

1 SUPPLEMENTARY METHODS

2

3 DNA extraction, shotgun metagenomic sequencing, and quality control

4 We extracted genomic DNA from the frozen fecal samples by the bead-beating method
5 using the GNome® DNA Isolation Kit (MP Biomedicals, Santa Ana, CA, USA). The quality
6 was assessed on a 4200 TapeStation (Agilent Technologies, Santa Clara, CA, USA). The
7 extracted DNAs were then subjected to shotgun metagenomic sequencing on an Illumina
8 HiSeq2500 platform with 150-bp paired-end read lengths and a targeted 5 Gbp
9 sequencing depth. The sequencing libraries were generated with the Nextera XT DNA
10 Library Prep Kit (Illumina, San Diego, CA, USA). The quality of the libraries was analyzed
11 by the 4200 TapeStation.

12

13 Metabolomics quantification of fecal samples

14 Fecal metabolites were extracted from ten milligrams of fresh thawed freeze-dried fecal
15 samples suspended in 400 μ L of 50% methanol in Milli-Q containing internal standards
16 (20 μ M each of methionine sulfone and D-camphor-10-sulfonic acid (CSA)[1]. The 3-mm
17 zirconia beads (BioSpec Products, Bartlesville, OK, USA) and 100 mg of 0.1-mm
18 zirconia/silica beads (BioSpec Products, Bartlesville, OK, USA) were added into the
19 mixture then subjected to vigorous shaking using Micro Smash (TOMY, Nerima, Tokyo,
20 Japan). The suspensions were centrifuged for 10 minutes at 1500 rpm, 10 times. The
21 supernatant was transferred to a 5-kDa-cutoff filter column (Ultrafree MC-PLHCC
22 250/pk) for Metabolome Analysis (Human Metabolome Technologies, Tsuruoka,
23 Yamagata, Japan). The flow-through was dried under vacuum and the residue then was
24 dissolved in 40 μ L of Milli-Q water containing reference compounds (200 μ M each of 3-
25 aminopyrrolidine and trimesate). The levels of extracted metabolites were measured in
26 both positive and negative modes by Capillary Electrophoresis Time-of-Flight Mass
27 Spectrometry (CE-TOFMS) as previously described[2]. The CE-TOFMS experiments were
28 carried out using an Agilent CE Capillary Electrophoresis System (Agilent Technologies,
29 Santa Clara, CA, USA).

30 The raw data were processed for metabolite quantification using automatic
31 integration software MasterHands (ver. 2.16.0.15)[3]. We generated annotations tables
32 based on the measurement of standard compounds and aligned with the datasets
33 according to the similar m/z values and normalized migration time. The peak areas were

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34 then normalized against those of the internal standards methionine sulfone and CSA for
35 cationic and anionic metabolites, respectively. Concentrations of each metabolite were
36 calculated based on their relative peak areas and the concentrations of the standard
37 compounds.

38 For the analysis, concentrations below the detection limit were substituted with
39 zero, and metabolites for which levels were below the detection limit in all of the samples
40 were excluded. The metabolite concentration (nmole) was normalized using the fecal
41 weight to obtain the amount of metabolite in each gram of sample (nmole/g)[1,2].

42

43 **Reads quality filtering**

44 Shotgun metagenomics sequencing was performed on an Illumina HiSeq2500 platform
45 with 150-bp read length to a targeted 5 Gbp sequencing depth. The sequence quality
46 filtering process involved several steps and started by removing reads that contained
47 ambiguous bases (**Supplementary Figure S1B**). The elimination of reads with *PhiX* DNA
48 contamination was then performed, followed by trimming the sequencing adapter and
49 3'-end low quality reads using cutadapt (version 1.9.1)[4]. The adapter sequences were
50 removed by cutadapt using the option of “-a
51 CTGTCTCTTATACACATCTCCGAGCCCACGAGAC -o 33” for the forward primer sequence
52 and “-a CTGTCTCTTATACACATCTGACGCTGCCGACGA -o 32” for the reverse primer
53 sequence.

54 We continued the filtering process by applying a read length and average quality
55 cut-off. The reads of less than 50 bp in length and an average quality score of less than 25
56 were removed. Furthermore, contaminating human DNA sequences were removed by
57 screening against the human genome
58 (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA/000/001/405/GCA_000001405.15_GRCh38/GCA000001405.15_GRCh38_assembly_structure/Primary_Assembly/assembled_chromosomes/FASTA/). We used Bowtie2 (version 2.2.9)[5] to eliminate reads with *PhiX*
60 and human genome contamination. Only paired-end reads were selected as high-quality
61 reads for further analysis.

62

64 **Taxonomic profiling by the mOTU and MetaPhlAn2 pipeline**

65 Taxonomic profiling was performed using the metagenomics operational taxonomic
66 units (mOTUs)[6] and MetaPhlAn2 pipeline[7] that will be explained below.

67 ***mOTU pipeline***

68 There were two processes involved in the abundance profiling of samples. First,
69 the high-quality reads were subjected to mapping against the mOTU.v1.padded database
70 using sequence identity and alignment cutoffs of 97% and 45 bp, respectively. The
71 mOTU.v1.padded database contains the marker gene (MG) sequences that have been
72 extracted from 3,496 reference genomes and 263 published human gut metagenomic
73 samples. The counts of respective mOTUs were increased by one for each read that
74 mapped to one or more MGs that belonged to the same mOTU. For each read that mapped
75 to MGs from n different mOTUs with the same alignment scores, the count of respective
76 mOTUs was increased by the fraction of unique mappers of these mOTUs. The reads
77 counts were then normalized to the gene length, scaled by the average gene length, and
78 rounded down to get the mOTU abundances.

79 Second, the taxa were annotated based on the RefMG.v1.padded database. The
80 RefMG.v1.padded database is a subset of the mOTU.v1.padded database, and contains
81 only MGs from 3,496 NCBI reference genomes. Taxa were merged if their NCBI species
82 annotations were the same. The final taxonomic abundance profiles were then stored in
83 the form of tab-delimited files with the sample IDs as the header and the NCBI species ID
84 as the rows. The relative abundances were calculated by dividing the relative abundances
85 by the total sum of relative abundances so that all taxa relative abundances totaled 1
86 (**Supplementary Figure S1B**). Species with average relative abundance exceed 0.001%
87 and appeared in at least 5% of samples number (five samples) were retrieved for
88 downstream analysis.

89

90 ***MetaPhlan2 pipeline***

91 In addition to mOTU annotation, we performed taxonomic annotation using MetaPhlan2
92 pipeline version 2.7.0 with default parameters[7]. High-quality reads were mapped to
93 unique clade-specific marker genes identified from ~17,000 reference genomes
94 (~13,500 bacterial and archaeal, ~3,500 viral, and ~110 eukaryotic). The output listed
95 the relative abundance for detected species level. We only used the bacterial with average
96 relative abundance that were exceed 0.1% in at least 5% of samples number (five
97 samples) for our downstream analysis.

98

99

100 Functional annotation of fecal metagenomes by *in-house* and HUMAnN2 pipeline

101 We performed our *in-house* and HUMAnN2[8] for functional annotations that will be
102 explained below. We employed the pipeline to generate the KEGG (Kyoto Encyclopedia
103 of Genes and Genome orthology) Orthology (KO) profile[9]. KO abundances were then
104 calculated for KEGG modules, a collection of manually defined functional units, using
105 omixer-rpm (default parameter)[10] that will select the modules that pass the defined
106 coverage (number of observed steps/number of defined steps) cutoff. The abundance
107 were derived for each modules by selecting the combination of KO that maximize the
108 KEGG modules abundance. The KEGG modules with average relative abundance of
109 0.0001% and appeared in at least 5% of samples number (five samples) were selected
110 for the downstream analysis.

111 *In-house pipeline*

112 Our *in-house* pipeline for functional annotation was written in Python 2.7
113 integrated with several tools that are publicly available. The high-quality reads were
114 subjected to the assembly process using the IDBA-UD assembler (version 1.1.1)[11]. To
115 obtain the scaffold for gene prediction, the parameter --mink 20 -maxk 120 --step 10 was
116 used. This parameter meant that the *k* values ranged from 20 to 120, with an increase in
117 the *k*-mer every 10 iterations.

118 Genes were predicted by MetaGeneMark (version 3.26) using the bacterial,
119 archaeal, and plant plastid genetic codes as the protein translation options and the
120 parameters for metagenome gene prediction (-g 11 -m MetaGeneMark_v1.mod)[12]. The
121 predicted genes with more than 50 bp of amino acid sequences were selected for gene
122 annotation. We mapped our predicted genes to the Kyoto Encyclopedia of Genes and
123 Genomes (KEGG) GENES database (as of 2017)[9] using DIAMOND[13] (version 0.9.10)
124 (cut-offs: sequence identity >40, bit score >70, coverage >80). The top hit with the highest
125 score was selected from the annotated genes, since one predicted gene can be annotated
126 to more than one gene. The top hit genes were then filtered by applying the cut-off values
127 of 40% identity and a score of 70. The final top hit of annotated gene abundance was
128 calculated by the number of hits weighing process. The calculation of predicted gene
129 abundance was started by determining the position of the high-quality reads within the
130 scaffold. The high-quality reads were mapped back to the scaffold using Bowtie2 (version
131 2.2.9)[5]. The number of bases in the reads that mapped in the positions of the predicted
132 genes were then summed to calculate the annotated gene abundances. The number of

133 bases was then divided by the length of the predicted genes to determine the gene
134 abundances. In other words, the predicted gene abundance was defined by the number
135 of reads that mapped to the predicted gene. After the abundance of the annotated gene
136 was calculated, total sum scaling normalization (TSS) was performed to account for
137 uneven sequencing depth across samples. The abundance value was divided by the total
138 abundance per sample to generate its relative abundance. The KEGG were then annotated
139 into the KEGG orthologous (KO) group profile[14].

140 ***HUMAnN2 pipeline***

141 Functional profiling was performed using HUMAnN2 v0.11.1 in UniRef90
142 mode[8]. High-quality reads were initially mapped to the pangenomes species identified
143 during the taxonomic profiling by MetaPhlan2 using Bowtie2. The pangenomes have
144 been pre-annotated to its respective UniRef90 families. Subsequently the unmapped
145 reads were mapped to UniRef90 by translated search using DIAMOND. The gene-level
146 outputs were produced in reads per kilobases units and stratified according to
147 known/unclassified community contribution to relative abundance unit. The gene
148 relative abundance was regrouped into its respective KO using the UniRef90-KEGG linked
149 file.

150

151 **Microbial community structure analysis**

152 To visualize the microbial community structure between the gastrectomy and the control
153 group, we calculated between-sample diversity score (Bray-Curtis distance) using the
154 relative abundances of taxa and the concentration of metabolites. We visualized the
155 separation using the Principal coordinates analysis (PCoA). The distance calculation and
156 PCoA analysis were performed by R package phyloseq[15]. Subsequently, we performed
157 permutational multivariate analysis of variance (PERMANOVA) ('adonis' function, vegan
158 package, R) on the matrices between-sample diversity score to test the differences of
159 microbial community between group. We also performed PERMANOVA using the other
160 predictor which are the medical history that were extracted from the questionnaire and
161 the clinical parameters (BMI, serum glucose and total cholesterol) from participants
162 records. For the medical history, we marked the participants who had a history of other
163 diseases or history of medications before the sample collections as "Yes". We excluded
164 the samples who did not have the data for particular parameter (label as NA) in the
165 PERMANOVA analysis. Our gastrectomy participants have undergone different types of

166 surgery (total and subtotal gastrectomy) and different gastrectomy reconstructions.
167 Thus, we also assess how those factors could explain the variance in the microbiome and
168 metabolome compositions in the gastrectomy subjects. The results were reported in
169 **Supplementary Table S4.**

170 Species richness and the Shannon diversity index were evaluated to estimate the
171 microbial diversity between the gastrectomy patients and controls participants. While
172 richness indicates the number of different species in a community, diversity takes into
173 account both the richness and evenness (relative abundance of species). The analysis was
174 carried out using the `skbio.diversity.alpha` module from the Python package of `scikit-bio`
175 (version 0.4.2) using the richness calculation metric of 'chao1' to estimate the Chao1
176 richness. The metric 'shannon' was used for defining the microbial Shannon-Wiener
177 alpha-diversity index. The two-sided Mann-Whitney U (MWU) test and significant
178 differences were stated if the P value was below 0.05.

179

180 **Different microbial features between the control and gastrectomy group**

181 The differences in relative abundance of the features (taxonomy, KO module, and
182 metabolites) were determined by linear discriminant analysis (LDA) effect size (LEfSe)
183 analysis, which emphasizes statistical significance, biological consistency, and effect
184 relevance[16]. LEfSe first identifies features that are statistically different between
185 control and gastrectomy groups using the non-parametric Kruskal-Wallis sum-rank test
186 ($P < 0.05$). We modified the default calculation by controlling the multiple testing using
187 Benjamini-Hochberg (BH) false discovery rate (FDR) correction procedure. The features
188 that pass the threshold ($q < 0.1$) were subsequently tested by a set of pairwise tests among
189 the sub-group using the Wilcoxon rank-sum test ($P < 0.05$) to investigate its biological
190 consistency. LDA coupled with effect size measurement was finally performed to identify
191 bacterial taxa, annotated functions, and metabolome whose sequences were
192 differentially abundant between the controls and gastrectomy participants. In addition
193 to detect significant features, LEfSe also ranks features by effect size, which places
194 features that can explain most of the biological difference at the top[16]. A log-
195 transformed LDA score of 2.0 (default parameter) was used as the threshold for
196 significance in the KEGG module, taxon and metabolites to narrow down the focus. For
197 taxonomic analysis, two types of tables were used for LEfSe analysis. The first table
198 contains hierarchical taxonomic data in which abundances are computed at different

199 taxonomic levels, such as kingdom as the highest level and species as the lowest level,
200 and the results are visualized as a cladogram. The second table only contains taxa at the
201 species level and the results are visualized as a histogram.

202 The P-value and FDR-value for each test are shown in **Supplementary Tables**
203 **S10, S11, S12 and S14**. A log₂-fold change in a feature was also calculated by dividing
204 the log of the relative abundance in gastrectomy patients to controls participants. A
205 pseudo-count was added to the relative abundances, which was the lowest relative
206 abundance observed for the entire data. This calculation was carried out to indicate in
207 which group the feature was over-represented to confirm the LefSe calculation.

208

209 **Associations of the detected features to the demographic data**

210 Patient demographic data was acquired from the questionnaire and from demographic
211 measurements (**Supplementary Table S2**) and dietary information (**Supplementary**
212 **Table S16**). First, statistical differences were tested for the demographic data between
213 control and gastrectomy group to test the nature of the possible confounding factors. The
214 two-sided MWU test (`scipy.stats.mannwhitneyu` version 0.18.1) was performed on
215 numerical data (such as BMI, age, and dietary component information) and Fisher's exact
216 test (`FisherExact` 1.4.2) was performed on categorical data (such as medical history,
217 gender, smoking status, and alcohol consumption status).

218 In addition, the associations coefficient (variance explained) between the
219 demographic data (such as age, gender), medical history (such as history of diseases and
220 drug usage) and clinical parameters (BMI, serum glucose and total cholesterol) as
221 explanatory variables and the detected microbial features as response were tested by the
222 multivariate associations with linear models (MaAsLin) R package (default parameters
223 except for the `fAllvAll=TRUE`, `dMinAbd=0.0`, `dMinSamp=0.05` and `dSignificanceLevel =`
224 `0.1` were applied). The relative abundance of species and KEGG modules and metabolite
225 concentrations were transformed by arcsin-square root transformation. The MaAsLin
226 package was assessing the association using boosted additive generalized linear models
227 with the calculation of significance level of multiple testing correction[17]. The significant
228 associations were reported, and the associations were stated as significant if the P value
229 was below 0.05 and FDR (q value) was below 0.1. Additionally, the variance explained
230 calculated using the participant's group as explanatory variables (defined as crude
231 coefficient) were also compared to those adjusted by potential confounder which are

232 (BMI, total cholesterol, diabetes medications status , and gastric acids medications, age,
233 and gender) (strForcedPredictors="BMI, total cholesterol, diabetes medications, and
234 gastric acids medications, age,gender" was applied).

235

236 **Oral microbes categorizations and microbial phenotype predictions**

237 To examine the effect of gastrointestinal reconstruction, we further categorized the
238 annotated microbiome by performing oral microbes categorizations and phenotype
239 predictions. The categorizations of oral microbes were performed based on the expanded
240 Human Oral Microbiome Database (eHOMD)[18]. The reference is in a tabularized view
241 of all human oral microbial taxa that are defined and curated by eHOMD. The list of
242 microbes retrieved from both mOTU and MetaPhlan2 pipeline was categorized as oral
243 microbes if it is listed on eHOMD and the non-listed species were categorized as others.
244 The total relative abundance of oral microbes in each participant was calculated and
245 compared between the gastrectomy (n=50) and control (n=56) groups using two-sided
246 MWU test.

247 To reveal the phenotypic properties regarding the oxygen requirement of the
248 species, we used the BugBase tool[19] on the metagenomics data. BugBase determines
249 the proportions for each microbiome sample of Gram positive, Gram negative, biofilm-
250 forming, pathogenic, potential mobile element-containing, oxygen utilizing, and oxidative
251 stress-tolerant microorganisms. For the metagenomics shotgun sequencing data, we
252 selected the OTUs using the IMG database before processing in BugBase. The detected
253 phenotypes were plotted and the statistical P-values from two-sided MWU test between
254 gastrectomy and control groups were also reported.

255

256 **Correlation between genus and species in each group by SparCC**

257 We estimated the microbial association (genus and species level) in each group using
258 SparCC (bootstrap n=5000)[20]. First, we selected the significantly different genus and
259 species between the gastrectomy and control groups that were overlapped in the mOTU
260 and MetaPhlan2 (LEfSe: P<0.05, q<0.1, LDA>2.0) pipeline annotation. SparCC correlation
261 value between the genus or species counts (mOTU annotated) were then calculated in
262 each group (control and gastrectomy) independently. SparCC has been widely used to
263 estimate the correlation values from compositional data. Significant co-occurrence and
264 co-excluding interaction (SparCC correlation scores $\rho < -0.2$ or $\rho > 0.2$ for genus and

265 $\rho < -0.4$ or $\rho > 0.4$ for species, with a $P < 0.05$) were visualized and analyzed using *igraph*.
266 We calculated the degree, betweenness and strength of each node to estimate its
267 importance to the network.

268

269 **Correlation between microbiome and metabolite in each group**

270 We performed Procrustes analysis to determine the congruence of two-dimensional
271 shapes produced by superimpositions of principal component analysis from microbiome
272 and metabolome datasets based on the Euclidean distances of eigenvalues from both
273 matrix (protest functions in vegan R package, n of permutations=1000)[21]. We selected
274 significantly different (LEfSe: $P < 0.05$; $q < 0.1$; $LDA > 2$ for comparison of
275 gastrectomy (n=50) vs control (n=56)) metabolites and species that were overlap
276 between mOTU and MetaPhlan2 annotations for the analysis. First, we generated
277 principal component from each measurement and then measured their inter-omics
278 associations. Procrustes analysis superimpose and scales principal component plots and
279 allows for quantification of non-random conformance between two different
280 measurements from similar participants (**Supplementary Figure S9**).

281 Additionally, to evaluate the possible microbe–metabolite associations a
282 Spearman rank-based correlation matrix was employed. Correlation analysis was
283 performed on the relative abundance of 16 statistically-significant genus (LEfSe: $P < 0.05$;
284 $q < 0.1$; $LDA \text{ score} \geq 2.0$ both confirmed by mOTU and MetaPhlan2 pipelines, genus relative
285 abundances annotated by mOTU pipeline were used for calculation) and the
286 concentrations of 34 statistically-significant metabolites (LEfSe: $P < 0.05$; $q < 0.1$; LDA
287 $\text{score} \geq 3.0$) among 44 gastrectomy patients and 54 controls participants. The correlation
288 analysis was performed in Python 2.7 using spearmanr modules from the scipy.stats
289 (version 0.18.0) package[22]. The P value of the correlation analysis was controlled by
290 the BH-FDR multiple correction test and was stated as the q-value. The correlation was
291 defined as significant if the P-value was below 0.05 and the FDR value was below 0.10.
292 The correlation coefficients were between -1 and 1, for which 0 implied no correlation,
293 while -1 or 1 implied an exact correlation. The cluster heatmap was created from the
294 spearman correlation matrices (without q value thresholding) using the
295 seaborn.clustermap based on the Euclidean distance metric. The results were visualized
296 as a heatmap in which the red color implied a positive correlation and the blue color
297 implied a negative correlation[23]. Significant correlations ($P < 0.05$; $q < 0.10$) were

298 indicated by an asterisk (*).

299

300 **Predicting the species contribution on metabolite by Model-based Integration of**
301 **Metabolite Observations and Species Abundances (MIMOSA)**

302 The functional contribution of each species was retrieved from the HUMAnN2
303 annotation. The table of KO relative abundance stratified by species information was
304 utilized to calculate the community metabolic potential (CMP) of each species based on
305 the KEGG reaction information. This method integrates information about gene
306 abundances in terms of KOs and KEGG reaction definition describing the quantitative
307 relationship between genes and metabolites to provide an estimate of the way the species
308 composition may impact each metabolite's abundance. The CMP score was then
309 compared to the measured metabolomic data using Mantel test for each metabolite. The
310 test assesses Spearman correlation between pairwise differences in CMP scores (across
311 all pairs of samples) and the corresponding pairwise differences in measured metabolite
312 concentration[24]. A significant positive pairwise correlation (Mantel test: $P \leq 0.01$;
313 $q \leq 0.01$) was determined as "consistent" while a negative correlation was determined as
314 "contrasting" trend. We also retrieved the information of key contributor species to the
315 synthesis or degradation process for each metabolite (**Supplementary Figure 10,**
316 **Supplementary Table 10**).

317

318

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