Rapid fucosylation of intestinal epithelium sustains host–commensal symbiosis in sickness

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Systemic infection induces conserved physiological responses that include both resistance and ‘tolerance of infection’ mechanisms1. Temporary anorexia associated with an infection is often beneficial2, reallocating energy from food foraging towards resistance3 or depriving pathogens of nutrients4. However, it imposes a stress on intestinal commensals, as they also experience reduced substrate availability; this affects host fitness owing to the loss of caloric intake and colonization resistance (protection from additional infections)5. We hypothesized that the host might utilize internal resources to support the gut microbiota during the acute phase of the disease. Here we show that systemic exposure to Toll-like receptor (TLR) ligands causes rapid α(1,2)-fucosylation of small intestine epithelial cells (IECs) in mice, which requires the sensing of TLR agonists, as well as the production of interleukin (IL)-23 by dendritic cells, activation of innate lymphoid cells and expression of fusocyltransferase 2 (Fut2) by IL-22-stimulated IECs. Fucosylated proteins are shed into the lumen and fucose is liberated and metabolized by the gut microbiota, as shown by reporter bacteria and community-wide analysis of microbial gene expression. Fucose affects the expression of microbial metabolic pathways and reduces the expression of bacterial virulence genes. It also improves host tolerance of the mild pathogen Citrobacter rodentium. Thus, rapid IEC fucosylation appears to be a protective mechanism that utilizes the host’s resources to maintain host–microbial interactions during pathogen-induced stress.

To maintain healthy gut microbiota during a systemic response induced by microbial products the host may use its internal resources. α-L-Fucosylation could present an example of such a resource: α-fucose attached to glycoproteins and glycolipids is accessible for microbial, but not for host energy harvest6. Constitutive α(1,2)fucosylation affects the microbial community in a diet-dependent manner7, serves as a substrate for pathogens during antibiotic exposure8 and for microbes colonizing the ileum of newborns or of adult germ-free (GF) mice9–11. Under normal conditions, however, the small intestine of specific pathogen-free (SPF) BALB/c mice is largely free of surface fucose. By contrast, a systemic injection of agonists of TLRs, such as lipopolysaccharide (LPS; TLR4 ligand) (Fig. 1), CpG DNA (TLR9 ligand), or Pam3CSK4 (TLR2 agonist), led to ubiquitous α(1,2)fucosylation of the small intestine in mice of different genetic backgrounds, which started within a few hours after LPS exposure and lasted for several days (Extended Data Fig. 1a–c). It did not result in differentiation of IECs into functional M cells12, which are permanently fucosylated and are involved in microbial sensing and translocation (Extended Data Fig. 1d). Induced fucosylation was independent of the gut microbiota (observed in GF mice), and was not induced by oral LPS (Extended Data Fig. 1e).

Global deletion of the TLR signalling adaptor molecule MyD88 prevented IEC fucosylation, and its conditional deletion from dendritic cells, but not from IECs, abrogated the process (Fig. 1). The inducible fucosylation pathway was similar to induction of antimicrobial peptides by a systemic microbial signal13: it required MyD88-expressing dendritic cells, production of IL-23, the transcriptional regulator RORγt and IL-22 (Fig. 1 and Extended Data Fig. 2a), and was induced by a direct injection of IL-22 into Myd88−/− mice (Fig. 1). IEC fucosylation in mice lacking T cells (Fig. 1) suggested that innate lymphoid cells (ILCs) were a sufficient source of IL-22. Salmonella enterica ssp. Typhimurium, known to spread systemically, induced small intestine IEC fucosylation (Extended Data Fig. 2b). The α(1,2)fucosyltransferase responsible for fucosylation of IECs in the small intestine was identified as Fut2 (Fig. 2a), which is inducible by stress conditions14,15 and constitutively expressed in the stomach and large intestine16. Genetic ablation of the Fut2 gene blocked IEC fucosylation in response to LPS (Fig. 2b, c). The overall chain of events is shown in Extended Data Fig. 3.

LPS injection caused marked sickness in BALB/c Fut2-deficient (Fut2−/−) and Fut2-sufficient (Fut2+/+ or Fut2+−) littermates hours after injection: mice displayed measurable anorexia, adipsia, reduced activity, diarrhoea and weight loss. Food consumption, weight loss, and production of inflammatory cytokines and antimicrobial peptides (Fig. 2d, e and Extended Data Fig. 4a, b) were similar in both groups. Although...
**Fut2**−/− mice have been shown to be healthy under normal SPF conditions, they were significantly slower than controls in recovering their body weight (Fig. 2e) after LPS injection. Importantly, food deprivation without LPS challenge did not cause small intestine fucosylation and the weight recovery was identical in Fut2-sufficient and Fut2-deficient animals (Extended Data Figs 1a and 1c). Thus, fucosylation of the small intestine was a response to activation by microbial stimuli and not to anorexia per se.

To understand the reason for the slow weight recovery in Fut2−/− mice, we tested whether fucosylation affected the function of host IEC proteins, which were identified by direct sequencing as secreted mucins and digestive enzymes (Fig. 3a). Fucosylation did not change the activity of several enzymes (Fig. 3b). Thus, changes in enzymatic activity are unlikely to explain the slow weight recovery in Fut2−/− mice, although the role of less abundant fucosylated proteins cannot be excluded.

The beneficial effect of fucosylation was dependent on the microbiota: the weight recovery after LPS challenge was delayed in GF mice and in Fut2−/− mice, we tested whether fucosylation affected the function of host IEC proteins, which were identified by direct sequencing as secreted mucins and digestive enzymes (Fig. 3a). Fucosylation did not change the activity of several enzymes (Fig. 3b). Thus, changes in enzymatic activity are unlikely to explain the slow weight recovery in Fut2−/− mice, although the role of less abundant fucosylated proteins cannot be excluded.

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**Figure 2 | Consequences of the loss of Fut-2-dependent fucosylation.** a, Expression of mouse α(1,2)fucosyltransferase genes (Fut2, Fut4 and Sec1) and control (villin-encoding gene, Vill) in the gut 24 h after LPS injection (semiquantitative polymerase chain reaction with reverse transcription [RT–PCR]). Caec, caecum; Col., colon; Sl, small intestine. b, Intestinal fucosylation (green) of Fut2-sufficient and Fut2-deficient mice. Red, propidium iodide. Scale bars = 100 μm. c, Fluorescence-activated cell sorting (FACS) histograms of small intestine IECs from PBS- (left) or LPS- (right) injected, Fut2+/+ (top) or Fut2−/− (bottom) mice. d, Food consumption in LPS-treated Fut2+/+ (n = 5, black bars) and Fut2−/− mice (n = 3, open bars) (mean ± standard error of the mean (s.e.m.)). e, Dependence of weight recovery after LPS challenge on the presence of microbiota and expression of Fut2 (mean ± s.e.m. of percentage of starting body weight, data combined from four experiments). *P < 0.05, one-way ANOVA. abx, antibiotics (ampicillin and vancomycin). All data are representative of at least two independent experiments.

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**Supplementary Table 1**. Analytical and environmental parameters for the study. **Operational taxonomic units (OTUs) significantly associated with host genotype before or after LPS treatment** (all were q > 0.05 for both comparisons; permutational multivariate analysis of variance (PERMANOVA) of unweighted UniFrac distances); **2 species-level operational taxonomic units (OTUs) significantly associated with host genotype before or after LPS treatment** (all were q > 0.05; analysis of variance (ANOVA)); or **3 significant differences in overall microbial diversity** (Extended Data Fig. 7c). However, we were able to detect a significantly increased abundance of B. acidifaciens after LPS treatment in Fut2-sufficient mice (P < 0.05, linear discriminant analysis (LDA) score > 4; linear discriminant analysis effect size (LeFSe) analysis of 16S profiles), consistent with its ability to utilize fucosylated glycans.

At the same time, LPS markedly altered community-wide gene expression in both Fut2+/+ and Fut2−/− mice (Fig. 3h), with multiple orthologous groups differentially expressed upon LPS treatment: 61 in Fut2+/+ mice and 56 in Fut2−/− animals. These changes were not due to altered community structure: only one differentially expressed orthologous group (K05351, xylulose reductase) also exhibited significant changes in gene abundance (Supplementary Table 2). As expected, we detected a significant upregulation of fucose permease (fucP; K02429) in Fut2-sufficient mice after exposure to LPS (Fig. 3i), and increased expression of metabolic modules for anaerobic respiration, protein and ATP synthesis, iso-}

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creased pathogen burden (resistance), or by an increase in pathogen tolerance. We tested this by infecting Fut2-sufficient and Fut2-deficient mice with a non-lethal intestinal pathogen, Vibrio cholerae K10953 and haemolysin III (K11068) (Supplementary Table 2). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways potentially involved in microbial pathogenesis (defined as flagellar peptidase, chemotaxis, plant/pathogen interaction and Vibrio cholerae infection) were overrepresented in Fut2-deficient mice (Extended Data Fig. 7d). We hypothesized that fucosylation induced by systemic microbial challenge might limit the effects of additional exogenous or endogenous pathogens. We tested this by infecting Fut2-sufficient and Fut2-deficient mice with a non-lethal intestinal pathogen, C. rodentium. Four days after infection, mice were treated with LPS. Infected Fut2-negative mice lost significantly more weight than Fut2-sufficient animals compared with respective LPS-treated non-infected controls (Fig. 4a). Thus, infection with a non-lethal pathogen further reduced the overall fitness of Fut2-deficient mice in response to LPS. C. rodentium did not induce small intestine IEC fucosylation and did not colonize the small intestine (Extended Data Fig. 8), indicating that systemic challenge by a microbial pathogen appears to affect gut microbial metabolism.

Importantly, LPS challenge led to the significantly increased expression of microbial virulence genes in Fut2-negative but not Fut2-sufficient mice, including rtxA (K10953) and haemolysin III (K11068) (Supplementary Table 2). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways potentially involved in microbial pathogenesis (defined as flagellar peptidase, chemotaxis, plant/pathogen interaction and Vibrio cholerae infection) were overrepresented in Fut2-deficient mice (Extended Data Fig. 7d). We hypothesized that fucosylation induced by systemic microbial challenge might limit the effects of additional exogenous or endogenous pathogens. We tested this by infecting Fut2-sufficient and Fut2-deficient mice with a non-lethal intestinal pathogen, C. rodentium. Four days after infection, mice were treated with LPS. Infected Fut2-negative mice lost significantly more weight than Fut2-sufficient animals compared with respective LPS-treated non-infected controls (Fig. 4a). Thus, infection with a non-lethal pathogen further reduced the overall fitness of Fut2-deficient mice in response to LPS. C. rodentium did not induce small intestine IEC fucosylation and did not colonize the small intestine (Extended Data Fig. 8), indicating that systemic challenge by a microbial pathogen was required to reveal the role of inducible fucosylation.

The fitness of infected animals can be maintained through either decreased pathogen burden (resistance), or by an increase in pathogen tolerance without a change in pathogen burden. We quantified the abundance of C. rodentium in the faeces of infected mice, as well as the adherence to IECs of C. rodentium-expressing luciferase (Fig. 4b, c). No differences in pathogen loads were found between Fut2-sufficient and Fut2-deficient animals treated with LPS. Thus, fucosylation of the small intestine upon systemic treatment with LPS probably enhances tolerance of the pathogen. Fut2-negative mice infected with C. rodentium and injected with LPS had more pronounced colonic hyperplasia (a trademark of this infection) compared with Fut2-sufficient mice or mice that did not receive LPS (Fig. 4d, e).

Thus, inducible IEC fucosylation might be viewed as an emergency measure taken by the host to support its gut commensals. Fucose used by microbes as an energy source may contribute to protection of the host from endogenous opportunistic pathogens, or it could increase tolerance of infection by regulating bacterial phyla across treatment groups and genotypes, as indicated by shotgun sequencing of community DNA. Phyla with a mean reads per kilobase per million mapped reads (RPKM) >40,000 are shown, including Actinobacteria (purple), Bacteroidetes (red), Firmicutes (blue), Fusobacteria (green), Proteobacteria (orange) and Tenericutes (yellow). 16S rRNA gene sequencing confirms these observations. Extended Data Fig. 7 shows 16S rRNA gene sequencing results. h, Differentially expressed KEGG orthologous groups following LPS treatment (paired glm edgeR analysis; q < 0.05, >2-fold change; see Supplementary Table 2 for complete list). i, Increased gut microbial expression of fucP (K02429) in Fut2-sufficient mice (mean ± s.e.m.; *P < 0.01, Mann–Whitney test).

For aminoglycan degradation (Extended Data Fig. 7d). Thus, intact host fucosylation appears to affect gut microbial metabolism.

Figure 3 | Commensals utilize fucose detached from proteins fucosylated by Fut2 upon systemic challenge with LPS. a, Silver-stained SDS-polyacrylamide gel electrophoresis (SDS–PAGE) of UEA-1-precipitated small intestine IEC protein from control or LPS-treated mouse. b, Ratios of digestive enzyme activities in small intestine IECs of LPS-treated to untreated Fut2-sufficient (black bars) or Fut2-deficient (open bars) mice 2 days after LPS injection. Mean ± s.e.m. of four combined experiments; four mice per group. NS, not significant (P > 0.05, two-tailed Student’s t-test). c, SDS–PAGE of intestinal contents blotted on nitrocellulose and stained with UEA-1–biotin reagent. Enzyme activities in small intestine IECs of LPS-treated to untreated Fut2-sufficient and Fut2-deficient mice were determined. d, Relative expression of fucA, fucP and fucZ upon systemic treatment with LPS (paired glm edgeR analysis; q < 0.05, >2-fold change; see Supplementary Table 2 for complete list). e, Increased gut microbial expression of fucP (K02429) in Fut2-sufficient mice (mean ± s.e.m.; *P < 0.01, Mann–Whitney test).
proteins—a resistance mechanism. Thus, the mechanisms of resistance and tolerance to pathogens could be evolutionarily linked together to increase the fitness of the host.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Information The DNA and RNA shotgun sequencing data have been deposited in the Gene Expression Omnibus database under accession number GSE60874; 16S rRNA gene sequencing reads have been deposited in MG-RAST under accession number 12094994. Reprints and permissions information is available at www.nature.com/reprints/. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.V.C. (achervon@bxd.upenn.edu).
METHODS

Mice. BALB/c, C57BL/6, NOD/Ltj, C3H/HeN, Rag1−/− (B6.129S7-Rag1tm1Mom/J), Fas−/− (B6.129X1-Fas−/−), Villin-Cre (B6.SL-Tg(Vil-Cre)997Gum/J), and CD1c-Cre (C57BL/6-Tg(Itgax-Cre-EFP)4097Ath/J) mice were purchased from The Jackson Laboratory. Fas−/− mice were backcrossed greater than seven generations to BALB/c. Knockout mice produced litters of mixed genotypes kept cohoused to homogenize their gut microbiota. B6 Myd88−/− mice were a gift from S. Akira. B6 Myd88−/− mice were described previously25, Rorc−/− (ref. 32), and Il12p19−/− (also known as Il23a−/−) mice were provided by Y.-X. Fu. Il22−/− mice26 were maintained at the Memorial Sloan-Kettering Cancer Center. Mice were housed in a specific pathogen-free facility and used in accordance with institutional guidelines for animal welfare. Six- to twelve-week-old male and female mice were used for randomization purposes. The numbers of mice per group were chosen as the minimum needed to obtain biologically significant results, based on previous experience. Evaluations were made in a blind fashion. Functional experiments were done with Fas2-negative mice on the BALB/c genetic background using Fas2-sufficient littermates as controls.

Metabolic studies. Mice were single-housed in TSE Systems LabMaster cages to monitor physical activity (x/y and z axes by infrared beams crossed), drinking, and feeding.

Fucosylation activation in vivo. LPS from S. enterica serp. Typhimurium (16615, Sigma-Aldrich) was injected i.p. at 1 μg g−1 body weight, or gavaged to GF mice at 1 mg in 400 μl sterile PBS. CpG (100 μg CpG-B ODN 1826, Coley Pharmaceutical Group) and Pam3CSK4 (100 μg, Invivogen), were injected i.p. Carrier-free recombinant mouse IL-22 (Biologend) was diluted in 1% BSA/PBS and 1.5 μg was given i.p.

Antibiotic treatment. Ampicillin (1 g l−1) and 200 mg g−1 vancomycin (Sigma-Aldrich) were 0.22 μm filtered and added to autoclaved drinking water starting 2 days before LPS treatment (day −2) and lasting for the duration of the experiment.

Lectin staining. For whole-mount staining, small intestine was removed, a 1 cm piece from the upper third was excised, opened, cleaned of mucus in cold PBS, and incubated with Ulex europeaeus agglutinin-1 (UEA-1) conjugated to FITC, TRITC or atto-594 (Vector Laboratories or Sigma-Aldrich) to label nuclei. Glutaraldehyde/0.1 M cacodylate buffer, transferred to cacodylate buffer overnight at room temperature. Cells were then stained with UEA-1–FITC (1 μg ml−1, Vector), and gated on the FSC/SSC high epithelial cell population.

Light microscopy. Fluorescence microscopy of whole-mounts and sections used a Leica DM LB microscope (Leica Camera AG) and Spot RT Slider camera and software (Diagnostic Instruments). Confocal microscopy of bacteria was performed with an Olympus DSU spinning dish microscope and Slidebook software (3i). All images in an experiment were taken using the same exposure settings.

Scanning electron microscopy. Tissues were fixed in 2% paraformaldehyde/2% glutaraldehyde/0.1 M caducate buffer, transferred to caducate buffer overnight and processed with the OTOTO procedure27. To dehydrate, samples were passed through increasing concentrations of acetone in water, followed by hexamethyldisilazane (Electron Microscopy Sciences). Samples were mounted with colloidal silver paste ( Electron Microscopy Sciences) and imaged with an FEI Nova NanoSEM 230 (FEI) at 5 kV.

Identification of fucosylated proteins. To visualize fucosylated proteins in the lumen contents, the intestinal contents were gently removed by squeezing and homogenized at 1 g sample per 5 ml of Tris-Triton-X-100 buffer (150 mM NaCl, 50 mM Tris pH 8, 1% Triton X-100, and protease inhibitor tablet (Roche)) on ice and spun at 17,000g for 20 min at 4 °C. Supernatant proteins were separated by 6% non-reducing SDS–PAGE, transferred to PVDF membrane (Bio-Rad), blocked with 0.5% gelatin in 0.05% Tween-20/ PBS, and visualized with UEA-1–HRP (1 μg ml−1, Sigma-Aldrich).

For sequencing of fucosylated epithelial cells, small intestinal epithelial cells were isolated as described earlier and lysed in Tris-Triton-X-100 buffer. Lysates were pre-cleared by incubating with unconjugated agarose beads (Vector) twice for 45 min at 4 °C. Cleared lysates were then incubated with washed UEA-1-conjugated beads (Vector) for 45 min. Beads were washed five times to remove unbound protein. To elute UEA-1-bound protein, beads were incubated with 200 mM M-fucose (Sigma–Aldrich) for 30 min. Eluted proteins were separated on a 4–15% gradient SDS–PAGE gel (Bio-Rad), silver-stained (Thermo Fisher Scientific), and bands were excised for identification by mass spectrometry at the Taplin Biological Mass Spectrometry Facility, Harvard Medical School.

Isolation of fucosylated-positive bacteria. Brain heart infusion (BHI) agar plates (Becton, Dickinson, and Co.) with added thiglycolic acid, menadione and hemin/histidine (Sigma–Aldrich) were incubated in anaerobic chamber with 2.5% hydrogen atmosphere at 37 °C, and spread with 40 μl of 5-bromo-4-chloro-3-indolyl-β-D-fucopyranoside (50 mM in DMF) (Carbosynth). Faecal pellets were homogenized in reduced 0.01% thiglycolic acid/PBS, plated, and grown for 3 days at 37 °C aerobically, then at 4 °C aerobically to develop colour. Bacteria were identified by sequencing of their 16s rDNA genes using primers 8F (5′-AGAGTTTGTATCCTGCGCC-3′) and 191R (5′-GACGGGCGGTG-WGATCRA-3′), and sequencing of their gyrB genes using primers gyrF B (5′-GAATCATCATGACGCGGTTGC-3′) and gyrB R (5′-ACAGGTTACGTCGATGGAGTGC-3′) for sequencing28. B. thetaiotaomicron VPI-5482 and B. uniformis (a gift from C. Nagler) were used as controls.

Measurement and inhibition of fucosidase activity. Faecal pellets were weighed and homogenized in 10 μl PBS per 1 mg sample, and centrifuged at 17,000g for 10 min. Fifty microfilters of this supernatant was incubated for 1 h at 37 °C with or without adding 0.5 μl of 50 mM 4-methylumbelliferyl β-D-fucopyranoside (in dimethyl-sulphoxide (DMSO), Sigma or Gold Biotechnology). The reaction was diluted 100-fold in 0.2 M glycine-NaOH buffer, pH 10.5, and fluorescence was measured at 365 nm excitation, 445 nm emission. Fluorescence of the no-substrate control was subtracted from the substrate-containing reaction. The amount of cleaved substrate and fucosidase activity was then calculated by comparison to a standard curve of 4-methylumbellifere (Sigma in glycine-NaOH buffer. Deoxyfuconojirimycin (DF); Enzo Life Sciences) was dissolved in PBS and gavaged to mice in 100 μl (5 μmol total).

Reporter E. coli. The pXDC94 plasmid29, containing the ptc promoter controlling mCherry expression and a multiple cloning site upstream of the promoter-less GFP gene, was a gift from H. Shuman. The promoter region upstream of the E. coli fucP genes was amplified using primers that added restriction sites (bold) to the 5′ and 3′ ends of the ampiclon, respectively: fucP-F 5′-TATGTTACCGGATCCTTTCGAATAAAATAAAA-3′; fucP-R 5′-TATGCCGGGGTACGTCCTTCGTCA-3′; and gyrB F 5′-ATGGATCGTCTAGGAGTACGGATGTGCGAGCC-3′; and gyrB R 5′-GGTACTCGAGCTGACTGAACTGTTCGGAAGGGCAGCG-3′ for sequencing36. B. thetaiotaomicron VPI-5482 and B. uniformis (a gift from C. Nagler) were used as controls.

Estimation of total bacterial loads. Fresh faecal pellets were placed in an anaerobic chamber and washed in 500 μl of reduced PBS containing 0.01% thiglycolic acid. Serial dilutions were made in reduced PBS and plated on pre-reduced Brucella blood agar plates (Becton, Dickinson, and Co.; for anaerobic counts, removed from the anaerobic chamber, and plated on tryptic soy agar (TSA)/5% sheep’s blood plates (Becton, Dickinson, and Co.; aerobic counts). Total colonies were counted after 2 days (aerobic) or 3 days (anaerobic) incubation at 37 °C.

For 16S copy number, faecal pellets were weighed, DNA was isolated by a bead beating and phenol/chloroform extraction method30, and qPCR performed and copy number determined as described47.

S. enterica Typhimurium and C. rodentium infection. For S. enterica Typhimurium and C. rodentium infection, mice were gavaged with 20 mg streptomycin (Sigma) in 100 μl sterile}

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water, 24 h before infection. *S. enterica* Typhimurium strain SL1344 was grown overnight in LB with streptomycin (50 μg ml⁻¹) and gavaged to mice at 5 × 10^5 c.f.u. in 100 μl volume.

*C. rodentium* strains DBS100 or DBS120 pler-lux were grown in LB overnight at 37 °C, then diluted 1:100 and grown for 2.5 h, centrifuged and resuspended in 0.01 volumes PBS, and mice were gavaged with 5 × 10^6 bacteria in 100 μl LB. To determine mouse colonization levels, a fresh faecal pellet or small intestines content (gently squeezed to remove, except last 3 cm of ileum) was weighed, washed in 500 μl of PBS, serially diluted, and plated on MacConkey (Becton, Dickinson, and Co.) or LB agar with 50 μg ml⁻¹ kanamycin. For luciferase measurements, the faecal homogenate was adjusted to 10 mg in 1 ml PBS in an Eppendorf tube and light measured in a Triathler scintillation counter (Hidex), before plating dilutions in PBS on agar with kanamycin. Colon-adherent bacteria were measured in a 1 cm piece from the middle of the colon. The piece was opened longitudinally, washed in 1 mM DTT/PBS by vortexing for 10 s, then washed in PBS, and placed in an Eppendorf in 500 μl PBS for light measurement as before. The piece was then washed and diluted and plated on agar with kanamycin.

**Histology.** Distal colon from *C. rodentium*-infected mice or uninfected controls was fixed in neutral formalin, then kept in 70% EtOH until being embedded in paraffin, and 5 μm sections were cut and stained with haematoxylin and eosin. Well-orientedcrypts were photographed and their lengths measured in Imagel, and the mean taken for each mouse.

**Serum cytokine ELISA.** Serum concentrations were measured by ELISA according to the manufacturer’s instructions (IL-1β: eBioscience; IL-6 and TNF-α: Becton, Dickinson and Co.).

**Short-chain fatty acid measurements.** For gavage experiments, food was removed and SPF mice were gavaged at 0, 3 and 6 h with 300 μl of 0.1 ML-fucose or 0.1 M D-galactose (Sigma-Aldrich) in autoclaved tap water, or water only. At 8 h, mice were killed and caecal contents removed and kept at −80 °C until processing.

Concentrations of the short-chain fatty acids in caecal contents were measured using the direct injection gas chromatography–mass spectrometry (GC–MS) method adapted from that described previously³⁴–⁴⁵. Caecal contents were extracted in two steps. Deionized water was added to the samples at the amount of 5 ml per 1 g of sample, followed by brief vortex-mixing and sonication for 15 min. In the second round of brief vortex-mixing and sonication for 15 min.

Extracted samples were centrifuged for 5 min at 17,000 g and 0.05 mM of 2-ethylbutyric acid (#109959, Aldrich). The mixtures were briefly vortex-mixed and centrifuged for 5 min at 17,000g and 1 μl of the obtained supernatants were analysed by direct injection GC–MS on an Agilent 6890N GC system equipped with a Mass Selective (MS) Detector 9793 (Agilent Technologies).

The instrument was used in a splitless mode with an online 1 μl split injector liner (#23308, Sky by Restek) and fused-silica column with polyethylene glycol stationary phase (INNOWax #19019FN-133, J&W Scientific, Agilent Technologies) 30 × 0.25 mm ID coated with 0.25 μm film. Flavier-Lifland carrier gas was supplied at 1.00 ml min⁻¹ flow rate. The injection port temperature was 260 °C. The initial oven temperature of 60 °C was maintained for 2 min, then increased to 150 °C at 10 °C min⁻¹ and further to 250 °C at 25 °C min⁻¹. The oven temperature at 4 min, bringing the total duration of the run to 19 min. The MS detector temperature was set at 245 °C.

**GC–MS data analysis were performed using the MSD ChemStation D.01.02.16.2 software (Agilent Technologies). Tetradeuteroacetic and 2-ethylbutyric acids served as internal standards.**

**Enzymatic activity of digestive enzymes.** Epithelial cells were dissociated from the upper half of the small intestine as described earlier. Haemapoietic cells were removed using anti-GD45-biotin (clone 30-F11, Biologend) and streptavidin magnetic beads with a MACS LS column (Milteny Biotec). Epithelial cells were diluted in 96-well plates in triplicate in appropriate substrate solutions (for sucrase, 60 mM sucrose in PBS; for maltase, 30 mM maltose in PBS; for aminopeptidase, 5 mM L-Leu-pNA (Enzo Life Sciences) in 50 mM TRIS, pH 7.4). Cells were incubated for 30– 60 min at 37 °C for aminopeptidase assay, absorbance was measured at 405 nm. For the sucrase and maltase assays, plates were centrifuged and 5 μl of supernatant was used for a colorimetric assay to measure liberated glucose (Cayman Chemical).

**Metagenomic library preparation and sequencing.** Methods for microbial community DNA and mRNA sequencing were as previously described³⁶–³⁹. Faecal pellets collected from mice before or after LPS treatment (pooled from 2 and 3 days post-injection) were kept at −80 °C until DNA and RNA isolation using the guanidinium thiocyanate/cæsium chloride gradient method⁴⁰ as described, except that crude particles were removed by centrifugation before overlaying the gradient. RNA was subjected to DNase treatment (Ambion), purification using MEGAClear columns (Ambion), and rRNA depletion via subtractive hybridization (MICROExpress, Ambion, in addition to custom depletion oligonucleotides). The presence of genomic DNA contamination was assessed by PCR with universal 16S rDNA primers. cDNA was synthesized using SuperScript II and random hexamers (Invitrogen), followed by second-strand synthesis with RNaseH and E.coli DNA polymerase (New England Biolabs). Samples were prepared for sequencing with an Illumina HiSeq instrument after enzymatic fragmentation (NEBNext40L/M0348S). Libraries were quantified by qPCR according to the Illumina protocol. qPCR assays were run using AbsoluteTDF QPCR SYBR Green ROX Mix (Thermo Scientific) on an MX3000P QPCR System instrument (Stratagene). The size distribution of each library was quantified on an Agilent HS-DNA chip (Agilent).

**16S rRNA gene sequencing and analysis.** Community DNA was PCR-amplified using universal bacterial primers targeting variable region 4 of the 16S rRNA gene with the following thermocycler protocol: denaturation at 94 °C for 3 min, 35 cycles of 94 °C for 45 s, 50 °C for 30 s, and 72 °C for 90 s, with a final extension at 72 °C for 10 min⁴⁻⁻⁵⁰. Triplicate reactions for each sample were pooled and amplified. Amplification was confirmed by 1.5% gel electrophoresis. 16S rRNA gene amplicons were cleaned with the Ampure XP kit (Agencourt) and quantified using the Quant-iT PicoGreen ds DNA Assay Kit (Invitrogen). Barcoded amplicons from different samples were pooled and sequenced using the Illumina HiSeq platform⁵¹. 16S rRNA gene sequences were analysed using the QIIME (Quantitative Insights Into Microbial Ecology)⁴² software package along with custom Perl scripts. Data sets were randomly subsampled before clustering analyses at a depth that retained all of the individual samples (180,000 sequences per sample). All sequences were used for the comparison of the relative abundance of bacterial taxonomic groups. OTUs were picked at 97% similarity against the Greengenes database⁴³ (constructed by the nested._gg_workshop.py QimeUtils script on 4 February 2011), which we trimmed to span only the 16S rRNA region flanked by our sequencing primers (positions 521–773). The LeSe package was used to identify taxonomic groups significantly associated with each treatment⁴⁴. LeSe was run on the sub-sampled data sets, after filtering out species-level OTUs with <100 sequences or present in only 1 sample. Statistical analyses were also performed using the QIIME scripts ‘otu_category_significance’ (ANOVA) and ‘compare_categories.py’ (PERMANOVA).

**Reference genome database.** A custom database was constructed from draft and finished reference genomes obtained from human-associated microbial isolates (538 genomes from the Human Microbiome Project Data Analysis and Coordination Center (http://www.hmpdacc.org)), in addition to the *Eggerthella lenta* DSM2243 reference genome. All predicted proteins from the reference genome database were annotated with KEGG orthologous groups (KO's) using the KEGG database (version 52; BLASTX e-value < 10⁻⁵, bit score > 50, and >50% identity)⁴⁵. For query genes with multiple matches, the annotated reference gene with the lowest e-value was used. When multiple annotated genes with an identical e-value were encountered after a BLAST query, we included all KO's assigned to those genes. Genes from the database with significant homology (BLASTN e-value < 10⁻²⁰) to non-coding transcripts from the 539 microbial genomes were excluded from subsequent analysis.

**Metagenomic sequence analysis.** DNA- and RNA-seq analysis was performed with our recently described pipeline⁴⁶. Briefly, high-quality reads (see Supplementary Table 1 for sequencing statistics) were mapped using SSAHA²² to our custom 539- genome *C. rodentium* genome and the Illumina 16S rRNA amplicon sequence data set. We then ran LeSe on the resulting KEGG module abundance file using the ‘-o 1000000’ flag. We used the edger package⁴⁷ to identify orthologous groups with significantly altered abundance or expression. Prior to analysis, we calculated the cumulative number of sequencing reads assigned to each sample for each KO (without RPMK normalization). We then used a paired t gm analysis to determine consistent changes within each animal following LPS treatment. Default parameters were used, with one exception: for the estimateGLMTrendedDisp step ‘min.n’ was set to 50/300 for within each animal following LPS treatment. Default parameters were used, with one exception: for the estimateGLMTrendedDisp step ‘min.n’ was set to 50/300 for each animal following LPS treatment.
GGC-3’, fut2R, 5’-TAACACCGGGAGACTGATCC-3’; sec1F, 5’-ATCCAAGCAGTGCTCCAGC-3’; sec1R, 5’-CAATATTGCCCATCTGGTTC-3’; villinF, 5’-GCTTGGCACAACCTTCTCCAG-3’, villinR, 5’-CTTGCTTGAAAGATAGCTCCGG-3’.  
Quantitative RT–PCR was performed on an Applied Biosystems StepOnePlus instrument with Universal Sybr Green Universal Supermix (Bio-Rad), and the following primers: E. coli fucA F, 5’-GGCGCGCAAGGAATAGAA-3’, E. coli fucA R, 5’-GATCCCCGCTATTCAATCAATGGA-3’, E. coli fucP F, 5’-CCAAATACGGTTCGGCGGTCGTGGT-3’, E. coli fucP R, 5’-ACCCATGACCGAGGTGACAA-3’, E. coli rpoA F, 5’-GGCGCTGTCTCTTCCGGAAT-3’, E. coli rpoA R, 5’-CCCGGTGCTGGTGTAATGCC-3’.  
Statistics. Statistical analyses were performed with GraphPad Prism 5 software (GraphPad Software).

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Extended Data Figure 1 | Requirements and kinetics for small intestine fucosylation induced by systemic injection of TLR ligands. a, Systemic injection of bacterial TLR ligands induces small intestine fucosylation, but simple starvation does not. UEA-1 staining (as in Fig. 1) after i.p. injection of CpG DNA, or Pam3CSK4, or food deprivation for 24 h of BALB/c SPF mouse. b, LPS injection causes small intestine fucosylation in various inbred mouse strains. SPF mice of the indicated strains were injected with LPS i.p. and the small intestine was stained with UEA-1 after 24 h, as in Fig. 1. c, Fucosylation peaks at 8 h after LPS injection and is still detectable at 96 h. d, M cells can be readily detected by scanning electron microscope and UEA-1 staining of the domes of the Peyer’s patches, but are rare in the villi and are not massively induced in the villi by LPS injection. UEA-1 staining and scanning electron microscopy were performed on adjacent pieces from the proximal one-third of the small intestine. Scale bars = 100 µm for UEA-1 staining, 50 µm for scanning electron microscope images. e, Small intestine fucosylation does not require the presence of endogenous microbiota (LPS injection in GF mouse) and is not induced by oral administration of LPS (1 mg). All data are representative of at least two independent experiments.
Extended Data Figure 2 | MyD88-dependent pathway for fucosylation of small intestine IECs in response to systemic stimulation of TLRs. a, FACS analysis of IECs from three segments of small intestine from the indicated mice. Cells are gated on the FSC/SSC high epithelial cell population. At least two mice per mutant genotype were stained along with two control mice in the experiments shown. b, SPF mice were pre-treated with 20 mg streptomycin and orally infected with S. enterica Typhimurium. The small intestine was stained at 24 h post-infection. MyD88 expression was necessary in CD11c+ cells but not villin+ IECs for S. enterica Typhimurium-induced fucosylation. Data are representative of at least two independent experiments.
A proposed model for the mechanisms linking inducible fucosylation to the gut microbiota. Systemic microbial agonists activate TLRs on CD11c⁺ dendritic cells (DCs), causing secretion of the cytokine IL-23, which in turn stimulates RORγt-dependent ILCs to secrete IL-22. IL-22 causes small intestine epithelial cells to upregulate Fut2. Fucosylated proteins are either secreted into the lumen or expressed on the cell surface and later shed into the lumen. Fucosidase-expressing bacteria (blue) liberate fucose residues, which they can utilize and share with other bacteria lacking the fucose-cleaving enzyme. Bacterial metabolism of fucose potentially produces metabolites such as short-chain fatty acids (SCFAs). Fucose also directly or indirectly downregulates virulence gene expression by pathobionts (red) or bona fide pathogens.\(^2\)
Extended Data Figure 4 | Consequences of LPS injection in Fut2-sufficient and Fut2-deficient BALB/c mice. a, Inflammatory cytokines IL-1β, IL-6 and TNF-α were measured by ELISA in sera of mice before or 2 h after injection with LPS (4 h for IL-1β), abx, mice on antibiotic water for 2 days before injection. Bars are mean ± s.e.m.; ND, not detected. Data are combined from three experiments.

b, Expression of RegIIIγ (also regulated by the MyD88–IL23–IL22 pathway). Measurement by qPCR of reg3g gene expression in mid-small-intestine tissue, relative to gapdh (ddCt method). Numbers indicate mean fold change ± s.e.m. in LPS-treated versus untreated mice. Differences between LPS-treated Fut2+/− and abx or Fut2−/− levels are not significant (P > 0.05, two-tailed Student’s t-test). Data are combined from three experiments.

c, Weight loss and recovery is not different in Fut2+/− and Fut2−/− mice after simple starvation (mean ± s.e.m., P > 0.05 at all time points, two-tailed Student’s t-test; NS, not significant).

d, Lack of direct toxic effect of antibiotics (abx) measured as the weight loss of BALB/c GF animals treated with LPS i.p (mean ± s.e.m., P > 0.05 by two-tailed Student’s t-test at all time points). Data are combined from two experiments.

e–g, Similar total bacterial loads in Fut2+/− and Fut2−/− mice before and after LPS injection and antibiotic treatment. Total bacterial loads in faeces were estimated by plating on aerobic (e) and anaerobic (f) non-selective media, and by qPCR for 16S gene copies (g). There were no significant differences between Fut2-sufficient (filled circles) and Fut2-deficient (open circles) mice before or after LPS treatment (two-tailed Student’s t-test). Circles indicate individual mice; horizontal lines indicate means; red circles indicate antibiotic-treated mice. Data are combined from three experiments.
Extended Data Figure 5 | Fucosylated protein in IECs and gut contents.
a, Proteins α(1,2)fucosylated in IECs after LPS injection identified by UEA-1 precipitation and mass spectrometry. Abundance is the number of peptide fragments attributed to each gene. b, IECs from Fut2−/− untreated, Fut2−/+ LPS-treated, or Fut2−/+ untreated mice were isolated, and lysates separated by SDS-PAGE. α(1,2)fucosylated proteins were detected by blotting with UEA-1 lectin conjugated to horseradish peroxidase (HRP). c, Identical gel stained with Coomassie blue for total protein content. d, Relative density of the boxed area of each lane from b divided by the relative density in c. e, UEA-1 staining of luminal proteins as in Fig. 3c. Blot is overexposed to show absence of luminal fucosylated proteins in the LPS-treated, Fut2−/− mouse. b–e, Data are representative of two independent experiments.
Extended Data Figure 6 | Generation of fucose-sensing reporter bacterial strains. a, Reporter E. coli were grown to stationary phase in minimal medium containing 10 mM glucose and the indicated concentrations of L-fucose (asterisk indicates promoter-less vector), and GFP fluorescence was measured. b, Fucosidase activity is dramatically reduced after 2 days of antibiotics (abx) treatment but recovers after cessation of treatment. Measurement of total α-L-fucosidase activity in faeces. Faecal supernatant was assayed for cleavage of 4-methylumbelliferyl-fucopyranoside substrate by fluorescence. n = 5 SPF antibiotics-treated, 3 GF mice. c, Faecal homogenates were plated anaerobically on BHIS agar containing 5-bromo-4-chloro-3-indolyl α-L-fucopyranoside, which forms a blue precipitate upon cleavage of the fucosyl residue. Both blue and white colonies are present. d, Pure cultures of Bacteroides species were streaked on the same medium as in c. B. uniformis (left) is not predicted to carry an α-L-fucosidase gene, and remains white; B. acidifaciens (middle) and B. thetaiotaomicron (right) both express fucosidase activity and develop blue colonies. e, Loss of B. acidifaciens from the faeces of mice treated with antibiotics (Abx) in water (PCR for the gyrB gene). C−, water control; C+, B. acidifaciens genomic DNA. f, Summary of reporter E. coli experiments in SPF mice (representative experiment is shown in Fig. 3e). Points are mean GFP fluorescence from all reporter bacteria measured in each of three independent experiments (n = 65 bacteria per mouse; *P < 0.05, Student’s t test).
Extended Data Figure 7 | Microbial community structure is impacted by cohousing yet robust to host fucosylation and LPS exposure, whereas microbial gene expression depends on Fut2. **a**, Stable relative abundance of bacterial phyla across treatment groups and genotypes, as indicated by 16S rRNA gene sequencing. Values represent the mean abundance of phyla found at >1% relative abundance in at least one sample. **b**, Unweighted UniFrac analysis of the gut microbiota of Fut2-deficient (no outline) and Fut2-sufficient (black outline) mice. Points are coloured based on kinship and labelled by time point (before or after LPS exposure). Results are based on 180,000 randomly selected 16S rRNA gene sequences per sample. **c**, Microbial diversity as measured by the Shannon diversity index (n = 178,100 sequences per sample). Values are mean ± s.e.m. (n = 3 Fut2+, 4 Fut2− mice per time point). **d**, KEGG modules and pathways expressed in microbiota at higher levels after LPS exposure in Fut2-positive (left) and Fut2-negative mice (right) (n = 3 per group; Humann/LefSe analysis; LDA >2).
Extended Data Figure 8 | Lack of indicible fucosylation and small intestine colonization in *C. rodentium*-infected mice. a, *C. rodentium* causes no small intestine fucosylation in SPF mice at day (d)3, day 7, or day 12 post-infection (p.i.). b, Small intestine colonization by *C. rodentium* is low regardless of Fut2 expression and LPS treatment. Small intestine contents were removed by gentle squeezing, homogenized in PBS, and plated. Data are shown as mean ± s.e.m.; n = 4. NS, not significant. Dotted line shows the limit of detection. Data are representative of two experiments.
**Extended Data Figure 9 | Effect of exogenous fucose on caecal short-chain fatty acid levels.** Caecal short-chain fatty acids were measured after gavaging starved mice with the indicated sugars (100 mM concentration). Fucose gavage leads to increased propionate production in SPF but not GF mice. Data are shown as mean ± s.e.m. **P < 0.01, Student’s two-tailed t-test. ND, not detected. Data are combined from three experiments.**