



OPEN ACCESS

## ORIGINAL ARTICLE

## Fungal microbiota dysbiosis in IBD

Harry Sokol,<sup>1,2,3,4</sup> Valentin Leducq,<sup>1,4</sup> Hugues Aschard,<sup>5</sup> Hang-Phuong Pham,<sup>6</sup> Sarah Jegou,<sup>1,4</sup> Cecilia Landman,<sup>3,4</sup> David Cohen,<sup>1,2</sup> Giuseppina Liguori,<sup>7</sup> Anne Bourrier,<sup>3,4</sup> Isabelle Nion-Larmurier,<sup>3,4</sup> Jacques Cosnes,<sup>3,4</sup> Philippe Seksik,<sup>3,4</sup> Philippe Langella,<sup>2,4</sup> David Skurnik,<sup>8,9</sup> Mathias L Richard,<sup>2,4</sup> Laurent Beaugerie<sup>3,4</sup>

► Additional material is published online only. To view please visit the journal online (<http://dx.doi.org/10.1136/gutjnl-2015-310746>).

For numbered affiliations see end of article.

## Correspondence to

Dr Harry Sokol, Service de Gastroentérologie et Nutrition, Hôpital Saint-Antoine, 184 rue du faubourg St Antoine, Paris 75571, cedex 12, France; [harry.sokol@aphp.fr](mailto:harry.sokol@aphp.fr)

Received 17 September 2015

Revised 12 January 2016

Accepted 14 January 2016

Published Online First

3 February 2016

## ABSTRACT

**Objective** The bacterial intestinal microbiota plays major roles in human physiology and IBDs. Although some data suggest a role of the fungal microbiota in IBD pathogenesis, the available data are scarce. The aim of our study was to characterise the faecal fungal microbiota in patients with IBD.

**Design** Bacterial and fungal composition of the faecal microbiota of 235 patients with IBD and 38 healthy subjects (HS) was determined using 16S and ITS2 sequencing, respectively. The obtained sequences were analysed using the Qiime pipeline to assess composition and diversity. Bacterial and fungal taxa associated with clinical parameters were identified using multivariate association with linear models. Correlation between bacterial and fungal microbiota was investigated using Spearman's test and distance correlation.

**Results** We observed that fungal microbiota is skewed in IBD, with an increased Basidiomycota/Ascomycota ratio, a decreased proportion of *Saccharomyces cerevisiae* and an increased proportion of *Candida albicans* compared with HS. We also identified disease-specific alterations in diversity, indicating that a Crohn's disease-specific gut environment may favour fungi at the expense of bacteria. The concomitant analysis of bacterial and fungal microbiota showed a dense and homogenous correlation network in HS but a dramatically unbalanced network in IBD, suggesting the existence of disease-specific inter-kingdom alterations.

**Conclusions** Besides bacterial dysbiosis, our study identifies a distinct fungal microbiota dysbiosis in IBD characterised by alterations in biodiversity and composition. Moreover, we unravel here disease-specific inter-kingdom network alterations in IBD, suggesting that, beyond bacteria, fungi might also play a role in IBD pathogenesis.

## INTRODUCTION

Crohn's disease (CD) and UC, the two primary types of IBD, are lifelong conditions that usually affect young subjects and substantially alter their quality of life. The exact pathogenesis of IBD remains unknown; however, studies over the last decade have demonstrated that IBD involves an altered immune response towards gut microbiota in genetically predisposed subjects and under the influence of environmental factors. The bacterial microbiota in IBD has been thoroughly investigated, and several groups worldwide observed a bacterial dysbiosis (an imbalance in composition)

## Significance of this study

## What is already known on this subject?

- The bacterial intestinal microbiota is unbalanced in IBDs and plays a role in its pathogenesis.
- The fungal microbiota has been poorly studied despite several clues of its role in IBD pathogenesis.
- Card9 and Dectin1, two key molecules involved in the innate immunity against fungi, strongly influence mice susceptibility to intestinal inflammation and the fungal microbiota.

## What are the new findings?

- The faecal fungal microbiota is imbalanced in patients with IBD.
- The concomitant analysis of the bacterial microbiota in the same subjects showed many correlations between bacterial and fungal components with differences between IBD and healthy subjects, suggesting the existence of disease-specific inter-kingdom alterations.

## How might it impact on clinical practice in the foreseeable future?

- These results support the role of fungal microbiota in IBD pathogenesis and indicate a new potential therapeutic target.

that is characterised by a reduced biodiversity, a decrease in some bacteria belonging to the Firmicutes phylum (such as *Faecalibacterium prausnitzii*) and an increase in bacteria belonging to the Proteobacteria phylum such as *Escherichia coli*.<sup>1–5</sup> However, other microorganism types colonising the human gut have not been thoroughly investigated. With the exception of a recent study highlighting the possible role of the enteric virome in IBD,<sup>6</sup> the data are scarce, particularly regarding fungal microbiota. Fungi have long been suspected in IBD pathogenesis. Many years ago, antibodies directed against *Saccharomyces cerevisiae* mannan (Anti *Saccharomyces cerevisiae* antibody (ASCA)) were shown to be associated with CD. Moreover, several IBD-associated genes, such as Card9, are involved in immune responses to fungi.<sup>7</sup> In mice, gut inflammation promotes fungi proliferation;<sup>8</sup> conversely, some fungi can modulate susceptibility



CrossMark

**To cite:** Sokol H, Leducq V, Aschard H, et al. *Gut* 2017;**66**:1039–1048.

to inflammation in a negative (*Candida albicans*) or positive (*Saccharomyces boulardii*) manner.<sup>8–11</sup> Finally, mice lacking major genes involved in fungi sensing, such as Dectin-1 or Card9, have an increased fungal microbiota load and are more susceptible to colitis.<sup>12–13</sup> These data suggest a link between fungal microbiota and IBD pathogenesis.

Here, we characterised the fungal microbiota in both healthy subjects (HS) and patients with well-phenotyped IBD using high-throughput sequencing technology. In the corresponding patients, we also determined the bacterial microbiota composition and the sequence of 22 single-nucleotide polymorphisms (SNPs) in genes known to be involved in fungal susceptibility. We observed a clear fungal dysbiosis in patients with IBD. Moreover, a correlation analysis suggested altered inter-kingdom relations in IBD. Finally, while somewhat lacking in power, our genotype–fungal microbiota analysis suggested that genes may be a driving factor of the fungal microbiota dysbiosis in IBD.

Overall, the data presented in this study represent the most comprehensive analysis of fungal microbiota in patients with IBD to date and provide a rationale to support the role of fungal microbiota in IBD pathogenesis. These data thus pave the way for intervention studies targeting fungal microbiota.

## RESULTS

### Bacterial dysbiosis in IBD

Our study population was composed of 235 patients with well-phenotyped IBD and 38 HS (see online supplementary 1). We first analysed the bacterial fraction of the microbiota using 16S sequencing. A beta diversity analysis showed a clustering of samples according to disease phenotypes (figure 1A, B, online supplementary figure S1). Compared with HS samples, the alpha diversity (assessed using four different indexes) was significantly decreased in UC and CD and particularly in samples from patients in flare (figure 1C, D, online supplementary figure S2). In all phenotypes, the bacterial microbiota was dominated by bacteria from Firmicutes, Bacteroidetes and Proteobacteria phyla (figure 1E, F, online supplementary figure S3). These data are in accordance with the published literature and validate the quality of our cohort and the methods used.

### Altered fungal microbiota diversity in IBD

Using ITS2 sequencing, we then assessed the composition of the fungal microbiota in our population. The clustering between the samples according to disease phenotype was weaker than with bacterial microbiota (figure 2A, B, online supplementary figure S4A). Notably, no statistically significant difference was observed between samples from patients with CD and UC or between samples from patients with IBD in remission and HS. However, a difference was observed between samples from patients with IBD in flare and HS ( $p=0.0008$ ) or patients with IBD in remission ( $p=0.0007$ ). This fungal microbiota-specific signal in flare was observed with a higher intensity in UC ( $p=0.0002$ ) than in CD ( $p=0.006$ ; see online supplementary figure S4B–C). Similar to the results found with the bacterial microbiota, the alpha diversity of the fungal microbiota was decreased in patients with IBD (figure 2C, online supplementary figure S5A–D). This feature was primarily found in samples from patients with UC, whereas fungi diversity was largely similar among the HS and patients with CD. To explore the equilibrium between bacteria and fungi diversity in the gut, we then determined the fungi-to-bacteria diversity ratio. This ratio was increased in IBD samples and particularly in CD and flares (figure 2D, online supplementary figure S5E–G). The highest ratio was found among patients with CD and demonstrated ileal involvement. In both

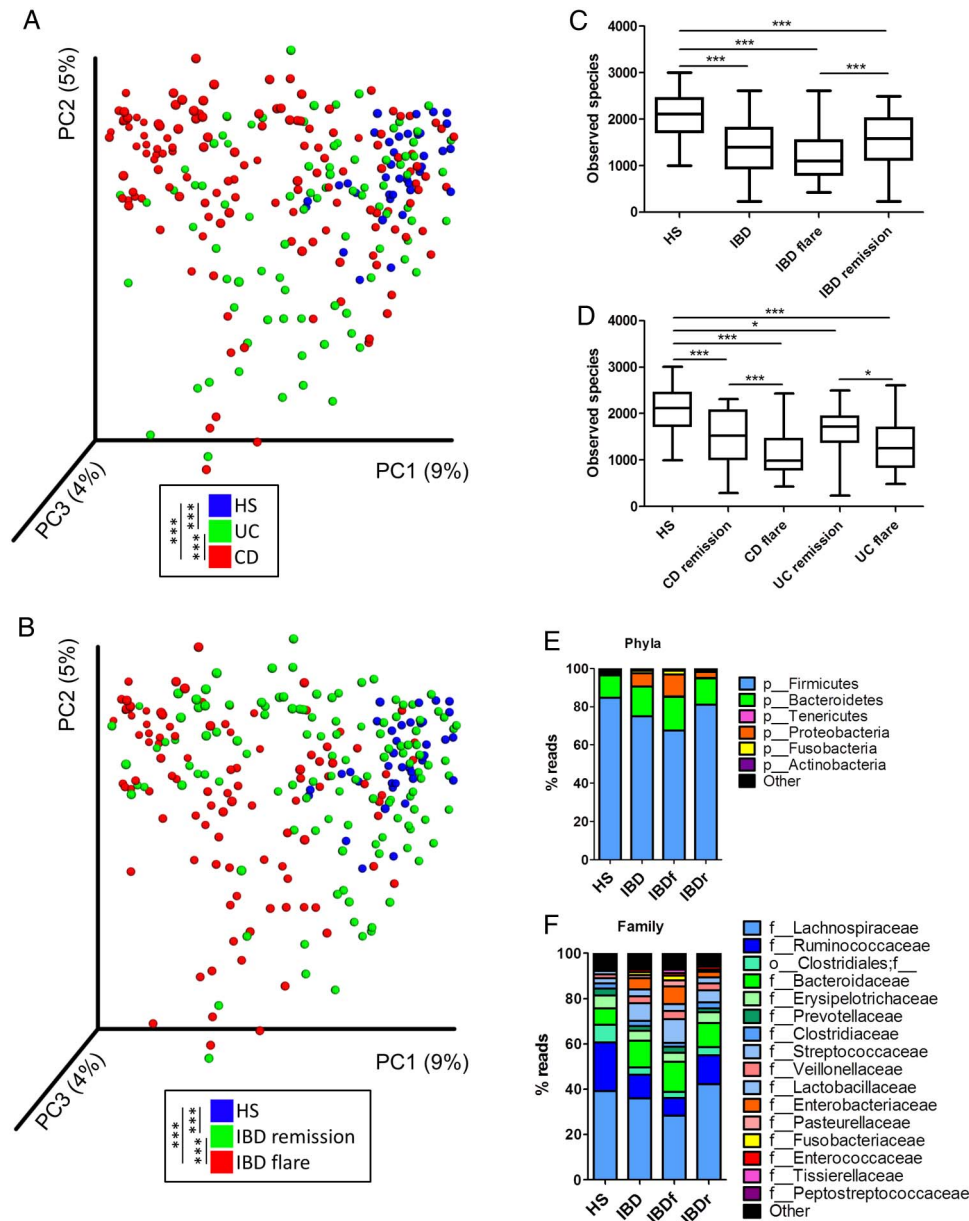
HS and patients with IBD, the fungal microbiota was dominated by fungi from the Ascomycota and Basidiomycota phyla with some variations according to disease phenotype (figure 2E, F, online supplementary figure S6). Among the most dominant genera were *Saccharomyces*, *Debaryomyces*, *Penicillium*, *Cluyveromyces* and *Candida*. Interestingly, *Saccharomyces*, *Debaryomyces* and *Cluyveromyces* are found in food (cheese, bread, beer notably); they might be routinely ingested in this French cohort, suggesting a possible influence of the diet habits, although a specific study is needed to explore this hypothesis.

These data show specific alterations in fungal microbiota diversity in parallel with modifications of the bacterial microbiota. Taken together, this suggests that the environmental changes during inflammation might affect differently fungi and bacteria and induce an altered fungal–bacterial inter-kingdom relationship in IBD.

### Distortion in bacterial and fungal microbiota composition in IBD

We identified the microbial features associated with disease phenotype and used a multivariate association test to control for the effects of potential confounding factors such as age, gender, smoking and treatment (multivariate association with linear models (MaAsLin); see ‘Materials and methods’ for details). Regarding bacterial microbiota, we observed an alteration in the abundance of several taxa in IBD compared with HS, in flare compared with remission and in IBD with ileal involvement compared with IBD without ileal involvement (figure 3A; see online supplementary figures S7A and 8, table S1 for full MaAsLin output). Many of these taxa have been reported in previous studies,<sup>1–5</sup> including Ruminococcaceae, Lachnospiraceae, Enterobacteriaceae, Pasteurellaceae, Rikenellaceae and Gemellaceae. Notably, *Ruminococcus*, *Coprococcus*, *Blautia*, *Eubacterium* and *Dorea* abundance were decreased in IBD, *Roseburia*, *Faecalibacterium*, *Dorea* and *Blautia* abundance were decreased in IBD flare and *Ruminococcus gnavus* was increased in ileal CD. In addition to confirming these already demonstrated associations, we also found new associations such as a decrease in *Anaerostipes* in IBD and particularly in flare and in ileal CD. We also found an increase of *Streptococcus anginosus* in IBD and an increase of *Aggregatibacter segnis* and *Actinobacillus* (two members of the Pasteurellaceae family) in IBD flare compared with remission.

When analysing fungal microbiota, we identified a lower number of associations with the disease phenotype (figure 3B; see online supplementary figure S7B, table S2 for full MaAsLin output). One of the most striking features was the increased abundance of Basidiomycota in IBD and particularly in flare, which was balanced by an equivalent decrease in Ascomycota. Among the decreased Ascomycota in IBD and in flare, *Malassezia sympodialis* was identified. However, the *Saccharomyces* genus and particularly *S. cerevisiae* species exhibited the strongest signals. We thus performed real-time qPCR targeting *S. cerevisiae* on the same samples and confirmed a clear decrease in *S. cerevisiae* both in the absolute number and regarding the proportion in IBD and particularly in flare (figure 3C, online supplementary figure S9A–B). Although *C. albicans* abundance is increased in CD,<sup>14</sup> *Candida* was not identified to be associated with disease phenotype in the multivariate analysis. We thus performed real-time qPCR targeting *C. albicans* on the same samples to specifically examine this microorganism. The *C. albicans* proportion and absolute number were significantly increased in IBD flare compared with IBD in remission (figure 3D, E, online supplementary figure S9C). We investigated correlations between their respective



**Figure 1** Altered bacterial microbiota biodiversity and composition in IBD. (A and B) Beta diversity. Principal coordinate analysis of Bray–Curtis distance with each sample coloured according to the disease phenotype. PC1, PC2 and PC3 represent the top three principal coordinates that captured most of the diversity. The fraction of diversity captured by the coordinate is given as a percentage. Groups were compared using Permanova method. (C and D) Observed species number describing the alpha diversity of the bacterial microbiota in the various groups studied (Kruskal–Wallis test with Dunn's multiple comparison test). (E and F) Global composition of bacterial microbiota at the phyla and family levels. Healthy subjects (HS) and patient subgroups are labelled on the x-axis and expressed as the relative operational taxonomic unit (OTUs) abundance for each group. In all panels: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . CD, Crohn's disease.

abundance. Basidiomycota and Ascomycota abundances exhibited a strong negative correlation with each other (see online supplementary figure S9D). Moreover, the Basidiomycota-to-Ascomycota abundance ratio was dramatically different according to the disease phenotype with higher values in IBD flare compared with IBD in remission and HS (figure 3F–G), suggesting that this ratio could represent a fungal dysbiosis index.

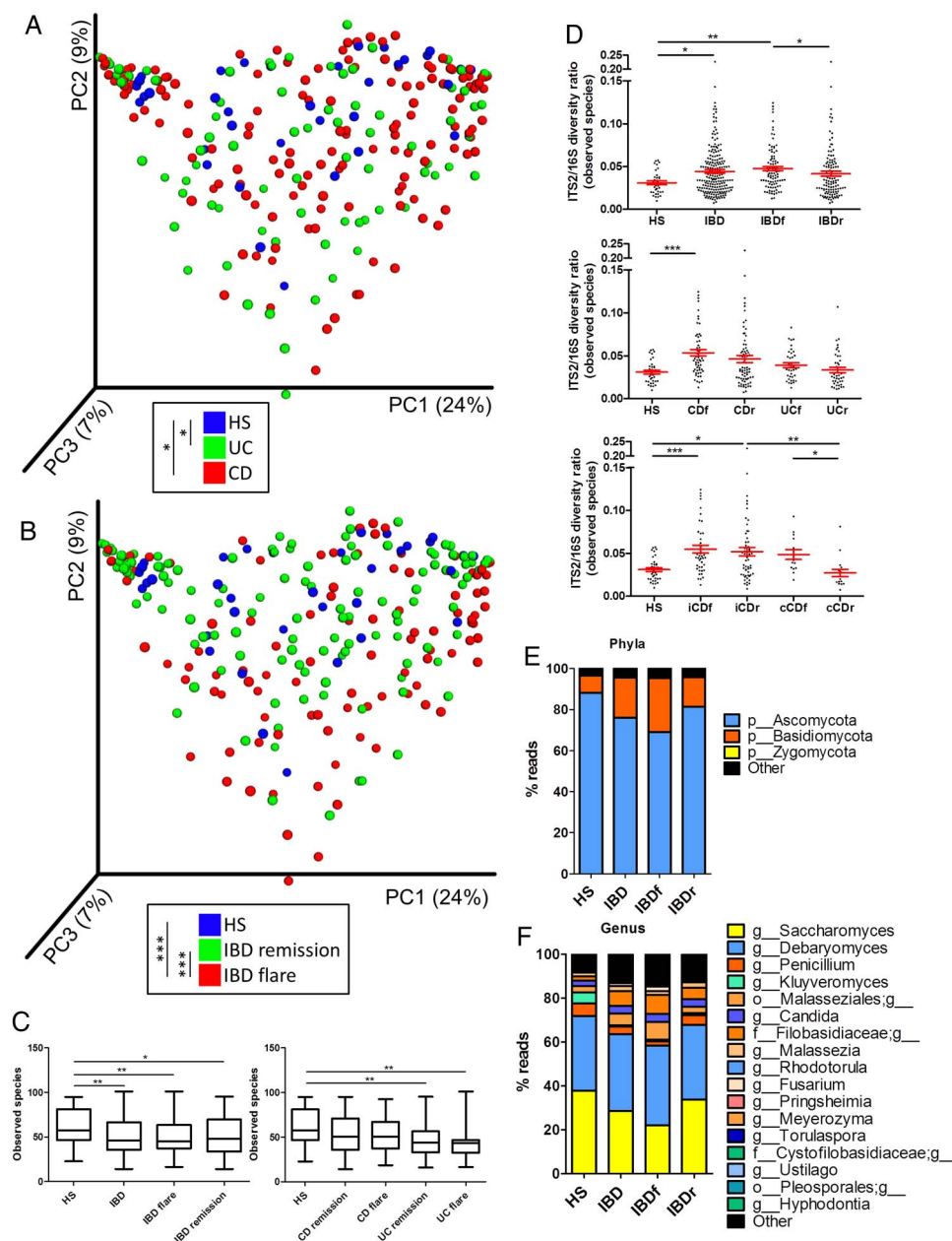
### *S. cerevisiae* induces a regulatory response of dendritic cells

Due to the sequencing and qPCR results, we hypothesised that *S. cerevisiae* and *C. albicans* could respectively exert a protective and worsening role in the inflammatory process. As a proof of principle, we assessed the interleukin (IL)6 and IL10 production

of murine bone marrow-derived dendritic cells (BMDCs) after stimulation with the two heat-killed yeast strains. The IL6 production was similar among *S. cerevisiae* and *C. albicans*; however, the production of the anti-inflammatory cytokine IL10 was significantly higher following stimulation with *S. cerevisiae*, suggesting an anti-inflammatory effect of *S. cerevisiae* compared with *C. albicans* (figure 4). Interestingly, the observed effects were Card9-dependent because cytokine production was nearly abolished in DC from Card9 KO mice. This confirmed the central role of this gene in host–fungi interactions.

### IBD microbiota show-specific bacteria–fungi associations

We next assessed whether the fungi microbiota composition was correlated with the bacterial composition. To address this, we



**Figure 2** Altered fungal microbiota biodiversity and composition in IBD. (A and B) Beta diversity. Principal coordinate analysis of Bray–Curtis distance with each sample coloured according to the disease phenotype. PC1, PC2 and PC3 represent the top three principal coordinates that captured most of the diversity. The fraction of diversity captured by the coordinate is given as a percentage. Groups were compared using Permanova method. (C) Observed species number describing the alpha diversity of the fungal microbiota in the various groups studied. (D) ITS2/16S observed species ratio (Kruskal–Wallis test with Dunn's multiple comparison test). (E and F) Global composition of fungal microbiota at the phyla and genus levels. Healthy subjects (HS) and patient subgroups are labelled on the x-axis and expressed as relative OTUs abundance for each group. In all panels: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ . CD, Crohn's disease.

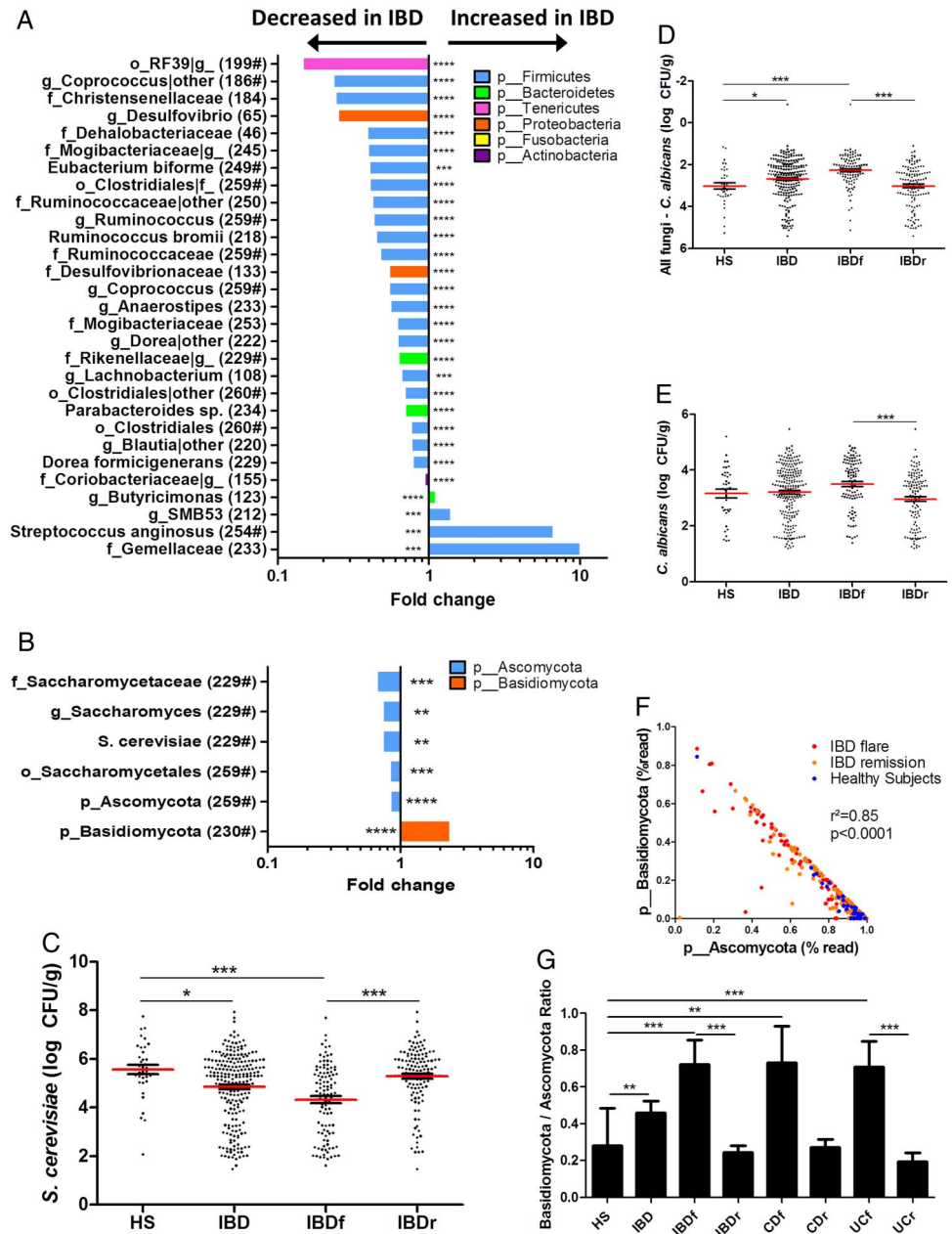
investigated a correlation at the genus level according to disease phenotype. We observed a disease-specific pattern with a higher number of significant correlations in UC compared with HS (figure 5). Although the number of correlations was similar in HS and in CD, the strength of the correlations was weaker in CD (figure 5).

Interestingly, in patients with IBD, there was a positive correlation between the abundance of *Saccharomyces* and the abundance of several bacteria that display reduced abundance in IBD such as *Bifidobacterium*, *Blautia*, *Roseburia* and *Ruminococcus*. On the other hand, unidentified *Malasseziales* followed an opposite pattern. Then, to demonstrate the global intra-

kingdom and inter-kingdom equilibrium according to disease phenotype, we built correlation networks at the genus level involving both bacteria and fungi (figure 6). In HS, the bacterial and fungi diversity was high (figures 1 and 2), with a network showing no marked foci of attractions with both positive and negative correlations distributed throughout the nodes. Strikingly, the CD and UC networks were dramatically different. Notably, many negative correlations connecting genera from the Proteobacteria phylum to members of the Firmicutes phylum were observed in IBD. Involvement of fungi genera from the Basidiomycota phylum, and particularly unidentified *Malasseziales*, in correlations was decreased in CD but increased



**Figure 3** Bacterial and fungal taxa associated with IBD. (A and B) Differences in abundance are shown for the bacterial and fungal taxa detected using a multivariate statistical approach (see 'Material and Methods'). The fold change for each taxon was calculated by dividing the mean abundance in the cases by that of the controls. The number of subjects that have any presence (>0) of the indicated taxon is indicated in brackets, and taxon with a mean abundance of >0.5% in at least one of the groups is indicated with '#'. (C) Absolute *Saccharomyces cerevisiae* abundance in the faecal microbiota quantified using qRT-PCR (mean  $\pm$  SEM). (D) Relative proportion of *Candida albicans* calculated by subtracting the log number of *C. albicans* from the log number of all fungi. (E) Absolute *C. albicans* abundance in the faecal microbiota quantified using qRT-PCR (mean  $\pm$  SEM). (F) Basidiomycota/Ascomycota relative abundance ratio in the various groups studied (Kruskal–Wallis test with Dunn's multiple comparison test, \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$ ). CD, Crohn's disease; HS, healthy subjects.



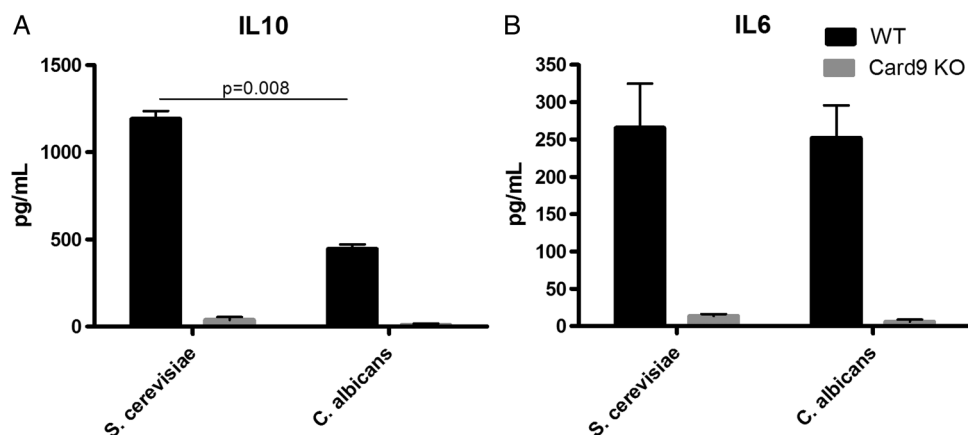
in UC compared with HS. Similar results were obtained when Spearman's test was used to build the correlation network (data not shown).

Taken together, these results suggest a complex relationship between the bacteria and fungi in the gut microbiota and that specific alterations are present in CD and UC.

### Fungal microbiota–genotype association

In mice, the gut bacterial microbiota has been shown to be influenced by host genes.<sup>15–16</sup> Substantial data suggest similar effects in humans.<sup>17–18</sup> However, no data are available regarding fungal microbiota. We thus tested for a correlation between the relative abundance of fungal taxa associated with the disease phenotype (Basidiomycota and Ascomycota phyla as well as *S. cerevisiae* and *M. sympodialis* species) and the CARD9 SNPs associated with IBD as well as several other SNPs that have been involved in defective responses to fungi.<sup>12–19–23</sup> We used standard linear regression and adjusted all analyses for age, gender, smoking

and treatment. With a total of 84 tests, the Bonferroni corrected  $p$  value threshold for an initial alpha of 5% was  $6 \times 10^{-4}$ . As shown in table 1, which presents the 10 most significant associations, no test passed that threshold. Such a stringent significance threshold led to only limited power for our population. For example, we had 80% power to detect SNP explaining 10% (or more) of the variance of the tested taxa, but <2% power to detect SNP explaining 1% of the variance. While the presence of taxa-associated SNPs and the magnitude of the SNP effect remain to be determined, large effect size (eg,  $\geq 10\%$ ) appear unlikely in regard of the marginal effect of these SNPs on IBD. However, we identified interesting trends: notably, a Dectin1 SNP associated with medically refractory UC (rs2078178, 'T' allele<sup>12</sup>) was negatively correlated with the abundance of *M. sympodialis* (itself decreased in IBD flare). In the same manner, the IBD-associated CARD9 SNP (rs10781499, 'A' allele<sup>21</sup>) was negatively correlated with the abundance of *S. cerevisiae* (itself decreased in IBD).



**Figure 4** *Saccharomyces cerevisiae* and *Candida albicans* induces distinct dendritic cell response. Interleukin (IL)10 (A) and IL6 (B) secretion by mouse bone marrow-derived dendritic cells following stimulation with *S. cerevisiae* and *C. albicans* (mean±SEM). The numbers of mice per experiment are n=5–15 (Mann–Whitney *U* test). KO, knockout; WT, wildtype.

## DISCUSSION

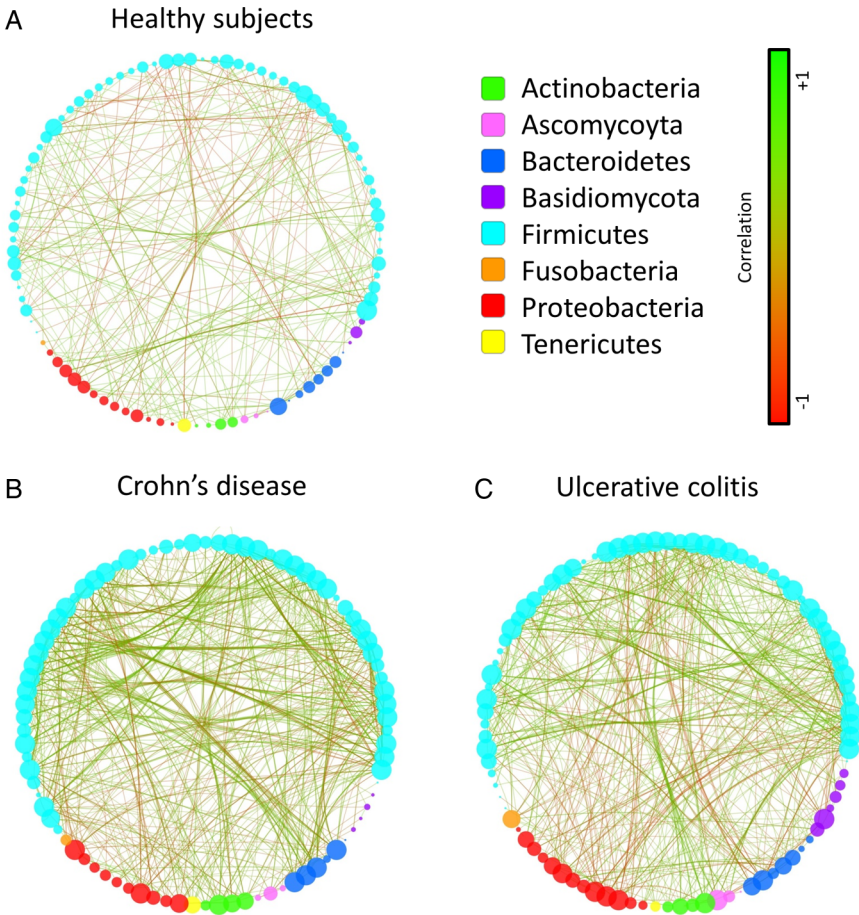
In this study, we showed that disease-specific changes in the fungal gut microbiota are present in IBD. The simultaneous

analysis of both the fungal and the bacterial microbiota enabled the elucidation of differences in the inter-kingdom equilibrium between patients with IBD and HS.

**Figure 5** Specific bacteria–fungi correlation pattern in IBD. Distance correlation plots of the relative abundance of fungi and bacteria genera. Healthy subjects (HS) (left), UC (middle) and Crohn's disease (CD) (right). Statistical significance was determined for all pairwise comparisons; only significant correlations (*p* value <0.05 after false discovery rate correction) are displayed. Positive values (blue squares) indicate positive correlations, and negative values (red squares) indicate inverse correlations. The shading of the square indicates the magnitude of the association; darker shades are more strongly associated than lighter shades. The sign of the correlation was determined using Spearman's method.



**Figure 6** Imbalance trans-kingdom network in IBD. Correlation network in healthy subjects (A), Crohn's disease (B) and UC (C) generated using Cytoscape. Each circle (node) represents a microbial genus, its colour represents the bacterial or fungal phylum it belongs to and its size represents the number of direct edges that it has. The edge colour indicates the magnitude of the distance correlation; green indicates positive correlation and red indicates negative correlation (determined using spearman test). Statistical significance was determined for all pairwise comparisons; only significant correlations (p value <0.05 after false discovery rate correction) are displayed.



Bacterial biodiversity was decreased in both CD and UC. However, fungal biodiversity was decreased only in UC. For CD, this suggests that CD-specific environmental changes may favour fungi at the expense of bacteria. These results were particularly observed in CD patients with ileal involvement. Notably, CD patients without ileal involvement behave like UC patients with decreased fungal biodiversity (see online supplementary figure S5A, D) and only small changes in their ITS2/16S biodiversity ratio (figure 2D). Decreases in fungal gut microbiota have recently been observed in paediatric patients with IBD.<sup>24</sup> However, the number of patients analysed in that study did not enable the authors to discriminate them according to disease phenotype and thus precluded the elucidation of CD patients' specificities. The ileum is a major actor in intestinal

physiology, notably by producing antimicrobial peptides and absorbing bile acids, two functions that are altered in ileal CD and with a potential high impact on luminal bacteria and fungi.<sup>25–28</sup> These alterations in ileal CD could be involved in the specific microbiota alterations observed.

In accordance with published data, the fungal faecal microbiota composition of both HS and patients with IBD were dominated by fungi from the Ascomycota and the Basidiomycota phyla.<sup>7 24 29 30</sup> Ascomycota and Basidiomycota abundances were strongly negatively correlated with each other and were among the most important discriminative features between IBD and HS microbiota as well as between IBD flare and remission. Logically, the Basidiomycota-to-Ascomycota abundance ratio differed between patients with IBD and HS. Compared with

**Table 1** Ten most significant associations between IBD-associated fungi taxa and tested single-nucleotide polymorphisms (SNPs)

Outcome	SNP	Coded allele	Gene	Beta	p Value
s__ <i>Malassezia sympodialis</i>	rs2078178	T	Dectin-1	−0.264	0.003
s__ <i>Malassezia sympodialis</i>	rs4833095	T	TLR1	0.198	0.019
p__Ascomycota	rs5743611	G	TLR1	0.545	0.036
s__ <i>Malassezia sympodialis</i>	rs3901533	T	Dectin-1	−0.204	0.044
p__Basidiomycota	rs5743611	G	TLR1	−0.504	0.050
p__Ascomycota	rs2287886	G	DC-SIGN	−0.197	0.058
s__ <i>Malassezia sympodialis</i>	rs5743618	T	TLR1	−0.139	0.072
s__ <i>Saccharomyces cerevisiae</i>	rs10781499	G	CARD9	0.193	0.072
s__ <i>Saccharomyces cerevisiae</i>	rs3775291	G	TLR3	0.193	0.079
s__ <i>Malassezia sympodialis</i>	rs10841845	G	Mincle	−0.146	0.081



HS, the ratio was high in patients with IBD in flare (either UC or CD) but was normal in remission, suggesting that this imbalance between Basidiomycota and Ascomycota may be either driven by inflammation or involved in the inflammatory process.

We showed that *S. cerevisiae* represents a major component of the normal fungal microbiota. Its decrease, confirmed via real-time qPCR, was independently associated with IBD (vs HS) and with flare (vs remission). *S. cerevisiae* has recently been shown to reduce colitis induced by adherent-invasive *E. coli*, a bacteria associated with ileal CD, in CEACAM6-expressing mice.<sup>31</sup> *S. boulardii*, a probiotic yeast closely related to *S. cerevisiae*,<sup>32</sup> has been shown to exhibit anti-inflammatory effects in several colitis models<sup>7</sup> as well as beneficial effects in the prevention of antibiotic-associated diarrhoea, acute diarrhoea, *Clostridium difficile* infection and enteral feeding-related diarrhoea.<sup>33</sup> Taken together, these data suggest that *S. cerevisiae* could be poorly adapted to an inflammatory environment and/or that it has an anti-inflammatory potential. This raises the possibility of using fungi in new therapeutic approaches in a manner similar to what is currently being developed with several bacterial types. The results obtained using an in vitro BMDC system suggest that *S. cerevisiae* may exhibit regulatory effects on the host, notably by inducing IL10 production. These results are in accordance with another study reporting an increased expression of IL10 in the colons of mice receiving *S. cerevisiae* in a colitis setting.<sup>34</sup> Further experiments with *S. cerevisiae* and other fungi, such as *M. sympodialis*, will be necessary to determine the role of these species in the gut and during intestinal inflammation.

Although *Candida* was among the most abundant genera in our studied population, it was not identified to be associated with disease phenotype in the multivariate analysis. This may be due to the high heterogeneity within the *Candida* genera and to the difficulty in identifying fungi at the species level using our sequencing approach. Indeed, the majority of the sequences identified as belonging to the *Candida* genera were not assigned to a specific species. Because several studies showed an increased level of *C. albicans* in patients with IBD,<sup>7 24 30</sup> we assessed its abundance in the same samples using real-time qPCR. We confirmed a significant increase of *C. albicans* abundance in patients with IBD and particularly in flare.

We herein identified a disease-specific fungal microbiota dysbiosis with shifts in composition involving the two dominant fungi phyla Ascomycota and Basidiomycota and several fungi species including *S. cerevisiae*, *M. sympodialis* and *C. albicans*. Although changes in the fungal microbiota of patients with IBD could be connected with their altered dietary habits,<sup>35</sup> these shifts potentially also play a role in the inflammatory process. Very little is known on the influence of *M. sympodialis* on intestinal inflammation. *Malassezia* is a genus found on the skin of mammals and associated with numerous conditions from dandruff to atopic eczema or pityriasis.<sup>36</sup> Despite that *Malassezia* clearly belongs to the human skin microbiota, species of this genus have been frequently identified in the human gut microbiota, suggesting possible colonisation of the gut.<sup>7</sup> Indeed, the genome of *Malassezia* shows the presence of genes coding for secreted enzymes similar to the well-known human pathogen *C. albicans*. Additionally, *M. sympodialis* is known to secrete potent allergens that might increase local inflammation in injured part of gut of patients with IBD.<sup>37</sup> Recent data show that *M. sympodialis* can activate mast cells to release cysteinyl leukotrienes and enhance the mast cell IgE response, which could contribute to inflammation.<sup>38</sup> Moreover, we found

alterations of the fungi–bacteria diversity balance in CD that might be explained by modified inter-kingdom interactions.

Fungi and bacteria coexist within the gut and may directly interact. Commensal fungi and bacteria can be found together in regions of the mouse gut.<sup>12</sup> Antibiotics treatment in mice leads to major fungi expansions that are then reduced following antibiotic cessation,<sup>39</sup> suggesting a balance between fungal and bacterial microbiota. In addition to differences in the ITS2/16S biodiversity ratio, we noted a disease-specific pattern for the inter-kingdom network. We performed a correlation analysis aiming at globally investigating the gut microbiota equilibrium. It showed an imbalanced microbial network in patients with IBD. In UC, the number and the intensity of the correlations between fungi and bacteria were increased. This suggested tighter interactions. On the other hand, the high ITS2/16S biodiversity ratio in CD associated with the weaker correlations between fungi and bacteria suggested a disconnection between the two kingdoms. Therefore, the role of the fungi microbiota may differ according to UC versus CD pathogenesis. In UC, the restricted biodiversity in bacteria and fungi is associated with new inter-kingdom interactions that may be involved in the inflammatory process. However, CD is characterised by disrupted connections between bacterial and fungal microbiota. This suggests that their respective effects on pathogenesis may be independent. Further studies are needed to elucidate more precisely the functional connections within and between kingdoms in the gut microbiota.

Finally, although our study was not of sufficient power to statistically demonstrate an association between genotype and fungal microbiota, we identified some trends suggesting that SNPs associated with IBD or IBD severity may influence fungal microbiota dysbiosis.

## CONCLUSION

In addition to elucidating bacterial dysbiosis, our study identified a distinct fungal microbiota dysbiosis in IBD that is characterised by alterations in biodiversity and composition. Moreover, here, we unravel disease-specific inter-kingdom network alterations in IBD, suggesting that, in addition to bacteria, fungi may also play a role in IBD pathogenesis. Identifying the key players of these inter-kingdom interactions and understanding how they influence or are influenced by gut inflammation are further research avenues to pursue.

## MATERIALS AND METHODS

### Patients and samples collection

All patients were recruited at the Gastroenterology Department of the Saint Antoine Hospital (Paris, France) and provided informed consent. A diagnosis of IBD was defined according to clinical, radiological, endoscopic and histological criteria. None of the study participants had taken antibiotics or used colon-cleansing products for at least two months prior to enrolment. Patient characteristics are presented in online supplementary table S1. Faecal samples were collected from 235 patients with IBD and 38 HS. Whole stools were collected in sterile boxes and immediately homogenised, and 0.2 g aliquots were frozen at  $-80^{\circ}\text{C}$  for further analysis.

### Microbiota analysis in healthy subjects and patients with IBD

Faecal samples were subjected to DNA extraction as previously described.<sup>3</sup> The DNA samples were then used for 16S and ITS2 gene sequencing and sequence analysis (see online supplementary materials and methods). For specific analysis of *C. albicans*



and *S. cerevisiae* population, real-time quantitative PCR on the faecal DNA was done (see online supplementary materials and methods).

### In vitro experiments on the response of dendritic cells to *S. cerevisiae* and *C. albicans*

BMDCs were generated as described in the online supplementary materials and methods. BMDCs were stimulated with heat-killed *C. albicans* or *S. cerevisiae* at a multiplicity of infection (MOI) of 10 during 18 h and the cell culture supernatant was used for ELISA analysis (see online supplementary materials and methods).

Genotyping is described in the online supplementary materials and methods.

### Statistical analysis

GraphPad Prism V6.0 (San Diego, California, USA) was used for all analyses and graph preparation. For all graph data, the results are expressed as mean±SEM, and statistical analyses were performed using the two-tailed non-parametric Mann–Whitney U test or Kruskal–Wallis test with Dunn's multiple comparison test. Statistical significance of sample grouping for beta diversity analysis was performed using Permanova method (9999 permutations). Differences with a *p* value <0.05 were considered significant.

MaAsLin, a multivariate statistical framework, was used to find associations between clinical metadata and microbial community abundance.<sup>14</sup>

Correlation within microbial taxa abundance data was measured by correlation proposed by Szekely and Rizzo<sup>40</sup> and recommended by Simon and Tibshirani.<sup>41</sup> It was used with success in other microbiota data analysis<sup>42</sup> as well as in human genetic to identify gene network.<sup>43</sup> It is scale free and allows detecting non-linear relationship. The distance correlation is bounded by 0 and 1 and is equal to 0 if there is independence between variables. A statistical test is provided to assess for the dependence between variables and is shown powerful and easy to compute.<sup>41</sup> In addition to the distance correlation, the Spearman's correlation sign was computed to describe heuristically the direction of association between microbial taxa. The distance correlation was computed under R-3.2.3 using the package *energy* v1.6.2. The *p* values were corrected using Benjamini and Hochberg to control false discovery rate. Correlation networks were built using Cytoscape 3.1.1.

To investigate correlations between fungi taxa and genotype, we performed a systematic association screening between the four major independent taxa that were previously identified to be associated with IBD (vs HS) or with flare (vs remission) (figure 3B, online supplementary figure S7B) and the 21 common candidate SNPs. All outcomes were standardised using a rank normal transformation using the function *rntransform* from the R package *GenABEL* (<http://cran.r-project.org/web/packages/GenABEL/index.html>). We used standard linear regression and adjusted all analyses for age, gender, smoking and treatment.

### Author affiliations

<sup>1</sup>Sorbonne University—UPMC Univ Paris 06, INSERM ERL 1157, Avenir Team Gut Microbiota and Immunity, UMR 7203, Saint-Antoine Hospital, AP-HP, UPMC Univ Paris 06, Paris, France

<sup>2</sup>Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, France

<sup>3</sup>Department of Gastroenterology, Saint Antoine Hospital, Paris, France

<sup>4</sup>Inflammation-Immunopathology-Biotherapy Department (DHU i2B), Paris, France

<sup>5</sup>Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts, USA

<sup>6</sup>ILTOO Pharma, iPEPS ICM, Hôpital Pitié Salpêtrière, Paris, France

<sup>7</sup>Department of Medical and Surgical Sciences, University of Bologna, Bologna, Italy

<sup>8</sup>Division of Infectious Diseases, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

<sup>9</sup>Massachusetts Technology and Analytics, Brookline, Massachusetts, USA

**Acknowledgements** The authors thank Christophe Hennequin from CH Saint Antoine for providing them with the *S. cerevisiae* strain. They also thank Elodie Drouet and the Clinical Research Assistant team of Unité de Recherche Clinique de l'Est Parisien (Assistance Publique—Hôpitaux de Paris and UPMC Paris 06) for their help in obtaining the IBD patient samples.

**Contributors** HS conceived and designed the study, performed data analysis and wrote the manuscript. VL and SJ conducted all experiments unless otherwise indicated below. HA performed the statistical analysis of genetic data. H-PP performed the statistical analysis of the pyrosequencing data. DC and MLR performed the in vitro experiments. CL, AB, IN-L, GL, JC, PS, PL, DS, MLR, LB, and HS discussed the experiments and results.

**Funding** This study was supported by the Broad Medical Research Program, the INSERM cohort grant and the Association Francois Aupetit.

**Competing interests** None declared.

**Patient consent** Obtained.

**Ethics approval** Local ethics committee of Comité de Protection des Personnes Ile-de-France IV, IRB 00003835, Suivitheque study, registration number 2012/05NICB.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Open Access** This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

### REFERENCES

- Morgan XC, Tickle TL, Sokol H, *et al.* Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol* 2012;13:R79.
- Sokol H, Pigneur B, Watterlot L, *et al.* Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci USA* 2008;105:16731–6.
- Sokol H, Seksik P, Furet JP, *et al.* Low counts of Faecalibacterium prausnitzii in colitis microbiota. *Inflamm Bowel Dis* 2009;15:1183–9.
- Willing BP, Dicksved J, Halfvarson J, *et al.* A pyrosequencing study in twins shows that gastrointestinal microbial profiles vary with inflammatory bowel disease phenotypes. *Gastroenterology* 2010;139:1844–54.e1.
- Gevers D, Kugathasan S, Denson LA, *et al.* The treatment-naïve microbiome in new-onset Crohn's disease. *Cell Host Microbe* 2014;15:382–92.
- Norman JM, Handley SA, Baldridge MT, *et al.* Disease-specific alterations in the enteric virome in inflammatory bowel disease. *Cell* 2015;160:447–60.
- Richard ML, Lamas B, Liguori G, *et al.* Gut fungal microbiota: the Yin and Yang of inflammatory bowel disease. *Inflamm Bowel Dis* 2015;21:656–65.
- Jawahara S, Thuru X, Standaert-Vitse A, *et al.* Colonization of mice by *Candida albicans* is promoted by chemically induced colitis and augments inflammatory responses through galectin-3. *J Infect Dis* 2008;197:972–80.
- Chen X, Yang G, Song JH, *et al.* Probiotic yeast inhibits VEGFR signaling and angiogenesis in intestinal inflammation. *PLoS ONE* 2013;8:e64227.
- Jawahara S, Poulain D. *Saccharomyces boulardii* decreases inflammation and intestinal colonization by *Candida albicans* in a mouse model of chemically-induced colitis. *Med Mycol* 2007;45:691–700.
- Zwolinska-Wcislo M, Brzozowski T, Budak A, *et al.* Effect of *Candida* colonization on human ulcerative colitis and the healing of inflammatory changes of the colon in the experimental model of colitis ulcerosa. *J Physiol Pharmacol* 2009;60:107–18.
- Iliev ID, Funari VA, Taylor KD, *et al.* Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis. *Science* 2012;336:1314–17.
- Sokol H, Conway KL, Zhang M, *et al.* Card9 mediates intestinal epithelial cell restitution, T-helper 17 responses, and control of bacterial infection in mice. *Gastroenterology* 2013;145:591–601.e3.
- Standaert-Vitse A, Sendid B, Joossens M, *et al.* *Candida albicans* colonization and ASCA in familial Crohn's disease. *Am J Gastroenterol* 2009;104:1745–53.
- Couturier-Maillard A, Secher T, Rehman A, *et al.* NOD2-mediated dysbiosis predisposes mice to transmissible colitis and colorectal cancer. *J Clin Invest* 2013;123:700–11.
- Larsson E, Tremaroli V, Lee YS, *et al.* Analysis of gut microbial regulation of host gene expression along the length of the gut and regulation of gut microbial ecology through MyD88. *Gut* 2012;61:1124–31.

- 17 Knights D, Silverberg MS, Weersma RK, *et al.* Complex host genetics influence the microbiome in inflammatory bowel disease. *Genome Med* 2014;6:107.
- 18 Sadaghian Sadabad M, Regeling A, de Goffau MC, *et al.* The ATG16L1-T300A allele impairs clearance of pathosymbionts in the inflamed ileal mucosa of Crohn's disease patients. *Gut* 2015;64:1546–52.
- 19 Caliz R, Canet LM, Lupianez CB, *et al.* Gender-specific effects of genetic variants within Th1 and Th17 cell-mediated immune response genes on the risk of developing rheumatoid arthritis. *PLoS ONE* 2013;8:e72732.
- 20 Ferwerda B, Ferwerda G, Plantinga TS, *et al.* Human dectin-1 deficiency and mucocutaneous fungal infections. *N Engl J Med* 2009;361:1760–7.
- 21 Jostins L, Ripke S, Weersma RK, *et al.* Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012;491:119–24.
- 22 Sainz J, Lupianez CB, Segura-Catena J, *et al.* Dectin-1 and DC-SIGN polymorphisms associated with invasive pulmonary Aspergillus infection. *PLoS ONE* 2012;7:e32273.
- 23 Ma X, Liu Y, Zhang H, *et al.* Evidence for genetic association of CARD9 and SNAPC4 with ankylosing spondylitis in a Chinese Han population. *J Rheumatol* 2014;41:318–24.
- 24 Chehoud C, Albenberg LG, Judge C, *et al.* Fungal signature in the Gut Microbiota of pediatric patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2015;21:1948–56.
- 25 Bajor A, Gillberg PG, Abrahamsson H. Bile acids: short and long term effects in the intestine. *Scand J Gastroenterol* 2010;45:645–64.
- 26 Duboc H, Rajca S, Rainteau D, *et al.* Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases. *Gut* 2013;62:531–9.
- 27 Selsted ME, Ouellette AJ. Mammalian defensins in the antimicrobial immune response. *Nat Immunol* 2005;6:551–7.
- 28 Wehkamp J, Schmid M, Stange EF. Defensins and other antimicrobial peptides in inflammatory bowel disease. *Curr Opin Gastroenterol* 2007;23:370–8.
- 29 Hoffmann C, Dollive S, Grunberg S, *et al.* Archaea and fungi of the human gut microbiome: correlations with diet and bacterial residents. *PLoS ONE* 2013;8:e66019.
- 30 Mukherjee PK, Sendid B, Hoarau G, *et al.* Mycobacteria in gastrointestinal diseases. *Nat Rev Gastroenterol Hepatol* 2015;12:77–87.
- 31 Sivignon A, de Vallee A, Barnich N, *et al.* Saccharomyces cerevisiae CNCM I-3856 prevents colitis induced by AIEC bacteria in the transgenic mouse model mimicking Crohn's disease. *Inflamm Bowel Dis* 2015;21:276–86.
- 32 Edwards-Ingram L, Gitsham P, Burton N, *et al.* Genotypic and physiological characterization of Saccharomyces boulardii, the probiotic strain of Saccharomyces cerevisiae. *Appl Environ Microbiol* 2007;73:2458–67.
- 33 McFarland LV. Systematic review and meta-analysis of Saccharomyces boulardii in adult patients. *World J Gastroenterol* 2010;16:2202–22.
- 34 Jawhara S, Habib K, Maggioro F, *et al.* Modulation of intestinal inflammation by yeasts and cell wall extracts: strain dependence and unexpected anti-inflammatory role of glucan fractions. *PLoS ONE* 2012;7:e40648.
- 35 Van Kruiningen HJ, Joossens M, Vermeire S, *et al.* Environmental factors in familial Crohn's disease in Belgium. *Inflamm Bowel Dis* 2005;11:360–5.
- 36 Saunders CW, Scheynius A, Heitman J. Malassezia fungi are specialized to live on skin and associated with dandruff, eczema, and other skin diseases. *PLoS Pathog* 2012;8:e1002701.
- 37 Buentke E, Heffler LC, Wilson JL, *et al.* Natural killer and dendritic cell contact in lesional atopic dermatitis skin—Malassezia-influenced cell interaction. *J Invest Dermatol* 2002;119:850–7.
- 38 Selander C, Engblom C, Nilsson G, *et al.* TLR2/MyD88-dependent and -independent activation of mast cell IgE responses by the skin commensal yeast Malassezia sympodialis. *J Immunol* 2009;182:4208–16.
- 39 Dollive S, Chen YY, Grunberg S, *et al.* Fungi of the murine gut: episodic variation and proliferation during antibiotic treatment. *PLoS ONE* 2013;8:e71806.
- 40 Szekely GJ, Rizzo ML. Brownian distance covariance. The Annals of Applied Statistics. *Ann Appl Stat* 2009;3:1236–65.
- 41 Simon N, Tibshirani R. Comment on "detecting novel associations in large data sets" by Reshef *et al.*, Science Dec 16, 2011. arXiv 2014;1401.
- 42 Liu R, Ge Y, Holden PA, *et al.* Analysis of soil bacteria susceptibility to manufactured nanoparticles via data visualization. *Beilstein J Nanotechnol* 2015;6:1635–51.
- 43 Zhang KK, Xiang M, Zhou L, *et al.* Gene-network and familial analyses uncover a gene network involving Tbx5/Osr1/Pcsk6 interaction in the second heart field for atrial septation. *Hum Mol Genet* Published Online First 2016.

## Supplementary Materials and Methods

### Fecal DNA extraction

Genomic DNA was extracted from 200 mg of feces as previously described (Sokol *et al.* 2009). Following microbial lysis involving both mechanical and chemical steps, nucleic acids were precipitated via isopropanol for 10 minutes at room temperature, followed by incubation for 15 minutes on ice and centrifugation for 30 minutes at 15,000 *g* and 4°C. Pellets were suspended in 112 µL of phosphate buffer and 12 µL of potassium acetate. After the RNase treatment and DNA precipitation, nucleic acids were recovered via centrifugation at 15,000 *g* and 4°C for 30 minutes. The DNA pellet was suspended in 100 µL of TE buffer.

### 16S rRNA gene sequencing

After extraction, the total DNA concentration was measured using PicoGreen (Invitrogen), and global 16S gene DNA copy numbers were measured using a qPCR method adapted from Maeda *et al.* (Maeda *et al.* 2003) allowing for inhibition effect estimation and DNA concentration adjustment. The sequence region of the 16S rRNA gene spanning the variable region V3-V5 was then amplified using the broad-range forward primer For16S\_519 (CAGCMGCCGCGGTAATAC) and reverse primer Rev16S\_926 (CCGTCAATTCMTTGTGAGTTT). Amplification reaction (initial activation step at 94°C for 1 min followed by 30 cycles of 94°C for 15 s, 43°C for 15 s and 68°C for 45 s plus final incubation at 68°C for 1 min) was performed in a total volume of 100 µL containing 1X PCR buffer, 2 mM MgSO<sub>4</sub>, 1 U of DNA High Fidelity Taq Polymerase (Invitrogen, Carlsbad, CA), 625 nM of each barcoded primer (IDT), 250 µM of each dNTP (Invitrogen) and the concentration-adjusted DNA sample. A bidirectional library was prepared using the One Touch2 Template Kit and sequenced on PGM Ion Torrent using the Ion PGM Sequencing 400 Kit (Life Technologies, Carlsbad, CA).



## **16S rRNA genes sequence analysis**

The sequences were demultiplexed and quality filtered using the Quantitative Insights Into Microbial Ecology (QIIME, version 1.8.0) software package (Caporaso *et al.* 2010). The sequences were trimmed for barcodes and PCR primers and were binned for a minimal sequence length of 200 pb. The sequences were then assigned to Operational Taxonomic Units (OTUs) using the UCLUST algorithm (Edgar 2010) with a 97% threshold pairwise identity and taxonomically classified using the Greengenes reference database (McDonald *et al.* 2012). Rarefaction was performed (2,041-83,162 sequences per sample; four samples with less than 10,000 sequences were excluded from analysis) and used to compare OTUs abundances across samples.

## **ITS2 sequencing**

Fungal diversity was determined for each sample via 454 pyrosequencing of Internal Transcribed Spacer 2 (ITS2). An ITS2 fragment of approximately 350 bases was amplified using the primers ITS2 (sense) 5'-GTGARTCATCGAATCTTT-3' and (antisense) 5'-GATATGCTTAAGTTCAGCGGGT-3' and the optimized and standardized ITS2 amplicon library preparation protocol (METABIOTE®, Genoscreen, Lille, France). Briefly, for each sample, diluted genomic DNA were used for a 25-μl PCR conducted under the following conditions: 94°C for 2 min, 35 cycles of 15 sec at 94°C, 52°C for 30 sec and 72°C for 45 sec, followed by 7 min at 72°C. The PCR products were purified using AMPure XP Beads (Beckman Coulters, Brea, CA) and quantified using the PicoGreen Staining Kit (Molecular Probes, Paris, France). A second PCR of 9 cycles was then conducted under similar PCR conditions with purified PCR products, and ten base pair multiplex identifiers (SIM identifiers) were added to the primers at the 5' position to specifically identify each sample and avoid PCR biases. Finally, the PCR products were purified and quantified as previously described. Sequencing was then performed on a GS FLX Titanium Sequencing System (Roche Life Science, Mannheim, Germany).

## ITS2 sequence analysis

The sequences were demultiplexed and quality-filtered using the Quantitative Insights Into Microbial Ecology (QIIME, version 1.8.0) software package (Caporaso *et al.* 2010). The sequences were trimmed for barcodes and PCR primers and were binned for a minimal sequence length of 150 pb, a minimal base quality threshold of 25 and a maximum homopolymer length of 7. The sequences were then assigned to OTUs using the UCLUST algorithm (Edgar 2010) with 97% threshold of pairwise identity and classified taxonomically using the UNITE ITS database (alpha version 12\_11) (Koljalg *et al.* 2013). Rarefaction was performed (540-5,648 sequences per sample; ten samples with less than 1,000 sequences were excluded from analysis) and used to compare the abundances of OTUs across samples. Principal component analyses (PCA) of the Bray Curtis distance with each sample colored according to the disease phenotype were built and used to assess the variation between experimental groups. The number of observed species, as well as the Shannon, Simpson and Chao1 diversity indexes were calculated using rarefied data (depth = 1,000 sequences/sample for ITS2 and depth = 10,000 sequences/sample for 16S) and used to characterize species diversity in a community.

## Real-time quantitative PCR

The quantitative analysis of the total fungal population, *Saccharomyces cerevisiae* and *Candida albicans* was performed on fecal DNA extracted from weighted human stool samples via real-time quantitative PCR using an ABI 7000 Sequence Detection System apparatus with 7000 system software v. 1.2.3 (Applied Biosystems, Foster City, CA, USA). Amplification and detection were carried out in 96-well plates and with Takyon<sup>TM</sup> SYBR Green PCR kit (Eurogentec, Liege, Belgium). Each reaction was performed in duplicate in a final volume of 25 µl with 10 µl of appropriate dilutions of the DNA sample. Amplifications were performed as follows: 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 64°C (*All Fungi*) / 58°C (*S. cerevisiae*) / 60°C (*C. albicans*) for 45 seconds, 72°C for 30 seconds with a final extension step of 5 minutes at 72°C. A dissociation step was added, and dissociation curves were analyzed to confirm the identity and

fidelity of the amplification products. The following primers were specific for the 18S rRNA gene and were used for all fungi quantifications: 5'-ATTGGAGGGCAAGTCTGGTG-3' and 5'-CCGATCCCTAGTCGGCATAG-3'. For *S. cerevisiae* quantification, primers specific of the D1/D2 domain of the 26S rRNA gene of *S. cerevisiae* were used: 5'-AGGAGTGCGGTTCTTTG-3' and 5'-TACTTACCGAGGCAAGCTACA-3'. For *C. albicans* quantification, primers specific of the ITS1-ITS2 region of *C. albicans* were used: 5'-TTTATCAACTTGTCACACCAGA-3' and 5'-ATCCCGCCTTACCACTACCG-3'. The threshold cycle of each sample was determined for each gene, and CT values were used to estimate the absolute quantity (CFU per gram of stool sample) of all fungi, *S. cerevisiae* and *C. albicans*, according to the standard curve method. For all fungi and *S. cerevisiae* absolute quantification, a *S. cerevisiae* purified DNA sample was used to design the standard curve. For *C. albicans* absolute quantification, a *C. albicans* purified DNA sample was used to design the standard curve. The relative proportion of *S. cerevisiae* and *C. albicans* was calculated by subtracting the log number of the targeted fungi from the log number of all fungi.

### **Preparation of murine bone marrow dendritic cells**

The protocols for animal handling were previously approved by our institutional Animal Ethics Committee (COMETHEA, protocol number 14\_45). Femurs were obtained from 6–12-week-old C57BL/6 wild-type and Card9KO strains. After euthanasia, the femurs and tibias were dissected, muscles connected to the bone were removed using clean gauze, and the femurs were placed into a polypropylene tube containing sterile Roswell Park Memorial Institute (RPMI) 1640 medium on ice. In a tissue culture hood, both epiphyses were removed using sterile scissors and forceps. The bones were flushed with a syringe filled with complete RPMI (RPMI 1640 supplemented with 20% fetal bovine serum (Lonza), 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine) to extrude bone marrow into a 50-mL sterile polypropylene tube. The bone marrow was homogenized on a 75-µm strainer and the strainer was washed. After centrifugation, the cells were resuspended in freezing media containing 90% fetal bovine serum and 10% DMSO. The cryotubes were frozen at -80°C for several weeks before use.



## **Differentiation of bone marrow-derived macrophages**

To thaw the cells, a cryovial was quickly transferred to a 37°C incubator until the suspension was completely thawed. The contents were then transferred to plastic tubes containing 10 ml of 37°C complete RPMI. The cells were centrifuged at 200 *g* for 5 minutes and resuspended in bone marrow differentiation media as described below.

The cells were resuspended in 10 ml bone marrow differentiation media (DC media): complete RPMI supplemented with 20 ng/mL of GM-CSF (BioLegend, CA, USA). The cells were seeded in tissue culture-treated Petri dishes and incubated at 37°C in a 5% CO<sub>2</sub> atmosphere. Two days after seeding the cells, an extra 5 ml of fresh DC media was added per plate and incubated for an additional 3 days. At Day 5, 5 ml of the media was removed, centrifuged and the cells were resuspended in fresh DC media. At Day 7, only the suspension cells were collected, counted, seeded in 96-well plates using 100 µL of cell suspension containing 500,000 cells per well and cultivated 12 hours prior to any further experimental procedure.

## **Fungal stimulation**

*Saccharomyces cerevisiae* (MYCOTQ 1146, human clinical isolate provided by Christophe Hennequin, Saint Antoine Hospital, APHP, Paris, France) and *Candida albicans* SC5314 were grown in YEPD media (2% glucose, 2% Bacto Peptone, 1% yeast extract) at 37°C for 19 hours. Yeast cells were killed via heat treatment of 1 h in a water bath at 65°C and resuspended in complete RPMI prior to stimulation of BMDC in 96-well plates at a multiplicity of infection (MOI) of 10. After 18 hours of incubation, the supernatant was collected for cytokine quantification using ELISA kits according to the manufacturer's instructions (IL10: Mabtech, Nacka Strand, Sweden; IL6: eBioscience, CA, USA).

## Genotyping

Patients with IBD with available genomic DNA were genotyped using Fluidigm technology (UMR CNRS 8199, Lille, France) for 21 common candidate SNPs (i.e., minor allele frequency > 5%) from 9 genes involving the IBD-associated *Card9* SNP as well as several other SNPs that have been involved in defective responses to fungi (Ferwerda *et al.* 2009; Iliev *et al.* 2012; Jostins *et al.* 2012; Sainz *et al.* 2012; Caliz *et al.* 2013; Ma *et al.* 2014) (Supplementary Table 2).

## References

- Caliz, R., L. M. Canet, C. B. Lupianez, et al. (2013). "Gender-specific effects of genetic variants within Th1 and Th17 cell-mediated immune response genes on the risk of developing rheumatoid arthritis." *PLoS One* 8(8): e72732.
- Caporaso, J. G., J. Kuczynski, J. Stombaugh, et al. (2010). "QIIME allows analysis of high-throughput community sequencing data." *Nat Methods* 7(5): 335-336.
- Edgar, R. C. (2010). "Search and clustering orders of magnitude faster than BLAST." *Bioinformatics* 26(19): 2460-2461.
- Ferwerda, B., G. Ferwerda, T. S. Plantinga, et al. (2009). "Human dectin-1 deficiency and mucocutaneous fungal infections." *N Engl J Med* 361(18): 1760-1767.
- Iliev, I. D., V. A. Funari, K. D. Taylor, et al. (2012). "Interactions between commensal fungi and the C-type lectin receptor Dectin-1 influence colitis." *Science* 336(6086): 1314-1317.
- Jostins, L., S. Ripke, R. K. Weersma, et al. (2012). "Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease." *Nature* 491(7422): 119-124.
- Koljal, U., R. H. Nilsson, K. Abarenkov, et al. (2013). "Towards a unified paradigm for sequence-based identification of fungi." *Mol Ecol* 22(21): 5271-5277.
- Ma, X., Y. Liu, H. Zhang, et al. (2014). "Evidence for genetic association of *CARD9* and *SNAPC4* with ankylosing spondylitis in a Chinese Han population." *J Rheumatol* 41(2): 318-324.

- Maeda, H., C. Fujimoto, Y. Haruki, et al. (2003). "Quantitative real-time PCR using TaqMan and SYBR Green for *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *tetQ* gene and total bacteria." *FEMS Immunol Med Microbiol* 39(1): 81-86.
- McDonald, D., M. N. Price, J. Goodrich, et al. (2012). "An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea." *ISME J* 6(3): 610-618.
- Sainz, J., C. B. Lupianez, J. Segura-Catena, et al. (2012). "Dectin-1 and DC-SIGN polymorphisms associated with invasive pulmonary Aspergillosis infection." *PLoS One* 7(2): e32273.
- Sokol, H., P. Seksik, J. P. Furet, et al. (2009). "Low counts of *Faecalibacterium prausnitzii* in colitis microbiota." *Inflamm Bowel Dis* 15(8): 1183-1189.



## Supplementary Figure Legends

### Supplementary Figure 1: Bacterial microbiota beta diversity in HS, CD and UC

**(A,B)** Beta diversity. Principal Coordinate Analysis of Bray Curtis distance with each sample colored according to the disease phenotype. PC1, PC2, and PC3 represent the top three principal coordinates that captured most of the diversity. The fraction of diversity captured by the coordinate is shown as a percentage. Groups were compared using Permanova method. \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ .

### Supplementary Figure 2: Bacterial microbiota alpha diversity in HS, CD and UC

**(A)** OTUs number and Chao1 index **(B, C, D)** describing the alpha diversity of the bacterial microbiota in the various groups studied (Kruskal-Wallis test with Dunn's Multiple Comparison Test, \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ ).

### Supplementary Figure 3: Bacterial microbiota composition in HS, CD and UC

Global composition of bacterial microbiota at the **(A)** phyla, **(B)** family and **(C)** genus levels. HS and patient sub-groups are labeled on the x-axis and expressed as relative OTUs abundance for each group.

### Supplementary Figure 4: Fungal microbiota Beta diversity in HS, CD and UC

**(A, B, C)** Beta diversity. Principal Coordinate Analysis of the Bray-Curtis distance with each sample colored according to the disease phenotype. PC1, PC2, and PC3 represent the top three principal coordinates that captured most of the diversity. The fraction of diversity captured by the coordinate is shown as a percentage. Groups were compared using Permanova method. In all panels : \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$

### Supplementary Figure 5: Fungal microbiota alpha diversity in HS, CD and UC

**(A)** OTUs number and Chao1 index **(B, C, D)** describing the alpha diversity of the fungal microbiota in the various groups studied. **(E, F, G)** ITS2/16S Chao1 index ratio (Kruskal-Wallis test with Dunn's Multiple Comparison Test, \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ).

### **Supplementary Figure 6: Fungal microbiota composition in HS, CD and UC**

Global composition of fungal microbiota at the **(A)** phyla, **(B)** family and **(C)** genus levels. HS and patient sub-groups are labeled on the x-axis and expressed as relative OTUs abundance for each group.

### **Supplementary Figure 7: Bacterial and fungal taxa associated with IBD flare**

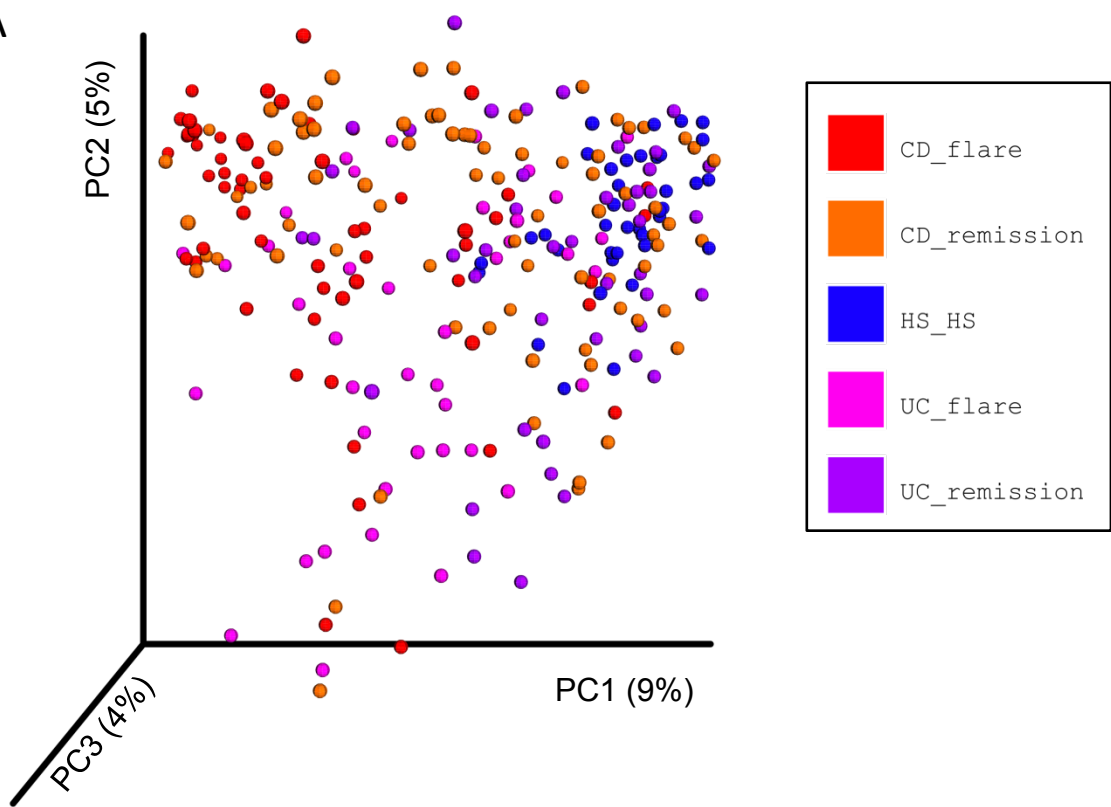
Differences in abundance are shown for the **(A)** bacterial and **(B)** fungal taxa using a multivariate statistical approach (see Experimental Procedures). The fold change for each taxon was calculated by dividing the mean abundance in the cases by that of the controls. The number of subjects that have any presence (>0) of the indicated taxon is indicated in brackets and taxon with a mean abundance of >0.5% in at least one of the groups is indicated with “#”. **(C)** *S. cerevisiae* levels in the fecal microbiota quantified using qRT-PCR (mean ± s.e.m.). In all panels : \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$

### **Supplementary Figure 8: Bacterial and Fungal taxa associated with ileal involvement in CD patients**

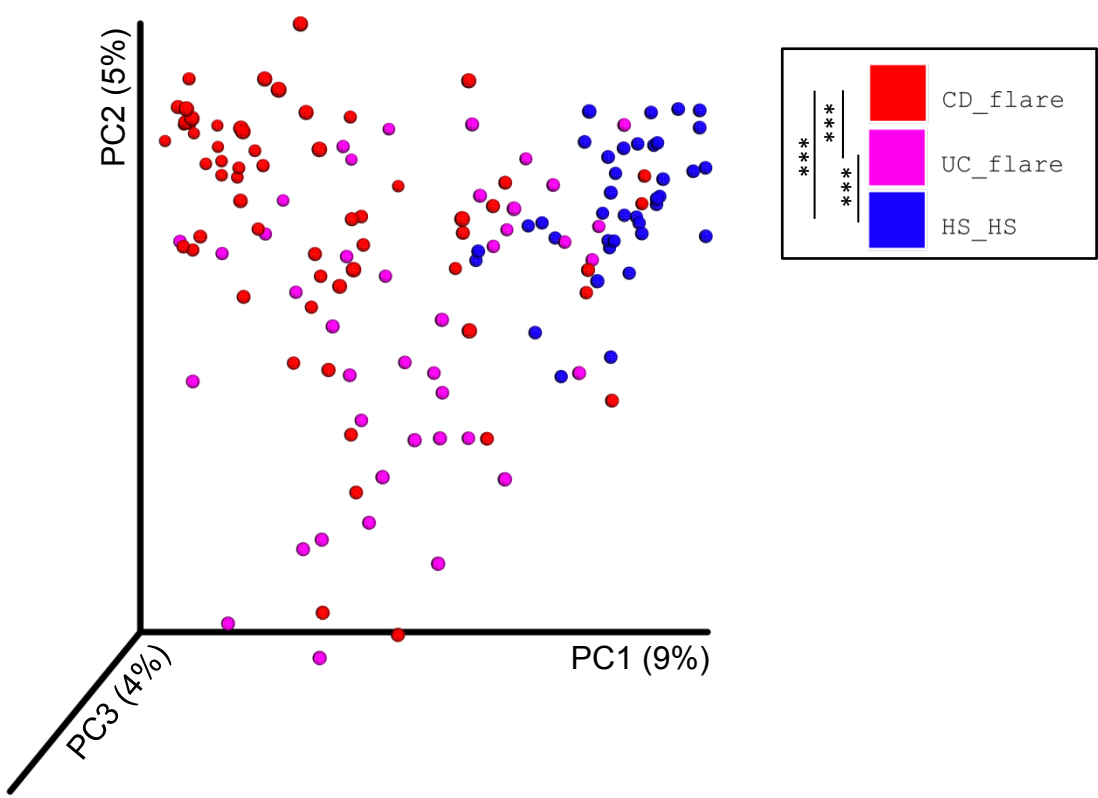
Differences in abundance are shown for the bacterial taxa detected using a multivariate statistical approach (see Experimental Procedures). The fold change for each taxon was calculated by dividing the mean abundance in the cases by that of the controls. The number of subjects that have any presence (>0) of the indicated taxon is indicated in brackets and taxon with a mean abundance of >0.5% in at least one of the groups is indicated with “#”. \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$

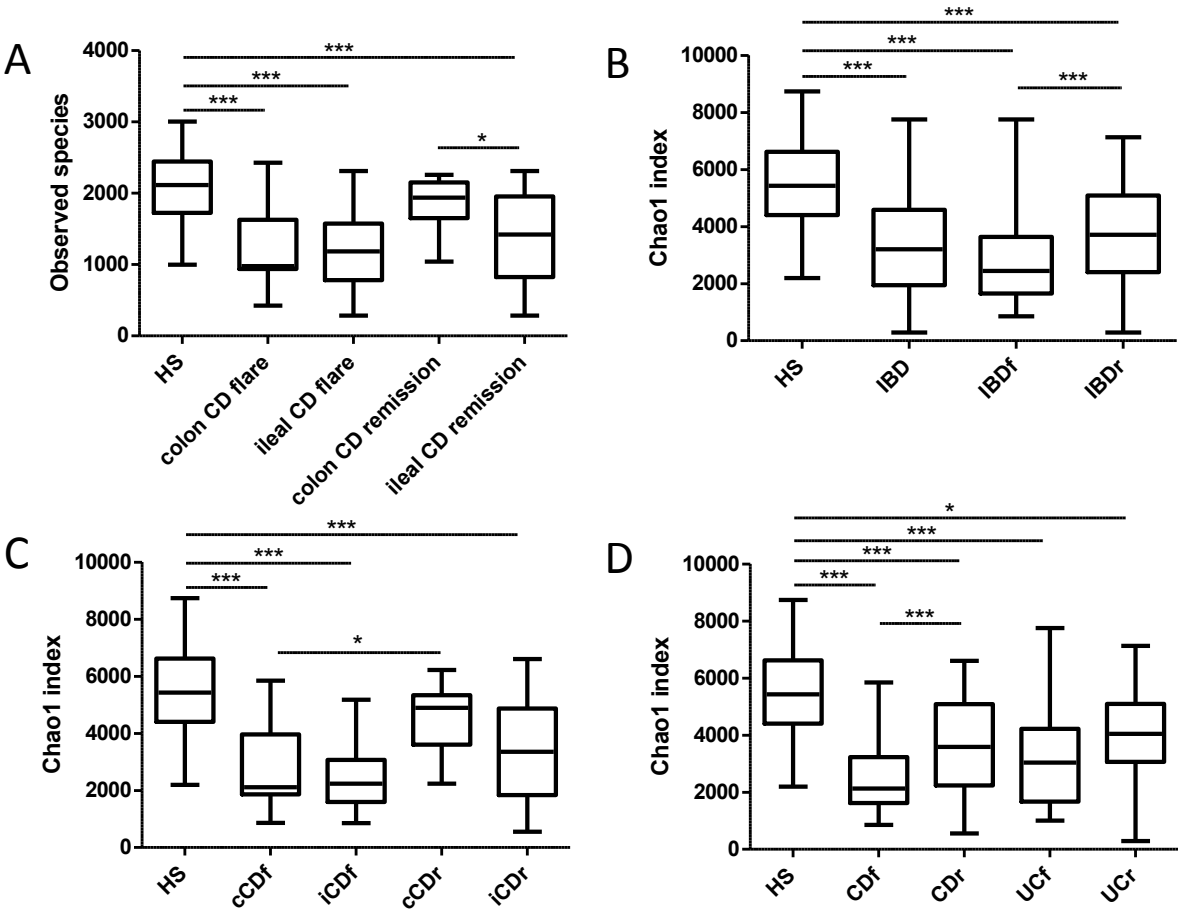
### **Supplementary Figure 9: Relative proportion of *S. cerevisiae* (A, B) and *C. albicans* (C) in the fecal microbiota quantified using qRT-PCR (mean ± s.e.m.). In all panels : \* = $p < 0.05$ ; \*\* = $p < 0.01$ ; \*\*\* = $p < 0.001$**

A

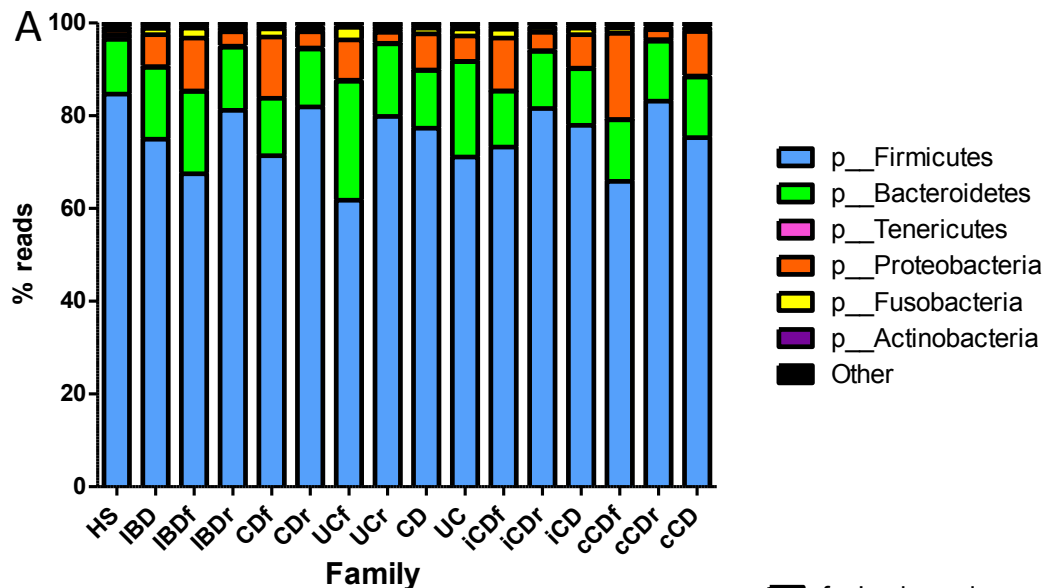


B

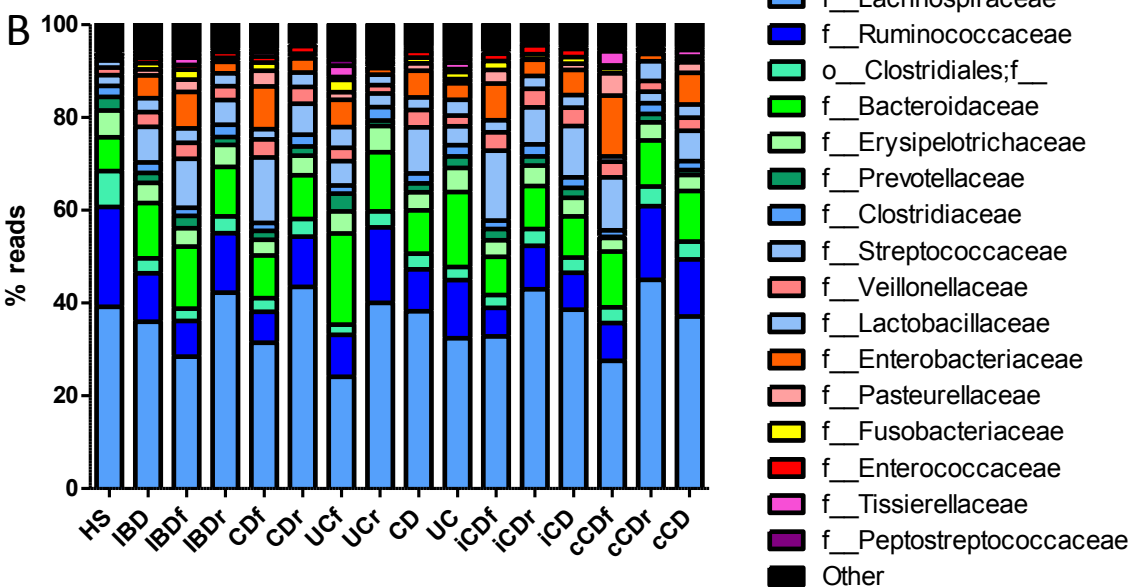




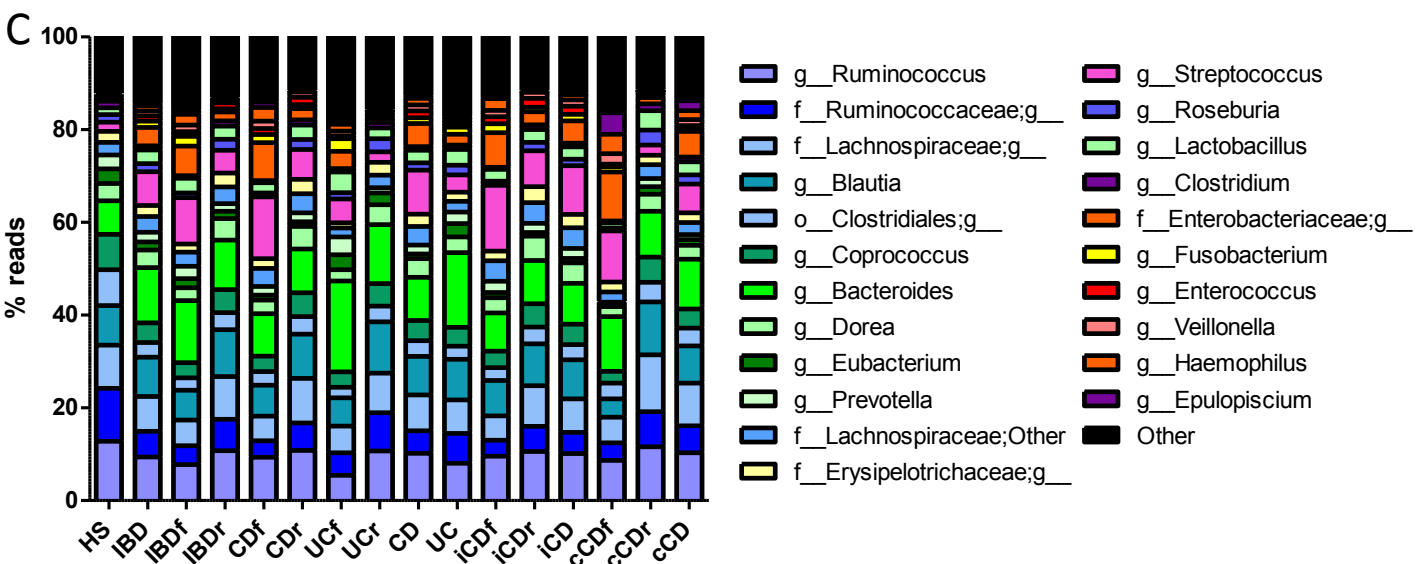
# Phyla



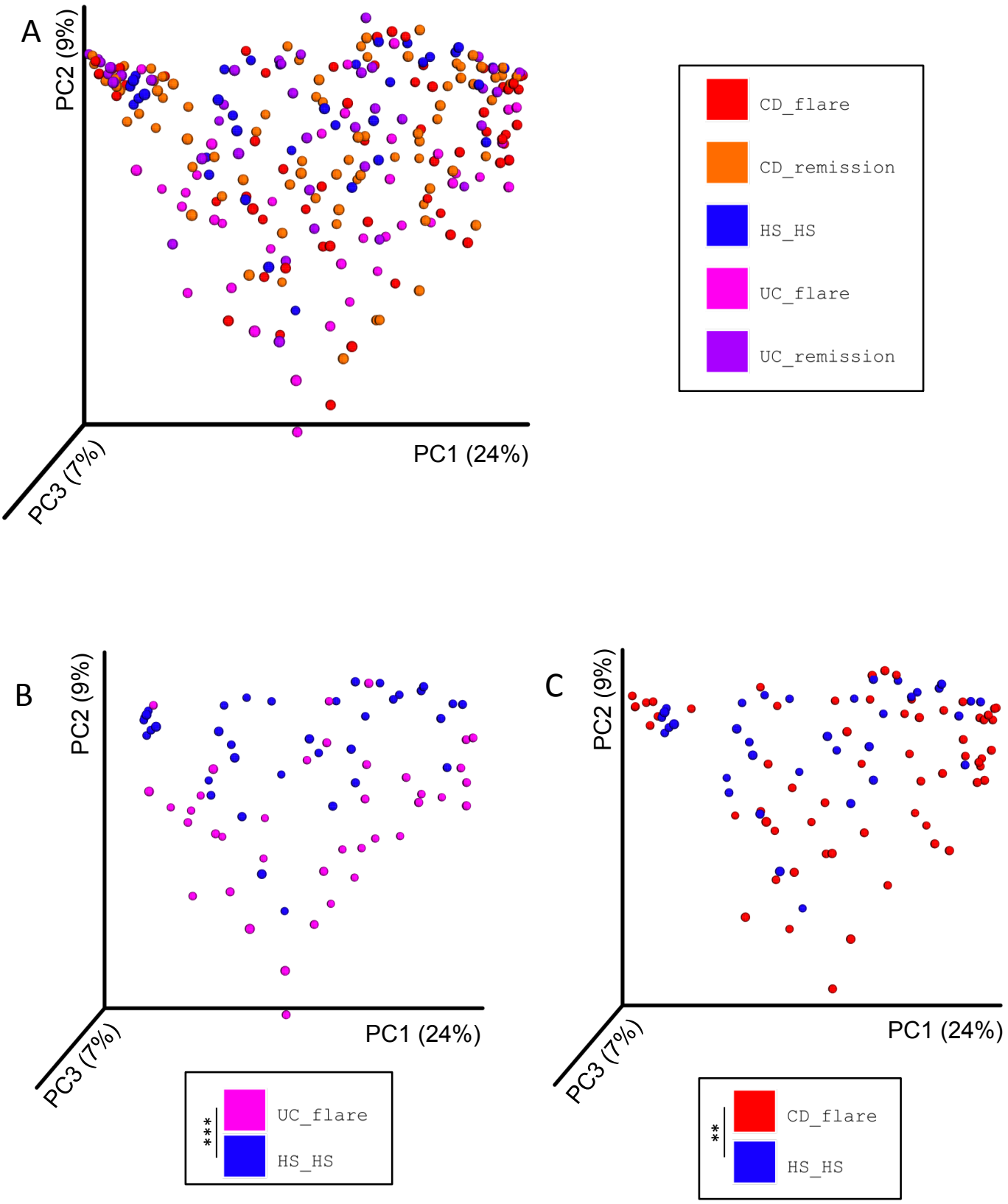
# Family

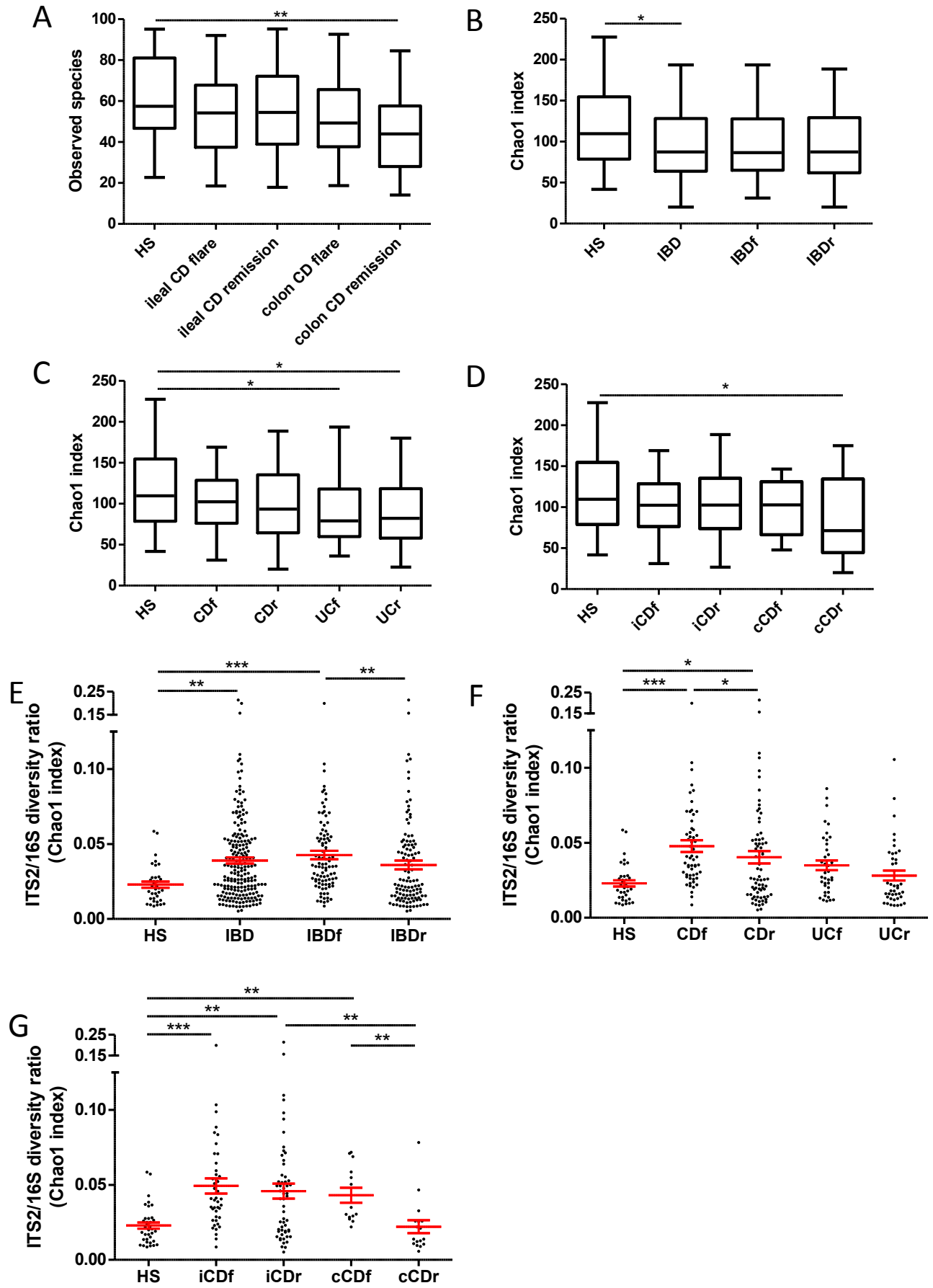


# Genus

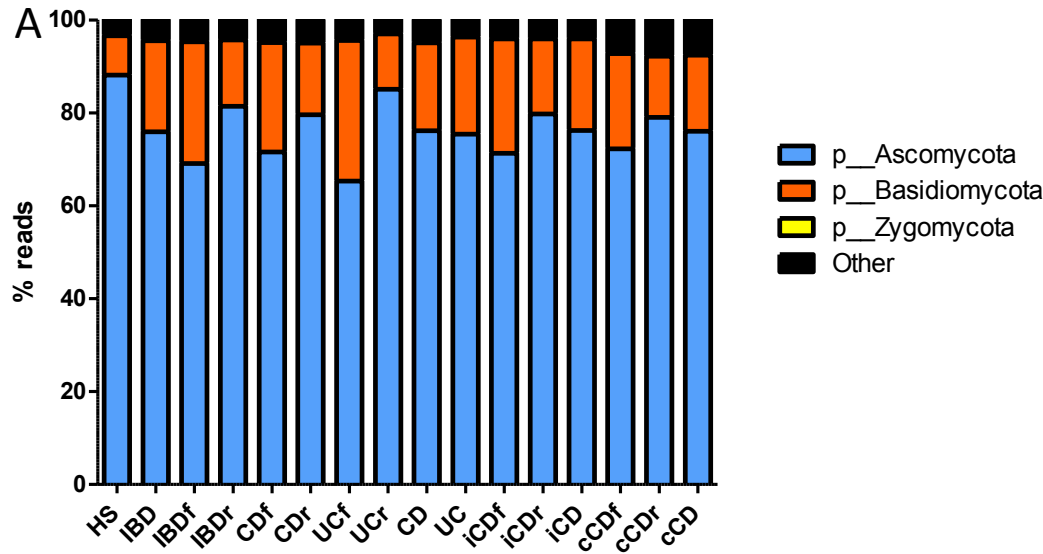




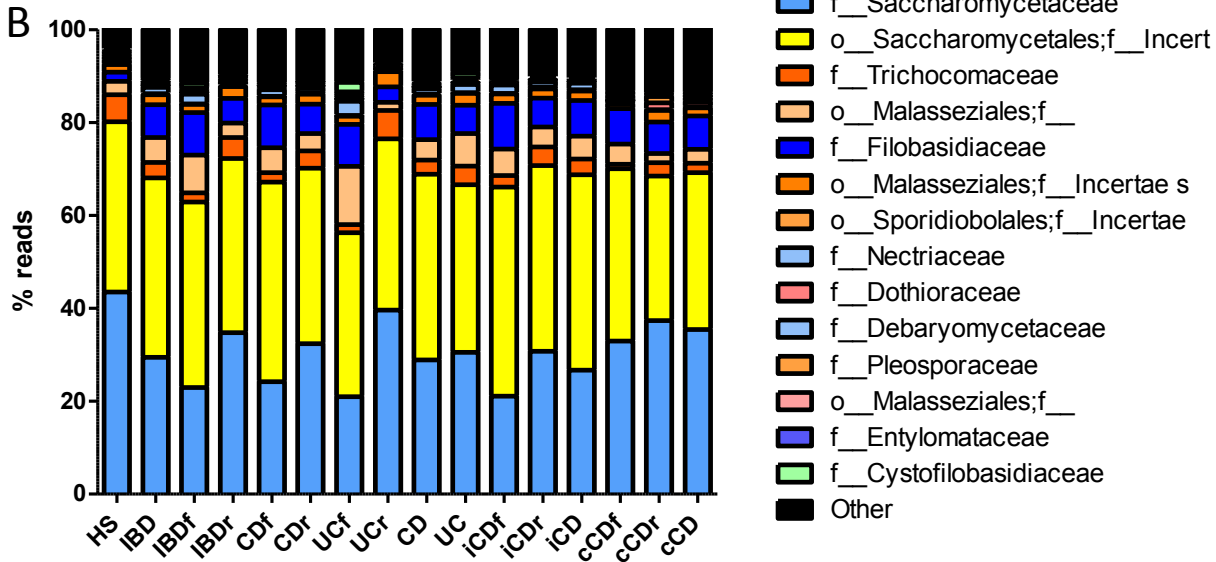




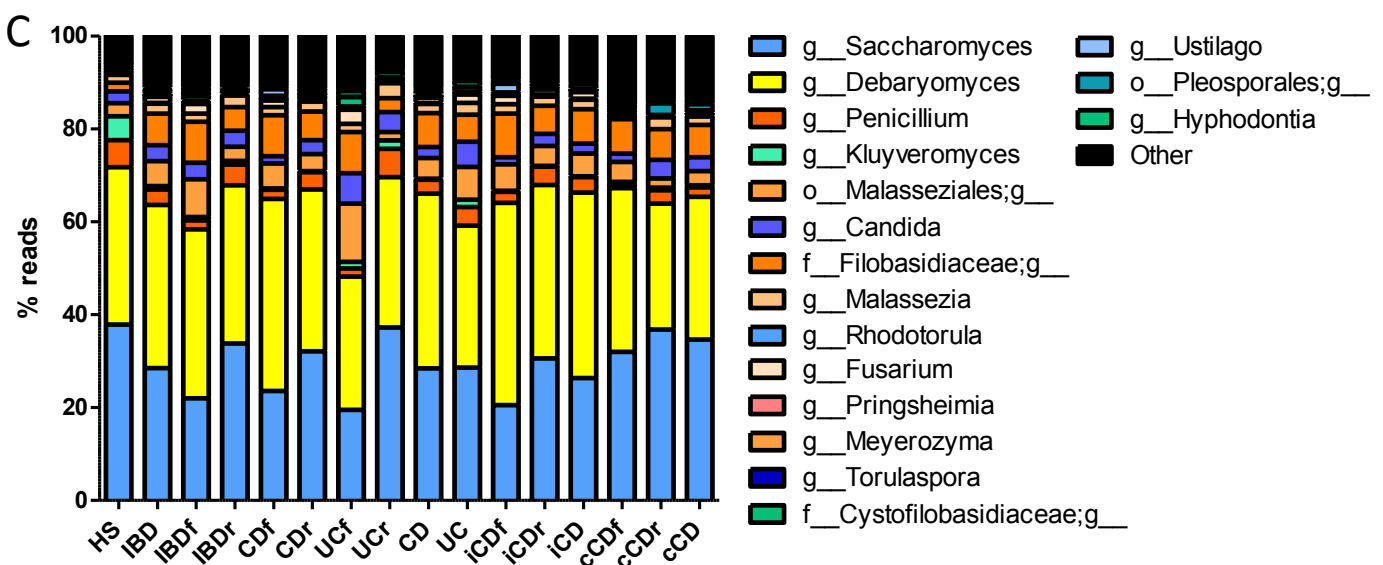
# Phyla



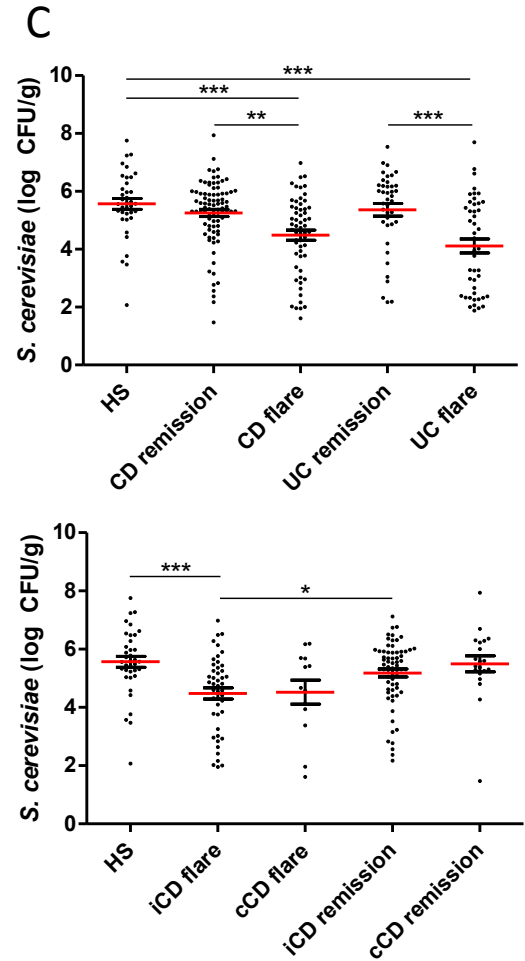
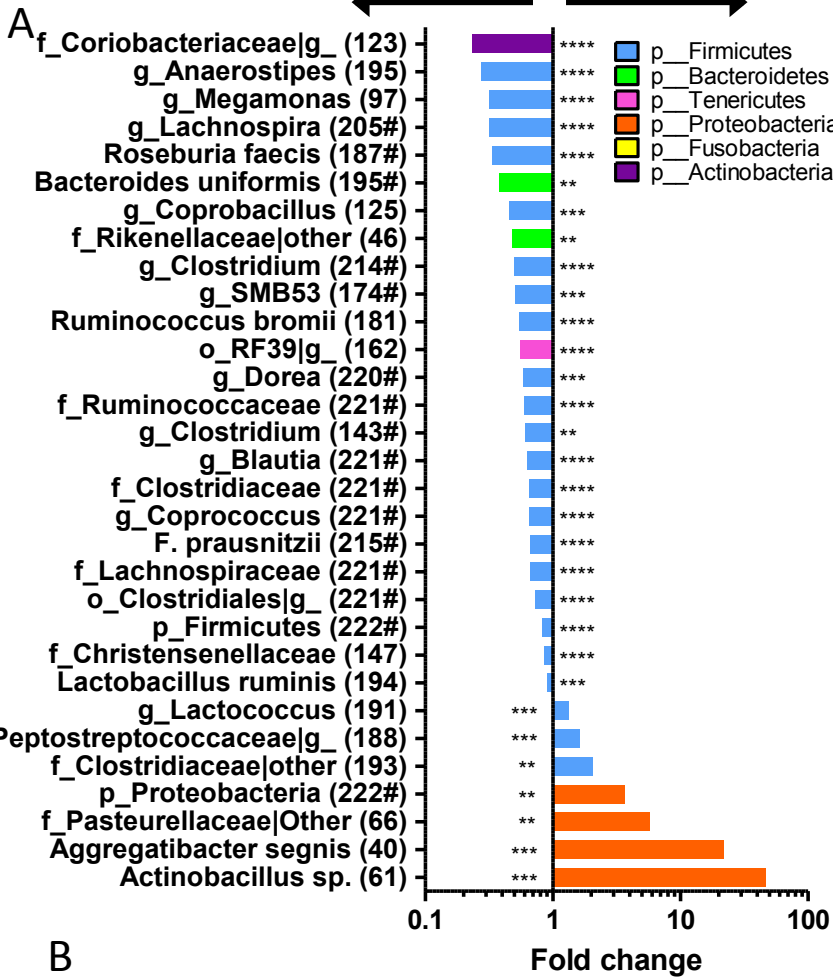
# Family

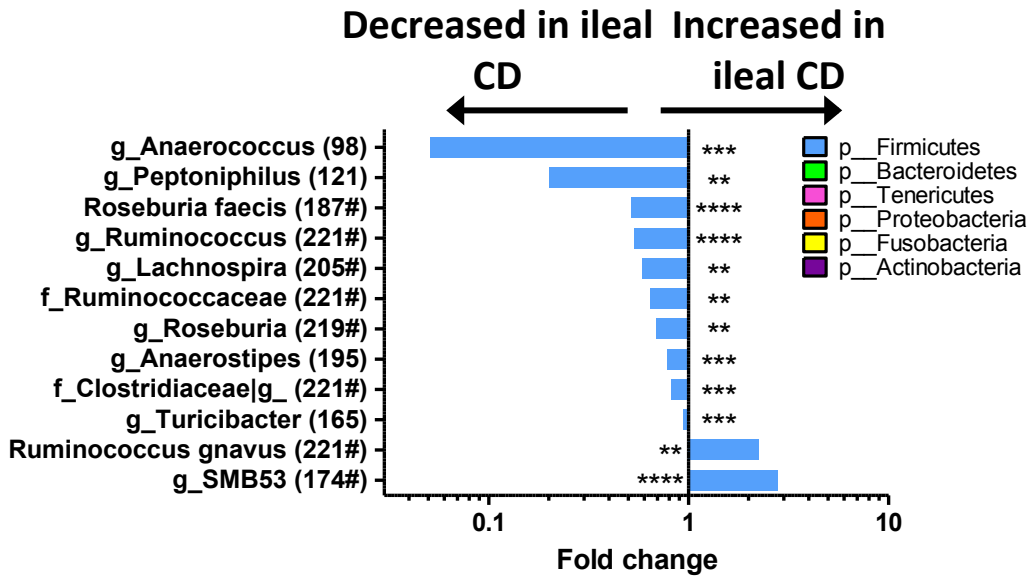


# Genus



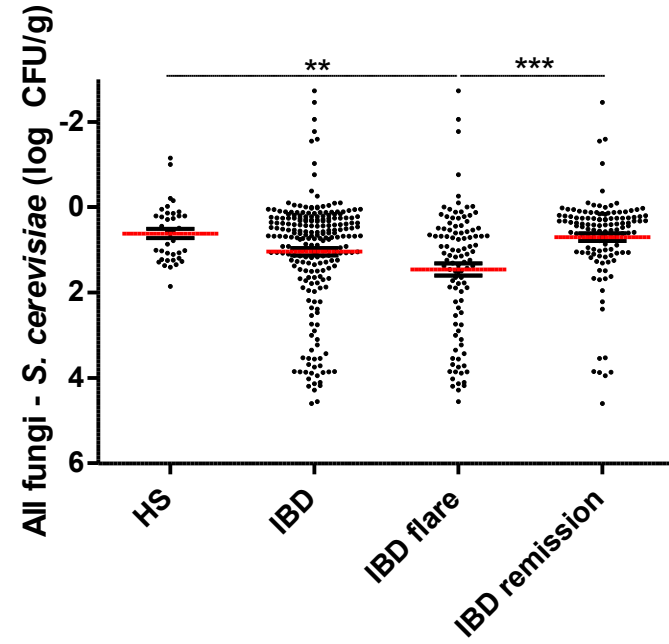
Decreased in flare      Increased in flare



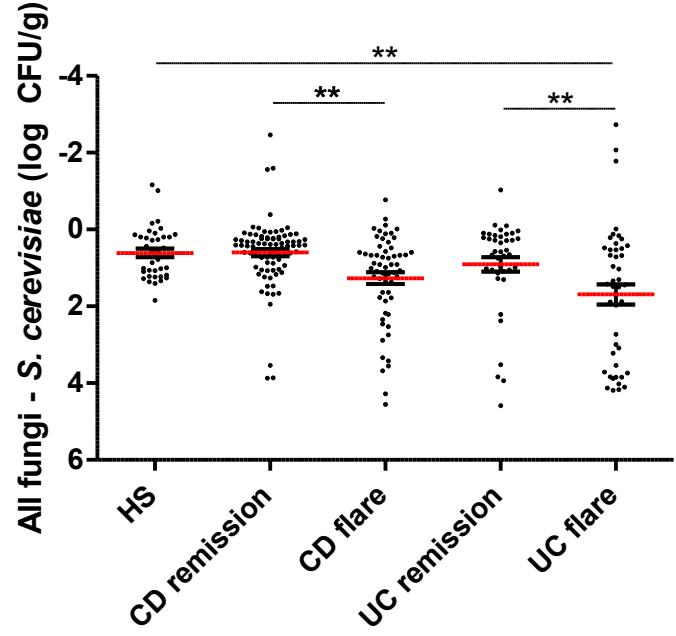




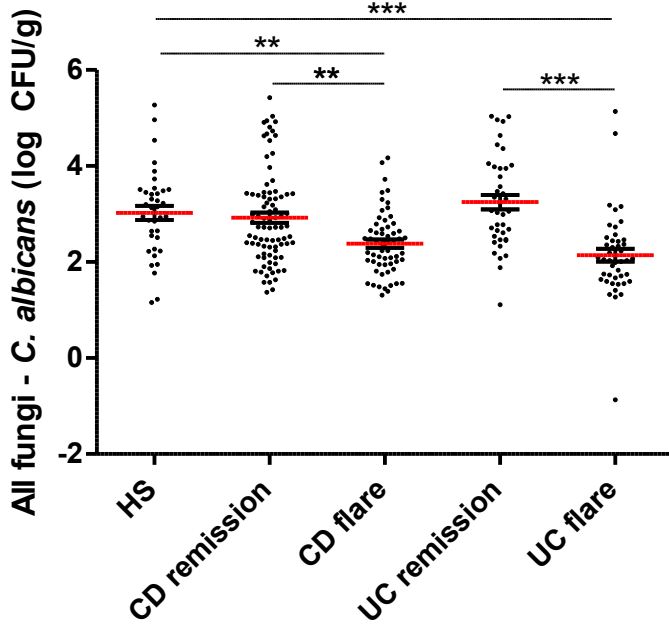
A



B



C



**Table S1: Study population**

	<b>IBD (n=235)</b>		<b>HS (n=38)</b>
Age: Year (mean +/- SD)	40.4 +/- 14.6		35.8 +/- 13.2
Male: n (%)	94 (40.0%)		17 (44.7%)
Active smoking: n (%)	55 (23.4%)		3 (7.9%)
Flare / remission	106 (45.1%) / 129 (54.9%)		NA
CD/UC	CD	UC	NA
n (%)	149 (63.4%)	86 (36.6%)	NA
<b>Montreal classification</b>			
A1 / A2 / A3 (n)	30 / 95 / 24	NA	NA
L1 / L2 / L3 (n)	35 / 42 / 72	NA	NA
B1 / B2 / B3 (n)	58 / 24 / 72	NA	NA
E1 / E2 / E3 (n)	NA	12 / 49 / 25	NA
<b>Treatment: n (%)</b>			
5-ASA	84 (35.7%)		0
Corticosteroids	45 (19.1%)		0
Thiopurine or Methotrexate	88 (37.4%)		0
anti-TNF alpha	109 (46.4%)		0
Antibiotics	0		0

**Supplementary Table 2: Genotype of patients with IBD**

	<b>G0</b>	<b>G1</b>	<b>G2</b>	<b>FrqG0</b>	<b>FrqG1</b>	<b>FrqG2</b>	<b>N</b>	<b>MAF</b>
<i>CARD9_rs10781499</i>	A:A	A:G	G:G	31	93	54	178	0.44
<i>CARD9_rs11145835</i>	A:A	A:G	G:G	135	41	2	178	0.13
<i>DC.SIGN_rs2287886</i>	A:A	A:G	G:G	34	68	74	176	0.39
<i>DC.SIGN_rs4804803</i>	A:A	A:G	G:G	117	55	6	178	0.19
<i>DC.SIGN_rs7248637</i>	A:A	A:G	G:G	26	42	113	181	0.26
<i>DC.SIGN_rs7252229</i>	C:C	C:G	G:G	25	45	109	179	0.27
<i>Dectin.1_rs16910526</i>	G:G	G:T	T:T	2	15	163	180	0.05
<i>Dectin.1_rs16910631</i>	C:C	C:T	T:T	154	25	0	179	0.07
<i>Dectin.1_rs2078178</i>	C:C	C:T	T:T	102	58	15	175	0.25
<i>Dectin.1_rs3901533</i>	G:G	G:T	T:T	108	65	7	180	0.22
<i>Dectin.1_rs7309123</i>	C:C	C:G	G:G	58	86	36	180	0.44
<i>Dectin.2_rs4264222</i>	C:C	C:T	T:T	103	64	12	179	0.25
<i>Dectin.2_rs4459385</i>	C:C	C:T	T:T	96	58	23	177	0.29
<i>Dectin.2_rs7134303</i>	A:A	A:G	G:G	115	53	12	180	0.21
<i>Mincle_rs10841845</i>	A:A	A:G	G:G	100	59	20	179	0.28
<i>TLR1_rs4833095</i>	C:C	C:T	T:T	26	80	69	175	0.38
<i>TLR1_rs5743611</i>	C:C	C:G	G:G	0	18	160	178	0.05
<i>TLR1_rs5743618</i>	G:G	G:T	T:T	56	75	47	178	0.47
<i>TLR2_rs5743708</i>	A:A	A:G	G:G	0	9	168	177	0.03
<i>TLR3_rs3775291</i>	A:A	A:G	G:G	16	61	99	176	0.26
<i>TLR4_rs4986790</i>	A:A	A:G	G:G	158	20	0	178	0.056
<i>TLR4_rs4986791</i>	C:C	C:T	T:T	161	18	0	179	0.05

MAF, minor allele frequency

Variable  
1 Sasaoral  
2 age  
3 antitnf  
4 antitnf  
5 antitnf  
6 antitnf  
7 corticoide  
8 corticoide  
9 corticoide  
10 F\_R  
11 F\_R  
12 F\_R  
13 F\_R  
14 F\_R  
15 F\_R  
16 F\_R  
17 F\_R  
18 F\_R  
19 F\_R  
20 F\_R  
21 F\_R  
22 F\_R  
23 F\_R  
24 F\_R  
25 F\_R  
26 F\_R  
27 F\_R  
28 F\_R  
29 F\_R  
30 F\_R  
31 F\_R  
32 F\_R  
33 F\_R  
34 F\_R  
35 F\_R  
36 F\_R  
37 F\_R  
38 F\_R  
39 F\_R  
40 F\_R  
41 F\_R  
42 F\_R  
43 F\_R  
44 F\_R  
45 F\_R

46 F\_R  
47 F\_R  
48 F\_R  
49 F\_R  
50 F\_R  
51 F\_R  
52 F\_R  
53 F\_R  
54 F\_R  
55 F\_R  
56 F\_R  
57 F\_R  
58 F\_R  
59 F\_R  
60 F\_R  
61 F\_R  
62 F\_R  
63 F\_R  
64 F\_R  
65 F\_R  
66 F\_R  
67 F\_R  
68 F\_R  
69 F\_R  
70 F\_R  
71 F\_R  
72 F\_R  
73 F\_R  
74 F\_R  
75 F\_R  
76 F\_R  
77 F\_R  
78 F\_R  
79 F\_R  
80 F\_R  
81 F\_R  
82 F\_R  
83 F\_R  
84 F\_R  
85 F\_R  
86 F\_R  
87 F\_R  
88 F\_R  
89 IBD\_HS  
90 IBD\_HS  
91 IBD\_HS



92 IBD\_HS  
93 IBD\_HS  
94 IBD\_HS  
95 IBD\_HS  
96 IBD\_HS  
97 IBD\_HS  
98 IBD\_HS  
99 IBD\_HS  
100 IBD\_HS  
101 IBD\_HS  
102 IBD\_HS  
103 IBD\_HS  
104 IBD\_HS  
105 IBD\_HS  
106 IBD\_HS  
107 IBD\_HS  
108 IBD\_HS  
109 IBD\_HS  
110 IBD\_HS  
111 IBD\_HS  
112 IBD\_HS  
113 IBD\_HS  
114 IBD\_HS  
115 IBD\_HS  
116 IBD\_HS  
117 IBD\_HS  
118 IBD\_HS  
119 IBD\_HS  
120 IBD\_HS  
121 IBD\_HS  
122 IBD\_HS  
123 IBD\_HS  
124 IBD\_HS  
125 IBD\_HS  
126 IBD\_HS  
127 IBD\_HS  
128 IBD\_HS  
129 IBD\_HS  
130 IBD\_HS  
131 IBD\_HS  
132 IBD\_HS  
133 IBD\_HS  
134 IBD\_HS  
135 IBD\_HS  
136 IBD\_HS  
137 IBD\_HS

138 IBD\_HS  
139 IBD\_HS  
140 IBD\_HS  
141 IBD\_HS  
142 IBD\_HS  
143 IBD\_HS  
144 IBD\_HS  
145 IBD\_HS  
146 IBD\_HS  
147 IBD\_HS  
148 IBD\_HS  
149 Ileum  
150 Ileum  
151 Ileum  
152 Ileum  
153 Ileum  
154 Ileum  
155 Ileum  
156 Ileum  
157 Ileum  
158 Ileum  
159 Ileum  
160 Ileum  
161 Ileum  
162 Ileum  
163 Ileum  
164 Ileum  
165 Ileum  
166 Ileum  
167 Ileum  
168 Ileum  
169 Ileum  
170 Ileum  
171 Ileum  
172 Ileum  
173 Ileum  
174 Ileum  
175 Ileum  
176 Ileum  
177 THIOPURINE\_MTX  
178 THIOPURINE\_MTX

## Feature

k\_\_Bacteria|p\_\_Bacteroidetes|c\_\_Bacteroidia|o\_\_Bacteroidales|f\_\_[Paraprevotellaceae]|g\_\_Para  
k\_\_Bacteria|p\_\_Actinobacteria|c\_\_Actinobacteria|o\_\_Bifidobacteriales|f\_\_Bifidobacteriaceae|g\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Bacilli|o\_\_Lactobacillales|f\_\_Enterococcaceae|g\_\_Enterococcus|s\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Clostridiaceae|g\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Clostridiaceae|g\_\_|s\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Peptostreptococcaceae|g\_\_|s\_\_  
k\_\_Bacteria|p\_\_Bacteroidetes|c\_\_Bacteroidia|o\_\_Bacteroidales|f\_\_Porphyromonadaceae|g\_\_Par  
k\_\_Bacteria|p\_\_Bacteroidetes|c\_\_Bacteroidia|o\_\_Bacteroidales|f\_\_Bacteroidaceae|g\_\_Bacteroid  
k\_\_Bacteria|p\_\_Bacteroidetes|c\_\_Bacteroidia|o\_\_Bacteroidales|f\_\_Porphyromonadaceae|g\_\_Par  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Anaerostipes  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Anaerostipes|s\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Roseburia|Othe  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Roseburia|s\_\_f  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Roseburia  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Roseburia|s\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Clostridiaceae|g\_\_Clostridium|s\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_|s\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Ruminococcaceae  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Ruminococcaceae|g\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Ruminococcaceae|g\_\_|s\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Clostridiaceae|g\_\_Clostridium  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Coprococcus  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Coprococcus|s\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Veillonellaceae|g\_\_Megamonas|s\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Veillonellaceae|g\_\_Megamonas  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Ruminococcaceae|g\_\_Ruminococcus  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Ruminococcaceae|g\_\_Ruminococcus  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Clostridiaceae  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Blautia|s\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Blautia  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Christensenellaceae|g\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Christensenellaceae|g\_\_|s\_\_  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Blautia|Other  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Lachnospira  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Lachnospira|s\_\_  
k\_\_Bacteria|p\_\_Actinobacteria|c\_\_Coriobacteriia|o\_\_Coriobacteriales|f\_\_Coriobacteriaceae|g\_\_|  
k\_\_Bacteria|p\_\_Actinobacteria|c\_\_Coriobacteriia|o\_\_Coriobacteriales|f\_\_Coriobacteriaceae|g\_\_  
k\_\_Bacteria|p\_\_Firmicutes  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Ruminococcaceae|g\_\_Ruminococcus  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Ruminococcaceae|Other  
k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Ruminococcaceae|Other|Other

k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Ruminococcaceae|g\_Faecalibacteri  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_|g\_\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_|g\_|s\_\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Ruminococcaceae|g\_Faecalibacteri  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Christensenellaceae  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|g\_Dorea  
 k\_Bacteria|p\_Firmicutes|c\_Bacilli|o\_Lactobacillales|f\_Lactobacillaceae|g\_Lactobacillus|s\_\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|g\_Dorea|s\_\_  
 k\_Bacteria|p\_Proteobacteria|c\_Gammaproteobacteria|o\_Pasteurellales|f\_Pasteurellaceae|  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Ruminococcaceae|g\_Ruminococcus  
 k\_Bacteria|p\_Firmicutes|c\_Bacilli|o\_Lactobacillales|f\_Streptococcaceae|g\_Lactococcus  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Peptostreptococcaceae|g\_\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|g\_Coproccoccus|s\_\_  
 k\_Bacteria|p\_Actinobacteria|c\_Coriobacteriia  
 k\_Bacteria|p\_Actinobacteria|c\_Coriobacteriia|o\_Coriobacteriales  
 k\_Bacteria|p\_Actinobacteria|c\_Coriobacteriia|o\_Coriobacteriales|f\_Coriobacteriaceae  
 k\_Bacteria|p\_Proteobacteria|c\_Gammaproteobacteria|o\_Pasteurellales|f\_Pasteurellaceae|  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Clostridiaceae|g\_SMB53  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Clostridiaceae|g\_SMB53|s\_\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|Other  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|Other|Other  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Peptostreptococcaceae|g\_|s\_\_  
 k\_Bacteria|p\_Firmicutes|c\_Erysipelotrichi|o\_Erysipelotrichales|f\_Erysipelotrichaceae|g\_Co  
 k\_Bacteria|p\_Tenericutes|c\_Mollicutes|o\_RF39  
 k\_Bacteria|p\_Tenericutes|c\_Mollicutes|o\_RF39|f\_|g\_\_  
 k\_Bacteria|p\_Tenericutes|c\_Mollicutes|o\_RF39|f\_|g\_|s\_\_  
 k\_Bacteria|p\_Tenericutes|c\_Mollicutes|o\_RF39|f\_\_  
 k\_Bacteria|p\_Firmicutes|c\_Erysipelotrichi|o\_Erysipelotrichales|f\_Erysipelotrichaceae|g\_Co  
 k\_Bacteria|p\_Proteobacteria|c\_Gammaproteobacteria|o\_Pasteurellales|f\_Pasteurellaceae|  
 k\_Bacteria|p\_Firmicutes|c\_Erysipelotrichi|o\_Erysipelotrichales|f\_Erysipelotrichaceae|g\_Cl  
 k\_Bacteria|p\_Firmicutes|c\_Erysipelotrichi|o\_Erysipelotrichales|f\_Erysipelotrichaceae|g\_Cl  
 k\_Bacteria|p\_Proteobacteria|c\_Gammaproteobacteria|o\_Pasteurellales|f\_Pasteurellaceae|  
 k\_Bacteria|p\_Bacteroidetes|c\_Bacteroidia|o\_Bacteroidales|f\_Bacteroidaceae|g\_Bacteroid  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|g\_Coproccoccus|O  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Clostridiaceae|Other  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Clostridiaceae|Other|Other  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|Other  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|Other|Other  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|Other|Other|Other  
 k\_Bacteria|p\_Proteobacteria  
 k\_Bacteria|p\_Bacteroidetes|c\_Bacteroidia|o\_Bacteroidales|f\_Rikenellaceae|Other  
 k\_Bacteria|p\_Bacteroidetes|c\_Bacteroidia|o\_Bacteroidales|f\_Rikenellaceae|Other|Other  
 k\_Bacteria|p\_Proteobacteria|c\_Deltaproteobacteria|o\_Desulfovibrionales|f\_Desulfovibriona  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_|g\_\_

k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_|g\_|s\_  
 k\_Bacteria|p\_Proteobacteria|c\_Deltaproteobacteria|o\_Desulfovibrionales|f\_Desulfovibrion  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|g\_Coproccoccus  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|g\_Anaerostipes  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|g\_Anaerostipes|s\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Ruminococcaceae|Other  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Ruminococcaceae|Other|Other  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|g\_Coproccoccus|s\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Christensenellaceae|g\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Christensenellaceae|g\_|s\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Christensenellaceae  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Ruminococcaceae|g\_Ruminococcus  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Ruminococcaceae|g\_Ruminococcus  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Ruminococcaceae|g\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Ruminococcaceae|g\_|s\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|Other  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|Other|Other  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|Other|Other|Other  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Ruminococcaceae|g\_Ruminococcus  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_[Mogibacteriaceae]|g\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_[Mogibacteriaceae]|g\_|s\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|g\_Coproccoccus|O  
 k\_Bacteria|p\_Tenericutes|c\_Mollicutes|o\_RF39  
 k\_Bacteria|p\_Tenericutes|c\_Mollicutes|o\_RF39|f\_|g\_  
 k\_Bacteria|p\_Tenericutes|c\_Mollicutes|o\_RF39|f\_|g\_|s\_  
 k\_Bacteria|p\_Tenericutes|c\_Mollicutes|o\_RF39|f\_  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Dehalobacteriaceae  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Ruminococcaceae  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|g\_Dorea|Other  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Ruminococcaceae|g\_Ruminococcus  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_[Mogibacteriaceae]  
 k\_Bacteria|p\_Tenericutes|c\_Mollicutes  
 k\_Bacteria|p\_Bacteroidetes|c\_Bacteroidia|o\_Bacteroidales|f\_Rikenellaceae  
 k\_Bacteria|p\_Bacteroidetes|c\_Bacteroidia|o\_Bacteroidales|f\_[Odoribacteriaceae]|g\_Butyri  
 k\_Bacteria|p\_Bacteroidetes|c\_Bacteroidia|o\_Bacteroidales|f\_[Odoribacteriaceae]|g\_Butyri  
 k\_Bacteria|p\_Tenericutes  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|g\_[Ruminococcus]  
 k\_Bacteria|p\_Proteobacteria|c\_Deltaproteobacteria|o\_Desulfovibrionales  
 k\_Bacteria|p\_Proteobacteria|c\_Deltaproteobacteria|o\_Desulfovibrionales|f\_Desulfovibrion  
 k\_Bacteria|p\_Proteobacteria|c\_Deltaproteobacteria  
 k\_Bacteria|p\_Bacteroidetes|c\_Bacteroidia|o\_Bacteroidales|f\_Rikenellaceae|g\_  
 k\_Bacteria|p\_Bacteroidetes|c\_Bacteroidia|o\_Bacteroidales|f\_Rikenellaceae|g\_|s\_  
 k\_Bacteria|p\_Bacteroidetes|c\_Bacteroidia|o\_Bacteroidales|f\_Porphyrimonadaceae|g\_Par  
 k\_Bacteria|p\_Firmicutes|c\_Clostridia|o\_Clostridiales|f\_Lachnospiraceae|g\_Dorea|s\_form



k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Blautia|Other  
 k\_\_Bacteria|p\_\_Actinobacteria|c\_\_Coriobacteriia|o\_\_Coriobacteriales|f\_\_Coriobacteriaceae|g\_\_  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Erysipelotrichi|o\_\_Erysipelotrichales|f\_\_Erysipelotrichaceae|g\_\_[Ei  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Lachnobacteriu  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Lachnobacteriu  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Clostridiaceae|g\_\_SMB53  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Clostridiaceae|g\_\_SMB53|s\_\_  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Bacilli|o\_\_Lactobacillales|f\_\_Streptococcaceae|g\_\_Streptococcus|s  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Bacilli|o\_\_Gemellales|f\_\_Gemellaceae  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Erysipelotrichi|o\_\_Erysipelotrichales|f\_\_Erysipelotrichaceae|g\_\_[Ei  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Bacilli|o\_\_Gemellales|f\_\_Gemellaceae|g\_\_  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Roseburia|s\_\_f  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Clostridiaceae|g\_\_SMB53  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Clostridiaceae|g\_\_SMB53|s\_\_  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Ruminococcaceae|g\_\_Ruminococcus  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Ruminococcaceae|g\_\_Ruminococcus  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Roseburia|Othe  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Bacilli|o\_\_Turicibacterales  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Bacilli|o\_\_Turicibacterales|f\_\_Turicibacteraceae  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Bacilli|o\_\_Turicibacterales|f\_\_Turicibacteraceae|g\_\_Turicibacter  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Bacilli|o\_\_Turicibacterales|f\_\_Turicibacteraceae|g\_\_Turicibacter|s  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_[Tissierellaceae]|g\_\_Anaerococcus  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_[Tissierellaceae]|g\_\_Anaerococcus|s  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Anaerostipes  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Anaerostipes|s  
 Unassigned|Other  
 Unassigned|Other|Other  
 Unassigned|Other|Other|Other|Other  
 Unassigned|Other|Other|Other|Other|Other  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Clostridiaceae|g\_\_  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Clostridiaceae|g\_\_|s\_\_  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_[Ruminococcus]  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Lachnospira  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Lachnospira|s\_\_  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Lachnospiraceae|g\_\_Roseburia  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Ruminococcaceae  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_[Tissierellaceae]|g\_\_Peptoniphilus  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_[Tissierellaceae]|g\_\_Peptoniphilus|s  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Veillonellaceae|g\_\_Megamonas  
 k\_\_Bacteria|p\_\_Firmicutes|c\_\_Clostridia|o\_\_Clostridiales|f\_\_Veillonellaceae|g\_\_Megamonas|s\_\_

Value	Coefficient	N	N.not.0	P.value
`5asaoral`NA	0.00330928359366297	260	65	0.000735049653141003
age	-6.16973635172476e-05	260	68	0.000906658677367852
antitnfyes	-0.0118977218896839	260	254	0.000699035842138117
antitnfyes	0.0104255533189508	260	259	0.00196524601948534
antitnfyes	0.0104255533189508	260	259	0.00196524601948534
antitnfyes	0.00740672523360804	260	222	0.00254860550503575
corticoideyes	0.0206417254618399	260	236	0.000155668124015778
corticoideyes	0.00547322875901667	260	173	0.00116162607554134
corticoideyes	0.00569309757436784	260	129	0.0024514906830929
F_Rremission	0.0279416711382131	260	233	4.02031618913011e-13
F_Rremission	0.0279416711382131	260	233	4.02031618913011e-13
F_Rremission	0.0293950406333476	260	216	4.61389089758771e-10
F_Rremission	0.0426550623077112	260	225	5.85483494810257e-10
F_Rremission	0.0577294079689456	260	257	6.62481950448716e-09
F_Rremission	0.0253481719608822	260	257	2.35193768828572e-08
F_Rremission	0.0366244497569332	260	251	9.67770156634439e-08
F_Rremission	0.0766713890940216	260	259	1.07836379677835e-07
F_Rremission	0.0766713890940216	260	259	1.07836379677835e-07
F_Rremission	0.0937495763546869	260	259	1.42562799330395e-07
F_Rremission	0.182328343315544	260	260	1.79911997211372e-07
F_Rremission	0.181576460797013	260	260	2.43288033396057e-07
F_Rremission	0.155385601571415	260	259	3.7705923303716e-07
F_Rremission	0.0638670789629989	260	259	6.55361379939375e-07
F_Rremission	0.0638670789629989	260	259	6.55361379939375e-07
F_Rremission	0.0340121804194754	260	252	8.91149713488754e-07
F_Rremission	0.0545809550167818	260	259	1.66906721671177e-06
F_Rremission	0.0530786318124208	260	259	2.53929636908439e-06
F_Rremission	0.0046567771145008	260	120	2.64907590345008e-06
F_Rremission	0.00448205868353029	260	121	5.11681307408498e-06
F_Rremission	0.0607995731416748	260	257	5.53478505479781e-06
F_Rremission	0.0601611144734055	260	257	6.00274171559339e-06
F_Rremission	0.032323831571929	260	259	7.85054341434534e-06
F_Rremission	0.0687047386389922	260	259	1.1375276773599e-05
F_Rremission	0.0693365944527671	260	259	1.28943803673415e-05
F_Rremission	0.0086825208336154	260	180	1.39825586825922e-05
F_Rremission	0.0086825208336154	260	180	1.39825586825922e-05
F_Rremission	0.00660594109432379	260	220	1.55121424744287e-05
F_Rremission	0.0184208919725374	260	242	1.55622537653329e-05
F_Rremission	0.0184208919725374	260	242	1.55622537653329e-05
F_Rremission	0.0110169275581025	260	155	1.87654531011438e-05
F_Rremission	0.0107721236653888	260	155	2.42515915501361e-05
F_Rremission	0.150440145025752	260	260	2.75347330886868e-05
F_Rremission	0.00893187151456076	260	218	2.8379997522377e-05
F_Rremission	0.0100528335722373	260	250	4.33071653554282e-05
F_Rremission	0.0100528335722373	260	250	4.33071653554282e-05

F_Remission	0.0229894709858891	260	253	4.66043536643208e-05
F_Remission	0.0356894491635197	260	259	4.83782562401373e-05
F_Remission	0.0356894491635197	260	259	4.83782562401373e-05
F_Remission	0.0356894491635197	260	259	4.83782562401373e-05
F_Remission	0.0228289015468383	260	254	5.0740552159259e-05
F_Remission	0.00820553584892316	260	184	5.49600038233891e-05
F_Remission	0.034583464646137	260	258	0.000170437422331501
F_Remission	0.00683060997300483	260	231	0.00020487365602605
F_Remission	0.0339175454570775	260	258	0.0002956604957649
F_Remission	-0.00766414684151014	260	42	0.000299321687530392
F_Remission	0.00879023420540545	260	234	0.000305386685678376
F_Remission	0.00832858090797622	260	225	0.000343971101666338
F_Remission	0.00877083933987701	260	222	0.000379096187289408
F_Remission	0.00571518545683837	260	201	0.000380154275495265
F_Remission	0.00885973864189734	260	203	0.00039086220583352
F_Remission	0.00885973864189734	260	203	0.00039086220583352
F_Remission	0.00885973864189734	260	203	0.00039086220583352
F_Remission	-0.00237378293130738	260	61	0.000518553608318883
F_Remission	0.0113965159036884	260	212	0.000600754439013932
F_Remission	0.0113965159036884	260	212	0.000600754439013932
F_Remission	0.0267443538198879	260	259	0.000620334895205207
F_Remission	0.0266587443317334	260	259	0.000626352996964133
F_Remission	0.00829010205400153	260	222	0.00072798876688411
F_Remission	0.00621245291253428	260	152	0.000749565143970108
F_Remission	0.00482234093973922	260	199	0.00090947238785967
F_Remission	0.00482234093973922	260	199	0.00090947238785967
F_Remission	0.00482234093973922	260	199	0.00090947238785967
F_Remission	0.00481999363292008	260	199	0.000921021732665704
F_Remission	0.00602942351962874	260	152	0.00106199066918976
F_Remission	-0.00240242124685183	260	70	0.00106317140245335
F_Remission	0.00712943331835652	260	143	0.00112621176614702
F_Remission	0.00712943331835652	260	143	0.00112621176614702
F_Remission	-0.00238621461967317	260	70	0.00117627111914863
F_Remission	0.0283686084818666	260	232	0.00132967341006616
F_Remission	0.00419704402654728	260	186	0.00156912718986697
F_Remission	0.00580465356570866	260	231	0.00159777427446337
F_Remission	0.00580465356570866	260	231	0.00159777427446337
F_Remission	0.0113307123395818	260	260	0.00187182635400342
F_Remission	0.0113307123395818	260	260	0.00187182635400342
F_Remission	0.0113307123395818	260	260	0.00187182635400342
F_Remission	-0.0198785454012118	260	260	0.00205176927555343
F_Remission	0.00224944601110198	260	54	0.00209266576158987
F_Remission	0.00224944601110198	260	54	0.00209266576158987
IBD_HSIBD	-0.00842466571246543	260	63	1.89620171758802e-16
IBD_HSIBD	-0.105657282069405	260	259	3.85142357520605e-16
IBD_HSIBD	-0.105657282069405	260	259	3.85142357520605e-16

IBD_HSIBD	-0.105657282069405	260	259	3.85142357520605e-16
IBD_HSIBD	-0.00910028823420094	260	65	4.03008882436228e-16
IBD_HSIBD	-0.122175676306574	260	259	3.63803187169564e-13
IBD_HSIBD	-0.0397551698005713	260	233	2.49220842163929e-12
IBD_HSIBD	-0.0397551698005713	260	233	2.49220842163929e-12
IBD_HSIBD	-0.0252684592990025	260	250	1.46406578806169e-11
IBD_HSIBD	-0.0252684592990025	260	250	1.46406578806169e-11
IBD_HSIBD	-0.111846864380486	260	259	7.63936379511196e-11
IBD_HSIBD	-0.0194825465948454	260	180	1.17184293240004e-10
IBD_HSIBD	-0.0194825465948454	260	180	1.17184293240004e-10
IBD_HSIBD	-0.019699494149026	260	184	1.67599779763441e-10
IBD_HSIBD	-0.129018614802871	260	257	1.67765816022091e-10
IBD_HSIBD	-0.126972298115984	260	257	2.40133051939931e-10
IBD_HSIBD	-0.319378838734554	260	260	4.26027193390369e-10
IBD_HSIBD	-0.149864679275397	260	259	4.55149527574688e-10
IBD_HSIBD	-0.149864679275397	260	259	4.55149527574688e-10
IBD_HSIBD	-0.320631913392283	260	260	5.14353977331787e-10
IBD_HSIBD	-0.0339204408843599	260	260	1.18615621026087e-09
IBD_HSIBD	-0.0339204408843599	260	260	1.18615621026087e-09
IBD_HSIBD	-0.0339204408843599	260	260	1.18615621026087e-09
IBD_HSIBD	-0.0222891547349334	260	234	1.24308633178649e-09
IBD_HSIBD	-0.0259733718441898	260	245	1.66477908746617e-09
IBD_HSIBD	-0.0259733718441898	260	245	1.66477908746617e-09
IBD_HSIBD	-0.0115330715183719	260	186	2.01183678776989e-09
IBD_HSIBD	-0.0125895727727741	260	199	2.89606527104224e-09
IBD_HSIBD	-0.0125895727727741	260	199	2.89606527104224e-09
IBD_HSIBD	-0.0125895727727741	260	199	2.89606527104224e-09
IBD_HSIBD	-0.0130875461866174	260	199	3.04405900201149e-09
IBD_HSIBD	-0.00498490388632822	260	46	3.93075959190465e-09
IBD_HSIBD	-0.194521114436633	260	259	4.25326526635348e-09
IBD_HSIBD	-0.0116784567358779	260	222	1.78443278309711e-08
IBD_HSIBD	-0.0178744023884196	260	218	2.31060195725668e-08
IBD_HSIBD	-0.0236622501638504	260	253	2.49851290708144e-08
IBD_HSIBD	-0.0135090913518048	260	200	3.53355461614784e-08
IBD_HSIBD	-0.0474702610555685	260	229	1.06843514025956e-07
IBD_HSIBD	-0.00992982645169203	260	123	1.31231089261016e-07
IBD_HSIBD	-0.00992982645169203	260	123	1.31231089261016e-07
IBD_HSIBD	-0.0123125565423926	260	200	3.69907258763729e-07
IBD_HSIBD	-0.0489061120884719	260	258	1.16594304769072e-06
IBD_HSIBD	-0.00906039096502017	260	133	1.20311385961738e-06
IBD_HSIBD	-0.00906039096502017	260	133	1.20311385961738e-06
IBD_HSIBD	-0.00904042708369952	260	136	1.25308749283145e-06
IBD_HSIBD	-0.0422717390936979	260	229	1.29208130741508e-06
IBD_HSIBD	-0.0422717390936979	260	229	1.29208130741508e-06
IBD_HSIBD	-0.022591636963921	260	234	7.86058757072743e-06
IBD_HSIBD	-0.0111188045436107	260	229	1.6402306922579e-05

IBD_HSIBD	-0.00932588085160324	260	220	3.12451902081243e-05
IBD_HSIBD	-0.0194312675493053	260	155	4.30174454251918e-05
IBD_HSIBD	-0.00981539309422297	260	171	4.47367784690171e-05
IBD_HSIBD	-0.00625163202204601	260	108	0.000131969647582831
IBD_HSIBD	-0.00625163202204601	260	108	0.000131969647582831
IBD_HSIBD	-0.017933888452386	260	212	0.000243031315595808
IBD_HSIBD	-0.017933888452386	260	212	0.000243031315595808
IBD_HSIBD	0.0129779945632353	260	254	0.000499771862159514
IBD_HSIBD	0.00910434199342279	260	233	0.000663358970799846
IBD_HSIBD	-0.0160335891816572	260	249	0.000777114243478313
IBD_HSIBD	0.00763534829707084	260	206	0.00156015144570876
Ileumyes	-0.0286783549766237	260	225	2.06006761984234e-05
Ileumyes	-0.0133206112851762	260	212	2.57479927265557e-05
Ileumyes	-0.0133206112851762	260	212	2.57479927265557e-05
Ileumyes	-0.0552463620387837	260	257	2.94443439210748e-05
Ileumyes	-0.054479133666507	260	257	4.21688029523119e-05
Ileumyes	-0.0184555450760818	260	216	6.05844927810569e-05
Ileumyes	-0.00661343862781175	260	196	0.000295034300589521
Ileumyes	-0.00661343862781175	260	196	0.000295034300589521
Ileumyes	-0.00661343862781175	260	196	0.000295034300589521
Ileumyes	-0.00661343862781175	260	196	0.000295034300589521
Ileumyes	-0.0043730834725772	260	109	0.000337310925803892
Ileumyes	-0.0043730834725772	260	109	0.000337310925803892
Ileumyes	-0.0131408922301706	260	233	0.00037320283380451
Ileumyes	-0.0131408922301706	260	233	0.00037320283380451
Ileumyes	-0.0100768216026271	260	260	0.000796190403992625
Ileumyes	-0.0100768216026271	260	260	0.000796190403992625
Ileumyes	-0.0100768216026271	260	260	0.000796190403992625
Ileumyes	-0.0100768216026271	260	260	0.000796190403992625
Ileumyes	-0.0100768216026271	260	260	0.000796190403992625
Ileumyes	-0.010888450377313	260	259	0.000875486937998124
Ileumyes	-0.010888450377313	260	259	0.000875486937998124
Ileumyes	0.0376603207603003	260	259	0.00157129289222564
Ileumyes	-0.013186057302148	260	242	0.00166710175659545
Ileumyes	-0.013186057302148	260	242	0.00166710175659545
Ileumyes	-0.0301997348658218	260	257	0.00184799164077319
Ileumyes	-0.0804720561227035	260	259	0.00190310310633624
Ileumyes	-0.00363966844573751	260	141	0.00263429396897023
Ileumyes	-0.00363966844573751	260	141	0.00263429396897023
THIOPURINE_MTXyes	0.00427576477549041	260	121	2.15517652635777e-05
THIOPURINE_MTXyes	0.00409386500098469	260	120	5.10749343651968e-05

Q.value

0.0172412381876456  
0.0194712544783379  
0.0166412263911985  
0.0366616070301651  
0.0366616070301651  
0.0461934747787729  
0.00472934586295554  
0.0239070140708185  
0.0446871730232362  
1.60310108041563e-10  
1.60310108041563e-10  
6.69014180150217e-08  
7.782051451853e-08  
5.56136163666159e-07  
1.82992225015401e-06  
7.01633363559968e-06  
7.31910747175092e-06  
7.31910747175092e-06  
9.09550659727922e-06  
1.12533190412603e-05  
1.49247851256427e-05  
2.22744250627508e-05  
3.73321928929751e-05  
3.73321928929751e-05  
4.98731155443706e-05  
8.31925690829772e-05  
0.000124620852575065  
0.000128038668666754  
0.000243621398601956  
0.000259646534188309  
0.00027751805902526  
0.000353172878177754  
0.000503987957052513  
0.000563466758518075  
0.000594724829299589  
0.000594724829299589  
0.000636456275787333  
0.000636456275787333  
0.000636456275787333  
0.000748272442408108  
0.000932079241505232  
0.00102134649480129  
0.00104059990915382  
0.00148548233853565  
0.00148548233853565

0.00156492513883351  
0.00157476160618406  
0.00157476160618406  
0.00157476160618406  
0.00162929040624978  
0.00173586546729318  
0.00512920167205177  
0.00610791553946823  
0.0082733068551757  
0.00830292333236479  
0.00839813385615535  
0.00922073793542536  
0.00985928568154387  
0.00985928568154387  
0.00989563838578516  
0.00989563838578516  
0.00989563838578516  
0.0129233282073222  
0.0147415896958034  
0.0147415896958034  
0.0151058650053787  
0.0151368640932999  
0.0172021049360023  
0.0174533781698149  
0.0194712544783379  
0.0194712544783379  
0.0194712544783379  
0.0195870621813573  
0.0223126103541197  
0.0223126103541197  
0.0233286722987597  
0.0233286722987597  
0.0240532363466932  
0.027016931070771  
0.0313276520387488  
0.0314623452810996  
0.0314623452810996  
0.0355424170789934  
0.0355424170789934  
0.0355424170789934  
0.038053162726834  
0.0383655389624809  
0.0383655389624809  
2.57119666994313e-13  
2.57119666994313e-13  
2.57119666994313e-13

2.57119666994313e-13  
2.57119666994313e-13  
1.60310108041563e-10  
7.95014486502934e-10  
7.95014486502934e-10  
3.89197488659734e-09  
3.89197488659734e-09  
1.87458234664671e-08  
2.49211930290408e-08  
2.49211930290408e-08  
3.14807619476747e-08  
3.14807619476747e-08  
4.25569130937989e-08  
6.69014180150217e-08  
6.69014180150217e-08  
6.69014180150217e-08  
7.13386603342783e-08  
1.40142159656747e-07  
1.40142159656747e-07  
1.40142159656747e-07  
1.41623049942818e-07  
1.77021509633903e-07  
1.77021509633903e-07  
2.07024495257612e-07  
2.71719065136022e-07  
2.71719065136022e-07  
2.71719065136022e-07  
2.77444234754762e-07  
3.48308974949329e-07  
3.66700437828854e-07  
1.45957450719995e-06  
1.82992225015401e-06  
1.89768004133091e-06  
2.62140447104921e-06  
7.31910747175092e-06  
8.54341172944165e-06  
8.54341172944165e-06  
2.2264229348232e-05  
6.39655535363243e-05  
6.39655535363243e-05  
6.39655535363243e-05  
6.54244344548271e-05  
6.54244344548271e-05  
6.54244344548271e-05  
0.000353172878177754  
0.000662321001050975



0.00111991187375187  
0.00148548233853565  
0.00151819492889537  
0.00404791515181951  
0.00404791515181951  
0.0071125678600975  
0.0071125678600975  
0.0125533247266839  
0.0159106399763271  
0.0177611705506047  
0.0313276520387488  
0.000811310581147787  
0.000966307021149563  
0.000966307021149563  
0.00106735746713896  
0.00148548233853565  
0.00189475031344678  
0.0082733068551757  
0.0082733068551757  
0.0082733068551757  
0.0082733068551757  
0.00911882926537641  
0.00911882926537641  
0.00983898380030072  
0.00983898380030072  
0.0177611705506047  
0.0177611705506047  
0.0177611705506047  
0.0177611705506047  
0.0177611705506047  
0.0192607126359587  
0.0192607126359587  
0.0313276520387488  
0.0324271622167041  
0.0324271622167041  
0.0355424170789934  
0.0359224787527373  
0.0472100997809834  
0.0472100997809834  
0.000838416234034304  
0.00162929040624978

Variable

- 1 F\_R
- 2 F\_R
- 3 F\_R
- 4 F\_R
- 5 F\_R
- 6 F\_R
- 7 F\_R
- 8 F\_R
- 9 F\_R
- 10 F\_R
- 11 F\_R
- 12 F\_R
- 13 IBD\_HS
- 14 IBD\_HS
- 15 IBD\_HS
- 16 IBD\_HS
- 17 IBD\_HS
- 18 IBD\_HS
- 19 IBD\_HS

## Feature

k\_\_Fungi|p\_\_Ascomycota

k\_\_Fungi|p\_\_Basidiomycota

k\_\_Fungi|p\_\_Basidiomycota|c\_\_Incertaedis|o\_\_Malasseziales|f\_\_Incertaedis|g\_\_Malassezia|

k\_\_Fungi|p\_\_Ascomycota|c\_\_Saccharomycetes|o\_\_Saccharomycetales|f\_\_Saccharomycetaceae

k\_\_Fungi|p\_\_Ascomycota|c\_\_Saccharomycetes|o\_\_Saccharomycetales|f\_\_Saccharomycetaceae|g

k\_\_Fungi|p\_\_Ascomycota|c\_\_Saccharomycetes|o\_\_Saccharomycetales|f\_\_Saccharomycetaceae|g

k\_\_Fungi|p\_\_Basidiomycota|Other

k\_\_Fungi|p\_\_Basidiomycota|Other|Other

k\_\_Fungi|p\_\_Basidiomycota|Other|Other|Other

k\_\_Fungi|p\_\_Basidiomycota|Other|Other|Other|Other

k\_\_Fungi|p\_\_Ascomycota|c\_\_Saccharomycetes|o\_\_Saccharomycetales

k\_\_Fungi|p\_\_Ascomycota|c\_\_Saccharomycetes

k\_\_Fungi|p\_\_Ascomycota

k\_\_Fungi|p\_\_Basidiomycota

k\_\_Fungi|p\_\_Ascomycota|c\_\_Saccharomycetes|o\_\_Saccharomycetales

k\_\_Fungi|p\_\_Ascomycota|c\_\_Saccharomycetes

k\_\_Fungi|p\_\_Ascomycota|c\_\_Saccharomycetes|o\_\_Saccharomycetales|f\_\_Saccharomycetaceae

k\_\_Fungi|p\_\_Ascomycota|c\_\_Saccharomycetes|o\_\_Saccharomycetales|f\_\_Saccharomycetaceae|g

k\_\_Fungi|p\_\_Ascomycota|c\_\_Saccharomycetes|o\_\_Saccharomycetales|f\_\_Saccharomycetaceae|g

Value	Coefficient	N	N.not.0	P.value
F_Rremission	0.161015981772809	259	259	1.22323327385739e-06
F_Rremission	-0.157801103147122	259	230	1.49376860316499e-05
F_Rremission	0.034159689629057	259	88	0.000189630487589262
F_Rremission	0.176942074123673	259	229	0.000288275517799094
F_Rremission	0.174691133923712	259	229	0.00030509985140341
F_Rremission	0.174691133923712	259	229	0.00030509985140341
F_Rremission	-0.0103548925729985	259	63	0.000747695359971366
F_Rremission	-0.0103548925729985	259	63	0.000747695359971366
F_Rremission	-0.0103548925729985	259	63	0.000747695359971366
F_Rremission	-0.0103548925729985	259	63	0.000747695359971366
F_Rremission	0.125335372324228	259	259	0.000861312783128581
F_Rremission	0.124443892219203	259	259	0.000973772070770371
IBD_HSIBD	-0.241999980240929	259	259	4.18893527499225e-07
IBD_HSIBD	0.260402329379016	259	230	7.80527621143302e-07
IBD_HSIBD	-0.207798158022306	259	259	0.000129095561360952
IBD_HSIBD	-0.214486699722227	259	259	0.000162630464221362
IBD_HSIBD	-0.255059916729299	259	229	0.000509649835251042
IBD_HSIBD	-0.22844734654855	259	229	0.0010848949865301
IBD_HSIBD	-0.22844734654855	259	229	0.0010848949865301

Q.value

0.000327418772969161

0.00299874047085372

0.0217533259334539

0.0244995180676938

0.0244995180676938

0.0244995180676938

0.0400266249371338

0.0400266249371338

0.0400266249371338

0.0400266249371338

0.0432271353032656

0.0458510881149298

0.000313381839889036

0.000313381839889036

0.0207327471545689

0.0217533259334539

0.0372044379733261

0.0458510881149298

0.0458510881149298