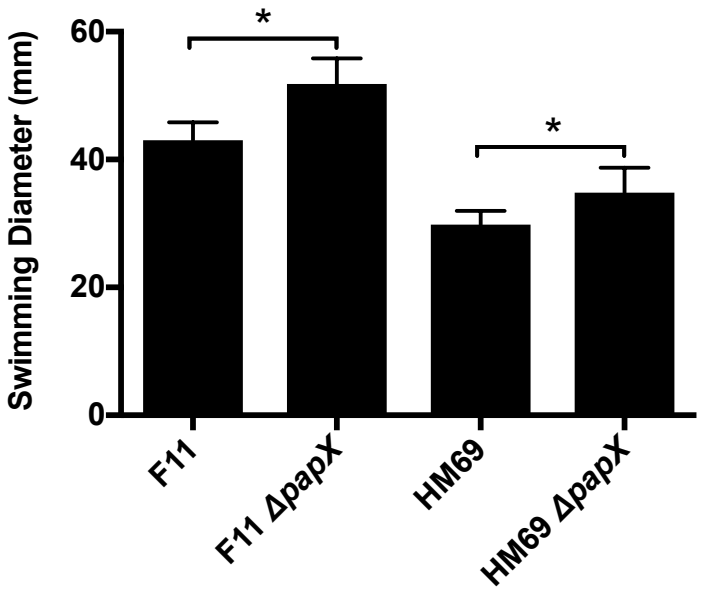

Fig. S1. The loss of *papX* in the cystitis UPEC isolates F11 and HM69 increases motility. Bars represent the average diameter (mm) of swimming motility of bacteria following a 16-18 hr incubation at 30ºC (N=6). The error bars represent the standard deviation, and a Students *t*-test was used for statistical analysis. *, $P < 0.05$

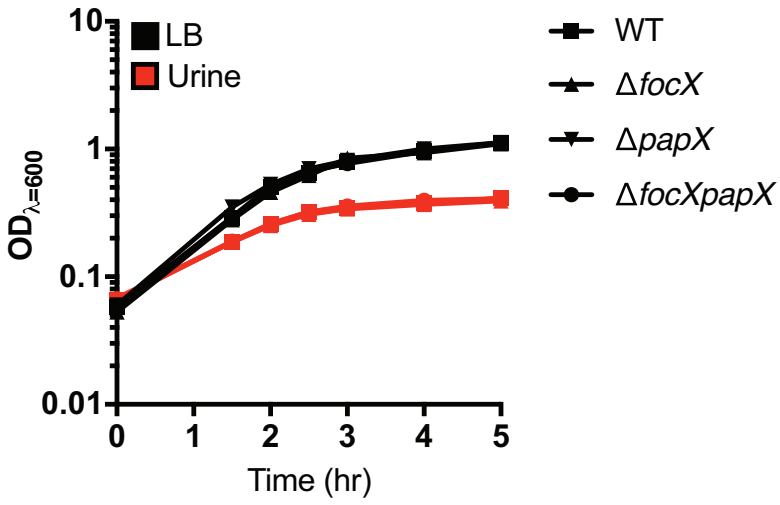

Fig. S2. Growth curves showing similar levels of bacterial growth between CFT073 wild type and the Δ*focX*, Δ*papX*, Δ*focXpapX* constructs. Bacteria were diluted 1:100 from overnight cultures into either fresh pooled, sterilized human urine (red) or LB medium (black) and cultured with aeration at 37°C for 5 hrs. Data represent the OD_{600} measurements taken at different time points.

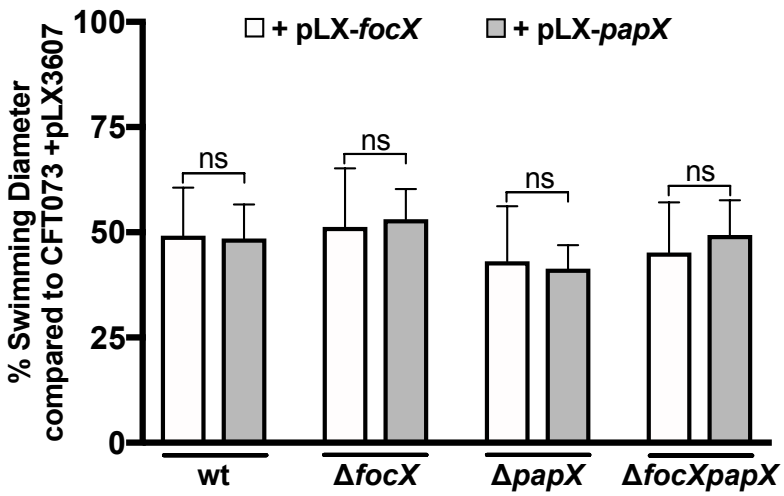

Fig. S3. Expression of either *focX* or *papX* represses motility. Bars represent the average diameter (mm) of swimming motility of CFT073, carrying either pLX-*focX* or pLX-*papX*, compared to CFT073 carrying an empty vector control following 16-18 hr incubation at 30°C. Data represent five biological replicates with the error bars showing the standard deviation. Tukey's multiple comparisons test following ANOVA was used for statistical analysis. *, $P < 0.05$; ns (not significant).

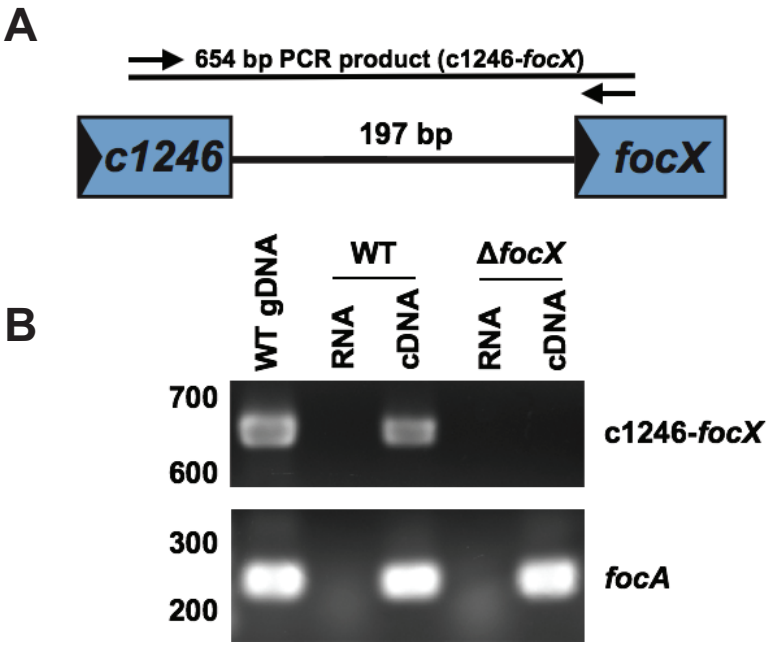

Fig. S4. *focX* is transcribed as part of the *foc* operon. (A) Schematic of the c1246 and *focX* genes in the *foc* operon of CFT073. *focX* is located 197-bp downstream of the c1246 allele. (B) PCR using Taq polymerase was performed to determine whether *focX* is transcribed with c1246. Arrows indicate the location of the primers (c1246-Fpcr/focX-Rpcr) used to PCR amplify a 654-bp product (c1246-*focX*) from either CFT073 or Δ*focX* cDNA. Additional primers (papA-Fpcr/papA-Rpcr) were included that amplified a 240-bp product from *focA*, which is included as a positive control confirming cDNA synthesis. CFT073 gDNA acts a positive control, and extracted RNA samples from CFT073 wild type and Δ*focX* were DNase treated and act as a negative control for contaminating gDNA.

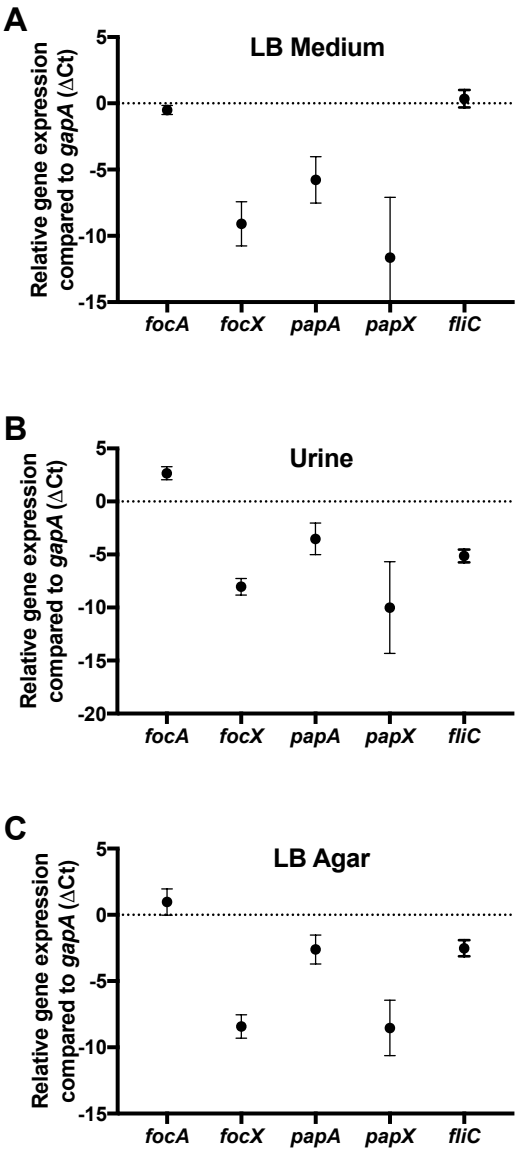

Fig. S5. Relative expression of *foc*, *pap*, and *fliC* compared to *gapA*. qPCR was conducted using cDNA collected from *E. coli* CFT073 wild type cultured to mid-logarithmic growth in either (A) LB medium, (B) sterilized and pooled human urine, or (C) from bacteria cultured on LB agar for 24 hr at 37°C. Data represent the average ΔCt values (reference gene *gapA* - gene of interest (GOI)) of *focA*, *focX*, *papA*, *papX*, and *fliC* with error bars showing the standard deviation. The dashed line at y=0 serves as a reference line for no difference between the Ct values of *gapA* and a GOI.

Table S1. Bacterial strains and plasmids used in this study

Strain	**Genotype/Resistance/Use[a]**	**Source**
CFT073	Pyelonephritis isolate (O6:K2:H1)	(1)
ΔfocX	CFT073 *ΔfocX::cat* (Cam^R)	This Study
ΔpapX	CFT073 *ΔpapX::kan* (Kan^R)	(2)
ΔfocXΔpapX	CFT073 *ΔfocX::cat ΔpapX::kan* (Cam^R, Kan^R)	This Study
E. coli F11	Cystitis isolate (O6:K2:H31)	(3)
E. coli F11 *ΔpapX*	F11 *ΔpapX::kan* (Kan^R)	This Study
E. coli HM69	Cystitis isolate	(4)
E. coli HM69 *ΔpapX*	HM69 *ΔpapX::kan* (Kan^R)	This Study
Plasmid	**Relevant Characteristics**	**References**
pLX3607	IPTG-inducible vector (Amp^R)	(5)
pLX-*focX*	*pLX3607* encoding *focX* at the multiple cloning site (Amp^R)	This Study
pLX-*papX*	pLX3607+*papX,* also known as pDRM001 (Amp^R)	(5)
pKD4	Vector carrying a FRT-flanked *kan* gene (Amp^R, Kan^R)	(6)
pKD3	Vector carrying a FRT-flanked *cat* gene (Amp^R, Cam^R)	(6)
pKD46	Vector carrying phage λ Red recombinase (Amp^R)	(6)

a: cam-chloramphenicol, kan-kanamycin, amp-ampicillin, R-resistant

Table S2. Primers used in this study

Name	Purpose	Sequence (5'-3')
scrnF	Screening by PCR	AGCCCTGTATCTGAGAAAACATGGC
scrnR	Screening by PCR	CACCAGGTCTTTCTGCAACACTACTGC
KO-F	Construction of Δ*focX* and Δ*focX*Δ*papX*	ATCTGTCCGCTGTGCCGGGATATCTCAGTT ATACAGTGTAGGCTGGAGCTGCTTC
KO-R	Construction of Δ*focX* and Δ*focX*Δ*papX*	CTTTATGAGCTGACATCATCAAGATGCGCC AGTAAATGGGAATTAGCCATGGTCC
KO-F2	Construction of Δ*focX*Δ*papX* in CFT073 and Δ*papX* in F11 and HM69	ATGCGCGCTTGTACACAGACAGTGTGTTTC AGTAAGTGTAGGCTGGAGCTGCTTC
KO-R2	Construction of Δ*focX*Δ*papX*	AATGACTTCAAACAGTTCCTGTTCATCATG GGTCAATGGGAATTAGCCATGGTCC9
scrnF2	Screening by PCR	AACGCTGCTCATGATATTGC
scrnR2	Screening by PCR	TCTGCAGGCGCAGCTGGATAAAC
scrnR	Screening by PCR in HM69 and F11 for Δ*papX*	TGAGCTGACATCATCAAGATG
pLXfocX-F	Construction of pLX-*focX*	NNNNCCATGGGGAATAACACAGACACATT
pLXfocX-R	Construction of pLX-*focX*	NNNNAAGCTTTTATGAGCTGACATCATCA AGATG
scrn-pLX-F	Screening by PCR of pLX3607	ATTTGCTTTGTGAGCGGATAAC
scrn-pLX-R	Screening by PCR of pLX3607	CTATCAACAGGAGTCCAAGCTC
gapA-F	qPCR	TGCCTGGCTCCGCTGGCTAA
gapA-R	qPCR	CGCCGCGCCAGTCTTTGTGA
flhD-F	qPCR	TCCGCCTATGTTTCGTCTCGGCATA
flhD-R	qPCR	ACCAGTTGATTGGTTTCTGCCAGC
fliA-F	qPCR	GCGGTATAGAGTGAATTCAC
fliA-R	qPCR	GCGTTTACGCCGCATCCGGC
fliC-F	qPCR	ACAGCCTCTCGCTGATCACTCAAA
fliC-R	qPCR	GCGCTGTTAATACGCAAGCCAGAA
gapA-F	qPCR2	ACCAACTGCCTGGCTCCGCTGGCTAAAGT
gapA-R	qPCR2	CGCGCCAGTCTTTGTGAGACGGGCCATCAA
papA-F	qPCR2	TGGTGCGACAGCAACAGGTGTTTCCTATTT
papA-R	qPCR2	TCGCAACTGCTGAGAAAGCACCTTCTGT
papX-F	qPCR2	TCCATACACTTCCGGCATACAGCG
papX-R	qPCR2	CCCCGCATATCCCTTTCG
focA-F	qPCR2	TGACTCGCAGGTGTCTGCTGGTGCAGGAAT
focX-R	qPCR2	CCGACGTTTGTTGCAGAACCCGCTGCAGAA
focX-F	qPCR2	AGCCCTGTATCTGAGAAAACATGGC
focX-R	qPCR2	CACCAGGTCTTTCTGCAACACTACTGC
fliC-F	qPCR2	TGGTGCGACTGCGGCTACGCTTGATGGTTT
fliC-R	qPCR2	AGTTGCAGCACCAGCATCAGCACCCGTTT
gsp1	5'RACE	ATACCTTCAGATGTCAG
gsp2	5'RACE	ATCCTCCCTGCTATCCATCC

nested_gsp2	5'RACE	CTGAAGGTGACAGACAATGACTCTCC
papA-Fpcr	PCR of *papA*	ACAAATGCGTCTGCTGTCAC
papA-Rpcr	PCR of *papA*	TGGGCCTGAAAAGACAATTC
c1246-Fpcr	PCR of c1246-*focX*	CCGGTGATGACCCCCTGTTA
focX-Rpcr	PCR of c1246-*focX*	TTTGCGCGAATACAGAGTTG

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