

**FIG S1** Alignment of SidC<sub>Llo</sub>, SidC<sub>Lpn</sub> and SdcA<sub>Lpn</sub>. (A) Domain analysis of 111 kDa SidC<sub>Llo</sub> and 106 kDa SidC<sub>Lpn</sub>. (B) Amino acid sequence alignment of SidC<sub>Llo</sub>, SidC<sub>Lpn</sub> and SdcA<sub>Lpn</sub> using the Clustal Omega algorithm. Full-length SidC<sub>Llo</sub>/SidC<sub>Lpn</sub> or the corresponding P4C domains share 40% or 45% identity, respectively. Identical or similar amino acids are marked in black or in gray.

Strain/plasmid	Properties	Reference
E. coli		
BL21(DE3)		Novagen
TOP10		Invitrogen
ST18	S17 $\lambda pir \Delta hem A$	(1)
L. longbeachae		
NSW150	L. longbeachae strain NSW150, serogroup 1	(2)
$\Delta dot A$	NSW150 $dotA$ ::Kan <sup>R</sup> ( $\Delta dotA$ )	(2)
IH02	NSW150 <i>llo3098</i> ::Kan <sup>R</sup> (Δ <i>sidC</i> )	This study
L. pneumophila		
JR32	L. pneumophila strain Philadelphia-1, serogroup 1	(3)
LELA3118	JR32 $dotA3118$ ::Kan <sup>R</sup> ( $\Delta dotA$ )	(3)
GS3011	JR32 $icmT3011$ ::Kan <sup>R</sup> ( $\Delta icmT$ )	(4)
CR01	JR32 <i>sidC-sdcA</i> ::Kan <sup>R</sup> ( $\Delta$ <i>sidC-sdcA</i> )	(5)
Plasmids		
pET28a(+)	N-terminal His <sub>6</sub> -fusion, P <sub>T7</sub> , Kan <sup>R</sup>	Novagen
pGEX4T-1	N-terminal GST-fusion, P <sub>tac</sub> , Amp <sup>R</sup>	Amersham
pMMB207-C-M45	pMMB207-C, Δ <i>mobC</i> , M45-(Gly) <sub>5</sub> , Cam <sup>R</sup>	(6)
pCaln-GFP	Pact15, calnexinA-RSSSKLK-GFP (S65T), G418	(7)
pCR01	pET28a(+), $His_6$ -sid $C_{Lpn}$ (P <sub>tac</sub> )	(6)
pCR02	pGEX4T-1, $GST$ -sid $C_{Lpn}$ (P <sub>tac</sub> )	(6)
pCR34	pMMB207-C, <i>M45-sidC</i> <sub>Lpn</sub> (P <sub>tac</sub> )	(6)
pCR77	pMMB207-C-P <sub>tac</sub> -RBS-dsred-RBS	(8)
pCR80	pMMB207-C-P <sub>tac</sub> -RBS-dsred-RBS-sidC <sub>Lpn</sub>	(8)
pGEX-PH <sub>FAPP1</sub>	pGEX4T-1, GST-PH <sub>FAPP1</sub> (P <sub>tac</sub> )	(9, 10)

TABLE S1. Bacterial strains and plasmids.

pHP56	pGEX4T-1, <i>GST-sidC</i> <sub>Lpn_609-776</sub> (P <sub>tac</sub> )	(5)
pIH33	pLAW344-upstream $sidC_{Llo}$ '-Kan <sup>R</sup> -downstream $sidC_{Llo}$ '	This study
pIH47	pMMB207-C-P <sub>tac</sub> -RBS-dsred-RBS-sdcA <sub>Lpn</sub>	This study
pIH60	pGEX4T-1, <i>GST-sidC</i> <sub>Lpn_1-608-Llo_609-969</sub>	This study
pLAW344	oriT (RK2), oriR (ColE1), sacB, Cam <sup>R</sup> , Amp <sup>R</sup>	(11)
pMH01	pGEX4T-1, <i>GST-sidC</i> <sub>Llo_1-608-Lpn_609-917</sub>	This study
pSD01	pET28a(+); $His_6$ - $sidC_{Llo}$ (P <sub>T7</sub> )	This study
pSD02	pET28a(+); $His_6$ -sidC <sub>Llo</sub> (P <sub>T7</sub> ), BamHI/SalI deleted in sidC	This study
pSD03	pGEX4T-1, $GST$ -sid $C_{\text{Llo}_{609-782}}$ (P <sub>tac</sub> )	This study
pSD04	pGEX4T-1, $GST$ -sid $C_{\text{Llo}_{1-340}}$ (P <sub>tac</sub> )	This study
pSD05	pGEX4T-1, GST-sidC <sub>Llo_341-608</sub> (P <sub>tac</sub> )	This study
pSD06	pGEX4T-1, <i>GST-sidC</i> <sub>Llo_783-969</sub> (P <sub>tac</sub> )	This study
pSD07	pGEX4T-1, $GST$ -sid $C_{Llo}$ (P <sub>tac</sub> )	This study
pSD13	pMMB207-C, <i>M45-sidC</i> <sub>Llo</sub> (P <sub>tac</sub> )	This study
pSD14	pMMB207-C-P <sub>tac</sub> -RBS-dsred-RBS-sidC <sub>Llo</sub>	This study
pSW001	pMMB207-C, $\Delta lacl^{q}$ , constitutive <i>dsred</i>	(12)
pUC4K	<i>oriR</i> (pBR322), Amp <sup>R</sup> , MCS::Kan <sup>R</sup>	Amersham

Abbreviations: ampicillin (Amp); chloramphenicol (Cam); kanamycin (Kan); geneticin (G418)

## REFERENCES

- Thoma S, Schobert M. 2009. An improved *Escherichia coli* donor strain for diparental mating. FEMS Microbiol Lett 294:127-132.
- Cazalet C, Gomez-Valero L, Rusniok C, Lomma M, Dervins-Ravault D, Newton HJ, Sansom FM, Jarraud S, Zidane N, Ma L, Bouchier C, Etienne J, Hartland EL, Buchrieser C. 2010. Analysis of the *Legionella longbeachae* genome and transcriptome uncovers unique strategies to cause Legionnaires' disease. PLoS Genetics 6:e1000851.

- Sadosky AB, Wiater LA, Shuman HA. 1993. Identification of *Legionella pneumophila* genes required for growth within and killing of human macrophages. Infect Immun 61:5361-5373.
- 4. **Segal G, Shuman HA.** 1998. Intracellular multiplication and human macrophage killing by *Legionella pneumophila* are inhibited by conjugal components of IncQ plasmid RSF1010. Mol Microbiol **30**:197-208.
- Ragaz C, Pietsch H, Urwyler S, Tiaden A, Weber SS, Hilbi H. 2008. The Legionella pneumophila phosphatidylinositol-4 phosphate-binding type IV substrate SidC recruits endoplasmic reticulum vesicles to a replication-permissive vacuole. Cell Microbiol 10:2416-2433.
- Weber SS, Ragaz C, Reus K, Nyfeler Y, Hilbi H. 2006. Legionella pneumophila exploits PI(4)P to anchor secreted effector proteins to the replicative vacuole. PLoS Pathog 2:e46.
- Müller-Taubenberger A, Lupas AN, Li H, Ecke M, Simmeth E, Gerisch G. 2001. Calreticulin and calnexin in the endoplasmic reticulum are important for phagocytosis. EMBO J 20:6772-6782.
- Finsel I, Ragaz C, Hoffmann C, Harrison CF, Weber S, van Rahden VA, Johannes L, Hilbi H. 2013. The *Legionella* effector RidL inhibits retrograde trafficking to promote intracellular replication. Cell Host Microbe 14:38-50.
- 9. Dowler S, Kular G, Alessi DR. 2002. Protein lipid overlay assay. Sci STKE 2002:PL6.
- Godi A, Di Campli A, Konstantakopoulos A, Di Tullio G, Alessi DR, Kular GS, Daniele T, Marra P, Lucocq JM, De Matteis MA. 2004. FAPPs control Golgi-to-cellsurface membrane traffic by binding to ARF and PtdIns(4)*P*. Nat Cell Biol 6:393-404.
- Wiater LA, Sadosky AB, Shuman HA. 1994. Mutagenesis of *Legionella pneumophila* using Tn903dll*lacZ*: identification of a growth-phase-regulated pigmentation gene. Mol Microbiol 11:641-653.
- Mampel J, Spirig T, Weber SS, Haagensen JAJ, Molin S, Hilbi H. 2006. Planktonic replication is essential for biofilm formation by *Legionella pneumophila* in a complex medium under static and dynamic flow conditions. Appl Environ Microbiol 72:2885-2895.

**TABLE S2.** Oligonucleotides used in this study.

Oligo	<b>Sequence</b> (5' - 3') <sup>a</sup>	Comments
oCR1	AAAAACGC <u>GGATCC</u> ATGGTGATAAACATGGTTGACG	5'of sidC, BamHI (Ref. 5)
oIH19	AAAAACGC <u>TCTAGA</u> TCCTCTACTACCTTGTGAGT	5' upstr. $sidC_{Llo}$ (fo), $XbaI$
oIH20	AAAAACGC <u>GGATCC</u> GAAACATTAAGAAGTGTGCG	3' upstream $sidC_{Llo}$ (re)
oIH21	AAAAACGC <u>GGATCC</u> ATCTGAATGAAGCAAAATTG	5' downstream $sidC_{Llo}$ (fo)
oIH22	AAAAACGC <u>TCTAGA</u> AATTTACTGACTTGCAATCA	3' downstream $sidC_{Llo}$ (re)
oIH23	ATCCAAACCATCGTCGTGTC	5' upstream oIH019 (fo)
oIH24	ATCCTGAGGAGTTGCAGAAAG	3' downstream oIH022 (re)
oIH39	AAAAACGC <u>CTGCAG</u> CTGACCAGGTAATTGC	3' of $sidC_{Lpn1-608}$ (re), $PstI$
oIH40	AAAAACGC <u>CTGCAG</u> TTATTTCCTGGGAAAC	5' of $sidC_{Llo609-969}$ (fo), $PstI$
oMH01	GGATCCACGCGGAACCAGATCCGATTTTGG	pGEX-4T-1 (re)
oMH02	CCGGAATTCCCGGGTCGACTCGAGCGGCCG	pGEX-4T-1 (fo)
oMH03	gttccgcgtggatccATGAGAGTCACTAAAATGCC	5' of $sidC_{Llo1-608}$ (fo)
oMH04	aggaatatttATTTTCTCTATTCACGTTTGC	3' of $sidC_{Llo1-608}$ (re)
oMH05	tagagaaaatAAATATTCCTCCAAGCCATTATTG	5' of $sidC_{Lpn609-917}$ (fo)
oMH06	acccgggaattccggCTATTTCTTTATAATTCCCGTGTAC	3' of $sidC_{Lpn609-917}$ (re)
oSD05	TTTTG <u>GGATCC</u> GAATATAGAGAACTAAAATG	5' flanking sequence $sidC_{Llo}$
		(fo), <i>Bam</i> HI
oSD06	TTTTT <u>GTCGAC</u> GAAATAAAGTTAAGTACGTGAATTAAA	3' flanking sequence $sidC_{Llo}$
	AGTACG	(re), <i>Pst</i> I
oSD07	TTTT <u>CATATG</u> ATGAGAGTCACTAAAATGCCTAAAGAC	5' flanking sequence $sidC_{Llo}$
		(fo), <i>Nde</i> I
oSD08	TTTTT <u>GCTAGC</u> TTAAGTACGTGAATTAAAAGTACGTCC	3' flanking sequence $sidC_{Llo}$
		(re), NheI
oSD16	TTTTG <u>GGATCC</u> ATGAGAGTCACTAAAATGCTTAAAGAC	5' of $sidC_{Llo1-340}$ (fo)
oSD17	TTTTT <u>GTCGAC</u> TCATCCAGTGATTTTTTCTACGTC	3' of $sidC_{Llo1-340}$ (re)
oSD18	TTTTG <u>GGATCC</u> GCTGCTGTTATTTCCTGGG	5' of $sidC_{Llo609-782}$ (fo)
oSD19	TTTTT <u>GTCGAC</u> TCATTCATTGAAAAAGTTAAGCGCTG	3' of $sidC_{Llo609-782}$ (re)
oSD22	GAAAATAATATCAAGGCATGGTCCACTGATCTTGAAGC	Quick Change sidC <sub>Llo</sub>
	AATCG	$(\Delta SalI)$ (fo)
oSD23	CGATTGCTTCAAGATCAGTGGACCATGCCTTGATATTA	Quick Change $sidC_{Llo}$
	TTTTC	$(\Delta Sal1)$ (re)
oSD24	AAACGC <u>GGATCC</u> GATGAATCACAGCAAAAGGAAGC	5' of $sidC_{Llo341-608}$ (fo)

oSD25	AAACGC <u>GTCGAC</u> TCAATTTTCTCTATTCACGTTTGCTGG	3' of $sidC_{Llo341-608}$ (re)
	AG	
oSD26	AAACGC <u>GGATCC</u> TTGTCTTTACATGAGGTGCTTAAAGT	5' of $sidC_{ m Llo783-969}$ (fo)
	AGC	
oSD27	AAACGC <u>GTCGAC</u> TTAAGTACGTGAATTAAAAGTACGTC	3' of $sidC_{Llo783-969}$ (re)
	С	
oSD28	GATGAGTTCTTTTTGATGGACCCCAATAGAAAAGG	Quick Change $sidC_{Llo}$ ( $\Delta Bam$ HI) (fo)
oSD29	CCTTTTCTATTGGGGTCCATCAAAAAGAACTCATC	Quick Change $sidC_{Llo}$ ( $\Delta Bam$ HI) (re)

<sup>a</sup> Restriction sites are underlined.