Title: Optimizing iPSC maturation towards small intestinal epithelial cells

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Abstract
In celiac disease, the small intestinal epithelium is damaged due to an autoimmune response to dietary gluten. Throughout the years animal models and immortalized cell lines have been important tools for gaining information about celiac disease. Currently, due to the poor comparability between humans and the models mentioned above, induced pluripotent stem cells (iPSCs) are used to create physiologically relevant preclinical models for celiac disease. The aim of this study was to optimize our protocol for iPSC maturation towards small intestinal epithelial cells (SIECs) into a more reproducible and effective one. The production of SIECs from iPSCs allows a patient-specific investigation on intestinal epithelial function and pathogenesis of celiac disease.

An in-house differentiation protocol was used as a base and a control for four different methods, which were modified from current iPSC-SIEC differentiation methods from Inui et al (2024) and Moerkens et al (2024). First, the iPSCs were differentiated into definitive enododerm and then to posterior definitive endoderm using the in-house method. After that, we proceeded to SIEC-maturation with four different methods and the control method. The cells were characterized using immunofluorescence. Further cell characterization with RNA-seq analysis is yet to be conducted.

The preliminary results show that the iPSCs are matured towards SIECS most effectively with the protocols modified from Morkens et al. (2024). Although the aim was to create a two-dimensional model, some villus-like structures might have also been formed. Yet, further analyzes and optimization must be conducted in order have an effective and reproducible maturation protocol.