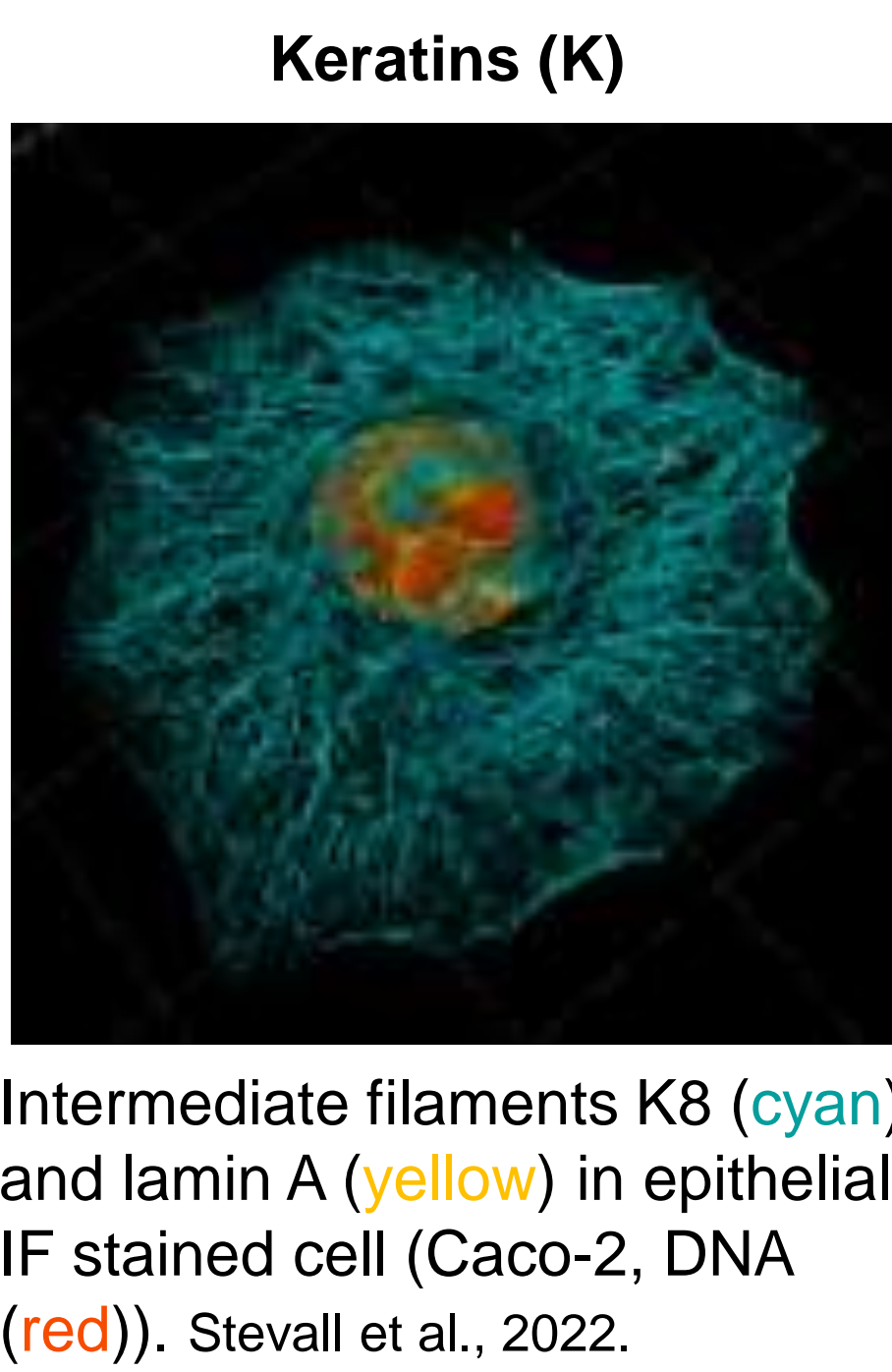


Background



Integrity

- Guardians of epithelial homeostasis

Protection

Protectors against cell stress:

- Mechanical stress
- Microbiota
- Aging

In normal cells

Effectors of cellular functions:

- Proliferation
- Apoptosis
- Differentiation

In inflammation

- Aberrant levels found in inflammatory disorders, especially in skin
- K7 is *de novo* expressed in IBD colon

In cancer

- Keratin patterns are changed in e.g breast and colon cancer

In diagnostics

- Keratins are well-established markers in pathological examinations
- Cell type and differentiation stage specific patterns and robust expression

Colonic keratins

- Main keratins: K8, K18-20
- K8, K18-19 are expressed throughout the whole crypt, while K20 is localized more at the crypt top
- K8 deficient mice exhibit inflammation phenotypes including diarrhea, crypt damage and epithelial hyper proliferation
- Downregulation of K8 and K19 alters differentiation of colon epithelial cells
- In some forms of colon cancer, K8 and K19 are upregulated, while K7 is *de novo* expressed
- K20 is a commonly used IHC marker for tumours of colonic origin

Hypothesis

- Keratin expression pattern in colon epithelium is altered in IBD and microscopic colitis
- Keratin levels correlate with inflammation severity
- Keratin could be utilized as biomarkers for specific colon inflammatory diseases

Materials and methods

Sample	Subtype/diagnosis
Inflammatory Bowel disease (IBD)	Ulcerative colitis (UC) n=14
	Crohn's disease (CD) n=12
Microscopic colitis	Collagenous colitis (CC) n=8
	Lymphocytic colitis (LC) n=10
Control (CTRL)	Non-inflammatory and non-neoplastic colon tissue n=12

IBD, microscopic colitis and control colon samples from human patients were received from Auria Biobank, Turku, Finland

Staining

Slides were stained with hematoxylin-eosin (HE) and immunostained with antibodies against K8, K18, K19 and K20 using DAB as chromogen

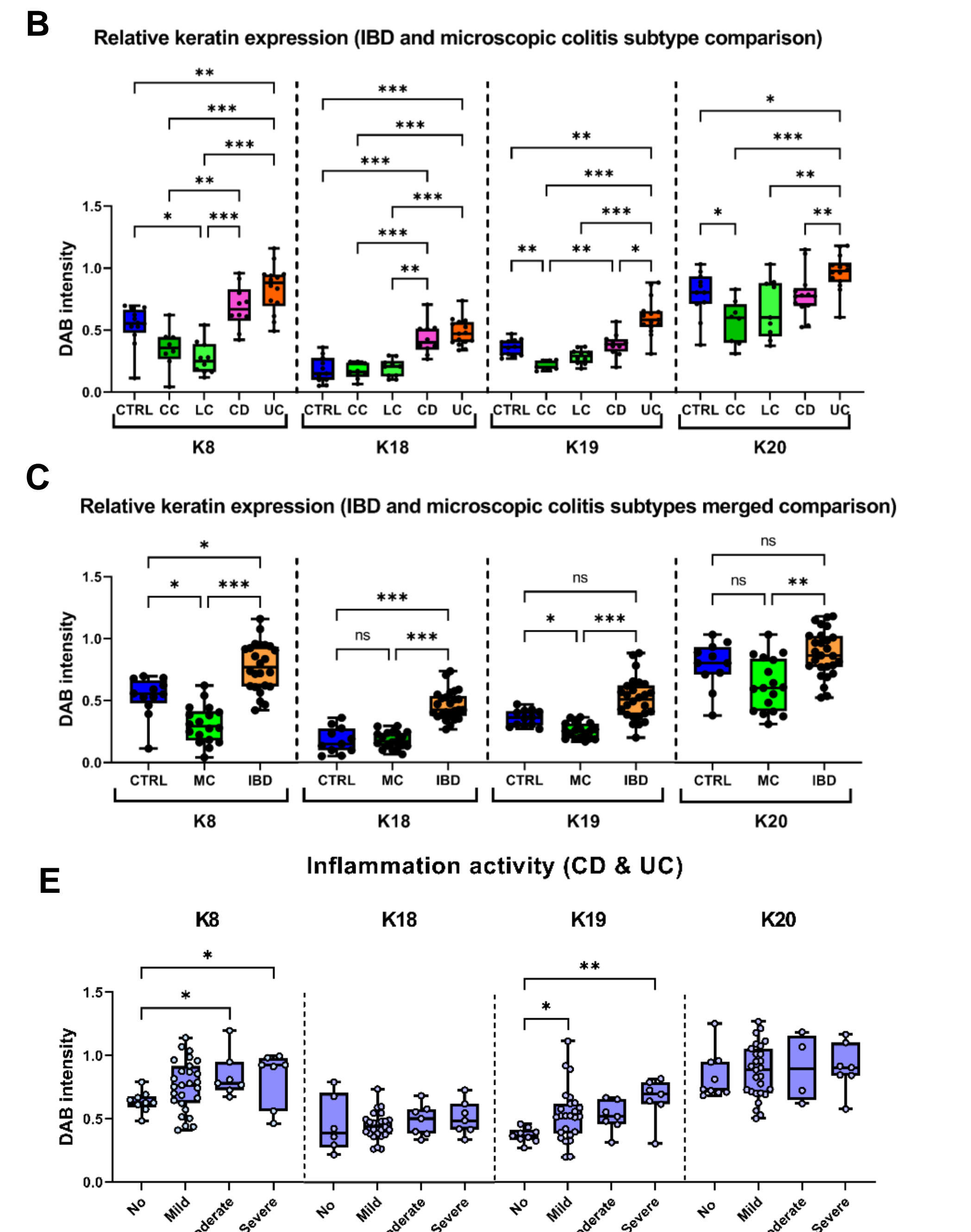
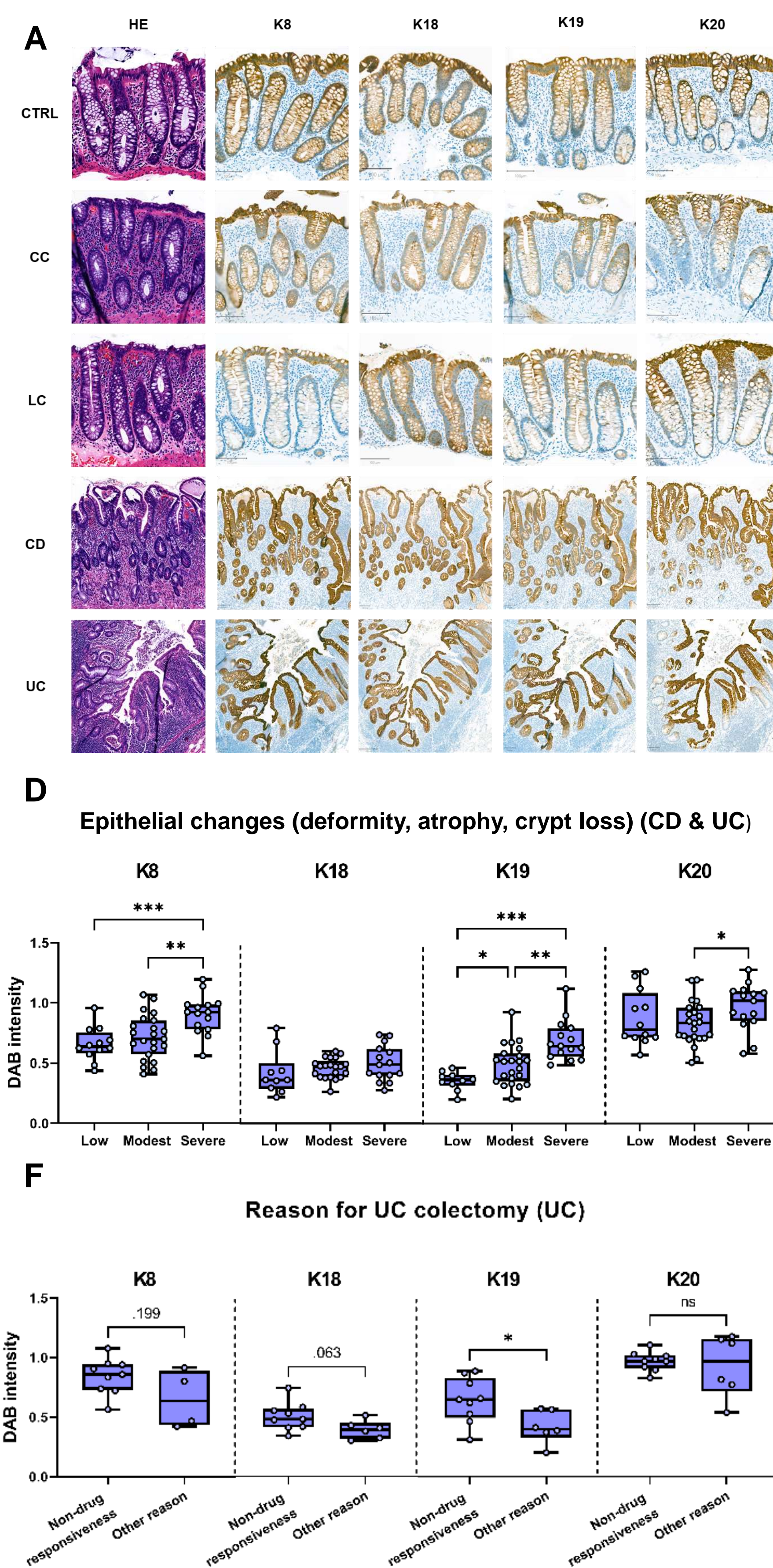
Digital image and statistical analysis

ROIs were scored for severity of IBD-related histopathologies from the HE stained slides. Relative intensity of keratin expression in epithelial cells were measured from these ROIs using Qupath software

Keratin staining intensity of different colon inflammatory diseases and healthy controls were compared and correlated with histopathological grades of the samples and clinical parameters of the patient.

Statistical analyses were performed using GraphPad Prism and Kruskal-Wallis test, followed by Dunn's test for measuring the difference between more than two groups and Mann-Whitney test for the difference between two groups.

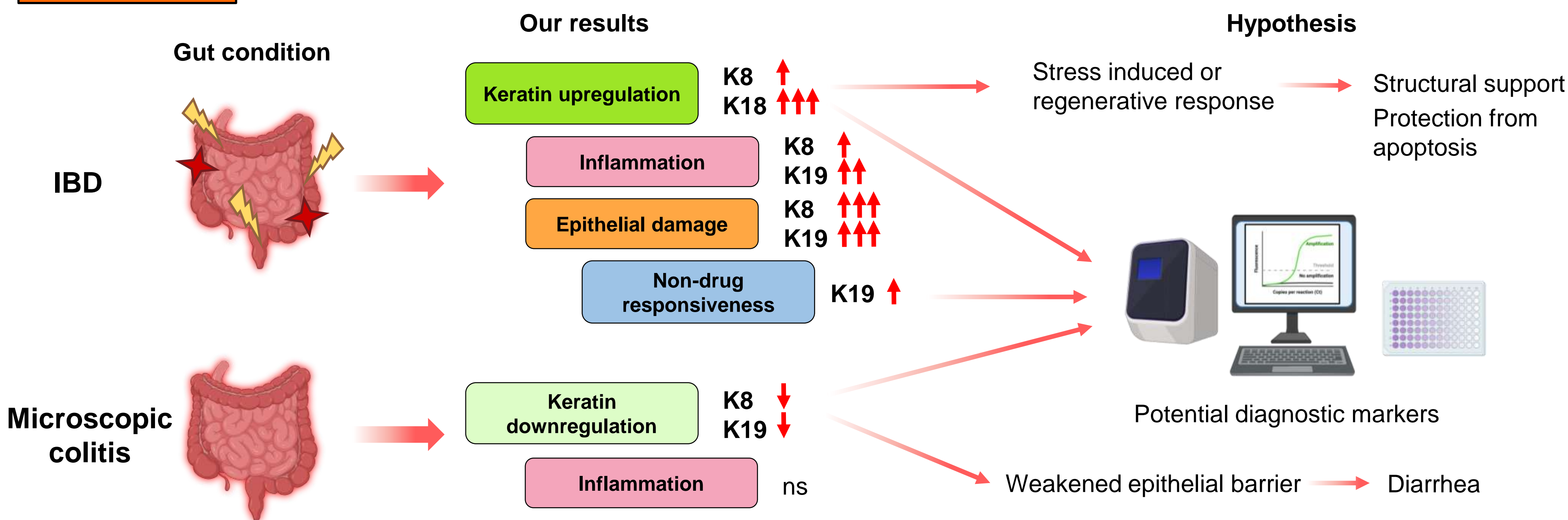
Results



Key points

- Expression of K8, K18, K19 and K20 are robustly upregulated in UC (Fig. A, B, C)
- Colonic keratins are downregulated in both subtypes of microscopic colitis, including K8 in LC and K19 and K20 in CC (Fig A, B, C).
- Epithelial areas with severe pathological changes and elevated inflammation activity are associated with higher K8 and K19 expression (Fig. D, E)
- UC-lesions from non-drug responsive UC patients have further increased levels of K8, K18 and K19 (Fig. F)

Conclusions



Next steps

- Investigate the interactions between inflammatory signaling and keratin expression in preclinical model**
- Develop *in vitro* organoid model for inflamed intestinal environment colon and immune cells
 - Study the correlation between levels of inflammatory signaling and the expression levels of different colonic keratins
- Keratins as noninvasive biomarkers**
- Develop noninvasive keratin detection assay based on PCR and/or ELISA
 - Investigate threshold keratins levels for IBD and microscopic colitis detection from fecal samples
- IBS studies**
- Study keratin expression levels in colon samples from IBS patients and compare the pattern with IBD and microscopic colitis results

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IHC stainings
TYKS pathology lab (Turku, Finland) and Satakunta pathology lab (Pori, Finland)

Samples
Auria Biobank (Turku, Finland)

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