




# Mechanism of Chikungunya Pathogenesis: Implications For Disease Interventions

Lisa F.P. Ng, PhD

Laboratory of Microbial Immunity

SIgN, A\*STAR, Singapore

This map has been adapted from *Fields Virology*  
5th ed., Vol. 1, Philadelphia, Lippincott Williams &  
Wilkins, 2008:1047.

 Countries or areas at risk

0 850 1700 3400 Kilometers

# Chikungunya Fever

- Caused by Chikungunya virus (CHIKV) – *Alphavirus*
- Mosquito-transmitted: *Aedes*
- Re-emerged in late 2005 and spread to several countries
- Acute infection lasts 1-10 days: sudden onset of high fever, rash, painful arthralgia > **incapacitating joint disease**

## Acute (2-10 days)



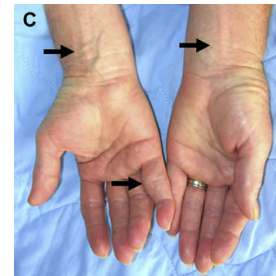
Edematous rash



Swollen and stiff joints

Simon et al., 2011 *Curr Infect Dis Rep*

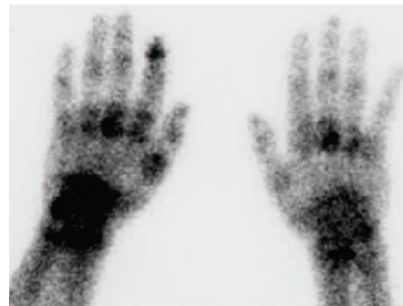
## Chronic (months to years)



Inflammatory osteoarthritis



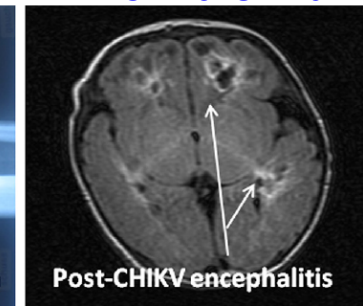
Arthralgia →



X-ray picture  
from an adult



MRI picture  
from a child

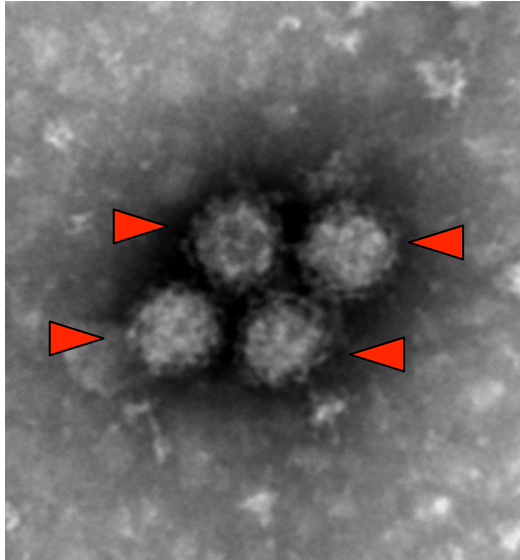


Jaffar-Bandjee et al., 2009 *Microbes Infect*

**No anti-virals and no licensed vaccines**

# ***Alphavirus* belonging to the *Togaviridae* family**

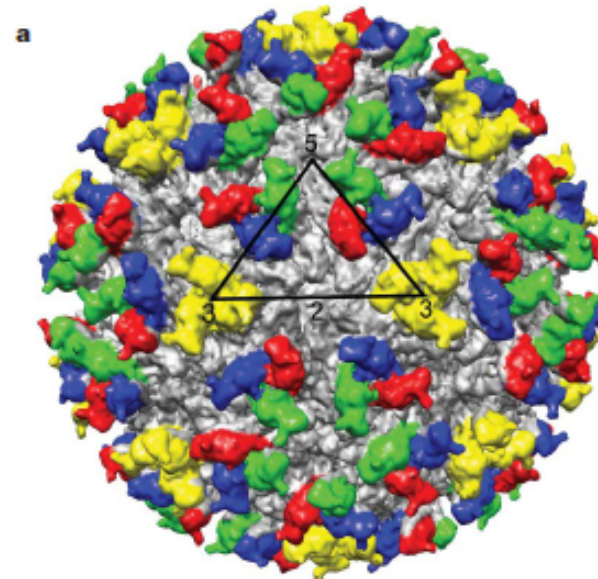
## **Electron Microscopy**



0.1 μm

*Her et al., 2009 Microbes Infect*

## **Cryo-EM density diameter ~ 700Å°**



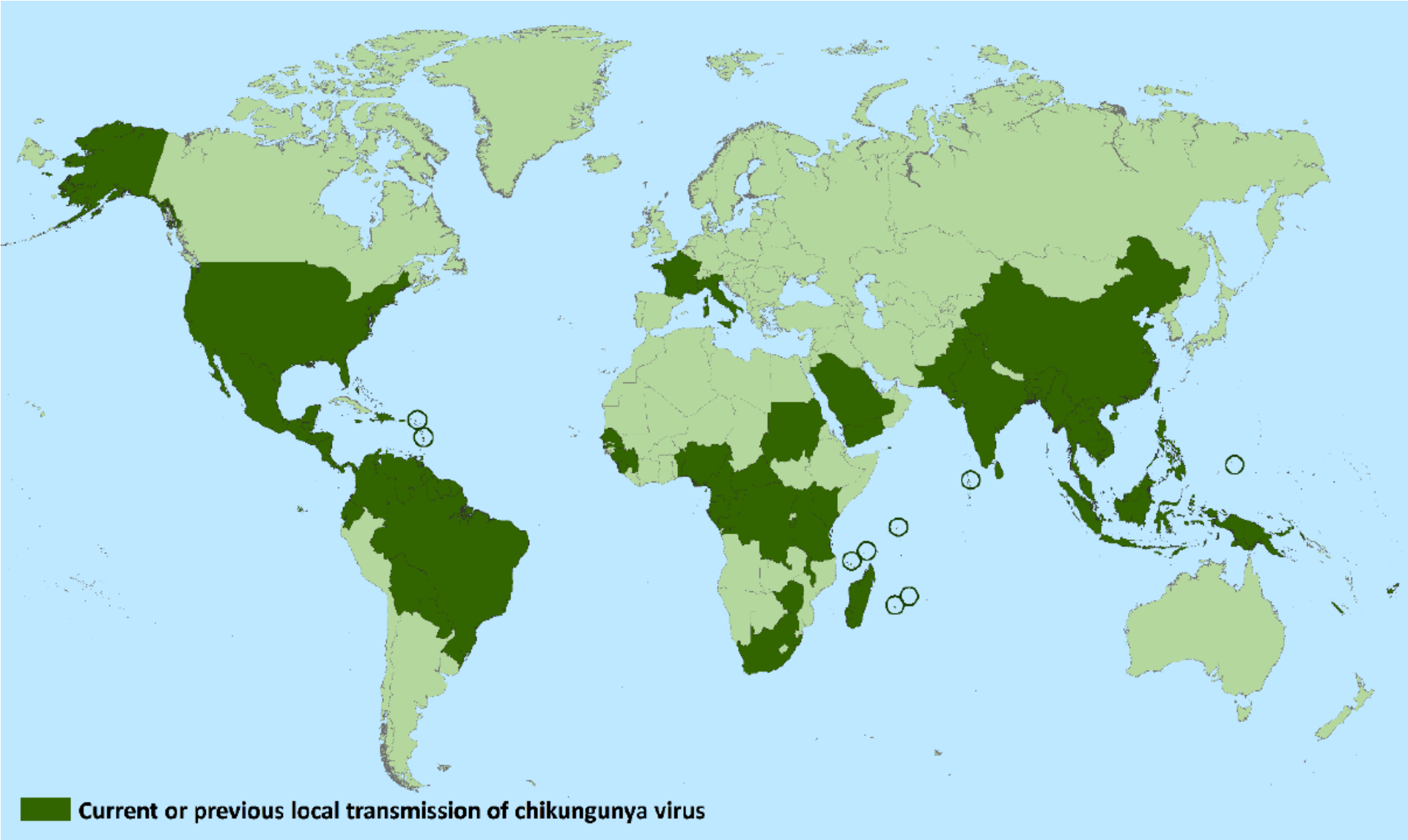
*Li et al., 2010 Nature*

## **Positive-strand RNA virus of 11.8 kb**

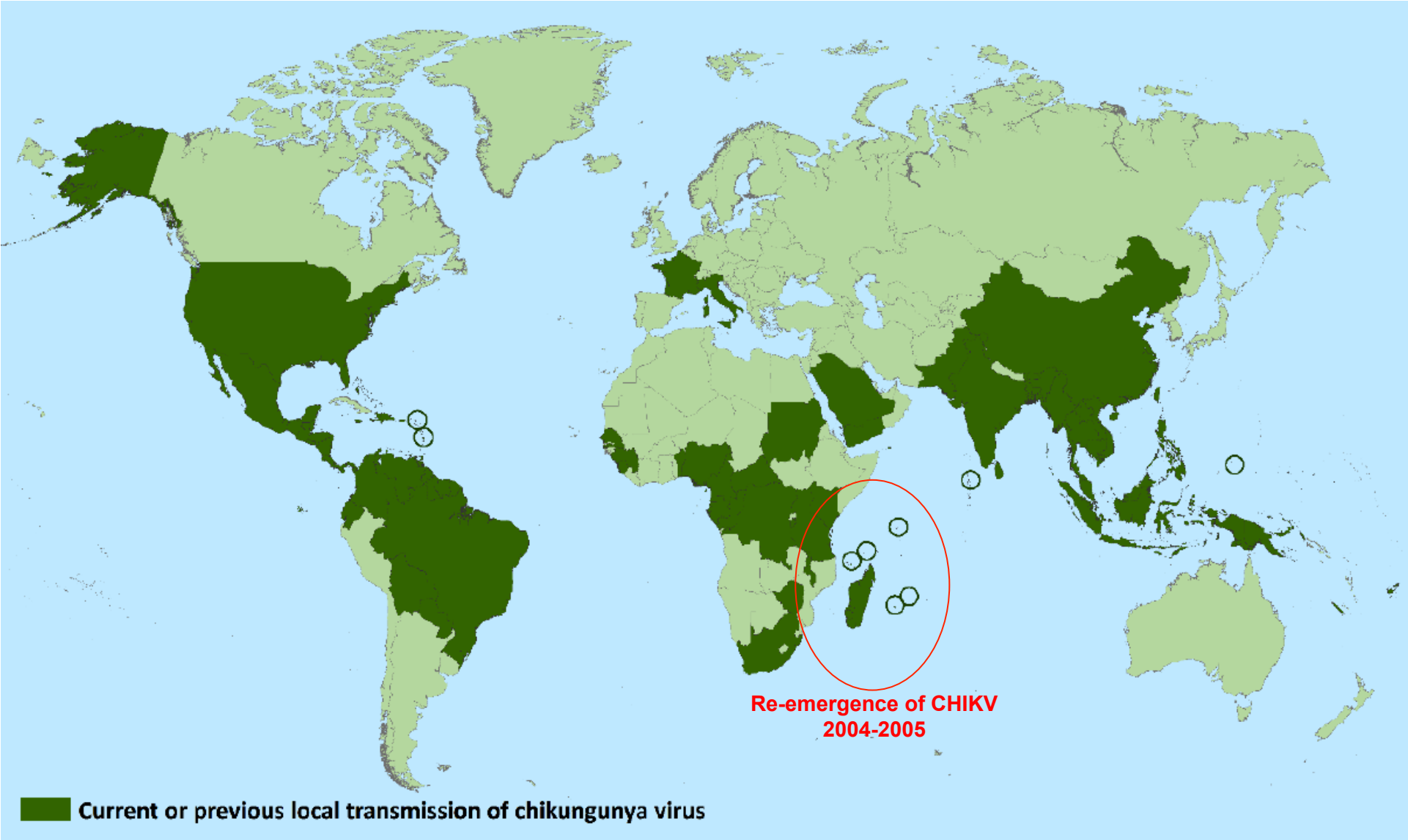


*Teng et al., 2011 Future Virol*

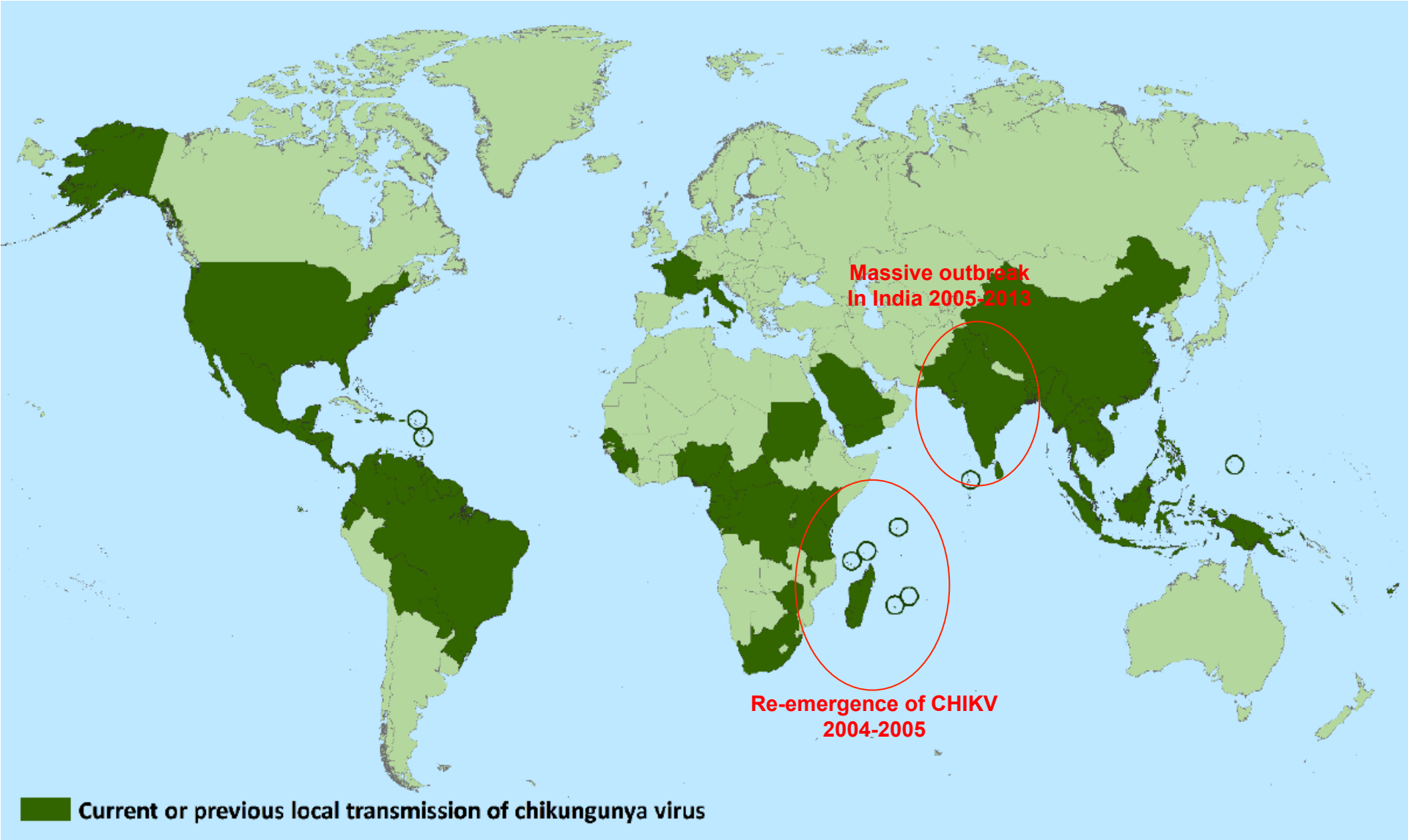
# CHIKF outbreaks



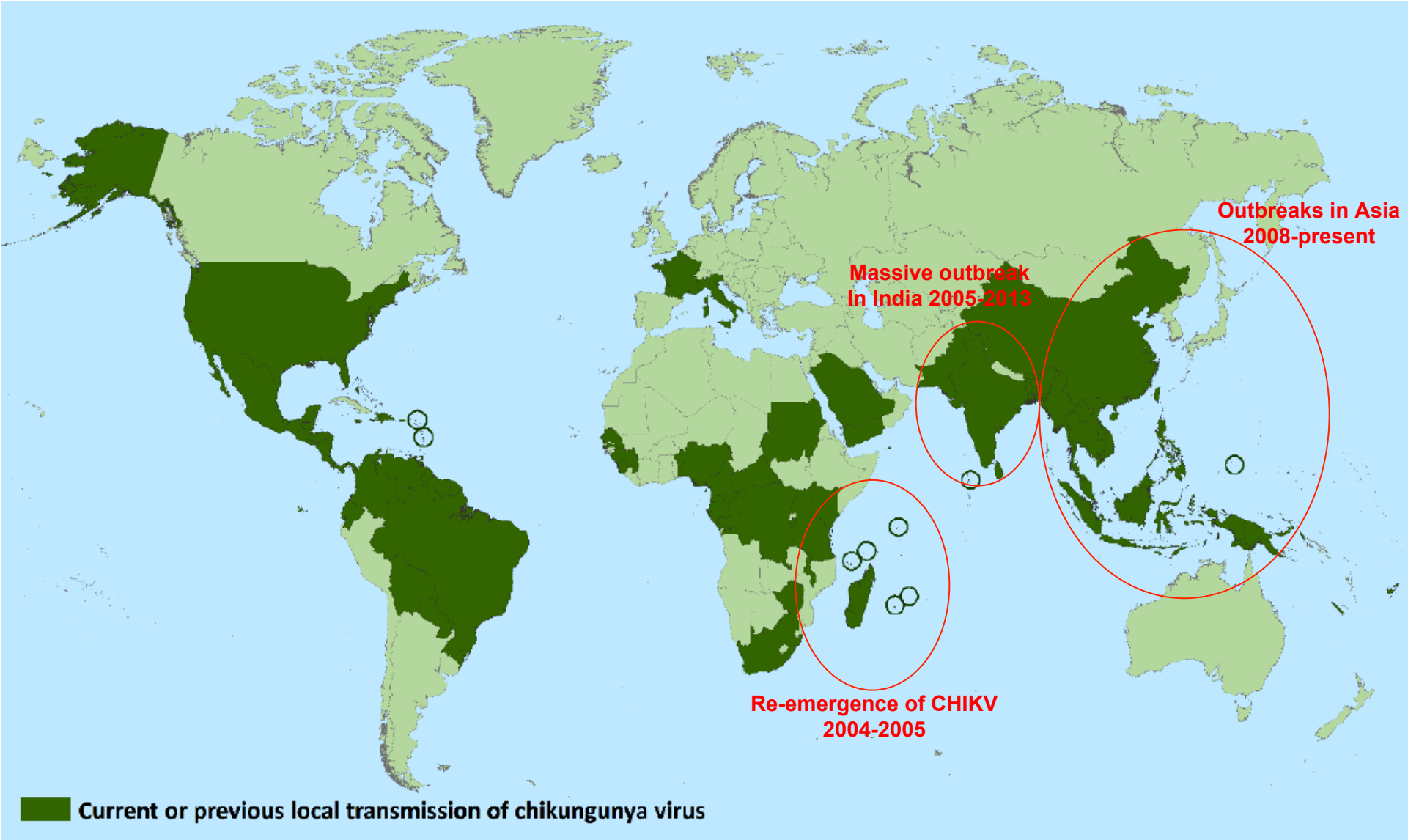
# CHIKF outbreaks



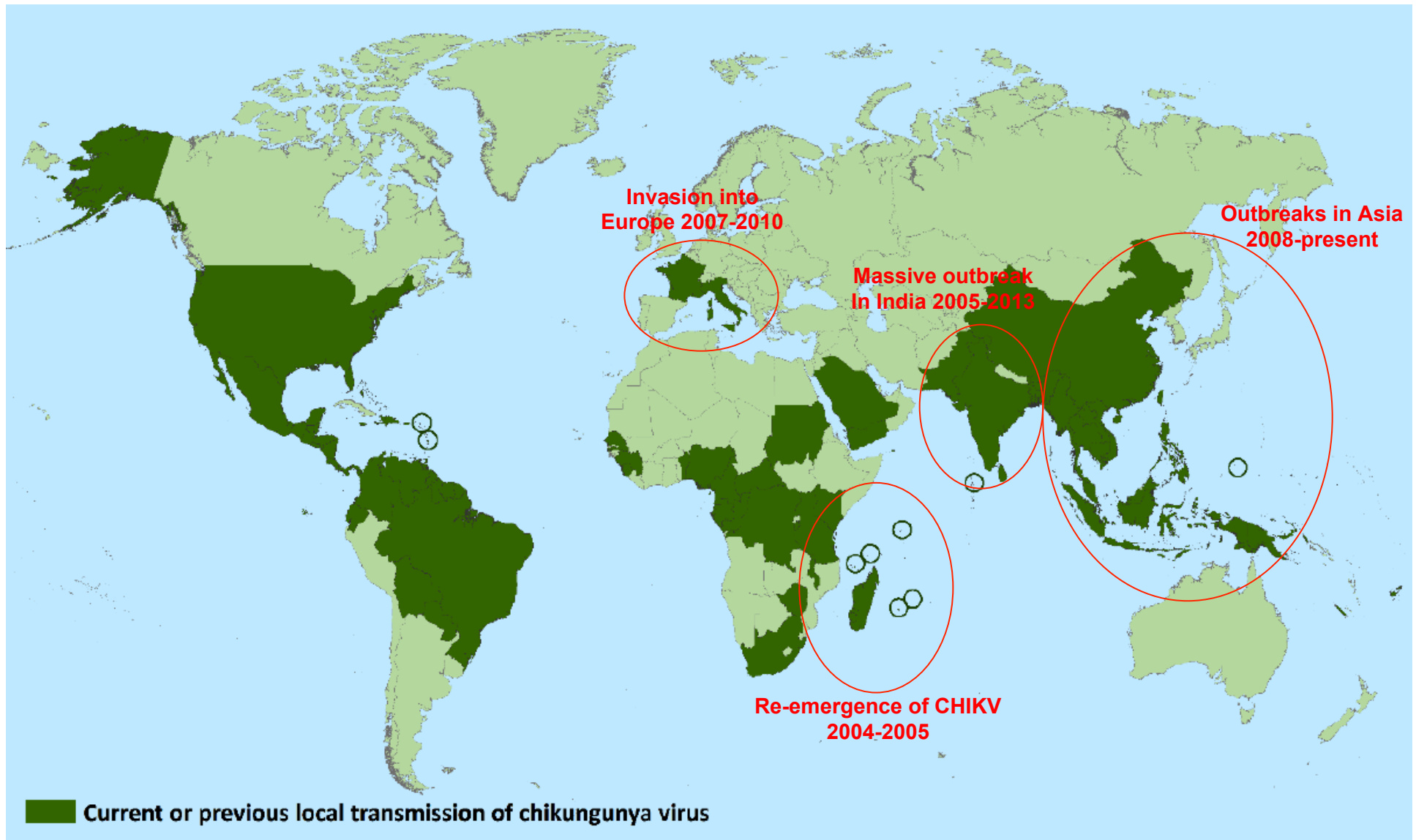
# CHIKF outbreaks



# CHIKF outbreaks

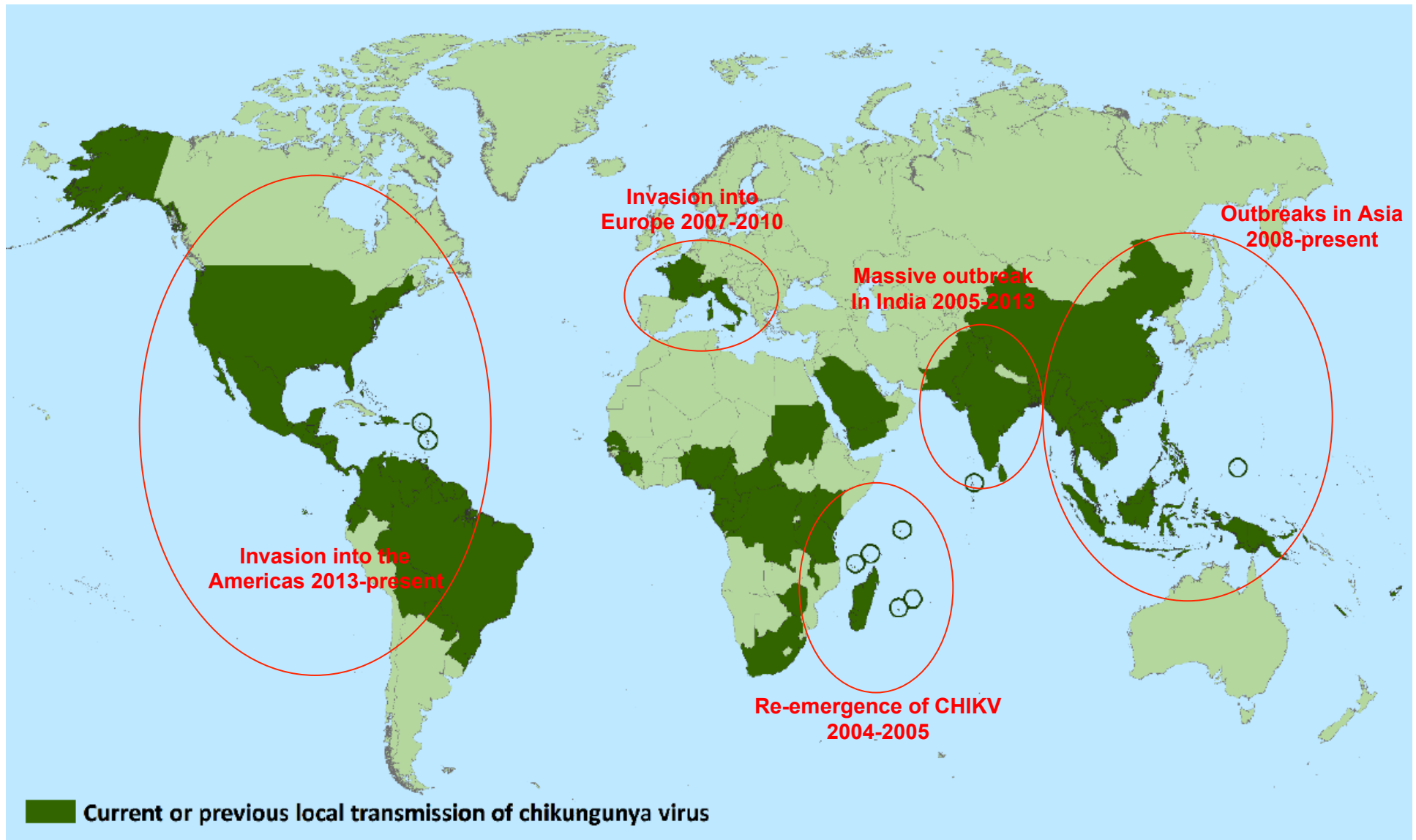


# CHIKF outbreaks



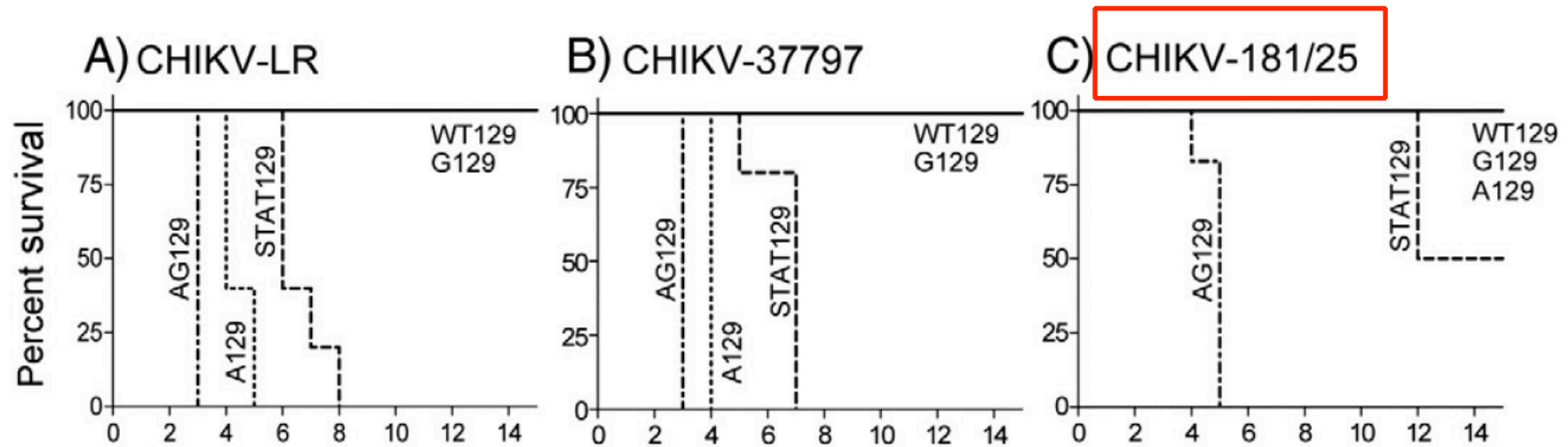


# CHIKF outbreaks



# CHIKV vaccines – where are we?

1st: Cell culture-adapted live-attenuated 181/25 vaccine

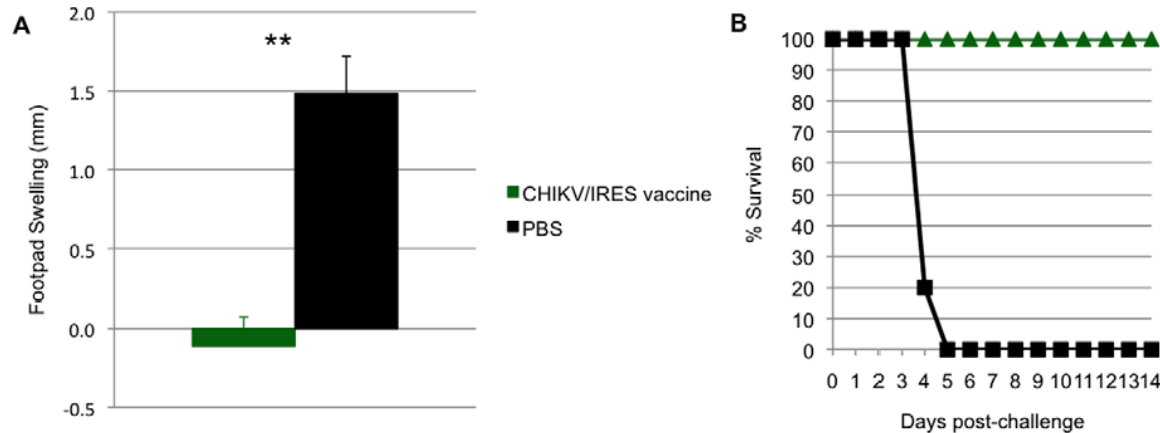


Gardner et al., 2011 *Virology*

No mortality or ruffling of the fur were observed in CHIKV-181/25-infected WT129 or A129 mice

# CHIKV vaccines – where are we?

## IRES (internal ribosomal entry site) - based CHIKV vaccine



Reduced foot swelling and protection in A129 mice

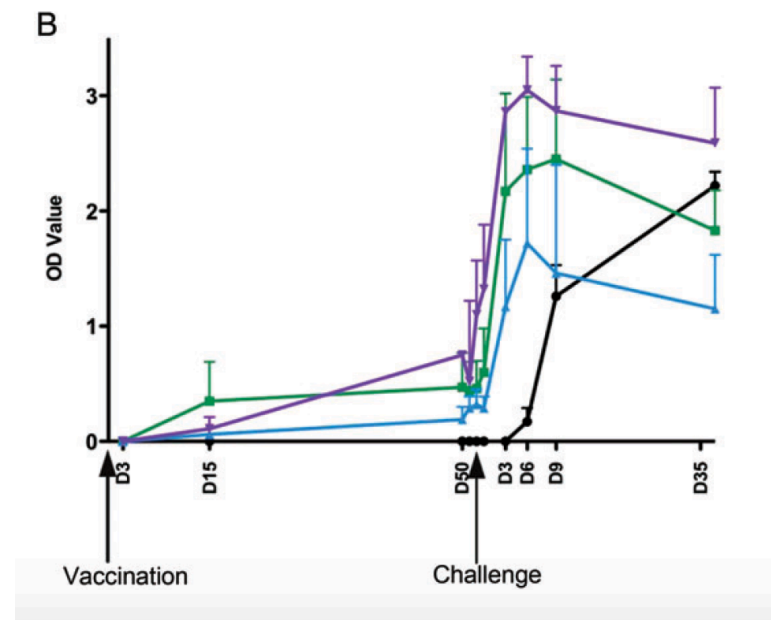
Plante *et al.*, 2011 *PLoS Patho*

Highly attenuated

Protects Nonhuman Primates

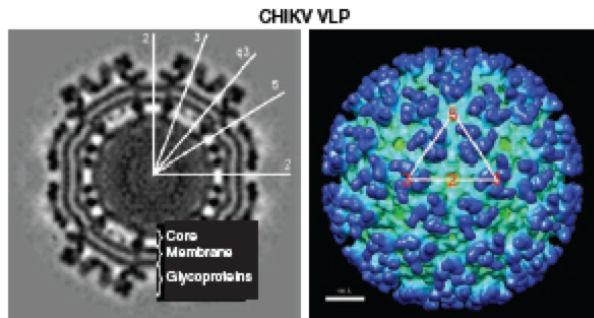
Elicits high IgG

Roy *et al.*, 2014 *J Infect Dis*



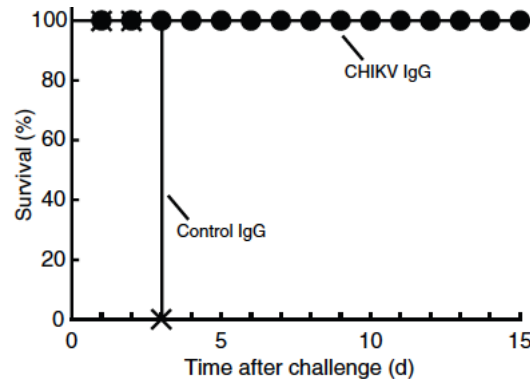
# CHIKV vaccines – where are we?

## Virus-like-particle (VLP) CHIKV vaccine

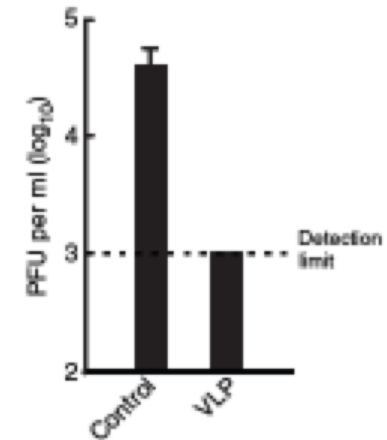


### CHIKV VLP

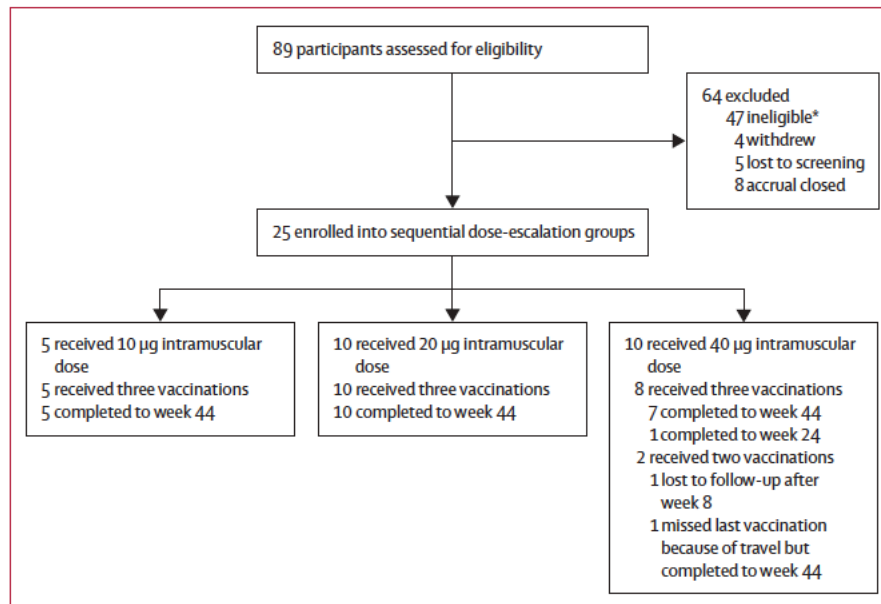
Akahata *et al.*, 2010 *Nat Med*



### Protection in mice



### Protection in monkeys (*Rhesus macaques*)



### VRC 311 – a phase 1 CHIKV vaccine

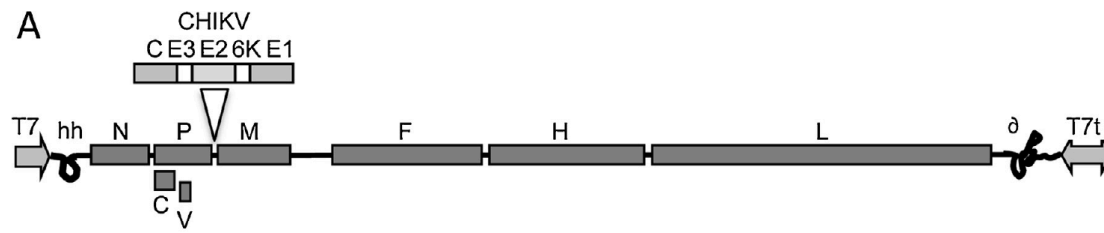
Neutralizing Abs detected in all gps

VLP vaccine was well-tolerated, safe

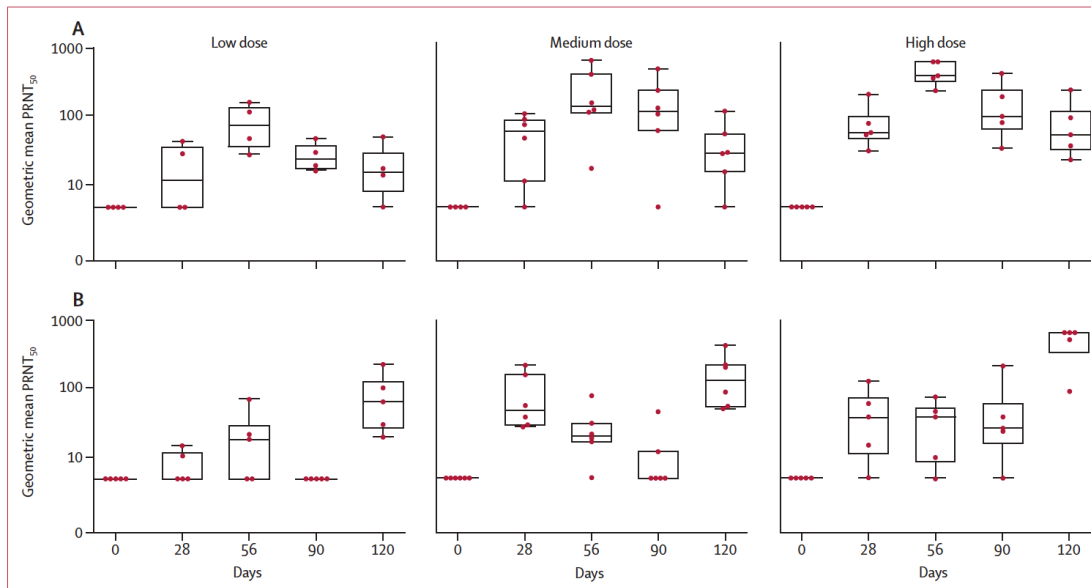
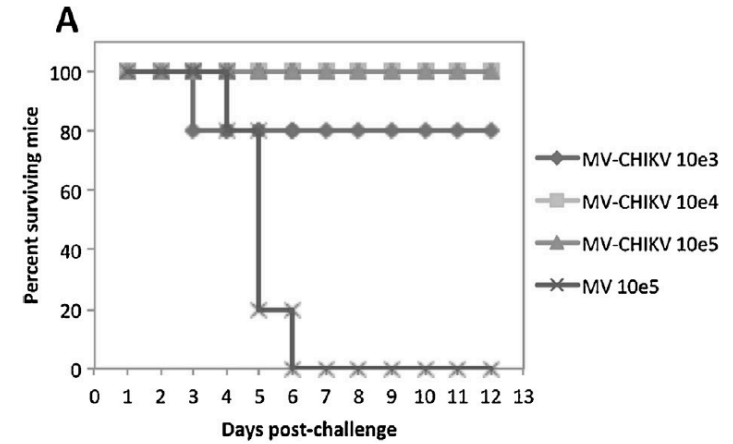
Chang *et al.*, 2014 *Lancet*

# CHIKV vaccines – where are we?

## Recombinant measles vaccine expressing CHIKV VLP



**MV-CHIKV vaccine protected all mice**



**1 dose clinical trial in Vienna**

**Funded buy Themis GmbH**

**42 participants**

**Protective anti-CHIKV Abs**

**No adverse effects**

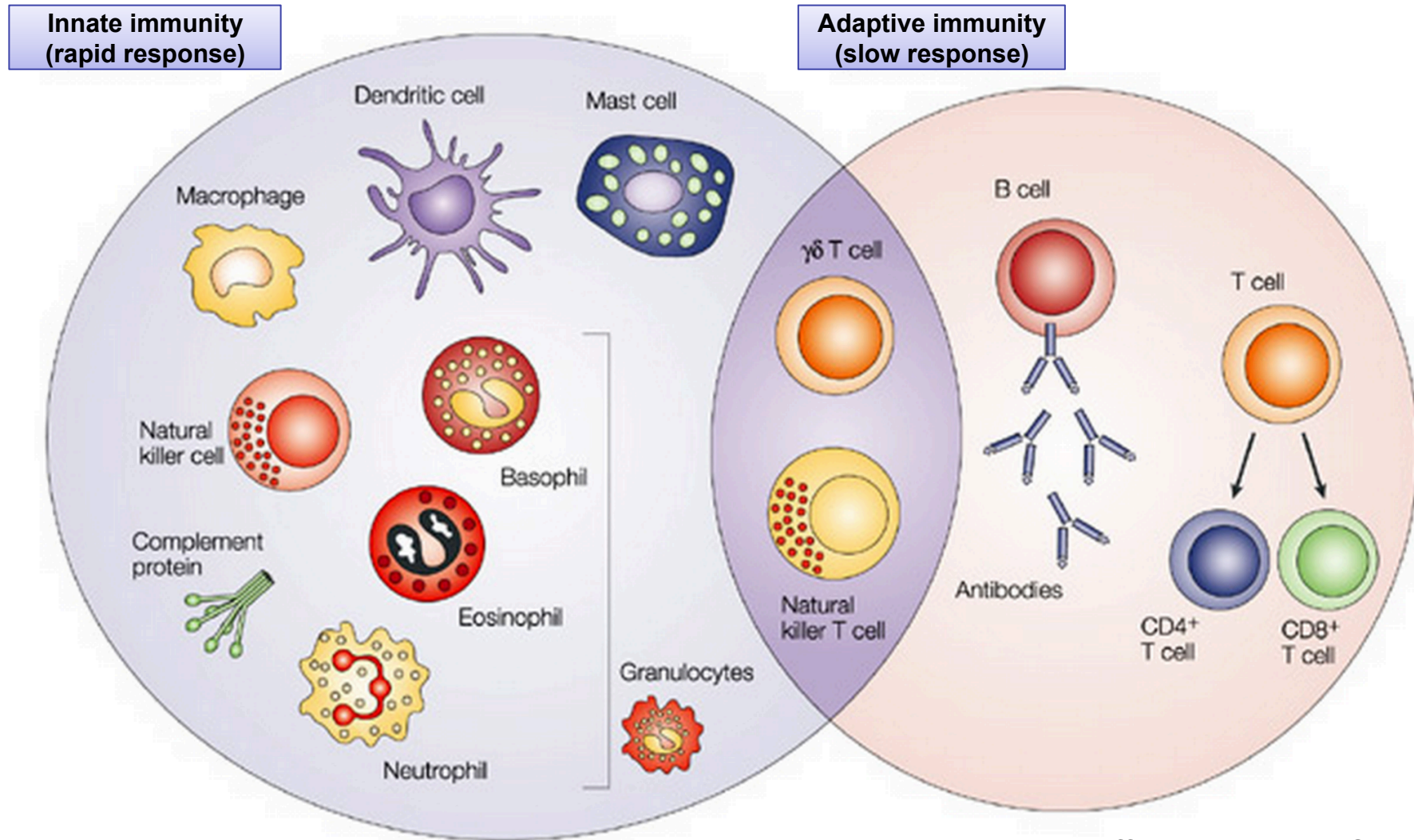
**Not based on the analyses of the immune response...**

# Aims

To gather fundamental knowledge on the immune responses mounted against CHIKV with a view to exploit this to develop new immune-based preventive and treatment strategies

- **Pathology to devise appropriate treatments**
- **Rationally-guided design in vaccine development**
- **Immune-based therapies**

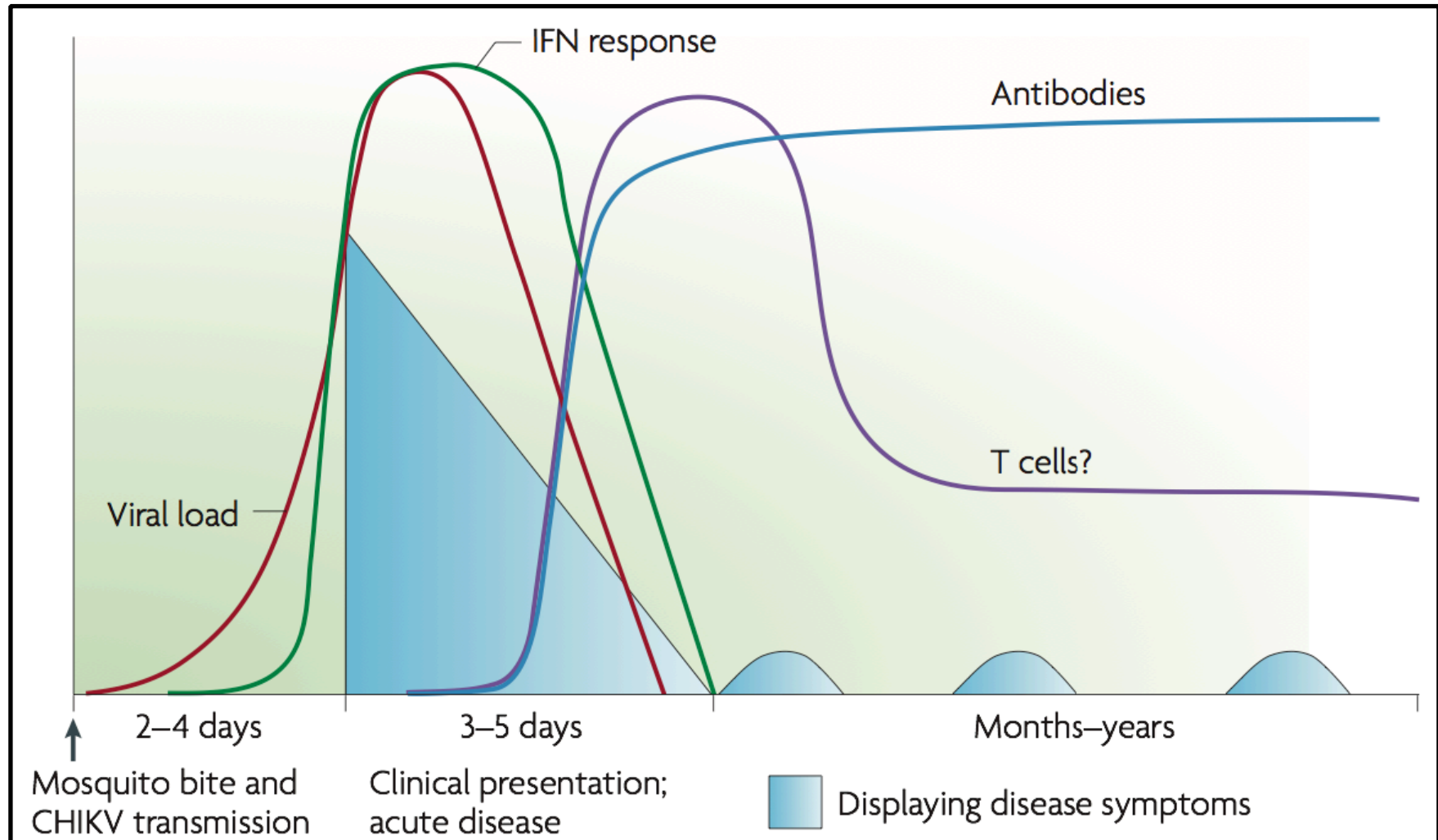
# The innate and adaptive immune response



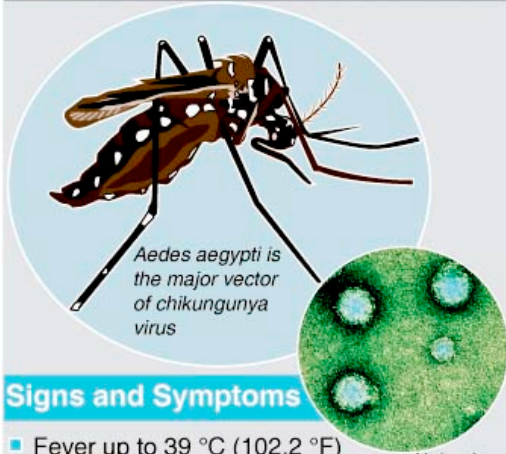
Dranoff, 2004 *Nat Rev Cancer*



# Immune response in CHIKV infection



# Chikungunya



*Aedes aegypti* is the major vector of chikungunya virus

*Alphavirus*

## Signs and Symptoms

- Fever up to 39 °C (102.2 °F)
- Maculopapular rash
- Arthralgia or arthritis affecting multiple joints
- Pigmentation on the face and externalities
- Nasal Erythema also known as redness of the nose
- The other symptoms include headache, conjunctival injection and slight photophobia



## Ayurvedic treatments

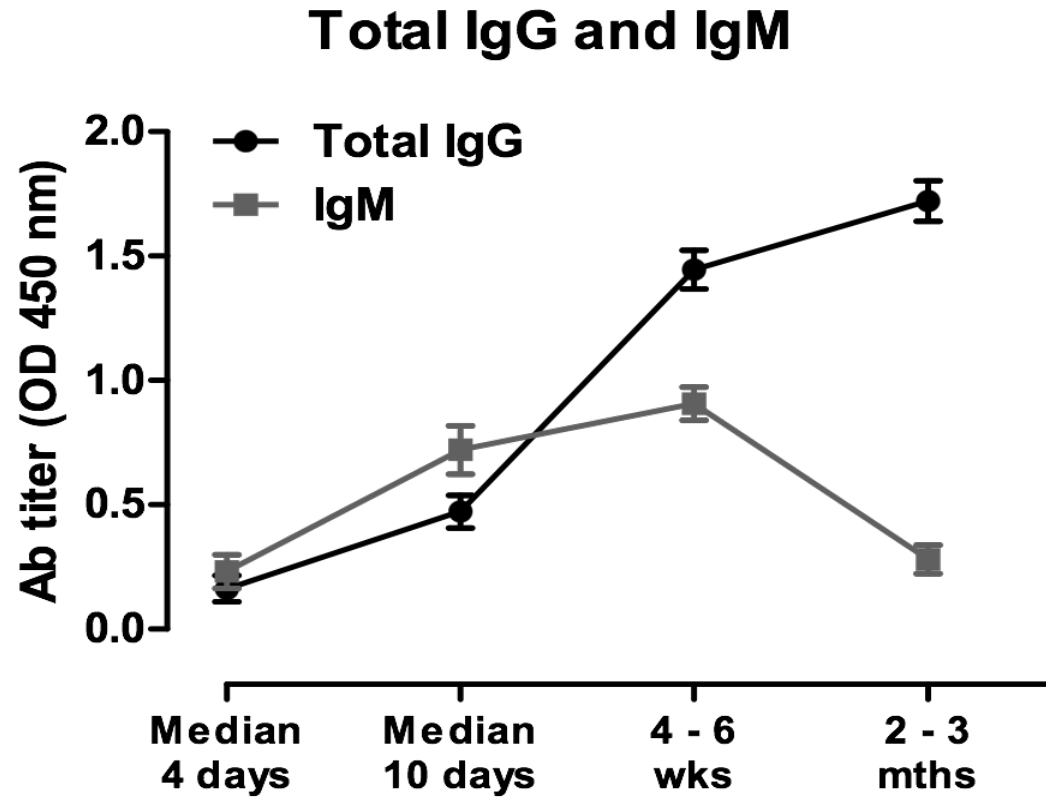
- Decoctions are made using herbs like Coriander for fever, Venivalgata
- Heen Araththa Ala is given as a pain killer
- The improvement of the immune system is done through ginger, Rasakinda, and Bin kohomba
- Swellings of the limbs are treated by Saarana and Devadara
- Also pills like Murthunjaya Rasa and Raambaana Rasa are also given to the patients

# CHIKV pathogenesis in humans



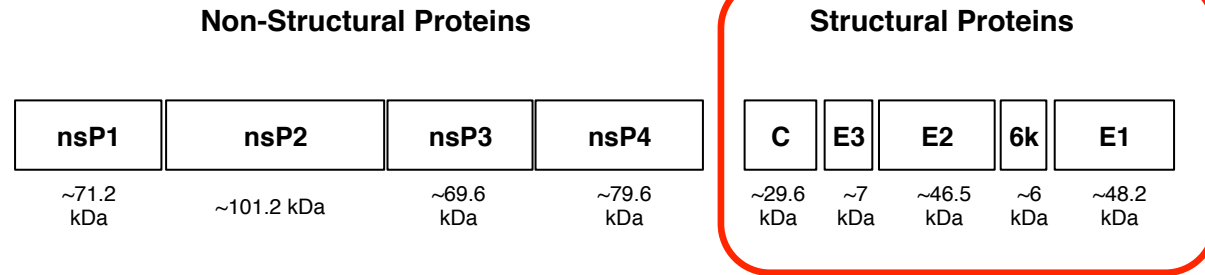
India, 2006 to 2007

# Timing of the Ab response

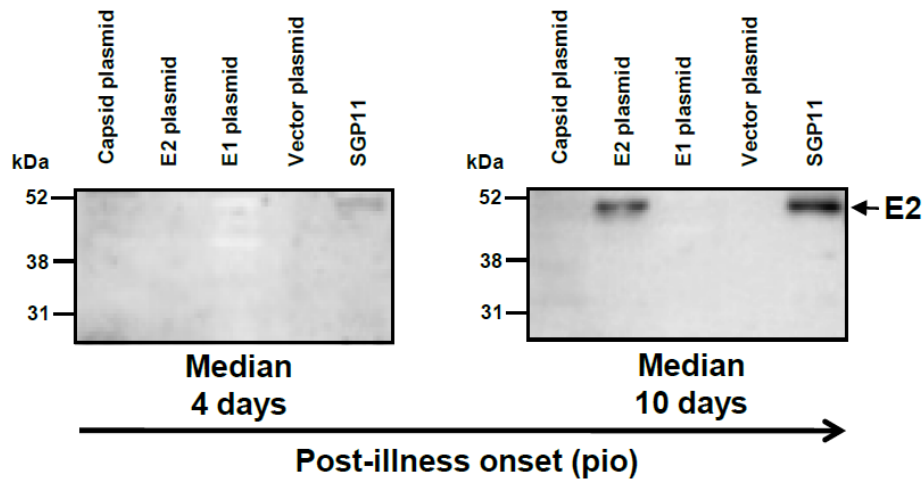


**CHIKV-specific response produces high IgG titres**

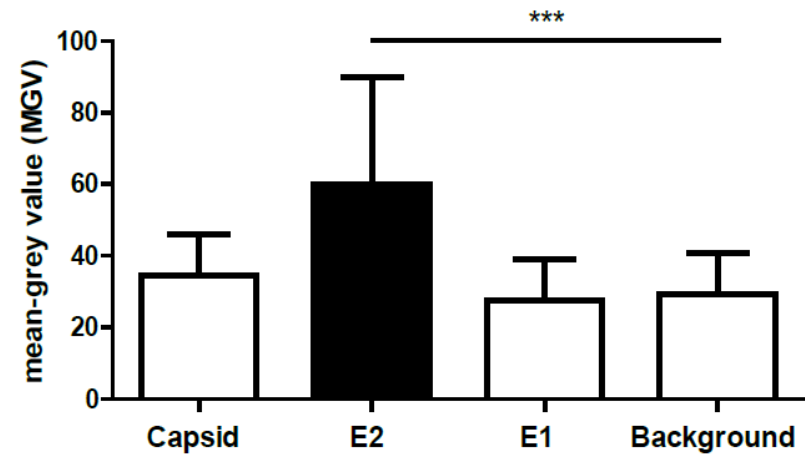
# Anti-CHIKV antibodies recognize E2 glycoprotein



IgG from CHIKV-infected patient



Antigenic profile (n = 30)



**E2 is the immunodominant viral protein upon CHIKV infection**

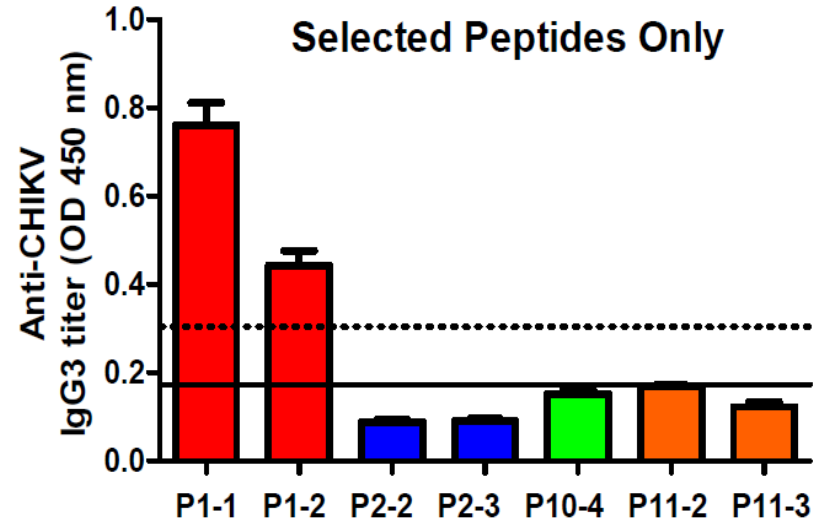
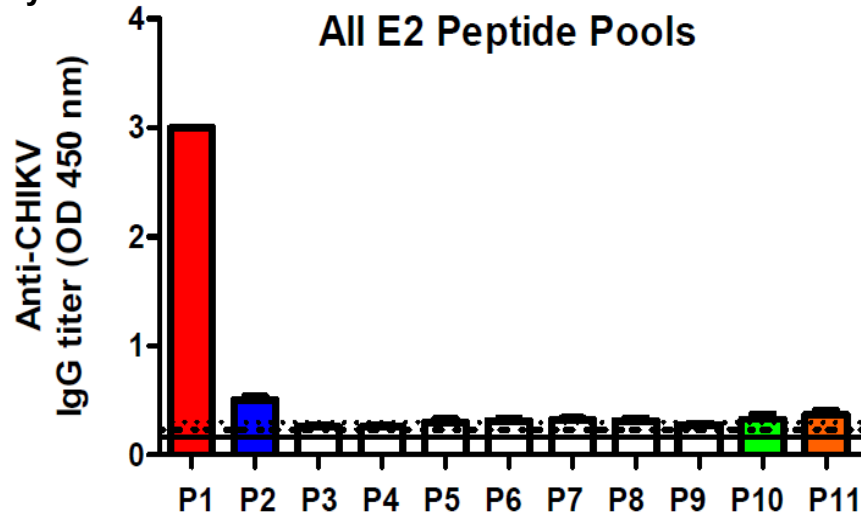


Wendy Lee

# B cell epitope mapping and localisation

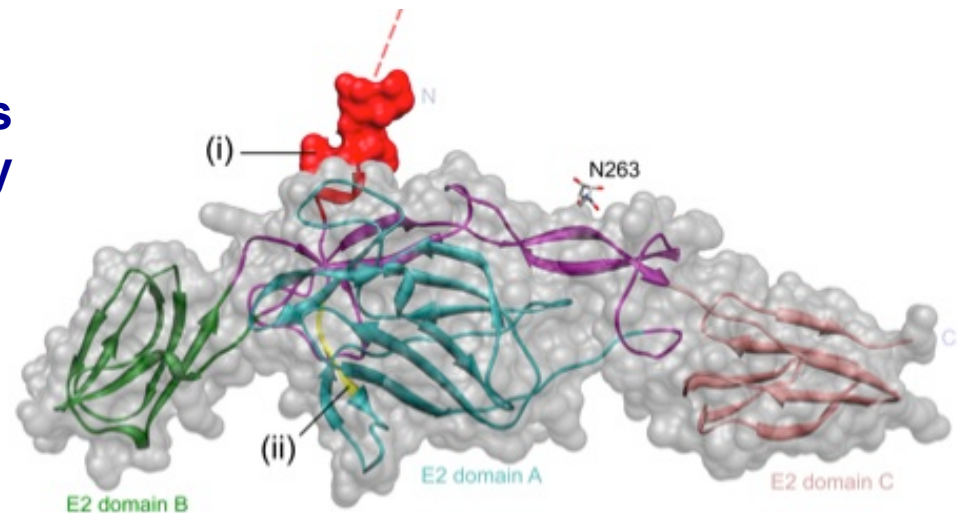


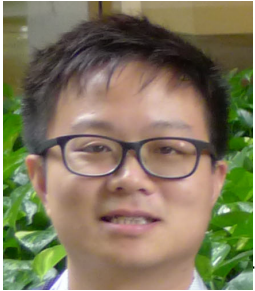
Jason Kam



Region at the N-terminal is recognised by CHIKV Abs at early points

E2EP3 is located at the surface of virion

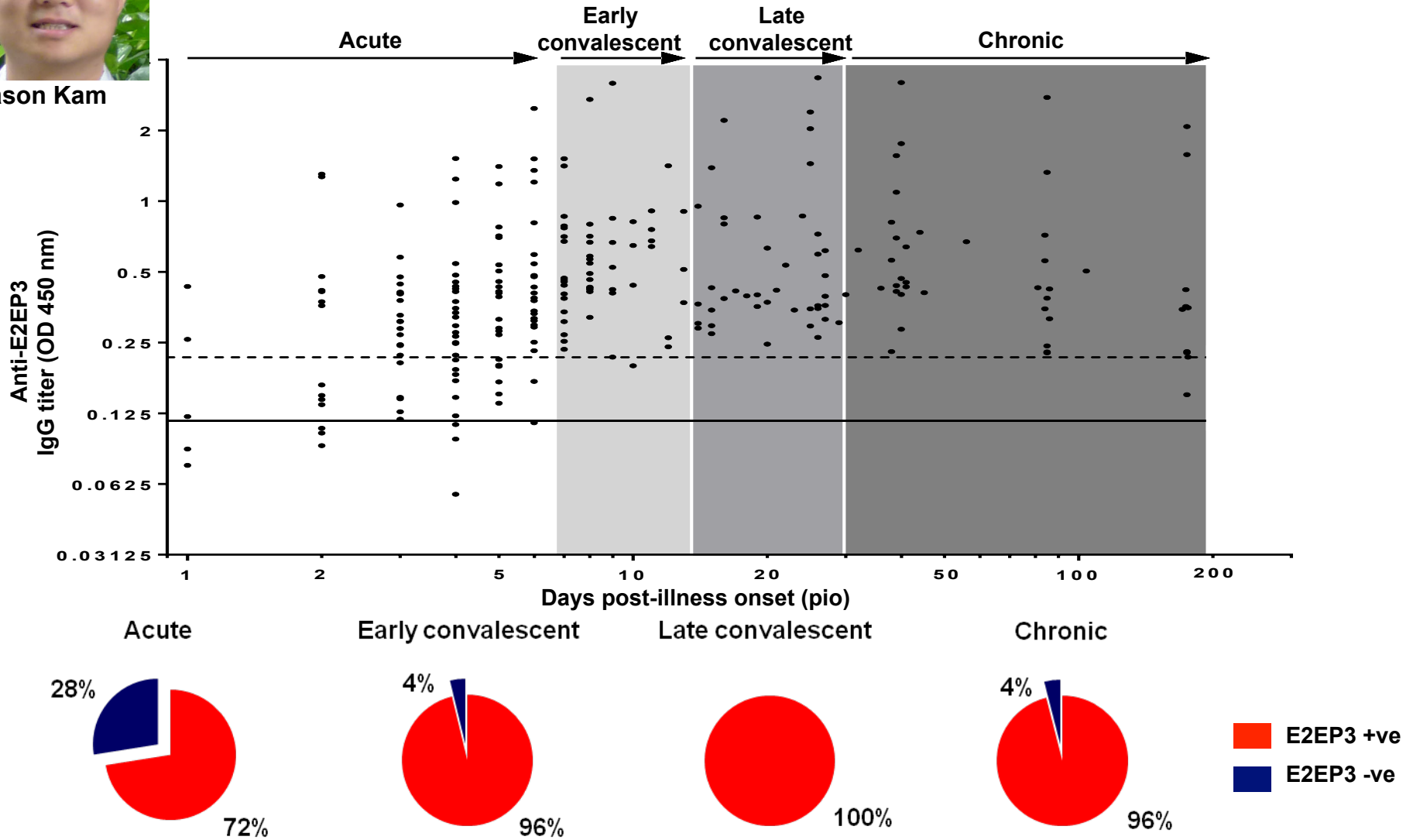




Jason Kam

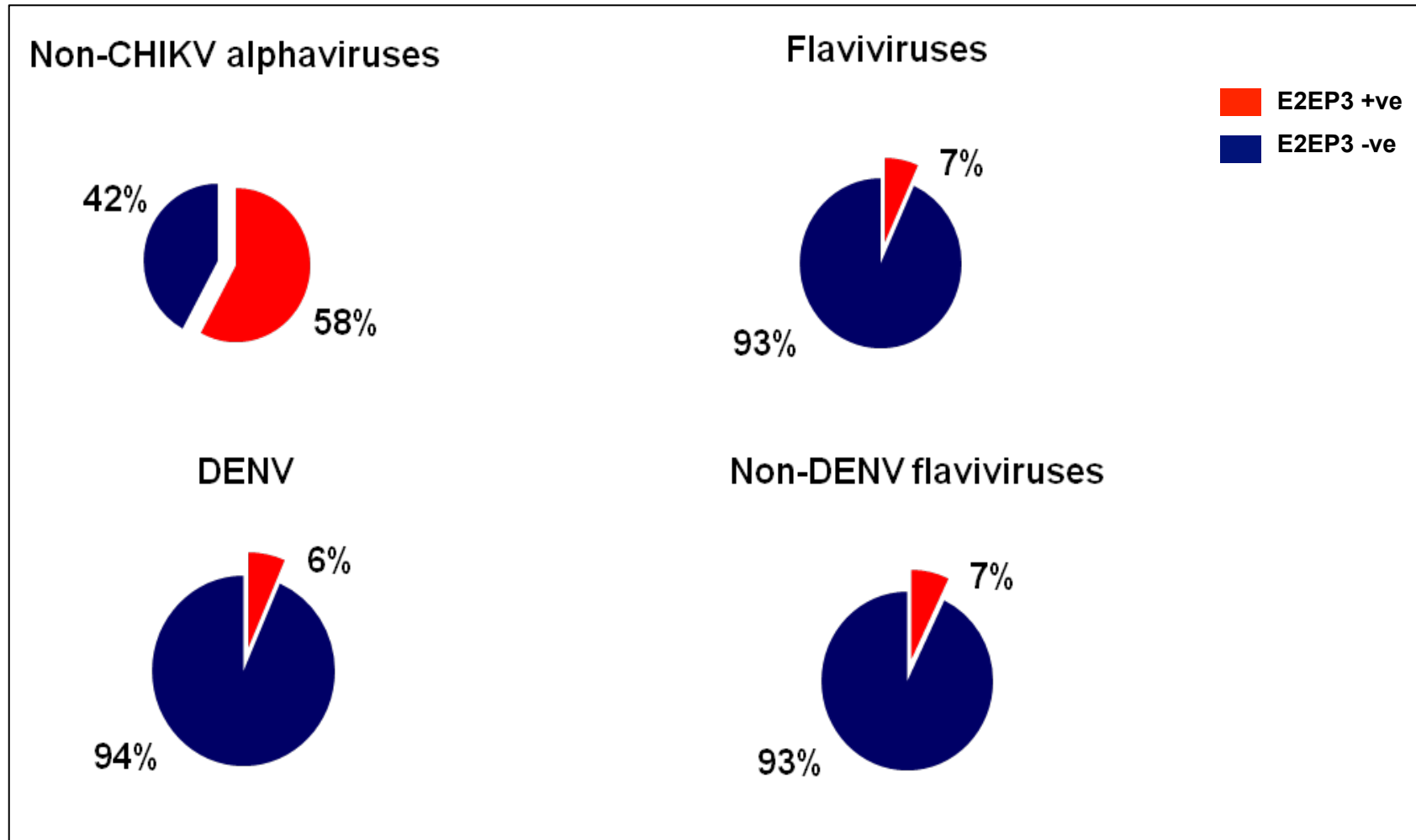
# Sero-epidemiological studies in population

## In collaboration with WHO arbovirus reference centre



**E2EP3 is a common detection serology marker for early CHIKV infections**

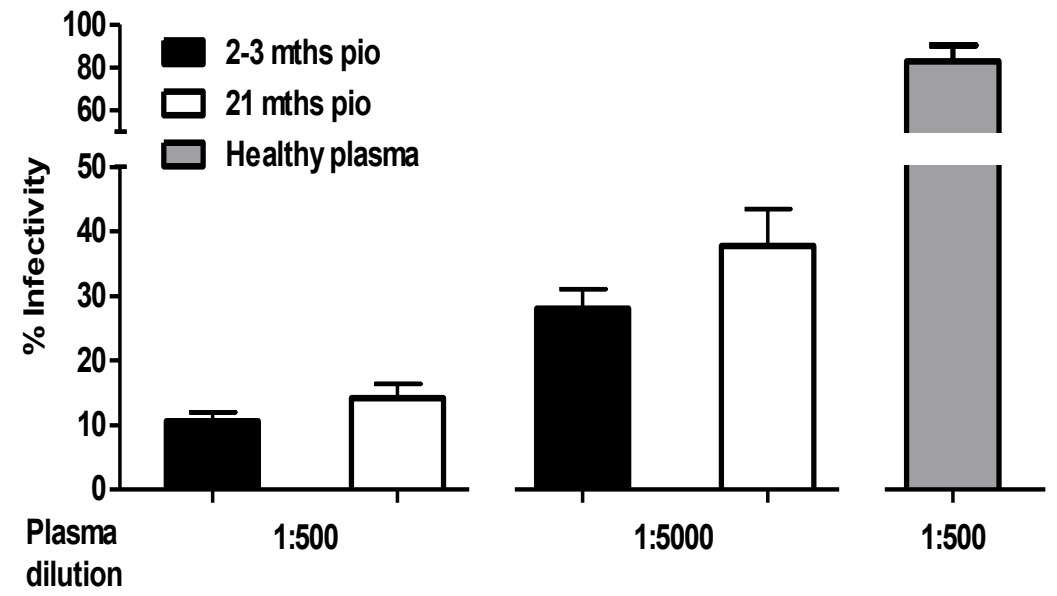
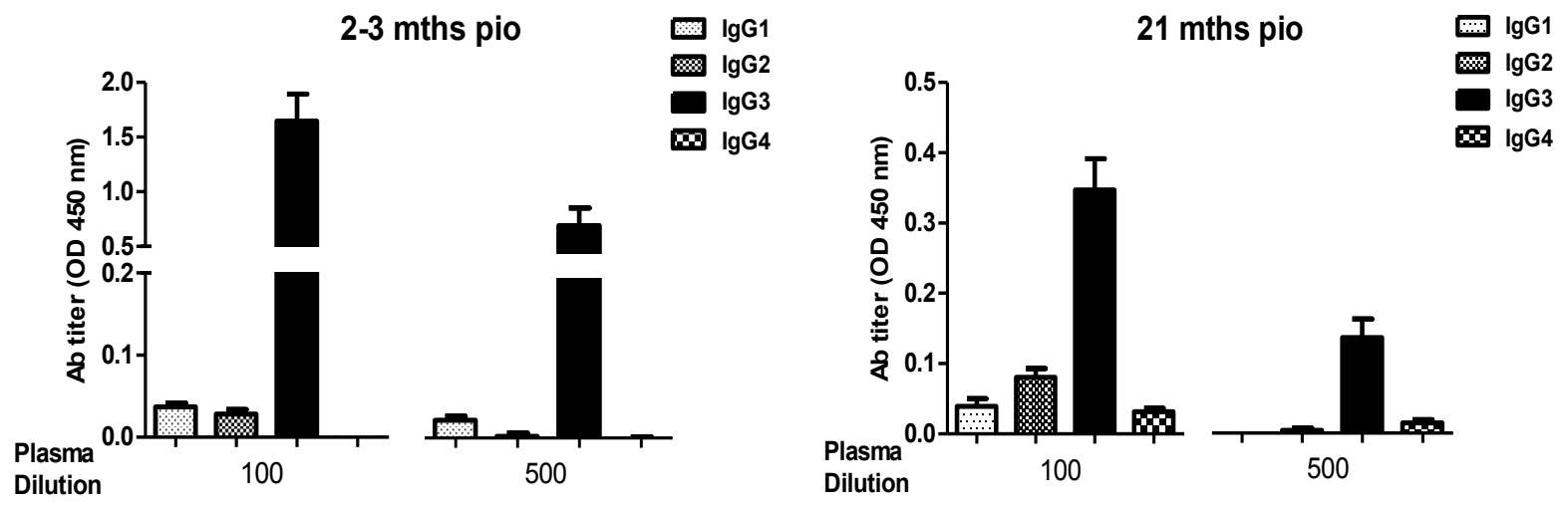
# What is the level of cross-reactivity with others?



Kam *et al.*, 2015 *PLoS NTD*

**Low level of cross-reactivity with DENV**

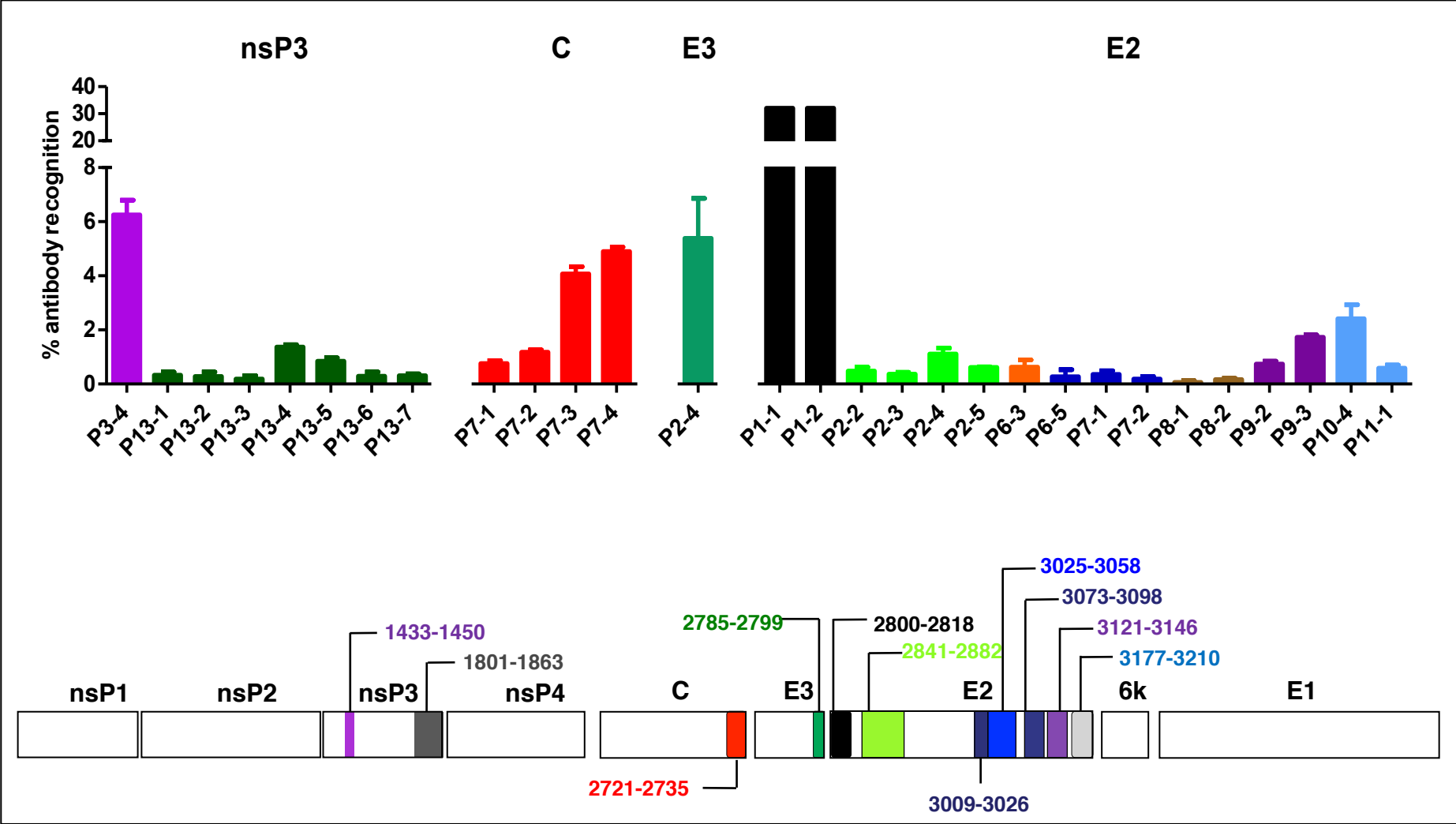
# Specificity of anti-CHIKV antibodies at late phase



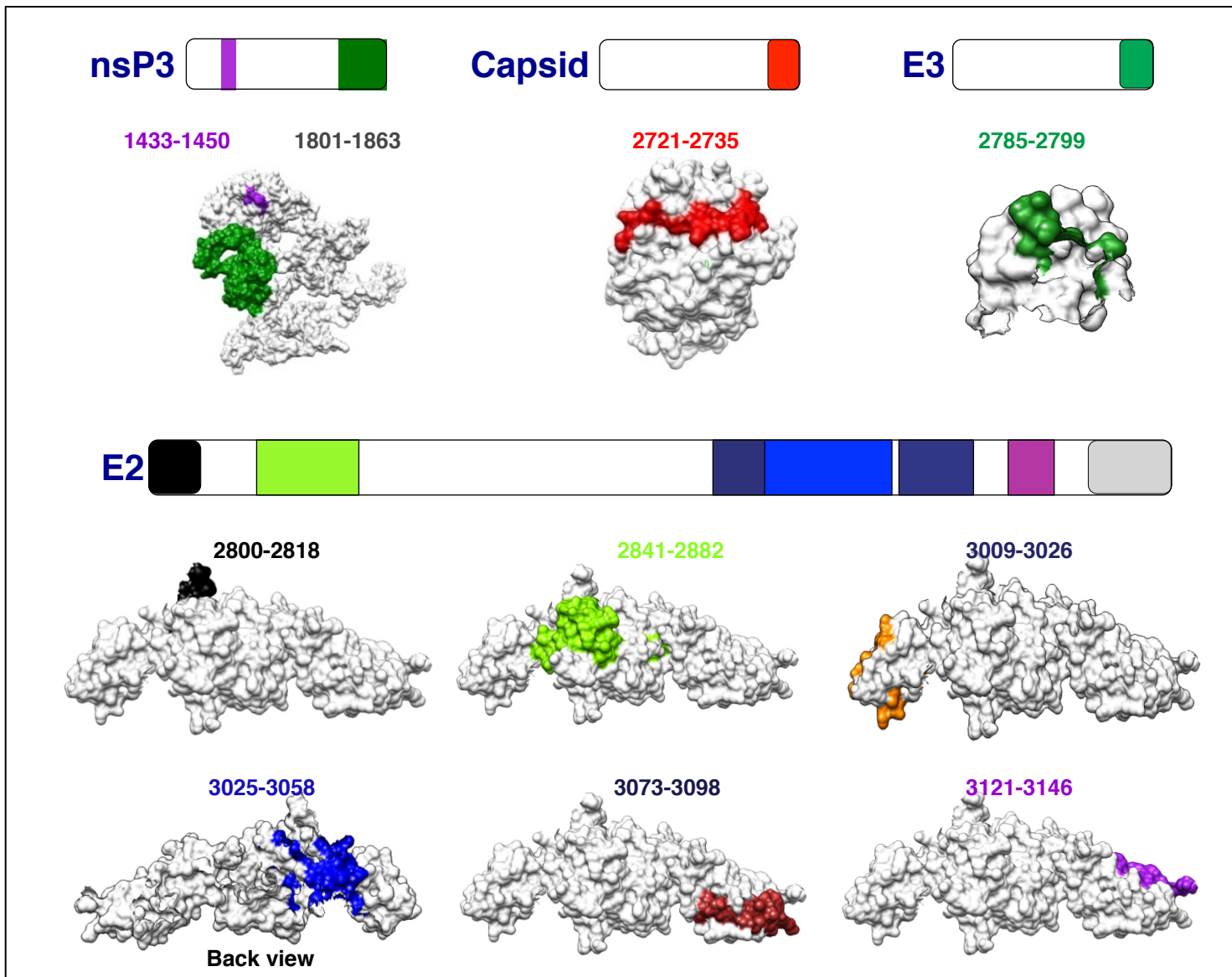
**Anti-CHIKV antibodies are still protective at 2 years post-infection**



# Mapping of other less dominant epitopes



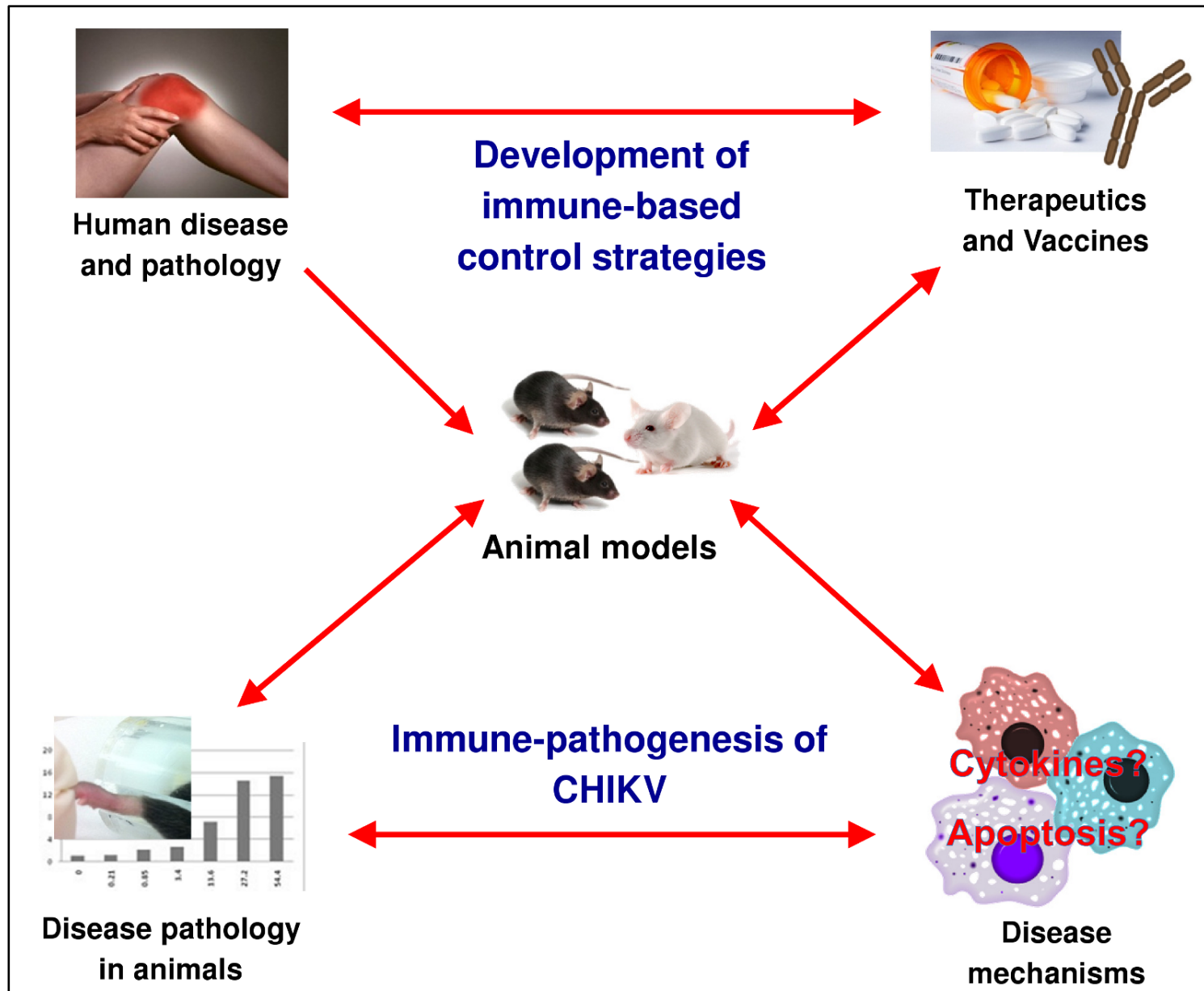
The E2 and E3 glycoproteins and the capsid and nsP3 proteins are targets



Kam\_Lee *et al.*, 2012 *J Virol*

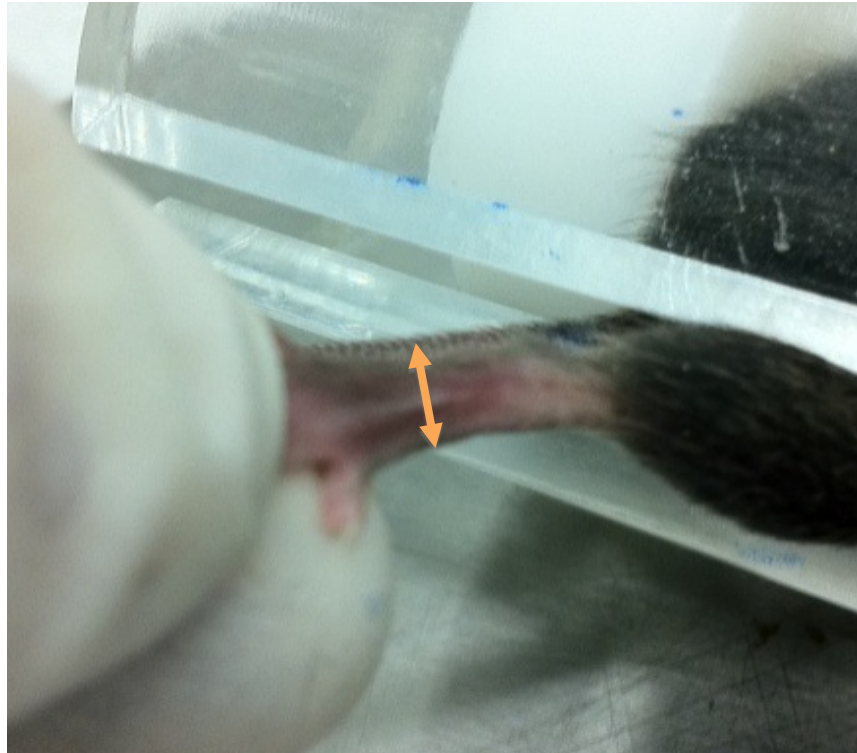
Important for neutralisation and can be used in vaccine designs, and sero-epidemiological studies

# *In vivo* models and pathology

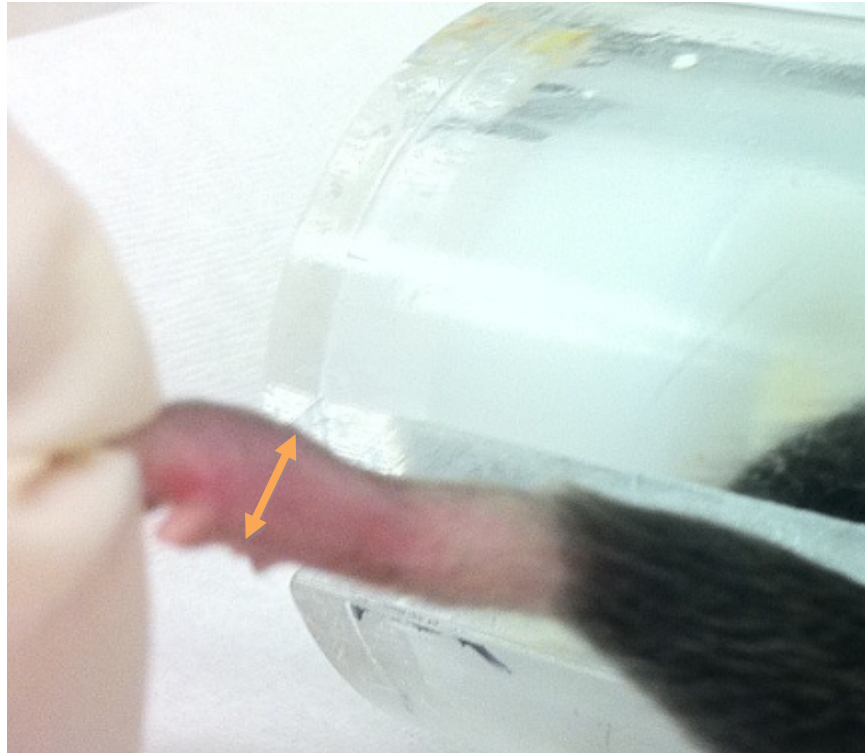


# A CHIKV Mouse Model

Adult WT C57BL/6 mice – 1<sup>st</sup> reported by Gardner *et al.*, 2010 *J Virol*

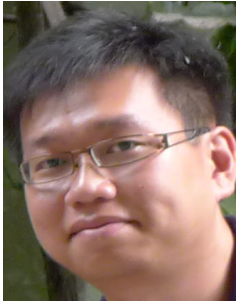


**PBS control**



**CHIKV**

**Mimics human infection in viremia and joint inflammation**

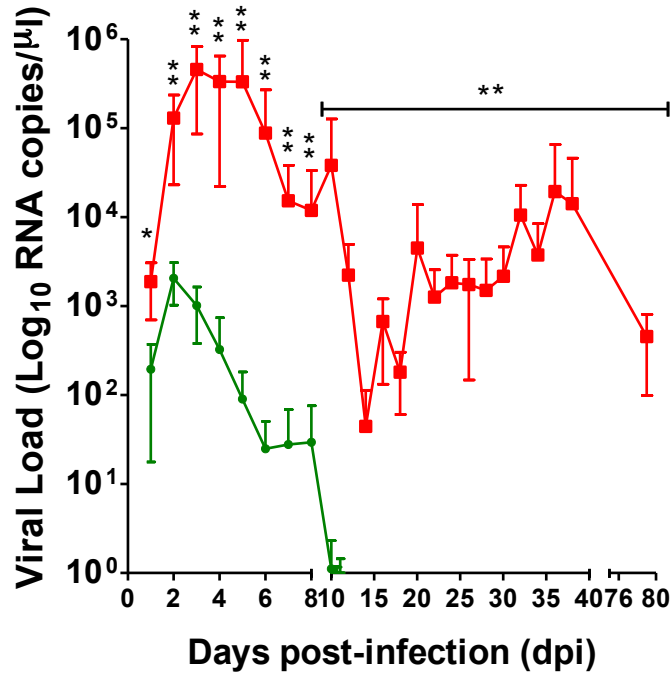


Fok-Moon Lum

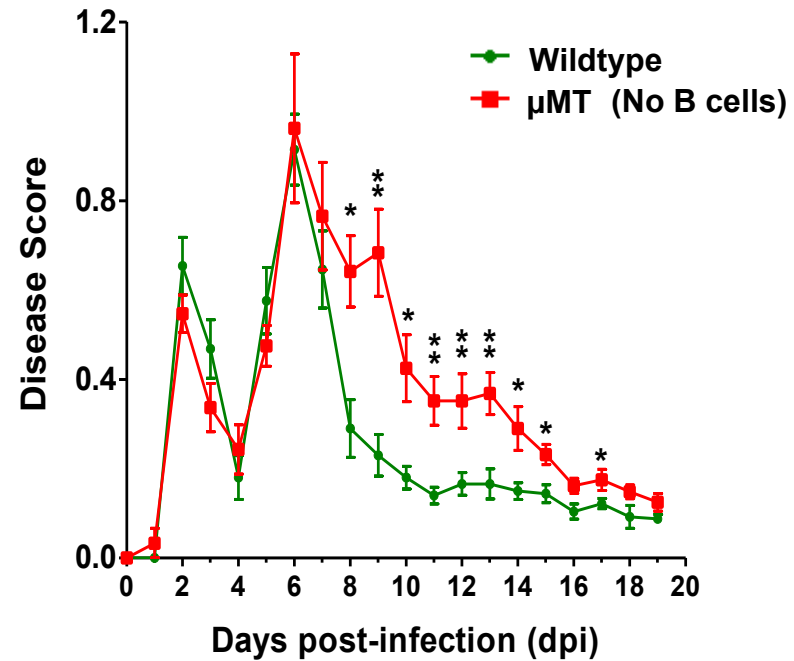
## B cells are essential for control of CHIKV



Teck-Hui Teo

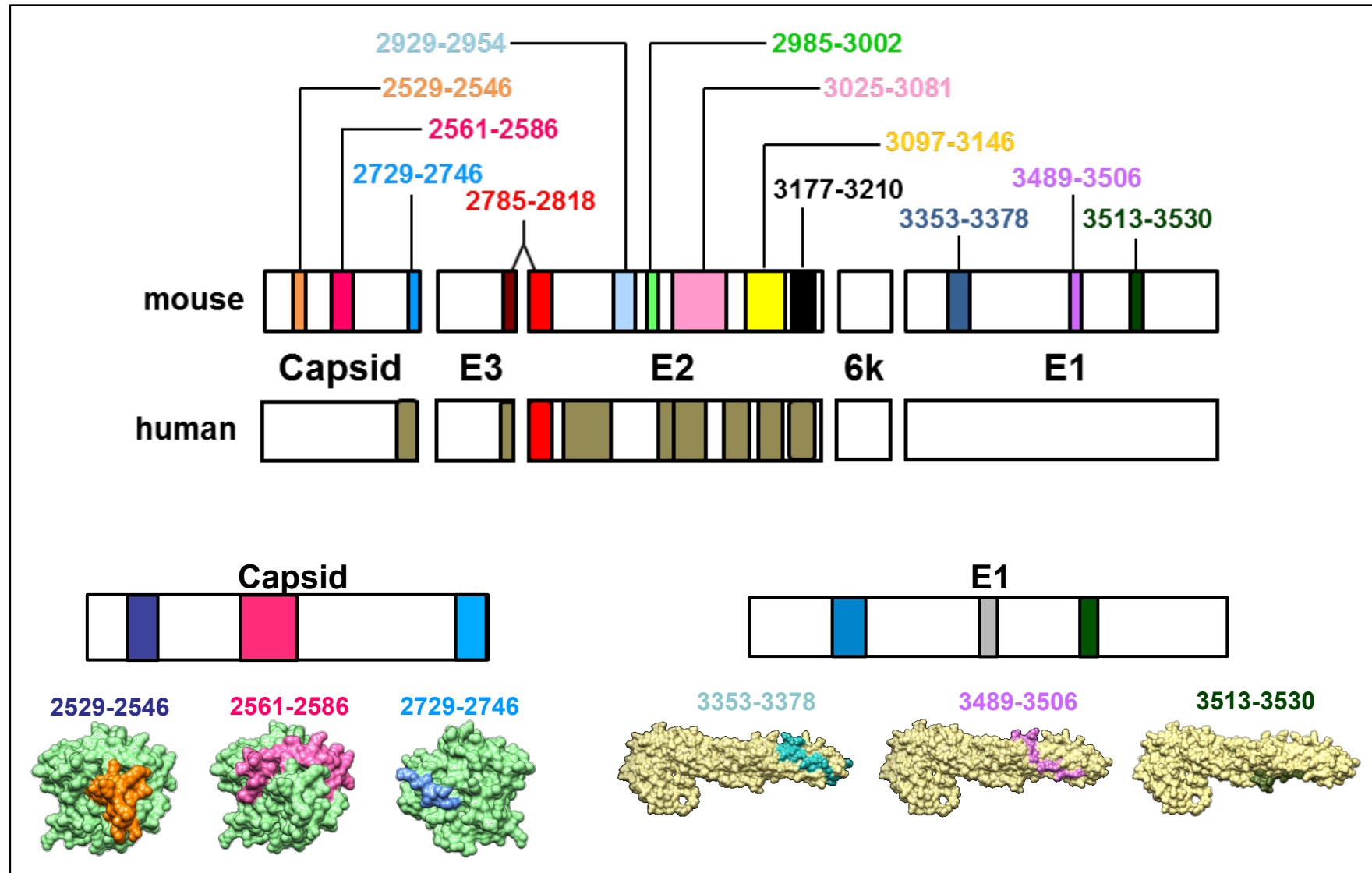


Lum *et al.*, 2013 *J Immunol*



**B cells mediate virus clearance and disease pathology in CHIKV infection**

# CHIKV B cell epitopes between mouse and human



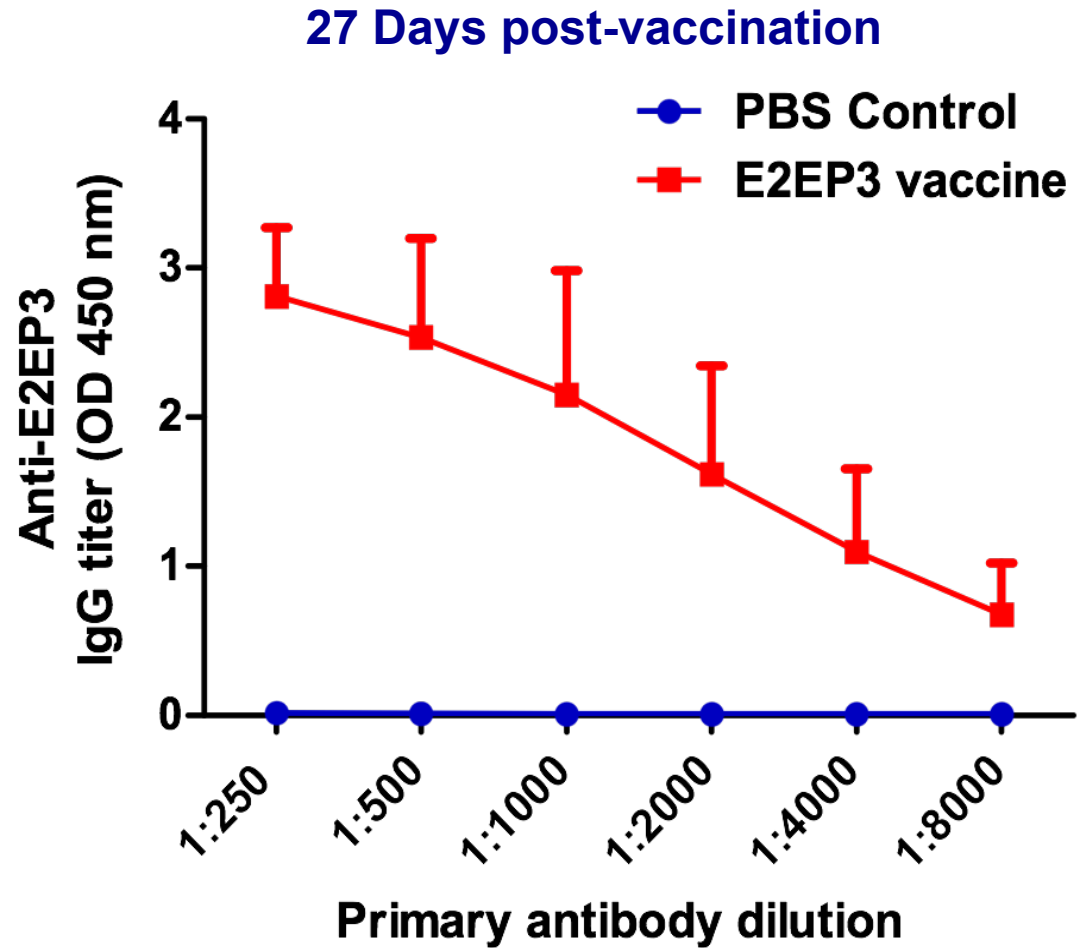
Lum et al., 2013 *J Immunol*

**Additional B cell epitopes recognized by mice**

# E2EP3 as a preclinical vaccine candidate?



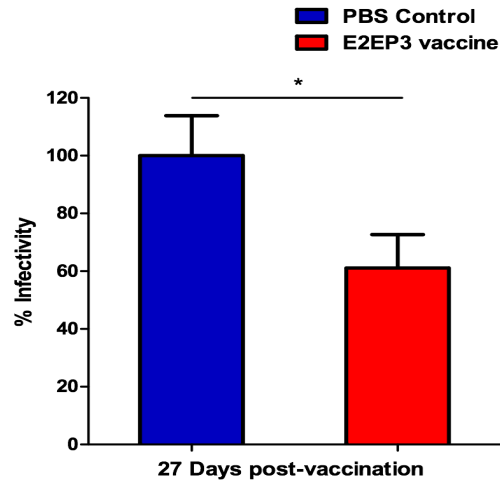
C57BL/6



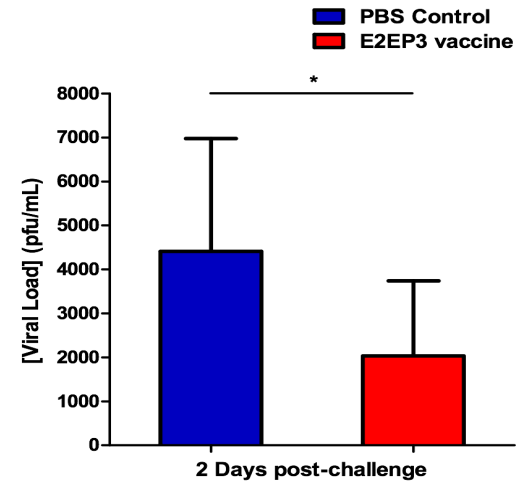
E2EP3-induced antibody response in vaccinated mice

# E2EP3 protects

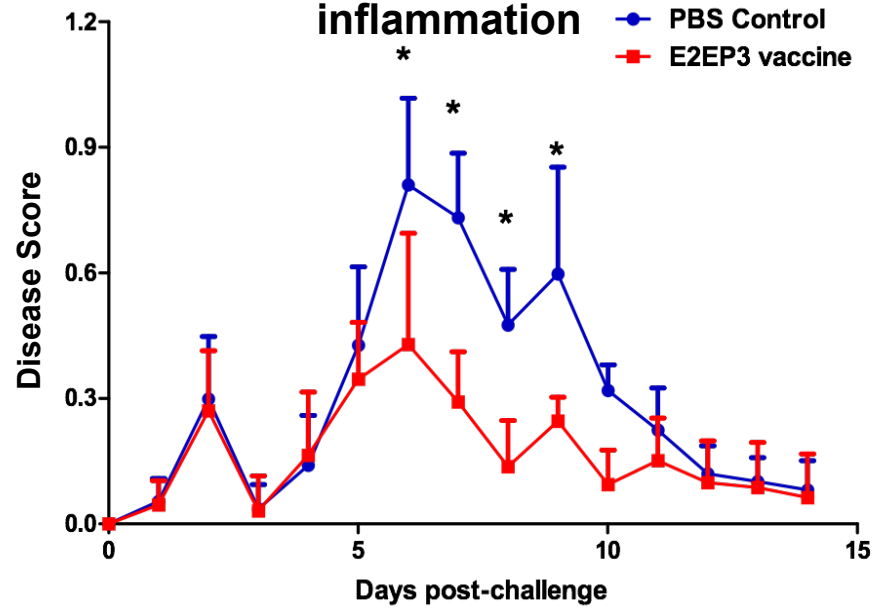
## In-vitro neutralization



## Viremia



## Footpad inflammation





# Mapping of epitopes using post-immunized mice sera

How would the epitope mapping look like with post-immunized mice sera?



David Hallengard



Peter Liljestrom

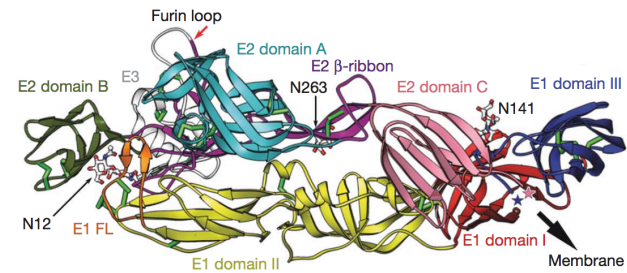
# Vaccine candidates

## Attenuated CHIKV ( $\Delta 5nsP3$ )



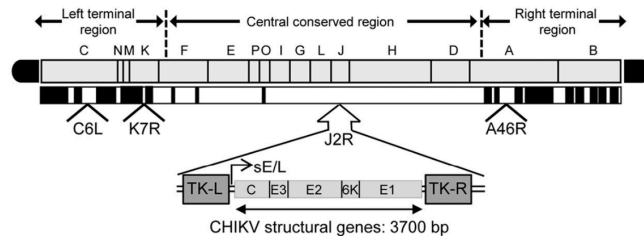
Hallengard et al., 2014 *J Virol*

## Recombinant protein (P62-E1)



Voss et al., 2010 *Nature*

## MVA coding for CHIKV structural proteins (MVA-CHIKV)



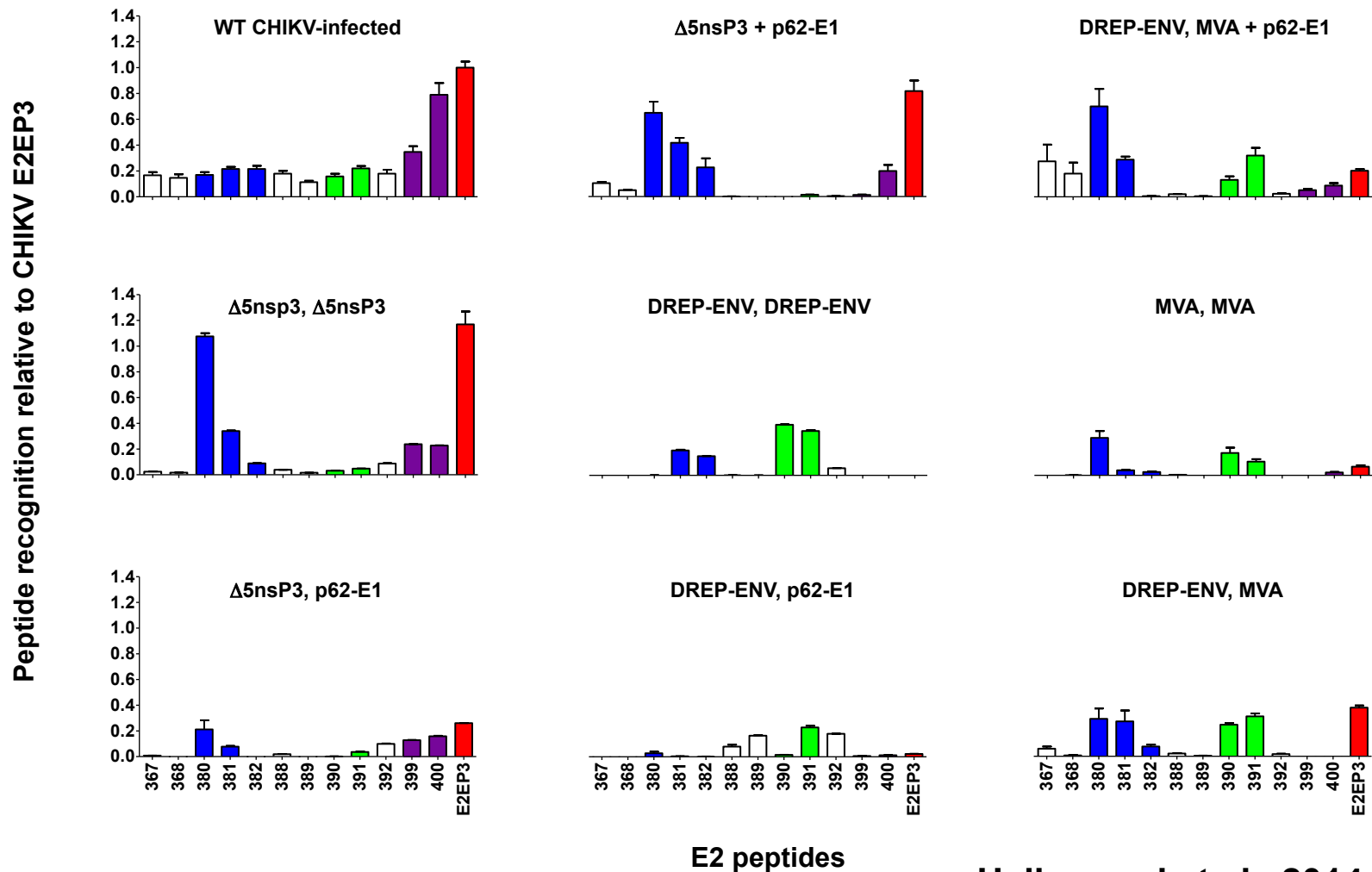
Garcia Arriaza et al., 2014 *J Virol*

## DNA replicon encoding CHIKV envelope proteins (DREP-ENV)



Knudsen et al., 2015 *PLoS One*

# Linear epitope mapping within CHIKV E2 glycoprotein



Hallengard et al., 2014 *J Virol*

Epitopes targeted were mapped on to E2 glycoprotein targeting mainly 4 regions: (1) Blue; acid sensitive region, (2) Green; solvent exposed of CHIKV E2, (3) Purple; Immuno-dominant in other studies, (4) Red; E2EP3 peptide

# Linear epitope mapping within CHIKV E2 glycoprotein

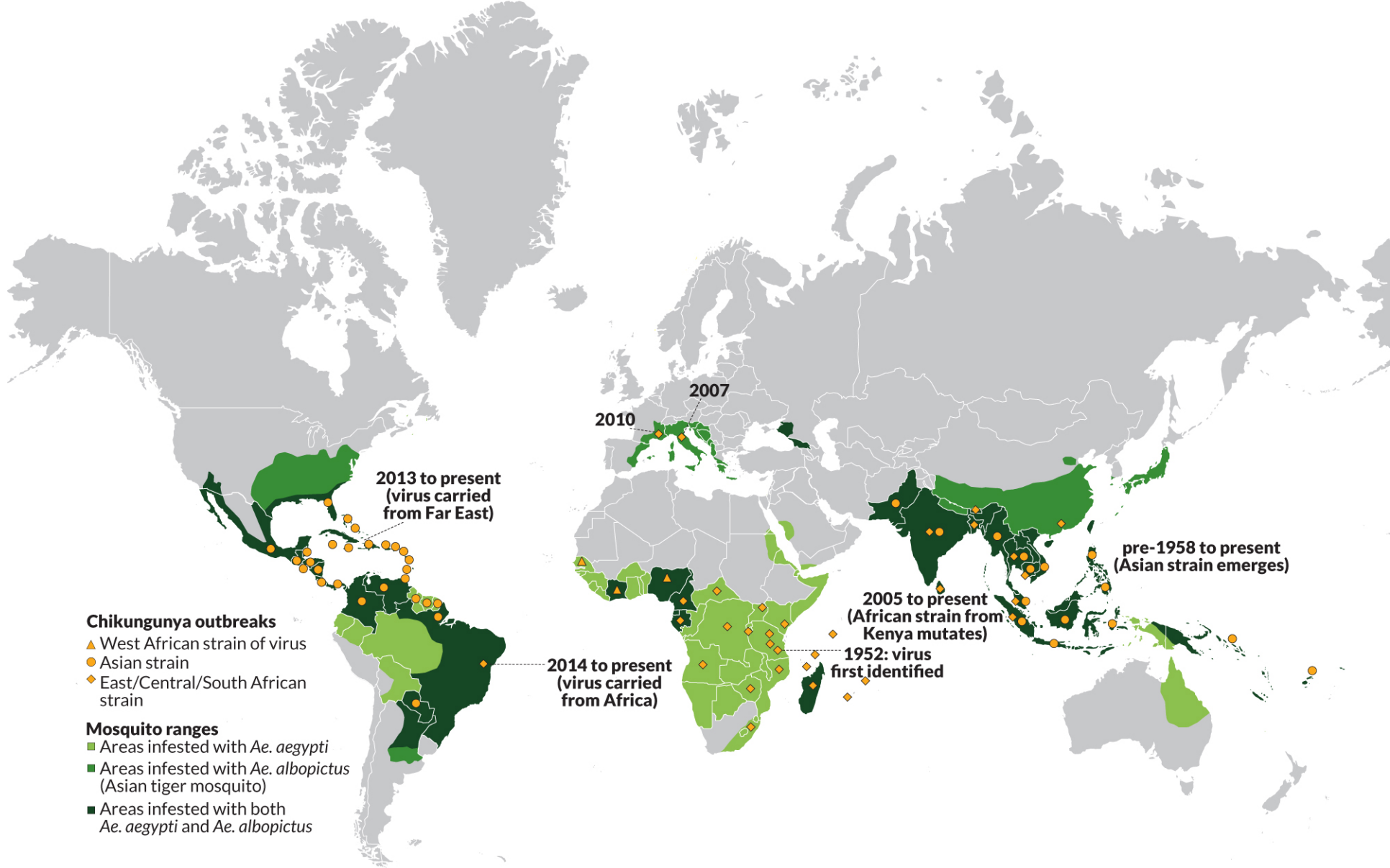
Peptides	Polypeptide sequence <sup>a</sup>	Amino acid sequence covered	Description	References
380-382 (region 1)	3033-3066	KWQYNSPLVPRNAELGDRKGKI HIPFPLANVTGR	Sits in the acid sensitive region, thought to play a role in virulence and immuno-regulation	(Akahata and Nabel, 2012; Gardner et al., 2014)
390-391 (region 2)	3113-3138	KKEVLTVPTEGLEVTWGNNEP YKYW	Immuno-dominant region mapped to the exposed region of the E2 glycoprotein	(Kam et al., 2012c)
399-400 (region 3)	3185-3210	AGMCMCARRRCITPYELTPGAT VPFL	Immuno-dominant region / no crystal structures available currently	(Kam et al., 2012c)
E2EP3 (region 4)	2800-2818	STKDNFNVYKATRPYLAHC	Highly recognized single linear epitope located proximally to a conserved furin E2/E3 cleavage site	(Kam et al., 2012b)

<sup>a</sup> These numbers correspond to the position of the stated amino acid along the CHIKV proteome. The first amino acid is from nsP1 and is annotated as 1.

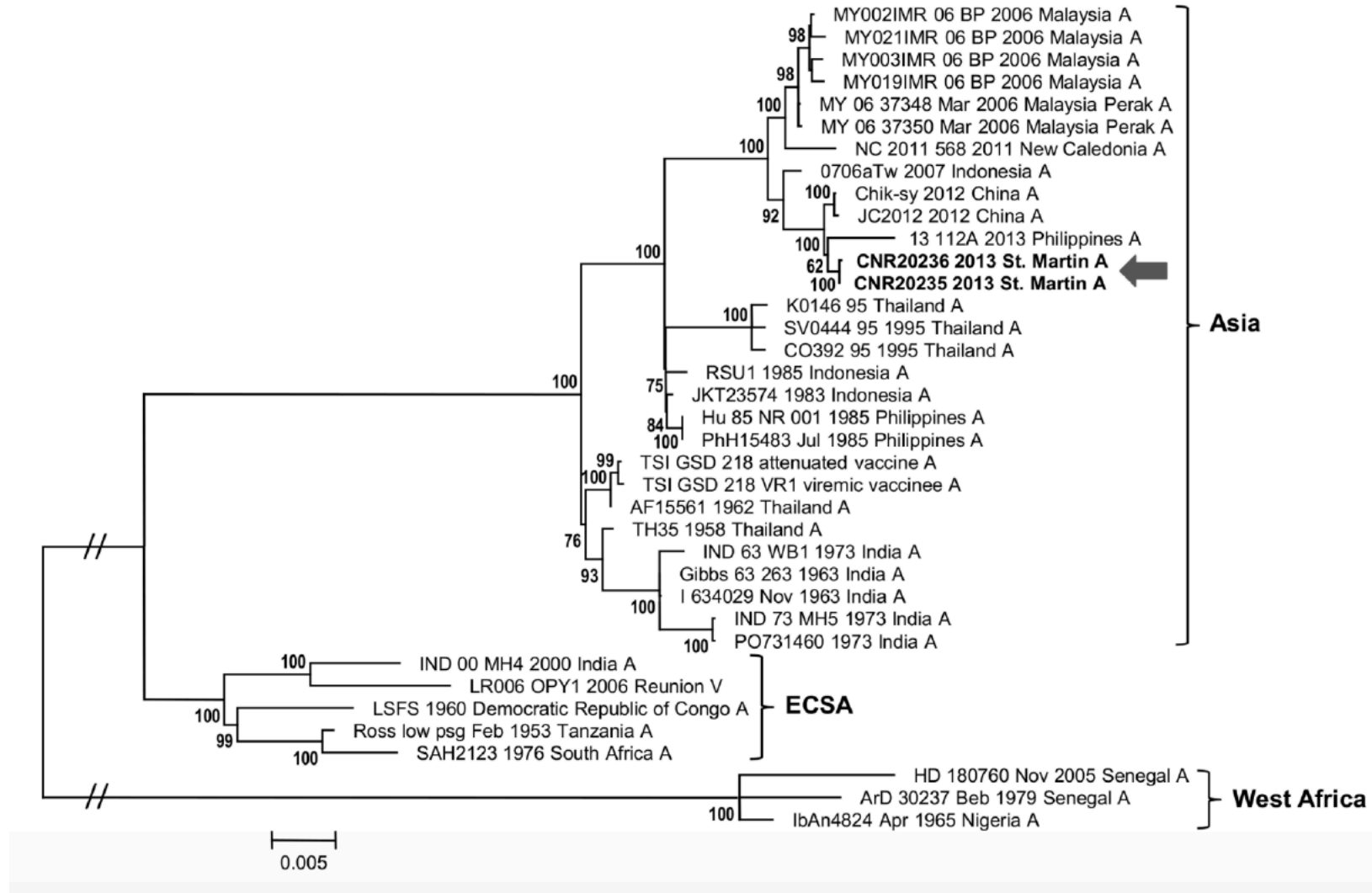
Hallengard et al., 2014 *J Virol*

**Do all vaccines generate protective immune responses against the CHIKV?**

# Increasing spread of CHIKV around the world



# Both viruses belong to different genotypes



Leparc-Goffart *et al.*, 2014, *Lancet*

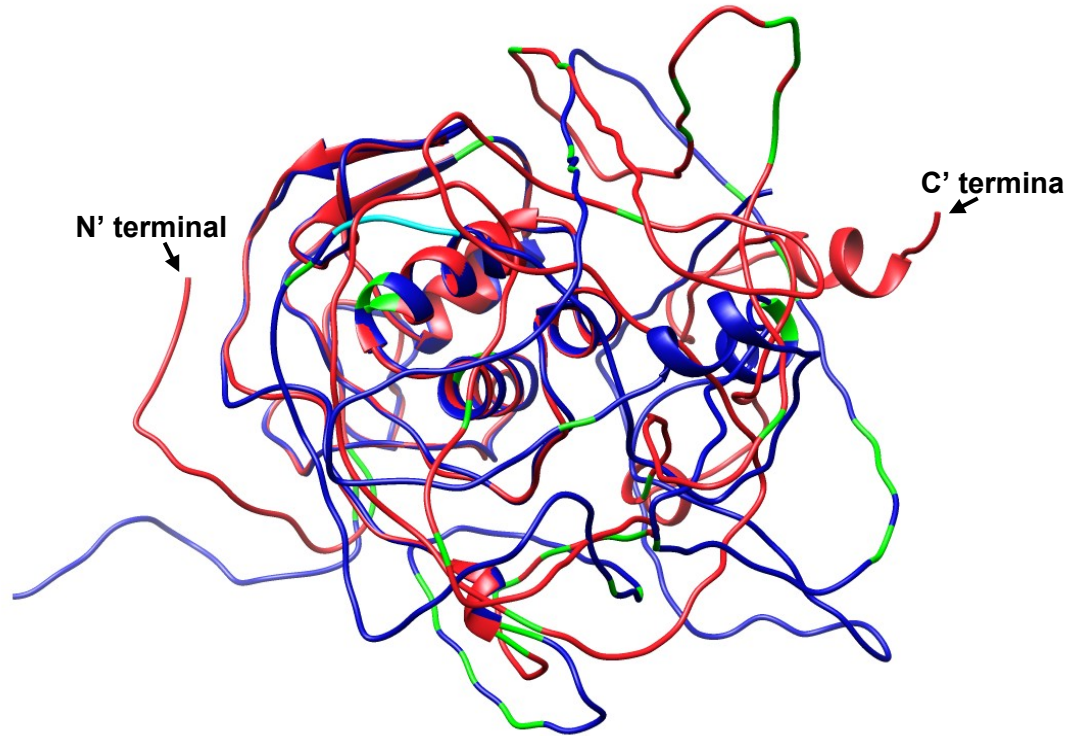


Teck-Hui Teo



Zhisheng Her

# Specific 4 amino acid deletion in the NSP3



■ LR2006 OPY1   
 ■ CNR20235   
 ■ Mutation positions   
 ■ Deletion positions

## Any differences in replication efficiencies?



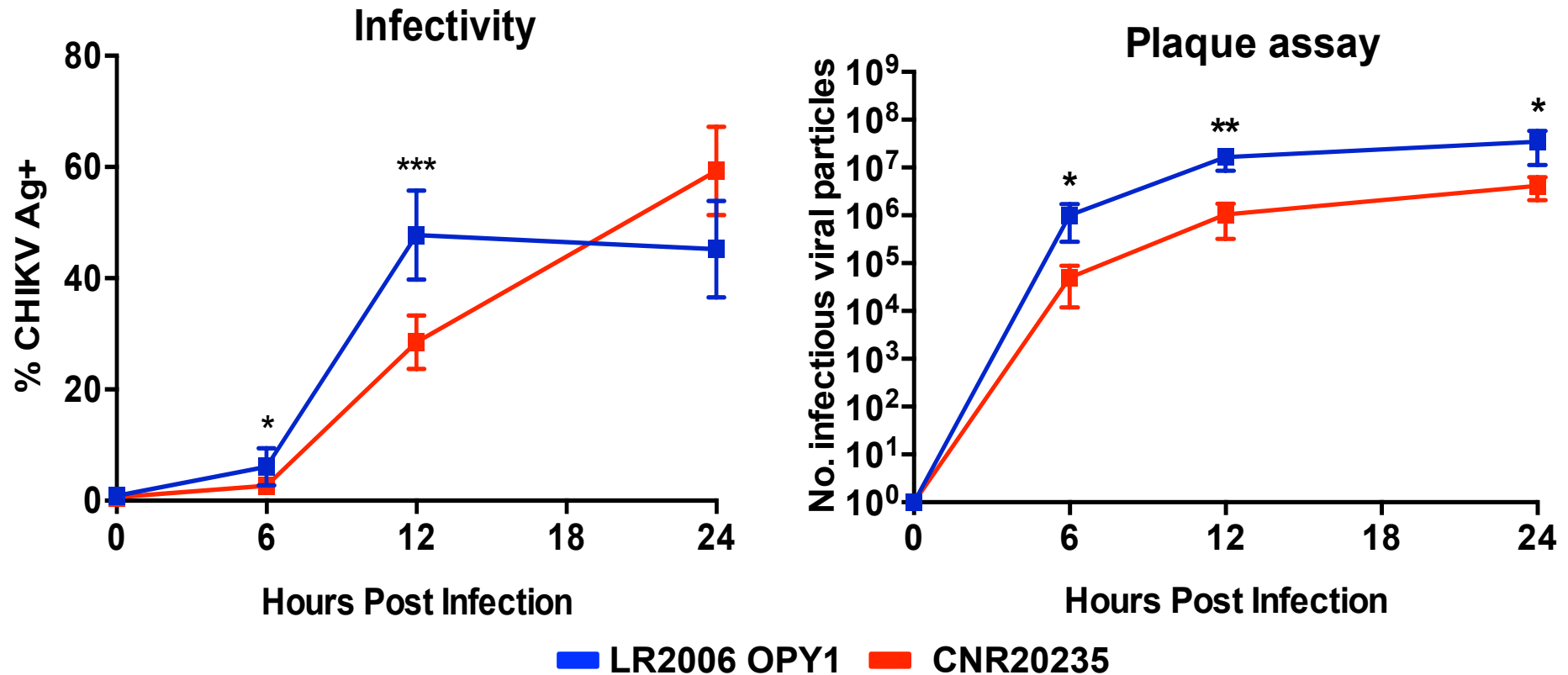
Fok-Moon Lum



Jeslin Tan

# *In vitro* infection kinetics of LR2006 OPY1 and CNR20235

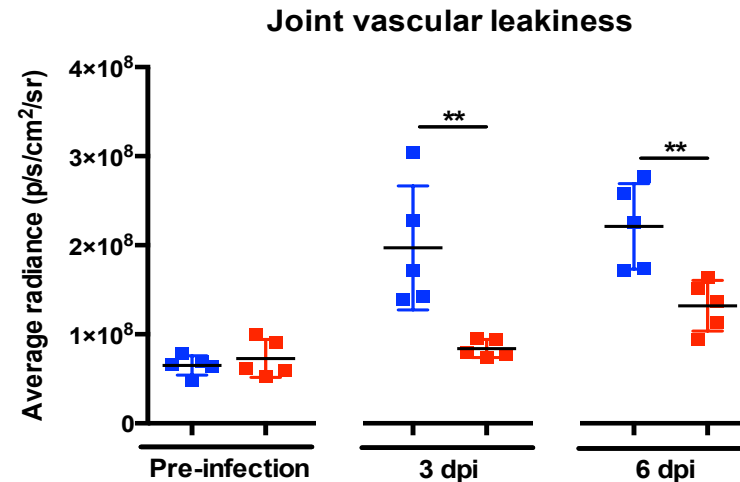
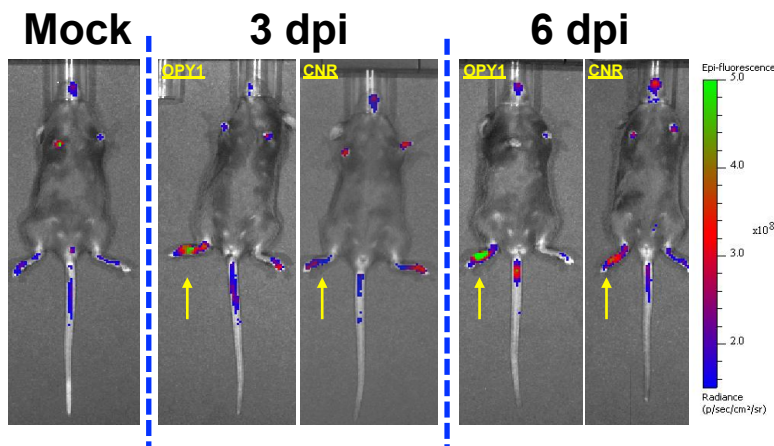
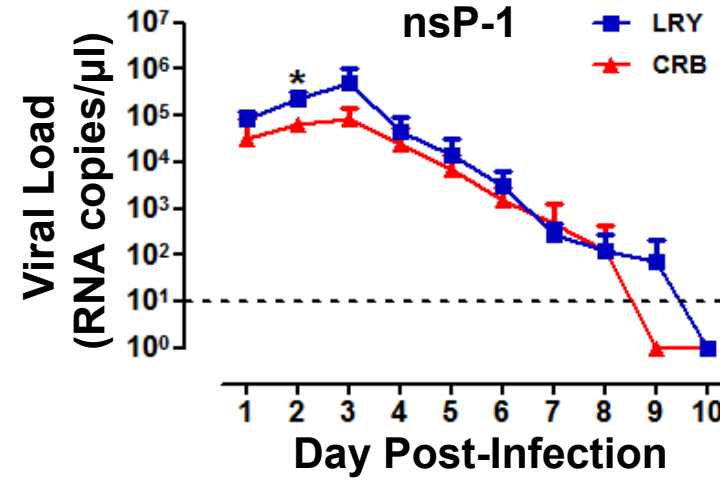
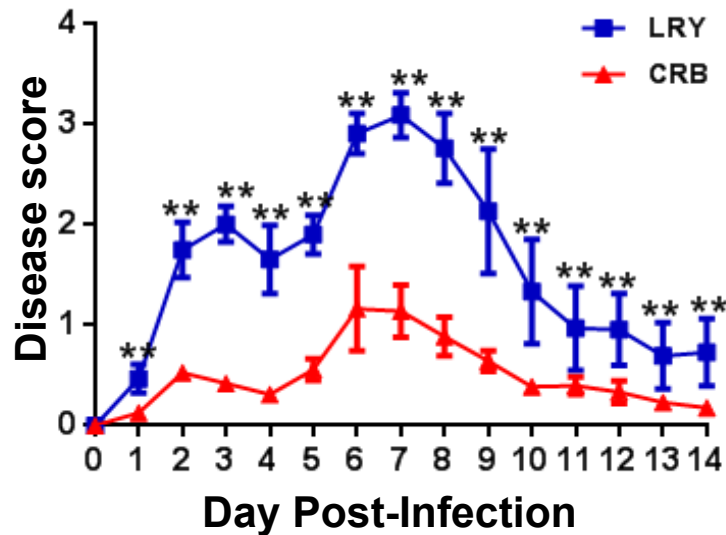
## Mouse tail fibroblasts



**CNR20235 has a lower early replicative capacity than LR2006 OPY**

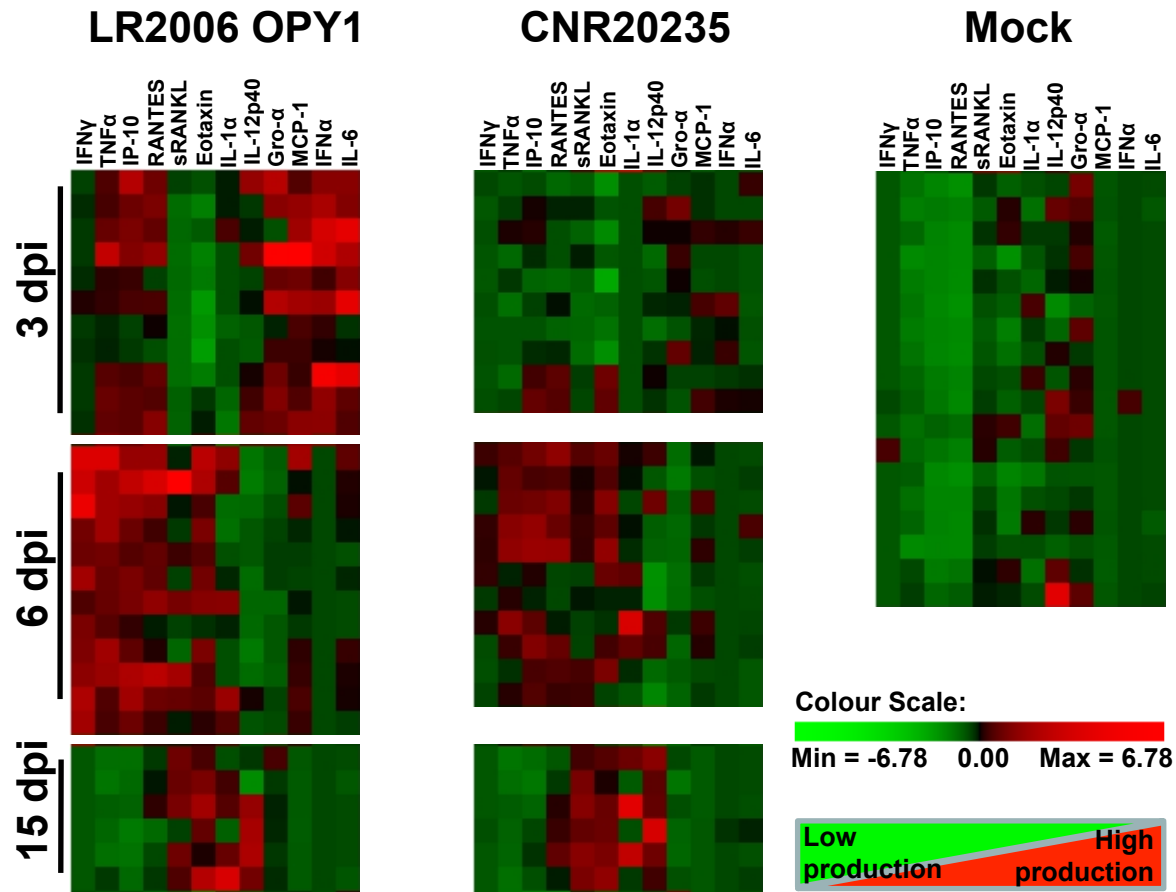


# Differential pathological disease outcome in mice



**CNR20235 induces a less pronounced pathology than LR2006 OPY1**

# Contrasting host inflammatory response in infected mice



Teo *et al.*, 2015, *J Virol*

**CNR20235 induces a milder systemic inflammatory response than LR2006 OPY1**

# Markers of Infection, Pathogenesis and Protection

1. High levels of IgM and IgG
2. Neutralising capacity of CHIKV-specific antibodies
3. E2EP3 and other epitopes as markers of CHIKV infection

**Form the basis for future translational studies**

**What has been happening?**

## **Flavivirus - Zika virus**

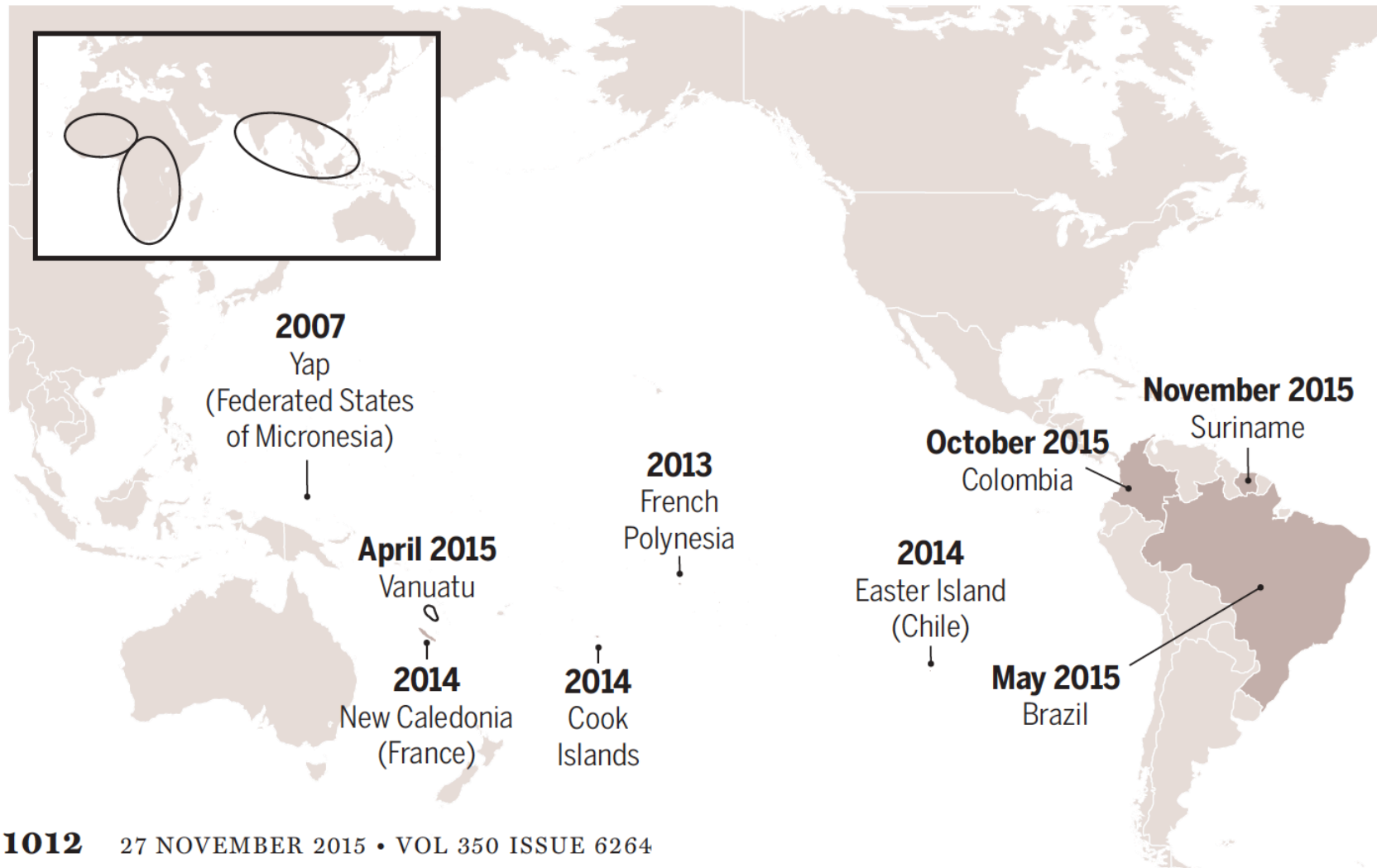
***An obscure mosquito-borne disease goes global***

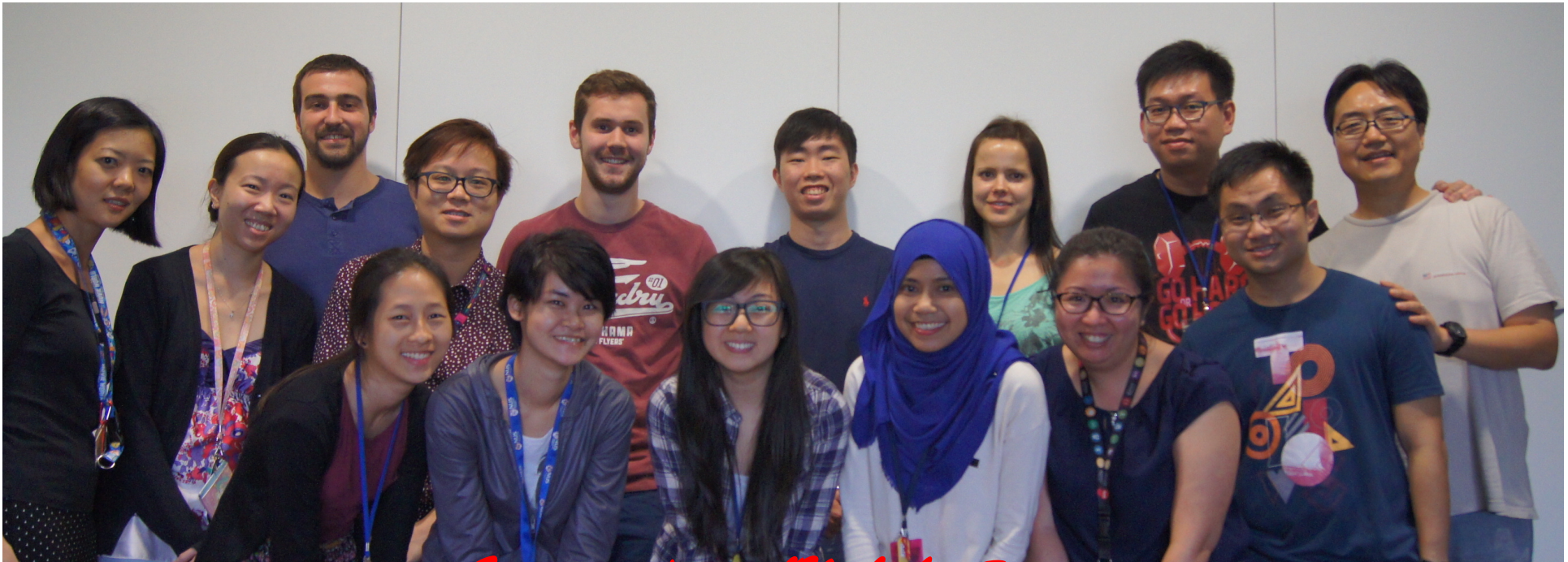
After racing through Oceania last year, the Zika virus is now spreading in the Americas



## Virus on the move

Zika, which occurred sporadically in Africa and Southeast Asia before 2007 (inset), has caused major outbreaks in Oceania and now has reached the Americas.





*Greetings from LN Lab, Singapore*



# Acknowledgements



*LN Lab*



*Integrated Chikungunya  
RESearch # 261202*



*Pierre Roques*



*Andres Merits*

*Anis Labi &  
Flow Cytometry*

*Mouse Core*

*Immune Monitoring*



*Xavier de Lamballerie  
Isabelle Leparc-Goffart*



*Ann Powers*



*Peter Liljeströme*

*Laurent Rénia  
Olaf Röttschke*

[lisa\\_ng@immunol.a-star.edu.sg](mailto:lisa_ng@immunol.a-star.edu.sg)



*Gardens by the Bay, Singapore*