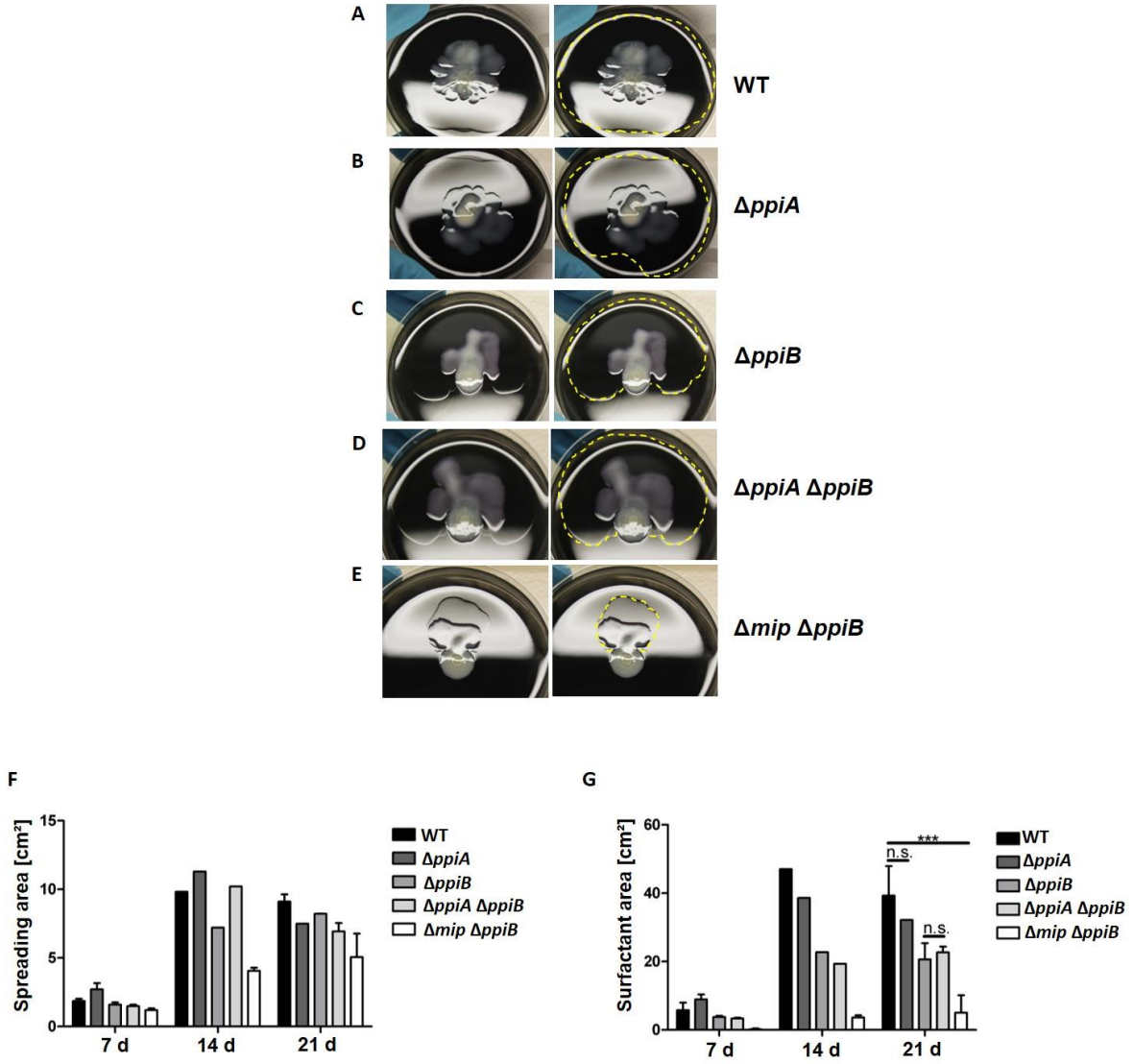
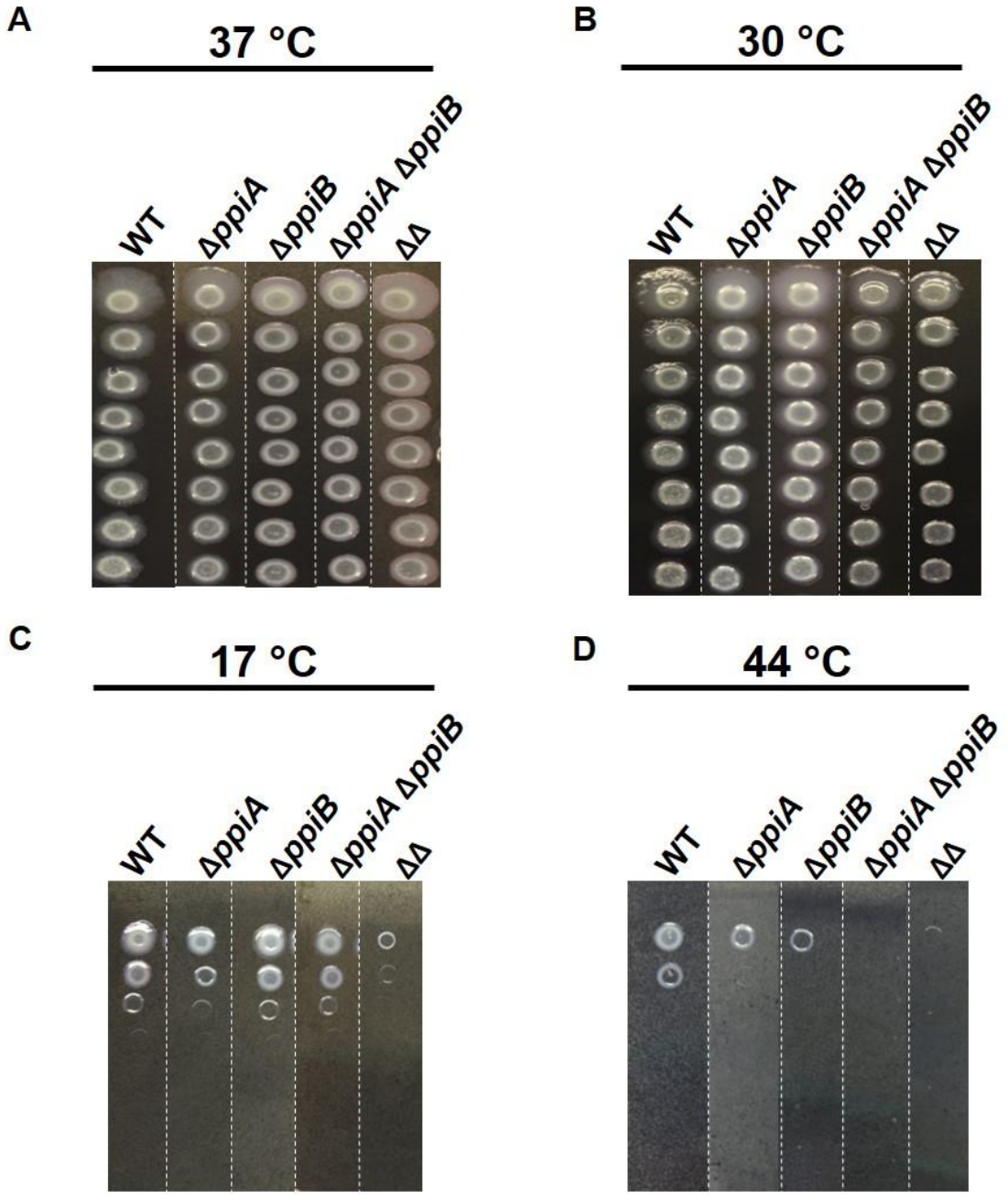
Supplementary Information

Supp. Fig. 1



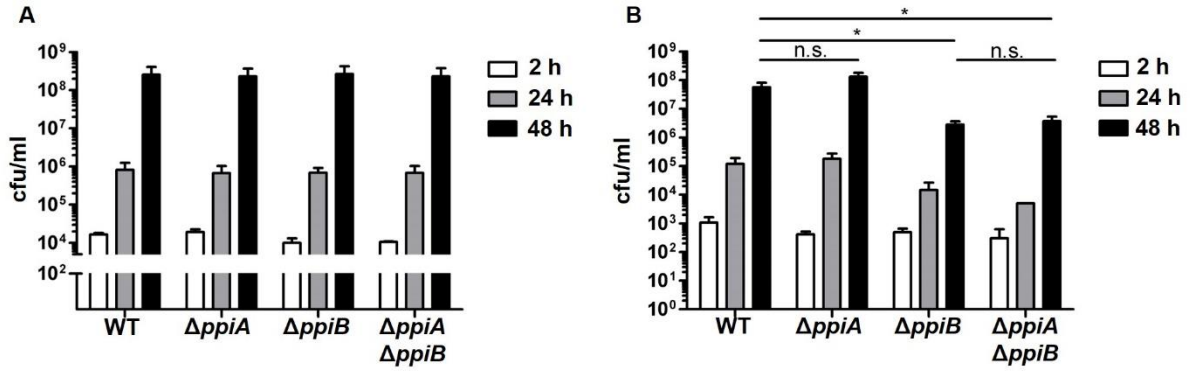
Supp. Fig. 1: PpiA has no influence on sliding motility of *L. pneumophila*. When grown on BCYE medium with 0.5 % (w/v) agar for 21 d at 30 °C no difference in the size of the surfactant film could be observed between the wild type strain **(A)** and its isogenic *ppiA*-deficient mutant **(B)**. The Δ*ppiB* **(C)** and the Δ*ppiA* Δ*ppiB* double mutant **(D)** produced comparably less surfactant, while there was no significant difference between both strains. The Δ*mip* Δ*ppiB* double mutant **(E)** produced a significantly smaller surfactant film. **(F)** The spreading area over the 21 d was not significantly different between the strains. **(G)** Quantitative evaluation of the surfactant area of wild the *L. pneumophila* and its PPIase mutants. Shown are mean and standard deviations of three independent experiments. Statistical significance was calculated using unpaired Student's *t*-test (***p≤0.001, n.s.=not significant).

Supp. Fig. 2



Supp. Fig. 2: Growth of different the *L. pneumophila* PpiA-negative mutant at suboptimal temperatures. No growth defect could be observed in the PpiA-deficient mutant at **(A)** 37 °C, **(B)** 30 °C or **(C)** 17 °C. **(D)** The mutant lacking both PpiA and PpiB was affected by heat stress similar to the Mip and PpiB deficient double mutant (ΔΔ). Representative sections of bacterial plates of three separate experiments are shown and aligned for a better comparison of the strains. Single sections originating from different plates are separated by white dashed lines.

Supp. Fig. 3



Supp. Fig. 3: PpiA has no influence on intracellular replication in *A. castellanii* or human machrophage-like THP-1 cells. (A) When *A. castellanii* was infected with wild type *L. pneumophila* Corby or its isogenic *ppiA* or *ppiB* single or double deletion mutants no significant differences could be observed in the outcome of the infections. **(B)** In THP-1 cells the Δ*ppiB* mutant is attenuated as it reaches significantly lower cfu counts in 48 h. In contrast, the Δ*ppiA* mutant reveals no significant difference compared to wild type. Similarly, the intracellular replication of the Δ*ppiB* and the Δ*ppiA* Δ*ppiB* double mutant do not significantly differ. Shown are the mean and standard deviations of three independent experiments performed in duplicates. Statistical significance was calculated using unpaired Student's *t*-test (*p≤0.05, n.s.=not significant).