# Gut microbiome structure and metabolic activity in IBD

Eric Franzosa

Research Scientist, Huttenhower Lab

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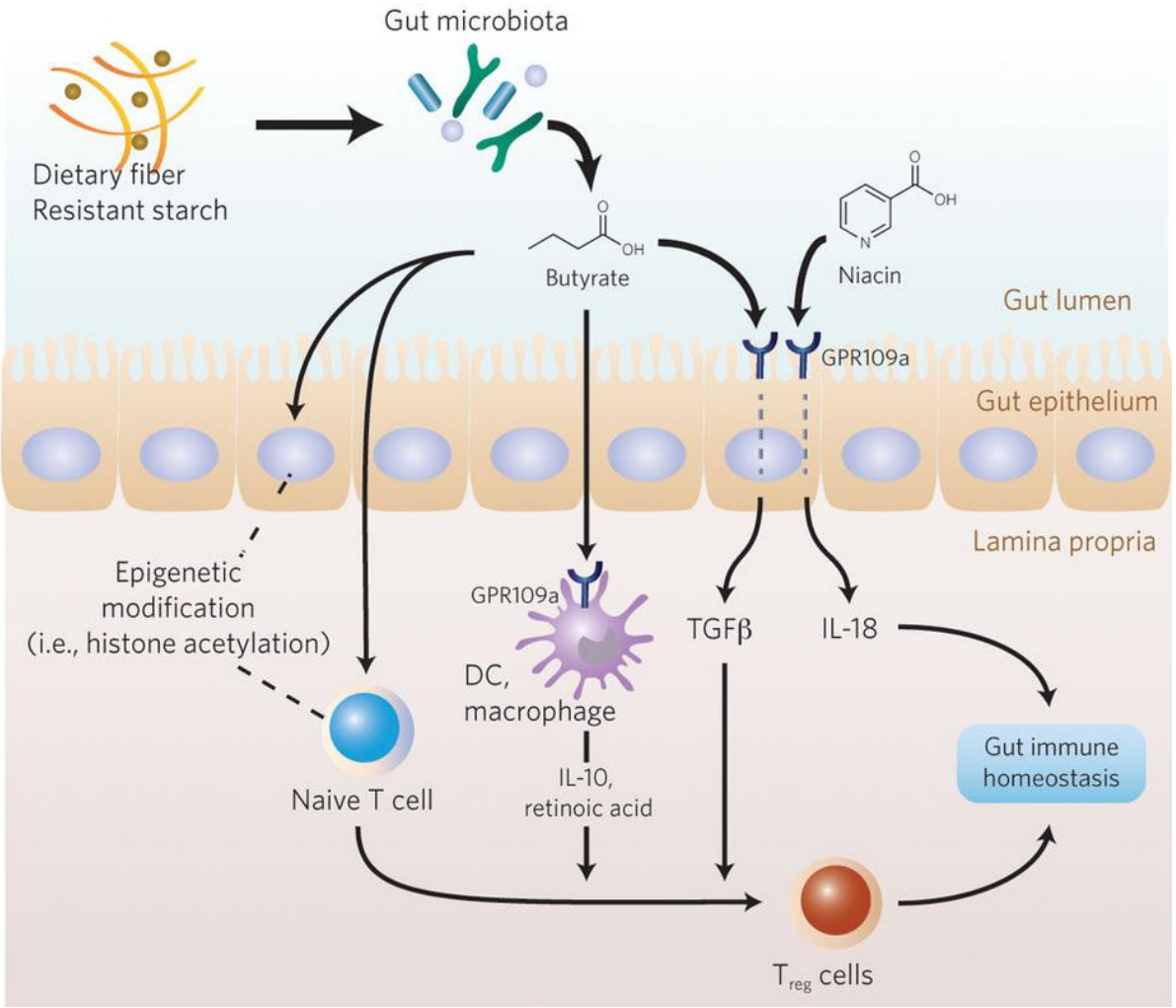
# The gut metabolome as a host-microbiome interface




Adapted from Won-Jae Lee & Koji Hase, *Nature Chemical Biology* 10, 416–424 (2014)

- Dietary compounds may directly promote/hinder the growth of certain microbes
- Microbes produce compounds with pro- and anti-inflammatory effects
- Most of what we know comes from targeted metabolomic surveys
- Potentially many microbially-derived, uncharacterized metabolites remain to be found

# Profiling metabolomes & metagenomes of the IBD gut

| | Non-IBD Control (control) | Crohn's Disease (CD) | Ulcerative Colitis (UC) |
|---|---|---|---|
| **Discovery cohort** (PRISM) | 34 | 68 | 53 |
| **Validation cohort** (NLIBD/LLDeep) | 22 | 20 | 23 |

**Multi'omic screening of stool samples**

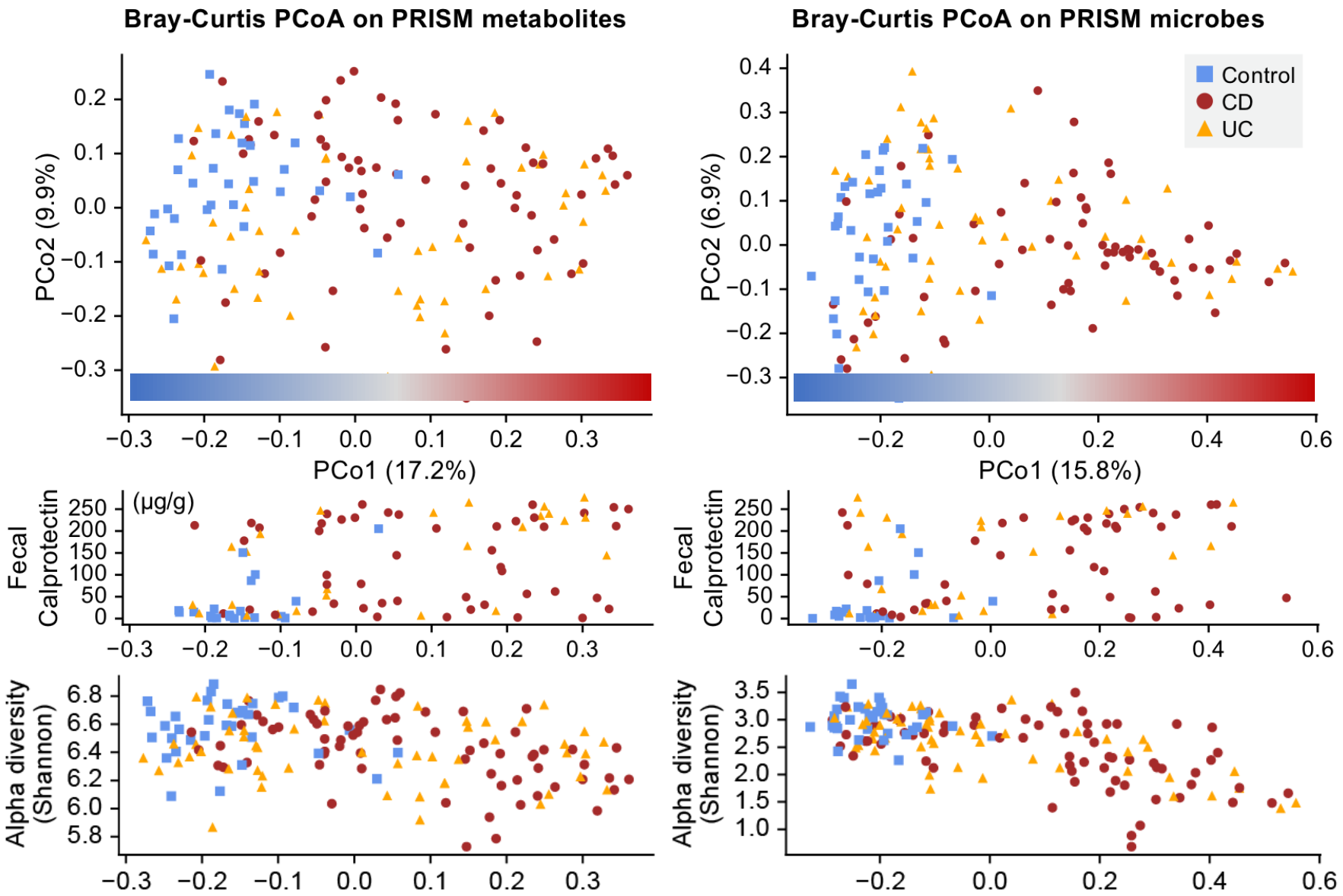
**Metagenomic shotgun sequencing**
Microbial taxa, genes, and pathways

+

**Four LC-MS metabolomics methods**
Lipids, polar metabolites, free fatty acids, & bile acids

- Metagenomes QC'ed by kneadData and profiled by MetaPhlAn2 + HUMAnN2
- ~8K measured metabolites from four untargeted metabolomics methods
- ~50% had approximate matches to HMDB (focus on molecular subclass)
- ~5% confidently matched standards from in-house compound library

# Broad ‘omics shifts correlate with host inflammation




Dominated by CD/control separation (UC is heterogeneous)

The two PCo1s were strongly correlated ($r_s$=0.66, $p$<$10^{-20}$)

PCo1s correlate with gut inflammation ($r_s$=0.49 and 0.44)

PCo1s anti-correlate with Shannon diversity ($r_s$=-0.32 and -0.57)

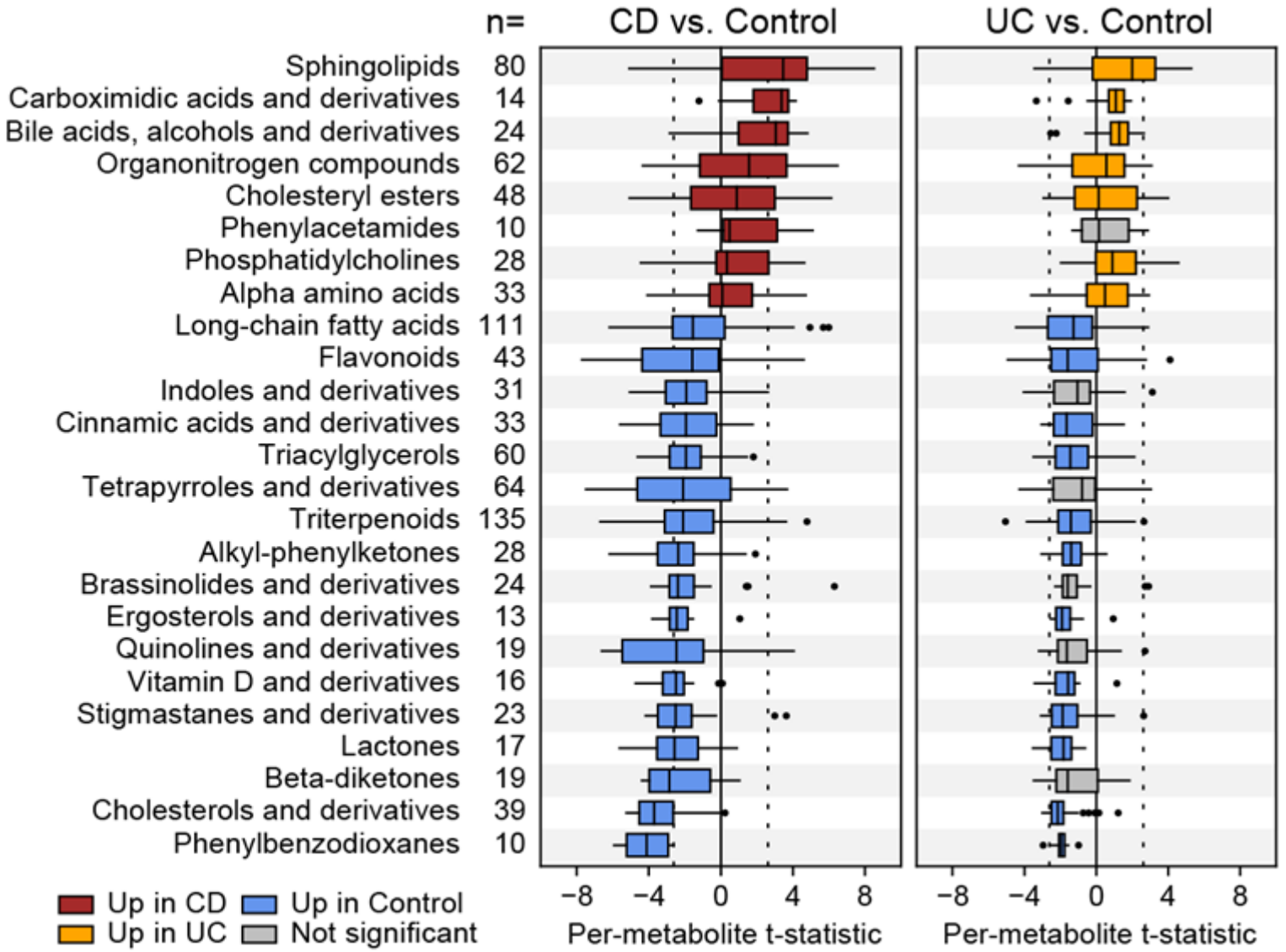
# Finding differentially abundant (DA) features in IBD

- Model (same for all features, sum-normalized within method):

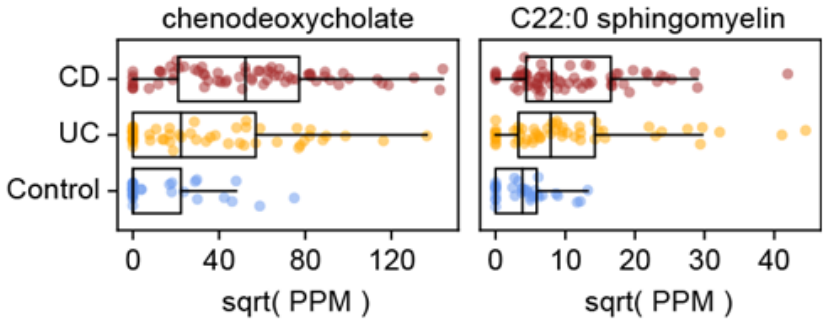
$$\log(\textbf{feature} + \epsilon) \sim \textbf{diagnosis} + \text{age} + \text{abx} + \text{mesalamine} + \text{immunosuppressants} + \text{steroids}$$

- Isolate features where diagnosis:CD or diagnosis:UC coefficient had Benjamini-Hochberg (FDR) $q$<0.05
- Save model residuals for downstream applications
- Metagenomic trends (species + enzymes) consistent with previous findings
  - We'll come back to these later…
- Many, many DA metabolites (~2.7K of 8K)
  - Used "GSEA" and clustering to simplify interpretation

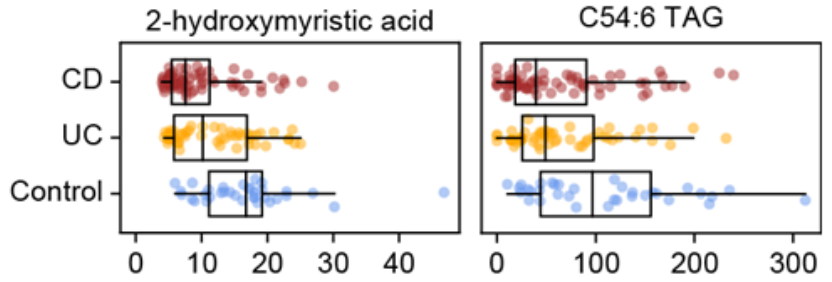
# Metabolite set enrichment analysis on model results



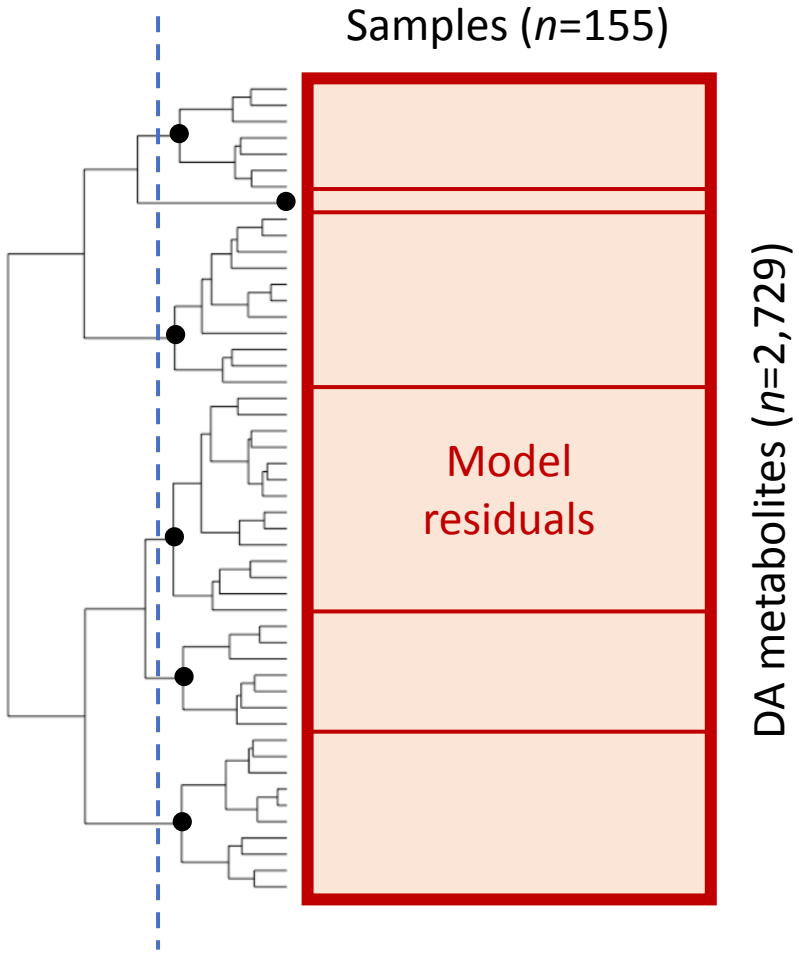

- UC trends are a weakened subset of CD trends
- Sphingolipids, bile acids up



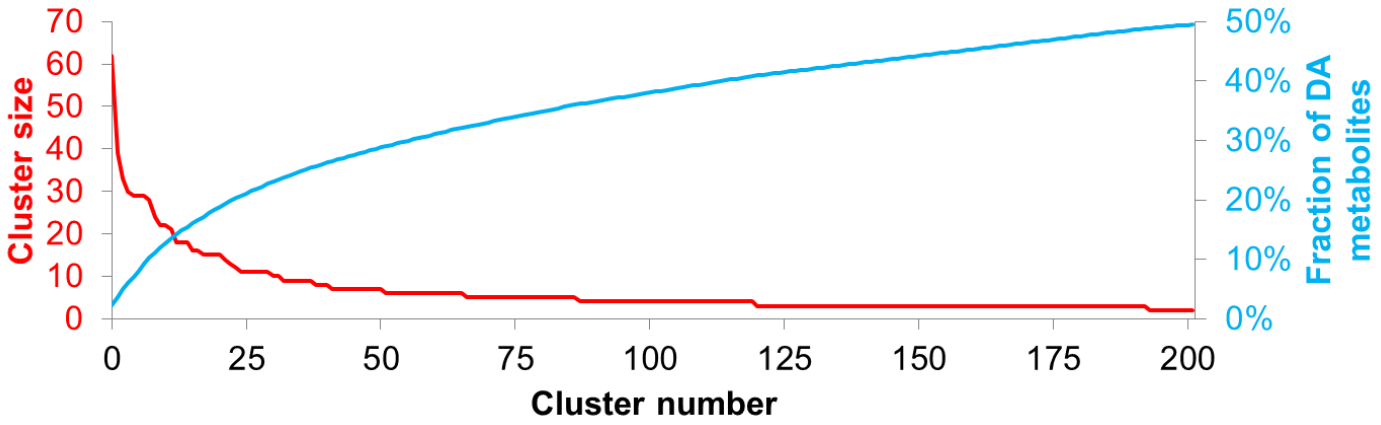

- Fatty acid subclasses down



# Finding clusters of co-varying, DA metabolites

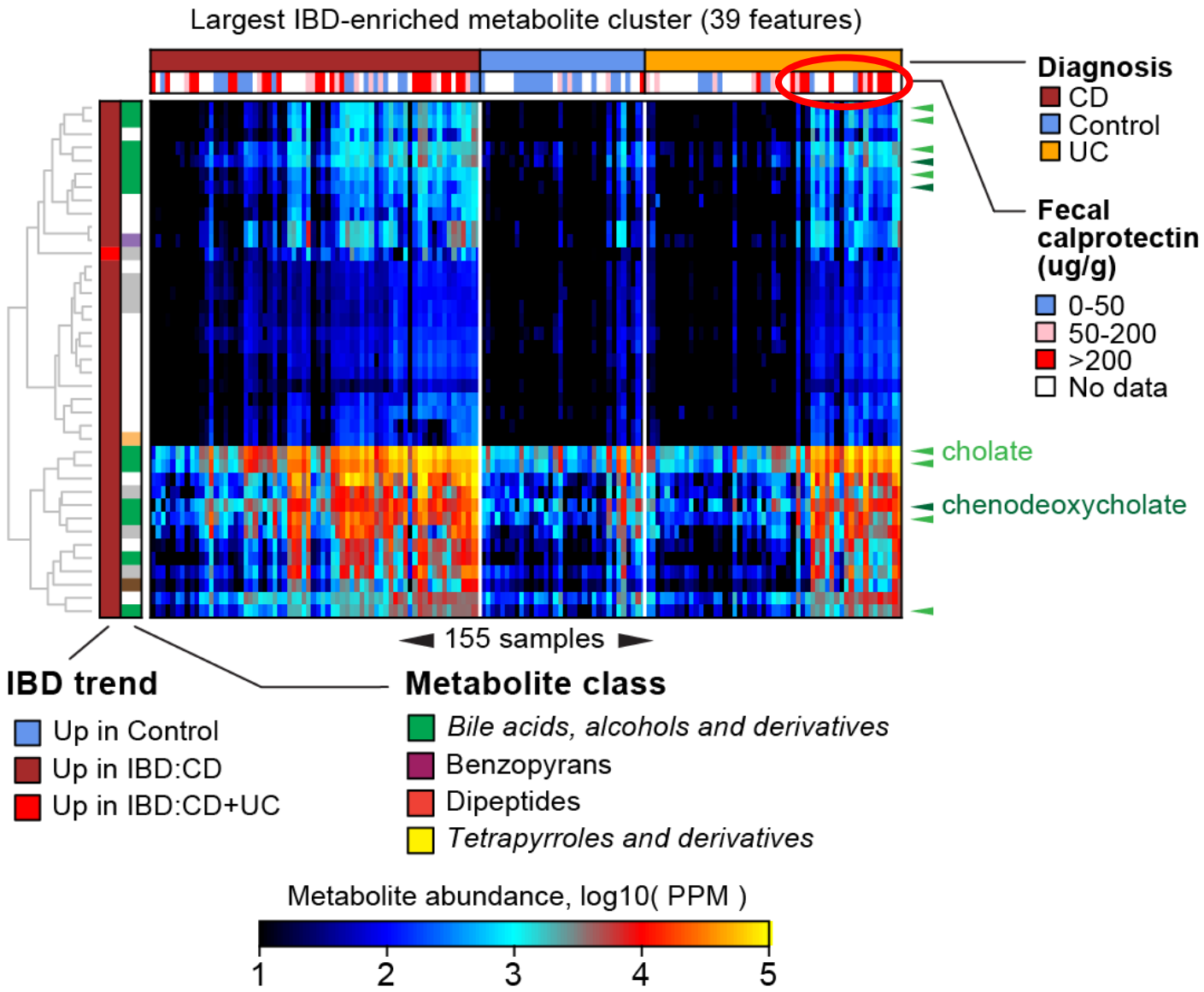



- Cluster DA metabolites based on residual abundance (Spearman as similarity measure)
- "Cut" hierarchy to define clusters with mean similarity $r_s$ = 0.7 (strongly correlated)



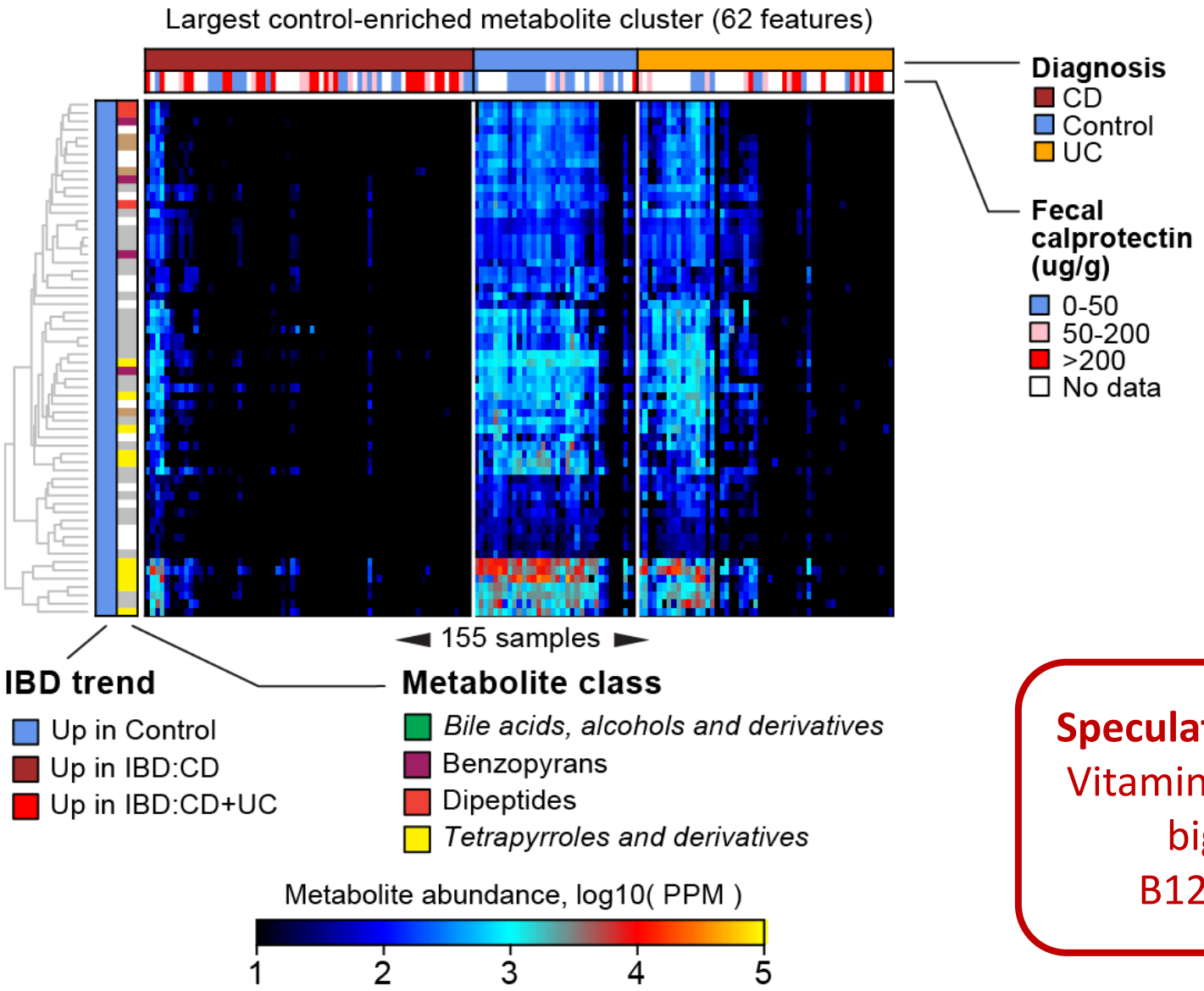

- Cluster members are **physicochemically similar**:
  - → 2.7x more similar retention times
  - → 3.0x more similar mass/charge ratios
  - → 15x more likely to be in same HMDB subclass

# The largest IBD-enriched cluster (mostly bile acids)




- Here, 39 bile-acid related metabolites co-vary strongly across samples
- All are elevated in CD
- Note substructure among UC patients, coinciding with high/low fecal calprotectin

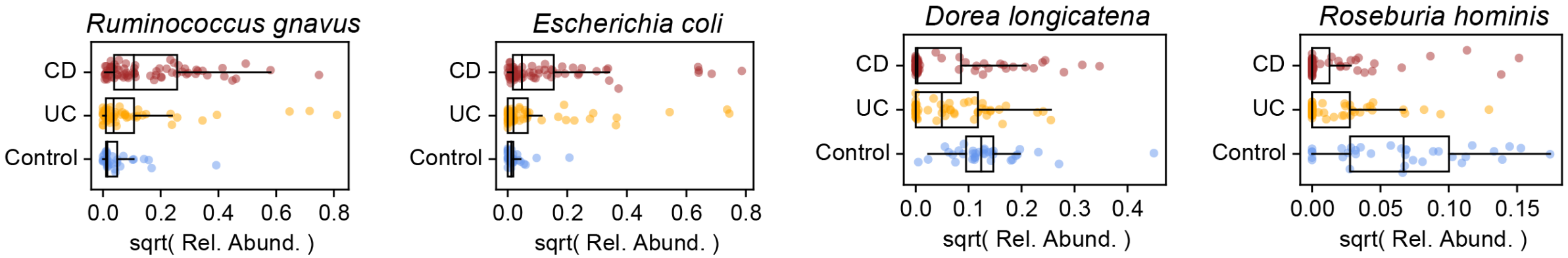
# The largest control-enriched cluster (mostly unknown)



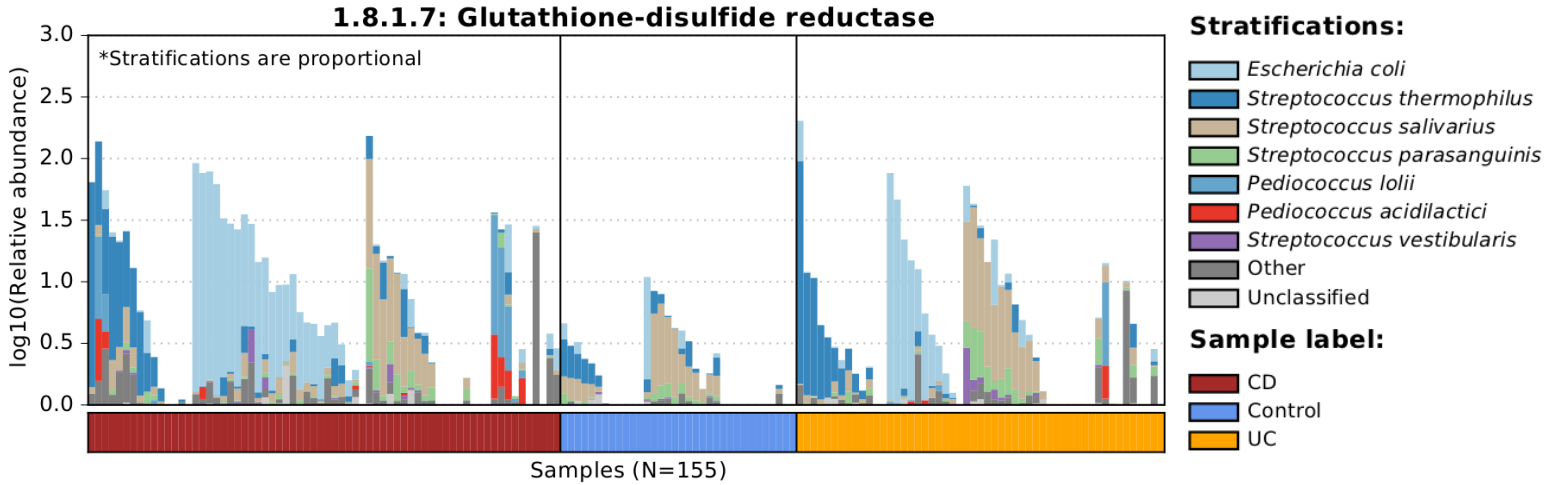

- This cluster (enriched in control subjects) contains no standard metabolites
- Could be enriched for uncharacterized microbially derived metabolites

**Speculation**: Lots of tetrapyrrole derivatives here. Vitamin B12 is a tetrapyrrole compound (but too big to be seen by these MS methods). B12-producing bugs are depleted in IBD.

# Metagenomic trends mostly follow previous findings



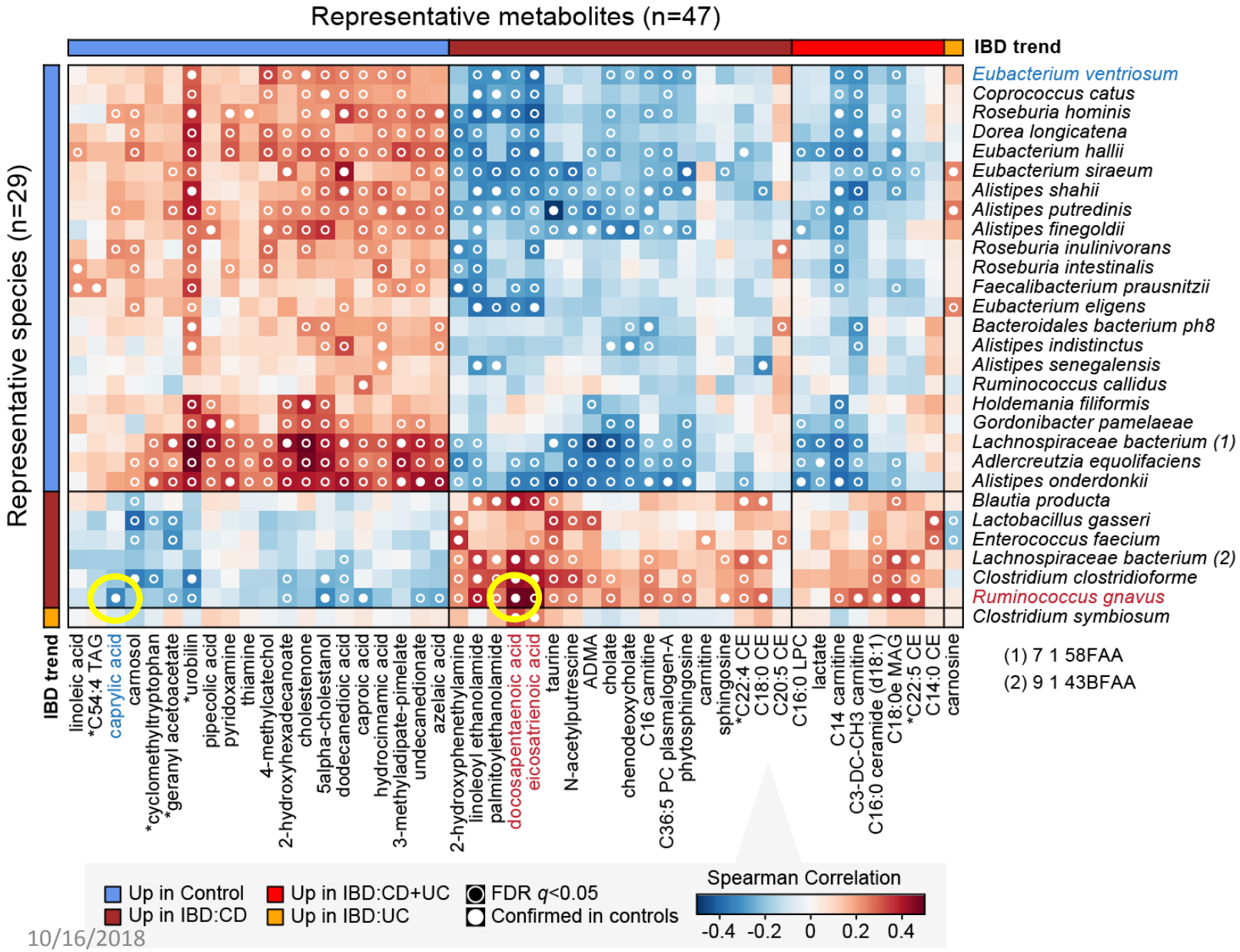

50 DA species, of which 35 were up in controls (reflects general loss of diversity in IBD)

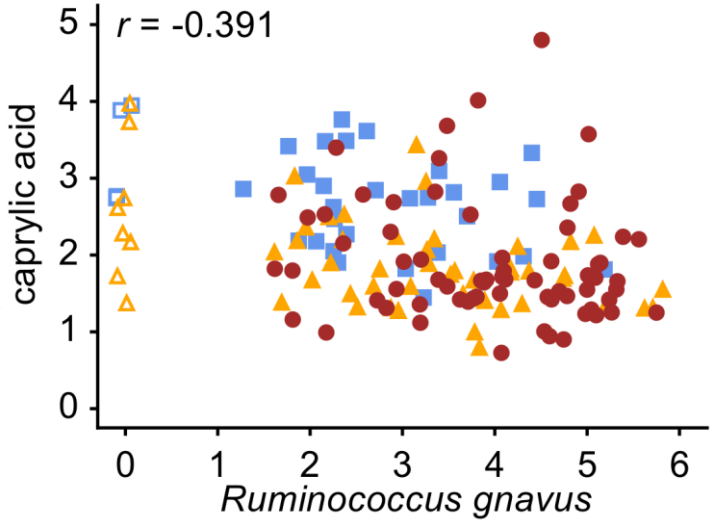


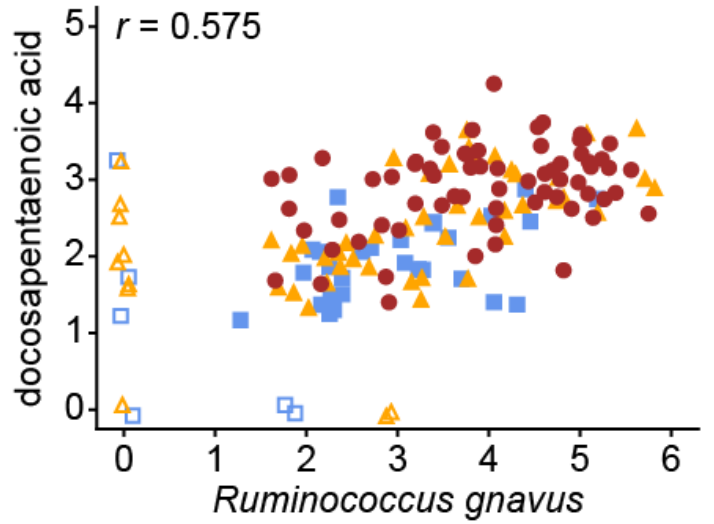

DA enzymes reflect community-level responses to a more aerobic environment in IBD (e.g. response to ox. stress)

***We focused on associating DA metagenomic features with DA metabolites!***

# Robust associations between metabolites & microbes

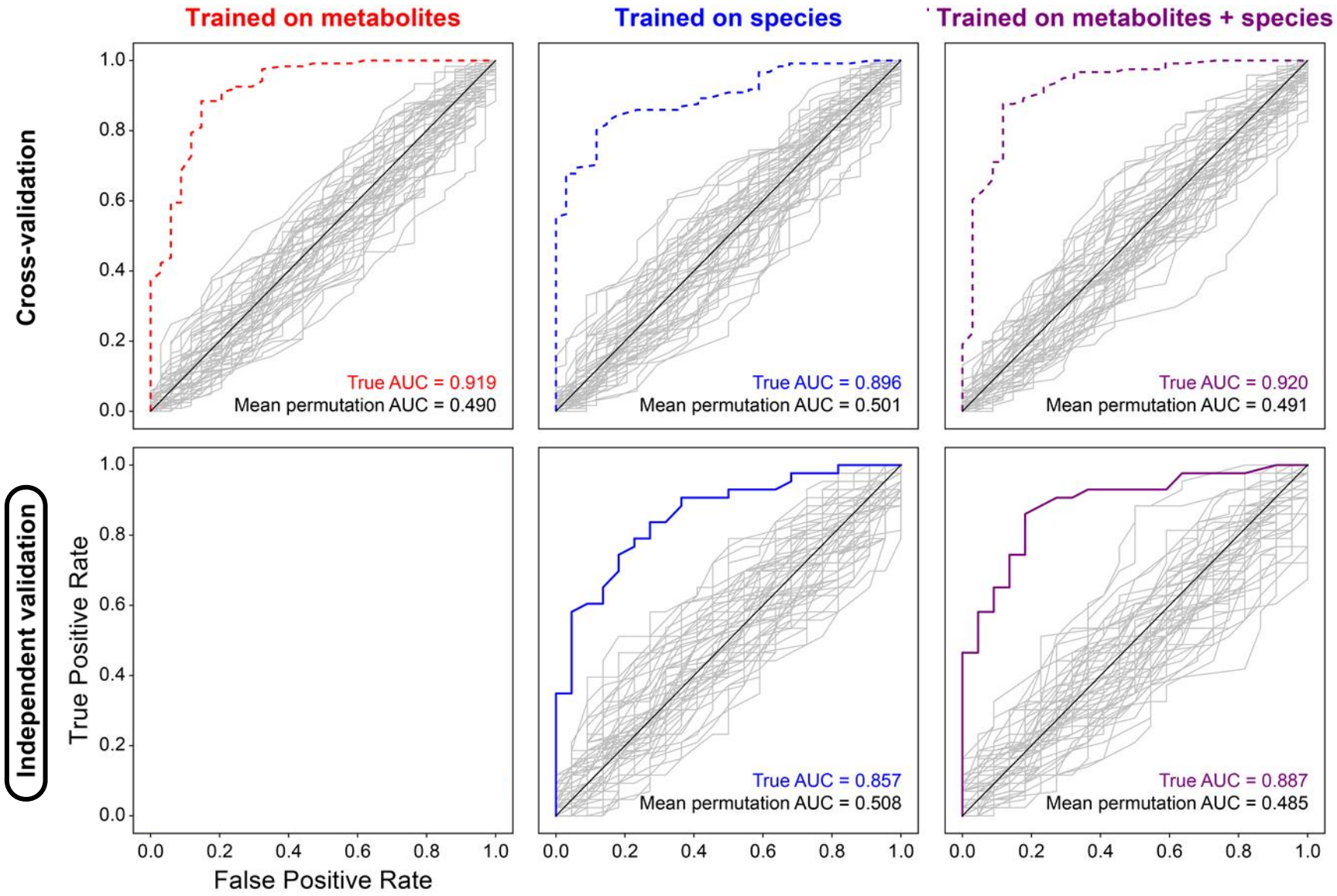



log10(PPM) units

# Random forest prediction of IBD status



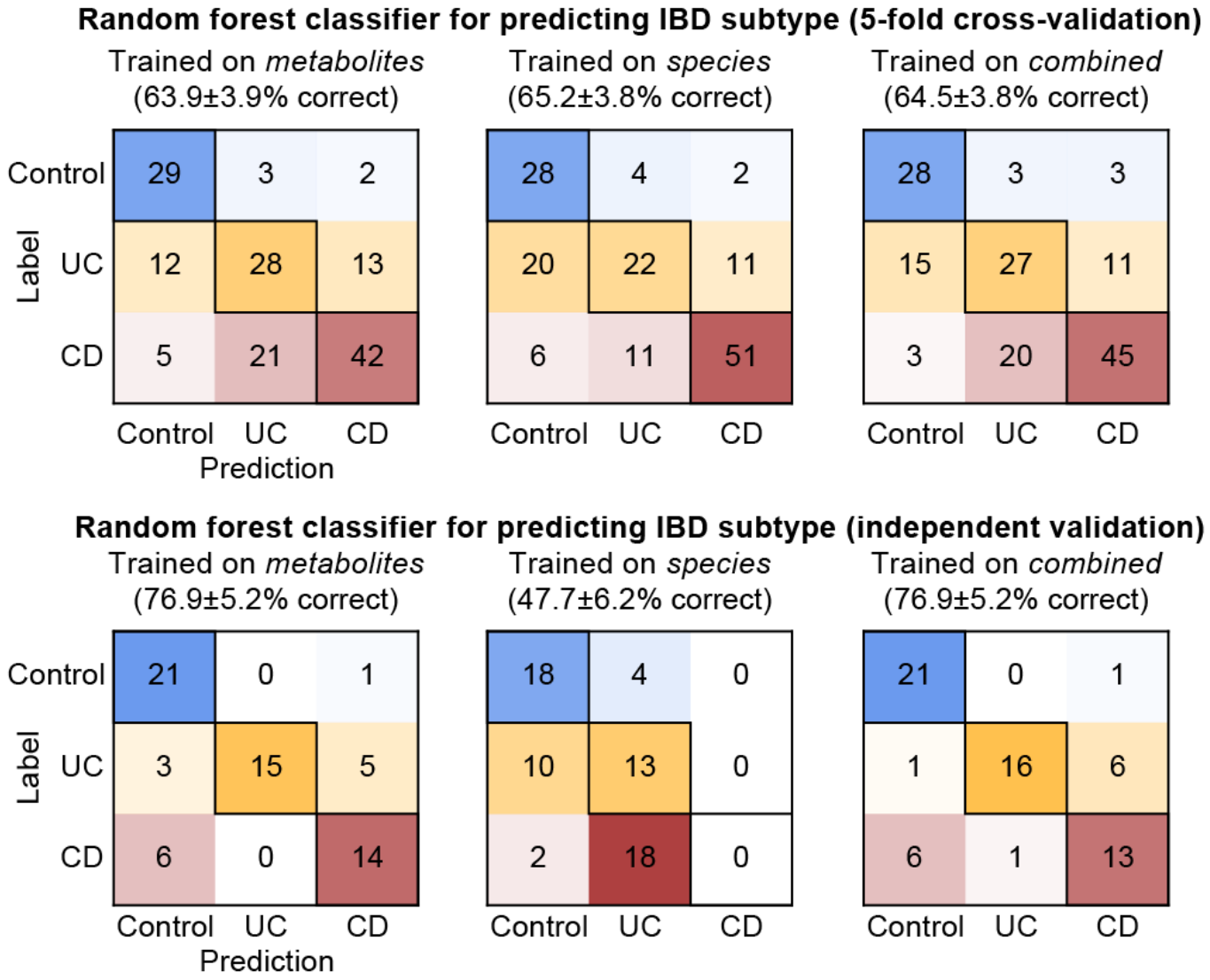

Metabolites predict IBD status well (AUC>0.9)

Predictor generalizes to new samples

Species also perform well, despite drawing on far fewer features

No marked boost in accuracy from combining metabolites and species

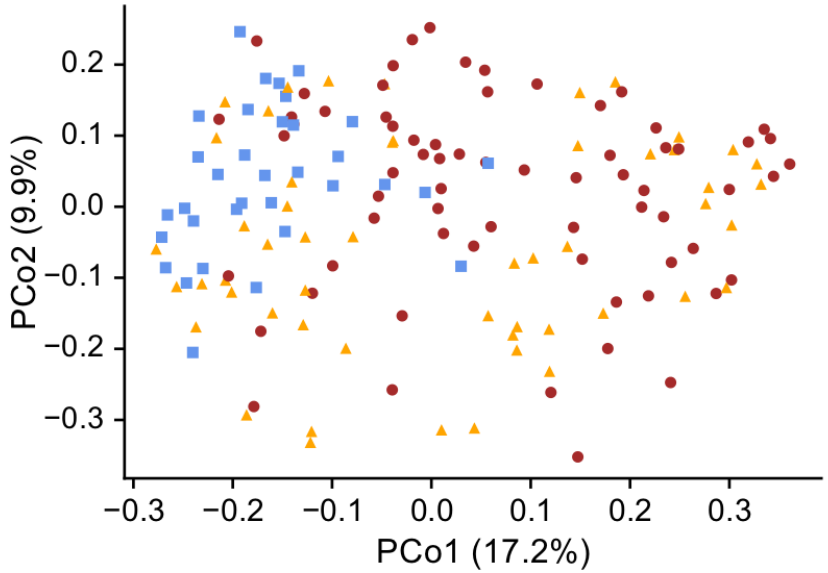
# Random forest prediction of IBD type: CD vs. UC



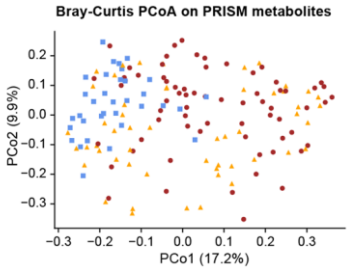

**Non-IBD** controls rarely classified as **IBD:CD** or **IBD:UC**

**IBD:CD** rarely classified as **Non-IBD** control, but sometimes as **UC**

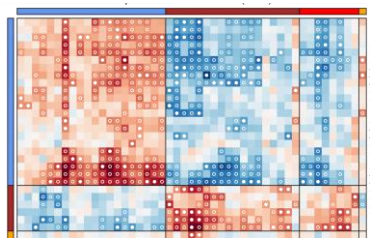
**IBD:UC** hard to get right



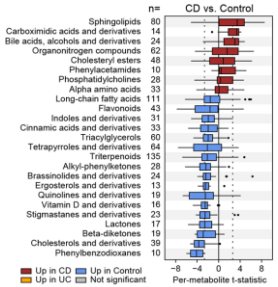
# Summary



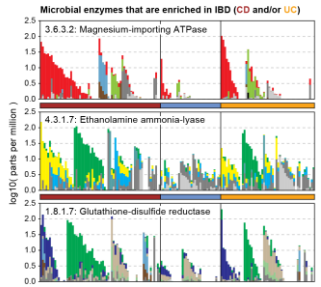

Metabolomes broadly stratified by subject inflammation level



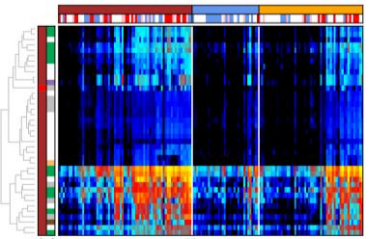
Putatively mechanistic microbe-metabolite associations



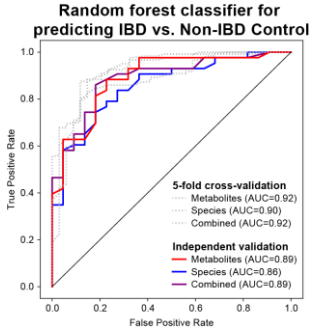

Many molecular classes are differentially abundant in IBD




Microbial functional adaptations to the IBD gut agree with earlier work



Some classes (clusters) are well understood, while others are largely uncharacterized




Metabolites and microbes can classify IBD status reasonably well, though subtype is harder

# Acknowledgements

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