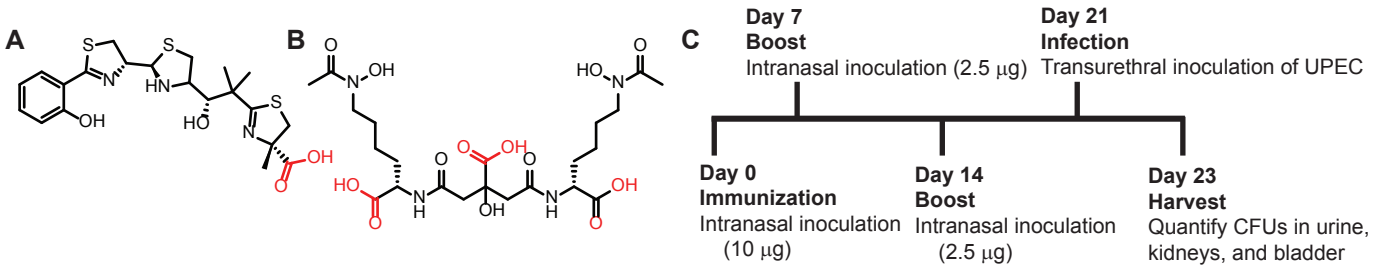
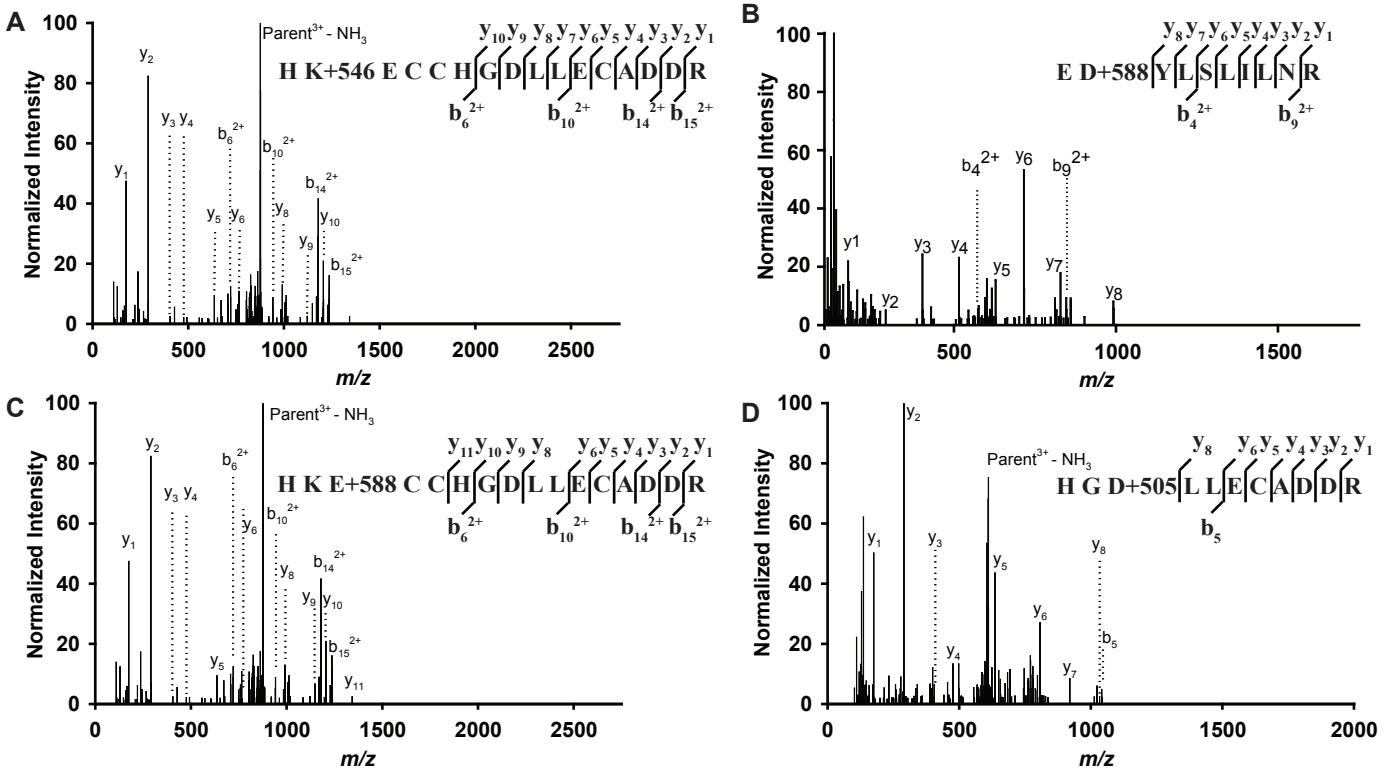
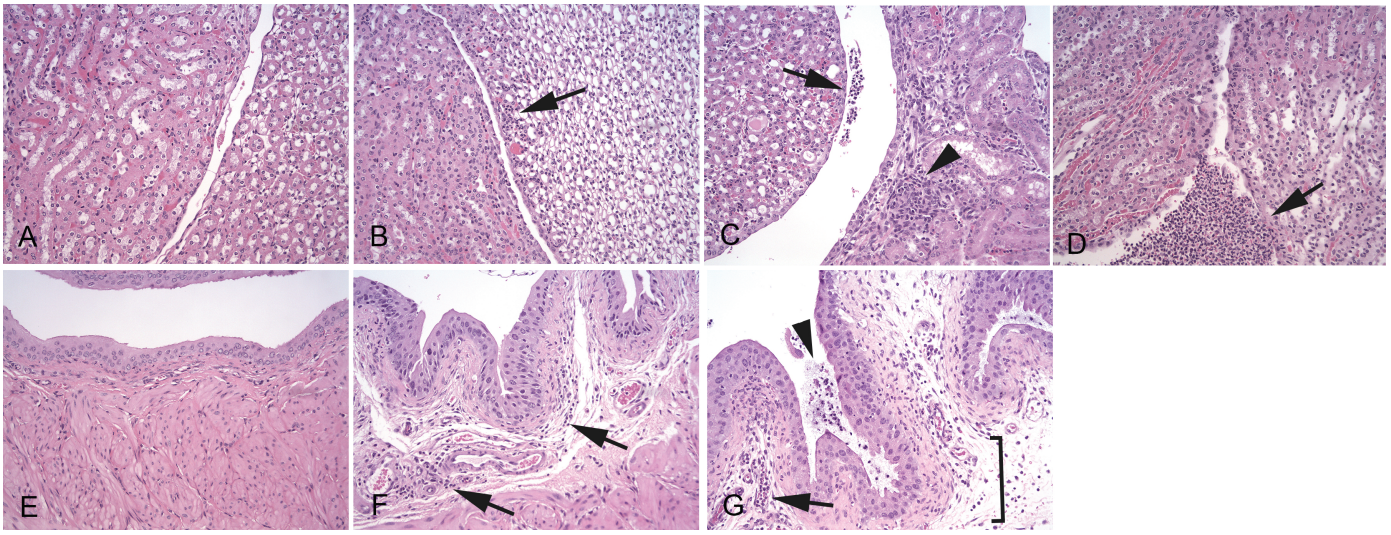
SI Appendix Figures



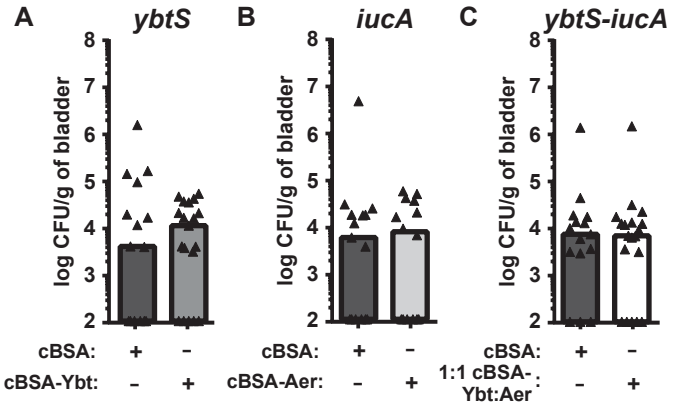
Supplemental Figure 1. Key components of the siderophore vaccine studies. Shown are the structures of (A) yersiniabactin (Ybt, MW 481.64) and (B) aerobactin (Aer, MW 564.55). The carboxylic acids used for conjugation to cBSA are highlighted in red. (C) The standard vaccine schedule is illustrated here. In brief, mice were intranasally immunized with 10 μg of vaccine prepared in 20 μL of PBS on day 0. On days 7 and 14 post-immunization, mice were intranasally boosted with 2.5 μg of vaccine prepared in 20 μL of PBS. The following week, mice were transurethrally inoculated with UPEC strain HM69. After 48 h, colony forming units (CFUs) in the urine, bladder, and kidneys were enumerated by serial dilution and plating on LB agar.



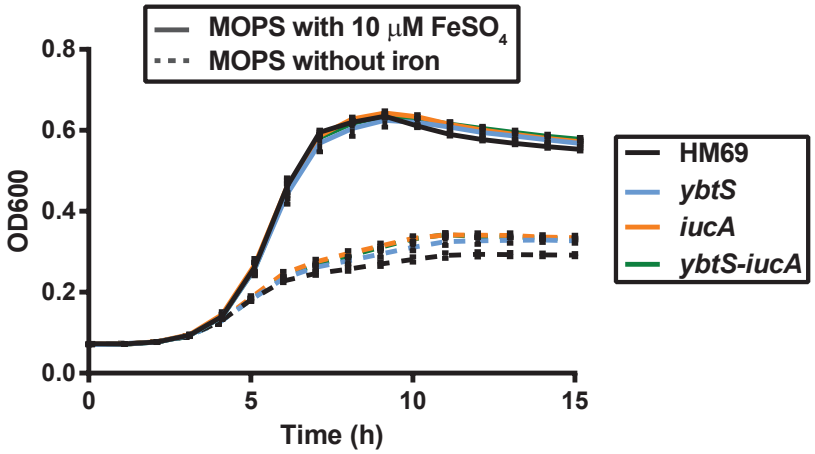
Supplemental Figure 2. Detection of siderophore-conjugated cBSA peptides by mass spectrometry. cBSA-siderophore conjugation was confirmed using in-gel trypsin digestion followed by nano-LC-MS/MS. Siderophore-modified peptide fragments were identified using Mascot by comparing peptides to cBSA reacted in the absence of siderophore. Detected Ybt (mass addition of 463) or Aer (mass addition of 546) modified lysine, aminoethyl-aspartate and aminoethyl-glutamate residues in cBSA are indicated in the annotated MS/MS of cBSA tryptic peptides. (A) The MS/MS spectrum confirms the presence of Aer on Lys_{266} from the b_6^{2+}, b_{10}^{2+}, b_{14}^{2+}, and b_{15}^{2+} ions. (B) The b_4^{2+} and b_9^{2+} ions confirm the presence of the Aer conjugated to Asp_{474}. (C) The b_6^{2+}, b_{10}^{2+}, b_{14}^{2+}, and b_{15}^{2+} ions confirm the presence of the Aer conjugated to Glu_{267}. (D) The b_5 ion confirms the presence of Ybt on Asp_{272}. Shown is a representative MS/MS scan for each peptide. Aminoethyl modification of aspartate and glutamate results in a mass addition of 42.



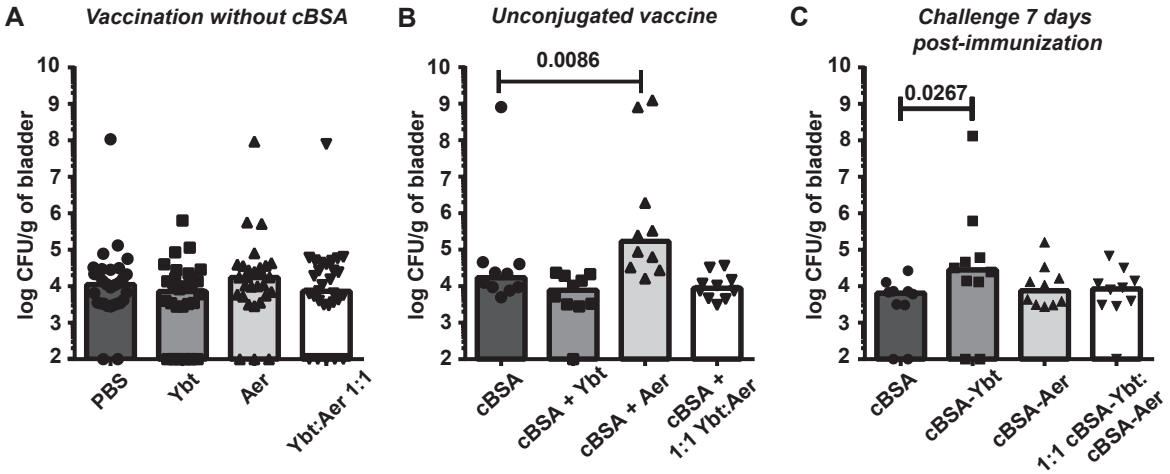
Supplemental Figure 3. Histologic scoring of kidney (A-D) and bladder (E-G) tissues in Figure 1. (A) Normal renal pelvis, score = 0; (B) mild infiltration of neutrophils (arrow), score = 1; (C) mild pyelonephritis with moderate infiltration of neutrophils in tissue and in the pelvic lumen (arrow), and mild peripelvic inflammation and fibrosis (arrowhead), score =2; (D) severe pyelonephritis with neutrophils packed in the pelvic lumen (arrow), score = 3; (E) normal urinary bladder, score = 0; (F) mild cystitis with scattered inflammatory cells surrounding vessels in the submucosa (arrows), score = 1; (G) moderate cystitis with submucosal edema (bracket) and dense clusters of inflammatory cells in the submucosa (arrow) and extending into the lumen (arrowhead).



Supplemental Figure 4. Bacterial burdens in the bladders of Figure 2 mice. CBA/J mice were vaccinated according to the protocol, but transurethrally inoculated with the corresponding siderophore biosynthesis mutant. (A) cBSA-Ybt vaccinated mice were inoculated with HM69 *ybtS*, (B) cBSA-Aer vaccinated mice were inoculated with HM69 *iucA*, and (C) 1:1 cBSA-Ybt:cBSA-Aer vaccinated mice were infected with HM69 *ybtS-iucA*. For each experiment, cBSA was used as the negative control (dark gray bars). In all instances, mice were transurethrally inoculated with 10^8 colony forming units (CFUs) of the indicated strain. After 48 h, CFUs in the bladders were enumerated by serial dilution and plating on LB agar. Statistical analyses using a two-tailed Mann-Whitney test identified no significant differences between negative control and siderophore-vaccinated groups. Limit of detection was 10^2 CFU per g of bladders; bars represent the median; and $n \geq 14$.



Supplemental Figure 5. *In vitro* growth of stealth siderophore mutants. The growth of wildtype HM69 (black), *ybtS* (blue), *iucA* (orange), and *ybtS-iucA* (green) was monitored at 600 nm (OD600) over the course of 15 h. Bacteria were washed in MOPS medium without iron before being inoculated into either MOPS medium with 10 μM iron sulfate (solid lines) or MOPS medium without iron (dashed lines). Shown is the mean and SEM for a combined three independent experiments, each comprised of nine replicates.



Supplemental Figure 6. Bacterial burdens in the bladders of Figure 3 mice. The requirement of the cBSA carrier protein was examined by intranasally administering the unconjugated apo-siderophores (A) in PBS and (B) with cBSA. (C) To determine if the vaccine requires time adequate for generating an adaptive immune response, mice were vaccinated with 10 µg of cBSA, cBSA-Ybt, cBSA-Aer, or 1:1 cBSA-Ybt:cBSA-Aer and transurethrally inoculated one week after immunization. No booster doses were administered. In all experiments, mice were transurethrally inoculated with 10^8 colony forming units (CFUs) of wildtype HM69. After 48 h, colony forming units (CFUs) in the bladders were enumerated by serial dilution and plating on LB agar. Statistical analyses were calculated using a two-tailed Mann-Whitney test. Limit of detection was 10^2 CFU per g of bladders; bars represent the median; and $n \geq 10$.

SI Appendix Tables

Supplemental Table 1. Expected *m/z* of peptide fragments confirming Aer linkage to Lys_{266}*†

B	B Ions	B+2H	B-H2O	Residue	Y Ions	Y+2H	Y-H2O	Y
1	138.07	69.54		H	2,645.15	1,323.08	2,627.14	16
2	812.38	406.69		**K+546**	2,508.09	1,254.55	2,490.08	15
3	983.48	*492.24*	965.47	E+42	1,833.78	917.39	1,815.77	14
4	1,143.51	*572.26*	1,125.50	C+57	1,662.68	831.84	1,644.67	13
5	1,303.54	*652.27*	1,285.53	C+57	1,502.65	751.83	1,484.64	12
6	1,440.60	*720.80*	1,422.59	H	1,342.62	671.81	1,324.61	11
7	1,497.62	*749.31*	1,479.61	G	1,205.56	603.28	1,187.55	10
8	1,654.71	*827.86*	1,636.70	D+42	1,148.54	574.77	1,130.53	9
9	1,767.79	*884.40*	1,749.78	L	991.45	496.23	973.44	8
10	1,880.87	*940.94*	1,862.86	L	878.37	439.69	860.36	7
11	2,009.92	*1,005.46*	1,991.91	E	765.28	383.15	747.27	6
12	2,169.95	*1,085.48*	2,151.94	C+57	636.24	318.62	618.23	5
13	2,240.98	*1,121.00*	2,222.97	A	476.21	238.61	458.20	4
14	2,356.01	*1,178.51*	2,338.00	D	405.17	203.09	387.16	3
15	2,471.04	*1,236.02*	2,453.03	D	290.15	145.58	272.14	2
16	2,645.15	1,323.08	2,627.14	R	175.12	88.06		1

*This fragmentation table corresponds to the MS/MS spectrum that shows the most supporting fragmentation ions after upload into Scaffold.

†The *m/z* of each ion identified by MS/MS is identified in gray boxes. Those that support linkage between Aer and Lys_{266} are emphasized in italics. The site of conjugation is highlighted in bold.

Supplemental Table 2. Expected *m/z* of peptide fragments confirming Aer linkage to aminoethyl-Asp_{474}*†

B	B Ions	B+2H	B-H2O	Residue	Y Ions	Y+2H	Y-H2O	Y
1	172.11	86.56	154.10	E+42	1,866.00	933.50	1,847.99	16
2	875.41	*438.21*	857.40	**D+588**	1,694.90	847.95	1,676.89	15
3	1,038.47	519.74	1,020.46	Y	991.59	496.30	973.58	14
4	1,151.56	*576.28*	1,133.55	L	828.53	414.77	810.52	13
5	1,238.59	619.80	1,220.58	S	715.45	358.23	697.44	12
6	1,351.67	676.34	1,333.66	L	628.41	314.71	610.40	11
7	1,464.76	732.88	1,446.75	I	515.33	258.17	497.32	10
8	1,577.84	789.42	1,559.83	L	402.25	201.63	384.24	9
9	1,691.89	*846.45*	1,673.87	N	289.16	145.08		8
10	1,866.00	933.50	1,847.99	R	175.12	88.06		7

*This fragmentation table corresponds to the MS/MS spectrum that shows the most supporting fragmentation ions after upload into Scaffold.

†The *m/z* of each ion identified by MS/MS is identified in gray boxes. Those that support linkage between Aer and aminoethyl-Asp_{474} are emphasized in italics. The site of conjugation is highlighted in bold.

Supplemental Table 3. Expected *m/z* of peptide fragments confirming Aer linkage to aminoethyl-Glu$_{267}$*†

B	B Ions	B+2H	B-H2O	Residue	Y Ions	Y+2H	Y-H2O	Y
1	138.07	69.54		H	2,645.15	1,323.08	2,627.14	16
2	*266.16*	133.58		K	2,508.09	1,254.55	2,490.08	15
3	983.48	*492.24*	965.47	**E+588**	2,380.00	1,190.50	1,815.77	14
4	1,143.51	*572.26*	1,125.50	C+57	1,662.68	831.84	1,644.67	13
5	1,303.54	*652.27*	1,285.53	C+57	1,502.65	751.83	1,484.64	12
6	1,440.60	*720.80*	1,422.59	H	1,342.62	671.81	1,324.61	11
7	1,497.62	*749.31*	1,479.61	G	1,205.56	603.28	1,187.55	10
8	1,654.71	*827.86*	1,636.70	D+42	1,148.54	574.77	1,130.53	9
9	1,767.79	*884.40*	1,749.78	L	991.45	496.23	973.44	8
10	1,880.87	*940.94*	1,862.86	L	878.37	439.69	860.36	7
11	2,009.92	*1,005.46*	1,991.91	E	765.28	383.15	747.27	6
12	2,169.95	*1,085.48*	2,151.94	C+57	636.24	318.62	618.23	5
13	2,240.98	*1,121.00*	2,222.97	A	476.21	238.61	458.20	4
14	2,356.01	*1,178.51*	2,338.00	D	405.17	203.09	387.16	3
15	2,471.04	*1,236.02*	2,453.03	D	290.15	145.58	272.14	2
16	2,645.15	1,323.08	2,627.14	R	175.12	88.06		1

*This fragmentation table corresponds to the MS/MS spectrum that shows the most supporting fragmentation ions after upload into Scaffold.

†The *m/z* of each ion identified by MS/MS is identified in gray boxes. Those that support linkage between Ybt and aminoethyl-Glu$_{267}$ are emphasized in italics. The site of conjugation is highlighted in bold.

Supplemental Table 4. Expected *m/z* of peptide fragments confirming Ybt linkage to aminoethyl-Asp$_{272}$*†

B	B Ions	B+2H	B-H2O	Residue	Y Ions	Y+2H	Y-H2O	Y
1	138.07	69.54		H	1,847.78	924.39	1,829.77	16
2	195.09	98.05		G	1,710.72	855.86	1,692.71	15
3	*815.28*	408.14	*797.27*	**D+505**	1,653.70	827.35	1,635.69	14
4	928.36	464.69	910.35	L	1,033.51	517.26	1,015.50	13
5	*1,041.45*	521.23	*1,023.44*	L	920.43	460.72	902.42	12
6	1,212.55	*606.78*	1,194.54	E+42	807.34	404.17	789.33	11
7	1,372.58	686.79	1,354.57	C+57	636.24	318.62	618.23	10
8	1,443.62	722.31	1,425.60	A	476.21	238.61	458.20	9
9	1,558.64	779.82	1,540.63	D	405.17	203.09	387.16	8
10	1,673.67	*837.34*	1,655.66	D	290.15	145.58	272.14	7
11	1,847.78	924.39	1,829.77	R	175.12	88.06		6

*This fragmentation table corresponds to the MS/MS spectrum that shows the most supporting fragmentation ions after upload into Scaffold.

†The *m/z* of each ion identified by MS/MS is identified in gray boxes. Those that support linkage between Ybt and aminoethyl-Asp$_{272}$ are emphasized in italics. The site of conjugation is highlighted in bold.

Supplemental Table 5. Expected *m/z* of peptide fragments confirming Ybt linkage to Lys_{437}*†#

B	B Ions	B+2H	B-H2O	Residue	Y Ions	Y+2H	Y-H2O	Y
1	115.05	58.03		N	3,231.73	1,616.37	3,213.72	24
2	186.09	93.55		A	3,117.69	1,559.35	3,099.68	23
3	299.17	150.09		L	3,046.65	1,523.83	3,028.64	22
4	412.26	206.63		I	2,933.57	1,467.29	2,915.56	21
5	511.32	256.17		V	2,820.48	1,410.75	2,802.47	20
6	667.42	334.22		R	2,721.41	1,361.21	2,703.40	19
7	830.49	415.75	812.48	Y	2,565.31	1,283.16	2,547.30	18
8	931.54	466.27	913.53	T	2,402.25	1,201.63	2,384.24	17
9	1,087.64	544.32	1,069.63	R	2,301.20	1,151.11	2,283.19	16
10	1,678.84	839.92	1,660.83	**K+463**	2,145.10	1,073.05	2,127.09	15
11	1,777.91	889.46	1,759.90	V	1,553.90	777.45	1,535.89	14
12	1,874.96	937.98	1,856.95	P	1,454.83	727.92	1,436.82	13
13	2,003.02	1,002.01	1,985.01	Q	1,357.78	679.39	1,339.77	12
14	2,102.09	1,051.55	2,084.08	V	1,229.72	615.36	1,211.71	11
15	2,189.12	1,095.06	2,171.11	S	1,130.65	565.83	1,112.64	10
16	2,290.17	1,145.59	2,272.16	T	1,043.62	522.31	1,025.61	9
17	2,387.22	1,194.11	2,369.21	P	942.57	471.79	924.56	8
18	2,488.27	1,244.64	2,470.26	T	845.52	423.26	827.51	7
19	2,601.35	1,301.18	2,583.34	L	744.47	372.74	726.46	6
20	2,700.42	1,350.71	2,682.41	V	631.39	316.20	613.38	5
21	2,871.52	1,436.26	2,853.51	E+42	532.32	266.66	514.31	4
22	2,970.59	1,485.80	2,952.58	V	361.22	181.11	343.21	3
23	3,057.62	1,529.31	3,039.61	S	262.15	131.58	244.14	2
24	3,231.73	1,616.37	3,213.72	R	175.12	88.06		1

*This fragmentation table corresponds to the MS/MS spectrum that shows the most supporting fragmentation ions after upload into Scaffold.

†The *m/z* of each ion identified by MS/MS is identified in gray boxes. The predicted site of conjugation is highlighted in bold.

#The parent peptide ion NALIVRYTRK437VPQVSTPTLVEVSR supported the linkage of Ybt to Lys_{437}, but MS/MS fragmentation did not encompass the Lys residue in question.

Supplemental Table 6. Bacterial strains and plasmids

Strain	Description	Source
HM69	UPEC isolate	(38)
iucA	HM69 *iucA::cam*	Present study
ybtS	HM69 *ybtS::kan*	Present study
ybtS-iucA	HM69 *ybtS::kan-iucA::cam*	Present study
Plasmids		
pKD46-*spec*	λ red recombineering	(63), modified by Dr. David Friedman
pKD3	Template for *cam*	(63)
pKD4	Template for *kan*	(63)

Supplemental Table 7. Oligonucleotides used in this study.

Sequence (5'→3')	Description*	Source
TTGATAATGAGAATCATTATTGACATAATTGTTATTAATGGGAATTAGCCATGGTCC	*5'-iucA*	This study
GACCTCCTGAGCCTGCAGCGGGTTAGCGAAATCGAAATAGGTGTAGGCTGGAGCTGCTTC	*3'-iucA*	This study
GAATTTCTACATCTGGCGTTACCAGAGGAACAATGGCTACGTGTAGGCTGGAGCTGCTTC	*5'-ybtS*	(33)
ACCATTAAATAGGGCGCAATGCTCGCTAATTTCTCCCGGGATGGGAATTAGCCATGGTCC	*3'-ybtS*	(33)
GATCTTCCGTCACAGGTAGG	*F-cam*	(63)
TTATACGCAAGGCGACAAGG	*R-cam*	(63)
CGGTGCCCTGAATGAACTGC	*F-kan*	(63)
CAGTCATAGCCGAATAGCCT	*R-kan*	(63)
GCAGGACAGTGGTTTTTGG	*F-iucA*	This study
CAGCCACCCTGTCGGCAG	*R-iucA*	This study
CAGTAGAACGCCAGATGAGT	*F-ybtS*	This study
GCTTATATCCGGCTGCATTC	*R-ybtS*	This study

*5' and 3' primer pairs were used for generating mutants using λ Red recombinase. F and R primers were used for confirming the mutants by PCR.

SI Materials and Methods

Bacterial strains and culture conditions

Bacterial strains and plasmids used in these studies are listed in SI Appendix, Table S6. *E. coli* strain HM69 was isolated from the urine of a female patient diagnosed with uncomplicated cystitis at the University of Michigan Health Services clinic in 2012 (1). All bacterial strains were cultured in lysogeny broth (LB) at 37 ºC, unless otherwise noted. When appropriate, antibiotics were added at the following concentrations, spectinomycin (50 μg/mL), chloramphenicol (20 μg/mL), and kanamycin (50 μg/mL).

Generation of isogenic mutants

Insertional mutations in the *ybtS* and *iucA* siderophore biosynthesis genes were generated using λ Red recombineering (2). All oligonucleotides for mutagenesis and confirmation of the targeted mutations are listed in SI Appendix, Table S7. Since *E. coli* HM69 is ampicillin-resistant, pKD46 containing a spectinomycin resistance cassette was used for recombineering. A chloramphenicol resistance cassette was used to disrupt *ybtS* and a kanamycin resistance cassette was inserted into *iucA*. Successful mutagenesis was confirmed by PCR, restriction digests (SnaBI), and Sanger sequencing (SI Appendix, Table S7).

In vitro growth curves

Wildtype HM69, *ybtS, iucA,* and *ybtS-iucA* were grown for 20 h in 3 ml of LB with shaking and aeration at 37 ºC. 1X MOPS without iron medium was prepared from 10X MOPS medium minus iron sulfate (Teknova) with the addition of 1.32 mM K_2HPO_4 and 0.2% glucose. All components were prepared in Chelex-treated (Bio-Rad) ddH_2O. 100 μl of culture were

pelleted and washed three times with 100 µl of MOPS without iron medium (Teknova). 100 µl/well of MOPS without iron or MOPS with 10 µM FeSO4 were aliquoted into Bioscreen honeycomb plates. 1 µl of the washed bacteria were inoculated into each well. The plates were incubated at 37 ºC with medium shaking in a Bioscreen C Automated Growth Curve Analysis System (Growth Curves USA). Growth was monitored at 600 nm every 15 minutes.

Vaccine conjugation and preparation

Apo-aerobactin (Aer, item #DES-AERO) and apo-yersiniabactin (Ybt, item #YER) were purchased from EMC Microcollection as white powders. HyperCarrier cBSA (item #786-092) was purchased from GBiosciences. 2-(N-morpholino)ethanesulfonic acid (MES) buffered saline (item #28390), EDC (item #PG82079), and 7 kDa Zeba spin desalting column (item #89892) were all purchased from ThermoScientific (Pierce). Water distilled using Barnstead MegaPure System (MP-6A) was used to make all buffers.

cBSA was reconstituted to 10 mg/mL in 0.1 M MES/0.9% NaCl, pH 4.7. Ybt and Aer were resuspended to 0.3 mg/mL in 0.1 M MES/0.9% NaCl, pH 4.7. Ybt required brief sonication to solubilize the siderophore. 200 µL of cBSA was added to either 500 µL of the desired siderophore or 500 µL of 0.1 MES/0.9% NaCl, pH 4.7 as a negative control. EDC was prepared to 10 mg/mL in distilled water and 100 µL of this solution was immediately added to each conjugation reaction. Reactions were incubated at ambient temperature, in the dark, for 3 h.

Zeba spin columns were equilibrated with sterile PBS pH 7.4 according to the manufacturer directions. Vaccine conjugates were purified and exchanged into PBS pH 7.4 by passing each reaction mixture through an equilibrated Zeba spin column at 1,000 x *g* for 2 min at

ambient temperature. Eluants were sterilized by filtration through 0.22 μm pore diameter membrane. Aliquots were flash frozen and stored at -80 ºC.

In-gel trypsin digestion and LC-MS/MS

The cBSA control (1 μg) and cBSA-siderophore conjugates (20 μg) were boiled in SDS-PAGE loading buffer for 10 min and loaded into individual wells of a 12.5% SDS-PAGE gel. Samples were migrated into the gel by electrophoresis at 150 V and then visualized using SimplyBlue SafeStain according to the manufacturer's instructions (Invitrogen, #LC6060). Protein bands were excised and subjected to in-gel trypsin digestion using a robot (ProGest, DigiLab) with the following protocol: washed with 25 mM ammonium bicarbonate followed by acetonitrile; reduced with 10 mM DTT at 60 ºC followed by alkylation with 50 mM iodoacetamide at ambient temperature; digested with trypsin (Promega) at 37 ºC for 4 h; and quenched with formic acid. Samples were concentrated by vacuum centrifugation to 60 μl prior to LC-MS/MS.

The samples were analyzed by nano HPLC-electrospray ionization tandem mass spectrometry (LC-MS/MS) on a Thermo Scientific Orbitrap Q-Exactive mass spectrometer. On-line HPLC separation was accomplished with a Waters NanoAcquity HPLC system. Peptides were loaded on a trapping column and eluted over a 75 μm analytical column at 350 nL/min; both columns were packed with Jupiter Proteo resin (Phenomenex). The injection volume was 30 μL. The mass spectrometer was operated in data-dependent mode, with the Orbitrap operating at 60,000 FWHM and 17,500 FWHM for MS and MS/MS, respectively.

The fifteen most abundant ions were selected for MS/MS. Mascot (Matrix Science) was used to search the spectra against the bovine subset of the Uniprot (for Lysine modification) and

SwissProt (Asp/Glu modification) databases concatenated with a database of reverse sequences as well common protein contaminants. Cysteine carbamidomethylation was set as a fixed modification and methionine oxidation, as well as reaction products of ethylenediamine (mass addition of 42.06 Da) with Glu and Asp side chains were considered as variable modifications. Ybt was detected as a mass addition of 505.16 Da for Asp/Glu modification and 463.11 for Lys modification, and Aer was detected as a mass addition of 588.28 Da for Asp/Glu modification and 546.22 for Lys modification. Trypsin (for Lys modification) or no enzyme (for Asp/Glu modification) were specified as the proteolytic enzyme, with two missed cleavage allowed. Peptide mass tolerance was 10 ppm and fragment mass tolerance 0.02 Da. Mascot DAT files were parsed into Scaffold (Proteome Software) for validation, filtering, and to create a non-redundant list per sample. Data were filtered using a minimum protein value of 90%, a minimum peptide value of 50% (Prophet scores), and requiring at least two unique peptides per protein.

Histologic scoring

Immediately following sacrifice, bladders and kidneys were harvested from the mice. Bladders were cut in half. One half was processed for bacterial burden and the other half was placed on a paper towel in a histology cassette. One kidney was cut sagittally and the other was cut transversely. One half of each kidney was placed cut side down in the same histology cassette. The other halves of the kidneys were homogenized for bacterial enumeration. All tissues were fixed in 10% neutral buffered formalin for >24 h. Tissues were paraffin embedded, hematoxylin and eosin stained, and sectioned by the *in-vivo* Animal Core Histology Lab at the University of Michigan. Sections were randomized and blindly scored.

Pyelonephritis scores were determined as follows: 0 = no significant lesions, 1 = very occasional PMNs in the tissue section, 2 = rafts of PMNs in the pelvis and/or scattered focal aggregates of PMNs in tissue, peripelvic inflammation, 3 = many PMNs in all fields of the tissue section, a single large focus of PMNs in one field, or extension of inflammation into the parenchyma (see Fig. S3 A-D for examples). Cystitis scores were determined as follows: 0 = no significant lesions, 1 = very rare PMNs in the stroma or lumen or occasional perivascular lymphoid cuffs, 2 = many PMNs and moderate edema (see Fig. S3 E-G for examples).

SI References

1. Subashchandrabose S, Hazen TH, Rasko DA, & Mobley HL (2013) Draft genome sequences of five recent human uropathogenic *Escherichia coli* isolates. *Pathogens and Disease* 69(1):66-70.
2. Datsenko KA & Wanner BL (2000) One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci USA* 97(12):6640-6645.