Biopsy-Derived Human Intestine-Chips to Investigate Region-Specific Barrier Responses

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October 11, 2023

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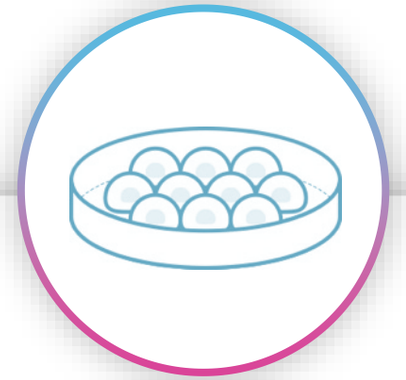
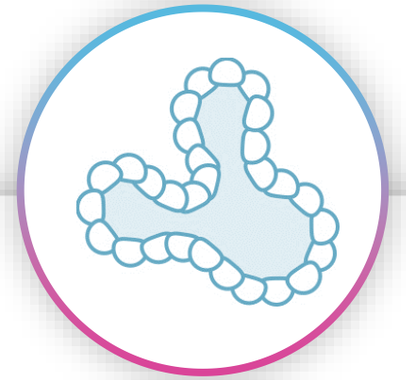
Challenges in Intestinal Research



2D cell models lack human-relevant cell differentiation, polarization, function, and transporter expression.



Organoids have limited cellular maturity, with a spherical structure that makes transporter studies and imaging difficult.

Animals have differences in drug metabolism, disease pathology, microbiome, etc.

Why do Existing Models Fall Short?

Conventional models fail to capture the complexity of the human intestine

In vivo Intestine		Conventional intestine cell models
Heterogenous range of cells	≠	Often 1 or 2 cell types
Close interaction with vasculature	≠	Often epithelial only
Region-specific characteristics	≠	Often uses Caco-2: Colonic origin but duodenal characteristics
Mechanical forces from fluid flow and peristalsis	≠	Static cell culture

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A Human-Centric Approach to Intestinal Modeling

Human Relevance

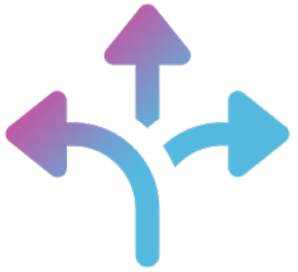
Cellular diversity with improved polarization, barrier function, and gene expression.

Tissue Specificity

Recreating tissue-specific characteristics and microenvironment.

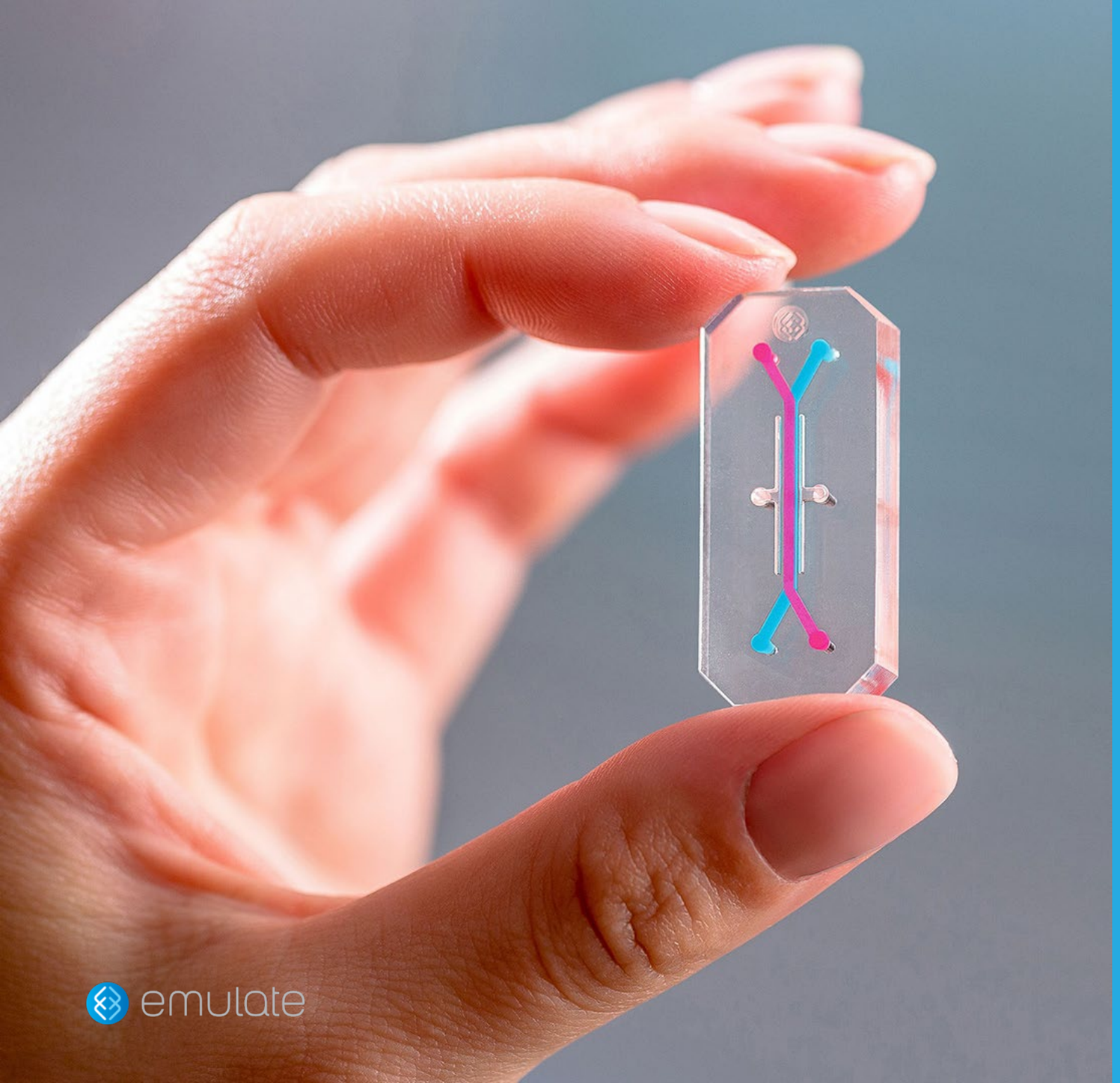
Broad Use Case Applicability

Applicability for ADME/Tox disease modeling, and efficacy testing.

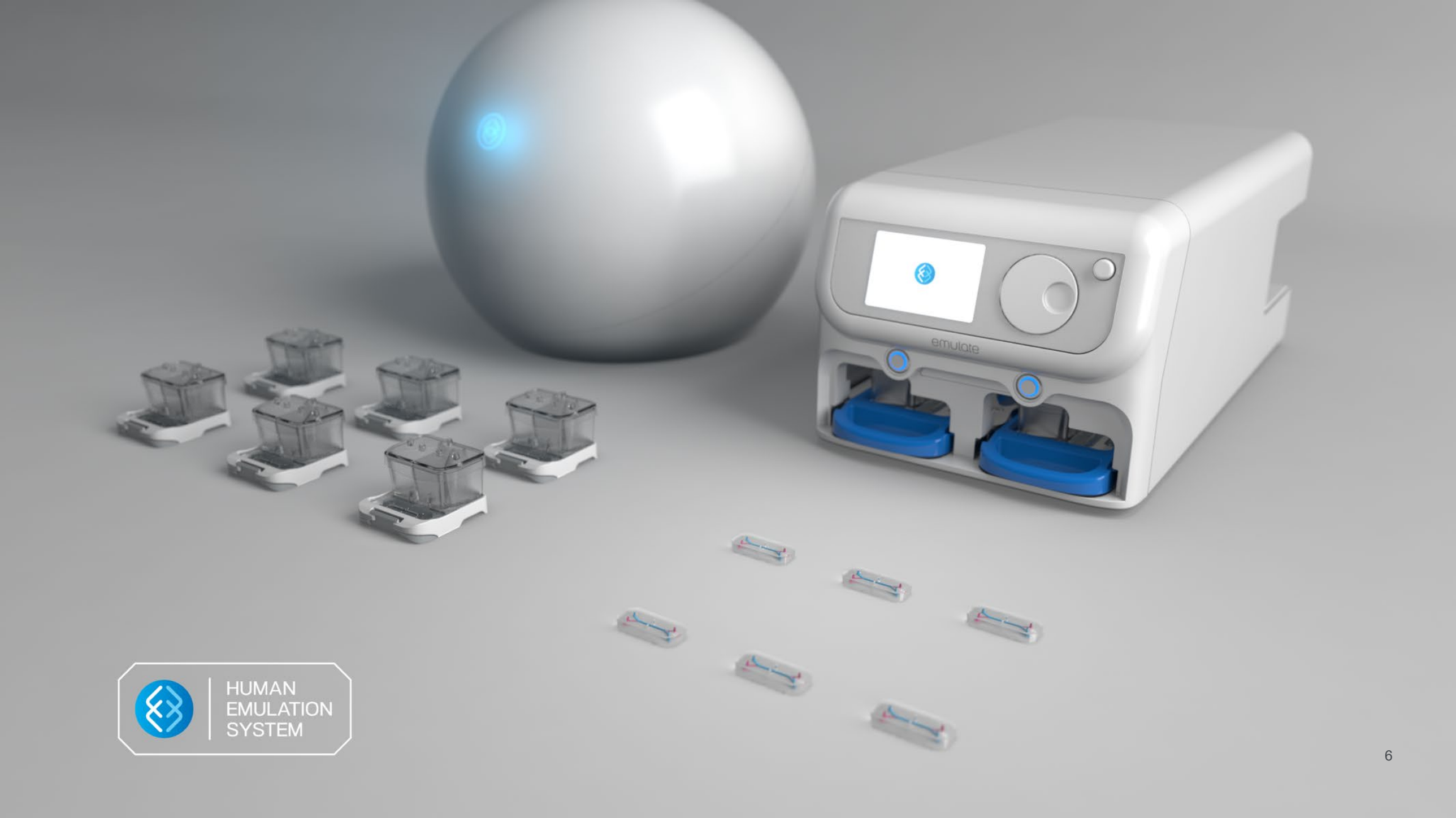


Model Flexibility

Tunable parameters, flexible cell sources, & scalable complexity

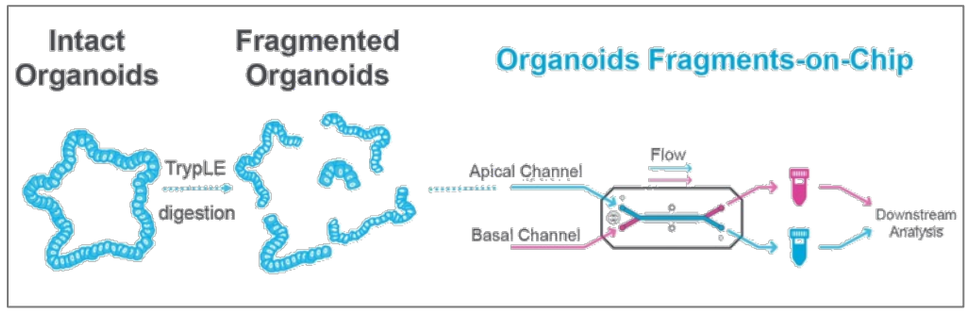


Modeling the Human Intestine with Organ-Chips



HUMAN
EMULATION
SYSTEM

Organ-on-a-Chip Technology Overcomes Organoid Limitations



Feature	Organoids	Emulate Organ-Chips
Media Flow	✕	✓
Mechanical Forces	✕	✓
In vivo-relevant cytoarchitecture	✕	✓
Consistent size & shape (robustness/reproducibility)	✕	✓
Easy to image	✕	✓
Easy to measure barrier function	✕	✓
Microbial co-culture	Requires microinjection	✓

Organoids and Organ-Chips are complementary technologies that can be combined for greater human relevance

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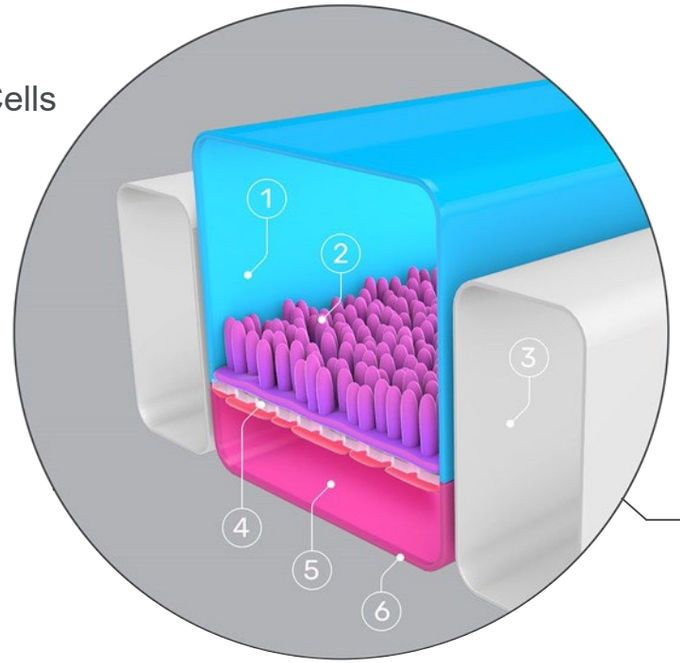
Modeling the Intestine with Organ-on-a-Chip Technology

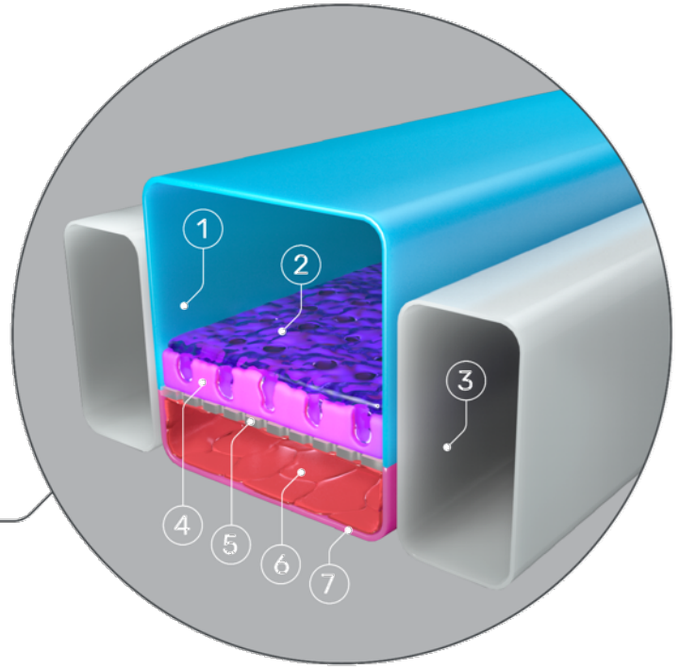
Duodenum Intestine-Chip

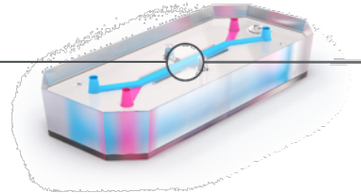
1. Top Channel
2. Intestine Epithelial Cells
3. Vacuum Channel
4. Porous Membrane
5. Endothelial Cells
6. Bottom Channel



Colon Intestine-Chip

1. Top Channel
2. Mucus
3. Vacuum Channel
4. Colon Epithelial Cells
5. Porous Membrane
6. Endothelial Cells
7. Bottom Channel





Human cells: Tissue-specific biopsy-derived organoids & primary endothelium

Mechanical forces: Tunable media flow rates and cyclic stretch

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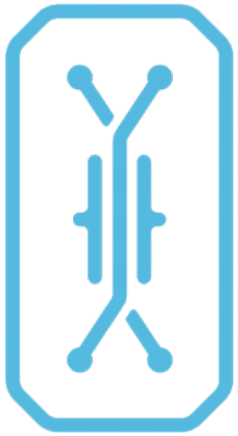


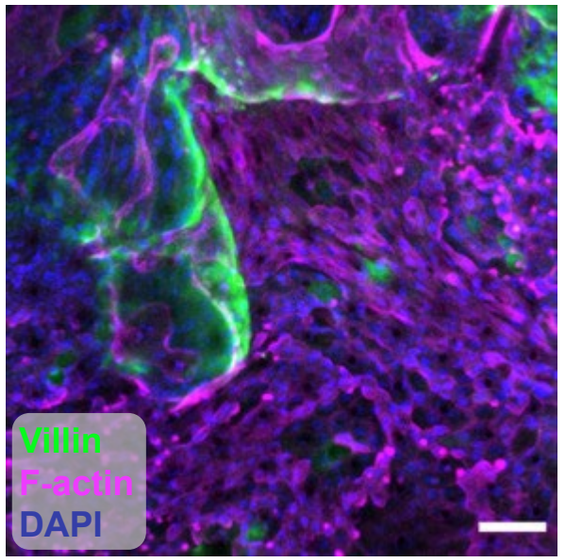
Duodenum Intestine-Chip

SEM micrograph of microvilli network in Colon Intestine-Chip

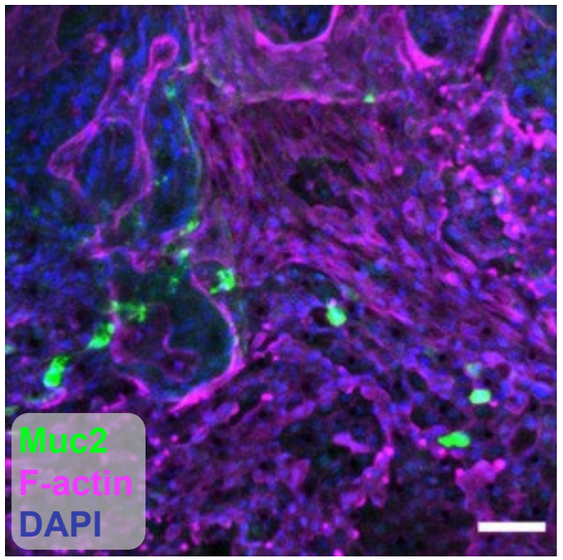
Major Epithelial Cell Subtypes Represented on-Chip

Representative Confocal Fluorescent Imaging

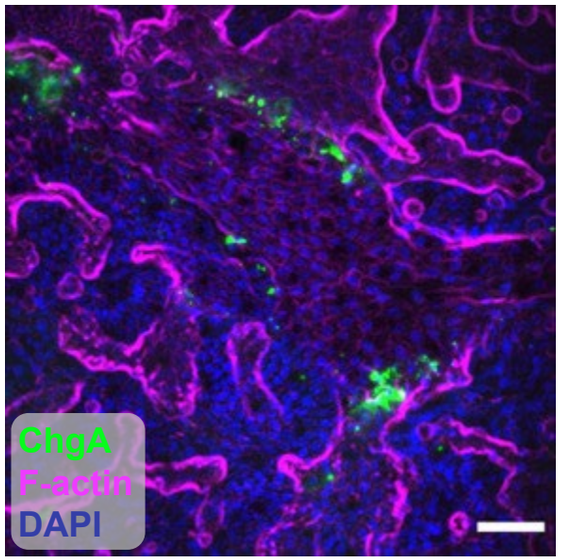
Absorptive Enterocytes



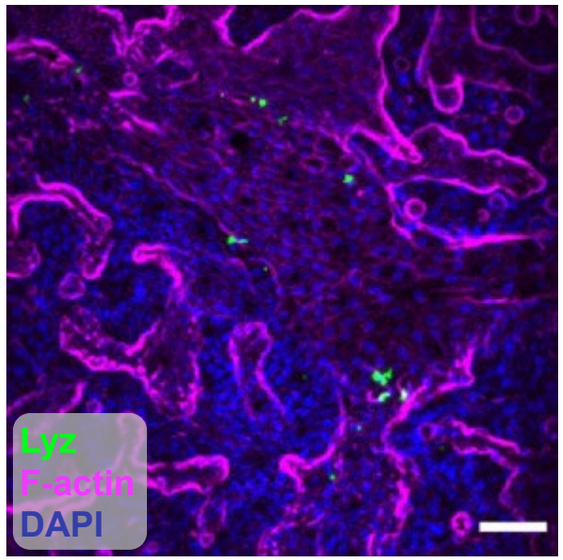
Goblet Cells



Enteroendocrine Cells



Paneth Cells



Scale bar: 50um

All major epithelial cell types present on-chip

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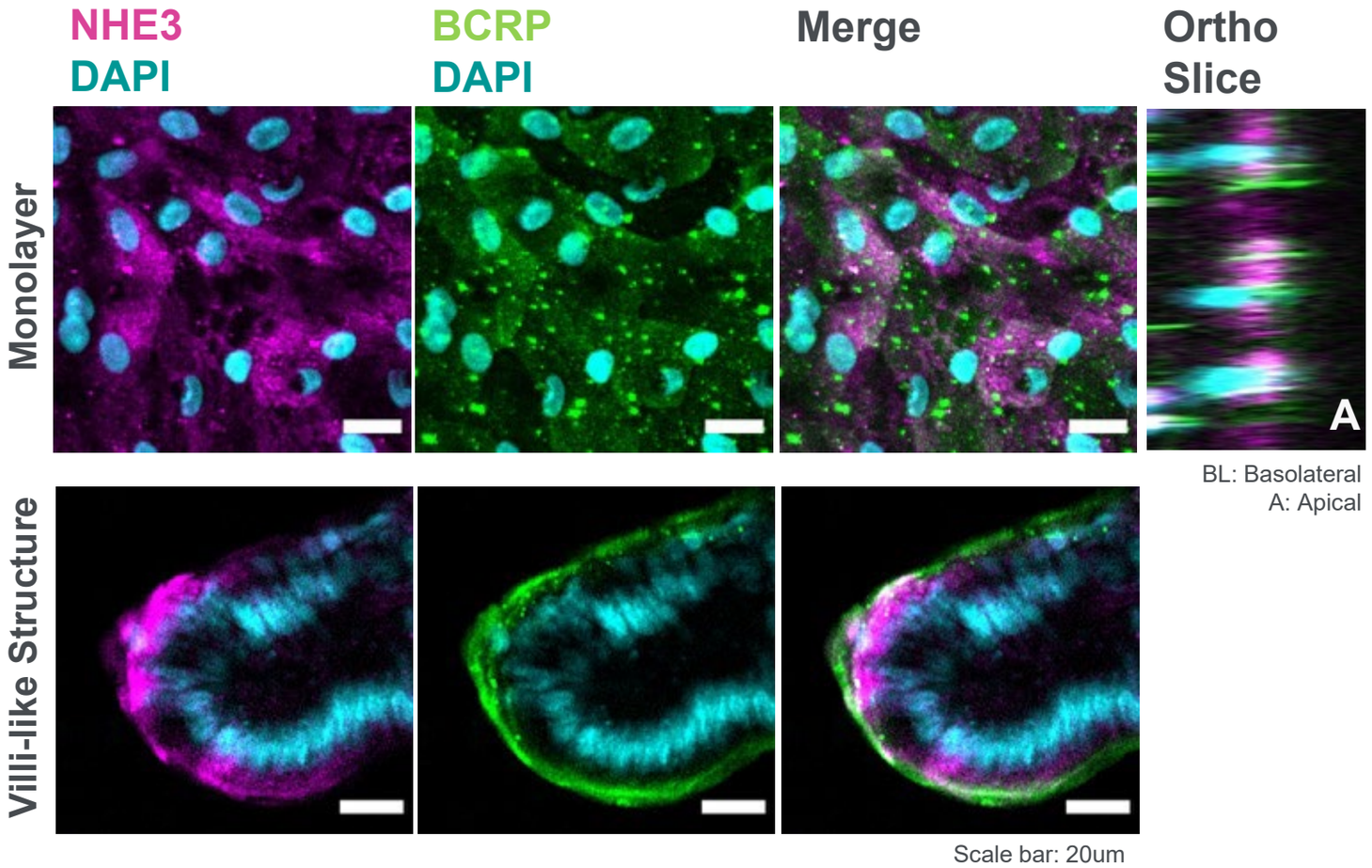
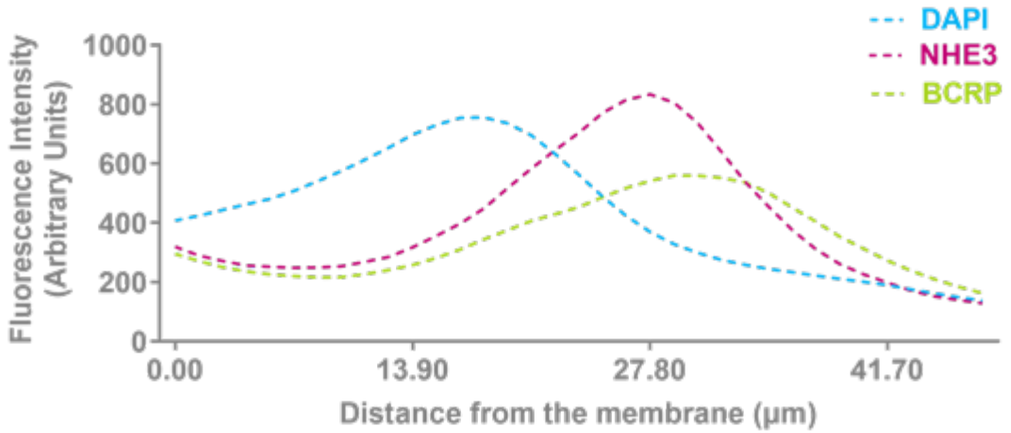
Day 8 of Culture

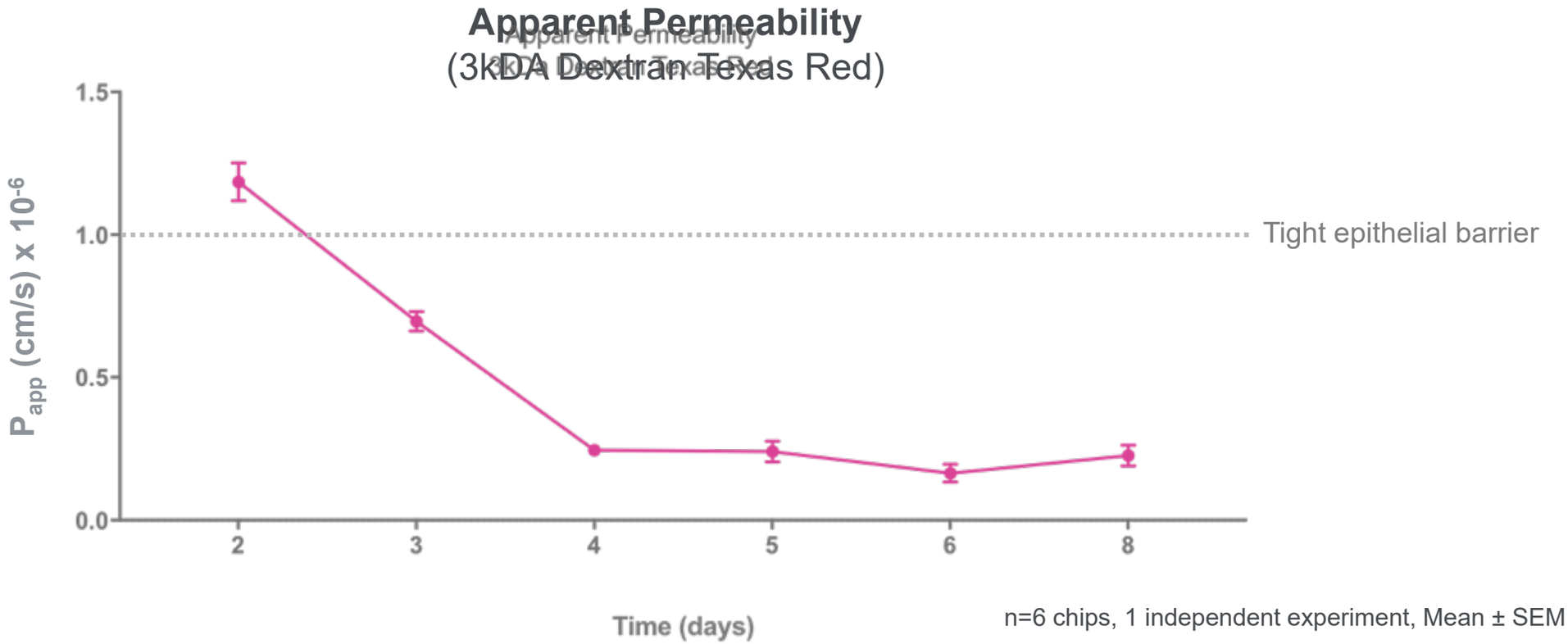
Proper Epithelial Polarization on-Chip





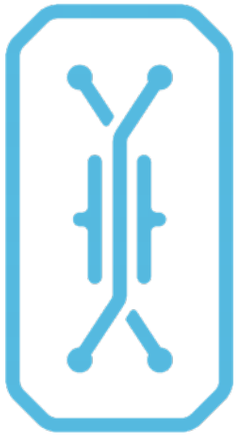
Duodenum Intestine-Chip demonstrates cellular polarization and correct localization of major intestinal transporters

Duodenum Intestine-Chip forms Tight Barrier



n=6 chips, 1 independent experiment, Mean ± SEM

Duodenum Intestine-Chip achieves strong intestinal barrier function to 3kDa dextran (~1x10^{-6} cm/s)



Colon Intestine-Chip

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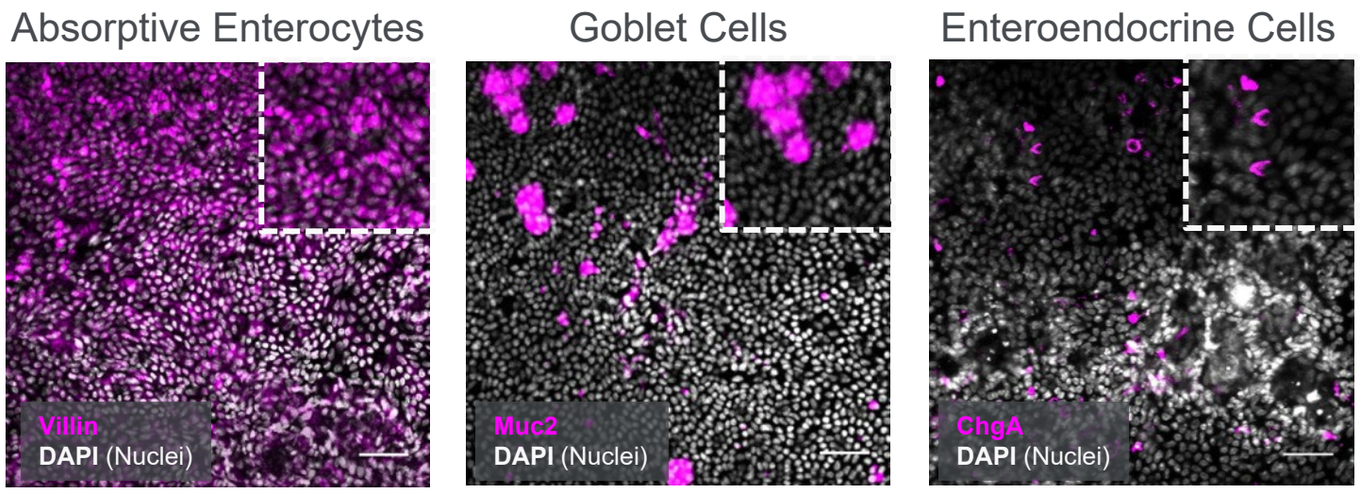
SEM micrograph of microvilli network in Colon Intestine-Chip

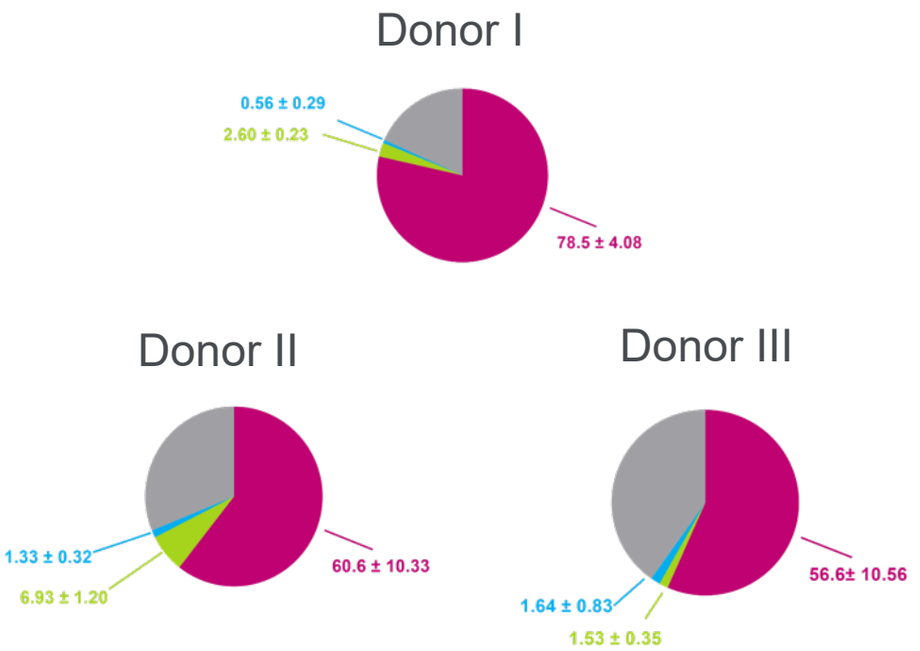
Major Epithelial Cell Subtypes Represented with Expected Donor Variability

Representative Confocal Fluorescent Imaging



Scale Bar: 50µm

Intestinal Epithelial Cell Types (%)



*5 FOVs acquired per chip
N=3 chips/ donor, Mean±SD

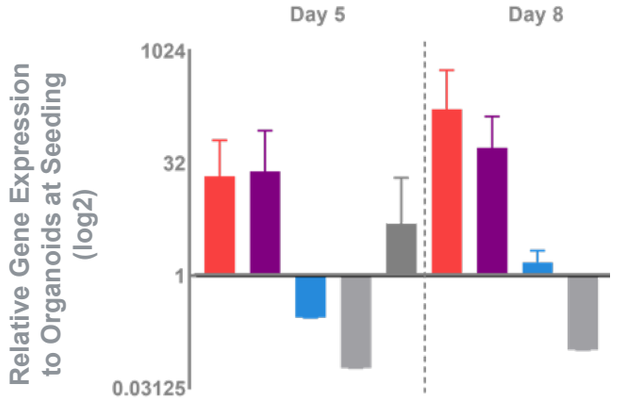
All major epithelial cell types are present on-chip, with expected donor-to-donor variability.

***In vivo* proportions:** ~70-80% absorptive enterocytes, ~15-15% goblet cells, ≤1% enteroendocrine cells (EECs)
Relative abundance of each cell type is expressed as the percentage of the positively stained cells for each marker over total number of nuclei.

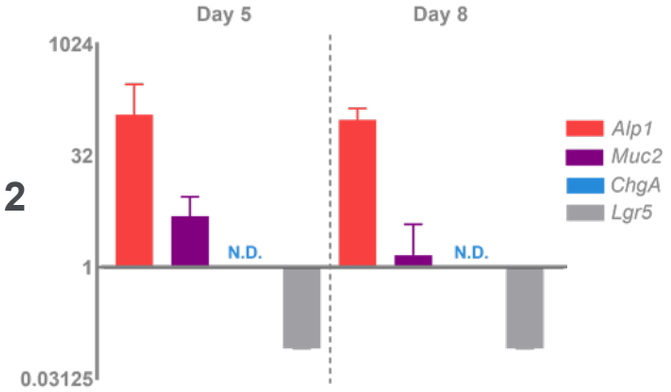
Apostolou, A., et. al. (2021). *Cellular and molecular gastroenterology and hepatology*.

On-Chip Culture Promotes Multilineage Differentiation of Epithelial Cells

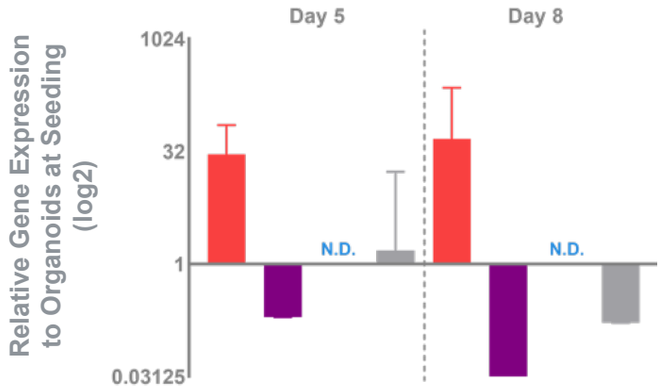
Donor 1



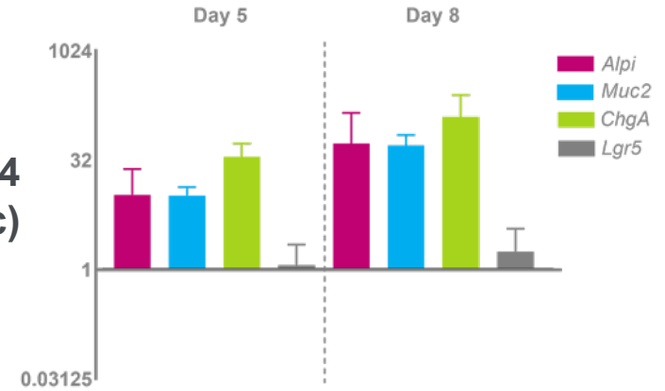
Donor 2



Donor 3



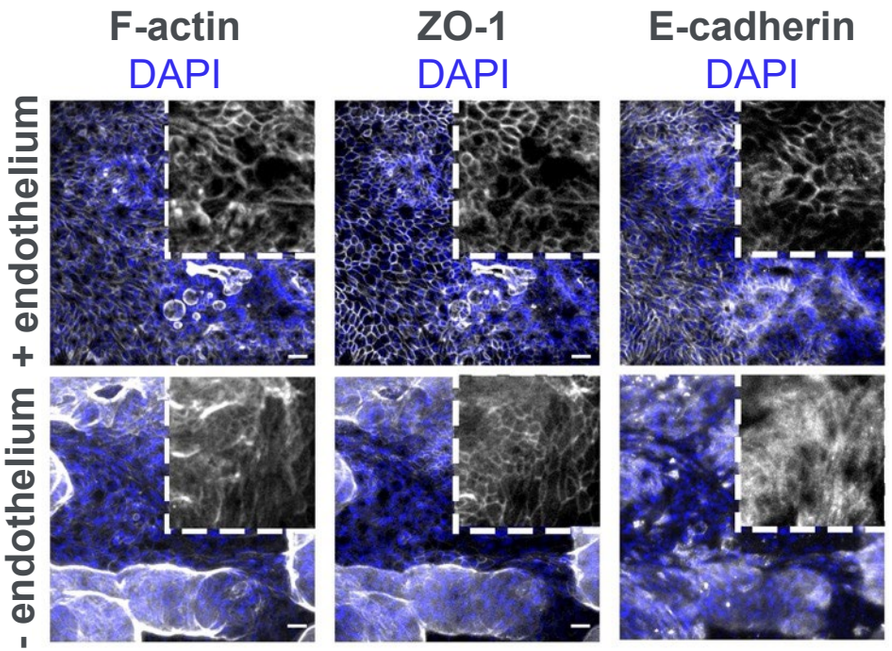
Donor 4 (Pediatric)



Colon Intestine-Chip allows for multilineage differentiation of organoid-derived epithelial cells and reflects expected interindividual variability

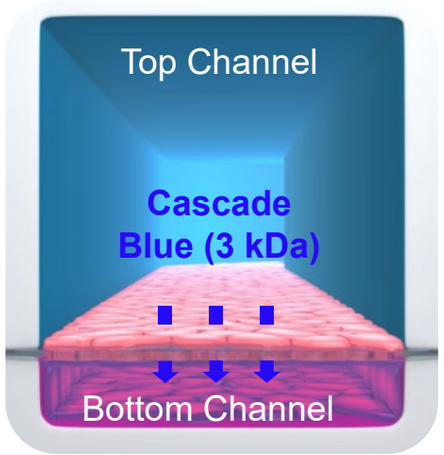
Donors 1-3: Apostolou, A., et. al. (2021). *Cellular and molecular gastroenterology and hepatology*.
Donor 4: Unpublished

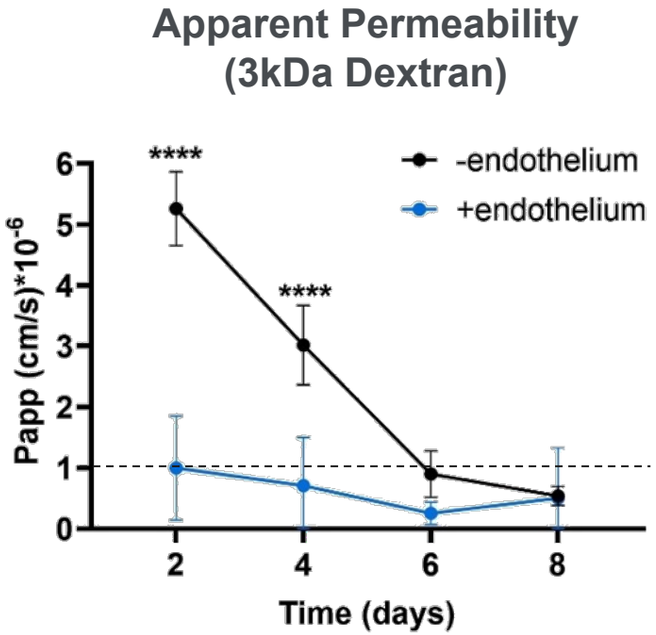
Barrier Formation Enhanced by Endothelial Co-Culture



Scale Bar: 50um

Apparent Permeability Assay (P_{app})



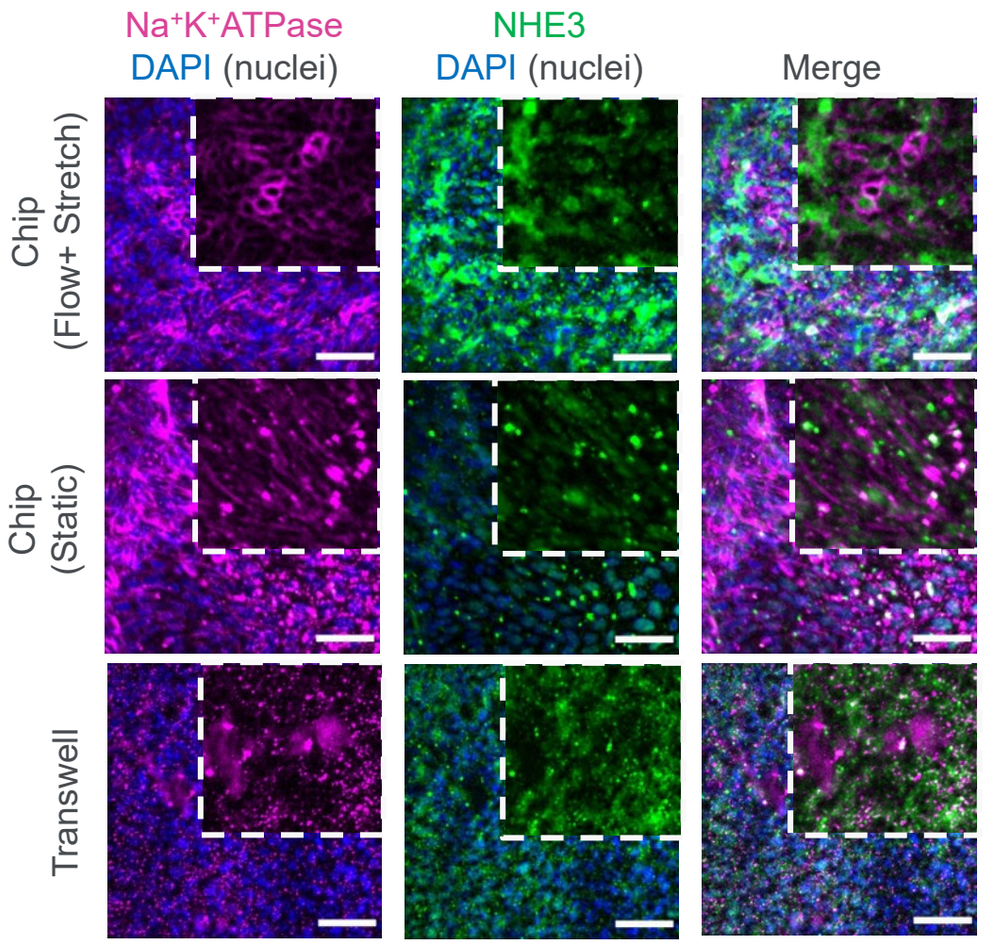


N = 3-11 chips/group, mean ± 95% CI
Two-way ANOVA, Tukey's post hoc test,
****: p<0.0001

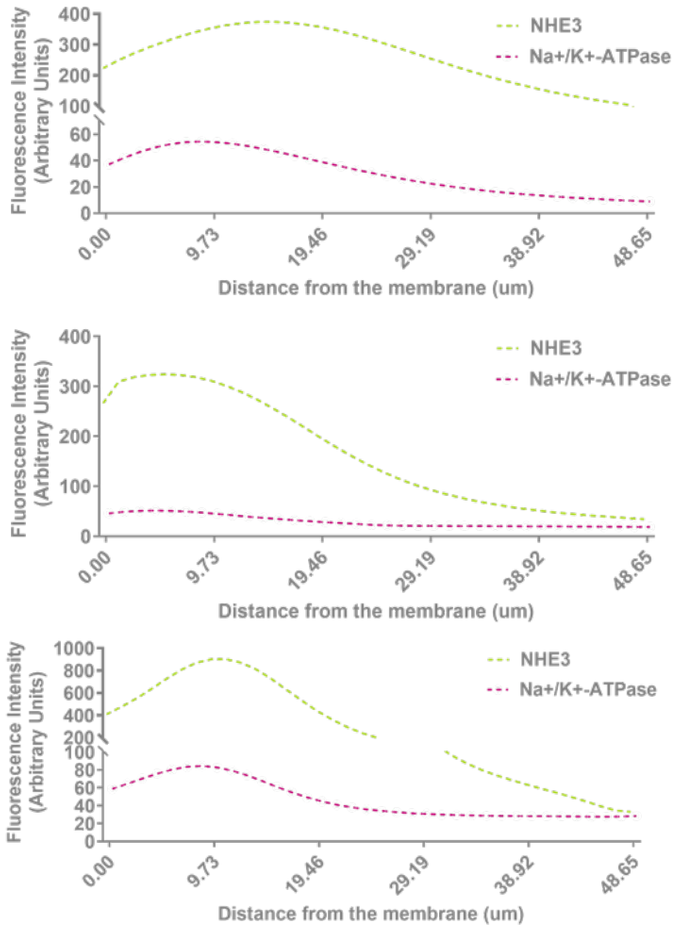
Endothelium enhances establishment of epithelial tight junctions and functional barrier

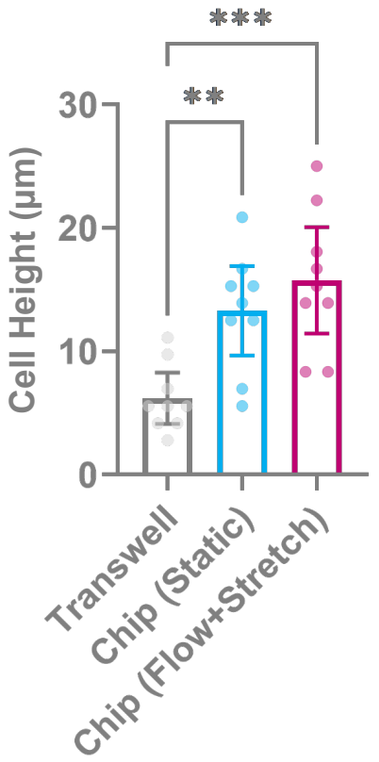
Apostolou et al., Cellular and Molecular Gastroenterology and Hepatology (2021)

Epithelial Polarity Improved by On-Chip Culture & Mechanical Forces



Scale bar: 50μm

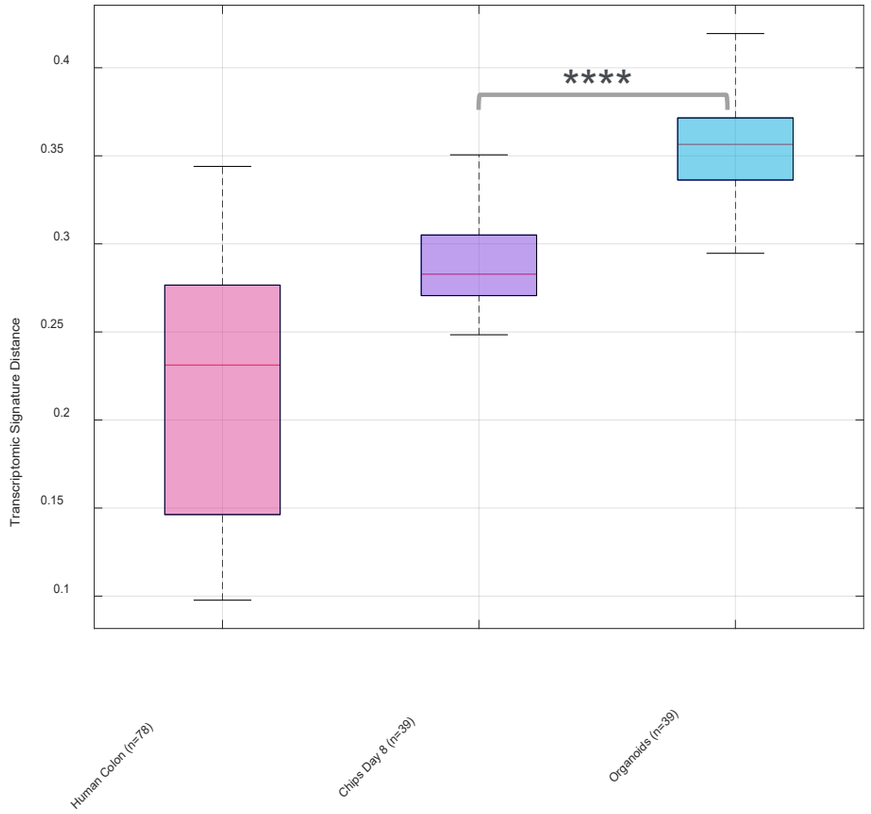




n= 3 chips/group, 3FOVs/chip, mean ± 95%CI,
one-way ANOVA, Tukey's post hoc test
ns: p>0.05, **: p<0.01, ***: p<0.001

Apostolou et al., Cellular and Molecular Gastroenterology and Hepatology (2021)

Transcriptome Profile Closer to *In Vivo* Than Organoids Alone



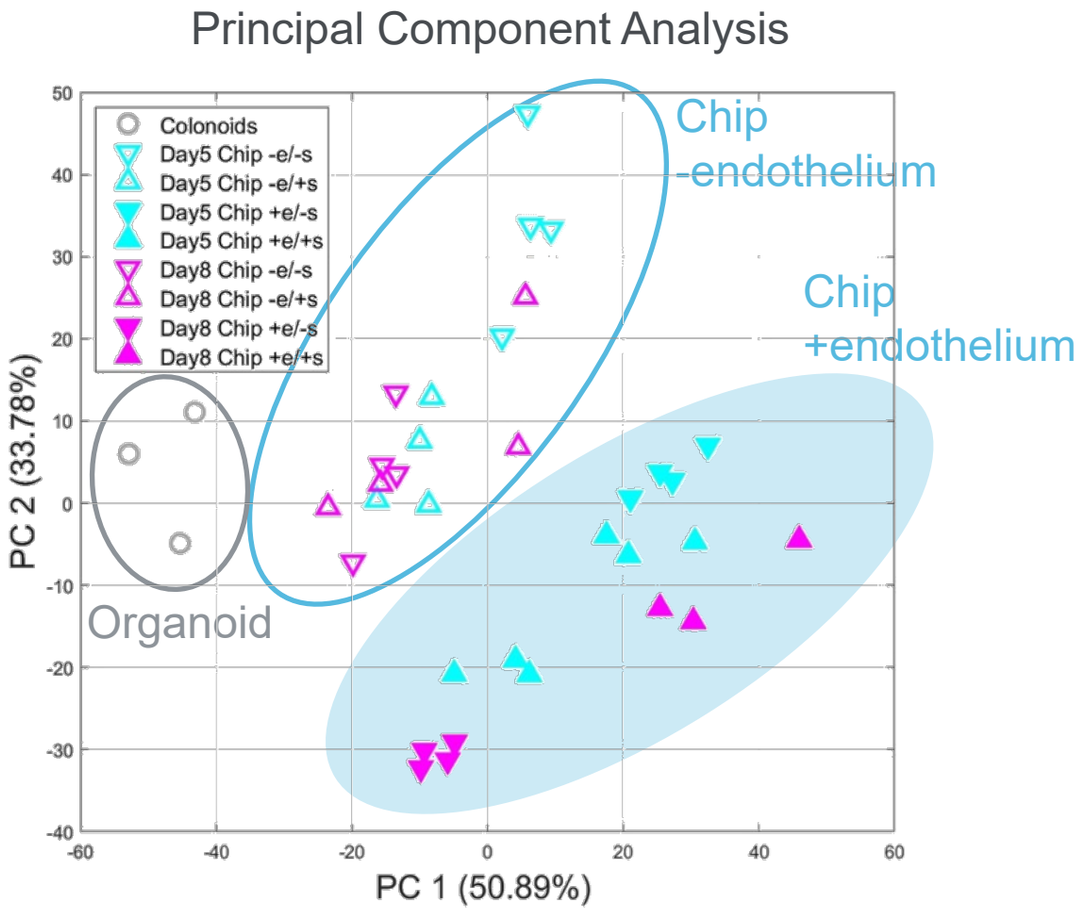
Transcriptomic Signature Distance[1]—a novel metric we published on in 2020—to assess transcriptomic similarity of the Colon Intestine-Chip and organoids in suspension to the human colon.

Transcriptome profile of Colon Intestine-Chip epithelium is significantly closer to *in vivo* colonic tissue than organoids in suspension culture

1. Manatakis, D., et. al. *Bioinformatics* (2020)
2. Apostolou et al., Cellular and Molecular Gastroenterology and Hepatology (2021)

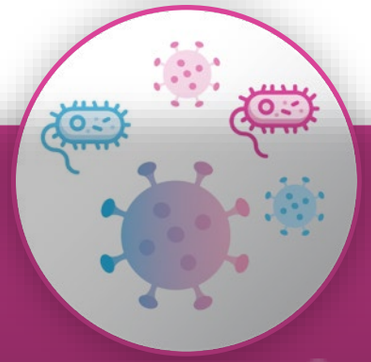
Effect of Endothelium on the Transcriptome Profile of the Epithelial Cells

Endothelium is a critical factor for differentiating the transcriptomic signature of the epithelial cells cultured on-chip, as compared to conventional organoids



Apostolou et al., Cellular and Molecular Gastroenterology and Hepatology (2021)

Applications of Emulate Intestine-Chip Models



Disease Pathology

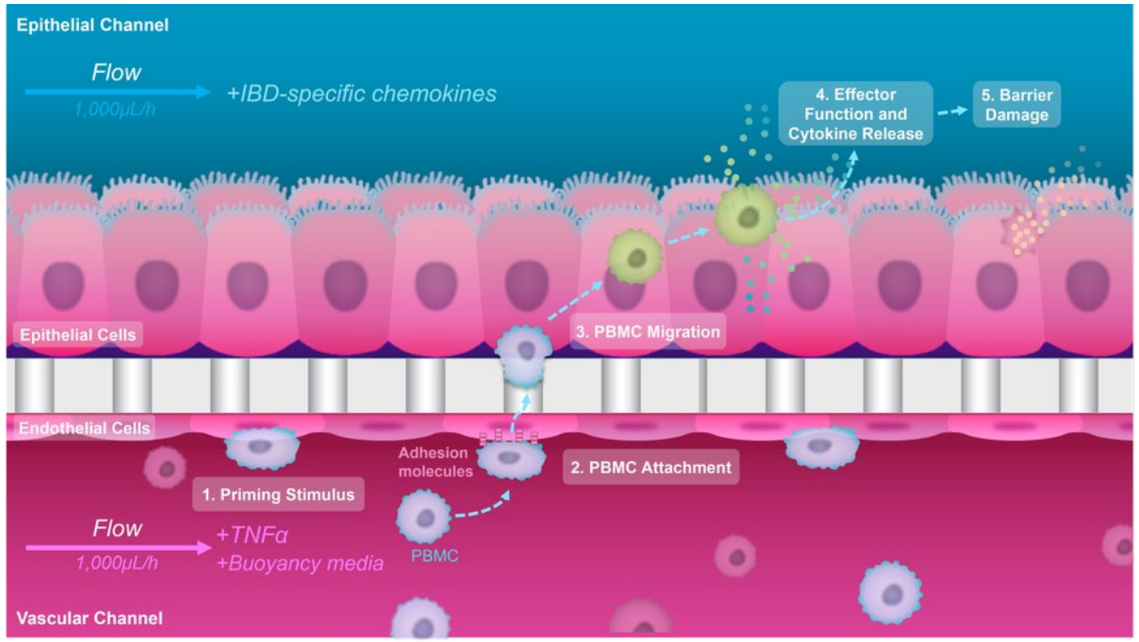
Therapeutic Efficacy

ADME / Tox Evaluation

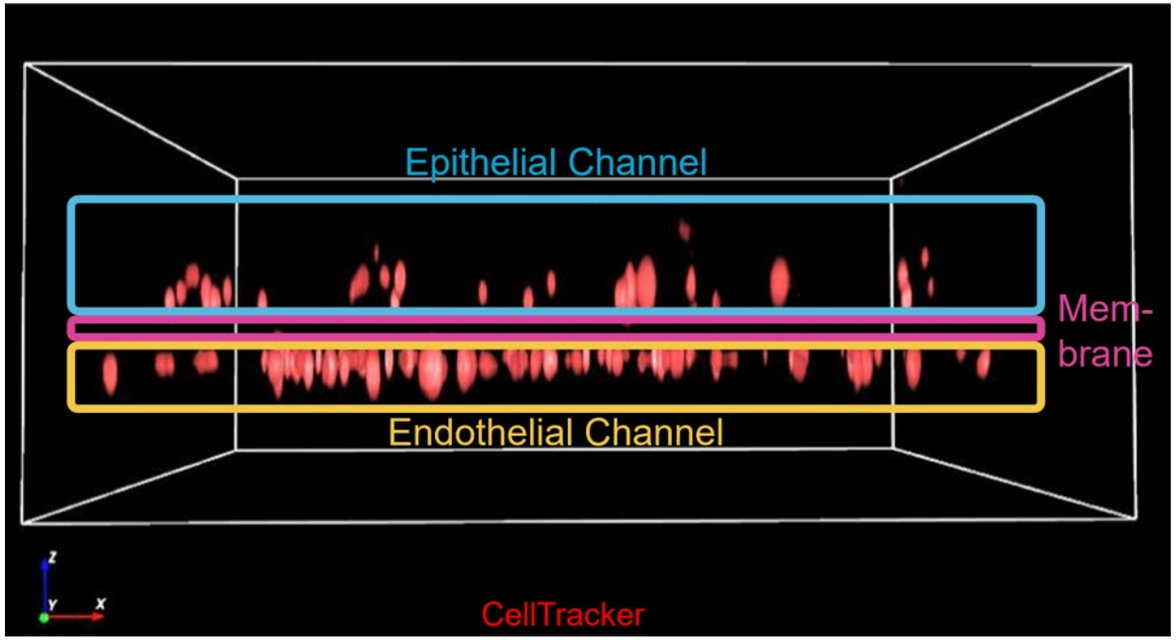
IBD-Specific Inflammatory Immune Cell Recruitment

To model the dysregulated immune cell recruitment seen in IBD, immune cells were administered to the vascular channel in the presence of IBD-specific inflammatory stimuli

Schematic of Immune Cell Recruitment (ICR)



3D Confocal Imaging of ICR on-Chip

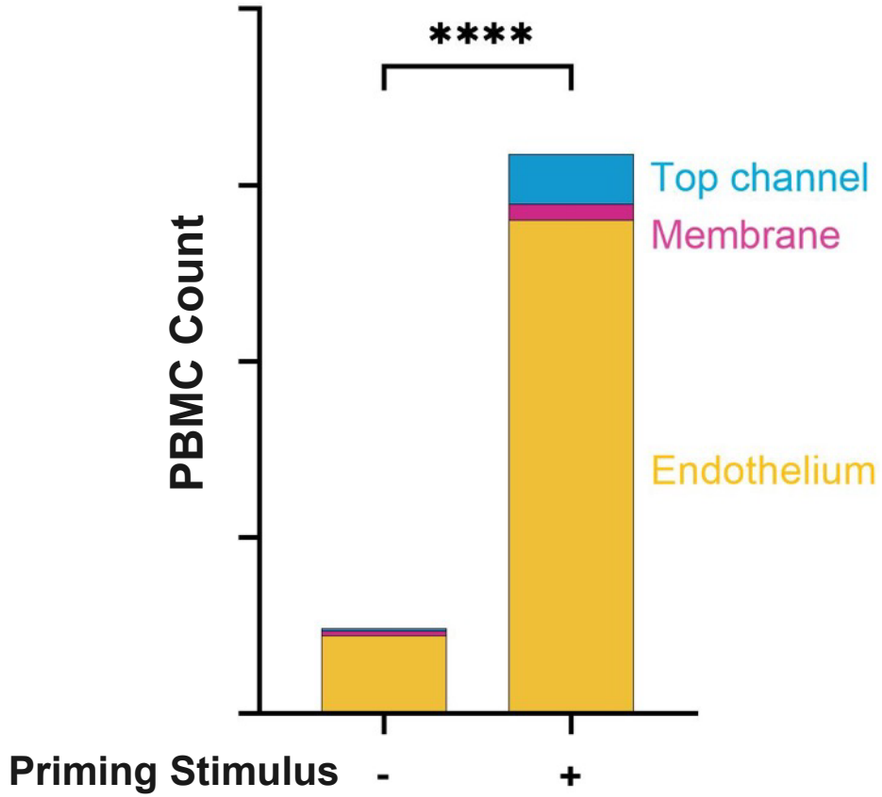


Robust recruitment of immune cells in the inflammation-primed Colon Intestine-Chip

DISEASE PATHOLOGY: COLON INTESTINE-CHIP

IBD-Specific Inflammatory Immune Cell Recruitment

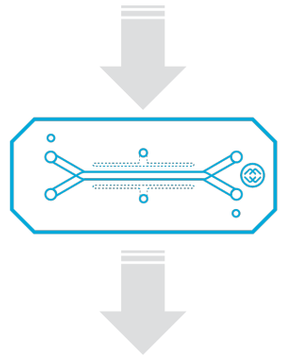
Inflammation-Specific Recruitment



Gut-Specific Enrichment

~20-30% α4β7+

Administered PBMCs



>60% α4β7+

PBMCs recruited to epithelial channel

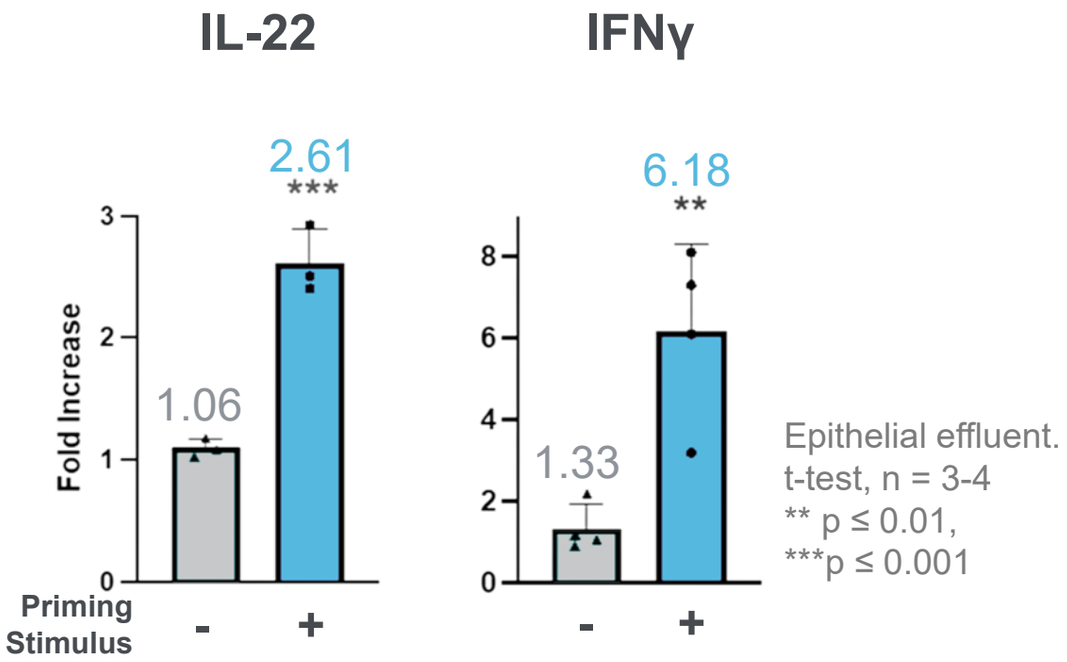
Inflammation- and gut-specific recruitment and migration

Unpublished Data

PBMCs: Peripheral Blood Mononuclear Cells

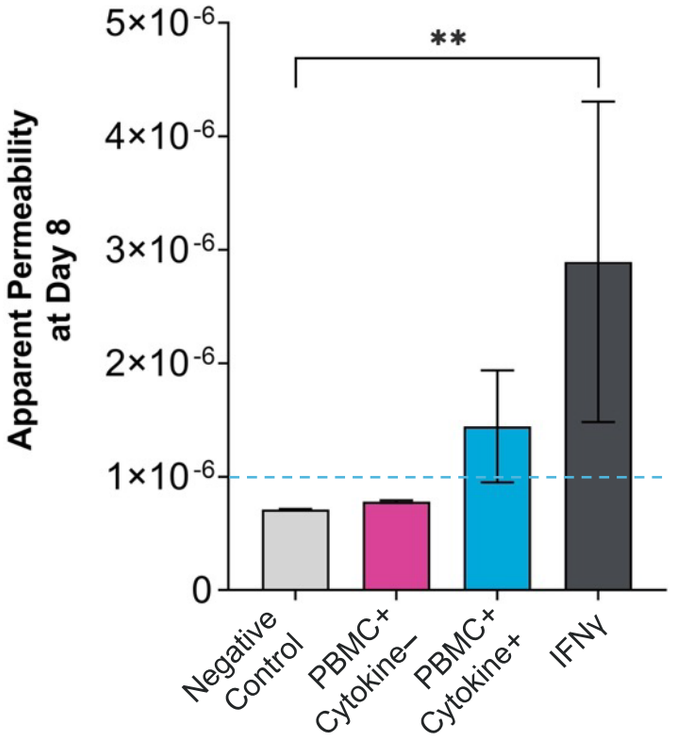
IBD-Specific Inflammatory Immune Cell Recruitment

PBMC-Dependent Cytokine Release



Epithelial effluent.
t-test, n = 3-4
** p ≤ 0.01,
***p ≤ 0.001

PBMC-Dependent Barrier Damage



3 kDa dextran Cascade Blue
n=5 chips/group, mean ± SEM
**p ≤ 0.01, compared to negative control, two-way ANOVA

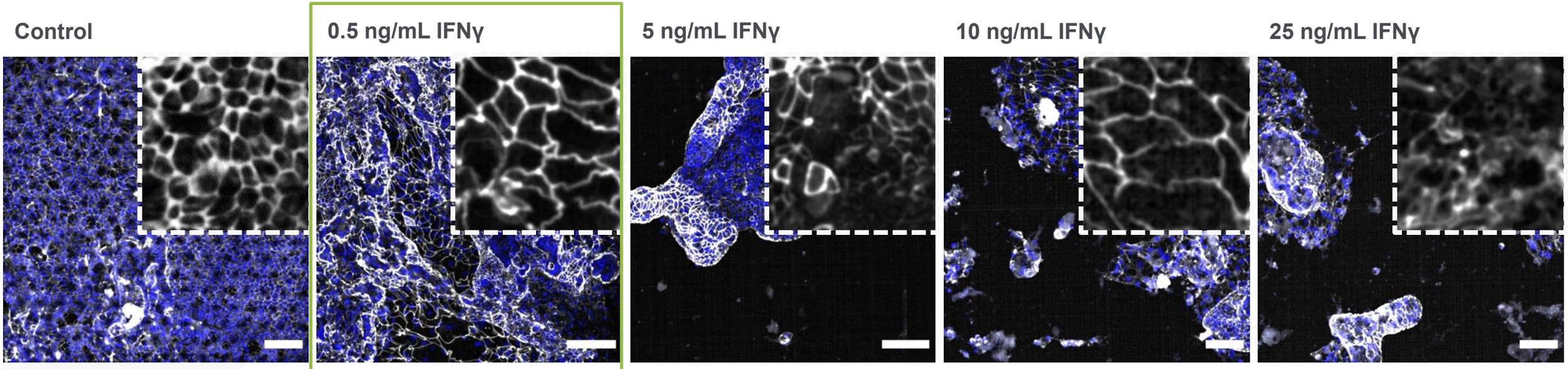
Model captures complex immune-mediated cytokine cascades and downstream barrier damage.

Unpublished Data

Endpoints assessed Day 8 post cell seeding

DISEASE PATHOLOGY: COLON INTESTINE-CHIP

IFNγ-Mediated Loss of Epithelial Barrier Function



When IFNγ is administered at a dose as low as 0.5ng/mL, epithelial tight junctions are deformed but the epithelial monolayer maintains confluency.

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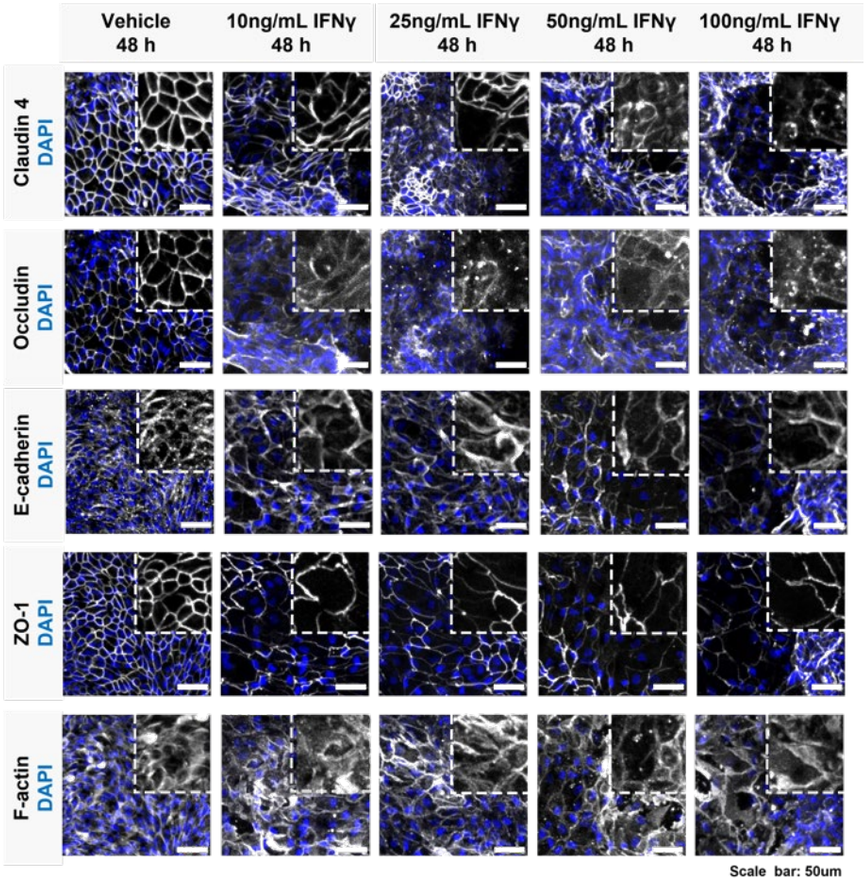
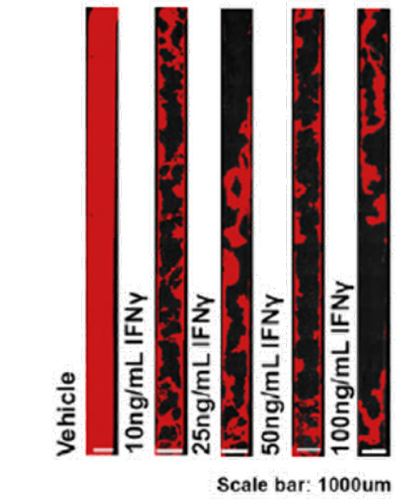
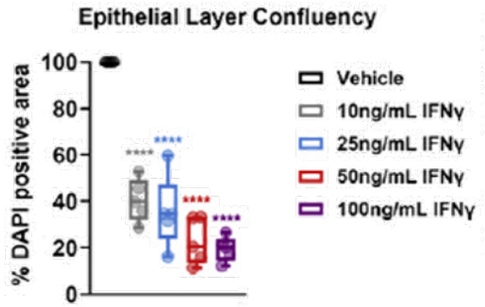
Phenocopying the IFNγ Mechanism of Action

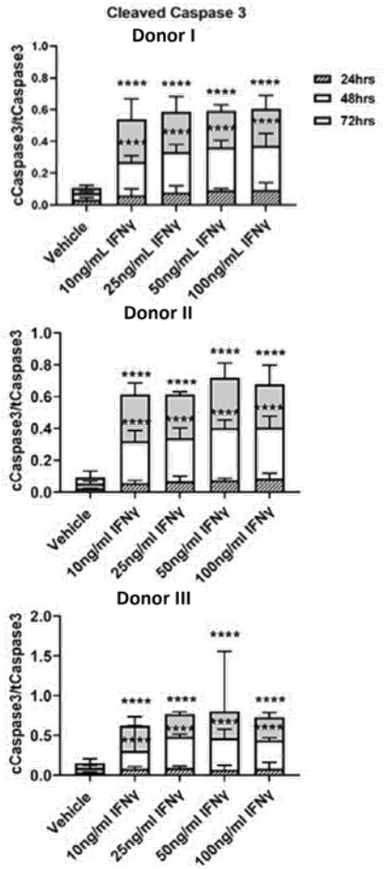
Degradation of Epithelial Tight and Adherens Junctions



Induction of Apoptosis

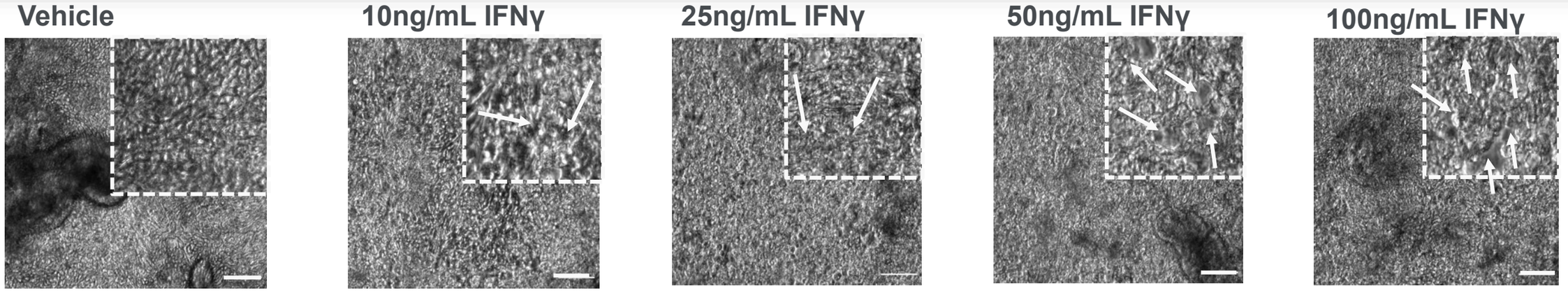






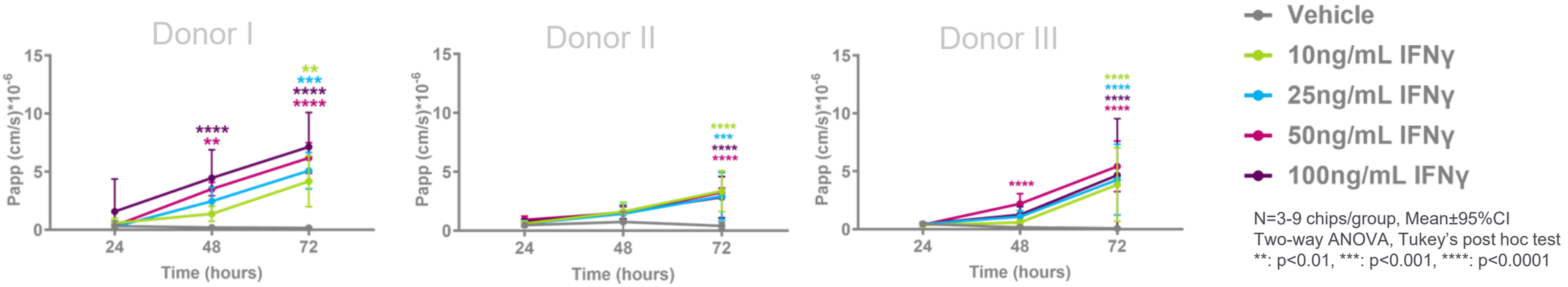
Apostolou et al., CMGH, 2021

Timing and Dosing the Donor-to-Donor Response to IFNγ



Scale bar: 100um

Apparent Permeability (3kDa Dextran Cascade Blue)



N=3-9 chips/group, Mean±95%CI
Two-way ANOVA, Tukey's post hoc test
**: p<0.01, ***: p<0.001, ****: p<0.0001

Compromised epithelial morphology is observed after 48 hours of IFNγ stimulation across all donors, aligned to the concentration and donor dependent increase of the epithelial apparent permeability.

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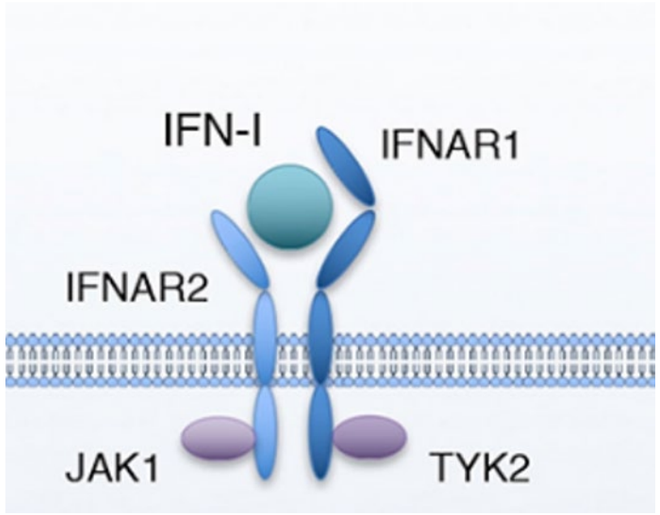
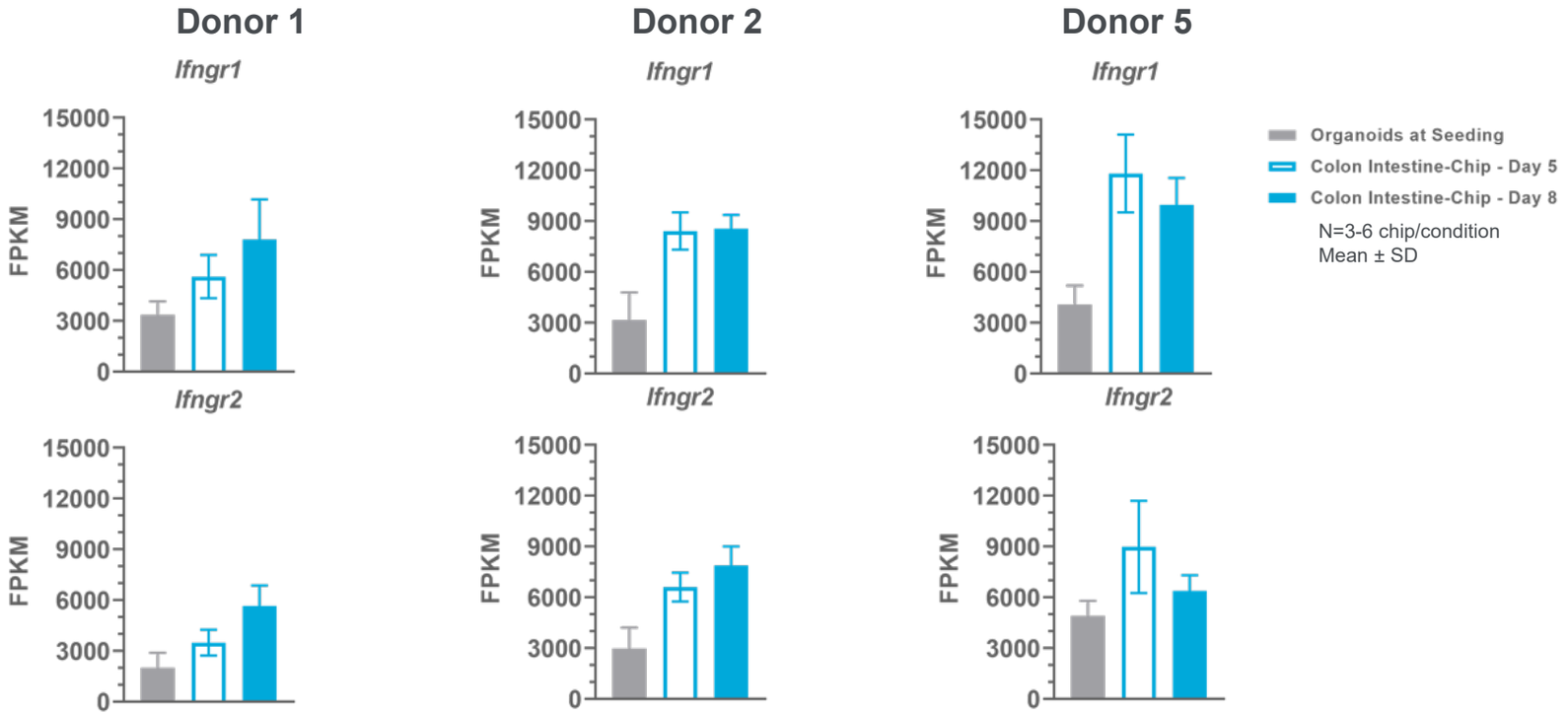
Apostolou A. et al, CMGH. 2021 Jul 17, 5:1719-1741

IFNγ Receptor Expression Across Organoid Donors



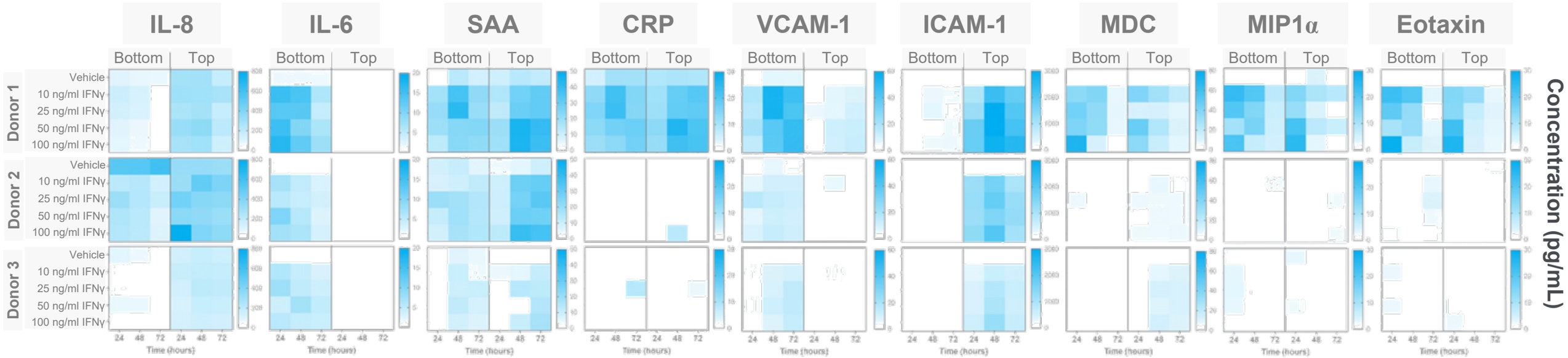
Martin-Hijano L. and Sainz Jr. B., Front.Immunol., 2020



	Donor 1	Donor 2	Donor 5
Min IFNγ Concentration (ng/mL)	10	10	0.5
Min IFNγ Concentration (h)	72	72	48

Colonic organoid donors express the IFNγ receptor subunits at variable levels both in the suspension and on-chip culture, and manifest different sensitivity to IFNγ.

IFNγ Induces Secretion of Proinflammatory Molecules



A concentration-, donor-, and time-dependent increase of proinflammatory molecule secretion was detected upon stimulation with IFNγ. The Colon Intestine-Chip captures the polarized secretion of cytokines and the interindividual variability as observed in clinic.

N=3-6chips/group, Mean, Two-way ANOVA, Tukey's post hoc test, *: p<0.05, **: p<0.01, ***: p<0.001, ****: p<0.0001, All groups are compared to the Control group

Picking Up the Patient Response to Standards of Care

JAK inhibition using tofacitinib for inflammatory bowel disease treatment: a hub for multiple inflammatory cytokines

Silvio Danese,[1] Matthew Grisham,[2] Jennifer Hodge,[3] and Jean-Baptiste Telliez[4]
[1]Division of Gastroenterology, Inflammatory Bowel Disease Center and Humanities Medical School, Milan, Italy;
[2]Department of Immunology and Molecular Microbiology, Texas Tech University Health Sciences Center, Lubbock, Texas;
[3]Pfizer Inc., New York, New York; and [4]Pfizer Inc., Cambridge, Massachusetts

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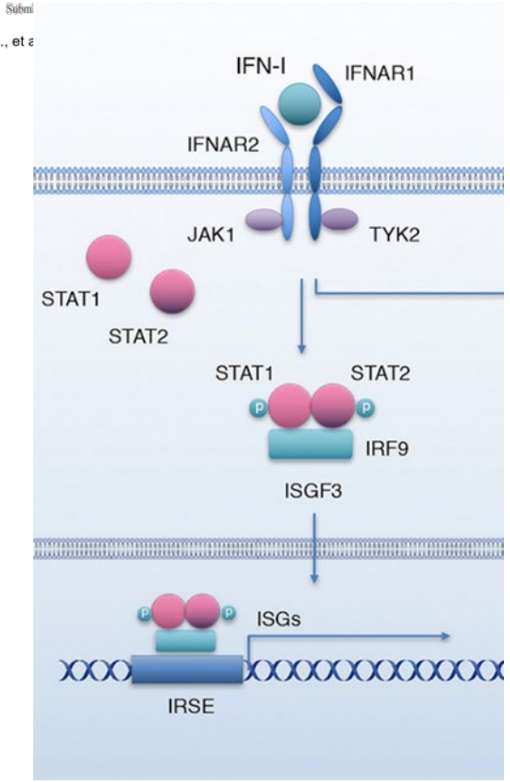
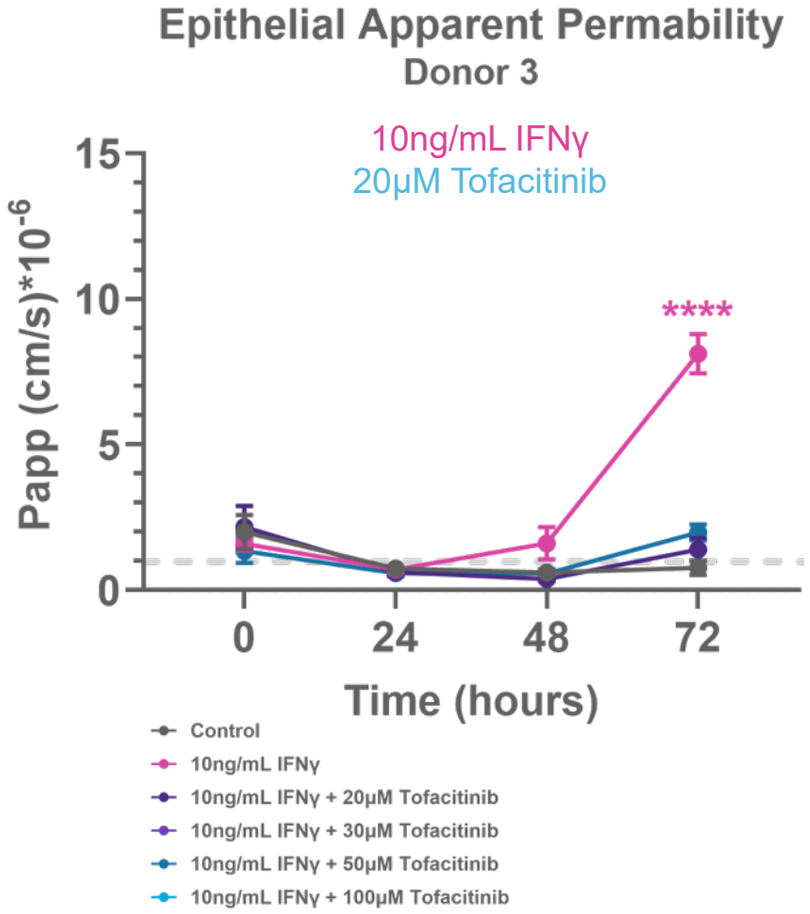
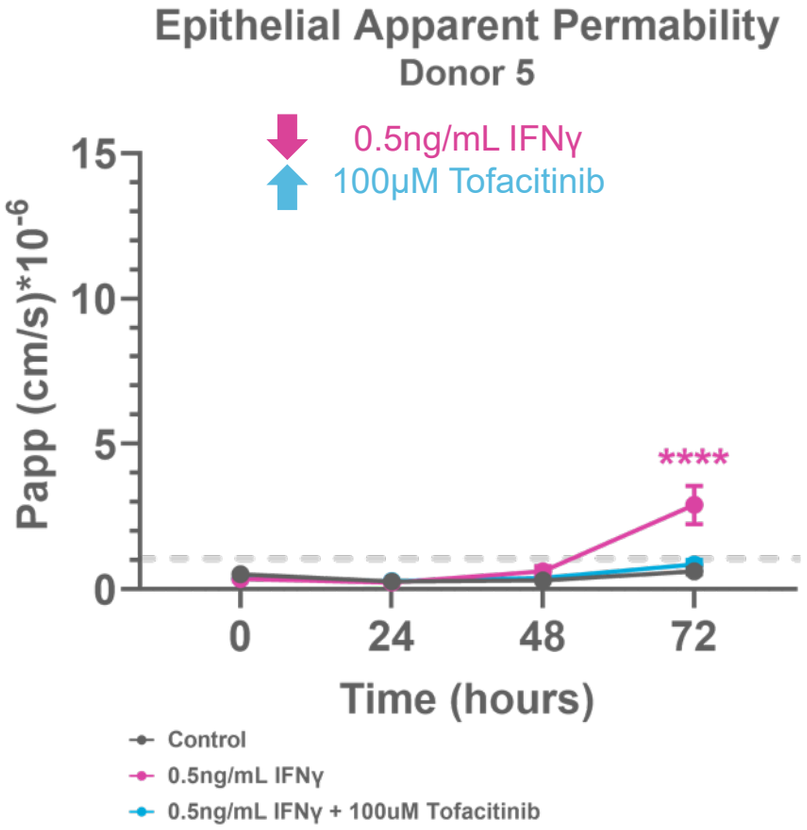
Danese S., et a



Martin-Hijano L. and Sainz Jr. B., Front.Immunol., 2020





The Colon Intestine-Chip captures variable sensitivity of Patient Derived Organoids (PDOs) to Tofacitinib, a Janus Kinase inhibitor, used as a standard of care therapeutic in IBD.

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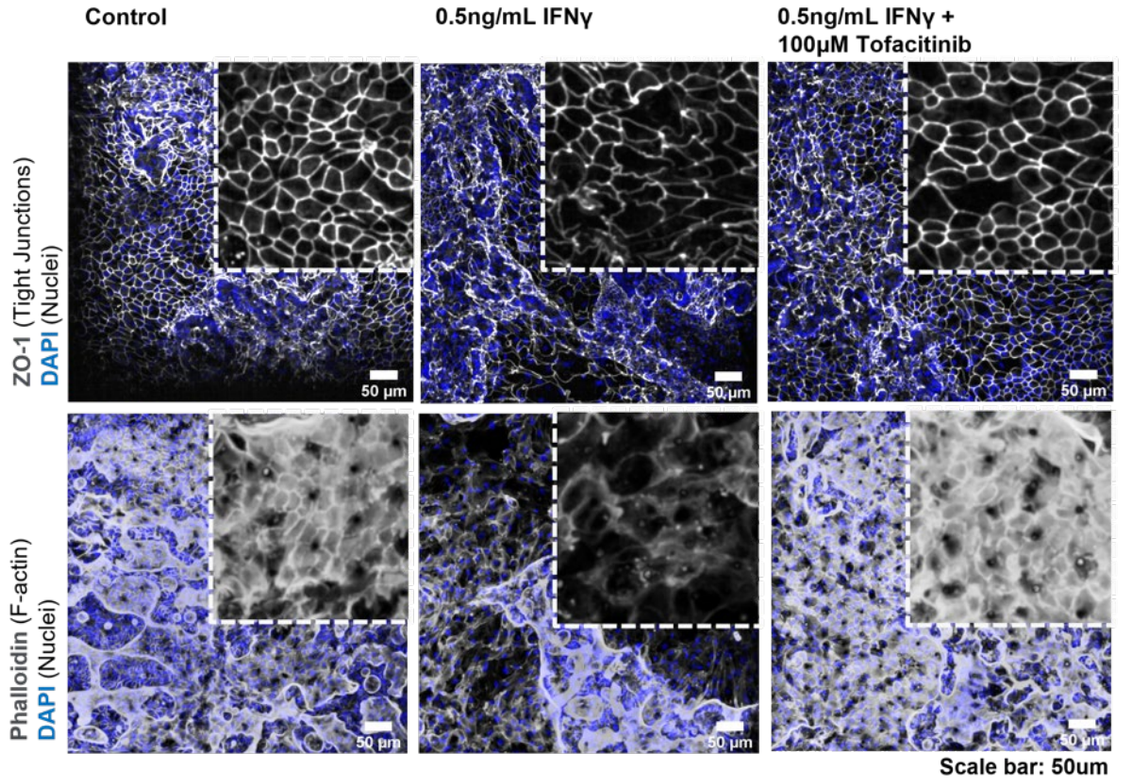
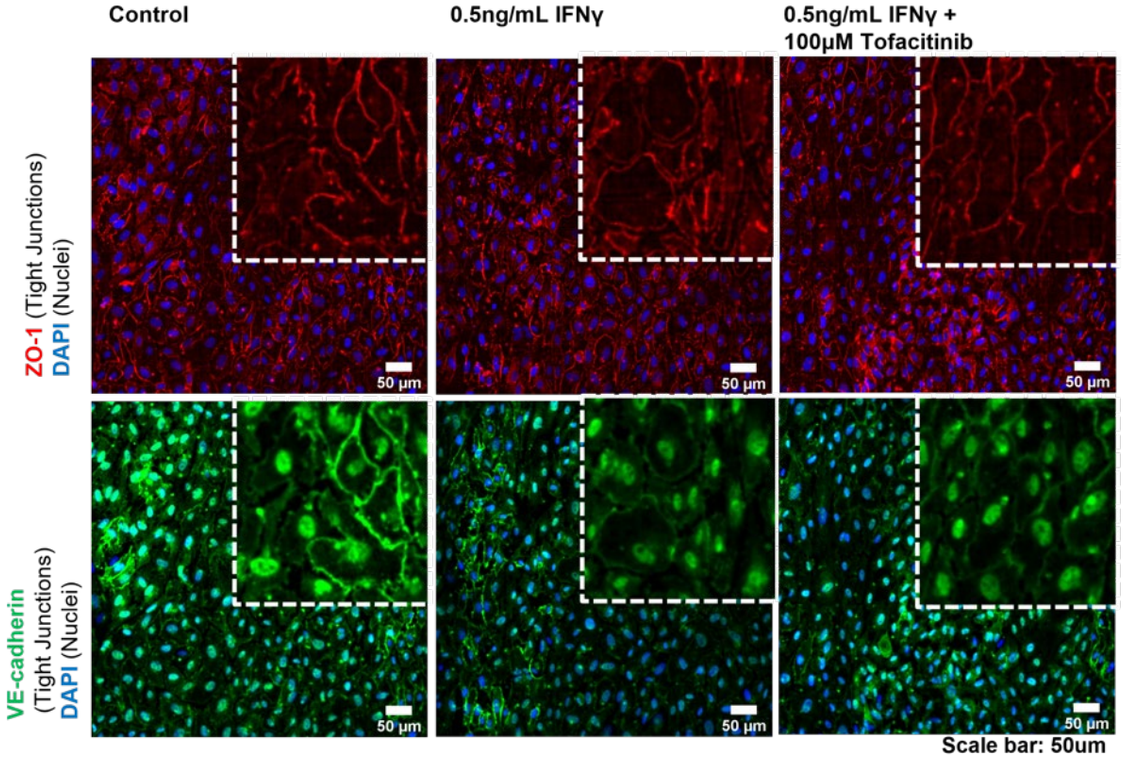
N=2-12chips/group, n=2-7 individual experiments, Mean ± SEM, Two-way ANOVA, Tukey's post hoc test, *: p<0.05,****: p<0.0001, All groups are compared to the Control group

Capturing the Therapeutic Effect of Tofacitinib

Tofacitinib supports the the epithelial cytoarchitecture and barrier integrity and ...

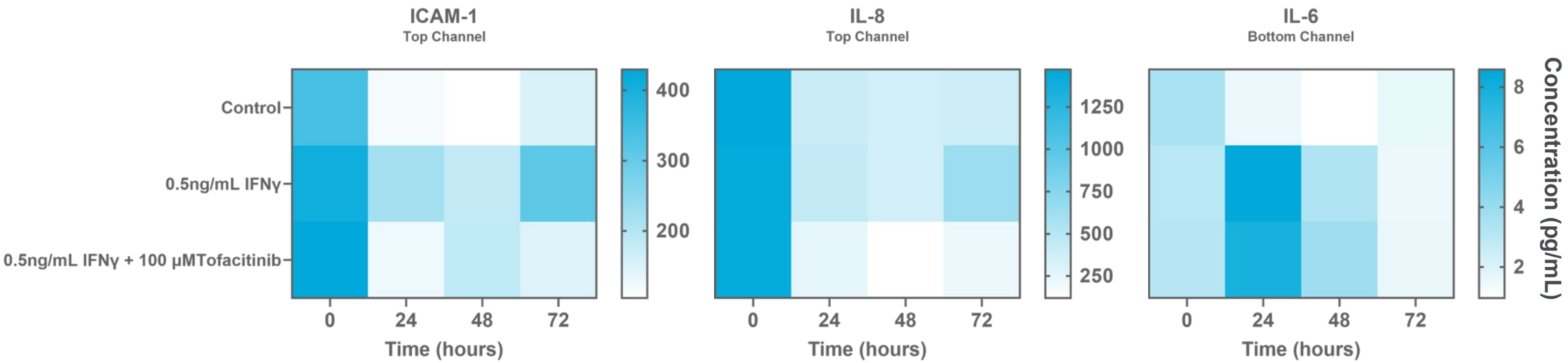


… attenuates the degeneration of the endothelial tight junctions.



THERAPEUTIC EFFICACY: COLON INTESTINE-CHIP

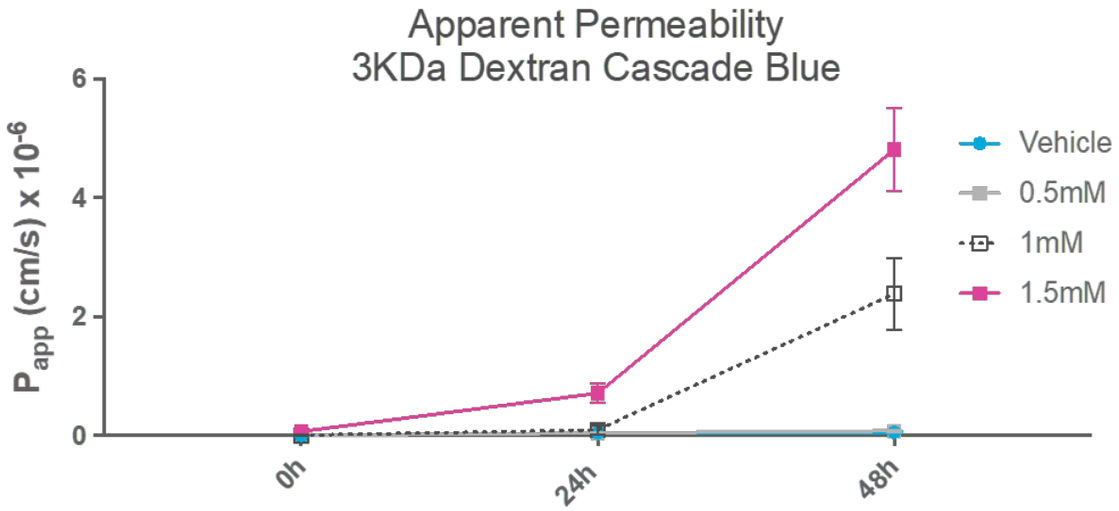
Tofacitinib Reduces Proinflammatory Signaling



Administration of 100 μM Tofacitinib reduces the secretion of ICAM-1, IL-8, and IL-6, proinflammatory cytokines and chemokines participating in the acute epithelial inflammatory response to IFNγ.

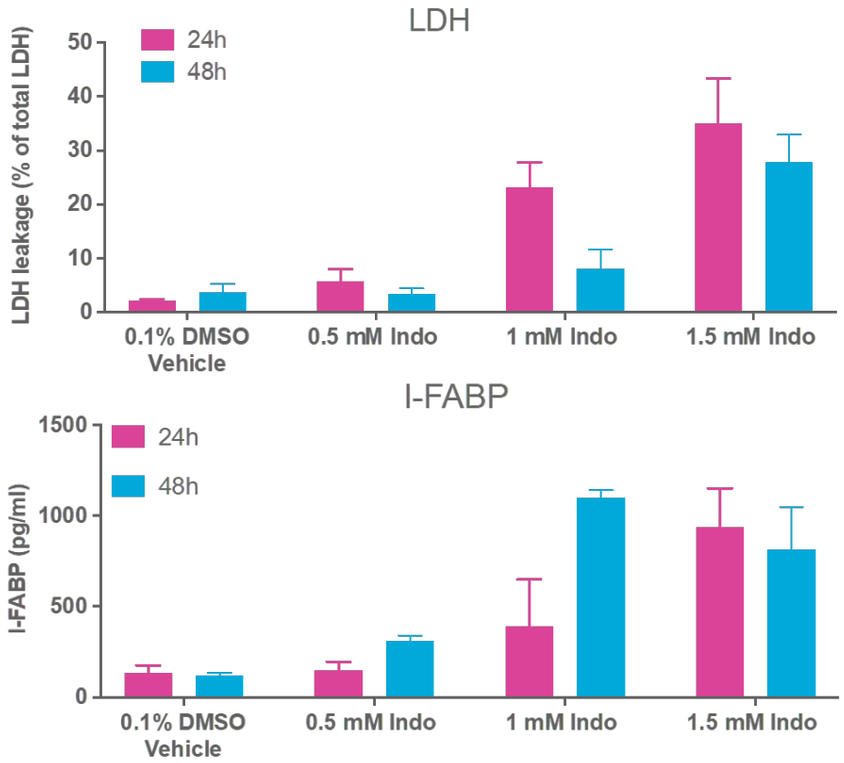
Indomethacin Toxicity

The Duodenum Intestine-Chip was treated with indomethacin, a known gastrointestinal (GI) toxicant, to determine whether it could detect GI toxicity.



Indomethacin treatment resulted in concentration-dependent increase in intestinal permeability and release of injury markers LDH and I-FABP and cellular apoptosis.

Cellular Injury Markers

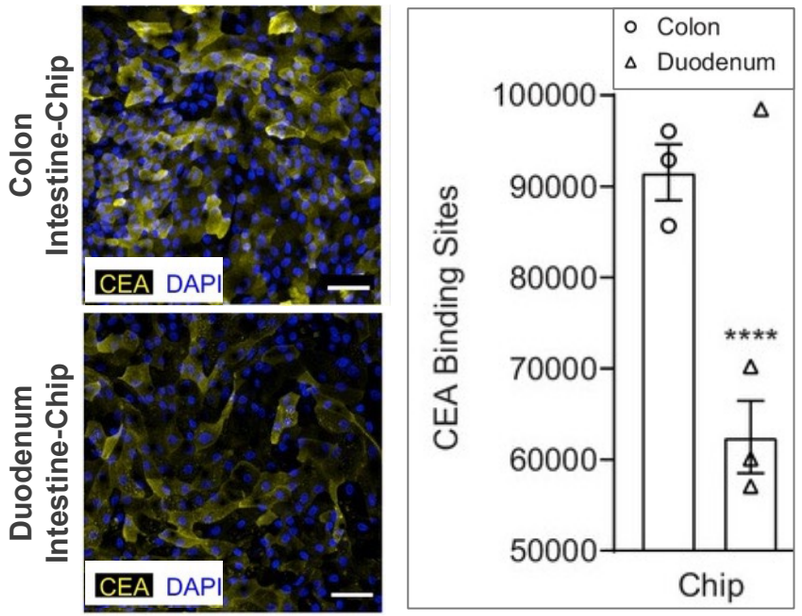


Unpublished Data

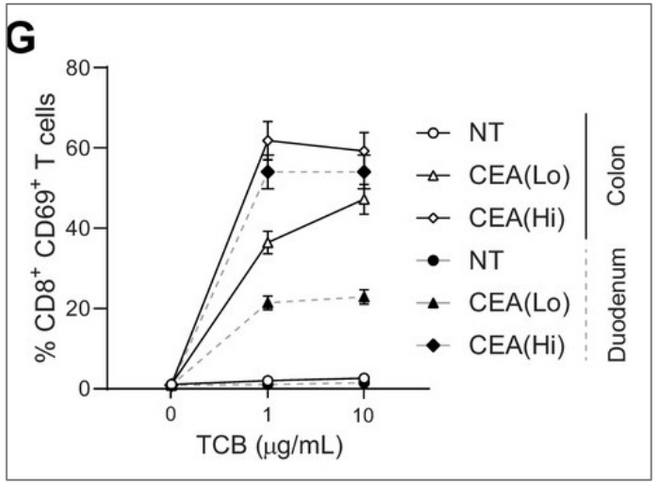
Immunotherapy Immunotoxicity

The Intestine-Chip was used to evaluate on-target, off-tumor safety risk of a candidate immunotherapy targeting a human-specific colorectal cancer antigen (CEA)

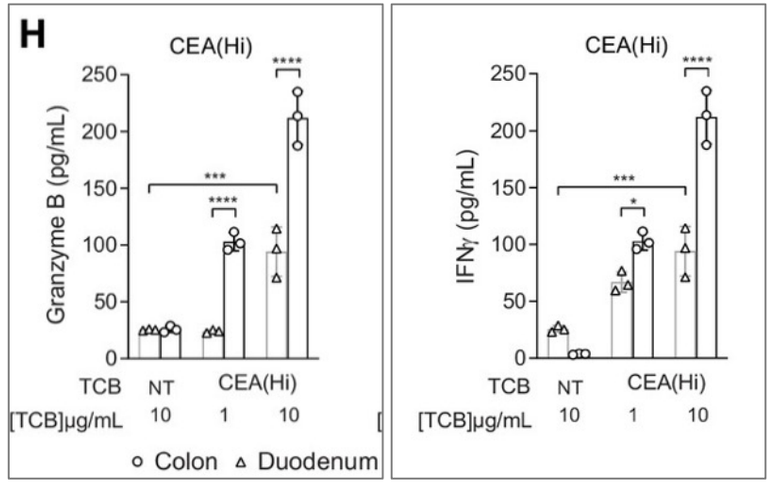
Region-specific expression of CEA



PBMC Attachment & Activation



Pro-inflammatory cytokine release



Organ-Chips accurately reflected tissue-dependent target expression and highlighted the safety liabilities of the TCB in a dose- and region-specific manner.

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Kerns, et al., eLife (2021)

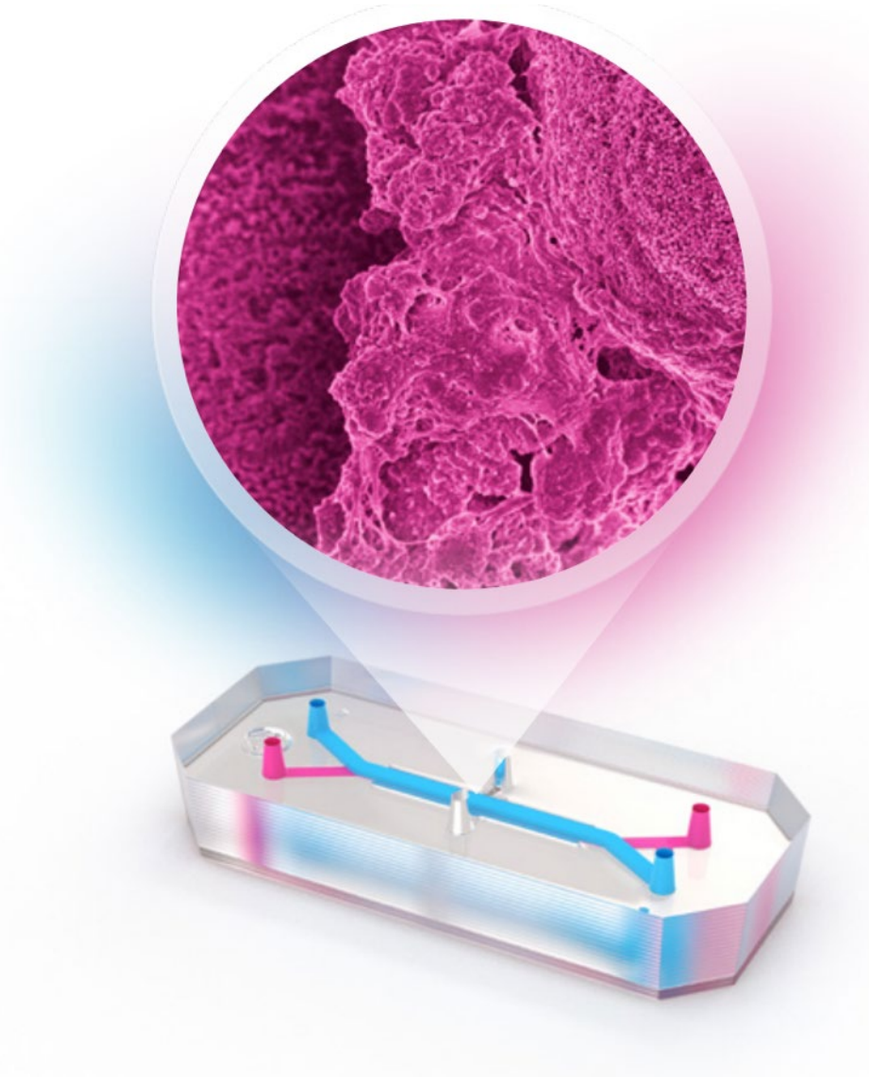
Intestine-Chips

Human-relevant, biologically complex models of the intestine

- Cellular diversity with improved differentiation
- Characteristic morphology with accurate polarization
- Tight barrier improved by endothelial co-culture
- Improved gene expression

Broad use case applicability

- Disease pathology
- Therapeutic efficacy
- ADME / Tox Evaluation



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Thank You