Supplementary Materials

Acidocalcisomes and Polyphosphate Granules Are Different Subcellular Structures in *Agrobacterium tumefaciens*

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Key words: Acidocalcisomes, polyphosphate, alpha-proteobacteria, polyphosphate kinase,

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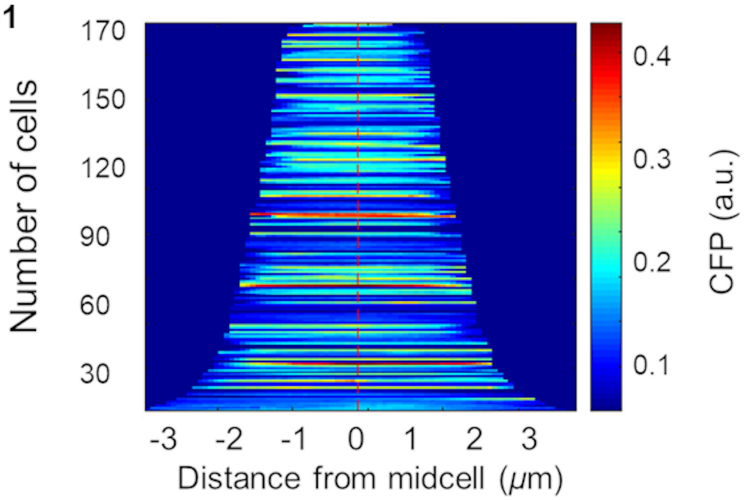
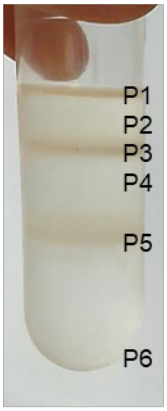
S1



Suppl. Materials, Fig. S1: Staining of *A. tumefaciens* with Lysosensor green DND-189. The demograph shows the localization of Lysosensor green DND-189 fluorescence dependent on cell length. Note the absence of any fluorescent foci in all cells (n=170).

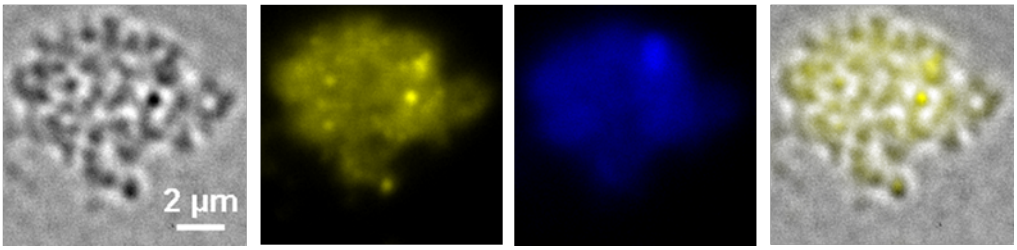
S2

A

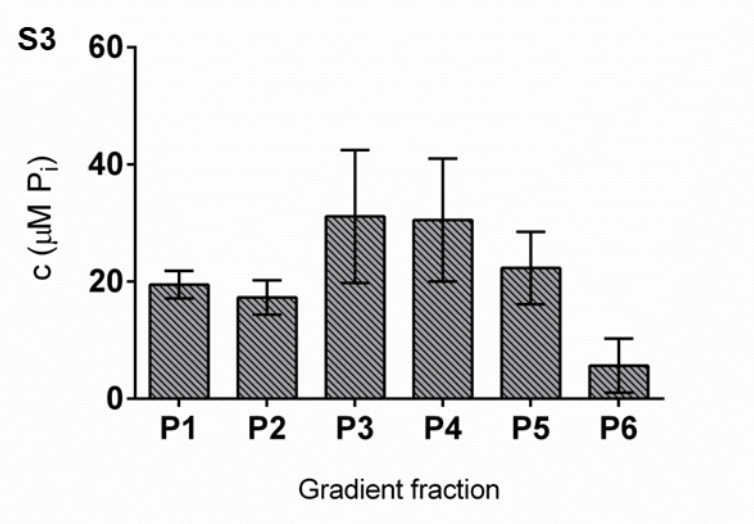


Iodixanol gradient:
20%
24%
28%
30%
35%
40%

B

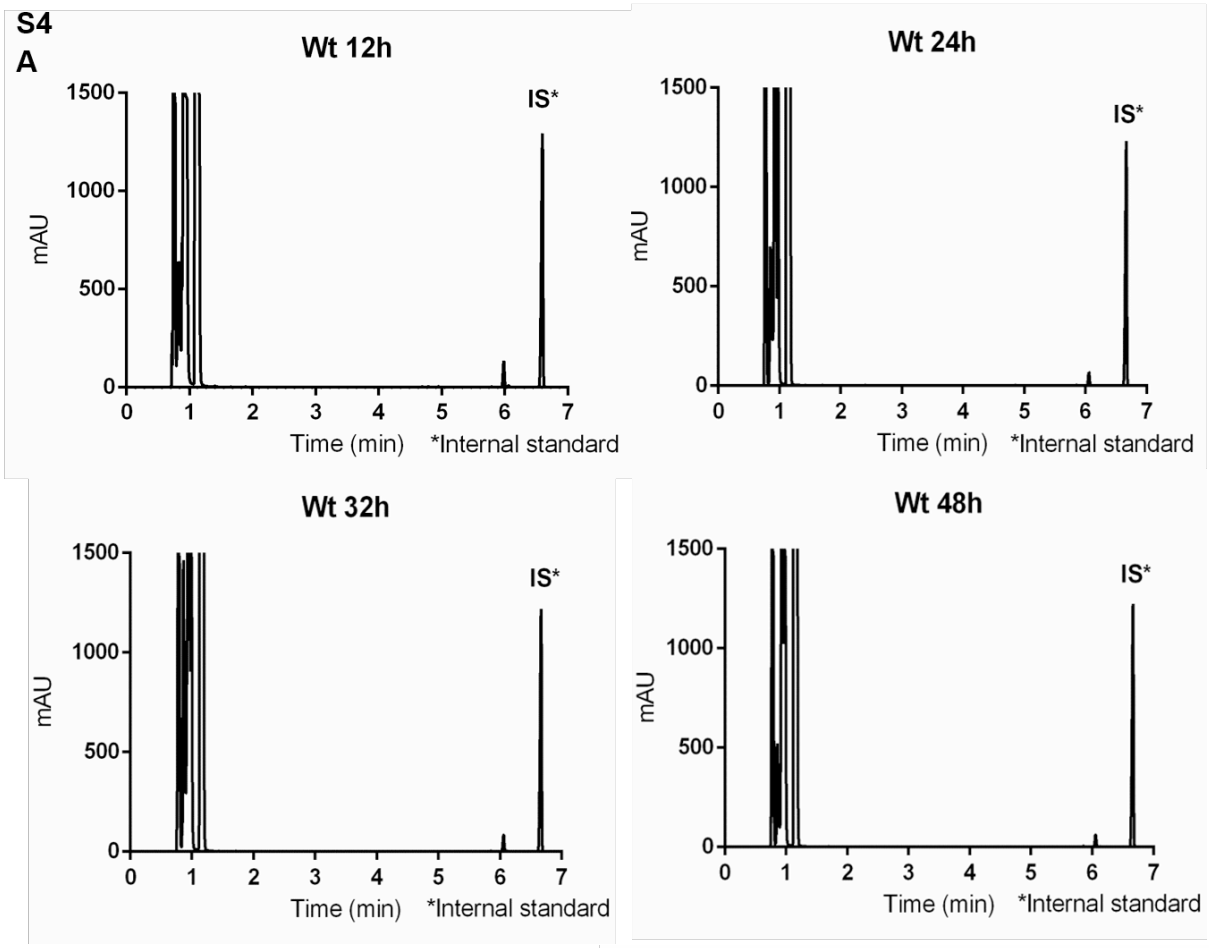


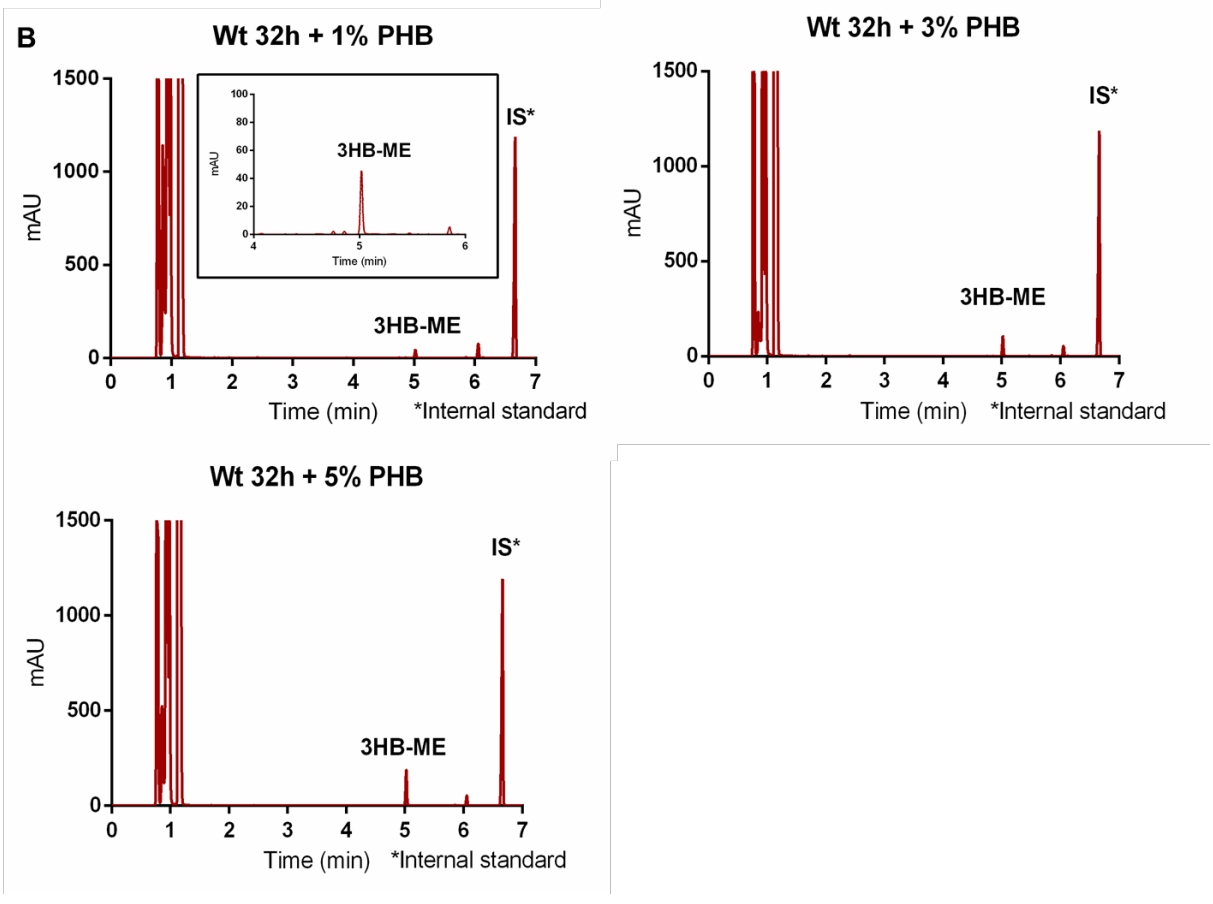
Suppl. materials, Fig. S2: Iodixanol density gradient centrifugation of *A. tumefaciens* cell extracts. Separate fractions as indicated (P1-P6) (**A**). Microscopical analysis of the bottom fraction P6 (**B**). The fraction P6 shows the presence of DAPI-polyP stainable structures. From left to right: bright field, DAPI-polyP, DAPI-DNA, merge of 1 and 2.

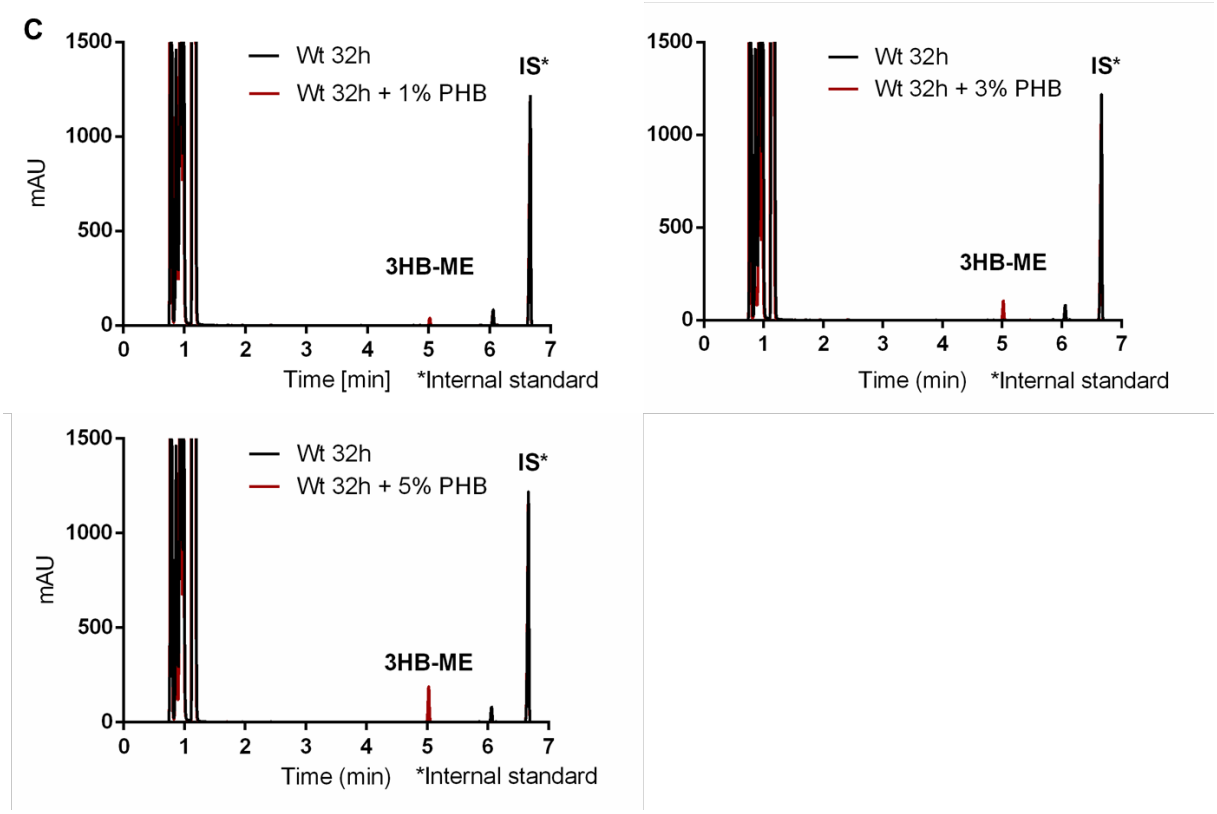


Suppl. materials, Fig. S3: Determination of polyP content in iodixanol fractions P1-P6. PolyP was digested with *E. coli* PPX and concentration of liberated phosphate (P_i) was determined by malachite green assay.

S4

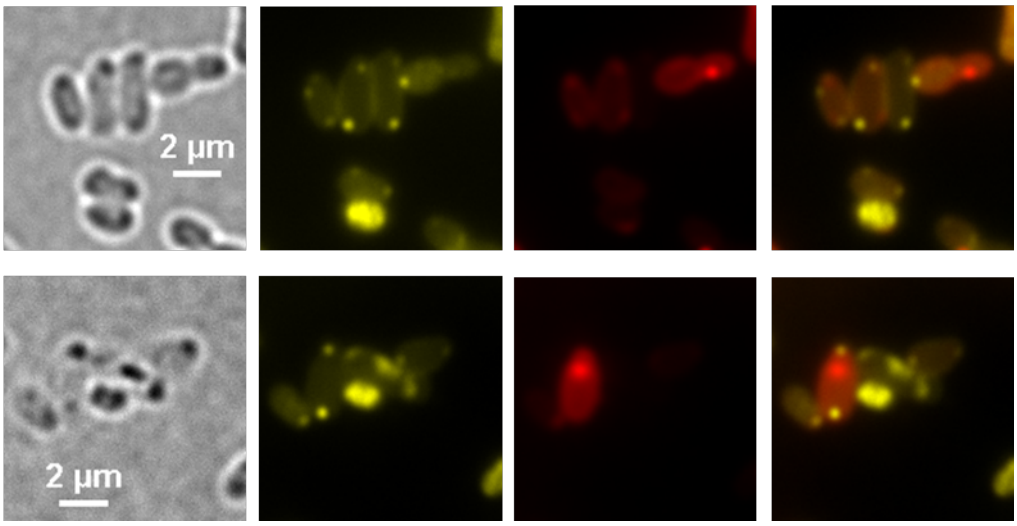

A
Wt 12h
Wt 24h
Wt 32h
Wt 48h
IS*
mAU
Time (min)
*Internal standard


B
Wt 32h + 1% PHB
Wt 32h + 3% PHB
Wt 32h + 5% PHB
IS*
3HB-ME
mAU
Time (min)
*Internal standard



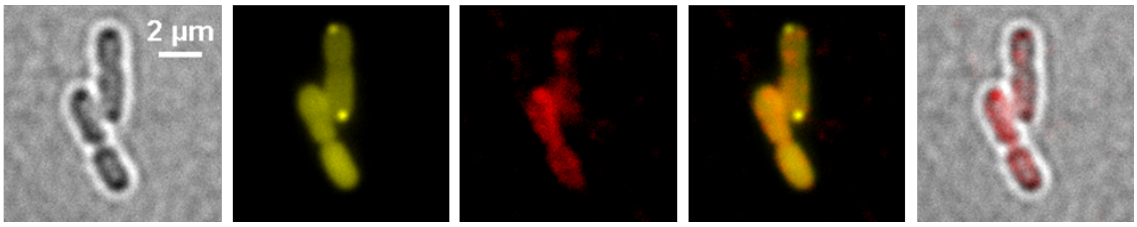
Suppl. materials, Fig. 4: PHB determination in samples from *A. tumefaciens* wild type by gas chromatography after acidic methanolysis. Chromatograms of samples taken after 12h, 24h, 32h and 48h show the absence of 3-hydroxybutyrate methylester (3HB-ME) (**A**). The chromatograms of the 32 h sample were spiked with 1, 3 or 5% of 3HB-ME of the cell dry weight to estimate the detection limit (**B**). The presence of 1% 3HB-ME (PHB) could be well detected as shown in the enlarged inlay figure. The overlay of the 32h time point sample in (**A**) with the spiked samples of (**B**) are shown in (**C**). IS* refers to the internal standard (methyl-benzoate).

S5



Suppl. materials Fig. 5: Fluorescence microscopy of *A. tumefaciens*. *A. tumefaciens* wild type was stained with DAPI and MitroTracker. DAPI-PolyP and MitoTracker foci occurred in close proximity in the cells, but no co-localisation was observed. From left to right: bright field, DAPI-polyP, MitoTracker, merge.

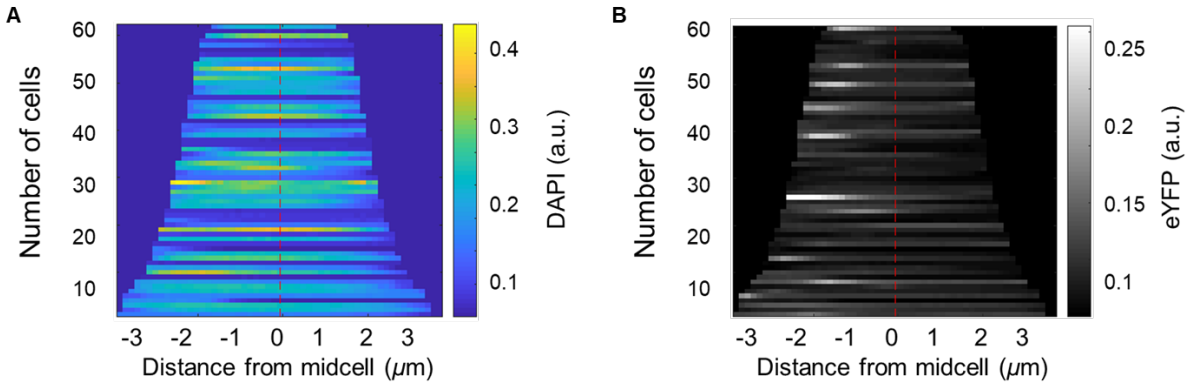
S6



Suppl. materials Fig. 6: Fluorescence microscopy of *A. tumefaciens* with DAPI and LysoTracker red DND-99. With LysoTracker the cells show an irregular staining. From left to right: bright field, DAPI-polyP, LysoTracker, merge.

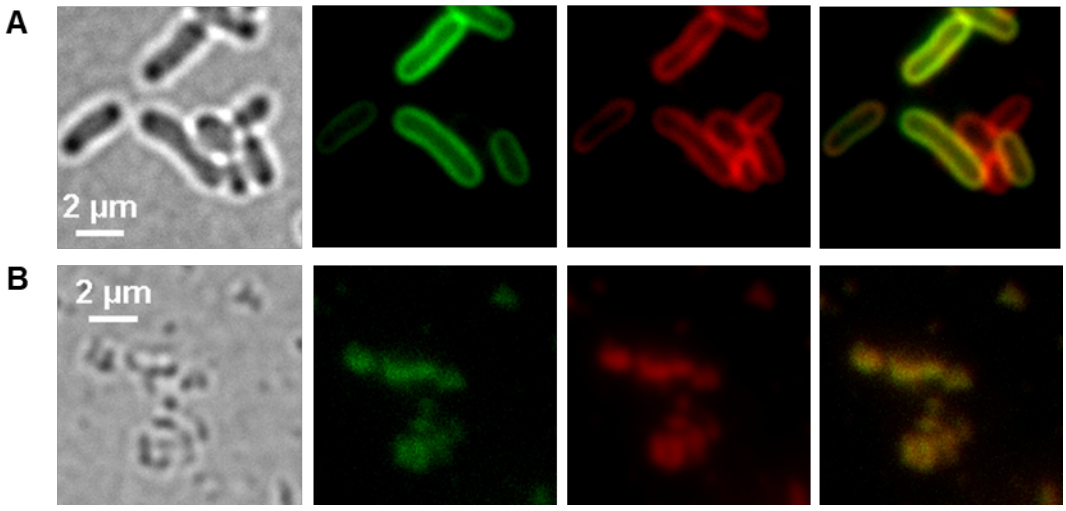
Suppl. materials, movie S7 AB: Time lapse experiment of *A. tumefaciens* harboring the pBBR1MCS2-P_{phaC}-*eyfp-hppA* plasmid. Bright field (**A**) and eYFP-HppA (**B**) channel show cell growth and the formation/localization of the eYFP-HppA fluorescence signal, respectively.

S8



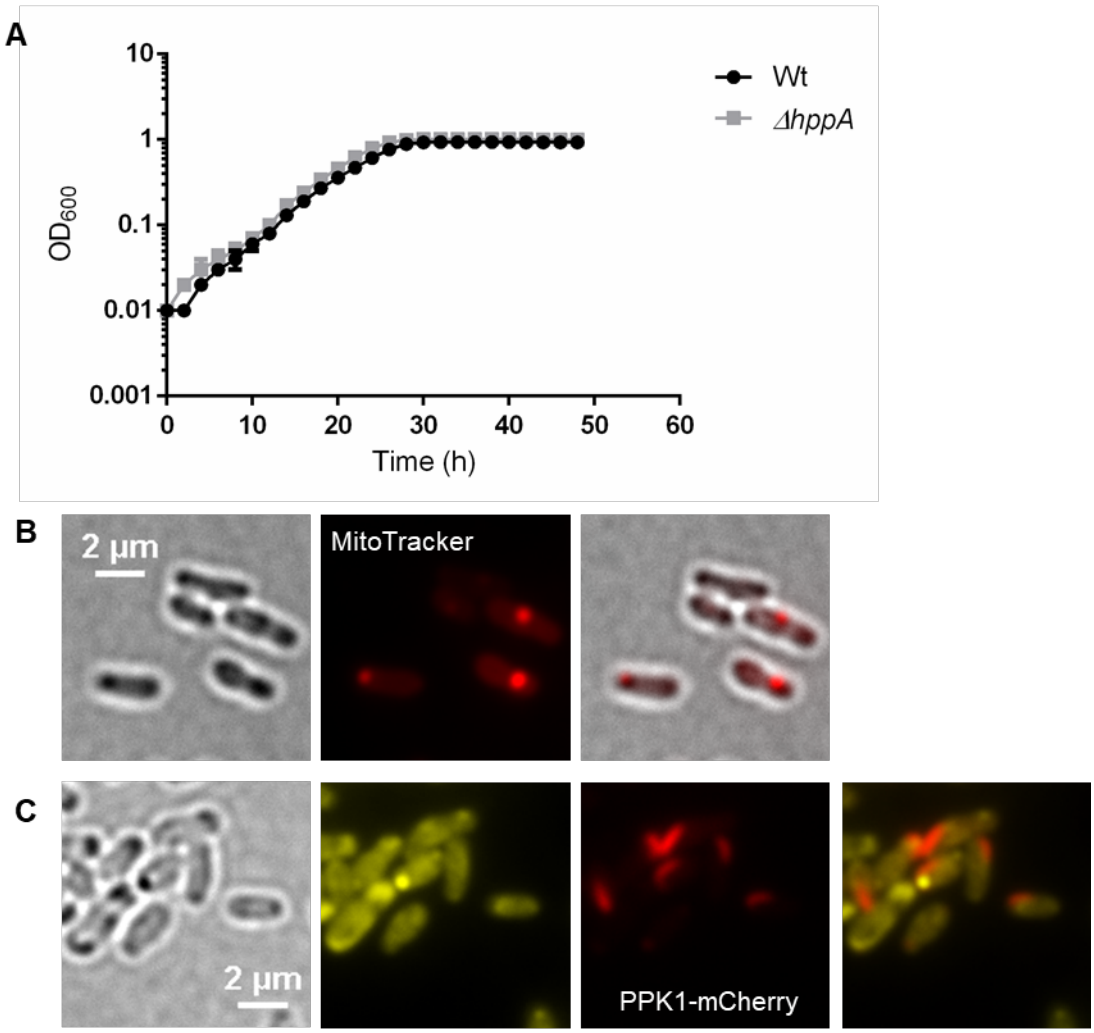
Suppl. materials Fig. 8: Detection of DAPI-polyP and eYFP-HppA foci in *A. tumefaciens*. The demographs show the distribution of the DAPI-polyP (**A**) and eYFP-HppA (**B**) signals for *A. tumefaciens* after 24h of growth on LB medium.

S9



Suppl. materials Fig. 9: Images of *A. tumefaciens* cells stained with FM dyes and of commercial fluorescent nanobeads. *A. tumefaciens* cells were stained with FM 1-43 and FM 4-64 (**A**). Note the absence of any misalignment of the green and red fluorescence signals. From left to right: bright field, MF1-43, FM4-64, merge. The images in (**B**) show nanobeads (90nm) after sonification. The beads still present mainly as agglomerates of different sizes. In no case could a misalignment of the signal at different wavelength be observed.

S10



Suppl. materials Fig. 10: Growth of *A. tumefaciens* in ATGN Medium. *A. tumefaciens* wild type and the Δ*hppA* mutant were grown in ATGN medium at 30°C in an Biotek microplate reader under continuous orbital shaking (282 cpm) (**A**). Turbidity (OD_{600}) was determined in regular intervals. Error bars show standard deviation of eight biological replicates.

Fluorescence microscopic images of *A. tumefaciens* Δ*hppA* mutant after staining with MitoTracker still showed the presence of fluorescent foci as the wild type (**Fig. 4** of the main publication) (**B**). From left to right: bright field, MitoTracker, merge.

Fluorescence microscopic images of *A. tumefaciens* Δ*hppA* harboring pBBR1MCS2-P_{phaC}-*ppk1-mcherry* (**C**) showed the formation of rod-shaped PPK1-mCherry signals which appeared close to the cell pole and in close proximity to the DAPI-polyP signal in cells with DAPI-polyP granules (compare with **Fig. 8** of the main publication). From left to right: bright field, DAPI-polyP, PPK1-mCherry, merge.