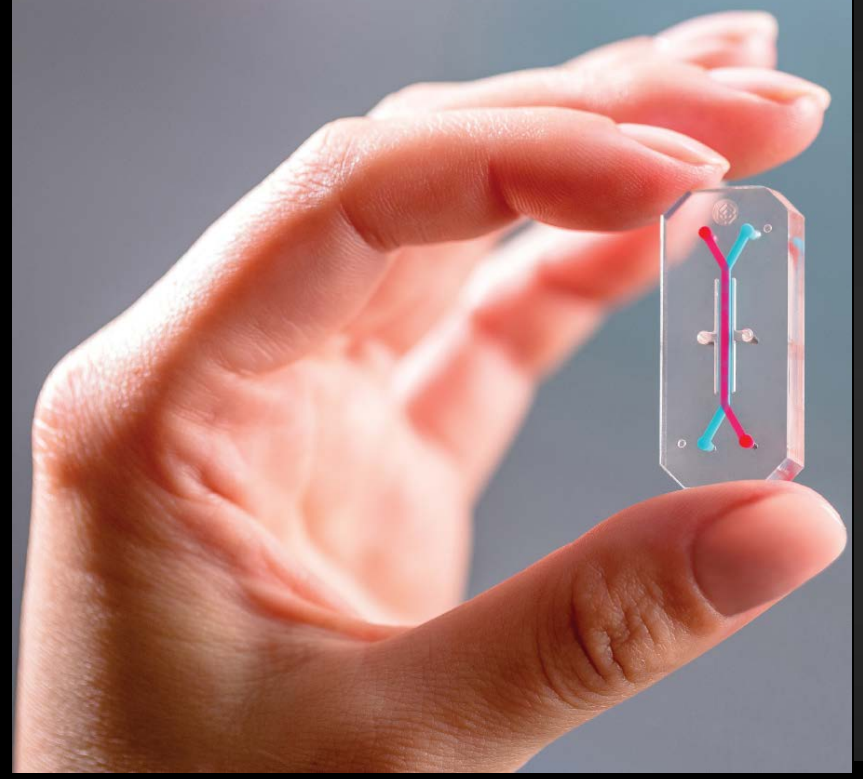

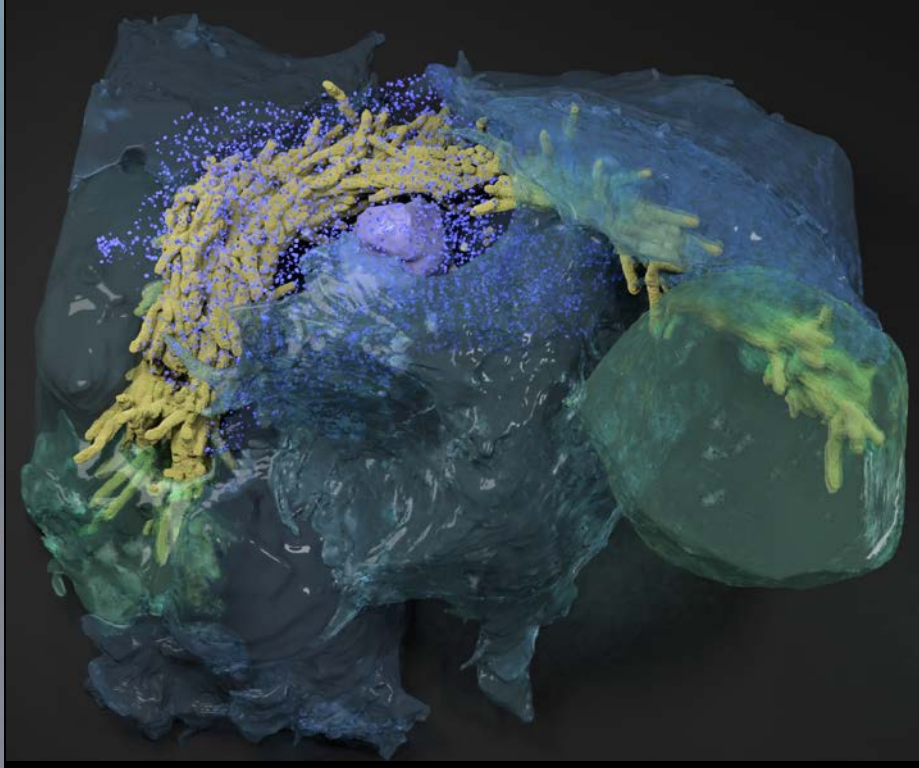

Lung-on-chip model systems to study host-pathogen interactions in respiratory infections

Vivek Thacker

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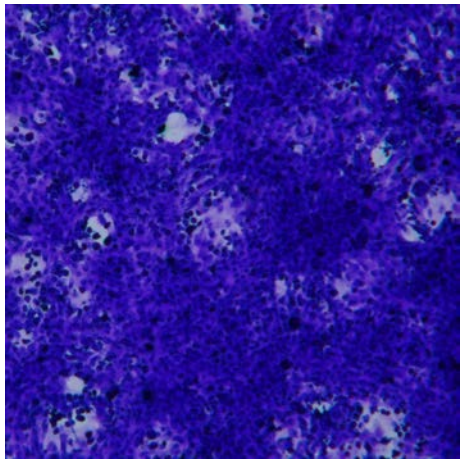
Why do we need new model systems? *Emerging* viruses

In vitro Host complexity ***In vivo***

~~Axenic culture~~ Monoculture infection model ~~Animal model~~



Dynamics

?

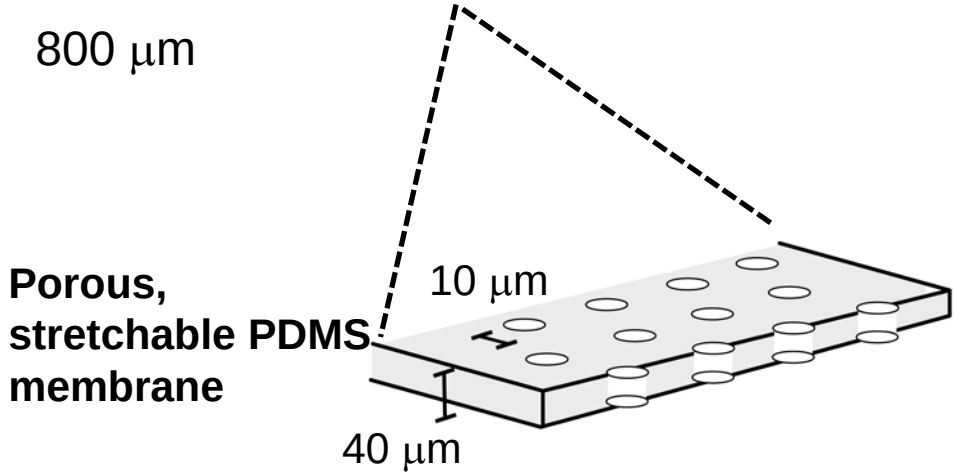
Individuality

Individuality	~~Yes (pathogen)~~	Yes (host and pathogen)	~~No~~
Cell-cell interactions	~~No~~	No	~~Complex~~
Tissue dynamics	~~No~~	No	~~Yes, but difficult to probe~~
Host	~~Absent~~	Simple	Complex

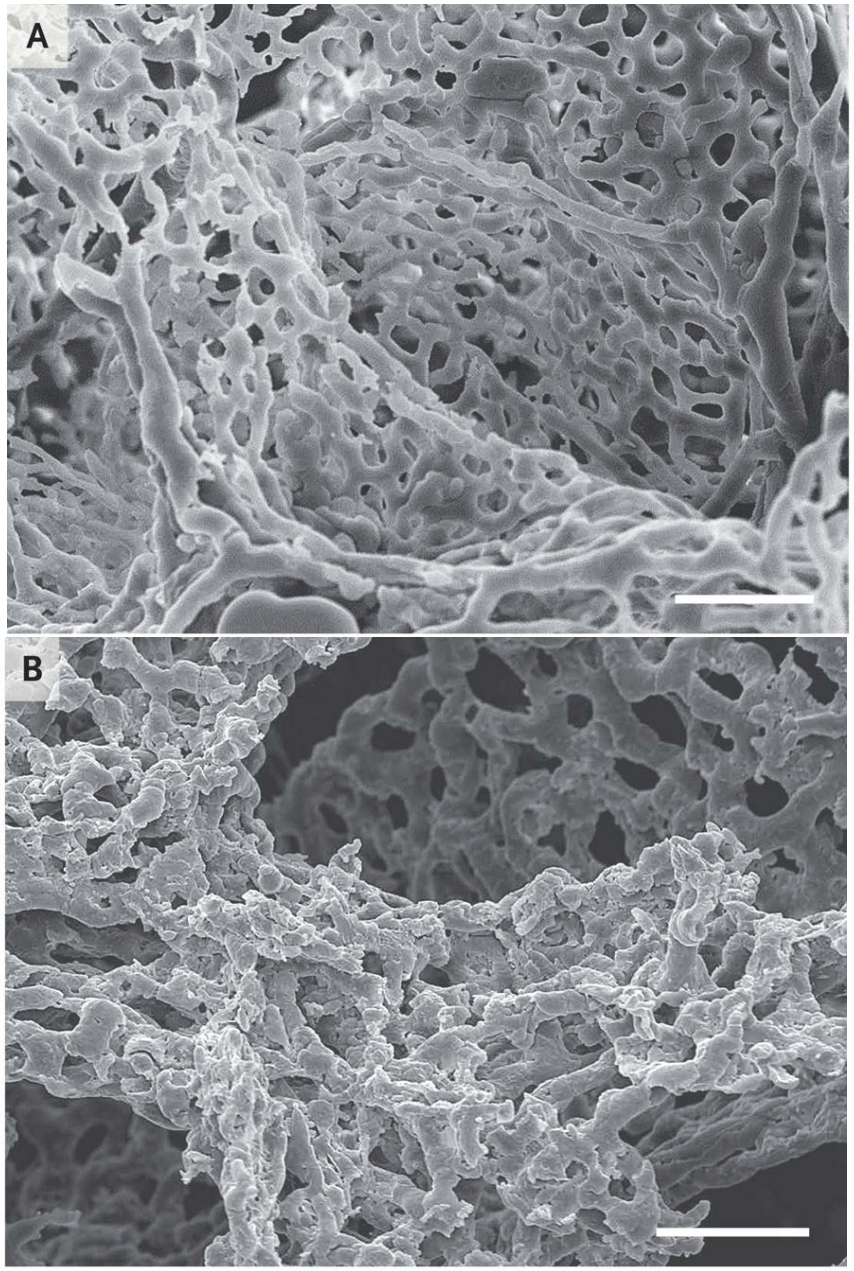
Emulate lung-on-chip (LoC) system

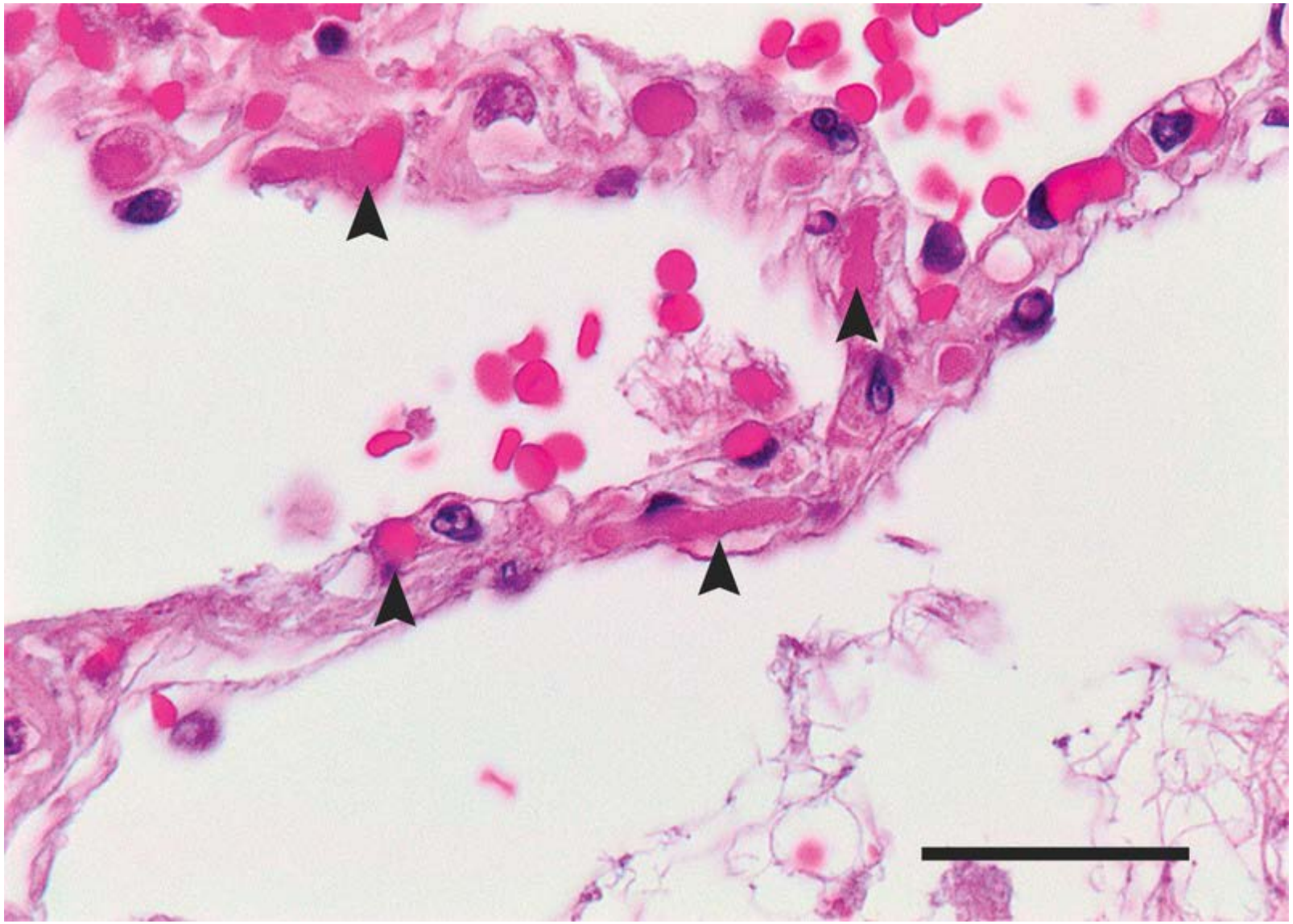
Top view

Cross-sectional view



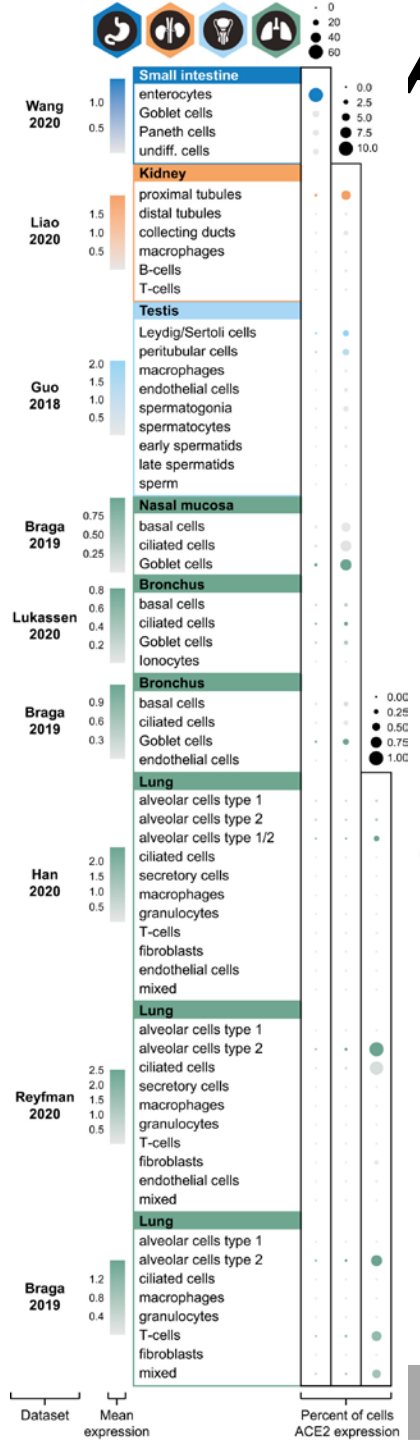
Microvasculature damage is a clinical hallmark of SARS-CoV-2



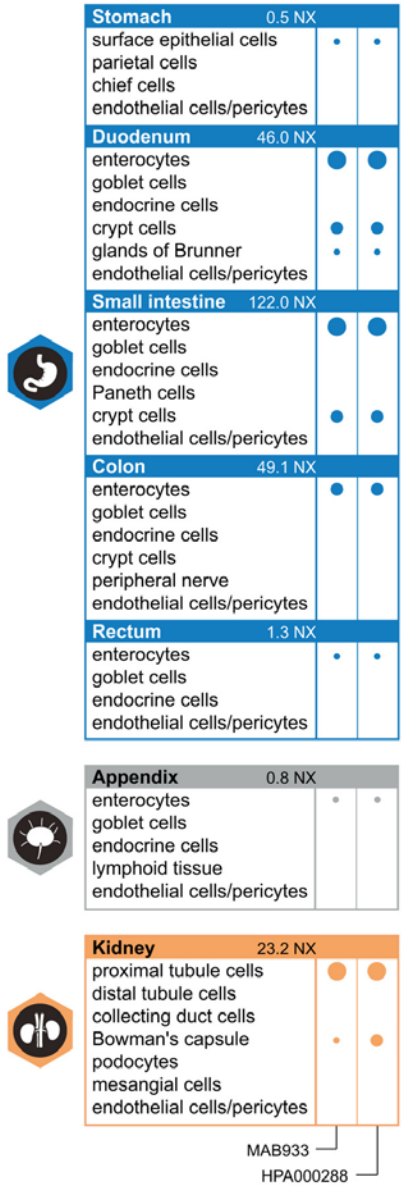


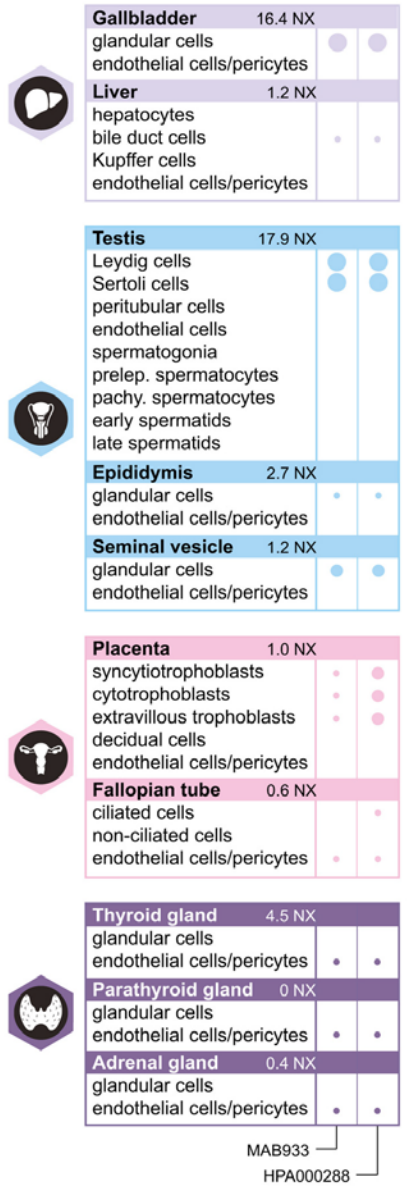
Images: Ackermann et al., **N Engl J Med** 2020; 383:120-128

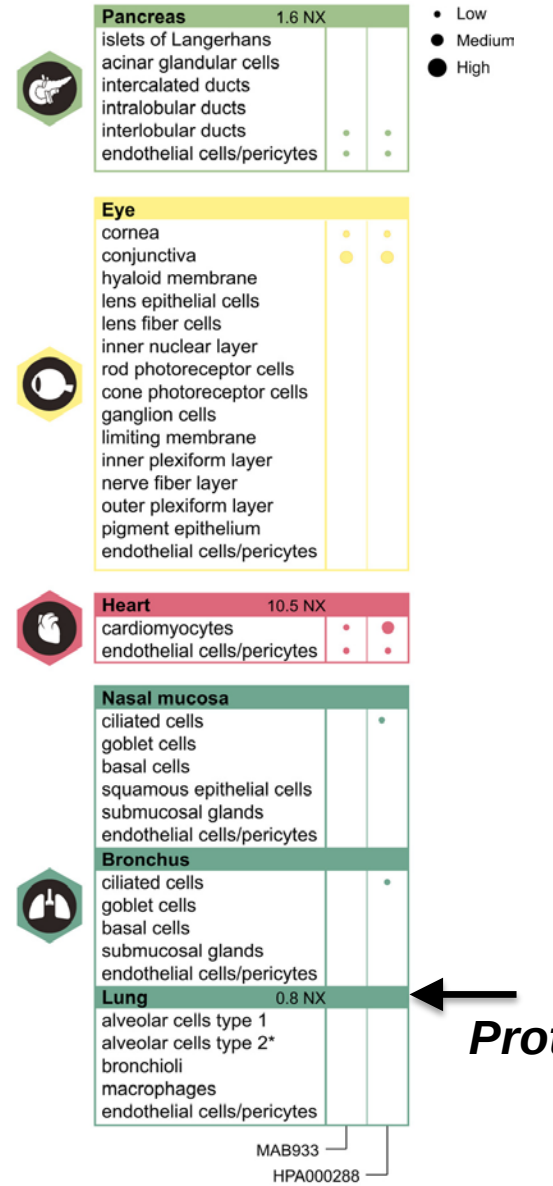
ACE2 expression is low in the alveolar space



Transcriptomics







Proteomics

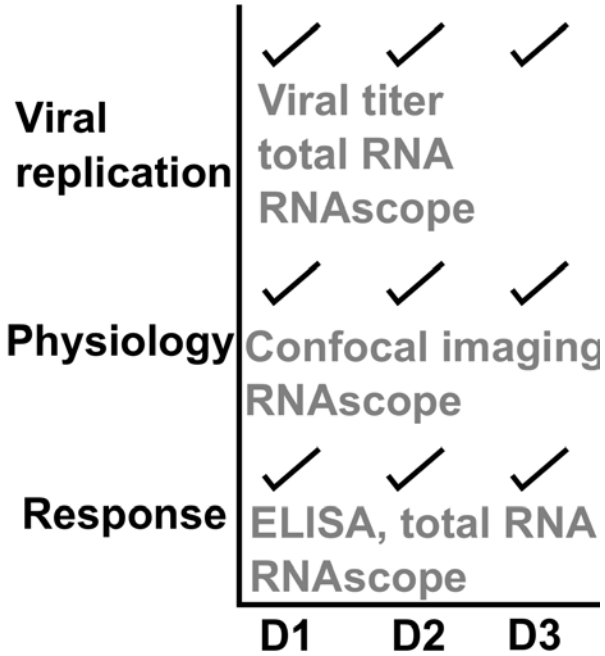
Data: Hilkmet *et al.*, **Mol Syst Biol** (2020)16:e9610

SARS-CoV-2 in the alveolar space

How does SARS-CoV-2 infection alter alveolar physiology?

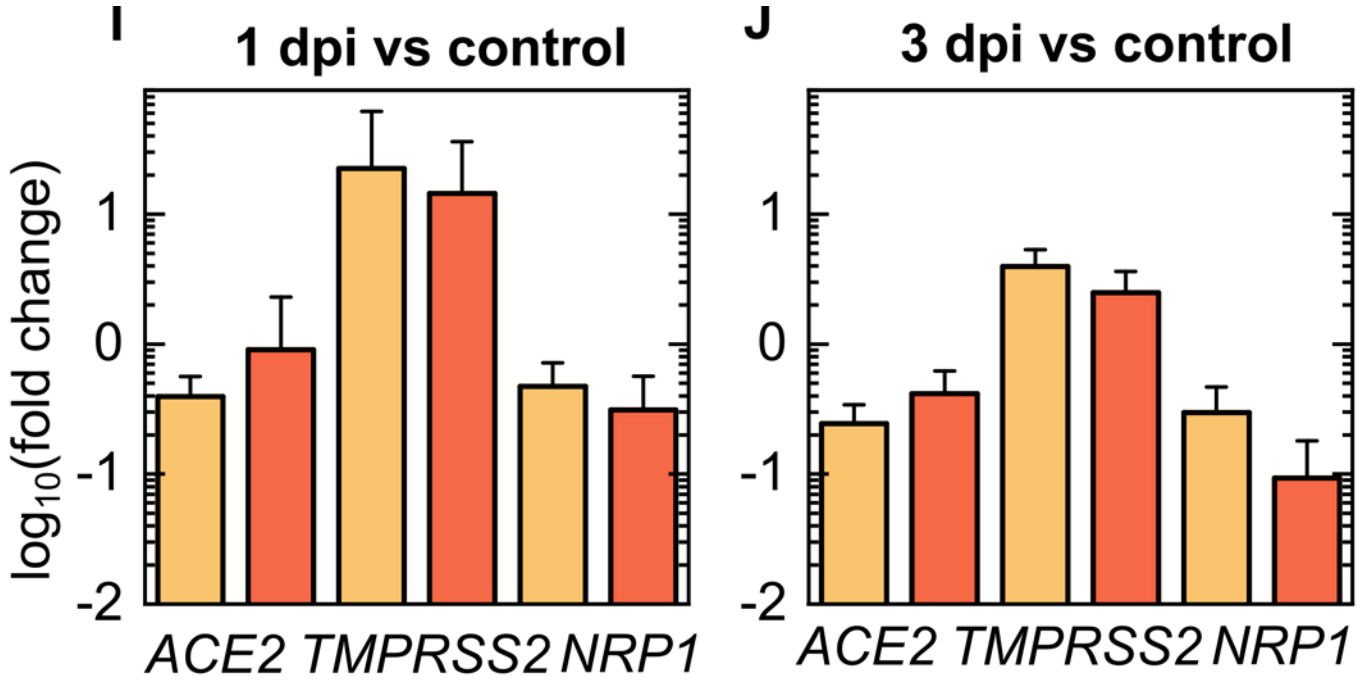
Why does severe disease manifest in the alveolar space despite low ACE2 levels?

LoC model for SARS-CoV-2



Preprint: VV Thacker *et al. (under review)*
bioarxiv:10.1101/2020.08.10.243220

Low *ACE2* expression, infection increases *TMPRSS2* expression



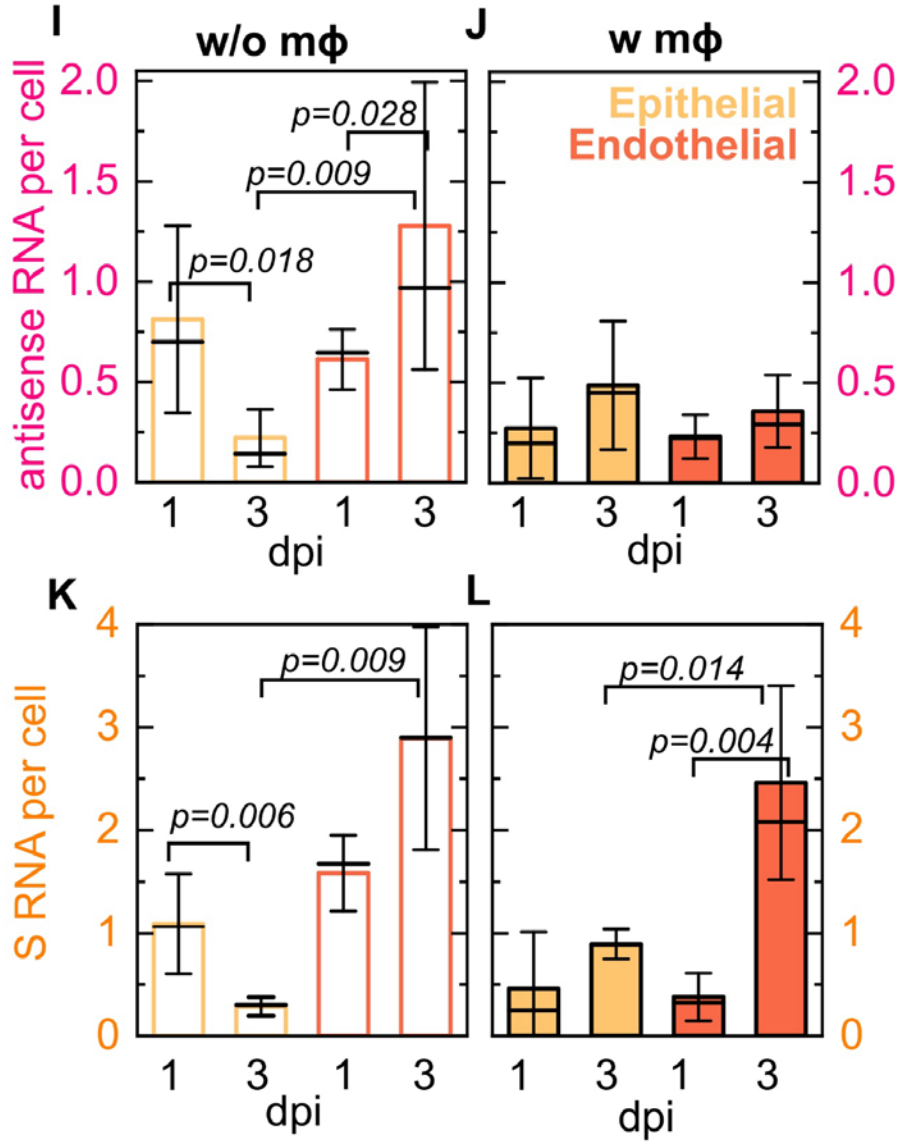
Low *ACE2*: examples of productive infection in alveolar epithelial cells are rare

High *NRP1* might represent an alternative entry route

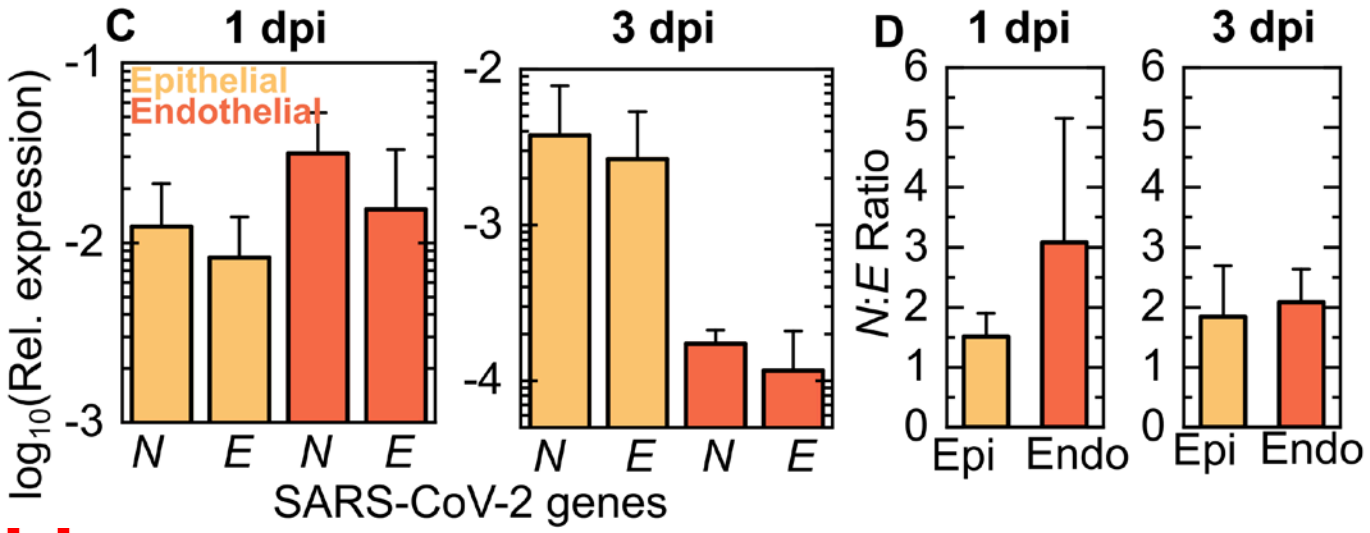
SARS-CoV-2 persists in individual epithelial and endothelial cells

Apical wash and vascular effluent

RNAscope



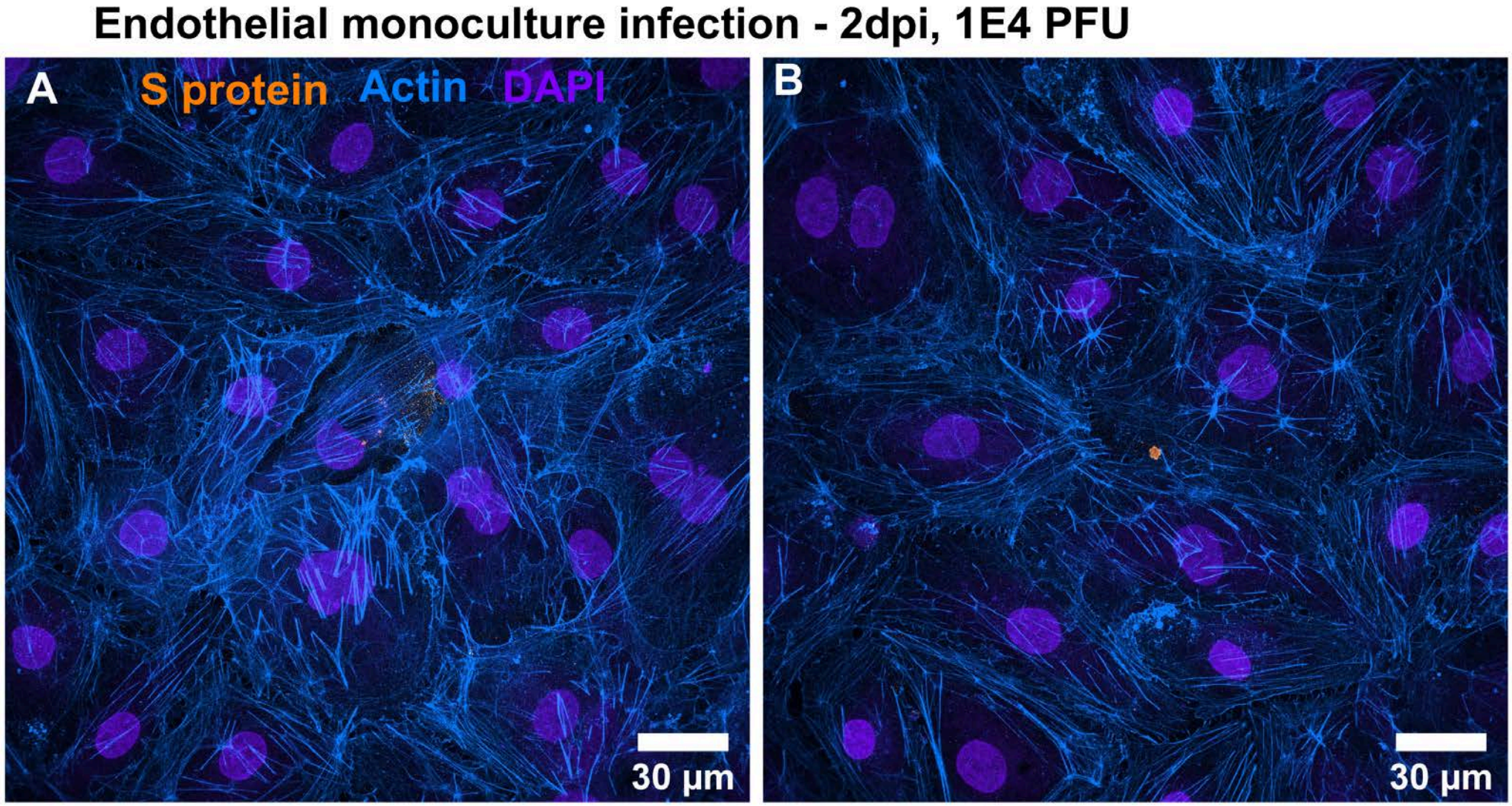
Intracellular RNA



Transient hyperplasia and loss of tight junctions

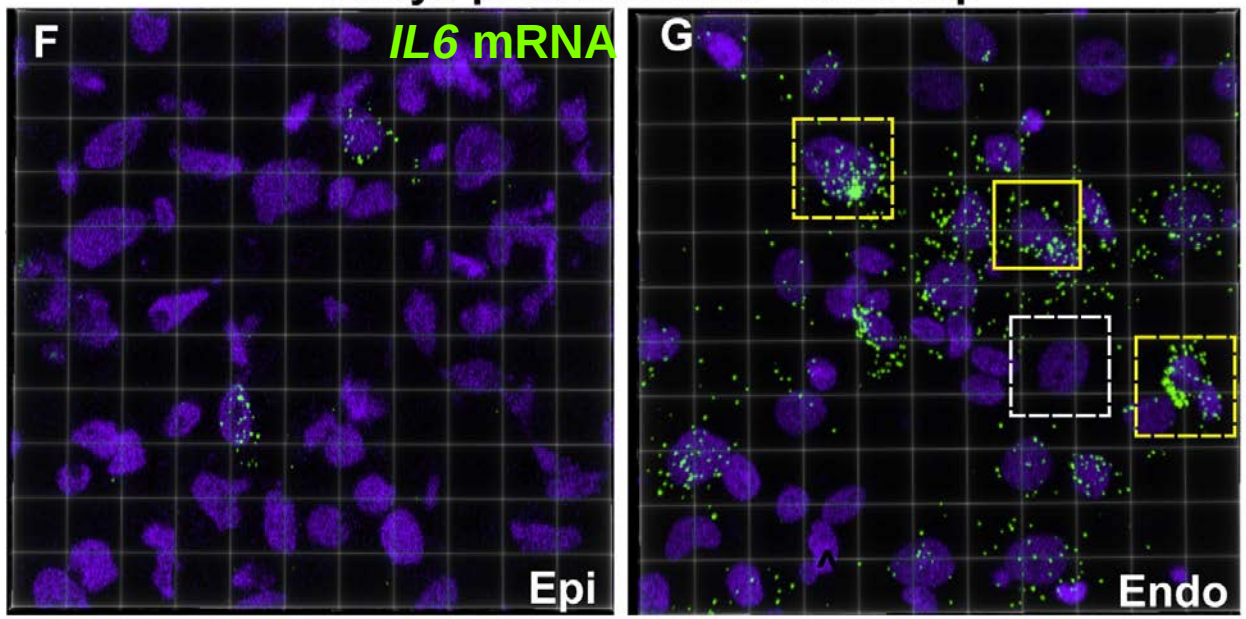
Endothelial inflammation generates a pro-coagulatory environment

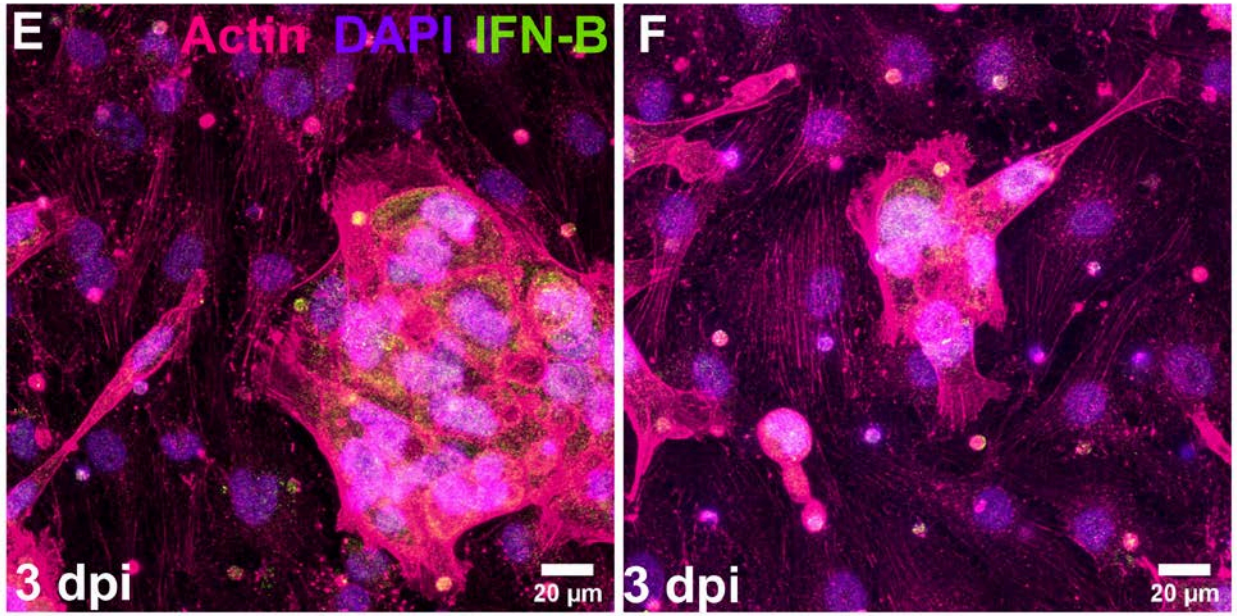
Infection of endothelial cell monocultures does not lead to inflammation



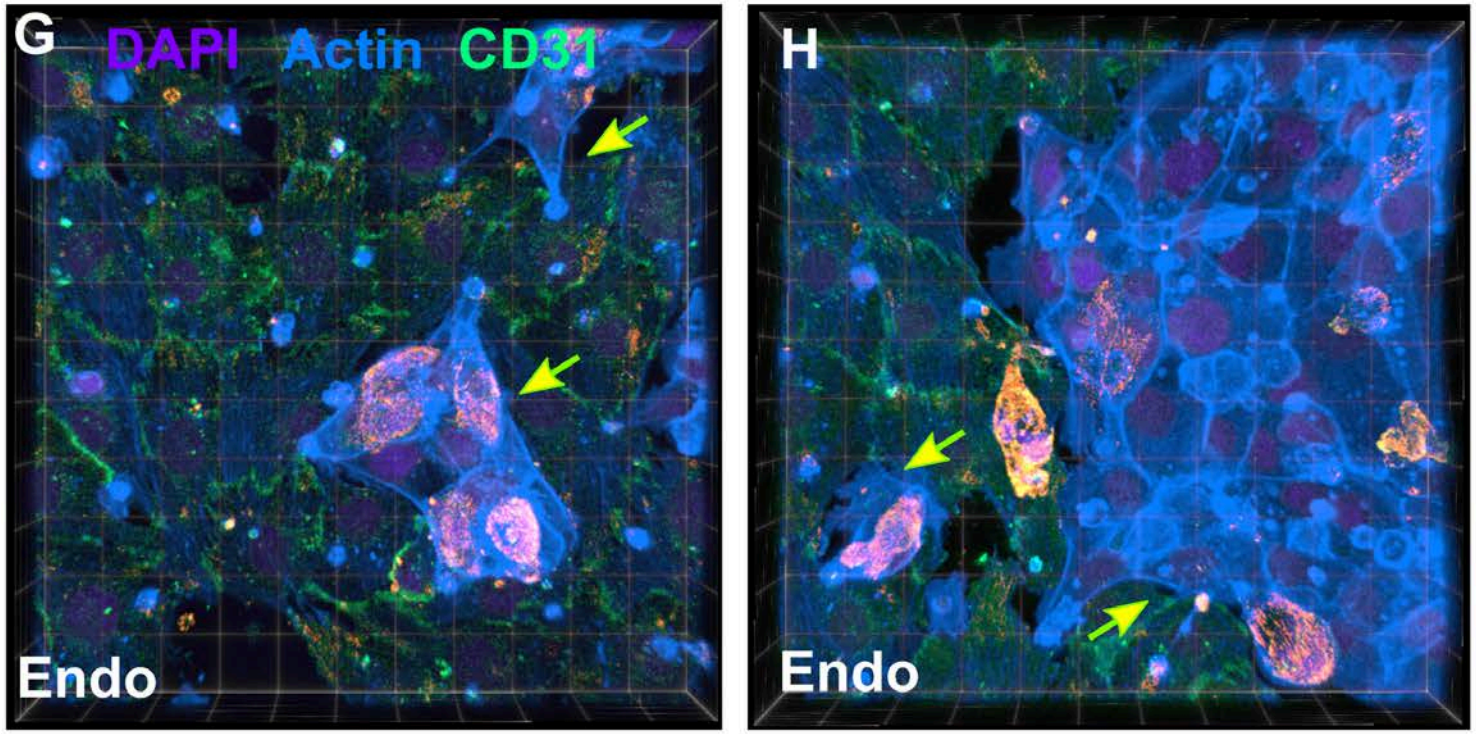
NF-KB inflammatory responses are cell-type specific

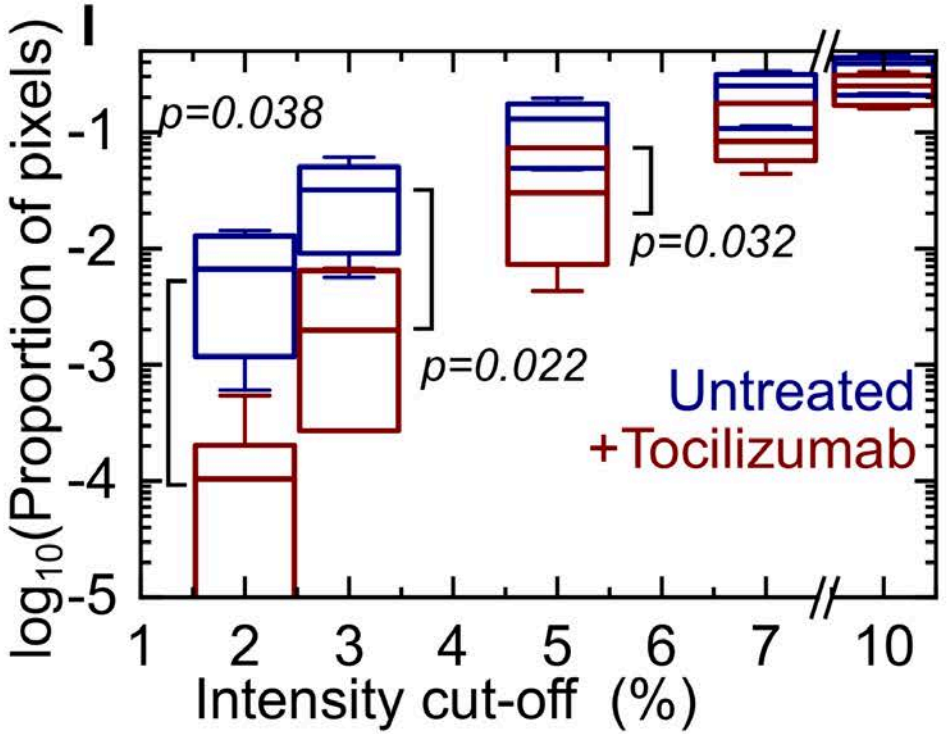
3 days post infection - with mϕ





Tocilizumab does not alleviate endothelial cell dysfunction





Tocilizumab – anti IL6R antibody being tested in clinical trials as a repurposed therapeutic

Administration slows down the loss of barrier function but does not prevent formation of cell clusters

Conclusions

1. **Replication kinetics** – SARS-CoV-2 does not replicate productively in the alveolar space, yet low levels of virions can persist and replicate in individual cells for up to 3 days.

2. **Physiology** – Rapid basolateral transmission despite low ACE2 expression. Transient hyperplasic phenotype. None of these changes occur in monoculture experiments where endothelial cells are inoculated with the virus apically.

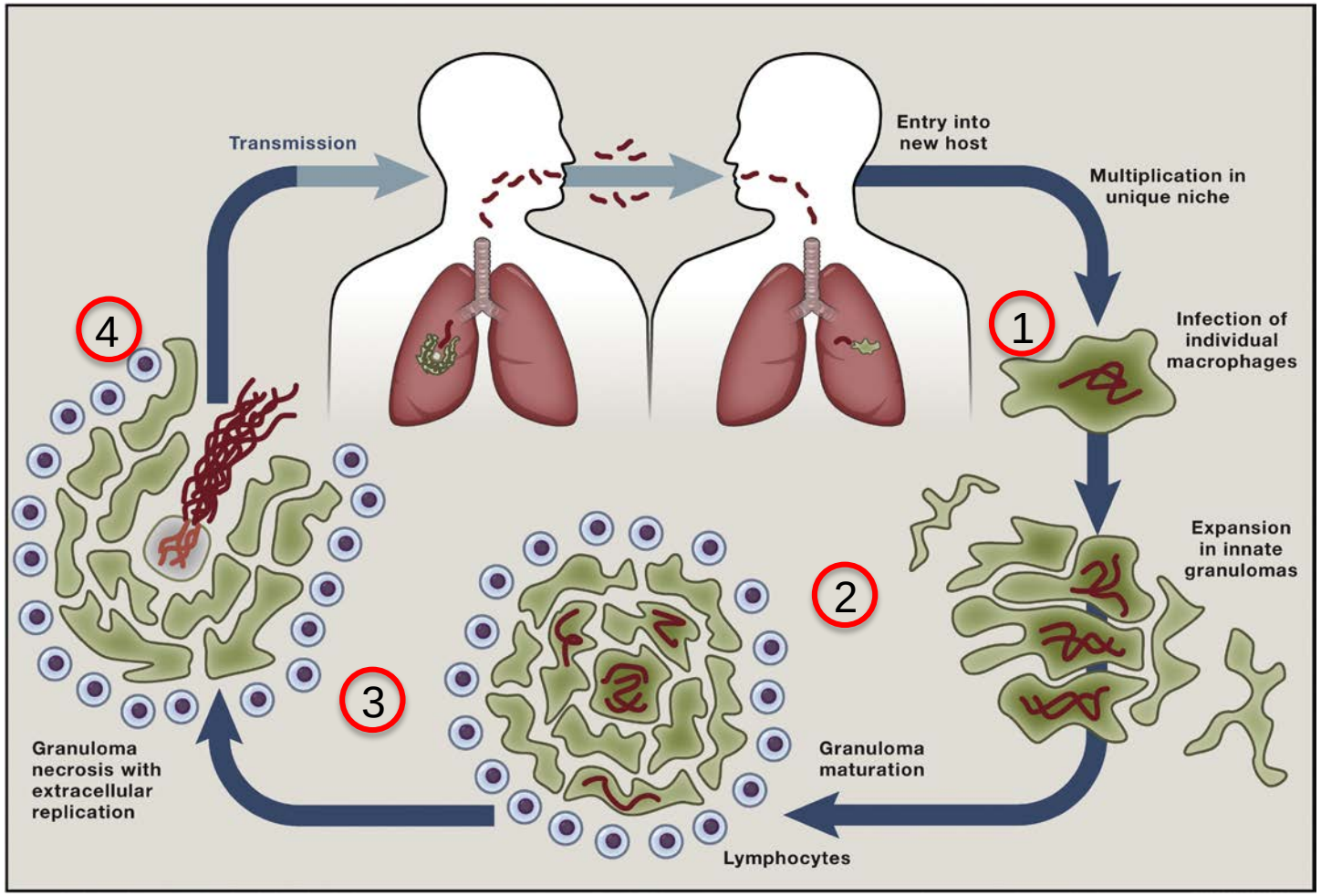
3. **Persistent nature of inflammation** – an NF-KB inflammatory response typified by IL-6 secretion and a weak antiviral interferon response is transient in epithelial cells but persistent in endothelial cells.

4. **Endothelial cells as a source for IL-6** – IL-6 secretion is independent of the presence or absence of resident immune cells, which shows that the damaged endothelium itself represents a source for this cytokine.

5. **Anti-IL6R therapy does not prevent inflammation** – Administration of Tocilizumab slows the loss of barrier integrity but does not reduce the occurrence of hyperplasia, which indicates that not all inflammation observed is IL-6 signalling dependent.

Tuberculosis: an extensive series of host-pathogen interactions

Current paradigm: Intracellular life-cycle of *M. tuberculosis*



Significance: Global (1.3 million deaths/year), long and expensive antibiotic treatment, **BCG vaccine is ineffective in adults**

Known Unknowns in Human Patients
Why do only 5-10% of people develop active disease?
What determines entry into and exit from latency?

Mouse Model
Permissive
No latency
No granulomas

Cynomolgus macaques
Closest to human
Expensive to work with

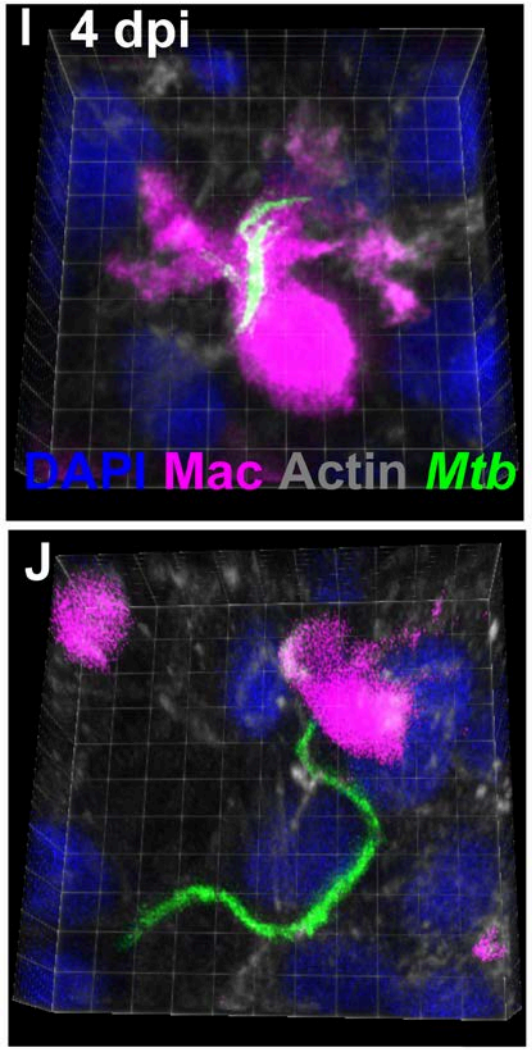
'First contact' with the host is extremely difficult to study in any animal model

Images: *Cell*, **157**, 1497-1509 (2014), Cold Spring Harb Perspect Med. 4(10): a021105, (2014), *Nat Med* **11**,638–644 (2005)

In vitro passaging alters surfactant expression in ATs

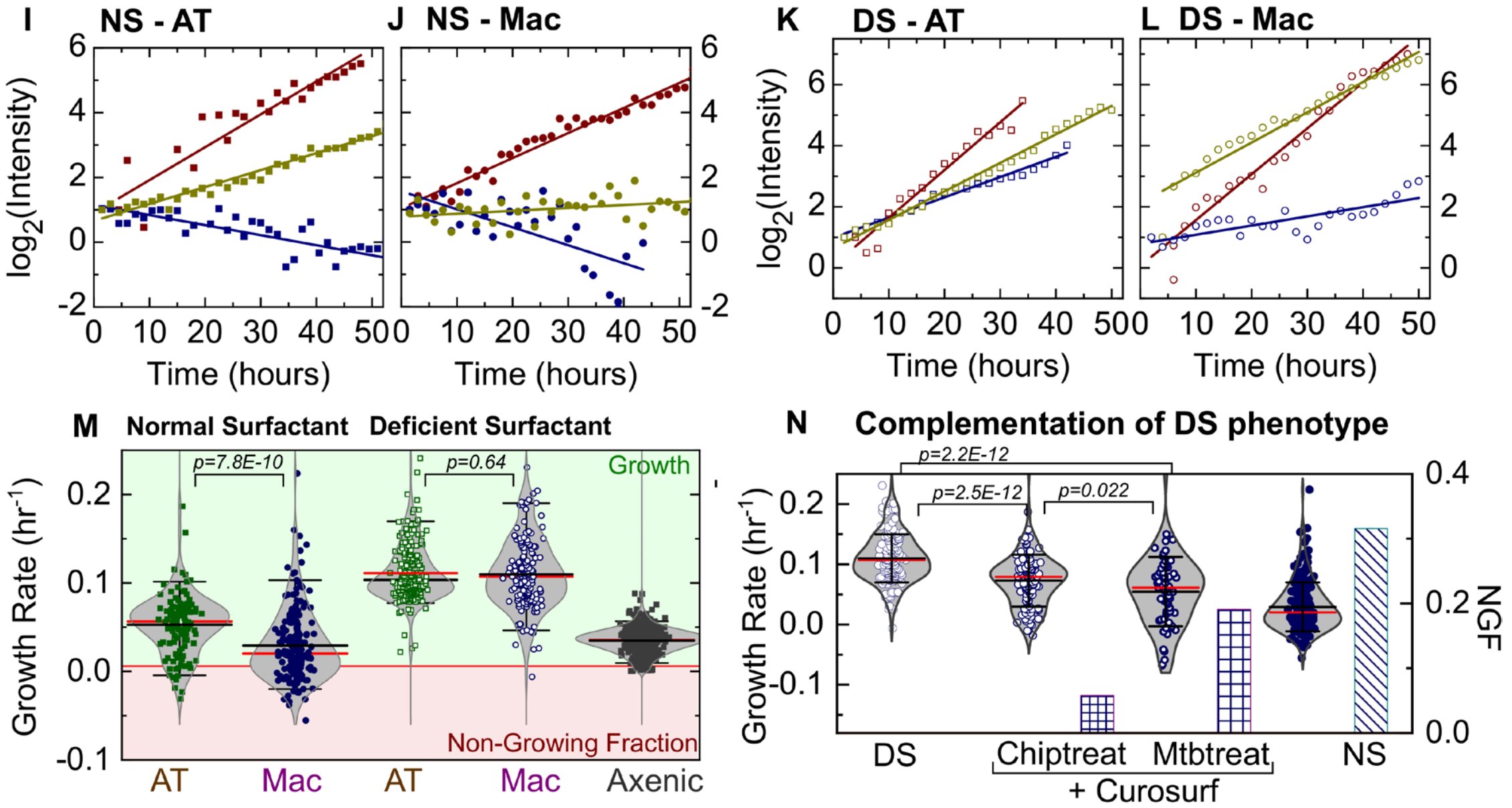
V.V. Thacker et al., eLife 2020, 9:e59961

I, II. Striking observations: role for surfactant and MTB cords

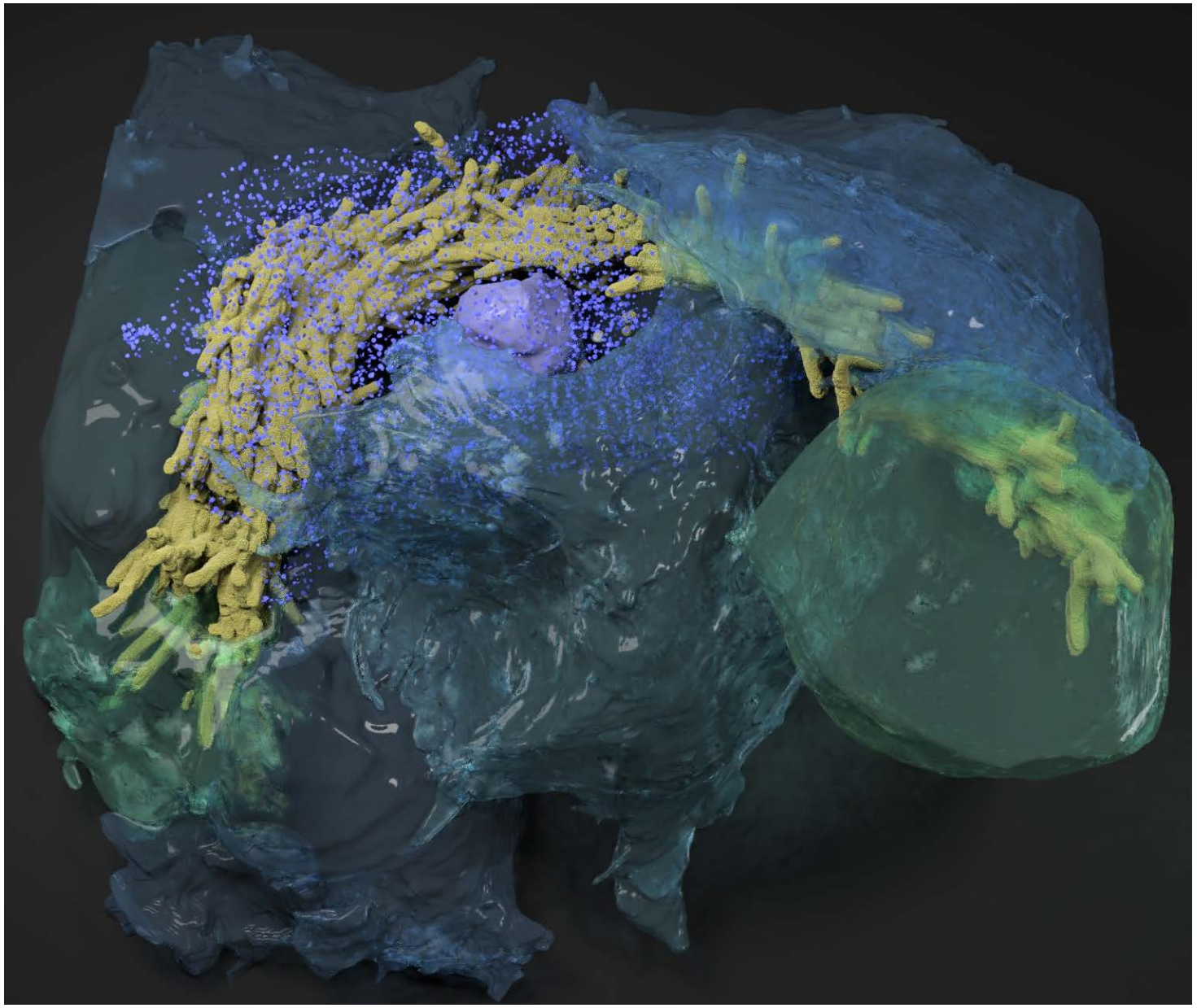


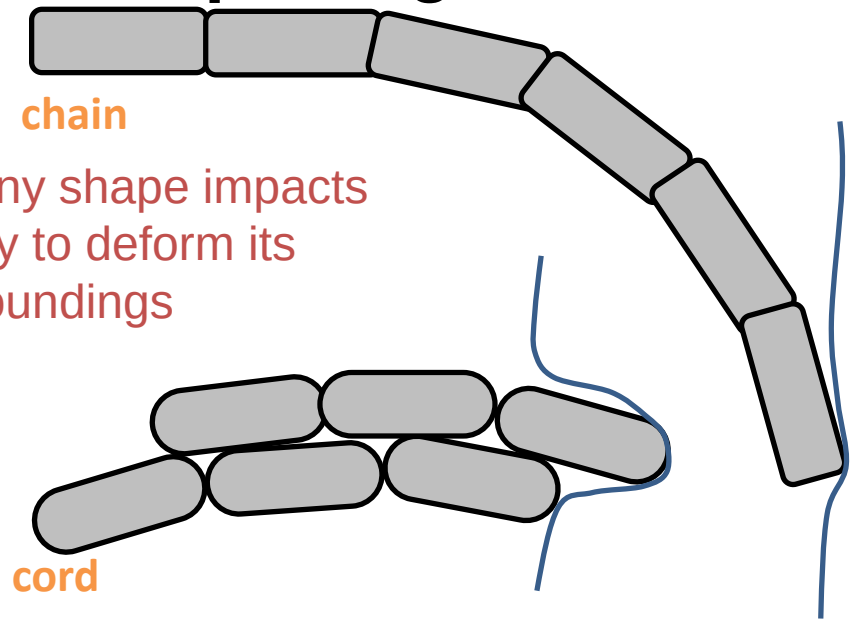
VV Thacker *et al., eLife* 2020 9:e59961

Single-cell growth dynamics in ATs vs. macrophages

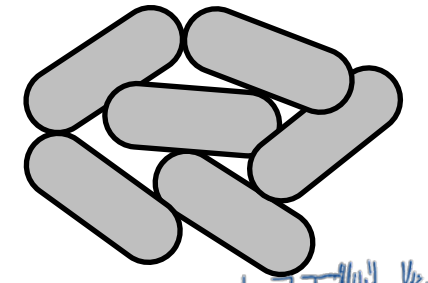


Biophysical role: Cords as “battering rams” to aid pathogenesis?





Colony shape impacts ability to deform its surroundings



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Jose Artacho

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EMBO Fellow

HFSP