

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>	moderate	<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].

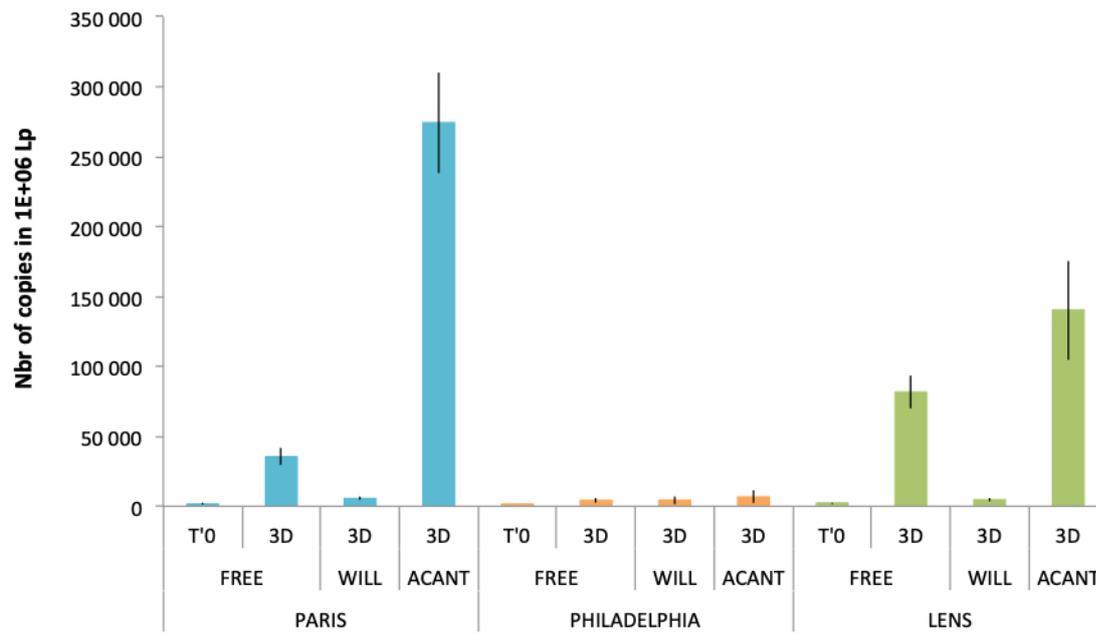
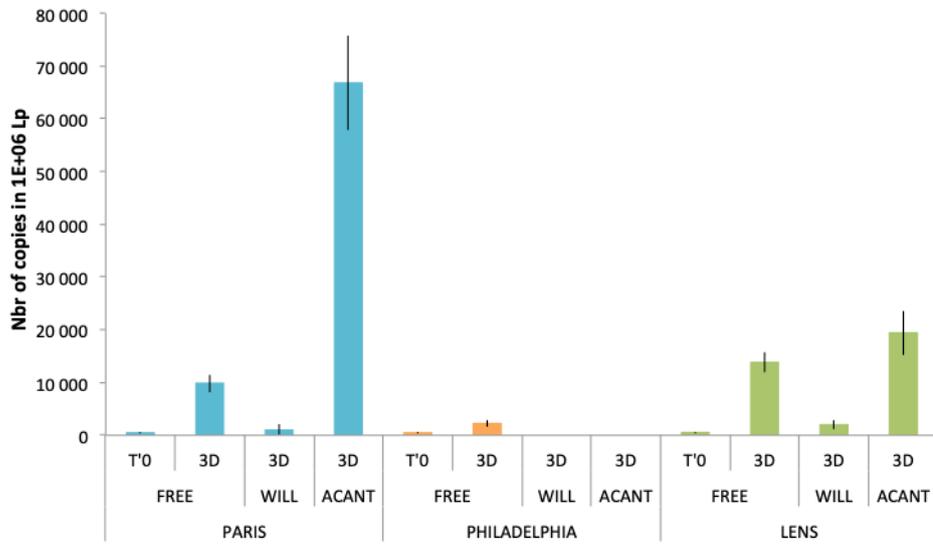


Figure S1: Level of *rspL* transcript in the different conditions, expressed as the number of copies in 10^6 *L. pneumophila* (Lp) \pm SD. FREE: *L. pneumophila* strains alone; WILL: *L. pneumophila* strains cocultured with *W. magna* C2c Maky; ACANTH: *L. pneumophila* strains cocultured with *A. castellanii*;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

ccmF

A – Linear scale



B – Logarithmic scale

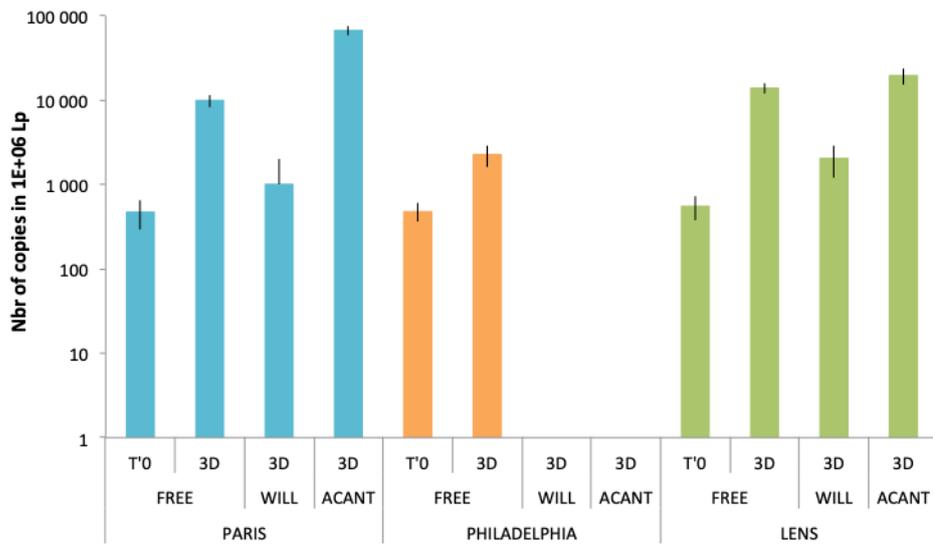
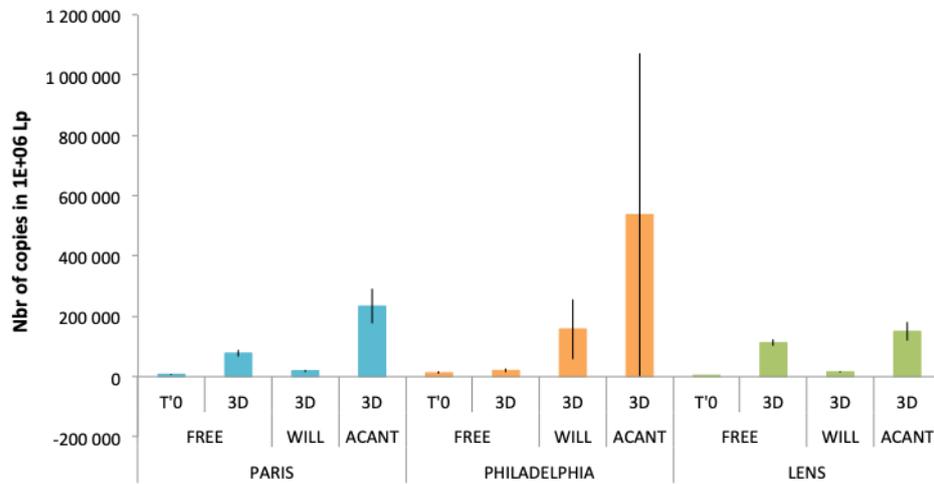


Figure S2: *ccmF* cDNA copy number measured in 10^6 *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean \pm SD ($n=6$, with $n=3$ in each of the two replicates).

gacA

A – Linear scale



B – Logarithmic scale

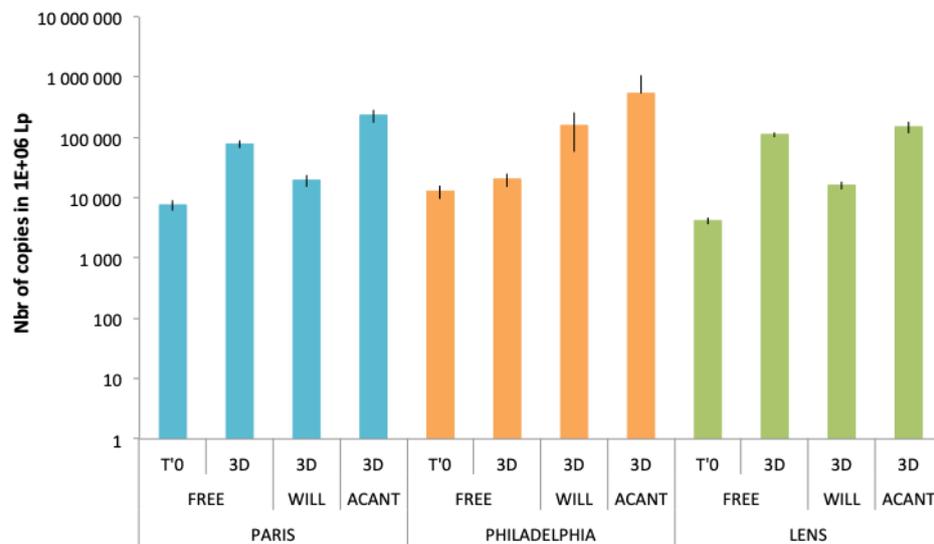
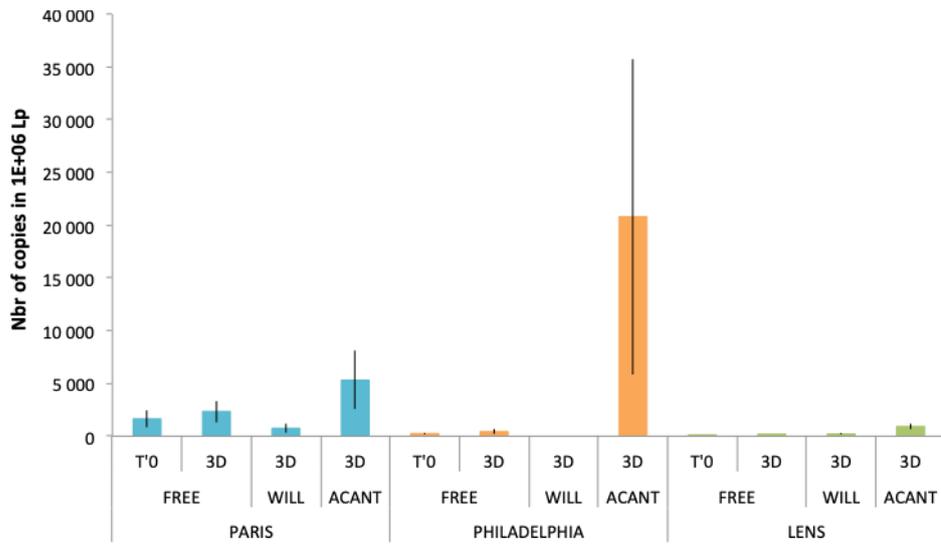


Figure S3: *gacA* cDNA copy number measured in 10^6 *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean \pm SD ($n=6$, with $n=3$ in each of the two replicates).

htpX

A – Linear scale



B – Logarithmic scale

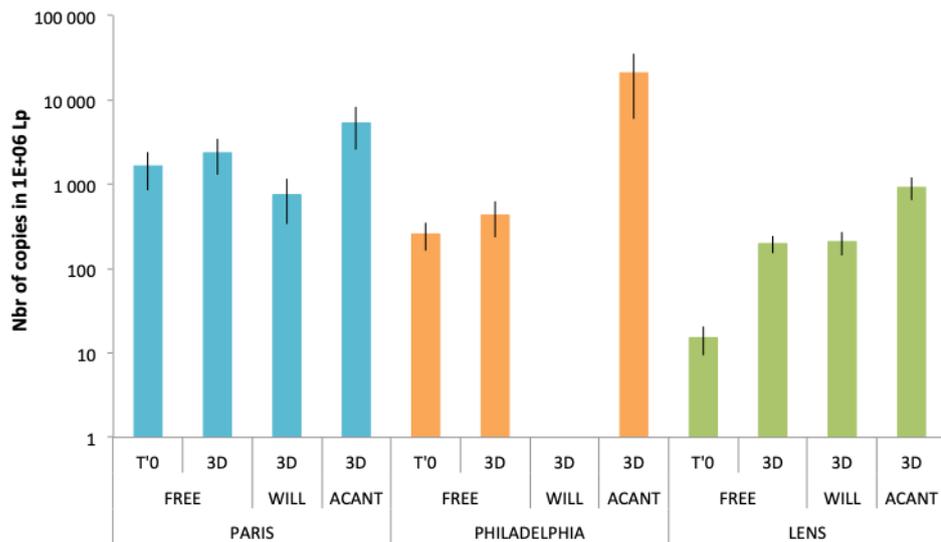
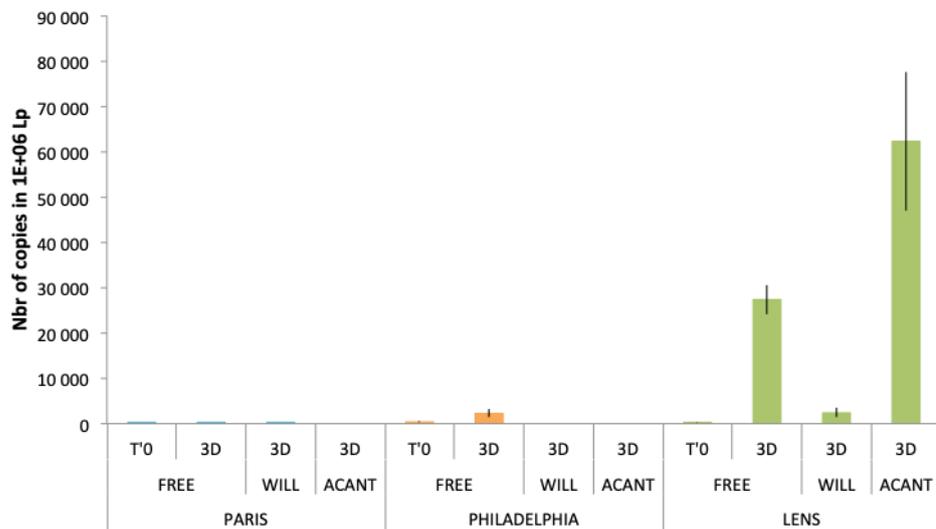


Figure S4: *htpX* cDNA copy number measured in 10^6 *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean \pm SD ($n=6$, with $n=3$ in each of the two replicates).

A – Linear scale

icmE



B – Logarithmic scale

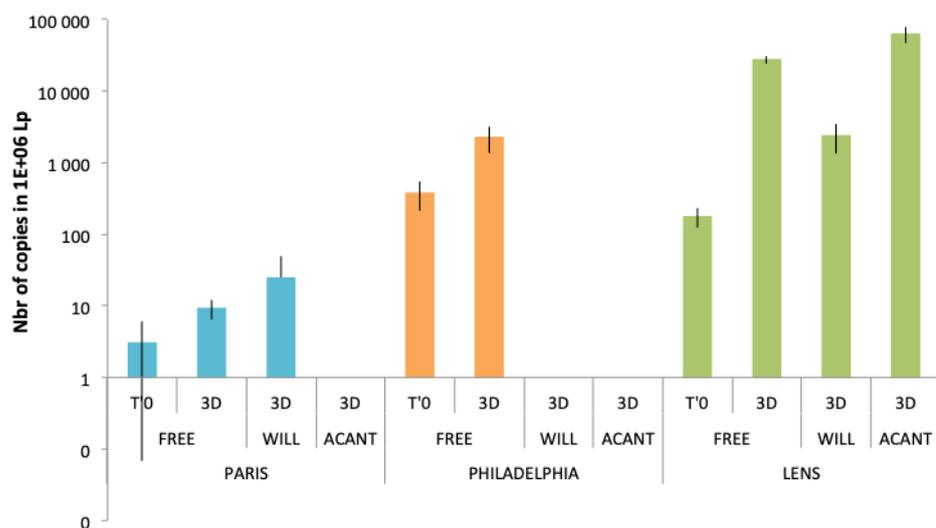
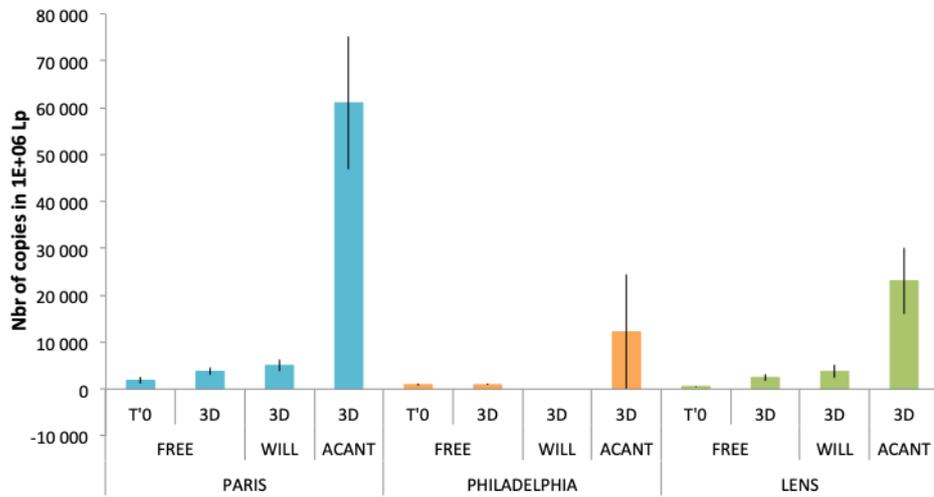


Figure S5: *icmE* cDNA copy number measured in 10^6 *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean \pm SD (n=6, with n=3 in each of the two replicates).

lirR

A – Linear scale



B – Logarithmic scale

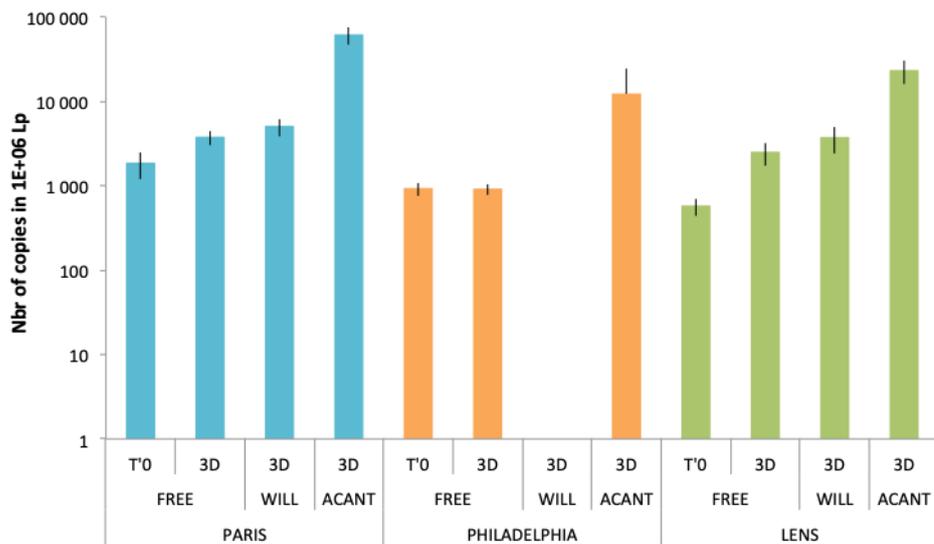
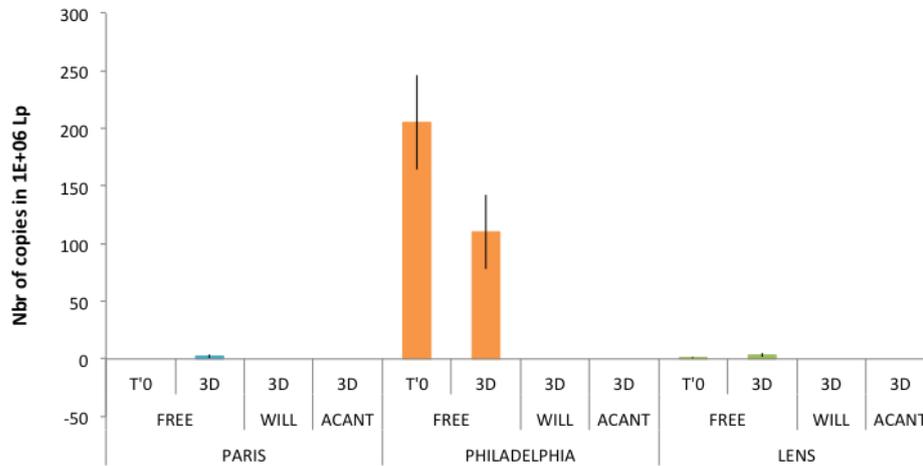


Figure S6: *lirR* cDNA copy number measured in 10^6 *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean \pm SD ($n=6$, with $n=3$ in each of the two replicates).

IvrE

A – Linear scale



B – Logarithmic scale

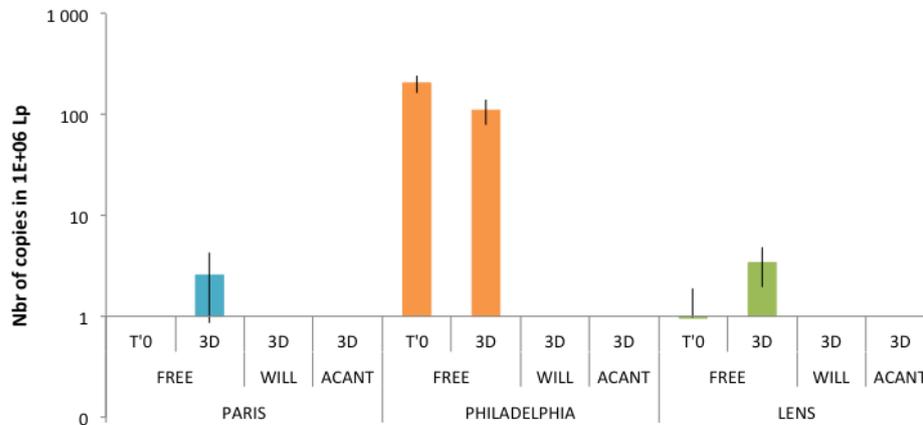
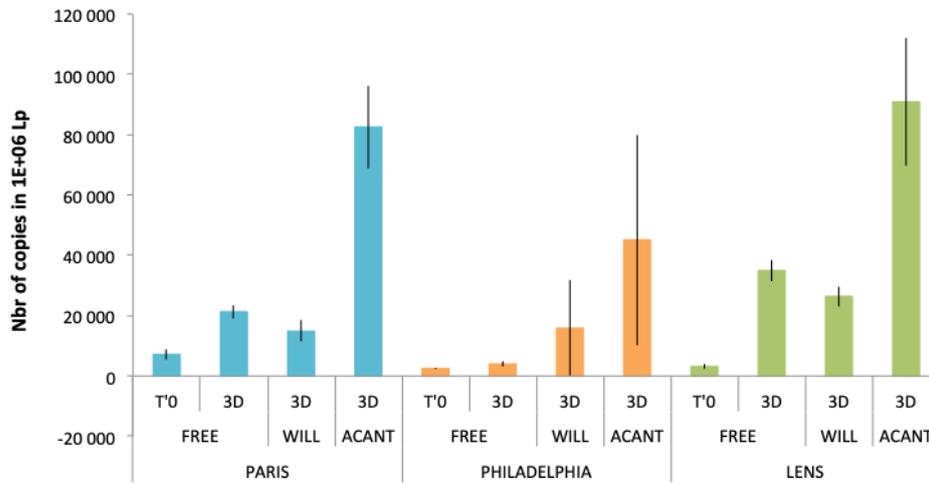


Figure S7: *IvrE* cDNA copy number measured in 10^6 *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean \pm SD (n=6, with n=3 in each of the two replicates).

tatB

A – Linear scale



B – Logarithmic scale

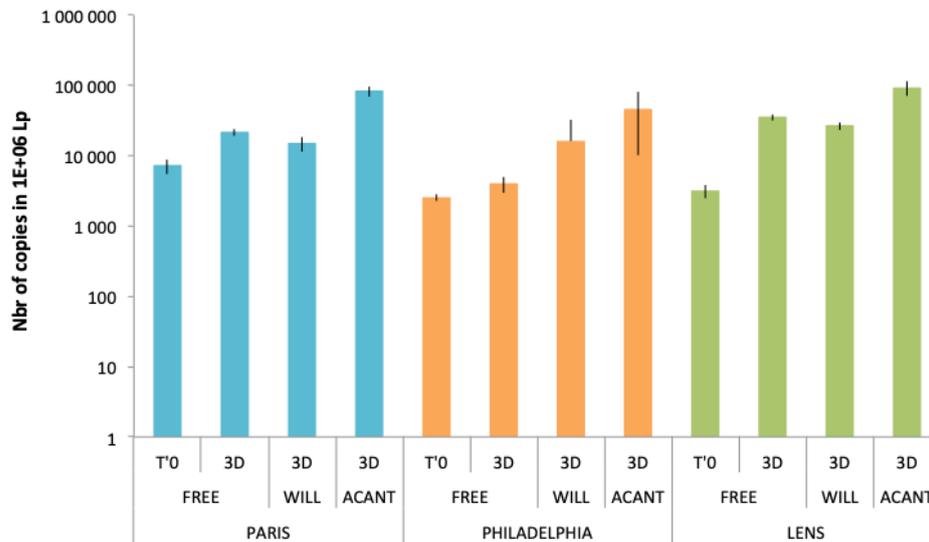
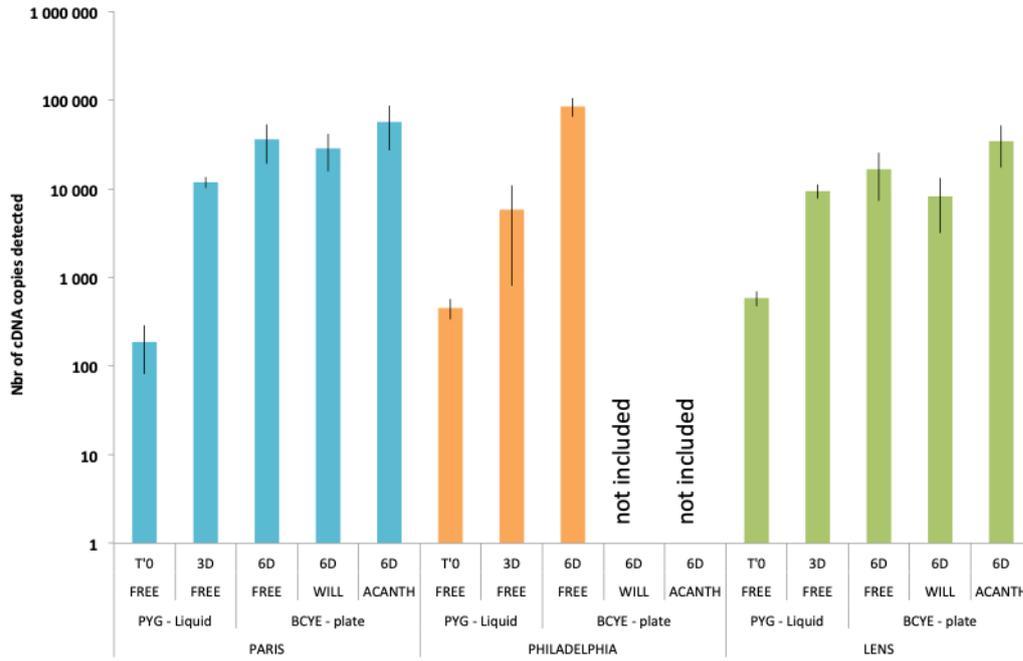


Figure S8: *tatB* cDNA copy number measured in 10^6 *L. pneumophila* for each strain and each culture condition (see Figure S13 for details). A: linear scale; B: logarithmic scale. Results are expressed as the mean \pm SD ($n=6$, with $n=3$ in each of the two replicates).

A – *ccmF*



B – *gacA*

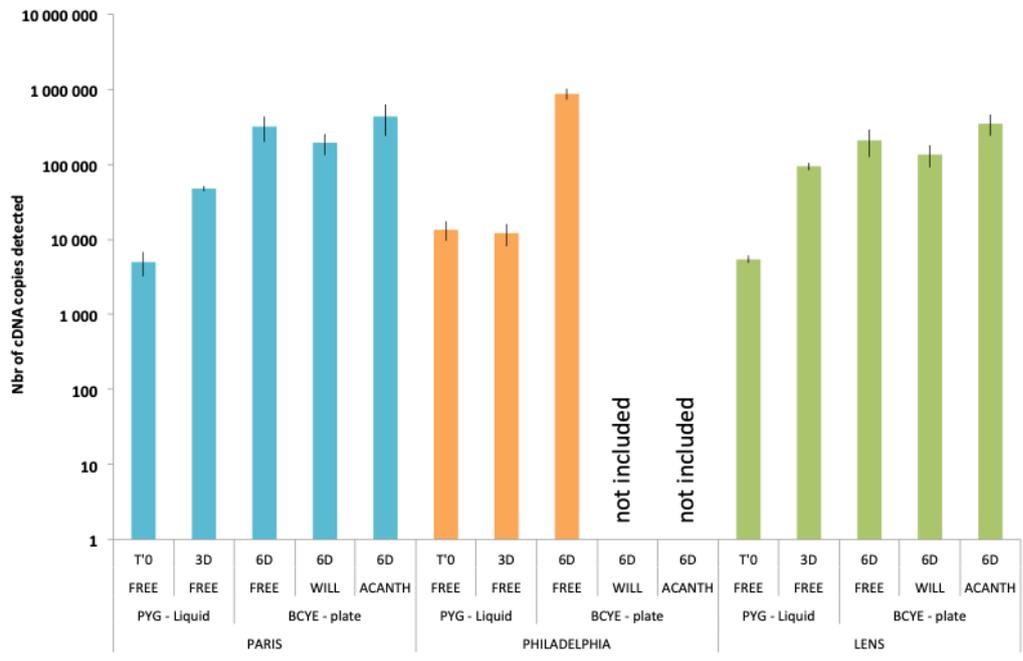
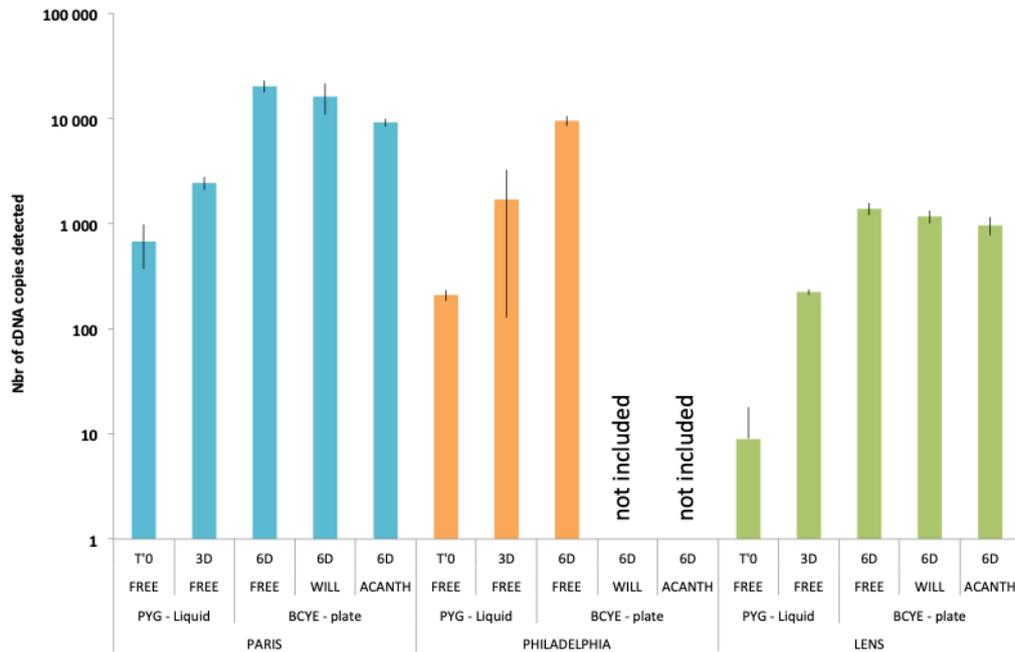


Figure S9: *ccmF* and *gacA* cDNA copy number measured in 10^6 *L. pneumophila* for each strain and each culture condition (see Figure S14 for details). A: *ccmF* gene; B: *gacA* gene. Results are expressed as the mean \pm SD (n=3).

A – *hptX*



B – *icmE*

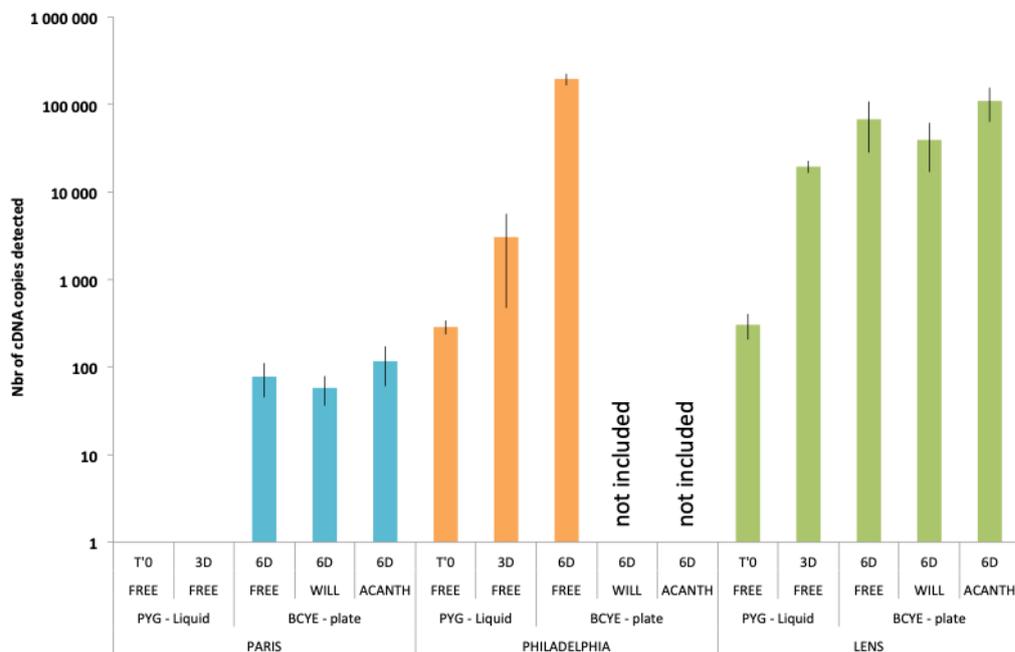
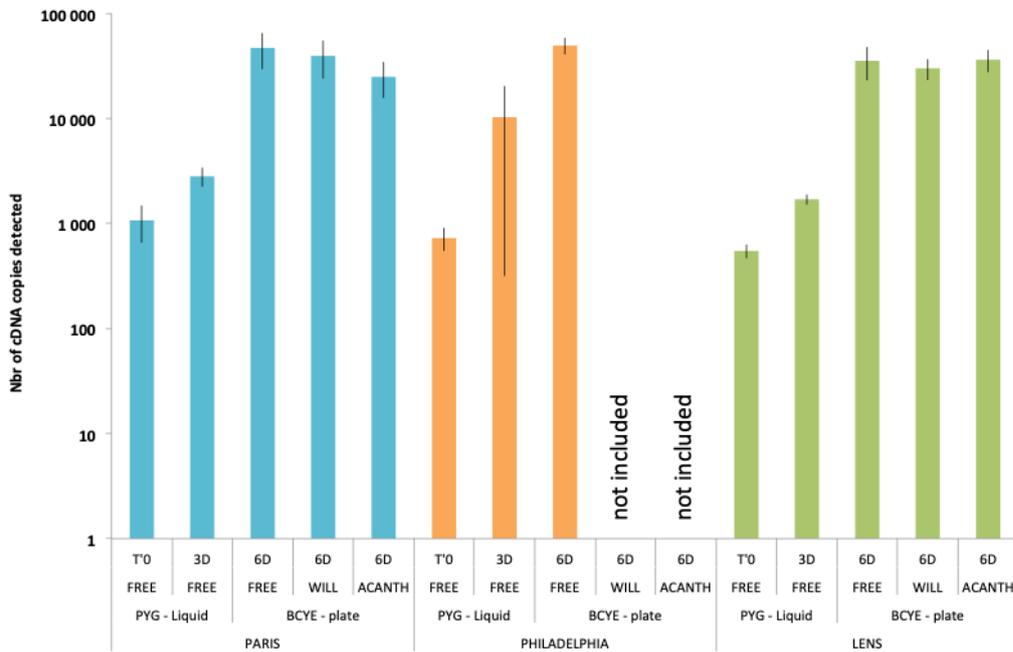


Figure S10: *hptX* and *icmE* cDNA copy number measured in 10^6 *L. pneumophila* for each strain and each culture condition (see Figure S14 for details). A: *hptX* gene; B: *icmE* gene. Results are expressed as the mean \pm SD (n=3).

A – *lirR*



B – *lvrE*

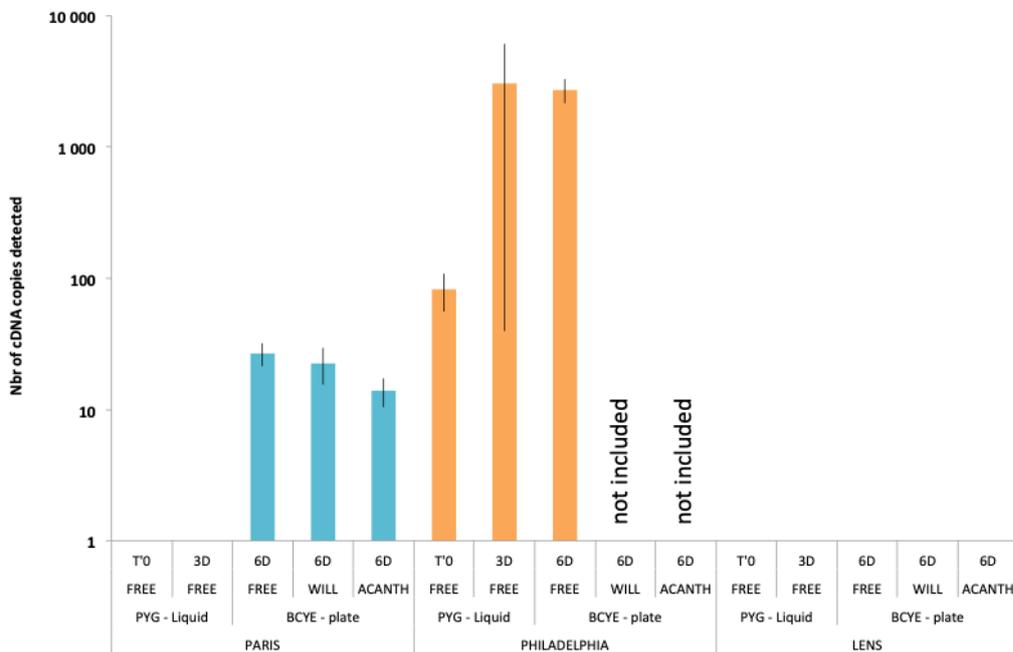


Figure S11: *lirR* and *lvrE* cDNA copy number measured in 10^6 *L. pneumophila* for each strain and each culture condition (see Figure S14 for details). A: *lirR* gene; B: *lvrE* gene. Results are expressed as the mean \pm SD (n=3).

tatB

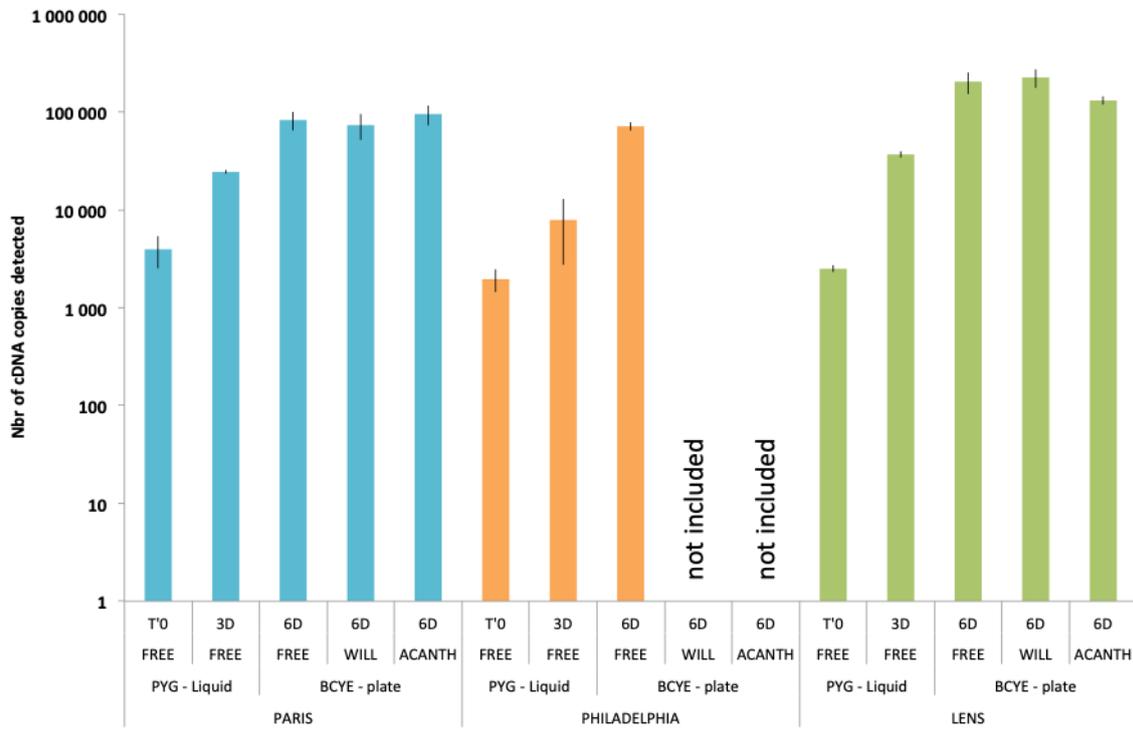


Figure S12: *tatB* cDNA copy number measured in 10^6 *L. pneumophila* for each strain and each culture condition (see Figure S14 for details). Results are expressed as the mean \pm SD (n=3).

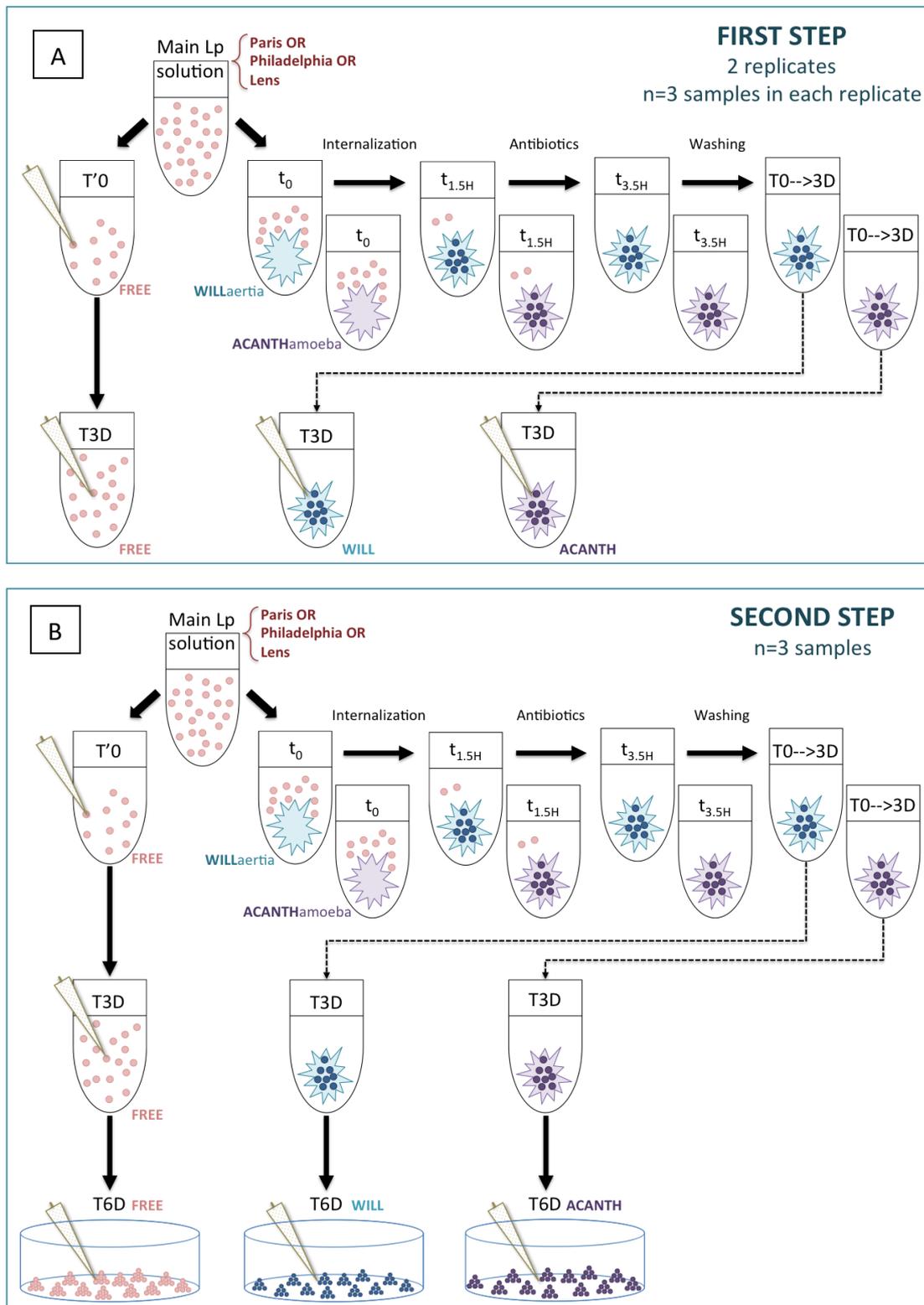


Figure S13: Synoptic diagram of the experiments. A: first step: for the cocultures, after a 1.5h of contact, extracellular *L. pneumophila* were eliminated by antibiotic action for 2h, then the cocultures were rinsed and incubated for 3 days; B: second step: after the 3-day incubation, coculture were 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.