

Table S1. Sequence of oligonucleotides used in this study

Primer Name	Target ^a	Sequence (5' - 3') ^b
L_WITS_1_cam_FW1	Cm ^r cassette	AAGCTTGGCGTGGGAGTG
R_WITS_cam_RV1	Cm ^r cassette	GAATTCCGCCCTTTAGTTCC
CC069	Cm ^r cassette control primer	ATATGTGCAGGGCGTATTGC
<i>flaB</i> _L1-FW1	<i>flaB</i> (CJM1_1294) left flank	AGCAGATATTAACCGCTTGATGG
<i>flaB</i> _L2-RV1	<i>flaB</i> (CJM1_1294) left flank	CACTCCCACGCCAAGCTTACCGCAT TGATAGCTGAAACC
<i>flaB</i> _R1-FW1	<i>flaB</i> (CJM1_1294) right flank	GGAACTAAAGGGCGGAATTCAAATAT AACATTCTTGACAATCAGGC
<i>flaB</i> _R2-RV1	<i>flaB</i> (CJM1_1294) right flank	TTCTTGCATGAAATTTTAGGACCT
<i>flaB</i> _A-RV1	<i>flaB</i> (CJM1_1294) control primer	ACAAGCTTGCCGTTTTTCATC
<i>pflA</i> _L1-FW1	<i>pflA</i> (CJM1_1501) left flank	TGAGAGTTTAAATGGGCTTTTGG
<i>pflA</i> _L2-RV1	<i>pflA</i> (CJM1_1501) left flank	CACTCCCACGCCAAGCTTGCTTGATT ATTTTCGCGACCC
<i>pflA</i> _R1-FW1	<i>pflA</i> (CJM1_1501) right flank	GGAACTAAAGGGCGGAATTCAATCA AAAGTGCTTCAAATTGGC
<i>pflA</i> _R2-RV1	<i>pflA</i> (CJM1_1501) right flank	AGAGTAGGTTCTGATATGAATGCT
<i>pflA</i> _A-RV1	<i>pflA</i> (CJM1_1501) control primer	AGCTCCAACATAAGGCAAACCTC
<i>flaD</i> _L1-FW1	<i>flaD</i> (CJM1_0851) left flank	AATCAAGATTTATCCGATCCAAATGT
<i>flaD</i> _L2-RV1	<i>flaD</i> (CJM1_0851) left flank	CACTCCCACGCCAAGCTTAAGCACTT TGACCGCCATA
<i>flaD</i> _R1-FW1	<i>flaD</i> (CJM1_0851) right flank	GGAACTAAAGGGCGGAATTCAGCTA GTACAACAATTTCCCAAC
<i>flaD</i> _R2-RV1	<i>flaD</i> (CJM1_0851) right flank	ATTCCACTCCCTGTATGCC
<i>flaD</i> _A-RV1	<i>flaD</i> (CJM1_0851) control primer	GCTTTCAGTTCAGCTCCAG
<i>flaB</i> *_L1-FW1	<i>flaB</i> (CJM1_1295) left flank	CAGAAACTGCTATAACAAATCTAGA
<i>flaB</i> *_L2-RV1	<i>flaB</i> (CJM1_1295) left flank	CTCACTCCCACGCCAAGCTTCCGAT GTTGGTGTTTATCCT
<i>flaB</i> *_A-RV1	<i>flaB</i> (CJM1_1295) control primer	GTTTGGTTTGAAGTTGCGCC
<i>flaA</i> _L1-FW2	<i>flaA</i> (CJM1_1296) left flank	TTTGCTTAGAACATGCTATAAGGTT
<i>flaA</i> _L2-RV2	<i>flaA</i> (CJM1_1296) left flank	CTCACTCCCACGCCAAGCTTTGCTTG AGATCTTAAGCTATCTGCT
<i>flaA</i> _R1-RV1	<i>flaA</i> (CJM1_1296) right flank	GGAACTAAAGGGCGGAATTCCTCAA GCAAATTCTAGTCAGCAA
<i>flaA</i> _R2-RV1	<i>flaA</i> (CJM1_1296) right flank	TTGCTGCTTGTGAACGCAAA
<i>flaA</i> _A-RV1	<i>flaA</i> (CJM1_1296) control primer	TCCTGAACCTTGTGCACTCA
<i>fliW</i> -L1-FW1	<i>fliW</i> (CJM1_1052) left flank	CAGCATTGCGTGAATTTCCAA
<i>fliW</i> -L2-RV1	<i>fliW</i> (CJM1_1052) left flank	CTCACTCCCACGCCAAGCTTGCATTT AACAGCTAGGGTCATG
<i>fliW</i> -R1-FW1	<i>fliW</i> (CJM1_1052) right flank	GGAACTAAAGGGCGGAATTCAGTAG ATACAGTTAATTATCCCGACT
<i>fliW</i> _R2-RV1	<i>fliW</i> (CJM1_1052) right flank	ATGGTGTGGTTGAAGCCTTG
<i>fliW</i> _A-RV1	<i>fliW</i> (CJM1_1052) control primer	TCAAGATTAATGACTACTGGAGCT
<i>fliW</i> _GC.XhoI_FW1	<i>fliW</i> genetic complementation	CAGCTCGAGACTTCACCTTGATTTAT ATTCTC

<i>fliW</i> _GC. <i>Bam</i> HI_RV1	<i>fliW</i> genetic complementation	<u>CTGCCTAGGG</u> GATCCATTTGCAAGTAT ATAGAGC
pSV009_GC <i>amplif</i> _FW1	genetic complementation region	TAATAGAAATTTCCCAAGTCCCA
pSV009_GC <i>amplif</i> _RV1	genetic complementation region	CTATTGCCATAGTAGCTCTTAGTGG
pSV009_seq_FW1	sequencing complemented gene	GGAGACATTCCTCCGTATCT
pSV009_seq_RV1	sequencing complemented gene	AGCGAGACAAAAACACTGAGC

^a Abbreviations for antibiotics: Cm^r, chloramphenicol resistance. ^b restriction sites are underlined.

Table S2 Overview of variants identified in CJM1cam compared to the M1 reference genome

Position ^a	Gene or intergenic	Gene	Description (C. jejuni M1 annotation)	Reference	Variant	Variant type	Feature Type	Variant effect	Predicted effect (protein level) ^b	Method of identification
60704	CJM1_0043	-	putative drug resistance transporter	A	C	SNP	transcript	stop lost, splice region	Ter351Gly; termination codon at position 351 changed to a glycine with the extension not containing a new stop codon	Illumina sequencing
130527	CJM1_0121	-	conserved hypothetical protein	T	G	SNP	coding	mis-sense	Cys418Gly	Illumina sequencing
243119	CJM1_0246	<i>zupT</i>	ZIP Zinc transporter family protein	-	A	Insertion	coding	frameshift	Asn144fs	Illumina sequencing
254704	CJM1_0259	<i>clpX</i>	ATP-dependent Clp protease, ATP-binding subunit ClpX	-	A	Insertion	coding	frameshift	Ile406fs	Illumina sequencing
328734	CJM1_0339	<i>lspA</i>	signal peptidase II	-	T	Insertion	coding	frameshift	Gly117fs	Illumina sequencing
345149	CJM1_0355	-	ATPase associated with various cellular activities family protein	-	A	Insertion	coding	frameshift	Val436fs	Illumina sequencing
538730	CJM1_0561	-	DNA polymerase III, delta subunit	T	-	Deletion	coding	frameshift	Leu172fs	Illumina sequencing
566518	CJM1_0588, CJM1_0589	<i>algI/-</i>	Alginate O-acetyltransferase AlgI/hypothetical protein	A	-	Deletion	coding	frameshift, frameshift	Leu5fs/Ala206fs	Illumina sequencing
620355	CJM1_0644	-	potassium-transporting ATPase A subunit	-	T	Insertion	coding	frameshift	Tyr249fs	Illumina sequencing
624999	CJM1_0648	<i>kdpD</i>	universal stress family protein	-	A	Insertion	coding	frameshift	Ser345fs	Illumina sequencing
635642	CJM1_0659	-	conserved hypothetical protein	C	-	Deletion	coding	frameshift	Gly134fs	Illumina sequencing
648786	CJM1_0674	-	conserved hypothetical protein	T	-	Deletion	coding	frameshift	Asn194fs	Illumina sequencing
684129	CJM1_0713	-	putative membrane protein	C	G	SNP	coding	mis-sense	Pro24Ala	Illumina sequencing
752411	CJM1_0777	-	mviN-like family protein	-	A	Insertion	coding	frameshift	Glu77fs	Illumina sequencing
790633	CJM1_0815	-	putative soluble lytic murein transglycosylase	A	G	SNP	coding	mis-sense	Ile291Thr	Illumina sequencing
813743	CJM1_0839	<i>astA</i>	arylsulfotransferase family protein	A	-	Deletion	coding	frameshift	Arg427fs	Illumina sequencing
813749	CJM1_0839	<i>astA</i>	arylsulfotransferase family protein	T	G	SNP	coding	mis-sense	Leu428Val	Illumina sequencing
817253	CJM1_0845, CJM1_0846	-, -	hypothetical protein, hypothetical protein	A	C	SNP	intergenic	-	-	Illumina sequencing
861927	CJM1_0891	<i>apt</i>	adenine phosphoribosyltransferase	C	T	SNP	coding	mis-sense	Leu46Phe	Illumina sequencing
878548	CJM1_0907	-	putative cju26	A	G	SNP	coding	mis-sense	Leu5Pro	Illumina sequencing
921927	CJM1_0954	-	M42 glutamyl aminopeptidase family protein	-	A	Insertion	coding	frameshift	Arg170fs	Illumina sequencing
939896	CJM1_0974	<i>rpoD</i>	RNA polymerase sigma factor RpoD	A	T	SNP	coding	mis-sense	Asp106Val	Illumina sequencing
961924	CJM1_0995	-	putative porin domain protein	-	T	Insertion	coding	frameshift	Asn44fs	Illumina sequencing
965941	CJM1_1000, CJM1_1001	-, <i>gyrA</i>	Putative lipoprotein, DNA gyrase subunit A	-	T	Insertion	coding	-	-	Illumina sequencing
972139	CJM1_1005	<i>cmeD</i>	outer membrane efflux family protein	A	G	SNP	coding	synonymous	Leu93Leu	Illumina sequencing
1002619	CJM1_1032, CJM1_1033	-, <i>murC</i>	integral membrane protein, UDP-N-acetylmuramate-L-alanine ligase	-	A	Insertion	intergenic	-	-	Illumina sequencing
1103635	CJM1_1139	-	rho termination factor, N-terminal domain protein	-	A	Insertion	coding	frameshift	Ala63fs	Illumina sequencing
1130876	CJM1_1170	<i>mnmG</i>	putative tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG	A	G	SNP	coding	mis-sense	Val438Ala	Illumina sequencing
1252081	CJM1_1289	<i>pseA</i>	putative flagellin modification protein, PseA	-	A	Insertion	coding	frameshift	Ter330fs	Illumina sequencing
1252105	CJM1_1289	<i>pseA</i>	putative flagellin modification protein, PseA	-	G	Insertion	coding	frameshift	Leu322fs	Illumina sequencing
1261071	CJM1_1295	-	flagellin B domain protein	-	T	Insertion	coding	frameshift, splice region	-	Sanger DNA sequencing
1261073	CJM1_1295	-	flagellin B domain protein	-	T	Insertion	coding	frameshift	Ter222fs	Sanger DNA sequencing
1261080	CJM1_1295	-	flagellin B domain protein	-	T	Insertion	coding	frameshift	Arg220fs	Sanger DNA sequencing
1271162	CJM1_1304, CJM1_1305	<i>cdsA/cdsA</i>	putative phosphatidate cytidyllyltransferase/cytidyllyltransferase family protein	-	C	Insertion	coding	frameshift, frameshift	Met9fs/Val117fs	Illumina sequencing
1300762	CJM1_1333, CJM1_1334	-, -	Iron-sulfur cluster-binding domain protein/membrane protein	T	G	SNP	coding	mis-sense, synonymous	Leu11Arg/Thr118Thr	Illumina sequencing
1300765	CJM1_1333, CJM1_1334	-, -	Iron-sulfur cluster-binding domain protein/membrane protein	T	-	Deletion	coding	frameshift, frameshift	Phe120fs/Leu13fs	Illumina sequencing
1341270	CJM1_1376	-	putative nucleotidyl-sugar dehydratase	-	T	Insertion	coding	frameshift	Phe290fs	Illumina sequencing
1514519	CJM1_1546, CJM1_1547	-, <i>pgpA</i>	sulfate adenylyltransferase, putative, phosphatidylglycerophosphatase A	-	T	Insertion	intergenic	-	-	Illumina sequencing
1544219	CJM1_1585	-	PDZ domain family protein	A	G	SNP	coding	mis-sense	Arg136Gly	Illumina sequencing
1577749	CJM1_1627, CJM1_1628	-, <i>bioB</i>	Putative biotin synthase	T	-	Deletion	coding	frameshift, frameshift	Lys2fs/Asn238fs	Illumina sequencing
1613795	CJM1_1679	<i>flgE-2</i>	putative flagellar hook protein FlgE	T	A	SNP	transcript	stop lost, splice region	Ter691Leu; termination codon at position 691 changed to a leucine with the extension not containing a new stop codon	Illumina sequencing

^a position in *C. jejuni* M1 reference genome as published in Friis *et al.*, 2010 PLoS one 5, e1225^b Based on Human Genome Variation Society (HGVS) notation (<http://www.hgvs.org/mutnomen/>); fs = frame shift

Table S3 Overview of variants identified in M1 wild-type *ΔfliW* (CJM1cam_1052) and *ΔfliW* (CJM1cam_1052) clone A

Position ^a	Gene or intergenic region	Gene	Description (C. jejuni M1cam annotation)	Reference	Variant	Variant type	Feature Type	Variant effect	Predicted effect (protein level) ^b	Note
C. jejuni M1 wild-type <i>ΔfliW</i> (CJM1cam_1052)										
78447	CJM1cam_0061	<i>fliY_2</i>	Flagellar motor switch protein FliY, partial	-	T	Insertion	coding	frameshift	Lys138fs	Unique to WT
87749	CJM1cam_0071, CJM1cam_0072	CJM1cam_0071, CJM1cam_0072	Glutathione synthase/Ribosomal protein S6 modification enzyme (glutaminyl transferase), non-heme iron protein, hemerythrin family	-	T	Insertion	intergenic	-	-	Also present in <i>fliW</i> mutant clone A
236030	CJM1cam_0238	CJM1cam_0238	Exonuclease III	-	T	Insertion	coding	frameshift	Lys45fs	Also present in <i>fliW</i> mutant clone A
275077	CJM1cam_0280	<i>modB</i>	ABC-type sulfate/molybdate transport system, permease component	A	T	SNP	coding	missense	Leu197His	Also present in <i>fliW</i> mutant clone A
279769	CJM1cam_0286	<i>bioA_1</i>	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase	-	A	Insertion	coding	frameshift	Ile138fs	Also present in <i>fliW</i> mutant clone A
730937	CJM1cam_0753, CJM1cam_0754	<i>uvrD, peb2</i>	DNA helicase II, Major antigenic peptide Peb2	-	A	Insertion	intergenic	-	-	Also present in <i>fliW</i> mutant clone A
744771	CJM1cam_0769, CJM1cam_0770	<i>flgS</i> , CJM1cam_0770	Two-component system sensor histidine kinase FlgS, Hypothetical protein CJM1cam_0770	C	T	SNP	intergenic	-	-	Also present in <i>fliW</i> mutant clone A
744795	CJM1cam_0769, CJM1cam_0770	<i>flgS</i> , CJM1cam_0770	Two-component system sensor histidine kinase FlgS, Hypothetical protein CJM1cam_0770	-	TT	Insertion	intergenic	-	-	Also present in <i>fliW</i> mutant clone A
801201	CJM1cam_0826, CJM1cam_0827	CJM1cam_0826, <i>folD</i>	Hypothetical protein CJM1cam_0826, Bifunctional protein FolD	-	A	Insertion	intergenic	-	-	Unique to WT
904409	CJM1cam_0934	<i>mnmE</i>	tRNA modification GTPase MnmE	A	T	SNP	coding	missense	Leu212Ile	Also present in <i>fliW</i> mutant clone A
917987	CJM1cam_0948	CJM1cam_0948	Non-heme iron protein, hemerythrin family	C	T	SNP	coding	synonymous	Tyr55Tyr	Also present in <i>fliW</i> mutant clone A
918173	CJM1cam_0948	CJM1cam_0948	Non-heme iron protein, hemerythrin family	C	T	SNP	coding	synonymous	His117His	Also present in <i>fliW</i> mutant clone A
976923	CJM1cam_1008	CJM1cam_1008	DnaJ like protein, partial	-	T	Insertion	coding	frameshift, splice region	-	Unique to WT
977445	CJM1cam_1009	CJM1cam_1009	Hypothetical protein CJM1cam_1009	-	G	Insertion	coding	frameshift	Leu95fs	Also present in <i>fliW</i> mutant clone A
1087523	CJM1cam_1123	CJM1cam_1123	Hypothetical protein CJM1cam_1123	-	T	Insertion	coding	frameshift	Lys383fs	Unique to WT
1263720	CJM1cam_1296, CJM1cam_1297	<i>flaA</i> and <i>maf-5</i>	Flagellin A, Motility accessory factor Maf-5	-	T, TT	Insertion	intergenic	-	-	TT variant also present in <i>fliW</i> mutant clone A
1445565	CJM1cam_1477	<i>galU</i>	UTP-glucose-1-phosphate uridylyltransferase GalU	G	A	SNP	coding	missense	Leu85Phe	Unique to WT
1449230	CJM1cam_1480	<i>cysW</i>	ABC-type tungstate transport system, periplasmic component	A	G	SNP	coding	missense	Val155Ala	Also present in <i>fliW</i> mutant clone A
1459202	CJM1cam_1491	CJM1cam_1491	Putative ATPase	A	T,G	SNP	transcript, transcript coding	stop gained, missense	Lysine 8 to a stop codon, Lys8Glu	G variant also present in <i>fliW</i> mutant clone A
1540983	CJM1cam_1579	<i>dnaG</i>	DNA primase DnaG	-	A	Insertion	coding	frameshift	Val482fs	
C. jejuni M1 <i>ΔfliW</i> (CJM1cam_1052) clone A										
87749	CJM1cam_0071, CJM1cam_0072	CJM1cam_0071, CJM1cam_0072	Glutathione synthase/Ribosomal protein S6 modification enzyme (glutaminyl transferase), non-heme iron protein, hemerythrin family	-	T	Insertion	intergenic	-	-	Also present in coupled WT
129257	CJM1cam_0120, CJM1cam_0121	CJM1cam_0120, CJM1cam_0121	Putative metalloprotease, hypothetical protein CJM1cam_0121	-	A	Insertion	intergenic	-	-	Unique to <i>fliW</i> mutant clone A
236030	CJM1cam_0238	CJM1cam_0238	Exonuclease III	-	T	Insertion	coding	frameshift	Lys45fs	Also present in coupled WT
275077	CJM1cam_0280	<i>modB</i>	ABC-type sulfate/molybdate transport system, permease component	A	T	SNP	coding	missense	Leu197His	Also present in coupled WT
279769	CJM1cam_0286	<i>bioA_1</i>	Adenosylmethionine-8-amino-7-oxononanoate aminotransferase	-	A	Insertion	coding	frameshift	Ile138fs	Also present in coupled WT
730937	CJM1cam_0753, CJM1cam_0754	<i>uvrD, peb2</i>	DNA helicase II, Major antigenic peptide Peb2	-	A	Insertion	intergenic	-	-	Also present in coupled WT
744771	CJM1cam_0769, CJM1cam_0770	<i>flgS</i> , CJM1cam_0770	Two-component system sensor histidine kinase FlgS, Hypothetical protein CJM1cam_0770	C	T	SNP	intergenic	-	-	Also present in coupled WT
744795	CJM1cam_0769, CJM1cam_0770	<i>flgS</i> , CJM1cam_0770	Two-component system sensor histidine kinase FlgS, Hypothetical protein CJM1cam_0770	-	TT	Insertion	intergenic	-	-	Also present in coupled WT
766440	CJM1cam_0791	CJM1cam_0791	Hypothetical protein CJM1cam_0791	G	A	SNP	coding	synonymous	Lys252Lys	Unique to <i>fliW</i> mutant clone A
904409	CJM1cam_0934	<i>mnmE</i>	tRNA modification GTPase MnmE	A	T	SNP	coding	missense	Leu212Ile	Also present in coupled WT
917987	CJM1cam_0948	CJM1cam_0948	Non-heme iron protein, hemerythrin family	C	T	SNP	coding	synonymous	Tyr55Tyr	Also present in coupled WT
918173	CJM1cam_0948	CJM1cam_0948	Non-heme iron protein, hemerythrin family	C	T	SNP	coding	synonymous	His117His	Also present in coupled WT
977445	CJM1cam_1009	CJM1cam_1009	Hypothetical protein CJM1cam_1009	-	G	Insertion	coding	frameshift	Leu95fs	Also present in coupled WT
1263720	CJM1cam_1296, CJM1cam_1297	<i>flaA, maf-5</i>	Flagellin A, Motility accessory factor Maf-5	-	T	Insertion	intergenic	-	-	TT variant also present in coupled WT
1449230	CJM1cam_1480	<i>cysW</i>	ABC-type tungstate transport system, periplasmic component	A	G	SNP	coding	missense	Val155Ala	Also present in coupled WT
1459202	CJM1cam_1491	CJM1cam_1491	Putative ATPase	A	G	SNP	coding	missense	Lys8Glu	G variant also present in coupled WT

^a position in C. jejuni CJM1cam reference genome (CP012149) submitted as part of this manuscript

^b Based on Human Genome Variation Society (HGVS) notation (<http://www.hgvs.org/mutnomen/>); fs = frame shift

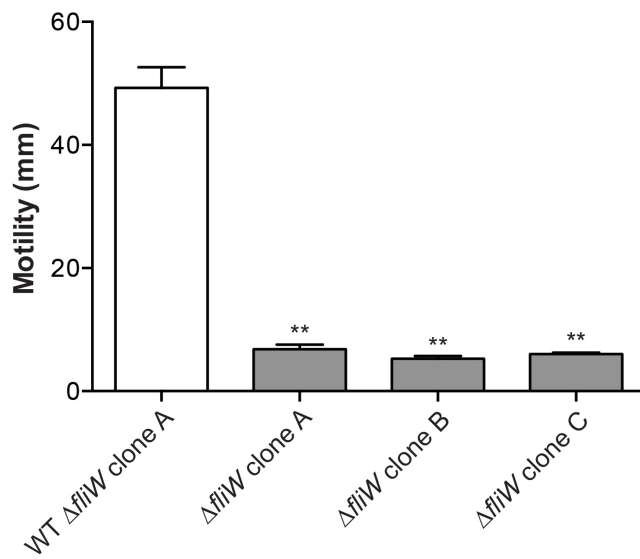


Fig. S1. Motility of *C. jejuni* M1 *fliW* defined gene deletion mutants. Motility of three *fliW* (CJM1_1052) deletion mutant clones ($n = 4$) along with their coupled wild-type strain ($n = 2$) was assayed on BHI plates with 0.4% (w/v) agar after overnight incubation. All three *fliW* (CJM1_1052) mutant clones were non-motile. Differential motility of *fliW* mutant clones was tested against the coupled wild-type (WT) strain with a Mann-Whitney test. Data shown are the mean and SEM, with ** $P < 0.01$.