Supporting Information

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SI Results and Discussion

Transcriptional Analysis of Nine-Member Artificial Community in HF/HS and LF/HPP Diet. We identified 96 enzyme commission numbers (ECs) that were differentially represented in fecal microbiomes as a function of diet (threshold cutoffs; fold-difference >2, posterior probability of differential expression (PPDE) > 0.95; Cyber-T; Dataset S3A). Many of these enzymes participate in various facets of carbohydrate metabolism. For example, the microbiota of mice fed the low-fat and high-plant-polysaccharides (LF/HPP) diet exhibited significantly higher expression of genes encoding ECs involved in (*i*) the breakdown of plant-derived polysaccharides present in this diet, including xylans (EC3.1.1.72, acetylxylan esterase), β-glucans (EC3.2.1.4, β-glucan hydrolase), pectins (EC3.2.1.67, polygalacturonate hydrolase), and arabinans (EC3.2.1.99, endo-arabinanase, and EC3.2.1.55, arabinofuranosidase) and (*ii*) metabolism of the resulting monosaccharides [arabinose present in arabinans and pectins (EC2.7.1.16, ribulokinase and EC5.1.3.4, L-ribulose 5-phosphate 4-epimerase) and galacturonic acid present in pectins (EC4.2.1.7, D-altronate dehydratase)] (Fig. S2*B*, Dataset S3*A*). In contrast, the microbiota of mice fed the high-fat and high-simple-sugar (HF/HS) diet exhibited higher levels of expression of genes involved in (*i*) the metabolism of sucrose (EC2.7.1.4, fructokinase), sorbitol (EC1.1.1.140, sorbitol dehydrogenase), glycerol (e.g., EC1.1.1.202, 1,3-propanediol dehydrogenase), and myo-inositol (EC1.1.1.18, myo-inositol dehydrogenase); (*ii*) the breakdown of host-derived mucus glycans (e.g., EC4.1.3.3, *N*-acetylneuraminate lyase; EC3.2.1.35, hyaluronidase); and (*iii*) the removal of sulfate from sulfated glycans (EC3.1.6.14, *N*-acetylglucosamine-6-sulfatase) (Fig. S2*B*, Dataset S3*A*).

The contributions of components of the nine-member artificial community to the pool of ECs differentially represented in the fecal metatranscriptomes of mice consuming the LF/HPP versus HF/HS diets are presented in Dataset S3*B*. Transcriptional changes in genes encoding enzymes predicted to be involved in the breakdown of dietary and host polysaccharides were largely driven by *Bacteroides* species; *Bacteroides ovatus*, and to a lesser extent *Bacteroides thetaiotaomicron*, made the biggest contribution to ECs involved in the breakdown of plant polysaccharides that were overrepresented in LF/HPP diet (e.g., EC3.2.1.4, β-glucan hydrolase, and EC3.2.1.99, endo-arabinanase), whereas transcripts from *Bacteroides caccae* and *B. thetaiotaomicron* drove the observed increase in the abundance of ECs predicted to break down host polysaccharides including sulfated mucins (e.g., EC4.1.3.3, *N*-acetylneuraminate lyase; EC3.2.1.35, hyaluronidase; EC3.1.6.14, *N*-acetylglucosamine-6-sulfatase) when mice were consuming the HF/HS diet.

Construction and in Vitro Validation of INSeq Library for *Desulfovibrio piger*. We constructed an isogenic library composed of ∼30,000 strains containing unique transposon mutants of *D. piger* (inter- and intragenic insertions). The library was generated under strict anaerobic conditions using a rich medium, allowing us to obtain mutants in genes involved in a wide range of metabolic functions. INsertion Sequencing (INSeq) analysis revealed that the library was composed of transposon insertions in 2,181 of the 2,487 predicted open reading frames (ORFs) in the *D. piger* GOR1 genome. Of the 306 ORFs without observed transposon insertions, 174 likely encode products that are essential for growth of *D. piger* on rich medium; they include genes involved in "core functions" such as cell division, protein translation, and cell wall biosynthesis (Dataset S4; see *SI Methods* for how we identified these "likely essential" ORFs).

We first characterized the mutant library in vitro, applying a growth selection in a fully defined medium containing all 20 amino acids, lactate (source of carbon and reducing equivalents), and sulfate (electron acceptor) (see *SI Methods* for details). We identified 266 genes that when disrupted by a transposon had significantly reduced fitness under these conditions ($P_{adj} < 0.05$, output–input ratio < 0.3). They included genes involved in pyrimidine and purine biosynthesis, lactate utilization, gluconeogenesis, and sulfate reduction (Dataset S5; Fig. S3 presents a pathway map for sulfate reduction showing fitness determinants disclosed by the transposon mutagenesis screen). With the exception of arginine, genes involved in amino acid biosynthesis were not required for growth in this amino acid–rich medium (Dataset S5).

Comparison of in Vitro and in Vivo *D. piger* Fitness Determinants. We subjected the *D. piger* mutant library to another set of selections in vitro, this time using various electron donors for sulfate reduction (formate, H_2, lactate, or pyruvate). We also tested fermentative growth (i.e., the ability to grow without sulfate using pyruvate as the sole carbon and energy source). INSeq revealed genes involved in numerous functions important for growth (e.g., sulfate reduction, purine and pyrimidine biosynthesis, and ATP synthesis) that were also critical for fitness in vivo (Dataset S9). Transposon insertions in the periplasmic [NiFeSe] hydrogenase genes (*DpigGOR1_1496–DpigGOR1_1497*) important for gut colonization resulted in in vitro growth defects in the presence of H_2 but not with the other electron donors. In contrast, genes required for optimal growth and survival in vitro with formate (e.g., formate dehydrogenase encoded by *DpigGOR1_0133–DpigGOR1_0135*) or lactate [e.g., the lactate transporter specified by *DpigGOR1_1075*; and lactate dehydrogenase (*DpigGOR1_0371*)] were not required for fitness in vivo. The finding that genes required for optimal growth in vivo do not overlap with those specifically required for optimal growth in vitro with formate and lactate suggests that *D. piger* either does not use these electron donors in vivo or uses several different electron donors, and/or that disruption of one pathway is compensated by another pathway.

The list of in vivo–specific fitness determinants included members of a locus that encodes rubredoxin–oxygen oxidoreductase (*DpigGOR1_1319*), rubredoxin (*DpigGOR1_1321*), and rubredoxin oxidoreductase (*DpigGOR1_1322*), and a locus encoding subunits of a cytochrome *bd* oxidase (*DpigGOR1_1865–DpigGOR1_1866*). These genes are known to be important for handling oxygen and oxidative stress (1–5). *D. piger* could experience varying degrees of oxidative stress during the process of gavage into gnotobiotic animals, during transit from the proximal to the distal gut, and/or as it associates with the gastrointestinal mucosa (compared with the intestinal lumen, the mucosa is exposed to higher oxygen levels due to the extensive capillary network that underlies it) (6–8).

Dataset S10 groups genes that have significant fitness effects in vivo but not in vitro into those that exhibit diet independence or diet dependence.

SI Methods

Multiplex Pyrosequencing of Amplicons Generated from the *aprA* Gene. DNA was isolated from frozen fecal specimens obtained from healthy adults living in the United States who were recruited to a previously described and completed study using protocols approved by the Washington University Human Research Protection Office (9, 10). An aliquot of fecal DNA was used for PCR amplification and sequencing of a conserved region of subunit

A of the adenosine-5′-phosphosulfate reductase gene (*aprA*) present in sulfate-reducing bacteria (SRB) using primers adapted from ref. 11. Amplicons (466 bp) were generated by using (*i*) modified primer AprA forward primer (5′-CCATCTCATCCCTGCGTGTCTCCGACTCAGNNNNNNNNNN*TGGCAGATMATGATYMACGG*-3′), which consists of 454 FLX Titanium Amplicon primer A (underlined), a sample-specific 10-mer barcode (N's) and the *aprA* primer (italics), and (*ii*) a modified *aprA* reverse primer (5′-CCTATCCCCTGTGTGCCTTGGCAGTCTCAG *GGGCCGTAACCGTCCTTGAA*, which contains 454 FLX Titanium amplicon primer B and the bacterial *aprA* primer. Three replicate polymerase chain reactions were performed for each fecal DNA sample: each 20 μL reaction contained 50 ng of purified fecal DNA (Qiaquick, Qiagen), 8 μL 2.5× HotMaster PCR Mix (Eppendorf), 0.25 μM of the forward primer, and 0.1 μM of the reverse primer. PCR conditions consisted of an initial denaturation step performed at 95 °C for 4 min, followed by 35 cycles of denaturation (95 °C for 20 s), and annealing and amplification (65 °C for 1 min). Amplicons generated from each set of three reactions were subsequently pooled and purified using Ampure magnetic beads (Agencourt). The amount of purified DNA obtained was quantified using Picogreen (Invitrogen), and equimolar amounts of barcoded samples were pooled for each subsequent multiplex 454 FLX pyrosequencer run. The *aprA* amplicon sequences were processed using the QIIME (v1.2) suite of software tools (12); fasta files and a mapping file indicating the sequence of the 10 nt barcode that corresponded to each sample were used as inputs.

COPRO-Seq. DNA isolated from feces (and cecal contents) was used to prepare libraries for shotgun Illumina sequencing (13). Briefly, 1 μg of DNA from each sample was fragmented by sonication to an average size of ∼500 bp and subjected to enzymatic blunting and adenine tailing. Customized Illumina adapters containing maximally distant 4 bp or 8 bp barcodes were ligated to the A-tailed DNA. Barcoded libraries were then pooled, subjected to gel electrophoresis for size selection (∼250 bp), and the size-selected DNA amplified by PCR using primers and cycling conditions recommended by Illumina. Amplicons were purified (QIAquick PCR Purification Kit, Qiagen) and sequenced using an Illumina GA-IIx or HiSeq2000 instrument, with libraries loaded onto the flow cell at a concentration of 2.0–2.5 pM. A previously described custom software pipeline was used to process and analyze the resulting COmmunity PROfiling by shotgun Sequencing (COPRO-Seq) datasets (13).

Quantitative PCR Measurements of *D. piger* Colonization. Quantitative PCR (qPCR) was performed by using an Mx3000P real-time PCR system (Stratagene). Reaction mixtures (25 μL) contained SYBR Green supermix (Bio-Rad), 400 nM *D. piger*–specific primers (see below), and 10 ng of DNA isolated from feces or cecal contents. Primer pairs targeted the 16S rRNA gene of *D. piger* (DpigGOR1_fwd 5′-AAAGGAAGCACCGGCTAACT-3′, DpigGOR1_rev 5′-CGGATTCAAGTCGTGCAGTA-3′). Amplification conditions were 55 °C for 2 min and 95 °C for 15 min, followed by 40 cycles of 95 °C (30 s), 55 °C (45 s), and 72 °C (30 s). Data were collected at 78 °C, 80 °C, 82 °C, and 84 °C. The amount of *D. piger* DNA from each genome in each PCR was calculated by comparison with a standard curve of genomic DNA prepared in the same manner from *D. piger* monocultures. Data were converted to genome equivalents (GEs) by calculating the mass of *D. piger* genomic DNA per cell ($\sim 3.4 \times 10^6$ fg) and normalized by fecal weight.

Microbial RNA-Seq. Fecal samples obtained from mice and from bacteria cultured under various defined nutrient conditions were immediately frozen at −80 °C and maintained at this temperature before processing. All samples were treated with RNAProtect (Qiagen). Each frozen sample was suspended in a solution containing 500 μL of acid-washed glass beads (Sigma-Aldrich), 500 μL of extraction buffer A (200 mM NaCl, 20 mM EDTA), 210 μL of 20% (wt/wt) SDS, and 500 μL of a mixture of phenol–chloroform–isoamyl alcohol (125:24:1, pH 4.5; Ambion), and then lysed by using a bead beater (BioSpec Products; maximal setting; 4 min at room temperature). Cellular debris was removed by centrifugation (8,000 × *g*; 3 min at 4 °C). The extraction was repeated, and nucleic acids were precipitated with isopropanol and sodium acetate (pH 5.5). Details about protocols used for removing residual DNA from RNA preparations, rRNA depletion, double-stranded cDNA synthesis, and multiplex sequencing with the Illumina HiSeq instrument, as well as our data analysis pipeline have been described previously (14, 15).

Host (Mouse) RNA-Seq Analysis. Transcriptional profiling of mouse samples was performed using procedures detailed elsewhere (16). Briefly, frozen proximal colon tissue was homogenized in 1 mL of TRIzol (Invitrogen), and total RNA was purified using the Qiagen RNeasy mini kit and two DNase treatments including one on-column DNase treatment (Qiagen) followed by the Zymo DNA-Free RNA kit (Zymo Research). mRNA was further purified using Dynabeads mRNA Purification Kit (Invitrogen), reverse-transcribed to ds cDNA, and Illumina libraries were generated using the NEBNext mRNA Sample Prep Reagent Set 1 following the manufacturer's protocol. In-house barcoded DNA adaptors were ligated to cDNA to allow multiplexing of seven libraries per lane of a HiSeq 2000 flow cell.

Construction of *D. piger* Transposon Mutagenesis Vector. To generate the *D. piger* GOR1 transposon mutant library, we modified the original INSeq vector, pSAM_Bt (17) by (*i*) switching the transposon's *ermG* antibiotic resistance gene with one known to work in *Desulfovibrio vulgaris* [*aadA* (spectinomycin resistance)], (*ii*) using the promoter region from a highly expressed *D. piger* gene to drive expression of the *mariner* transposase, and (*iii*) optimizing codon use for the transposase based on the *D. piger* genome. This effort involved the following procedures. *aadA* was PCR amplified from pMO719 (18), kindly provided by Judy Wall (University of Missouri Columbia, MO), using primers MfeI *aadA* 5′ (5′-GGGAATTCCAATTGAGACCAGCCAGGACAGAAATGCC) and XbaI *aadA* 3′ (5′-CTAGTCTAGACGGGGTCTGACGCTCAGTGGAACG). The resulting PCR fragment was digested with MfeI and XbaI, and ligated into pSAM_Bt (17) after excision of its *ermG* gene with MfeI and XbaI, creating pSAM–*aadA*. The *mariner* transposase gene was synthesized (GenScript) using codon sequences optimized to the *D. piger* GOR1 genome, and a 1,052 bp fragment containing this gene was excised with NdeI and NotI from the pUC57 vector into which it had been originally cloned. The *D. piger* codon-optimized *mariner* transposase was then ligated to the linearized pSAM–*aadA*, creating pSAM-*aadA**. Finally, we recovered the 5′ proximal region of a highly expressed *D. piger* gene (*DpigGOR1_2316*) that encodes the α subunit of sulfite reductase using PCR primers BamHIDpig23165′ (5′-ACGCGGATCCGGGCGCTCCCGCAGGGGACAGCGG) and Dpig2316prom3 (5′-GCCATACCTCCACATGGTTTGTTGTATCAC) and *D. piger* GOR1 genomic DNA. The resulting amplicon was digested with BamHI and ligated into pSAM-*aadA**, which had been initially cut with NdeI and blunt ended by filling in the 5′ overhang using T4 DNA polymerase and then digested with BamHI, yielding pSAM–aadA*–2316.

Transposon Mutagenesis of *D. piger* GOR1. We used the following procedure to mutagenize *D. piger* GOR1 via anaerobic conjugation with a diaminopimelic acid (DAP) auxotrophic strain of *Escherichia coli*, β2163 (19), harboring pSAM–*aadA**–2316. Aliquots (1.25 OD_{600} units) of exponential phase cultures of *D. piger* GOR1, grown anaerobically at 37 °C in SRB641 medium

(Dataset S15), and the *E. coli* mating strain (β2163/pSAM–aadA*–2316), grown aerobically at 37 °C in LB medium containing 100 μg ampicillin/mL and 300 μg diaminopimelic acid (DAP)/mL, were combined on a filter that was then transferred to MegaMedium 2.0 (Dataset S15) containing DAP (300 μg/mL) and DTT (0.5 g/L) in lieu of cysteine as the reductant (the oxidized form of cysteine, cystine, competes with DAP for cellular uptake and can inhibit growth of the DAP auxotrophic strain) (20). We incubated the filter matings overnight at 37 °C under strictly anaerobic conditions (atmosphere of 5% H_2, 20% CO_2, and 75% N_2), and then resuspended the cells in 2.5 mL of MegaMedium 2.0. To obtain isolated *D. piger* transconjugants, we diluted the cell suspension 1:3 in MegaMedium 2.0 and plated 300 μL aliquots onto large Petri dish plates (150 × 15 mm, Falcon) containing MegaMedium 2.0/agar supplemented with spectinomycin (300 μg/mL). These plates lacked DAP and contained cysteine instead of DTT to counterselect against growth of the *E. coli* donor strain. Plates were incubated at 37 °C under strictly anaerobic conditions for 2 d to allow spectinomycin-resistant transconjugants of *D. piger* GOR1 to grow. Colonies (~40,000) were scraped from plates and pooled together in MegaMedium 2.0 with 20% glycerol and frozen at −80 °C in 0.5 mL aliquots (in cryovials).

In Vitro INSeq Analysis of the *D. piger* Mutant Library. A 0.5 mL aliquot of the *D. piger* transposon mutant library was diluted in SRB Base medium (Dataset S15) to OD_{600} ~6 under anaerobic conditions, and 0.5 mL aliquots were then introduced into duplicate flasks containing 500 mL of SRB medium (see next paragraph). The resulting culture was incubated at 37 °C under anaerobic conditions until it reached late exponential phase (OD_{600}~0.5). Aliquots (2 mL) were then inoculated into duplicate flasks containing 500 mL of fresh SRB medium. Growth of this second set of flasks was monitored and samples were harvested during the late exponential phase of growth (OD_{600}~0.5) for INSeq analysis.

We used SRB 20 amino acid medium (Dataset S15) supplemented with lactate (30 mM) and sulfate (14 mM Na_2SO_4, 4.1 mM $MgSO_4$), or the SRB Base medium (Dataset S15) with yeast extract and NH_4Cl, supplemented with (*i*) pyruvate alone (60 mM final concentration) or (*ii*) pyruvate (60 mM final concentration) and sulfate (14 mM Na_2SO_4, 4.1 mM $MgSO_4$) or (*iii*) lactate (30 mM) and sulfate (14 mM Na_2SO_4, 4.1 mM $MgSO_4$) or (*iv*) formate (60 mM), acetate (10 mM), and sulfate (14 mM Na_2SO_4, 4.1 mM $MgSO_4$) or (*v*) acetate (10 mM) and sulfate (14 mM Na_2SO_4, 4.1 mM $MgSO_4$). The last condition was used for testing H_2 as the electron donor and performed in 125 mL serum bottles filled with 50 mL of medium that were incubated with a headspace of 80% H_2/20% CO_2 (30 psi of pressure) at 37 °C.

Library Preparation for INSeq. The procedure involves the following steps: (*i*) isolation and purification of DNA, (*ii*) linear PCR enrichment of the transposon/chromosomal junction, (*iii*) purification and double-strand synthesis of the PCR product, (*iv*) digestion with restriction enzymes for DNA size selection, (*v*) barcode ligation, (*vi*) PCR amplification, and (*vii*) Illumina DNA sequencing. We followed the DNA preparation and INSeq protocol as previously described (21) with the following exceptions. Linear PCR was performed using 2× Pfx buffer (20 μL/100 μL PCR) and the linear PCR was run on a thermocycler using the following conditions: 94 °C for 2 min, followed by 50 cycles of 94 °C for 15 s, 60 °C for 30 s, and 68 °C for 30 s The final PCR amplification was run on a thermocycler at 94 °C for 2 min, followed by 20 cycles of 94 °C for 15 s, 55 °C for 1 min, 68 °C for 30 s, and then 68 °C for 4 min. Amplicons were sequenced using an Illumina HiSeq instrument. Sequencing data were analyzed using customized perl scripts (21) and the DESeq package (22).

Identification of Essential Genes. We identified a list of *D. piger* genes likely to be essential through the following method. We performed five independent sequencing runs of the input INSeq mutant library. We then assembled read counts for each TA dinucleotide site of transposon insertion. Each insertion site needed more than three reads to be counted as an insertion. Additionally, only insertions located within the first 80% of the coding region (relative to the 5′ end) were considered, as those would likely disrupt gene function. From these data we compiled a list of "putative essential" genes based on two criteria: there were no insertions located within the 80% proximal region of the gene, and the gene had a significant probability of having a transposon insertion, but did not have any insertions (P value < 0.05). The probability that a given gene with n TA sites has k insertions follows a binominal distribution with a success probability θ, in which θ was conservatively estimated to be the fraction of TA sites containing insertions in the entire genome. To assess the statistical significance of the observed gene without disrupted insertions, the P value was calculated as $P(k;n,\theta)=\binom{n}{k}\theta^k(1-\theta)^{(n-k)}$.

Gas Chromatography–Mass Spectroscopy. ***Targeted GC-MS of short chain fatty acid measurements.*** Cecal contents or fecal pellets were weighed in 4 mL polytetrafluoroethylene (PTFE) screw cap vials and 10 μL of a mixture of internal standards (20 mM of acetic acid-$^{13}C_2$,D_4, propionic acid-D_6, butyric acid-$^{13}C_4$, lactic acid-3,3,3-D_3, and succinic acid-$^{13}C_4$) was subsequently added to each vial, followed by 20 μL of 33% HCl and 1 mL diethyl ether. The mixture was vortexed vigorously for 10 min and then centrifuged (4,000 × *g*, 5 min). The upper organic layer was transferred to another vial and a second diethyl ether extraction was performed. After combining the two ether extracts, a 60 μL aliquot was removed, combined with 20 μL *N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) in a GC auto-sampler vial with a 200 μL glass insert, and incubated for 2 h at room temperature.

Samples were analyzed in a randomized order. Derivatized samples (1 μL) were injected with 15:1 split into an Agilent 7890A gas chromatography system coupled with 5975C mass spectrometer detector (Agilent). Analyses were carried on a HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness, Agilent J & W Scientific) using electronic impact (70 eV) as ionization mode. Helium was used as a carrier gas at a constant flow rate of 1.26 mL/min, and the solvent delay time was set to 3.5 min. The column head pressure was 10 psi. The temperatures of injector, transfer line, and quadrupole were 270 °C, 280 °C, and 150 °C, respectively. The GC oven was programmed as follows: 45 °C held for 2.25 min, increased to 200 °C at a rate of 20 °C/min, increased to 300 °C at a rate of 100 °C/min, and finally held for 3 min.

Quantification of short chain fatty acid (SCFA) was performed by isotope dilution GC-MS using selected ion monitoring (SIM). For SIM analysis, the *m/z* for native and labeled molecular peaks for SCFA quantified were 117 and 122 (acetate), 131 and 136 (propionate), 145 and 149 (butyrate), 261 and 264 (lactate), and 289 and 293 (succinate), respectively. Various concentrations of standards were spiked into control samples to prepare the calibration curves for quantification.

Targeted GC-MS of hydrogen sulfide. Sample preparation was based on a previously described procedure (23) with some modifications. Frozen cecal contents were cut on dry ice into 10 mg aliquots and weighed in 2 mL screw cap vials. We added 150 μL of 5 mM benzalkonium chloride in oxygen-free water, saturated with sodium tetraborate, to each vial, followed by 400 μL of 20 mM of pentafluorobenzylbromide in toluene and 100 μL of ethyl acetate containing 15 μM 4-chloro-benzyl methyl sulfide (internal standard). Vials were closed tightly with a PTFE-coated cap,

and the mixture was shaken in a 55 °C oven for 4 h. A saturated solution of potassium dihydrogenphosphate (in water) was added (200 μL), and the mixture was vigorously vortexed for 1 min. The organic and inorganic layers were separated by centrifugation (3,220 × *g* for 10 min at 4 °C).

Samples were analyzed in a randomized order. Samples (1 μL) were injected without split into an Agilent 7890A gas chromatography system coupled with 5975C mass spectrometer detector. Analyses were carried on a HP-5MS capillary column (see above) using electronic impact (70 eV) as ionization mode. Helium was used as a carrier gas at a constant flow rate of 1.1 mL/min, and the solvent delay time was set to 5.5 min. The column head pressure was 8.23 psi. The temperatures of the injector, transfer line, and quadrupole were 250 °C, 280 °C, and 150 °C, respectively. The GC oven was programmed as follows: 100 °C held for 1 min, increased to 250 °C at a rate of 8 °C/min, increased to 300 °C at a rate of 50 °C/min, and finally held for 3 min.

Nontargeted GC-MS analysis. Cecal contents or fecal pellets were weighed and 20 volumes of HPLC grade water were added. Homogenization was performed using a bead beater (Biospec Products) without beads for 2 min. After centrifugation (20,800 × *g* for 10 min at 4 °C), a 200 μL aliquot of the supernatant was transferred to a clean tube. Ice-cold methanol (400 μL) was added to each sample. The mixture was vortexed and subsequently centrifuged at 20,800 × *g* for 10 min at 4 °C. A 500 μL aliquot of the resulting supernatant together with 10 μL of lysine-$^{13}C_6$,$^{15}N_2$ (2 mM) was evaporated to dryness using a speed vacuum. Derivatization of all dried supernatants followed a method adapted with modifications from ref. 24. Briefly, 80 μL of a solution of methoxylamine (15 mg/mL in pyridine) was added to methoximate reactive carbonyls (incubation for 16 h for 37 °C), followed by replacement of exchangeable protons with trimethylsilyl groups using *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide with a 1% vol/vol catalytic admixture of trimethylchlorosilane (Thermo-Fisher Scientific) (incubation at 70 °C for 1 h). Finally, 160 μL heptane was added to the derivatives before injection.

We injected 1 μL aliquot of each derivatized sample without split into the GC-MS system described above. Analyses were carried on a HP-5MS capillary column (see above) using electronic impact (70 eV) as ionization mode. Helium was used as a carrier gas at a constant flow rate of 1 mL/min; the solvent delay time was set to 5.5 min. The column head pressure was 8.23 psi. Temperatures of the injector, transfer line, and source were 250 °C, 290 °C, and 230 °C, respectively. The GC oven was programmed as follows: 60 °C held for 2 min, increased to 140 °C at a rate of 10 °C/min, increased to 240 °C at a rate of 4 °C/min, increased to 300 °C at a rate of 10 °C/min, and finally held at 300 °C for 8 min. Metabolite identification was done by co-characterization of standards.

Data in instrument specific format (.D) were converted to common data format (.cdf) files using MSD ChemStation (E02.01, Agilent); the .cdf files were extracted using Bioinformatics Toolbox in the MATLAB 7.1 (The MathWorks, Inc.), along with custom scripts (25) for alignment of data in the time domain, automatic integration, and extraction of peak intensities. The resulting three dimension dataset included sample information, peak retention time, and peak intensities. Data were then mean centered and unit variance scaled for multivariate analysis.

Ultra high-performance liquid chromatography–mass spectrometry. Frozen cecal samples were combined with 20 volumes of cold methanol, one volume of cysteine $^{13}C_6$,$^{15}N_2$ (4 mM), and mixed for 2 min in a Biospec Beadbeater (maximal setting; no beads added). Samples were then incubated at –20 °C for 1 h and subsequently centrifuged 10 min at 20,800 × *g*. The supernatant (300 μL) was collected and dried in a SpeedVac at room temperature. Dried samples were resuspended in 100 μL of 95:5 water–ethanol, clarified for 5 min by centrifugation at 20,800 × *g* for 10 min at 4 °C, and the supernatant was separated for ultra high-performance liquid chromatography–mass spectrometry (UPLC-MS).

Analyses were performed on a Waters Acquity I Class UPLC system (Waters Corp.) coupled to an LTQ-Orbitrap Discovery (Thermo Fisher Corporation). A 5 μL injection volume and flow rate of 0.3 mL/min were used for both the Discovery HS F5 PFPP column (150 mm × 2.1 mm, 3 μm particle size; Sigma-Aldrich) and the 150 mm × 2.1 mm Waters BEH C18 1.7 μm particle column. Mobile phases for positive ion mode were (*i*) 0.1% formic acid in water and (*ii*) 0.1% formic acid in acetonitrile. Negative ion mode used (*i*) 5 mM ammonium bicarbonate in water and (*ii*) 5 mM ammonium bicarbonate in 95/5 acetonitrile/water. The capillary column was maintained at 325 °C with a sheath gas flow of 40 (arbitrary units), an aux gas flow of 5 (arbitrary units) and a sweep gas flow of 3 (arbitrary units) for both positive and negative injections. The spray voltage for the positive ion injection was 4.5 kV and 4 kV for the negative ion injection.

Quality Control of Metabolomics Data. Pooled quality control (QC) samples were prepared from 20 μL of each sample and analyzed together with the other samples. The QC samples were also inserted and analyzed in every 10 samples. To exclude false positives, raw data for metabolites that exhibited statistically significant differences in their concentration were reevaluated in MSD ChemStation E.02.01.1177 (Agilent).

In Vitro Cross-Feeding Between *B. thetaiotaomicron* and *D. piger*. Exponential phase cultures of *B. thetaiotaomicron* Δ*bt0238* and the isogenic wild-type parental strain (26) (kindly provided by Eric Martens, University of Michigan, Ann Arbor, MI, and Olivier Berteau, Institut National de la Recherche Agronomique, Jouy-en-Josas, France), grown in Mega Medium 2.0, were inoculated under anaerobic conditions (atmosphere of 5% H_2, 20% CO_2, and 75% N_2) into Balch tubes containing minimal medium supplemented with either 0.5% (wt/vol) chondroitin sulfate purified from shark cartilage (Sigma) or 0.5% fructose (Sigma) (n = 6 tubes/carbon substrate/strain). Anaerobic cultures were incubated at 37 °C, and growth was monitored at OD_{600} until cells reached late exponential phase (with the exception of *B. thetaiotaomicron* Δ*bt0238*, which failed to grow in minimal medium plus chondroitin sulfate). Samples were taken and immediately frozen in liquid nitrogen for GC-MS analysis to provide the background levels of H_2S before *D. piger* growth. Cultures representing the same strain and carbon substrate were combined, and bacteria were pelleted by centrifugation at 3,200 × *g* at 4 °C for 20 min. The supernatant was removed and sterilized by passage through a 0.22 μm filter (Fisher). To allow for potential *D. piger* growth, we added lactate (to a final concentration of 30 mM), yeast extract (to 1 mg/mL), NH_4Cl (to 20 mM), and a mixture of vitamins and minerals (ATCC; 1× final concentration). The pH of the conditioned medium was adjusted to ∼7.0 using potassium phosphate buffer (pH 7.2).

One half of each conditioned medium preparation was used to fill anaerobic Balch tubes (in triplicate), and sulfate (14 mM Na_2SO_4 and 4.1 mM $MgSO_4$) was added to the remaining conditioned medium before filling the tubes (in triplicate). A 100 μL aliquot of a late exponential phase culture of *D. piger* GOR1 (grown in SRB641 medium) was added to each tube containing the conditioned medium, and the tubes were incubated at 37 °C. Samples were taken during exponential phase (OD_{600} = 0.28–0.44) for those cultures with growth and at this same time point for cultures without growth, and immediately frozen in liquid nitrogen for GC-MS analysis of H_2S levels.

Ammonia Measurements. Ammonia levels in feces and cecal contents were quantified using an assay kit from Abcam (ab83360) and a protocol described by the manufacturer.

Bromodeoxyuridine Staining. Bromodeoxyuridine (BrdU) staining was performed following instructions from the manufacturer (BrdU Flow Kit, BD Pharmingen). Briefly 200 μL of BrdU (10 mg/mL) was injected intraperitoneally to mice. Animals were killed 2 h later. The proximal half of the colon was harvested, opened along its cephalocaudal axis, washed by shaking vigorously in PBS/0.1% BSA, chopped into small pieces using a razor blade, and placed in PBS/0.1% BSA on ice before further processing.

PBS/0.1% BSA containing tissue was passed through a stainless steel kitchen strainer and collected in a sterile beaker. Tissue was then collected from the strainer, placed in 20 mL of prewarmed (37 °C) RPMI 1640 (Gibco) supplemented with FBS (3%, vol/vol; Gibco), HEPES (20 mM; Cellgro), L-glutamine (2 mM; Cellgro), penicillin (100 U/mL; Gibco), streptomycin (100 μg/mL; Gibco), EDTA (5 mM; Cellgro), and DTT (0.94 mM; Sigma), and mixed for 20 min at 37 °C using a magnetic stir plate. The stirred solution was passed through a kitchen strainer. Residual tissue was again recovered from the strainer and placed in 15 mL of RPMI 1640 (Gibco) supplemented as before but without DTT and EDTA at 2 mM rather than 5 mM. Tissue was shaken vigorously for 30 s and then passed through a kitchen strainer. This step was repeated one more time and all three collections of cells isolated using RPMI 1640 that were passed through the strainer were pooled and pelleted by centrifugation at 453 × *g* for 5 min. The supernatant was discarded and cells in the pellet were resuspended by flicking the tube.

To assess BrdU incorporation, cells were first incubated in a solution of PBS/0.1% BSA containing anti-CD16/CD32 (BD) for 20 min at 4 °C to prevent nonspecific antibody binding in subsequent steps. Cells were subsequently surfaced stained with anti-CD45 and anti-EpCam antibodies diluted in PBS/0.1% BSA (20 min at 4 °C). Cells were washed twice by addition of 200 μL of PBS/0.1% BSA, pelleted by centrifugation (453 × *g*), and then treated according to the "BrdU Flow Kit" manual (BD Pharmingen). In brief, cells were fixed by addition of 100 μL of BD Cytofix/Cytoperm, incubated for 15 min at room temperature in the dark, and washed twice (each wash involved addition of 200 μL of fresh BD Perm/Wash Buffer followed by centrifugation at 652 × *g*). Washed cells were stored overnight in PBS/0.1% BSA at 4 °C, pelleted (centrifugation at 652 × *g*), and incubated in 100 μL BD Cytoperm Permeabilization Buffer Plus for 10 min on ice while being protected from light. Cells were washed twice (200 μL BD Perm/Wash Buffer; centrifugation at 652 × *g*) and refixed by addition of 100 μL BD Cytofix/Cytoperm buffer and incubated at room temperature for 5 min in the dark. Cells were washed twice more in BD Perm/Wash Buffer, treated with 100 μL of DNase (300 μg/mL) for 1 h at 37 °C, washed twice again, and stained for BrdU incorporation by incubation in BD Perm/Wash buffer containing an anti-BrdU antibody for 20 min at room temperature in the dark. Cells were washed twice and acquired on a BD FACS Aria III flow cytometer and analyzed using FlowJo software.

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Fig. S1. SRB in the fecal microbiota of healthy adult humans. The adenosine-5′-phosphosulfate reductase alpha subunit gene (*aprA*) was amplified by PCR from fecal samples obtained from human subjects previously identified as SRB carriers (10). Amplicons were subjected to multiplex pyrosequencing, and sequences were analyzed using QIIME pipeline software tools. Reads were classified into operational taxonomic units (OTUs) on the basis of sequence similarity; we specified that species-level phylotypes share ≥94% identity over the sequenced region.

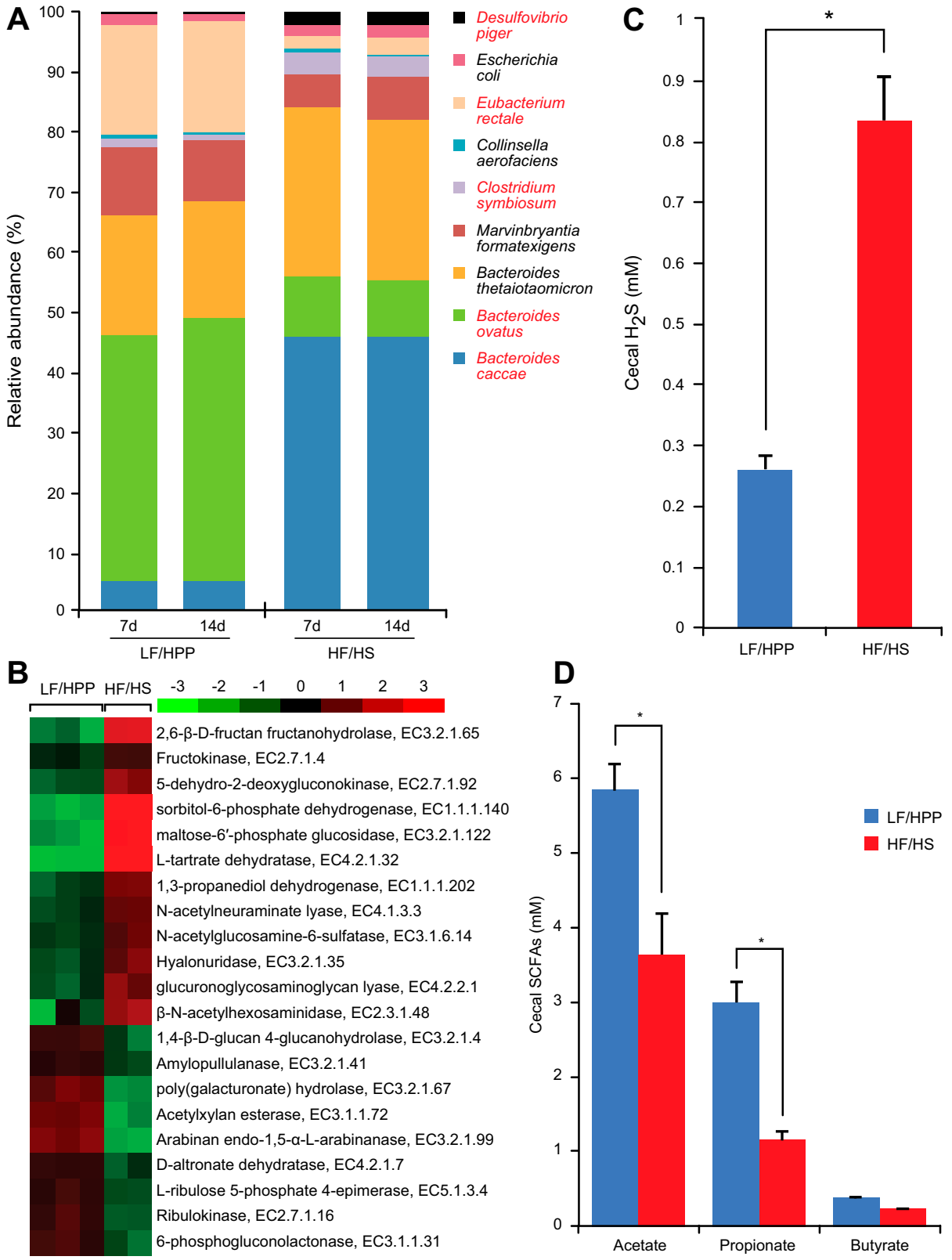

Fig. S2. Diet shapes the structure, metatranscriptome, and metabolic output of a defined human gut microbiota. (*A*) Relative abundance of bacterial species in the feces of mice fed a LF/HPP diet or a HF/HS diet. Abundance was defined by shotgun sequencing of fecal DNA (COPRO-Seq) 7 and 14 d after gavage with a consortium of nine sequenced members of the human gut microbiota ($n = 4$–5 animals/diet). Bacterial species that exhibited a significant difference in their abundance in the fecal microbiota of mice consuming one or the other diet for both time points examined are highlighted in red ($P < 0.05$, two-tailed Student *t* test followed by Bonferroni correction). Fecal biomass was 3.1-fold higher in mice fed LF/HPP ($P < 0.05$, Student *t* test). (*B*) Selected results from microbial RNA-Seq analysis of the fecal metatranscriptome. The heat map shows a subset of mRNAs encoding ECs whose expression was significantly different as a function of

Legend continued on following page

host diet (fold-difference ≤2 or >2; $P < 0.01$, PPDE > 0.95). The maximal relative expression across a row is red; the minimum is green. Each column represents a different mouse in the indicated treatment group. (*C* and *D*) Targeted GC-MS analysis of hydrogen sulfide (*C*) and SCFAs (*D*) in cecal contents as a function of diet ($n = 4$–5 animals/diet). Mean values ± SEM are plotted. *$P < 0.05$ (Student *t* test). [Comparison of two groups of mice fed the HF/HS diet and colonized with either the nine-member artificial community or the same assemblage minus *D. piger* revealed that the presence of *D. piger* was associated with a statistically significant 1.8 ± 0.3-fold higher level of H_2S in cecal contents ($n = 5$ mice/treatment group; $P < 0.05$, two-tailed *t* test)].

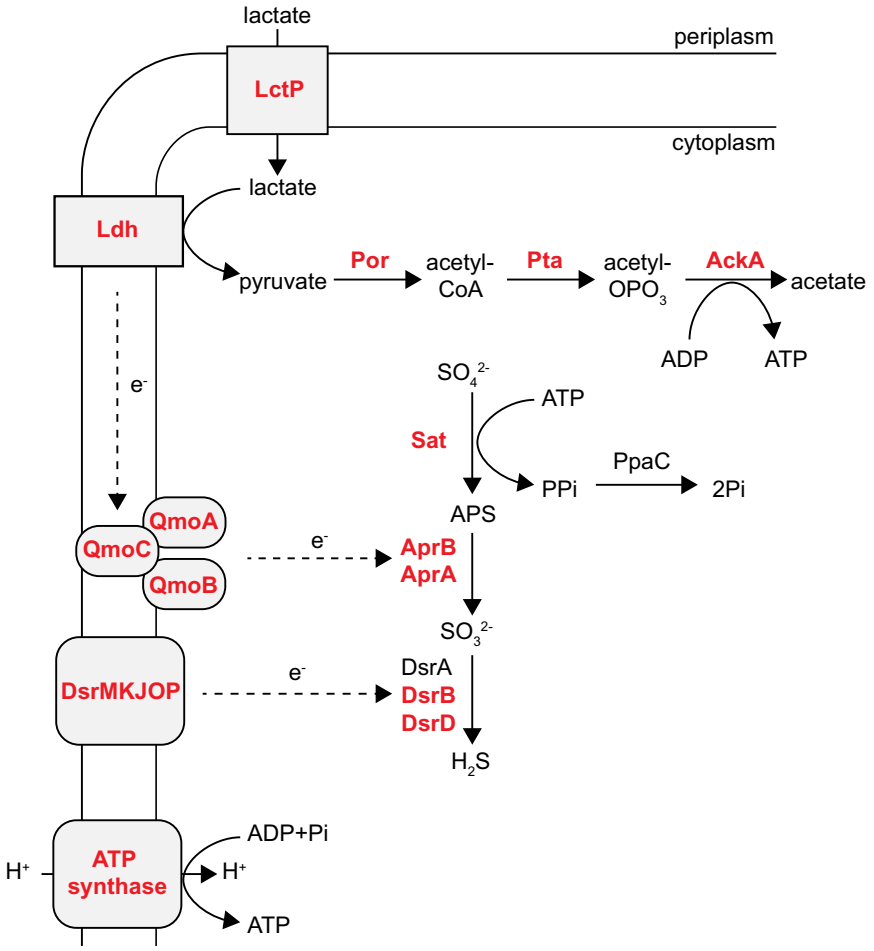

Fig. S3. Fitness determinants identified by INSeq analysis of *D. piger* GOR1 grown in vitro using lactate as the electron donor and sulfate as the electron acceptor. Growth of *D. piger* in a fully defined medium containing lactate as an electron donor and sulfate as electron acceptor occurs through the uptake and oxidation of lactate, which supplies electrons for sulfate reduction. This pathway generates a proton gradient that is used to generate energy via an F-type ATP synthase. Solid arrows represent enzyme reaction steps, and dashed arrows represent electron transfer steps (e^-). Proteins and protein complexes involved in these reactions are noted, with those identified as statistically significant fitness determinants highlighted in red. LctP, lactate permease (encoded by *DpigGOR1_1075*); AckA, acetate kinase (*DpigGOR1_1329*); AprA, adenosine 5′-phosphosulfate reductase alpha subunit (*DpigGOR1_0793*); AprB, adenosine-5′-phosphosulfate reductase beta subunit (*DpigGOR1_0794*); ATP synthase *(DpigGOR1_0309-DpigGOR1_0315*); DsrA, dissimilatory sulfite reductase alpha subunit (*DpigGOR1_2316*); DsrB, dissimilatory sulfite reductase beta subunit (*DpigGOR1_2317*); DsrD, dissimilatory sulfite reductase delta subunit (*DpigGOR1_2318*); DsrMKJOP (*DpigGOR1_0174-DpigGOR1_0170*); Ldh, lactate dehydrogenase (*DpigGOR1_0371* and *DpigGOR1_1628*); Por, pyruvate-ferredoxin oxidoreductase (*DpigGOR1_1331*); PpaC, pyrophosphatase (*DpigGOR1_2264*); Pta, phosphate acetyltransferase (*DpigGOR1_1330*); QmoA, quinone-interacting membrane-bound oxidoreductase flavin protein (*DpigGOR1_0792*); QmoB, quinone-interacting membrane-bound oxidoreductase flavin protein (*DpigGOR1_0791*); QmoC, quinone-interacting membrane-bound oxidoreductase membrane FeS protein (*DpigGOR1_0790*); Sat, sulfate adenylyltransferase (*DpigGOR1_0178*).

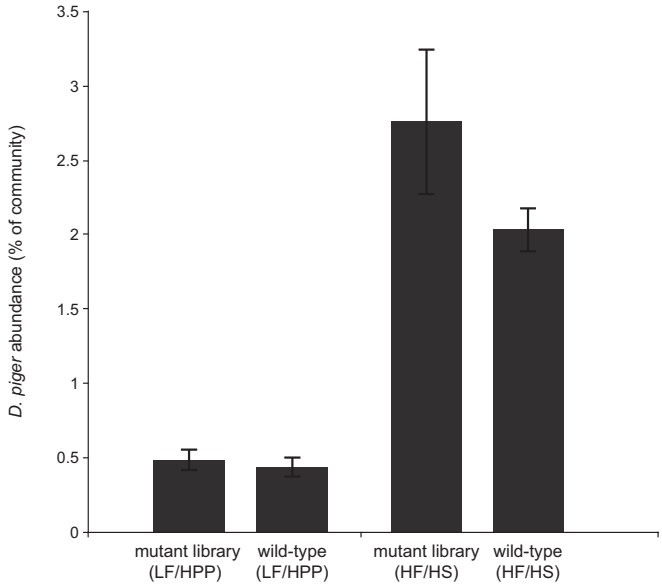

Fig. S4. Levels of wild-type *D. piger* versus the aggregate *D. piger* library of transposon mutants in the fecal microbiota of gnotobiotic mice harboring the nine-member artificial human gut community and fed the LF/HPP versus HF/HS diet. The relative abundance of the *D. piger* INSeq library was defined using COPRO-Seq. Samples were taken 7 d after gavage of mice already colonized with the eight-member consortium with the *D. piger* Tn mutant library ($n = 4$ animals/diet). Also shown is the relative abundance of wild-type (WT) *D. piger* from Fig. S2 ($n = 4$–5 mice/diet). Note that there are no statistically significant differences between the levels of the aggregate INSeq library and wild-type *D. piger* in groups of mice consuming the same diet (Student *t* test). Mean values ± SEM are plotted.

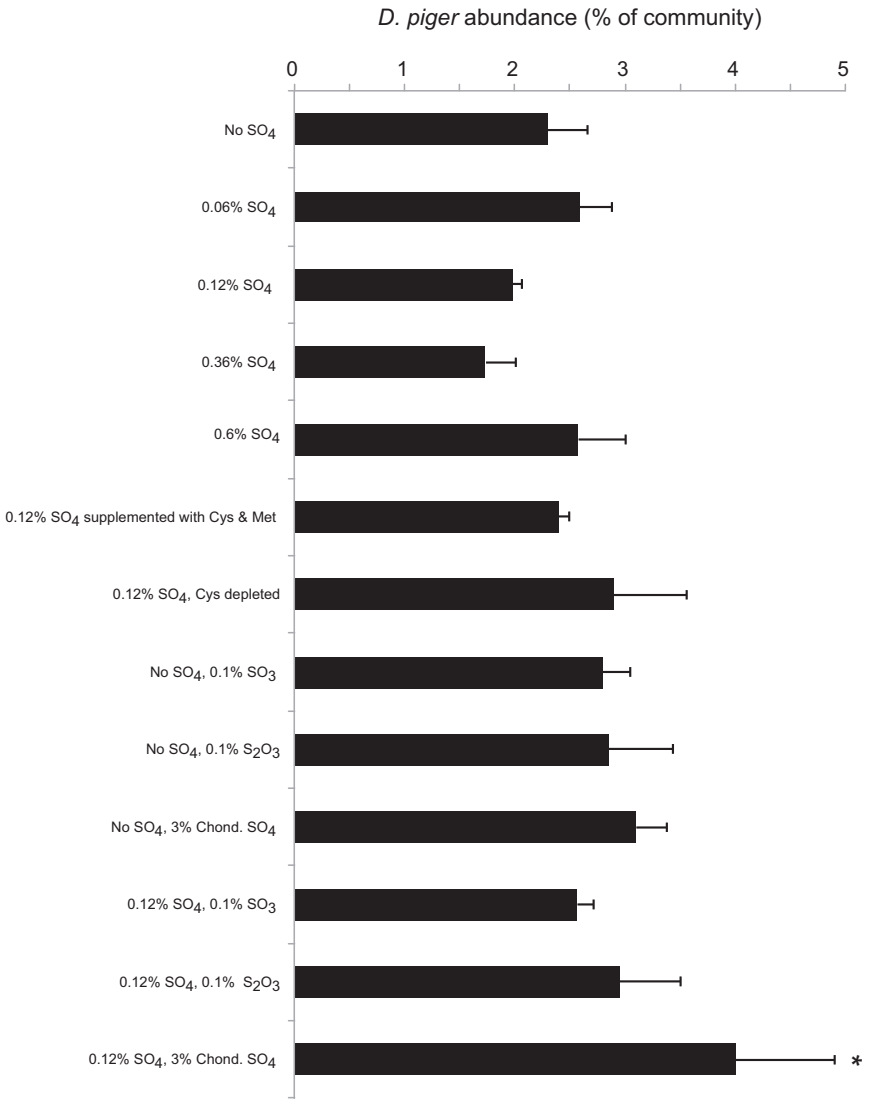

Fig. S5. Effects of different levels and types of sulfur-containing diet supplements on levels of *D. piger*. The relative abundance of *D. piger* was determined by shotgun sequencing of fecal DNA (COPRO-Seq). Six groups, each composed of two co-housed mice colonized with the nine-member artificial human gut community, were fed one of 13 diets, all based on the HF/HS diet (0.12% wt/wt SO_4; see Dataset S2 for diet compositions). Each group of mice was started on the HF/HS diet and then given a sequence of four diets with differing sulfur content, each for a 7-d period. The sequence of presentation of the four diets was randomized so that that each diet was eventually fed to two different groups of cohoused animals. Mean values ± SEM are plotted. *$P < 0.05$ compared to the HF/HS base diet, which contained 0.12% SO_4 (one-way ANOVA; Dunnett's Multiple Comparison Test). Chond. SO_4, chondroitin sulfate; Cys, cysteine; Met, methionine; S_2O_3, thiosulfate; SO_3, sulfite; SO_4, sulfate.

Dataset S1. List of genes predicted to encode carbohydrate active enzymes (*A*) and proteolytic enzymes (*B*) in the artificial nine-member human gut microbiota

Dataset S1 (XLSX)

Dataset S2. Composition of mouse diets

Dataset S2 (XLSX)

Dataset S3. Artificial human gut microbial community's metatranscriptome (*A*) and individual species contributions to statistically significant changes in expression of genes encoding ECs (*B*)

Dataset S3 (XLSX)

Dataset S4. ***D. piger*** **genes without identified mutations in the INSeq library that are "presumably essential" for** ***D. piger*** **survival in rich medium**

Dataset S4 (XLSX)

Dataset S5. ***D. piger*** **fitness determinants identified by INSeq during growth in a fully defined medium**

Dataset S5 (XLSX)

Dataset S6. ***D. piger*** **fitness determinants that exhibit diet independence**

Dataset S6 (XLSX)

Dataset S7. All ***D. piger*** **genes with fitness effects in the cecal and/or fecal microbiota of mice consuming the LF/HPP and/or HF/HS diets**

Dataset S7 (XLSX)

Dataset S8. ***D. piger*** **fitness determinants that exhibit diet specificity**

Dataset S8 (XLSX)

Dataset S9. ***D. piger*** **fitness factors in vitro and in vivo**

Dataset S9 (XLSX)

Dataset S10. ***D. piger*** **fitness determinants that exhibit in vivo specificity**

Dataset S10 (XLSX)

Dataset S11. Design for diet oscillation experiments

Dataset S11 (XLSX)

Dataset S12. ***D. piger*** **fitness determinants identified in mice fed the HF/HS diet lacking sulfate**

Dataset S12 (XLSX)

Dataset S13. Effect of ***D. piger*** **on the metatranscriptome of the artificial microbial gut community**

Dataset S13 (XLSX)

Dataset S14. Effects of the presence or absence of ***D. piger*** **on mouse gene expression in the proximal colon**

Dataset S14 (XLSX)

Dataset S15. Media used for bacterial cultures

Dataset S15 (XLSX)