



# *A novel vaccine technology platform Plasmid **L** launched **L**ive **A**ttenuated **V**irus (PLLAV) vaccines*

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[www.antivirals.be](http://www.antivirals.be)

[www.facebook.com/NeytsLab](https://www.facebook.com/NeytsLab)

## The Neyts-lab of Virology, Antiviral Drug & Vaccine Research

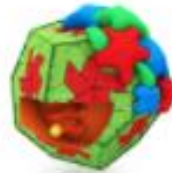
Our Mission : The development of (i) antiviral strategies against a range of (RNA) viruses and (ii) a game-changing novel vaccine technology platform



[Flaviviridae](#)



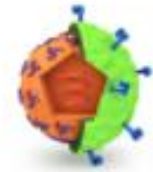
[Picornaviridae](#)



[Norovirus](#)



[Respiratory syncytial virus](#)



[Chikungunya virus](#)



[Coronavirus](#)



[Hepatitis E virus](#)

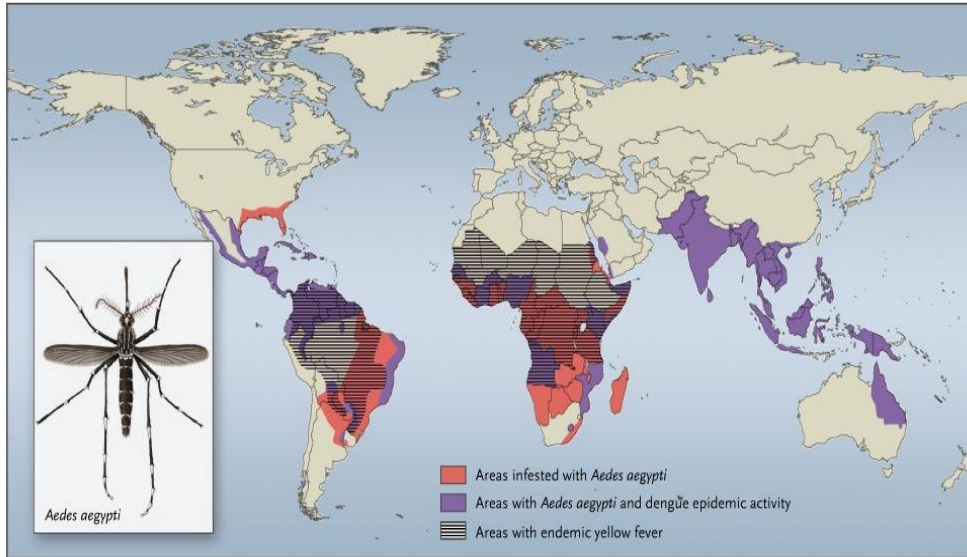


[Bunyaviridae](#)

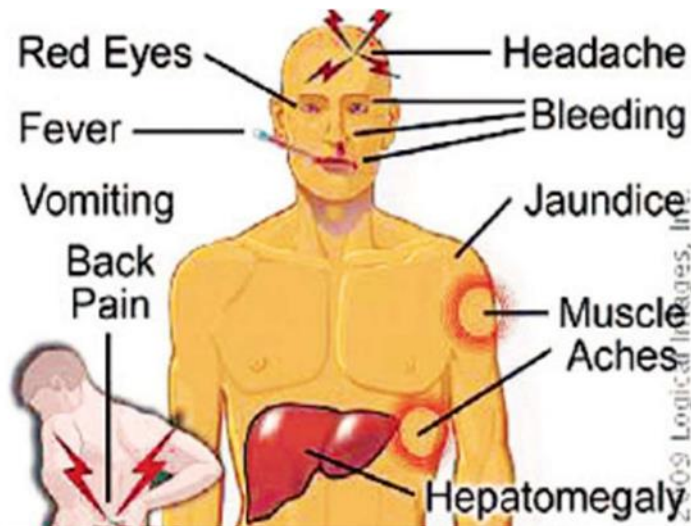


[Hepatitis B virus](#)

# Yellow fever



A man from West Darfur is treated for yellow fever during another outbreak in November 2012. Albert Gonzalez/REUTERS



~200.000 cases/year

~ 30.000 deaths/year

PUBLIC HEALTH

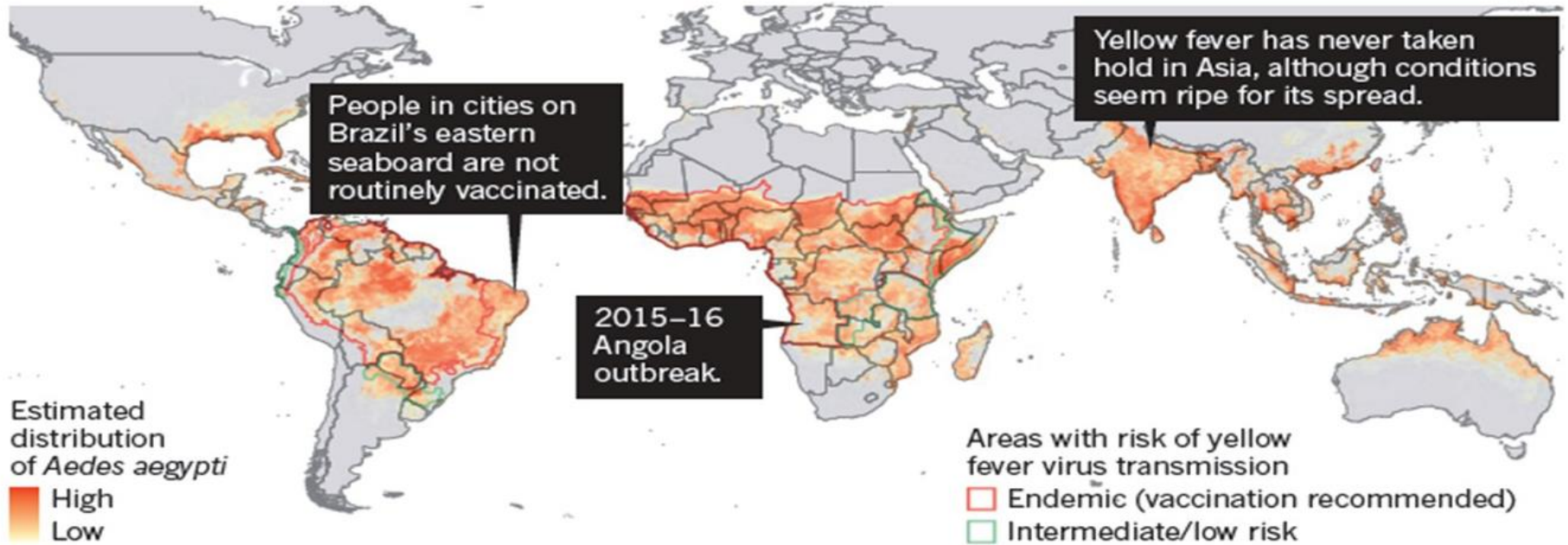
# Fears rise over yellow fever's next move

Darfour (2012/13): 2M  
Angola (2015/16): 6M  
Congo (2016): 11M  
Brazil (2016/17): 3.5M  
USA July 2017: breakdown supply  
Nigeria (2018) : 25M

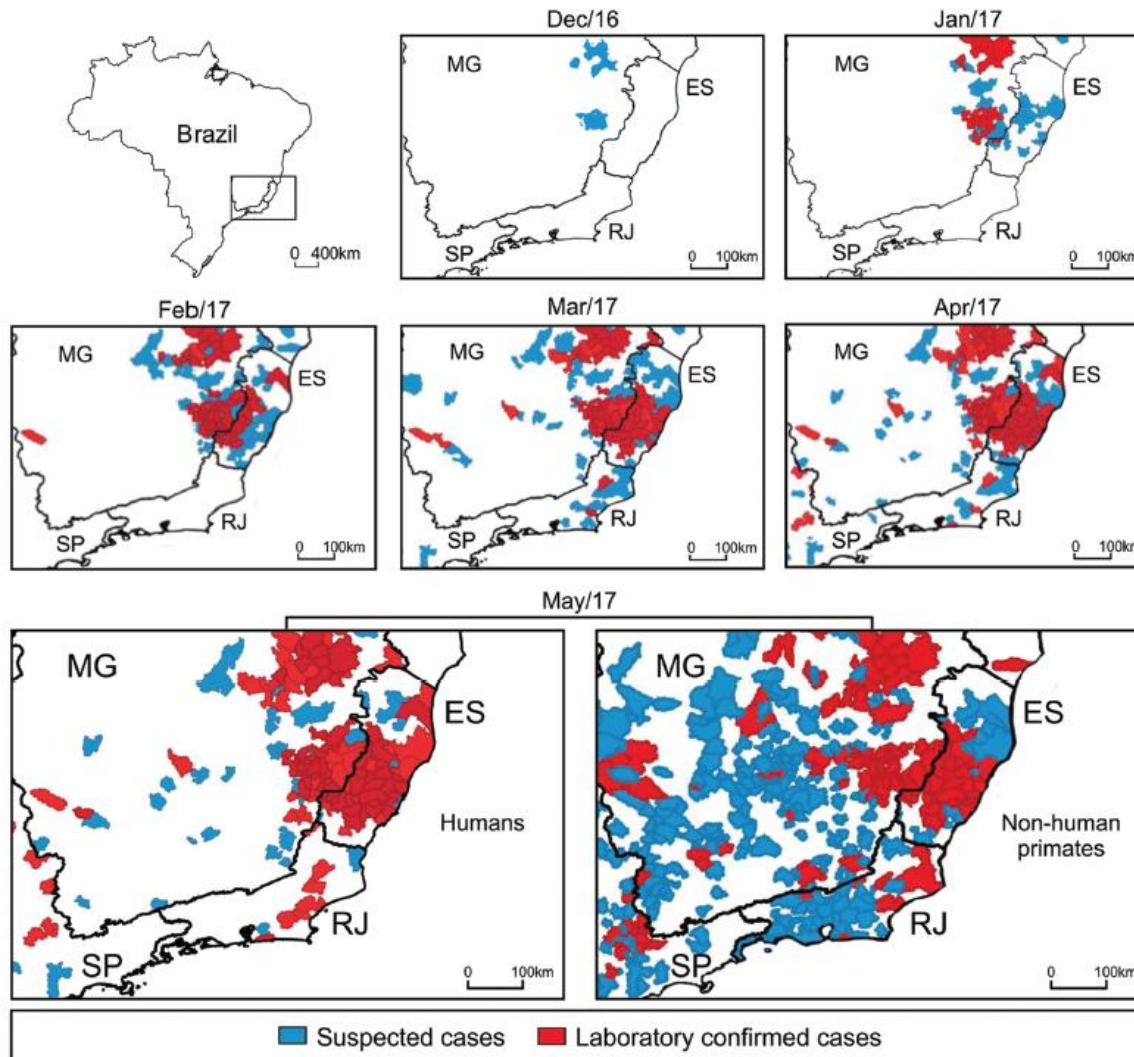
Scientists warn vaccine stocks would be overwhelmed in the event of large urban outbreaks.

## WHERE MIGHT YELLOW FEVER GO NEXT?

An ongoing outbreak of yellow fever in Angola has scientists worried that the virus might spread to cities that harbour its urban carrier, the *Aedes aegypti* mosquito.



# The recent outbreak in Brazil



# The Live-attenuated YFV vaccine (YFV-17D)

## PROS

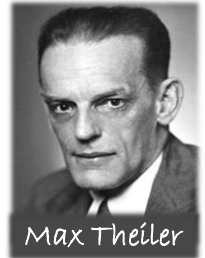


### Safe

- ✓ Massively used since 1938
- ✓ Rare complications

### Efficient

- ✓ Fast neutralizing immune response
- ✓ One dose; long-lasting protection



Max Theiler

## CONS



### Production

- ✓ Need for embryonated egg
- ✓ Q/C & batch release time

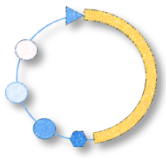
### Storage & transport

- ✓ Need for strict cold-chain

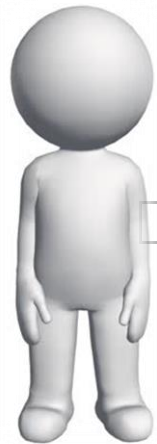


*Workers from MSF cross a river in the DRC. On their 10-hour tour of villages, they must keep the vaccine perfectly chilled.  
(Source : National Geographic)*





# PLLAV: Plasmid-Launched Live-Attenuated Viral vaccine



Live-attenuated virus route



entry (1)

translation (2)

transcription

infection and amplification in surrounding cells

assembly and release (4)

viral RNA replication (3)

transfection  
PLLAV route

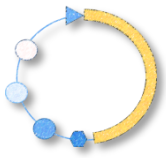


Replicating LAV in mammalian cell



Jet-injector



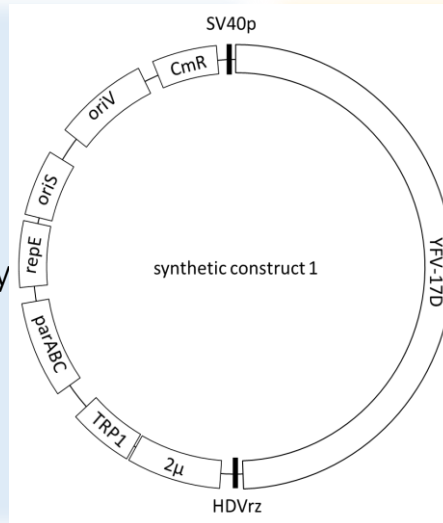


# PLLAV: Plasmid-launched live-attenuated viral vaccine

## Proprietary BAC shuttle vector:

- Stable maintenance in *E.coli*
- Inducible high yield production
- Modularity and high vector capacity
- No cold chain required
- Needle-free administration

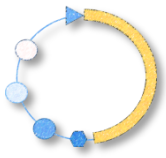
### ADVANTAGES OF DNA VACCINE



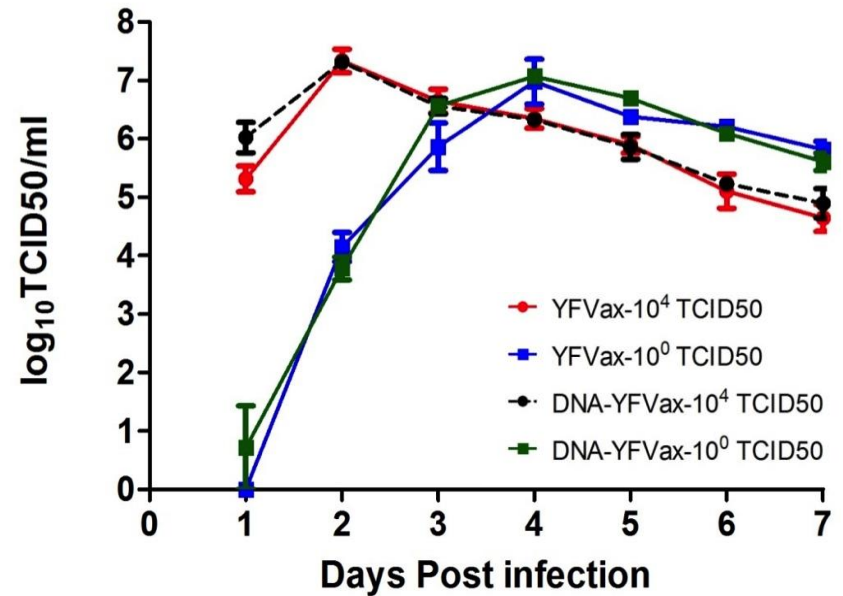
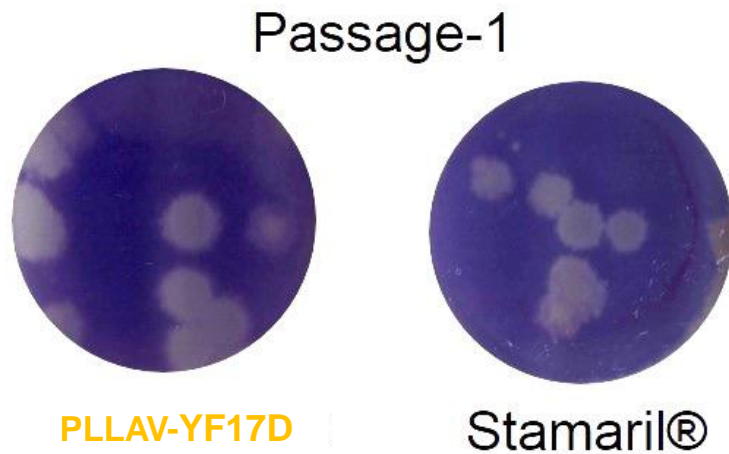
## Live-attenuated YFV vaccine strain (cDNA):

- replicates in mammalian cells
- induction of strong immune response
- insertion of foreign antigens possible

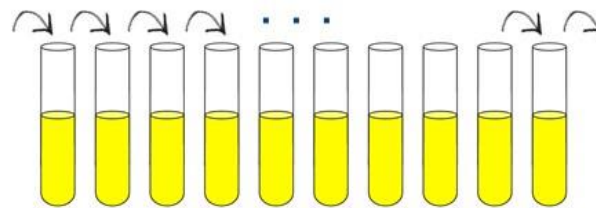
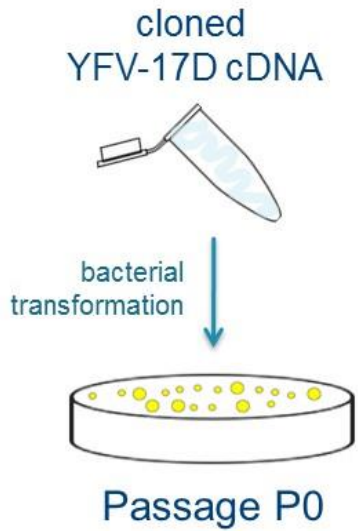
### ADVANTAGES OF LAV



# Identical virus replication of Stamaril® and PLLAV-YF17D in tissue culture

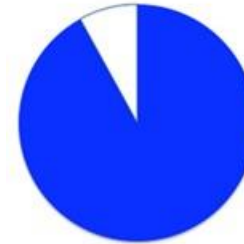


# PLLAV-YF17D is highly stable in *E. coli*



10x overnight 1:100

High copy n° plasmid



**92%**

- **large deletions** (> 1000 bp)
- restriction pattern changed



**PLLAV-YF17D**

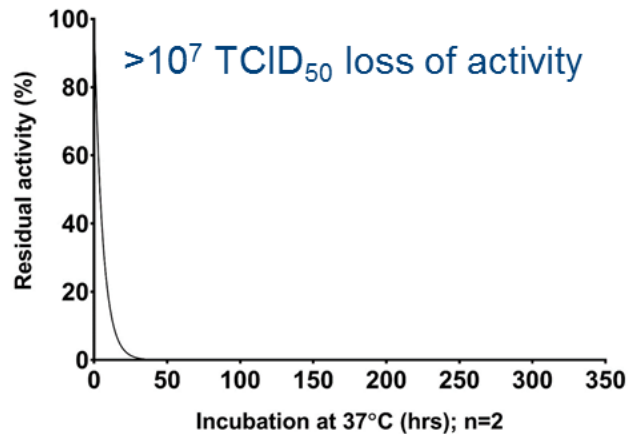


**4%**

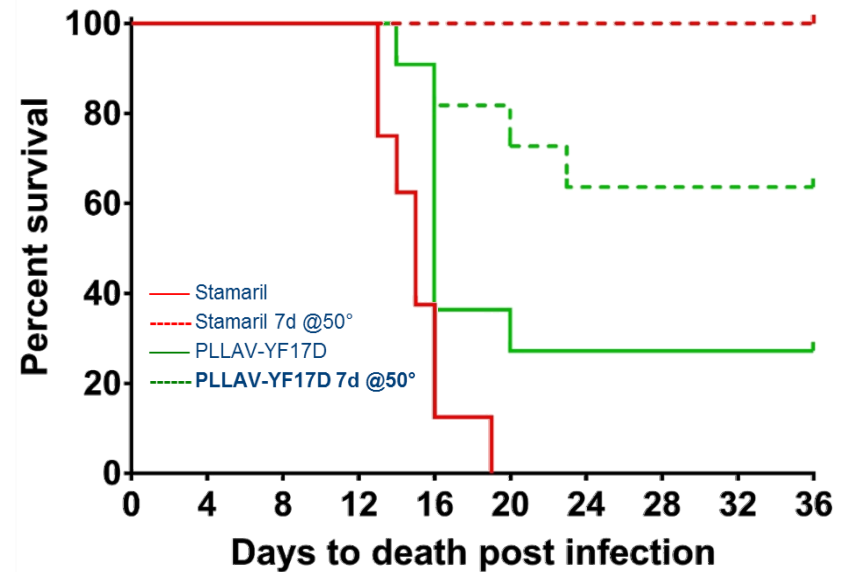
- non synonymous
- **ORF remains open**

# PLLAV-YF17D is highly thermostable

## Stamaril® *in vitro*

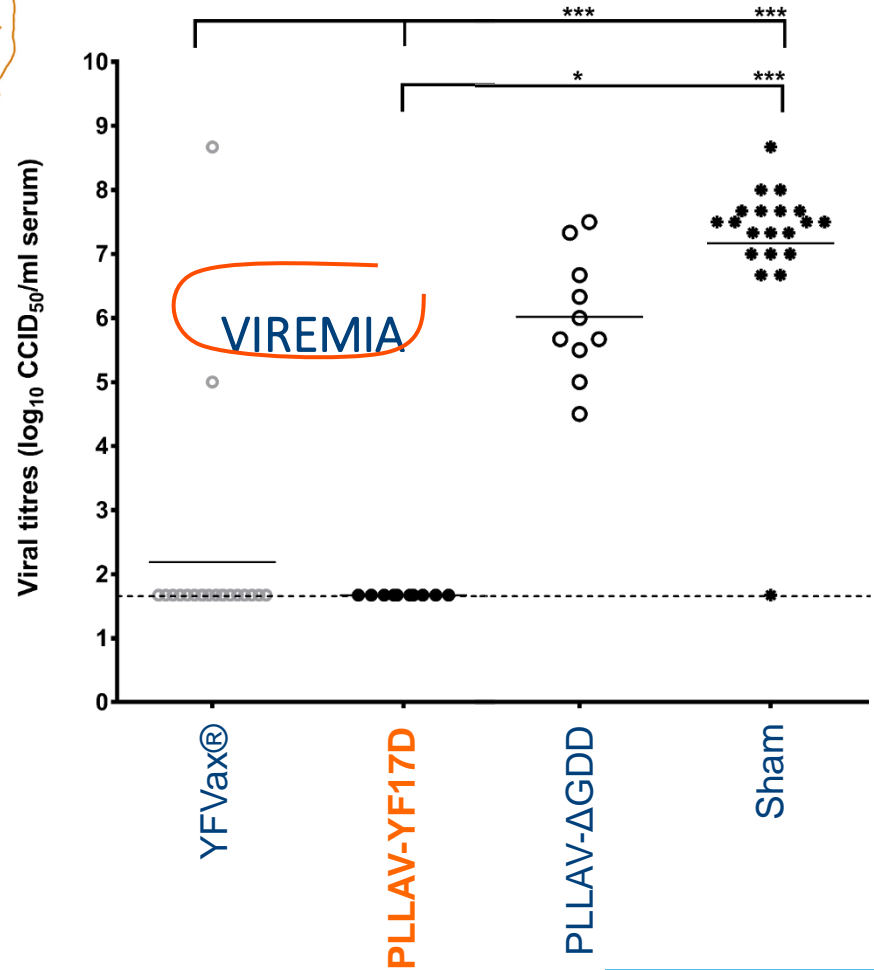
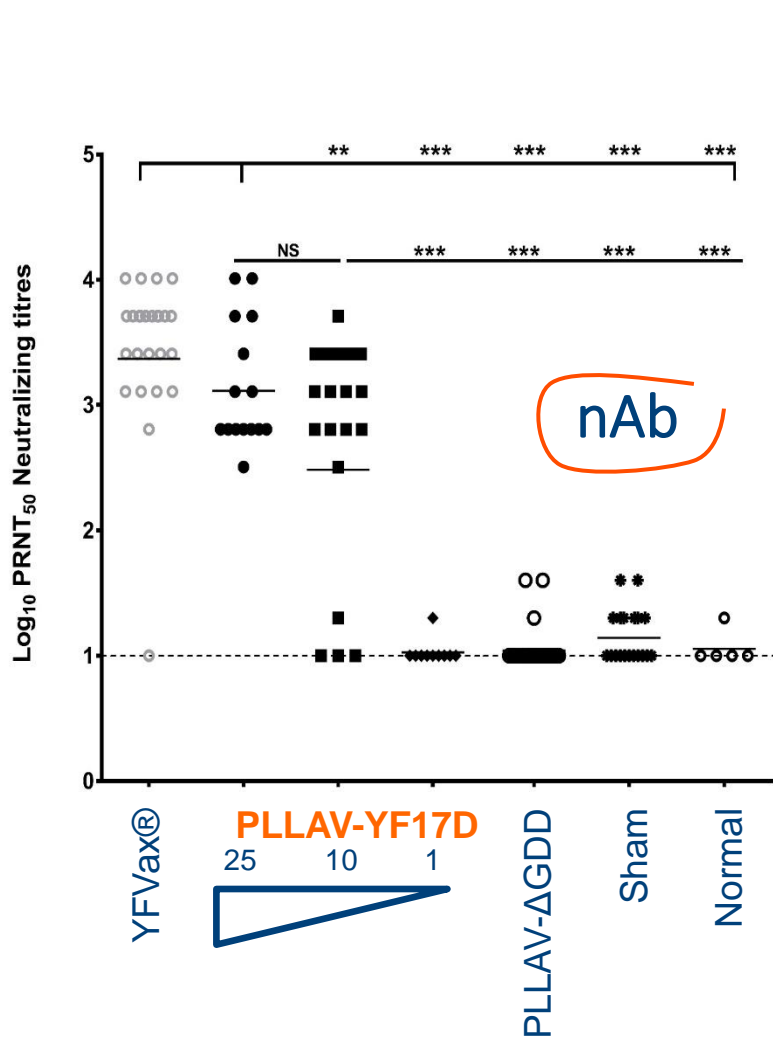


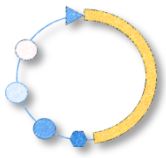
## Stamaril® versus PLLAV-YF-17D



AG129 (IFN-R KO)

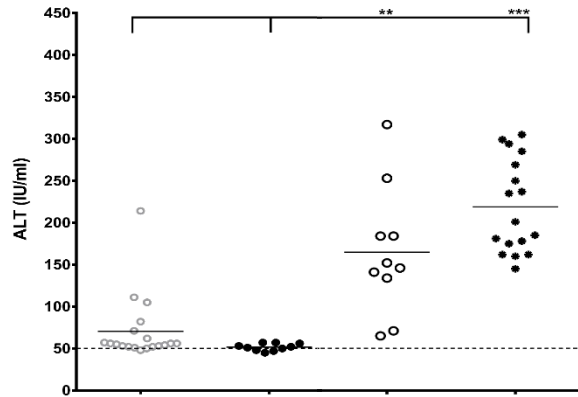
# Immunogenicity and protection from challenge viremia



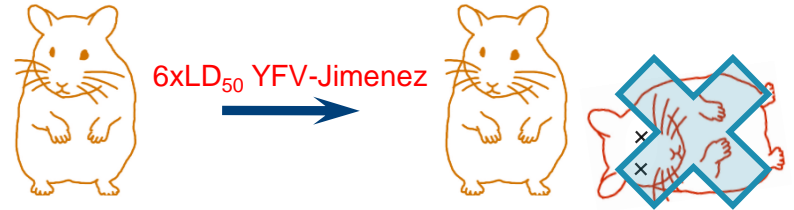
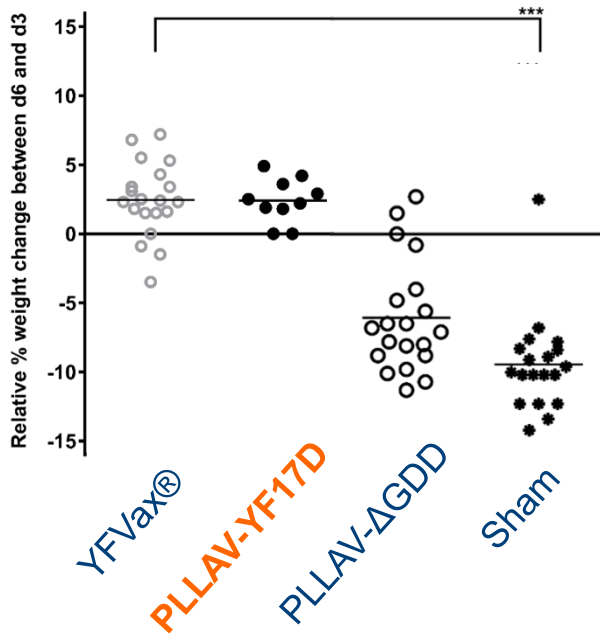


# Protection from disease and mortality

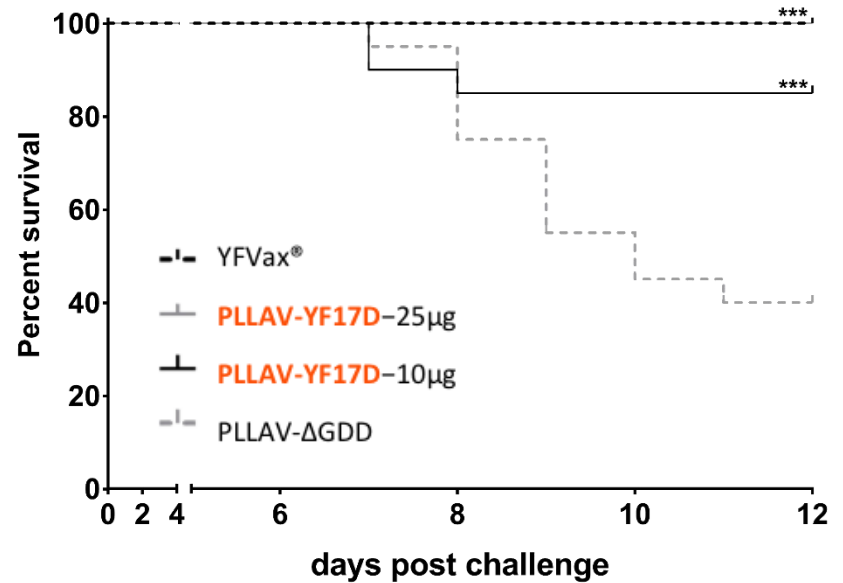
LIVER

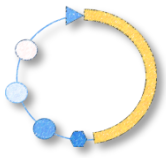


WEIGHT



SURVIVAL



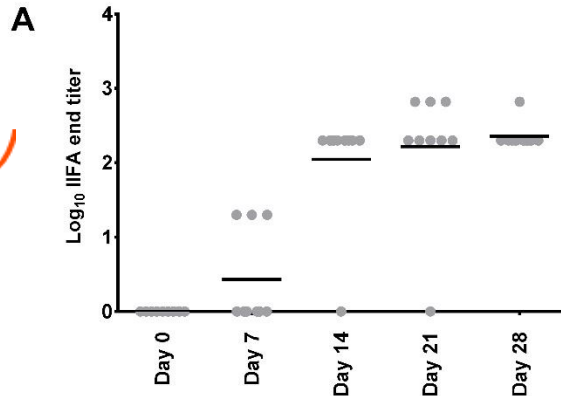


# Rapid antibody response in hamsters

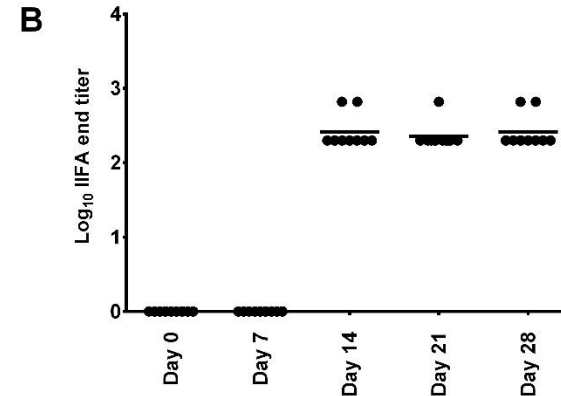


anti-YFV IgG

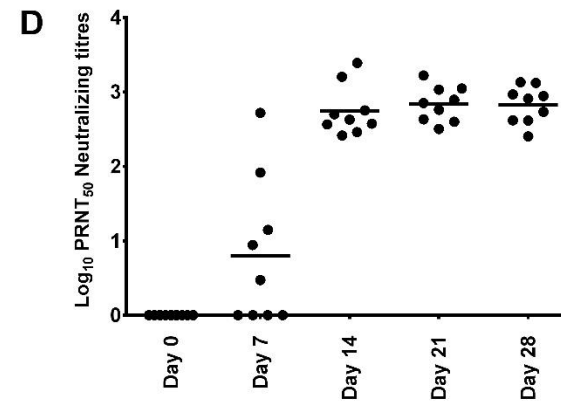
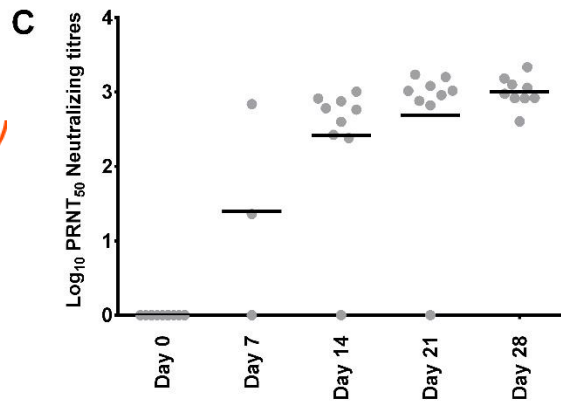
Stamaril®



PLLAV-YF17D

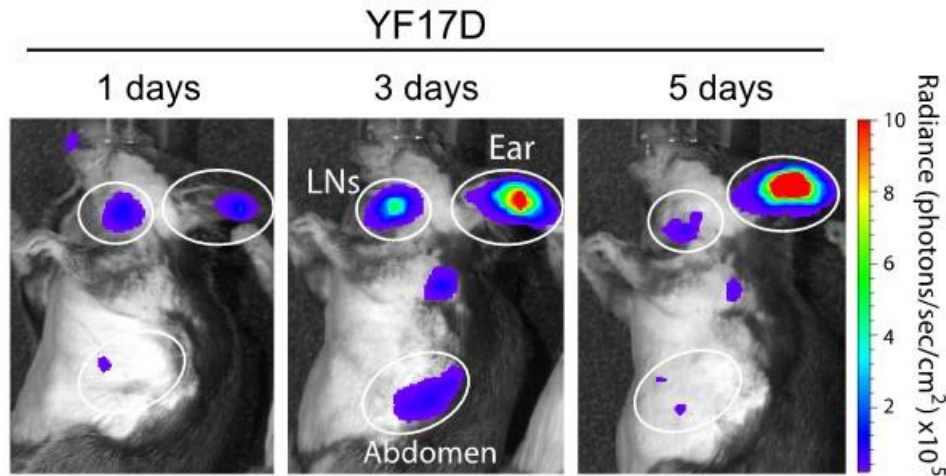


nAb

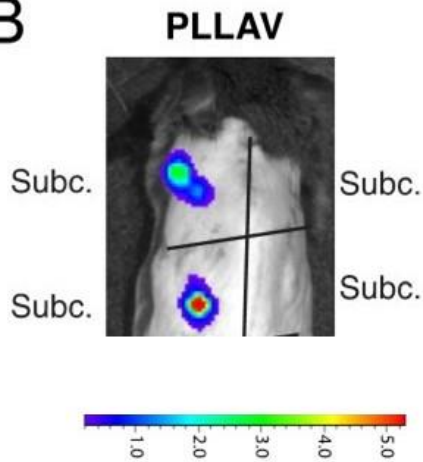


# Towards the best route of administration

A

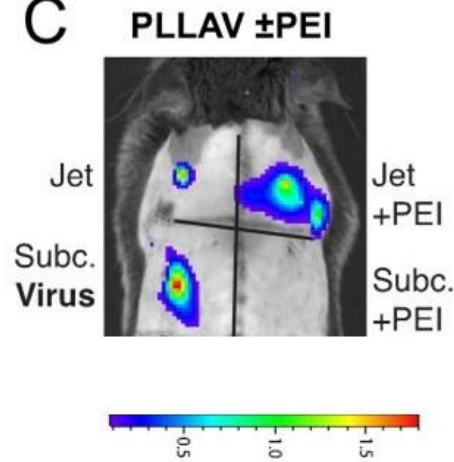


B



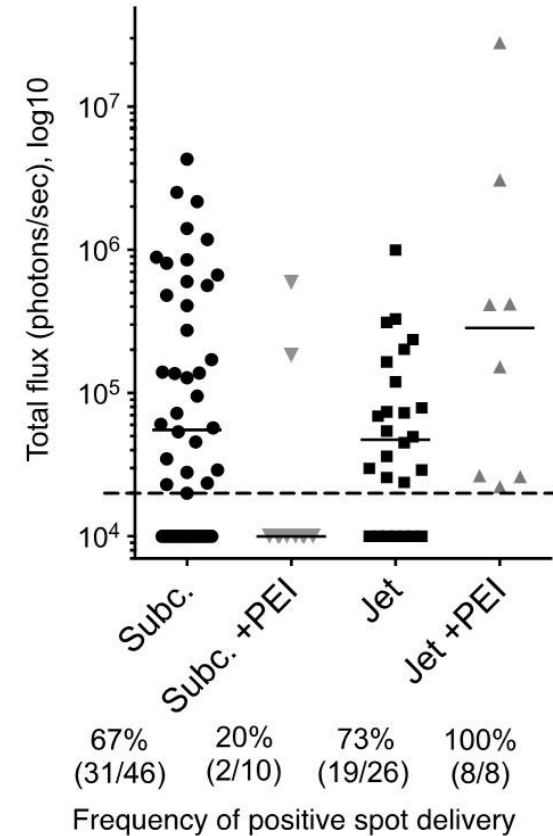
PLLAV : 2,5µg

C



Stamaril® 10<sup>4</sup> PFU

D



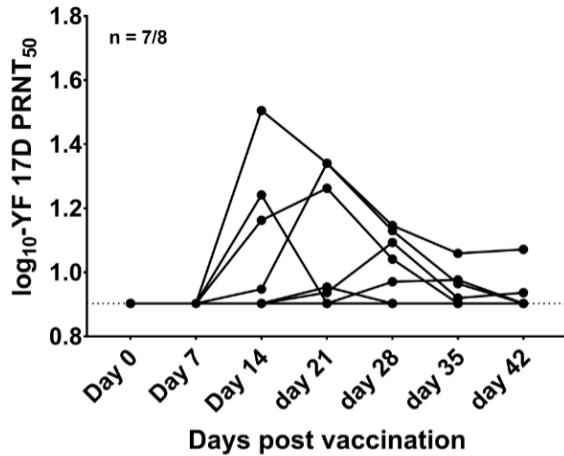
KU LEUVEN



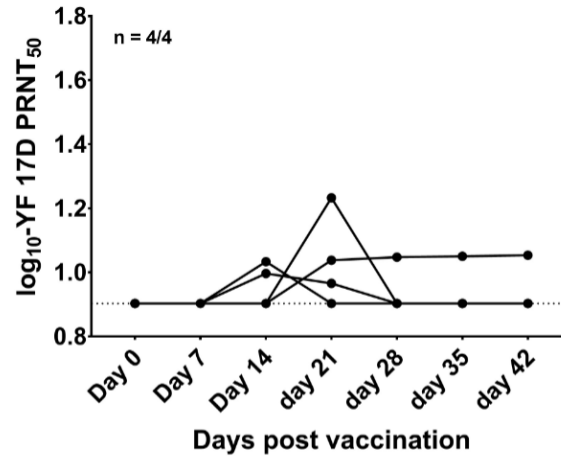
# PLLAV-YF17D can be administered to pigs



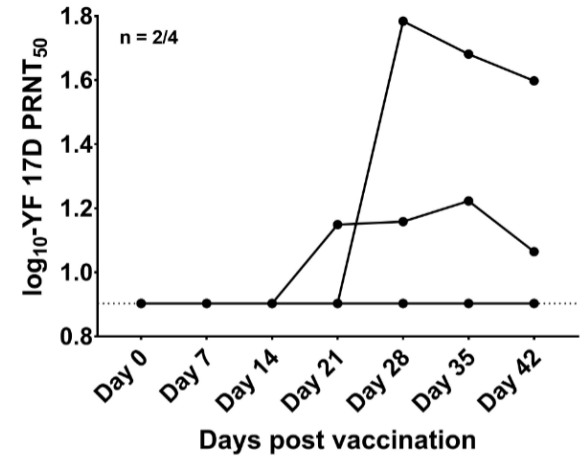
100 $\mu$ g-Jet

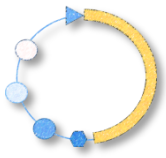


10 $\mu$ g-Jet

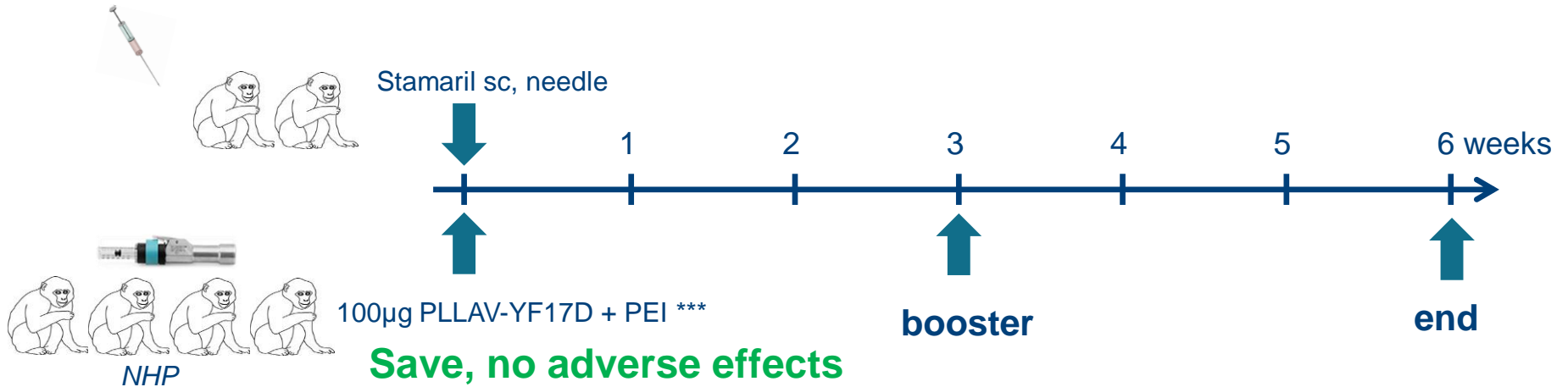


Stamaril®



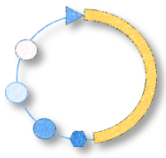


# Proof-of-concept: Immunogenicity of PLLAV-YF17D in rhesus macaques



Seroconversion	Stamaril (n=2)	PLLAV-YF17D (n=4)
Anti-YFV IgM/IgG (IIFA)	2/2	1/4
Neutralizing titer (CPNT)	1 : 500 1 : 800	1 : 1500

CoP by serum transfer to AG129 mice



# PLLAV as a platform technology

## Chimeric flavivirus vaccines

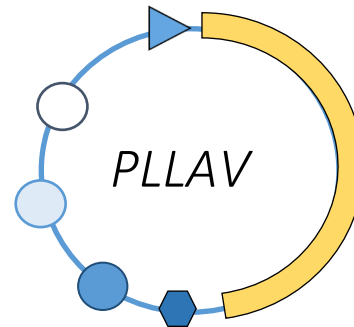


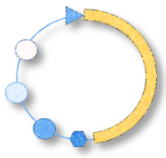
*Japanese encephalitis virus*  
*Zika virus*

## Transgenic vaccines



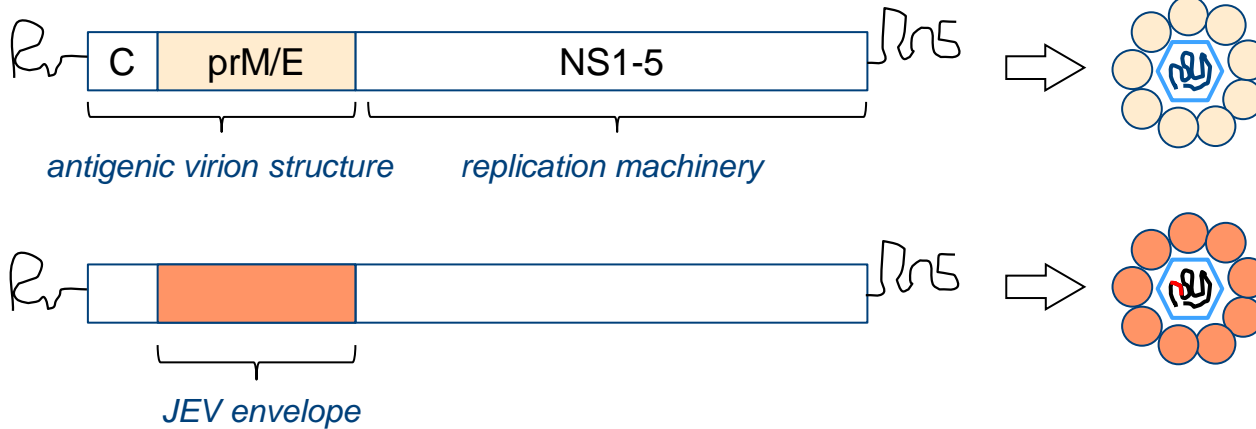
*Ebola*  
*Rabies*  
*Influenza*  
*Leishmania*  
*HBV*  
...



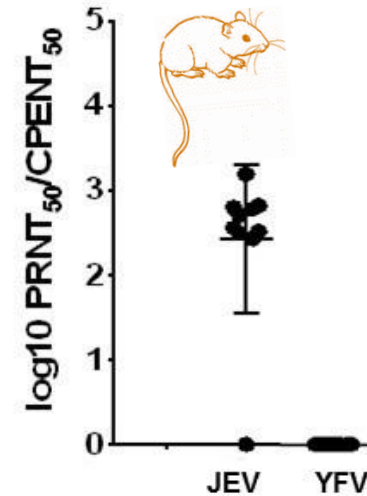


# PLLAV as platform for chimeric vaccines

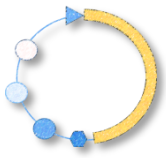
Japanese encephalitis



[www.xinhuanet.com](http://www.xinhuanet.com)



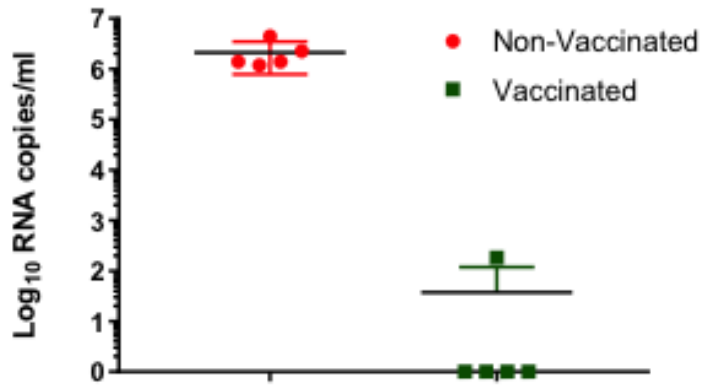
KU LEUVEN



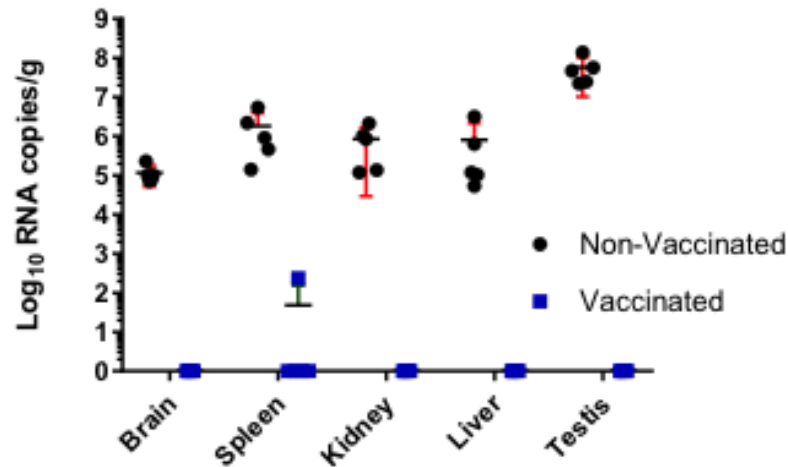
# A very safe and highly efficient ZIKV vaccine



VIREMIA



ORGANS



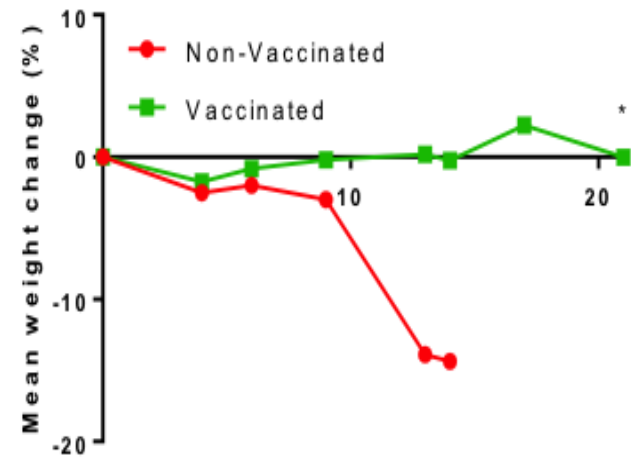
1x vax 10<sup>2</sup> PFU



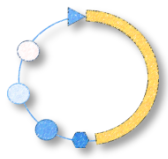
10<sup>5</sup> × LD<sub>50</sub>  
ZIKV MR766



SURVIVAL

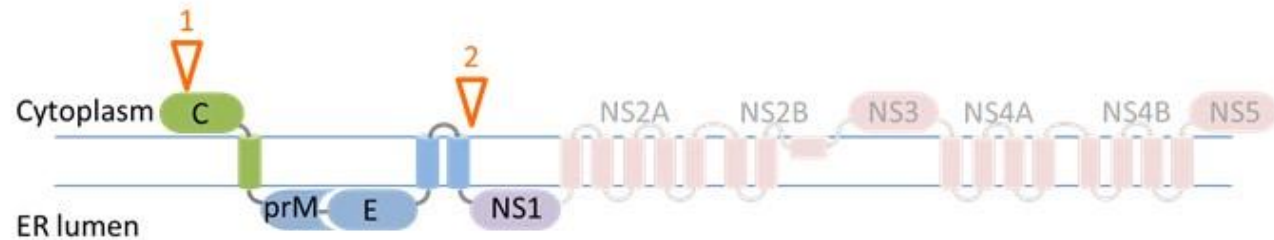
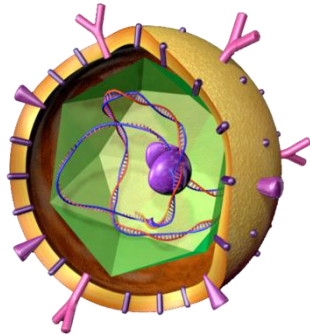


KU LEUVEN

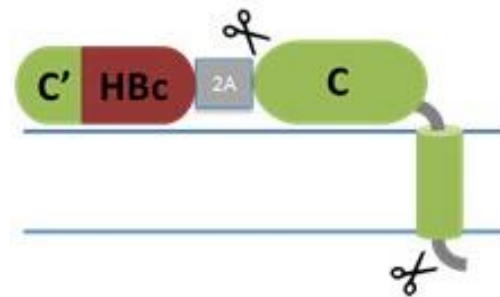


# PLLAV as platform for transgenic vaccines (HBV)

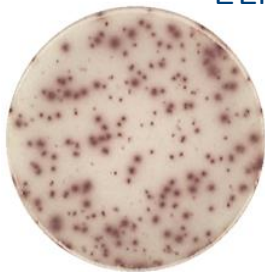
HBV



insertion site 1



ELISPOT

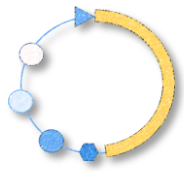


YFV-17D/Hbc

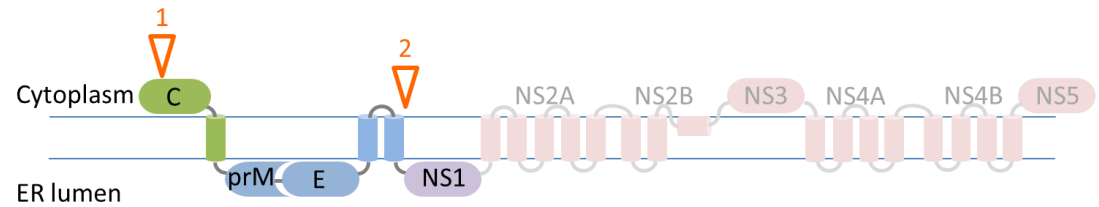
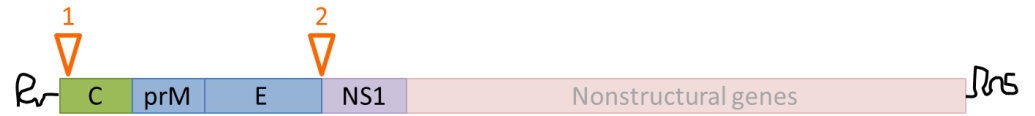
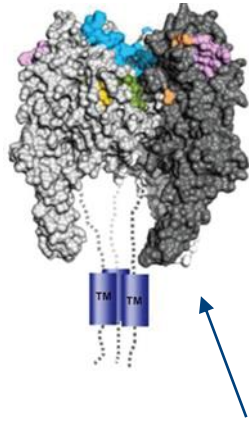


YFV-17D

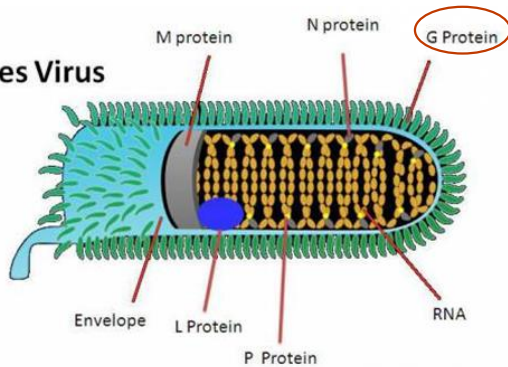
Induction of T-cell response to HBV core



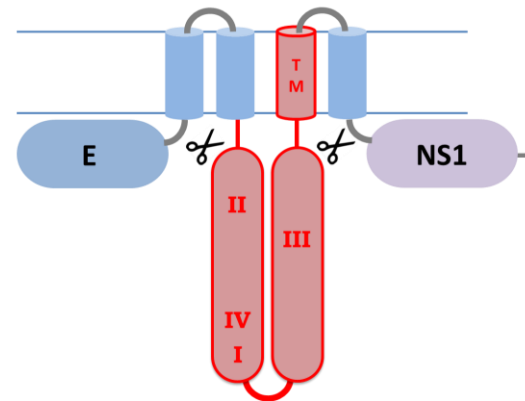
# PLLAV as platform for transgenic vaccines (rabies)

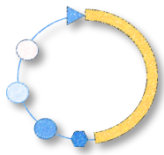


## Rabies Virus



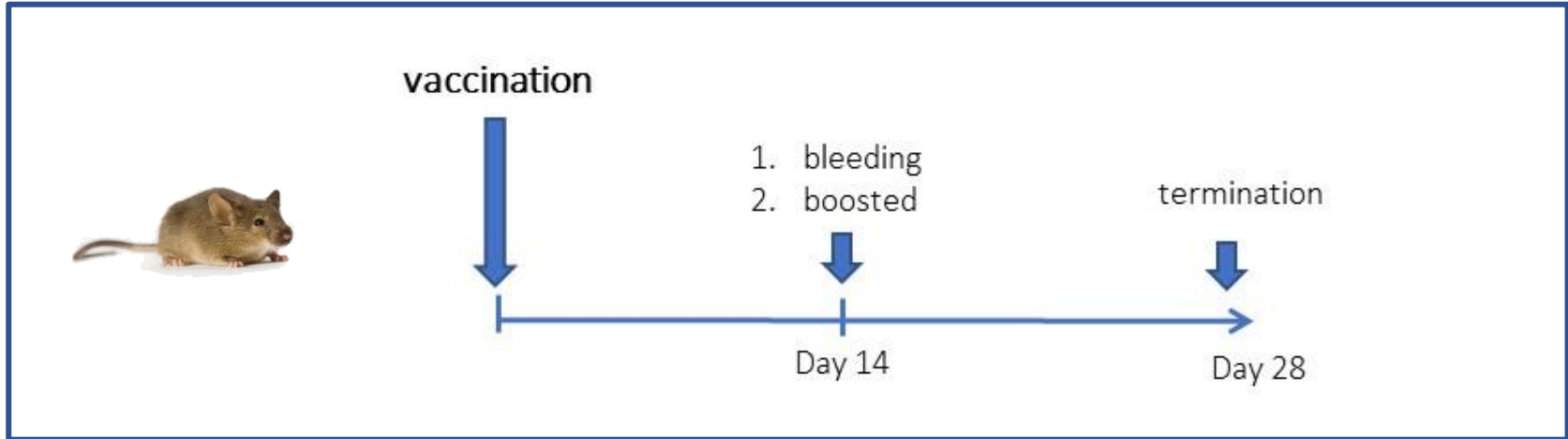
## insertion site 2





# PLLAV as platform for transgenic vaccines (rabies)

RabG insertion between E and NS1 (PLLAV-YF/RabG)



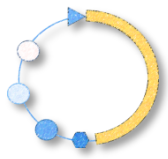
## Day 14

YFV seroconversion 5/5  
RABV seroconversion 5/5  
RABV nAb: 1.5 [0.25-3.3]

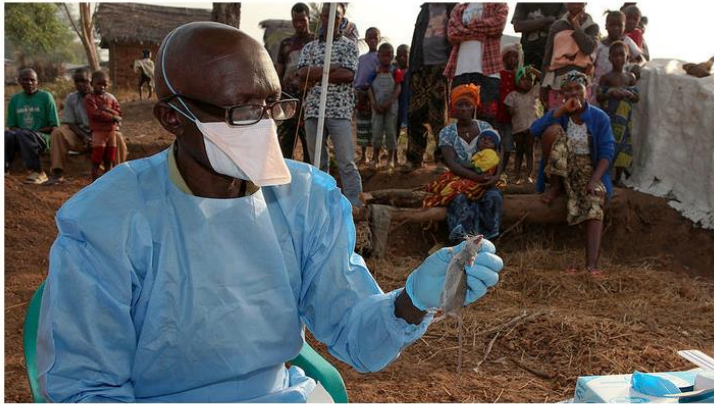
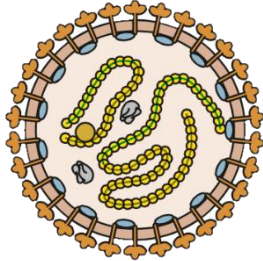
## Day 28

YFV seroconversion 5/5  
RABV seroconversion 5/5  
RABV nAb: 5.3 [0.54- 11.5]





# PLLAV as platform for transgenic vaccines (Lassa)

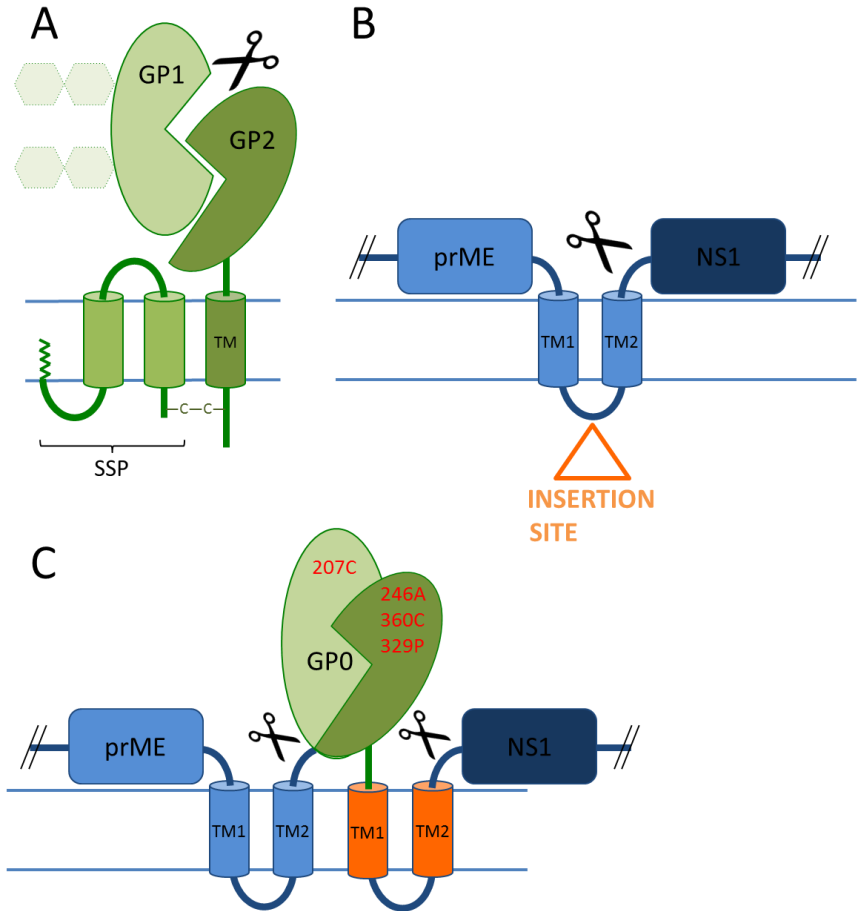


This year, the rats that carry Lassa fever may be more numerous, or more likely to harbor the virus. REUTERS/SIMON AKAM

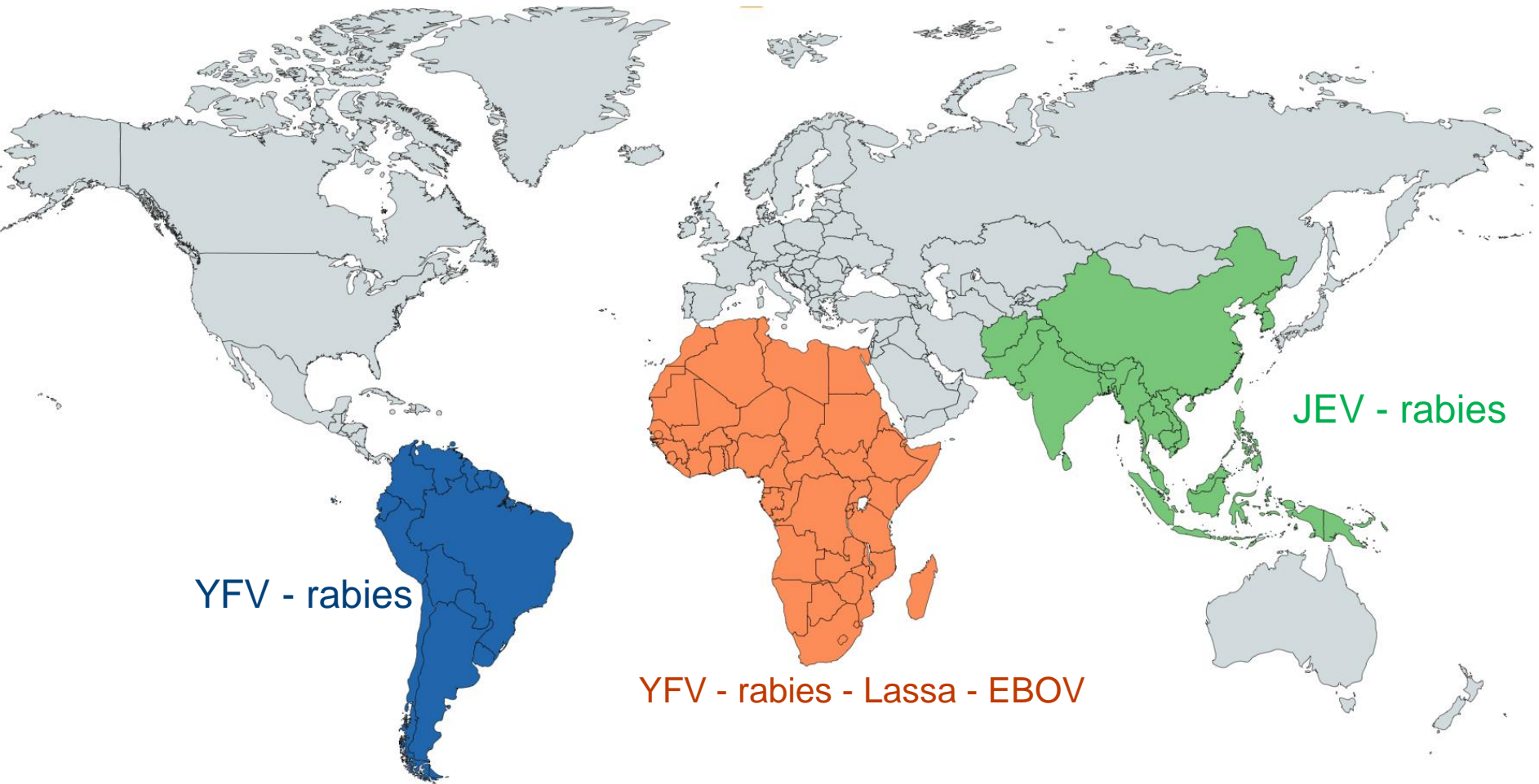
## Health workers scramble to contain deadly rat-borne fever in Nigeria

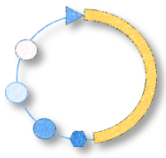
By Leslie Roberts | Mar. 12, 2018, 4:20 PM

Science, March 2018



# Towards region specific vaccines



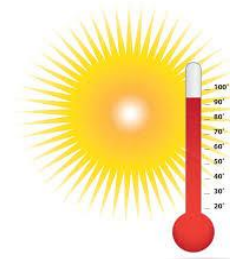


To summarize...

Commercial YFV-17D vaccine



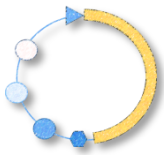
PLLAV-17D



OR



**KU LEUVEN**



# PL LAV team



Kai Dallmeier



Niraj Mishra



Lorena Sanchez Felipe



Robbert Boudewijns



Dieudonne Buh Kum



Michael Schmid



Katrien Geerts



Ji Ma



Sapna Sharma



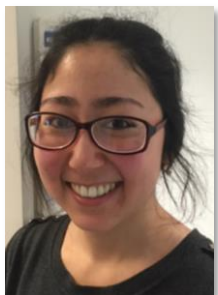
Sarah Debaveye



Raphael Marquez



Catherina Coun



Madina Rosulova



Lotte Coelmont



Pieter Leyssen



Hendrik Thibaut



## **The Neyts-lab of Virology, Antiviral Drug & Vaccine Research**

Our Mission : The development of (i) antiviral strategies against a range of (RNA) viruses and (ii) a game-changing novel vaccine technology platform

# Acknowledgements



This project has received funding from the European Union's Horizon 2020 research and innovation programme under RABYD-VAX grant agreement No 733176.



National Institute of  
Allergy and  
Infectious Diseases

KU LEUVEN

🏠 LUVAC | LEUVEN UNIVERSITAIR VACCINOLOGIE CENTRUM



Animal &  
Plant Health  
Agency



KU LEUVEN

## PLLAV, PLASMID-LAUNCHED LIVE VACCINES