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Spatial single-cell multiomics reveals peripheral immune dysfunction in Parkinson's and inflammatory bowel disease

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Parkinson's disease (PD) is the fastest-growing neurodegenerative disease in the world¹. Gastrointestinal (GI) dysfunction can occur decades before motor impairments and in up to 80% of individuals living with PD^{2–4}. We investigated peripheral relationships that may underlie mechanisms along the gut–blood axis that contribute to PD progression. Single-cell multiomic spatial molecular imaging (SMI) of colonic tissue localized and identified inflammatory injury within epithelial cells that appear to be associated with iron mishandling in both inflammatory bowel disease (IBD) and PD biosamples. We found that both the single-cell SMI of RNA and protein revealed parallel cross-modal dysregulation in the gut epithelium, in both IBD and PD biosamples. These data are accompanied by plasma (PD) and stool (IBD) protein depletion of CCL22. Our findings suggest iron mishandling along the gut barrier likely contributes to systemic inflammation, which may be one catalyst that primes circulating immune cells to body-first PD progression.

Parkinson's disease (PD) is a heterogeneous spectrum disorder¹. As a result, there has been no molecular mechanism or therapeutic identified that “cures” this life-altering disease^{2–4}. Recent efforts to prevent the progression of PD have looked outside of the brain and stereotypical motor symptoms that are used to diagnose this neurodegenerative disease. Specifically, a recent wealth of literature focuses on non-motor autonomic symptoms of PD, including anosmia, sleep disturbance, and gastrointestinal (GI) dysfunction that can occur decades before motor impairment^{2,5}. The delayed timeline from non-motor symptoms to

motor symptom presentation has prompted intense investigation into the possibility that environmental barrier sites outside of the brain could be the nexus of PD pathogenesis.

The rapid growth in PD diagnoses in the last decade is likely due in part to an increase in environmental risk factors such as unhealthy diets, pesticides, and pollutants related to industrialization that have all been implicated in PD risk⁶. One such peripheral site is the GI tract and its role in body-first PD⁶. The body-first PD hypothesis poses that two of the likely many subtypes of PD include: 1) periphery first

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phenotype of PD, where autonomic damage (such as sleep and GI dysfunction) precedes pathology in the brain and 2) a central nervous system phenotype where damage occurs in the substantia nigra prior to any autonomic dysfunction^{7,8}. Gut dysfunction, such as constipation, often occurs decades prior to motor deficits and in up to 80% of those living with PD⁵. It is also known that those living with inflammatory bowel disease (IBD), the most common subtype of GI dysfunction, have a 30% increased risk of being diagnosed with PD⁷. Importantly, not all individuals with IBD develop PD, and vice versa, but individuals with IBD may be predisposed to the development of body-first PD as opposed to brain-first PD⁹.

The molecular basis connecting gut dysfunction in IBD to increased risk for PD is still not understood. A primary issue with investigating the gut-brain axis is the heterogeneity in biological features and validated clinical symptom presentation of early-phase gut dysfunction, such as in IBD^{8,10}. Therefore, to our knowledge, we have evaluated the largest sigmoid colonic biopsy cohort of both IBD and PD to date (IBD, in endoscopic remission $N = 13$; PD $N = 12$; NHC $N = 8$) to appreciate the spatial single-cell targeted transcriptome and proteomic landscape, and thus, represent as much of this heterogeneous population as possible.

It is known that the epithelial lining of the gut separates the host's intestinal lamina propria, where gut-resident immune cells reside, from bacterial-derived products and opportunistic pathogens; when this barrier is breached or compromised, there is increased mucosal permeability to harmful antigens¹¹. IBD (including both ulcerative colitis and Crohn's disease) is associated with chronic relapsing and often transmural inflammation of the intestine¹². This inflammatory cascade drives the breakdown of the intestinal lining and contributes to the development of a "leaky gut" and uncontrolled release of chemokines and cytokines that prompt the release of apoptotic and necrotic cell factors¹³. A leaky gut drives a pro-inflammatory immune response by increasing mucosal immune-cell engagement with antigens present in the gut and allows for microbial products present in the gut to leak into deeper layers of the intestine within the peritoneal cavity¹⁴. This relapsing-remitting cycle of cytokines drives the activation of the innate and adaptive immune systems¹⁵, leading to a feed-forward mechanism of further cytokine and pro-inflammatory product release. Of note, those living with IBD who are prescribed anti-TNF biologics, common immune-targeted brain-permeant anti-inflammatory treatments, have a ~75% decreased risk of developing PD compared to those living with IBD not on anti-TNF medication¹⁶. These epidemiological associations and anti-inflammatory intervention outcomes suggest that the peripheral immune system is an important messenger in the gut-brain axis in the context of PD.

There has yet to be an identified biomarker(s) indicating a molecular mechanism that links these two diseases of inflammation, likely due to the breadth of existing literature that places a heavy emphasis on a brain-centric approach to neurodegenerative disease. We, as well as others^{17,18}, hypothesize that PD pathogenesis may be initiated in the gut decades prior to the development of classic PD motor phenotypes in part due to disruptions in central-peripheral neuroimmune crosstalk¹⁵.

We interrogated the unique versus shared cellular and molecular features of mechanisms associated with dysregulated gut immunity and gut inflammation in IBD, PD, and neurologically healthy controls (NHCs). We employed NanoString CosMx™ Spatial Molecular Imaging (SMI) to conduct multiplexed spatial imaging of both the targeted transcriptome and proteome to profile the heterogeneous mosaic of the colon and immune-interacting cellular partners within the colonic microenvironment. The use of multiplexed protein immunoassays of biospecimens at additional peripheral sites (plasma and stool) allowed for additional in-depth and cross-correlational analyses of the gut-blood axis in individuals with IBD (in endoscopic remission), PD, or NHCs. Our findings provide an open-access data repository that can be leveraged to identify peripheral biomarkers of PD progression along the gut-blood axis (Table 1 for code and dataset access).

Results

Clustering analysis identifies cell-type abundance and mapping of spatial organization unique to PD and IBD in sigmoid colonic biopsies

Colonic biopsies from PD ($N = 12$), IBD in endoscopic remission ($N = 13$, including $n = 9$ diagnosed Crohn's disease, $n = 3$ diagnosed ulcerative colitis and $n = 1$ IBD with no defined diagnosis) and NHCs ($N = 8$) were collected to interrogate intrinsic immune and inflammatory features within each compartment (Supplementary Table 1; Supplementary Table 3; Supplementary Fig. 1). It is important to note that all biopsies collected were deemed endoscopically non-inflamed by a gastroenterologist at the time of collection, due to this classification all individuals diagnosed with ulcerative colitis or Crohn's disease were pooled together into one IBD cohort. Compartments consisting of primary cell-types (epithelial, B and plasma cells, stromal, T cells, myeloid cells) (Fig. 1D; Supplementary Table 2; Supplementary Fig. 2) were isolated in silico to generate higher resolution of refined subpopulations (Fig. 1B; Supplementary Fig. 3 and Supplementary Fig. 4). Annotated refined populations were then digitally mapped onto tissue. The spatial distribution of relevant cell-types appeared to have the expected characteristics of sigmoid colon (Fig. 1C) and was unique to IBD, PD or NHC (Fig. 1D). Additionally, refined cell-type abundance differed by disease (Fig. 1E). However, there was a significant increase in the abundance of colonocytes in both IBD and PD patients (Fig. 1E); but interestingly, there was a significant decrease in CD4 + T cells in those living with IBD and an increase in CD8 + T cells in those living with PD, compared to NHC (Fig. 1E). A subsequent slide was then analyzed for protein regulation, however only to the depth of subpopulations. Epithelial cells along the colonic mucosal lining are known to proliferate in response to injury, especially in IBD¹⁶. Colonic biopsies from either those living with IBD (in endoscopic remission) or PD displayed a significant enrichment of proteins associated with epithelial and T-cell subpopulations (Fig. 6C), perhaps as a result of chronic injury and repair cycles, which is a hallmark of IBD but is a novel finding for PD.

Differential gene expression identifies inflammatory pathways associated with immune signaling dysfunction in the sigmoid colon in both PD and IBD

Dysregulation of iron absorption is a known outcome of PD in the brain¹⁹ and can be a marker of inflammation and autoimmune dysfunction in circulating peripheral immune cells²⁰. The role gut inflammation plays in peripheral iron handling and its link to PD has yet to be elucidated. Volcano plots depicting differentially expressed genes comparing epithelial cell clusters in PD or IBD vs. NHC revealed enriched markers of inflammation in both IBD and PD colonic biopsies (Fig. 2A, B). Here, we observed a significant enrichment of one of the genes that encode for the major iron storage protein ferritin, the ferritin heavy chain 1 (*FTH1*), in pooled epithelial IBD biopsies (Fig. 2A); and in pooled epithelial PD biopsies depletion of *FTH1* and the gene that encodes for ferroportin (*SLC40A1*), the protein that drives ferritin export (Fig. 2B), relative to NHC biopsies. Visual spatial distribution of cells annotated as epithelial (orange) and *FTH1* single-cell gene expression (yellow) revealed significantly enriched *FTH1* expression in epithelial cells in those living with IBD in endoscopic remission (Fig. 2Ai, Aii, Bi, Bii). In colonocytes from IBD biopsies, we observed a depletion in protein exporter gene *SLC40A1* and enrichment in genes associated with cell proliferation and migration including *MTOR* (mammalian target of rapamycin), *LGALS3* (lectin, galactoside-binding, soluble, 3) and *TIMP1* (TIMP metalloproteinase inhibitor 1) (Fig. 2C). In colonocytes from PD biopsies, *FTH1* and *SLC40A1* were significantly depleted (Fig. 2D). *MT2A*, a critical marker of metal homeostasis and immune regulation, was depleted in both IBD and PD colonocytes as compared to NHC (Fig. 2C, D). Relative density plot of total *FTH1* expression by disease and all refined cell-types indicated that colonocytes contained the highest abundance of *FTH1* in the sigmoid colon regardless of disease status (Fig. 2E). Similarly, *SLC40A1*

Table 1 | Open Science Key Resource Table

Resource type	Resource name	Source	Identifier	New or reused	Additional information
Dataset	CosMx™ Human Colon RNA	Zenodo	https://doi.org/10.5281/zenodo.14851478	New	Zenodo doi for associated code and README file: https://doi.org/10.5281/zenodo.17537303
Dataset	CosMx™ Human Colon protein	Zenodo	https://doi.org/10.5281/zenodo.14851272	New	Zenodo doi for associated code and README file: https://doi.org/10.5281/zenodo.17537303
Dataset	Ibd-bcn_single_celldataset	Github Ibd-bcn data repository	https://github.com/ibd-bcn/ibd-bcn_single_cell.git	Reuse	Dataset was uploaded to CosMx™ RNA dataset as reference for cell-types
Dataset	Meso Scale Discovery Human Plasma	Zenodo	https://doi.org/10.5281/zenodo.17652663	New	README file for dataset within upload.
Dataset	Meso Scale Discovery Human Stool	Zenodo	https://doi.org/10.5281/zenodo.17652370	New	README file for dataset within upload.
Software/code	Ibd-bcn_single_cell	Github	Github.com/ibd-bcn/ibd-bcn_single_cell	Reuse	
Software/code	Seurat Version 5.1.0	Seurat	RRID: SCR_007322	Reuse	http://seurat.r-forge.r-project.org/
Software/code	Python Version ≥ 3.8.10	Python	RRID:SCR_008394	Reuse	https://www.python.org/downloads/release/python-360/
Software/code	R Version ≥ 4.3.2	R studio	RRID: SCR_000432	Reuse	r-project.org
Software/code	CellTalkDB Version 1.0	Git Hub	https://github.com/ZJUFanLab/CellTalkDB	Reuse	
Software/code	SCOTIA	Git Hub	https://github.com/Caochris/SCOTIA?tab=readmeov-file	Reuse	
Software/code	CosMx™ human Colon RNA code	Zenodo	https://doi.org/10.5281/zenodo.17537303	New	Git Hub: https://github.com/ibd-bcn/CosMx-Bolen/tree/main/analysis/CosMx_RNA The above Github link will take you to all code for CosMx™ RNA analysis and README files.
Software/code	CosMx™ human Colon Protein code	Zenodo	https://doi.org/10.5281/zenodo.17537303		Git Hub: https://github.com/ibd-bcn/CosMx-Bolen/tree/main/analysis/CosMx_Protein The above Github link will take you to all code for CosMx™ Protein analysis and README files.
Software/code	InsituType Cell phenotyping Version 1.0	Git Hub	RRID:SCR_025976	Reuse	https://github.com/Nanostring-Biostats/CosMx-Cell-Profiles/tree/main/Human
Protocol	CosMx™ Tissue Microarray preparation	Protocols IO	https://doi.org/10.17504/protocols.io.eq2ly6pppgx9/v1	New	
Protocol	CosMx™ spatial transcriptomics workflow	Protocols IO	https://doi.org/10.17504/protocols.io.3byl41mrolo5/v2	Reuse	Note this is per the manufacturer's instructions, which are provided with assay purchase
Protocol	CosMx™ protein slide preparation	Protocols IO	https://doi.org/10.17504/protocols.io.bp2l62bokqge/v1	Reuse	Note this is per the manufacturer's instructions, which are provided with assay purchase
Protocol	Meso Scale Discovery workflow	Protocols IO	https://doi.org/10.17504/protocols.io.rm7vzxd98gx1/v1	Reuse	
Critical commercial assay	CMx Hs Univ Cell Panel RNA Kit EA	NanoString	CAT# 121500002	Reuse	
Critical commercial assay	CMx Hs IO Panel Protein Kit	NanoString	CAT# 121500010	Reuse	
Other	Pierce™ BCA Protein Assay Kit	ThermoFisher scientific	CAT# 23225	Reuse	
Critical commercial assay	U-PLEX custom pro-inflammatory human panel	Meso scale Discovery	MSD#; K15067M-1	Reuse	
Critical commercial assay	R-PLEX human ferritin	Meso scale Discovery	MSD#; F21ADA-3	Reuse	
Other	Meso Scale Discovery lysis buffer	Meso Scale Discovery	CAT # R60TX-3	Reuse	
Other	Protease inhibitor	Roche	CAT # 04693116001	Reuse	
Other	CosMx™ single image of slide to visualize morphology markers	Zenodo		New	Bolen CosMx human morph image is an image of the total tissue microarray (TMA).

relative density plot revealed high abundance in colonocytes, and M2 macrophages and neutrophils in a disease-specific manner, with PD being the highest (Fig. 2F). Gene ontology (GO) biological process (BP), cellular component (CC) and molecular function (MF) analysis revealed several cellular mechanisms and resulting products that were significantly down- or up-regulated (Fig. 3). IBD epithelial cells displayed a

marked decrease in expression of genes involved in cell chemotaxis, cytokine activity, leukocyte migration (Fig. 3A: blue); and a significant increase in genes involved in amoeboid cell migration, response to extracellular stimulus and oxygen levels, as well as inflammatory responses (Fig. 3A: red). Epithelial cells from those living with PD displayed enrichment in GO pathways associated with neurogenesis, glial

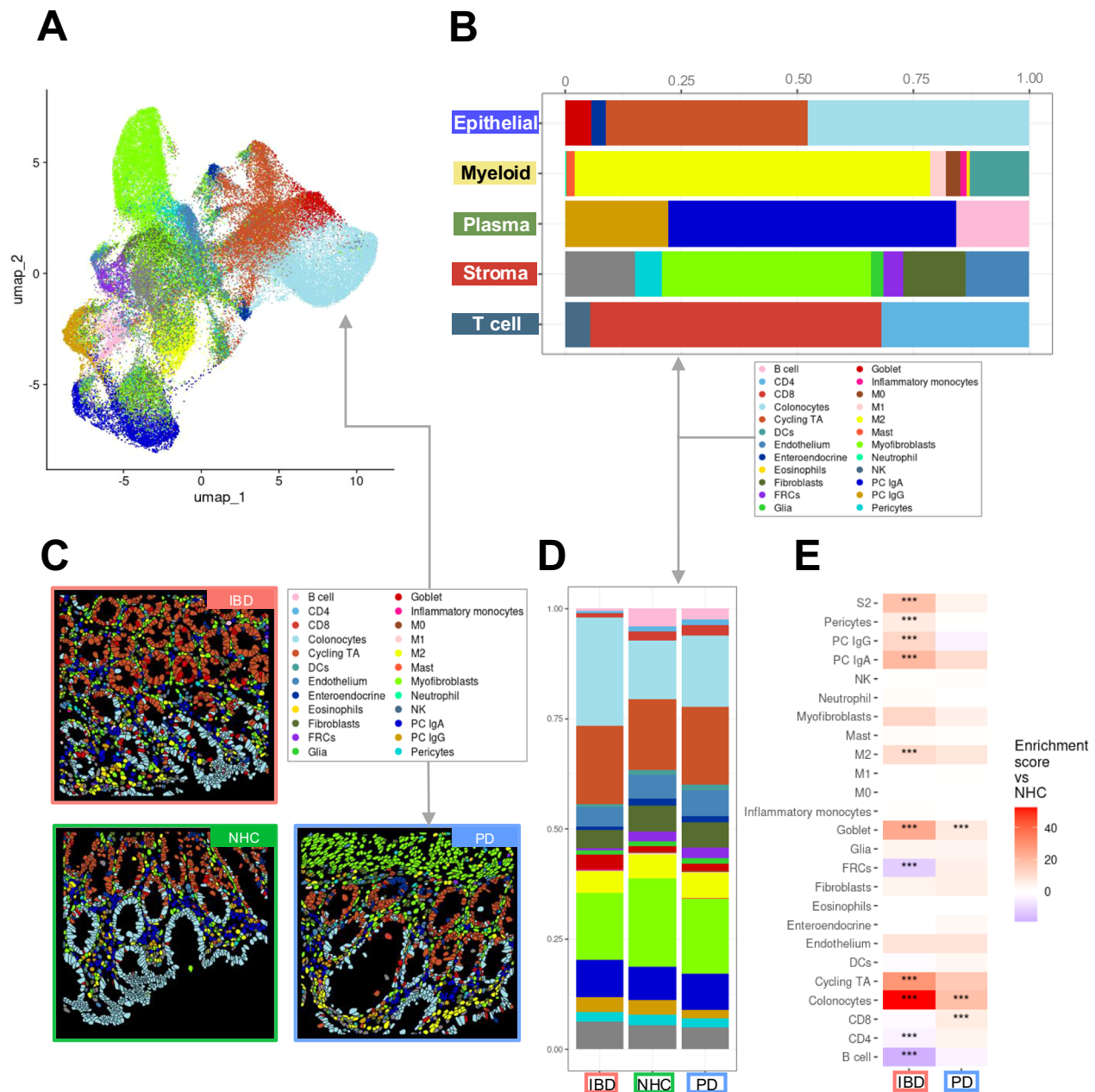


Fig. 1 | Spatial molecular imaging (SMI) enables deep immune-phenotyping of sigmoid colon biopsies from those living with inflammatory bowel disease (IBD), Parkinson's disease (PD), or neurologically healthy controls (NHC). **A** In situ uniform manifold approximation and projection (UMAP) clustering of all cells identified within sigmoid colon tissue samples ($N = 13$ IBD, $N = 12$ PD, $N = 8$ NHC). Cells are colored by all annotated refined clusters identified (epithelial, myeloid, plasma, stroma and T cells). Cells were only included if the probability of being correctly annotated within the respective cluster was greater than 0.75. **B** Bar plots of SMI data are colored by group cluster identifier and shaded by refined subset population within each

primary cluster. Each bar plot represents the proportion of refined subpopulation in each primary cluster of cells. **C** CosMx™ SMI visualization indicating cell-type-specific spatial distribution of all refined populations annotated. **D** Bar plot representation of refined subcluster proportion of cells by group. Source data may be found in Supplemental Table 2. **E** Heatmap depicting cell-type abundance by IBD (red, x-axis) or PD (blue, x axis) as compared to NHC. Statistical analysis completed by Chi-Square (χ^2) test with p value adjustment using Benjamini–Yekutieli test. Only results with a fold change >1.5 or <0.66 are considered significant after adjustment. * = adjusted p value <0.05, ** = adjusted p value <0.01, *** = adjusted p value <0.001.

cell development and gliogenesis, as compared to NHC (Fig. 3B: red). IBD colonocyte GO pathways indicated depletion in chemotaxis signaling, leukocyte proliferation, and cytokine activation with an increase in regulation of response to external stimulus and amoeboid cell migration (Fig. 3C). Additionally, colonocytes from those living with IBD but are in endoscopic remission revealed a significant increase in gene expression involved in the negative regulation of inflammatory and general immune response (Fig. 3C: red). GO pathway analysis of PD colonocytes revealed significant depletion in genes involved with cell adhesion, T-cell migration, and myeloid differentiation (Fig. 3D: blue). Additional GO

pathways evaluating thematic gene enrichment of PD colonocytes revealed significant enrichment in genes associated with response to external stimulus, T-cell activation and leukocyte proliferation, with a decrease in migratory regulation and chemokine activity (Fig. 3D). Collectively, these data suggested a dampened immune activation status characterized by dampened cytokine expression in colonic leukocytes (i.e., neutrophils, lymphocytes, monocytes, eosinophils and basophils) (Fig. 3). These data suggested the presence of an inflammatory phenotype characterized by deficient expression of genes that promote and sustain immune-cell recruitment at the level of the sigmoid colon in those living

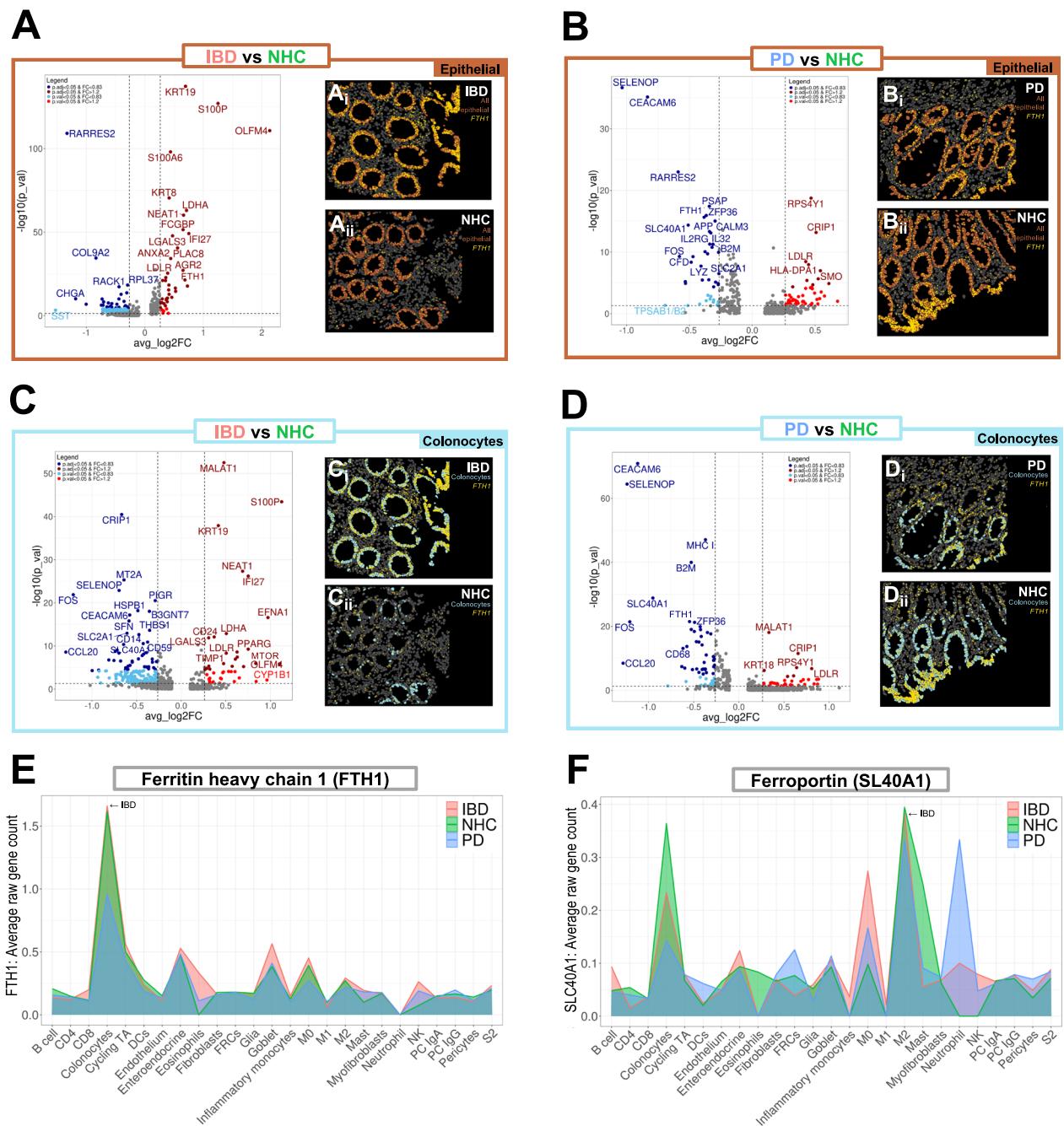


Fig. 2 | Epithelial gene profiles reveal enhanced inflammatory regulation in IBD and reduced inflammatory regulation in PD colonic biopsies relative to NHC. A–D Volcano plots of differential gene expression between IBD (red), PD (blue) or NHC (green) in all epithelial cells (brown box) or colonocytes (light blue box). Light blue indicates p value < 0.05 & FC < 0.83 , dark blue indicates adjusted p value < 0.05 & FC < 0.83 , light red indicates p value < 0.05 , and dark red indicates adjusted

p value < 0.05 & FC > 1.2 . A–D (i and ii) Visual spatial representation of one representative FOV from each disease group of epithelial cells (brown) or colonocytes (light blue) with gene counts of ferritin heavy chain 1 (*FTH1*) spatially resolved in yellow. E, F Cell-type-specific density plot by average raw gene count of ferritin heavy chain 1 (*FTH1*) or ferroportin (*SLC40A1*) by disease group (IBD red, NHC green, PD blue).

with PD. Several other pathways appeared within GO pathway analysis as significantly upregulated in IBD and downregulated in PD (Fig. 3), however they are not discussed herein in great detail due to being outside of the scope of this manuscript.

Spatial transcriptomics analysis reveals dysregulation in cell–cell crosstalk and neighborhoods at the level of the sigmoid colon in both IBD and PD

When assessing cell–cell communication, SCOTIA was used to generate chord plots and leverage ligand–receptor spatial analysis between source and target cells. Colonocytes positive for *FTH1* (green) or colonocytes

negative for *FTH1* (blue) from individuals living with IBD in endoscopic remission, PD, or NHC were assessed based on frequency of ligand–receptor interactions with other cells (Fig. 4A). Number of colonocyte cell–cell interactions was then quantified, wherein we found that there were significantly more interactions between *FTH1*-positive (*FTH1*+) cells and other cell-types in IBD and PD colonic biopsies, but not NHC (Fig. 4B). Spatial connection maps displayed representative FOVs of colonocyte communication to *FTH1*+ cells (blue) and colonocyte communication to *FTH1*-negative (*FTH1*–) cells (red) (Fig. 4Ci, ii). Those living with IBD and PD had significantly more *FTH1*+ colonocyte ligand–receptor interactions with other cells than *FTH1*+ colonocytes from NHC (Fig. 4A, C,

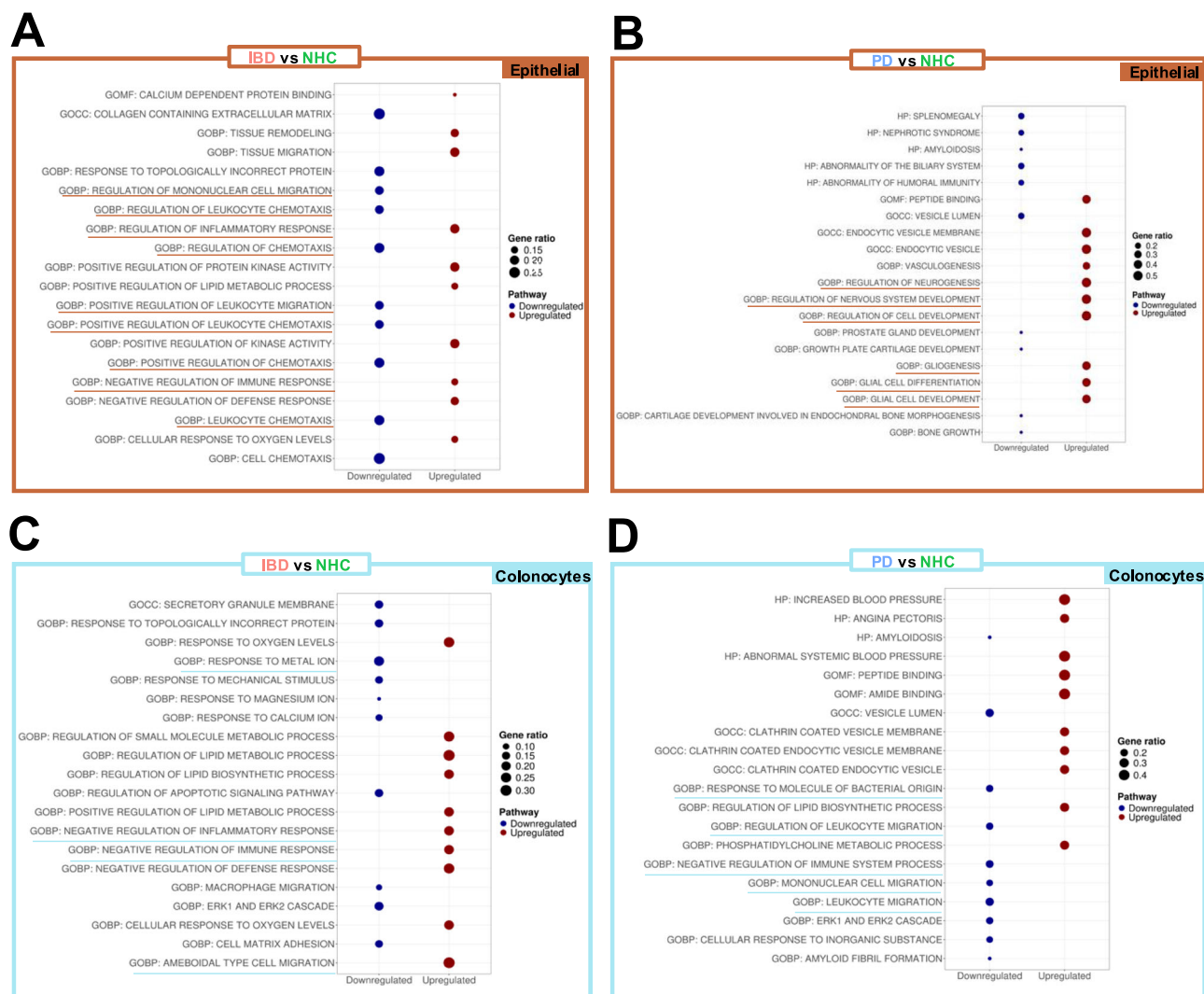


Fig. 3 | Colonocytes gene ontology pathway profile indicate enhanced inflammatory regulation in IBD and reduced inflammatory regulation in PD relative to NHC. A–D Gene Ontology (GO) Human Category 5 specific pathways are compared between IBD, PD and NHC within epithelial (brown box) or colonocyte (blue box) populations to understand the mechanism behind broader pathway

implications. The top 10 upregulated and downregulated pathways are displayed, ranked via the most significant adjusted p value (adjusted via false discovery rate, Benjamini–Yekutieli procedure). BP biological process, MF molecular function, and CC cellular component.

Ci). Additionally, colonocytes that are *FTH1* + in those living with IBD that are in endoscopic remission displayed the most significant depletion in *CRP1* (cysteine-rich protein 1), *MT2A* (metallothionein 2 A), and *FOS* (Fos proto-oncogene, AP-1 transcription factor subunit) (Fig. 4D), as compared to NHC, all of which are immune and stress response genes that should aid in an anti-inflammatory protection response^{21–23}, suggesting aberrant mucosal homeostasis. Colonocytes that are *FTH1* + in those living with PD displayed a significant depletion in *MHC I*, as compared to NHC, which is involved in antigen presentation to cytotoxic T cells²⁴ (Fig. 4Di). Colonocytes positive for *FTH1* in those living with IBD in endoscopic remission or those living with PD displayed a significant depletion of *CLDN4* (claudin-4), a tight junction protein known to be reduced in IBD patients and as a result of depletion, increased mucosal barrier permeability²⁵. Additionally, *SLC40A1*, the primary iron export gene, was significantly depleted in colonocytes positive for *FTH1* in those living with IBD in endoscopic remission or those living with PD (as compared to NHC); indicating iron may be trapped within these cells. In support of this, *CCL20* (C-C motif ligand 20), which is known to significantly decrease in the presence of excess iron²⁶, was significantly depleted in colonocytes positive for *FTH1* in those living with IBD in endoscopic remission or those living with PD, as compared to NHC.

Inflammatory factor immunoassays reveal a dysfunctional immune phenotype in both IBD and PD cohorts relative to NHC

One critical function of cytokines and chemokines is to recruit adaptive immune cells to sites of injury²⁷. Multiplexed immunoassays on the Meso Scale Discovery (MSD) platform were leveraged to assess cytokine and chemokine signaling in the blood and stool from the same cohort of participants. It is important to note that the stool and blood biosample cohorts are slightly larger and not identical to the colonic biopsies due to sample availability. C-C motif chemokine 22 (CCL22), also known as macrophage-derived chemokine (MDC), displayed a significant DC-released T-cell recruitment protein, and was significantly depleted in plasma from those living with PD as compared to NHC (Fig. 5A). A linear regression analysis identified a significant negative relationship between CCL22 protein abundance and PD disease duration where individuals with longer disease duration had reduced levels of CCL22 ($R^2 = 0.32$, $p = 0.03$) (Fig. 5Ai). Linear regressions assessing the relationship between CCL22 content and ferritin protein abundance separated by disease group revealed a substantial relationship in IBD ($R^2 = 0.60$, $p = 0.0020$) and PD ($R^2 = 0.51$, $p = 0.006$) plasma samples, where individuals with low CCL22 also had low ferritin, but this relationship did not exist in NHC ($R^2 = 0.19$, $p = 0.21$) (Fig. 5Aii). CCL22 total protein abundance was also measured in stool samples from the same

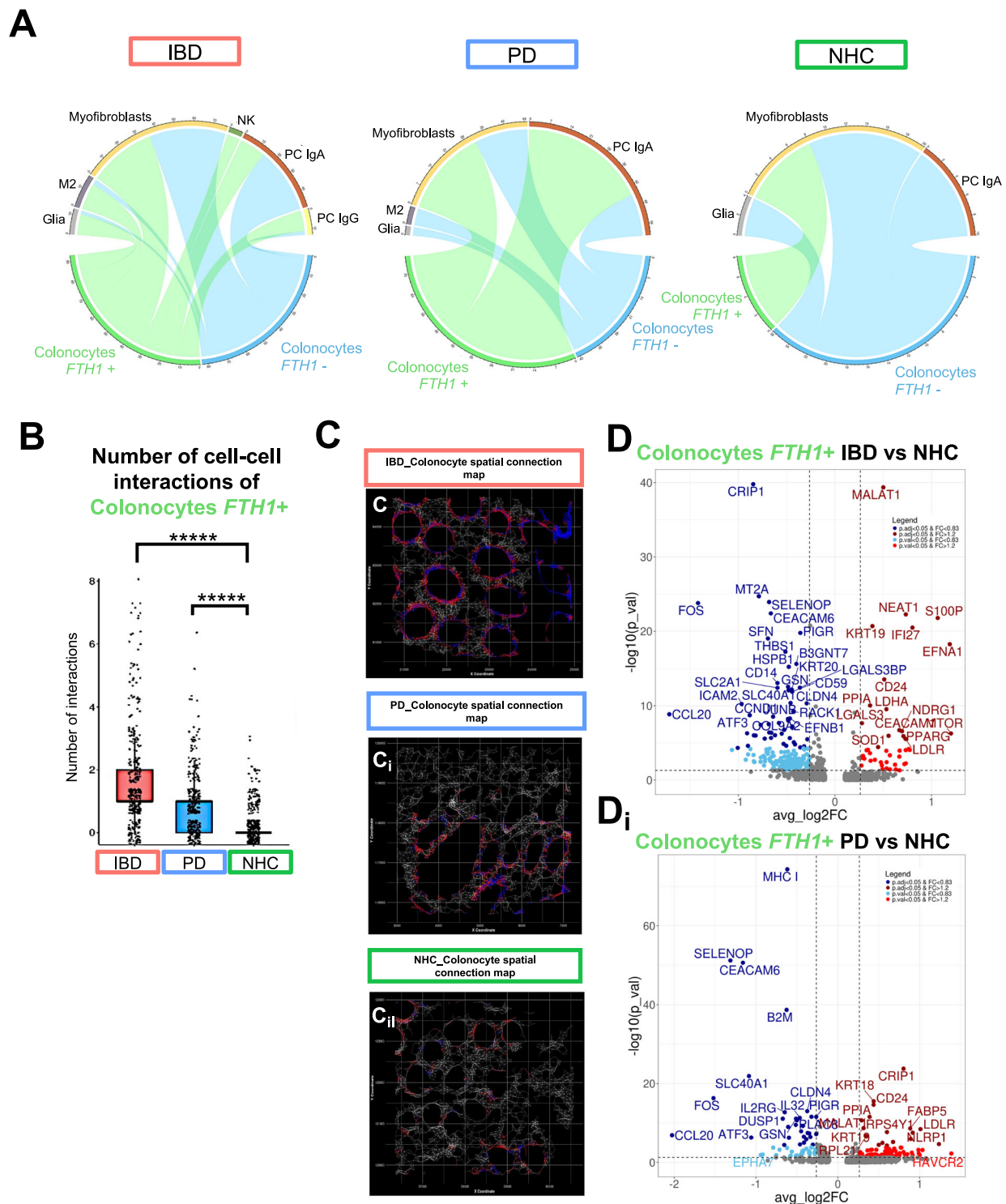


Fig. 4 | Spatial distribution of immune cells differs between IBD and PD relative to NHC. A, Aii Cell-cell communication of colonocytes, either positive for ferritin heavy chain 1 (*FTH1*) (green) or negative for *FTH1* (blue) ligand-receptor interactions to all other refined cell populations based on group, generated via SCOTIA ligand-receptor analysis. **B** Bar plots depicting the number of significant interactions between the number of colonocytes that are *FTH1* + interacting with other cells by group. **C, Cii** Gene spatial connection map, white lines represent all communication

between all cells within 25 μ M radius, red = colonocytes connection to a ferritin heavy chain 1 (*FTH1*) negative cell, blue = colonocyte communication to *FTH1* positive cell. **D, Di** Volcano plots of differential gene expression between IBD colonocytes positive for *FTH1*, as compared to NHC and PD colonocytes positive for *FTH1*, as compared to NHC. Light blue indicates p value < 0.05 & FC < 0.83, dark blue indicates adjusted p value < 0.05 & FC < 0.83, light red indicates p value < 0.05, and dark red indicates adjusted p value < 0.05 & FC > 1.2.

participants (Fig. 5B). CCL22 was significantly depleted in stool samples from those living with IBD as compared to NHC (Fig. 5B). Linear regression analysis did not identify any relationship between CCL22 protein abundance and PD disease duration ($R^2 = 0.21$, $p = 0.09$) (Fig. 5Bi). Linear

regressions assessing a relationship between CCL22 content and ferritin protein abundance parsed by disease group revealed a significant relationship in IBD ($R^2 = 0.56$, $p = 0.0020$) and PD ($R^2 = 0.38$, $p = 0.014$) and NHC ($R^2 = 0.42$, $p = 0.042$) where individuals with low CCL22 also had low

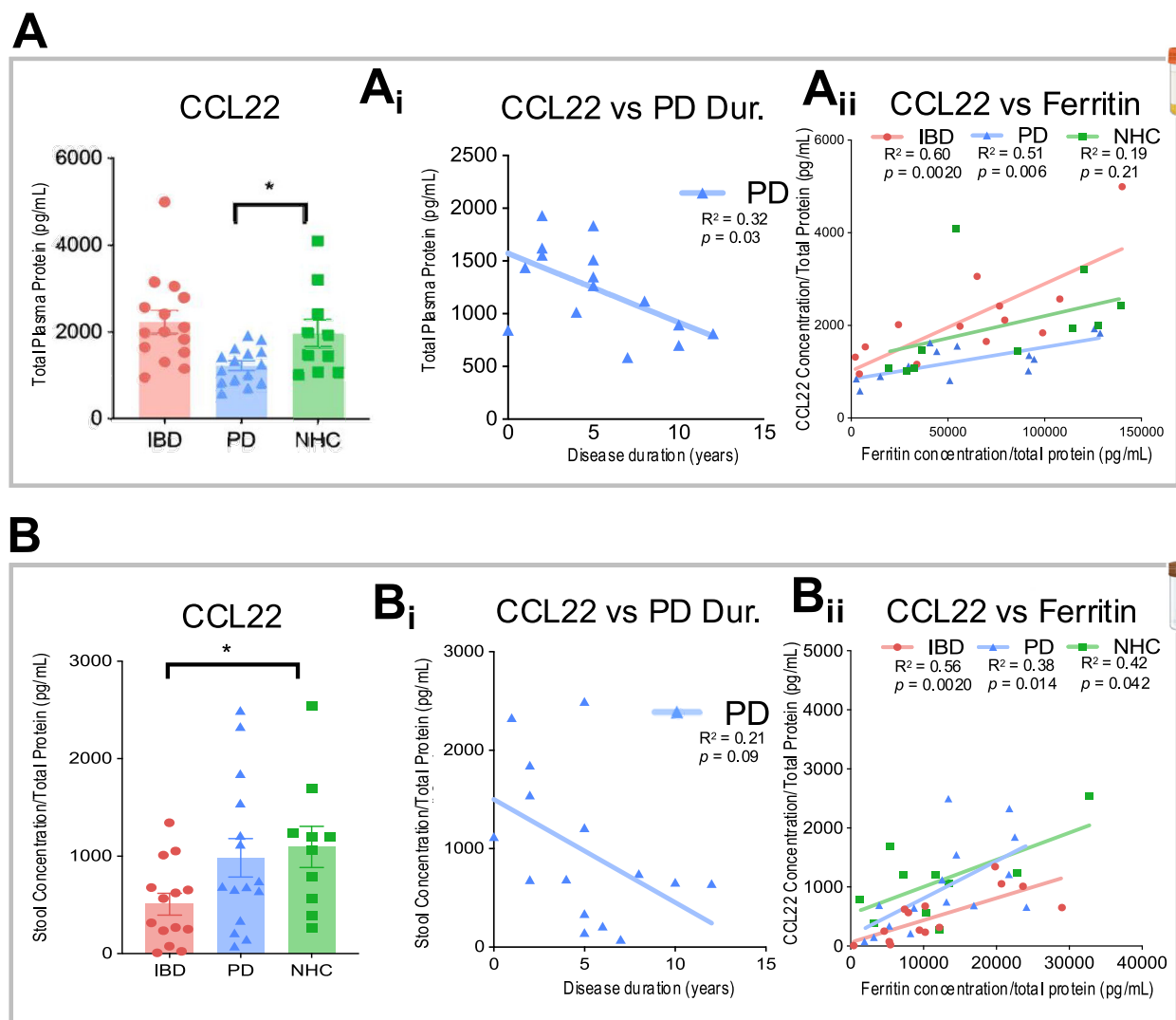


Fig. 5 | Metabolic analyses reveal alterations in markers of chemotaxis in plasma and stool in individuals living with IBD or PD relative to NHC. A, Aii In plasma samples ($N = 15$ IBD, $N = 14$ PD, $N = 10$ NHC), C-C motif chemokine ligand 22 (CCL22) is significantly depleted in PD as compared to NHC. There is no significant change observed between IBD and NHC. **B, Bii** In stool samples ($N = 15$ IBD, $N = 14$ PD, $N = 10$ NHC), CCL22 is significantly depleted in IBD as compared to NHC.

There is no significant change observed between PD and NHC. Linear regressions identify a significant correlation between an increase in PD disease duration) and a decrease in CCL22 in plasma from those living with PD. Linear regression also displays a significant relationship with an increase in CCL22 load and an increase in ferritin load in both IBD and PD patients that does not appear in NHC ($* = P < 0.05$). $* = p$ value < 0.05 , $** = p$ value < 0.01 , $*** = p$ value < 0.001 , $**** = p$ value < 0.0001 .

ferritin protein across all participant groups (Fig. 5Bii). The full panel of cytokines and chemokines completed on the MSD platform is listed within the Methods.

Single-cell targeted proteomic analysis successfully integrates with scRNA-seq and depicts multimodal immune dysregulation across multiple biological outputs of human sigmoid colonic biopsies

To obtain the highest quality cell-type annotation of the targeted CosMx[™] SMI proteomics dataset, we integrated an existing single-cell RNA sequencing (scRNA-seq) dataset of human intestine biopsies⁸ using MaxFuse²⁸ (FOV visualization Fig. 6A, B; Supplementary Fig. 4; Supplementary Fig. 5). Weak links between protein and RNA overlay were chosen to generate UMAP clustering of 5 cell subpopulations (T cells, plasma cells, fibroblasts, epithelial cells and B cells) (Fig. 6Bi; Supplementary Fig. 6). Additionally, abundance analysis identified significant enrichment of epithelial, plasma and T cells in those living with PD or IBD, as compared to NHC (Fig. 6C). To interrogate the cell-cell communication, SCOTIA ligand-receptor analysis was leveraged to identify

potential cell-type-specific interactions. This analysis revealed an enrichment in communication between T cells in both those living with IBD or PD to epithelial cells as compared to NHC (Fig. 6D). We then analyzed immune checkpoint markers known to regulate immune responses in the context of inflammation and cancer²⁹ and found a significant difference in PD-1 as well as CTLA4, GITR, GZMA, Ki-67, LAG3 and TIM3 in T cells housed in the colon of individuals living with PD, as compared to NHC (Fig. 6E). In contrast, T cells from those living with IBD in endoscopic remission only displayed a significant difference in PD-1, GZMA, LAG3 and TIM3 (Fig. 6E).

Discussion

It is known that the gut microbiome and health of the GI tract directly shape the circulating immune system¹⁴. Peripheral immune dysfunction influences blood-brain barrier permeability and can thereby compromise brain function via alterations that include chemotactic signaling^{18,30,31}. Individuals living with PD commonly report GI dysfunction decades prior to the onset of motor symptoms². Additionally, individuals living with PD have been reported to have a dysbiotic gut microbiome distinct from NHC^{32–35}. IBD is a

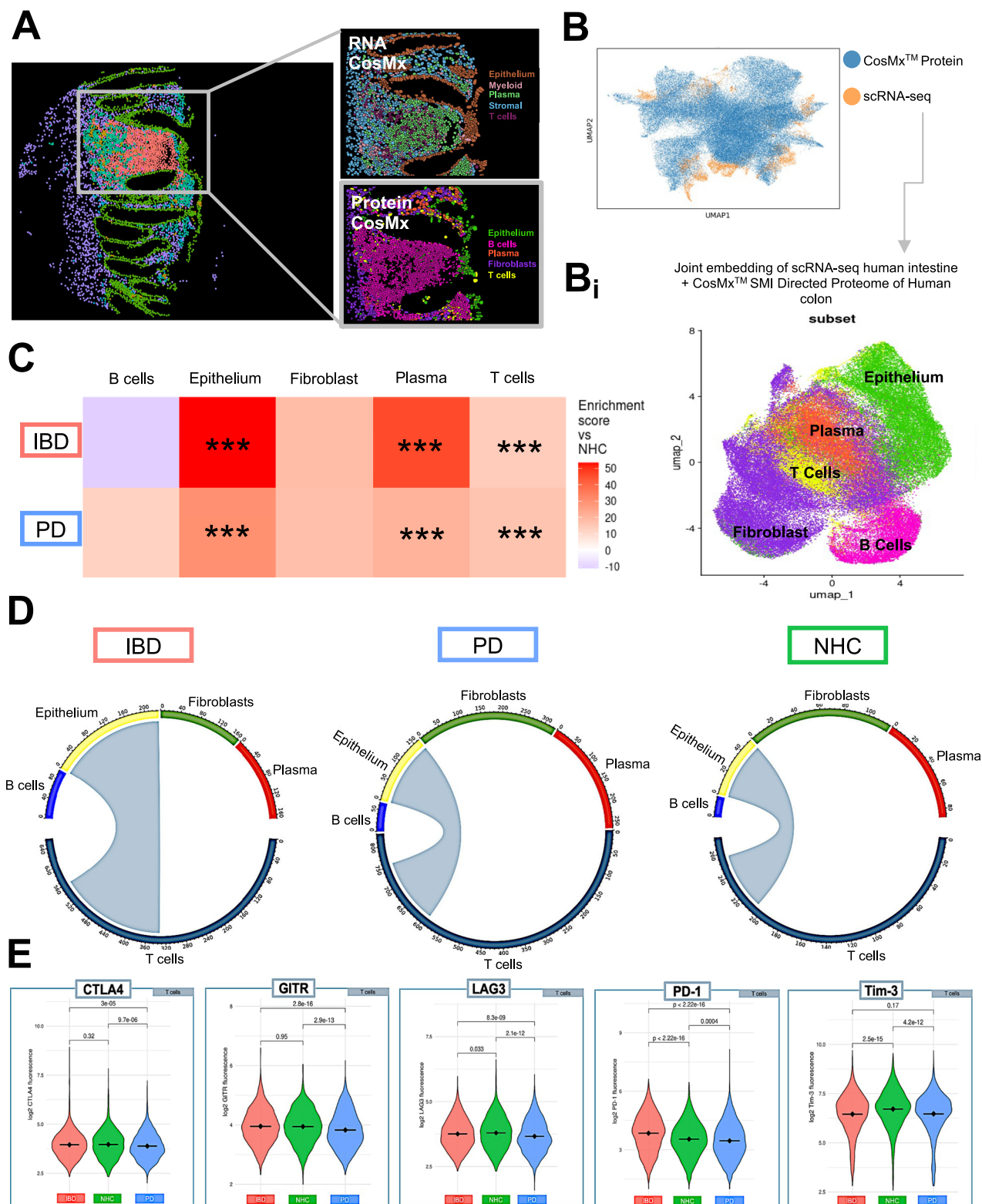


Fig. 6 | Integration of CosMx™ SMI proteomic and transcriptomic profiles reveals alterations in epithelial and T-cell communication. **A** CosMx™ protein and CosMx™ RNA images depicting the spatial distribution of all annotated subpopulations. **B** Uniform manifold approximation and projection (UMAP) of individual cell distribution and dataset overlap between scRNA-seq (dataset obtained from Garrido-Trigo et al., 2023) and protein dataset. **Bi** UMAP combining scRNA-seq and CosMx™ SMI data to link gene expression to protein regulation and annotate cell-types. Gene and protein datasets were linked using MaxFuse. **C** Heatmap depicting cell-type abundance by IBD or PD as compared to NHC. Statistical

analysis completed by Chi-Square (χ^2) test with p value adjusted using Benjamini–Yekutieli. Only results with a fold change >1.5 or <0.66 are considered significant after adjustment. * = adjusted p value <0.05 , ** = adjusted p value <0.01 , *** = adjusted p value <0.001 . **D** T cells (gray) are the source, and epithelial cells are the target, based on inflammatory disease presentation. Chord plots generated via SCOTIA analysis. **E** Violin plots comparing raw fluorescence of individual proteins expressed in T cells, parsed by disease group. Violin plot significance values are generated via Wilcoxon test with Bonferroni p value correction.

commonly occurring condition in the US¹¹, which catalyzes chronic systemic inflammation and has been epidemiologically linked to PD^{36–38}. To investigate the molecular underpinnings of this association, we analyzed the microenvironments of the colonic luminal lining, peripheral blood and stool from individuals with IBD in endoscopic remission, or with idiopathic PD, or NHC to determine the extent of shared versus unique pathways that could shed light on potential molecular mechanisms linking mid-life chronic gut dysfunction arising from IBD to the increased risk for development of PD later in life.

First, single-cell targeted transcriptomic profiles of sigmoid colonic biopsies revealed cell-type-specific phenotypes unique to PD and IBD. Second, differential gene expression analyses of these clusters identified inflammatory pathways associated with immune signaling dysfunction in both PD and IBD colonic biopsies, as compared to NHC. Third, spatial transcriptomics analysis of these colonic biopsies revealed dysregulation in cell-cell crosstalk and neighborhoods in both IBD and PD, which were, importantly, confirmed and validated at the protein level by proteomic analysis. Finally, multiplexed inflammatory factor immunoassays from stool and plasma revealed a unique immune phenotype in both IBD (stool) and PD (plasma) cohorts, where CCL22 correlated with both PD disease duration and ferritin load, as compared to NHC. Together with literature describing alterations in the abundance of circulating adaptive immune cells in those living with IBD³⁹ or PD^{40,41} and shared depletion of key short-chain fatty acid (SCFA)-producing gut bacteria in IBD and PD⁴², our novel findings suggest that a subset of individuals living with IBD may be at increased risk for PD pathogenesis due to chronic systemic inflammation, likely initiated in the GI tract due to gut dysbiosis and loss of SCFA-producing microbes. Specifically, the chronic peripheral (blood and gut) inflammation in IBD would be expected to impair the ability of the peripheral immune system to mount proper responses to antigenic challenges over time, which is a critical requirement to distinguish between self and non-self and maintain immune tolerance. It is well-known that inflammatory challenges in vivo hasten upregulation of α -synuclein in various cell-types^{43–46}, and in the case of α -synuclein-expressing enteroendocrine cells, increasing the likelihood of transfer and propagation to enteric ganglia and vagal efferent connections where it becomes pathogenic⁴⁷.

Although the mucosal lining is the largest immune-antigen interface in the body⁴⁸, to our knowledge, this is the first single-cell spatial resolution investigation of the colonic immune mosaic from those living with PD or IBD to look for mechanism-based clues to support the gut-first hypothesis of PD and explain the epidemiological association between these conditions.

As a result of GI injury and resulting intestinal epithelial breakdown, up to 79% of IBD patients 18–25 years of age and 90–100% of patients >65 years of age experience iron dysregulation⁴⁹. Iron dysregulation is also observed in PD, where total iron content in the brain often increases^{50–52} and a reported decrease in ferritin is observed in the blood^{53,54}. These data suggested to us that iron mishandling may also link these two chronic systemic diseases of inflammation⁵⁵. Therefore, we herein further characterized this mechanism at the only anatomical site of iron absorption—the gut mucosal lining. Additionally, a recently published study identified enrichment of ferroptotic pathways in gut-innervating neurons upon inflammatory stimuli that mimic colitis⁵⁶. Our findings revealed disease-specific differences in transcript accumulation for *FTH1*, the ferritin heavy chain gene critical for primary iron storage, within several immune cell-types (Fig. 2E); specifically, we detected significantly increased transcript accumulation of *FTH1* in epithelial cells of IBD patients and significantly decreased in epithelial cells of PD patients compared to NHC (Fig. 2A, B). We also observed the largest difference in *FTH1* accumulation in colonocytes, a subtype of epithelial cells (Fig. 2). We speculate this finding may be related to the inflammatory peripheral stress response associated with PD, which can directly support systemic iron withdrawal by limiting dietary iron absorption⁵⁷. Additionally, *SLC40A1*, which encodes ferroportin, the primary iron exporter that releases iron into circulation for systemic use, was significantly downregulated in colonocytes from both those living with IBD and PD, as compared to NHC (Fig. 2F). We posit there are multiple catalysts that

contribute to *FTH1* enrichment and *SLC40A1* depletion in colonocytes. One possibility for *SLC40A1* depletion is as a possible consequence of the host defense response to gut inflammation^{58,59}. *SLC40A1* depletion in colonocytes could also indicate a response to the energy demands of rapidly proliferating epithelial cells^{60,61}. Interestingly, our dataset also revealed that neutrophils in the PD gut are loaded with *SLC40A1* (Fig. 2F), which would give them the ability to dump any iron picked up in the gut as they travel to other organs (including the brain) or into the circulation. Given that plasma iron is often found to be deficient in PD, we speculate the iron accumulation in the PD brain may be the result of peripheral immune cells (such as neutrophils) that have a limited ability to export iron via a decrease in *SLC40A1* expression, infiltrate into the CNS, which has been reported in PD animal modeling⁶². At the single-cell level, we found that those living with IBD (in endoscopic remission) and PD had significantly more *FTH1*+ colonocytes interacting with other cell-types, in particular IgA-producing plasma cells, relative to NHC colonocytes, which primarily interacted with myofibroblasts (Fig. 4A, C–Ci). Additionally, colonocytes that were *FTH1*+ in those living with IBD in endoscopic remission displayed significant enrichment in *MTOR* and *LGALS* transcripts (Fig. 4D), indicating active inflammatory signaling. Colonocytes that were *FTH1*+ in those living with PD displayed a substantial depletion in *MHC I*, as compared to *FTH1*+ colonocytes from NHC. *MHC I* expression is critical for adaptive immune-cell recruitment, specifically cytotoxic CD8 T-cell differentiation⁶³. It is known that depletion in *MHC I* expression can indicate an impaired immune response specifically via dysregulation of immune surveillance and altered T cell recruitment^{64–66}. Therefore, altered *MHC I* expression in *FTH1*+ epithelial cells could be an indicator of a decreased ability to initiate immune cell recruitment, decreasing the peripheral immune response to fight against infection or invasion of opportunistic pathogens in individuals living with PD. In parallel with the dysregulated immune phenotype displayed in subsets of colonocytes by spatial transcriptomics, GO pathway analyses revealed both shared and distinct features in IBD versus PD relative to NHC. Specifically, while there was shared upregulation of lipid biosynthetic processes in IBD and PD colonocytes, IBD colonocytes displayed upregulation of pathways involved in negative regulation of immune and inflammatory responses relative to NHC, while PD colonocytes displayed downregulation of the same processes relative to NHC (Fig. 3).

Chronic inflammation associated with tumor microenvironments triggers immune cell dysfunction—where immune cell function greatly decreases, and over time cells become unresponsive to invading pathogens and other stimuli⁶⁷. Interestingly, we identified a depletion in a key T-cell recruitment chemokine CCL22 in IBD stool and PD plasma, as compared to NHC (Fig. 5). The decrease in CCL22 expression in stool and plasma may be the result of dysregulation of immune-related proteins in colonic T-cell populations in IBD (in endoscopic remission) and PD, as compared to NHC (Fig. 6E). These markers of reduced immune competence may contribute to increased susceptibility to the effects of chronic infections and risk for age-related disease^{68–71}. Adaptive immune cells rely on iron metabolism to perform effector functions—such as proliferation and migration⁷². Circulating T cells from the blood of individuals living with PD have been reported to display decreased migratory capacity and proliferative phenotypes⁷³. Interestingly, iron-deficient mice display a reduction in T-cell proliferation and an impaired immune response that is not recapitulated in mice experiencing iron overload⁷⁴. Furthermore, iron dyshomeostasis can promote a leaky gut as well as impair immune function; this may account for the phenotype of immune signaling dysfunction within T cells we observed near the epithelial barrier (Fig. 6C–E). It is known that chronic systemic inflammation increases the risk of PD⁷⁰. One possible hypothesis is the downregulation of the adaptive immune response, which could be a protective peripheral mechanism to deplete a chronic cytotoxic inflammatory response.

In summary, it is known that the clinical manifestations of PD can remain dormant for decades and appear long after non-motor symptoms start. We speculate that chronic immune activation in the gut due to gut

dysbiosis, invading pathogens, pesticide/toxicant exposure, IBD, and/or diets rich in carbohydrates represent environmental triggers that synergize in a subset of individuals to promote a leaky gut. This can induce the dampening of an immune response and is further catalyzed by iron mis-handling, which can result in inappropriate immune signaling along the gut-brain axis in individuals at risk for the development of PD⁷⁵. It is important to note that while the IBD colonic biopsies were obtained at a time when the participant was not undergoing a flare-up, remittent gut inflammation experienced throughout the disease course of those living with IBD is associated epidemiologically with an increase in PD risk^{7,76,77}. Additionally, there are shared features of a dysbiotic microbiome, specifically depletion in SCFA-producing bacteria, between individuals living with IBD or PD^{42,78}. We posit this association is due to both remittent gut inflammation and microbial dysbiosis that drives metabolomic alterations, which may contribute to increases in aggregated α -synuclein protein along the gut-brain axis.

Together, our data support a model in which sustained chronic inflammation in several peripheral compartments likely converges and creates the perfect storm that accelerates the transition from a prodromal pre-clinical pre-motor stage of PD to a clinical motor stage. These datasets set the stage for future investigation and therapeutic discovery that targets the integrity of the gut mucosal lining. It is important to note that the potential utility of gut immune phenotyping to identify individuals at high risk for development and/or progression of PD is still not clinically validated. Development of an SAA protocol for gut biopsy material is a pursuit of interest by multiple groups and will also enable crucial investigations into the direct effects of an inflamed gut microenvironment on α -synuclein pathology. Leveraging an IBD population that is not in endoscopic remission and/or including PD patients who report specific gut dysfunction prior to movement disorder onset will be critical for development and enrollment into prevention-based PD clinical trials. These findings bolster the investigation of peripheral inflammatory cascades that are likely to increase the risk for PD development and progression.

Methods

Participant recruitment

The University of Florida Institutional Review Board (IRB 201902459) reviewed and approved this study. Individuals living with PD were recruited from the University of Florida Neuromedicine Clinic at the Fixel Institute for Neurological Diseases. Individuals living with IBD who were already preparing for routine colonoscopy were recruited from the UF GI Clinic. All participants consented to this study and signed an informed consent document prior to sample collection. Participation within this cross-sectional study included inclusion criteria of reported age between 40 and 80 years, diagnosis of PD (based on MDS score), IBD (ulcerative colitis and/or Crohn's disease specified if applicable), or no diagnosis in the case of the NHC cohort. Individuals were excluded if they lived with PD and reported use of immunosuppressants for active infections or active autoimmune or chronic inflammatory conditions. Individuals were also excluded if they reported a history of antibiotic use within a month of recruitment. Additional exclusion criteria applicable included if an individual reported being pregnant, if the individual reported a history of a blood transfusion within 4 weeks of recruitment, or reported body weight <110 lbs. Additional health history collected included: assessment of disease duration, MoCA and Schwab scores, past or current endocrine dysfunction, and cancer history, age, sex, race, lifestyle habits such as smoking, over-the-counter or prescribed medication use. Primary selection criteria for this cohort included age, then sex matching as close to the NHC as possible; therefore, due to cost and assay size, not all patients who provided biopsies were used within this series of assays. Despite intense efforts toward recruiting a diverse population of participants, we were unable to obtain quality representation of non-white ethnic groups within our study. Future studies will focus on the recruitment of a more diverse population. Of importance and unique to this cohort, all IBD and PD participants within this

study were in endoscopic remission (not actively inflamed at the level of the gut), confirmed by UF GI clinic gastroenterologists.

Sample collection and preparation

Sigmoid colonic biopsies were collected via sigmoidoscopy in individuals with PD or NHC and via colonoscopy in individuals with IBD by a board-certified UF gastroenterologist via protocols approved by the UF IRB and fixed in formalin and paraffin-embedded (FFPE) to be used for CosMx™ SMI. Within these data, $N = 12$ PD biopsies, $N = 13$ IBD biopsies, $N = 8$ NHC biopsies (Supplementary Table 1; Supplementary Table 3). Two 5 mm FOVs were drawn per biopsy, aiming to obtain the most surface area of the total core. In brief, FFPE sample preparation for imaging included punching 1 mm tissue microarray (TMA) cores from each patient sample block to generate a TMA at 5 μ m section thickness and allow for all samples to be run on one slide in the same run with the same lot numbered reagents (Supplementary Fig. 5). A more detailed description of the protocol designed by the Tansey lab for TMA production using FFPE prepared tissue may be found in Table 1. Blood and stool samples were also collected from this participant cohort for protein analysis, however it's important to note that these additional biosamples do not include all of the same participants due to biosample availability. All protocols, software/code, and datasets may be obtained via the Open Science Key Resource Table located in Table 1.

Spatial molecular imaging (SMI) analysis: targeted transcript and proteomics

CosMx™ SMI: directed transcriptomics and proteomics. All protocols for single-cell spatial workflow were followed exactly to manufacturer instruction with no adaptation, as described in CosMx™ manual provided, as well as⁸. Via the use of multiplexed capture probes and reporter probes, NanoString CosMx™ can spatially resolve up to 1000 transcripts (CMx Hs Univ Cell Panel RNA Kit EA: CAT# 121500002) and 64 proteins (CMx Hs IO Panel Protein Kit: CAT# 121500010). A custom spike-in for the following 12 genes labeled following the HUGO gene nomenclature were included in the RNA assay: *SLC6A4* (solute carrier family 6 member 4), *CHGA* (chromogranin A), *SLC11A2* (solute carrier family 11 member 2), *FTH1* (ferritin heavy chain 1), *HAMP* (hepcidin antimicrobial peptide), *IREB2* (iron responsive element binding protein 2), *ITPKB* (Inositol-Trisphosphate 3-Kinase B), *NDUFB1* (NADH: Ubiquinone Oxidoreductase Subunit B1), *PINK1* (PTEN-induced kinase 1), *PYY* (peptide YY), *RAB8A* (RAB8A, member RAS oncogene family), *TF* (transferrin). It is important to note that due to assay restrictions, the protein sections are 5 μ m from RNA sections and thus were run on separate slides. We used morphology markers CD45, PanCK, and CD3 to visualize the tissue and picked two 0.5 mm fields of view (FOV) per sample (Supplementary Fig. 5). To obtain cell count matrices for each slide, cell boundaries were generated via morphology antibodies described above and localization of transcript expression, as previously described⁷⁹. Individual analysis pipelines for either RNA or protein are described below.

Quality control and processing of CosMx™ RNA. Cells were flagged if: the cell had <20 counts, if 10% of the counts were negative probes, total counts did not exceed the number of detected genes, the cell had <10 features, the cells had area outliers based on Grubb's test p value(0.01) and the cell had a mean of 0.5 negative counts. Flagged cells were then removed from downstream analysis. An average of 89.29% cells passed quality control (QC). After QC, a total of 75,170 cells were used in downstream analysis. Then SC Transform (Seurat package, version 5.1) was leveraged for normalization (Table 1).

Cell-type assessment and annotation for CosMx™ RNA. Supervised clustering was performed using an existing scRNA-seq intestine database⁸ as a reference. Immunoglobulins (Igs) were removed from downstream analysis due to high expression within all cell-types. Cell subset

classification was then performed using immunofluorescence data obtained from the morphology markers described above (PanCK, CD45, mean DAPI, mean CD68) as well as gene expression, and the reference scRNA-seq object using InSituType (version 1.0). Supervised clustering, then generated five primary clusters: epithelial, myeloid, plasma, stroma and T cell. Cells with 75% or more probability to be that specific subset were used for downstream analysis. Within each subset, the same clustering methodology was performed to identify refined (i.e., from T cells parsing CD4 and CD8) (Table 1).

QC and processing of CosMx™ Protein

Cells were used in downstream analysis if 50% or more of the proteins were in the 90th percentile or higher of expression. Additionally, cells were removed from downstream analysis if the cell contained <10 proteins that were also in the 50th percentile or lower of expression. Cells were also removed from downstream analysis if the cells' negative probe threshold was lower than 2 and above the upper threshold of 15 and/or contained area outliers based on Grubbs' test p -value (0.01). After QC, 51.25% of cells were kept, which resulted in 77,057 total cells. For cell normalization, average total intensity scaling and arsinh transformation with a cofactor of 50 were implemented.

Cell-type assessment and annotation for CosMx™ protein. Due to the size of the CosMx™ Human Protein Immuno-Oncology (64 total proteins) panel and to provide information from multiple biological modalities, we used MaxFuse²⁸ to integrate an existing scRNA-seq dataset of the intestinal mucosa from NHC⁸ to most accurately annotate cell types. Annotations were then curated by using only the weak links between the 63 proteins and genes present in the scRNA-seq dataset (Supplementary Fig. 4). These data, as well as detailed annotation methods, are available in Table 1.

Abundance analysis. Aggregation of total cell counts per patient was leveraged to identify the abundance of cell-type by cluster by health status (Fig. 1E, RNA; Fig. 6C, protein). Cluster frequencies were compared using Chi-squared tests (χ^2) for enrichment and the Wilcoxon rank-sum test to account for patient variability. Fold changes were calculated, and p -values were adjusted via the Benjamini–Yekutieli method (adj $p < 0.05$) where significant clusters were annotated with a FC > 1.5 or < 0.66 (Fig. 6C).

Cell-to-cell interaction. CellTalkDB (version 1.0) was accessed to understand ligand–receptor interactions. Then the SCOTIA python package (version 3.13) was used to obtain cell–cell communication by disease (Fig. 4B, D, F RNA; Fig. 6D protein; Table 1).

Multiplex immunoassays on MSD platform. Protein was isolated from stool samples using MSD lysis buffer (MSD; R60TX-3), 1 tablet complete protease inhibitor (Roche; CAT # 04693116001) and 5 mm stainless steel bead (Qiagen). After solids were removed by separating protein supernatant, protein concentration was determined via the BCA Protein Assay Kit (ThermoFisher scientific; CAT# 23225), according to the manufacturer's instructions. Duplicates of stool and plasma (25 μ l) were diluted 1:1 and used to quantify chemokines and cytokines via U-PLEX custom pro-inflammatory human panel (Eotaxin, Eotaxin-3, IFN- γ , IL-1 β , IL-6, IL-8, CCL22 (MDC), TNF- α (MSD#; K15067M-1) and R-PLEX human ferritin (MSD#; F21ADA-3) on the Quickplex MSD instrument, according to the manufacturer's protocol. The MSD data are openly available on Zenodo under Tansey lab MSD stool (Table 1) and Tansey lab MSD plasma (Table 1).

Data availability

The data, code, protocols, and key lab materials used and generated in this study are listed in a Key Resource Table alongside their persistent identifiers in Table links located in Table 1. Additionally, these data are also accessible via the ASAP CRN Cloud dataset at <https://doi.org/10.5281/zenodo.17917771>.

17917788 (RNA) and <https://doi.org/10.5281/zenodo.17917771> (protein), expected to release in early 2026.

Code availability

Code for custom CosMx™ SMI pipelines and figure generation are accessible via Github: <https://github.com/ibd-bcn/CosMx-Bolen> (Table 1). All Code for scRNAseq used for cell phenotyping is available on the Salas lab Github (Table 1).

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Author contributions

M.L.B. wrote and conceived the manuscript with associated assays, as well as interpreted data and generated figures. MB performed computational analysis for CosMx™ SMI. J.S. trained M.L.B. and aided in the CosMx™ SMI workflow as well as optimized experimental CosMx™ SMI design. H.S. trained M.L.B. on MSD and optimized the experimental design of MSD. J.M.K. reviewed the manuscript and helped with CosMx™ SMI workflow. P.A.E. provided funding support and helped with sample preparation for the assay. I.G.P. and R.N. helped with experimental design and sample preparation. S.D.A. helped obtain samples during recruitment. Q.S.X. helped optimize TMA and prepare colonic tissue for CosMx™ SMI. N.R.M., E.M.Z., and C.E.F. helped obtain samples and funding during participant recruitment. K.B.M. helped with all experimental design, revised and edited the manuscript. A.S. supervised and advised data analysis for CosMx™ SMI and reviewed the manuscript. EAT supervised and advised the assay workflow of CosMx™ SMI. M.G.T. conceived the idea, supervised and advised experimental design and data analysis, as well as reviewed and edited the manuscript. All authors read and approved this manuscript.

Competing interests

M.G.T., the corresponding author, is editor-in-chief of NPJ PD. M.G.T. was blinded from the evaluation of this manuscript by NPJ PD for objective acceptance and approval.

Additional information

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