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DISEASE MODEL INNOVATION

Guidance and properties of Def-INT cells cultured in a monolayer

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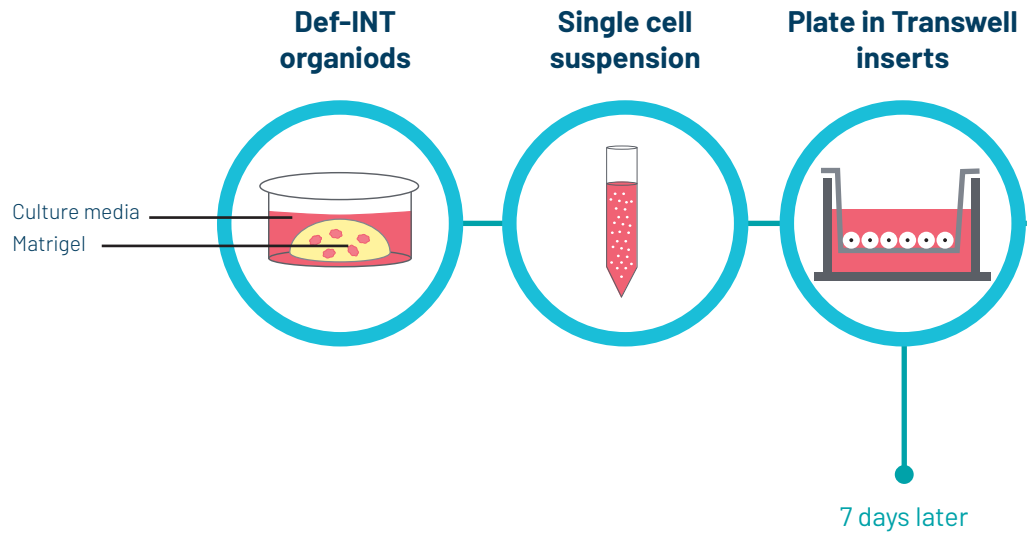
1. Introduction

Generation of a monolayer of Def-INT cells follows a simple, completely standardized and reproducible protocol. Single cells grown in a Transwell insert after 7 days show a homogeneous monolayer with tight junctions and characteristics that mimic the intestinal epithelium in a similar way to Caco-2.



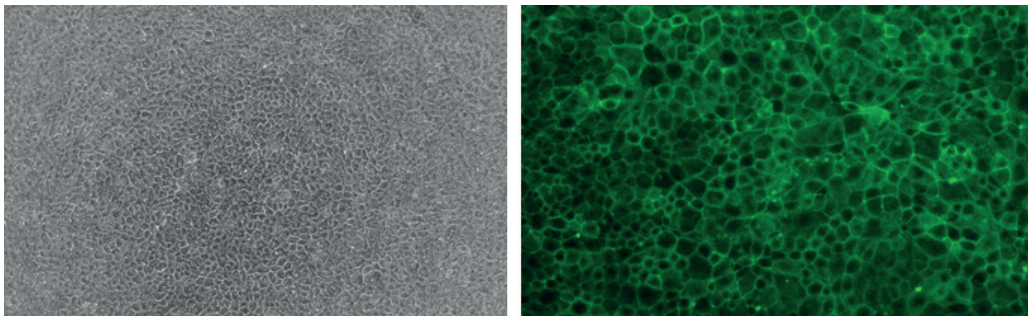


Figure 1. Def-INT monolayer formation. A) Diagram showing the simple process to obtain a monolayer from Def-INT organoids. B) Def-INT monolayer after 7 days of culture showing epithelial morphology and ZO-1 staining.

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Monolayer integrity and reproducibility

Measurement of transepithelial electrical resistance (TEER) shows Def-INT are able to form a tight monolayer with resistance values closer to the ones found in human small intestine. This suggests a better ability to predict permeability of hydrophilic drugs or compounds absorbed through the paracellular route than other models such as Caco-2 that usually underpredict the absorption of those compounds.

Def-INT cells can generate a tight monolayer after only 7 days of culture, but they are stable at least up to 12 days after plating. They present very little well to well variability and high reproducibility between experiments.

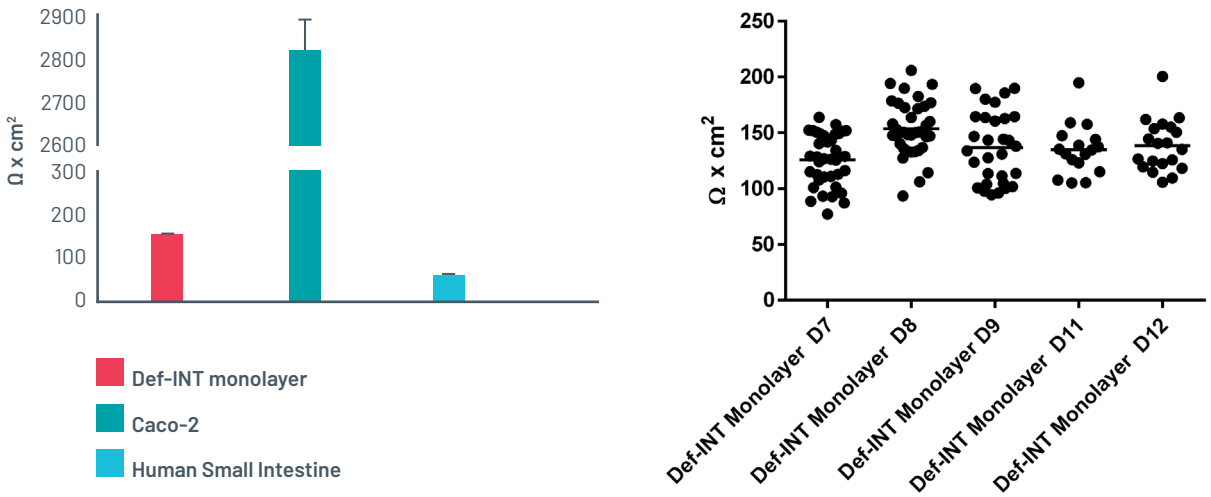

Figure 2. TEER evaluation. A) Def-INT monolayer show TEER values around 150 Ω x cm2. B) Cells are consistent throughout experiments and they show optimal performance between d7 and d12 after plating.

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Drug permeability

Def-INT monolayer can predict permeability of marketed drugs similarly to Caco-2 and can be used to categorize them in high permeability (Eg. Carbamazepine) or low permeability (Eg. Atenolol). The physical characteristics of the monolayer and the activity of several phase I and phase II enzymes makes them a highly predictive model. The range of compounds that can be studied with these cells also includes substrates of efflux transporters as Def-INT monolayer presents transporter activity including MDR1, BCRP and MRP2.

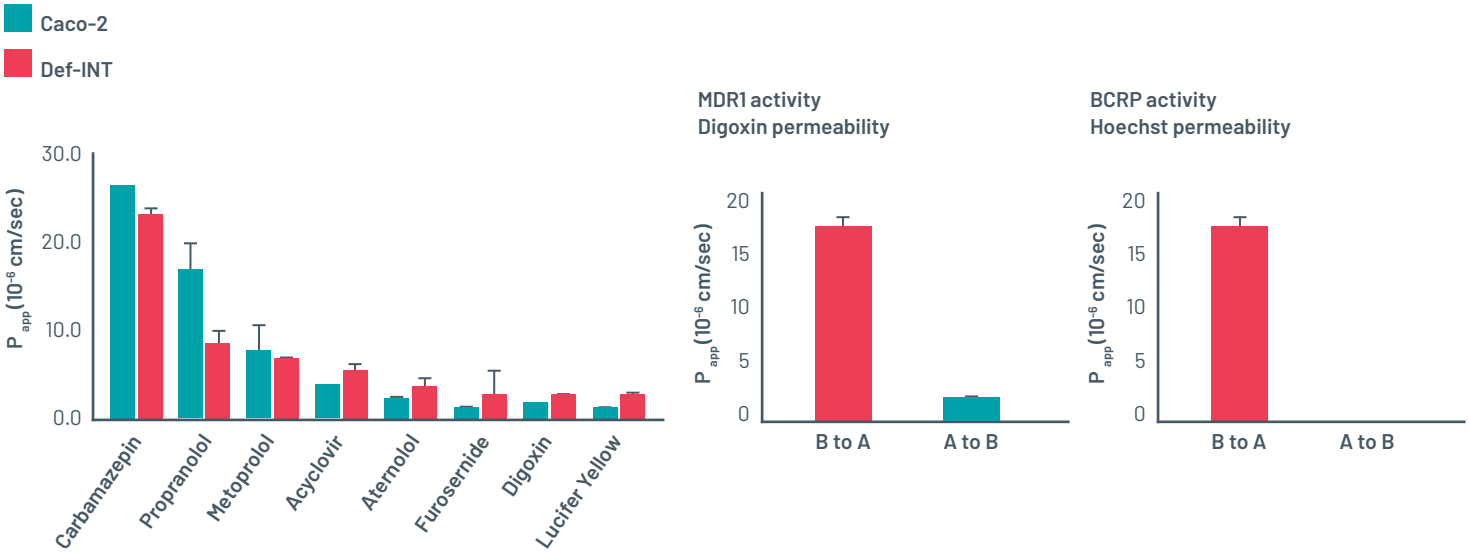

Figure 3. Def-INT monolayer can be used to predict drug permeability. A) Drugs tested in Def-INT monolayer show Papp values that correspond to their known level of permeability throughout the intestinal epithelium. This includes high, medium and low permeability compounds and substrates for efflux transporters. B) Cell polarization with MDR1 and BCRP activity shown with the different permeability coefficients obtained when measuring from Apical to Basolateral or Basolateral to Apical (A to B/B to A).

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Drug transport and metabolism

Def-INT monolayer cells show expression of several transporters and enzymes related to drug absorption and metabolism to levels closer to human small intestine than Caco-2. Uptake transporters such as PEPT1 (SLC15A1), phase I enzymes such as CYP3A4/5, CYP2C9 and CES2 and phase II enzymes such as UGT2B7 and GSTM2 are expressed in Def-INT monolayer but are negligible in other models like Caco-2.

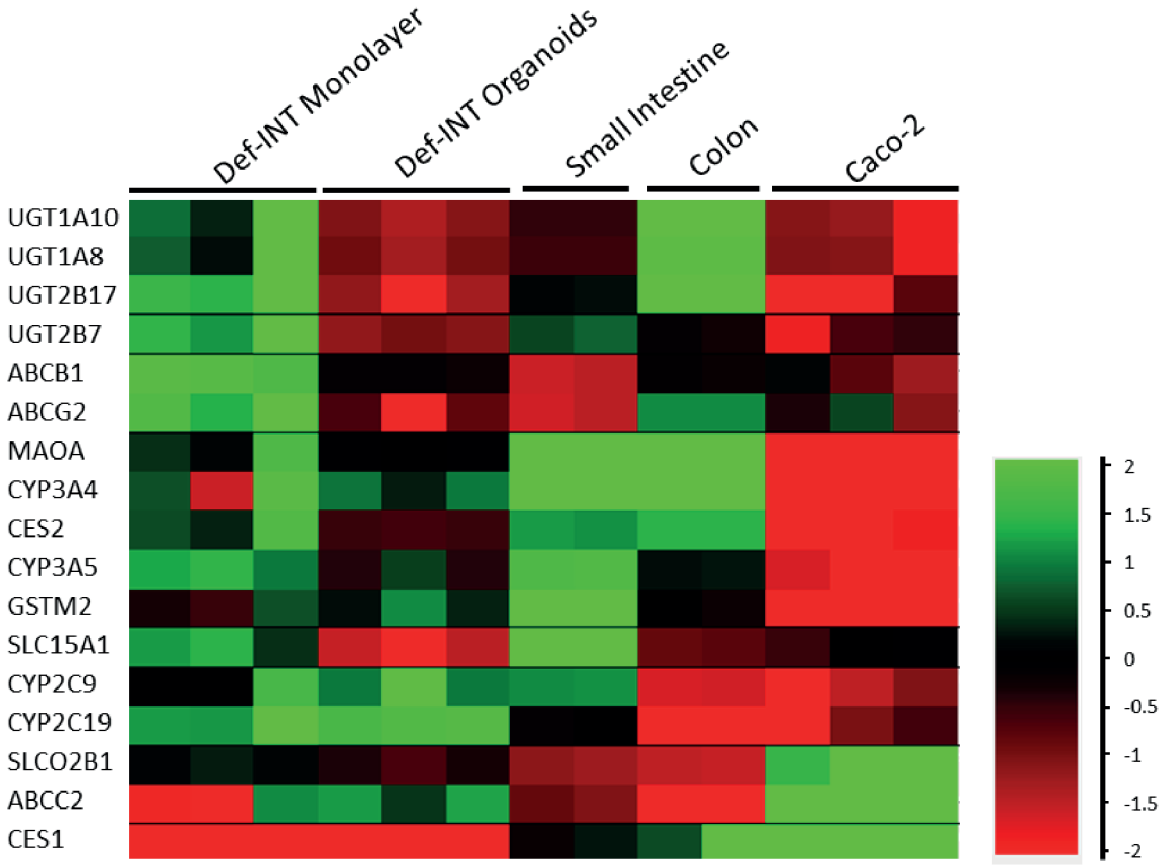

Figure 4. Heatmap for gene expression of relevant genes for drug transport and metabolism.

Def-INT monolayer cells have CYP3A4 activity that can be measured with the hydroxylation of testosterone. Moreover, this activity can be induced several time-fold via PXR and VDR nuclear receptors with common inducers such as Rifampicin and VD3. Other members of the CYP450 family relevant to the intestine are also present in Def-INT cells.

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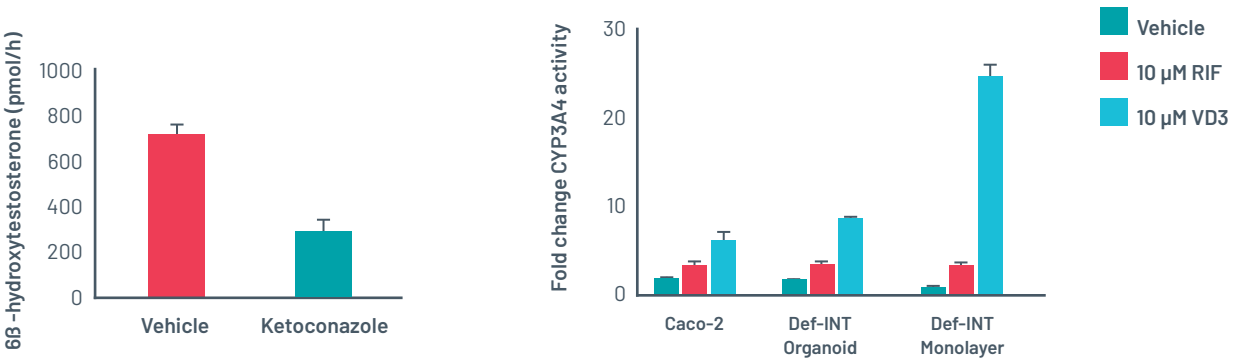
CYP34 activity



CYP450 gene expression

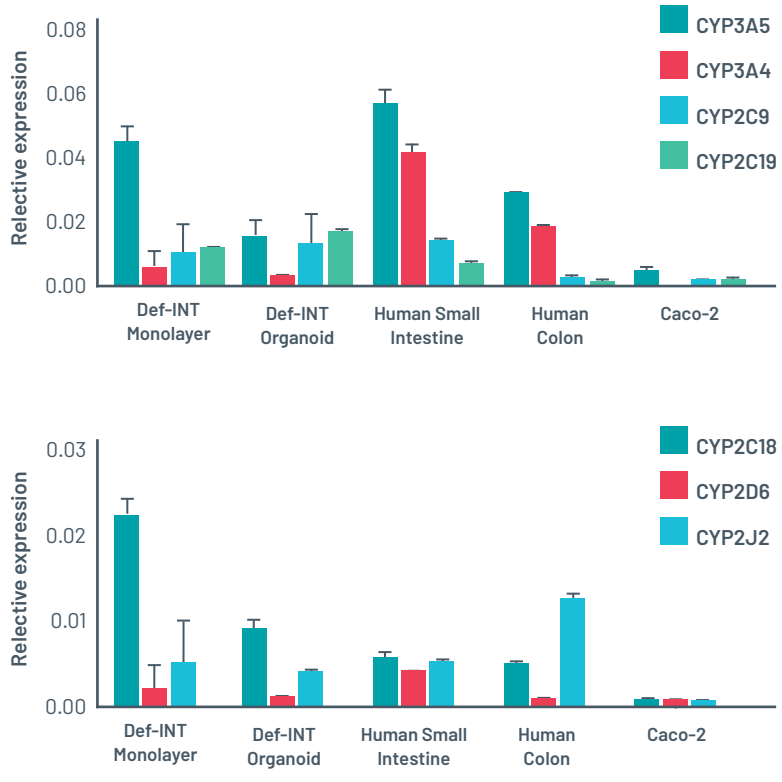


Figure 5. CYP450 expression and activity. A) Def-INT monolayer shows specific CYP3A4 activity measured by the metabolization of Testosterone into 6B-hydroxytestosterone that can be inhibited by Ketoconazole. B) Fold change in CYP3A4 activity upon induction with VD3 and RIF. C) Gene expression of the most important isoforms of the CYP3 and CYP2 family in the human intestine. D) Gene expression of other key CYP2 isoforms found in human intestine.

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Expression of the main transporters can be found in Def-INT monolayer cells after 7 days in culture. Levels of ABCB1, SLC15A1 and ABCG2 increase throughout the culture process while ABCC2 and SLCO2B1 reach their maximum expression as soon as day 7. Although the expression levels for some of these genes may vary slightly from day 7 to day 12, they are always within the level of variability expected amongst different human specimens or along the proximal/distal axis in the intestine.

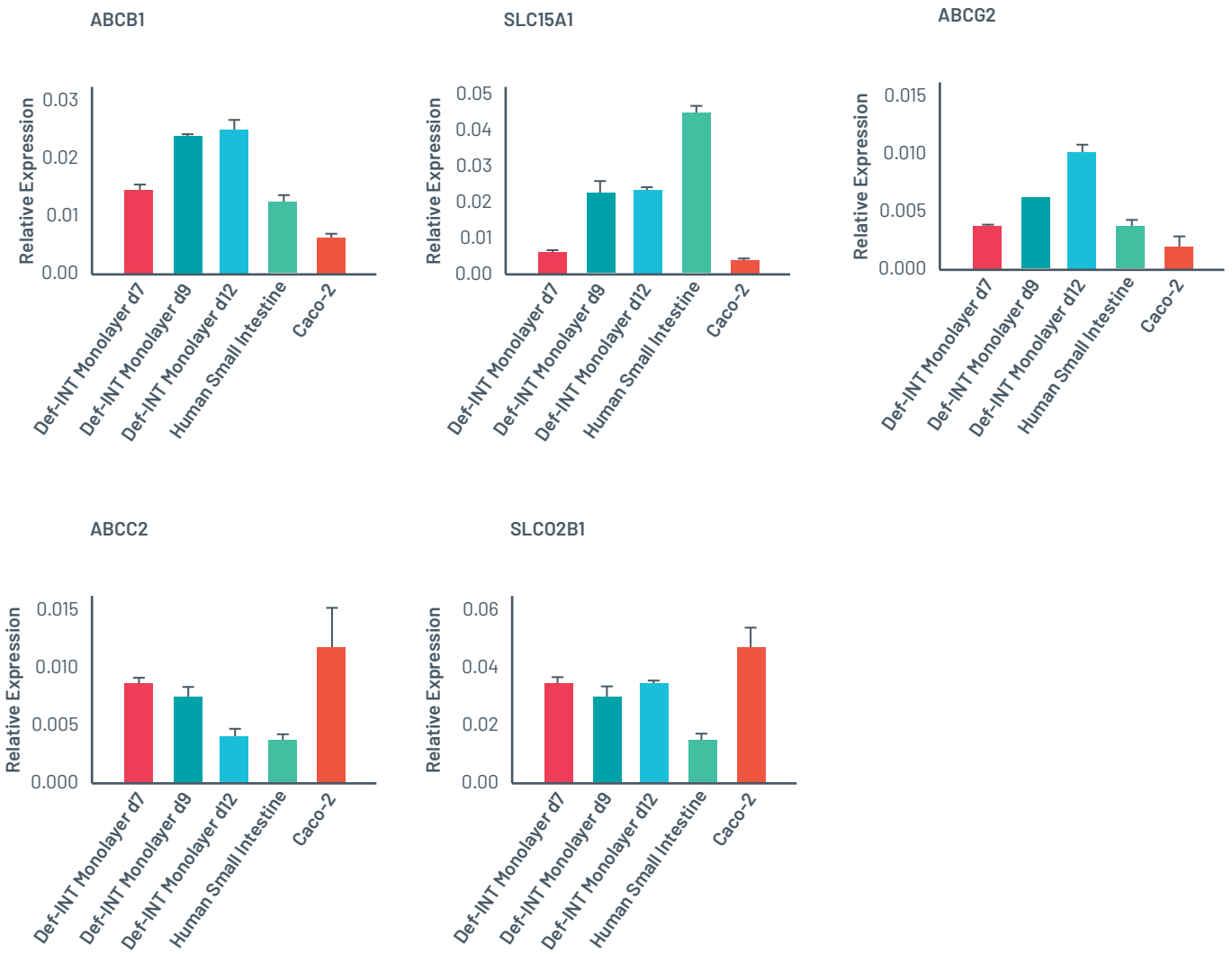

Figure 6. Gene expression of the main transporters involved in drug efflux and uptake.

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Physiologically relevant model

Def-INT cells are derived from multicellular organoids and when cultured in a monolayer format they maintain the expression of several proteins present in the human intestine. Muc2 is the main protein for mucus formation and can have an impact in drug absorption. Other enzymes such as lysozyme or enteroendocrine peptides are also produced by Def-INT cells, making them a model that mimics closely the human physiology of the intestine.

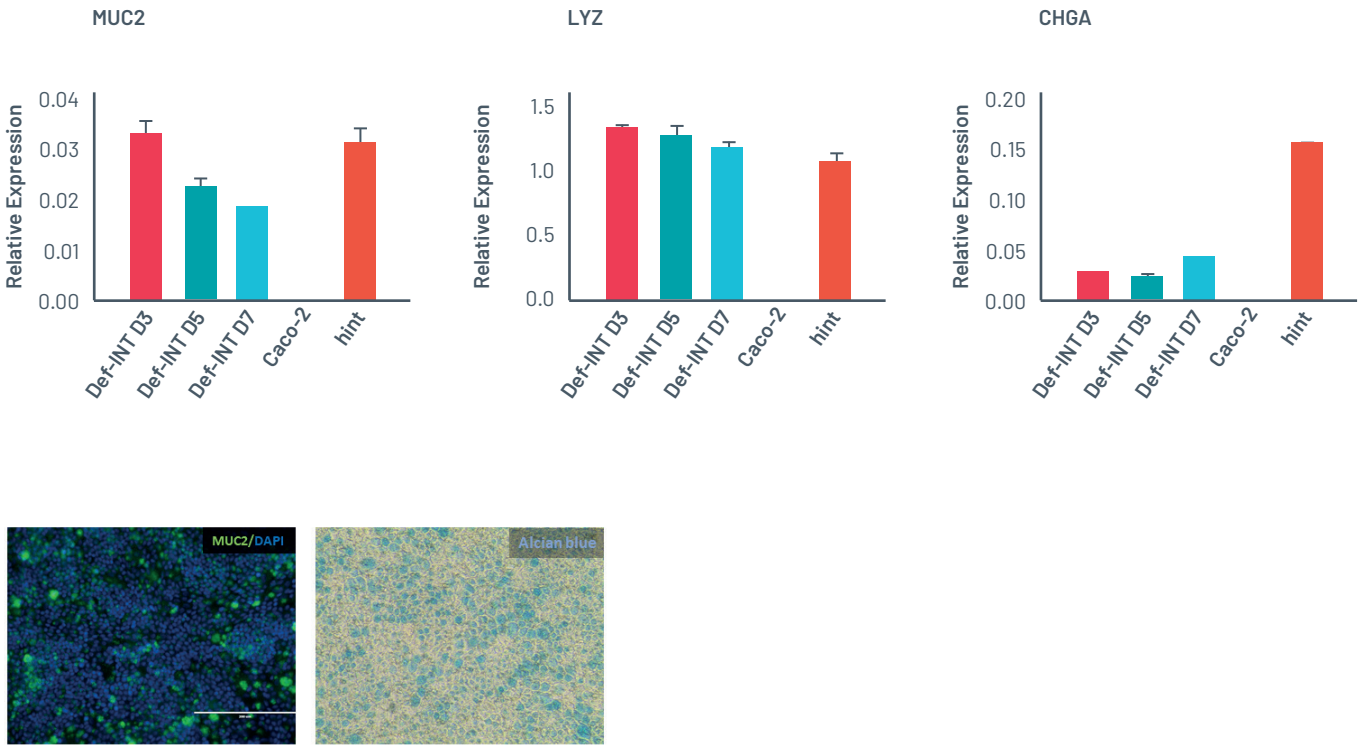

Fig 7. Def-INT monolayer is a physiologically relevant model. A) Gene expression of markers for secretive lineages within the intestine. B) Protein localization of MUC2, the main mucus-forming protein in the intestine. C) Alcian blue staining showing the presence of acid mucins in the Def-INT monolayer.

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