Supplementary Materials for

Aggravating genetic interactions allow a solution to redundancy in a bacterial pathogen

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This PDF file includes:

Materials and Methods

Bacterial strains, cultured cells and growth media. *L. pneumophila* strains were grown in liquid ACES buffered yeast extract (AYE) media (*28*) or on solid charcoal buffered yeast extract (CYE) media (*29*) containing 0.1 mg/ml thymidine (Sigma) (CYET), 40 ug/ml kanamycin, 5% sucrose, or 5 ug/ml chloramphenicol when appropriate. For intracellular growth assays, the chromosomal *thyA*$^{-}$ allele in each *L. pneumophila* strain analyzed was replaced with the *thyA*$^{+}$ allele by allelic exchange as described (*30*). *E. coli* strains were grown in liquid Luria broth (LB) or on solid LB plates supplemented with 50 ug/ml ampicillin, 50 ug/ml kanamycin, 50 ug/ml streptomycin, 10 ug/ml tetracycline or 17 ug/ml chloramphenicol when appropriate. Cloning was performed using the *E. coli* strain DH5α λpir (*31*). Strains are summarized in Table S2.

Drosophila Kc167 cells were cultured in Schneider's media (Gibco) containing 10% fetal bovine serum (Gibco) as described (*9*). Primary bone marrow-derived macrophages from A/J mice were prepared and cultured as described in (*32*). The human monocytic cell line U937 (ATCC CRL 1593.2) were cultured as per the manufacturer's instructions. *D. discoideum* Ax4 was a kind gift from Richard H. Gomer. *D. discoideum* Ax4 and *A. castellanii* (ATCC30234) strains were cultured as described in (*33*) and (*14*), respectively. For the *D. discoideum* Ax4 strain containing the GFP-HDEL expression construct (*34*), the plasmid was maintained by supplementing media with 20 ug/ml G418 (Gibco).

iMAD Screen

Construction of dsRNA for RNA interference

Genomic DNA was isolated from *Drosophila* Kc167 cells using a DNeasy Blood and Tissue kit (QIAGEN). Internal fragments of *sar1*, *arf1*, *bet5*, *sec22* and *rab1* were amplified by PCR using the appropriate primers (Table S2) and used as template to generate dsRNA using a MEGAscript RNAi kit (Ambion) following the manufacturer's instructions.

RT-PCR to test target depletion by RNAi

RNA was isolated from *Drosophila* cells (untreated or dsRNA treated) using an RNeasy kit (QIAGEN). In a 20 ul volume, 1ug of RNA and 5 ug of oligodT (Table S2) were combined in ddH_2O and heated to 70°C for 10 min then reverse transcribed to generate cDNA using a Superscript II Reverse Transcriptase kit (Invitrogen) following the manufacturer's instructions. 1 ul of the cDNA reaction was then used to amplify each gene of interest and actin using the appropriate primers (Table S2). Samples were separated in 2% agarose and the corresponding bands were quantified using Kodak 1D 3.6 software.

Growth of L. pneumophila *in RNAi treated* Drosophila *cells*

10^7 *Drosophila melanogaster* Kc167 cells were treated with 50 ug of *sar1*, *arf1*, *sec22*, *rab1* or *bet5* specific dsRNA in Schneider's media lacking serum for 45 minutes. FBS serum was then added to a final concentration of 10% and cells were incubated at 25°C for 48 (*sar1* and *arf1*) or 72 (*sec22*, *rab1*, *bet5*) hours. An aliquot of the *L. pneumophila* transposon mutant library (~10^{10} bacteria) was diluted in AYE media then cultured overnight at 37°C to post-exponential phase and used to challenge either

untreated or dsRNA treated *Drosophila* Kc167 cells at an MOI=1 for 1 hour. Cells were then rinsed 3 times with fresh media and incubated at 25°C for 29 hours. Bacterial cultures used to challenge *Drosophila* cells were diluted and plated for single colonies on solid CYET media containing kanamycin and grown at 37°C for four days. Thirty hours post infection, equivalent to a single round of infection and a 10-fold increase in bacterial colony forming units (cfus) for wild-type bacteria, *Drosophila* cells were lysed with 0.02% saponin at 25°C for 10 minutes and bacteria were harvested by plating supernatants on solid CYET media containing kanamycin and grown at 37°C for four days. An estimated 100,000-150,000 bacterial colonies were harvested, resuspended in AYE (~2500 cfus/ml) and mixed to homogeneity for bacteria plated prior to and post challenge of *Drosophila* cells. From these suspensions, triplicate aliquots of $2x10^9$ bacteria were used to isolate genomic DNA using a Qiagen DNeasy kit, including proteinase K and RNAse digestion steps, and then pooled. This procedure was performed in duplicate with three separate aliquots of the library for a total of six individual replicates. The relative abundance of each mutant before and after challenge of *Drosophila* cells was determined using transposon site hybridization as described (*16*) using a custom *Legionella* microarray (*16*). The average output/input probe ratio for each gene from two replicates of three individual experiments was calculated. For each dsRNA treatment, the logarithmic base 10 values for the average output/input ratio for each gene was plotted as a histogram and the value at which the dataset deviated from a Gaussian distribution was used to define a growth disadvantage (or growth advantage). This value corresponded to 3.7 ± 0.2-fold growth defect or 1.75 ± 0.1 standard deviations from the mean, which varied depending on the dsRNA used.

Construction of *L. pneumophila* deletion mutants. Null mutations in individual genes were constructed in *L. pneumophila* Philadelphia 1 strain Lp02 using a double recombination strategy employing the suicide vector pSR47s as described (*30*) using 1000 bp segments directly upstream and downstream of each gene deleted for homologous recombination. Primers used in plasmid construction are listed in Table S2. All plasmid constructs (Table S2) were verified by sequencing prior to use. Strains encoding the appropriate null mutations were identified by PCR and the genomes of those Δ*lidA*, Δ*wipB*Δ*lidA*, Δ*lpg2395*Δ*lidA* and Δ*mavP*Δ*lidA* mutants used in subsequent analyses were verified by sequencing as described (*16*).

Construction of gene expression plasmids for *in trans* complementation. *L. pneumophila* Philadelphia 1 strain Lp02 genomic DNA was isolated using a DNeasy Blood and Tissue Kit (QIAGEN). Individual genes were amplified by PCR from genomic DNA using the appropriate primers (Table S2) then cloned as *Sac*I-*Pst*I fragments into similarly digested pJB908. The resulting plasmids were verified by sequencing prior to use.

***In vitro* growth assays.** *L. pneumophila* strains were inoculated into AYE medium from patches grown on solid CYE medium and diluted to $0.3x10^9$ bacteria/ml. Growth at 37°C, with shaking, was monitored over 20 hours by measuring absorbance at 600 nm every 15 minutes in a Synergy HT plate reader (BioTek) using KC4 Data Analysis software (BioTek).

Intracellular growth assays. Growth of *L. pneumophila* in bone marrow-derived murine A/J macrophages, *D. discoideum* and *A. castellanii* was performed as described in (*32*), (*34*) and (*14*), respectively. For growth in *Drosophila*, cultured *Drosophila melanogaster* Kc167 cells were plated at $2x10^5$ cells per well in a 96 well tissue culture plate in Schneider's media containing 10% FBS and 0.4 mg/ml thymidine and incubated at 25°C overnight. Cells were then challenged at an MOI=1with *L. pneumophila* strains grown to post-exponential phase. Cells were incubated for 1 hour at 25°C then rinsed three times with fresh media. At 1 and 48 hours post infection, *Drosophila* Kc167 cells were lysed with 0.02% saponin and growth of *L. pneumophila* was determined by plating host cell lysates on solid CYET media and counting bacterial cfus.

Infectious Center Assays. *A. castellanii* cells were preincubated in Ac buffer (*35*) for 2 hours at 37°C. *L. pneumophila* strains containing the *gfp* expressing plasmid, pMMB207Δ267:*gfp* were grown at 37°C in AYE containing 1 uM IPTG and 12.5 ug/ml chloramphenicol to post-exponential phase then used to challenge $5x10^5$ *A. castellanii* cells at an MOI=10. Cells were incubated at 37°C for 1 hour then rinsed with Ac buffer. At 1, 6, 8 and 10 hours post infection, cells were harvested, rinsed twice with Ac buffer then fixed in 4**%** paraformaldehye in 1x PBS containing 1.25% sucrose and 7.5 mM D-lysine (Sigma) in 1x PBS for 30 min at room temperature in the dark. Cells were then rinsed twice with Ac buffer and mounted on agarose pads and visualized by fluorescence microscopy.

D. discoideum ectopically expressing GFP-HDEL were plated on poly-L-lysine coated coverslips and preincubated in MB buffer (*33*) for 2 hours at 25°C. *L. pneumophila* strains containing the *rfp* expressing plasmid, pMMB207Δ267:*rfp* were grown at 37°C in AYE containing 1 uM IPTG and 12.5 ug/ml chloramphenicol to post-exponential phase then used to challenge $2x10^5$ *D. discoideum* cells at an MOI=1. Cells were incubated at 25°C for 1 hour then rinsed three times with MB buffer. At 1, 2 and 4 hours post infection, cells were fixed in 4**%** paraformaldehye containing 1.25% sucrose and 7.5 mM D-lysine (Sigma) in 1x PBS for 30 minutes at room temperature in the dark. Cells were then rinsed three times with 1xPBS and visualized by fluorescence microscopy.

For A/J macrophages, $2x10^5$ cells were plated on coverslips in a 24 well tissue culture plate and incubated overnight at 37°C. *L. pneumophila* strains were grown at 37°C in AYE to post-exponential phase then used to challenge macrophages at an MOI=1 for 1 hour. Cells were rinsed three times with culture medium. At 1, 6, 8 and 10 hours post infection, cells were fixed in 4**%** paraformaldehyde in 1x PBS for 30 min at room temperature in the dark, permeabilized with ice cold methanol then stained with a rabbit α- *Legionella* antibody in 1x PBS containing 4% goat serum (Gibco) followed by α-rabbit IgG Alexa Fluor 488 (Molecular Probes). For Galectin-3 colocalization, cells were infected with *L. pneumophila* for 6 or 8 hours, fixed and permeabilized as above then stained with rabbit α-*Legionella* and rat α-Galectin-3 (Santa Cruz Biotechnology) followed by goat α-rabbit IgG Alexa Fluor 594 (Molecular Probes) and goat α-rat IgG Alexa Fluor 488 (Molcular Probes). Aberrant bacterial and infected host cell nuclear morphology was examined and quantified as described (*21*).

Image optimization of fluorescence micrographs was performed by subjecting each of the images in a single channel to the same linear transformation.

Cyclase reporter assay. The *lpg2395* gene was amplified by PCR using primers 2395cyaF and 2395rev (Table S2) and cloned into *Bam*HI-*Pst*I digested pJB2581 to generated plasmid pTO126. Translocation of CyaA and the CyaA:Lpg2395 fusion protein by wild-type *L. pneumophila* and a *dotA* mutant into U937 macrophages was performed as described (*14*).

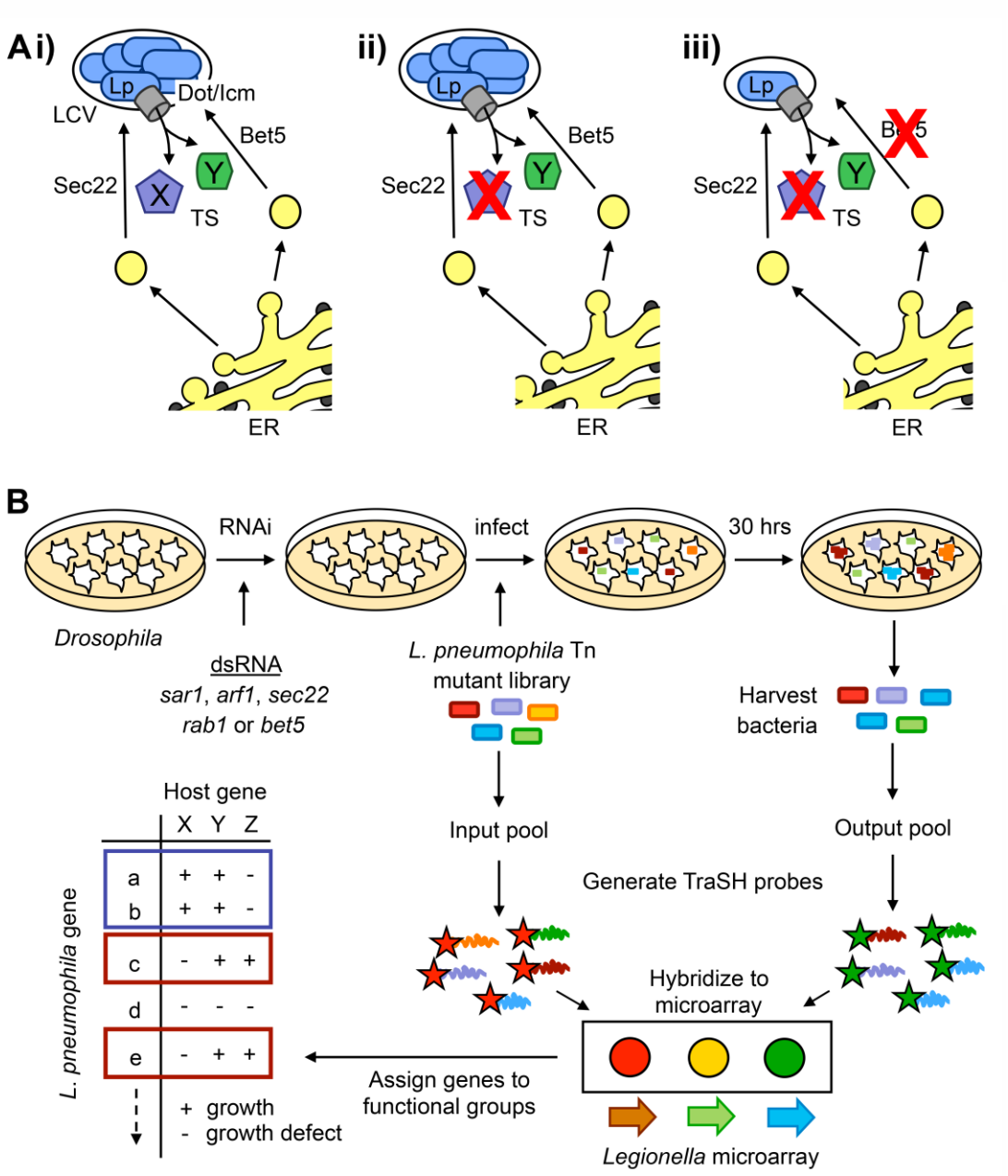

Fig. S1. Defining functional relationships amongst *L. pneumophila* Dot/Icm translocated substrates. (**A**) Strategy for identifying functional relationships between sets of Dot/Icm translocated substrates (TS). Simultaneous depletion of the host factors Sec22 and Bet5 by RNAi in *Drosophila* cells impairs intracellular growth of wild-type *L. pneumophila*. This implies that *L. pneumophila* targets two independent host pathways to remodel its vacuole, a Sec22-dependent pathway and a Bet5-dependent pathway. If *L. pneumophila* translocates two proteins, X and Y, into the host that each target one of these pathways (i), a *L. pneumophila* mutant lacking X would not be defective for growth because Y could compensate through the modulation of the Bet5-dependent pathway (ii). Similarly a mutant lacking Y would not be attenuate for growth due to the activity of X.

However, if the host cell pathway targeted by Y was disrupted by depleted the cell of Bet5, Y would no longer be able to compensate for lack of X (iii). Thus, a bacterial mutant with a mutation in the gene encoding protein X would exhibit a growth defect in a host cell depleted of Bet5. (**B**) iMAD genetic screen. *Drosophila* cells were treated with dsRNA targeting either *sar1*, *arf1*, *sec22*, *rab1* or *bet5*. Cells were then infected with a *L. pneumophila himar1* transposon mutant library (input pool) for 30 hours, equivalent to a single round of infection. Host cells were then lysed and bacteria were harvested and plated on solid bacteriological media (output pool). Mutants containing a transposon insertion in a gene that is important for intracellular replication under the specific host condition will be underrepresented in the output pool compared to the input pool. For each host condition examined, the relative abundance of each mutant in both the input and output pools was determined by transposon site hybridization using a custom *Legionella* microarray (*16*). Genes in which mutations resulted in a significant growth advantage or disadvantage (Table S1) were determined as described (*16*) (Materials and Methods). Those mutants with similar phenotypic patterns across all conditions were clustered into distinct functional groups and used to define gene sets target redundant host pathways through genetic interaction mapping.

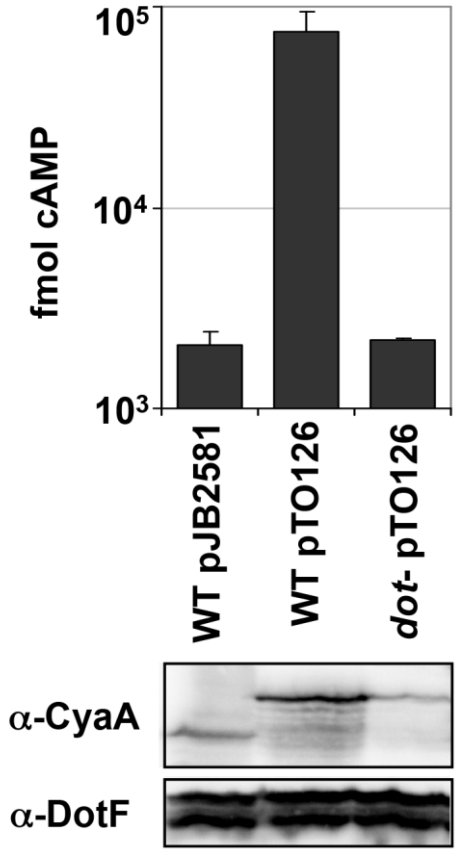

Fig. S2. *lpg2395* encodes a novel Dot/Icm translocated substrate. (**A**) Cyclase A reporter assay. The CyaA:Lpg2395 fusion protein is translocated into *L. pneumophila*-infected human monocytic U937 cells as measured by enhanced cAMP production relative to CyaA alone. CyaA:Lpg2395 translocation was dependent on the Dot/Icm translocation system as a mutation in *dotA* (*dot*-), which impairs Dot/Icm translocation, caused reduced cAMP production compared to that observed with wild-type (WT) bacteria. cAMP production was normalized to the relative abundance of CyaA or CyaA fusion protein in each strain as measured in (B). (**B**) Western analysis of CyaA and CyaA:Lpg2395 protein levels. An aliquot of bacteria used to infect U937 cells were lysed in SDS-PAGE loading buffer by boiling. Protein levels in bacterial lysates were examined by Western analysis using antibodies against CyaA and DotF. Bands were visualized using a Syngene system with GeneSys software (Syngene) and band intensities were quantitated using GeneTools software (Syngene). The relative abundance of CyaA or CyaA:Lpg2395 was normalized to DotF.

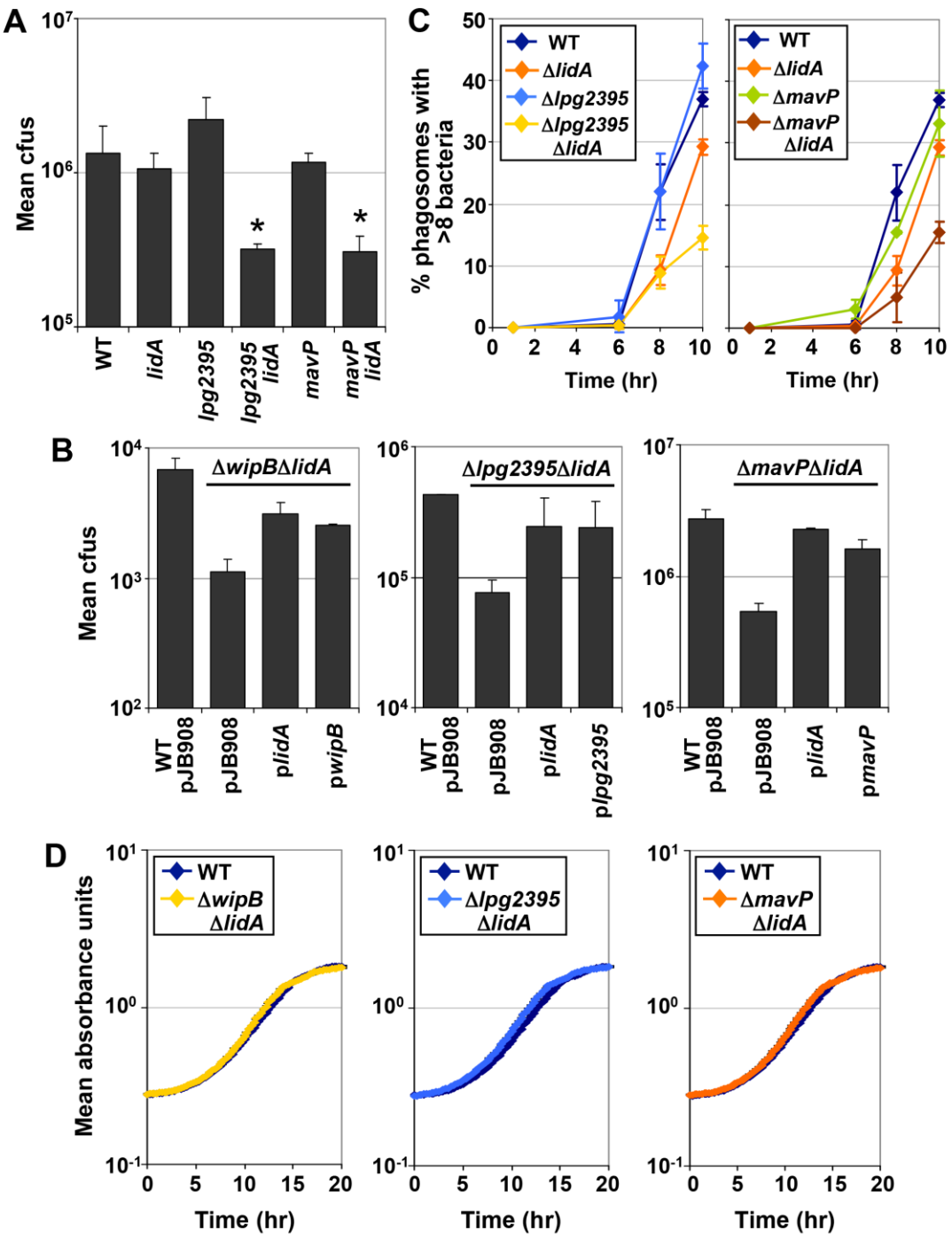

Fig. S3. Impaired growth of the Δ*lpg2395*Δ*lidA* and Δ*mavP*Δ*lidA* mutants in *A. castellanii*. (**A**) Growth of the Δ*lpg2395*Δ*lidA* and Δ*mavP*Δ*lidA* mutants in *A. castellanii* was impaired relative to the wild-type (WT) and corresponding single deletion mutant strains. (**B**) *In trans* complementation of *L. pneumophila* double mutant phenotypes in *A. castellanii*. Intracellular growth of the Δ*wipB*Δ*lidA*, Δ*lpg2395*Δ*lidA* and Δ*mavP*Δ*lidA* mutants was rescued by introducing either *lidA* (p*lidA*) or *wipB* (p*wipB*), *lpg2395* (p*lpg2395*) or *mavP* (p*mavP*), respectively, on a self-replicating plasmid but not the

empty vector, pJB908 alone. (**C**) The Δ*lpg2395*Δ*lidA* and Δ*mavP*Δ*lidA* mutants were defective in the accumulation of vacuoles containing large numbers of bacteria. *A. castellanii* were infected with *L. pneumophila* expressing GFP and the number of bacteria per phagosome 1, 6, 8 and 10 hours post infection was scored using fluorescence microscopy. (**D**) Growth of the Δ*wipB*Δ*lidA*, Δ*lpg2395*Δ*lidA*, and Δ*mavP*Δ*lidA* mutants in nutrient rich media is comparable to that of the wild-type strain. Bacterial growth in nutrient rich AYE medium at 37°C was monitored by measuring absorbance at 600 nm every 15 minutes for 20 hours. (A, B) Bacterial growth based on recovered cfus on solid media from lysed host cells was monitored over three days. The total bacterial yield at 48 hours post infection is plotted normalized to the wild-type strain at 1 hour post infection. *p <0.05 relative to the wild-type strain. (A-D) Data are representative of 2 independent experiments ± standard deviation of 3 replicates.

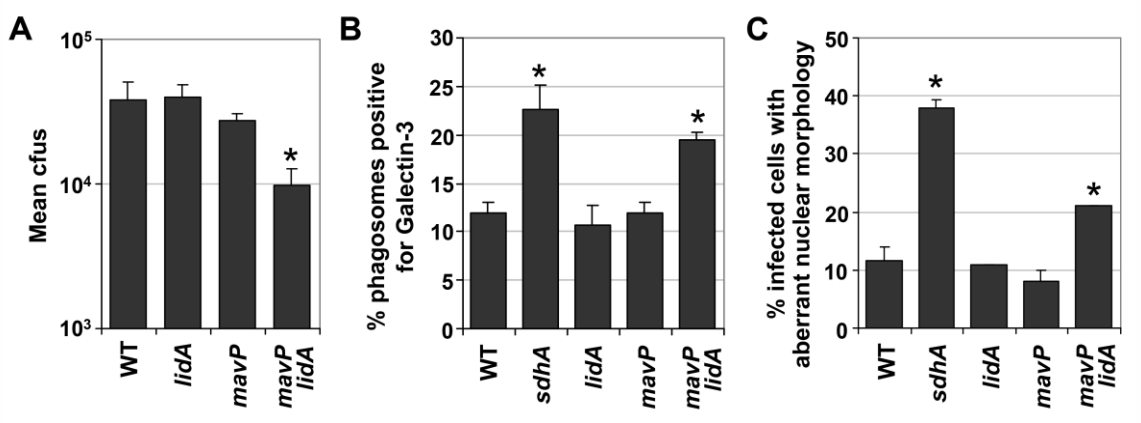

Fig. S4. Vacuole disruption of Δ*mavP*Δ*lidA* mutant-containing vacuoles in macrophages. (**A**) Growth of the Δ*mavP*Δ*lidA* mutant was reduced in macrophages compared to the wild-type (WT) and single deletion mutant strains. Bacterial growth in macrophages infected with the indicated *L. pneumophila* strains was monitored over three days based on recovered cfus on solid media from lysed host cells. The total bacterial yield 48 hours post infection was plotted normalized to the wild-type strain at 2 hours post infection. (**B**) The Δ*mavP*Δ*lidA* mutant shows enhanced recruitment of Galectin-3. Macrophages were infected with *L. pneumophila* for 6 hours, stained for *Legionella* and Galectin-3 then visualized by fluorescence microscopy. The number of *Legionella*-containing vacuoles colocalizing with Galectin-3 were scored. (**C**) Cells infected with the Δ*mavP*Δ*lidA* mutant exhibited increased host cell death based on aberrant nuclear morphology characteristic of apoptosis. Macrophages were infected with *L. pneumophila* for 8 hours, fixed and stained for *Legionella* then with Hoechst and visualized by fluorescence microscopy. (A-C) Data are representative of 2 independent experiments ± standard deviation of 3 replicates, scoring 100 vacuoles (B) or infected cells (C) per replicate. *p <0.05 relative to the wild-type strain.

Table S2: Stains, Plasmids and Oligonucleotides

Strains

Strain	Genotype	Description	Reference
L. pneumophila			
Lp02	Philadelphia 1, *thyA rpsL, hsdR*	wild type strain	(*32*)
Lp03	*thyA rpsL hsdR dotA03*	Dot/Icm translocation deficient	(*32*)
T646	Δ*lpg0940*	Δ*lidA* mutant	this work
TO279	Δ*lpg0642*	Δ*wipB* mutant	this work
TO288	Δ*lpg2395*	Δ*lpg2395* mutant	this work
TO473	Δ*lpg2884*	Δ*mavP* mutant	this work
TO651	Δ*lpg0642,* Δ*lpg0940*	Δ*wipB*, Δ*lidA* double mutant	this work
TO623	Δ*lpg2395,* Δ*lpg0940*	Δ*lpg2395*, Δ*lidA* double mutant	this work
TO626	Δ*lpg2884,* Δ*lpg0940*	Δ*mavP*, Δ*lidA* double mutant	this work
TO495	Lp02, *thyA*$^+$		(*16*)
TO497	Lp03, *thyA*$^+$		(*16*)
TO694	Δ*lpg0940, thyA*$^+$	Δ*lidA*, thy+	this work
TO501	Δ*lpg0642, thyA*$^+$	Δ*wipB*, thy+	this work
TO503	Δ*lpg2395, thyA*$^+$	Δ*lpg2395*, thy+	this work
TO517	Δ*lpg2884, thyA*$^+$	Δ*mavP*, thy+	this work
TO710	Δ*lpg0642,* Δ*lpg0940, thyA*$^+$	Δ*wipB*, Δ*lidA*, thy+	this work
TO690	Δ*lpg2395,* Δ*lpg0940, thyA*$^+$	Δ*lpg2395*, Δ*lidA*, thy+	this work
TO692	Δ*lpg2884,* Δ*lpg0940, thyA*$^+$	Δ*mavP*, Δ*lidA*, thy+	this work
TO348	Δ*lpg2504*	Δ*ceg33*/*sidI* mutant	this work
TO432	Δ*lpg2182*	Δ*lpg2182* mutant	this work
TO442	Δ*lpg0642,* Δ*lpg2182*	Δ*wipB*, Δ*lpg2182* double mutant	this work
TO326	Δ*lpg1098*	Δ*lpg1098* mutant	this work
TO584	Δ*lpg2395,* Δ*lpg1098*	Δ*lpg2395*, Δ*lpg1098* double mutant	this work
TO351	Δ*lpg2862*	Δ*legC8*/*lgt2* mutant	this work
TO468	Δ*lpg2884,* Δ*lpg2862*	Δ*mavP*, Δ*legC8*/*lgt2* double mutant	this work
TO340	Δ*lpg0642,* Δ*lpg2395*	Δ*wipB*, Δ*lpg2395* double mutant	this work
TO222	Lp02, pJB908		this work

TO183	Lp03, pJB908		this work
TO727	Δ*lpg0940,* pJB908	Δ*lidA*, thy+	this work
TO300	Δ*lpg0642,* pJB908	Δ*wipB*, thy+	this work
TO302	Δ*lpg2395,* pJB908	Δ*lpg2395*, thy+	this work
TO551	Δ*lpg2884,* pJB908	Δ*mavP*, thy+	this work
TO720	Δ*lpg0642,* Δ*lpg0940,* pJB908	Δ*wipB*, Δ*lidA*, pJB908	this work
TO722	Δ*lpg0642,* Δ*lpg0940,* p*lidA*	Δ*wipB*, Δ*lidA*, pJB908:lidA	this work
TO724	Δ*lpg0642,* Δ*lpg0940,* pTO89	Δ*wipB*, Δ*lidA*, pJB908:wipB	this work
TO678	Δ*lpg2395,* Δ*lpg0940,* pJB908	Δ*lpg2395*, Δ*lidA*, pJB908	this work
TO680	Δ*lpg2395,* Δ*lpg0940,* p*lidA*	Δ*lpg2395*, Δ*lidA*, pJB908:*lidA*	this work
TO682	Δ*lpg2395,* Δ*lpg0940,* pTO90	Δ*lpg2395*, Δ*lidA*, pJB908:*lpg2395*	this work
TO684	Δ*lpg2884,* Δ*lpg0940,* pJB908	Δ*mavP*, Δ*lidA*, pJB908	this work
TO686	Δ*lpg2884,* Δ*lpg0940,* p*lidA*	Δ*mavP*, Δ*lidA*, pJB908:*lidA*	this work
TO688	Δ*lpg2884,* Δ*lpg0940,* pTO133	Δ*mavP*, Δ*lidA*, pJB908:*mavP*	this work
TO628	Lp02, *thyA*$^+$, pGFP	*gfp* expression strain	this work
TO629	Lp03, *thyA*$^+$, pGFP	*gfp* expression strain	this work
TO712	Δ*lpg0940, thyA*$^+$, pGFP	*gfp* expression strain	this work
TO631	Δ*lpg0642, thyA*$^+$, pGFP	*gfp* expression strain	this work
TO633	Δ*lpg2395, thyA*$^+$, pGFP	*gfp* expression strain	this work
TO635	Δ*lpg2884, thyA*$^+$, pGFP	*gfp* expression strain	this work
TO718	Δ*lpg0642,* Δ*lpg0940, thyA*$^+$, pGFP	*gfp* expression strain	this work
TO714	Δ*lpg2395,* Δ*lpg0940, thyA*$^+$, pGFP	*gfp* expression strain	this work
TO716	Δ*lpg2884,* Δ*lpg0940, thyA*$^+$, pGFP	*gfp* expression strain	this work
MHL37	Lp02, pJB2581	p*cyaA*	(*36*)
TO427	Lp02, pTO126	p*cyaA*:*lpg2395*	this work
TO428	Lp03, pTO126	p*cyaA*:*lpg2395*	this work
E. coli			
DH5α λpir	*endA1 glnV44 thi*-1 *recA1 relA1 gyrA96 deoR nupG* (Φ80d*lac* Δ*lacZ*) M15 (Δ*lacZYA-argF*)U169, *hsdR17*(r_K^- m_K^+), (λ*pir*)		(*31*)

Plasmids

Plasmid	Features	Description	Reference
pTO100	*mariner himar*-1 transposon, R6K, km^R, *sacB*, Ap^R, C9 transposase	*Legionella* mariner *himar*1 transposon	(*16*)
pGFP	pMMB207Δ267 (*mobA*$^-$), *gfp*, cm^R	GFP expression plasmid	Sina Mohammadi, Elizabeth Creasey
pRFP	pMMB207Δ267 (*mobA*$^-$), *rfp*, cm^R	RFP expression plasmid	Sina Mohammadi, Elizabeth Creasey
pJB3395	*thyA*$^+$, amp^R	*thyA* allelic exchange vector	(*14*)
pJB908	RSF1010 ori, *tdΔi*, Δ*oriT, thyA*$^+$, amp^R, p*tac*	*in trans* complementation empty vector	(*37*)
p*lidA*	pJB908:*sidM*p::*lidA*, amp^R	*lidA* complementation plasmid	(*25*)
pJB2581	*cyaA*, Cm^R	cyclase reporter plasmid	(*14*)
pTO89	pJB908:*lpg0642*, amp^R	*wipB* complementation plasmid	this work
pTO90	pJB908:*lpg2395*, amp^R	*lpg2395* complementation plasmid	this work
pTO133	pJB908:*lpg2884*, amp^R	*mavP* complementation plasmid	this work
pSR47S	R6K, *sacB*, Km^R	*Legionella* suicide vector	(*30*)
pZLΔ*lidA*	pSR47s:*lpg0940*, Km^R	*lidA* deletion plasmid	Zhao-Qing Luo
pTO79	pSR47s:*lpg0642*, Km^R	*wipB* deletion plasmid	this work
pTO81	pSR47s:*lpg2395*, Km^R	*lpg2395* deletion plasmid	this work
pTO84	pSR47s:*lpg2884*, Km^R	*mavP* deletion plasmid	this work
pTO92	pSR47s:*lpg2182*, Km^R	*lpg2182* deletion plasmid	this work
pTO87	pSR47s:*lpg1098*, Km^R	*lpg1098* deletion plasmid	this work
pTO88	pSR47s:*lpg2862*, Km^R	*legC8*/*lgt2* deletion plasmid	this work
pTO126	pJB2581:*lpg2395*	*cyaA*:*lpg2395* expression plasmid	this work

Oligonucleotides

Name	Sequence
dsRNA generation	
sar1R	TAATACGACTCACTATAGGGAGACCACGACGCGTCTGGAAGGACTA
sar1S	TAATACGACTCACTATAGGGAGACCACGGTTGTTAGCTGATACAGTCC
arf1R	TAATACGACTCACTATAGGGAGACCACCTGCCAAATGCAATGAACGC

arf1S	TAATACGACTCACTATAGGGAGACCACTAGCGATTAGCGTTCTTCAATTGG
sec22bR	TAATACGACTCACTATAGGGAGACCACTAATCGAGAACGACGTCTGC
sec22bS	TAATACGACTCACTATAGGGAGACCACCTGTGCCACGTTGGAGCAC
rab1R	TAATACGACTCACTATAGGGAGACCACCACCATCACGTCTTCATATTAT
rab1S	TAATACGACTCACTATAGGGAGACCACTGGTGTGGTCGACTACTTTC
bet5R	TAATACGACTCACTATAGGGAGACCACGCATTCCATAACTTCGCC
bet5S	TAATACGACTCACTATAGGGAGACCACATCCAGAGGGAGTTTCC
act5F	ATGTGTGACGAAGAAGTTG
act5R	AGTCCAGAACGATACCG

Gene deletion plasmid construction

lpg0642up1	GGTGGAGCTCACACGACAGCTCGTCAAGTG
lpg0642up2	GAGTGGATCCTTATTTCTGATATAAACAACCATTATG
lpg0642dn1	CTTGGGATCCTCCTTTTACCTTGTTTTTATTATTGTTCC
lpg0642dn2	CAAGGTCGACCGGAGCCGGTACAGAATTT
lpg2395up1	ATTAGTCGACTTTCTGGAAAGGCAGATGCT
lpg2395up2	TCCCGGATCCATTAATGCAAATCTTGAGTTAAATGCAAATG
lpg2395dn1	GTATGGATCCTTAACAGGACATCCTTACTTAACC
lpg2395dn2	GCATGAGCTCGGCTTGTTTGATGTCATGGA
lpg2884up1	GTTTGTCGACATCAAGCGAATGAAGCAGGT
lpg2884up2	TTAAGGATCCTTTTTTACTCCAAGGTTATAAATCGG
lpg2884dn1	CTCAGGATCCTATAAAAATATTACAAGCAAGTGGTC
lpg2884dn2	TCCAGAGCTCTCATCCCCACAAACGCTAAT
lpg2182up1	GAACGAGCTCCAAAATACACTGGGTGGCCAG
lpg2182up2	CCAGGGATCCTAATCATTGTAAGGGAAGATATAC
lpg2182dn1	TATCGGATCCCTCAAGGTGTTAGGAGGCAATAC
lpg2182dn2	CAACGTCGACTTGTTTGCCATGCATGAAAG
lpg1098up1	TTTTGAGCTCGGGCGGTATATGGTGGAAGG
lpg1098up2	GACCGGATCCCGTAAAACTCCTGTTACCTTTTG
lpg1098dn1	AGGAGGATCCAAGCAACTGGGAATCAGTTTATTTTTC
lpg1098dn2	ATTAGTCGACAACTTGGCCATTGTTTCCTGGTG

lpg2862up1	TTTTGAGCTCGAGGTTGCGAAAGGAGTCTG
lpg2862up2	GTTCGGATCCAATATCTCCAAATAGACGCCAATTTGG
lpg2862dn1	ATCAGGATCCGTTTAATTGAATAAGTATAACTTC
lpg2862dn2	CCACGTCGACTTGCAGAAGCAGCAGCTAAACTGC
lpg2504up1	TAGCGAGCTCGGGCAATCGCTCTTGTTTTAAC
lpg2504up2	TATTGCGGCCGCAAATTACTTTAGTAAAATTTTTGTCATGG
lpg2504dn1	CTGGGCGGCCGCTATGATAAAAGGAAAACTTATGCCCAAC
lpg2504dn2	GTCCGTCGACAAATACAGCGACCTGGAAGC

Complementation plasmid construction

lpg0642F	CATTGAGCTCATAATGGTTGTTTATATCAG
lpg0642R	GAACCTGCAGGAGCCGCCTTTAAAAGACATAATG
lpg2395F	GAAAGAGCTCTTTGCATTTAACTCAAGATTTGC
lpg2395R	GAAACTGCAGGAGGTTAGGGGCTTTTCTTTTAGG
lpg2884F	TTGCGAGCTCCTGCTCCGATTTATAACCTTGGAGTAAAAAAATG
lpg2884R	ATTTCTGCAGTTTGGATTGTGGATTGGTGAAACGCTGG

Cyclase reporter plasmid construction

lpg2395cyaF	TTGCGGATCCCTGAATGGGACTCACAAGGAAAAGG
lpg2395rev	GAAACTGCAGGAGGTTAGGGGCTTTTCTTTTAGG

[1]Restriction enzyme sites are underlined

Additional Data Table S1 (separate file)
Table S1. *L. pneumophila* genes for which mutations cause a growth disadvantage in untreated or host factor depleted *Drosophila* cells.