

Fecal metabolic signals are associated with changes in microbiota and systemic metabolic pathways in Crohn's disease

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One Sentence Summary: Changes in fecal metabolites are associated with microbial signals and host metabolic pathways in the pathogenesis of Crohn's Disease (CD).

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Supplementary Materials

Table S1. The number of overlapping samples in each omics' pair in Fig. 2 and Fig. S1 .	
Datasets	N samples
Serum metabolomics & Fecal metabolomics	117
Serum metabolomics & 16S	133
Fecal metabolomics & 16S	229
Fecal metabolomics & MGX	129

Supplementary datasets (Excel files)

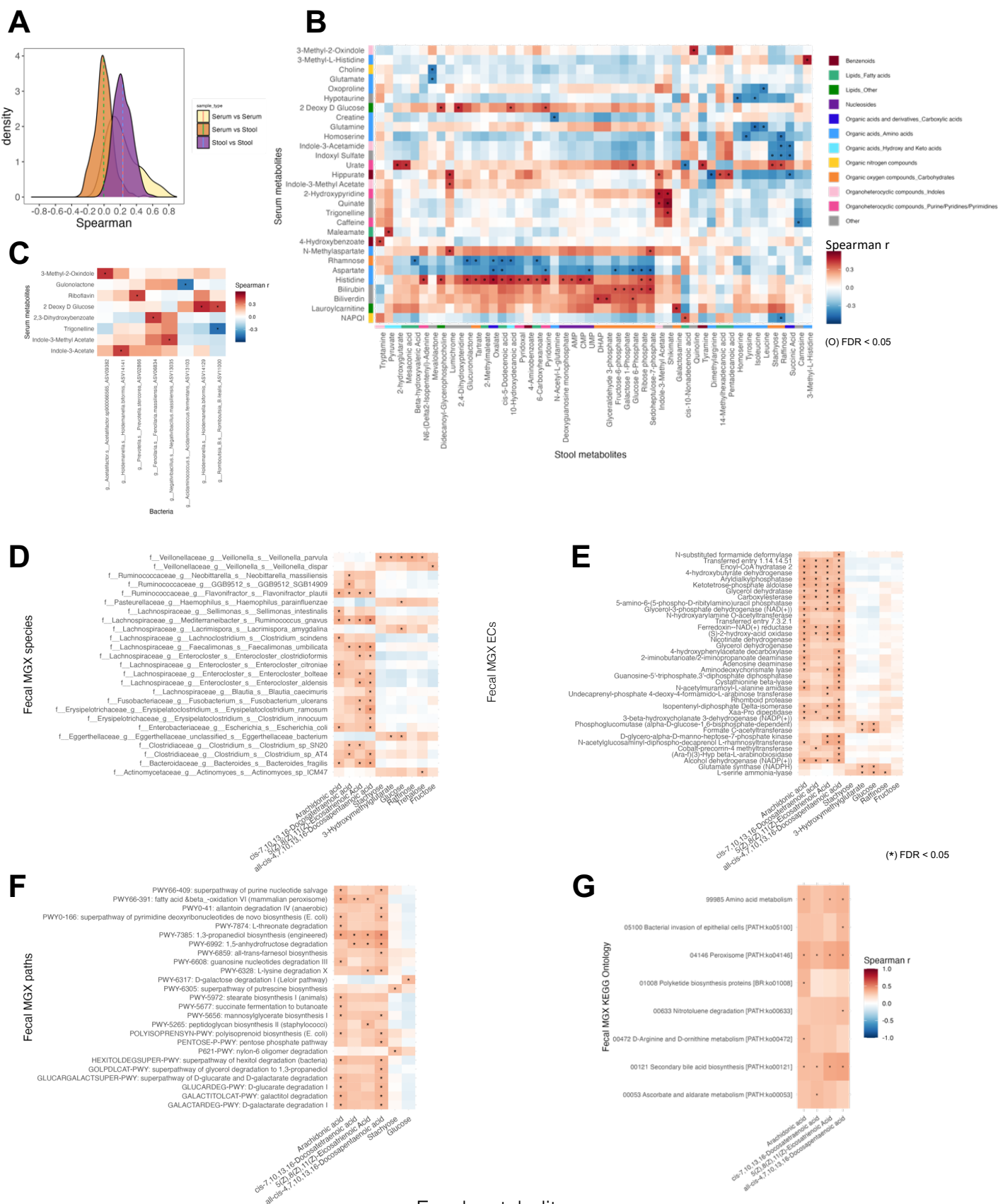
Dataset S1: Datasets of the different omics, and their classifications/taxonomy.

Dataset S2: Multi-omics associations, as presented in Fig. 2 and Fig. S1.

Dataset S3: MaAsLin differential expression of serum metabolomics, fecal metabolomics, and 16S, in CD vs. controls, and the corresponding Metaboanalyst pathway enrichment in CD vs. controls, in serum and feces, as presented in Fig. 3 and Fig. S3-S4.

Dataset S4: MaAsLin differentially abundant serum and fecal metabolites, in clinically active CD (vs. CD in remission), fecal calprotectin (FCP) > 100 (vs. FCP ≤100), elevated CRP (>5 vs. CRP ≤5), as well as significant correlations of fecal metabolites with alpha diversity (faith pd) and CD dysbiosis index, and the corresponding Metaboanalyst pathway enrichment in clinically active CD, in serum and feces, as presented in Fig. 4 and Fig. S5.

Supplementary Materials



Fecal metabolites

Fig. S1. Multi-omics associations. A. Histogram plots showing the distributions of Spearman rho correlations of the same metabolite within and between the different omic datasets. Spearman rho was calculated for pairs of samples obtained from the same subject [serum (yellow, n=294 pairs) and fecal (purple, n=617 pairs)] in different sampling time points, while metabolites correlation between serum and feces (orange) was calculated from pairs of samples obtained from the same subject and timepoint (n=117 pairs). The dotted lines represent the mean Spearman rho. **B-G.** HAllA (Hierarchical All-against-All Associations) based heatmaps showing Spearman correlations between serum metabolomics vs. fecal metabolomics (**B**), serum metabolomics vs. 16S microbial amplicon sequence variant (ASV) taxa (**C**), and fecal metabolomics vs. shotgun metagenomics (MGX) bacterial species (**D**), vs. MGX bacterial pathways (**E**), vs. MGX bacterial enzymes (ECs) (**F**), or vs. MGX KEGG level 3 ontology (**G**). *Indicates $FDR \leq 0.05$. The complete results are in **Dataset S2**. **Table S1** indicates the numbers of overlapping omics' pairs of samples included in each analysis.

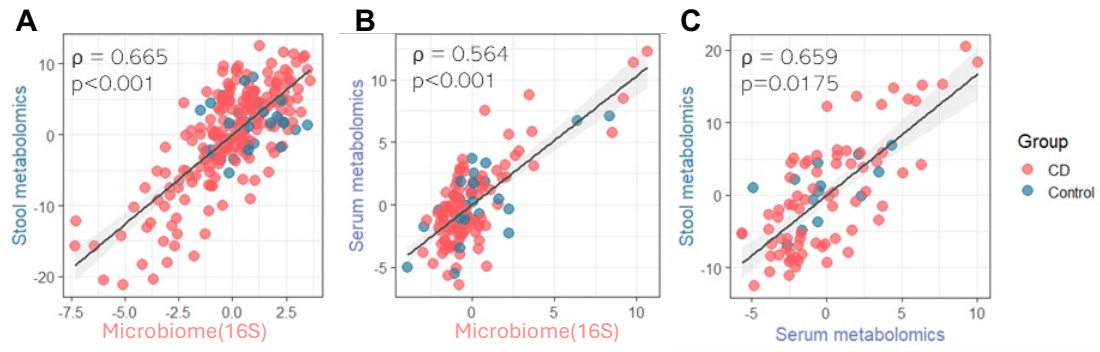


Fig. S2. Relationship between 16S dataset and metabolomic profiles. Spearman correlation between the first PLS component of (A) 16S microbial bacteria and fecal metabolomics ($r = 0.665$, $p = 2.2 \times 10^{-16}$), (B) 16S microbial bacteria and serum metabolomics ($r = 0.564$, $p = 2.2 \times 10^{-16}$), and (C) fecal and serum metabolomics ($r = 0.659$, $p = 0.0175$).

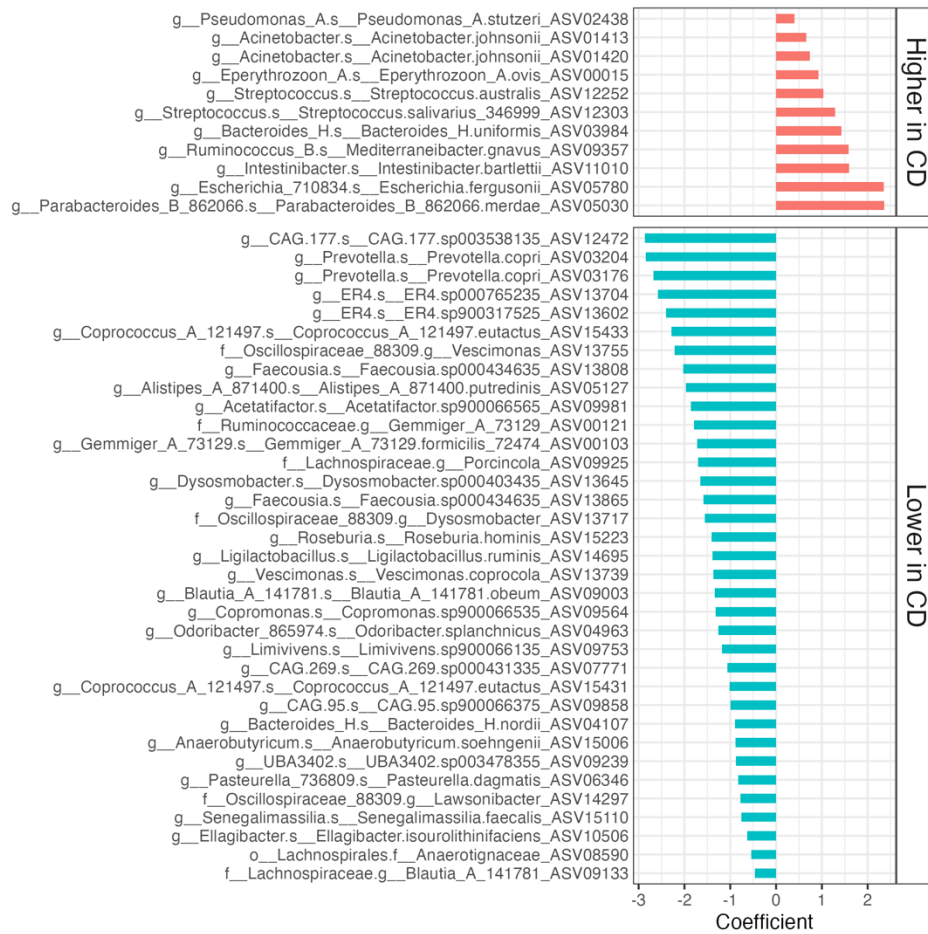


Fig. S3. Differentially abundant 16S microbial taxa in CD vs. controls. Coefficient values of 16S ASVs taxa that were differentially abundant ($FDR \leq 0.25$) between CD samples and healthy controls (sample $n=258$), based on MaAsLin multivariate analyses using patient ID as the random variable and controlling for age and gender. Colors represent the direction of the coefficient as indicated. The complete results are in **Dataset S3**.



Fig. S4. Serum and fecal metabolites and metabolic pathways that differed between CD and control samples. Pathway enrichment ratio and FDR of all serum (**A**) and fecal (**B**) significantly enriched pathways ($FDR \leq 0.1$) in CD vs. controls, based on Quantitative Enrichment Analysis in Metaboanalyst and using the differentially abundant metabolites presented in **Fig. 3B-C**. The complete results are in **Dataset S3**. **C**. Serum vs. fecal metabolites coefficients in CD vs. controls, for all differentially abundant metabolites ($FDR \leq 0.25$) in either serum or feces presented in **Fig.**

3B-C. Each metabolite (dot) is colored based on its HMDB classification. The complete results are in **Dataset S3**.

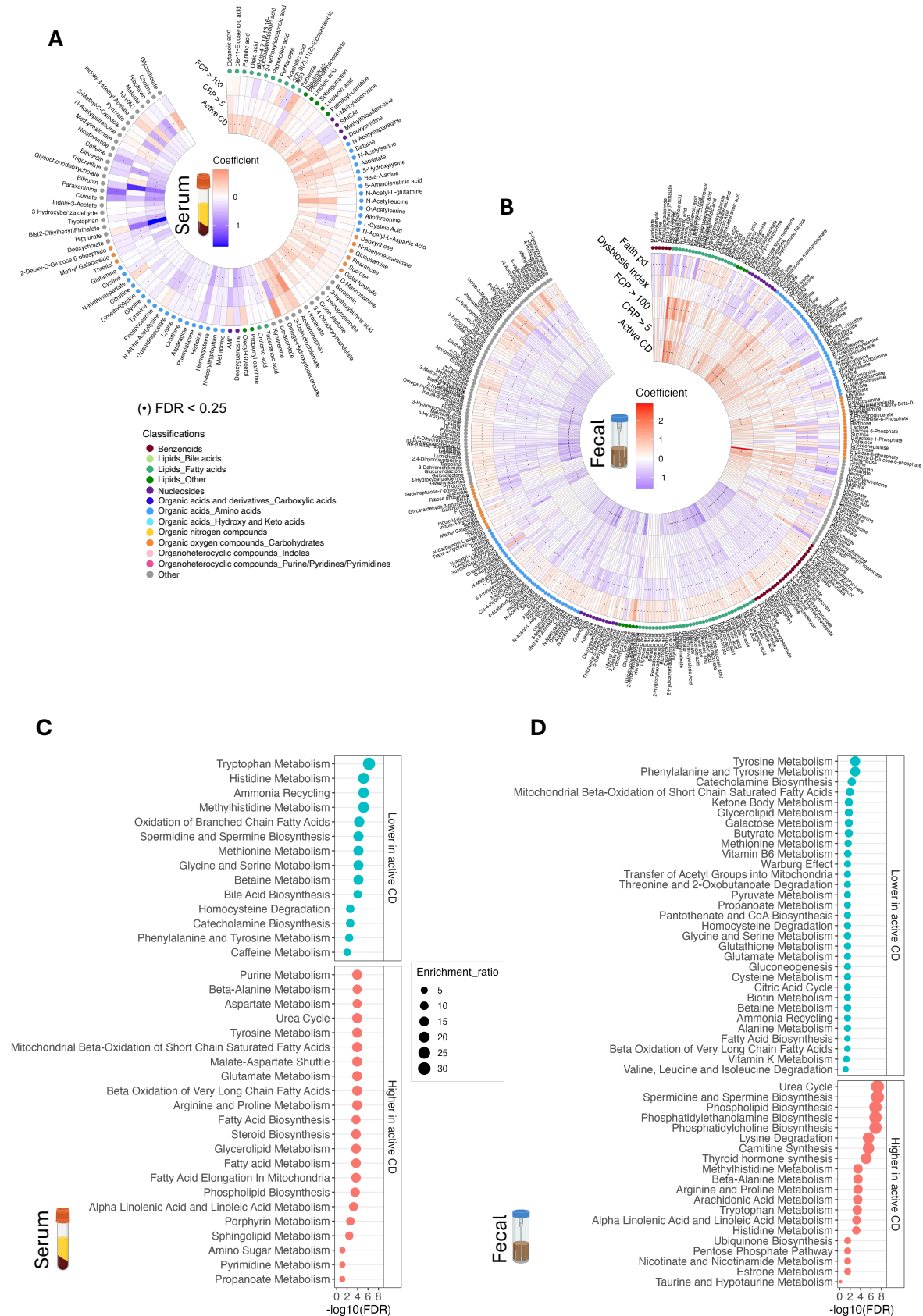


Fig. S5. Serum and fecal metabolites and metabolic pathways significantly associated with

disease activity. A. Complete heatmap of **Fig. 4C**, showing all serum metabolites significantly associated with at least one disease activity indicator (clinically active CD; CRP >5; FCP >100). Outer dots are colored by HMDB classifications. • indicates $FDR \leq 0.25$. The complete results are in **Dataset S4. B.** Complete heatmap of **Fig. 4D**, showing all fecal metabolites significantly associated with at least one disease activity indicator (clinically active CD; CRP >5; FCP >100; faith pd, and dysbiosis index). Outer dots are colored by HMDB classifications. • Indicates $FDR \leq 0.25$. The complete results are in **Dataset S4. C.** Pathway enrichment ratio and FDR of all significantly enriched pathways ($FDR \leq 0.1$) based on Quantitative Enrichment Analysis in Metaboanalyst and using the differentially abundant serum metabolites presented in **Fig. 4C. D.** Same as C, using the differentially abundant fecal metabolites presented in **Fig. 4D**. The complete results are in **Dataset S4.**