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1 **Intestinal dysbiosis in Inflammatory Bowel Disease associated with primary**
2 **immunodeficiency.**

3 Harry Sokol, MD, PhD^{1,2,3,4}, Nizar Mahlaoui, MD^{4,5,17}, Claire Aguilar, MD, PhD^{6,8,17}, Perrine
4 Bach, MSc⁴, Olivier Join-Lambert, MD, PhD⁷, Aurélie Garraffo⁶, Philippe Seksik, MD, PhD
5 ^{1,3}, François Danion, MD,⁶ Sarah Jegou, MSc¹, Marjolene Straube, MSc², Christelle Lenoir,
6 MSc,⁸ Bénédicte Neven, MD, PhD^{5,8,9}, Despina Moshous^{5,8,9} MD, PhD, Stéphane Blanche,
7 MD, PhD^{5,9}, Bénédicte Pigneur, MD, PhD^{8,10}, Olivier Goulet, MD, PhD^{8,10}, Frank Ruemmele,
8 MD, PhD^{8,10}, Felipe Suarez, MD, PhD^{4,8,12}, Laurent Beaugerie, MD, PhD³, Stéphanie Pannier,
9 MD, PhD^{11,13}, Françoise Mazingue, MD, PhD¹⁴, Olivier Lortholary, MD, PhD^{4,6,9}, Lionel
10 Galicier, MD, PhD¹⁵, Capucine Picard, MD, PhD^{8,9,11,4}, Geneviève de Saint Basile, MD, PhD
11 ^{8,9,11}, Sylvain Latour, PhD^{8,9}, Alain Fischer, MD, PhD^{4,5,8,9,16}

12

13 ¹ Sorbonne Universités, UPMC Univ. Paris 06, École normale supérieure, PSL Research
14 University, CNRS, INSERM, APHP, Laboratoire des Biomolécules (LBM), 27 rue de
15 Chaligny, 75005 Paris, France

16 ² INRA, UMR1319 Micalis & AgroParisTech, Jouy en Josas, France

17 ³ Department of Gastroenterology, Saint Antoine Hospital, AP-HP, UPMC Univ Paris 06, Paris,
18 France

19 ⁴ French National Reference Center for Primary Immune Deficiency (CEREDIH), Necker
20 Enfants Malades University Hospital, Assistance Publique-Hôpitaux de Paris (APHP), Paris,
21 France

22 ⁵ Paediatric Haematology-Immunology and Rheumatology Department, Hôpital Necker-
23 Enfants Malades, Assistance Publique-Hôpitaux de Paris (AP-HP), Paris, France.

24 ⁶ Paris Descartes University, Infectious Diseases and Tropical Medicine Department Necker-
25 Pasteur Infectious Diseases Center, Necker-Enfants Malades Hospital AP-HP, Paris, France.

26 ⁷ Paris Descartes University, Microbiology Department, Necker-Enfants Malades Hospital AP-
27 HP, Paris, France

28 ⁸ INSERM UMR 1163, Paris, France, Imagine Institute, Paris, France.

29 ⁹ Paris Descartes University, Sorbonne Paris Cité, Imagine Institute, Paris, France.

30 ¹⁰ Paris Descartes University, Paediatric Gastroenterology Hepatology and Nutrition
31 Department, Hôpital Necker-Enfants Malades, AP-HP, Paris, France

32 ¹¹ Study Center for Primary Immunodeficiencies, Necker-Enfants Malades University Hospital,
33 Assistance Publique Hôpitaux de Paris (APHP), Paris, France.

34 ¹²Paris Descartes University, Hematology Department, Necker-Enfants Malades University
35 Hospital, Assistance Publique Hôpitaux de Paris (APHP), Paris, France.

36 ¹³ Department of Orthopedic Pediatrics, Necker-Enfants Malades University Hospital,
37 Assistance Publique Hôpitaux de Paris (APHP), Paris, France.

38 ¹⁴ Department of Pediatrics, Hôpital Jeanne de Flandre, University Hospital of Lille, Lille,
39 France.

40 ¹⁵ Department of Clinical Immunology, Saint-Louis Hospital, Assistance Publique Hôpitaux de
41 Paris (APHP), Paris, France.

42 ¹⁶Collège de France, Paris, France.

43 ¹⁷These authors contributed equally to this work.

44

45 Corresponding authors :

46 Professor Alain Fischer

47 Institut Imagine

48 24 Bld Montparnasse, 75015, Paris, France.

49 alain.fischer@aphp.fr

50

51 Professor Harry Sokol

52 Gastroenterology Department, Hôpital Saint-Antoine

53 184 rue du Faubourg Saint-Antoine, 75571 Paris CEDEX 12, France

54 harry.sokol@aphp.fr

55

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59 **DECLARATION OF INTERESTS**

60 The authors declare no competing financial interests.

61

62

63 **CAPSULE SUMMARY:** The gut microbiota plays a key role in host physiology and is an
64 actor in inflammatory bowel disease pathogenesis. Patients with primary immunodeficiency
65 causing intestinal inflammation have disease-specific dysbiosis.

66

67 **Keywords:** gut microbiota; inflammatory bowel disease; primary immunodeficiency

68

69 *To the Editor:*

70 The gut microbiota is composed of a huge amount and diversity of microorganisms playing
71 major roles in physiological and pathological settings such as inflammatory bowel disease
72 (IBD). The triggering role of the gut microbiota on intestinal inflammation is known and an
73 intestinal dysbiosis (i.e. an imbalance in the microbiota composition and function) has been
74 pointed out in IBD 15 years ago. Moreover, a positive efficacy signal has been observed in
75 studies evaluating fecal microbiota transplantation in IBD demonstrating that the gut microbiota
76 alterations is an actor in the inflammatory process and not simple consequence¹.

77 Environmental factors have an important effect on gut microbiota composition, but host genetic
78 has an impact too. This effect has been demonstrated in knockout mouse for innate immunity
79 genes. Microbiome genome-wide association studies have identified several associations
80 between genetic polymorphisms and the gut microbiota composition offering some clues for
81 the effects of genes on microbiota composition in humans². However, the effect of major
82 specific disease-causing genetic defects on gut microbiota has never been studied in humans
83 yet.

84 Here we characterized the fecal microbiota composition of patients with three types of rare
85 primary immunodeficiency (PID) causing IBD conditions, chronic granulomatous disease
86 (CGD, 11 samples), X-linked inhibitor of apoptosis (XIAP, 7 samples) deficiency and partial
87 Tetratricopeptide Repeat Domain 7A (TTC7A, 7 samples) deficiency, in comparison to patients
88 with non-genetic determined IBD (18 samples) and healthy subjects (HS, 23 samples, Suppl
89 Table 1).

90

91 The microbiota composition was assessed by 16S sequencing. As seen previously, gut
92 microbiota composition was influenced by age, antifungal and antibiotic treatment³ (Suppl

93 Figure 1). Beta diversity analysis showed a remarkable clustering of samples according to
94 disease phenotypes (Figure 1A). The difference with HS was statistically significant for CGD
95 and TTC7A groups while it did not reach significance for XIAP (Figure 1 A-D). Interestingly
96 the difference with IBD patients was statistically significant for all three PID patients groups
97 (Figure 1A). Age was very heterogeneous in patients from TTC7A and XIAP groups (0.62 to
98 18 and 1.3 to 34 years respectively). The gut microbiota composition changes constantly
99 between birth and the age of 3 years when it reaches an adult-like configuration⁴. We thus
100 performed the same analysis after segregating the patients according to their age (younger or
101 older than 3 years). The difference with HS was maintained for TTC7A patients in both age
102 groups and reached significance in XIAP patients older than 3 (only 2 XIAP patients younger
103 than 3) (Suppl Figure 2).

104

105 Patients with CGD, XIAP and TTC7A deficiency can experience intestinal inflammation
106 mimicking IBD⁵. In our study population, samples were taken from patients with (PID_{IBD}) or
107 without (PID_{no-IBD}) IBD involvement. In addition, PID patients could be in active phase (flare)
108 or remission of IBD (PID, IBD flare/inactive). Microbiota from patients with active intestinal
109 inflammation (PID_{IBD (flare)}) was significantly different from the one of PID_{no-IBD} patients
110 (Figure 1E). We observed a significant diversity reduction in patients with PID_{IBD} compared to
111 HS (Figure 1F, Suppl Figure 3A). Moreover, this reduction was even stronger in patients with
112 active intestinal inflammation (Figure 1G, Suppl Figure 3B). These effects were seen in the
113 three-studied PID although the numbers were too low to reach statistical significance when
114 analyzing each PID independently (Suppl Figure 4).

115 In accordance with the published literature, the bacterial microbiota of all groups was
116 dominated by bacteria from Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria
117 phyla. Differences were noticed between PID patients and HS with notably a dramatic increase

118 in Proteobacteria from the Enterobacteriaceae family in the TTC7A group and an increase
119 proportion in bacteria from the Bacteroidetes phylum and from the Clostridiaceae family in the
120 CGD and XIAP groups respectively (Figure 1 H, Suppl Figure 3C). These alterations were
121 found both in patients younger and older than 3 years (Suppl Figure 4 B, D) and were clearly
122 stronger in patients with IBD and PID_{IBD} with active inflammation (Figure 1I; Suppl Figure 4
123 C, E). We then looked for the microbial features associated with the three studied PID and used
124 a multivariate association test (MaAsLin) to control for potential confounding factors such as
125 age, gender, smoking, intestinal inflammation and treatment. We observed several alterations
126 specific to each PID compared to HS. Patients with TTC7A deficiency exhibited an increased
127 abundance of Proteobacteria involving several components of the Gammaproteobacteria and
128 Epsilonproteobacteria classes (Figure 2A). On the other hand, bacteria from the
129 Ruminococcaceae family and notably the *Oscillospira* genera were decreased. Patients with
130 XIAP deficiency exhibited an increased abundance of several bacterial taxa including members
131 of the Proteobacteria, Firmicutes, Actinobacteria and Fusobacteria phyla (Figure 2B). Four of
132 these taxa (*Scardovia*, *Fusobacterium*, *Rothia dentocariosa*, *Veillonella*) are not usually found
133 in the gut but are known members of the oral microbiota that are implicated in dental caries,
134 but also IBD, colorectal cancer, and liver diseases⁶. Interestingly, an increased abundance of
135 oral bacteria in the gut has been observed in several intestinal and extra-intestinal diseases⁷.
136 Moreover, a recent study showed that some members of the oral microbiota can have pro-
137 inflammatory effects when colonizing the gut⁷ suggesting a possible direct effect of these oral
138 bacteria in the intestinal inflammatory phenotype observed in patients with XIAP deficiency.
139 Another important specificity in XIAP patients was the presence of *Lactococcus garvieae*
140 which is a highly virulent pathogen affecting saltwater fish⁸ and is rarely involved in human
141 infection. *L. garvieae* was found at a high level (0.23-0.5% of all reads) in XIAP patients with
142 active intestinal inflammation but not in all other patients in this study. Patients with CGD

143 exhibited an increased abundance of *Ruminococcus gnavus* that has also been associated with
144 ileal Crohn's disease⁹ (Figure 2C).

145

146 In summary, we showed that the gut microbiota of patients with CGD, TTC7A and XIAP
147 deficiency have distinct alterations suggesting a primary defect in host immune system as a
148 basis of dysbiosis. Although it remains to be experimentally documented, the microbial
149 alterations induced by the host genetic defect might play a role in some aspects of the PID
150 phenotype and particularly intestinal involvement. The number of subjects studied was low
151 because of the extreme rarity of the studied diseases. However, independent and ideally larger
152 and longitudinal studies are required to confirm our findings. Controlling for potential
153 confounding factors such as diet, treatment, age, delivery mode, socio-economic features,
154 gastrointestinal symptoms, is particularly difficult in studies aiming at discriminating genetic
155 factors from environmental and inflammation influences. Similarly, identification of ideal
156 controls is not trivial. Although non-affected healthy siblings sharing similar lifestyles would
157 be attractive, there are obvious limitations in term of feasibility.

158 Finally, if these observations are confirmed, the alteration in gut microbiota composition might
159 have clinical interest as diagnosis biomarkers.

160

161 Harry Sokol^{1,2,3,4}, Nizar Mahlaoui^{4,5,17}, Claire Aguilar^{6,8,17}, Perrine Bach⁴, Olivier Join-
162 Lambert⁷, Aurélie Garraffo⁶, Philippe Seksik^{1,3}, François Danion⁶, Sarah Jegou¹, Marjolene
163 Straube², Christelle Lenoir⁸, Bénédicte Neven^{5,8,9}, Despina Moshous^{5,8,9}, Stéphane Blanche^{5,9},
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167 Fischer^{4,5,8,9,16}

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171 Chaligny, 75005 Paris, France

172 ² INRA, UMR1319 Micalis & AgroParisTech, Jouy en Josas, France

173 ³ Department of Gastroenterology, Saint Antoine Hospital, AP-HP, UPMC Univ Paris 06, Paris,
174 France

175 ⁴ French National Reference Center for Primary Immune Deficiency (CEREDIH), Necker
176 Enfants Malades University Hospital, Assistance Publique-Hôpitaux de Paris (APHP), Paris,
177 France

178 ⁵ Paediatric Haematology-Immunology and Rheumatology Department, Hôpital Necker-
179 Enfants Malades, Assistance Publique-Hôpitaux de Paris (AP-HP), Paris, France.

180 ⁶ Paris Descartes University, Infectious Diseases and Tropical Medicine Department Necker-
181 Pasteur Infectious Diseases Center, Necker-Enfants Malades Hospital AP-HP, Paris, France.

182 ⁷ Paris Descartes University, Microbiology Department, Necker-Enfants Malades Hospital AP-
183 HP, Paris, France

184 ⁸ INSERM UMR 1163, Paris, France, Imagine Institute, Paris, France.

185 ⁹ Paris Descartes University, Sorbonne Paris Cité, Imagine Institute, Paris, France.

186 ¹⁰ Paris Descartes University, Paediatric Gastroenterology Hepatology and Nutrition
187 Department, Hôpital Necker-Enfants Malades, AP-HP, Paris, France

188 ¹¹ Study Center for Primary Immunodeficiencies, Necker-Enfants Malades University Hospital,
189 Assistance Publique Hôpitaux de Paris (APHP), Paris, France.

190 ¹²Paris Descartes University, Hematology Department, Necker-Enfants Malades University
191 Hospital, Assistance Publique Hôpitaux de Paris (APHP), Paris, France.

192 ¹³ Department of Orthopedic Pediatrics, Necker-Enfants Malades University Hospital,
193 Assistance Publique Hôpitaux de Paris (APHP), Paris, France.

194 ¹⁴ Department of Pediatrics, Hôpital Jeanne de Flandre, University Hospital of Lille, Lille,
195 France.

196 ¹⁵ Department of Clinical Immunology, Saint-Louis Hospital, Assistance Publique Hôpitaux de
197 Paris (APHP), Paris, France.

198 ¹⁶Collège de France, Paris, France.

199 ¹⁷These authors contributed equally to this work.

200

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204 **AUTHOR CONTRIBUTION**

205 Recruited patients with PID: Alain Fischer, Nizar Mahlaoui, Perrine Bach, Bénédicte Neven,
206 Despina Moshous, Stéphane Blanche, Françoise Mazingue, Lionel Galicier, Claire Aguilar,
207 Aurélie Garraffo, François Danion, Olivier Lortholary, Felipe Suarez.

208 Recruited patients with IBD: Harry Sokol, Laurent Beaugerie, Philippe Seksik, Olivier Goulet,
209 Frank Ruemmele, Benedicte Pigneur.

210 Recruited controls: Stéphanie Pannier, Perrine Bach, Francois Danion

211 Genetic diagnosis of patients with PID: Geneviève de Saint Basile, Capucine Picard, Sylvain
212 Latour, Christelle Lenoir

213 Original study developed by Sylvain Latour, Claire Aguilar and Alain Fischer

214 Analysis of the data and manuscript writing: Harry Sokol, Alain Fischer, Nizar Mahlaoui.

215

216

217 **REFERENCES**

- 218 1. Pigneur B, Sokol H. Fecal microbiota transplantation in inflammatory bowel disease: the
219 quest for the holy grail. *Mucosal Immunol.* 2016;9:1360–5.
- 220 2. Hall AB, Tolonen AC, Xavier RJ. Human genetic variation and the gut microbiome in
221 disease. *Nat Rev Genet.* 2017;18:690–9.
- 222 3. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, et al. Dysfunction
223 of the intestinal microbiome in inflammatory bowel disease and treatment. *Genome Biol.*
224 2012;13:R79.

- 225 4. Tamburini S, Shen N, Wu HC, Clemente JC. The microbiome in early life: implications
226 for health outcomes. *Nat Med.* 2016;22:713–22.
- 227 5. Uhlig HH. Monogenic diseases associated with intestinal inflammation: implications for
228 the understanding of inflammatory bowel disease. *Gut.* 2013;62:1795–805.
- 229 6. Kummen M, Holm K, Anmarkrud JA, Nygård S, Vesterhus M, Høivik ML, et al. The
230 gut microbial profile in patients with primary sclerosing cholangitis is distinct from
231 patients with ulcerative colitis without biliary disease and healthy controls. *Gut.*
232 2017;66:611–9.
- 233 7. Atarashi K, Suda W, Luo C, Kawaguchi T, Motoo I, Narushima S, et al. Ectopic
234 colonization of oral bacteria in the intestine drives TH1 cell induction and inflammation.
235 *Science.* 2017;358:359–365.
- 236 8. Vendrell D, Balcázar JL, Ruiz-Zarzuola I, de Blas I, Gironés O, Múzquiz JL.
237 *Lactococcus garvieae* in fish: a review. *Comp Immunol Microbiol Infect Dis.*
238 2006;29:177–98.
- 239 9. Sokol H, Leducq V, Aschard H, Pham H-P, Jegou S, Landman C, et al. Fungal
240 microbiota dysbiosis in IBD. *Gut.* 2016;

241

242 **FIGURE LEGENDS**

243 **Figure 1: Abnormal bacterial microbiota composition and diversity in PID.** Principal
244 coordinate analysis of Bray–Curtis distance with each sample colored according to the studied
245 group. PC1, PC2 and PC3 represent the top three principal coordinates that captured most of
246 the diversity. The fraction of diversity captured by the coordinate is given as a percentage.
247 Groups were compared using ANOSIM method (9999 permutations). **(A)** All the studied
248 groups plotted together. **(B)** CGD group compared to healthy subjects. **(C)** TTC7A group
249 compared to healthy subjects. **(D)** XIAP group compared to healthy subjects. **(E)** Patients with
250 active IBD involvement ($PID_{IBD(flare)}$) compared to non-active IBD involvement ($PID_{IBD(non-}$
251 $active)$) and without IBD involvement (PID_{no-IBD}). **(F,G)** Bacterial diversity based on the Shannon
252 index in the fecal samples of indicated groups. Statistical significance was assessed using
253 ANOVA with a post hoc Dunn’s test. **(H,I)** Global composition of bacterial microbiota at the
254 phylum levels for the indicated groups *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$.

255

256 **Figure 2: Bacterial taxa associated with TTC7A, XIAP and CGD.** Bacterial taxa associated
257 with TTC7A deficiency (**A**), XIAP deficiency (**B**) and CGD (**C**). Differences in abundance are
258 shown for the bacterial taxa detected using a multivariate statistical approach (see ‘Material and
259 Methods’). All the indicated differences are statistically significant with $p < 0.05$ after correction
260 for multiple testing. The fold change for each taxon was calculated by dividing the mean
261 abundance in the cases by that of the controls.

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263

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Figure 1

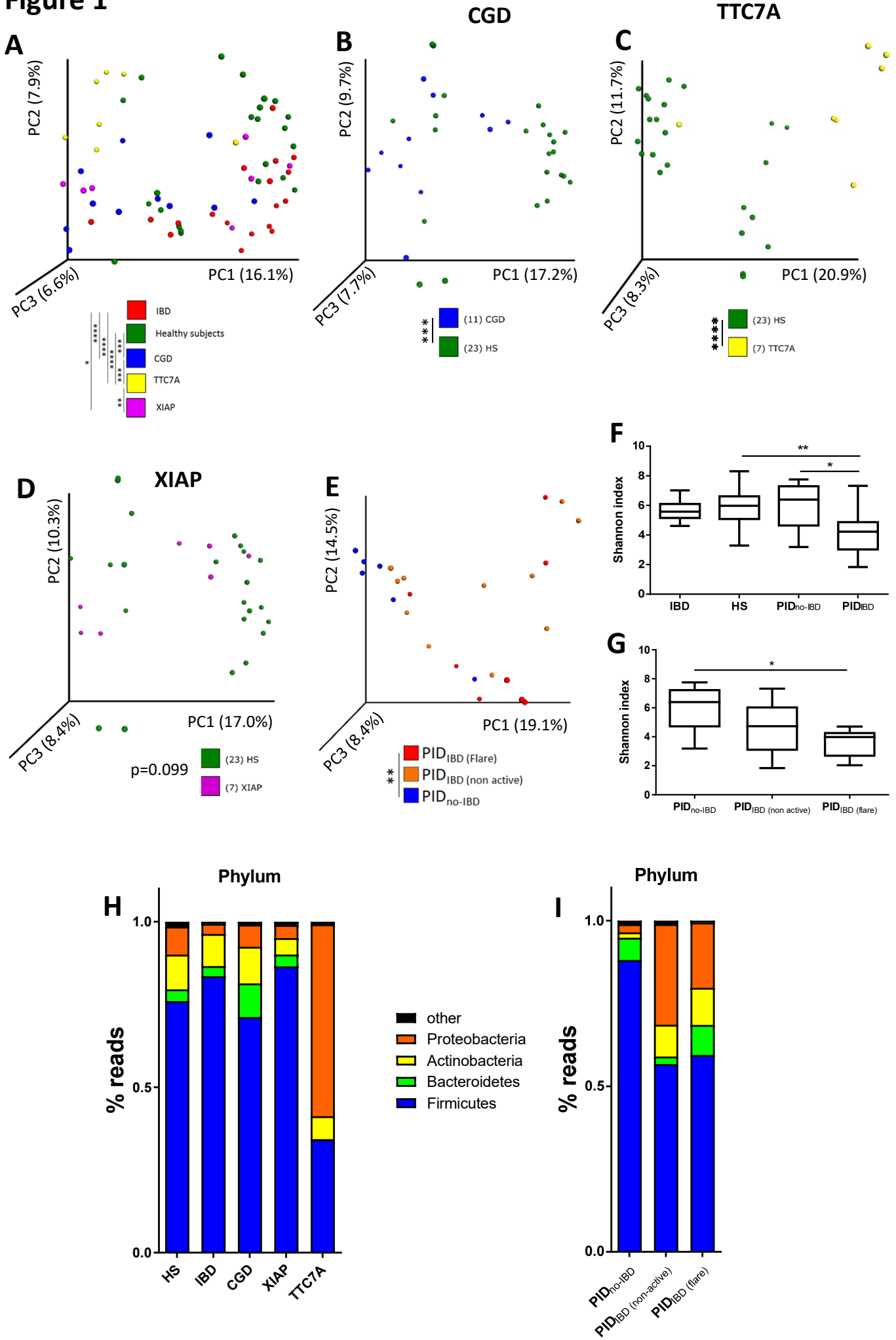
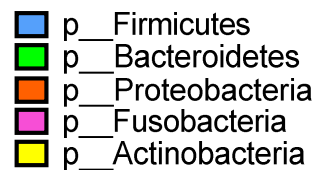
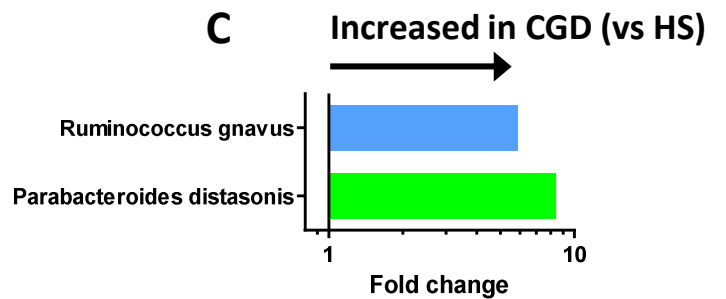
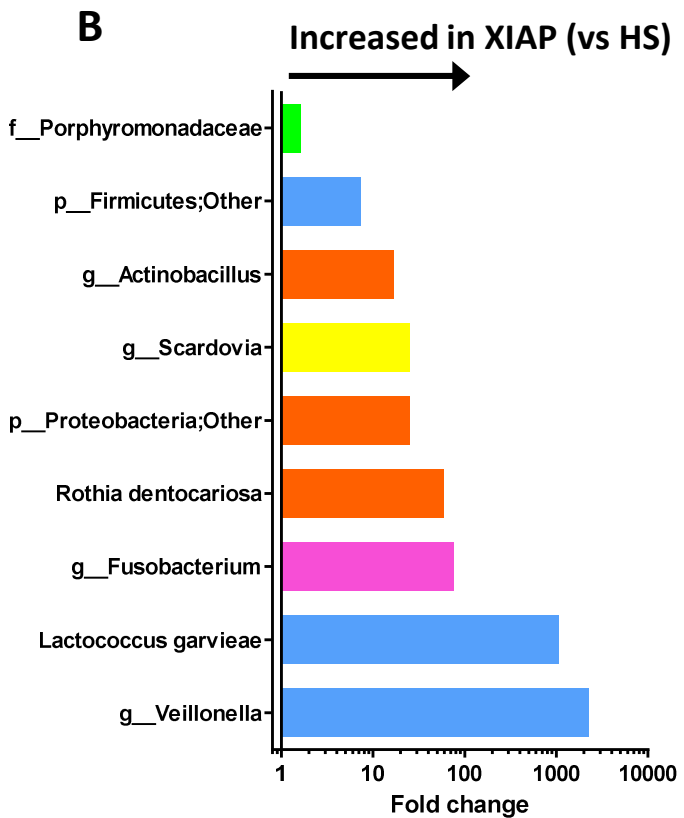
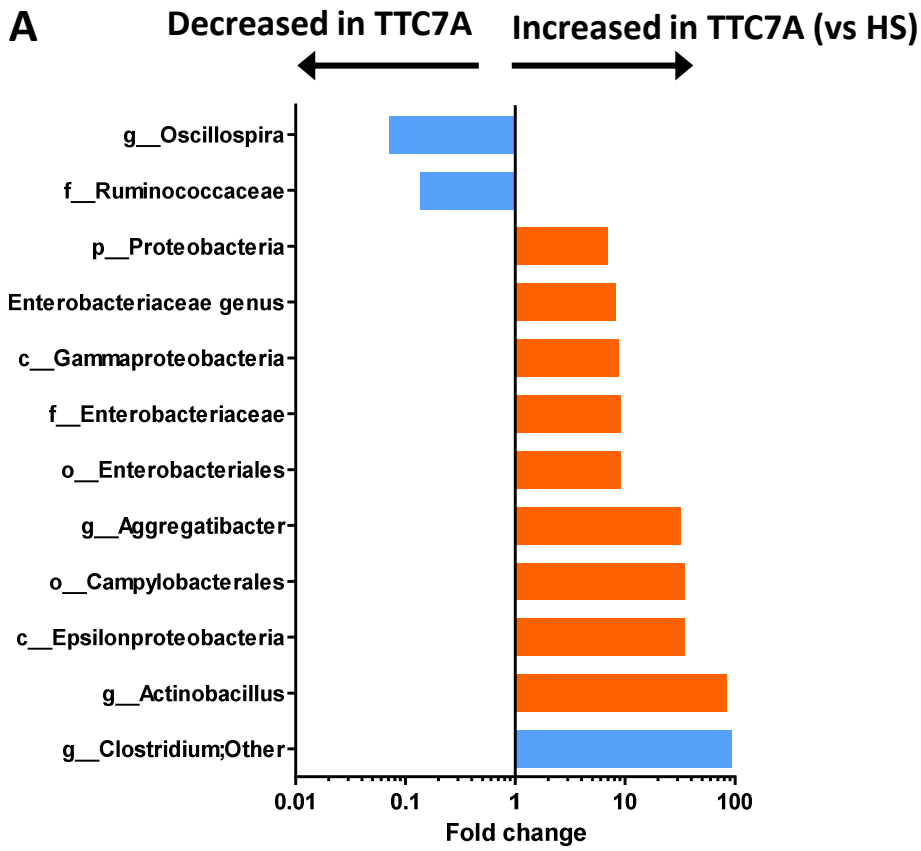
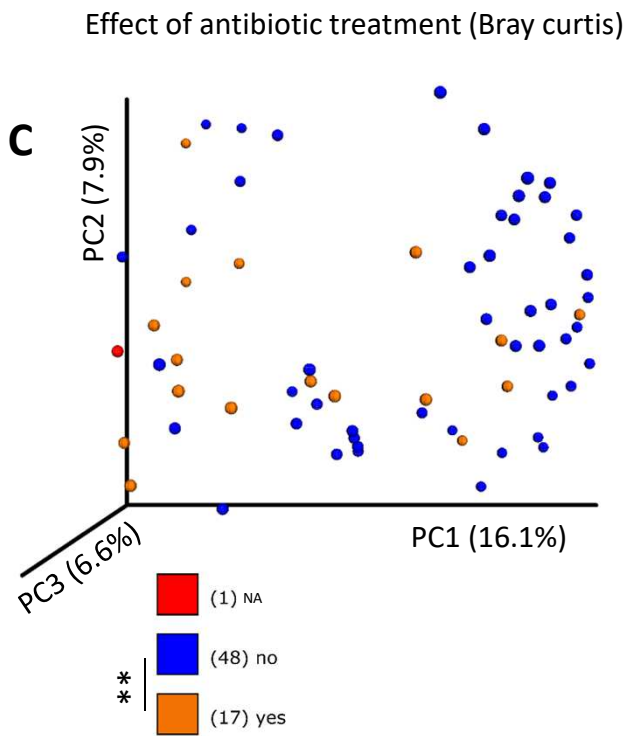
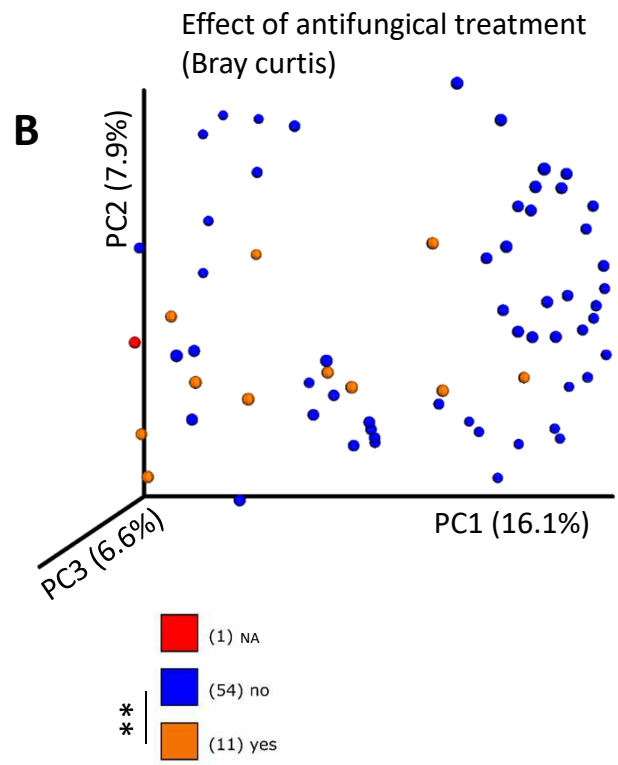
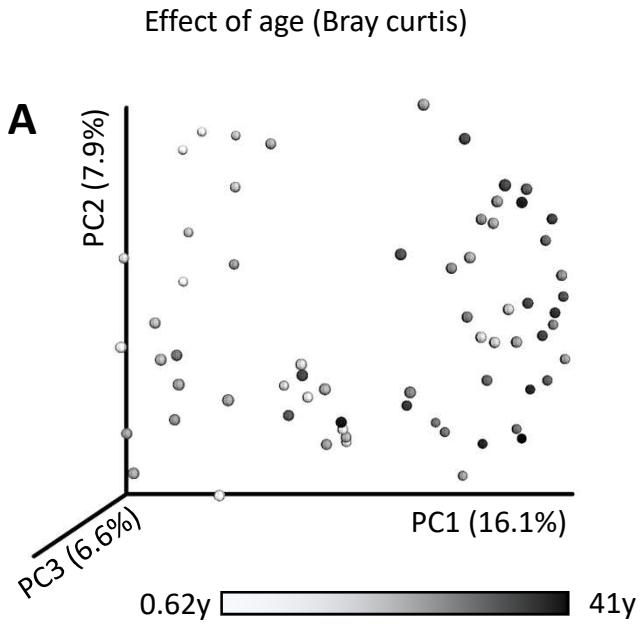


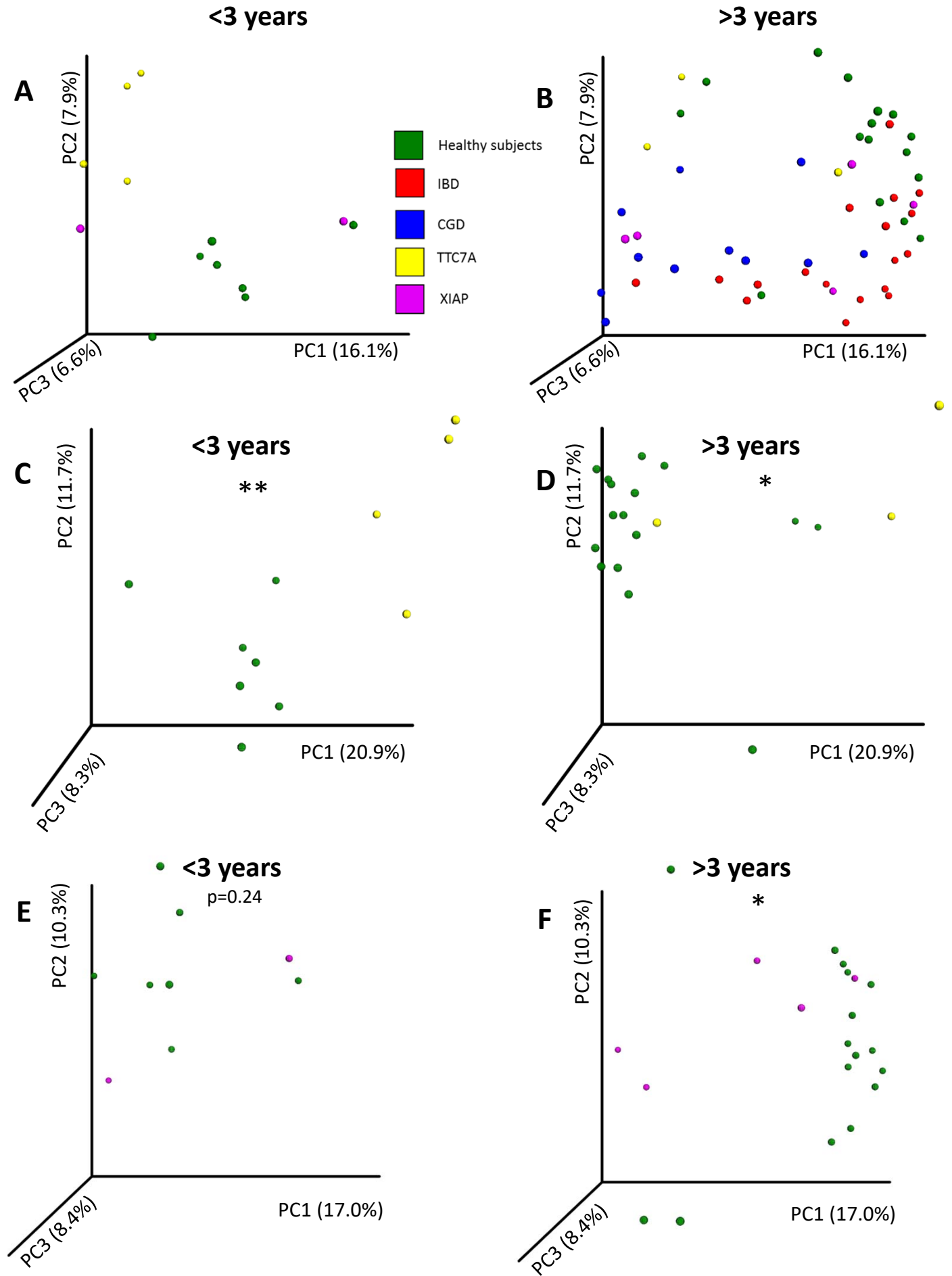
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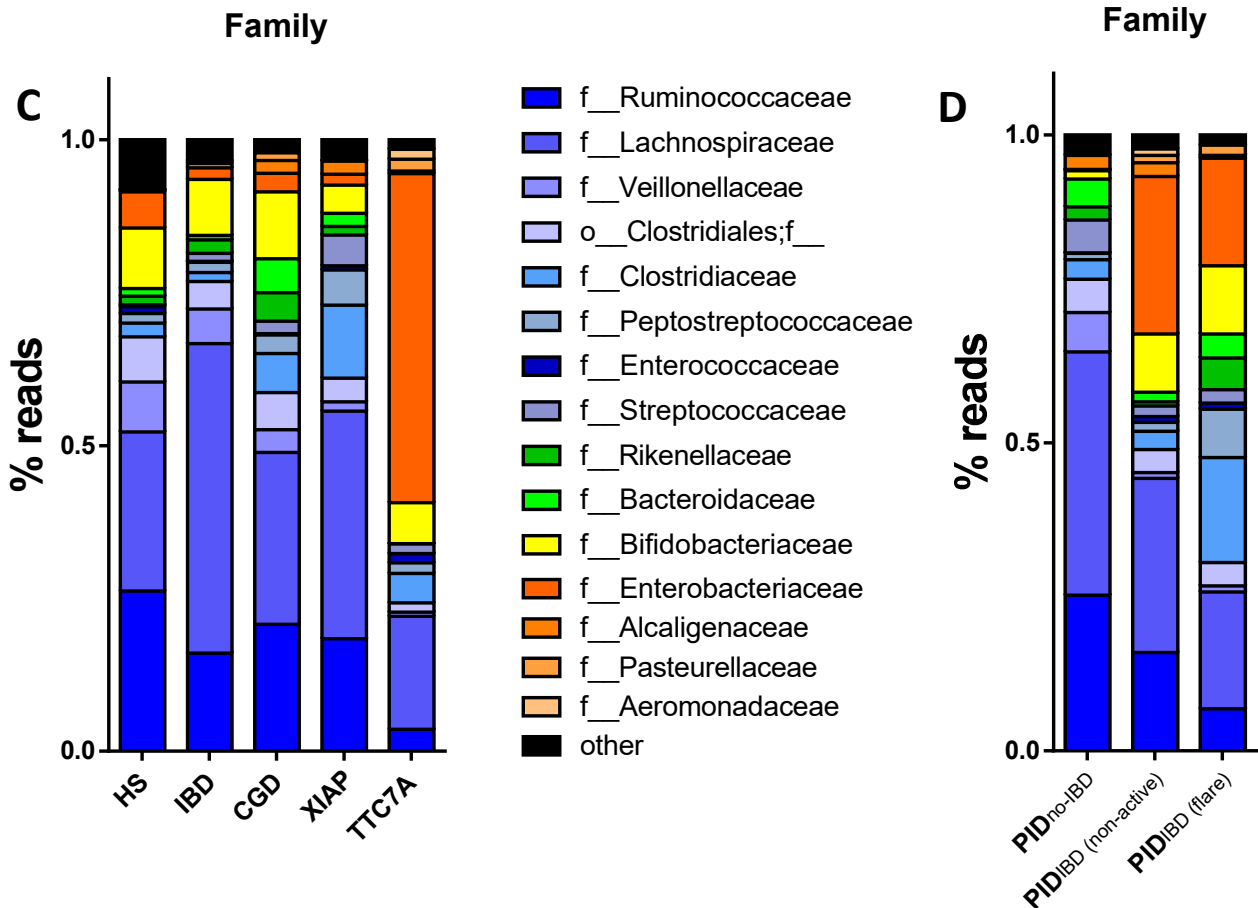
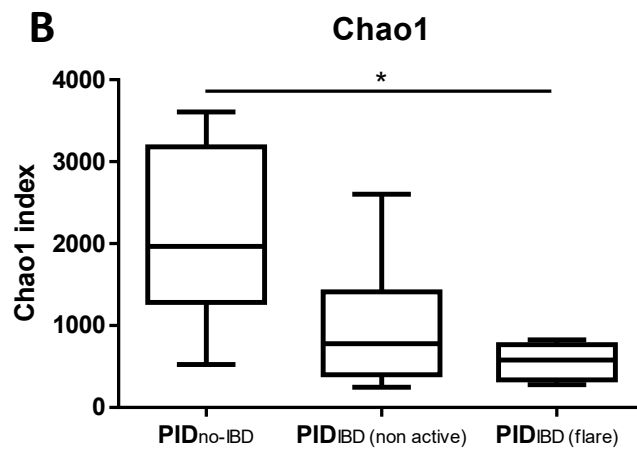
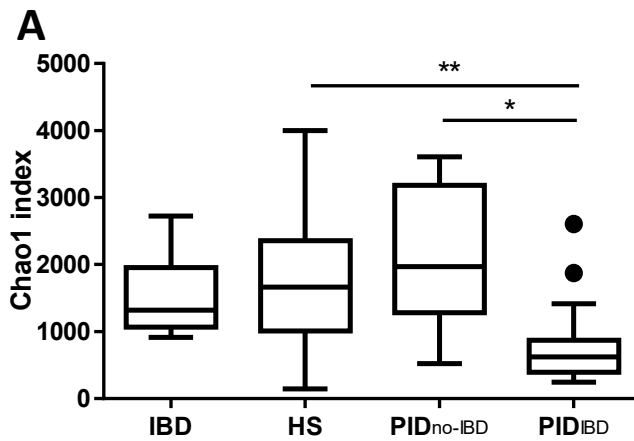
Supplementary Figure 1



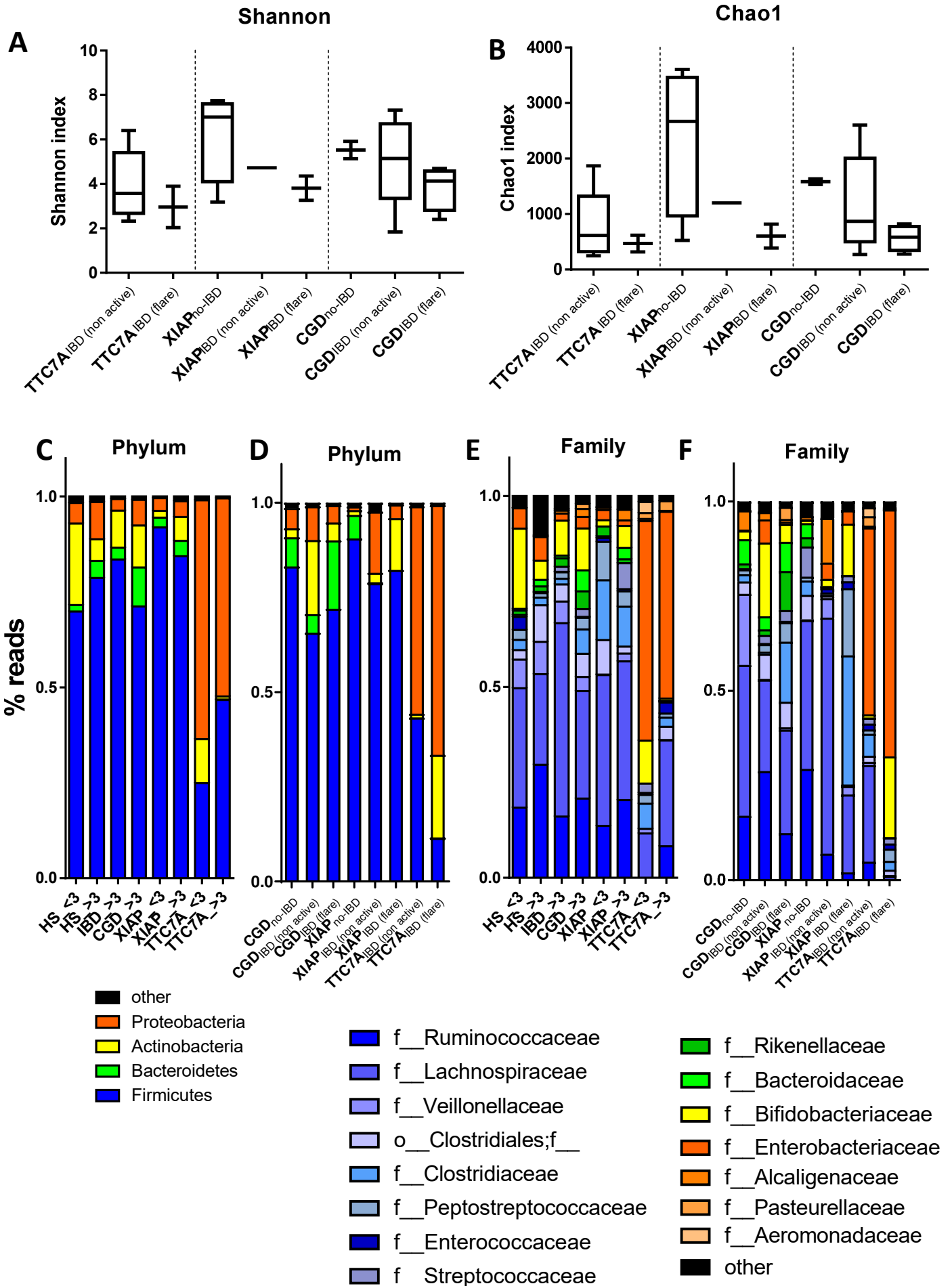
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Table 1 : Characteristics of patients

Patients n	HS 23	IBD 18	CGD 10	TTC7A 5	XIAP 6
Sample n	23	18	11	7	7
Age: median (min-max)	9 (0.83-32)	28.5 (10-41)	10 (6-31)	1.8 (0.62-18)	8 (1.3-34)
Male Gender % (n)	61% (14)	89% (16)	100% (11)	43% (3)	100% (7)
Gut inflammation history	0	100% (18)	82% (9)	100% (7)	43% (3)
Active gut inflammation	0	0	64% (7)	71% (5)	71% (5)
Smoking	4% (1)	22% (4)	0	0	43% (3)
Sulfamethoxazole / trimethoprim	0	0	100% (11)	29% (2)	50% (3)
Other antibiotics	0	0	27% (3)	0	29% (2)
Itraconazole	0	0	91% (10)	0	0
Posaconazole	0	0	9% (1)	0	0
5-ASA	0	33% (6)	18.2% (2)	0	0
Corticosteroid	0	0	0	0	33% (2)
Azathioprin	0	50% (9)	0	0	0
Methotrexate	0	0	9% (1)	0	17% (1)
Anti-TNF α	0	61% (11)	0	0	0
Anti IL1 therapy	0	0	0	0	17% (1)
Natalizumab (anti α 4) therapy	0	0	0	0	17% (1)
Any immunosuppressant	0	61% (11)	9% (1)	0	50% (3)
PPI	0	0	0	14% (1)	0
Ig substitution	0	0	0	100% (7)	17% (1)
Enteral nutrition	0	0	0	43% (3)	0
Hydroxychloroquine	0	0	9% (1)	0	0

5-ASA: 5 aminosaclylate ; PPI: proton pump inhibitor ; Ig: Immunoglobulin substitution

1 CGD patient, 1 XIAP patient and 2 TTC7A patients experienced flare during the follow up and were then sampled twice

SUPPLEMENTARY INFORMATION

MATERIAL AND METHODS

Patients and sample collection

Patients with primary immunodeficiency were recruited at Necker-Enfants malades University Hospital, Paris, France (Pediatric Immunology-Hematology-Rheumatology Unit, Adult Hematology Unit, Adult infectious Disease Unit), Saint-Louis Hospital, Paris, France and Lille Regional University Hospital, Lille, France and provided informed consent. Pediatric healthy subjects were recruited from the pediatric orthopedic surgery unit, Necker-Enfants malades University Hospital. Approval was obtained from the local ethics committee (Comité de Protection des Personnes III (Ref.: 3149, 2014, June 10th, Dipobiota study, ClinicalTrials.gov Identifier: NCT02909244). Patients with IBD in remission were recruited at the Gastroenterology Department of the Saint Antoine Hospital (Paris, France) and provided informed consent (local ethics committee: Comité de Protection des Personnes Ile-de-France IV, Suivitheque study). None of the IBD patients and the healthy subjects reported having taken antibiotics, probiotics or using colon-cleansing products for at least 1 months prior to enrollment. Patient characteristics are presented in Table 1. Whole stools were collected in sterile boxes and immediately homogenized, and 0.2 g aliquots were frozen at -80°C for further analysis. Patients' data were collected, including clinical, treatment, immunological and genetic diagnosis. Enrolled patients with PID had damaging causal mutation respectively in either the TTC7A gene "ELA syndrome"²⁶, BIRC4 (XIAP deficiency) and CYBBNox2 (X-linked CGD). None of the subjects received probiotics.

Fecal DNA extraction

Genomic DNA was extracted from 200 mg of feces as previously described.²⁷ . Following microbial lysis with both mechanical and chemical steps, nucleic acids were precipitated in isopropanol for 10 minutes at room temperature, incubated for 15 minutes on ice and

centrifuged 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 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

Fecal DNA was extracted from the weighted feces before and during the infection as previously described²⁷. Microbial diversity was determined for each sample by targeting a portion of the ribosomal genes. A 16S rRNA gene fragment comprising V3 and V4 hypervariable regions (16S; 5'-TACGGRAGGCAGCAG-3' and 5'-CTACCNGGGTATCTAAT-3') was amplified using an optimized and standardized 16S-amplicon-library preparation protocol (Metabiotec, GenoScreen). Briefly, 16S rRNA gene PCR was performed using 5 ng genomic DNA according to the manufacturer's protocol (Metabiotec) using 192 bar-coded primers (Metabiotec MiSeq Primers, GenoScreen) at final concentrations of 0.2 µM and an annealing temperature of 50°C for 30 cycles. The PCR products were purified using an Agencourt AMPure XP-PCR Purification system (Beckman Coulter), quantified according to the manufacturer's protocol, and multiplexed at equal concentrations. Sequencing was performed using a 250-bp paired-end sequencing protocol on an Illumina MiSeq platform (Illumina) at GenoScreen. Raw paired-end reads were subjected to the following process: (1) quality filtering using the PRINSEQ-lite PERL script⁵ by truncating the bases from the 3' end that did not exhibit a quality < 30 based on the Phred algorithm; (2) paired-end read assembly using FLASH (fast length adjustment of short reads to improve genome assemblies) with a minimum overlap of 30 bases and a 97% overlap identity; and (3) searching and removing both forward and reverse primer sequences using CutAdapt, with no mismatches allowed in the primers sequences. Assembled sequences for which perfect forward and reverse primers were not found were eliminated.

16S rRNA gene sequence analysis

The sequences were demultiplexed and quality filtered using the Quantitative Insights Into Microbial Ecology (QIIME, version 1.9.1) software package ²⁸, and the forward and reverse Illumina reads were joined using the fastq-join method (<http://code.google.com/p/ea-utils>). The sequences were assigned to OTUs using the UCLUST algorithm ²⁹ with a 97% threshold of pairwise identity and classified taxonomically using the Greengenes reference database ³⁰. Principal component analyses of the Bray Curtis distance were built and used to assess the variation between experimental groups (beta diversity). Significance was assessed using ANOSIM (9999 permutations). The Shannon and Chao1 diversity index were calculated using rarefied data (depth = 17,000 sequences/sample) and used to characterize species diversity in a community.

Statistical analysis

GraphPad Prism version 6.0 (San Diego, CA) was used for all analyses and graph preparation. For all graph data, the results are expressed as the mean \pm SEM, and statistical analyses were performed using the 2-tailed nonparametric 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 Anosim method (9999 permutations). Differences with a p value less than 0.05 were considered significant.

Multivariate Analysis by Linear Models (MaAsLin), a multivariate statistical framework, was used to find associations between clinical metadata and microbial community abundance ³¹. We took into account as much factor as possible in the analysis to control for the effects of potential confounding factors including: age, gender, smoking, intestinal inflammation and treatment and enteral nutrition.

SUPPLEMENTARY FIGURE LEGEND

Supplementary Figure 1: Beta diversity analysis reveals effect of age, antibiotics and antifungics on the microbiota composition. Principal coordinate analysis of Bray–Curtis distance with each sample colored according to the studied group. 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 ANOSIM method (9999 permutations). All the studied samples are plotted together and colored according to age (**A**), antifungic treatment (**B**), and antibiotic treatment (**C**). *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

Supplementary Figure 2: Beta diversity analysis according to age. Principal coordinate analysis of Bray–Curtis distance with each sample colored according to the studied group and stratified by age (< or > 3 years). 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 ANOSIM method (9999 permutations). (**A, B**) all the studied groups plotted together. (**C, D**) TTC7A group compared to healthy subjects. (**E, F**) XIAP group compared to healthy subjects. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$. In brackets, number of samples.

Supplementary Figure 3: Abnormal microbiota composition at Family level in PID.

Bacterial diversity based on Chao1 index (**A, B**) in the fecal samples of indicated groups. Statistical significance was assessed using ANOVA with a post hoc Dunn's test. *: $p < 0.05$; **:

p<0.01; ***: p<0.001; ****: p<0.0001. Global composition of bacterial microbiota at the levels for the indicated groups (C, D).

Supplementary Figure 4: Alpha diversity and microbiota composition according to age and IBD intestinal inflammation. Bacterial diversity based on the Shannon index (A) and Chao1 index (B) in the fecal samples of indicated groups. Global composition of bacterial microbiota at the phylum (C, D) and family (E, F) levels for the indicated groups.