Multi-omics phenotyping characterizes molecular divergence underlying different clinical scenarios of inflammatory bowel disease

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ABSTRACT (270 words)

Background: Clinically heterogeneous spectrum and molecular phenotypes of inflammatory bowel disease (IBD) remain to be comprehensively elucidated. This study set out to explore the serum molecular profiles (I) underlying the disease subtypes, in association with (II) elevated fecal calprotectin and (III) disease activity states, (IV) upon treatment escalation, and (V) in patients who needed treatment escalation. **Methods:** The serum proteome, metabolome, and lipidome of 75 treated IBD patients were profiled. Following robust annotation, single- and multi-omic data analysis was performed to determine differential analytes and integrative biosignatures. **Results:** In (I), chronic inflammation, phosphatidylcholines, and bile acid homeostasis disturbances underlined the differences between Crohn's disease (CD) and ulcerative colitis. For (II), fecal calprotectin was associated with elevation of inflammatory proteins and sphingomyelins (SM) and decrease in bile acids, amino acids, and triacylglycerols (TG). (III) Relative to patient remission, active disease state was characterized by decreased SMs and increased inflammatory proteins and TGs. In (IV), treatment escalation was associated with augmented levels of inflammatory response-related proteins and reduced levels of amino acids. Notably, TGs increased consistently in the post-treatment escalation group. Moreover, needed-treatment-escalation patients had down-regulated TGs in (V). They also showed increased SMs and decreased signaling receptor binding proteins. Integrative signatures captured the differences between groups of five scenarios through cross-validation procedures. NFASC was selected as a biomarker in 4 of 5 scenarios with consistently lower levels in CD, elevated-calprotectin, active-disease-state, and needed-treatment-escalation patients. NFASC also increased in the post-treatment escalation group. **Conclusion:** Disturbances in immune response, bile acid homeostasis, amino acids, and lipids alteration

potentially underlie the clinically heterogeneous spectrum of IBD. NFASC and TGs hold promise as potential biomarkers for multi-purpose IBD management.

Keywords: IBD; deep phenotyping; molecular phenotype; multi-omics; DIABLO

1. INTRODUCTION

Inflammatory bowel disease (IBD) poses a significant health burden worldwide, impacting millions of individuals [1,2]. The disease emerges from a multifaceted interplay involving genetic predisposition, the gut microbiota, diverse environmental exposures, and immunological disturbances [3,4]. Upon diagnosis, IBD is traditionally classified as Crohn's disease (CD) and ulcerative colitis (UC) [5]. A knowledge gap persists regarding the underlying molecular basis leading to the distinct clinical presentations of CD and UC. Additional heterogeneity across the spectrum of IBD beyond these subtypes has also been characterized [4-6]. Treatment plans are tailored based on disease activity, aiming to mitigate inflammation, alleviate symptoms, and enhance the patient's quality of life [7]. Multiple evaluation methods are needed to achieve this goal, e.g., biomarkers including fecal calprotectin serve as a surrogate endpoint of gastrointestinal tract inflammation, and clinicians implement symptomology to manage the patients [7]. Failure of therapy requires treatment escalation where biologics target key immunoregulatory-related processes to reduce inflammation, improve symptoms, and promote healing for IBD patients [8,9]. However, disease heterogeneity, inadequate drug concentration, and immunogenicity development can compromise biologics's efficacy [4]. Therefore, a deeper understanding of molecular profiles associated with the disease pathogenesis, inflammation level, disease activity state, and molecular profiles upon treatment escalation is imperative [5].

Deep molecular phenotyping represents a paradigm shift in disease characterization and refers to a comprehensive and highly detailed approach beyond solely relying on clinical, biochemical, and imaging findings [10,11]. Delving into the deeper layer of phenotype is the valuable contribution of multi-omics data, which shows a potential application for complex diseases like IBD. Omics-based studies in IBD have been widely conducted, and the multi-omic approach has become a

cornerstone of IBD research [12-20]. Not only for biomarker research, but the approach has also supported elucidating the intricate interplay of molecular perturbations driving disease pathophysiology, including barrier dysfunction, chronic immunological response, microbial dysbiosis, and consequences alteration in tricarboxylic acid cycle metabolites, amino acid and lipid metabolism, and oxidative pathways. However, gaps persist in current IBD omics-based research, e.g., limited independent validation and a small number of multi-omic studies [19]. Additionally, most (multi)-omics studies focused on molecular profiling for IBD subtypes, with a limited number of research studies on other clinical scenarios, including disease activity, inflammation level, and treatment response. As a result, the multi-omics research holds significant promise for advancing our understanding of IBD. Omics-based approaches enable us to understand the response of the disease at the molecular level and establish the association between molecular profiles and different clinically meaningful scenarios toward IBD diagnosis, stratification, and treatment optimization.

This study aimed to investigate the differences in serum molecular profiles of proteome, metabolome, and lipidome underpinning different clinical scenarios of the under-treatment IBD population. Specifically, we characterized the single- and multi-ome differences among patients diagnosed with either CD or UC, those with elevated or normal fecal calprotectin levels, and individuals who presented an active or remission disease state. Moreover, we investigated the molecular perturbations in patients who underwent treatment escalation with samples collected before and after the treatment initiation, as well as those who needed treatment escalation and those who did not. The study provided comprehensive insights into the functional multi-ome profiles of IBD patients across five distinct clinical scenarios and laid a foundation for opening new doors for disease management and treatment optimization.

2. METHODS

2.1. Ethical statement and study population

The study protocol was reviewed and approved by the Institutional Review Board of Busan Paik Hospital (No. BPIRB 2024-02-046) and was conducted following the Helsinki Declaration. Before enrollment, all subjects provided written informed consent and were apprised of their right to withdraw. Participants were excluded if they had severe comorbidities or extraintestinal manifestations. Previously diagnosed IBD patients who underwent extensive treatment were recruited in the current study. Endoscopy was performed to classify patients into UC and CD subtypes at enrolment, which yielded 18 and 57 patients, respectively (Table 1). It was measured in fecal samples and was used to categorize participants into elevated or normal groups using a 150 µg/g cut-off [21,22]. Two participants with unavailable fecal calprotectin were imputed using a measured median value. The C-reactive proteins were significantly higher in the CD than in the UC group and in the elevated fecal calprotectin group compared to the normal counterpart (Table 1, Supplementary Table S1). The MAYO score cut-off of 1 and CD activity index (CDAI) score cut-off of 150 concerning CD and UC were used to reflect the disease status as either active state (i.e., mild, moderate, or severe conditions) or remission. Remission-state patients tended to have a longer illness duration (Supplementary Table S2). Patients with samples collected before and after the treatment escalation were assigned to the pseudo-pre- and post-treatment escalation design as an unpaired comparison (Supplementary Table S3). The former group (i.e., pre-treatment escalation) also served as patients who needed treatment escalation, compared to those who did not need it (Supplementary Table S4). These constituted five clinically meaningful scenarios, which are schematized in Figure 1, to investigate the differences in the serum multi-ome profile of IBD patients.

2.2. Sample preparation for metabolomics and lipidomics

Metabolomics and lipidomics experiments followed our previously established procedure [23-25]. For metabolite extraction, 50 μL of the serum sample (−80 °C) was first thawed on ice for about 30 min and then vortexed extensively for 10 s. Afterward, 150 μL precooled extraction solvent, which was the pre-dissolved of the 6 internal standards in MeOH, was pipetted into each sample. The mixtures were vortexed thoroughly for 30 s and then centrifuged at 14,000 *rcf* and 4 ºC for 2 min. Next, 150 μL of supernatant was taken and evaporated using a nitrogen gas flow. The dried samples were resuspended in 200 μL of 50% MeOH and then vortexed vigorously. The samples were centrifuged at 14,000 *rcf* and 4 ºC for 2 min before obtaining 150 μL of supernatant and 20 μL of supernatant for pooled quality control (QC) sample to inject into the LC-MS.

In lipid extraction, pre-aliquoted serum samples (50 μL at −80 °C) were initially thawed on ice for approximately 30 min. Subsequently, 10 μL of a pre-mixed IS of SPLASH and Deuterated Ceramide LIPIDOMIX (Alabama, USA) was spiked to each sample, followed by brief vortexing. After a 20-min incubation on ice and underwent vortexing, 300 μL of methanol (−20 °C) and 1000 μL of MTBE (−20 °C) were put in. The mixtures were vortexed for 10 s and incubated in a shaker for 20 min at 1500 *rpm* and 4 °C. Subsequently, 250 μL of water was added, vortexed for 20 s, and incubated for 10 min on ice. The samples were centrifuged for 2 min at 4 °C and 14,000 *rcf*. Next, 500 μL of supernatants were collected from each sample and dried under a nitrogen gas flow. Methanol/toluene (9:1, *v/v*) of 200 μL was used to resuspend the dried lipid extracts. The samples were then introduced into a centrifuge for 2 min at 4 °C and 14,000 *rcf* to obtain 150 μL of supernatant for instrumental analysis. Additionally, post-extraction pooled quality control (QC) samples were created by mixing and vortexing 20 μL of supernatant from each sample.

2.3. Metabolomics and lipidomics instrumental analysis and data acquisition

The instrumental analysis consisting of the Shimadzu Nexera LC system (Kyoto, Japan) and the X500R Quadrupole Time-of-Flight mass spectrometer combined with Turbo V™ ion source and TwinSpray probe (SCIEX, MA, USA) was utilized [23-25]. For metabolomics, ACQUITY UPLC HSS T3 column (50 mm × 2.1 mm; 1.8 μm) coupled with ACQUITY UPLC HSS T3 VanGuard pre-column (5 mm × 2.1 mm; 1.8 μm) was used. Mobile phase A of 100% water with 0.2% FA and mobile phase B of 100% ACN with 0.1% FA were implemented [26]. The injection volume for positive (POS) and negative (NEG) ion modes was 3 μL. For lipidomics, the stationary phase of ACQUITY UPLC BEH C18 column (50 mm × 2.1 mm; 1.7 μm) connected to an ACQUITY UPLC BEH C18 VanGuard pre-column (5 mm × 2.1 mm; 1.7 μm) was used. Mobile phase A was ACN/water (60:40, *v/v*) with 10 mM ammonium formate and 0.1% FA, and mobile phase B was IPA/ACN (90:10, *v/v*) containing 10 mM ammonium formate and 0.1% FA [26,27]. Within the POS, 1 μL of the samples were injected. The injection volume for the NEG was 2 μL. Mass calibration was performed after every five injections during the analysis, and data was acquired via data-dependent mode.

2.4. Proteomics sample preparation and data acquisition

For proteomics analysis, Olink® panels of Explore 384 Inflammation and Target 96 Cardiovascular III (Olink, Uppsala, Sweden) were used. Sample treatment for protein extraction followed Olink's established procedure. This method utilizes a highly specific antibody probe labeled with two oligonucleotides to bind the target protein. The real-time PCR amplification of the oligonucleotide sequence then allows for quantitative measurement of the resulting DNA sequence. The experiment was conducted by DNALink (Seoul, Republic of Korea).

2.5. Data preprocessing, data treatment, and normalization

MS-DIAL (version 4.9.0) was used for MS data processing via a data-dependent module [28]. In-house libraries of authentic standards, MS-DIAL in-built libraries, and public libraries were used to annotate metabolites and lipids [24,25]. Next, we exported the aligned data and formatted it according to the *MetaboAnalystR* package version 4.0.0 requirements in R 4.2.3 [29]. Only metabolites and lipids with reliable annotations were subjected to subsequent analyses. The normalized protein expression values were used for proteomics data, and the values recorded below the limit of detection were considered missing. Shared proteins between 2 panels were retained in the Olink® Target 96 Cardiovascular III for downstream analyses.

For data treatment, analytes (metabolites, lipids, and proteins) with a missing rate ≥ 50% were excluded, whereas the retained missing values were inputted via the k-nearest neighbors algorithm. For metabolomics and lipidomics, metabolites whose relative standard deviation was > 25% in the pooled QCs samples were also filtered out.

2.6. Single-omics differential analysis

For differential analysis, the *limma* method was used with adjustment for age and body mass index in five investigated scenarios. The analysis was achieved in the *MetaboAnalystR* package version 4.0.0 in R 4.2.3 for each omics data separately [29]. Pre-treated and normalized data without scaling was used to obtain differential analytes (DAs) of metabolites, lipids, and proteins between groups of five comparisons. A raw P-value of 0.05 was considered for the statistical cut-off. The heatmap of DAs of each omics was visualized via the *ComplexHeatmap* version 2.14.0 in R 4.2.3 [30].

2.7. Integrative multi-omics analysis and biosignature identification

Regarding integrative multi-omics analysis, we employed the data integration analysis for biomarker discovery using the latent components (DIABLO) method, which was implemented via

the *mixOmics* package version 6.23.4 in R 4.2.3 [31,32]. Before the analysis, each omics data was normalized and transformed in line with differential analysis. The scaling was achieved internally by DIABLO. For model tuning, we first explored the weight and chose the value of 0.5 to achieve a trade-off between correlation and discriminability, given our study cohort. Next, the number of components and the distance method were selected via a 10-fold cross-validation (CV). Afterward, the number of features within each data layer was determined via a 10-fold 10-repeated CV procedure. The performance of selected signatures to discriminate groups of interest was obtained separately via a 10-fold 10-repeated CV. The area under the receiver operating characteristic curve (AUC) was calculated by averaging sequentially over 10-fold cross-validation, 10-repeated, and three data blocks [31,32].

3. RESULTS

To investigate the molecular perturbation underlying different clinical scenarios of IBD, we stratified the patient cohort using their diagnostic results, fecal calprotectin level, disease activity score, and treatment escalation information (Figure 1).

3.1. Treated UC and CD patients presented divergence in molecular perturbations linked to chronic inflammation, phosphatidylcholines, and bile acid homeostasis

UC and CD are traditionally the two main subtypes of IBD, which are macro-phenotyped based on endoscopy results and clinical manifestations (Figure 2A). Herein, we determined molecules that were differentially expressed or had differential abundance between UC *vs.* CD. Employing an age- and sex-adjusted linear model, we identified 44 (32 up-regulated, 12 down-regulated), 14 (11 up-regulated, 3 down-regulated), and 31 (8 up-regulated, 23 down-regulated) differential proteins, metabolites, and lipids, respectively. These differential analytes (DAs) showed relatively straightforward patterns of differences across three data modalities, especially lipids, and proteins (Figure 2B-D). Most differential proteins (e.g., IFNG, IL1R1, IL12RB1, CCL20, AGER) were linked to inflammation and immune signaling pathways (Figure 2B). We also observed a consistent up-regulation of cell adhesion molecules, including ITGB2, ITGB6, ITGA11, LAMA4, and ADA, among others. Regarding metabolomics modality, changes in bile acid biosynthesis were observed where four conjugated bile acids of glycocholate, glycochenodeoxycholate, taurocholate, and taurodeoxycholate increased 2-4 times in the UC compared to the CD group. Two amino acids of histidine and 5-hydroxylysine increased nearly 2-fold. Metabolites involved in the purine metabolism (i.e., hypoxanthine and inosine) and an immunoregulatory metabolite (itaconate) decreased about 1.5 times in the UC group (Figure 2C). Lipidomics analyses showed that phosphatidylcholines (PC) and its derivatives of lysoPC (LPC) and ether-linked PC [PC(O-)]

and LPC(O-) were the major altered species. LPC, lysophosphatidylethanolamines (LPE), ceramides (Cer), and triacylglycerols (TG) were consistently down-regulated, whereas ether-linked phosphatidylethanolamine [PE(O-)] was consistently up-regulated in the UC group. PCs showed up- and down-regulation patterns (Figure 2D).

Utilizing an integrative omics approach leveraging DIABLO, we assessed the correlation of the data layers and determined the highly correlated and discriminant features across the three data blocks. The proteomics modality correlated well with two others (Figure 2E, Supplementary Figure S1). Proteins significantly differentiated the IBD sub-type (Figure 2E). Among 25 DIABLO-identified proteins, mostly belonging to cell adhesion, 18 were statistically up-regulated in UC. Proteins (especially differential proteins of ITGB2, CD200, and PRELP) correlated negatively with metabolites and lipids, particularly hypoxanthine (Figure 2E). Most highly correlated and discriminant metabolites (e.g., taurodeoxycholate, hypoxanthine, 2,3-dihydroxybenzoate, and itaconate) were statistically different. A panel of 4 lipid species with a significantly different abundance between the two IBD subtypes was also identified. LPC(16:0-SN1) and LPC(O-18:1) demonstrated a strong positive correlation with hypoxanthine.

3.2. Elevated fecal calprotectin associated with increased inflammation-related proteins, SMs, and LPCs; decreased bile acids, amino acids, and TGs

Fecal calprotectin has emerged as a valuable tool in managing IBD, including screening, diagnosis, disease activity and severity assessment, treatment monitoring, and relapse prediction of IBD patients [7,33]. We explored the molecular perturbations of two IBD subpopulations, i.e., elevated and normal fecal calprotectin levels (Figure 3A). We identified 34 differentially expressed proteins between the two groups. Most of them (32 proteins) were up-regulated in patients with elevated fecal calprotectin levels (Figure 3B). These differential proteins belonged to some of the

innate and adaptive immune responses, such as TNF, MAPK, IL-17, and NF-κB signaling pathways. Change in proteins involved in the sphingolipid signaling (TNFRSF1A, TRAF2) and PI3K-Akt signaling (CSF1, HGF, OSM, TGFA) was also observed. For metabolites, 12 up-regulated and 8 down-regulated differential metabolites were identified (Figure 3C). While conjugated bile acids (glycocholate, taurodeoxycholate, glycochenodeoxycholate, and glycodeoxycholate) consistently decreased (about 2.5-fold), lithocholate increased slightly in the elevated group. Ascorbate, homogentisate, and pyrocatechol were altered significantly, implying the perturbations in the tyrosine metabolism. Alteration of DNA metabolism-related metabolites was also observed with slight up-regulation of hypoxanthine, urate, deoxyguanosine monophosphate, and cytidine. Glutathione metabolism-related metabolites of oxoproline were also up-regulated. In all of these pathways, we observed a slight increase of glutamate in the elevated calprotectin group. Lipid, especially lysoPC, was the most class detected in the metabolomics modality, implying the potential findings of the lipidomics analysis. The lipid modality contributed the most significant number of DAs, with 52 differential lipids (Figure 3C). They presented a clear contrast mostly linked to the low abundance of TG and high abundance of SM and PC [and its derivatives of LPC, LPC(O-)] in the elevated calprotectin group. Among 19 detected TGs, 17 were down-regulated, while all SMs were up-regulated. We also observed the consistent augmentation of Cer, and cholesteryl esters (CE).

Integrative omics analysis revealed a good correlation between different data blocks, in which negative correlations were generally observable between proteins with two other layers (Figure 3E, Supplementary Figure S1). Proteins also served as the major modality to distinguish 2 groups (Figure 3E). In 30 and 16 highly correlated and discriminant proteins and metabolites, only 5 (glutamate, ascorbate, folate, creatinine, and oxoproline) and 4 (CCL23, TFPI, CLEC4G, and

MZB1) molecules were differential analytes, respectively. DIABLO-exclusively identified proteins were related to immune activation. Besides, DIABLO also identified DNA-related metabolites (hypoxanthine, urate), potential bacterial-source metabolites (pyrocatechol, 3-dehydroshikimate), chenodeoxycholate, and 2 lipids. Six selected lipids belonged to the Cer, SM, and PC species, all of which were differential lipids.

3.3. Active IBD disease state was characterized by an upregulation of inflammation-related proteins, a decrease in SM, and an increase in TG levels

The molecular response underlying patients in active or remission states was investigated (Figure 4A). While lipids showed a significant contribution with 44 differential analytes, 18 and 6 differential proteins and metabolites were observed. Overall, patients with active disease were characterized by the upregulation of all inflammatory-related proteins, a decrease in SM, and an increase in TG levels compared to the remission group (Figure 4B-D). We observed that patients who did not need treatment escalation contributed mainly to the augmented levels of TGs in the active group, whereas TGs were down-regulated in the remission group regardless of biologics status (Figure 4D). The significantly altered proteins, such as CXCL9, CXCL1, IL17A, TNFSF11, ITGB2, and ADGRE2, were prominently related to activated immune response. Regarding differential metabolites, beta-muricholate and taurocholate were elevated (2-fold), whereas coprocholate was slightly declined. Lactate, proline, and two acylcarnitines were decreased slightly. Lipidomics analysis revealed that the abundance of three PEs was augmented, and PC and Cer were decreased.

Thirty integrative signatures of proteins were identified. Most were up-regulated in the active group, and 11 proteins were differentially expressed (Figure 4E). DIABLO-selected metabolites were not differential analytes. There were 5 exclusive metabolites, 2 of which were acylcarnitines

[CAR(14:1), CAR(18:1)]. Within the lipidomics layer, all 23 identified integrative signatures belonged to TG and SM subclasses whose up- and down-regulated consistently in the active group, respectively. Among them, 17 (5 SMs and 12 TGs) lipid species had a differential abundance. A pair-wise correlation from DIABLO showed a good correlation between omics layers (Supplementary Figure S1). Both strong positive and negative correlations were observed, which mainly related to the relationship between proteins (mostly belonging to PGF, PRSS8, CTSZ, BSG, and EPHA1) and TGs; and TGs and metabolites [CAR(14:1), CAR(18:1), and 3-dehydroshikimate]. Lipids and proteins might be major drivers for differentiating active and remission disease states (Figure 4E).

3.4. Treatment escalation promoted upregulation of inflammatory response-related proteins, TGs, PCs, and decreased amino acids, purine metabolites

We explored molecular perturbations between patients who received treatment escalation with samples collected before and after the therapeutic initiation (pseudo pre- and post-treatment design) (Figure 5A). Treatment escalation triggered significant alterations in proteome and metabolome, including lipidome response in IBD patients (Figure 5B-D). We observed 16, 30, and 30 differential proteins, metabolites, and lipids. The majority of altered proteins, which were up-regulated, were linked to the perturbations of the cellular receptor signaling pathway, e.g., COL1A1, FLT3LG, ITGB6, and LAMA4 were related to disturbances in PI3K-Akt signaling pathway as well as in focal adhesion (Figure 5B). A consistent decrease in levels of differential metabolites was observed (Figure 5C). They were linked to lipid metabolism [e.g., LPC(O-16:0), LPC(O-16:1), and LPE(O-18:1)], arginine biosynthesis (arginine, aspartate, ornithine, and glutamine), pentose phosphate pathway (hexose-monophosphate, gluconate, and glycerate), purine metabolism (xanthine, glutamine, and deoxyguanosine monophosphate), and amino acid

alterations (e.g., creatinine, DOPA, histidine, and oxoproline). Lipidomics modality showed a consistent upregulation (Figure 5D). Among 26 up-regulated lipids, TG contributed mainly (2-3-fold increased levels), followed by PC and PE species. Ceramides derivates and PC(O-) were down-regulated.

A high correlation of metabolites and proteins, along with good separation between the two groups, was indicated by the distinction in the centroids of each group, yet an observable moderate amount of overlap between each sample (Figure 5E, Supplementary Figure S1). Most proteins (12/25) and metabolites (13/14, primarily amino acids) signatures were also differential analytes. Treatment escalation decreased creatinine, oxoproline, lithocholate, phosphorate, and CAR(12:0;O), which correlated negatively with ITGB6, NME3, OMD, MMP2, TNFSF13B, and PC(36:2) species.

3.5. Patients who needed to escalate their treatment showed downregulation of signaling receptor binding proteins, lower levels of TGs, higher levels of SMs and PCs

The multi-ome profiles of patients who needed treatment escalation and those who did not were compared (Figure 6A). The adjusted linear model revealed 25 proteins, mostly linked to signaling receptor binding, differentially expressed (11 up- and 14 down-regulated) between two groups (Figure 6B). Proteins linked to cytokine-cytokine receptor interaction were down-regulated (2 to 10-fold; CXCL6, CD40LG, CCL13, TNFRSF14, and TNFSF13B) in patients who needed treatment escalation, except CXCL9 increased 6-fold. Four proteins involved in MAPK and PI3K-Akt signaling pathways (i.e., FLT3LG, EGF, EGFR, and PDGFA) were also consistently down-regulated. Changes in the proteins mentioned above might also indicate the alteration of NF-κB and JAK-STAT signaling pathways. For metabolomics data, we determined 14 differential metabolites (8 up- and 6 down-regulated) (Figure 6C). A marginal increase in the abundance of

three amino acids (DOPA, arginine, and ornithine) was observed, which might imply a change in arginine metabolism. Two bile acids were also statistically different. Lithocholate elevated slightly, and urosodeoxycholate declined by 2.5-fold. Regarding lipidome alteration, 25 lipids were statistically significant. The highest number belonged to TG species, with the lower level (2-3-fold) in patients who required treatment escalation. The second rank was PC(O-), such as PC(O-34:1), PC(O-34:2), and PC(O-38:6), followed by Cer with increased abundance.

The high correlation between proteome, metabolome, and lipidome profiles was observed within two subpopulations (Figure 6E, Supplementary Figure S1). The negative pattern was mainly observed, leaving the positive correlation attributed primarily to the relationship between 3-dehydroshikimate and SM species. Other lipid species correlated negatively with pyrocatechol, LPE(20:5), NFASC, and ADGRE2. Among 18 and 21 DIABLO-selected proteins and lipids, only 1 (ANGPTL4) and 4 (belonging to Cer and PC(O-) species) molecules were differentially expressed between the two groups. None of the 6 DIABLO-selected metabolites were differential metabolites. Exclusively, proteins identified by DIABLO, which consistently down-regulated, were mainly linked to the immune response. While metabolites range from DNA-related (hypoxanthine) to bile acids (chenodeoxycholate) and bacterial-sourced metabolites (pyrocatechol, 3-dehydroshikimate), altered lipids mainly were linked to SM and PC species, whose higher abundance in patients needed treatment escalation.

3.6. Integrative signatures captured differences between groups of five scenarios

The predictive performance of selected multi-ome signatures is shown in Table 2. Differences between CD and UC groups, as well as between individuals with elevated and normal fecal calprotectin levels, could be captured by the selected multi-ome signatures (AUC = 0.8021, P-value = 0.0032; AUC = 0.7358, P-value = 0.0172; respectively). The model showed an acceptable

ability to differentiate between active and remission disease state groups, yet it could be by chance (AUC = 0.6192, P-value = 0.2040). Regarding treatment escalation, biologics triggered significant differences in the multi-ome profiles of IBD patients, leading to an excellent result in distinguishing between post- and pre-treatment escalation groups (AUC = 0.9228, P-value = 0.0075). Additionally, a good capability was observed to differentiate between patients needing to escalate their treatment and those not (AUC = 0.7695, P-value = 0.0174). Among these integrative signatures, NFASC was selected as a biomarker in 4 of 5 situations except the pre- and post-treatment escalation (Supplementary Figure S2). NFASC expression decreased in patients diagnosed with CD, presented elevated fecal calprotectin levels, manifested active disease state, and needed biologics treatment (Supplementary Figure S3). We also observed that NFASC expression increased in the post- compared to the pre-treatment escalation group. Moreover, Cer(42:2;2O)|Cer(18:1;2O/24:1), hypoxanthine, 3-dehydroshikimate, ANGPTL4, MILR1, and ISM1 were also observed in 3 of 5 investigated situations. Altogether, the results provide a *priori* predictability of integrative signatures for subsequent validation.

4. DISCUSSION

Owing to the heterogeneous nature of IBD, the current classification, routinely called UC and CD, could preclude the endeavors to understand the pathophysiology of the disease and to improve the patient's quality of life. Because of the complex pathophysiology involving cross-talk between multiple and interactive processes, a signature panel should be prioritized instead of a single biomarker. Thus, a classification comprising multiple biomarkers that reflect the IBD spectrum holds promise to account for many manifestations of IBD at the bedside [5]. Common alterations underlying different clinical scenarios might be a potential target for multiple applications. Nonetheless, individual omics data might not be sufficient to describe the complexity and capture holistic change in the IBD spectrum [19,20]. Hence, using multi-ome molecular profiles may be a better approach. Our study set out to investigate five commonly seen clinical scenarios and provide the first evidence about the molecular differences of the comparable groups at the functional multi-ome data. It pinpointed the serum multi-ome profiles underlying the disease and associated with either the fecal calprotectin levels or disease activity states, as well as molecular profiles upon treatment escalation.

We first aimed to elucidate the molecular differences between UC and CD, the two main subtypes of IBD. Barrier dysfunction is known to be the primary mechanism underlying IBD pathophysiology [4]. In UC, the injury only extends to the mucosal layer, whereas transmural inflammation occurs in CD [13]. Previous studies demonstrated higher expression levels of integrins (ITGB2 and ITGB6) in patients with IBD, emphasizing their relevance to the disease process [34]. Notably, *ITGB6* knockout can mitigate the disruption of tight junctions between colonic epithelial cells in colitis induced by dextran sulfate sodium [35]. In our study, cell adhesion-related proteins were consistently down-regulated in CD compared to UC, suggesting the importance and potential

utility of these proteins. Additionally, the decreased expression of ITGB2, CD200, and PRELP negatively correlated with the higher abundance of hypoxanthine in serum. Gut microbiota-derived purines play a significant role in intestinal energetics and barrier integrity [36]. Therefore, the alteration of hypoxanthine suggests the impaired barrier function. Over-time loss of epithelial barrier function promotes tissue destruction and angiogenesis, which also induces chronic inflammation and, in turn, exacerbates mucus layer injury [37]. Further, multiple immune pathways were also perturbed in our study. Besides, the alteration of PC species and their derivatives might suggest the disruption of the gut mucus layer. Previous research consistently showed that PCs and LPCs species decreased in UC vs. CD, whereas we observed a consistent decrease of LPC species and PCs showed both tendencies [17]. Notably, the changes in levels of LPC(16:0-SN1) and LPC(O-18:1) also correlated highly with low abundance of hypoxanthine. The shift of phospholipid composition in intestinal mucus could lead to an increase in the permeability of the intestinal mucus, promoting mucosal inflammation [38]. Bile acids are mainly re-absorbed via the ileum, a well-known location for CD compared to UC. Changes in bile acid homeostasis indicated by altered levels of bile acids and FGF19 expression between two disease subtypes also might induce chronic inflammation, resulting in an impaired mucosal barrier [39,40].

IBD activity evaluation benefits from a multi-faced approach, with each method offering specific valuable insights [7]. On the one hand, fecal calprotectin could be used as a surrogate endpoint presenting gastrointestinal tract inflammation in IBD. Mechanistically, non-apoptotic epithelial cell death, oxidative stress, and homeostasis pathway dysregulation result in an excessive release of calprotectin, promoting inflammation [41]. Establishing the association between serum multi-ome profiles and elevated fecal calprotectin levels to shed light on the systemic molecular alterations upon inflammation. Compared to the normal level group, patients with elevated fecal

calprotectin presented up-regulation of proteins involved in inflammation-promoting signaling pathways. Inflammation and microbiome dysbiosis might lead to malabsorption and disrupt bile acid function in the gastrointestinal tract. This might result in systemic changes in amino acids and DNA-related metabolites, as observed in our study [15,16]. Clinicians are aware of and implement symptomatic signs and quality-of-life assessments to manage IBD patients [7]. Determining the relationship between serum multi-omic profiles and disease activity could illuminate systemic molecular alterations associated with active disease manifestations. In turn, active disease was characterized by up-regulation of Th1 and Th17 immune activation-related proteins such as CXCL9, CXCL1, ITGB2, and IL17A. Moreover, ITGB2, B4GALT1, and CTSZ are shown to have a significant role in lysosomal function, critical for maintaining intestinal barrier integrity and mucosal homeostasis in IBD. Studies on *B4GALT1*-deficient mice revealed the role of oligosaccharide modification in colitis pathogenesis, indicating that altered oligosaccharide structures can mitigate colitis induction in mice lacking beta-1,4-galactosyltransferase [42,43]. Between these two scenarios, we observed differences, yet significance in the direction of change of TGs and SMs. This might originate from the nature of how each index has been established and what clinically they have been valued for. Moreover, several confounding factors have not been controlled in the analysis. In the gut, the immunomodulatory role of SMs, either of host- or microbiome-origin, has been recognized as contributing to cell growth, differentiation, apoptosis, and membrane structure integrity [17,44,45]. In addition, intestinal spontaneous inflammations could result from reduced bacterial sphingolipids. IBD patients suffer from lipid abnormalities that correlate with disease activity [46]. The risk of atherosclerosis increased in IBD patients, which highlights the role of lipid biomarkers in determining high-risk patients for timely intervention. Accordingly, we observed consistent up-regulated levels of TGs in the active disease state.

We also designed the pre- and post-treatment scenarios to investigate the molecular perturbations upon treatment escalation. Treatment escalation promoted augmentations of inflammatory response-related proteins, TGs, and PCs; and a decrease of amino acids and purine metabolites. The up-regulation of TGs could be linked to the down-regulation of TGs in the elevated fecal calprotectin as a response to inflammation alleviation. Moreover, needed-treatment-escalation patients had down-regulated TGs, as characterized in the fifth scenario. It should be noted that increased TGs in the active-disease-state patients were attributed predominantly to individuals who did not need treatment escalation. Therefore, biologics might support inflammation alleviation and disease control and (thus) reduce the risk of atherosclerotic cardiovascular disease. TGs might also have the potential for treatment monitoring and complication detection. However, a more sophisticated analysis in the context of improved study design and sample size is warranted to establish the association [47]. Patients required to escalate their treatment tend to suffer more severity of disease and thus need to escalate their treatment. Nevertheless, disease severity assessment is a hard-to-implement process requiring multiple criteria [33]. Therefore, fast and convenient biosignatures might open the door for early treatment intervention. We also observed the down-regulation of signaling pathways-related proteins and higher levels of SMs and PCs. Interestingly, ANGPTL4, known for its regulatory role in inflammatory responses across various diseases, was up-regulated [48]. In IBD, studies suggested that the plasma levels of ANGPTL4 were elevated in patients with CD and UC during mild to moderate stages of the disease but returned to normal levels in severe cases of IBD [49]. Moreover, increased ANGPTL4 expression in the inflamed colons of IBD patients correlated with the reduced infiltration of CD8+ cells, suggesting a potential regulatory role for ANGPTL4 in modulating immune responses within the gut microenvironment [49].

Integrative biosignature holds the potential to account for diverse manifestations and capture the holistic change of IBD at the bedside. We evaluated predictive performance to give an idea about the potential of the proposed biosignature. This *priori* information might be of potential use for further studies. Our findings also showed that neurofascin (NFASC), which was only detected by integrative analysis, was a biomarker among 4 of 5 investigated scenarios with consistently lower levels in CD, elevated-calprotectin, active-disease-state, and needed-treatment-escalation patients. NFASC expression also tended to be normalized in the post-treatment escalation group. Neurofascin is known for its mechanistic and clinical role in peripheral neuropathy, which is one of the most common yet unrecognized neurological disorders in the IBD population, especially in CD patients [50-54]. Ani-TNF drugs were also associated with neuropathy, which could exacerbate this clinical complication [54]. In our study, we excluded patients presenting extraintestinal manifestations. Therefore, early complications of IBD might be captured by changes in neurofascin expression. NFASC might be a protective factor for IBD and could be used as the early indicator of clinical severity and complications as well as for treatment selection and monitoring.

Some limitations in our study should be noted. First, the genetic background information was unavailable. Second, the limited number of participants might restrict the generalizability of results and decrease the statistical power to detect subtle but potentially important differences in the multi-ome profiles. This is particularly relevant in a complex disease like IBD, where significant heterogeneity exists. It, along with treated patients recruited in our study, could introduce noise to the data and preclude the isolation of the effects of the underlying disease itself and the effects of the treatment. Although the molecular profiles reported in our study were comprehensive, the

cross-sectional and convenient sampling strategy made it necessary to improve the study design to draw the causative relationship.

5. CONCLUSION

This study wielded a functional multi-omics approach toward deep molecular phenotyping of IBD. This comprehensive analysis revealed distinct molecular signatures across five typical IBD scenarios. Our findings provide deeper insights into serum multi-ome profiles underlying the disease and in association with either the fecal calprotectin levels or disease activity states, and molecular profiles upon treatment escalation. These biosignatures pointed towards disturbances in key pathophysiological processes like immune response, bile acid homeostasis, amino acids, and lipids alteration, potentially underlying the heterogeneous spectrum of IBD. Notably, NFASC and TGs hold promise as potential biomarkers for early detection of disease severity and complications as well as for treatment selection and monitoring. Future validation in more extensive and longitudinal cohorts and translation into clinical tools can pave the way for a more precise approach to IBD diagnosis, stratification, and treatment.

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CRediT authorship contribution statement

Nguyen Tran Nam Tien: Data curation, Methodology, Investigation, Formal analysis, Validation, Writing – Original Draft, Writing – Review & Editing. **Eun Jeong Choi**: Data curation, Methodology, Investigation, Formal analysis, Visualization, Writing – Review & Editing. **Nguyen Quang Thu**: Data curation, Methodology, Validation, Writing – Original Draft, Writing – Review & Editing. **Seung Jung Yu**: Data curation, Investigation, Validation, Writing – Review & Editing. **Duc Ninh Nguyen**: Methodology, Supervision, Validation, Writing – Review & Editing. **Dong Hyun Kim**: Conceptualization, Investigation, Supervision, Resources, Writing – Review & Editing, Funding acquisition. **Nguyen Phuoc Long**: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Supervision, Resources, Writing – Original draft, Writing – Review & Editing, Funding acquisition. **Hong Sub Lee**: Conceptualization, Methodology, Investigation, Formal analysis, Validation, Resources, Supervision, Writing – Original draft, Writing – Review & Editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

The data supporting this study's findings are available upon reasonable request.

REFERENCES

1. Alatab S, Sepanlou SG, Ikuta K, *et al.* The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2020;**5**:17–30.
2. Kaplan GG, Windsor JW. The four epidemiological stages in the global evolution of inflammatory bowel disease. *Nat Rev Gastroenterol Hepatol* 2021;**18**:56–66.
3. Ananthakrishnan AN. Epidemiology and risk factors for IBD. *Nat Rev Gastroenterol Hepatol* 2015;**12**:205–17.
4. Chang JT. Pathophysiology of Inflammatory Bowel Diseases. *New Engl J Med* 2020;**383**:2652–64.
5. Verstockt B, Bressler B, Martinez-Lozano H, McGovern D, Silverberg MS. Time to Revisit Disease Classification in Inflammatory Bowel Disease: Is the Current Classification of Inflammatory Bowel Disease Good Enough for Optimal Clinical Management? *Gastroenterology* 2022;**162**:1370–82.
6. Gonzalez CG, Mills RH, Zhu Q, *et al.* Location-specific signatures of Crohn's disease at a multi-omics scale. *Microbiome* 2022;**10**:133.
7. Walsh AJ, Bryant RV, Travis SPL. Current best practice for disease activity assessment in IBD. *Nat Rev Gastroenterol Hepatol* 2016;**13**:567–79.
8. Danese S, Vuitton L, Peyrin-Biroulet L. Biologic agents for IBD: practical insights. *Nat Rev Gastroenterol Hepatol* 2015;**12**:537–45.
9. Juillerat P, Grueber MM, Ruetsch R, *et al.* Positioning biologics in the treatment of IBD: A practical guide – Which mechanism of action for whom? *Curr Res Pharmacol Drug Discov* 2022;**3**:100104.
10. Robinson PN. Deep phenotyping for precision medicine. *Hum Mutat* 2012;**33**:777–80.
11. Delude CM. Deep phenotyping: The details of disease. *Nature* 2015;**527**:S14–S5.
12. Gisbert JP, Chaparro M. Clinical Usefulness of Proteomics in Inflammatory Bowel Disease: A Comprehensive Review. *J Crohns Colitis* 2018;**13**:374–84.
13. Titz B, Gadaleta RM, Lo Sasso G, *et al.* Proteomics and Lipidomics in Inflammatory Bowel Disease Research: From Mechanistic Insights to Biomarker Identification. *Int J Mol Sci* 2018;**19**:2775.
14. Bourgonje AR, Feelisch M, Faber KN, *et al.* Oxidative Stress and Redox-Modulating Therapeutics in Inflammatory Bowel Disease. *Trends Mol Med* 2020;**26**:1034–46.
15. Gallagher K, Catesson A, Griffin JL, Holmes E, Williams HRT. Metabolomic Analysis in Inflammatory Bowel Disease: A Systematic Review. *J Crohns Colitis* 2021;**15**:813–26.
16. Aldars-García L, Gisbert JP, Chaparro M. Metabolomics Insights into Inflammatory Bowel Disease: A Comprehensive Review. *Pharmaceuticals* 2021;**14**:1190.
17. Lee EG, Yoon YC, Yoon J, *et al.* Systematic Review of Recent Lipidomics Approaches Toward Inflammatory Bowel Disease. *Biomol Ther (Seoul)* 2021;**29**:582–95.
18. Agrawal M, Allin KH, Petralia F, Colombel J-F, Jess T. Multiomics to elucidate inflammatory bowel disease risk factors and pathways. *Nat Rev Gastroenterol Hepatol* 2022;**19**:399–409.
19. Sudhakar P, Alsoud D, Wellens J, *et al.* Tailoring Multi-omics to Inflammatory Bowel Diseases: All for One and One for All. *J Crohns Colitis* 2022;**16**:1306–20.

20. Fiocchi C. Omics and Multi-Omics in IBD: No Integration, No Breakthroughs. *Int J Mol Sci* 2023;**24**:14912.
21. Costa F, Mumolo MG, Ceccarelli L, *et al.* Calprotectin is a stronger predictive marker of relapse in ulcerative colitis than in Crohn's disease. *Gut* 2005;**54**:364.
22. Gisbert JP, Bermejo F, Pérez-Calle J-L, *et al.* Fecal Calprotectin and Lactoferrin for the Prediction of Inflammatory Bowel Disease Relapse. *Inflamm Bowel Dis* 2009;**15**:1190-8.
23. Long NP, Anh NK, Yen NTH, *et al.* Comprehensive lipid and lipid-related gene investigations of host immune responses to characterize metabolism-centric biomarkers for pulmonary tuberculosis. *Sci Rep* 2022;**12**:13395.
24. Yen NTH, Anh NK, Jayanti RP, *et al.* Multimodal plasma metabolomics and lipidomics in elucidating metabolic perturbations in tuberculosis patients with concurrent type 2 diabetes. *Biochimie* 2023;**211**:153–63.
25. Anh NK, Yen NTH, Tien NTN, *et al.* Metabolic phenotyping and global functional analysis facilitate metabolic signature discovery for tuberculosis treatment monitoring. *Biochim Biophys Acta Mol Basis Dis* 2024;**1870**:167064.
26. Cajka T, Hricko J, Rudl Kulhava L, *et al.* Optimization of Mobile Phase Modifiers for Fast LC-MS-Based Untargeted Metabolomics and Lipidomics. *Int J Mol Sci* 2023;**24**:1987.
27. Nguyen BT, Le Q-V, Ahn J, *et al.* Omics analysis unveils changes in the metabolome and lipidome of Caenorhabditis elegans upon polydopamine exposure. *J Pharm Biomed Anal* 2024;**244**:116126.
28. Tsugawa H, Cajka T, Kind T, *et al.* MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nat Methods* 2015;**12**:523–6.
29. Pang Z, Chong J, Zhou G, *et al.* MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Res* 2021;**49**:W388–W96.
30. Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 2016;**32**:2847–9.
31. Rohart F, Gautier B, Singh A, KA LC. mixOmics: An R package for 'omics feature selection and multiple data integration. *PLoS Comput Biol* 2017;**13**:e1005752.
32. Singh A, Shannon CP, Gautier B, *et al.* DIABLO: an integrative approach for identifying key molecular drivers from multi-omics assays. *Bioinformatics* 2019;**35**:3055–62.
33. Peyrin-Biroulet L, Panés J, Sandborn WJ, *et al.* Defining Disease Severity in Inflammatory Bowel Diseases: Current and Future Directions. *Clin Gastroenterol Hepatol* 2016;**14**:348–54.
34. Dotan I, Allez M, Danese S, *et al.* The role of integrins in the pathogenesis of inflammatory bowel disease: Approved and investigational anti-integrin therapies. *Med Res Rev* 2020;**40**:245–62.
35. Sun Q, Lu Z, Ma L, *et al.* Integrin β6 deficiency protects mice from experimental colitis and colitis-associated carcinoma by altering macrophage polarization. *Front Oncol* 2023;**13**:1190229.
36. Lee JS, Wang RX, Alexeev EE, Colgan SP. Intestinal Inflammation as a Dysbiosis of Energy Procurement: New Insights into an Old Topic. *Gut Microbes* 2021;**13**:1880241.
37. Britzen-Laurent N, Weidinger C, Stürzl M. Contribution of Blood Vessel Activation, Remodeling and Barrier Function to Inflammatory Bowel Diseases. *Int J Mol Sci* 2023;**24**:5517.
38. Bischoff SC, Barbara G, Buurman W, *et al.* Intestinal permeability – a new target for disease prevention and therapy. *BMC Gastroenterol* 2014;**14**:189.

39. Gadaleta RM, Garcia-Irigoyen O, Cariello M, *et al.* Fibroblast Growth Factor 19 modulates intestinal microbiota and inflammation in presence of Farnesoid X Receptor. *EBioMedicine* 2020;**54**:102719.
40. Di Ciaula A, Bonfrate L, Khalil M, Portincasa P. The interaction of bile acids and gut inflammation influences the pathogenesis of inflammatory bowel disease. *Intern Emerg Med* 2023;**18**:2181–97.
41. Boyapati RK, Rossi AG, Satsangi J, Ho GT. Gut mucosal DAMPs in IBD: from mechanisms to therapeutic implications. *Mucosal Immunol* 2016;**9**:567-82.
42. Shinzaki S, Iijima H, Fujii H, *et al.* Altered Oligosaccharide Structures Reduce Colitis Induction in Mice Defective in β-1,4-Galactosyltransferase. *Gastroenterology* 2012;**142**:1172–82.
43. Vanhooren V, Vandenbroucke RE, Dewaele S, *et al.* Mice overexpressing β-1,4-Galactosyltransferase I are resistant to TNF-induced inflammation and DSS-induced colitis. *PLoS One* 2013;**8**:e79883.
44. Boldyreva LV, Morozova MV, Saydakova SS, Kozhevnikova EN. Fat of the Gut: Epithelial Phospholipids in Inflammatory Bowel Diseases. *Int J Mol Sci* 2021;**22**:11682.
45. Kayama H, Takeda K. Emerging roles of host and microbial bioactive lipids in inflammatory bowel diseases. *Eur J Immunol* 2023;**53**:2249866.
46. Tews HC, Schmelter F, Kandulski A, *et al.* Unique Metabolomic and Lipidomic Profile in Serum From Patients With Crohn's Disease and Ulcerative Colitis Compared With Healthy Control Individuals. *Inflamm Bowel Dis* 2023.
47. Cainzos-Achirica M, Glassner K, Zawahir HS, *et al.* Inflammatory Bowel Disease and Atherosclerotic Cardiovascular Disease: JACC Review Topic of the Week. *J Am Coll Cardiol* 2020;**76**:2895-905.
48. Zuo Y, He Z, Chen Y, Dai L. Dual role of ANGPTL4 in inflammation. *Inflammation Res* 2023;**72**:1303–13.
49. Zhang X, Tu J, Ding S, *et al.* Increased angiopoietin-like 4 expression ameliorates inflammatory bowel diseases via suppressing CD8+ T cell activities. *Biochem Biophys Res Commun* 2022;**612**:37–43.
50. Ng JKM, Malotka J, Kawakami N, *et al.* Neurofascin as a target for autoantibodies in peripheral neuropathies. *Neurology* 2012;**79**:2241–8.
51. Koike H, Katsuno M. Pathophysiology of Chronic Inflammatory Demyelinating Polyneuropathy: Insights into Classification and Therapeutic Strategy. *Neurol Ther* 2020;**9**:213–27.
52. Gao Y, Kong L, Liu S, Liu K, Zhu J. Impact of Neurofascin on Chronic Inflammatory Demyelinating Polyneuropathy via Changing the Node of Ranvier Function: A Review. *Front Mol Neurosci* 2021;**14**.
53. Helmar Christoph L, David B, Satoshi K. Chronic inflammatory demyelinating polyneuropathy: update on diagnosis, immunopathogenesis and treatment. *J Neurol Neurosurg Psychiatry* 2019;**90**:981.
54. García-Cabo C, Morís G. Peripheral neuropathy: An underreported neurologic manifestation of inflammatory bowel disease. *Eur J Intern Med* 2015;**26**:468–75.

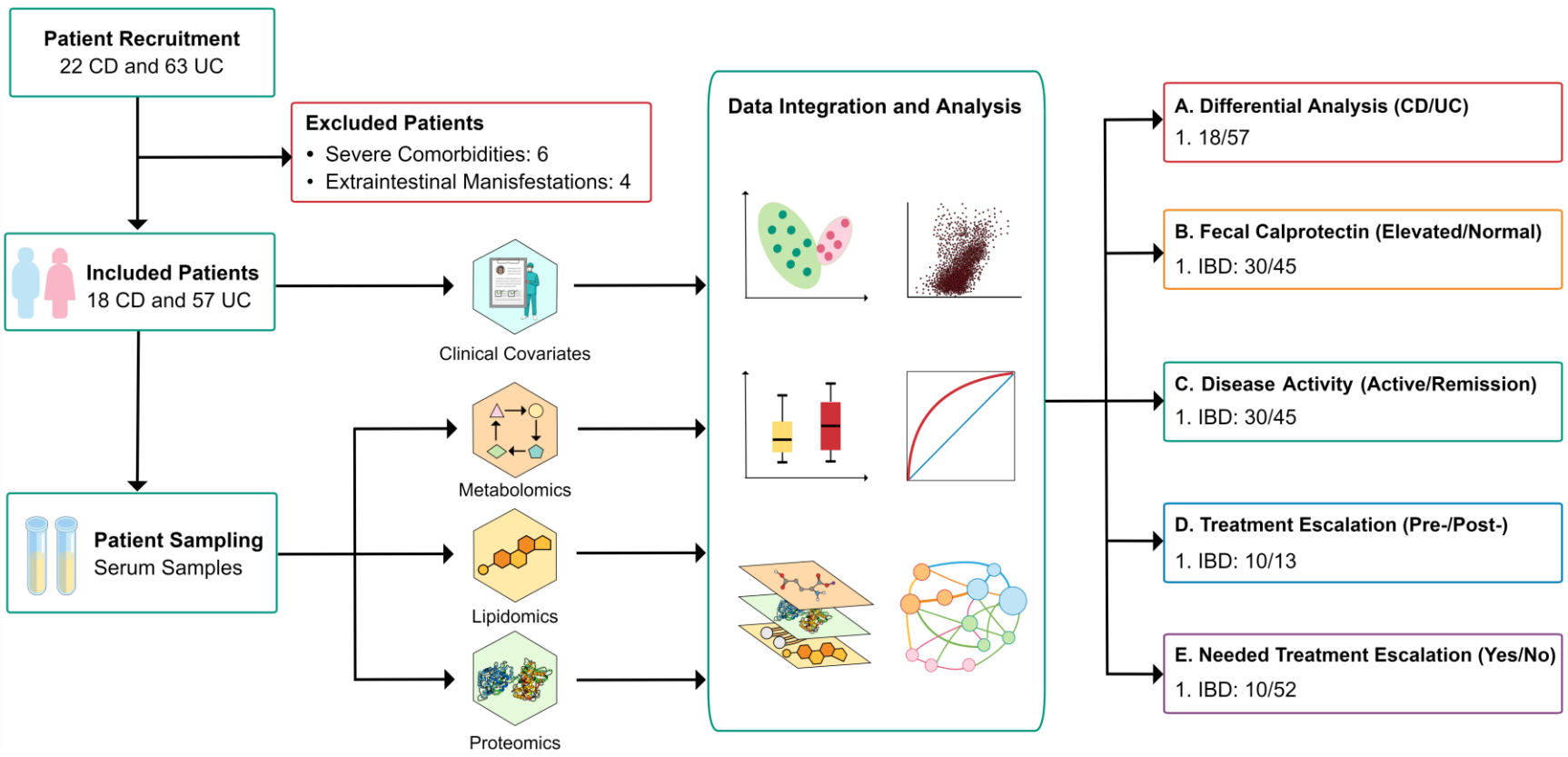


Figure 1. Overall study workflow spanning from patient recruitment, sampling, multi-ome profiling, and data integration, analysis, and interpretation of five clinically meaningful scenarios of treated inflammatory bowel disease patients.

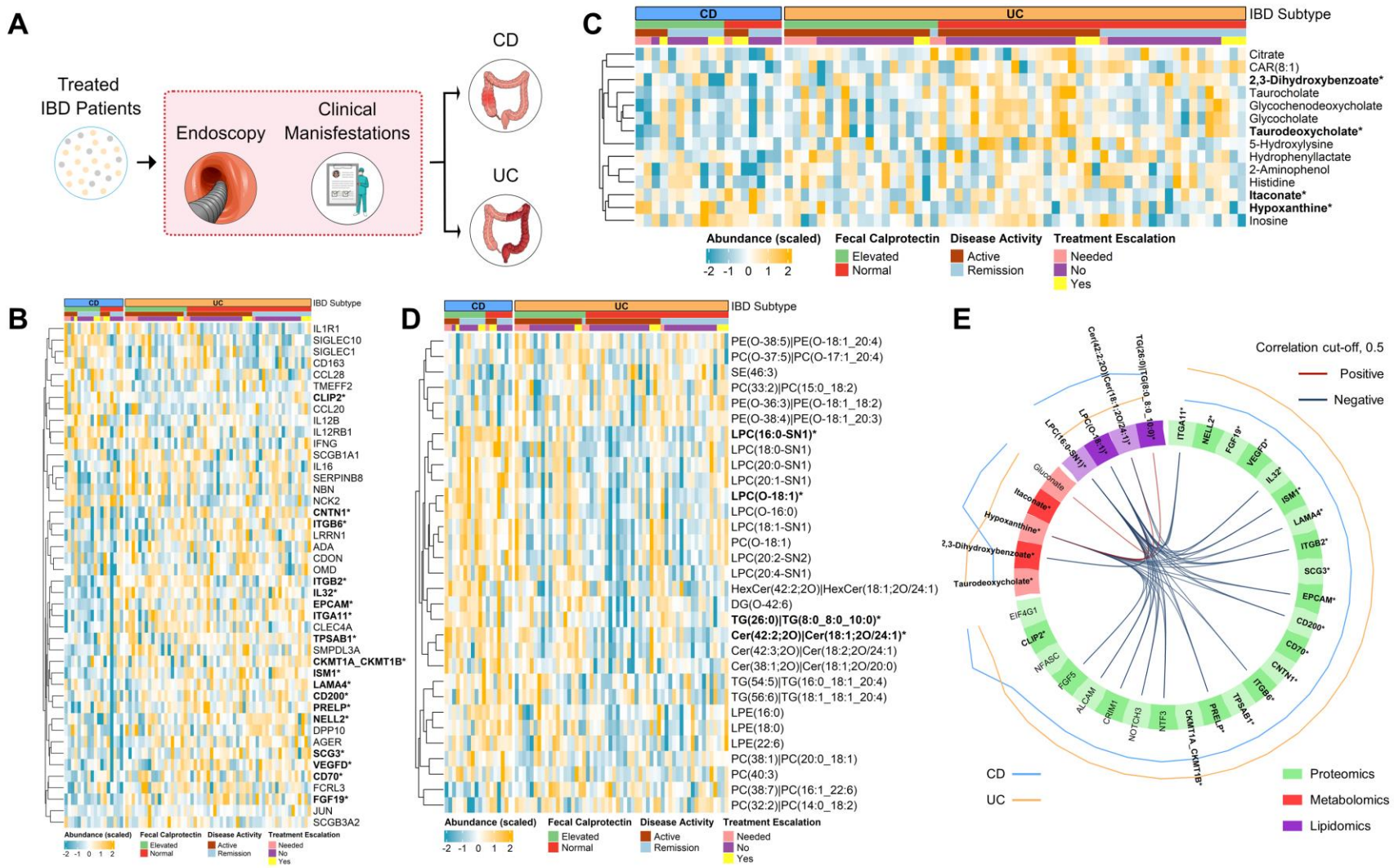
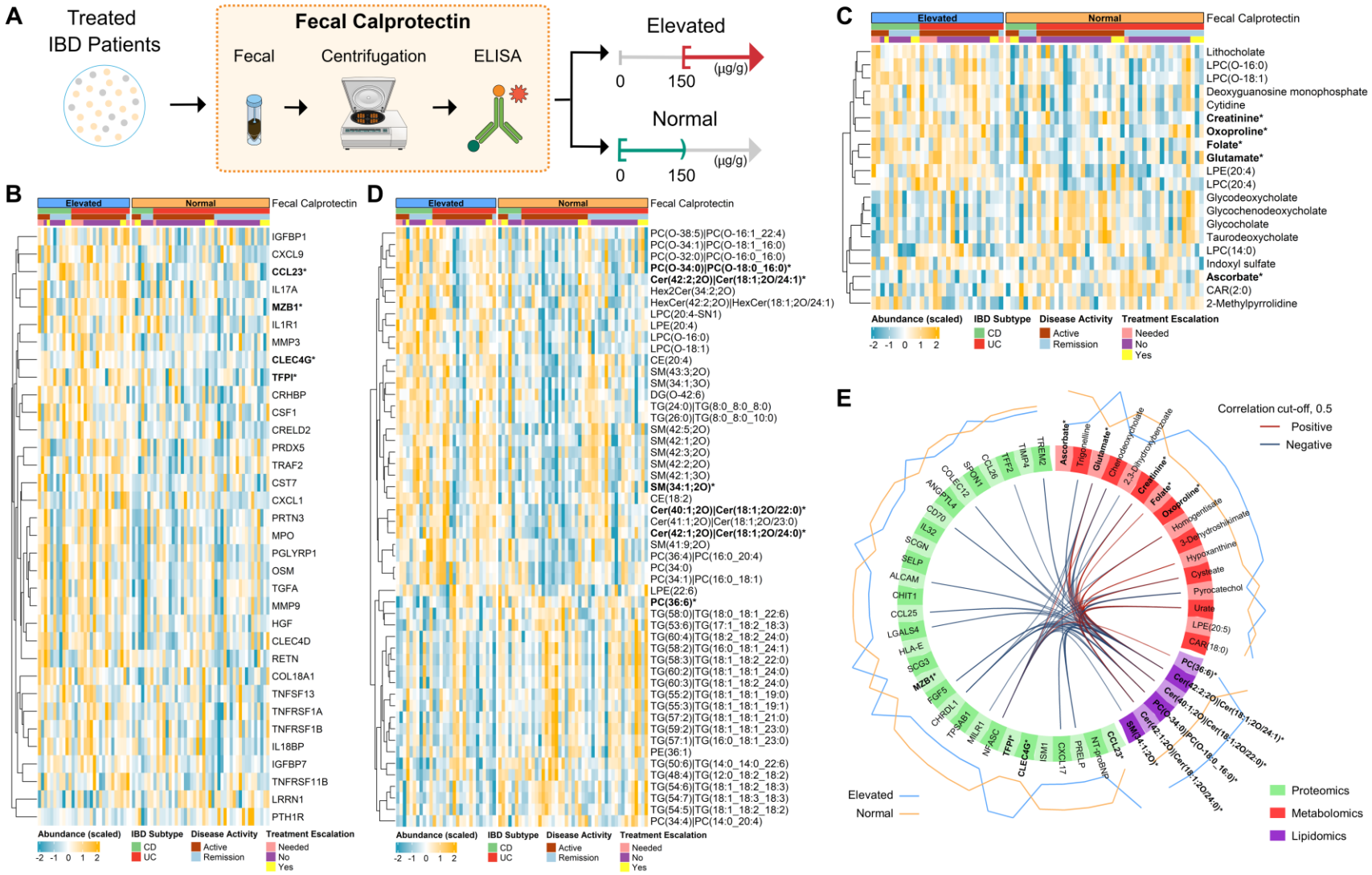


Figure 2. Single and integrative omics analysis presenting molecular perturbances between treated UC and CD patients. (A) Sample collection and grouping. Heatmap of differential (B) proteins, (C) metabolites, and (D) lipids. (D) DIABLO-selected

signatures. Abbreviations: IBD: inflammatory bowel disease, UC: ulcerative colitis, CD: Crohn's disease. * and bold: analytes that detected in both single- and multi-omics data analysis.


A
Treated IBD Patients
Fecal Calprotectin
Fecal
Centrifugation
ELISA
Elevated
0
150
(µg/g)
Normal
0
150
(µg/g)
B
Elevated
Normal
Fecal Calprotectin
IGFBP1
CXCL9
CCL23*
IL17A
MZB1*
IL1R1
MMP3
CLEC4G*
TFPI*
CRHBP
CSF1
CRELD2
PRDX5
TRAF2
CST7
CXCL1
PRTN3
MPO
PGLYRP1
OSM
TGFA
MMP9
HGF
CLEC4D
RETN
COL18A1
TNFSF13
TNFRSF1A
TNFRSF1B
IL18BP
IGFBP7
TNFRSF11B
LRRN1
PTH1R
Abundance (scaled)
-2 -1 0 1 2
IBD Subtype
CD
UC
Disease Activity
Active
Remission
Treatment Escalation
Needed
No
Yes
D
Elevated
Normal
Fecal Calprotectin
PC(O-38:5)|PC(O-16:1_22:4)
PC(O-34:1)|PC(O-18:1_16:0)
PC(O-32:0)|PC(O-16:0_16:0)
PC(O-34:0)|PC(O-18:0_16:0)*
Cer(42:2;2O)|Cer(18:1;2O/24:1)*
Hex2Cer(34:2;2O)
HexCer(42:2;2O)|HexCer(18:1;2O/24:1)
LPC(20:4-SN1)
LPE(20:4)
LPC(O-16:0)
LPC(O-18:1)
CE(20:4)
SM(43:3;2O)
SM(34:1;3O)
DG(O-42:6)
TG(24:0)|TG(8:0_8:0_8:0)
TG(26:0)|TG(8:0_8:0_10:0)
SM(42:5;2O)
SM(42:1;2O)
SM(42:3;2O)
SM(42:2;2O)
SM(42:1;3O)
SM(34:1;2O)*
CE(18:2)
Cer(40:1;2O)|Cer(18:1;2O/22:0)*
Cer(41:1;2O)|Cer(18:1;2O/23:0)
Cer(42:1;2O)|Cer(18:1;2O/24:0)*
SM(41:9;2O)
PC(36:4)|PC(16:0_20:4)
PC(34:0)
PC(34:1)|PC(16:0_18:1)
LPE(22:6)
PC(36:6)*
TG(58:0)|TG(18:0_18:1_22:6)
TG(53:6)|TG(17:1_18:2_18:3)
TG(60:4)|TG(18:2_18:2_24:0)
TG(58:2)|TG(16:0_18:1_24:1)
TG(58:3)|TG(18:1_18:2_22:0)
TG(60:2)|TG(18:1_18:1_24:0)
TG(60:3)|TG(18:1_18:2_24:0)
TG(55:2)|TG(18:1_18:1_19:0)
TG(55:3)|TG(18:1_18:1_19:1)
TG(57:2)|TG(18:1_18:1_21:0)
TG(59:2)|TG(18:1_18:1_23:0)
TG(57:1)|TG(16:0_18:1_23:0)
PE(36:1)
TG(50:6)|TG(14:0_14:0_22:6)
TG(48:4)|TG(12:0_18:2_18:2)
TG(54:6)|TG(18:1_18:2_18:3)
TG(54:7)|TG(18:1_18:3_18:3)
TG(54:5)|TG(18:1_18:2_18:2)
PC(34:4)|PC(14:0_20:4)
Abundance (scaled)
-2 -1 0 1 2
IBD Subtype
CD
UC
Disease Activity
Active
Remission
Treatment Escalation
Needed
No
Yes
C
Elevated
Normal
Fecal Calprotectin
Lithocholate
LPC(O-16:0)
LPC(O-18:1)
Deoxyguanosine monophosphate
Cytidine
Creatinine*
Oxoproline*
Folate*
Glutamate*
LPE(20:4)
LPC(20:4)
Glycodeoxycholate
Glycochenodeoxycholate
Glycocholate
Taurodeoxycholate
LPC(14:0)
Indoxyl sulfate
Ascorbate*
CAR(2:0)
2-Methylpyrrolidine
Abundance (scaled)
-2 -1 0 1 2
IBD Subtype
CD
UC
Disease Activity
Active
Remission
Treatment Escalation
Needed
No
Yes
E
Correlation cut-off, 0.5
Positive
Negative
Elevated
Normal
Proteomics
Metabolomics
Lipidomics

651

Figure 3. Single and integrative omics analysis presenting molecular perturbances associated with fecal calprotectin levels. (A) Sample collection and grouping. Heatmap of differential (B) proteins, (C) metabolites, and (D) lipids. (D) DIABLO-selected signatures. Abbreviations: IBD: inflammatory bowel disease, UC: ulcerative colitis, CD: Crohn's disease. * and bold: analytes that detected in both single- and multi-omics data analysis.

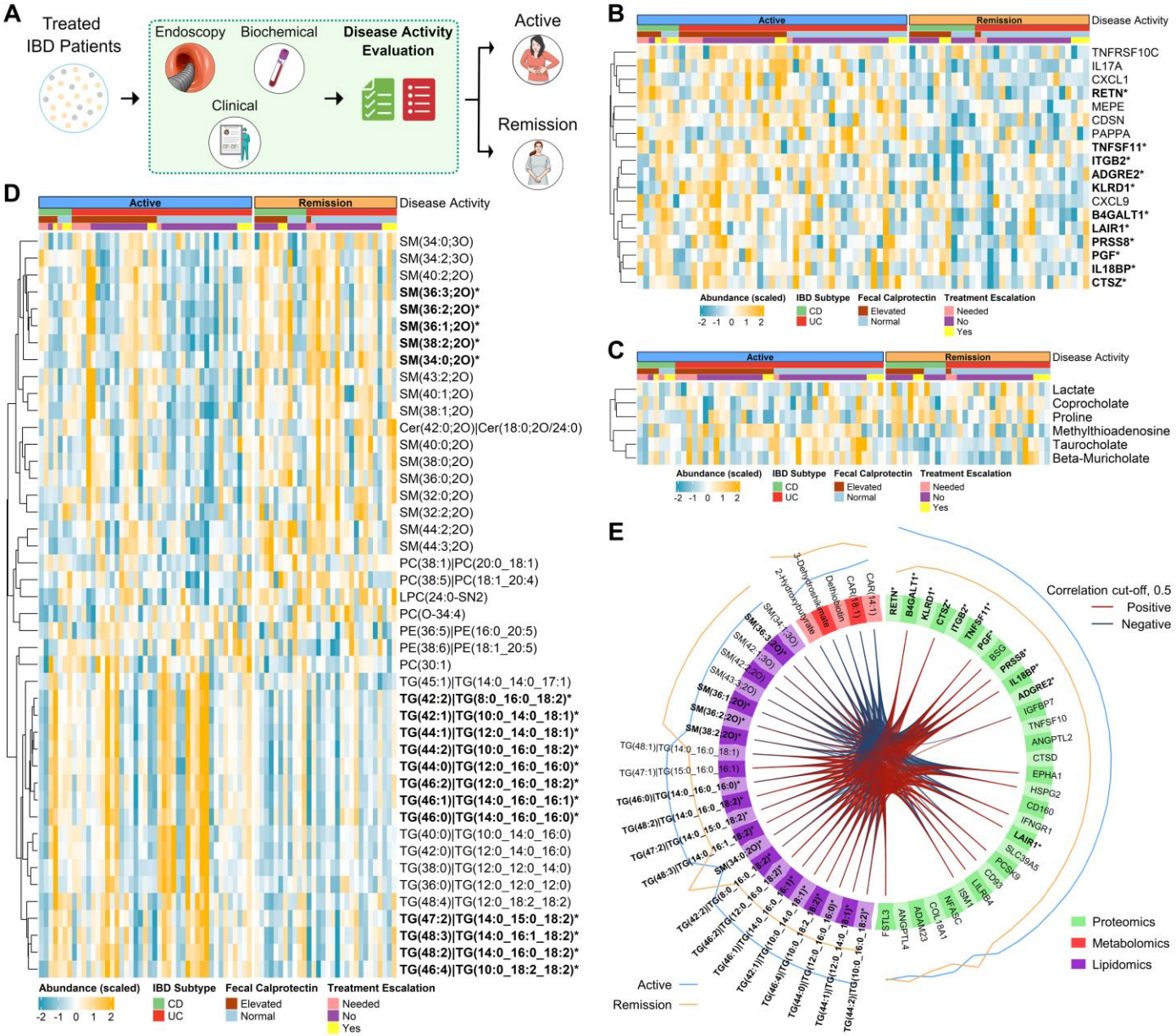

A
Treated IBD Patients
Endoscopy
Biochemical
Clinical
Disease Activity Evaluation
Active
Remission
B
Active
Remission
Disease Activity
TNFRSF10C
IL17A
CXCL1
RETN*
MEPE
CDSN
PAPPA
TNFSF11*
ITGB2*
ADGRE2*
KLRD1*
CXCL9
B4GALT1*
LAIR1*
PRSS8*
PGF*
IL18BP*
CTSZ*
Abundance (scaled)
IBD Subtype
Fecal Calprotectin
Treatment Escalation
CD
UC
Elevated
Normal
Needed
No
Yes
C
Active
Remission
Disease Activity
Lactate
Coprocholate
Proline
Methylthioadenosine
Taurocholate
Beta-Muricholate
Abundance (scaled)
IBD Subtype
Fecal Calprotectin
Treatment Escalation
CD
UC
Elevated
Normal
Needed
No
Yes
D
Active
Remission
Disease Activity
SM(34:0;3O)
SM(34:2;3O)
SM(40:2;2O)
SM(36:3;2O)*
SM(36:2;2O)*
SM(36:1;2O)*
SM(38:2;2O)*
SM(34:0;2O)*
SM(43:2;2O)
SM(40:1;2O)
SM(38:1;2O)
Cer(42:0;2O)|Cer(18:0;2O/24:0)
SM(40:0;2O)
SM(38:0;2O)
SM(36:0;2O)
SM(32:0;2O)
SM(32:2;2O)
SM(44:2;2O)
SM(44:3;2O)
PC(38:1)|PC(20:0_18:1)
PC(38:5)|PC(18:1_20:4)
LPC(24:0-SN2)
PC(O-34:4)
PE(36:5)|PE(16:0_20:5)
PE(38:6)|PE(18:1_20:5)
PC(30:1)
TG(45:1)|TG(14:0_14:0_17:1)
TG(42:2)|TG(8:0_16:0_18:2)*
TG(42:1)|TG(10:0_14:0_18:1)*
TG(44:1)|TG(12:0_14:0_18:1)*
TG(44:2)|TG(10:0_16:0_18:2)*
TG(44:0)|TG(12:0_16:0_16:0)*
TG(46:2)|TG(12:0_16:0_18:2)*
TG(46:1)|TG(14:0_16:0_16:1)*
TG(46:0)|TG(14:0_16:0_16:0)*
TG(40:0)|TG(10:0_14:0_16:0)
TG(42:0)|TG(12:0_14:0_16:0)
TG(38:0)|TG(12:0_12:0_14:0)
TG(36:0)|TG(12:0_12:0_12:0)
TG(48:4)|TG(12:0_18:2_18:2)
TG(47:2)|TG(14:0_15:0_18:2)*
TG(48:3)|TG(14:0_16:1_18:2)*
TG(48:2)|TG(14:0_16:0_18:2)*
TG(46:4)|TG(10:0_18:2_18:2)*
Abundance (scaled)
IBD Subtype
Fecal Calprotectin
Treatment Escalation
CD
UC
Elevated
Normal
Needed
No
Yes
E
Correlation cut-off, 0.5
Positive
Negative
Proteomics
Metabolomics
Lipidomics
Active
Remission

Figure 4. Single and integrative omics analysis presenting molecular perturbances associated with disease activity states. (A) Sample collection and grouping. Heatmap of differential (B) proteins, (C) metabolites, and (D) lipids. (D) DIABLO-selected signatures. Abbreviations: IBD: inflammatory bowel disease, UC: ulcerative colitis, CD: Crohn's disease. * and bold: analytes that detected in both single- and multi-omics data analysis.

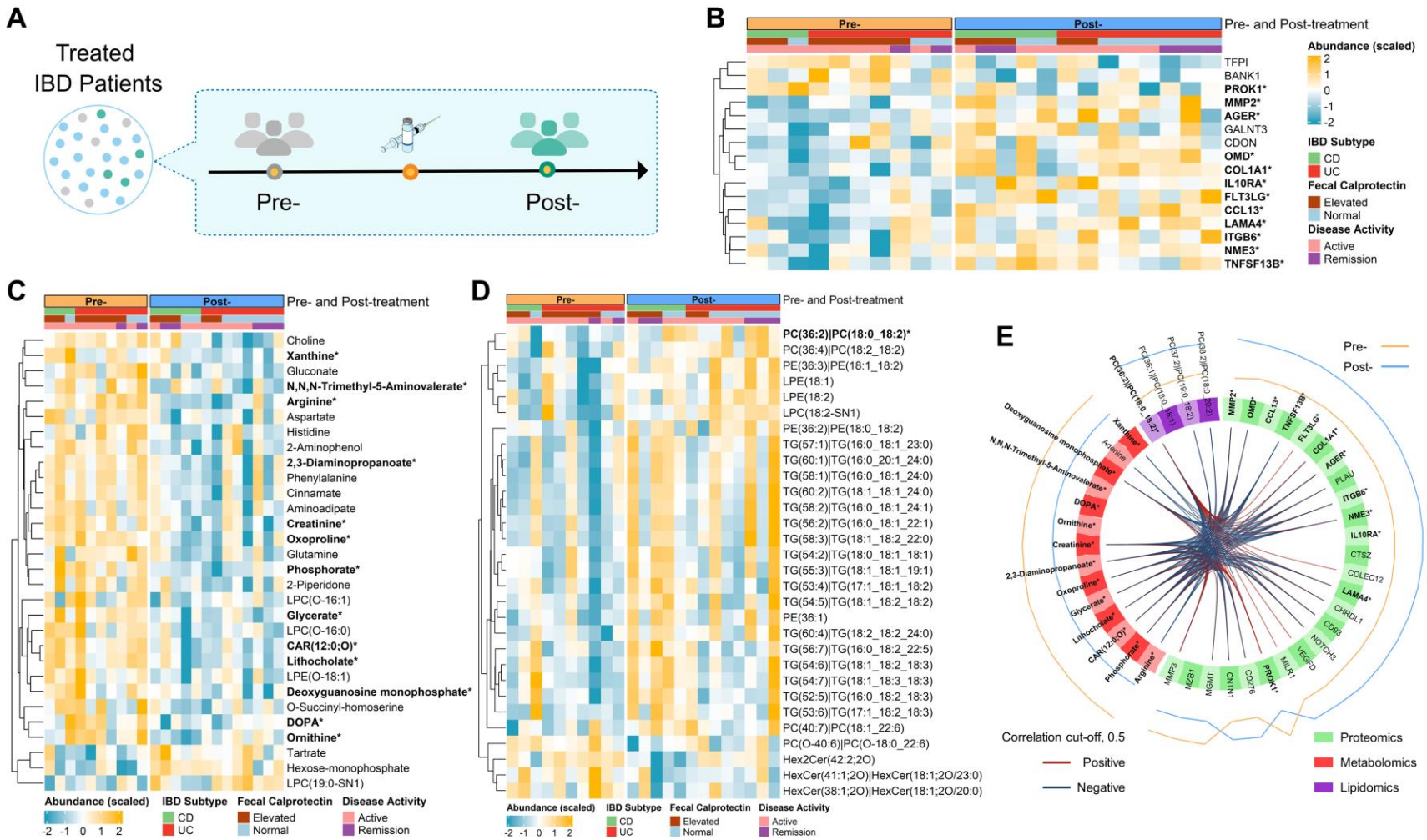


Figure 5. Single and integrative omics analysis presenting molecular perturbances for the pseudo pre- and post-treatment escalation design. (A) Sample collection and grouping. Heatmap of differential (B) proteins, (C) metabolites, and (D) lipids. (D)

DIABLO-selected signatures. Abbreviations: IBD: inflammatory bowel disease, UC: ulcerative colitis, CD: Crohn's disease. * and bold: analytes that detected in both single- and multi-omics data analysis.

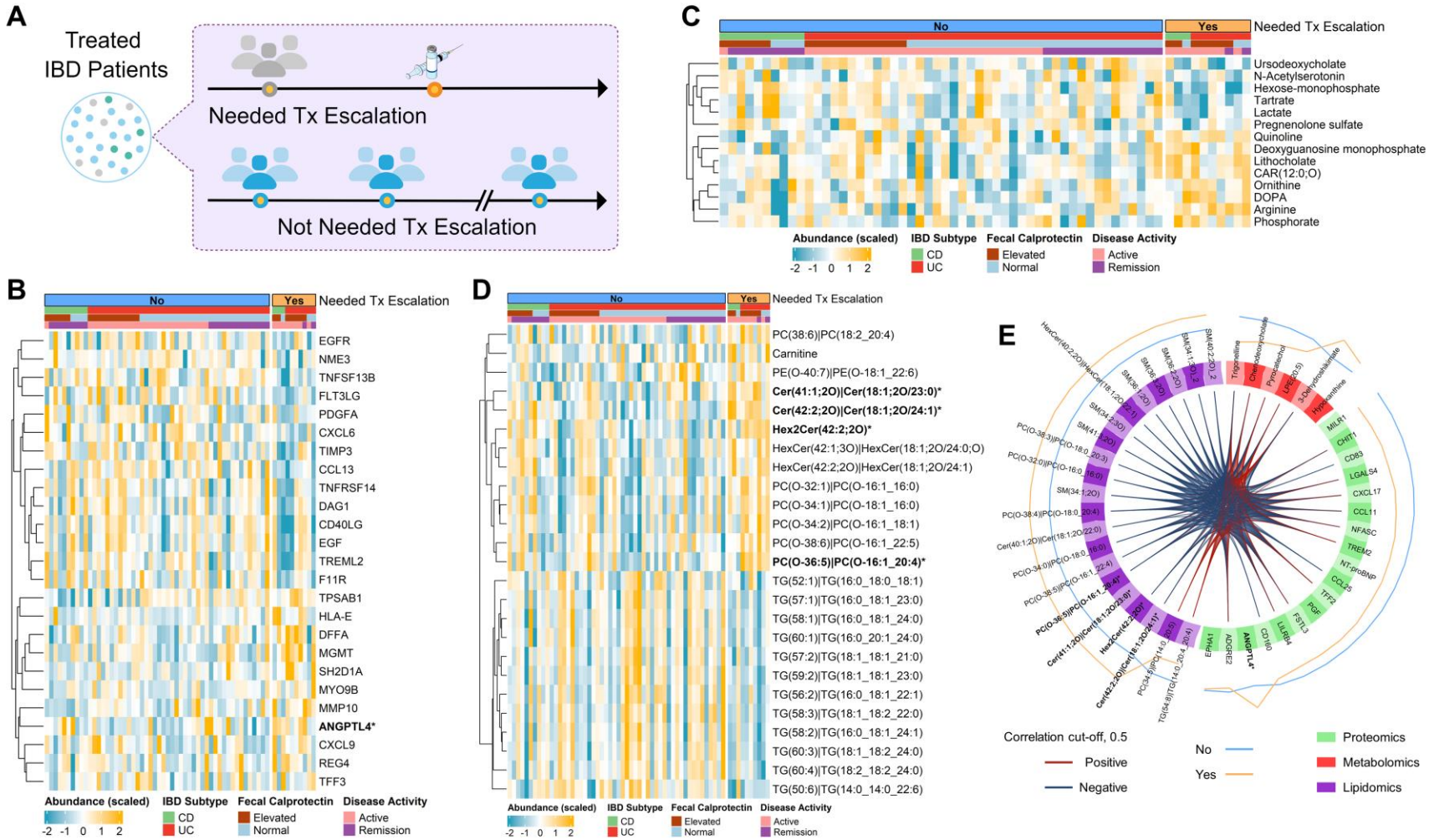


Figure 6. Single and integrative omics analysis presenting molecular perturbances between IBD patients who needed treatment escalation and those who did not. (A) Sample collection and grouping. Heatmap of differential (B) proteins, (C) metabolites, and

(D) lipids. (D) DIABLO-selected signatures. Abbreviations: IBD: inflammatory bowel diseases, Tx escalation: treatment escalation, UC: ulcerative colitis, CD: Crohn's disease. * and bold: analytes that detected in both single- and multi-omics data analysis.

Table 1. Clinical characteristics by disease subtypes.

	CD, N = 18[1]	UC, N = 57[1]
Age at recruitment [years]	30 (25, 53)	41 (27, 55)
BMI [kg.m^{-2}]	23.6 (20.8, 25.4)	22.0 (20.7, 23.8)
Sex, females	5 (28%)	22 (39%)
Time since diagnosis [years]	1.08 (0.32, 2.07)	1.48 (0.50, 2.71)
Smokers		
-	11 (61%)	41 (72%)
No	6 (33%)	12 (21%)
Yes	1 (6%)	4 (7%)
Disease state[2]		
Active	7 (39%)	38 (67%)
Remission	11 (61%)	19 (33%)
Fecal calprotectin[3]		
Elevated	11 (61%)	19 (33%)
Normal	7 (39%)	38 (67%)
CRP [mg/L]	0.28 (0.08, 1.01)	0.08 (0.04, 0.19)*
5-aminosalicylic acid, Yes	17 (94%)	56 (98%)
Steroids, Yes	3 (17%)	15 (26%)
Azathioprine, Yes	12 (67%)	18 (32%)*
Biologics, Yes	8 (44%)	15 (26%)

* $p < 0.05$. Wilcoxon rank-sum test and Fisher's exact test were used for continuous and categorical variables.

[1] Data are presented as median (interquartile range) or n (%).

[2] The MAYO score cut-off of 1 and CD activity index (CDAI) score cut-off of 150 concerning CD and UC were used to reflect the disease activity in either active (i.e., mild, moderate, or severe conditions) or remission.

[3] A 150 µg/g cut-off was used to categorize participants into elevated or normal groups.

Table 2. Predictability of selected integrative biosignatures in five scenarios.

	AUC[1]	P-value
IBD subtypes, CD *vs.* UC	0.8021	0.0032
Fecal calprotectin, elevated *vs.* normal	0.7358	0.0172
Disease state, active *vs.* remission	0.6192	0.204
Post- *vs.* pre-treatment escalation	0.9228	0.0075
Needed *vs.* not needed treatment escalation	0.7695	0.0174

[1] Average AUC via cross-validation procedure.