Zika Virus Animal Models
Suitability for Vaccine Testing

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Desired Properties of Zika Animal Model

- Infection results in measurable pathogenesis
- For vaccine testing, pathogenesis should be apparent in older animals due to amount of time needed to prime, boost, and challenge
- Animal is capable of mounting immune response to vaccine antigen
- Pathogenesis and immune responses resemble what is seen in humans

Zika Animal Models

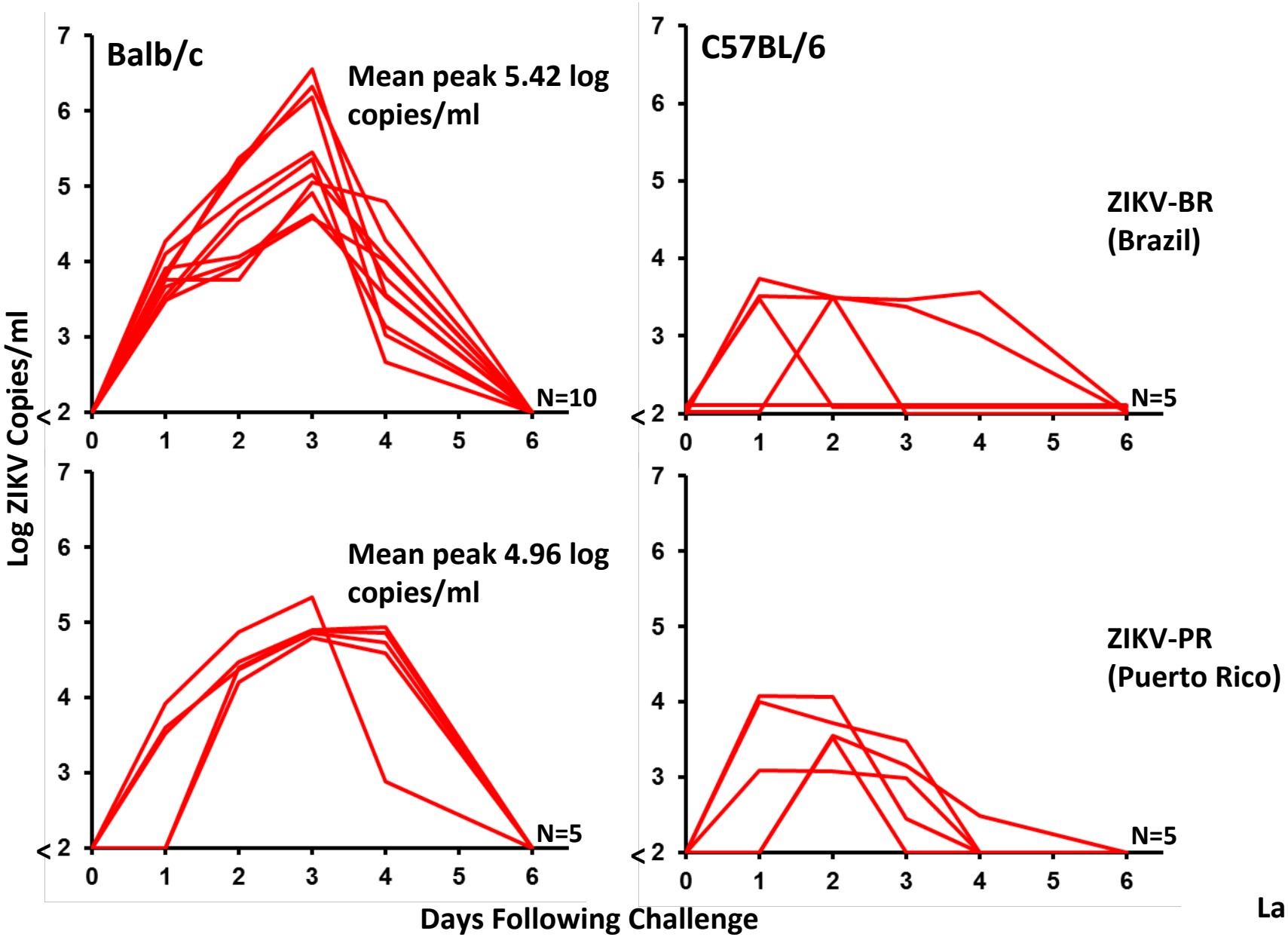
- Mouse
  - Wild type (immune competent) strains
  - Strains lacking components of innate immunity
- Hamster (Morrey, Julander, Utah State Univ.)
  - STAT2 KO
- Non-human Primate (David O'Connor, U. of Wisconsin, Madison)
  - Rhesus Macaques

Mouse Models

- Immune competent strains
  - CD1, C57Bl6 do not exhibit Zika virus induced pathogenesis (unless infected at 1 week of age)
    - Do not show fetal abnormalities when infected during pregnancy

      (Rossi et al., 2016; Lazear et al., 2016; Cugola et al., 2016)
  - SJL (Cugola et al., 2016; Kurtz et al., 1995), Brazilian isolate of Zika highly pathogenic in SJL mice, virus infected placenta and fetus. Need evaluation in older animals
  - Unpublished data from D. Barouch: viremia observed in adult Balb/c mice

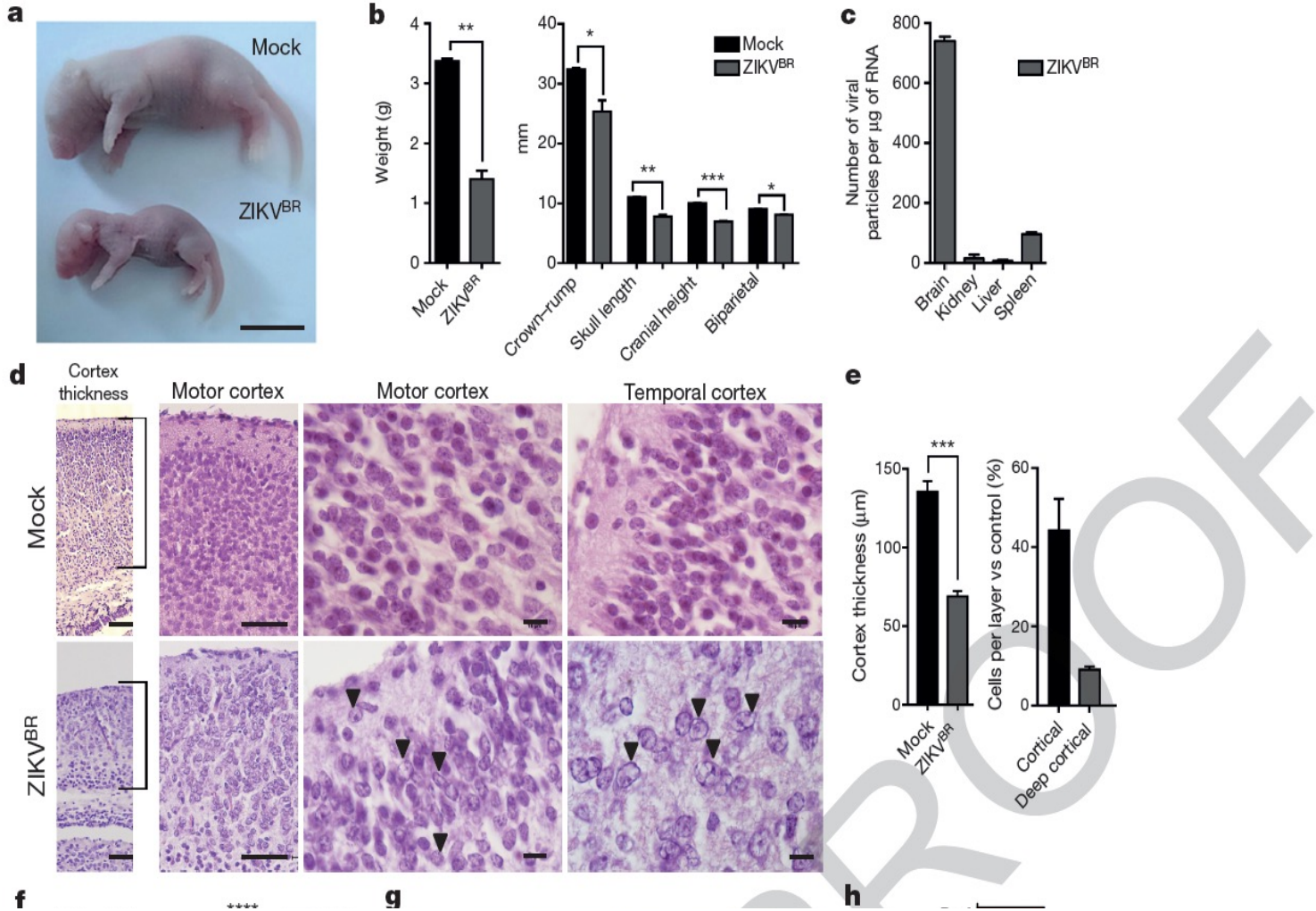
Robust ZIKV Replication in Wildtype Balb/c Mice (Brazil Stock > Puerto Rico Stock)



Larocca et al., 2016

Zika infection of pregnant, immune competent SJL mice results in IUGR and cortical malformations in the brain

RESEARCH LETTER



Cugola et al., 2016

Mouse Models

Immunocompromised mice lacking components of innate immunity

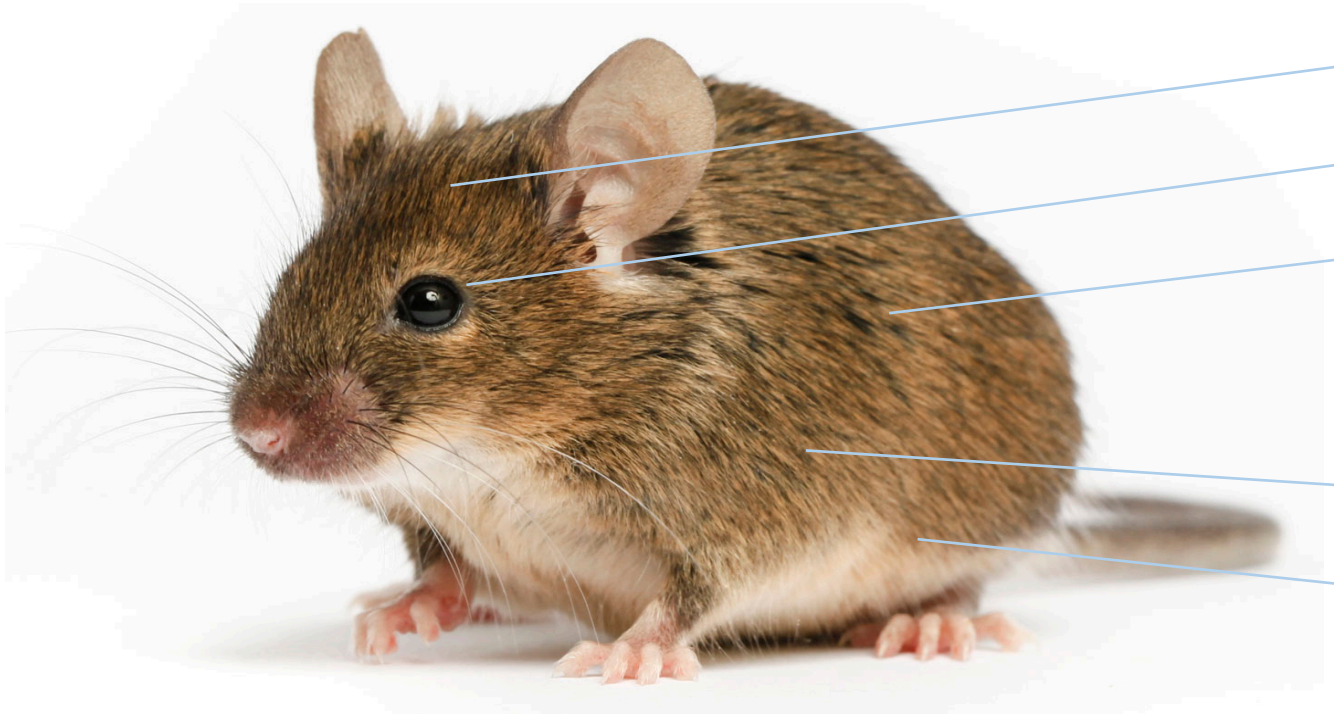
- A129 (Rossi et al., 2016; Lazear et al., 2016)
    - Lack receptor for type 1 IFN (IFNα/β), virus titers in brain, spinal cord, and testes, mice up to 6 months old susceptible, need evaluation in pregnancy model
- Type I IFN-blocking monoclonal antibody (Lazear et al., 2016): in adult mice viral replication is seen, but no clinical signs are observed. In pregnant mice, infection of fetus occurred, but was not severe. No fetal demise
- $Irf3^{-/-}$ $Irf5^{-/-}$ $Irf7^{-/-}$ triple knock out, produce little type I IFN (Lazear et al., 2016), more susceptible to Zika than A129 mice, needs further evaluation esp. in pregnancy model and in older animals

Mouse Models

Immunocompromised strains lacking components of innate immunity

- AG129 (Aliota et al. 2016; Rossi et al., 2016; Zmurko, et al.; 2016; Julander et al., unpublished data; review by Sarathy et al. 2015 (Dengue))
  - Lack receptors for types I and II IFN (IFNα/β and IFNγ)
  - Significant pathogenesis and clinical signs evident in both young and older (2 month old) mice (Aliota et al., 2016)
  - Evaluation of Zika infection needed in pregnant dams and in older (≥3 month pregnant dams) to account for time needed to prime, boost and challenge
- A129 females crossed with wild type males produce heterozygote fetuses with largely intact Type I IFN response (Miner et al., 2016)
  - Zika infects pregnant dams and placenta and results in damage to placental barrier and infection of fetus, severe cases lead to fetal demise

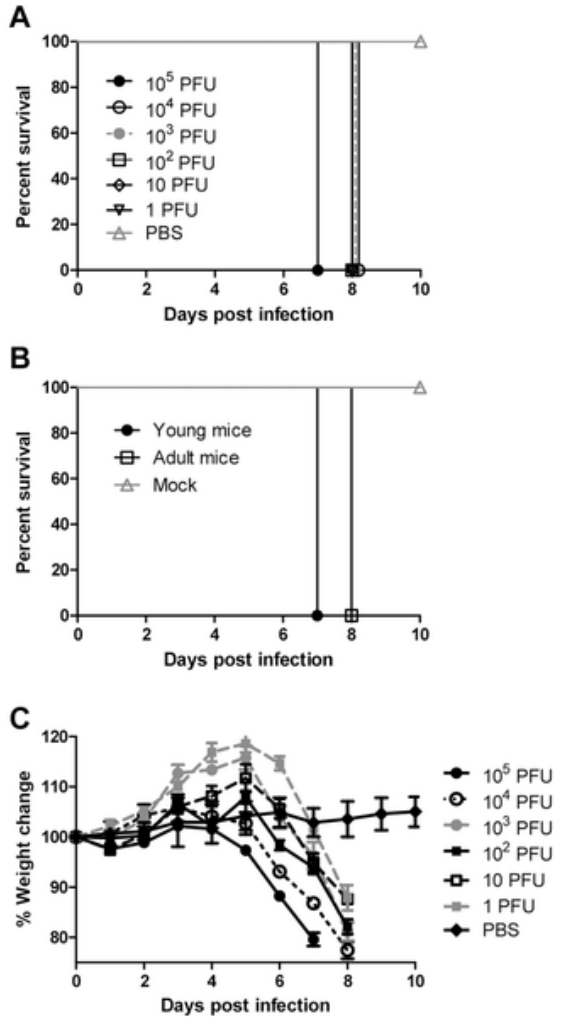
ZIKV AG129 Mouse Model



- **Neurologic disease**
- **Conjunctivitis**
- **Hunching, lethargy, and excitability @ late stage**
- **Measurable viremia**
- **Hindlimb paralysis**

Appropriate for antiviral and vaccine studies except evaluation of interferon pathway agents

Fig 1. ZIKV causes mortality and morbidity in young and adult AG129 mice



Aliota MT, Caine EA, Walker EC, Larkin KE, Camacho E, et al. (2016) Characterization of Lethal Zika Virus Infection in AG129 Mice. PLoS Negl Trop Dis 10(4): e0004682. doi:10.1371/journal.pntd.0004682
http://journals.plos.org/plosntds/article?id=info:doi/10.1371/journal.pntd.0004682

Fig 2. Young and adult mice have high serum viremia early during infection.

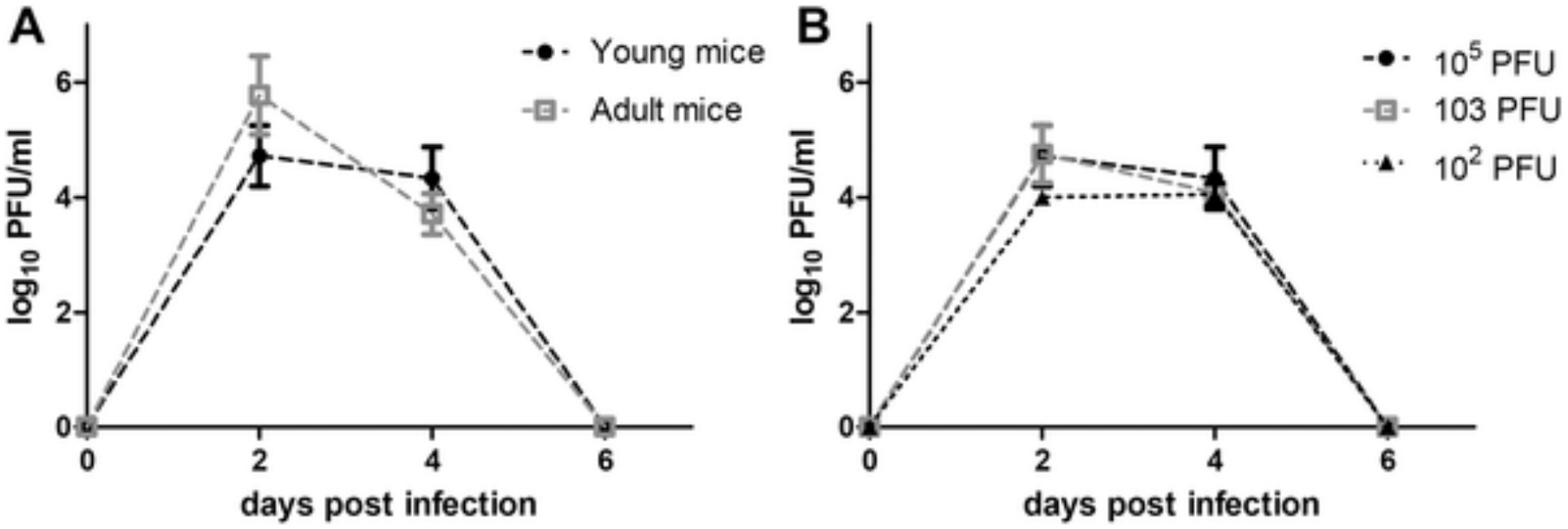


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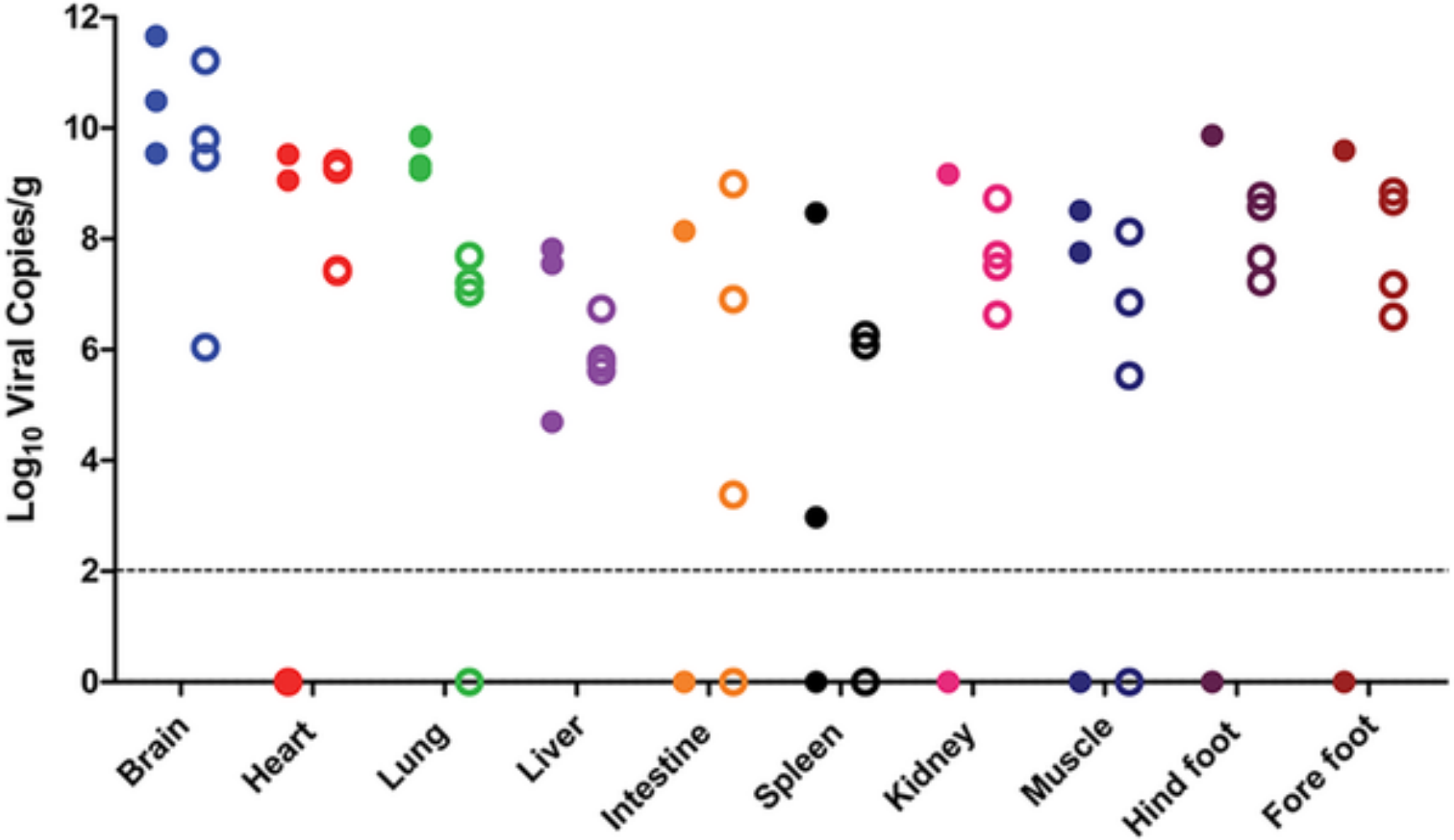
Fig 3. Young and adult mice have high tissue viral loads 7 days post infection.



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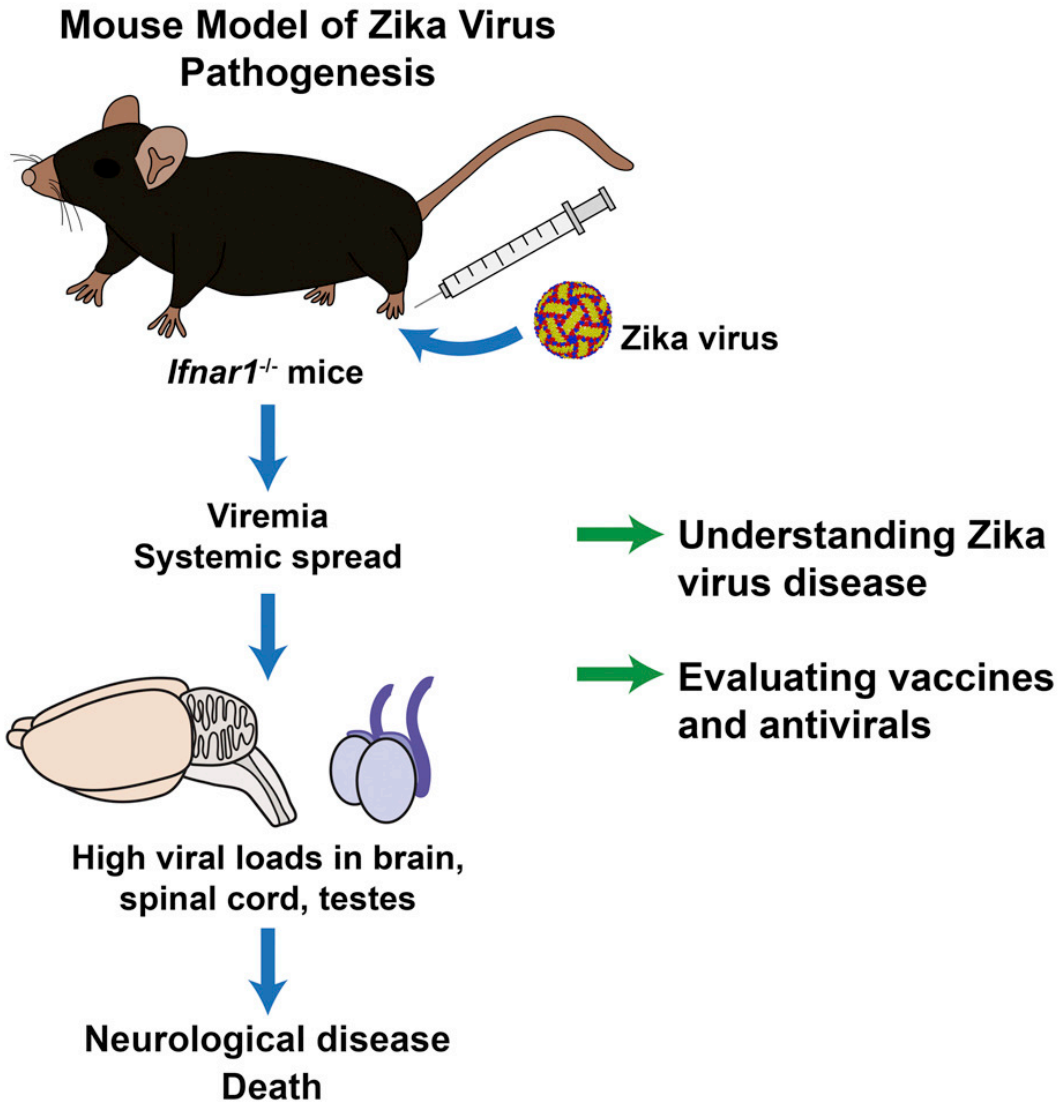
Zika/AG129 Mouse Model

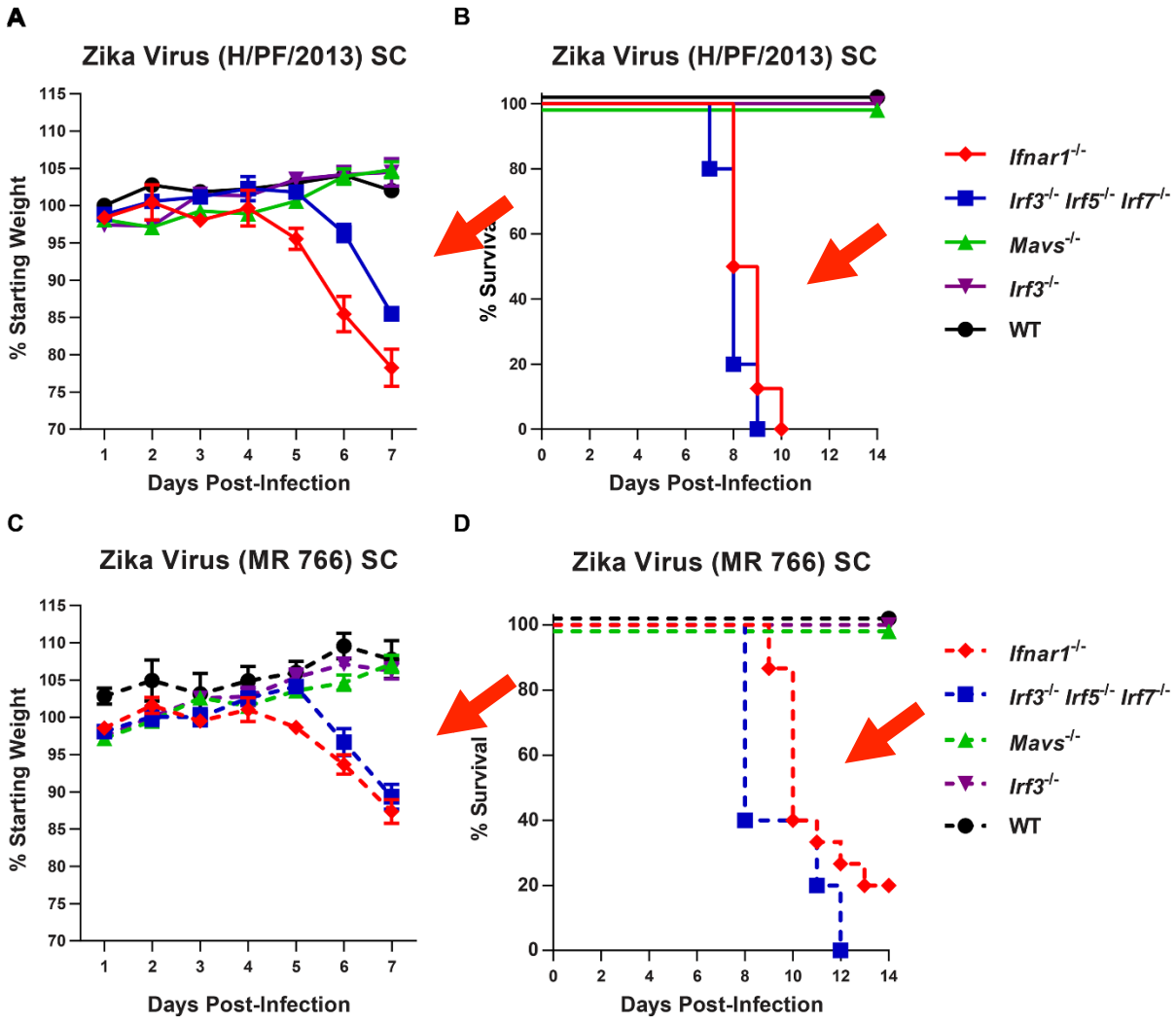
- Pathogenesis more severe than that experienced in humans
- Virus infects fetus resulting in fetal abnormalities
- No significant differences in pathogenesis between young (4 week) and adult (8 week) animals
- Mice that survive exposure to virus are completely protected from re-challenge
- Caveat: AG129 mice lack receptors for Type 1 and Type II IFN therefore not appropriate for testing vaccines that rely on intact IFN pathways

A129 (IFNAR -/-) Mice Develop Neurological Disease and Succumb to Infection



Lazear Cell Host Microbe 2016

Zika virus can cause lethal disease in adult mice lacking type I Interferon immunity (but not in WT mice)



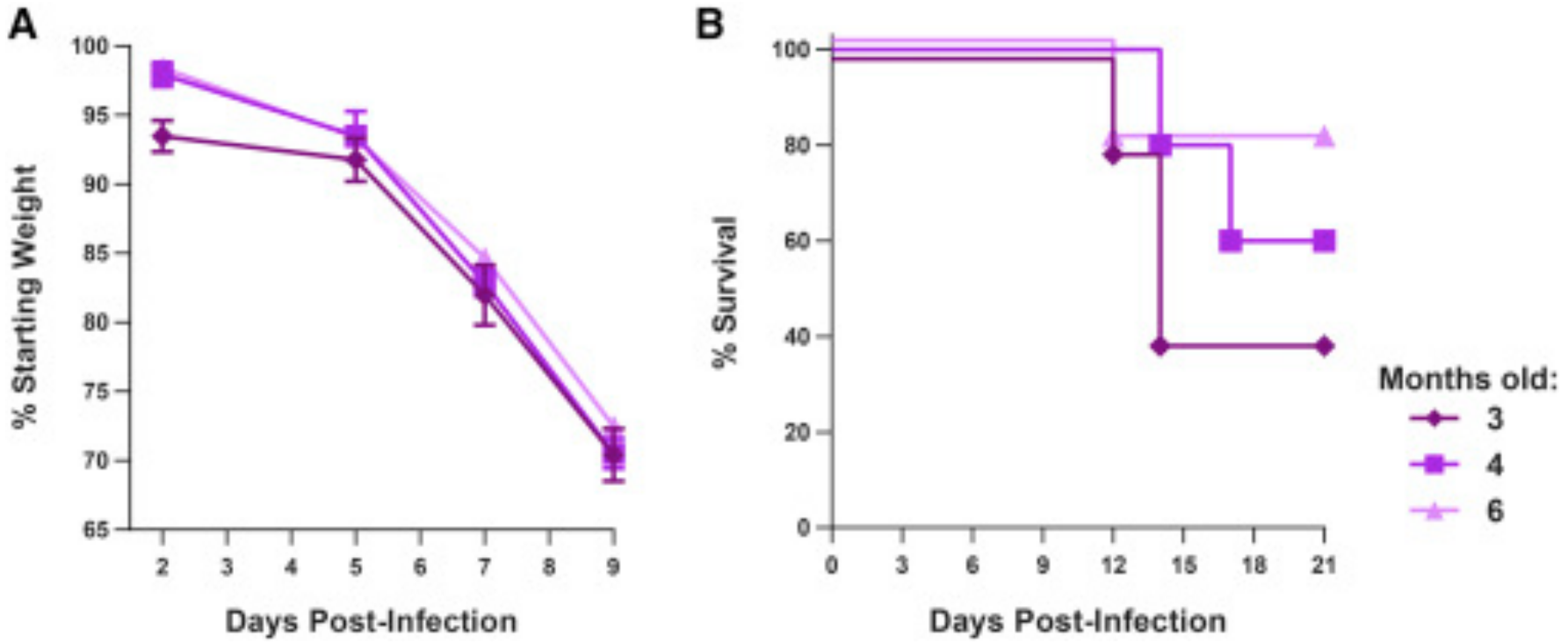
Ifnar1$^{-/-}$ and Irf3$^{-/-}$ Irf5$^{-/-}$ Irf7$^{-/-}$ C57BL/6 mice

Strains: MR 766 (Uganda 1947)
H/PF/2013 (French Polynesia 2013)

Causes paralysis and encephalitis

Lazear Cell Host Microbe 2016

Older A129 (IFNAR1 -/-) Mice Remain Susceptible to Infection



Lazear Cell Host Microbe 2016

Creating a model of *in utero* transmission of Zika virus infection

A129
IFNAR KO

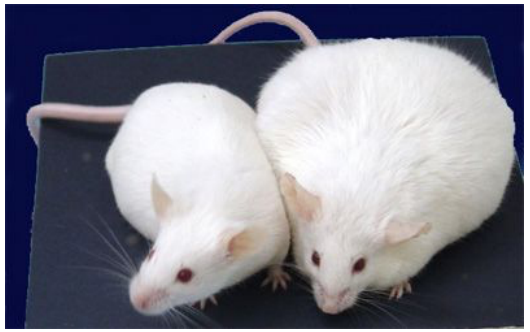
♀

X

WT

♂

Pregnant mice



Infect with Zika virus

Different days
E6, E7, E10

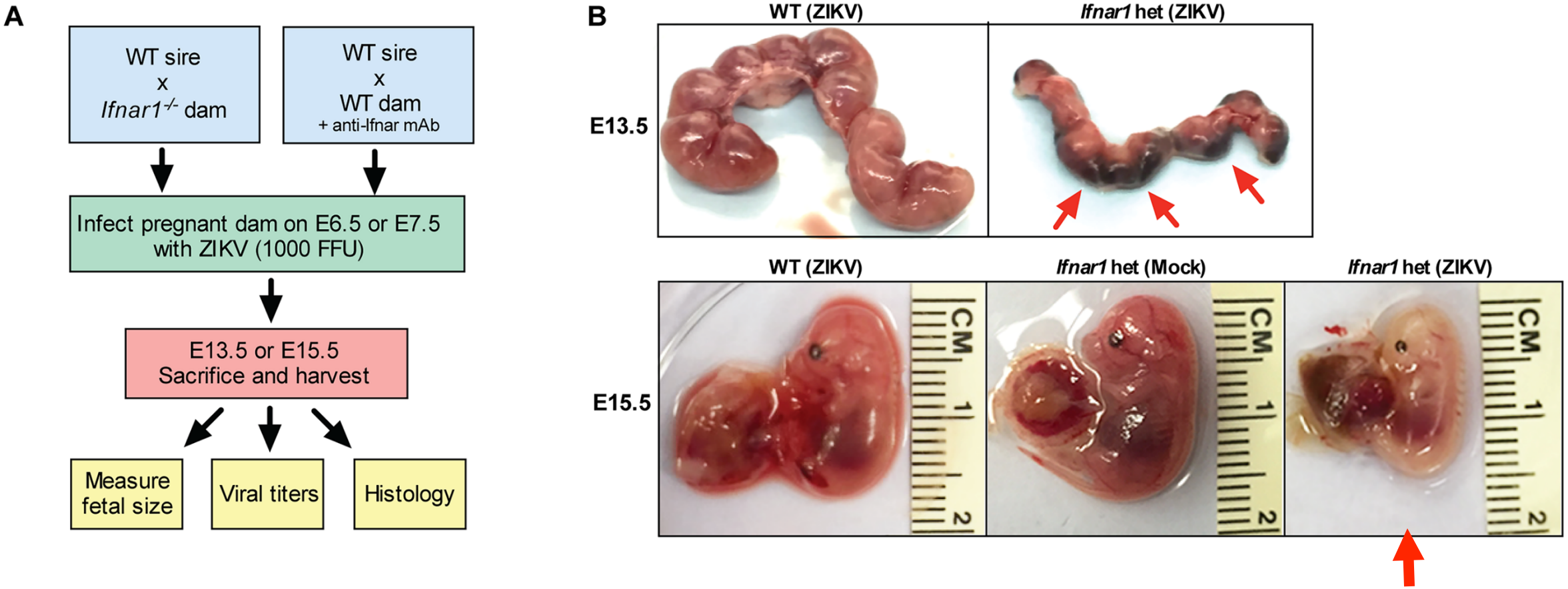
Analyze
newborn or fetal
IFNAR+/- mice

(young mice susceptible)

Readouts: survival, pathology, virology, immune infiltrates, mother/fetus/neonate

Miner Cell 2016

Zika virus infection of pregnant dams results in fetal resorption



Infect E7.5, Harvest E15.5

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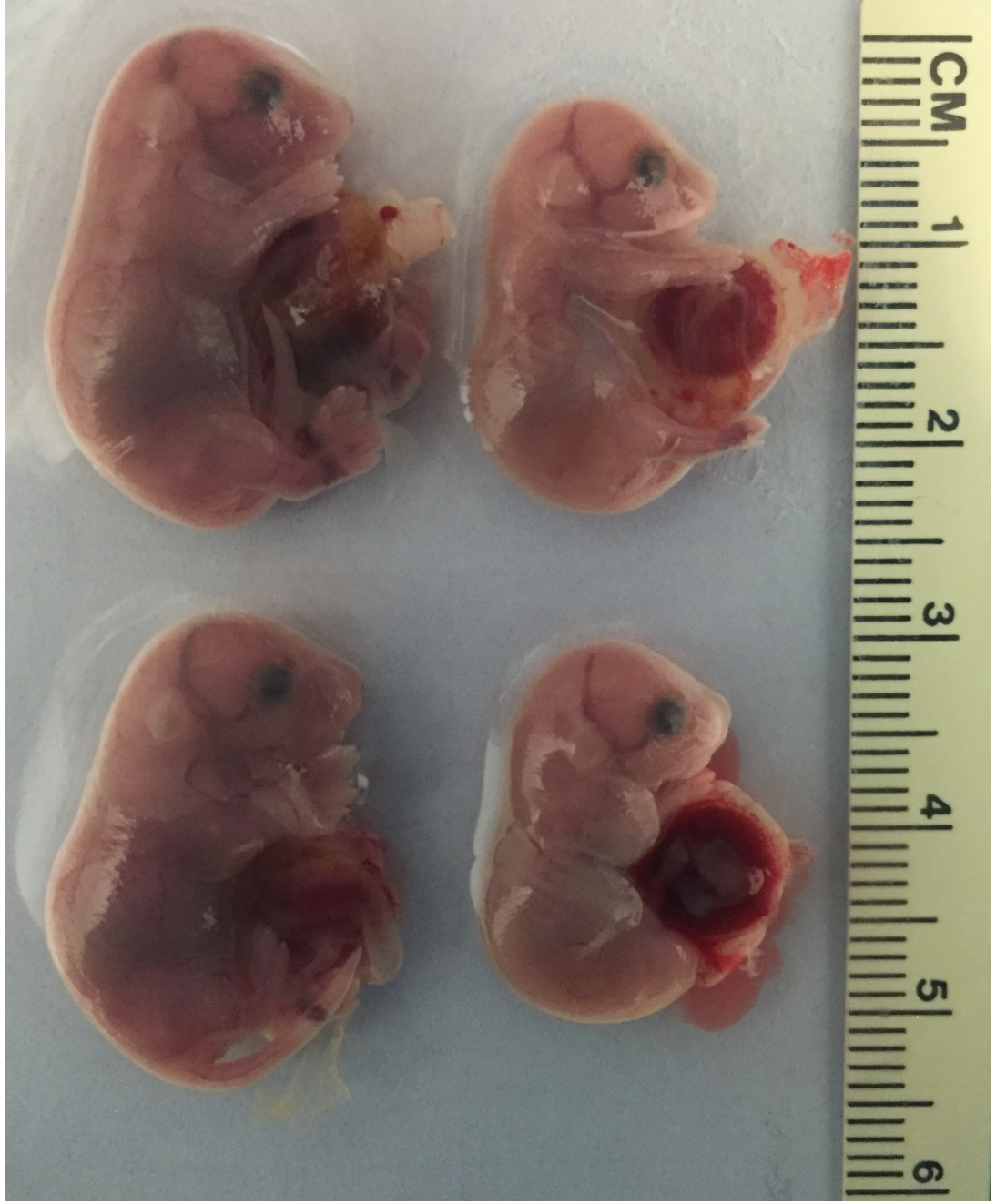

Ifnar1 het - MOCK Ifnar1 het - ZIKV

Infect E12, Harvest E18.5

Infect later in pregnancy

- **No fetal demise**
- **+++ Intrauterine growth retardation**

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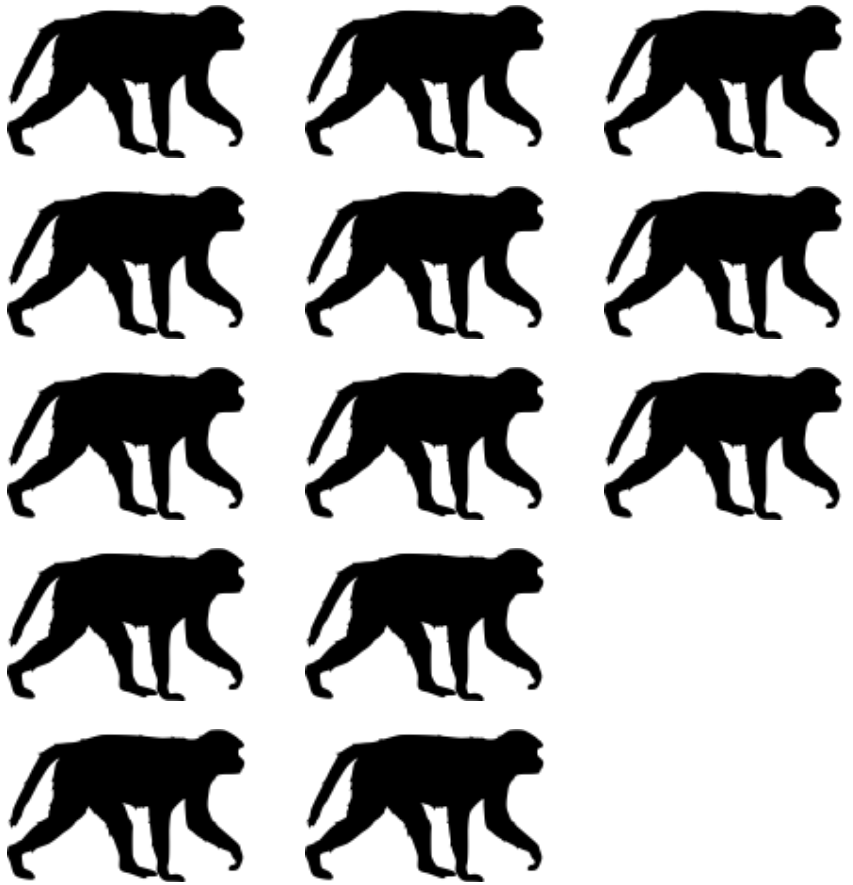
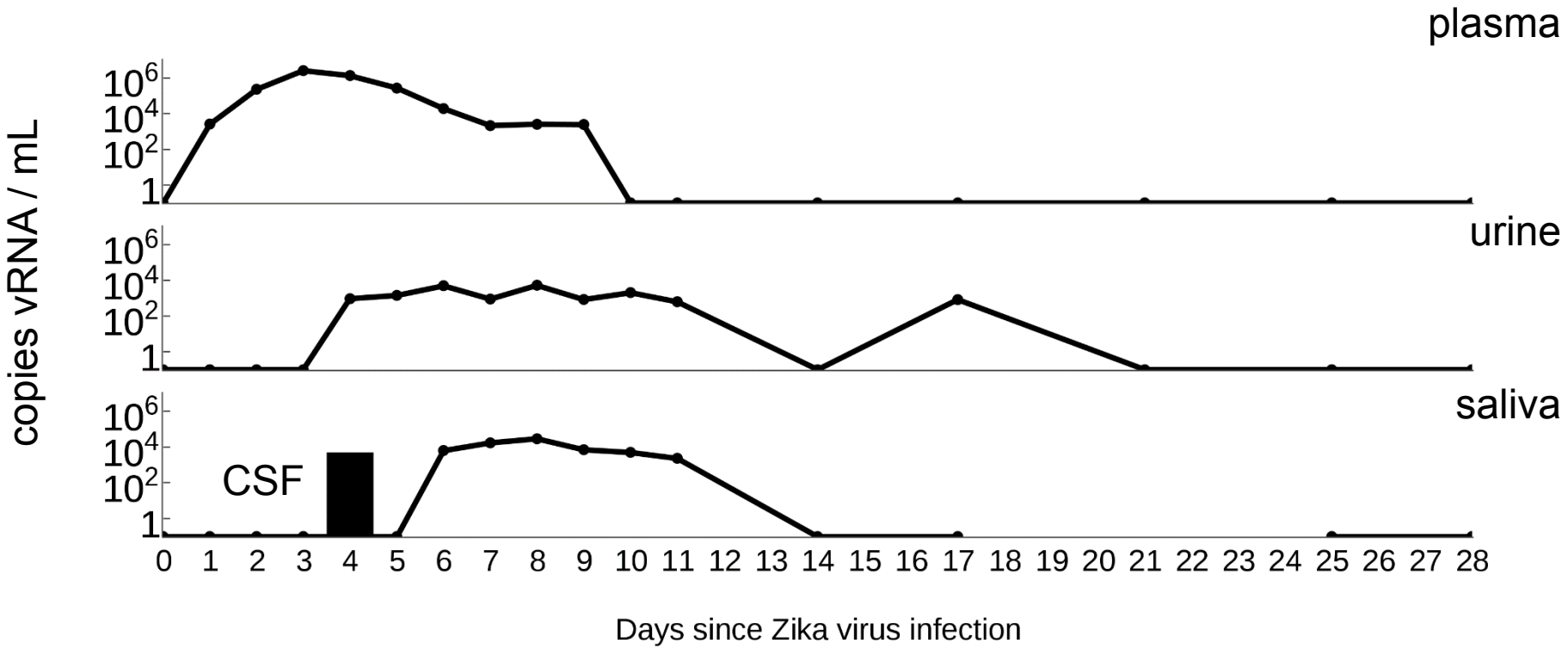
Rhesus macaques model of Zika
Dave O'Connor-U. Wisconsin

- Can macaques be infected with Zika virus?
  - via physiologic routes and with physiologic doses of virus?
  - with strains similar to those circulating in the Americas?
- Do macaques develop disease similar to humans?
  - rash, asymptomatic infection in non-pregnant macaques?
    - rare complications such as GBS hard to detect
  - fetal abnormalities in pregnant macaques?

All challenged animals had detectable virus in blood

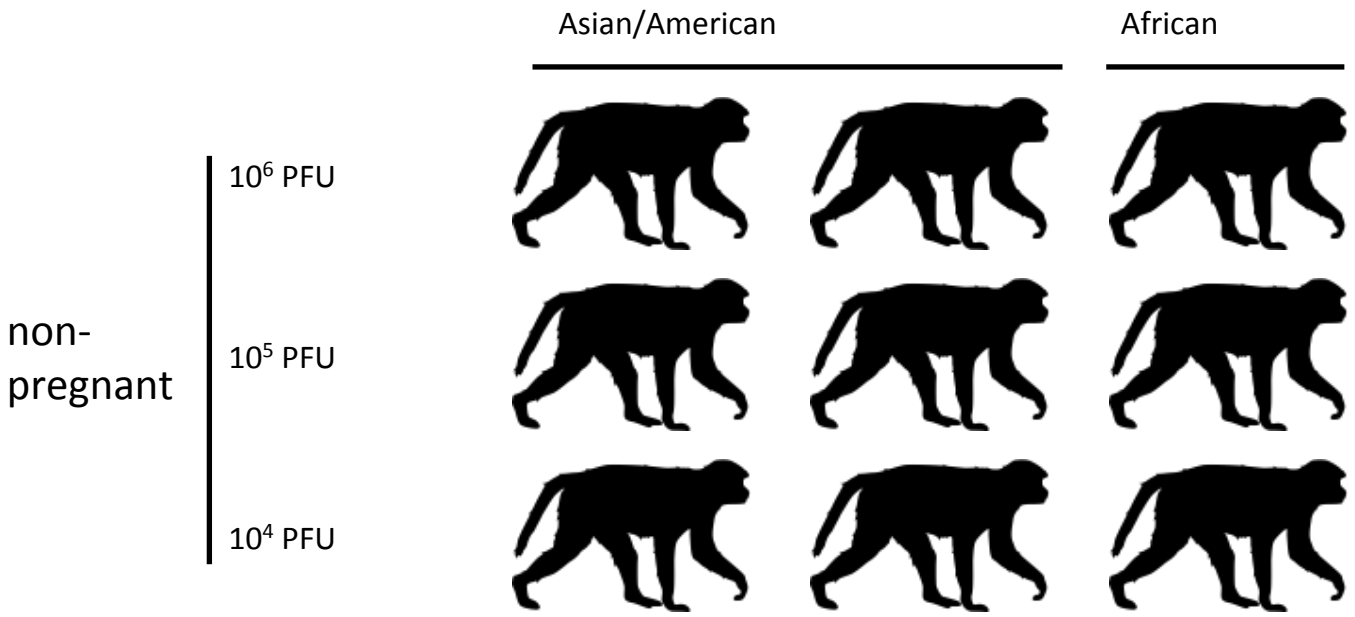


Zika virus is detectable in blood, saliva, urine, and CSF



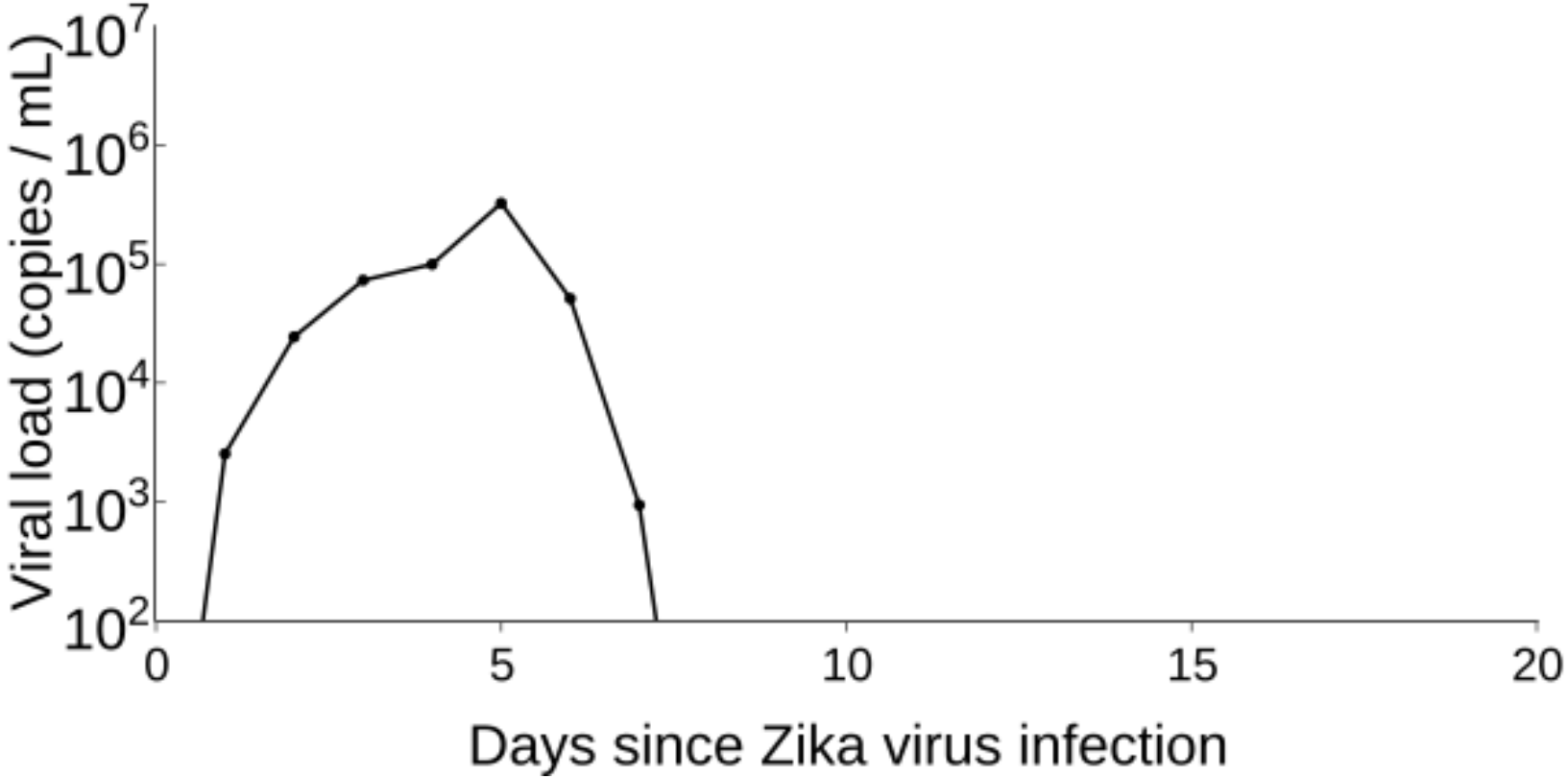
also detected low levels in vaginal swabs; semen not yet tested

Mild, asymptomatic infection in non-pregnant macaques



- animals appeared healthy throughout experiments
- mild rash observed at infection site in few WNPRC animals

Plasma viremia lasts approximately 10 days in non-pregnant macaques



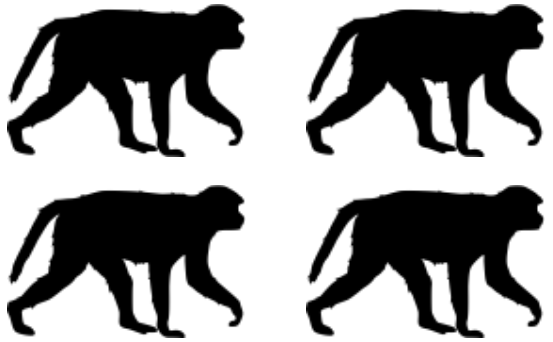
Infection of pregnant macaques

- mothers remained healthy throughout pregnancy
- placental calcification in one macaque at 42 dpi

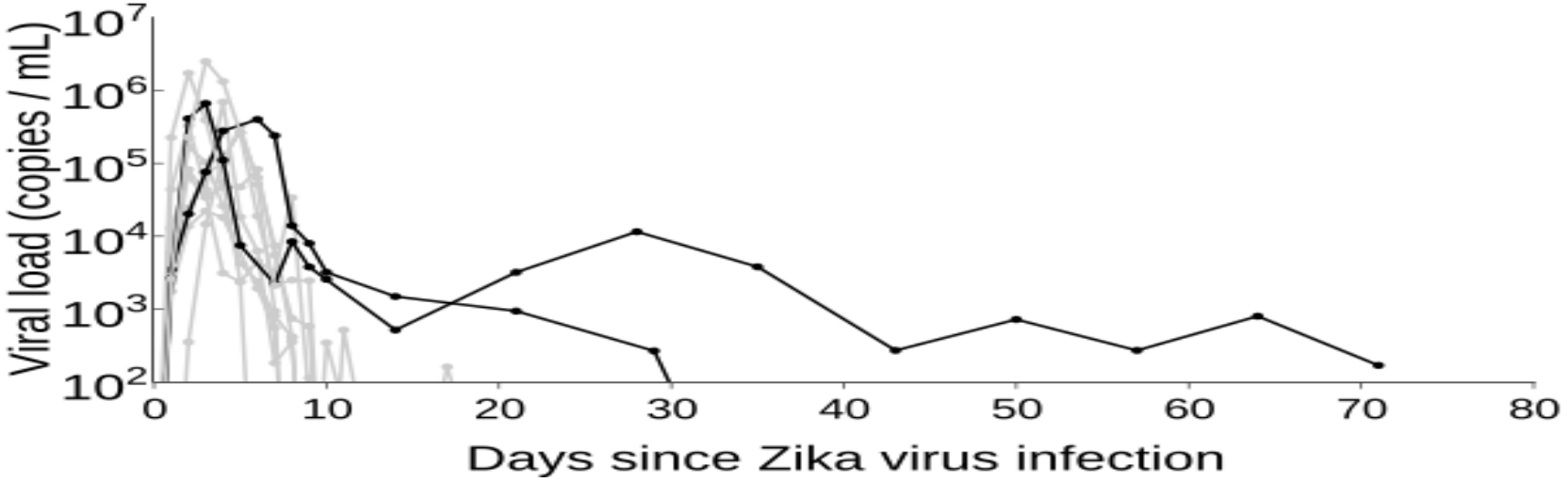
Asian

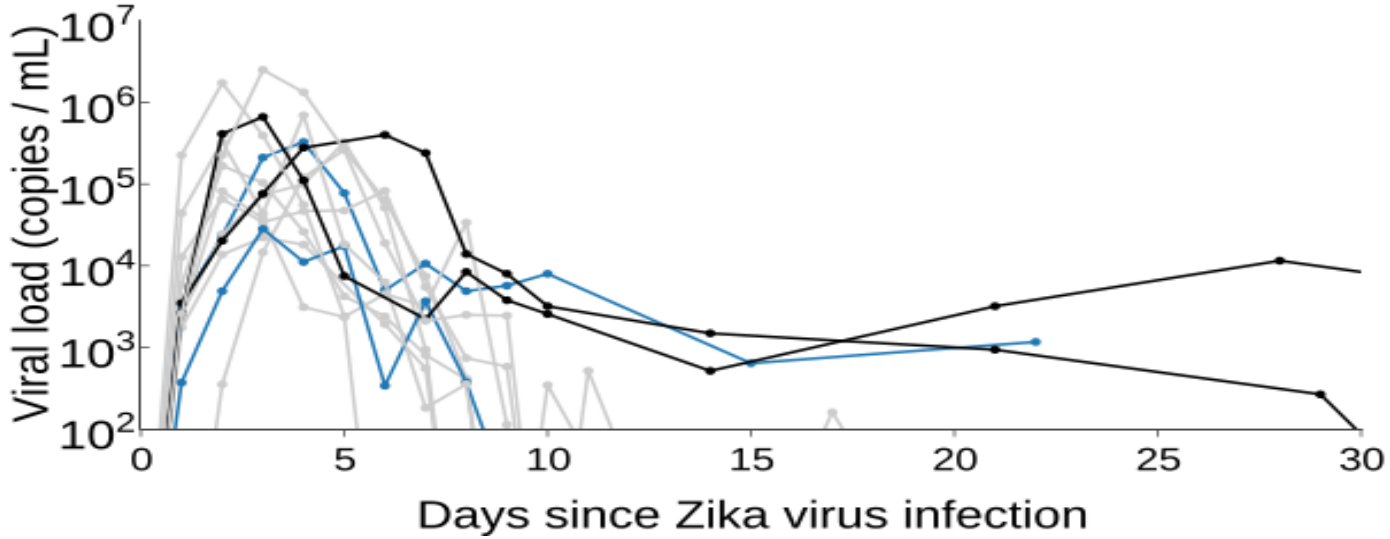
first trimester 10^4 PFU

third trimester 10^4 PFU

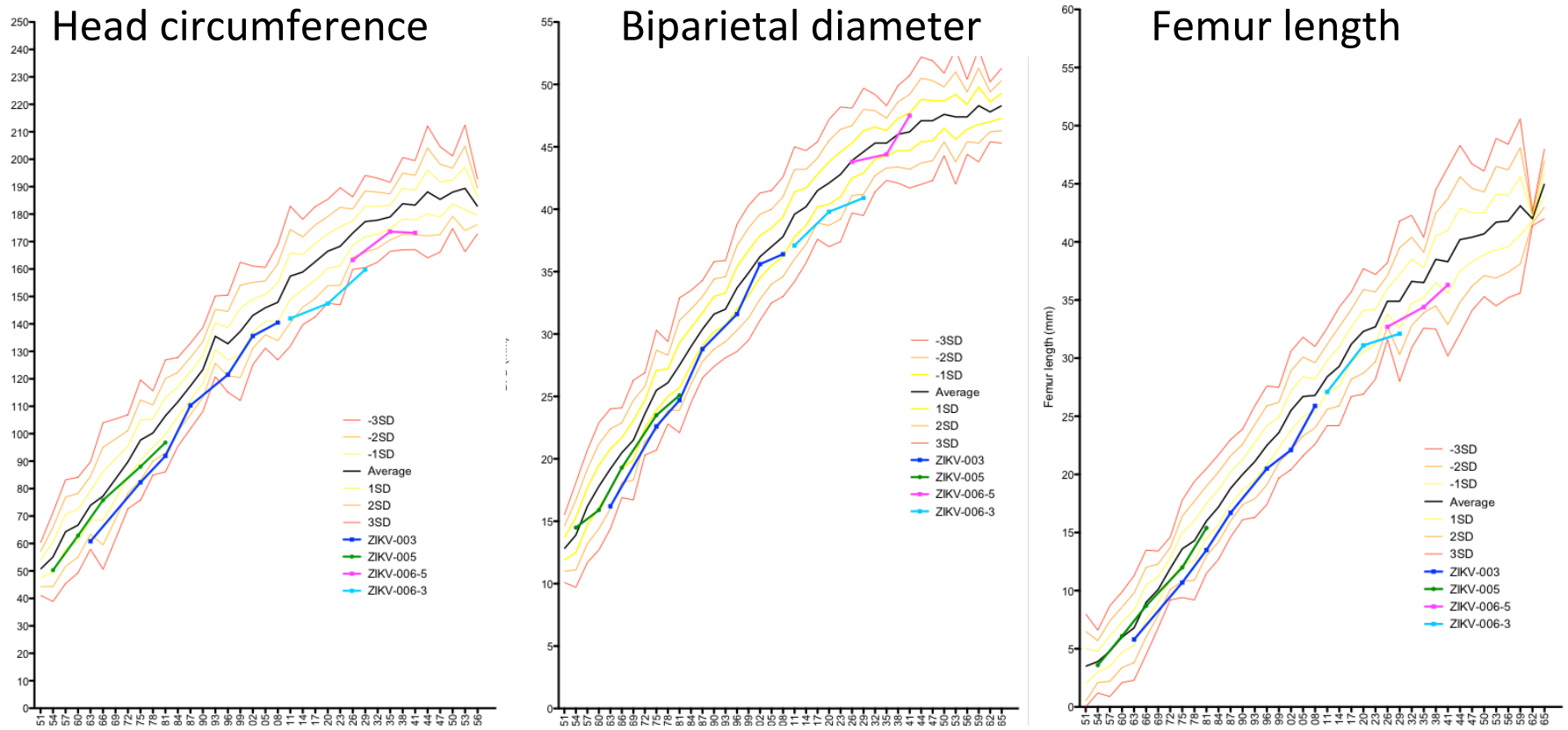


Plasma viremia is extended in pregnant macaques infected in the first trimester and variable when infected in third trimester

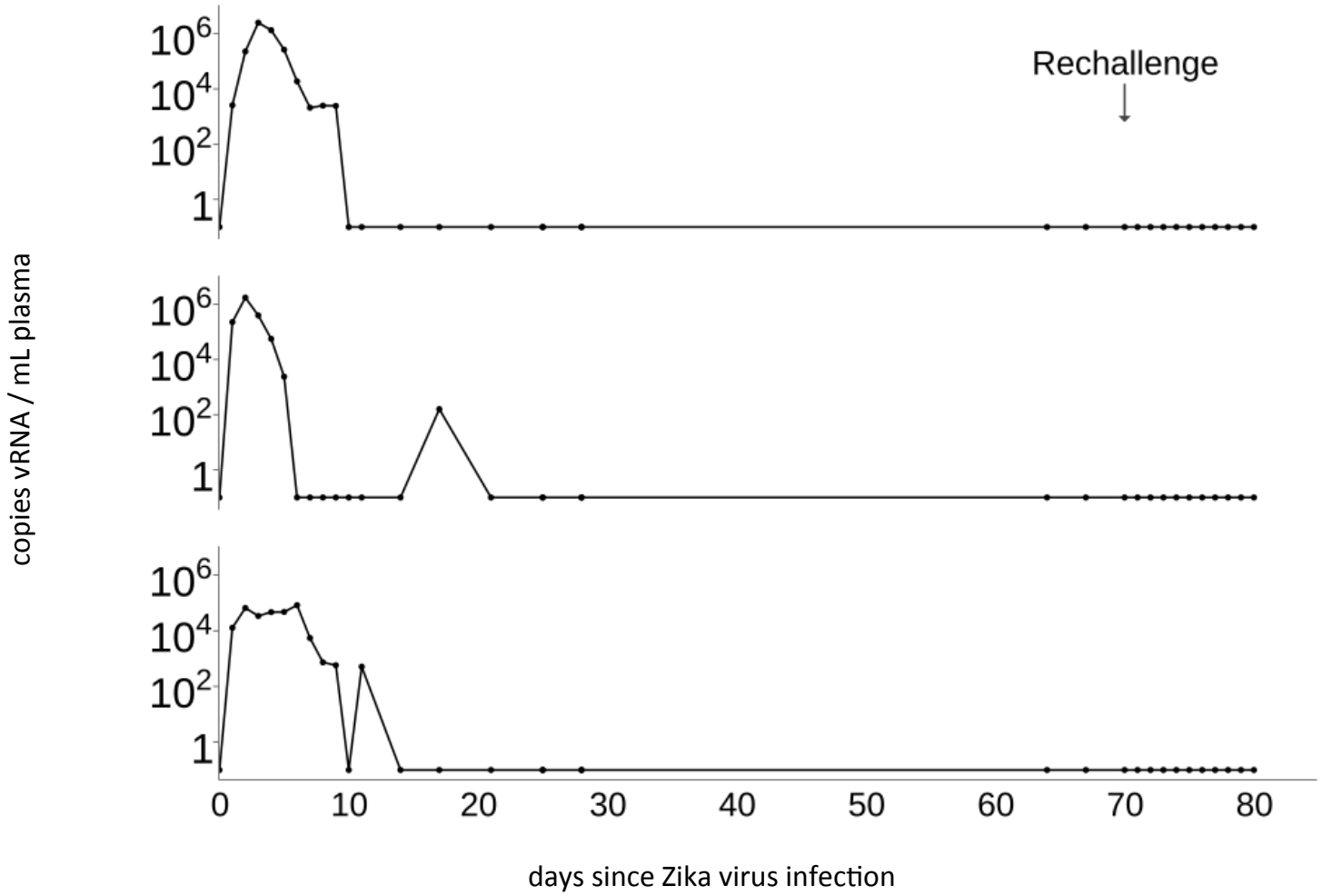




Fetuses from 1st trimester infections are smaller than average

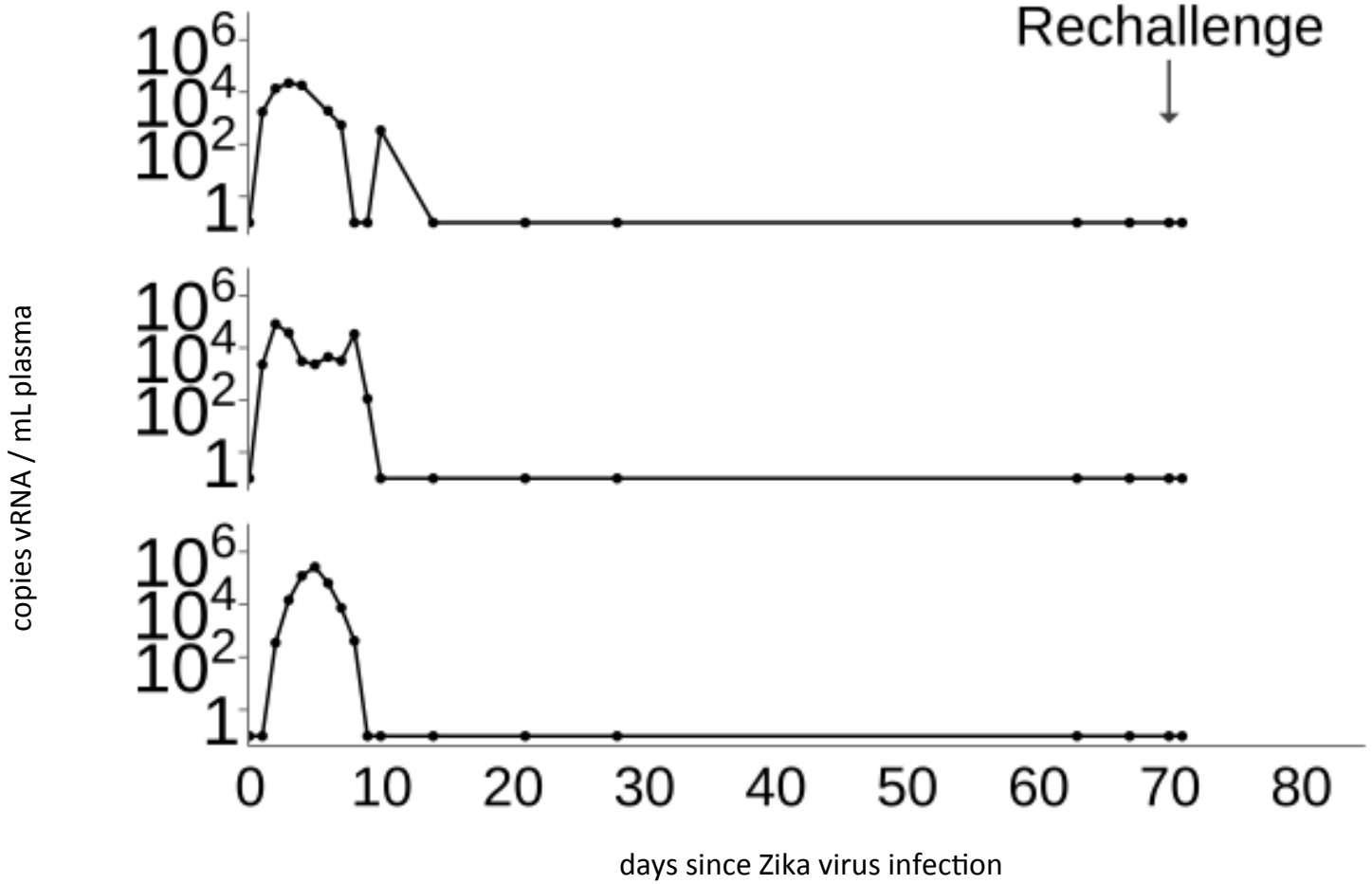


Primary immunity completely protects against homologous rechallenge



Challenged with French Polynesia Zika, rechallenged 10 weeks later

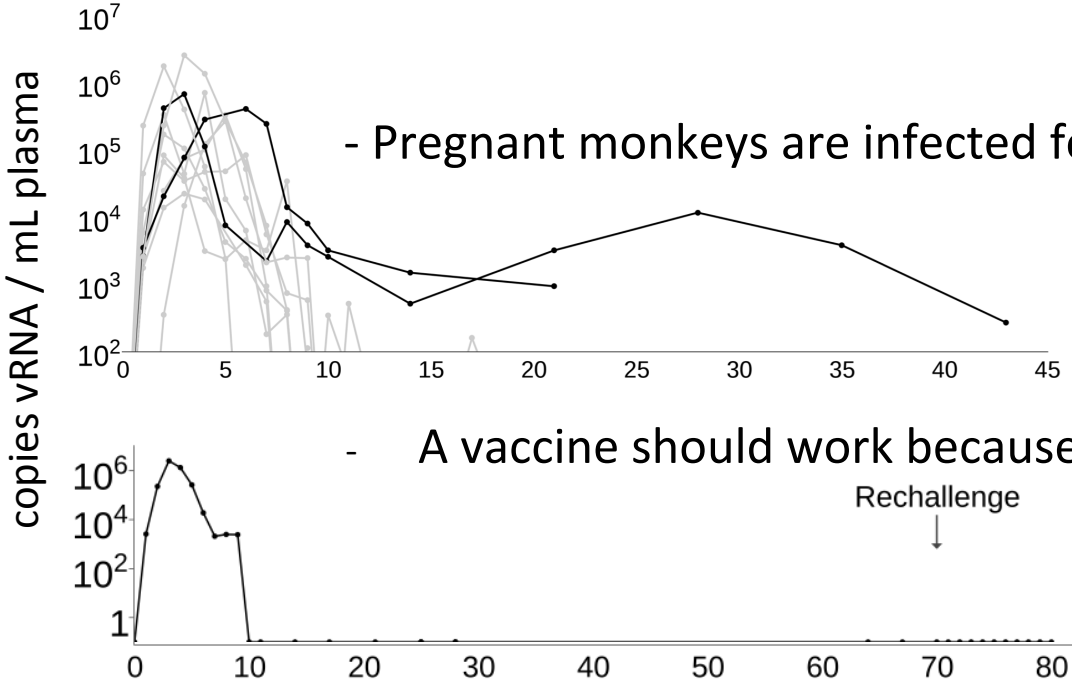
Primary immunity apparently protects against heterologous rechallenge



Initially challenged with African lineage Zika, 10 weeks later rechallenged with Asian lineage

Progress towards a Zika virus vaccine using monkeys

- Macaque monkeys are susceptible to infection with Zika virus

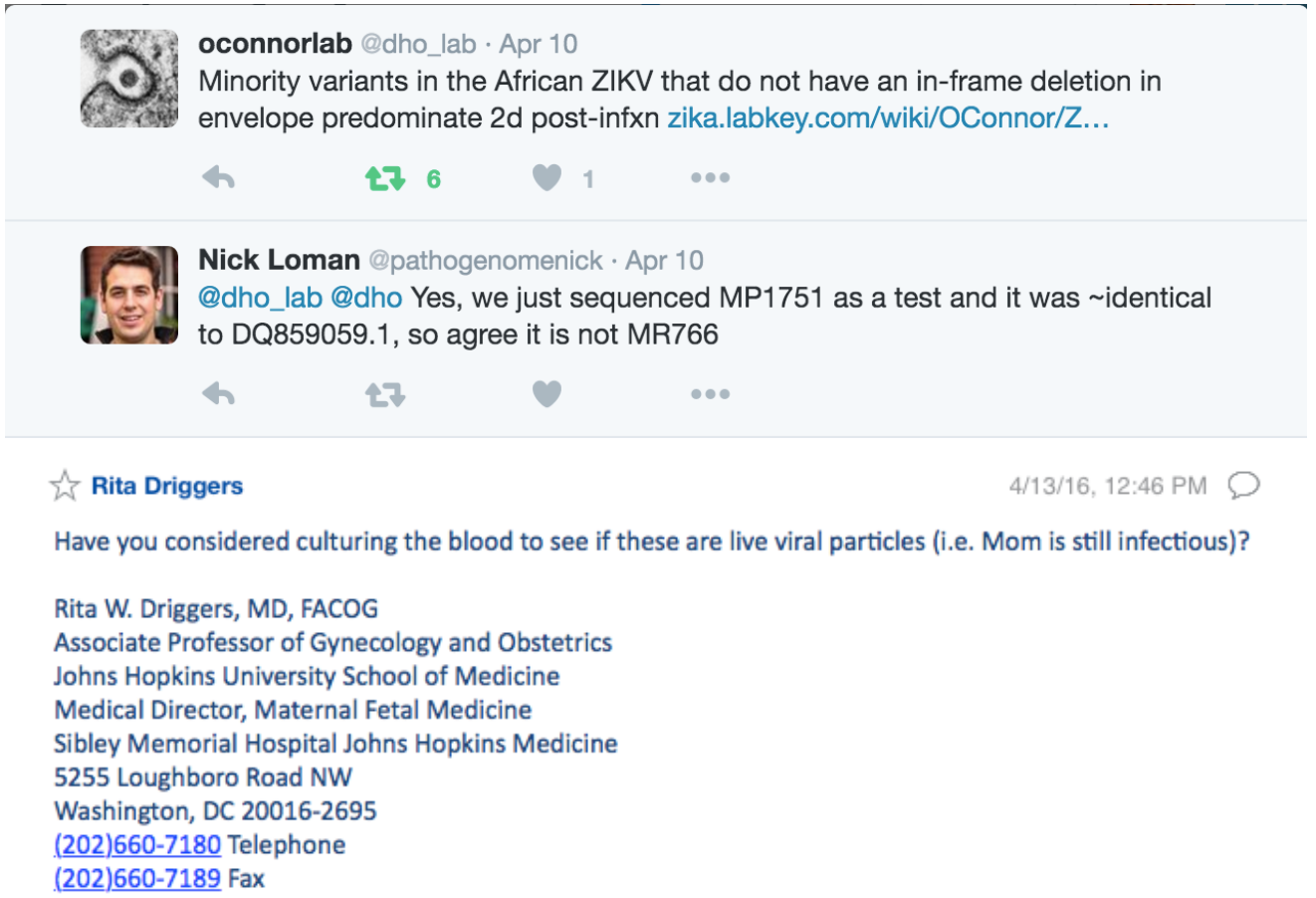


- Pregnant monkeys are infected for an unusually long time

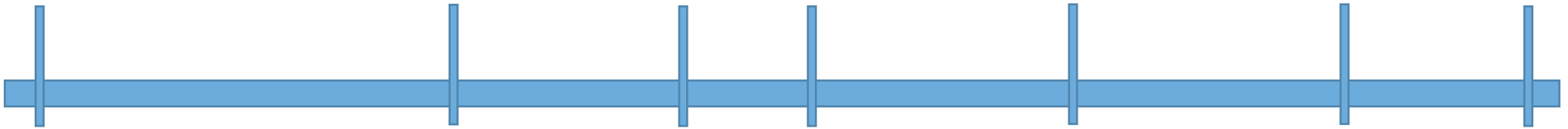
- A vaccine should work because monkeys resist reinfection

http://zika.labkey.com

- Enables stakeholders, scientists, and community to engage in experiments – leads to better, faster research



Zika Animal Models



Range from no disease (C57Bl6)
to highly susceptible (SJL)

Sensitive to infection, but lacks IFN signaling to prime the innate and adaptive immune response

- Zika/NHP model recapitulates human disease very well
- Effects of viral infection on fetuses will be known in the coming weeks
- Macaques exposed to virus are protected from re-challenge
- Promising model for vaccine testing