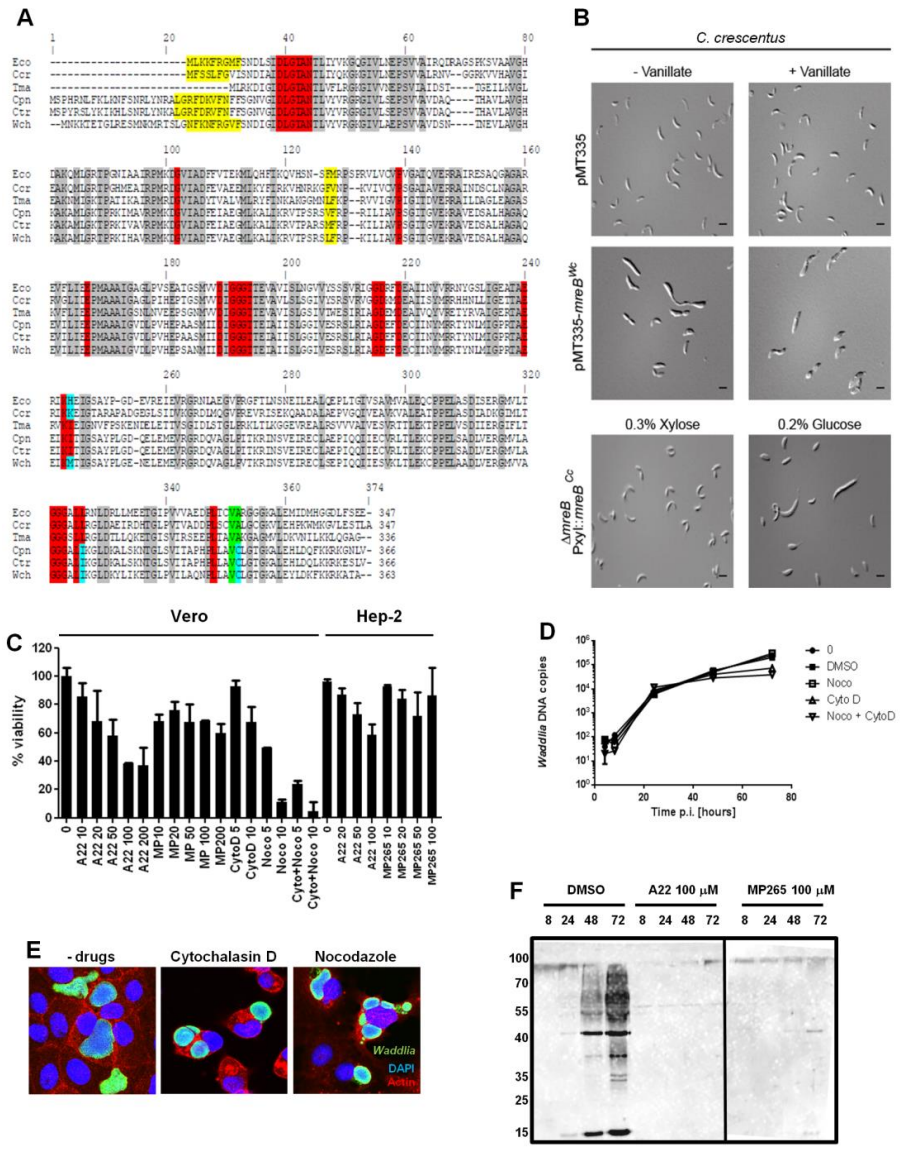
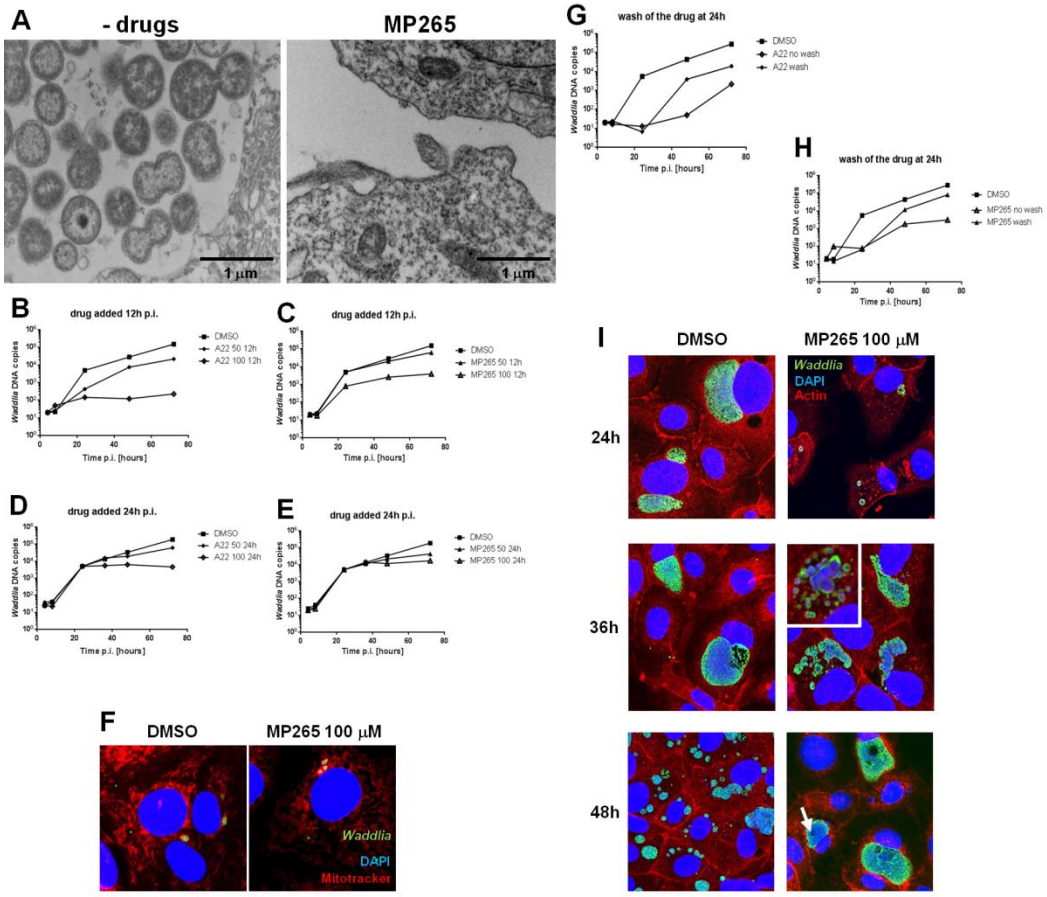
Supplementary figures:

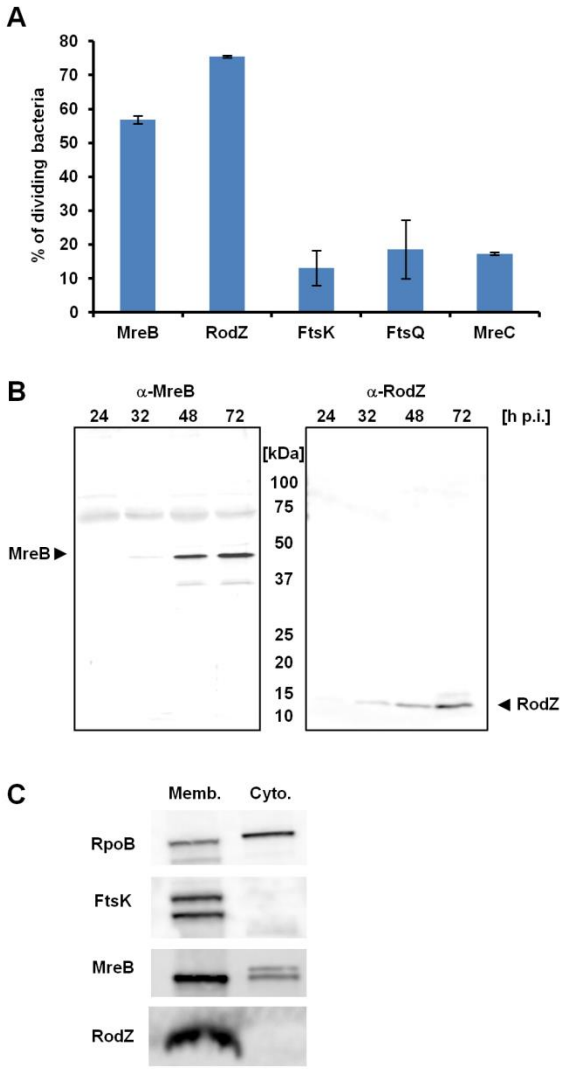


Supplementary Figure 1: A) Amino acid sequences of MreB from 6 different bacteria, *Escherichia coli* (Eco), *Caulobacter crescentus* (Ccr), *Thermotoga maritima* (Tma), *Chlamydia pneumoniae* (Cpn), *Chlamydia trachomatis* (Ctr) and *Waddlia chondrophila* (Wch) were aligned using BLASTP. Amino acids are highlighted: in red for conserved active site residues, in grey for totally conserved residues among the 6 species, in green for residues important for A22 sensitivity and in yellow for residues involved in membrane association. Residues not conserved in important sites are highlighted in blue. B) *C. crescentus* strains harboring the empty pMT335 vector and the recombinant pMT335-*mreB*^{Wc} were grown over-night at 30°C, then diluted to a starting OD_{600nm}=~ 0.05. Growth of the cultures was monitored until they reached an OD_{600nm} of 0.3-0.4 then cultures were splitted and vanillate 0.05 mM was added. After 6

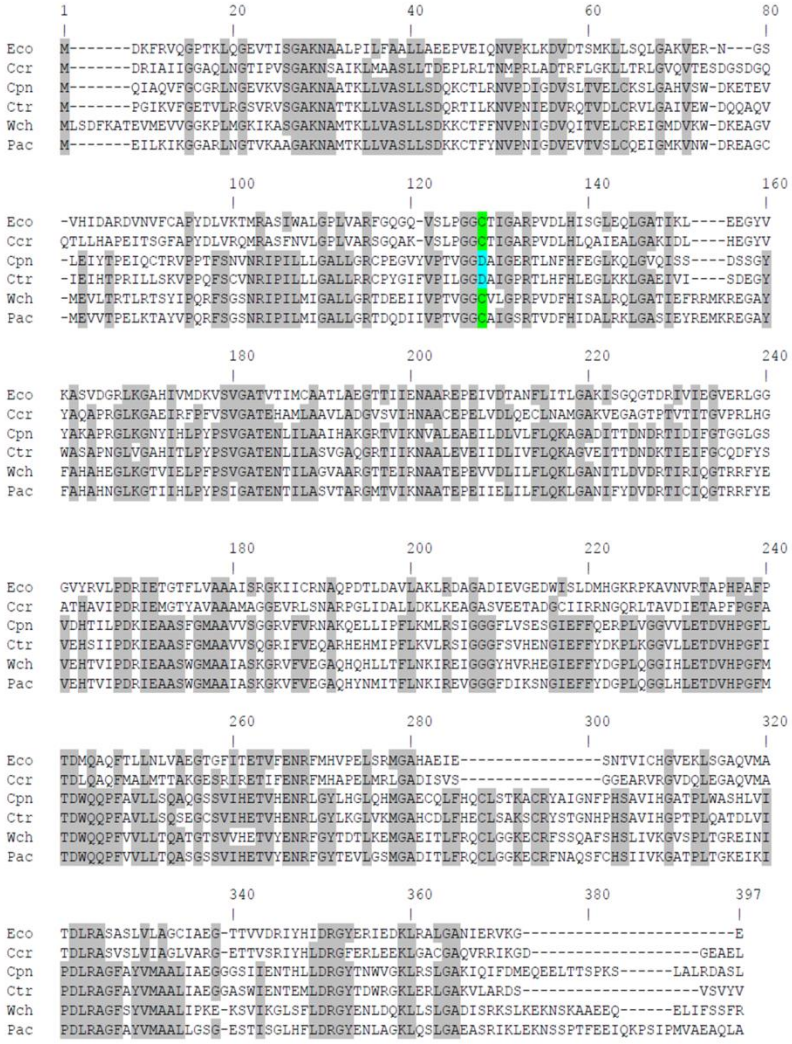
hours images of the cultures were collected. The *C. crescentus mreB* mutant strain harboring a copy of *mreB*^{cc} under a xylose promoter was grown overnight at 30°C in PYEX (Xylose 0.3 %), then it was diluted to a starting OD_{600nm} =~ 0.05, growth of the culture was monitored until they reached an OD_{600nm} of 0.3-0.4 then 1 mL of the culture was washed thrice in PYE. The resulting pellet was resuspended in 1 mL of PYE and diluted into 5 mL of fresh PYEX or PYEG (Glucose 0.2 %). DIC images were collected after an overnight growth at 30°C. Scale bar is 4 µm. C) Cells were treated for 24 hours with the indicated drugs (µM) and viability was then determined using Rezasurin as described. 5 or 10 µM of nocodazole (Noco) and of cytochalasin D (Cyto D) were used. D) Vero cells were treated with the indicated drugs 2 hours post-infection and *Waddlia* DNA was quantified by qPCR. 10 µM of nocodazole (Noco) and of cytochalasin D (Cyto D) were used. E) Cells from (B) were fixed and prepared for immunofluorescence using anti-*Waddlia* antibodies, anti-Actin antibodies and DAPI. They were then observed by confocal microscopy. F) Bacterial protein accumulation is lowered by A22 and MP265. Harvested Vero cells were resuspended in loading buffer and analyzed by Western blotting with an anti-*Waddlia* antibody. Bands observed represent different immunogenic proteins of the outer membrane of *W. chondrophila*.



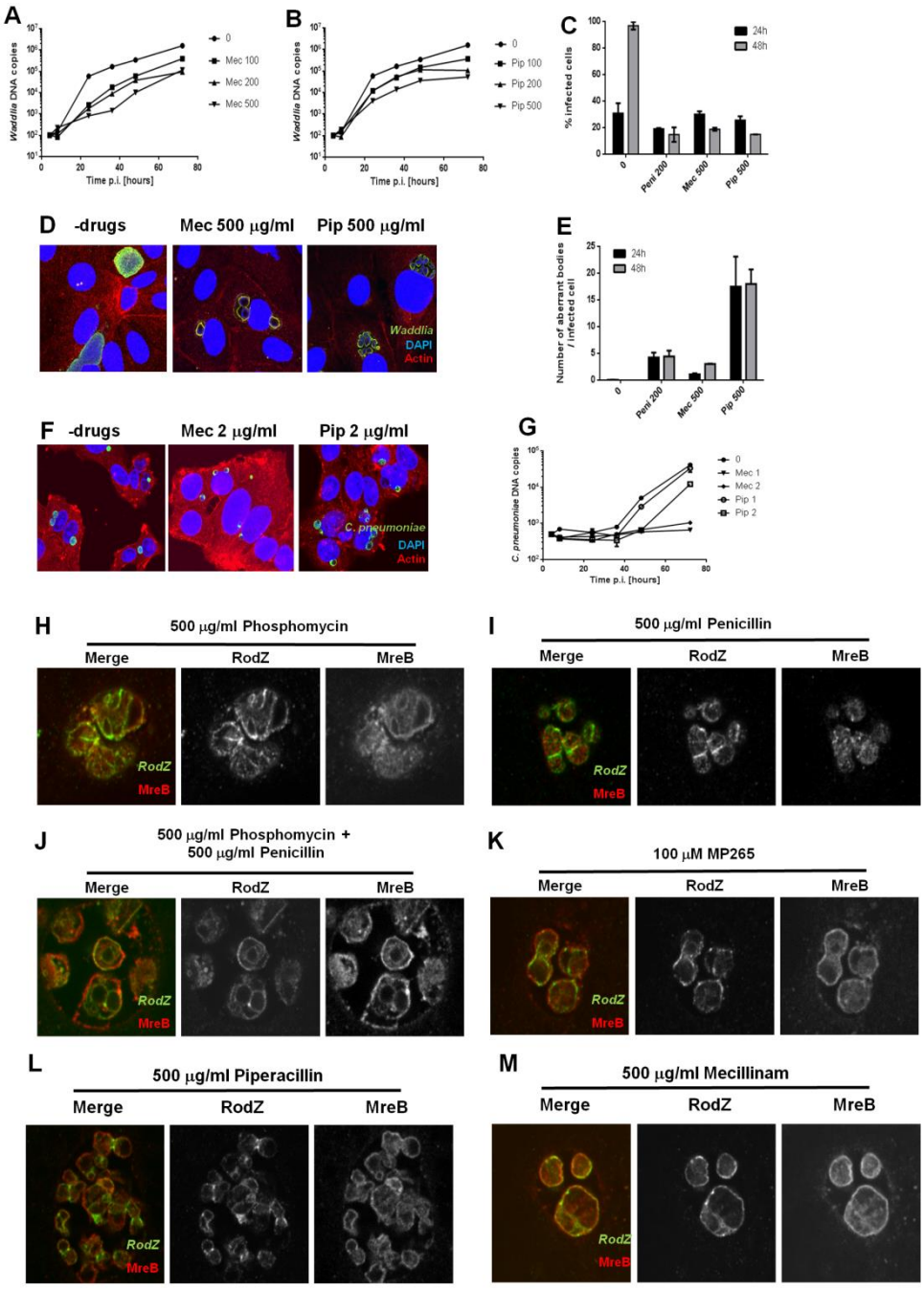
Supplementary Figure 2: A) *C. pneumoniae* infected Hep-2 cells were treated with MP265 2 h p.i. Cells were harvested 24h p.i. and subsequently prepared for electron microscopy as described. B-E) Waddlia infected Vero cells were treated with A22, MP265 or DMSO 12 h p.i. (B-C) or 24h p.i. (D-E). Samples were taken at the indicated time points, DNA was extracted and *Waddlia* DNA was quantified by qPCR. F) Vero Cells were treated with the indicated drugs at time of infection with *Waddlia*. 8 hours post-infection, cells were labeled with Mitotracker as described and fixed for immunofluorescence using anti-*Waddlia* antibodies and DAPI. Cells were then observed by confocal microscopy. G-H) Infected cells were treated with A22, MP265 or DMSO 2h p.i. Cells were washed 24h p.i. and fresh media without inhibitor was added (wash). For comparison, cells were not washed and further incubated with the inhibitor (no wash). Infection was quantified by qPCR. I) The same treatment as for E-F was performed and cells were fixed and prepared for immunofluorescence. Remaining aberrant bodies are magnified in boxes or shown by arrows.



Supplementary Figure 3: Antibodies raised against MreB and RodZ are specific. A) Antibodies raised against MreB and RodZ, but not against FtsZ, FtsQ and MreC target proteins localized at the mid-cell. *Waddlia* infected Vero cells were prepared for immunofluorescence 24h p.i. and incubated with the indicated antibodies. Accumulation of the protein at mid-cell in dividing cells was observed and quantified by confocal microscopy (n=100). B) Western blot was performed as described in Fig. 2B, and whole membrane pictures are presented, showing no crossreactivity between both antibodies. C) *Waddlia* infected Vero cells were harvested 24h p.i. and lysed in alkaline EDTA as described. Membrane and cytosol fractions were separated by ultracentrifugation and analyzed by Western blot.

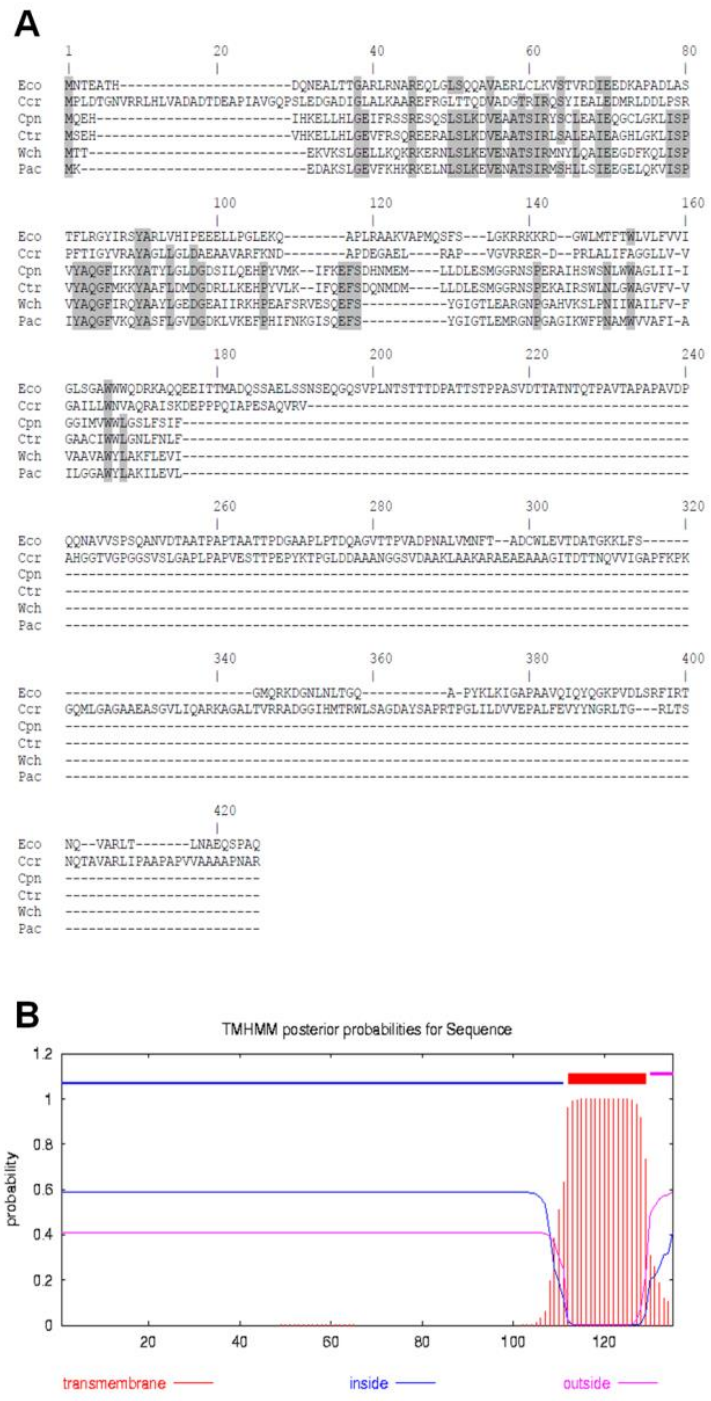


Supplementary Figure 4: MurA is conserved in *Chlamydiales*. Amino acid sequences of MurA from 6 different bacteria, *Escherichia coli* (Eco), *Caulobacter crescentus* (Ccr), *Chlamydia pneumoniae* (Cpn), *Chlamydia trachomatis* (Ctr), *Waddlia chondrophila* (Wch) and *Parachlamydia acanthamoebae* were aligned using BLASTP. Amino acids are highlighted in grey for totally conserved residues among the *Chlamydiales* or in at least 5 of the 6 species. The important residue for phosphomycin resistance (corresponding to Cys115 in *E. coli*) is highlighted in green and the mutation causing resistance in blue.



Supplementary Figure 5: A-B) *Waddlia* infected Vero cells were treated with the indicated concentration of the drugs piperacillin (Pip) or mecillinam (Mec) 2h post infection, DNA was extracted at the given time points and *Waddlia* DNA was quantified by qPCR. C) Percentage of *Waddlia* infected Vero cells were quantified by immunofluorescence in presence of the indicated drugs, penicillin (Peni), piperacillin (Pip) or mecillinam (Mec) (N=100). D) Infected Vero cells treated with the indicated drugs were fixed, treated for immunofluorescence and observed by confocal microscopy. E) The number of aberrant bodies per cell was quantified at the indicated times p.i. (n=100). F) Hep-2 cells were infected with *C. pneumoniae*. Indicated drugs were added 2 hours post-infection. Cells were fixed and labeled for immunofluorescence using DAPI, anti-chlamydial LPS and anti-actin antibodies and observed by confocal microscopy. G) Hep-

2 cells were infected with *C. pneumoniae*. Indicated drugs were added 2 hours post-infection (1-2 μg/ml mecillinam or 1-2 μg/ml piperacillin). DNA was extracted at the indicated time points and *C. pneumoniae* DNA was quantified by qPCR. H-M) Vero cells infected with *W. chondrophila* were treated with the indicated drugs 2h p.i. Cells were fixed 24h p.i., labeled with antibodies against RodZ and MreB and observed by confocal microscopy.



Supplementary Figure 6: A) Only the N-terminal part of RodZ is conserved in *Chlamydiales*. Amino acid sequences of RodZ from 6 different bacteria, *Escherichia coli* (Eco), *Caulobacter crescentus* (Ccr), *Chlamydia pneumoniae* (Cpn), *Chlamydia trachomatis* (Ctr), *Waddlia chondrophila* (Wch) and *Parachlamydia acanthamoebae* were aligned using BLASTP. Amino acids are highlighted in grey for totally conserved residues among the *Chlamydiales* or in at least 5 of the 6 species. B) The *W. chondrophila* RodZ is composed of a N-terminal cytoplasmic domain and a C-terminal transmembrane domain. Topology and transmembrane domains predictions was performed using the online software TMHMM server.

Supplementary table 1: Percentage of identity of proteins of the PG biosynthesis pathway compared to *C. pneumoniae*.

	C. trachomatis	*W. chondrophila*	*E. coli*
GlmS	61.41	45.35	41.61
GlmM	79.47	59.24	47.66
GlmU	64.2	45.96	27.62
MurA	70.51	56.29	34.63
MurB	58.04	44.44	27.22
MurC-ddl	55.31	40.32	33.26
MurD	50.74	37.05	31.24
MurE	58.63	42.91	36.95
MurF	55.94	27.11	27.46
MraY	50.32	40.32	38.26
MurG	53.37	33.87	30.56
pbp2	58.76	38.6	22.55
pbp3	66.25	44.68	27.72
dacC	63.34	32.73	29.52
dapA	44.24	28.57	28.37
dapB	42.8	25.87	28.1
dapL	56.3	45.85	26.97
dapF	50	32.3	33.33

Supplementary table 2: E-values for table S1. Low E-values indicate that the probability of the proteins to be orthologues is high.

	C. trachomatis	*W. chondrophila*	*E. coli*
GlmS	0.00E+000	6.00E-154	5.00E-126
GlmM	0.00E+000	6.00E-160	8.00E-105
GlmU	3.00E-065	2.00E-037	5.00E-009
MurA	1.00E-175	5.00E-135	2.00E-055
MurB	3.00E-085	6.00E-058	2.00E-009
MurC	0.00E+000	3.00E-089	7.00E-058
MurD	1.00E-111	5.00E-058	2.00E-034
MurE	3.00E-160	1.00E-098	1.00E-058
MurF	2.00E-139	1.00E-032	6.00E-025
MraY	8.00E-081	5.00E-060	1.00E-049
MurG	3.00E-099	6.00E-040	8.00E-023
pbp2	0.00E+000	6.00E-157	4.00E-016
pbp3	0.00E+000	1.00E-160	6.00E-041
dacC	5.00E-107	8.00E-50	5.00E-13
dapA	1.00E-80	5.00E-26	1.00E-26
dapB	1.00E-58	1.00E-12	3.00E-22
dapL	1.00E-159	6.00E-111	7.00E-25
dapF	3.00E-84	6.00E-30	3.00E-22

Supplementary table 3: Primers and probes used in this study.

Name	Sequence
WadF4	5'-GGCCCTTGGGTCGTAAAGTTCT-3'
WadR4	5'-CGGAGTTAGCCGGTGCTTCT-3'
WadS2	5'-FAM-CATGGGAACAAGAGAAGGATG-BHQ1-3'
CpnF	5'-CATGGTGTCATTCGCCAAGT-3'
CpnR	5'-CGTGTCGTCCAGCCATTTTA-3'
CpnS	5'-FAM-TCTACGTTGCCTCTAAGAGAAAACTTCAAGTTGGA-BHQ1-3'
MreB RT F	5'-CGCCTTGCCCGTCCCTAAGC-3'
MreB RT R	5'-ATGGTTGTTGCCGGAGGCGG-3'
RodZ RT F	5'-GCAGCTCATCTCTCCGGTTT-3'
RodZ RT R	5'-CGCTCCAGGATTGCCTCTAG-3'
MreB Nde	5'-AAAAAACATATGAATAAAAAAACGGAAACC-3'
MreB Eco	5'-AAAAAAGAATTCTATCTAGCTCTTTTTCTAGG-3'
RodZ fwd	5'-CACCATGACGACAGAAAAAG-3'
RodZ rev	5'-CTAAATGACCTCGAGGAATTTT-3'