

Spatial and Temporal Analysis of the Stomach and Small Intestinal Microbiota in Fasted Healthy Humans

Authors: Anna M. Seekatz, PhD^{1*}, Matthew K. Schnizlein^{2*}, Mark J. Koenigskecht, PhD^{1,3*}, Jason R. Baker, PhD⁴, William L. Hasler, MD⁴, Barry E. Bleske, PharmD⁵, Vincent B. Young, MD, PhD^{1,2†}, Duxin Sun, PhD^{3†}

¹*Department of Internal Medicine, Division of Infectious Disease, University of Michigan, Ann Arbor, MI 48109, USA*

²*Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI 48109, USA*

³*Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, USA*

⁴*Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of Michigan, Ann Arbor, MI 48109, USA*

⁵*Department of Pharmacy Practice and Administrative Sciences, University of New Mexico, Albuquerque, NM 87131*

*These authors contributed equally

†Corresponding authors

Contact information:

Duxin Sun, PhD

Department of Pharmaceutical Sciences, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, USA, duxins@umich.edu

Vincent B. Young, MD, PhD

Department of Internal Medicine, Division of Infectious Disease, University of Michigan, Ann Arbor, MI 48109, USA, youngvi@umich.edu

Manuscript word count: 3105

Patient consent: Informed consent was obtained from individuals prior to the time of sampling. IRB approved on 02/04/2015.

Conflicts of Interest: None

1 **Abstract**

2 Although the microbiota in the proximal gastrointestinal (GI) tract has been
3 implicated in health and disease, much of these microbes remains understudied
4 compared to the distal GI tract. This study characterized the microbiota across
5 multiple proximal GI sites over time in healthy individuals.

6 As part of a study of the pharmacokinetics of oral mesalamine
7 administration, healthy, fasted volunteers (N=8; 10 observation periods total) were
8 orally intubated with a four-lumen catheter with multiple aspiration ports. Samples
9 were taken from stomach, duodenal, and multiple jejunal sites, sampling hourly (≤ 7
10 hours) to measure mesalamine (administered at $t=0$), pH, and 16S rRNA gene-based
11 composition.

12 We observed a predominance of Firmicutes across proximal GI sites, with
13 significant variation compared to stool. The microbiota was more similar within
14 individuals over time than between subjects, with the fecal microbiota being unique
15 from that of the small intestine. The stomach and duodenal microbiota displayed
16 highest intra-individual variability compared to jejunal sites, which were more
17 stable across time. We observed significant correlations in the duodenal microbial
18 composition with changes in pH; linear mixed models identified positive
19 correlations with multiple *Streptococcus* operational taxonomic units (OTU) and
20 negative correlations with multiple *Prevotella* and *Pasteurellaceae* OTUs. Few OTUs
21 correlated with mesalamine concentration.

22 The stomach and duodenal microbiota exhibited greater compositional
23 dynamics compared to the jejunum. Short-term fluctuations in the duodenal

24 microbiota was correlated with pH. Given the unique characteristics and dynamics
25 of the proximal GI tract microbiota, it is important to consider these local
26 environments in health and disease states.

27

28

29 INTRODUCTION

30 The microbiota of the proximal gastrointestinal tract in humans represent an
31 understudied yet highly relevant microbial community.¹ Physiological processes
32 such as gastric emptying, bile acid secretion, and the transit of food can influence
33 the proximal gastrointestinal (GI) tract and disease development.²⁻⁵ However, our
34 current understanding of how the processes and microbiota in different regions of
35 the proximal GI tract relate to health and disease remains limited compared to other
36 areas of the GI tract.

37 Much of our knowledge about the involvement of the human GI microbiota in
38 health and disease has relied on fecal sampling, a non-invasive sampling method
39 that is largely representative of the large intestine.^{6,7} Although it is known that the
40 microbiota across the GI tract varies in composition and density,⁸⁻¹⁰ studying the
41 microbiota at these sites is difficult, limiting our knowledge to invasive procedures,
42 specific patient populations, or single time points.¹ Analyses of mucosal samples
43 from autopsies, endoscopies, and colonoscopies have revealed that *Streptococci* and
44 *Lactobacilli*, both members of the oral and esophageal microbiota, are abundant
45 members of the jejunal and ileal microbiota.¹¹⁻¹⁷ Studies using naso-ileal catheters
46 and ileostoma effluent, which allow collection over time, have supported these
47 conclusions and revealed that the small intestinal microbiota is highly dynamic over
48 short time courses, likely reflective of physiological processes at the stomach-small
49 intestine interface.¹⁸⁻²¹

50 Understanding how these microbiota along the GI tract are related is of
51 physiological relevance, particularly in relation to intestinal homeostasis and

52 disease. Recent evidence suggests that the drug mesalamine, designed to reach high
53 concentrations in the GI tract as treatment for irritable bowel disease (IBD), may
54 directly target the microbiota in addition to host effectors.^{22,23} It is possible that
55 some of the effectiveness of mesalamine treatment for IBD is mediated by the
56 microbiota, potentiating the need to characterize these microbial communities to a
57 fuller extent in the context of mesalamine administration.

58 This study investigated the bacterial composition across the intact upper GI
59 tract in the same healthy, fasted adults over time. We used a multi-lumen tube
60 designed to sample multiple sites along the upper GI tract. As part of a previously
61 published study aimed at measuring mesalamine dissolution, subjects were given a
62 dose of mesalamine and the proximal GI tract lumen was sampled over time.²⁴ We
63 used these samples to 1) characterize and compare microbial community dynamics
64 over time at multiple upper GI sites within an individual and 2) identify how
65 environmental factors, such as pH and the acute effect of mesalamine, shaped the
66 microbiota. To the best of our knowledge, this is the first study to characterize the
67 luminal microbiota across multiple upper GI sites over time within the same
68 individual.

69

70 **METHODS**

71 **Study recruitment**

72 Healthy individuals (age 18-55) were included who were free of medications
73 for the past two weeks, passed routine health screening, had a BMI 18.5-35, and had
74 no significant clinical illness within three weeks. Health screening included a review

75 of medical history and a physical examination (checking vital signs,
76 electrocardiography, and clinical laboratory tests) described in Yu et al.²⁴

77 **Catheter design and sterilization**

78 A customized multi-channel catheter was constructed by Arndorfer Inc.
79 (Greendale, WI), consisting of independent aspiration ports located 50 cm apart.
80 The catheter had a channel to fit a (0.035 in x 450 cm) guidewire (Boston Scientific,
81 Marlborough, MA), a channel connected to a balloon that could be filled with 7 ml of
82 water to assist tube placement, and an end that was weighted with 7.75 grams of
83 tungsten. Each single-use catheter was sterilized according to guidelines set by the
84 American Society for Gastrointestinal Endoscopy at the University of Michigan prior
85 to insertion (Supplemental Methods).²⁵

86 **Collection of GI fluid samples**

87 The full details of catheter placement have been previously described.²⁴
88 Briefly, catheter placement occurred approximately 12 hours before sample
89 collection. The catheter was orally inserted into the GI tract with aspiration ports
90 located in the stomach, duodenum, and the proximal, mid and distal jejunum,
91 confirmed by fluoroscopy. Subjects were given a light liquid snack approximately 11
92 hours before sample collection and fasted overnight for 10 hours prior to sample
93 collection. At 0 hours, a mesalamine formulation was administered to each subject
94 (Table 1). Luminal GI fluid samples (approximately 1.0 ml) were collected from up
95 to four sites of the upper GI tract hourly up to 7 hours. Samples were collected by
96 syringe, transferred to sterile tubes, and placed at -80°C until sample processing. A

97 paired sample was collected to detect pH using a calibrated micro pH electrode
98 (Thermo Scientific (Waltham, MA) Orion pH probe catalog no. 9810BN).

99 **DNA extraction and Illumina MiSeq sequencing**

100 The detailed protocol for DNA extraction and Illumina MiSeq sequencing was
101 followed as previously described with modifications (Supplemental Methods).²⁶
102 Briefly, 0.2 ml of GI fluid or 20 mg of stool was used for DNA isolation using a Qiagen
103 (Germantown, MD) MagAttract Powermag microbiome DNA isolation kit (catalog
104 no. 27500-4-EP). Barcoded dual-index primers specific to the V4 region of the 16S
105 rRNA gene were used to amplify the DNA,²⁷ using a “touchdown PCR” protocol
106 (Supplemental Methods). Multiple negative controls were run parallel to each PCR
107 reaction. PCR reactions were normalized, pooled and quantified.²⁸ Libraries were
108 prepared and sequenced using the 500 cycle MiSeq V2 Reagent kit (Illumina, San
109 Diego, CA, catalog no. MS-102-2003). Raw FASTQ files, including those for negative
110 controls, were deposited in the Sequence Read Archive database (BioProjectID:
111 PRJNA495320; BioSampleIDs: SAMN10224451-SAMN10224634).

112 **Data processing and microbiota analysis**

113 Analysis of the V4 region of the 16S rRNA gene was done using mothur
114 (v1.39.3).^{27,29} Full methods, including detailed processing steps, raw processed data,
115 and code for each analysis, are described in:
116 https://github.com/aseekatz/SI_mesalamine. Briefly, following assembly, quality
117 filtering, and trimming, reads were aligned to the SILVA 16S rRNA sequence
118 database (v128).³⁰ Chimeric sequences were removed using UCHIME.³¹ Prior to
119 analysis, both mock and negative control samples (water) were assessed for

120 potential contamination; samples with < 2500 sequences were excluded (Table S1).
121 Sequences were binned into operational taxonomic units (OTUs), 97% similarity,
122 using the optclust algorithm.³² The Ribosomal Database Project (v16) was used to
123 classify OTUs or sequences directly for compositional analyses (> 80% confidence
124 score).³³ Alpha and beta diversity measures (inverse Simpson index; the Yue &
125 Clayton dissimilarity index, θ_{YC})³⁴ were calculated from unfiltered OTU data. Basic R
126 commands were used to visualize results, calculate % OTUs shared between
127 samples, and conduct statistics, using packages plyr, dplyr, gplots, tidyr, and
128 tidyverse. The nonparametric Kruskal-Wallis test, using Dunn's test for multiple
129 comparisons and adjusting *p*-values with the Benjamini-Hochberg method when
130 indicated, was used for multi-group comparisons. The R packages lme4³⁵ and
131 lmerTest³⁶ were used for mixed linear models between OTU relative abundance
132 (filtered to include OTUs present in at least half of samples collected from a subject,
133 per site) and pH or mesalamine.

134

135 **RESULTS**

136 **Study population**

137 Using a multi-channel catheter with multiple aspiration points,²⁴ samples
138 collected from the upper GI tract of 8 healthy subjects during 10 different study
139 visits were processed for 16S microbial community analysis (Supplemental
140 Methods, Table 1, Table S1). Samples were collected hourly over the course of 7
141 hours primarily from the proximal GI tract in the following possible locations: the
142 stomach (n=44), duodenum (n=64), proximal/mid/distal jejunum (n=46), and stool

143 (n=3). At the beginning of the study, subjects were given one form of mesalamine
144 (Table 1). One of the seven subjects was studied three times over the course of 10
145 months; for most analyses, each study visit from this subject was considered
146 independently.

147

148 **The proximal GI microbiota is dominated by Firmicutes and is distinct from** 149 **the fecal microbiota**

150 Analysis of the relative abundances of 16S rRNA-encoding genes from the GI
151 tract across all timepoints and individuals demonstrated that the small intestinal
152 microbiota was compositionally unique compared to stool (Fig. 1A). At all four sites
153 in the proximal GI tract, Firmicutes composed the most abundant phyla (i.e.
154 *Streptococcus*, *Veillonella*, and *Gemella* sp.). Higher levels of Bacteroidetes species
155 (*Prevotella*) were detected in the stomach and duodenum. Proteobacteria and
156 Actinobacteria predominated the remainder of the community at all sites. Diversity
157 of the microbiota (inverse Simpson index) was decreased in sites of the upper GI
158 tract compared to stool, which were enriched in Firmicutes (*Blautia*,
159 Ruminococcaceae sp., and *Faecalibacterium*) and depleted in Bacteroidetes in these
160 individuals (n=3) (Fig. 1B).

161

162 **The proximal GI microbiota is individualized and variable over time**

163 To compare the microbiota across the proximal GI tract within and across
164 individuals, we assessed pairwise community dissimilarity using the Yue & Clayton
165 dissimilarity index, θ_{YC} , which takes into account relative abundance of OTU

166 compositional data. Both across (inter-individual) and within (intra-individual)
167 subjects, stool was highly dissimilar to any proximal GI site (Figure 2A, 2B). Across
168 proximal GI sites, subjects were more similar to their own samples than samples
169 across other individuals (Figure 2A-D). The stomach microbiota was highly
170 dissimilar across individuals compared to the duodenum or any part of the jejunum,
171 which exhibited the least amount of dissimilarity (Figure 2C). A similar degree of
172 dissimilarity was observed within an individual in the stomach, duodenum, and
173 combined parts of the jejunum (Figure 2D).

174 Using a dissimilarity measure such as θ_{YC} allows us to assess stability based
175 on changes in the relative abundance of OTUs. It is possible that certain GI sites
176 fluctuate more in total OTUs. To measure whether any site had a higher rate of flux
177 in their community, i.e. a higher rate of OTU turnover, we calculated the % OTUs
178 detected at a given timepoint from the total number of OTUs detected within that
179 individual at a given site. We observed that for each proximal GI site, a mean of
180 36.6% of the OTUs ever detected in that subject at a given site (mean number of
181 total OTUs ever detected per subject per site = 135; range 78-212) were detectable
182 at a given timepoint (Figure 3A). Similarly, we calculated the number of OTUs that
183 were consistently present in all samples collected at that site within an individual
184 (mean number of consistently detected OTUs per subject per site = 14.1; range 2-
185 45). Overall, only 28.7% of the total OTUs ever detected at a given time point within
186 an individual at a given site were represented by these consistently prevalent OTUs
187 (Figure 3B). However, these prevalent OTUs explained an average of 72.0% of the
188 relative abundance observed in the samples (Figure 3C). Of all sites, the relative

189 abundance explained by the individual's most prevalent OTUs in the stomach was
190 lowest, followed by the duodenum, suggesting more variation at these sites
191 compared to the jejunum (Kruskal-Wallis, $p < 0.05$).

192 One subject (M046) returned three times over the course of 10 months,
193 allowing us to compare long-term changes. Across the sites that were sampled
194 during multiple visits (the duodenum and mid-jejunum), prevalent OTUs were still
195 detected during all three visits, explaining 74.4% and 66.1% OTUs in the duodenum
196 and mid-jejunum, respectively (Fig. S1).

197

198 **Large fluctuations in the duodenal microbiota are associated with pH but not** 199 **mesalamine**

200 We next investigated how these compositional trends changed over time
201 across the subjects. We focused on the duodenum and stomach since these sites
202 were highly sampled across and within individuals and demonstrated variable pH.
203 In the duodenum, we observed large fluctuations in genus-level composition across
204 hourly timepoints within individuals (Figure 4, Figure S2, S3). Specifically, the
205 relative abundance of *Streptococcus*, *Prevotella*, and an unclassified Pasteurellaceae
206 species fluctuated in all individuals. We hypothesized that these fluctuations could
207 be driven by mesalamine, administered in different forms to each subject at study
208 onset. However, no visible pattern was observed with mesalamine levels.
209 Interestingly, we observed that these compositional changes tracked with pH
210 fluctuations (Figure 4). These patterns were less apparent in the stomach, where
211 individuals displayed variable dynamics and highly individualized compositional

212 patterns independent of mesalamine levels or pH, or in the jejunum of the subject
213 with three different admissions, where pH fluctuated less (Figure S1, S2).

214 To identify whether any singular OTUs correlated with changes in pH, we
215 applied a generalized linear mixed model approach that takes into account subject-
216 specificity.³⁷⁻³⁹ Within duodenal samples (n=56), we observed 15 OTUs that
217 significantly correlated with pH changes. Linear regression of pH and relative
218 abundance of these OTUs was significant across all samples (Figure 5; Table S2). Of
219 the negatively correlated OTUs, six OTUs were classified as Bacteroidetes, mainly
220 *Prevotella*, and two OTUs were classified as Pasteurellaceae sp. (Proteobacteria).
221 The majority of the OTUs that were positively correlated with pH were Firmicutes,
222 mainly *Streptococcus*, alongside an *Actinomyces* OTU (Actinobacteria). Only one OTU
223 in the duodenum was significantly correlated to mesalamine (Table S2). We
224 identified 17 OTUs that correlated with pH or mesalamine in the stomach; however,
225 these were not representative at all sites (Table S2).

226

227 **DISCUSSION**

228 Our results demonstrate that the microbial communities inhabiting the GI
229 tract are distinct and dynamic across different sites within the proximal GI tract. Our
230 sampling procedure provided us with an opportunity to longitudinally characterize
231 such microbial populations in conjunction with the administration of a commonly
232 used drug, mesalamine. We observed high stability of the microbiota in the jejunum
233 compared to the stomach or duodenum, indicating that the indigenous microbiota
234 residing in more proximal regions of the GI tract may experience greater changes.

235 While we did not observe strong correlations between mesalamine concentration
236 and particular microbiota members at any site, we did observe a strong correlation
237 between the microbiota composition and pH, particularly in the duodenum.

238 In this report, we describe the use of a multi-lumen catheter design with
239 unique aspiration ports that enabled sampling of small intestinal content over the
240 course of seven hours.²⁴ Many studies aimed at investigating the microbiota of the
241 proximal GI have overcome sampling difficulty in this region by using ileostoma
242 effluent, samples from newly deceased individuals, or naso-ileal tubes. Although
243 easy to access, ileostoma effluent does not fully recapitulate the distal small
244 intestine, as it more closely resembles the colon than the small intestine due to
245 increased oxygen concentrations near the stoma.⁴⁰⁻⁴³ Single lumen naso-ileal tubes
246 are unable to sample multiple sites simultaneously.^{18,20,21,44} GI fluid collected with
247 our methodology was sufficient for determining mesalamine concentration,
248 assaying fluid pH, and isolating microbial DNA across time and GI sites, which has
249 not been previously described.²⁴

250 Our results support previous observations that the small intestine is dynamic
251 with higher inter-individual than intra-individual variability.^{18,21,45} However, the
252 mid-distal small intestine also contains a resilient microbial community composed
253 of several highly abundant OTUs. This resilience is demonstrated by the shift from
254 an altered to a normal ileal microbiota following the resolution of an ileostoma.⁴⁶
255 This mirrors the colonic microbiota, which also has a small community which is
256 stable over long periods of time.^{42,47,48}

257 This and other studies have shown that the jejunal and proximal ileal
258 microbiota are distinct from the colonic microbiota.^{10,49} Despite changes in overall
259 community structure and an overall decrease in microbial diversity across the
260 stomach and small intestine compared stool, many of the same organisms
261 commonly observed in stool were also present in the upper GI tract, albeit at very
262 different abundances.¹⁰ Interestingly, colonic resection and ileal pouch-anal
263 anastomosis has been shown to shift the terminal ileum microbiota to a state similar
264 to the colon, suggesting that a colonic community structure can develop at these
265 sites given the right conditions.^{21,43,49-51}

266 Many of the abundant microbes observed in our study, *Streptococcus*,
267 *Veillonella*, *Gemella*, and Pasteurellaceae species, are also common residents of the
268 oral cavity, which reflects the proximity of these locations in the GI tract.
269 Populations of Proteobacteria, such as Pasteurellaceae, have also been observed
270 consistently in the small intestinal microbiota in other studies, particularly in
271 patients with IBD.^{14,52-54} In our study, *Streptococcus* and *Veillonella* were correlated
272 with pH in duodenal samples. It is possible that growth of these organisms drives a
273 decrease in pH via metabolism of short-chain fatty acids, an observed functional
274 capacity of these genera.^{21,55} Conversely, large fluctuations in environmental pH
275 may select for genera like *Streptococcus*, which have evolved a variety of
276 mechanisms to control pH intracellularly.⁵⁶⁻⁵⁹ In any case, our data suggests a
277 relationship between microbial dynamics and environmental physiology of the
278 duodenum, which is an important observation to consider when comparing this site
279 across individuals.

280 We observed little association between mesalamine concentration and
281 changes in microbial relative abundance in our cohort. Several studies have
282 reported differences in the fecal microbiota of patients with or without IBD, in
283 particular Crohn's disease, which can affect the small intestine.⁵² Compositional
284 shifts in the small intestine have been reported during IBD, specifically increased
285 levels of Enterobacteriaceae species, such as *Enterococcus*, *Fusobacterium*, or
286 *Haemophilus*.^{14,53,54} It has been hypothesized that mesalamine's ability to reduce
287 inflammation in patients with ulcerative colitis could be by altering the
288 microbiota.^{22,23} While acute effects of mesalamine on the microbiota have not
289 previously been reported, earlier work has demonstrated that mesalamine
290 decreases bacterial polyphosphate accumulation and pathogen fitness, suggesting
291 an influence on the microbiota.²³ We did not observe strong correlations between
292 mesalamine concentration and the microbiota here. However, our study was small,
293 used different doses of mesalamine that may be metabolized differently across GI
294 sites, and was conducted in healthy individuals.²⁴ It is possible that mesalamine is
295 less likely to impact the small intestinal microbiota, which historically has lower
296 efficacy in treating active Crohn's Disease, which manifests in the small intestine,
297 compared to ulcerative colitis, which manifests in the large intestine.^{22,60,61} As
298 indicated by the variability of mesalamine in the subjects in this study, the effects of
299 mesalamine on the small intestinal microbiota may be highly individualized.^{24,62-64}
300 Furthermore, individuals with disease may harbor a distinct microbiota that
301 responds to mesalamine differently.

302 Despite the opportunity provided by our method to describe the microbiota
303 across the GI tract, our study has some lingering questions. Movement by the subject
304 during the study can result in movement of each sampling port, particularly
305 between the distal stomach and antrum. This may explain the inconsistent pH
306 values and severe fluctuations of the microbiota observed in the stomach. Similarly,
307 the shorter length of the sampling device, as compared to a naso-ileal catheter,
308 prevented reliable collection of fluid from the distal small intestine, limiting our
309 sampling to the proximal region. We also were limited to three concurrent fecal
310 samples, each of which was low in Bacteroidetes, a profile generally observed in
311 individuals on low fat-high fiber, non-Western diets.⁶⁵

312 The use of a novel catheter allowed us to assess the microbiota across several
313 proximal GI sites overtime, representing a powerful clinical and/or investigative
314 tool for studying the small intestinal microbiota. Future studies on the upper GI
315 microbiota should collect concurrent oral swab/sputum and fecal samples to
316 strengthen the ability to “track” microbial populations across the GI tract,
317 potentiating our ability to correlate the microbiota from fecal sampling, a more
318 convenient method to study the microbiota, to other sites of the GI tract.

319 **Declarations:**

320 **Acknowledgements:**

321 This research was funded by FDA grant HHSF223201000082C. Clinical
322 samples collected with help from Michigan Institute for Clinical & Health Research
323 (MICHR) NIH grant UL1TR000433. The authors would also like to thank the Host
324 Microbiome Initiative and the Microbial Systems Molecular Biology Laboratory at

325 the University of Michigan for their support with the 16s rRNA sequencing, and
326 Krishna Rao and Rose Putler for their assistance with the modelling and statistical
327 analyses. This research was funded by the FDA(HHSF223201000082C) and the NIH
328 (5U01AI124255-03).

329

330 **Availability of data and material:**

331 SRA: BioProjectID: PRJNA495320; BioSampleIDs: SAMN10224451-

332 SAMN10224634

333 GitHub: https://github.com/aseekatz/SI_mesalamine

334

335 **Authors' contributions:**

336 MJK, DS, BEB, AMS, VBY, MKS - Conception or design of the work

337 MJK, BEB, WLH, JRB - Data collection

338 AMS, MJK, MKS - Data analysis and interpretation

339 MKS, AMS - Drafting the article

340 MKS, AMS, DS, VBY - Critical revision of the article

341 AMS, MKS, MJK, JRB, WLH, BEB, VBY, DS - Final approval of the version to be

342 published

343

344 **Ethics approval and consent to participate:**

345 Samples collected in this study were part of clinical trial NCT01999400. The

346 institutional review boards at the University of Michigan (IRBMED) and the

347 Department of Health and Human Services, Food and Drug Administration

348 (Research Involving Human Subjects Committee/RIHSC) both approved the study
349 protocol. All subjects provided written informed consent in order to participate.

350 **Consent for publication:**

351 Not applicable

352

353 **Competing interests:**

354 None

355 **Table 1: Subject Recruitment.** Selected metadata and sample collections for 10
356 admissions (subject M046 was admitted for three visits).

357

358 **Figure 1: Bacterial community relative abundance and diversity in the upper**
359 **GI tract. A)** The mean relative abundance of genera at each GI site (sample n
360 indicated). **B)** Boxplots of the inverse Simpson Index measuring community
361 diversity across the GI tract (median, with first and third inter-quartile ranges).
362 Statistical analysis: Kruskal-Wallis test (ns).

363

364 **Figure 2: Dissimilarity of the proximal GI tract within and across individuals.**
365 Heatmap of the Yue & Clayton dissimilarity index, θ_{YC} , comparing different proximal
366 GI sites and stool **A)** across individuals (inter-individual pairwise comparisons) and
367 **C)** within individuals (intra-individual pairwise comparisons). **C)** Inter-individual
368 and **D)** intra-individual dissimilarity in the stomach, duodenum, and jejunum (sites
369 combined). Statistical analysis: Kruskal-Wallis test (will add p values to graph).
370 Statistical analysis: Dunn's test for multiple comparisons with a Benjamini-
371 Hochberg p-value adjustment (* $p < 0.01$; ** $p < 0.001$; *** $p < 0.0001$).

372

373 **Figure 3: Fluctuations in prevalent OTUs observed within an individual across**
374 **the proximal GI tract. A)** Boxplots of the percentage of OTUs detected in a given
375 sample out of all OTUs detected (all OTUs possible for that individual) at a subject-
376 site. **B)** Boxplots of the percentage of OTUs that were consistently detected at a
377 subject-site out of the total OTUs detected in a given sample. **C)** The percent of

378 relative abundance explained by prevalent OTUs at a subject-site in a given sample.

379 Statistical analysis: Kruskal-Wallis test.

380

381 **Figure 4: Longitudinal compositional dynamics, mesalamine levels, and pH in**
382 **the duodenum.** Streamplots of genus-level composition over time in the duodenum
383 of six individuals (% , left y-axis; genera coded in legend). White lines indicate pH
384 measurements (black y-axis labels on right) and red lines indicate mesalamine
385 concentration (red y-axis labels on right).

386

387 **Figure 5: Relative abundance of significant OTUs vs. pH.** Log relative abundance
388 ($\log(\text{RA})$) as a function of pH of OTUs found to be significantly correlated with pH
389 using linear mixed models (all samples with measurable pH). Lines represent linear
390 fit per OTU. OTUs classified as **A)** Firmicutes, **B)** Bacteroidetes, **C)** Proteobacteria,
391 and **D)** Actinobacteria are depicted (genus-level OTU classification coded by
392 legends).

393

394

395

396

397 **REFERENCES**

- 398 1 El Aidy, S., van den Bogert, B. & Kleerebezem, M. The small intestine
399 microbiota, nutritional modulation and relevance for health. *Current Opinion*
400 *in Biotechnology* **32**, 14-20, doi:10.1016/j.copbio.2014.09.005 (2015).
- 401 2 Poulakos, L. & Kent, T. H. Gastric Emptying and Small Intestinal Propulsion in
402 Fed and Fasted Rats. *Gastroenterology* **64**, 962-967, doi:10.1016/S0016-
403 5085(73)80008-3 (1973).
- 404 3 Ridlon, J. M., Kang, D. J., Hylemon, P. B. & Bajaj, J. S. Bile acids and the gut
405 microbiome. *Current opinion in gastroenterology* **30**, 332-338,
406 doi:10.1097/mog.000000000000057 (2014).
- 407 4 Araújo, J. R., Tomas, J., Brenner, C. & Sansonetti, P. J. Impact of high-fat diet on
408 the intestinal microbiota and small intestinal physiology before and after the
409 onset of obesity. *Biochimie* **141**, 97-106, doi:10.1016/j.biochi.2017.05.019
410 (2017).
- 411 5 Martinez-Guryn, K. *et al.* Small Intestine Microbiota Regulate Host Digestive
412 and Absorptive Adaptive Responses to Dietary Lipids. *Cell Host & Microbe* **23**,
413 458-469.e455, doi:10.1016/j.chom.2018.03.011 (2018).
- 414 6 Flynn, K. J., Ruffin, M. T., Turgeon, D. K. & Schloss, P. D. Spatial Variation of the
415 Native Colon Microbiota in Healthy Adults. *Cancer Prevention Research* **11**,
416 393-402, doi:10.1158/1940-6207.Capr-17-0370 (2018).
- 417 7 Falony, G., Vieira-Silva, S. & Raes, J. Richness and ecosystem development
418 across faecal snapshots of the gut microbiota. *Nature microbiology* **3**, 526-
419 528, doi:10.1038/s41564-018-0143-5 (2018).
- 420 8 Sekirov, I., Russell, S. L., Antunes, L. C. M. & Finlay, B. B. Gut Microbiota in
421 Health and Disease. *Physiological Reviews* **90**, 859-904,
422 doi:10.1152/physrev.00045.2009 (2010).
- 423 9 Donaldson, G. P., Lee, S. M. & Mazmanian, S. K. Gut biogeography of the
424 bacterial microbiota. *Nature Reviews Microbiology* **14**, 20,
425 doi:10.1038/nrmicro3552 (2015).
- 426 10 Zmora, N. *et al.* Personalized Gut Mucosal Colonization Resistance to Empiric
427 Probiotics Is Associated with Unique Host and Microbiome Features. *Cell*
428 **174**, 1388-1405.e1321, doi:10.1016/j.cell.2018.08.041 (2018).
- 429 11 Hayashi, H., Takahashi, R., Nishi, T., Sakamoto, M. & Benno, Y. Molecular
430 analysis of jejunal, ileal, caecal and recto-sigmoidal human colonic microbiota
431 using 16S rRNA gene libraries and terminal restriction fragment length
432 polymorphism. *Journal of medical microbiology* **54**, 1093-1101,
433 doi:10.1099/jmm.0.45935-0 (2005).
- 434 12 Wang, M., Ahrné, S., Jeppsson, B. & Molin, G. Comparison of bacterial diversity
435 along the human intestinal tract by direct cloning and sequencing of 16S
436 rRNA genes. *FEMS Microbiology Ecology* **54**, 219-231,
437 doi:10.1016/j.femsec.2005.03.012 (2005).
- 438 13 Wang, X., Heazlewood, S. P., Krause, D. O. & Florin, T. H. J. Molecular
439 characterization of the microbial species that colonize human ileal and
440 colonic mucosa by using 16S rDNA sequence analysis. *Journal of Applied*
441 *Microbiology* **95**, 508-520, doi:10.1046/j.1365-2672.2003.02005.x (2003).

- 442 14 Dey, N., Soergel, D. A., Repo, S. & Brenner, S. E. Association of gut microbiota
443 with post-operative clinical course in Crohn's disease. *BMC gastroenterology*
444 **13**, 131, doi:10.1186/1471-230x-13-131 (2013).
- 445 15 Barrett, E. *et al.* Microbiota diversity and stability of the preterm neonatal
446 ileum and colon of two infants. *MicrobiologyOpen* **2**, 215-225,
447 doi:10.1002/mbo3.64 (2013).
- 448 16 Di Pilato, V. *et al.* The esophageal microbiota in health and disease. *Annals of*
449 *the New York Academy of Sciences* **1381**, 21-33, doi:10.1111/nyas.13127
450 (2016).
- 451 17 Verma, D., Garg, P. K. & Dubey, A. K. Insights into the human oral microbiome.
452 *Archives of Microbiology* **200**, 525-540, doi:10.1007/s00203-018-1505-3
453 (2018).
- 454 18 Booijink, C. C. G. M. *et al.* High temporal and inter - individual variation
455 detected in the human ileal microbiota. *Environmental Microbiology* **12**,
456 3213-3227, doi:10.1111/j.1462-2920.2010.02294.x (2010).
- 457 19 den Bogert, B. v. *et al.* Diversity of human small intestinal Streptococcus and
458 Veillonella populations. *FEMS Microbiology Ecology* **85**, 376-388,
459 doi:10.1111/1574-6941.12127 (2013).
- 460 20 Angelakis, E. *et al.* A Metagenomic Investigation of the Duodenal Microbiota
461 Reveals Links with Obesity. *PLOS ONE* **10**, e0137784,
462 doi:10.1371/journal.pone.0137784 (2015).
- 463 21 Zoetendal, E. G. *et al.* The human small intestinal microbiota is driven by
464 rapid uptake and conversion of simple carbohydrates. *The Isme Journal* **6**,
465 1415, doi:10.1038/ismej.2011.212 (2012).
- 466 22 Hauso, Ø., Martinsen, T. C. & Waldum, H. 5-Aminosalicylic acid, a specific drug
467 for ulcerative colitis. *Scandinavian journal of gastroenterology* **50**, 933-941,
468 doi:10.3109/00365521.2015.1018937 (2015).
- 469 23 Dahl, J.-U. *et al.* The anti-inflammatory drug mesalamine targets bacterial
470 polyphosphate accumulation. *Nature microbiology* **2**, 16267,
471 doi:10.1038/nmicrobiol.2016.267 (2017).
- 472 24 Yu, A. *et al.* Measurement of in vivo Gastrointestinal Release and Dissolution
473 of Three Locally Acting Mesalamine Formulations in Regions of the Human
474 Gastrointestinal Tract. *Molecular Pharmaceutics* **14**, 345-358,
475 doi:10.1021/acs.molpharmaceut.6b00641 (2017).
- 476 25 Committee, A. Q. A. I. E. *et al.* Multisociety guideline on reprocessing flexible
477 gastrointestinal endoscopes: 2011. *Gastrointest Endosc* **73**, 1075-1084,
478 doi:10.1016/j.gie.2011.03.1183 (2011).
- 479 26 Seekatz, A. M. *et al.* Fecal Microbiota Transplantation Eliminates Clostridium
480 difficile in a Murine Model of Relapsing Disease. *Infection and immunity* **83**,
481 3838-3846, doi:10.1128/IAI.00459-15 (2015).
- 482 27 Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K. & Schloss, P. D.
483 Development of a dual-index sequencing strategy and curation pipeline for
484 analyzing amplicon sequence data on the MiSeq Illumina sequencing
485 platform. *Appl Environ Microbiol* **79**, 5112-5120, doi:10.1128/AEM.01043-13
486 (2013).

- 487 28 Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K. & Schloss, P. D.
488 Development of a dual-index sequencing strategy and curation pipeline for
489 analyzing amplicon sequence data on the MiSeq Illumina sequencing
490 platform. *Appl Environ Microbiol* **79**, doi:10.1128/aem.01043-13 (2013).
- 491 29 Schloss, P. D. *et al.* Introducing mothur: open-source, platform-independent,
492 community-supported software for describing and comparing microbial
493 communities. *Appl Environ Microbiol* **75**, 7537-7541,
494 doi:10.1128/AEM.01541-09 (2009).
- 495 30 Pruesse, E. *et al.* SILVA: a comprehensive online resource for quality checked
496 and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic
497 Acids Res* **35**, 7188-7196, doi:10.1093/nar/gkm864 (2007).
- 498 31 Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C. & Knight, R. UCHIME
499 improves sensitivity and speed of chimera detection. *Bioinformatics* **27**,
500 2194-2200, doi:10.1093/bioinformatics/btr381 (2011).
- 501 32 Westcott, S. L. & Schloss, P. D. OptiClust, an Improved Method for Assigning
502 Amplicon-Based Sequence Data to Operational Taxonomic Units. *mSphere* **2**,
503 doi:10.1128/mSphereDirect.00073-17 (2017).
- 504 33 Cole, J. R. *et al.* The Ribosomal Database Project: improved alignments and
505 new tools for rRNA analysis. *Nucleic Acids Res* **37**, D141-145,
506 doi:10.1093/nar/gkn879 (2009).
- 507 34 Yue, J. C. & Clayton, M. K. A Similarity Measure Based on Species Proportions.
508 *Communications in Statistics - Theory and Methods* **34**, 2123-2131,
509 doi:10.1080/STA-200066418 (2005).
- 510 35 Bates, D., Mächler, M., Bolker, B. & Walker, S. Fitting Linear Mixed-Effects
511 Models Using lme4. *Journal of Statistical Software; Vol 1, Issue 1 (2015)*
512 (2015).
- 513 36 Kuznetsova, A., Brockhoff, P. B. & Christensen, R. H. B. lmerTest Package:
514 Tests in Linear Mixed Effects Models. *Journal of Statistical Software; Vol 1,
515 Issue 13 (2017) (2017)*.
- 516 37 Xia, Y. & Sun, J. Hypothesis testing and statistical analysis of microbiome.
517 *Genes & Diseases* **4**, 138-148, doi:10.1016/j.gendis.2017.06.001 (2017).
- 518 38 Gajer, P. *et al.* Temporal dynamics of the human vaginal microbiota. *Sci Transl
519 Med* **4**, 132ra152, doi:10.1126/scitranslmed.3003605 (2012).
- 520 39 Mehta, S. D. *et al.* The vaginal microbiota over an 8- to 10-year period in a
521 cohort of HIV-infected and HIV-uninfected women. *PLoS One* **10**, e0116894,
522 doi:10.1371/journal.pone.0116894 (2015).
- 523 40 Heimesaat, M. M. *et al.* Comprehensive Postmortem Analyses of Intestinal
524 Microbiota Changes and Bacterial Translocation in Human Flora Associated
525 Mice. *PLoS ONE* **7**, e40758, doi:10.1371/journal.pone.0040758 (2012).
- 526 41 DeBruyn, J. M. & Hauther, K. A. Postmortem succession of gut microbial
527 communities in deceased human subjects. *PeerJ* **5**, e3437,
528 doi:10.7717/peerj.3437 (2017).
- 529 42 Fukuyama, J. *et al.* Multidomain analyses of a longitudinal human microbiome
530 intestinal cleanout perturbation experiment. *PLOS Computational Biology* **13**,
531 e1005706, doi:10.1371/journal.pcbi.1005706 (2017).

- 532 43 Young, V. B. *et al.* Multiphasic analysis of the temporal development of the
533 distal gut microbiota in patients following ileal pouch anal anastomosis.
534 *Microbiome* **1**, 9, doi:10.1186/2049-2618-1-9 (2013).
- 535 44 Muller-Lissner, S. A. *et al.* Effect of gastric and transpyloric tubes on gastric
536 emptying and duodenogastric reflux. *Gastroenterology* **83**, 1276 (1982).
- 537 45 Onishi, J. C. *et al.* Bacterial communities in the small intestine respond
538 differently to those in the caecum and colon in mice fed low- and high-fat
539 diets. *Microbiology (Reading, England)* **163**, 1189-1197,
540 doi:10.1099/mic.0.000496 (2017).
- 541 46 Hartman, A. L. *et al.* Human gut microbiome adopts an alternative state
542 following small bowel transplantation. *Proceedings of the National Academy
543 of Sciences of the United States of America* **106**, 17187-17192,
544 doi:10.1073/pnas.0904847106 (2009).
- 545 47 Caporaso, J. G. *et al.* Moving pictures of the human microbiome. *Genome
546 Biology* **12**, R50, doi:10.1186/gb-2011-12-5-r50 (2011).
- 547 48 Dethlefsen, L. & Relman, D. A. Incomplete recovery and individualized
548 responses of the human distal gut microbiota to repeated antibiotic
549 perturbation. *Proceedings of the National Academy of Sciences* **108**, 4554-
550 4561, doi:10.1073/pnas.1000087107 (2011).
- 551 49 Villmones, H. C. *et al.* Species Level Description of the Human Ileal Bacterial
552 Microbiota. *Scientific Reports* **8**, 4736, doi:10.1038/s41598-018-23198-5
553 (2018).
- 554 50 Hinata, M. *et al.* A Shift from Colon- to Ileum-Predominant Bacteria in Ileal-
555 Pouch Feces Following Total Proctocolectomy. *Digestive diseases and sciences*
556 **57**, 2965-2974, doi:10.1007/s10620-012-2165-9 (2012).
- 557 51 Maharshak, N. *et al.* Alterations of Enteric Microbiota in Patients with a
558 Normal Ileal Pouch Are Predictive of Pouchitis. *Journal of Crohn's and Colitis*
559 **11**, 314-320, doi:10.1093/ecco-jcc/jjw157 (2017).
- 560 52 Harris, K. G. & Chang, E. B. The intestinal microbiota in the pathogenesis of
561 inflammatory bowel diseases: new insights into complex disease. *Clinical
562 science (London, England : 1979)* **132**, 2013-2028, doi:10.1042/cs20171110
563 (2018).
- 564 53 De Cruz, P. *et al.* Association between specific mucosa-associated microbiota
565 in Crohn's disease at the time of resection and subsequent disease
566 recurrence: a pilot study. *J Gastroenterol Hepatol* **30**, 268-278,
567 doi:10.1111/jgh.12694 (2015).
- 568 54 Gevers, D. *et al.* The treatment-naive microbiome in new-onset Crohn's
569 disease. *Cell Host Microbe* **15**, 382-392, doi:10.1016/j.chom.2014.02.005
570 (2014).
- 571 55 Pancholi, V. & Caparon, C. in *Streptococcus pyogenes : Basic Biology to Clinical
572 Manifestations* (eds J. J. Ferretti, D. L. Stevens, & V. A. Fischetti) Ch.
573 *Streptococcus pyogenes Metabolism*, (University of Oklahoma Health
574 Sciences Center
575 (c) The University of Oklahoma Health Sciences Center., 2016).

- 576 56 Scott, K. P., Gratz, S. W., Sheridan, P. O., Flint, H. J. & Duncan, S. H. The
577 influence of diet on the gut microbiota. *Pharmacological Research* **69**, 52-60,
578 doi:10.1016/j.phrs.2012.10.020 (2013).
- 579 57 Abuhelwa, A. Y., Williams, D. B., Upton, R. N. & Foster, D. J. R. Food,
580 gastrointestinal pH, and models of oral drug absorption. *European Journal of*
581 *Pharmaceutics and Biopharmaceutics* **112**, 234-248,
582 doi:10.1016/j.ejpb.2016.11.034 (2017).
- 583 58 Lund, P., Tramonti, A. & De Biase, D. Coping with low pH: molecular strategies
584 in neutralophilic bacteria. *FEMS Microbiology Reviews* **38**, 1091-1125,
585 doi:10.1111/1574-6976.12076 (2014).
- 586 59 Bradshaw, D. & Marsh, P. D. Analysis of pH-Driven Disruption of Oral
587 Microbial Communities in vitro. *Caries Research* **32**, 456-462 (1998).
- 588 60 Wright, E. K. *et al.* Recent Advances in Characterizing the Gastrointestinal
589 Microbiome in Crohn's Disease: A Systematic Review. *Inflammatory bowel*
590 *diseases* **21**, 1219-1228, doi:10.1097/MIB.0000000000000382 (2015).
- 591 61 Lim, W. C., Wang, Y., MacDonald, J. K. & Hanauer, S. Aminosalicylates for
592 induction of remission or response in Crohn's disease. *The Cochrane database*
593 *of systematic reviews* **7**, Cd008870, doi:10.1002/14651858.CD008870.pub2
594 (2016).
- 595 62 Allocca, M. *et al.* Effectiveness of Mesalazine, Thiopurines and Tumour
596 Necrosis Factor Antagonists in Preventing Post-Operative Crohn's Disease
597 Recurrence in a Real-Life Setting. *Digestion* **96**, 166-172 (2017).
- 598 63 Hanauer, S. B. *et al.* Postoperative maintenance of Crohn's disease remission
599 with 6-mercaptopurine, mesalamine, or placebo: A 2-year trial.
600 *Gastroenterology* **127**, 723-729, doi:10.1053/j.gastro.2004.06.002 (2004).
- 601 64 Singh, S. & Nguyen, G. C. Management of Crohn's Disease After Surgical
602 Resection. *Gastroenterology clinics of North America* **46**, 563-575,
603 doi:10.1016/j.gtc.2017.05.011 (2017).
- 604 65 Smits, S. A. *et al.* Seasonal cycling in the gut microbiome of the Hadza hunter-
605 gatherers of Tanzania. *Science* **357**, 802-806, doi:10.1126/science.aan4834
606 (2017).
607

| Subject ID* | Mesalamine Formulation† | Age | BMI | Sex | Stomach | Duodenum | Jejunum | | | Stool | Total |
|----------------|----------------------------|----------------|----------------|--------------|-----------|-----------|-----------|-----------|----------|----------|------------|
| | | | | | | | Proximal | Mid | Distal | | |
| M046-A | Pentasa | 38 | 21.2 | M | 1 | 8 | - | 7 | - | 1 | 17 |
| M046-B | Apriso | 38 | 21.3 | M | - | 8 | - | 5 | 6 | - | 19 |
| M046-C | Lialda | 38 | 21.7 | M | 8 | 6 | - | 7 | - | 1 | 22 |
| M047 | Pentasa | 36 | 21.1 | M | - | 8 | 6 | - | - | - | 14 |
| M048 | Apriso | 51 | 34.3 | F | 5 | 7 | - | - | - | - | 12 |
| M053 | Apriso | 34 | 25.2 | F | 1 | - | 7 | 3 | - | - | 11 |
| M061 | Pentasa | 51 | 21.6 | M | 7 | 8 | - | - | - | - | 15 |
| M062 | Pentasa | 37 | 27.3 | M | 7 | 7 | - | - | - | 1 | 15 |
| M063 | Lialda | 26 | 28.6 | M | 7 | 5 | - | 5 | - | - | 17 |
| M064 | Lialda | 25 | 27.5 | F | 8 | 7 | - | - | - | - | 15 |
| Summary | 40% P, 30% A, 30% L | 37 ±8.6 | 25 ±4.4 | 70% M | 44 | 64 | 13 | 27 | 6 | 3 | 157 |

*All subjects were caucasian and none identified as hispanic/latinx.

†Pentasa = Immediate release in stomach acid; Apriso = Extended release at pH > 6; Lialda = Extended release at pH > 7









