SUPPLEMENTARY MATERIAL

Supplemental Materials and Methods

Gateway® IDTS library construction

The Invitrogen Gateway® system was used to clone of 159 *L. pneumophila* gene products. Genes were amplified using a forward primer designed to anneal to 25 bases of gene-specific sequence at the 5' end of each gene and 25 bases of *attB* specific base pairs for N-terminal fusions (GGGGACAAGTTTGTACAAAAAAGCAGGCTTC) and a reverse primer had 25 bases of gene-specific sequence at the 3' end of each gene followed by 25 bases of *attB* specific base pairs containing a stop codon (GGGGACCACTTTGTACAAGAAAGCTGG GTC**CTA**). PCR products of the correct size were purified according to manufacturer's instructions (Invitrogen) and recombined with pDONR221 (Invitrogen) using BP Clonase Enzyme Mix. The

plasmids derived in this fashion were sequenced, and the appropriate recombinants were subjected to a second recombination reaction using LR clonase II enzyme mix (Invitrogen) to introduce the *L. pneumophila* gene into pDEST53 (Invitrogen; mammalian CMV-GFP fusion vector). Correct plasmids constructs were verified by restriction digest and purified using Ultra Pure Miniprep kits (Qiagen) for use in transfections.

Nod1 psiRNA knock-down experiments

PAGE purified oligos (IDT, Coralville, IA) were annealed, 5'-phosophorylated, and ligated into pRNAT-U6.1/Neo according to manufactures instruction (GenScript; Table S2). Plasmids were prepared using Qiagen endofree maxiprep and 100 ng of each was transfected into ~2 x10^4 HEK293T in 96-well plates. 24 hours after transfection, the cell culture medium was changed and cells were transfected with 200 ng pNFκB-luciferase. After 16 hours, cells were infected as previously described for the NF-κB reporter assay with *L. pneumophila* infection. To confirm knockdown of Nod1 in HEK293T cells, cells were co-transfected with 10 ng pmyc-Nod1 and 200 ng of siRNA plasmids for 48 hrs. Cells were collected and then analyzed by Western blotting with anti-myc antibody (Clontech) for expression of myc-Nod1. For construction of pmyc-Nod1, Nod1 was amplified from cDNA isolated from U937 cells using primers Nod1F-myc and Nod1R and then inserted into plasmid pmyc using the *Eco*RI and *Xho*I sites. Proper construction of the plasmid was confirmed by sequencing.

Supplementary Figures

Figure S1. TLR-independent induction of p65 translocation dependent on the presence of the Icm/Dot translocation system. Bone marrow macrophages from C57BL/6 (black bars) and C57BL/6 *trif* $^{-/-}$ *myd88* $^{-/-}$ double knockout (DKO) mice (white bars) were incubated with the Dot^{+}-*flaA*$^{-}$, or *dotA*$^{-}$ *flaA*$^{-}$ strains at MOI=1 for noted times. The percentage of infected cells with NF-κB p65 staining in the nucleus was quantitated by immunofluorescence microscopy (Losick *et al.*, 2006).

Figure S2. *L. pneumophila* Gateway® IDTS library

(A) Gateway® cloning scheme. *L. pneumophila* genes were PCR amplified with *attB* sites. Products were introduced into the entry vector (pDONR221) by site-specific recombination and then introduced by a second site-specific recombination reaction into an expression plasmid that allows generation of GFP fusions in pDEST53 (Experimental Procedures). (B) HEK293T cells were transfected with the resulting GFP fusion plasmids for 24 h. Cell lysates were assayed for GFP-protein expression by Western blot with anti-GFP antibodies. The expected molecular weight of the GFP fusions is marked by a white dot.

Figure S3. Nod1 silencing in HEK293T cells reduces NF-κB activation in response to *L. pneumophila*

(A) HEK293T cells harboring the pNFκB-luciferase reporter plasmid were transfected with the indicated siRNA plasmids and then challenged with wild-type *L. pneumophila* Lp02 at MOI = 1 and assayed for luminescence at 8 hours post infection. Fold NF-κB activity is the ratio of the relative light units of cells challenged with *L. pneumophila* compared to cells incubated in absence of bacteria. Data represent the mean $\pm$ SE of triplicate samples from a representative experiment, performed in triplicate. * *T*-test p-value ≤ 0.05. (B) To verify Nod1 knockdown, HEK293T cells were co-transfected with 100 ng of the indicated siRNA plasmid and 10 ng

pmyc-Nod1. Samples were then assayed by Western blot for myc-Nod1 expression. Tubulin was used as a loading control.

Table S1. Complete list of all the *L. pneumophila* genes included in the Gateway® library.

Table S2. Primers used in this study.

Table S3. Plasmids used in this study.

Table S4. Strains used in this study.

Table S1. Gateway® Library of *Legionella pneumophila* known and putative IDTS

lpg#	*Gene*	Domain/ paralogs[a]	Evidence for Translocation[b]	Expression by Blot (293T) [c]	References
lpg0035	*ceg1*	-	ND	-	
lpg0038	*legA10*	AR	BLA	+	de Felipe *et al.*, 2008
lpg0059	*ceg2*	-	UA	+	Huang, unpublished
lpg0080	*ceg3*	-	CyaA	+	Burstein *et al.*, 2009
lpg0096	*ceg04*	lpg1101 paralog	UA, CyaA	+	Huang, unpublished; Burstein *et al.*, 2009
lpg0103	*vipF*	N-acetyltransferase	CyaA	+	Shohdy *et al.*, 2005
lpg0126	*cegC2*	Lpg2433(*ceg30*), lpg 1120, lpg1484 (*ceg22*) paralog	CyaA	-	Altman and Segal, 2008
lpg0135	*sdhB*	Lpg2829 (SidH) paralog	ND	+	
lpg0171	*legU1*	F-box	BLA	+	de Felipe *et al.*, 2008
lpg0208	*ceg6/ pkn5*	ser/thr kinase	ND	-	
lpg0209	-	-	ND	+	
lpg0234	*sidE*	-	*Cre/loxP*, CyaA	+	Cambronne and Roy, 2007; Luo and Isberg, 2004
lpg0240	*ceg8*	-	CyaA	+	Burstein *et al.*, 2009
lpg0275	*sdbA*	lipase	UA	+	Huang, unpublished
lpg0276	*legG2*	Ras GEF	BLA, CyaA	+	de Felipe *et al.*, 2008; de Felipe *et al.*, 2005
lpg0279	-	-	ND	+	
lpg0294	-	-	CyaA	ND	Kubori *et al.*, 2008; Zusman *et al.*, 2007
lpg0390	*vipA*	CC	CyaA	+	Shohdy *et al.*, 2005
lpg0401	*ceg11*	-	UA	+	Huang, unpublished
lpg0402	*legA9/ceg12*	AR	UA, CyaA	+	de Felipe *et al.*, 2008; Pan *et al.*, 2008
lpg0403	*legA7*	AR	UA, CyaA	-	de Felipe *et al.*, 2008; Pan *et al.*, 2008
lpg0404	*ceg13*	-	ND	-	
lpg0436	*legA11*	-	BLA	+	de Felipe *et al.*, 2008; Pan *et al.*, 2008
lpg0437	*ceg14*	-	CyaA	-	Burstein *et al.*, 2009
lpg0439	*ceg15*	Phospholipase?	UA	-	Huang, unpublished
lpg0483	*legA12*	AR	BLA	+	de Felipe *et al.*, 2008; Habyarimana *et al.*, 2008

lpg0515	*legD2*	phytanoyl-CoA dioxygenase	-	+	de Felipe *et al.*, 2008
lpg0519	*ceg17*	-	CyaA	-	{Burstein, 2009 #116
lpg0550	-	-	ND	+	
lpg0563	-	-	ND	+	
lpg0581	-	-	ND	+	
lpg0621	*sidA*	CC	Cre/*loxP*, CyaA	+	Cambronne and Roy, 2007; Luo and Isberg, 2004
lpg0634	-	-	UA, CyaA	-	Huang, unpublished; Kubori *et al.*, 2008
lpg0642	*wipB*	-	CyaA	+	Ninio *et al.*, 2005
lpg0645	-	-	ND	+	
lpg0693	*ligA*	-	ND	+	
lpg0695	*legA8*	AR	BLA, CyaA	+	de Felipe *et al.*, 2008; Habyarimana *et al.*, 2008; Pan *et al.*, 2008
lpg0717	-	-	ND	+	
lpg0733	-	CC	UA	+	Huang, unpublished
lpg0823	-	-	ND	-	
lpg0921	-	-	ND	+	
lpg0926	-	-	UA	+	Huang, unpublished
lpg0940	*lidA*	CC	IF	+	Derre and Isberg, 2005; Conover *et al.*, 2003
lpg0945	*legL1*	LRR	BLA	+	de Felipe *et al.*, 2008
lpg0952	-	VipD paralog	ND	+	
lpg1098	*vrrB*	-	ND	+	
lpg1101	*sdmA*	lpg2464 (SidM) paralog	CyaA	+	Burstein *et al.*, 2009
lpg1108	-	Esterase	UA	+	Huang, unpublished
lpg1111	-	lpg0940 (LidA) paralog	UA	+	Huang, unpublished
lpg1120	-	lpg2433 paralog	UA, CyaA	-	Huang, unpublished; Burstein *et al.*, 2009
lpg1121	*ceg19*	-	UA, CyaA	+	Huang, unpublished; Burstein *et al.*, 2009; Heidtman *et al.*, 2008
lpg1137	-	-	ND	+	
lpg1144	*cegC3*	CC	CyaA	+	Altman and Segal, 2008
lpg1151	-	loose lpg0234 (SidE) paralog	ND	+	
lpg1158	-	-	UA, CyaA	+	Huang, unpublished; Kubori *et al.*, 2008

lpg1171	*ceg21*	-	ND	+	
lpg1227	*vpdB*	Phospholipase	IF	+	VanRheenen *et al.*, 2006
lpg1290	-	CC	CyaA,UA	-	Huang, unpublished; Burstein *et al.*, 2009
lpg1316	-	-	UA	+	Huang, unpublished
lpg1328	*legT*	thaumatin domain	-	+	de Felipe *et al.*, 2008
lpg1340	*flaA/ fliC*	-	ND	+	
lpg1355	*sidG*	CC	Cre/*loxP*, CyaA	-	Cambronne and Roy, 2007; Ninio *et al.*, 2005; Luo and Isberg, 2004
lpg1368	*lgt1*	DxD motif/toxin domain	ND	-	
lpg1377	-	-	ND	+	
lpg1483	*legK1*	ser/thr kinase	CyaA, BLA	+/-	This study, Ge *et al.*, 2009; de Felipe *et al.*, 2008; Shin *et al.*, 2008; de Felipe *et al.*, 2005
lpg1484	-	lpg1483 (LegK1) paralog	ND	+	
lpg1488	*legC5*	CC	BLA	-	de Felipe *et al.*, 2008
lpg1489	-	-	UA	+	Huang, unpublished
lpg1588	*legC6*	CC	UA, CyaA, BLA	+	Huang, unpublished; de Felipe *et al.*, 2008; Kubori *et al.*, 2008
lpg1602	*legL2*	LRR	BLA, UA	+	Huang, unpublished; de Felipe *et al.*, 2008; de Felipe *et al.*, 2005
lpg1642	*sidB*	RTX (repeat in toxin) cytotoxin	Cre/*loxP*, CyaA	+	Cambronne and Roy, 2007; Luo and Isberg, 2004
lpg1660	*legL3*	LRR	CyaA, BLA	-	de Felipe *et al.*, 2008; de Felipe *et al.*, 2005
lpg1663	-	-	ND	+	
lpg1666	*ceg24*	-	ND	+	
lpg1701	*legC3*	CC	BLA	+	Ninio *et al.*, 2009; de Felipe *et al.*, 2008
lpg1718	*legAS4*	AR/ SET	BLA	+	de Felipe *et al.*, 2008; Habyarimana *et al.*, 2008
lpg1798	-	Rho GAP?	ND	+	
lpg1836	*ceg25*	CC	ND	+	
lpg1884	*legC2/ylfB*	CC	CyaA, BLA	+	de Felipe *et al.*, 2008; Cambronne and Roy, 2007; Campodonico *et al.*, 2005

lpg1890	*legLC8*	LRR/CC	CyaA, BLA	+	de Felipe *et al.*, 2008; Kubori *et al.*, 2008; de Felipe *et al.*, 2005
lpg1925	-	-	ND	-	
lpg1930	-	-	ND	-	
lpg1931	*ceg26*	AR	ND	+	
lpg1948	*legLC4*	LRR/CC	CyaA, BLA	+	de Felipe *et al.*, 2008; de Felipe *et al.*, 2005
lpg1950	*ralF*	sec7	CyaA, IF	+	Nagai *et al.*, 2005; Nagai *et al.*, 2002
lpg1953	*legC4*	CC	BLA	+	de Felipe *et al.*, 2008
lpg1958	*legL5*	LRR	CyaA, BLA	+	de Felipe *et al.*, 2008; Kubori *et al.*, 2008; de Felipe *et al.*, 2005
lpg1959	-	-	ND	-	
lpg1976	*legG1*	RCC1 domain	CyaA, BLA, UA	-	Huang, unpublished; Ninio *et al.*, 2009; de Felipe *et al.*, 2008
lpg1978	*setA*	glucosyltransferase	UA, CyaA	+	Huang, unpublished; Heidtman *et al.*, 2008
lpg2073	-	-	ND	+	
lpg2131	*legA6*	AR	-	+	de Felipe *et al.*, 2008
lpg2137	*legK2*	ser/thr kinase	UA, BLA	+	Huang, unpublished; de Felipe *et al.*, 2008; Shin *et al.*, 2008
lpg2144	*legAU13*	AR/ Fbox	BLA	+	Al-Khodor *et al.*, 2008; de Felipe *et al.*, 2008; Zusman *et al.*, 2007
lpg2154	-	lpg0234 (SidE) paralog	ND	-	
lpg2155	*sidJ*	-	IF, CyaA	+	Kubori *et al.*, 2008; Cambronne and Roy, 2007; Liu and Luo, 2007
lpg2156	*sdeB*	lpg0234 (SidE) paralog	ND	+	
lpg2157	*sdeA*	lpg0234 (SidE) paralog	CyaA	-	Bardill *et al.*, 2005
lpg2160	-	lpg2638 paralog	ND	+	
lpg2176	*legS2*	sphingosine 1-P lyase	BLA	+	de Felipe *et al.*, 2008
lpg2206	*wipC*	-	ND	+	
lpg2207	-	-	ND	-	
lpg2210	-	-	ND	+	
lpg2215	*legA2*	AR	BLA	+	de Felipe *et al.*, 2008

lpg2248	-	lpg0227 (Ceg7) paralog	UA, CyaA	+	Huang, unpublished; Burstein *et al.*, 2009
lpg2266	-	-	ND	-	
lpg2298	*legC7/ylfA*	CC	CyaA, IF, BLA	+	de Felipe *et al.*, 2008; Cambronne and Roy, 2007; Campodonico *et al.*, 2005
lpg2300	*legA3*	AR	BLA, CyaA	+	de Felipe *et al.*, 2008; Pan *et al.*, 2008
lpg2322	*legA5*	AR	BLA, UA	+	Huang, unpublished; de Felipe *et al.*, 2008
lpg2370	-	-	ND	+	
lpg2391	*sdbC*	lpg1642 (SidB) paralog	ND	+	
lpg2392	*legL6*	LRR	UA	+	Huang, unpublished; de Felipe *et al.*, 2008
lpg2395	-	Cdc2 similarity	ND	-	
lpg2400	*legL7*	LRR	CyaA, BLA	+	de Felipe *et al.*, 2008; de Felipe *et al.*, 2005
lpg2409	*ceg29*	-	CyaA	+	Zusman *et al.*, 2007
lpg2410	*vpdA*	phospholipase	UA, IF	-	VanRheenen *et al.*, 2006
lpg2416	*legA1*	AR	-	+	de Felipe *et al.*, 2008
lpg2420	-	lpg2247 paralog	ND	+	
lpg2425	-	-	UA	+	Huang, unpublished
lpg2433	*ceg30*	-	CyaA	-	Burstein *et al.*, 2009
lpg2452	*legA14*	AR	BLA, CyaA	+	de Felipe *et al.*, 2008
lpg2455	-	-	ND	+	
lpg2456	*legA15*	AR	BLA, CyaA	+	de Felipe *et al.*, 2008
lpg2461	-	-	ND	-	
lpg2464	*sidM/ drrA*	Rab1 GEF/ GDF	IF, CyaA	+	Ingmundson *et al.*, 2007; Machner and Isberg, 2006; Murata *et al.*, 2006
lpg2465	*sidD*	-	Cre/*loxP*, CyaA	+	Luo and Isberg, 2004
lpg2482	*sdbB*	Lipase	ND	+	
lpg2504	*ceg32*	-	*Cre/loxP*, UA, CyaA, IF	-	Huang, unpublished; Burstein *et al.*, 2009; Shen *et al.*, 2009
lpg2508	*sdjA*	lpg2155 (SidJ) paralog	IF, CyaA	+	Kubori *et al.*, 2008; Liu and Luo, 2007
lpg2509	*sdeD*	lpg0234 (SidE) paralog	ND	+	

lpg2510	*sdcA*	lpg2511 (SidC) paralog, CC	ND	+	
lpg2511	*sidC*	CC	Cre/*loxP*, IF, CyaA	+	Ragaz *et al.*, 2008; Cambronne and Roy, 2007; Luo and Isberg, 2004
lpg2523	-	-	CyaA	+	Burstein *et al.*, 2009
lpg2525	-	F-box?	UA	-	Huang, unpublished
lpg2527	*lnaB*	CC, lpg0437 (Ceg14) paralog	CyaA	+	This Study, Kubori *et al.*, 2008
lpg2552	-	-	ND	+	
lpg2556	*legK3*	ser/thr kinase	BLA	+	de Felipe *et al.*, 2008; Shin *et al.*, 2008
lpg2586	-	-	ND	+	
lpg2591	*ceg33*	-	CyaA	+	Altman and Segal, 2008
lpg2603	*sdmB*	lpg2464 (SidM) paralog	UA, CyaA	+	Huang, unpublished; Burstein *et al.*, 2009
lpg2638	-	lpg2160 paralog	ND	+	
lpg2678	-	lpg0563 paralog	ND	+	
lpg2694	*legD1*	-	-	-	de Felipe *et al.*, 2008
lpg2718	*wipA*	-	CyaA	+	Ninio *et al.*, 2005
lpg2720	*legN*	cNMP binding domain	-	+	de Felipe *et al.*, 2008
lpg2806	-	-	ND	-	
lpg2813	*vipE*	-	ND	+	
lpg2815	*dimB*	-	UA	-	Huang, unpublished
lpg2826	*ceg34*	AR	CyaA	-	Burstein *et al.*, 2009
lpg2829	*sidH*	-	Cre/*loxP*, UA, CyaA	-	Cambronne and Roy, 2007; Laguna *et al.*, 2006; Ninio *et al.*, 2005; Luo and Isberg, 2004
lpg2830	*legU2/ lubX*	U-box	CyaA, BLA	+	de Felipe *et al.*, 2008; Kubori *et al.*, 2008
lpg2831	*vipD*	phospholipase	CyaA, UA	+	VanRheenen *et al.*, 2006; Shohdy *et al.*, 2005
lpg2862	*legC8*	CC	BLA	-	de Felipe *et al.*, 2008
lpg2874	-	-	ND	+	
lpg2884	-	-	UA	+	Huang, unpublished
lpg2907	-	-	ND	-	
lpg2952	*ceg35*	-	ND	-	
lpg2975	-	-	UA	-	Huang, unpublished
lpg2999	*legP*	astacin protease	BLA	+	de Felipe *et al.*, 2008

Lpg#, *Legionella pneumophila* Philadelphia gene annotation. [a]Domain, AR, ankryin repeat; LRR, leucine rich repeats; CC, coiled-coil. [b]Translocation, assay used to demonstrate whether protein is an Icm/Dot secretion system substrate. IF, immunofluorescence; UA, unpublished fusion assay (Huang and Isberg, data not shown). CyaA fusion, adenylate cyclase reporter assay. BLA, β-lactamase fusion assay. Cre/*loxP*, interbacterial transfer assay. [c]Protein expression in 293T, + or -, indicates detection of the full-length GFP-fusions by Western blot.

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Table S2. Primers used in this study

Primer Name	Restriction site	Sequence
Nod1F-myc	EcoRI	CCG**GAATTC**GGGAAGAGCAGGGCCACAGT
Nod1R	XhoI	CCG**CTCGAG**CGAGTGGCATTTGCTGCTGA
siEGFPF	BamHI/HindIII	**GATCC**CGTGAACTTCAGGGTCAGCTTGCTTGATATCCGGCAAG CTGACCCTGAAGTTCATTTTTTCCAA**A**
siEGFPR	BamHI/HindIII	**AGCTT**TTGGAAAAAATGAACTTCAGGGTCAGCTTGCCGGATAT CAAGCAAGCTGACCCTGAAGTTCACG**G**
siNod1F1	BamHI/HindIII	**GATCC**CGTCAAAGGCAGCACGGAAGTGCTTGATATCCGGCACT TCCGTGCTGCCTTTGATTTTTTCCAA**A**
siNod1R1	BamHI/HindIII	**AGCTT**TTGGAAAAAAGGATGAAGATGGTCTCACCCTCGGATAT CAAAGGGTGAGACCATCTTCATCCG**G**
siNod1F2	BamHI/HindIII	**GATCC**CAGTCATTCTTCAGCAAGTTGTTTGATATCCGACAACTT GCTGAAGAATGACTTTTTTTCCAA**A**
siNod1R2	BamHI/HindIII	**AGCTT**TTGGAAAAAAAGTCATTCTTCAGCAAGTTGTCGGATATC AAACAACTTGCTGAAGAATGACTG**G**
1483F-cyaA	BamHI	GC**GGATCC**ATGCCTCGTACCATGTTTTT
2527F-cyaA	BamHI	GC**GGATCC**TTGTTATATTTTGTACTATGTG
1950F-cyaA	BamHI	GC**GGATCC**ATGCATCCAGAAATTGAAAAAGC
1950R	SalI	ACGC**GTCGAC**TTAAAATTTTAATTGTCTACCTT
2527F1-KO	BamHI	ACGGGATCCCCCATAAAGAGCAAACAACACA
2527R1-KO	SalI	ACGC**GTCGAC**GCGCTGACAGCCAGTTTTTCGC
2527F2-KO	SacI	ATC**GAGCTC**GTCATAATGAATGATATCAATG
2527R2-KO	BamHI	ACG**GGATCC**GTTTCTGCGGTGCTTGTATGTA
1483F1-KO	SacI	CAGTAC**GAGCTC**ACATCAGCTCTTTACCTAATACG
1483R1-KO	BamHI	CG**GGATCC**GGGTACGAGGCATGATA
1483F2-KO	BamHI	CG**GGATCC**CTGTATTTCCCGGATTAT
1483R2-KO	SalI	CAGCTA**GTCGAC**TTGATAAGCAACTCTGCTTTCAG
2527F EGFP	EcoRI	TC**GAATTC**TTTGTTATATTTTGTACTATGTG
2527R2	BamHI	GA**GGATCC**CTCATGATGCATCGCTGATTG
2527R3	BamHI	GA**GGATCC**ATTCAGTGCCTCGCGATGGCG
2527F3	EcoRI	TC**GAATTC**TGAGTTACAAGAGCGATTTAAG
2527F4	BamHI	CT**GGATCC**AATAAATTAAACGAGGTATTT
2527R4	BamHI	CT**GGATCC**CTATTGTAAACGAGCGCTTTTG

In bold are the restriction enzyme sites.

Table S3. Plasmids used in this study

Name	Relevant Genotype	Primers used	Reference or Source
pSR47s	ori R6K ori TRP4 Kan^R *sacB*		(Merriam et al., 1997)
pJB-CyaA	pJB2581 CM^R, Amp^R		(Bardill et al., 2005)
pEGFP-C1	KAN^R		Clonetech
pRNAT.U6.1	Amp^R		Genescript
pNFκB-luciferase	Amp^R		Stratagene
pMyc	Amp^R		Clonetech
pMyc-Nod1	pMyc +Human Nod1	Nod1F-myc/Nod1R	This study
psiEGFP	pRNAT.U6.1-siEGFP	siEGFPF/siEGRPR	This study
psiNod1	pRNAT.U6.1-siNod1	siNod1F/siNod1R	This study
pJB-CyaA-RalF	pJB2581+RalF (lpg1950)	1950F-cyaA/1950R	This study (Bardill et al., 2005)
pJB-CyaA-LegK1	pJB2581+LegK1 (lpg1483)	1483F-cyaA/1483R	This study
pJB-CyaA-LnaB	pJB2581+LnaB (lpg2527)	2527F-cyaA/2527R	This study
pSR47s-*ΔlegK1*	*legK1* flanking regions	1483F1-KO/1483R1-KO and 1483F2-KO/1483R2-KO	This study
pSR47s-*ΔlnaB*	*lnaB* flanking regions	2527F1-KO/2527R1-KO and 2527F2-KO/2527R2-KO	This study
pSR47s-LnaB	pSR47s +LnaB (lpg2527)	2527F1-KO/2527R2-KO	This study
pEGFP-LnaB	pEGFP + LnaB (lpg2527)	2527F-EGFP/2527R4	This study
pEGFP-$LnaB_{1-361}$		2527F-EGFP/2527R2	This study
pEGFP-$LnaB_{1-401}$		2527F-EGFP/2527R3	This study
pEGFP-$LnaB_{361-401}$		2527F3/2527R3	This study
pEGFP-$LnaB_{361-558}$		2527F3/2527R4	This study
pEGFP-$LnaB_{401-558}$		2527F4/2527R4	This study
pEGFP-LnaBΔCC		2527F-EGFP/2527R2 and 2527F4/2527R4	This study
Gateway® Plasmids			
pDEST17	pDEST17 (His) Amp^R		Invitrogen
pDEST53	pDEST53 (GFP) Amp^R		Invitrogen
pDONR221	pDONR221 KAN^R		Invitrogen

Table S4. Bacterial strains used in this study

Name	Strain	Relevant genotype	Reference or source
***E.coli* strains**			
	DH5 α λ pir	DH5 α (λ pir) *tet*::Mu	(Kolter et al., 1978)
	DH5 α		
	Helper *E. coli*	MT607 pRK600	(Finan et al., 1986)
***L. pneumophila* strains**			
	Lp02 or wild-type	Lp01 *rpsL hsdR thyA*	(Berger and Isberg, 1993)
	dotA	Lp02 *dotA3*	(Berger and Isberg, 1993)
	Lp02 *ΔflaA*	Lp02 *ΔflaA*	(Ren et al., 2006)
	dotA Δfla	Lp02 *dotA3 ΔflaA*	Creasey, E (Ren et al., 2006)
VPL1	*ΔlegK1*	Lp02 *ΔlegK1*	This study
VPL2	*ΔlnaB*	Lp02 *ΔlnaB*	This study
VPL3	Lp02 pJB-cyaA		This study
VPL4	Lp03 pJB-cyaA		This study
VPL5	Lp02 pJB-cyaA-RalF		This study
VPL6	Lp03 pJB-cyaA-RalF		This study
VPL7	Lp02 pJB-cyaA-LegK1		This study
VPL8	Lp03 pJB-cyaA-LegK1		This study
VPL9	Lp02 pJB-cyaA-LnaB		This study
VPL10	Lp03 pJB-cyaA-LnaB		This study
VPL11	Lp02::SR47s		This study
VPL12	*ΔlnaB*::SR47s	Lp02 *ΔlnaB*	This study
VPL13	*ΔlnaB*::SR47s-LnaB^{+}		This study