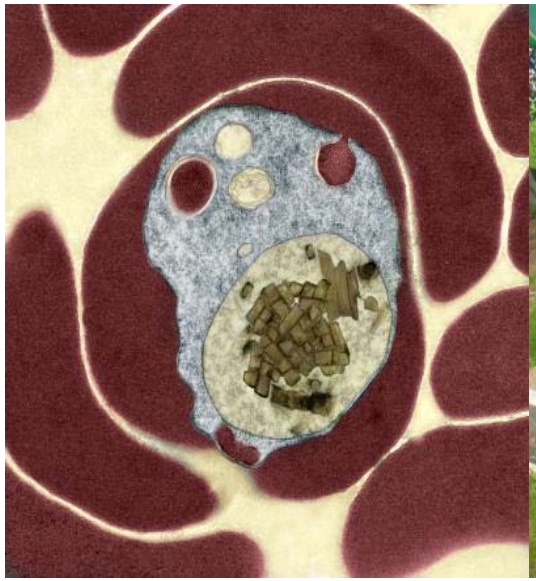
May 20th, 10:45 AM - 12:15 PM

Lessons from the Field: Parasite DNA Drives the Innate Immune Response to Malaria (it's not just the caiparinhas)

Douglas Golenbock
University of Massachusetts Medical School

Lessons from the field: parasite DNA drives the innate immune response to malaria (it's not just the caiparinhas)

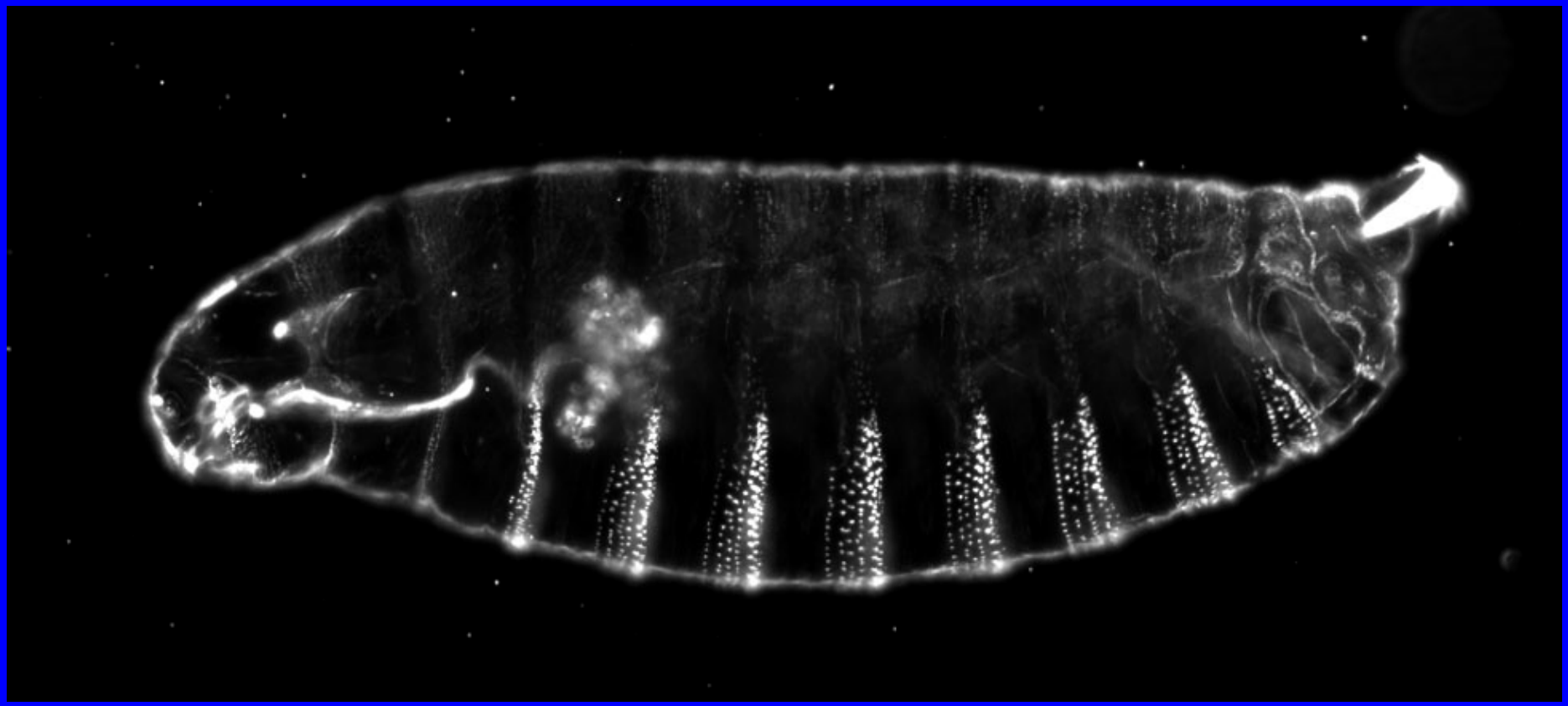


University of Massachusetts Medical School
UMASS

Douglas Golenbock, MD

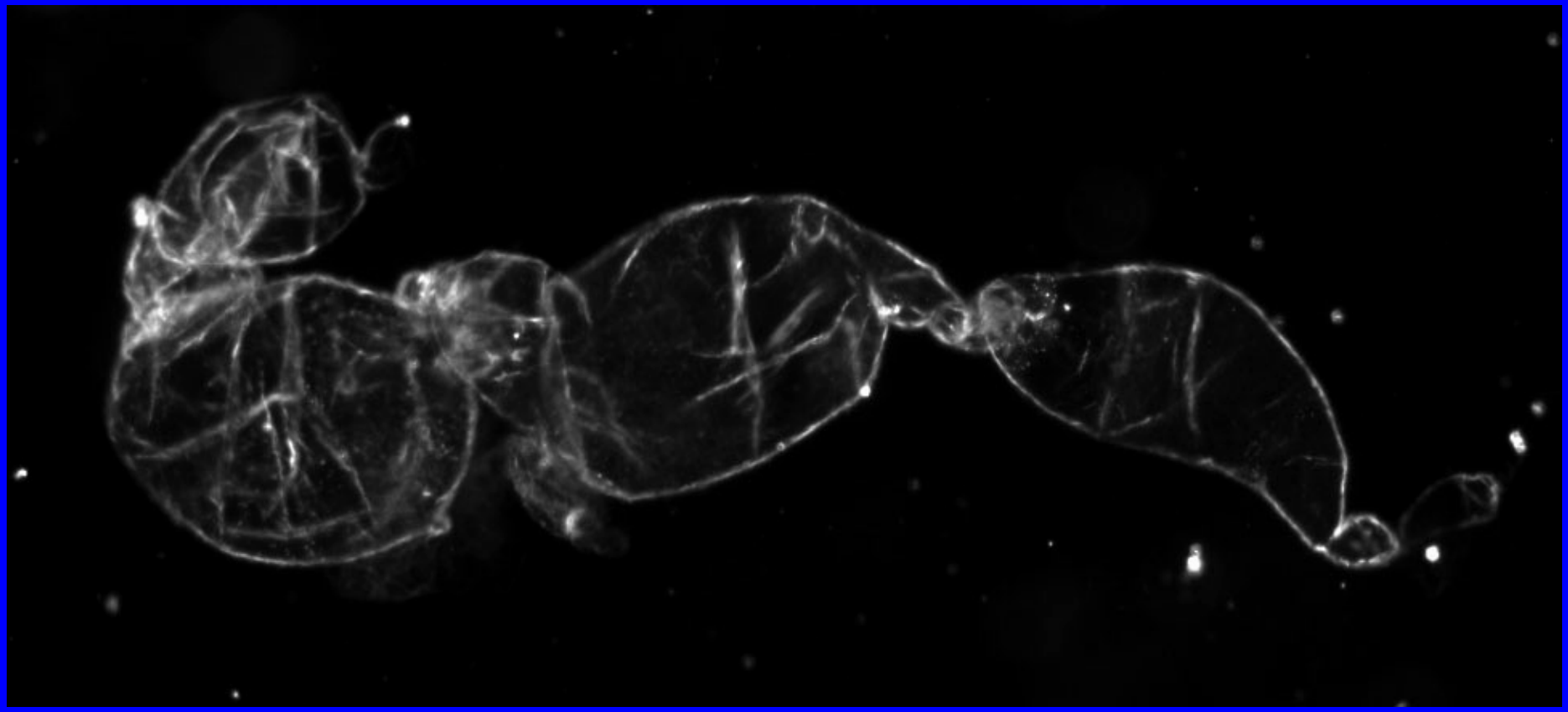
Chief, Infectious Diseases

Drosophila melanogaster larvae



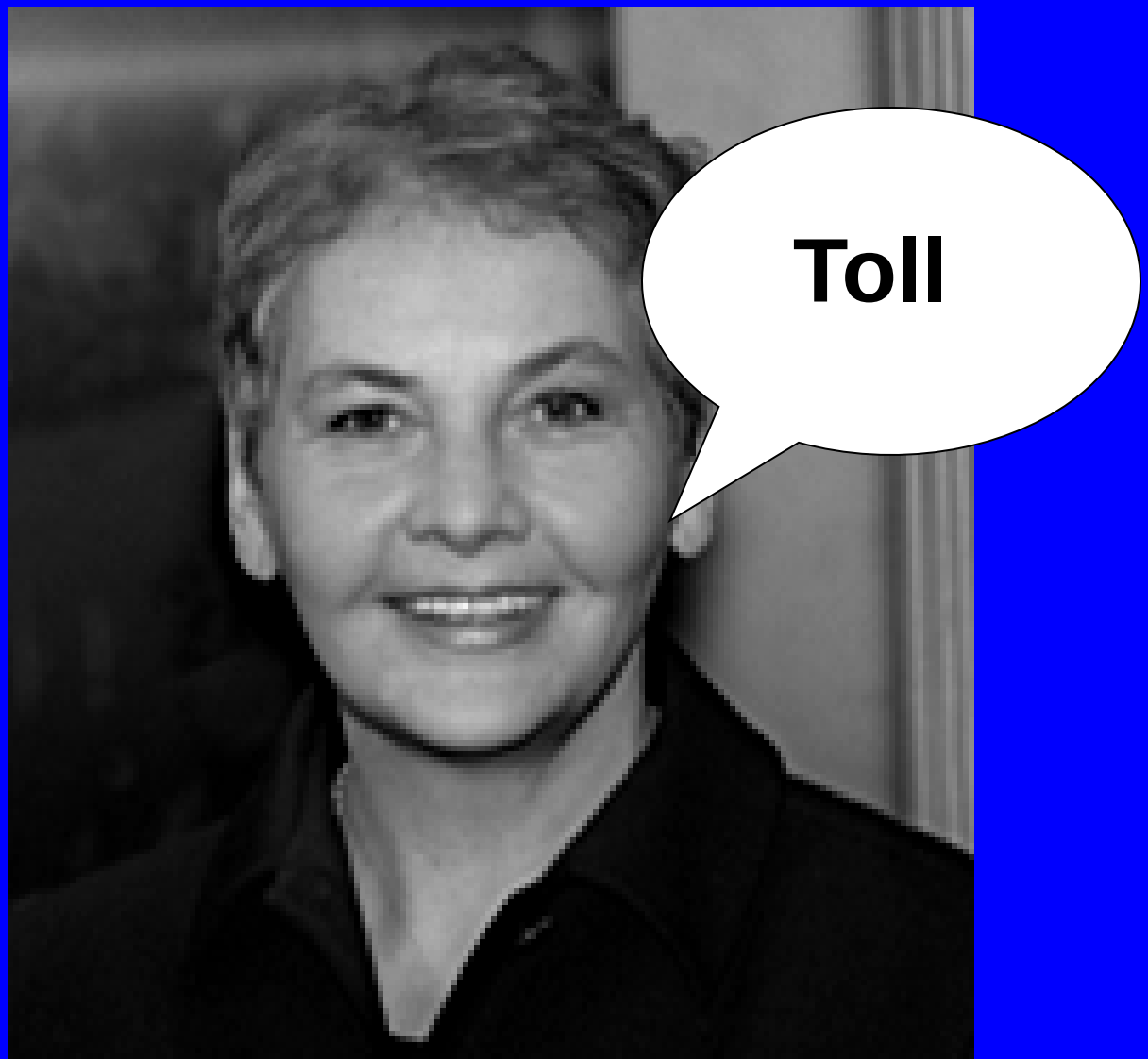
Christiane Nüsslein-Volhard, Nobel Laureate (Medicine, 1995)

mutant *Drosophila melanogaster* larvae



Anderson *et al. Cell* 1985; 42: 791-98

Christiane Nüsslein-Volhard, Nobel Laureate (Medicine, 1995)



Christiane Nüsslein-Volhard, Nobel Laureate (Medicine, 1995)

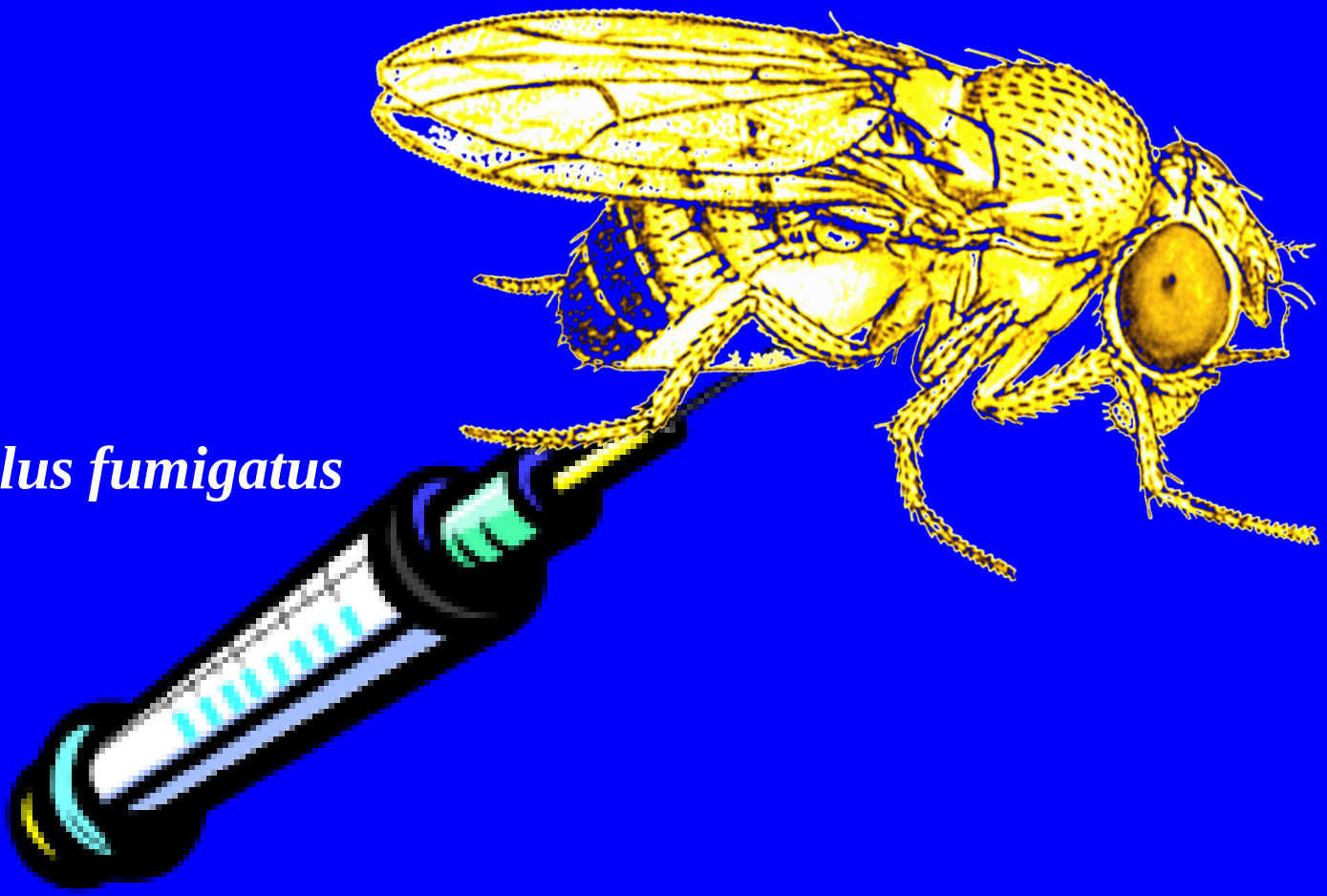
TOLL

Amazing
Bodacious
Cool
Corky
Crazy
Frantic
Furious
Great
Like blazes
Mad
Madcap
Screaming
Super
Wild
Wicked
Wow

Cloning of *Toll*:

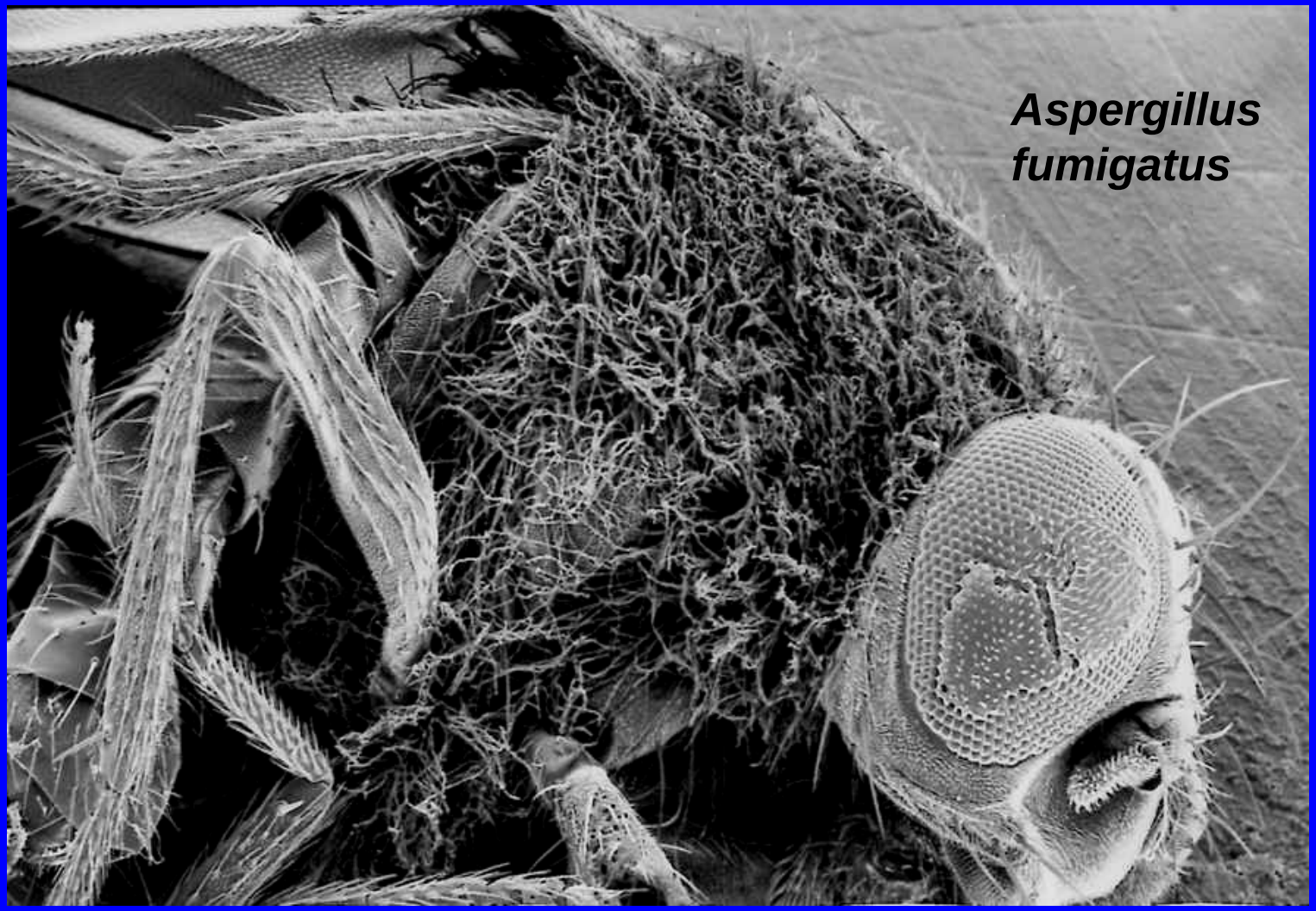
homology to the human IL-1 receptor

Lemaitre, Hoffmann *et al. Cell* 1996; 86: 973-83



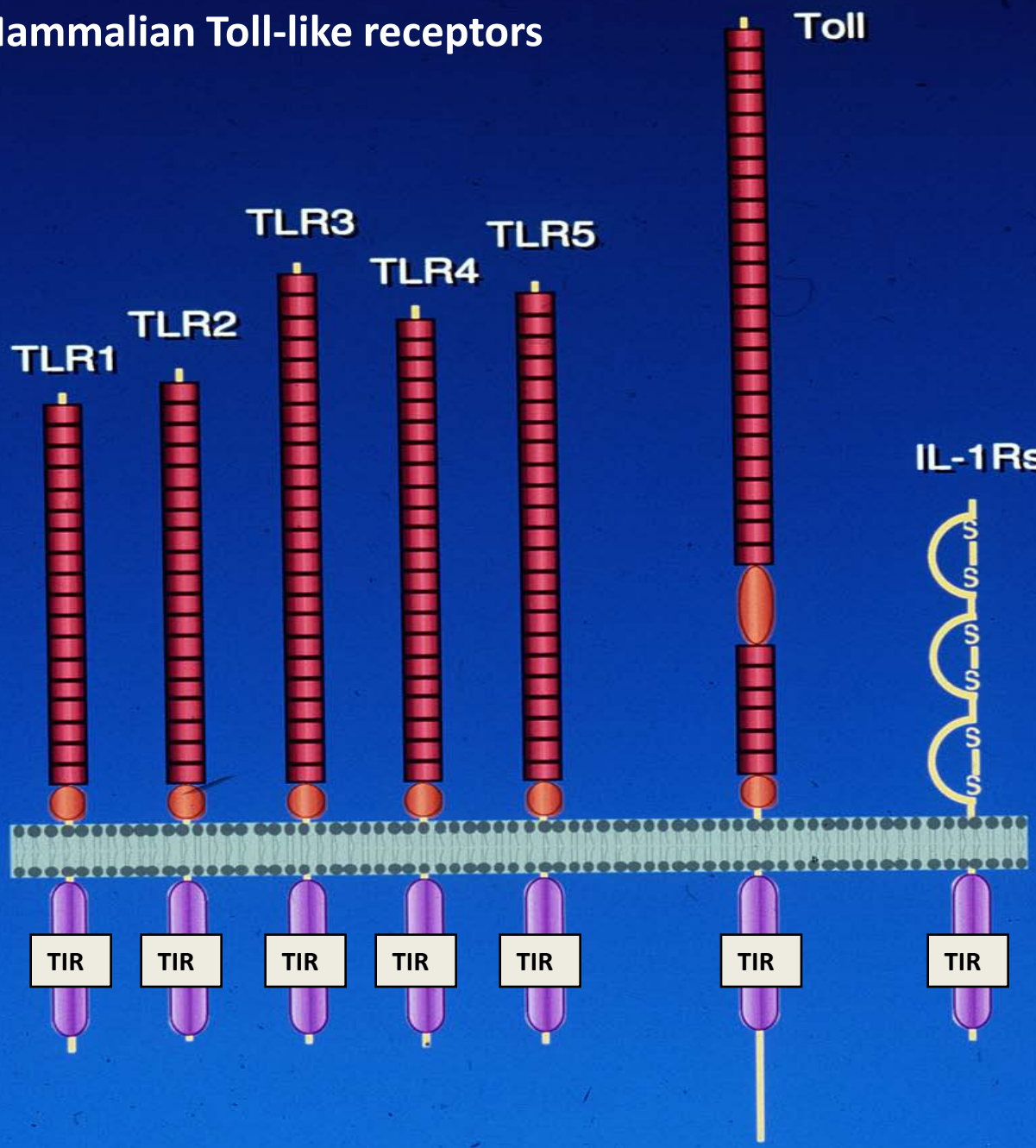
Aspergillus fumigatus

Mutations in *Toll* predispose to fungal infections



Lemaitre *et al*. *Cell* 1996; 86: 973-83

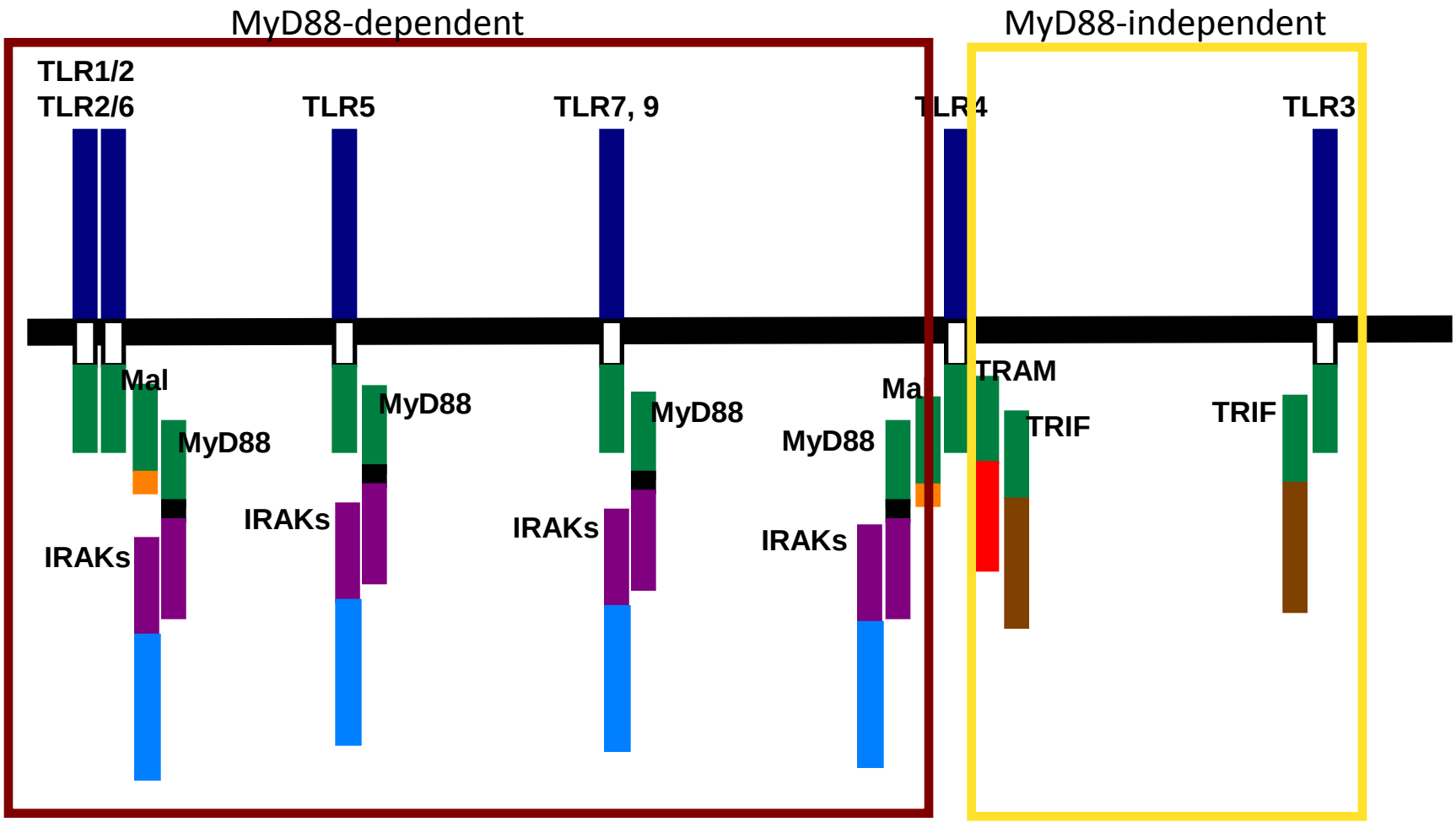
Mammalian Toll-like receptors



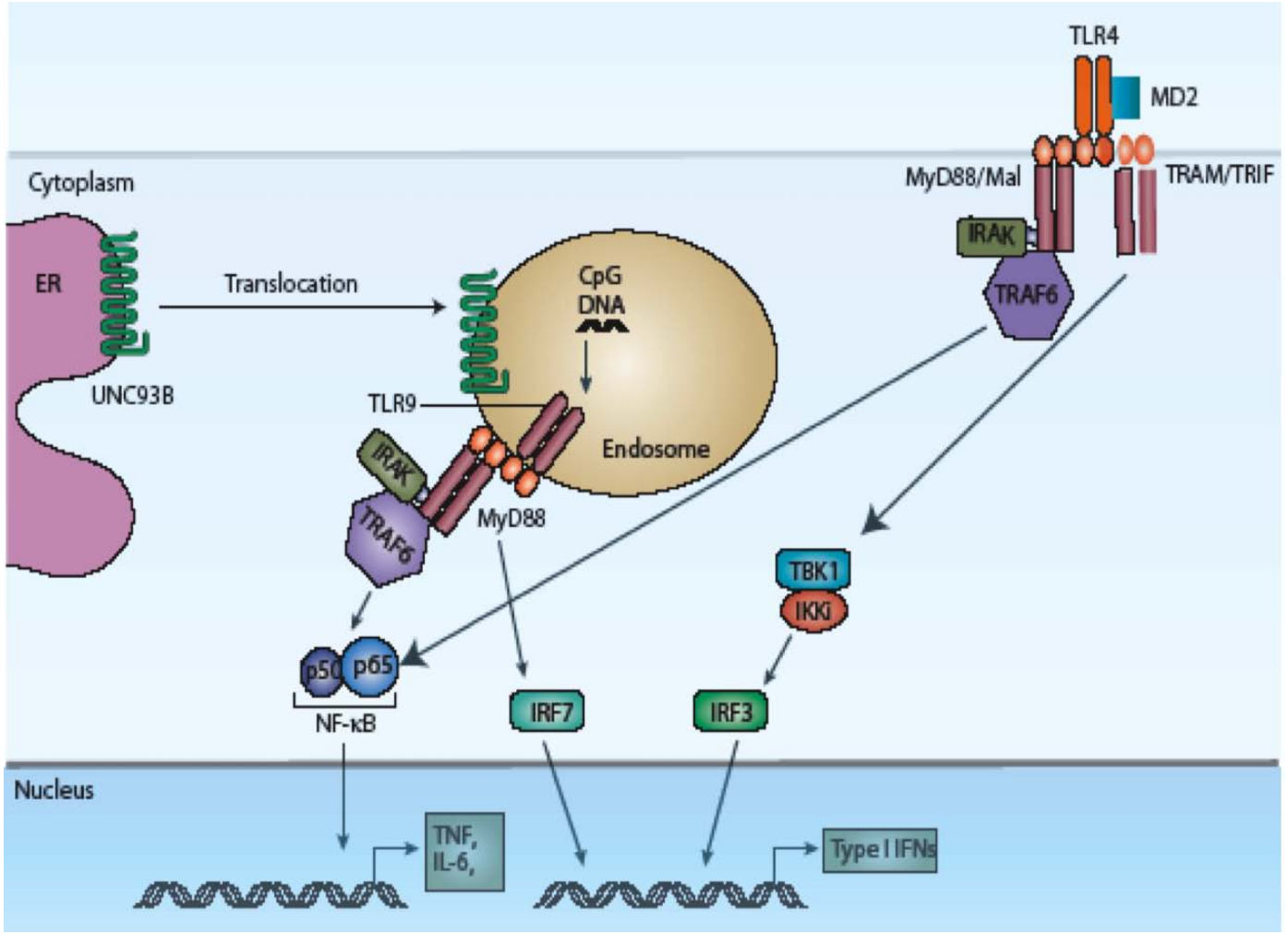
10 human TLRs

4 adapter TLRs

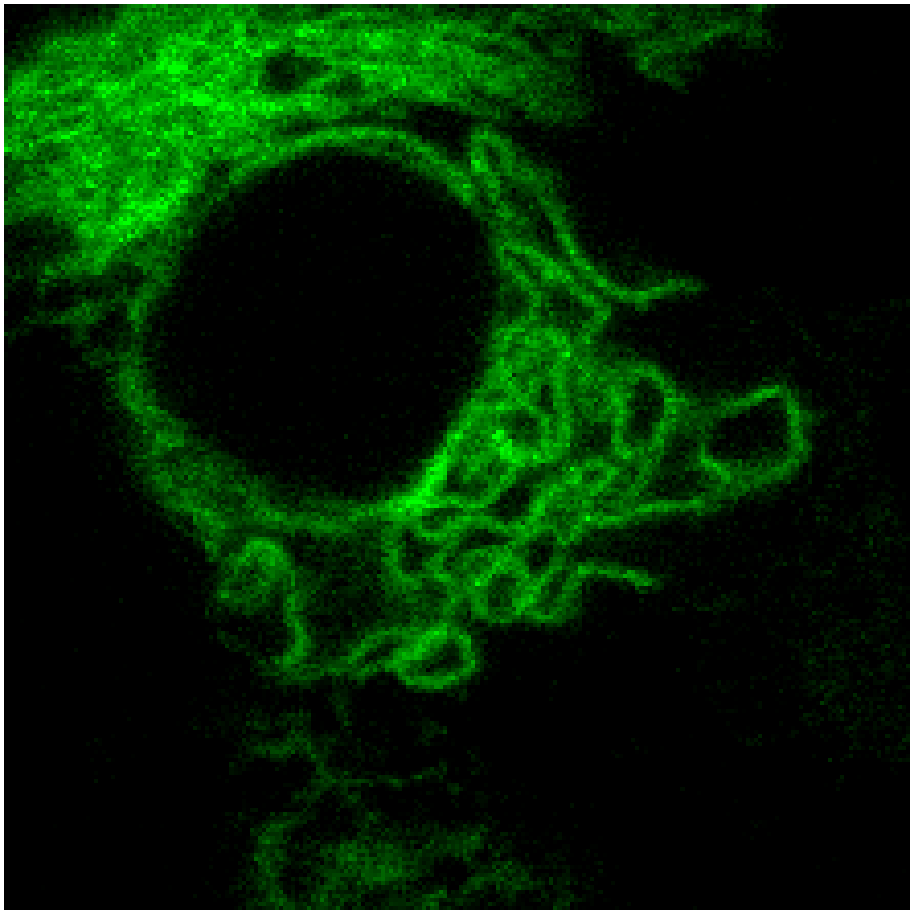
There are 4 TLR adapter molecules



There are two broad classes of TLRs: endosomal and surface TLRs.

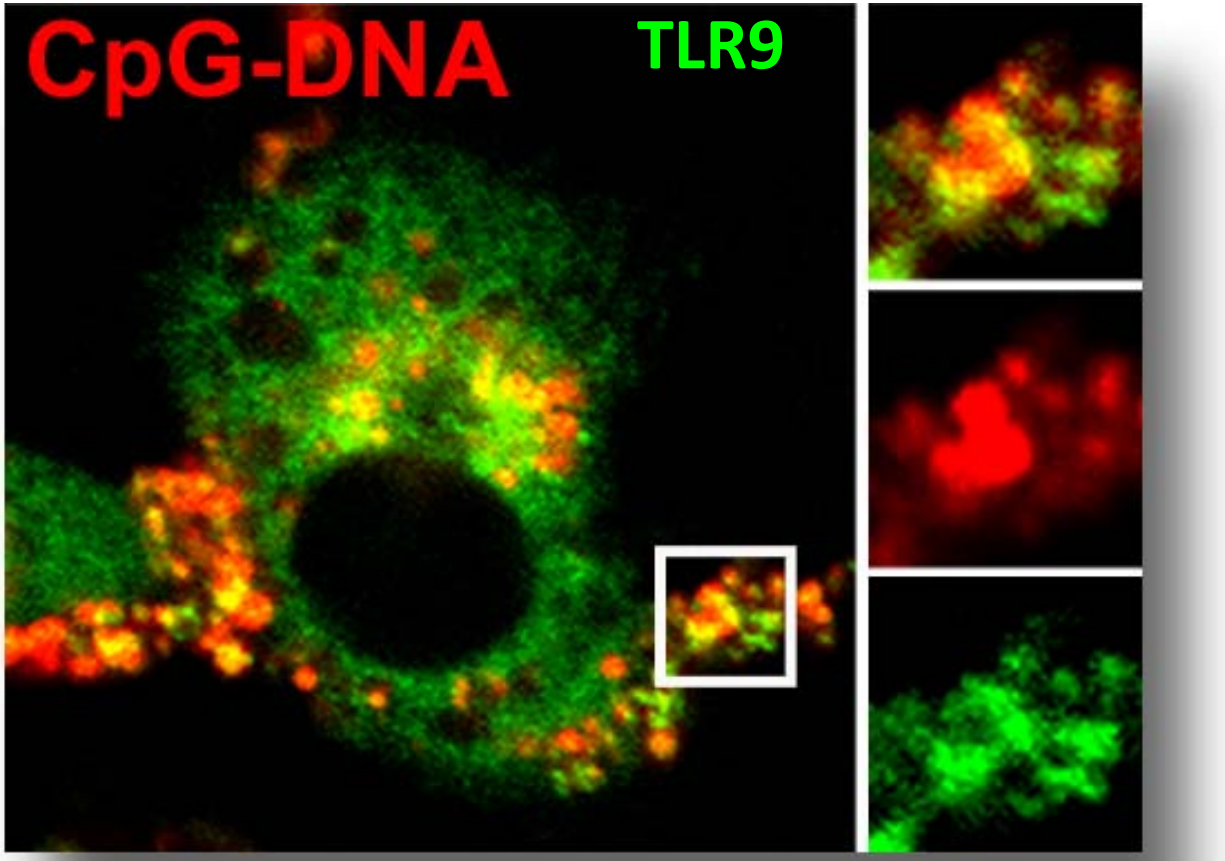


TLR9 is the prototypical endosomal TLR.

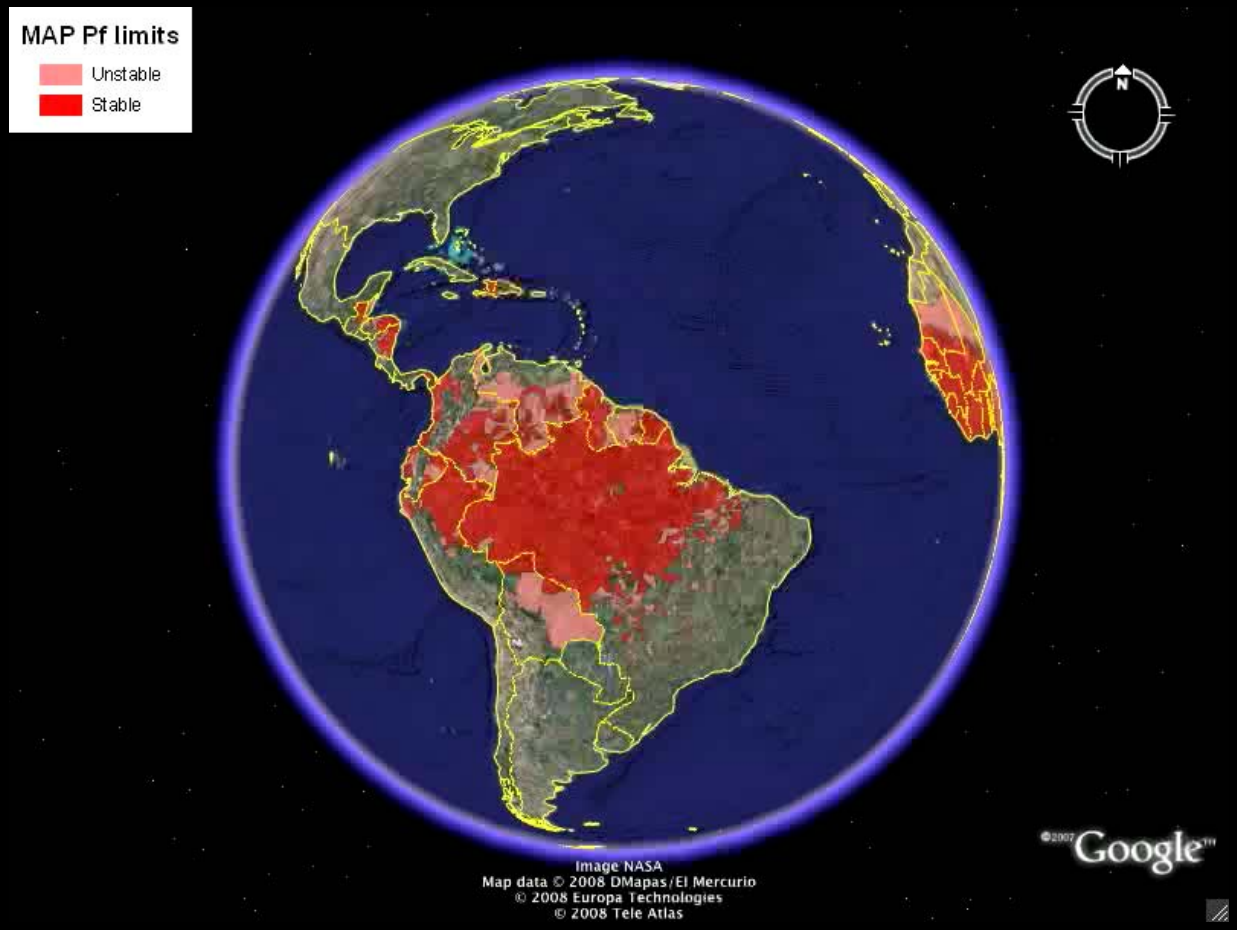


TLR9 must move from the ER to the endosome to encounter its ligand (CpG DNA).

Mouse dendritic cells after 5 min



Malaria: the facts in 2010

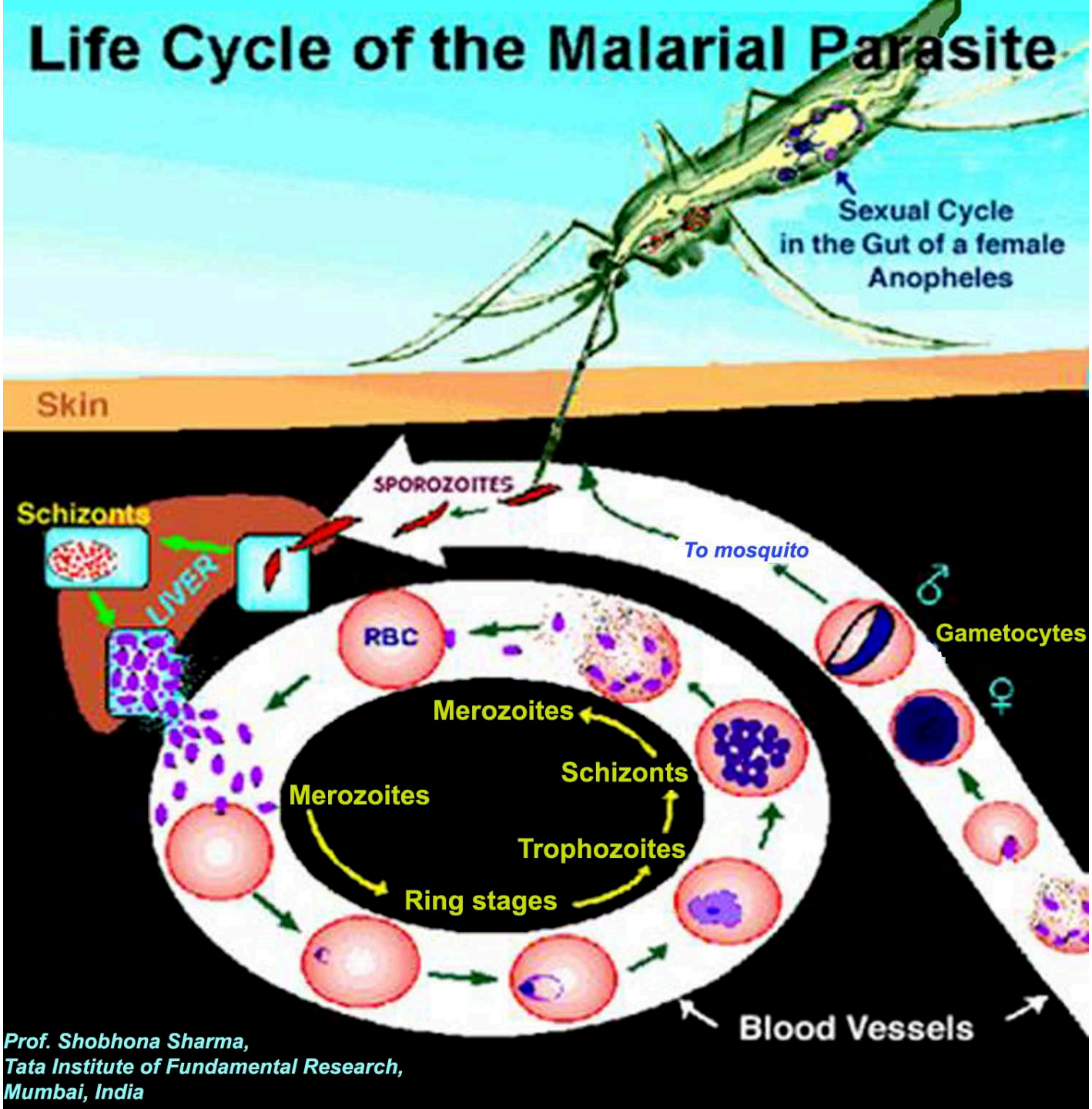


35% of the worlds population is at risk

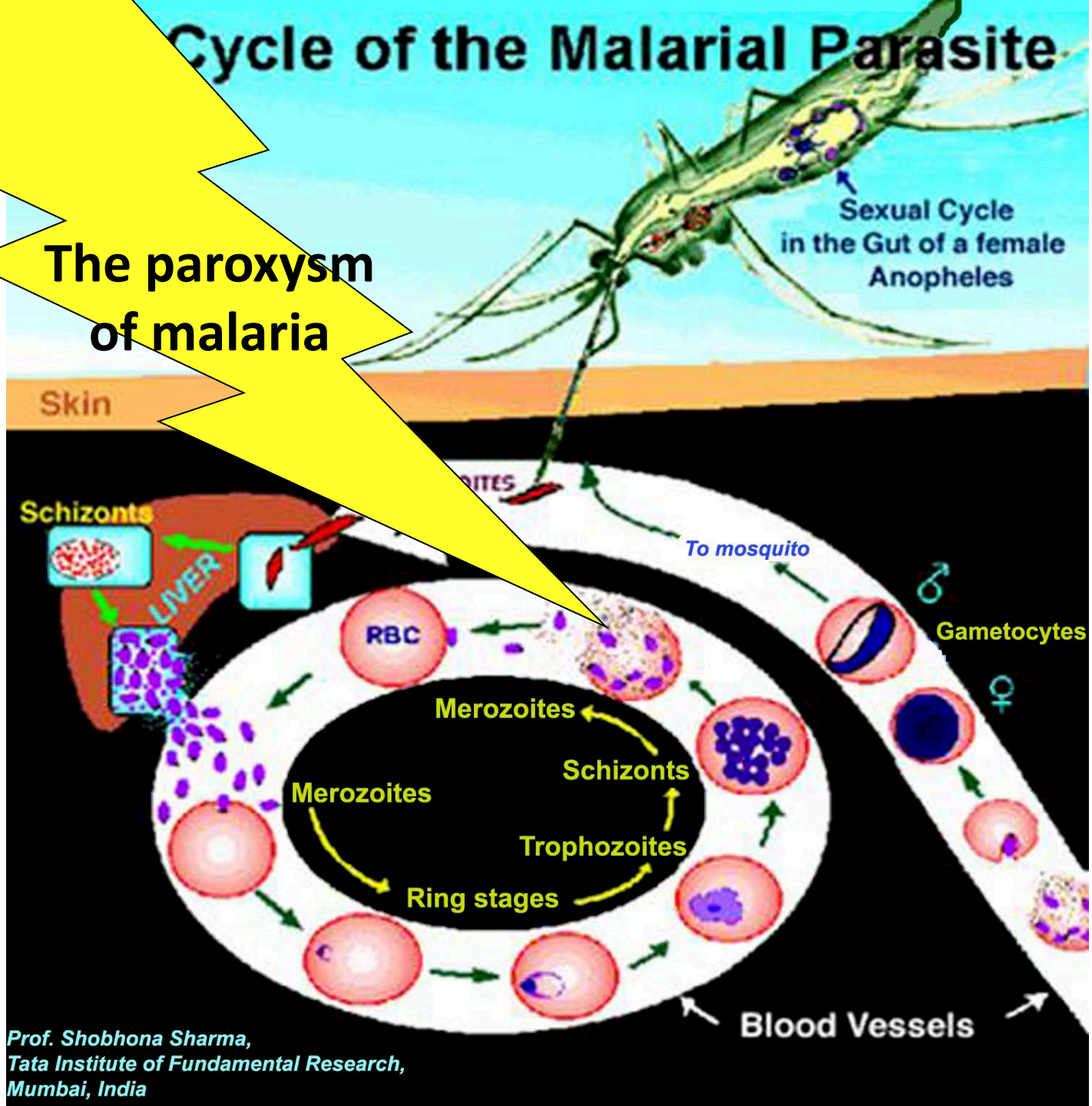
http://www.map.ox.ac.uk

Epidemiology

- Malaria is generally considered to be a tropical disease.
- Four major species of plasmodium, but *P. falciparum* and *P. vivax* are by far the most important.
- *P. falciparum* is worldwide; *P. vivax* is not frequent in Africa but frequent in South America and Asia.


Life Cycle of the Malarial Parasite
Sexual Cycle
in the Gut of a female
Anopheles
Skin
SPOROZOITES
Schizonts
LIVER
To mosquito
♂
Gametocytes
♀
RBC
Merozoites
Schizonts
Merozoites
Trophozoites
Ring stages
Blood Vessels
Prof. Shobhona Sharma,
Tata Institute of Fundamental Research,
Mumbai, India

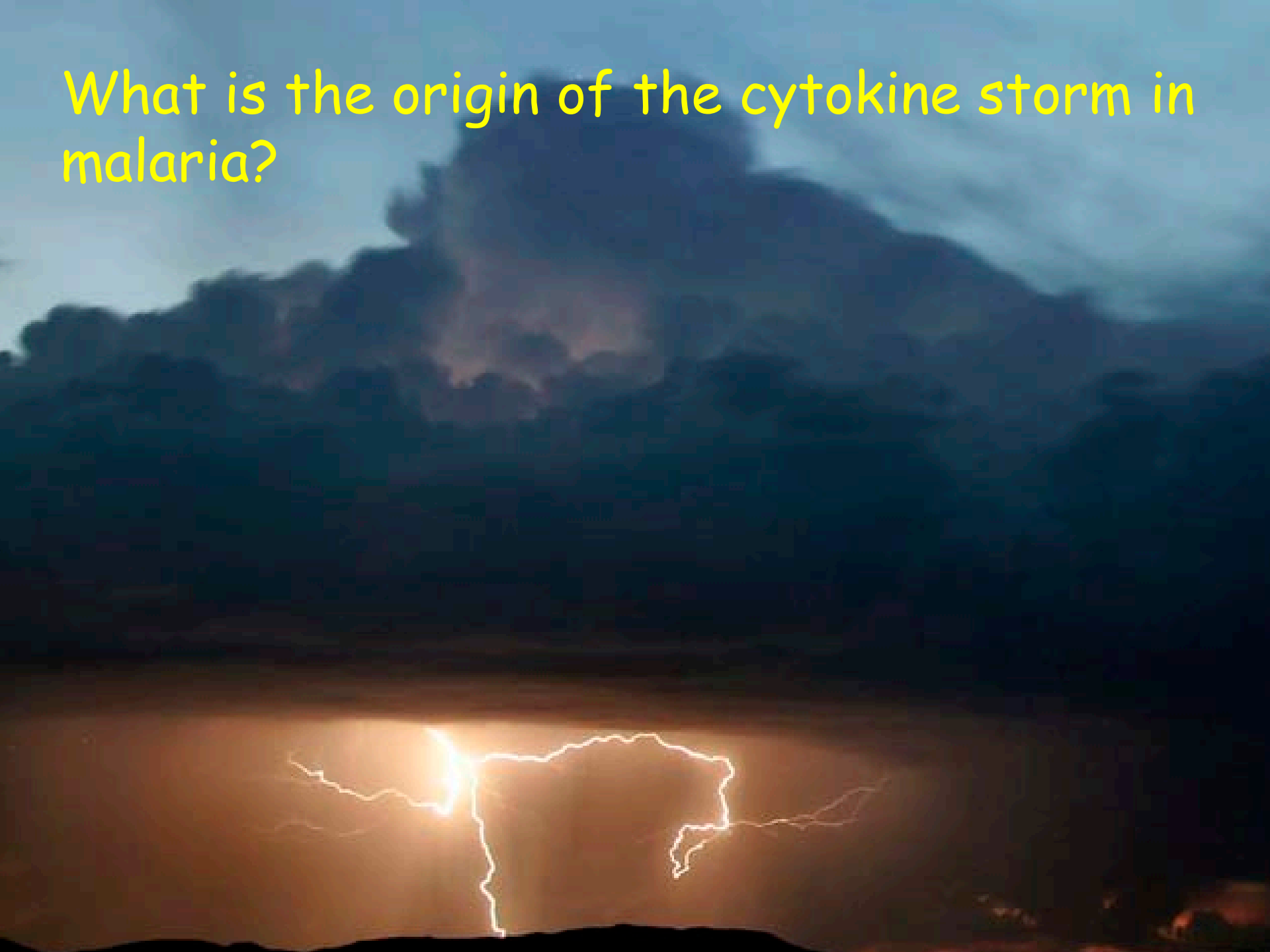
The paroxysm of malaria



The paroxysm of malaria represents an inflammatory cytokine storm

- Fever (up to 104°F or 40°C)
- Rigor
- Headache
- Myalgias
  - During the paroxysm, extremely high levels of TNFα and IL1β have been measured
- The paroxysm is often followed by a period of extreme fatigue

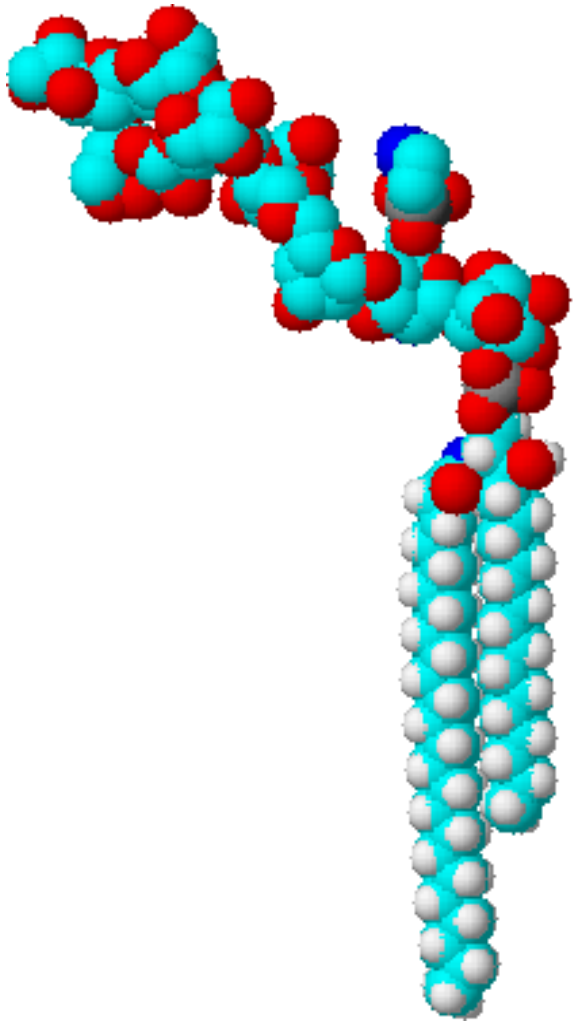
What is the origin of the cytokine storm in malaria?



Our initial hypothesis

- The fever in malaria is caused by the activation of a TLR.

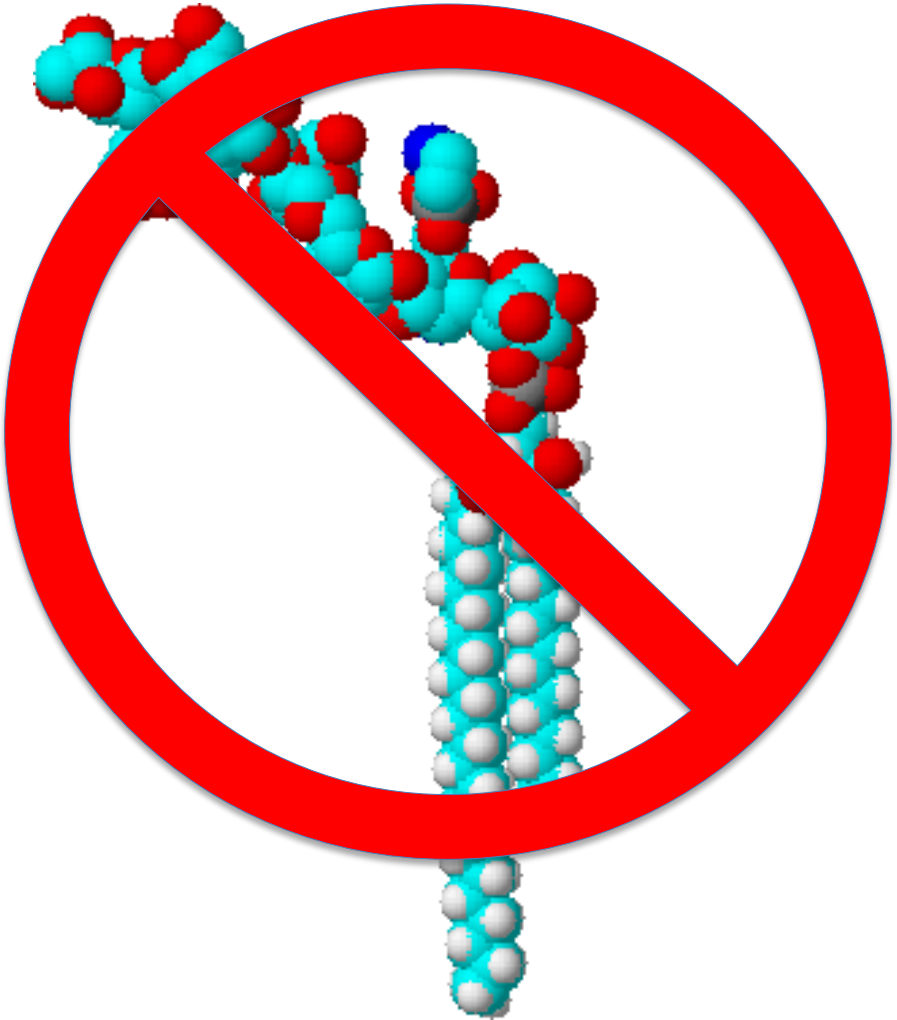
The Malarial Parasite is Coated with a Glycosylphosphatidyl Inositol Anchor (GPI)



Gowda *et al*., 2010. J Immunol.

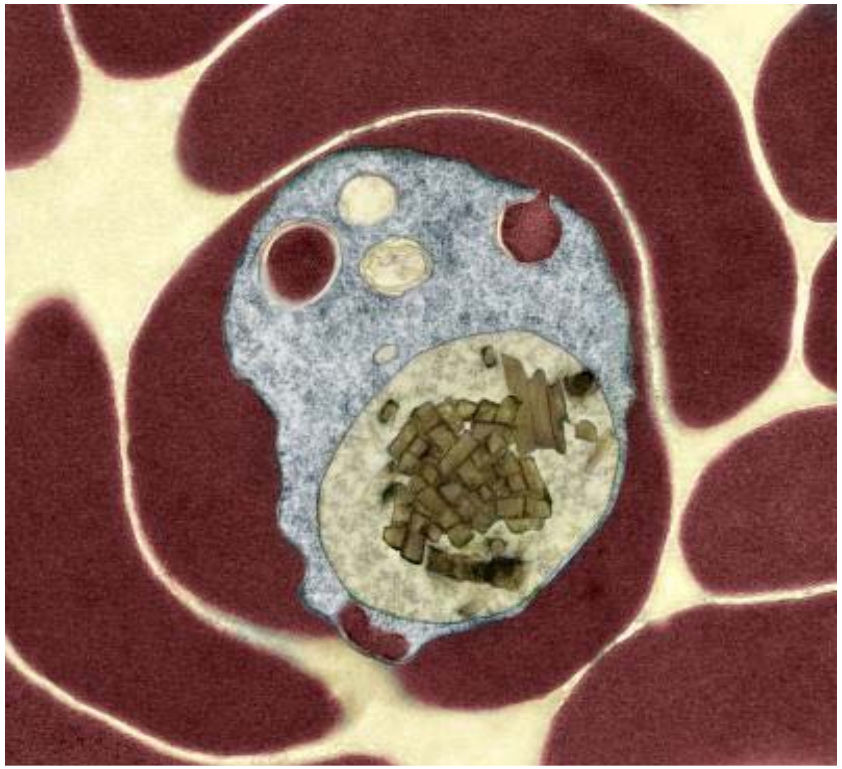
The concentration of GPI on the surface of merozoites is too low to account for the ability of parasite extracts to stimulate cytokine production.

GPI is not likely to be an important cause of cytokine stimulation in malaria.



Hemozoin

- *P. falciparum* metabolizes hemoglobin into hemin, which is subsequently detoxified by forming the inert crystal, hemozoin.



Hemozoin in parasitophorous vacuole

Shizuo Akira

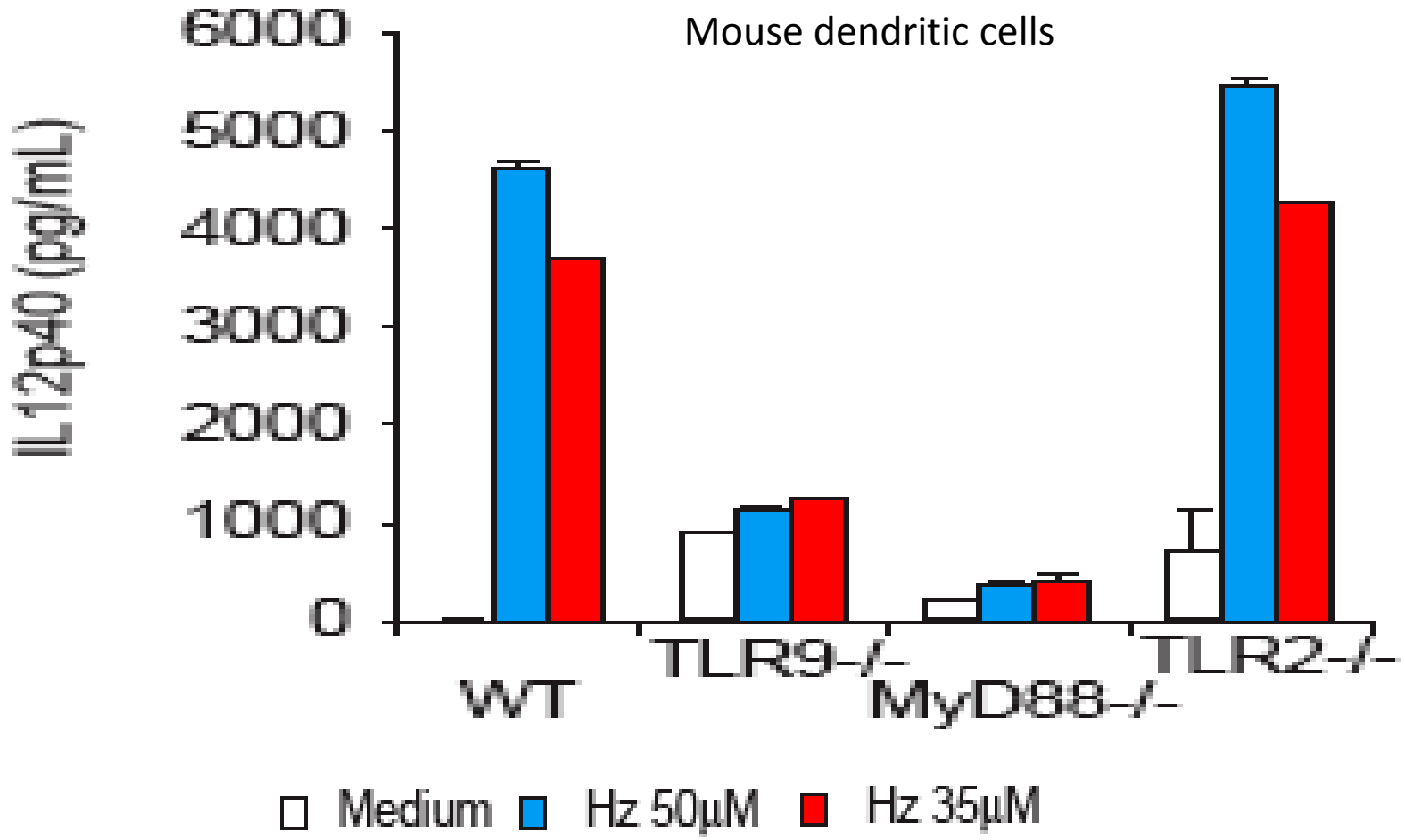
Akira *et al.*

Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin.

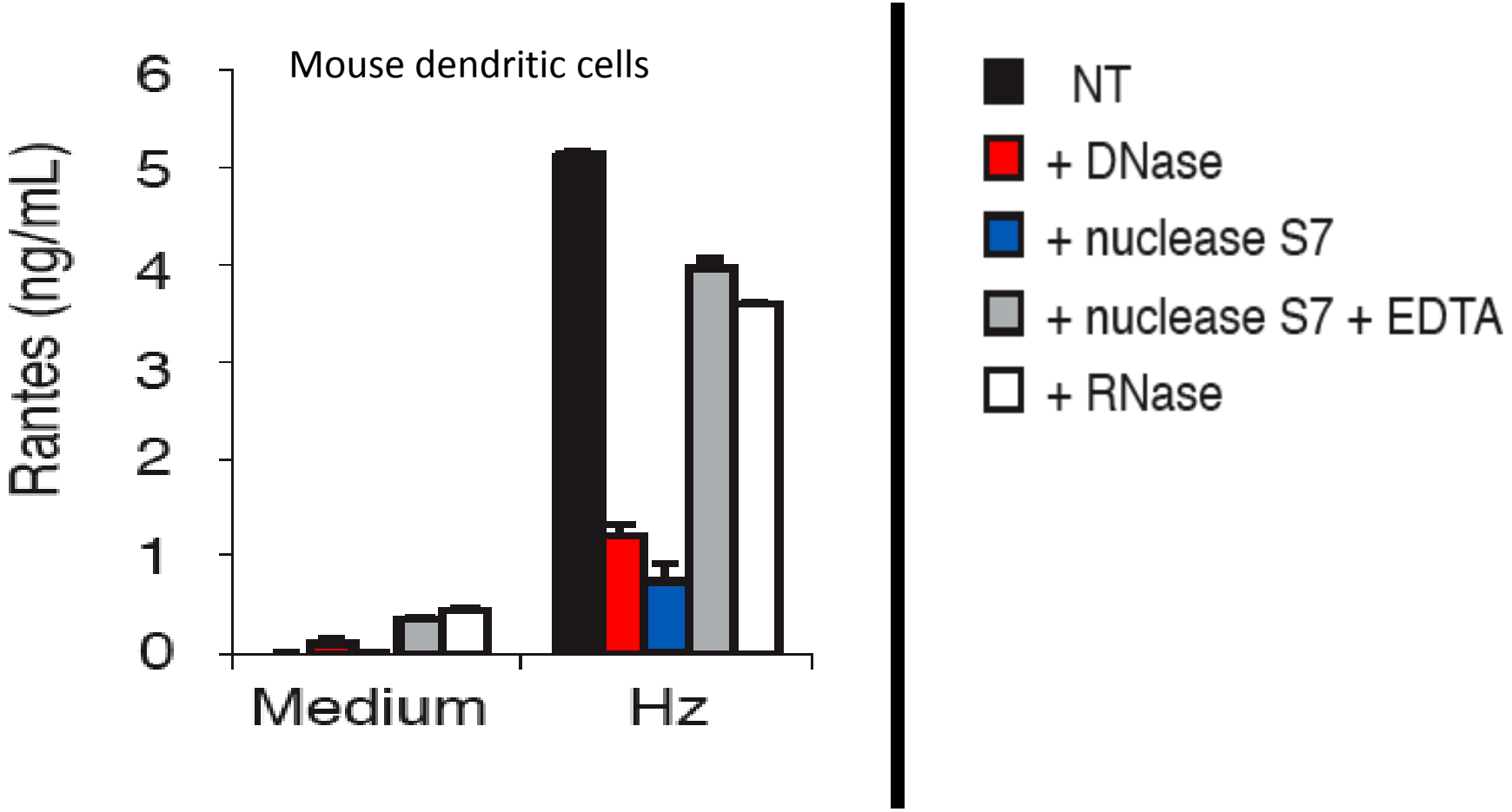
J Exp Med. 2005;201(1):19-25.


Scooped by Akira…again!
DTG

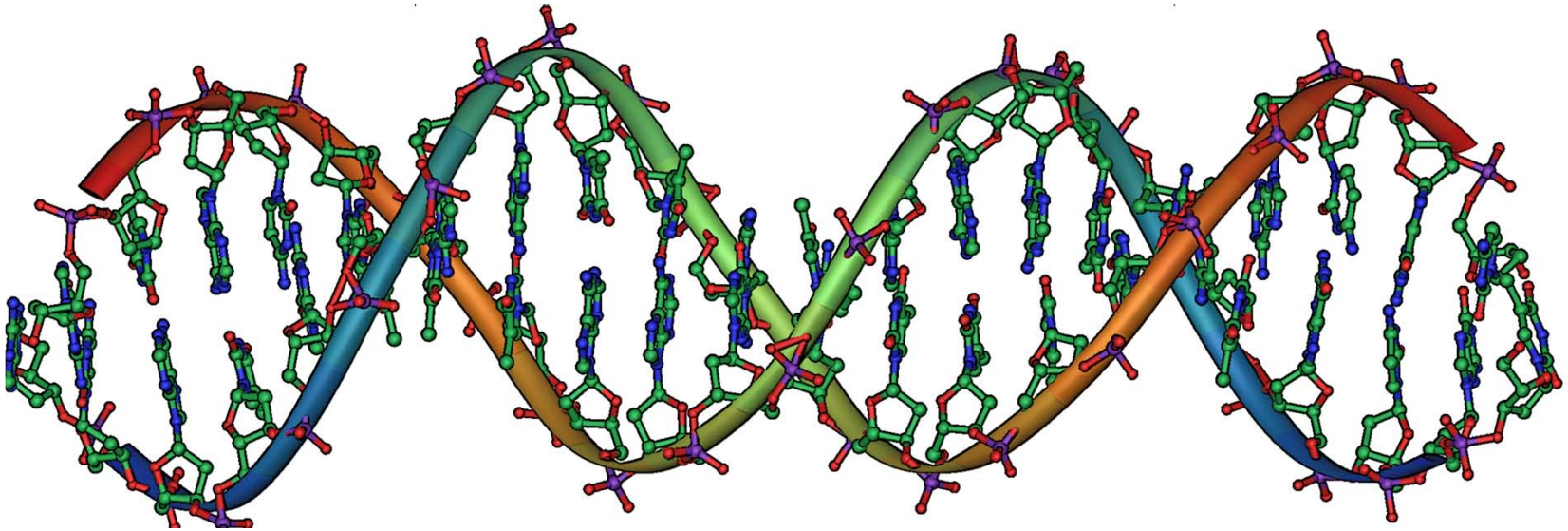
Natural Hemozoin Activates Cytokine Production via TLR9 and its adapter protein, MyD88



The stimulatory activity of hemozoin was destroyed by Dnase.

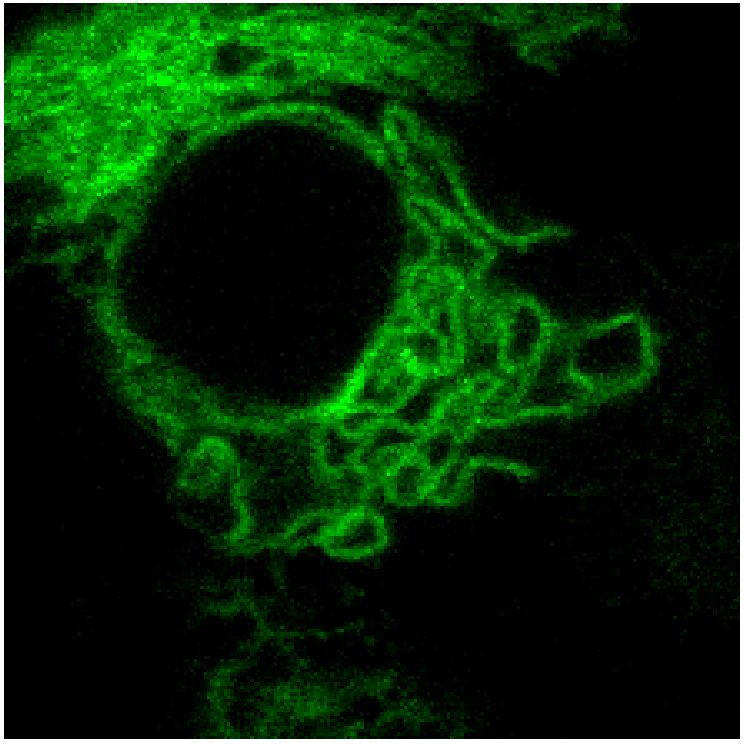


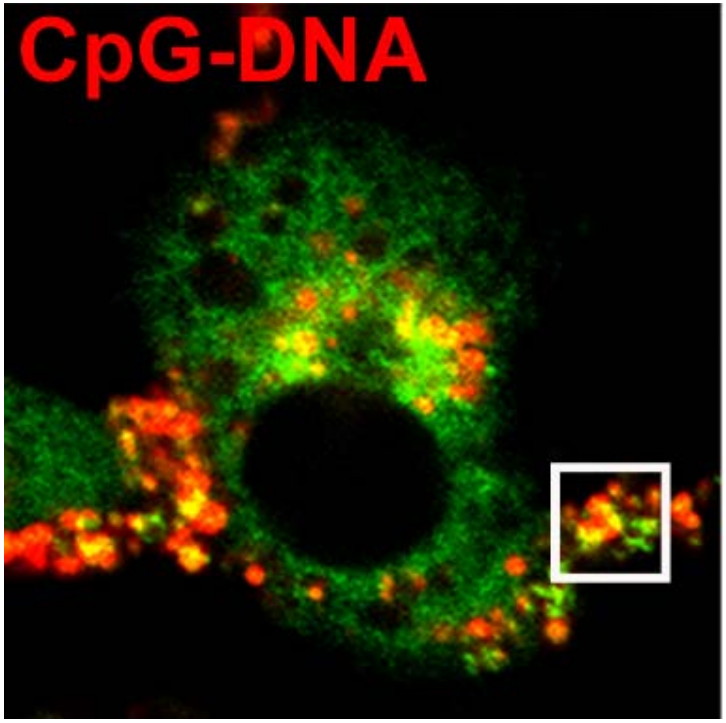
Thus, the cytokine inducing component of hemozoin is (plasmodial) DNA



Current hypothesis:

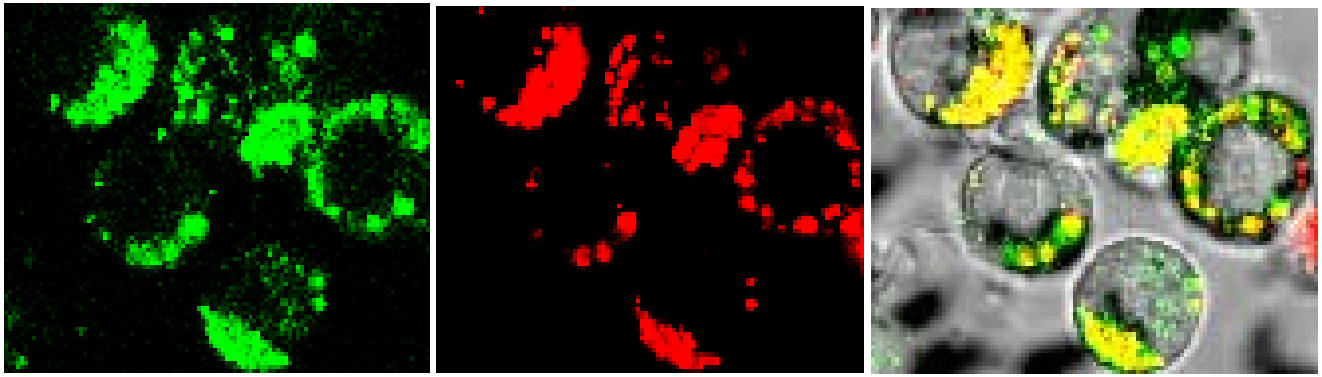
Hemozoin functions to traffic DNA into an intracellular compartment to which TLR9 can be recruited





Parisa Kalantari, Ph.D.

Hemozoin/DNA complexes are rapidly internalized into a lysosomal compartment in macrophages



100 ug of sHz and 30ug of CpG-Alexa 642-

Adding CpG-Alexa 642 and sHz in a microcentrifuge tube

↓

Rocked for 2 hours at RT

↓

Washed 4 times with PBS

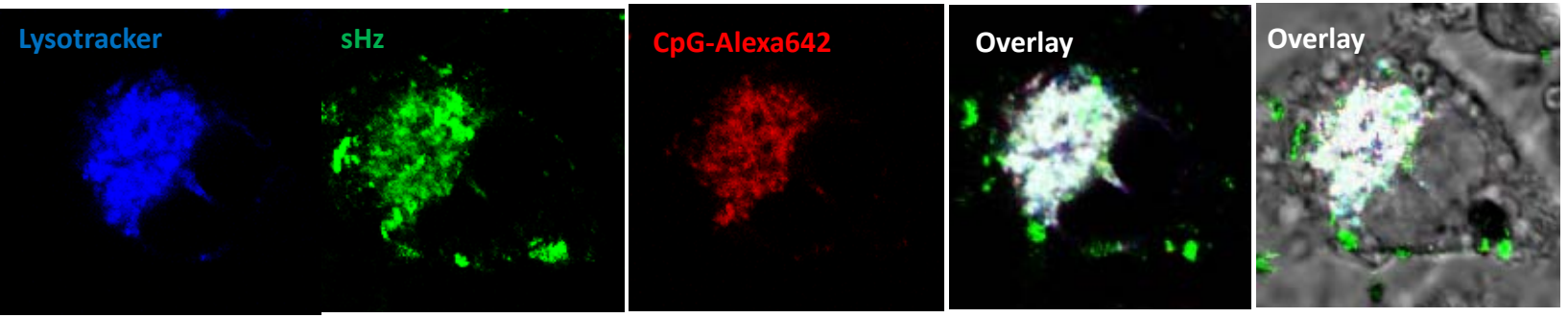
↓

Added to the cells

↓

Confocal Microscopy

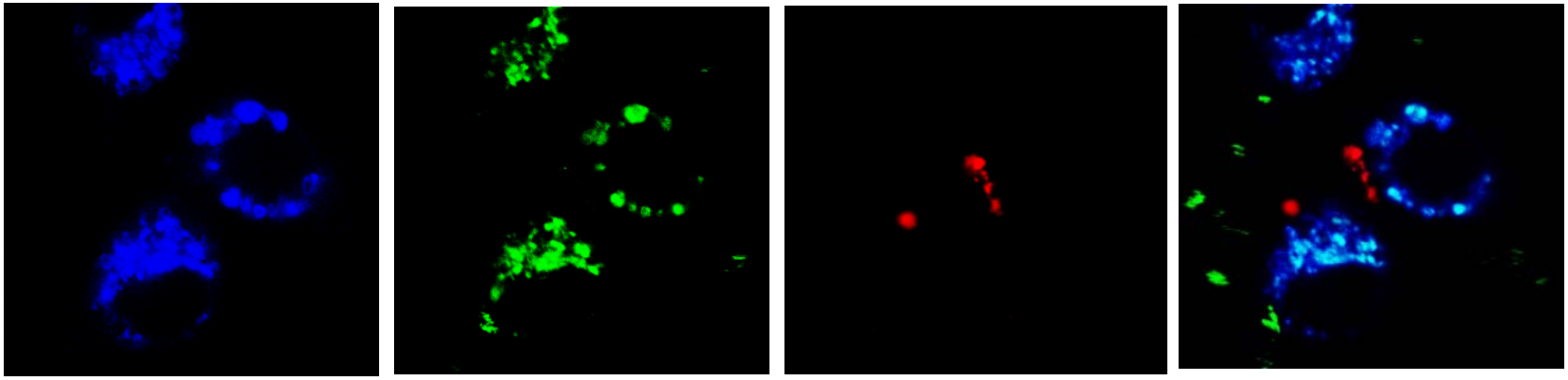
CpG DNA is internalized into lysosomes when complexed to Hemozoin



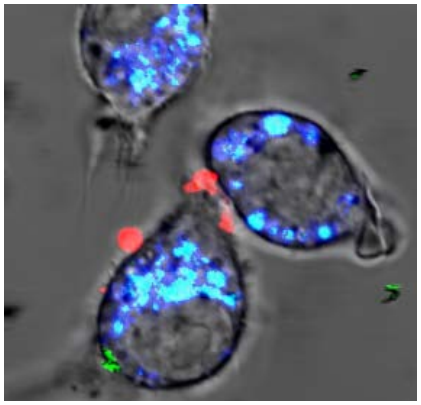
30 min

Free CpG DNA (unlike hemozoin-bound DNA) has delayed internalization into a lysosomal compartment.

Lysotracker | Reflection (SHz) | CpG-Alexa 642 | Overlay



Hz and CpG added to macrophages- No prebinding



30 minutes

Lysotracker:
Ex:380

The concept that plasmodial DNA is introduced into cells by hemozoin to activate TLR9 via CpG DNA motifs was compelling, except for the fact that plasmodial DNA is highly AT-rich!

- Daniele Bartholomeu

The concept that plasmodial DNA is introduced into cells by hemozoin to activate TLR9 via CpG DNA motifs was compelling, except for the fact that plasmodial DNA is highly AT-rich!

- The *P. falciparum* genome contains a stem loop motif: ATTTTTAC over 6000 times!

- Daniele Bartholomeu

The AT-r stem-loop motif is found in a variety of other organisms

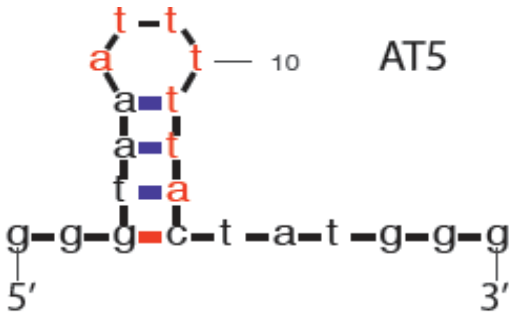
Genome (size)	AT content (%)	Number of putative AT-ODN motifs
***Plasmodium falciparum* (22.8Mb)**	82%	6130
Variola (371.1kb)	68%	24
Vaccinia (389.4kb)	67%	25
***Listeria monocytogenes* (5.8Mb)**	61%	480
***Homo sapiens* (3.08 Gb)**	56%	268828
***Human adenovirus 5* (35.9kb)**	45%	5
***Leishmania major* (32.8Mb)**	38%	184
***Human herpesvirus 2* (154.7kb)**	30%	2

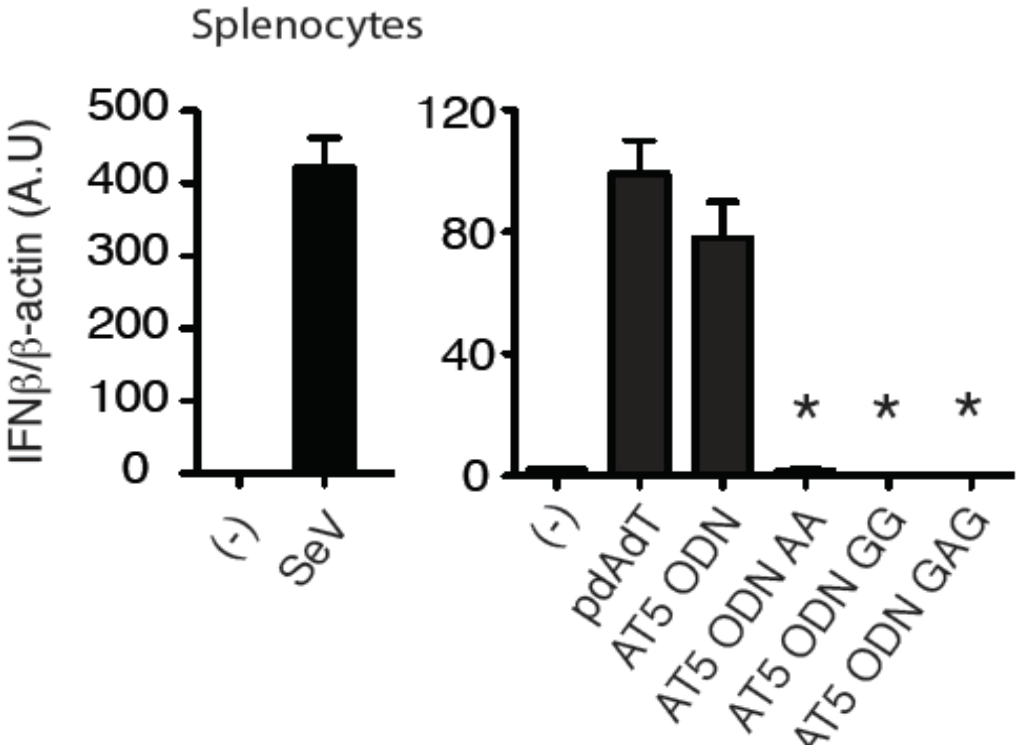
Shruti Sharma

One can study the AT-r stem-loop motif using synthetic ODN

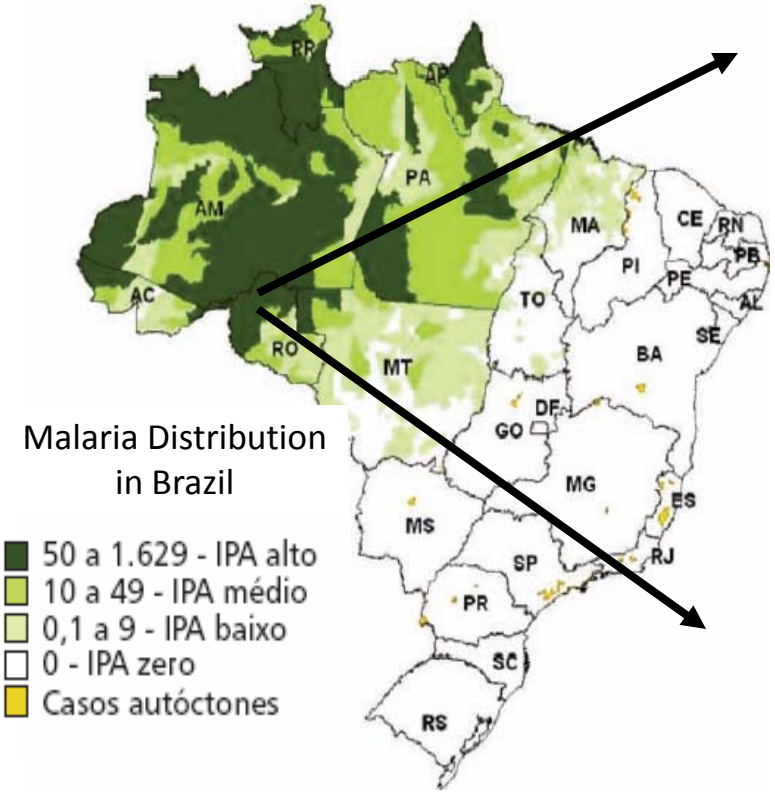
- AT-2: GCACAC**ATTTTTAC**TAAAAC

The stem-loop structure is *essential* for innate immune activity



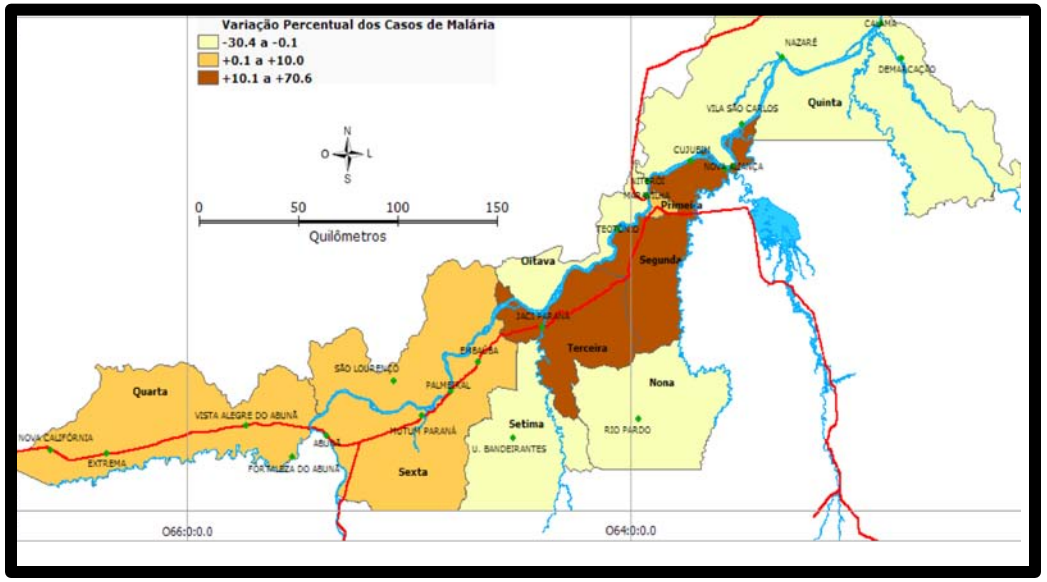


Malaria Clinical Research Site in Porto Velho, Rondônia, Brazil



Malaria Distribution in Brazil

Malaria Distribution in Porto Velho - 2010



Porto Velho is the capital city of **Rondonia State**; it is the most rapidly growing major city in Brazil and currently has a population of about 400,000 persons.

The ideal caiparinha comes from caipira (“hillbilly”)- an example of T3 research

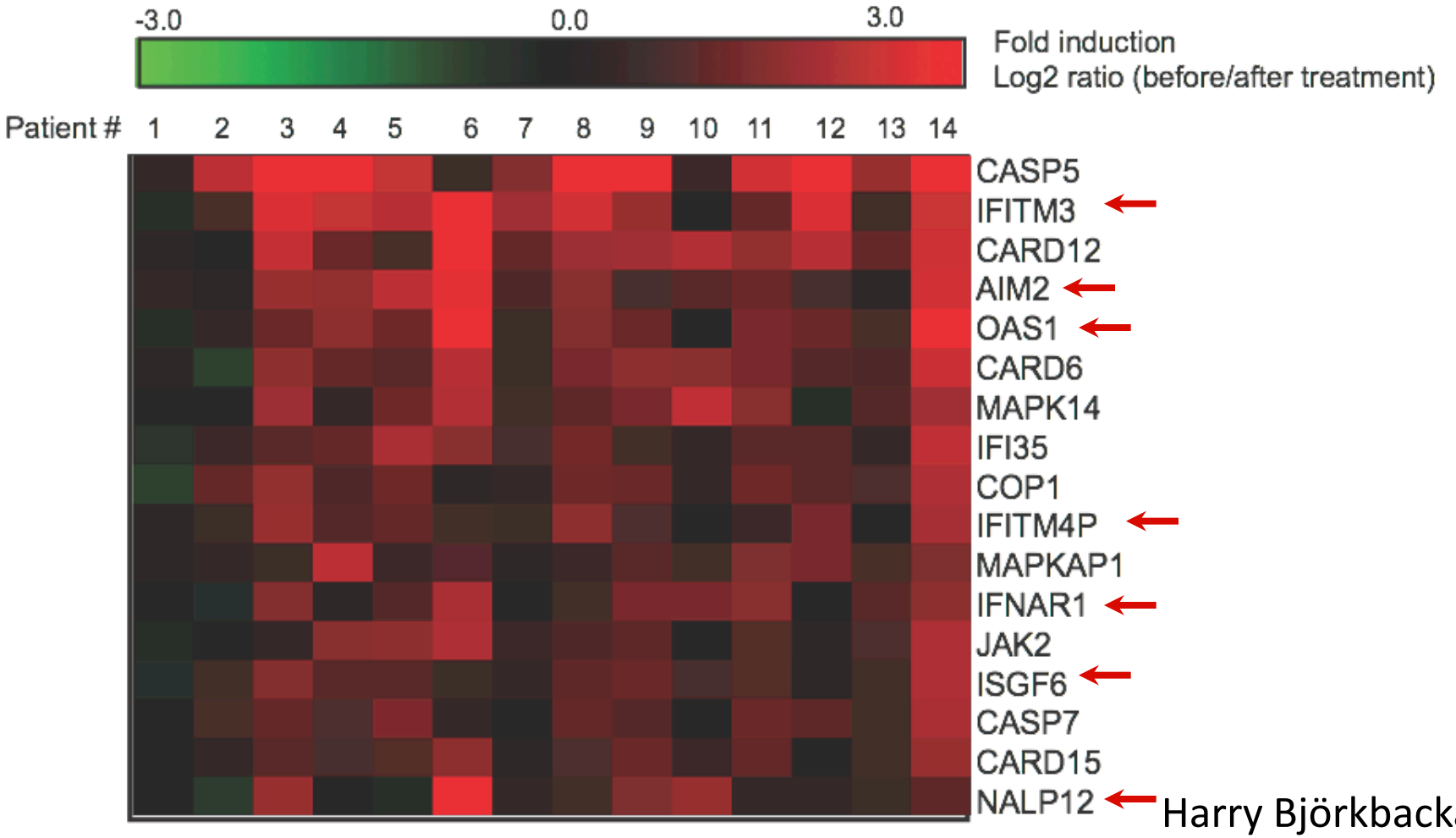
Ingredients

* 1/2 lime, quartered
* 1 teaspoon white sugar
* 2 1/2 fluid ounces cachaca
* 1 cup ice cubes

Directions

1. In a large rocks glass squeeze and drop in 2 eighths of lime. Add sugar, crush and mix with a spoon. Pour in the cachaca and plenty of ice. Stir well.

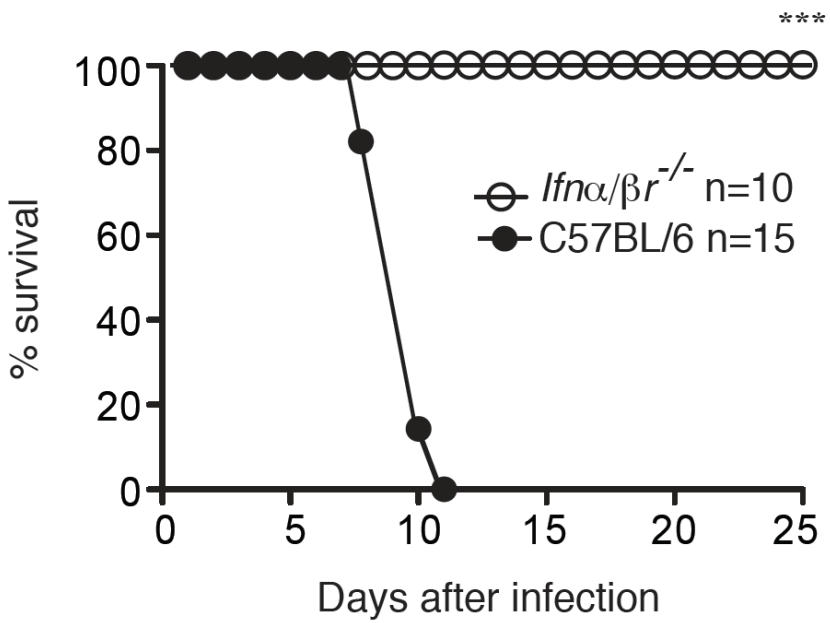
Studies of febrile malaria patients (*P. falciparum*) demonstrated that IFN-stimulated genes (ISG) are up-regulated during malaria



Harry Björkbacka
Peggy Parroche

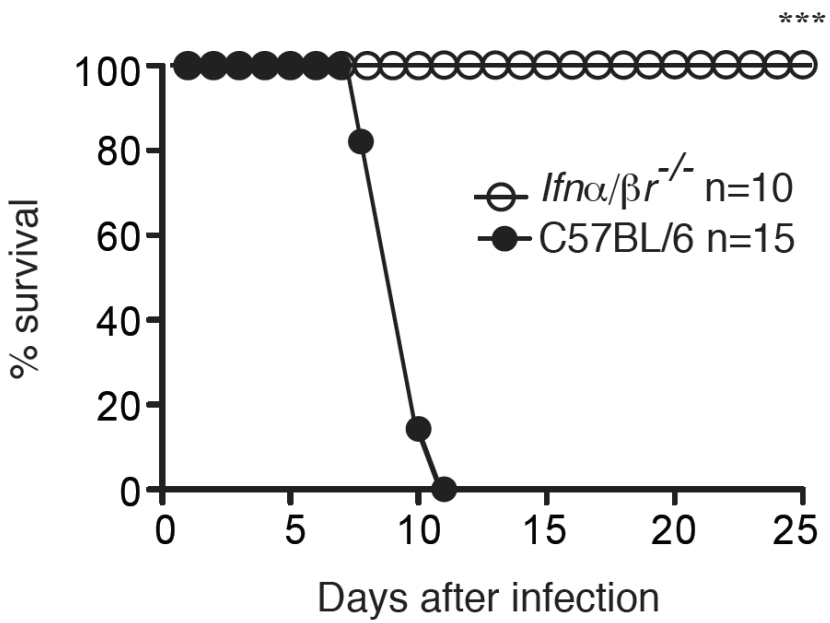
Rosane de Oliveira

IFN alpha/beta receptor (IFNAR) knockouts are highly resistant to mouse cerebral malaria



Rosane DeOliveira

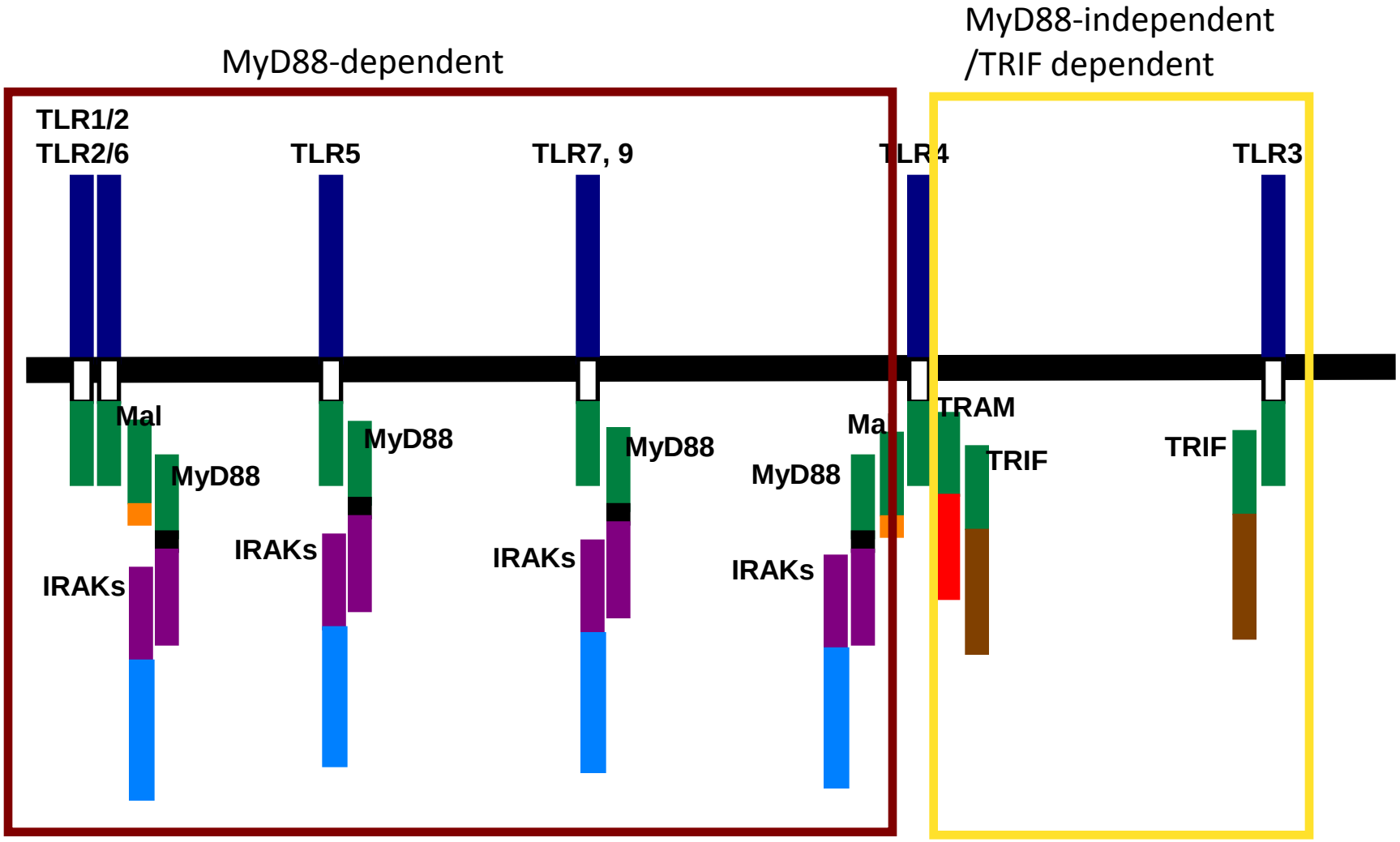
Hence, type I IFNs appear to be an essential part of the inflammation seen in malaria



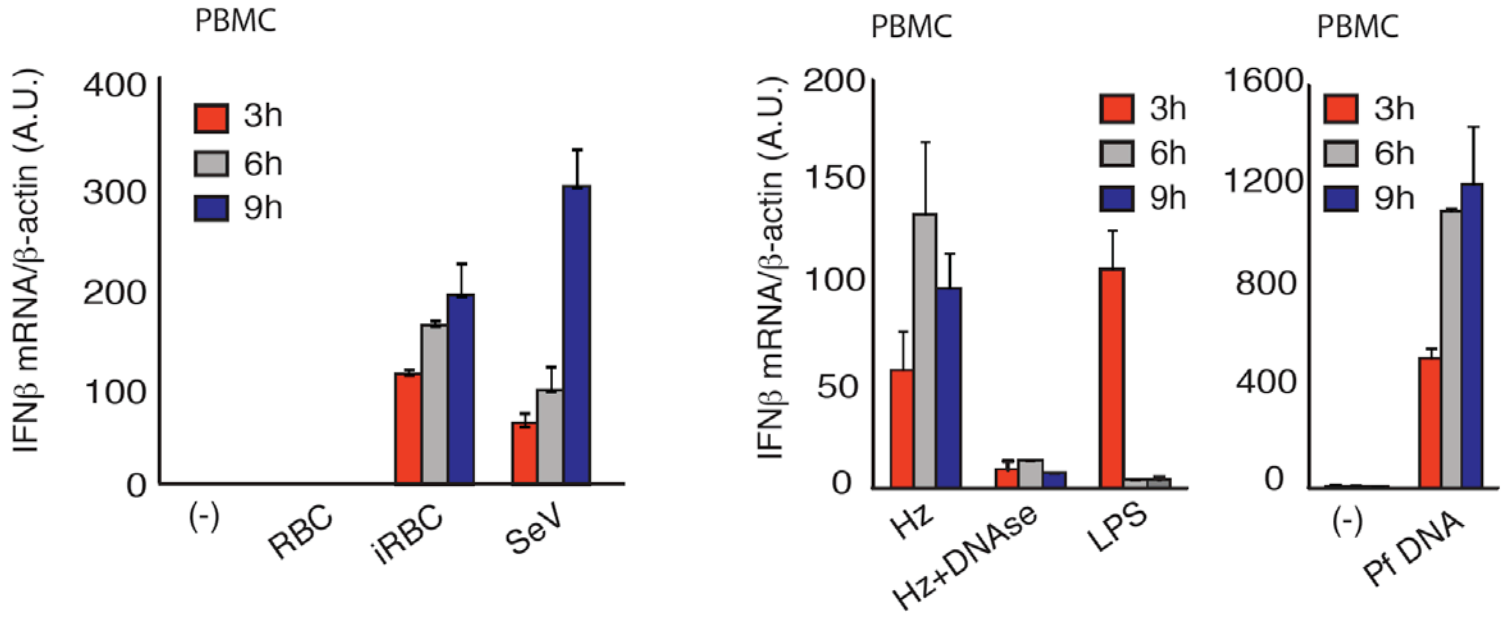
Rosane DeOliveira

RECAP

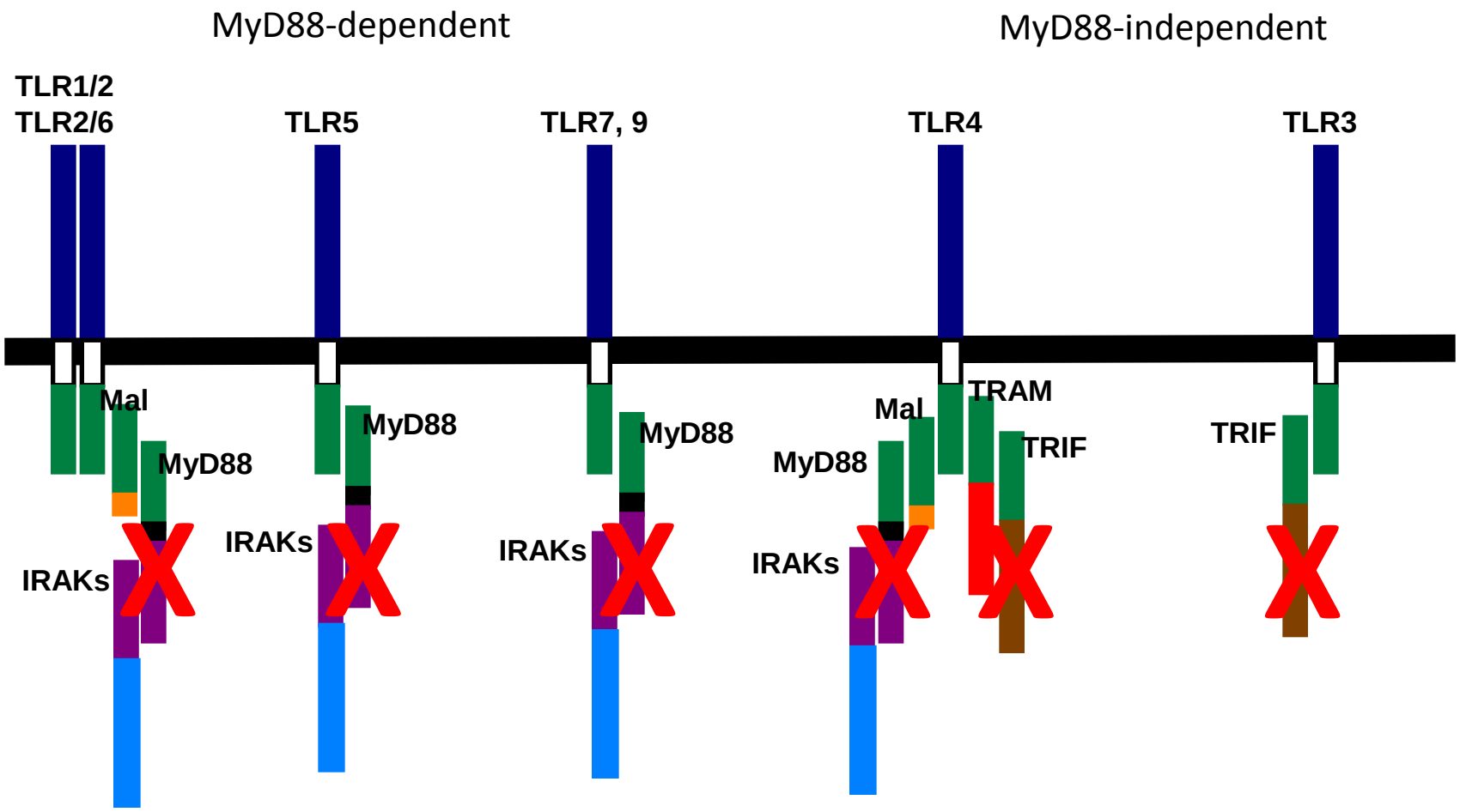
There two major TLR signaling pathways



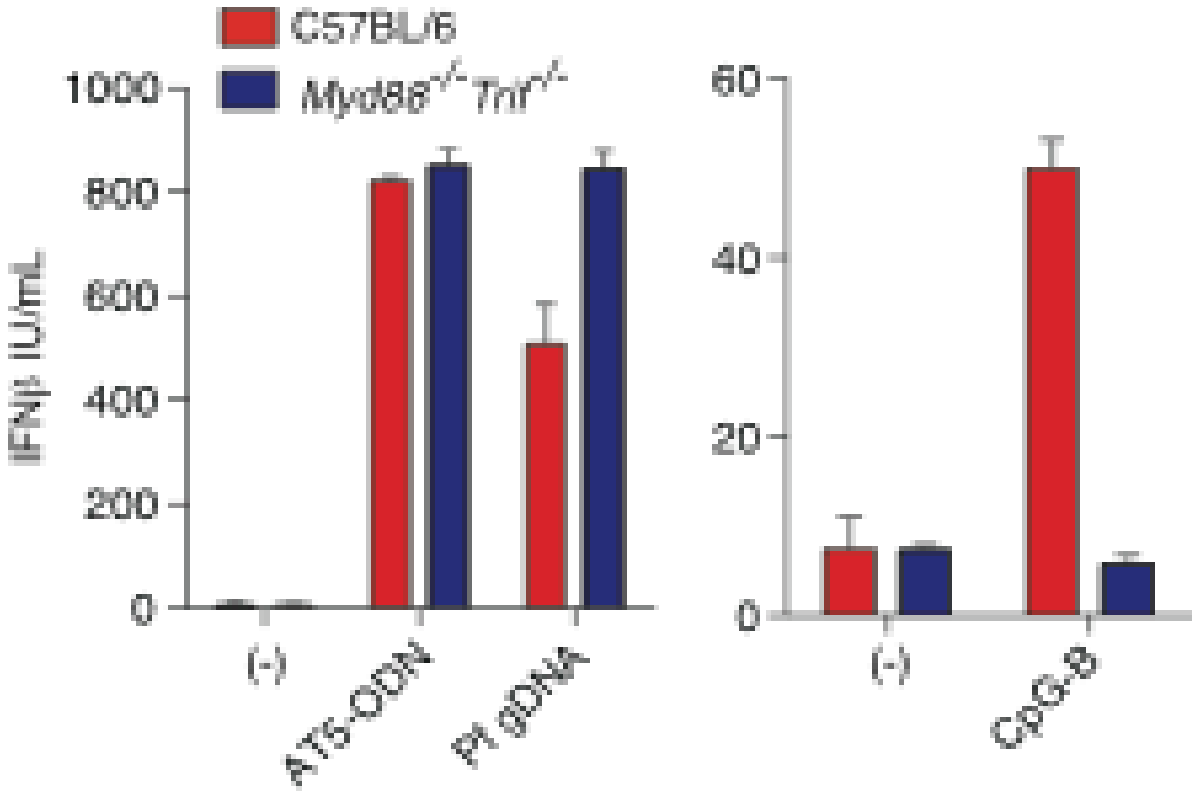
P. falciparum infected rbc, hemozoin/DNA and purified malaria DNA activate a type I interferon response



TRIF/MyD88 DKO have no TLR function

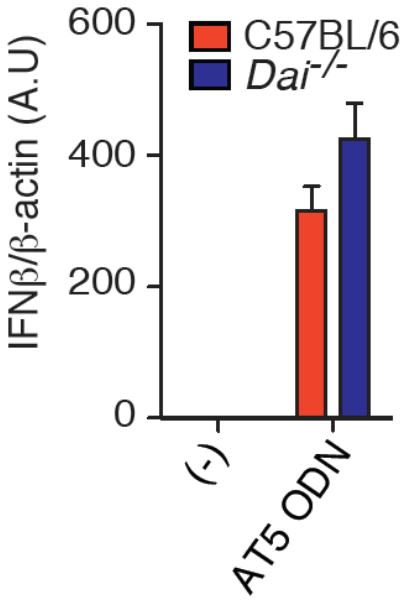


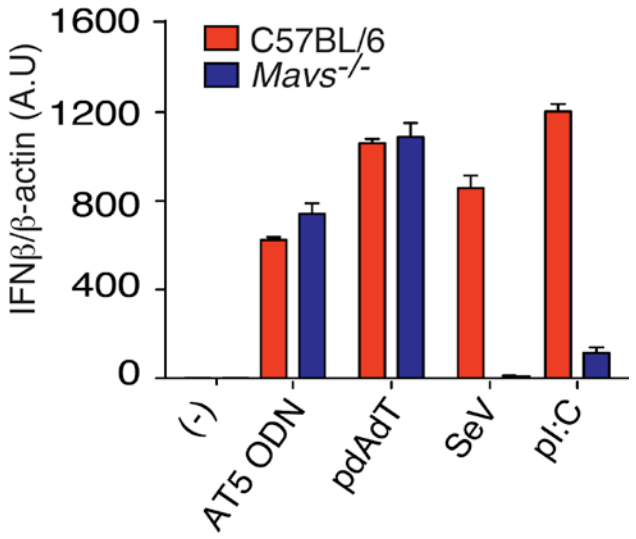
Neither AT-r ODN nor plasmodial genomic DNA induce IFN production via TLRs!

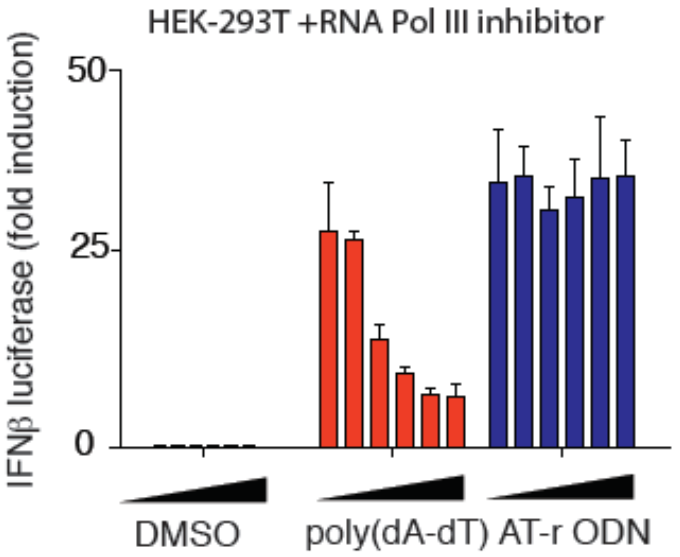


AT-r activation of type I interferons is not due to activation of known nucleotide sensors

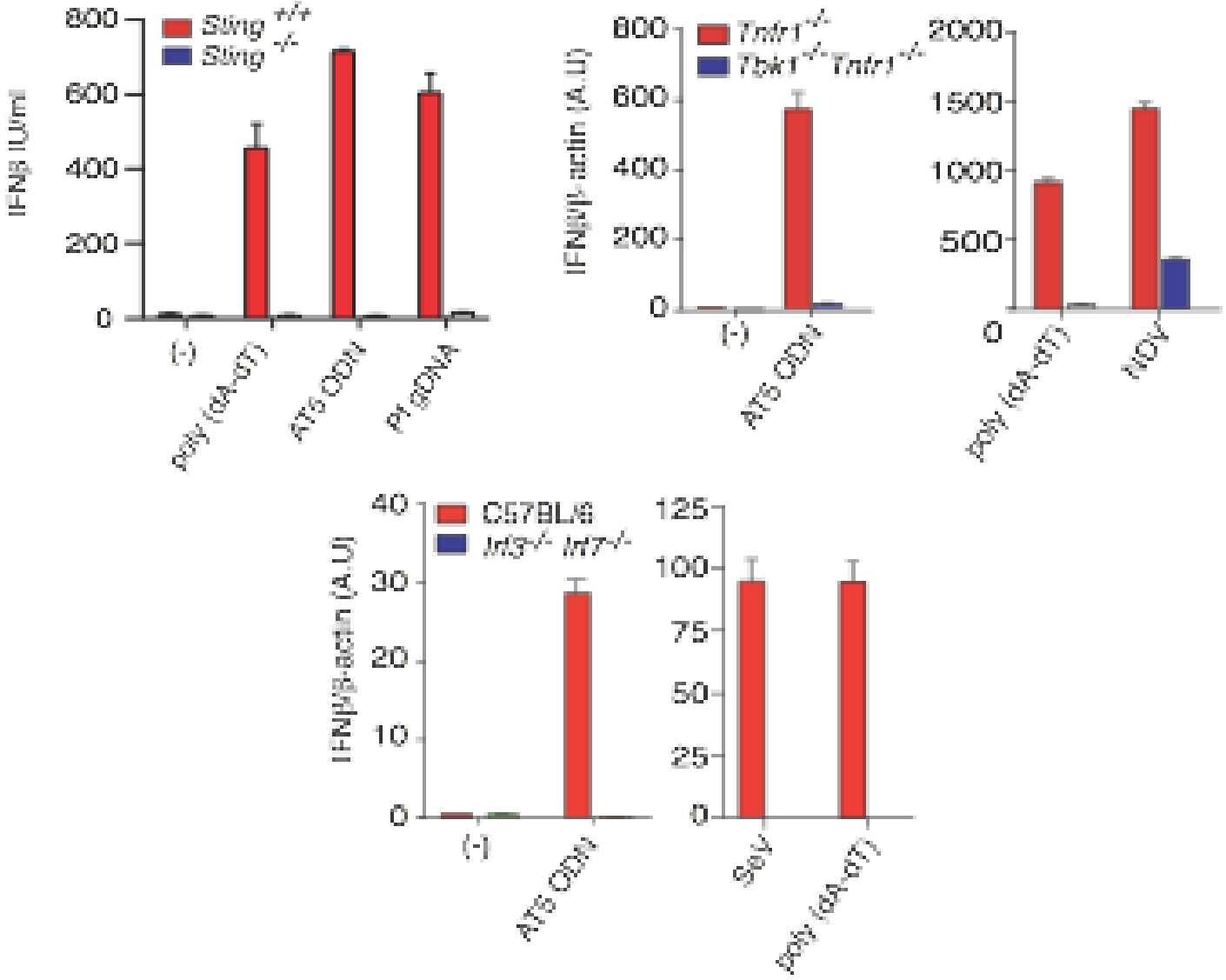
- DAI
- RNA helicases
- RNA Polymerase III



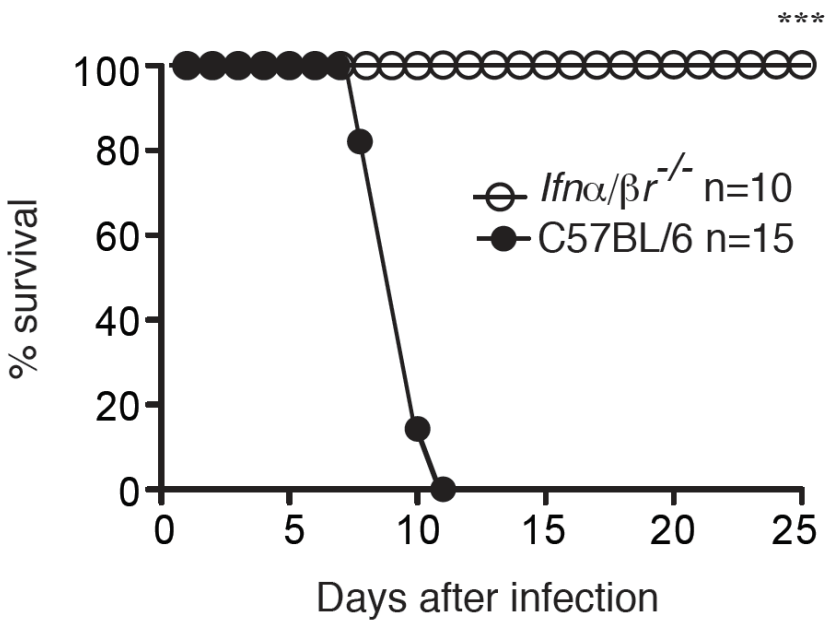


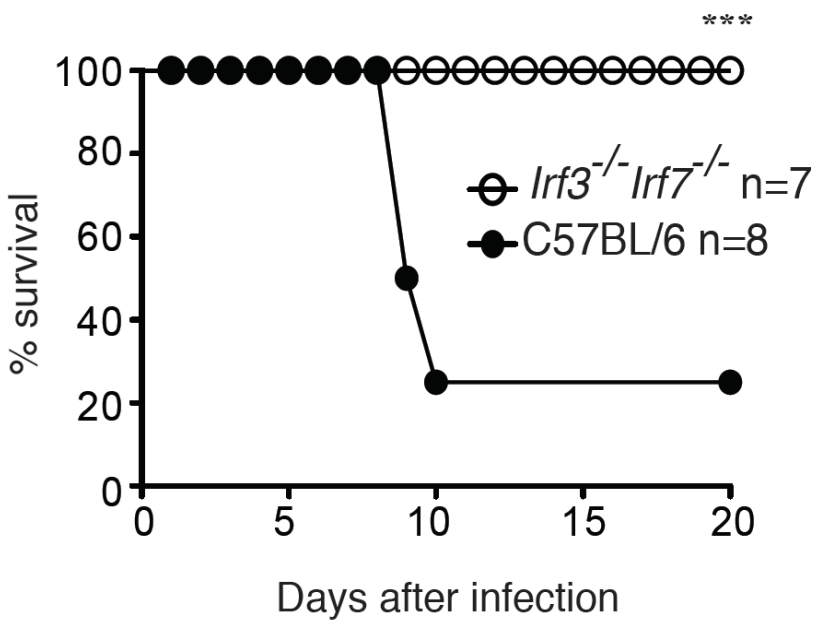


STING/TBK1 and IRF3/7 are critical components of AT-r DNA sensing pathway.



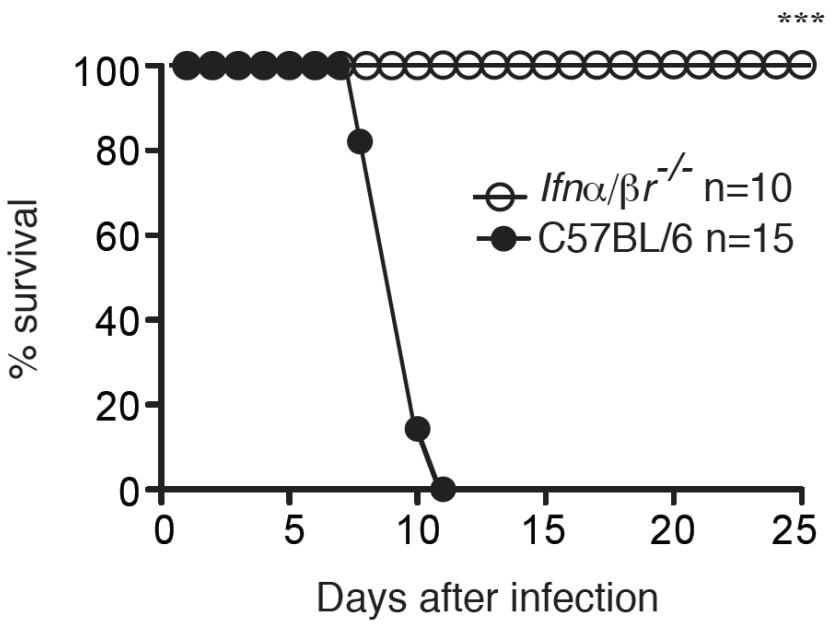
Like the IFNAR KOs, IRF3/7 double knockouts are similarly resistant to cerebral malaria

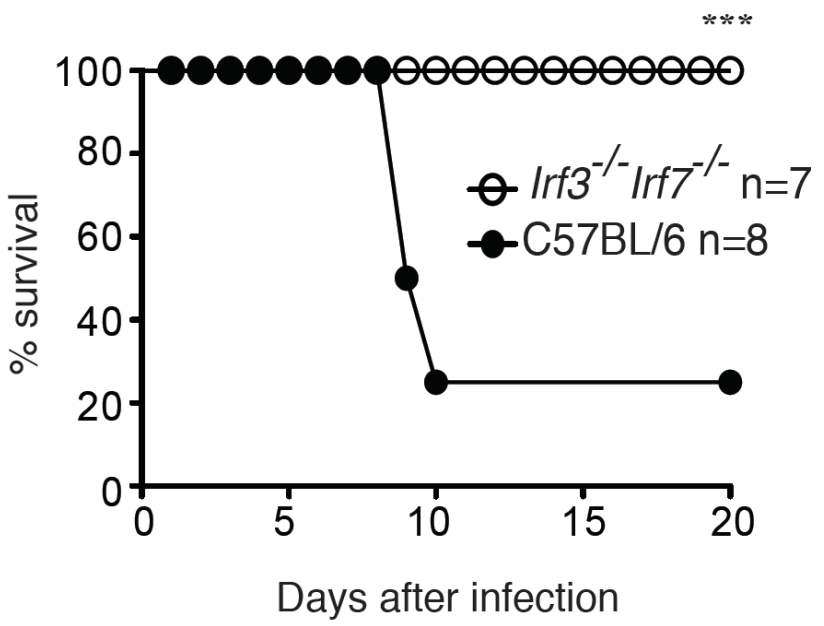




Rosane DeOliveira

Both STING KOs and TBK1 hypomorphic mice appear to be resistant to cerebral malaria



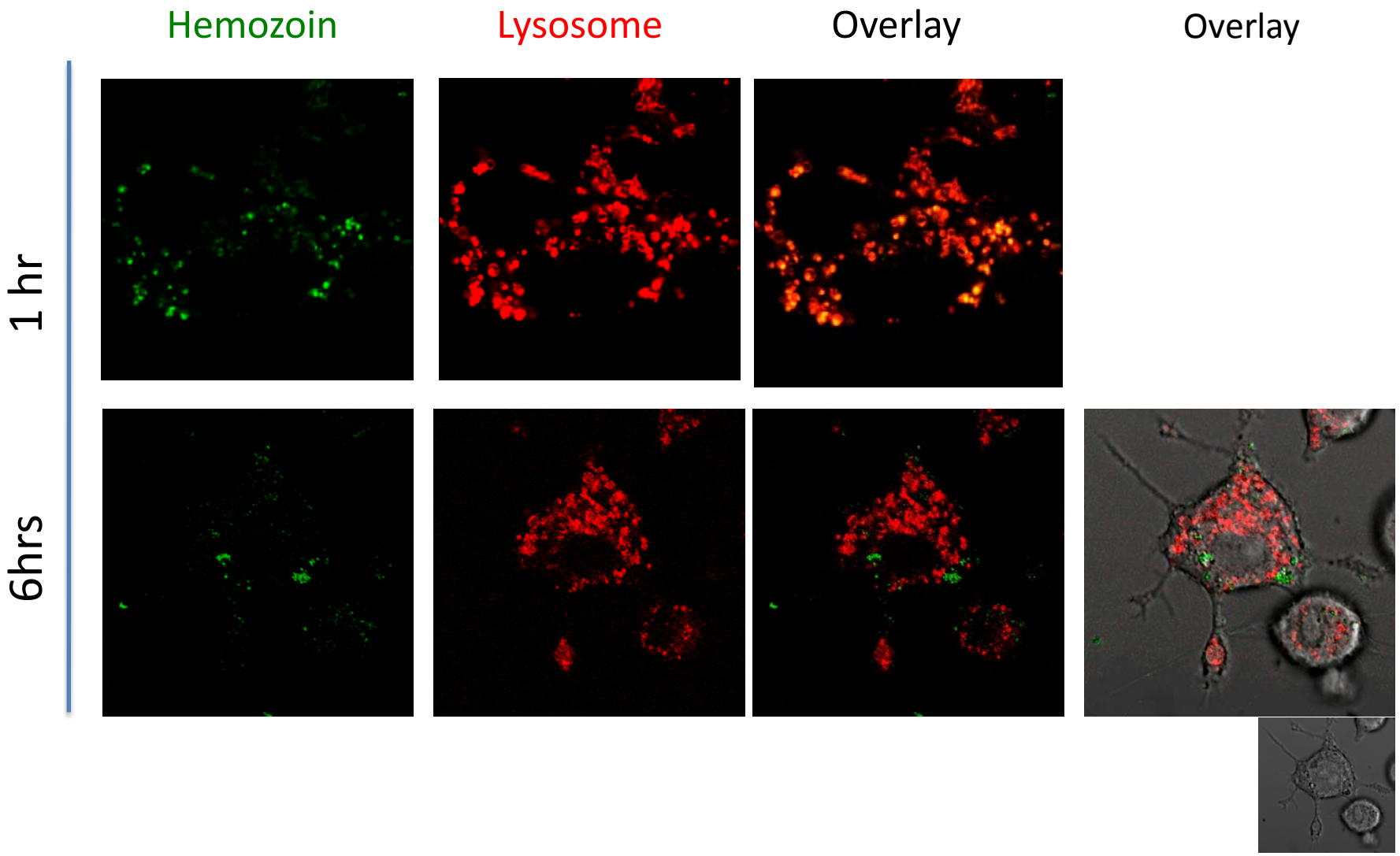


Rosane DeOliveira

AT-rich DNA must be in the cytosol of cells to activate IFN or pro-inflammatory cytokines.

How does the DNA on the surface of hemozoin move from the phagosome to the cytosol?

Hemozoin traffics into the phagolysosome and then into the cytosol



The phagocytosis of inert particles results in phagolysosomal leakage.

- Silicic acid
- Urate
- Asbestos

The phagocytosis of inert particles results in phagolysosomal leakage.

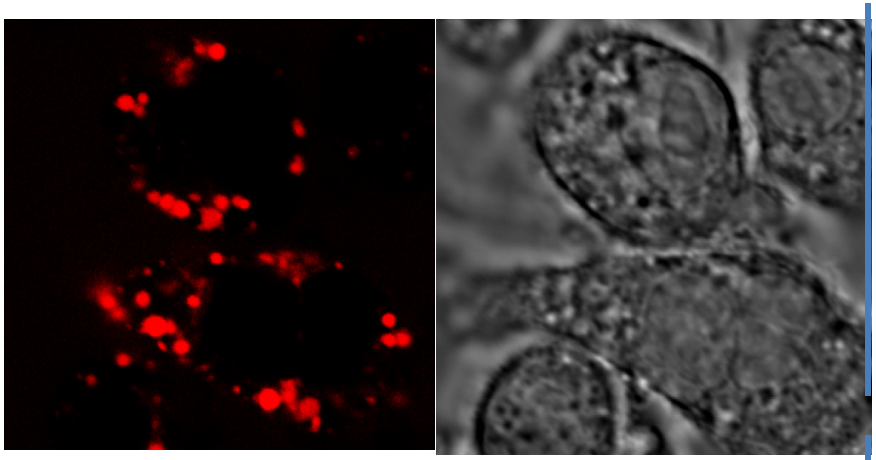
- Silicic acid
- Urate
- Asbestos
- Hemozoin

Hemozoin crystals lead to lysosomal rupture and leakage of lysosomal content into the cystosol

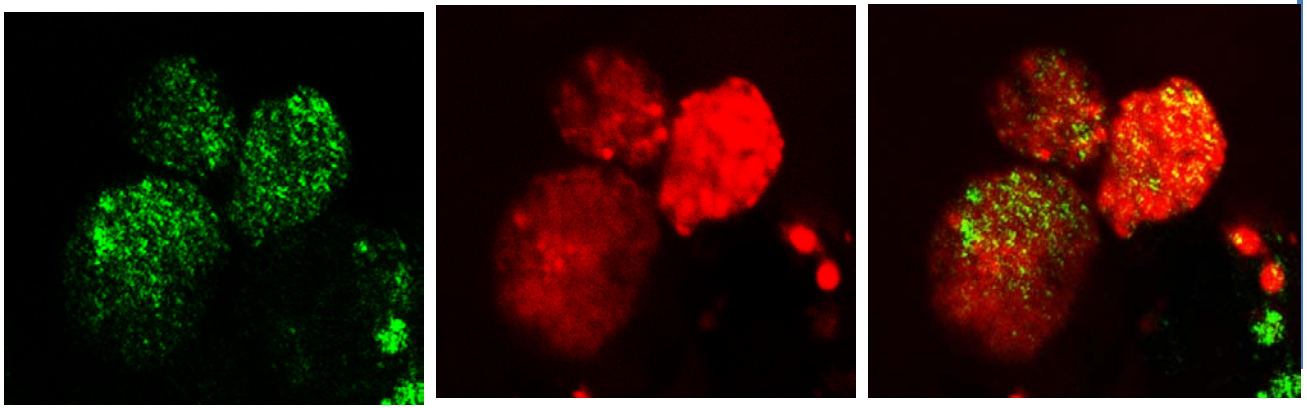
Hemozoin (reflection)

Dextran 10kDa

Overlay

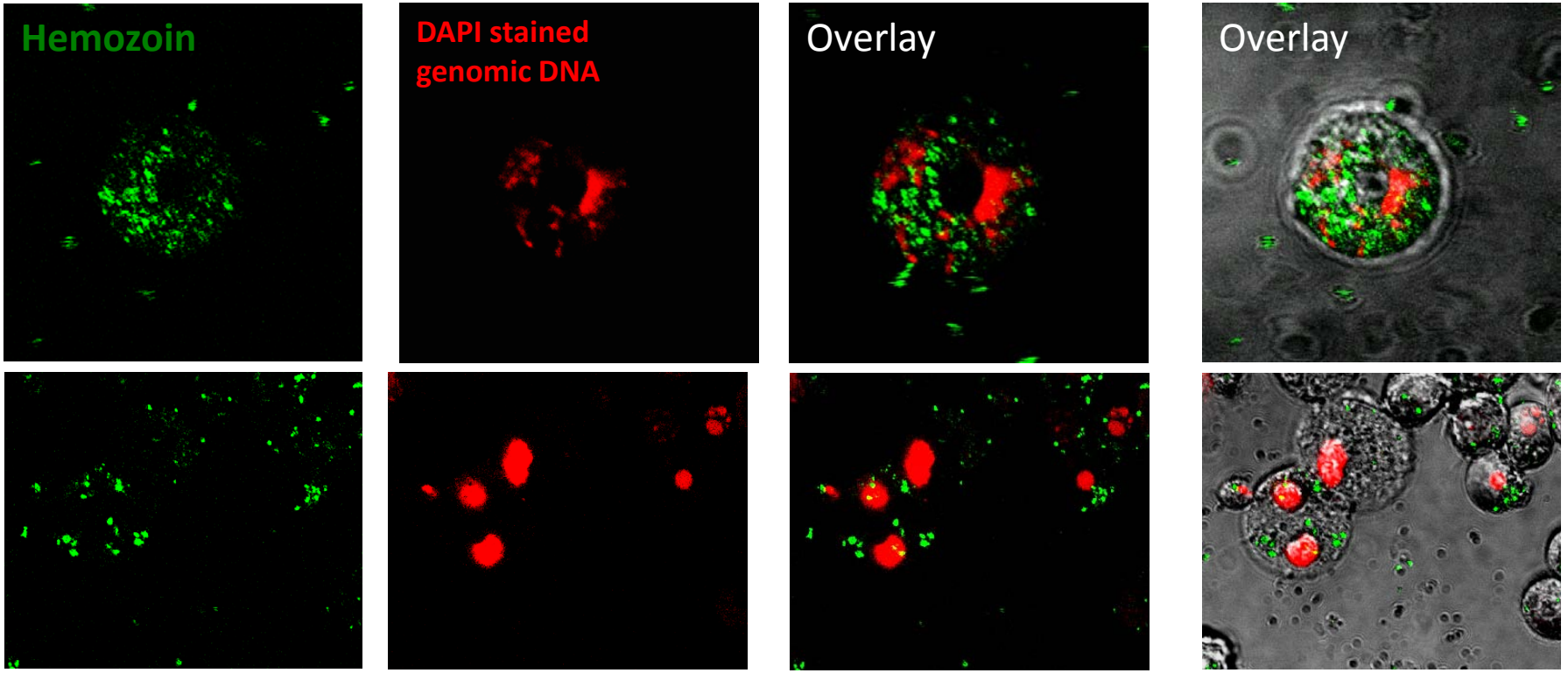


No Hemozoin



Hemozoin

Genomic DNA dissociates from the surface of hemozoin and is released into the cytosol



Recap

- **DNA recognition appears to be a major cause of inflammation in malaria.**
- **There appear to be several ways that the human host responds to plasmodial DNA:**
  - As DNA traffics through the lysosomal compartment, it engages TLR9 via CpG motifs.
  - When DNA gains access to the cytosol, an AT-rich motif activates an as yet unknown receptor via a TBK-1, IRF3/7 and STING dependent mechanism to produce type I interferons.
  - (Direct activation of inflammasomes)
- **Hemozoin promotes innate immune activation.**
  - By carrying DNA into the phagolysosomal compartment, where it engages TLR9, and then by allowing the DNA to escape to the cytosol.
  - (By activating the NLRP3 inflammasome activation)

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- STING ko mice