Human stem-cell derived intestinal organoids as a tool to evaluate human oral exposure and presystemic metabolism

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Patrik Lundquist, Department of Pharmacy, Uppsala University, Sweden

Trust Your Gut: Establishing Confidence in Gastrointestinal Models, NIH, October 11-12, 2023

RISK HUNT3R

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ADME profiling

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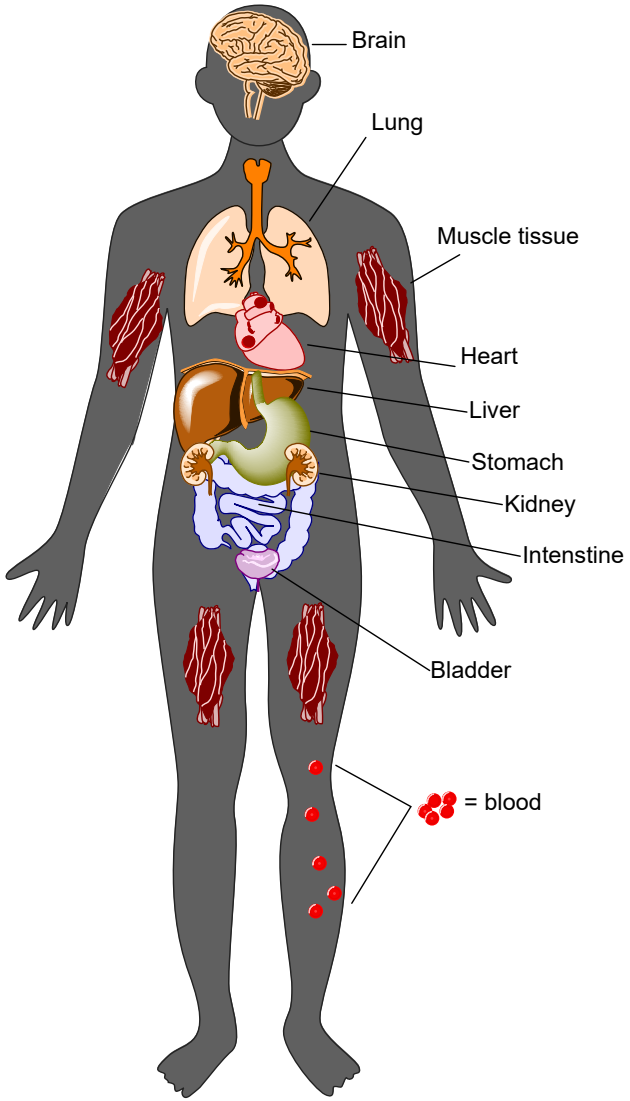
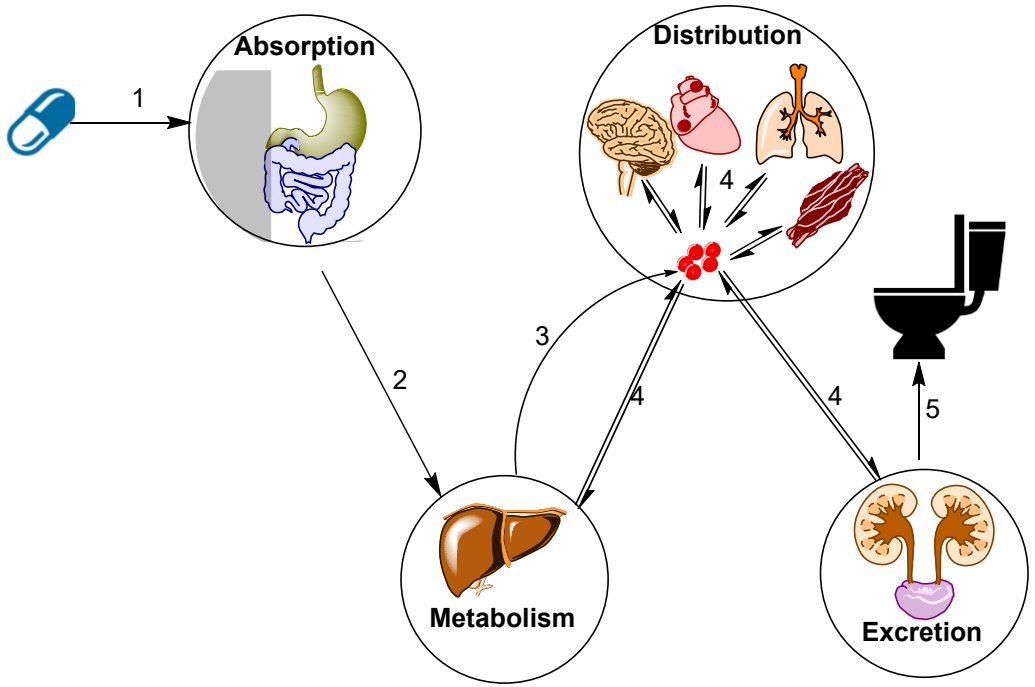
Absorption, Distribution, Metabolism, Excretion (ADME)





Drug development
Preferable PK properties
Right human dose
Low risk for DDIs
Good safety margins

NAM
Exposure-based risk assessment, toxikokinetics

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Impact of ADME on pharmaco/toxicokinetics

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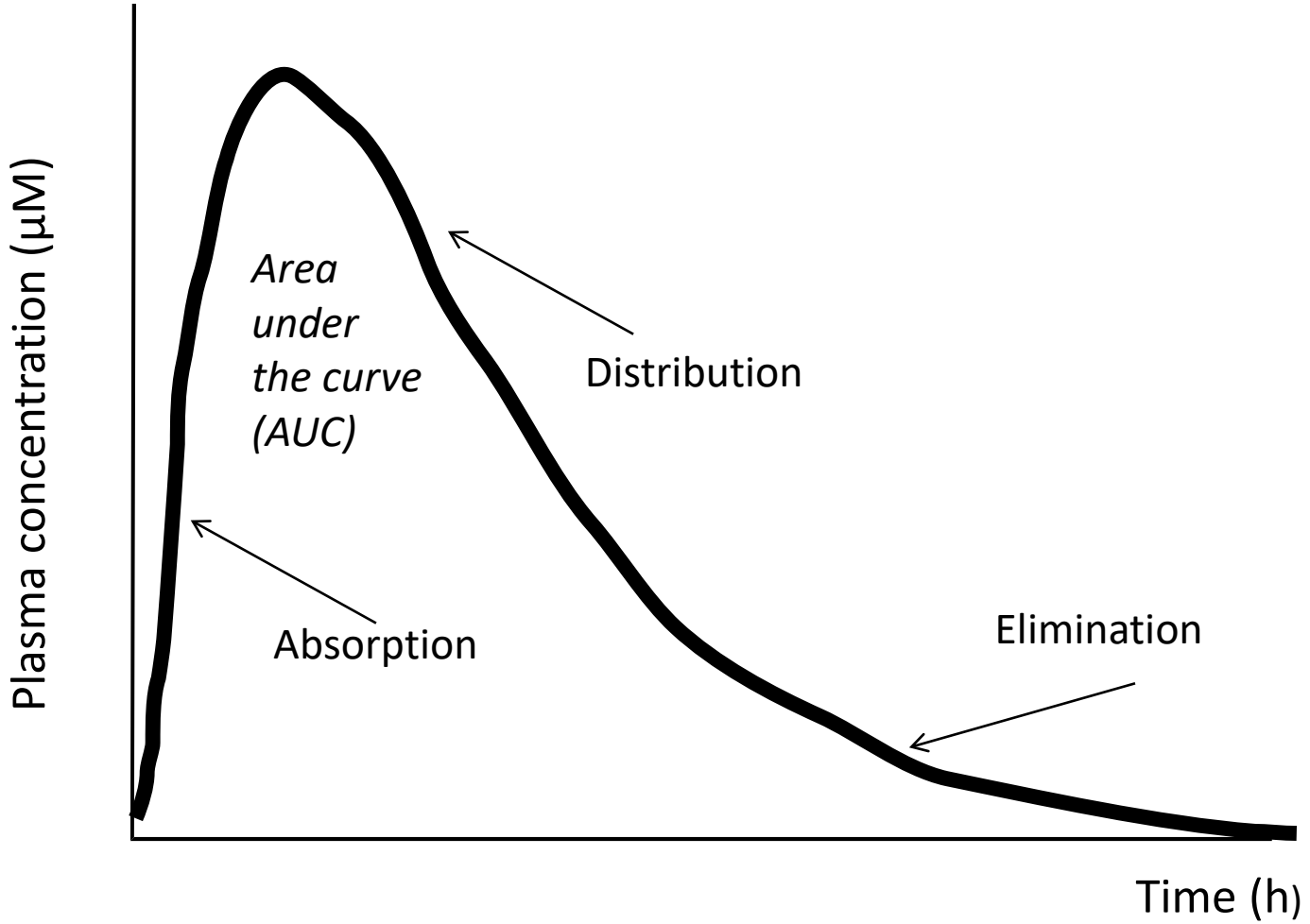

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Bioavailability in animal species versus oral bioavailability in humans

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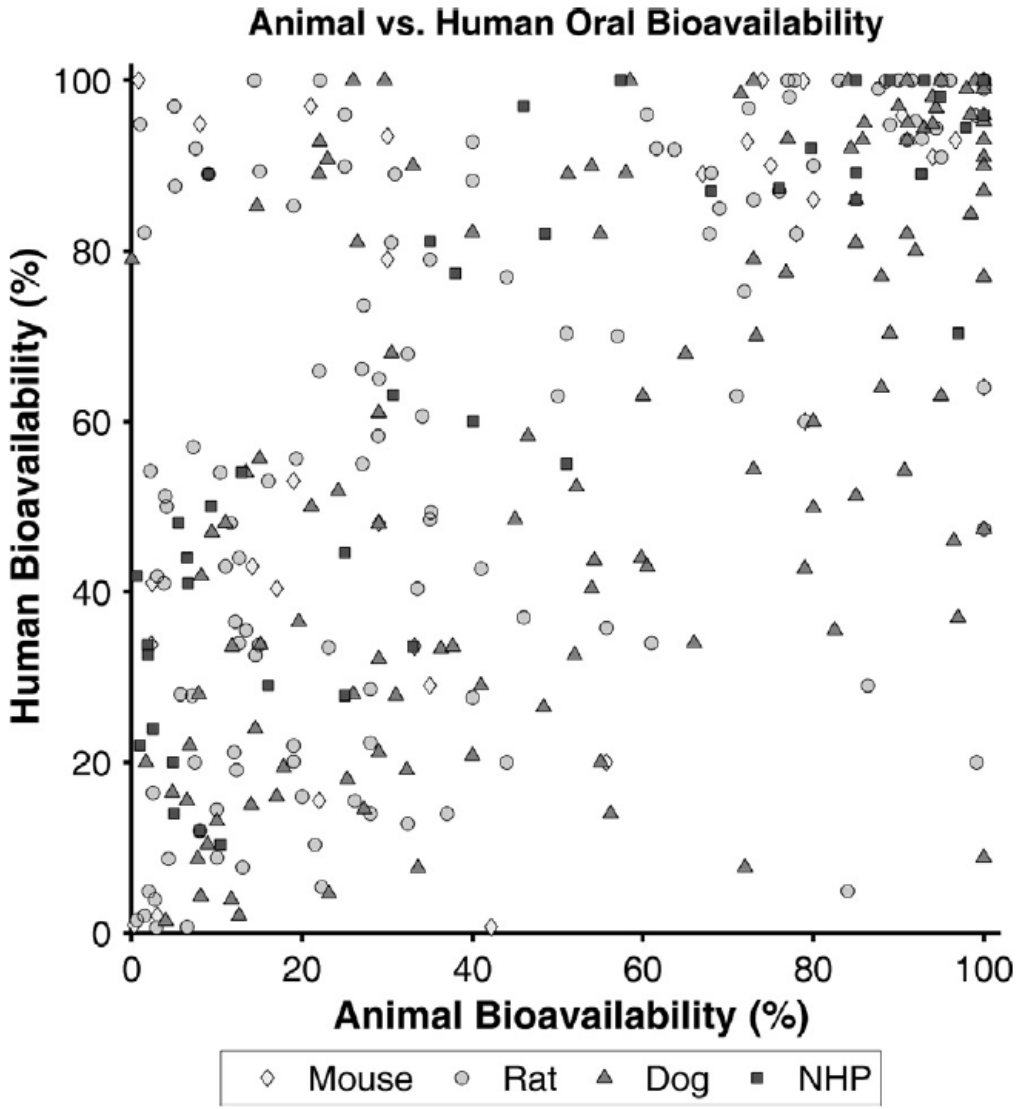

Husther et al. *Eur J Pharm Sci*, 2013

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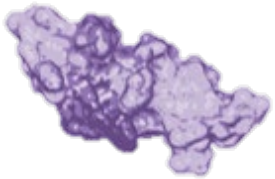
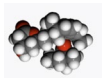
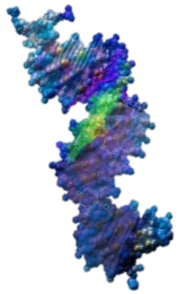
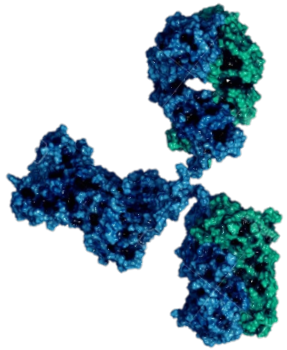
Our research workflow

Compound properties



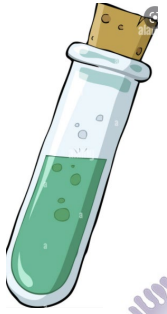


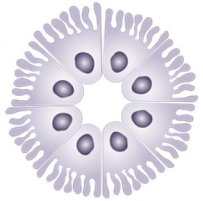




Over, *Nat Chem Biol*, 2016,
Matsson *J Med Chem*; 2018

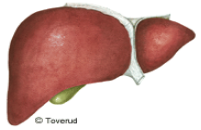
Model properties and quality

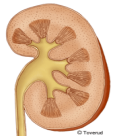


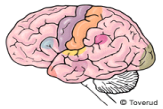


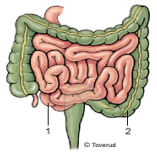
Ölander, *Arch Toxicol*, 2019
Handin, *iScience*, 2022

Tissue properties and quality



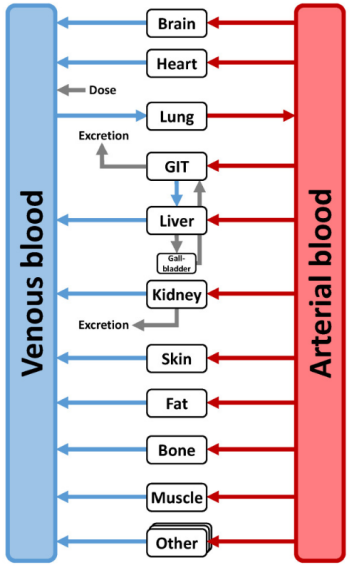






Wegler, *NAR Genom Bioinform*, 2019; *CPT* 2022

Parameter for PBPK



Filppula, *Sci Rep*, 2017
Treyer, *AAPS J*, 2019

Prediction outcome

Wegler, *Clin Pharmacol Ther*, 2021

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Caco-2 model of intestinal epithelial permeability

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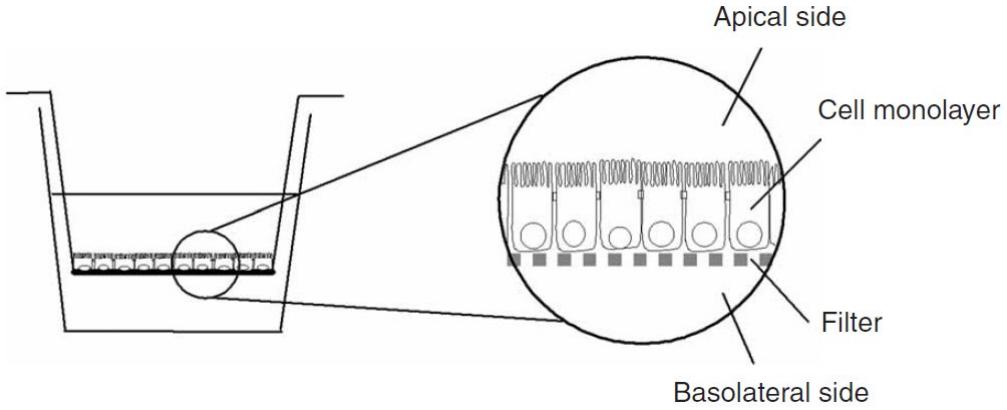
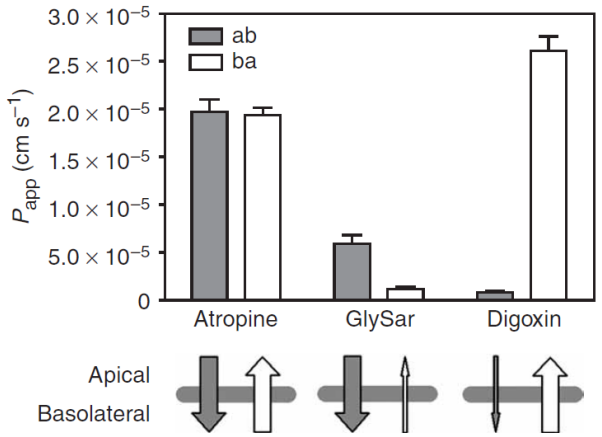
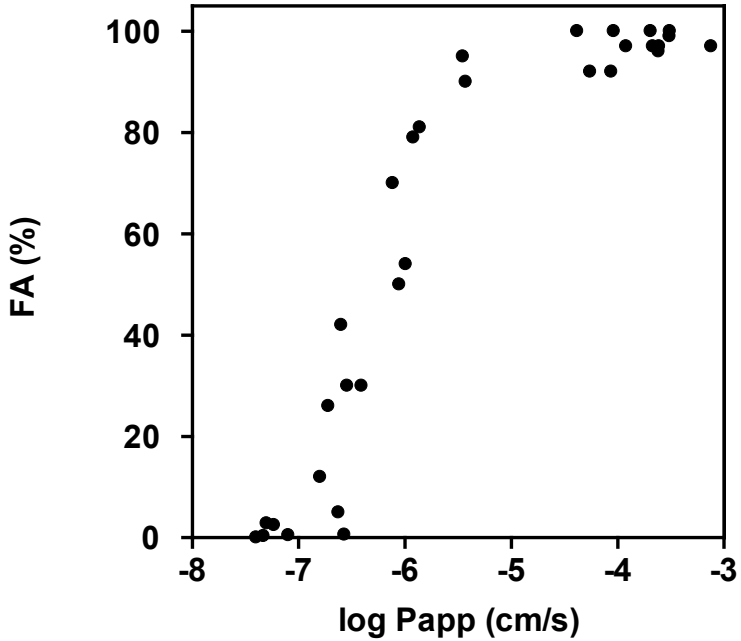




- Widely used to predict intestinal permeability
- Derived from a human colon carcinoma
- Expresses key intestinal Solute Carrier (SLC) uptake transporters and ATP-Binding Cassette (ABC) efflux transporters
- Intestinal drug metabolizing enzymes not expressed



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New: Simultaneous determination of cellular exposure and permeability

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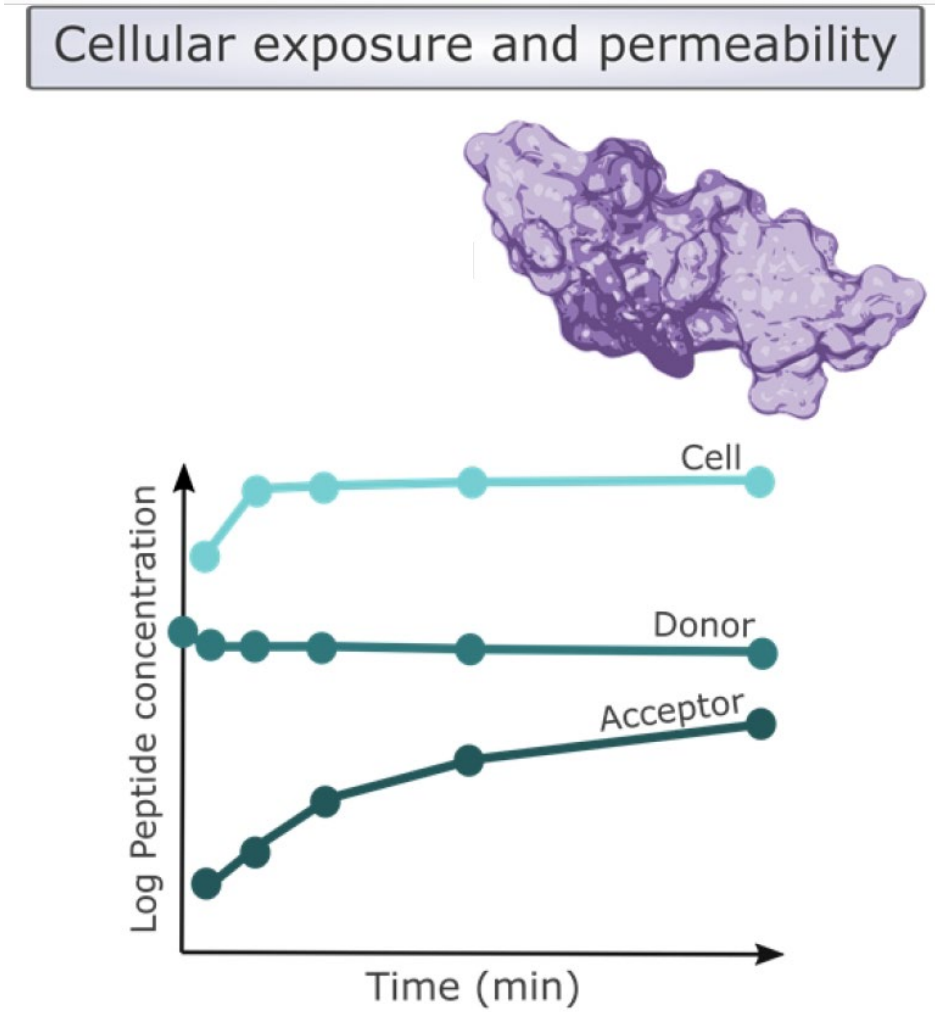
- Many lipophilic compounds have activity against intracellular (cytoplasmic) targets …
- …but they are not orally absorbed
- Up to a 1000-fold higher cellular accumulation than permeation
- Distribution into phospholipids



Lundquist, *manuscript in preparation*, 2023

Isolation of human jejunal enterocytes

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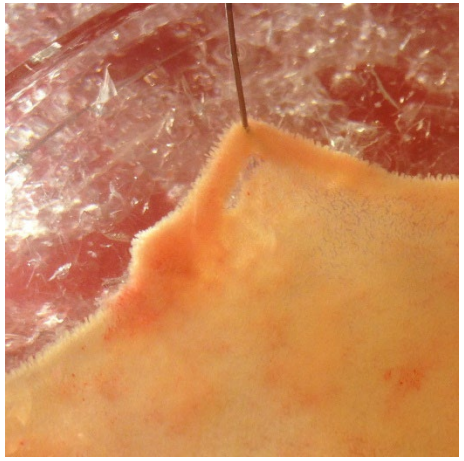
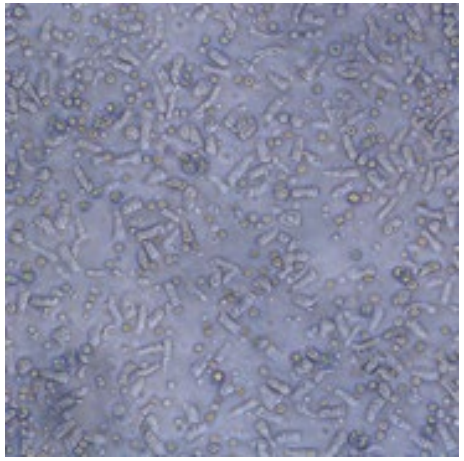
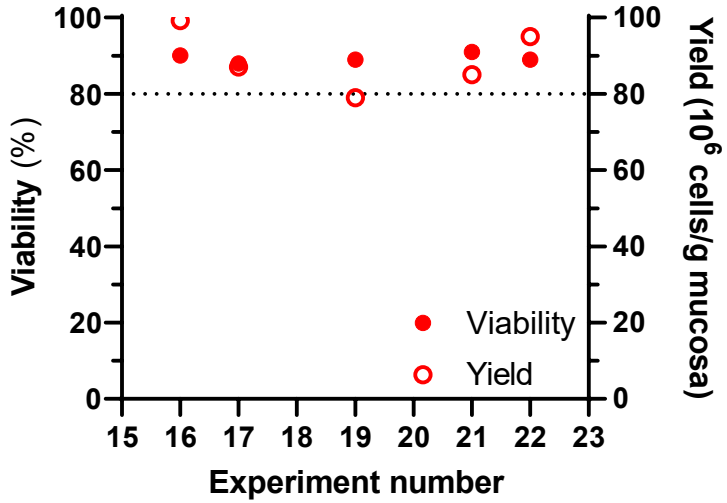


Enterocytes

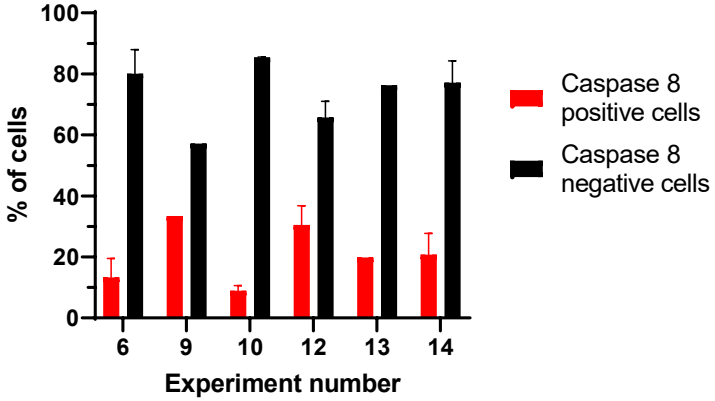


Enterocyte

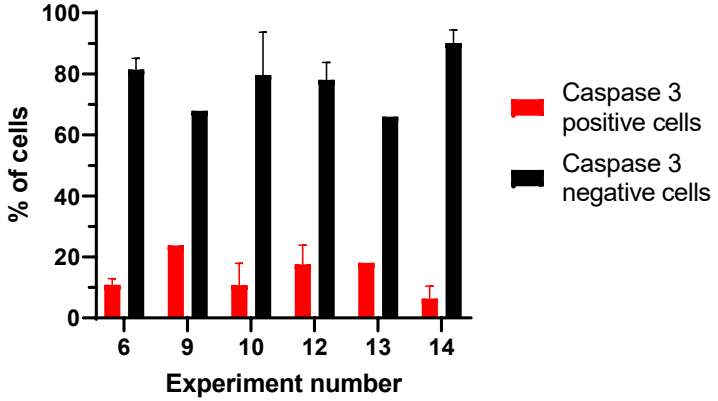
Yield and viability



Early apoptosis



Late apoptosis



Human jejunal enterocytes can be isolated with high yield and viability.

Enterocyte metabolism

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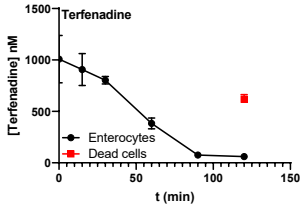
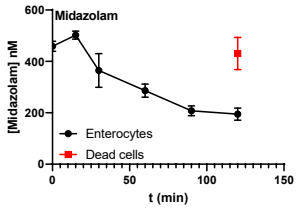
Compound	Metabolic pathway	CLint (µl/min/10^6 cells)	Metabolites identified
Midazolam	CYP3A4	5.3	1OH- and 4OH-midazolam
Loxapine	CYP1A2	2.2	7OH-loxapine
Lidocain	CYP1A2/CYP3A4	2.8	To be determined
Genistein	Glucoronidation/ Sulfation (CYP)	8.6	To be determined
Propylparaben	Esterase/ Glucoronidation	1.8	To be determined
Propranolol	CYP2D6/ Glucoronidation	0	None (so far)
Haloperidol	Glucoronidation CYP2D6	2.7	To be determined
Diclofenac	CYP2C9	5.9	4OH-diclofenac
Tolbutamide	CYP2C9	0	None (so far)
Caffeine	CYP1A2	0	None detected
Nicotine	CYP2A6	0	None detected
Terfenadine	CYP3A4	6	Fexofenadine
Bufuralol	CYP2D6	0	1OH-bufuralol

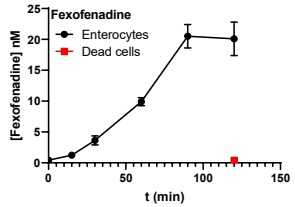
CYP3A4 – Terfenadine / Midazolam

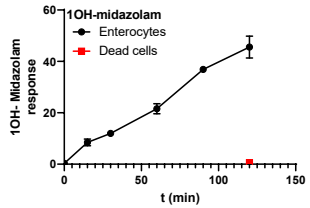
Substrate

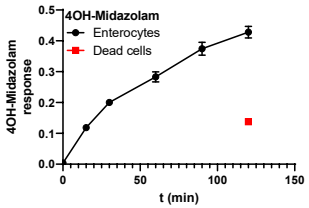




Metabolite(s)

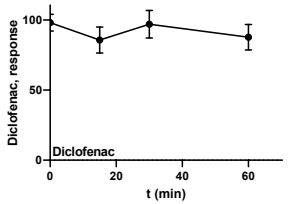




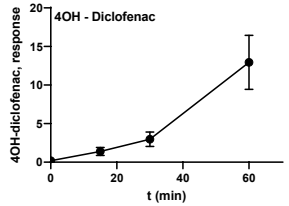


CYP2C9 - Diclofenac

Substrate



Metabolite(s)



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3D enteroids and colonoids in our lab

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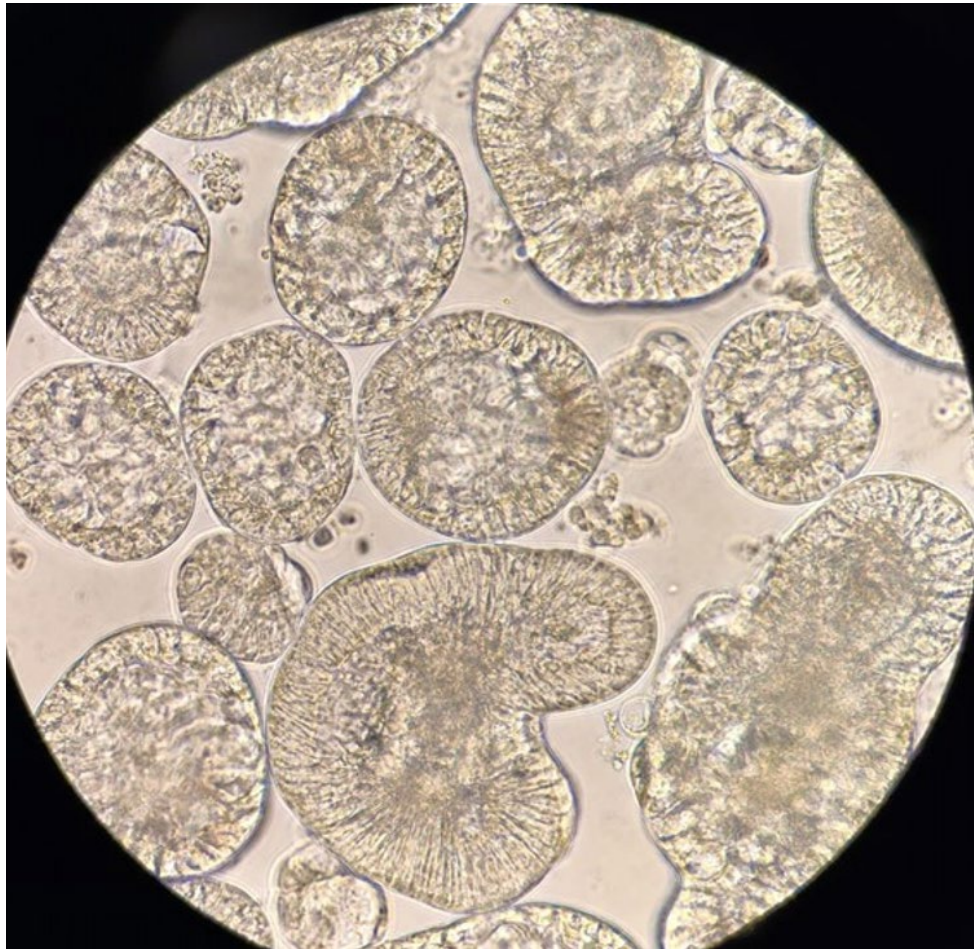
- Merve Ceylan, PhD student
  - Small intestinal (jejunal) organoids and primary cells and tissue
- Rebekkah Hammar, PhD student
  - Colon organoids and primary cells and tissue
- Daisy Hjelmqvist, PhD, Scientist

Apical-out enteroids



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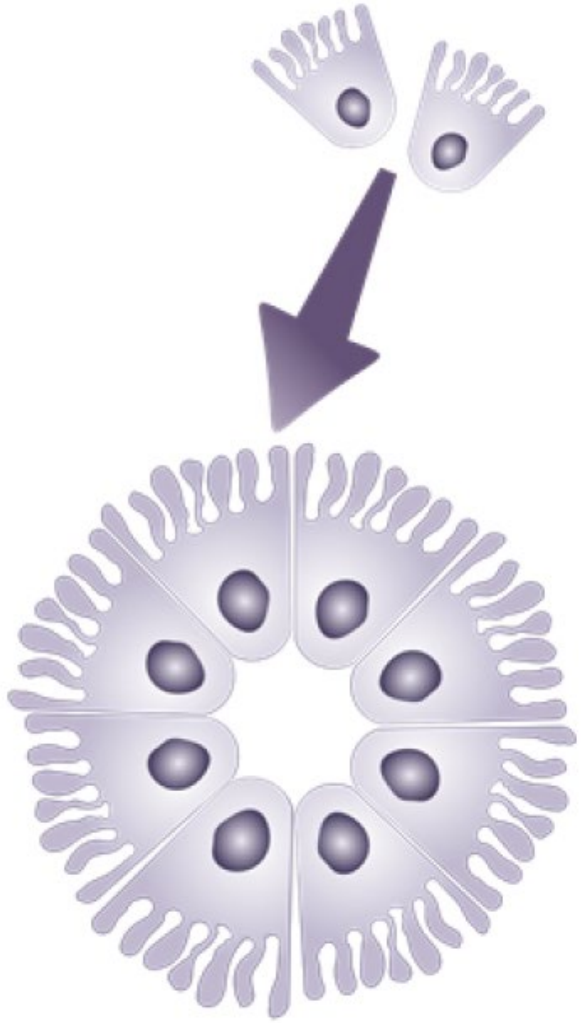
3D organoids for assessment of oral exposure

- Derived from human intestinal stem cells
- Standard technology: grow in extracellular matrix –basal side out
- New technology: remove extracellular matrix (matrigel) – apical side out
- Suitability for transport and permeability studies?
- Colon tissue (colonoids): cancer patients
- Jejunal tissue (enteroids) : Gastric bypass patients



Co *Cell Rep* 2019

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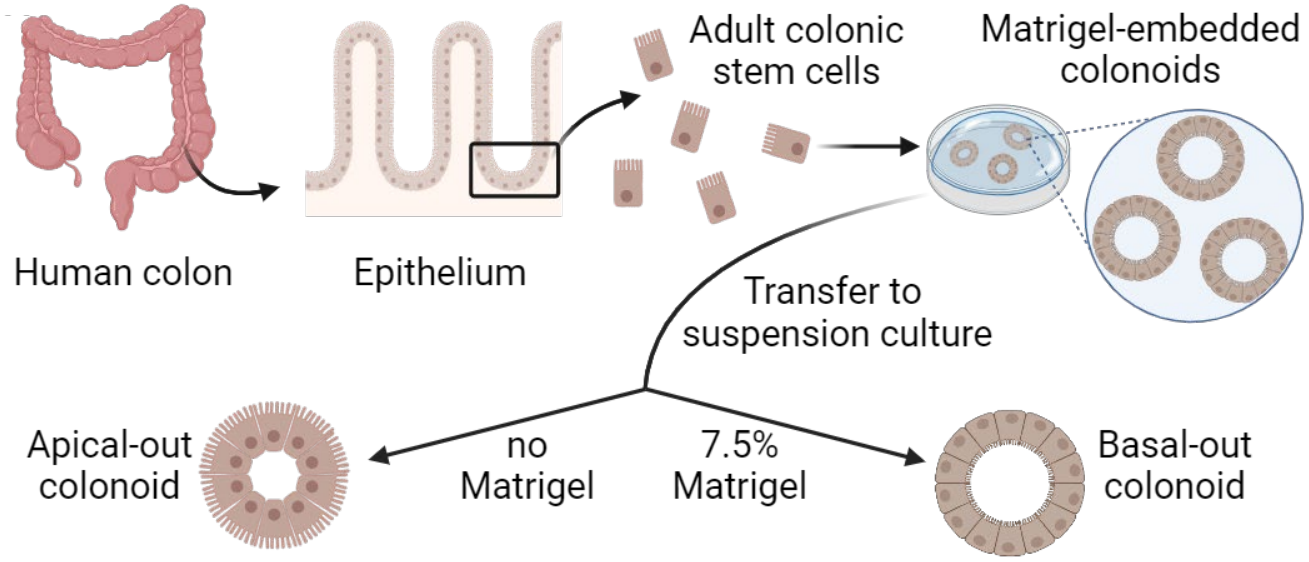
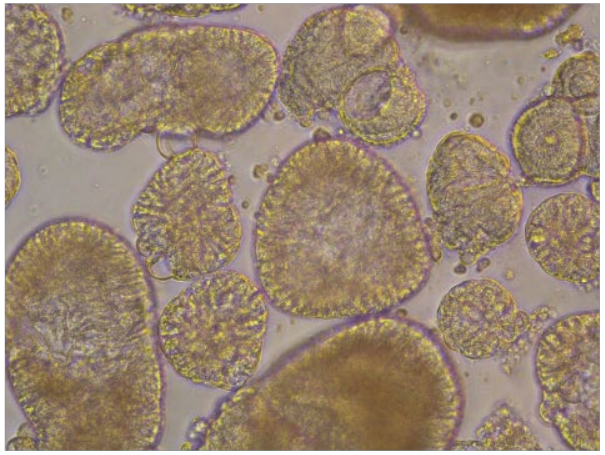
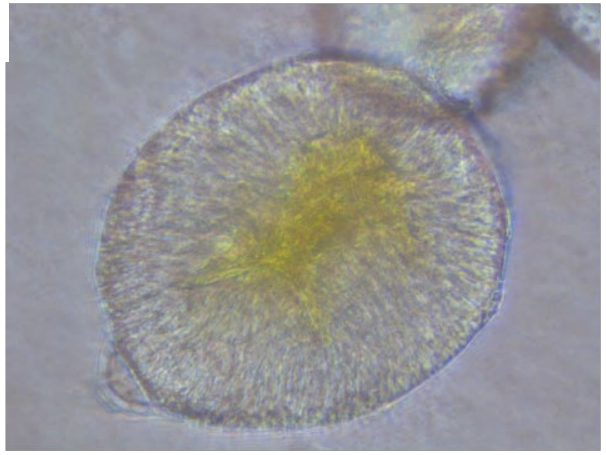
Establishment of organoids







Enteroids are derived from jejunal tissue using a similar protocol

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Apical-out versus basal-out

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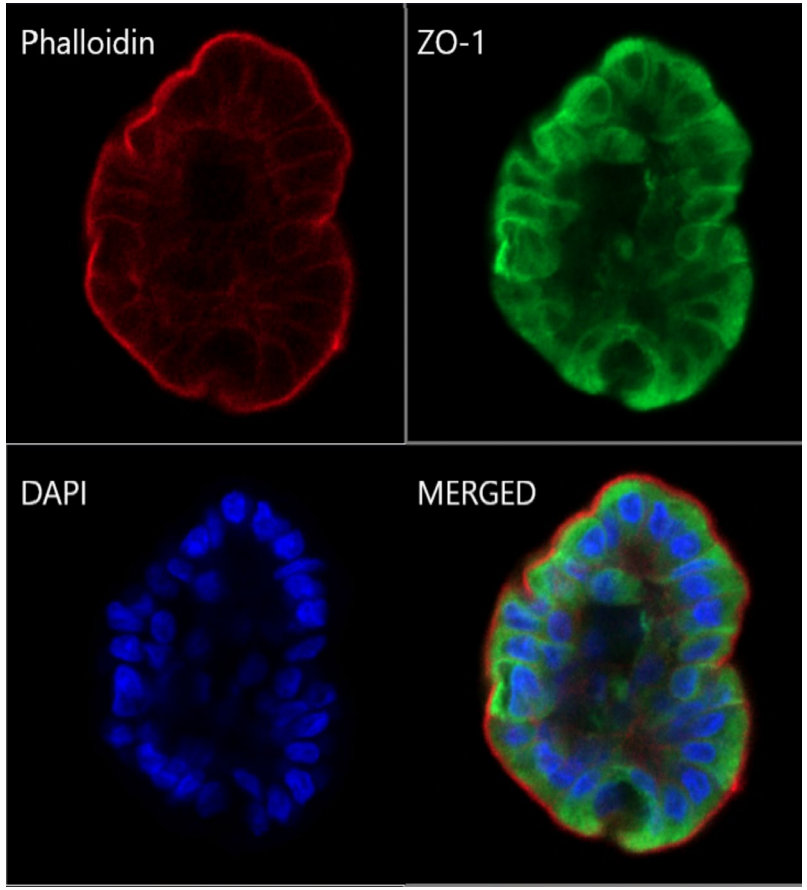
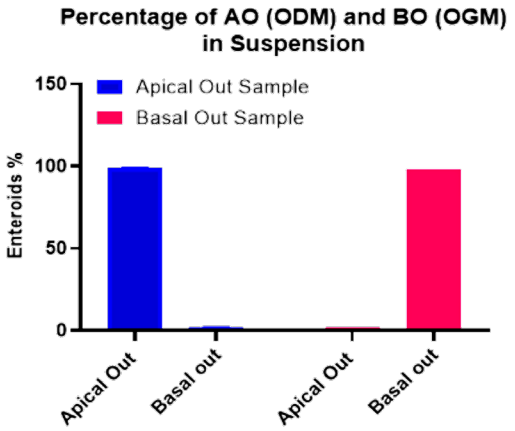
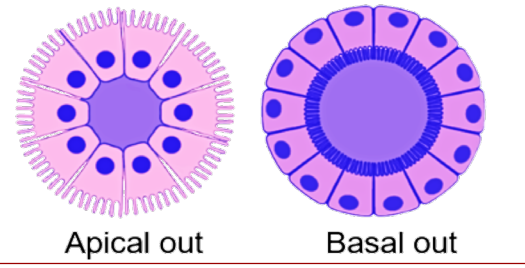
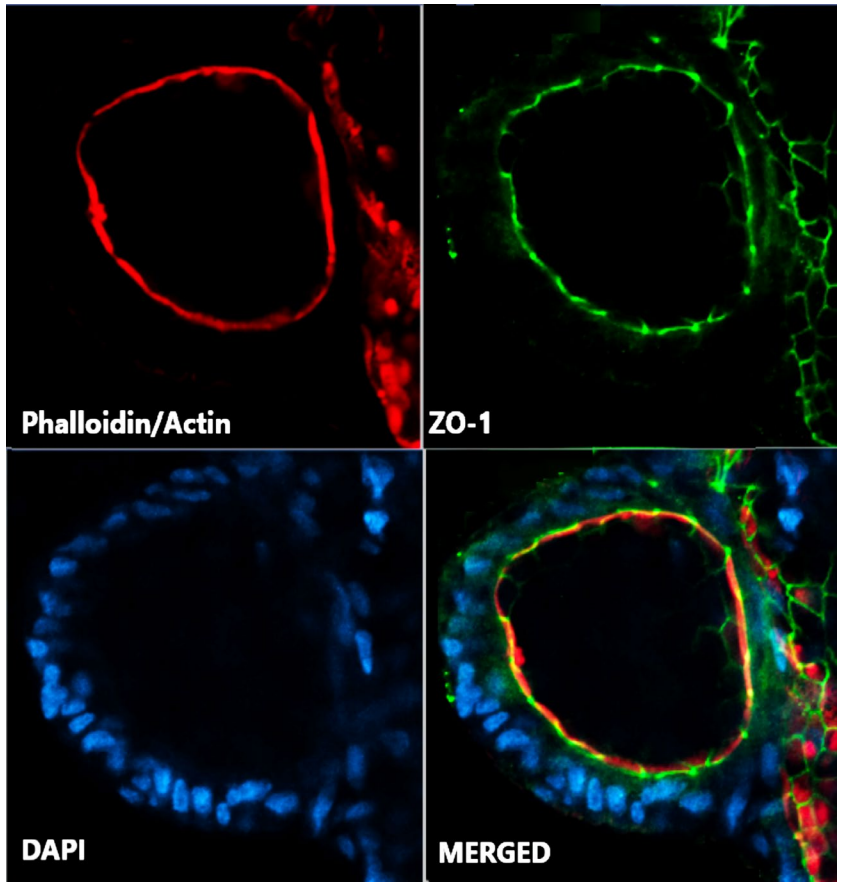


Enteroids: Donor no.: Hu18.9 jej
AO: Apical out in Differentiation medium
BO: Basal out in Growth medium
Passage 7, Day6







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Apical – out enteroids

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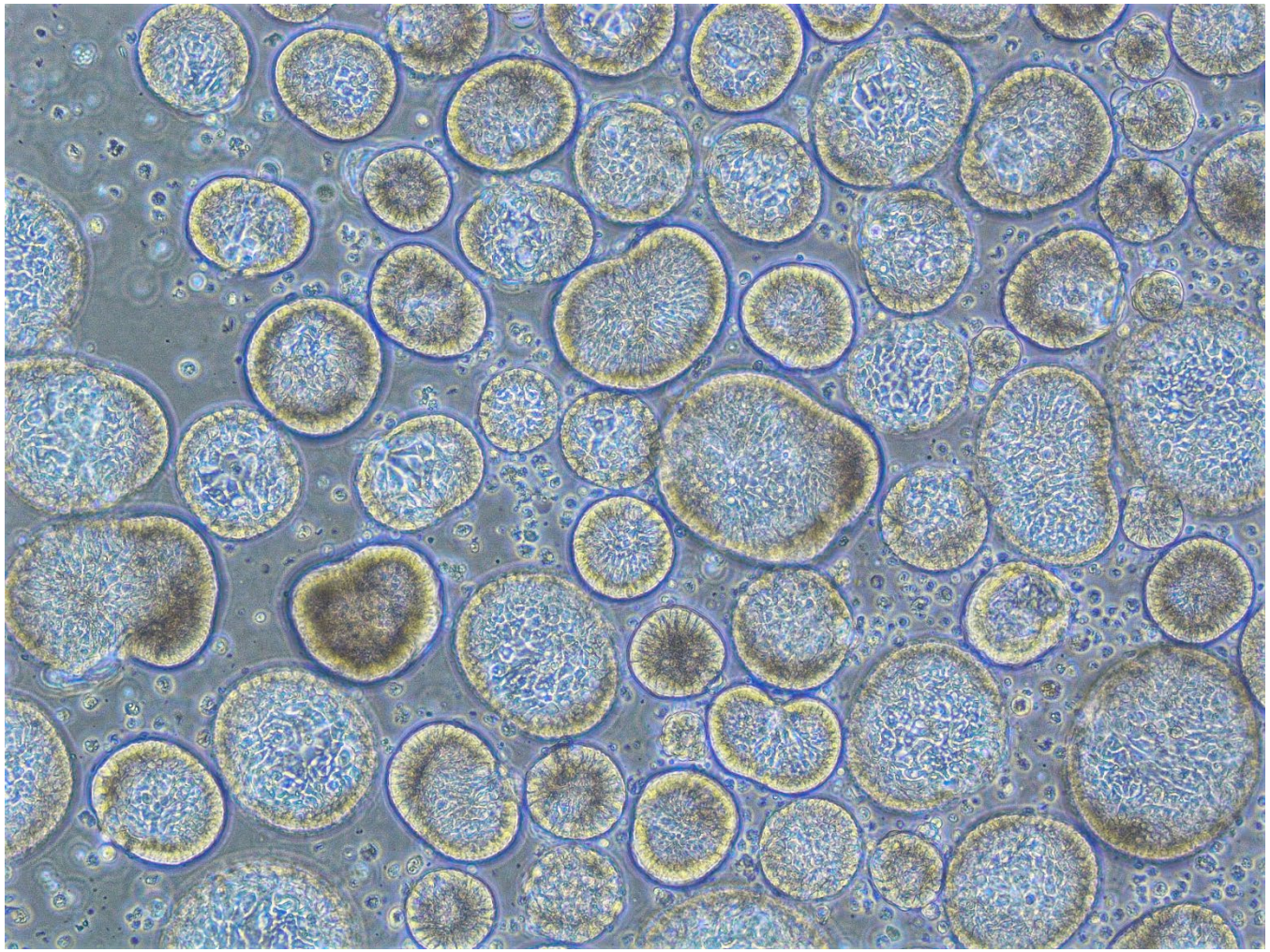

After six days of differentiation in AO configuration.

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Apical out enteroids - SEM

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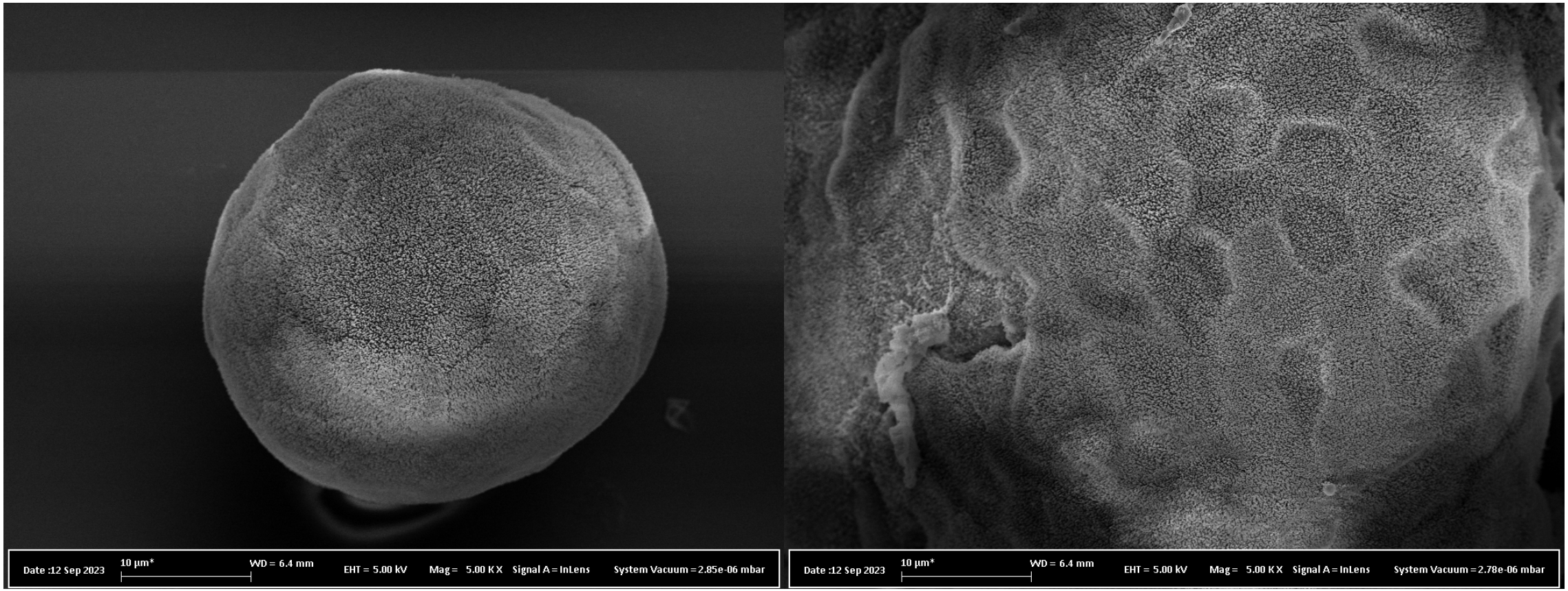

Brush border membrane apparent

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Mucus expression in enteroids

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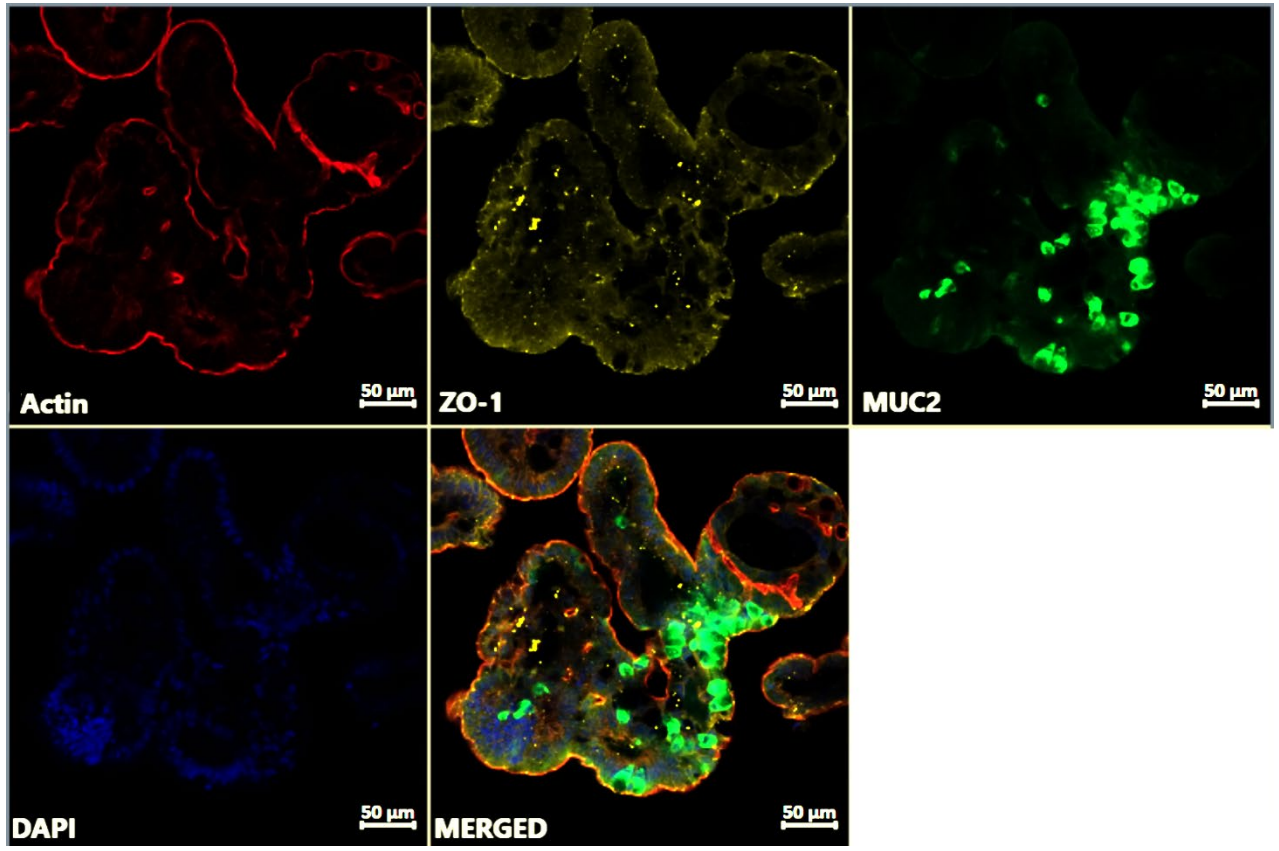

- MUC2 is expressed by a subset of cells in enteroids.
- MUC2 is a secreted mucin not expressed in Caco-2 cells.
- The presence of a mucus layer around the enteroids remains to be characterized.

Enteroids form a polarized barrier

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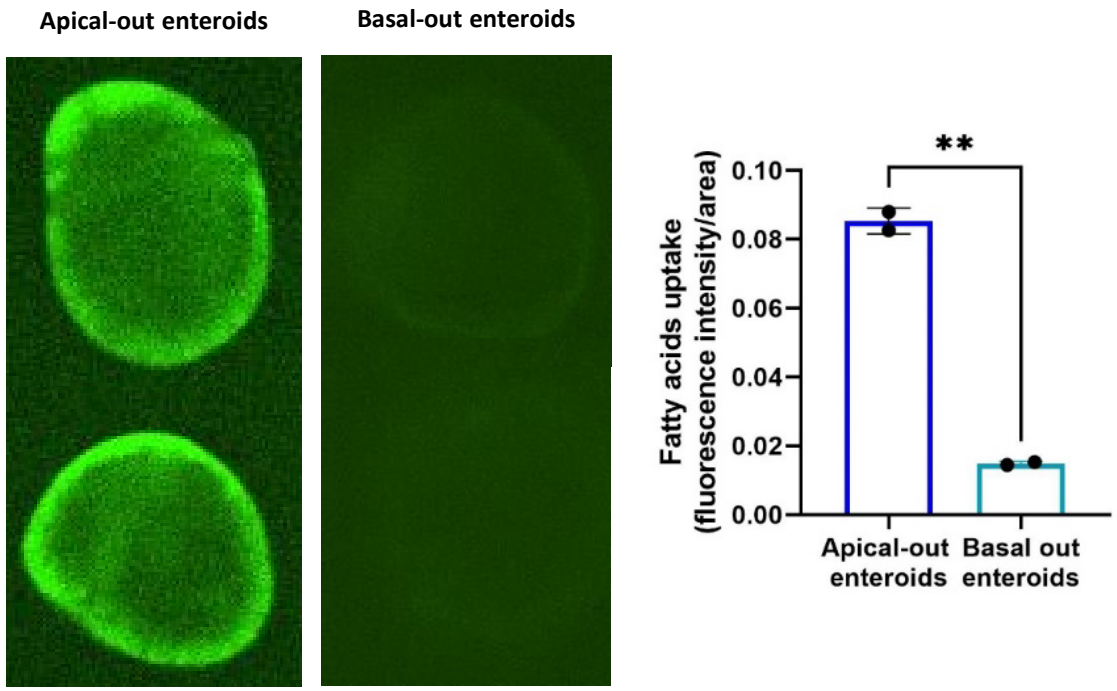
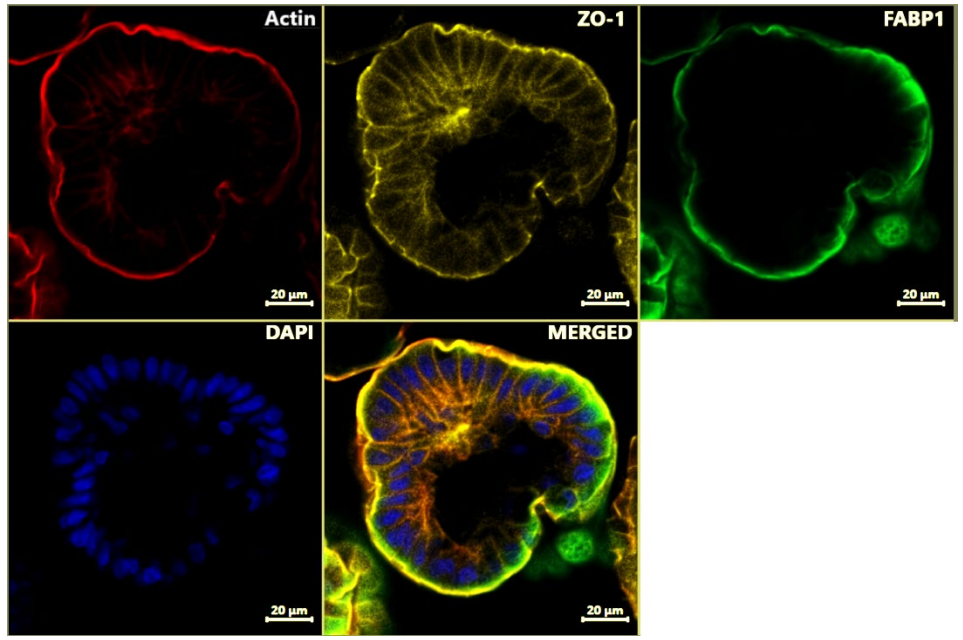
Fatty acid uptake



Expression of FABP1



- Enteroids show a polarized expression and activity of fatty-acid transport machinery.
- Experiments with barrier function and ABC transporter activity (P-gp) is on-going

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Enteroid CYP metabolism

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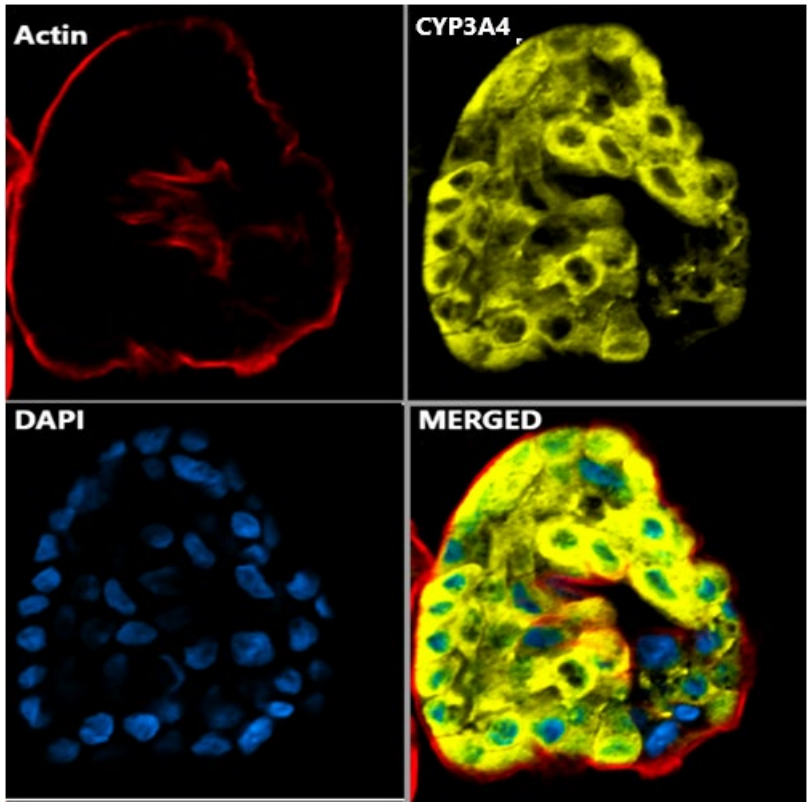
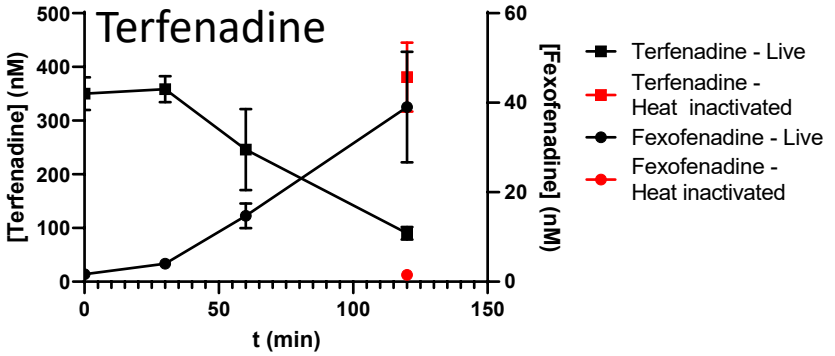
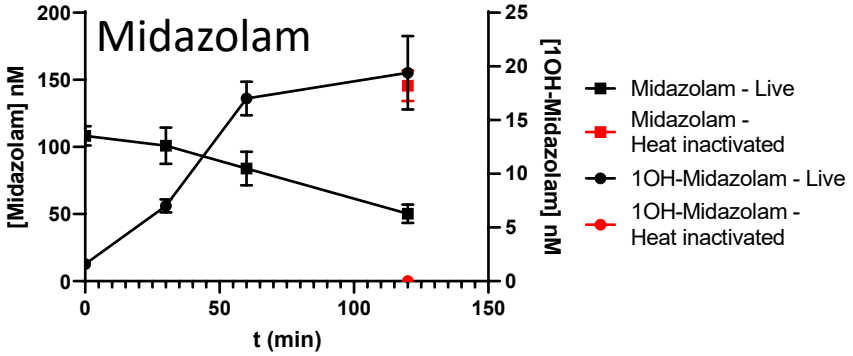
CYP3A4 expression



CYP3A4 activity





- CYP3A4 activity and expression can be detected in enteroids.
- Activity is approximately 1/3 of the activity seen in primary enterocytes.
- Acitvity of CYP2C9 and CYP2D6 can also be detected (so far).

Enteroid ADME protein expression

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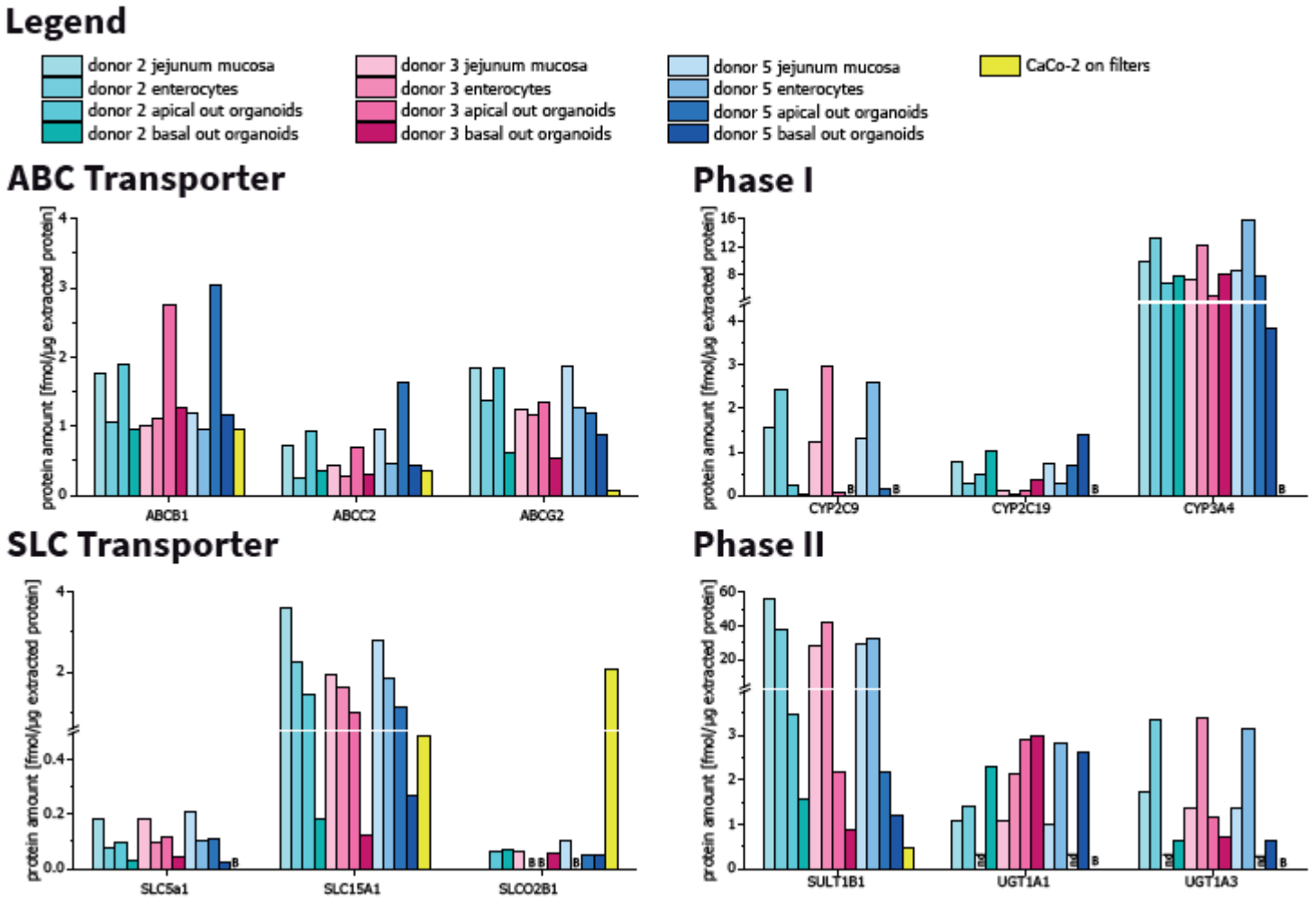

- Enteroids express in vivo-like levels of many ADME proteins
- Donor variations are in some cases carried over into enteroid expression levels.

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Conclusions

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- Caco-2 monolayers remains a robust assay for permeability studies if its identified weaknesses are taken into account
- Stem cell derived organoids from human intestine show more in vivo like expression profiles than Caco-2 cells and can be cultivated in new 3D configurations.
- Intestinal organoids can be used for studies of intestinal metabolism of compounds.
- Method optimization for transport and permeability studies is ongoing.

Acknowledgments

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- RISKHUNT3R
- Signatope
- Mikael Sellin and colleagues

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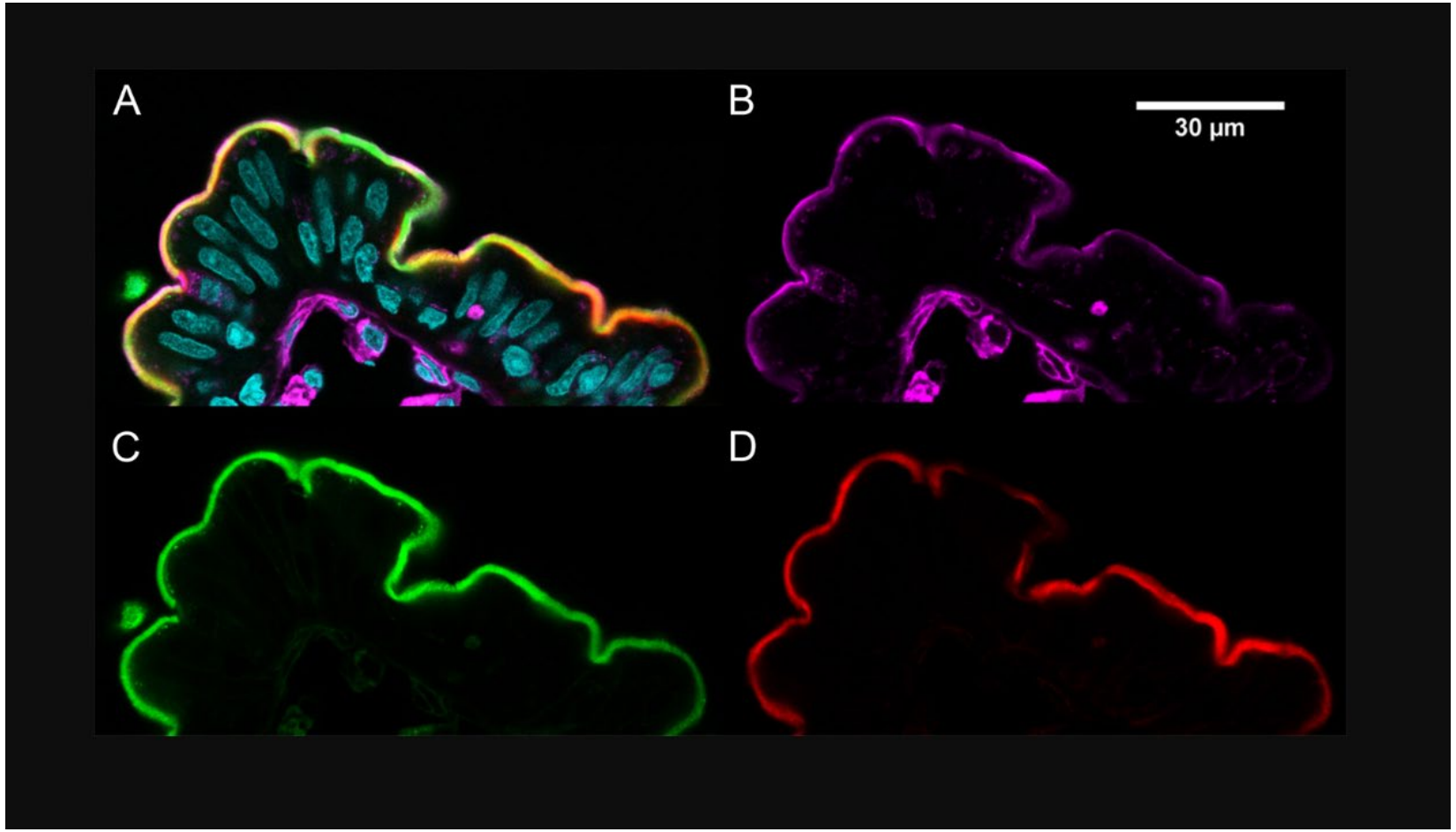

Lundquist, ACS Nano, 2022

Thank you for your attention!