Virginia Tech

Development and Characterization of a Sublethal Sequelae Mouse Model of EEEV Infection

Presenter: Shannon Carney, MPH, PhD Candidate

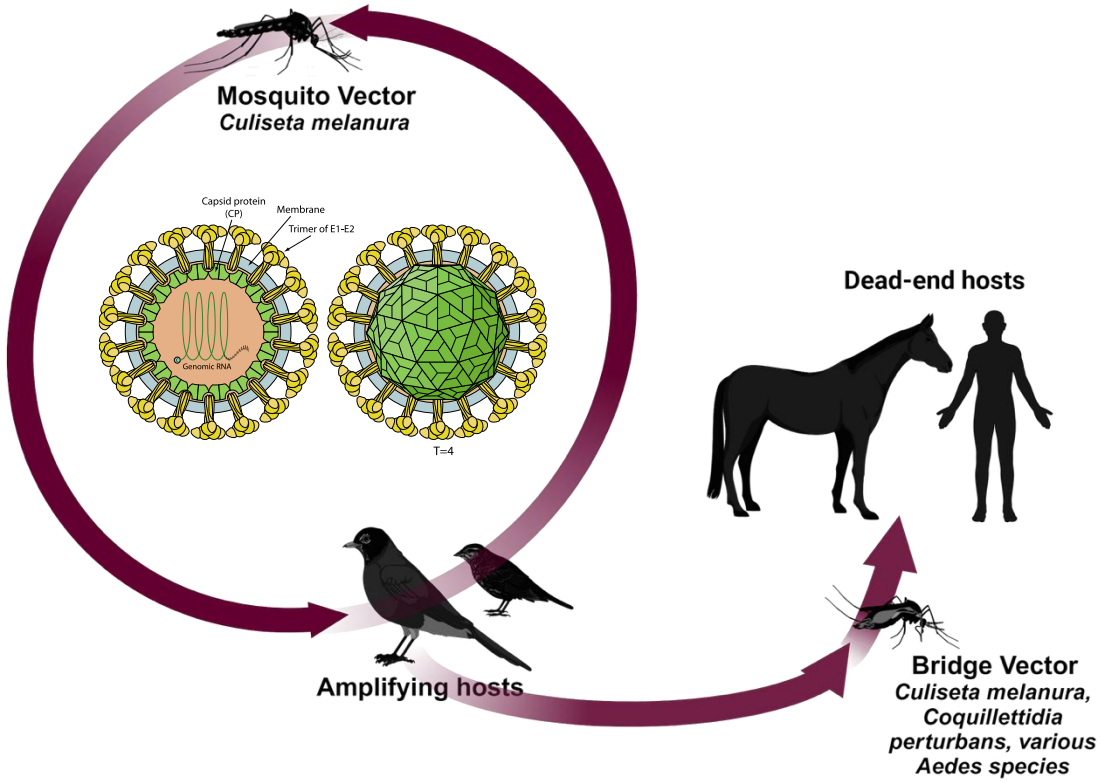
Military Health System Research Symposium 2026
MHSRS One Health in Uniform: Veterinary Medicine Supporting Warfighter Readiness
Wednesday, August 5th, 2026, 0800-1000

Non-disclosure

- **Shannon Carney, MPH, has no conflicts to report.**

Eastern equine encephalitis virus (EEEV)

- Togavirus—positive-sense RNA virus
- Transmitted by mosquitoes
- Endemic in and around freshwater swamps in the Atlantic and Gulf Coast states and the Great Lakes region
- Exposure risk occurs during outdoor activities/field operations
- Human infection is rare but unpredictable



EEEV is a pathogen of concern for both naturally occurring outbreaks and biodefense because of its high lethality and history of offensive biological weapons research

BACKGROUND

EEEV Neurological Sequelae Mouse Model Development

PROBLEM OVERVIEW

VIRAL EXPOSURE	ACUTE CLINICAL DISEASE	CHRONIC DISEASE
EEEV Bite of an infected arthropod Har… infectio…		…al sequelae: …s: …ability …avioral changes Long term impacts: • Strain on healthcare system (money, time resources) • Strain on caregivers & patients (money, quality of life)

Currently, … … infection in humans

GAP IN KNOWLEDGE:
The long-term cognitive impact of EEEV infection and its correlated neuropathology

GOAL:
Develop a neuroinvasive & sublethal mouse model of EEEV infection to study long term impacts of virally induced neuropathology

PROJECT OBJECTIVE

Development and …
of a sub-lethal mo…
EEEV infection to s…
long-term impacts

…OUTCOMES

- …ogy following EEEV infection
- …erlying EEEV-induced disease
- Model for testing efficacy of potential therapeutics and vaccines

EEEV strains used in these studies *(backbone is FL93-939)*

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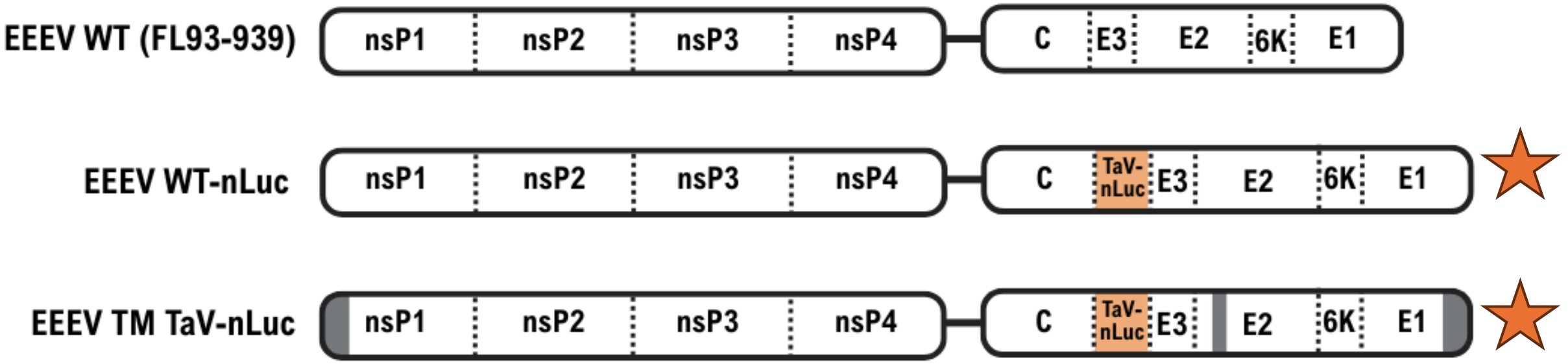

Due to its lethality, EEEV WT and WT-nLuc can only serve to characterize the acute phase of infection

First step: Pilot study to assess and characterize survival, clinical symptoms, neuropathology, viral replication in acute phase of disease between viral strains

Model info:
Source: Jackson Labs
Strain: C57BL/6J
Age: 12 weeks @ D0pi
Sex: Males only
EEEV challenge doses (PFU/mouse):
- WT-nLuc: 10^3 (n=10)
- TM-nLuc: 10^5 (n=20)

IVIS imaging:
- IP injection with FFz
- 5-10m post FFz, mice are anesthetized and imaged
- Photograph+luminescent overlay allows us to visualize viral replication

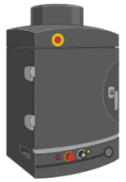

Image subsets of WT and TM groups on days 2, 4, 6, 8, 14, and 21 post-infection

Days post-infection with EEEV

Day -5
Days post-infection

IN infection

Day 0
STUDY START

Day 2 Day 4 Day 6 Day 8 Day 14 Day 21 Day 28

STUDY END

Days 1-28 post-infection for surviving groups:
Daily weights, body temperatures, clinical observations
Monitor for survival

Validation of EEEV TM-nLuc

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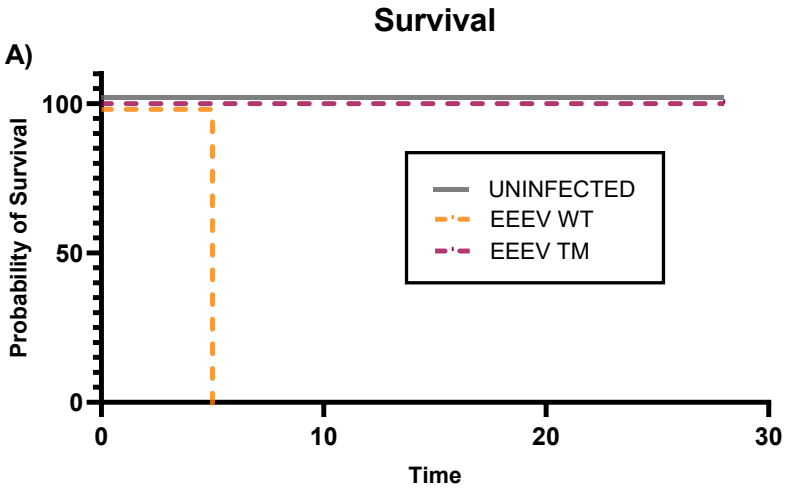
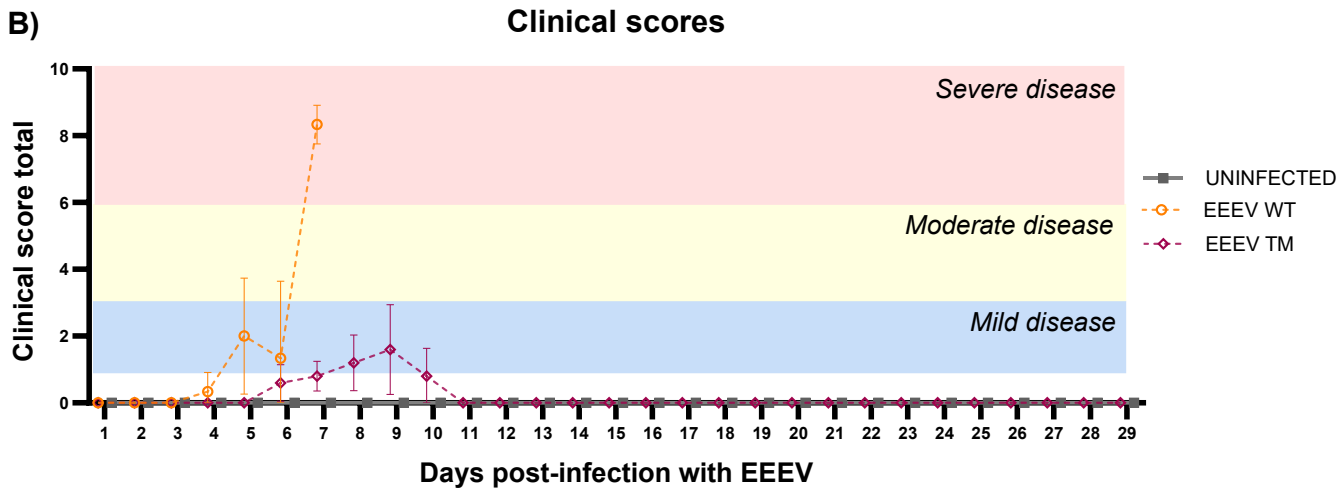
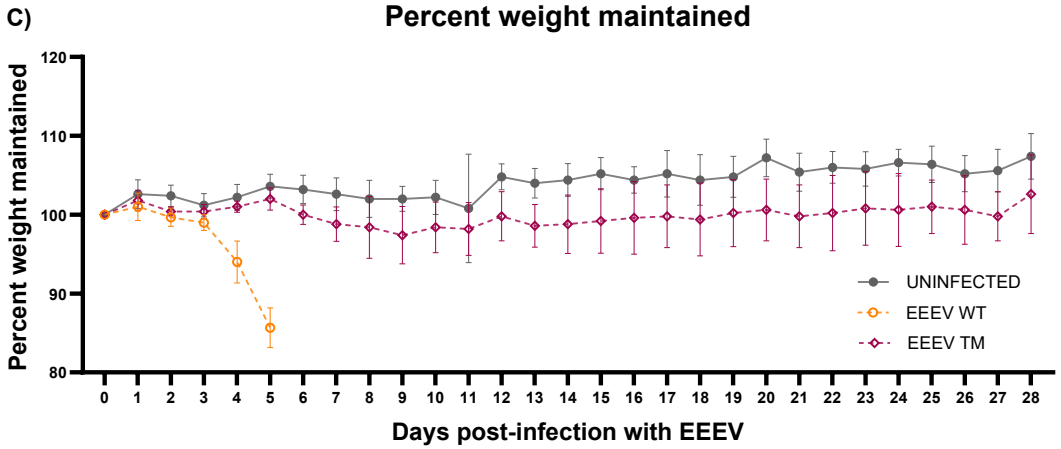






Mice infected with EEEV TM-nLuc have 100% survival, less weight loss, and decreased clinical scores compared to mice infected with EEEV WT-nLuc

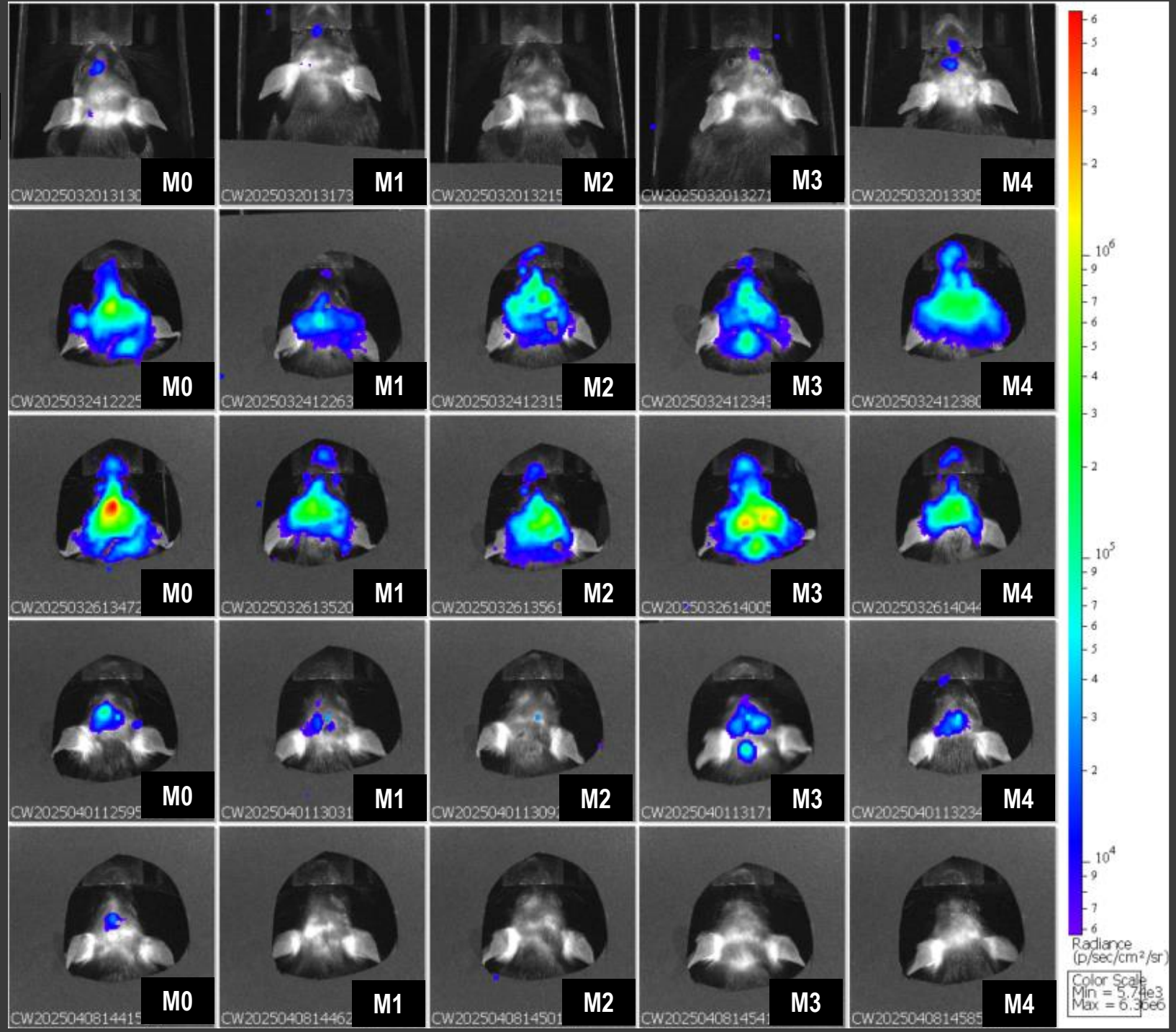
Day 2pi

Day 6pi

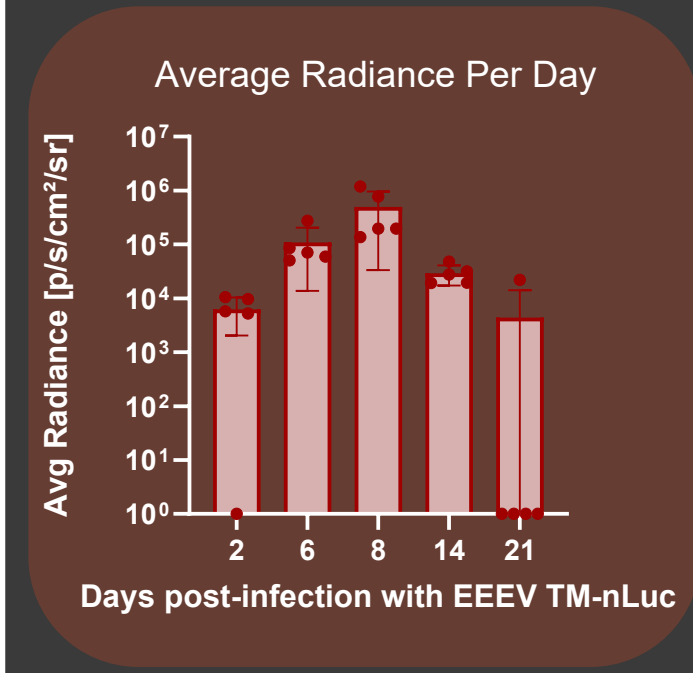
Day 8pi

Day 14pi

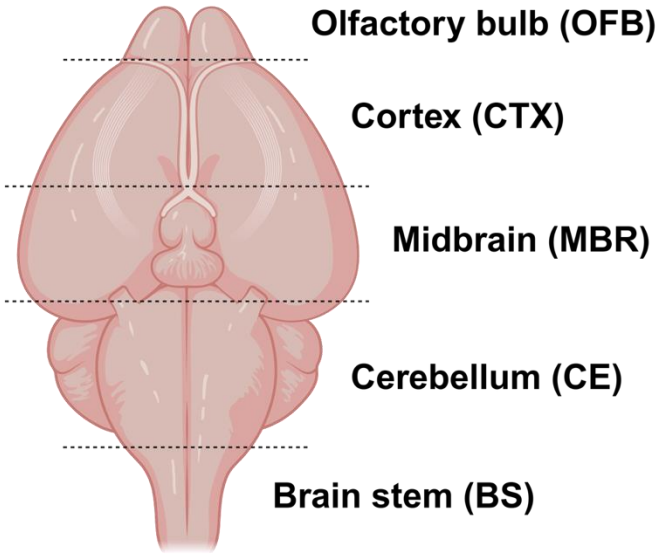
Day 21pi

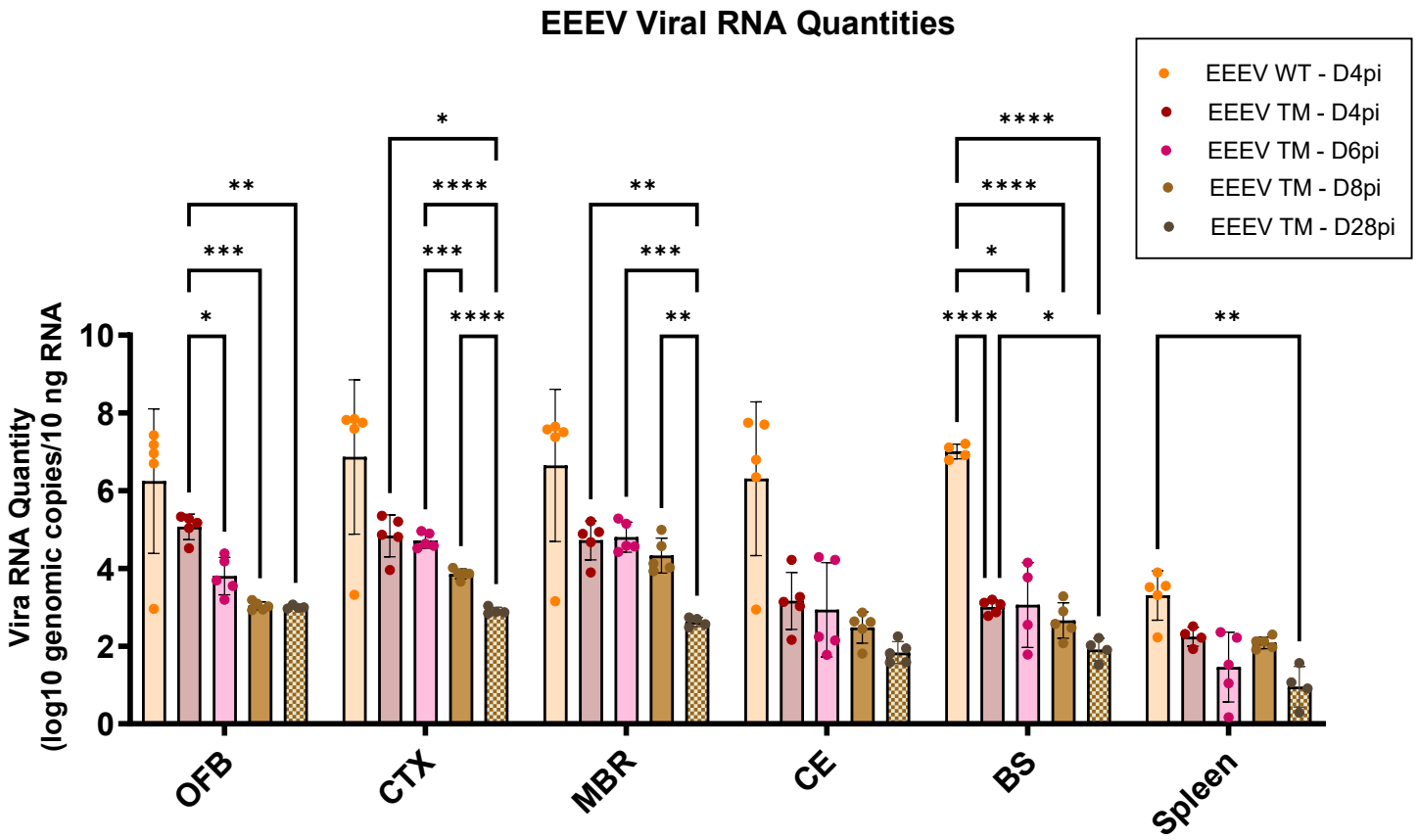


TM-nLuc-infected mice show persistence of viral replication out to day 21 post-infection



EEEV Viral RNA





TM-nLuc replicates more slowly and never reaches the same levels as WT-nLuc in all brain regions and the spleen

Lower viral RNA quantities in TM-nLuc groups at days 6 and 8

Gene Expression Analysis

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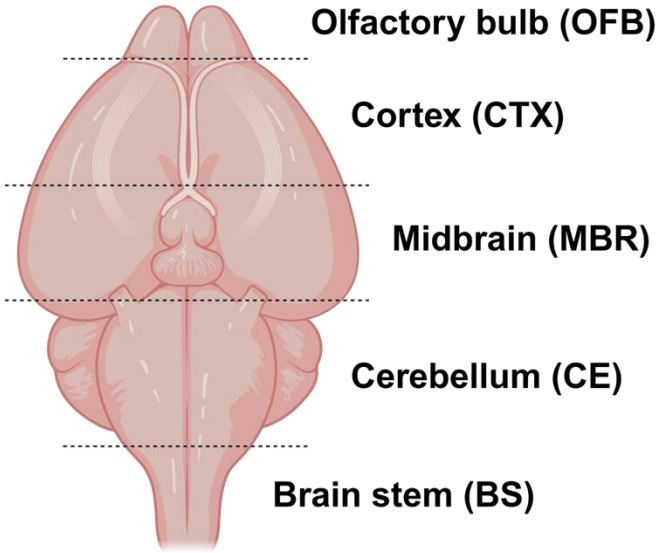
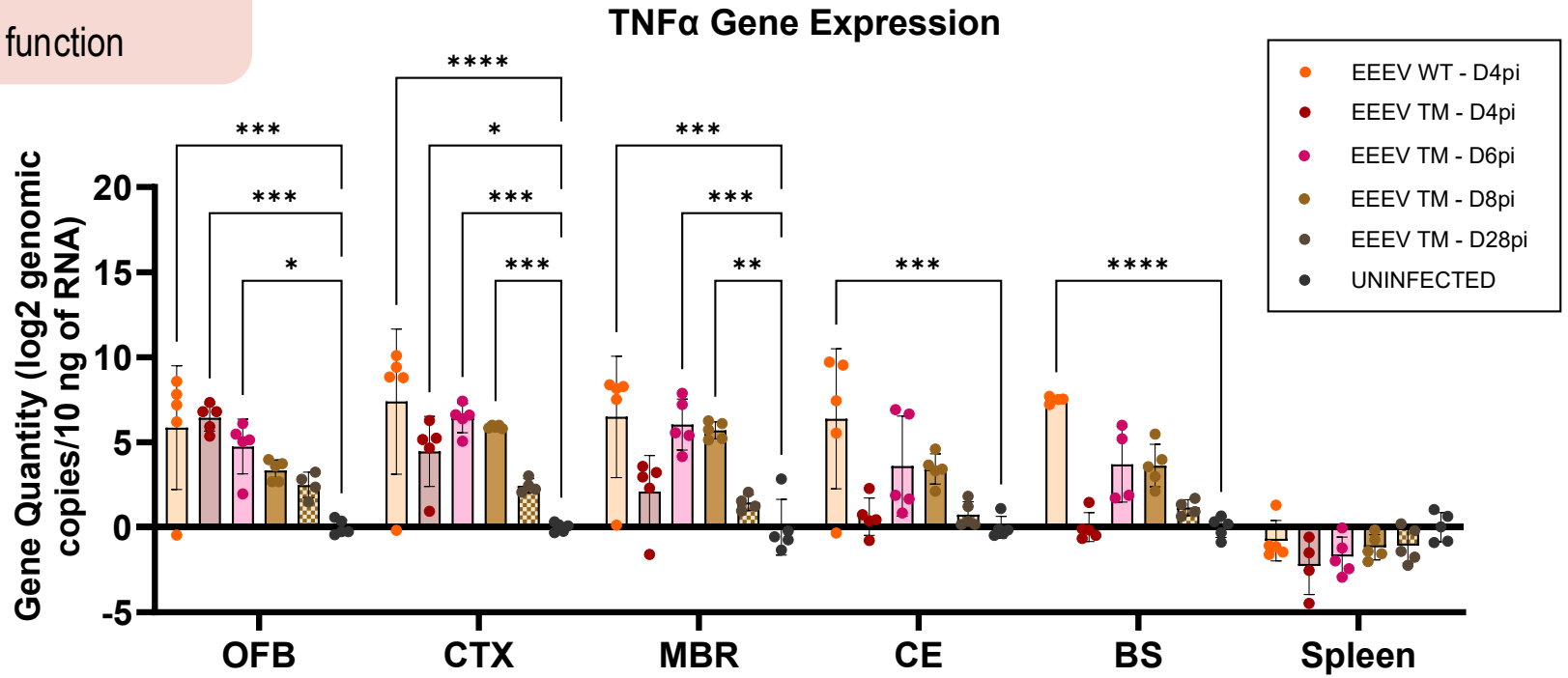
TNFα

Tumor necrosis factor-alpha

Cytokine that plays a role in inflammation and immune function





WT: Early, widespread TNFα induction throughout the brain (D4pi)

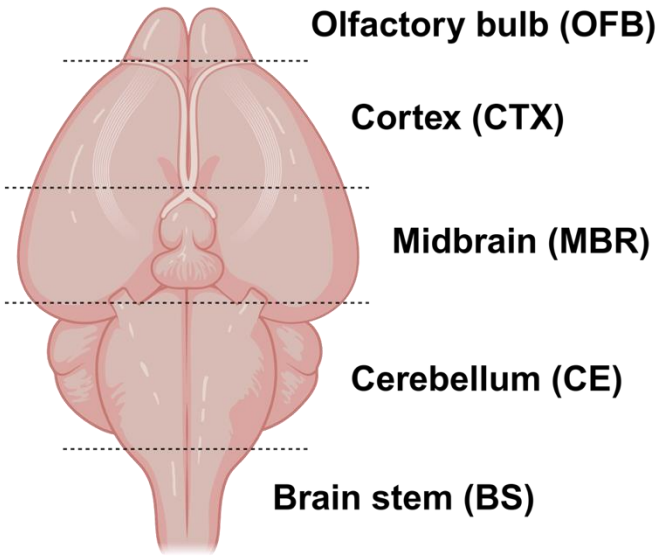
TM: Delayed TNFα response that begins in the olfactory bulb/cortex and progresses caudally over time, reaching levels comparable to WT and fading by d28pi

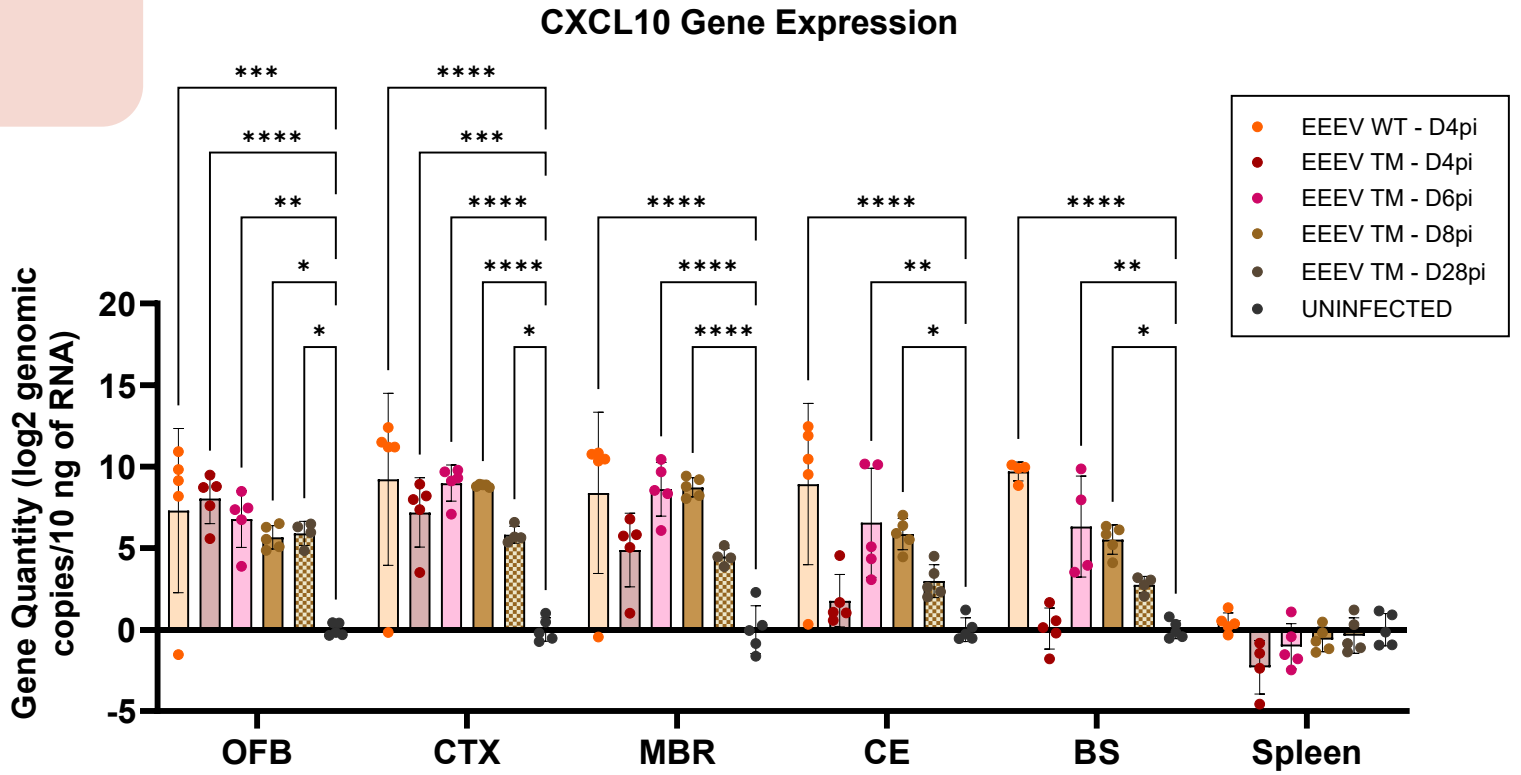
Gene Expression Analysis

Center for Emerging, Zoonotic, and Arthropod-borne Pathogens

CXCL10

Interferon gamma-induced protein 10
Circulating inflammatory marker
Mediates immune response

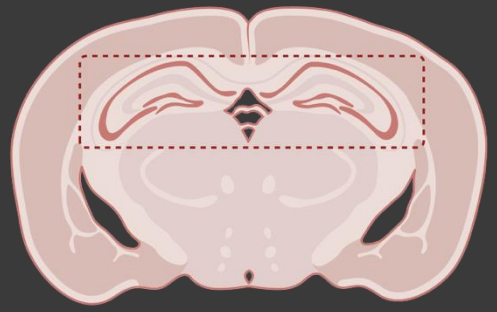




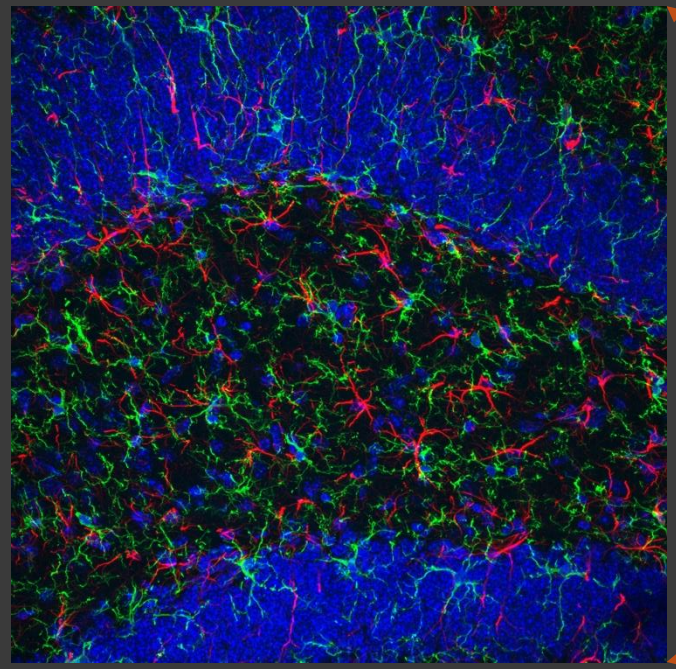
WT: CXCL10 robustly induces throughout the brain by d4pi

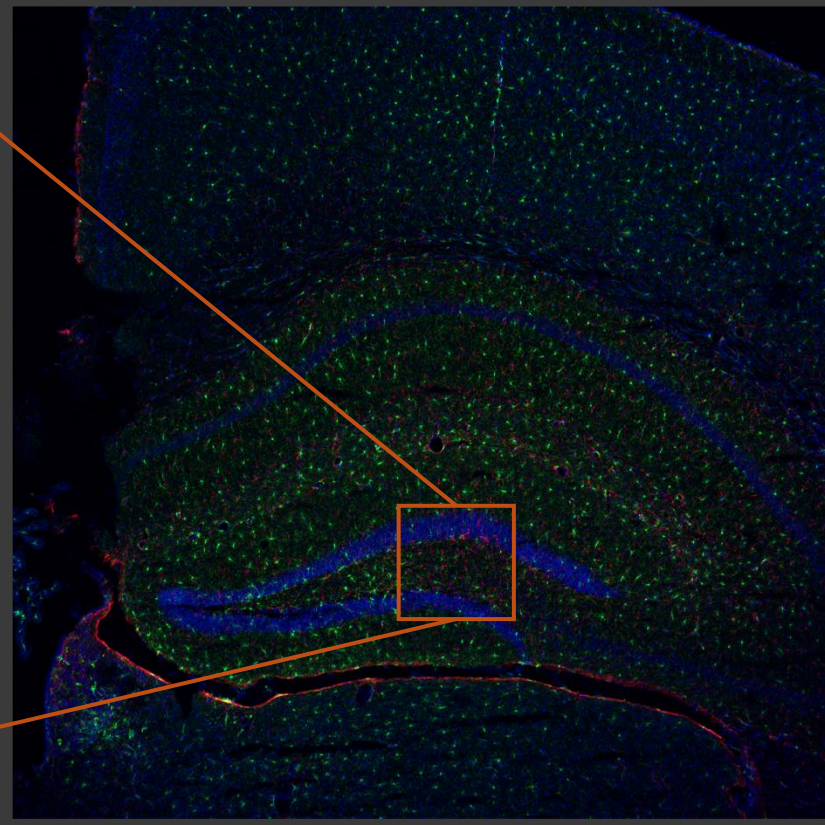
TM: CXCL10 induction followed a delayed, rostrocaudal pattern over time and persisted in the OFB through d28pi

The hippocampus



- Known target of EEEV neuropathology
- Central to memory, learning, and emotional regulation
- Clinically relevant to the cognitive and behavioral sequelae reported in EEEV survivors
- Site of neuronal survival and glial activation assessments





TM D28pi brain pathology

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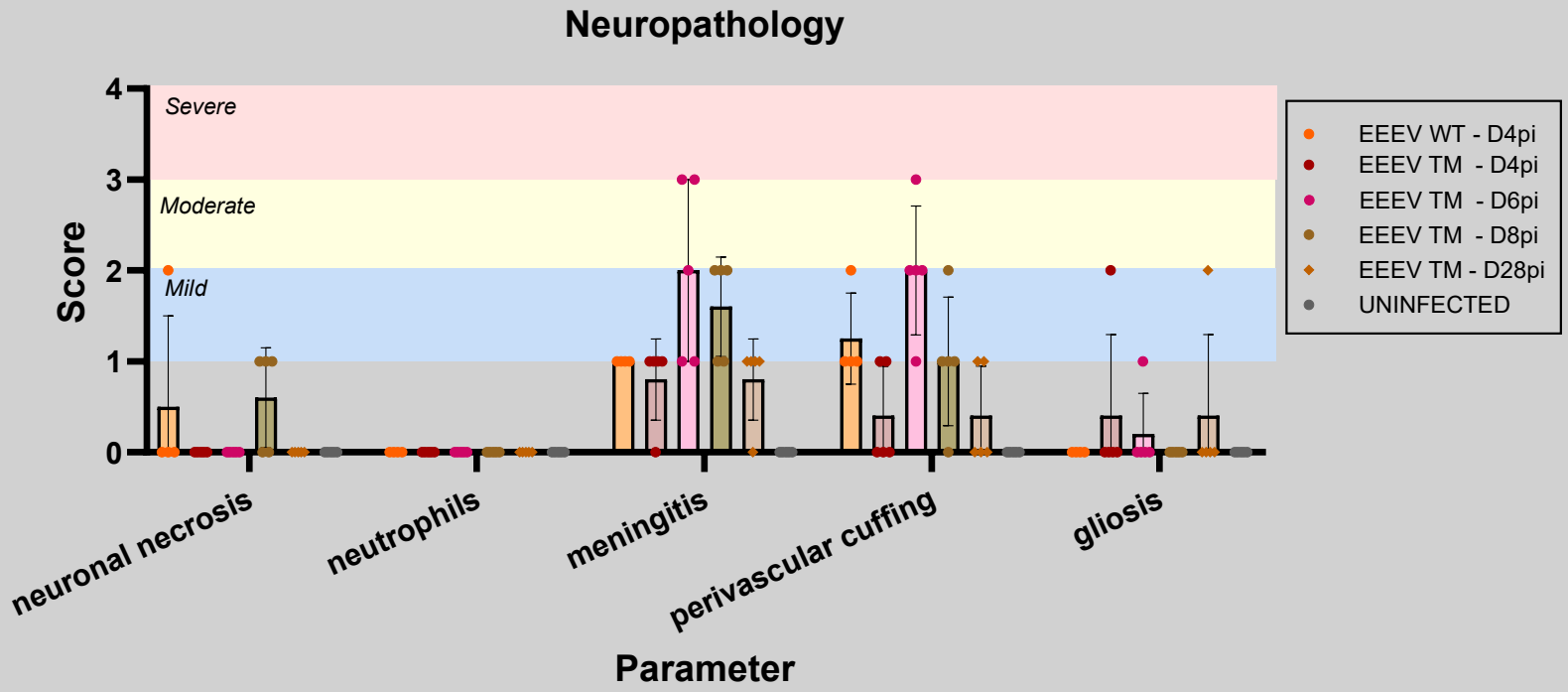
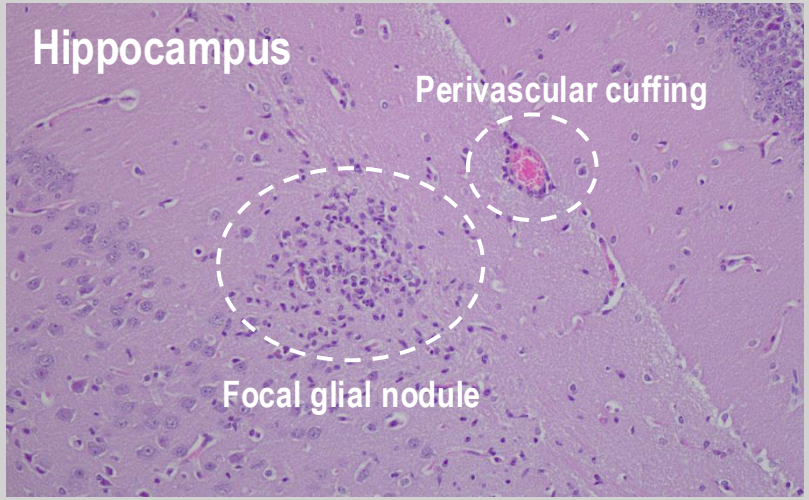
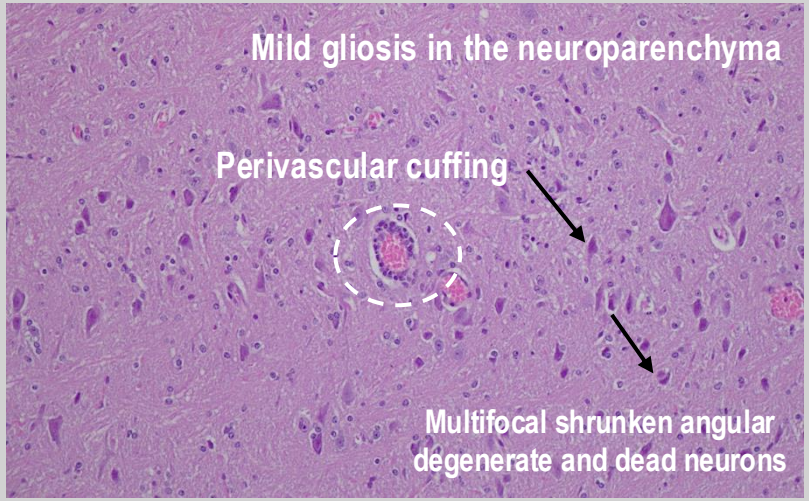
Mice infected with EEEV TM have persistent neuropathology at day 28 post-infection

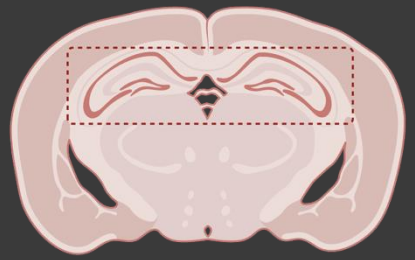


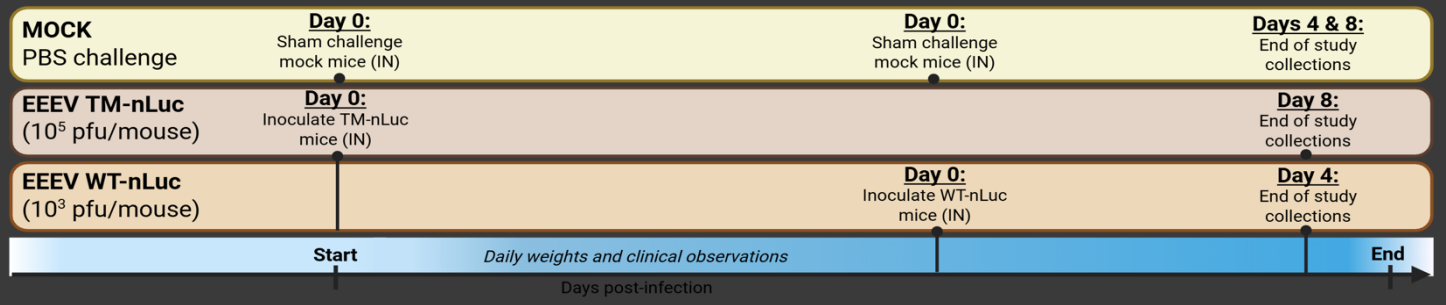
Day 28 post-infection – TM infected





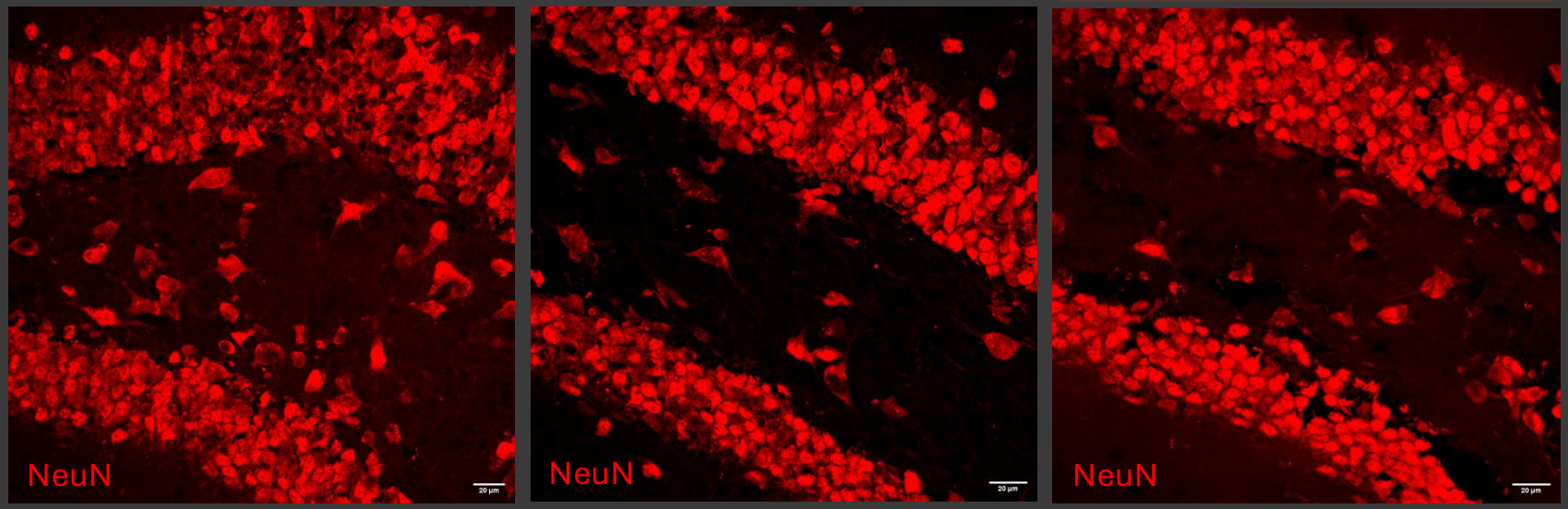
Interneurons in the hilus at acute timepoints

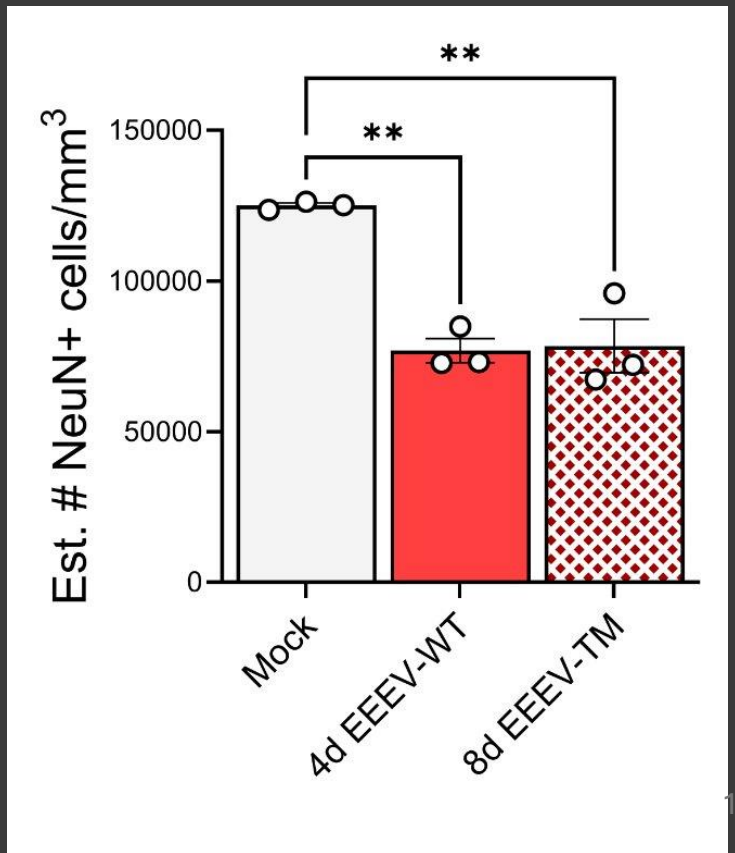




A

MOCK	WT d4pi	TM d8pi

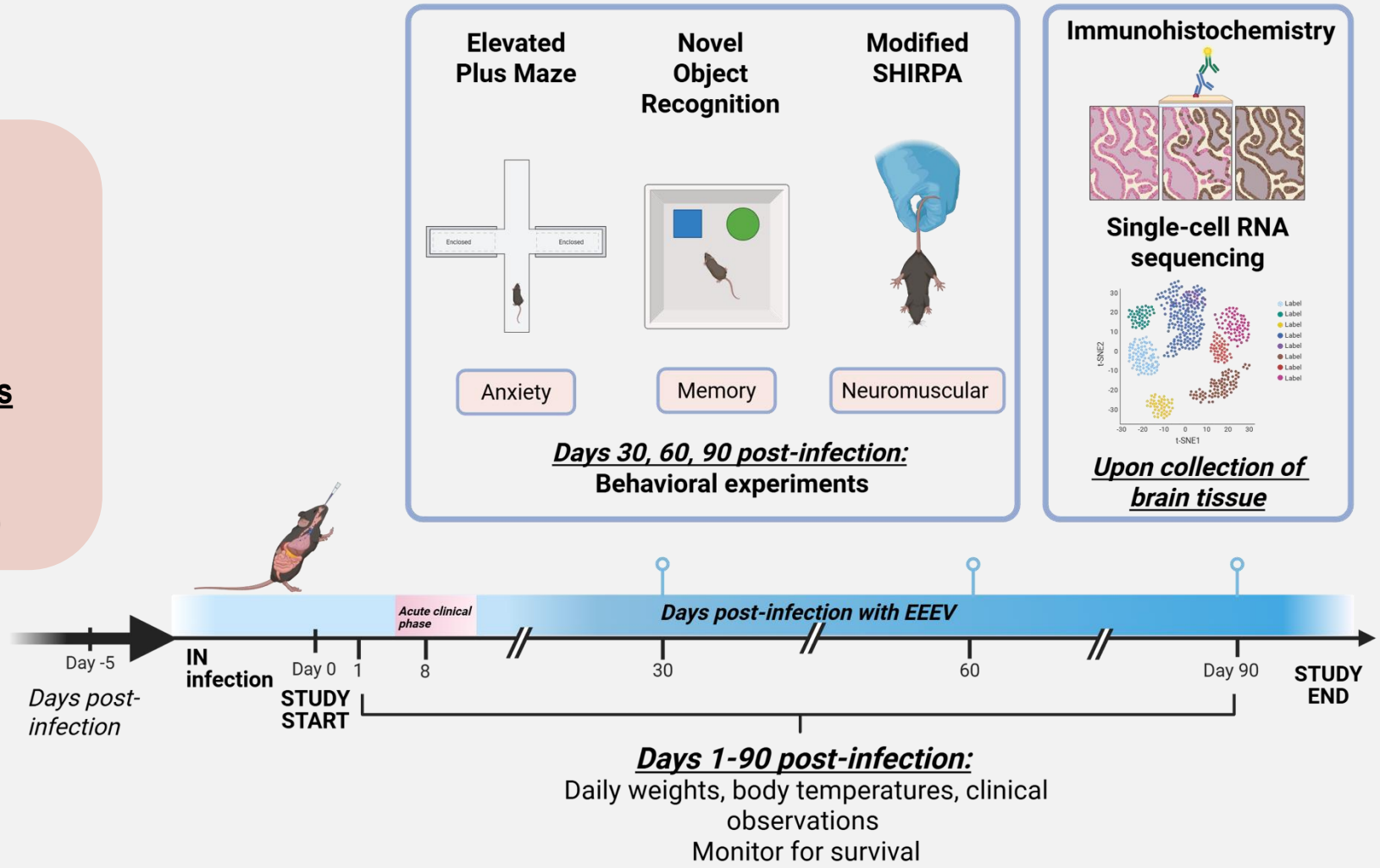




Levels of mature neurons in the hilus are comparable between WT and TM at acute timepoints, but are significantly decreased as compared to a mock control

Study design: Characterize long-term behavioral and neuropathological alterations in mice infected with EEEV TM

Model info:
Source: Jackson Labs
Strain: C57BL/6J
Age: 13 weeks @ D0pi
Sex: Males only
EEEV challenge doses (PFU/mouse):
- MOCK: PBS (n=8)
- TM-nLuc: 10^5 (n=8)



Semi-quantitative modified SHIRPA testing

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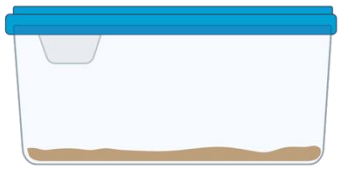
Body tone (muscular, agitation)

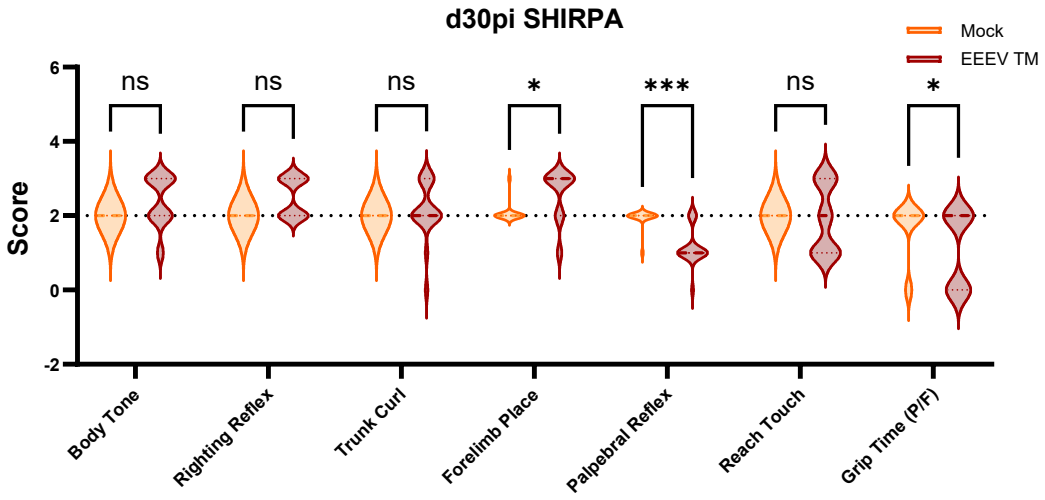
Trunk curl (muscular)

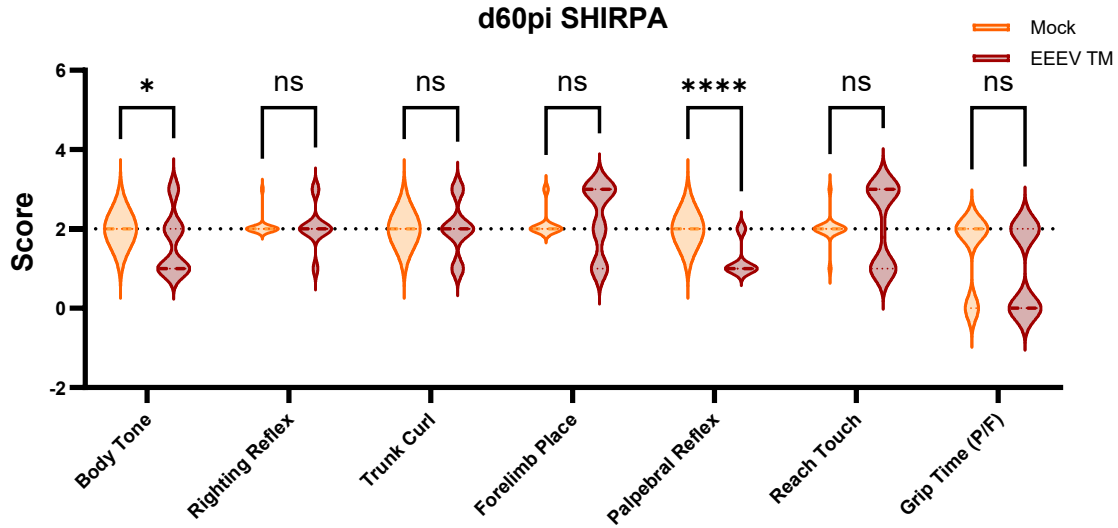
Righting reflex (muscular)

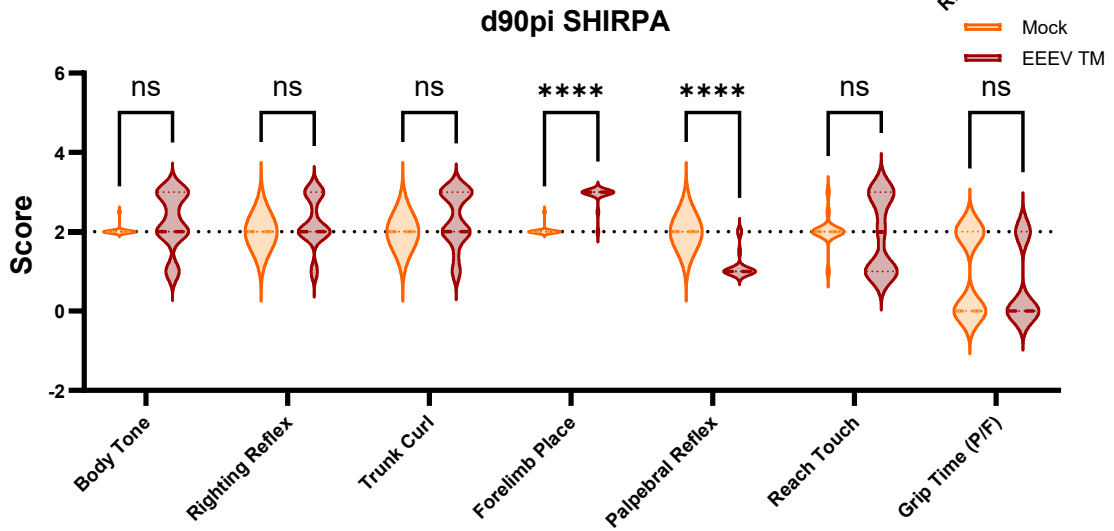
	0	1	2 (Normal)	3
Body Tone (muscular, agitation)	Flaccid, no reaction	Depression to floor	Slight resistance to touch	Hunches back to resist depression, irritable
Trunk Curl (muscular)	No response or hindlimb clasping	Curls <90°	Curls ≥90°	Climbs up tail
Righting Reflex (muscular)	Does not right	Struggles to right	Rights itself	Hyperactive response
Forelimb Place (muscular)	Leg stays where placed	Slow or incomplete return to position	Promptly returns to position	Hyperactive response, does not allow adjustment
Palpebral Reflex (visual)	No response	Slow blink	Quick blink	Continues blinking upon removal of stimulus
Reach Touch (visual)	Does not reach, clasps hindlimbs	Does not reach until whiskers touch surface	Reaches as approaches ground before whiskers touch surface	Hyperactive, tail climbing
Grip Time (muscular)	< 60 seconds (fail)		> 60 seconds (pass)	



SHIRPA







EEEV TM-infected mice displayed altered neuromuscular function as compared to mock-infected controls

Conclusions

Neuroinvasion with the EEEV TM confirmed and replication may persist to day 21 post-infection.

EEEV TM induces similar expression of **CXCL10** and **TNFα** in mice, with CXCL10 expression persisting out to d28pi.

Significant **pathology** observed in mice infected with EEEV TM out to d28pi.

Significant **loss of mature neurons** observed in mice infected with EEEV TM at acute timepoints.

Altered neuromuscular phenotype seen in mice infected with EEEV TM..

Future work

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Determine EEEV-induced acute and long-term **neuropathological alterations** using the EEEV TM and WT and **IHC** (astrocyte and microglial activation, presence of viral RNA, BBB integrity, neuronal degradation, etc.).

Continue analyzing behavioral tests to identify potential **cognitive deficits** post-infection.

Use **scRNAseq** to correlate gene expression alterations with neuropathological and behavioral changes.

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Laboratory Members

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Committee Members

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- Michelle Theus, Ph.D.
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Collaborators

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- Tanya LeRoith, DVM

Virginia-Maryland College of Veterinary Medicine

CZAP Center for Emerging, Zoonotic, and Arthropod-borne Pathogens

VIRGINIA TECH

Thank you for listening!

Any Questions ?

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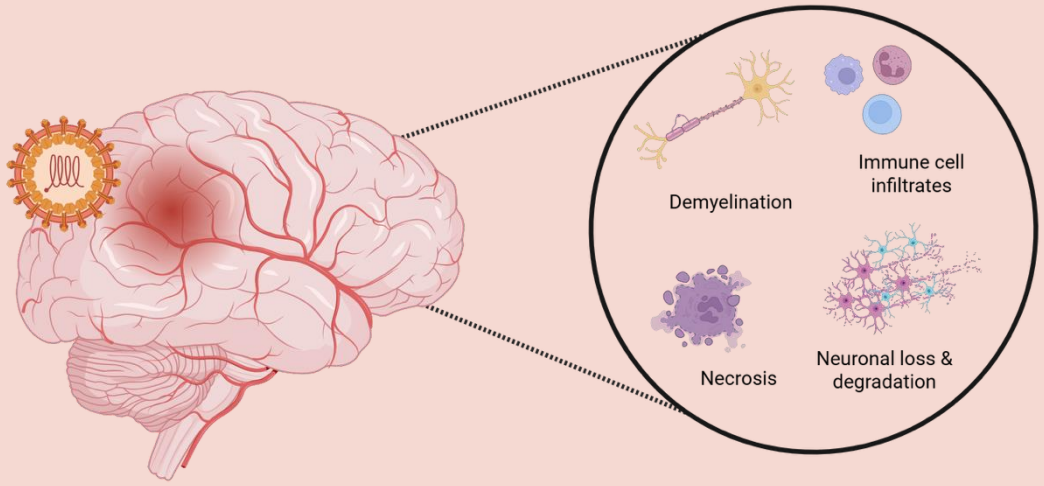
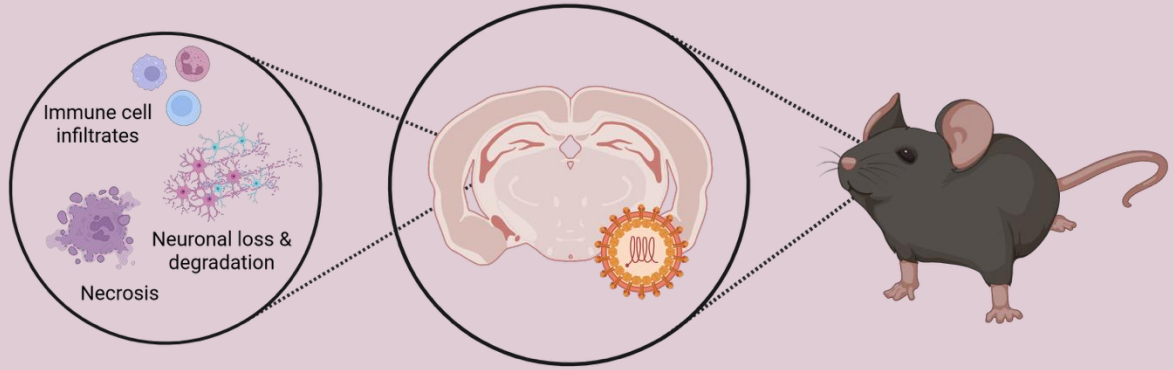
EEEV Neuropathology in Humans vs. Mice





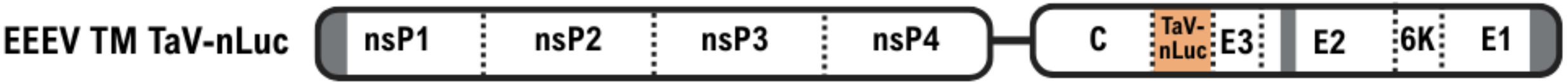
Parenchymal, meningeal, and perivascular **infiltrates containing monocytes/macrophages, neutrophils, and lymphocytes** have been observed

Studies in CD-1 mice found that EEEV replicates primarily in **neurons** with some detection found in glial cells

Fatal infections display **neuronal injury** with vasculitis and thrombosis, demyelination, **necrosis**, and caspase 3 activation

Mice display **encephalitis**, paralysis, **neuronal necrosis**, and inflammation as indicated by **infiltration of eosinophils and neutrophils**

EEEV strains used in these studies *(backbone is FL93-939)*



5' UTR mutation

- Nucleotides 4 and 6 mutated from **guanine** to **adenine**
- **Goal**: to disrupt the 5' terminal stem-loop structure similar to the VEEV TC-83 live attenuated vaccine 5' UTR

Increase sensitivity to IFIT-1

E2 mutation

- Three **lysine** residues at amino acids 71, 74, and 77 were mutated to **alanine**
- **Goal**: to disrupt binding of virus particles to cell surface heparan sulfate receptors

Increase viral replication in lymphoid tissues and reduce viral spread in the CNS

3' UTR mutation

- **260 nucleotides deleted**
- **Goal**: to remove the miR-142-3p binding sites

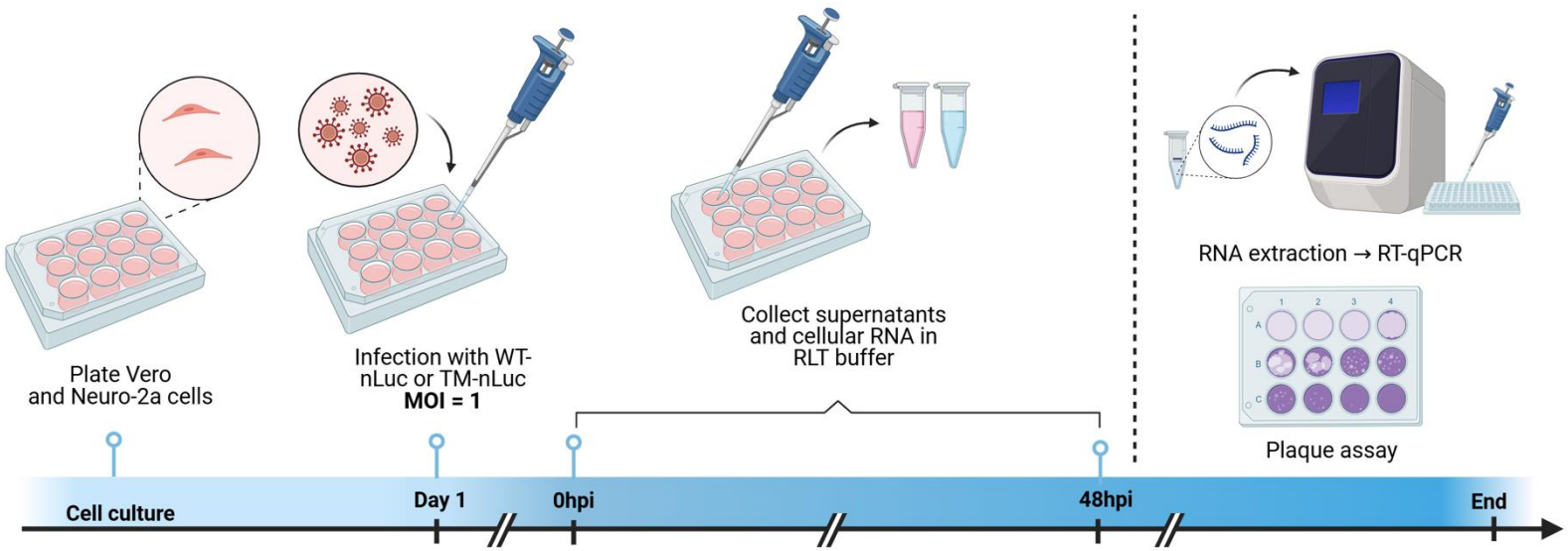
Allow efficient replication in myeloid cells and induce systemic IFN-α/β

IFIT-1: A human gene that encodes for an interferon-stimulated antiviral protein that binds to non-self viral RNA

Reduced HS interaction limits efficient attachment to vascular endothelium, decreasing the likelihood of BBB engagement

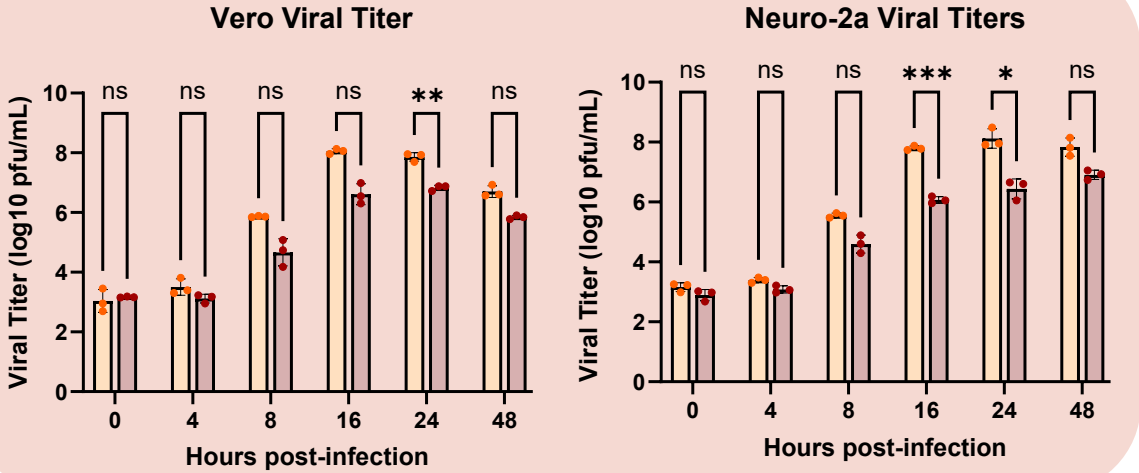
3' UTR miRNA binding sites restrict viral replication in innate immune cells that would normally act as sentinels and induce interferon

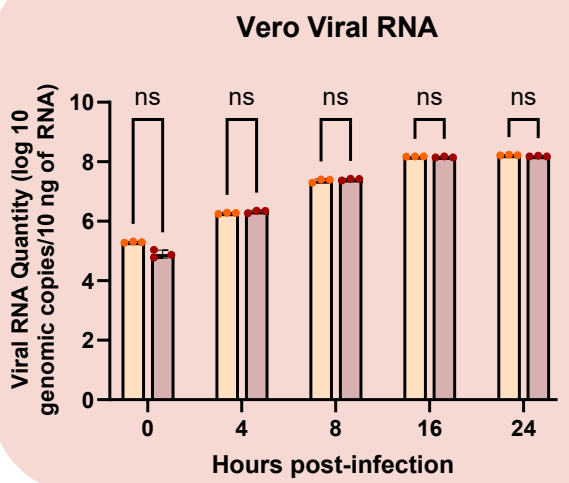
EEEV WT-nLuc v. TM-nLuc viral kinetics

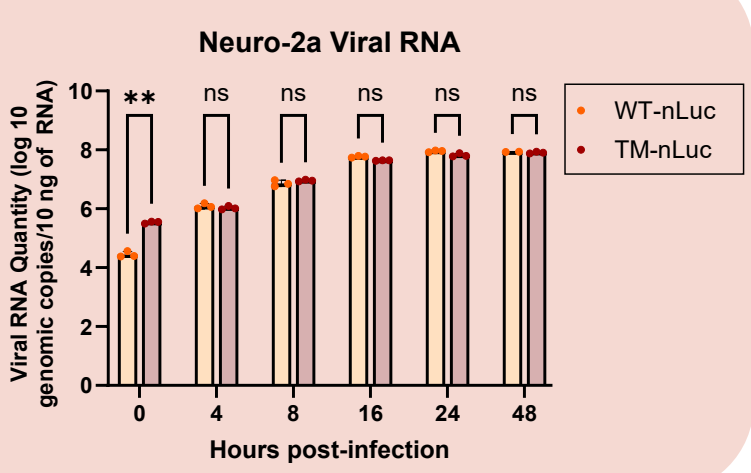


Veros: African green monkey kidney epithelial cells
Neuro-2a: Mouse neuroblastoma cell line

While similar amounts of viral RNA are produced between the two viruses, significantly less infectious virus is produced by TM-nLuc at 24hpi in Veros and 16 and 24hpi in Neuro-2a cells







2A: Characterize behavioral changes and correlate with transcriptomic and morphologic changes

Modified SHIRPA	Elevated plus maze (EPM)	Novel object recognition (NOR)
Used to screen for neuromuscular and neurological abnormalities	Used to assess anxiety-like behavior	Used to measure cognitive function, specifically short-term memory