Organoids as translational tools: Patient-matched transcriptomics reveals similarities between *in vitro* and *in vivo* epithelial responses in ulcerative colitis

Gunnar Andreas Enerhaug Walaas[#,1], Arun Sridhar[#,1,2], Lusie Frostvoll Kuraas[1,2], Siri Sæterstad[1], Atle Van Beelen Granlund[1,2,4], Arne Kristian Sandvik[1,2], Ann Elisabet Østvik[1,2], Ingunn Bakke[$,1,3], & Torunn Bruland[$,1,2]

[1]Department of Clinical and Molecular Medicine (IKOM), NTNU - Norwegian University of Science and Technology, Trondheim, Norway; [2]Department of Gastroenterology and Hepatology, Clinic of Medicine, St. Olav's University Hospital, Trondheim, Norway; [3]Clinic of Laboratory Medicine, St. Olav's University Hospital, Trondheim, Norway; [4]Department of Pathology, St. Olav's University Hospital, Trondheim, Norway

[#]Shared first authorship; [$]Shared senior authorship

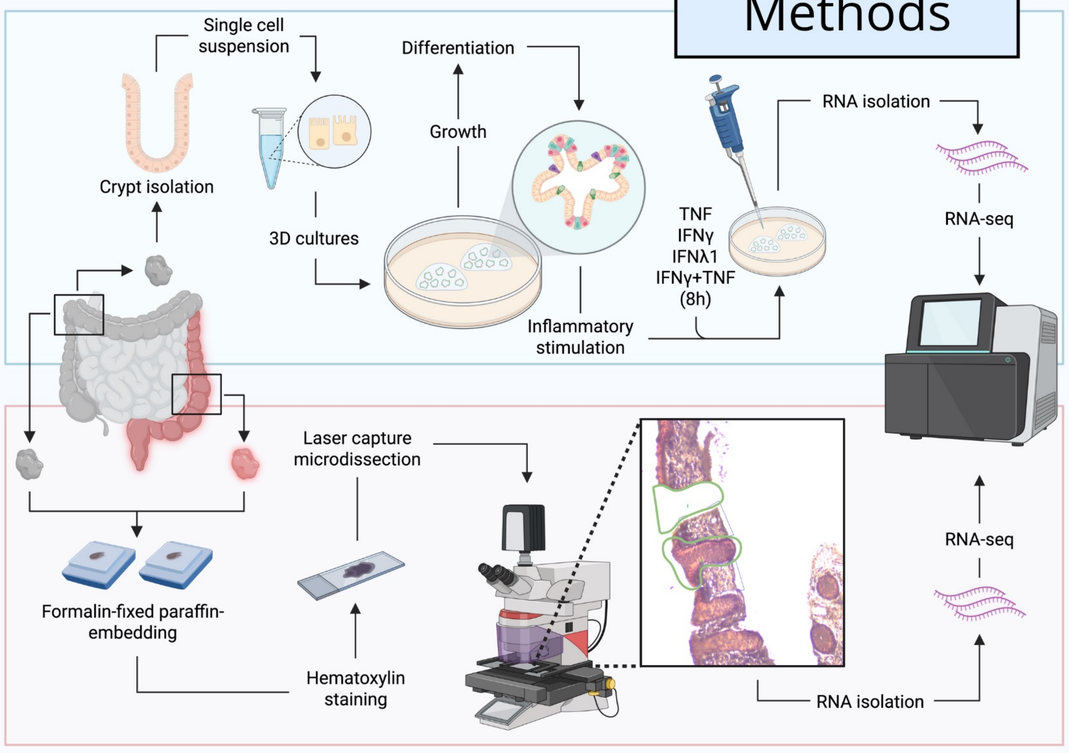
Background

Ulcerative colitis (UC) is a chronic inflammation in the colon. Effective mucosal healing in UC relies on restoring epithelial integrity, making it crucial to understand the mechanisms underlying epithelial dysfunction. Colonic epithelial organoids, or colonoids, offer a promising *in vitro* model for studying UC, as they closely replicate the architecture and function of the intestinal epithelium while preserving the donor's genetic background. These models enable controlled investigation of epithelial responses to inflammatory stimuli. However, the extent to which colonoids mirror the complex *in vivo* inflammatory environment of UC remains insufficiently understood, highlighting the need for further research to validate their translational relevance.

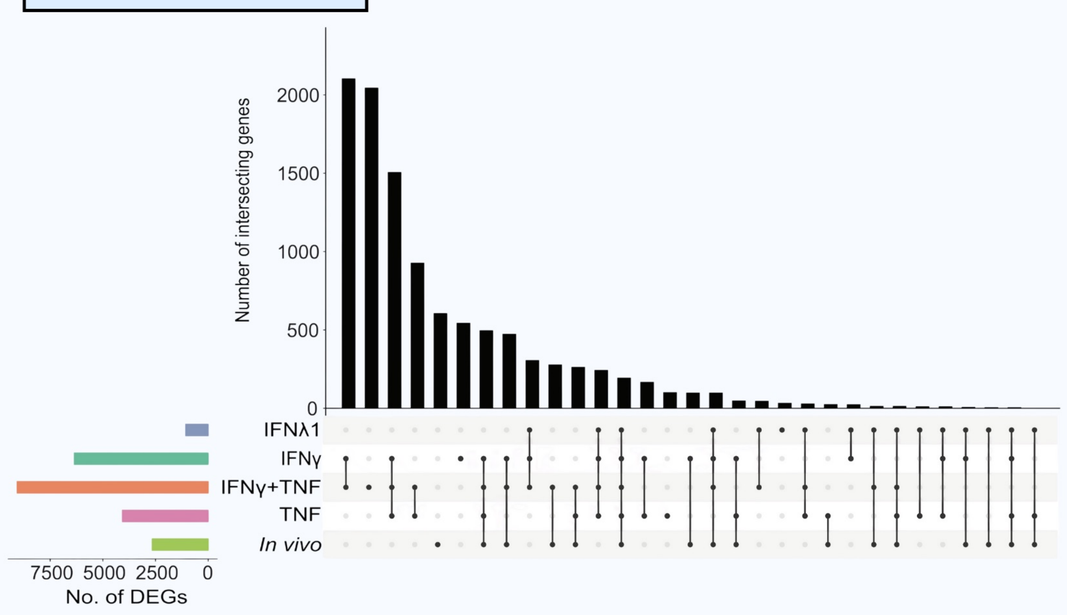
Aims

- Investigate how cytokine-stimulated IEOs reflect epithelial inflammation observed *in vivo* in UC patients.
- Identify cytokine combinations that best recapitulate patient-specific transcriptional inflammatory signatures.
- Explore the potential of patient-matched IEOs as a tool for advancing precision medicine in UC.

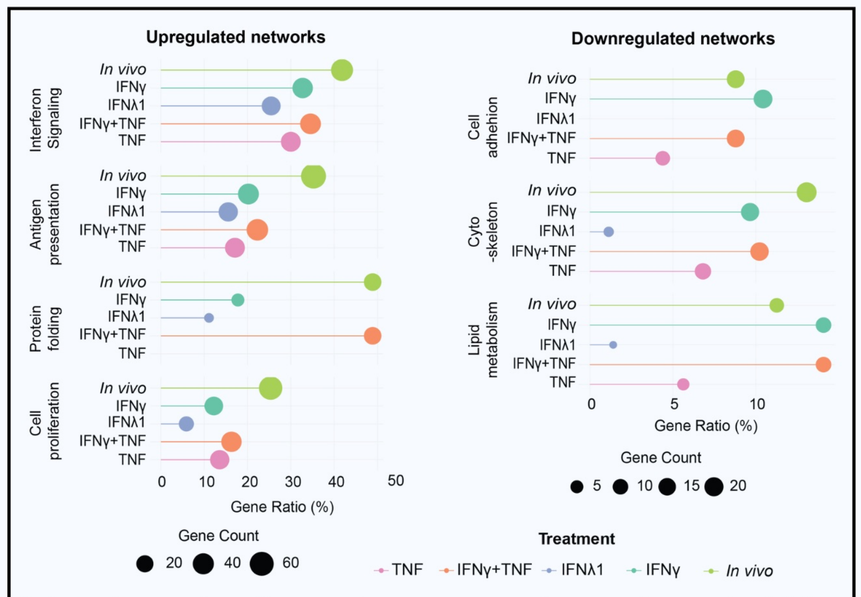
Methods



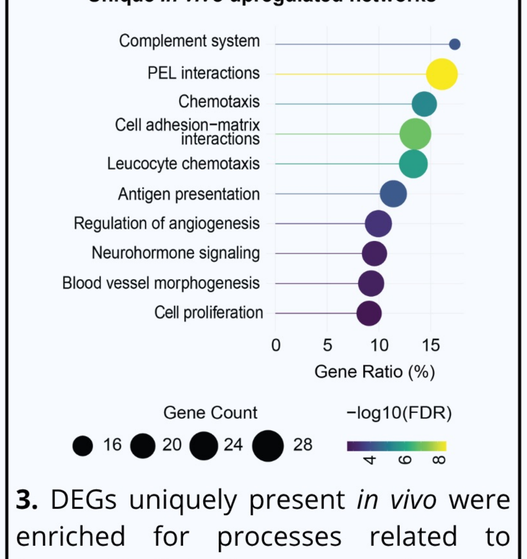
Results



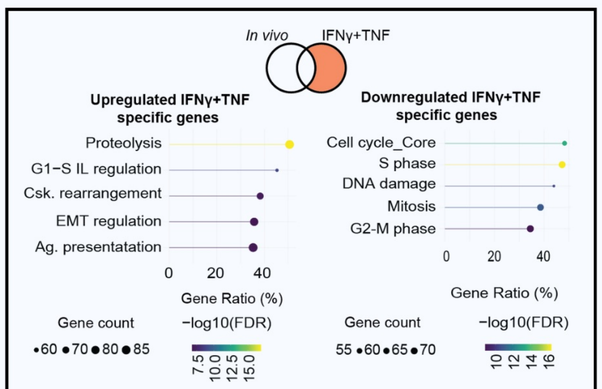
1. The UpSet plot illustrates that IFNγ+TNF stimulation triggers the strongest transcriptional response, with IFNγ and IFNγ+TNF combination sharing the largest set of DEGs, and IFNγ+TNF combination alone contributing the second largest set.



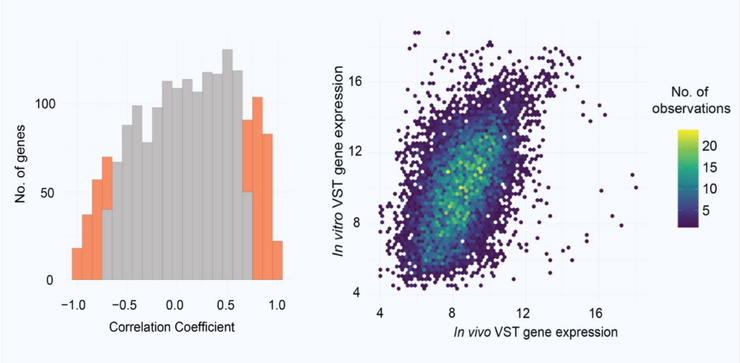
2. Interferon signaling, antigen presentation, and protein folding were consistently upregulated across all *in vitro* conditions. Downregulation of cell adhesion, cytoskeleton organization, and lipid metabolism was observed both *in vivo* and *in vitro*, with IFNγ being the main driver *in vitro*.



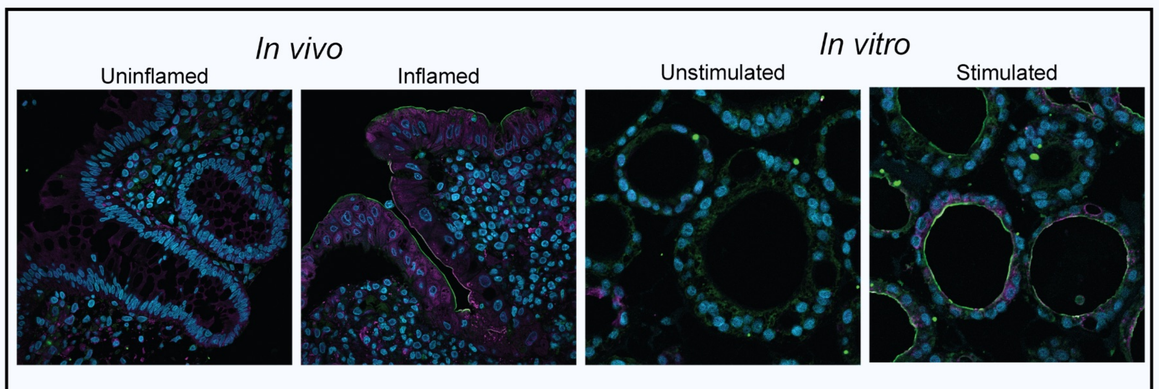
3. DEGs uniquely present *in vivo* were enriched for processes related to intraepithelial immune cells and mesenchymal cells, such as platelet-endothelium-leucocytes interaction, leucocyte chemotaxis, and cell matrix interactions.



4. Genes upregulated by IFNγ + TNF uniquely were enriched in proteolysis, G1-S transition, cytoskeleton remodeling, ECM regulation, and antigen presentation. Downregulated pathways included S phase, mitosis, DNA repair, and core cell cycle functions.



5. Correlation analysis between *in vitro* IFNγ+TNF stimulation and *in vivo* inflamed epithelium revealed 250 strongly positive correlating genes (r > 0.7) and 142 strongly negative correlating genes (r < -0.7). The relationship between *in vivo* and *in vitro* expression appeared linear.



6. DUOX2 and iNOS exhibit consistent expression patterns in both *in vivo* and *in vitro* models, indicating that IEOs reliably mimic patient-specific inflammatory responses at the protein level. This supports their potential as a platform for precision medicine.

Conclusions

1. Cytokine-treated IEOs acquired from uninflamed colonic areas successfully replicate active UC epithelial inflammation
2. The combination of IFNγ + TNF induces inflammatory signatures most similar to *in vivo* conditions
3. Transcriptional patterns observed *in vitro* are reflected at the protein level, confirming translational relevance

CAG-IBD

CAG-IBD Precision medicine in Inflammatory bowel disease

NTNU | Department of Clinical and Molecular Medicine

ST. OLAVS HOSPITAL TRONDHEIM UNIVERSITY HOSPITAL

Acknowledgements:
We thank Linn-Karina M. Selvik for help with RNA isolation. The RNA-seq method and bioinformatics analyses were carried out in collaboration with the Genomics Core Facility (GCF) at the Norwegian University of Science and Technology (NTNU).

The Research Council of Norway

Conflict of Interest:
The authors declare no conflict of interest.