Strain	Length (AA) ^a	% Identity ^b
NCTC 11168	572	93.9
81116	576	94.6
81-176	576	94.8
Rm1221	573	95.3
F38011	580	90.0
CF93-6	572	93.7
260.94	577	93.6
84-25	572	93.9

a. AA, amino acids.

b. Percent identity calculated from amino acid sequence.

Supplemental Table 2. Primers used in this study

Primer Name	Sequence $5' > 3^{1a}$
MEK 1001	ATA <u>CCGCGG</u> GAACTTGTTAAATGCTATATCGTTAC
MEK 1011	ATA <u>GAGCTC</u> CTTTAAGCACGATAGATTTGAGTG
MEK 1332	ATATA <u>CCGCGG</u> ATGGGATTTCGTATTAACACC
MEK 1334	ATATA <u>CCGCGG</u> ATGGGTTTTAGGATAAACACC
MEK 1611	ATATA <u>GAGCTC</u> TAAAACTTGAATATCTTTTTGCTTGC
MEK 1612	ATATA <u>CCGCGG</u> TCCTTTTAAAAAAATTCAAGCTCATCC
MEK 1613	ATATA <u>GAGCTC</u> TGAGTAGCGCAGGAAGTGG
MEK 1614	ATATA <u>CCGCGG</u> TCCTTTCAAAATATTGCATCGAAAAG
MEK 1655 ^b	GATATTGGTGTTAATACGAAATCC
MEK 1656 ^b	GCAGCTTTAAATGCAAAAGCAAAC
MEK 1657 ^b	ACCAACATTGGTGTTAATACG
MEK 1658 ^b	GCTTTAAATGCAAAAGCAAACG
MEK 1659 ^b	ATGTGCATTTAAAGCTGCAACATTG
MEK 1660 ^b	GCAAACGCTGATTTAAATAGTAAAAG
MEK 1661 ^b	AACAGCGTTTGCTTTTGCATTTAAAG
MEK 1662 ^b	TTAAATAGTAAAAGTTTAGATGCTTCTTTAAG
MEK 1663 ^b	AACATCAGCGTTTGCTTTTGC
MEK 1664 ^b	AATAGTAAAAGTTTAGATGCTTC
MEK 1665 ^b	TTCTTTACTATTTAAATCAGCGTTTG
MEK 1666 ^b	TTAGATGCTTCTTTAAGCAGAC
MEK 1667 ^b	CTTATCTAAACTTTTACTATTTAAATCAG
MEK 1668 ^b	TCTTTAAGCAGACTTAGTTCAGG
MEK 1844	ATATA <u>CTCGAG</u> TATGCAAATAAAGCTATTATATTTGTGC
MEK 1851	ATATA <u>GGATCC</u> AAACTTTTACAATAATCTTTCTAAAGAGC
MEK 1858 ^b	ATATA <u>TCTAGA</u> TCAAATCAGAGCCGACATTGG
MEK 1859 ^b	ATATA <u>TCTAGA</u> TTTGTTATAGCTGTTTCAGC
MEK 1860 ^b	ATATA <u>TCTAGA</u> TCAAATCAGAGCAGACATTGG
MEK 1861 ^b	ATATA <u>TCTAGA</u> TTTGTTGTGGCTGTTTCAGC
MEK 2015	ATTA <u>GAATTC</u> TATTGCATCGAAAAGATTAAAGC
MEK 2025	ATTA <u>GAATTCC</u> ATAGAACTTTTAGAAGTTCAAG
MEK 2255 ^b	CAGCATTAAATGCACATGCAAATTC
MEK 2256 ^b	CGATGTTGGTGTTTATCCTAAAAC
MEK 2257 ^b	ATGTTAATGCTAGAGAACTGGATAAG
MEK 2258 ^b	CTGAATTTGCATGTGCATTTAATGC
MEK 2259 ^b	TTAAATGCTAGAGAACTGGATAAGTC
MEK 2260 ^b	AACTGAATTTGCATGTGCATTTAATG
MEK 2391 ^b	AAGATCAGCGTTTGCTTTTGC
MEK 2392 ^b	CAAATCAGCGTTTGCTTTTGC
MEK 2393 ^b	TAGATCAGCGTTTGCTTTTGC
MEK 2394 ^b	GAGATCAGCGTTTGCTTTTGC
MEK 2395 ^b	CAGATCAGCGTTTGCTTTTGC

a. Restriction endonuclease cleavage sites are underlined.

b. Primers with 5' phosphorylation.

Supplemental Figure 1. Alignment of *C. jejuni* NCTC 11168 FlaA and FlaB with *S. typhimurium* LT2 FliC. *C. jejuni* NCTC 11168 FlaA and FlaB were aligned with *S. typhimurium* LT2 FliC at the peptide level using the ClustalW algorithm. A consensus sequence is displayed above the three peptide sequences. Residues that are identical among the three sequences are highlighted in gray.

LT2 FliC NCTC 11168 FlaA NCTC 11168 FlaB	MGFRINTNXXALNAXANXXXXLDXSLSRLSSGLRINSAADDASGMAIADSLRSQAXTLGQAIXNGND 10 20 30 40 50 60 70 MAQVINTNSLSLTQNINKSQSALGTAIERLSSGLRINSAKDDAAGQAIANRFTANIKGITQASRNAND MGFRINTNVAALNAKANADLNSKSLDASLSRLSSGLRINSAADDASGMAIADSLRSQANTLGQAISNGND MGFRINTNIGALNAKANADLNSKSLDASLSRLSSGLRINSAADDASGMAIADSLRSQAATLGQAISNGND MGFRINTNIGALNAHANSVVNARELDKSLSRLSSGLRINSAADDASGMAIADSLRSQAATLGQAINNGND
LT2 FliC NCTC 11168 FlaA NCTC 11168 FlaB	AIGILQTADKAMDEQLKILDTIKTKATQAAQDGQSLKTRTMLQADINRLMEELDNIANTTSFNGKQLLSG 80 90 100 110 120 130 140 GISIAQTTEGALNEINNNIQRVRELAVQSANSTNSQSDLDSIQAEITQRLNEIDRVSGQTOFNGVKVLAQ ALGILQTADKAMDEQLKILDTIKTKATQAAQDGQSLKTRTMLQADINRLMEELDNIANTTSFNGKQLLSG AIGILQTADKAMDEQLKILDTIKTKATQAAQDGQSLKTRTMLQADINRLMEELDNIANTTSFNGKQLLSG
LT2 FliC NCTC 11168 FlaA NCTC 11168 FlaB	NFINQEFQIGASSNQTIKATIGATQSSKICITRFETGGRISXSGEVQFTLKNYNGIDDFQFQKVVISTSV 150 160 170 180 190 200 210 DNT-LTIQVGANDGETIDIDLKQINSQTLGLDTLNVQQKYKVS-DTAATVTGY-ADTTIALDNSTFKAS NFINQEFQIGASSNQTVKATIGATQSSKIGLTRFETGGRISTSGEVQFTLKNVNGIDFQFQKVVISTSV NFINQEFQIGASSNQTVKATIGATQSSKIGLTRFETGGRISTSGEVQFTLKNVNGIDFQFQKVVISTSV
LT2 FliC NCTC 11168 FlaA NCTC 11168 FlaB	GTGLGALAXEINKXADKTGVRATFTVETRGIAAVRAGTTSDTFAINGVXIGXVXXXDGDGNGALVAAINS 220 230 240 250 260 270 280 ATGLGGTDQKIDGDLKFDDTTGKYYAKVTVT-GGTGKD GGYEV GTGLGALADEINKNADKTGVRATFTVETRGIAAVRAGATSDTFAINGVKIGKVDYKDGDANGALVAAINS GTGLGALAEEINKSADKTGVRATFTVETRGIAAVRAGATSDTFAINGVKIGQVAYEDGDGNGALVAAINS
LT2 FliC NCTC 11168 FlaA NCTC 11168 FlaB	VKDTTGVEASIDANGQLLLTSREGRGIKIDGNIGGGAFINADMKENYGRLSLVKNDGKDILISGSNLSSA 290 300 310 320 330 340 350 SVDKINGEVTLAGGATSPLTGGLPATATEDVKNVQVANADLTEAKAALTAA VKDTTGVEASIDANGQLLLTSREGRGIKIDGNIGGGAFINADMKENYGRLSLVKNDGKDILISGSNLSSA VKDTTGVEASIDANGQLLLTSREGRGIKIDGNIGGGAFINADMKENYGRLSLVKNDGKDILISGSNLSSA
LT2 FliC NCTC 11168 FlaA NCTC 11168 FlaB	GFGATQFISQASVSLRESKGXIDANIADAMCFGSANKGVVLGCYSSVSAYMSSAGSGFSSGSGYSVGSGK 360 370 380 300 400 410 420 GVTGTASVVKMSYTDNNGK-TIDGGLAVKVGDDYSATQNKDCSISINTTKYTADDCTSKTALNKLG GFGATQFISQASVSLRESKQIDANIADAMGFGSANKGVVLGCYSSVSAYMSSAGSGFSSGSGYSVGSGK GFGATQFISQASVSLRESKGRFDANIADAMGFGSANKGVVLGCYSSVSAYMSSAGSGFSSGSGYSVCSGK
LT2 FliC NCTC 11168 FlaA NCTC 11168 FlaB	NYSTOFANAIAISASQLSTVYNVSAGSGFSSGSTLSQFATMKTTAFGVKDETAGVTTLKGAMAVMDIAE 430 440 450 470 480 490 CADGKTEVVSIGGKTYAASKAECHNFKAQPDIAEAAATTENPLQKIDA NYSTGFANAIAISAASQLSTVYNVSAGSGFSSGSTLSQFATMKTTAFGVKDETAGVTTLKGAMAVMDIAE NYSTGFANAIAISAASQLSTVYNVSAGSGFSSGSTLSQFATMKTTAFGVKDETAGVTTLKGAMAVMDIAE
LT2 FliC NCTC 11168 FlaA NCTC 11168 FlaB	TAXTNLDQIRADICSVQNQXXSTINNITVTQVNVKAAESXIRDVDFAAESANXSKANILAQSGSYAMAQA 500 510 520 530 540 550 -aLAQVDTLRSDLGAVQNRFNSAITNLGNTVNNLTSARSRIEDSDYATEVSNMSRAQILQQAGTSVLAQA TAITNLDQIRADICSVQNQVTSTINNITVTQVNVKAAESQIRDVDFAAESANYSKANILAQSGSYAMAQA TATTNLDQIRADICSVQNQLQVTINNITVTQVNVKAAESTIRDVDFAAESANYSKANILAQSGSYAMAQA
LT2 FliC NCTC 11168 FlaA NCTC 11168 FlaB	NXVQQNVLXLLQ- 570 NQVPQNVLSLLR. NSVQQNVLRLLQ. NAVQQNVLKLLQ.

Supplemental Figure 2. Immunoblots of wild-type, *flaAB* mutant, and promoter swap strains. Analysis of FlaA and FlaB protein synthesis in the *C. jejuni* F38011 wild-type strain, F38011 *flaAB* mutant, and F38011 *flaAB* mutant transformed with *PflaA-flaA*. Whole cell lysates were prepared from broth cultures of *C. jejuni* in mid-exponential growth phase, and separated in 12% SDS-polyacrylamide gels. Panels: A)Whole cell lysates; and B) Supernatants probed with a polyclonal serum that recognizes both FlaA and FlaB. The samples in each lane are the: 1) *C. jejuni* F38011 wild-type strain; 2) *C. jejuni* F38011 *flaAB* mutant; and 3) *C. jejuni* F38011 *flaAB* mutant harboring *PflaA-flaA*. The protein loads in the immunoblots (Panels A and B) are normalized according to culture density, so the levels of flagellin can be directly compared.



Supplemental Figure 3. Phase variation motility plates.

C. jejuni F38011 wild-type and the *flaAB* mutant harboring *flaA* expressed from the *flaA* promoter were grown for 48 h on MHB agar plates and diluted in MH soft agar (0.4% agar) to a density that would yield ~30 CFUs when plated. After 48 h incubation, the motility of 500 CFU were observed to determine the percentage of the population that were non-motile. A representative plate of the *flaAB* mutant harboring *flaA* expressed from the *flaA* promoter is shown, which contains only motile colonies. The percentage of non-motile colonies observed for the *C. jejuni* F38011 wild-type strain and the *C. jejuni flaAB* mutant harboring pRY111 containing *flaA* expressed from the *flaA* promoter were 2% and 1.4%, respectively.

