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Supplemental Material to:

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Functional characterization of exopolyphosphatase/ guanosine pentaphosphate phosphohydrolase (PPX/ GPPA) of *Campylobacter jejuni*

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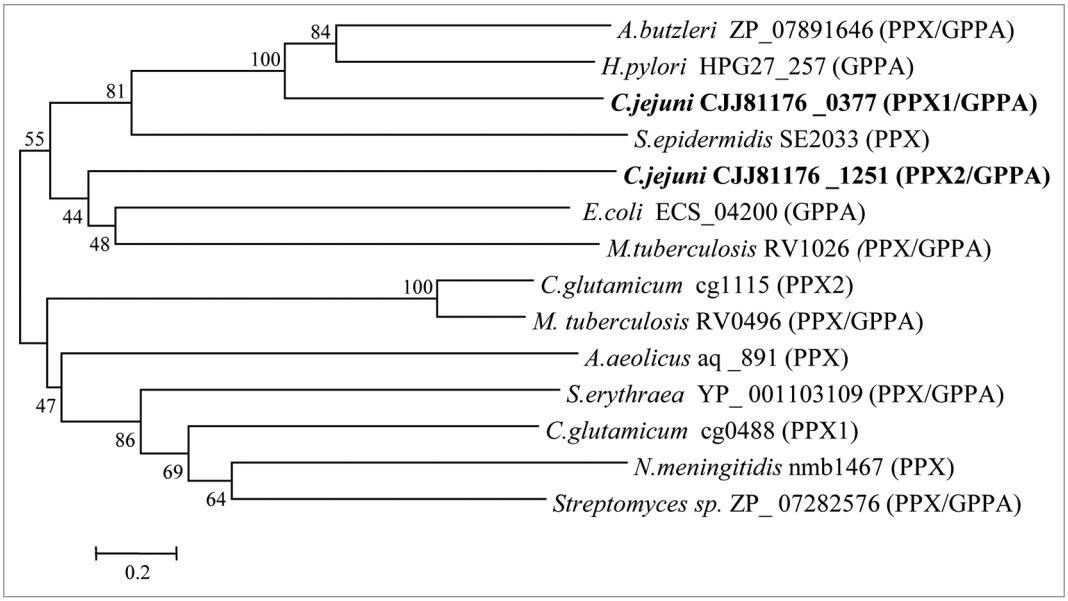
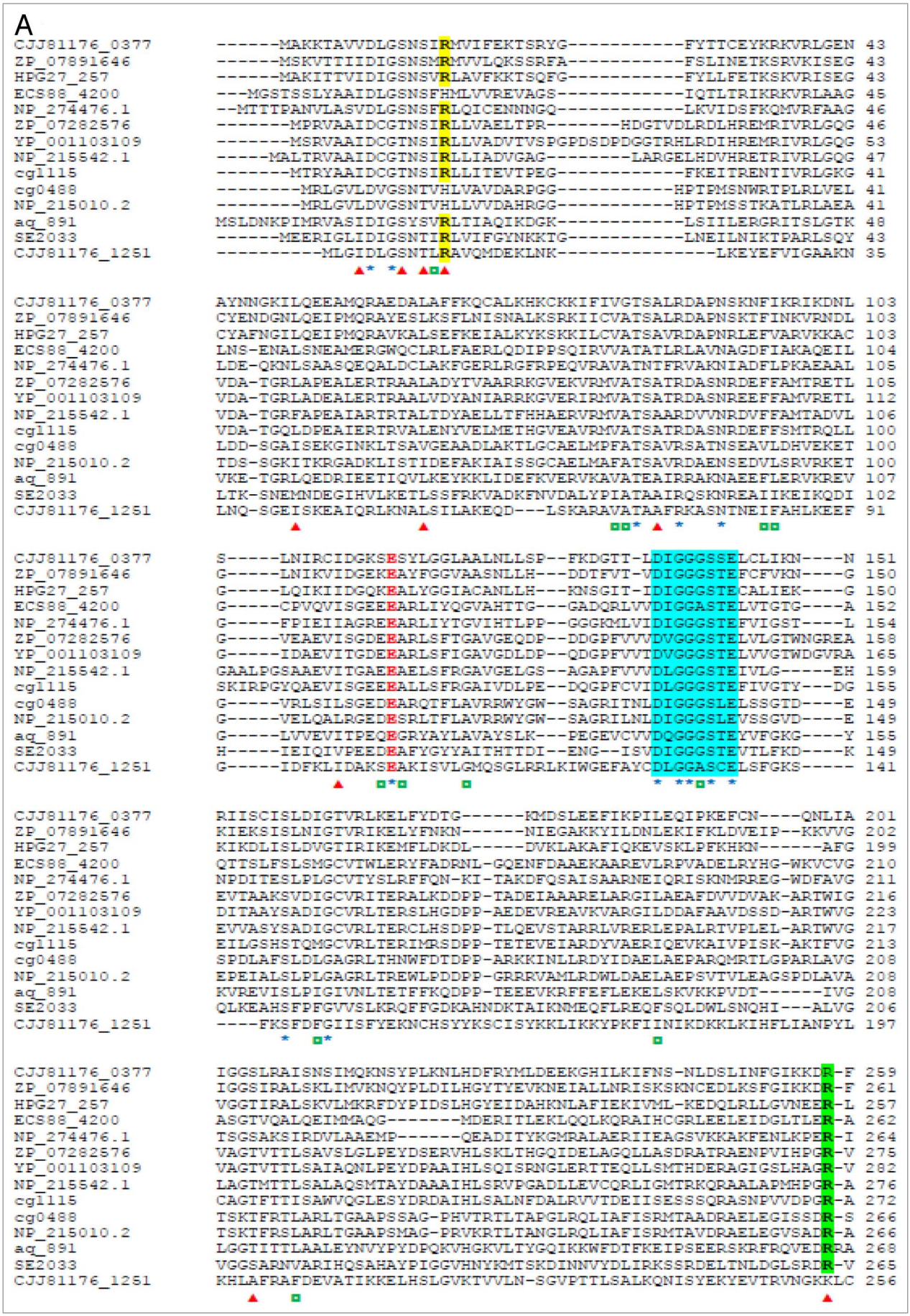


Figure S1. Phylogenetic tree of *C. jejuni* PPX/GPPA enzymes. MEGA-5 software was used to construct the phylogenetic tree. Branch lengths are indicated next to the protein name and are proportional to the predicted evolutionary change. The number at the branch node indicates the cophenetic correlation coefficient.

Figure S2A (See next page). Structure-based sequence alignment of PPX/GPAA proteins. (**A**) Alignment of PPX/GPPA domains of *C. jejuni* and related bacteria. Sequence alignment was performed using ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html). * indicates positions which have a single, fully conserved residue; ▲, the residues strongly conserved; ■, the residues weakly conserved. Catalytic residue required for phosphatase activity is highlighted in bold letters in red color and the residues required for guanosine pentaphosphate specificity are highlighted by bold black letters on yellow and green background. The conserved amino acids sequence highlighted in sky blue indicates the Walker B motif. *C. jejuni* CJJ81176_0377-(PPX1/GPPA); *A. butzleri* JV22 ZP_07891646-(PPX/GPPA); *H. pylori* HPG27_257-(GPPA); *E. coli* ECS88_4200-(GPPA); *N. meningitidis* MC58 nmb1467-NP_274476.1 (PPX); *Streptomyces* spp. AA4 ZP_07282576-(PPX/GPPA); *S. erythraea* NRRL2338 YP_001103109-(PPX/GPPA); *M. tuberculosis* RV1026-NP_215542.1(PPX/GPPA); *C. glutamicum* ATCC 13032 cg1115-(PPX2); *C. glutamicum* cg0488-(PPX1); *M. tuberculosis* RV0496- NP_215010.2 (PPX/GPPA); *A. aeolicus* aq_891-(PPX); *S. epidermidis* SE2033-(PPX); *C. jejuni* CJJ81176_1251-(PPX2/GPPA). For (**B and C**), see following page.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cg1115 -------MTRYAAIDCGTNSIRLLITEVTPEG----------FKEITRENTIVRLGKG 41
cg0488 --------MRLGVLDVGSNTVHLVAVDARPGG----------HPTPMSNWRTPLRLVEL 41
NP_215010.2 --------MRLGVLDVGSNTVHLLVVDAHRGG----------HPTPMSSTKATLRLAEA 41
aq_891 MSLDNKPIMRVASIDIGSYSVRLTIAQIKDGK----------LSIILERGRITSLGTK 48
SE2033 ------MEERIGLIDIGSNTIRLVIFGYNKKTG----------LNEILNIKTPARLSQY 43
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HPG27_257 CYAFNGILQEIPMQRAVKALSEFKEIALKYKSKKILCVATSAVRDAPNRLEFVARVKKAC 103
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HPG27_257 KIKDLISLDVGTIRIKEMFLDKDL-----DVKLAKAFIQKEVSKLPFKHKN------AFG 199
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aq_891 KVREVISLPIGIVNLTETFFKQDPP-TEEEVKRFFEFLEKELSKVKKPVDT------IVG 208
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CJJ81176_1251 ----FKSFDFGIISFYEKNCHSYYKSCISYKKLIKKYPKFIINIKDKKLKIHFLIANPYL 197
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HPG27_257 VGGTIRALSKVLMKRFDYPIDSLHGYEIDAHKNLAFIEKIVML-KEDQLRLLGVNEER-L 257
ECS88_4200 ASGTVQALQEIMMAQG-------MDERITLEKLQQLKQRAIHCGRLEELEIDGLTLER-A 262
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ZP_07282576 VAGTVTTLSAVSLGLPEYDSERVHLSKLTHGQIDELAGQLLASDRATRAENPVIHPGR-V 275
YP_001103109 VAGTVTTLSAIAQNLPEYDPAAIHLSQISRNGLERTTEQLLSMTHDERAGIGSLHAGR-V 282
NP_215542.1 LAGTMTTLSALAQSMTAYDAAAIHLSRVPGADLLEVCQRLIGMTRKQRAALAPMHPGR-A 276
cg1115 CAGTFTTISAWVQGLESYDRDAIHLSALNFDALRVVTDEIISESSSQRASNPVVDPGR-A 272
cg0488 TSKTFRTLARLTGAAPSSAG-PHVTRTLTAPGLRQLIAFISRMTAADRAELEGISSIR-S 266
NP_215010.2 TSKTFRSLARLTGAAPSMAG-PRVKRTLTANGLRQLIAFISRMTAVDRAELEGVSADR-A 266
aq_891 LGGTITTLAALEYNVYPYDPQKVHGKVLTYGQIKKWFDTFKEIPSEERSKRFRQVEIRRA 268
SE2033 VGGSARNVARIHQSAHAYPIGGVHNYKMTSKDINNVYDLIRKSSRDELTNLDGLSRIR-V 265
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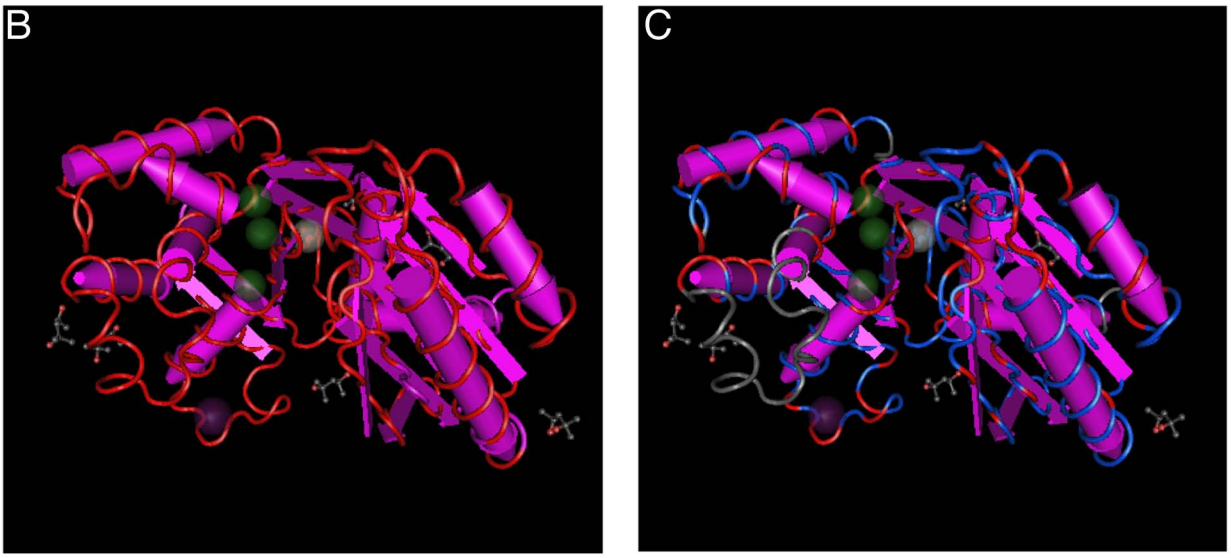

Figure S2B and C. Structure-based sequence alignment of PPX/GPAA proteins. (**B and C**) Predicted 3D structure of *C. jejuni* PPX/GPPA proteins. 3D structure was identified with CBLAST, 3D structure alignment search tool using *A. aeolicus* PPX/GPPA as reference (http://www.ncbi.nlm.nih.gov/Structure/CBLAST). (**B**) 3D structural alignment of *C. jejuni* PPX1/GPPA. (**C**) 3D structural alignment of *C. jejuni* PPX2/GPPA. The region in pink or red indicates *C. jejuni* PPX/GPPA residues identical to *A. aeolicus* PPX/GPPA, while the region with no homology is indicated in blue. The region in grey indicates unaligned sequences of *C. jejuni* PPX/GPPA. For (**A**), see previous page.

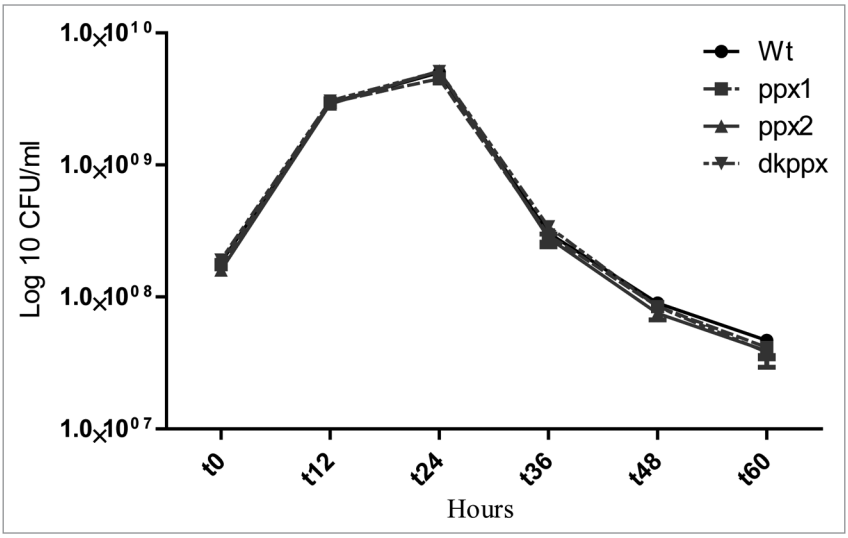

Figure S3. Growth kinetics of *C. jejuni* Δppx mutants. Mid-log phase grown cultures were used to adjust an OD_{600} of 0.05 in 5 ml of MH broth and incubated microaerobically at 42 °C with shaking at 200 rpm. The growth was assessed by CFU determination at different time points.

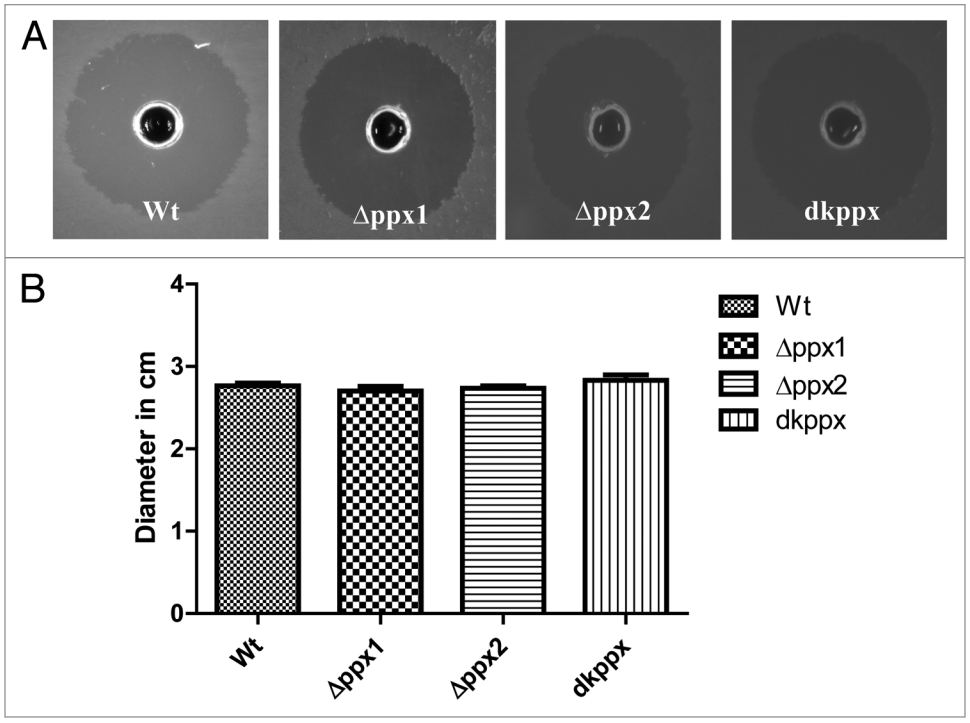

Figure S4. Sensitivity of Δppx mutants to oxidative stress. (**A**) Sensitivity to oxidative stress was determined by disc diffusion assay using 0.3% hydrogen peroxide or 20 mM paraquat. (**B**) Quantification of oxidative stress, the zone of inhibition was measured after 24 h of incubation and expressed in centimeter. Each bar represents the mean ± SE from 3 independent experiments performed in triplicate each time.

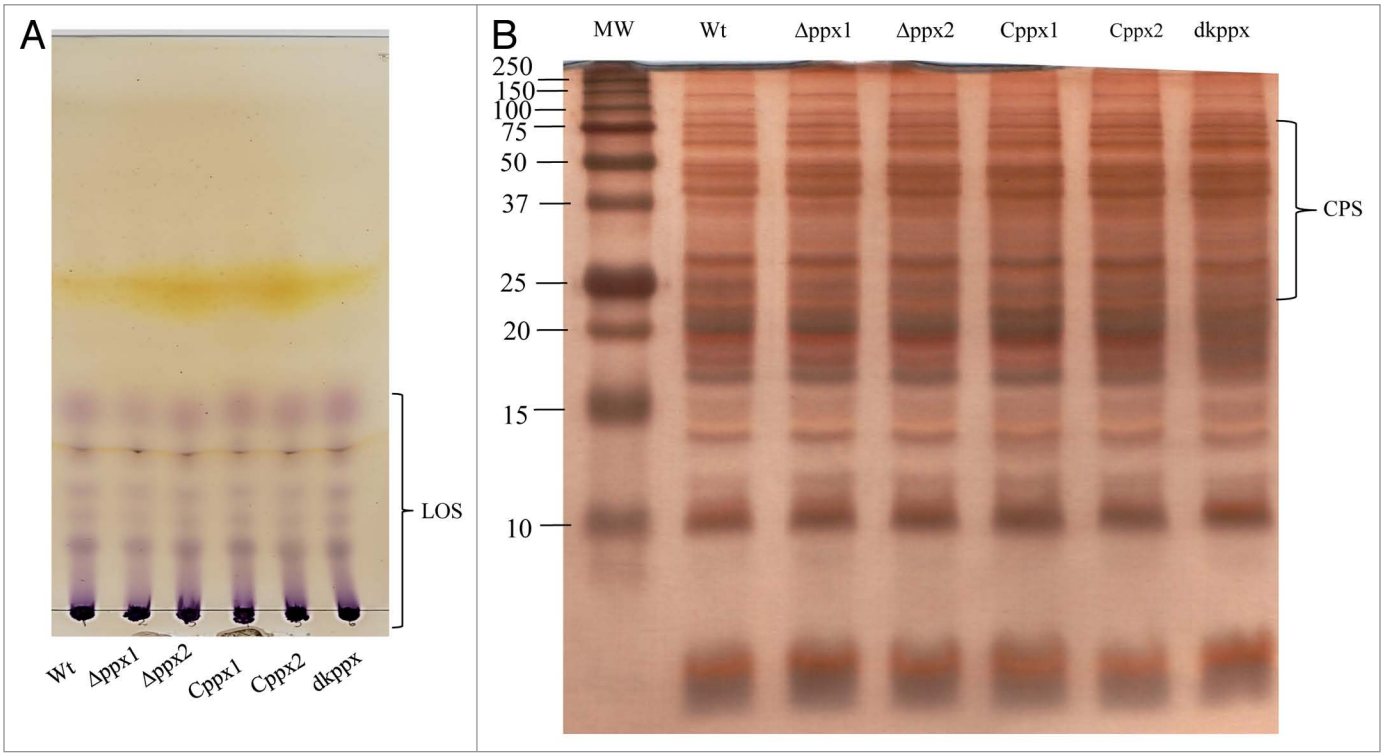

Figure S5. LOS profile of Δppx/gppa mutants. (**A**) Whole cell lysate (50 μg protein) was spotted on TLC plate and developed with n-propanol-water-25% NH_4OH (60:30:10-v/v/v) solvent system and visualized by using 10% sulfuric acid in ethanol. (**B**) CPS whole cell lysates (20 μg protein) were separated by 15% SDS-PAGE and stained with periodic acid silver nitrate (PAS) stain.

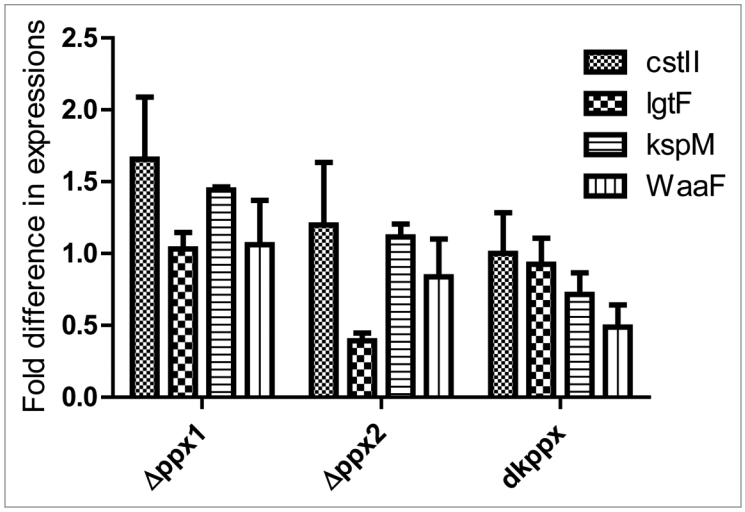

Figure S6. qRT-PCR analysis of *C. jejuni* Δ*ppx* mutants for genes involved in LOS and CPS biosynthesis. Fold difference in transcript level was assessed by ΔΔCT method after normalizing the expression to 16s-*rRNA* and compared to wild-type expression level. Each bar represents the mean ±SE of the relative fold change in expression from 4 independent experiments performed in duplicates each time. *$P \leq 0.05$.

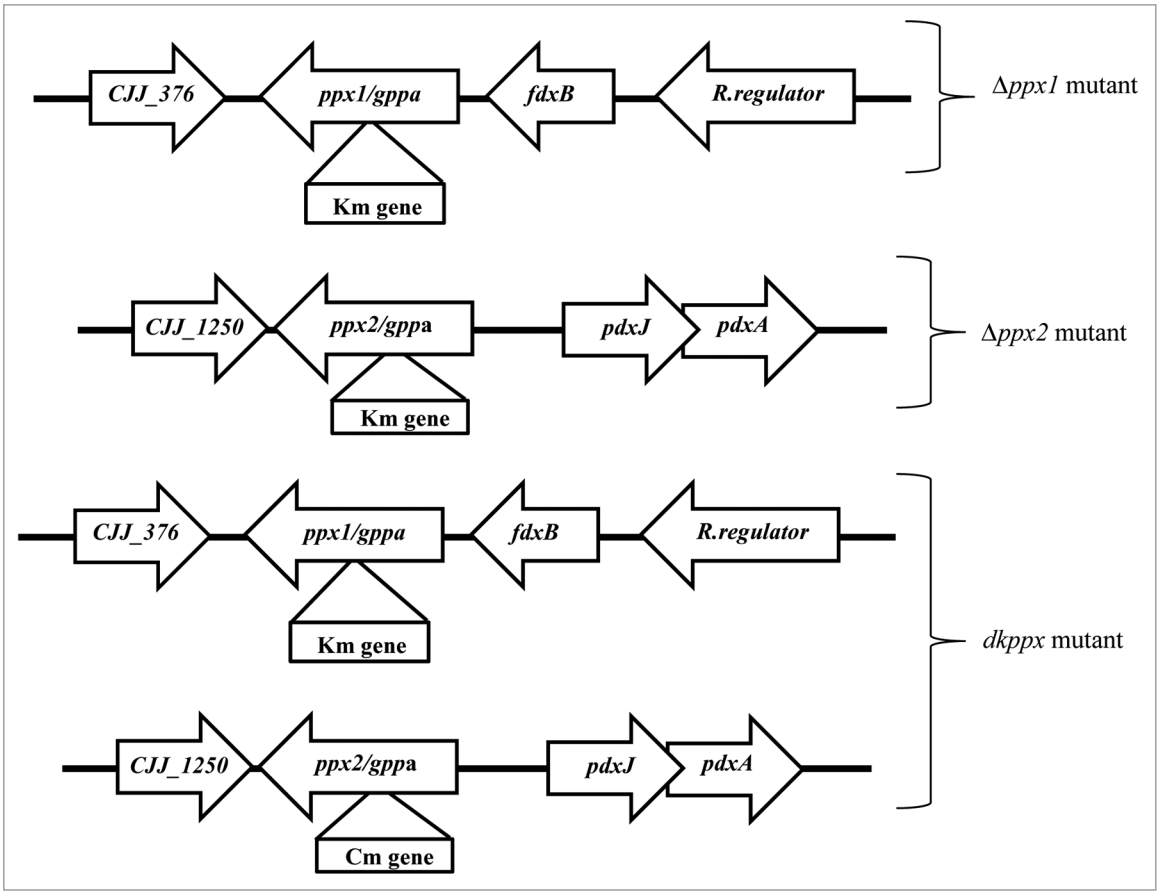

Figure S7. Genetic organization of *ppx1/gppa* and *ppx2/gppa* genes in *C. jejuni* genome. Schematic representation (not drawn to scale) of approximate location of kanamycin, and chloramphenicol antibiotic markers in Δ*ppx* deletion mutants of *C. jejuni* 81-176 strain. The *fdxB* encodes ferredoxin, *pdxJ* encodes for pyridoxine 5′-phosphate synthase, and the *pdxA* encodes 4-hydroxythreonine-4-phosphate dehydrogenase.

Table S1. List of primers used in this study.

Name	Sequence (5′-3′)	Description
PPX1/GPPA_F	AAATTA**GGTA CC**ATATGAGT GTAGCGGAGC TT	Used for *ppx1/gppa* gene amplification along with 1 kb up and downstream sequences
PPX1/GPPA_R	AAATTA**CTGC AG**GGTTATCA AACCGATTCT TC	
PPX1/INV_F	TTAATT**GGAT CC**TCTCTATG TTTGAGTTTT AA	Used for *ppx1/gppa* gene deletion by inverse PCR
PPX1/INV_R	AATTAA**GGAT CC**CTCTATAA GATTTTGAAT TC	
PPX1C_F	TTAATT**GGAT CC**TCTCTATG TTTGAGTTTT AA	Used for *ppx1/gppa* gene amplification for complementation
PPX1C_R	GATAT**GGATC C**GAAGCTATT TATGAAAATA G	
PPX2/GPPA_F	AAATTA**GGTA CC**AAACAAGT TGATCCTTTG GA	Used for *ppx2/gppa* gene amplification along with 1 kb up and downstream sequences
PPX2/GPPA_R	AAATTA**CTCG AG**GTAAGCTT TCGTGGATAA AA	
PPX2/INV_F	TAATAA**GGAT CC**AGTATTTG AACCAAGATC TA	Used for *ppx2/gppa* gene deletion by inverse PCR
PPX2/INV_R	AATTAA**GGAT CC**ACTTTCGT AAGTTTTAAT GT	
PPX2C_F	ATGC**GGTACC** CTTGCTTTAA TGGATTTTGG	Used for *ppx2/gppa* gene amplification for complementation
PPX2C_R	ATAAA**GGATC C**CCGTGTTTA GCTGCTATAA A	
SpoT F	GTAACCACTC GCACAATATC	Used for qRT-PCR
SpoT R	GATGTCGCAG TTTATTCTCC	
PPK1 F	TGAAGCAAGT ATGGAAGGAG	Used for qRT-PCR
PPK1 R	ATATAGGAGT CATAAGTTCT AAGC	
PPK2 F	ATCTAATACT CCAACTTGTC	Used for qRT-PCR
PPK2 R	TTCTTCTTCT CCACTACG	
LgtF F	ACCTCAGTGC AAGGGAAGAA GGAA	Used for qRT-PCR
LgtF R	TCTCATGCTC GGTACCATCA GCTT	
CstII_F	AGCTCACATC CTCGTATTCC ACCA	Used for qRT-PCR
CstII_R	AGAAGCGTCG GGTCTTCTCG CT	
KpsM_F	CCCTAAAGCA AAAGCTGAGC	Used for qRT-PCR
KpsM_R	TTAGCCTATA AACCTGTAAA ACCTATA	
WaaF_F	ATCACAAATG ACAGTGGACC T	Used for qRT-PCR
WaaF_R	GCCAAGGTGA AGTTTGAGTA AAT	
16s RNA_F	GTCTCTTGTG AAATCTAATG	Used as qRT-PCR internal control
16s RNA_R	GTATTCTTGG TGATATCTAC	

Bold sequence indicate the restriction enzymes site.