Targeting the Wnt/β-catenin pathway as a broad-spectrum antiviral strategy

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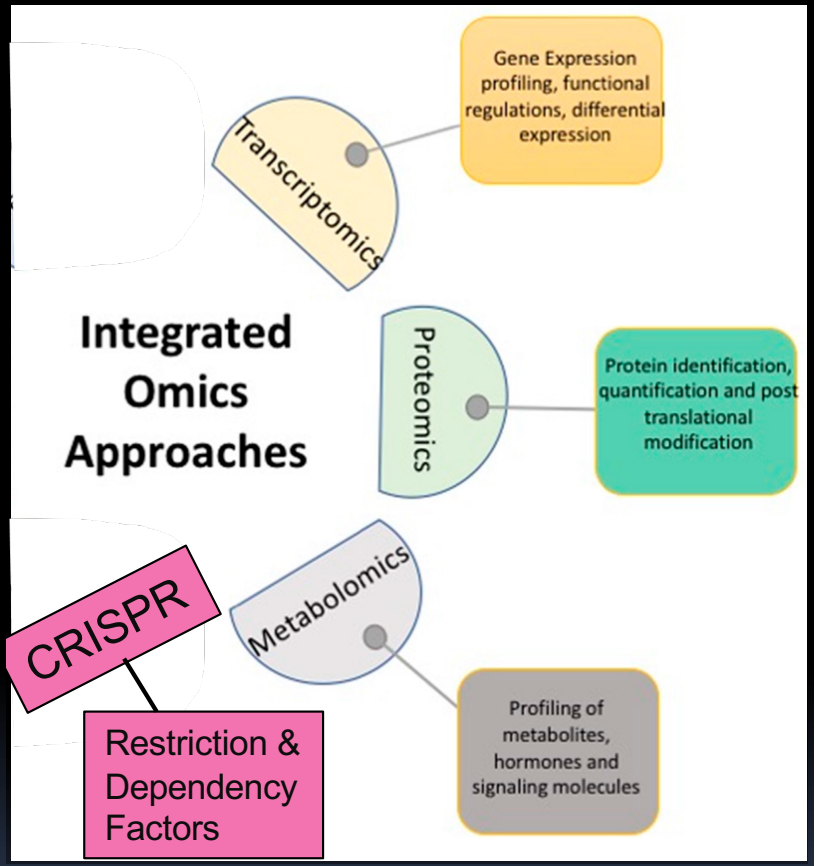
Disclosures

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

Research Focus

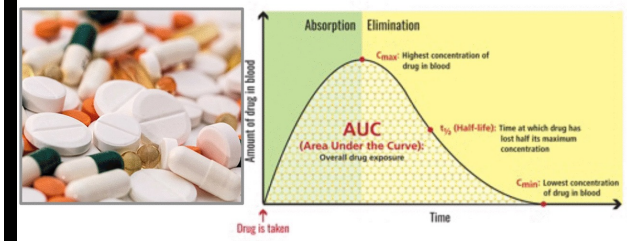
- Identification of key host factors/pathways that are utilized or affected by <u>multiple RNA viruses</u>

- Pharmacological targeting of these host factors/pathways should result in broad-spectrum antiviral activity

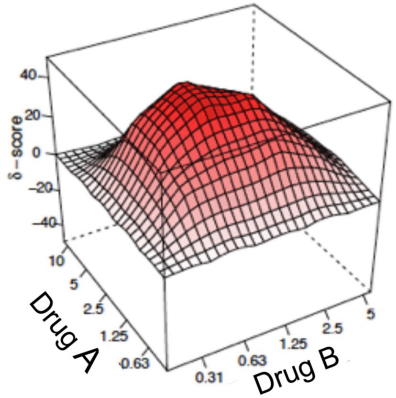


"For the present pandemic response, and for future pandemics …. the scientific community must be ready with an arsenal of easily self-administered drugs that can be tested in rapid, efficient clinical trials immediately after the causative viral agent is identified."

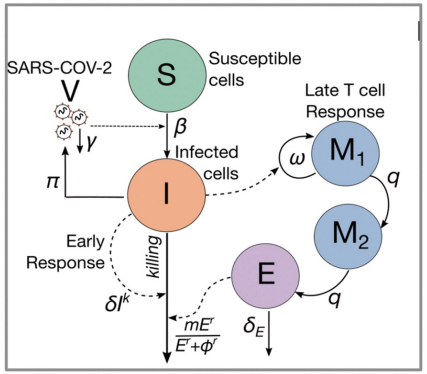
1. Prioritize oral and inhaled drugs active against members of a given virus family *(see legend for criteria)*



2. Assess potency of drug combinations in relevant cells



3. Model human *in vivo* potency based on PK/PD



4. Test predicted doses in animals

and

5. Design clinical study

6. Assess safety & PK of drug combinations in humans

ClinicalTrials.gov

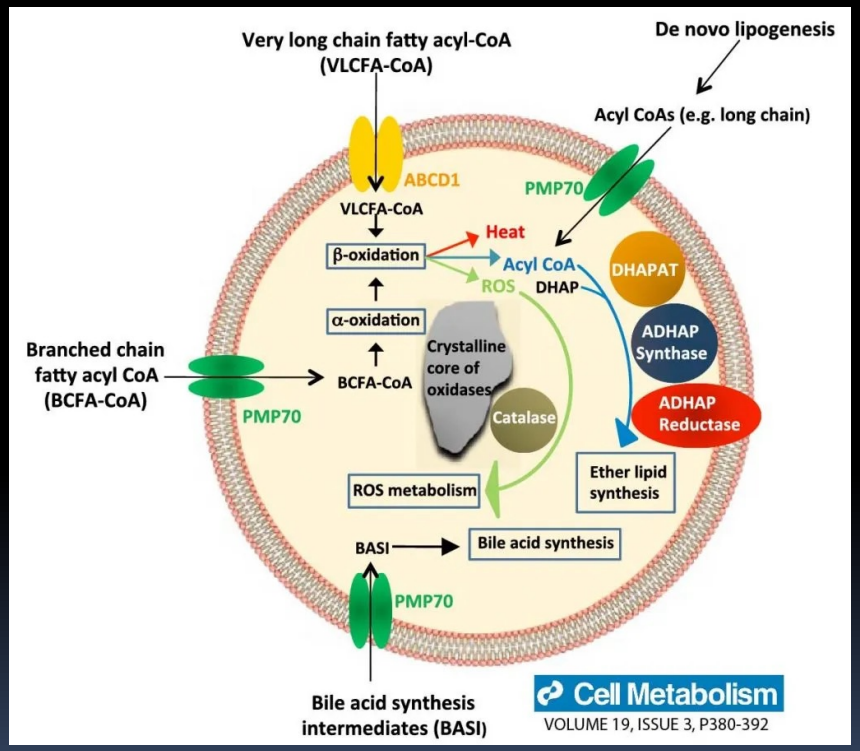
7. Stockpile Drug Cocktails
- **Pan-coronavirus**
- **Pan category A-C viruses**

White et al, 2021

Host-targeted antivirals to be part of this arsenal?

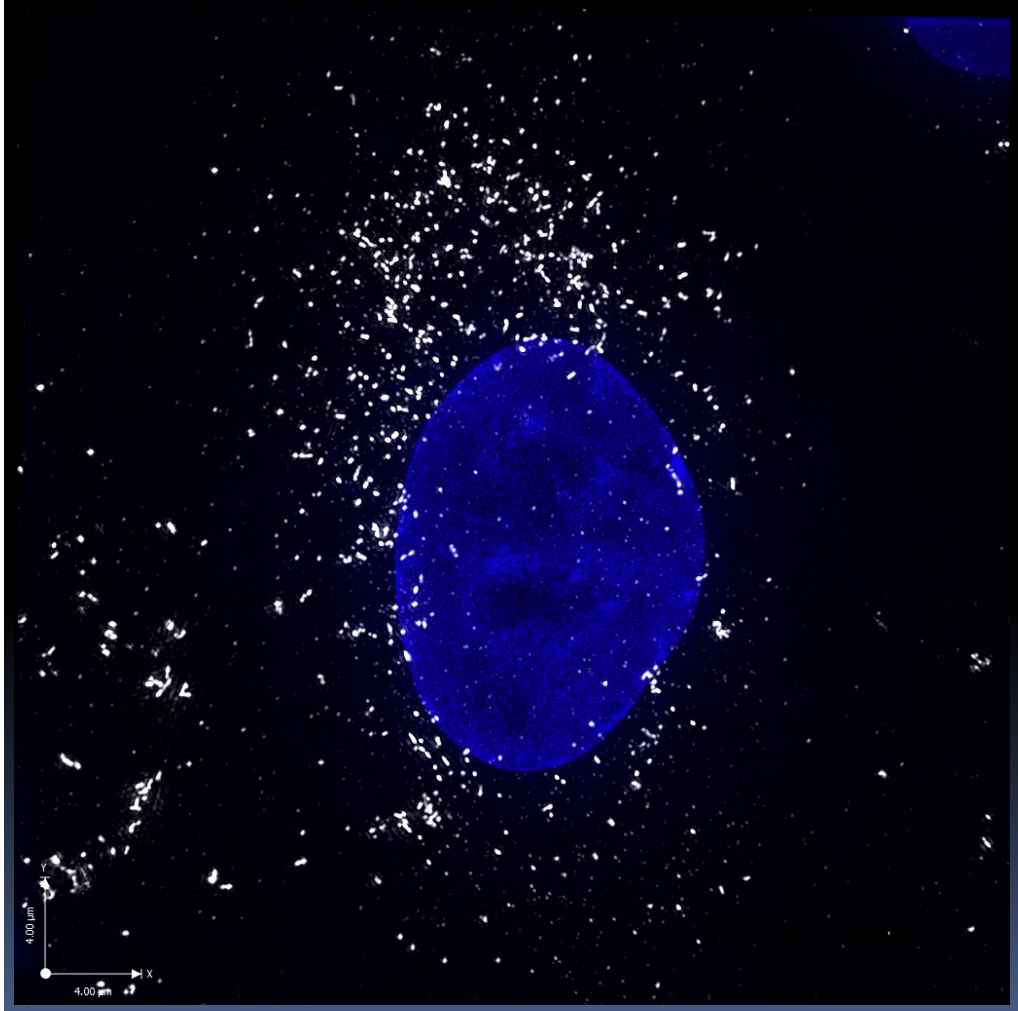
Peroxisomes are targeted during RNA virus infection

- Abundant metabolic organelles in the cytoplasm
  - Catabolize very long chain fatty acids
  - Regulate reactive oxygen species
  - Produce specialized phospholipids (e.g. plasmalogens)



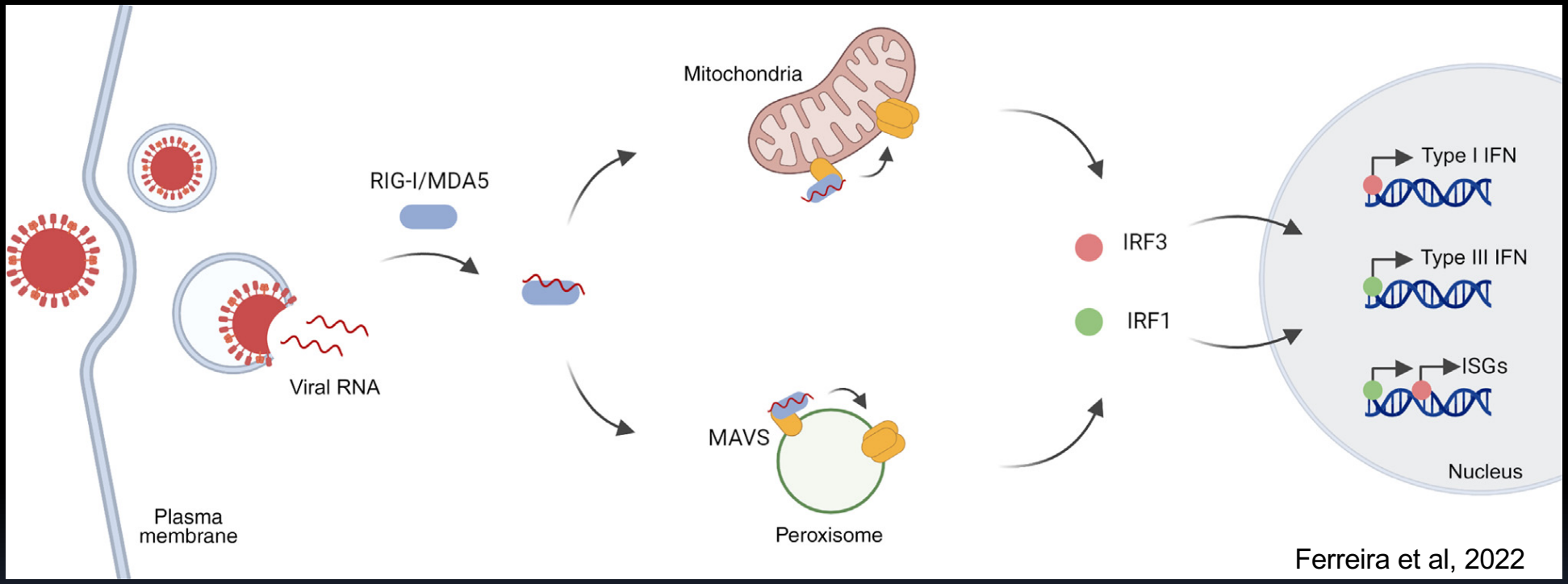
Flavivirus infection results in loss of peroxisomes

Mock



You, Hou *et al*. 2015

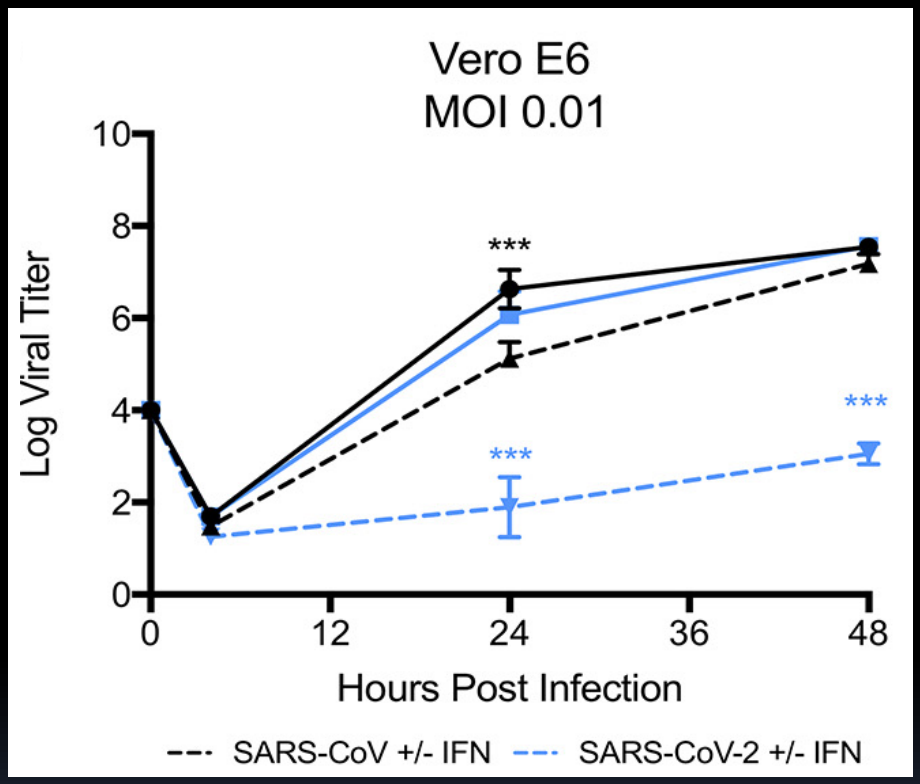
Why would a virus want to deplete the peroxisome pool?

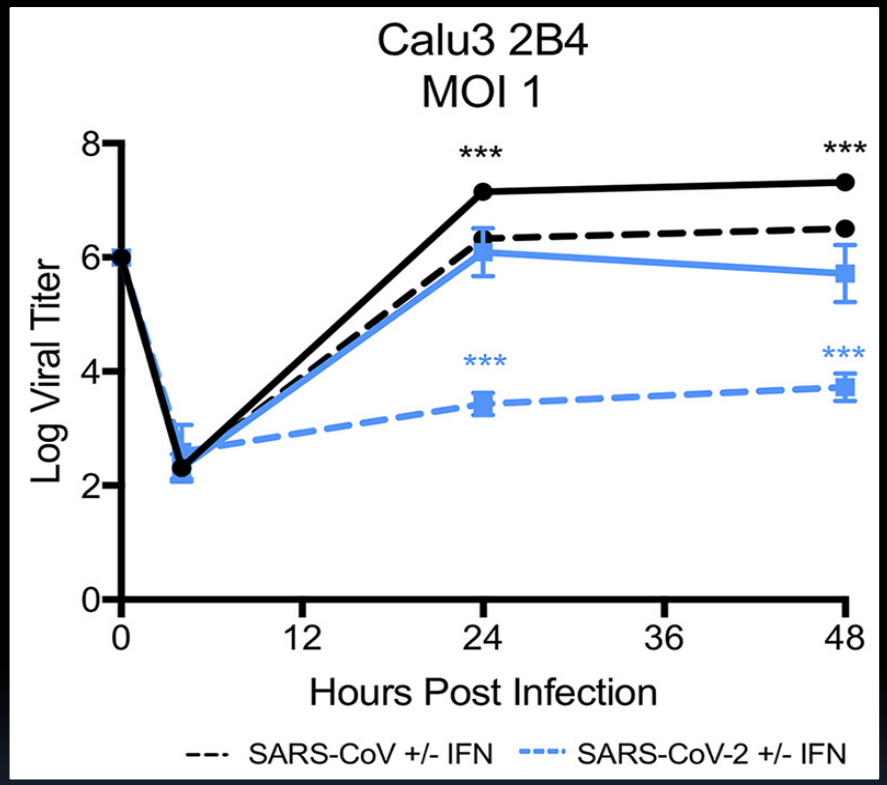


Ferreira et al, 2022

>>Peroxisomes are antiviral signaling platforms that facilitate induction of type I and III interferons (IFN)

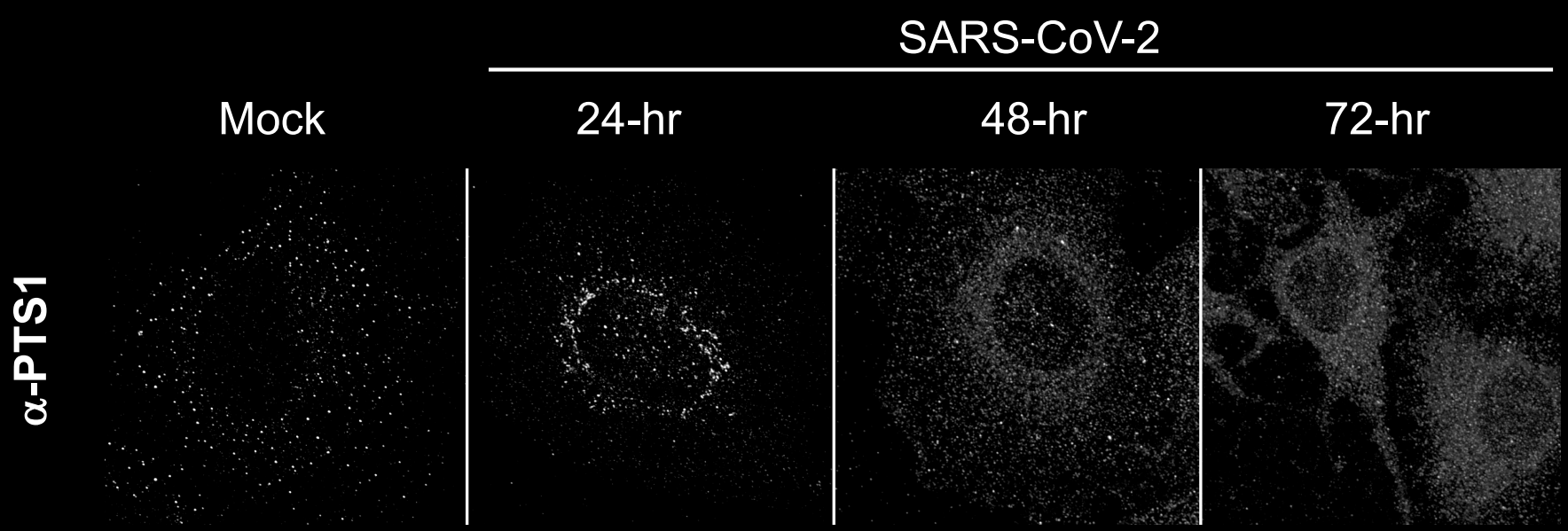
SARS-CoV-2 is highly sensitive to Interferon (IFN)

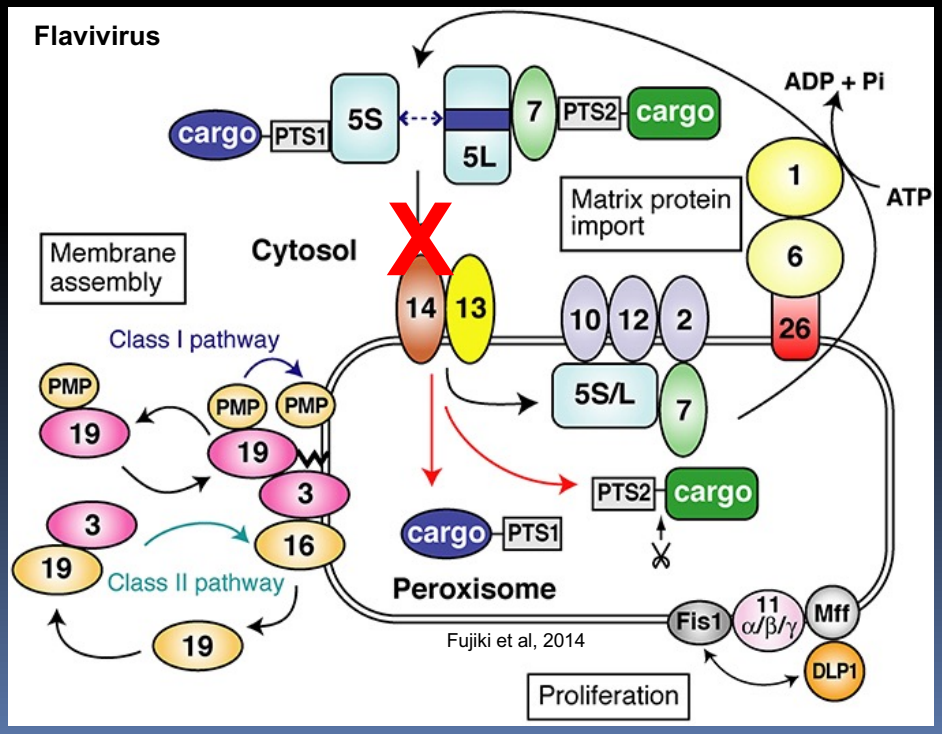




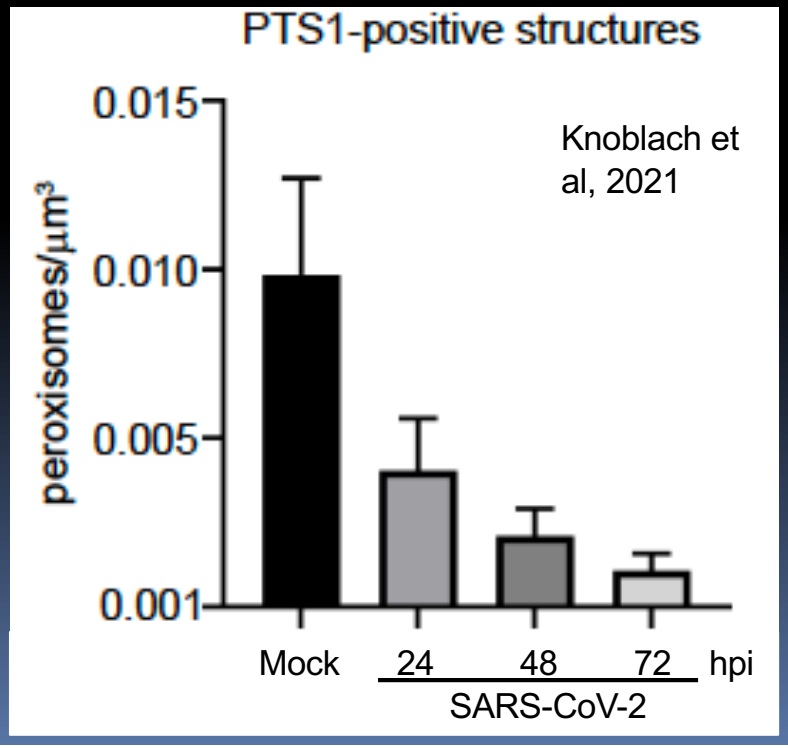
Lokugamage *et al*, 2020

SARS-CoV-2 depletes functional peroxisomes



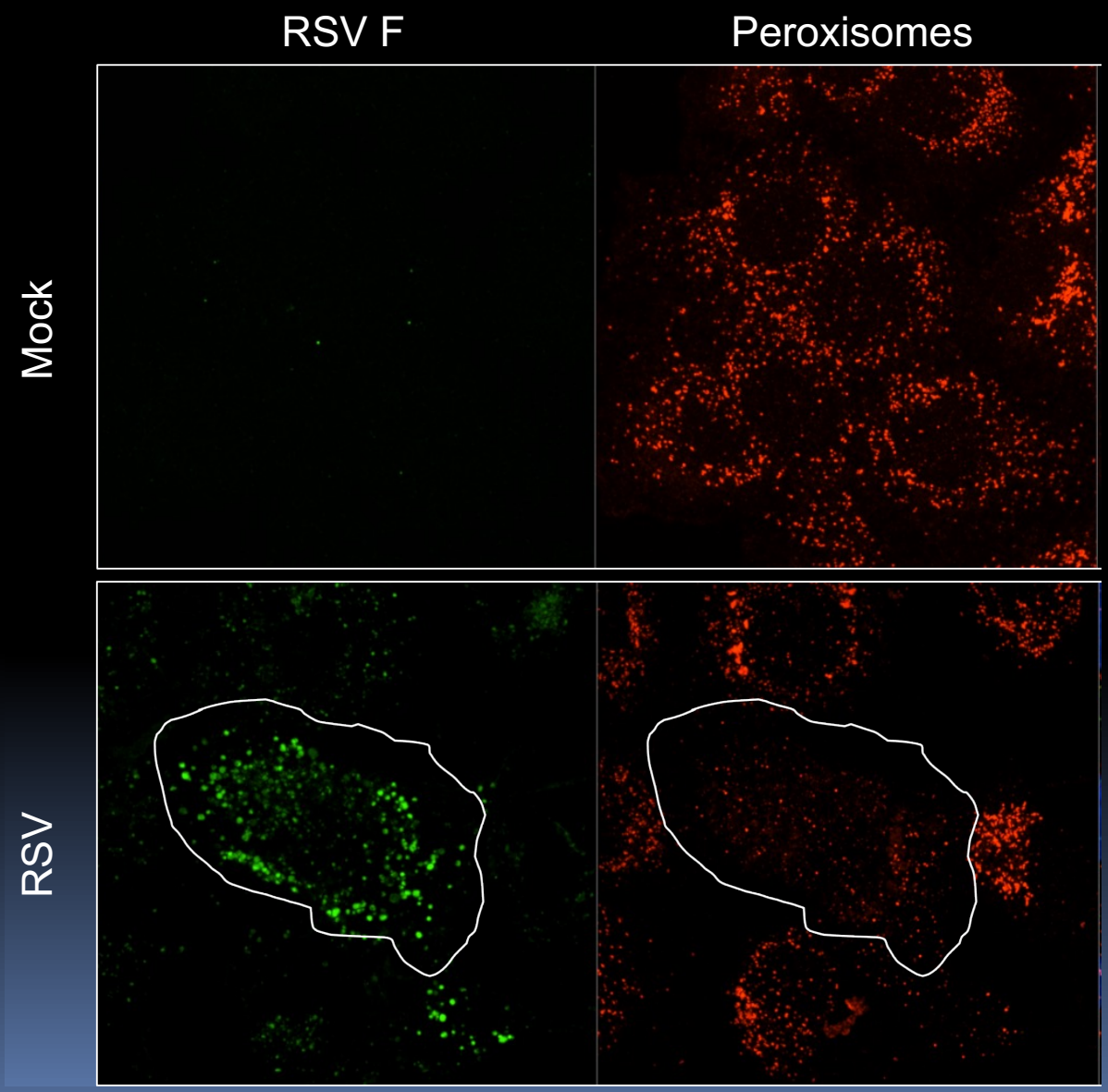


Fujiki et al, 2014

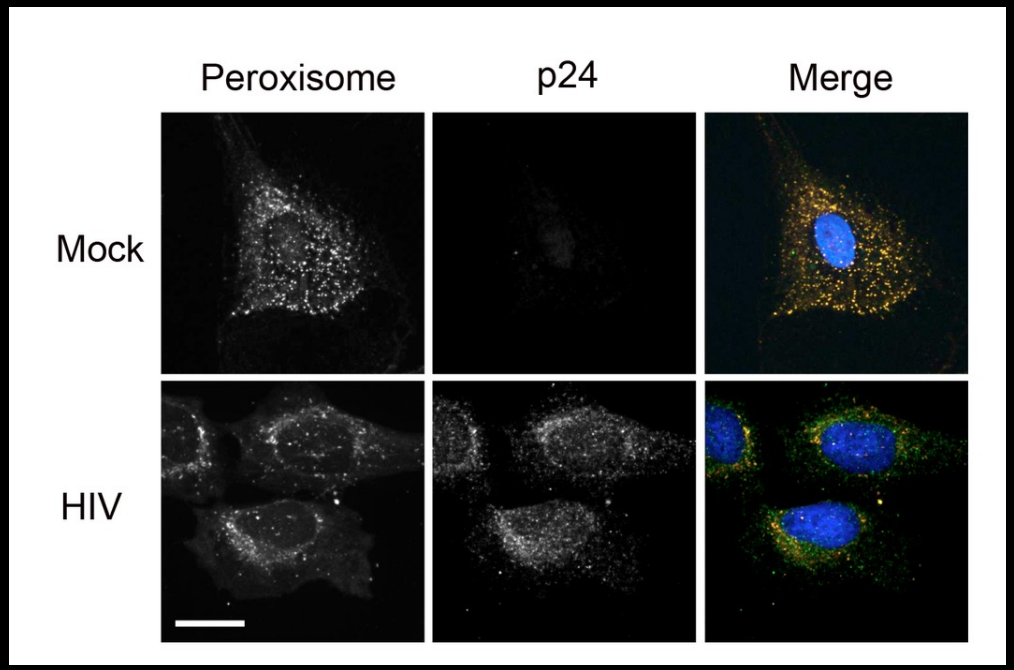


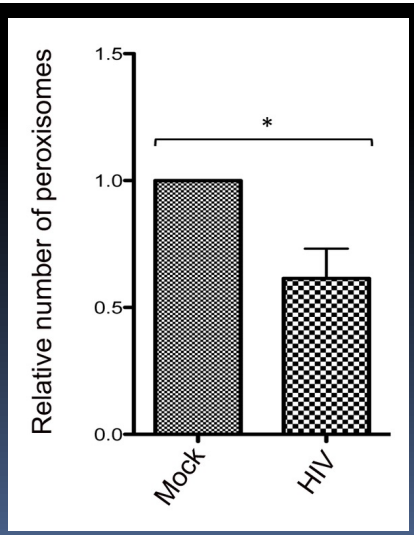
Knoblach et al, 2021

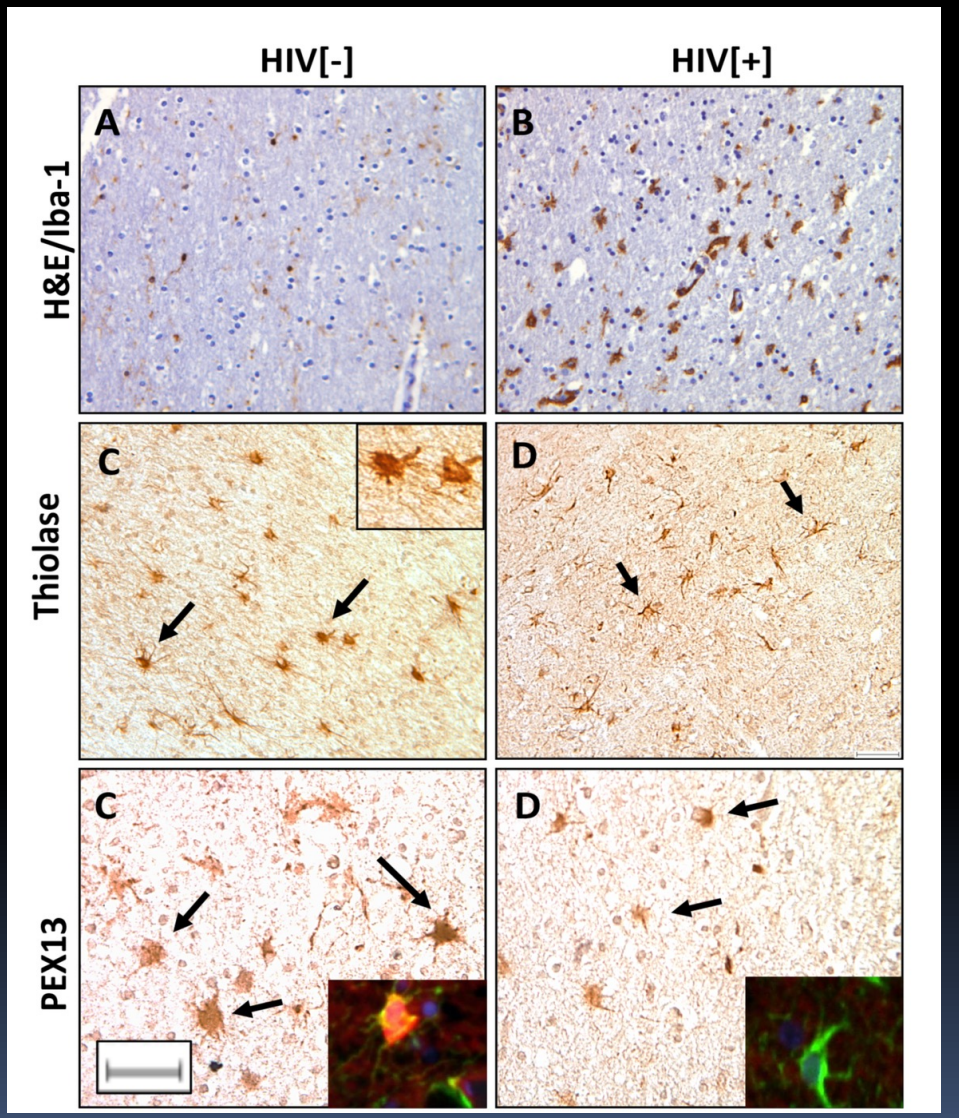
Respiratory Syncytial Virus (RSV) also reduces peroxisome pool



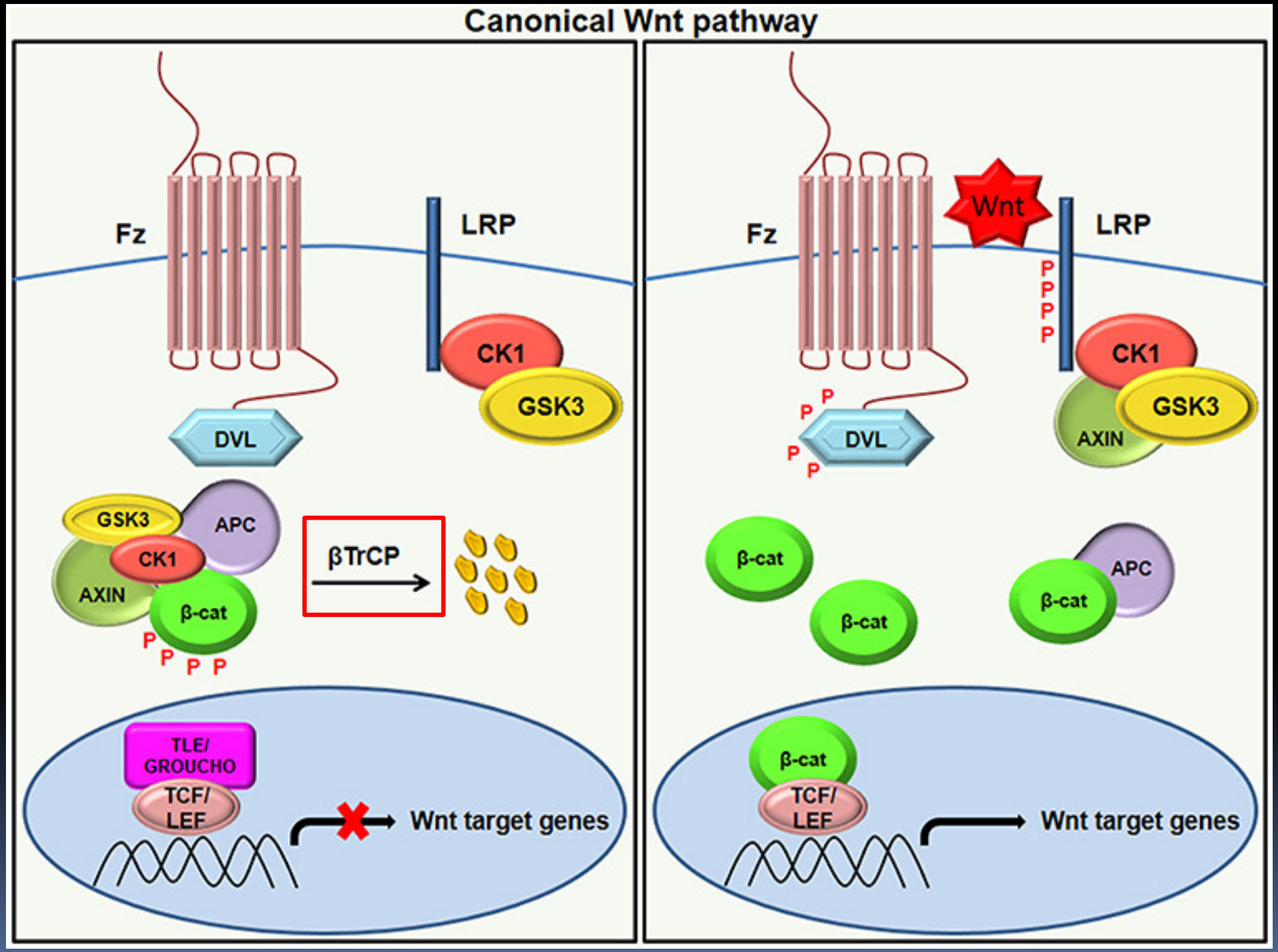
As does HIV….



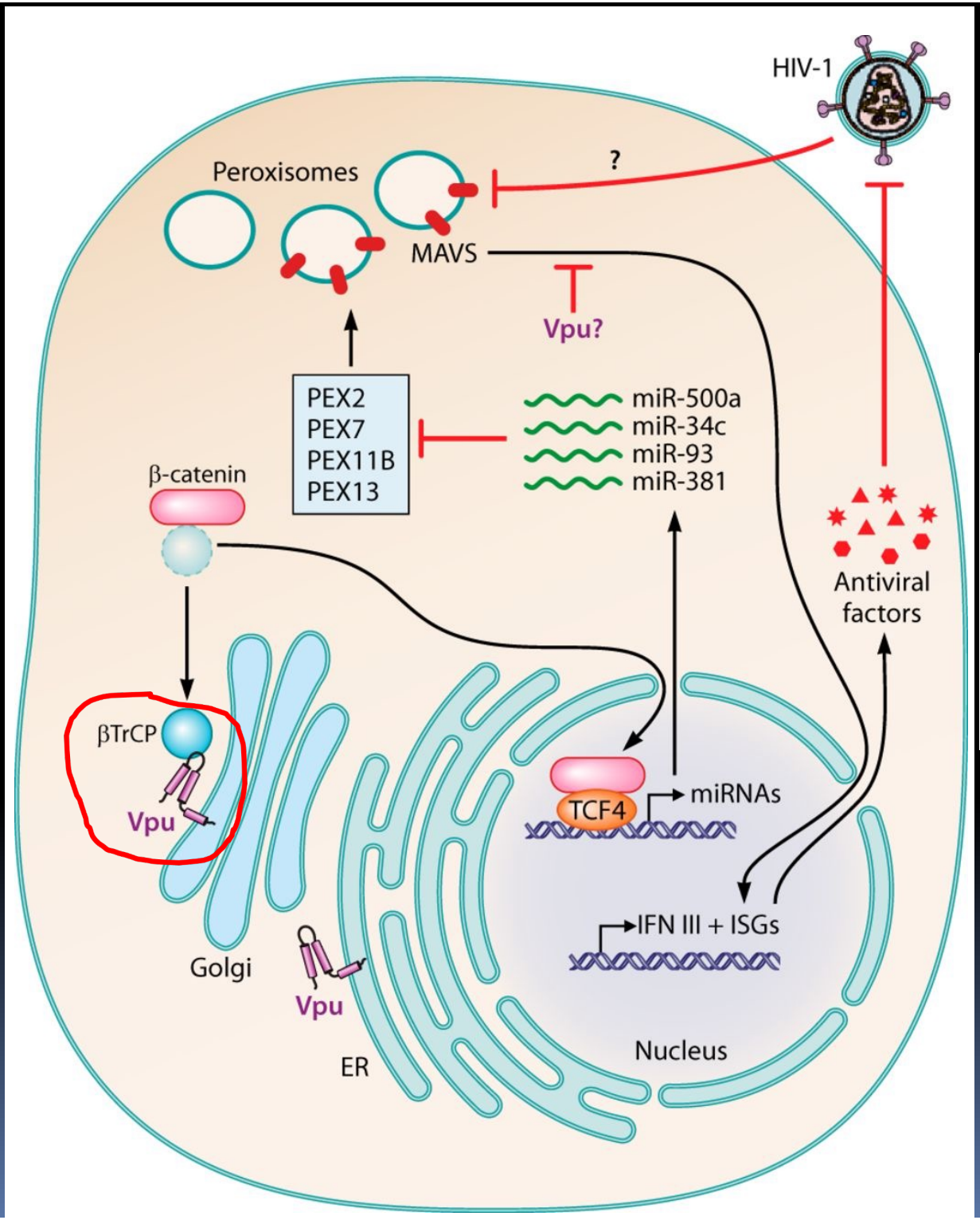




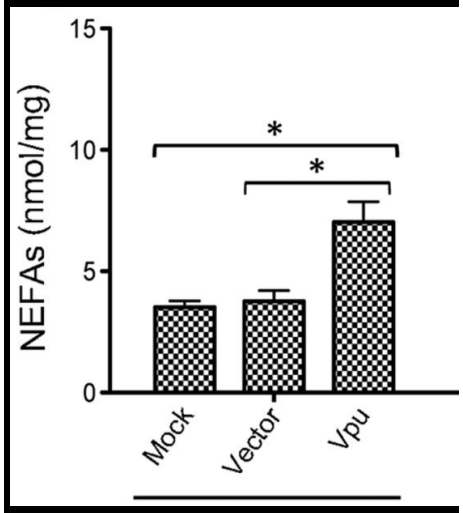
Xu et al, 2017



Patel et al, 2020



Vpu reduces metabolic activity of peroxisomes



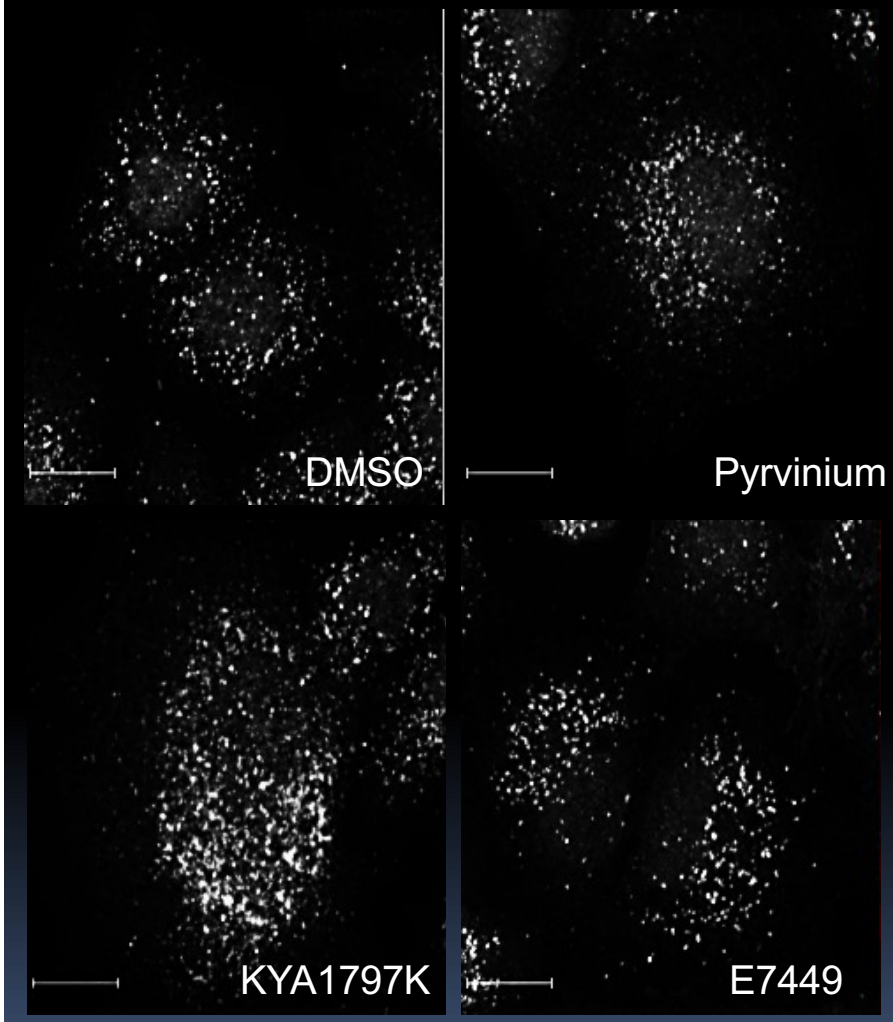
Hopfensperger et al, 2020

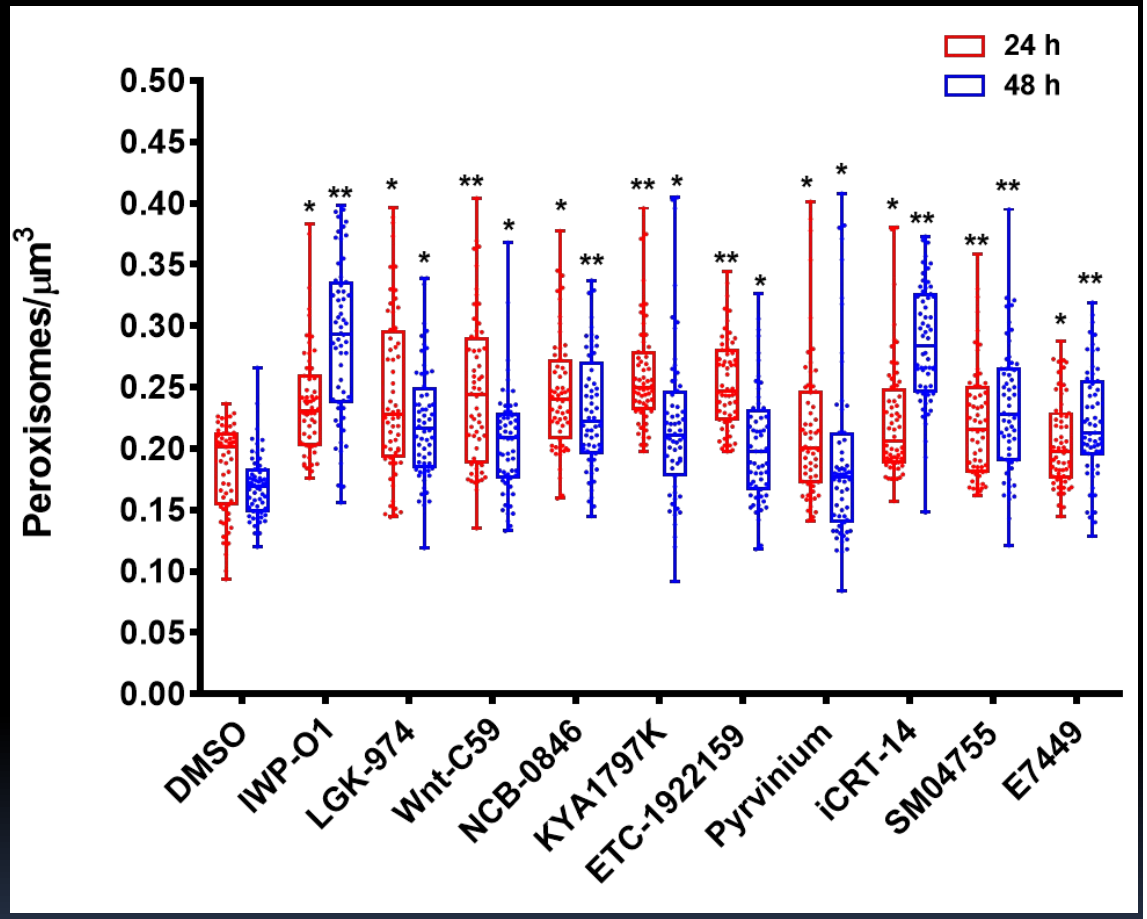
- Wnt/β pathway inhibits peroxisome biogenesis

Hypothesis: Inhibiting Wnt/β pathway will induce peroxisomes and reduce virus replication via enhanced IFN response

Test effects of Wnt/β pathway inhibitors on peroxisome density

Wnt/β-catenin inhibitors increase peroxisome density

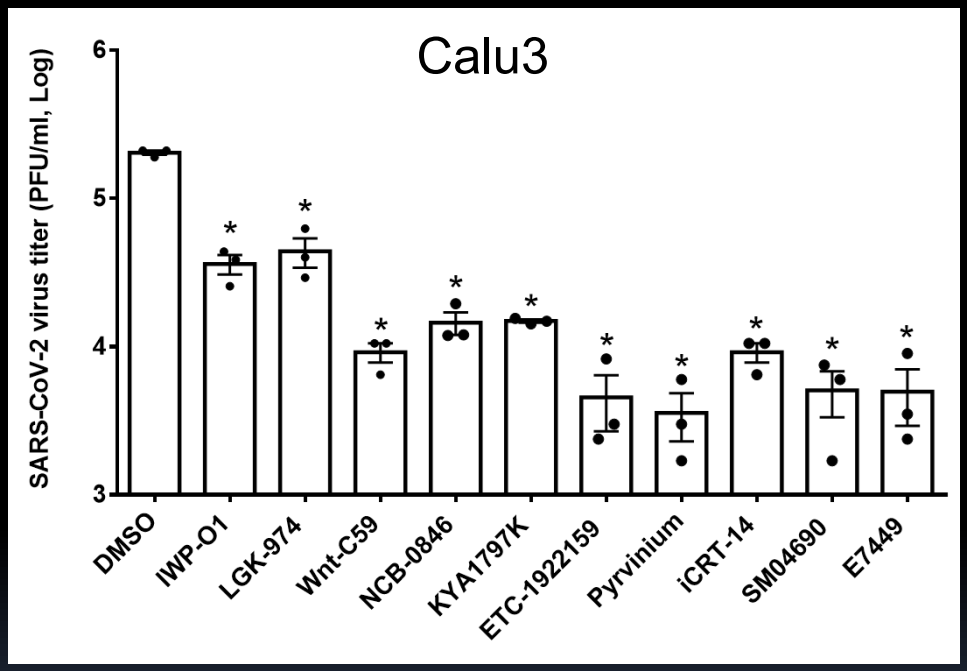


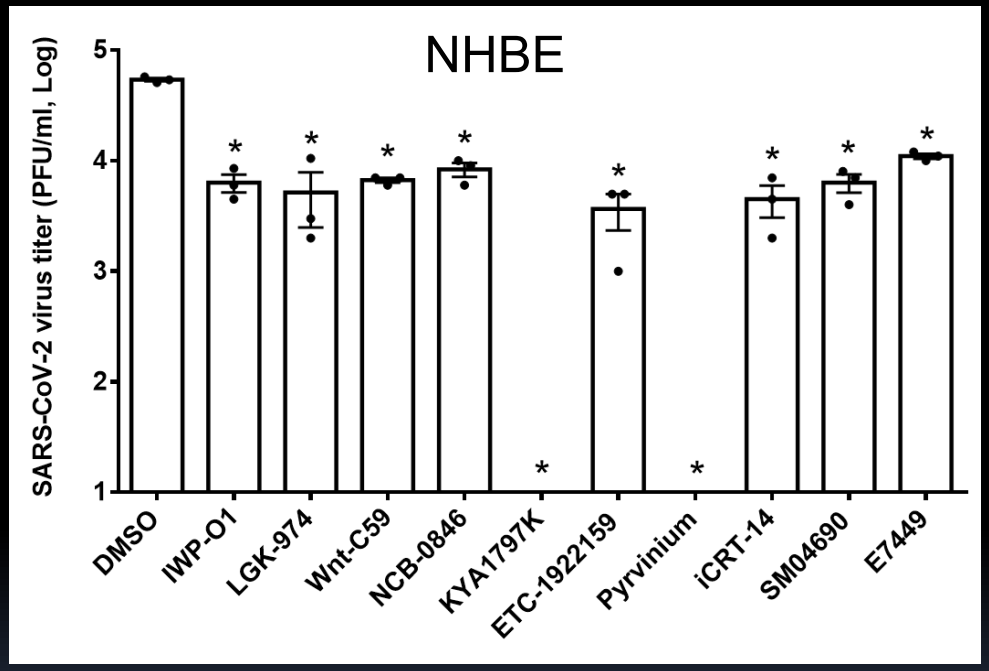


Xu, Elaish, Wong et al, in preparation

But do they inhibit virus replication?

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

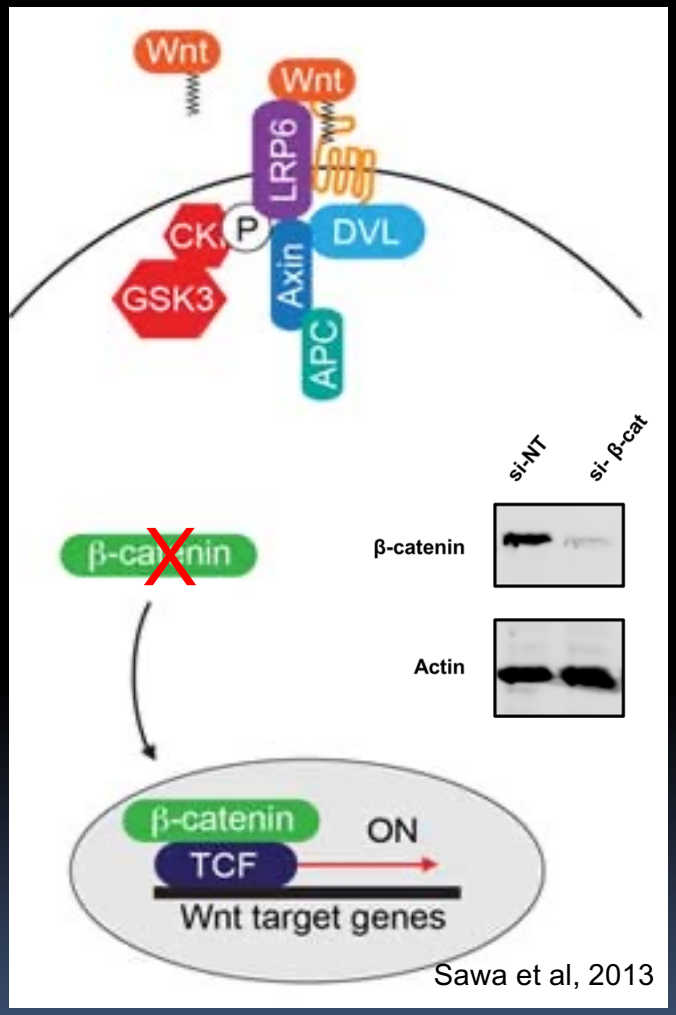




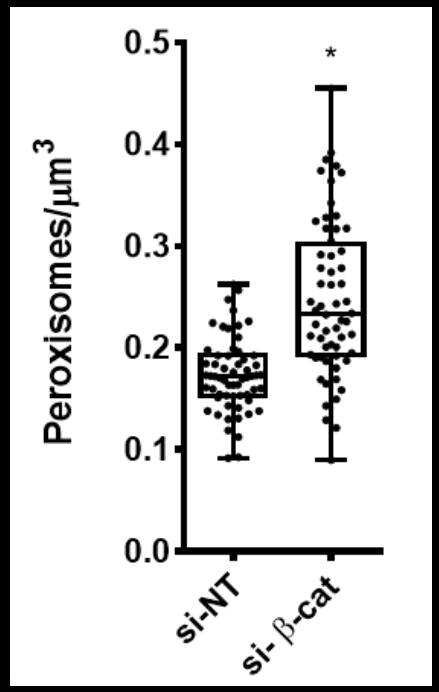
Calu3-human lung adenocarcinoma
NHBE-normal human bronchial epithelial cells

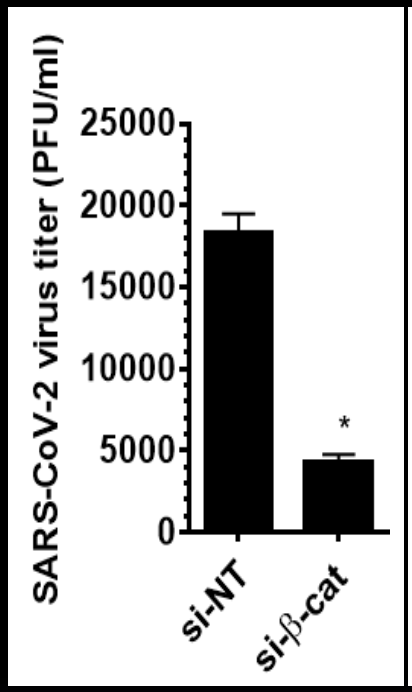
Xu, Elaish, Wong et al, in preparation

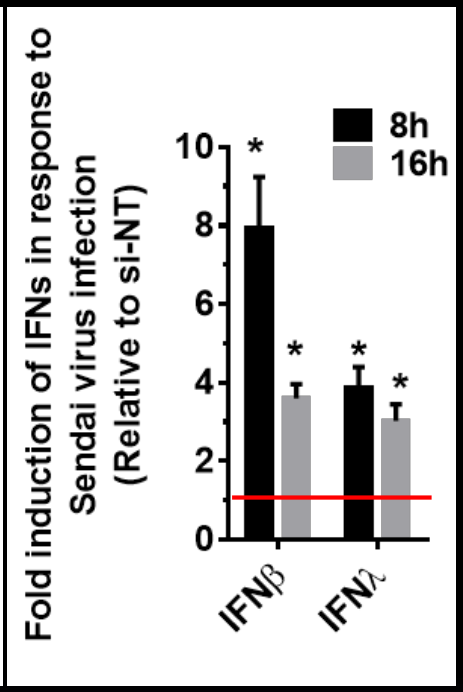
Reducing β-catenin levels induces peroxisome proliferation and enhances IFN response



Sawa et al, 2013



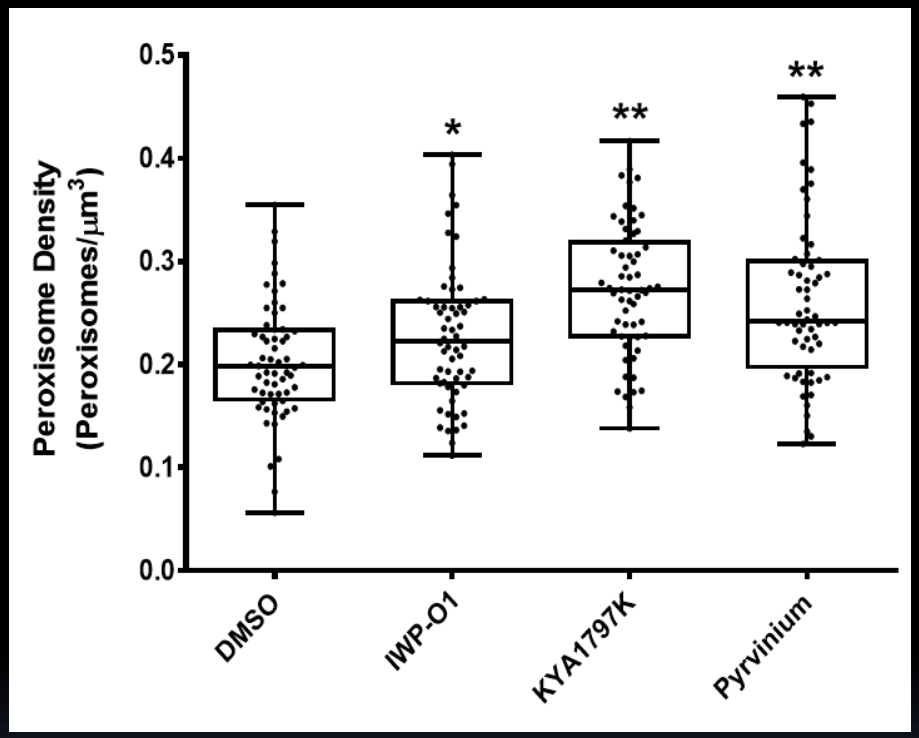


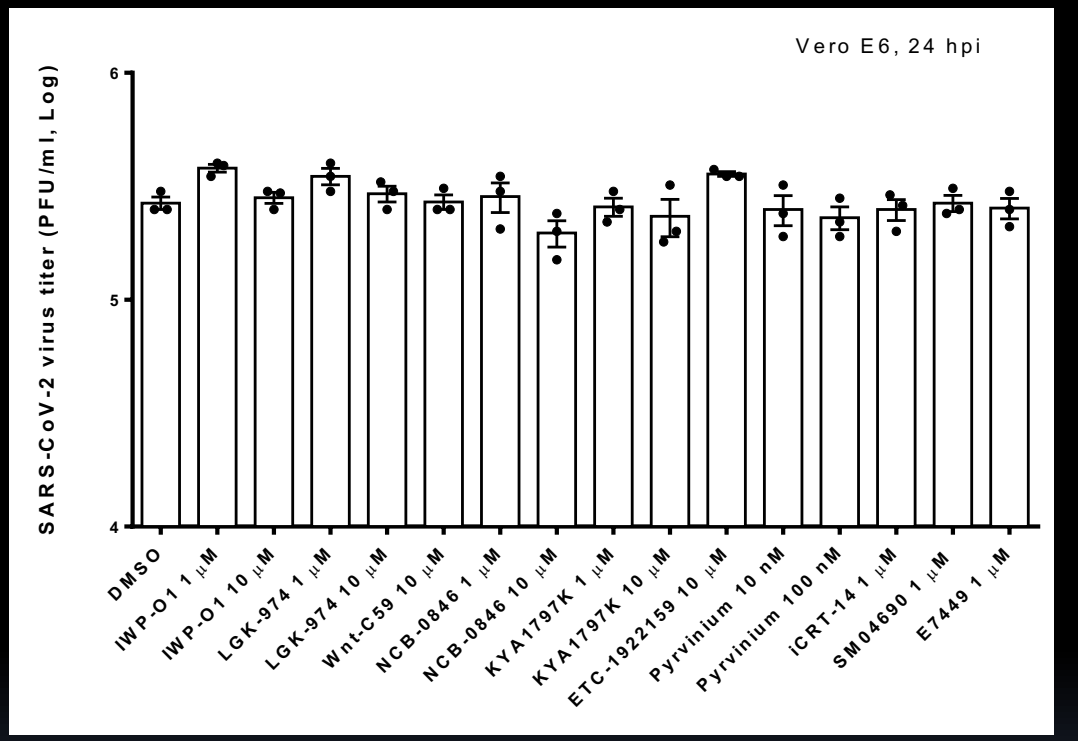


Suggests that antiviral effects of Wnt/β catenin inhibitors is not due to off target effects

Xu, Elaish, Wong et al, in preparation

Wnt/β-catenin inhibitors increase peroxisome density in Vero cells but do not reduce virus replication

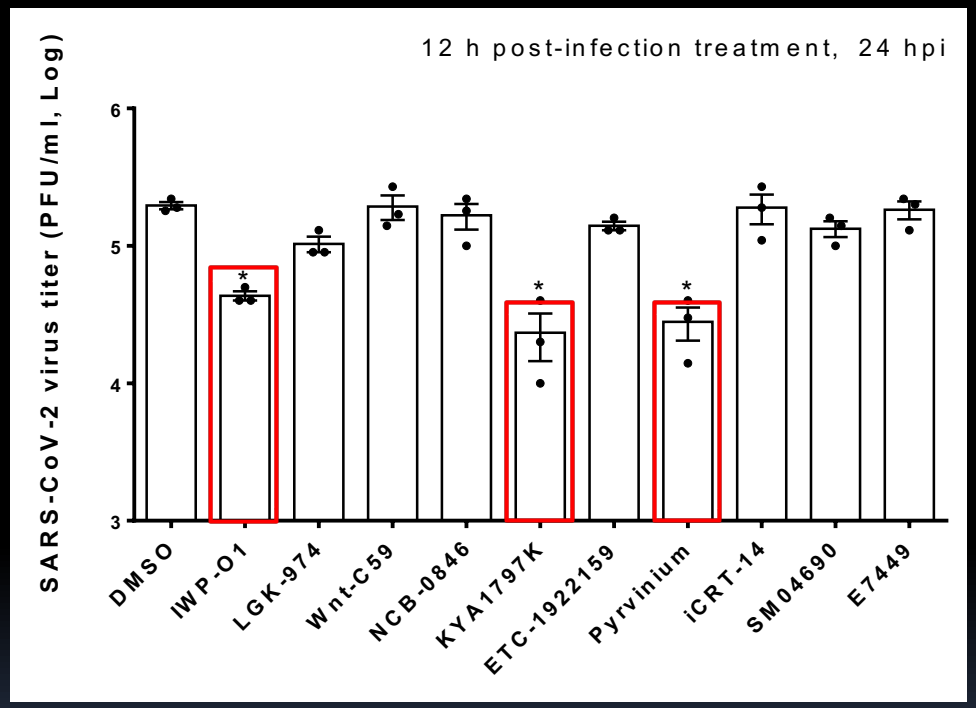


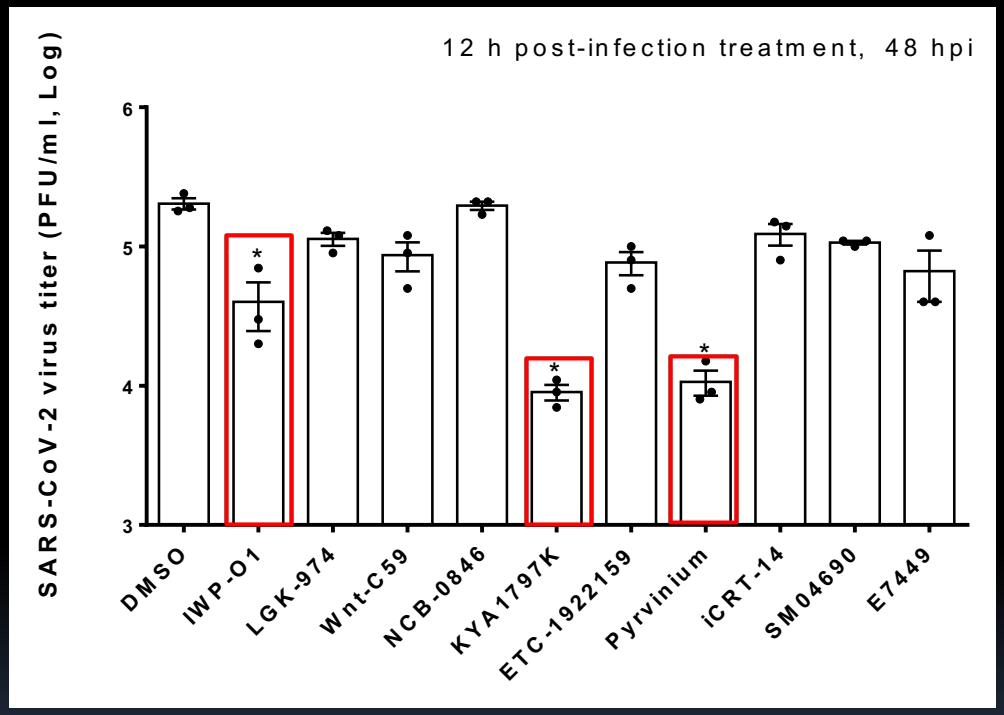


Consistent with model that antiviral effects of these drugs are IFN-dependent

Xu, Elaish, Wong et al, in preparation

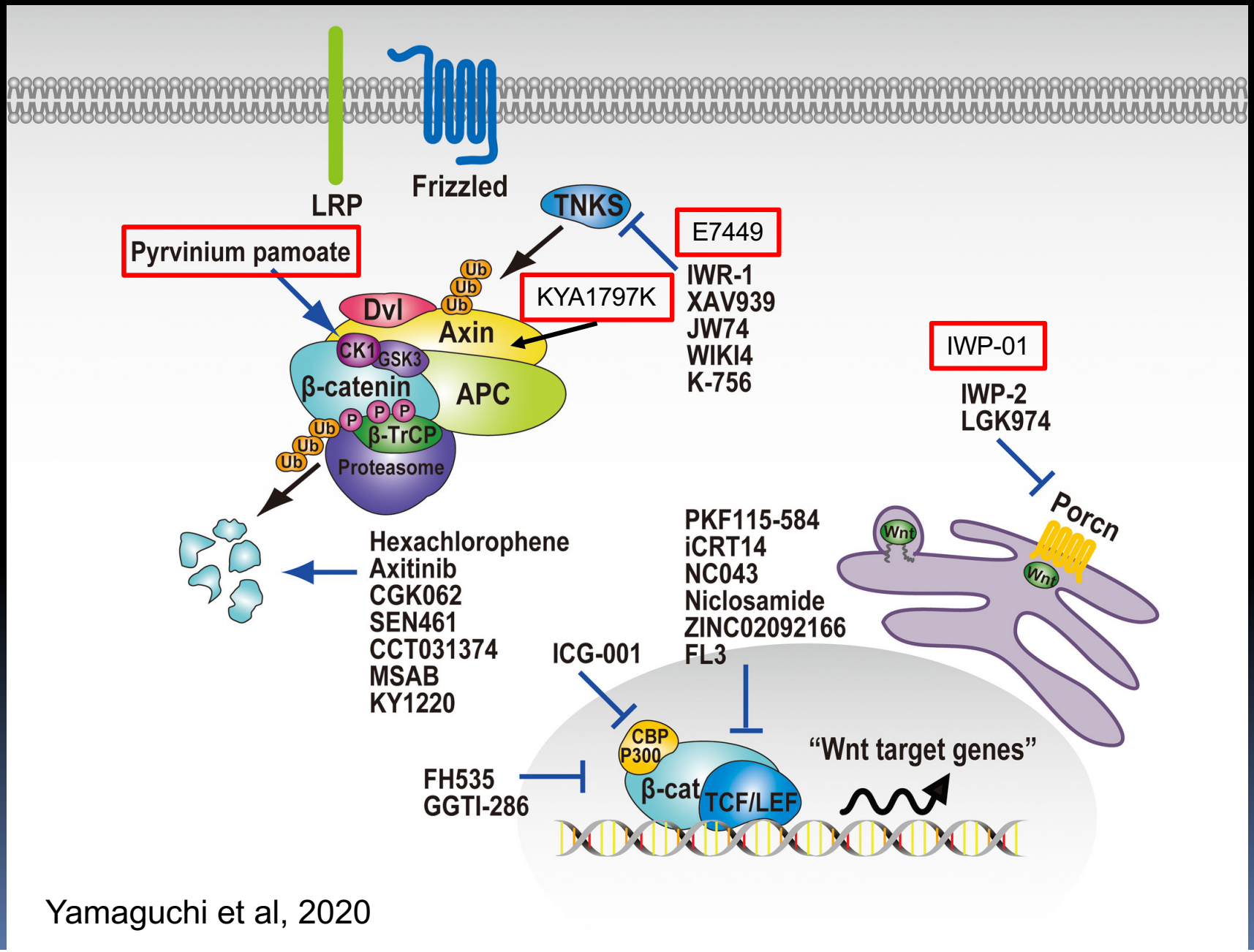
Some Wnt/β-catenin inhibitors decrease virus replication when added post-infection





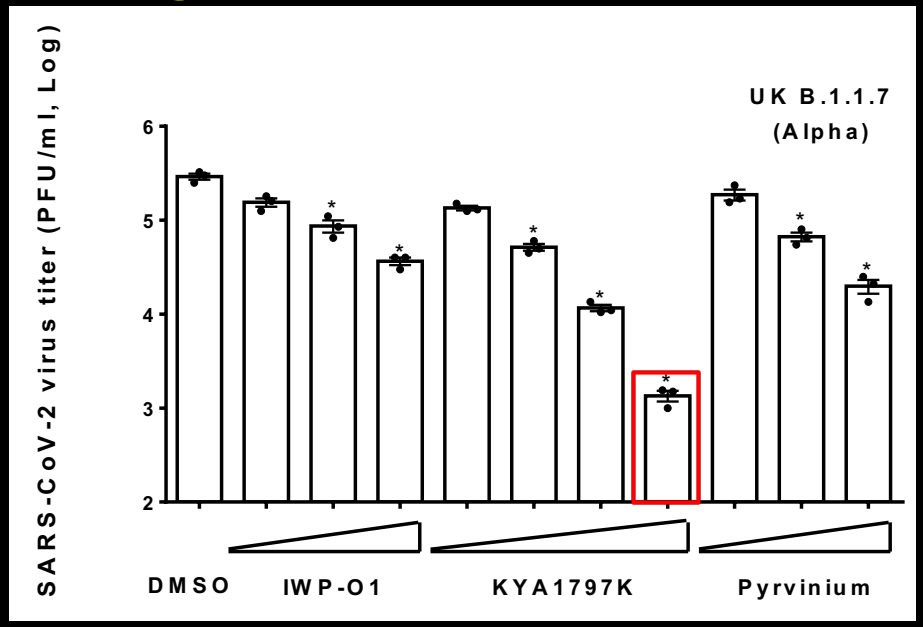
Xu, Elaish, Wong et al, in preparation

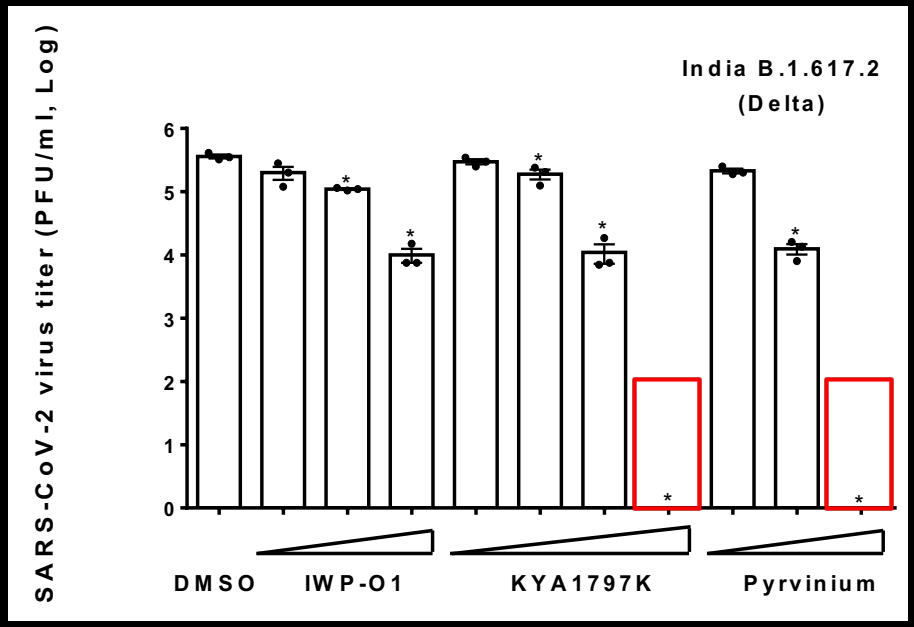
Drugs with high SIs chosen for testing against Variants of Concern and small animal studies

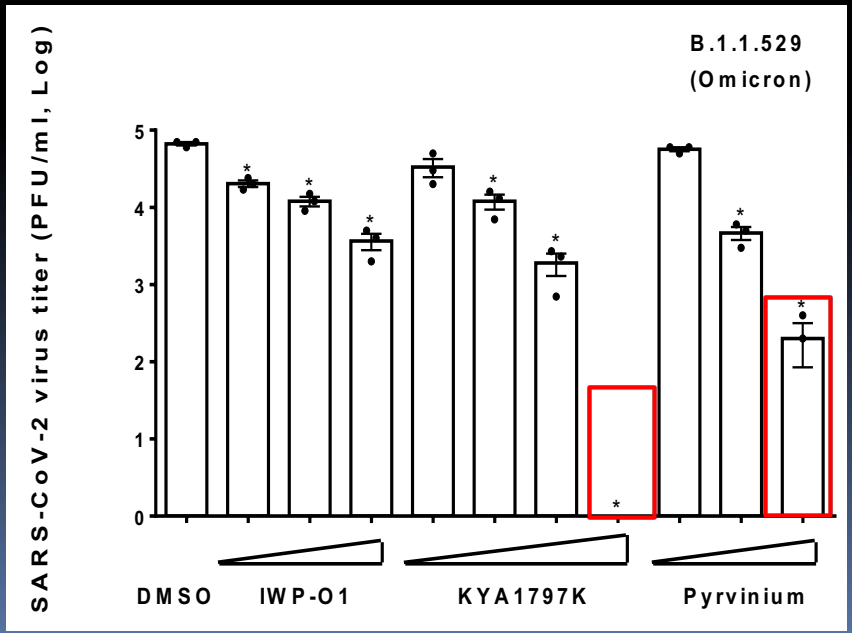


Yamaguchi et al, 2020

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

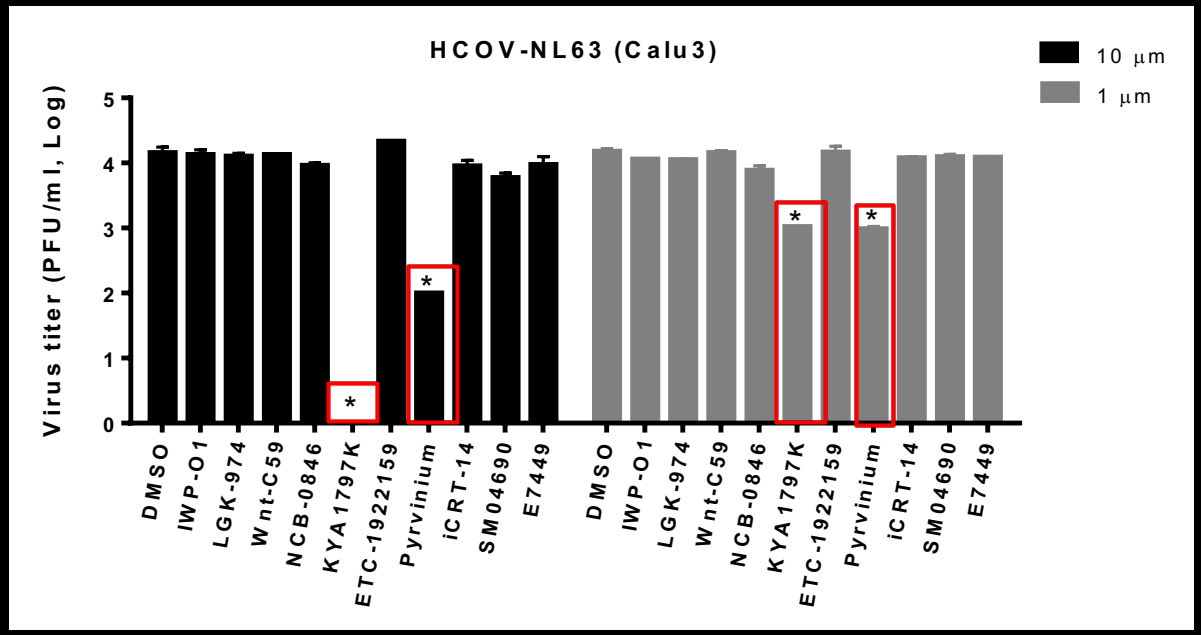


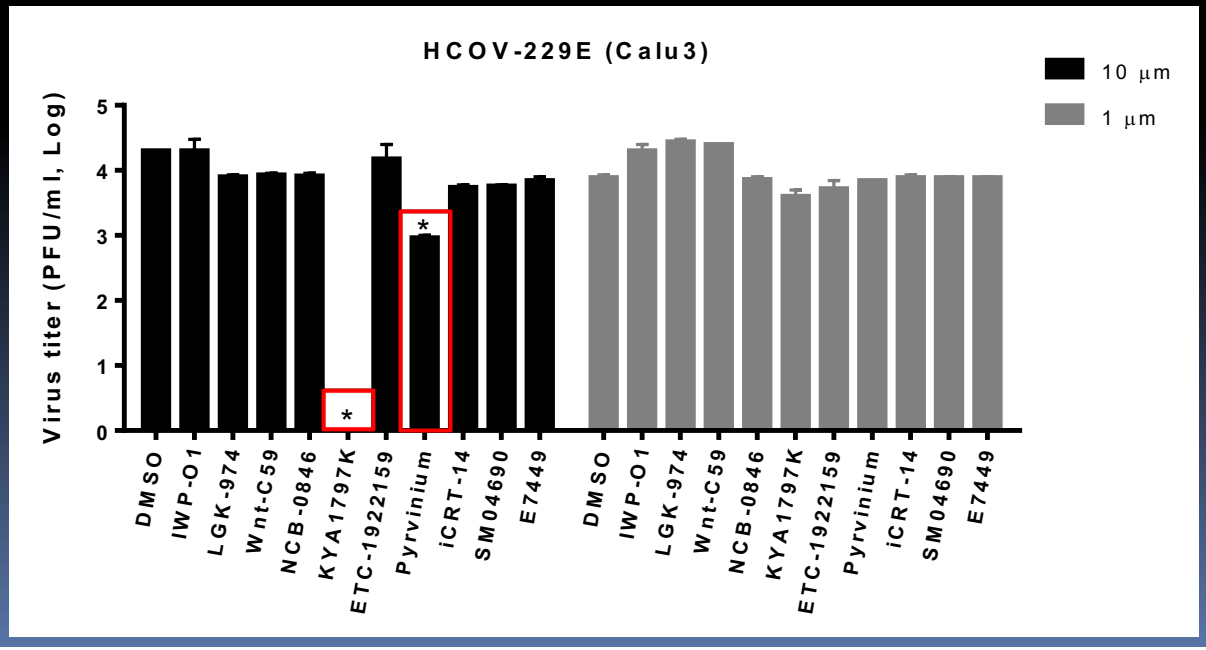




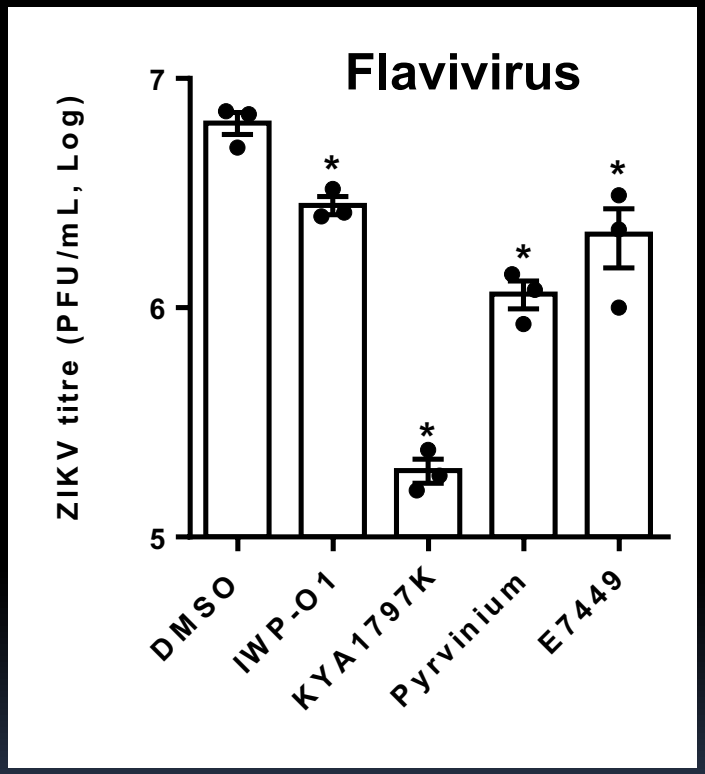
Xu, Elaish, Wong et al, in preparation

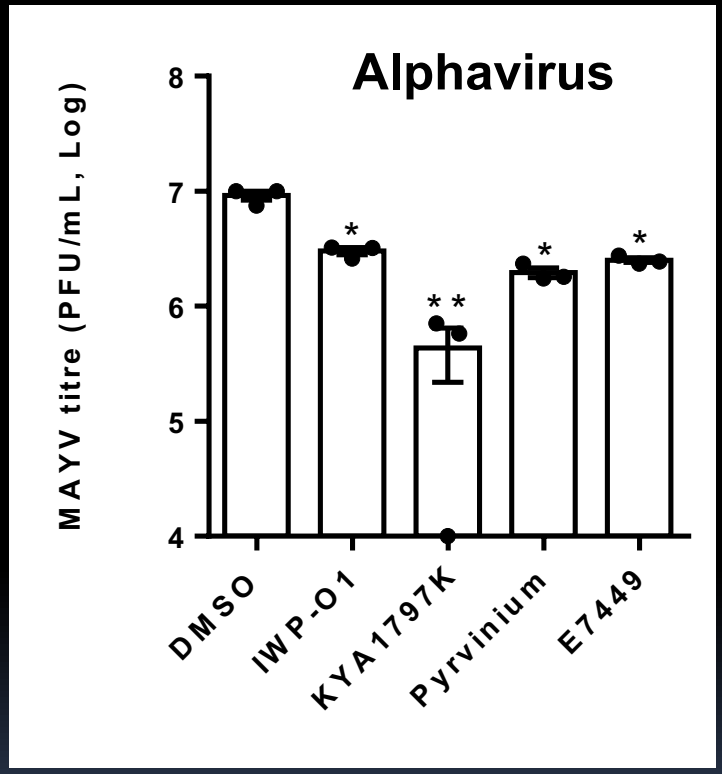
Some Wnt/β-catenin inhibitors reduce replication of other human coronaviruses





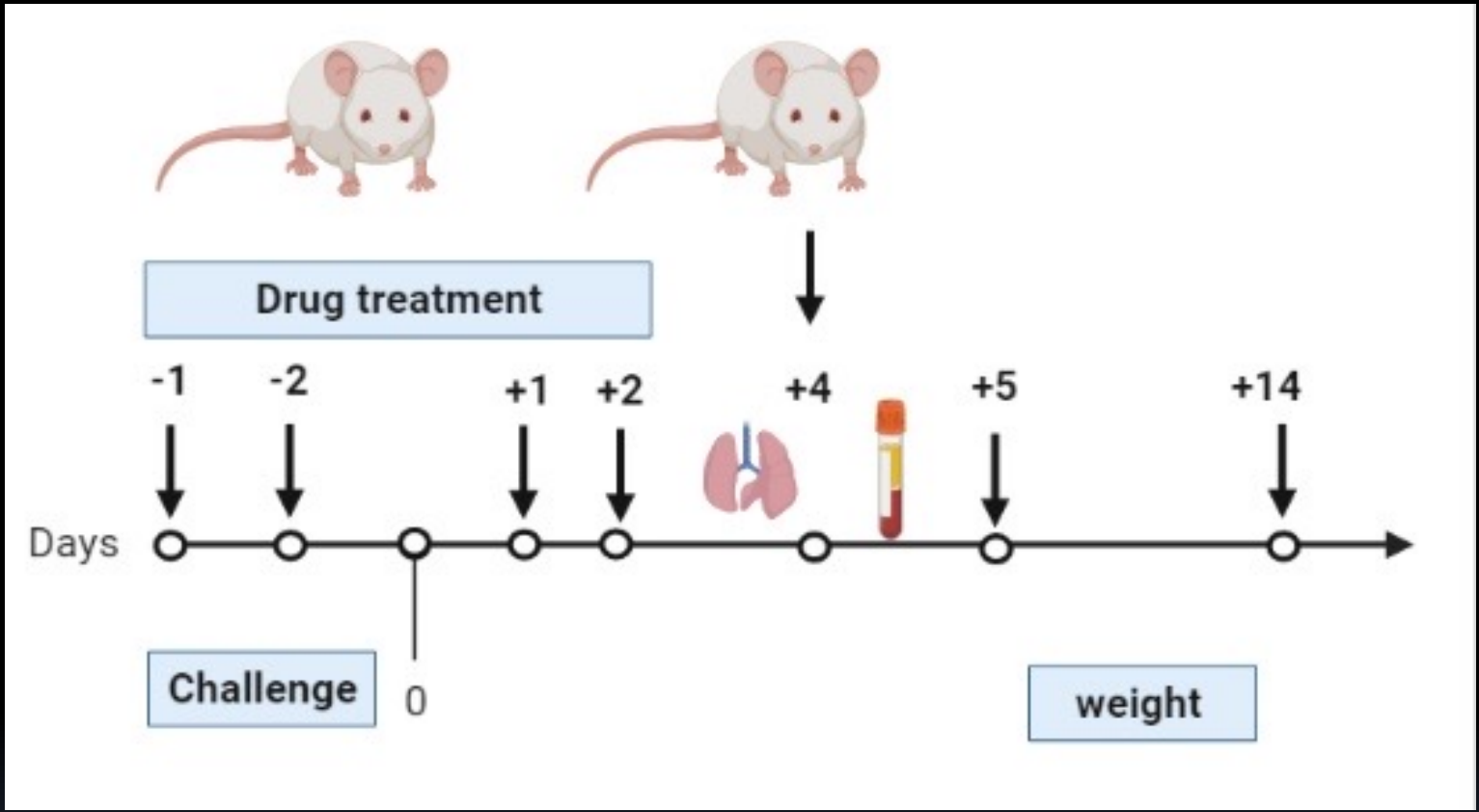
Wnt/β-catenin inhibitors reduce replication of other RNA viruses





Xu, Elaish, Wong et al, in preparation

In vivo testing of Wnt inhibitors

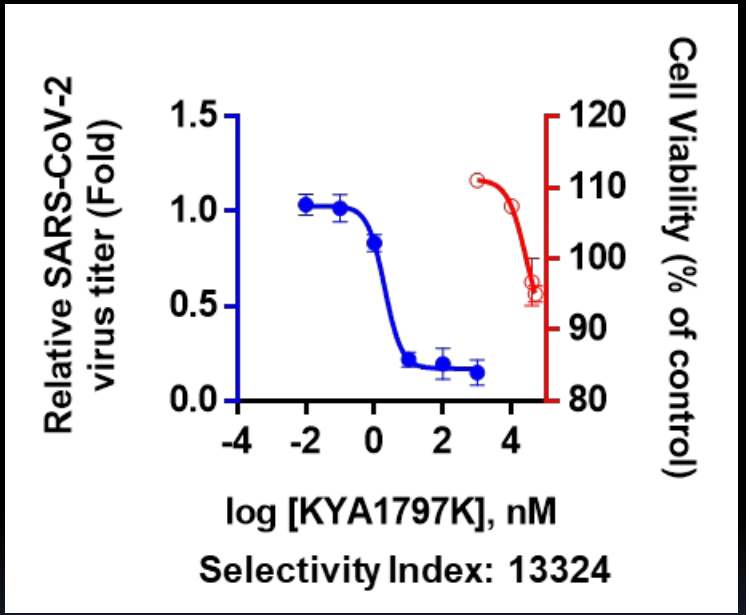


Drugs administered intranasally to female BALB/c mice (5 in each group)

Intranasal challenge with 5 x 10^3 pfu of mouse-adapted SARS-CoV-2

KYA1797K

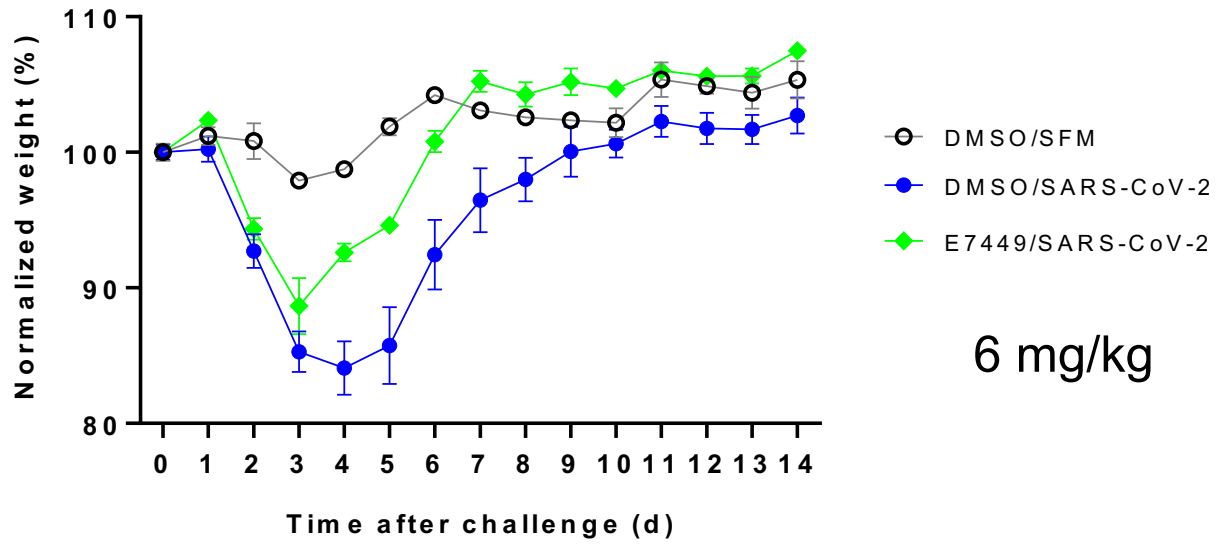
- Destabilizes β-catenin by activating Axin-GSK3β complex
- Tested via IP administration in mice (20 mg/kg/day)
  - Here limited to 3 mg/kg due to solubility

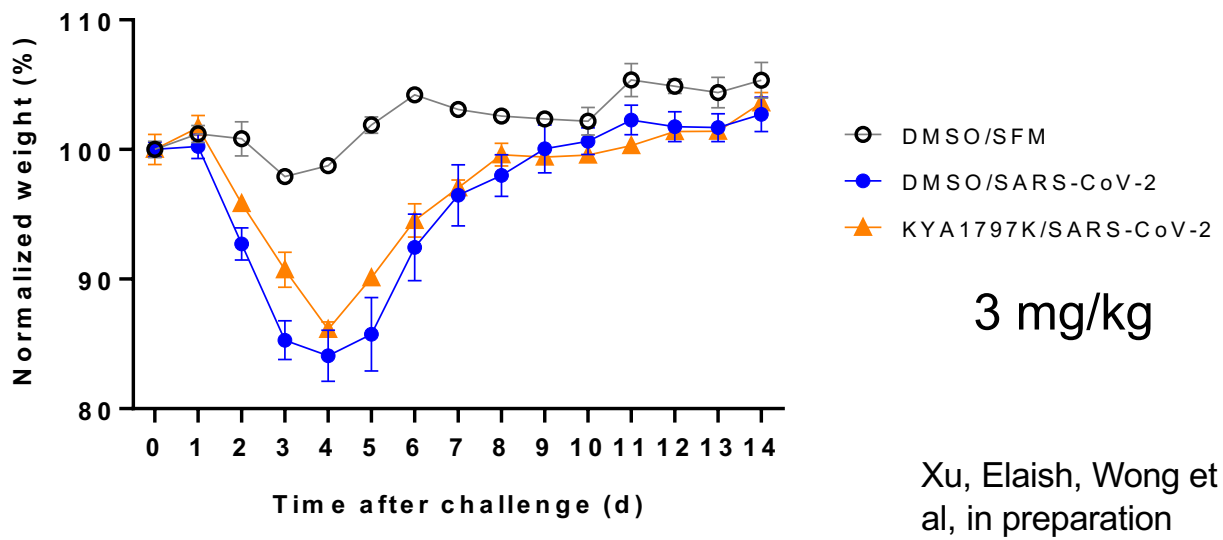


E7449

- Other names: Stenoparib
- Dual inhibitor of PARP1/2 & tankyrase1/2
- Orally bioavailable
- Phase1/2 study for cancer indications
  - Well tolerated (50-800 mg dosing) in humans
  - 0% cytotoxicity in human cells at 10 μM *in vitro*

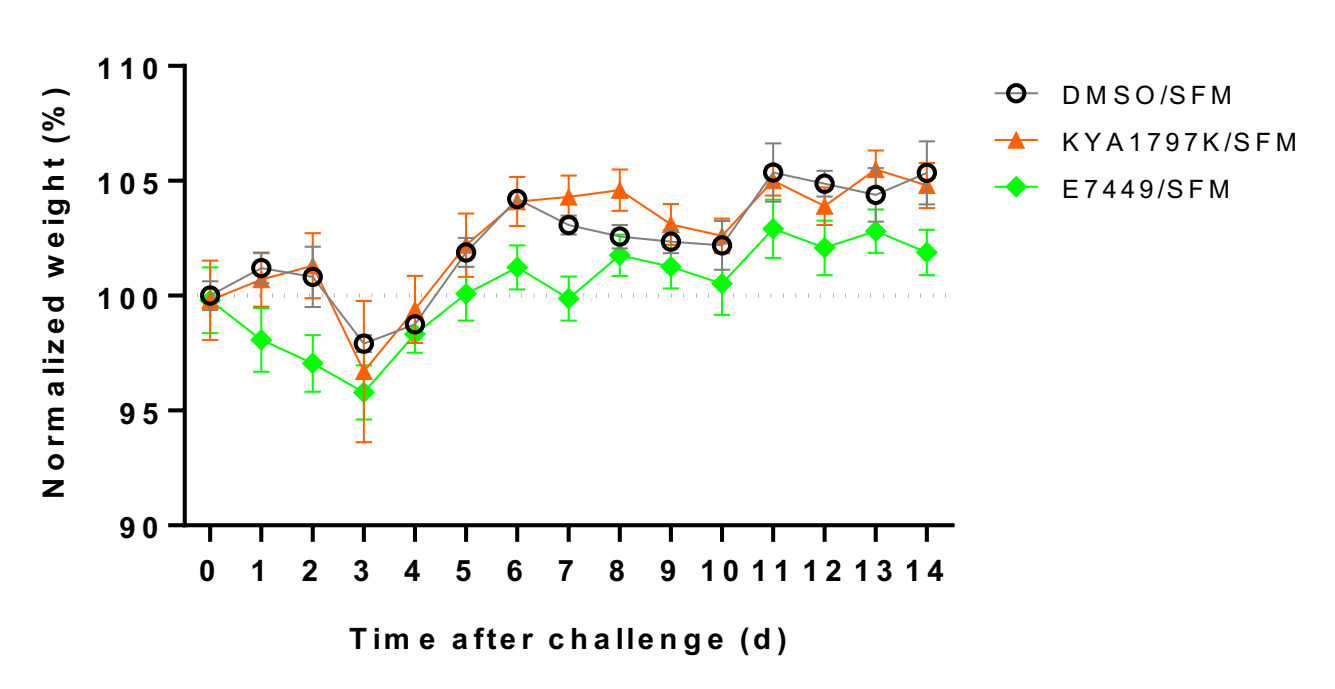
Wnt inhibitors have modest effect on virus-induced weight loss



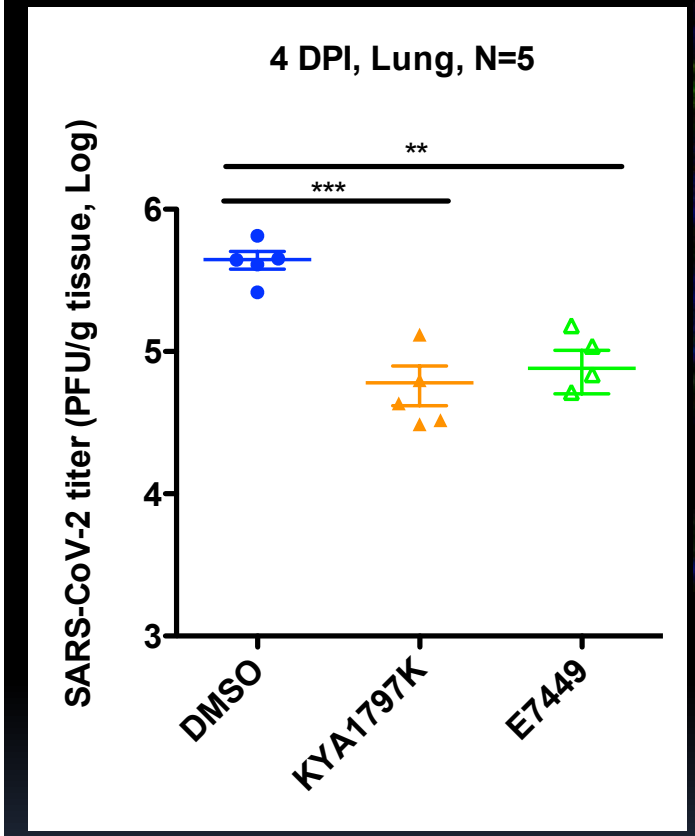


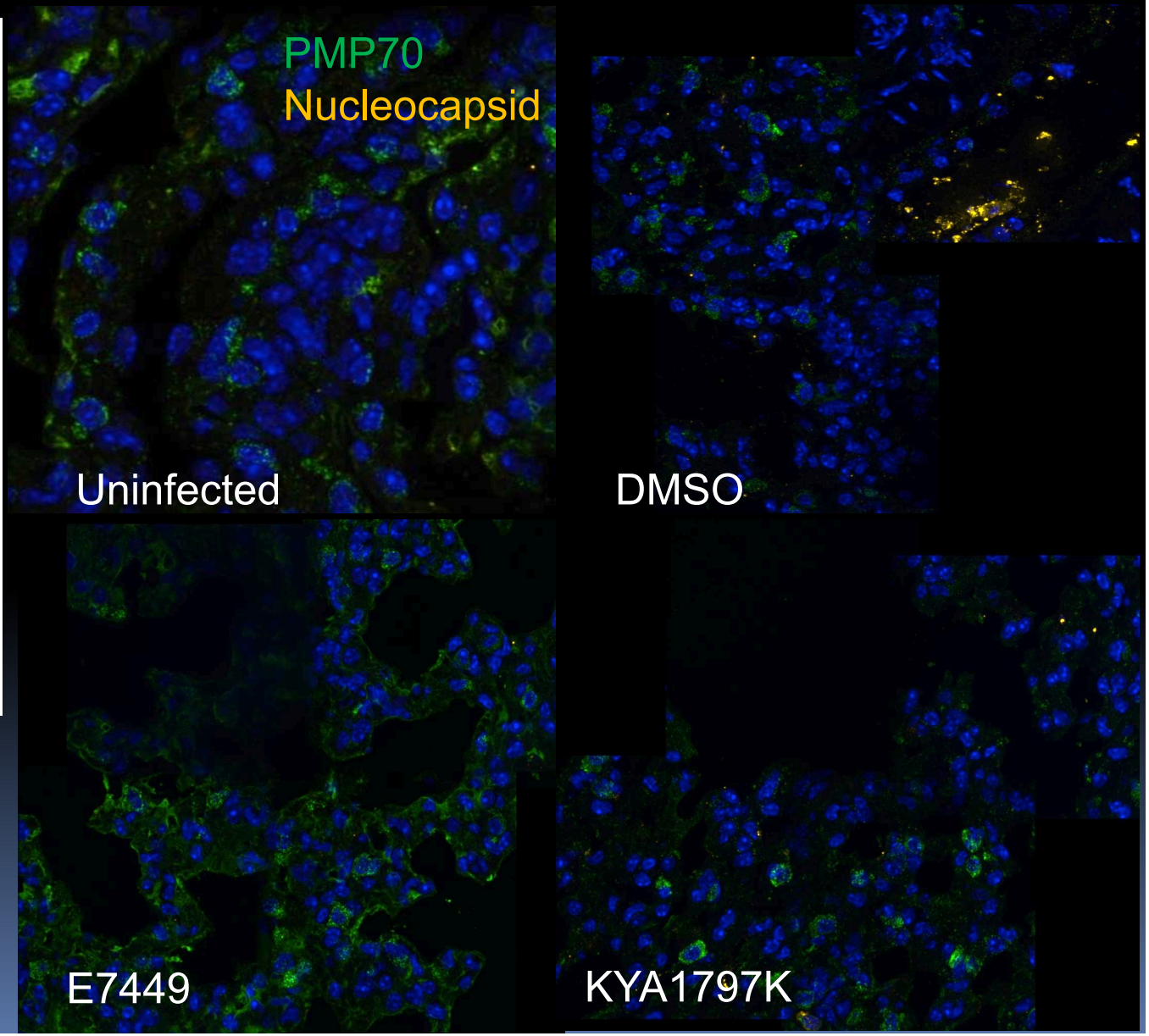
Xu, Elaish, Wong et al, in preparation

Anesthesia/IN drug administration causes mild transient weight loss



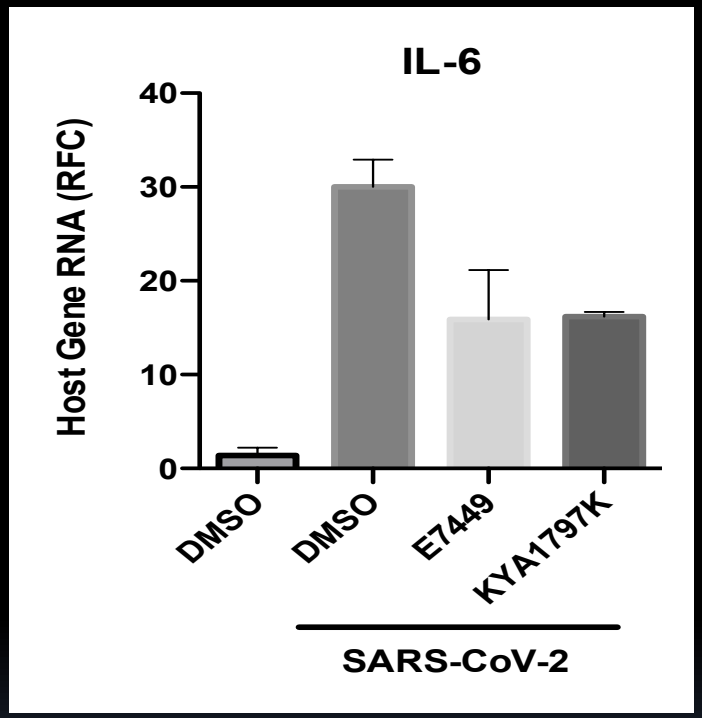
Wnt inhibitors reduce viral load in lungs

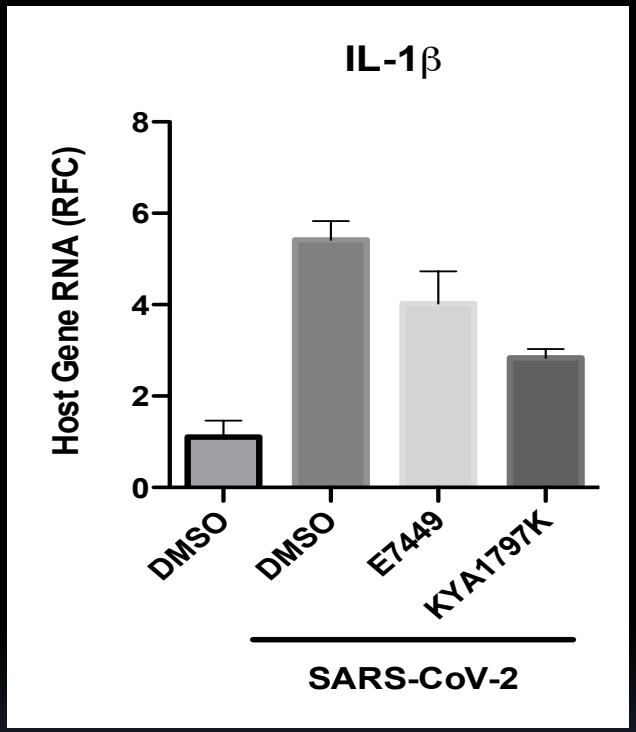




Xu, Elaish, Wong et al, in preparation

Wnt inhibitors reduce proinflammatory markers in lungs

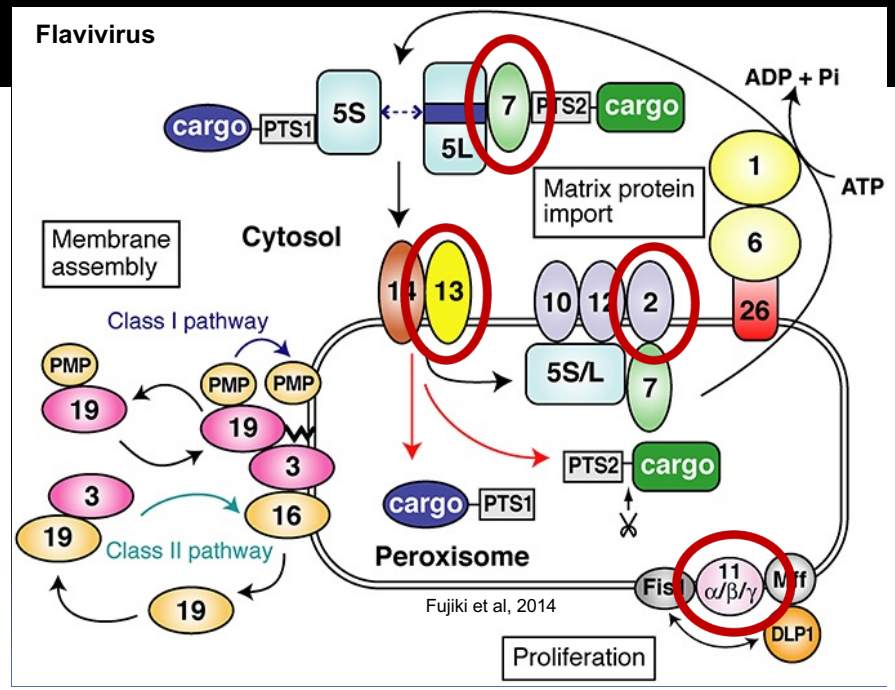


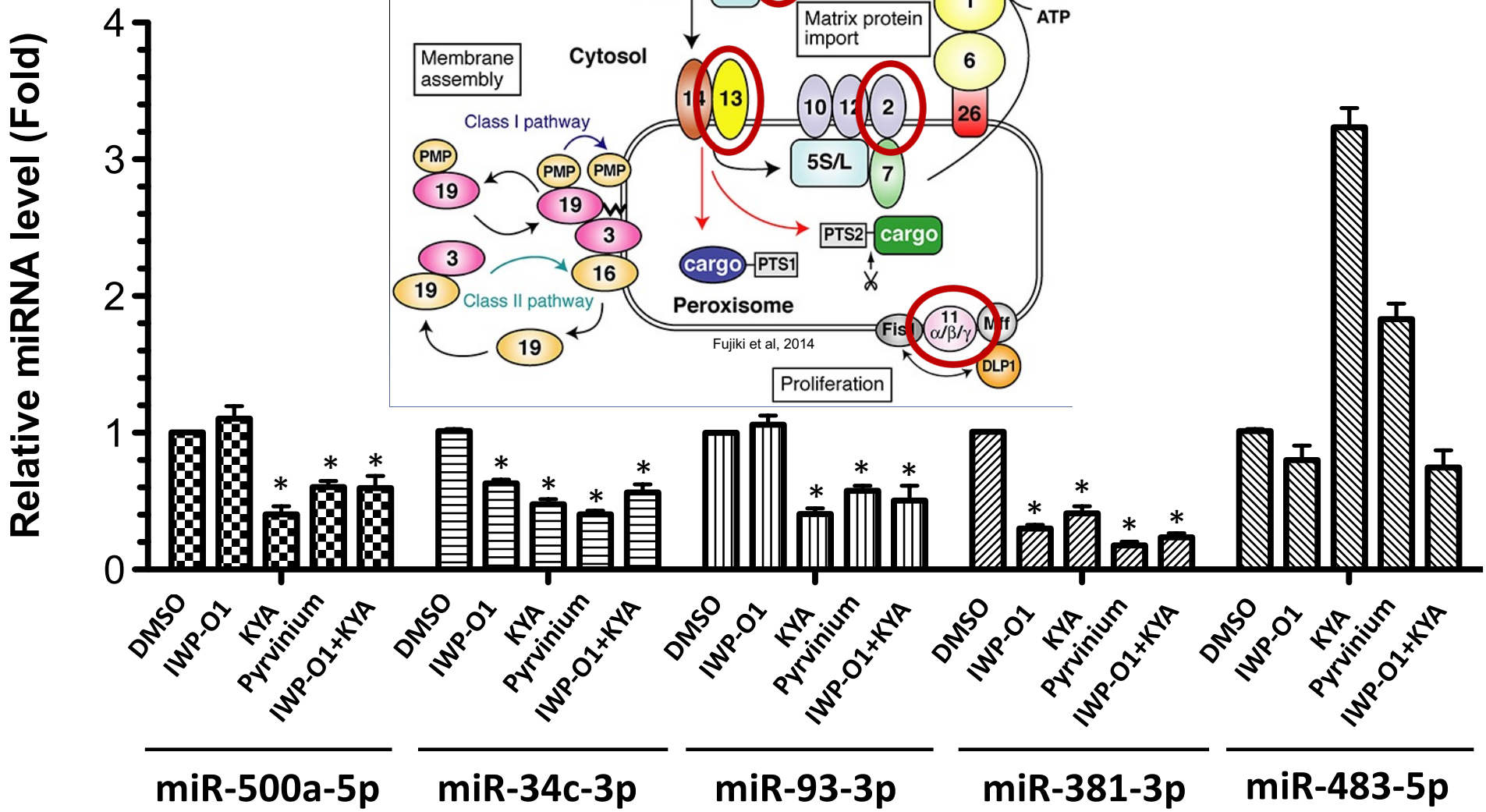


4-days post-infection

Xu, Elaish, Wong et al, in preparation

Wnt inhibitors reduce expression of miRNAs that suppress peroxisome biogenesis





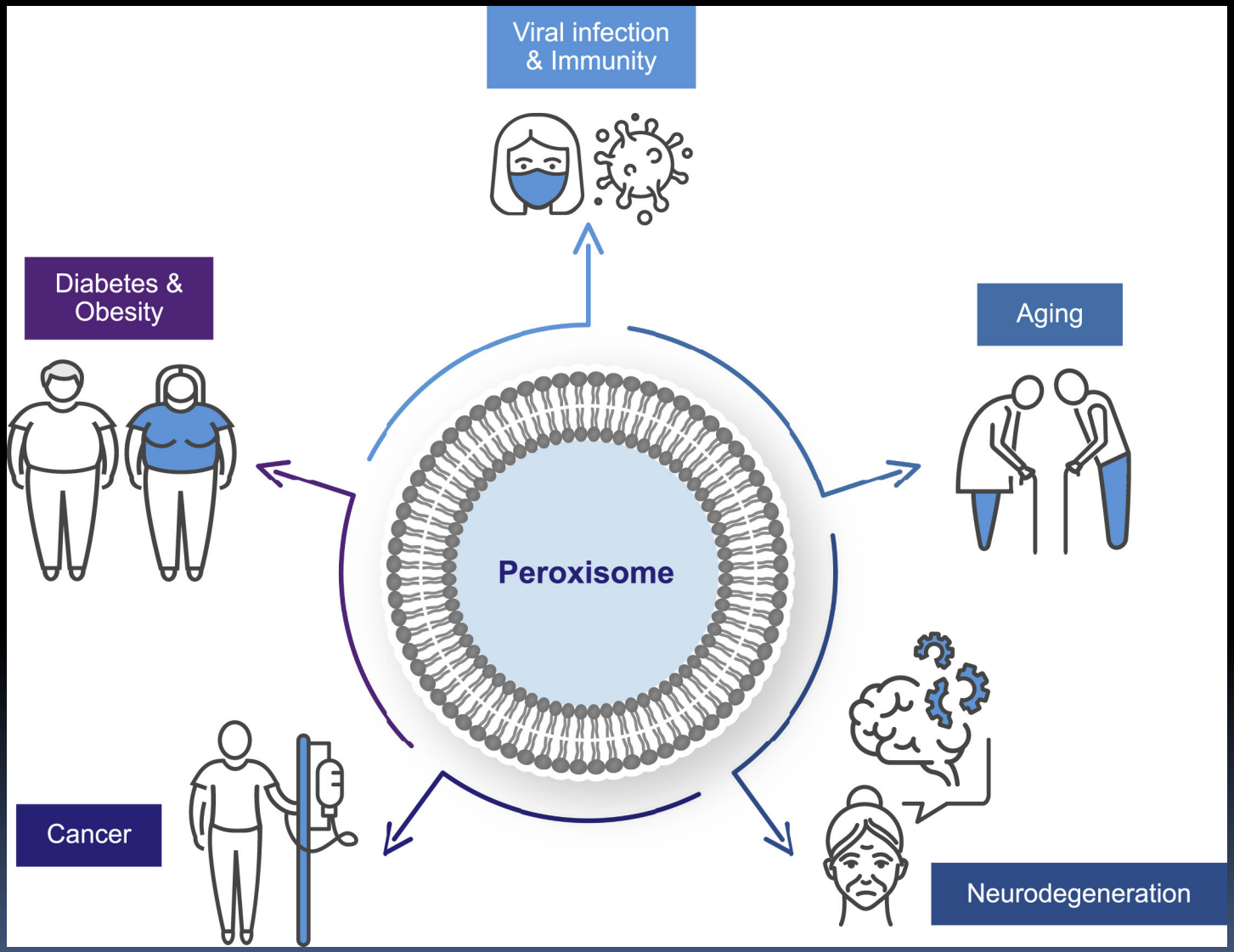
Summary

- Inhibition of Wnt/β catenin pathway induces peroxisomes and enhances IFN response during viral infection
- Significantly reduces SARS-CoV-2 replication *in vitro* and *in vivo*
- Broad-spectrum activity against other RNA viruses

Potential benefits of targeting peroxisomes for antiviral therapy

- Drug candidates with good safety profiles
  - Wnt inhibitors
  - Peroxisome proliferator-activated receptor agonists
- Reduce inflammation?
- Prophylactic and early therapeutic use?
- *Do not induce IFN in absence of viral infection*

Inducing peroxisome biogenesis may have multiple health benefits



Zalckvar and Schuldiner, 2022

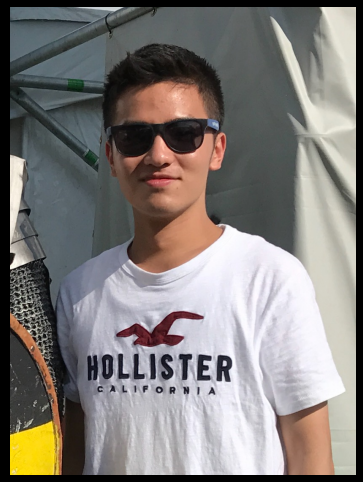
Ongoing/Future studies

- *In vivo* efficacy of post-infection administration of Wnt/β catenin inhibitors
- Increase bioavailability of drugs?
  - Oral?
  - Nebulizer?
  - Derivatives?
- Testing other Wnt/β catenin inhibitors and peroxisome proliferators alone and in combination
- High throughput screening for novel peroxisome-inducing drugs

Acknowledgements

Dr. Zaikun Xu

Dr. Mohamed Elaish



Dr. Jason Wong

Wil Branton
Dr. David Evans
Ray Ishida
Dr. Barbara Knoblach
Dr. Joaquin Lopez Orozco
Dr. Lara Mahal
Dr. Irv Mayers
Dr. Ryan Noyce

Dr. Natacha Ogando
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