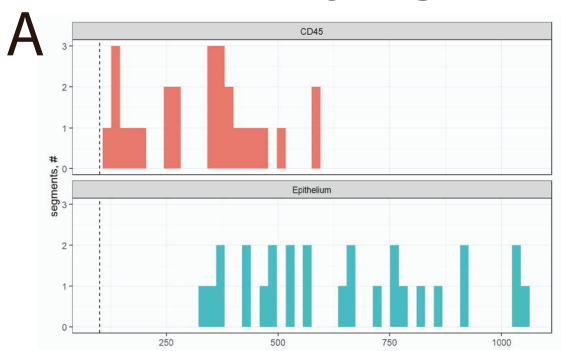


**Supplemental information**

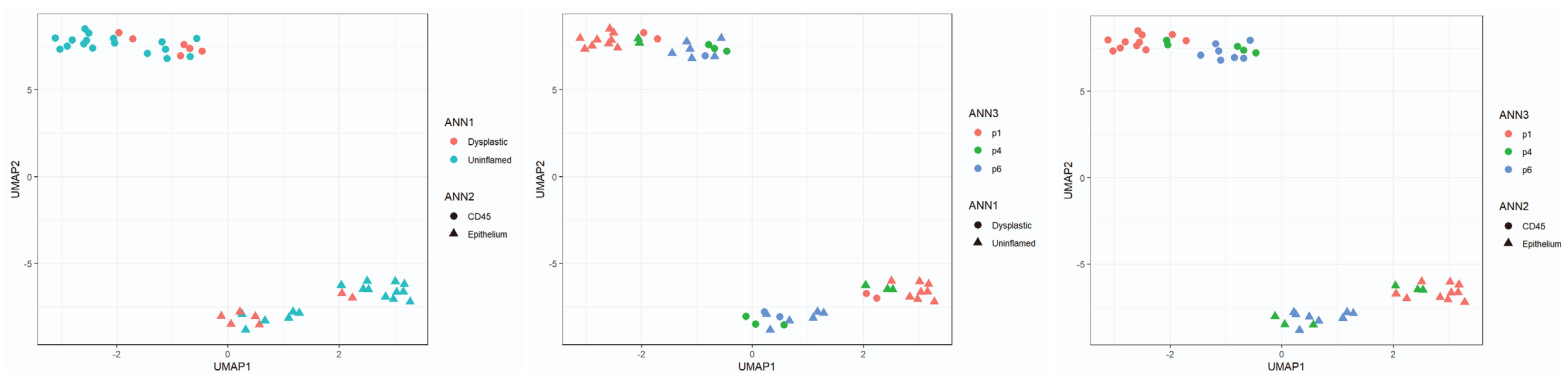
**Multiplex spatial omics reveals changes  
in immune-epithelial crosstalk during inflammation  
and dysplasia development in chronic IBD patients**

**Matthijs J.D. Baars, Evelien Floor, Neeraj Sinha, José J.M. ter Linde, Stephanie van Dam, Mojtaba Amini, Isaïc J. Nijman, Joren R. ten Hove, Julia Drylewicz, G.Johan A. Offerhaus, Miangela M. Laclé, Bas Oldenburg, and Yvonne Vercoulen**

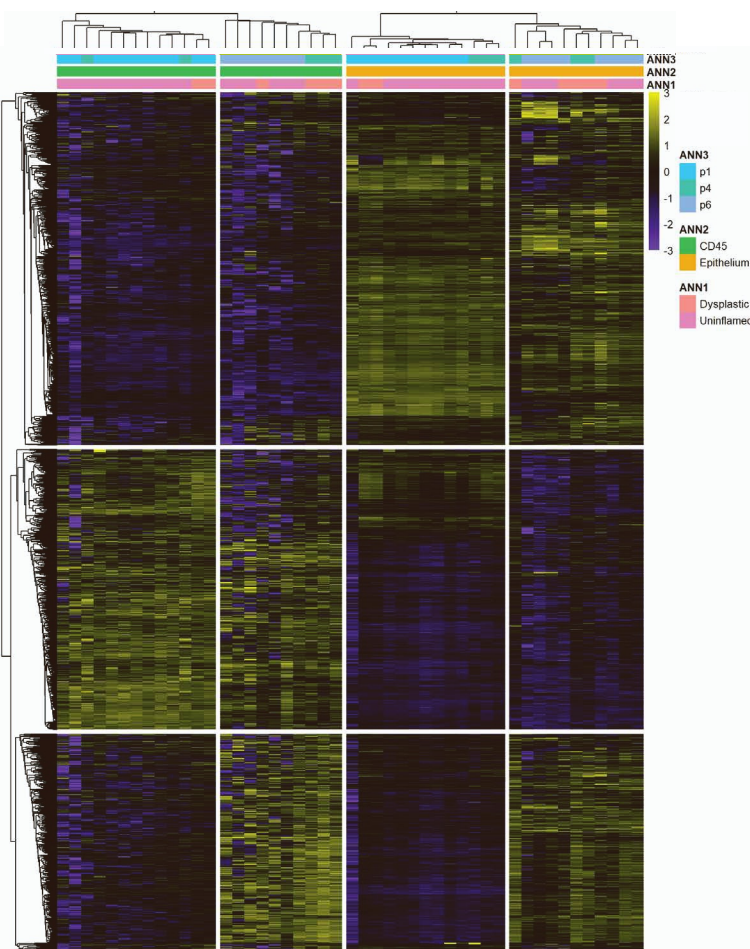
# Supplementary Figures



**B**



**C**

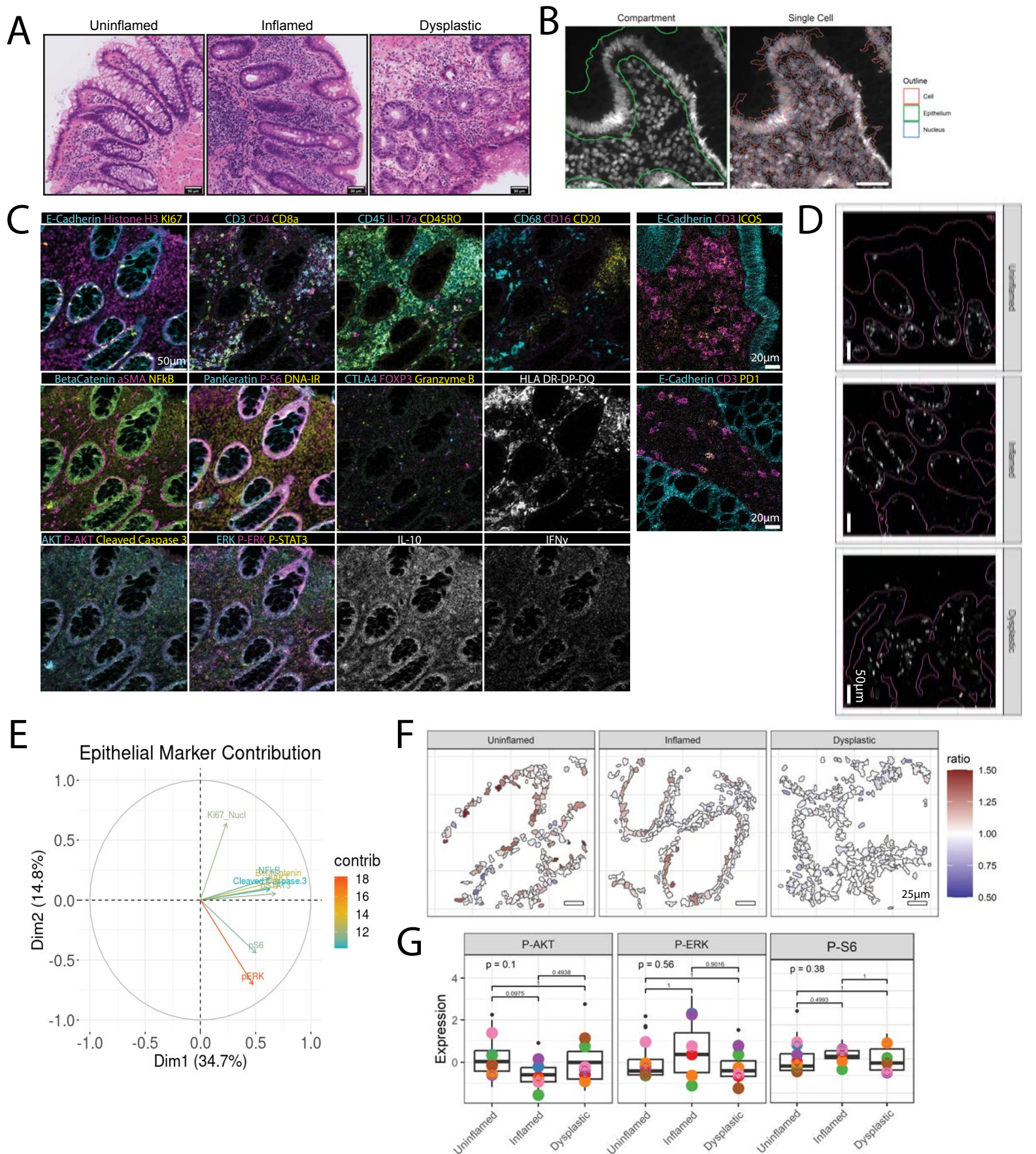


**Supplementary Figure 1. Spatial transcriptomics reveals differential gene expression in dysplasia,** related to Figure 1 and Tables S1A-B.

A) Numbers of cells included in GeoMx DSP analysis per segment (ROI), expressed in nuclei counts for CD45+ immune cells (top) and panCK+ epithelium (bottom).

B) UMAP clustering of samples: separate clusters of epithelial and immune cell segments, dysplasia/uninflamed control, and per patient.

C) The coefficient of variation (CV) for each gene (g) was calculated using formula  $Cvg/SDg/meang$  to identify genes with high CVs that should have large differences across various profiled segments (x-axis) in the study. Displayed as a heatmap is the unsupervised clustering.



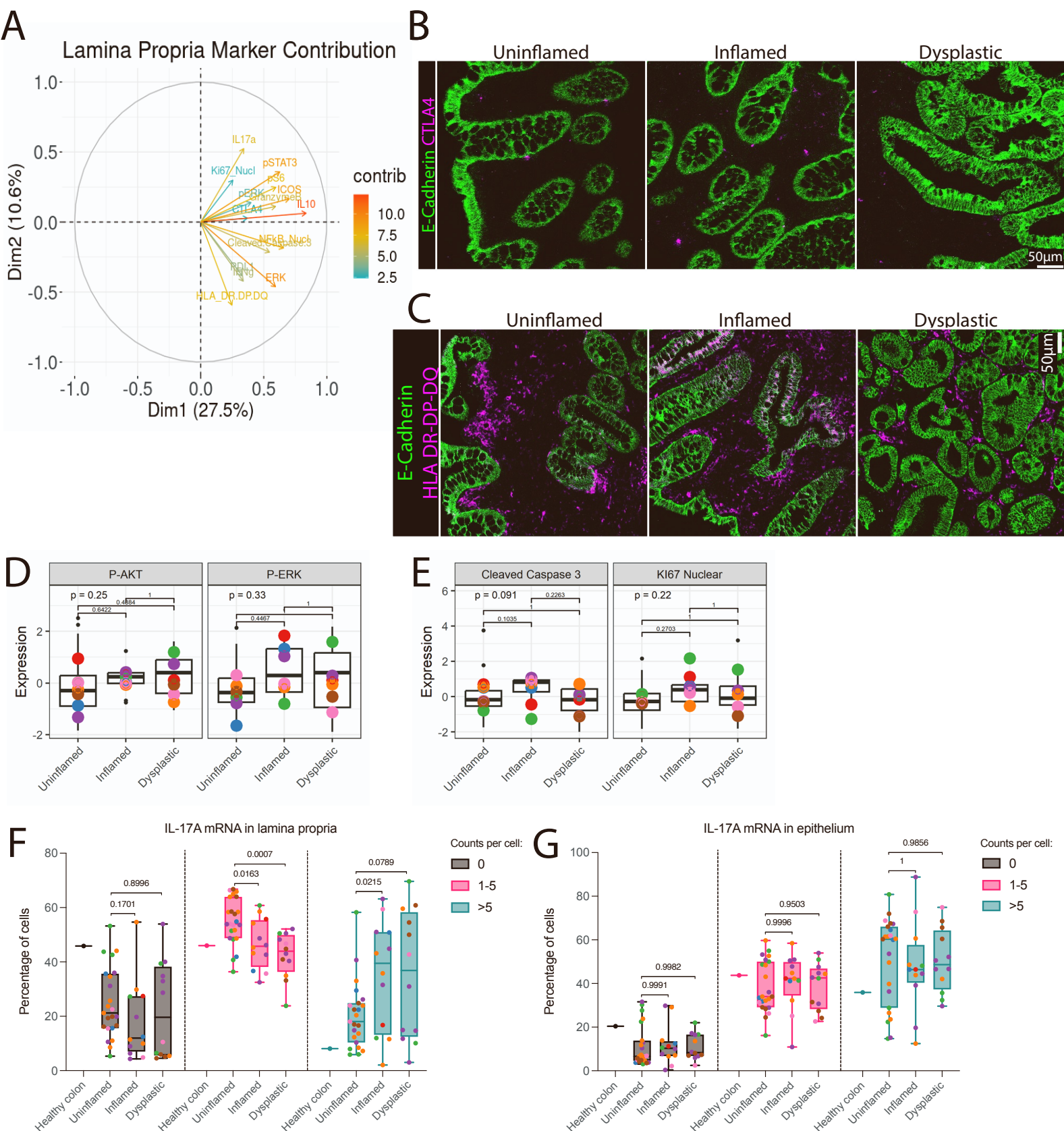
**Supplementary Figure 2. The epithelial compartment displays reduced inflammatory signaling in dysplasia, related to Figure 2 and Tables S2-3.**

Representative images of: (A) H&E tissue with disease state annotations: uninflamed, inflamed, and dysplastic. B) DAPI with segmentation outlines for both compartment (left), and single-cell segmentation (right), C) IMC tissue staining displayed per marker, D) KI67: the epithelial compartment border is indicated with a purple outline.

E) PCA plot showing the relative contribution of expression of each marker to variance observed in marker expression across biopsies.

F) Representative spatial display of the NFkB ratio for individual nuclei indicated by color gradient.

G) Boxplots showing median expression of P-AKT, P-ERK, and P-S6 in uninflamed, inflamed, and dysplastic epithelium.



**Supplementary Figure 3. Lamina propria cells display elevated expression of immune checkpoints, cytokines and active signaling in inflammation and early dysplasia, related to Figure 3.**

A) PCA plot showing the relative contribution of expression of each marker to variance observed in marker expression across biopsies.

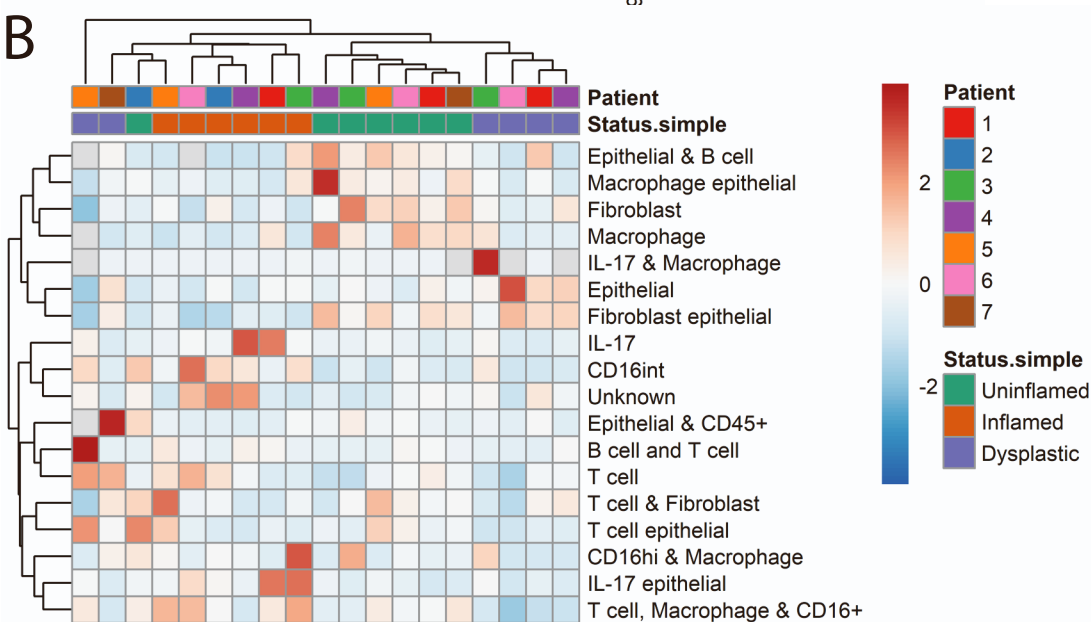
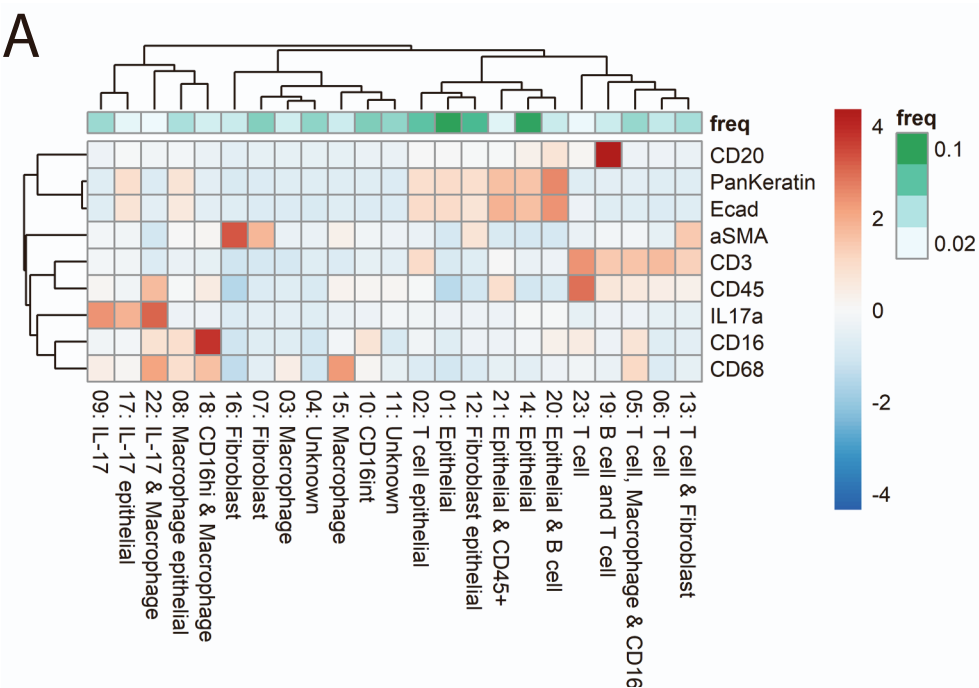
B) Representative images showing CTLA-4 (magenta) and E-cadherin (green).

C) Representative images showing HLA-DR-DP-DQ (magenta) and E-cadherin (green).

D) Boxplots showing median expression of P-ERK and P-AKT in lamina propria in different disease states (uninflamed, inflamed, colitis-associated dysplasia).

E) Median expression is displayed for Cleaved Caspase 3, and KI67 in lamina propria in different disease states (uninflamed, inflamed, colitis-associated dysplasia).

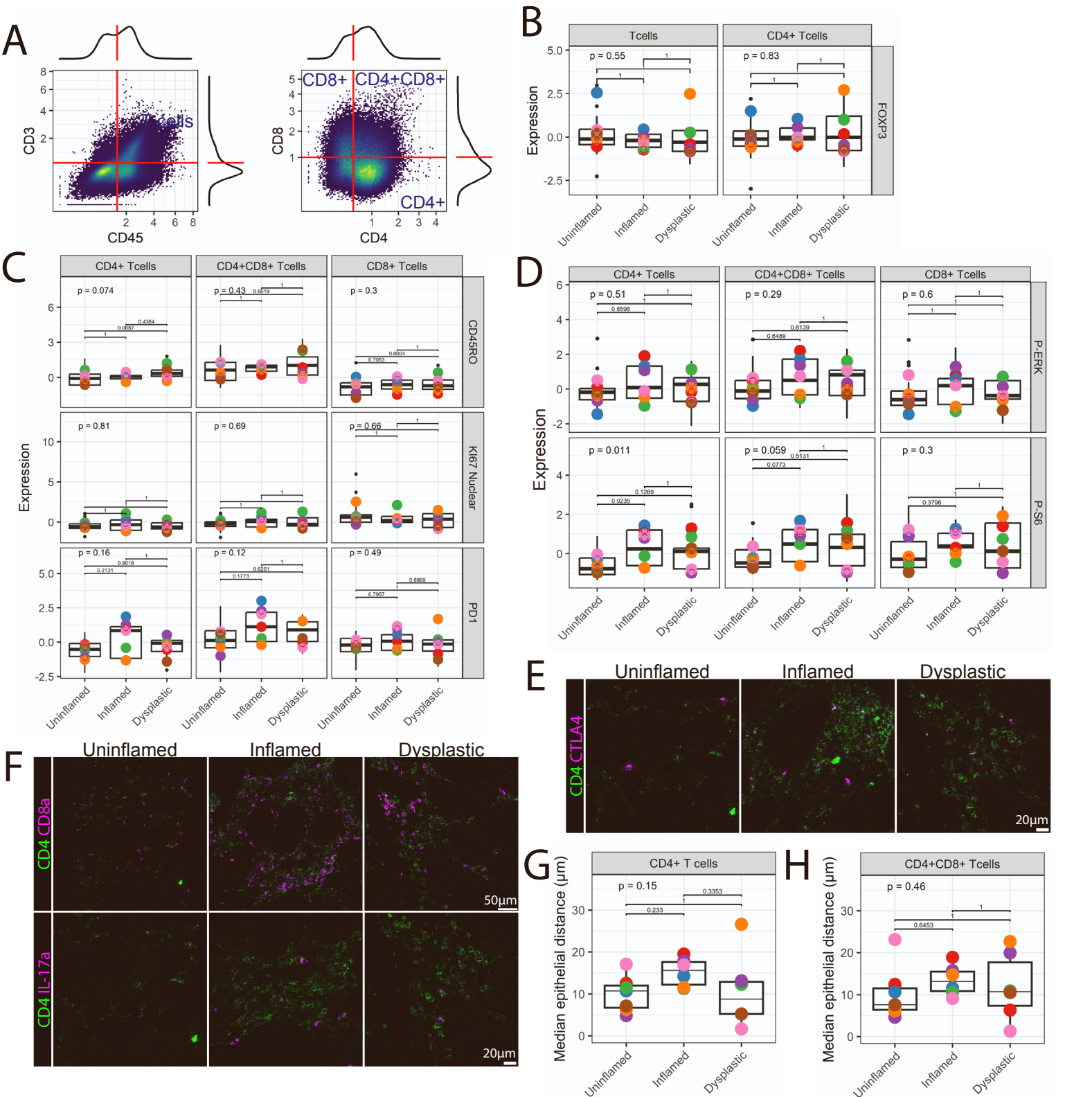
F-G) Percentage of cells in lamina propria and epithelium with 0, 1-5 or >5 IL-17A counts per cell. Healthy colon value was obtained from a TMA of healthy colon tissue. Two-way ANOVA with main effects only was performed, with Tukey HSD post-test. Colored points represent the percentage of cells per tissue biopsy.



**Supplementary Figure 4. Changed cellular composition of the tissue during dysplasia development, related to Figure 4.**

A) Hierarchical clustering of phenoclusters, displaying immune cell lineage markers expression. Heatmap displaying median expression values of lineage markers per phenocluster. Top row gradient in green indicates the frequency of cells per cluster.

B) Hierarchical clustering heatmap showing clustering per patient and disease state based on relative abundance of cell types.



**Supplementary Figure 5. T cell subsets**, related to Figure 5.

A) Gating strategy for T cell subsets. Total T cells (CD45+CD3+) were separated into subsets: negative, single-positive CD4 (CD4+ T cell), single-positive CD8 (CD8+ T cell), and double positive (CD4+CD8+ DP).

B-D) Boxplot graphs showing median expression in T cell subsets of B) FOXP3, C) CD45RO, KI67, PD-1, and D) P-ERK and P-S6. Kruskal-Wallis with Dunn's post-test and Bonferroni multiple testing correction was performed.

E-F) Representative images of E) CD4, CTLA-4 F) CD4, CD8a, and IL17A expression.

G-H) Analysis of localization of CD4+ and CD4+CD8+ T cells in the tissue, quantified as distance from the epithelium. Bins indicate "intra-epithelial" ( $\leq 0 \mu\text{m}$ ), "proximal" (1–25  $\mu\text{m}$ ), and "distal" (>25  $\mu\text{m}$ ). Distance was clipped to 75  $\mu\text{m}$ . ANOVA statistical test was performed, with Tukey HSD post-test. P-values are indicated in the graphs.



# Supplementary Tables

| Patient | Status.simple | Status                            | nBiopsy | nEvents IMC |
|---------|---------------|-----------------------------------|---------|-------------|
| 1       | Uninflamed    | Uninflamed                        | 3       | 37471       |
| 1       | Inflamed      | Actively inflamed                 | 1       | 14016       |
| 1       | Dysplastic    | Actively inflamed & dysplastic    | 1       | 9400        |
| 1       | Dysplastic    | Dysplastic                        | 1       | 13921       |
| 2       | Uninflamed    | Past inflammation                 | 1       | 1820        |
| 2       | Uninflamed    | Uninflamed                        | 1       | 2944        |
| 2       | Inflamed      | Chronically inflamed              | 1       | 13958       |
| 3       | Uninflamed    | Past inflammation                 | 4       | 23470       |
| 3       | Uninflamed    | Uninflamed                        | 1       | 3305        |
| 3       | Inflamed      | Actively & chronically inflamed   | 1       | 5772        |
| 3       | Dysplastic    | Chronically inflamed & dysplastic | 1       | 11392       |
| 3       | Dysplastic    | Dysplastic                        | 1       | 6070        |
| 4       | Uninflamed    | Past inflammation                 | 1       | 5801        |
| 4       | Uninflamed    | Uninflamed                        | 1       | 5417        |
| 4       | Inflamed      | Actively & chronically inflamed   | 1       | 29966       |
| 4       | Inflamed      | Chronically inflamed              | 2       | 20709       |
| 4       | Dysplastic    | Dysplastic                        | 2       | 19134       |
| 5       | Uninflamed    | Uninflamed                        | 6       | 40640       |
| 5       | Inflamed      | Chronically inflamed              | 3       | 22261       |
| 5       | Dysplastic    | Dysplastic                        | 1       | 3630        |
| 6       | Uninflamed    | Past inflammation                 | 6       | 29826       |
| 6       | Inflamed      | Actively & chronically inflamed   | 1       | 8723        |
| 6       | Dysplastic    | Dysplastic                        | 1       | 5951        |
| 7       | Uninflamed    | Past inflammation                 | 3       | 13004       |
| 7       | Uninflamed    | Uninflamed                        | 3       | 16726       |
| 7       | Dysplastic    | Dysplastic                        | 2       | 28805       |

**Supplementary Table 2**, related to Figure 2.  
Sample characteristics Imaging Mass Cytometry



| Target Name   | Isotope      | Clone      | Supplier                  | Cat #      | Dilution |
|---|--------------|------------|---------------------------|------------|----------|
| CTLA4 (primary)   | -            | CAL49      | Abcam                     | ab237712   | 1:100    |
| Goat anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody | 149Sm        |            | Invitrogen                | A16112     | 1:100    |
| E-Cadherin  | 142Nd        | 24E10      | Cell Signaling Technology | CST3195BF  | 1:150    |
| CD20  | 143Nd        | H1         | BD                        | 555677     | 1:300    |
| non-phospho (active) beta catenin                                   | 147Sm        | D13A1      | Cell Signaling Technology | CST8814BF  | 1:200    |
| PanKeratin  | 148Nd        | C11        | Cell Signaling Technology | CST4545BF  | 1:200    |
| IFN-γ   | 151Eu        | D3H2       | Cell Signaling Technology | CST8455BF  | 1:100    |
| CD45RO  | 152Sm        | UCHL1      | Cell Signaling Technology | CST55618BF | 1:100    |
| Akt (pan)   | 153Eu        | 40D4       | Cell Signaling Technology | CST2920BF  | 1:100    |
| HLA-DR+DP+DQ  | 154Sm        | CR3/43     | Abcam                     | ab7856     | 1:100    |
| FOXP3   | 155Gd        | 236A/E7    | Abcam                     | ab96048    | 1:50     |
| CD4   | 156Gd        | EPR6855    | Abcam                     | ab181724   | 1:100    |
| pSTAT3 (Tyr705)   | 158Gd        | D3A7       | Cell Signaling Technology | CST9145BF  | 1:100    |
| IL-10   | 160Gd        | polyclonal | R&D Systems               | AF-217-NA  | 1:150    |
| CD45  | 161Dy        | D9M8I      | Cell Signaling Technology | CST13917   | 1:100    |
| CD8a  | 162Dy        | C8/144B    | Thermo Fisher Scientific  | 14-0085-82 | 1:200    |
| ICOS  | 163Dy        | D1K2T      | Cell Signaling Technology | CST89601BF | 1:150    |
| pS6 (Ser235/236)  | 164Dy        | D57.2.2E   | Cell Signaling Technology | CST4858BF  | 1:100    |
| PD1   | 165Ho        | EPR4877(2) | Abcam                     | ab186928   | 1:100    |
| NFκB  | 166Er        | l8F6       | Cell Signaling Technology | CST6956BF  | 1:100    |
| IL-17a  | 167Er        | polyclonal | Bio-Techne                | AF-317-NA  | 1:100    |
| Ki-67   | 168Er        | B56        | BD                        | 550609     | 1:200    |
| GranzymeB   | 169Tm        | D6E9W      | Cell Signaling Technology | CST46890BF | 1:100    |
| CD3   | 170Er        | Polyclonal | DAKO                      | A045229-2  | 1:100    |
| pERK (Thr202/Tyr204)  | 171Yb        | D13.14.4E  | Cell Signaling Technology | CST4370BF  | 1:400    |
| Cl_Casp3  | 172Yb        | 5A1E       | Cell Signaling Technology | CST9664BF  | 1:100    |
| ERK (Pan)   | 173Yb        | 137F5      | Cell Signaling Technology | CST4695BF  | 1:100    |
| pAKT (Ser473)   | 175Lu        | D9E        | Cell Signaling Technology | CST4060BF  | 1:100    |
| H3  | 176Yb        | D1H2       | Cell Signaling Technology | CST4499BF  | 1:600    |
| aSMA  | 113In; 115In | 1A4        | Invitrogen                | 14-9760-82 | 1:100    |
| CD16  | 146Nd        | EPR16784   | Abcam                     | ab215977   | 1:100    |
| CD68  | 159Tb        | KP1        | Thermo Fisher Scientific  | 14-0688-82 | 1:100    |
| Intercalator  | 191Ir; 193Ir |            | Fluidigm                  | 201192B    | 1:300    |

**Supplementary Table 3**, related to STAR Methods.

Antibody panel Imaging Mass Cytometry

Patients used for IHC staining:

### Dysplasia

| Patient ID | Diagnosis | Sex | Age <sup>1</sup> | Disease duration (years) <sup>1</sup> | Montreal class | Maximal extent of inflammation |                   | IBD medication <sup>1</sup> |
|------------|-----------|-----|------------------|---------------------------------------|----------------|--------------------------------|-------------------|-----------------------------|
|            |           |     |                  |                                       |                | endoscopic                     | histological      |                             |
| 2          | CD        | F   | 51-53            | 20-22                                 | A2L2B1         | left-sided colitis             | pancolitis        | 5-ASA, AZA                  |
| 79         | UC        | F   | 48-56            | 9-16                                  | E3             | pancolitis                     | pancolitis        | AZA                         |
| 69         | UC+PSC    | M   | 61-62            | 43-44                                 | E2             | pancolitis                     | pancolitis        | 5-ASA                       |
| 14         | CD        | M   | 53-58            | 27-32                                 | A2L3B1         | left-sided colitis             | extensive colitis | 5-ASA                       |
| 50         | UC        | M   | 29-34            | 11-16                                 | E3             | pancolitis                     | pancolitis        | 5-ASA, AZA                  |
| 18         | CD        | M   | 49-55            | 28-34                                 | A2L3B1         | extensive colitis              | pancolitis        | 5-ASA, AZA                  |
| 72         | CD        | M   | 67-72            | 48-53                                 | A2L3B1         | extensive colitis              | pancolitis        | 5-ASA                       |

<sup>1</sup> at the time of sample collection

CD=Crohn's Disease; UC=ulcerative colitis; AZA=azathioprine; 5-ASA=5-aminosalicylates

### No dysplasia

| Patient ID | Diagnosis | Sex | Age <sup>1</sup> | Disease duration (years) <sup>1</sup> | Montreal class | Maximal extent of inflammation |              | IBD medication <sup>1</sup> |
|------------|-----------|-----|------------------|---------------------------------------|----------------|--------------------------------|--------------|-----------------------------|
|            |           |     |                  |                                       |                | endoscopic                     | histological |                             |
| 229        | UC        | M   | 68-72            | 17-22                                 | E2             | left-sided colitis             | pancolitis   | 5-ASA                       |
| 205        | CD        | F   | 57-59            | 32-35                                 | A2L2/4B2       | left-sided colitis             | pancolitis   | 5-ASA                       |
| 164        | UC        | M   | 33-36            | 11-14                                 | E3             | pancolitis                     | pancolitis   | 5-ASA, AZA                  |
| 214        | CD        | F   | 64-65            | 13-15                                 | A3L2B1         | pancolitis                     | pancolitis   | 5-ASA, AZA                  |
| 151        | UC        | F   | 56-58            | 20-21                                 | E3             | pancolitis                     | pancolitis   | AZA                         |
| 63         | CD        | M   | 64-66            | 45-46                                 | A2L2B1         | pancolitis                     | pancolitis   | 5-ASA                       |
| 111        | UC        | F   | 57-63            | 25-30                                 | E3             | extensive colitis              | pancolitis   | 5-ASA, AZA                  |

<sup>1</sup> at the time of sample collection

CD=Crohn's Disease; UC=ulcerative colitis; AZA=azathioprine; 5-ASA=5-aminosalicylates

**Supplementary Table 4**, related to Figure 6.

| Target name            | Clone   | Supplier                  | Cat #     | Dilution |
|------------------------|---------|---------------------------|-----------|----------|
| CD8a                   | C8/144B | Cell Signaling Technology | 70306     | 1:200    |
| IL-17/IL-17A           | NA      | Bio-Techne                | AF-317-NA | 1:200    |
| HLA DR + DP + DQ       | CR3/43  | Abcam                     | ab7856    | 1:200    |
| Phospho-Stat3 (Tyr705) | D3A7    | Cell Signaling Technology | 9145      | 1:100    |

**Supplementary Table 5**, related to STAR Methods.

Antibodies IHC