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 (CCGTCAATTCMTTTGAGTTT). 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 $MgSO_4$, 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™ 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_2 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.

Koljalg, 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.