Supplementary Information

Supplementary Methods

DNA manipulation and genetic techniques. Oligonucleotides were synthesized by Integrated DNA Technologies. Plasmid DNA was isolated using the QIAprep Spin Miniprep kit (Qiagen). Chromosomal DNA was purified using the Wizard Genomic DNA Purification Kit (Promega). PCR amplification was performed using KAPA HiFi polymerase (Kapa Biosystems). PCR products and other DNA fragments were purified using the QIAquick PCR Purification kit or the QIAquick Gel Extraction kit (Qiagen). Restriction endonucleases, T4 polynucleotide kinase, T4 ligase and T4 polymerase treatment were performed following the manufacturer’s recommendations (New England Biolabs). Nucleic acid quantification was performed using a Nano-Drop 2000 Spectrophotometer (Thermo Scientific). DNA sequencing was performed using Big Dye Terminator Sequencing v3.1 Cycle Sequencing (Applied Biosystems) at the Australian Equine Genomic Research Centre (AEGRC), University of Queensland.

Bioinformatic analysis. An E. coli genome database was generated by downloading up to 100 randomly selected sequence assemblies from each of the top 83 E. coli sequence types in the E. coli collection on EnteroBase (www.enterobase.warwick.ac.uk), a large publicly available Enterobacteriaceae genome sequence database [1]. This resulted in a collection of 8,247 assemblies (downloaded in January 2019) that we refer to as the 83ST database. The presence of the individual uclA-B-C-D genes in each genome was assessed by tblastn, using the amino acid sequence as a query, and employing a cut-off at 70 % identity and 95 % coverage. The nucleotide sequence of each gene in each genome was extracted using blastdbcmd and curated manually for false-positive hits. All alignments were constructed using ClustalO v1.2.4 [2] with default settings. Maximum-likelihood trees were produced with IQTree v1.6.8 using ModelFinder with default settings and supported by a bootstrap value of 100 [3]. Trees and metadata were visualised in Evolview [4].

Genetic mutagenesis of bacteria. Gene disruption mutants were generated using λ-Red recombinase-mediated homologous recombination as described previously [5]. Mutant strains F11ucl, UTI89ucl, S77ECucl and HVM1299ucl were generated via a previously described three-way PCR procedure [6] to amplify a chloramphenicol (cm) resistance cassette from pKD3 with a 500-bp
homology region to the ucl genes. UTI89lacI-Z was generated by amplifying the gfp-cm cassette with a 700-bp homology region using primers 4057 (5′- tcgtcttcatcctgctcttc) and 4058 (5′-getaaatgccgaatgggt), and the chloramphenicol resistance cassette was subsequently removed using the FLP flippase-encoding pCP20 [5]. Single-nucleotide switching of F11 and UTI to F11-PulcT-78G and UTI89-PulcG-78T was performed using pORTMAGE vectors as described previously [7].

**Protein preparation, immunoblotting and whole-cell ELISA.** Strains to be assessed were grown overnight in LB broth, supplemented with the necessary antibiotics. Whole-cell lysates were prepared by centrifuging 1 ml of overnight cultures standardised to OD_{600nm} = 1.0. Cell pellets were resuspended in 40 μl water and 5 μl of 2 M HCl and boiled for 10 min before being neutralised with 5 μl of 2 M NaOH, followed by adding 50 μl of 2×SDS loading buffer (100 mM Tris-HCl, 4 % w/v SDS, 20 % v/v glycerol, 0.2 % w/v bromophenol blue, pH 6.8). Samples were boiled for 10 min, prior to electrophoresis; a volume of 10 μl was routinely analysed. SDS-PAGE and transfer of proteins to a PVDF membrane for western-blot analysis was performed as described previously [8]. Rabbit polyclonal antibody generated against UclA, UclD or UcaD was utilised as primary antibody and detected with commercially purchased alkaline phosphatase conjugated anti-rabbit antibodies (Sigma Aldrich). SIGMAFAST BCIP/NBT (Sigma-Aldrich) were used as the substrate for detection. Western blots were scanned using the Bio-Rad GS-800 calibrated imaging densitometer.

To detect Ucl surface expression, cells were suspended in 100 mM sodium carbonate (pH 9.5) and standardised to an OD_{600} of 1. 100 μl of culture was used to coat each well of a MaxiSorp 96-well ELISA plate (Thermo Fisher 44-2404-21) overnight at 4 °C. Wells were blocked with 5 % skim milk in PBS for 1 hour at room temperature. Antibody incubations were performed in 100 μl PBS with 0.05 % Tween-20 for 1 hour at room temperature. Ucl fimbriae were probed with α-UclA, while α-E. coli (Life Research B65001R) was used as a cell-loading control, and secondary antibody was α-rabbit IgG, AP conjugated (Sigma A3687). All wash steps were performed three times with 250 μl PBS with 0.05 % Tween-20. The reaction was developed with 100 μl of substrate pNPP (Sigma P7998) in the absence of light and optical density was measured at 420 nm after 30 minutes.

**β-galactosidase assay.** β-galactosidase assays were performed essentially as described previously [9]. Briefly, strains to be assessed were grown overnight in LB broth, supplemented with the necessary antibiotics. Cultures were diluted in Z buffer (60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 50 mM β-mercaptoethanol, 10 mM KCl, 1 mM MgSO₄, pH 7) with 0.004 % SDS and 16 % chloroform added. Samples were vortexed and incubated at 28 °C to permeabilise the cells. The substrate o-nitrophenyl-β-D-galactopyranoside (ONPG) was added to initiate the reaction, which was

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subsequently stopped with sodium bicarbonate. β-galactosidase activity was assessed in quadruplicate for each strain, by measuring the absorbance at 420 nm. All experiments were performed as three independent replicates. Statistical analysis of β-galactosidase levels between F11lacI-Z and UTI89lacI-Z carrying each of the pQF50 construct was performed using an unpaired, one-way ANOVA and Sidak’s multiple comparison test.

**Rapid amplification of cDNA ends (5′ RACE).** The transcription start site of *ucl* was determined using 5′ RACE (Version 2.0; Invitrogen) [10]. Exponentially growing cells (OD$_{600nm}$ = 0.6) were stabilized with two-volumes of RNaprotect Bacteria Reagent (Qiagen), prior to RNA extraction using the RNeasy Mini Kit (Qiagen) and treated with rDNase I (Ambion) to remove contaminating DNA. First-strand cDNA was synthesized and PCR-amplified using the following gene specific primers: 6973 (5′-ctgagcactattcatacc) and 6974 (5′-gaatggcaaagggtgtcag), following the manufacturer’s specification. Amplified cDNA ends were sequenced to determine the transcription start site.

**Mouse gut colonization assays.** Competitive gut colonization assays were carried out as previously described, with slight modifications [11, 12]. Seven- to eight-week-old female Specific Pathogen Free C3H/HeN mice (Charles River Labs) were first inoculated via oral gavage with 100 uL of streptomycin (1000 mg/kg in water) 24 hours prior to introduction of the F11 strains. Bacteria were grown statically from frozen stocks at 37°C for 24 hours in 250 ml flasks containing 20 ml LB and then diluted 1:1000 into fresh LB and grown for another 24 hours. Prior to gavage, 6 mL of each culture was spun down at 8,000 x g for 8 minutes and pellets were washed twice and then resuspended in phosphate-buffered saline (PBS). Each mouse was inoculated with 50 µL PBS containing a total of ~5 x 10$^7$ CFU, comprised of a 1:1 mix of F11::kan and F11::cm (control) or F11::kan and F11-Puef$^78G$ (cm resistant). At the indicated time points post-inoculation, individual mice were briefly (3 to 10 min) placed into unused takeout boxes for weighing and feces collection. Freshly deposited feces were recovered and directly added to 1 ml of 0.7 % NaCl, weighed, and set on ice. Fecal pellets were broken up by homogenization and samples were then briefly centrifuged to pellet any large insoluble debris. Supernatants were serially diluted and spread onto LB agar plates containing either chloramphenicol (20 µg/ml) or kanamycin (50 µg/ml) to select for growth of the relevant bacterial strains. Plating assays confirmed that the mice contained no endogenous bacteria that were resistant to chloramphenicol or kanamycin prior to introduction of the ExPEC strains. Mice were housed 3 to 5 per cage and were allowed to eat (irradiated Teklad Global Soy Protein-Free Extruded chow) and drink antibiotic-free water *ad libitum*. Competitive indices (CI) were calculated as the ratio of the cm
resistant over kan resistant bacteria recovered from the feces divided by the ratio of the same strains within the inoculum.

**Cloning, expression and purification of UclD<sup>LD</sup> and UcaD<sup>LD</sup>.** The structures of the lectin domains of UclD and UcaD were predicted using Phyre (http://www.sbg.bio.ic.ac.uk/phyre2/html/) [13]. The coding sequences for the *uclD* and *ucaD* lectin domains were amplified from *E. coli* strain F11 and *P. mirabilis* PM54 clinical isolate [14] genomic DNAs. The amplicons were cloned into pET22b, which encodes a C-terminal His-tag. Epoch Life Science made and confirmed by sequencing the pET22b::*uclD* and pET22b::*ucaD* constructs.

*E. coli* BL21 (DE3) pLys harbouring pET22b::*uclD* and pET22b::*ucaD* were grown at 37 °C in LB media supplemented with 100 μg/mL ampicilllin. Cells were induced at OD<sub>600nm</sub> of 0.5 with 1 mM IPTG at 37 °C. Periplasmic extractions by cold osmotic shock of cells incubated overnight were carried out. UclD<sup>LD</sup> and UcaD<sup>LD</sup> were purified using a HiTrap nickel column (GE Healthcare). Proteins were eluted in a gradient of 0-400 mM imidazole in a buffer containing 20 mM Tris-HCl (pH 7.5) and 150 mM NaCl. Fractions containing the UclD or UcaD, as judged by SDS-PAGE, were pooled and dialysed against a buffer containing 20 mM Tris-HCl (pH 7.5) and 150 mM NaCl overnight at 4 °C. Size-exclusion chromatography (HiPrep 16/60 Sephacryl S-200 HR GE Healthcare) in 20 mM Tris-HCl (pH 7.5) and 150 mM NaCl was used to further purify UclD and UcaD, as assessed by SDS-PAGE.

**Glycan array analysis.** Glycan array analysis was carried out using methods previously described [15, 16]. Briefly, 2 μg of UclD and UcaD, in a final volume of 500 μL of array PBS (PBS with 2 mM MgCl<sub>2</sub> and 2 mM CaCl<sub>2</sub>), were pre-complexed with a mouse anti-his antibody and detected with fluorescent Alexafluor555 secondary and tertiary antibodies in a molar ration of 4:2:1 for 10 mins prior to application to the array. Slides were previously pre-blocked for 15 mins in array PBS with 0.5 % BSA. Slides were rinsed in PBS and dried by centrifugation. Protein was hybridised to the array for 15 min at room temperature in the dark. After 15 min, the slide was immersed in array PBS with 0.2% BSA and washed for 2 min. The slide was then placed in a 50 mL falcon tube and washed in array PBS for 2 min, before rinsing in clean PBS then drying by centrifugation at 200 x g for 5 min. The array was scanned by a ProScan Array scanner, and the results analysed by ScanArray Express software program. Binding was classified as RFU (relative fluorescence units) structure reported as positive had a value above mean background (defined as mean background plus 3 standard deviations), and had a P value of < 0.005.
**SPR analysis.** SPR analysis was carried out using a Biacore T200 system (Cytivia) as previously described [15] with minor modifications. Briefly, UclD and UcaD were immobilized onto flow cells of a CM5 sensor chip using amine coupling at 20 µg/mL in 10 mM sodium acetate pH 4.0 and at a flow rate of 5 µL/min for 10 minutes. Glycans were chosen based on positive glycan array results for each of the proteins with each glycan run across a dilution range starting at a maximum concentration of 100 µM and minimum concentrations tested being 1.6 nM using single cycle kinetics. Results were analysed using the Biacore T200 evaluation software.

**Crystallization and crystal structure determination.**

**UclD**<sup>LD</sup>: UclD<sup>LD</sup> crystals were produced using the hanging drop method, with drops containing 1 µL of protein (10 mg/mL) and 1 µL of well solution (20-25% w/v PEG 3350, 0.1 M Bis-Tris propane pH 6.5, 0.2 M sodium iodide). The crystals appeared within 1-5 days. The crystals were cryoprotected in glycerol (80% well solution and 20% (v/v) glycerol) before flash-cooling in liquid nitrogen. X-ray diffraction data were collected from a single crystal at the Australian Synchrotron MX1 beamline, using a wavelength of 0.9537 Å. Data collection was performed using Blu-Ice software [17], indexed and integrated using MOSFLM and scaled with AIMLESS within the CCP4 suite [18]. Molecular replacement was initially attempted using several published fimbrial adhesin structures as templates, but a solution could not be obtained. Because the crystallisation condition contained sodium iodide, we determined the UclD<sup>LD</sup> structure by SAD phasing using the CRANK2 [19] pipeline of the CCP4 suite and a dataset (2.85 Å resolution) collected at wavelength of 1.3776 Å, where the anomalous scattering properties of iodide are still significant. Iodine atoms were located by SHELXD [20], and automatic model building was performed using Buccaneer [21] and Refmac [22]. The higher resolution UclD<sup>LD</sup> structure was subsequently solved by molecular replacement using PHASER [23]. The model was refined using Phenix [24], with iterative model building carried out between rounds of refinement using Coot [25]. Structure validation was performed using MolProbity [26]. The structure was refined to final $R_{work}/R_{free}$ values of 24.8%/30.6% (Table A in S1 Text). The moderate quality of the UclD<sup>LD</sup> dataset ($R_{meas}$ of 27.9%, Table A in S1 Text) is a likely reason for the high $R_{free}$ value. The final UclD<sup>LD</sup> model contains residues 21-215. Electron density was not observed for residues 43-48, suggesting that these regions have a disordered or flexible conformation in the crystals. The coordinates and structure factors have been deposited in the PDB with ID 7MZP.

**UcaD**<sup>LD</sup>: UcaD<sup>LD</sup> crystals were produced using the hanging drop method with drops containing 1 µL of protein (8-16 mg/mL) and 1 µL of well solution (0.1 M sodium citrate buffer pH 4.5-5.5, 2-3 M NaCl). The crystals appeared within 3-5 days. The crystals were cryoprotected in Paratone-N, before
flash-cooling in liquid nitrogen. X-ray diffraction data were collected from a single crystal at the Australian Synchrotron MX2 beamline, using a wavelength of 0.9537 Å. Data collection was performed using Blu-Ice software [17], indexed and integrated using MOSFLM and scaled with AIMLESS within the CCP4 suite [18]. The structure was solved by molecular replacement using PHASER [23] and UclDLD as the template. The model was refined using Phenix [24] and structure validation was performed using MolProbity [26]. The structure was refined to final R_work/R_free values of 16.9%/19.2%, respectively (Table A in S1 Text). The final UcaDLD model contains residues 21-217. Electron density was not observed for residues 44-47, suggesting that these regions have a disordered or flexible conformation in the crystals. Coordinates and structure factors have been deposited in the PDB with ID 7MZO.

UcaDLD:monosaccharide complexes: Pre-formed UcaDLD and UclDLD crystals were soaked with 0.1-0.2 M of the monosaccharides Fuc, Glc, Gal, GlcNAc, GalNAc or Neu5Ac for 48 hours in the crystallisation solution (0.1 M sodium citrate buffer pH 4.5-5.5, 2-3 M NaCl). The crystals were cryoprotected in Paratone-N and flash-cooled at 100 K. X-ray diffraction data were collected from single crystals on the MX2 beamline at the Australian Synchrotron, using a wavelength of 0.9537 Å. The data-sets was processed using either MOSFLM (Gal complex), or XDS (Fuc and Glc complexes) [27] and scaled using AIMLESS in the CCP4 suite [18]. The structures were solved by molecular replacement using PHASER [23] and ligand-free UcaDLD as the template. The models were refined using Phenix [24], and structure validations were performed using MolProbity [26]. Coordinates and structure factors have been deposited in the PDB with IDs 7MZQ (Fuc complex), 7MZR (Glc complex), and 7MZS (Gal complex).

Molecular docking and molecular dynamics simulations. The initial structure for MD simulations was obtained by molecular docking of lacto-N-fucopentose VI with AutoDock Vina [28], as implemented in the YASARA molecular modelling package (Ver. 16.46) [29]. A grid box covering the entire monosaccharide-binding site and surroundings was used to place lacto-N-fucopentose VI. The docked structure with the best superimposition between the fucose moiety of lacto-N-fucopentose VI and the fucose molecule observed in the UcaDLD:Fuc complex was then subjected to further optimization by a 40 ns MD simulation using the AMBER force-field implemented in the YASARA software suite [29]. A representative energy-minimised snapshot from the MD trajectory was used for the analyses.
Supplementary Figures

Fig A. The Ucl fimbrial operon is most frequently found in phylogroup B2 strains. Percentage of strains encoding *uclABCD* for each sequence type in the 83ST database. The bars are split to show the presence of specific *uclA* alleles; the least common *uclA* variants are summarised by the “other” designation (other: 12 variants observed; 33/404, ~8%); see Fig B in S1 Text. The *ucl* genes were most frequently found in ExPEC strains from ST12, ST73, ST127, ST131 and ST141 in the pathogenic B2 phylogroup (360/900; 40%), compared to strains from STs in phylogroups D (15/600; 2.5%) and F (3/500; 0.6%), and rarely found in strains from STs in other *E. coli* phylogroups.
Fig B. Maximum likelihood phylogeny of \textit{uclA} variants found in the 83ST database, with the number of each \textit{uclA} variant indicated in the bar graph. A total of 15 \textit{uclA} allelic variants that differed by up to 27\% at the nucleotide level were identified. The most common allelic variant was \textit{uclA}-10 (212/404; 52.5\%), followed by \textit{uclA}-5 (88/404; 21.8\%) and \textit{uclA}-13 (71/363; 17.6\%); other allelic variants were infrequent.
**Fig C.** Pair-wise sequence comparison of GI-leuX region of UTI89 (top), F11 (middle) and MG1655 (bottom). The integase gene adjacent to tRNA-leuX is coloured blue, *uclABCD* are coloured red. The grey regions connecting the genomes represent blastn output with the percent of conservation shown in the scale bar.
**Fig D. Analysis of the ucl promoter.** a) Left, schematic diagram of the ucl promoter region from F11 and UTI89 cloned in the reporter plasmid pQF50. Indicated are the TSS, -10 and -35 promoter elements, and OxyR binding site. Right, β-galactosidase activity (measured in Miller units) for each Pucl-lacZ fusion construct in UTI89lacZ. Plasmid pQF50-PucF11-lacZ possessed a higher β-galactosidase activity as compared to pQF50-PucFUTI89-lacZ (p < 0.0001; one-way ANOVA with Sidak’s multiple comparisons test). b) Promoter region of ucl operon from F11. The transcription start site is indicated as +1, with the predicted -10 and -35 core promoter elements indicated accordingly. Bolded T with an arrow indicates the single nucleotide that differed in F11, where a G is present at this position in UTI89, S77EC and HVM1299. The region from F11 and UTI89 cloned into the pQF50 lacZ reporter plasmid is indicated by arrows denoting the 5’ ends of primers 6571-6572.
**Fig E. 5’ RACE of uclA to map the transcription start site.** Top: sequence, indicating the ATG start codon of the uclA gene and the +1 transcription start site. Bottom: sequence chromatogram.
Fig F. a) Whole-cell lysate western-blot analysis of F11<sup>T-78G</sup> and UTI89<sup>G-78T</sup>. Higher expression of UclA was observed in UTI89<sup>G-78T</sup> mutants and lower in abundance in F11<sup>T-78G</sup>, compared to that of their wild-type strains. b) Qualitative analysis of 100 cells assessed for Ucl fimbriation using α-UclA immuno-gold labelling. c) Representative UclA immunogold-labelled TEM images for F11, F11<sup>ΔuclA</sup> and F11-Puc<sup>T-78G</sup>.
**Fig G. Anti UclA whole-cell ELISA.** a) Whole-cell ELISA demonstrating expression of Ucl fimbriae on wild-type F11 (wt), F11Δucl (ucl) and F11-Pucl<sup>T-78G</sup> (T-78G), as well as wild-type UTI89 (wt), UTI89Δucl (ucl) and UTI89-Pucl<sup>G-78T</sup>. Ucl fimbriae were detected using UclA-specific polyclonal antibody. b) Control whole cell ELISA of wild-type F11 (wt), F11Δucl (ucl) and F11-Pucl<sup>T-78G</sup> (T-78G), as well as wild-type UTI89 (wt), UTI89Δucl (ucl) and UTI89-Pucl<sup>G-78T</sup> employing a general *E. coli* antibody (Life Research B65001R). The black dots show individual measurements for four technical replicates from three biological replicates (n=12); the grey bar indicates the mean. Statistical analyses were performed by one-way ANOVA with Sidak’s multiple comparisons test.
**Fig H. Binding of OxyR to the ucl promoter region (Pucl).** a, Coomassie-stained SDS-PAGE of OxyR-6xHis. Lane M: PageRuler Prestained Protein Ladder (Life Technologies, catalogue no. 26616), Lane 2: Nickel-affinity purified OxyR-6xHis. b, Schematic diagram of the uclABCD operon, indicating the transcription start site (TSS), -10 and -35 promoter region, and OxyR binding sequence containing the T(-78) in F11 and the G(-78) in UTI89. Also indicated are the 261 bp Pucl PCR fragment and the 240 bp uclC PCR fragment (negative control) used in the gel shift assay. c, Electrophoretic mobility shift assay of the Cy3-PuclT(-78T) (top) and Cy3-PuclT(-78G) (bottom) fragments with OxyR and increasing concentrations of unlabelled competitor (Pucl-UL) DNA.
Figure I. Conservation of the *Pucl* OxyR binding site in ST127. A total of 845 genomes from ST127 strains on Enterobase were downloaded, 698 of which were positive for the Ucl fimbriae genes. The OxyR binding site was extracted from each *Pucl* sequence and aligned to generate the DNA logo shown at the top of the figure. Twenty-seven unique OxyR binding sites were identified, with nucleotide sequence changes shown below the consensus sequence. The number of times each unique sequence was identified in the dataset is indicated. The OxyR binding site consensus sequence was found most frequently (n=486), while the F11 T-78G SNP was also common (n=96). In total, 31% of the *Pucl* OxyR binding sites contained at least one SNP compared to the consensus sequence, with the F11 T-78G SNP most prevalent (14%). Sequences containing the G-78T and T-76C are indicated.
**Fig J. UclA expression is increased in ECOR60.** a) OxyR binding site sequences from UTI89 (consensus), F11 and ECOR60, highlighting the C-76T nucleotide sequence change in ECOR60. b) Whole-cell lysate western-blot analysis of ECOR60, ECOR63, F11 (WT) and F11-Pucl<sup>T-78G</sup> employing a UclA-specific antibody. Higher expression of UclA was observed in ECOR60 and F11 (WT) compared to ECOR63 and F11-Pucl<sup>T-78G</sup>. The promoter region of the ucl operon from ECOR63 and UTI89 is identical.
Fig K. Ucl fimbriae do not impact colonisation of the mouse gut in single infection experiments. Mice were inoculated wild-type F11 (tagged with a chloramphenicol resistance cassette; black circles), F11-$\Delta$ucl (green squares) and an F11-$\Delta$ucl mutant (orange triangles). Each group contained 10-11 mice infected and monitored during two independent experiments. Bacterial loads were assessed over a 2-week period.
Fig 1. Architecture of Ucl fimbriae demonstrated by co-immunogold labelled electron microscopy. a) Electron micrograph demonstrating immunogold labelled UclA major subunit (left; 5 nm gold particles) and UclD tip adhesin (right; 10 nm gold particles) of Ucl fimbriae. b) Cartoon model of Ucl fimbriae architecture, depicting the UclA major subunit repeating protein (green), UclD tip adhesin (orange), UclC usher (yellow) and UclB chaperone (red). Also labelled are the inner membrane (IM) and outer membrane (OM) of the cell.
**Fig M. Prevalence of the uclABCD genes in Proteus species.** Genomes were assessed from the NCBI database. The analysis was performed using tblastn for UcaA, UcaB, UcaC and UcaD, with a positive result determined for blast hits with >70% identity and >80% coverage for all four proteins.
**Fig N.** Amino acid sequence alignment of the UcID (NCBI protein entry EEZ6997767), UcaD (CAR41289.1) and GafD (Q47341) adhesins. Identical amino acids are shaded in black; similar amino acids are shaded in grey. The consensus sequence is indicated.
**Fig O.** A) Crystal packing analysis of UcaD$^{LD}$ (PDB: 7MZO). The molecules are shown in cartoon representation. The asymmetric unit consists of one UcaD$^{LD}$ molecule shown in cyan, while symmetry-related molecules are colored orange. The monosaccharide binding region of the asymmetric unit is circled and highlighted in magenta. B) Comparison of the monosaccharide binding site region in 3 different crystal forms of ligand-free UcaD$^{LD}$. C) Comparison of the monosaccharide binding site in the UcaD$^{LD}$:Fuc, UcaD$^{LD}$ and UclD$^{LD}$ crystal structures.
**Fig P.** Electron density map of the fucose binding site in the UcaD<sup>LD</sup>:Fuc complex structure.

Composite (2Fo - Fc, blue, contoured at 2σ) and difference (Fo - Fc, green/red, contoured at 3.0σ) electron density map of the Fuc binding site after refinement. Positive difference density adjacent to C4 suggests that a minor fraction of the Fuc molecules in the crystal adopts an alternate conformation.
**Fig Q. UcaD^{LD}:Fuc interactions in the MD derived structure.** Interactions between the fucose residue of lacto-N-fucopentose VI (yellow) and residues of the binding pocket of ≤3.6 Å are shown as dashed lines. The binding model of the fucose molecule observed in the UcaD^{LD}:Fuc crystal structure is highlighted in green stick representation, for comparison.
### Table A. Crystallographic data.

|                     | UcID\textsuperscript{LD} Dataset 1 | UcID\textsuperscript{LD} Dataset 2 | UcaID\textsuperscript{LD} Galactose | UcaID\textsuperscript{LD} Fucose | UcaID\textsuperscript{LD} Glucose |
|---------------------|-------------------------------------|-------------------------------------|------------------------------------|----------------------------------|---------------------------------|
| **Data collection** |                                     |                                     |                                   |                                  |                                 |
| Detector            | ADSC                                | ADSC                                | ADSC                              | ADSC                            | ADSC                            |
|                     | Quantum 210r CCD                    | Quantum 210r CCD                    | Quantum 315r CCD                  | ADSC                            | Dectris Eiger                   |
|                     | 0.9537                              | 1.3776                              | 0.9537                            | 0.9537                           | 0.9537                          |
| Wavelength (Å)      | 376.36, 79.07, 90.00                | 375.91, 79.07, 90.00                | 376.36, 79.07, 90.00              | 374.97, 79.47, 90.00            | 379.07, 79.07, 90.00            |
| Temperature (K)     | 100                                 | 100                                 | 100                               | 100                              | 100                             |
| Rotation range per image (°) | 0.5                               | 0.5                                 | 0.5                               | 0.5                              | 0.5                             |
| Exposure time per image (s) | 0.5                               | 0.5                                 | 1.0                               | 1.0                              | 1.0                             |
| Space group         | P 2_1_2_1                          | P 2_1_2_1                          | P 2_1_2_1                        | P 2_1_2_1                       | X 16M                           |
|                     | 1 + 1 + 1                           | 1 + 1 + 1                           | 1 + 1 + 1                        | 1 + 1 + 1                       | 1 + 1 + 1                       |
| a, b, c (Å)         | 39.04, 58.12, 90.00                 | 39.03, 58.26, 90.00                 | 77.39, 77.39, 70.91              | 79.36, 79.36, 70.36             | 79.97, 79.47, 70.23             |
| a, β, γ (°)         | 90.00, 90.00, 90.00                 | 90.00, 90.00, 90.00                 | 90.00, 90.00, 90.00              | 90.00, 90.00, 90.00             | 90.00, 90.00, 90.00             |
| Average mosaicity (°)\textsuperscript{b} | 0.76                              | 0.71                               | 0.88                              | 0.84                            | 0.12                            |
| Resolution range (Å) | 58.12-2.20                         | 87.93-2.85                         | 54.72-1.62                       | 56.12-1.72                      | 39.74-1.50                      |
|                      | (2.27-2.20)                         | (3.01-2.85)                         | (1.65-1.62)                      | (1.75-1.72)                     | (1.53-1.50)                     |
| Total no. of reflections | 147408                            | 259840                             | 185132                           | 83446 (4474)                    | 475741                          |
|                     | (12817) (33746) (9197)              | (21842) (13975)                    |                                  |                                  |                                 |
| No. of unique reflections | 21160 (1794)                       | 9966                               | 26588                            | 23071 (1237)                    | 34590 (1653)                    |
|                     | (1412) (1305)                       |                                    |                                  |                                  | 20791 (1137)                    |
|Completeness (%)     | 100.0 (100.0)                       | 99.7 (99.6)                        | 99.8 (99.9)                      | 99.4 (99.7)                     | 99.8 (97.0)                     |
| Multiplicity        | 7.0 (7.1)                           | 26.1 (23.9)                        | 7.0 (7.0)                        | 3.6 (3.6)                       | 13.8 (13.2)                     |
| Mean I/σ(f)         | 5.4 (1.5)                           | 14.1 (6.1)                         | 11.6 (1.4)                       | 8.4 (1.5)                       | 18.3 (2.5)                      |
| R\textsuperscript{meas} (%)\textsuperscript{c} | 27.9 (143.7)                       | 19.0 (50.9)                        | 11.9 (155.9)                     | 15.1 (131.9)                    | 6.6 (69.4)                      |
| R\textsuperscript{pim} (%)\textsuperscript{d} | 10.5 (53.2)                        | 3.7 (10.2)                         | 4.5 (58.4)                       | 7.7 (68.7)                      | 1.8 (18.7)                      |
| CC\textsubscript{12}\textsuperscript{b}   | 0.990 (0.748)                      | 0.997 (0.985)                      | 0.998 (0.634)                    | 0.990 (0.291)                   | 0.999 (0.887)                   |
| Resolution range (Å) | 43.85-2.20                         | 38.70-1.62                         | 39.68-1.72                       | 39.74-1.50                      | 39.54-1.78                      |
| R\textsuperscript{work} (%)\textsuperscript{e} | 24.8                              | 16.9                               | 17.3                             | 16.3                            | 16.2                            |
| R\textsuperscript{free} (%)\textsuperscript{f} | 30.6                              | 19.2                               | 20.3                             | 17.6                            | 19.7                            |
| No. of non-H atoms  | 2920                               | 1709                               | 1689                             | 1678                            | 1683                            |
| Total               | 2785                               | 1457                               | 148                              | 1483                            | 1479                            |
| Non-solvent         | 129                                | 247                                | 236                              | 195                             | 204                             |
| Water               | 29.1                               | 20.8                               | 16.6                             | 22.0                            | 20.0                            |
| R.m.s.d. from ideal geometry | 0.002                             | 0.009                              | 0.009                            | 0.008                           | 0.008                           |
| Bond lengths (Å)    | 0.553                              | 0.957                              | 0.970                            | 0.936                           | 0.936                           |
| Ramachandran plot, residues in (%) | 96.19                             | 97.35                              | 97.31                            | 97.35                           | 97.34                           |
| Favored regions     | 3.81                               | 2.65                               | 2.69                             | 2.65                            | 2.66                            |
| Outlier regions     | 0                                  | 0                                  | 0                                | 0                               | 0                               |

\textsuperscript{a} The values in parentheses are for the highest-resolution shell.

\textsuperscript{b} Calculated with AIMLESS [42].

\textsuperscript{c} R\textsuperscript{meas} = \frac{\sum|hkl|\sqrt{N(hkl)}{\sum|hkl|}}, where \sqrt{N(hkl)} is the intensity of the \textsuperscript{ith} measurement of an equivalent reflection with indices hkl.

\textsuperscript{d} R\textsuperscript{pim} = \frac{\sum|hkl|\sqrt{|F_{obs}|}{\sum|hkl|\sqrt{|F_{calc}|}}, where |F_{obs}| and |F_{calc}| are the observed and calculated structure factor amplitudes.

\textsuperscript{e} R\textsuperscript{work} is equivalent to R\textsuperscript{work} but calculated with reflections (5-10%) omitted from the refinement process.

\textsuperscript{f} R\textsuperscript{free} is equivalent to R\textsuperscript{work} but calculated with reflections (5-10%) omitted from the refinement process.
Table B. Polar interactions in the UcaD\textsuperscript{1-D}: monosaccharide complexes\textsuperscript{1}.

| PROTEIN ATOM | Ligand atom | Distance (Å) | Ligand atom | Distance (Å) | Ligand atom | Distance (Å) |
|--------------|-------------|--------------|-------------|--------------|-------------|--------------|
| N63 NE2      | O4          | 3            | O2          | 2.9          | O5          | 3.3          |
| N63 NE2      | -           | -            | -           | -            | O6          | 2.9          |
| N63 O        | O3          | 2.7          | O3          | 2.8          | O1          | 2.7          |
| W124 NE1     | O5          | 3.2          | O5          | 3.1          | O4          | 2.8          |
| W124 NE1     | O4          | 3.1          | -           | -            | -           | -            |
| S157 OG      | -           | -            | O2          | 2.8          | O1          | 2.8          |
| G159 N       | O3          | 3            | O3          | 3.1          | O1          | 3.4          |
| G159 N       | O2          | 2.9          | O4          | 2.9          | O2          | 2.8          |
| HOH1\textsuperscript{2} | O2     | 2.6          | O4          | 2.6          | O2          | 2.6          |

\textsuperscript{1}Bond distances are based on the distances between nitrogen and oxygen atoms and do not include hydrogen atoms. \textsuperscript{2}The water molecule is displayed in Figure 5.
**Table C: List of strains and plasmids used in this study.**

| Strains or plasmids | Relevant description | Reference(s) or source |
|---------------------|----------------------|------------------------|
| **Strains**         |                      |                        |
| MS528               | *E. coli* K-12 MG1655::fim::flu | [30]                   |
| BL21(DE3) pLys      |                      |                        |
| F11                 | UPEC cystitis isolate | [30-32]                |
| F11::lacI-Z        |                      | This study             |
| F11::lacI-Z-Puc::lacZ |                      | This study             |
| F11::Puc<sup>TIRG</sup> |                      | This study             |
| F11::lacI-Z-Puc<sup>TIRG</sup>::lacZ |                      | This study             |
| F11oxyR             |                      | This study             |
| F11::Puc<sup>TIRG</sup> oxyR |                      | This study             |
| UTI89               | UPEC cystitis isolate | [33, 34]               |
| UTI89::ucl         | UTI89::uclA-D::cm; Cm<sup>r</sup> | This study             |
| UTI89::lacI-Z      | UTI89::lacI-Z::gfp   | This study             |
| UTI89::Puc<sup>C88T</sup> | UTI89::Puc<sup>C88T</sup> | This study             |
| S77EC               | Clinical ST131 isolate | [35]                   |
| S77EC::ucl         | S77EC::uclA-D::cm; Cm<sup>r</sup> | This study             |
| HVM1299             | Clinical ST131 isolate | [35]                   |
| HVM1299::ucl       | HVM1299::uclA-D::cm; Cm<sup>r</sup> | This study             |
| TOP10 pSU2718::uclABCD | *E. coli* TOP10 + pSU2718::uclABCD (pUcl) | This study             |
| MS528 pSU2718::uclABCD | *E. coli* K-12 MG1655::fim::flu pSU2718::uclABCD (pUcl) | This study             |
| *P. mirabilis* PMS4 | UTI clinical isolate | [36]                   |
| **Plasmids**        |                      |                        |
| pKOPEG              | λ-Red recombinase expression vector | [37]                   |
| pCP20               | FLP flipase expression vector | [37]                   |
| pSU2718             | pACYC184-derived cloning plasmid | [38]                   |
| pQF50               | Promoterless lacZ reporter plasmid | [39]                   |
| pQF50::Puc<sub>f11</sub> | ucl promoter region from F11 cloned in pQF50 | This study             |
| pQF50::Puc<sub>UTI89</sub> | ucl promoter region from UTI89 cloned in pQF50 | This study             |
| pSU2718::uclABCD (pUcl) | uclABCD operon from F11 cloned into pSU2718 | This study             |
| pBAD/myc-HisA       | Arabinose-inducible promoter, Amp<sup>+</sup> | [40]                   |
| pOxyR               | oxyR gene from *E. coli* MG1655 in pBAD/myc-HisA (pMGJ1) | [41]                   |
| pOxyR-6xHis         | pOxyR modified to encode OxyR containing C-terminal 6xHistag | This study             |
| pET22b              | Expression vector, T7 promoter, N-ter pelB signal sequence, C-ter 6xHis tag, Amp<sup>+</sup> | Novagen                |
| pET22b::uclD       | Lectin binding domain of uclD from F11 cloned into pET22b | This study             |
| pET22b::ucaD       | Lectin binding domain of ucaD from PMS4 cloned into pET22b | This study             |
Table D: List of primers used in this study.

| Primer name | Sequence (5’ to 3’) |
|-------------|---------------------|
| **Mutant generation** | |
| 6310-ucl.cm.1 | tagcaagcgtgacgtaacag |
| 6311-ucl.cm.2 | cacagcagaacaataagtcag |
| 6312-ucl.cm.3 | gcatgtgcctatgtttggtgcgtgattcttgctctattcc |
| 6313-ucl.cm.4 | cacttcaggtgtgctacgacaaagtcgtgctgtgagtcctagcctagt |
| 6314-ucl.cm.5 | ttgctgatgcaactacacctg |
| 6315-ucl.cm.6 | tagtcgtcttgcgtatcct |
| 5080-oxyR.1 | caactcttcctgcagcagca |
| 5078-oxyR.2 | gcaggtgatggtgtcagaa |
| 3842-oxyR.3 | ggaataggaactaaggagtgcacacgtactcaagat |
| 3843-oxyR.4 | ctctacacaaactgcagcacaataagatctagctg |
| 5079-oxyR.5 | aggtcaacagcatgtatcag |
| 5081-oxyR.6 | actgtgcgctcattgcag |
| 4057-lacIZ::cm-gfp 700bp F | tegcttcctcctccttct |
| 4058-lacIZ::cm-gfp 700bp R | getaataagcggaggtgctg |
| 7900_uclAprom_TtoG | a*t*ggtttttgatcgtttttcgcagtcaatcatgataGaGgtgaactctacatagctggtttagttagctgtaaaatctattt*g |
| 7901_uclAprom_GtoT | a*t*ggtttttgatcgtttttcgcagtcaatcatgataTtgtaactctacatagctggtttagttagctgtaaaatctattt*g |
| **5’ RACE** | |
| 6973-PuclA-5’ RACE GSP1 | cttgcgactattcatacc |
| 6974-PuclA-5’ RACE GSP2 | gaaigcgaaggggtcgag |
| **Cloning** | |
| 6571-BamHI-Pucl-F | cgcgggatatcgccgggtcgaggtggtgtaat |
| 6572-HindIII-Pucl-R | cccgggagaacgatcaggtatcttctggtgagc |
| 6568-uclABCD-F | cgcgggactttgtggtggtggtgagtcg |
| 6569-uclABCD-R | cccggtcttagattgtgatcaggtaggaagataagtgg |
| 11190_OxyR_ceterGG6xHis_F | ggccgggtcatcactcactcatcactcactcactcactcactcactcact |
| 11191_OxyR_ceterGG6xHis_R | aacgeactgttaaaactcact |
| **Gel shift assay** | |
| 11206_PuclA-F2 | aataggtgcctttcagag |
| 11207_PuclA-F2-cy3 | cy3-aattaggtgcctttcagag |
| 11208_PuclA-R2 | cagcagaaaaaattcactg |
| 11193_uClC-F | tcatgcctgattgcattg |
| 11196_uClC-R2 | aataggtgcctttcagag |


### Table E. Glycan array analysis of UclD and UcaD

| Glycan structure                  | ID | UclD  | UcaD  |
|-----------------------------------|----|-------|-------|
| **Monosaccharides**               |    |       |       |
| Fucα-sp3                          | 1  | <1    | <1    |
| Galα-sp3                          | 2  | <1    | <1    |
| Galβ-sp3                          | 3  | <1    | <1    |
| GalNAcα-sp0                       | 4  | <1    | <1    |
| GalNAcα-sp3                       | 5  | <1    | <1    |
| GalNAcβ-sp3                       | 6  | <1    | <1    |
| Glcα-sp3                          | 7  | <1    | <1    |
| Glcβ-sp3                          | 9  | <1    | <1    |
| GlcNAcβ-sp3                       | 10 | <1    | <1    |
| GlcN(Glc)β-sp4                    | 14 | <1    | <1    |
| HOCH2(HOCH)4CH2NH2                | 15 | <1    | <1    |
| Manα-sp3                          | 16 | <1    | <1    |
| Manβ-sp4                          | 18 | <1    | <1    |
| ManNAcβ-sp4                       | 19 | <1    | <1    |
| Rhaα-sp3                          | 20 | <1    | <1    |
| GlcNAcβ-sp4                       | 22 | <1    | <1    |
| 3-O-Su-Galβ-sp3                   | 37 | <1    | <1    |
| 3-O-Su-GalNAcα-sp3                | 38 | <1    | <1    |
| 6-O-Su-GlcNAcβ-sp3                | 43 | <1    | <1    |
| GlcAα-sp3                         | 44 | <1    | <1    |
| GlcAβ-sp3                         | 45 | <1    | <1    |
| 6-H2PO3Glcβ-sp4                   | 46 | <1    | <1    |
| 6-H2PO3Manα-sp3                   | 47 | <1    | <1    |
| Neu5Aca-sp3                       | 48 | <1    | <1    |
| Neu5Aca-sp9                       | 49 | <1    | <1    |
| Neu5Gcα-sp3                       | 52 | <1    | <1    |
| 9-Nac-Neu5Acaα-sp3                | 54 | <1    | <1    |
| 3-O-Su-GlcNAcβ-sp3                | 55 | <1    | <1    |
| **Terminal Galactose**            |    |       |       |
| Galα1-2Galβ-sp3                   | 75 | <1    | <1    |
| Galα1-3Galβ-sp3                   | 76 | <1    | <1    |
| Galα1-3GalNAcβ-sp3                | 77 | <1    | <1    |
| Galα1-3GalNAcα-sp3                | 78 | <1    | <1    |
| Galα1-3GlcNAcβ-sp3                | 80 | <1    | <1    |
| Galα1-4GlcNAcβ-sp3                | 81 | <1    | <1    |
| Galα1-6Glcβ-sp4                   | 83 | <1    | <1    |
| Galβ1-2Galβ-sp3                   | 84 | <1    | <1    |
| Galβ1-3GlcNaAcβ-sp3               | 85 | <1    | <1    |
| Galβ1-3Galβ-sp3                   | 87 | <1    | <1    |
| Galβ1-3GalNAcβ-sp3                | 88 | <1    | <1    |
| Galβ1-3GalNAcα-sp3                | 89 | <1    | <1    |
| Galβ1-4Glcβ-sp4                   | 93 | <1    | <1    |
| Galβ1-4Galβ-sp4                   | 94 | <1    | <1    |
| Galβ1-4GlcNAcβ-sp3                | 97 | <1    | <1    |
| Galβ1-6Galβ-sp4                   | 100| <1    | <1    |
| Galβ1-3(6-O-Su)GlcNAcβ-sp3        | 145| <1    | <1    |
| Galβ1-4(6-O-Su)Glcβ-sp2           | 146| <1    | <1    |
| Linkage | Branching | Value   | p-value |
|---------|-----------|---------|---------|
| Galβ1-4(6-O-Su)GlcNAcβ-sp3 | 147 | <1 | <1 |
| 3-O-Su-Galβ1-3GlcNAcα-sp3 | 150 | <1 | <1 |
| 6-O-Su-Galβ1-3GlcNAcα-sp3 | 151 | <1 | <1 |
| 3-O-Su-Galβ1-4Glcβ-sp2 | 152 | <1 | <1 |
| 6-O-Su-Galβ1-4Glcβ-sp2 | 153 | <1 | <1 |
| 3-O-Su-Galβ1-3GlcNAcβ-sp3 | 155 | <1 | <1 |
| 3-O-Su-Galβ1-4GlcNAcβ-sp3 | 157 | <1 | <1 |
| 4-O-Su-Galβ1-4GlcNAcβ-sp3 | 159 | <1 | <1 |
| 6-O-Su-Galβ1-3GlcNAcβ-sp3 | 161 | <1 | <1 |
| 6-O-Su-Galβ1-4GlcNAcβ-sp3 | 163 | <1 | <1 |
| 3-O-Su-Galβ1-4(6-O-Su)Glcβ-sp2 | 176 | <1 | <1 |
| 3-O-Su-Galβ1-4(6-O-Su)GlcNAcβ-sp2 | 177 | <1 | <1 |
| 6-O-Su-Galβ1-4(6-O-Su)Glcβ-sp2 | 178 | <1 | <1 |
| 6-O-Su-Galβ1-3(6-O-Su)GlcNAcβ-sp2 | 179 | <1 | <1 |
| 6-O-Su-Galβ1-4(6-O-Su)GlcNAcβ-sp2 | 180 | <1 | <1 |
| 3,4-O-Su2-Galβ1-4GlcNAcβ-sp3 | 181 | <1 | <1 |
| 3,6-O-Su2-Galβ1-4GlcNAcβ-sp2 | 182 | <1 | <1 |
| 4,6-O-Su2-Galβ1-4GlcNAcβ-sp2 | 183 | <1 | <1 |
| 4,6-O-Su2-Galβ1-4GlcNAcβ-sp3 | 184 | <1 | <1 |
| 3,6-O-Su2-Galβ1-4(6-O-Su)GlcNAcβ-sp2 | 189 | <1 | <1 |
| 3,4-O-Su2-Galβ1-4GlcNAcβ-sp3 | 201 | <1 | <1 |
| Galβ1-4(6-O-Su)GlcNAcβ-sp2 | 203 | <1 | <1 |
| Galα1-3Galβ1-4Glcβ-sp2 | 220 | <1 | <1 |
| Galα1-3Galβ1-4GlcNAcβ-sp3 | 222 | <1 | <1 |
| Galα1-4Galβ1-4Glcβ-sp3 | 224 | <1 | <1 |
| Galα1-4Galβ1-4GlcNAc-sp2 | 225 | <1 | <1 |
| Galβ1-2Galα1-4GlcNAcβ-sp4 | 228 | <1 | <1 |
| Galβ1-3Galβ1-4GlcNAcβ-sp4 | 229 | <1 | <1 |
| Galβ1-4GlcNAcβ1-3GlcNAcα-sp3 | 231 | <1 | 5.882±1.12 |
| Galβ1-4GlcNAcβ1-6GlcNAcα-sp3 | 232 | <1 | <1 |
| Galβ1-3(GlcNAcβ1-6)GlcNAcα-sp3 | 254 | <1 | <1 |
| Galβ1-3GlcNAcβ1-3Glc-sp4 | 262 | <1 | 4.944±0.98 |
| Galβ1-4Galβ1-4GlcNAc-sp3 | 264 | <1 | <1 |
| Galα1-3Galβ1-4GlcNAcβ1-3Galβ-sp3 | 373 | <1 | <1 |
| Galα1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp3 | 375 | <1 | <1 |
| Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ-sp4 | 376 | <1 | <1 |
| Galβ1-3GlcNAcβ1-3Galβ1-3GlcNAcβ-sp2 | 377 | <1 | <1 |
| Galβ1-3GlcNAcα1-3Galβ1-4GlcNAcβ-sp3 | 378 | <1 | <1 |
| Galβ1-3GlcNAcα1-3Galβ1-4GlcNAcβ-sp3 | 379 | <1 | <1 |
| Galβ1-3GlcNAcα1-6Galβ1-4GlcNAcβ-sp2 | 380 | <1 | <1 |
| Galβ1-3GlcNAcα1-6Galβ1-4GlcNAcβ-sp2 | 381 | <1 | <1 |
| Galβ1-3GlcNAcβ1-4Galβ1-4Glcβ-sp3 | 382 | <1 | <1 |
| Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ-sp2 | 383 | <1 | <1 |
| Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp3 | 385 | <1 | <1 |
| Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAcβ-sp2 | 387 | <1 | <1 |
| Galβ1-4GlcNAcβ1-6(Galβ1-3)GlcNAcα-sp3 | 388 | <1 | <1 |
| Galβ1-4GlcNAcβ1-6Galβ1-4GlcNAcβ1-3)GlcNAcα-sp3 | 488 | <1 | 5.566±1.08 |
| Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)GlcNAcα-sp3 | 504 | <1 | <1 |
| Galβ1-3GlcNAc | 1A | <1 | <1 |
| Galβ1-4GlcNAc | 1B | <1 | <1 |
| Galβ1-4Gal | 1C | <1 | 2.886±0.77 |
| Galβ1-6GlcNAc | 1D | <1 | <1 |
Galβ1-3GalNAc  
Galβ1-3GlcNAcβ1-4Galβ1-4Glc  
Galβ1-3GlcNAcβ1-3Galβ1-4Glc  
Galβ1-4GlcNAcβ1-3Galβ1-4Glc  
Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3)Galβ1-4Glc  
Galβ1-4GlcNAcβ1-6(Galβ1-3GlcNAcβ1-3)Galβ1-4Glc  
Gala1-4Galβ1-4Glc  
GalNAcα1-O-Ser  
Galβ1-3GalNAcα1-O-Ser  
Gala1-3Gal  
Gala1-3Galβ1-4GlcNAc  
Gala1-3Galβ1-4Glc  
Gala1-3Galβ1-4Galα1-3Gal  
Galβ1-6Gal  
GalNAcβ1-3Gal  
GalNAcβ1-4Gal  
Gala1-4Galβ1-4GlcNAc  
GalNAcα1-3Galβ1-4Glc  
Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-6(Galβ1-3GlcNAcβ1-3)Galβ1-4Glc  
Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc  
Galβ1-3GalNAcβ1-4Galα1-4Galβ1-4Glc  
Galβ1-3GalNAcβ1-3Gal  
Galβ1-4Glc  
Galβ1-4Gal  
Galβ1-6Gal  

Terminal N-Acetylgalactosamine
GalNAcα1-3GalNAcβ-sp3  
GalNAcα1-3Galβ-sp3  
GalNAcα1-3GalNAcα-sp3  
GalNAcβ1-3Galβ-sp3  
GalNAcβ1-4GlcNAcβ-sp3  
GalNAcβ1-4(6-O-Su)GlcNAcβ-sp3  
3-O-Su-GalNAcβ1-4GlcNAcβ-sp3  
6-O-Su-GalNAcβ1-4GlcNAcβ-sp3  
6-O-Su-GalNAcβ1-4(3-O-Su)GlcNAcβ-sp3  
3-O-Su-GalNAcβ1-4(3-O-Su)-GlcNAcβ-sp3  
3,6-O-Su2-GalNAcβ1-4GlcNAcβ-sp3  
4,6-O-Su2-GalNAcβ1-4GlcNAcβ-sp3  
4,6-O-Su2-GalNAcβ1-4(3-O-Ac)GlcNAcβ-sp3  
4-O-Su-GalNAcβ1-4GlcNAcβ-sp3  
6-O-Su-GalNAcβ1-4(6-O-Su)GlcNAcβ-sp3  
4-O-Su-GalNAcβ1-4GlcNAcβ-sp2  
GalNAcβ1-4Galβ1-4Glcβ-sp3  
GalNAcβ1-3Galα1-4Galβ1-4Glcβ-sp3  
GalNAcα1-O-Ser  
GalNAcβ1-3Gal  
GalNAcβ1-4Gal  
GalNAcα1-3Galβ1-4Glc  

Terminal N-Acetylgalactosamine
GlcNAcβ1-3GalNAcα-sp3  
GlcNAcβ1-3Manβ-sp4  

30
| Structured Formulation | Masses (Da) | Error (ppm) |
|------------------------|-------------|-------------|
| Galβ1-3(Fucα1-4)GlcNAcβ-sp3 | 233 | <1 |
| Fucα1-3(Galβ1-4)GlcNAcβ-sp3 | 234 | <1 |
| Fucα1-2(GalNAcα1-3)Galβ-sp3 | 235 | <1 |
| 3-O-Su-Galβ1-3(Fucα1-4)GlcNAcβ-sp3 | 287 | <1 |
| Fucα1-3(3-O-Su-Galβ1-4)GlcNAcβ-sp3 | 288 | <1 |
| Fucα1-2(Galα1-3)Galβ1-3GlcNAcβ-sp3 | 359 | <1 |
| Fucα1-2(Galα1-3)Galβ1-4GlcNAcβ-sp3 | 360 | <1 |
| Fucα1-2(Galα1-3)Galβ1-3GalNAcα-sp3 | 362 | <1 |
| Fucα1-2(Galα1-3)Galβ1-3GalNAcβ-sp3 | 363 | <1 |
| Fucα1-2(Galα1-3)Galβ1-4GlcNAcβ-sp3 | 364 | <1 |
| Fucα1-2(GalNAcα1-3)Galβ1-3GlcNAcβ-sp3 | 366 | <1 |
| Fucα1-2(GalNAcα1-3)Galβ1-4GlcNAcβ-sp3 | 368 | <1 |
| Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ-sp3 | 371 | <1 |
| Fucα1-3(Fucα1-2Galβ1-4)GlcNAcβ-sp3 | 372 | <1 |
| Fucα1-2(GalNAcα1-3)Galβ1-3GalNAcα-sp3 | 392 | <1 |
| Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ-sp4 | 479 | <1 |
| Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ-sp2 | 480 | <1 |
| Fucα1-3(Fucα1-2(Gala1-3)Galβ1-4)GlcNAcβ-sp3 | 483 | <1 |
| Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glcβ-sp4 | 496 | <1 |
| Fucα1-3(Fucα1-2Galβ1-4)GlcNAcβ1-3Galβ1-4Glcβ-sp4 | 497 | <1 |
| Lex1-6(Lec1-3')Lac-sp4 | 538 | <1 |
| LacNAc1-6'(Led1-3')Lac-sp4 | 539 | <1 |
| Lex1-6'(Ledi-3')Lac-sp4 | 541 | <1 |
| LecLex1-6'(Leci-3')Lac-sp4 | 542 | <1 |
| Lex1-6'(Lebi-3')Lac-sp4 | 543 | <1 |
| Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc | 7A | <1 |
| Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc | 7B | <1 |
| Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc | 7C | <1 |
| Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc | 7D | <1 |
| Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc | 7E | <1 |
| Fucα1-2Gal | 7F | <1 |
| Fucα1-2Galβ1-4Glc | 7G | <1 |
| Galβ1-4(Fucα1-3)Glc | 7H | <1 |
| Galβ1-4(Fucα1-3)GlcNAc | 7I | <1 |
| Galβ1-3(Fucα1-4)GlcNAc | 7J | <1 |
| GalNAcα1-3(Fucα1-2)Gal | 7K | <1 |
| Fucα1-2Galβ1-4(Fucα1-3)Glc | 7L | <1 |
| Galβ1-3(Fucα1-2)Glc | 7M | <1 |
| Fucα1-2Galβ1-4(Fucα1-3)GlcNAc | 7N | <1 |
| Fucα1-2Galβ1-3GlcNAc | 7O | <1 |
| Fucα1-2Galβ1-3(Fucα1-4)GlcNAc | 7P | <1 |
| SO3-3Galβ1-3(Fucα1-4)GlcNAc | 8A | <1 |
| SO3-3Galβ1-4(Fucα1-3)GlcNAc | 8B | <1 |
| Galβ1-3GlcNAcβ1-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc | 8C | <1 |
| Galβ1-4(Fucα1-3)GlcNAcβ1-6(Galβ1-3GlcNAcβ1-3)Galβ1-4Glc | 8D | <1 |
| Galβ1-4(Fucα1-3)GlcNAcβ1-6(Fucα1-2Galβ1-3GlcNAcβ1-3)Galβ1-4Glc | 8E | <1 |
| Galβ1-4(Fucα1-3)GlcNAcβ1-6(Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3)Galβ1-4Glc | 8F | <1 |
| Galβ1-4GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc | 8G | <1 |
Fuca1-2Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc  
Fuca1-3Galβ1-4GlcNAcβ1-3Galβ1-4(Fuca1-3)Gle  
Fuca1-2Galβ1-4(Fuca1-3)GlcNAcβ1-3(Fuca1-2)Galβ1-4Gle  
Galβ1-4(Fuca1-3)GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3)Galβ1-4Gle  
Galβ1-4(Fuca1-3)GlcNAcβ1-6(Galβ1-4(Fuca1-3)GlcNAcβ1-3)Galβ1-4Gle  
Galβ1-4(Fuca1-3)GlcNAcβ1-6(9Galβ1-4(Fuca1-3)GlcNAcβ1-3)Galβ1-4Gle  
8H <1 <1

Fuca1-2Galβ1-4(Fuca1-3)GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3)Galβ1-4Gle  
Galβ1-3GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-6(Galβ1-3GlcNAcβ1-3)Galβ1-4Gle  
8M <1 <1

Fuca1-2Galβ1-3GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-6(Galβ1-3GlcNAcβ1-3)Galβ1-4Gle  
GalNAcβ1-3(Fuca1-2)Galβ1-4Gle  
8P <1 <1

Fuca1-3(Fuca1-2)Galβ1-4(Fuca1-3)GlcNAcβ1-6(Galβ1-2Galβ1-3GlcNAcβ1-3)Galβ1-4Gle  
Galα1-3(Fuca1-2)Galβ1-4Gle  
9B <1 <1

Sialylated  
Neu5Acα2-3Galβ-sp3  
169 <1 <1

Neu5Acα2-6Galβ-sp3  
170 <1 <1

Neu5Acα2-3GlcNAcα-sp3  
171 <1 <1

Neu5Acα2-6GlcNAcα-sp3  
172 <1 <1

Neu5Gcaα2-6GlcNAcα-sp3  
174 <1 <1

Neu5Acα2-8Neu5Acα2-sp3  
186 <1 <1

Neu5Acα2-6GlcNAcβ-sp3  
205 <1 <1

Neu5Gcaα2-3Galα-sp3  
206 <1 <1

Galα1-3(Neu5Acα2-6)GlcNAcα-sp3  
289 <1 <1

Galβ1-3(Neu5Acα2-6)GlcNAcα-sp3  
290 <1 <1

Neu5Acα2-3Galβ1-3GlcNAcα-sp3  
292 <1 <1

Neu5Acα2-3Galβ1-4Glcβ-sp3  
293 <1 <1

Neu5Acα2-3Galβ1-4Glcβ-sp4  
294 <1 <1

Neu5Acα2-6Galβ1-4Glcβ-sp2  
295 <1 <1

Neu5Acα2-3Galβ1-4GlcNAcβ-sp3  
298 <1 <1

Neu5Acα2-3Galβ1-3GlcNAcβ-sp3  
299 <1 <1

Neu5Acα2-6Galβ1-4GlcNAcβ-sp3  
300 <1 <1

Neu5Gcaα2-3Galβ1-4GlcNAcβ-sp3  
303 <1 <1

Neu5Gcaα2-6Galβ1-4GlcNAcβ-sp3  
304 <1 <1

9-NAc-Neu5Acα2-6Galβ1-4GlcNAcβ-sp3  
306 <1 <1

Neu5Acα2-3Galβ1-4-(6-O-Su)GlcNAcβ-sp3  
315 <1 <1

Neu5Acα2-3Galβ1-3-(6-O-Su)GlcNAcβ-sp3  
317 <1 <1

Neu5Acα2-3Galβ1-4-(6-O-Su)GlcNAcβ-sp3  
318 <1 <1

Neu5Acα2-3-(6-O-Su)Galβ1-4GlcNAcβ-sp3  
319 <1 <1

9A <1 <1

(9A) <1 <1

Fucα1-3(Neu5Acα2-3Galβ1-4)GlcNAcβ-sp3  
423 <1 <1

Neu5Acα2-3Galβ1-3(Fuca1-4)GlcNAcβ-sp3  
426 <1 <1

Fuca1-3(Neu5Acα2-3Galβ1-4)6-O-Su-GlcNAcβ-sp3  
428 <1 <1

9B <1 <1

L224±0.38
Fuca1-3(Neu5Aca2-3(6-O-Su)Galβ1-4)GlcNAcβ-sp3 429 <1 6.012±1.14
Neu5Aca2-3Galβ1-3(Neu5Aca2-6)GalNAcα-sp3 433 <1 <1
Neu5Aca2-8Neu5Aca2-3Galβ1-4Glcβ-sp4 434 <1 <1
Neu5Aca2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp2 527 <1 <1
Fuca1-3(Neu5Aca2-3Galβ1-4)GlcNAcβ1-3Galβ3-sp3 528 <1 <1
Neu5Aca2-6Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ-sp4 529 <1 <1
GalNAcβ1-4(Neu5Aca2-8Neu5Aca2-3)Galβ1-4Glc-sp2 531 <1 <1
Neu5Aca2-8Neu5Aca2-8Neu5Aca2-3Galβ1-4Glc-sp2 532 <1 6.882±1.44
(Neu5Aca2-8)2Neu5Aca2-3(GalNAcβ1-4)Galβ1-4Glc-sp2 533 <1 <1
Neu5Aca2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-sp3 534 <1 <1
Neu5Aca2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ-sp4 536 <1 <1
Neu5Aca2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ-sp4 537 <1 <1
Lex1-6(6'SLN1-3')Lac-sp4 540 <1 <1
Neu5Aca2-3Galβ1-3(Fuca1-4)GlcNAc 10A <1 2.437±0.58
Neu5Aca2-3Galβ1-4(Fuca1-3)GlcNAc 10B 1.211±0.38 <1
Neu5Aca2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc 10C <1 <1
Galβ1-4(Fuca1-3GlcNAcβ1-6(Neu5Aca2-6Galβ1-4GlcNAcβ1-3)Galβ1-4Glc 10D <1 <1
Neu5Aca2-3Galβ1-3(Neu5Aca2-6)GalNAc 10E <1 <1
Neu5Aca2-6Galβ1-3GlcNAcβ1-3Galβ1-4(Fuca1-3)Glc 10H 6.445±0.84 <1
Galβ1-3GlcNAcβ1-3(Neu5Aca2-6Galβ1-4GlcNAcβ1-3)Galβ1-4Glc 10I <1 <1
Neu5Aca2-6Galβ1-3GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc 10J <1 <1
Neu5Aca2-3Galβ1-4GlcNAc 10K <1 <1
Neu5Aca2-6Galβ1-4GlcNAc 10L <1 <1
Neu5Aca2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc 10M <1 <1
Galβ1-3(Neu5Aca2-6)GlcNAcβ1-3Galβ1-4Glc 10N <1 <1
Neu5Aca2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc 10O 1.291±0.64 <1
Neu5Aca2-3Galβ1-3(Neu5Aca2-6)GlcNAcβ1-3Galβ1-4Glc 10P <1 <1
Neu5Aca2-3Galβ1-4Glc 11A <1 <1
Neu5Aca2-6Galβ1-4Glc 11B <1 <1
(Neu5Aca2-8Neu5Aca)n (n<50) 11C <1 <1

**Gluco**

Glcα1-4Glcβ-sp3 110 <1 <1
Glcβ1-4Glcβ-sp4 111 <1 <1
Glcβ1-6Glcβ-sp4 112 <1 <1
GlcAβ1-3GlcNAcβ-sp3 164 <1 <1
GlcAβ1-3Glcβ-sp3 165 <1 <1
GlcAβ1-6Glcβ-sp3 166 <1 <1
(Glcα1-4)β-sp4 240 <1 <1
(Glcα1-6)β-sp4 241 <1 <1
(Glcα1-4)β-sp4 390 <1 <1
(Glcα1-6)β-sp4 391 <1 <1
(Glcα1-6)β-sp4 492 <1 <1
(Glcα1-6)β-sp4 502 <1 <1
GlcA 18I <1 <1
6-O-(H2PO4)-Glc 18J <1 <1
Glcα1-4Glcα1-4 19O <1 <1
Glcα1-4Glcα1-4 19P <1 <1

**LMW GAGs**

Neocarratetraose-41, 3-di-O-sulphate (Na+) 12A <1 <1
Neocarratetraose-41-O-sulphate (Na+) 12B 1 1
Neocarraxehaxoase-24,41, 3, 5-tetra-O-sulphate (Na+) 12C 1 1
Neocarraxehaxoase-41, 3, 5-tri-O-sulphate (Na+) 12D 1 1
Neocarroaetaose-41, 3, 5, 7-tetra-O-sulphate (Na+) 12E 1 1
Neocarradecacoae-41, 3, 5, 7, 9-penta-O-sulphate (Na+) 12F 1 1
ΔUA-2S-GlcNS-6S 12G 1 1
ΔUA-GlucNS-6S 12H 1 1
ΔUA-2S-GlucNS 12I 1 1
ΔUA-2S-GlcNac-6S 12J 1 1
ΔUA-GlcNac-6S 12K 1 1
ΔUA-2S-GlcNAc 12L 1 1
ΔUA-GlcNac 12M 1 1
ΔUA-GalNac-4S (Delta Di-4S) 12N 1 1
ΔUA-GalNAc-6S (Delta Di-6S) 12O 1 1
ΔUA-GalNAc-4s,6S (Delta Di-disE) 12P 1 1
ΔUA-2S-GalNAc-4S (Delta Di-disB) 13A 1 1
ΔUA-2S-GalNAc-6S (Delta Di-disB) 13B 1 1
ΔUA-2S-GalNAc-4S-6S (Delta Di-tisS) 13C 1 1
ΔUA-2S-GalNAc-6S (Delta Di-UA2S) 13D 1 1
ΔUA-GlcNac (Delta Di-HA) 13E 1 1
ΔUA→2S-GlcN-6S 14M 1 1
ΔUA→GlcN-6S 14N 1 1
ΔUA→2S-GlcN 14O 1 1
ΔUA→GlcN 14P 1 1

**HMW GAGs**

(GlcAβ1-4GlcNAcβ1-3)s-NH2-ol 625 1 1
(GlcAβ1-3GlcNAcβ1-4)n (n=4) 13F 1 1
(GlcAβ1-3GlcNAcβ1-4)n (n=8) 13G 1 1
(GlcAβ1-3GlcNAcβ1-4)n (n=10) 13H 1 1
(GlcAβ1-3GlcNAcβ1-4)n (n=12) 13I 1 1
(GlcA/IdoAα/β1-4GlcNAcα1-4)n (n=200) 13J 1 1
(GlcA/IdoAβ1-3(±/4/6S)GalNAcβ1-4)n (n<250) 13K 1 1
((±2S)GlcA/IdoAα/β1-3(±4S)GalNAcβ1-4)n (n<250) 13L 1 1
(GlcA/IdoAβ1-3(±6S)GalNAcβ1-4)n (n<250) 13M 1 1
HA - 4 10mM 13N 1 1
HA - 6 10mM 13O 1 1
HA - 8 9.7mM 13P 1 1
HA 10 7.83mM 14A 1 1
HA-12 6.5mM 14B 1 1
HA-14 5.6mM 14C 1 1
HA-16 4.9mM 14D 1 1
HA 30000 da 2.5mg/ml 14E 1 1
HA 107000 da 2.5mg/ml 14F 1 1
HA 190000 da 2.5 mg/ml 14G 1 1
HA 220000 da 2.5 mg/ml 14H 1 1
HA 1600000 da 2.5 mg/ml 14I 1 1
Heparin sulfate 5 mg/ml 14J 1 1
β1-3Glucan 14K 1 1

**Complex N-glycans**

(Sia2-6A-GN-M)-3,6-M-GN-GNβ-sp4 627 1 1
Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6Man)β1-4GlcNAcβ1-4(Fucα1-6)GlcNAc 19A 1 1
Galβ1-4GlcNAcβ1-2(Galβ1-4GlcNAcβ1-4)Manα1-3(Galβ1-4GlcNAcβ1-2(Galβ1-4GlcNAcβ1-6)Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc
Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc
Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc
Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc
Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc
Neu5Acα2-6Galβ1-4GlcNAcβ1-2(Neu5Acα2-6Galβ1-4GlcNAcβ1-4)Manα1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc
GlcNAcβ1-2(GlcNAcβ1-4)Manα1-3(GlcNAcβ1-2Manα1-6)GlcNAcβ1-4Manβ1-4GlcNAcβ1-4GlcNAc

19B <1 <1
19C <1 <1
19D <1 <1
19E <1 <1
19F <1 <1
19G <1 <1
19H <1 <1

Red indicates binding as shown in Fig 5. Binding indicates a value of greater than 1-fold of average background plus 3 standard deviations as described in the MIRAGE table (Table F).
Table F. Supplementary glycan microarray document based on MIRAGE guidelines DOI: 10.1093/glycob/cww118.

| Classification | Guidelines |
|----------------|------------|
| **1. Sample: Glycan Binding Sample** | |
| Description of Sample | Sample names: UclD<sup>LD</sup> and UcaD<sup>LD</sup>  
**Origin:** Produced as a recombinant protein in *E. coli*.  
**Method of preparation:**  
The preparation of UclD and UcaD are explained in the Materials and Methods section. |
| Sample modifications | UclD<sup>LD</sup> and UcaD<sup>LD</sup> are His-Tagged proteins. |
| Assay protocol | See *Materials and Methods*. |
| **2. Glycan Library** | |
| Glycan description for defined glycans | Glycans in this study are listed in Table E in S1 Text and as a published library in doi: 10.1371/journal.pntd.0004120. |
| Glycan description for undefined glycans | N/A. |
| Glycan modifications | Glycans were prepared in one of two ways for printing:  
1. Glycans (with IDs in number/letter format; e.g. 1A, 4C, 7K) were sourced commercially from Dextra Laboratories, Elicityl and Carbosythn and were |
made into glycoamines using the protocol published in Day et al 2009 (doi: 10.1371/journal.pone.0004927).

2. Glycans (with IDs in number only format) were obtained from Prof Nicolai Bovin and were modified with spacers as per DOI: 10.1073/pnas.0407902101. The library of these glycans was first published in DOI: 10.1016/j.molimm.2009.06.010

### 3. Printing Surface; e.g., Microarray Slide

| Description of surface | Epoxy activated glass microarray slides. |
|-----------------------|-----------------------------------------|
| Manufacturer          | ArrayIt SuperEpoxy 3 (SME3).            |
| Custom preparation of surface | N/A.                                      |
| Non-covalent Immobilisation | N/A.                                    |

### 4. Arrayer (Printer)

| Description of Arrayer | SpotBot® Extreme Protein Microarray Spotter (ArrayIt, California, USA). |
|------------------------|-------------------------------------------------------------------------|
| Dispensing mechanism   | Contact printing using 946NS6 pins with a 6 pin in a 3 columns x 2 rows configuration. |
| Glycan deposition      | Approximately 1.8 nl per spot is printed according to manufactures guidelines. Glycan were at 500 µM in 50:50 DMF:DMSO. |
| Printing conditions    | Array were printed with dehumidification at a maximum humidity of 60% relative humidity (Standard laboratory starting humidity of 75-90%) at 22°C. Glycans were left to react with the slide for at least 8 hours after the print was completed. |
### 5. Glycan Microarray with “Map”

**Array layout**
The array consists of a single array of glycans split between 6 pins (3 columns x 2 rows) with 4500μm row and column spacing. Each pin printed a 20 columns x 16 rows with 200μm spot spacing (centre to centre) with a minimum spot size of 100μm. Each sample is printed in quadruplicate with each of the 6 print areas including at least three negative control samples (print solution only) and two positive control samples consisting of one sample of fluoroscienamine and one sample of a mixture of rabbit anti-mouse antibody labelled with Alexa 555 and Alexa 647. Positive controls provide proof of successful immobilization of the amine reagents and provides for orientation for analysis. The antibodies also can provide controls for secondary antibodies used in experiments (if applicable).

**Glycan identification and quality control**
Arrays are quality controlled by a range of measures. 1. Each printed array is post print scanned to confirm deposition of the glycans on the array surface prior to neutralization of the remaining slide surface. 2. Post neutralized slides are scanned again to monitor for remaining autofluorescence. 3. Slides are assayed with fluorescently labeled lectins: WGA-Texas Red (EY Laboratories) and ConA-FITC (EY Laboratories).

### 6. Detector and Data Processing

| Scanning hardware | ProScanArray 4 laser (488 nM, 532 nM, 595 nM, 647 nM) scanner (Perkin Elmer). |
|-------------------|--------------------------------------------------------------------------------|
| **Scanner settings** | Scanning resolution: 10μM  
Laser channel: 532nM excitation / emission filter.  
PMT: 70% gain  
Scan powers: 100% laser power. |
| Image analysis software | ScanArray Express (Perkin Elmer). |
| Data processing | Data was exported as a CSV file and exported to Microsoft Excel. |

### 7. Glycan Microarray Data Presentation
**Data presentation**

Binding data is presented in Fig 5 together with SPR data. The yes/no binding including glycan identification is shown in Table E in S1 Text.

| **8. Interpretation and Conclusion from Microarray Data** |
|----------------------------------------------------------|
| **Data interpretation**                                  |
| We only use glycan arrays as a yes/no binding tool. Due to this we look only at binding that is unambiguously above background vs lack of binding above background. Average background + 3x standard deviation of the background of 20 sets of 4 spots of DMF:DMSO only spots is applied to determine if binding observed is significantly above background. Only spots with values equal to or greater than this value were considered as binding from data of any tested slide. These values are slide dependent. |
| **Conclusions**                                          |
| UcaD\(^{LD}\) and UclD\(^{LD}\) both recognize glycans but even though they are highly similar the glycans recognized are different. |
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