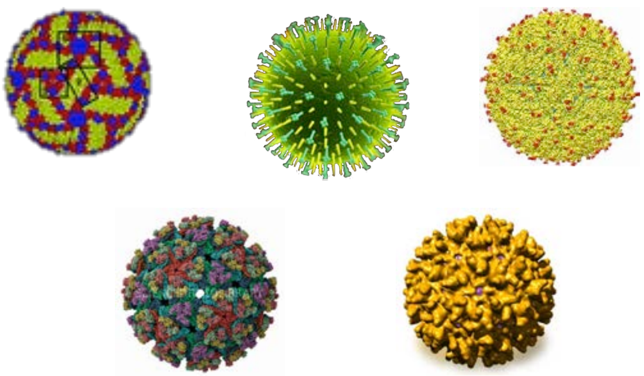
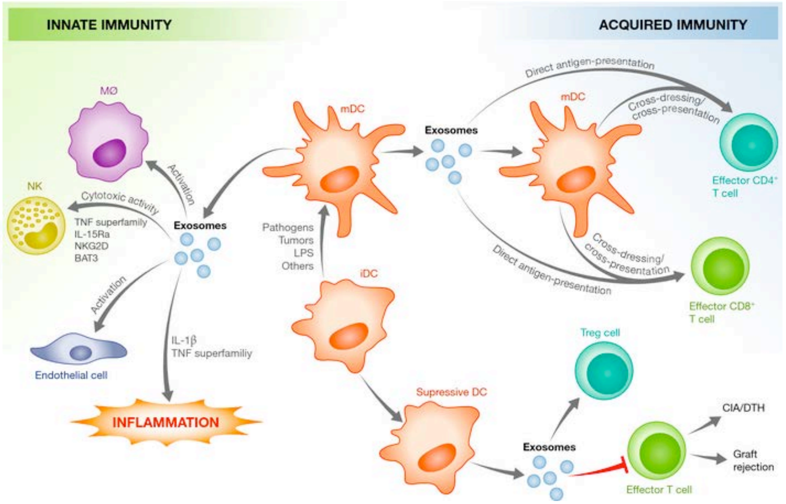
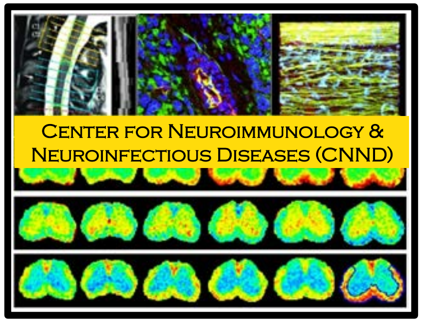




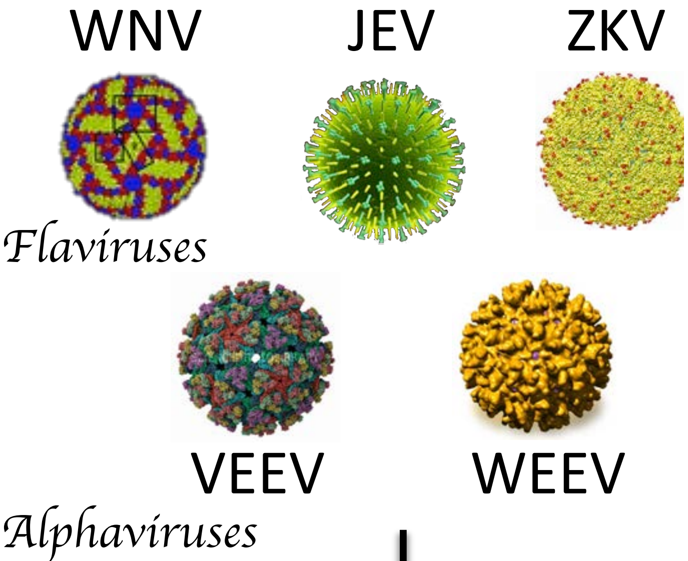
Virus-mediated Neurocognitive Disorders

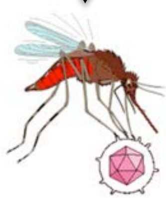


Robyn S. Klein, MD, PhD
Associate Dean for Graduate Education
Director, Center for Neuroimmunology & Neuroinfectious Diseases
Professor of Medicine, Pathology & Immunology, Neuroscience

Washington University
School of Medicine

Neurotropic Arboviruses





Neuroimmune interactions

Focus of research:

Virus sensing at brain endothelial barriers

Virologic control within the CNS

Post-infectious cognitive dysfunction ✔

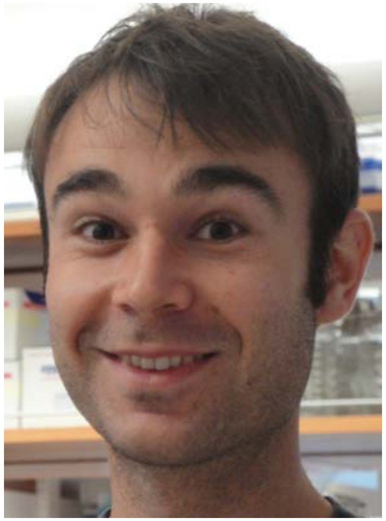
Today's talk:

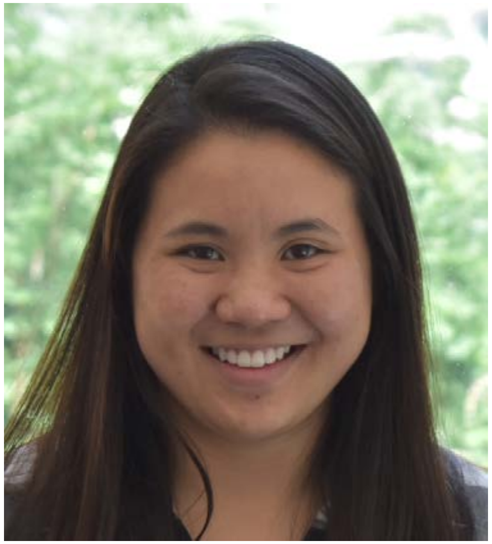
1. Introduction to cognitive sequelae after viral CNS infections: Focus on flaviviridae

2. Recovery from WNV – genetic signatures identify potential therapeutic targets

3. Murine models of WNV and ZIKV recovery and spatial learning – Trm-derived IFNγ: the most proximal signal for microglial activation

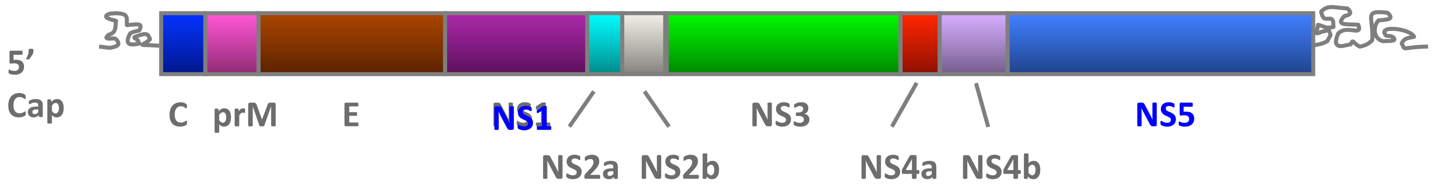
Acknowledgements:



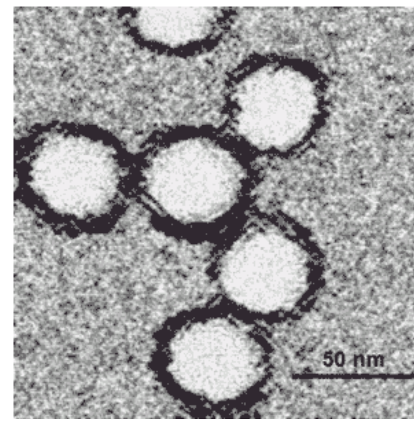


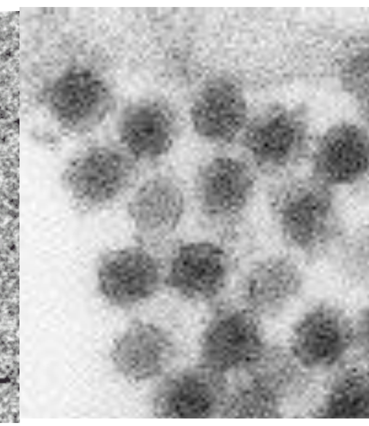
Mike Vasek, PhD **Charise Garber, PhD** **Allison Soung**

Flaviviruses



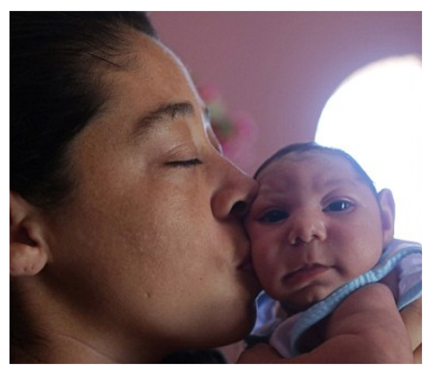
- *Flaviviridae*; Flavivirus, (Hepacivirus)
- Enveloped virus
- (+) ssRNA genome (~10.7kb), single ORF





West Nile virus (WNV)
Japanese encephalitis virus
Dengue virus
Zika virus
Yellow fever virus
Tick-borne encephalitis virus

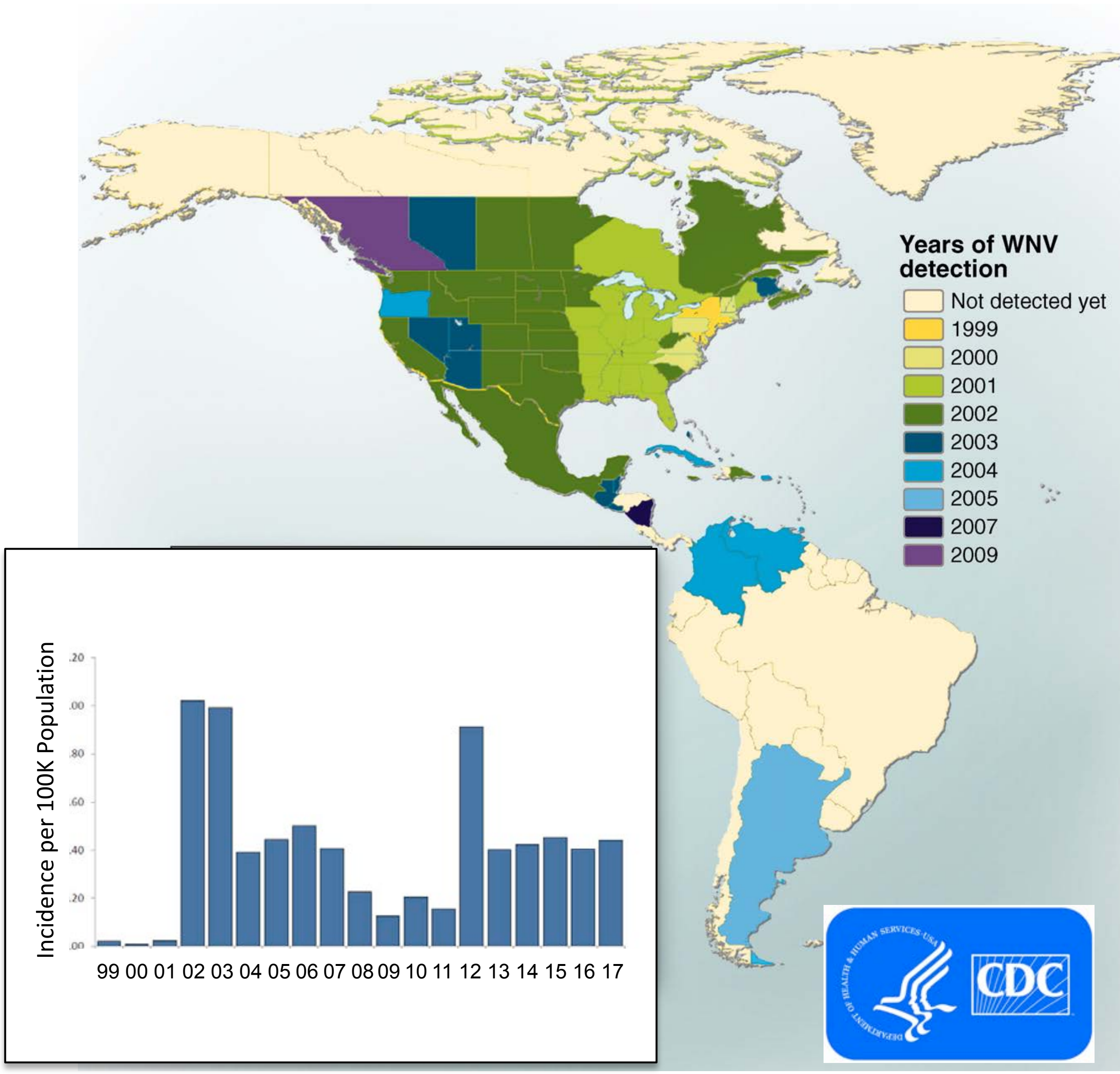
JEV: 180,000 infections/year



Zika-induced microcephaly

WNV Worldwide

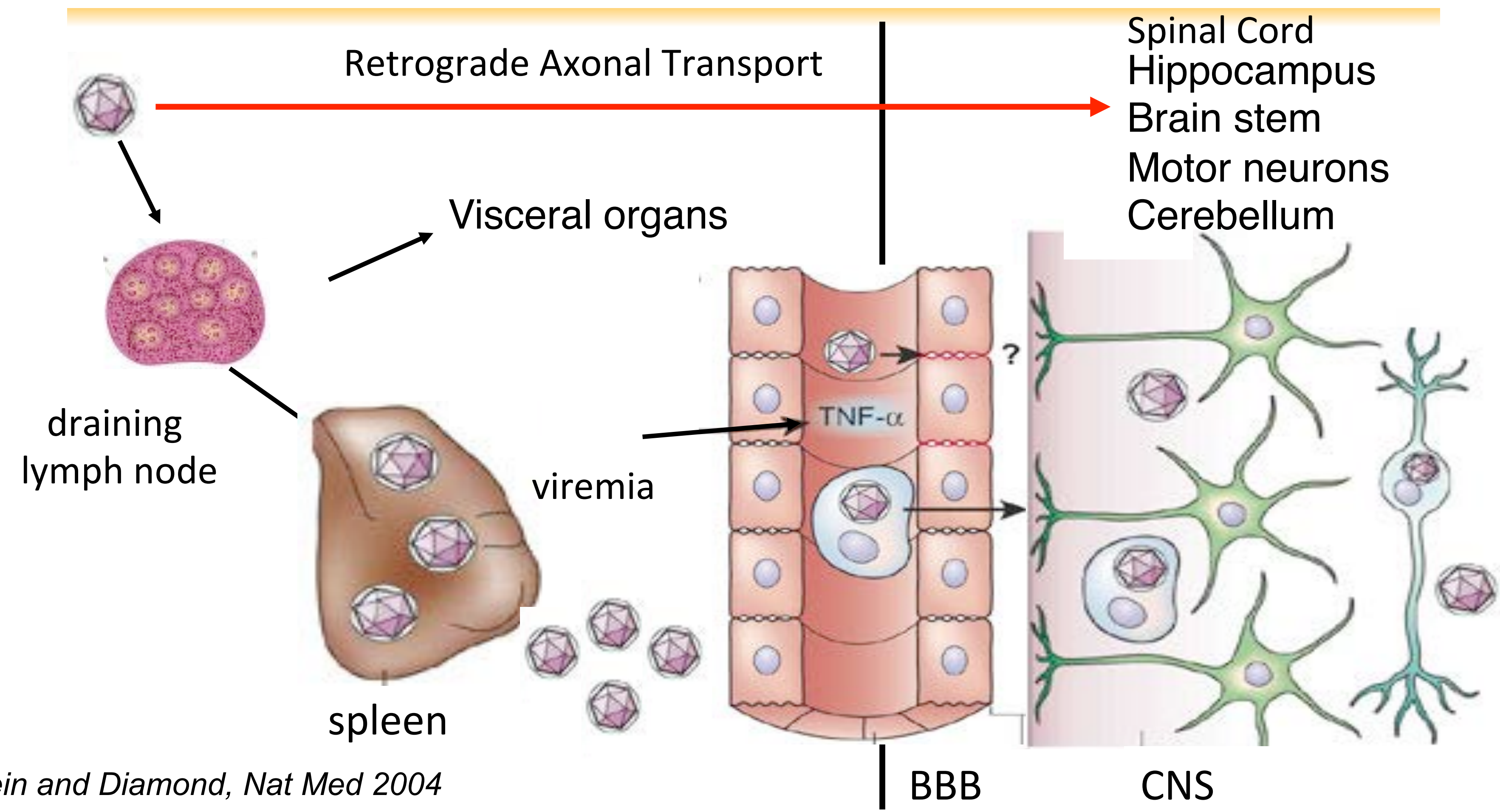
Spread of the West Nile virus



- Crossed US in 4 years 1999-2017:
  - Est 8.5M infections
  - >50K illnesses
  - 22,999 WNVE/M
  - 2163 deaths (90% surv)
  - Donor blood/organ screening
  - >50% loss of certain bird species
- NY99→WN02, others
  - > transm. by *Culex*
  - > virulence
  - > epidemic potential

Pathogenesis of Neurotropic Flavivirus Infection

1. Mosquito inoculation of skin (DC infection?)
2. Spread to local Lymph Node
3. Viremia
4. Hematogenous/retrograde spread to CNS (neuron infection)

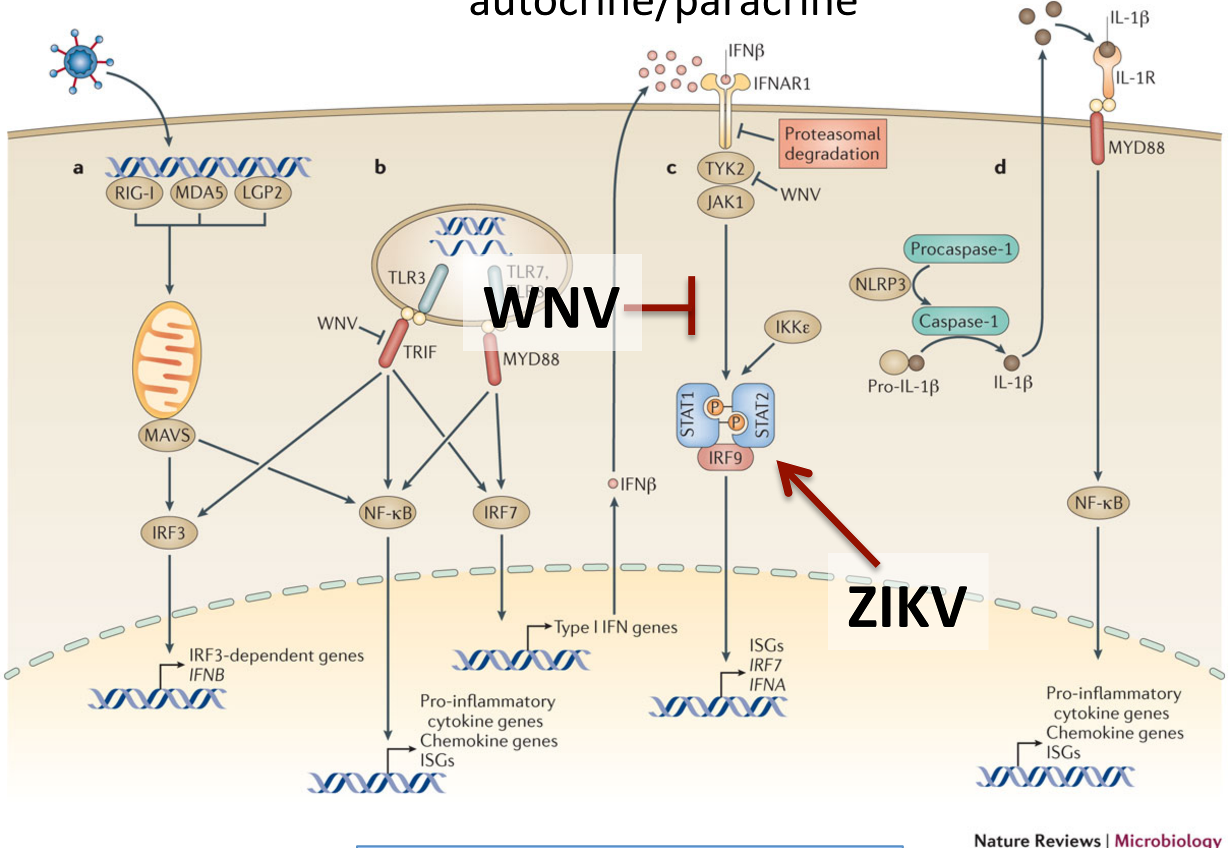


Klein and Diamond, Nat Med 2004

Innate immune response to flavivirus infection

- **Viruses detected by four classes of pattern recognition receptors (PRRS):**
    - **Toll-like receptors (TLRs)**
    - **Retinoic acid-inducible gene-like receptors (RIG-I, MDA-5, LGP2)**
    - **NOD-like receptor family, pyrin domain-containing protein 3 (NLRP3) inflammasome**
    - **DNA sensors (cGAS, IFI16)**

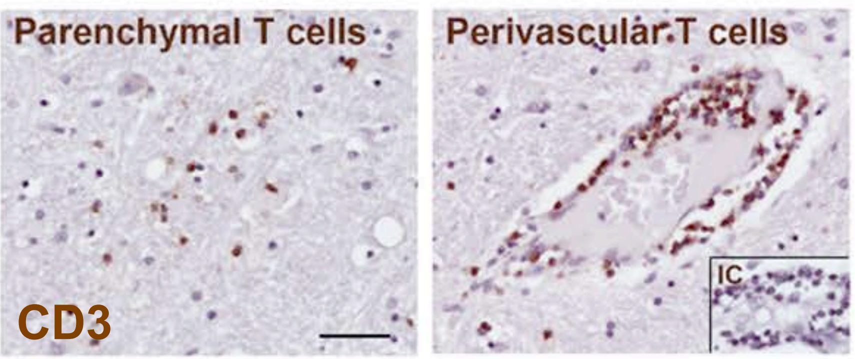
autocrine/paracrine



Cruz-Orengo, J Clin Invest. 2014; Daniels, mBio. 2014; Lazear, Daniels, Sci Transl Med. 2015; Miner, Nat Med. 2015; Daniels, J Clin Invest. 2017; Salimi, mBio. 2020

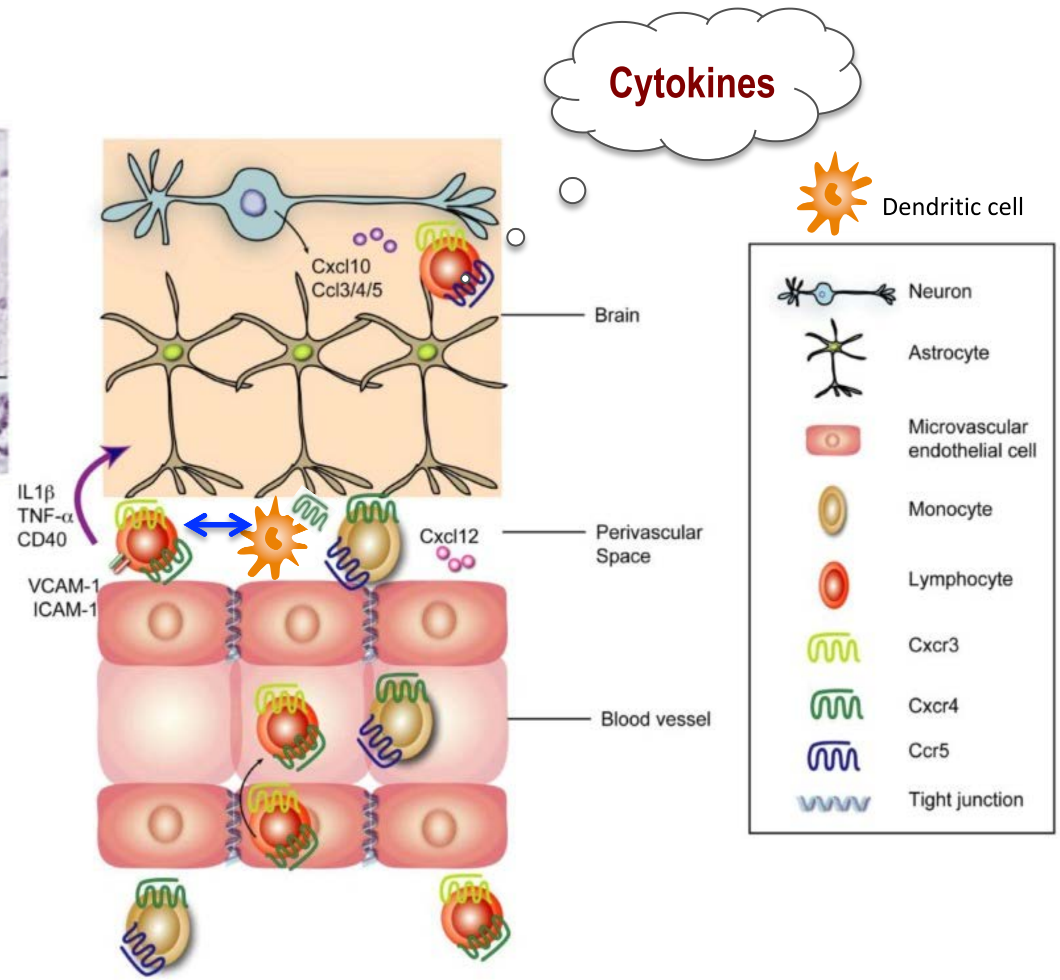
Stabilization of TJ/AJ: IFN I and III, SIP1
Destabliization: TNFa, IL-1, S1PR2

Survival from Flavivirus encephalitis: CNS T cell entry



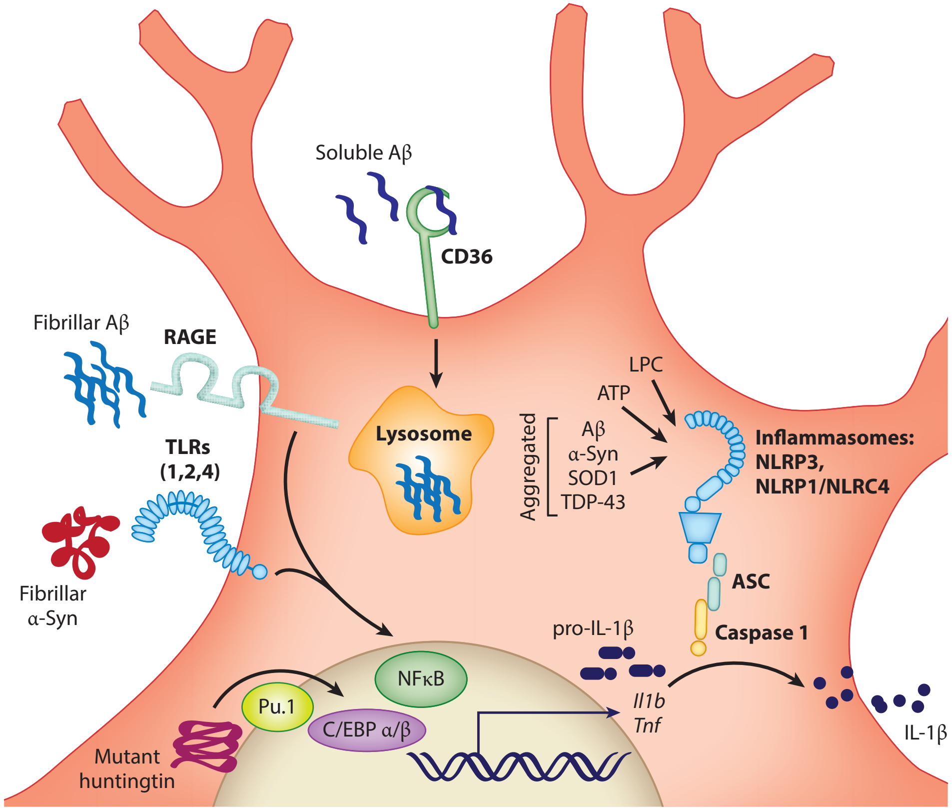
T cell localization and migration:

- chemokines expressed by BBB and infected neurons
- T cells persist after recovery



Klein et al. 2005, JV*I;* Glass et al. 2005, JEM; Sitati et al. 2007, JVI; Zhang et al. 2008, JVI; Shrestha et al. 2008, JVI; McCandless et al. 2008, PNAS; Durrant et al, 2013, JEM; Durrant et al, 2014, JI

PRR Activation by Protein Aggregates in Neurodegeneration



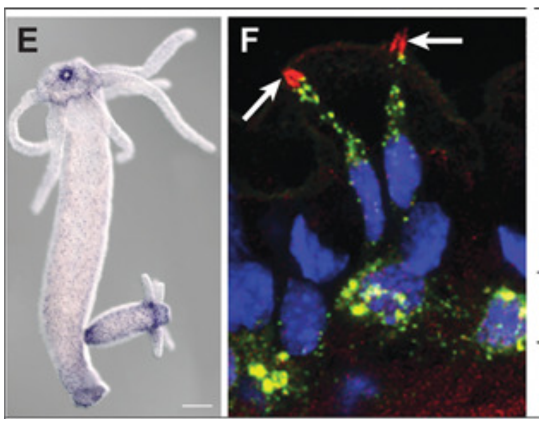
Annu Rev Med. 2017 Nov 6

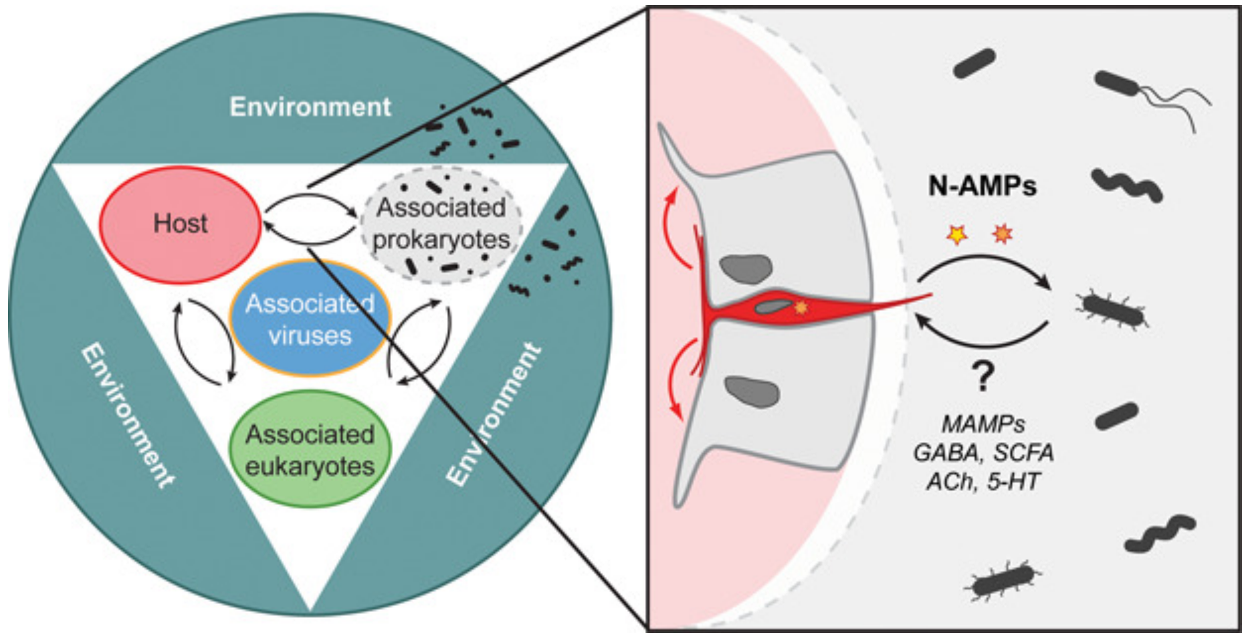
- **Toll-like and other surface receptors can recognize Aβ, α-syn or DAMPs → cytokines**
- **Protein aggregates activate inflammasome NLRP family proteins → Caspase 1**
- **Recruitment/Activation of T cells**

SUGGESTS COMMON INNATE IMMUNE MECHANISMS MAY UNDERLIE MEMORY DISORDERS

Nervous System = Neuroimmune System

Hydra Holobiont – hydra and its microbiome



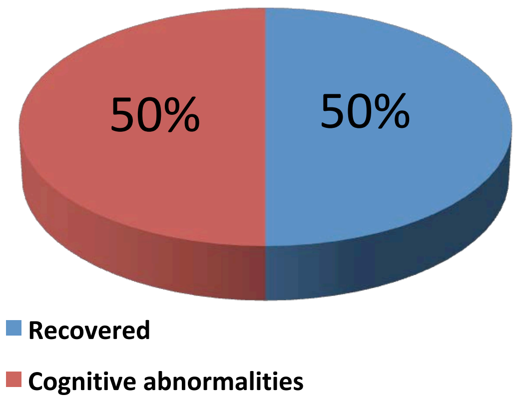


BioEssays, Volume: 40, Issue: 9, First published: 10 July 2018

Suggests nervous and immune systems evolved as single system and validates hydra as a relevant model

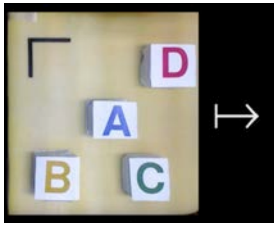
New Syndromes: Recovery from WNF and WNND

- 40% - 70% of patients
  - Confusion, memory impairments, muscle weakness, concentration difficulties, parkinsonism
  - Long-term: Months to 5+ years after infection: increases over time



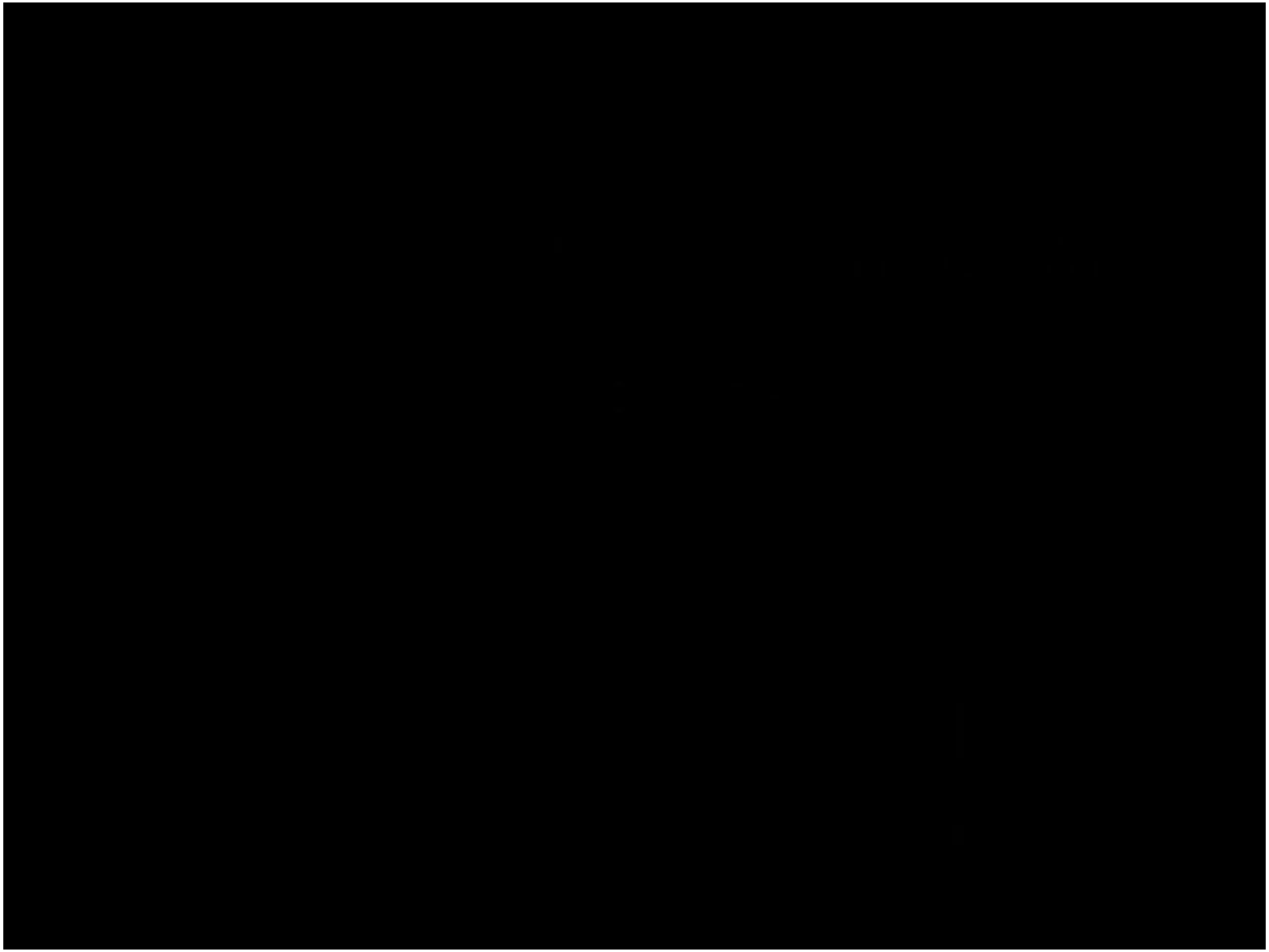
Modified from Klee et al., *Emer. Inf. Dis.* (2004)

- Neuropsychological testing (Sadek et al., *J. Clin. Exp. Neuropsych.* 2010; Samaan et al. PLOSOne, 2016)
  - Psychomotor speed (24-56% patients impaired)
    - Fingertap speed, Gooved Pegboard
  - Memory (11-49% patients impaired)
    - Hopkins Verbal Learning Test
  - Visuospatial (3-27% patients impaired)
    - Rey Complex Figure Copy and recall



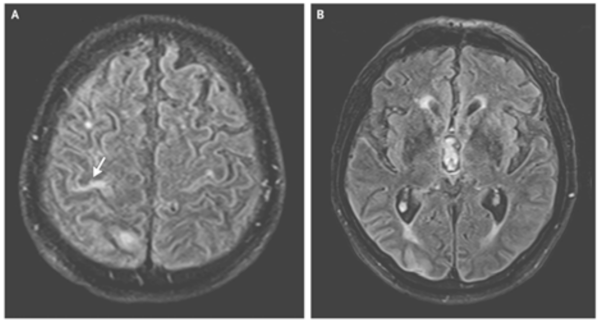
CANARY
SHOES
EAGLE
BLOUSE
NAILS
CROW
BLUEBIRD
SCREWDRIVER
PANTS
CHISEL
SKIRT
WRENCH

New Syndromes: Recovery from Flavivirus Encelphalitides



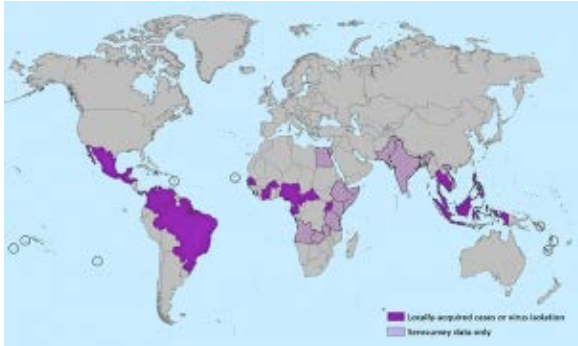
Emerging Syndrome: Zika Virus-Associated Cognitive Impairment

ZIKV in adults:
Guillain Barre
meningoencephalitis



N Engl J Med 2016; 374:159

CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™



EMERGING INFECTIOUS DISEASES®

Volume 22, Number 12—December 2016

Letter

Zika Virus Infection in the Central Nervous System and Female Genital Tract

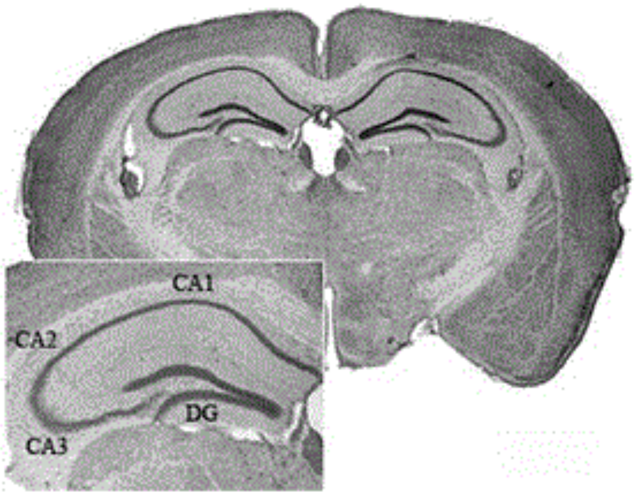
Volume 23, Number 6—June 2017

Research Letter

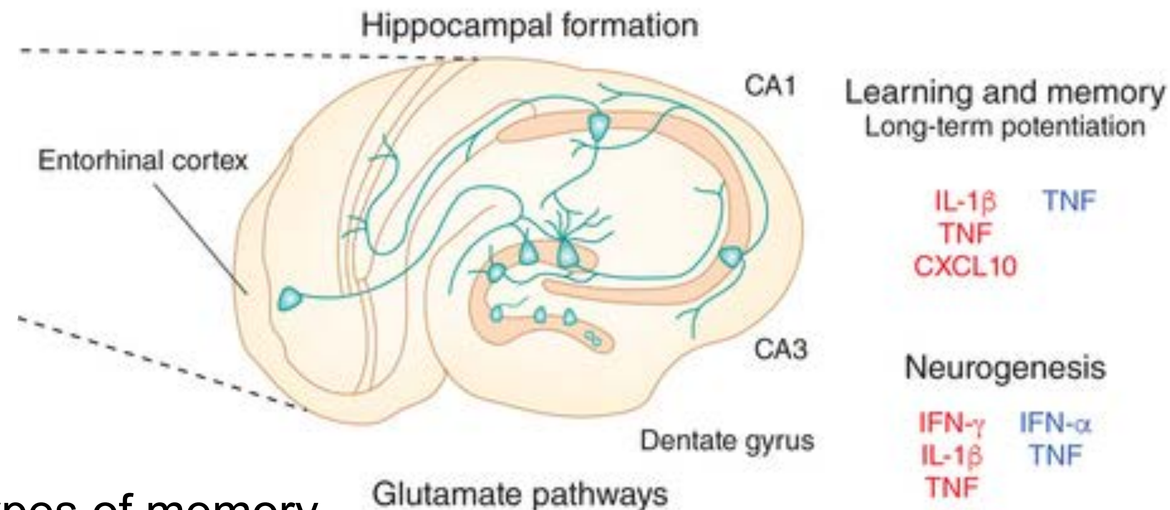
Zika Virus–Associated Cognitive Impairment in Adolescent,

Do events triggered in the acute setting lead to neurologic sequelae?

Spatial learning occurs in the hippocampus



Homeostatic vs pathological role of neuroimmune communication



Klein et al, *Nature Immunology* 2017

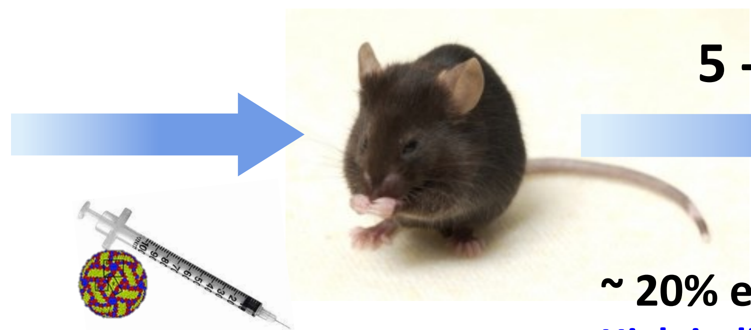
Hippocampus is critical for forming and recovering certain types of memory.

Neural correlates of learning:

1. SGZ neurogenesis - Formation of episodic and spatial memory (DG and CA3) (Zhao and Gage, *Cell.* 2008)

2. Activity dependent strengthening of synapses

No models to study WNV-induced memory dysfunction before 2016



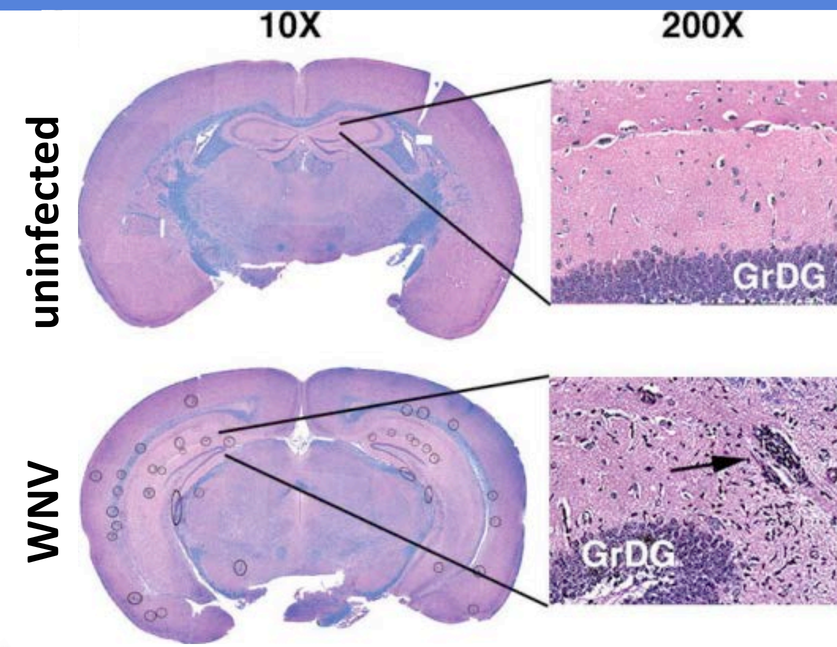
New York, 1999

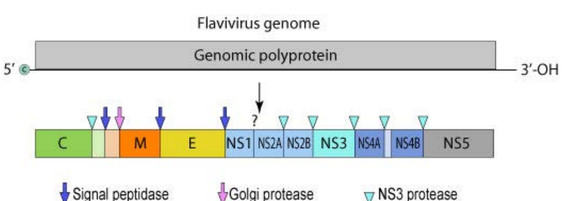
5 - 7 days

~ 20% encephalitis

High individual variablity

20-50% mortality





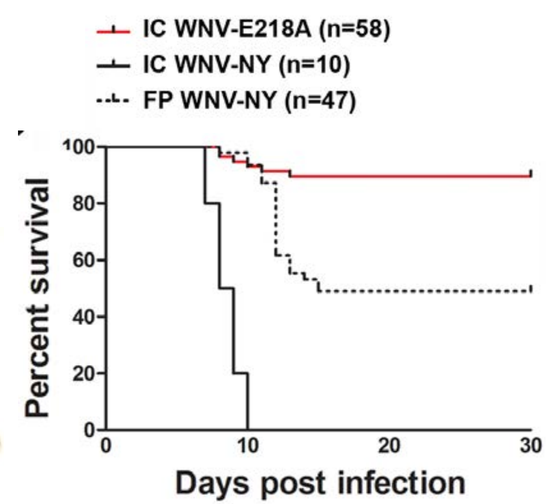
m7GppA-RNA

m7GpppAm-RNA

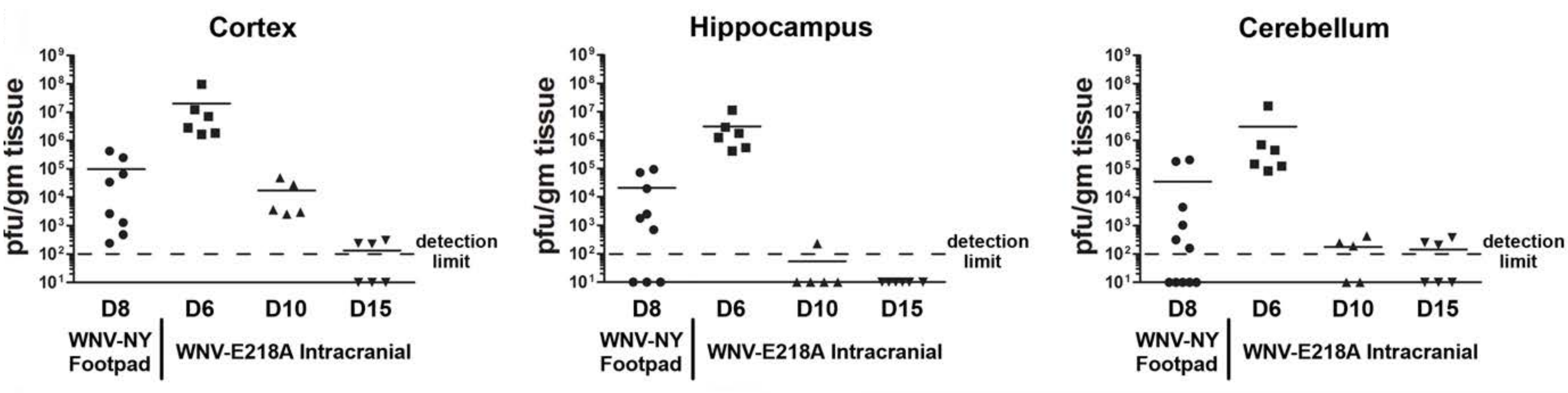
2'-O Methyltransferase

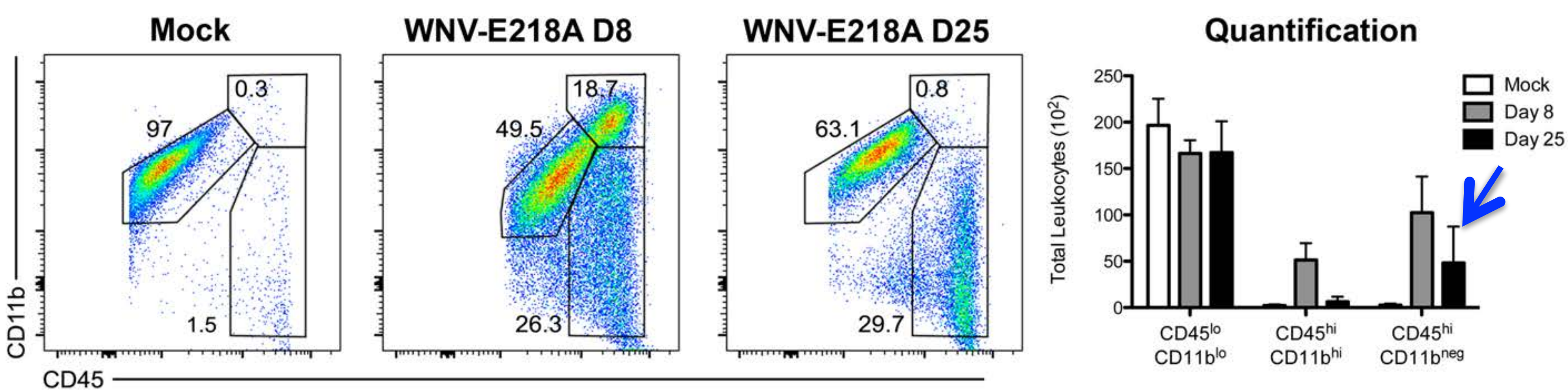
Blocks binding to IFIT1

NS5-E218A



i.c. WNV-NS5-E218A: more uniform CNS viral loads with CD8 T cell-mediated clearance and recovery



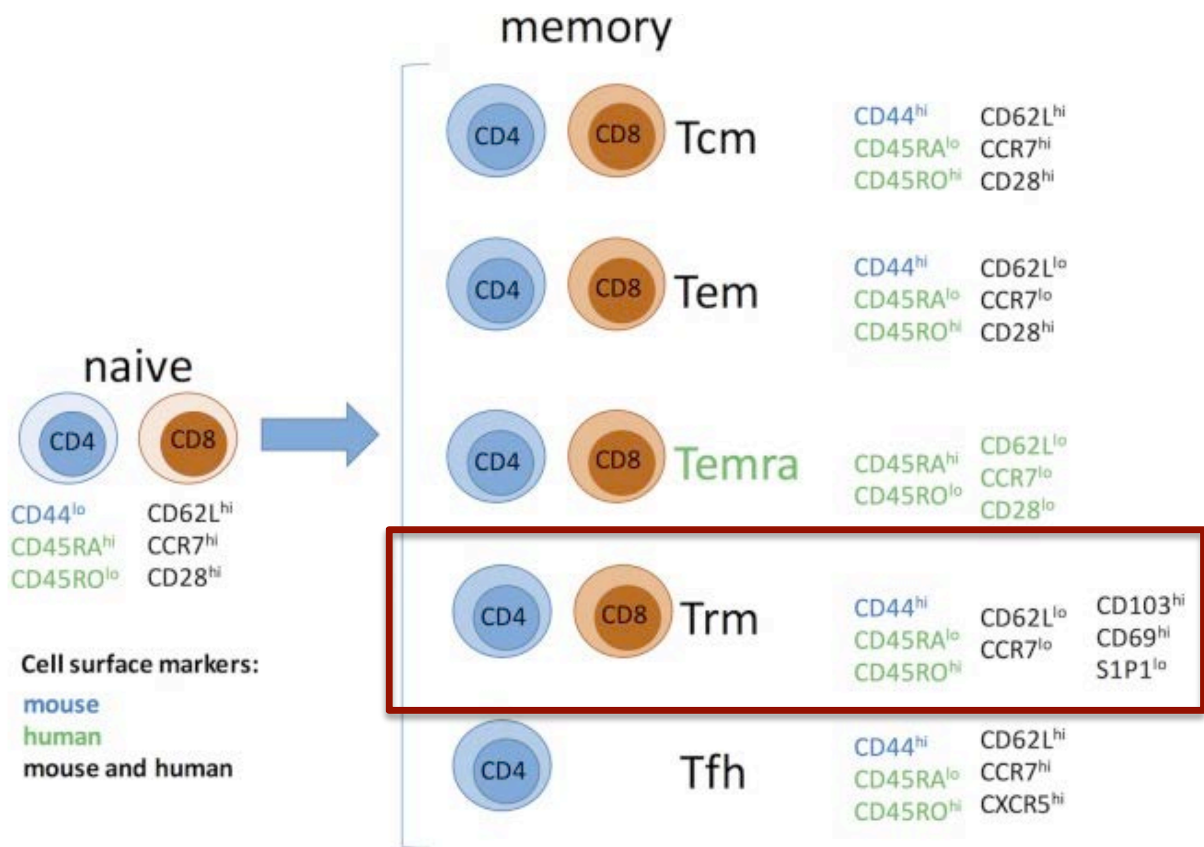


Vasek et al. Nature 2016

Memory T cells Persist in Tissues after Infectious Diseases

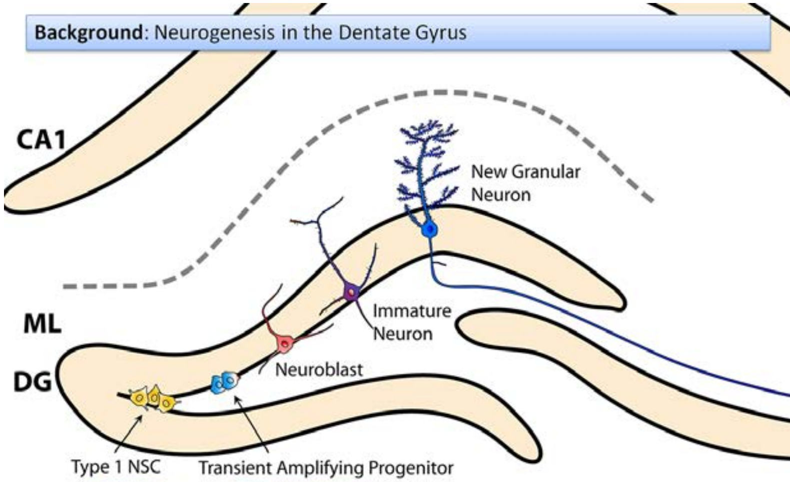
- Observed after initial infection with a pathogen
- Secondary challenge with antigen leads to rapid activation of much higher magnitude than naïve response.

- Central→lymphoid homing
- Effector→peripheral tissue homing
- **Resident→tissue specific homing, non-recirculating**

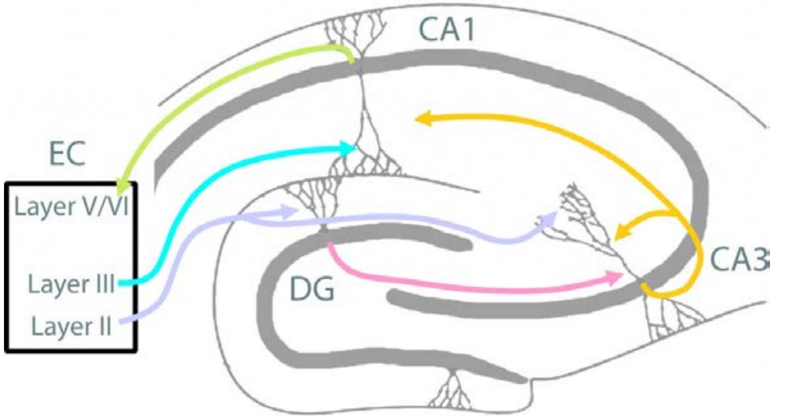


Hypothesis regarding mechanisms of Flavivirus-induced memory disorders

1. Virus or T lymphocytes destroy neurons

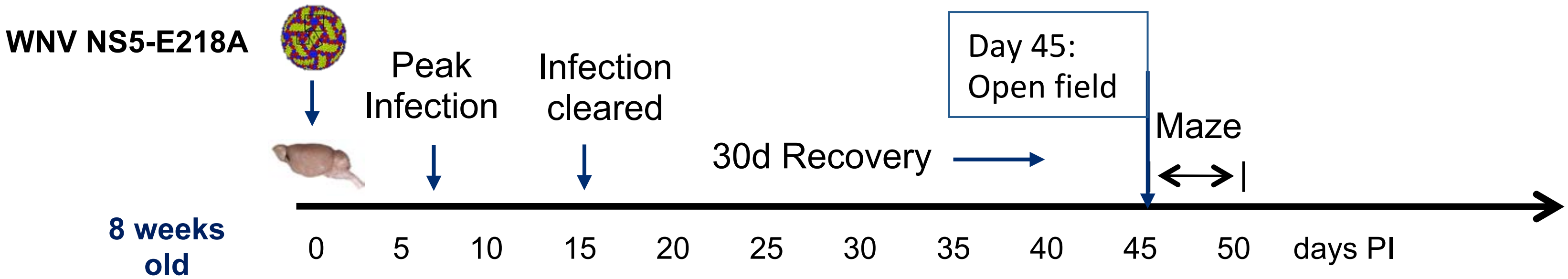


2. Virus or inflammation prevent repair of neurons



3. Virus or inflammation eliminates communication between neurons

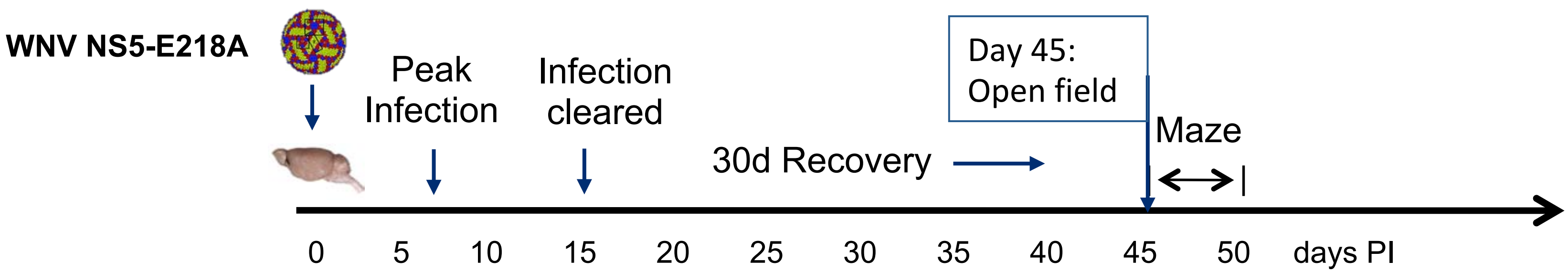
Model of post-infectious cognitive recovery

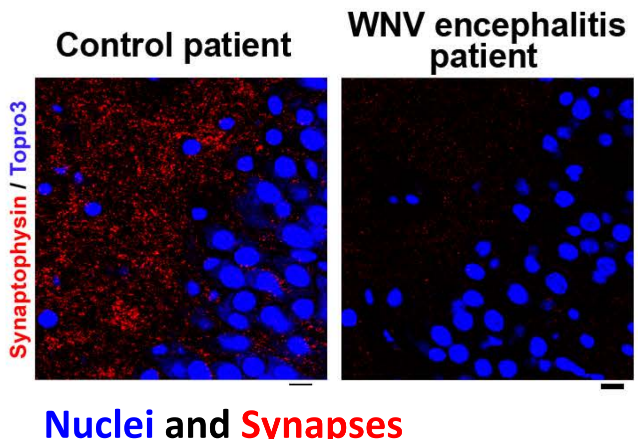


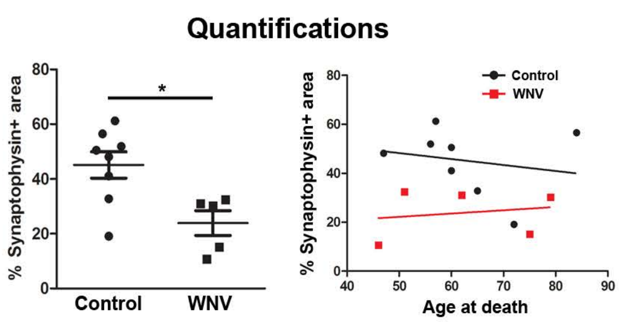
MOCK INFECTED

WNV E218A

Model of post-infectious cognitive recovery







Findings:

Uniform CNS viral loads

Persistently activated microglia (→ 52 dpi)

Elimination of presynaptic termini (C1q)

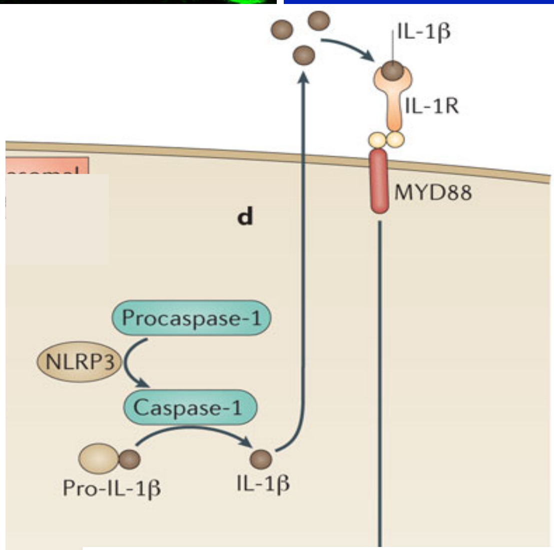
Decrease in adult neurogenesis (IL-1R1)

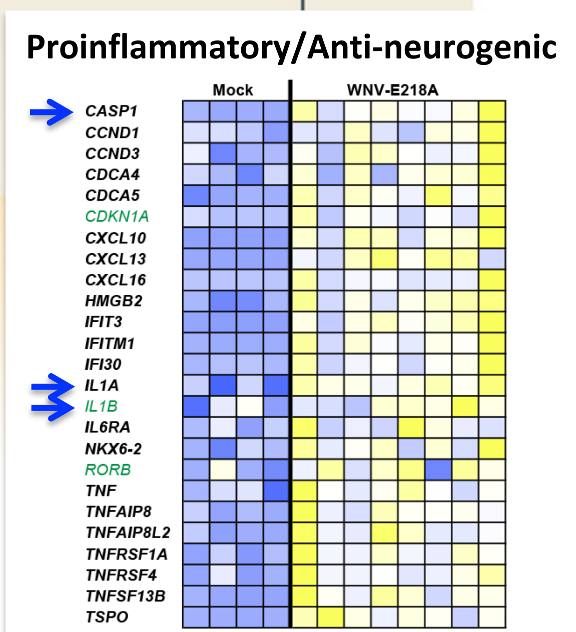
Increase in IL-1+ reactive astrocytes

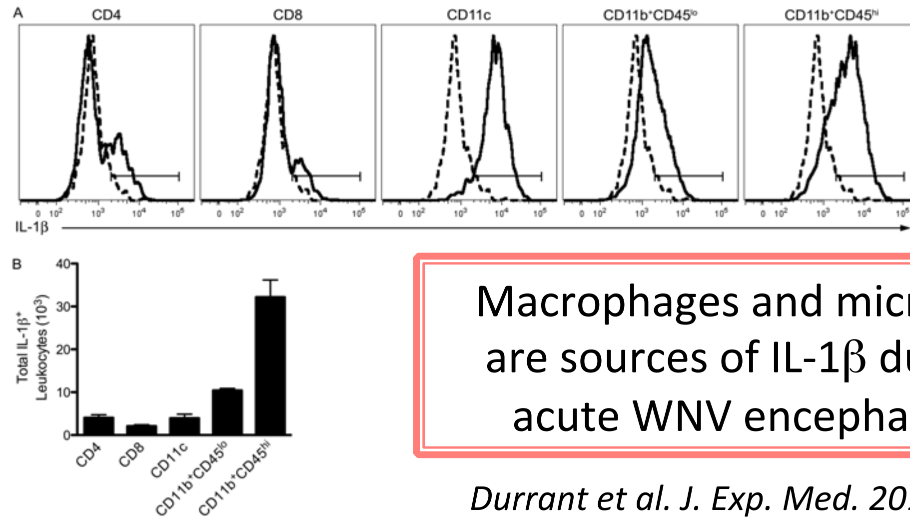
Spatial learning deficits

Vasek et al, Nature, 2016; Garber et al. Nat. Immunol. 2017
Garber and Soung et al., Nat. Neurosci., 2019

IL-1 pathway in good and poor learners

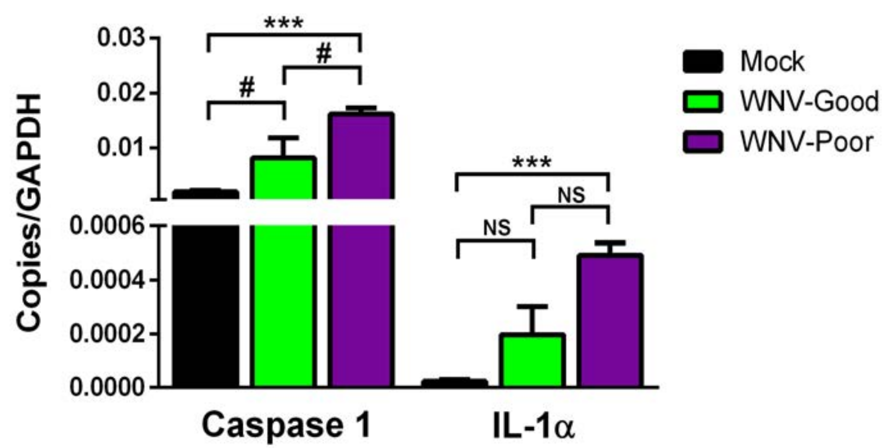






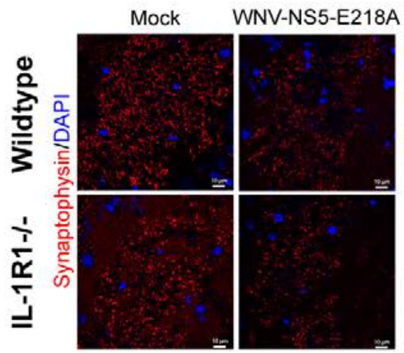
Macrophages and microglia are sources of IL-1β during acute WNV encephalitis

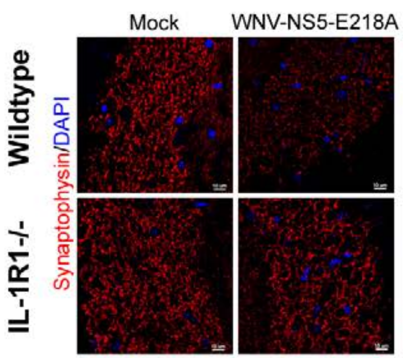
Durrant et al. J. Exp. Med. 2013
Durrant et al. J. Immunol. 2014

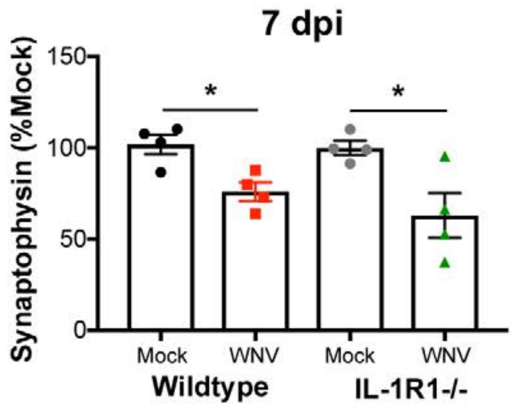


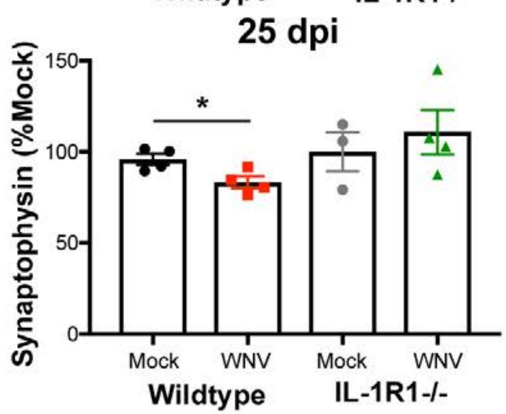
Garber et al., Nat. Immunol. 2018

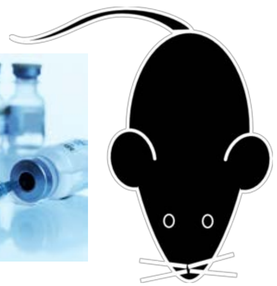
IL-1R1 inactivation, synapse repair and spatial learning



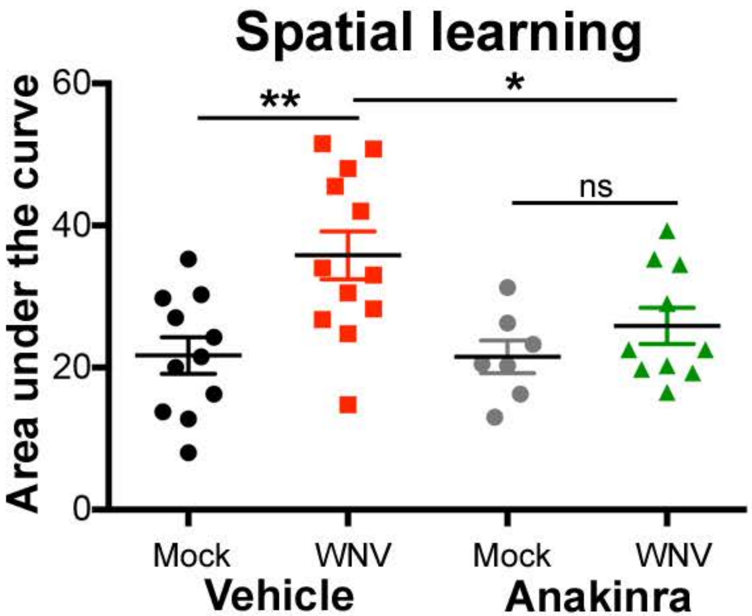






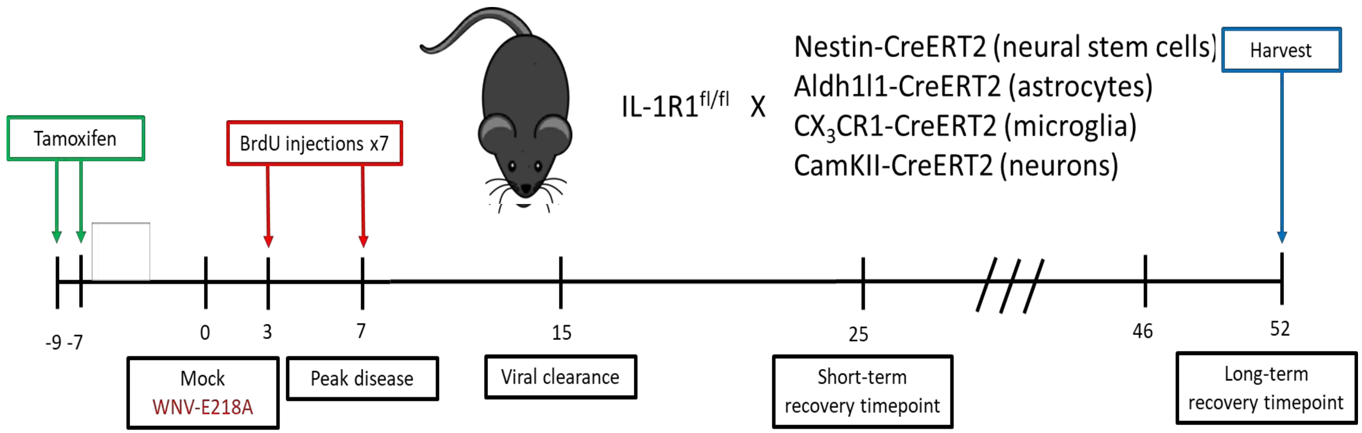


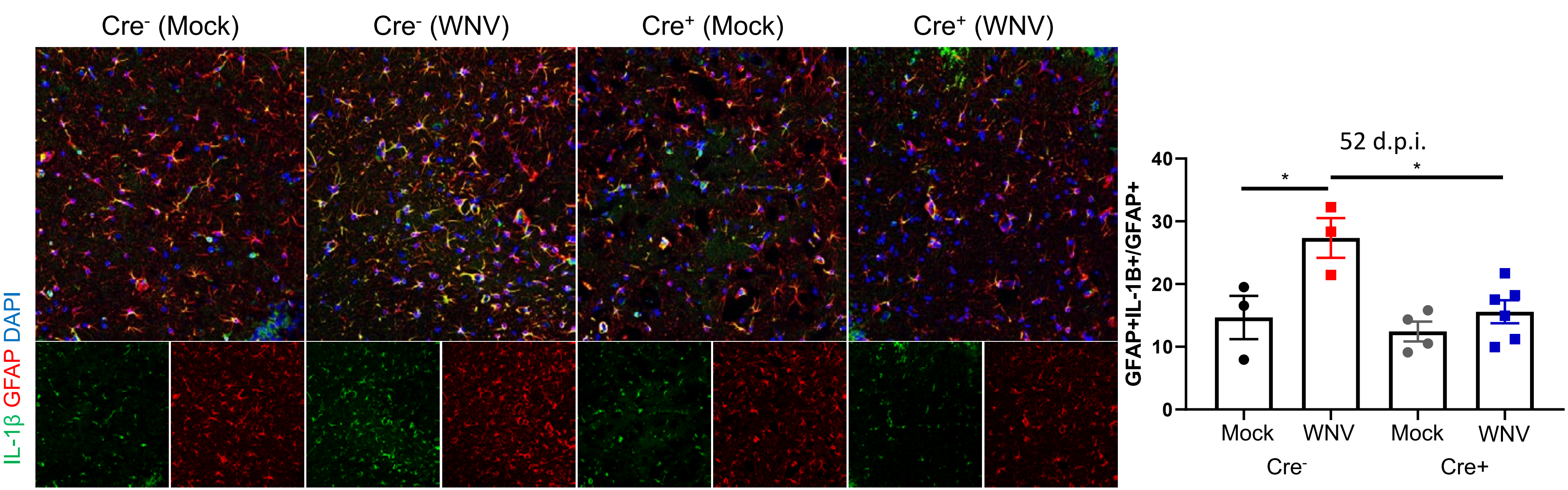
i.p. anakinra
100mg/kg/dy
10-15 dpi



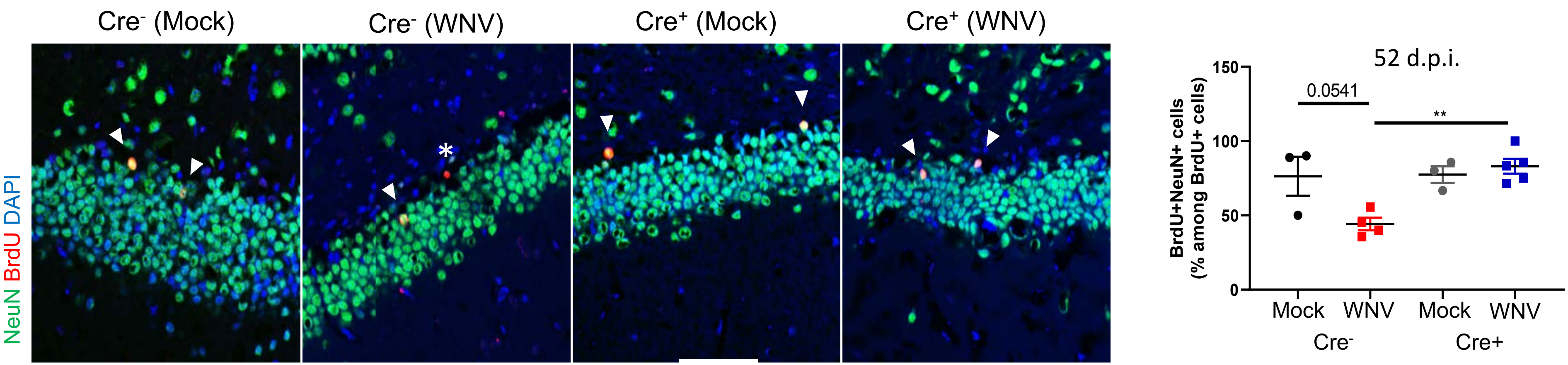
Garber et al., Nat. Immunol. 2018

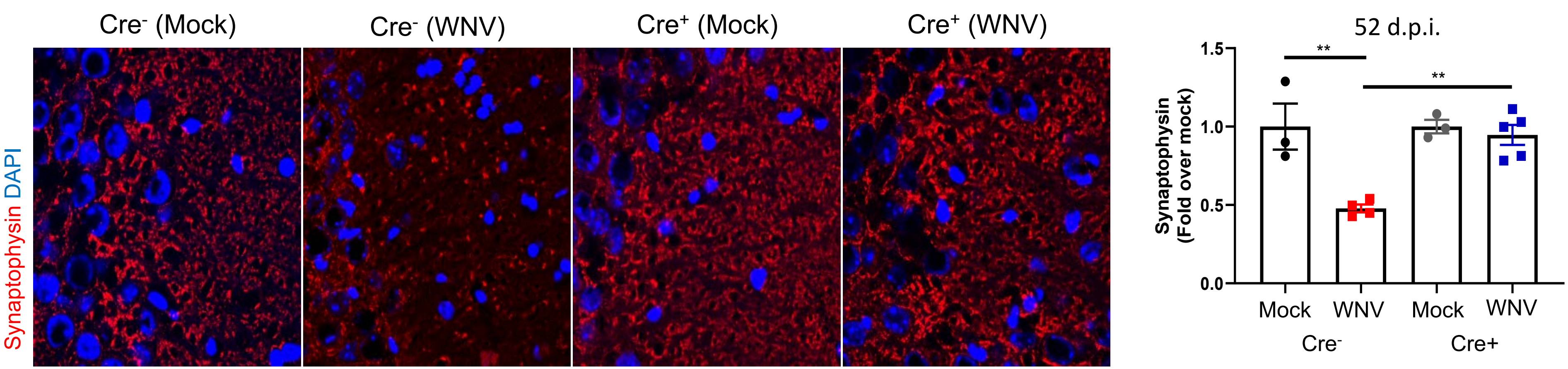
Astrocyte production of IL-1β is attenuated during recovery when IL-1R1 is deleted from NSCs



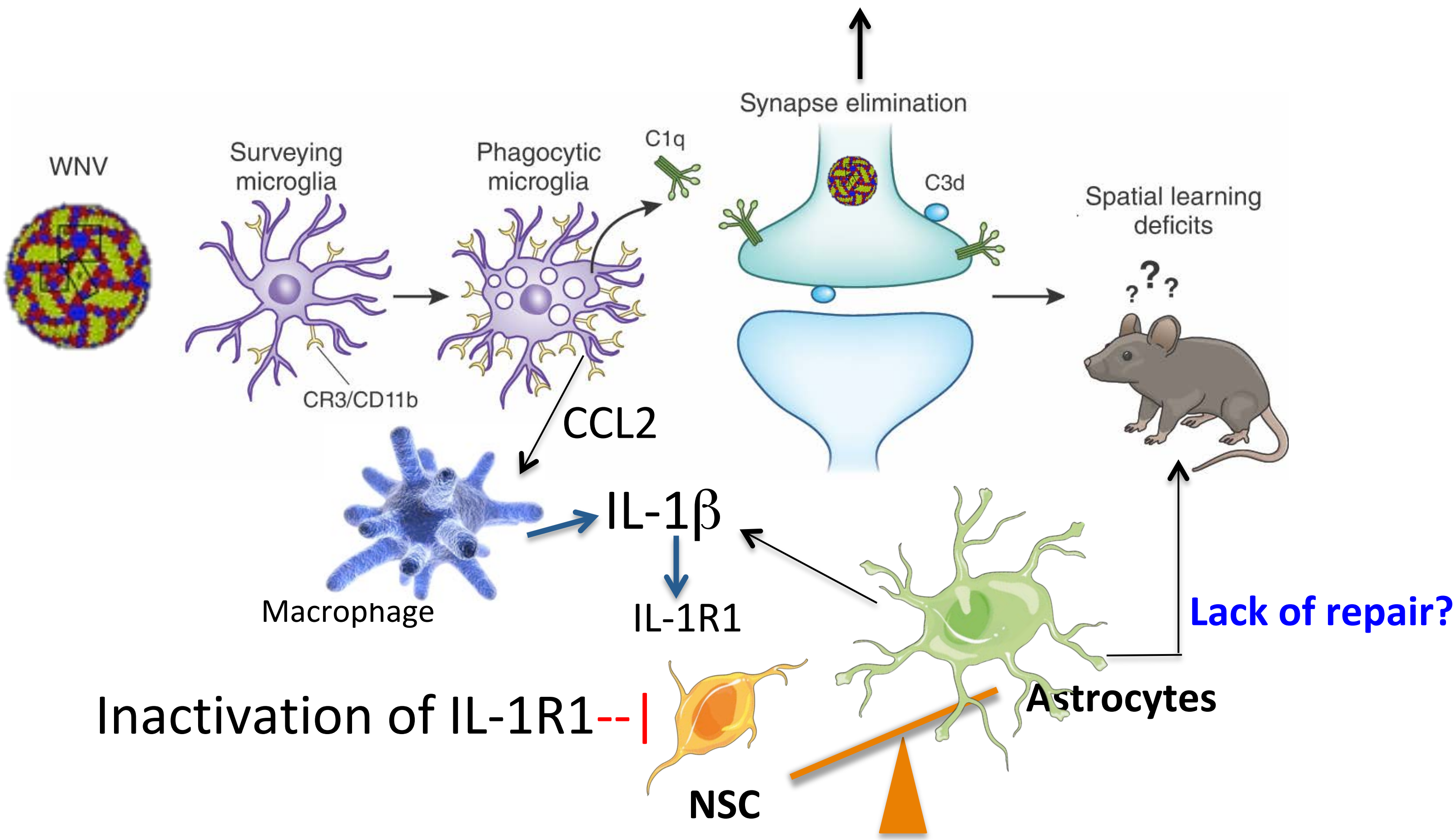


IL-1β signaling in neural stem cells limits adult neurogenesis and synaptic repair





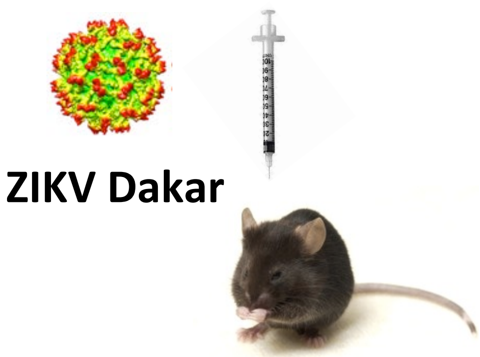
WNV Recovery: Model for synapse elimination/recovery

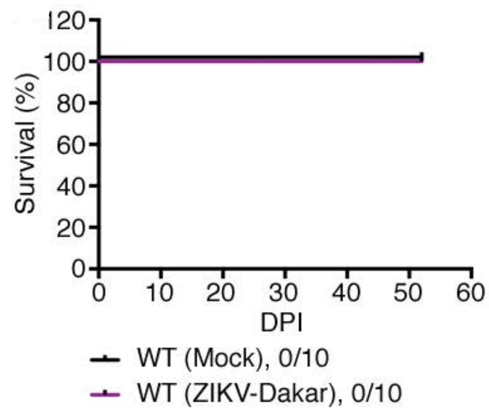


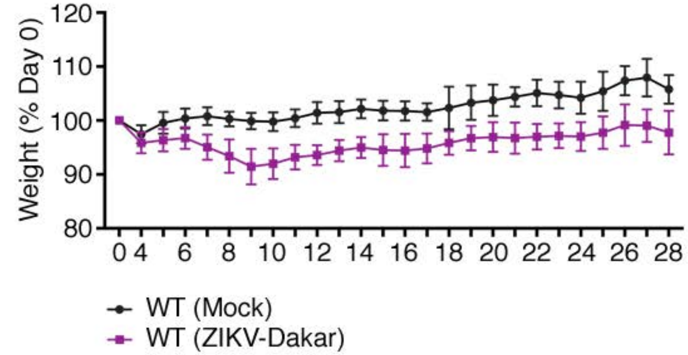
Question: Cause of prolonged activation of microglia and delayed cognitive recovery? How does ZIKV cause cognitive dysfunction in recovered adult animals?

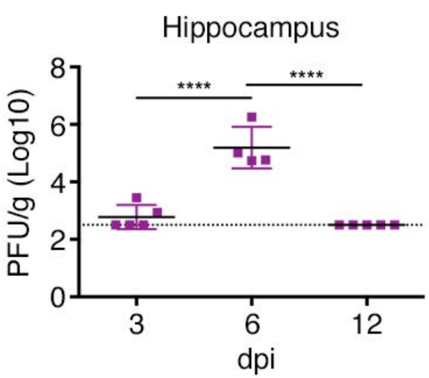
Vasek et al. Nature 2016; Klein et al. Nature Immunology, 2017;Garber, et al. Nature Immunology 2018; Garber, Soung, Nature Neuroscience 2019

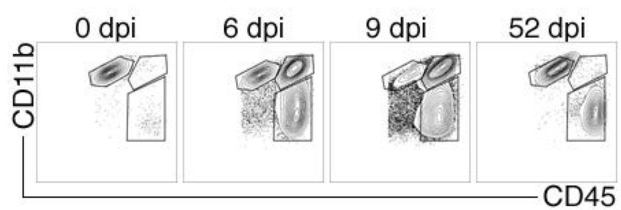
ZIKV-recovered animals exhibit profound spatial learning defects and chronic inflammation

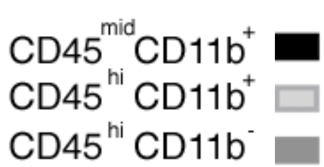


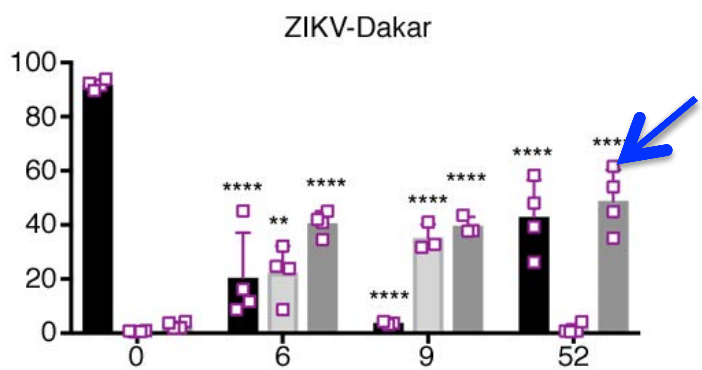


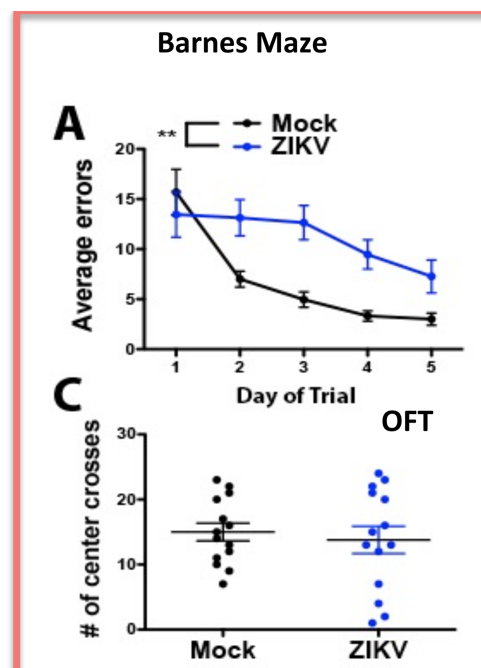






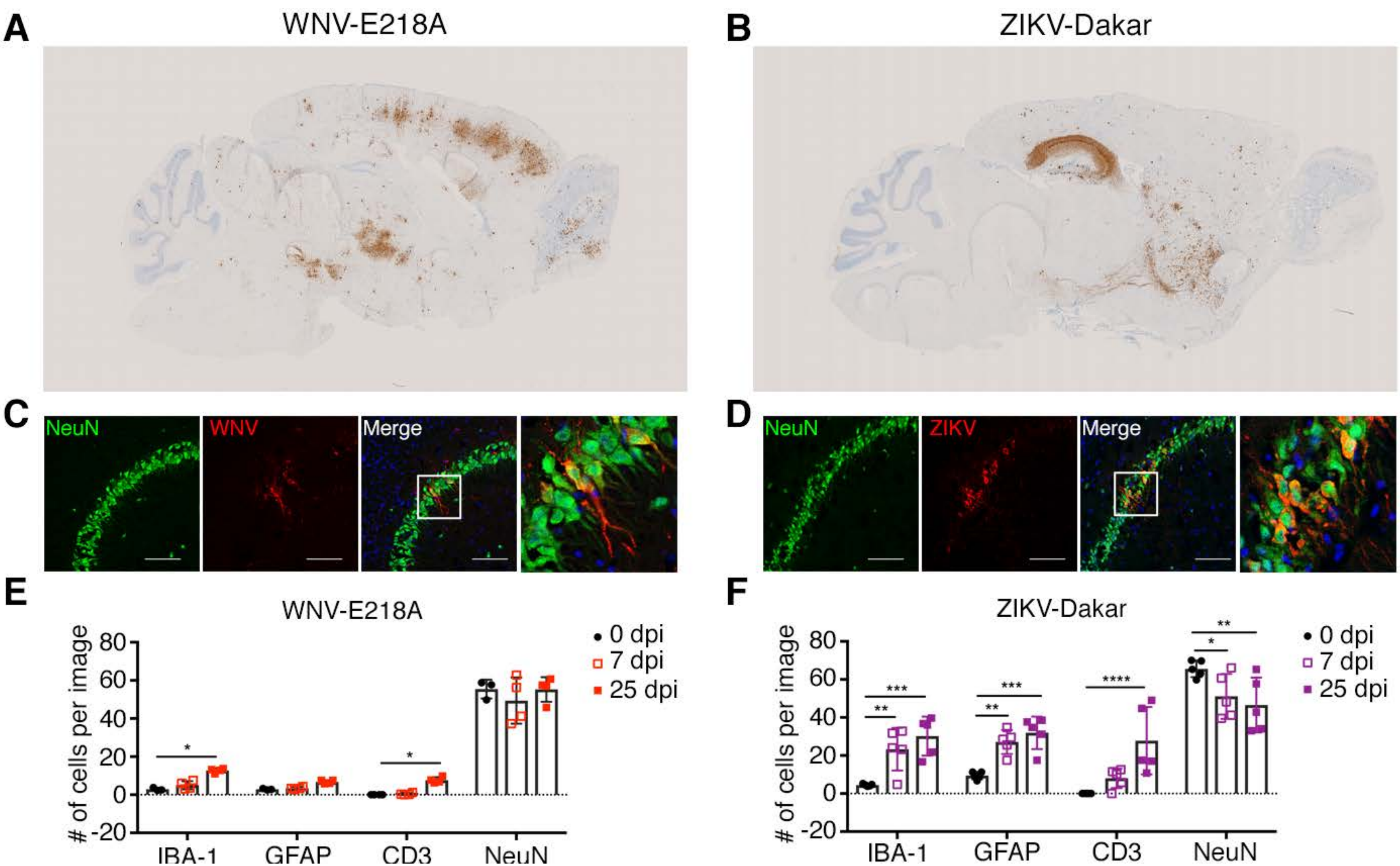






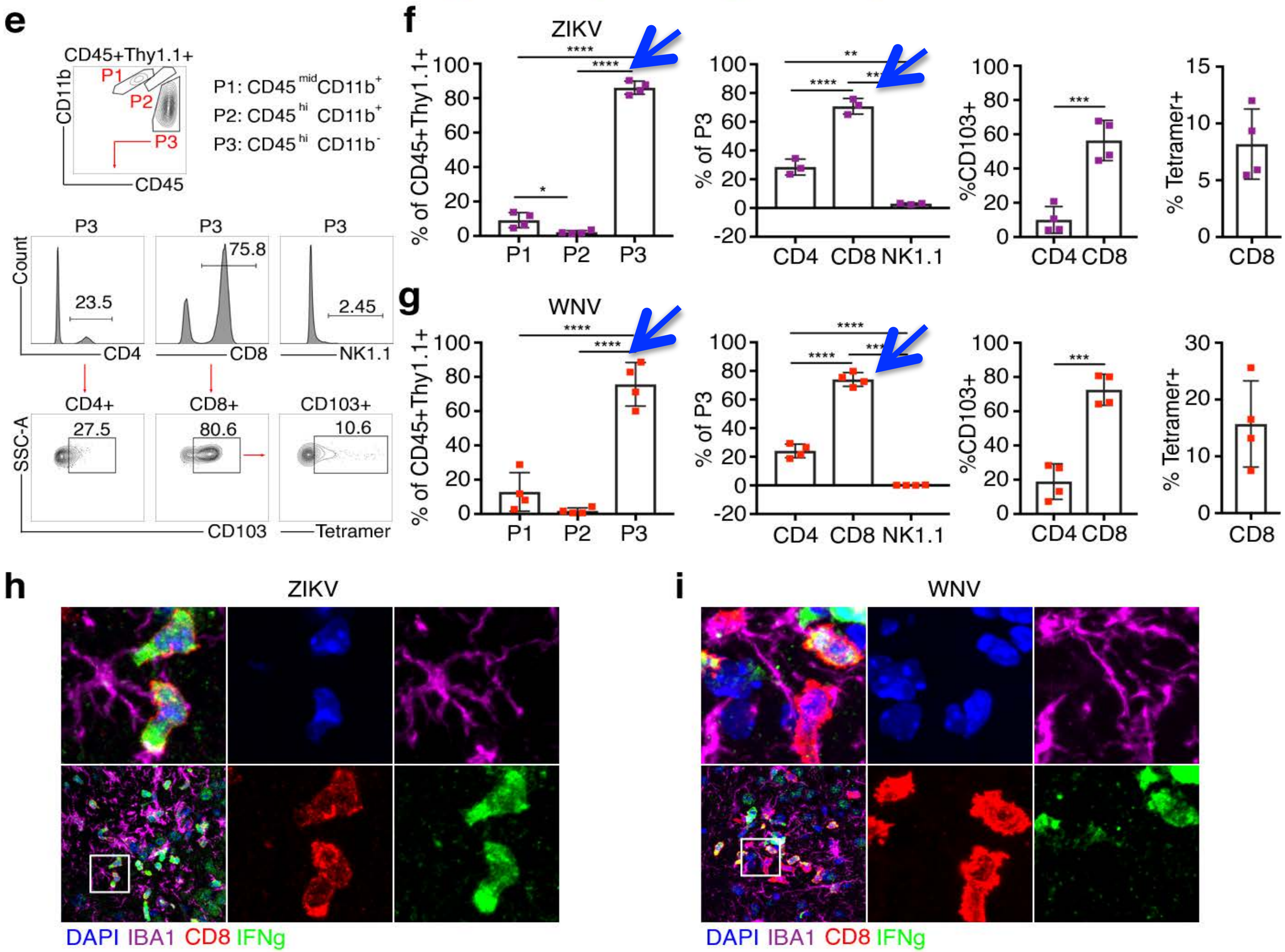
Garber and Soung et al., Nat. Neurosci., 2019

ZIKV targets the hippocampus and induces neuronal loss

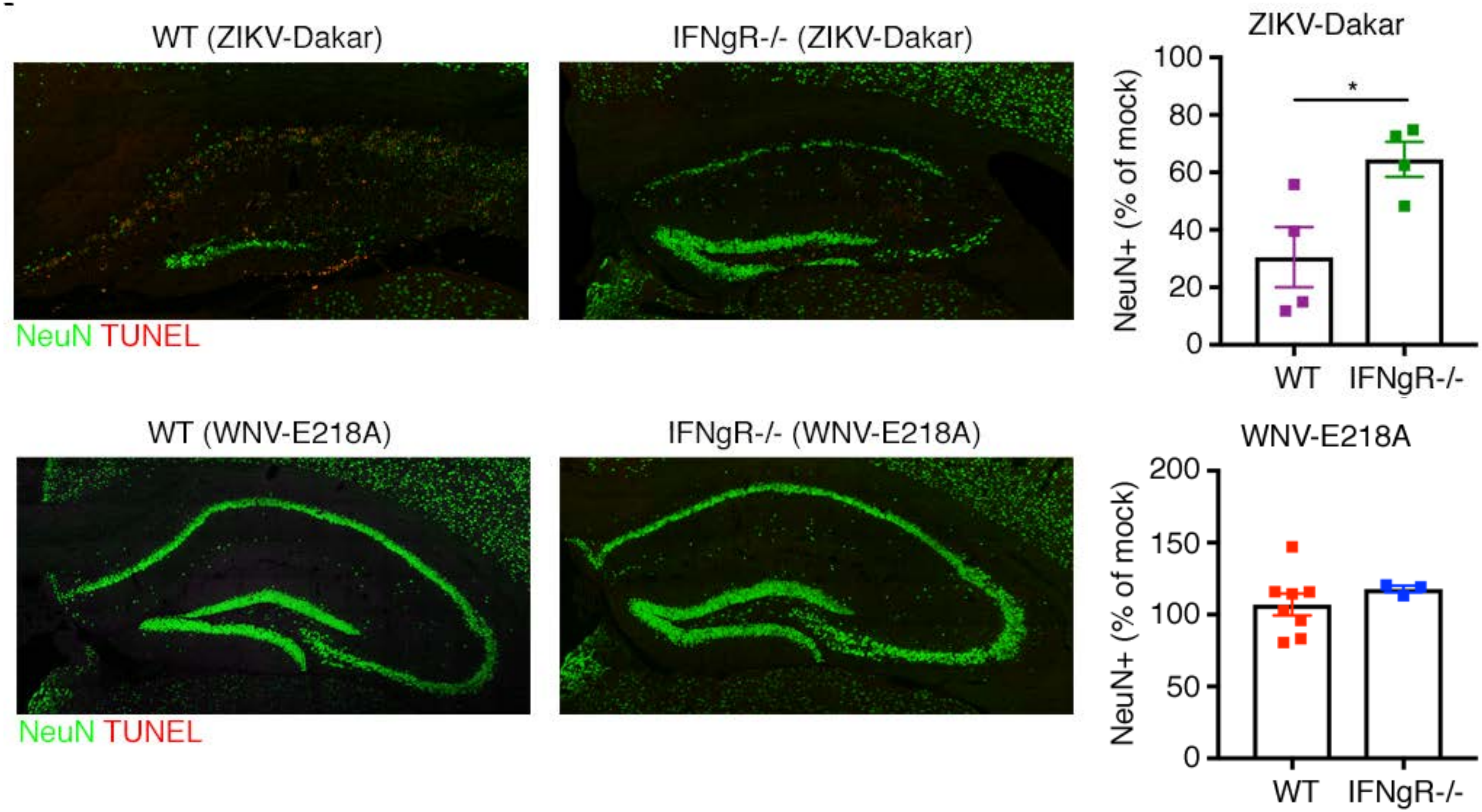


Garber and Soung et al., Nat. Neurosci., 2019

IFNγ+CD8+ cells persist and a subset express CD103 and are virus-specific

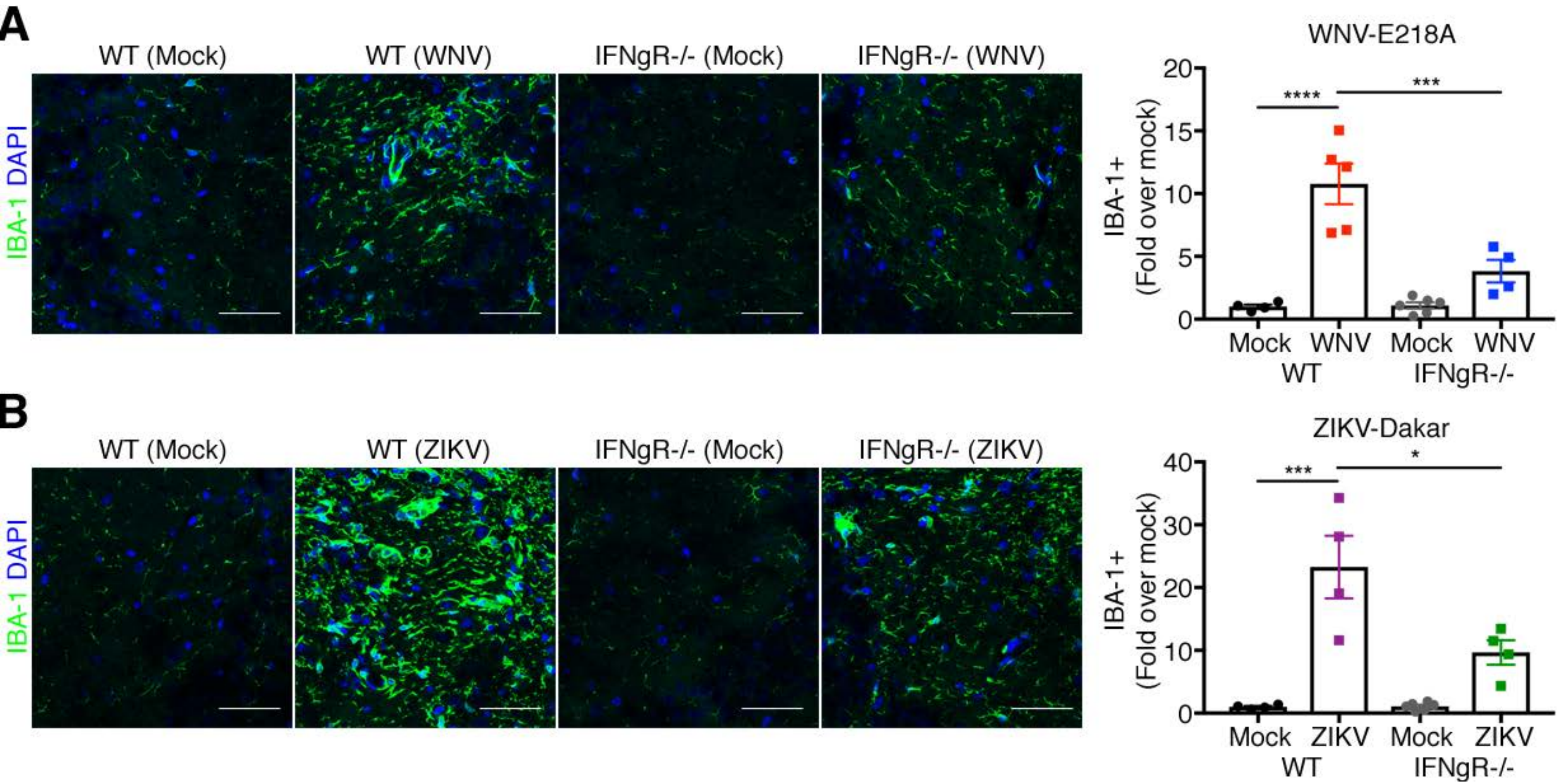


IFNγ leads to loss of neurons within the hippocampi of ZIKV-recovered animals – 25 dpi



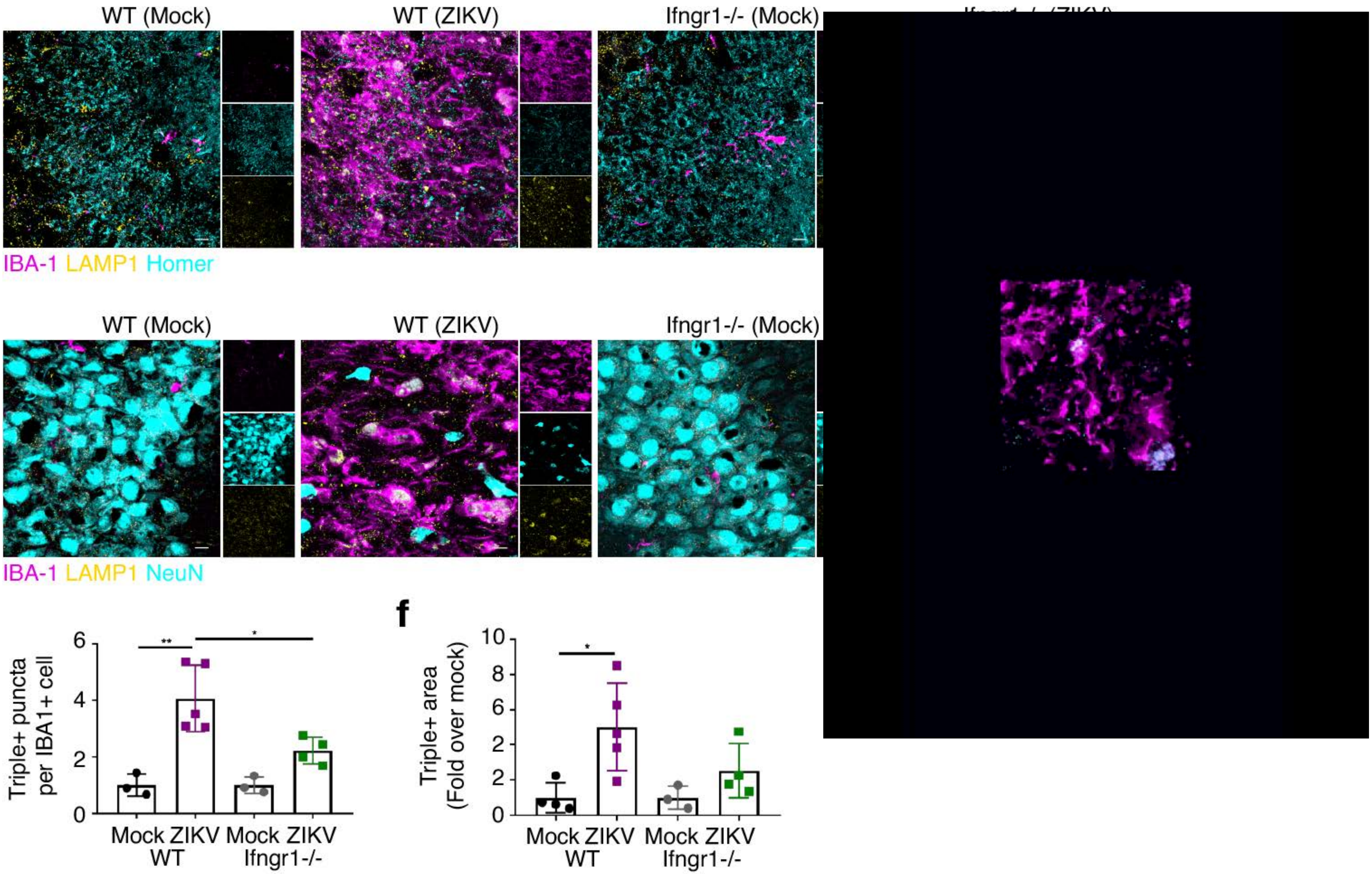
Garber and Soung et al., Nat. Neurosci., 2019

IFNγ induces microglial activation in WNV and ZIKV-recovered animals – 52 dpi

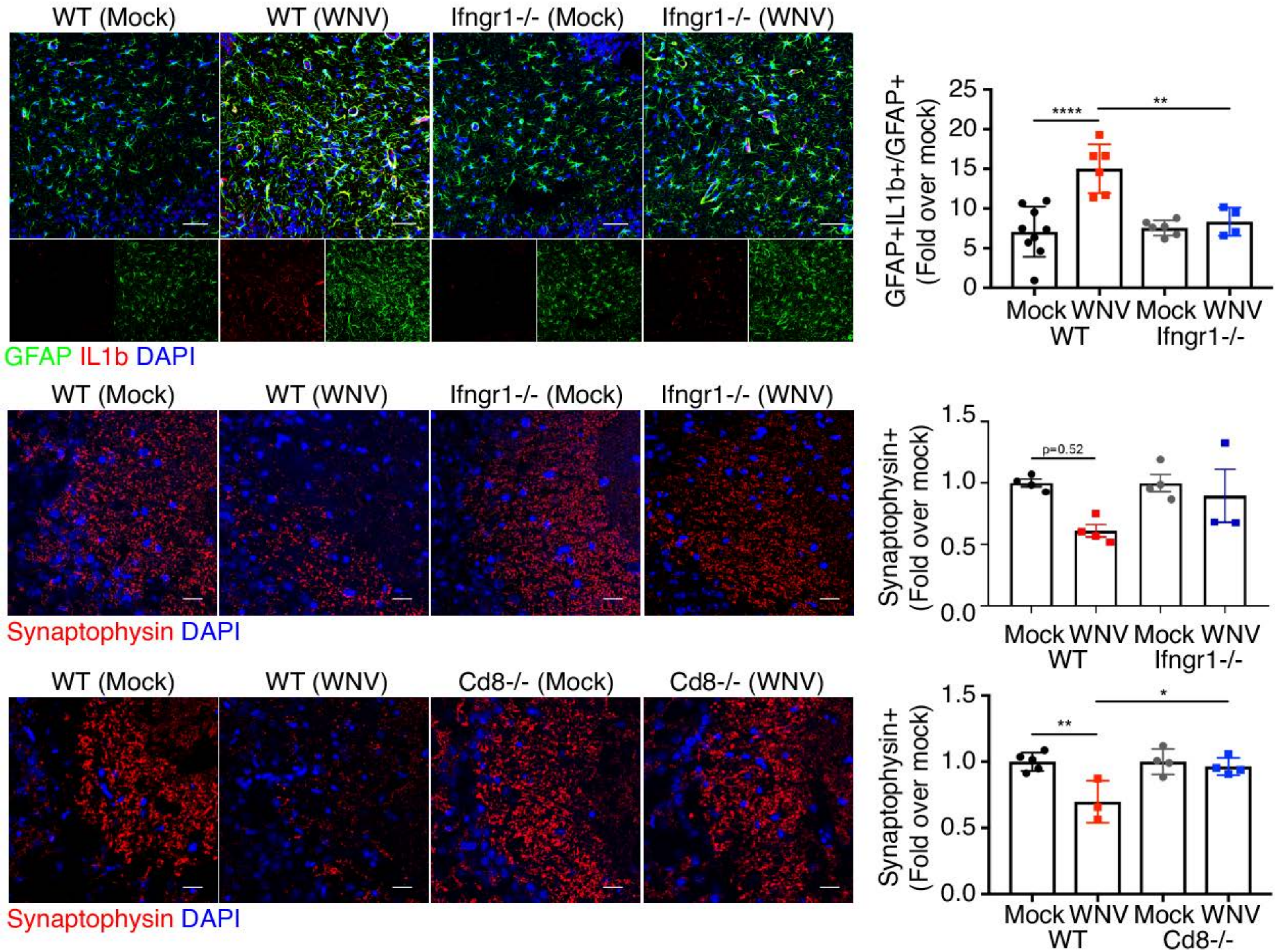


Garber and Soung et al., Nat. Neurosci., 2019

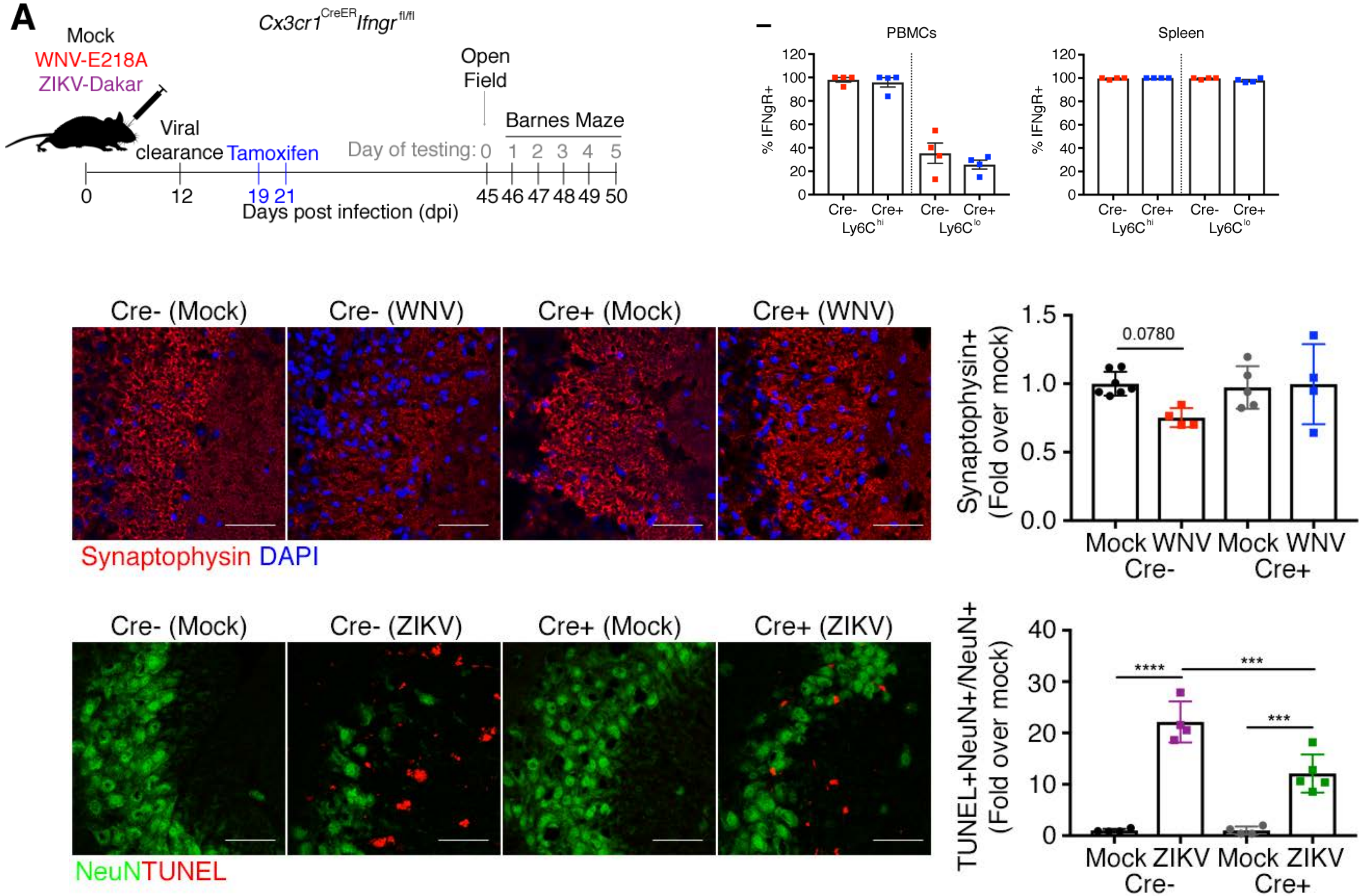
Microglial-mediated loss of post-synaptic termini in ZIKV-recovered animals



IFNγ and CD8: synapse elimination via upregulation of astrocyte IL-1 during WNV recovery

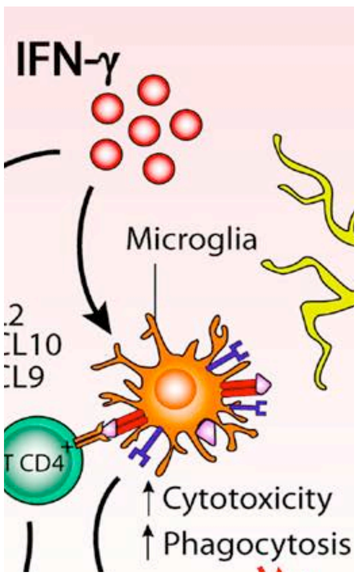


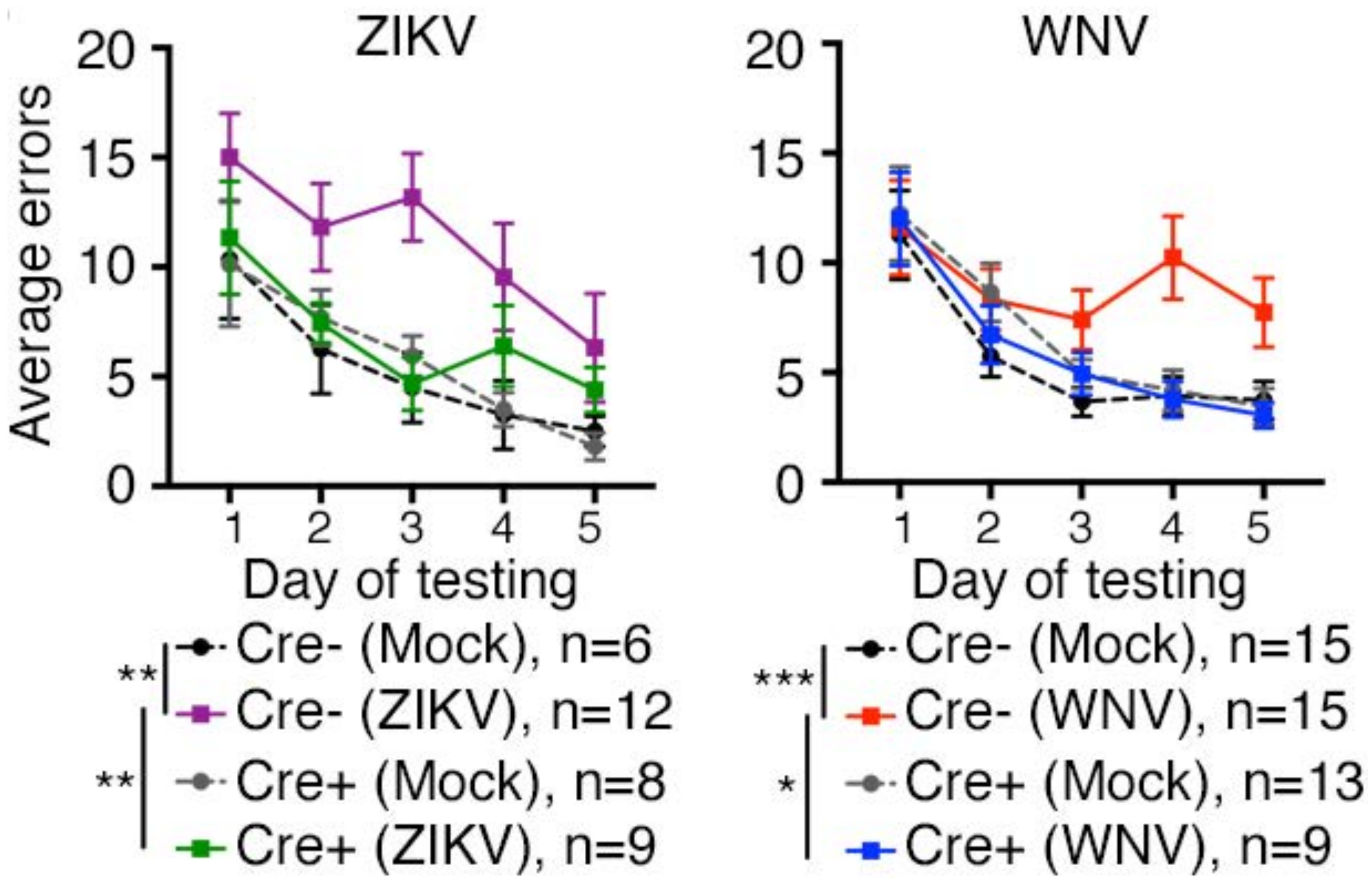
Loss of IFNγ signaling in microglia during recovery prevents synapse elimination and neuronal loss



Garber and Soung et al., Nat. Neurosci., 2019

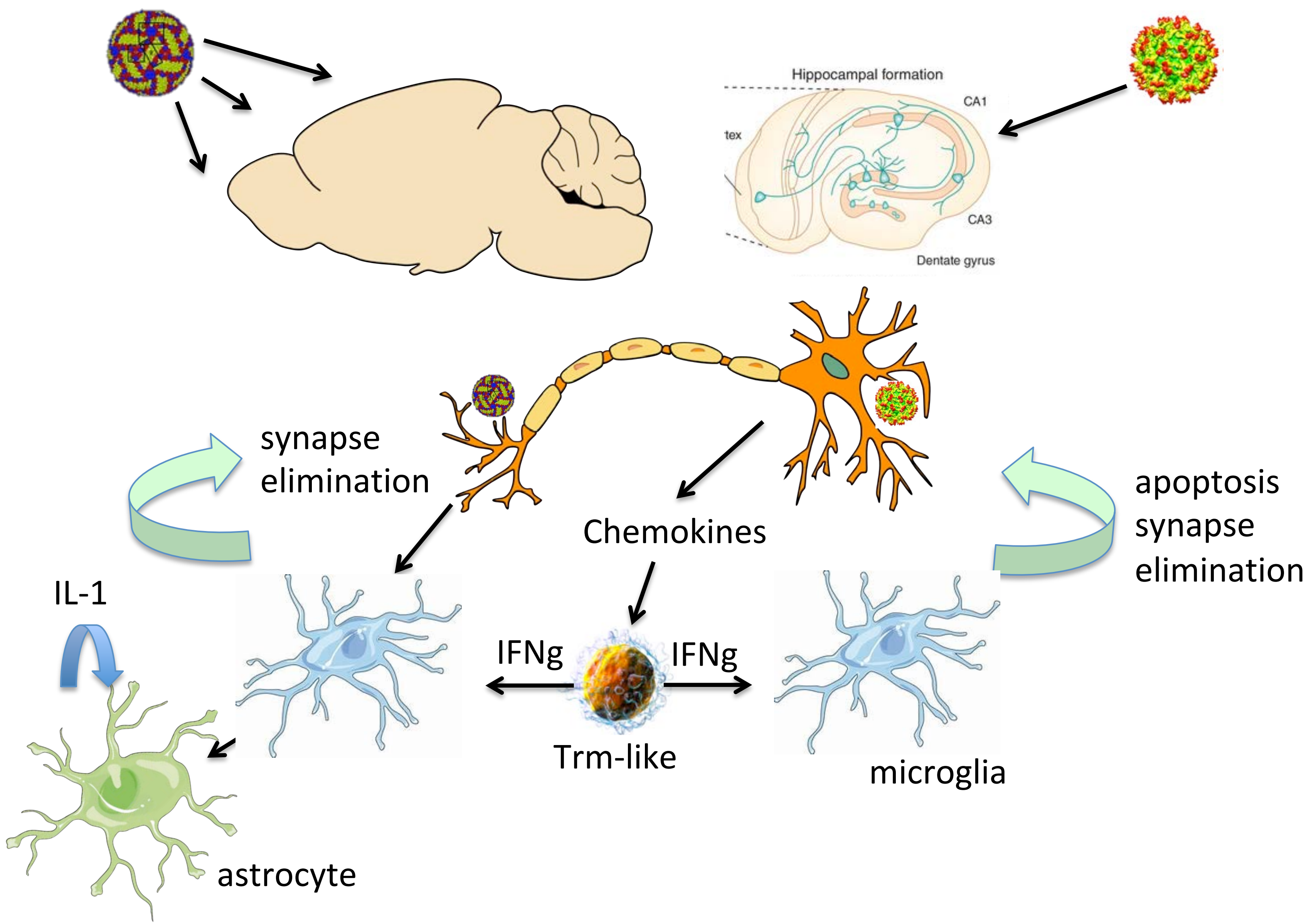
Loss of IFNγ signaling in microglia during recovery protects animals from spatial learning deficits





Garber and Soung et al., Nat. Neurosci., 2019

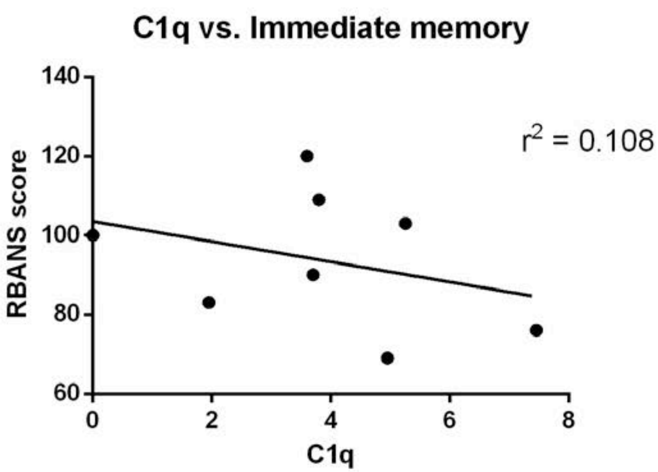
WNV and ZIKV differentially induce cognitive dysfunction

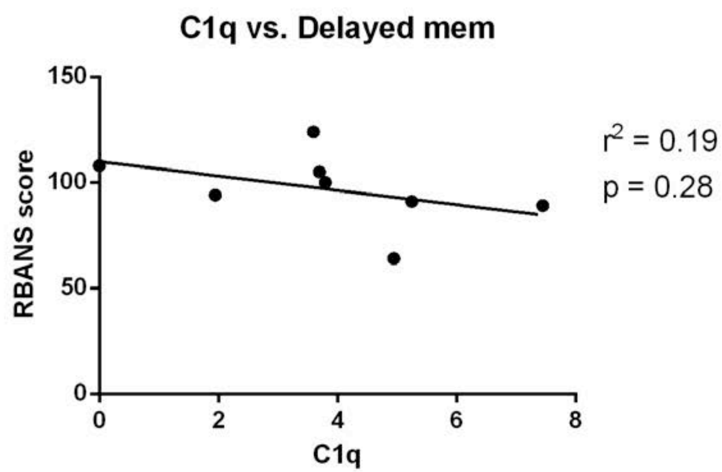


Translation to Humans 1

CSF Biomarkers – important to determine risk/benefit of perturbing cytokine systems

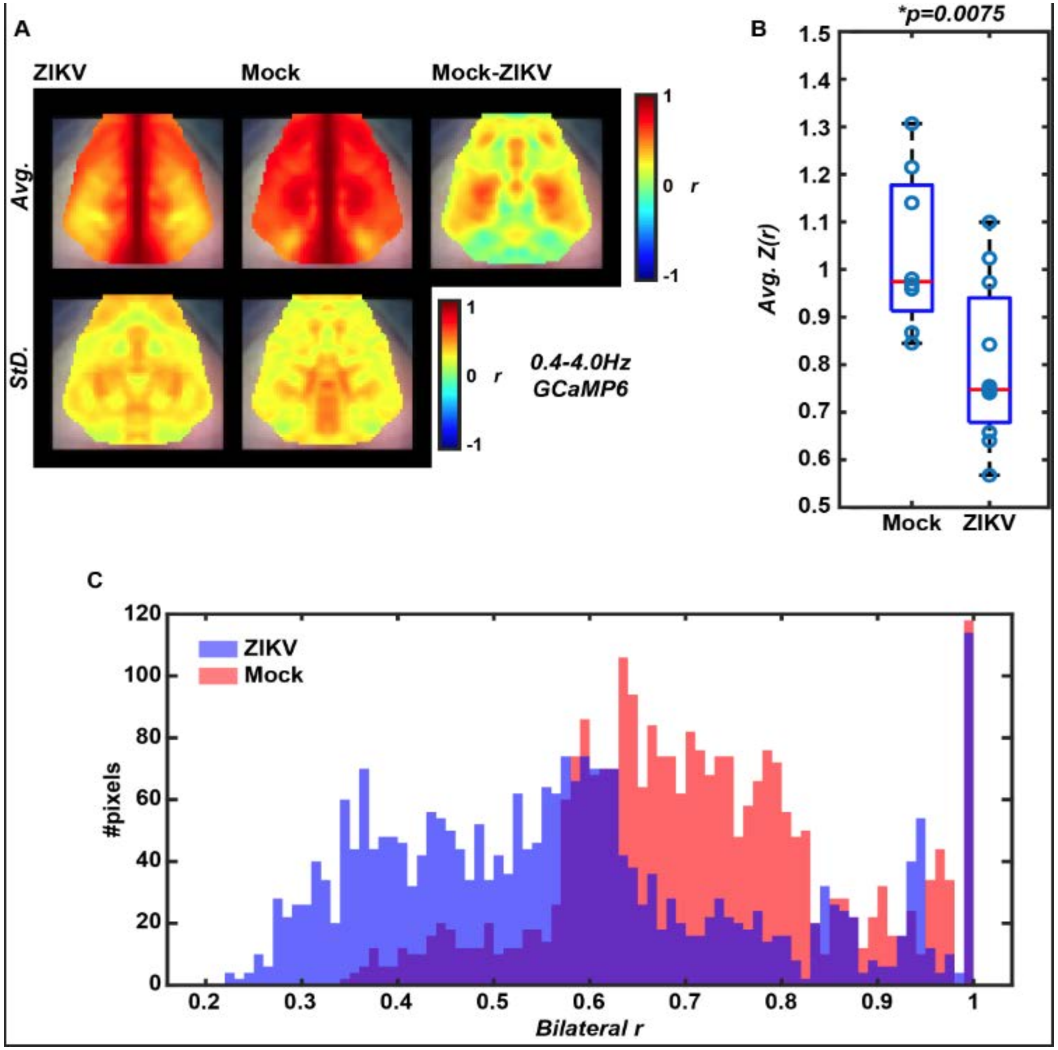
Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)





Rodrigo Hasbun, UT-Houston

Translation to Humans 2 – Functional Imaging Reveals Deficits in Cortical Connectivity



A. Deficits in bilateral functional connectivity in ZIKV- compared to mock-infected mice, particularly in parietal/ somatosensory cortical regions

B. Significant deficits in bilateral FC strength appeared to spatially vary in ZIKV infected mice

C. The distribution of each individual pixel's bilateral FC strength demonstrates separation of the two populations sampled.

Lindsey Brier, Culver Lab

Summary

- **Development of new mouse models of memory dysfunction after recovery from acute Flavivirus encephalitis**

- **Most severe spatial learning defects are associated with alterations in expression of genes that promote microglial activation, synapse loss, and lack of repair.**

- **Identification of new therapeutic targets**
  - **Demonstration of *in vivo* effect of gene deletion**
  - **Demonstration of *in vivo* effect of repurposed drug**

Path to Translation

- **Utility of mouse model of WNV-induced memory disorders:**
  - **Study spatial and other learning defects**
  - **Test novel therapeutics**
  - **Identify new mechanisms of disease**
  - **Examine links to other dementing illnesses**
  - **Identify biomarkers (CSF, Neuroimaging)**

Unanswered Questions

- How do viruses differentially impact on synapse elimination?

- Why are synapses eliminated? Does this prevent spread of viruses? Are new synapses functional?

- What maintains T cell presence within the parenchyma?
- Which T cells are associated with cognitive deficits?

- Will targeting cytokines prevent viral clearance?

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Douglas Durrant, PhD
Charise Garber, PhD
Jesscia Williams, PhD
Mike Vasek, PhD

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National Institute of Allergy and Infectious Diseases

National Institute of Neurological Disorders and Stroke

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