

The virus-host interface: A treasure trove of novel antiviral targets

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Disclosures

- Received research funding from Tonix Pharmaceuticals
- Licensing agreement with Tonix Pharmaceuticals

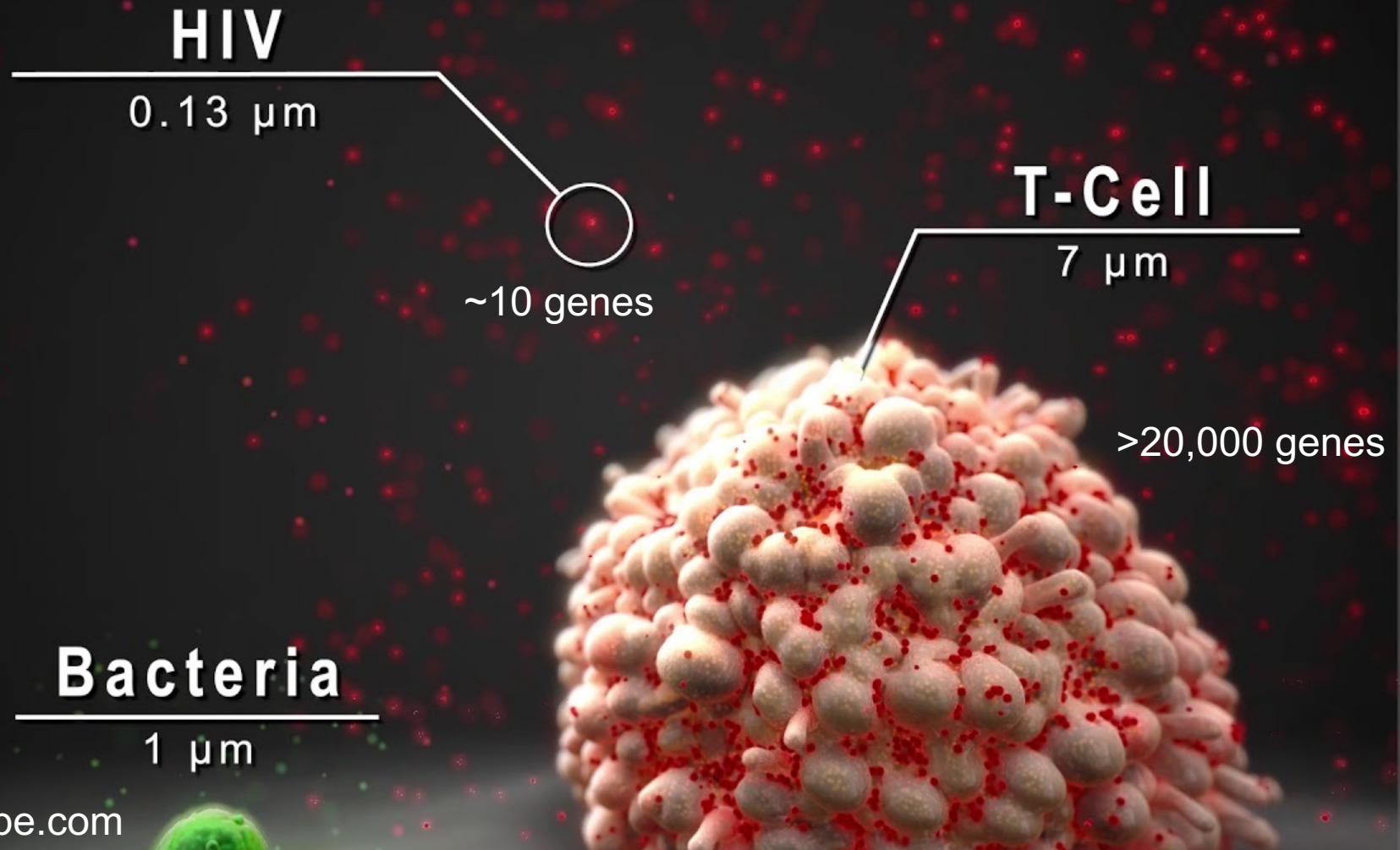
Burden of epidemic/pandemic viruses

- Smallpox virus , influenza virus, Dengue virus, HIV, chikungunya virus, Ebola virus, HCV, HBV, Zika virus, SARS-CoV-2
- Billions of people infected since 19th century
- Hundreds of millions of deaths
- Enormous economic and social impact
 - >\$82 trillion for COVID-19

Paucity of antivirals for emerging viruses

- Conventional antivirals target viral proteins
 - Take years to develop
 - Often highly specific
- Vaccines are most effective in preventing viral diseases
 - Highly specific
- Need broad spectrum antivirals as first line of defense against emerging viruses

Taking advantage of the matchup

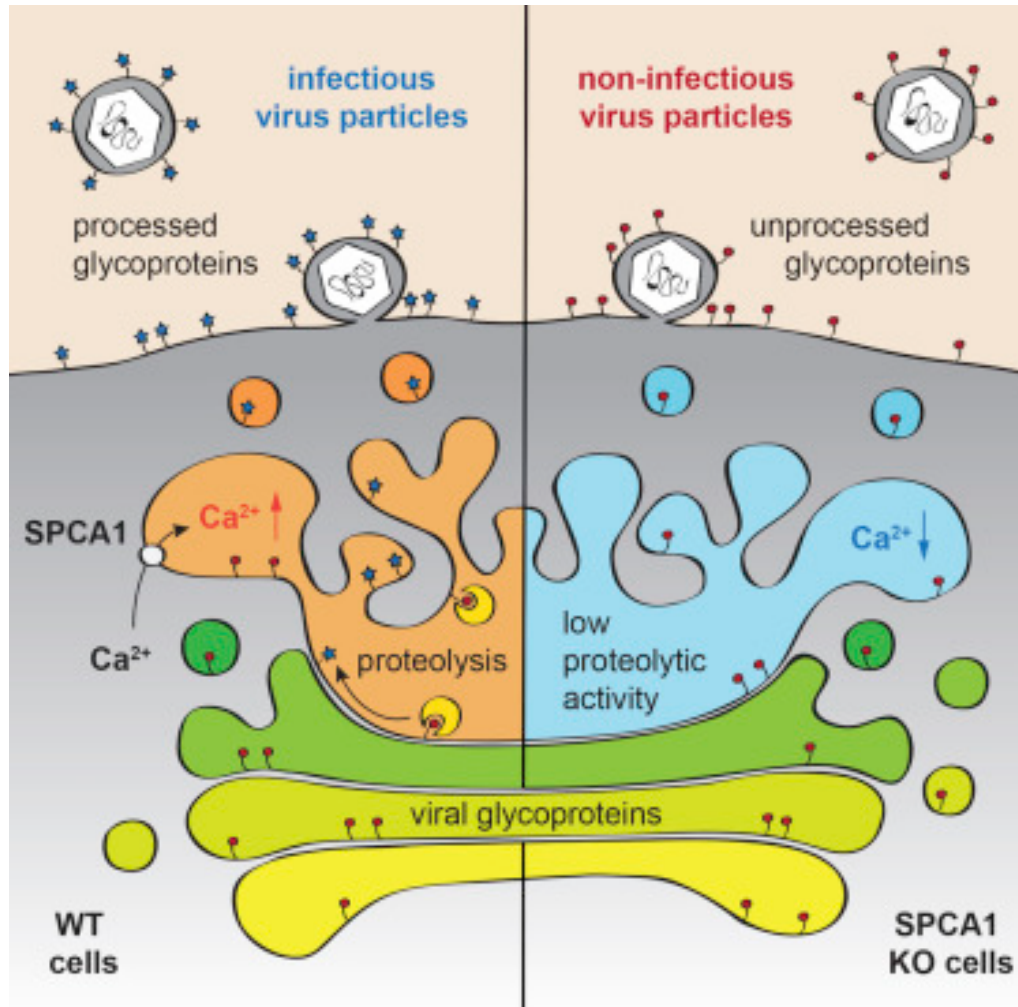


Targeting host-dependency factors is an exciting new frontier for antivirals

- Viruses are wholly reliant on host cellular pathways for replication & assembly
- Vulnerabilities
 - Conserved pathways that can be targeted
 - Multiple viruses affected
 - Less chance of developing resistance

















Proof of principle

The calcium pump SPCA1 is required for infectivity of multiple RNA viruses including measles, Dengue, West Nile, Zika and chikungunya viruses

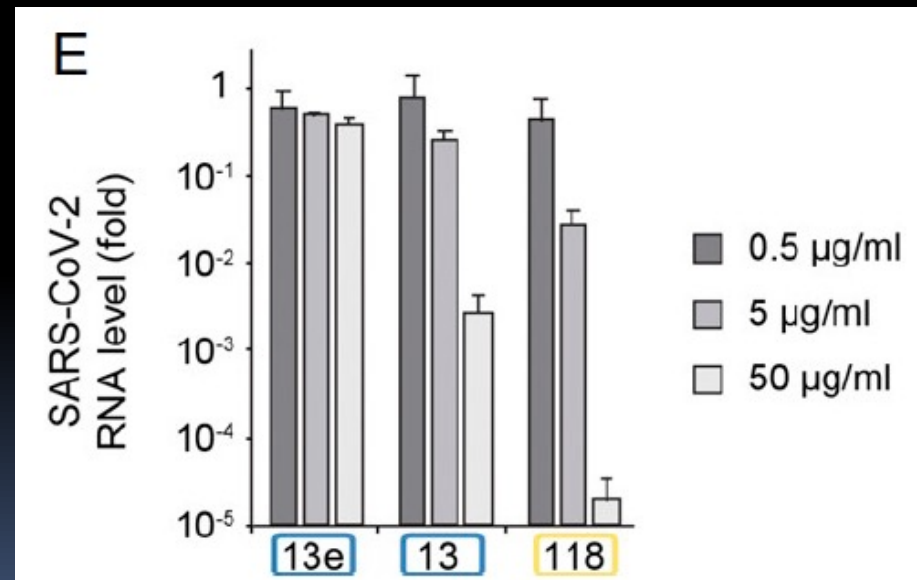
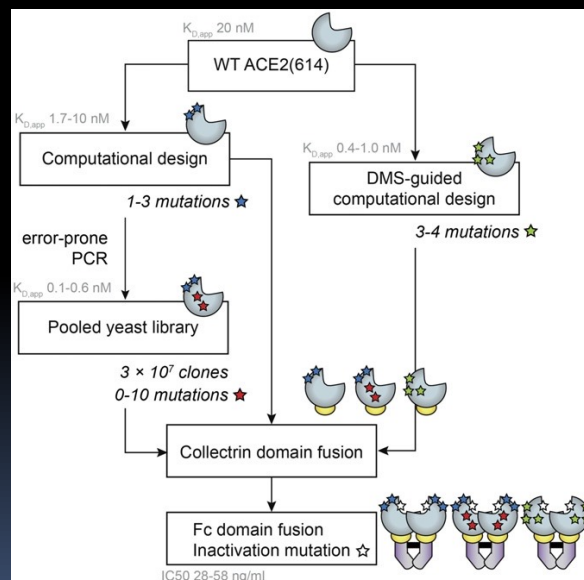
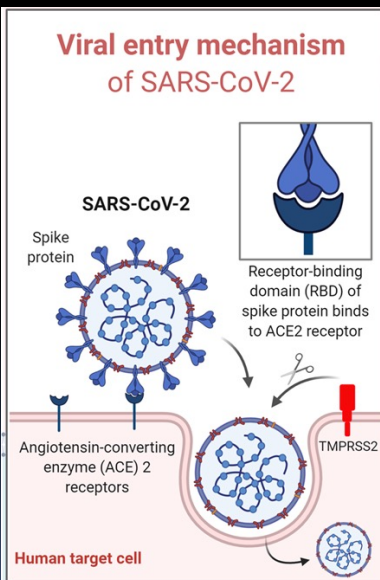


Hoffmann et al, 2017
Cell Host Microbe

Engineered ACE2 receptor traps potentially neutralize SARS-CoV-2

Anum Glasgow^{a,1} , Jeff Glasgow^{b,1} , **Daniel Limonta^{c,d}** , Paige Solomon^b, Irene Lui^b , Yang Zhang^a , Matthew A. Nix^e , Nicholas J. Rettko^b , Shoshana Zha^f , Rachel Yamin^g, Kevin Kao^g, Oren S. Rosenberg^f , Jeffrey V. Ravetch^g , Arun P. Wiita^e , Kevin K. Leung^b , Shion A. Lim^b , Xin X. Zhou^b , **Tom C. Hobman^{c,d,h}** , Tanja Kortemme^a, and James A. Wells^{b,i,2} 

28046–28055 | PNAS | November 10, 2020 | vol. 117 | no. 45

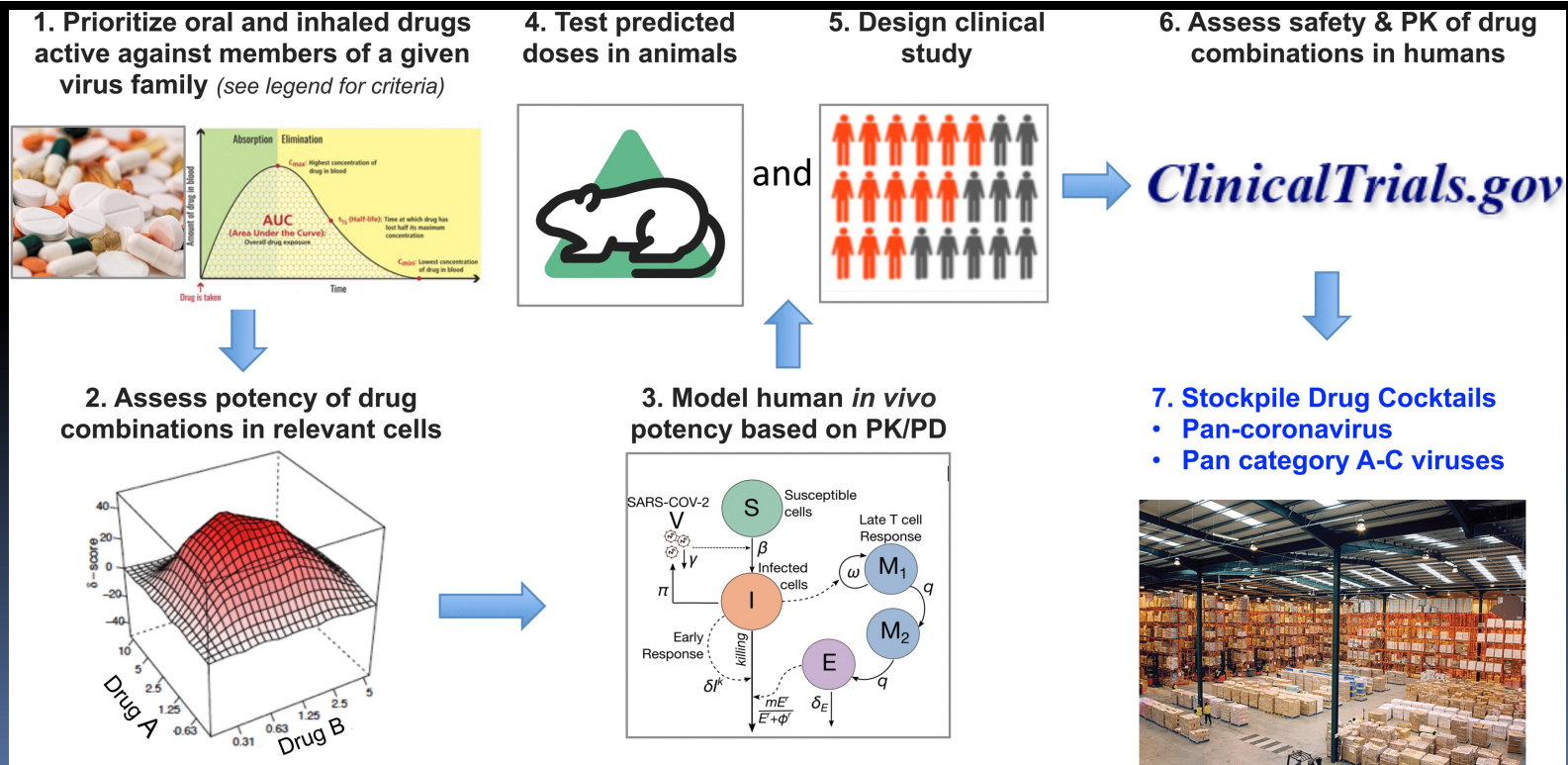


>50,000-fold reduction in SARS-CoV-2 replication

Drug Combinations as a First Line of Defense against Coronaviruses and Other Emerging Viruses

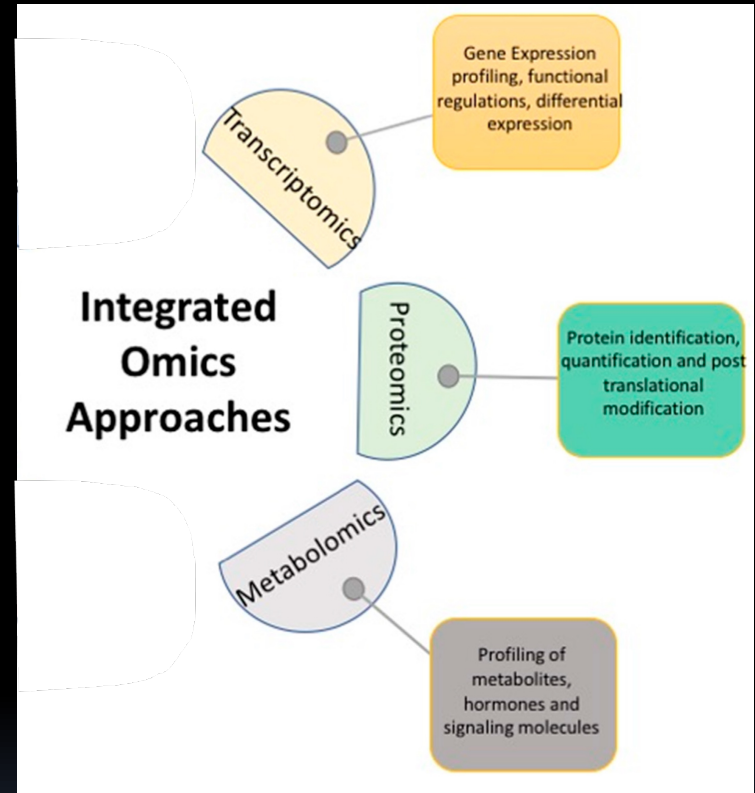
^{a,b} Judith M. White, ^{c,d} Joshua T. Schiffer, ^{c,d} Rachel A. Bender Ignacio, ^{c,d} Shuang Xu, ^{e,f,g} Denis Kainov, ^{e,f,g} Aleksandr Ianevski, ^{e,g} Tero Aittokallio, ^{g,h,i} ^j Matthew Frieman, ^j Gene G. Olinger, ^k ^{l,m,n} Stephen J. Polyak

“For the present pandemic response, and for future pandemics the scientific community must be ready with an arsenal of easily self-administered drugs that can be tested in rapid, efficient clinical trials immediately after the causative viral agent is identified.”



Experimental approaches

- Flaviviruses
- Alphaviruses
- HIV
- Coronaviruses

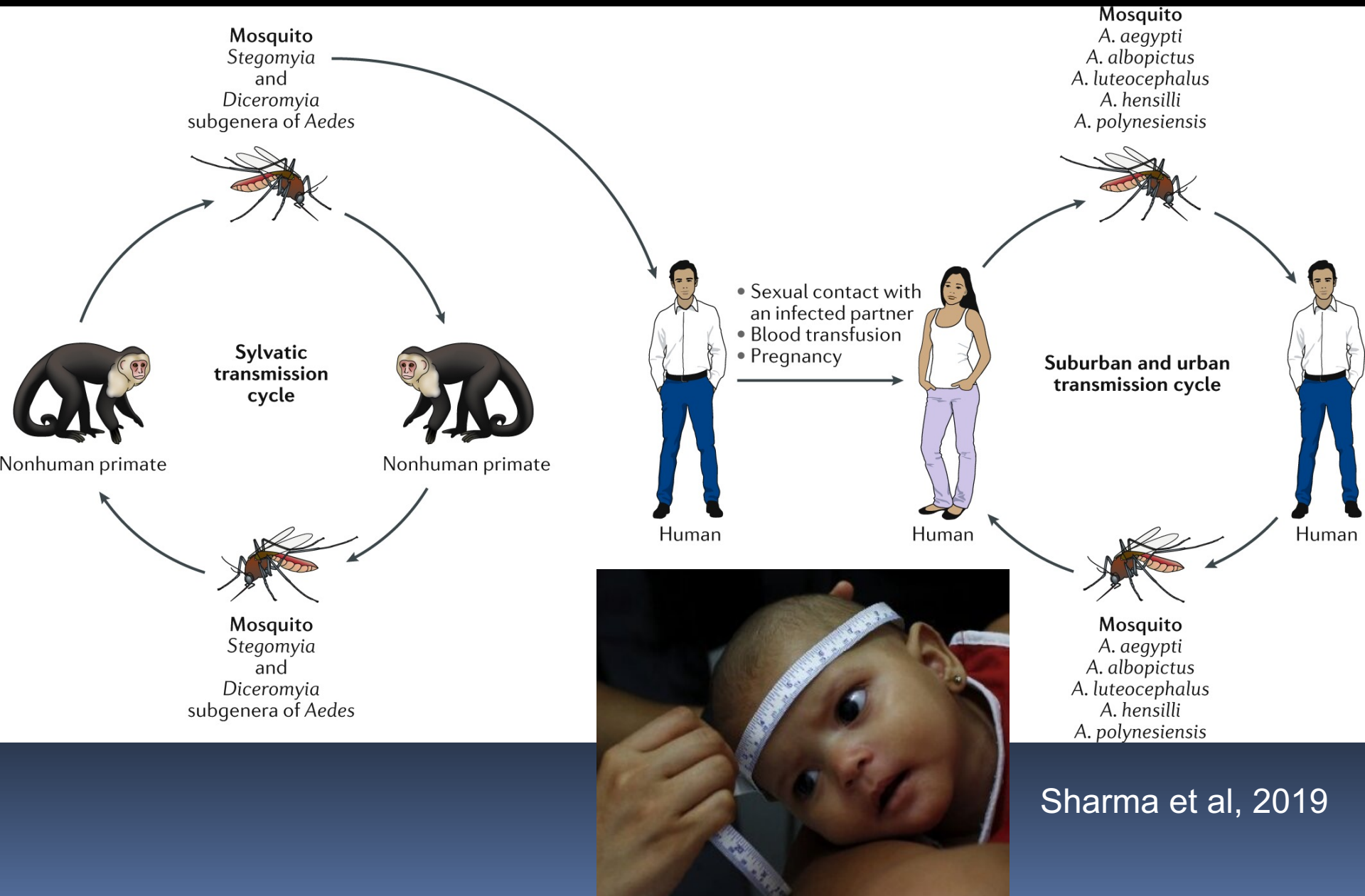


- Determine which cellular pathways are perturbed or activated
- Test effects of pathway agonists/inhibitors on virus replication

Zika virus (ZIKV) distribution

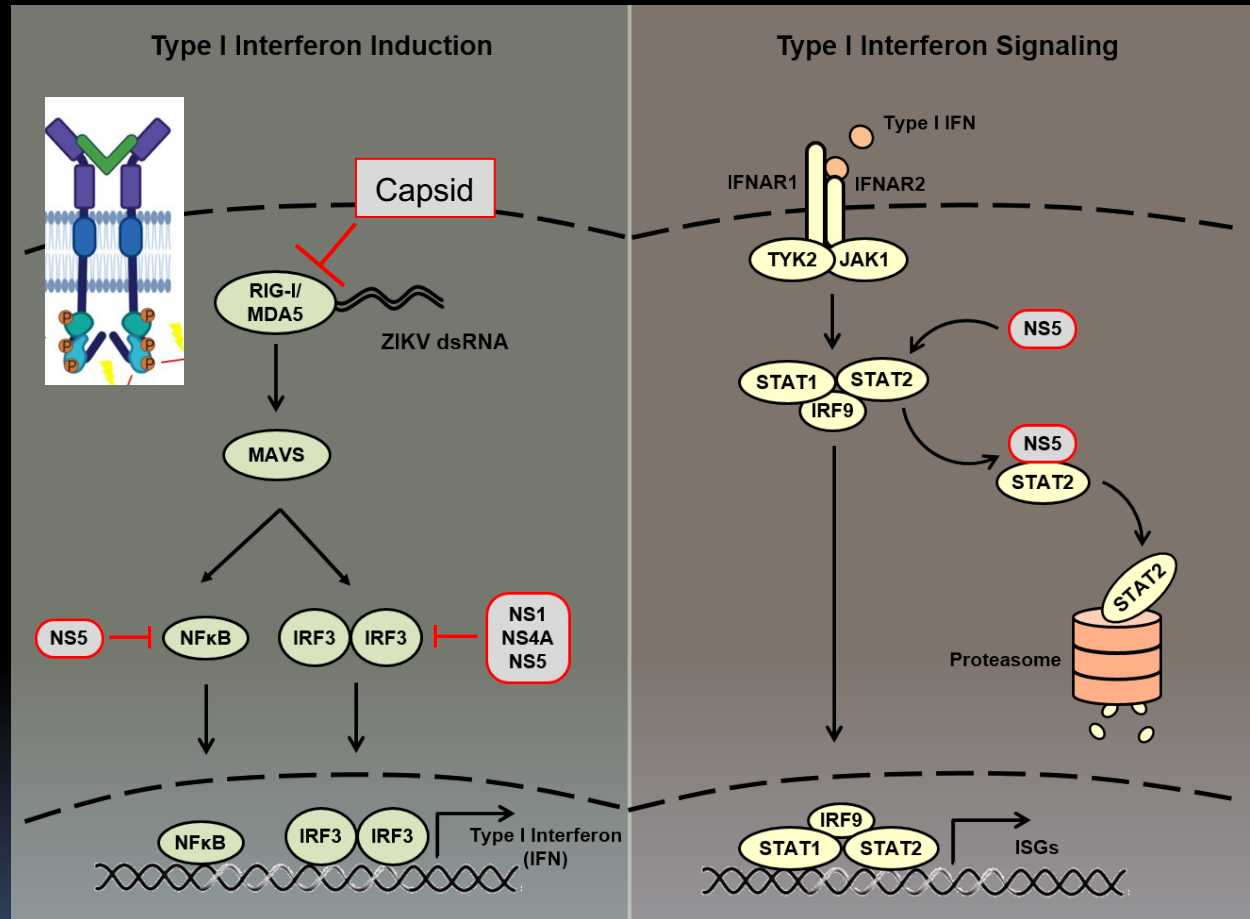


ZIKV transmission cycles



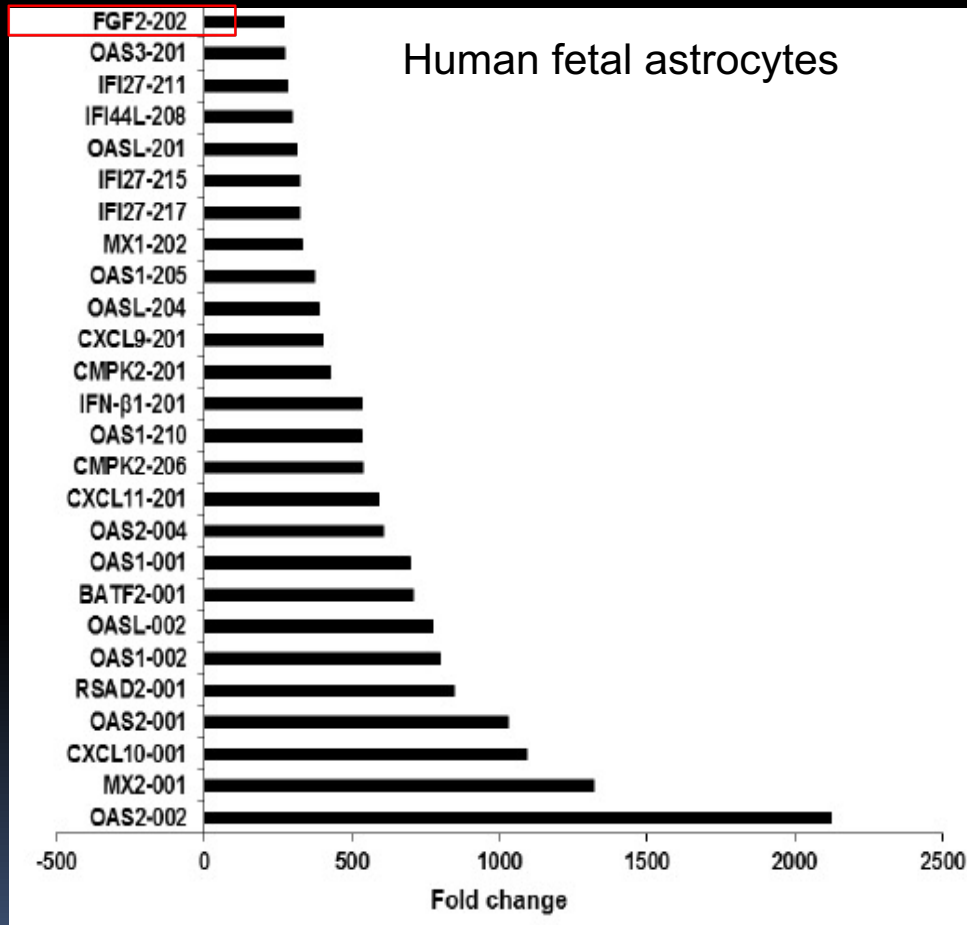
Sharma et al, 2019

ZIKV causes persistent infection and uses multiple strategies to antagonize the interferon response



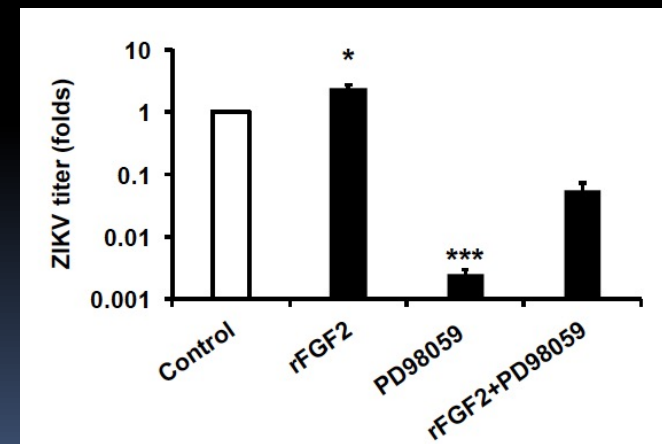
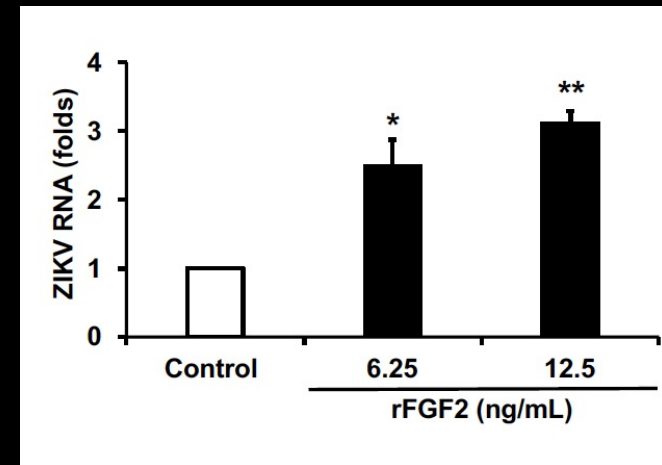
Kumar, Hou et al, 2016; Kumar et al, 2018; Limonta et al, 2019; Airo et al, 2022

Fibroblast growth factor-2 (FGF2) mRNA is upregulated 270-8600X during ZIKV infection



Kumar et al, 2018 (Sertoli cells)

Limonta et al, 2019 (Fetal astrocytes)



- FGF2 signaling suppresses IFN!

Canadian pioneers in regulation of the IFN response

The EMBO Journal Vol.17 No.12 pp.3351–3362, 1998

The molecular basis of viral oncolysis: usurpation of the Ras signaling pathway by reovirus

**James E.Strong, Matthew C.Coffey,
Damu Tang, Pauline Sabinin and
Patrick W.K.Lee¹**

Department of Microbiology and Infectious Diseases, University of
Calgary Health Sciences Centre, Calgary, Alberta, Canada T2N 4N1

JOURNAL OF VIROLOGY, May 2006, p. 4422–4430
0022-538X/06/\$08.00+0 doi:10.1128/JVI.80.9.4422–4430.2006
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Vol. 80, No. 9

Negative Regulation of the Alpha Interferon-Induced Antiviral Response by the Ras/Raf/MEK Pathway

Sarah M. Battcock, Thaddeus W. Collier, Dong Zu, and Kensuke Hirasawa*

Division of Basic Medical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Canada

JOURNAL OF VIROLOGY, July 2009, p. 6717–6726
0022-538X/09/\$08.00+0 doi:10.1128/JVI.02213-08
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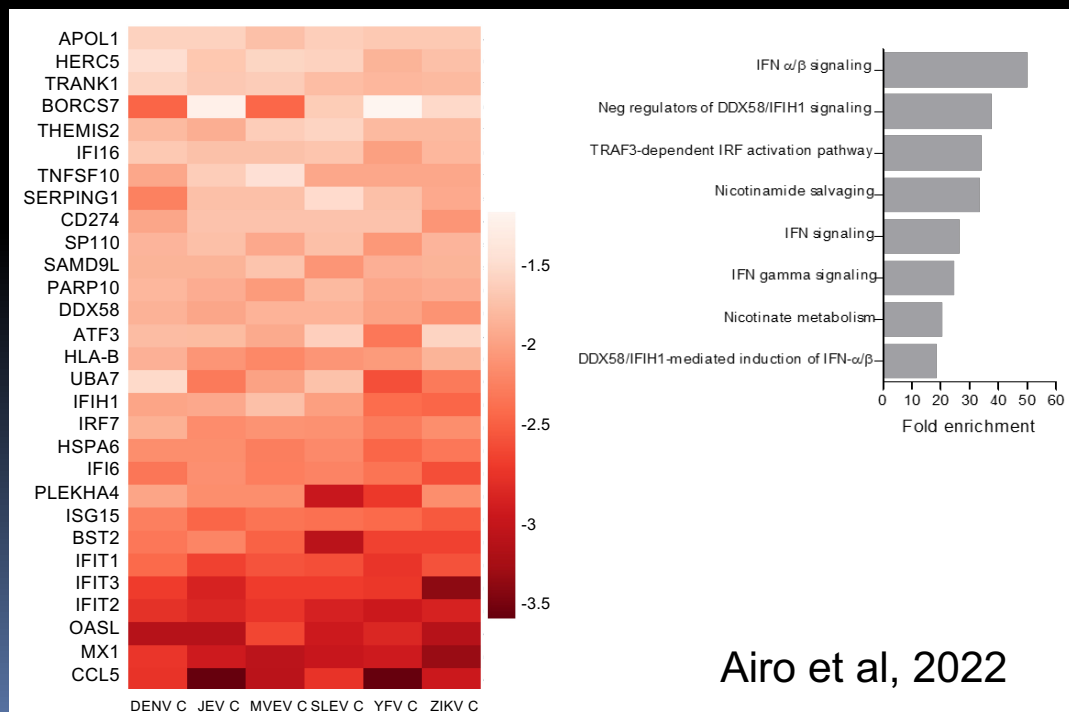
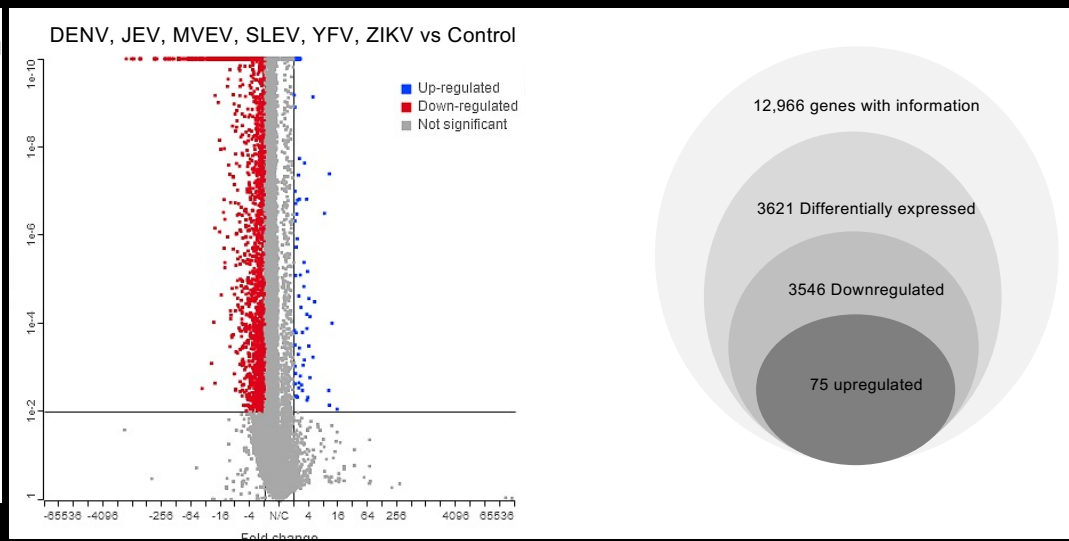
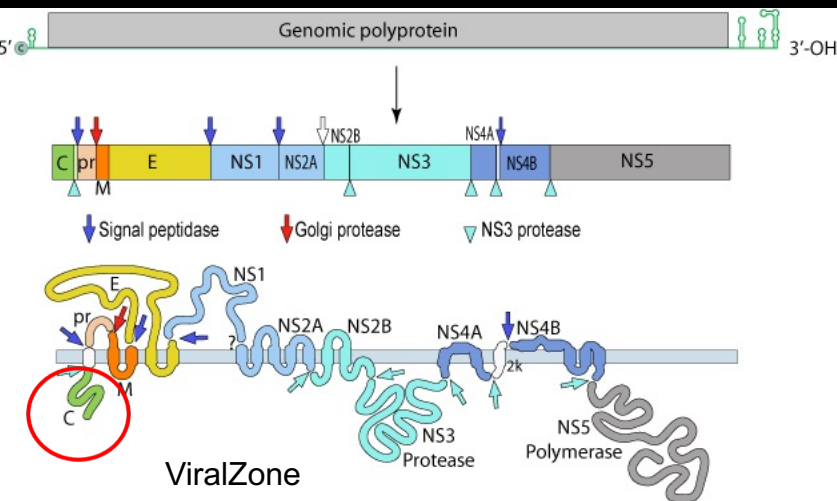
Vol. 83, No. 13

Activated Ras/MEK Inhibits the Antiviral Response of Alpha Interferon by Reducing STAT2 Levels^{▽†}

Sherri L. Christian, Thaddeus W. Collier, Dong Zu, Maria Licursi,
Chris M. Hough, and Kensuke Hirasawa*

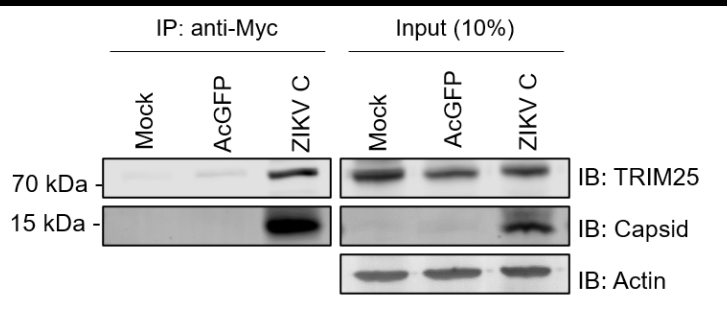
Division of BioMedical Sciences, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Canada

Flavivirus capsids downregulate the IFN response

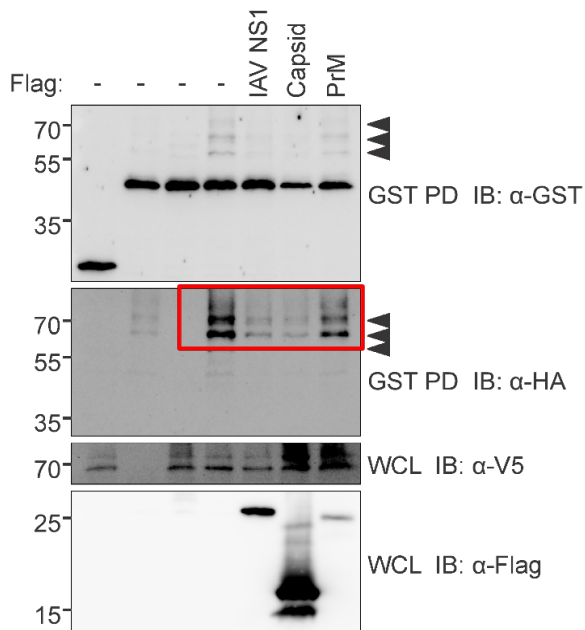


Airo et al, 2022

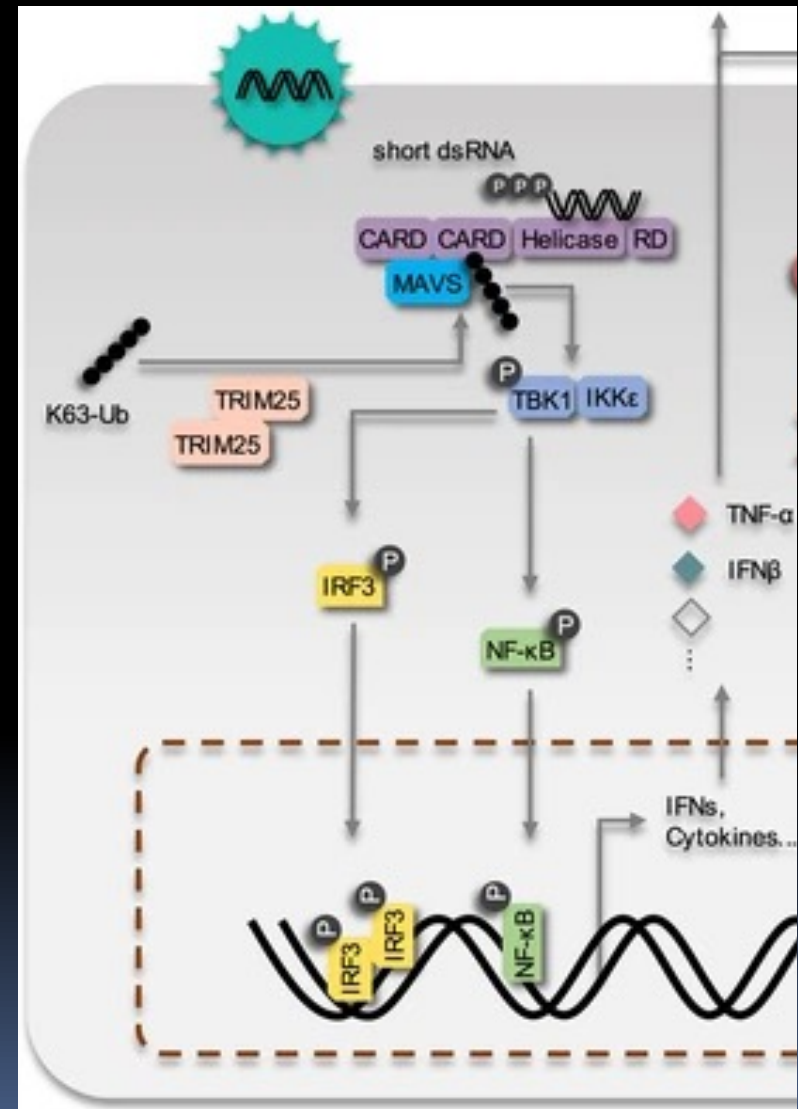
ZIKV capsid inhibits RIG-I ubiquitination by TRIM25



GST:	+	-	-	-	-	-	-
GST-h2CARD:	-	+	+	+	+	+	+
hTRIM25-V5:	+	-	+	+	+	+	+
HA-Ub:	-	+	-	+	+	+	+



Airo et al, 2022



Yang et al, 2021

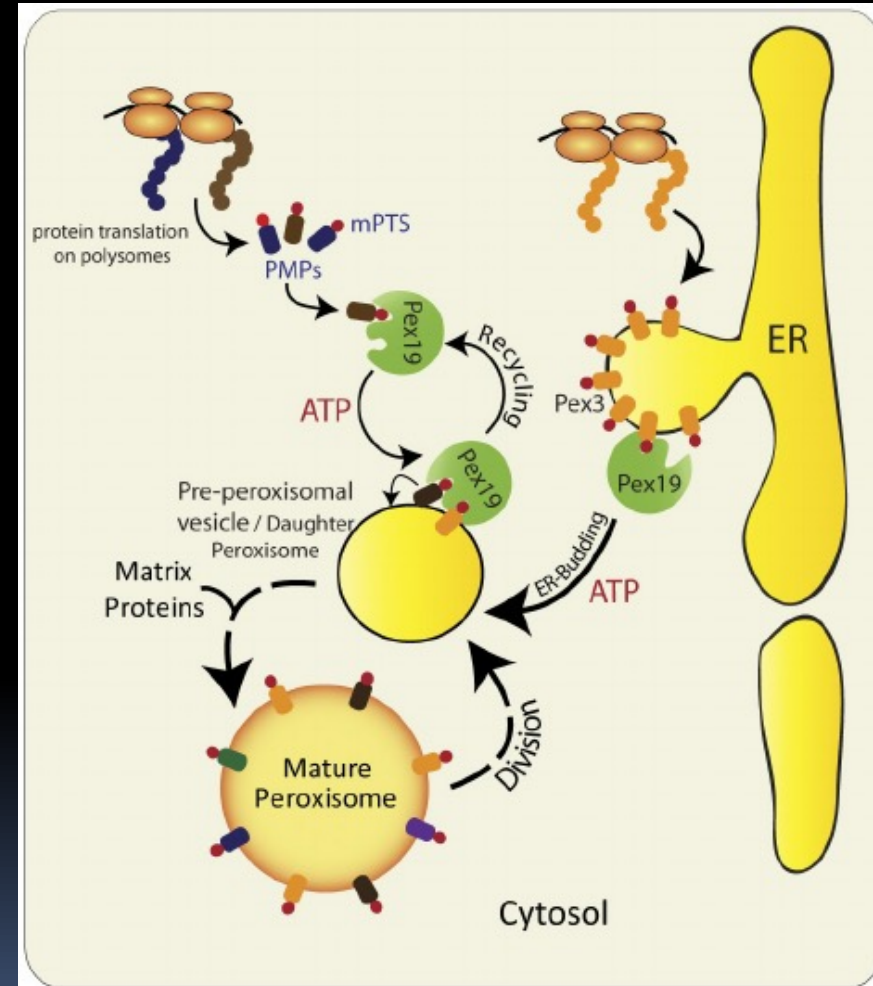
Flaviviruses use many strategies to inhibit IFN response

- Non-structural proteins block IFN induction and signaling by targeting $\text{NF}\kappa\text{B}$, IRF3 and STAT2
 - Mechanisms known in many cases
- Upregulated FGF signaling blocks IFN induction and ISG expression
 - MEK-dependent pathway
- Capsid proteins block IFN induction
 - Inhibits TRIM25-mediated activation of RIG-I
 - Conserved mechanism among flaviviruses
 - Occurs before NS proteins act?
- Others?

Conserved interactions between flavivirus capsid proteins and host proteins

	FLAG-DENV				FLAG-WNV				FLAG-UL137			
	Method 1		Method 2		Method 1		Method 2		Method 1		Method 2	
	UP	SC	UP	SC	UP	SC	UP	SC	UP	SC	UP	SC
DENV	1	1	4	17	0	0	0	0	0	0	0	0
WNV	0	0	0	0	1	1	5	6	0	0	0	0
Pex 19	3	4	5	9	5	5	6	8	0	0	0	0

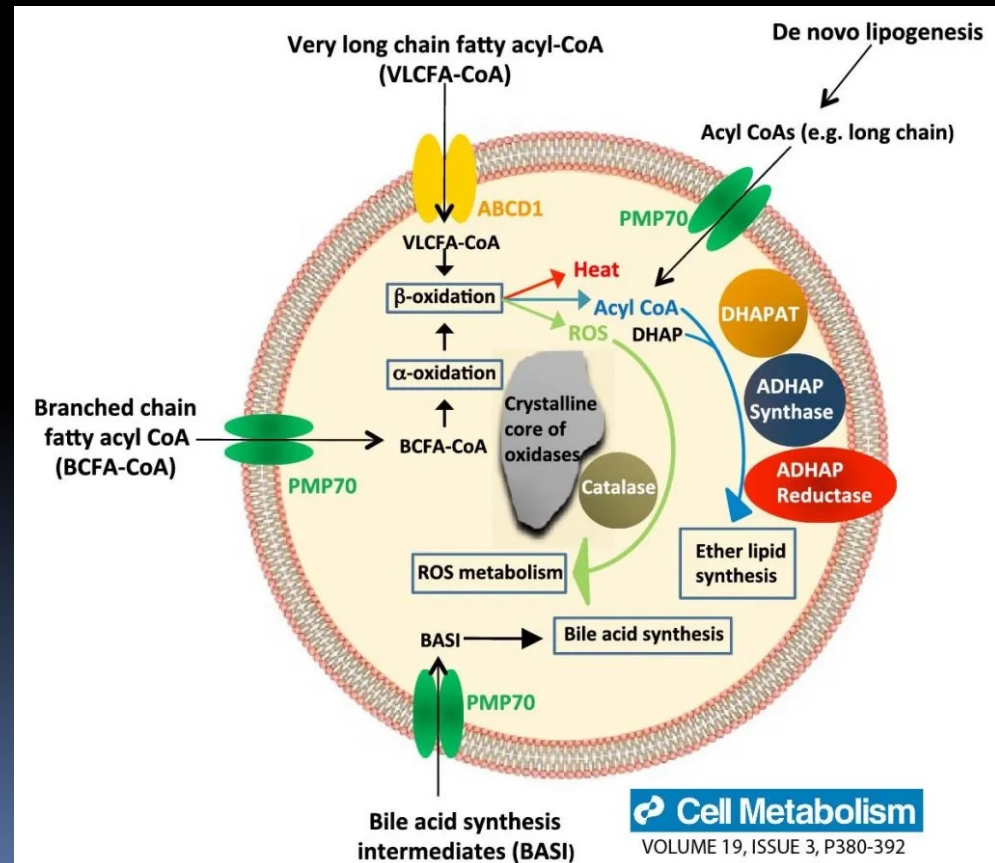
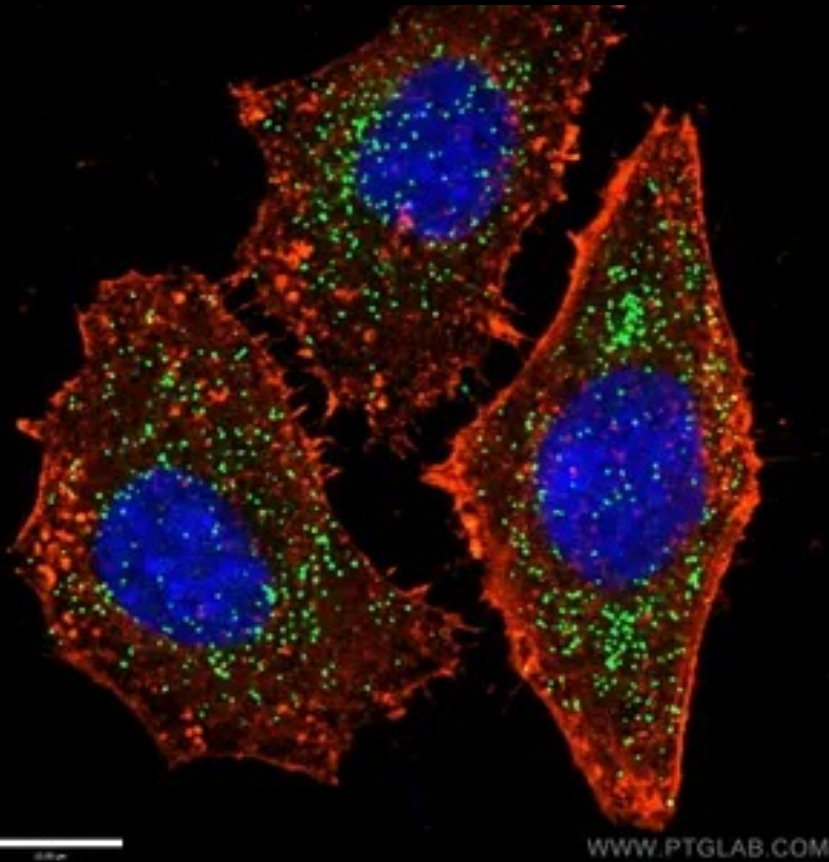
You, Hou et al, 2015



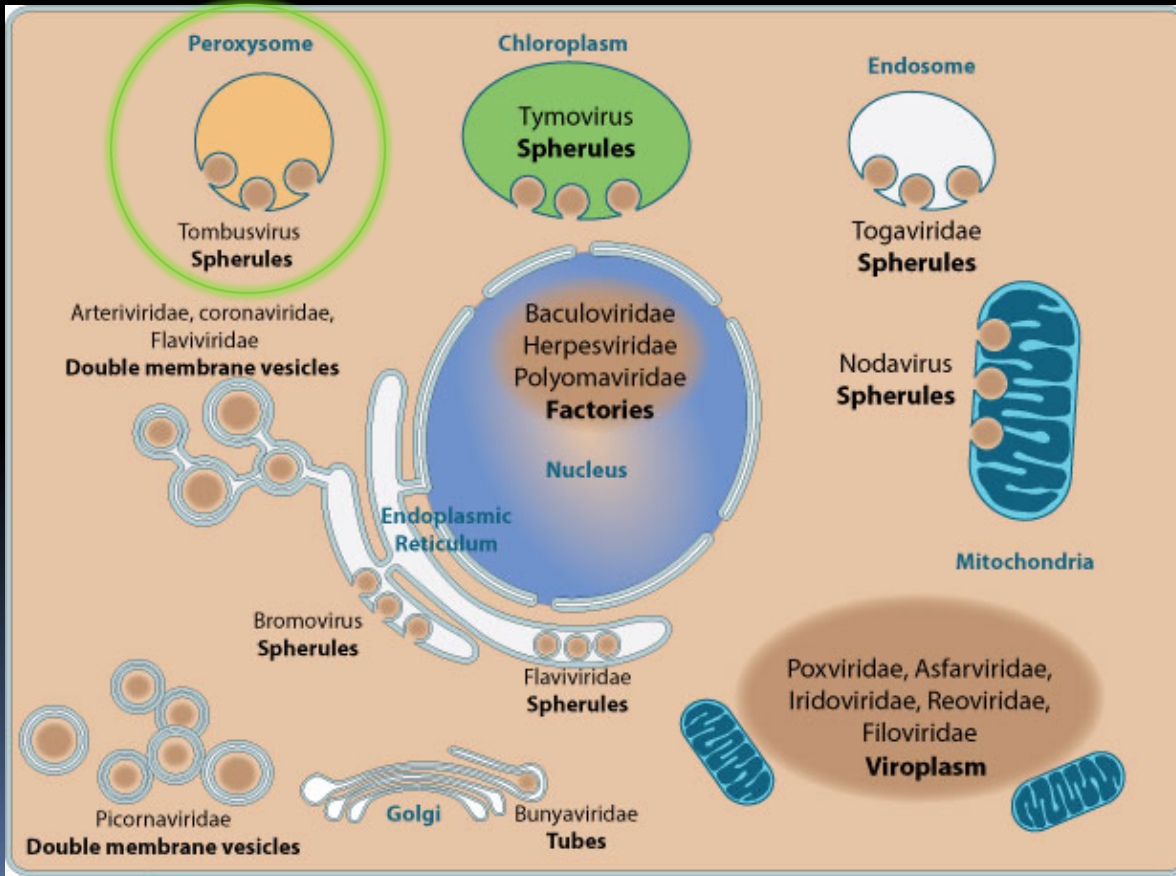
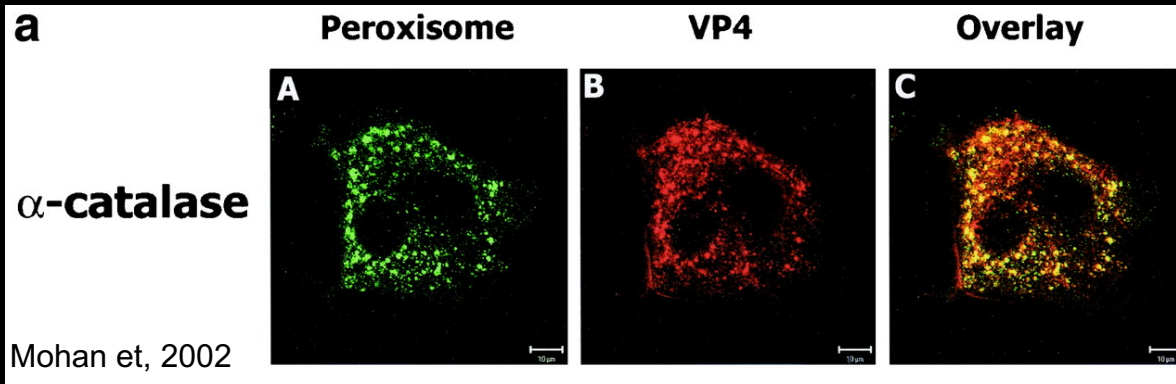
Ma et al, 2011

Peroxisomes are membranous organelles that:

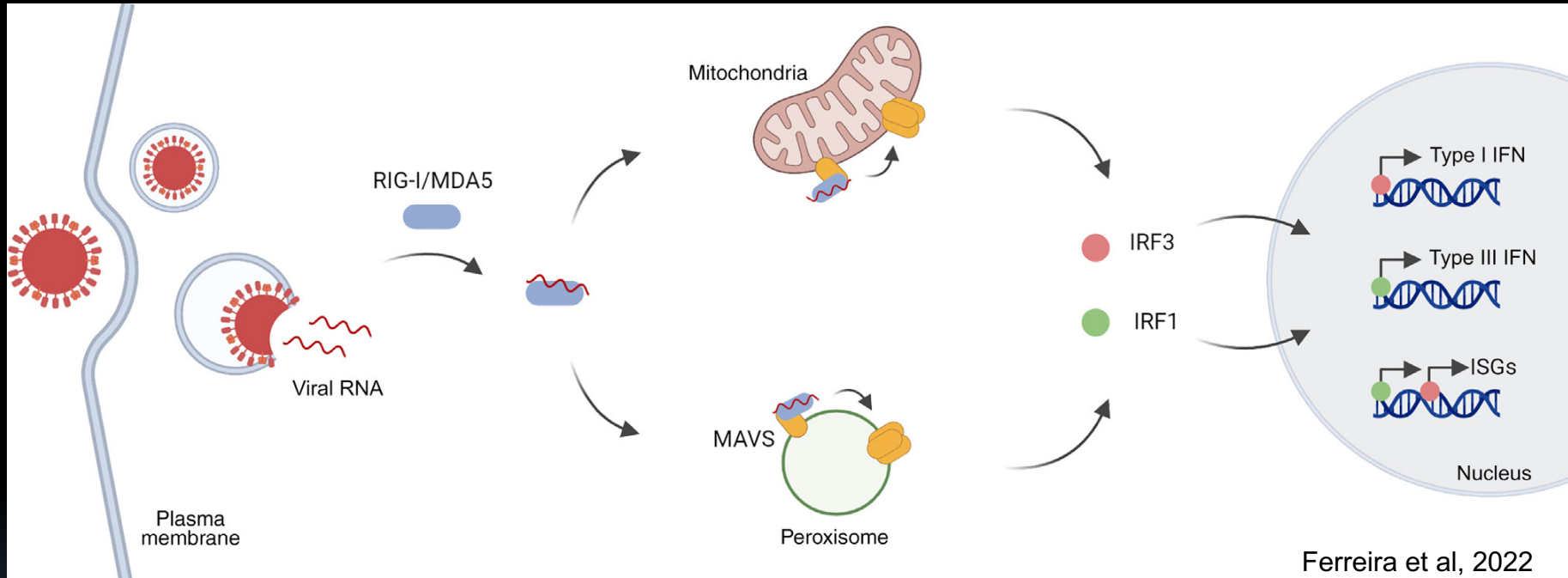
- Catabolize very long chain fatty acids
- Regulate reactive oxygen species
- Are sites of biosynthesis for specialized phospholipids
- Control inflammation



Knowledge gap regarding viruses and peroxisomes



Peroxisomes are Antiviral Signaling Platforms

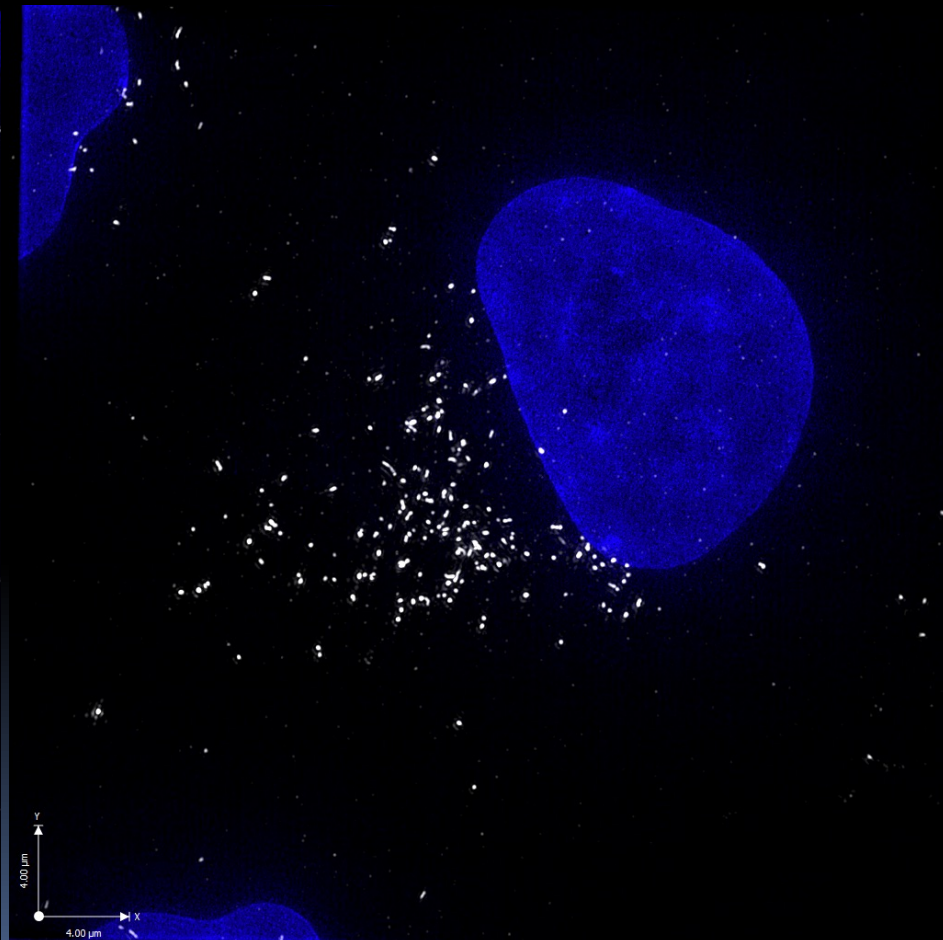
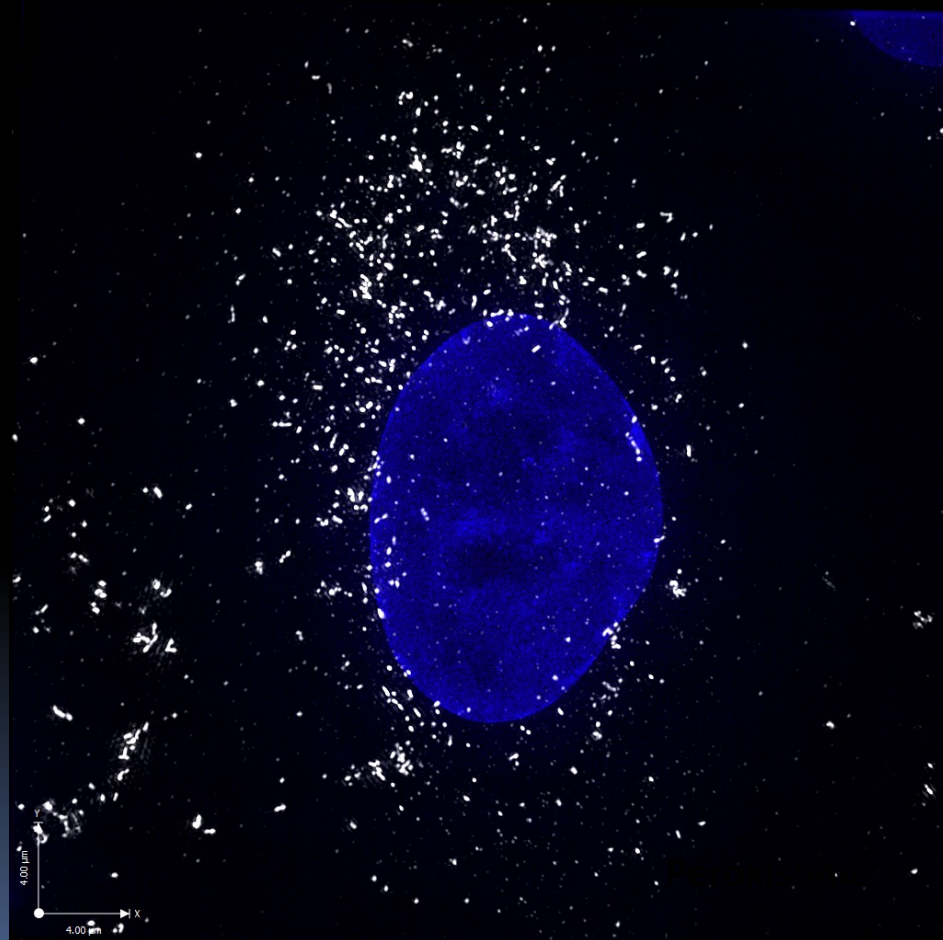


What happens to peroxisomes
during viral infection?

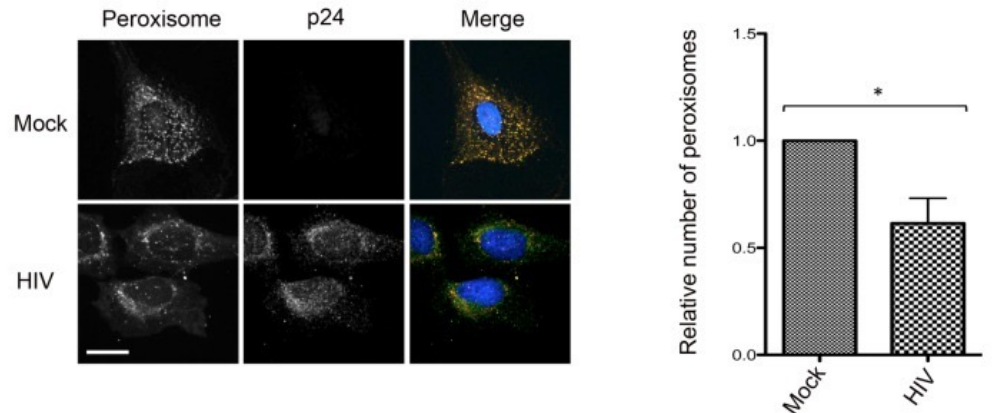
Flavivirus infection results in loss of peroxisomes

Mock

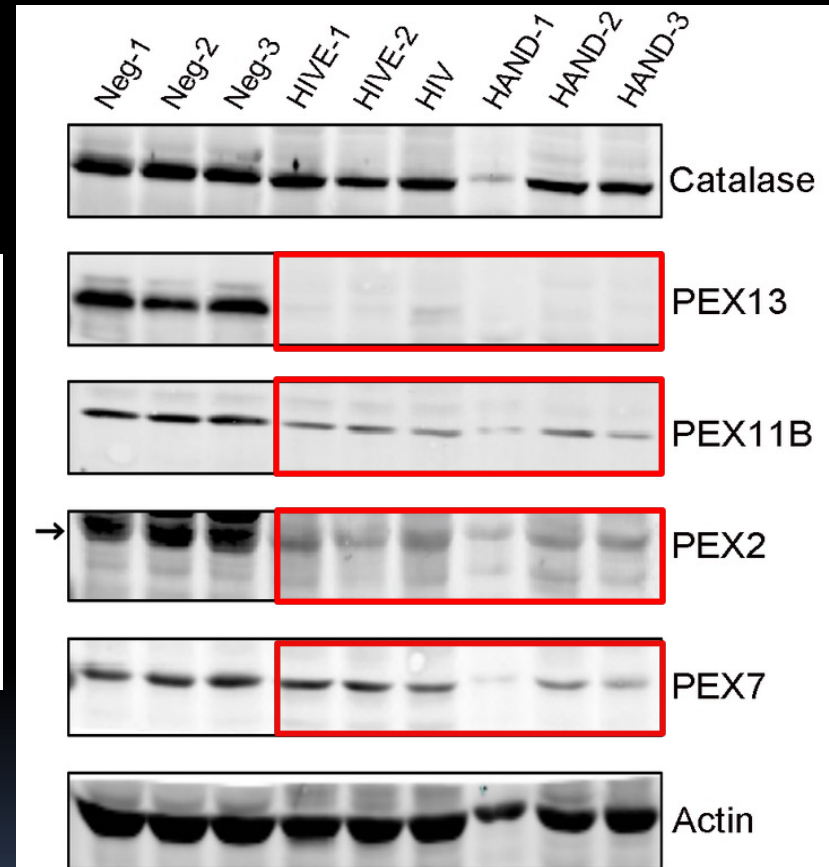
WNV



HIV-1 infection also depletes peroxisomes



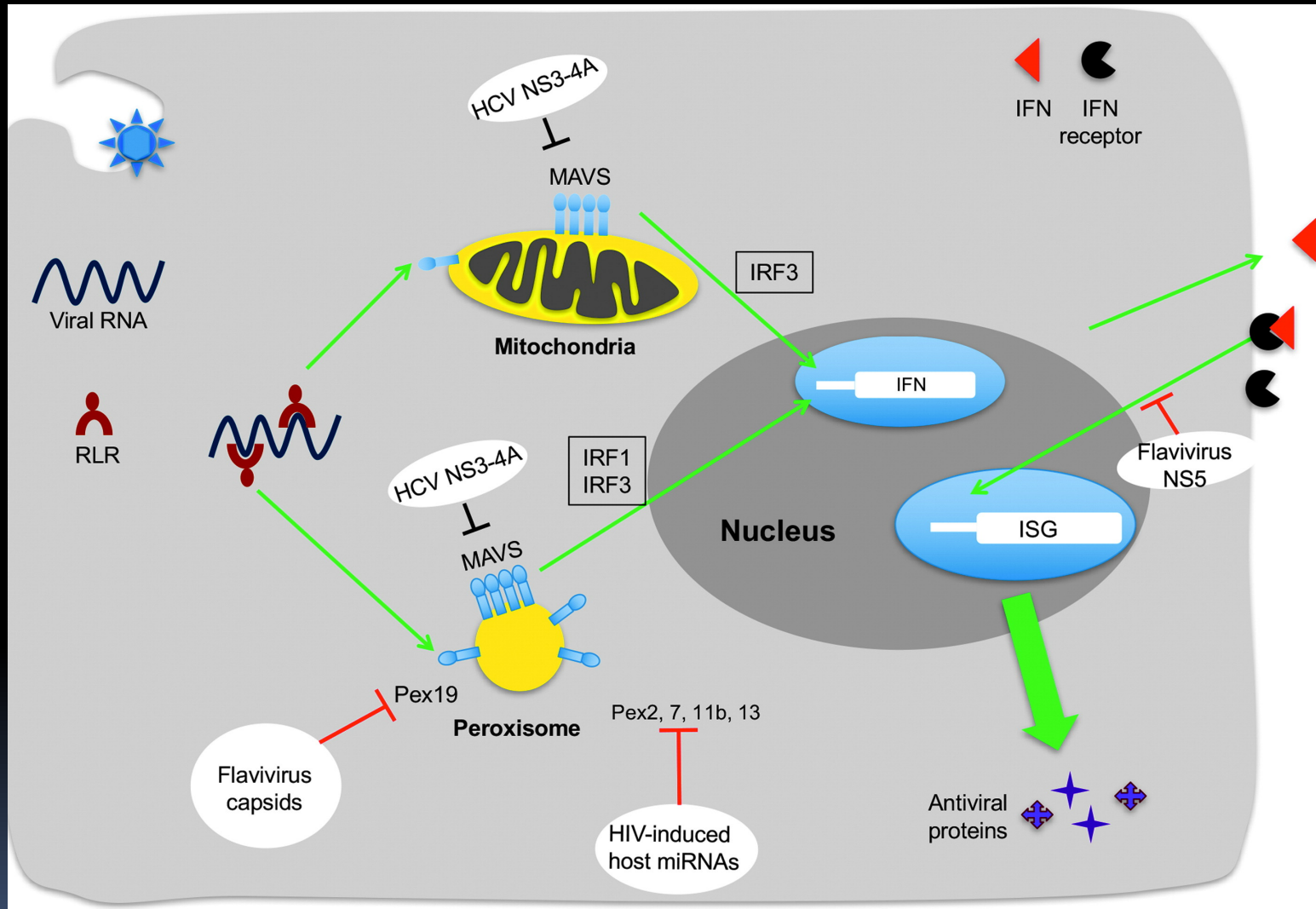
Xu *et al*, 2017



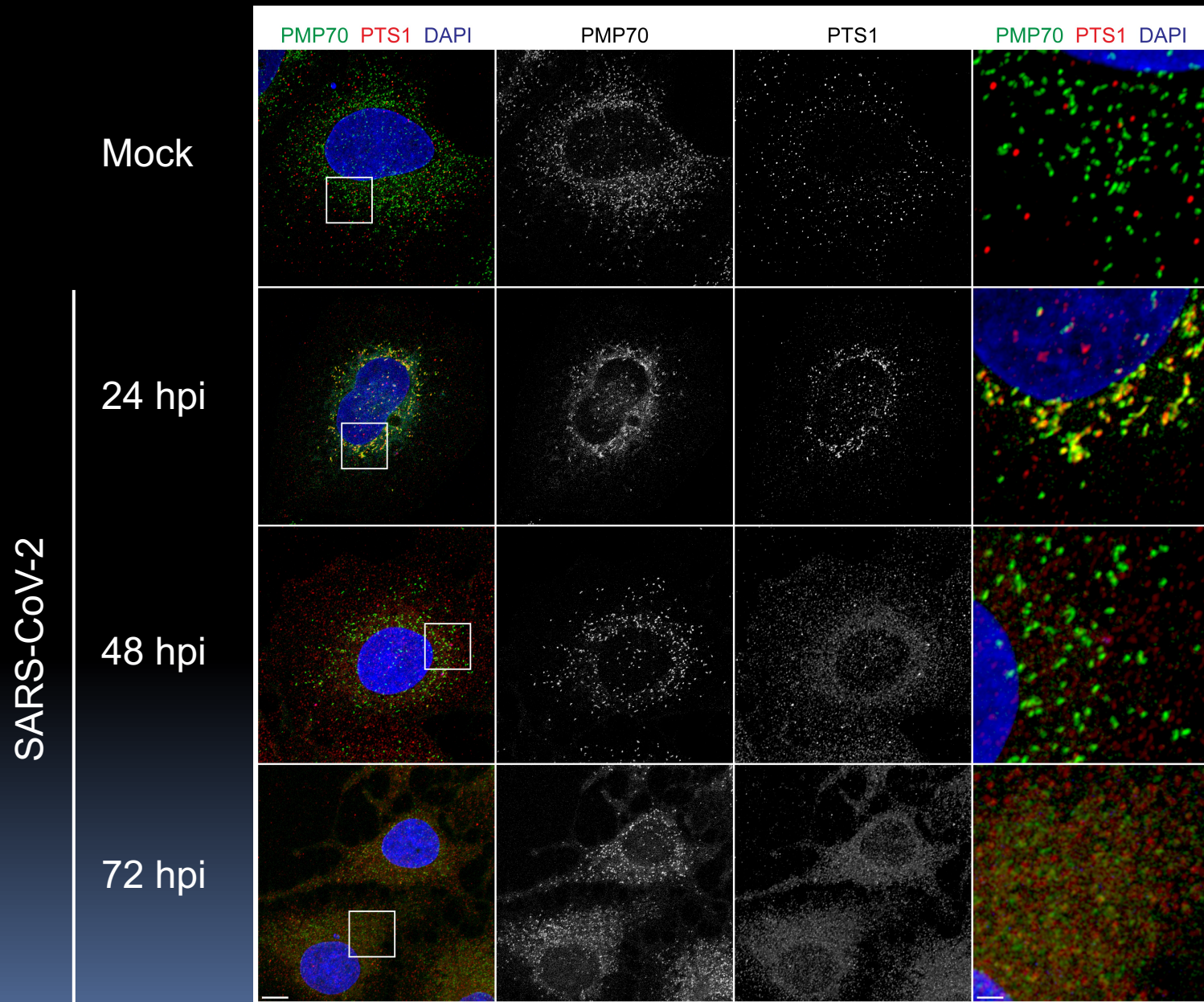
HIVE-HIV with encephalitis

HAND-HIV-Associated Neurocognitive Disorder

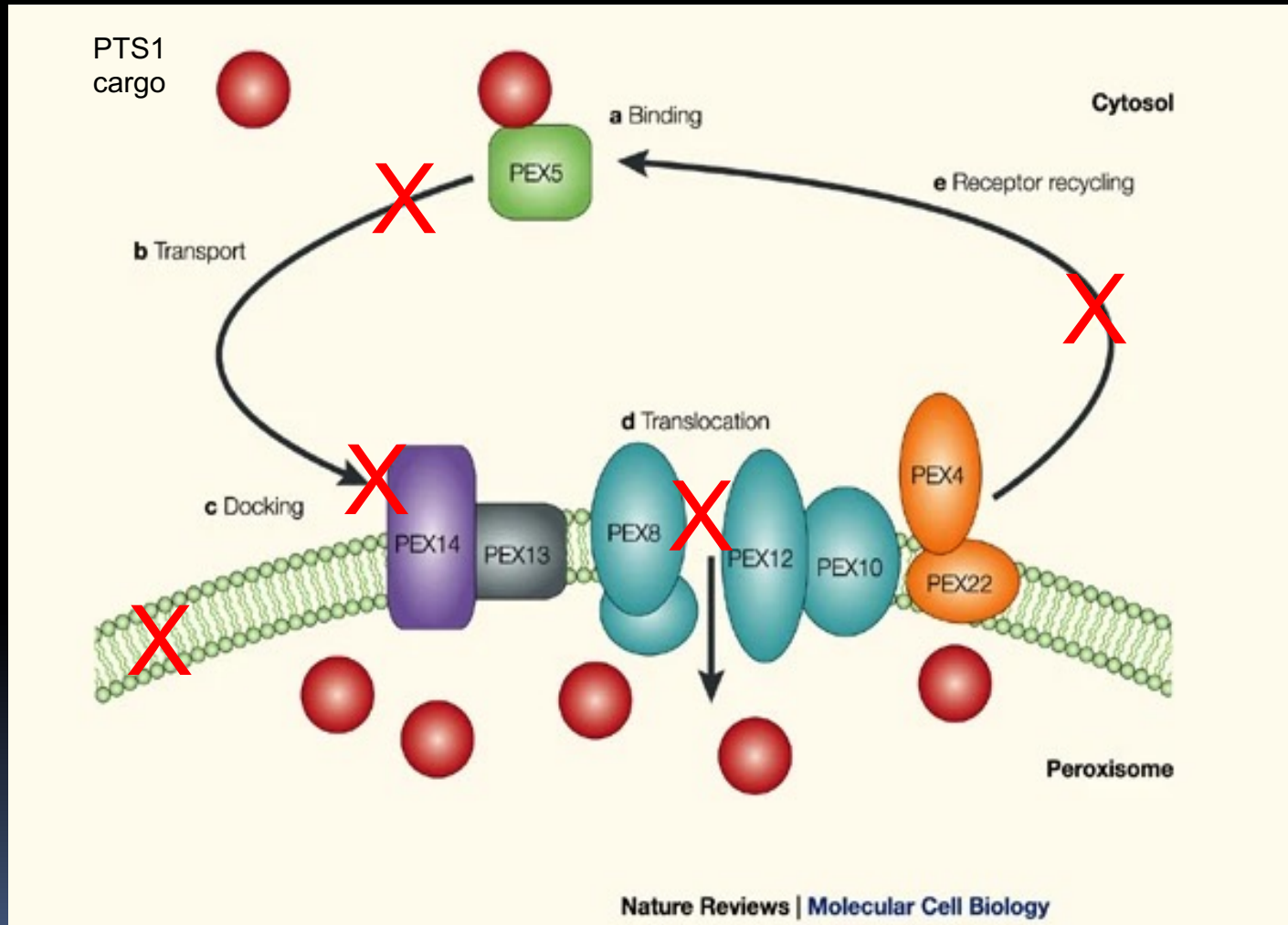
Different mechanisms, same result....



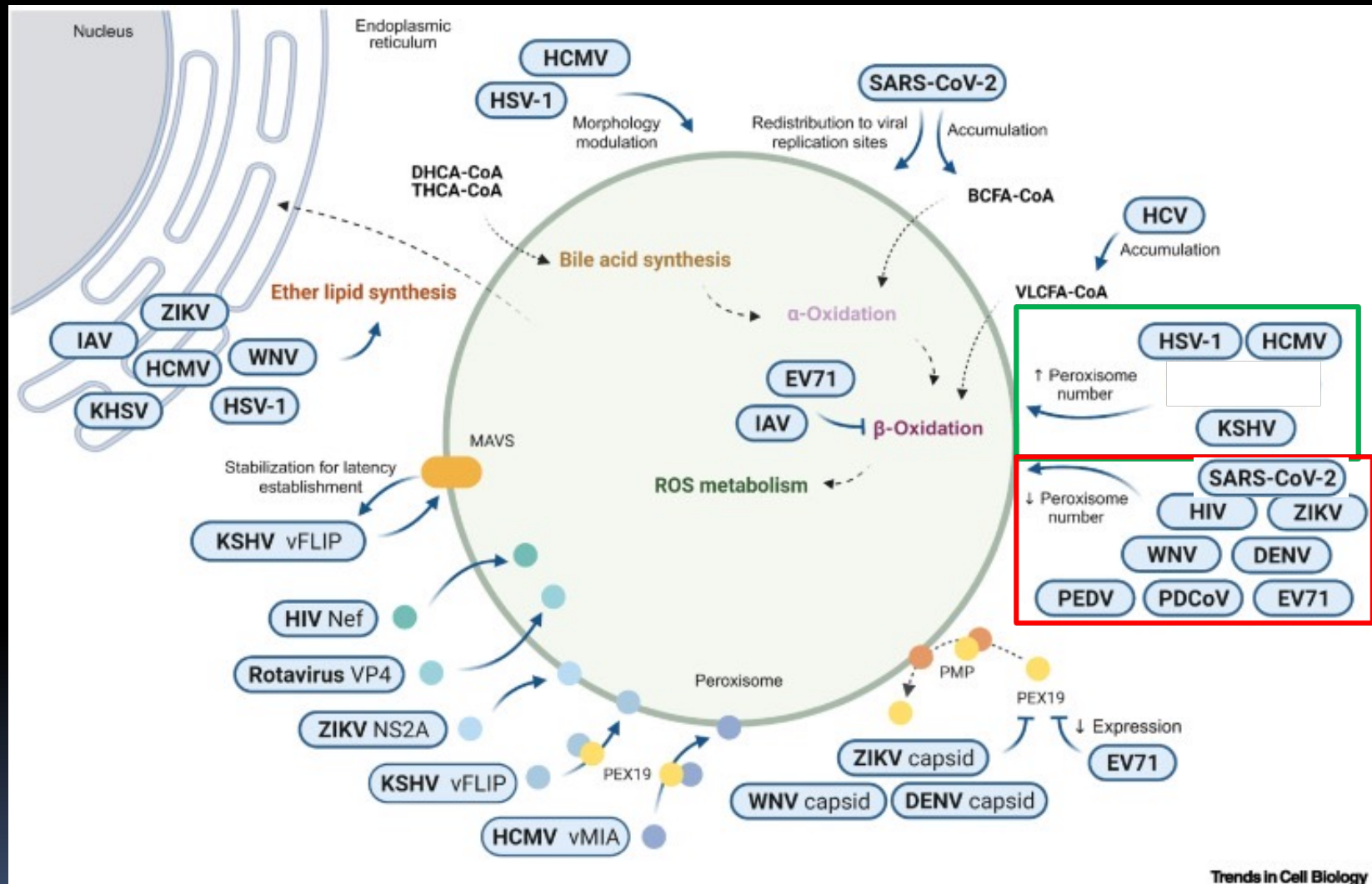
SARS-CoV-2 infection depletes functional peroxisomes



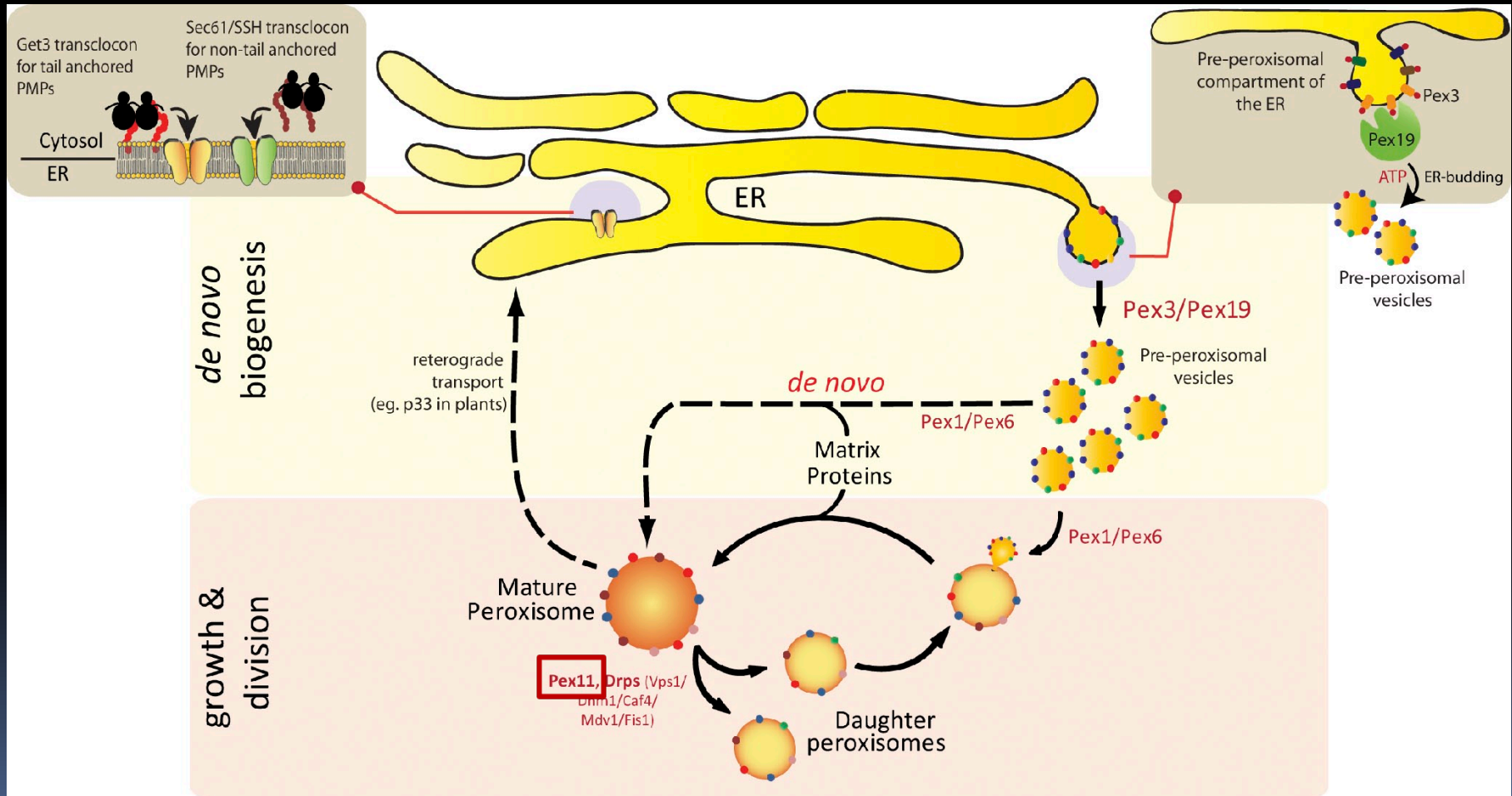
SARS-CoV-2 infection impairs import of matrix proteins



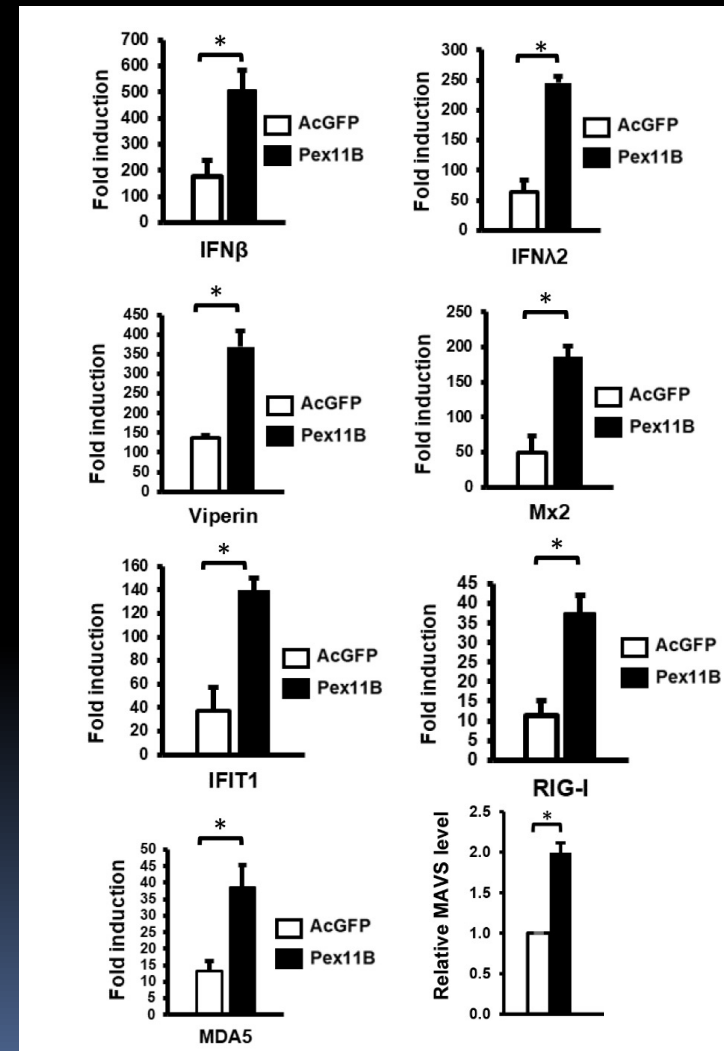
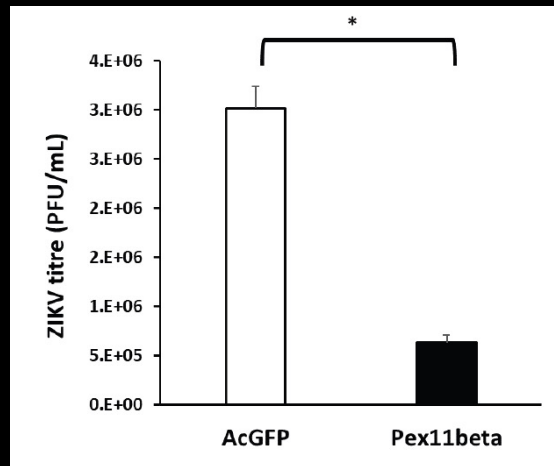
Some viruses upregulate peroxisomes



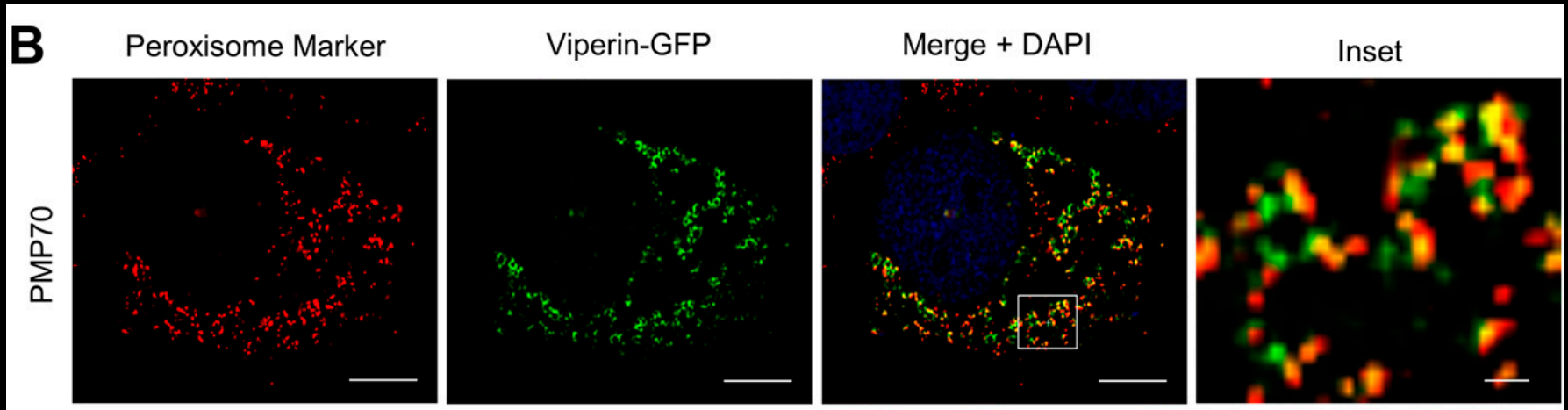
Peroxisomes pool is maintained by 2 main pathways



Genetic induction of peroxisomes by Pex11 inhibits ZIKV replication by enhancing IFN response



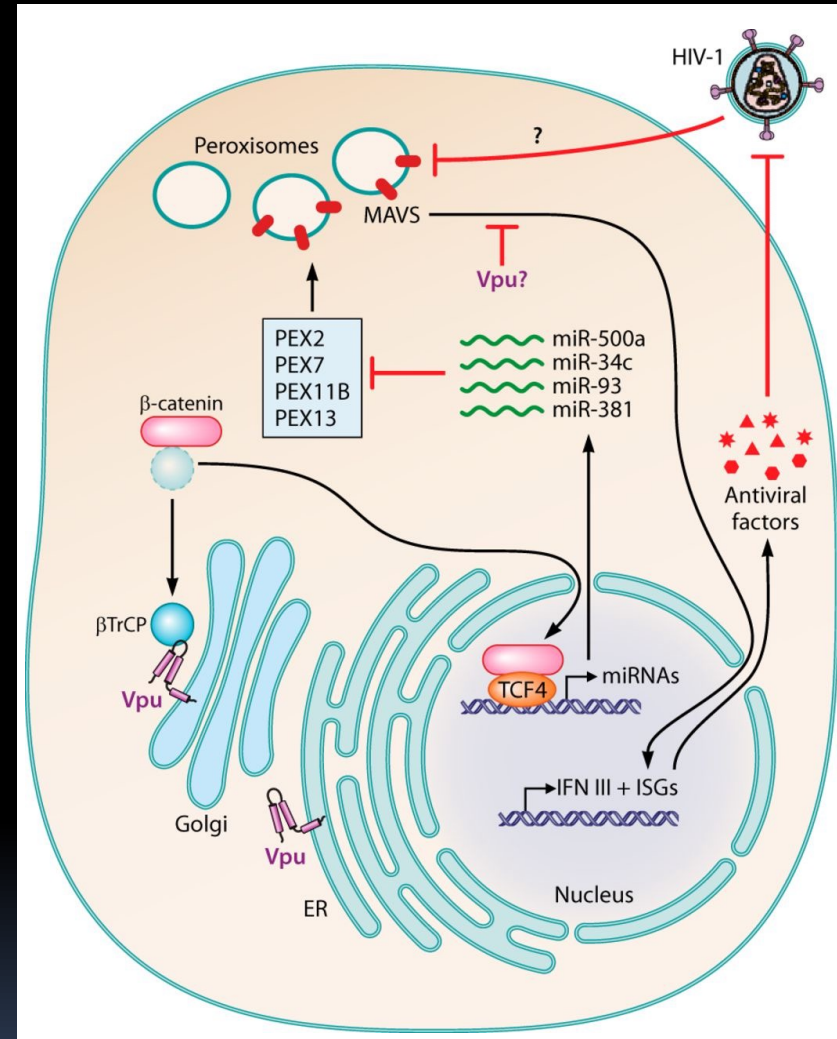
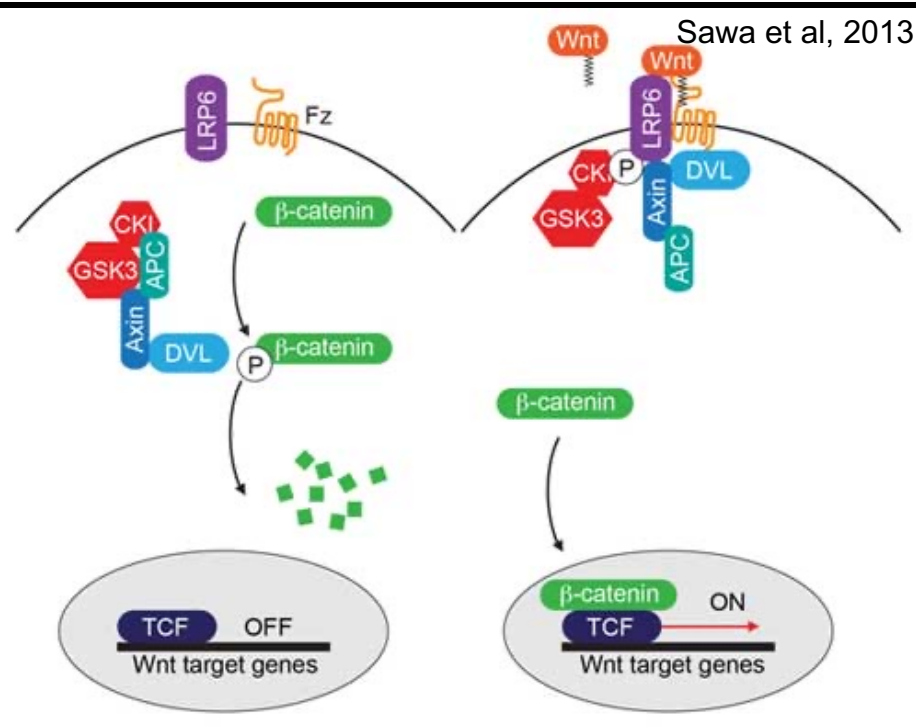
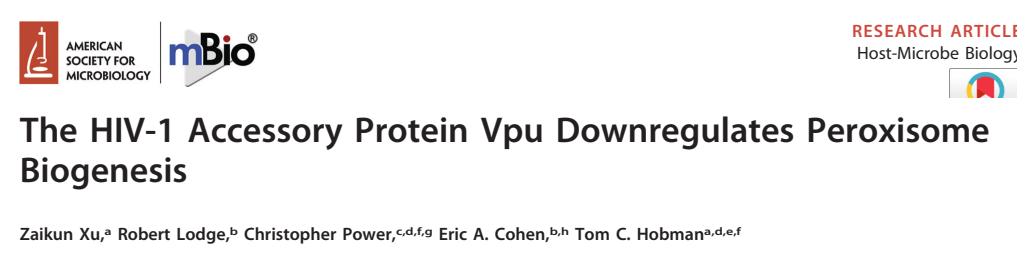
The ISG Viperin associates with peroxisomes



Khantisitthiporn et al, 2021

- Potentiates the innate antiviral response
- Positive feedback mechanism?
- Positioning peroxisomes near the mitochondrial/MAM MAVS signaling synapse?

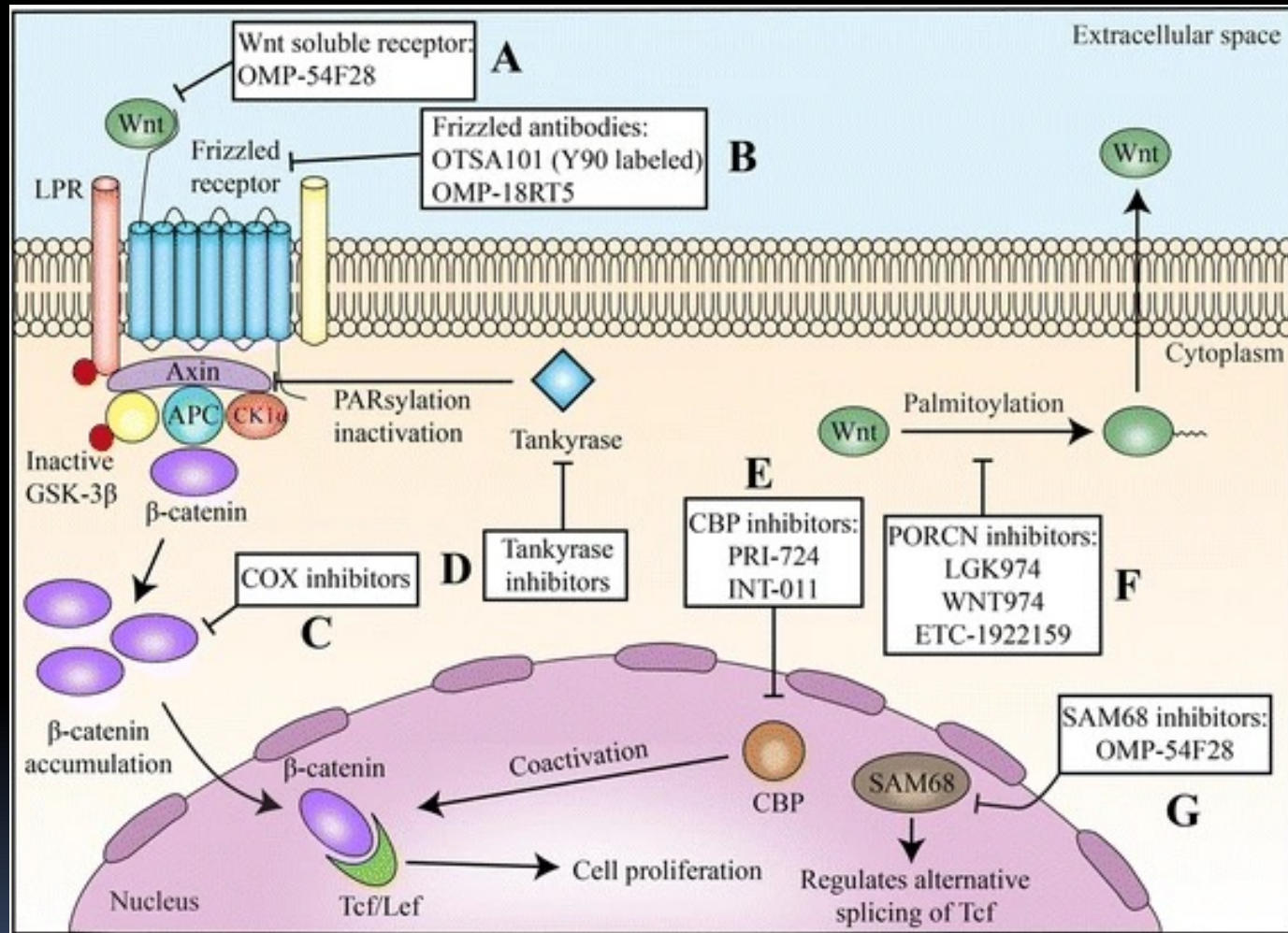
The power and serendipity of collaboration.....



Hopfensperger et al, 2020

So what?

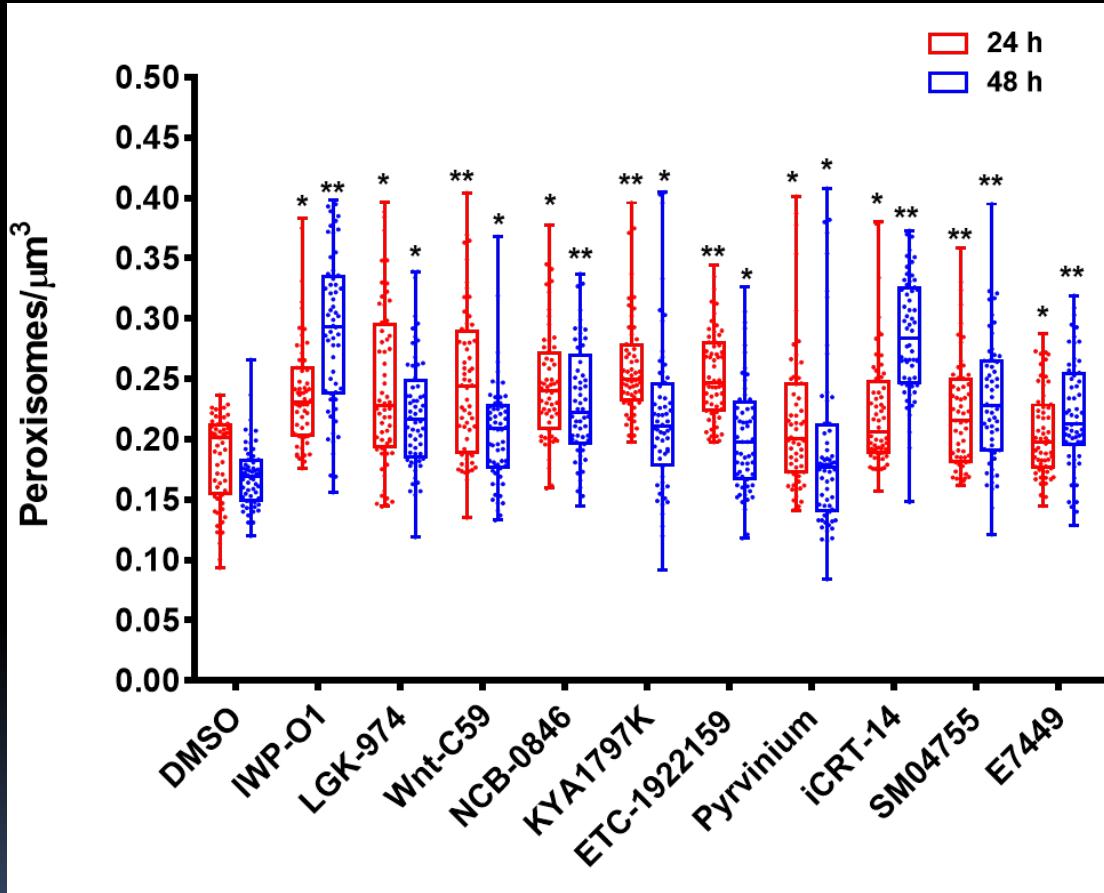
Lots of drugs that inhibit Wnt signaling pathway



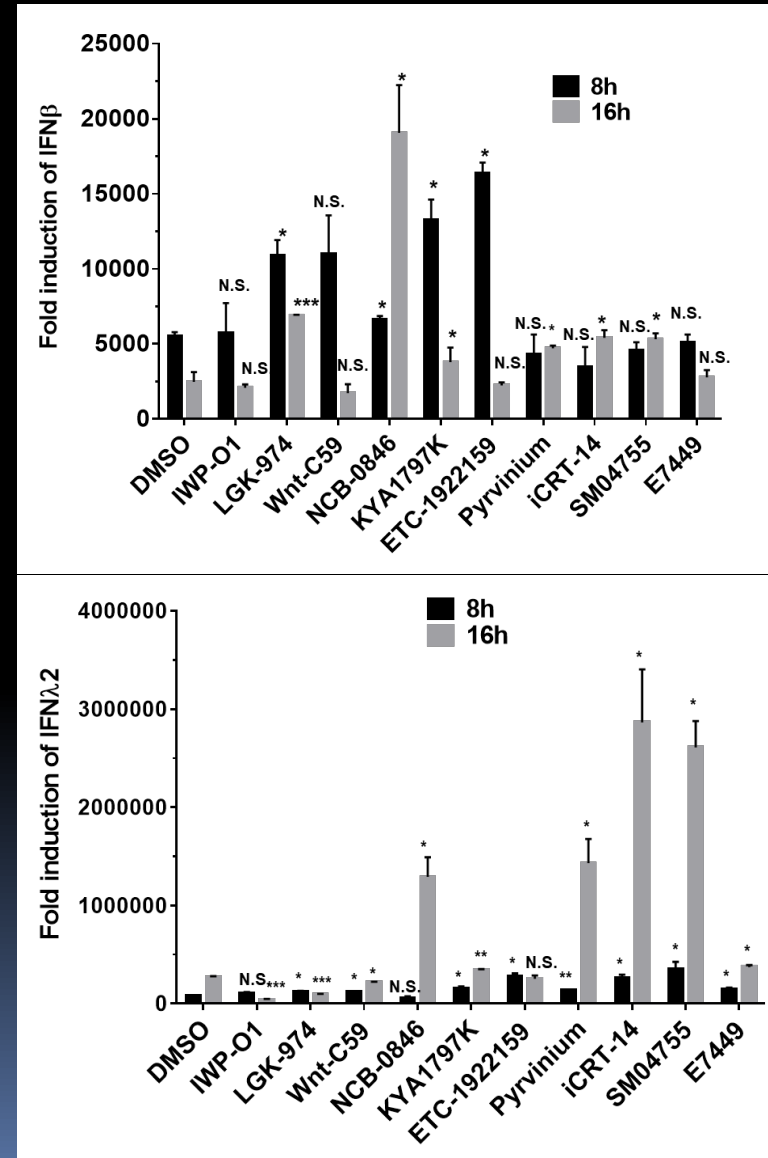
Pai et al, 2017

Do these types of drugs induce peroxisomes??

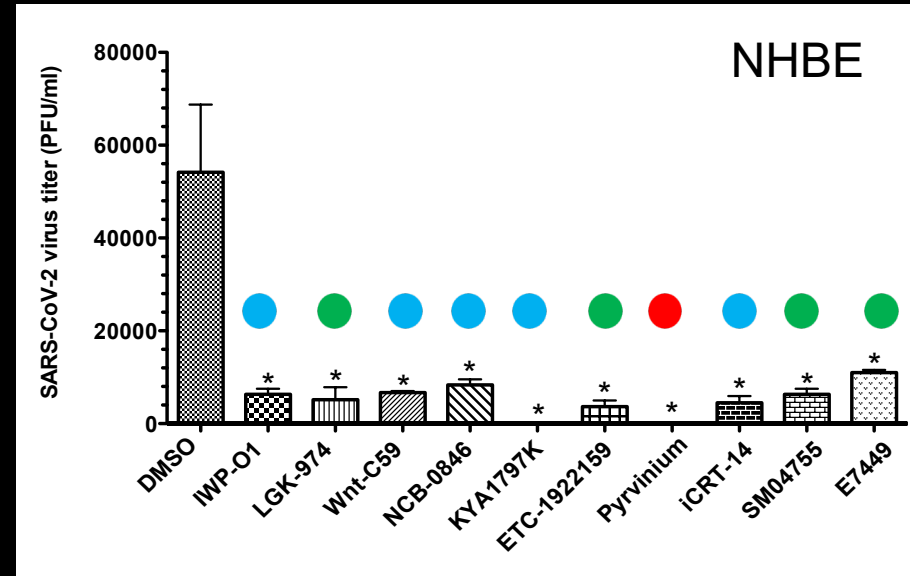
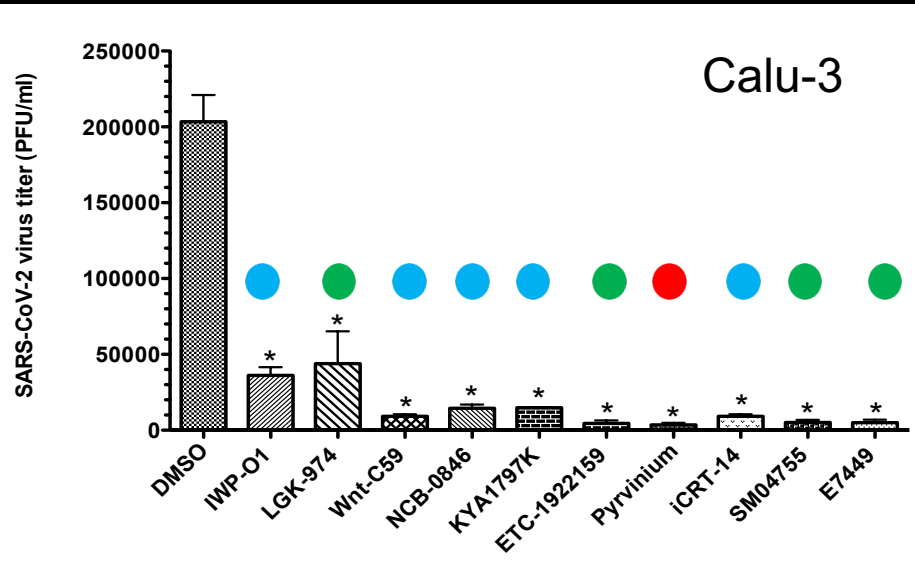
Wnt/ β -catenin inhibitors increase peroxisome density and potentiate the IFN response during viral infection



Xu, Wong et al, in preparation



Wnt/ β -catenin inhibitors inhibit SARS-CoV-2 replication in multiple cell types

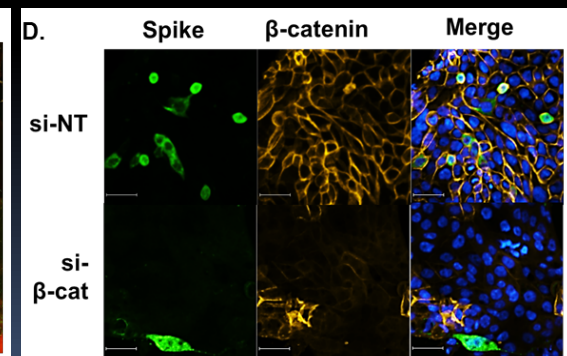
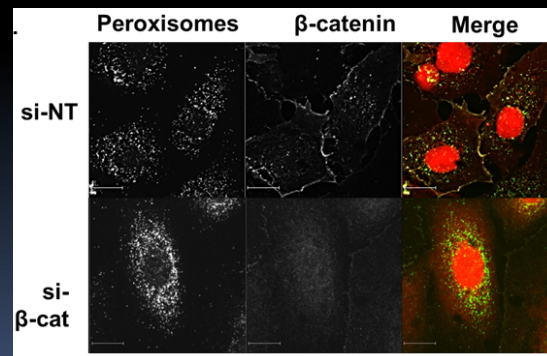
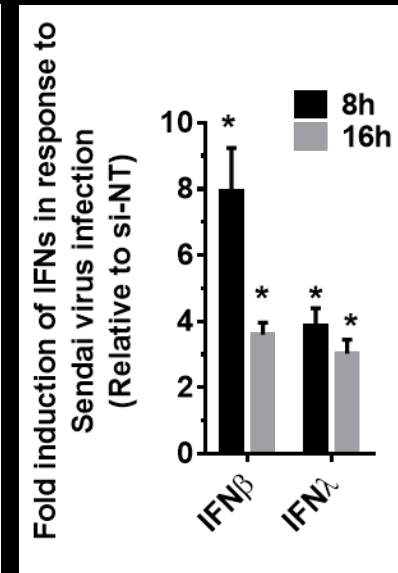
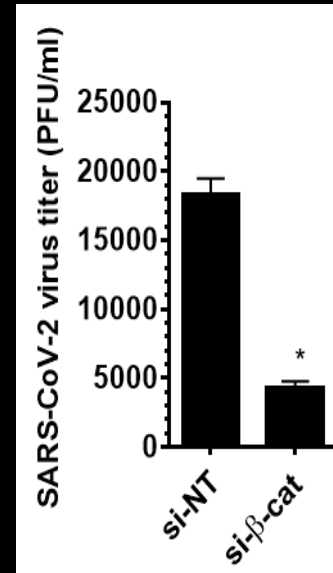
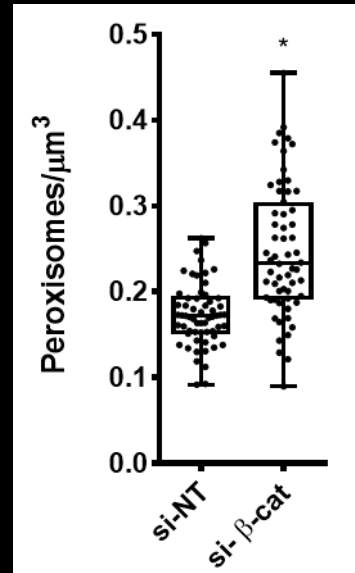
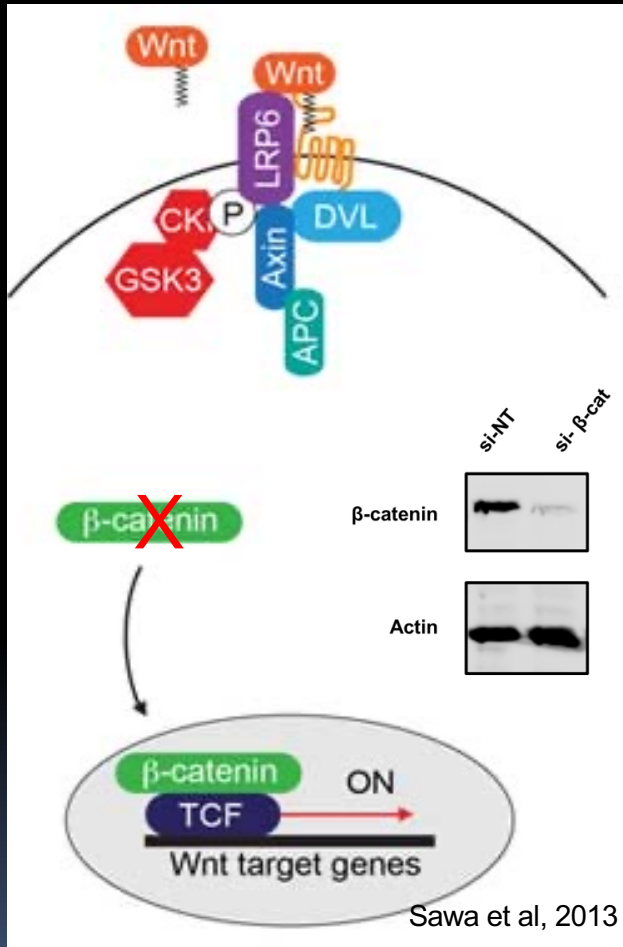


Xu, Wong et al, in preparation

- Licensed for human use
- Phase I or II
- Preclinical

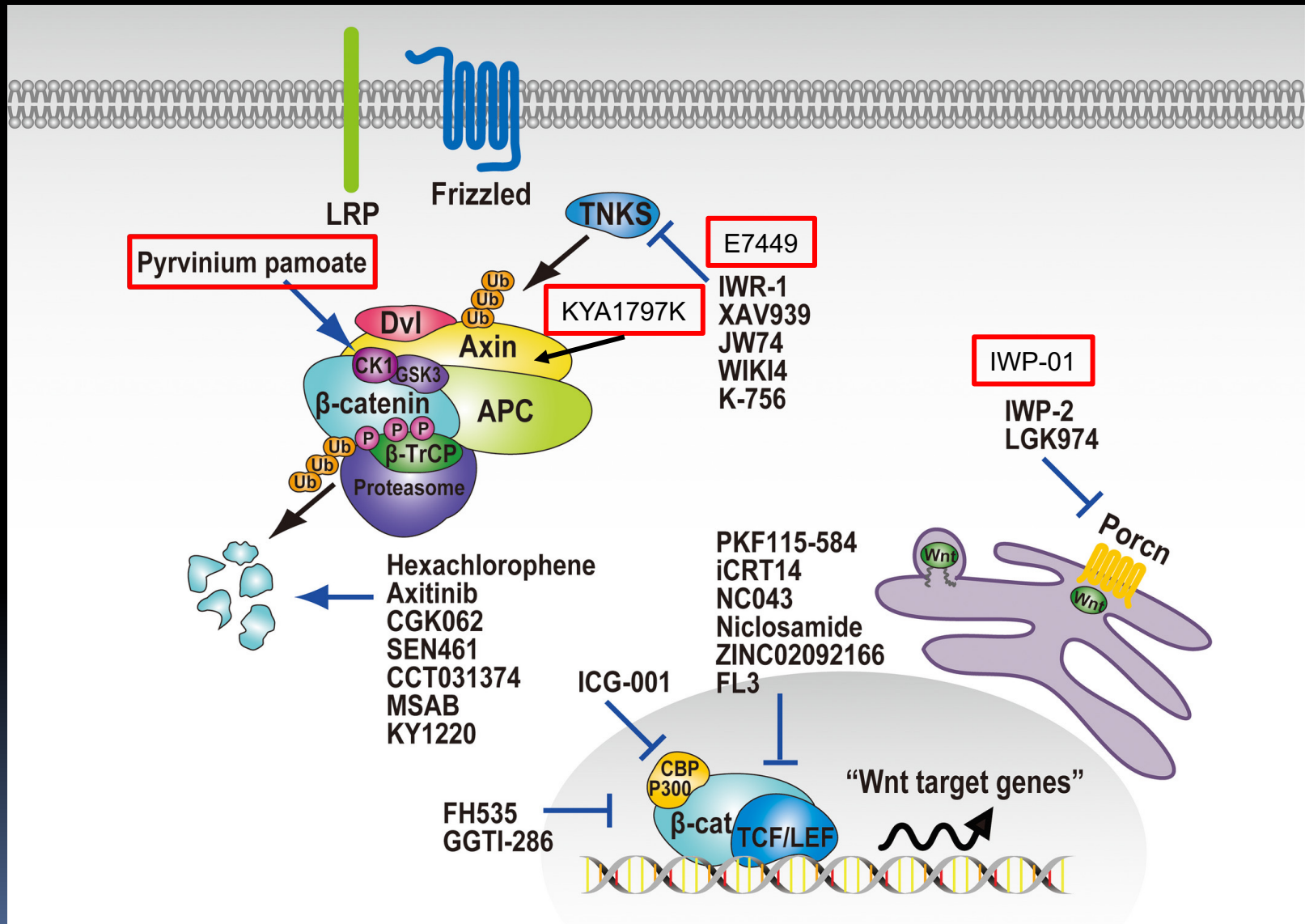
- No effect in Vero cells
- IFN requirement?

Reducing β -catenin levels induces peroxisome proliferation and enhances IFN response

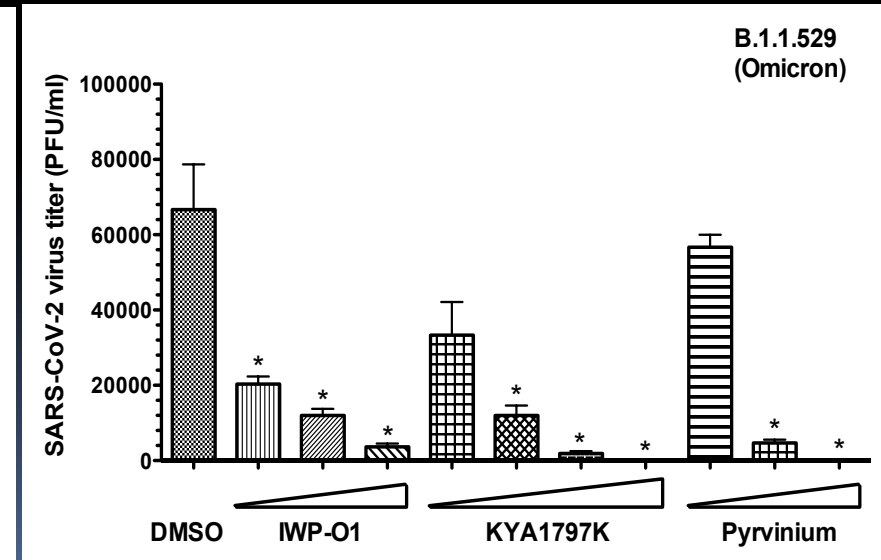
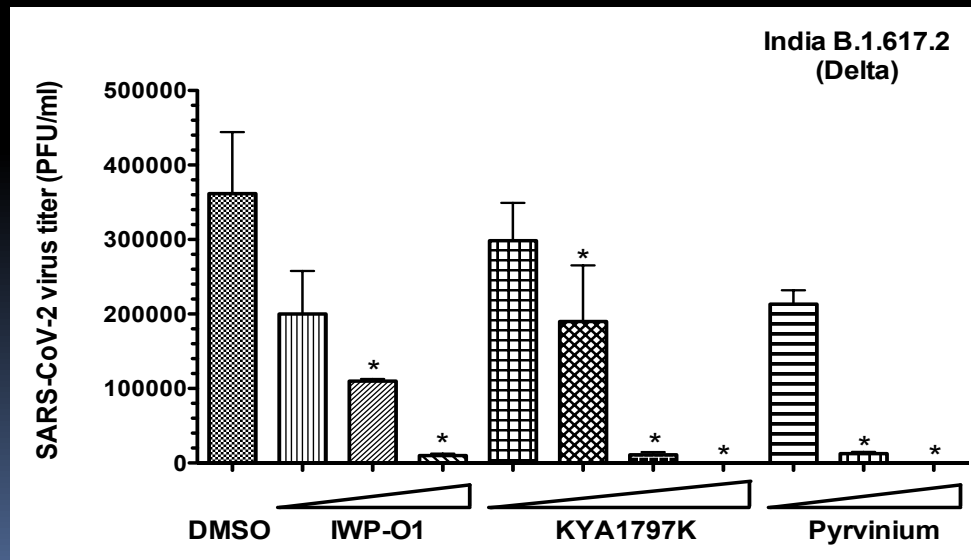
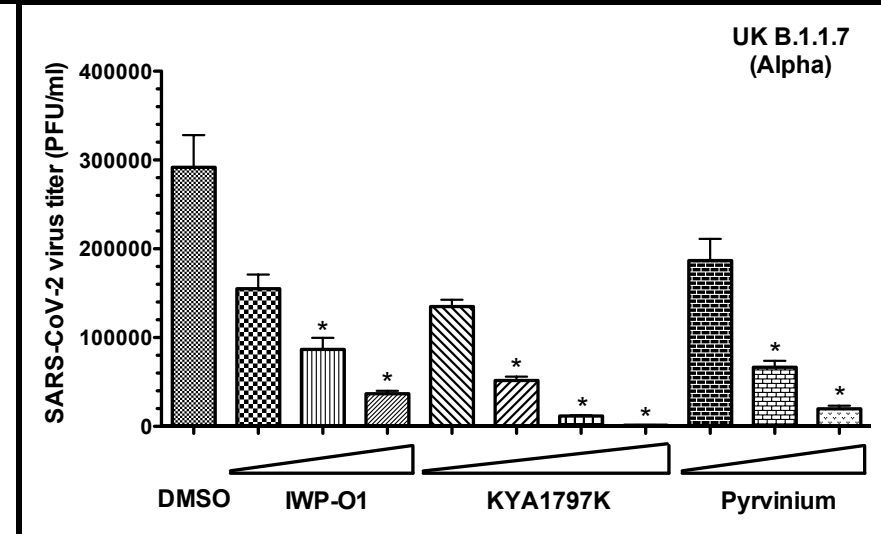
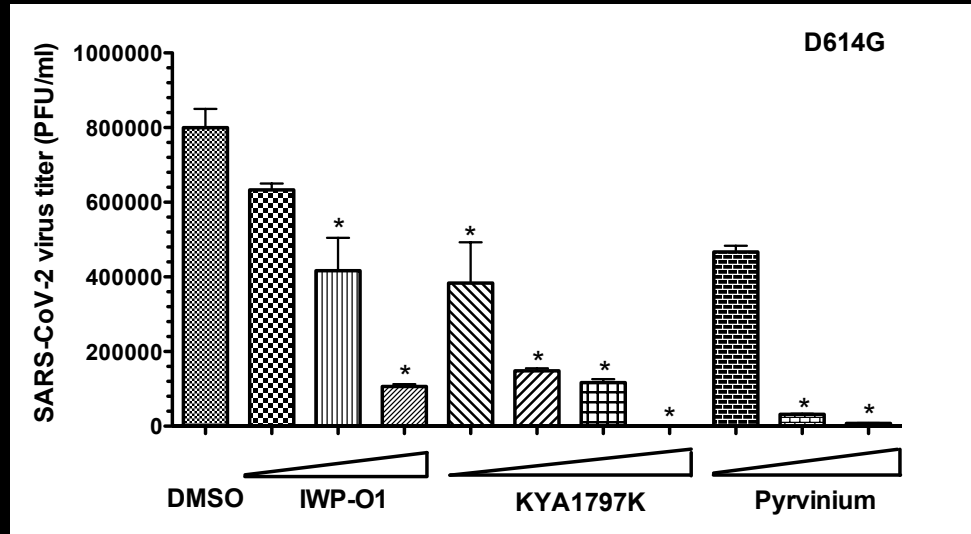


Xu, Wong et al, in preparation

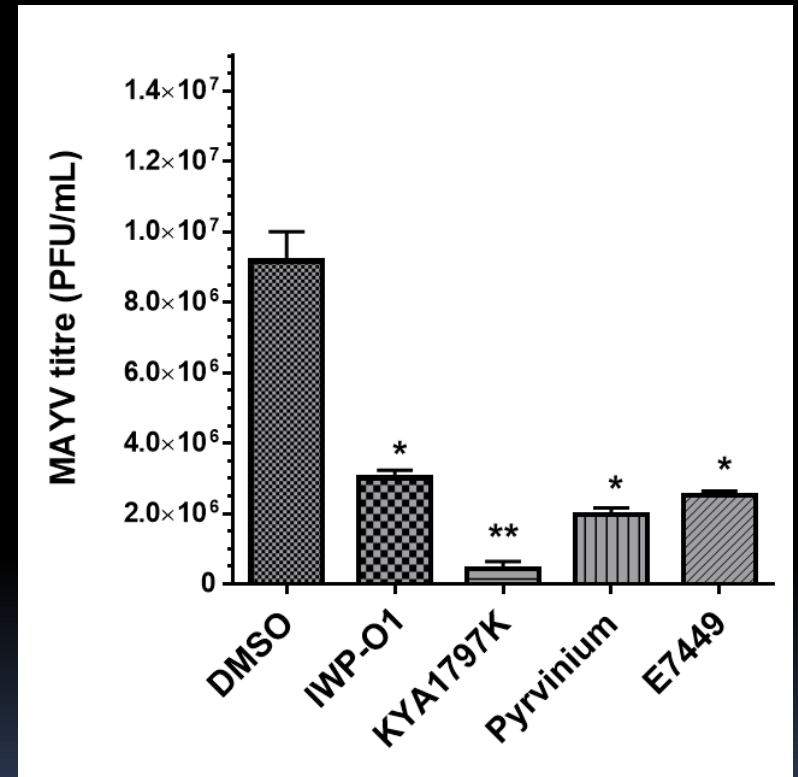
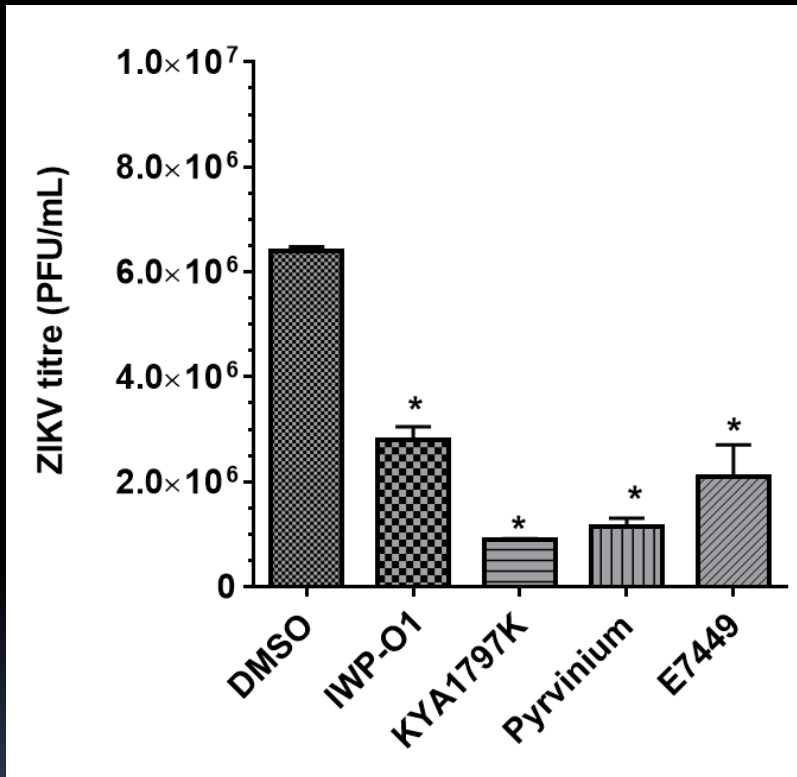
Drugs with high SIs chosen for testing against Variants of Concern and small animal studies



Peroxisome-modulating drugs are effective against SARS-CoV-2 Variants of Concern

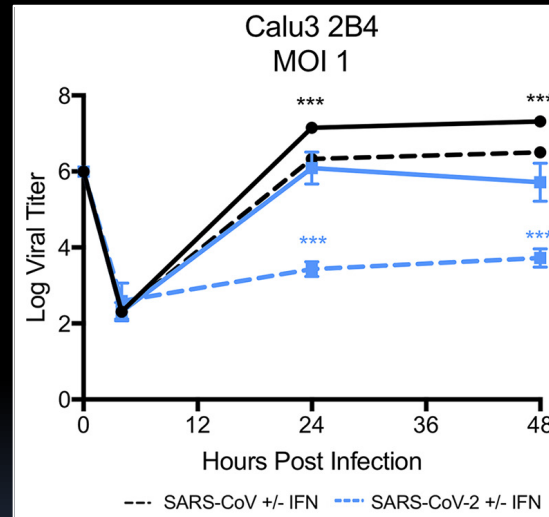
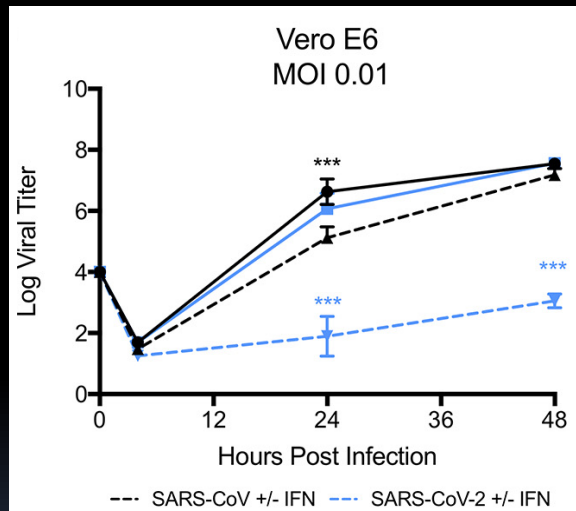


Peroxisome-inducing drugs reduce replication of other RNA viruses

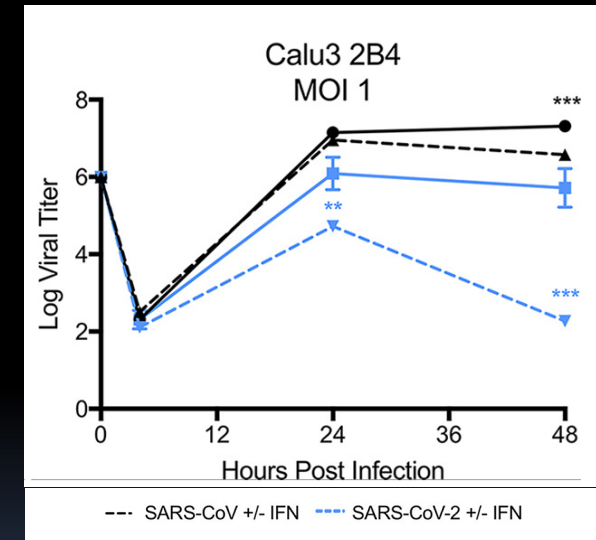


SARS-CoV-2 is highly sensitive to Interferon

IFN added pre-infection



IFN added post-infection

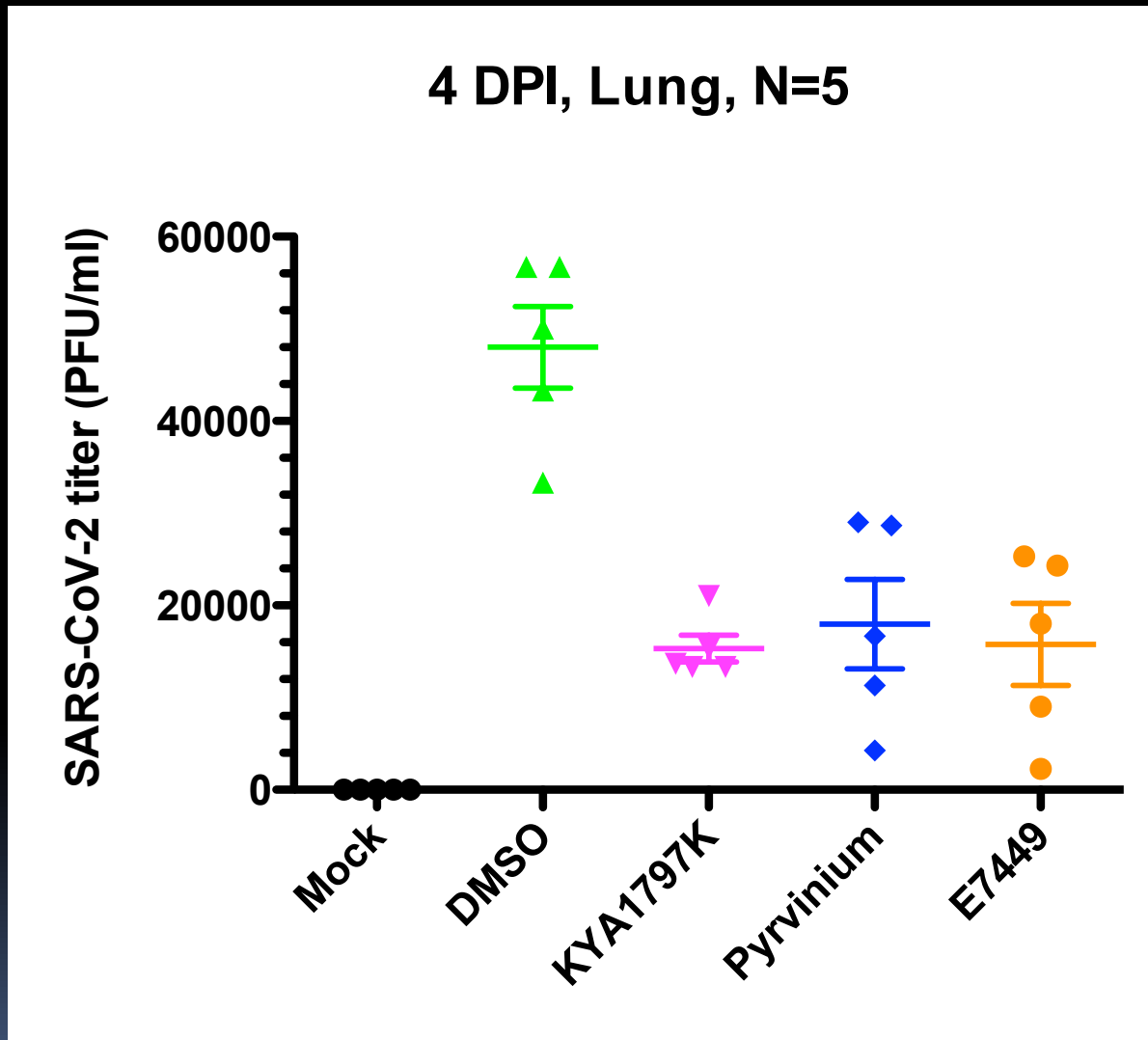


Lokugamage *et al*, 2020

In vivo testing of Wnt inhibitors



Promising trend.....



- Mice infected with 10^3 pfu of mouse-adapted SARS-CoV-2
- Drugs administered IP pre- and post-infection

Summary

- RNA viruses employ a highly diverse array of tactics to block innate immune signaling
- This includes novel mechanisms to disrupt biogenesis and/or function of peroxisomes during infection
- Wnt signaling plays a role in peroxisome homeostasis
- Genetic or pharmacological upregulation of peroxisomes enhances the IFN response during viral infection

Potential benefits of targeting peroxisomes for antiviral therapy

- Drugslots of them!
 - Wnt inhibitors
 - Peroxisome proliferator-activated receptor agonists
 - Good safety profiles
 - Bench to bedside route shorter
- Broad-spectrum antiviral activity
- Reduce inflammation?
- Prophylactic and early therapeutic use?

Ongoing studies and future directions

- Determine how SARS-CoV-2 depletes peroxisomes
 - Candidate viral proteins and interactome studies
- *In vivo* studies
 - Intranasal and oral delivery routes
- Testing antiviral activity of Wnt inhibitors in combination with other drugs
- Screening libraries for additional peroxisome-inducing compounds/drugs
- Metabolic consequences of peroxisome loss?
 - Inflammation and lipotoxicity

Acknowledgements

