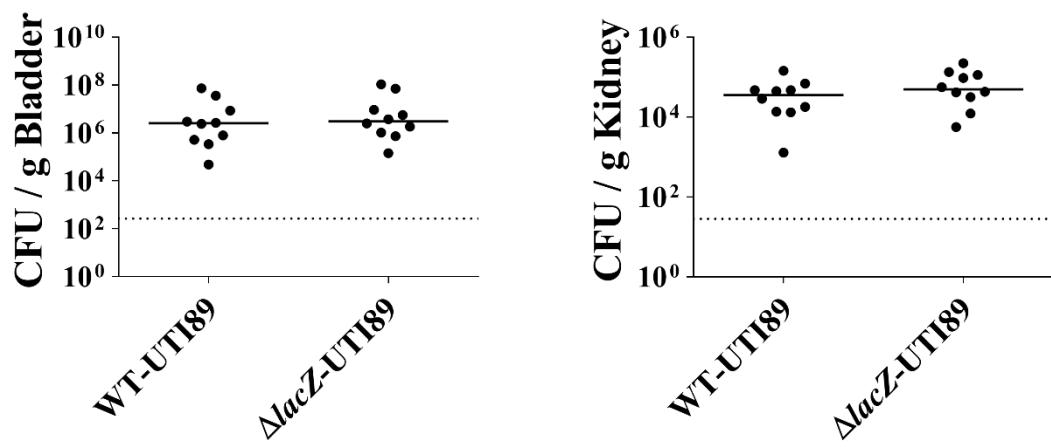
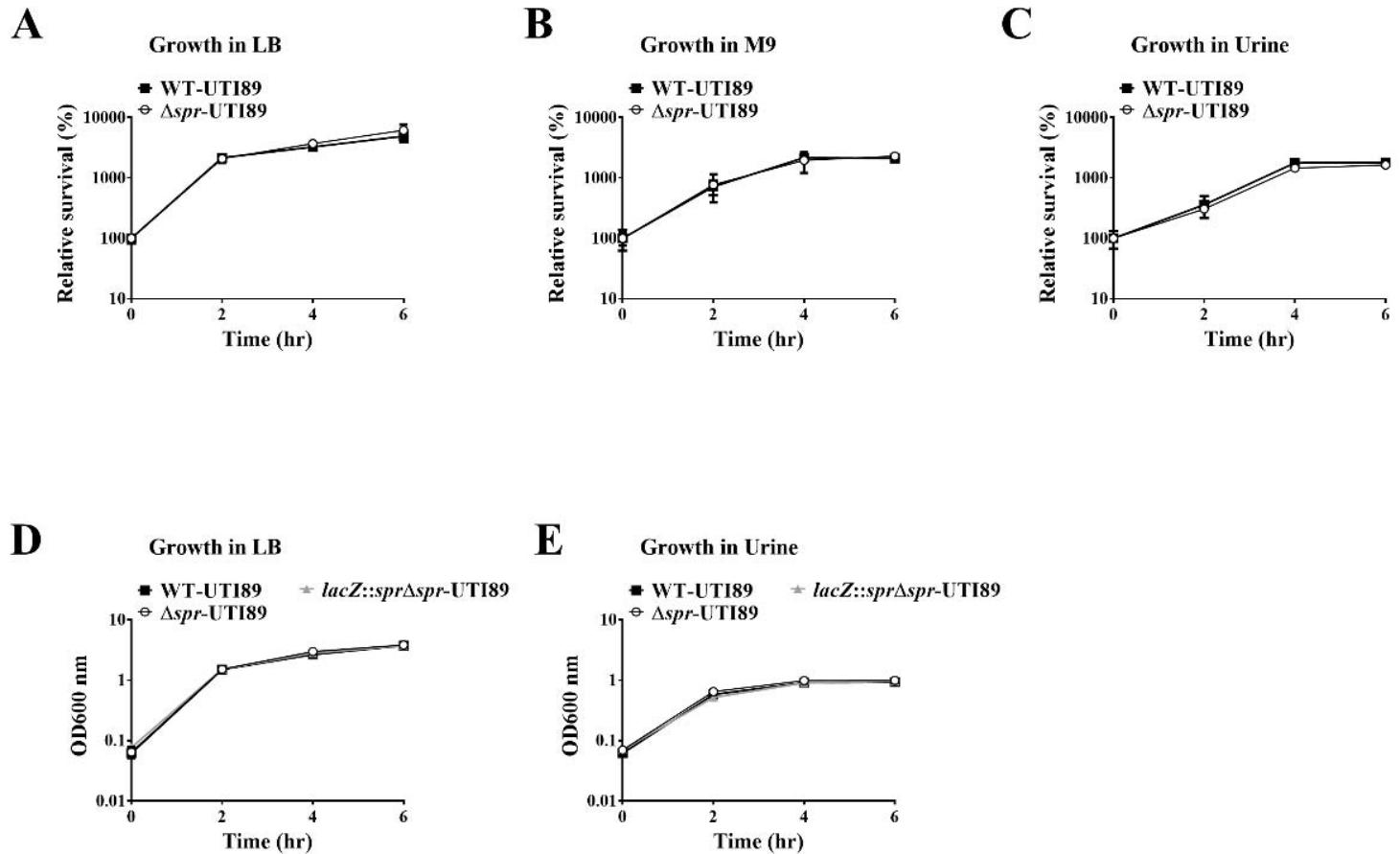


### Supplementary Figure S1



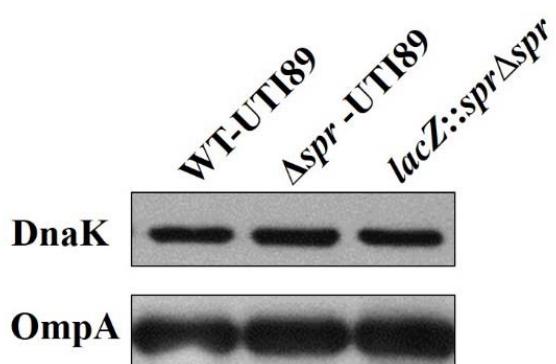
**Supplementary Figure S1.** Deletion of *lacZ* did not affect the ability of UPEC to cause UTIs. The bacterial counts of WT-UTI89 and  $\Delta lacZ$ -UTI89 in the bladders and kidneys at 48 h after transurethral coinoculation with equal amounts of these strains ( $5 \times 10^7$  CFU/mouse for each strain). Animal numbers (N) = 10.

## Supplementary Figure S2

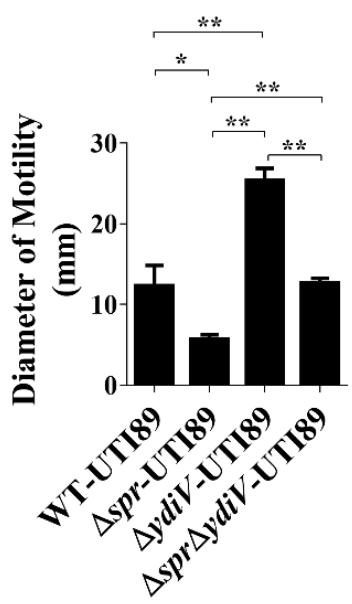


**Supplementary Figure S2.** The growth curves of UIT89 strains in different media. (A), (B), and (C) WT-UTI89 and  $\Delta spr$ -UTI89 were co-cultured in LB broth (A), M9 medium (B), and urine (C). Equal numbers of the bacterial strains ( $1 \times 10^6$  CFU/strain) were inoculated in 100  $\mu$ l of fresh media and incubated at 37°C. The bacterial counts were determined by plating the culture on LB agar plates with and without chloramphenicol to differentiate the wild type (chloramphenicol sensitive) and the *spr* mutant (chloramphenicol resistant). The growth curves were presented as relative growth rates compared to those at 0 h of incubation. (D) and (E) The independent growth curves of the indicated UIT89 strains in LB broth (D), and urine (E). 16-h bacterial cultures were diluted 1:100 in 5 ml LB broth or urine, and incubated at 37°C. The growth of the strains was determined by measuring the values of O.D.600 at the indicated time points using a spectrophotometer. Data are shown as the mean  $\pm$  SD of three independent experiments.

**Supplementary Figure S3**

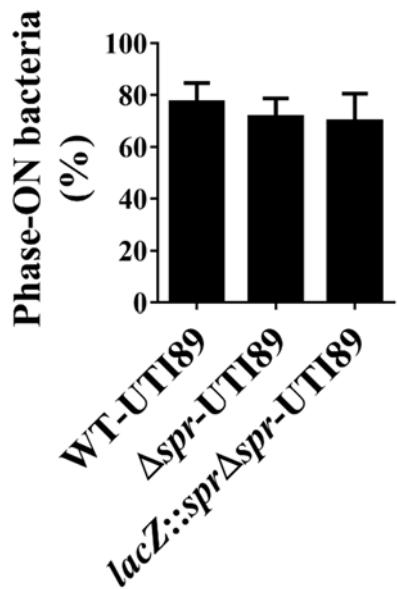


**Supplementary Figure S3.** Deletion of *spr* did not affect the levels of DnaK.



**Supplementary Figure S4.** Deletion of *spr* still decreased motility in the *ydiV* deletion background.

### Supplementary Figure S5



**Supplementary Figure S5.** The ratios of type 1 fimbria Phase-ON bacteria in WT-UTI89,  $\Delta spr$ -UTI89, and *lacZ::sprΔspr*-UTI89. Bacteria from 16-h cultures in LB medium were subjected to invertible element orientations assay as described previously (Chen et al., 2014) to determine the ratios of the type 1 fimbria Phase-ON bacteria in the cultures of the three strains. Data are shown as the mean  $\pm$  SD of experiments in triplicate. There were no significant difference in the Phase-ON ratios among the cultures of the three strains, suggesting that deletion of *spr* does not affect type 1 fimbria expression.

### Reference

Chen, Y.W., Teng, C.H., Ho, Y.H., Jessica Ho, T.Y., Huang, W.C., Hashimoto, M., Chiang, I.Y., and Chen, C.S. (2014). Identification of bacterial factors involved in type 1 fimbria expression using an *Escherichia coli* K12 proteome chip. *Mol Cell Proteomics* 13, 1485-1494.

**Supplementary Table S1.** Primers used in this study.

Primers	Sequence (5'→3')
<b>Gene knockout</b>	
NK- <i>lacZ</i> -F	GTCGTGACTGGAAAACCTGGCGTTACCC AACTTAATCGCATATCAATATCCTCCTTAG
NK- <i>lacZ</i> -R	CAACTGGTAATGGTAGCGACC GGCGCTCAG CTGGAATTCCGTGTAGGCTGGAGCTGCTTC
NK- <i>spr</i> -F	TTGTCGTTAAGGACTTCAAGGGAAAACAA ACAACATGGTCCATATGAATATCCTCCTTAG
NK- <i>spr</i> -R	GAGAACCCGGCGTGCCTCGTTGTAACGCTT CTTCCAGTATGTGTAGGCTGGAGCTGCTTC
NK- <i>flhDC</i> -F	AGAAATGGCGACAACGTTAGCGGCACTGA CTCTTCCGCAAATAGGAATATCCTCCTTAG
NK- <i>flhDC</i> -R	T GTGGGATAATATCGGCAGGATTCTGGGAA AGTTTACGTCTTGTGTAGGCTGGAGCTGCT
<b>Complementary <i>spr</i> gene at the <i>lacZ</i> gene chromosomal locus</b>	
The upstream region	
FD- <i>lacZ</i> -F	CCGGAAGAGAGTCAATTCAAG
LacI-500-R	CATATGATAAGC ATCGCTGCTCATACCAAAG CCGTTGATGGGTGTCTGGTCAG
The <i>spr</i> -3xFlag-Cm region	
lacZ- <i>spr</i> -F	CTTGATGTCTCTGACCAGACACCCATCAAC GTTGCTGACGCCATTAAGTG
New P1	TGTGTAGGCTGGAGCTGCTT
The downstream region	
LacZ down 500-F	AAGCAGCTCCAGCCTACACAGAGCTCCTGC ACTGGATGGTG
141-30-2	AGACCAACTGGTAATGGTAG
<b>The <i>spr</i> point mutation strain (Spr-C68A)</b>	
The upstream region of C68A	
Spr promoter-F	GTTGCTGACGCCATTAAGTG
Spr-C68A-R	GAAACCAGACGCATCGATACCTTTTTAGT GC
The downstream region of C68A	
Spr-C68A-F	GTATCGATGCGTCTGGTTTCGTACAGCGTAC
spr500-R	GTAAGATGCCATATCGACG

---

### The flagellar genes' promoter-lacZ fusion strains

The upstream region

check-no lacI-F                    GCTGTGCAACACAATACTGC  
no lacI-UP500-R                    TGACGCCGAAGTGAGATTAAAATGCTGA  
                                       C

The chloramphenicol-resistance cassette

no lacIp-Cm-F                    GACCTGGCGTCAGCATTAAATCTCACTTC  
                                      GGCCTCATGTGTAGGCTGGAGCTGCTTCG  
                                      ATAGGAATATCCTCCTTAGTTC

NEW-P2

*fkhD-lacZ*

Cm-fkhDp-F

CGGAATAGGAACTAAGGAGGATATTCTTA  
TTGATGTGTCCCTTACTGGC

Cm-fkhDp-R

TCCTGTGTGAAATTGTTATCGCTCACAAATT  
TTATGCGGTCTCACCGCAC

*fliA-lacZ*

Cm-fliAp-F

CGGAATAGGAACTAAGGAGGATATTCTTAT  
CGACCGAAGTGTCCAACATG

Cm-fliAp-R

TCCTGTGTGAAATTGTTATCGCTCACAAATC  
GGCATGATTATCCGTTCTGC

*fkhA-lacZ*

Cm-fkhAp-F

CGGAATAGGAACTAAGGAGGATATTCTTAT  
GTCAGTTGCTGATGGTGTG

Cm-fkhAp-R

CTGTGTGAAATTGTTATCGCTCACAAATAAG  
CGTAAATGATGCCAGAG

*fliM-lacZ*

Cm-fliMp-F

CGGAATAGGAACTAAGGAGGATATTCTTATA  
CACCAAGACACAGCCACTGC

Cm-fliMp-R

CTGTGTGAAATTGTTATCGCTCACAAATCGG  
CTAATTATCCTGCGTCTTG

*fliT-lacZ*

Cm-fliTp-F

CGGAATAGGAACTAAGGAGGATATTCTTATA  
TACGCAAGCCAGAACAGACAG

Cm-fliTp-R

CTGTGTGAAATTGTTATCGCTCACAAATGAA  
TTGCGCAAAGTTACG

*fliC-lacZ*

Cm-fliCp-F

CGGAATAGGAACTAAGGAGGATATTCTTATA  
GATGGGTGACGCTGATGGTG

Cm-fliCp-R

TCCTGTGTGAAATTGTTATCGCTCACAAATA

---

---

	ACCCCTGTTATCGTCTGTCG
The downstream region	
lacZ-500-F	ATTGTGAGCGAATAACAATTCACACAG
lacZ-500-R	ACCACAGATGAAACGCCGAG
<b>qPCR</b>	
gyrB-RT-F	CTATAAAGTGTCCGGCGGTC
gyrB-RT-R	CGGAGTTGAGGAACGACAAC
flhD-RT-F	TCCGCTATGTTCGTCTCGGCATA
flhD-RT-R	ACCAGTTGATTGGTTCTGCCAGC
fliA-RT-F	AACGCTATGACGCCCTACAAGGAA
fliA-RT-R	AGTTCCCTGCTCCAGTTGCCCTATT
flgE-RT-F	CACGTTAGCCTGAGCTTCC
flgE-RT-R	CAACCGTACCGTCATCATTG
flhA-RT-F	ACGAGAAACCGACCCATGAG
flhA-RT-R	CCATCATCGACAAGATCAAC
fliF-RT-F	AATGCGACTGCAGCCCAGAC
fliF-RT-R	AGGATCAGTGCACCATGAC
fliM-RT-F	GATAACGACATGGGCGATAG
fliM-RT-R	CACTTCGCCGCTAACACTG
fliE-RT-F	GTTATCAGCCAGTTACAGGC
fliE-RT-R	TGTGTATCGCTTATGCGATC
fliT-RT-F	ATTTCGCCCTGGCAACAACTC
fliT-RT-R	TGCACCGCATTCACATACGC
flgM-RT-F	GAGTATTGATCGCACTTCGC
flgM-RT-R	ACGTCACACTGGTGCTGGTG
fliC-RT-F	ACAGCCTCTCGCTGATCACTCAA
fliC-RT-R	GCGCTGTTAACAGCAAGCCAGA
motA-RT-F	GCGATTAAGGCACGCTGAAGG
motA-RT-R	GAAGGTGTTCATGTGACCGCTG
<b>Plasmid pUC18-FlhDC</b>	
<i>flhDC</i> promoter-F	GCGATAGATACCGCTTTGCCAGCAGTTGC
<i>flhDC</i> promoter-R	TCCAGCATAATCTGGAACATCATATGGATA
HA- <i>flhD</i> -F	CACCCAGAATAACCAACTTATTTTATGC
<i>flhC</i> -R	TATCCATATGATGTTCCAGATTATGCTGGA
	ATAATGCATACCTCCGAG
	CCGGGGATCCTAACGATGATGATGATGAT
	GAACAGCCTGTACTCTCTGTTG

---