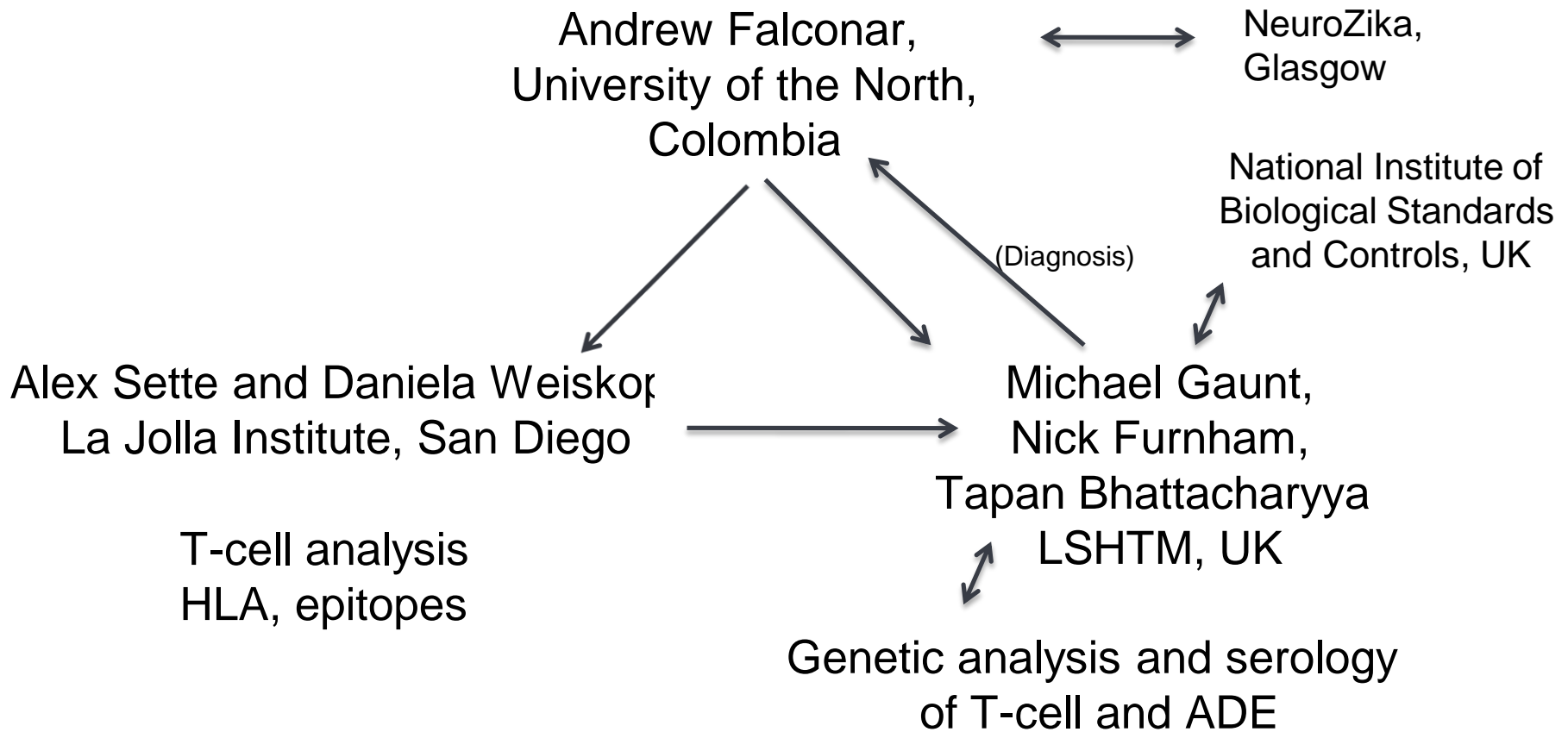# INVADE: Investigating Antibody Dependent Enhancement
Finding the mutations

Andrew K. Falconar, Michael W. Gaunt, Daniela Weiskopf and Alexander Sette

Insert Logo of partner authoring the document here

12/10/2016

Preparedness Latin American Network

Zika**PLAN**

General setup: WP 6, INVADE

Andrew Falconar,
University of the North,
Colombia

←——————→ NeuroZika,
Glasgow

National Institute of
Biological Standards
and Controls, UK

(Diagnosis)

Alex Sette and Daniela Weiskopf
La Jolla Institute, San Diego

Michael Gaunt,
Nick Furnham,
Tapan Bhattacharyya
LSHTM, UK

T-cell analysis
HLA, epitopes

Genetic analysis and serology
of T-cell and ADE

Zika virus and Evolution

Gaunt *et al*. 2001, reviewed Ash, 2001
Science

Lancet Gould and Solomon 2008 *Lancet*
371, 500–509,
+ 8 additional publications
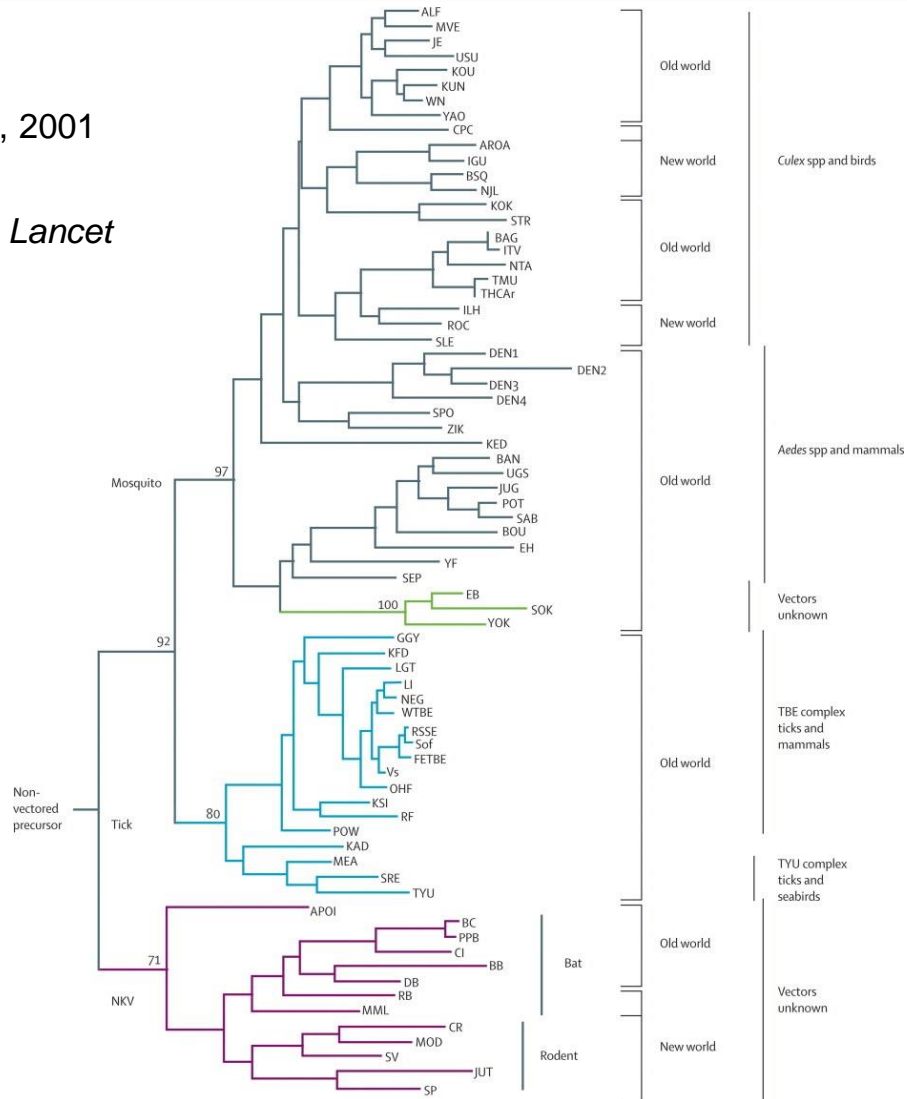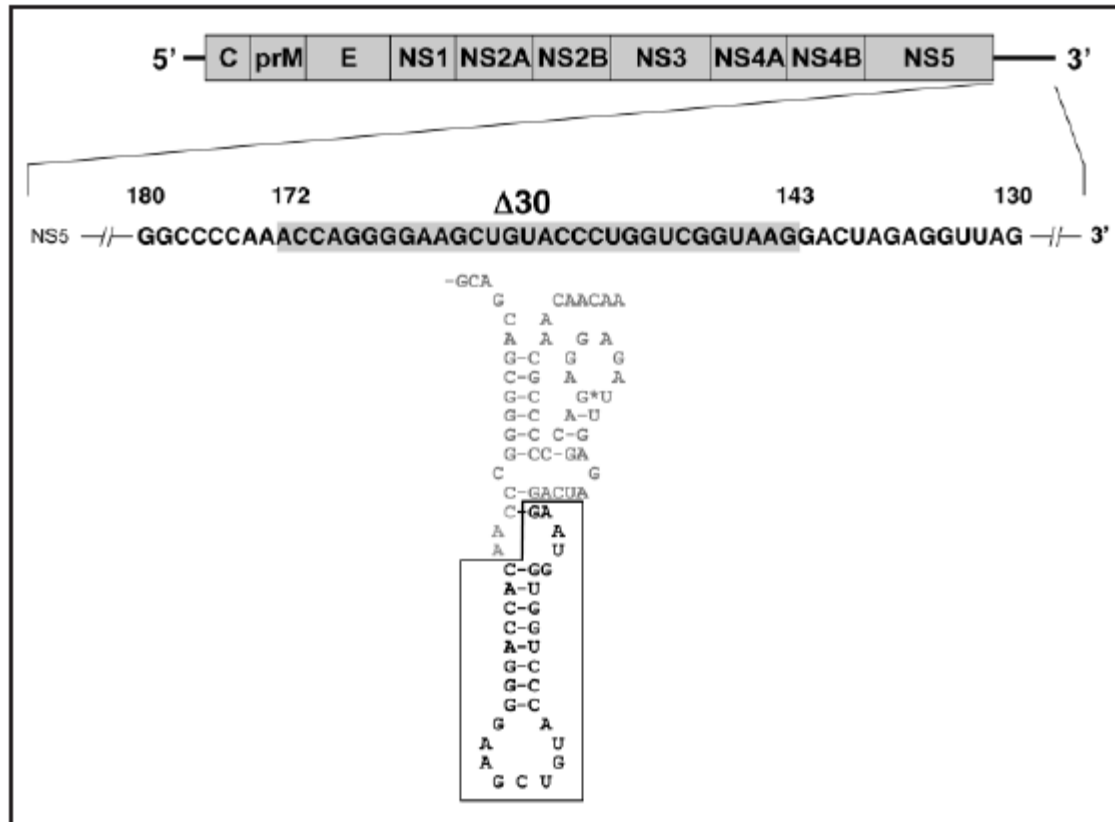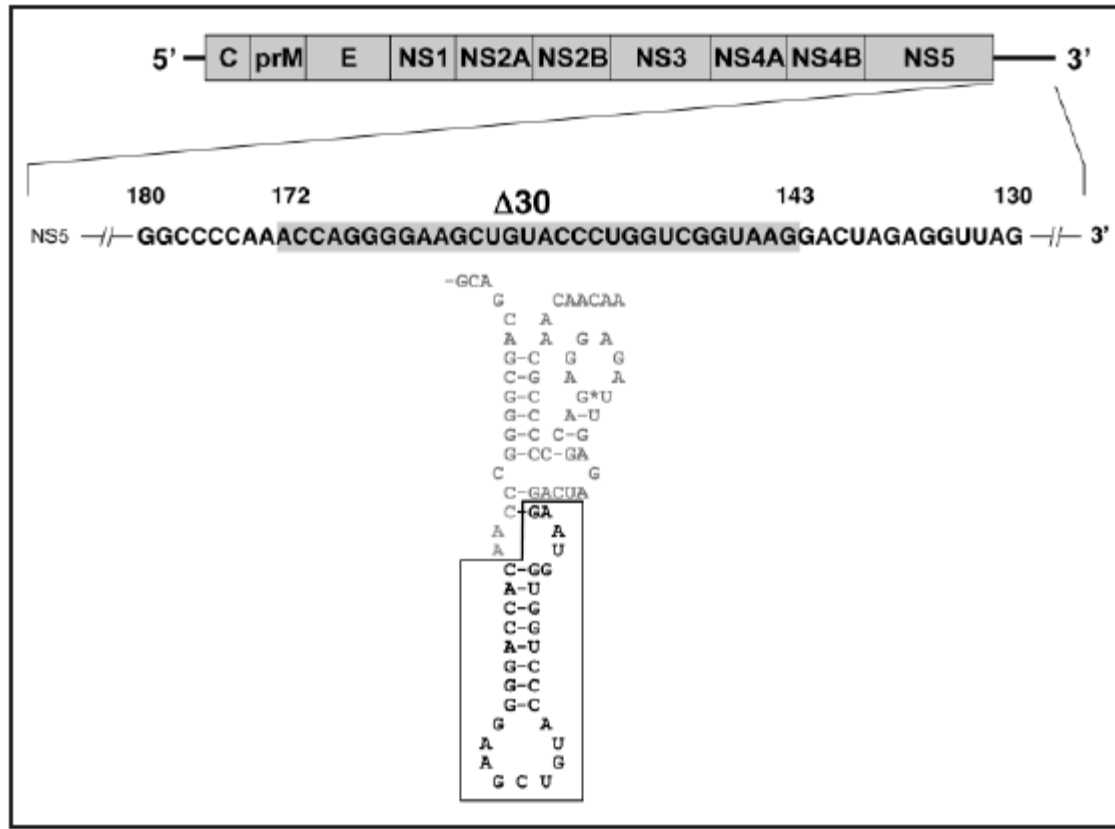
Molecular basis to Butantan vaccine

Figure 1. The Δ30 mutation is located in the 3'UTR region of the DEN1 genome. The highlighted nucleotides represent those included in the deletion. Below the sequence is the predicted secondary structure of the TL2 region of DEN1 with the deleted nucleotides in the highlighted sequence boxed and in bolder type.

Whitehead *et al.* (2007)

Molecular basis to Butantan vaccine

Copyright
http://weallseqt
oseq.blogspot.de
/2013/09/niaid-
dvi-tv003-live-
attenuated.html

Figure 1. The Δ30 mutation is located in the 3'UTR region of the DEN1 genome. The highlighted nucleotides represent those included in the deletion. Below the sequence is the predicted secondary structure of the TL2 region of DEN1 with the deleted nucleotides in the highlighted sequence boxed and in bolder type.
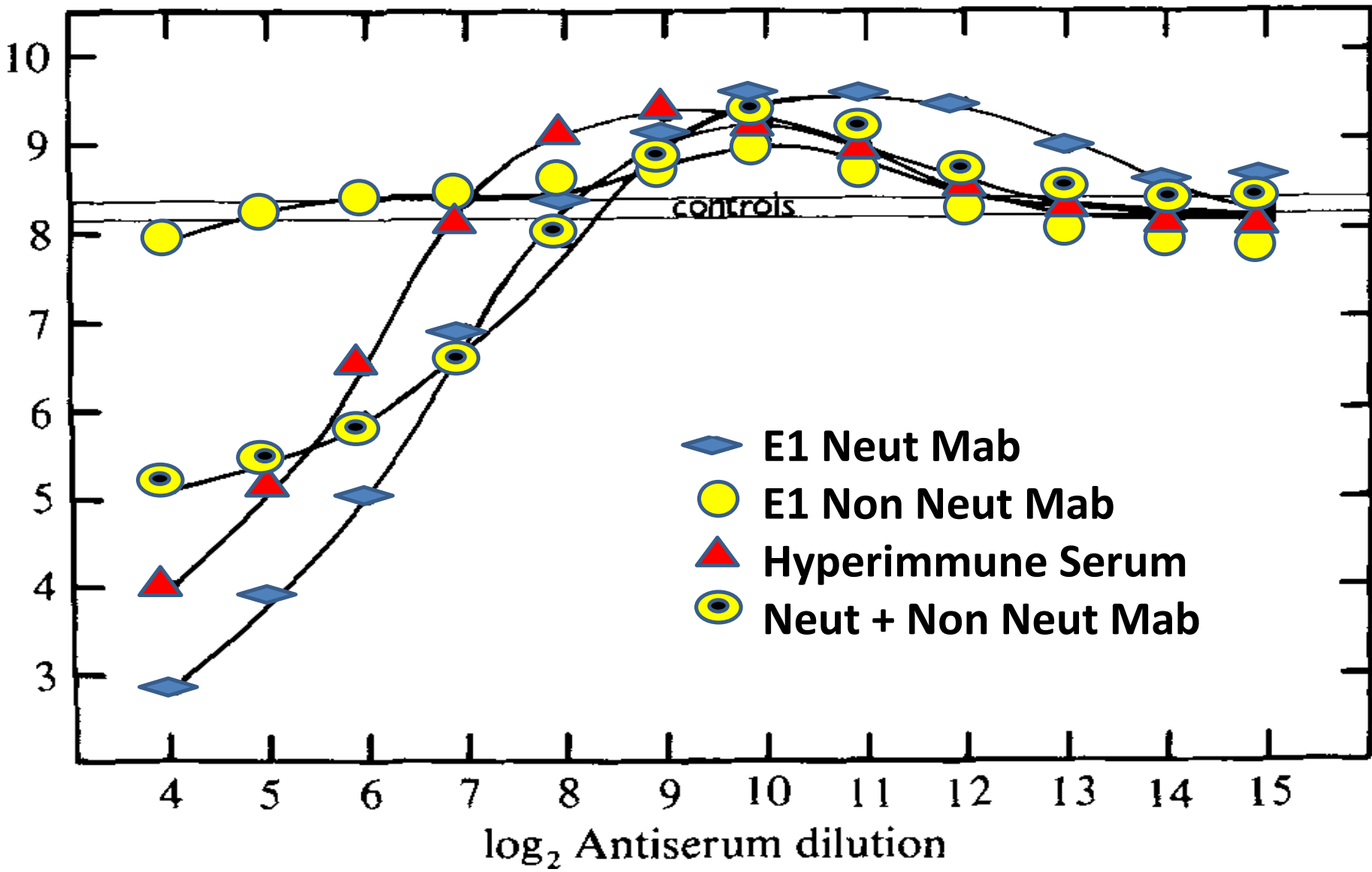
Whitehead *et al.* (2007)

Antibody dependent enhancement *in vitro*
First described by Halstead
and developed as an *in vitro* test by Peiris and Porterfield

Nimmerjahn and Lux (2014). PNAS 111: 2404-2405
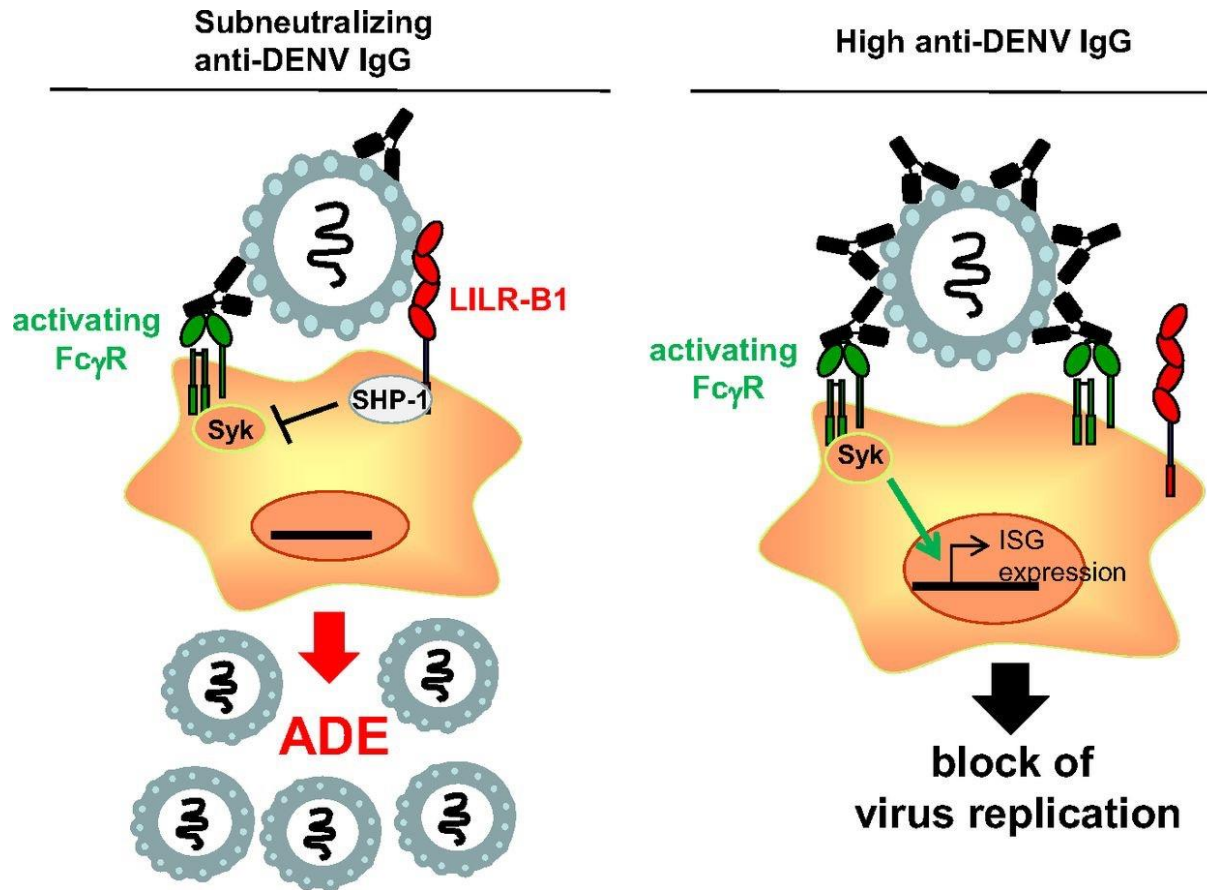
Antibody dependent enhancement P388D1 cells (mouse macrophage)

ADE Macaque Study

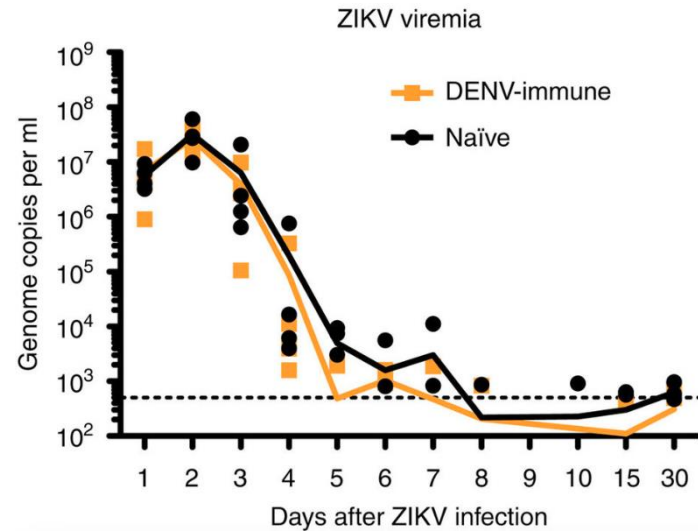- **Four monkeys challenged with DENV1 or DENV2 2.8 years before Zika virus challenge**

- **Four controls**
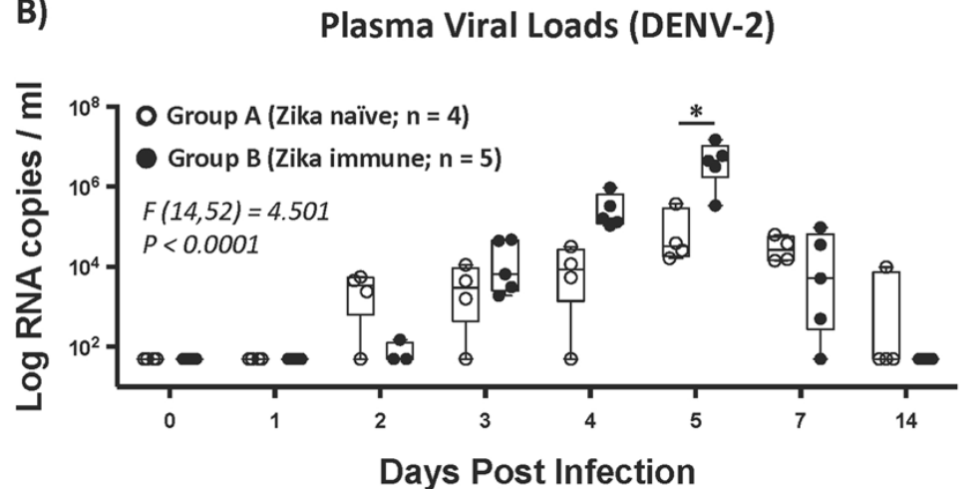
    Pantoja *et al*. (2017)

    Nature Comm. 8: 15674

ZIKV viremia

- **Five monkeys challenged with ZIKV before DENV2 challenge**

    George *et al*. (2017) Scientific

    Reports. 8: 15674

B)

Plasma Viral Loads (DENV-2)

$F_{(14,52)} = 4.501$
$P < 0.0001$

- ○ Group A (Zika naïve; n = 4)
- ● Group B (Zika immune; n = 5)

22/06/2018

Sample base to date

- **650 ZIKV sera from humans ~400 RT-PCR for Zika virus from Caribbean (400ul)**

- **~50 DENV IgM sera from the Caribbean**

- **Control ZIKV sera is available from travel patients without exposure to DENV**

- **All samples have relevant epidemiological information for age, gender and location**

- **TIGHTEN THE PHYSIOLOGICAL CONDITIONS OF THE ASSAY**

Preparedness
Latin American Network

ZikaPLAN

Bioinformatics of attenuating mutations for ADE

Virus	Monoclonal antibody		
	294	427	126
YF-FNV	7·0 ± 0·3	5·2 ± 0·3	5·4 ± 0·3
YF-Asibi	7·7 ± 0·2	6·6 ± 0·2	6·8 ± 0·1
YF-B11	8·8 ± 0·5	6·0 ± 0·7	7·4 ± 0·1
YF-B1	7·6 ± 0·7	8·0 ± 0·2	7·6 ± 0·3
YF-B5	9·2 ± 0·7	P	P
YF-B7	9·3 ± 0·3	10·8 ± 0·5	10·3 ± 0·6
YF-B9	8·3 ± 0·3	8·6 ± 1·1	8·8 ± 0·6
YF-B15	8·4 ± 0·5	8·1 ± 0·3	NT
YF-17D-UK	12·3 ± 0·3	P	P
YF-17D-SA	8·4 ± 0·4	P	P
YF-17DD-Braz	9·5 ± 0·4	P	9·8 ± 0·4
YF-9026	10·2 ± 0·3	9·8 ± 0·6	NT
JE	5·4 ± 0·2	5·9 ± 0·3	5·9 ± 0·4

Butantan vaccine: When modelling worked

- **Tetravalent vaccine tested at the Butantan Institute, Brazil**
- **Attenuated mutations introduced in the 3' UTR**
- **Phase 3 clinical trails in Brazil**
- **Will induce a strong Th1 response**
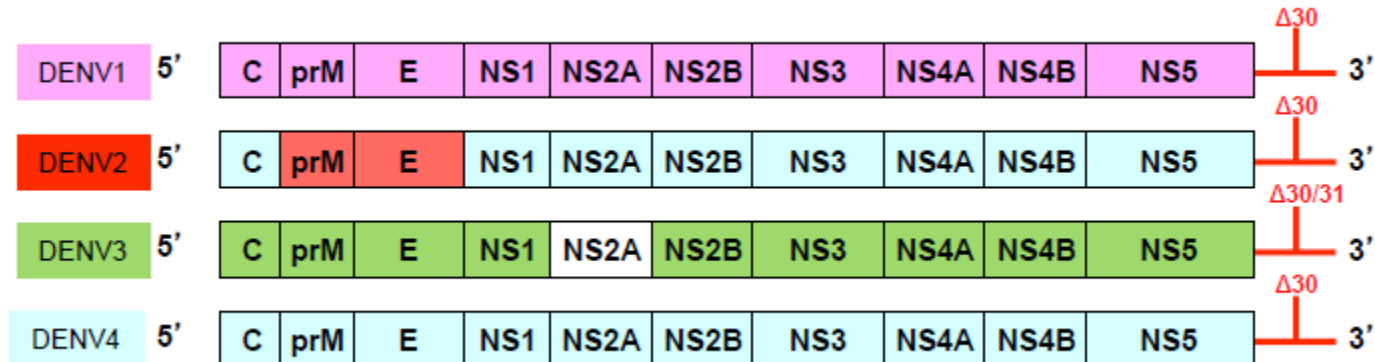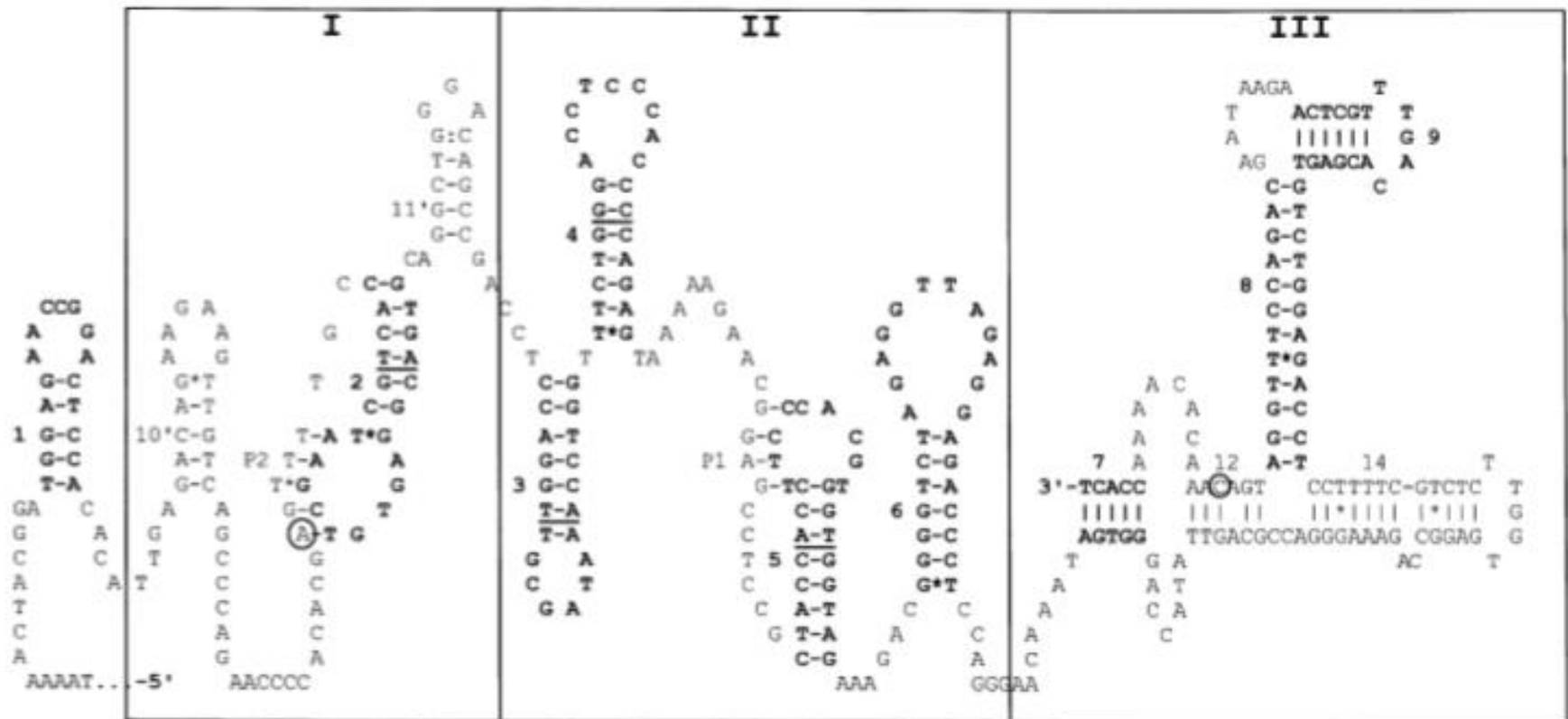

Whitehead *et al.* (2007)

When modelling worked

3' UTR secondary structure: wild type yellow fever virus
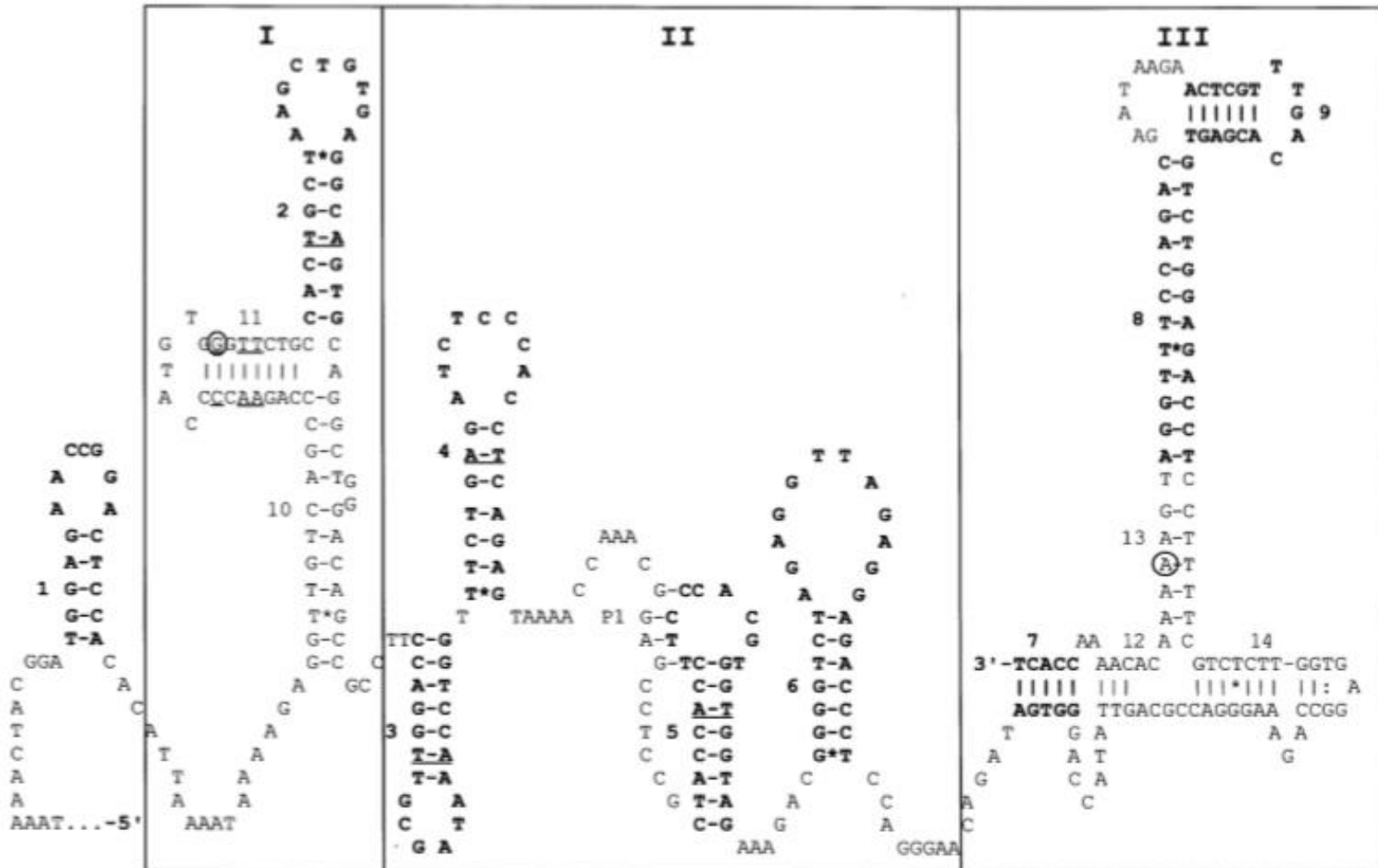
When modelling worked

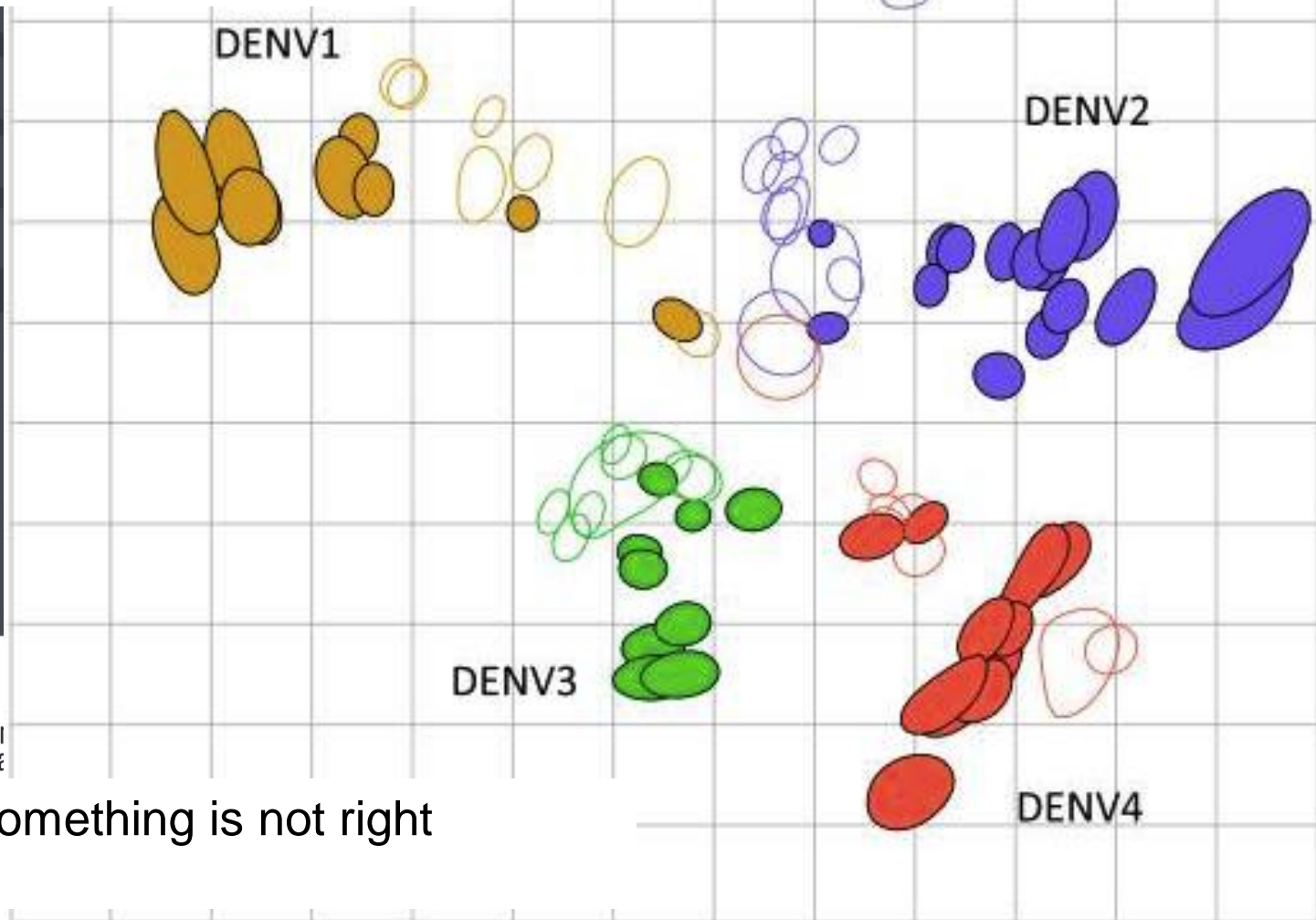## 3' UTR secondary structure: vaccine yellow fever virus

Antigenic map using PRNT for acute DENV sera

Serological analysis for cross-reactivity
(PRNT) in a mouse model
KATZELNICK et al (2015) *Science 349: 1338-1343*

Something is not right

What is the molecular mechanism of ADE?

Hinge regions, or conformational plasticity?

- **DENV E-protein: Domain II (fusion protein) relates to DI and DIII as a 'hinge' or hairpin**
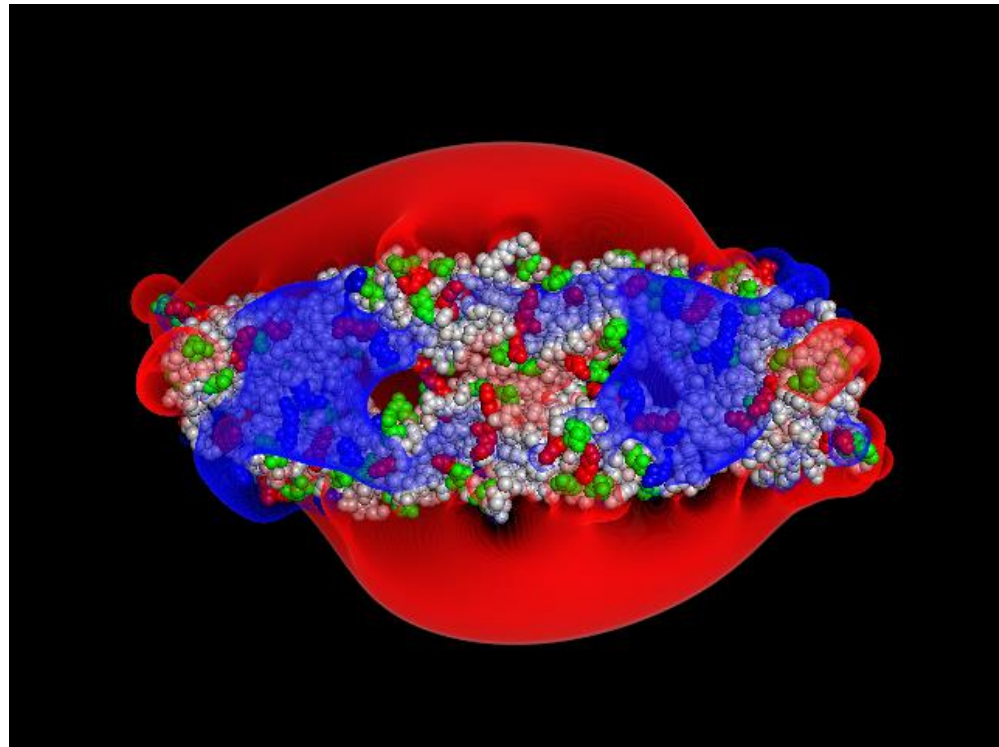
Work in collaboration with
Nick Furnham
(Sir Tom Blundell's FRS alumini)
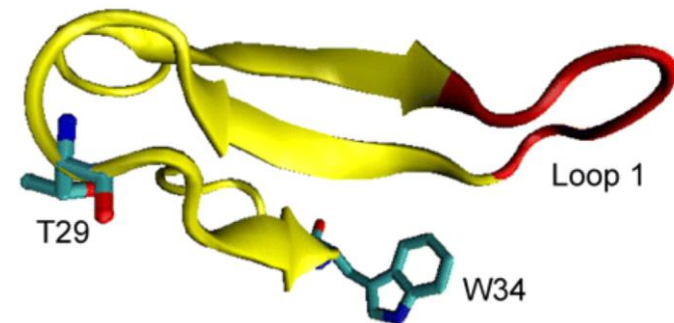
Modern protein theory: two wrongs make one right

Co-variance "plus" amino acid mutation modelling

- **E.g. Burger and Nimwegen approaches**
  - Techniques to model protein structures from co-evolving mutation frequencies (co-variance)
  - If co-evolving amino acids are identified mutating residue destabilises the protein structure and shifts its antigenicity, BUT mutating the second residue recovers the structure (and the antigenicity)
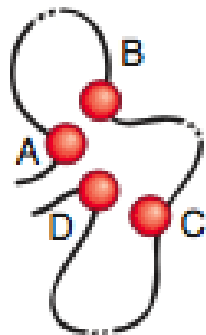
Physical contacts — Observed correlations — Predicted contacts
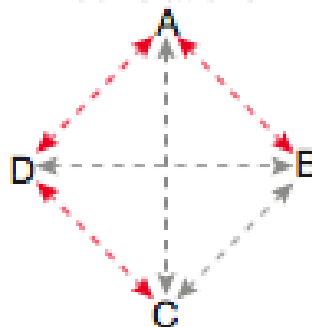
■ Causative   ■ Transitive

Modern protein theory:
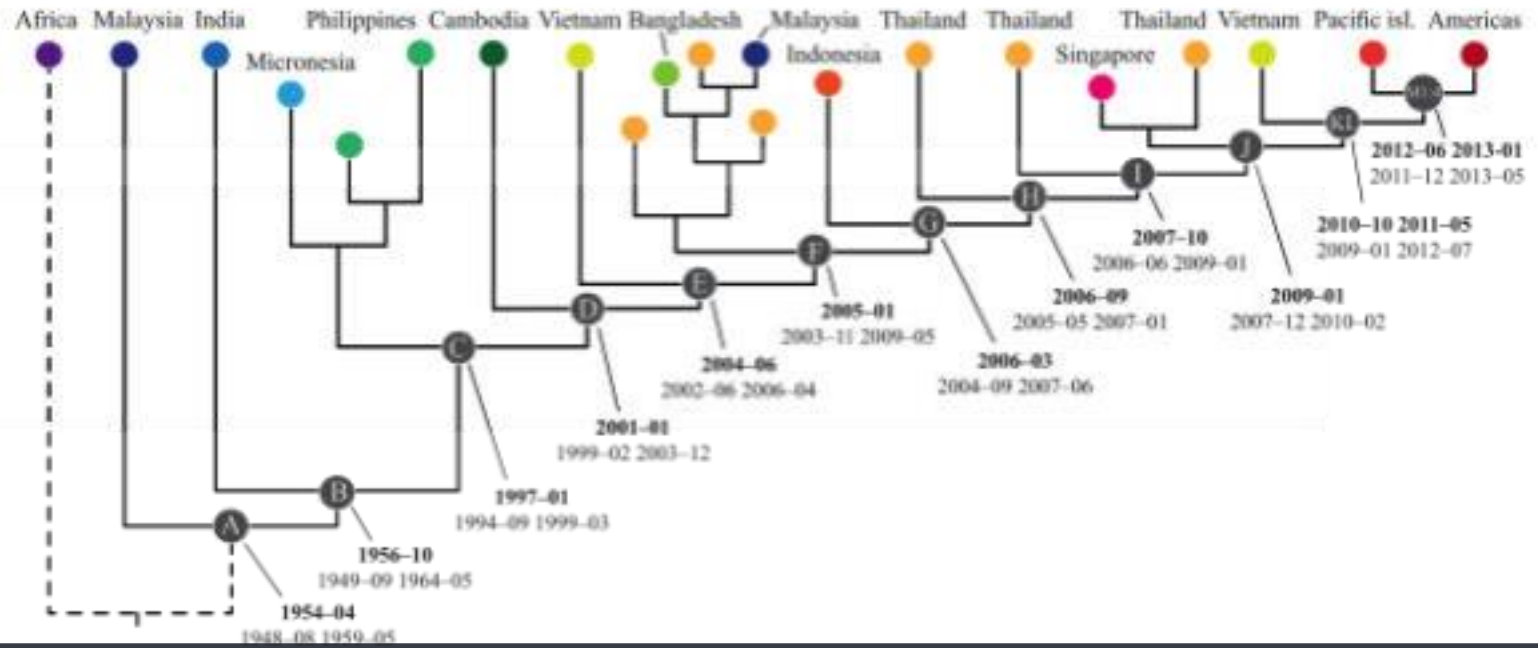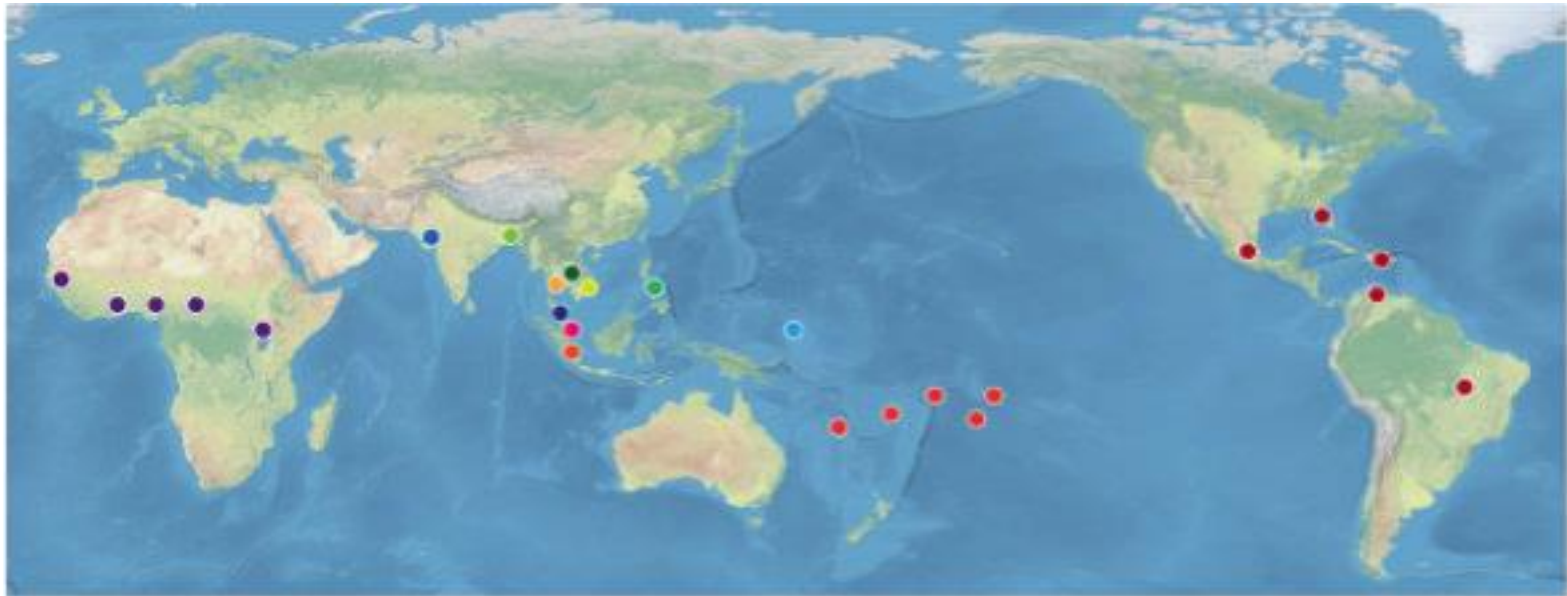# two wrongs make one right

The state of the art protein modelling methods identify a loop (10 aa) in N-term. E protein showing clear co-evolution of residues and associated with ADE

prM and NS1 have remained elusive to this approach
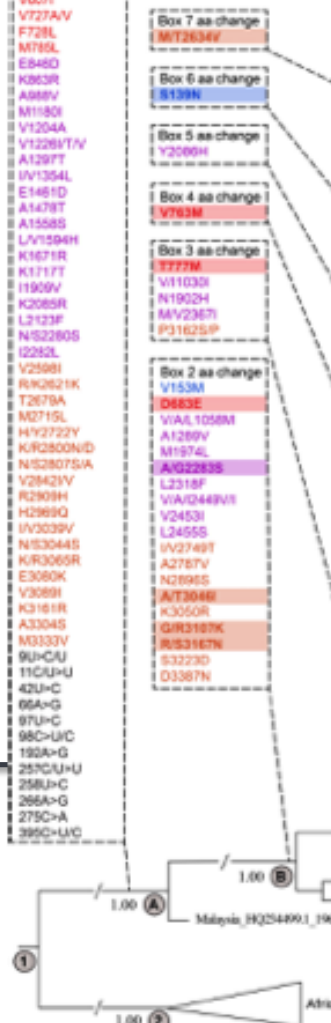
Tested using infectious clones
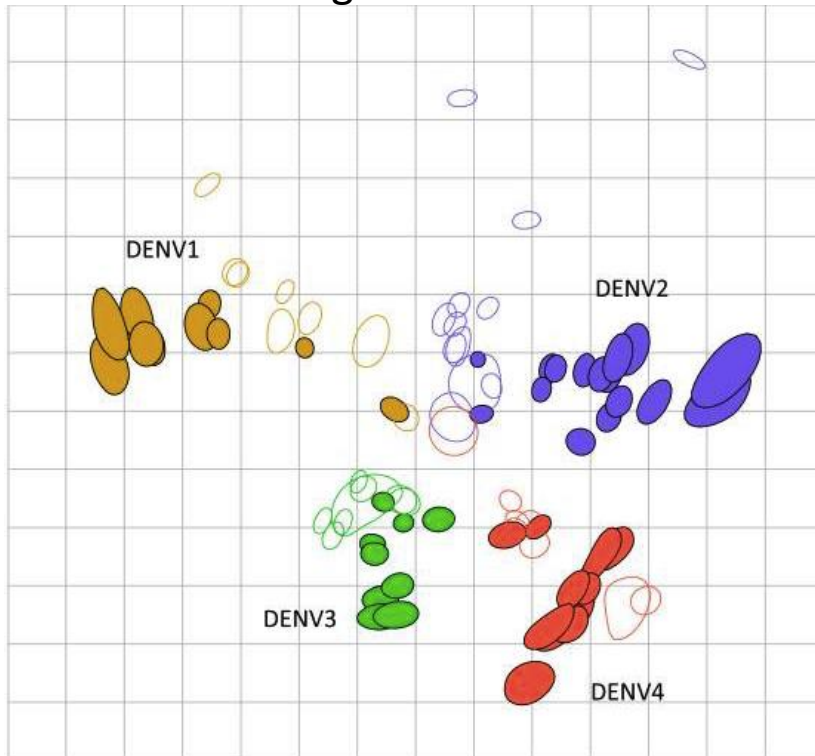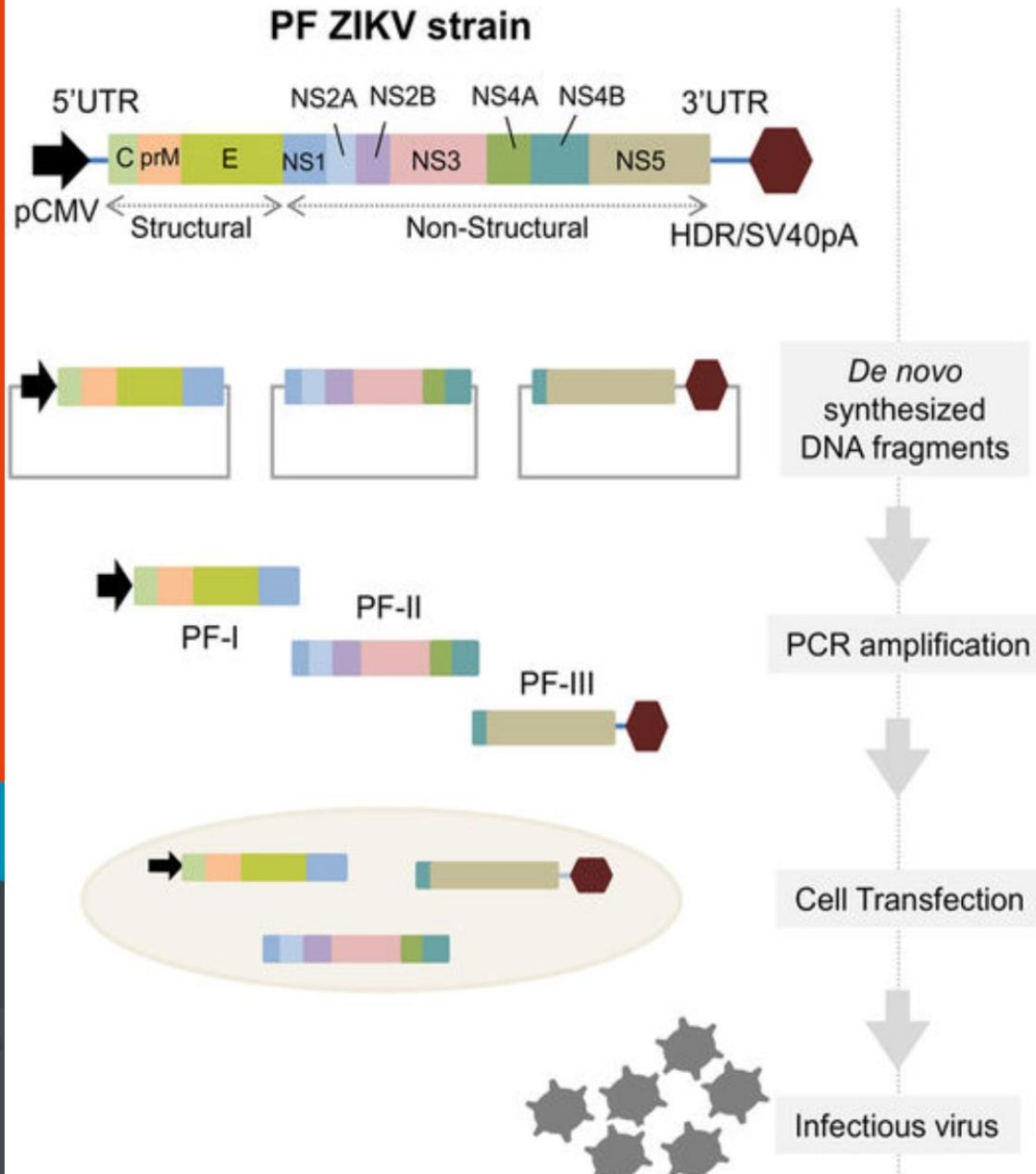
Zika virus evolution: Africa to SE Asia

Zika virus evolution: Africa to SE Asia

Zika virus reverse genetics ISA

Gibson assembly

Acknowledgements

- **Ernie Gould, Centre of Ecology, Oxford, UK**

- **Annelies Wilder-Smith – robust project management**

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- **Kevin Arien, ITM, Belgium (ZikaPLAN)**

- **Phil Minor, Mark Page, James Ashall, NIBSC, UK**

- **Suzanna Kaptein – help with mouse model**

- **Ludmila Lobkowicz, Nick Furnham, Tapan Bhattacharyya, LSHTM**