SUPPLEMENTARY RESULTS

Associations of clinical parameter with microbiome and metabolome

We have collected available serum glucose and total cholesterol levels from patients' medical records. On average, our participants were under the category of normal BMI (18.5<BMI<25.0). However, 16 participants (control, n=12; gastrectomy, n=4) fall to the overweight category (BMI>25.0)[1]. The control group also tends to have higher BMI (two-sided Mann-Whitney *U* (MWU) test: $P=1.224\times10^{-5}$). Serum glucose level did not differ significantly (two-sided Mann-Whitney *U* (MWU) test: P=0.147) between gastrectomy (n=50) and control (n=42) patients. In 35 subjects (gastrectomy, n=14; control, n=21), serum glucose levels were higher than normal (69–104 mg/dL). Total cholesterol level was significantly higher (two-sided MWU test: P=0.0145) in control (n=40) compared to gastrectomy (n=50) patients. The average amount of cholesterol in each group, however, remained within the normal range (128–219 mg/dL). Twenty-two participants (control, n=15; gastrectomy, n=7) have high cholesterol level (over 219 mg/dL).

To account for possible confounding effects of those clinical factors, we performed Permutational Multivariate Analysis of Variance (PERMANOVA) on the microbiome and metabolome between samples distance (Bray-curtis) using the clinical parameters (BMI, serum glucose, and total cholesterol level) as the predictor. The microbiome compositions were significantly varied along with the BMI (adonis: $R^2=0.0171$, P=0.0289; $R^2=0.0240$, $P=9.99\times10^{-4}$ for mOTU and MetaPhlAn2, respectively) but not in metabolome (adonis: $R^2=0.0120$, P=0.0699). However, the participants grouping explained the variance of microbiome better compare to the BMI (**Supplementary Table S4**). Therefore, the significantly different microbiome compositions along with the BMI, might be potentially explained by the participants' grouping (control and gastrectomy). Overall microbiome and metabolome composition did not vary significantly (adonis: P>0.05) in relation to the serum glucose and total cholesterol level (**Supplementary Table S4**).

Additionally, we tested associations of each microbial features (species, functional modules, and metabolite) with the clinical parameters (BMI, serum glucose, and total cholesterol) using the Multivariate associations with Linear Models (MaAsLin) R package. Among the species that were reported to be differentially enriched between control (n=56) and gastrectomy (n=50), we found that *Roseburia hominis*, *Eubacterium eligens*

34 that were enriched in gastrectomy (both by mOTU and MetaPhlan2 annotations) were
35 found to be negatively associated with the BMI and *Ruminococcus gnavus* that were
36 enriched in control were positively correlated to BMI (**Supplementary Tables S10 and**
37 **S11**). Similarly, to the PERMANOVA results, the associations values (explained variance)
38 were higher when we used the participants' grouping (control and gastrectomy) as the
39 predictor rather than the BMI (**Supplementary Tables S10 and S11**). We also calculated
40 the associations values between participants status and each species with and without
41 adjustment of BMI (**Supplementary Materials**). We found that the explained variances
42 were increased by 10% or more after adjustment for *Roseburia hominis* and
43 *Ruminococcus gnavus*. Therefore, the BMI might also affect the differential abundance of
44 these species in control and gastrectomy groups (**Supplementary Table S10 and S11**).
45 We did not observe differentially enriched species (**Supplementary Table S10 and S11**),
46 KEGG modules (**Supplementary Table 12**), and metabolites (**Supplementary Table**
47 **S14**) between gastrectomy and control group had significant associations with the serum
48 glucose and total cholesterol (MaAsLin: P>0.05, q>0.1).
49 In the function modules, we did not find overlap between KEGG modules annotated
50 by *in house* and HUMAnN2 pipeline that were in significant association with the clinical
51 parameters. Metabolites' association with the participants' demographic parameters by
52 MaAsLin showed that cholate enrichment in the control group might be affected by BMI,
53 whereas phenyl-lactate and arginine enrichment might be affected by total cholesterol
54 (**Supplementary Results** and **Supplementary Table S14**).
55
56 **Associations of medical history with the microbiome and metabolome**
57 Underlying comorbidities and concurrent medication of the participants may influence
58 gut microbiota. Therefore, we extracted information about the medical history of 23
59 diseases (e.g., hypertension, diabetes, dyslipidemia) and the history of usage of eight
60 drugs (e.g., diabetes medication, gastric acid-suppression medication, cholesterol
61 medication), which were obtained from questionnaires (**Supplementary Tables S2**). No
62 significant (Fisher's exact test: P>0.05) difference was observed in the distribution of
63 individuals with any disease history between gastrectomy and control groups (**Table 1**).
64 However, we found a significantly higher (Fisher's exact test: P<0.05) number of subjects
65 with a history of diabetes medication or gastric acid-suppression medication in the
66 control group. Thus, we performed PERMANOVA to assess how gastric acid-suppression

67 medication or diabetes medication contributed to variations in microbial community
68 data. The microbiome and metabolome compositions were not significantly different
69 between the users of these medications (adonis: P>0.05) (**Supplementary Table S4**). To
70 eliminate the possible confounding effect of gastric acid-suppression medication or
71 diabetes medication, we performed two-step analysis in addition to the associations
72 between microbial profiles (species, functions, metabolite) and drug usage by MaAsLin
73 (**Supplementary Methods**). First, we performed a comparison between participants
74 who took and did not take diabetes medication or gastric acid-suppression medication
75 within the control group to specifically assess the effect of drug usage. Second, we re-
76 evaluated the significantly different microbiome and metabolome between the two
77 groups after excluding users of these drugs and compared them with those before
78 exclusions, whom we referred to as "originally reported".
79
80 ***Gastric acid-suppression medication***
81 There were significant different distributions (Fisher's exact test: P=0.0375) of the user
82 of gastric acid-suppression medication in the gastrectomy (n=4) and control (n=13)
83 groups. PERMANOVA analysis showed that the microbiome and metabolome
84 composition did not vary significantly (P>0.05) between users (n=17) and non-users
85 (n=89) of gastric acid-suppression medication (**Supplementary Table S4**). Association
86 analysis by MaAsLin found that differentially enriched fecal microbial features (species,
87 KEGG modules, metabolites) between control (n=56) and gastrectomy (n=50) patients
88 were not associated with gastric acid-suppression medication (P>0.05, q>0.1)
89 (**Supplementary Tables S10, S11, S12, and S14**).
90 Furthermore, we compared the microbiome and metabolome compositions between
91 control participants who took (n=13) and did not take (n=43) gastric acid-suppression
92 medication usage by PERMANOVA and LEfSe. PERMANOVA analysis showed that the
93 composition of the mOTU-annotated species, MetaPhlan2-annotated species, and
94 metabolome (R^2=0.0195, P=0.337; R^2=0.0255, P=0.0829; R^2=0.0100, P=0.829,
95 respectively) (**Supplementary Table S4f**) were not significantly varied along with the
96 gastric acid-suppression medication in our control participants. LEfSe results showed
97 eight mOTU-annotated species and five MetaPhlAn2-annotated species that were
98 differentially enriched (LEfSe: P<0.05, q<0.1, LDA>2.0) between control participants who
99 took (n=13) and did not take (n=43) gastric acid-suppression medication

100 (**Supplementary Table S8**). Among them, *Streptococcus mutans* and *Haemophilus*
101 *parainfluenzae* were significantly enriched in the participants with gastric acid-
102 suppression medication both in mOTU and MetaPhlan2 species annotations. Twenty-two
103 and twenty-three KEGG modules annotated by *in-house* pipeline and HUMAnN2 pipeline,
104 respectively, were also significantly enriched (LEfSe: P<0.05, q<0.1, LDA>2.0) in the
105 participants with gastric acid-suppression medication. Among them, fourteen were
106 overlapped between two functional annotations. Interestingly, some of the microbiome
107 features that were enriched in gastric acid-suppression users overlapped with those
108 enriched in gastrectomy individuals (**Supplementary Table S8**). Those included two
109 nutrient transporter (M00229, "arginine transport system"; M00317, "manganese/iron
110 transport system"), two two-component regulatory system (M00447, "CpxA-CpxR
111 (envelope stress response) two-component regulatory system"; M00456, "ArcB-ArcA
112 (anoxic redox control) two-component regulatory system"), Cationic antimicrobial
113 peptide (CAMP) resistance (M00728), and "Pyruvate oxidation" (M00307) that were
114 significantly enriched in the gastrectomy. These findings might indicate some of the
115 microbiome features may be influenced by reduced gastric acid. We did not observe any
116 metabolites that were differentially enriched between the participants with or without
117 history of gastric acid-suppression medication.

118 Additionally, we examined the possible confounding effect of the gastric acid-
119 suppression medication by re-performing LEfSe on the subset of the two groups
120 excluding those with gastric acid-suppression medication (control, n=43; gastrectomy,
121 n=46) to confirm that the originally reported gastrectomy enriched microbiome and
122 metabolome signatures were not affected by the exclusion. In the species level, 31 out of
123 38 differentially enriched species (LEfSe: P<0.05, q<0.1, LDA>2.0) between control
124 (n=56) and gastrectomy (n= 50) groups before exclusion that overlap in annotation
125 based on MetaPhlAn2 and mOTU were at similar enrichment after exclusion
126 (**Supplementary Table S6**). From this analysis, we also observed the different
127 enrichment pattern of *Streptococcus mutans* before and after exclusion. The
128 *Streptococcus mutans* enrichment in the control group before exclusion might be
129 contributed by the participants with gastric acid-suppression medication. In fact, we
130 observed that *Streptococcus mutans* was enriched in the gastric acid-suppression
131 medication in the control group (**Supplementary Table S8**). In the functional modules
132 level, we detected 32 KEGG modules that were differentially enriched (LEfSe: P<0.05,

q<0.1, LDA>2.0) between control (n=56) and gastrectomy (n= 50) before exclusion and were overlapped in the uniref90 and KEGG gene-based annotation. Among them, 26 KEGG modules were conserved before and after exclusion (**Supplementary Table S6**). Similarly, large portions of significantly different metabolites (86 out of 104) between control and gastrectomy groups were conserved before and after exclusion (**Supplementary Table S6**). Thus, it may reflect that the differences of microbial features were mostly driven by gastrectomy rather than the gastric acid-suppression medication.

Diabetes medication

We compared the microbiome and metabolome compositions between control participants who took (n=12) and did not take (n=43) diabetes medication by PERMANOVA and LEfSe. The compositions of mOTU-annotated species by PERMANOVA were significantly varied along with diabetes medication (adonis: R^2=0.0342, P=0.0220) but it was not significantly different in the MetaPhlan2-annotated species and metabolome (R^2=0.0251, P=0.114; R^2=0.0178, P=0.477, respectively) (**Supplementary Table S4f**). LEfSe results showed eight mOTU-annotated species and one MetaPhlAn2-annotated species that were differentially enriched (LEfSe: P<0.05, q<0.1, LDA>2.0) between control participants who took (n=12) and did not take (n=43) diabetes medication (**Supplementary Table S9**). Among them, *Mitsuokella multacida* was significantly enriched (LEfSe: P=0.00159, q=0.0687, LDA=3.23; P=9.93×10^{-5}, q=0.0236, LDA=3.357, in mOTU and MetaPhlAn2 annotation, respectively) in the participants who took diabetes medication. We did not detect significantly different (LEfSe: P<0.05, q<0.1, LDA>2.0) functional modules that were overlapped based on the annotation by our *in-house* pipeline and HUMAnN2 and metabolites between these two groups (**Supplementary Table S9**).

Additionally, we re-performed LEfSe on the subset of the two groups excluding participants who took diabetes medication (control, n=43; gastrectomy, n=48) to confirm that the originally reported gastrectomy enriched microbiome and metabolome signatures were not affected by the exclusion. In the species level, 35 out of 38 differentially enriched species (LEfSe: P<0.05, q<0.1, LDA>2.0) between control (n=56) and gastrectomy (n= 50) group before exclusion that were annotated based on MetaPhlAn2 and mOTU were at similar enrichment after exclusion (**Supplementary Table S7**). In the functional modules level, we detected 32 KEGG modules that were

166 differentially enriched (LEfSe: P<0.05, q<0.1, LDA>2.0) between control (n=56) and
167 gastrectomy (n= 50) before exclusion and were overlapped in the uniref90 and KEGG
168 gene based annotation. Among them, 21 KEGG modules were conserved before and after
169 exclusion (**Supplementary Table S7**). Similarly, large portions of significantly different
170 metabolites (85 out of 94) between control and gastrectomy groups were conserved
171 before and after exclusion (**Supplementary Table S7**). We also did not find the
172 associations between each of microbial features (species, functional modules, and
173 metabolites) with the diabetes medications (MaAsLin: P>0.05, q>0.1). Thus, it may reflect
174 that the differences of microbial features were driven by gastrectomy rather than
175 diabetes medication.
176
177 **Microbiome, functional potential and metabolome differences in different types of**
178 **gastrectomy**
179 The post-gastrectomy patients in the present study were underwent different types of
180 gastrectomy (total gastrectomy, n=12 and subtotal gastrectomy, n=38) and followed by
181 different types of reconstructions (*Stomach-stomach anastomosis*, n=1; *Billroth I*, n=2;
182 *Jejunal interpositions*, n=6; *Pylorus-preserving gastrectomy,* n=8; *Roux-en-Y*, n=29). The
183 overall profiles analysis by PERMANOVA revealed a tendency towards different species
184 and metabolite composition between different types of and reconstructions (adonis:
185 P<0.05) (**Supplementary Table S4**). We additionally performed the LEfSe analysis to
186 compare the species, functional modules, and metabolites compositions in the different
187 types of gastrectomy. Regarding different types of surgical reconstructions, we were
188 limited by a small number of patients for each reconstruction. Therefore, any statistical
189 analysis may not be powerful enough to detect microbiome and metabolome differences
190 across reconstructions. To partially address this issue, we provided analysis on the
191 microbial features comparison between control (n=56) and patients with Roux-en Y
192 reconstruction (n=29). We also discuss the microbial features of interest and their
193 distribution in different types of surgery and reconstruction
194
195 ***Surgery types***
196 The overall profiles revealed a tendency towards different species and metabolite
197 composition between different types of surgery (total gastrectomy, n=12; subtotal
198 gastrectomy=38) (adonis: R^2=0.0318, P=0.0709; R^2=0.0370, P=0.175 for mOTU-

199 annotated species and metabolome, respectively); however, only MetaPhlAn2-annotated
200 species reached a statistically significant level (adonis: R^2=0.0337, P=0.0360)
201 (**Supplementary Table S4e**). Based on species categorizations, we did not observe
202 significantly different (P>0.05) compositions of oral microbes, aerobes, and facultative
203 anaerobes between total gastrectomy (n=12) and subtotal gastrectomy (n=38) groups
204 (**Supplementary Figure S2**). Species richness (Chao1 index) and diversity (Shannon-
205 Wiener alpha-diversity index) also not significantly varied (P>0.05) between those two
206 groups. However, the total relative abundance of oral species and aerobes tended to differ
207 between both types of gastrectomy (subtotal and total gastrectomy) and control groups
208 (**Supplementary Figure S2**).

209 Most of post-gastrectomy patients (38 of 50) underwent subtotal gastrectomy. Thus,
210 we performed LEfSe pairwise comparison between control (n=56) and each type of
211 surgery to analyze whether the detected microbiome features (species, functional
212 modules, and metabolites) were mainly represented in subtotal gastrectomy. From this
213 analysis, we recovered several microbiome features that mutually enriched in subtotal
214 and total gastrectomies in comparison to the control group (**Supplementary Table S5**).
215 Among 27 species that were significantly enriched (LEfSe: P<0.05, q<0.1, LDA>2.0) in the
216 gastrectomy group (n=50) compared to the control group (n=56) and overlapped in
217 between mOTU and MetaPhlAn2 annotations, seven species were mutually enriched in
218 the total and subtotal gastrectomy compared to the control group (**Supplementary**
219 **Table S5B and S5C**). In addition, ten species were enriched in the subtotal gastrectomy
220 (**Supplementary Table S5B**) and three species were enriched in the total gastrectomy
221 (**Supplementary Table S5C**) compared to the control group. Similar pattern was
222 observed in the metabolites enrichment in the gastrectomy. The majority of gastrectomy-
223 enriched metabolites were found to be enriched in the subtotal gastrectomy (43 out of
224 46). However, the different patterns were observed in the functional modules. There
225 were 8 out of 26 of the gastrectomy-enriched features were mutually enriched in both
226 types of gastrectomy (total and subtotal gastrectomy) compared to control. The majority
227 of modules which were thirteen functional modules were enriched in the total
228 gastrectomy, while seven functional modules were enriched in the subtotal gastrectomy
229 compared to the control group (**Supplementary Table S5**).

230 In addition, LEfSe analysis of total gastrectomy *versus* subtotal gastrectomy showed
231 that *Fusobacterium nucleatum* was enriched in the former (LEfSe: P=5.58×10^{-5}, q=0.0150,

232 LDA=2.34; P=1.45×10^{-5}, q=0.00366, LDA=2.14, for mOTU and MetaPhlAn2 annotations,
233 respectively) (**Supplementary Table S5**). A comparison between total gastrectomy
234 (n=12) and control (n=56) groups further confirmed enrichment of *F. nucleatum* in total
235 gastrectomy (LEfSe: P=1.53×10^{-5}, q=0.00205, LDA=2.87; P=4.12×10^{-6}, q=3.34×10^{-4},
236 LDA=1.43, in mOTU and MetaPhlAn2 annotations, respectively). Additionally, MaAsLin
237 confirmed positive associations between total gastrectomy and *F. nucleatum* (MaAsLin:
238 P=3.17×10^{-12}, q=4.58×10^{-8}, r=0.0122; P=3.78×10^{-5}, q=0.0143, r=0.00776, in mOTU and
239 MetaPhlAn2 annotations, respectively). Its enrichment might be reflecting its survival in
240 the higher pH environment following total gastrectomy. This is worth noting because *F.*
241 *nucleatum* has long been considered as an opportunistic pathogen which is important
242 during the development of the plaque biofilm and recently associated with
243 gastrointestinal related diseases such as colorectal cancer (CRC)[3]. None of the KEGG
244 modules and metabolites were significantly (LEfSe: P<0.05; q<0.1, LDA>2.0) enriched
245 either in total or subtotal gastrectomy (**Supplementary Table S5**). Therefore, the
246 majority type of gastrectomy might not highly affect the observed gastrectomy-enriched
247 signatures.
248
249 ***Reconstructions Type***
250 The post-gastrectomy patients underwent different types of reconstructions (*Stomach-*
251 *stomach anastomosis*, n=1; *Billroth I*, n=2; *Jejunal interpositions*, n=6, *Pylorus-preserving*
252 *gastrectomy*, n=8; *Roux-en-Y*, n=29). The overall species compositions (mOTU and
253 MetaPhlAn2-annotated species) were significantly different in different types of
254 reconstruction but not in metabolite profiles (adonis: R^2=0.123, P=0.0150; R^2=0.121,
255 P=0.0119; R^2=0.0986, P=0.483, for mOTU-annotated species, MetaPhlAn2-annotated
256 species, and metabolome, respectively, **Supplementary Table S4**). However, we were
257 limited by a small number of patients for each reconstruction types, thus, any statistical
258 analysis may not be powerful enough to detect microbiome and metabolome.
259 To partially address this issue, we show distribution patterns of microbial features
260 of interest (species, functional modules, and metabolites). First, in terms of predominant
261 species in post gastrectomy patients across different reconstructions, we observed two
262 patterns. The species enrichment that might reflect the Roux-en Y reconstructions
263 (**Pattern I**) and those that might be driven by other reconstructions (**Pattern II**). The
264 majority of predominantly enriched species in gastrectomy came into **Pattern I**. For

265 instances, in **Pattern I,** we observed that *Streptococcus anginosus*, *Streptococcus*
266 *parasanguinis*, *Streptococcus vestibularis* and *Streptococcus salivarius* were enriched in
267 patients undergoing Roux-en Y reconstruction, when we compared the control (n=56)
268 and Roux-en Y groups (n=29) (**Supplementary Table S18**). The distribution pattern also
269 showed that those species were more abundant among patients undergoing Roux-en Y
270 reconstruction (**Supplementary Figure S3**). Similar patterns were observed in three
271 species of *Veillonella* (**Supplementary Figure S3**). In contrast, three species of
272 *Lactobacillus* (*Lactobacillus gasseri*, *Lactobacillus oris* and *Lactobacillus salivarius*) were
273 more abundant in Billroth I reconstruction (**Supplementary Figure S3**). When we
274 compared control (n=50) and post-gastrectomy patients undergone Roux-en Y
275 reconstruction (n=29) we did not find that these three species were differentially
276 enriched (**Supplementary Table S18**). Thus, enrichment of those three species might be
277 driven by Billroth I reconstructions.
278 Different distribution pattern of CRC-related species such as *Atopobium parvulum*
279 and *F. nucleatum* were also observed in different reconstructions. *A. parvulum* were
280 observed to be more abundant in the Roux-en Y reconstructions both in total and subtotal
281 gastrectomies, while *F. nucleatum* were enriched in Roux-en Y reconstructions in patients
282 undergoing total gastrectomy (**Supplementary Figure S3**). These results were in
283 accordant with our analyses between total and subtotal gastrectomies (**Supplementary**
284 **Table S5**). We also observed different distribution pattern of metabolites that were
285 associated with CRC (**Supplementary Figure S4**).
286
287 ***Control versus Roux-en Y reconstructions***
288 Majority of post-gastrectomy patients (29 of 50) underwent Roux-en Y reconstruction
289 following the surgery. Thus, we performed LEfSe analysis to analyze whether the
290 detected microbiome features (species, functional modules, and metabolites) were
291 mainly represented in patients undergoing Roux-en Y reconstruction. We did recover
292 high number of microbial features that were overlapped when we compared the control
293 group (n=56) with the gastrectomy group (n=50) in a subset of post-gastrectomy patients
294 who underwent R-Y reconstruction (n=29). For instances, in the species level, 26 out of
295 38 of differentially enriched species (LEfSe: $P<0.05$, $q<0.1$, LDA>2.0) between control
296 (n=56) and gastrectomy (n= 50) groups that were overlapped in annotation based on
297 MetaPhlAn2 and mOTU were at similar enrichment in the comparison between control

298 (n=56) and Roux-en Y (n=29) (**Supplementary Table S18**). In the functional modules
299 level, the 32 KEGG modules that were differentially enriched (LEfSe: P<0.05, q<0.1,
300 LDA>2.0) between control (n=56) and gastrectomy (n= 50) and overlap in two
301 annotations pipelines (uniref90 and KEGG gene based-annotation) were also retained in
302 the comparison between control (n=56) and Roux-en Y (n=29) (**Supplementary Table**
303 **S18**). Similarly, large portions of significantly different metabolites (86 out of 104)
304 between control and gastrectomy groups were conserved before and after exclusion
305 (**Supplementary Table S18**). The non-overlap features might possibly come from other
306 reconstructions. The number of subjects in the other surgery reconstructions were
307 relatively low compared to the control to give a statistical power to account for the effects
308 of reconstructions to the microbiome and metabolome.
309
310 **Observed gastrointestinal complications following gastrectomy**
311 Information about any gastrointestinal complications (e.g., diarrhea, dumping syndrome,
312 anemia) was available for 47 out of 50 gastrectomy patients from their medical records
313 (**Supplementary Table S2**). Among 29 patients who had gastrointestinal complications
314 after gastrectomy, 15 subjects experienced dumping syndrome. After gastrectomy
315 patients were divided into those with (n=15) and without (n=32) dumping syndrome,
316 overall microbiome and metabolome profiles revealed a generally different composition,
317 but this was not statistically significant (adonis: R2=0.0557, P=0.0629; R2=0.0518,
318 P=0.0959; R2=0.0476, P=0.442, for mOTU-annotated species, MetaPhlAn2-annotated
319 species, and metabolome, respectively, **Supplementary Table S4e**). In addition, we did
320 not observe any significant difference (LEfSe: P>0.05, q>0.1) regarding the abundance of
321 species, functional modules, and metabolites between patients with and without
322 dumping syndrome (**Supplementary Table S17**). Notably, the diagnosis of dumping
323 syndrome depends mainly on the individual clinician's perspective.
324 Furthermore, we observed a high rate of dumping syndrome in patients with total
325 gastrectomy (8 out of 12 patients, 66.7%) compared to subtotal gastrectomy (7 out of 38,
326 18.4%), which does not deviate from previous studies such as a Japanese large-scale
327 investigation (n=1,153) reported by Mine S *et al.* (79.6% early and 48.7% late dumping
328 syndrome for total gastrectomy)[4]. In general, the frequency of postsurgical dumping
329 syndrome is estimated at 25-50%[5]. Such a wide range might be explained by diagnosis

330 for dumping syndrome being highly dependent on the individual clinician's
331 perspective[6].
332

333 Differences of species-species correlations between control and gastrectomy
334 groups

335 We performed the microbes correlations in the species level, owing to the different
336 characteristics of different species in the same genus. We observed that several co-
337 occurrence and co-excluding patterns that appeared at genus level were present also at
338 species level. The number of edges was higher ($\rho>0.4$; $\rho<-0.4$) in the control group (co-
339 occurrence, 31; co-excluding, 8) compared to the gastrectomy group (co-occurrence, 25;
340 co-excluding, 1). *Veillonella*, which formed the hub of the network in the gastrectomy
341 group, was confirmed at species-level network (**Supplementary Figure S8**). Loss of
342 edges between *Veillonella* and *Lactobacillus* as well as *Anaerotruncus* and *Alistipes* in the
343 microbes' network in the gastrectomy group was observed also at species level. In the
344 species-level network, the species in the same genus tended to form a common cluster,
345 such as the cluster of species from *Streptococcus* and *Veillonella* genera. It should be
346 noted, however, that some patterns have been lost in the species-level network during
347 network construction process as only differentially abundant species between the
348 gastrectomy and control groups, which overlapped in mOTU and MetaPhlAn2 pipelines,
349 were used. This was the case, for example, of the genus *Coprobacillus*, which disappeared
350 in the species network.

351 **SUPPLEMENTARY TABLES**

352 **Supplementary Table S1.**

353 Clinical characteristics of participants in post-gastrectomy and control groups

354 **Supplementary Table S2.**

355 Clinical characteristics of each participant based on medical records

356 **Supplementary Table S3.**

357 Quality control and annotation profile of each sample

358 **Supplementary Table S4.**

359 PERMANOVA analysis between microbiome and metabolome based on clinical
360 parameters, demographic data, and medical history

361 **Supplementary Table S5.**

362 Microbiome and metabolome enrichment in pairwise comparisons between different
363 types of gastrectomy and the control group

364 **Supplementary Table S6.**

365 Microbiome and metabolome profiles after exclusion of gastric acid-suppression
366 medication users (control, n=43; gastrectomy, n=46)

367 **Supplementary Table S7.**

368 Microbiome and metabolome profiles after exclusion of diabetes therapeutic medication
369 users (control, n=43; gastrectomy, n=48)

370 **Supplementary Table S8.**

371 Effect of gastric acid-suppression medication on microbiome and metabolome profiles in
372 the control group (user, n=13; non-user, n=43)

373 **Supplementary Table S9.**

374 Effect of diabetes therapeutic drugs on microbiome and metabolome profiles in the
375 control group (user, n=12; non-user, n=43)

376 **Supplementary Table S10.**

377 Significantly different taxa between gastrectomy and control groups and their associated
378 clinical information (annotation with mOTU)

379 **Supplementary Table S11.**

380 Significantly different taxa between gastrectomy and control groups and their associated
381 clinical information (annotation with MetaPhlAn2)

382 **Supplementary Table S12.**

383 Significantly different KEGG modules between gastrectomy and control groups and their
384 associated demographic information (annotation by *in house* pipeline and HUMAnN2)
385 **Supplementary Table S13.**
386 Species alpha-diversity and richness of KEGG modules contributor
387 **Supplementary Table S14.**
388 Significantly different metabolites between gastrectomy and control groups and their
389 associated demographic information
390 **Supplementary Table S15.**
391 MIMOSA output showing the species predicted to contribute to each metabolite
392 **Supplementary Table S16.**
393 Consumption of each dietary component in post-gastrectomy and control groups
394 **Supplementary Table S17.**
395 Microbiome and metabolome profiles in participants with (n=15) and without (n=32)
396 dumping syndrome
397 **Supplementary Table S18.**
398 Microbiome and metabolome profiles between control (n=56) and post-gastrectomy
399 patients undergoing Roux-en-Y reconstruction (n=29)
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413 **SUPPLEMENTARY FIGURES**

414 **Supplementary Figure S1. Study participants' overview and analysis workflow**

415 (**A**) Sample collection and general analysis workflow. (**B**) Detailed workflow for our
416 metagenome pipeline including quality filtering, functional annotation, and taxonomic
417 annotation.

418

419 **Supplementary Figure S2. Microbiome and metabolome composition in different**
420 **types of surgery (total gastrectomy *versus* subtotal gastrectomy)**

421 Principal coordinates analysis (PCoA) with Bray-Curtis distance (**A**) was performed to
422 assess the community structure of species' relative abundance obtained by mOTU and
423 MetaPhlAn2, and metabolites in the subtotal gastrectomy group (n=38) (**red**) and in the
424 total gastrectomy group (n=12) (**blue**). Species richness was measured using the Chao1
425 index (**B**) calculated from the species annotated by mOTU and MetaPhlAn2. Species
426 alpha-diversity was measured using the Shannon-Wiener index (**C**) based on mOTU and
427 MetaPhlAn2 annotation. The summed relative abundances of oral microbes (**D**) were
428 compared between the control (n=50), subtotal gastrectomy (n=38), and total
429 gastrectomy (n=12) groups based on species annotated by mOTU and MetaPhlAn2
430 annotation. The summed relative abundances of aerobes (**E**) and facultative anaerobes
431 (**F**) were also compared between the three groups.

432

433 **Supplementary Figure S3. Species distributions in different types of surgery and**
434 **reconstructions**

435 Relative abundances (log10) were plotted as boxplots to shows the distribution of each
436 species of interests. Two distributions patterns were observed. Most species followed
437 Pattern I which reflects the Roux-en-Y reconstructions (A). Several species followed
438 Pattern II and they might be driven by other reconstructions (B). CRC-enriched species
439 that were enriched in the gastrectomy group also showed different distribution patterns
440 across reconstructions (C). Different types of reconstructions are labeled with different
441 colors (see legends in the figures). The distributions were also divided into control
442 (n=50), subtotal gastrectomy (n=38), and total gastrectomy (n=12).

443

444 **Supplementary Figure S4. Metabolites distributions in different types of surgery**
445 **and reconstructions**

The concentrations of metabolites (nmol/g) were plotted as boxplots to shows the distributions of each predominant metabolite that were associated to colorectal cancer and were enriched in the gastrectomy group (**A**). Different types of reconstructions are labeled with different colors (**see legends in the figures**). The distributions were also divided into control (n=50), subtotal gastrectomy (n=38), and total gastrectomy (n=12).

Supplementary Figure S5. Comparison between the variance explained calculated in terms of the participants' control or gastrectomy groups (crude coefficient) and those adjusted by possible confounder and demographic data (adjusted coefficient) as explanatory variable

Crude and adjusted coefficient (variance explained) are shown as scatter plots. The adjusted coefficients were calculated by adjusting for possible confounders (BMI, total cholesterol, status of diabetes medication, gastric acids-suppression medication) in addition to the demographic variables (age and gender) as explanatory in (**A-E**). Each dot represents the response variable (species, KEGG modules, metabolites). The **red** dot represents the features which are significantly (LEfSe: P<0.05, q<0.1, LDA>2.0) different between control (n=56) and gastrectomy (n=50) groups based on species annotated by mOTU (**A**), species annotated by MetaPhlAn2 (**B**), functional modules annotated by the *in-house* pipeline (**C**), functional modules annotated by the HUMAnN2 pipeline (**D**), and metabolites (**E**)

Supplementary Figure S6. Differences in KEGG modules between control (n=56) and gastrectomy (n=50) groups

(**A**) Relative abundance and LDA score (log10) of KEGG modules annotated by our *in-house* pipeline (LEfSe: P<0.05, q<0.1, LDA>2.0). (**B**) Relative abundance and LDA score (log10) of KEGG modules annotated by HUMAnN2 (LEfSe: P<0.05, q<0.1, LDA>2.0).

Supplementary Figure S7. Species contribution to KEGG modules

KEGG modules involved in phosphate transport (**A**) and manganese/zinc/iron/ transport (**B**) that were differentially abundant between the gastrectomy (n=50) and control (n=56) groups are annotated by their taxonomic contributor (**see legends in the figure**). The KEGG modules' relative abundances are represented by the top value of each stack of bars. Samples were subsequently sorted according to the dominant contributor to a

479 module and then grouped as either gastrectomy or control (sample in order differs
480 between panels).
481
482 **Supplementary Figure S8. Species-species correlations**
483 Co-occurrence (**red**) and co-excluding (**green**) relationships between species (SparCC: -
484 0.4< ρ<0.4, $P<0.05$) in gastrectomy (n=44) (**A**) and control (n=54) (**B**) groups. The edge
485 width corresponds to the SparCC correlation coefficients. The nodes' size is scaled based
486 on the genus relative abundance averaged over participants within each group. Nodes'
487 color represents enrichment of the genus in gastrectomy (**orange**) and control (**blue**)
488 participants.
489
490 **Supplementary Figure S9. Procrustes analysis between species and metabolites**
491 Principal component analysis (PCA) plots for mOTU-annotated species (**A**), MetaPhlAn2-
492 annotated species (**B**), and metabolites profile (**C**). Procrustes analysis was performed
493 between metabolite profiles and species annotated by mOTU (**D**) and MetaPhlAn2 (**E**).
494
495 **Supplementary Figure S10. Genus contribution to metabolites based on MIMOSA**
496 **analysis**
497 Each table cell in the matrix represents the contribution of a particular genus to a
498 metabolite (**see legends in the figure**). Table cells are colored based on the community
499 metabolic potential (CMP) score calculated from the KEGG reaction data and KO relative
500 abundances stratified by species information. The species were later summarized at the
501 genus level. Each metabolite is given a prediction level, which represents how the
502 observed metabolite values are consistent or contrasting with the predicted metabolite-
503 producing potential. High prediction scores indicate that a metabolite is enriched in the
504 gastrectomy group, and it is predicted to be produced in sufficient amounts by a certain
505 genus. Based on reaction information, MIMOSA predicted metabolite enrichment in one
506 of the groups and compared that enrichment trend to those observed in the actual
507 quantification. A positive value (**green**) shows a consistent trend and a negative value
508 (**orange**) shows a contrasting trend compared to the measured metabolite.

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