

## SUPPLEMENTARY INFORMATION

**Table S1.** *C. jejuni* 488 strain genome coordinates.

TSS System	Coordinates
TssA	<b>525818..527047</b>
TssB	<b>525264..525749</b>
TssC	<b>523808..525262</b>
TssD	<b>531562..532077</b>
TssE	<b>523413..523805</b>
TssF	<b>522589..523416</b>
TssG	<b>520972..521265</b>
TssH	<b>529563..530219</b>
TssI	<b>520821..519097</b>
TssJ	<b>527048..527473</b>
TssK	<b>527483..528880</b>
TssL	<b>528877..529683</b>
TssM	<b>530216..531526</b>

**Table S2. Antimicrobial susceptibility testing.**

<b>Antibiotics</b>	<b>488 wild-type</b>	<b>488 <i>tssD</i> mutant</b>	<b>488 <i>tssD</i> complement</b>
Ampicillin (10 µg)	S (31)	S (31)	S (31)
Amoxycillin/Clavulonic acid (30 µg)	S (30)	S (30)	S (30)
Tetracycline (30 µg)	S (40)	S (40)	S (40)
Polymyxin B (300 units)	S (20)	S (20)	S (20)
Vancomycin (MIC, µg/ml)	256	256	256

Antimicrobial susceptibility of 488 wild-type, 488 *tssD* mutant, and 488 *tssD* complement strains. The disk diffusion assay was performed with ampicillin (10 µg), amoxycillin/clavulonic acid (2:1, 30 µg), tetracycline (30 µg), and polymyxin B (300 units) disks (Oxoid) following method published by the European Society of Clinical Microbiology (EUCAST) [67]; zones of growth inhibition were measured in millimetres and sensitivity (S) determined based on EUCAST guidelines. Broth microdilution was performed with vancomycin (Sigma) and the minimum inhibitory concentration (MIC, µg/ml) was determined according to method published by Wiegand *et al.* [68]. The experiments were performed on three independent occasions.

**Table S3.** *Campylobacter jejuni* strains used in this study.

<i>C. jejuni</i> strains	Description	Source/Reference
T6SS-positive		
488	Wild-type human isolate from Brazil	This study
488 <i>tssB</i>	488 <i>tssB</i> :: <i>Cm</i>	This study
488 <i>tssC</i>	488 <i>tssC</i> :: <i>Km</i>	This study
488 <i>tssBC</i>	488 <i>tssB</i> :: <i>Cm</i> <i>tssC</i> :: <i>Km</i>	This study
488 <i>tssD</i>	488 <i>tssD</i> :: <i>Km</i>	This study
488 <i>tssD</i> complement	488 <i>tssD</i> :: <i>Km</i> complemented with a copy of <i>tssD</i> in pRRC plasmid	This study
43431	Wild-type human isolate from Canada	[35]
T6SS-negative		
81-176	Wild-type human isolate from the United States	[69]

**Table S4.** *Escherichia coli* strains used in this study.

<i>E. coli</i> strains	Description	Source/Reference
SCS110	Competent cells deficient in Dam and Dcm methylases	Agilent Technologies
XL2-Blue MRF	Competent cells for cloning methylated DNA	Agilent Technologies

**Table S5. Primers used in this study.**

Primer Name	Sequences	Source
Mutagenesis		
<i>tssB</i> F	TGACAAAGAACATACACTACAAG	This study
<i>tssB</i> R	AATCTAAGTCCACGCC	This study
<i>tssB</i> IPCRM F	GGGAGATCTGAACAGGAGCTGAAGAGC	This study
<i>tssC</i> IPCRM R	GGGAGATCTTGTTATATTGAGTTTC	This study
<i>tssC</i> F	AAGCAGTTGATATGCC	This study
<i>tssC</i> R	AATGAGGTCGGACAC	This study
<i>tssC</i> IPCRM F	GGGAGATCTGACTGTTAAGGATCTGCCTAC	This study
<i>tssC</i> IPCRM R	GGGAGATCTCACACCACATCTATTTAGC	This study
<i>tssD</i> F	ATTGAAGGTTCCACACAAGG	This study
<i>tssD</i> R	GTTGATAATCTCCAAT	This study
<i>tssD</i> complement F	CCCTCTAGAATGAAGCGAGTAGATTTGCCAAGCATTA AATA	This study
<i>tssD</i> complement R	CCCTCTAGATTAAATTACGATACAATCC	This study
Kan <sup>R</sup> F out	TGGGTTCAAGCATTAGTCCATGCAAG	[48]
Kan <sup>R</sup> R out	GTGGTATGACATTGCCTCTGCG	[48]
Cam <sup>R</sup> F out	CGATTGATGATCGTTGTA	[48]
Cam <sup>R</sup> R out	TACAGCAGACTATACTG	[48]
RT-PCR		
<i>tssB</i> F	TGAGGATGTGGAGTTG	This study
<i>tssB</i> R	ATTACCCATAGGACCT	This study
<i>tssC</i> F	TTGATGAAATGATAGC	This study

<i>tssC</i> R	GTTGATAATCTCCAAT	This study
<i>tssD</i> F	TGAAGGTTCCACACAAGG	This study
<i>tssD</i> R	CACTTGTGCGGTTCTAA	This study
qRT-PCR		
<i>katA</i> F	AACAAGCTGCCTTAGTCCAAG	This study
<i>katA</i> R	CATAGCACCAAGCGACATTGTAAG	This study
<i>sodB</i> F	GTGGCTGTGGCGGTTCATGTC	This study
<i>sodB</i> R	CTGCGTTGAAGTACCTACA	This study
<i>ahpC</i> F	ATCAAGGTGGTATTGGTCAG	This study
<i>ahpC</i> R	TAACCACAGCATGGCGAACTG	This study
<i>gyrA</i> F	GTTATTATAGGTCGTGCTTT	This study
<i>gyrA</i> R	CTATGAGGTGGGATGTTTGT	This study

(IPCRM = Inverse PCR mutagenesis. Kan = Kanamycin. Cam = Chloramphenicol.)