## Supplementary Data

# Comparative immunophenotyping of *Saccharomyces cerevisiae* and *Candida spp.* strains from Crohn's disease patients and their interactions with the gut microbiome

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## Supplementary Text

### Clinical data of IBD patients

By using calprotectin, we scored 18 patients for the presence of clear mucosal inflammation (high levels of calprotectin) and 16 with mucosal healing (low levels of calprotectin). These numbers changed when clinical scoring indexes such as PCDAI were used, resulting 14 CD patients in active disease and 20 CD in remission. For UC, 2 out of the 4 patients with active disease had a clear mucosal inflammation (high levels of fecal calprotectin), while in 10 out of 23 UC in clinical remission displayed no mucosal inflammation (low levels of calprotectin).

### Mice infection and cytokine detection.

Fungal growth was higher with the SK1 isolate, lower with the BB1533 and BT2240 isolates and restrained with the YUC22, YH3 and YP4 isolates. The mice eventually cleared the infection, as no yeasts could be recovered a week after infection.

To define the functional role, if any, of these cytokines in yeast colonization and/or infection, we comparatively evaluated parameters of inflammatory pathology in the colon of C57BL/6 wild- type (WT) or cytokine-deficient mice inoculated with the different isolates.

The histology analysis on tissues of mice infected with different *S. cerevisiae* strains revealed that, in contrast with infection with *C. albicans*, acanthosis and hyperkeratosis of the stratified squamous epithelium were rarely observed. Colonization with *Saccharomyces* isolates was instead associated with

inflammatory influx of the mucosa, particularly with the YUC22, YP4, YH3, BT2240 and BB1533 isolates. Interestingly, inflammation and hypertrophy of the colon mucosa were drastically reduced in IL17a-/- mice inoculated with the IL-17A-producing isolates YUC22, YH3 and YP4 and, similarly, in IFNg-/- mice inoculated with the IFN-γ-producing isolates BB1533 and BT2240. As expected, increased inflammation was observed in IL10-/- mice, as opposed to WT mice, inoculated with the IL-10-producing SK1.

## Supplementary Tables

**Table S1**: List of *S. cerevisiae* and *Candida spp.* strains used in this study. For each strain, we report here the source of isolation and the assays in which the strain was tested. CD=Crohn's disease; UC= Ulcerative colitis; HC= Healthy Children

| | | | ***In vitro* Immunological tests** | | | |
|---|---|---|---|---|---|---|
| **Strain ID** | **Yeast species** | **Origin** | **healthy donors' PBMCs** | **CD patients' PBMCs** | **healthy donors' DCs** | **cell wall sugar component** |
| YA5 | *S. cerevisiae* | CD-feces | x | x | x | x |
| YA8 | *S. cerevisiae* | CD-feces | x | | x | |
| YB7 | *S. cerevisiae* | CD-feces | x | | x | x |
| YB8 | *S. cerevisiae* | CD-feces | x | x | x | x |
| YD1 | *S. cerevisiae* | CD-feces | x | x | x | x |
| YE5 | *S. cerevisiae* | CD-feces | x | x | x | |
| YH1 | *S. cerevisiae* | CD-feces | x | x | x | x |
| YH2 | *S. cerevisiae* | CD-feces | | | x | |

| | | | | | | |
|---|---|---|---|---|---|---|
| YH3 | *S. cerevisiae* | CD-feces | | | x | |
| YP1 | *S. cerevisiae* | CD-feces | x | x | x | |
| YP4 | *S. cerevisiae* | CD-feces | x | | x | x |
| YUC22 | *S. cerevisiae* | UC- feces | x | x | x | x |
| Y13EU | *S. cerevisiae* | HC-feces | x | x | x | x |
| YN19 | *S. cerevisiae* | HC-feces | | | x | |
| SG60 | *S. cerevisiae* | Grape | | | x | |
| SGU165 | *S. cerevisiae* | Grape | | | x | x |
| SGU421 | *S. cerevisiae* | Grape | x | | x | x |
| SGU52 | *S. cerevisiae* | Grape | x | | x | |
| BB1533 | *S. cerevisiae* | Natural | x | | x | x |
| BB2148 | *S. cerevisiae* | Natural | | | x | |
| BT2240 | *S. cerevisiae* | Natural | x | | x | x |
| BY4741 | *S. cerevisiae* | Laboratory | | | x | |
| SK1 | *S. cerevisiae* | Laboratory-Rotting fig | x | x | x | x |
| YD11 | *C. albicans* | CD-feces | | | x | |
| YL1 | *C. albicans* | CD-feces | | | x | x |
| YM1 | *C. albicans* | CD-feces | | | x | |
| YQ2 | *C. albicans* | CD-feces | x | | x | x |
| YR1 | *C. albicans* | CD-feces | x | | x | |

| | | | | | | |
|---|---|---|---|---|---|---|
| YN1 | *C. albicans* | HC-feces | x | | x | |
| YN22 | *C. albicans* | HC-feces | | | x | |
| YN7 | *C. albicans* | HC-feces | | | x | |
| SC5314 | *C. albicans* | laboratory | x | | x | x |
| YUC14 | *C. albicans* | UC- feces | | | x | |
| YUC17 | *C. albicans* | UC- feces | | | x | |
| YUC26 | *C. albicans* | UC- feces | | | x | |
| YA4 | *C. parapsilosis* | CD-feces | x | | x | x |
| YB1 | *C. parapsilosis* | CD-feces | x | | x | x |
| YB3 | *C. parapsilosis* | CD-feces | | | x | |
| YB4 | *C. parapsilosis* | CD-feces | | | x | |
| YV1 | *C. parapsilosis* | CD-feces | | | x | |
| YUC23 | *C. glabrata* | UC- feces | x | | x | |

**Table S3.** *S. cerevisiae* strains and clinical features of the IBD and healthy children from whose fecal samples the strains were isolated. CD=Crohn's disease; UC= Ulcerative colitis.

| ***S. cerevisiae* strain** | **Subject** | **ASCA** | **Inflammation indexes** | | | **Intestinal mucosa s tatus (by calprotectin dosage)** | **Clinical status** |
|---|---|---|---|---|---|---|---|
| | | | **PCDAI** | **PUCAI** | **calprotectin** | | |
| | | | **<10 CD remission** | **<10 UC remission** | **<100 no inflammation** | | |
| **YA5** | A CD patient | + | 12.5 | | 1100 | Inflammation | Active |
| **YB8** | B CD patient | - | 15 | | 1300 | Inflammation | Active |
| **YP1** | P CD patient | + | 0 | | 280 | Inflammation | Remission |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **YD1** | D CD patient | - | 12.5 | | 2600 | Inflammation | Active |
| **YE5** | E CD patient | - | 0 | | 80 | no inflammation | Remission |
| **YH1** | H CD patient | + | 5 | | 600 | Inflammation | Remission |
| **YUC22** | 22 UC patient | - | | 0 | 27 | no inflammation | Remission |
| **Y13EU** | 13 healthy child | ND | NA | NA | NA | NA | Healthy |

## Supplementary Figures

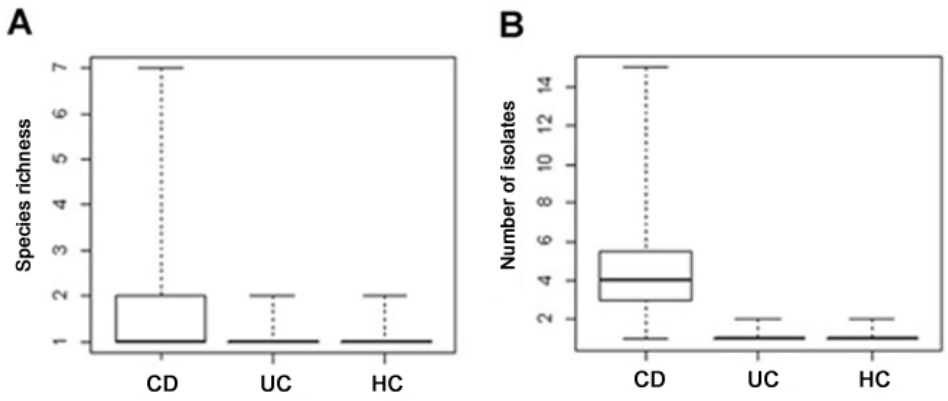


**Figure S1.** Richness and biodiversity of cultivable mycobiota. Comparison of species richness (A) and number of isolates (B) among IBD and HC fecal mycobiota for the most abundant isolated species. CD= Crohn's disease patients; UC= ulcerative colitis patients; HC= healthy children. Kruskal-Wallis test: Species richness, p= 0.004; number of isolates p= 9.267x10-8.

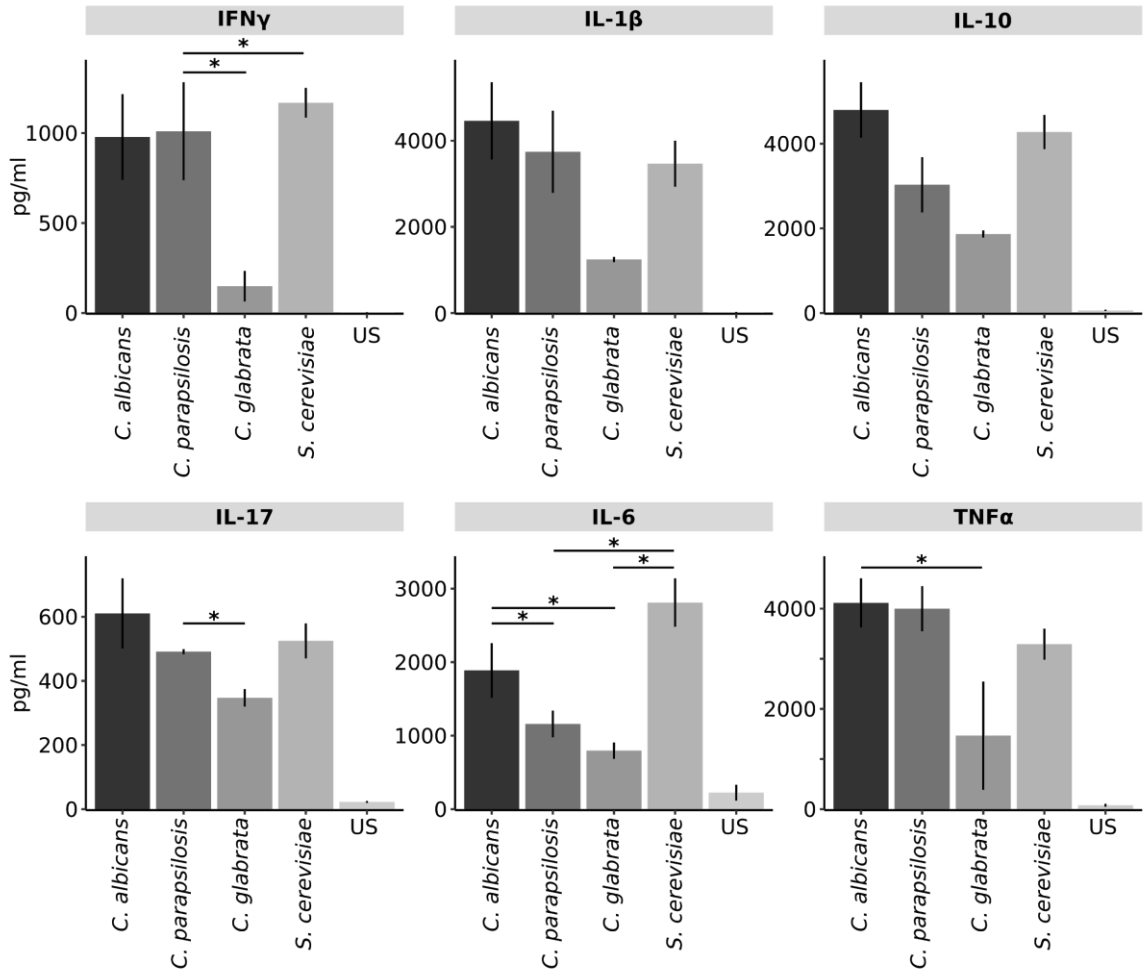


**Figure S2.** PBMCs immune response to *S. cerevisiae* and *Candida* spp. species. PBMCs from healthy donors (n=6) were stimulated with cells for 24 hours or 5 days and cultures supernatants used for IL-6, IL-1β, TNF-α, IFN-γ, IL-17A and IL-10 measurements. Average of cytokine levels for each tested fungal species are represented, error bars show the standard error. *p<0.05, **p<0.001, ***p<0.0001 by Wilcoxon test.

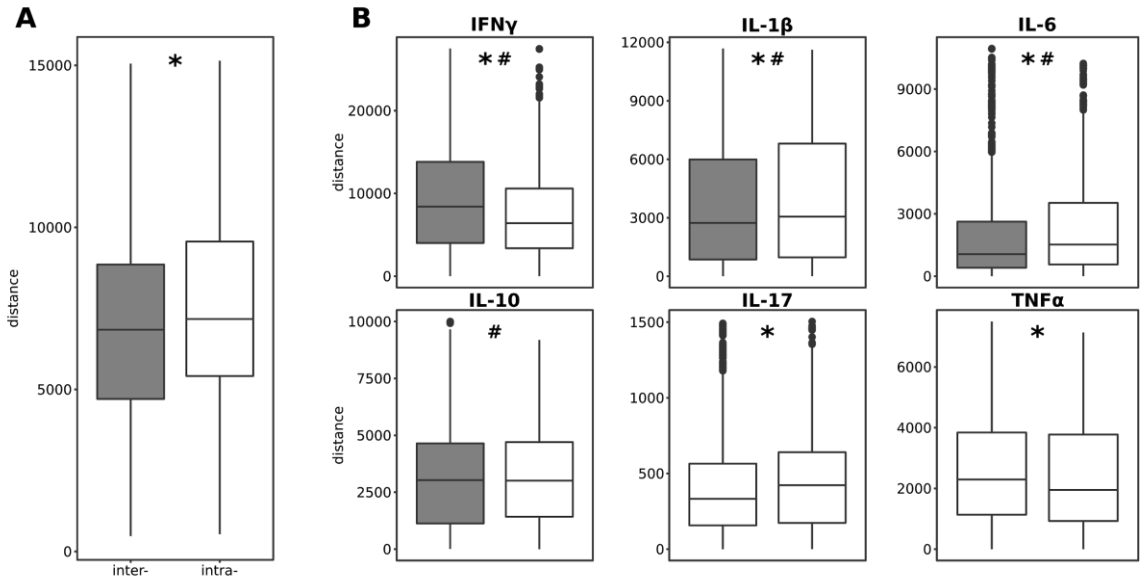


**Figure S3.** Comparison of inter- and intra- species PBMCs immune response to *S. cerevisiae* and *Candida* spp. isolates. PBMCs from healthy donors (n=6) were stimulated with cells for 24 hours or 5 days and cultures supernatants used for IL-6, IL-1β, TNF-α, IFN-γ, IL-17A and IL-10 measurements. Distances were calculated among strains-induced cytokine release by PBMCs, either considering all the cytokines (A) or for each cytokine separately (B). Wilcoxon (* p-value<0.05) and Levene (# p-value<0.05) tests were calculated on distances grouped as 'inter-species' (indicated as 'inter', among strains of different species) or 'intra-species' (indicated as 'intra', among strains of the same species).

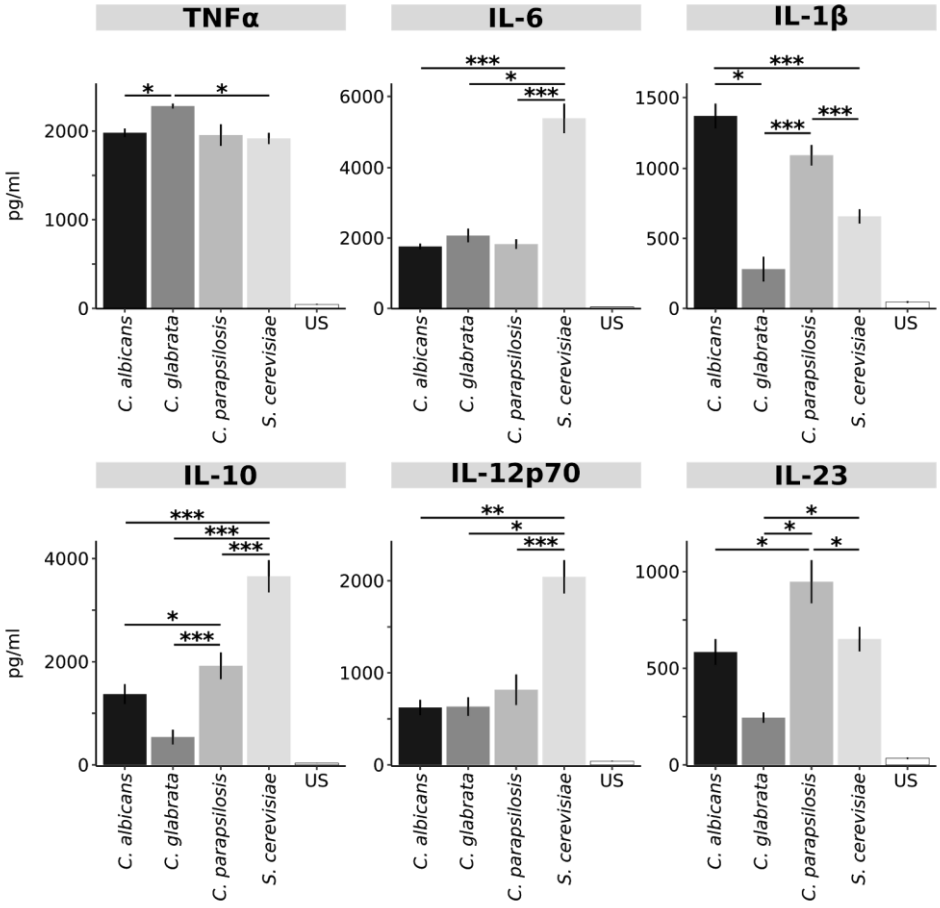


**Figure S4.** Dendritic cells immune response to *S. cerevisiae* and *Candida* spp. isolates. DCs from healthy donors (n=6) were stimulated with fungal cells for 24 hours and culture supernatants used for TNF-α, IL-10, IL-1β, IL-6, IL-12p70 and IL-23 quantification. Averages of cytokine levels for each tested fungal species are reported, error bars indicate the standard errors *p<0.05; **p<0.001; ***p<0.0001 by Wilcoxon fdr corrected. Every treatment with fungi induced a significantly different level of cytokine compared to the US control.

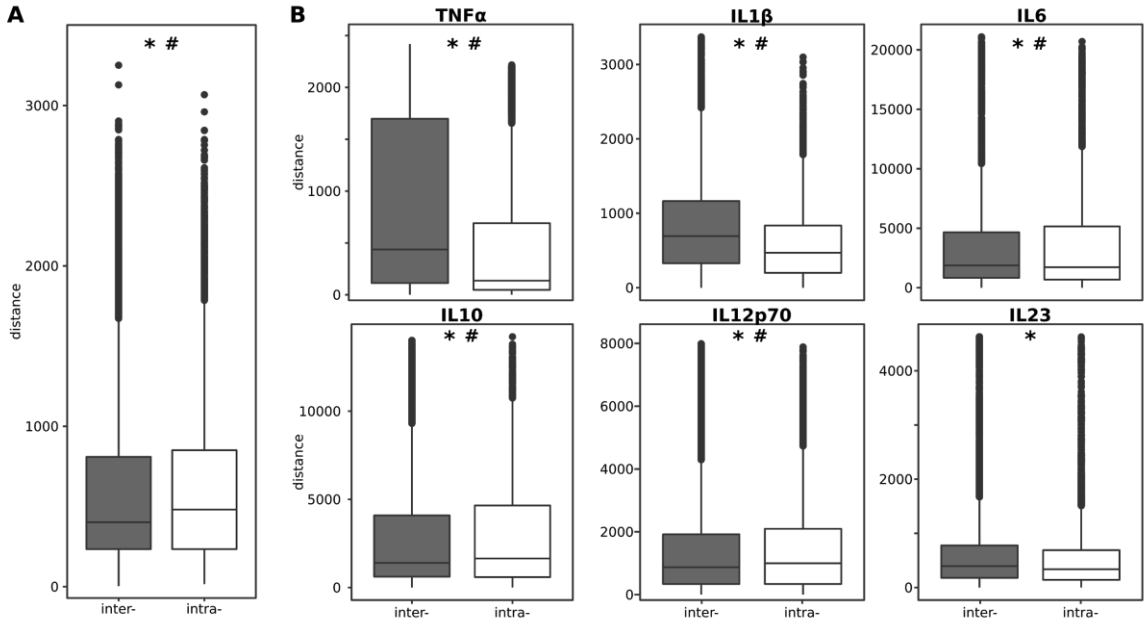


**Figure S5.** Comparison of inter- and intra- species DCs immune response to *S. cerevisiae* and *Candida* spp. isolates. DCs from healthy donors (n=6) were stimulated with fungal cells for 24 hours and culture supernatants used for TNF-α, IL-10, IL-1β, IL-6, IL-12p70 and IL-23 quantification. Distances were calculated among strains-induced cytokine release by DCs, either considering all the cytokines (A) or for each cytokine separately (B). Wilcoxon (* p-value<0.05) and Levene (# p-value<0.05) tests were calculated on distances grouped as 'inter-species' (indicated as 'inter', among strains of different species) or 'intra-species' (indicated as 'intra', among strains of the same species).

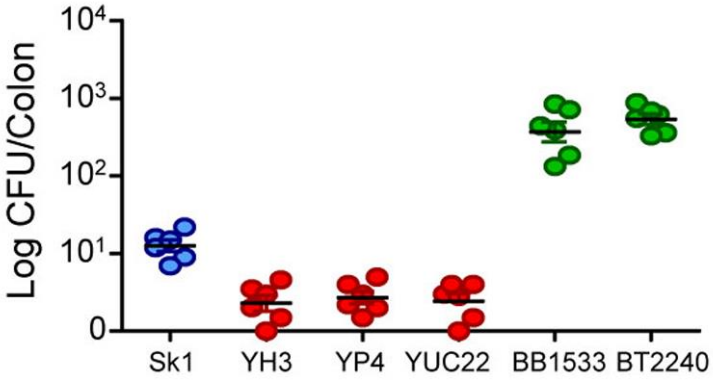


**Figure S6.** Fungal growth (measured as log CFU) in the colon of C57BL/6 mice infected with selected *S. cerevisiae* strains isolated from CD (red) and natural sources (green), in comparison with SK1 reference strain (blue).

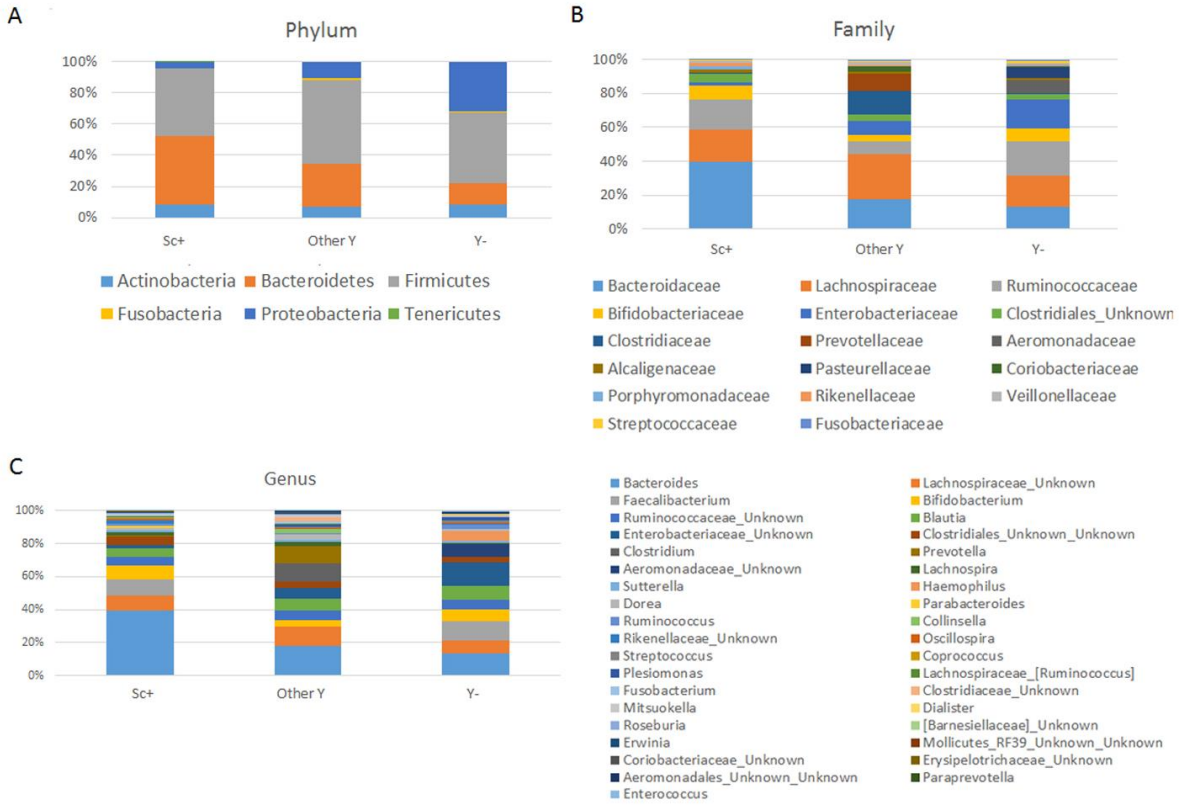


**Figure S7.** Stacked bar-plot representation of the relative abundances at (A) phylum, (B) family and (C) genus level of the fecal microbiota of CD patients from metagenomics analysis distributed according to presence/absence of fungal isolates (Other Y= *Candida spp*. isolates; Sc+= S. cerevisiae; Y-= no fungal isolates) in fecal samples.

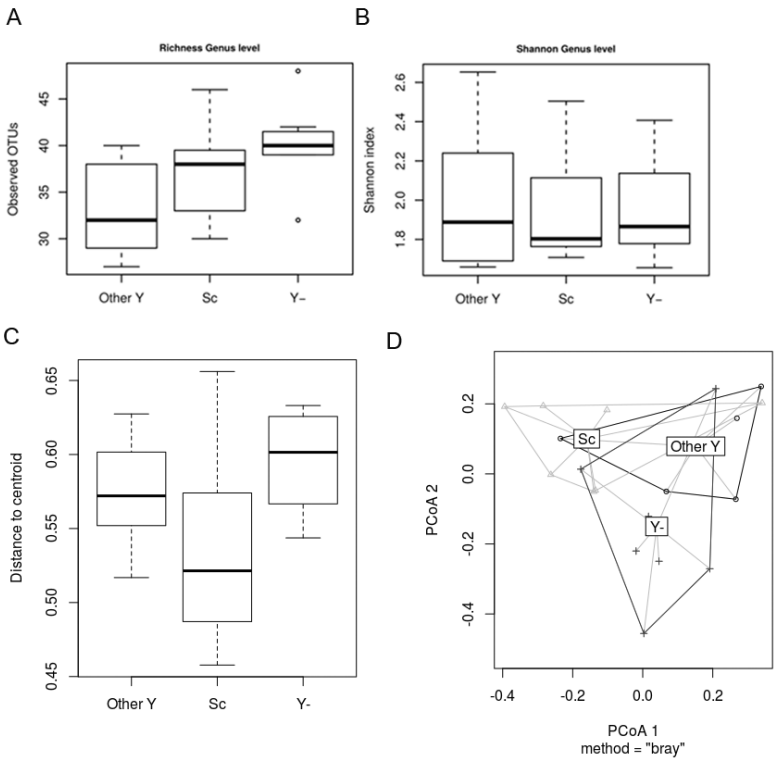


**Figure S8.** Alpha and beta diversity of gut bacterial community. (A-B) Alpha diversity of gut microbiota in presence/absence of fungal isolates. Box plots of species richness measured as number of observed OTUs and Shannon index at genus level, considering presence/absence of fungal isolates in fecal samples (Other Y= *Candida* spp.; Sc=*S. cerevisiae*; Y- = absence of fungi). (C-D) Beta dispersion analysis. (C) Distances from centroids calculated for each group of samples. (E) Principal Coordinate Analysis (PCoA) calculated on Bray-Curtis distances considering presence/absence of fungal isolates in fecal samples (Other Y= *Candida* spp.; Sc= *S. cerevisiae*; Y- = absence of fungi). Networks represent distances of each point from centroid.

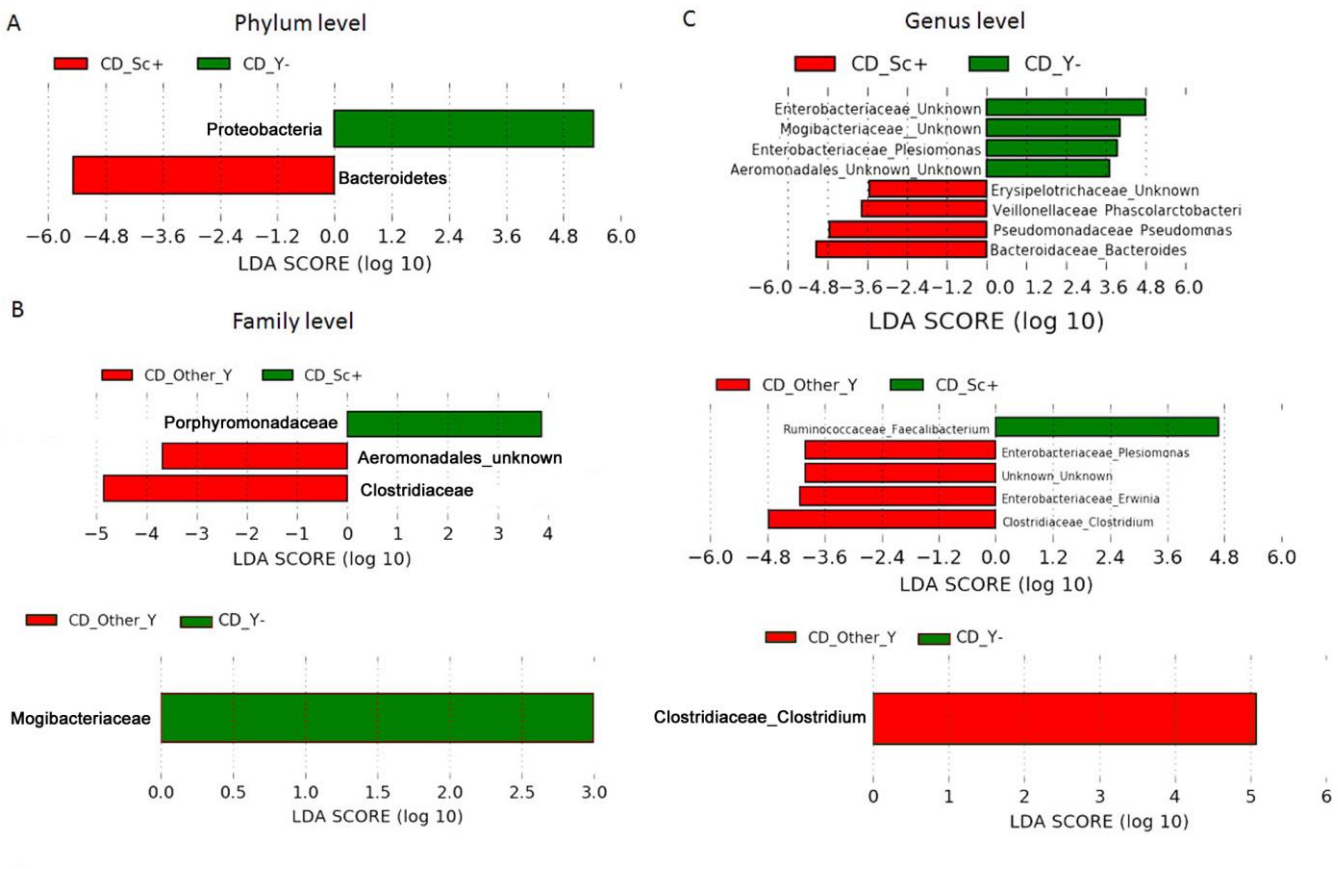


**Figure S9.** Differences in bacterial taxa in gut microbial communities of fecal samples related to the presence/absence of cultivable fungi. LEfSe analysis shows a statistically significant enrichment of bacterial (A) phylum, (B) family and (C) genera associated to the presence of *S. cerevisiae* (CD_Sc+) or other fungi (CD_Other_Y), and absence of fungi (CD_Y-) in CD patients' faecal samples. LEfSe results indicate a sequentially significant ranking among groups (Alpha value=0.05 for the factorial Kruskal-Wallis test among classes). The threshold for the logarithmic LDA score was 2.0.