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=1.224×10^{-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=9.99×10^{-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...
that were enriched in gastrectomy (both by mOTU and MetaPhlan2 annotations) were found to be negatively associated with the BMI and *Ruminococcus gnatus* that were enriched in control were positively correlated to BMI ([Supplementary Tables S10 and S11](#)). Similarly, to the PERMANOVA results, the associations values (explained variance) were higher when we used the participants' grouping (control and gastrectomy) as the predictor rather than the BMI ([Supplementary Tables S10 and S11](#)). We also calculated the associations values between participants status and each species with and without adjustment of BMI ([Supplementary Materials](#)). We found that the explained variances were increased by 10% or more after adjustment for *Roseburia hominis* and *Ruminococcus gnatus*. Therefore, the BMI might also affect the differential abundance of these species in control and gastrectomy groups ([Supplementary Table S10 and S11](#)). We did not observe differentially enriched species ([Supplementary Table S10 and S11](#)), KEGG modules ([Supplementary Table 12](#)), and metabolites ([Supplementary Table S14](#)) between gastrectomy and control group had significant associations with the serum glucose and total cholesterol (MaAsLin: P>0.05, q>0.1).

In the function modules, we did not find overlap between KEGG modules annotated by *in house* and HUMAnN2 pipeline that were in significant association with the clinical parameters. Metabolites' association with the participants’ demographic parameters by MaAsLin showed that cholate enrichment in the control group might be affected by BMI, whereas phenyl-lactate and arginine enrichment might be affected by total cholesterol ([Supplementary Results](#) and [Supplementary Table S14](#)).

**Associations of medical history with the microbiome and metabolome**

Underlying comorbidities and concurrent medication of the participants may influence gut microbiota. Therefore, we extracted information about the medical history of 23 diseases (e.g., hypertension, diabetes, dyslipidemia) and the history of usage of eight drugs (e.g., diabetes medication, gastric acid-suppression medication, cholesterol medication), which were obtained from questionnaires ([Supplementary Tables S2](#)). No significant (Fisher’s exact test: P>0.05) difference was observed in the distribution of individuals with any disease history between gastrectomy and control groups ([Table 1](#)). However, we found a significantly higher (Fisher’s exact test: P<0.05) number of subjects with a history of diabetes medication or gastric acid-suppression medication in the control group. Thus, we performed PERMANOVA to assess how gastric acid-suppression
medication or diabetes medication contributed to variations in microbial community data. The microbiome and metabolome compositions were not significantly different between the users of these medications (adonis: P>0.05) (Supplementary Table S4). To eliminate the possible confounding effect of gastric acid-suppression medication or diabetes medication, we performed two-step analysis in addition to the associations between microbial profiles (species, functions, metabolite) and drug usage by MaAsLin (Supplementary Methods). First, we performed a comparison between participants who took and did not take diabetes medication or gastric acid-suppression medication within the control group to specifically assess the effect of drug usage. Second, we re-evaluated the significantly different microbiome and metabolome between the two groups after excluding users of these drugs and compared them with those before exclusions, whom we referred to as “originally reported”.

**Gastric acid-suppression medication**

There were significant different distributions (Fisher’s exact test: P=0.0375) of the user of gastric acid-suppression medication in the gastrectomy (n=4) and control (n=13) groups. PERMANOVA analysis showed that the microbiome and metabolome composition did not vary significantly (P>0.05) between users (n=17) and non-users (n=89) of gastric acid-suppression medication (Supplementary Table S4). Association analysis by MaAsLin found that differentially enriched fecal microbial features (species, KEGG modules, metabolites) between control (n=56) and gastrectomy (n=50) patients were not associated with gastric acid-suppression medication (P>0.05, q>0.1) (Supplementary Tables S10, S11, S12, and S14).

Furthermore, we compared the microbiome and metabolome compositions between control participants who took (n=13) and did not take (n=43) gastric acid-suppression medication usage by PERMANOVA and LEfSe. PERMANOVA analysis showed that the composition of the mOTU-annotated species, MetaPhlan2-annotated species, and metabolome (R²=0.0195, P=0.337; R²=0.0255, P=0.0829; R²=0.0100, P=0.829, respectively) (Supplementary Table S4f) were not significantly varied along with the gastric acid-suppression medication in our control participants. LEfSe results showed eight mOTU-annotated species and five MetaPhAn2-annotated species that were differentially enriched (LEfSe: P<0.05, q<0.1, LDA>2.0) between control participants who took (n=13) and did not take (n=43) gastric acid-suppression medication.
Among them, *Streptococcus mutans* and *Haemophilus parainfluenzae* were significantly enriched in the participants with gastric acid-suppression medication both in mOTU and MetaPhlan2 species annotations. Twenty-two and twenty-three KEGG modules annotated by *in-house* pipeline and HUMAnN2 pipeline, respectively, were also significantly enriched (LEfSe: P<0.05, q<0.1, LDA>2.0) in the participants with gastric acid-suppression medication. Among them, fourteen were overlapped between two functional annotations. Interestingly, some of the microbiome features that were enriched in gastric acid-suppression users overlapped with those enriched in gastrectomy individuals (**Supplementary Table S8**). Those included two nutrient transporter (M00229, "arginine transport system"; M00317, "manganese/iron transport system"), two two-component regulatory system (M00447, "CpxA-CpxR (envelope stress response) two-component regulatory system"; M00456, "ArcB-ArcA (anoxic redox control) two-component regulatory system"), Cationic antimicrobial peptide (CAMP) resistance (M00728), and "Pyruvate oxidation" (M00307) that were significantly enriched in the gastrectomy. These findings might indicate some of the microbiome features may be influenced by reduced gastric acid. We did not observe any metabolites that were differentially enriched between the participants with or without history of gastric acid-suppression medication.

Additionally, we examined the possible confounding effect of the gastric acid-suppression medication by re-performing LEfSe on the subset of the two groups excluding those with gastric acid-suppression medication (control, n=43; gastrectomy, n=46) to confirm that the originally reported gastrectomy enriched microbiome and metabolome signatures were not affected by the exclusion. In the species level, 31 out of 38 differentially enriched species (LEfSe: P<0.05, q<0.1, LDA>2.0) between control (n=56) and gastrectomy (n= 50) groups before exclusion that overlap in annotation based on MetaPhlAn2 and mOTU were at similar enrichment after exclusion (**Supplementary Table S6**). From this analysis, we also observed the different enrichment pattern of *Streptococcus mutans* before and after exclusion. The *Streptococcus mutans* enrichment in the control group before exclusion might be contributed by the participants with gastric acid-suppression medication. In fact, we observed that *Streptococcus mutans* was enriched in the gastric acid-suppression medication in the control group (**Supplementary Table S8**). In the functional modules 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: R²=0.0342, P=0.0220) but it was not significantly different in the MetaPhlAn2-annotated species and metabolome (R²=0.0251, P=0.114; R²=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
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, 21 KEGG modules were conserved before and after exclusion (Supplementary Table S7). Similarly, large portions of significantly different metabolites (85 out of 94) between control and gastrectomy groups were conserved before and after exclusion (Supplementary Table S7). We also did not find the associations between each of microbial features (species, functional modules, and metabolites) with the diabetes medications (MaAsLin: P>0.05, q>0.1). Thus, it may reflect that the differences of microbial features were driven by gastrectomy rather than diabetes medication.

Microbiome, functional potential and metabolome differences in different types of gastrectomy

The post-gastrectomy patients in the present study were underwent different types of gastrectomy (total gastrectomy, n=12 and subtotal gastrectomy, n=38) and followed by different types of reconstructions (Stomach-stomach anastomosis, n=1; Billroth I, n=2; Jejunal interpositions, n=6; Pylorus-preserving gastrectomy, n=8; Roux-en-Y, n=29). The overall profiles analysis by PERMANOVA revealed a tendency towards different species and metabolite composition between different types of and reconstructions (adonis: P<0.05) (Supplementary Table S4). We additionally performed the LEfSe analysis to compare the species, functional modules, and metabolites compositions in the different types of gastrectomy. Regarding different types of surgical reconstructions, we were limited by a small number of patients for each reconstruction. Therefore, any statistical analysis may not be powerful enough to detect microbiome and metabolome differences across reconstructions. To partially address this issue, we provided analysis on the microbial features comparison between control (n=56) and patients with Roux-en Y reconstruction (n=29). We also discuss the microbial features of interest and their distribution in different types of surgery and reconstruction.

Surgery types

The overall profiles revealed a tendency towards different species and metabolite composition between different types of surgery (total gastrectomy, n=12; subtotal gastrectomy=38) (adonis: $R^2=0.0318$, P=0.0709; $R^2=0.0370$, P=0.175 for mOTU-
annotated species and metabolome, respectively); however, only MetaPhlAn2-annotated species reached a statistically significant level (adonis: $R^2=0.0337$, $P=0.0360$) (Supplementary Table S4e). Based on species categorizations, we did not observe significantly different ($P>0.05$) compositions of oral microbes, aerobes, and facultative anaerobes between total gastrectomy ($n=12$) and subtotal gastrectomy ($n=38$) groups (Supplementary Figure S2). Species richness (Chao1 index) and diversity (Shannon-Wiener alpha-diversity index) also not significantly varied ($P>0.05$) between those two groups. However, the total relative abundance of oral species and aerobes tended to differ between both types of gastrectomy (subtotal and total gastrectomy) and control groups.

Most of post-gastrectomy patients (38 of 50) underwent subtotal gastrectomy. Thus, we performed LEfSe pairwise comparison between control ($n=56$) and each type of surgery to analyze whether the detected microbiome features (species, functional modules, and metabolites) were mainly represented in subtotal gastrectomy. From this analysis, we recovered several microbiome features that mutually enriched in subtotal and total gastrectomies in comparison to the control group (Supplementary Table S5).

Among 27 species that were significantly enriched (LEfSe: $P<0.05$, $q<0.1$, LDA>2.0) in the gastrectomy group ($n=50$) compared to the control group ($n=56$) and overlapped in between mOTU and MetaPhlAn2 annotations, seven species were mutually enriched in the total and subtotal gastrectomy compared to the control group (Supplementary Table S5B and S5C). In addition, ten species were enriched in the subtotal gastrectomy (Supplementary Table S5B) and three species were enriched in the total gastrectomy (Supplementary Table S5C) compared to the control group. Similar pattern was observed in the metabolites enrichment in the gastrectomy. The majority of gastrectomy-enriched metabolites were found to be enriched in the subtotal gastrectomy (43 out of 46). However, the different patterns were observed in the functional modules. There were 8 out of 26 of the gastrectomy-enriched features were mutually enriched in both types of gastrectomy (total and subtotal gastrectomy) compared to control. The majority of modules which were thirteen functional modules were enriched in the total gastrectomy, while seven functional modules were enriched in the subtotal gastrectomy compared to the control group (Supplementary Table S5).

In addition, LEfSe analysis of total gastrectomy versus subtotal gastrectomy showed that *Fusobacterium nucleatum* was enriched in the former (LEfSe: $P=5.58\times10^{-5}$, $q=0.0150$,
LDA=2.34; P=1.45×10⁻⁵, q=0.00366, LDA=2.14, for mOTU and MetaPhlAn2 annotations, respectively) (Supplementary Table S5). A comparison between total gastrectomy (n=12) and control (n=56) groups further confirmed enrichment of *F. nucleatum* in total gastrectomy (LEfSe: P=1.53×10⁻⁵, q=0.00205, LDA=2.87; P=4.12×10⁻⁶, q=3.34×10⁻⁴, LDA=1.43, in mOTU and MetaPhlAn2 annotations, respectively). Additionally, MaAsLin confirmed positive associations between total gastrectomy and *F. nucleatum* (MaAsLin: 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 MetaPhlAn2 annotations, respectively). Its enrichment might be reflecting its survival in the higher pH environment following total gastrectomy. This is worth noting because *F. nucleatum* has long been considered as an opportunistic pathogen which is important during the development of the plaque biofilm and recently associated with gastrointestinal related diseases such as colorectal cancer (CRC)[3]. None of the KEGG modules and metabolites were significantly (LEfSe: P<0.05; q<0.1, LDA>2.0) enriched either in total or subtotal gastrectomy (Supplementary Table S5). Therefore, the majority type of gastrectomy might not highly affect the observed gastrectomy-enriched signatures.

Reconstructions Type

The post-gastrectomy patients underwent different types of reconstructions (*Stomach-stomach anastomosis*, n=1; *Billroth I*, n=2; *Jejunal interpositions*, n=6, *Pylorus-preserving gastrectomy*, n=8; *Roux-en-Y*, n=29). The overall species compositions (mOTU and MetaPhlAn2-annotated species) were significantly different in different types of reconstruction but not in metabolite profiles (adonis: R²=0.123, P=0.0150; R²=0.121, P=0.0119; R²=0.0986, P=0.483, for mOTU-annotated species, MetaPhlAn2-annotated species, and metabolome, respectively, Supplementary Table S4). However, we were limited by a small number of patients for each reconstruction types, thus, any statistical analysis may not be powerful enough to detect microbiome and metabolome.

To partially address this issue, we show distribution patterns of microbial features of interest (species, functional modules, and metabolites). First, in terms of predominant species in post gastrectomy patients across different reconstructions, we observed two patterns. The species enrichment that might reflect the Roux-en-Y reconstructions (Pattern I) and those that might be driven by other reconstructions (Pattern II). The majority of predominantly enriched species in gastrectomy came into Pattern I. For
instances, in **Pattern I**, we observed that *Streptococcus anginosus, Streptococcus parasanguinis, Streptococcus vestibularis* and *Streptococcus salivarius* were enriched in patients undergoing Roux-en Y reconstruction, when we compared the control (n=56) and Roux-en Y groups (n=29) (**Supplementary Table S18**). The distribution pattern also showed that those species were more abundant among patients undergoing Roux-en Y reconstruction (**Supplementary Figure S3**). Similar patterns were observed in three species of *Veillonella* (**Supplementary Figure S3**). In contrast, three species of *Lactobacillus* (*Lactobacillus gasseri, Lactobacillus oris* and *Lactobacillus salivarius*) were more abundant in Billroth I reconstruction (**Supplementary Figure S3**). When we compared control (n=50) and post-gastrectomy patients undergone Roux-en Y reconstruction (n=29) we did not find that these three species were differentially enriched (**Supplementary Table S18**). Thus, enrichment of those three species might be driven by Billroth I reconstructions.

Different distribution pattern of CRC-related species such as *Atopobium parvulum* and *F. nucleatum* were also observed in different reconstructions. *A. parvulum* were observed to be more abundant in the Roux-en Y reconstructions both in total and subtotal gastrectomies, while *F. nucleatum* were enriched in Roux-en Y reconstructions in patients undergoing total gastrectomy (**Supplementary Figure S3**). These results were in accordant with our analyses between total and subtotal gastrectomies (**Supplementary Table S5**). We also observed different distribution pattern of metabolites that were associated with CRC (**Supplementary Figure S4**).

**Control versus Roux-en Y reconstructions**

Majority of post-gastrectomy patients (29 of 50) underwent Roux-en Y reconstruction following the surgery. Thus, we performed LEfSe analysis to analyze whether the detected microbiome features (species, functional modules, and metabolites) were mainly represented in patients undergoing Roux-en Y reconstruction. We did recover high number of microbial features that were overlapped when we compared the control group (n=56) with the gastrectomy group (n=50) in a subset of post-gastrectomy patients who underwent R-Y reconstruction (n=29). For instances, in the species level, 26 out of 38 of differentially enriched species (LEfSe: P<0.05, q<0.1, LDA>2.0) between control (n=56) and gastrectomy (n= 50) groups that were overlapped in annotation based on MetaPhlAn2 and mOTU were at similar enrichment in the comparison between control
(n=56) and Roux-en Y (n=29) (Supplementary Table S18). In the functional modules level, the 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) and overlap in two annotations pipelines (uniref90 and KEGG gene based-annotation) were also retained in the comparison between control (n=56) and Roux-en Y (n=29) (Supplementary Table S18). Similarly, large portions of significantly different metabolites (86 out of 104) between control and gastrectomy groups were conserved before and after exclusion (Supplementary Table S18). The non-overlap features might possibly come from other reconstructions. The number of subjects in the other surgery reconstructions were relatively low compared to the control to give a statistical power to account for the effects of reconstructions to the microbiome and metabolome.

Observed gastrointestinal complications following gastrectomy

Information about any gastrointestinal complications (e.g., diarrhea, dumping syndrome, anemia) was available for 47 out of 50 gastrectomy patients from their medical records (Supplementary Table S2). Among 29 patients who had gastrointestinal complications after gastrectomy, 15 subjects experienced dumping syndrome. After gastrectomy patients were divided into those with (n=15) and without (n=32) dumping syndrome, overall microbiome and metabolome profiles revealed a generally different composition, but this was not statistically significant (adonis: R2=0.0557, P=0.0629; R2=0.0518, P=0.0959; R2=0.0476, P=0.442, for mOTU-annotated species, MetaPhlAn2-annotated species, and metabolome, respectively, Supplementary Table S4e). In addition, we did not observe any significant difference (LEfSe: P>0.05, q>0.1) regarding the abundance of species, functional modules, and metabolites between patients with and without dumping syndrome (Supplementary Table S17). Notably, the diagnosis of dumping syndrome depends mainly on the individual clinician’s perspective.

Furthermore, we observed a high rate of dumping syndrome in patients with total gastrectomy (8 out of 12 patients, 66.7%) compared to subtotal gastrectomy (7 out of 38, 18.4%), which does not deviate from previous studies such as a Japanese large-scale investigation (n=1,153) reported by Mine S et al. (79.6% early and 48.7% late dumping syndrome for total gastrectomy)[4]. In general, the frequency of postsurgical dumping syndrome is estimated at 25-50%[5]. Such a wide range might be explained by diagnosis
for dumping syndrome being highly dependent on the individual clinician’s perspective[6].

Differences of species-species correlations between control and gastrectomy groups

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

Supplementary Table S1. Clinical characteristics of participants in post-gastrectomy and control groups

Supplementary Table S2. Clinical characteristics of each participant based on medical records

Supplementary Table S3. Quality control and annotation profile of each sample

Supplementary Table S4. PERMANOVA analysis between microbiome and metabolome based on clinical parameters, demographic data, and medical history

Supplementary Table S5. Microbiome and metabolome enrichment in pairwise comparisons between different types of gastrectomy and the control group

Supplementary Table S6. Microbiome and metabolome profiles after exclusion of gastric acid-suppression medication users (control, n=43; gastrectomy, n=46)

Supplementary Table S7. Microbiome and metabolome profiles after exclusion of diabetes therapeutic medication users (control, n=43; gastrectomy, n=48)

Supplementary Table S8. Effect of gastric acid-suppression medication on microbiome and metabolome profiles in the control group (user, n=13; non-user, n=43)

Supplementary Table S9. Effect of diabetes therapeutic drugs on microbiome and metabolome profiles in the control group (user, n=12; non-user, n=43)

Supplementary Table S10. Significantly different taxa between gastrectomy and control groups and their associated clinical information (annotation with mOTU)

Supplementary Table S11. Significantly different taxa between gastrectomy and control groups and their associated clinical information (annotation with MetaPhlAn2)

Supplementary Table S12.
Significantly different KEGG modules between gastrectomy and control groups and their associated demographic information (annotation by in house pipeline and HUMAnN2)

Supplementary Table S13.

Species alpha-diversity and richness of KEGG modules contributor

Supplementary Table S14.

Significantly different metabolites between gastrectomy and control groups and their associated demographic information

Supplementary Table S15.

MIMOSA output showing the species predicted to contribute to each metabolite

Supplementary Table S16.

Consumption of each dietary component in post-gastrectomy and control groups

Supplementary Table S17.

Microbiome and metabolome profiles in participants with (n=15) and without (n=32) dumping syndrome

Supplementary Table S18.

Microbiome and metabolome profiles between control (n=56) and post-gastrectomy patients undergoing Roux-en-Y reconstruction (n=29)
SUPPLEMENTARY FIGURES

Supplementary Figure S1. Study participants’ overview and analysis workflow

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

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

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

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

Relative abundances (log10) were plotted as boxplots to shows the distribution of each species of interests. Two distributions patterns were observed. Most species followed Pattern I which reflects the Roux-en-Y reconstructions (A). Several species followed Pattern II and they might be driven by other reconstructions (B). CRC-enriched species that were enriched in the gastrectomy group also showed different distribution patterns across reconstructions (C). 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 S4. Metabolites distributions in different types of surgery and reconstructions
The concentrations of metabolites (nmol/g) were plotted as boxplots to show 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
module and then grouped as either gastrectomy or control (sample in order differs between panels).

**Supplementary Figure S8. Species-species correlations**

Co-occurrence (red) and co-excluding (green) relationships between species (SparCC: $-0.4 < r < 0.4$, $P<0.05$) in gastrectomy (n=44) (A) and control (n=54) (B) groups. The edge width corresponds to the SparCC correlation coefficients. The nodes' size is scaled based on the genus relative abundance averaged over participants within each group. Nodes’ color represents enrichment of the genus in gastrectomy (orange) and control (blue) participants.

**Supplementary Figure S9. Procrustes analysis between species and metabolites**

Principal component analysis (PCA) plots for mOTU-annotated species (A), MetaPhlAn2-annotated species (B), and metabolites profile (C). Procrustes analysis was performed between metabolite profiles and species annotated by mOTU (D) and MetaPhlAn2 (E).

**Supplementary Figure S10. Genus contribution to metabolites based on MIMOSA analysis**

Each table cell in the matrix represents the contribution of a particular genus to a metabolite (see legends in the figure). Table cells are colored based on the community metabolic potential (CMP) score calculated from the KEGG reaction data and KO relative abundances stratified by species information. The species were later summarized at the genus level. Each metabolite is given a prediction level, which represents how the observed metabolite values are consistent or contrasting with the predicted metabolite-producing potential. High prediction scores indicate that a metabolite is enriched in the gastrectomy group, and it is predicted to be produced in sufficient amounts by a certain genus. Based on reaction information, MIMOSA predicted metabolite enrichment in one of the groups and compared that enrichment trend to those observed in the actual quantification. A positive value (green) shows a consistent trend and a negative value (orange) shows a contrasting trend compared to the measured metabolite.
References


