

# Towards high-throughput modelling of inflammatory bowel disease using human intestinal organoids

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## INTRODUCTION

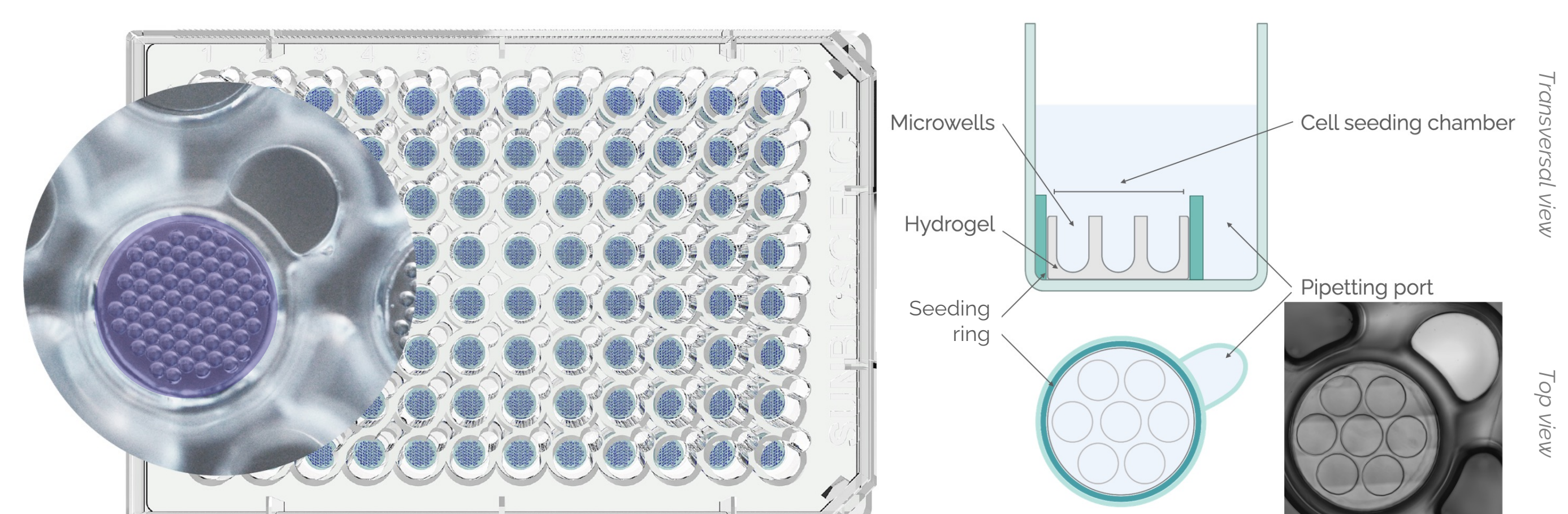
Inflammatory bowel diseases (IBD) are characterized by chronic inflammations of the gastrointestinal tract during which the intestinal mucosal barrier gets damaged. Until today, mice models have been used to unravel the complex interactions involved in IBD, yet they often fail to predict human responses<sup>1</sup>. In recent years, organoids have emerged as a game-changer for disease modelling and drug screening<sup>2</sup>. These organoids are three-dimensional (3D) miniaturized versions of an organ that mimic some of the key features of the native tissue *in vitro*. Traditional organoid culture methods consist of embedding these 3D structures in solidified extracellular matrix (ECM), thus introducing an intrinsic lack of reproducibility and creating highly heterogeneous organoid populations<sup>3</sup>. To overcome these challenges, we use our innovative technology Gri3D<sup>®</sup>, a ready-to-use platform for high-throughput and reproducible 3D cultures<sup>4</sup>. Combined with the ImageXpress<sup>®</sup> Micro Confocal High-Content Imaging System, organoids were monitored at a single-organoid level. We report the induction of an IBD-like phenotype on healthy human rectal organoids using pro-inflammatory cytokines and demonstrate the use of Gri3D<sup>®</sup> as a robust and high-throughput *in vitro* platform for organoid-based disease modelling.

## METHODS

Human rectal organoids are generated in Gri3D<sup>®</sup> g6WP imaging-bottom 500 µm microwells (SUN bioscience) starting from a single cell suspension and cultured for 7 days. At day 3, a pro-inflammatory cocktail composed of TNF-α and IL-1β is added and refreshed at day 5. After 96 hours of exposure, Live/Dead assay is performed and imaged on an ImageXpress<sup>®</sup> Micro Confocal system (Molecular Devices). Results are analysed using a 3D Custom Module Editor on MetaXpress<sup>®</sup>. Cultures are then fixed for immunostaining assay targeting filamentous actin (F-actin, phalloidin) and nuclei (DAPI).



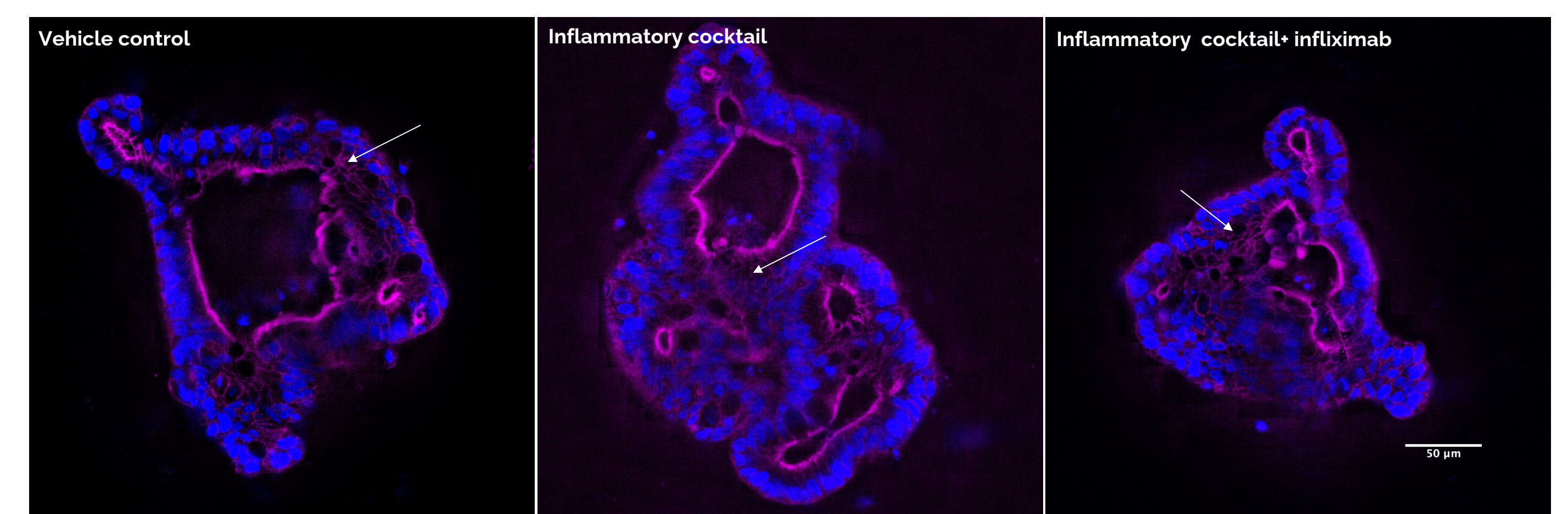
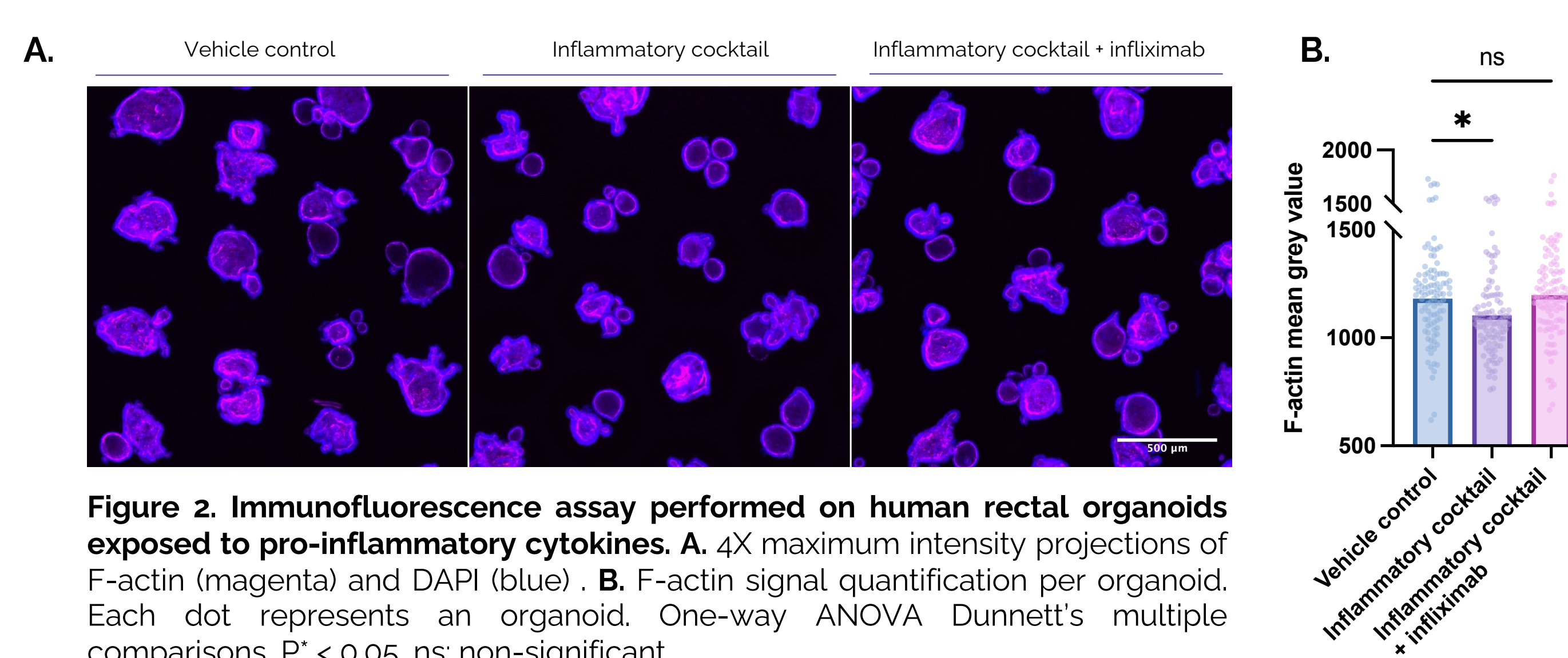
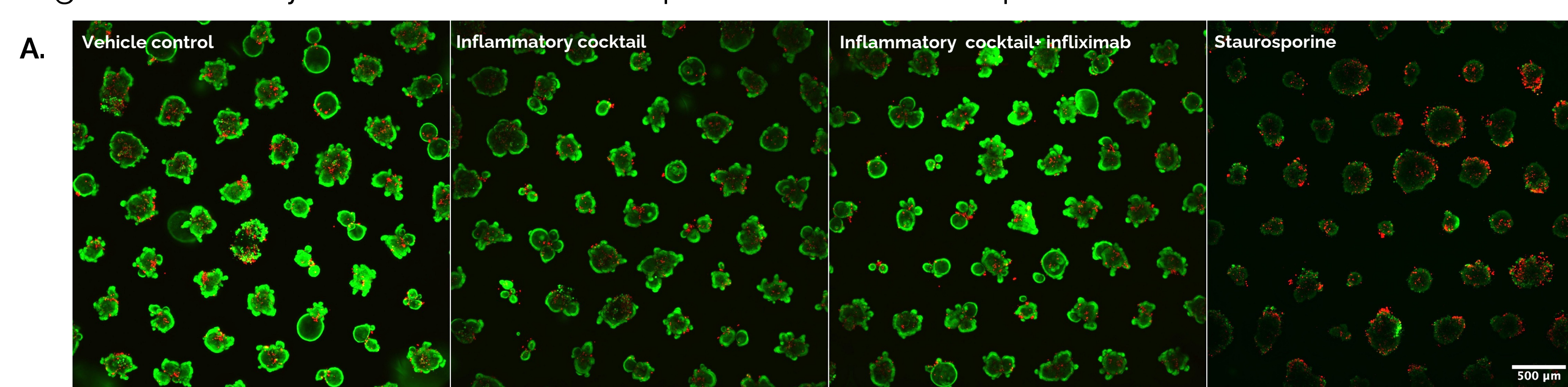
## GRI3D<sup>®</sup> TECHNOLOGY



Gri3D<sup>®</sup> is a ready-to-use platform for high-throughput and reproducible organoid culture. Based on an array of ultra-dense U-bottom microwells in a hydrogel, single organoids are robustly generated in each microcavity and grown in suspension-like culture without a solid ECM.

## RESULTS

Human rectal organoids cultured are robustly generated on Gri3D<sup>®</sup>. At day 3 upon lumen formation, cultures are exposed to a pro-inflammatory cocktail composed of TNF-α and IL-1β, key-player cytokines in IBD. After 96 hours of exposure, Live/Dead assay shows a viability decrease of organoids as seen by lower Calcein AM intensity and higher dead area per organoid (Fig. 1). Anti-inflammatory treatment infliximab reverts the phenotype caused by the cytokines. We then assess the 3D architecture of organoids by immunofluorescence assay (Figs. 2 and 3). F-actin signal decreases upon cytokine exposure, a phenotype that is reversible upon treatment with infliximab (Fig. 2 B). A close up look shows delocalization of F-actin in treated organoids (Fig. 3). We hypothesize that impaired organoid viability could be related to epithelial barrier disruption.



## CONCLUSIONS

- Gri3D<sup>®</sup> is a front-to-end solution for image-based organoid assays enabling robust viability readouts and phenotypic analyses.
- We show that hallmarks of IBD disease can be recapitulated using human rectal organoids and analysed in a standard and high-throughput manner.
- The combination of Gri3D<sup>®</sup> technology and a high content imaging system provides new insights on disease development.
- Our innovative approach is a powerful tool that has high potential in solving key challenges related to 3D cultures and disease modelling at large scale using patient-derived samples.

## REFERENCES

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