

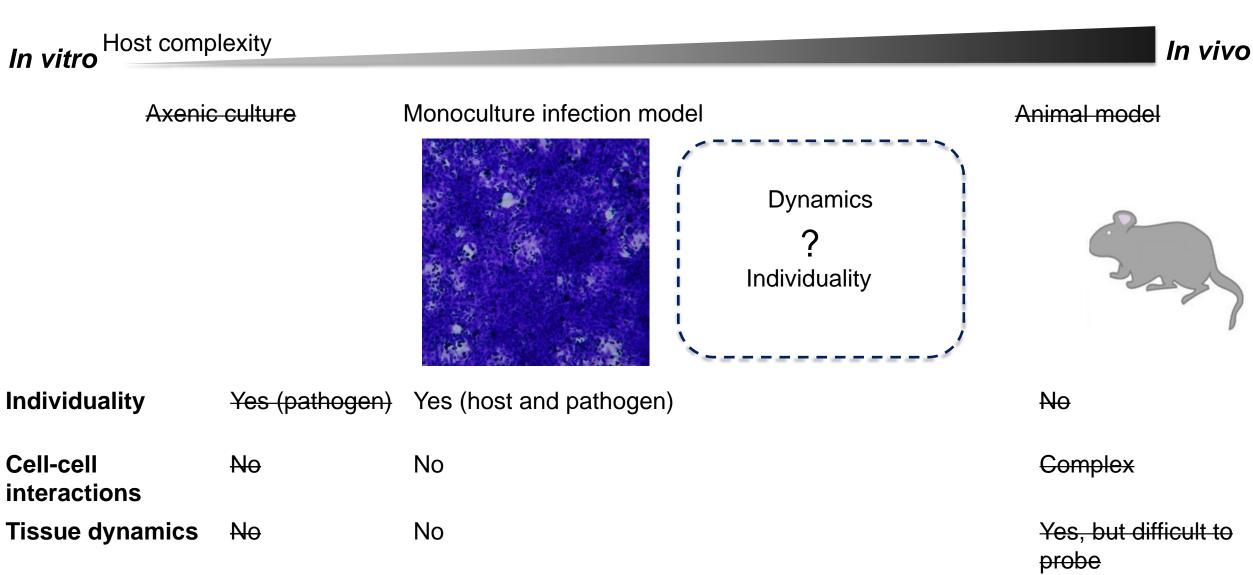
Lung-on-chip model systems to study host-pathogen interactions in respiratory infections

Vivek Thacker

vivek.thacker@epfl.ch

@DrVivekThacker

Why do we need new model systems? Emerging viruses





Absent

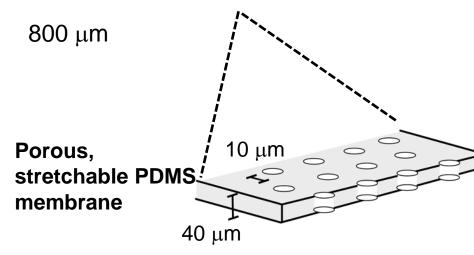
Simple

Host

Complex

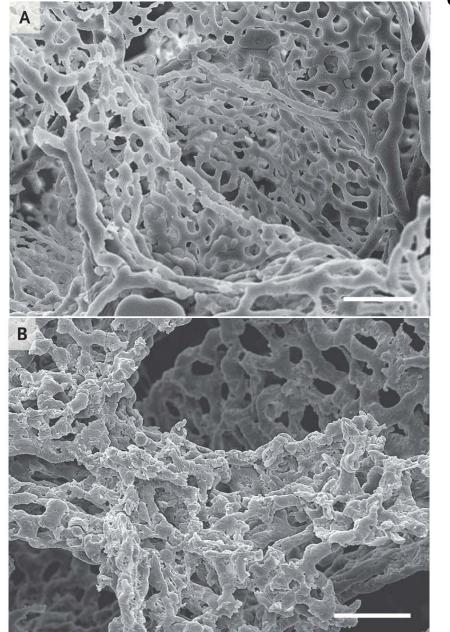


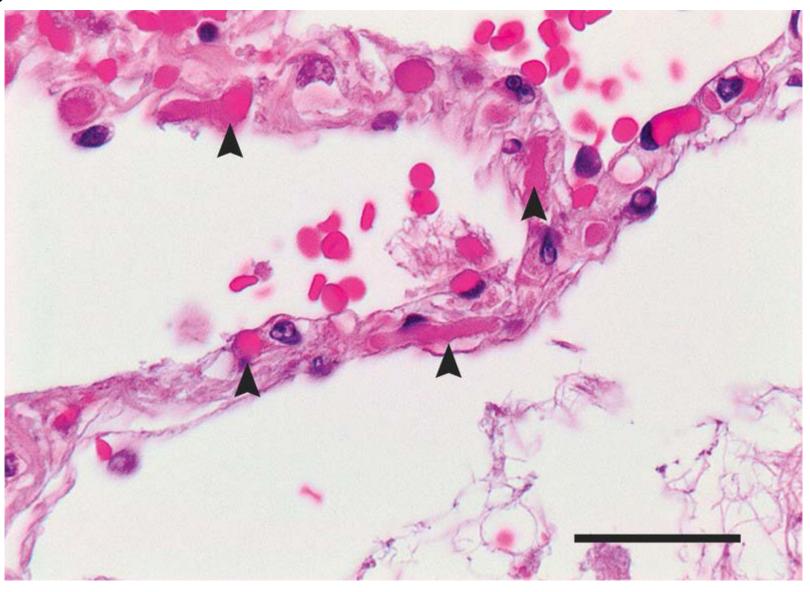






Microvasculature damage is a clinical hallmark of SARS-CoV-2

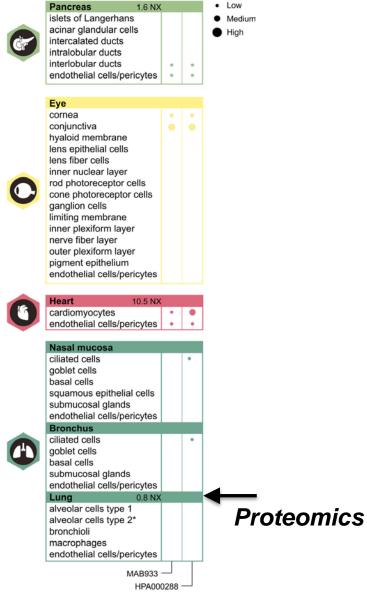




ACE2 expression is low in the alveolar space Goblet cells 5.0 2020 Paneth cells ● 7.5 10.0 undiff, cells Stomach Gallbladder proximal tubules surface epithelial cells glandular cells distal tubules parietal cells endothelial cells/pericytes collecting ducts 2020 chief cells Liver 1.2 NX macrophages endothelial cells/pericytes hepatocytes B-cells T-cells Duodenum 46.0 NX bile duct cells enterocytes Kupffer cells Leydia/Sertoli cells goblet cells endothelial cells/pericytes peritubular cells endocrine cells macrophages crypt cells 1.5 Testis 17.9 NX glands of Brunner spermatogonia Leydig cells endothelial cells/pericytes Sertoli cells early spermatids Small intestine 122.0 NX peritubular cells enterocytes endothelial cells goblet cells spermatogonia endocrine cells basal cells prelep. spermatocytes 0.50 2019 ciliated cells Paneth cells pachy, spermatocytes 0.25 Goblet cells crypt cells early spermatids endothelial cells/pericytes late spermatids Lukassen 49.1 NX ciliated cells **Epididymis** 2.7 NX Goblet cells enterocytes glandular cells Ionocytes goblet cells endothelial cells/pericytes endocrine cells Seminal vesicle basal cells • 0.25 crypt cells ciliated cells glandular cells 0.50 peripheral nerve Goblet cells 0.75 endothelial cells/pericytes endothelial cells/pericytes 1.00 endothelial cells Rectum alveolar cells type 1 1.0 NX Placenta enterocytes alveolar cells type 2 syncytiotrophoblasts goblet cells alveolar cells type 1/2 cytotrophoblasts ciliated cells endocrine cells extravillous trophoblasts secretory cells endothelial cells/pericytes 2020 macrophages decidual cells granulocytes endothelial cells/pericytes T-cells Appendix 0.8 NX Fallopian tube fibroblasts enterocytes ciliated cells endothelial cells goblet cells non-ciliated cells mixed endocrine cells endothelial cells/pericytes lymphoid tissue alveolar cells type 1 endothelial cells/pericytes alveolar cells type 2 ciliated cells Thyroid gland secretory cells glandular cells macrophages endothelial cells/pericytes granulocytes proximal tubule cells T-cells Parathyroid gland 0 NX distal tubule cells fibroblasts glandular cells collecting duct cells endothelial cells **Transcriptomics** endothelial cells/pericytes Bowman's capsule podocytes Adrenal gland mesangial cells alveolar cells type 1 glandular cells alveolar cells type 2 endothelial cells/pericytes endothelial cells/pericytes ciliated cells macrophages MAB933 MAB933 granulocytes HPA000288 HPA000288 T-cells fibroblasts mixed Data: Hilkmet et al., Mol Syst Biol (2020)16:e9610 Dataset

ACE2 expression

expression



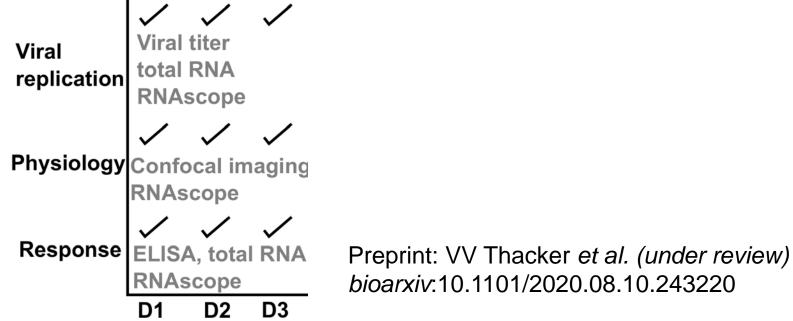
SARS-CoV-2 in the alveolar space

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

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

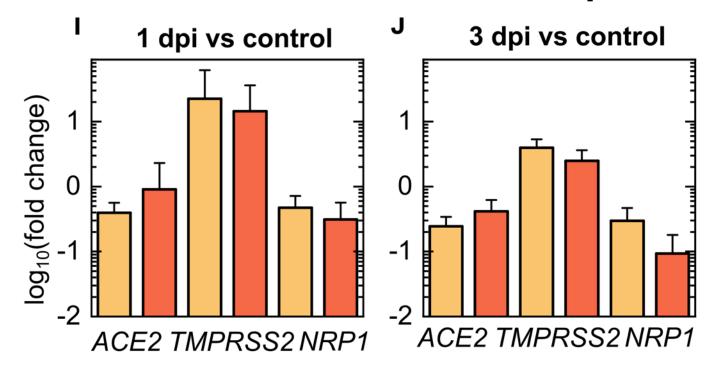


LoC model for SARS-CoV-2





Low ACE2 expression, infection increases TMPRSS2 expression



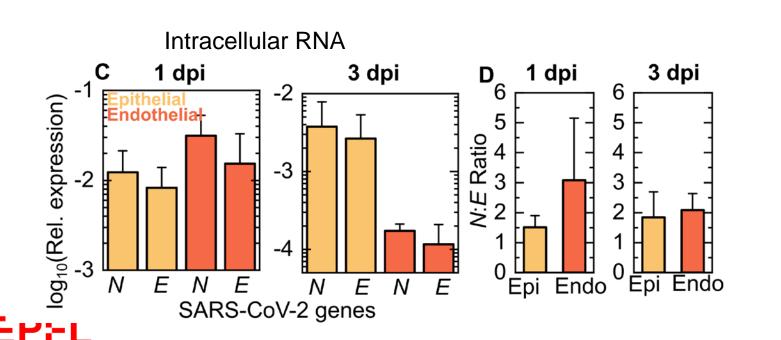
Low ACE2: examples of productive infection in alveolar epithelial cells are rare

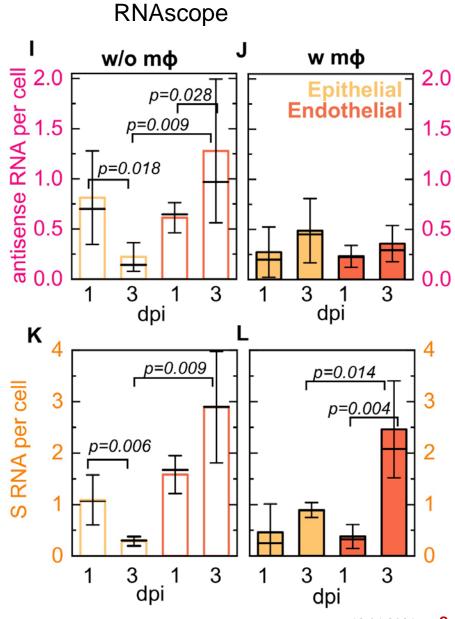
High NRP1 might represent an alternative entry route



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

Apical wash and vascular effluent





Transient hyperplasia and loss of tight junctions

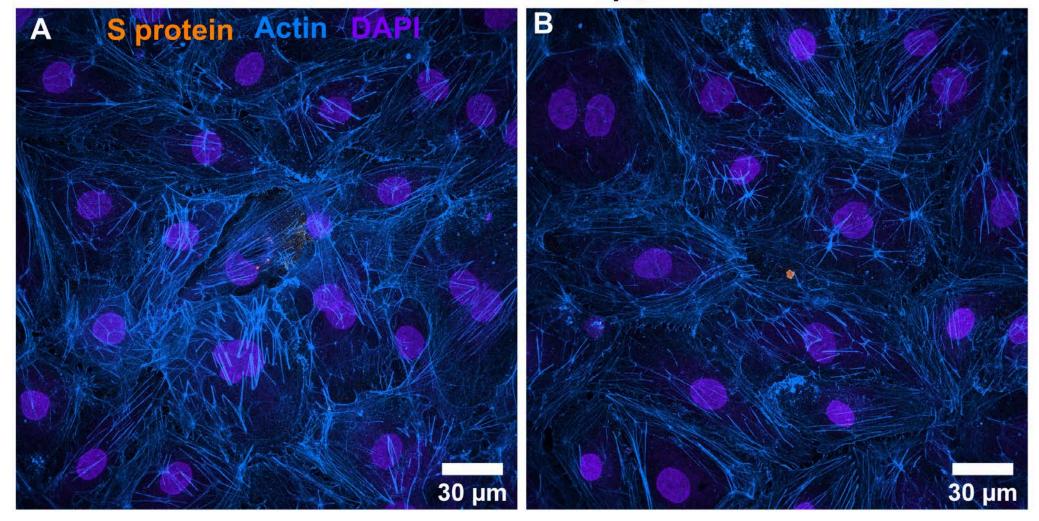


Endothelial inflammation generates a pro-coagulatory environment



Infection of endothelial cell monocultures does not lead to inflammation

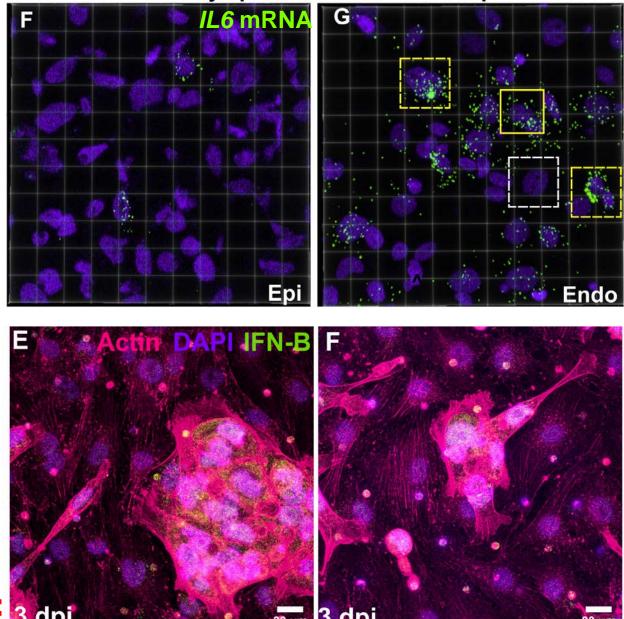
Endothelial monoculture infection - 2dpi, 1E4 PFU



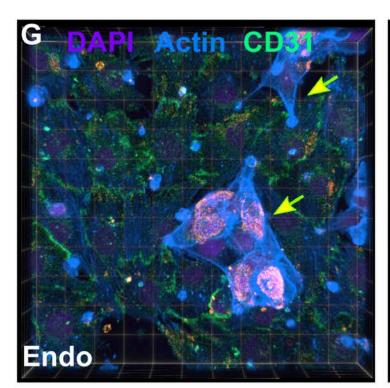


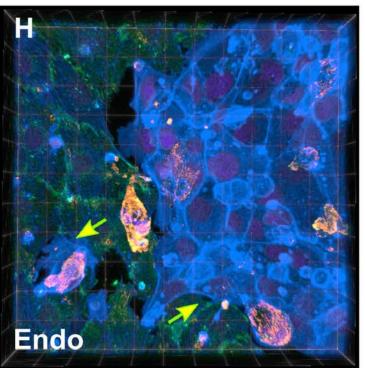
NF-KB inflammatory responses are cell-type specific

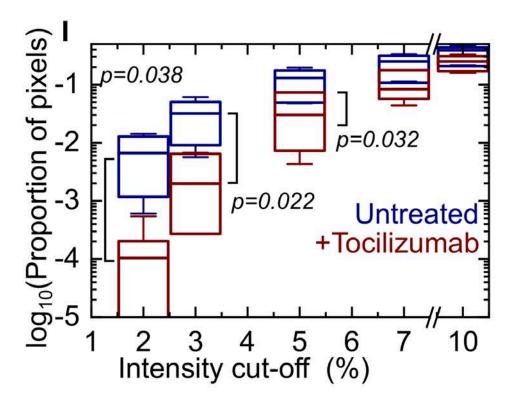
3 days post infection - with mф



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



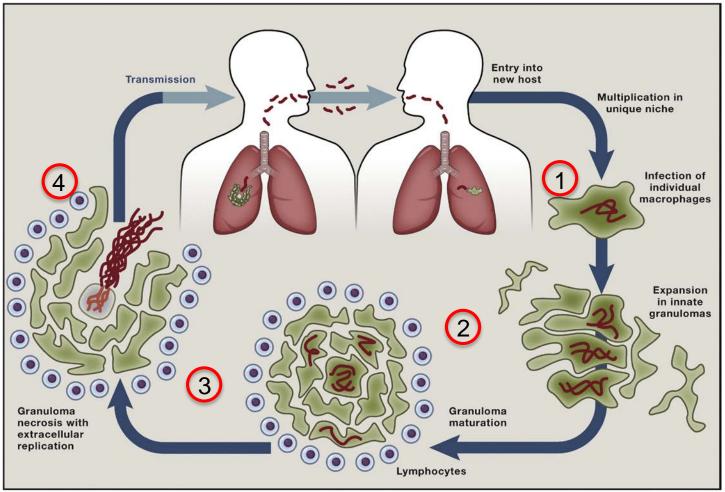
Conclusions

- **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

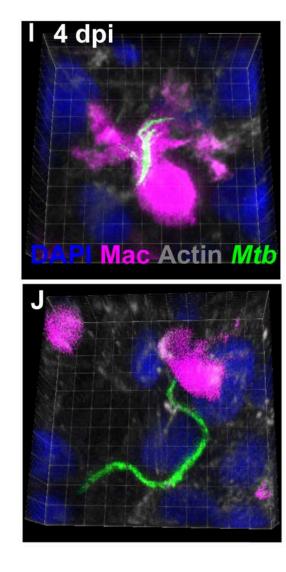
EPFL Im

19.01.2021 **16**

In vitro passaging alters surfactant expression in ATs

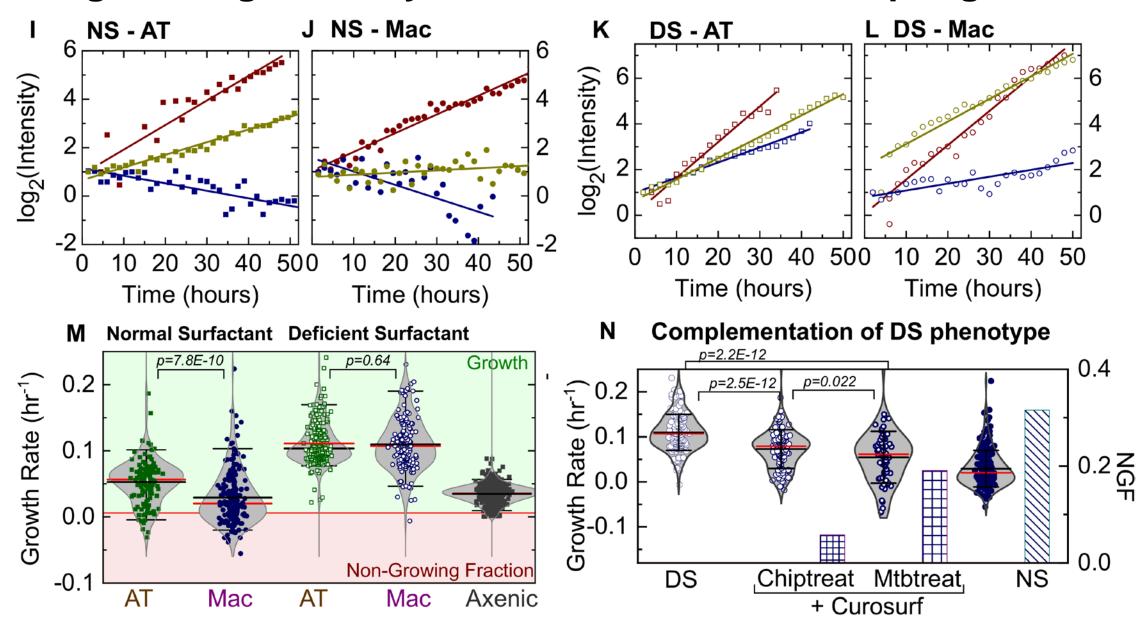


I, II. Striking observations: role for surfactant and MTB cords



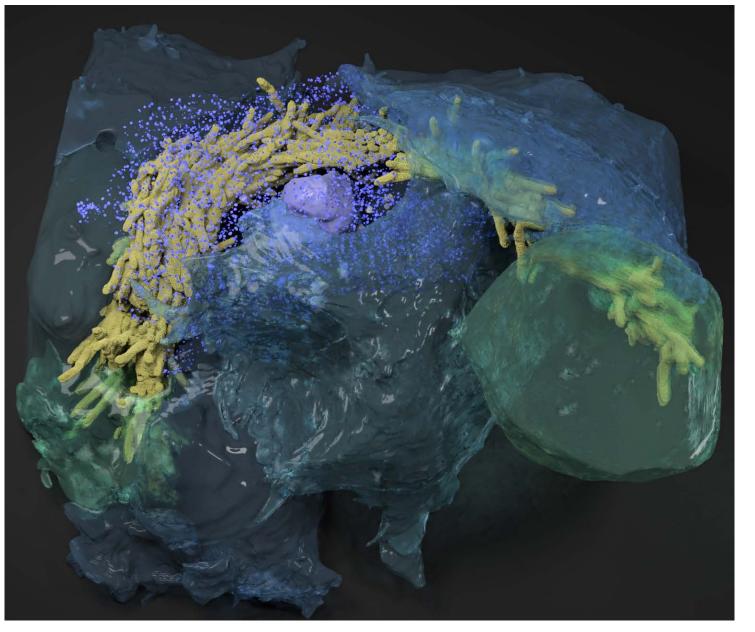


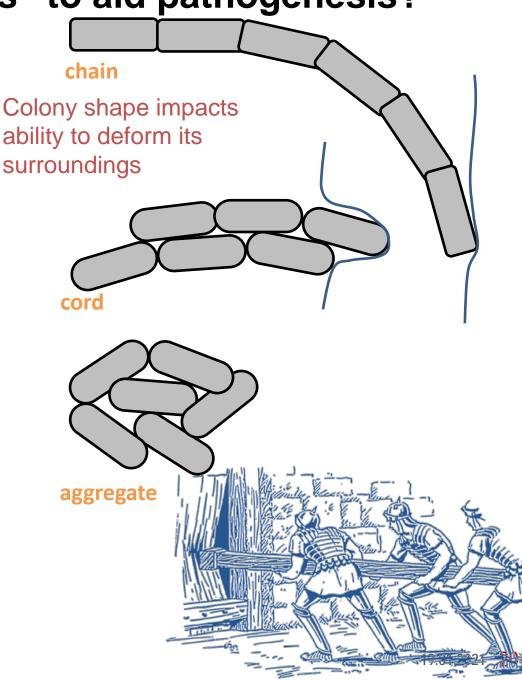
Single-cell growth dynamics in ATs vs. macrophages





Biophysical role: Cords as "battering rams" to aid pathogenesis?





Acknowledgements McKinney lab

Kunal Sharma

Neeraj Dhar

Melanie Hannabelle

Histology Facility

Jessica Sordet-Dessimoz

Gian-Fillipo Mancini

BioEM Facility

Graham Knott

Anaelle Dubois

University of Geneva

Carolyn Tapparel

Valeria Cagno

BIOP facility

Arne Seitz

Jose Artacho

Thierry Laroche
EPFL





