Supplemental Material

Metabolism of *myo*-inositol by *Legionella pneumophila* promotes infection of amoeba and macrophages

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Running title: Inositol metabolism by *L. pneumophila*

Key words: amoeba, bipartite metabolism, Legionnaires' disease, macrophage, nutrition, pathogen vacuole, phytate, transport, type IV secretion

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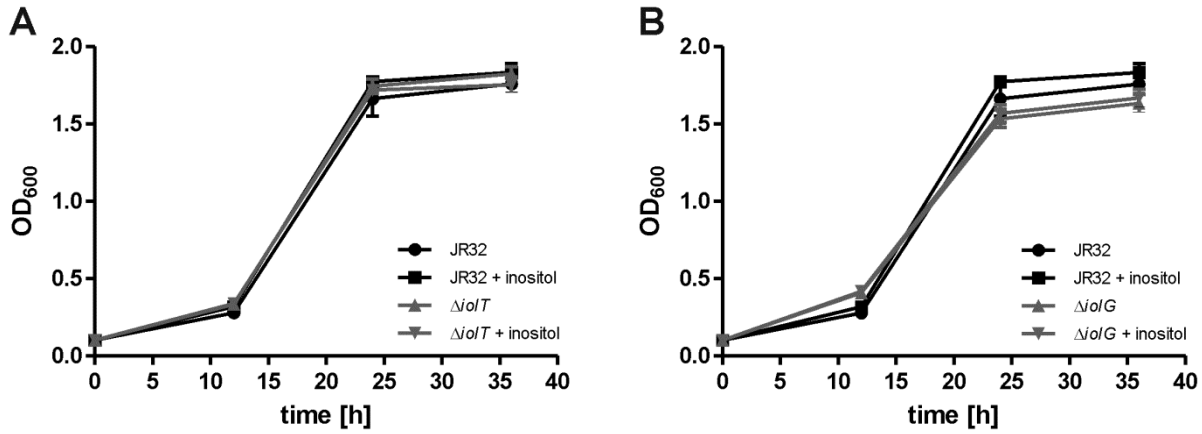

FIG S1 Growth of the *L. pneumophila* mutant strains Δ*iolT* and Δ*iolG* in CDM. Extracellular growth of *L. pneumophila* JR32, Δ*iolT* or Δ*iolG* in CDM with and without 10 mM inositol was assessed. Optical density at 600 nm was determined at the time points indicated.

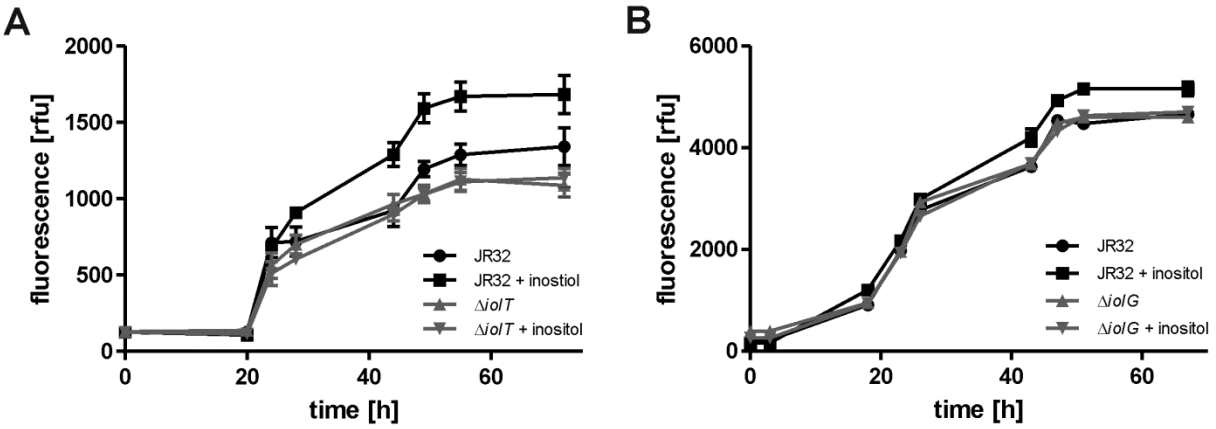

FIG S2 Inositol promotes intracellular growth of *L. pneumophila* at 4 h post infection. *A. castellanii* was infected (MOI 20) with *L. pneumophila* JR32, (A) Δ*iolT* or (B) Δ*iolG* harboring plasmid pNT28 (constitutive GFP production). 20 mM inositol was added 4 h post infection, and replication was determined by fluorescence. Mean and SD of triplicates are shown (Student´s *t*-test; A, > 44 h: $p < 0.01$; B, > 26 h: $p < 0.05$). Data are representative of three independent experiments.

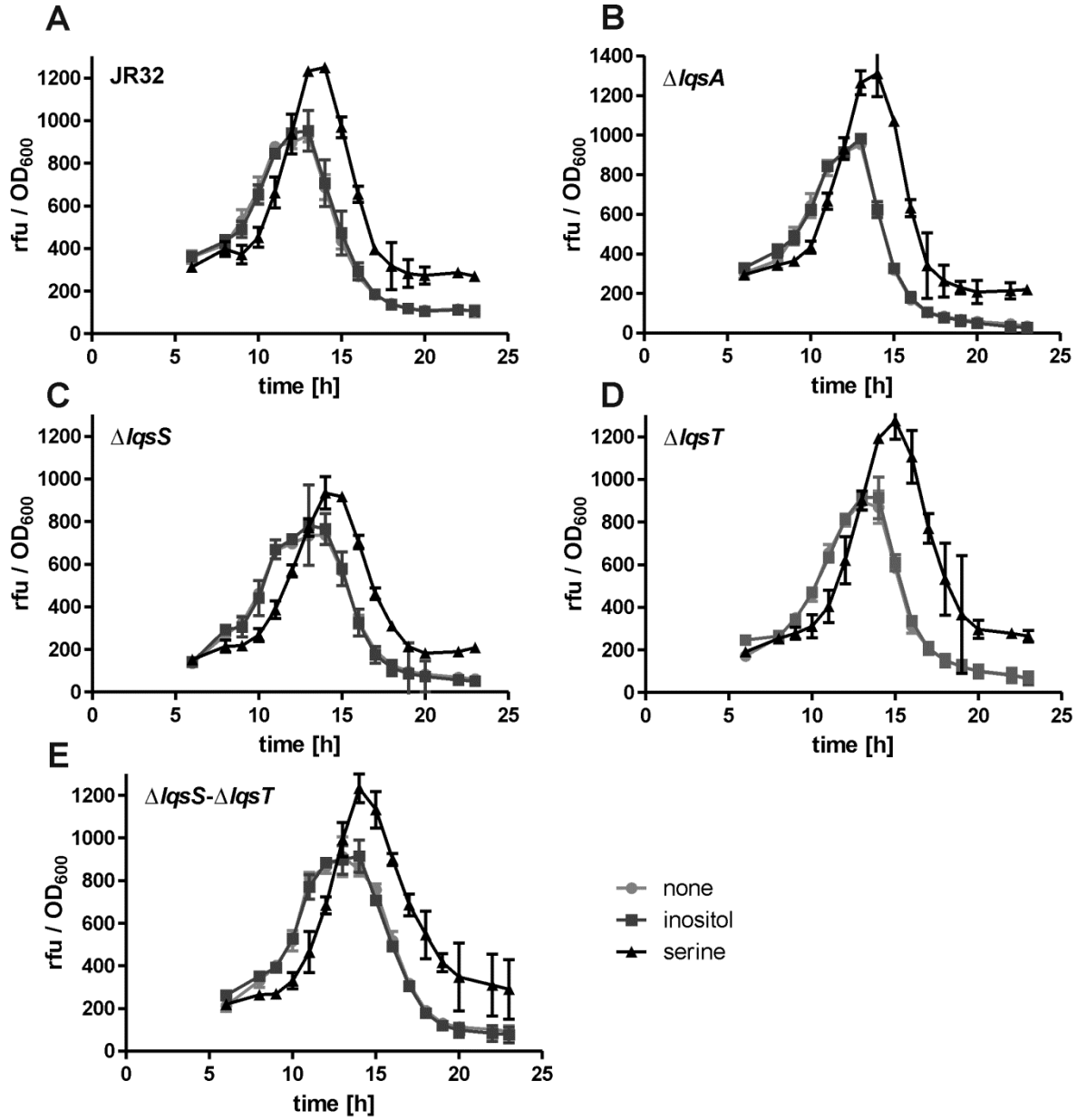

FIG S3 Expression of P_{iol} is not regulated by *lqs* genes. Exponentially growing cultures of (A) *L. pneumophila* JR32, (B) Δ*lqsA*, (C) Δ*lqsS*, (D) Δ*lqsT* or (E) Δ*lqsS*-Δ*lqsT* harboring plasmid pCM007 (unstable GFP (ASV) under control of P_{iol}) were diluted to a starting OD_{600} of 0.1 in AYE broth. The bacteria were grown at 37°C with 10 mM inositol, 6 mM serine or without additional nutrients (none). Optical density at 600 nm and GFP fluorescence was measured every hour for 24 h, and results were plotted with RFU as function of OD_{600} over time. Mean and SD of triplicates are shown (Student´s *t*-test; A-E [serine *versus* none], > 14 h: $p < 0.05$). Data are representative of three independent experiments.

TABLE S1 Oligonucleotides used in this study.

Oligonucleotide	**Sequence (5' – 3')**, restriction sites underlined	**Description**
iol-660-fo-Seq	TACTGGGGAGTAGTTTGTGTGC	seq. primer of *iolT*
iol-1347-fo-Seq	TCAGTGGTACTTTGATCGCA	seq. primer of *iolT*
iol-2051-fo-Seq	CATCTCGAACAAATTATTACAGCG	seq. primer of *iolT*
iol-2744-fo-Seq	GATGGACTACAAGCCTTGCG	seq. primer of *iolT*
iol-3459-fo-Seq	TATGCGATTTGATTGTTGGG	seq. primer of *iolT*
iol-7042-re-Seq	GAAGTCGTAAGCAATTCGGC	seq. primer of *iolT*
iol-6351-re-Seq	CCCAATCCCTCTGCGTACT	seq. primer of *iolT*
iol-5655-re-Seq	AAAGCCCATTTGGAAGCTGT	seq. primer of *iolT*
iol-4942-re-Seq	TTTTTGTTTGGCATAGGCG	seq. primer of *iolT*
iol-4241-re-Seq	AAAATAAATTTTCGTTTTCCGTTTC	seq. primer of *iolT*
iolG-1400up-fo	ATGAACAAGGGAATGAAC	seq. primer of *iolG*
iolG-511-fo	ATATTTATGGACATGTCT	seq. primer of *iolG*
iolG-451-fo	CGAATTACTTCTCGTGAT	seq. primer of *iolG*
iolG-301-re	TATCTCTTCTTCATTCAA	seq. primer of *iolG*
iolG-1400down-re	GTCTAACTCAAGCATATTAGA	seq. primer of *iolG*
iolG-850bp-fo	CCGCTGGATAATGATTAA	seq. primer of *iolG*
iolG-850bp-re	GATTTTGCCTGTAATAGA	seq. primer of *iolG*
iolG-LB-XbaI-fo	GGCCAGTCTAGAATAATATTCAGCAGGAATTG	800 bp 5'-flanking region of *iolG*
iolG-LB-SalI-re	TTTCATGTCGACTCCATAATTAAATTAAATTTTGC	800 bp 5'-flanking region of *iolG*
iolG-RB-SalI-fo	AGTTGAGTCGACTATGAAACAGTTTCCCGG	800 bp 3'-flanking region of *iolG*
iolG-RB-XbaI-re	CGAGCATCTAGATCTCCTAATTTAACAAC	800 bp 5'-flanking region of *iolG*
iolT-400bp-SacI-fo	GACGATGAGCTCTTTTGTGTTCAAACGGCAGA	P_{iolT} 400 bp for pCM007
iolT-400bp-XbaI-re	TCCCTTTCTAGAGTAACTATCTGTCCCTAATG	P_{iolT} 400 bp for pCM007

iolT-BamHI-fo-comp	ACAGATGGATCCATGAACAAGGGAATGAAC	*iolT* for complementation
iolT-SalI-re-comp	TCTTTTGTCGACTTAATCCATAATTAAATTAAATTTTGC	*iolT* for complementation
iolG-BamHI-fo-comp	TATGGAGGATCCATGAAAAAGAAAATATGTCGAATTGGG	*iolG* for complementation
iolG-SalI-re-comp	TTCATAGTCGACTCAACTCAATACTACTGGCAG	*iolG* for complementation
iolT-LB-XbaI-fo	AATATGTCTAGACAATTGTCTTTGGCAGCAGA	500 bp 5'-flanking region of *iolT*
iolT-LB-SalI-re	CTGTTCGTCGACGATGTTATCACGGCCAGGTT	500 bp 5'-flanking region of *iolT*
iolT-RB-SalI-fo	TCGAATGTCGACAGTAGGTGCGGGAAGAATTG	500 bp 3'-flanking region of *iolT*
iolT-RB-XbaI-re	TCAAGGTCTAGATATTGAGGTTTTGCGCCATC	500 bp 3'-flanking region of *iolT*