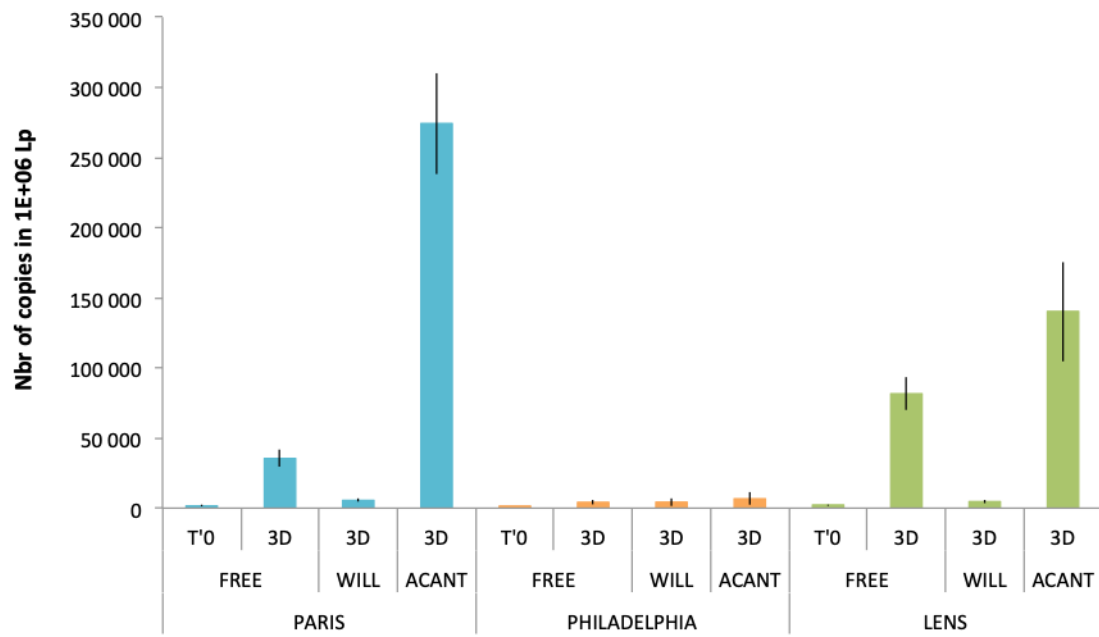


| Short-term exposure: 3-6 h for CuO-NPs and 4 h for Gw   |                         |                         |
|---|-------------------------|-------------------------|
| Gene  | CuO                     | Gw                      |
| <i>cegC1</i>  | x10 (high)              | no change               |
| <i>csrA</i>   | no change               | x40                     |
| <i>dotA</i>   | x20 (high)              | <i>not investigated</i> |
| <i>enhC</i>   | high                    | <i>not investigated</i> |
| <i>htpX</i>   | low                     | <i>not investigated</i> |
| <i>icmE</i>   | moderate                | <i>not investigated</i> |
| <i>icmV</i>   | x16 (high)              | <i>not investigated</i> |
| <i>icmW</i>   | high                    | <i>not investigated</i> |
| <i>lepA</i>   | moderate                | <i>not investigated</i> |
| <i>lolA</i>   | <i>not investigated</i> | x6                      |
| <i>pla</i>  | <i>not investigated</i> | x7                      |
| <i>pvcA</i>   | high                    | <i>not investigated</i> |
| <i>sidF</i>   | high                    | x25                     |
| <i>umuD</i>   | moderate                | <i>not investigated</i> |
| Long-term exposure: 24 h for CuO-NPs and 24-48 h for Gw |                         |                         |
| Gene  | CuO                     | Gw                      |
| <i>ccmF</i>   | <i>not investigated</i> | x5                      |
| <i>cegC1</i>  | x10 (high)              | no change               |
| <i>dotA</i>   | x20 (high)              | <i>not investigated</i> |
| <i>enhC</i>   | high                    | <i>not investigated</i> |
| <i>gacA</i>   | <i>not investigated</i> | x2.5                    |
| <i>htpX</i>   | low                     | <i>not investigated</i> |
| <i>icmE</i>   | moderte                 | <i>not investigated</i> |
| <i>lirR</i>   | <i>not investigated</i> | x15                     |
| <i>lvrB</i>   | <i>not investigated</i> | x65                     |
| <i>lvrE</i>   | <i>not investigated</i> | x15                     |
| <i>pvcA</i>   | high                    | <i>not investigated</i> |
| <i>sidF</i>   | high                    | no change               |
| <i>tatB</i>   | <i>not investigated</i> | x3.5                    |

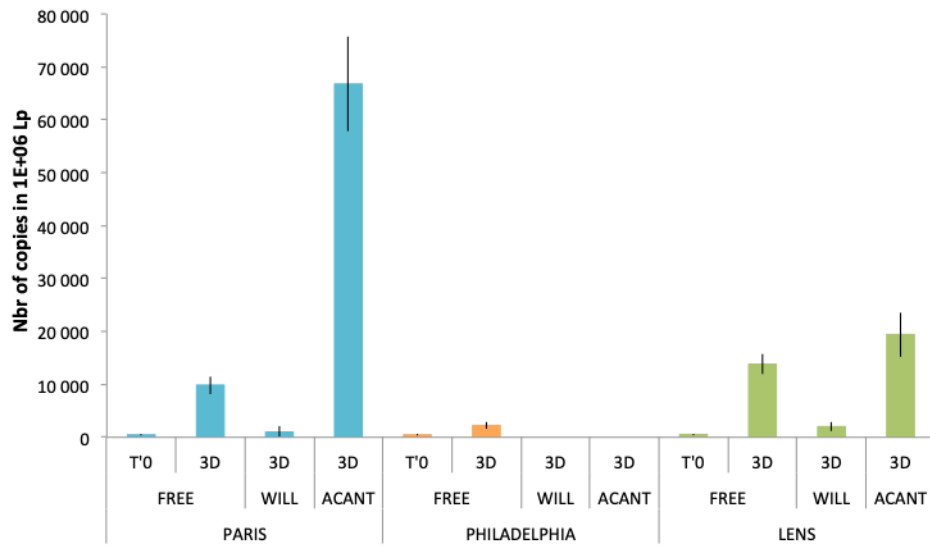
**Table S1:** Fold changes of gene expression after 3-48h exposure of *L. pneumophila* Philadelphia to CuO nanoparticles (CuO-NPs) or synthetic Gray water (Gw). Note that changes in *sidF* were only transient in GW, and that *csrA* and *cegC1* were not modified after exposure to CuO-NPs and Gw, respectively. Adapted from Lu et al., 2013 and Buse et al., 2015 [18-19].



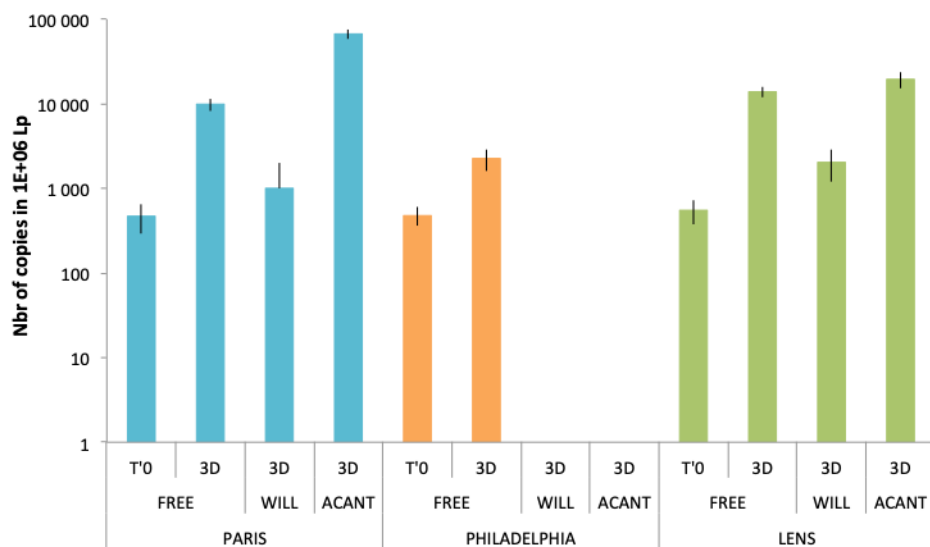
**Figure S1:** Level of *rspL* transcript in the different conditions, expressed as the number of copies in  $10^6$  *L. pneumophila* (Lp)  $\pm$  SD. FREE: *L. pneumophila* strains alone; WILL: *L. pneumophila* strains cocultured with *W. magna* C2c Maky; ACANTH: *L. pneumophila* strains cocultured with *A. castellanii*; T'0: reference transcript level ; 3D: transcript level after 3 days.

## A – Linear scale

**ccmF**



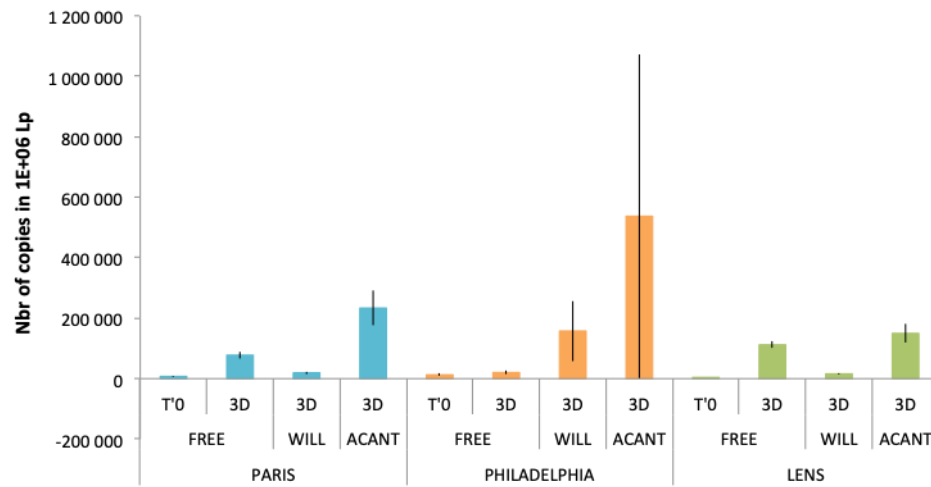
## B – Logarithmic scale



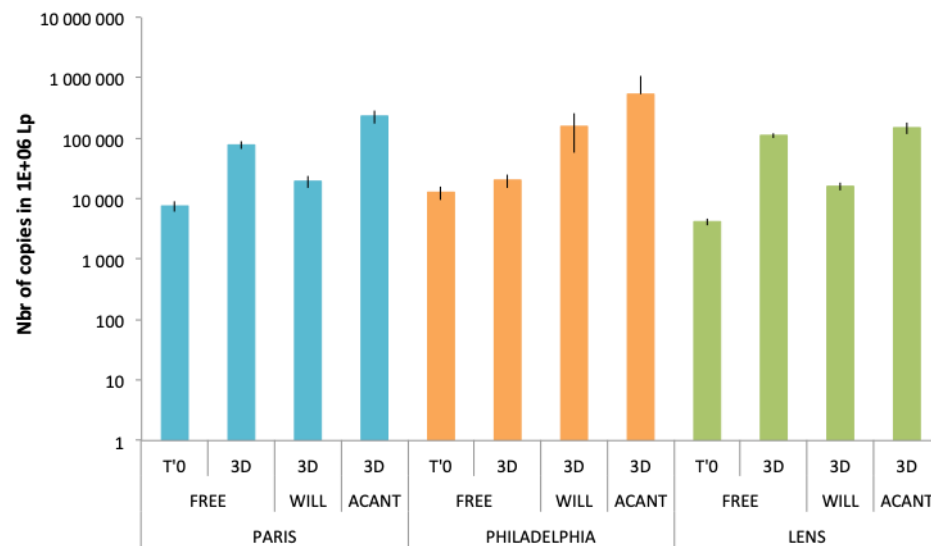
**Figure S2:** *ccmF* cDNA copy number measured in  $10^6$  *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean  $\pm$  SD ( $n=6$ , with  $n=3$  in each of the two replicates).

## A – Linear scale

***gacA***



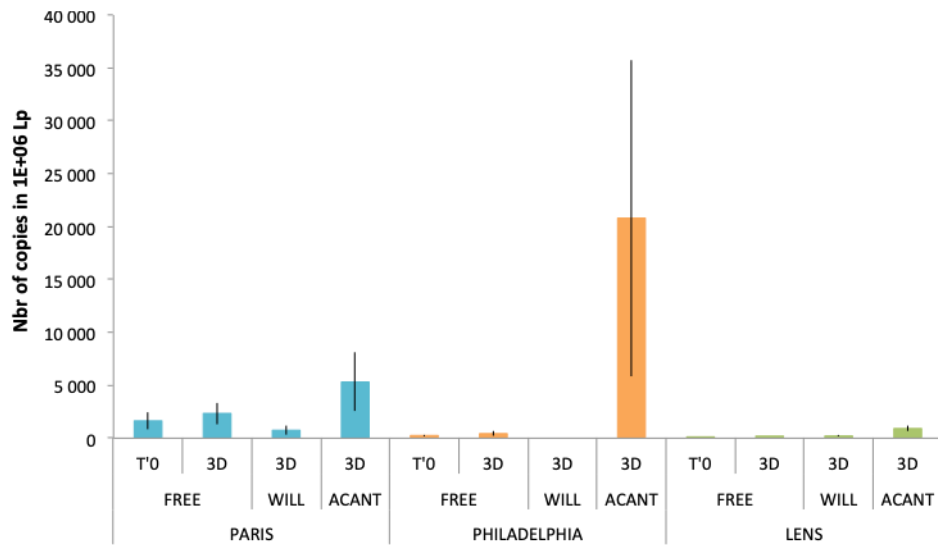
## B – Logarithmic scale



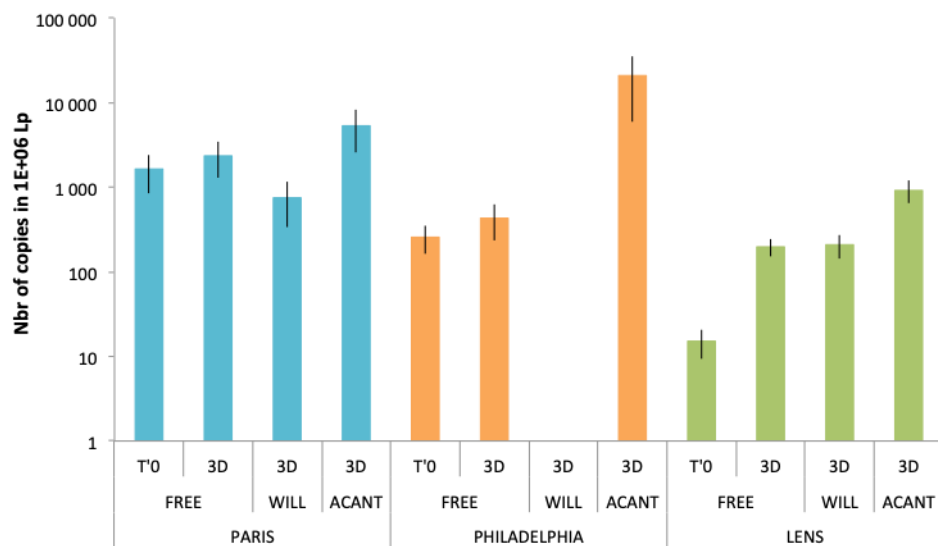
**Figure S3:** *gacA* cDNA copy number measured in  $10^6$  *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean  $\pm$  SD ( $n=6$ , with  $n=3$  in each of the two replicates).

# htpX

## A – Linear scale

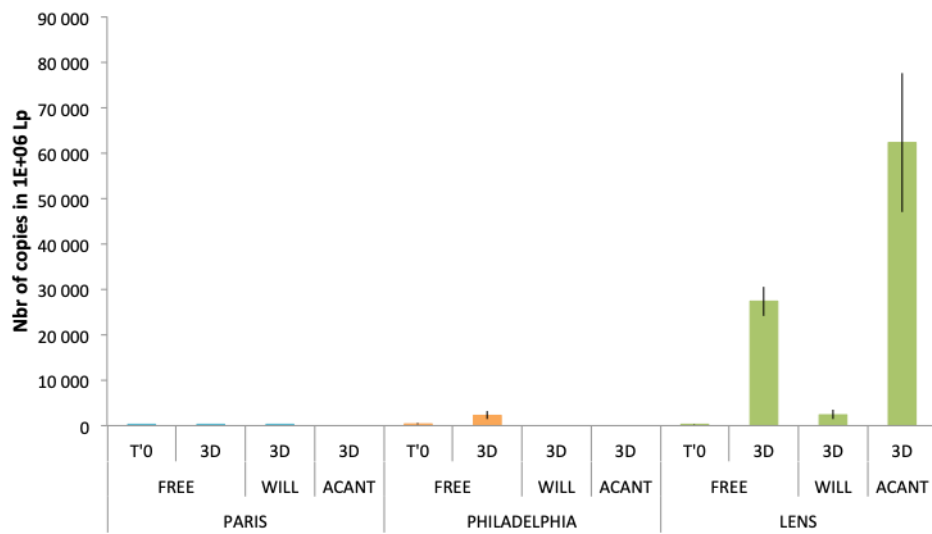


## B – Logarithmic scale

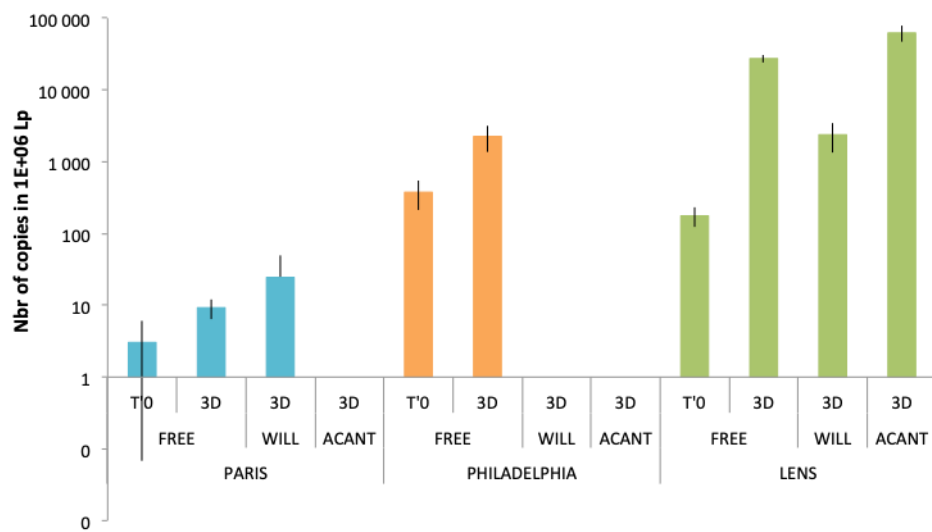


**Figure S4:** *htpX* cDNA copy number measured in  $10^6$  *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean  $\pm$  SD ( $n=6$ , with  $n=3$  in each of the two replicates).

## A – Linear scale



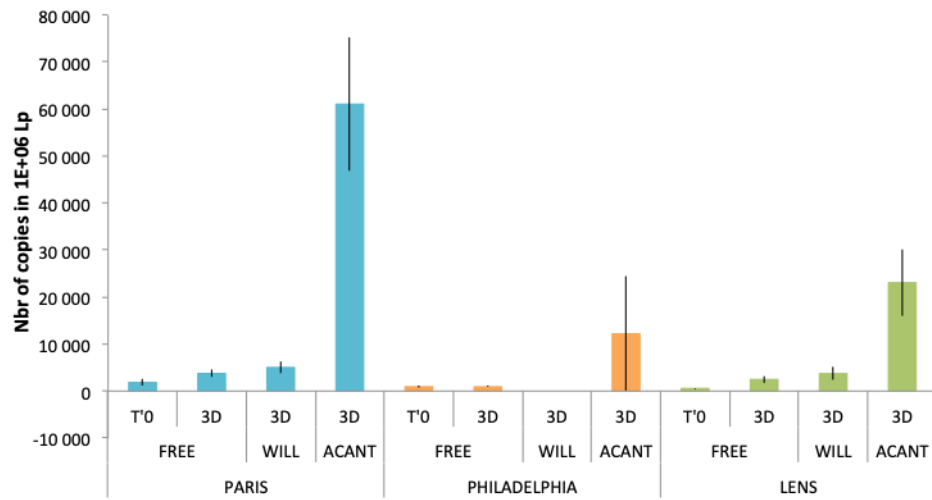
## B – Logarithmic scale



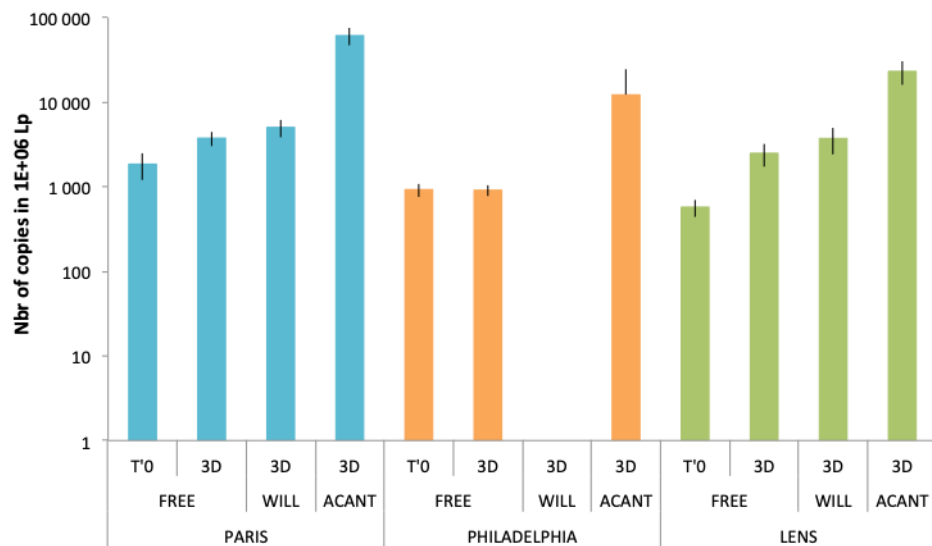
**Figure S5:** *icmE* cDNA copy number measured in  $10^6$  *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean  $\pm$  SD (n=6, with n=3 in each of the two replicates).

# lirR

## A – Linear scale



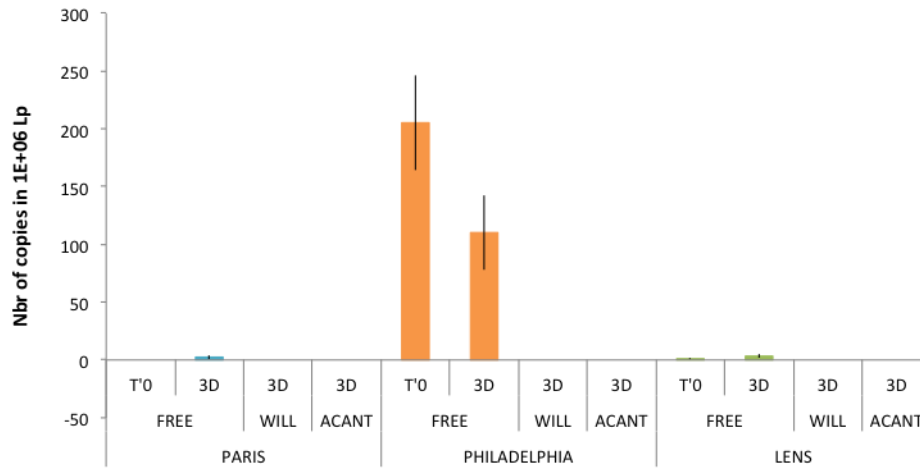
## B – Logarithmic scale



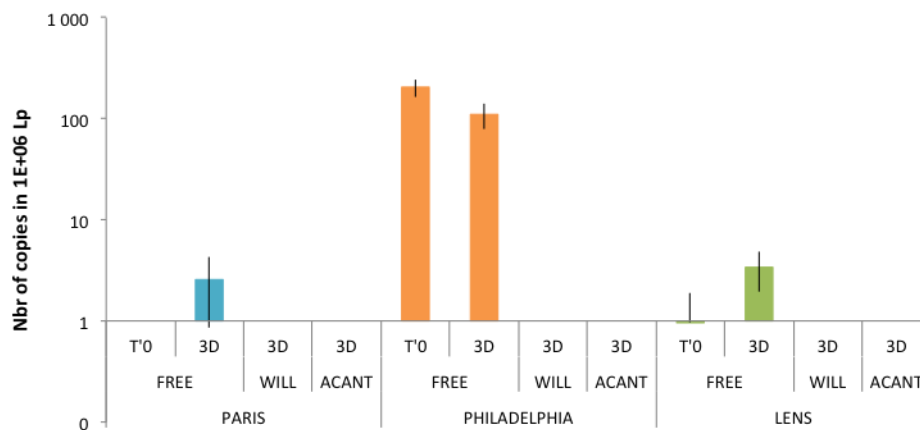
**Figure S6:** *lirR* cDNA copy number measured in  $10^6$  *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean  $\pm$  SD ( $n=6$ , with  $n=3$  in each of the two replicates).

# IvrE

## A – Linear scale



## B – Logarithmic scale

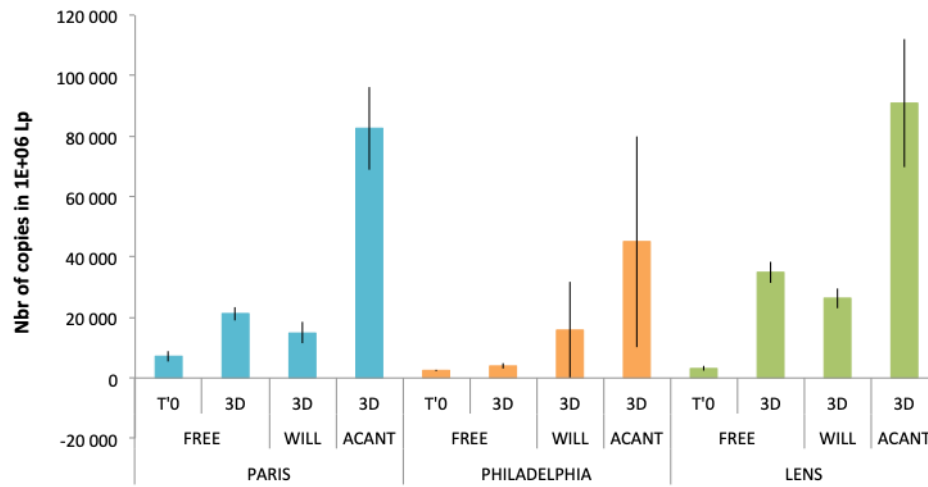


**Figure S7:** *IvrE* cDNA copy number measured in  $10^6$  *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean  $\pm$  SD ( $n=6$ , with  $n=3$  in each of the two replicates).

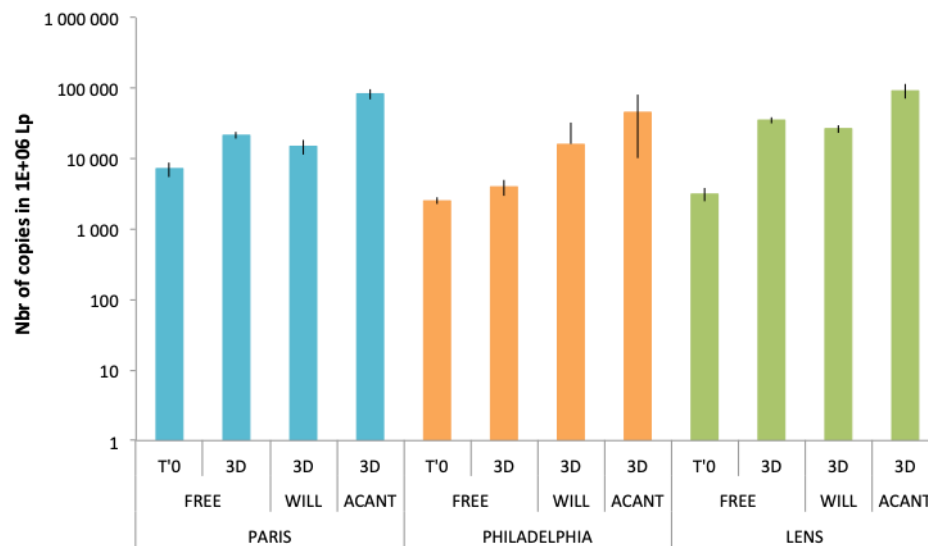


## A – Linear scale

**tatB**

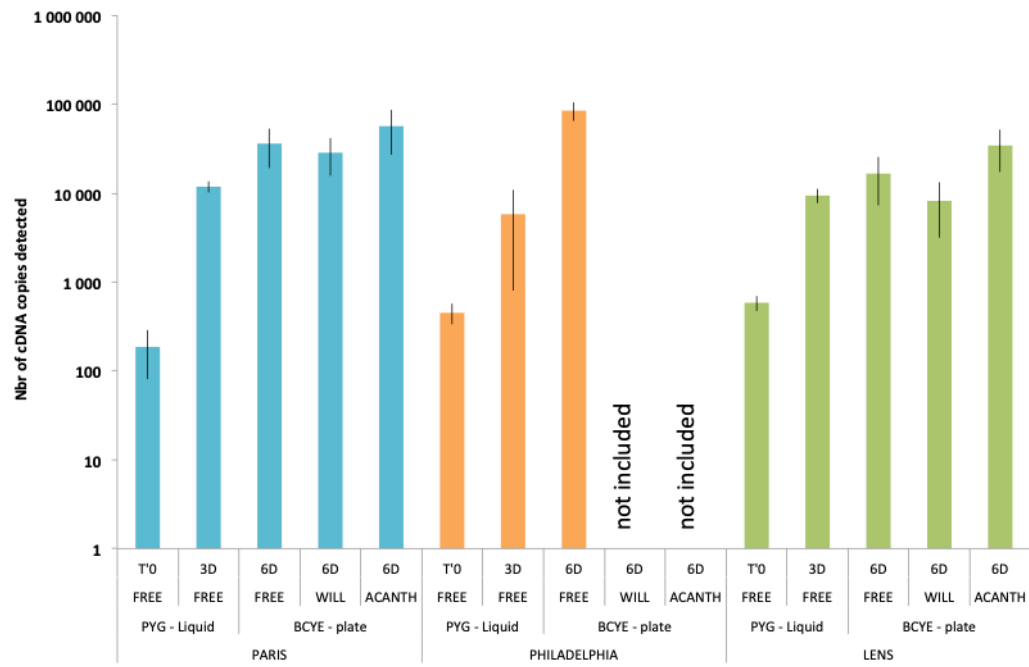


## B – Logarithmic scale

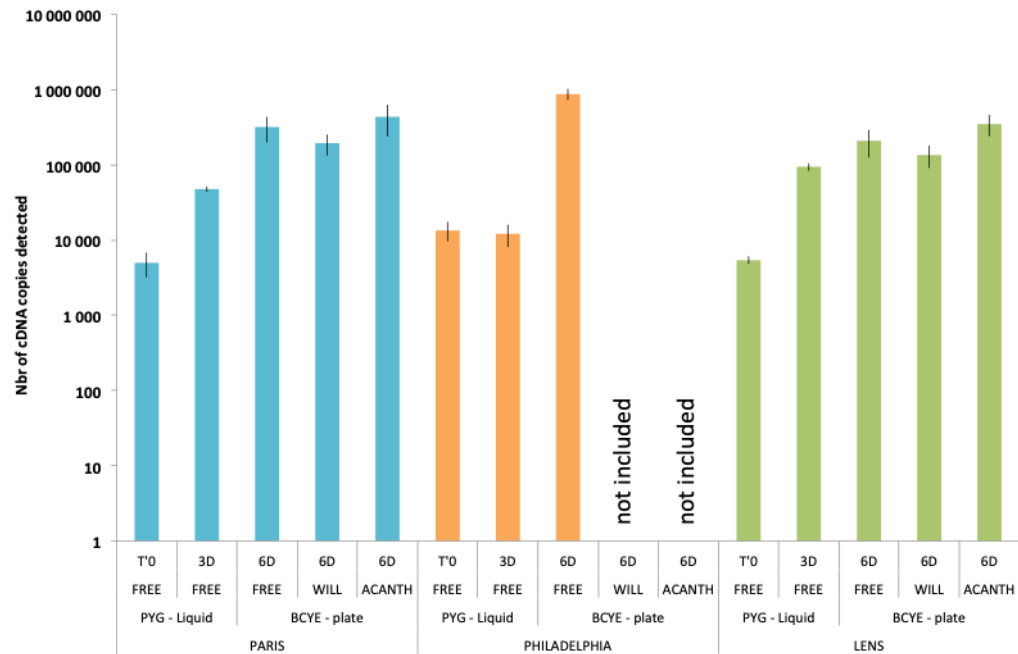


**Figure S8:** *tatB* cDNA copy number measured in  $10^6$  *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean  $\pm$  SD ( $n=6$ , with  $n=3$  in each of the two replicates).

## A – *ccmF*

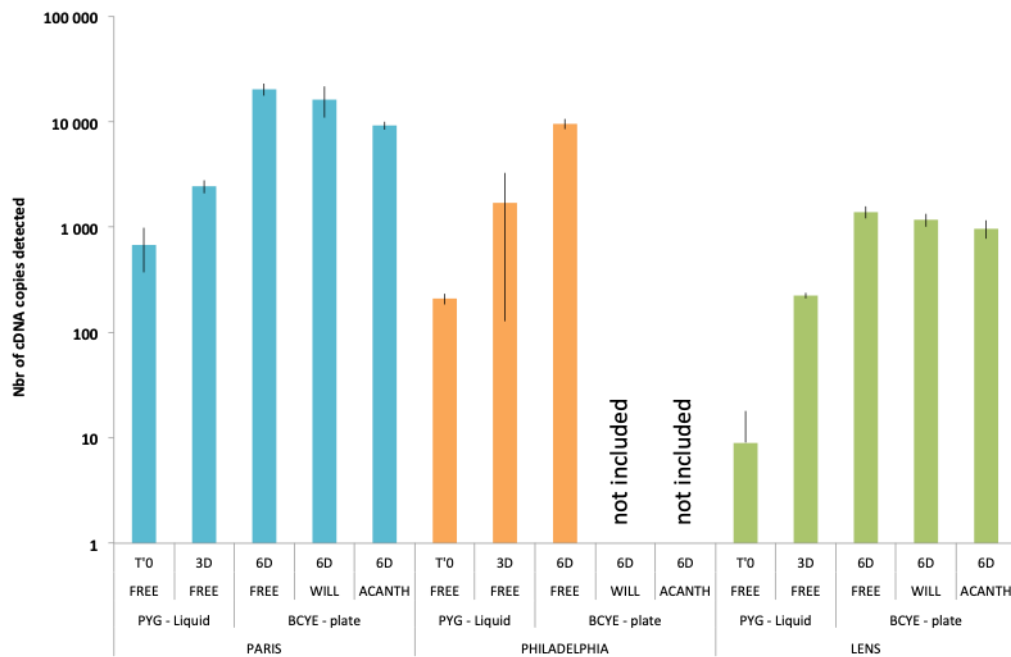


## B – *gacA*

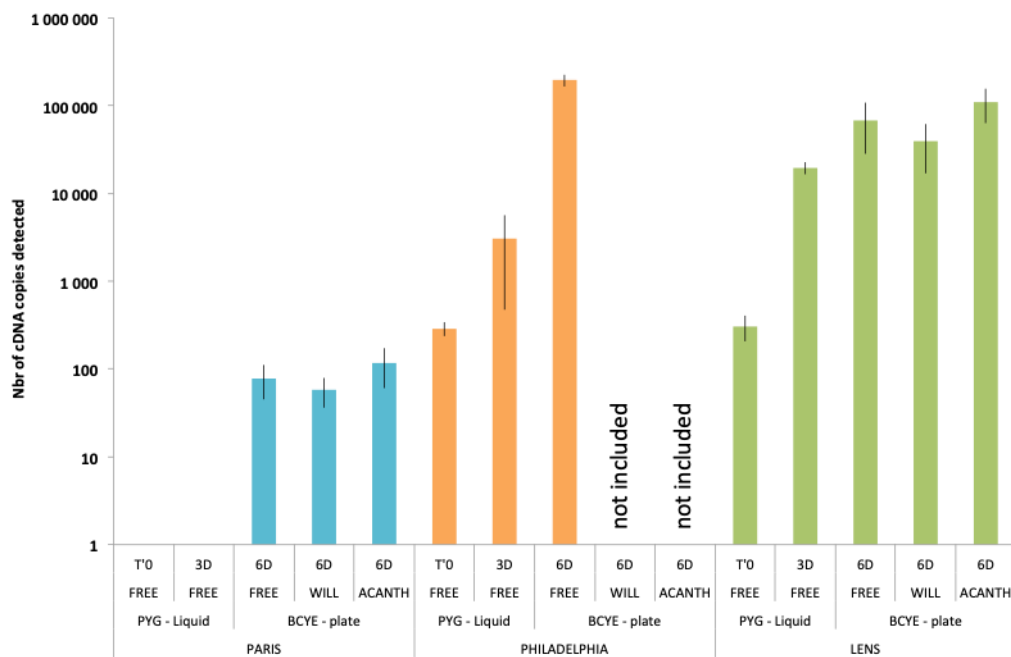


**Figure S9:** *ccmF* and *gacA* cDNA copy number measured in  $10^6$  *L. pneumophila* for each strain and each culture condition (see Figure S14 for details). A: *ccmF* gene; B: *gacA* gene. Results are expressed as the mean  $\pm$  SD (n=3).

## A – hptX

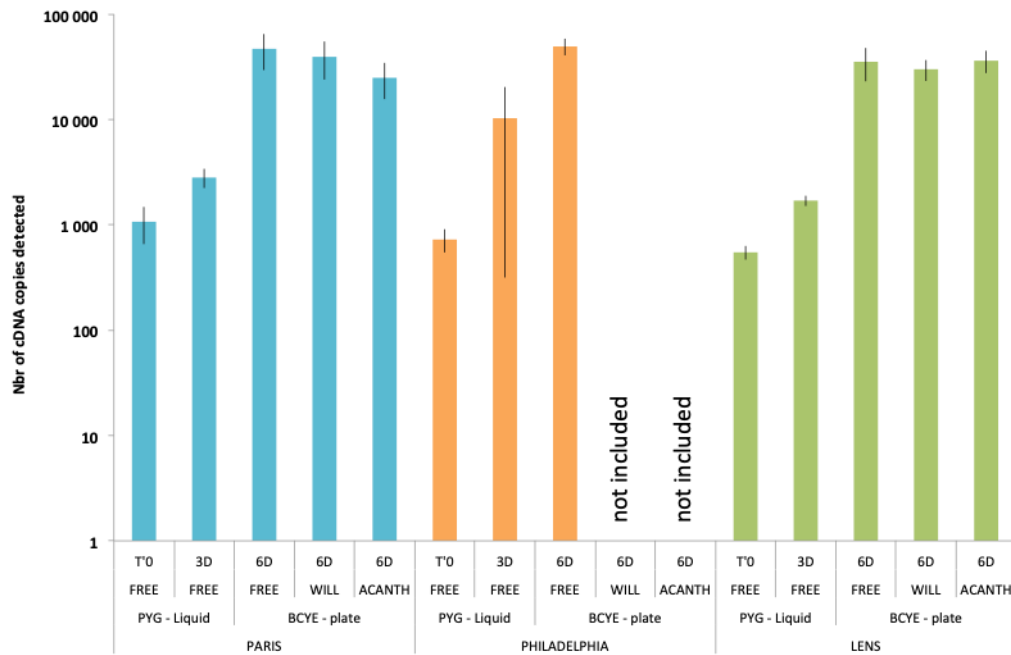


## B – icmE

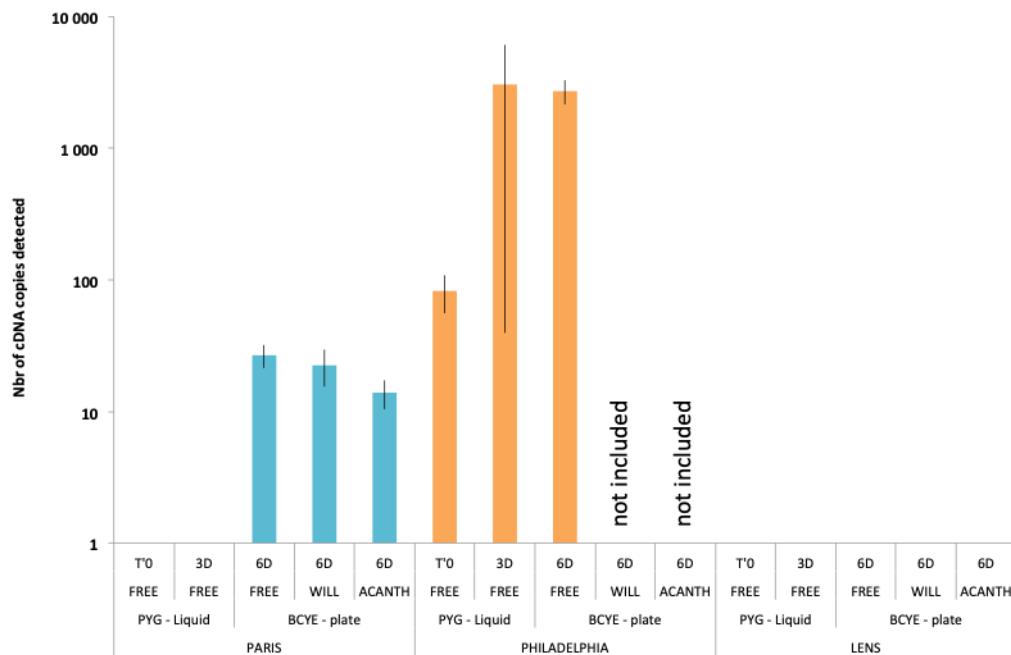


**Figure S10:** *hptX* and *icmE* cDNA copy number measured in  $10^6$  *L. pneumophila* for each strain and each culture condition (see Figure S14 for details). A: *hptX* gene; B: *icmE* gene. Results are expressed as the mean  $\pm$  SD (n=3).

## A – *lirR*

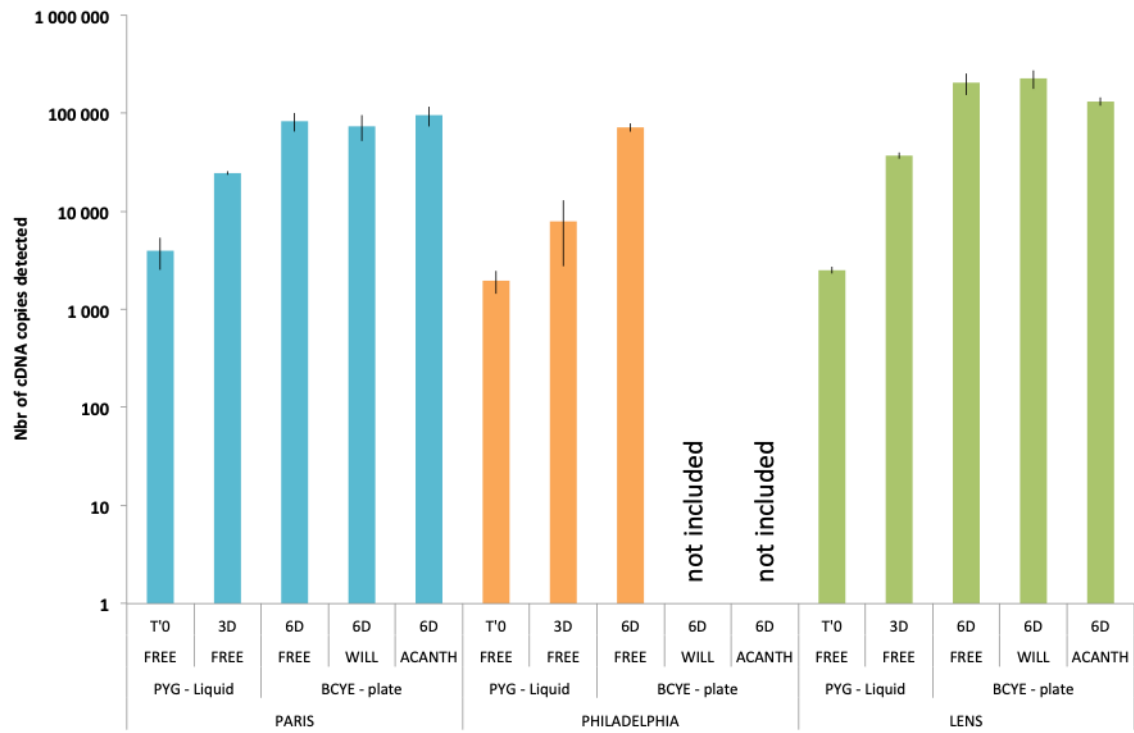


## B – *lvrE*

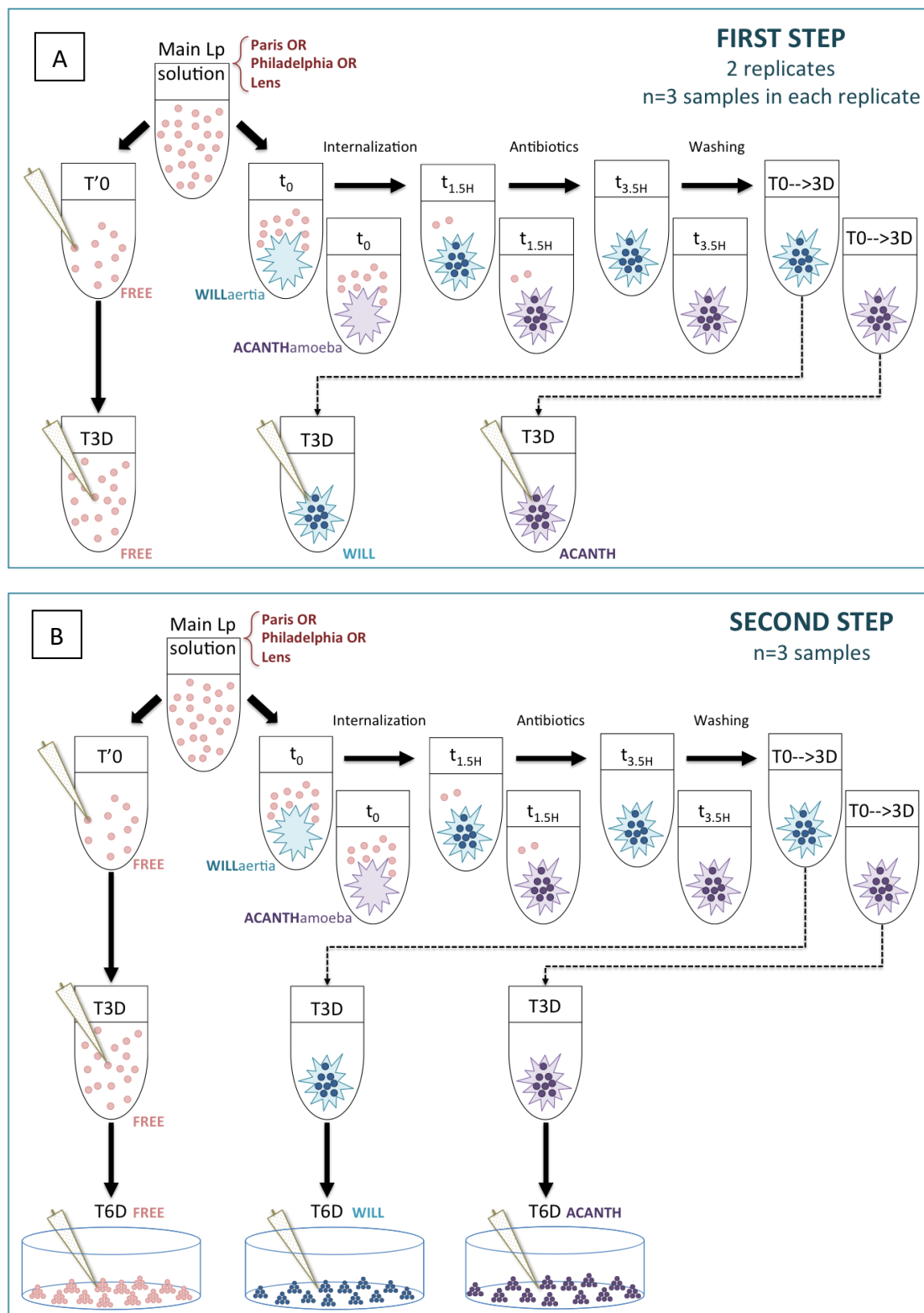


**Figure S11:** *lirR* and *lvrE* cDNA copy number measured in  $10^6$  *L. pneumophila* for each strain and each culture condition (see Figure S14 for details). A: *lirR* gene; B: *lvrE* gene. Results are expressed as the mean  $\pm$  SD (n=3).

## tatB



**Figure S12:** *tatB* cDNA copy number measured in  $10^6$  *L. pneumophila* for each strain and each culture condition (see Figure S14 for details). Results are expressed as the mean  $\pm$  SD (n=3).



**Figure S13:** Synoptic diagram of the experiments. A: first step: for the cocultures, after a 1.5h of contact, extracellular *L. pneumophila* were eliminated by antibiotic action for 2h, then the cocultures were rinsed and incubated for 3 days; B: second step: after the 3-day incubation, coculture were spread on BCYE plates and incubated for 3 additional days. Abbreviations: FREE: *L. pneumophila* strains alone; WILL: *W. magna* C2c Maky; ACANTH: *A. castellanii*; T'0: reference sample; T3D: samples collected after 3 days; T6D: samples collected after 6 days, 3 days in liquid medium and 3 days on BCYE plates.