

1 **Reduction of Short-Chain Fatty Acid-Producing Gut Microbiota Leads to**  
2 **Transition from Rapid-Eye-Movement Sleep Behavior Disorder to Parkinson's**  
3 **Disease**

4  
5 Hiroshi Nishiwaki,<sup>a</sup> Tomonari Hamaguchi,<sup>a</sup> Mikako Ito,<sup>a</sup> Tomohiro Ishida,<sup>b</sup> Tetsuya Maeda,<sup>c</sup>  
6 Kenichi Kashihara,<sup>d</sup> Yoshio Tsuboi,<sup>e</sup> Jun Ueyama,<sup>b</sup> Teppei Shimamura,<sup>f</sup> Hiroshi Mori,<sup>g</sup> Ken  
7 Kurokawa,<sup>g</sup> Masahisa Katsuno,<sup>h</sup> Masaaki Hirayama,<sup>b</sup> Kinji Ohno<sup>a</sup>

8  
9 <sup>a</sup>Division of Neurogenetics, Center for Neurological Diseases and Cancer, Nagoya University  
10 Graduate School of Medicine, Nagoya, Japan

11 <sup>b</sup>Department of Pathophysiological Laboratory Sciences, Nagoya University Graduate School of  
12 Medicine, Nagoya, Japan

13 <sup>c</sup>Division of Neurology and Gerontology, Department of Internal Medicine, School of Medicine,  
14 Iwate Medical University, Iwate, Japan

15 <sup>d</sup>Department of Neurology, Okayama Kyokuto Hospital, Okayama, Japan

16 <sup>e</sup>Department of Neurology, Fukuoka University, Fukuoka, Japan

17 <sup>f</sup>Division of Systems Biology, Center for Neurological Diseases and Cancer, Nagoya University  
18 Graduate School of Medicine, Nagoya, Japan

19 <sup>g</sup>Genome Evolution Laboratory, Department of Informatics, National Institute of Genetics,  
20 Mishima, Japan

21 <sup>h</sup>Department of Neurology, Nagoya University Graduate School of Medicine, Japan

22

23 **Corresponding authors:** Kinji Ohno, MD, PhD. Division of Neurogenetics, Center for  
24 Neurological Diseases and Cancer, Nagoya University Graduate School of Medicine, 65  
25 Tsurumai, Showa-ku, Nagoya 466-8550, Japan (ohnok@med.nagoya-u.ac.jp)  
26 Masaaki Hirayama, MD, PhD. Department of Pathophysiological Laboratory Sciences, Nagoya  
27 University Graduate School of Medicine, 1-1-20 Daiko-Minami, Higashi-ku, Nagoya 461-8673,  
28 Japan (hirasan@met.nagoya-u.ac.jp)  
29  
30 **Keywords:** rapid-eye-movement behavior disorder; gut microbiota; meta-analysis; Parkinson's  
31 disease; topic model  
32

33 **Abstract**

34 Gut dysbiosis has been reported repeatedly in Parkinson's disease (PD), but once in rapid-eye-  
35 movement sleep behavior disorder (RBD) from Germany. Abnormal aggregation of  $\alpha$ -synuclein  
36 fibrils causing PD possibly starts from the intestine. RBD patients frequently develop PD. Early-  
37 stage gut dysbiosis that is causally associated with PD is thus expected to be observed in RBD.  
38 We analyzed gut microbiota in 26 RBD patients and 137 controls by 16S rRNA-seq. Our RBD  
39 dataset was meta-analyzed with the German RBD dataset, and was compared with gut microbiota  
40 in 223 PD patients. Unsupervised clustering of gut microbiota by LIGER, a topic model-based  
41 tool for single-cell RNA-seq analysis, revealed four enterotypes in controls, RBD, and PD. Short-  
42 chain fatty acid (SCFA)-producing bacteria were conserved in an enterotype observed in controls  
43 and RBD, whereas they were less in enterotypes observed in PD. Genus *Akkermansia* and family  
44 *Akkermansiaceae* were consistently increased in both RBD in two countries and PD in five  
45 countries. No short-chain fatty acid (SCFA)-producing bacteria were significantly changed in  
46 RBD in two counties. In contrast, we previously reported that recognized and putative SCFA-  
47 producing genera *Faecalibacterium*, *Roseburia*, and *Lachnospiraceae* ND3007 group were  
48 consistently decreased in PD in five countries. Increased mucin-layer-degrading genus  
49 *Akkermansia* possibly accounts for the development of RBD, and an additional decrease of  
50 SCFA-producing genera is likely to be associated with the transition from RBD to PD.

51 **Importance** Nineteen studies have been reported on gut microbiota in PD, whereas only one  
52 study has been reported in RBD from Germany. RBD has the highest likelihood ratio to develop  
53 PD. Our meta-analysis of RBD in Japan and Germany revealed increased mucin-layer-degrading  
54 genus *Akkermansia* in RBD. Genus *Akkermansia* may increase the intestinal permeability, as we  
55 previously observed in PD patients, and make the intestinal neural plexus exposed to oxidative

56 stress, which can lead to abnormal aggregation of prion-like  $\alpha$ -synuclein fibrils in the intestine. In  
57 contrast to PD, SCFA-producing bacteria were not decreased in RBD. As SCFA induces Treg  
58 cells, a decrease of SCFA-producing bacteria may be a prerequisite for the development of PD.  
59 We propose that prebiotic and/or probiotic therapeutic strategies to increase the intestinal mucin  
60 layer and to increase intestinal SCFA potentially retard the development of RBD and PD.  
61

## 62 **Introduction**

63           Parkinson's disease (PD) is a progressive neurodegenerative disease exhibiting four  
64 major motor deficits of tremor, slowness of movement, rigidity, and postural instability (1). PD  
65 also exhibits non-motor symptoms that are characterized by dysautonomia (constipation,  
66 vomiting, orthostatic hypotension, excessive sweating, and dysuria) and mental disorders  
67 (depression, anxiety disorder, visual hallucination, and dementia) (1). Turning our eyes to the  
68 pathophysiology of PD, PD is caused by loss of the dopaminergic neurons in the substantia nigra,  
69 which is induced by abnormally aggregated  $\alpha$ -synuclein fibrils (Lewy bodies) in the neuronal  
70 cells. Abnormal aggregation of  $\alpha$ -synuclein fibrils behaves like prions, and is propagated to other  
71 neuronal cells probably via synapses (2). Lewy bodies are also observed in the cerebral cortex,  
72 the lower brainstem (3), the olfactory bulb (4), the autonomic nervous system (5), the salivary  
73 glands (6), the skin (7), and the intestine (6, 8, 9). Abnormal aggregation of  $\alpha$ -synuclein fibrils  
74 possibly initiates in the intestinal neural plexus and ascends to the substantia nigra (4).  
75 Constipation, rapid-eye-movement sleep behavior disorder (RBD), and depression are frequently  
76 predisposed to the development of motor symptoms in PD in this order, which is in accordance  
77 with the ascending  $\alpha$ -synucleinopathy (1). A total of 19 studies had been reported by us (10, 11)  
78 and others (12-28) for gut microbiota in PD. Our recent report included the largest cohort of PD  
79 patients, and the development of a novel nonparametric meta-analysis method that was applied to  
80 analyze gut microbiota in PD in five countries (11). Our meta-analysis revealed that increased  
81 genus *Akkermansia* and decreased genera *Roseburia* and *Faecalibacterium* were shared in PD  
82 across countries. In addition, these taxonomic changes were independent of the confounding  
83 effects of constipation, body mass index (BMI), sex, age and catechol-O-methyl transferase  
84 (COMT) inhibitor intake.

85 RBD is characterized by dream-enactment behaviors during the rapid eye movement  
86 (REM) sleep, when normal people lose muscle tone, called a state of atonia (29). The prevalence  
87 of RBD is estimated to be 0.5 to 2 percent (30, 31). RBD frequently predisposes to  $\alpha$ -  
88 synucleinopathy including PD, dementia with Lewy bodies (DLB), and multiple system atrophy  
89 (MSA) (29). RBD patients sometimes have subtle sensory, motor, and cognitive deficits, as well  
90 as constipation, before the onset of PD and other  $\alpha$ -synucleinopathies (29). PD has been classified  
91 into three group according as the disease progresses: preclinical PD (no overt symptoms even in  
92 the presence of neurodegeneration), prodromal PD (overt symptoms but lacking the criteria of  
93 PD), and clinical PD (overt symptoms satisfying the criteria of PD) (32). RBD is the most  
94 dependable hallmark of prodromal PD (32). Similarly, the likelihood ratio of RBD to develop PD  
95 is as high as 130 (33). Thus, therapeutic intervention to prevent transition from RBD to PD has a  
96 potential to become a causative treatment for PD (34).

97 In contrast to as many as 19 studies reported on gut microbiota in PD as stated above,  
98 only one study has been reported on 21 RBD patients along with 76 PD patients from Germany  
99 (22). They reported that gut microbiota in RBD was similar to that in PD. We recently reported  
100 increased genus *Akkermansia*, and decreased short-chain fatty acid (SCFA)-producing taxa in PD  
101 in five countries including the German dataset (11, 22). We here performed 16S rRNA-seq  
102 analysis of 26 RBD patients and 137 controls. We also meta-analyzed our dataset with the  
103 German dataset using a nonparametric meta-analysis method that we developed previously to  
104 identify shared taxonomic changes between the two countries, and compared RBD-associated  
105 taxonomic changes in two countries with PD-associated changes in five countries.

106

107 **Results**

108 *PCoA plot to analyze the overall composition of gut microbiota in controls, RBD, and PDs*

109 We performed 16S rRNA-seq analysis of gut microbiota in 26 patients with idiopathic  
110 RBD and 137 healthy controls. All RBD patients were diagnosed by International Classification  
111 of Sleep Disorders Criteria-Third Edition (35). We conducted PCoA analysis of gut microbiota in  
112 controls and RBD in our dataset, as well as gut microbiota in our previously reported PD subjects  
113 (Hoehn & Yahr scales 1-5) (11). The centers of gravity moved from the upper left to the lower  
114 right with disease progression from controls, RBD, to Hoehn & Yahr scales 1-5 (Fig. 1A). RBD  
115 was positioned close to the mildest form of PD with Hoehn & Yahr scale 1.

116  
117 *LIGER analysis to reveal unsupervised enterotypes in a combined dataset of controls, RBD, and*  
118 *PD*

119 We applied LIGER that was developed for topic model-based single-cell RNA-seq  
120 analysis (36) to make unsupervised clustering of gut microbiota in controls, RBD, and PD. Each  
121 cluster should represent an enterotype. LIGER revealed four enterotypes of A, B, C, and D (Fig.  
122 1B). Examination of the proportion of controls, RBD, and Hoehn & Yahr scales 1-5 in each  
123 enterotype revealed that the proportion of controls was decreased in the order of enterotypes A to  
124 D, while the proportions of Hoehn & Yahr scales 3-5 were increased in the same order (Fig. 1C).  
125 The proportion of RBD was the highest in enterotype A. In factorization by LIGER, the first factor  
126 contributes most to differentiate enterotypes A to D, and genera with high loadings in the first  
127 factor are major determinants of enterotypes. Color coding of the first factor in each subject  
128 showed a gradual decrease of the first factor from enterotypes A to D (Fig. 1D). The top ten  
129 genera with the highest loadings in the first factor are indicated in Table S1 in the supplemental  
130 material. It was interesting to note that, among the ten genera, nine produce SCFA and one

131 putatively produces SCFA (*Lachnospiraceae ND3007 group*). Scatter plots of the ten genera in  
132 each enterotype showed that the abundances of these genera were also decreased in the order of  
133 enterotypes A to D (see Fig. S1 in the supplemental material). Among the ten genera,  
134 *Faecalibacterium*, *Roseburia*, and *Lachnospiraceae ND3007 group* were exactly the three genera  
135 that were decreased in PD in our previous meta-analysis of five countries (11). To summarize,  
136 unsupervised clustering of enterotypes revealed that enterotypes were shifted from A to D with  
137 transition from control, RBD, to Hoehn & Yahr scales 1-5, and that SCFA-producing genera  
138 were decreased from enterotypes A to D.

139

#### 140 *Analysis of each taxon between controls and RBD in our dataset*

141 We examined taxonomic differences between controls and RBD in our dataset using  
142 Analysis of Composition of Microbiomes (ANCOM) (37) and Wilcoxon rank sum test (Table  
143 S2a for the genus level analysis, and S2b for the family level analysis in the supplemental  
144 material). ANCOM was developed to reduce false discoveries by exploiting microbial  
145 compositional constraints (37, 38). The analyses revealed that seven genera were increased in  
146 RBD (*Ruminococcus 2*, *Alistipes*, *Akkermansia*, *Ruminococcaceae UCG-005*, *Ruminococcaceae*  
147 *UCG-004*, *[Eubacterium] coprostanoligenes group*, and *Family XIII AD3011 group*); two  
148 families were increased in RBD (*Rikenellaceae* and *Akkermansiaceae*); and no genera or families  
149 were decreased in RBD. The top ten increased or decreased genera in RBD by ANCOM were  
150 compared to those in PD in our previous report (11). Seven out of the top ten increased genera  
151 were shared between RBD and PD, whereas only one of the top ten decreased genera was shared  
152 between RBD and PD (Fig. 2).

153



154 *Differences in demographic and clinical features between controls and RBD in our dataset*

155 To search for possible confounding factors, we compared six features (age, sex, BMI,  
156 constipation, proton pump inhibitor (PPI), and H<sub>2</sub> blocker) between RBD and controls in our  
157 dataset (Table 1). Compared to controls, RBD patients had higher ages and higher BMI, and  
158 included more males. Similarly, the ratios of constipation and PPI intake were higher in RBD  
159 patients.

160  
161 *PERMANOVA to evaluate the differences in the overall composition of gut microbiota in controls*  
162 *and RBD, as well as in RBD and Hoehn & Yahr 1 scale*

163 We next performed PERMANOVA to evaluate the difference in the overall composition  
164 of gut microbiota in controls and RBD (Table 2). The overall composition of gut microbiota  
165 between controls and RBD was statistically different by all three distance metrics, and the  
166 difference was not accounted for by age, sex, BMI, constipation, or PPI. Similarly, the overall  
167 composition of gut microbiota between RBD and Hoehn & Yahr scale 1 was also statistically  
168 different by Chao and weighted UniFrac but not by unweighted UniFrac. Again, the difference  
169 was not accounted for by any covariates.

170  
171 *Possible confounding factors in our dataset for nine taxa that were significantly changed in RBD*  
172 *in our dataset*

173 We next asked whether any of the nine taxonomic changes in RBD were due to  
174 confounding factor(s). We thus performed GLMM analysis with constipation, BMI, sex, age, and  
175 PPI. We found that five genera (*Ruminococcus 2*, *Alistipes*, *Akkermansia*, *Ruminococcaceae*  
176 *UCG-004*, and *Family XIII AD3011 group*) and two families (*Rikenellaceae* and

177 *Akkermansiaceae*) were changed in RBD after adjusting for constipation, BMI, sex, age, and PPI  
178 (Fig. 3 and bold letters in Table 3). In contrast, two genera (*Ruminococcaceae* UCG-005 and  
179 *Family XIII AD3011 group*) were increased by age (Fig. 3 and underlines in Table 3). Three  
180 genera (*Akkermansia*, *Ruminococcaceae* UCG-004 and *Family XIII AD3011 group*) and one  
181 family (*Akkermansiaceae*) were decreased by BMI (Fig. 3 and underlines in Table 3). Two  
182 genera (*Ruminococcaceae* UCG-004 and *Family XIII AD3011 group*) were increased by  
183 constipation (Fig. 3 and underlines in Table 3).

184

#### 185 *Meta-analysis of the Japanese and German datasets*

186         Meta-analysis of gut microbiota was performed using the Japanese and German datasets  
187 (22). The effect size and relative abundance of 132 genera and 39 families are collated in Tables  
188 S3a and S3b, respectively, in the supplemental material. Our putative criteria ( $I^2 < 25\%$  and  $p$ -  
189 values of both FEM and REM after Bonferroni correction  $< 0.05$ ) showed that four genera  
190 (*Ruminococcaceae* UCG-004, *Alistipes*, *Family XIII AD3011 group*, and *Akkermansia*) and two  
191 families (*Rikenellaceae* and *Akkermansiaceae*) were increased in RBD (Fig. 4, Table 4). These  
192 six taxa were a subset of the seven taxa that were significantly changed in RBD after adjusting  
193 for confounding factors in our dataset (Fig. 3 and bold letters in Table 3). Among the seven taxa,  
194 genus *Ruminococcus* 2 was increased in Japan but not in Germany, and was excluded from forest  
195 plots (Fig. 4A). Forest plots of the six taxa in RBD in two countries along with in PD in five  
196 countries showed that all taxa tended to be increased in PD, and the most homogenous and  
197 significant increases were observed in genus *Akkermansia* and family *Akkermansiaceae* (Fig.  
198 4A).

199 We previously reported that two SCFA-producing genera (*Faecalibacterium* and  
200 *Roseburia*) and one putative SCFA-producing genus (*Lachnospiraceae ND3007 group*) were  
201 decreased in PD across countries (11). We assumed that genus *Lachnospiraceae ND3007 group*  
202 is a putative SCFA producer, because most genera in family *Lachnospiraceae* produce SCFA.  
203 None of the three recognized or putative SCFA-producing genera were decreased in RBD in our  
204 meta-analysis. However, forest plots of the three genera showed that genus *Faecalibacterium*  
205 tended to be decreased in RBD, but genera *Roseburia* and *Lachnospiraceae ND3007 group* were  
206 not (Fig. 4B).

207

#### 208 *Relative abundances of four genera in progression of $\alpha$ -synucleinopathy*

209 Plots of relative abundances of genera *Akkermansia*, *Faecalibacterium*, *Roseburia* and  
210 *Lachnospiraceae ND3007 group* in controls, RBD, and Hoehn & Yahr scales 1-5 showed that  
211 genus *Akkermansia* gradually increased, and genera *Faecalibacterium*, *Roseburia*, and  
212 *Lachnospiraceae ND3007 group* gradually decreased with progression of  $\alpha$ -synucleinopathy  
213 (Fig. 5). Comparison of controls and RBD showed that genus *Akkermansia* was significantly  
214 increased in RBD. In contrast, genus *Faecalibacterium*, but not *Roseburia* or *Lachnospiraceae*  
215 *ND3007 group*, tended to be decreased in RBD (Fig. 5).

216

## 217 **Discussion**

218 We analyzed gut microbiota in RBD in our dataset, and meta-analyzed the Japanese and  
219 Germany datasets (22). We first observed by PCoA analysis that the overall compositions of gut  
220 microbiota were gradually changing in controls, RBD, Hoehn & Yahr scales 1-5 in this order  
221 (Fig. 1A). We next applied LIGER (36) to 16S rRNA-seq for the first time. LIGER, which was

222 developed for single-cell RNA-seq analysis, enables integrative non-negative matrix factorization  
223 by exploiting a topic model. Topic modeling that has been developed for text mining may be able  
224 to be applied to the analysis of gut microbiota (39, 40). LIGER revealed four enterotypes in  
225 controls, RBD, and Hoehn & Yahr scales 1-5 in an unsupervised manner (Fig. 1B). Enterotypes  
226 were shifted with transition from control, RBD, to Hoehn & Yahr scales 1-5 (Fig. 1C). SCFA-  
227 producing genera were similarly decreased with the shift in enterotypes (see Fig. S1 in the  
228 supplemental material). We showed that genus *Akkermansia* was increased in RBD in this  
229 communication and in PD in our previous report (11). Genus *Akkermansia*, however, was not  
230 detected in factorization by LIGER. Genus *Akkermansia* was likely to be underestimated by  
231 LIGER, because multiple SCFA-producing genera were coordinately decreased in PD, whereas  
232 genus *Akkermansia* was increased alone without any accompanying genera, which reduced a  
233 chance of detecting genus *Akkermansia* by topic modeling by LIGER. Both PCoA and LIGER  
234 indicate that gut dysbiosis advances with progression of  $\alpha$ -synucleinopathy. Alternatively,  
235 patients with  $\alpha$ -synucleinopathy with marked gut dysbiosis may progress faster than those with  
236 mild gut dysbiosis.

237 We additionally analyzed the overall compositions of gut microbiota in controls and  
238 RBD by PERMANOVA. Evaluation of the effects of covariates by PERMANOVA showed that  
239 the overall compositions of gut microbiota were statistically different between controls and RBD  
240 by all three distance metrics, and the difference was not due to covariates (Table 2).  
241 PERMANOVA similarly showed that the overall compositions of gut microbiota were  
242 statistically different between RBD and Hoehn & Yahr scale 1 by Chao and weighted UniFrac,  
243 but not by unweighted UniFrac (Table 2). Again, the difference was not due to covariates.  
244 Weighted UniFrac takes read counts into consideration to calculate the distance so that the effects

245 of low-abundance taxa become small, whereas low-abundance taxa have more effects on  
246 unweighted UniFrac (41). Thus, RBD and Hoehn & Yahr scale 1 may have large differences in  
247 major taxa but not in minor taxa.

248 Analysis of individual taxa by ANCOM and the Wilcoxon rank sum test revealed that  
249 seven genera (see Tables S2a in the supplemental material) and two families (see Tables S2b in  
250 the supplemental material) were increased in RBD. Adjustment for possible confounding factors  
251 for the seven genera and two families by GLMM showed that increases of five genera  
252 (*Ruminococcus 2*, *Alistipes*, *Akkermansia*, *Ruminococcaceae UCG-004* and *Family XIII AD3011*  
253 *group*) and two families (*Rikenellaceae* and *Akkermansiaceae*) were indeed accounted for by  
254 RBD (yellow arrows in Fig. 3), although age, BMI, and constipation had additional confounding  
255 effects on three genera (*Akkermansia*, *Ruminococcaceae UCG-004*, *Family XIII AD3011 group*)  
256 and one family (*Akkermansiaceae*). Among the five genera and two families, only genus  
257 *Akkermansia* and family *Akkermansiaceae* were also changed in PD in our meta-analysis of five  
258 countries (11).

259 Meta-analysis of the Japanese and German datasets revealed that four genera  
260 (*Ruminococcaceae UCG-004*, *Alistipes*, *Family XIII AD3011 group* and *Akkermansia*) and two  
261 families (*Rikenellaceae* and *Akkermansiaceae*) were increased in RBD (Fig. 4A, Table 4).  
262 Among these six taxa, we previously reported that genus *Akkermansia* and family  
263 *Akkermansiaceae* were consistently increased in PD across countries (11). We found that relative  
264 abundances of genus *Akkermansia* gradually increased from RBD to Hoehn & Yahr scales 1-5  
265 (Fig. 5). *Akkermansia muciniphila* degrades the mucus layer of the gut (42), and erodes the  
266 mucus layer in the lack of dietary fibers (43). Indeed, intestinal permeability is increased in PD  
267 (44), and the serum lipopolysaccharide-binding protein levels are decreased in PD (10, 44).

268 Reduced expression of a tight junction protein, occludin, in colonic biopsies in PD is similarly in  
269 accordance with the reduced mucus layer (45). Increased intestinal permeability may expose the  
270 intestinal neural plexus to oxidative stress and pesticide/herbicide (46). which subsequently  
271 allows the formation of abnormal  $\alpha$ -synuclein aggregates in the intestine. Moreover, in the  
272 presence of other gut microbiota, *Akkermansia muciniphila* in mouse intestine enhances  
273 differentiation of follicular T cells, which mediate humoral immunity by B cells (47, 48). A high  
274 prevalence (20%) of autoimmune diseases in female patients with RBD compared to 5% in  
275 general population is in accordance with the *Akkermansia*-mediated increased humoral immunity  
276 (49). Similarly, RBD is sometimes associated with neuronal autoimmune diseases including  
277 narcolepsy, anti-IgLON5 disease, Kleine–Levin syndrome, multiple sclerosis, Guillain–Barré  
278 syndrome, anti-Ma2 encephalitis, LGI1 limbic encephalitis, Morvan’s syndrome, paraneoplastic  
279 cerebellar degeneration, and anti-NMDA receptor encephalitis (50).

280           Meta-analysis of the Japanese and German datasets also showed that no SCFA-  
281 producing genera were decreased in RBD (Fig. 4B, Table 4). We previously reported that three  
282 recognized and putative SCFA-producing genera (*Faecalibacterium*, *Roseburia*, and  
283 *Lachnospiraceae ND3007 group*) were consistently decreased in PD across countries (11).  
284 Although genus *Faecalibacterium* tended to be decreased in RBD, no significance was observed  
285 (Fig. 5). In contrast, genera *Roseburia* and *Lachnospiraceae ND3007 group* were not decreased  
286 in RBD (Fig. 5). Preservation of most of SCFA-producing bacteria in RBD was also implicated  
287 in the LIGER analysis, which showed that both controls and RBD were enriched in enterotype A  
288 (Fig. 1C), in which SCFA-producing bacteria were high (Fig. 1D). Major constituents of gut  
289 SCFAs, butyrate and propionate, induce anti-inflammatory Treg cells by inhibiting histone  
290 deacetylase (51, 52), and by binding to G protein-coupled receptors of GPR41, GPR43, and

291 GPR109A (53, 54). Indeed, in mouse models of PD, SCFAs may (55-57) or may not (58) have  
292 beneficial effects on PD symptoms. In addition, in another German cohort, fecal SCFA  
293 concentrations were decreased in PD (14). Our analysis suggests that reduced fecal SCFA  
294 concentrations may be a prerequisite for the development of PD but not of RBD. Reduction of  
295 SCFA-producing bacteria culminating in the development of PD may start from genus  
296 *Faecalibacterium*. A decrease of genus *Faecalibacterium* may thus be a hallmark to predict  
297 transition from RBD to PD. We expect that administration of SCFA and probiotics/prebiotics to  
298 increase the intestinal SCFA possibly retard the progression of  $\alpha$ -synucleinopathy at the stage of  
299 RBD.

300

## 301 **Materials and Methods**

### 302 *Patients in our dataset*

303 All studies were approved by the Ethical Review Committees of the Nagoya University  
304 Graduate School of Medicine (approval #2016-0151), Iwate Medical College Hospital (H28-  
305 123), Okayama Kyokuto Hospital (approval #kyoIR-2016002), and Fukuoka University, School  
306 of Medicine (approval #2016M027). We obtained written informed consent from all patients and  
307 controls.

308 We recruited 26 patients with idiopathic RBD and their 137 healthy controls from four  
309 hospitals to participate in this study from September 2015 to February 2018. Among the 137  
310 healthy controls, 8 were healthy spouses of RBD patients. All RBD patients were diagnosed by  
311 International Classification of Sleep Disorders Criteria-Third Edition (35). Subjects with diabetes  
312 mellitus, heart failure, liver cirrhosis, any malignancy, hematological diseases, or autoimmune

313 diseases were excluded from our study. Subjects who have taken any antibiotics in the past one  
314 month were similarly excluded.

315

### 316 *DNA isolation and 16S rRNA V3-V4 sequencing in our dataset*

317 The detailed transportation procedures of a fecal sample from participant's home to the  
318 Nagoya University, freeze-drying of the fecal sample (26), and DNA isolation were described  
319 previously (11). The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified  
320 by primer 341F, 5'-CCTACGGGNGGCWGCAG-3' and primer 805R, 5'-  
321 GACTACHVGGGTATCTAATCC-3'. Paired-end sequencing of 300-nucleotide fragments was  
322 performed using the MiSeq reagent kit V3 on a MiSeq System (Illumina). Taxonomic analysis  
323 was performed with QIIME2 (59). Operational taxonomic units (OTUs) were generated using  
324 DADA2 and the SILVA taxonomy database release 132 (60) was used for taxonomic  
325 identification.

326

### 327 *Analysis of the overall gut microbiota between controls, RBD, and PD in our dataset*

328 For the overall analysis of gut microbiota, PD samples in our previous report (11) were  
329 included. We first performed principal coordinate analysis (PCoA) of each subject, and the  
330 centers of gravity and standard errors in seven categories of controls, RBD, and Hoehn & Yahr  
331 scales 1-5 were plotted.

332 We next employed the Linked Inference of Genomic Experimental Relationships  
333 (LIGER) (36), which uses integrative non-negative matrix factorization (iNMF) for single-cell  
334 RNA-seq analysis, for unsupervised clustering of gut microbiota of controls, RBD, and Hoehn &



335 Yahr scales 1-5. LIGER enabled us to identify four enterotypes, each of which was comprised of  
336 a set of bacteria that were synchronously changed in each subject.

337

338 *Analysis of each taxon between controls and RBD in our dataset*

339 Taxa were filtered at the genus and family levels using the following conditions. For  
340 each taxon, we counted the number of samples in which the relative abundance of the taxon of  
341 interest was greater than  $1E-4$ . The number of such samples should be 17 or more (more than  
342 ~10% of all samples). We thereby chose 50 families and 168 genera.

343 The difference in the abundance of each taxon between RBD and controls was analyzed  
344 by Analysis of Composition of Microbiomes (ANCOM) (37), as well as by the Wilcoxon rank-  
345 sum test. ANCOM was performed on R  
346 (<https://github.com/antagomir/scripts/tree/master/R/ancom>). The Wilcoxon rank-sum test was  
347 performed with the mannwhitneyu functionality of scipy.stat on Python 3.6.5. The threshold of  $W$   
348 calculated in ANCOM was set to more than  $0.6 \times N$ , where  $N$  is the number of taxa. The  
349 difference in the abundance of each taxon between RBD and controls was also analyzed by the  
350 Wilcoxon rank-sum test followed by calculation of the false discovery rate (FDR) using the  
351 Benjamini-Hochberg procedure. The FDR threshold was set to 0.05. Bacterial taxa filtered for  
352 both  $W$  and FDR were assumed to be significant.

353

354 *Possible confounding factors in our dataset for nine taxa that were significantly changed in our*  
355 *dataset*

356 Six demographic and clinical features [age, sex, body mass index (BMI), constipation,  
357 proton pump inhibitor intake, and H<sub>2</sub> blocker intake] were compared between RBD and controls

358 in our dataset using either Student's *t*-test or Fisher's exact test. Subjects with the stool frequency  
359 twice a week or less were defined to be constipated (61). Constipation and BMI were statistically  
360 different between RBD and controls.

361 We examined lack of multicollinearity between RBD, constipation, BMI, sex, and age  
362 by calculating the variance inflation factor (VIF) using the R package HH version 3.1-40. We  
363 verified that the VIFs were all less than 2, indicating that there was no multicollinearity between  
364 RBD, constipation, BMI, sex, and age.

365 Next, we analyzed the effects on the overall composition of gut microbiota of (i) RBD or  
366 controls, and (ii) RBD or controls, age, sex, BMI, constipation, and PPI in our dataset comprised  
367 of controls and RBD using PERMANOVA (62). We similarly analyzed the effects on the overall  
368 composition of gut microbiota of (iii) RBD or Hoehn & Yahr 1 scale, and (iv) RBD or Hoehn &  
369 Yahr 1 scale, age, sex, BMI, constipation, and PPI in a combined dataset comprised of our  
370 current RBD subjects and PD subjects with Hoehn & Yahr 1 scale in our previous report (11)  
371 using PERMANOVA (62). All genera were included in this analysis. The effects were evaluated  
372 by three distance metrics of Chao (63), unweighted-UniFrac (64), and weighted-UniFrac (64).  
373 Chao and unweighted/weighted-UniFrac distances were calculated using the R package vegan  
374 and QIIME2, respectively.

375 Seven genera and two families that were identified in our dataset were subjected to  
376 GLMM (Generalized Linear Mixed Model) analysis using the function "glmer.nb" of the R  
377 package lme4 by setting an option to accept taxonomic variations from subject to subject.

378

379 *Meta-analysis of the Japanese and German datasets*

380 Our Japanese dataset was comprised of 26 RBD patients and 137 healthy controls,  
381 whereas the German dataset was comprised of 20 RBD patients and 38 healthy controls (22). We  
382 first collated the experimental methods and demographic features (see Table S4 in the  
383 supplemental material), as well as statistical measures of sequencing depths (see Table S5 in the  
384 supplemental material) of the two datasets. The read count of each sample was all more than  
385 10,000 in the two datasets, and no sample was excluded from our meta-analysis. For each taxon,  
386 we counted the number of samples in which the relative abundance of the taxon was more than  
387  $1E-4$ . We then filtered 39 families and 132 genera, in which the number of such samples was  
388 more than 10% (17/163 and 6/58) in both datasets.

389 In the meta-analysis, we applied two criteria that we used in our previous report (11) to  
390 identify homogeneously and significantly changed taxa in the Japanese and German datasets. The  
391 two criteria were that  $I^2$ , representing heterogeneity in meta-analysis, was below 25% (65) and  
392 that the  $p$ -values after Bonferroni correction for FEM and REM were both less than 0.05.

393  
394 *Data availability*  
395 FASTQ files of our RBD dataset are available at the DNA Data Bank of Japan (DDBJ) under the  
396 accession number DRA009322 (<https://www.ncbi.nlm.nih.gov/sra/?term=DRA009322>). FASTQ  
397 files of our PD dataset was previously deposited at DDBJ under the accession number  
398 DRA009229 (<https://www.ncbi.nlm.nih.gov/sra/?term=DRA009229>).

399  
400 **Conflict of Interest Disclosures:** None reported.

401  
402 **Funding/Support:** This study was supported by Grants-in-Aid from the Japan Society for the  
403 Promotion of Science (JP17K07094, JP19K16516, and JP20H03561); the Ministry of Health,

404 Labour and Welfare of Japan (20FC1036); the Japan Agency for Medical Research and  
405 Development (20gm1010002, 20ek0109281, and 20bm0804005), the National Center of  
406 Neurology and Psychiatry (2-5), the Smoking Research Foundation, and the Hori Sciences and  
407 Arts Foundation.

408  
409 **Acknowledgements:** We thank Keiichi Takimoto, Anzu Suzuki, Rino Asai, Yukina Matsuzaki,  
410 Sayaka Inagaki, Yuka Mishima, Yurika Muramatsu, and Tomomi Yamada at the Nagoya  
411 University Graduate School of Medicine for preparing DNA from fecal samples.

412

### 413 **References**

- 414 1. Kalia LV, Lang AE. 2015. Parkinson's disease. *The Lancet* 386:896-912.
- 415 2. Burre J, Sharma M, Sudhof TC. 2018. Cell Biology and Pathophysiology of alpha-  
416 Synuclein. *Cold Spring Harb Perspect Med* 8.
- 417 3. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. 2003. Staging of  
418 brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 24:197-211.
- 419 4. Chiang HL, Lin CH. 2019. Altered Gut Microbiome and Intestinal Pathology in Parkinson's  
420 Disease. *J Mov Disord* 12:67-83.
- 421 5. Bloch A, Probst A, Bissig H, Adams H, Tolnay M. 2006. Alpha-synuclein pathology of the  
422 spinal and peripheral autonomic nervous system in neurologically unimpaired elderly  
423 subjects. *Neuropathol Appl Neurobiol* 32:284-95.
- 424 6. Cersosimo MG. 2015. Gastrointestinal Biopsies for the Diagnosis of Alpha-Synuclein  
425 Pathology in Parkinson's Disease. *Gastroenterol Res Pract* 2015:476041.

- 426 7. Gibbons CH, Garcia J, Wang N, Shih LC, Freeman R. 2016. The diagnostic discrimination  
427 of cutaneous alpha-synuclein deposition in Parkinson disease. *Neurology* 87:505-12.
- 428 8. Braak H, de Vos RA, Bohl J, Del Tredici K. 2006. Gastric alpha-synuclein immunoreactive  
429 inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-  
430 related brain pathology. *Neurosci Lett* 396:67-72.
- 431 9. Shannon KM, Keshavarzian A, Dodiya HB, Jakate S, Kordower JH. 2012. Is alpha-  
432 synuclein in the colon a biomarker for premotor Parkinson's disease? Evidence from 3  
433 cases. *Mov Disord* 27:716-9.
- 434 10. Hasegawa S, Goto S, Tsuji H, Okuno T, Asahara T, Nomoto K, Shibata A, Fujisawa Y,  
435 Minato T, Okamoto A, Ohno K, Hirayama M. 2015. Intestinal Dysbiosis and Lowered  
436 Serum Lipopolysaccharide-Binding Protein in Parkinson's Disease. *PLoS One*  
437 10:e0142164.
- 438 11. Nishiwaki H, Ito M, Ishida T, Hamaguchi T, Maeda T, Kashihara K, Tsuboi Y, Ueyama J,  
439 Shimamura T, Mori H, Kurokawa K, Katsuno M, Hirayama M, Ohno K. 2020. Meta-  
440 Analysis of Gut Dysbiosis in Parkinson's Disease. *Mov Disord* doi:10.1002/mds.28119.
- 441 12. Scheperjans F, Aho V, Pereira PA, Koskinen K, Paulin L, Pekkonen E, Haapaniemi E,  
442 Kaakkola S, Eerola-Rautio J, Pohja M, Kinnunen E, Murros K, Auvinen P. 2015. Gut  
443 microbiota are related to Parkinson's disease and clinical phenotype. *Mov Disord* 30:350-8.
- 444 13. Keshavarzian A, Green SJ, Engen PA, Voigt RM, Naqib A, Forsyth CB, Mutlu E, Shannon  
445 KM. 2015. Colonic bacterial composition in Parkinson's disease. *Mov Disord* 30:1351-60.
- 446 14. Unger MM, Spiegel J, Dillmann KU, Grundmann D, Philippeit H, Burmann J, Fassbender  
447 K, Schwiertz A, Schafer KH. 2016. Short chain fatty acids and gut microbiota differ

- 448 between patients with Parkinson's disease and age-matched controls. *Parkinsonism Relat*  
449 *Disord* 32:66-72.
- 450 15. Hill-Burns EM, Debelius JW, Morton JT, Wissemann WT, Lewis MR, Wallen ZD, Peddada  
451 SD, Factor SA, Molho E, Zabetian CP, Knight R, Payami H. 2017. Parkinson's disease and  
452 Parkinson's disease medications have distinct signatures of the gut microbiome. *Mov*  
453 *Disord* 32:739-749.
- 454 16. Petrov VA, Saltykova IV, Zhukova IA, Alifirova VM, Zhukova NG, Dorofeeva YB, Tyakht  
455 AV, Kovarsky BA, Alekseev DG, Kostryukova ES, Mironova YS, Izhboldina OP, Nikitina  
456 MA, Perevozchikova TV, Fait EA, Babenko VV, Vakhitova MT, Govorun VM, Sazonov  
457 AE. 2017. Analysis of Gut Microbiota in Patients with Parkinson's Disease. *Bull Exp Biol*  
458 *Med* 162:734-737.
- 459 17. Bedarf JR, Hildebrand F, Coelho LP, Sunagawa S, Bahram M, Goeser F, Bork P, Wullner  
460 U. 2017. Functional implications of microbial and viral gut metagenome changes in early  
461 stage L-DOPA-naive Parkinson's disease patients. *Genome Med* 9:39.
- 462 18. Hopfner F, Kunstner A, Muller SH, Kunzel S, Zeuner KE, Margraf NG, Deuschl G, Baines  
463 JF, Kuhlenbaumer G. 2017. Gut microbiota in Parkinson disease in a northern German  
464 cohort. *Brain Res* 1667:41-45.
- 465 19. Li W, Wu X, Hu X, Wang T, Liang S, Duan Y, Jin F, Qin B. 2017. Structural changes of gut  
466 microbiota in Parkinson's disease and its correlation with clinical features. *Sci China Life*  
467 *Sci* 60:1223-1233.
- 468 20. Qian Y, Yang X, Xu S, Wu C, Song Y, Qin N, Chen SD, Xiao Q. 2018. Alteration of the  
469 fecal microbiota in Chinese patients with Parkinson's disease. *Brain Behav Immun* 70:194-  
470 202.

- 471 21. Lin A, Zheng W, He Y, Tang W, Wei X, He R, Huang W, Su Y, Huang Y, Zhou H, Xie H.  
472 2018. Gut microbiota in patients with Parkinson's disease in southern China. *Parkinsonism*  
473 *Relat Disord* 53:82-88.
- 474 22. Heintz-Buschart A, Pandey U, Wicke T, Sixel-Doring F, Janzen A, Sittig-Wiegand E,  
475 Trenkwalder C, Oertel WH, Mollenhauer B, Wilmes P. 2018. The nasal and gut microbiome  
476 in Parkinson's disease and idiopathic rapid eye movement sleep behavior disorder. *Mov*  
477 *Disord* 33:88-98.
- 478 23. Tan AH, Chong CW, Teh CSJ, Yap IKS, Loke MF, Bowman J, Song SL, Tan JY, Ang BH,  
479 Tan YQ, Yong HS, Lang AE, Mahadeva S, Lim SY. 2018. Unveiling the function of altered  
480 gut microbiota composition in Parkinson's disease. *Movement Disorders* 33:S783-S784.
- 481 24. Barichella M, Severgnini M, Cilia R, Cassani E, Bolliri C, Caronni S, Ferri V, Canello R,  
482 Ceccarani C, Faierman S, Pinelli G, De Bellis G, Zecca L, Cereda E, Consolandi C, Pezzoli  
483 G. 2019. Unraveling gut microbiota in Parkinson's disease and atypical parkinsonism. *Mov*  
484 *Disord* 34:396-405.
- 485 25. Schirinzi T, Sancesario GM, Di Lazzaro G, Biticchi B, Colona VL, Mercuri NB, Bernardini  
486 S, Pisani A. 2019. CSF alpha-synuclein inversely correlates with non-motor symptoms in a  
487 cohort of PD patients. *Parkinsonism Relat Disord* 61:203-206.
- 488 26. Ueyama J, Oda M, Hirayama M, Sugitate K, Sakui N, Hamada R, Ito M, Saito I, Ohno K.  
489 2020. Freeze-drying enables homogeneous and stable sample preparation for determination  
490 of fecal short-chain fatty acids. *Anal Biochem* 589:113508.
- 491 27. Pietrucci D, Cerroni R, Unida V, Farcomeni A, Pierantozzi M, Mercuri NB, Biocca S,  
492 Stefani A, Desideri A. 2019. Dysbiosis of gut microbiota in a selected population of  
493 Parkinson's patients. *Parkinsonism Relat Disord* doi:10.1016/j.parkreldis.2019.06.003.

- 494 28. Cirstea MS, Yu AC, Golz E, Sundvick K, Kliger D, Radisavljevic N, Foulger LH,  
495 Mackenzie M, Huan T, Finlay BB, Appel-Cresswell S. 2020. Microbiota Composition and  
496 Metabolism Are Associated With Gut Function in Parkinson's Disease. *Mov Disord*  
497 35:1208-1217.
- 498 29. Boeve BF. 2010. REM sleep behavior disorder: Updated review of the core features, the  
499 REM sleep behavior disorder-neurodegenerative disease association, evolving concepts,  
500 controversies, and future directions. *Ann N Y Acad Sci* 1184:15-54.
- 501 30. Kang SH, Yoon IY, Lee SD, Han JW, Kim TH, Kim KW. 2013. REM sleep behavior  
502 disorder in the Korean elderly population: prevalence and clinical characteristics. *Sleep*  
503 36:1147-52.
- 504 31. Haba-Rubio J, Frauscher B, Marques-Vidal P, Toriel J, Tobback N, Andries D, Preisig M,  
505 Vollenweider P, Postuma R, Heinzer R. 2018. Prevalence and determinants of rapid eye  
506 movement sleep behavior disorder in the general population. *Sleep* 41.
- 507 32. Berg D, Postuma RB, Adler CH, Bloem BR, Chan P, Dubois B, Gasser T, Goetz CG,  
508 Halliday G, Joseph L, Lang AE, Liepelt-Scarfone I, Litvan I, Marek K, Obeso J, Oertel W,  
509 Olanow CW, Poewe W, Stern M, Deuschl G. 2015. MDS research criteria for prodromal  
510 Parkinson's disease. *Mov Disord* 30:1600-11.
- 511 33. Postuma RB, Berg D. 2016. Advances in markers of prodromal Parkinson disease. *Nat Rev*  
512 *Neurol* 12:622-634.
- 513 34. Postuma RB, Gagnon JF, Montplaisir JY. 2013. REM Sleep Behavior Disorder and  
514 Prodromal Neurodegeneration - Where Are We Headed? *Tremor Other Hyperkinet Mov (N*  
515 *Y)* 3.



- 516 35. Sateia MJ. 2014. International classification of sleep disorders-third edition: highlights and  
517 modifications. *Chest* 146:1387-1394.
- 518 36. Welch JD, Kozareva V, Ferreira A, Vanderburg C, Martin C, Macosko EZ. 2019. Single-  
519 Cell Multi-omic Integration Compares and Contrasts Features of Brain Cell Identity. *Cell*  
520 177:1873-1887 e17.
- 521 37. Mandal S, Van Treuren W, White RA, Eggesbo M, Knight R, Peddada SD. 2015. Analysis  
522 of composition of microbiomes: a novel method for studying microbial composition.  
523 *Microb Ecol Health Dis* 26:27663.
- 524 38. Weiss S, Xu ZZ, Peddada S, Amir A, Bittinger K, Gonzalez A, Lozupone C, Zaneveld JR,  
525 Vazquez-Baeza Y, Birmingham A, Hyde ER, Knight R. 2017. Normalization and microbial  
526 differential abundance strategies depend upon data characteristics. *Microbiome* 5:27.
- 527 39. Almugbel R, Hung LH, Hu J, Almutairy A, Ortogero N, Tamta Y, Yeung KY. 2018.  
528 Reproducible Bioconductor workflows using browser-based interactive notebooks and  
529 containers. *J Am Med Inform Assoc* 25:4-12.
- 530 40. Anderson L, Close GL, Morgans R, Hambly C, Speakman JR, Drust B, Morton JP. 2019.  
531 Assessment of Energy Expenditure of a Professional Goalkeeper From the English Premier  
532 League Using the Doubly Labeled Water Method. *Int J Sports Physiol Perform* 14:681-684.
- 533 41. Chen LL, Madhavan R, Rapoport BI, Anderson WS. 2011. A method for real-time cortical  
534 oscillation detection and phase-locked stimulation. *Conf Proc IEEE Eng Med Biol Soc*  
535 2011:3087-90.
- 536 42. Derrien M, Vaughan EE, Plugge CM, de Vos WM. 2004. *Akkermansia muciniphila* gen.  
537 nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int J Syst Evol Microbiol*  
538 54:1469-76.

- 539 43. Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, Pudlo NA,  
540 Kitamoto S, Terrapon N, Muller A, Young VB, Henrissat B, Wilmes P, Stappenbeck TS,  
541 Nunez G, Martens EC. 2016. A Dietary Fiber-Deprived Gut Microbiota Degrades the  
542 Colonic Mucus Barrier and Enhances Pathogen Susceptibility. *Cell* 167:1339-1353 e21.
- 543 44. Forsyth CB, Shannon KM, Kordower JH, Voigt RM, Shaikh M, Jaglin JA, Estes JD,  
544 Dodiya HB, Keshavarzian A. 2011. Increased intestinal permeability correlates with  
545 sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early  
546 Parkinson's disease. *PLoS One* 6:e28032.
- 547 45. Clairembault T, Leclair-Visonneau L, Coron E, Bourreille A, Le Dily S, Vavasseur F,  
548 Heymann MF, Neunlist M, Derkinderen P. 2015. Structural alterations of the intestinal  
549 epithelial barrier in Parkinson's disease. *Acta Neuropathol Commun* 3:12.
- 550 46. Ascherio A, Chen H, Weisskopf MG, O'Reilly E, McCullough ML, Calle EE,  
551 Schwarzschild MA, Thun MJ. 2006. Pesticide exposure and risk for Parkinson's disease.  
552 *Ann Neurol* 60:197-203.
- 553 47. Ansaldo E, Slayden LC, Ching KL, Koch MA, Wolf NK, Plichta DR, Brown EM, Graham  
554 DB, Xavier RJ, Moon JJ, Barton GM. 2019. *Akkermansia muciniphila* induces intestinal  
555 adaptive immune responses during homeostasis. *Science* 364:1179-1184.
- 556 48. Cekanaviciute E, Yoo BB, Runia TF, Debelius JW, Singh S, Nelson CA, Kanner R,  
557 Bencosme Y, Lee YK, Hauser SL, Crabtree-Hartman E, Sand IK, Gacias M, Zhu Y,  
558 Casaccia P, Cree BAC, Knight R, Mazmanian SK, Baranzini SE. 2017. Gut bacteria from  
559 multiple sclerosis patients modulate human T cells and exacerbate symptoms in mouse  
560 models. *Proc Natl Acad Sci U S A* 114:10713-10718.

- 561 49. Ju YE, Larson-Prior L, Duntley S. 2011. Changing demographics in REM sleep behavior  
562 disorder: possible effect of autoimmunity and antidepressants. *Sleep Med* 12:278-83.
- 563 50. Iranzo A. 2020. Sleep and neurological autoimmune diseases. *Neuropsychopharmacology*  
564 45:129-140.
- 565 51. Canani RB, Costanzo MD, Leone L, Pedata M, Meli R, Calignano A. 2011. Potential  
566 beneficial effects of butyrate in intestinal and extraintestinal diseases. *World J Gastroenterol*  
567 17:1519-28.
- 568 52. Louis P, Hold GL, Flint HJ. 2014. The gut microbiota, bacterial metabolites and colorectal  
569 cancer. *Nat Rev Microbiol* 12:661-72.
- 570 53. Koh A, De Vadder F, Kovatcheva-Datchary P, Backhed F. 2016. From Dietary Fiber to Host  
571 Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* 165:1332-1345.
- 572 54. Nakajima A, Nakatani A, Hasegawa S, Irie J, Ozawa K, Tsujimoto G, Suganami T, Itoh H,  
573 Kimura I. 2017. The short chain fatty acid receptor GPR43 regulates inflammatory signals  
574 in adipose tissue M2-type macrophages. *PLoS One* 12:e0179696.
- 575 55. Kidd SK, Schneider JS. 2010. Protection of dopaminergic cells from MPP+-mediated  
576 toxicity by histone deacetylase inhibition. *Brain Res* 1354:172-8.
- 577 56. St Laurent R, O'Brien LM, Ahmad ST. 2013. Sodium butyrate improves locomotor  
578 impairment and early mortality in a rotenone-induced *Drosophila* model of Parkinson's  
579 disease. *Neuroscience* 246:382-90.
- 580 57. Sharma S, Taliyan R, Singh S. 2015. Beneficial effects of sodium butyrate in 6-OHDA  
581 induced neurotoxicity and behavioral abnormalities: Modulation of histone deacetylase  
582 activity. *Behav Brain Res* 291:306-314.

- 583 58. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, Challis C, Schretter  
584 CE, Rocha S, Gradinaru V, Chesselet MF, Keshavarzian A, Shannon KM, Krajmalnik-  
585 Brown R, Wittung-Stafshede P, Knight R, Mazmanian SK. 2016. Gut Microbiota Regulate  
586 Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell* 167:1469-  
587 1480 e12.
- 588 59. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H,  
589 Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ,  
590 Brown CT, Callahan BJ, Caraballo-Rodriguez AM, Chase J, Cope EK, Da Silva R, Diener  
591 C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki  
592 M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J,  
593 Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK,  
594 Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, et al. 2019.  
595 Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2.  
596 *Nat Biotechnol* 37:852-857.
- 597 60. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO. 2013.  
598 The SILVA ribosomal RNA gene database project: improved data processing and web-  
599 based tools. *Nucleic Acids Res* 41:D590-6.
- 600 61. Jamshed N, Lee ZE, Olden KW. 2011. Diagnostic approach to chronic constipation in  
601 adults. *Am Fam Physician* 84:299-306.
- 602 62. Anderson MJ. 2001. A new method for non - parametric multivariate analysis of variance.  
603 *Austral Ecology* 26:32-46.

- 604 63. Chao A, Chazdon RL, Colwell RK, Shen TJ. 2005. A new statistical approach for assessing  
605 similarity of species composition with incidence and abundance data. *Ecology Letters*  
606 8:148-159.
- 607 64. Lozupone C, Knight R. 2005. UniFrac: a new phylogenetic method for comparing  
608 microbial communities. *Appl Environ Microbiol* 71:8228-35.
- 609 65. Higgins JP, Thompson SG, Deeks JJ, Altman DG. 2003. Measuring inconsistency in meta-  
610 analyses. *BMJ* 327:557-60.

611 **Table 1. Demographic and clinical features of RBD and controls in our dataset**

612

	RBD patients ( <i>n</i> = 26)	Controls ( <i>n</i> = 137)	<i>p</i> -value
Age (years) <sup>a</sup>	74.5 ± 6.4	68.3 ± 9.8	*2.2E-3
Sex (males/females) <sup>b</sup>	20/6 (76.9% males)	62/71 (45.3% males)	*5.2E-3
Body mass index (BMI) <sup>a</sup>	24.4 ± 2.4	22.9 ± 3.1	*0.018
# Constipation (less than or equal to twice a week) <sup>b</sup>	9 (34.6%)	6 (4.4%)	*5.7E-5
# Proton pump inhibitor <sup>b</sup>	7 (26.9%)	12 (8.8%)	*0.018
# H <sub>2</sub> blocker <sup>b</sup>	1 (3.8%)	8 (5.8%)	1.000

613

614 <sup>a</sup>Mean and SD are indicated, and Student's *t*-test was applied. <sup>b</sup>Fisher's exact test was applied. \**p*-

615 value < 0.05.

616

617 **Table 2. PERMANOVA to examine the effect of each factor on the overall bacterial**  
 618 **composition in our dataset**

619

	# RBD patients	# Controls	<i>p</i> -value (Chao)	<i>p</i> -value (weighted UniFrac)	<i>p</i> -value (unweighted UniFrac)
<b>(A)</b>	26	137			
RBD vs controls			*4.3E-03	*0.010	*3.2E-03
<b>(B)</b>	26	133 <sup>a</sup>			
RBD vs controls			*3.6E-03	*0.011	*5.7E-03
Age			*0.012	0.45	0.14
Sex			0.14	0.8	0.44
BMI			0.49	0.53	0.48
Constipation			0.12	0.1	0.62
PPI			0.092	0.26	0.26
	# RBD patients	# Hoehn Yahr 1 scale	<i>p</i> -value (Chao)	<i>p</i> -value (weighted UniFrac)	<i>p</i> -value (unweighted UniFrac)
<b>(C)</b>	26	30			
RBD vs Hoehn Yahr 1 scale			*0.011	*4.6E-03	0.44
<b>(D)</b>	26	30			
RBD vs Hoehn Yahr 1 scale			*0.046	*7.4E-03	0.48
Age			0.46	0.45	0.43
Sex			0.46	0.42	0.63
BMI			0.90	0.79	0.24
Constipation			0.52	0.46	0.85
PPI			0.80	0.75	0.39

620

621 *P*-values of three distance metrics (Chao, unweighted-UniFrac, and weighted-UniFrac) by

622 PERMANOVA are indicated. PERMANOVA to examine the effect of “RBD vs Controls” (A),

623 and “RBD vs Hoehn and Yahr 1 scale” (C), on the overall microbial composition without

624 considering covariates. The effects of “RBD vs Controls” (**B**), and “RBD vs Hoehn and Yahr 1  
625 scale” (**D**) were evaluated in the presence of the effects of sex, age, BMI, constipation, and PPI  
626 by PERMANOVA. In (**B**) and (**D**), the six features were equally evaluated as covariates affecting  
627 the overall microbial composition. <sup>a</sup>Four controls lacking demographic features were excluded  
628 from the analysis. \**P*-value < 0.05.



629 **Table 3. Generalized linear mixed model (GLMM) analysis of our dataset for nine bacterial taxa that were significantly**  
 630 **changed in RBD in our dataset**

631

	W (ANCO M)	P-value (Wilcoxon rank sum test)	P-value of RBD	P-value of age	P-value of sex	P-value of BMI	P-value of constipation	P-value of PPI
<b>Increased at the genus level</b>								
<i>D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__<b>Ruminococcus 2</b></i>	157	*7.7E-4	<b>*0.031</b>	0.23	0.90	0.37	0.054	0.2
<i>D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Rikenellaceae;D_5__<b>Alistipes</b><sup>a</sup></i>	139	*2.7E-5	<b>*2.9E-3</b>	0.93	0.35	0.12	0.34	0.28
<i>D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Akkermansiaceae;D_5__<b>Akkermansia</b><sup>a</sup></i>	130	*6.0E-4	<b>*0.025</b>	0.32	0.31	<u>*0.035</u>	0.15	0.43
<i>D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__<u>Ruminococcaceae UCG-005</u></i>	117	*2.2E-3	0.13	<u>*2.2E-3</u>	0.76	0.23	0.18	0.58
<i>D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__<b>Ruminococcaceae UCG-004</b><sup>a</sup></i>	112	*7.6E-5	<b>*0.028</b>	0.18	0.36	<u>*0.012</u>	<u>*0.023</u>	0.23
<i>D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__[Eubacterium] coprostanoligenes group</i>	109	*1.5E-3	0.051	0.45	0.43	0.65	0.25	0.17
<i>D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Family XIII;D_5__<b>Family XIII AD3011 group</b><sup>a</sup></i>	104	*7.5E-4	<b>*0.034</b>	<u>*0.036</u>	0.62	<u>*0.024</u>	<u>*0.036</u>	0.21
<b>Decreased at the genus level</b>								
(no bacteria)								
<b>Increased at the family level</b>								

<i>D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Rikenellaceae</i> <sup>a</sup>	38	*5.2E-5	* <b>3.3E-3</b>	0.78	0.19	0.12	0.42	0.34
<i>D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Akkermansiaceae</i> <sup>a</sup>	36	*6.0E-4	* <b>0.025</b>	0.32	0.31	* <u>0.035</u>	0.15	0.43

**Decreased at the family level**

(no bacteria)

632

633 <sup>a</sup>Six taxa were also significantly changed in RBD in the meta-analysis of the Japanese and German datasets. *P*-values were obtained by  
634 GLMM analysis for each confounding factor. *P*-values are plotted in Fig. 3. Taxa and their *p*-values that were changed in RBD after  
635 adjusting for the effects of age, sex, BMI, constipation and PPI are indicated in bold (also indicated by orange arrows in Fig. 3). Taxa  
636 and their *p*-values that were changed by age, BMI, and constipation are underlined (also indicated by red, green, and black arrows,  
637 respectively, in Fig. 3). \**p* < 0.05.

638

639 **Table 4. Statistical measures of meta-analysis of bacterial taxa in RBD in two datasets (Japan and Germany) and in PD in five**  
640 **datasets (Japan, USA, Finland, Russia, and Germany).**

641

	RBD				PD			
	<i>p</i> -value (FEM)	<i>p</i> -value (REM)	<i>I</i> <sup>2</sup> (%)	Relative abundance (%)	<i>p</i> -value (FEM)	<i>p</i> -value (REM)	<i>I</i> <sup>2</sup> (%)	Relative abundance (%)
<b>A. Significantly increased in RBD at the genus level (plotted in Fig. 4A)</b>								
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-004	1.2E-06	1.2E-06	0	0.27	2.9E-07	0.029	74	0.13
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Rikenellaceae;D_5__Alistipes	1.4E-05	1.4E-05	0	2.6	4.8E-08	1.2E-04	45	2.7
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Family XIII;D_5__Family XIII AD3011 group	2.5E-04	2.5E-04	0	0.27	6.3E-06	0.026	67	0.13
D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Akkermansiaceae;D_5__Akkermansia	3.5E-04	3.5E-04	0	1.0	1.9E-08	1.9E-08	0	2.7
<b>B. Significantly decreased in RBD at the genus level</b>								
No bacteria								
<b>C. Significantly increased in RBD at the family level (plotted in Fig. 4A)</b>								
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Rikenellaceae	2.5E-05	2.5E-05	0	2.7	2.0E-08	3.8E-05	41	2.8
D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Akkermansiaceae	3.5E-04	3.5E-04	0	1.0	1.0E-08	1.9E-08	0	2.7
<b>D. Significantly decreased in RBD at the family level</b>								
No bacteria								
<b>E. Significantly decreased in PD at the genus level (plotted in Fig. 4B)</b>								

D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Faecalibacterium	0.052	0.052	0	6.9	4.4E-11	7.2E-10	0	4.8
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Roseburia	0.64	0.64	0	1.4	1.0E-11	1.0E-11	12	0.57
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospiraceae ND3007 group	0.41	0.41	0	0.76	2.0E-10	6.5E-10	4.5	0.20

642

643 **(A-D)** Four genera and two families were significantly increased in RBD (Bonferroni-corrected  $p$ -value < 0.05 and the homogeneity

644 index  $I^2$  < 25%) in the Japanese and German datasets. Statistical measures of these taxa in PD in five datasets in our previous report

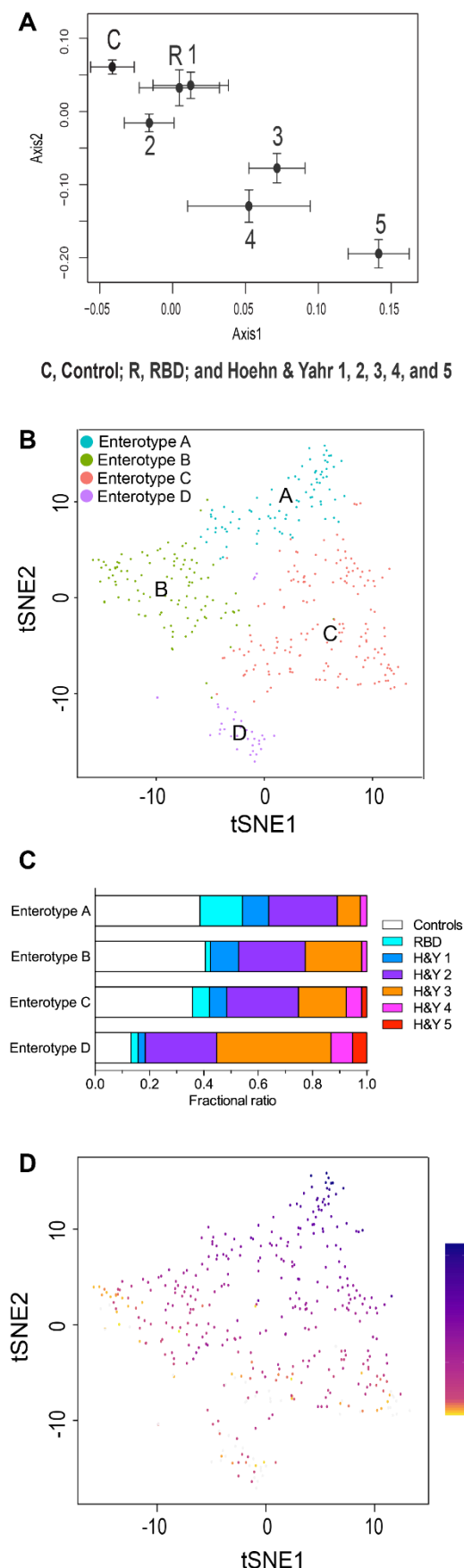
645 (11) are indicated. Forest plots of these taxa are indicated in Fig. 4A.

646 **(E)** Two recognized SCFA-producing genera (*Faecalibacterium* and *Roseburia*) and one putative SCFA-producing genus

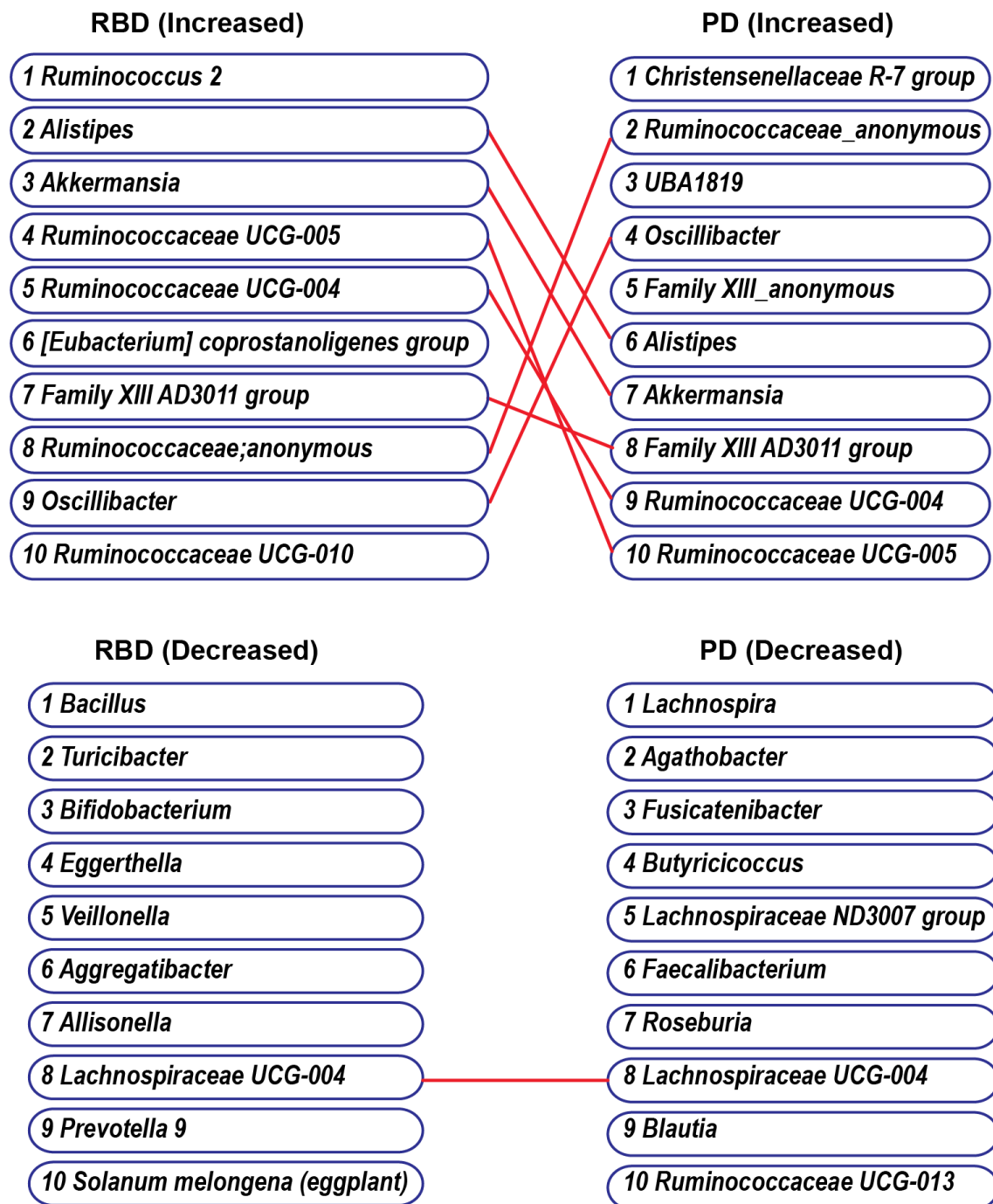
647 (*Lachnospiraceae* ND3007 group) were significantly decreased in PD in five datasets in our previous report (11). Statistical measures

648 of these taxa in RBD in two datasets in this communication are indicated. Forest plots of these taxa are indicated in Fig. 4B.

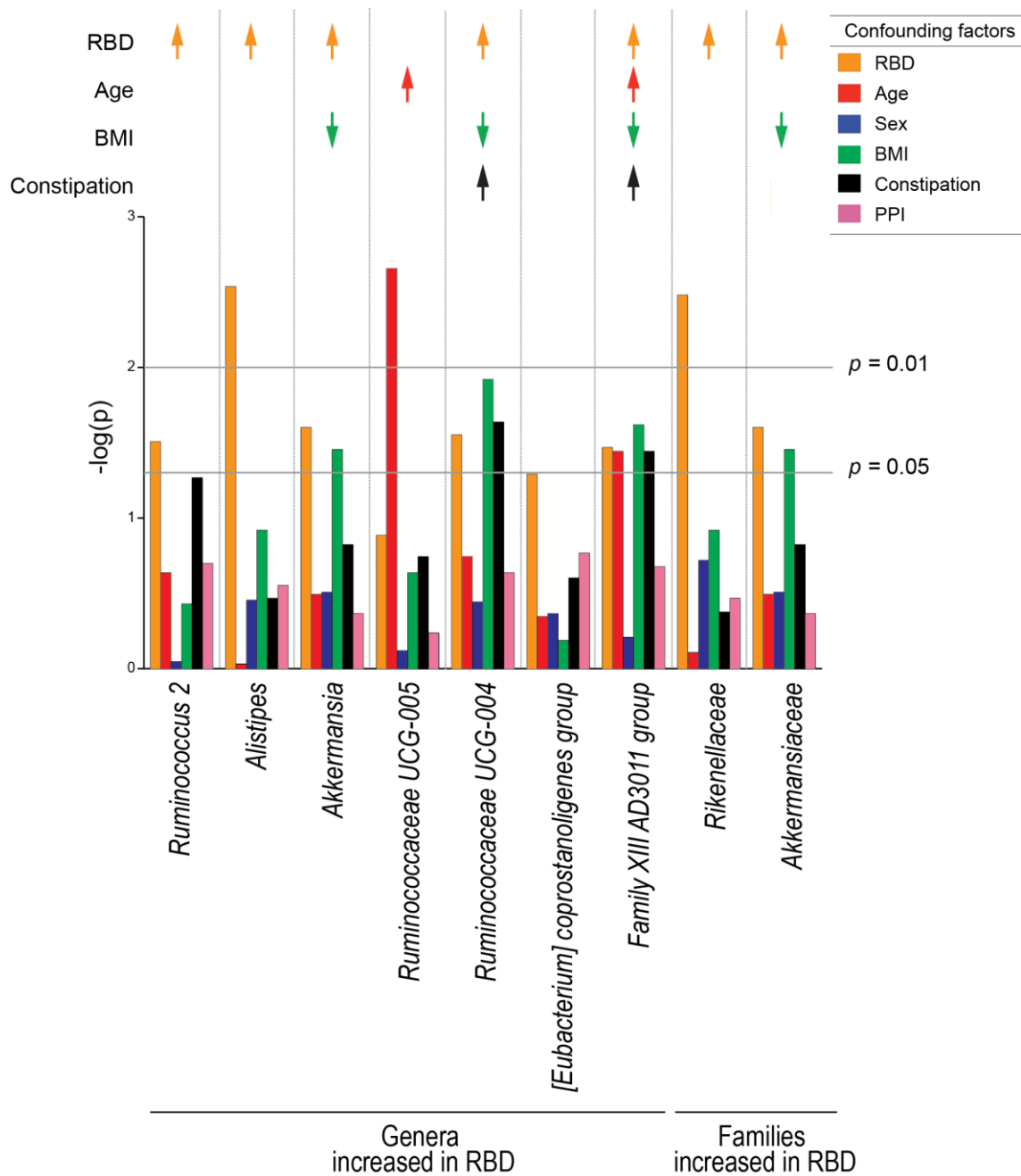
649 FEM, the fixed-effects model. REM, the random-effects model.



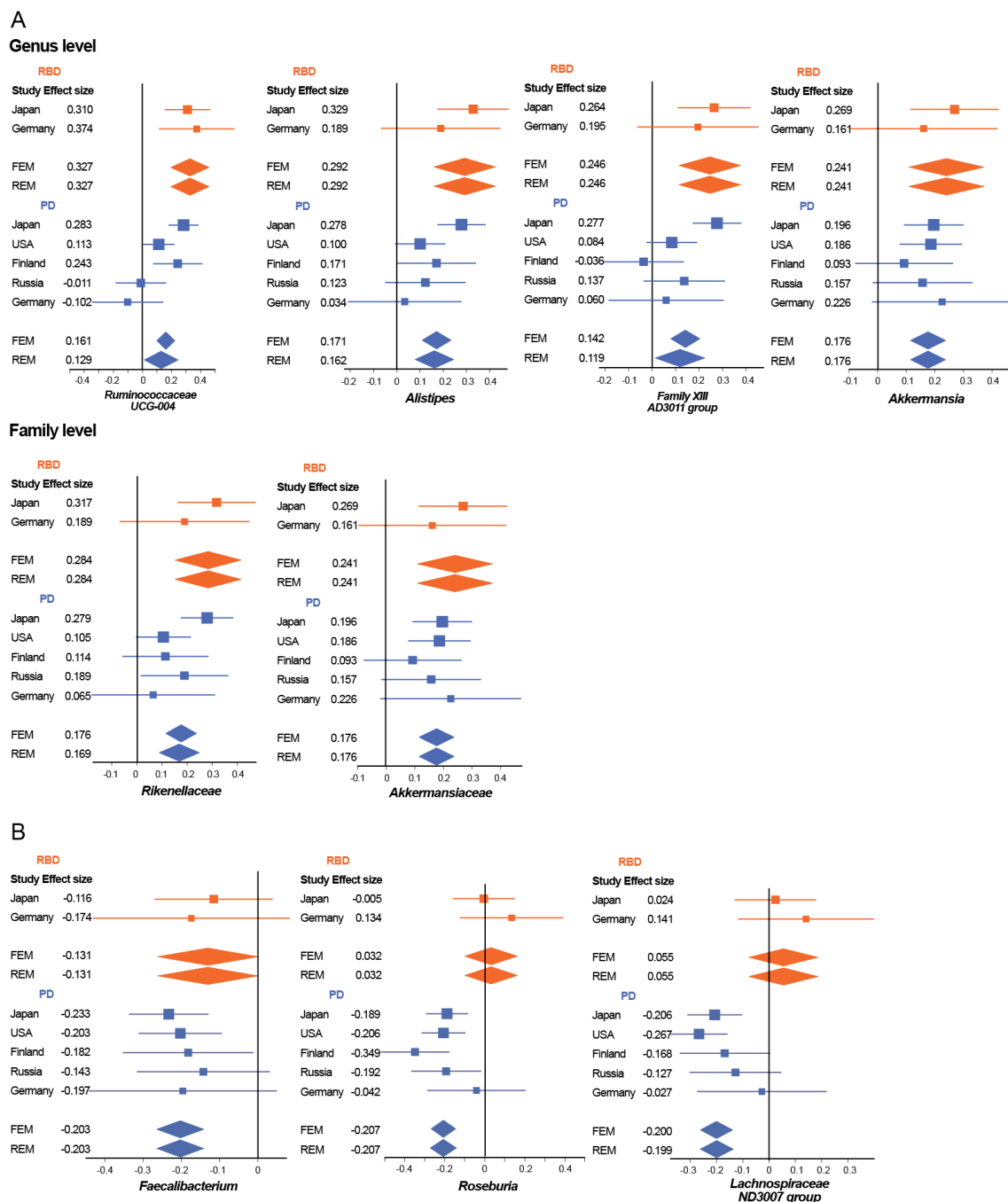
**Figure 1.** Overall compositions of gut microbiota in controls, RBD, and PD in our dataset. (A) PCoA plot showing the center of gravity of the overall compositions of gut microbiota in seven morbidity categories. The number of subjects in controls, RBD, Hoehn & Yahr scales 1-5 were 137, 26, 30, 99, 73, 16, and 5, respectively. Chao is used as a distance metric. Standard errors are indicated. (B) Unsupervised clustering of overall compositions of gut microbiota in controls, RBD, and PD by LIGER yielded four enterotypes. tSNE was adopted to visualize four clusters representing enterotypes A-D. (C) Fractional ratios of controls, RBD, and Hoehn & Yahr (H&Y) scales 1-5 in each enterotype. (D) Bacterial abundances in a total of 386 subjects were factorized into multiple factors. The first factor is color-coded in each subject on a tSNE plot indicated in (B). As SCFA-producing bacteria have high loadings in the first factor (see Table S1 in the supplemental material), individuals colored in blue carry a high proportion of SCFA-producing bacteria.



**Figure 2.** The 10 most increased and the 10 most decreased genera by ANCOM in RBD and PD both in our datasets. Identical genera are connected by a red line.

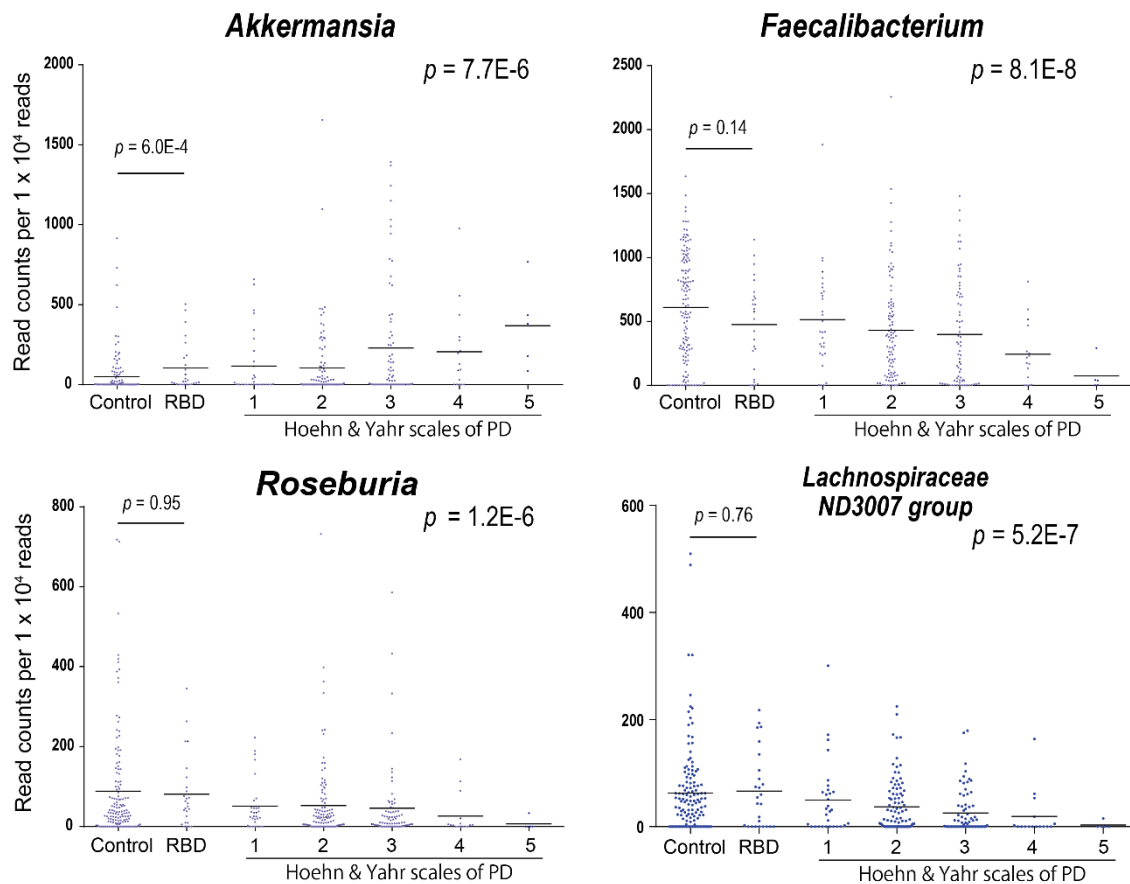


**Figure 3.** Generalized linear mixed model (GLMM) analysis to evaluate confounding factors of seven genera and two families that were significantly changed between RBD and controls in our dataset. The effects of RBD, age, sex, body mass index (BMI), constipation, and PPI were individually analyzed by mutually adjusting for covariates by GLMM. Arrows indicate taxa that were significantly changed by RBD (orange arrows), age (red arrows), BMI (green arrows) and constipation (black arrows) after adjusting for the other confounding factors. Upward and downward arrows indicate increased and decreased taxa, respectively. Exact  $p$ -values are indicated in Table 3.



**Figure 4. (A)** Forest plots of four genera and two families that were significantly and homogenously changed in RBD in the Japanese and German datasets. Forest plots of PD in five datasets are also indicated in parallel. **(B)** Forest plots of two recognized and one putative SCFA-producing genera that were significantly and homogenously decreased in PD in five countries in our previous report (11). Forest plots of RBD in the Japanese and German datasets are also indicated in parallel. An effect size of each dataset, as well as the overall effect sizes by the fixed-effects model (FEM) and the random-effects model (REM), are indicated. Both lines and diamonds indicate 95% confidence intervals. Orange and blue symbols represent RBD and PD, respectively.





**Figure 5.** Reads counts of genera *Akkermansia*, *Faecalibacterium*, *Roseburia*, and *Lachnospiraceae ND3007* group normalized for 1 x 10<sup>4</sup> reads in controls, RBD, and Hoehn & Yahr scales 1 to 5. Bar indicates an average in each category. *P*-values of Jonckheere-Terpstra trend test are shown on the right to indicate whether the genus increases or decreases monotonically. *P*-values of Wilcoxon rank sum test between controls and RBD (Table S2a in the supplemental material) are indicated on the left. Reads counts of genera *Akkermansia*, *Faecalibacterium*, and *Roseburia* in Hoehn & Yahr scales 1-5 were previously reported (11).

**Supplementary Table S1. The top 10 genera with the highest loadings in the first factor by LIGER analysis**

<b>Genus</b>	<b>Factor loading</b>
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Fusicatenibacter	33.0
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospiraceae UCG-004	29.6
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Faecalibacterium	27.8
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Butyricoccus	26.4
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Anaerostipes	26.2
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospiraceae ND3007 group	24.9
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Bacteroidaceae;D_5__Bacteroides	24.7
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Roseburia	24.6
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Blautia	24.0
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospira	23.3

## Supplementary Table S2a. Genera changed in RBD in our dataset

Genus	W	p-value	q-value	Relative abundance in RBD (%)	Increased (+) or decreased (-)
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__ <b>Ruminococcus 2</b>	157	7.7E-04	0.014	5.4	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Rikenellaceae;D_5__ <b>Alistipes</b>	139	2.7E-05	4.6E-03	2.6	+
D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Akkermansiaaceae;D_5__ <b>Akkermansia</b>	130	6.0E-04	0.014	1.0	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__ <b>Ruminococcaceae UCG-005</b>	117	2.2E-03	0.030	0.68	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__ <b>Ruminococcaceae UCG-004</b>	112	7.6E-05	6.4E-03	0.27	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__[ <b>Eubacterium] coprostanoligenes group</b>	109	1.5E-03	0.023	1.7	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Family XIII;D_5__ <b>Family XIII AD3011 group</b>	104	7.5E-04	0.014	0.27	+
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Bacillaceae;D_5__Bacillus	100	3.8E-03	0.046	0.011	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__	96	7.3E-04	0.014	1.2	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Oscillibacter	95	9.5E-04	0.016	0.42	+
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Turicibacter	91	9.2E-03	0.091	0.020	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-010	79	7.0E-04	0.014	0.092	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Bifidobacteriales;D_4__Bifidobacteriaceae;D_5__Bifidobacterium	70	0.018	0.14	3.1	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Christensenellaceae;D_5__Christensenellaceae R-7 group	65	7.4E-03	0.077	0.69	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacteriia;D_3__Coriobacteriales;D_4__Eggerthellaceae;D_5__Eggerthella	60	0.032	0.17	0.083	-
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Veillonellaceae;D_5__Veillonella	56	0.021	0.15	0.093	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Eubacteriaceae;D_5__Eubacterium	40	7.7E-04	0.014	0.073	+
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pasteurellales;D_4__Pasteurellaceae;D_5__Aggregatibacter	40	0.026	0.16	0.020	-

D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__uncultured	39	2.5E-03	0.032	0.23	+
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Veillonellaceae;D_5__Allisonella	38	0.027	0.16	6.0E-3	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Peptococcaceae;D_5__uncultured	22	5.7E-04	0.014	0.015	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospiraceae UCG-004	20	0.077	0.28	0.048	-
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__Prevotella 9	17	0.17	0.47	1.3	-
D_0__Bacteria;D_1__Cyanobacteria;D_2__Oxyphotobacteria;D_3__Chloroplast;D_4__Solanum melongena (eggplant);D_5__Solanum melongena (eggplant)	16	0.23	0.58	0.015	-
D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia;D_3__Saccharimonadales;D_4__Saccharimonadaceae;D_5__TM7 phylum sp. canine oral taxon 250	16	0.20	0.52	2.1E-03	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Coproccoccus 2	15	0.41	0.76	0.11	-
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__Alloprevotella	14	0.51	0.79	0.041	-
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Enterococcaceae;D_5__Enterococcus	14	0.066	0.26	0.024	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Butyricoccus	14	0.062	0.26	0.33	-
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__;	13	0.44	0.77	4.5E-03	-
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Family XI;D_5__Gemella	12	0.25	0.62	5.2E-03	-
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Merdibacter	12	0.64	0.89	7.3E-03	-
D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rickettsiales;D_4__Mitochondria;D_5__	11	0.59	0.86	3.2E-03	-
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii;D_3__Coriobacteriales;D_4__Atopobiaceae;D_5__Olsenella	10	0.96	0.98	0.023	-
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii;D_3__Coriobacteriales;D_4__Eggerthellaceae;Ambiguous_taxa	10	1.0	1.0	4.7E-03	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii;D_3__Coriobacteriales;D_4__Eggerthellaceae;D_5__uncultured	10	0.72	0.94	0.020	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Tyzzerella 3	10	0.44	0.77	0.014	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Candidatus Soleaferrea	10	0.72	0.94	4.4E-03	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-009	10	0.36	0.72	0.011	+
D_0__Bacteria;D_1__;	10	0.53	0.81	5.4E-03	-

D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacteriia;D_3__Coriobacteriales;D_4__Eggerthellaceae;D_5__Gordonibacter	9	0.89	0.98	0.013	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospiraceae UCG-010	9	0.37	0.72	0.025	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__GCA-900066225	9	0.84	0.98	0.019	-
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Coprobacillus	9	0.82	0.97	0.011	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lactonifactor	8	0.69	0.94	0.016	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Eubacteriaceae;D_5__Anaerofustis	7	0.80	0.96	6.0E-03	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Family XIII;D_5__Family XIII UCG-001	7	0.85	0.98	0.011	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Hungatella	7	0.74	0.94	9.8E-03	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Caproiciproducens	7	0.51	0.79	0.016	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Phocea	7	0.98	0.99	4.3E-03	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-003	6	0.64	0.89	0.067	-
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__[Clostridium] innocuum group	6	0.48	0.79	0.050	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Defluviitaleaceae;D_5__Defluviitaleaceae UCG-011	5	4.5E-03	0.050	1.3E-02	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Negativibacillus	5	0.015	0.13	0.12	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteriia;D_3__Micrococcales;D_4__Micrococcaceae;D_5__Rothia	3	0.72	0.94	8.7E-03	-
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacteriia;D_3__Coriobacteriales;D_4__Eggerthellaceae;D_5__Enterorhabdus	3	0.63	0.89	0.012	+
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Streptococcaceae;D_5__Lactococcus	3	0.62	0.88	0.20	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Christensenellaceae;__	3	0.043	0.21	0.019	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Oscillospira	3	0.92	0.98	0.020	+
D_0__Bacteria;D_1__Firmicutes;__;__;__;__	3	0.93	0.98	0.017	-
D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Desulfovibrionales;D_4__Desulfovibrionaceae;D_5__Bilophila	3	0.023	0.15	0.14	+

D_0__Archaea;D_1__Euryarchaeota;D_2__Methanobact eria;D_3__Methanobacteriales;D_4__Methanobacteriace ae;D_5__Methanobrevibacter	2	0.016	0.13	0.30	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Christensenellaceae;D_5__uncultured	2	0.038	0.19	0.016	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Family XIII;D_5__[Eubacterium] nodatum group	2	0.10	0.33	0.014	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Fusicatenibac ter	2	0.059	0.26	1.2	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__UC5-1-2E3	2	0.057	0.25	0.011	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Peptococcaceae;D_5__Peptococcus	2	0.038	0.19	0.10	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Ruminococcaceae;D_5__Intestinimo nas	2	0.022	0.15	0.094	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Ruminococcaceae;D_5__Ruminiclost ridium 6	2	0.017	0.13	0.34	+
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia; D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__ Holdemania	2	0.031	0.17	0.040	+
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia; D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__ uncultured	2	0.020	0.15	0.040	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D _3__Bacteroidales;D_4__Prevotellaceae;D_5__Prevotell aceae NK3B31 group	1	0.57	0.85	0.079	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Family XIII;D_5__[Eubacterium] brachy group	1	0.071	0.27	0.060	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Blautia	1	0.65	0.89	6.4	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospirac eae UCG-008	1	0.94	0.98	0.020	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Ruminococcaceae;D_5__DTU089	1	0.066	0.26	0.033	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococ caceae NK4A214 group	1	0.030	0.17	0.25	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Ruminococcaceae;D_5__UBA1819	1	0.034	0.18	0.14	+
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia; D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__ Erysipelotrichaceae UCG-003	1	0.47	0.78	0.64	-
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia; D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;__	1	0.59	0.86	0.017	+
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D _3__Selenomonadales;D_4__Veillonellaceae;D_5__Diali ster	1	0.15	0.44	0.26	-
D_0__Bacteria;D_1__Fusobacteria;D_2__Fusobacteriia; D_3__Fusobacteriales;D_4__Fusobacteriaceae;D_5__Fus obacterium	1	0.31	0.66	0.36	-

D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Actinomycetales;D_4__Actinomycetaceae;D_5__Actinomyces	0	0.83	0.98	0.049	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii;D_3__Coriobacteriales;D_4__Coriobacteriaceae;D_5__Collinsella	0	0.30	0.65	1.4	-
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii;D_3__Coriobacteriales;D_4__Coriobacteriales Incertae Sedis;D_5__uncultured	0	0.46	0.77	0.045	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii;D_3__Coriobacteriales;D_4__Eggerthellaceae;D_5__Senechalimassilia	0	0.96	0.98	0.054	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii;D_3__Coriobacteriales;D_4__Eggerthellaceae;D_5__Slackia	0	0.29	0.65	0.065	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii;D_3__Coriobacteriales;D_4__Eggerthellaceae;D_5__	0	0.27	0.62	0.024	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Bacteroidaceae;D_5__Bacteroides	0	0.41	0.76	20.9	-
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Barnesiellaceae;D_5__Barnesiella	0	0.30	0.65	0.63	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Barnesiellaceae;D_5__Coproacter	0	0.55	0.82	0.12	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Barnesiellaceae;D_5__uncultured	0	0.20	0.52	0.041	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Marinifilaceae;D_5__Butyrimonas	0	0.51	0.79	0.062	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Marinifilaceae;D_5__Odoribacter	0	0.085	0.30	0.28	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Marinifilaceae;D_5__	0	0.19	0.51	0.028	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__Paraprevotella	0	0.071	0.27	0.37	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__Prevotella 2	0	0.095	0.31	0.58	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__uncultured	0	0.37	0.72	0.046	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__	0	0.47	0.78	0.16	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Rikenellaceae;D_5__uncultured Rikenella sp.	0	0.069	0.27	0.044	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Tannerellaceae;D_5__Parabacteroides	0	0.25	0.62	2.0	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_5__	0	0.77	0.94	0.078	+
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Carnobacteriaceae;D_5__Granulicatella	0	0.96	0.98	9.0E-03	-

D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Lactobacillaceae;D_5__Lactobacillus	0	0.71	0.94	1.1	+
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Leuconostocaceae;D_5__Weissella	0	0.24	0.60	0.17	+
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Streptococcaceae;D_5__Streptococcus	0	0.41	0.76	3.6	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Clostridiaceae 1;D_5__Clostridium sensu stricto 1	0	0.86	0.98	0.59	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Agathobacter	0	0.88	0.98	1.4	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Anaerostipes	0	0.85	0.98	1.6	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__CAG-56	0	0.49	0.79	0.086	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Coprococcus 1	0	0.77	0.94	0.22	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Dorea	0	0.82	0.97	0.75	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Eisenbergiella	0	0.095	0.31	0.032	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__GCA-900066575	0	0.29	0.65	0.055	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnoclostridium	0	0.16	0.46	0.56	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospira	0	0.93	0.98	0.73	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospiraceae FCS020 group	0	0.75	0.94	0.054	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospiraceae NC2004 group	0	0.013	0.12	0.031	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospiraceae ND3007 group	0	0.76	0.94	0.67	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospiraceae NK4A136 group	0	0.75	0.94	0.27	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Marvinbryantia	0	0.31	0.66	0.068	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Moryella	0	0.45	0.77	0.022	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Roseburia	0	0.95	0.98	1.3	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Sellimonas	0	0.61	0.88	0.23	+



D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Tyzzerella	0	0.61	0.88	0.083	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Tyzzerella 4	0	0.96	0.98	0.32	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Eubacterium] eligens group	0	0.11	0.35	0.55	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Eubacterium] fissicatena group	0	0.13	0.39	0.013	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Eubacterium] hallii group	0	0.97	0.98	1.2	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Eubacterium] ruminantium group	0	0.18	0.49	0.19	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Eubacterium] ventriosum group	0	0.76	0.94	0.23	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Eubacterium] xylanophilum group	0	0.057	0.25	0.037	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Ruminococcus] gauvreauii group	0	0.44	0.77	0.41	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Ruminococcus] gnavus group	0	0.52	0.80	0.50	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Ruminococcus] torques group	0	0.16	0.46	1.3	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__uncultured	0	0.79	0.96	0.24	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;__	0	0.26	0.62	2.4	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Peptostreptococcaceae;D_5__Rombo utsia	0	0.35	0.72	0.030	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Peptostreptococcaceae;D_5__Terrisporobacter	0	0.70	0.94	0.082	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Peptostreptococcaceae;__	0	0.93	0.98	1.3	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Anaerotruncus	0	0.16	0.46	0.010	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Faecalibacterium	0	0.14	0.43	4.8	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Flavonifractor	0	0.51	0.79	0.16	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Fournierella	0	0.95	0.98	0.031	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminiclostridium 5	0	0.27	0.62	0.50	+

D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminoclostridium 9	0	0.90	0.98	0.079	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-002	0	0.046	0.21	1.1	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-013	0	0.77	0.94	0.34	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-014	0	0.41	0.76	0.59	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcus 1	0	0.45	0.77	1.5	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Subdoligranulum	0	0.95	0.98	2.3	-
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcus 1	0	0.092	0.31	0.015	+
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Catenibacterium	0	0.72	0.94	0.26	-
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Erysipelatoclostridium	0	0.45	0.77	0.52	+
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Faecalitalea	0	0.29	0.65	0.26	+
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Holdemanella	0	0.41	0.76	0.87	-
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Acidaminococcaceae;D_5__Acidaminococcus	0	0.41	0.76	0.057	-
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Acidaminococcaceae;D_5__Phascolarctobacterium	0	0.35	0.72	0.89	+
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Veillonellaceae;D_5__Megasphaera	0	0.88	0.98	0.42	-
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Veillonellaceae;D_5__Megasphaera	0	0.71	0.94	0.19	-
D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Desulfovibrionales;D_4__Desulfovibrionaceae;D_5__Desulfovibrio	0	0.20	0.52	0.051	+
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Parasutterella	0	0.92	0.98	0.13	-
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Sutterella	0	0.85	0.98	0.32	-
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;D_5__Sutterella	0	0.082	0.29	0.15	+
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Escherichia-Shigella	0	0.46	0.77	2.2	-
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Klebsiella	0	0.37	0.72	0.46	-

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;__	0	0.52	0.79	0.051	-
D_0__Bacteria;D_1__Synergistetes;D_2__Synergistia;D_3__Synergistales;D_4__Synergistaceae;D_5__Cloacibacillus	0	0.30	0.65	0.033	+

---

W indicates a statistical measure generated by ANCOM. *P*-value was calculated by Wilcoxon rank sum test. *Q*-value was calculated by the Benjamini-Hochberg method. We set the significance thresholds of  $W > 0.6 \times N$  (*N* is the number of taxa tested) and *q*-value  $< 0.05$ . Significant taxa are indicated in bold.

## Supplementary Table S2b. Families changed in RBD in our dataset

Family	W	p-value	q-value	Relative abundance in RBD (%)	Increased or decreased
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__ <b>Rikenellaceae</b>	38	5.2E-05	2.6E-03	2.7	+
D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__ <b>Akkermansiaceae</b>	36	6.0E-04	0.010	1.0	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Christensenellaceae	28	6.2E-03	0.031	0.74	+
D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Desulfovibrionales;D_4__Desulfovibrionaceae	28	8.5E-04	0.011	0.22	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Peptococcaceae	26	1.3E-04	3.1E-03	0.12	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Family XIII	23	1.1E-03	0.011	0.36	+
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pasteurellales;D_4__Pasteurellaceae	20	0.010	0.045	0.020	+
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Bacillaceae	18	3.8E-03	0.024	0.011	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Eubacteriaceae	18	3.0E-03	0.022	0.079	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Bifidobacteriales;D_4__Bifidobacteriaceae	16	0.018	0.077	3.1	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae	15	1.7E-03	0.014	23	+
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Enterococcaceae	10	0.066	0.21	0.024	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacteriia;D_3__Coriobacteriales;D_4__Atopobiaceae	9	0.26	0.58	0.026	+
D_0__Bacteria;D_1__Cyanobacteria;D_2__Oxyphotobacteria;D_3__Chloroplast;D_4__Solanum melongena (eggplant)	9	0.23	0.58	0.015	+
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Veillonellaceae	9	0.037	0.13	1.0	+
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Family XI	8	0.25	0.58	5.2E-03	+
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__	8	0.44	0.70	4.5E-03	+
D_0__Bacteria;D_1__Proteobacteria;D_2__Alphaproteobacteria;D_3__Rickettsiales;D_4__Mitochondria	8	0.59	0.79	3.2E-03	+

D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Deffluviitaleaceae	7	4.5E-03	0.025	0.013	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae	7	0.44	0.70	23	+
D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia;D_3__Saccharimonadales;D_4__Saccharimonadaceae	7	0.62	0.80	3.7E-03	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacteriia;D_3__Coriobacteriales;D_4__Eggerthellaceae	6	0.46	0.70	0.29	+
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae	6	0.58	0.79	2.8	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Micrococcales;D_4__Micrococcaceae	5	0.72	0.88	8.7E-03	+
D_0__Bacteria;D_1__Fusobacteria;D_2__Fusobacteriia;D_3__Fusobacteriales;D_4__Fusobacteriaceae	5	0.27	0.58	0.36	+
D_0__Bacteria;__;__;__;__	5	0.53	0.76	5.4E-03	+
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Carnobacteriaceae	3	0.96	0.98	9.0E-3	+
D_0__Bacteria;D_1__Synergistetes;D_2__Synergistia;D_3__Synergistales;D_4__Synergistaceae	3	0.38	0.70	0.058	+
D_0__Archaea;D_1__Euryarchaeota;D_2__Methanobacteria;D_3__Methanobacteriales;D_4__Methanobacteriaceae	2	0.030	0.11	0.31	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;__	2	0.77	0.90	0.080	+
D_0__Bacteria;D_1__Firmicutes;__;__;__	2	0.93	0.96	0.017	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Bacteroidaceae	1	0.41	0.70	21	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Marinifilaceae	1	0.05	0.15	0.38	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Actinomycetales;D_4__Actinomycetaceae	0	0.87	0.95	0.049	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacteriia;D_3__Coriobacteriales;D_4__Coriobacteriaceae	0	0.30	0.62	1.4	+
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacteriia;D_3__Coriobacteriales;D_4__Coriobacteriales Incertae Sedis	0	0.74	0.88	0.046	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Barnesiellaceae	0	0.35	0.68	0.79	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae	0	0.48	0.70	2.9	+
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Tannerellaceae	0	0.25	0.58	2.0	+
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Lactobacillaceae	0	0.72	0.88	1.1	+

D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Leuconostocaceae	0	0.34	0.68	0.19	+
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Streptococcaceae	0	0.42	0.70	3.8	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Clostridiaceae 1	0	0.87	0.95	0.59	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Clostridiales vadinBB60 group	0	0.09	0.25	0.044	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Peptostreptococcaceae	0	0.89	0.95	1.4	+
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;__	0	0.092	0.25	0.015	+
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Acidaminococcaceae	0	0.84	0.95	0.95	+
D_0__Bacteria;D_1__Lentisphaerae;D_2__Lentisphaeria;D_3__Victivallales;D_4__Victivallaceae	0	0.60	0.79	0.032	+
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae	0	0.99	0.99	0.6	+
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae	0	0.46	0.70	2.7	+

W indicates a statistical measure generated by ANCOM. *P*-value was calculated by Wilcoxon rank sum test. *Q*-value was calculated by the Benjamini-Hochberg method. We set the significance thresholds of  $W > 0.6 \times N$  (*N* is the number of taxa tested) and *q*-value  $< 0.05$ . Significant taxa are indicated in bold.

**Supplementary Table S3a. Effect sizes and relative abundances of all filtered genera in RBD in the meta-analysis of the Japanese and German datasets**

<b>Genus</b>	<b>Effect size</b>	<b>Relative abundance in RBD (%)</b>
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-004	0.33	0.18
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Rikenellaceae;D_5__Alistipes	0.29	2.7
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Family XIII;D_5__Family XIII AD3011 group	0.25	0.24
D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Akkermansiaceae;D_5__Akkermansia	0.24	2.0
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__UBA1819	0.24	0.14
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__[Eubacterium] coprostanoligenes group	0.23	1.4
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__uncultured	0.21	0.26
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Oscillibacter	0.21	0.39
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Bacillaceae;D_5__Bacillus	-0.20	8.6E-03
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcus 2	0.20	3.8
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Eisenbergiella	0.20	0.028
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae NK4A214 group	0.19	0.37
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Anaerotruncus	0.19	0.022
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__	0.19	2.2
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-010	0.19	0.12

D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Negativibacillus	0.19	0.18
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Holdemania	0.18	0.035
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Christensenellaceae;D_5__Christensenellaceae R-7 group	0.18	2.1
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospiraceae NC2004 group	0.18	0.070
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Turicibacter	-0.18	0.025
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__UC5-1-2E3	0.17	0.011
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__Prevotella 9	-0.17	1.7
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-005	0.17	0.55
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminiclostridium 6	0.17	0.57
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__DTU089	0.16	0.027
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Ruminococcus] torques group	0.16	1.3
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Eubacterium] xylanophilum group	0.16	0.093
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Bifidobacteriales;D_4__Bifidobacteriaceae;D_5__Bifidobacterium	-0.16	2.4
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Family XIII;D_5__[Eubacterium] nodatum group	0.14	0.010
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Faecalibacterium	-0.13	6.9
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-002	0.13	1.1
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Veillonellaceae;D_5__Dialister	-0.1	0.17



D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnoclostridium	0.13	0.61
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospiraceae UCG-004	-0.13	0.14
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Marinifilaceae;__	0.13	0.054
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminiclostridium 5	0.13	0.35
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae;__	0.12	0.62
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;__	0.12	4.1
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Tannerellaceae;D_5__Parabacteroides	0.11	2.1
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Coproccoccus 2	-0.11	0.24
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;__;__	0.11	0.27
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Marvinbryantia	0.11	0.10
D_0__Archaea;D_1__Euryarchaeota;D_2__Methanobacteria;D_3__Methanobacteriales;D_4__Methanobacteriaceae;D_5__Methanobrevibacter	0.11	0.21
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Marinifilaceae;D_5__Butyricimonas	0.11	0.14
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Butyricoccus	-0.10	0.29
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Barnesiellaceae;D_5__Barnesiella	0.10	0.68
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Erysipelatoclostridium	0.10	0.33
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Flavonifractor	0.10	0.16
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Intestinimonas	0.091	0.079
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Marinifilaceae;D_5__Odoribacter	0.090	0.31

D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia; D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__ _uncultured	0.089	0.027
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia; D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;__	0.089	0.037
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii a;D_3__Coriobacteriales;D_4__Eggerthellaceae;D_5__Sl ackia	0.085	0.053
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococc aceae UCG-014	0.081	0.48
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii a;D_3__Coriobacteriales;D_4__Eggerthellaceae;__	0.079	0.067
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Sellimonas	0.078	0.14
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D _3__Bacteroidales;D_4__Prevotellaceae;D_5__Prevotella 2	0.077	0.37
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D _3__Bacteroidales;D_4__Barnesiellaceae;D_5__unculture d	0.077	0.083
D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimon adia;D_3__Saccharimonadales;D_4__Saccharimonadacea e;D_5__TM7 phylum sp. canine oral taxon 250	-0.073	2.5E-03
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__[Eubacterium] eligans group	0.072	0.53
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Peptostreptococcaceae;D_5__Rombou tsia	0.072	0.018
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Coproccus 1	0.072	0.23
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D _3__Bacteroidales;D_4__Prevotellaceae;D_5__Paraprevo tella	0.071	0.27
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__ Selenomonadales;D_4__Veillonellaceae;D_5__Megas phaera	-0.071	0.11
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospirac eae UCG-008	-0.066	0.042
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Blautia	0.066	4.7
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii a;D_3__Coriobacteriales;D_4__Eggerthellaceae;D_5__un cultured	-0.066	0.043
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Ruminococcaceae;D_5__Candidatus Soleaferrea	-0.064	4.6E-03

D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia; D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__ _Catenibacterium	-0.064	0.28
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia; D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__ _Merdibacter	-0.063	6.7E-03
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococc aceae UCG-003	-0.062	0.12
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Clostridiaceae 1;D_5__Clostridium sensu stricto 1	-0.062	0.40
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lac tobacillales;D_4__Streptococcaceae;D_5__Streptococcus	0.059	2.2
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D _3__Bacteroidales;D_4__Barnesiellaceae;D_5__Coproba cter	0.059	0.087
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospira	-0.058	0.66
D_0__Bacteria;D_1__Synergistetes;D_2__Synergistia;D _3__Synergistales;D_4__Synergistaceae;D_5__Cloacibacil lus	0.056	0.034
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Fusicatenibact er	-0.056	1.1
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospirac eae ND3007 group	0.055	0.76
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Ruminococcaceae;D_5__GCA- 900066225	0.054	0.013
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii a;D_3__Coriobacteriales;D_4__Coriobacteriaceae;D_5__ Collinsella	-0.051	1.1
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Tyzzerella	0.048	0.061
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospirac eae UCG-010	-0.048	0.039
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia; D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__ _Erysipelotrichaceae UCG-003	-0.048	0.48
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii a;D_3__Coriobacteriales;D_4__Eggerthellaceae;D_5__Eg gerthella	-0.048	0.091
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii a;D_3__Coriobacteriales;D_4__Coriobacteriales Incertae Sedis;D_5__uncultured	0.047	0.040

D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Ruminococcus] gnavus group	0.047	0.35
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__[Clostridium] innocuum group	0.047	0.049
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Eubacterium] ruminantium group	0.046	0.38
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Faecalitalea	0.046	0.17
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Dorea	-0.043	0.63
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Phoceae	0.043	5.0E-03
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae;D_5__Klebsiella	-0.043	0.28
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Ruminococcus] gauvreauii group	0.043	0.38
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Peptostreptococcaceae;D_5__	-0.043	0.75
D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Desulfovibrionales;D_4__Desulfovibrionaceae;D_5__Desulfovibrio	0.040	0.29
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-009	0.039	1.1E-02
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Acidaminococcaceae;D_5__Phascolarctobacterium	0.032	0.86
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcaceae UCG-013	0.032	0.24
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Agathobacter	-0.032	1.7
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Roseburia	0.032	1.4
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Eubacterium] hallii group	0.031	1.2
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__Coprobacillus	0.030	0.019
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__	-0.028	0.27

D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Streptococcaceae;D_5__Lactococcus	0.027	0.12
D_0__Bacteria;D_1__Fusobacteria;D_2__Fusobacteriia;D_3__Fusobacteriales;D_4__Fusobacteriaceae;D_5__Fusobacterium	-0.027	0.20
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__[Eubacterium] ventriosum group	0.026	0.14
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminiclostridium 9	-0.024	0.11
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__Alloprevotella	-0.024	0.12
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteriia;D_3__Actinomycetales;D_4__Actinomycetaceae;D_5__Actinomyces	0.023	0.033
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacteriia;D_3__Coriobacteriales;D_4__Eggerthellaceae;D_5__Gordonibacter	0.023	0.012
D_0__Bacteria;__;__;__;__	-0.023	0.076
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae;D_5__uncultured	0.023	0.073
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Lactobacillaceae;D_5__Lactobacillus	0.021	0.91
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Moryella	0.021	0.019
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Tyzzerella 3	-0.019	0.040
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;__;__	0.018	0.42
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Peptostreptococcaceae;D_5__Terrisporobacter	-0.017	0.062
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae;D_5__Ruminococcus 1	-0.016	1.2
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospiraceae FCS020 group	0.012	0.077
D_0__Bacteria;D_1__Firmicutes;__;__;__;__	0.012	0.26
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacteriia;D_3__Coriobacteriales;D_4__Eggerthellaceae;D_5__Senechalimassilia	-7.7E-03	0.05
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae;D_5__CAG-56	7.0E-03	0.11

D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia; D_3__Erysipelotrichales;D_4__Erysipelotrichaceae;D_5__ _Holdemanella	6.8E-03	0.75
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Lachnospira eae NK4A136 group	-6.3E-03	0.47
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Ruminococcaceae;D_5__Oscillospira	5.5E-03	0.045
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__Anaerostipes	-4.7E-03	1.3
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacterii a;D_3__Coriobacteriales;D_4__Atopobiaceae;D_5__Olse nella	-4.6E-03	0.023
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D _3__Bacteroidales;D_4__Bacteroidaceae;D_5__Bacteroid es	4.2E-03	19
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__uncultured	-4.1E-03	0.17
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteo bacteria;D_3__Enterobacteriales;D_4__Enterobacteriace e;__	7.2E-04	0.03
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Ruminococcaceae;D_5__Subdoligran ulum	7.1E-04	2.2
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__ Clostridiales;D_4__Lachnospiraceae;D_5__GCA- 900066575	9.5E-05	0.059

---

**Supplementary Table S3b. Effect sizes and relative abundances of all filtered families in RBD in the meta-analysis of the Japanese and German datasets**

Family	Effect size	Relative abundance (%)
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Rikenellaceae	0.28	2.8
D_0__Bacteria;D_1__Verrucomicrobia;D_2__Verrucomicrobiae;D_3__Verrucomicrobiales;D_4__Akkermansiaceae	0.24	2.0
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Family XIII	0.21	0.31
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Bacillales;D_4__Bacillaceae	-0.20	8.6E-03
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Veillonellaceae	-0.19	0.63
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Christensenellaceae	0.18	2.1
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Pasteurellales;D_4__Pasteurellaceae	-0.18	0.020
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Marinifilaceae	0.17	0.52
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Ruminococcaceae	0.17	24
D_0__Bacteria;D_1__Proteobacteria;D_2__Deltaproteobacteria;D_3__Desulfovibrionales;D_4__Desulfovibrionaceae	0.16	0.40
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Bifidobacteriales;D_4__Bifidobacteriaceae	-0.16	2.4
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Clostridiales vadinBB60 group	0.12	0.080
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__	0.11	0.27
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Tannerellaceae	0.10	2.1
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Barnesiellaceae	0.087	0.86
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacteriia;D_3__Coriobacteriales;D_4__Atopobiaceae	-0.087	0.034
D_0__Archaea;D_1__Euryarchaeota;D_2__Methanobacteria;D_3__Methanobacteriales;D_4__Methanobacteriaceae	0.083	0.21
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacteriia;D_3__Coriobacteriales;D_4__Eggerthellaceae	-0.075	0.34

D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Betaproteobacteriales;D_4__Burkholderiaceae	-0.069	1.0
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Clostridiaceae 1	-0.062	0.40
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Streptococcaceae	0.061	2.3
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Prevotellaceae	-0.056	3.1
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacteriia;D_3__Coriobacteriales;D_4__Coriobacteriaceae	-0.051	1.1
D_0__Bacteria;D_1__Synergistetes;D_2__Synergistia;D_3__Synergistales;D_4__Synergistaceae	0.050	0.053
D_0__Bacteria;D_1__Fusobacteria;D_2__Fusobacteriia;D_3__Fusobacteriales;D_4__Fusobacteriaceae	-0.032	0.20
D_0__Bacteria;D_1__Lentisphaerae;D_2__Lentisphaeria;D_3__Victivallales;D_4__Victivallaceae	0.029	0.050
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Peptostreptococcaceae	-0.027	1.0
D_0__Bacteria;D_1__Actinobacteria;D_2__Coriobacteriia;D_3__Coriobacteriales;D_4__Coriobacteriales Incertae Sedis	0.026	0.043
D_0__Bacteria;D_1__Firmicutes;D_2__Clostridia;D_3__Clostridiales;D_4__Lachnospiraceae	0.025	24
D_0__Bacteria;__;__;__;	-0.023	0.076
D_0__Bacteria;D_1__Patescibacteria;D_2__Saccharimonadia;D_3__Saccharimonadales;D_4__Saccharimonadaceae	-0.021	3.6E-03
D_0__Bacteria;D_1__Firmicutes;D_2__Bacilli;D_3__Lactobacillales;D_4__Lactobacillaceae	0.019	0.91
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;__	0.018	0.42
D_0__Bacteria;D_1__Actinobacteria;D_2__Actinobacteria;D_3__Actinomycetales;D_4__Actinomycetaceae	0.016	0.033
D_0__Bacteria;D_1__Firmicutes;__;__;__	0.012	0.26
D_0__Bacteria;D_1__Proteobacteria;D_2__Gammaproteobacteria;D_3__Enterobacteriales;D_4__Enterobacteriaceae	-6.7E-03	2.1
D_0__Bacteria;D_1__Firmicutes;D_2__Negativicutes;D_3__Selenomonadales;D_4__Acidaminococcaceae	-6.0E-03	1.1
D_0__Bacteria;D_1__Firmicutes;D_2__Erysipelotrichia;D_3__Erysipelotrichales;D_4__Erysipelotrichaceae	4.4E-03	2.3
D_0__Bacteria;D_1__Bacteroidetes;D_2__Bacteroidia;D_3__Bacteroidales;D_4__Bacteroidaceae	4.2E-03	19

---



**Supplementary Table S4. Experimental methods and disease durations of the Japanese and German datasets**

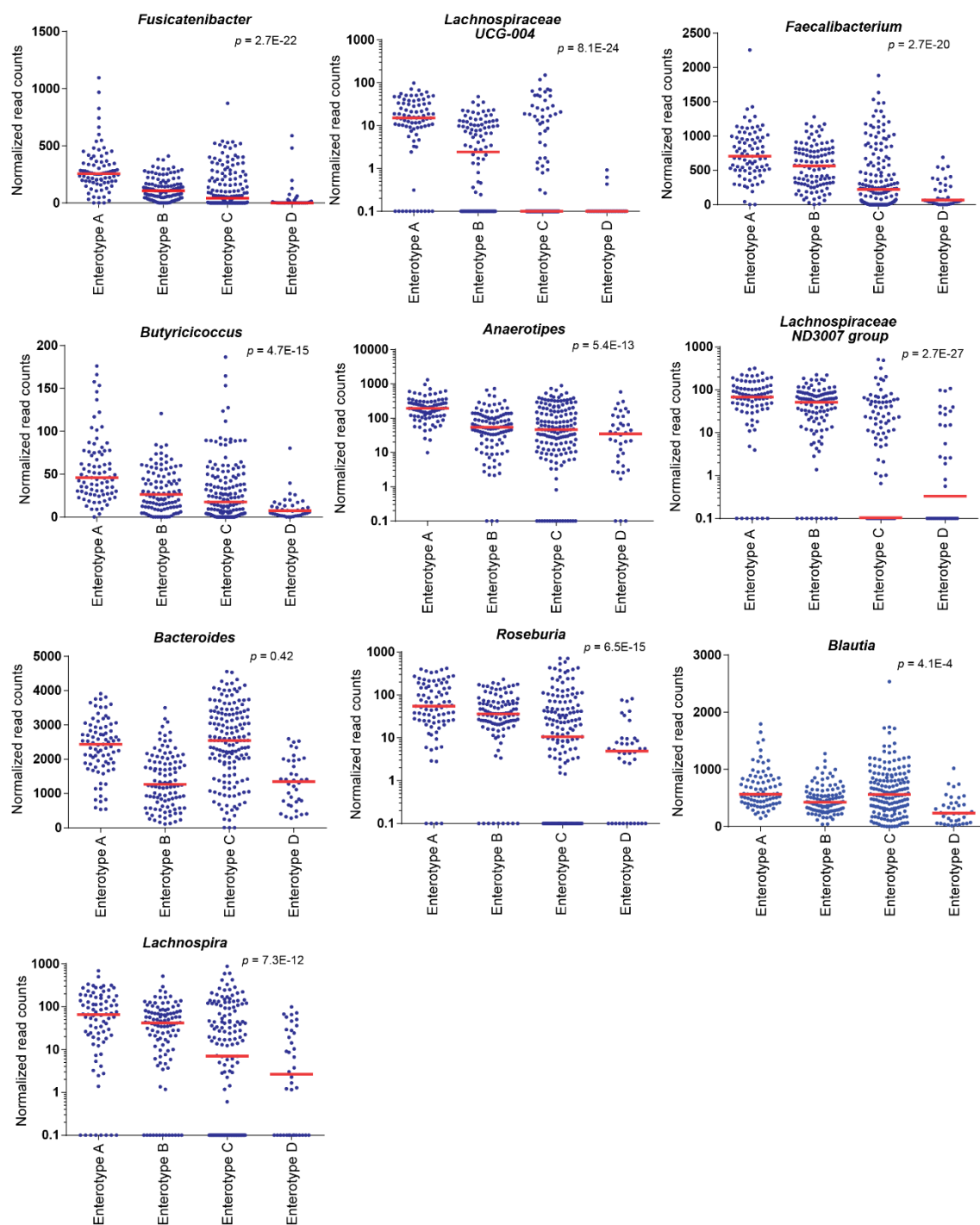
<b>Country</b>	<b>Transportation temperature</b>	<b>Storage method</b>	<b>Stool DNA stabilizer</b>	<b>Sequencing</b>	<b>Primers</b>	<b>Disease duration (years)<sup>a</sup></b>
Japan	0°C	Freeze dry	no	16S rRNA V3-4	341F/805R	6.4 ± 4.8 (Max 20, Min 0.1)
Germany	On dry ice	-80°C	no	16S rRNA V4	515F/805R	n.a.

<sup>a</sup>Mean and SD. n.a., not available.

## Supplementary Table S5. The numbers of read counts in the Japanese and

### German datasets

	(x 1000 read counts)				
<b>Country (sample size)</b>	<b>Average</b>	<b>SD</b>	<b>Median</b>	<b>Max</b>	<b>Min</b>
Japan (163)	53.5	18.7	50.8	98.0	13.8
Germany (58)	155.7	39.5	164.8	261.4	63.7



**Supplementary Figure S1.** Relative abundances of ten genera with the highest loadings in the first factor (Supplementary Table S3) are plotted against enterotypes A to D generated by LIGER. Bar indicates the median value. *P*-values of Jonckheere-Terpstra trend test are indicated to show whether the genus increases or decreases monotonically.