Macrophage and neutrophil heterogeneity at single-cell spatial resolution in inflammatory bowel disease

Case Study

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A single cell, spatial resolved map of healthy and diseased colon enabled with the CosMx™ Spatial Molecular Imager

Results

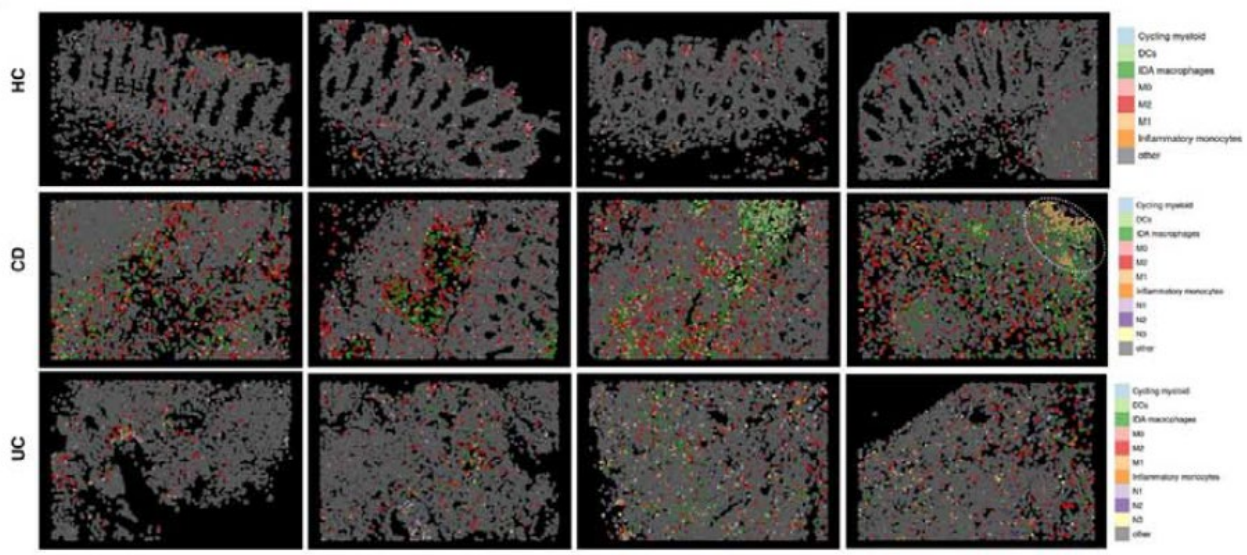
Resident M0 and M2 macrophages as well as activated macrophages such as classical M1 and new inflammation-dependent alternative (IDA) types were found in IBD samples. Intestinal neutrophils were found in three transcriptional states. Subepithelial IDA macrophages expressed NRG1, which promotes epithelial differentiation, whereas NRG1^{low} IDA macrophages were found within the submucosa and in granulomas, close to inflammatory fibroblasts, which may promote macrophage activation.

Conclusions

The most diversity of cell types was found in the myeloid compartment of macrophages and neutrophils. Disease heterogeneity may be due to the response of macrophages to unique tissue microenvironments.

Why CosMx?

"…available scRNA-seq datasets lack information on tissue distribution and spatially relevant cell-to-cell interactions. To fill this critical gap, highly multiplexed spatial technologies are rapidly evolving. **Our study is the first to provide combined scRNA-seq data with spatial transcriptomics at single-cell resolution to start unraveling patient-dependent disease mechanisms**."



SMI images showing spatial distribution of the different myeloid cell populations in representative fields of view of colonic tissue of two HC, one inflamed CD and two inflamed UC patients.

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What is the transcriptional and cellular makeup of tissue from Ulcerative Colitis and Crohn's Disease?

Background

- The molecular basis for the heterogeneity of ulcerative colitis (UC) and Crohn's Disease (CD) remains uncharacterized and there is remarkable variability in disease severity, progression, and treatment response

Research question

- What is the cellular distribution and gene signature makeup of normal and inflamed colon tissue?

Experimental design

- Use single cell Spatial Molecular Imaging of 1000 RNAs in combination with scRNA-seq data to profile the tissue distribution of different immune cell populations in IBD

Study Details	
Research area:	Autoimmune Disease
Organism and tissue:	Human Colon
Sample type:	FFPE
Instrument:	CosMx™ SMI
Analyte:	RNA
Assay:	Universal Cell Characterization RNA Panel
Journal, Year:	BioRxiv, 2022

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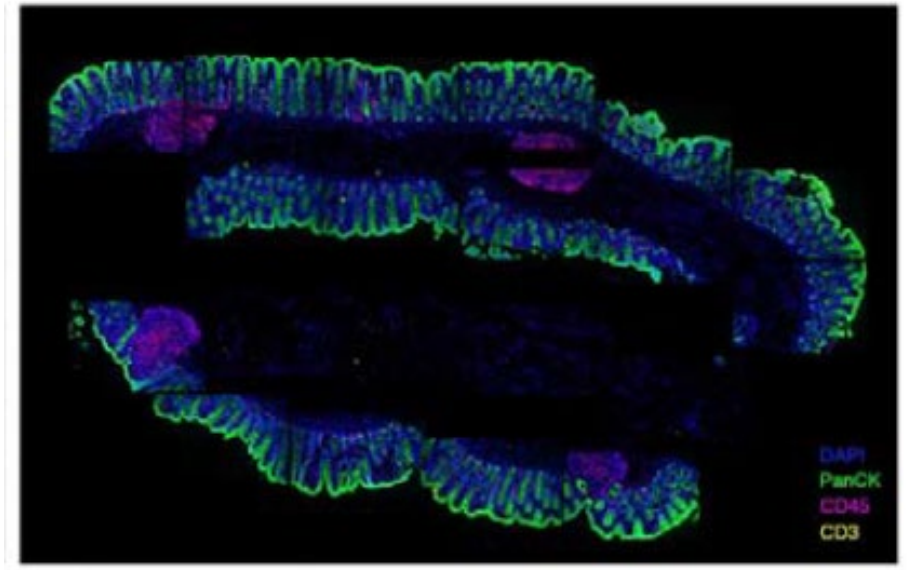
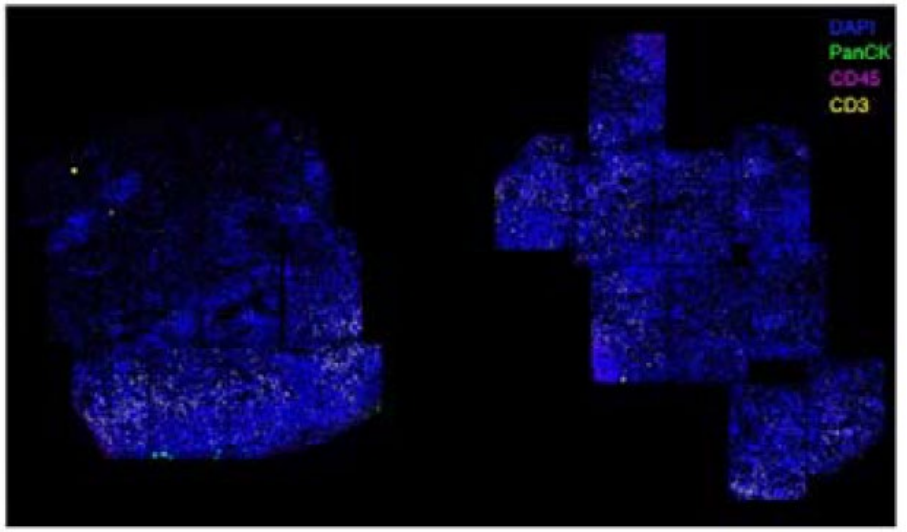
Cell Segmentation Markers

Segment	Target	Channel
T Cells	CD3	647nm
Immune Compartment	CD45	594nm
Nucleus	Nucleic Acids (DAPI)	461nm
Epithelial Cells	PanCK	532nm





Healthy (top) and diseased (bottom) UC colon tissue stained with cell segmentation markers

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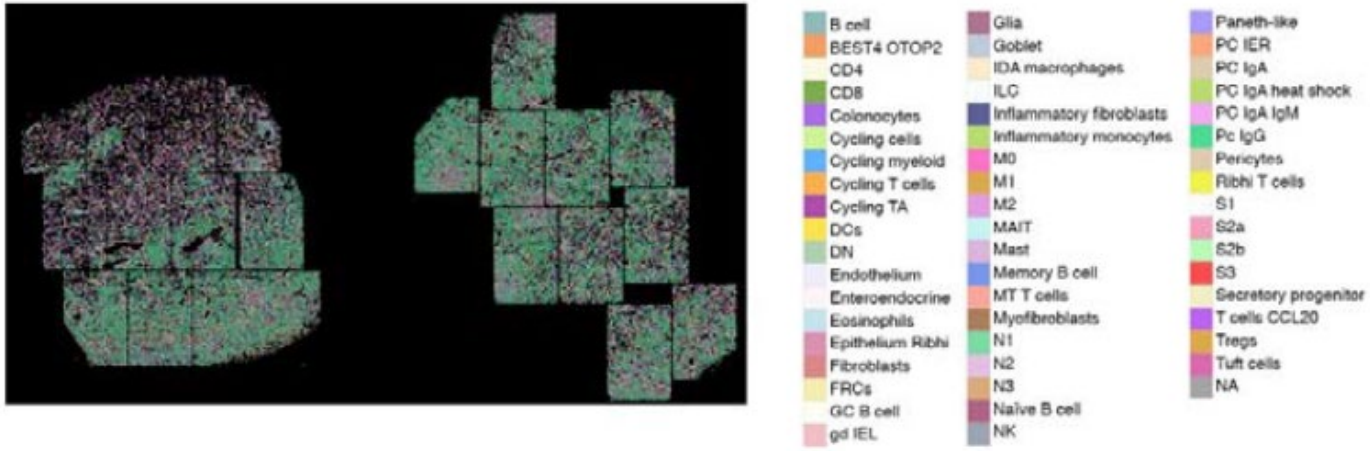
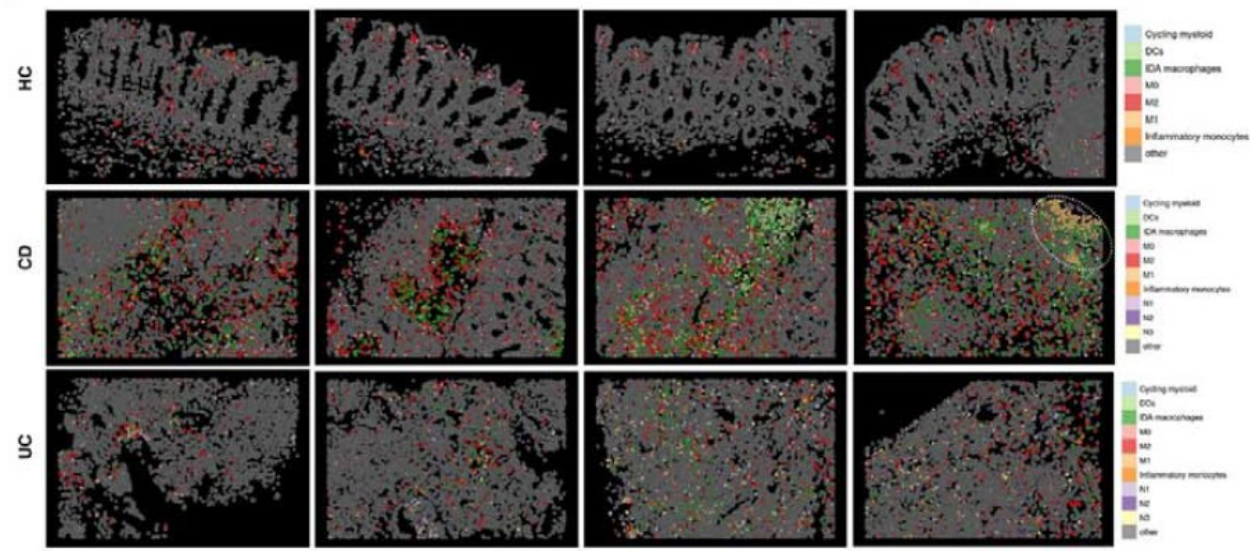
Inflammatory/activated macrophages in IBD exist in two different states

- M0 and M2 subsets of macrophages were found in healthy colon samples in agreement with sc-RNA-seq
- Inflammatory/activated macrophages found in IBD samples in at least two different states: two different M1 populations (M1 CXCL5 and M1 ACOD1) and a newly identified inflammation-dependent alternative (IDA) macrophage as well as inflammatory monocytes
- Markers of IDA macrophages include EGFR family ligands (including Neuregulin 1;NRG1)
- NRG1 promotes the expansion of the transit-amplifying epithelial compartment, which could play a role in the regeneration of the epithelium
- IDA macrophages present in the inflamed colon display differential NRG1 expression depending on tissue location





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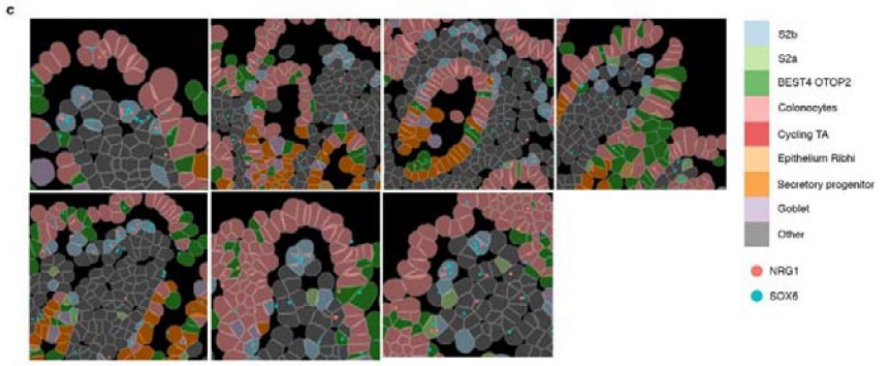
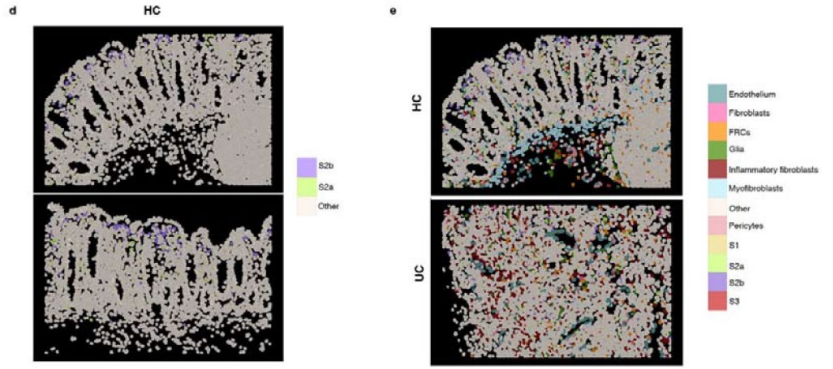
S2b fibroblasts overexpress NRG1 in IBD, particularly in UC patients

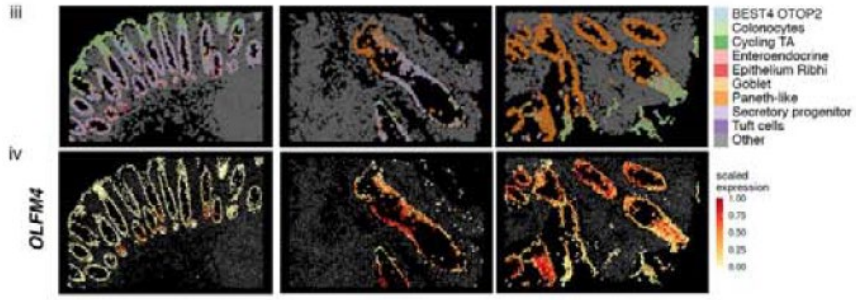
- NRG1 mRNA is expressed on abundant CD68+ macrophages in IBD
- NRG1 expression in HC was more specific to a population underlying the surface epithelium, not CD68+ macrophages
- CosMx SMI spatial analysis shows that S2b (SOX6+) (localized at the most apical area), but not S2a pericryptal fibroblasts also express NRG1
- Fibroblast-expression of NRG1 was also markedly increased in UC
- Neuregulin 1 significantly decreases expression of the stem cell marker LRG5 and upregulated OLFM4, without inducing changes in the proliferation marker MKI67
- Expression of OLFM4 is mostly restricted to a progenitor cell type in healthy tissues (Epithelium Ribhi, Secretory Progenitors) while it is dramatically increased in UC and CD



(c) SMI spatial analysis of S2a and S2b pericryptal fibroblasts showing expression of NRG1 and SOX6 (S2 marker) in representative images of a healthy colon. S2b localizes at the most apical area.



(d) S2a and S2b differential spatial distribution showed by SMI in 2 representative FoVs from healthy colon



(iii) SMI visualization and localization of the different epithelial cell subsets described by scRNA-seq, from left to right in a HC and two UC representative Fields of View (FoVs) and (iv) mean expression of OLFM4 in each of those cells

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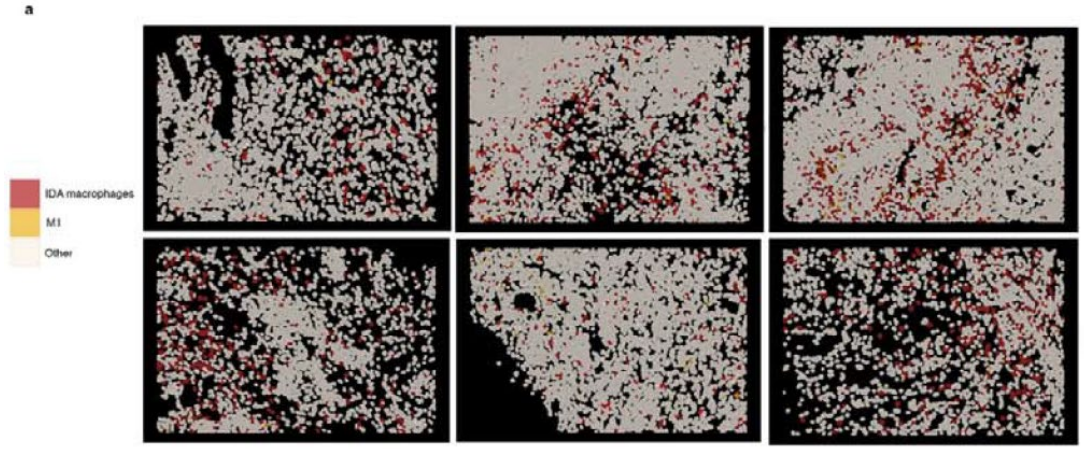
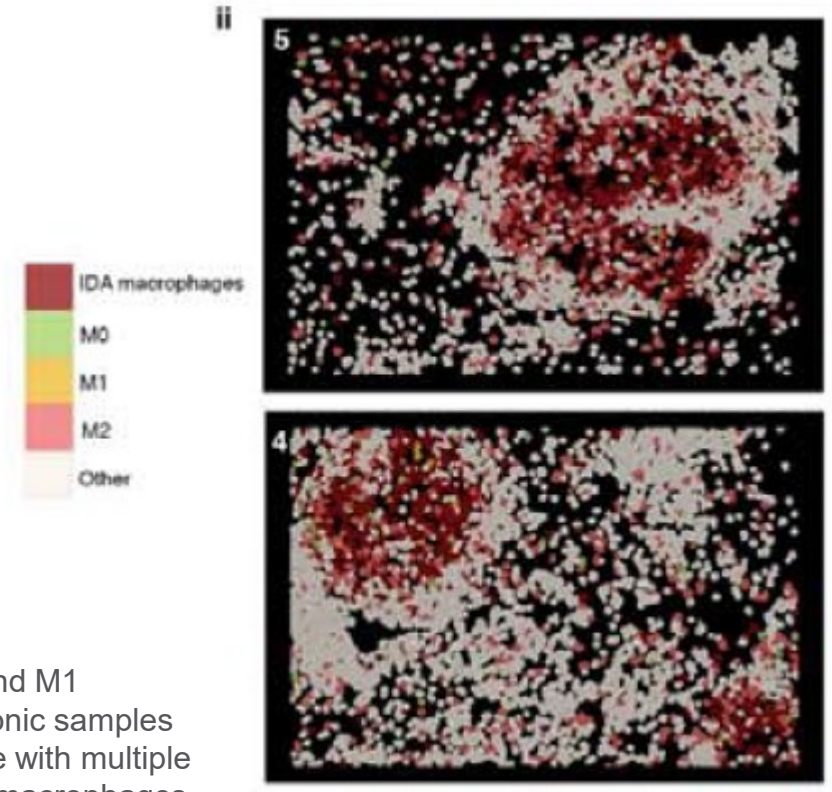
Tissue distribution of IDA macrophages in IBD samples

- IDA macrophages are found in UC and CD colon samples
- One CD patient sample had aggregates of macrophages, a granuloma
- IDA macrophages, together with some M2, and a few M0 and M1 macrophages, were the predominant macrophage state within granulomas
- NRG1 expression within the granulomas was low
- NRG1^{low} alternatively activated macrophages accumulate within granulomas in CD and in the submucosa of both UC and CD patients, suggesting independent functions





SMI distribution of IDA and M1 macrophages in IBD colonic samples (Left). CD colonic sample with multiple granulomas (Right) with macrophages shown
by SMI.

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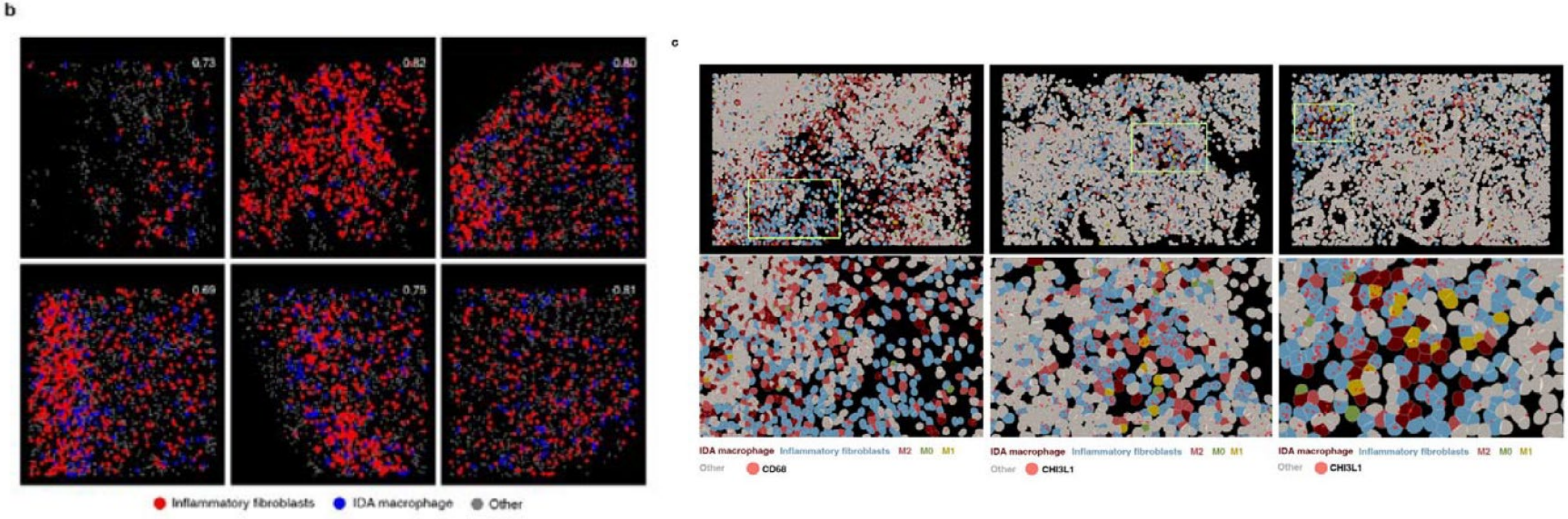
Crosstalk between inflammatory fibroblasts and IDA macrophages may occur in IBD



(b) Representative FoVs of co-localization analysis between IDA macrophages and inflammatory fibroblasts in inflamed UC tissue. Co-localization scores are indicated in white for each FoV. (c) Representative FoVs of IBD inflamed tissues containing IDA macrophages and inflammatory fibroblasts. Expression of CD68 (macrophages) or CHI3L1 (inflammatory fibroblasts) is shown as red dots. Each dot represents a single mRNA molecule

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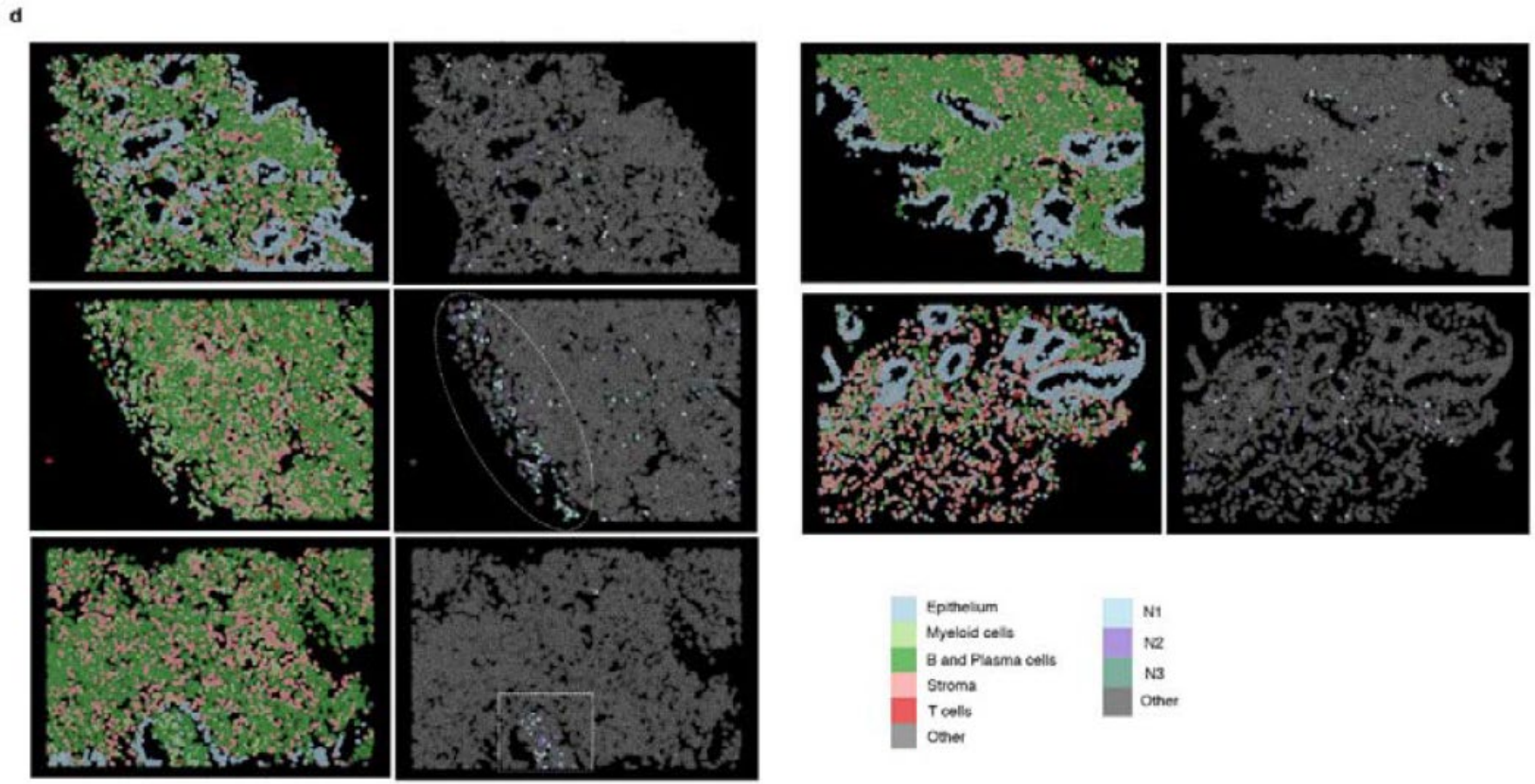
Distribution of neutrophils within IBD inflamed tissue

- IDA Granulocytes, including eosinophils and neutrophils, increased in IBD and expressed distinct membrane protein markers (CD62L, CD193, CD69) compared to their peripheral counterparts, indicating different states of activation
- Intestinal neutrophils were found in 3 unique states (annotated as N1, N2 and N3) whose relative abundance varied on individual patients and disease type
- Compared to N1 and N3, N2 neutrophils, instead expressed higher levels of CCL3, LGALS3 and CXCR4, while N3 neutrophils displayed a marked IFN523 response signature (e.g., GBP1, IRF1 and FCGR1A)



Representative SMI images of IBD inflamed tissue showing the spatial location of N1, N2 and N3 neutrophil subsets. Circle shows the surface of an ulcer, and a square shape is used to indicate a crypt abscess

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Patient-to-patient heterogeneity in IBD may be defined by the response of macrophages to the tissue microenvironment

Results

- Resident M0 and M2 macrophages as well as activated macrophages such as classical M1 and new inflammation-dependent alternative (IDA) types were found in IBD samples. M0 macrophages have never been described before in the intestine.
- Intestinal neutrophils were found in three transcriptional states.
- Subepithelial IDA macrophages expressed NRG1, which promotes epithelial differentiation, whereas NRG1low IDA macrophages were found within the submucosa and in granulomas, close to inflammatory fibroblasts, which may promote macrophage activation.

Conclusions

- The most diversity of cell types was found in the myeloid compartment of macrophages and neutrophils
- Macrophages can adopt diverse transcriptional signatures that are heterogenous between patients and associate with different types of cells such as fibroblasts
- The authors generated the first single cell, spatial map of healthy and diseased colon by integrated sc-RNA-seq data with CosMx SMI data

Why CosMx?

- "…available sc-RNA-seq datasets lack information on tissue distribution and spatially relevant cell-to-cell interactions. To fill this critical gap, highly multiplexed spatial technologies are rapidly evolving. Our study is the first to provide combined sc-RNA-seq data with spatial transcriptomics at single-cell resolution to start unraveling patient-dependent disease mechanisms."

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