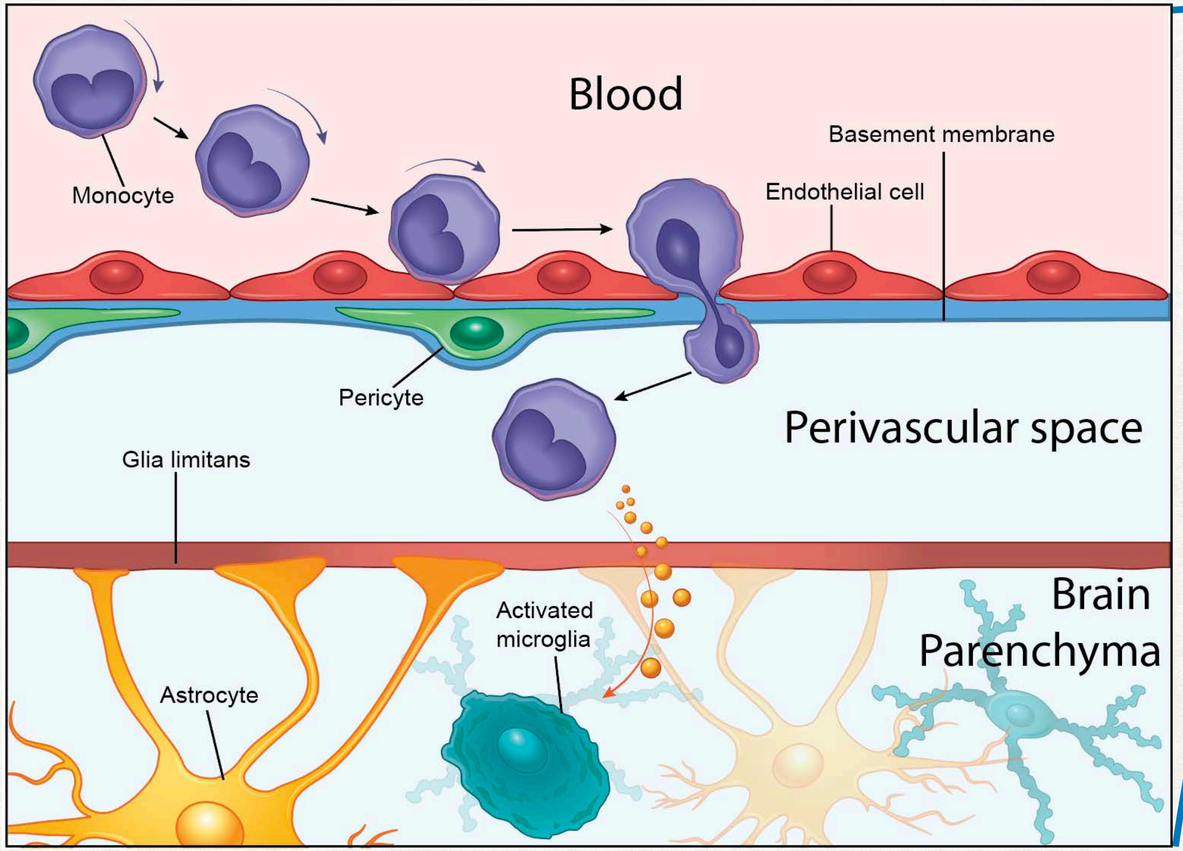
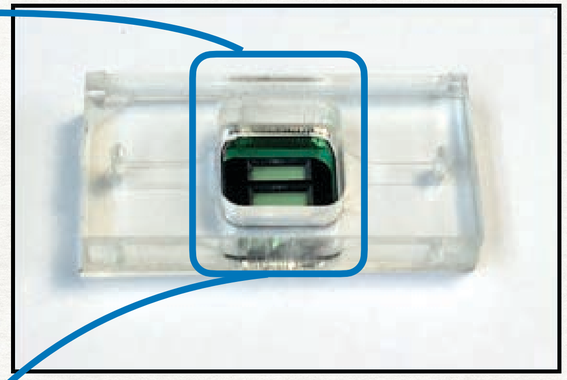
The µSiM-*h*NVU – a Human BBB Platform for the Study of Brain Injury Mechanisms in Sepsis





Trans-Agency Blood-Brain Interface Program

NIH National Heart, Lung, and Blood Institute

R61/R33 HL154249

1850 MELIORA

RIT

UNIVERSITY OF MICHIGAN

UNIVERSITÄT BERN

COGNITIVE DECLINE AS AN OUTCOME OF SYSTEMIC INFLAMMATION

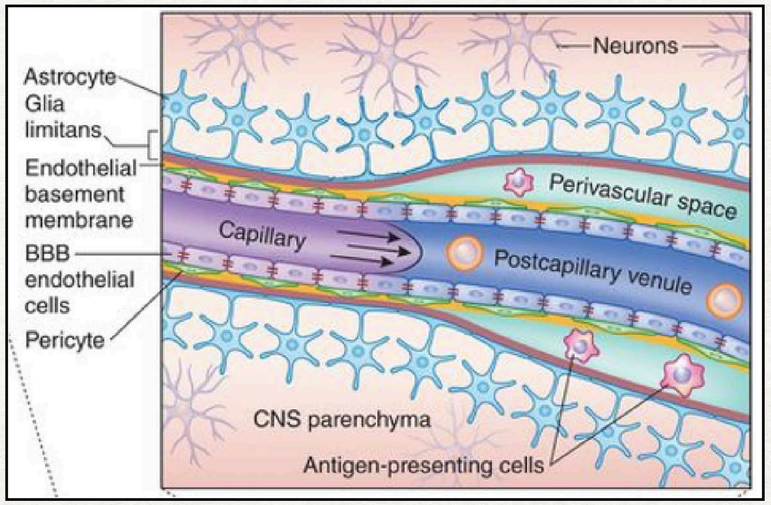
Cognitive decline in sepsis survivors

- Sepsis accounts for more than 1.5M hospital stays in the US and is the leading cause of death in hospitals.
- No treatments for sepsis beyond supportive care.
- Mortality rate remains high (25%) but improvements in supportive care is leading to an increasing number of sepsis survivors.
- More than 70% of sepsis survivors experience long term cognitive impairment.
- Systemic inflammation leading to cognitive impairment appears in post-operative delirium, long-COVID, and more.

Circulating factors of interest

- Cytokines
- Infiltrating leukocytes
- DAMPS
- glycocalyx reducing factors
- cell-free hemoglobin

Post - capillary venues



Engelhardt, B., et al., 2017. Nat Immunol, 18: 123-31.

James McGrath, PhD
Richard Waugh, PhD
Jonathan Flax, MD
Minsoo Kim, PhD
Anthony Pietropaolii, MD.

Trainees

Molly McCloskey (μSiM development, BBB models, permeability)
Chloe Chen (Flow Adoption @ UR)
Dan Ahmad (Transmigration)

Staff

Julie Kuebel (glycocalyx, AFM)

Collaborator

Angela Glading, PhD
Feyone La (Student in Glading group)

Influence of Shear Stress on Cerebral Cavernous Malformations (CCMs - KRIT1)

Tom Gaborski, PhD
Vinay Abhyankar, PhD
Steven Day, PhD

Trainees

Louis Widom (Basement membrane)
Mehran Mansouri (Flow module)

Britta Engelhardt, PhD

Trainee

Pelin Kasap (iPSC tri-culture)

Staff

Yury Belyaev (Microscopy)

Collaborator Anuska Andjelkovic-Zochowski, MD, PhD

Role of BBB Gap junction proteins in stroke and cerebral amyloid antipathy (CAA)

Trainees

Muyu Situ (Student in Andjelkovic group)
Howard Su (Digital ELISA)

Benjamin Singer, MD, PhD
Katsuo Kurabayashi, PhD

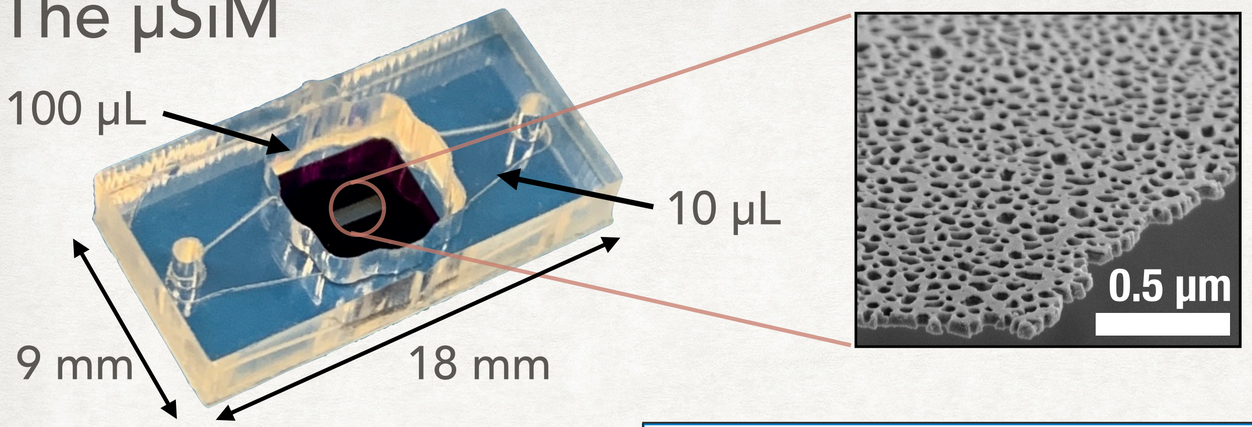
AARHUS UNIVERSITY

Collaborators

Morten Nielson, PhD
Diana Hundzec, PhD

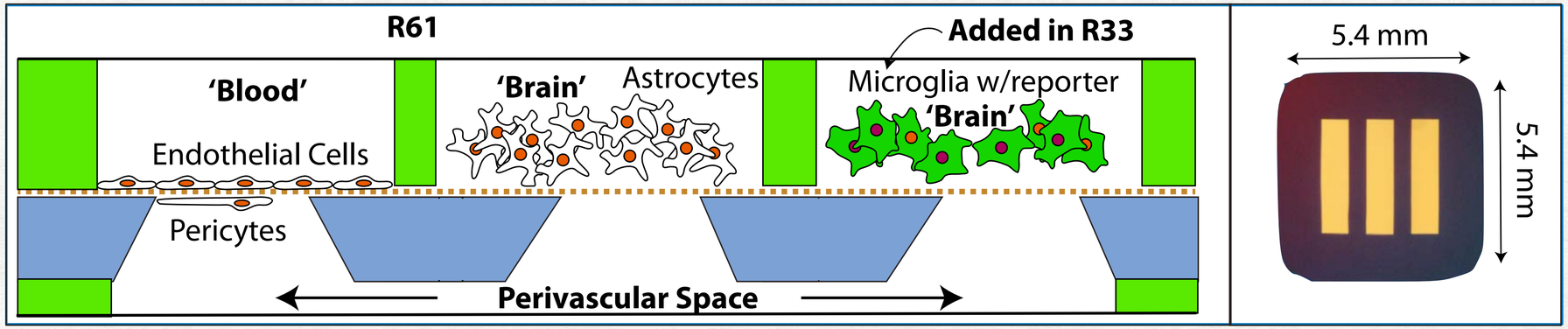
High Resolution Imaging of molecular trafficking across the BBB

The µSiM

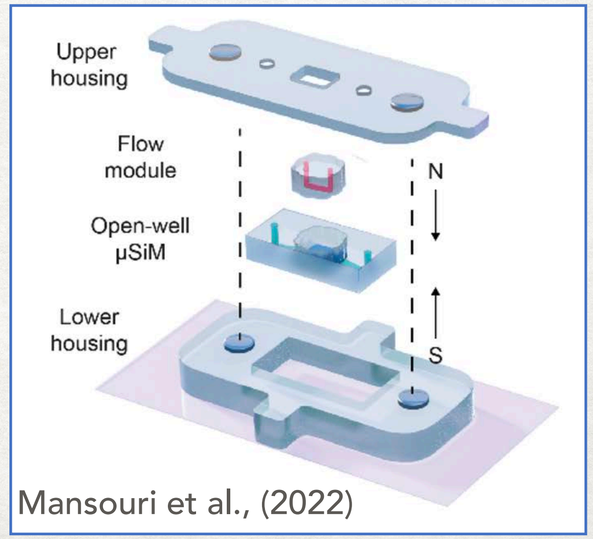


- Ultrathin (< 100 nm) and highly porous nanomembrane
- Optically transparent
- No hindrance to small molecule exchange between compartments

microphysiological system enabled by a **si**licon **m**embrane



- Modular design allows for easy assembly by any user.
- Mass production of components for distribution, not devices.
- Distributed components for more than 4000 devices during R61.
- Locally reconfigurable using swappable components and plug-ins.



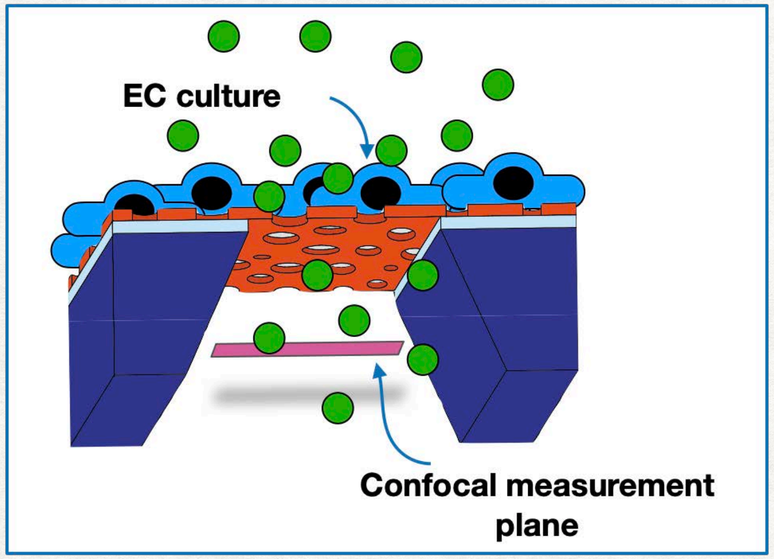
Mansouri et al., (2022)

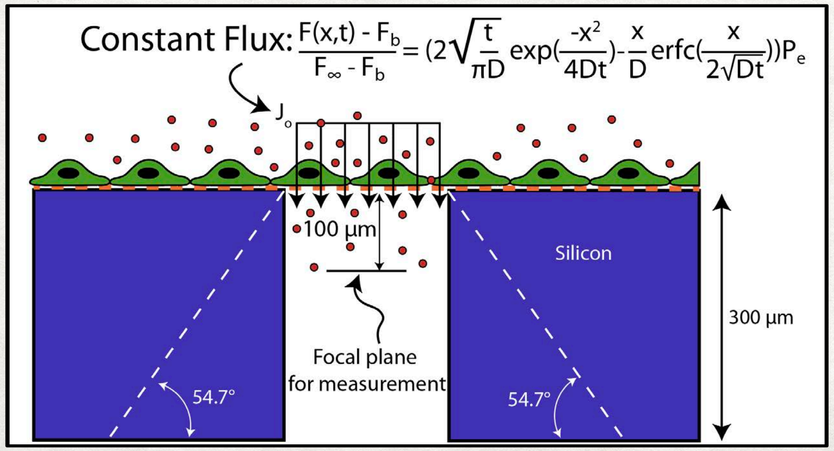
1. McCloskey et al., (2022) Adv. Healthcare Mater., 202200804
2. Mansouri et al., (2022) Adv. Healthcare Mater., 202200802

SMALL MOLECULE PERMEABILITY

IN SITU METHOD

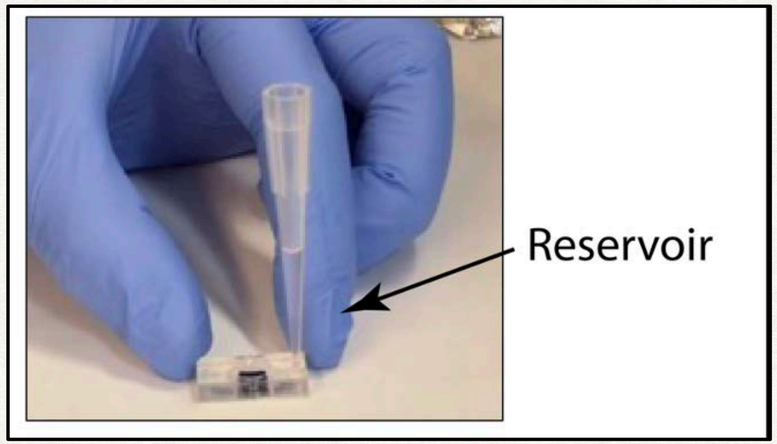
- Live imaging of molecules diffusing to a focus 100 µm below monolayer
- Fast (10 min) measurement

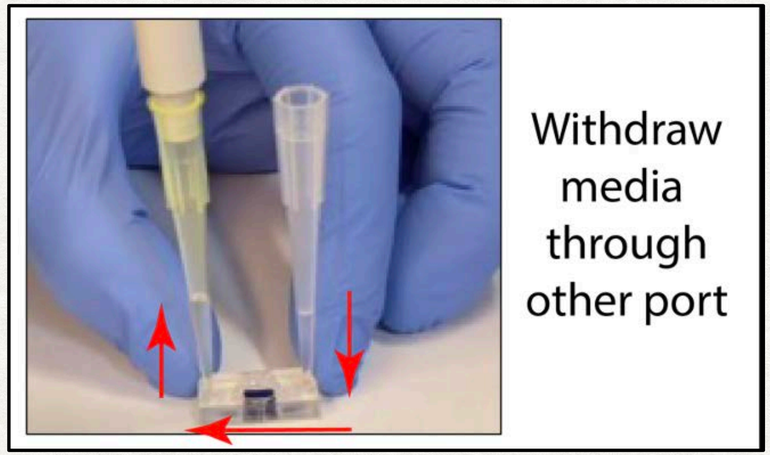


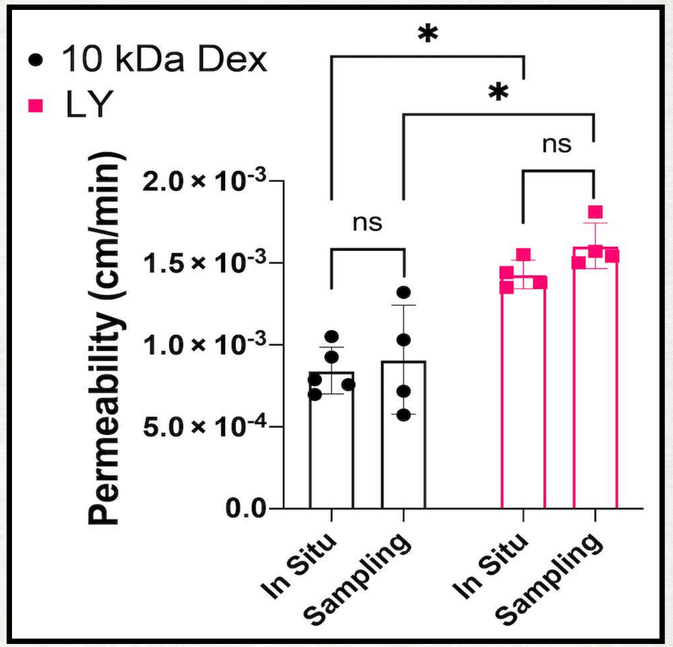


SAMPLING METHOD

- Uses familiar “Transwell®” style calculations for permeability.
- 50 µL extraction recovers > 99% of molecules in ‘receiver’ channel.







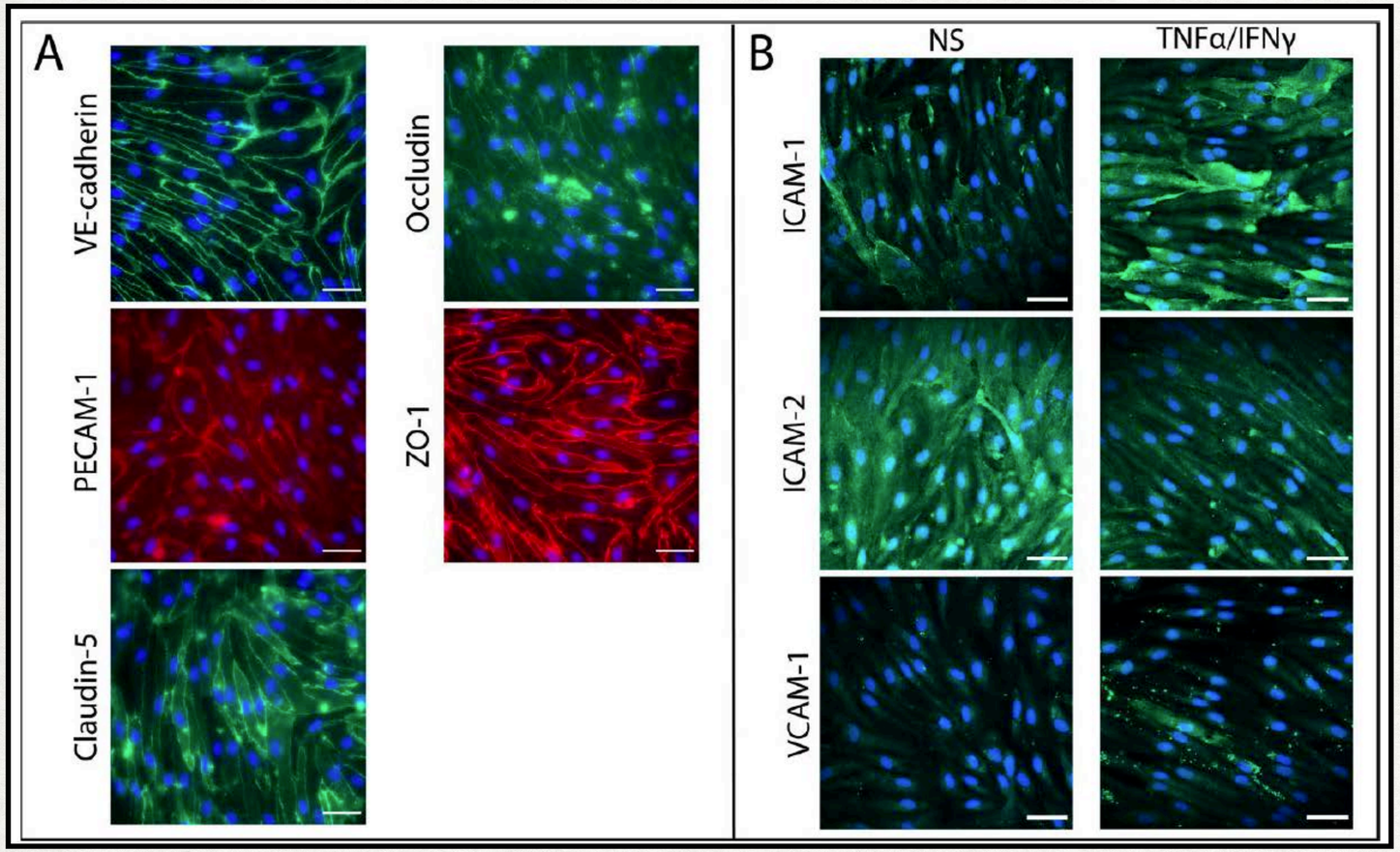
AGREEMENT BETWEEN METHODS

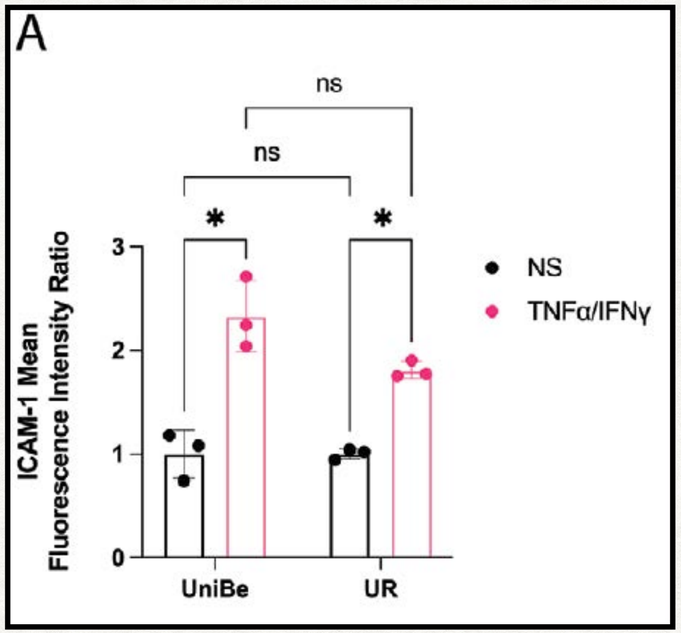
McCloskey et al., (2022) Adv. Healthcare Mater., 202200804

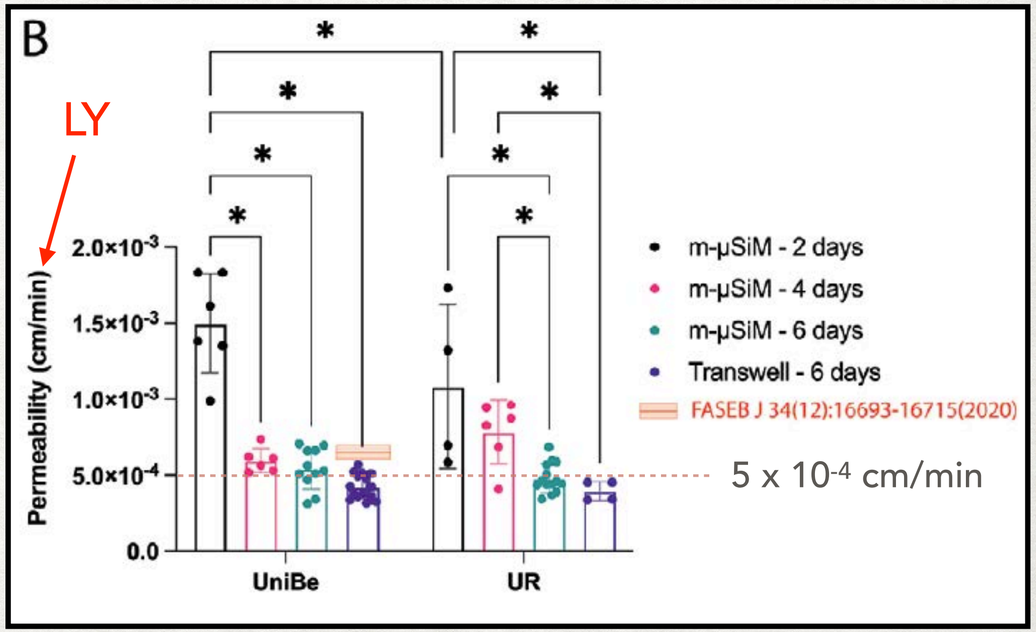
AGREEMENT BETWEEN LABS

All data for IMR90-4 derived BMECs by EECM

McCloskey et al., (2022) Adv. Healthcare Mater., 202200804







Lesson learned!

- Many more devices than data points.
- Training, troubleshooting, and protocol refinement!
- Reproducibility implies more than the final data - everything upstream too.

MACHINE LEARNING FOR AUTOMATED MIGRATION ANALYSIS OF LEUKOCYTE TRAFFICKING ACROSS EC MONOLAYERS

Images

ML Model

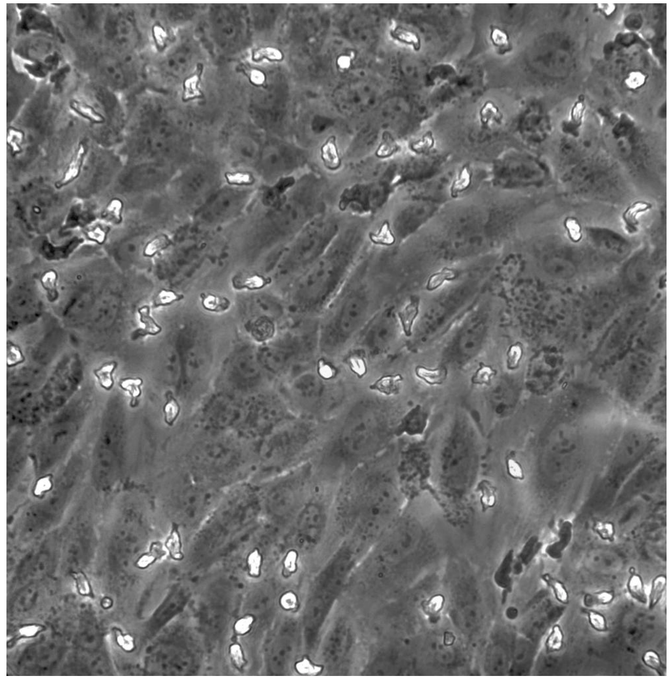
Ground Truth Labels

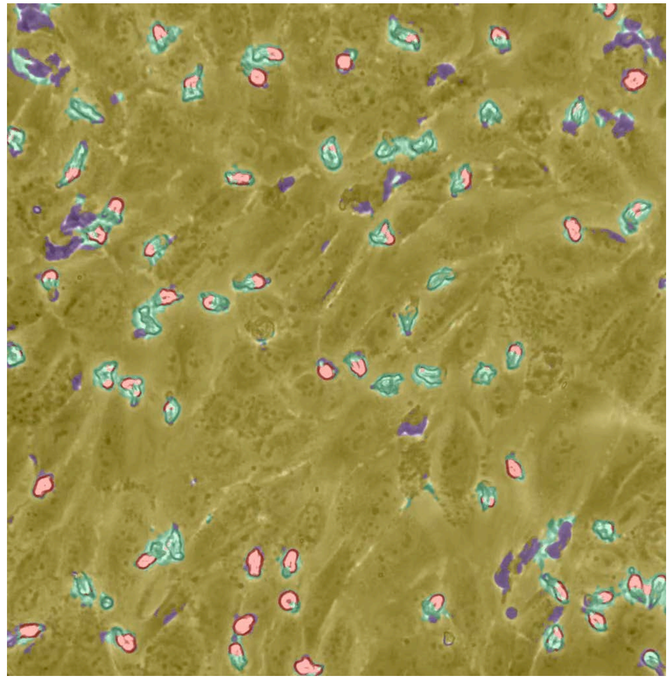
+

Features/ Convolutions + Random Forest Algorithm

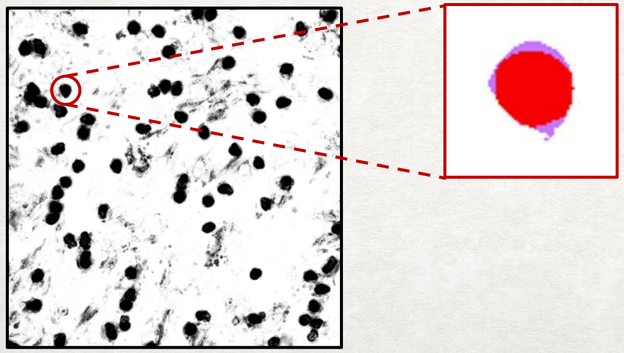
+Post-Hoc Analysis w/ ML model via LENet

+Script analysis of centroids and nearest neighbors





Ex. 10 nM fMLP

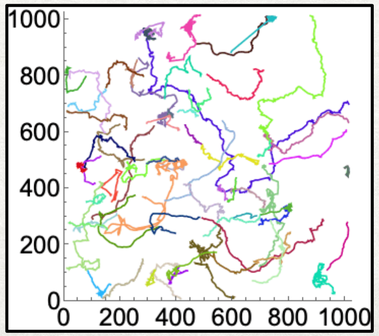


1. States …

- Apical
- Basal
- Probing
- ECs

2. Counts

- Total
- Per state



3. Migration Parameters …

- Speeds
- Persistance

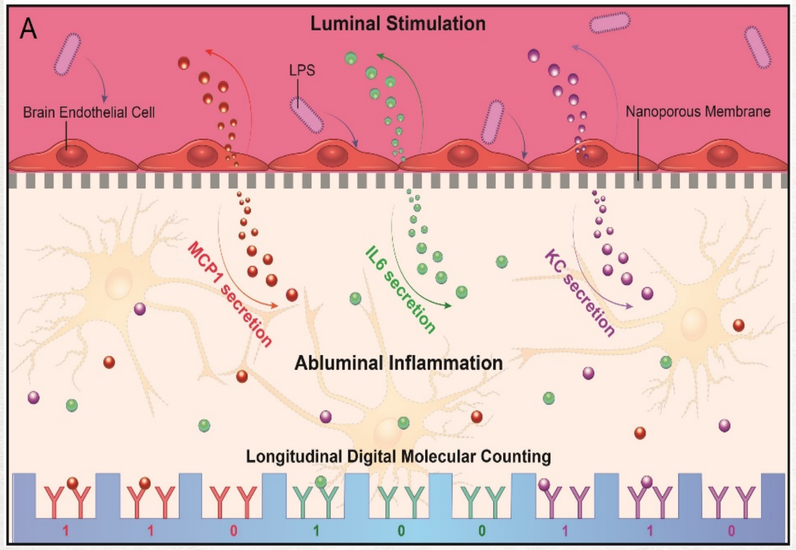
Apical Migration

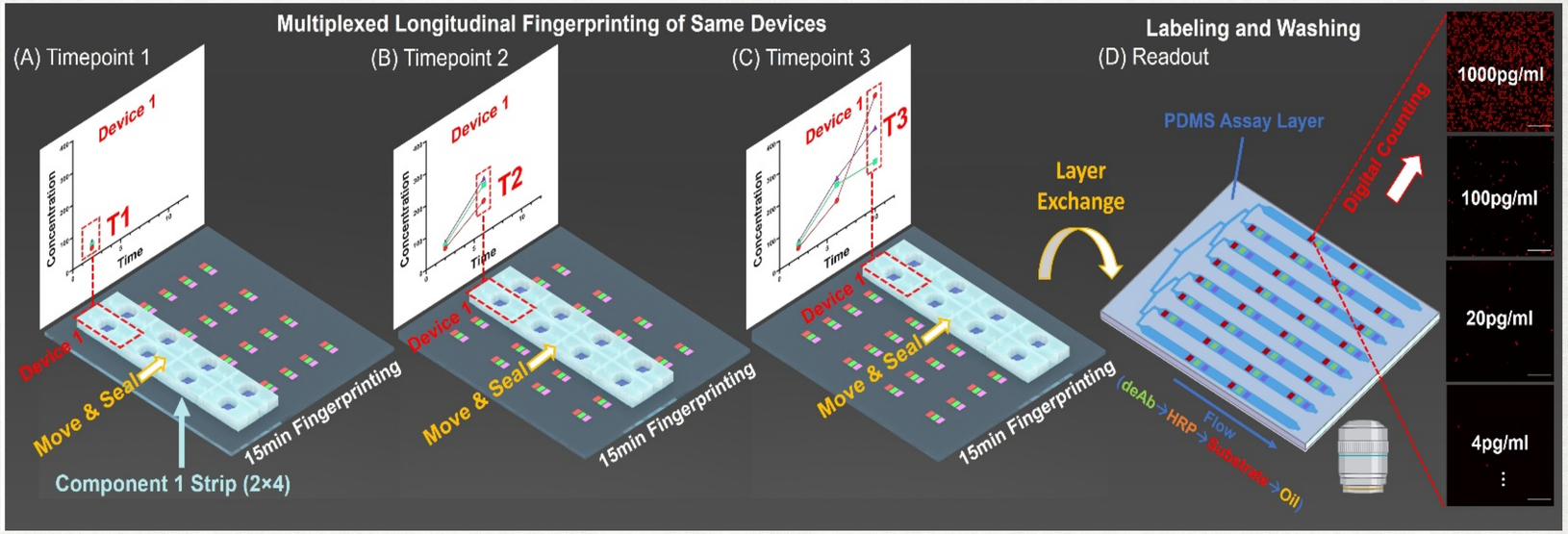
Basal Migration

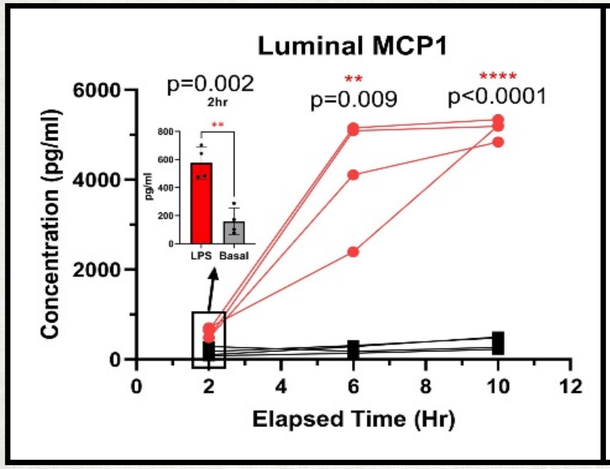
INTEGRATED DIGITAL ELISA REVEALS A POLARIZED SECRETORY RESPONSE

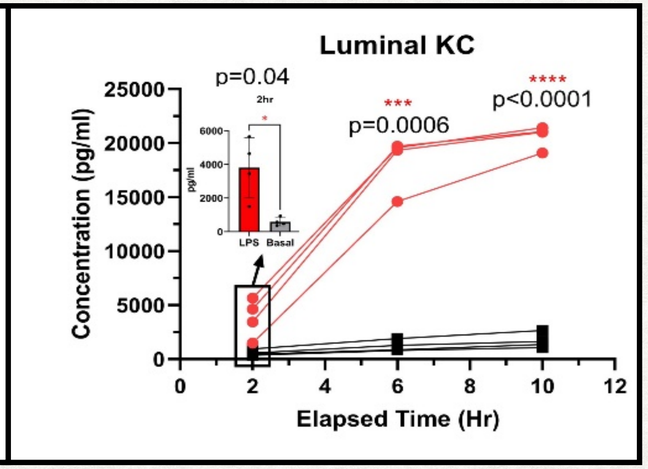
KURABAYASHI AND SINGER LABS

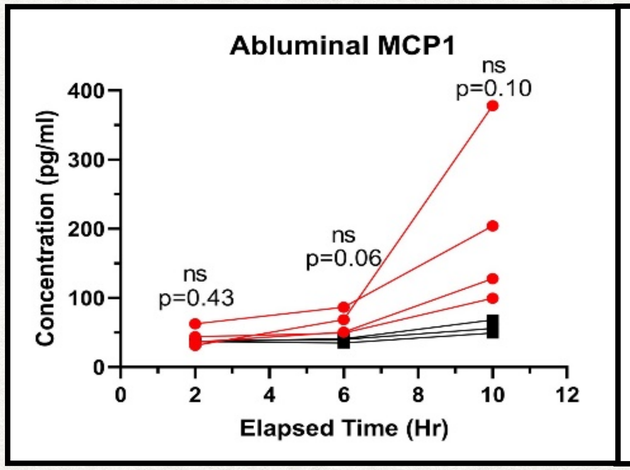
UNIVERSITY OF MICHIGAN

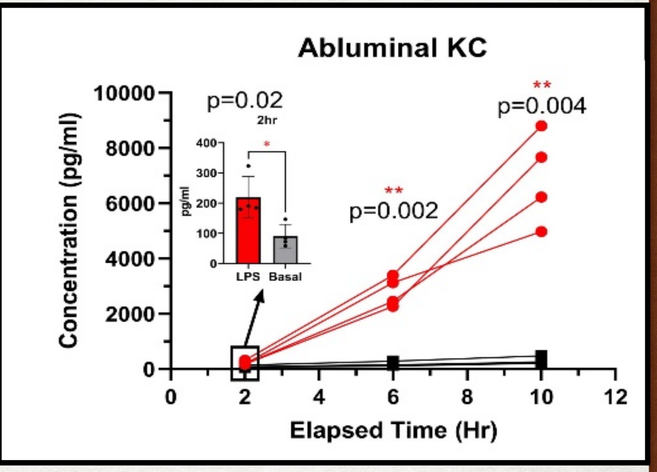






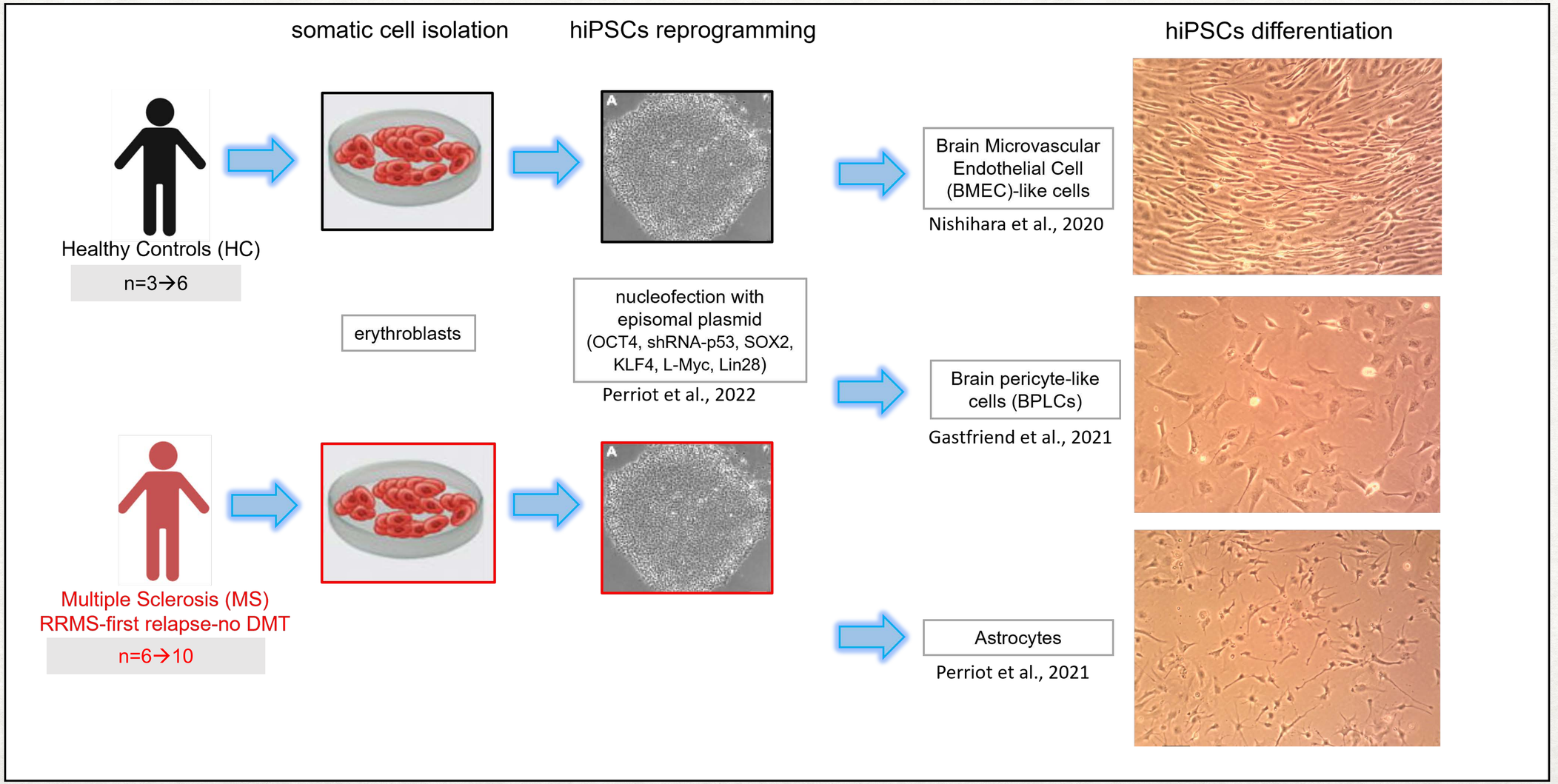




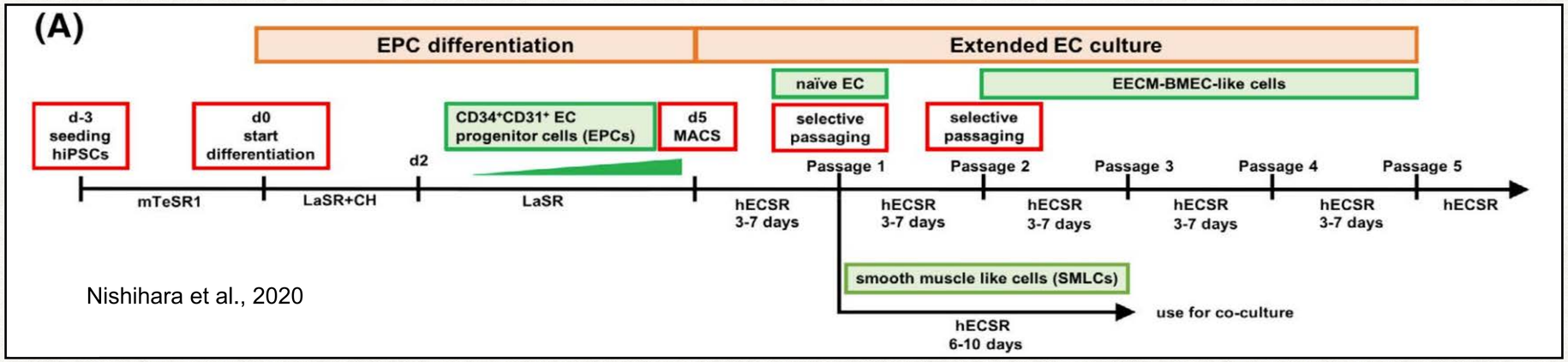


- Secretory response to LPS is fast and asymmetric
- Asymmetric chemokine release will establish gradients that may drive (or suppress) leukocyte transmigration across the BBB

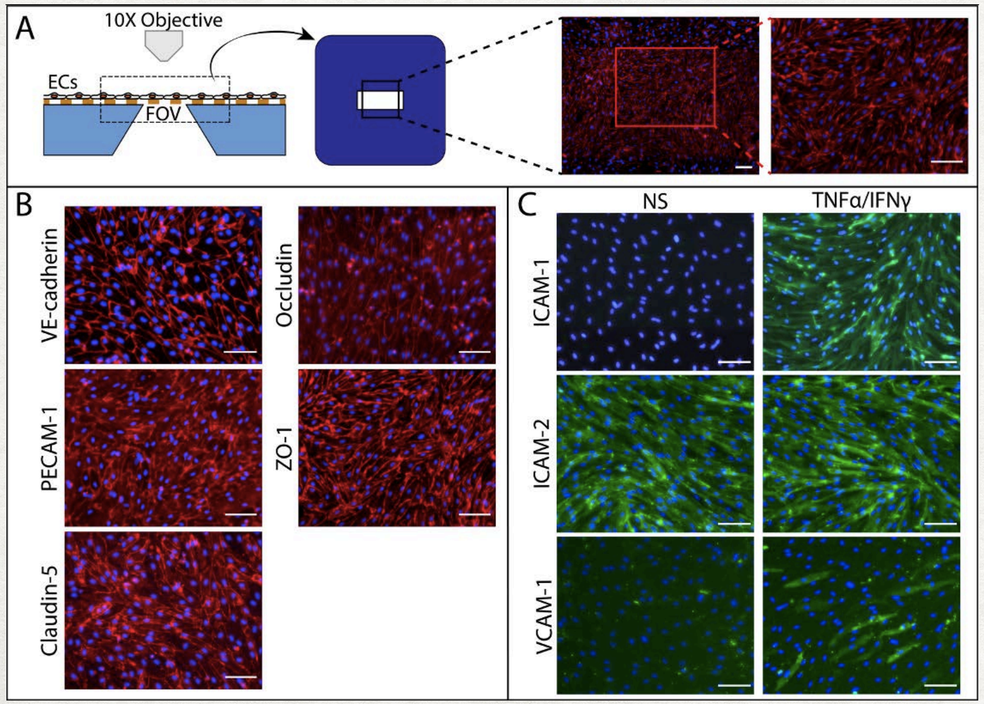
DIFFERENTIATION OF THE CELLS OF THE HUMAN NEUROVASCULAR UNIT



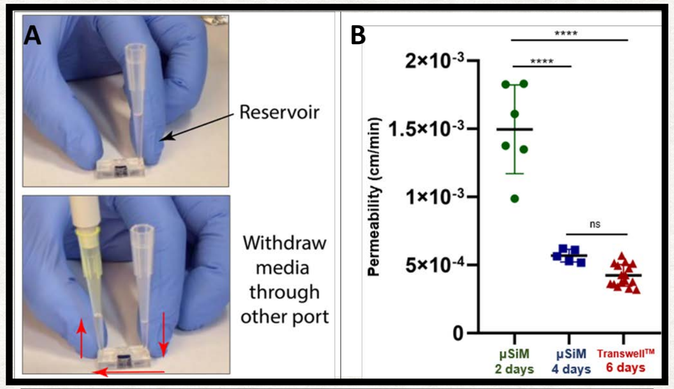
BUILDING THE hNVU: DIFFERENTIATION AND VALIDATION OF EECM-BMEC-LIKE CELLS

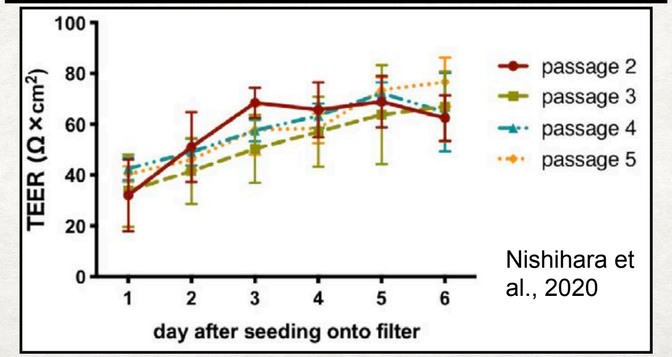


Nishihara et al., 2020

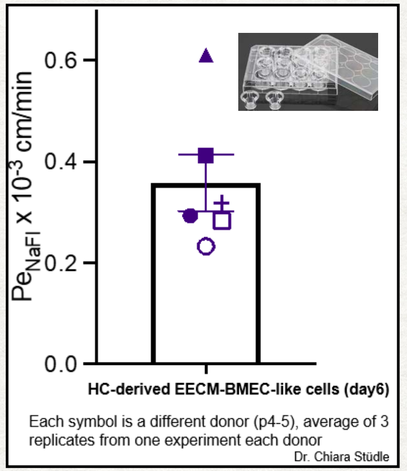


McCloskey et al., (2022) Adv. Healthcare Mater., 202200804



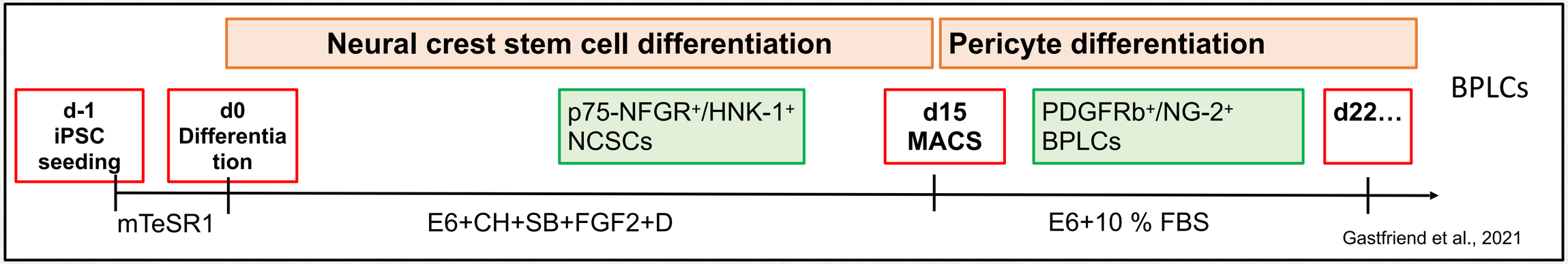


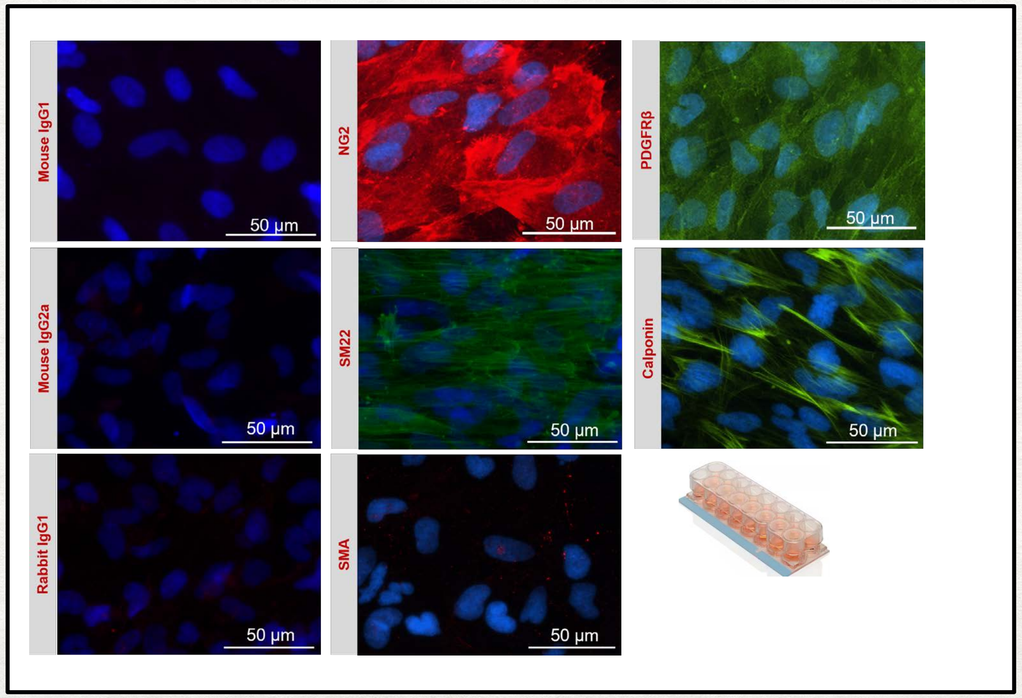
Nishihara et al., 2020

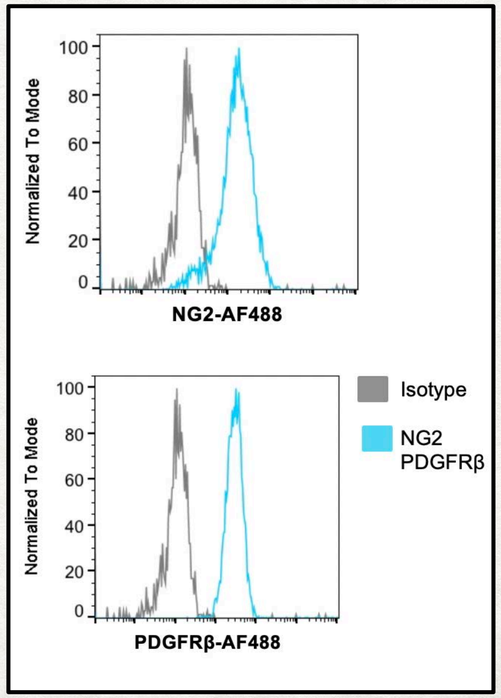


UNIVERSITÄT BERN

BUILDING THE hNVU: DIFFERENTIATION AND VALIDATION OF BRAIN PERICYTE-LIKE CELLS

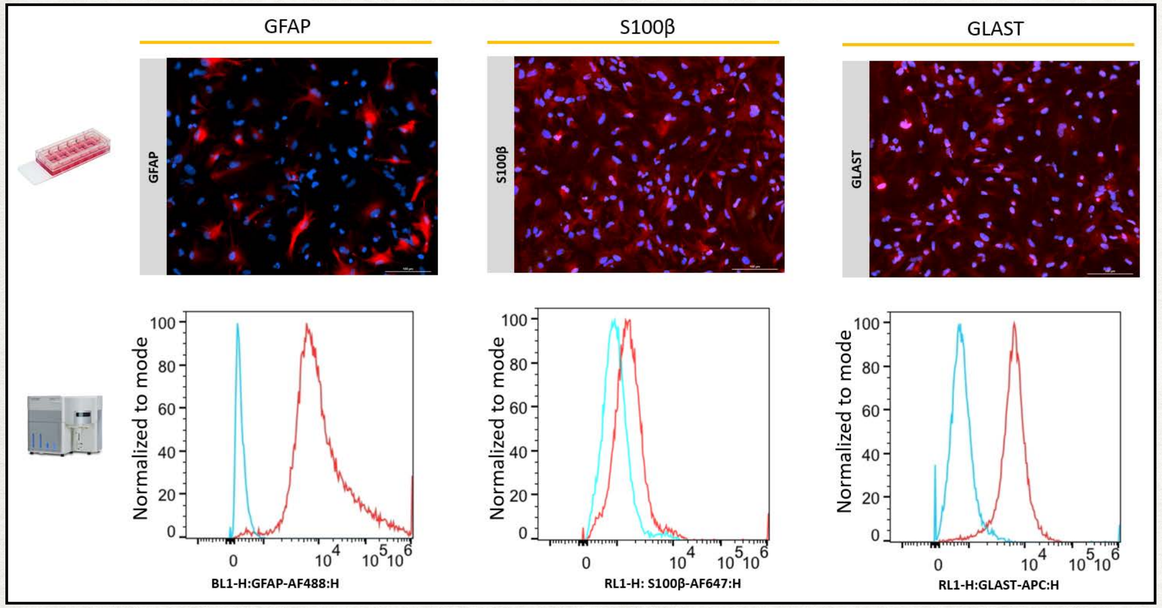


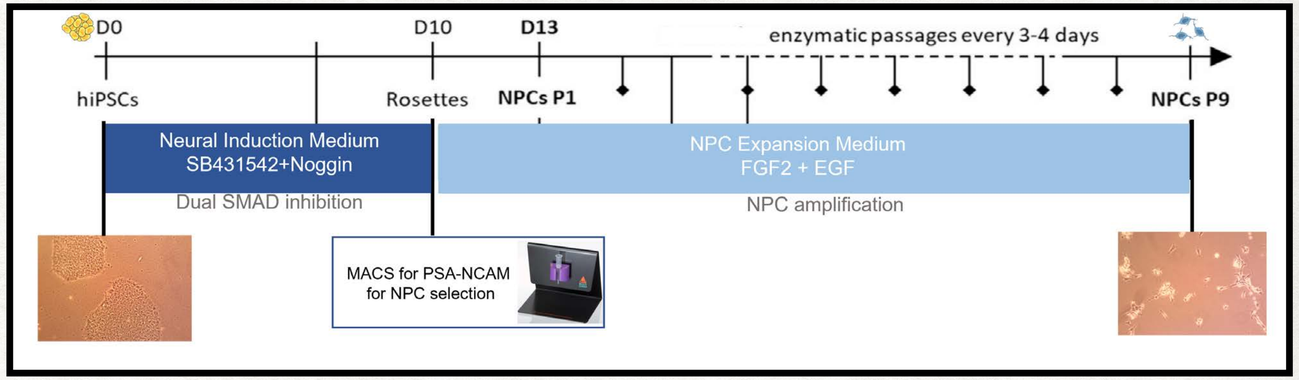


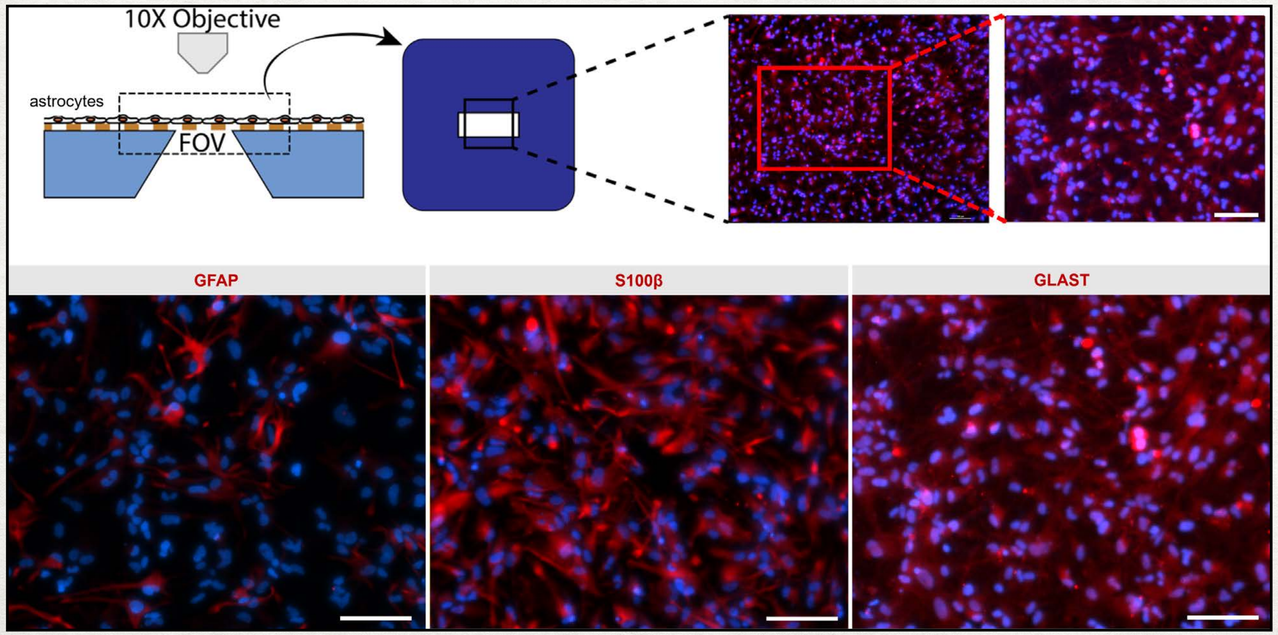


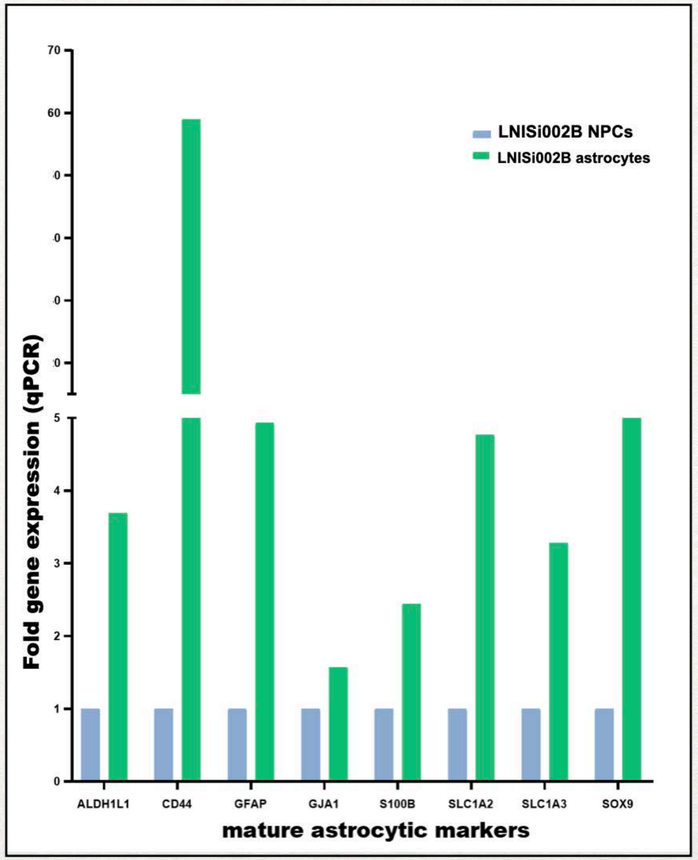
UNIVERSITÄT BERN

BUILDING THE hNVU: DIFFERENTIATION OF iPSC-DERIVED ASTROCYTES









Perriot S, Canales M, Mathias A, Du Pasquier R.STAR Protoc. 2021 Oct 20;2(4):100902

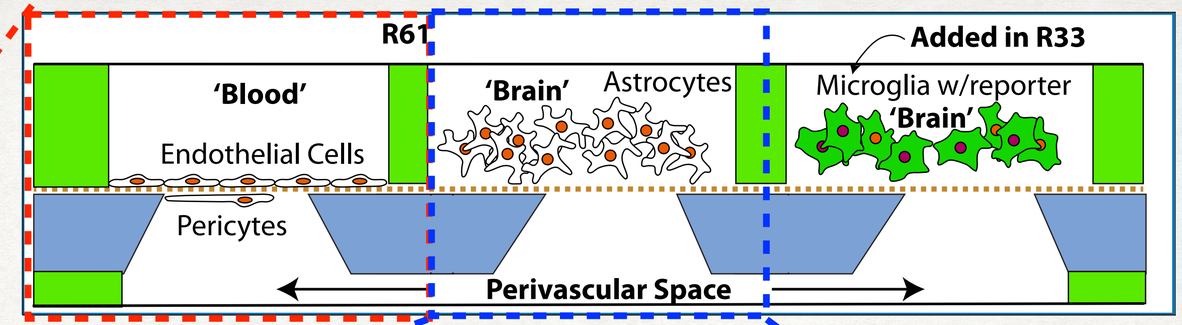
UNIVERSITÄT BERN

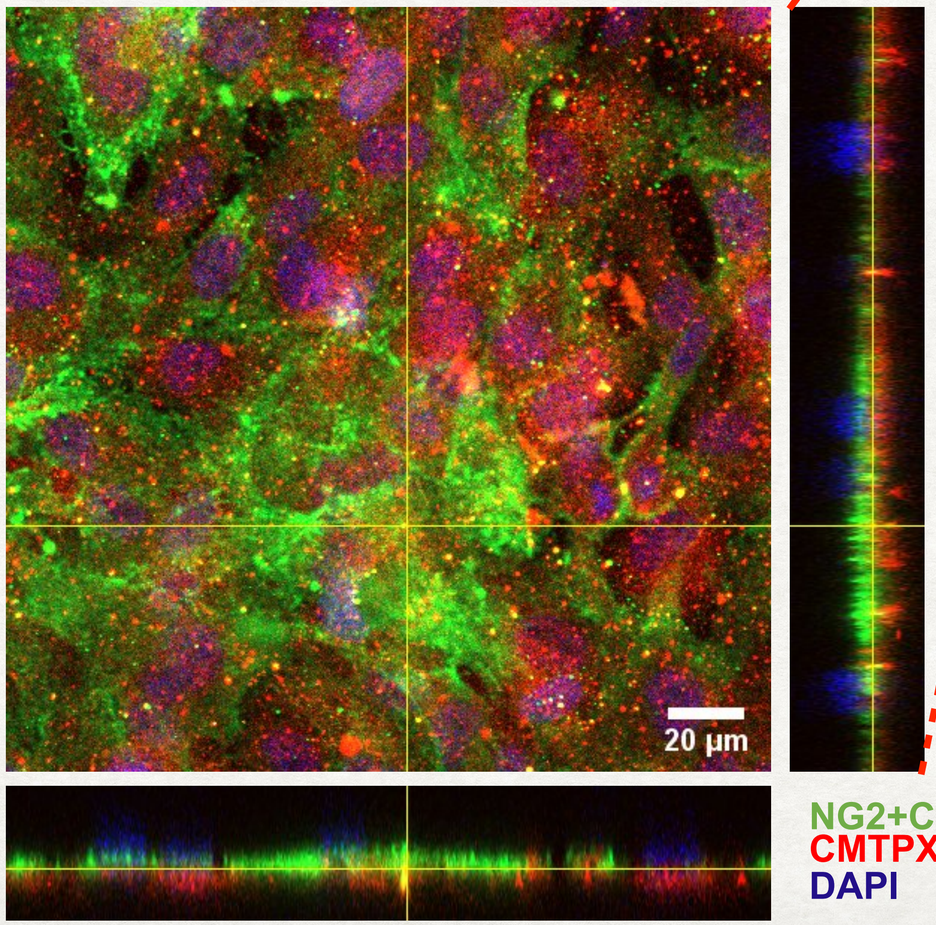
BUILDING hNVU IN THE μSIM: TRI-CULTURE

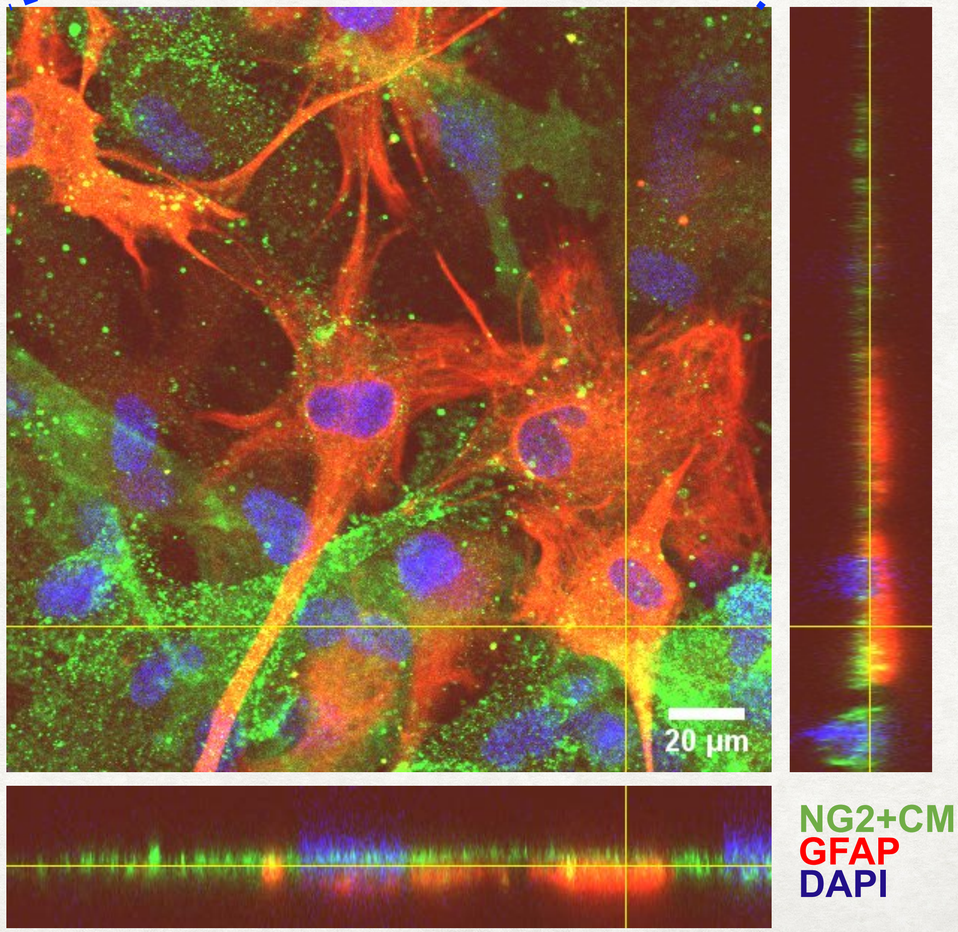
BPLCs: bottom channel
- Labelled with 32 μM CMFDA pre-seeding
- Immunostained with NG2+AF488

EECM-BMEC-like cells: left compartment of the well
- Labelled with 32 μM CMTPX pre-seeding

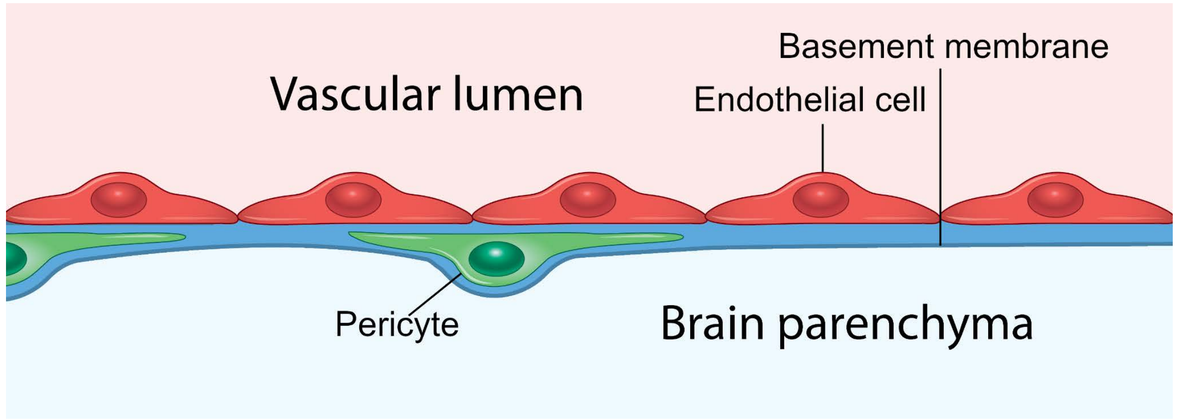
Astrocytes: right compartment of the well
- Immunostained with GFAP+Cy3





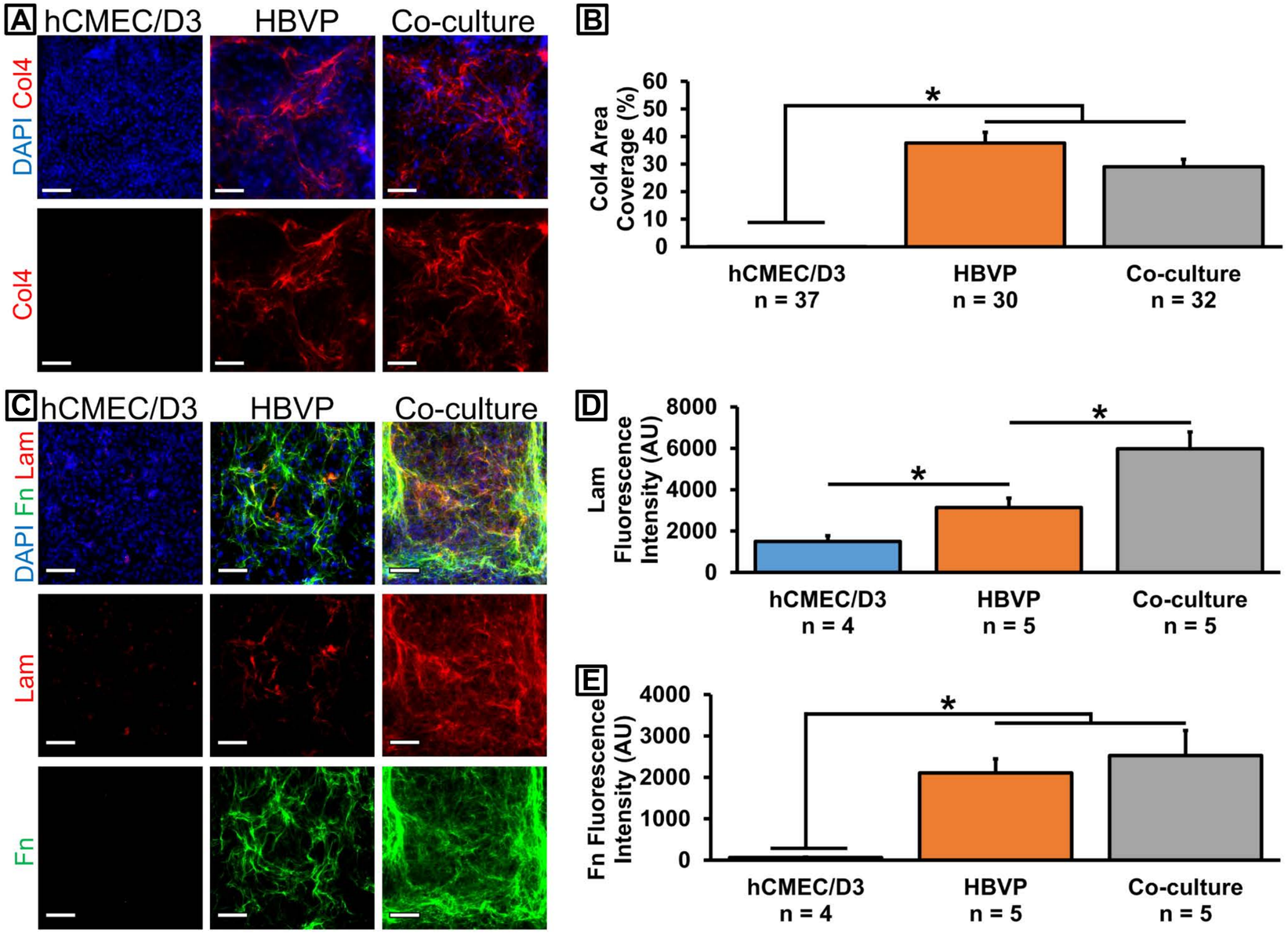


Formation of a Vascular Basement Membrane (BM)

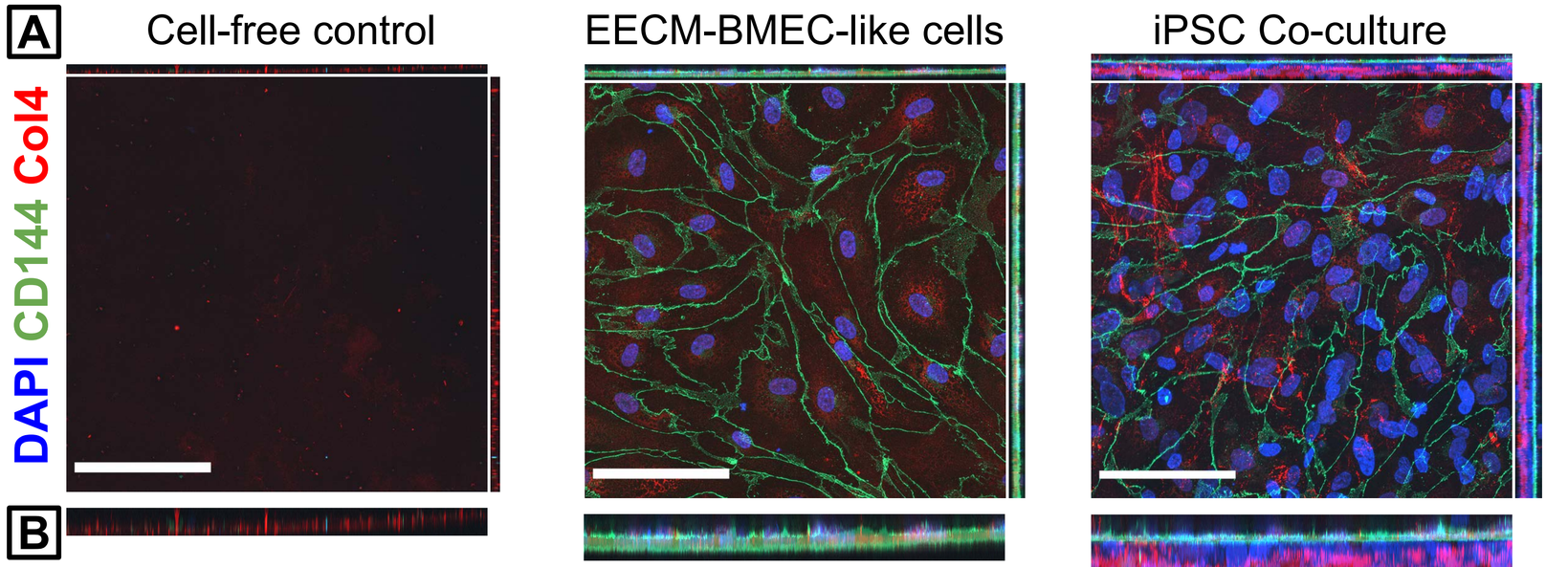


Immunostained image of cell nuclei (blue), VE-cadherin (green) and collagen IV (red) taken on an Andor Dragonfly Spinning Disk Confocal Microscope. Bottom left and exploded view = side view obtained from 3D projection. Image taken at 40x magnification with a 0.21 μm step interval. Scale bar = 100 μm.

Pericytes Contribute the Majority of BM Proteins



A. Immunofluorescence images of collagen IV (Col4) 7-10 days after HBVP seeding and 6-8 days after hCMEC/D3 seeding in monocultures and co-cultures. **B.** Barplot of collagen IV area coverage in immunostained images. Error bars = SEM. **C.** Immunofluorescence images of fibronectin (Fn) and laminin (Lam) 8 days after HBVP seeding and 7 days after hCMEC/D3 seeding in monocultures and co-cultures. **D.** Barplot of laminin fluorescence intensity. Error bars = StDev. **E.** Barplot of fibronectin fluorescence intensity. Error bars = StDev. Scale bars = 100 μm. * = $p < 0.001$. One-way ANOVA performed on $\log_{10}$ transformed data with Tukey post-hoc test.

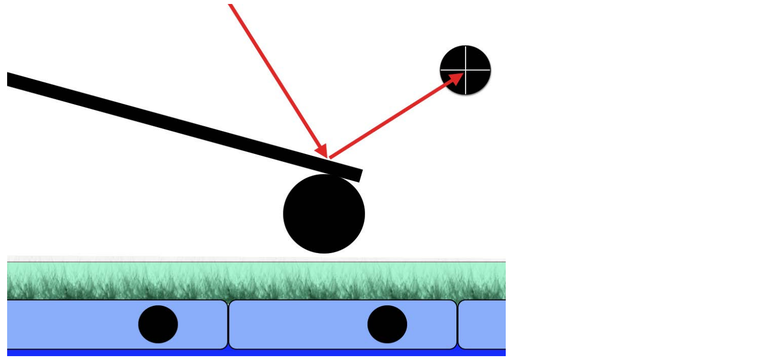


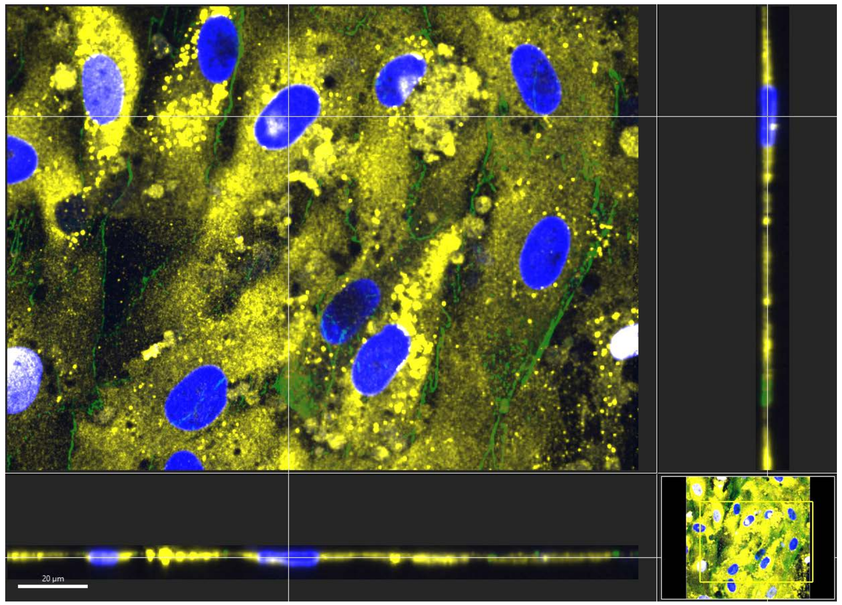
iPSC-Derived BBB Cells Deposit BM Proteins in a Similar Manner to the hCMEC/D3s and HBVPs.

A. Immunofluorescence images of collagen IV (Col4) and VE-cadherin (CD144) 7 days after iPSC-pericyte seeding and 6 days after EECM-BMEC-like cell seeding in monocultures and cocultures. A cell-free membrane was immunostained as a control. **B.** Images of x-axis 3D projections from panel A distorted to highlight the relative z-positions of the cells and collagen IV. **C.** Immunofluorescence images of fibronectin (Fn) 7 days after iPSC-pericyte seeding and 6 days after EECM-BMEC-like cell seeding in monocultures and co-cultures. A cell-free membrane was immunostained as a control. **D.** Images of x-axis 3D projections from panel C distorted to highlight the relative z-positions of the cells and fibronectin. The endothelial cell nuclei are indicated by the orange dashed lines in the co-culture image. Images were taken at 40x magnification on an Andor Dragonfly Spinning Disc Confocal Microscope with a 0.2 μm step interval. Scale bar = 100 μm.

GLYCOCALYX CHANGES IN RESPONSE TO INFLAMMATORY STIMULUS

All measurements on EECM-BMECs (IMR90)





iPSC-EC's grown statically 48 hours,
treated with TNF-α (10 ng/ml, last 4 hours)
(Hyaluronic Acid Binding Protein, Hoechst, ZO-1)

	EGL Thickness (nm) ± Std Err of Mean (n)
Grown under flow w/o treatment	**151** ± 19 **(16)**
Grown under flow w/ TNF	**48** ± 11 **(21)**
Grown statically	**52** ± 13 **(13)**

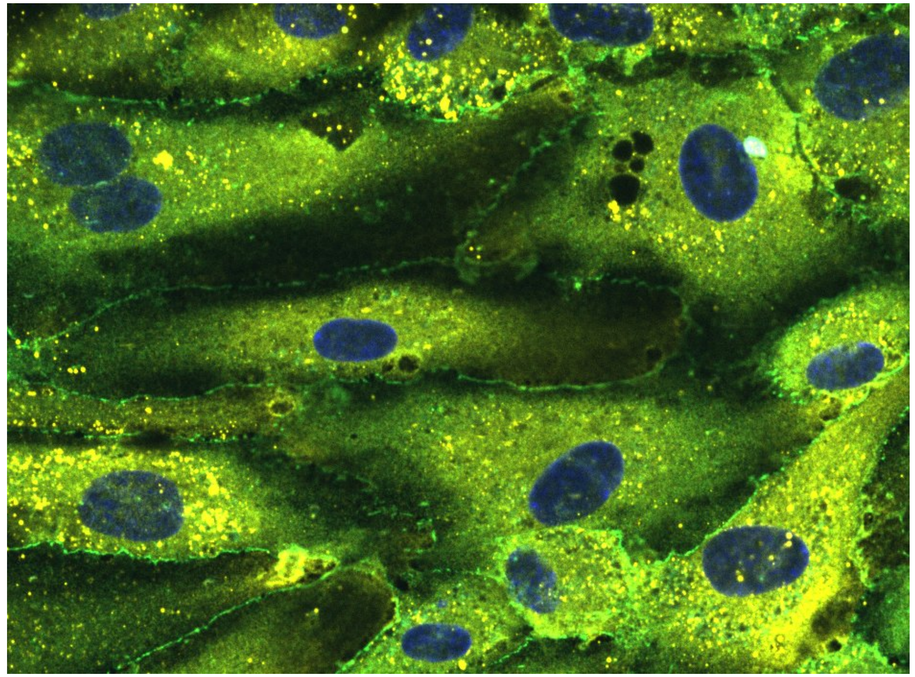
AFM Conclusions:

- Flow required for glycocalyx formation
- Thickness reduced by TNF-α

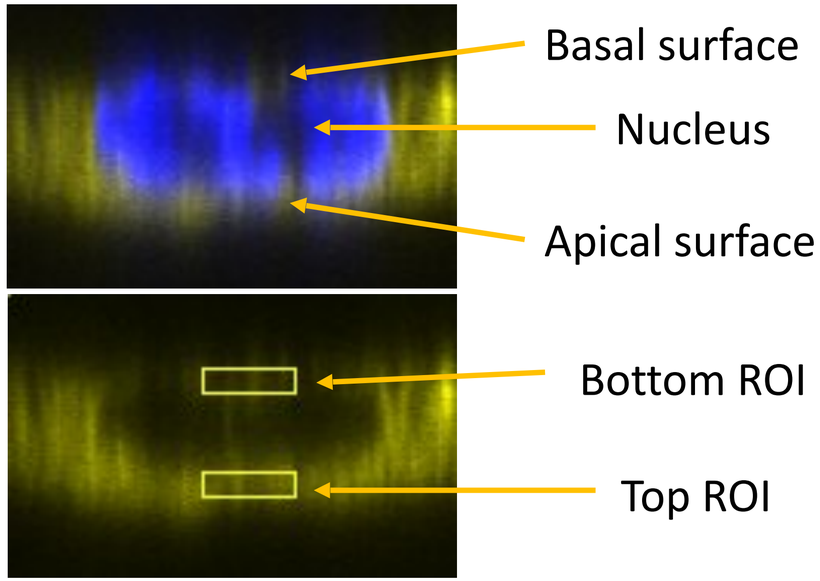
Cytosolic labeling of HA confounds immunohistochemical confirmation.

GLYCOCALYX CHANGES IN RESPONSE TO INFLAMMATORY STIMULUS

Reliable measures of luminal glycocalyx fluorescence require confocal imaging



Control cells, HUVEC:
73 hours 10 dyne/cm^2
HA: Yellow
ZO-1: Green
Hoechst: Blue
Inflammatory stimulus: 20 hours flow with Cytomix:
Equal parts by weight: TNF-α, Interferon-γ, IL-1β



Characteristic intensity of lognormal distribution

	Control	Cytomix treated	p < 0.05
Top	58.9	49.7	*
Bottom	38.0	45.6	*