SUPPLEMENTAL MATERIALS & METHODS

Construction of *C. jejuni* 81-176 Δ*1228* and Δ*1228c* complemented strain.

The *cjj81176_1228* gene was PCR amplified with iProof (Biorad) from 81-176 genomic DNA using primers 1228-L2 and 1228-R2. A polyA tag was added to the PCR product and it was ligated to a commercially available pGEM-T vector (Promega). The resulting construct pGEMT-*1228* was verified by restriction enzyme digestion and sequencing. Inverse PCR was performed on the resulting plasmid with primers 1228-IL1 (*SacI*) and 1228-IR1 (*HincII*) deleting 1014 bp of the gene. The inverse PCR product was digested with *SacI* and *HincII* encoded by the primers and ligated to the similarly digested non-polar kanamycin resistance cassette (*aphA-3*) digested out of pUC18K-2 (1), forming construct pGEMT-*pgp1*Km. The construct was verified by restriction enzyme digestion and naturally transformed into *C. jejuni* 81-176. 81-176 Δ*1228* mutant strains were selected by a Km^R phenotype and verified by PCR and restriction enzyme digestion.

For complementation of *1228*, the gene was PCR-amplified from 81-176 genomic DNA and cloned into the pRRC integration vector (2) to form pEF48. The *1228* gene and 330 bp of the upstream sequence containing the putative promoter were PCR amplified with primers 1228-1 (*XbaI*) and 1228-2 (*XbaI*). The PCR product was digested with *XbaI* and ligated to pRRC digested with *XbaI* and dephosphorylated. The resulting construct was verified for orientation by PCR with the *1228* gene in the same orientation as the antibiotic resistance cassette encoded by the vector, and sequenced. Plasmids were inserted into Δ*1228* by natural transformation, and transformants selected on the appropriate antibiotics. Single insertions into the rRNA spacer region were verified by PCR with primers ak233, ak234, ak235 (2) and cat-2 for pRRC.

Confirmation of the Δ*1228c* complemented strain in Mueller-Hinton (MH) and Minimum Essential Medium (MEM) broth: morphology and *1228* expression levels.

Overnight cultures of the 81-176 wild type, Δ*1228* mutant and Δ*1228c* complemented strains were grown in MH biphasic flasks. These were then used to inoculate MH or MEM cultures to an OD_{600} of 0.1. After 4 hours growth, 10 μl samples were removed, formalin fixed and stained stained using *Campylobacter jejuni* specific antibodies (Abcam) directly on the glass slides, and imaged at 1000 x. RNA was extracted from the remainder of the sample using a hot-phenol RNA extraction as described elsewhere (3). cDNA was synthesized from each RNA sample using "All-in-one RT Mastermix" (ABM) and qPCR was performed using IQ-SYBR green supermix (Bio-rad). Expression of 1228 was analyzed relative to *C. jejuni* 16sRNA using Bio-rad CFX software

Table S1: Bacterial strains or plasmids used in this study.

Strain or Plasmid	Genotype, serotype or description	Reference or Source
C. jejuni		
81-176	Wild type isolated from a diarrheic patient	(4)
Δ*pgp1*	81-176 *pgp1*::*aphA3*; Km^R	(5)
Δ*pgp2*	81-176 Δ*pgp2*	(6)
Δ*pgp1c*	81-176 Δ*1344 rrn::pgp1* (from pEF35R)	(5)
Δ*pgp2c*	81-176 Δ*pgp2 rrn::pgp2* (from pJV4)	(6)
Δ*1228*	81-176 *1228*::*aphA3*; Km^R	This study
Δ*1228c*	81-176 Δ*1228 rrn::1228* (from pEF48)	This study
E. coli		
DH5α	F^-, ϕ80d *deoR lacZΔM15 endA1 recA1 hsdR17*(r_K-m_K+) *supE44 thi-1 gyrA96 relA1 Δ(lacZYA-argF) U169*	Invitrogen
Plasmids		
pGEM-T	PCR cloning vector; Ap^R	Promega
pUC18-K2	Source of non-polar *aphA3* cassette; Ap^RKm^R	(1)
pRRC	*C. jejuni* rRNA spacer integration vector; Cm^R	(2)
pEF35R	pRRC-1344-1348 coding for 81-76 *pgp1* and 3816 bp upstream of *pgp1* cloned in the reverse orientation as the *cat* cassette; Cm^R	(5)
pJV4	pRRC containing *pgp2* cloned with 196 bp of upstream sequence cloned in the same orientation as the *cat* cassette; Cm^R	(6)
pGEMT-*1228*	pGEM-T ligated to *1228* amplified with 1228-L2 and 1228-R2; Ap^R	This study
pGEMT-*1228*-Km	pGEMT-*1228* with the *1228* gene disrupted with the *aphA3* cassette; Ap^RKm^R	This study
pEF48	pRRC containing *pgp2* cloned with 330 bp of upstream sequence in the same orientation as the *cat* cassette; Cm^R	This study

Table S2: Primers used in this study

Primer	Sequence 5′ to 3′	Restriction Site	Reference
ak233	GCAAGAGTTTTGCTTATGTTAGCAC		(2)
ak234	GAAATGGGCAGAGTGTATTCTCCG		(2)
ak235	GTGCGGATAATGTTGTTTCTG		(2)
cat-2	GTTTTTTGGATGAATTACAAGA		(5)
1228-L2	TATAACACTTGCTCTAGCGATGATA	*XbaI*	This study
1228-R2	GTGCTAAAAATTATGAAATAGAT	*XbaI*	This study
1228-IL1	tggagctccCAAGGTATCTCCATTATCCCAAGTTAGCTC TTCAAC	*SacI*	This study
1228-IR1	gaagtcgacAATCAACCTAATCCCCCAAAAGAA	*HincII*	This study
1228-1	gctctagaTATAACACTTGCTCTAGCGATGATA	*XbaI*	This study
1228-2	CAATTAATTGATCTAGATTTTTGTGAAATTAGTG	*XbaI*	This study
cjj1228F	TTCGTGCTTTTCGAGGAAGT		This study
cjj1228R	ACCCCAAAGTTTACCCATGC		This study
s-Cjej-F	AAGTCGAACGATGAAGCTCC		(7)
s-Cjej-R	CCTACTCAACTTGTGTTAAGC		(7)

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Supplemental Figure 1. Intracellular survival and IL-8 stimulation by *C. jejuni* cell shape mutants Δ*pgp1* and Δ*pgp2*

(A) Invasion and intracellular survival of the *C. jejuni* Δ*pgp1* and Δ*pgp2* in the INT407 epithelial cell line was assessed by the gentamicin (Gm) protection assay, as carried out previously (5, 6). The Δ*pgp1* and Δ*pgp2* infection profile resembled that of wild type. Gm was added 3 h post-infection. After 2 h, the Gm was washed off and the cells were incubated with fresh MEM containing 3% FBS and a low dose of Gm. At each time point, CFUs were determined for each well by lysing the cells with water and plating the dilutions onto MH-TV plates. Data represent the mean ± SEM of three replicates and are representative of three independent experiments. (B) ELISA was used to quantify IL-8 levels secreted by uninfected INT407 epithelial cell lines and cells infected for 8 and 24h with *C. jejuni* wild type 81-176, Δ*pgp1* and Δ*pgp2* strains. As previously published, infection with the Δ*pgp1* mutant increased IL-8 secretion, and the Δ*pgp2* mutant triggered IL-8 secretion levels similar to wild type (5, 6). Data represent the mean ± SEM of three replicates and are representative of three independent experiments. The asterisk (*) indicates a statistically significant difference using the unpaired Student's t-test, with * indicating $p < 0.05$.

Supplemental Figure 2. Loss of Δ*1228* mutant complementation in MEM.

(A) Wild type *C. jejuni* 81-76 maintains a consistent, helical shape in both nutrient-rich MH medium as well as the nutrient-restricted MEM medium (Top panels). The Δ*1228* defect is evident in both media, with a broader spiral, or "C" shape to the cell (Middle panels). The *Δ1228c* complemented strain shows the restored wild type helical shape in MH medium, but not in MEM medium (Bottom panels). Immunofluorescent images were taken under 1000x magnification. (B) Despite phenotypic differences between media, there were no significant differences in *1228* gene transcription between the wild type and complement strains. The experiment was performed in duplicate and analyzed using Bio-rad software.

Supplemental Figure 3. Enhanced Imaging of *C. jejuni* mucus colonization.

Formalin-fixed, paraffin embedded tissue sections of ceca obtained from *Sigirr*$^{-/-}$ mice infected with wild type and mutant strains, 7 days post-infection at 1000 x magnification. Cell nuclei are stained with DAPI (blue), the goblet cells and secreted mucus are stained with antibodies specific to Muc2 (Green), and *C. jejuni* with *C. jejuni* specific antibodies (red). Both the wild type and *Δ1228* mutants are clearly visible within the mucus-filled crypt, whereas the *Δpgp1* and *Δpgp2* mutants remain on the outside of the mucus layer and the intestinal crypts.

SUPPLEMENTAL FIGURES

FIG. S1

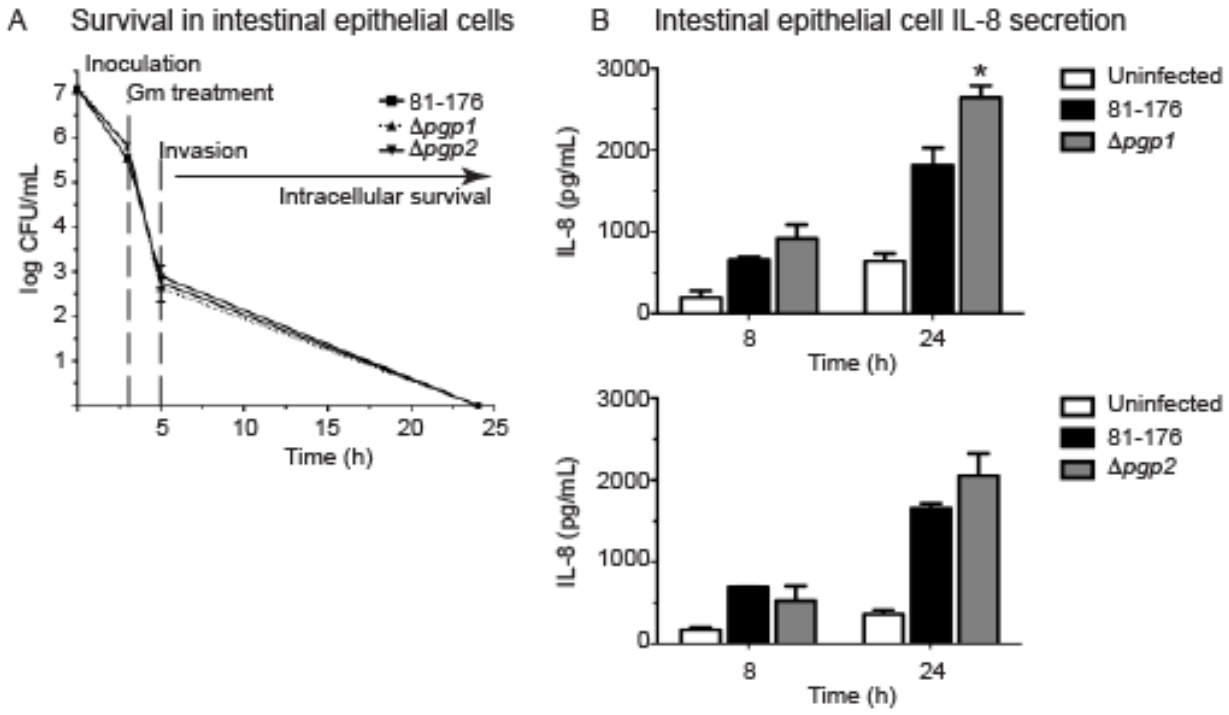

FIG. S2

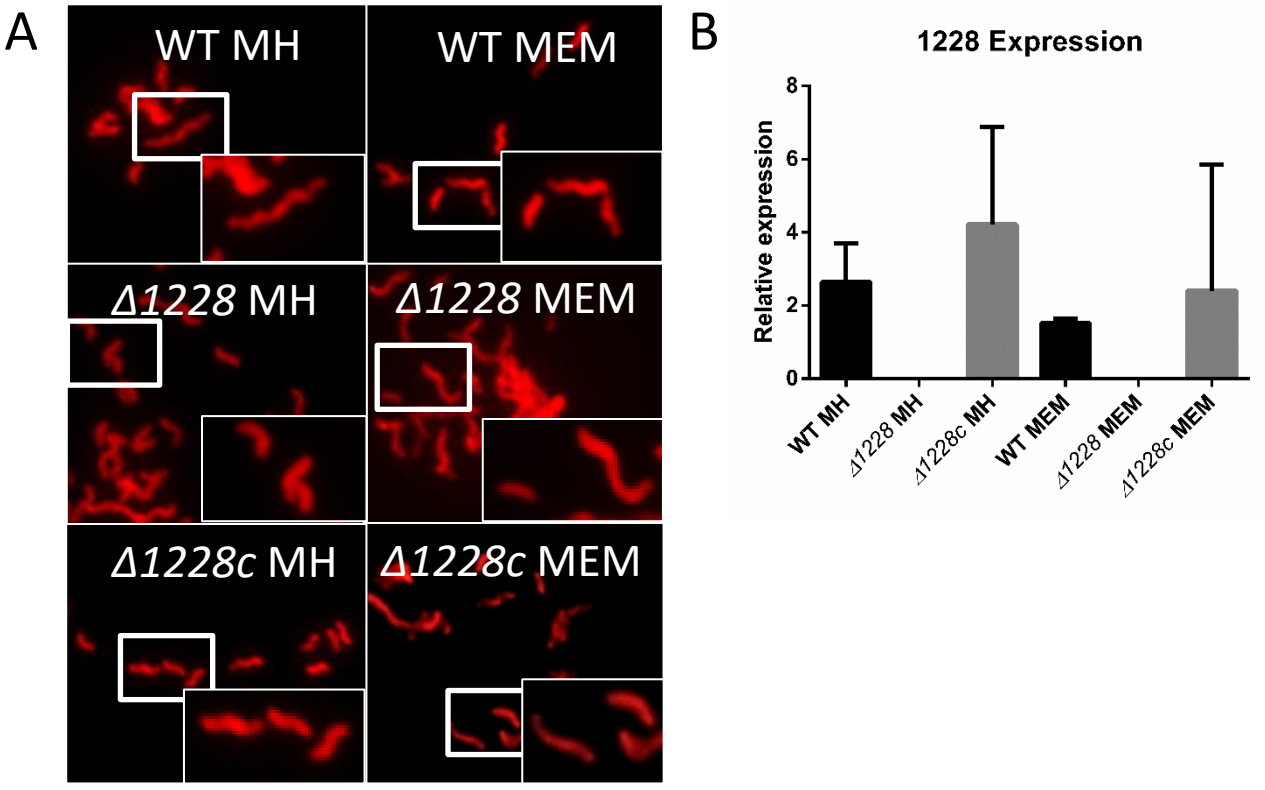
A
WT MH
WT MEM
Δ1228 MH
Δ1228 MEM
Δ1228c MH
Δ1228c MEM
B
1228 Expression
Relative expression
0
2
4
6
8
WT MH
Δ1228 MH
Δ1228c MH
WT MEM
Δ1228 MEM
Δ1228c MEM

FIG. S3