Expanded View Figures

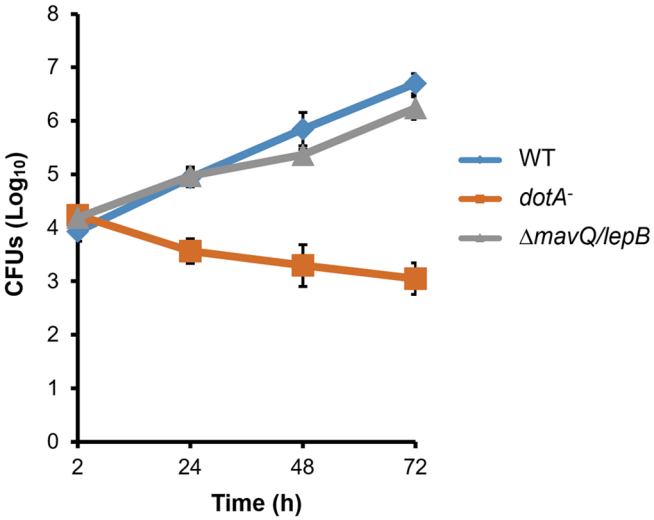

Figure EV1. The lack of *mavQ* and *lepB* does not affect the intracellular replication of *L. pneumophila*.

BMDMs infected with indicated *L. pneumophila* strains were lysed with saponin at 2, 24, 48, and 72 h post-infection. The saponin-solubilized lysates of infected cells were plated on CYE plates and grown at 37°C for 4 days to determine the CFUs. Results are shown as mean ± SD (technical replicates, $n = 3$) and are representative of three independent experiments (biological replicates, $n = 3$).

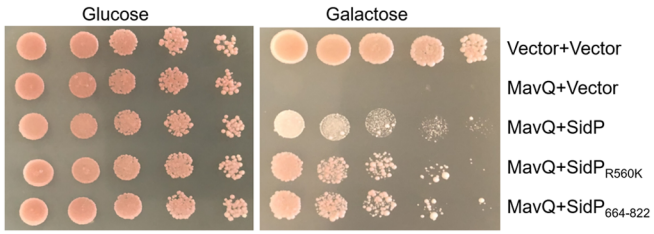

Figure EV2. SidP suppresses the yeast toxicity of MavQ.

The yeast strain expressing Flag-MavQ guided by a galactose-inducible promoter was transformed with plasmids carrying wild-type SidP, catalytically inactive mutant $SidP_{R560K,}$ or the carboxyl fragment of SidP ($SidP_{664–822}$) under the glyceraldehyde-3-phosphate dehydrogenase (GPD) promoter. Serial diluted yeast cells were spotted onto selective media plates with 2% glucose or 2% galactose. Images were taken after 3 days of incubation at 30°C. Results shown are from one representative of three independent experiments (biological replicates, $n = 3$).

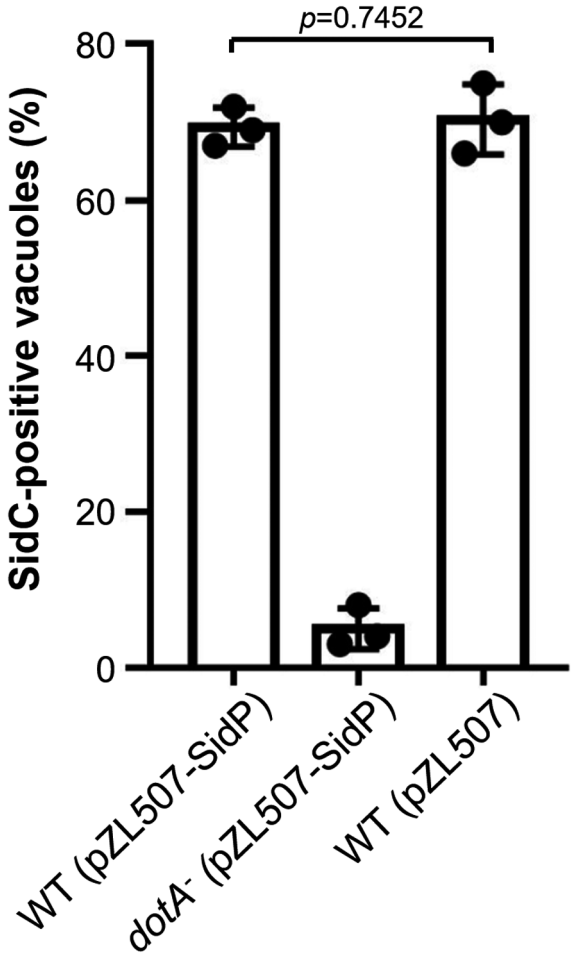

Figure EV3. Overexpression of SidP in *L. pneumophila* does not affect the association of SidC on the LCV.

BMDMs were infected with indicated *L. pneumophila* strains at an MOI of 2 for 2 h. The anchoring of SidC on the LCV was detected by immunostaining with antibody specific for SidC. At least 100 phagosomes ($n \geq 100$) were counted for each sample. Results shown are the mean ± SD from three independent experiments (biological replicates, $n = 3$). Unpaired two-tailed Student's *t*-test, $p < 0.05$ indicates significant difference.

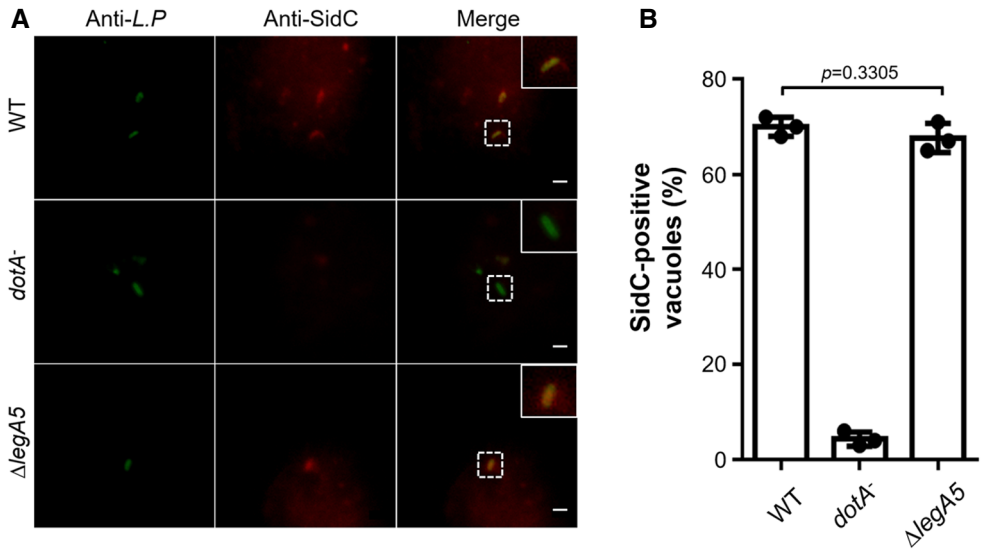

Figure EV4.

Figure EV4. LegA5 is not required for the anchoring of SidC to the LCV.

A Representative immunofluorescence images of anti-*Legionella* and anti-SidC staining of bacterial phagosomes. BMDMs were infected with the indicated *L. pneumophila* stains at an MOI of 2 for 2 h. SidC association on the bacterial phagosomes was detected as described in Fig 5. Insets represent 3× magnification of regions defined by dash lines. Scale bar, 5 μm.

B Percentages of SidC-positive LCVs. At least 100 phagosomes ($n \geq 100$) were scored for each sample. Results are shown as mean ± SD of three independent experiments (biological replicates, $n = 3$). Unpaired two-tailed Student's *t*-test, $p < 0.05$ indicates significant difference.

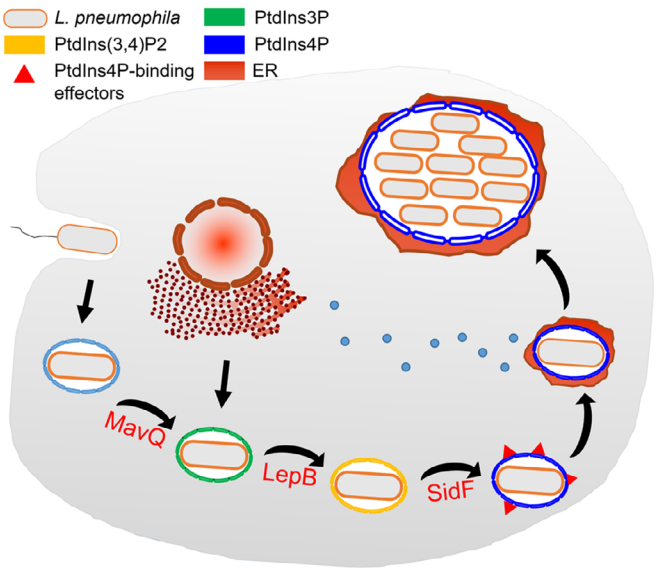

Figure EV5. A model for *de novo* biosynthesis of PtdIns4P from PtdIns by MavQ, LepB, and SidF on the phagosome membrane.

Once being internalized by phagocytosis, *L. pneumophila* resides in the LCV that has intimate communication with ER-derived vesicles. The LCV develops into a PtdIns4P enriched compartment which is permissive for bacterial replication. At least three Dot/Icm effector proteins have been established to contribute to the PI metabolism on the LCV. MavQ catalyzes the conversion of PtdIns into PtdIns3P, which is then phosphorylated by the PI4K LepB to yield PtdIns(3,4)P2; the PI 3-phosphatase SidF removes the 3-phosphate from PtdIns(3,4)P2 to generate PtdIns4P. The accumulation of PtdIns4P is critical for the specific anchoring of Dot/Icm effectors on the LCV, such as SidM/DrrA, SidC, SdcA, Lpg1101, and Lpg2603.