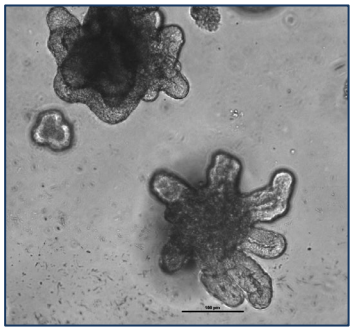
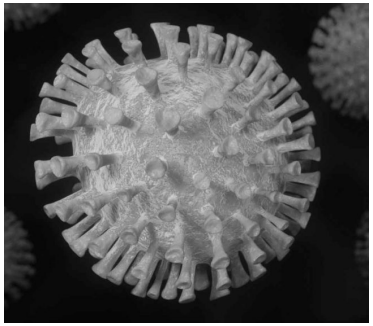
Bat and human gastrointestinal organoids as *in vitro* models to define pathogenic and protective responses to SARS-CoV-2 infection



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Associate Professor of Microbiology and Immunology

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Department of Microbiology & Immunology

Diane Bimczok

Mucosal Immunology

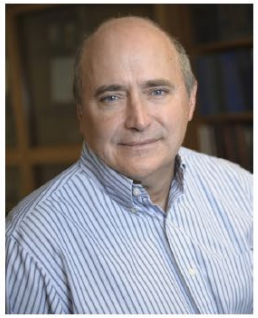
Marziah Hashimi
Michelle Cherne
Andy Sebrell
Katrina Lyon

Seth Walk

Gut Microbiome

Brittany Jenkins

Mark Jutila



Immunology

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Amanda Robison

Chemical & Biological Engineering Department

Connie Chang

Drop-based Microfluidics

Humberto Sanchez
Emma Loveday

James Wilking

Soft Matter Physics

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Vincent Munster, NIH
Christiane Wobus, U Michigan

BAT ONE HEALTH

Raina Plowright, MSU

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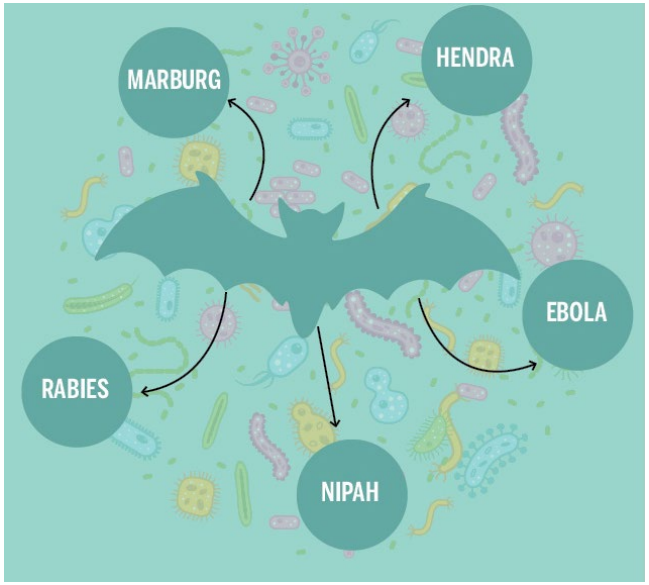
Tony Schountz

Bats are key reservoir hosts for multiple zoonotic viruses

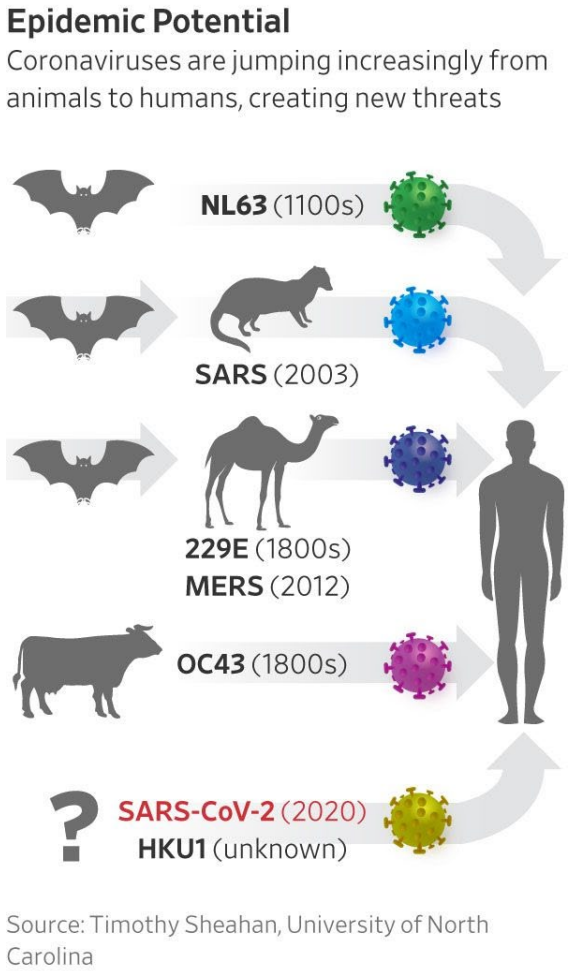
- Bats are key reservoirs for zoonotic viruses including filoviruses, henipahviruses and coronaviruses (Letko, Nature Rev. Microbiol. 2020)
- Bats harbor a large number of coronaviruses in their guts without developing disease (Watanabe 2010, Banerjee 2019).
- Bats have unique antiviral response pathways that enable them to tolerate viral infections (Schountz, Viruses 2014)



➢ **Understanding the pathways involved in viral tolerance in bat cells may point to new treatment approaches in humans**

Bats as reservoir hosts for SARS-CoV-2?

- Bats (Chinese horseshoe bats) are thought to be the reservoir host for SARS-CoV-2 (Li, Infect Genet Evol. 2020).
- Bat coronavirus RaTG13 is closely related to SARS-CoV-2 – 96% sequence identity (Zhou, Nature, 2020).
- Other zoonotic coronaviruses have been traced back to bats (Letko, Nat Rev. Microbiol. 2020).



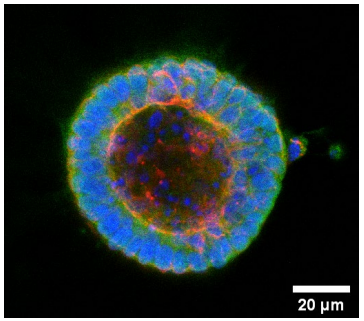
Hurdles to investigating infectious diseases in bats

- Regulatory hurdles in working with wild bats
- Few research colonies, specific requirements
- Very few cell models available
- No commercially available antibodies and reagents

Development of a microphysiological model to study bat responses to SARS-CoV-2

1. Bat gastrointestinal organoids

- Organoids are permanent 3-D cultures of non-transformed organ-specific cell types
- Intestinal organoids from horseshoe bats have been shown to support SARS-CoV-2 infection in a landmark study from China (Zhou et al. Nature Med. 2020)

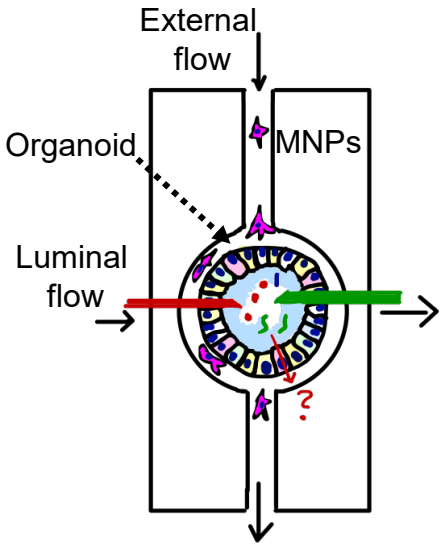


2. Modeling SARS-CoV-2 infection in a microphysiological platform

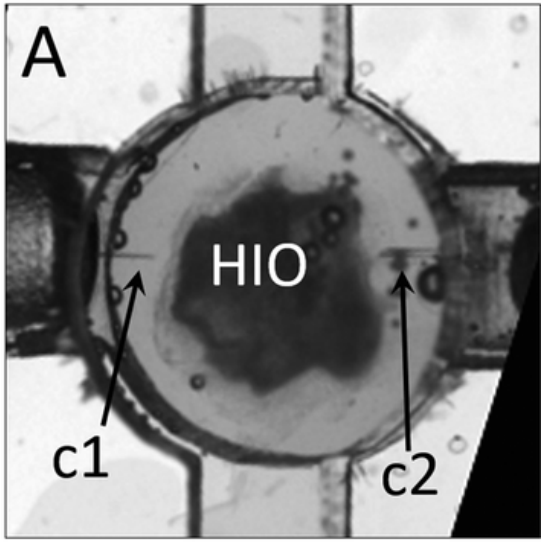
- Fluidics enable controlled application and sampling of virus, mediators, potential treatments, etc.
- Co-culture of immune cells with mucosal epithelium enables analysis of complex tissue responses

The GoFlowChip platform

- Incorporates 3-D gastrointestinal organoids in their natural conformation
- Organoids embedded in extracellular matrix material

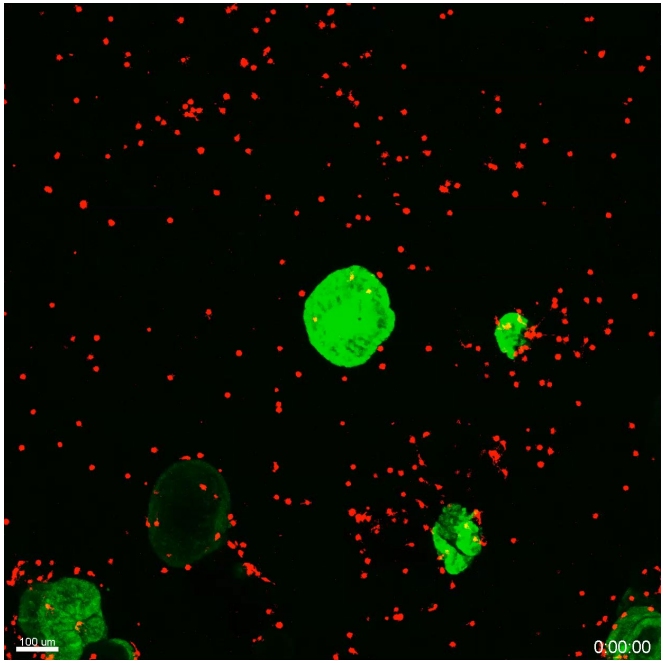


Original design with internal and external flow

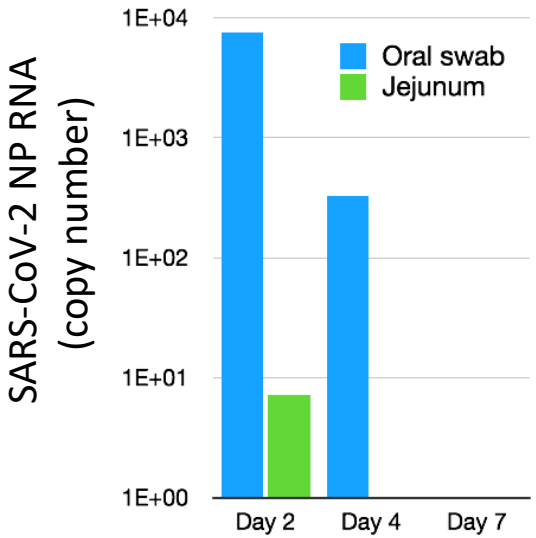


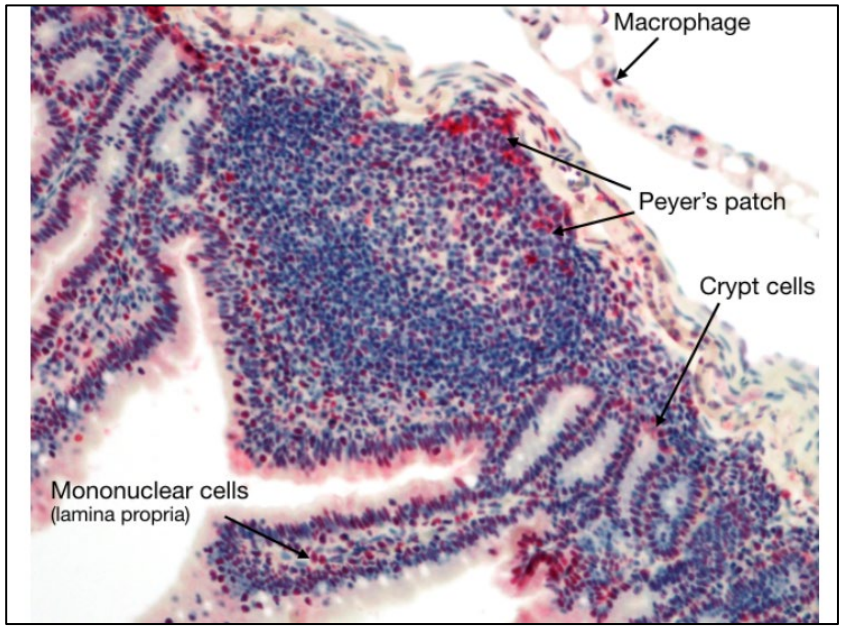
Sidar et al. LabChip 2019

Incorporation of mononuclear phagocytes



Jamaican Fruit Bats are Susceptible SARS-CoV-2 Infection



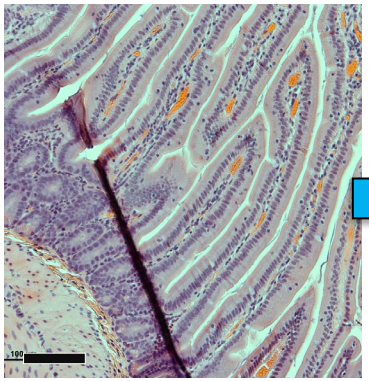


Data kindly provided by Dr. Tony Schountz, Colorado State University

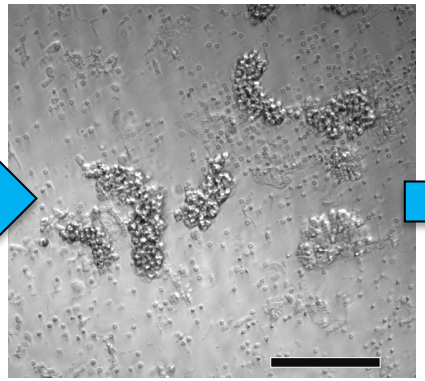
Key growth factors for gut organoids are highly conserved in mammals

Optimization of bat small intestinal organoid derivation and growth

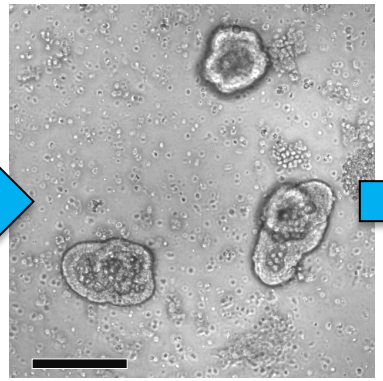
Small intestinal tissue



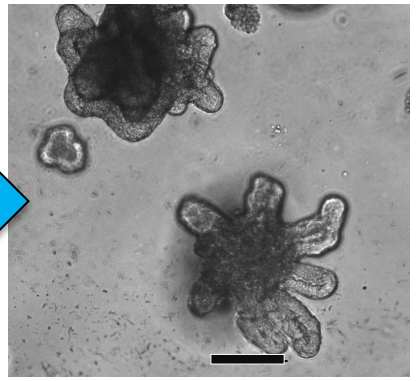
Isolated crypts



Organoid formation



Passage and differentiation



Crypt isolation

Growth media

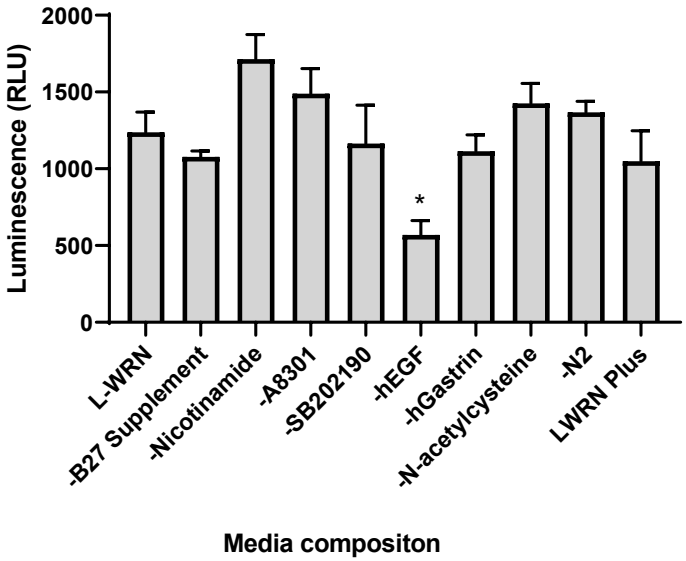
Differentiation protocols

Optimization of Jamaican fruit bat organoids derivation and growth

Organoid lines derived

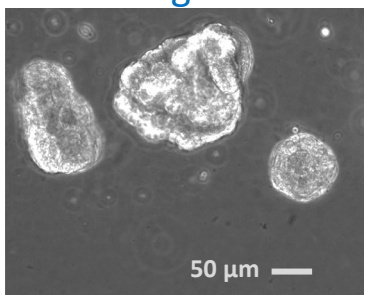
Bat #	Sex	Organs	Sample type	Max. passage
Bat001	M	Stomach Prox. SI Dist. SI	Fresh	29
Bat002	M		Fresh & frozen	13
Bat003	F		Fresh & frozen	13

Optimization of growth media

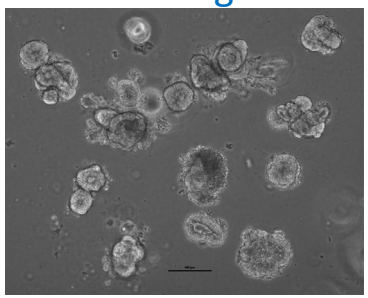


Successful reconstitution after freezing/thawing

Gastric organoids

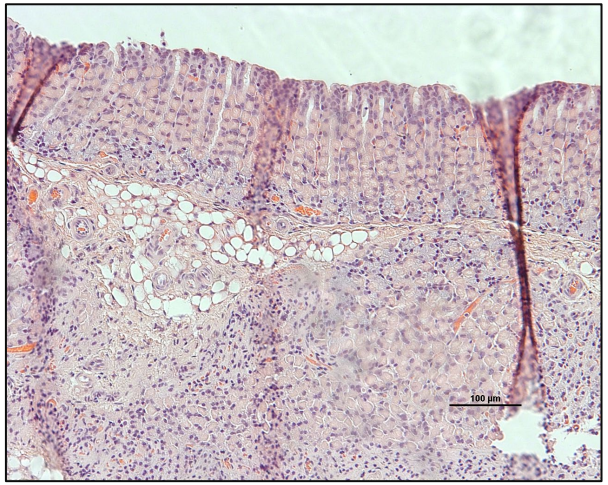


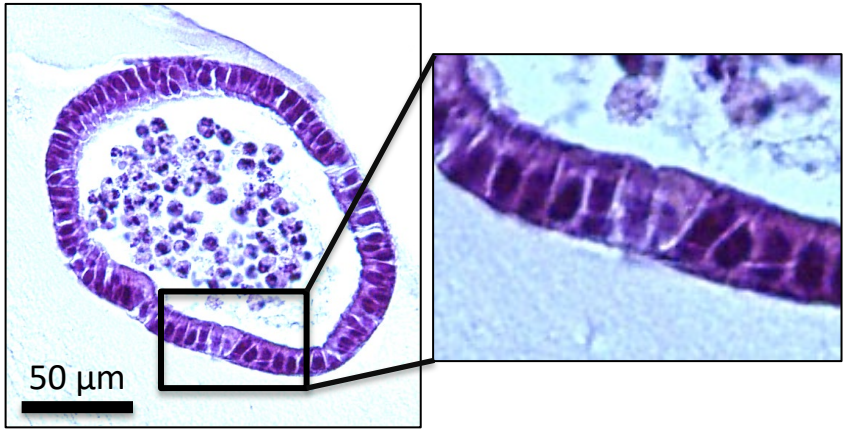
Intestinal organoids



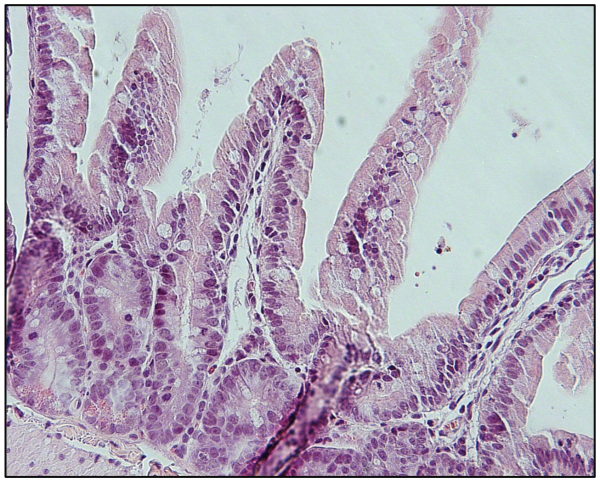
Morphology of fruit bat organoids

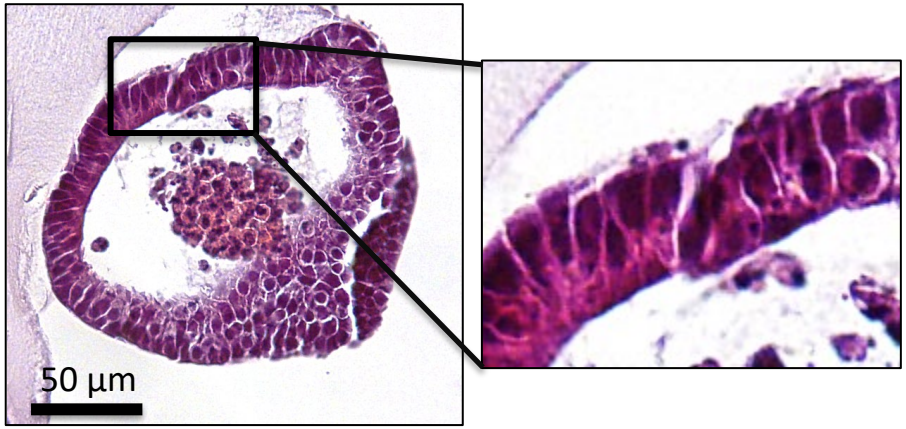
Stomach





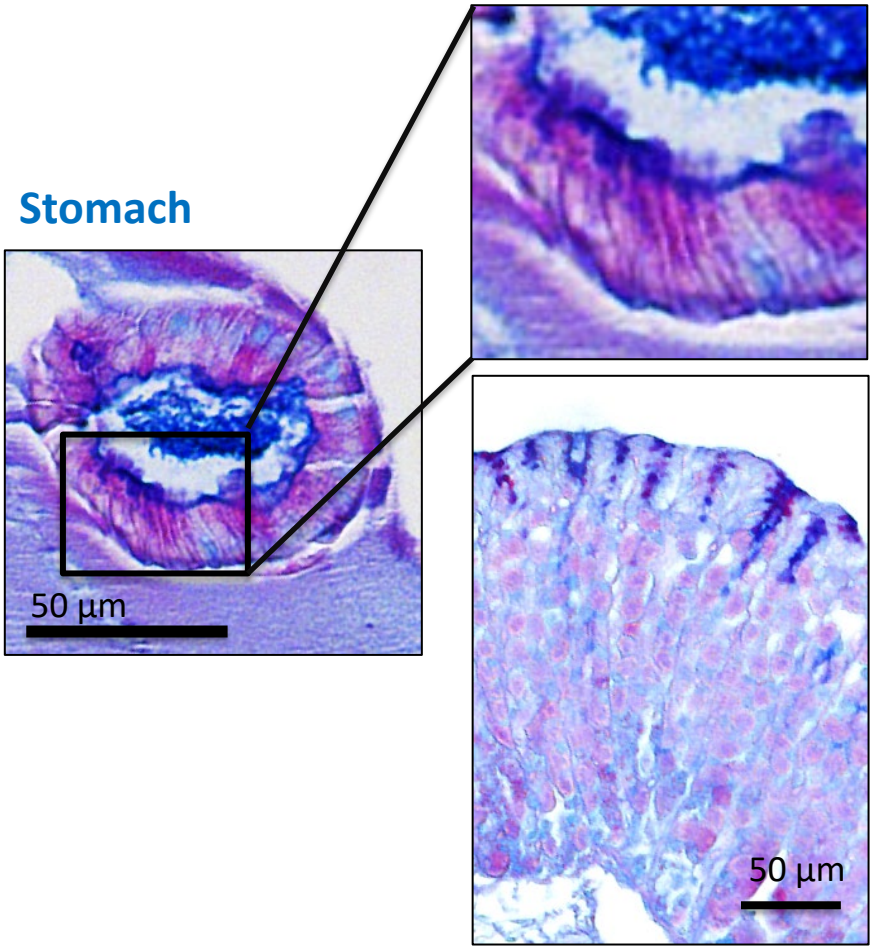
Small intestine



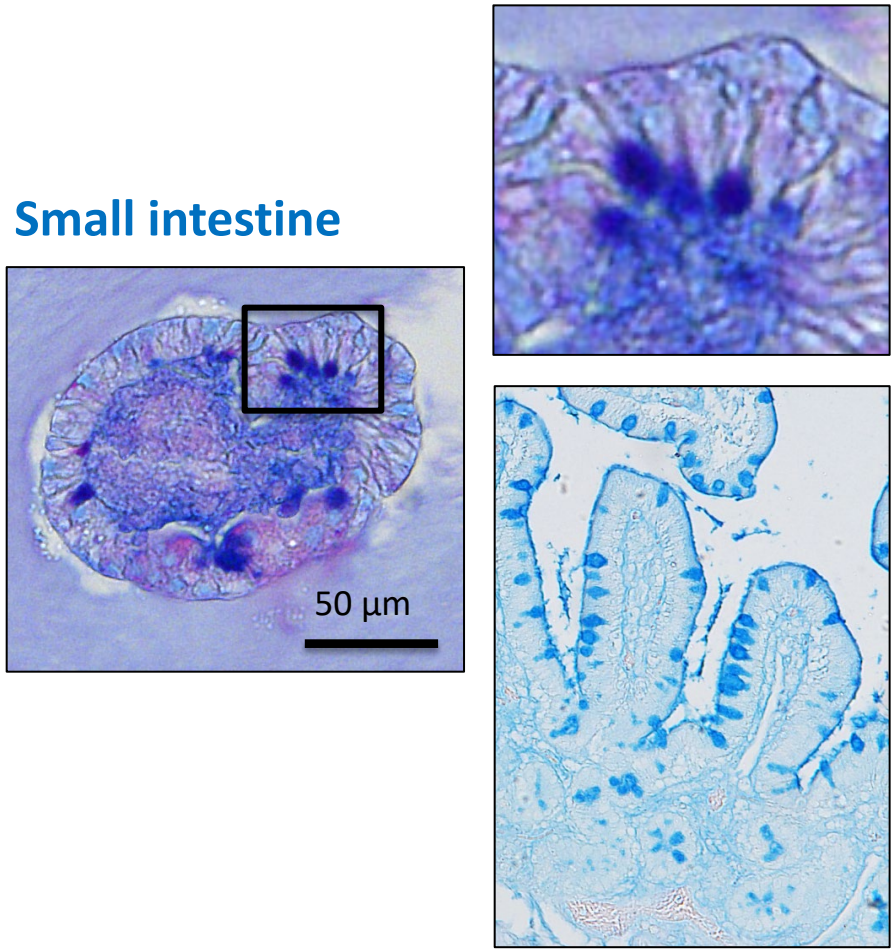


Tissue-specific differentiation of mucus-producing cells – PAS staining

Stomach

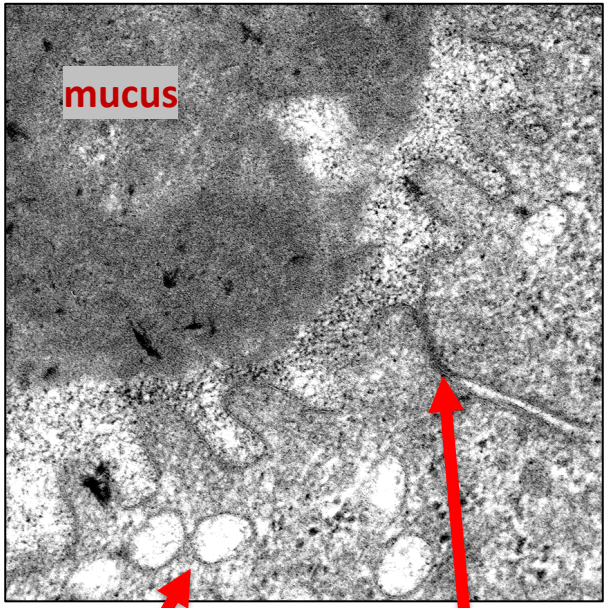


Small intestine



Electron microscopy reveals microvilli, tight junctions and secretory vesicles

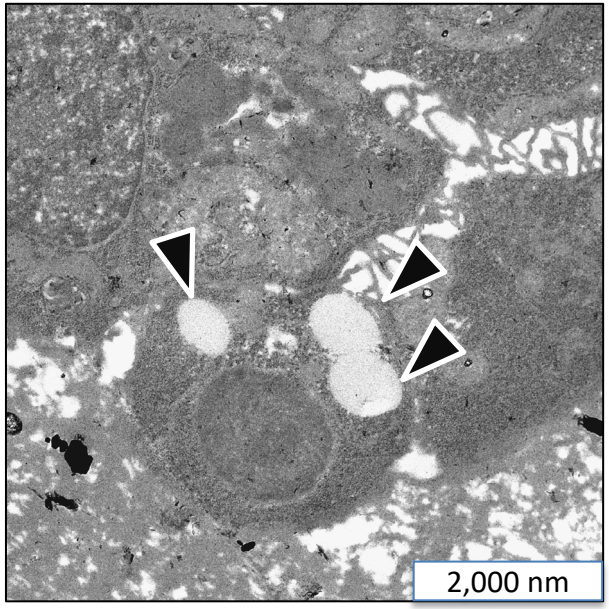
Stomach



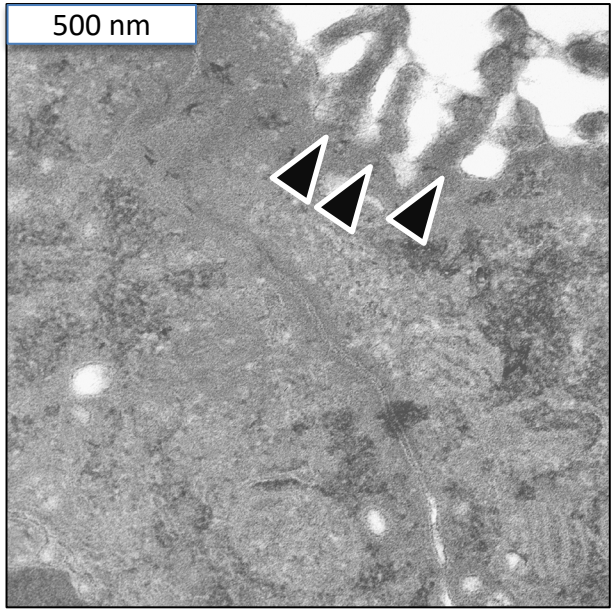
Vesicles

Tight junction

Distal small intestine

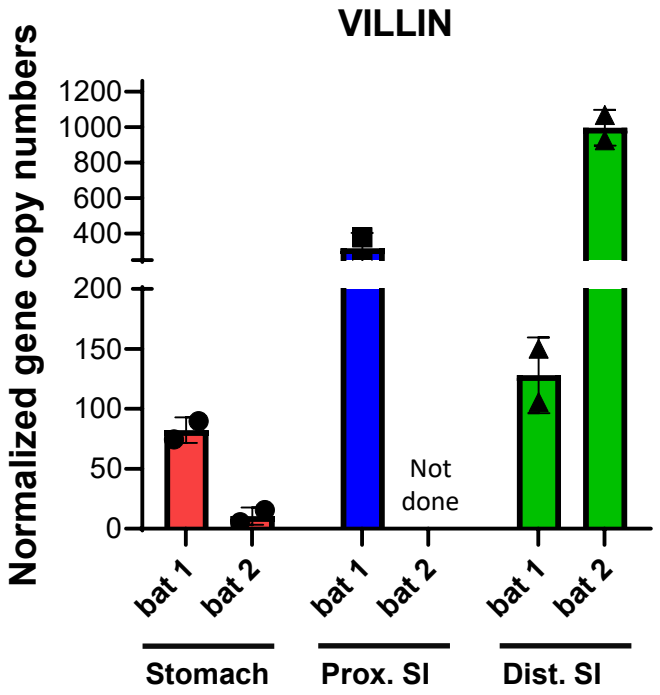


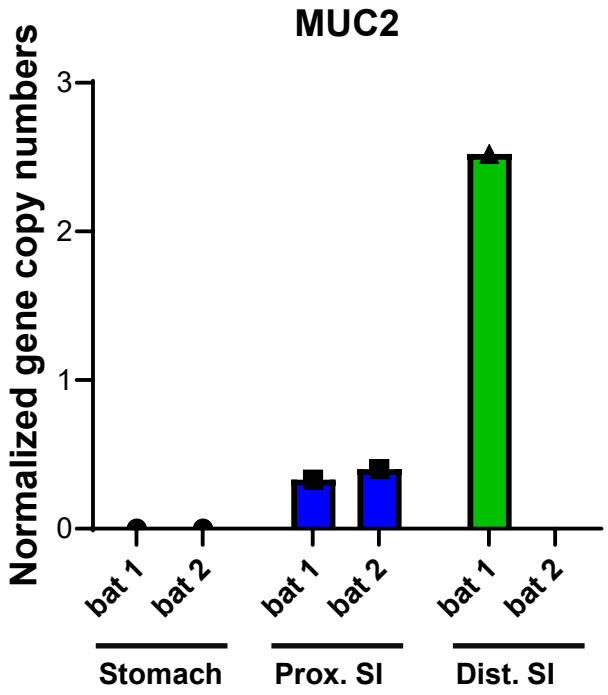
Secretory vesicles



Microvilli

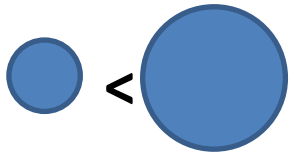
Bat organoids show tissue-specific gene expression patterns

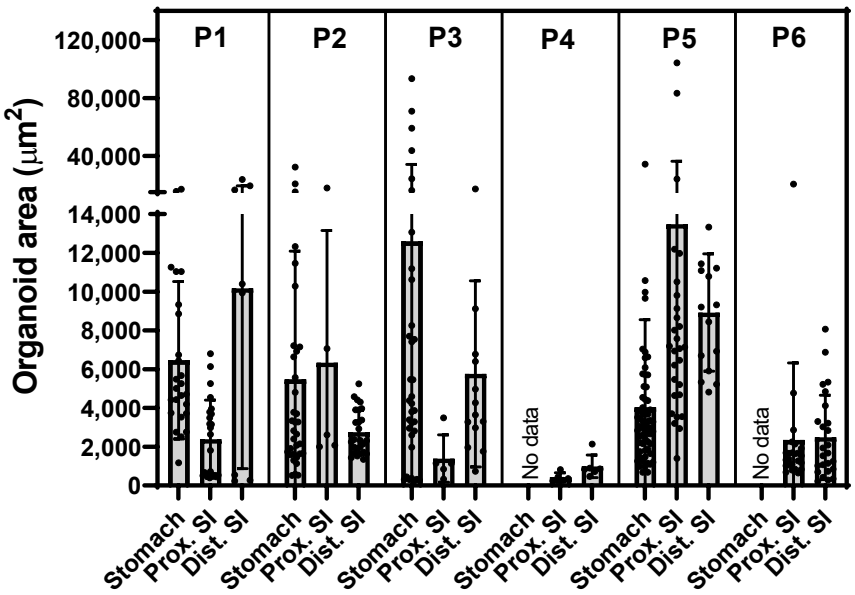




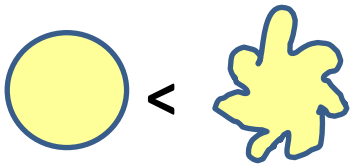
Bat organoids remain stable over multiple passages

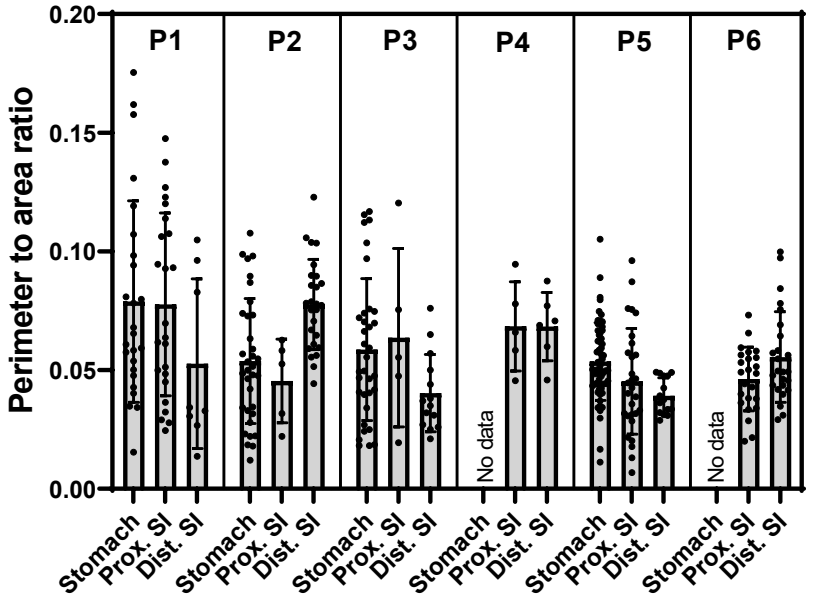
Area



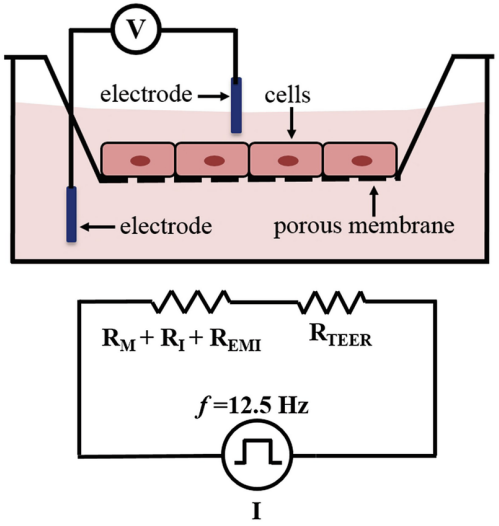


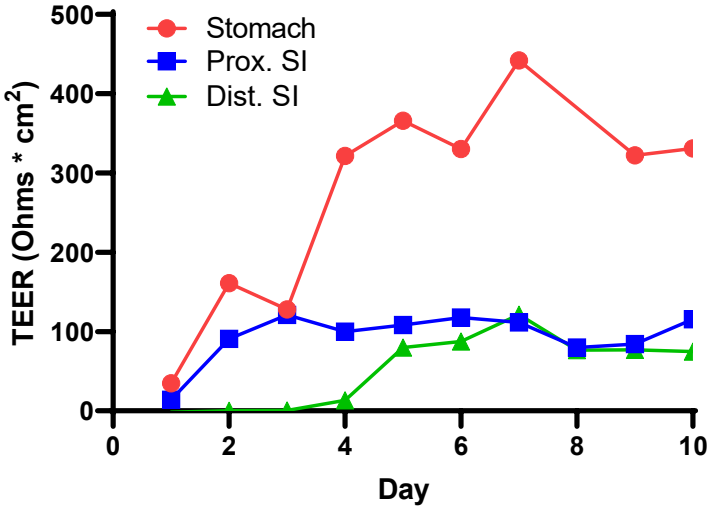
Perimeter vs. area

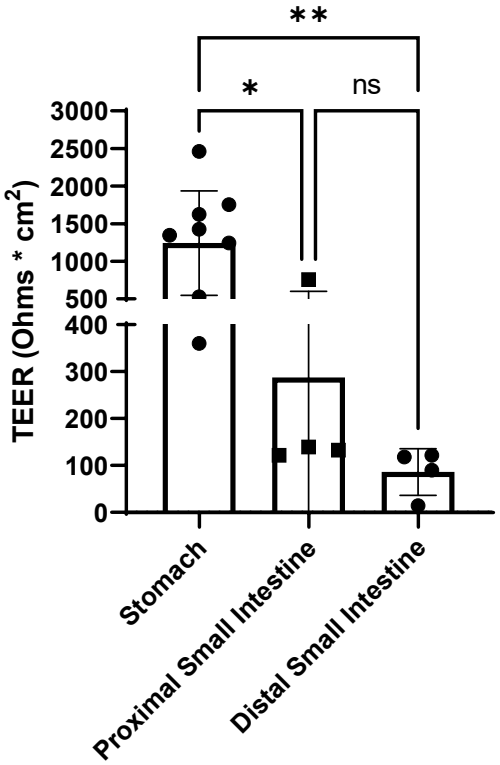




Evidence for epithelial barrier function in bat organoids

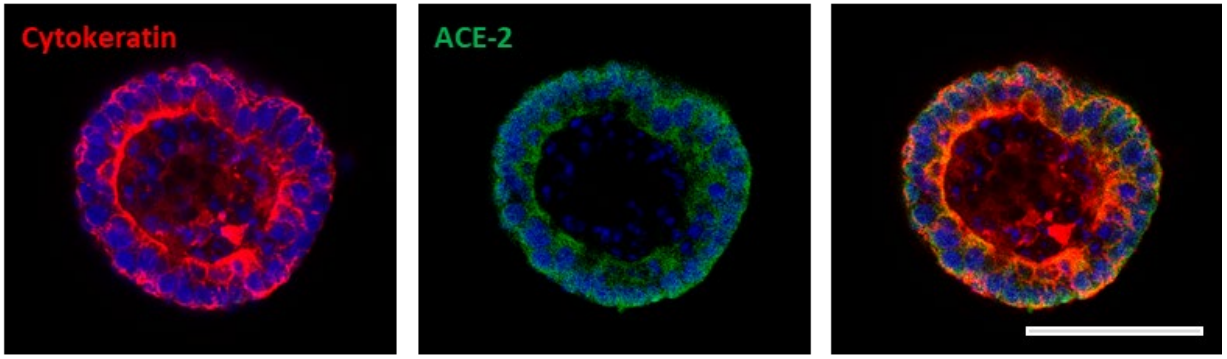


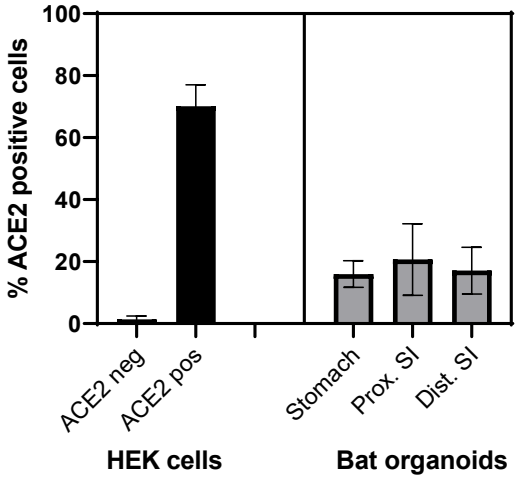




Are bat gastrointestinal organoids susceptible to SARS-CoV-2 infection?

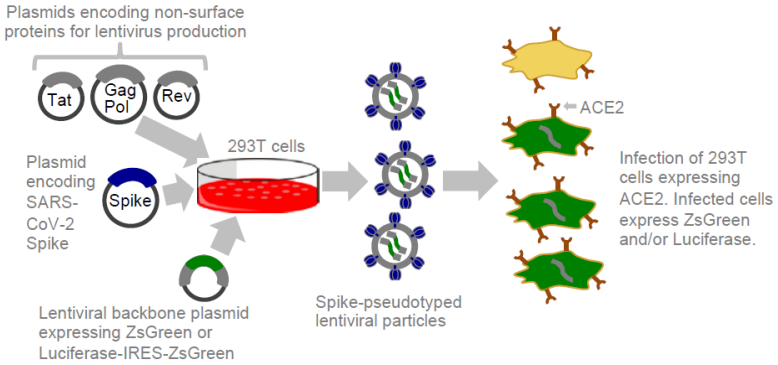
ACE-2 expression

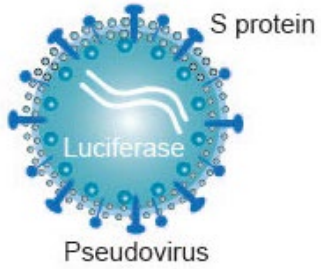


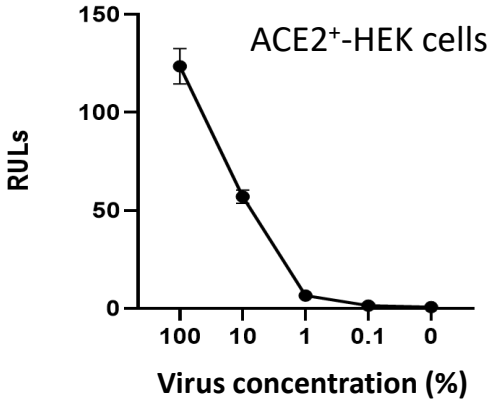


SARS-CoV-2-spike lentiviral reporter pseudovirus

(Crawford et al., Viruses 2020, 12, 513)







SUMMARY

- Developed and characterized gastrointestinal organoids from Jamaican fruit bats.
- Optimizing SARS-CoV-2 infection protocol of human and bat organoids on and off chip
- **Goal:** Compare primary gastrointestinal epithelial and immune responses of bat and human cells to SARS-CoV-2