

P078: Physiological hypoxia improves growth and functional differentiation of human intestinal epithelial organoids

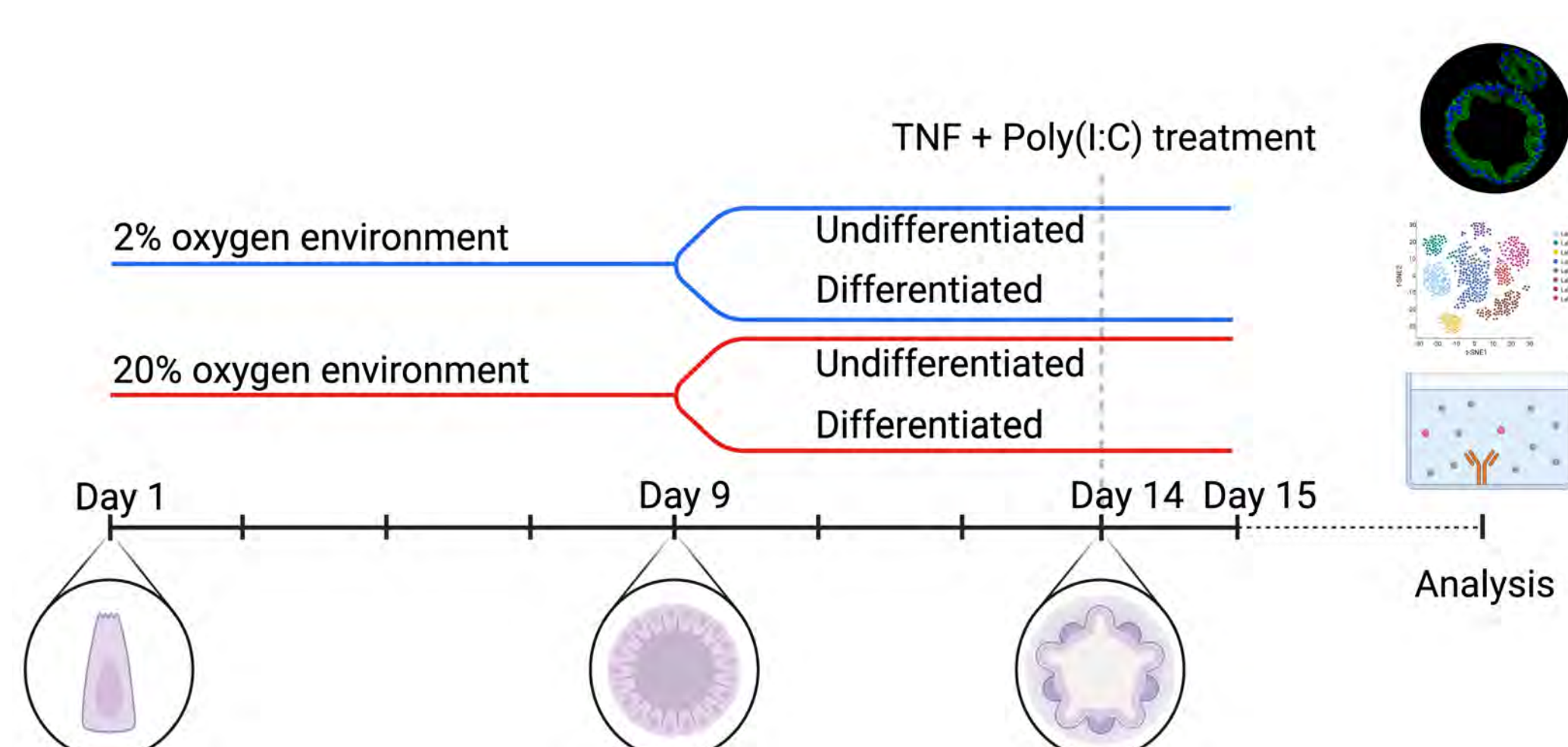
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INTRODUCTION

Intestinal epithelial organoids from the colon (colonoids) can be used for disease modeling and personalized drug screening¹. Colonoids are usually cultured at 18-21% O₂ without accounting for the physiological hypoxia (physioxia) in the colonic epithelium (3% - <1%)². We hypothesized that recapitulating the *in vivo* O₂-environment will enhance the translational value of colonoids as pre-clinical models.

METHODS



- Colonic stem cells were cultured in parallel in 2% and 20% O₂ into colonoids.
- Growth was monitored by brightfield images and evaluated with a linear mixed model. Cell composition was identified by fluorescent staining of cell markers and single-cell RNA-sequencing (scRNA-seq.)
- Enrichment analysis was used to identify transcriptomic differences within cell populations.
- Pro-inflammatory treatment-induced chemokines and NGAL release were analyzed by Multiplex profiling and ELISA.
- Direct response to a lower oxygen level was analyzed by enrichment analysis of bulk RNA sequencing data.

CONCLUSION

Our results suggest that colonoid studies can and should be performed in physioxia when the resemblance to *in vivo* conditions is important.

This work is recently published in *Frontiers in Immunology*³.

References:

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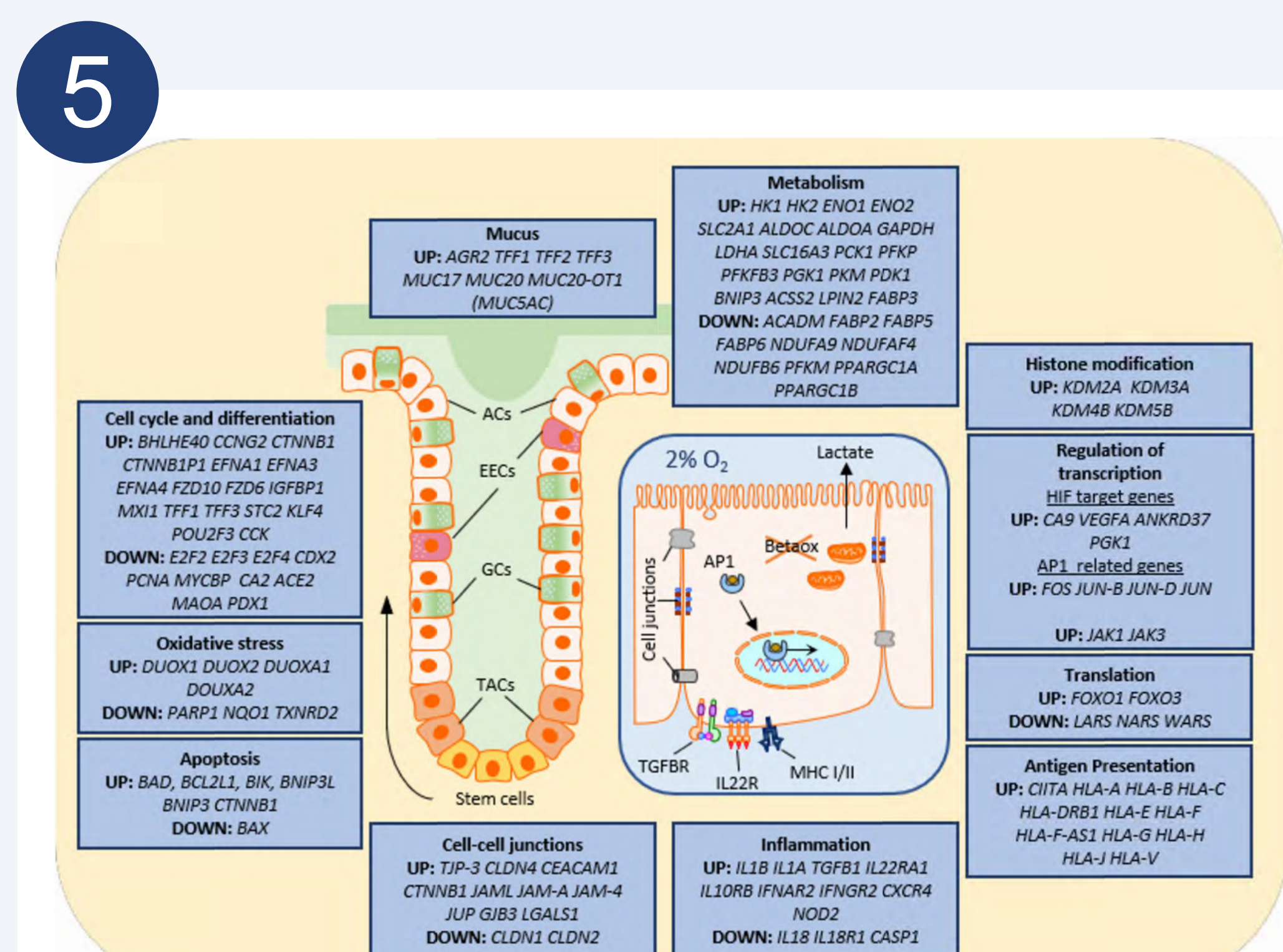
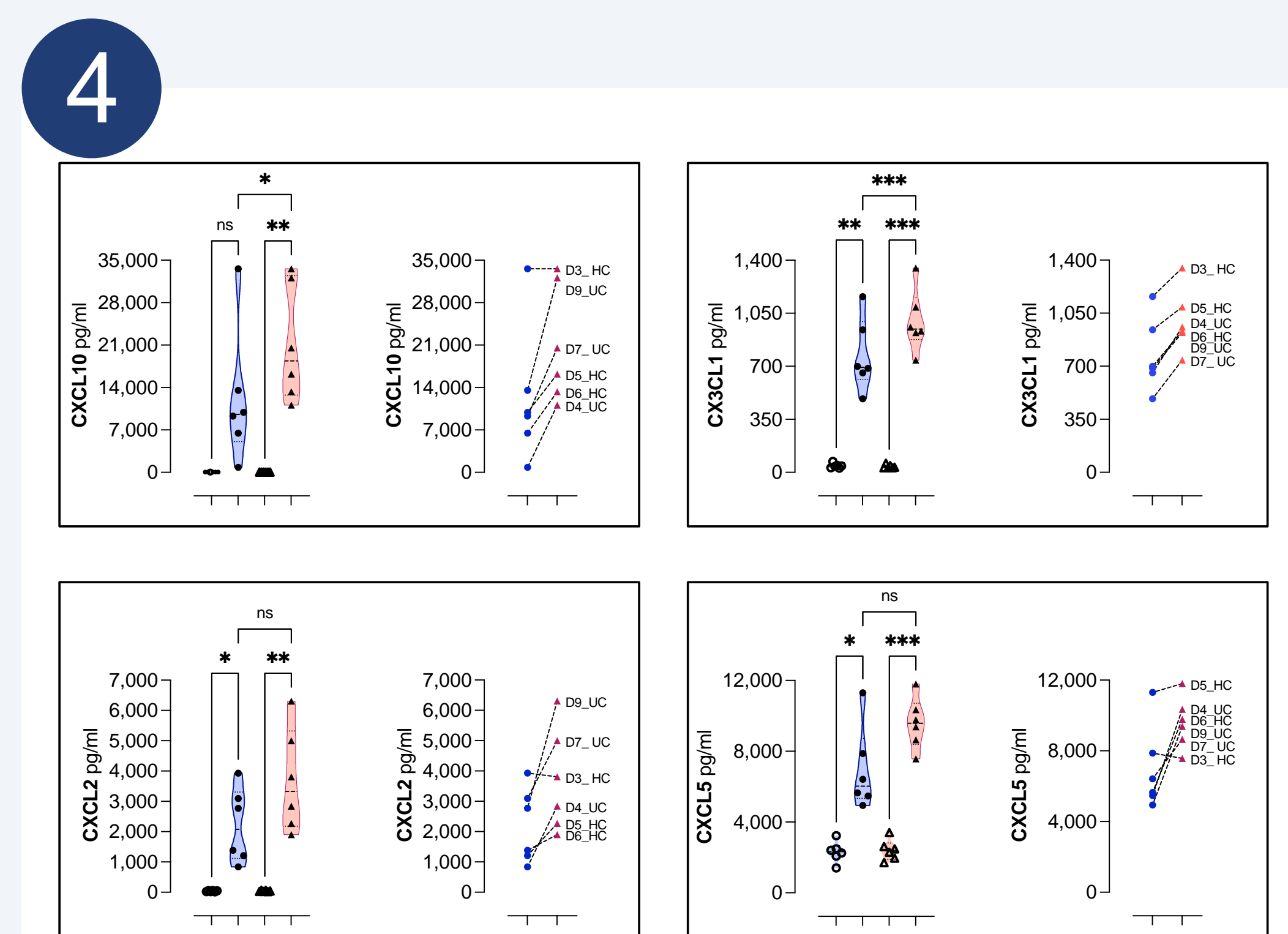
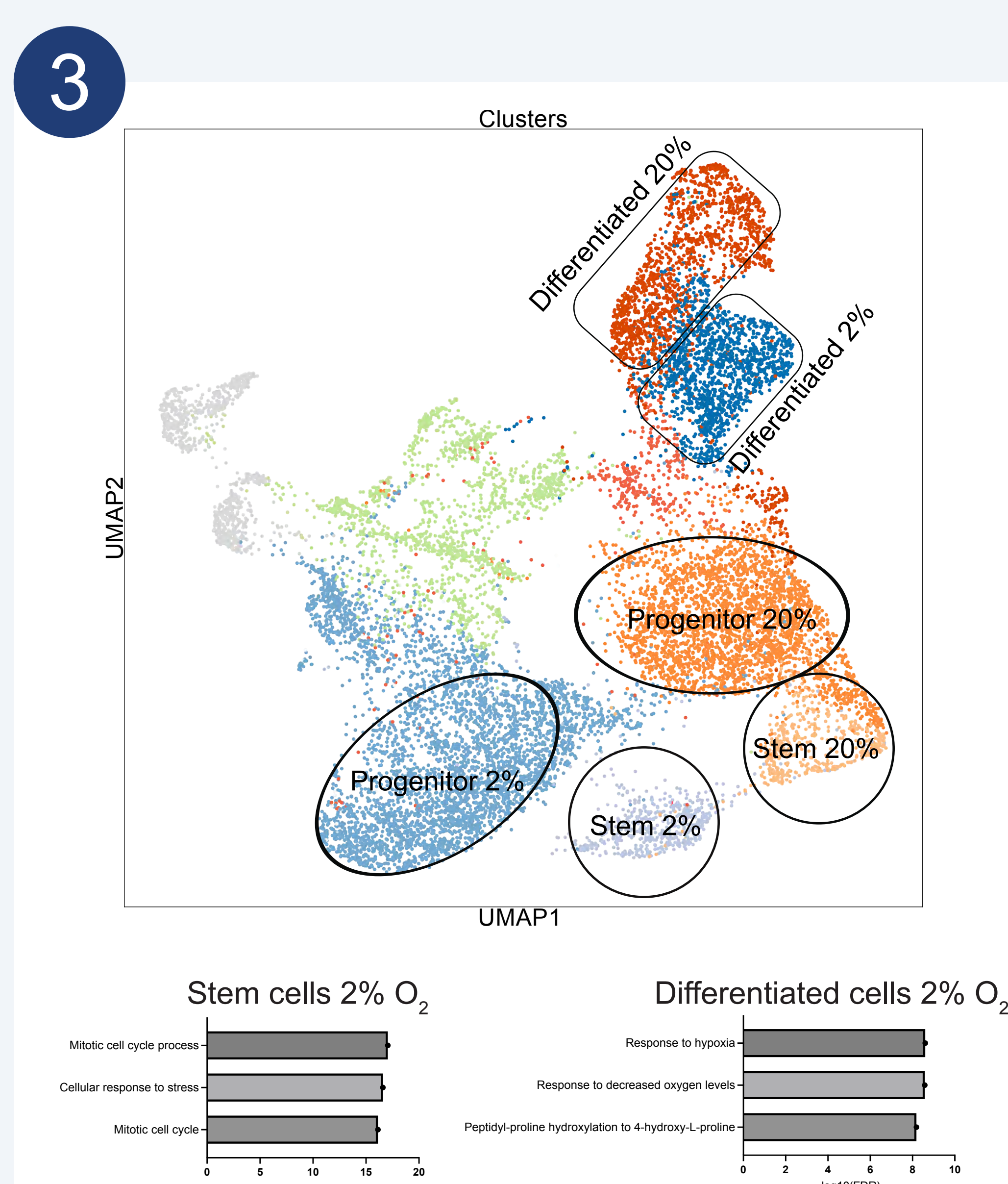
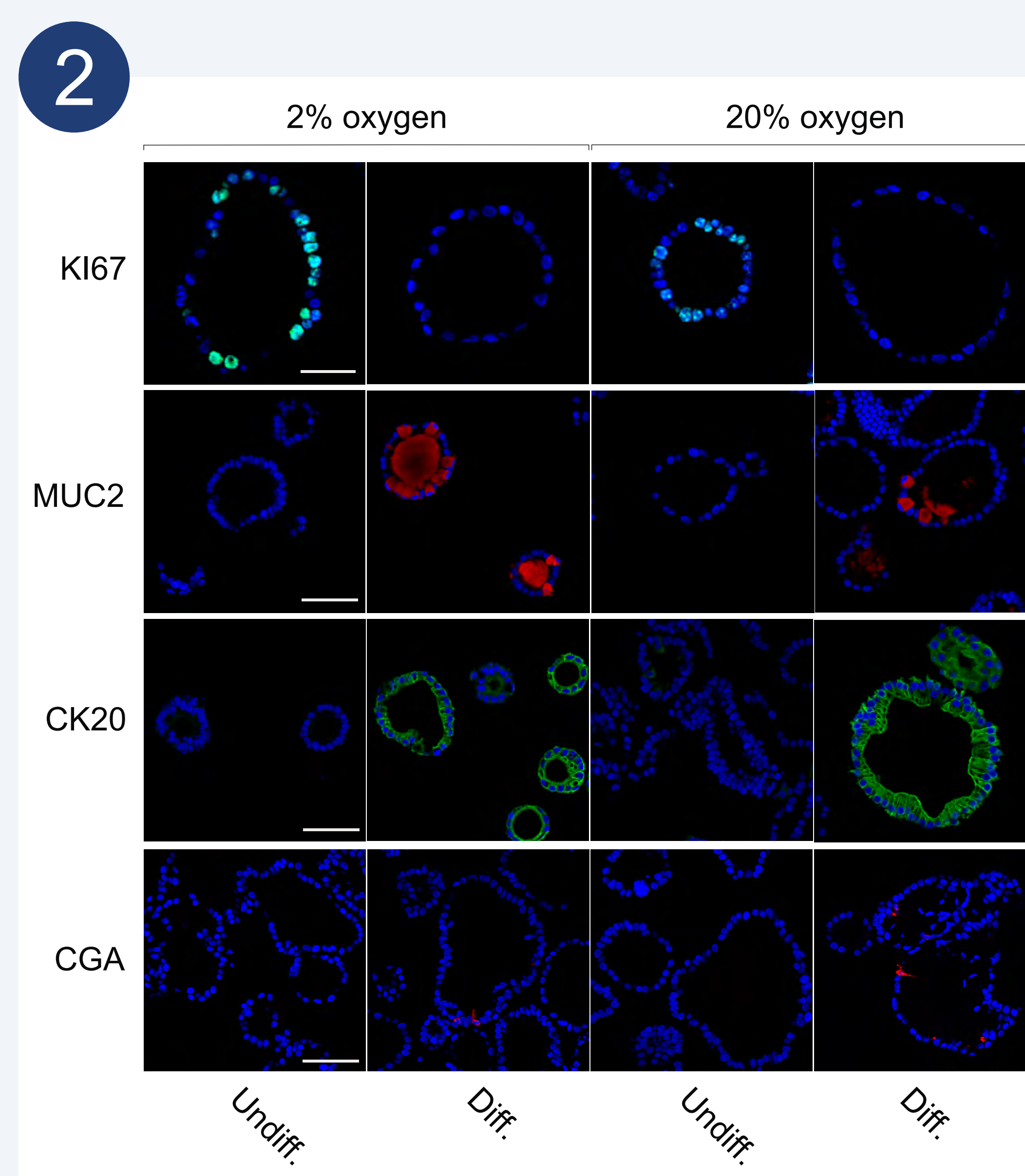
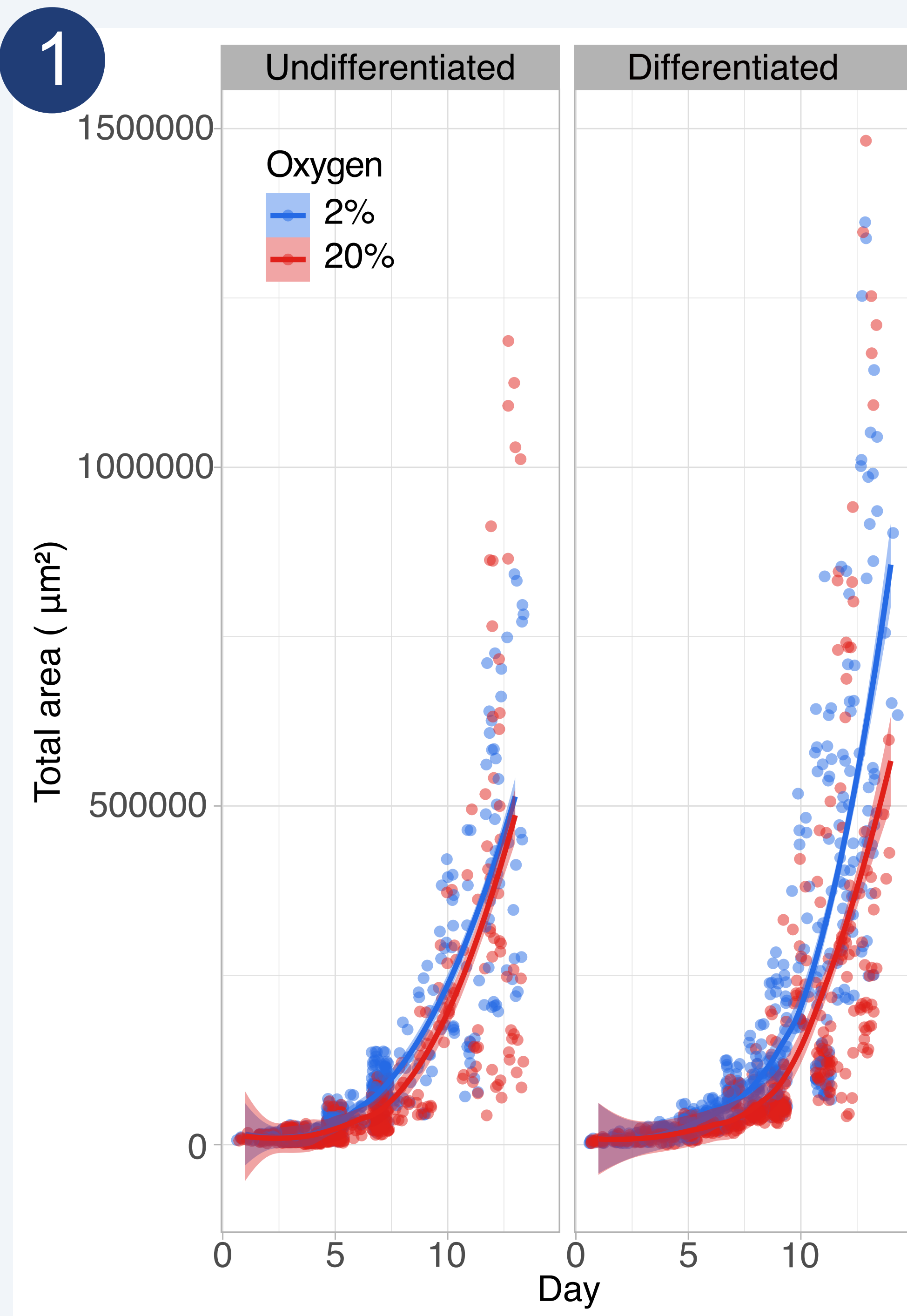
RESULTS

1-2) Colonoids established in 2% O₂ acquired a larger mass, without altering the expression of cell marker proteins.

3) The scRNA-seq analysis identified differences in the transcriptome within stem-, progenitor- and differentiated cell clusters.

4) Colonoids grown at 2% and 20% O₂ secreted chemokines and NGAL upon TNF + poly(I:C) treatment, but there appeared to be a tendency towards lower pro-inflammatory response in 2% O₂.

5) Reducing the O₂ environment from 20% to 2% in differentiated colonoids altered the expression of genes related to differentiation, metabolism, mucus lining, and immune networks.



Conflict of interests:

The authors have no potential conflict of interest to disclose