

# **Imipramine Inhibits Chikungunya Virus Replication in Human Skin Fibroblasts through Interference with Intracellular Cholesterol Trafficking**

**SINEEWANLAYA WICHIT**

**Faculty of Medical Technology**

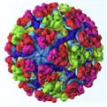
**Mahidol University**

JOINT INTERNATIONAL TROPICAL MEDICINE MEETING 2017

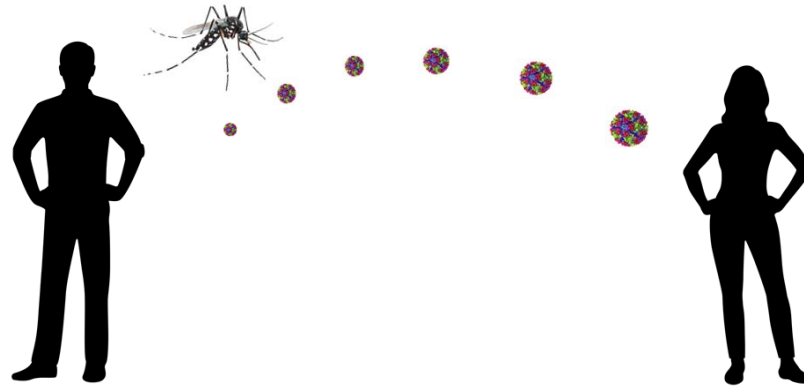
Amari Watergate Bangkok, Thailand

7<sup>th</sup> December 2017





# ARBOVIRUS: (Arthropod-Borne Virus)



“Viruses that are transmitted to vertebrate hosts by hematophagous arthropod vectors”



Mosquito



Sand fly



Midges



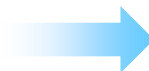
Tick

*Flaviviridae: Flavivirus*

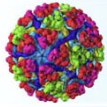


Dengue and Zika virus

*Togaviridae: Alphavirus*



Chikungunya virus



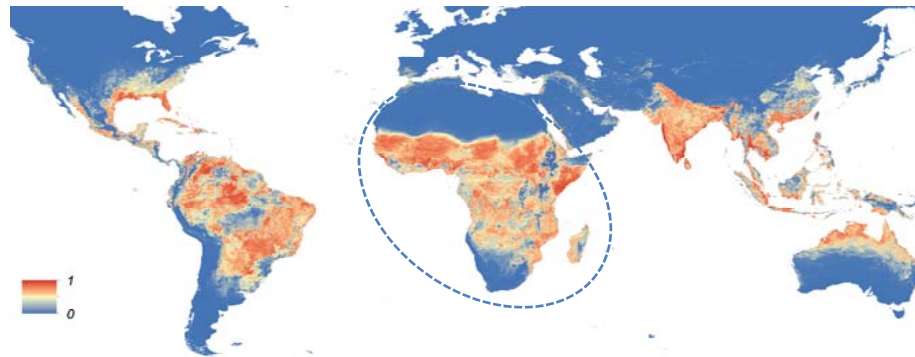
## ARBOVIRUS: Mosquito Vector



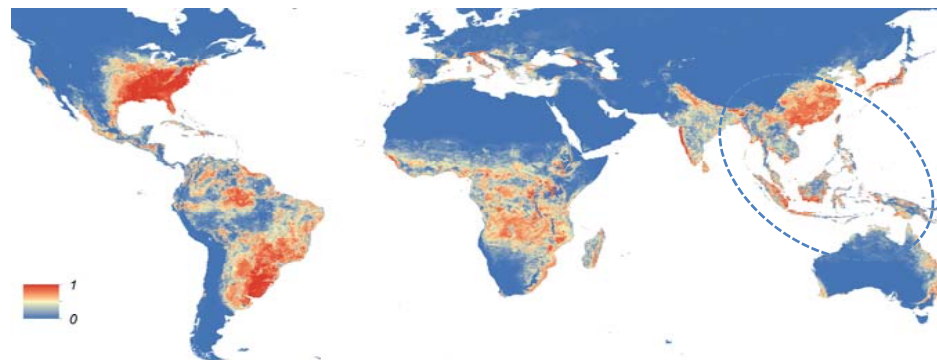
*Aedes aegypti*



*Aedes albopictus*

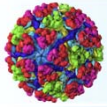


- Originated in sub-Saharan Africa, spread throughout the tropics centuries ago

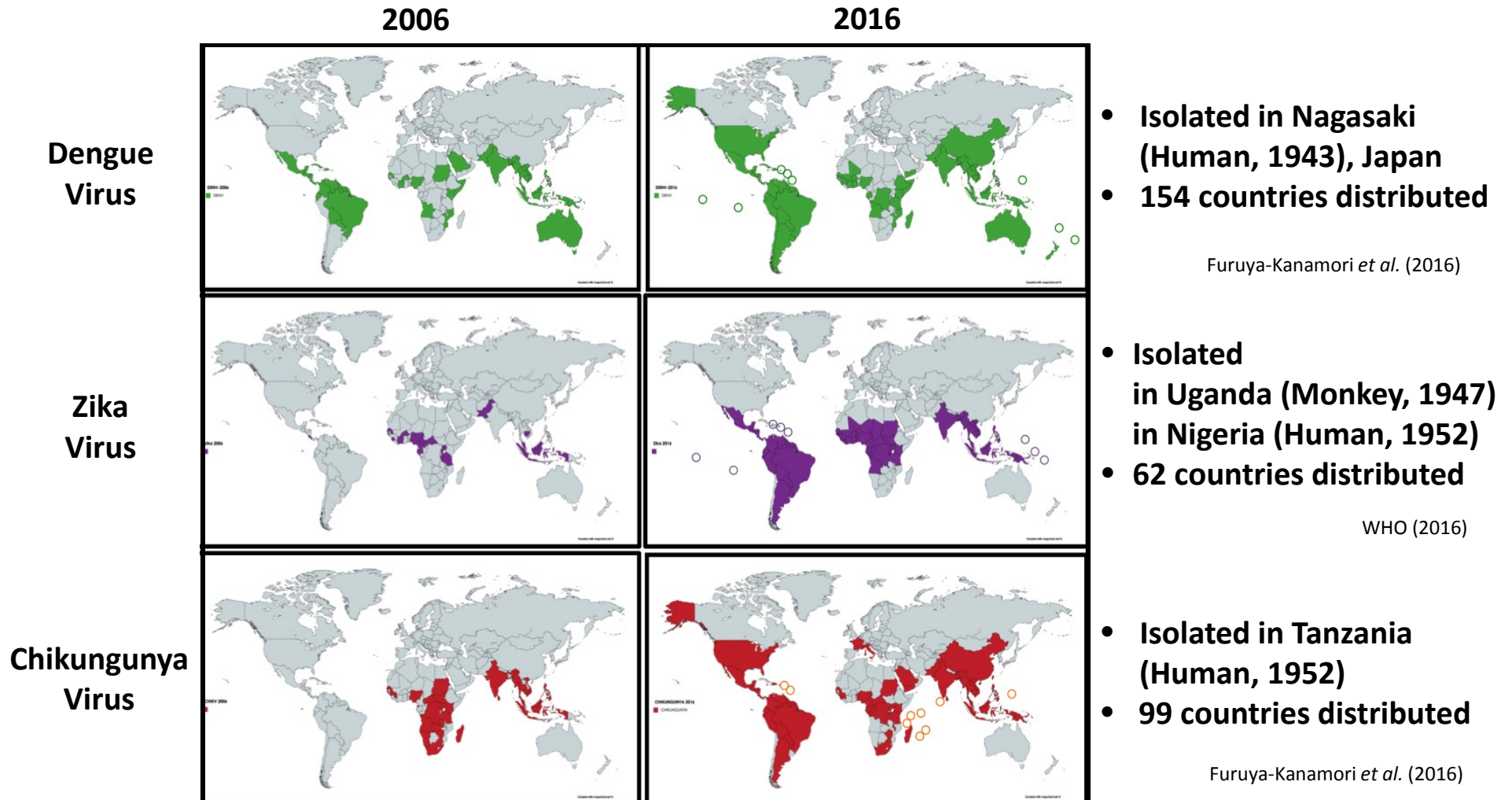


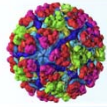
- Originated in Asia, spread to the Americas, Africa and Europe beginning in 1985

**3 billion people living in *Aedes*-infested regions** (Kraemer *et al.*, 2015)

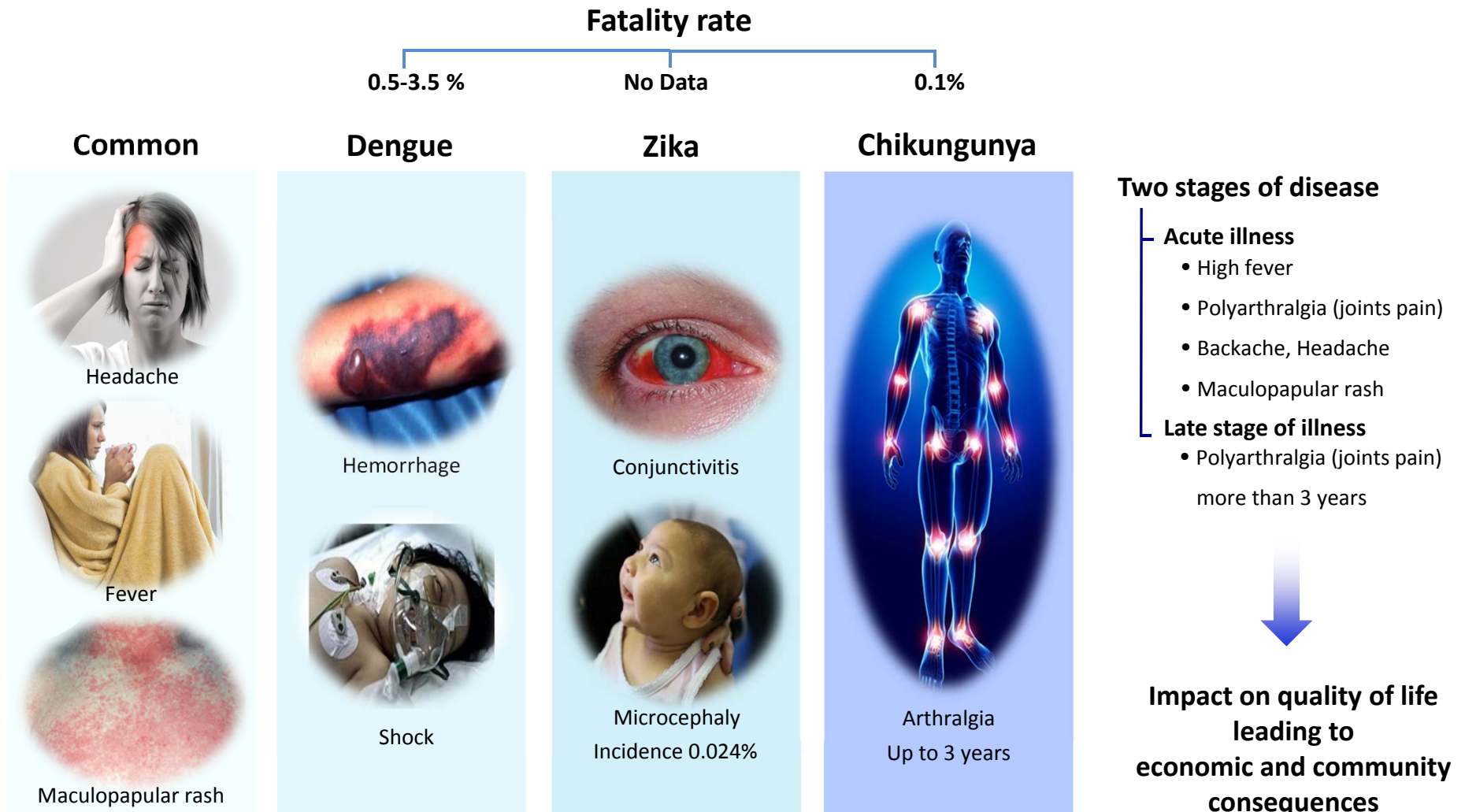


# ARBOVIRUS: Geographic distribution

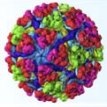




# ARBOVIRUS: Clinical manifestation

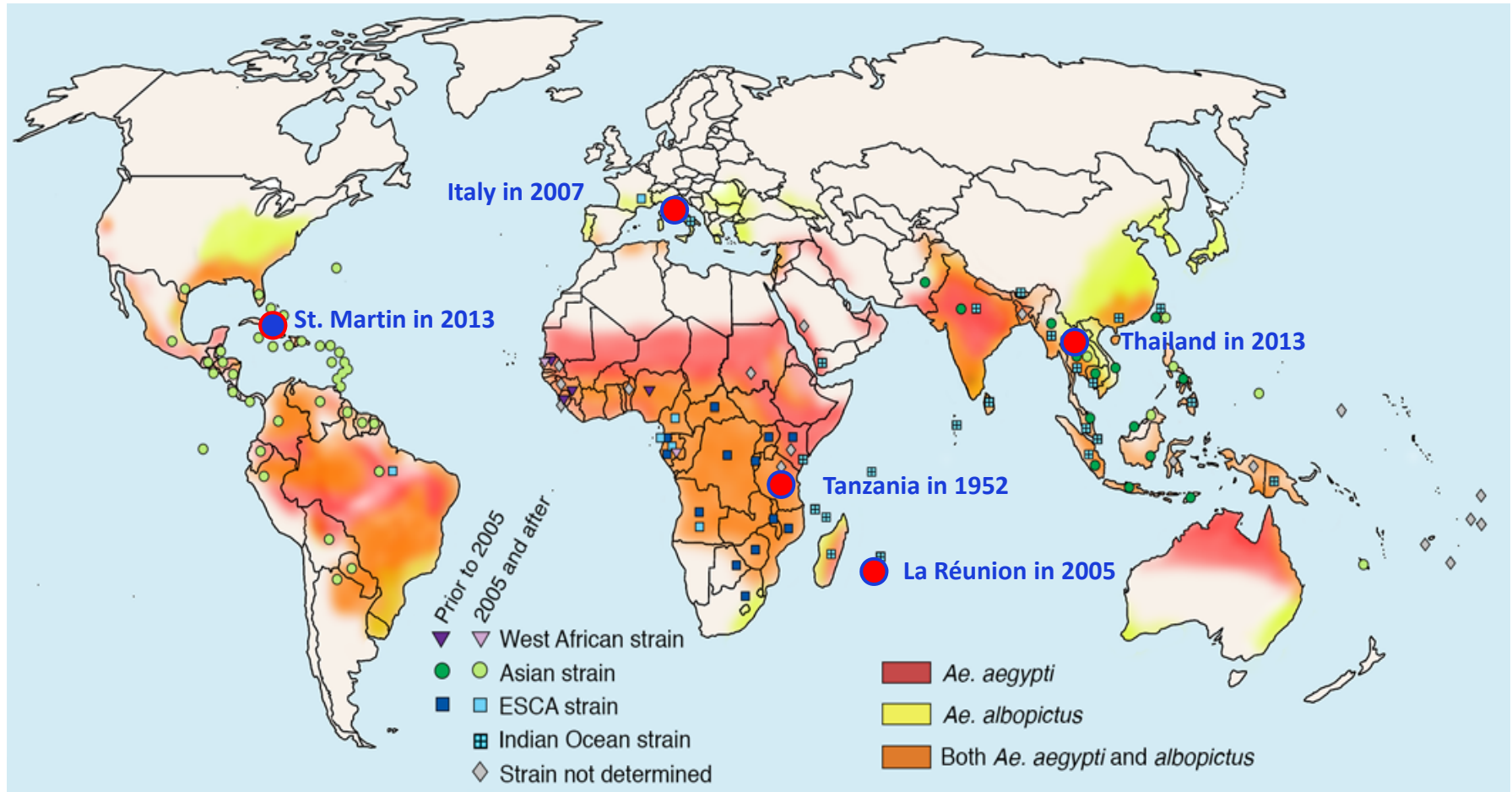


Furuya-Kanamori *et al.* (2016), loos *et al.* (2014), Patterson *et al.* (2016)

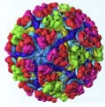


# CHIKUNGUNYA VIRUS: Geographic distribution

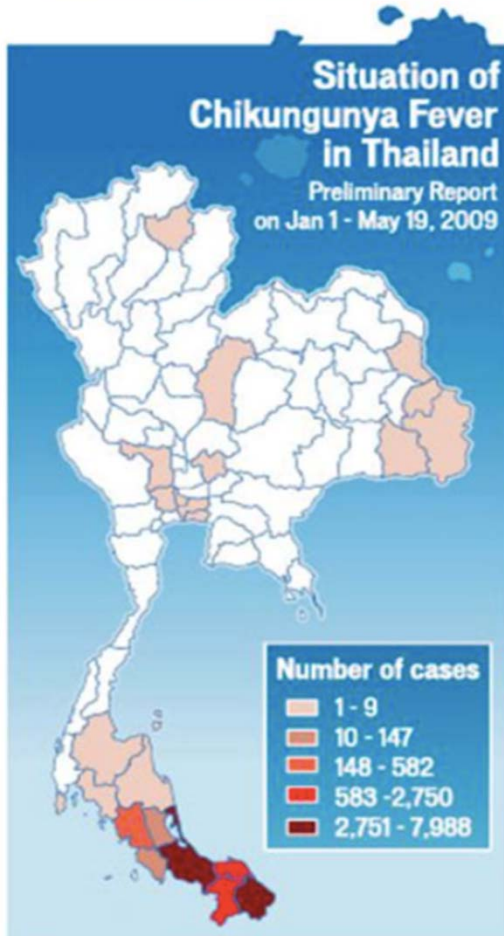
CHIKUNGUNYA becomes one of the most important arboviral diseases



(Silva and Dermody, 2017)



# CHIKUNGUNYA VIRUS: Geographic distribution

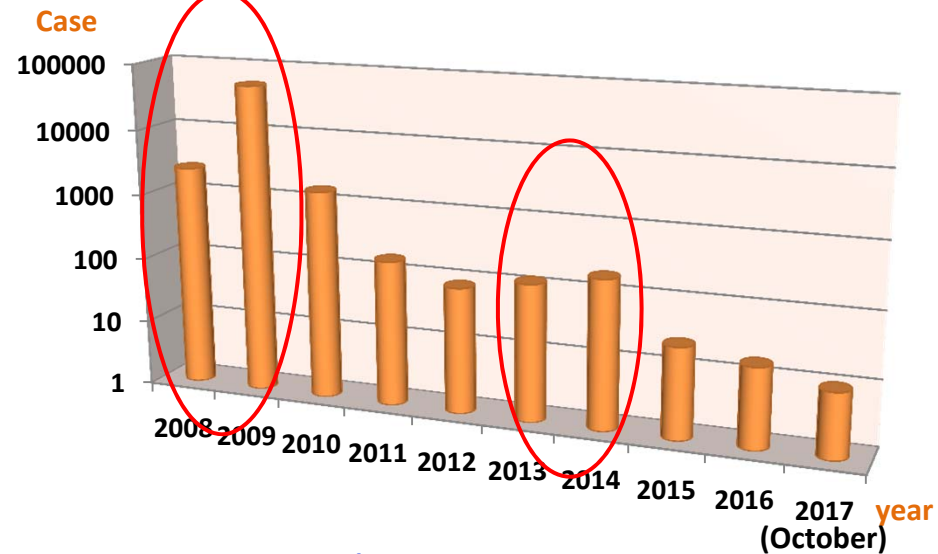


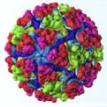
Volume 20, Number 8—August 2014

EMERGING INFECTIOUS DISEASES\*

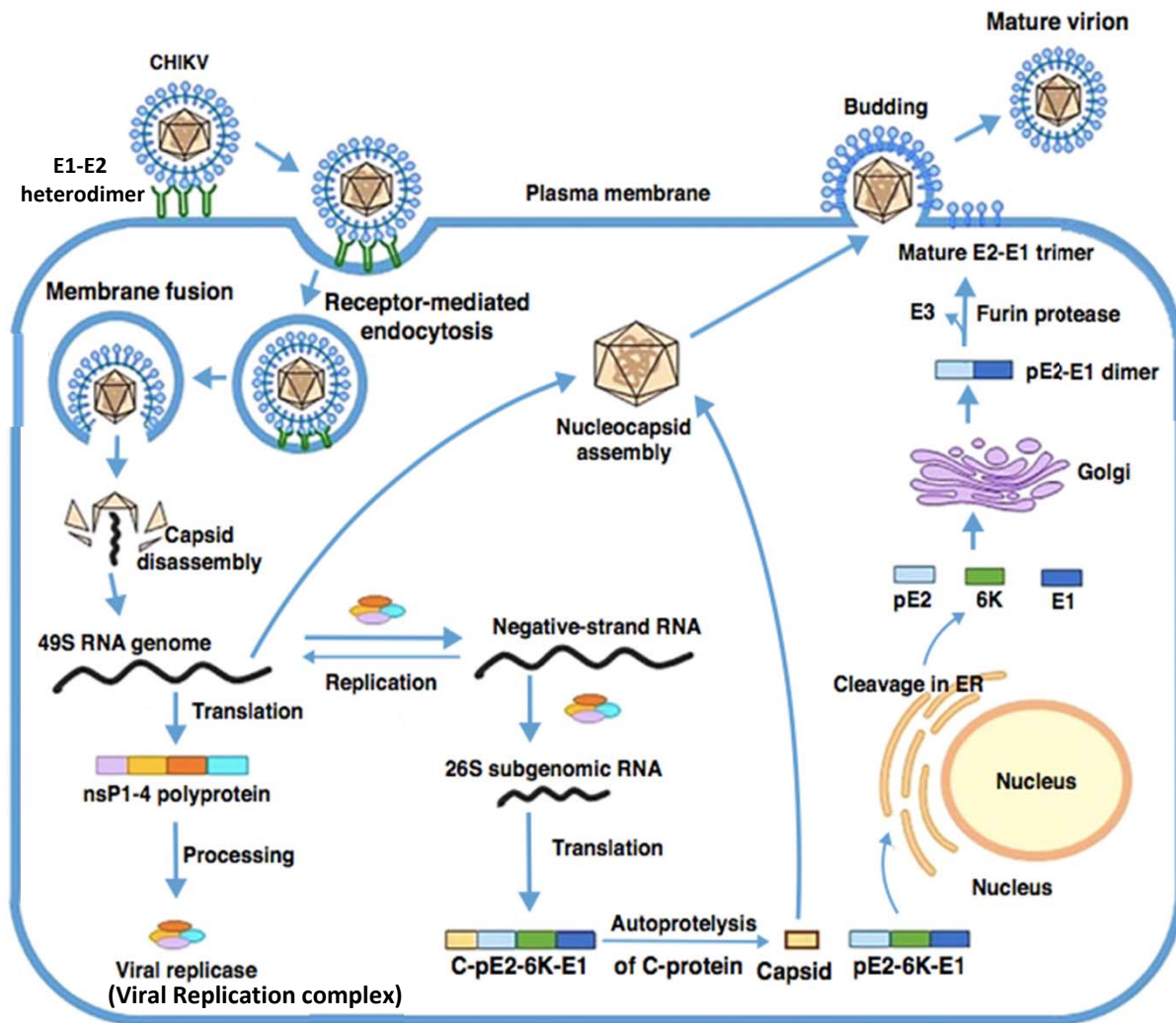
Letter

Chikungunya Outbreak in Bueng Kan Province, Thailand, 2013





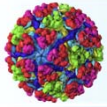
# CHIKUNGUNYA VIRUS: Replication cycle



Johan et al. (2017)

- Virus enters host cell by endocytosis
- Two rounds of translation occur to produce
  - 4 non-structural proteins (nsP1, 2, 3 and 4)
  - 5 structural proteins (C, E3, E2, 6k, E1)
- Assembly occurs at the cell surface
- New viral particles ready to buds from host cell membrane

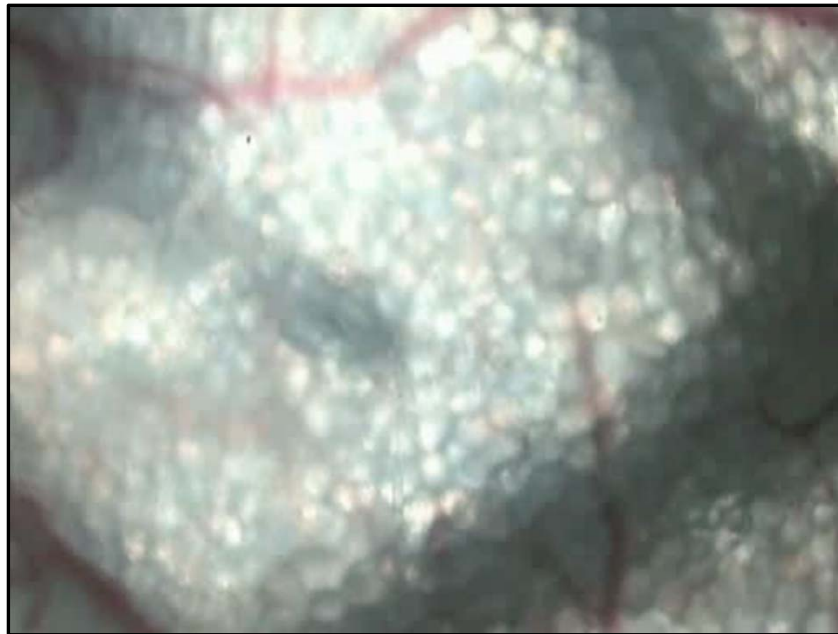




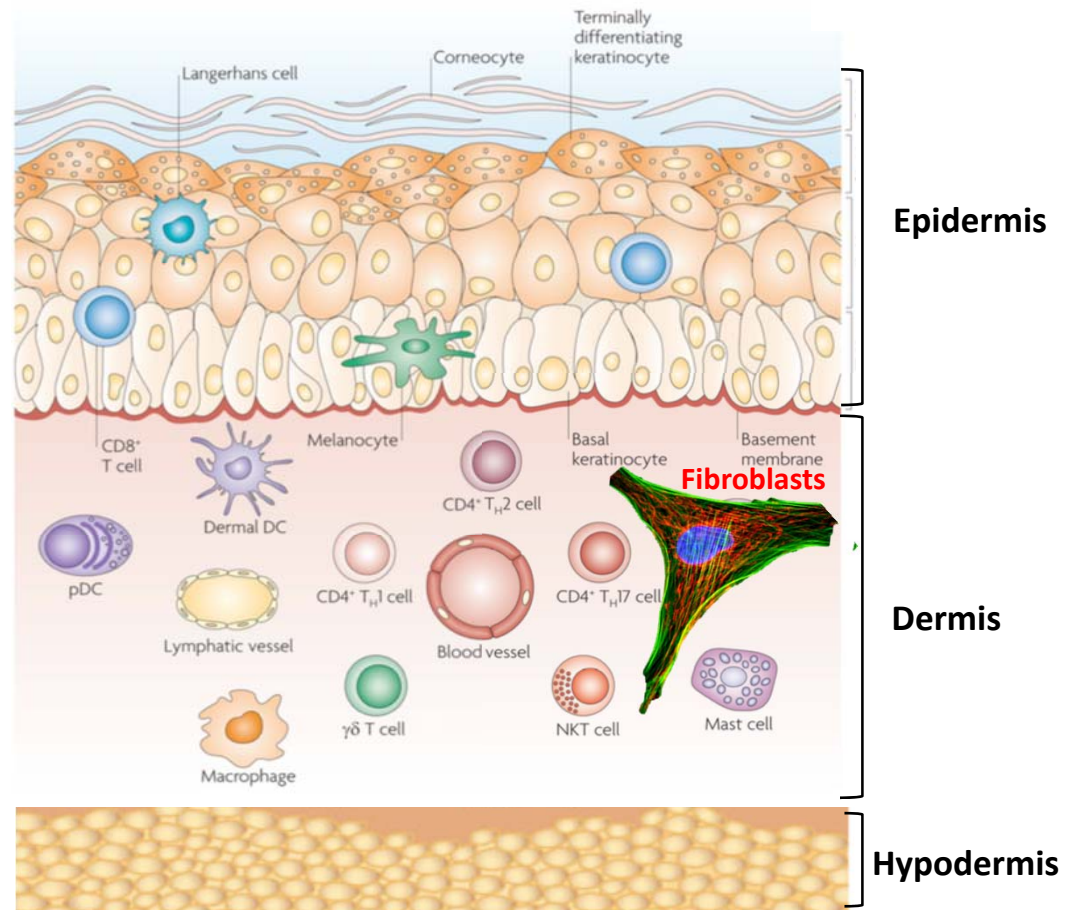
# Human Skin Fibroblasts

## Human skin fibroblasts are permissive to CHIKV and ZIKV

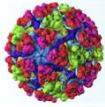
(Ekchariyawat *et al.*, 2015 and Hamel *et al.*, 2015)



Missé *et al.* (2014)



Nestle *et al.* (2009)



# Cholesterol and Niemann-Pick type C Protein (NPC)

JOURNAL OF VIROLOGY, July 2008, p. 6470-6480  
0022-538X/08/\$08.00+0 doi:10.1128/JVI.00117-08  
Copyright © 2008, American Society for Microbiology. All Rights Reserved.

Vol. 82, No. 13

## Cholesterol Effectively Blocks Entry of Flavivirus<sup>∇</sup>

Chyan-Jang Lee,<sup>1</sup> Hui-Ru Lin,<sup>1</sup> Ching-Len Liao,<sup>2</sup> and Yi-Ling Lin<sup>1,2,3\*</sup>

*Institute of Biomedical Sciences,<sup>1</sup> Genomics Research Center,<sup>3</sup> Academia Sinica, and Department of Microbiology and Immunology, National Defense Medical Center,<sup>2</sup> Taipei, Taiwan, Republic of China*

2008

*Virology*, 2009 Jun 20;389(1-2):8-19. doi: 10.1016/j.virol.2009.03.025. Epub 2009 May 5.

## Cholesterol biosynthesis modulation regulates dengue viral replication.

Rothwell C,<sup>1</sup> Lebreton A, Young Ng C, Lim JY, Liu W, Vasudevan S, Labow M, Gu F, Gaither LA.

2009

OPEN ACCESS Freely available online



## Endocytosis of Chikungunya Virus into Mammalian Cells: Role of Clathrin and Early Endosomal Compartments

Eric Bernard<sup>1,3</sup>, Maxime Solignat<sup>1,3</sup>, Bernard Gay<sup>1</sup>, Nathalie Chazal<sup>1</sup>, Stephen Higgs<sup>2</sup>, Christian Devaux<sup>1</sup>, Laurence Briant<sup>1\*</sup>

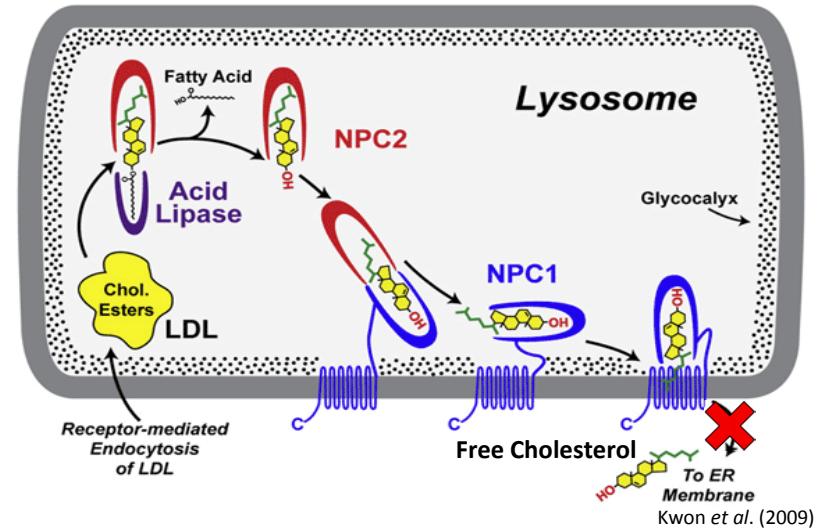
2010



Abnormalities in cholesterol metabolism

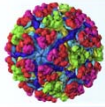


**Inhibits CHIKV infection**



## Niemann-Pick C (NPC) disease

- **Dysfunctional NPC activity causes NPC disease type C**
  - a rare, autosomal recessive, neurodegenerative disorder
- **Composes of NPC1 and NPC2**
- **Causes cholesterol accumulation in LE/Ls**



# U18666A and IMIPRAMINE

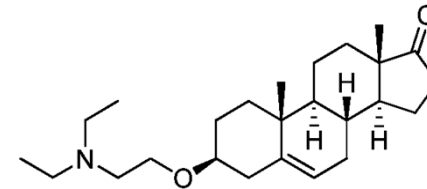
- A class II amphiphilic hydrophobic amine
- Inhibits of cholesterol transport from late endosomes/lysosomes to ER
- Induces a Neimmann-Pick disease type C or NPC-like phenotype

- **U18666A** inhibits the replication of

**EBOV** (Carette *et al.*, 2011, Shoemaker *et al.*, 2013)

**DENV** (Poh *et al.*, 2012)

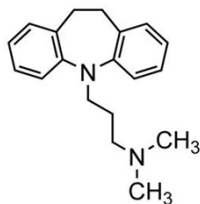
**HIV-1** (Tang, *et al.*, 2009)



- **IMIPRAMINE** (Tricyclic antidepressant ,TCA)

Inhibits the production of EBOV in human umbilical vein epithelial cells

(Herbert *et al.*, 2015)

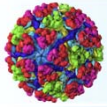


NDC 0781-1762-01  
**Imipramine HCl**  
**Tablets, USP**  
**10 mg**  
ATTENTION: DISPENSE WITH MEDICATION GUIDE.  
Rx only  
100 Tablets  
**SANDOZ**

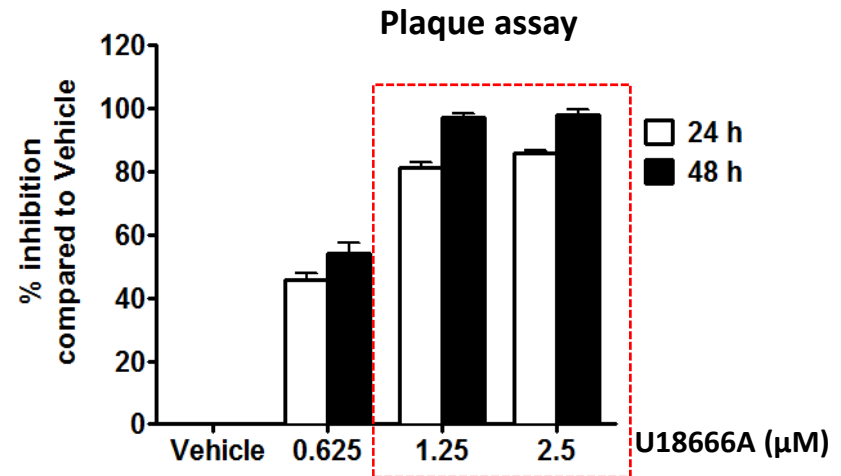
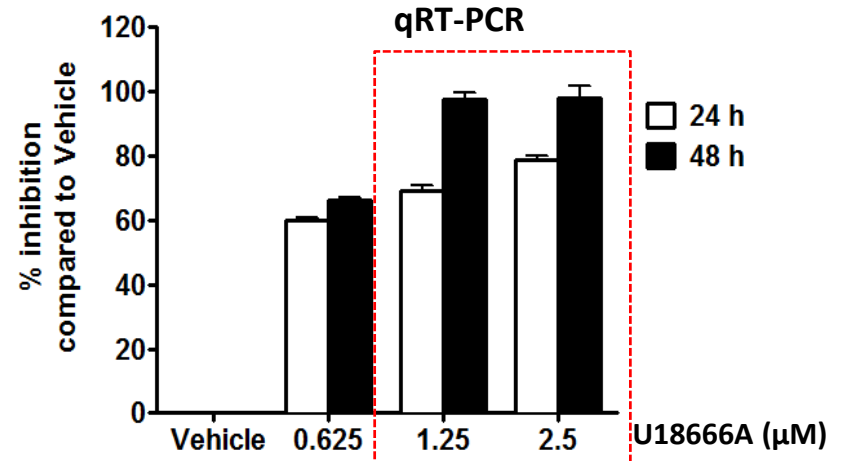
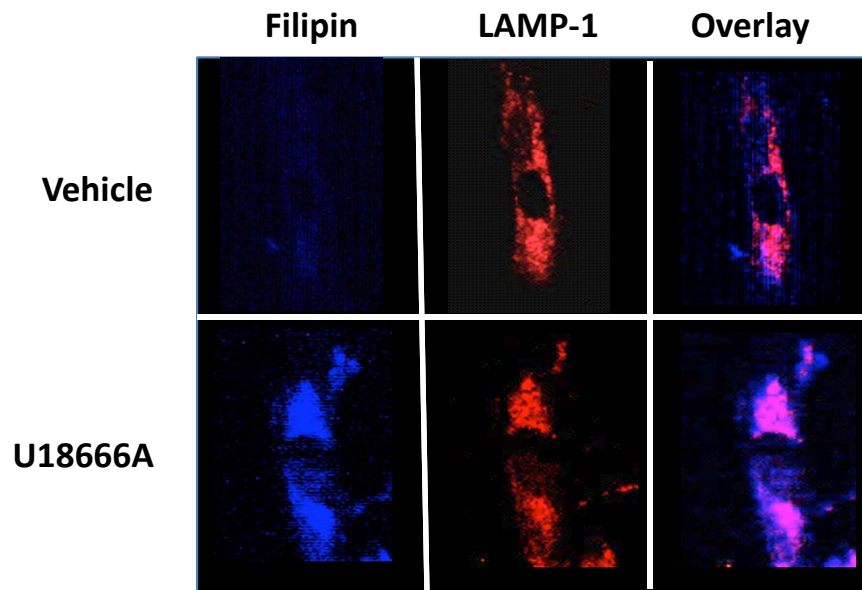
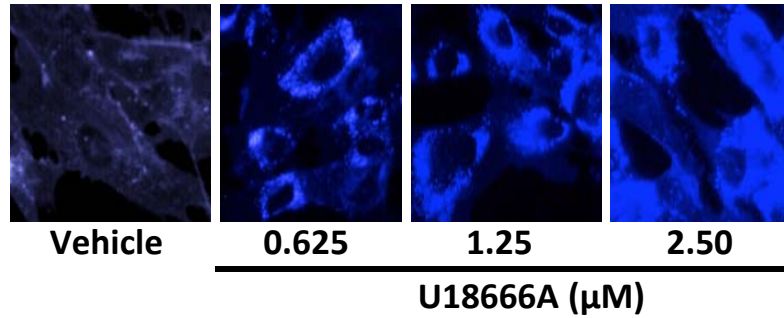
Each tablet contains:  
Imipramine HCl, USP 10 mg  
Usual Dosage: See package insert.  
Store at 20°-25°C (68°-77°F) (see USP Controlled Room Temperature). Dispense in a tight, light-resistant container. KEEP THIS AND ALL DRUGS OUT OF THE REACH OF CHILDREN.  
Sandoz Inc., Princeton, NJ 08540  
Rev. 01-2007M



**Tofranil 25**  
Imipramin HCl 25 mg  
buy-mocafinil-uk  
Antidepressan  
50 draje  
NOVARTIS

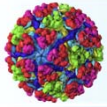


# U18666A and CHIKV replication

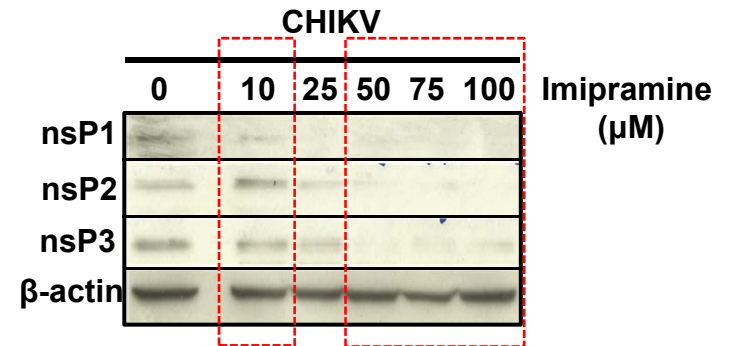
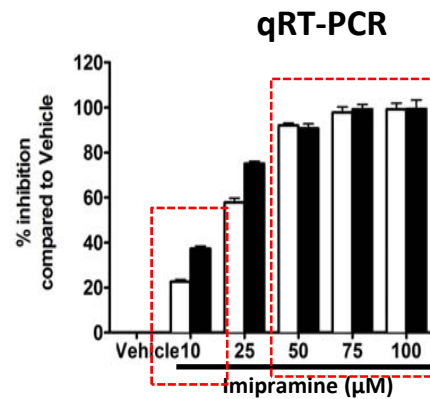
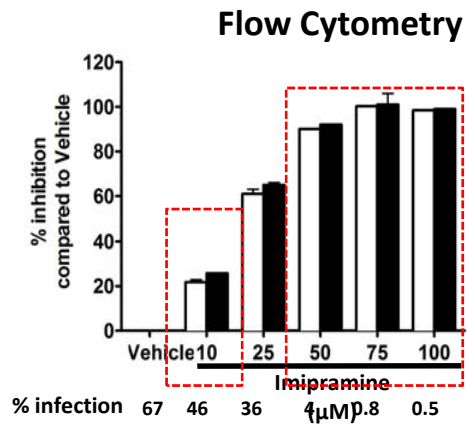
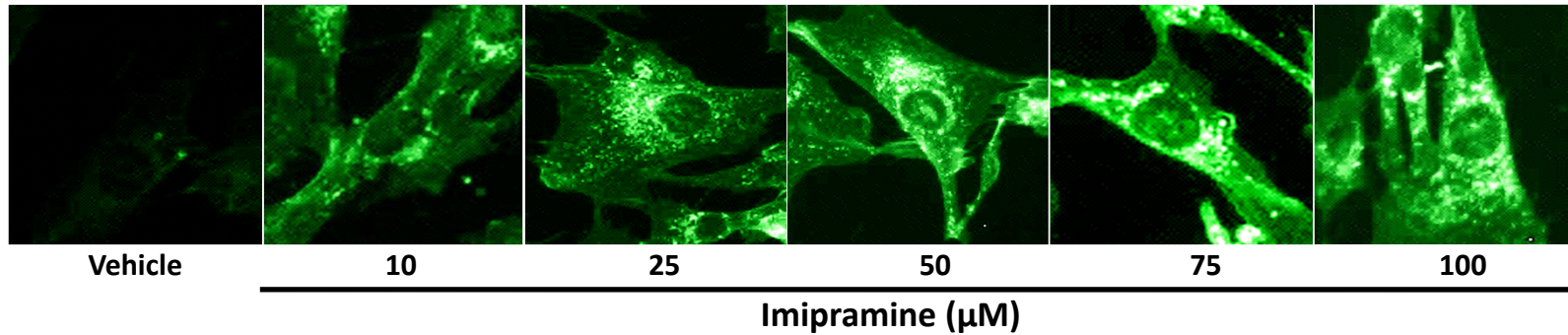


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**U18666A causes accumulation of cholesterol in the LE/Ls  
and inhibits CHIKV replication**

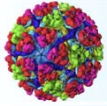


# Imipramine and CHIKV replication



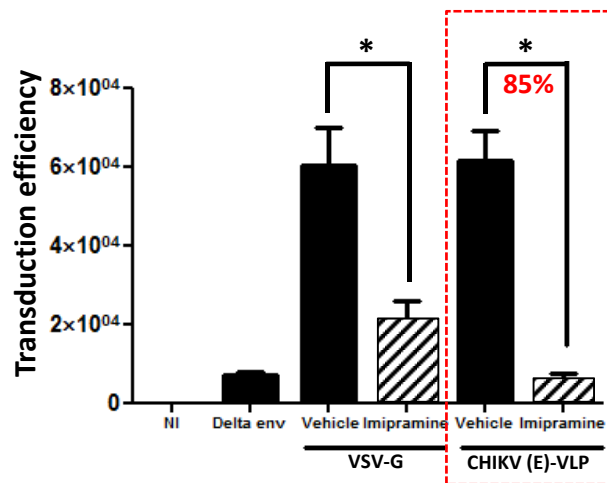
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**Imipramine causes accumulation of cholesterol in the LE/Ls  
and inhibits CHIKV replication**

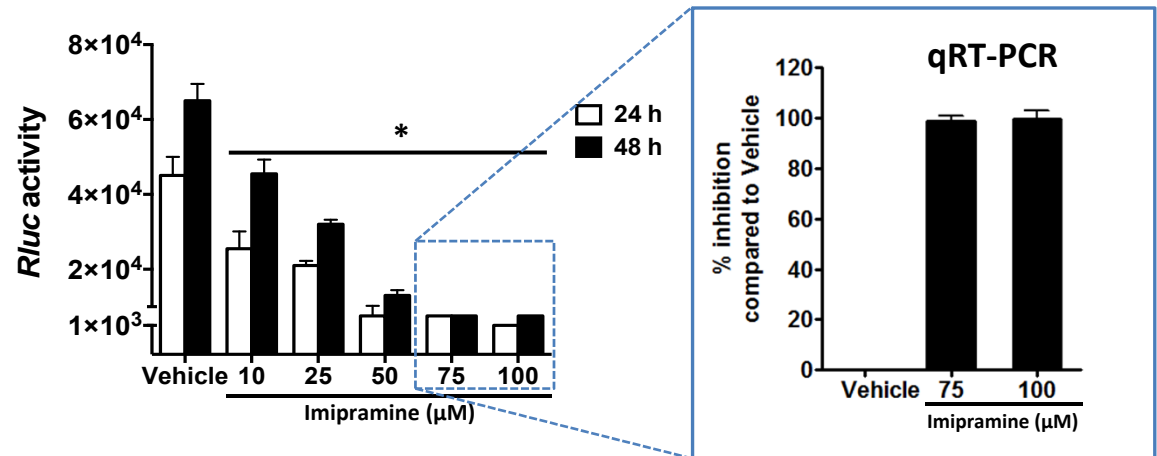


# Imipramine and CHIKV replication cycle

## Fusion/entry step

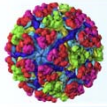


## Post-entry step (HUH-7 CHIKV replicon)

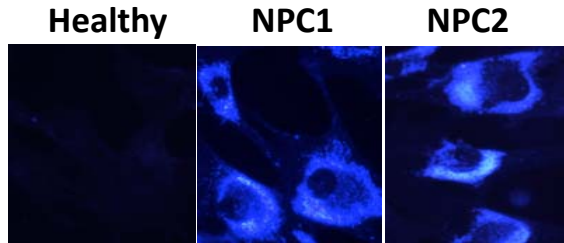


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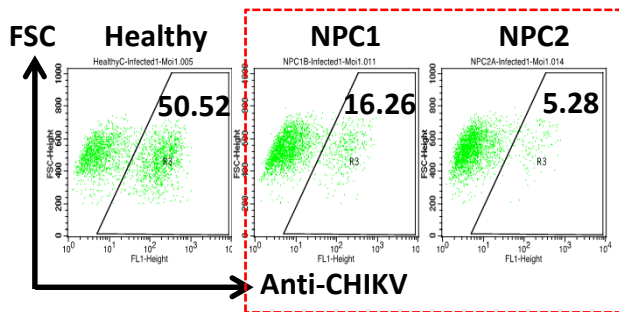
Imipramine exerts its inhibitory effects at least two different stages of the CHIKV infection cycle



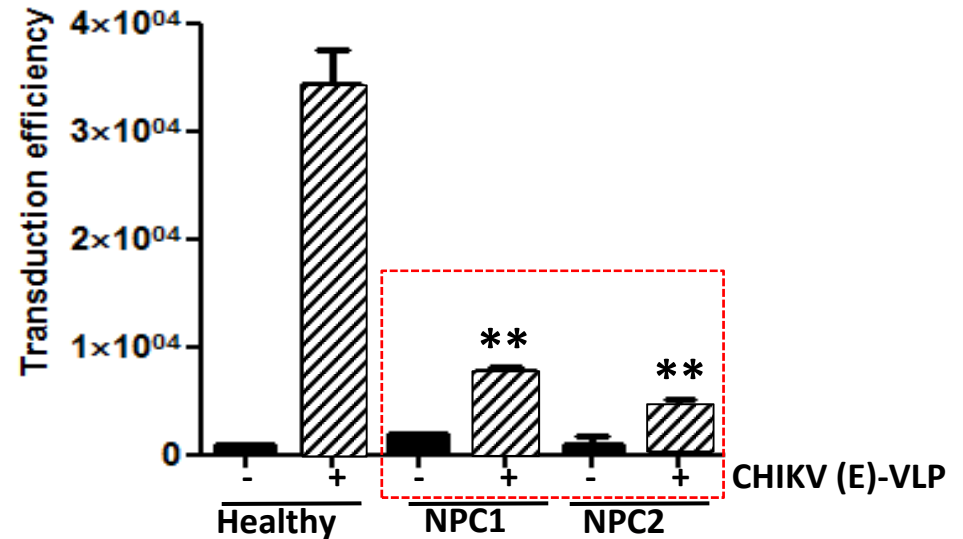
# NPC proteins are crucial for CHIKV replication



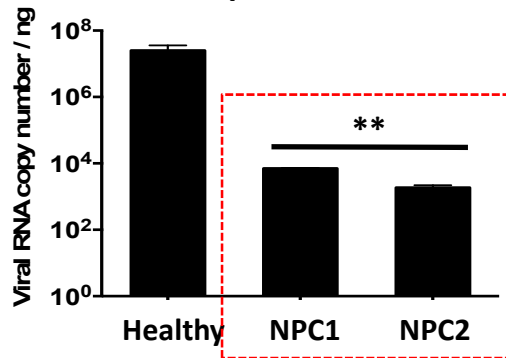
## Flow cytometry



## NPCs-deficient cells impaired CHIKV fusion

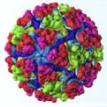


## qRT-PCR

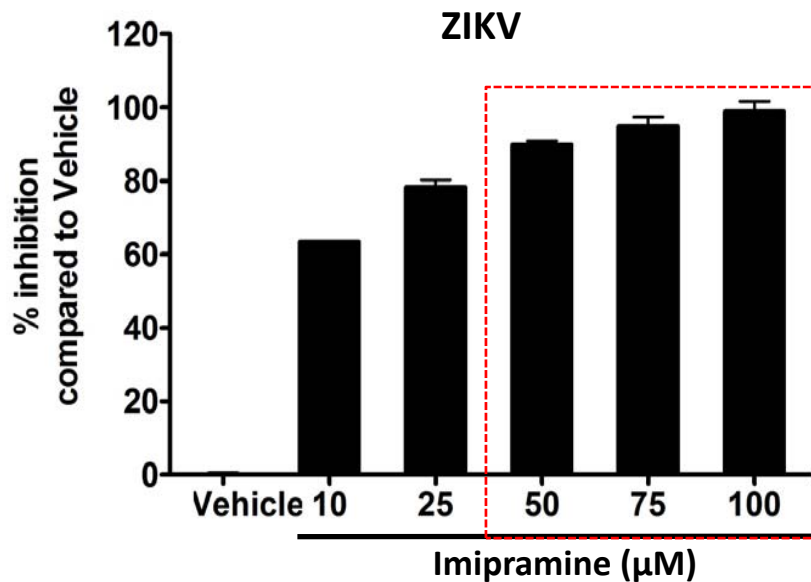
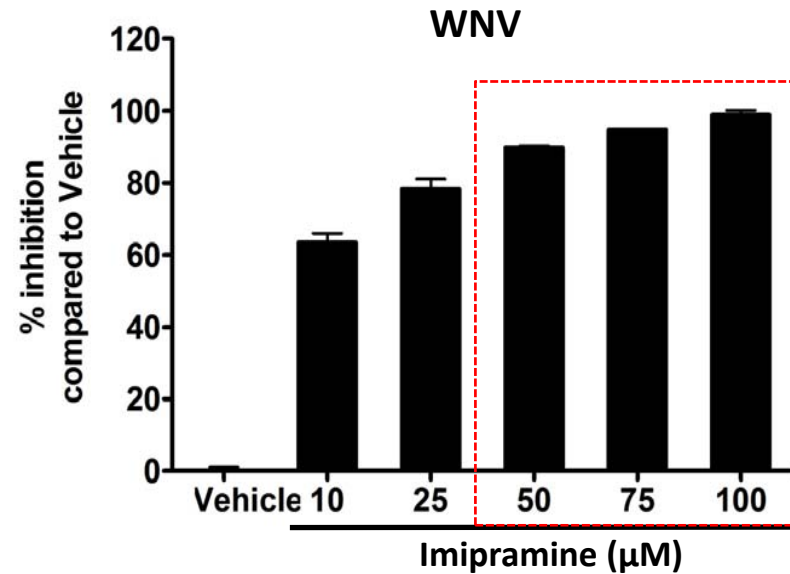
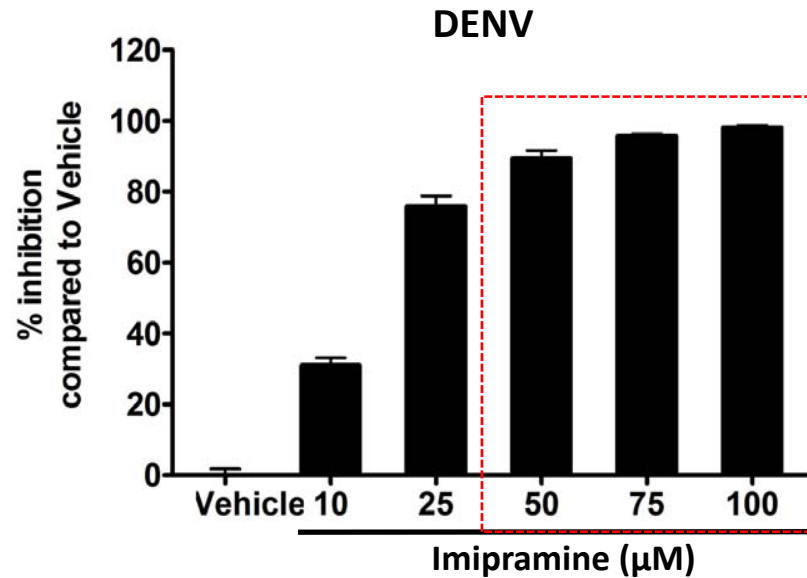


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NPC1 or NPC2 deficiency has a strong impact on the entry and/or fusion step of the CHIKV life cycle

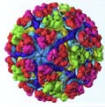


# Imipramine exerts antiviral activity against Flaviviruses

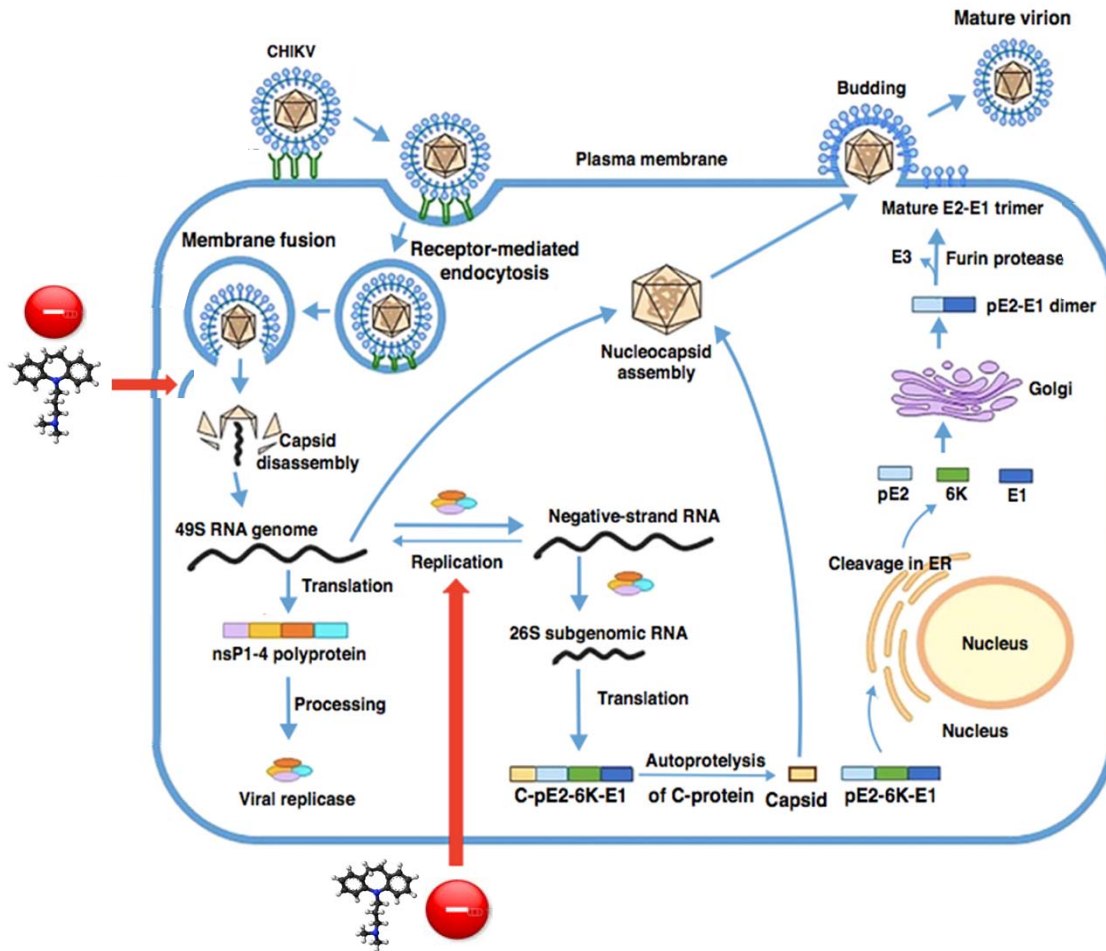


**\*\*\***  
Imipramine strongly inhibited, in a dose-dependent manner, the replication of DENV, WNV and ZIKV





# CONCLUSIONS



Wichit S. et al., *Sci Rep.* 2017 Jun 9;7(1):3145.  
<https://www.ncbi.nlm.nih.gov/pubmed/28600536>

## “IMIPRAMINE”

- I. Causes accumulation of cholesterol in the LE/Ls
- II. Exerts its inhibitory effects at least two different stages of the CHIKV infection cycle
- III. Exerts broad-spectrum antiviral activity against Alphavirus and Flavivirus

# Acknowledgements

## Dorothee MISSÉ's Team:

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Dr. Pornapat SURASOMBATPATTANA  
Loïc TALIGNANI  
Deborah GARCIA  
All internship students



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