





В







Lp02 Lp02∆*lpg2644* pGFP pGFP

| Code | Primer | Amplification | Sequence 5' to 3' |
|------|------------------|---------------|--|
| | | target | |
| 1 | <i>lpg2644</i> F | lpg2644 | GAAATAAAGAATGATACATCGA |
| 2 | <i>lpg2644</i> R | | GCAAAGCGAATTTATGAACA |
| 3 | lpg2644 | | TGCGTA <u>TCATGA</u> TACATCGAAATAAAGTC |
| | Bsph1 F | | |
| 4 | lpg2644 | | GTTA <u>CGAATTC</u> TTAAAAGGCTCTTACAGCAC |
| | EcoR1 R | | |

Figure S1. Lcl promotes divalent cation dependent sedimentation in *E.coli* and that is inhibited by fucoidan. (A) Sedimentation kinetics of *E.coli* plcl in deionized water in the presence of 1000μ M, 500μ M and 100μ M MgCl₂. (B) Sedimentation of *E. coli* plcl in deionized water with 1000μ M MgCl₂ in the absence or presence of 0.25 mg/ml fucoidan.

Figure S2. Lcl does not influence intracellular replication rate of *L. pneumophila* in *A. castellanii*. Infection of *A. castellanii* by Lp02 pGFP, Lp02 Δ *lpg2644* pGFP and Lp02 Δ *lpg2644 clpg2644* pGFP in deionized water in the absence or presence of 500µM MgCl₂ measured by microscopy (A) after centrifugation (grey bars) and (C) in non-centrifuged conditions (white bars). Infection of *H. vermiformis* in deionized water in the absence or presence of 500µM MgCl₂ measured by flow cytometry (B) after centrifugation (grey bars) and (D) in noncentrifuged conditions (white bars). Release of Lp02 pGFP (black diamonds), Lp02 Δ *lpg2644* pGFP (grey squares) and Lp02 Δ *lpg2644 clpg2644* pGFP (white triangles) into the supernatant measured by flow cytometry during *A. castellanii* infections performed in (E) deionized water and in (F) deionized water with 500µM MgCl₂. (G) Quantification of the number of *L. pneumophila* per infected *A. castellanii* in non-centrifuged conditions in the absence or presence of 500µM MgCl₂ water in deionized water.

Figure S3. Fucoidan inhibits *L. pneumophila* sedimentation in a dose dependent manner.

Sedimentation kinetics of Lp02 in deionized water 500μ M MgCl₂ with the addition of 0, $2.5X10^{-5}$, $2.5X10^{-4}$ and $2.5X10^{-3}$ mg /ml fucoidan.

Figure S4. Deletion of Lcl does not impact flagella expression. Anti-FlaA immunoblots with

cell lysate from Lp02 pGFP and Lp02 $\Delta lpg2644$ pGFP.

 Table S1. Primers used in this study