Selective depletion of uropathogenic \textit{E. coli} from the gut by a FimH antagonist

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Urinary tract infections (UTIs) caused by uropathogenic \textit{Escherichia coli} (UPEC) affect 150 million people annually\textsuperscript{1,2}. Despite effective antibiotic therapy, 30–50\% of patients experience recurrent UTIs\textsuperscript{1}. In addition, the growing prevalence of UPEC that are resistant to last-line antibiotic treatments, and more recently to carabapenems and colistin, make UTI a prime example of the antibiotic-resistance crisis and emphasize the need for new approaches to treat and prevent bacterial infections\textsuperscript{3–5}. UPEC strains establish reservoirs in the gut from which they are shed in the faeces, and can colonize the periurethral area or vagina and subsequently ascend through the urethra to the urinary tract, where they cause UTIs\textsuperscript{6}. UPEC isolates encode up to 16 distinct chaperone-usher pathway pili, and each pilus type may enable colonization of a habitat in the host or environment\textsuperscript{2}. For example, the type 1 pilus adhesin FimH binds mannose on the bladder surface, and mediates colonization of the bladder. However, little is known about the mechanisms underlying UPEC persistence in the gut\textsuperscript{7}. Here, using a mouse model, we show that F17-like and type 1 pili promote intestinal colonization and show distinct binding to epithelial cells distributed along colonic crypts. Phylogenomic and structural analyses reveal that F17-like pili are closely related to pilus types carried by intestinal pathogens, but are restricted to extra-intestinal pathogenic \textit{E. coli}. Moreover, we show that targeting FimH with M4284, a high-affinity inhibitory mannose analogue (mannoside), reduces intestinal colonization of genetically diverse UPEC isolates, while simultaneously treating UTI, without notably disrupting the structural configuration of the gut microbiota. By selectively depleting intestinal UPEC reservoirs, mannoses could markedly reduce the rate of UTIs and recurrent UTIs.

The genome of UTI89, a human cystitis isolate, contains nine distinct functional chaperone-usher pathway (CUP) pili. To determine whether any of these CUP pili promote intestinal colonization, we used a streptomycin mouse model of UPEC intestinal colonization\textsuperscript{8} to co-colonize C3H/HeN mice with wild-type UTI89 and one of nine mutant strains, each lacking a single CUP operon (Extended Data Fig. 1). Deletion of each of the seven operons yfc, yeh, yad, pap, sfa, yqi and mat had no effect on UTI89 intestinal fitness compared to the isogenic wild-type strain (Fig. 1a–g). However, deletion of the \textit{fim} or \textit{ucf} pilus operons, which encode type 1 or F17-like pili, respectively, produced significant defects in colonization (up to 100- and 1,000-fold, respectively; Fig. 1h, i). Loss of FimH, the type 1 pilus adhesin, mirrored the defect caused by deletion of the full type 1 pilus operon (Extended Data Fig. 2a). Deletion of both pilus types in a single strain produced a fitness defect greater than either individual deletion alone, suggesting that these two pilus types do not have redundant roles (Fig. 1j, k).

In a mouse model, type 1 pilus-mediated binding to mannosylated receptors is indispensable for bladder colonization and invasion of urothelial cells lining the bladder lumen\textsuperscript{2,5}. Once inside urothelial cells, a single bacterium rapidly divides, forming an intracellular bacterial community (IBC)\textsuperscript{2,5}. Furthermore, UPEC can access underlying transitional cells, forming quiescent intracellular reservoirs\textsuperscript{1–3}. Mutations in \textit{fimH} abolish the ability of UPEC to colonize the bladder, form IBCs and quiescent intracellular reservoirs\textsuperscript{2,5,9}. By contrast, no role was observed for F17-like pili in the rate or severity of bladder infection after individual or concurrent transurethral inoculations of UTI89 and UTI89\textit{Δucf} strains into the bladders of female C3H/HeN mice (Extended Data Fig. 3). Differences between mouse and human bladders or the overexpression of F17-like pili \textit{in vitro} may account for the inconsistency with another study that showed a role for F17-like pili in binding to desquamated epithelial cells obtained from human urine\textsuperscript{10}.

The \textit{fim} and \textit{ucf} operons encode two-domain tip adhesin proteins, FimH and UcID, respectively. The adhesin lectin domain contains the ligand-binding site, and the pilin domain joins the adhesin to the pilus rod\textsuperscript{7}. Purified FimH lectin domain (FimH\textit{LD}) bound to more differenti-ated epithelial cells located in the upper portion of crypts and in ‘surface epithelial cuffs’ (the colonic homologues of small intestinal villi) (Fig. 1l). FimH binding was prevented by pretreating tissue sections with peptide-N-glycosidase F (PNGase F), which cleaves \textit{N}-linked oligosaccharides. FimH\textit{LD} also bound to Caco-2 cells (an immortalized human enterocyte-like cell line derived from colorectal carcinoma); binding was inhibited by d-mannose and a high-affinity mannose analogue (mannoside), M4284 (Extended Data Fig. 2b)\textsuperscript{11}. The UcID lectin domain (UcID\textit{LD}) also bound colonic epithelial cells in tissue sections; binding was inhibited by pretreating tissue sections with O-glycosidase, an enzyme that cleaves O-linked oligosaccharides, suggesting that the UcID ligand is contained within an O-glycan (Fig. 1m).

CUP pili are highly conserved throughout Proteobacteria and are assembled by dedicated chaperone-usher assembly machines encoded by each respective CUP operon along with the various subunit types comprising the pilus fibre\textsuperscript{5,6}. The sequence identity between usher genes of distinct CUP pilus types is greater than the identity of genes that encode other CUP pilus proteins and thus can be compared to determine evolutionary relationships of CUP pili among Proteobacteria\textsuperscript{7,12}. A homology search of a database of \gamma-Proteobacteria genomes revealed that the UTI89 F17-like usher gene sequence (\textit{ucfL}) shared highest identity with other \textit{E. coli ucfL} sequences and with orthologous usher sequences of \textit{Proteus mirabilis}, a bacterium that can colonize the gut, and...
Type 1 and F17-like pili promote UPEC intestinal colonization. a–k, C3H/HeN mice pretreated with streptomycin were concurrently (a–j) or singly (k) colonized with wild-type UTI89 and/or UTI89 lacking one or more CUP operons (ycf (a), yeh (b), yad (c), pap (d), sfa (e), yqi (f), mat (g), fim (h), ucl (i) and both fim and ucl (j)). 1, m, Purified adhesin lectin domains FimHLD (type 1 pili) and UclDLD (F17-like pili) were tested for binding to mouse colonic sections. Sections were stained with Hoechst (blue) and antibodies to the mucus-associated glycoprotein Muc2 (green). Binding of FimHLD and UclDLD was lost by pretreating tissue sections (blue) and antibodies to the mucus-associated glycoprotein Muc2 (green). b, Structural analysis of UclDLD. a, Left, superposition of the P21, UclDLD (green) and F17GLD (cyan) structures. b, Comparison of residue positioning and electrostatic surface potential of the putative binding site between the UclDLD structures and the known binding site of F17GLD c, Structural alignment of UclDLD and F17GLD amino acid sequences. Residues in the putative UclDLD binding site are highlighted in purple. Insertions in the UclDLD structure are highlighted in orange and yellow. Starred residues are proposed to mediate UclD ligand binding. β-strands (red arrows), 3₁₀ helices (coils) and an α-helix (cylinder) are shown.

Figure 2 | Structural analysis of UclDLD. a, Left, superposition of the P21, UclDLD (green) and P21, UclDLD (grey) crystal structures. Middle, F17GLD adhesin crystal structure (PDB accession 1OIO; ref. 16). Right, superposition of P21, UclDLD (green) and F17GLD (cyan) structures. b, Comparison of residue positioning and electrostatic surface potential of the putative binding site between the UclDLD structures and the known binding site of F17GLD c, Structural alignment of UclDLD and F17GLD amino acid sequences. Residues in the putative UclDLD binding site are highlighted in purple. Insertions in the UclDLD structure are highlighted in orange and yellow. Starred residues are proposed to mediate UclD ligand binding. β-strands (red arrows), 3₁₀ helices (coils) and an α-helix (cylinder) are shown.

Salmonella enterica, an intestinal pathogen (Supplementary Table 1). The uclC usher gene was also closely related to usher genes in F17 (thus the derivation of the name, F17-like), pVir99 and ECs1278 pili. A phylogenetic analysis showed clustering of these E. coli and Proteus species ushers into a distinct sub-branch within the broader F17 group usher phylogeny, suggesting that they share a common ancestor (Extended Data Fig. 4). F17-like pili are present in only 10% of E. coli strains; these strains are almost exclusively in the B2 clade, which contains most extraintestinal pathogenic E. coli (ExPEC) and UPEC.
D-mannose intestinal segments. UTI89 was introduced into the gut of C3H/HeN adhesin F17G, it is almost invariant across all strains encoding it control), or d-mannose (100 mg kg⁻¹), suggesting that there is a single, distinct ligand for UTI events were caused by B2 strains(17,18) (Supplementary Table 2). Of these 14 strains, 13 encoded F17-like pili (approximately 93%) (Extended Data Fig. 5). By contrast, F17-like pili have been found in less than 50% of all B2 strains(10) (E. coli reference collection; ECOR), suggesting that F17-like pili might be associated with UPEC persistence in women with recurrent UTI owing to their ability to promote maintenance of a UPEC intestinal reservoir.

To further characterize F17-like pili, we solved two X-ray crystal structures of UclD(13). The structures in the P2₁ (green) and P2₁;2₁;2₁ (grey) space groups were resolved to 1.05 Å and 1.60 Å resolution, respectively, and are nearly identical (Fig. 2a (left); Supplementary Table 3). Despite low primary sequence identity (~25%), the structural characteristics of UclD(13) and the F17 adhesin F17G(13) are conserved(16) (Fig. 2a–c). This includes the presence of a transverse putative binding site in UclD(13) located at a similar position to the GlcNAc-binding site on F17G (Fig. 2a (right), b, c)(16). Structural and sequence alignments

Figure 3 | Mannoside simultaneously reduces the UPEC intestinal reservoir and treats UTI. a, M4284 concentration in mouse faeces after one dose (100 mg kg⁻¹ administered by oral gavage; PO, per os). EC₉₀, 90% effective concentration to inhibit haemagglutination in vitro. b, C3H/HeN mice were intestinally colonized with UTI89 and given three oral doses of M4284 (100 mg kg⁻¹), vehicle alone (10% cyclodextrin, control), or d-mannose (100 mg kg⁻¹). c, d, UTI89 levels in the faeces and intestinal segments. e, UTI89 was introduced into the gut of C3H/HeN mice by oral gavage and into the bladder by transurethral inoculation before receiving three doses of M4284. Cm, chloramphenicol; Kn, kanamycin. f, g, UTI89 levels in the gut and urinary tract were assessed. Error bars represent median (a), geometric mean ± s.d. (c, d, f); and geometric mean (g). *P < 0.05, **P < 0.01 by Mann–Whitney U test. n = 3 mice, 1 replicate (a); n = 14 mice (control); n = 15 mice (M4284); 3 replicates (c); n = 10 mice, 2 replicates (d); n = 9 mice, 2 replicates (f, g). All replicates are biological (a–g).

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reveal two large conserved insertions in UclD relative to F17G (Fig. 2c), whose direct proximity to the putative binding pocket suggests that they are involved in UclD receptor binding. The six conserved residues that make up the candidate binding pocket in UclD are chemically distinct from their F17G counterparts (Fig. 2b, c), providing further evidence that UclD binds a distinct ligand.

In light of the role of FimH and UclD in gut colonization, we conducted a study designed to reduce the UPEC intestinal reservoir with an adhesin-directed therapeutic. M4284 is a high-affinity biphenyl adhesin-directed therapeutic. M4284 (100 mg kg\(^{-1}\)), (ii) cyclodextrin (cyclo; 10%), (iii) ciprofloxacin (cipro; 15 mg kg\(^{-1}\)), or (iv) none (untreated). Faecal community structure was defined by sequencing bacterial 16S rRNA gene amplicons. a. For each treatment performed on C3H/HeN mice from Envigo or Charles River Laboratories (CRL), the change in microbiota configuration was determined by measuring the unweighted UniFrac distance between samples obtained from each animal before treatment and 24 h after the last dose (larger UniFrac distance equates to a larger shift in community structure). b–f. Mice were colonized by oral gavage (PO) of one of four different UPEC strains and given three doses of M4284. g, h. The ability of M4284 to target UTI89 in C3H/HeN mice from CRL (g) and C57BL/6 mice from Envigo (h) was also assessed. Error bars represent median (a) or geometric mean ± s.d. (c–h). \(*P < 0.05, **P < 0.01, ***P < 0.001\) by Mann–Whitney U test. n = 5 mice per vendor, 1 replicate (a); n = 10 mice, 2 replicates (c–e, g); n = 14 (control, 10% cyclodextrin); n = 15 (M4284); 3 replicates (f). n = 10 mice (control, 10% cyclodextrin); n = 9 mice (M4284); 2 replicates (h). All replicates are biological (a–h).

The infectious dose required to cause cystitis in 50% of mice (ID\(_{50}\)) in the UTI mouse model is \(10^5\) colony-forming units (CFU)\(^{21}\). Furthermore, decreasing the dose of UPEC introduced into the bladder from \(10^9\) to \(10^6\) CFU significantly reduced the rate of UTI, suggesting that the 1–1.5 log (or 90–95%) mannose-driven reduction in faecal UPEC levels would reduce the numbers of bacteria available to access the urinary tract and probably reduce the rate of UTI and/or recurrent UTI (Extended Data Fig. 7). Indeed, we found that M4284 simultaneously reduces UTI89 levels in the gut and urinary tracts of mice that were concurrently colonized with UTI89 in the gut and bladder (Fig. 3e–g).

We sequenced bacterial 16S rRNA gene amplicons generated from faecal samples of C3H/HeN mice that had not been given streptomycin or infected with UPEC but were treated with three doses of M4284 or vehicle alone. We found that M4284 produced no significant changes in the overall phylogenetic configuration of the microbiota as judged by the unweighted UniFrac dissimilarity metric, in contrast to the significant perturbations produced by treatment with ciprofloxacin, a fluorquinolone antibiotic (Fig. 4a, Extended Data Fig. 8a). Using

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M4284 treatment of mice colonized with three additional genetically diverse uPEC intestinal isolates (EC958 ref. (23), 41.4p ref. (18) and CFT073 ref. (24)), reduced the levels of each uPEC strain by a similar percentage in the faeces, caecum and colon (Fig. 4b–f, Extended Data Fig. 10a). Furthermore, we found that M4284 treatment reduced UTI89 levels in C3H/HeN and C57BL/6 mice from different vendors, containing distinct gut microbial communities. In each case tested, the percentage reduction in UPEC levels in caecum, colon and faeces did not vary significantly between the different treatment groups (Fig. 4f–h, Extended Data Figs 8c, 10b). We concluded that M4284 treatment has activity against different UPEC strains in different host genetic backgrounds and gut microbial community contexts.

As the prevalence of antibiotic-resistant pathogens continues to rise, the need to develop highly targeted/specific therapeutic approaches has gained increased urgency. Furthermore, an increasing number of studies are finding that disruption of the gut microbiota by orally administered antibiotics, especially during childhood, may affect its functional properties in ways that are deleterious to the host, not only in the short term but also for more protracted periods of time. Therefore, developing therapeutic agents, such as mannosides, that specifically target a pathogen without disrupting the remainder of a microbial community has important ramifications not only for UPEC but also potentially for other infections, including those caused by Enterobacteriaceae (Extended Data Fig. 8a, b), suggesting that these treatments could be stratified for epidemiological studies of risk for recurrent disease as well as for proof-of-concept clinical studies of the efficacy of CUP-directed treatment regimens.

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METHODS

Ethics statement. The Washington University Animal Studies Committee approved all procedures used for the mouse experiments described in the present study. Overall care of the animals was consistent with The Guide for the Care and Use of Laboratory Animals from the National Research Council and the USDA Animal Care Resource Guide. For collection of colonic tissues for adhesion binding studies, mice were euthanized according to institutional, national and European animal regulations, using protocols that were also approved by the animal ethics committee of Ghent University.

Bacterial strains. CUP operon and adhesion deletions in UTI89 were engineered by replacing the gene(s) of interest with antibiotic-resistance markers using the λ Red Recombinase system22. Earlier reports described wild-type UTI89 and its isogenic fim and fimH mutants21,22 as well as EC958 (ref. 23), 41.4p (ref. 18) and CFT073 (ref. 24).

Colonization of mice with upec strains. Six-week-old female C3H/HeN mice were obtained from Envigo or Charles River Laboratories (CRL). Six-week-old female C57BL/6 mice were also obtained from Envigo. Animals were maintained in a single room in our vivarium for no more than 2 days before treatment. Before and after treatment all animals received Picolab Rodent Diet 20 (Purina ad libitum). All animals were maintained under a strict light cycle (lights on at 06:00, off at 18:00). For competitive infections, if a phenotype was observed after testing five mice (1 biological replicate), the experiment was repeated 1–2 times (total of n = 10–16 mice, 2–3 biological replicates). For 16S rRNA analyses, 4–5 mice were examined (1 biological replicate). For all other experiments, 9–16 mice were tested and the experiment was repeated 2–3 times (2–3 biological replicates). Exclusion criteria for mice were pre-established; (i) both introduced strains in competitive infections became undetectable during the course of a 14-day experiment, and (ii) mice died or lost more than 20% of their body weight. No mice in this study met these criteria. Mice were acquired from indicated vendors and maintained by the animal husbandry for the first 4 weeks before pre-plating. Mice were pre-plated for mycoplasma (n = 5 mice per cage) by employees of Washington University’s Division of Comparative Medicine; no additional methods for randomization were used to determine how animals were allocated to experimental groups. Investigators were not blinded to group allocation during experiments. Animals received a single dose of streptomycin (1,000 mg kg\(^{-1}\) in 100\(\mu\)l water by oral gavage) followed 24 h later by an oral gavage of approximately 10\(^6\) CFU upec in 100\(\mu\)l PBS. Bladder infections were performed via transurethral inoculation20. upec strains were prepared for inoculation as described previously20. In brief, a single UTI89 colony was inoculated in 20 ml of Luria Broth (LB) and incubated at 37°C under static conditions for 24 h. Bacteria were then diluted (1:1,000) into fresh LB and incubated at 37°C under static conditions for 18–24 h. Bacteria were subsequently washed three times with PBS and then concentrated to approximately 1 × 10\(^5\) CFU per 100\(\mu\)l for intestinal infections and 1 × 10\(^5\) CFU per 50\(\mu\)l for bladder infections. In all cases, faecal and urine samples were collected directly from each animal at the indicated time points. Faecal samples were immediately weighed and homogenized in 1 ml PBS. Urine samples were immediately diluted 1:10 before plating. Mice were euthanized via cervical dislocation under isoflurane anaesthesia, and their organs were removed and processed under aseptic conditions. Intestinal segments (caecum and colon) were weighed before homogenization and plating on LB supplemented with the appropriate antibiotics.

Enumeration of bladder intracellular bacterial communities. Six-week-old C3H/HeN mice were given a single oral dose of either M4284 (100 mg kg\(^{-1}\)) or vehicle control (10% cyclodextrin) 30 min before transurethral inoculation with UTI89. To count accurately the number of IBCs, mice were euthanized 6 h after infection. Bladders were removed aseptically, bisected, splayed on silicone plates and fixed in 4% (v/v) paraformaldehyde. IBCs, readily discernable as punctate foci within the uropathogenic E. coli titres after treatment was terminated, mice were euthanized 5 days after the last dose of mannoside. To test the effect of additional doses on M4284 treatment on upec titres, mice were given five doses of mannoside; the first three doses were administered 8 h apart, followed 12 h later by the fourth dose, and 24 h after the fifth dose. Mice were euthanized 24 h after the fifth dose.

Carriage of FimH-like pili. We examined 43 available upec isolates (Supplementary Table 2). These isolates originated from a clinical study of 14 women who experienced at least two episodes of a UTI (an initial UTI and one or more recurrent UTIs) during the 90-day study window27. The isolates used in this work were sequenced in a previous study28 (Bioproject ID PRJNA269984) and include (i) 14 isolates collected at enrollment, (ii) 18 isolates collected during recurrent UTI (10 women experienced a single recurrent UTI while four women experienced two recurrent UTI events), and (iii) 11 isolates collected in the days leading up to a recurrent UTI. The distribution of the F17-like operon in these clinical E. coli isolates was determined using BLAST and the F17-like operon from UTI89 as the query sequence. A hit was considered as any genome sequence that matched the entire length of the query sequence with more than 75% identity. As a control to prevent false negatives in the BLAST search of draft genomes, DNA sequencing reads from each clinical upec isolate were mapped against a reference sequence constructed by concatenating all the ucl genes with 100 N-separators using Geneious v6.1.7 (ref. 33).

Phylogenetic analyses and sequence alignments. Amino acid alignments of full-length UTI89 UcdD, P. mirabilis UcdD, S. enterica UchH, and ETEC F17G were conducted using the MAFFT L-INS-i iterative refinement method and the default parameters (Supplementary Table 1). MAFFT collected up to 100 homologues with E values of less than 1 × 10\(^{-10}\) to each sequence. The alignment was visualized using Geneious33. A homology search of the coding sequence database of the European Nucleotide Archive (ENA) was conducted using the basic Local Alignment Search Tool (BLAST)15 using the UTI89 ucdC (ENA accession ABE10308) and EDL933 ECS1278 (ENA accession AIG67653) as queries. Sequences that matched either gene sequence with more than 50% identity were downloaded and then filtered to remove partial hits (less than 80% length of query sequence) and sequences with nonsense mutations, which resulted in a total of 659 sequences (Supplementary Table 1). Duplicate sequences were removed, resulting in a list of 122 unique, representative sequences. These sequences were then aligned with the UTI89 fimH uchH sequence (ENA accession ABEI04147) as an outgroup using the MAFFT MAFFT L-INS-i alignment method and the 200PAM scoring matrix14. The phylogenetic relationship between gene sequences was then estimated using RAxML v1.8.13 with the GTRCAT model and supported with 1000 bootstrap replicates; the tree was visualized using the tool interactive Tree of Life (iTOL) v3 (ref. 36).

ELISA targeting FimH. Caco-2 cells (ATCC number HTB-37) were cultured in minimum essential medium (MEM) supplemented with 20% FBS. Cells cultures were tested negative for mycoplasma. Cells were split into 48-well plates, grown to 100% confluence and then fixed with paraformaldehyde for 15 min followed by treatment with blocking buffer (PBS containing 2% BSA) for 2 h. A truncated FimH, corresponding to residues 1–178 of the mature FimH adhesin (FimH\(^{1-178}\)) was expressed in E. coli and purified as described previously27,28. Briefly, 2 ml of a bacterial culture were centrifuged at 10 000 × g for 10 min and the supernatant was removed. The bacterial pellet was resuspended in 2 ml of buffer (50 mM Tris, pH 8.0, or 20 mM Tris plus water or 10% cyclodextrin, respectively) and then filtered through a 0.2 μm filter. The antibody was bound to 1 μl of immobilized FimH\(^{1-178}\) and washed three times with PBS before counterstaining with biotin-zine (Hoechst dye) (1:1000 in PBS) for 10 min at room temperature. Finally, slides were incubated with FimH\(^{1-178}\) or UcdD\(^{1-172}\) (P2) protein, labelled with NHS 650 nm Dylight, in blocking buffer at 4°C overnight.
1 M H$_2$SO$_4$. Binding was assessed by measuring the absorbance at 450 nm on a TECAN infinite 2 PRO plate reader. Wells lacking protein were used as control. All conditions were examined in quadruplicate.

**Effect of antibiotic exposure on the microbiota.** Six-week-old female C3H/HeN mice from Envigo and CRL were subjected to the following treatments: (i) none (naive control mice, untreated); (ii) three doses of M4284 (100 mg kg$^{-1}$; in 10% cyclodextrin) or with 10% cyclodextrin alone; (iii) faecal samples collected before treatment but after exposure to streptomycin and UTI89 and 24 h after the last dose of each treatment. Another group of four C3H/HeN mice from Envigo (1 biological replicate) were pretreated with streptomycin and colonized with UTI89 before receiving treatment with either three doses of M4284 (100 mg kg$^{-1}$) or with 10% cyclodextrin alone; faecal samples were collected before treatment but after exposure to streptomycin and UTI89 and 24 h after the last dose of each treatment. Data were indexed and processed with XDS$^{42}$, scaled and merged using HKL-2000 in the CCP4 suite$^{43}$ and phased with the Single anomalous dispersion (SAD) method using phenix.autosol, and refined with phenix.refine$^{44}$. Structural alignments can be found in Supplementary Table 3. r.m.s.d. values were calculating using the DALI server$^{45}$. Structural alignments were performed in PROMALS3D using the default settings. Secondary structure assignments for Ucd$^{12}$ were completed using DSSP.

**Differential scanning fluorimetry.** Purified Ucd$^{12}$ (1.4 μg per well) was incubated with 5× Sypro orange fluorescent dye in 20 mM Tris, pH 8.0, with or without 10 mM monosaccharide in a total volume of 70 μl. Samples were heated from 20°C to 100°C in 30-s/0.5°C increments using a Bio-Rad C1000 thermocycler with CTA96 RT-PCR attachment. The reported melting temperatures were determined by the inflection point of the sigmoidal graph.

**Data availability.** Bacterial V4-16S rRNA data sets have been deposited in the European Nucleotide Archive (ENA) under accession number PRJEB19121. Sequences used to examine the carriage of F17-like pili in clinical recurrent UTI isolates were previously published$^{18}$ and are deposited in the NCBI under the BioProject accession PRJNA269984. Crystalllography data have been deposited in the Protein Data Bank (PDB) under accession codes 5NW (P2_1_2_1 lysozyme) and 5VQ (P2_1_2_1). All other data are available from the corresponding author upon reasonable request.

**Code availability.** No new code was generated for this study. All software was obtained from publicly available sources; papers describing the software are cited in the text.

**Statistical analysis.** No statistical methods were used to predetermine sample size. The statistical significance of differences between groups in experiments (excluding competitive infections) was determined by a Mann–Whitney U test. The competitive index was defined as: (CFU output strain A/CFU output strain B)/CFU input strain A/CFU input strain B. For competitive infections, statistical significance was determined by a Wilcoxon signed-rank test. Statistical analyses were performed using Graphpad Prism 7.
Extended Data Figure 1 | Streptomycin treatment allows for persistent UTI89 colonization of the caecum and colon in female C3H/HeN and C57BL/6 mice. 

a. Mice were pretreated with streptomycin and subsequently colonized via oral gavage (PO) with UTI89, a prototypical human UPEC cystitis isolate. 

b–e. Colonization of UTI89 in C3H/HeN (b, c) or C57BL/6 (d, e) mice from Envigo was assessed by quantifying CFU in faecal samples collected over the course of 21 days from mice who did not receive streptomycin (white circles) or mice pretreated with the antibiotic (black circles). CFU analysis of levels of colonization in the caecum and colon were defined by analysing tissue homogenates prepared 21 days after colonization. Symbols represent geometric mean ± s.d. 

*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 by Mann–Whitney U test. n = 15 mice, 3 biological replicates (b–e).
The FimH adhesin is required for type 1 pilus-dependent colonization of the mouse gut and for binding to human intestinal epithelial cells. 

**a**. C3H/HeN mice from Envigo were pretreated with streptomycin and concurrently colonized with $1 \times 10^8$ CFU of wild-type UTI89 and UTI89ΔfimH. The wild-type strain outcompetes the strain lacking the FimH adhesin. 

**b**. The ability of FimHLD to bind to Caco-2 cells was assessed by a FimH ELISA. Pre-incubation of FimHLD with d-mannose (1 mM) or M4284 (1 mM) results in significant reductions in FimH binding to Caco-2 cells while 10% cyclodextrin (M4284 vehicle) had no significant effect. All data shown are normalized to wells that were not exposed to the purified adhesin. Data in a are mean ± s.e.m, and bars in b represent the median. *P < 0.05, **P < 0.01, ***P < 0.001 by Wilcoxon signed-rank test (a). n = 14 mice, 3 biological replicates (a); n = 4 wells examining FimH binding to Caco-2 cells per protein concentration, 4 technical replicates (b).
Extended Data Figure 3 | F17-like pili are not required for UTI in mice. C3H/HeN mice received a transurethral inoculation of wild-type UTI89 and UTI89Δucl, concurrently (a, b), or individually (c–e). a, UTI89Δucl and wild-type strains persist at similar levels in the urine over 28 days in competitive infections. b, The two strains are also present at equal levels in the bladder and kidney at the time of euthanization (28 days after infection). c, Single infection with the wild-type strain (black circles) or the F17-like mutant strain (white circles) produces similar levels of bacteruria over 28 days. d, Single strain infection also produces similar levels of viable cells in homogenates of whole bladder or kidneys collected at the time of euthanization (28 days after infection). There was no statistically significant difference in the number of mice that resolved bacteriuria while maintaining bladder-associated CFUs after transurethral infection with either wild-type UTI89 or UTI89Δucl (highlighted in red in d), suggesting that both strains are capable of forming similar numbers of quiescent intracellular reservoirs. e, Mice infected transurethrally with wild-type or Δucl strains of UTI89 exhibit a similar number of IBCs at 6 h in the bladder, indicating that loss of the ucl operon does not alter the ability of UTI89 to form IBCs. Error bars represent mean ± s.e.m. (a, b), geometric mean (c, d) or median (e). No significant difference was detected between any samples by Wilcoxon signed-rank test (a, b) or Mann–Whitney U test (c–e). n = 10 mice, 2 biological replicates (a, b, e); n = 16 mice, 3 biological replicates (c, d).
Extended Data Figure 4 | Distribution of F17 usher homologues in members of Enterobacteriaceae. The phylogenetic relationships between F17 homologues were estimated using the sequence of the usher genes. Branch colours indicate host strain and pilus identity, and coloured symbols indicate the annotated pathotype of the *E. coli* strain for each sequence as determined by publically available annotations. Stars indicate extraintestinal pathogenic *E. coli* (ExPEC) strains, and circles indicate intestinal pathogenic *E. coli* strains. Carriage of F17-like pili is enriched in UPEC strains, whereas F17 and ECs1278 pili are more common in intestinal pathogens such as ETEC and EHEC, respectively. The strain names for each sequence and ENA accessions are given. Numbers beneath the branches indicate the percentage of support from 1,000 bootstrap replicates (numbers greater than 80% are shown).
Extended Data Figure 5 | Phylogenetic distribution of F17-like carriage in UPEC from patients with recurrent UTI. The phylogeny of a set of clinical UPEC strains (n = 43 with taxon labels highlighted in green, orange or grey) was contextualized with reference E. coli strains (n = 46, unhighlighted taxon labels) by comparing the concatenated single-copy, core genes of the strains using the RAxML algorithm and the GTRCAT model. Highlighted taxon labels indicate UPEC isolates collected at enrolment (green) and during recurrent UTI (orange). In all cases, patients cleared each infection before recurrence, no patient exhibited signs of asymptomatic bacteriuria. The study design also allowed for the collection, from cohort participants, of E. coli isolates present in the urine in the days leading up to their clinical visit and recurrent UTI diagnosis (highlighted in grey). Branch lines indicate phylogenetic background for strains from clade B2 (red branch lines) and non-B2 clades (blue branch lines). Carriage of F17-like pili (black stars) was limited to the B2 clade and enriched within recurrent UTI UPEC isolates. Bootstrap supports are indicated at internal nodes. Bootstrap values greater than 95 have been removed. The clade to which each strain belongs is indicated in brackets to the right.
Extended Data Figure 6  | Testing the effects of more prolonged dosing of M4284 and analysis of the duration of its effects. a, Experimental design. b, Animals treated as in a show a continued decrease in UT189 levels in their faeces (samples were processed after 3, 4 and 5 doses of M4284), and at the time of euthanization in the caecum and colon, compared to control mice treated with vehicle alone (control, 10% cyclodextrin). c, d, The effects of mannoside treatment persist 5 days after M4284 exposure. Data are geometric mean ± s.d. *P < 0.05, **P < 0.01 by Mann–Whitney U test. n = 9 mice (control); n = 10 mice (M4284), 2 biological replicates (b); n = 16 mice, 3 biological replicates (d).
Extended Data Figure 7 | The severity of UTI outcome is directly linked to the dose of UTI89 inoculated into the urinary tract. C3H/HeN mice (Envigo) were given an experimental UTI via transurethral inoculation of either $10^6$ or $10^8$ CFU of UTI89. The doses were chosen to represent the reduction observed in intestinal UTI89 titres before and after treatment with the M4284 mannoside. Mice were euthanized 24 h after inoculation, and UTI89 titres in urine, bladder and kidneys were defined by quantifying CFU. Mice receiving the $10^6$ dose of UTI89 had significantly fewer bacteria in all three biospecimen types, indicating an important relationship between the number of bacteria introduced into the urinary tract and the severity of UTI outcome. Bars represent geometric means. $***P < 0.01$, $****P < 0.001$ by Mann–Whitney U test. $n = 10$ mice, 2 biological replicates.
Extended Data Figure 8 | See next page for caption.
Extended Data Figure 8 | 16S rRNA-based comparison of faecal bacterial communities in mice obtained from Envigo and CRL and mice of different genetic backgrounds from a common vendor.

a, C3H/HeN mice were treated with M4284 (100 mg kg\(^{-1}\), three doses over 24 h), vehicle alone (10% cyclodextrin, three doses over 24 h), or ciprofloxacin (15 mg kg\(^{-1}\), two doses over 24 h). Untreated mice served as reference controls. Heat maps show the effect of each of the treatments on animals from CRL and Envigo. Each row represents a species-level bacterial taxon, while each column represents a mouse sampled 24 h after the termination of the indicated treatment. Coloured boxes next to the taxon names indicate species whose relative abundance was significantly changed by ciprofloxacin treatment (\(P < 0.05\); Wilcoxon signed-rank test with false discovery rate (FDR) correction). Individual comparisons between untreated and other treatment types did not disclose changes that were statistically significant by Wilcoxon signed-rank test with FDR correction. (as shown in Extended Data Fig. 8a) were homogenized, diluted serially, and plated on MacConkey medium. The abundance of bacteria capable of growing on the selective medium was similar between faecal samples taken from untreated mice and those collected 24 h after treatment with cyclodextrin and M4284. No colonies were detected from faecal samples collected 24 h after ciprofloxacin treatment. 

b, Corresponding faecal samples collected 24 h after treatments were homogenized, diluted serially, and plated on MacConkey medium. The abundance of bacteria capable of growing on the selective medium was similar between faecal samples taken from untreated mice and those collected 24 h after treatment with cyclodextrin and M4284. No colonies were detected from faecal samples collected 24 h after ciprofloxacin treatment. 

c, Comparison of the representation of bacterial taxa in the faecal microbiota of untreated mice obtained from different vendors or representing different genetic backgrounds. Each row in the heat map represents a species-level taxon, while each column represents a mouse of the indicated genetic background from the indicated vendor. Coloured boxes indicate species whose relative abundances were significantly different (\(P < 0.05\)) between all three groups of animals (Kruskal–Wallis test with FDR correction). Rows of each heat map were hierarchically clustered according to pair-wise distances using Pearson correlation. \(n = 5\) mice per treatment type, 1 biological replicate (a); \(n = 5\) mice, 1 biological replicate (b); \(n = 5\) mice per vendor/mouse strain, 1 biological replicate (c). Bars denote median. **\(P < 0.001\), Mann–Whitney U test (b).
Extended Data Figure 9 | The configuration of the faecal microbiota of C3H/HeN mice pretreated with streptomycin and colonized with UTI89 is minimally altered by M4284 treatment. a, C3H/HeN mice from Envigo were pretreated with streptomycin and 24 h later colonized with UTI89 by oral gavage. Three days after inoculation, animals were treated with three doses of M4284 (100 mg kg\(^{-1}\), three doses over 24 h) or vehicle alone (10% cyclodextrin, 3 doses over 24 h). Faecal samples were collected 24 h after the last dose of M4284 or vehicle. b, Heat map showing the effect of each treatment type. Each row represents a bacterial species-level taxon, while each column represents a mouse 24 h after the indicated treatment. Rows of the heat map were hierarchically clustered according to pair-wise distances using Pearson correlation. No treatments produced changes that were statistically significant, as judged by Wilcoxon signed-rank test with FDR correction. \(n = 4\) mice per treatment type, 1 biological replicate.

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Extended Data Figure 10 | The percentage reduction in strains by M4284 treatment is similar in mice colonized with genetically distinct human isolates, and in multiple strains of mice colonized with UTI89. 

(a) The percentage reduction in CFU for the indicated UPEC strains from M4284-treated versus untreated control C3H/HeN mice obtained from Envigo (based on data in Fig. 4c–f). 

(b) CFU data obtained from C3H/HeN mice from Envigo and CRL and C57BL/6 mice from Envigo (based on data in Fig. 4f–h). P values calculated using Kruskal–Wallis test. 

\( n = 14 \) mice, 3 biological replicates (UTI89); \( n = 10 \) mice, 2 biological replicates (CFT073, EC958 and 41.4p) (a); \( n = 14 \) mice, 3 biological replicates (C3H/HeN from Envigo); \( n = 10 \) mice, 2 biological replicates (C3H/HeN from CRL); and \( n = 9 \) mice, 2 biological replicates (C57BL/6 from Envigo) (b).