

REGULATION OF THE ANTIVIRAL RESPONSE DURING DENGUE VIRUS INFECTION

Innovative Training Network



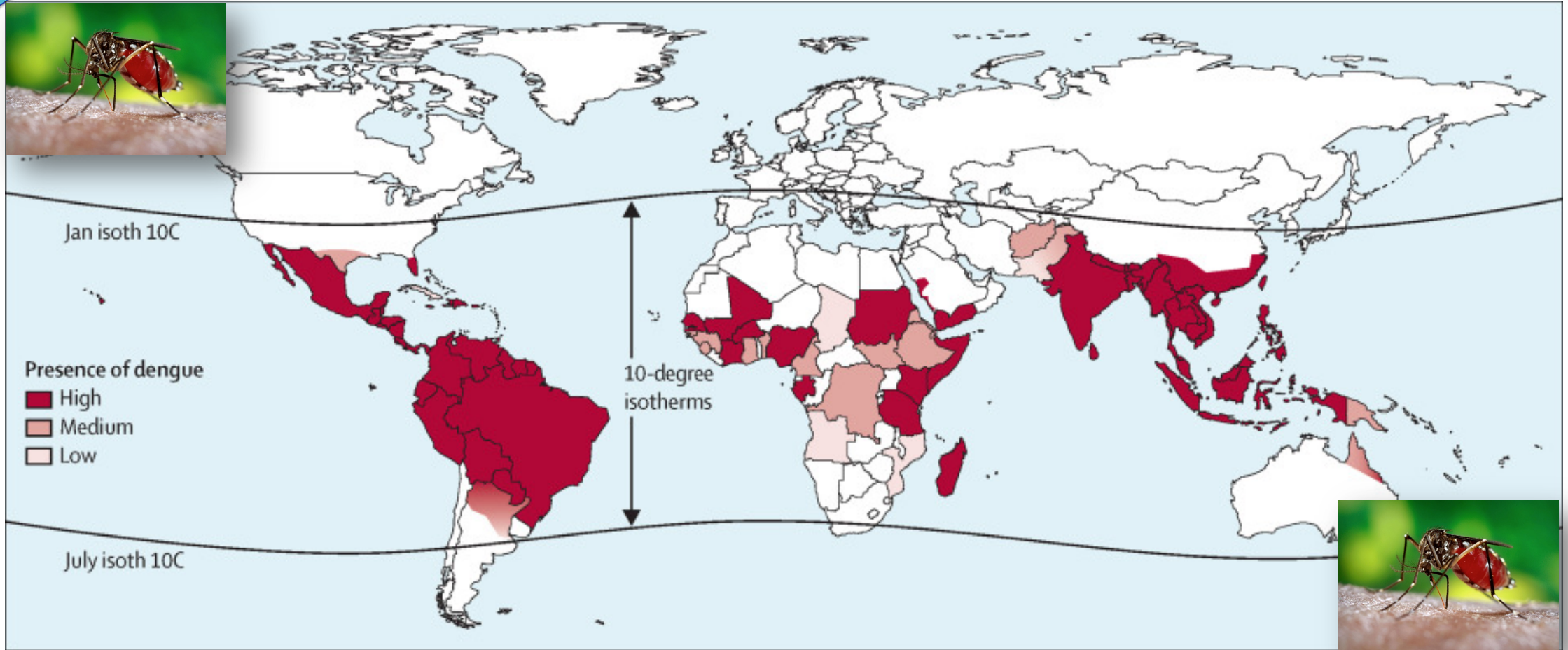
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Epidemiology & pathogenesis of dengue virus infection

- **Most common arthropod-borne viral pathogen**
- transmitted to humans by *Aedes aegypti* and *Aedes albopictus*
- **2.5 billion people at risk in tropical regions**
- **Estimated 300 million infections per year, with 25,000-50,000 deaths annually**
- **Primary infection: often a self limiting acute infection ('breakbone fever')**
- **Secondary heterologous infection generally lead to more severe immunopathogenic disease with risk of dengue hemorrhagic fever or shock syndrome (DHF/DSS)**
- **No effective antiviral agents and no effective vaccine to treat or prevent dengue infection**



Global Distribution of Dengue



Found in tropical and subtropical regions of the world, 2.5 billion at risk

Endemic in more than 100 countries in the WHO regions:

- Africa, Americas, Eastern Mediterranean, South-East Asia, and Western Pacific
- South-East Asia and South American regions are the most seriously affected

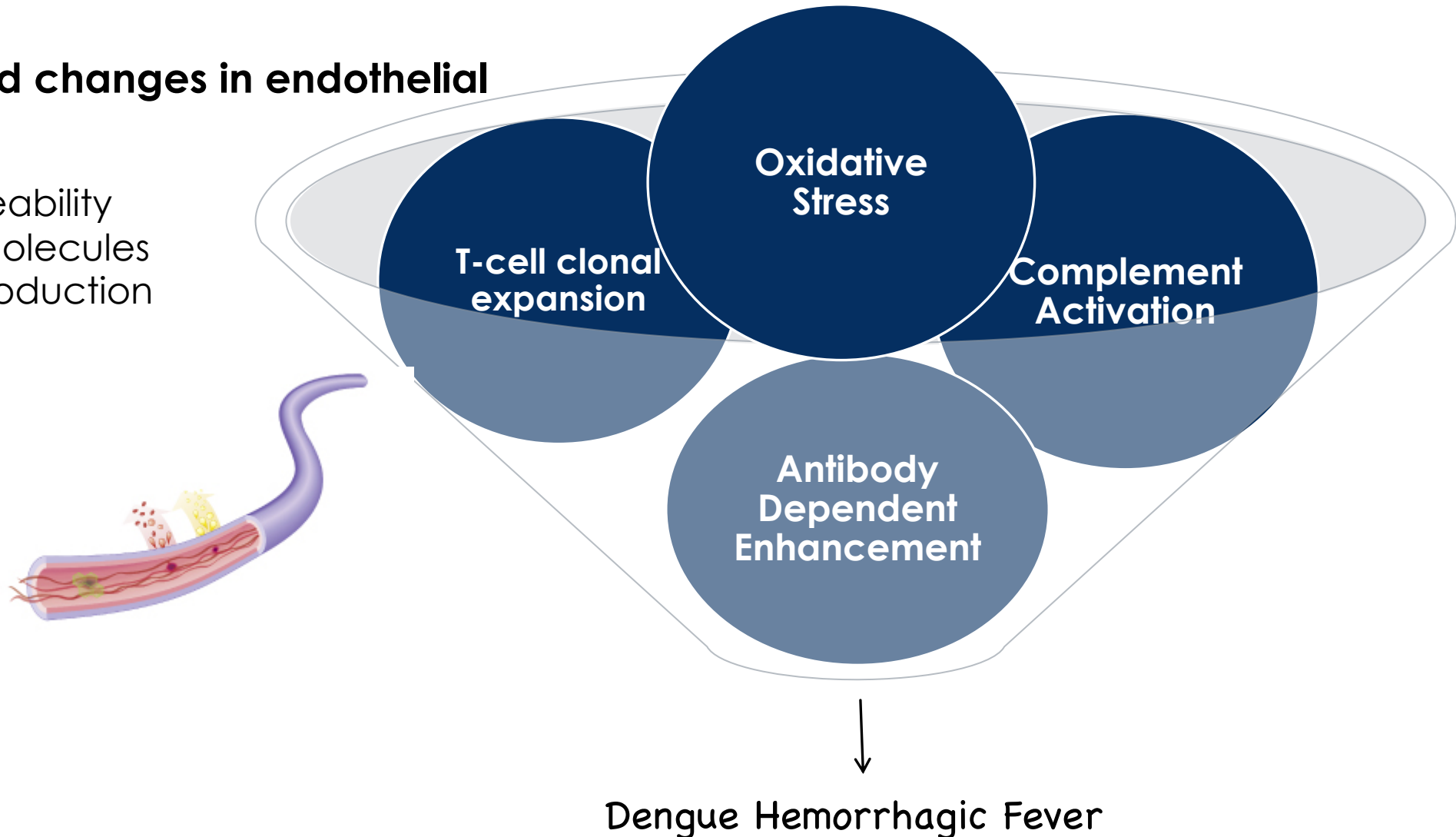
PATHOPHYSIOLOGY of severe dengue - DHF/DSS

Cytokine storm induced changes in endothelial morphology:

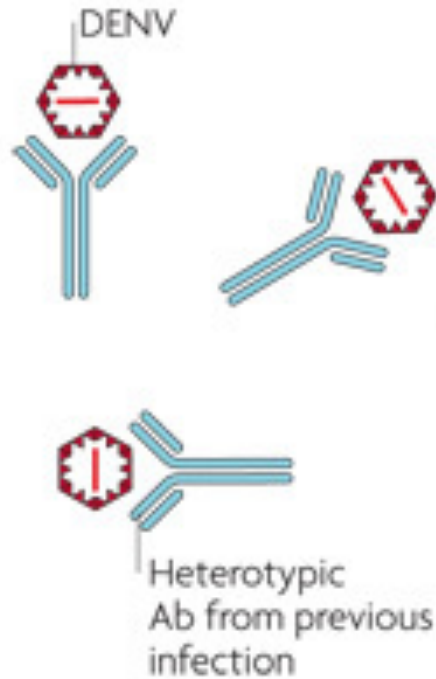
- Loss of vascular permeability
- Increased adhesion molecules
- Increased cytokine production

Consequences:

- Plasma leakage
- Edema
- Hypovolemic shock
- Thrombocytopenia



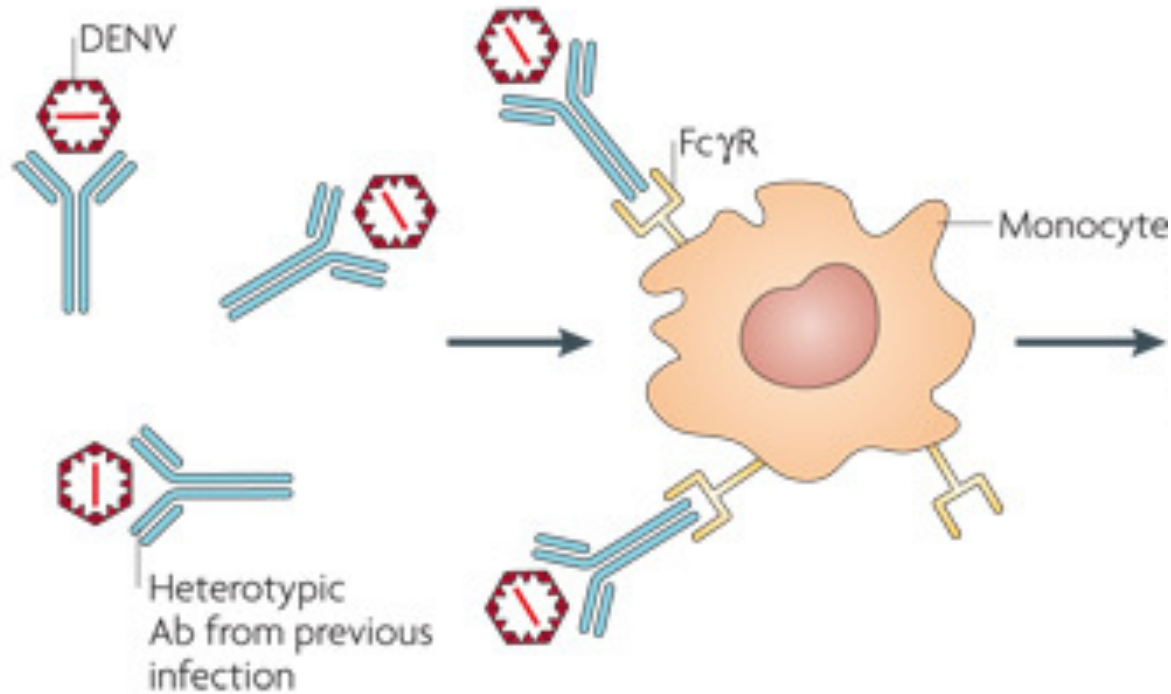
PATHOPHYSIOLOGY- Antibody Dependent



Heterotypic antibodies

- Low concentration
- Partial neutralization
- μM

PATHOPHYSIOLOGY- ADE Activation



Heterotypic antibodies

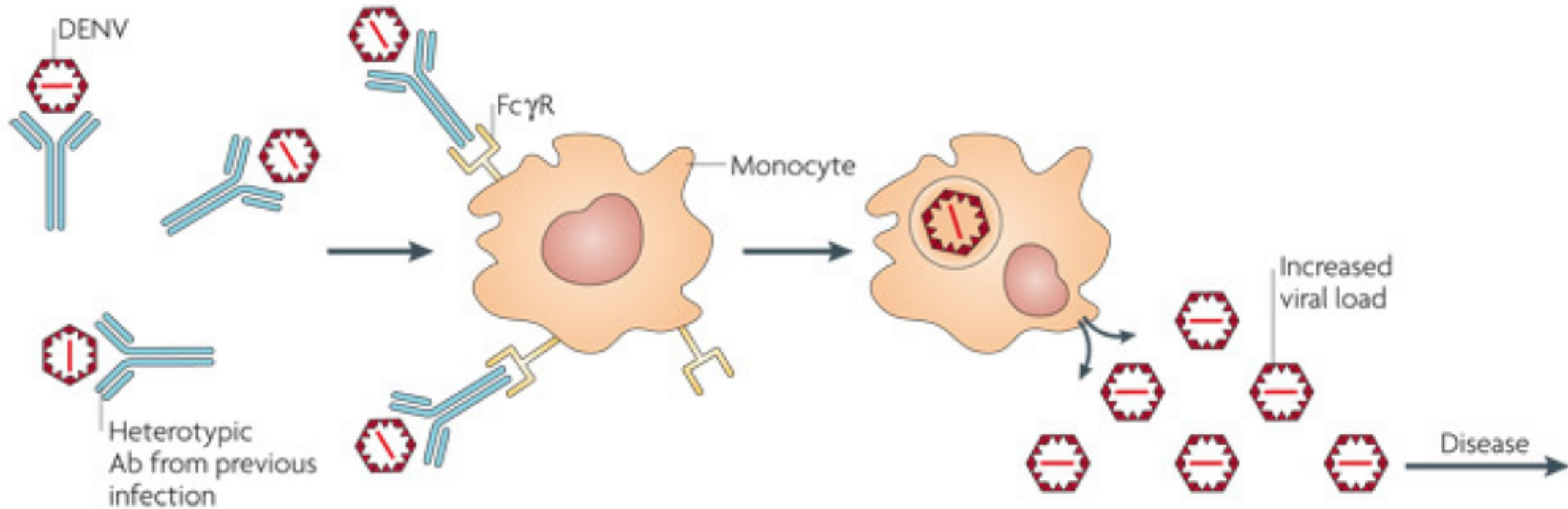
- Low concentration
- Partial neutralization
- Ie. prM

Virus uptake

- Delivery to Fcγ receptor cells

PATHOPHYSIOLOGY- ADE Activation

Increase in virus replication and number of infected cells



Heterotypic antibodies

- Low concentration
- Partial neutralization
- prM

Virus uptake

- Delivery to Fcγ receptor cells

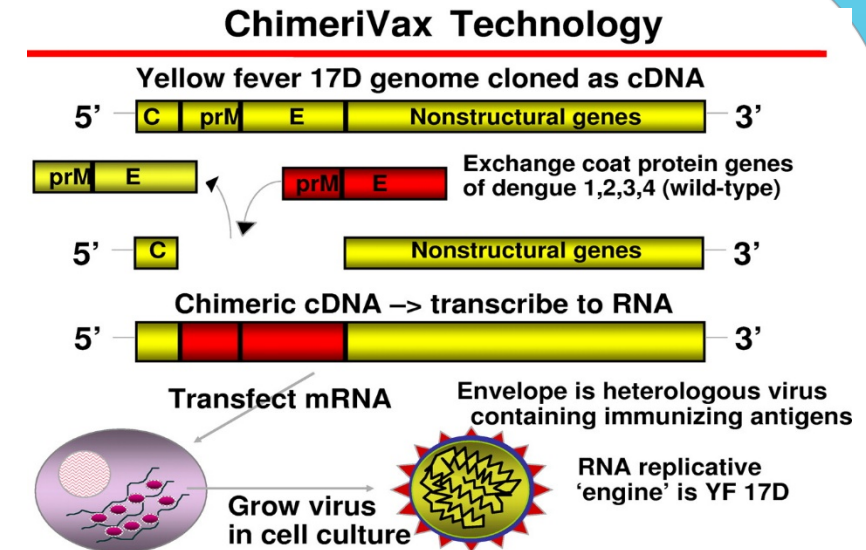
Replication

- Increased viral replication compared to virus alone

Chimeric Live-attenuated Vaccination

ChimeriVax Vaccine (Sanofi Pasteur)

- uses 17D yellow fever vaccine virus
- Phase I, II, III
 - shows 57% overall efficacy
 - reduction of hospitalization by 80%
 - 89% reduction of dengue haemorrhagic fever

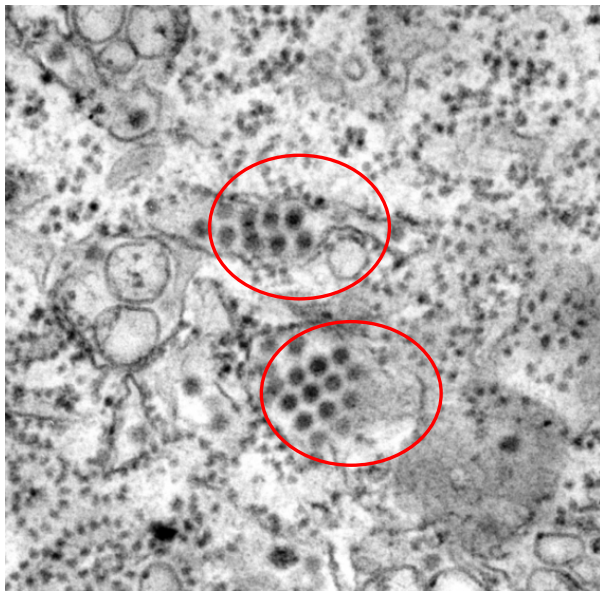


Dengvaxia program launched a school based vaccination program in the Philippines in April 2016 (~700,00 immunizations)

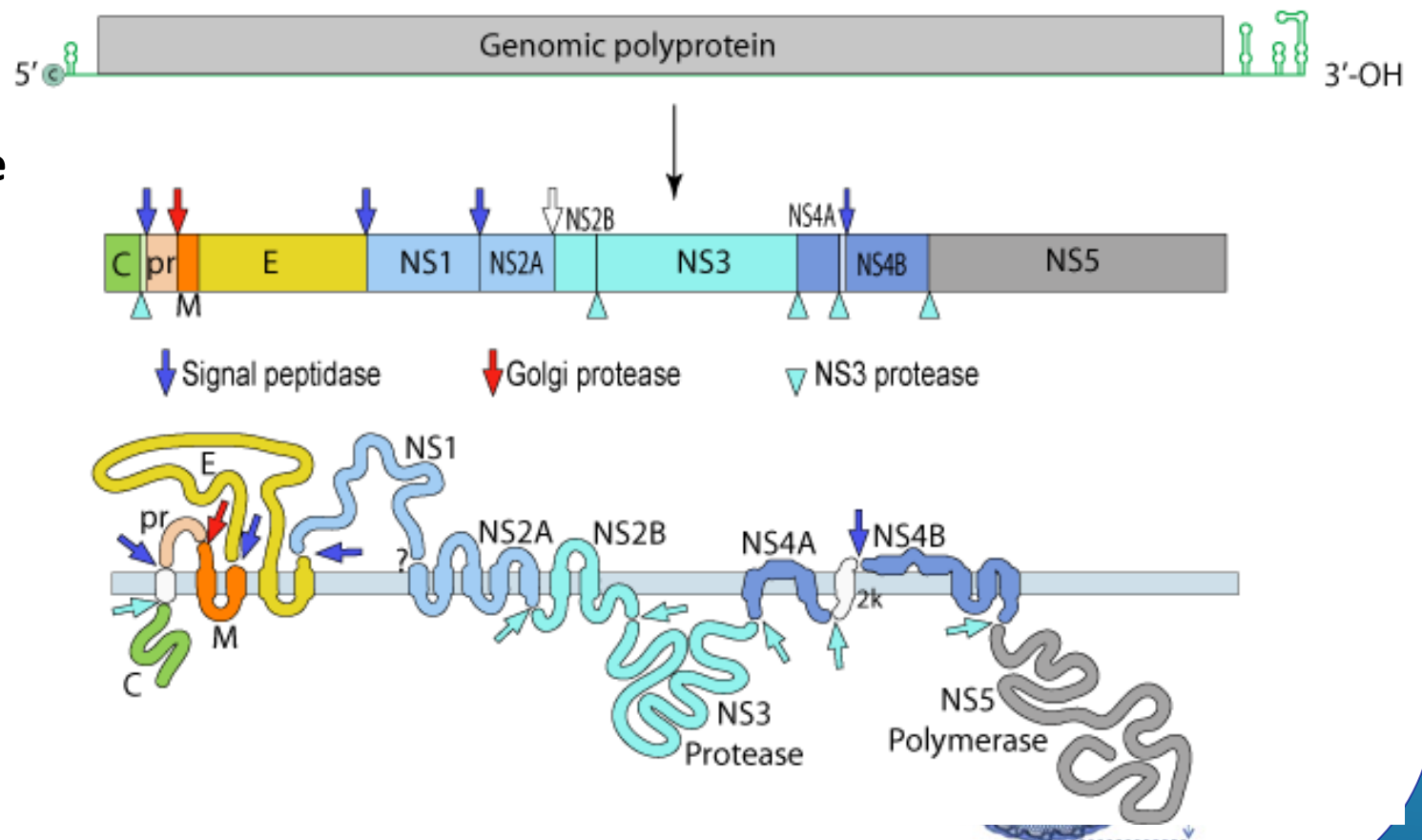
Program suspended (November 2017) after the death of 64 children with no prior dengue infection

Virus structure & components

- *Flaviviridae* family, genus *flavivirus*
- Icosahedral nucleocapsid
- Lipid envelope
- 4 serotypes (1-4) which all can cause severe forms of infection



- Positive-sense, single-stranded RNA
- Approximately, 11Kb in length



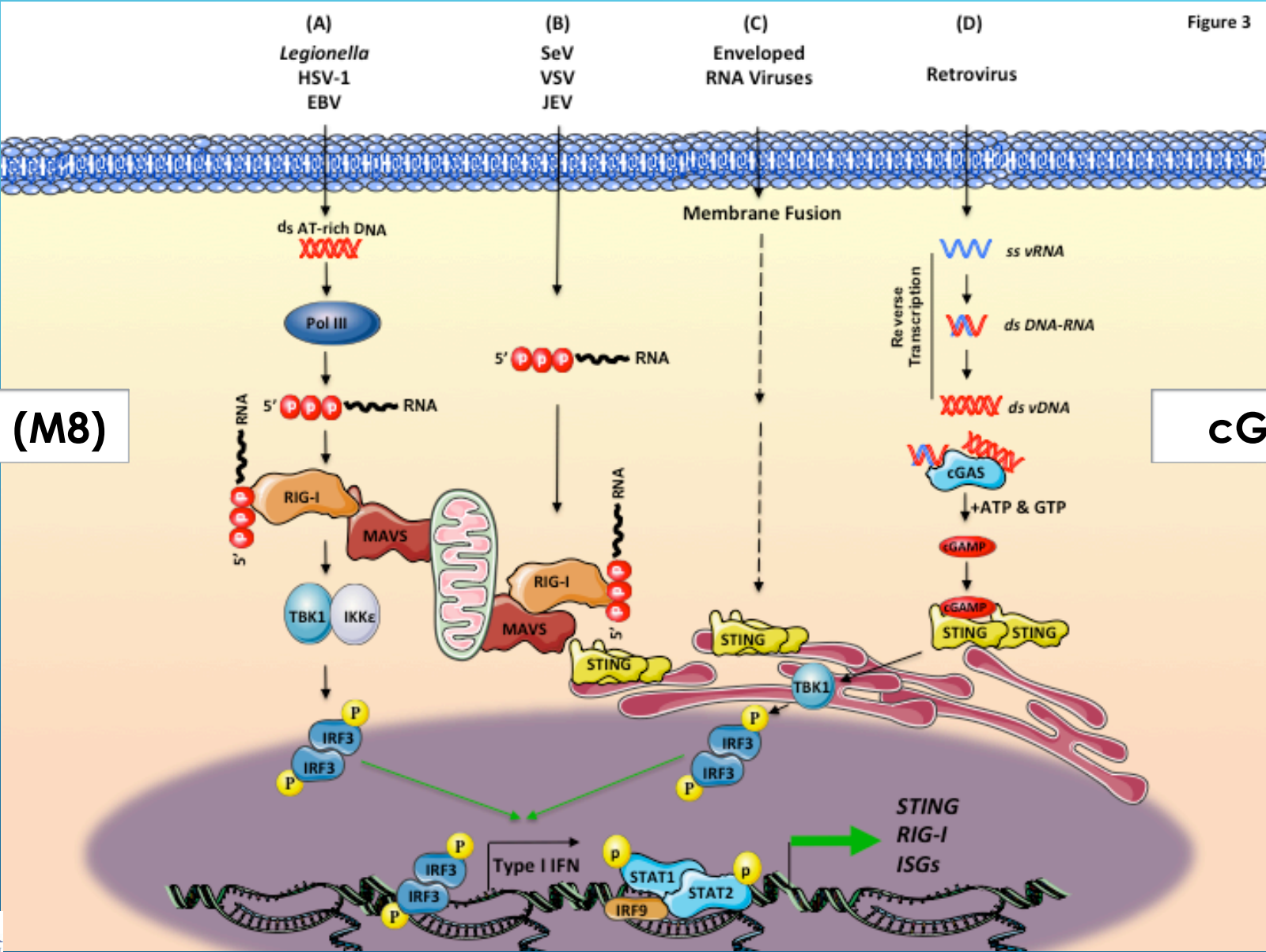
Cross-talk between RIG-I and cGAS-STING pathways

RIG-I pathway

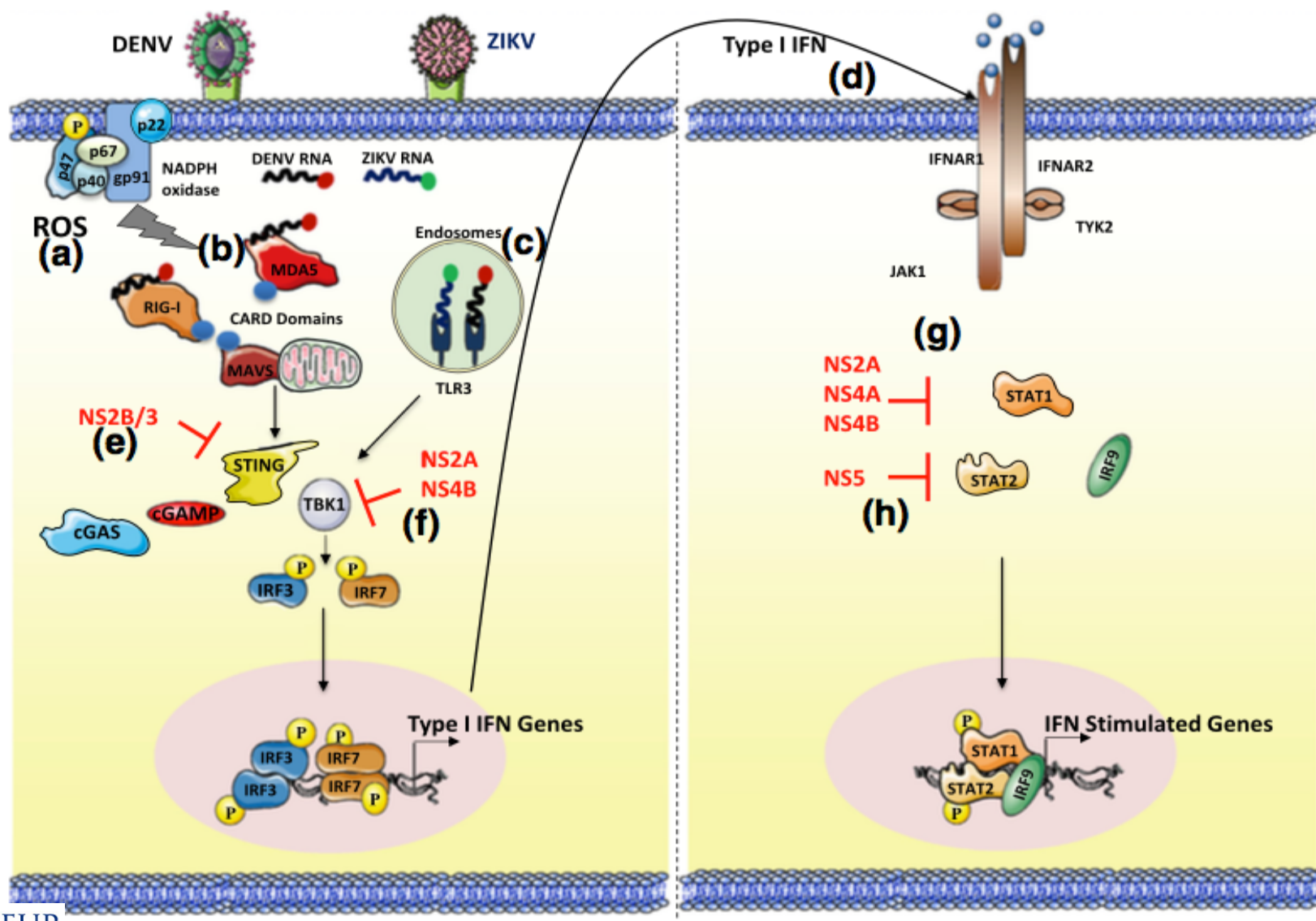
cGAS-STING pathway

5'ppp RNA (M8)

cGAMP



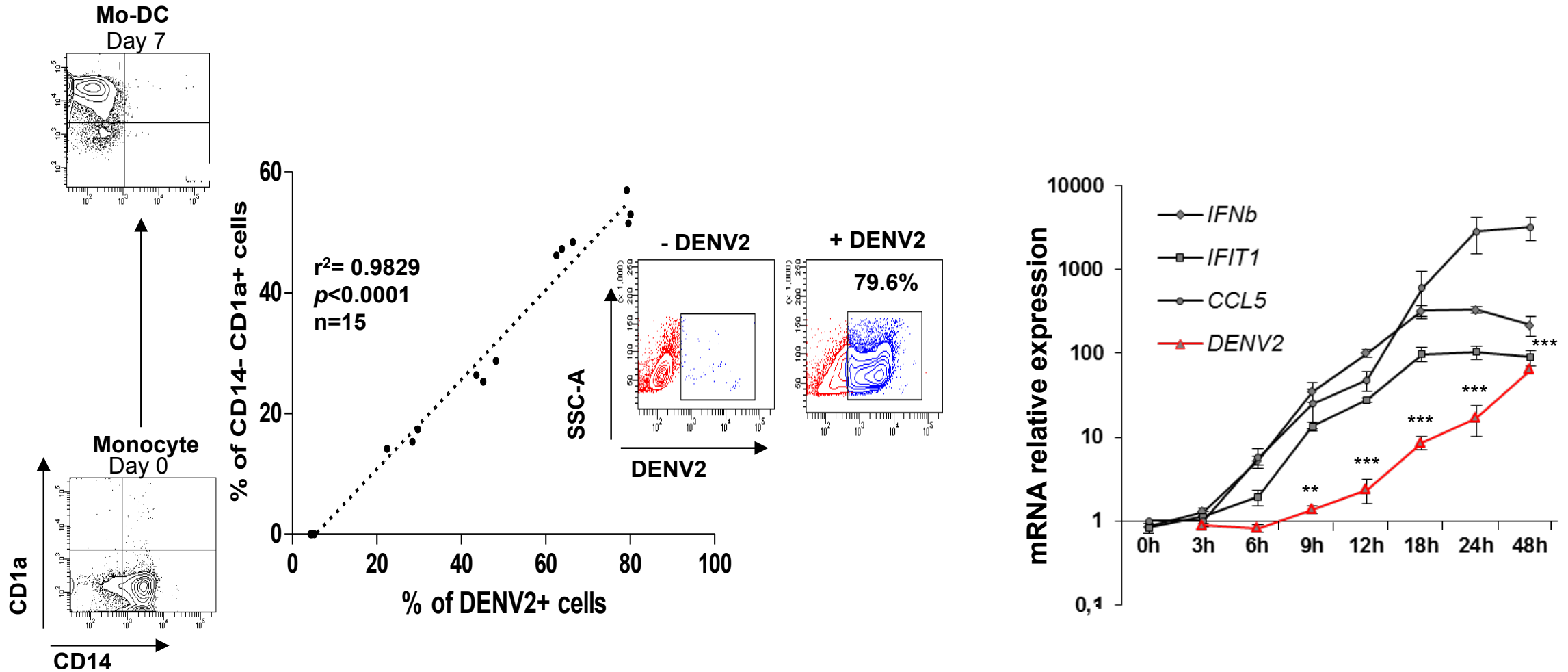
Dengue infection disrupts antiviral immunity



Dendritic cells and dengue virus

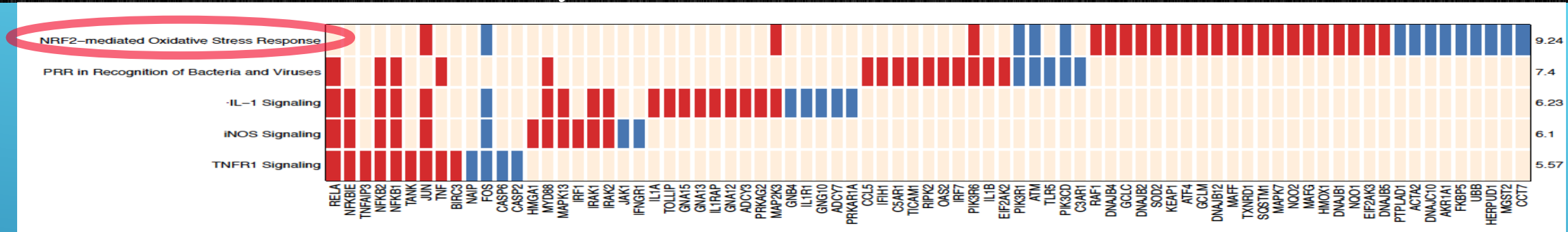
- Dendritic cells and skin-resident Langerhans cells are the initial targets for infection after the bite of a mosquito
- DC are able to mount a rapid antiviral & inflammatory response to DENV
- Interaction between DENV and DC is crucial in both the control of infection and in the evolution of disease severity
- DENV-infected DC are subject to viral evasion mechanisms (interference with STAT signaling; cleavage of STING)

DENV infects myeloid cells during differentiation to mature Mo-DC, triggering antiviral, inflammatory, and oxidative stress pathways

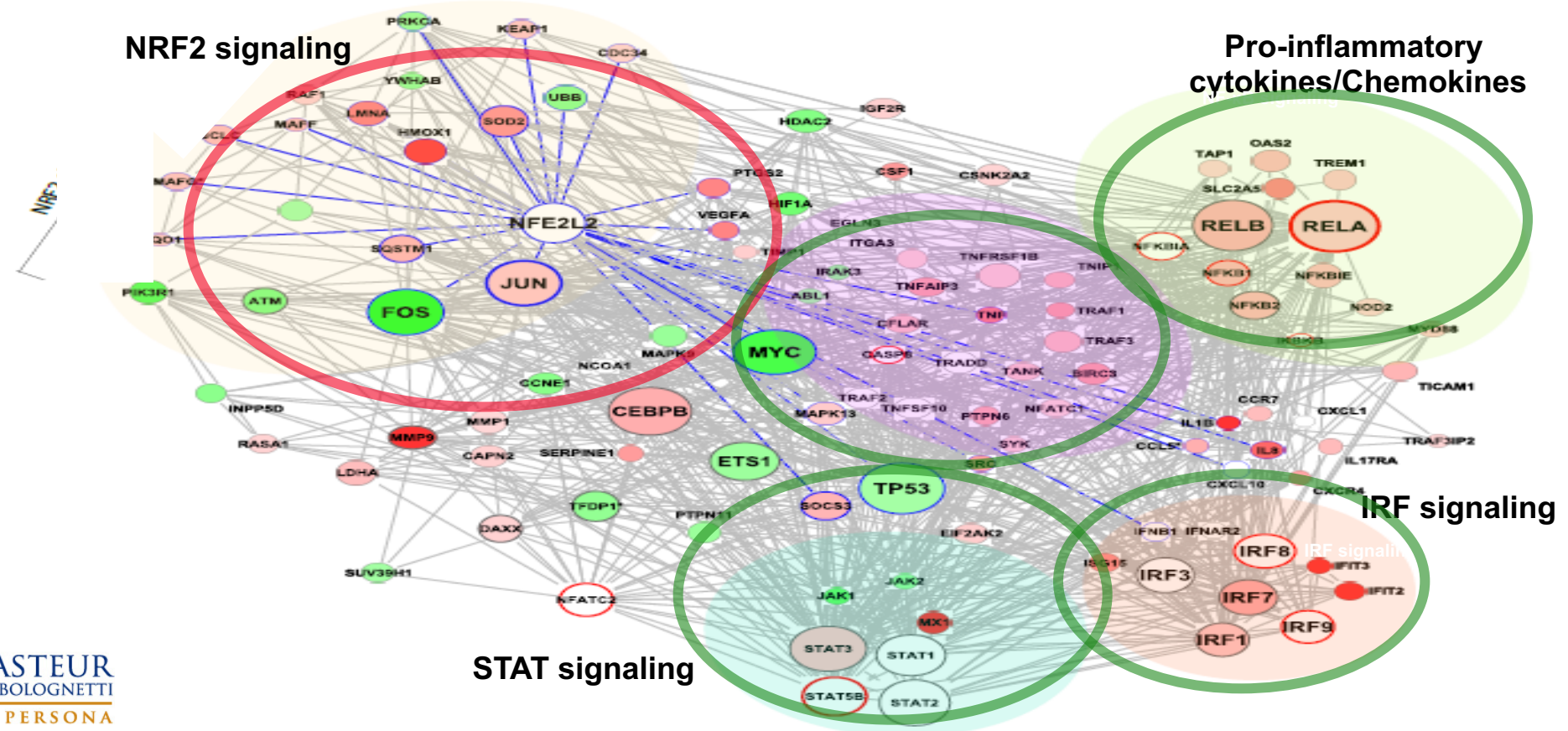


Primary human monocytes differentiated with GM-CSF+IL4; infected with DenV NGC (3 MOI/cell)

Nrf2-antioxidant response pathway is highly enriched early after DENV2 infection of primary DC

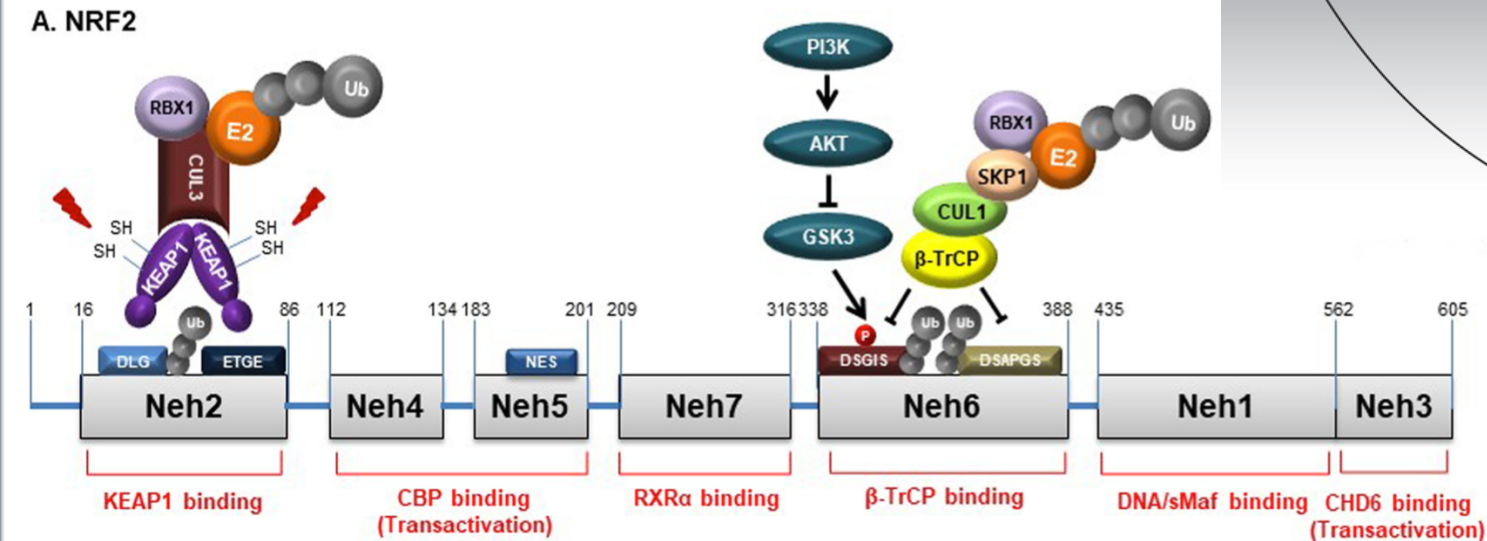
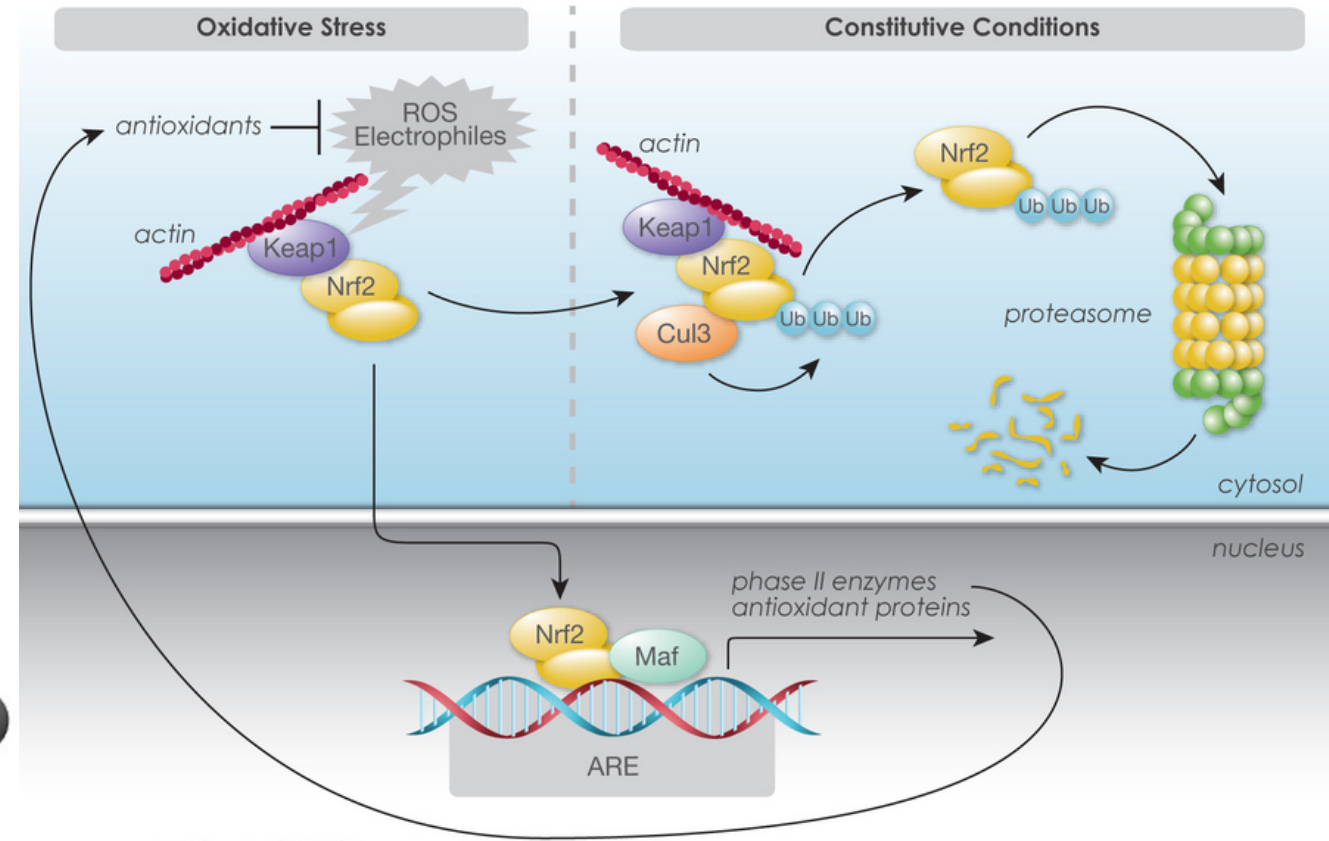


6 hrs



Nuclear factor erythroid 2 related factor 2 (Nrf-2)

- *bZIP* transcription factor
- Involved in redox homeostasis & in protection against oxidative stress
- Regulates expression of cytoprotective & anti-oxidant genes



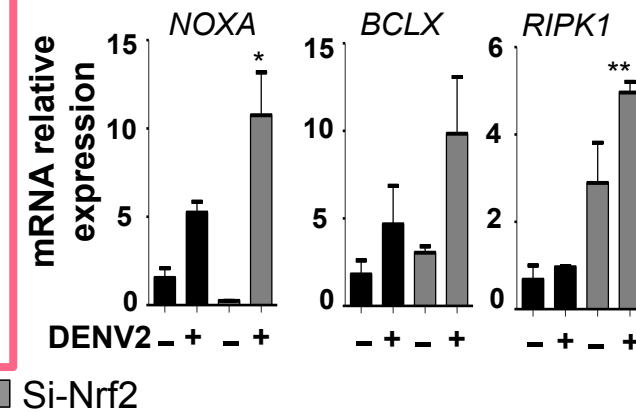
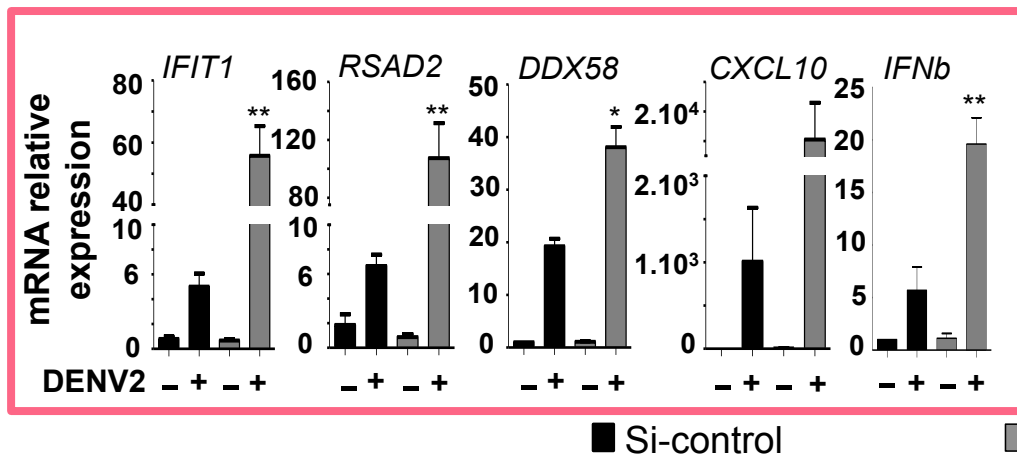
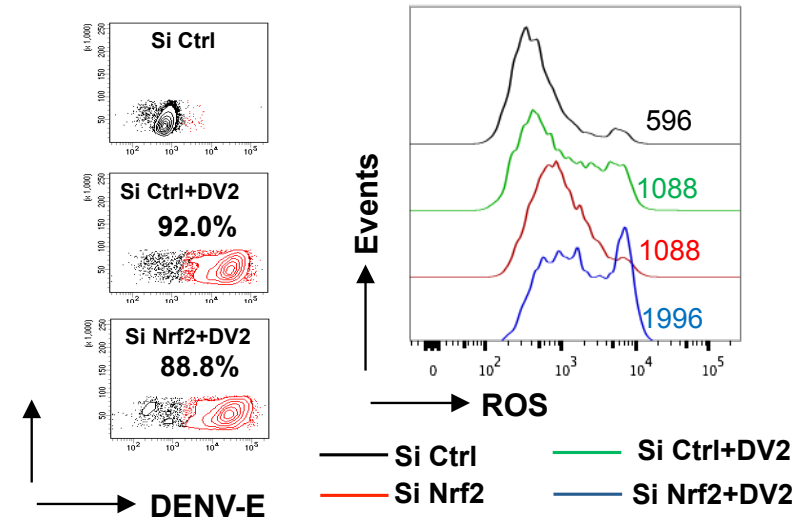
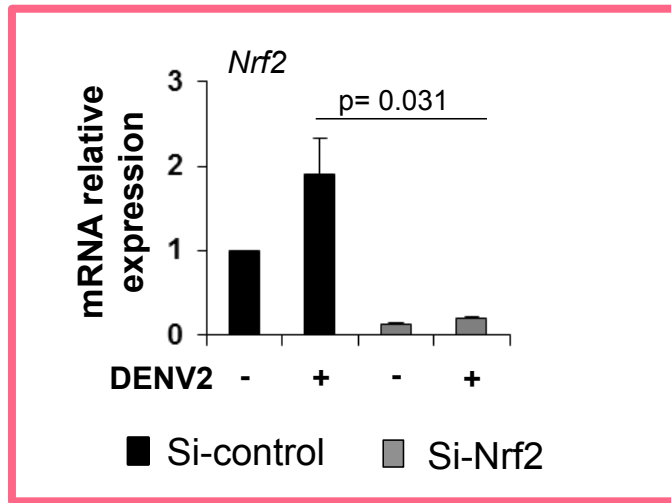
Structural domains of Nrf2

Questions

Does ROS accumulation & Nrf2 activation impact innate immune responses?



Nrf2 silencing increases ROS production, antiviral, inflammatory & apoptotic gene expression



Questions & Answers

Does ROS accumulation & Nrf2 activation impact innate immune responses?

ROS-mediated induction of Nrf2 limits antiviral & inflammatory responses.



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Can Nrf2 activation modulate metabolic function in DENV infection?



Regulation of metabolic pathways by Nrf2

Nrf2 contributes to increased glucose uptake rates & aerobic glycolysis

Activation of Nrf2 increases glucose uptake & directs it to the pentose phosphate pathway by controlling expression of enzymes such as:

- glucose 6 phosphate dehydrogenase
- 6-phosphogluconate dehydrogenase
- transketolase
- transaldolase

Enzymes associated with NADPH synthesis:

- malic enzyme
- isocitrate dehydrogenase

Questions & Answers

Does ROS accumulation & Nrf2 activation impact innate immune responses?

ROS-mediated induction of Nrf2 limits antiviral & inflammatory responses.

Can Nrf2 activation modulate metabolic function in DENV infection?

Nrf2 regulates numerous enzymatic functions involved in glucose metabolism.

A series of three parallel white diagonal lines extending from the bottom right corner towards the center of the slide.

Questions & Answers

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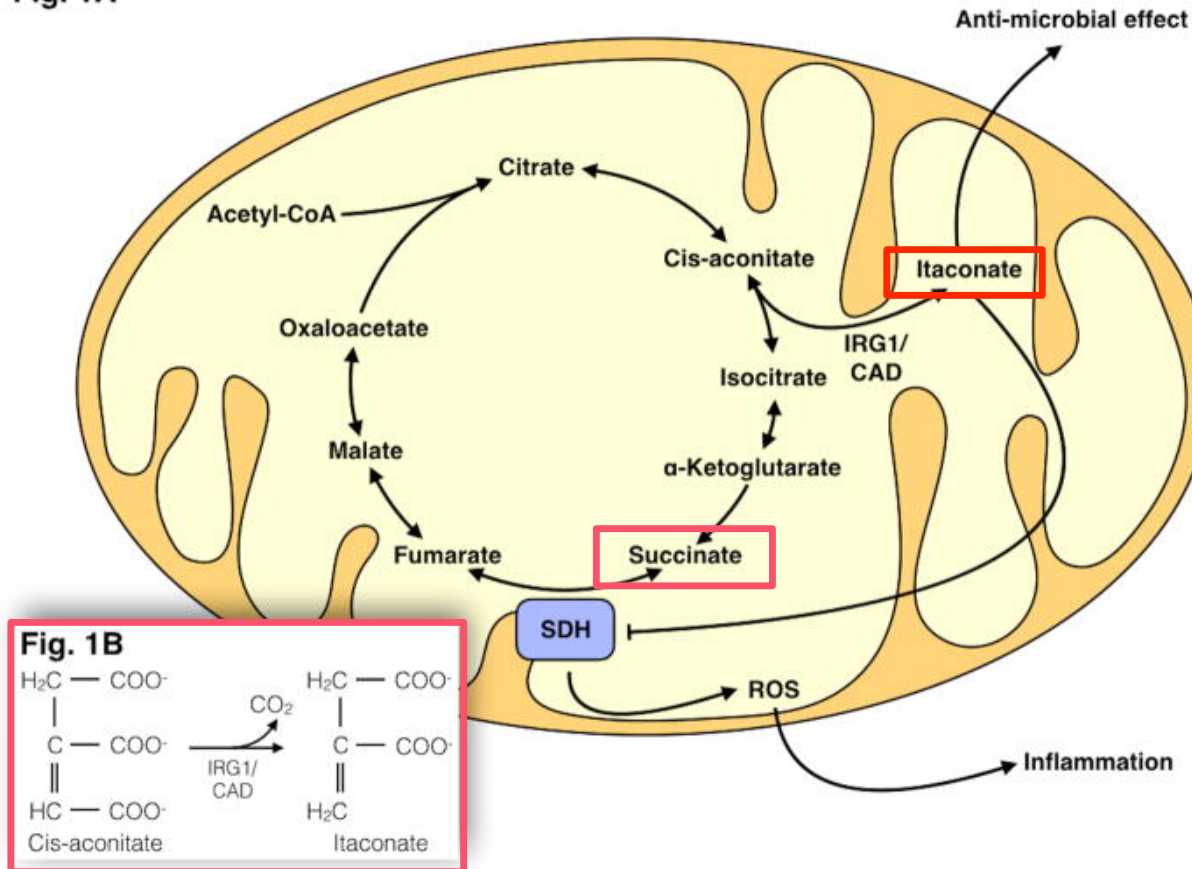
Nrf2 regulates numerous enzymatic functions involved in glucose metabolism.

Does metabolic stimulation of Nrf2 modulate antiviral innate response?

Three white lines of varying lengths and slopes are positioned in the bottom right corner of the slide, extending from the right edge towards the center.

Krebs cycle metabolic byproduct Itaconate activates Nrf2 to limit antiviral signaling

Fig. 1A

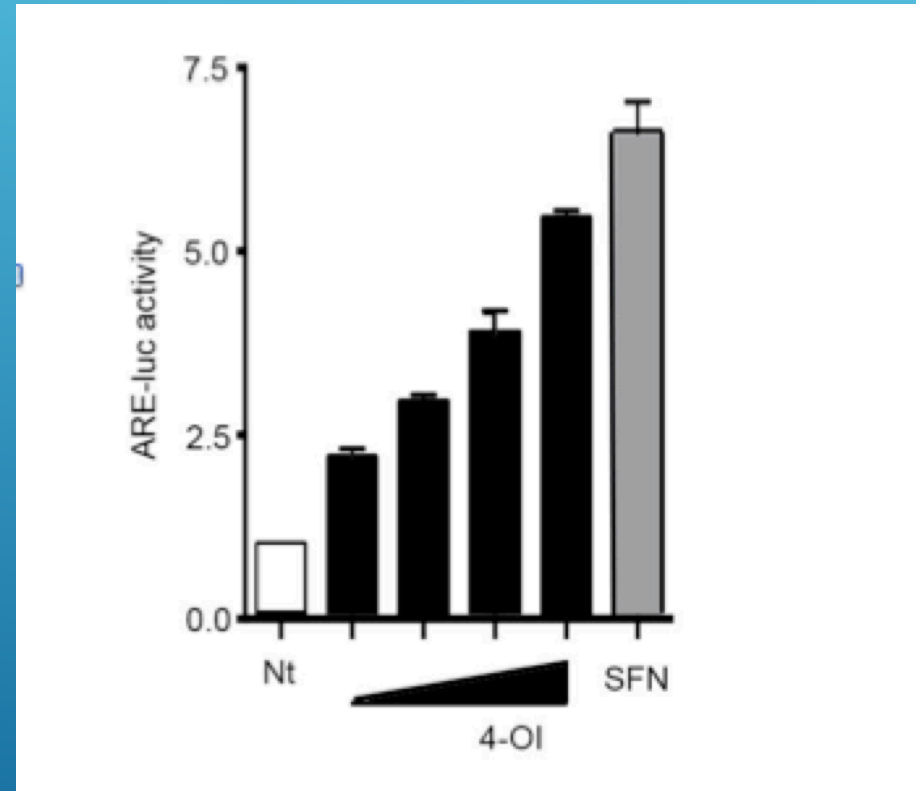


Itaconate – suppresses inflammation via induction of the transcription factor Nrf2 through direct binding of the Nrf2 repressor Keap1

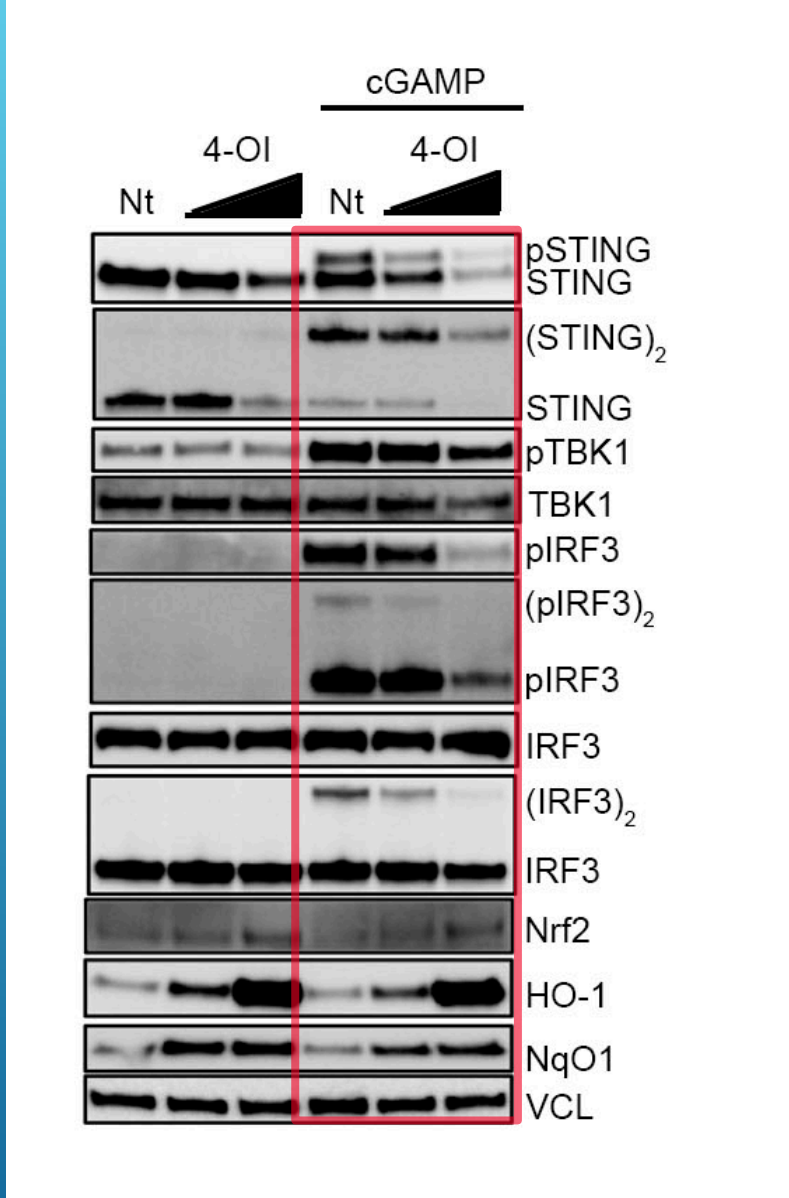
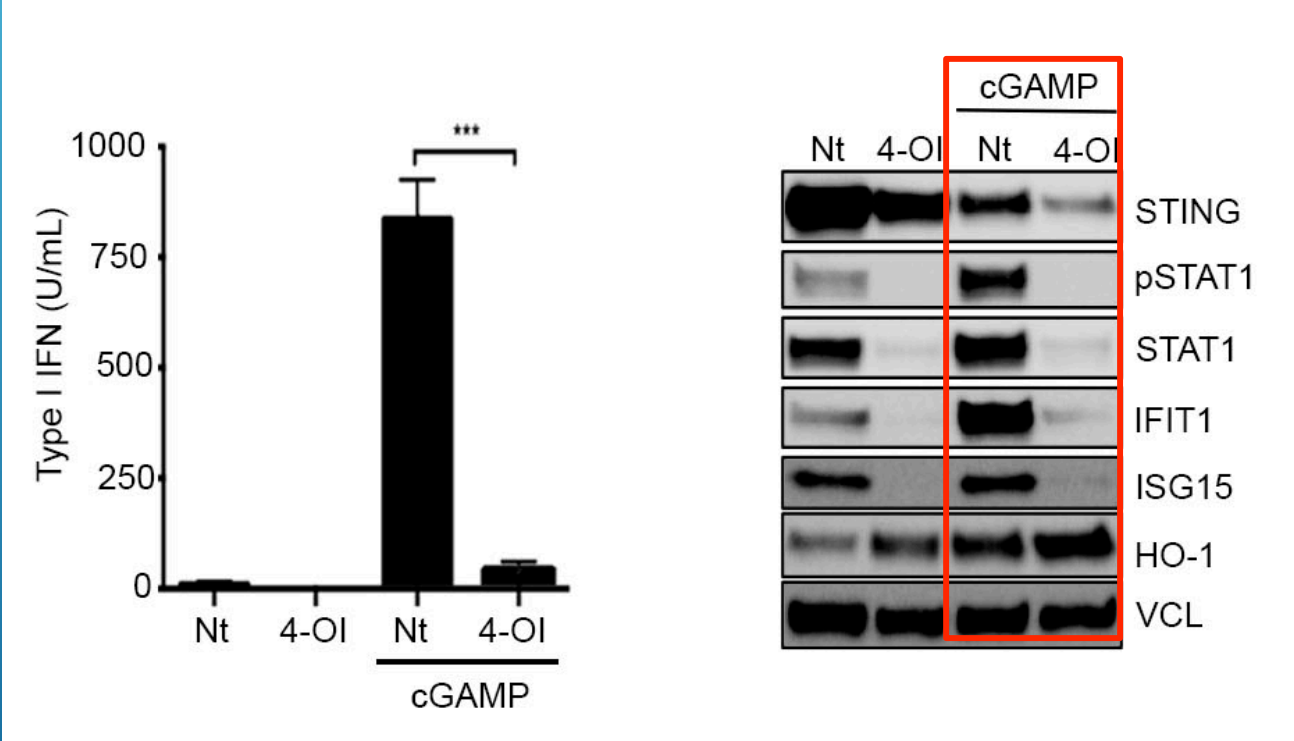
Itaconate links inhibition of succinate dehydrogenase with macrophage remodeling & regulation of inflammation
Lampropoulou et al, Cell Metabolism (2016)

Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1
Mills et al, Nature (2018)

Itaconate & sulforaphane activate the Nrf2 anti-oxidant response element

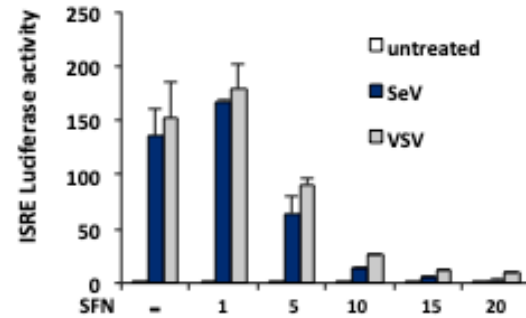


Itaconate (& SFN) inhibits cGAMP induced IFN signaling & production

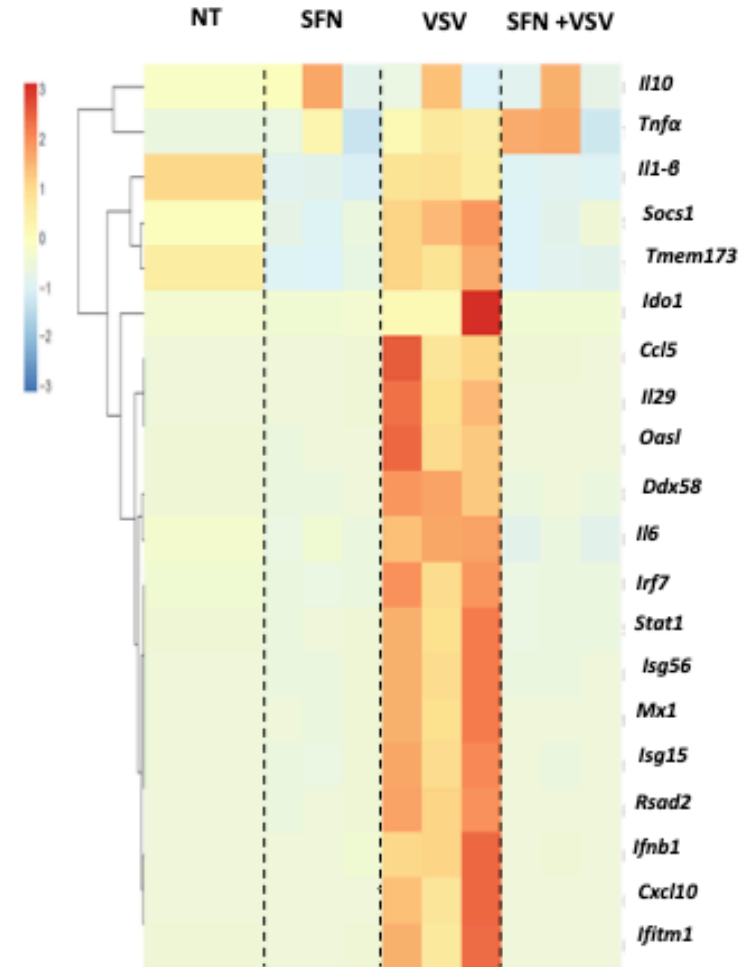
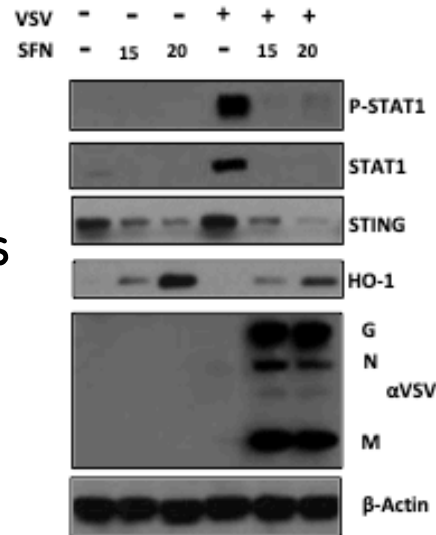


Sulforaphane interferes with antiviral immunity

SFN inhibits
ISRE activity



...and enhances virus
replication



SFN inhibits
Antiviral ISGs

Questions & Answers

Does ROS accumulation & Nrf2 activation impact innate immune responses?

ROS-mediated induction of Nrf2 limits antiviral & inflammatory responses.

Can Nrf2 activation modulate metabolic function in DENV infection?

Nrf2 regulates numerous enzymatic functions involved in glucose metabolism.

How does metabolic stimulation of Nrf2 modulate antiviral innate response?

Krebs cycle metabolic byproduct itaconate & sulfoxaphane induces Nrf2 via Keap1 alkylation, resulting in downregulation of antiviral responses.

Questions & Answers

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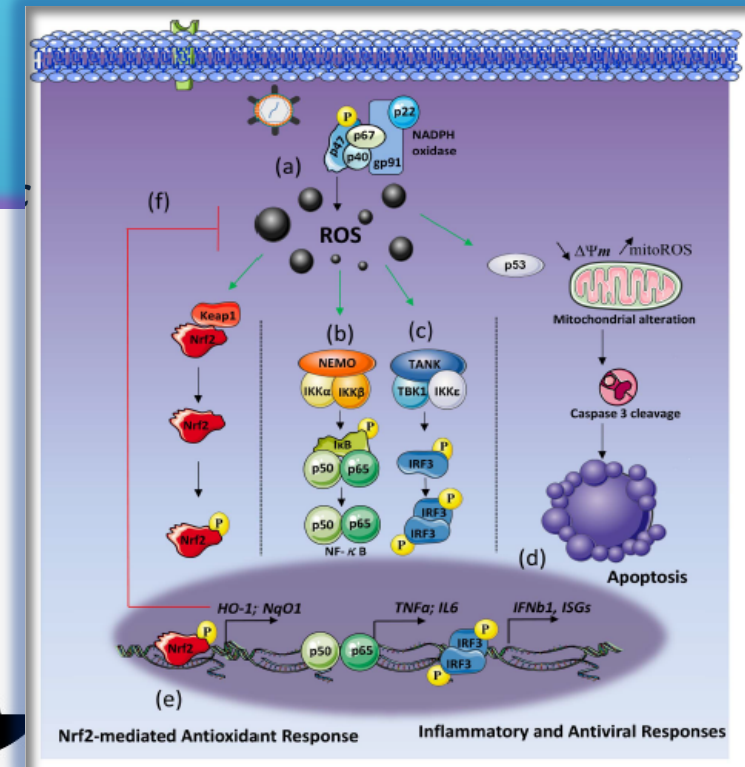
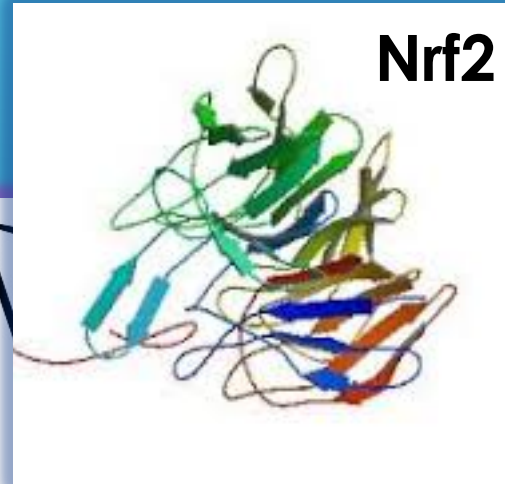
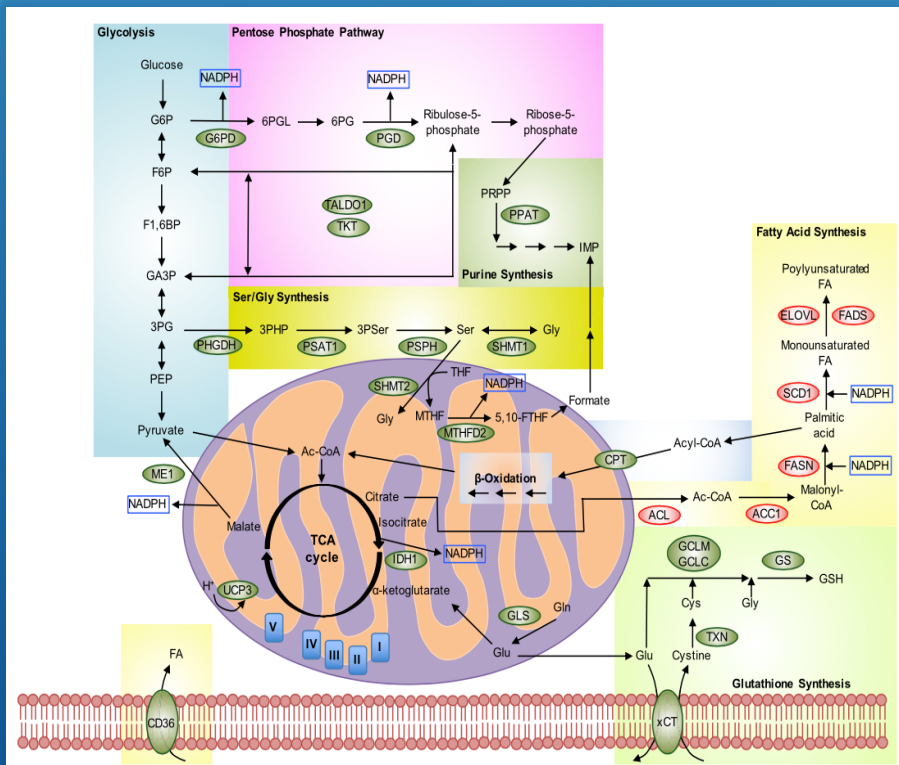
Does DENV infection alter Nrf2 activation?

DENV NS2B3 targets Nrf2 for degradation late in DENV infection.

A line graph with two curves plotted against time on the x-axis (0 to 24 hours) and an unlabeled y-axis. The first curve, labeled 'ROS' in red, is a purple-filled area that peaks early, around 6 hours, and then declines. The second curve, labeled 'Nrf2 activity' in red, is a green-filled area that starts rising after 6 hours, peaks around 18 hours, and then declines. The two curves intersect at approximately 12 hours.



Nrf2 anti-oxidant network at the interface of metabolism and antiviral immune response



Conclusions

Nrf2 anti-oxidant network sits at the interface between metabolic and innate immune regulation

DENV targeting of Nrf2 by NS3 may reflect a viral evasion mechanism to disrupt metabolic-antiviral responses in DENV infection.

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