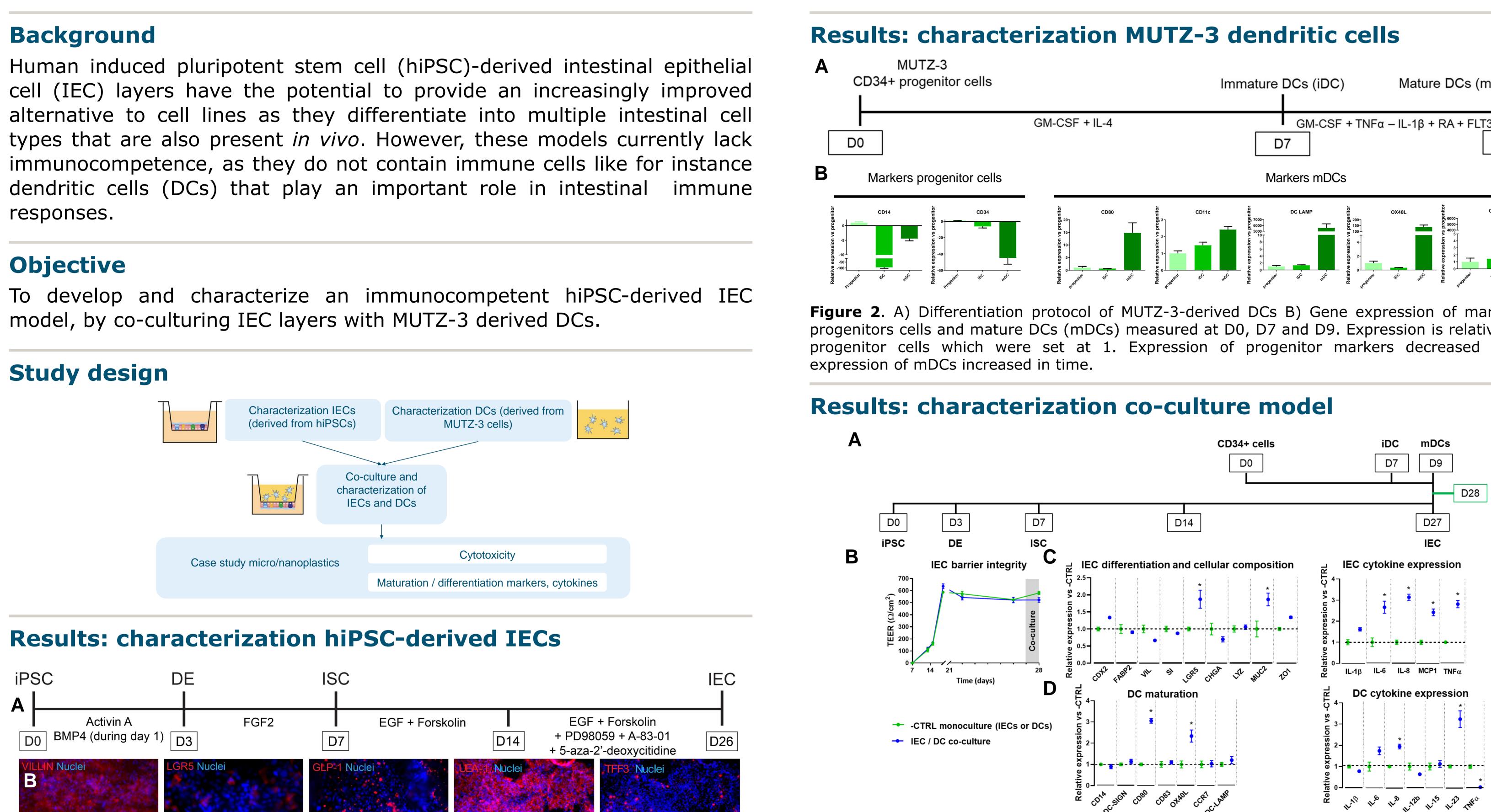


## Development and application of an immunocompetent human induced pluripotent stem cell-derived intestinal epithelial cell model for hazard assessment of the oral exposure route

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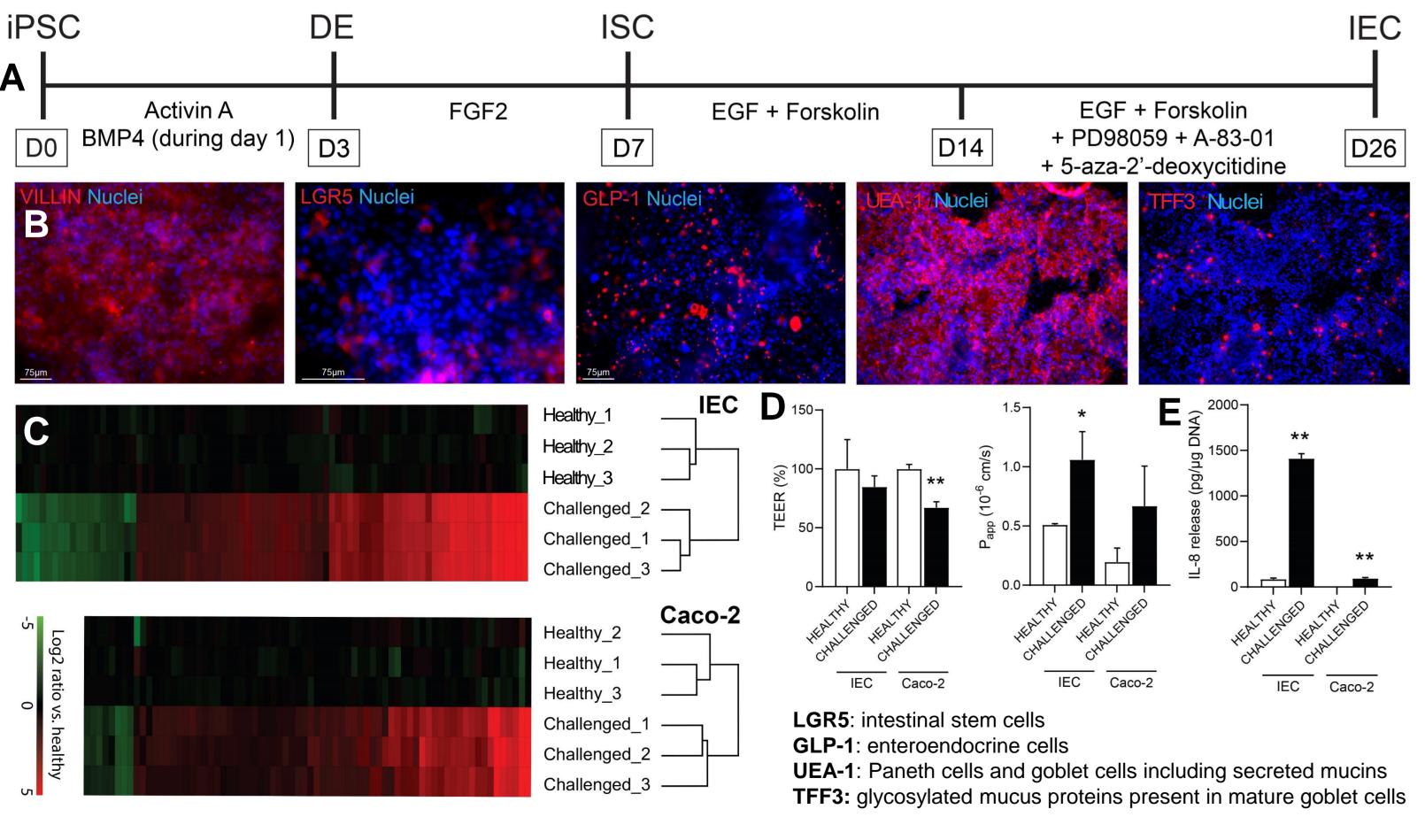




Figure 2. A) Differentiation protocol of MUTZ-3-derived DCs B) Gene expression of markers for progenitors cells and mature DCs (mDCs) measured at D0, D7 and D9. Expression is relative to the progenitor cells which were set at 1. Expression of progenitor markers decreased whereas

Figure 3. A) hiPSCs and MUTZ-3 cells were differentiated into IECs and mDCs separately and cocultured when fully differentiated. After 24h of co-culture the differentiation status of the IECs and DCs and the expression of cytokines was evaluated and compared to their respective monocultures before co-culture. B) Barrier integrity of the IEC layer. C) Gene expression of various markers for differentiation and cellular composition and cytokine expression in the IEC layer. D) Gene expression of maturation markers and cytokines in mDCs. In general, co-culturing has little effect on cellular composition, but increases cytokine expression.

← Figure 1. A) Differentiation protocol hiPSC-derived IECs B) Immunohistochemistry showing the presence of multiple IEC types. Immune responsiveness of IECs after stimulation with a proinflammatory cocktail of INFy, IL1B and TNFa shown as C) expression of genes in the "cytokine" storm pathway", as D) barrier integrity (TEER and permeability of lucifer yellow), and as E) IL-8 excretion (ELISA) in comparison with Caco-2 cells.

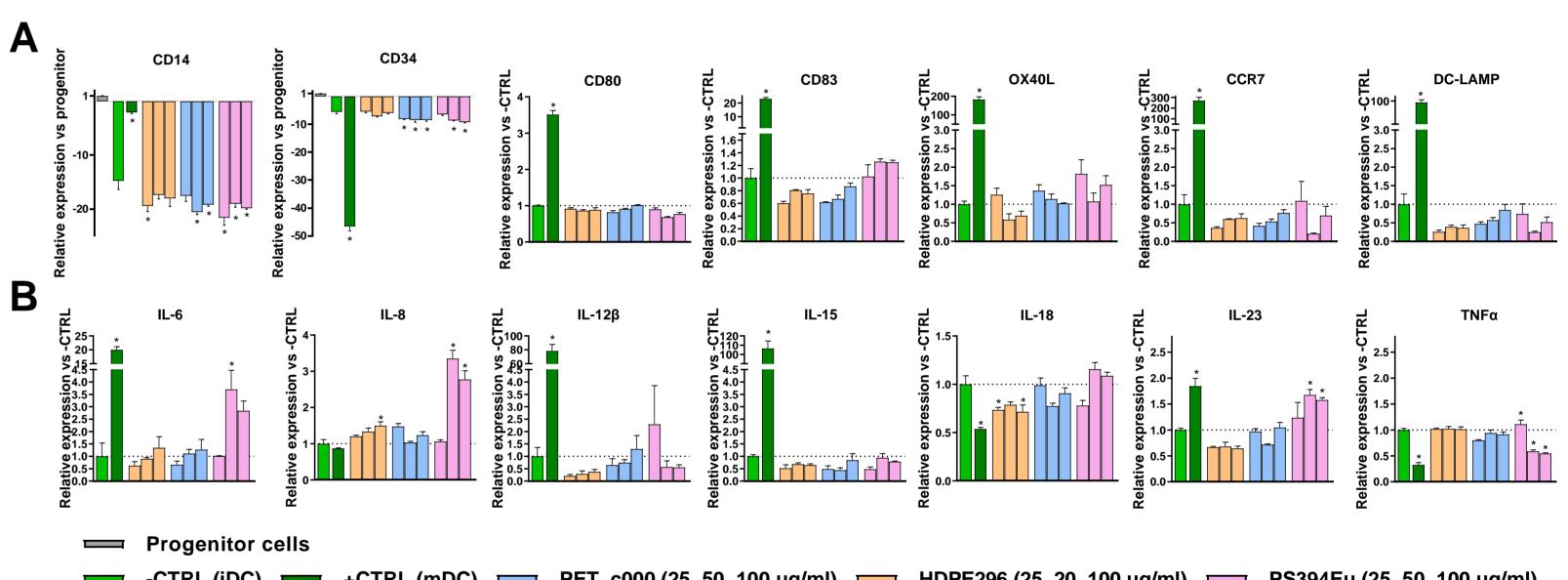


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# Mature DCs (mDC) GM-CSF + TNF $\alpha$ – IL-1 $\beta$ + RA + FLT3L

## **Results: exposure of iDCs to microplastics**

Abbreviation	Main polymer composition	Additives	Particle size distribution	Particle shape
HDPE269	High density polyethylene	No	X <sub>10;3</sub> 1.8 μm / X <sub>50;3</sub> 4.9 μm / X <sub>90;3</sub> 8.7 μm	Round
PET_c000	Polyethylene terephthalate	No	X <sub>10;3</sub> 57 nm / X <sub>50;3</sub> 90 nm / X <sub>90;3</sub> 144 nm	Round
PS394Eu	Europium doped polystyrene	No	300 nm	Round



**Figure 4.** Gene expression in iDCs after 48h exposure to micro/nano plastics at three different concentrations. A) Effects of the micro/nanoplastics on gene expression of markers for maturation of iDCs into mDCs and B) on cytokine expression. No significant effects were observed after 24h exposure to the micro/nanoplastics and no cytotoxicity was observed (data not shown). After 48h exposure to the PS394Eu plastics mild effects on cytokine expression were observed.

### Conclusions

- the intestine *in vivo*
- differentiated
- an inverted IEC culture technique
- expression in both cell models



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**Table 1.** Physicochemical data of the micro/nanoplastics for the cell exposure experiment.

-CTRL (iDC) - +CTRL (mDC) - PET\_c000 (25, 50, 100 µg/ml) + HDPE296 (25, 20, 100 µg/ml) - PS394Eu (25, 50, 100 µg/ml)

• hiPSC-derived IEC layers consist of multiple cell types that are present in

• hiPSC-derived IEC layers have the capacity to induce pro-inflammatory responses when exposed to a pro-inflammatory stimulus

• MUTZ-3-derived DCs express (immature/mature) DC markers when

• Co-culture of separately differentiated IECs and DCs was successful using

• 24h co-culture of IECs and DCs did not appear to negatively affect the differentiation status of the cells and significantly increased cytokine

• 24h and 48h exposure of iDCs to micro/nanoplastics did not result in strong immunomodulatory effects of any of the materials tested

