Mechanistic Insights into Anti-TNF Response in Ulcerative Colitis

Gunnar Andreas Walaas[1#], Lusie Frostvoll Kuraas[1], Arun Sridahr[1], Siri Sæterstad[1], Ann-Therese Chattergoon Ali[1], Arne Kristian Sandvik[1], Ann Elisabeth Østvik[1,3], Torunn Bruland[1,3], Ingunn Bakke[1,2]
[1]Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU), Trondheim 7491, Norway; [2]Clinic of Laboratory Medicine, St. Olav's University Hospital, Trondheim, Norway; [3]Department of Gastroenterology and Hepatology, Clinic of Medicine, St. Olav's University Hospital, Trondheim, Norway;
[#]presenting author

INTRODUCTION

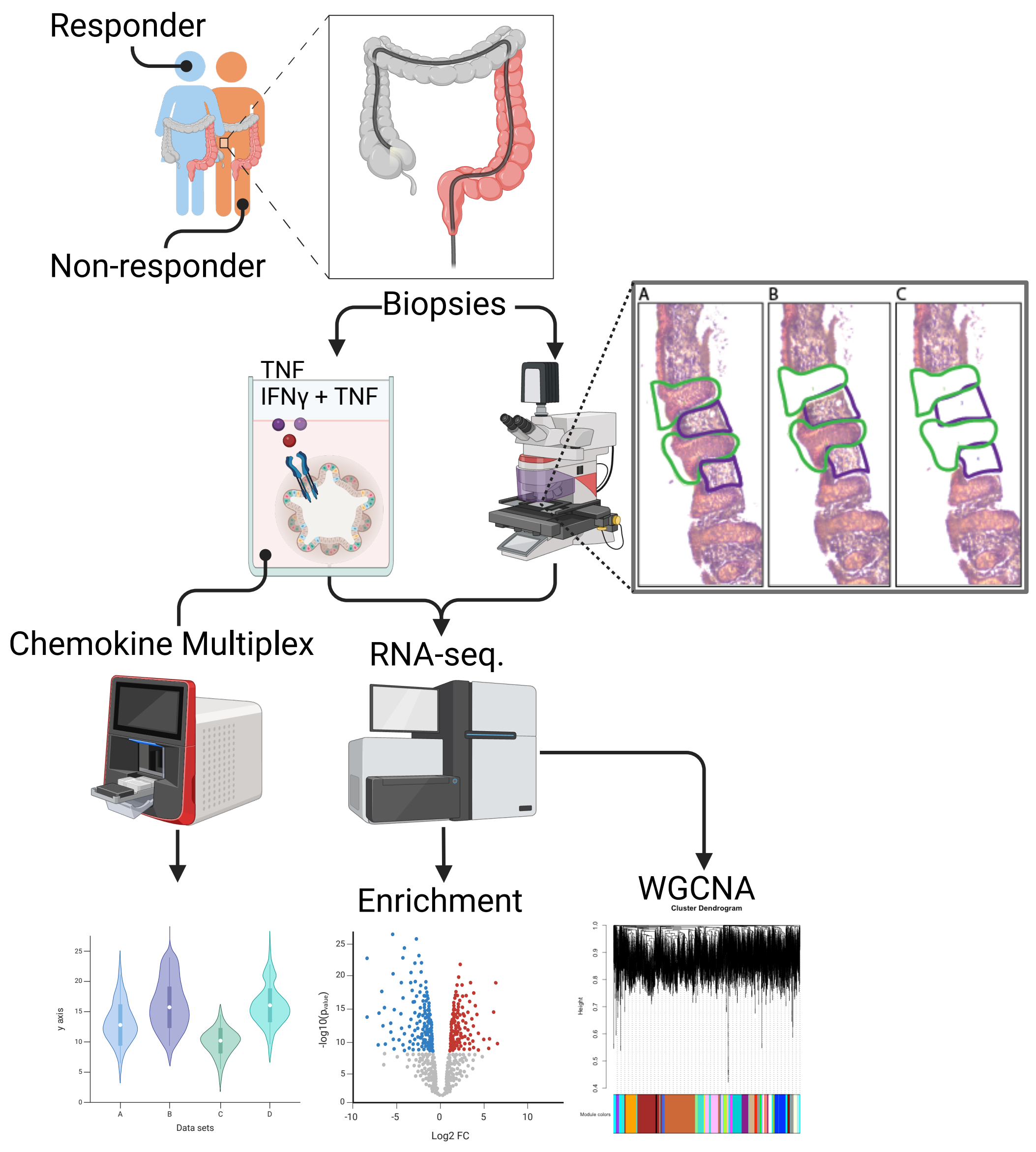
Ulcerative colitis (UC) is a chronic inflammatory bowel disease predominantly affecting adolescents and young adults. Despite available treatments, many patients experience prolonged symptoms due to the challenge of predicting drug efficacy. Anti-tumor necrosis factor (anti-TNF) therapy is the primary treatment for moderate to severe cases. However, 20-40% of patients do not respond to this intervention.

The molecular mechanisms underlying anti-TNF efficacy remain incompletely understood, despite its widespread use. The drug has been shown to modulate lamina propria's macrophage composition. Yet this observation is only able explain a fraction of non-response. The epithelium, previously overlooked, is recognised as a central component to the mucosal immune response which is why spatial information is key.

This investigation aims to:

1. Characterize inflammatory phenotypes in the epithelium and lamina propria associated with treatment response.
2. Study epithelial pro-inflammatory mechanisms in responders (R) and non-responders (NR) derived colonoids

METHODS



Biopsies were collected from inflamed and uninflamed colonic regions of UC patients prior to anti-TNF therapy.

Epithelium and lamina propria were isolated from FFPE sections using laser capture microdissection and analyzed by total RNA sequencing.

Patient-derived colonoids, from the same patients, were established and treated with pro-inflammatory cytokines. Conditioned media from these colonoids were analyzed for chemokines, and the colonoids themselves were sequenced.

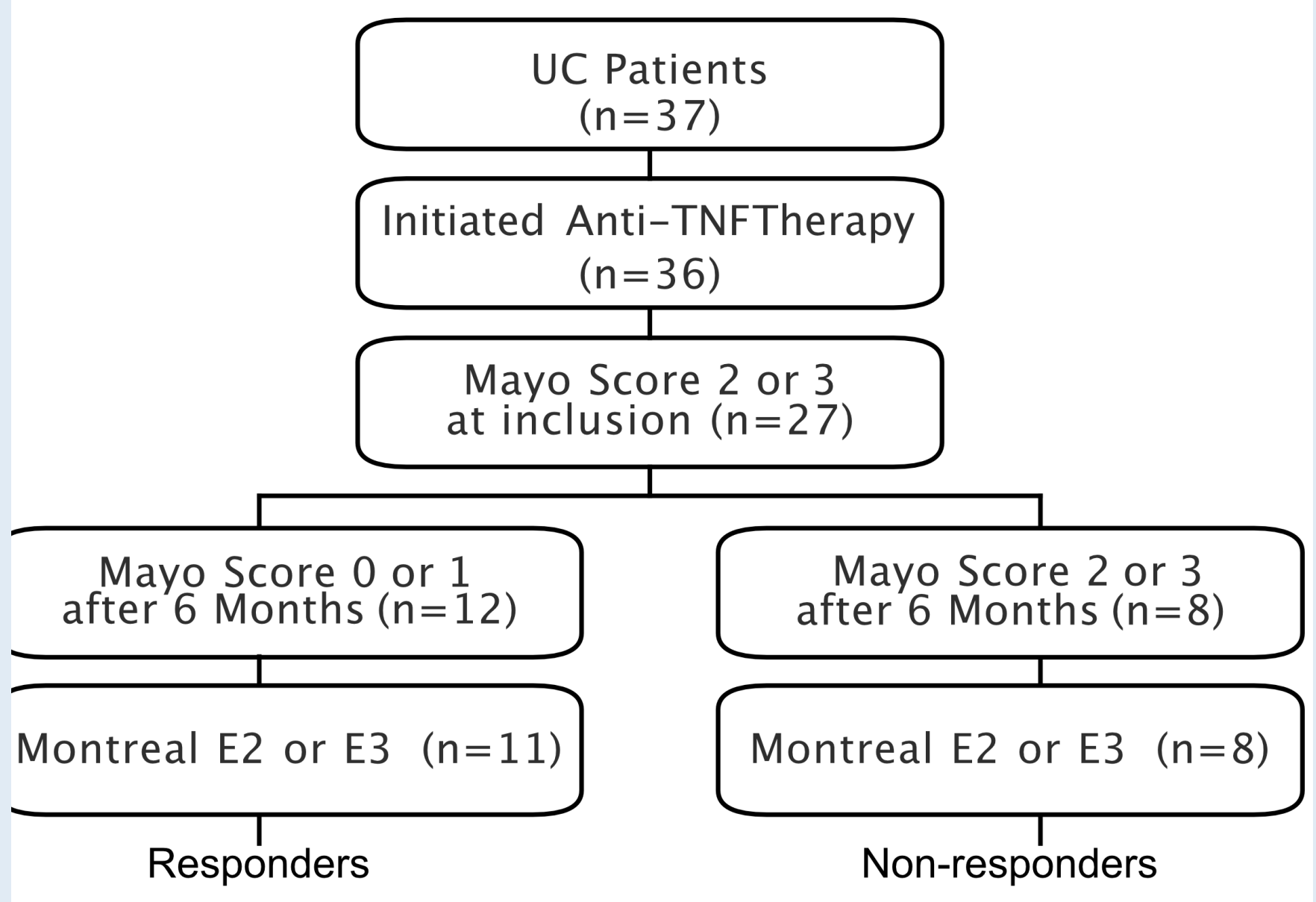
CONCLUSION

Inflammation dominated the epithelial and lamina propria derived expression profiles. However, non-inflamed lamina propria samples displays divergence between R and NR with indications of a pre-inflammatory state in NR. These are preliminary results which will need to be confirmed on a protein level.

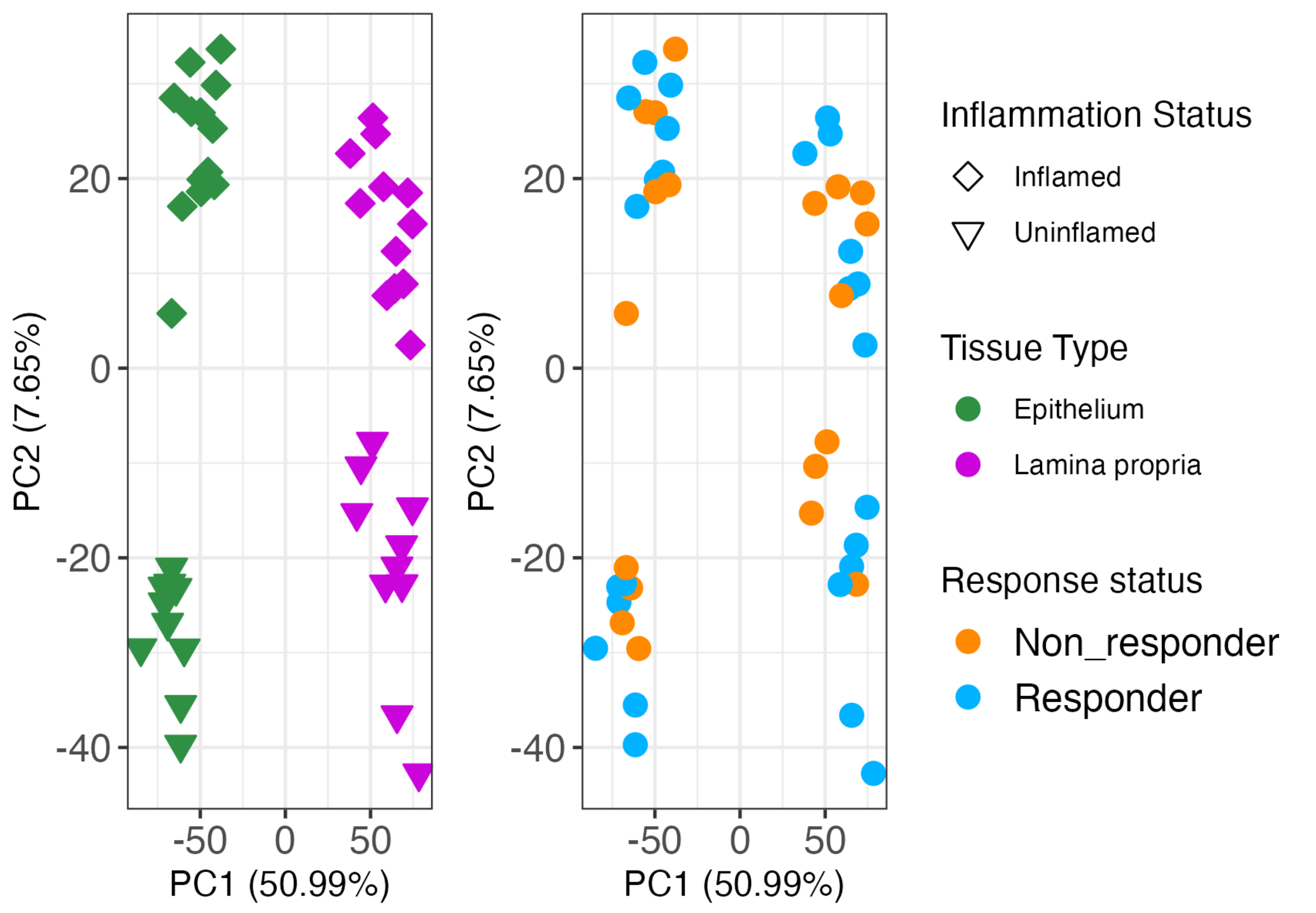
Colonoids respond to pro-inflammatory treatment, but a difference between responders and non-responders could only be detected after IFNg+TNF treatment with Z-transformed values.

Acknowledgements
This work was performed in collaboration with the Gastrointestinal Endoscopy Unit at the Department of Gastroenterology and Hepatology, St. Olav's University Hospital. The RNA-seq and bioinformatics analyses were carried out in collaboration with the Genomics Core Facility (GCF) at the Norwegian University of Science and Technology (NTNU). We thank Arnar Flatberg for valuable support with bioinformatics and data analysis and Liv Ryan for performing the multiplex analysis.

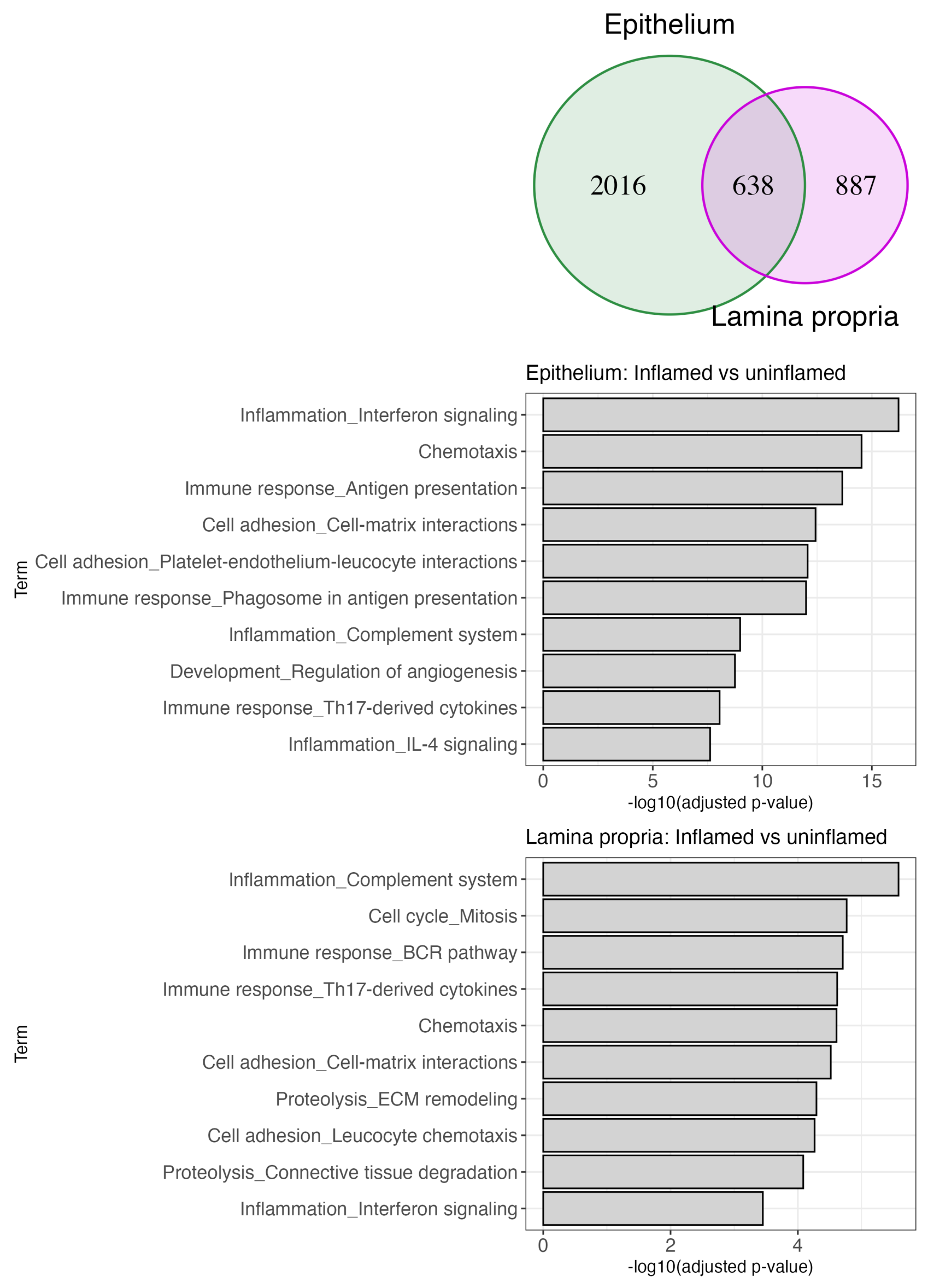
RESULTS



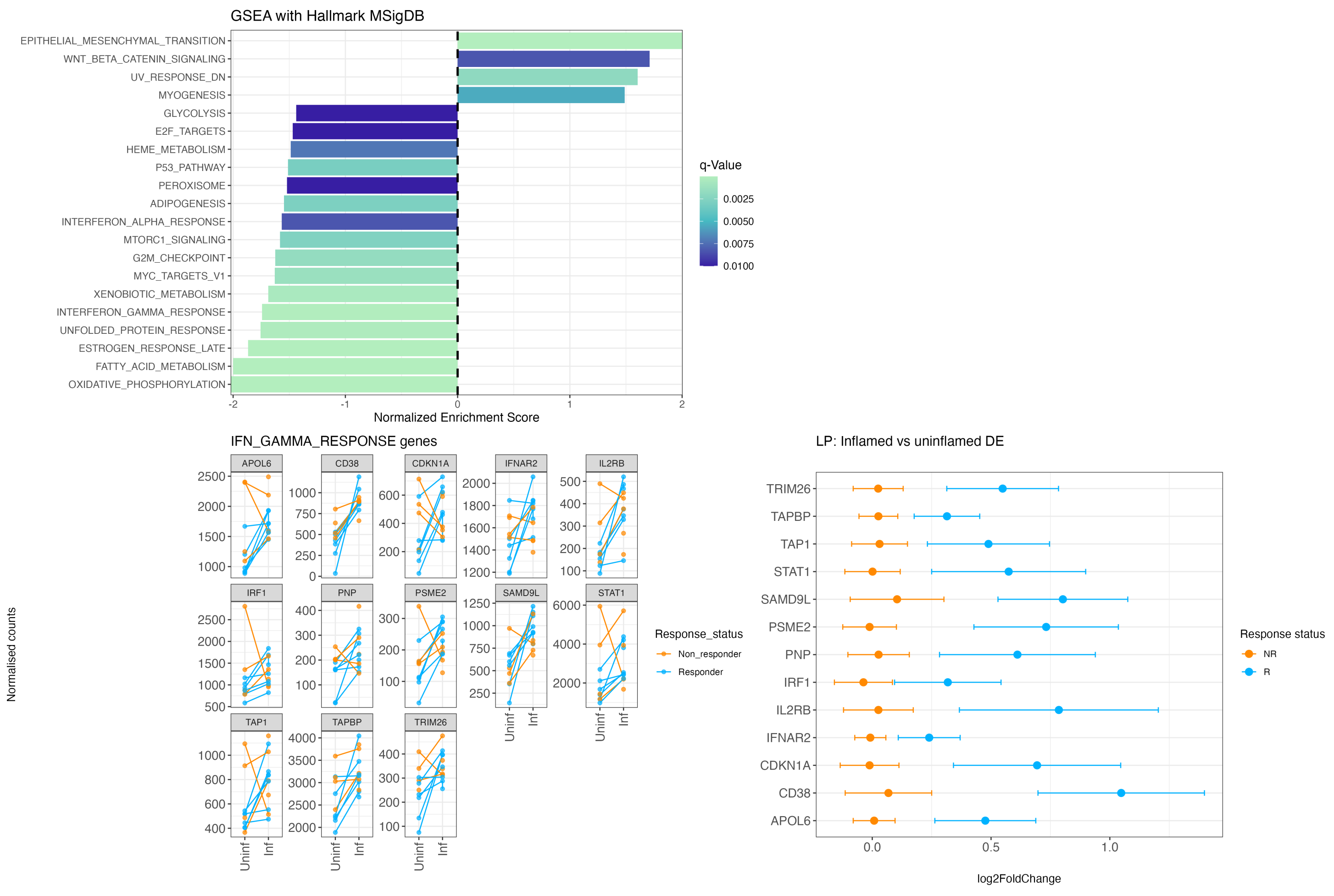
1) The included UC patients had a baseline endoscopic mayo score of 2 or 3. Response was defined based on the endoscopic Mayo after 6 months. Responders had a score of 0/1 and non-responders a score of 2/3. Ulcerative proctitis (E1) patients were excluded.



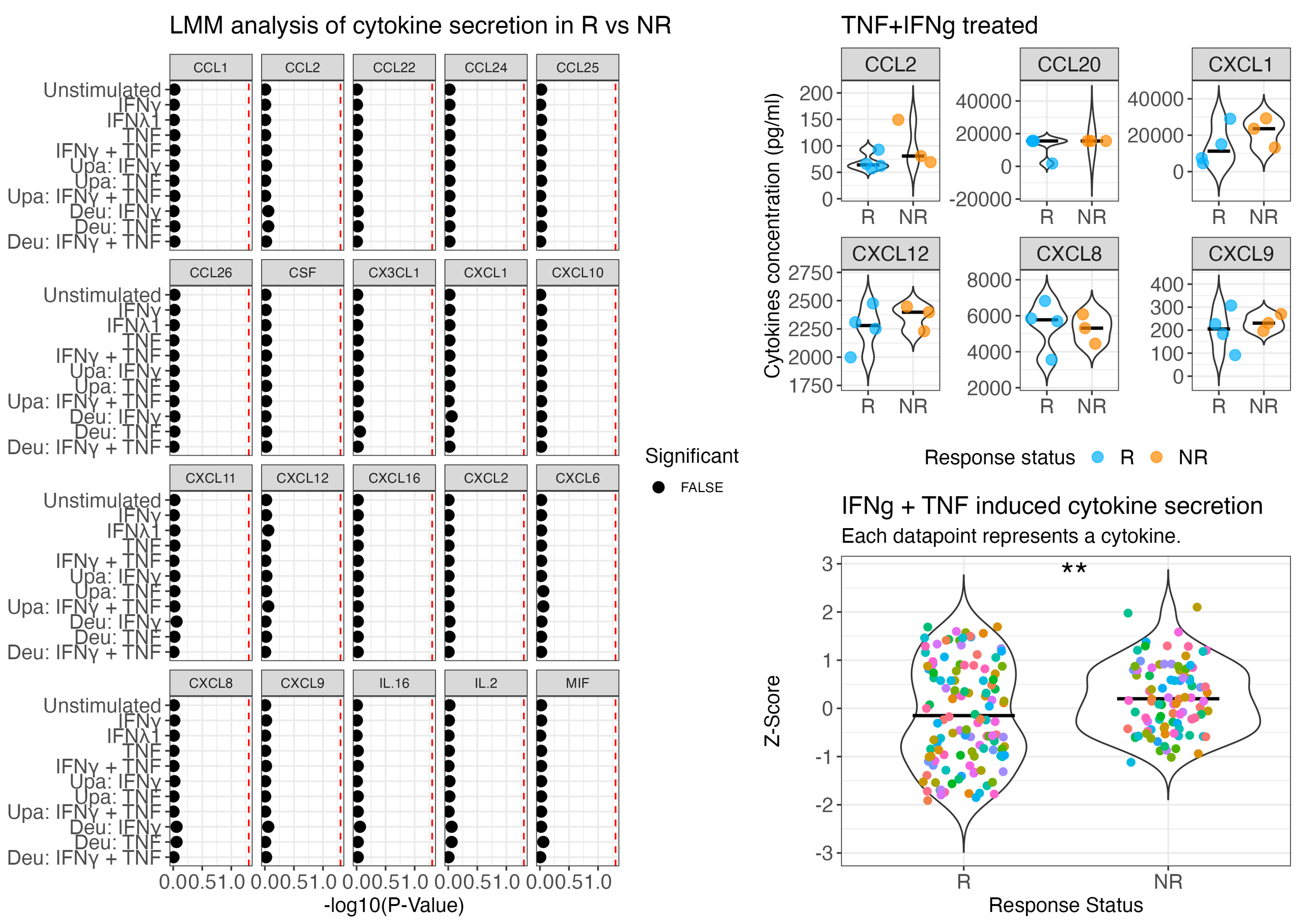
2) Principal component analysis plot of microdissected patients samples. Tissue type and inflammatory status were the dominating characteristics. The difference in responders and non-responders were most evident in uninflamed lamina propria samples.



3) Inflamed vs uninflamed samples in the epithelium and lamina propria regardless of response status. Venn diagram showing the amount of unique and common inflammatory genes. Bar plots highlighting the inflammatory networks. (Threshold: log2 fold change > 0.3, padj < 0.05.)



4) Gene set enrichment analyses (GSEA) with a log2 fold change * -log10(padj) ranked gene list. Q-value represents the false dicovery rate. Responders vs Non-responders in uninflamed lamina propria samples. Several interesting pathways are upregulated in non-responders, including IFNγ- and IFNα response. Several IFNg related genes have a higher uninflamed expression in non-responders. The expression difference between uninflamed and inflamed samples is increased in responders for these genes.



5) Pro-inflammatory treatment assay of colonoids from responders and non-responders.

Linear mixed model (LMM) analysis of cytokine concentrations in the conditioned media after pro-inflammatory treatment. The analysis did not show any difference between responders and non-responders.

Cytokines with known regulation in the epithelium. The cytokine concentrations overlap between responders and non-responder, but with a higher mean concentration in non-responders for some cytokines.

Z transformed cytokines. Z transformation enables comparisons of values with different magnitudes like cytokine concentrations. The overall cytokine level is higher in non-responders compared to responders after IFNg + TNF treatment.