Gastric metabolomics analysis supports *H. pylori*'s catabolism of organic and amino acids in both the corpus and antrum

Short title: Gastric metabolomic changes with *H. pylori* infection

Daniela Keilberg¹, Nina Steele^{2#}, Sili Fan³, Christina Yang¹, Yana Zavros^{2@}, Karen M. Ottemann¹

1: Department of Microbiology and Environmental Toxicology, University of California, Santa Cruz, Santa Cruz, CA 95060 USA.

2: Department of Pathology and Lab Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA

3: West Coast Metabolomics Center, University of California Davis, Davis, CA, USA

Present address: Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, Michigan, USA

@ Present address: Department of Cellular and Molecular Medicine, University of Arizona, Tucson AZ, USA

* Corresponding author at ottemann@ucsc.edu

Abstract

Helicobacter pylori is a chronic bacterial pathogen that thrives in several regions of the stomach, causing inflammation that can vary by site and result in distinct disease outcomes. It is not known, however, whether the host-derived metabolites differ between the two main regions, the corpus and antrum. We thus characterized the metabolomes of mouse gastric corpus and antrum organoids and tissue. The secreted organoid metabolites differed significantly between the corpus and antrum in only seven metabolites: lactic acid, malic acid, phosphoethanolamine, alanine, uridine, glycerol, and isoleucine, with only lactic acid and phosphoethanolamine exceeding two-fold differences. Multiple chemicals were depleted upon *H. pylori* infection including urea, cholesterol, glutamine, fumaric acid, lactic acid, citric acid, malic acid, and multiple non-essential amino acids. Many are known *H. pylori* nutrients and did not differ between the two regions. When tissue was examined, there was little overlap with the organoid metabolites. The two tissue regions were distinct, however, including in antrum-elevated 5-methoxytryptamine, lactic acid, and caprylic acid, and corpus-elevated phospholipid products. Over an 8 month infection time course, the corpus and antrum remained distinct. The antrum displayed no significant changes, in contrast to the corpus which exhibited metabolite changes indicating stress, tissue damage, and depletion of key nutrients such as glutamine and fructose-6-phosphate. Overall, our results suggest the *H. pylori* preferentially uses carboxylic acids and amino acids in complex environments, and these are found in both the corpus and antrum.

Introduction

The mammalian body is colonized by a rich diversity of bacteria that can utilize a range of nutrients. To establish a long-term colonization, bacteria must sense and adapt to nutrient conditions that likely vary temporally and spatially. One bacterium that can establish a chronic infection is *Helicobacter pylori*, which colonizes two main stomach regions, the corpus, also called the fundus in mice, and the antrum. Little is known about the nutrients *H. pylori* utilizes in these two regions and how they vary over space and time.

H. pylori colonizes the stomachs of many people: 50% worldwide and 35% in the United States (1). The infection is acquired in childhood, and becomes chronic and persistent unless treated with antibiotics. Approximately 85% of infected people are asymptomatic, developing only a low-level inflammation. However, around 15% of infected people will develop serious *H. pylori* triggered disease during their life, of either a gastric or duodenal ulcer or gastric cancer (2, 3).

The corpus and antrum are distinct from each other in several ways. In each of these regions, the epithelium is highly invaginated into gastric glands that each contain stem cells (4, 5). These stem cells give rise to a distinct set of epithelial cells in each area. Both areas contain cells that secrete mucus apically, but the corpus is specialized for digestive functions while the antrum is specialized for endocrine ones (6). The corpus digestive functions are created via acid-secreting parietal cells along with pepsinogen- and lipase-secreting chief/zymogenic cells, all secreted apically. The antrum endocrine function is created by gastrin-producing G cells, which is secreted basolaterally. Overall, these different cell types create a distinct milieu in each region, which presumably requires distinct *H. pylori* abilities for colonization. There is some support for

this idea: *H. pylori* mutants lacking chemotaxis have a greater colonization defect in the antrum as compared with the corpus (7-9), while mutants lacking the cytotoxin-associated pathogenicity island (*cagPAI*) genes *cagA* or *cagY* have a greater colonization defect in the corpus as compared to the antrum (10). However, the nature of the differences that *H. pylori* encounters in these two regions is not known.

Several studies have compared *H. pylori* colonization dynamics between the mouse corpus and antrum (8, 9). *H. pylori* infections begin corpus dominant, by 5-10-fold higher amounts compared with the antrum. Over the first two months, however, *H. pylori* multiplies to a great extent in the antrum, such that the infection becomes antral dominant from one week post infection to ~ 2 months post infection (8, 9). After two months of infection, the *H. pylori* numbers decline more-so in the antrum and the infection becomes corpus dominant again. These experiments suggest the antrum switches between a challenging and favorable environment for *H. pylori* growth. In agreement with this idea, bacterial chemotaxis, the ability to sense environmental conditions and move toward beneficial ones and away from harmful ones, is particularly critical in the antrum (7, 9). Overall, these colonization patterns suggest that host metabolites might differ between the corpus and the antrum, and possibly change over the course of the infection.

Knowledge about the corpus and antrum conditions is important because *H. pylori*-triggered diseases differ in these two areas. Inflammation localized to the antrum leads mainly to ulcers and few cancers, inflammation in the corpus correlates with intestinal-type gastric cancer, and inflammation throughout the stomach increases the risk of diffuse-type gastric cancer (3, 11, 12). It is not known whether there are metabolites differences between these

regions. Metabolomics studies of gastric tissue have been limited. The total mouse gastric metabolome was analyzed over an *H. pylori* infection time course, but the separate corpus and antrum metabolites were not characterized (13). They did find evidence of upregulation of glycolysis, TCA and choline pathway intermediates, but the findings were not consistent across time points. Other studies have compared the metabolomes of human gastric cancerous and normal tissue (Reviewed in (14)), in part with the idea that these metabolites might be biomarkers of disease (15). Those studies were noted to have limitations, but *in toto* suggested that cancerous tissue has elevated amounts lactic acid, citric acid, fumaric acid, glutamine, glutamate, and valine as well as several free fatty acids. To help fill gaps in our knowledge, we thus embarked on a study of the corpus and antrum metabolites, to describe them and evaluate how *H. pylori* infection affected them. We report here that a handful of metabolites differ between these compartments. Several are depleted upon *H. pylori* infection, and thus may represent preferred nutrient sources for *H. pylori*. We additionally find that the antrum is surprisingly stable pre- and post-infection, while the corpus undergoes substantial alterations. Overall, our results are consistent with *H. pylori* preferential catabolism of carboxylic acids and amino acids in both the corpus and the antrum.

Results

Secreted metabolites of antrum and corpus gastric organoids are similar

We investigated the differences in corpus/fundus and antrum first using a mouse organoid system, because this approach would allow us to isolate secreted metabolites that would be accessible to *H. pylori*. For this experiment, mouse gastric glands were isolated from

the corpus or antrum, cultured as spherical organoids. This culture method results in representation of all standard stomach epithelial cells, including surface pit, mucous neck, chief, endocrine and parietal cells (16). After development of spheroids, the organoids were transferred to collagen-coated dishes to allow them to flatten out as two-dimensional (2D) organoids. For the 2D organoid culture, the medium is very rich and contains tissue culture medium (DMEM), fetal bovine serum, and multiple growth factor supplements. For metabolomics analysis, the culture supernatant was collected, filtered to remove cells, and analyzed using gas chromatography time of flight (GC-TOF) mass spectroscopy for various metabolites, including carbohydrates, sugar phosphates, amino acids, hydroxyl acids, free fatty acids, purines, and pyrimidines.

Using this approach, a total of 352 compounds were found to be secreted by the organoids, of which 132 (38%) were identified chemicals, while the other 220 (62%) were unknown chemical structures (Supplemental Table 1). Principal component analysis suggested that the organoids differed only minimally from the media (Fig. 1A), which was consistent with the richness of the organoid media. Specifically, the corpus organoids had no significant differences from media, while the antrum organoids differed significantly in 13 compounds, of which 12 were identified chemicals (Supplemental Table 2). Nine chemicals were decreased in the antral organoids as compared to the media, suggesting they may be used by the antral organoid cells. These nine included the amino acids aspartic acid, leucine, serine, isoleucine, phenylalanine, and valine, and the chemicals glycerol, ethanolamine and nicotinamide (Supplemental Table 2). Three metabolites, however, were elevated 1.7-3.9 fold by the antral organoid system, suggesting they may be excreted by the cells: the carboxylic acids lactic acid

and malic acid, and the phospholipid component phosphoethanolamine. This analysis thus suggests that media collected after exposure to corpus organoids did not change much in composition, while exposure to antral organoids lead to a significant increase of lactic acid, malic acid and phosphoethanolamine.

We next compared the antrum and corpus organoids to each other (Fig. 1A). 26 named compounds differed between the two, with seven of these significant (Table 1). Antral organoids were significantly enriched over the corpus organoids for lactic acid, malic acid, and phosphoethanolamine, as well as the amino acid alanine. Corpus organoids, on the other hand, were enriched for the pyrimidine nucleoside uridine, glycerol, and the amino acid isoleucine. For the most part, these differences were not more than 2-fold, but a few compounds were more substantial. The antrum was over 2-fold enriched for lactic acid and phosphoethanolamine; fumaric acid was also highly enriched but did not reach significance (Table 1). ChemRICH chemical similarity analysis (17) using the larger set of non-FDR corrected significant compounds and fold changes identified that the corpus-antrum organoid differences were dominated by differences in general amino acids, branched chain amino acids, and dicarboxylic acids (Fig. 1B and Supplemental Table 3A). These analyses show that corpus and antrum organoids display some differences between each other, dominated by the antral presence of carboxylic acids and phosphoethanolamine.

Antrum and corpus organoid supernatants show a metabolomic response to *H. pylori* infection

We next examined how the antrum and corpus organoid supernatants changed after *H. pylori* infection. Both types of organoids support *H. pylori* colonization (18, 19). After preparation,

the 2D organoids were infected by adding the mouse-adapted wild-type *H. pylori* strain SS1 to the media. Infections proceeded for 24 hours, and then the supernatant was collected, filtered, and analyzed as above. Out of 132 identified chemicals, infection resulted in a change of 46 and 51 identified chemicals in the antrum and corpus, respectively (Table 2-3; Supplemental Table 4-5). Changes in both types of organoid supernatants mapped to multiple categories of metabolites, but most substantially to amino acids and dicarboxylic acids, and additionally sugar alcohols in the corpus (Fig. 1D-E, Supplemental Table 3). These changes resulted in a substantial shift in the principal components between uninfected and infected samples (Fig. 1A).

Several of the changed metabolites were depleted with *H. pylori* infection, and these metabolites are generally consistent with what is known about *H. pylori* metabolism. For example, urea was highly depleted in both corpus and antrum systems (Table 2; Fig. 2-3). This decrease agrees with *H. pylori*'s robust urease and its known ability to find urea via chemotaxis (Huang et al. 2015). Similarly, glutamine was highly depleted, consistent with *H. pylori*'s gamma glutamyl transferase (GGT) which breaks glutamine into glutamate and ammonia (20, 21). Lactic acid was highly depleted in each system too, consistent with *H. pylori*'s ability to use lactic acid as a carbon or energy source (22). Additional amino acids or amino acid derivatives were substantially depleted as well: serine, N-acetylglutamate, aspartic acid, oxoproline/pyroglutamic acid, and tyrosine. Only one of these is an essential amino acid—serine, and only for some strains (23). The other amino acids are likely used as *H. pylori* carbon and nitrogen source, as has been shown for aspartic acid (24). Also used up were several additional carboxylic acids including malic acid, fumaric acid, and citric acid.

Overall, the metabolite usage was similar but non identical between the corpus and antrum organoids, suggesting *H. pylori* has a fairly similar metabolism in both regions. There were some differences, however. For example, cholesterol, a requirement for *H. pylori* growth (25, 26), was substantially depleted in infected antrum organoids but not corpus ones (Fig. 3), although present in both. Additionally, there were several compounds that were lowered more in the corpus, including the carboxamide lactic acid derivative lactamide, the saturated medium chain fatty acid capric acid (also called decanoic acid), benzoic acid, the sugar alcohol threitol, and the purine guanosine. Again, these compounds were present in both organoids, but not highly used in the antrum. Overall, these results suggest that *H. pylori* depletes multiple metabolites in both corpus and antrum organoids that appear to be key nutrients and growth substrates, with some region-specific utilization.

Several compounds were increased in infected organoids as compared to uninfected ones (Table 2, Table 3). The vast majority of these compounds appeared to come from *H. pylori*, as they were also present in bacteria alone samples (Fig. 2). Indeed, when we compared the infected antral organoids to the *H. pylori* samples, only one compound was significantly increased: the malic acid analog citramalic acid (Fig. 2).

After infection, corpus and antrum organoids appeared more distinct than before infection as seen by a shift and separation of the principle components (Fig. 1A), although the number of significantly different metabolites were about the same before and after infection (Table 1, Table 4). After infection, there were five significantly differing compounds that were all found in higher levels in the antrum than in the corpus. The most striking change was in lactic acid, which was heavily used in the corpus and became 150-fold more abundant in the antrum.

Phosphoethanolamine also remained enriched in the antrum, staying at about 2-fold over the corpus. The antrum was newly enriched for lactamide, uracil, and citramalic acid (Table 4). Employing ChemRICH enrichment analysis suggested that substantial differences in amino acids were lost, and that differences were dominated by those in dicarboxylic acids (Fig. 1C). Overall, these analyses suggest that *H. pylori* uses a discrete set of organoid-secreted metabolites, dominated by urea, glutamine, lactic acid, fumaric acid, citric acid, and specific amino acids. For the most part, these are used similarly but non-identically between the types of organoids with more utilization in the corpus. This differential resulted in the post- infection metabolomes differing from the pre-infection ones.

The uninfected mouse tissue differs between antrum and corpus

We next assessed how uninfected mouse gastric tissue compared with the gastric organoids analyzed above. First, mouse gastric tissue had substantially fewer total compounds as compared with organoids: 194 total compounds in the gastric tissue as compared with 352 in the organoids. A somewhat higher percentage of the tissue metabolites were known: 52% in the tissue as compared to 38% in the organoids (Suppl. Table 7). Between the two regions, there were 15 identified compounds that differed significantly between the corpus and the antrum tissue (Table 5), a number that was about 2-fold greater than the number of metabolites that were different between the uninfected antrum and corpus organoids (Table 1). Generally, most of these differing compounds were enriched in the antrum. Specifically, the antrum was enriched for several compounds used by *H. pylori* in the organoids, including lactic acid, glutamine, capric acid, and benzoic acid (Fig. 3), as well as other *H. pylori* nutrients such as pyruvic acid. The corpus was

enriched for a few other compounds used up in the organoid system, malic acid and fumaric acid, (Table 5). Overall, the majority of the differing compounds were elevated in the antrum.

When comparing the metabolites of the gastric tissue to the organoids, there was not substantial overlap (Table 1 and Table 5). This lack of similarity is perhaps not surprising, as the mouse tissue sample was whole sample, not restricted to secreted molecules and was cultured in the lab for multiple days. The cultured organoid cells are fed predominantly glucose and glutamine, which may be different than their in vivo metabolism. Therefore, it may be that *H. pylori* is exposed to different metabolites in the two systems.

The corpus and antrum mouse tissue remain distinct post infection

We then infected mice and evaluated how the metabolomes changed over time. Samples were collected from mice infected for 3 days, 28 days, and 8 months, representing early and chronic infections and confirmed for infection (Supplemental Fig. 1). We then used these samples to see whether the corpus and antrum metabolite differences were stable across this time course. Generally, as the infection proceeded, there were fewer compounds that differed between the two regions. Using the full set of different compounds (not FDR corrected), the differing metabolites decreased from 37, to 14, 25, and 29 at the 3-day, 28-day, and 8-month timepoints respectively (Table 6). Compounds that were consistently distinct between the corpus and antrum included the antrum-elevated 5-methoxytryptamine, lactic acid, and the fatty acid caprylic/octanoic acid. Chemicals that were regularly high in the corpus included multiple products that derive from phospholipids, including arachidonic acid, linoleic acid, oleic acid, and glycerol alpha phosphate (Table 6). Lastly, several compounds were distinct at the outset, before

infection, but became more similar between regions after infection, including monoacylglycerol 2-monopalmitin (also called Glyceryl 2-palmitate), the sugars glucose and sucrose, and the carboxylic acids pyruvic acid and succinic acid. Taken together, the data suggests that the corpus and antrum remain distinct throughout the infection, with relatively modest changes, but may become slightly more similar over time.

Tissue changes over the course of an infection

As described above, we collected tissue from mice infected for varying lengths of time. Initially, we hoped to compare all these samples, but were not able to do this because the metabolomics analyses were run on separate days and no normalization methods could remove the analysis-day bias. We therefore compared age-matched uninfected with 8 month-infected samples, and 3 day with 28 day infected samples because these sample metabolomes were analyzed together. Overall, the antral tissue remained stable, with no significant metabolite changes, between 0-8 months and 3-28 days. The only differences detected were in the corpus, specifically comparing uninfected tissue to 8 month infected tissue. In this case, there were 37 identified compounds that differed significantly (Table 7) and 61 total (Supplemental Table 8). Compounds that substantially increased during this time included a nearly 5-fold increase in adenosine, several lipids including those related to phospholipids (arachidonic acid, its product arachidic acid, oleic acid, linoleic acid), the lipid 1-monoolein, and the cholesterol precursor squalene. Additionally, there were ~1.5-2-fold increases in the ATP product inosine, and the inorganic compound hydroxylamine. Compounds that decreased included the purine derivative uric acid, several amino acids (glutamine, aspartic acid, ornithine, aminomalonate) and the sugar fructose-6-

phosphate. These data suggest that the antrum metabolome remains remarkably stable in the face of an *H. pylori* infection. The corpus, in contrast, displays several changes between the uninfected and chronically infected mice. These include indicators of tissue damage at the later time points, as well as depletion of key nutrients such as glutamine and fructose-6-phosphate that support cell proliferation.

Discussion

We report the metabolomic differences that exist between gastric corpus and antral organoid-secreted products and gastric tissues, and how these two metabolomes respond to *H. pylori* infection. The gastric organoid data is the most straightforward to interpret because we were able to analyze the extracellular molecules. We find that *H. pylori* infection leads to depletion of specific metabolites in both the corpus and antrum, and these lost metabolites do not differ much between these regions. Our results suggest the preferred carbon and energy sources of *H. pylori* are the carboxylic acids lactic acid, lactamide, fumaric acid, malic acid and citric acid as well as the amino acids glutamine, serine, N-acetylglutamate, aspartic acid, oxoproline/pyroglutamic acid, and tyrosine. The simplest interpretation is that these compounds are used by *H. pylori*, an idea that is supported by the decrease in compounds that are known to be used by *H. pylori* such as urea, glutamine, cholesterol, lactic acid, and fumaric acid, although it's possible that they are actively depleted by the mammalian cells after *H. pylori* infection.

Surprisingly, little is known about the preferred nutrient and energy sources of *H. pylori* when confronted with a complex mixture. Several studies support that the microbe does not substantially rely on sugars such as glucose, and instead relies on amino acids and carboxylic acids

(27-29). Previous work supports the catabolism of carboxylic acids that we also show are used here. *H. pylori* can catabolize fumaric acid (30), malic acid (24), and lactic acid (22). Catabolism of carboxylic acids requires first their uptake. *H. pylori* has a C4-dicarboxylate transport protein that takes up fumaric acid and malic acid but not the larger citric acid (31). Citric acid complexes with iron, and there are several known ferric and ferrous citrate uptake systems in that have been characterized for their iron uptake properties but also presumably bring citrate into the cell as well (32). Another interpretation is that citric acid is depleted from the supernatant because it is used as a source of iron. Lactic acid has a separate uptake system, the LctP lactate permease. *H. pylori* actually has two lactate permease genes, *lctP1* (HP0140) and *lctP2* (HP0141), which are encoded next to each other in the genome (22). Studies have shown these two proteins are needed for lactate uptake, but have not yet determined if they play distinct roles.

Another finding from this work is that amino acids are depleted upon *H. pylori* infection. Amino acids can act as carbon, nitrogen, and energy sources. Work using defined medium determined that there are several *H. pylori* essential amino acids (e.g. arginine, histidine, leucine, methionine, phenylalanine, and valine), with others showing cross-study variation (alanine, cysteine, proline, serine and isoleucine) (23) but—for the most part—these were not the ones that were depleted here. Instead, it seems most likely that our depleted amino acids are carbon/nitrogen or energy sources. This idea agrees well with findings that *H. pylori* can catabolize amino acids as sole carbon/energy sources for *H. pylori* growth (29). Specifically, *H. pylori* catabolizes a large number of amino acids with the most substantial being alanine, arginine, asparagine, aspartate, glutamate, glutamine, proline, and serine, with studies reporting variation in the use of alanine, glycine, and valine (24, 29, 33, 34). This list is consistent with the

amino acids depleted in this work although we did not detect depletion of all of these, and additionally found depletion of tyrosine and the glutamate derivatives N-acetylglutamate and pyroglutamic acid/oxoproline. *H. pylori* has several transporters that can take up amino acids including GltS for glutamate and DcuA for aspartate (35). These same transporters also take up glutamine and asparagine after they are deamidated to glutamate and aspartate. Other known transporters include those for peptides, Dpp and Opp (36, 37). There remain several unknowns. For serine, there is a predicted but as-yet uncharacterized serine uptake transporter (*sdaC*/HP0133), there is no predicted tyrosine transporter, and it is not yet known whether GltS can take up glutamate derivatives like N-acetylglutamate or pyroglutamic acid.

The mouse gastric tissue analysis uncovered that the antral metabolome is surprisingly stable over the course of an 8 month infection while the corpus showed significant changes. Another study analyzed the mouse gastric metabolome over an infection time course, but did not separate the corpus and the antrum (13). These authors also found relatively minimal changes in the mouse gastric metabolome over time. Our data suggest the infected corpus tissue has hallmarks of damage including elevated phospholipid products and adenosine, as well as depletion of important nutrients including the amino acids glutamine, aspartic acid and the sugar fructose-6-phosphate. *H. pylori* has been shown to use chemotaxis to preferentially colonize sites of gastric injury, further suggesting that these changes may lead to accumulation of *H. pylori* in the corpus region (38). Taken together, the metabolomics supports the idea that the corpus is undergoing atrophy, a known *H. pylori*-associated condition (39), and indeed that released phospholipids may be early indicators of carcinogenesis (40). Adenosine has immunosuppressive properties, and also has been associated with cancer development (41, 42). Thus the data might

suggest that the *H. pylori* mouse model develops signs of carcinogenesis, cell damage, immunosuppression that can be detected using metabolomics.

In summary, our studies suggest that *H. pylori* utilizes multiple carboxylic acids and amino acids in both the corpus and the antral organoids, with minimal difference in metabolite usage between the two epithelia. We also find that there is very little overlap between the metabolites detected in the gastric organoid and tissue systems, reflecting the challenges of comparing the two systems. Lastly, we report that the antral metabolome is surprisingly stable over an 8 month *H. pylori* infection period, while the corpus displays significant changes that may indicate an early carcinogenic process.

Acknowledgements

The authors are grateful to Eitaro Aihara and Chip Montrose (Univ. of Cincinnati) for gastric organoid culture training and advice; Andrew Liu and Kyle Kaminski (UC Santa Cruz) for experimental assistance; Kevin Johnson and Shuai Hu (UC Santa Cruz) for comments on the manuscript. The work described here was supported by National Institutes of Health National Institute of Allergy and Infectious Disease grant RO1AI116946 (to K.M.O.), the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases grant R01DK083402 (to Y.Z.), the California Cancer Research Coordinating Committee grant CRC- 15-380545 (to K.M.O.) and a postdoctoral fellowship from Leopoldina (to D.K.). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Material and Methods

H. pylori strains and growth conditions

H. pylori strain SS1 with the GFP-expressing plasmid pTM115 was used for all studies (9, 43, 44). *H. pylori* were grown at 37°C, 5% O₂, and 10% CO₂ (balance N₂). For solid media, we used Columbia blood agar (BD Diagnostics, Fisher Scientific) with 5% defibrinated horse blood (Hemostat Labs, Dixon, California), 50 µg/ml cycloheximide (VWR), 10 µg/ml vancomycin, 5 µg/ml cefsulodin, 2.5 Units/ml polymyxin B (all from Gold Biotechnology, St Louis, MO), and 0.2% (wt/vol) β-cyclodextrin (Spectrum Labs, Gardena, CA) (CHBA). For liquid media, we used Brucella Broth (BBL) media supplemented with 10% heat-inactivated fetal bovine serum (FBS, Life Technologies) (BB10). For metabolomics analysis, *H. pylori* was grown for 24 hours in BB10 broth, and then filtered to remove bacterial cells as described below.

Organoid preparation.

All organoids and tissues were prepared from female C57Bl/6N mice (Helicobacter-free; Charles River). The University of California, Santa Cruz (UCSC) Institutional Animal Care and Use Committee approved all animal protocols and experiments (Protocol #OTTEK1505). All animal procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals. Mice were housed at the UCSC animal facility and given free access to food and water. For gastric organoids, 12 week old mice were sacrificed by CO₂ narcosis, and the stomach was removed by cutting at the stomach-esophageal junction and the antrum-duodenum sphincter. The forestomach was removed and the stomach opened along the lesser curvature using scissors. The stomach was gently rinsed by moving back and forth in 25 ml ice cold phosphate buffered

saline (PBS). Blood vessels and the muscle layer were removed from the non-luminal side of the tissue using microscissors (Kelly Scientific) and tweezers. The corpus was separated from the antrum with a scalpel, based on the difference in tissue coloration as a marker for the border between these regions, excluding a ~ 5mm section between the two tissues to ensure purity. Each piece was further divided into thirds to be used for organoid preparation, bacterial enumeration, or flash frozen in liquid N₂ for metabolomics analysis.

For organoids, we next isolated using a protocol adapted from Mahe *et al.* (45). Dissected gastric tissue was cut into 1 mm² pieces and incubated with slight shaking in Dulbeccos Phosphate buffered saline (DPBS, Millipore) with 5mM EDTA at 4°C for 2 hours. After this period, the tissue was transferred into ice-cold DBPS containing 1% sucrose and 1.5% sorbitol, and shaken roughly by hand for 2 minutes. The remaining large tissue pieces were allowed to settle, and 2 ml of the solution containing the glands was removed. Glands were washed with cold DPBS, and mixed into 50 µl of matrigel (BD Biosciences). Matrigel and glands were placed into a 24-well plate if being prepared for flat organoids and incubated for 10 minutes at 37°C, until the Matrigel solidified. 3D-gastric organoid media was then added, to cover the Matrigel and the glands. 3D-gastric organoid media contains Advanced DMEM/F12 (Invitrogen) supplemented with 2mM GlutaMax (Invitrogen), 10 mM HEPES (Sigma), 100 U/mL penicillin/100 µg/mL streptomycin (Invitrogen), 1× N2 supplement (Invitrogen), 1× B27 supplement (Invitrogen), 1 µg/mL mouse R-spondin (R&D systems), 100 ng/mL Noggin (Peprotech), 50 ng/mL Epidermal Growth Factor (EGF, Peprotech). Media was exchanged with fresh media every 2 days for up to 7 days.

To create 2D organoids, the spheroid gastric organoids were collected with ice-cold DPBS, the matrigel removed by a washing and centrifugation at 195xg for 5 minutes at 4°C. Organoids

were collected and resuspended in 500 µl 2D culture media (DMEMF12, Advanced-Base Medium, 10% Fetal Bovine Serum, 10 mM HEPES, 2mM Glutamine, 1X N2 supplement, 1X B27 supplement, 10 µM Y-compound (Y-27632, Sigma), 50 ng/ml EGF) and placed into a collagen-coated 24 well plate for attachment. After 24 hours, the media was replaced with fresh 2D culture media plus 1×10^7 *H. pylori* SS1 pTM115 per well. The *H. pylori* was grown in BB10 as above. After 24 hours of infection, media was collected from the organoids or bacterial cells, passed through a 0.2 µm filter to remove organoid and bacterial cells from the media, and quick frozen in liquid N₂. For this analysis, we had 6 independent samples for most groups (corpus infected, corpus uninfected, antrum infected, antrum uninfected and the *H. pylori* alone control) and 3 independent samples for the media alone.

Mouse tissue collection and preparation

Female C57Bl6/N mice (Charles River) were infected at 4-6 weeks of age with 500 µl *H. pylori* SS1 pTM115 grown in BB10 media to mid-exponential phase (OD₆₀₀ ~0.3). Infection was accomplished by oral infection with a 20-gauge × 1.5 inch feeding needle (Popper). At the desired time, the mouse stomachs were collected as for organoids and divided into corpus and antrum. For metabolomics, pieces were flash frozen in liquid N₂. For bacterial number determination, the piece was weighed, homogenized using the Bullet Blender (Next Advance) with 1.0-mm zirconium silicate beads, diluted, and plated on CHBA with the addition of 20 µg/ml bacitracin, 10 µg/ml nalidixic acid, and 15 µg/ml kanamycin.

Metabolomic profiling

Samples for metabolomics were quick frozen in liquid N₂, stored at -80°C, and shipped on dry ice to the UC Davis NIH West Coast Metabolomics Center (<https://metabolomics.ucdavis.edu>). An untargeted analysis of primary metabolites was performed using automatic liner exchange chromatography and cold injection gas chromatography (GC) time of flight (TOF) mass spectrometry methods as detailed in Fiehn *et al.* (46). Briefly, the GC column was a GC Restek corporation Rtx-5Sil MS column with a mobile phase of helium with automatic liner exchanges after each set of 10 injections. Mass spectrometry parameters were as follows: a Leco Pegasus IV mass spectrometer is used with unit mass resolution at 17 spectra s⁻¹ from 80-500 Da at -70 eV ionization energy and 1800 V detector voltage with a 230°C transfer line and a 250°C ion source. ChromaTOF vs. 2.32 was used for data preprocessing without smoothing, 3 s peak width, baseline subtraction just above the noise level, and automatic mass spectral deconvolution and peak detection at signal/noise levels of 5:1 throughout the chromatogram. Apex masses are reported for use in the BinBase algorithm. Result *.txt files are exported to a data server with absolute spectra intensities and further processed by a filtering algorithm implemented in the metabolomics BinBase database. The BinBase algorithm (rtx5) used the settings: validity of chromatogram (<10 peaks with intensity >10⁷ counts s⁻¹), unbiased retention index marker detection (MS similarity>800, validity of intensity range for high m/z marker ions), retention index calculation by 5th order polynomial regression. Spectra are cut to 5% base peak abundance and matched to database entries from most to least abundant spectra using the following matching filters: retention index window ±2,000 units (equivalent to about ±2 s retention time), validation of unique ions and apex masses (unique ion must be included in apexing masses and

present at >3% of base peak abundance), mass spectrum similarity must fit criteria dependent on peak purity and signal/noise ratios and a final isomer filter. Failed spectra are automatically entered as new database entries if s/n >25, purity <1s.0 and presence in the biological study design class was >80%. All thresholds reflect settings for ChromaTOF v. 2.32. Quantitative data are reported as relative peak intensities (for example, normalized to the peak intensities found in quality control samples). Data is given with the following information: (1) Binbase name, which includes a name if the peak has been identified, or just a Binbase number/BBID if it is not identified; (2) Retention index (ret index); (3) 'quant mz' column details the m/z value that was used to quantify the peak height of each entry.(4) The 'mass spec' column details the complete mass spectrum of the metabolite given as mz: intensity values, separated by spaces. (5) for identified compounds, external database identifiers such as InChI key, defined by the IUPAC and NIST consortia; PubChem ID, the unique identifier of a metabolite in the PubChem database; and KEGG ID gives the unique identifier associated with an identified metabolite in the community database KEGG LIGAND DB. The 'internal standard' addition within the BinBase name clarifies if a specific chemical has been added into the extraction solvent as internal standard. These internal standards serve as retention time alignment markers, for quality control purposes and for quantification corrections. The full organoid metabolomics data is given in **Supplemental Data Set 1** and the full mouse tissue metabolomics is given as **Supplemental Data Set 2**.

Metabolomics Statistical Analysis.

For normalization, a variant of a 'vector normalization' in which the sum of all peak heights for all identified metabolites was calculated for each sample to create the value called mTIC (47). All

mTIC averages were then compared. If these averages differ by $p < 0.05$, data was normalized to the average mTIC of each group; this was the case for the mouse tissue data. If averages between treatment groups are not different, data was normalized to the total average mTIC; this was the case for the organoid data. For principal component analysis (PCA), we applied log10 transformation and auto-scaling to the data. Difference comparisons between multiple groups was made using a one-way analysis of variance (ANOVA) or between two groups using the Students T test. To control the false discovery rate (FDR), we adjusted the p-values using Benjamini-Hochberg procedure (48). The mean fold change was calculated for each pair of two groups using non-transformed data. To generate the cluster analysis heat map shown in Fig. 2, the known chemicals peak height from the organoid data was submitted to Metaboanalyst (<https://www.metaboanalyst.ca/>) heatmap and clustering analysis. The following parameters were used: concentrations, samples in columns unpaired, data normalization by sum, and auto-scaling. For output, we chose the top 40 by ANOVA.

Enrichment Analysis

Chemical similarly enrichment analysis was performed using the ChemRICH approach (<http://chemrich.us>) (17) with the Mann-Whitney U test p-values and median fold-changes of our identified metabolites. ChemRICH was implemented using the Kolmogorov–Smirnov test on the identified clusters to evaluate whether a metabolite cluster was represented more than expected by chance.

References

1. **Choy M, Switzer P, De Martel C, Parsonnet J.** 2008. Estimating disease prevalence using census data. *Epidemiol Infect* **136**:1253–1260.
2. **Brown LM.** 2000. *Helicobacter pylori*: epidemiology and routes of transmission. *Epidemiol Rev* **22**:283–297.
3. **Wroblewski LE, Peek RM, Wilson KT.** 2010. *Helicobacter pylori* and gastric cancer: factors that modulate disease risk. *Clinical Microbiology Reviews* **23**:713–739.
4. **Yang C, Ottemann KM.** 2018. Control of bacterial colonization in the glands and crypts. *Curr Opin Microbiol* **47**:38–44.
5. **Mills JC, Shivdasani RA.** 2011. Gastric epithelial stem cells. *Gastroenterology* **140**:412–424.
6. **Ding L, El-Zaatari M, Merchant JL.** 2016. Recapitulating Human Gastric Cancer Pathogenesis: Experimental Models of Gastric Cancer. *Adv Exp Med Biol* **908**:441–478.
7. **Terry K, Williams SM, Connolly LL, Ottemann KM.** 2005. Chemotaxis plays multiple roles during *Helicobacter pylori* animal infection. *Infect Immun* **73**:803–811.
8. **Rolig AS, Shanks J, Carter JE, Ottemann KM.** 2012. *Helicobacter pylori* requires TlpD-driven chemotaxis to proliferate in the antrum. *Infect Immun* **80**:3713–3720.

- 497 9. **Keilberg D, Zavros Y, Shepherd B, Salama NR, Ottemann KM.** 2016. Spatial and
498 Temporal Shifts in Bacterial Biogeography and Gland Occupation during the
499 Development of a Chronic Infection. *mBio* **7**:e01705–16.
- 500 10. **Rieder G, Merchant J, Haas R.** 2005. *Helicobacter pylori* cag-Type IV Secretion System
501 Facilitates Corpus Colonization to Induce Precancerous Conditions in Mongolian Gerbils.
502 *Gastroenterology* **128**:1229–1242.
- 503 11. **Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M,**
504 **Taniyama K, Sasaki N, Schlemper RJ.** 2001. *Helicobacter pylori* infection and the
505 development of gastric cancer. *N Engl J Med* **345**:784–789.
- 506 12. **Atherton JC.** 2006. The pathogenesis of *Helicobacter pylori*-induced gastro-duodenal
507 diseases. *Annu Rev Pathol* **1**:63–96.
- 508 13. **Nishiumi S, Yoshida M, Azuma T.** 2017. Alterations in metabolic pathways in stomach of
509 mice infected with *Helicobacter pylori*. *Microb Pathog* **109**:78–85.
- 510 14. **Huang S, Guo Y, Li Z, Zhang Y, Zhou T, You W, Pan K, Li W.** 2020. A systematic review of
511 metabolomic profiling of gastric cancer and esophageal cancer. *Cancer Biol Med* **17**:181–
512 198.
- 513 15. **Chan AW, Gill RS, Schiller D, Sawyer MB.** 2014. Potential role of metabolomics in
514 diagnosis and surveillance of gastric cancer. *World J Gastroenterol* **20**:12874–12882.

16. **Schumacher MA, Aihara E, Feng R, Engevik A, Shroyer NF, Ottemann KM, Worrell RT, Montrose MH, Shivdasani RA, Zavros Y.** 2015. The use of murine-derived fundic organoids in studies of gastric physiology. *J Physiol (Lond)*.
17. **Barupal DK, Fiehn O.** 2017. Chemical Similarity Enrichment Analysis (ChemRICH) as alternative to biochemical pathway mapping for metabolomic datasets. *Sci Rep* **7**:14567.
18. **Bertaux-Skeirik N, Feng R, Schumacher MA, Li J, Mahé MM, Engevik AC, Javier JE, Peek RM, Ottemann K, Orian-Rousseau V, Boivin GP, Helmrath MA, Zavros Y.** 2015. CD44 Plays a Functional Role in *Helicobacter pylori*-induced Epithelial Cell Proliferation. *PLoS Pathog* **11**:e1004663.
19. **Chakrabarti J, Zavros Y.** 2020. Generation and use of gastric organoids for the study of *Helicobacter pylori* pathogenesis. *Methods Cell Biol* **159**:23–46.
20. **Chevalier C, Thiberge JM, Ferrero RL, Labigne A.** 1999. Essential role of *Helicobacter pylori* gamma-glutamyltranspeptidase for the colonization of the gastric mucosa of mice. *Mol Microbiol* **31**:1359–1372.
21. **Shibayama K, Wachino J-I, Arakawa Y, Saidijam M, Rutherford NG, Henderson PJF.** 2007. Metabolism of glutamine and glutathione via gamma-glutamyltranspeptidase and glutamate transport in *Helicobacter pylori*: possible significance in the pathophysiology of the organism. *Mol Microbiol* **64**:396–406.

- 533 22. **Iwatani S, Nagashima H, Reddy R, Shiota S, Graham DY, Yamaoka Y.** 2014. Identification
534 of the genes that contribute to lactate utilization in *Helicobacter pylori*. PLoS ONE
535 9:e103506.
- 536 23. **Testerman TL, Conn PB, Mobley HLT, McGee DJ.** 2006. Nutritional requirements and
537 antibiotic resistance patterns of *Helicobacter* species in chemically defined media. J Clin
538 Microbiol **44**:1650–1658.
- 539 24. **Lee WC, Goh KL, Loke MF, Vadivelu J.** 2017. Elucidation of the Metabolic Network of
540 *Helicobacter pylori* J99 and Malaysian Clinical Strains by Phenotype Microarray.
541 *Helicobacter* **22**.
- 542 25. **Testerman TL, McGee DJ, Mobley HL.** 2001. *Helicobacter pylori* growth and urease
543 detection in the chemically defined medium Ham's F-12 nutrient mixture. J Clin Microbiol
544 **39**:3842–3850.
- 545 26. **Hirai Y, Haque M, Yoshida T, Yokota K, Yasuda T, Oguma K.** 1995. Unique cholesteryl
546 glucosides in *Helicobacter pylori*: composition and structural analysis. J Bacteriol
547 **177**:5327–5333.
- 548 27. **Kelly DJ.** 1998. The physiology and metabolism of the human gastric pathogen
549 *Helicobacter pylori*. Adv Microb Physiol **40**:137–189.
- 550 28. **Marais A, Mendz GL, Hazell SL, Mégraud F.** 1999. Metabolism and genetics of
551 *Helicobacter pylori*: the genome era. Microbiol Mol Biol Rev **63**:642–674.

- 552 29. **Mendz GL, Hazell SL.** 1995. Aminoacid utilization by *Helicobacter pylori*. Int J Biochem
553 Cell Biol **27**:1085–1093.
- 554 30. **Mendz GL, Hazell SL.** 1993. Fumarate catabolism in *Helicobacter pylori*. Biochem Mol Biol
555 Int **31**:325–332.
- 556 31. **Mendz GL, Meek DJ, Hazell SL.** 1998. Characterization of fumarate transport in
557 *Helicobacter pylori*. J Membr Biol **165**:65–76.
- 558 32. **Velayudhan J, Hughes NJ, McColm AA, Bagshaw J, Clayton CL, Andrews SC, Kelly DJ.**
559 2000. Iron acquisition and virulence in *Helicobacter pylori*: a major role for FeoB, a high-
560 affinity ferrous iron transporter. Mol Microbiol **37**:274–286.
- 561 33. **Stark RM, Suleiman MS, Hassan IJ, Greenman J, Millar MR.** 1997. Amino acid utilisation
562 and deamination of glutamine and asparagine by *Helicobacter pylori*. J Med Microbiol
563 **46**:793–800.
- 564 34. **Nagata K, Nagata Y, Sato T, Fujino MA, Nakajima K, Tamura T.** 2003. L-Serine, D- and L-
565 proline and alanine as respiratory substrates of *Helicobacter pylori*: correlation between
566 in vitro and in vivo amino acid levels. Microbiology (Reading, Engl) **149**:2023–2030.
- 567 35. **Leduc D, Gallaud J, Stingl K, de Reuse H.** 2010. Coupled amino acid deamidase-transport
568 systems essential for *Helicobacter pylori* colonization. Infect Immun **78**:2782–2792.

- 569 36. **Rahman MM, Machuca MA, Khan MF, Barlow CK, Schittenhelm RB, Roujeinikova A.**
570 2019. Molecular Basis of Unexpected Specificity of ABC Transporter-Associated
571 Substrate-Binding Protein DppA from *Helicobacter pylori*. J Bacteriol **201**.
- 572 37. **Weinberg MV, Maier RJ.** 2007. Peptide transport in *Helicobacter pylori*: roles of dpp and
573 opp systems and evidence for additional peptide transporters. J Bacteriol **189**:3392–
574 3402.
- 575 38. **Aihara E, Closson C, Matthis AL, Schumacher MA, Engevik AC, Zavros Y, Ottemann KM,**
576 **Montrose MH.** 2014. Motility and chemotaxis mediate the preferential colonization of
577 gastric injury sites by *Helicobacter pylori*. PLoS Pathog **10**:e1004275.
- 578 39. **Nookaew I, Thorell K, Worah K, Wang S, Hibberd ML, Sjövall H, Pettersson S, Nielsen J,**
579 **Lundin SB.** 2013. Transcriptome signatures in *Helicobacter pylori*-infected mucosa
580 identifies acidic mammalian chitinase loss as a corpus atrophy marker. BMC Med
581 Genomics **6**:41–14.
- 582 40. **Johnson CH, Santidrian AF, LeBoeuf SE, Kurczy ME, Rattray NJW, Rattray Z, Warth B,**
583 **Ritland M, Hoang LT, Lorient C, Higa J, Hansen JE, Felding BH, Siuzdak G.** 2017.
584 Metabolomics guided pathway analysis reveals link between cancer metastasis,
585 cholesterol sulfate, and phospholipids. Cancer Metab **5**:9–9.
- 586 41. **Harvey JB, Phan LH, Villarreal OE, Bowser JL.** 2020. CD73's Potential as an
587 Immunotherapy Target in Gastrointestinal Cancers. Front Immunol **11**:508.

- 588 42. **Colgan SP, Fennimore B, Ehrentauf SF.** 2013. Adenosine and gastrointestinal
589 inflammation. *J Mol Med* **91**:157–164.
- 590 43. **Lee A, O'Rourke J, De Ungria MC, Robertson B, Daskalopoulos G, Dixon MF.** 1997. A
591 standardized mouse model of *Helicobacter pylori* infection: introducing the Sydney
592 strain. *Gastroenterology* **112**:1386–1397.
- 593 44. **Amieva MR, Vogelmann R, Covacci A, Tompkins LS, Nelson WJ, Falkow S.** 2003.
594 Disruption of the epithelial apical-junctional complex by *Helicobacter pylori* CagA.
595 *Science* **300**:1430–1434.
- 596 45. **Mahé MM, Aihara E, Schumacher MA, Zavros Y, Montrose MH, Helmrath MA, Sato T,**
597 **Shroyer NF.** 2013. Establishment of Gastrointestinal Epithelial Organoids. *Curr Protoc*
598 *Mouse Biol* **3**:217–240.
- 599 46. **Fiehn O, Wohlgemuth G, Scholz M, Kind T, Lee DY, Lu Y, Moon S, Nikolau B.** 2008.
600 Quality control for plant metabolomics: reporting MSI-compliant studies. *Plant J* **53**:691–
601 704.
- 602 47. **Fiehn O.** 2016. Metabolomics by Gas Chromatography-Mass Spectrometry: Combined
603 Targeted and Untargeted Profiling. *Current Protocols in Molecular Biology* **114**:30.4.1–
604 30.4.32.
- 605 48. **Benjamini Y, Hochberg Y.** 1995. Controlling the False Discovery Rate: A Practical and
606 Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B*
607 (Methodological) **57**:289–300.

612

613 **Fig. 1.** Comparison of metabolites from 2D organoid culture supernatants of mouse gastric
 614 antrum and corpus, with and without infection by *H. pylori* SS1. Control samples include organoid
 615 media alone and *H. pylori* alone. A. Principle Component Analysis (PCA). B-E. ChemRICH
 616 enrichment analysis for pairwise comparison as indicated. The Y axis shows the most significantly
 617 altered clusters at the top, with enrichment p-values given by the Kolmogorov–Smirnov-test.
 618 Only enrichment clusters are shown that are significantly different at $p < 0.05$. The X axis shows
 619 the clusters similarities to each other. Each node reflects a significantly altered cluster of
 620 metabolites. Node sizes represent the total number of metabolites in each cluster set. The node
 621 color scale shows the proportion of increased (red), decreased (blue) compounds, or both
 622 increased and decreased metabolites (purple). For each sample, N = 6 except media alone where
 623 N = 3.

624

625 **Fig. 2**

626
627
628 Fig. 2. Heat map showing metabolite alterations in organoid supernatant following *H. pylori*
629 infection. Heat map was generated using Metaboanalyst to show the top 40 most significantly
630 changed compounds identified by ANOVA across groups. Left side labels indicate a description of
631 the major nodes.

632

Fig. 3.

Fig. 3. Comparison of supernatant compounds depleted upon infection in corpus and antrum organoids.

640 ***Table 1.***

641

Metabolite	Ratio Antrum/Corpus organoid supernatant	Dominant in	adjusted p value: antrum uninfected * corpus uninfected
beta-sitosterol	0.22	Corpus	0.23883
URIDINE	0.52	Corpus	0.00542
aspartic acid	0.54	Corpus	0.15790
glutamine	0.60	Corpus	0.32847
citramalic acid	0.65	Corpus	0.33555
GLYCEROL	0.69	Corpus	0.01772
serine	0.74	Corpus	0.21067
pyruvic acid	0.77	Corpus	0.33275
palmitoleic acid	0.77	Corpus	0.33275
ethanolamine	0.78	Corpus	0.29324
leucine	0.82	Corpus	0.20584
ISOLEUCINE	0.84	Corpus	0.04030
hypoxanthine	0.84	Corpus	0.33275
2-ketoisocaproic acid	0.85	Corpus	0.27656

tyrosine	0.86	Corpus	0.25415
valine	0.87	Corpus	0.23883
threonine	0.87	Corpus	0.33755
citric acid	1.11	Antrum	0.25876
succinic acid	1.21	Antrum	0.23883
ALANINE	1.38	Antrum	0.02691
5-methoxytryptamine	1.39	Antrum	0.20584
fumaric acid	1.54	Antrum	0.07430
MALIC ACID	1.82	Antrum	0.01772
1-monoolein	2.46	Antrum	0.29324
PHOSPHOETHANOLAMINE	2.65	Antrum	0.00265
LACTIC ACID	3.72	Antrum	0.00743

642

643 Table 1. Uninfected gastric organoid supernatant was collected and analyzed for total
644 metabolites. Compounds that differed between corpus and antrum organoids, ranked by fold
645 difference. Red capital letter text indicates those that are highly significant in the whole data set
646 after applying false discovery parameters, but all compounds that were significant when
647 comparing just these two samples are shown. N= 6 for each group.

648 **Table 2.**

649

Metabolite	Ratio Infected/Uninfected	FDR corrected P value
urea	0.04	0.00000
glutamine	0.10	0.00010
malic acid	0.23	0.00001
n-acetylglutamate	0.23	0.00036
serine	0.34	0.00007
fumaric acid	0.36	0.00010
lactic acid	0.40	0.05266
citric acid	0.44	0.00000
cholesterol	0.51	0.00612
aspartic acid	0.65	0.03809
oxoproline	0.74	0.03809
tyrosine	0.78	0.01238
isoleucine	1.45	0.00314
inosine	1.50	0.01138
threonine	1.53	0.00040
myristic acid	1.55	0.03049
ethanolamine	1.59	0.00218

asparagine	1.64	0.00011
tryptophan	1.67	0.00727
valine	1.67	0.00091
ribose	1.88	0.00001
5-methoxytryptamine	1.89	0.00230
glyceric acid	1.89	0.01138
succinic acid	2.03	0.00001
pseudo uridine	2.05	0.03397
phenylalanine	2.07	0.00009
alanine	2.09	0.00002
leucine	2.11	0.00001
glycine	2.26	0.00064
glycerol-alpha-phosphate	2.54	0.02841
citramalic acid	2.76	0.00010
uracil	3.00	0.00262
pyruvic acid	3.06	0.00010
thymidine	3.13	0.00006
glycerol	3.57	0.00002
uric acid	4.41	0.00006
homoserine	5.02	0.00001

cysteine	5.34	0.00603
uridine	5.81	0.00000
cystine	5.82	0.00006
xanthine	7.58	0.00225
lysine	9.08	0.00120
glutamate	9.68	0.00000
histidine	12.79	0.00007
methionine	14.41	0.01977
taurine	43.87	0.00719

650

651 Table 2. Compounds that differed significantly between infected and uninfected antral organoid

652 supernatant. Antral organoids were cultured as 2D organoids and infected with wild-type *H.*

653 *pylori* SS1 for 24 hours. After infection, the culture supernatant was collected and filtered to

654 remove bacteria. P-values are corrected for multiple comparison problem using Benjamini-

655 Hochberg procedure. N = 6 samples/organoid group and 3 for the *H. pylori* and media controls.

656 ** indicates the only increased compound that was different from the *H. pylori* alone samples.

657

658 **Table 3.**

Metabolite	Ratio Infected/Uninfected	FDR corrected P value
lactic acid	0.01	0.00000
urea	0.02	0.00000
glutamine	0.06	0.00000
aspartic acid	0.17	0.00001
lactamide	0.27	0.00001
n-acetylglutamate	0.31	0.00035
malic acid	0.32	0.00000
serine	0.36	0.00006
fumaric acid	0.39	0.00193
capric acid	0.52	0.01265
citric acid	0.53	0.00007
tyrosine	0.60	0.00042
benzoic acid	0.62	0.01764
oxoproline	0.65	0.00027
threitol	0.81	0.02178
guanosine	0.81	0.02083
isoleucine	1.17	0.02577

threonine	1.21	0.02849
oxalic acid	1.22	0.04623
stearic acid	1.23	0.04208
inosine	1.33	0.00723
1-monostearin	1.34	0.04585
valine	1.36	0.00072
phenylalanine	1.45	0.03142
myristic acid	1.48	0.01723
phosphoethanolamine	1.51	0.00293
hypoxanthine	1.52	0.03807
glycine	1.53	0.00049
glycerol-alpha-phosphate	1.56	0.00738
glyceric acid	1.61	0.00098
pseudo uridine	1.61	0.00788
leucine	1.65	0.00065
sucrose	1.91	0.01583
glycerol	1.99	0.03715
5-methoxytryptamine	2.00	0.00009
ribose	2.01	0.00024
2-hydroxyglutaric acid	2.06	0.00392

alanine	2.17	0.00002
succinic acid	2.19	0.00002
thymidine	2.48	0.00006
uric acid	2.52	0.04075
uracil	2.56	0.01117
homoserine	3.39	0.00039
thymine	3.81	0.00000
uridine	3.94	0.00002
cystine	7.75	0.00000
xanthine	7.83	0.00008
glutamate	11.52	0.00000
taurine	11.87	0.00077
lysine	12.66	0.00000
histidine	16.62	0.00000

659

660 Table 3. Compounds that differed significantly between infected and uninfected corpus organoid

661 supernatant. Corpus organoids were cultured as 2D organoids and infected with wild-type *H.*

662 *pylori* SS1 for 24 hours. After infection, the culture supernatant was collected and filtered to

663 remove bacteria. P-values are corrected for multiple comparison problem using Benjamini-

664 Hochberg procedure. N = 6 samples/organoid group and 3 for the *H. pylori* and media controls.

665 Note: of the increased compounds, none differed between the infected corpus organoids and *H.*

666 *pylori* alone.

Table 4.

Metabolite	Fold change antrum infected / corpus infected	FDR corrected P value
uracil	1.71	0.0071
phosphoethanolamine	1.97	0.00018
citramalic acid	2.06	0.0071
lactamide	5.37	0.00599
lactic acid	151.68	0.00018

Table 4. Comparison of supernatant of infected antrum and corpus organoids.

673 **Table 5.**

674

Metabolite	Ratio Antrum/Corpus gastric tissue	Dominant in	FDR correct p value
ARACHIDONIC ACID	0.06	Corpus	0.00500
LINOLEIC ACID	0.14	Corpus	0.00600
OLEIC ACID	0.18	Corpus	0.00700
GLYCEROL-ALPHA-PHOSPHATE	0.37	Corpus	0.00600
ETHANOLAMINE**	0.42	Corpus	0.00100
adenosine-5-monophosphate	0.43	Corpus	0.06200
1-monoolein	0.45	Corpus	0.05500
SQUALENE	0.66	Corpus	0.03300
fumaric acid	0.74	Corpus	0.10600
inosine	0.75	Corpus	0.06900
malic acid	0.8	Corpus	0.10600
threonine	1.24	Antrum	0.07600
phosphate	1.29	Antrum	0.06200
creatinine	1.43	Antrum	0.10700

palmitic acid	1.51	Antrum	0.07100
caprylic acid	1.53	Antrum	0.10600
capric acid	1.54	Antrum	0.08600
pelargonic acid	1.56	Antrum	0.08800
FUCOSE	1.61	Antrum	0.00800
benzoic acid	1.66	Antrum	0.06200
oxalic acid	1.69	Antrum	0.06900
uridine	1.73	Antrum	0.10700
MANNITOL	1.74	Antrum	0.00800
URIC ACID	1.76	Antrum	0.01300
LACTIC ACID**	1.79	Antrum	0.01200
glutamine	1.83	Antrum	0.06000
succinic acid**	1.84	Antrum	0.10500
hydroxylamine	2.01	Antrum	0.09700
DODECANOL	2.03	Antrum	0.04400
fructose-6-phosphate	2.13	Antrum	0.06000
hexose-6-phosphate	2.3	Antrum	0.07000
OCTADECANOL	2.43	Antrum	0.03300
glucose-6-phosphate	2.56	Antrum	0.08000
2-MONOPALMITIN	3.5	Antrum	0.01100
glucose	4.37	Antrum	0.06200

SUCROSE	4.89	Antrum	0.01200
5-METHOXYTRYPTAMINE**	6.06	Antrum	0.00600
pyruvic acid	6.7	Antrum	0.05600

Table. 5. Uninfected mouse gastric tissue was collected from female C57Bl6/N mice and analyzed for total metabolites. Shown are all compounds that differed when comparing the two groups, with capital letters indicating differences that were highly significant in the whole data after applying false discovery corrections. ** indicates similarly different in the corpus or antrum organoids. N = six mice for each group.

683 **Table 6.**

	Ratio Antrum/Corpus			
Metabolite	Uninfected	3 day	28 days	8 months
1-monoolein (Monooleoylglycerol)	1.93	1.07	0.43	
2-monopalmitin	3.50			
3-hydroxybutyric acid				1.89
3-phosphoglycerate			3.03	3.71
5-methoxytryptamine	6.06	4.06	5.54	3.63
adenosine-5- monophosphate	0.43		0.56	
arachidic acid			1.38	
arachidonic acid	0.06	0.29	0.22	0.17
aspartic acid				0.57
benzoic acid	1.66			1.75
capric acid	1.54			
caprylic (octanoic) acid	1.53		1.22	1.24
creatinine			1.42	
cysteine			1.57	0.61
dodecanol	2.03			1.62
ethanolamine	0.42			0.48

fructose-6-phosphate	2.13		2.23	
fucose	1.61		4.07	
fumaric acid	0.74			0.74
glucose	4.37			
glucose-6-phosphate	2.56		2.50	
glutamine	1.83			0.51
glycerol-alpha-phosphate	0.37	0.60	0.46	
glycolic acid				1.43
hexose-6-phosphate	2.30		2.58	
hydroxylamine	2.01			2.64
hypoxanthine				0.55
inosine	0.75			
isoleucine		1.37		
lactic acid	1.79	1.54	1.51	
lauric acid				2.31
leucine		1.39		
lignoceric acid				2.01
linoleic acid	0.14	0.33	0.20	0.30
lysine			0.60	
malic acid	0.80			0.68
mannitol	1.74			2.01

methionine				0.77
octadecanol	2.43			1.95
oleic acid	0.18	0.36	0.18	
ornithine				0.62
oxalic acid	1.69		1.26	
palmitic acid	1.51			
palmitoleic acid		0.60		
pelargonic acid	1.56			1.72
phosphate	1.29			1.24
pyruvic acid	6.70			
ribulose-5-phosphate		1.52	1.88	
serine		1.52	1.56	
squalene	0.66	0.69	0.72	
succinic acid	1.84			
sucrose	4.89			
taurine			7.34	
threonine	1.24		1.32	
tocopherol alpha-				1.67
tyrosine				0.78
valine		1.30		
uracil				0.70

urea			0.62	
uric acid	1.76			0.70
uridine	1.73			2.15
xanthine				0.49
xylitol			0.49	0.62
Number filled:	37.00	14.00	26.00	32.00

684

685 **Table 6.** Ratios in the mouse antrum and corpus tissue over time post infection. For each time
686 point, all named metabolites that differed using a non-FDR corrected P value (T test, two
687 sample) were used. Orange color indicates up in antrum. Blue indicates down in antrum. Italics
688 indicates one time point.

689

690 **Table 7.**

691

Metabolite	Fold change	FDR adjusted p
	Uninfected/8 mos	value:
uric acid	0.22	0.001
glutamine	0.32	0.007
fructose-6-phosphate	0.35	0.037
xanthine	0.36	0.001
aspartic acid	0.39	0.004
aminomalonate	0.44	0.001
ornithine	0.46	0.001
cysteine	0.50	0.010
fucose	0.53	0.010
urea	0.55	0.002
lactic acid	0.57	0.010
oxoproline	0.58	0.005
glycine	0.58	0.001
serine	0.60	0.015
methionine	0.63	0.003
alanine	0.63	0.010
threonine	0.65	0.001

1,5-anhydroglucitol	0.70	0.033
creatinine	0.70	0.005
putrescine	0.72	0.010
valine	0.72	0.028
hypoxanthine	0.80	0.024
isoleucine	0.84	0.011
palmitic acid	1.27	0.013
phosphate	1.30	0.011
caprylic acid	1.32	0.031
stearic acid	1.35	0.035
arachidic acid	1.44	0.015
cholesterol	1.64	0.001
inosine	1.68	0.001
hydroxylamine	1.96	0.030
squalene	2.32	0.001
arachidonic acid	2.70	0.024
oleic acid	3.26	0.025
1-monoolein	3.26	0.019
linoleic acid	3.61	0.021
adenosine	4.88	0.001

Table 7. Differences in the corpus tissue between 0 and 8 months

Supplemental Fig. 1

Supplemental Fig. 1. *H. pylori* SS1 pTM115 infection levels were determined by homogenizing and plating *H. pylori* tissue samples on CHBA plates, as described in (9).

Supplemental Table 1. All compounds detected in corpus and antrum organoids, ordered by most significantly different in the uninfected tissue.

Metabolite	Uninfected Antrum * Corpus (Adjusted P)	Infected Antrum * Corpus (adjusted P)
phosphoethanolamine	0.002651985	0.000180099
uridine	0.005419144	0.464113525
lactic acid	0.007430126	0.000180099
104906	0.017715615	0.086153073
malic acid	0.017715615	0.268980032
glycerol	0.017715615	0.582983261
alanine	0.02691136	0.205852591
isoleucine	0.040300273	0.836884308
1029	0.047459149	0.231705706
fumaric acid	0.07430243	0.356836096
aspartic acid	0.157896458	0.086153073
5-methoxytryptamine	0.205835259	0.284930795
leucine	0.205835259	0.81471968
serine	0.210671869	0.284930795
110628	0.223964174	0.191110038
31272	0.238827659	0.191110038
110624	0.238827659	0.231705706
beta-sitosterol	0.238827659	0.242916418
21704	0.238827659	0.343995331
2011	0.238827659	0.505913205
succinic acid	0.238827659	0.517839725
171297	0.238827659	0.728199809
valine	0.238827659	0.782301967
124844	0.238827659	0.936919893
tyrosine	0.254154621	0.51753482
citric acid	0.258763862	0.636757531
2-ketoisocaproic acid	0.276555644	0.642081065
84213	0.293241669	0.018516393
18488	0.293241669	0.22578453
592	0.293241669	0.284260336
4937	0.293241669	0.446579436
1-monoolein	0.293241669	0.482490002

1702	0.293241669	0.515564206
106936	0.293241669	0.519845974
ethanolamine	0.293241669	0.782301967
146957	0.293241669	0.81471968
453	0.293241669	0.81471968
1721	0.293241669	0.946540754
4901	0.293241669	0.968800142
110607	0.31434439	0.191110038
glutamine	0.328468904	0.908297593
hypoxanthine	0.332745395	0.065661824
pyruvic acid	0.332745395	0.071370692
110611	0.332745395	0.284930795
palmitoleic acid	0.332745395	0.512257418
41891	0.332745395	0.936919893
citramalic acid	0.335550733	0.007095858
2438	0.337553399	0.191110038
43100	0.337553399	0.22578453
threonine	0.337553399	0.549194862
4746	0.356526648	0.812487739
34001	0.377827315	0.631327492
134408	0.380200283	0.510040491
phenylalanine	0.380200283	0.572210196
glutamate	0.384886914	0.836884308
84181	0.400166069	0.643917535
117998	0.406236611	0.618230449
palmitic acid	0.423562874	0.715981087
6767	0.426301631	0.936919893
xylose	0.439560043	0.83201964
stearic acid	0.443205758	0.836884308
glucose	0.459811251	0.231705706
84088	0.459811251	0.284930795
95422	0.459811251	0.333148932
guanosine	0.478340166	0.284260336
glycine	0.478340166	0.436484516
1684	0.478340166	0.836110331
linoleic acid	0.478340166	0.87510423
110610	0.480381473	0.231705706
110604	0.480381473	0.923206508
307	0.484483702	0.367975944

110268	0.484483702	0.936919893
106629	0.507178508	0.691984508
1,5-anhydroglucitol	0.510210459	0.975336286
39	0.518000515	0.59526644
46128	0.519371643	0.924926144
42525	0.529503528	0.631327492
4793	0.537723431	0.936919893
110606	0.537723431	0.970472622
heptadecanoic acid	0.553906672	0.833861163
104179	0.563207313	0.280690651
21706	0.563207313	0.296516958
oxalic acid	0.563207313	0.790834054
121002	0.563207313	0.81471968
462	0.563207313	0.857100411
lignoceric acid	0.563207313	0.989881797
n-acetylglutamate	0.565556245	0.313838668
UDP-glucuronic acid	0.565556245	0.482490002
137	0.565556245	0.515564206
18305	0.565556245	0.587183915
121890	0.565556245	0.686669057
257	0.565556245	0.774723994
62304	0.565556245	0.836884308
5242	0.566168823	0.933465375
171305	0.579019438	0.328437176
129225	0.579019438	0.918317387
oxoproline	0.579019438	0.985655378
104131	0.592096735	0.642081065
91421	0.592096735	0.654916617
16747	0.595132533	0.191110038
1-monostearin	0.595132533	0.505913205
126397	0.595132533	0.734162534
3361	0.595132533	0.736200584
asparagine	0.595132533	0.789090761
42913	0.595132533	0.864098289
ribulose-5-phosphate	0.595132533	0.896738468
inosine	0.595132533	0.927157981
133514	0.595132533	0.970472622
4766	0.601231218	0.81471968
uracil	0.603297379	0.007095858

110315	0.603297379	0.512257418
ribose	0.603297379	0.691984508
5085	0.603297379	0.970472622
88583	0.610618327	0.766586764
7490	0.614657762	0.284930795
84383	0.631546366	0.877738085
5837	0.634587824	0.284930795
816	0.634587824	0.936919893
109981	0.634587824	0.950234967
3-hydroxybutyric acid	0.646217257	0.231705706
98	0.646217257	0.338345742
124826	0.646217257	0.686730698
39801	0.646217257	0.857100411
4976	0.651752253	0.715981087
nicotinamide	0.656059674	0.367975944
sucrose	0.675162143	0.462239268
sorbitol	0.675162143	0.808176059
47358	0.675162143	0.81471968
myo-inositol	0.675162143	0.827285838
105554	0.675162143	0.936919893
50367	0.679604667	0.78171695
97455	0.679604667	0.946540754
17651	0.701216227	0.018073951
299	0.701216227	0.22578453
capric acid	0.701657334	0.231705706
squalene	0.701657334	0.512257418
urea	0.750285799	0.284930795
133242	0.750285799	0.287308954
110618	0.751982904	0.284260336
103886	0.751982904	0.519845974
110284	0.751982904	0.579843648
arachidonic acid	0.751982904	0.737061058
threonic acid	0.751982904	0.78171695
171823	0.751982904	0.81471968
pantothenic acid	0.751982904	0.836110331
ornithine	0.751982904	0.908297593
adenosine	0.751982904	0.923068234
128034	0.753901495	0.086153073
2242	0.753901495	0.81471968

1704	0.753901495	0.895894859
methionine	0.756140424	0.314027382
124903	0.756140424	0.435620298
131620	0.756140424	0.505913205
glucose-6-phosphate	0.756140424	0.506557439
pelargonic acid	0.756140424	0.734162534
84116	0.756140424	0.963604634
18520	0.762520561	0.231705706
lyxitol	0.763146274	0.528294835
benzoic acid	0.763442027	0.242916418
1872	0.769085302	0.284930795
106693	0.769085302	0.416839778
beta-alanine	0.769085302	0.927157981
124825	0.769085302	0.946540754
110550	0.769085302	0.968800142
31563	0.779838994	0.22578453
2061	0.779838994	0.242916418
133244	0.779838994	0.284930795
47	0.779838994	0.333148932
84682	0.779838994	0.605733645
4797	0.779838994	0.631327492
102716	0.779838994	0.886543646
isothreonine acid	0.785478385	0.946540754
1708	0.786113038	0.462239268
fucose	0.786113038	0.578472168
5269	0.786113038	0.636757531
111776	0.786113038	0.720121509
cystine	0.786133924	0.288992597
octadecanol	0.786133924	0.45964984
levoglucosan	0.786133924	0.482490002
pseudo uridine	0.786133924	0.578472168
121028	0.786133924	0.636757531
trans-4-hydroxyproline	0.786133924	0.836884308
N-acetylmannosamine	0.786133924	0.836884308
133348	0.786133924	0.946540754
1713	0.792617072	0.789090761
108936	0.811127089	0.284260336
hexitol	0.811127089	0.284930795
171564	0.811127089	0.512257418

146430	0.811127089	0.598662621
172625	0.811127089	0.946540754
methanolphosphate	0.811127089	0.985655378
taurine	0.81569458	0.38642342
hexose-6-phosphate	0.818819529	0.231705706
124881	0.825851935	0.312885721
adenosine-5-monophosphate	0.825851935	0.482490002
100253	0.825851935	0.517839725
270	0.825851935	0.568298427
1709	0.825851935	0.628058033
112533	0.825851935	0.812487739
171303	0.825851935	0.836110331
fructose	0.825851935	0.836884308
4945	0.83000812	0.782301967
102711	0.83000812	0.968800142
17068	0.83715613	0.528294835
creatinine	0.839148162	0.836884308
31359	0.839285803	0.284260336
34089	0.839285803	0.691567949
171970	0.839285803	0.774723994
62	0.839285803	0.946540754
110	0.839334928	0.517839725
glyceric acid	0.839334928	0.823780019
97332	0.839334928	0.886543646
myristic acid	0.85588809	0.578472168
99	0.85617772	0.636757531
48428	0.87391282	0.836884308
lactamide	0.87416202	0.005989406
glycerol-alpha-phosphate	0.87416202	0.231705706
42007	0.87416202	0.284930795
31357	0.87416202	0.365962254
110321	0.87416202	0.536608647
2575	0.87416202	0.636757531
135610	0.87416202	0.737061058
124868	0.87416202	0.812487739
47262	0.87416202	0.836884308
N-methylalanine	0.87416202	0.836884308
135384	0.87416202	0.933465375

26062	0.87416202	0.936919893
490	0.87416202	0.946540754
1-monopalmitin	0.875212416	0.818181154
glycolic acid	0.875212416	0.832383276
aminomalonate	0.875212416	0.936919893
1173	0.889444819	0.393398981
17253	0.889788777	0.284930795
119025	0.889788777	0.568298427
892	0.889788777	0.636757531
thymine	0.889788777	0.809589193
1981	0.889788777	0.946540754
caprylic acid	0.898189959	0.342276844
tocopherol alpha-	0.898189959	0.515564206
42914	0.898189959	0.51753482
127640	0.898189959	0.683562764
1912	0.898189959	0.700163732
172624	0.90612736	0.268980032
135260	0.916141921	0.968800142
41989	0.918622025	0.505913205
54	0.928348555	0.367975944
122191	0.928348555	0.450712839
42003	0.928348555	0.515564206
161878	0.929313	0.517839725
4546	0.929313	0.678307385
88502	0.930909724	0.231705706
3781	0.930909724	0.578472168
ribitol	0.930909724	0.582983261
tryptophan	0.930909724	0.695679906
121402	0.930909724	0.860865955
glucose-1-phosphate	0.932457697	0.642081065
84922	0.932457697	0.756172104
lauric acid	0.932457697	0.81471968
erythritol	0.932457697	0.836884308
21683	0.932457697	0.836884308
124870	0.932457697	0.936919893
maltose	0.941148991	0.981645483
1941	0.947643987	0.519845974
18252	0.947643987	0.836884308
46292	0.947775612	0.231705706

arachidic acid	0.947775612	0.923206508
108950	0.947775612	0.946540754
47170	0.95069283	0.81471968
1862	0.951213059	0.284930795
hydroxylamine	0.951213059	0.51753482
17664	0.951213059	0.631327492
inositol-4-monophosphate	0.951213059	0.642081065
170841	0.951213059	0.913905374
5990	0.952898391	0.634108425
xylitol	0.952898391	0.81471968
4531	0.95541352	0.946540754
6066	0.957595176	0.268980032
171285	0.957595176	0.496561445
galactinol	0.957595176	0.886543646
oleic acid	0.957595176	0.902484377
17044	0.957595176	0.936919893
gluconic acid	0.96053486	0.242916418
1878	0.96053486	0.284930795
103102	0.96053486	0.482490002
41811	0.96053486	0.756172104
132143	0.96053486	0.837533359
16572	0.96053486	0.946540754
1700	0.96053486	0.971569691
110355	0.965659677	0.915047353
mannitol	0.971697205	0.578472168
methionine sulfoxide	0.971697205	0.601233104
100730	0.981967674	0.231705706
cholesterol	0.981967674	0.280690651
34059	0.981967674	0.284260336
homoserine	0.981967674	0.284930795
uric acid	0.981967674	0.296516958
22423	0.981967674	0.367975944
fructose-6-phosphate	0.981967674	0.367975944
threitol	0.981967674	0.38642342
657	0.981967674	0.397180154
5576	0.981967674	0.438967876
UDP-N-acetylglucosamine	0.981967674	0.446579436
3-phosphoglycerate	0.981967674	0.446579436
thymidine	0.981967674	0.462239268

1735	0.981967674	0.505913205
120744	0.981967674	0.517839725
31285	0.981967674	0.530137261
41808	0.981967674	0.555578447
6104	0.981967674	0.578472168
134336	0.981967674	0.642081065
10962	0.981967674	0.654916617
111263	0.981967674	0.664222466
lysine	0.981967674	0.691984508
5346	0.981967674	0.691984508
573	0.981967674	0.691984508
2,5-dihydroxypyrazine NIST	0.981967674	0.717523417
histidine	0.981967674	0.72318169
3258	0.981967674	0.734162534
trehalose	0.981967674	0.737061058
134	0.981967674	0.774723994
hydroxycarbamate NIST	0.981967674	0.790834054
2-monopalmitin	0.981967674	0.790834054
phosphate	0.981967674	0.81471968
1875	0.981967674	0.81471968
130396	0.981967674	0.833861163
dodecanol	0.981967674	0.836884308
beta-glycerolphosphate	0.981967674	0.836884308
41683	0.981967674	0.844644327
n-acetyl-d-hexosamine	0.981967674	0.84921018
xanthine	0.981967674	0.864098289
1746	0.981967674	0.880867478
42424	0.981967674	0.91312373
120562	0.981967674	0.918317387
1064	0.981967674	0.927157981
pyrophosphate	0.981967674	0.944180589
133907	0.981967674	0.946540754
121404	0.981967674	0.963604634
443	0.981967674	0.989881797
106498	0.983353659	0.435620298
2-monoolein	0.983353659	0.981645483
23635	0.983699575	0.284930795
cysteine	0.988054349	0.249768926

21666	0.988054349	0.936919893
133712	0.988054349	0.946540754
putrescine	0.989151077	0.880867478
103870	0.993269431	0.284260336
2-hydroxyglutaric acid	0.993882527	0.191110038
2936	0.993882527	0.284260336
170728	0.993882527	0.496411576
1717	0.993882527	0.636757531
41985	0.993882527	0.75375956
31224	0.993882527	0.902484377

705

706

Supplemental Table 2. Compounds that differed between antrum organoids and media alone

label	adjusted p value: antrum uninfected * media control	mean fold change: antrum uninfected / media control
aspartic acid	0.049010772	0.524503499
171970	0.049010772	0.579202532
leucine	0.004304608	0.655079514
glycerol	0.047063192	0.664713484
ethanolamine	0.049010772	0.679878146
serine	0.046193691	0.681502683
phenylalanine	0.049010772	0.696430862
isoleucine	0.010060097	0.747946073
nicotinamide	0.047063192	0.761139839
valine	0.047063192	0.771265241
malic acid	0.049010772	1.722378988
phosphoethanolamine	0.049010772	2.554285714
lactic acid	0.010060097	3.92864641

Supplemental Table 3: Enrichment analysis comparing corpus and antrum in uninfected and infected tissues.

A. Uninfected corpus versus antrum organoids .

Cluster name	Cluster size	p-values	FDR	Key compound	Altered metabolites	Increased	Decreased
Amino Acids, Branched-Chain	3	0.00000	0.00005	isoleucine	3	0	3
Dicarboxylic Acids	7	0.0049	0.047	malic acid	4	3	1
Amino Acids	6	0.011	0.067	alanine	3	1	2
Amino Acids, Acidic	3	1	1	aspartic acid	1	0	1
Amino Acids, Aromatic	3	1	1	tyrosine	1	0	1
Amino Acids, Basic	4	1	1	glutamine	1	0	1
Amino Acids, Sulfur	4	1	1	methionine	0	0	0
Butyrates	3	1	1	3-hydroxybutyric acid	0	0	0
Disaccharides	3	1	1	sucrose	0	0	0
Hexosephosphates	4	1	1	glucose-6-phosphate	0	0	0
Hexoses	5	1	1	glucose	0	0	0
Purine Nucleosides	5	1	1	guanosine	0	0	0
Purinones	3	1	1	hypoxanthine	1	0	1
Pyrimidine Nucleosides	3	1	1	uridine	1	0	1
Saturated FA	10	1	1	palmitic acid	0	0	0
Saturated_Glycerides	3	1	1	1-monostearin	0	0	0
Sugar Acids	3	1	1	glyceric acid	0	0	0
Sugar Alcohols	10	1	1	glycerol	1	0	1
UnSaturated FA	4	1	1	palmitoleic acid	1	0	1

B. Infected corpus versus antrum

Cluster name	Cluster size	p-values	FDR	Key compound	Altered metabolites	Increased	Decreased
Dicarboxylic Acids	7	0.014	0.27	citramalic acid	3	3	0
Amino Acids	6	1	1	alanine	1	1	0
Amino Acids, Acidic	3	1	1	aspartic acid	1	1	0
Amino Acids, Aromatic	3	1	1	tyrosine	0	0	0
Amino Acids, Basic	4	1	1	lysine	0	0	0
Amino Acids, Branched-Chain	3	1	1	valine	0	0	0

Amino Acids, Sulfur	4	1	1	cysteine	1	1	0
Butyrates	3	1	1	3-hydroxybutyric acid	1	1	0
Disaccharides	3	1	1	sucrose	0	0	0
Hexosephosphates	4	1	1	hexose-6-phosphate	1	1	0
Hexoses	5	1	1	glucose	1	0	1
Purine Nucleosides	5	1	1	guanosine	1	1	0
Purinones	3	1	1	hypoxanthine	1	0	1
Pyrimidine Nucleosides	3	1	1	thymidine	0	0	0
Saturated FA	10	1	1	capric acid	1	1	0
Saturated_Glycerides	3	1	1	1-monostearin	0	0	0
Sugar Acids	3	1	1	gluconic acid	1	0	1
Sugar Alcohols	10	1	1	hexitol	0	0	0
UnSaturated FA	4	1	1	palmitoleic acid	0	0	0

715
716
717

Supplemental Table 4. All compounds that change in the antrum organoids upon infection, ordered by P value

Label	adjusted p value: antrum infected * antrum uninfected
21706	2.70849E-08
1708	2.70849E-08
1746	2.96284E-08
urea	5.7285E-08
glutamate	1.26084E-07
128034	1.26084E-07
citric acid	3.77645E-07
1721	4.8323E-07
1713	8.86461E-07
18520	1.11747E-06
uridine	3.06326E-06
133907	3.06326E-06
1717	4.15844E-06
270	4.92879E-06
malic acid	9.29347E-06
ribose	1.20005E-05
leucine	1.22013E-05
109981	1.22013E-05
homoserine	1.3636E-05
34001	1.3636E-05
4746	1.3636E-05
succinic acid	1.37595E-05
108950	1.69307E-05
alanine	1.69441E-05
glycerol	2.00477E-05
135260	2.65798E-05
84213	3.49234E-05
cystine	5.53993E-05
uric acid	6.01137E-05
thymidine	6.01137E-05
170728	6.01137E-05
serine	6.78232E-05
histidine	7.23439E-05
phenylalanine	8.76571E-05
fumaric acid	9.75406E-05
glutamine	9.75978E-05
citramalic acid	9.75978E-05

31272	9.79653E-05
pyruvic acid	0.000101866
asparagine	0.000110508
110355	0.000150299
97332	0.000193405
110315	0.000336396
1700	0.000351946
n-acetylglutamate	0.000363761
threonine	0.000397385
glycine	0.000636786
453	0.0006819
34059	0.000790494
valine	0.000912187
84088	0.001025989
43100	0.001142457
3361	0.001142457
lysine	0.001201798
171970	0.00165698
ethanolamine	0.002184853
xanthine	0.002247063
5-methoxytryptamine	0.002300687
uracil	0.002623214
7490	0.002920079
isoleucine	0.003141742
100730	0.004886865
110321	0.005167197
cysteine	0.0060314
cholesterol	0.006123783
105554	0.006963892
taurine	0.007193771
tryptophan	0.007265821
111776	0.008882999
95422	0.011249879
inosine	0.011381472
glyceric acid	0.011381472
tyrosine	0.012384678
120562	0.012645068
104906	0.015896638
methionine	0.019772261
42003	0.022147104
1029	0.026186395
glycerol-alpha-phosphate	0.028414301

22423	0.028414301
myristic acid	0.030490244
pseudo uridine	0.033967137
oxoproline	0.038094748
aspartic acid	0.038094748
133244	0.039519768
108936	0.045954398
lactic acid	0.052656987

721

722

Supplemental Table 5. All compounds that changed significantly in the corpus organoids upon infection, ordered by P value.

Label	p value: corpus infected v. corpus uninfected
glutamate	3.74E-10
lactic acid	9.67E-10
21706	3.39E-09
1717	3.86E-09
128034	5.04E-09
urea	7.22E-09
histidine	1.76E-08
1708	2.07E-08
1721	2.47E-08
1713	4.27E-08
111776	6.05E-08
34001	9.38E-08
thymine	1.39E-07
18520	4.22E-07
133907	5.61E-07
glutamine	6.32E-07
170728	7.98E-07
4746	9.74E-07
malic acid	1.61E-06
lysine	1.68E-06
31272	3.89E-06
cystine	4.62E-06
1746	4.91E-06
aspartic acid	5.69E-06
lactamide	6.95E-06
110355	9.22E-06
alanine	1.74E-05
uridine	2.01E-05
succinic acid	2.42E-05
270	2.63E-05
108950	4.03E-05
34059	4.81E-05
thymidine	5.51E-05
serine	5.57E-05
97332	5.99E-05

citric acid	6.61E-05
xanthine	8.00E-05
5-methoxytryptamine	9.00E-05
110315	0.000132126
ribose	0.000242953
oxoproline	0.000268929
n-acetylglutamate	0.000348905
109981	0.000384971
homoserine	0.000391185
tyrosine	0.000419087
16747	0.000411155
103870	0.000405677
glycine	0.000487647
leucine	0.000646362
valine	0.000721547
1700	0.000737462
taurine	0.000767655
glyceric acid	0.000981752
fumaric acid	0.001929187
84116	0.002489667
phosphoethanolamine	0.002931908
91421	0.002913907
2-hydroxyglutaric acid	0.003921997
135260	0.004018039
18488	0.005343896
3361	0.005681771
105554	0.005708205
inosine	0.007234357
glycerol-alpha-phosphate	0.00737794
pseudo uridine	0.007881359
1862	0.008825195
307	0.008986552
uracil	0.01117066
84213	0.011931764
108936	0.012702965
capric acid	0.012654162
104906	0.015530113
sucrose	0.015830734
myristic acid	0.017232189
benzoic acid	0.017639154
120562	0.019925609
guanosine	0.020832231

threitol	0.021783766
462	0.024509492
isoleucine	0.02577406
42007	0.025508016
threonine	0.028488815
21704	0.029161316
phenylalanine	0.031415725
161878	0.031092587
glycerol	0.037154183
134	0.037667293
hypoxanthine	0.038068542
1735	0.03952012
uric acid	0.040747738
1029	0.04022119
5085	0.040856257
17253	0.04138124
stearic acid	0.042076969
171297	0.043429052
oxalic acid	0.046228133
1-monostearin	0.045849482
3781	0.047841843
146957	0.048610532
110607	0.049164642

726

727

728 **Supplemental Table 6. Enrichment analysis comparing uninfected and infected tissues**

Cluster name	Cluster size	p-values	FDR	Key compound	Altered metabolites	Increase	Decrease	Increase ratio	Altered Ratio
Amino Acids, Branched-Chain	3	1.6E-10	3.1E-09	leucine	3	3	0	1	1
Amino Acids, Aromatic	3	0.000000017	0.0000016	phenylalanine	3	2	1	0.7	1
Dicarboxylic Acids	7	0.00000015	0.0000093	malic acid	5	3	2	0.6	0.7
Pyrimidine Nucleosides	3	0.0000005	0.0000024	uridine	3	3	0	1	1
Amino Acids, Acidic	3	0.00000075	0.0000029	glutamate	3	1	2	0.3	1
Amino Acids	6	0.0000021	0.0000068	homoserine	5	4	1	0.8	0.8
Sugar Acids	3	0.0014	0.0038	glyceric acid	2	2	0	1	0.7
Amino Acids, Sulfur	4	0.0017	0.0041	cystine	3	3	0	1	0.8
Amino Acids, Basic	4	0.0039	0.0083	glutamine	3	2	1	0.7	0.8
Purinones	3	0.037	0.071	uric acid	2	2	0	1	0.7
Hexoses	5	0.092	0.16	levoglucosan	2	1	1	0.5	0.4

729
730

Cluster name	Cluster size	p-values	FDR	Key compound	Altered metabolites	Increased
Amino Acids, Acidic	3	4.2E-11	8.1E-10	glutamate	3	1
Pyrimidine Nucleosides	3	0.00000049	0.0000047	uridine	3	3
Amino Acids, Branched-Chain	3	0.000017	0.00011	leucine	3	3
Amino Acids	6	0.000055	0.00026	alanine	5	4
Purinones	3	0.000068	0.00026	xanthine	3	3
Dicarboxylic Acids	7	0.00066	0.0021	malic acid	5	3
Amino Acids, Basic	4	0.021	0.056	glutamine	2	1
Sugar Alcohols**	10	0.036	0.085	threitol	2	1
Amino Acids, Aromatic	3	0.05	0.11	tyrosine	2	1

Purine Nucleosides	5	0.19	0.35	inosine	2	1
Saturated FA	10	0.22	0.39	capric acid	3	2
Amino Acids, Sulfur	4	1	1	cystine	1	1
Butyrates	3	1	1	isothreonic acid	0	0
Disaccharides	3	1	1	sucrose	1	1
Hexosephosphates	4	1	1	fructose-6-phosphate	0	0
Hexoses	5	1	1	1,5-anhydroglucitol	0	0
Saturated_Glycerides	3	1	1	1-monostearin	1	1
Sugar Acids	3	1	1	glyceric acid	1	1
UnSaturated FA	4	1	1	linoleic acid	0	0

731
732

Supplemental Table 7. All chemicals that differed between uninfected antrum and corpus mouse tissue

Metabolite	p value	FDR corrected p value
573	0.000	0.001
121402	0.000	0.001
ethanolamine	0.000	0.001
97455	0.000	0.001
50367	0.000	0.003
31357	0.000	0.003
arachidonic acid	0.000	0.005
5-methoxytryptamine	0.000	0.006
1875	0.000	0.006
linoleic acid	0.000	0.006
5990	0.000	0.006
glycerol-alpha-phosphate	0.000	0.006
111263	0.000	0.006
oleic acid	0.000	0.007
fucose	0.001	0.008
31563	0.001	0.008
mannitol	0.001	0.008
41808	0.001	0.008
2-monopalmitin	0.001	0.011
lactic acid	0.001	0.012
sucrose	0.001	0.012
135260	0.002	0.013
uric acid	0.002	0.013
21683	0.002	0.018
84922	0.002	0.018
1862	0.003	0.021
21706	0.004	0.027
443	0.004	0.029
100253	0.005	0.033
6066	0.006	0.033
146957	0.006	0.033
2061	0.006	0.033
octadecanol	0.006	0.033
squalene	0.006	0.033

16572	0.006	0.036
dodecanol	0.008	0.044
2936	0.008	0.044
892	0.010	0.050
42003	0.010	0.051
31285	0.011	0.051
106936	0.012	0.054
84181	0.012	0.054
84383	0.012	0.054
1-monoolein	0.012	0.055
103870	0.013	0.056
pyruvic acid	0.013	0.056
62304	0.014	0.057
134336	0.014	0.058
4901	0.015	0.060
102711	0.015	0.060
fructose-6-phosphate	0.016	0.060
1029	0.016	0.060
glutamine	0.016	0.060
glucose	0.017	0.062
110604	0.018	0.062
benzoic acid	0.019	0.062
adenosine-5-monophosphate	0.019	0.062
42007	0.020	0.062
phosphate	0.020	0.062
17068	0.020	0.062
1700	0.020	0.062
1878	0.020	0.062
307	0.023	0.069
46292	0.023	0.069
oxalic acid	0.023	0.069
inosine	0.023	0.069
hexose-6-phosphate	0.024	0.070
palmitic acid	0.025	0.071
17651	0.027	0.076
threonine	0.028	0.076
100730	0.029	0.078
31359	0.029	0.079
glucose-6-phosphate	0.030	0.080
capric acid	0.033	0.086
pelargonic acid	0.034	0.088
1709	0.035	0.089

10962	0.036	0.089
hydroxycarbamate NIST	0.036	0.089
3258	0.037	0.090
5346	0.038	0.091
1912	0.038	0.091
hydroxylamine	0.041	0.097
657	0.043	0.100
131620	0.045	0.103
succinic acid	0.046	0.105
malic acid	0.048	0.106
caprylic acid	0.048	0.106
fumaric acid	0.049	0.106
112533	0.049	0.106
uridine	0.050	0.107
creatinine	0.050	0.107

736

737

738 **Supplemental Table 8. Compounds that changed between uninfected and 8 month infected**
 739 **corpus mouse tissue**

label	p value: uninfected * 8 months @corpus tissue	adjusted p value: uninfected * 8 months @corpus tissue
threonine	0.00000878	0.000506869
cholesterol	0.0000154	0.000506869
inosine	0.0000161	0.000506869
glycine	0.000023	0.000506869
xanthine	0.0000235	0.000506869
uric acid	0.000019	0.000506869
adenosine	0.0000105	0.000506869
46128	0.00000824	0.000506869
squalene	0.0000201	0.000506869
54	0.0000462	0.000896776
257	0.0000548	0.000967324
aminomalonate	0.0000853	0.001378536
ornithine	0.0000968	0.001444545
urea	0.000153078	0.002121219
146957	0.00020686	0.002508179
methionine	0.00026525	0.003026965
aspartic acid	0.000405264	0.004367841
oxoproline	0.000464323	0.004740981
creatinine	0.00056276	0.005458773
111263	0.000726901	0.006715183
112533	0.000848875	0.006861743
97455	0.000789351	0.006861743
glutamine	0.000847102	0.006861743
573	0.001219807	0.009465704
42914	0.001275955	0.009520586
putrescine	0.001347462	0.009681766
alanine	0.001534679	0.01015553
fucose	0.001489801	0.01015553
2061	0.001570443	0.01015553
cysteine	0.001652174	0.01033941
lactic acid	0.001790385	0.01039079
5990	0.001874627	0.01039079
121402	0.001821545	0.01039079

50367	0.001831048	0.01039079
1872	0.002138795	0.011388238
isoleucine	0.0022465	0.011388238
1709	0.002289388	0.011388238
phosphate	0.002252143	0.011388238
palmitic acid	0.00261724	0.012693614
arachidic acid	0.003124121	0.014782426
serine	0.003259147	0.015054154
1-monoolein	0.004247252	0.019162021
linoleic acid	0.004709791	0.020765897
arachidonic acid	0.005676693	0.023940836
47	0.005556421	0.023940836
hypoxanthine	0.005846673	0.024133074
131620	0.005989265	0.024206614
84383	0.006173727	0.024442917
oleic acid	0.00653424	0.02535285
31359	0.006897578	0.026237847
valine	0.007432717	0.027639269
171305	0.007550934	0.027639269
31357	0.008206771	0.029483586
5576	0.008573751	0.030048426
hydroxylamine	0.008673772	0.030048426
caprylic acid	0.009114464	0.031021159
1,5-anhydroglucitol	0.009965453	0.032767761
5242	0.009934638	0.032767761
stearic acid	0.010922961	0.035317573
fructose-6-phosphate	0.011504352	0.036587612

740

741

- 742 **Supplemental Data Set 1, Organoid Data and Mouse Data from 3d and 28d: MX269377**
743 **Supplemental Data Set 2. Mouse Data: uninfected and 8 month infected**