DefiniGEN
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DefiniGEN Intestinal Organoids: A novel iPSC-derived *in vitro* system that models the human intestine

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1. Introduction

Drug-induced gastrointestinal toxicity is an underestimated adverse effect associated with a variety of symptoms ranging from asymptomatic histological changes in the gastrointestinal tissues to fatal complications. Alongside drug-induced liver injury, drug-induced gastrointestinal toxicity is a reason for drug discontinuation during drug development. However, and despite the severity of the problem, the mechanisms of drug-induced gastrointestinal toxicity are poorly understood, mainly due to the lack of relevant in vitro models able to recapitulate intestinal physiology. DefiniGEN intestinal organoids provide a unique in vitro system to model the human intestine. The organoids harbor a combination of cell types normally present in the primary intestinal epithelial in vivo, including goblet cells, Paneth cells, enterocytes, stem cells, and enteroendocrine cells. Our cells can offer a suitable and physiologically relevant in vitro model for drug absorption and metabolism, efflux transporter studies, as well as the modelling of infectious diseases.

2.1 Cell Morphology

Typical intestinal organoid morphology is observed in our iPSC-derived Intestinal organoids (Def-INT). The organoids initially form spheroid structures, which over successive passages develop the crypt architecture, characteristic of primary human intestinal organoids.

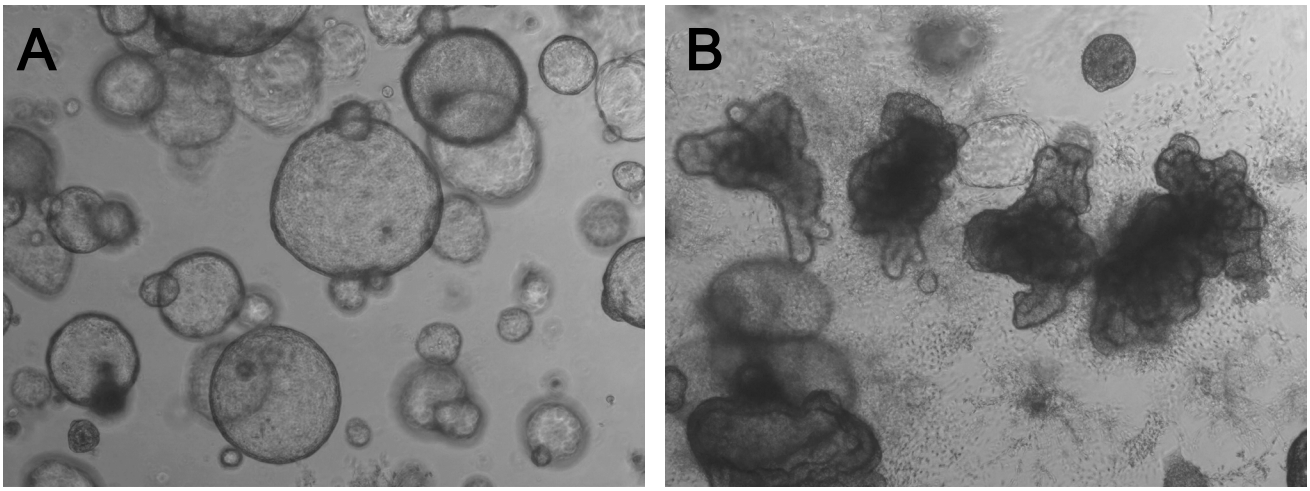

Figure 1. Def-INT morphology following two (A) and eight (B) passages encapsulated in matrigel.

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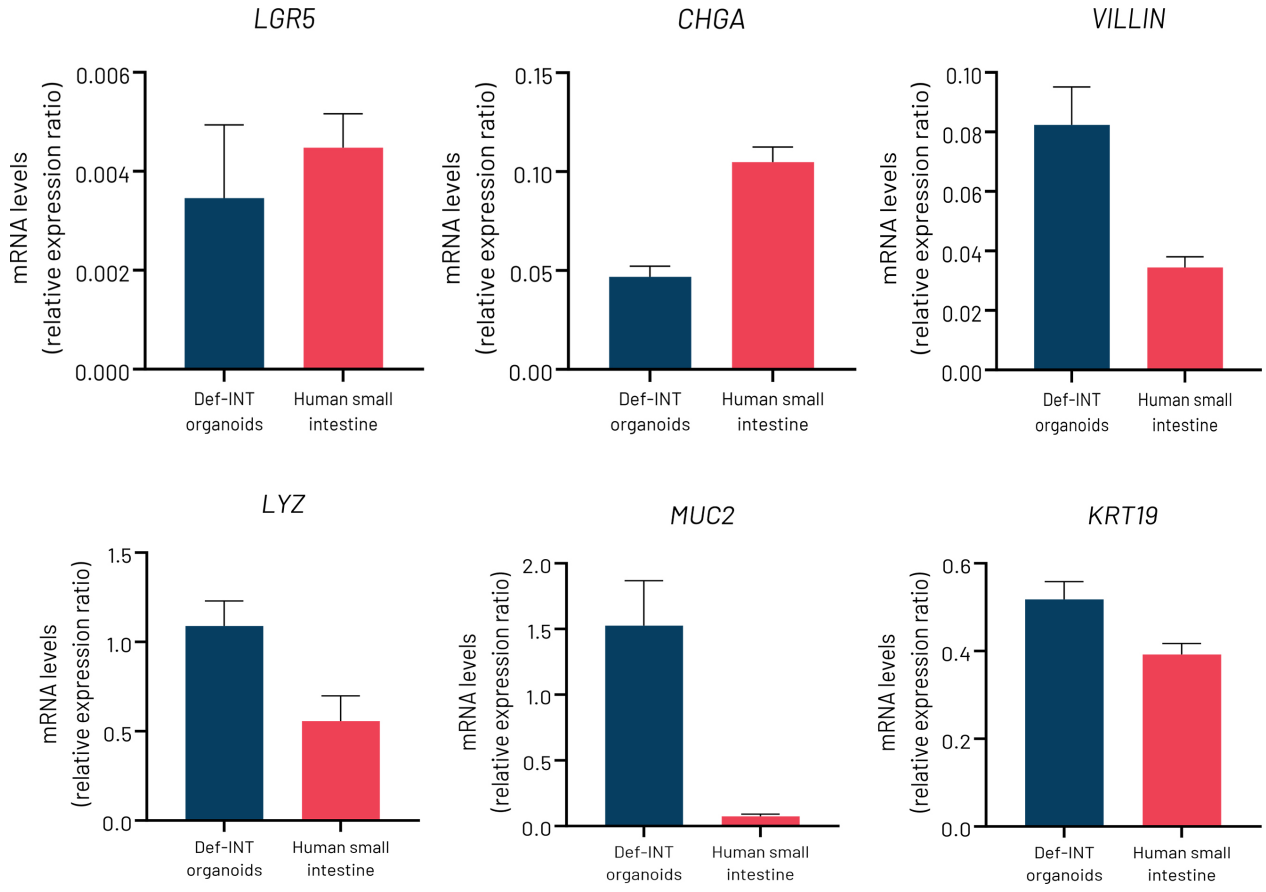
2.2 Def-INT express key intestinal cell markers at mRNA and protein level

Characterisation analysis has demonstrated that DefiniGEN intestinal organoids display a polarized epithelium and are composed of differentiated cell types with distinct morphologies. The organoids contain absorptive enterocytes as well as the major secretory lineage cell types, including Paneth cells, Goblet cells, stem cells, and enteroendocrine cells.

A



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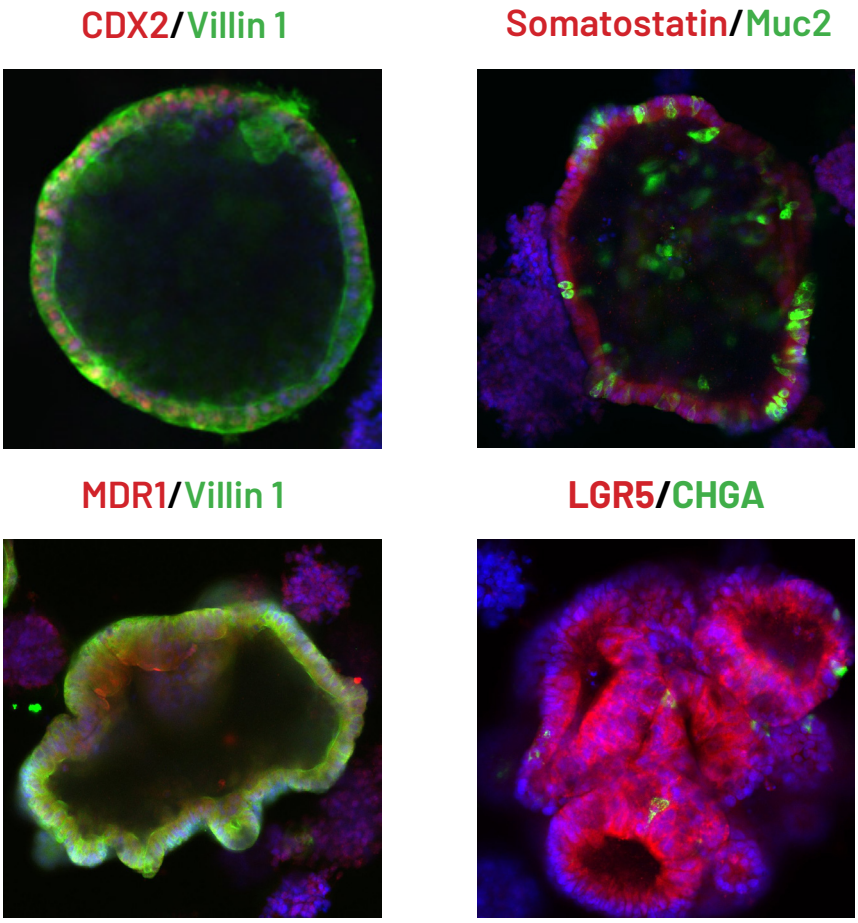

Figure 2. A) DefiniGEN intestinal organoids (Def-INT) demonstrate comparable gene expression levels of key intestinal markers (LGR5, CHGA, VILLIN, MUC2, LYZ, KRT19) to human small intestine tissues. B) DefiniGEN intestinal organoids express key intestinal protein markers present in different cell populations (enterocytes, Paneth cells, Goblet cells, enteroendocrine cells, stem cells) using immunocytochemistry. mRNA data were normalized to housekeeping gene GAPDH and are presented as mean±SEM of n=4 independent experiments.

2.3 Def-INT transcriptional profile

DefiniGEN intestinal organoids demonstrate a similar transcriptional profile to primary human intestinal tissues compared to carcinoma cell lines.

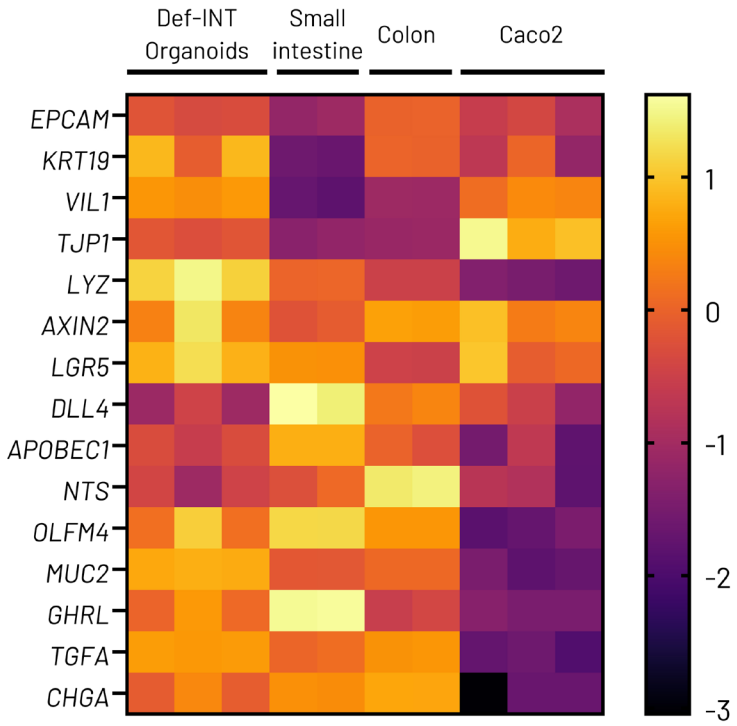

Figure 3. Heat map following bulk RNA-sequencing with the expression levels of key intestinal markers expressed in different cell populations (enterocytes, Paneth cells, Goblet cells, stem cells, enteroendocrine cells), revealing a similar transcriptional profile between DefiniGEN intestinal organoids (Def-INT), primary human small intestine, and primary human colon tissues. Distinct transcriptional differences are observed between the first three intestinal models and Caco2 carcinoma cells.

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2.4 Def-INT express key metabolism markers

DefiniGEN intestinal organoids express key drug and metabolism markers including CYP3A4, CYP2J2, CYP2D6, UG2B7, SLCO2B1, ABCB1.

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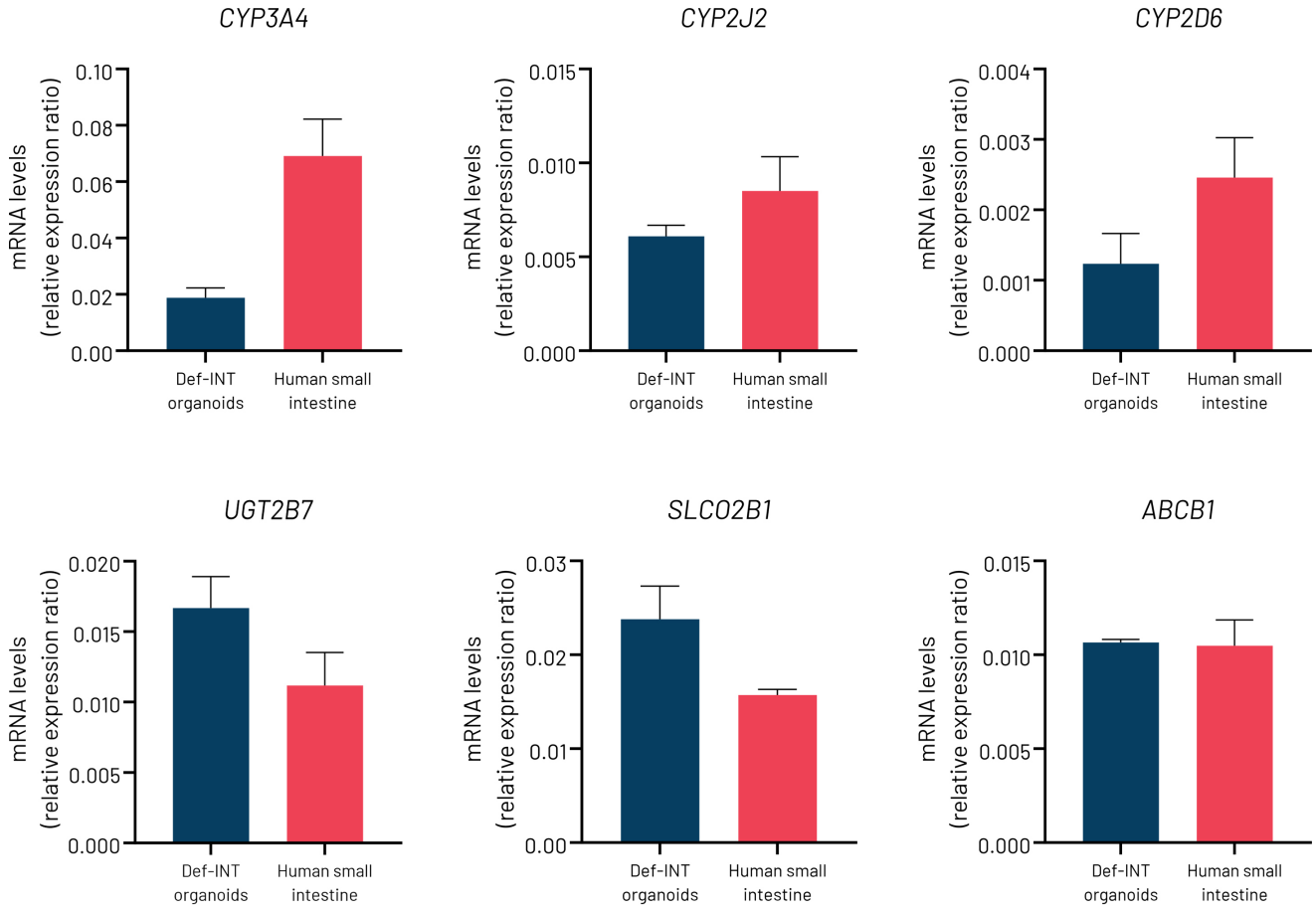

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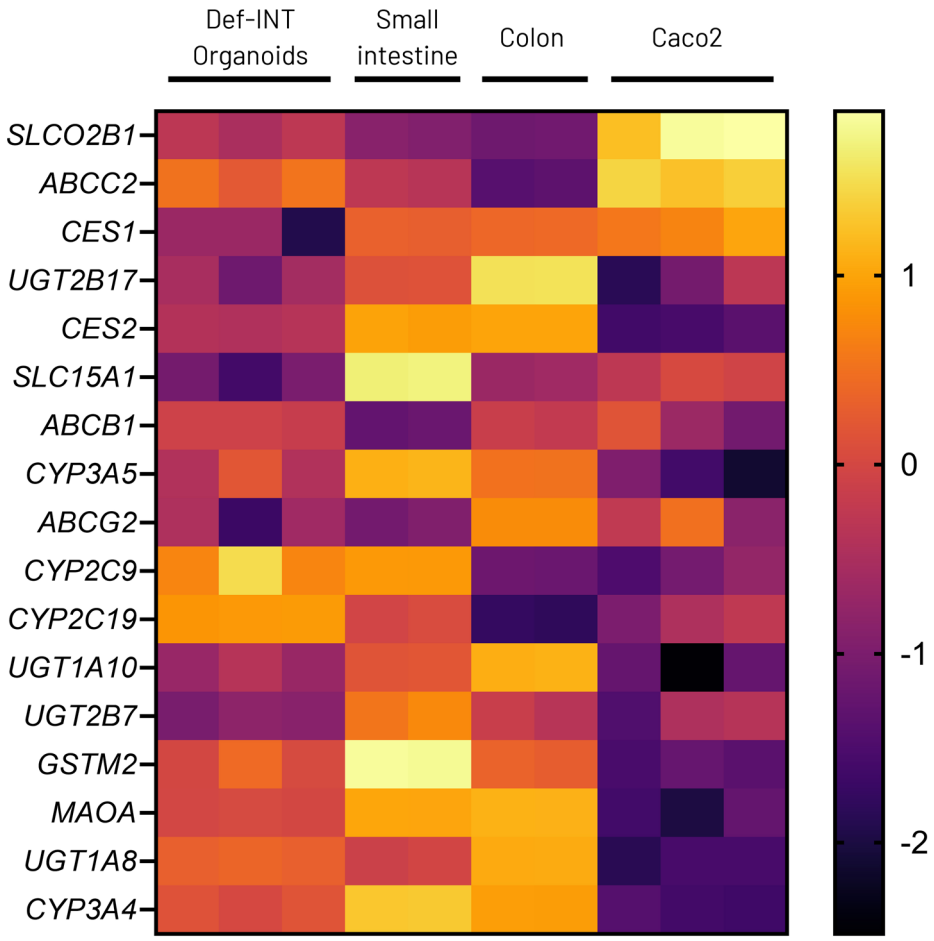

Figure 4. A) DefiniGEN intestinal organoids (Def-INT) demonstrate comparable gene expression levels of key drug metabolism and transporter markers (CYP3A4, CYP2J2, CYP2D6, ABCB1, SLCO2B1, UGT2B7) to human small intestine tissues. B) Heat map following bulk RNA-sequencing with the expression levels of key drug metabolism and transporter markers revealing a similar transcriptional profile between DefiniGEN intestinal organoids (Def-INT), primary human small intestine, and primary human colon tissues. Distinct transcriptional differences are observed between the first three intestinal models and Caco2 carcinoma cells. mRNA data were normalized to housekeeping gene GAPDH and are presented as mean±SEM of n=4 independent experiments.

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2.5 Def-INT demonstrate CYP450 activity

DefiniGEN intestinal organoids, Def-INT, demonstrate functional CYP450 induction, detoxification and transporter activity

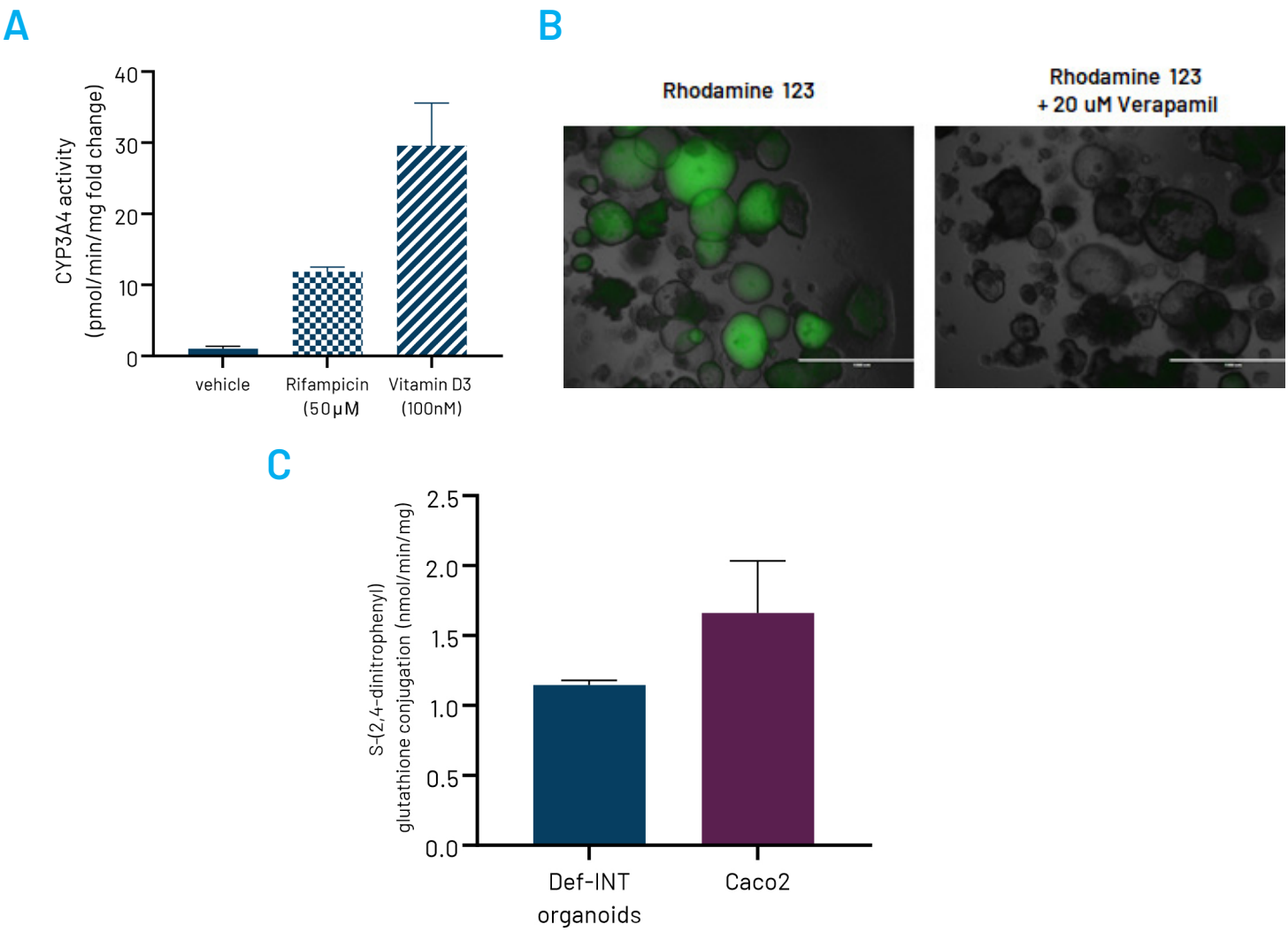

Figure 5. A) CYP3A4 mRNA induction in DefiniGEN intestinal organoids following 72 hours of treatment with vehicle, 50µM Rifampicin, or 100nM vitamin D3 (CYP3A4 inducers). B) DefiniGEN intestinal organoids demonstrate functional MDR1 (ABCB1) activity following organoid treatment with Rhodamine 123 (MDR substrate; green), and this effect can be reversed following co-treatment with Verapamil (MDR1 inhibitor; 20µM). C) DefiniGEN intestinal organoids demonstrate functional detoxification pathways, as shown by comparable Glutathione-S-Transferase (GST) activity to that observed in colorectal adenocarcinoma Caco2 cells. Data are presented as mean±SEM of n=2-3 independent experiments.

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3.1 DefiniGEN intestinal organoids can be cultured in monolayer to investigate drug permeability and absorption

Despite the benefits in using intestinal organoids to study physiological intestinal epithelium function, these are often not suitable when absorption and/or permeability of drug candidates with high hydrophilicity need to be investigated. DefiniGEN intestinal organoids can help overcome this issue, as they can be used to generate monolayer intestinal epithelial cell cultures that are polarized, thus allowing the direct access to the apical side of the cells.

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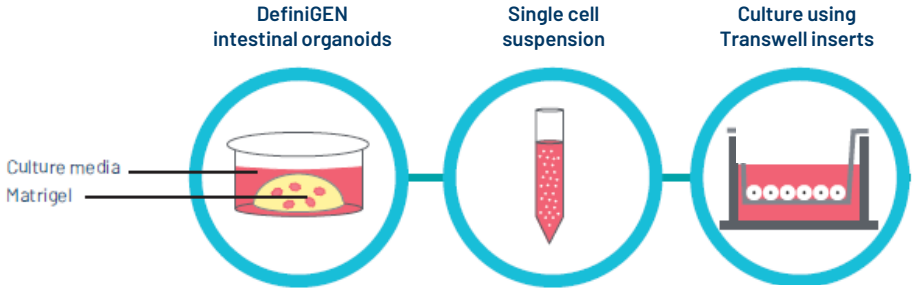

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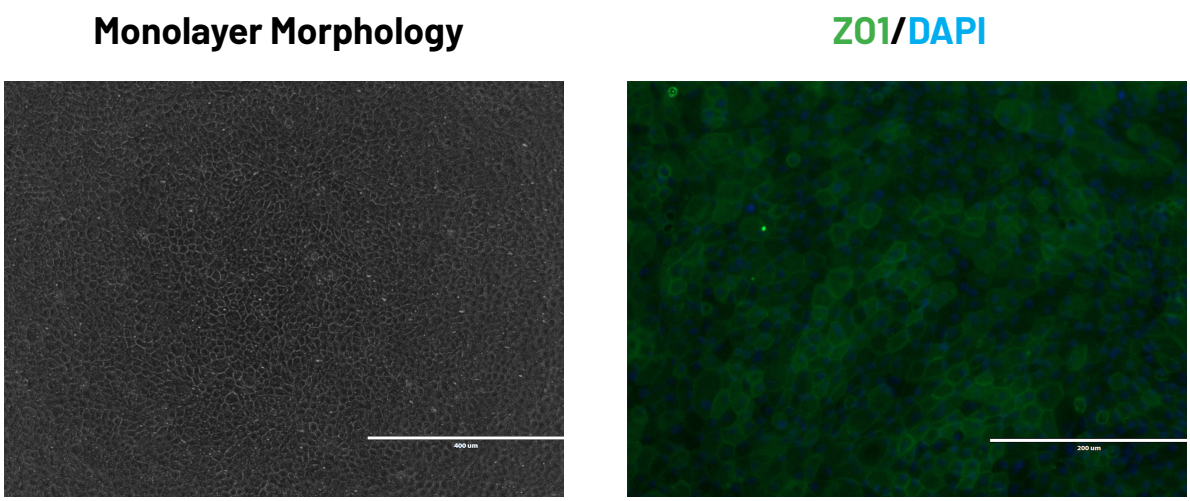

Figure 6. A) Simplified schematic demonstrating the process towards the generation of intestinal epithelial cell monolayer using DefiniGEN intestinal organoids. B) DefiniGEN intestinal epithelial cell monolayer following 7 days of culture in Transwell inserts, revealing the expected epithelial morphology and polarization following staining with the apical marker ZO-1 (green). Cells were counterstained with DAPI.

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3.2 Def-INT epithelial monolayers maintain their intestinal signature

Compared to human intestine tissues, DefiniGEN intestinal epithelial monolayers (Def-INT) demonstrate comparable gene expression levels of key intestinal markers.

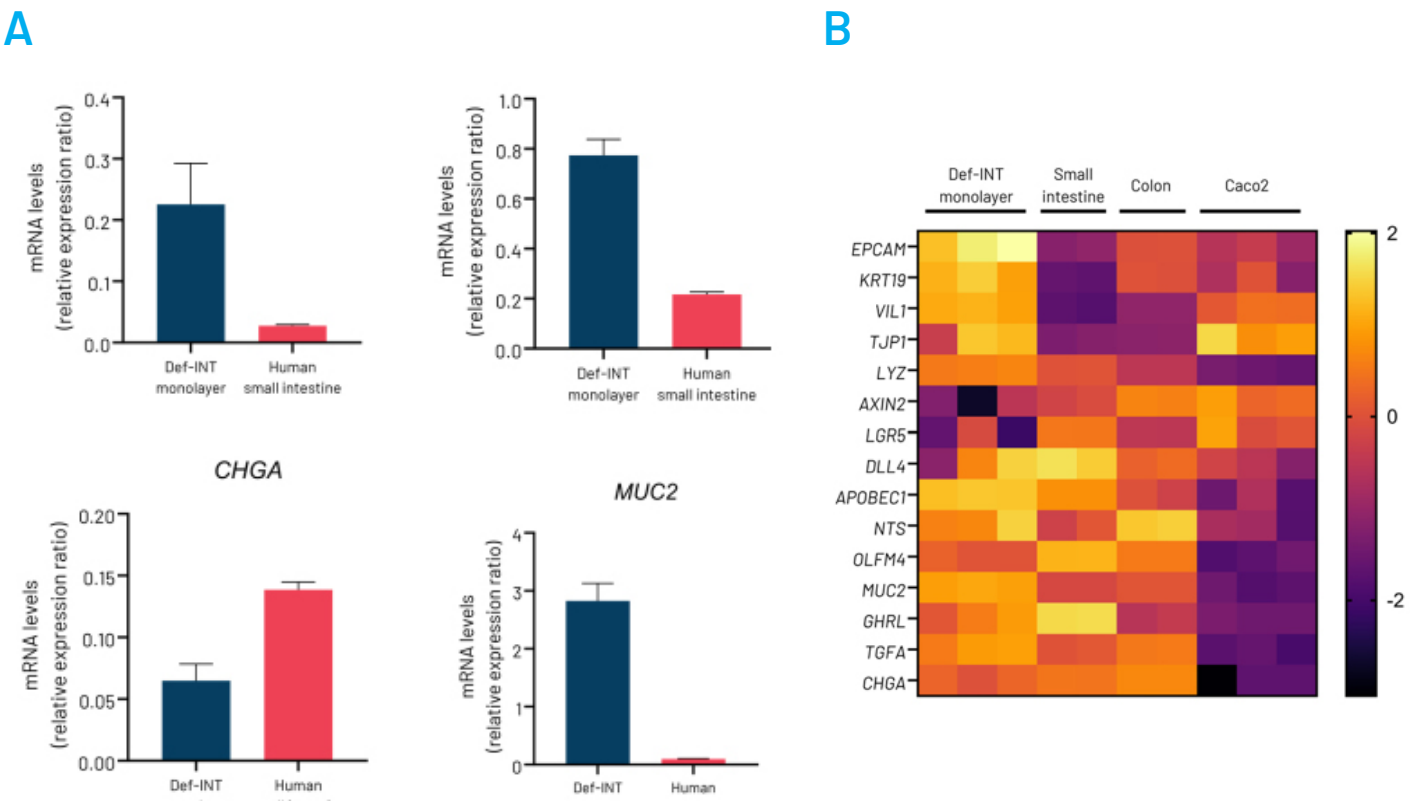

Figure 7. A) DefiniGEN intestinal epithelial monolayers (Def-INT) demonstrate comparable gene expression levels of key intestinal markers (VILLIN, LYZ, CHGA, MUC2) to human small intestine tissues. B) Heat map following bulk RNA-sequencing with the expression levels of key intestinal markers revealing a similar transcriptional profile between DefiniGEN intestinal epithelial monolayers (Def-INT), primary human small intestine, and primary human colon tissues. Distinct transcriptional differences are observed between the first three intestinal models and Caco2 carcinoma cells. mRNA data were normalized to housekeeping gene GAPDH and are presented as mean±SEM of n=4 independent experiments.

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3.3 DefiniGEN intestinal epithelial monolayers demonstrate high monolayer integrity and enhanced reproducibility

Measurement of transepithelial electrical resistance (TEER) demonstrates the ability of DefiniGEN intestinal cells (Def-INT) to form a tight monolayer with resistance values closer to those observed in human small intestine. These data reveal the ability of Def-INT to better predict permeability of hydrophilic drugs or compounds absorbed through the paracellular route compared to the currently available in vitro intestinal models (e.g., Caco2 cells). Def-INT cells can generate a tight monolayer after only 7 days of culture, and they are stable for at least 12 days post-seeding. They present minimal well-to-well variability and high reproducibility between different experiments.

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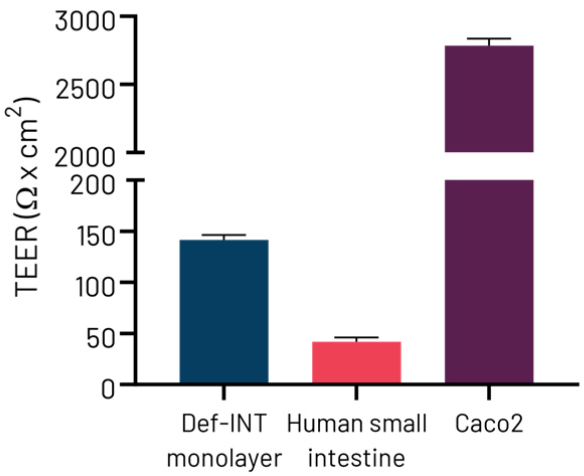

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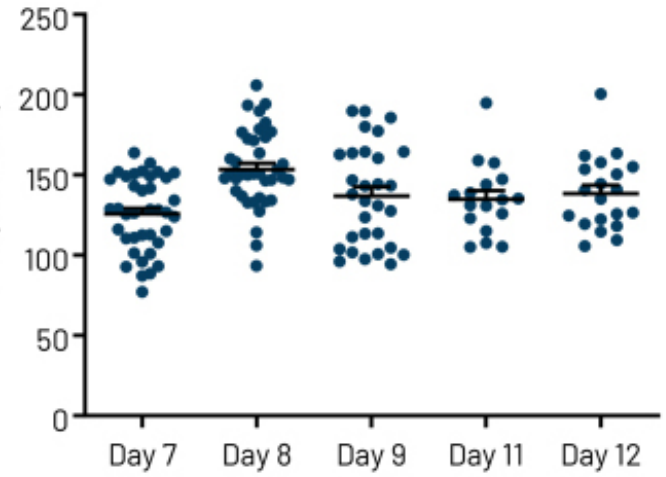

Figure 8. A) DeÞniGEN intestinal (Def-INT) epithelial monolayers demonstrate comparable TEER values to those observed in human small intestinal monolayers. TEER data are presented as mean±SEM of n=3 independent experiments. B) DeÞniGEN intestinal (Def-INT) epithelial monolayers demonstrate enhanced reproducibility and stability with comparable TEER values across >15 independent experiments between 7- and 12- days post cell-seeding.

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3.4 DefiniGEN intestinal epithelial monolayers express key membrane transporter and drug metabolism markers

Def-INT epithelial monolayers express key membrane transporter and drug metabolism markers including *SLCO2B1, UGT2B7, ABCB1, BCRP, CYP2J2, CYP2D6*

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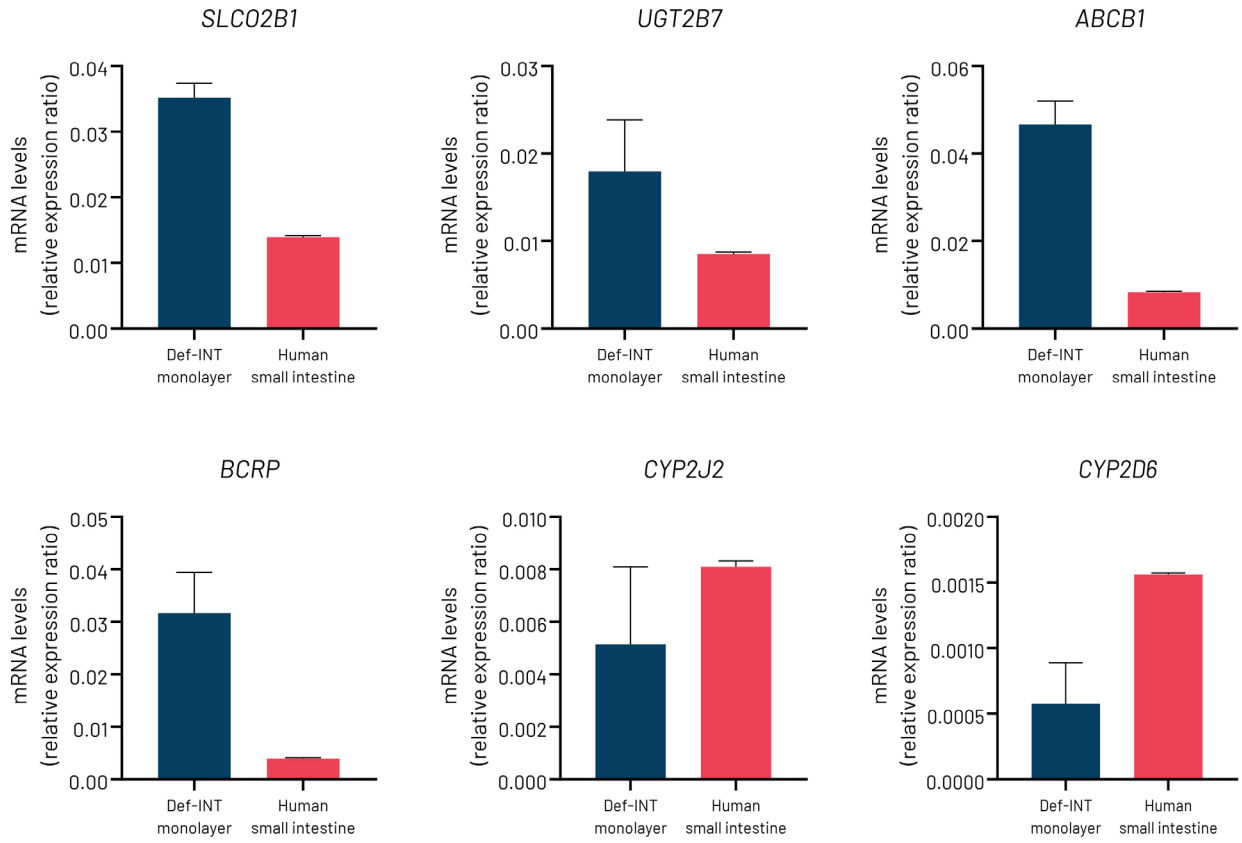

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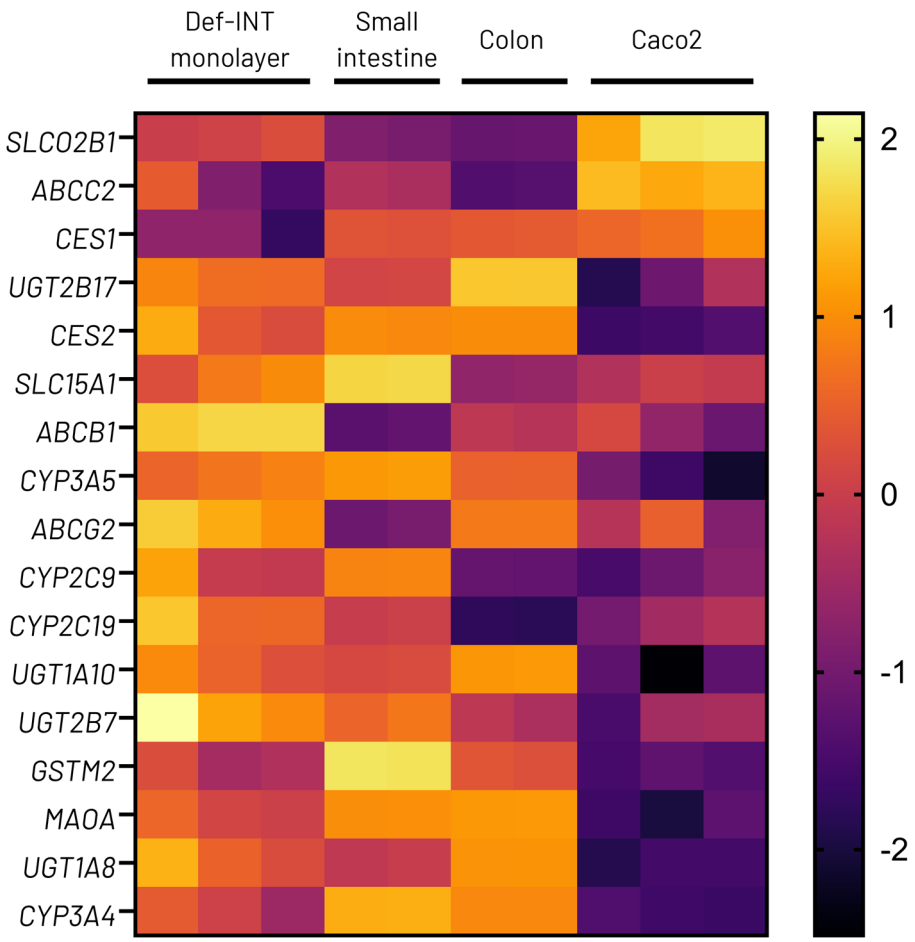

Figure 9. A) DefiniGEN intestinal (Def-INT) epithelial monolayer cultures demonstrate comparable gene expression levels of key membrane transporter and drug metabolism markers (SLCO2B1, UGT2B7, ABCB1, BCRP, CYP2J2, CYP2D6) to human small intestine tissues. B) Heat map following bulk RNA-sequencing with the expression levels of key membrane transporter and drug metabolism markers revealing a similar transcriptional profile between DefiniGEN intestinal (Def-INT) epithelial monolayer cultures, primary human small intestine, and primary human colon tissues. Distinct transcriptional differences are observed between the first three intestinal models and Caco2 carcinoma cells. mRNA data were normalized to housekeeping gene GAPDH and are presented as mean±SEM of n=4 independent experiments.

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3.5 DefiniGEN intestinal epithelial monolayer demonstrates efficient drug permeability

DefiniGEN intestinal epithelial monolayer can predict permeability of marketed drugs similarly to colorectal adenocarcinoma Caco2 cells and can be used to categorize these based on permeability rate. The physical characteristics of the Def-INT monolayer, in combination with the activity of several phase I and phase II enzymes, makes these cells a highly predictive model. Importantly, the range of compounds that can be studied using the Def-INT cells includes substrates of various efflux transporters, including MDR1, BCRP and MRP2.

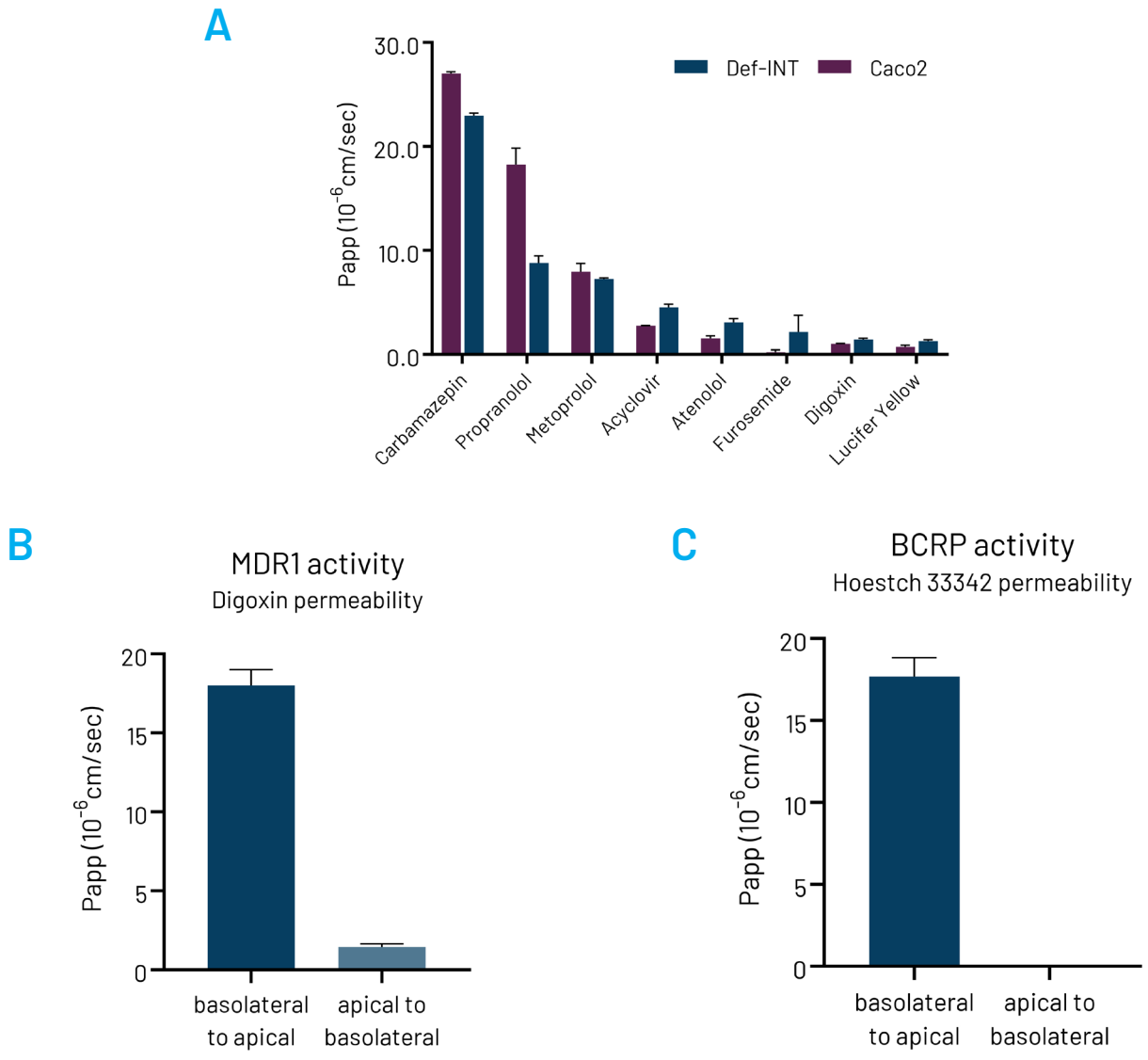

Figure 10. A) Permeability rate of high, medium and low permeability compounds tested in DefiniGEN intestinal (Def-INT) epithelial monolayer, revealing Papp values that accurately correspond to their known level of permeability throughout the intestinal epithelium. B-C) Def-INT monolayers demonstrate proper cellular polarization, as shown by MDR1 and BCRP activity when measuring different permeability coefficients secreted from either the apical-to-basolateral or basolateral-to-apical sides. Data are presented as mean±SEM of n=3 independent experiments.

DefiniGEN
Babraham Research Campus, Babraham,
Cambridge, CB22 3AT, United Kingdom

For more information please contact us:
+44 (0) 1223 497 106
info@definigen.com

www.definigen.com

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