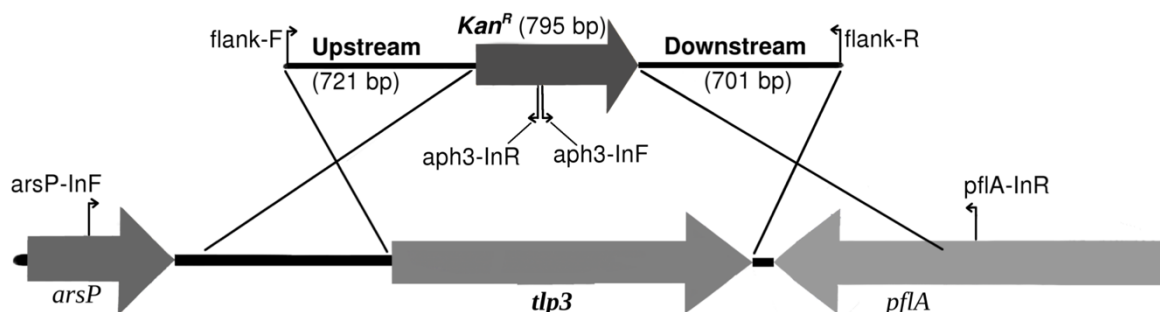
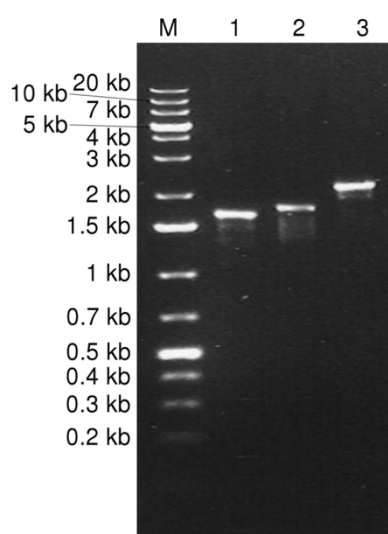


Supplementary information

A



B



Supplementary Figure 1. Generation of isogenic *tlp3* mutant in *C. jejuni* NCTC 11168. (A) Schematics showing the chromosomal locus of the target gene *tlp3* (bottom) and the design of the mutagenic construct (top) containing the *C. coli* kanamycin resistance gene *aphA3* flanked by *C. jejuni* chromosomal DNA sequences corresponding to the regions immediately upstream and downstream of *tlp3*. The crosses indicate anticipated crossing-over of the homologous gene regions annotated 'upstream' and 'downstream', resulting in the deletion of *tlp3* and introduction of kanamycin resistance. The positions of PCR primers used for colony screening are indicated with bent arrows. Primer sequences were as follows: *arsP-InF*, GCTGTGCAAATACTCAAGAT; *aph3-InR*, AGCTATTCGTATAGGGACA; *flank-F*, GGTCAATCAAGACTTTCAAGAC; *aph3-InF*, TACGAATAGCTTAGACAGC; *flank-R*, CTAATGTTGAACAAGCACTTG; *pflA-InR*, TGCAAGAGCAGCTATGAGT. (B) Confirmation of the homologous recombination event generating the *Δtlp3::kan* mutant by PCR. The chromosomal DNA extracted using GenElute™ Bacterial DNA extraction kit (Sigma-Aldrich) was used as a template. Lanes 1 to 3 correspond to the PCR products for the primer pairs *arsP/aph3-InR*, *aph3-InF/pflA-InR* and *flank-F/flank-R*, respectively.

Supplementary Table S1. Tlp3 LBD thermal shift assay results with the compounds from the Biolog PM1, PM3B and PM5 screens. The ligands that induced a stabilising effect are highlighted in yellow.

PM1 plate (carbon sources)					
Well	Compound	ΔT_m (°C)	Well	Compound	ΔT_m (°C)
A1	Negative Control (water)	0.0 ± 0.1	D3	D-Glucosaminic Acid *	-0.1 ± 0.4
A2	L-Arabinose	0.2 ± 1.1	D4	1,2-Propanediol	-0.5 ± 0.1
A3	N-Acetyl-D-Glucosamine	0.3 ± 0.5	D5	Tween 40	0.1 ± 0.1
A4	D-Saccharic Acid	0.0 ± 0.2	D6	α -Keto-Glutaric Acid*	-0.1 ± 0.0
A5	Succinic Acid *	-0.1 ± 0.6	D7	α -Keto-Butyric Acid	0.0 ± 0.6
A6	D-Galactose	0.0 ± 0.7	D8	α -Methyl-D-Galactoside	0.3 ± 0.4
A7	L-Aspartic Acid *	-0.2 ± 0.7	D9	α -D-Lactose	0.2 ± 0.5
A8	L-Proline	0.3 ± 0.3	D10	Lactulose	-0.2 ± 0.6
A9	D-Alanine	0.0 ± 0.2	D11	Sucrose	-0.4 ± 0.2
A10	D-Trehalose	0.2 ± 0.3	D12	Uridine	-0.5 ± 0.4
A11	D-Mannose	-0.1 ± 0.7	E1	L-Glutamine	0.3 ± 0.8
A12	Dulcitol	0.1 ± 0.1	E2	m-Tartaric Acid	-0.6 ± 0.3
B1	D-Serine	0.0 ± 0.5	E3	D-Glucose-1-Phosphate	-0.9 ± 0.2
B2	D-Sorbitol	0.2 ± 0.3	E4	D-Fructose-6-Phosphate	-0.8 ± 0.2
B3	Glycerol	-0.3 ± 0.7	E5	Tween 80	0.1 ± 0.1
B4	L-Fucose	0.1 ± 0.6	E6	α -Hydroxy Glutaric Acid- γ -Lactone	-0.7 ± 0.5
B5	D-Glucuronic Acid	-0.7 ± 0.5	E7	α -Hydroxybutyric Acid	0.2 ± 0.7
B6	D-Gluconic Acid	-0.7 ± 0.3	E8	β -Methyl-D-Glucoside	0.5 ± 0.7
B7	D,L- α -Glycerol-Phosphate	-1.1 ± 0.1	E9	Adonitol	-0.3 ± 0.3
B8	D-Xylose	-1.2 ± 0.3	E10	Maltotriose	-0.2 ± 0.3
B9	L-Lactic Acid	-0.7 ± 0.2	E11	2-Deoxy Adenosine	-0.3 ± 0.2
B10	Formic Acid	-0.5 ± 0.4	E12	Adenosine	-0.1 ± 0.5
B11	D-Mannitol	-0.7 ± 0.4	F1	Glycyl-L-Aspartic Acid	-0.6 ± 0.4
B12	L-Glutamic Acid	-0.7 ± 0.0	F2	Citric Acid	-0.3 ± 0.7
C1	D-Glucose-6-Phosphate	-0.9 ± 0.5	F3	m-Inositol	-0.3 ± 0.7
C2	D-Galactonic Acid- γ -Lactone	-1.1 ± 0.4	F4	D-Threonine	-0.6 ± 0.2
C3	D,L-Malic Acid *	-0.6 ± 0.6	F5	Fumaric Acid	-0.9 ± 0.6
C4	D-Ribose	-0.7 ± 0.5	F6	Bromo Succinic Acid	-1.4 ± 0.7
C5	Tween 20	-0.2 ± 0.1	F7	Propionic Acid	-0.9 ± 0.2
C6	L-Rhamnose	-0.8 ± 0.3	F8	Mucic Acid	-0.8 ± 0.4
C7	D-Fructose	-0.7 ± 0.7	F9	Glycolic Acid	-0.7 ± 0.9
C8	Acetic Acid	0.0 ± 0.3	F10	Glyoxylic Acid	-0.7 ± 0.1
C9	α -D-Glucose	-0.1 ± 0.3	F11	D-Cellobiose	-1.0 ± 0.5
C10	Maltose	0.2 ± 0.4	F12	Inosine	-1.2 ± 0.3
C11	D-Melibiose	-0.1 ± 0.4	G1	Glycyl-L-Glutamic Acid	-0.7 ± 1.0
C12	Thymidine	0.2 ± 0.2	G2	Tricarballic Acid	-1.1 ± 1.0
D1	L-Asparagine	-0.2 ± 0.1	G3	L-Serine	-1.1 ± 0.2
D2	D-Aspartic Acid *	-0.2 ± 0.4	G4	L-Threonine	-0.7 ± 0.6

PM1 plate (carbon sources)					
Well	Compound	ΔT_m (°C)	Well	Compound	ΔT_m (°C)
G5	L-Alanine	-0.6 ± 0.7	H3	m-Hydroxy Phenyl Acetic Acid	-1.3 ± 0.5
G6	L-Alanyl-Glycine	-0.6 ± 0.4	H4	Tyramine	-0.8 ± 0.7
G7	Acetoacetic Acid	-0.8 ± 0.1	H5	D-Psicose	-0.9 ± 0.4
G8	N-Acetyl- β -D-Mannosamine	-1.1 ± 0.2	H6	L-Lyxose	-1.3 ± 0.2
G9	Mono Methyl Succinate	-0.8 ± 0.6	H7	Glucuronamide	-0.5 ± 1.2
G10	Methyl Pyruvate	-1.8 ± 0.0	H8	Pyruvic Acid	0.1 ± 0.2
G11	D-Malic Acid *	-1.2 ± 0.8	H9	L-Galactonic Acid- γ -Lactone	0.4 ± 0.3
G12	L-Malic Acid *	-0.8 ± 0.4	H10	D-Galacturonic Acid	0.4 ± 0.4
H1	Glycyl-L-Proline	-1.2 ± 0.2	H11	Phenylethylamine	0.1 ± 0.1
H2	p-Hydroxy Phenyl Acetic Acid	-1.4 ± 0.8	H12	2-Aminoethanol	-0.3 ± 0.1
PM3 plate (nitrogen sources)					
A1	Negative control (water)	0.0 ± 0.1	C5	D-Aspartic Acid *	-0.7 ± 0.2
A2	Ammonia	-0.4 ± 0.9	C6	D-Glutamic Acid	-0.9 ± 0.2
A3	Nitrite	-0.2 ± 0.2	C7	D-Lysine *	-0.4 ± 0.4
A4	Nitrate	-1.0 ± 0.3	C8	D-Serine	-0.1 ± 0.4
A5	Urea	-0.8 ± 0.1	C9	D-Valine	0.1 ± 0.3
A6	Biuret	-0.9 ± 0.2	C10	L-Citrulline	0.2 ± 0.1
A7	L-Alanine	-0.8 ± 0.3	C11	L-Homoserine	-0.2 ± 0.4
A8	L-Arginine *	-1.0 ± 0.3	C12	L-Ornithine	-0.1 ± 0.2
A9	L-Asparagine	-1.1 ± 0.1	D1	N-Acetyl-L-Glutamic Acid	0.9 ± 0.4
A10	L-Aspartic Acid *	-0.4 ± 0.3	D2	N-Phthaloyl-L-Glutamic Acid	-0.3 ± 0.2
A11	L-Cysteine	-0.5 ± 0.9	D3	L-Pyroglutamic Acid	0.0 ± 0.7
A12	L-Glutamic Acid	0.2 ± 0.5	D4	Hydroxylamine	-0.4 ± 0.2
B1	L-Glutamine	-0.8 ± 0.5	D5	Methylamine	-0.5 ± 0.3
B2	Glycine	-0.4 ± 0.3	D6	N-Amylamine	0.1 ± 0.4
B3	L-Histidine	-0.4 ± 0.2	D7	N-Butylamine	0.0 ± 0.5
B4	L-Isoleucine	2.1 ± 0.8	D8	Ethylamine	0.3 ± 0.4
B5	L-Leucine	2.4 ± 0.2	D9	Ethanolamine	0.0 ± 0.3
B6	L-Lysine *	-0.7 ± 0.0	D10	Ethylenediamine	-0.2 ± 0.2
B7	L-Methionine	-0.3 ± 0.3	D11	Putrescine	-0.5 ± 0.5
B8	L-Phenylalanine	-0.2 ± 0.0	D12	Agmatine	-0.8 ± 0.8
B9	L-Proline	-0.9 ± 0.2	E1	Histamine	-0.9 ± 0.6
B10	L-Serine	-0.9 ± 0.3	E2	β -Phenylethylamine	-1.2 ± 0.2
B11	L-Threonine	-0.8 ± 0.2	E3	Tyramine	-0.4 ± 0.3
B12	L-Tryptophan	0.3 ± 0.5	E4	Acetamide	-0.1 ± 0.4
C1	L-Tyrosine	0.0 ± 0.5	E5	Formamide	-0.4 ± 0.5
C2	L-Valine	1.3 ± 0.1	E6	Glucuronamide	-0.4 ± 0.5
C3	D-Alanine	-0.5 ± 0.7	E7	D,L-Lactamide	-0.1 ± 0.8
C4	D-Asparagine	-0.2 ± 0.6	E8	D-Glucosamine *	-0.4 ± 0.3

PM3 plate (nitrogen sources)					
Well	Compound	ΔT_m (°C)	Well	Compound	ΔT_m (°C)
E9	D-Galactosamine	-1.4 ± 1.2	G5	Allantoin	-0.1 ± 0.5
E10	D-Mannosamine	-0.3 ± 0.3	G6	Parabanic Acid	-0.3 ± 0.3
E11	N-Acetyl-D-Glucosamine	0.3 ± 0.3	G7	D,L- α -Amino-N-Butyric Acid	0.0 ± 0.1
E12	N-Acetyl-D-Galactosamine	-0.6 ± 0.1	G8	γ -Amino-N-Butyric Acid	0.1 ± 0.2
F1	N-Acetyl-D-Mannosamine	0.1 ± 0.5	G9	ϵ -Amino -N-Caproic Acid	0.1 ± 0.2
F2	Adenine	-0.4 ± 0.4	G10	D,L- α -Amino-Caprylic Acid	0.2 ± 0.8
F3	Adenosine	-0.8 ± 0.2	G11	δ -Amino-N-Valeric Acid	-0.4 ± 0.2
F4	Cytidine	-0.1 ± 1.2	G12	α -Amino-N-Valeric Acid	1.4 ± 0.3
F5	Cytosine	-0.3 ± 0.7	H1	Ala-Asp	-0.4 ± 0.3
F6	Guanine	-1.1 ± 0.2	H2	Ala-Gln	-0.1 ± 0.7
F7	Guanosine	-0.3 ± 0.4	H3	Ala-Glu	0.2 ± 0.3
F8	Thymine	0.2 ± 0.9	H4	Ala-Gly	-0.6 ± 0.2
F9	Thymidine	-0.2 ± 0.3	H5	Ala-His	-0.3 ± 0.2
F10	Uracil	-1.1 ± 0.2	H6	Ala-Leu	0.1 ± 0.2
F11	Uridine	-0.4 ± 0.4	H7	Ala-Thr	1.1 ± 0.3
F12	Inosine	-0.1 ± 0.9	H8	Gly-Asn	-0.3 ± 0.4
G1	Xanthine	-0.5 ± 0.2	H9	Gly-Gln	-0.4 ± 0.4
G2	Xanthosine	-0.1 ± 0.5	H10	Gly-Glu	-0.5 ± 0.1
G3	Uric Acid	-0.2 ± 0.1	H11	Gly-Met	-0.3 ± 0.0
G4	Alloxan	0.0 ± 0.9	H12	Met-Ala	0.0 ± 0.3
PM5 plate (nutrient supplements)					
A1	Negative control (water)	0.0 ± 0.1	B6	L-Lysine *	-0.5 ± 0.1
A3	L-Alanine	-0.5 ± 0.1	B7	L-Methionine	0.3 ± 0.5
A4	L-Arginine *	0.1 ± 0.2	B8	L-Phenylalanine	-0.3 ± 0.4
A5	L-Asparagine	-0.2 ± 0.6	B9	Guanosine-3',5'-cyclic Monophosphate	0.2 ± 0.5
A6	L-Aspartic Acid *	-0.7 ± 0.3	B10	Guanine	-0.3 ± 0.6
A7	L-Cysteine	-0.2 ± 0.4	B11	Guanosine	-0.4 ± 0.1
A8	L-Glutamic Acid	0.0 ± 0.8	B12	2'-Deoxy Guanosine	0.0 ± 0.3
A9	Adenosine-3',5'-cyclicmonophosphate	0.0 ± 0.5	C1	L-Proline	-0.6 ± 0.1
A10	Adenine	-0.3 ± 0.6	C2	L-Serine	-0.3 ± 0.3
A11	Adenosine	0.1 ± 0.6	C3	L-Threonine	-0.4 ± 0.4
A12	2'-Deoxy Adenosine	-0.2 ± 0.1	C4	L-Tryptophan	0.0 ± 0.2
B1	L-Glutamine	-0.2 ± 0.3	C5	L-Tyrosine	-0.2 ± 0.5
B2	Glycine	-0.3 ± 0.3	C6	L-Valine	0.7 ± 0.4
B3	L-Histidine	0.2 ± 0.5	C7	L-isoleucine + L-valine	-0.3 ± 0.2
B4	L- Isoleucine	1.1 ± 0.1	C8	trans-4-Hydroxy L-Proline	-0.7 ± 0.3
B5	L-Leucine	0.6 ± 0.3	C9	(5) 4-Amino-Imidazole-4(5)-Carboxamide	-0.6 ± 0.6

PM5 plate (nutrient supplements)					
Well	Compound	ΔT_m (°C)	Well	Compound	ΔT_m (°C)
C10	Hypoxanthine	-0.5 ± 0.3	F6	Hematin	-0.2 ± 0.2
C11	Inosine	-0.3 ± 0.3	F7	Deferoxamine Mesylate	-0.3 ± 0.2
C12	2'-Deoxy Inosine	-1.0 ± 0.3	F8	D-(+)-Glucose	-0.2 ± 0.1
D1	L-Ornithine	-0.7 ± 0.5	F9	N-Acetyl D-Glucosamine	-0.4 ± 0.2
D2	L-Citrulline	-0.4 ± 0.2	F10	Thymine	-0.3 ± 0.1
D3	Chorismic Acid	-1.0 ± 0.1	F11	Glutathione (reduced form)	0.1 ± 0.4
D4	(-)-Shikimic Acid	-1.1 ± 0.2	F12	Thymidine	-0.1 ± 0.1
D5	L-Homoserine Lactone	-0.6 ± 0.1	G1	Oxaloacetic Acid	-0.3 ± 0.2
D6	D-Alanine	-0.4 ± 0.1	G2	D-Biotin	-0.3 ± 0.1
D7	D-Aspartic Acid *	-0.5 ± 0.1	G3	Cyano-Cobalamine	-0.3 ± 0.2
D8	D-Glutamic Acid	-0.8 ± 0.2	G4	p-Amino-Benzoic Acid	-0.1 ± 0.2
D9	D,L- α,ϵ -Diaminopimelic Acid	-0.7 ± 0.4	G5	Folic Acid	-0.7 ± 0.4
D10	Cytosine	-0.7 ± 0.1	G6	Inosine + Thiamine	-0.7 ± 0.3
D11	Cytidine	-0.4 ± 0.4	G7	Thiamine *	-0.1 ± 0.4
D12	2'-Deoxy Cytidine	1.4 ± 0.8	G8	Thiamine Pyrophosphate	0.0 ± 0.0
E1	Putrescine	-0.3 ± 0.1	G9	Riboflavin	-0.1 ± 0.2
E2	Spermidine	-0.2 ± 0.0	G10	Pyrrolo-Quinoline Quinone	-0.1 ± 0.5
E3	Spermine	-0.4 ± 0.3	G11	Menadione	-0.5 ± 0.7
E4	Pyridoxine	0.0 ± 0.2	G12	m-Inositol	-0.2 ± 0.4
E5	Pyridoxal	0.0 ± 0.4	H1	Butyric Acid	-0.4 ± 0.2
E6	Pyridoxamine	-0.4 ± 0.3	H2	D,L- α -Hydroxy-Butyric Acid	-0.4 ± 0.3
E7	β -Alanine	-0.4 ± 0.2	H3	α -Keto-Butyric Acid	0.0 ± 0.3
E8	D-Pantothenic Acid	-0.4 ± 0.3	H4	Caprylic Acid	-0.4 ± 0.8
E9	Orotic Acid	0.1 ± 0.3	H5	D,L- α -Lipoic Acid (oxidized form)	-0.2 ± 0.4
E10	Uracil	-0.3 ± 0.3	H6	D,L-Mevalonic Acid	0.0 ± 0.6
E11	Uridine	0.0 ± 0.6	H7	D,L-Carnitine	-0.1 ± 0.6
E12	2'-Deoxy Uridine	-0.1 ± 0.2	H8	Choline	-0.1 ± 0.3
F1	Quinolinic Acid	0.1 ± 0.3	H9	Tween 20	-0.2 ± 0.1
F2	Nicotinic Acid	0.1 ± 0.2	H10	Tween 40	0.1 ± 0.3
F3	Nicotinamide	0.2 ± 0.2	H11	Tween 60	-0.1 ± 0.1
F4	β -Nicotilamide Adenine Dinucleotide	-0.4 ± 0.1	H12	Tween 80	0.1 ± 0.2
F5	δ -Amino-Levulinic Acid	-0.1 ± 0.2			

* Molecules previously suggested to signal through Tlp3 [1].

Reference

- [1] Rahman, H.; King, R.M.; Shewell, L.K.; Semchenko, E.A.; Hartley-Tassell, L.E.; Wilson, J.C.; Day, C.J.; Korolik, V. Characterisation of a multi-ligand binding chemoreceptor CcmL (Tlp3) of *Campylobacter jejuni*. *PLoS Path.* **2014**, *10*, e1003822.