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Supporting Information

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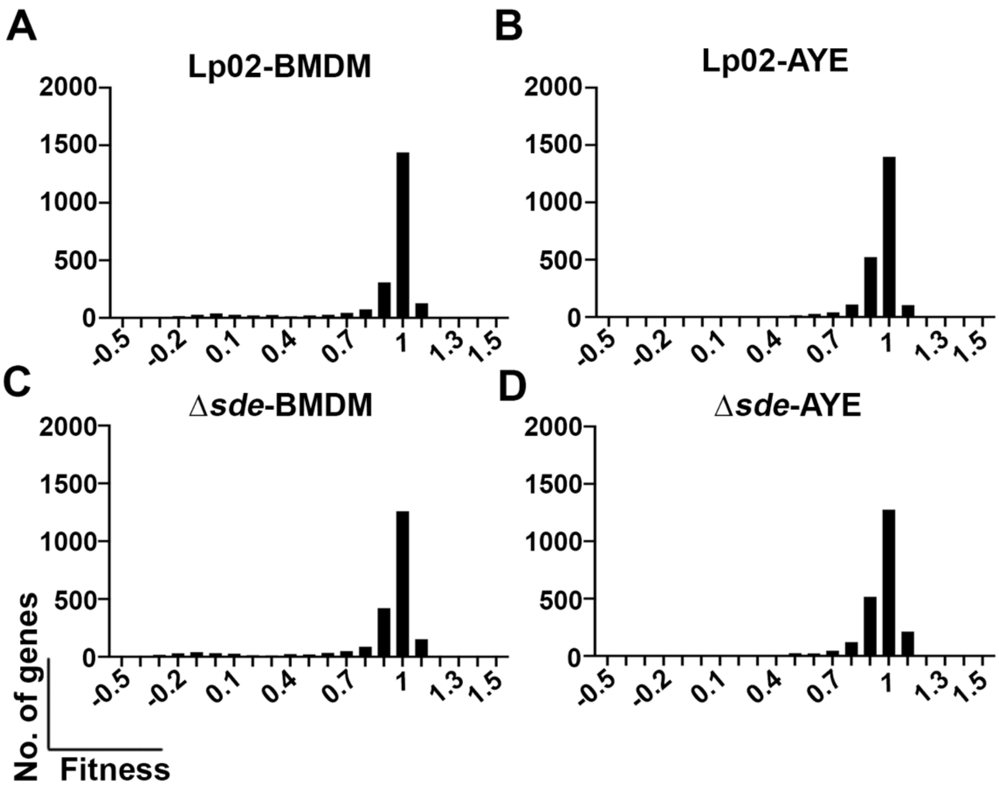

Fig. S1 (Linked to Fig.1). Histogram plots of fitness for all *L. pneumophila* genes represented on Tn-seq.

Histogram of WT (SK01) Tn-seq pool following either infection in BMDM (A) or growth in nutrient-rich AYE medium (B). Histogram of Δ*sde* (SK02) Tn-seq pool following infection in BMDM (C) or growth in nutrient-rich AYE medium (D).

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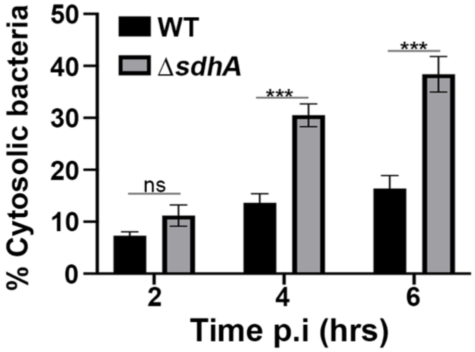

Fig. S2 (Linked to Fig.3). The integrity of LCVs harboring Δ*sdhA* strains after challenge with *L. pneumophila*.

Percent cytosolic bacteria was quantified based on antibody accessibility. BMDMs were infected with either WT or Δ*sdhA* strains for 2, 4, and 6 hr, fixed, and stained with antibodies. The internalized bacteria in the absence of permeabilization were calculated relative to the total infected population (mean ± SEM; three biological replicates were performed and 100 LCVs were counted per biological replicate). Statistical analysis was conducted using unpaired two-tailed Student's t test (ns, not significant; ***p < 0.001).

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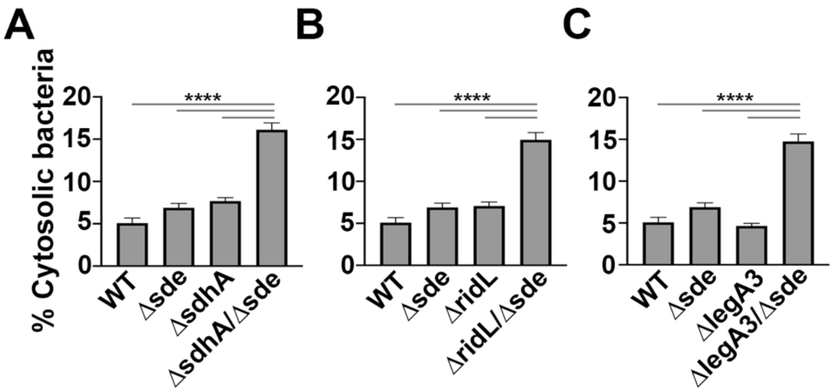

Fig. S3 (Linked to Fig. 3). The loss of *sdhA*, *ridL* and *legA3* aggravated vacuole disruption in Δ*sde* strain.

Vacuole integrity was measured based on antibody accessibility. BMDMs in a 96 well plate were infected with the indicated strains for 2 hr, fixed and stained with antibodies. The images were taken by Lionheart automatic microscope using 10X magnification objective. The internalized bacteria in the absence of permeabilization were calculated relative to total infected population to determine fraction of disrupted vacuoles (mean ± SEM; three biological replicates were performed and 1000-3000 LCVs were counted per biological replicate). Statistical significance was tested using one-way ANOVA with Tukey's multiple comparisons; ***p <0.001.

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Table S1. Strains, Plasmids and Oligonucleotides used in this study

Strains

Name	Genotype	Description	Reference
L.pneumophila			
Lp02	Philadelphia 1 *thyA*$^-$ *rpsL hsdR*	Wild type strain	(1)
SK01	Lp02 *thyA*$^+$	Wild type strain *thyA*$^+$	This work
Lp03	*thyA*$^+$ *rpsL hsdR dotA03*	Icm/Dot translocation deficient	(1)
JV6113	Lp02 Δ*sidE* Δ*sdeC* Δ*sdeBA* (Δ*lpg0234* Δ*lpg2153* Δlpg*2156-2157*)	*sidE* family deletion mutant	(2)
SK02	JV6113 *thyA*$^+$	JV6113 strain *thyA*$^+$	This work
SK03	Lp02 *thyA*$^+$Δ*sdhA*	*sdhA* deletion mutant	This work
SK04	JV6113 *thyA*$^+$ Δ*sdhA*	*sdhAsidE* family deletion mutant	This work
SK05	Lp02 *thyA*$^+$ Δ*ridL*	*ridL* deletion mutant	This work
SK06	JV6113 *thyA*$^+$ Δ*ridL*	*ridLsidE* family deletion mutant	This work
SK07	Lp02 *thyA*$^+$ Δ*legA3*	*legA3* deletion mutant	This work
SK08	JV6113 *thyA*$^+$ Δ*legA3*	*legA3sidE* family deletion mutant	This work
SK09	Lp02 *thyA*$^+$ Δ*sdhA* Δ*ridL*	*sdhAridL* deletion mutant	This work
SK10	Lp02 *thyA*$^+$ Δ*sdhA* Δ*legA3*	*sdhA legA3* deletion mutant	This work
SK11	Lp02 *thyA*$^+$ Δ*ridL* Δ*legA3*	*ridL legA3* deletion mutant	This work
SK12	Lp02 *thyA*$^+$ *kan*^{R} P_{ahpc}::*lux*	wild type strain Lux$^+$	This work
SK13	JV6113 *thyA*$^+$ *kan*^{R} P_{ahpc}::*lux*	*sidE* family deletion mutant Lux$^+$	This work
SK14	SK02 *kan*^{R} P_{ahpc}::*lux*	*sdhA* deletion mutant Lux$^+$	This work
SK15	SK03 *kan*^{R} P_{ahpc}::*lux*	*sdhAsidE* family deletion mutant Lux$^+$	This work
SK16	SK04 *kan*^{R} P_{ahpc}::*lux*	*ridL* deletion mutant Lux$^+$	This work
SK17	SK05 *kan*^{R} P_{ahpc}::*lux*	*ridLsidE* family deletion mutant	This work
SK18	SK06 *kan*^{R} P_{ahpc}::*lux*	*legA3* deletion mutant Lux$^+$	This work
SK19	SK07 *kan*^{R} P_{ahpc}::*lux*	*legA3 sidE* family deletion mutant Lux$^+$	This work
Lp03 lux$^+$	Lp03 *kan*^{R} P_{ahpc}::*lux*	Icm/Dot translocation deficient Lux$^+$	(3)
JV4487	Δ*sidJ*	*sidJ* deletion mutant	(2)
SK20	Lp02 Δ*sdhA*	*sdhA* deletion mutant	This work
SK21	Lp02 Δ*sdhA* Δ*sidJ*	*sdhAsidJ* deletion mutant	This work
SK22	SK01+ pMMB207Δ267		This work
SK23	SK02+ pMMB207Δ267		This work
SK24	SK03+ pMMB207Δ267		This work
SK25	SK04+ pMMB207Δ267		This work

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Plasmids

Name	Features	Description	Reference
pTO100MmeI	R6K*ori* *kan*^{R}, *sacB*, *ampR*, *himar1*-MmeI, C9 transposase	Tn-seq transposon mutagenesis plasmid	(5)
pSR47S	R6K*ori* *sacB*, *kan*^{R}	suicide vector	(6)
pSR47S-P_{ahpc}::lux	R6K*ori* *sacB*, *kan*^{R} P_{ahpc}::lux	pSR47 containing *P. luminescens* lux operon	(7)
pJB3395	pBluescript*::thyA*$^+$ *amp*^{R}	*thyA* allelic exchange vector	J. Vogel
pTO243	pbluescript:: PolyHis-*attR1*-[*Kan*^{R}-*Kan*^{R}- *ccdB*]-*attR2*		O'Connor Tamara
pSK01	pSR47S:: Δ*sdhA*	*sdhA* deletion plasmid	
pSK02	pSR47S:: Δ*ridL*	*ridL* deletion plasmid	
pMMB207	*OriR* (RSF1010), Cm^R		(8)
pMMB207Δ267	*OriR* (RSF1010), Cm^R, Δ267	pMMB207 lacking 267 bps of N- terminal *mobA*	Elizabeth Creasey
pSK03	pMMB207Δ267::PolyHis-*attR1*-[*Kan*^{R}-*Kan*^{R}-*ccdB*]-*attR2*	Gateway destination version of pMMB207Δ267	This work
pSK04	pMMB207Δ267::PolyHis-*attB1*-*sdeA*-*attB2*	*sdeA* complementation plasmid	This work
pSK05	pMMB207Δ267::PolyHis-*attB1*-*sdeB*-*attB2*	*sdeB* complementation plasmid	This work
pSK06	pMMB207Δ267::PolyHis-*attB1*-*sdeC*-*attB2*	*sdeC* complementation plasmid	This work
pTO198	pSR47S::Δ*legA3*	*legA3* deletion plasmid	(9)

E. coli

DH5α	*supE44 ΔlacU169*(*Φ80lacZDM15*) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*		
DH5α λpir	DH5α (λ*pir*) *tet*::Mu recA		(12)
BL21 DE3	F$^+$ *ompT hsdSB dcm* (*DE3*)		

Oligonucleotides

Name	Sequences (5' to 3')
Construction of sdhA mutant	
SK1	GGCGCTAATTGCTGAAATCATTTCAATATTAAAAAAATTAAC
SK2	CCGGGGGATGAACAATTTACCCCCTG
SK3	GATTTCAGCAATTAGCGCCATCCGCATAAAAATATTTG
SK4	GAACTAGGGCGTAGGCGTTGACCATTAAAAG
pSR47s_sdhA_F	TTGTTCATCCCCCGGGCTGCAGGAAT
pSR47s_sdhA_R	CCTACGCCCTAGTTCTAGAGCGGCCGCC
Construction of ridL mutant	
SK5	TCATTATTATTATGTGTTCATTTTAAGCCAAAAAAC

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SK6	AGCCCGGGGGGTTATTACTGAAGTCGTGAC
SK7	CTAGAACTAGGATACTGGTGGATTGTCG
SK8	TGAACACATAATAATAATGACTTTGGCTCTC
pSR47s_ridL_F	CAGTAATAACCCCCCGGGCTGCAGGAAT
pSR47s_ridL_R	CACCAGTATCCTAGTTCTAGAGCGGCCGCC

Confirmation of recombinant plasmid

pSR47s_conF	GGGAACAAAAGCTGGAGC
pSR47s_conR	GTGAACGGCAGGTATATGT

qRT-PCR

Name	Sequences (5’ to 3’)
rRNA_F	AGAGATGCATTAGTGCCTTCGGGA
rRNA_R	ACTAAGGATAAGGGTTGCGCTCGT
ridL_F	GTCCTCTGAAGGATAGCGAAAC
ridL_R	GTGTAAGTTCCCGCAACAAATC
sidE_F	GCCTAAGTACGTTGAAGGGATAG
sidE_R	GCCTGTCAAGAGCACCTTTA
sdeC_F	AAATCAGGAGAAGCGGTTAGG
sdeC_R	CGTGAGAGCCGGGATAATTT
sdeB_F	CCAGGCTTCACTCACTTGATAA
sdeB_R	CCTCTCGATACCTACTGTGTCT
sdeA_F	CCCACTGCACCACAAGATAA
sdeA_R	GGTATACGGTTTGCCCAGATAG
sdhA_F	GGAAGGCAGGATTCTCCATTTA
sdhA_R	AGCTCTGAGTTCAGGAGGTAT
legA3_F	CTCCGCTCTTTCCAGATGAC
legA3_R	GAGTGGGTCGAGTGGGATAA
sidJ_F	GTTGTTCCTACCCAACCTGG
sidJ_R	CAGAGAGGTGTCATGAGTGC

Mariner Tn-seq sequencing library construction

Name	Sequences (5’ to 3’)	Index
First PCR		
Nextera 2A-R	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG	
1st_TnR	GTAATACGACTCACTATAGGGTCTAGAG	
Second PCR- Leftward Mariner specific Nextera Indexed primers		
mar147	AATGATACGGCGACCACCGAGATCTACACGCAGGCGGCGTTGACCGGGGACTTATCAGCCAACCTGTTA	GCAGGCGG
mar148	AATGATACGGCGACCACCGAGATCTACACAGGCAGAACGTTGACCGGGGACTTATCAGCCAACCTGTTA	AGGCAGAA
mar149	AATGATACGGCGACCACCGAGATCTACACCAGAGAGGCGTTGACCGGGGACTTATCAGCCAACCTGTTA	CAGAGAGG
mar150	AATGATACGGCGACCACCGAGATCTACACCGAGGCTGC	CGAGGCTG

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	GTTGACCGGGGACTTATCAGCCAACCTGTTA	
mar151	AATGATACGGCGACCACCGAGATCTACACAAGAGGCACGTTGACCGGGGACTTATCAGCCAACCTGTTA	AAGAGGCA
mar152	AATGATACGGCGACCACCGAGATCTACACGAGGAGCCCGTTGACCGGGGACTTATCAGCCAACCTGTTA	GAGGAGCC
Second PCR- Rightward Mariner specific Nextera Indexed primers		
olk141	CAAGCAGAAGACGGCATACGAGATCCGCCTGCGTCTCGTGGGCTCGGAGATGTG	GCAGGCGG
N703 index	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGTG	AGGCAGAA
Reconditioning		
P1	AATGATACGGCGACCACCGA	
P2	CAAGCAGAAGACGGCATACGA	
Sequencing		
mar512	CGTTGACCGGGGACTTATCAGCCAACCTGTTA	

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