Supplemental material for:

Functional interaction between the cytoplasmic ABC protein LptB and the inner membrane LptC protein, components of the lipopolysaccharide transport machinery in *Escherichia coli*

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Running Head: Functional interaction between LptB and LptC

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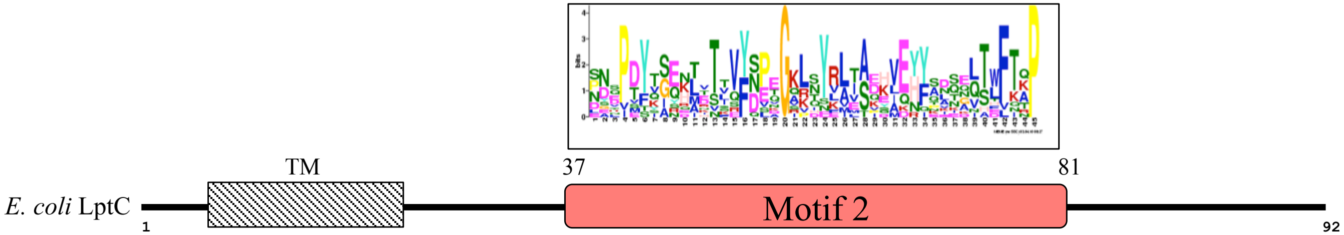
Table S1. Oligonucleotides

Name	Sequence (5'-3')^{a}	Use and/or description
AP23	CGATCCCCGCGGTTATAGCAAAAGCAGAGAAC	Amplification of Tn-SS2 left junctions in ST-190, with FG1676
AP63	**gtgatcacatctagat***CAGTGGTGGTGGTGGTG* AGGCTGAGTTTGTTTGTTTG	Construction of His tagged chimeras; *Xba*I
AP91	CATCGGCTCGTATAATGTG	Construction of pGS200 derivatives
AP92	CTGCGTTCTGATTTAATCTG	Construction of pGS200 derivatives
AP175	**cgagagaggaattcacc**atgccgaagacactacgc	*Pa*-LptC and *Pa*-LptC-H construction for pGS111, pGS200, pGS403 and pGS456; *Eco*RI
AP176	**aagcttctaga**ttaacgaacctcatgctgacc	*Pa*-LptC construction for pGS111, pGS403 and pGS456; XbaI
AP179	CAATTTCCCCGGAGTTG	Amplification of Tn-SS2 right junctions in ST-190, with FG690
AP182	TATTACCCTTCTTCTGTG	Sequencing of Tn-SS2 right junction in ST-190
AP191	AAGGATAAAATCCCGACAtggcacatccagagcgcc	CPP construction
AP192	ggcgctctggatgtgccaTGTCGGGATTTTATCCTT	CPP construction
AP193	CCCGACAAGAATTATGCAacctctgaagacctcgtc	CPC and PPC construction
AP194	gacgaggtcttcagaggtTGCATAATTCTTGTCGGG	CPC, and PPC construction
AP195	CGTGGCAACGTGCAACCCtggtccgtaaaagcagat	PCC construction
AP196	atctgcttttacggaccaGGGTTGCACGTTGCCACG	PCC construction
AP197	CTGGTGACGCAGGATGTT cagaccgagcaagccgtt	PCP and CCP construction
AP198	aacggcttgctcggtctg AACATCCTGCGTCACCAG	PCP and CCP construction
AP237	**aagcttctaga**TCA*GTGGTGGTGGTGGTGGTG* acgaacctcatgctgacct	*Pa*-LptC-H construction for pGS200; *Xba*I
AP317	GCGCATCCACTGTTGATCCCG	Sequencing of Tn-SS2 left junction in ST-190
AP329	**gaattcacc**ATGAAATTCAAAACAAACAAACTC	LptA-HA construction for pBAD24LptHA; *Eco*RI
AP330	**aagctt**TTA*AGCGTAATCTGGAACATCGTATGGGTA*ATTACCC TTCTTCTGTGCCGGGG	LptA-HA construction for pBAD24LptHA; *Hin*dIII
AP361	**cccaagcttctaga**TCA*GTGGTGGTGGTGGTGGTG*C*TG*AACATC CTGCGTCACC	CC-H construction for pGS208H; *Hin*dIII
AP363	**cccaagcttctaga**TCAAACATCCTGCGTCACC	CC- construction for pGS208; *Hin*dIII
AP467	CAGGAAGCCTCCATTGCCCGTCGCCTCAGCG	pGS429-LptB^{F90A} and pGS431-LptB^{F90A} construction, with AP468

AP468	CGCTGAGGCGACGGGCAATGGAGGCTTCCTG	pGS429-LptB^{F90A} and pGS431-LptB^{F90A} construction, with AP467
AP469	CAGGAAGCCTCCATTTACCGTCGCCTCAGCG	pGS429-LptB^{F90Y} and pGS431-LptB^{F90Y} construction, with AP470
AP470	CGCTGAGGCGACGGTAAATGGAGGCTTCCTG	pGS429-LptB^{F90Y} and pGS431-LptB^{F90Y} construction, with AP469
AP471	CGAAATTTATTCTGCTCGACCAACCGTTTGCCGGGG	pGS429-LptB^{E163Q} and pGS431-LptB^{E163Q} construction, with AP472
AP472	CCCCGGCAAACGGTTGGTCGAGCAGAATAAATTTCG	pGS429-LptB^{E163Q} and pGS431-LptB^{E163Q} construction, with AP471
FG575	CAACTCTCTACTGTTTCTCCATACCC	Sequencing of Tn-SS2 right junction in ST-190
FG687	TTGTGCCCAGTCATAGCCGAATAGCCT	Sequencing of Tn-SS2 left junction in ST-190
FG690	GCCAGTTAAGCCATTCATGCCAGTAGG	Amplification of Tn-SS2 right junctions in ST-190, with FG690
FG1676	CCACAGTCGATGAATCCAGA	Amplification of Tn-SS2 left junctions in ST-190, with FG1676
FG2935	**catattcgtctcgtcgacacc**ATGAAATTCAAAACAAACAAACTCAGCC	*lptA* and *lptAB* construction for pGS407, pGS413, pGS414 and pGS415; *Esp*3I-*Sal*I
FG2936	**ggccttcgtctcaagctt**TTAATTACCCTTCTTCTGTGCCG	*lptA* construction for pGS407; *Esp*3I-*Hin*dIII
FG2978	**catattcgtctcgaattcacc**ATGAGTAAAGCCAGACGTTGGG	*lptC190N* and *lptC*$^{\Delta 139-191}$ construction for pGS408, pGS411, pGS417, pGS418 and pGS419; *Esp*3I-*Eco*RI
FG2979	**caggttcgtctctctaga**TCAATCAATCACCGGATCCCC	*lptC190N* construction for pGS408 and pGS411; *Esp*3I-*Xba*I
FG3058	**ccttcgtctcaagctt**TCAGAGTCTGAAGTCTTCCCC	*lptAB* construction for pGS413, pGS414 and pGS415; *Esp*3I-*Hin*dIII
FG3088	**caggttcgtctctctaga**TTAGAGATTGATCTGCGCGTTATC	*lptC*$^{\Delta 139-191}$ construction for pGS417, pGS418, pGS419, pGS431; *Esp*3I-*Xba*I

[a] Upper case*, E. coli* genomic sequence; lower case, *P. aeruginosa* genomic sequence; bold lower case, oligonucleotide tail; lower bold underlined case, restriction site; italic upper case, HA or 6xHis coding sequence

Fig. S1A



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01EcLptC  MSKA-RRWVI IVLSLAVLVM IGINMA---E KDD-TAQVVV NNN-DPTYK- --SEHTDTLV YNPEGALSYR LIAQHVEYYS DQAVSWFTQP VLTTFD---- KDK------I ----P
02SeLptC  MSKT-RRWVI ILLSLAILVL IGINLA---D KDD-PAAVMV NSN-DPTYK- --SEHTDTVV YSPEGALSYR LIAQHVEYFS DQAVSWFTQP VLTTFD---- KDK------V ----P
03PaLptC  MSKT-KRWLT AFLALLVLIL IGWNIA---D D-D-T-VVAP DAN-DPAVPV YTSEKTNTQV YSPAGKLSYR LISEKAEYFN DEQLSWFTTP VATLFN---- EQG------T ----A
04YeLptC  MSKT-RLWIT LSLALIALAL IGWNIS-GFN QQD-T-QATI DDN-EPSSQ- --SQHTVTVV FNPVGQLNYK LVAEEVQNFS AQQLTWFTRP VMTMFD---- ENA------V ----A
05PlLptC  MDRI-KSWLV ITLVLIILAL IGWNLS---D SDEIKSSSAT DNS-QPTYQ- --SQEAITFV YDPTGKLTYK LVADDVQNYA ESKLTWFTKP VLITYD---- SNA------I ----A
06HiLptC  MN-I-R-WNV ILGVIALCAL A-WFYSLNQE TAD-LSELVK KPD-SPDYV- --GYKMETTV FSPEGKKQYL ALSDKIEHYT VNEQTLFTAP LVYLYPTTSN EKEQNPNQNV DFFST
07PmLptC  MN-I-R-WNI ILAGLALALL A-WFYSLNQG EPS-LNTLIK KPD-SPEYV- --GQKMKTTV FSPTGKKHYV AMSDSVEHFA LSGQTHFQKP EVYLFEL--- ETE---NQ-L S--NK
08ApLptC  MN-I-R-LNI IL-LIIVAAL AGWYVSQQKP ENDGLDQLIK KEG-NPDYT- --GNRITTSV YDLKGKPQYF AEAKEIKRYQ NTELTEFIQP LVELFDA--- ETA------L ----K
09VcLptC  MSLS-RIVYV LLLFIASWSL Y-YLLG-QEQ DSK-IQVAPN LE--LPMFS- --GENLENIS YDEQGIRNYV ITSIHLDHYA KSGNTLFKAP ILKVY-R--- EGT------L ----Q
10AsLptC  MSRQ-TLLFA LL-FLVALAA --WQIG-DV- DLT-PEPTTK AENYQPDFT- --ARNLVTTR FDVNGRRTER LESEYAEYYQ IIEQATFNKP VVYMFDK--- QGE------A -----
11SoLptC  MSRV-TLAII AF-FGTALVL Y-WQVQ-QKR GGD-TDDSLN VSD-RPDYI- --IEDLRSIE FNEQGQVNSR VTAKHMEHYE LKNQTDFTAP VYLVYPD--- QGK------A -----
12PaLptC  MPKTLRQKLL LALIALLLIV AGYYW--NVG LELFNEQPTR PGQ-DNTIDY Y-AENAHSLQ YQEDGSLDYE MTAVKLEHQK ATDITFVTTP DLLLF----- RGN------V ----Q
13LpLptC  MNIT-KQAIW LFLTLLILAG SGWYYA-HFN QAIINKK-TL SNEVDTTISQ L-TVK----Q FNSEGVLINL LTTPQVEYIP NNNLHLLKNP HIIIK----- QDN------- ----Q
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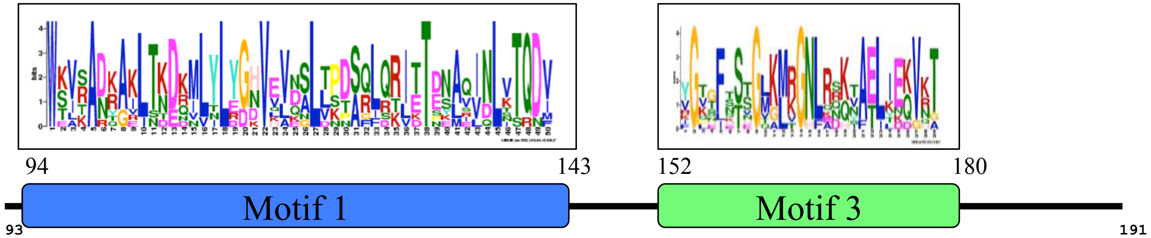

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01EcLptC  T-WSVKADKA KL-TNDRM-L YLYGHVEV-N ALVPDSQLRR ITTDNAQINL VTQDVTSEDL VTLYGTTFNS SGLKMRGNLR SKNAELIEKV RTSY-E--I- -Q-NKQTQP
02SeLptC  T-WSIKADKA KL-TNDRM-L YLYGHVEV-N ALVPDAQLRR ITTDNAQINL VTQDVTSNDL VTLYGTTFNS SGLKMRGNLR SKNAELIEKV RTSY-E--I- -Q-NKQTQP
03PaLptC  T-WSVRADRA KL-TKDKM-L YLYGHVEV-N SLTKDAQLQR ITTNNAQVNL VTQDVSSDDE VTLYGTSFTS SGMKMRGNLR NKTAELIEKV KTSY-E--I- -Q-N-Q
04YeLptC  T-WTVRADRA KL-TDDKM-L YLYGHVEV-D SLTPDAQLQK IKTDNAQVNL ITQDVSSDDE VTLFGVGFTS NGMKMRGNLR DKTAELIEKV KTSY-E--I- -Q-K
05PlLptC  T-WTVRANKA KL-TNDKM-L YLYGDVQV-D SLTNTSQLQR ITTENAIINL TTQDVSSDDQ VTLIGVGLKS VGMKMRGNLR NKTAELIEKV NTYY-E--I- -Q-HEKQNP
06HiLptC  QNWKLSANQA RL-TKDQI-L YLEGNVVV-Q SLTSDSRLQR IETESAVVNL KTQDMTSETQ VKIKGKNFSS TGLKLVGNLR QQVATLKEQV KTYY-E--VS KQ
07PmLptC  ENWKASANKA IL-TKENM-L YLEGNVVV-E SLLSPSRLQR VETESAVINL STQDISSEHA VKIYGQHFHS TGLKLTGNLQ QQIATLKEQV NTYY-E--IN K
08ApLptC  Q-WKVSADYA EI-TKEKI-L NLDGNVKL-Q ALDKNARLQK IETDKLRIDL NTQDISTESV VKSVGLGIST TGTGLKGNLK RQVATITKDV KTYL-EPTII KQSNDEQKN
09VcLptC  E-WEITARRG IL-SKDQV-L TLYDDVLA-K NLLPDSGFDT LTTSEMSIQL KSRDFWADKP VELRGPQFET HGQAMKGNFA DHSAELYNQV QGRY-E--TL TP
10AsLptC  E-WKVTADTG VL-NTDDN-V ILRDKVHL-D GLLPTSFIST LDTPYLELDL VTQNVRSNQH VSIVGKEFQT EGVGLKGHLE RKYFELLDQG HAIYFN--EK R
11SoLptC  K-WQIRADQG RL-NKDTGKV VLENNVII-D AISPGEPIQT LSTSFLELDL NTMIMTSDRI IYVTGKDFNI QGQGLYADLN AQEVKLTSQV VGTY-E--AK
12PaLptC  P-WHIQSARA EVGPKGKE-V ELIDDVRVAR TDAKG-QPSI LTTTRLTVFP DKNYAQTEQA VKIDAANGVT TAVGMKAYLK DSRMHLLSNV RGQH-E--VR
13LpLptC  PAWEINSLRA KSFDGGKQ-I TFIEQVVVHQ NPGENTREST LKTEEITYYP KEKKATTSLF VTFEQPGNII QSTGMNAYLD EKRVELLHKA RGSY-DP-AK G
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Fig. S1B

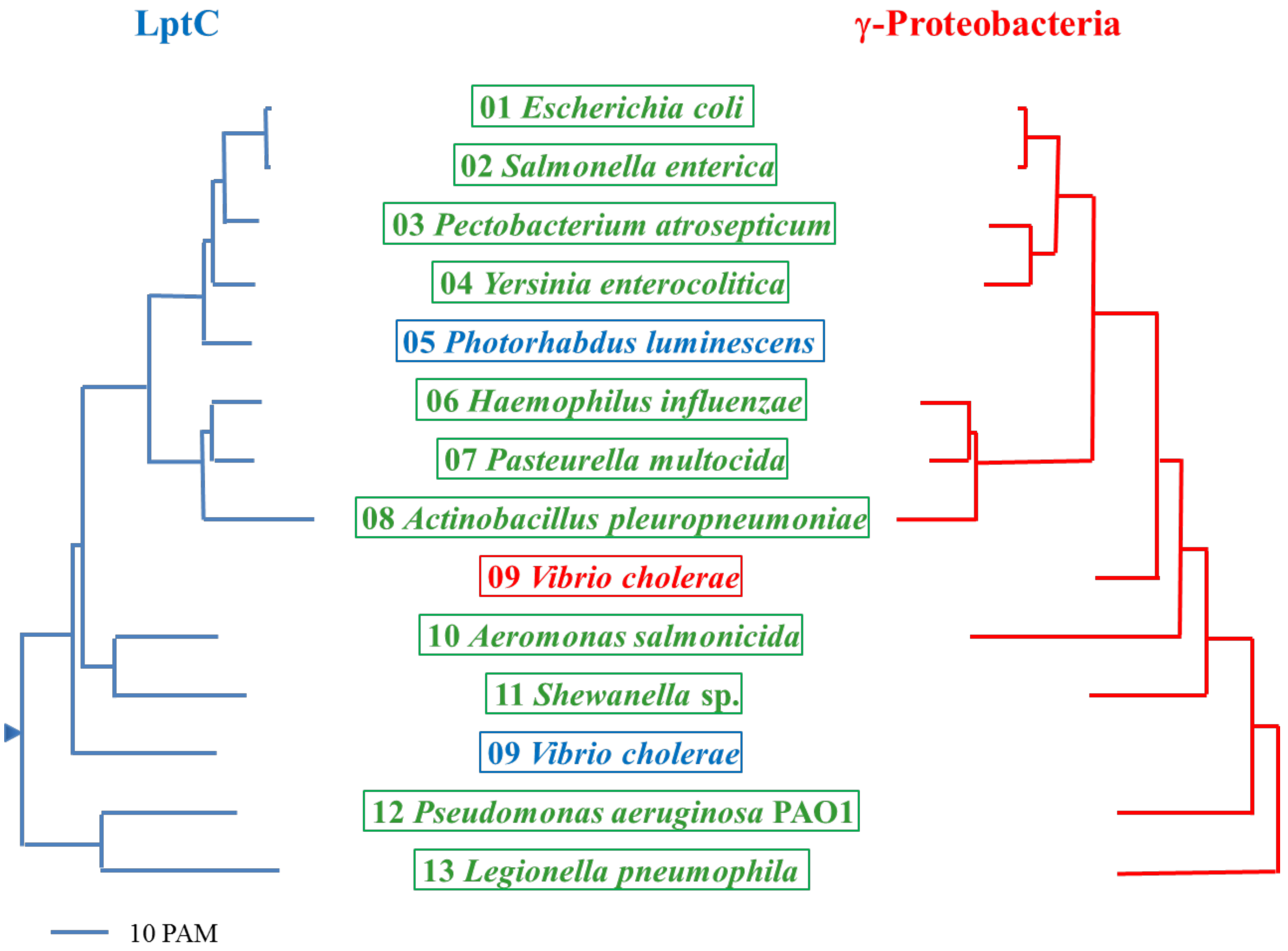
LptC
γ-Proteobacteria
01 Escherichia coli
02 Salmonella enterica
03 Pectobacterium atrosepticum
04 Yersinia enterocolitica
05 Photorhabdus luminescens
06 Haemophilus influenzae
07 Pasteurella multocida
08 Actinobacillus pleuropneumoniae
09 Vibrio cholerae
10 Aeromonas salmonicida
11 Shewanella sp.
09 Vibrio cholerae
12 Pseudomonas aeruginosa PAO1
13 Legionella pneumophila
10 PAM

Figure S1. Features of LptC homologues from representative γ-Proteobacteria.

(A) Motifs predicted in *E. coli* LptC by MM algorithm. Multiple sequence alignment between LptC orthologues of 13 γ-Proteobacteria by Multalin and the Motifs predicted in *E. coli* LptC by MM algorithm are shown (see Materials and Methods for details). Sequence codes and Uniprot Accession Numbers are provided in brackets as follow: *E. coli* (01EcLptC, LPTC_ECOLI), *S. enterica* (02SeLptC, A9N767_SALPB)*, Pectobacterium atrosepticum* (03PaLptC, Q6DAG2_PECAS)*, Yersinia enterocolitica* (04YeLptC, A1JRC3_YERE8)*, Photorhabdus luminescens* (05PlLptC, Q7N059_PHOLL)*, Haemophilus influenzae* (06HiLptC, Q4QLE6_HAEI8)*, Pasteurella multocida* (07PmLptC, V4PYQ4_PASMD), *Actinobacillus pleuropneumoniae* (08ApLptC, A3MZ56_ACTP2)*, Vibrio cholerae* O1 (09VcLptC, C3NV80_VIBCJ)*, Aeromonas salmonicida* (10AsLptC, A4SHX4_AERS4), *Shewanella* sp. (11SoLptC, Q0HRC7_SHESR)*, P. aeruginosa PAO1* (12PaLptC, Q9HVV8_PSEAE), *Legionella pneumophila* (13LpLptC, D5TAY1_LEGP2). Conserved (50%) and highly conserved (90%) residues are depicted in orange and red, respectively. TM (Transmembrane Region, from residue 7 to 25), Motif 2 (from residue 37 to 81), Motif 1 (from residue 94 to 143), Motif 3 (from 152 to 180) are indicated. Consensus polypeptides from each motif are represented as a sequence logo graphic in which the relative size of the letters correlates with their frequency in the sequences.

(B) Dendrograms of LptC homologues genetic distance and phylogeny of representative γ-Proteobacteria. Genetic distance between LptC homologues was derived from the multialignment depicted above (S1A) using blosum62 as substitution matrix. The arrowhead points to the root of the LptC tree (left). The minimal distance between sequences is 20 PAM (percent accepted mutation). The phylogenetic tree of the representative γ-Proteobacteria (right) is from (1). In this tree the genus *Photorhabdus*, an insect pathogen living in an entomopathogenic nematode and belonging to the Enterobacteriaceae, was omitted in order to stabilize the phylogenetic tree. Notwithstanding some topological difference in the dendrograms (see for example *Vibrio cholerae*) the LptC homologues genetic distances fit the phylogenetic distances of the γ-Proteobacteria.

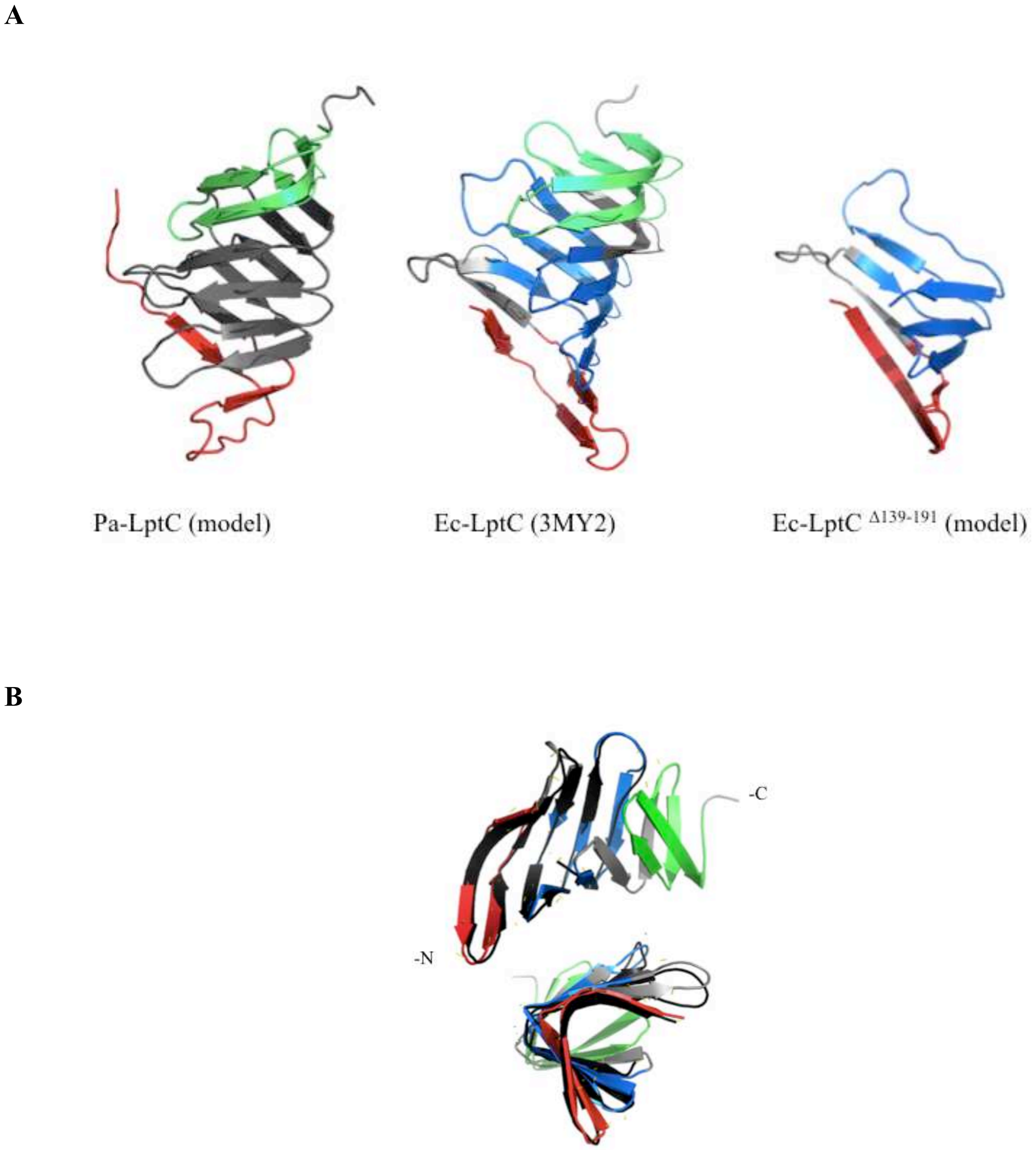
Pa-LptC (model) Ec-LptC (3MY2) Ec-LptC $^{\Delta139\text{-}191}$ (model)

Figure S2. Crystal structure of *E. coli* LptC and structure prediction of the C-terminally truncated Ec-LptC and of the *P. aeruginosa* LptC orthologue. Panel A. Structure of *Pa*-LptC (PA4459) and of Ec-LptC$^{\Delta139\text{-}191}$ predicted by I-TASSER is shown next to the *Ec*-LptC structure 3MY2 (2). Both Ec-LptC$^{\Delta139\text{-}191}$ and Pa-LptC are modeled from residue 59, mirroring the lack of structural information in Ec-LptC X-ray structure. Regions corresponding to MEME motifs (Fig. S1A) are coloured as blue, motif 1; red, motif 2; green, motif 3. The non-recognized motif 1 in *Pa*-LptC is indicated in grey. Panel B. Ec-LptC $^{\Delta139\text{-}191}$ (model, depicted in black) and Ec-LptC wild type superimposition (RMSD 0.82 Å)

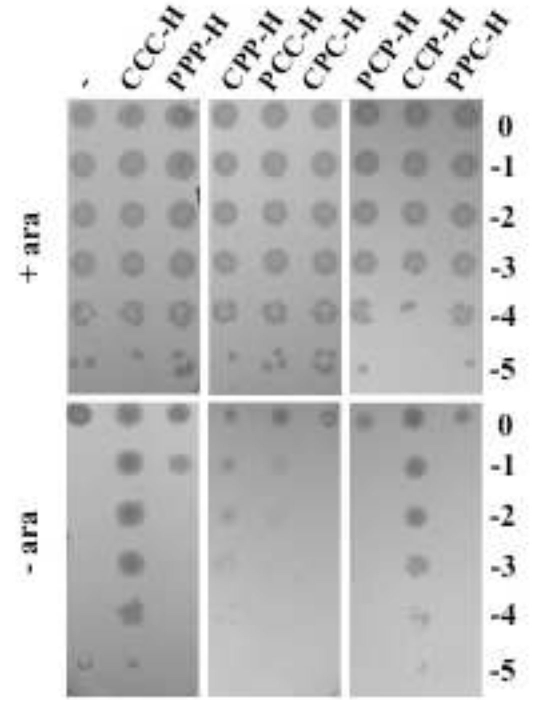

Figure S3. Complementation test of LptC depletion mutants with *E. coli* and *P. aeruginosa* wild type or chimeric LptC proteins fused to 6xHis-tag. Cultures of FL905 (*araB*p-*lptC*) strains freshly transformed with pGS100 derivatives expressing Ec-LptC-H (pGS108, CCC-H), Pa-LptC (pGS200, PPP-H), LptC chimeras fused to 6xHis-tag (pGS201H, CPP-H; pGS202H, PCC-H; pGS203H, CPC-H; pGS204H, PCP-H; pGS206H, CCP-H; pGS207H, PPC-H) and grown in LD-chloramphenicol-arabinose were serially diluted 1:10 in microtiter wells and replica plated in agar plates with (+ ara) or without (- ara) arabinose. The log of the serial dilutions is indicated on the right of the panel.

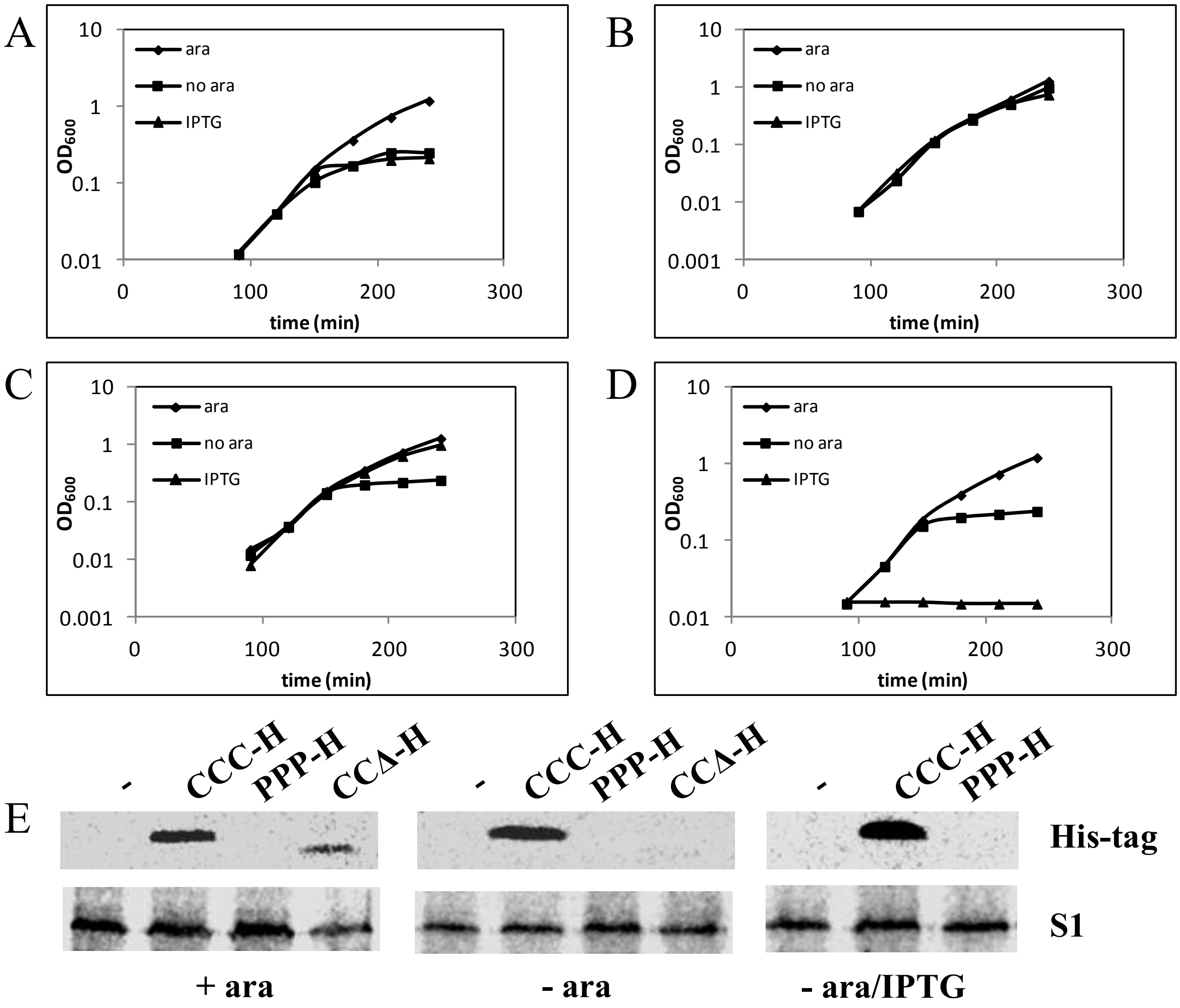

Figure S4. Growth curve and LptC protein level of FL905 cells complemented with different His tagged wild type or C-terminally truncated LptC proteins. Bacterial cultures grown in LD-arabinose up to an OD 0.2 were harvested by centrifugation, washed in LD and diluted 500 fold in LD with arabinose (ara) or without arabinose (no ara) or with IPTG (IPTG). FL905 cells were complemented with: pGS100 (-) (panel A), or pGS108 (CCC-H) (panel B), or pGS200 (PPP-H) (panel C) or pGS208H (CCΔ-H) (panel D). Samples collected 240 min after the shift to the different conditions (panel E) were analyzed by Western blotting using anti-His antibodies to detect the different His-tagged LptC forms. The level of S1 protein was used as loading control using anti-S1 antibodies.

References

1. **Williams KP, Gillespie JJ, Sobral BW, Nordberg EK, Snyder EE, Shallom JM, Dickerman AW.** 2010. Phylogeny of gammaproteobacteria. J Bacteriol **192:**2305-2314.
2. **Tran AX, Dong C, Whitfield C.** 2010. Structure and functional analysis of LptC, a conserved membrane protein involved in the lipopolysaccharide export pathway in *Escherichia coli*. J Biol Chem **285:**33529-33539.