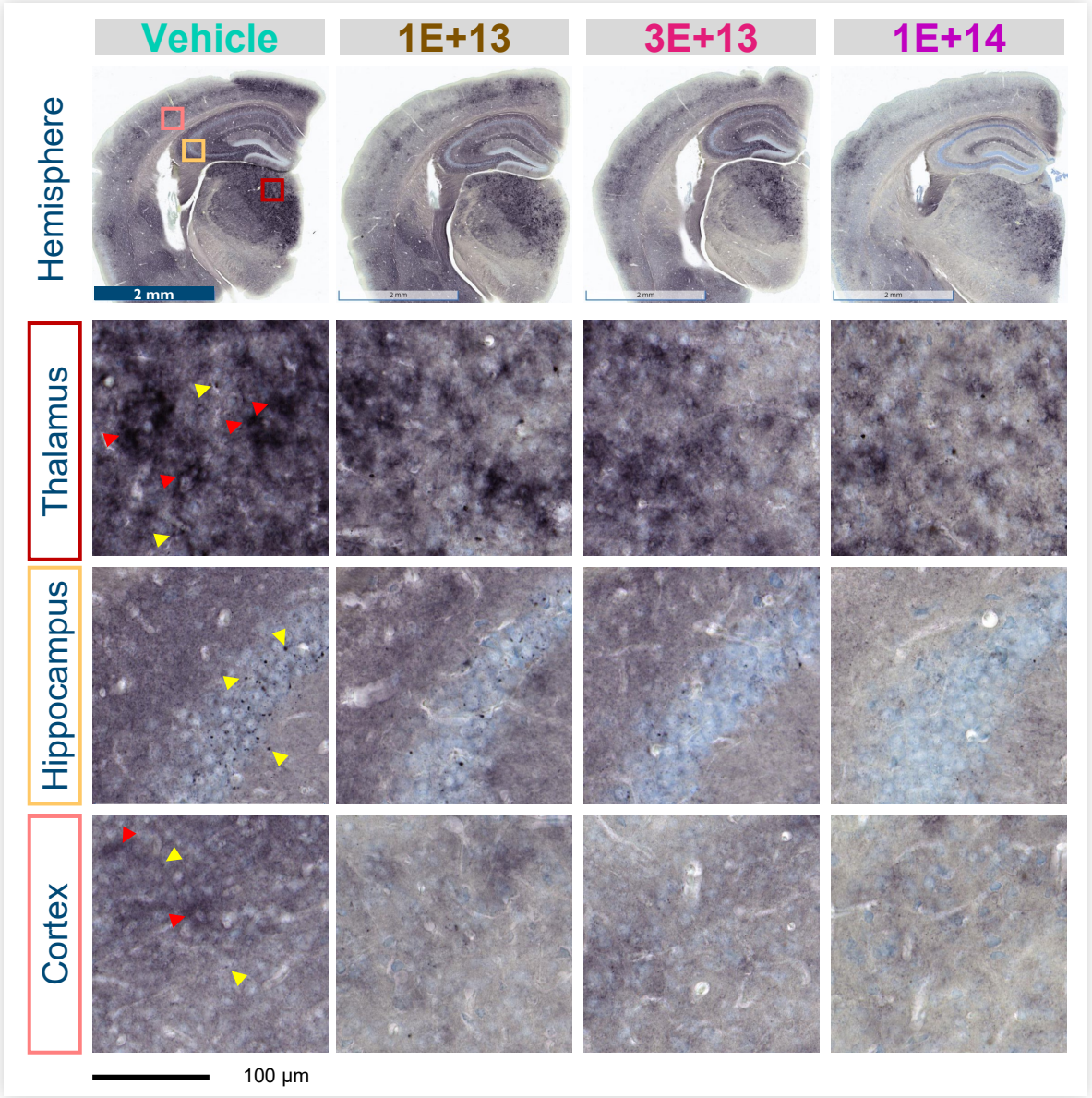
Dose-dependent repression of PrP in brain and CSF of RML mouse model of prion disease







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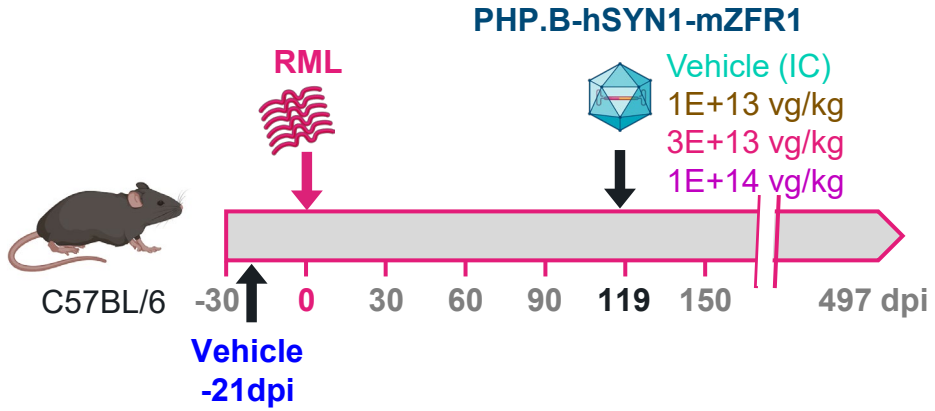
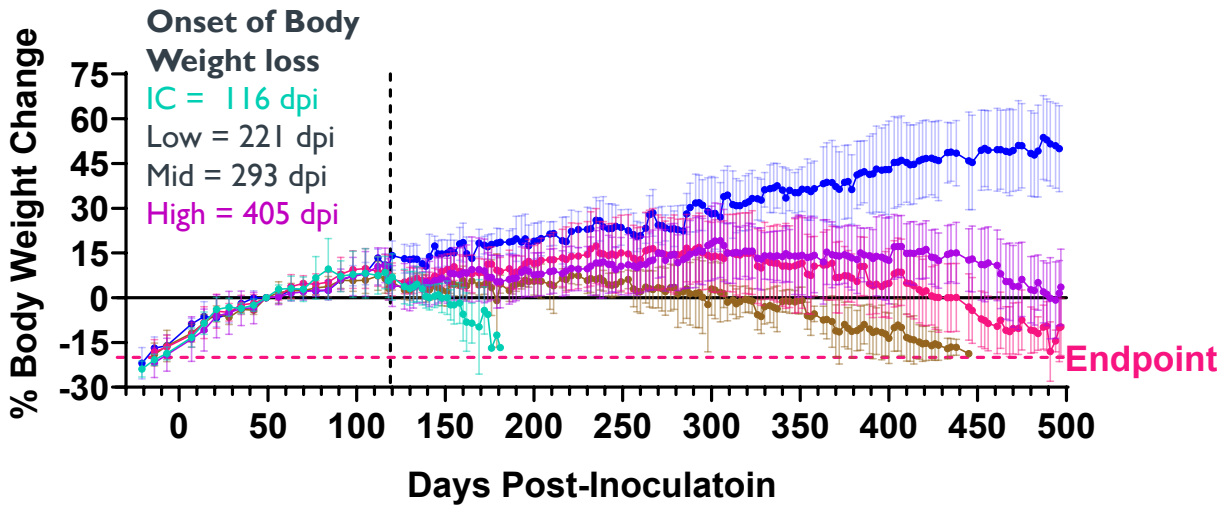
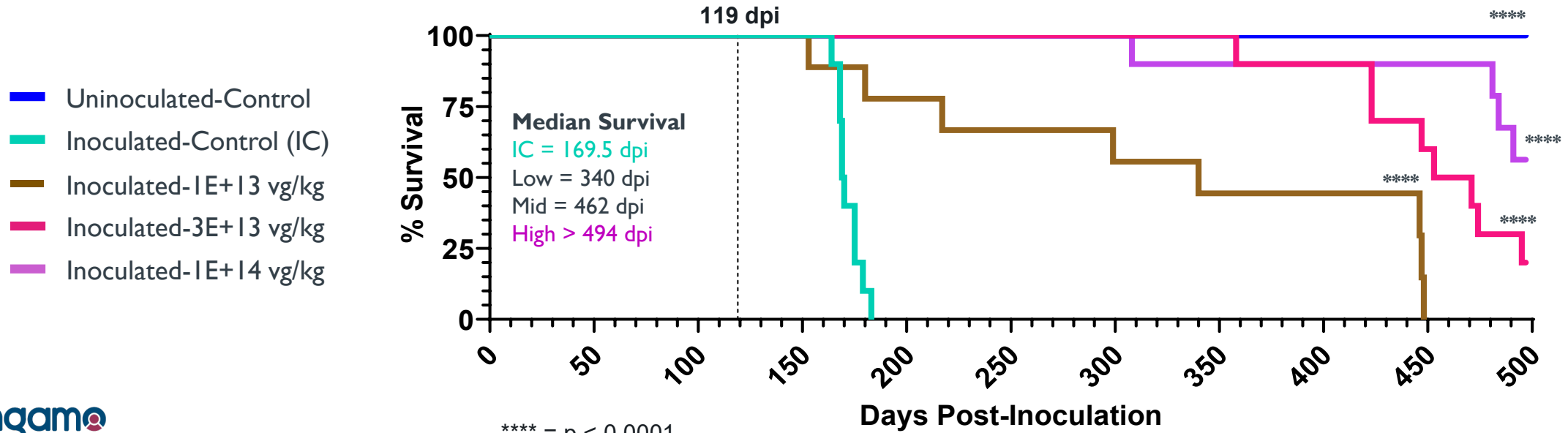
Surrogate ZFR mediates profound extension of life, and delay in body weight loss in post-symptomatically treated RML mouse model of prion disease







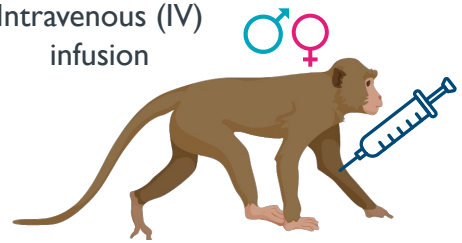
**** = p < 0.0001

4-week dose range-finding study following a single intravenous administration of ST-506 to cynomolgus monkeys

ST-506

Vehicle
3E+13 vg/kg
1E+14 vg/kg

Intravenous (IV) infusion



Cynomolgus Monkey

No immunosuppression
Screened for Anti-STAC-BBB Antibody
N = 3 / group

Dose
Day 1

Day 29

In-life Procedures → **Necropsy**

- Mortality/ morbidity
- Body weight
- Food consumption
- Clinical observations
- Clinical pathology

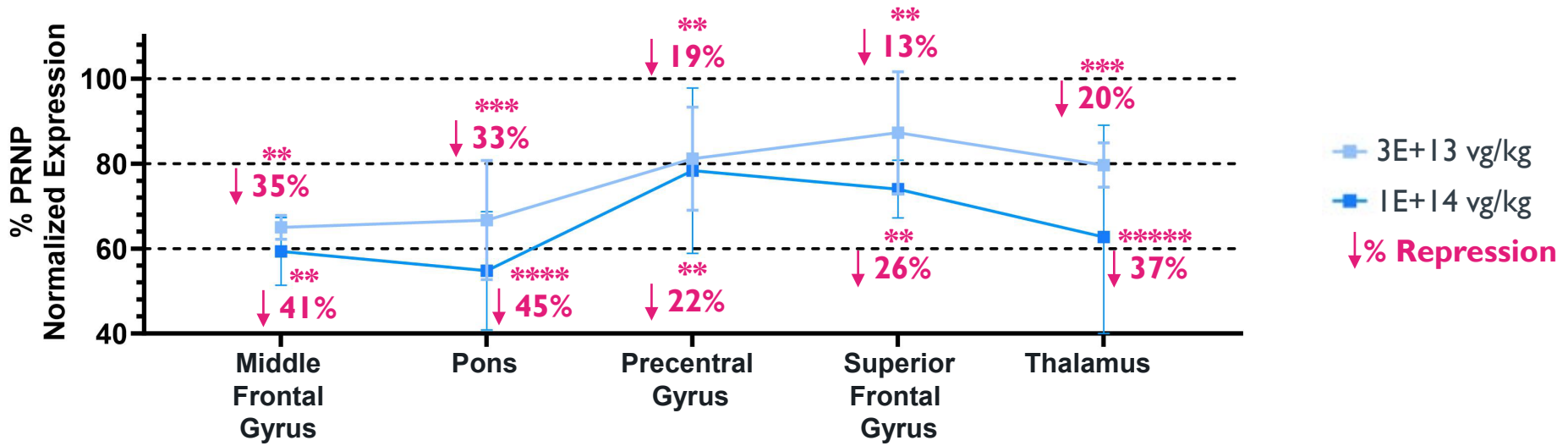
Terminal Procedures and Collections

Molecular Assessments:
- Vector tissue distribution tissues
- Brain vector distribution (various regions)
- Brain *PRNP* expression (various regions)

Safety Assessments:
- Gross necropsy
- Organ weight
- Histopathology
  - Brain
  - DRG
  - Spinal cord
  - Major peripheral organs including gonads

Single IV infusion of ST-506 shows dose-dependent *PRNP* repression in NHP brain

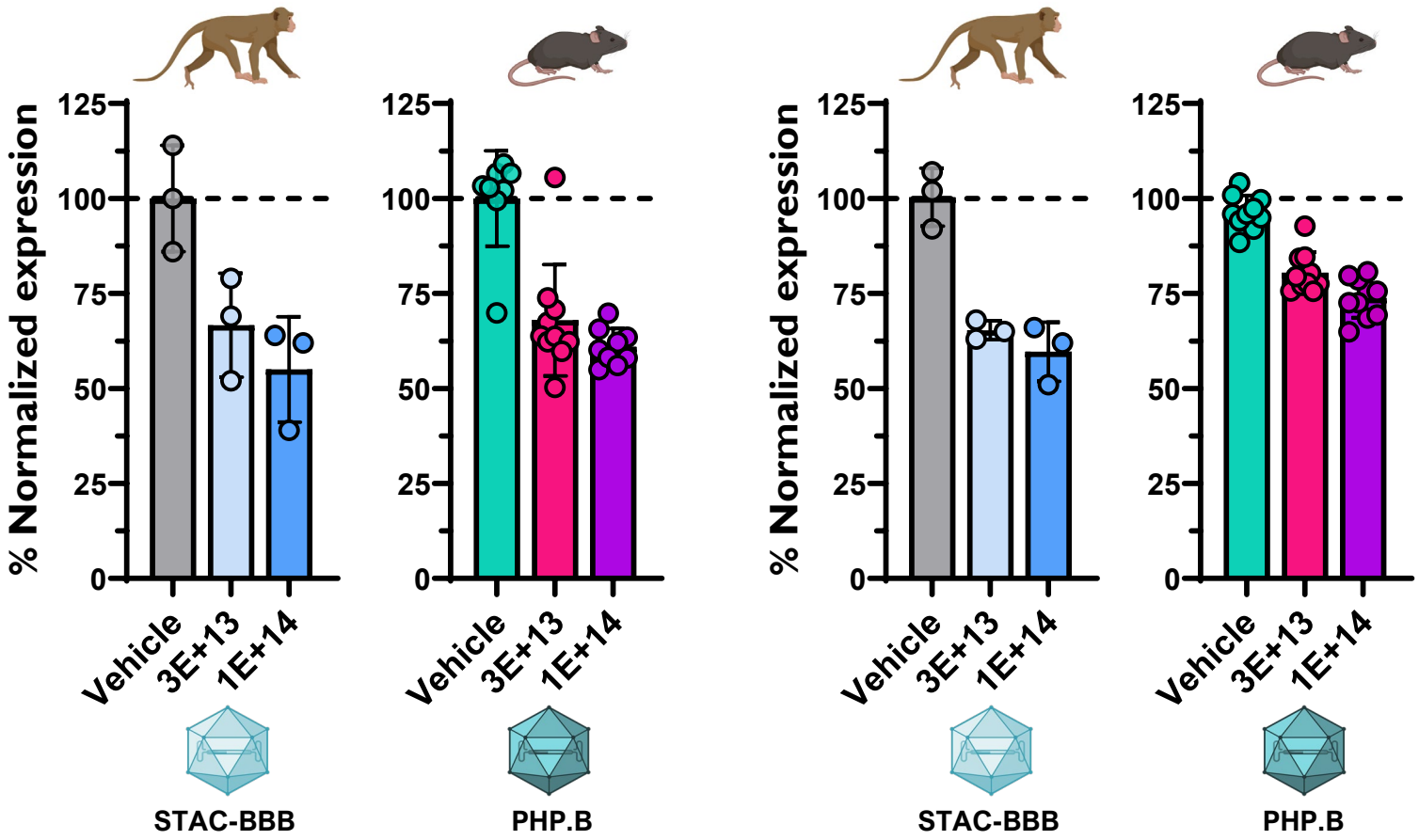
PRNP mRNA expression in brain normalized to vehicle control

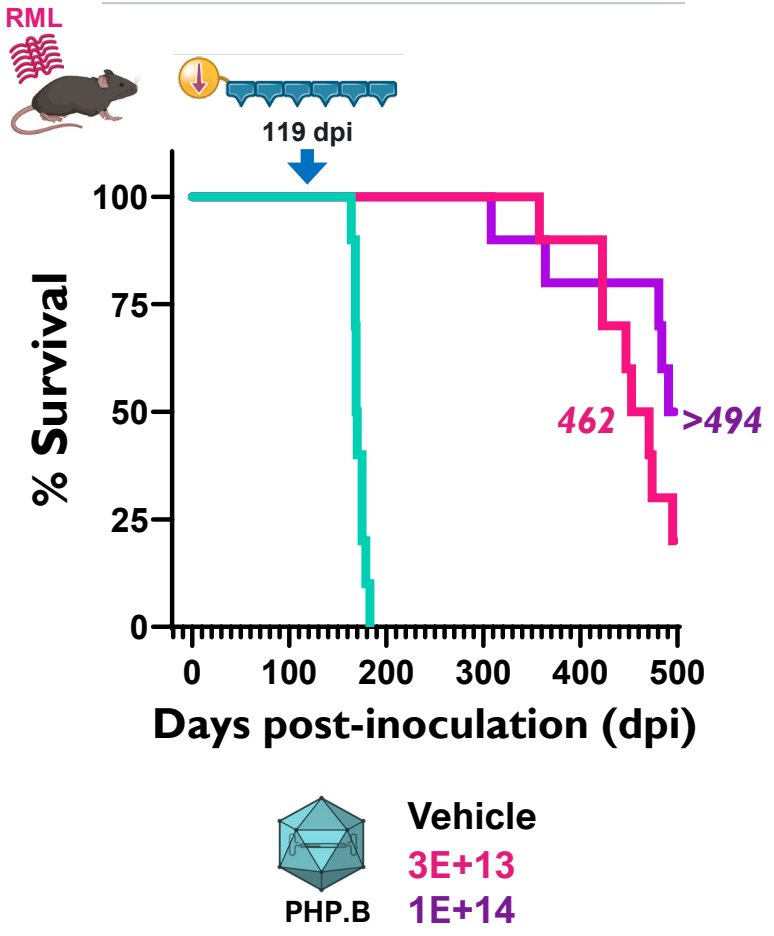


- Brain-wide distribution of ZFR
- Significant repression in both cortical and deep regions
- Repression ranged from 13% to 45% depending on dose and region

** = p < 0.01 *** = p < 0.001 **** = p < 0.0001 ***** = p < 0.00001

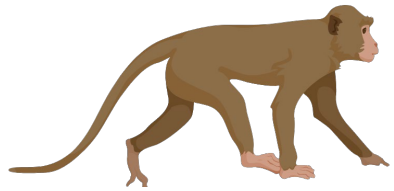
ST-506 achieves *PRNP* repression levels in NHPs needed for extension of survival as seen in prion disease mouse model





For mice: samples were collected 168 days post administration to wild type mice. N=7-10 per group. Brainstem and cortex shown
For NHPs: Mean values of multiple punches from pons and middle frontal gyrus shown.

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



In-life observations:

- Mortality/morbidity
- Clinical signs, including behavior assessment
- Food consumption and Body weight
- Clinical pathology
  - Hematology
  - Chemistry
  - Coagulation

Terminal observations

- Organ weights
- Gross necropsy
- Histopathology of CNS, PNS, and Non-CNS Tissues:
  - Brain
  - DRGs (4 levels)
  - Spinal cord (3 levles)
  - Trigeminal ganglia
  - Sciatic nerve
  - Adrenal gland
  - Epididymis
  - Heart
  - Kidney
  - Intestine
  - Liver
  - Lung
  - Lymph node
  - Ovary
  - Pancreas
  - Skeletal muscle
  - Spleen
  - Stomach
  - Testes
  - Thymus
  - Uterus

No Adverse effects

✓ All in-life observations were normal

No macroscopic & microscopic finding

✓ All terminal observations were normal

✓ No ST-506 related findings

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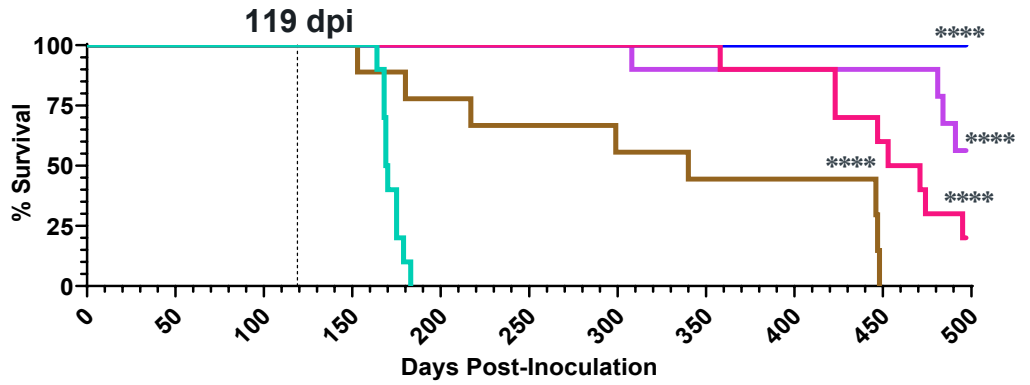
Summary and conclusions

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

- Profound, dose-dependent survival benefit in a mouse disease model when dosed post-symptomatically
- Prion repression sustained for at least 17 months in wild-type mice and well-tolerated
- ST-506 achieves brain-wide *PRNP* repression levels in nonhuman primate needed for extension of survival
- ST-506 was safe in nonhuman primates at the highest dose tested: 1E+14 vg/kg given IV

Chou et al., 2025.
Zinc Finger Repressors mediate widespread PRNP lowering in the nonhuman primate brain and profoundly extend survival in prion disease mice



Anticipate start of ST-506 clinical study for the treatment of prion disease in 2026

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Thank You

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evotec

SRI

NSA NeuroScience Associates www.NSALabs.com

Toufan Parman, PhD, DABT

Senior Director of Nonclinical Safety Evaluation

Email: tparman@sangamo.com