Supplementary Figure 1. Localization of chlamydial MreB_GFPsw proteins in *C. trachomatis***.** HeLa cells were infected with *C. trachomatis* transformants containing aTcinducible vectors encoding MreB_GFPsw proteins. At 12 hpi, expression of the GFP sandwich fusions was induced with 10 nM aTc, and the samples were fixed (3.2% Formaldehyde, 0.022% Glutaraldehyde in 1X PBS) at 16 hpi for 2 min and permeabilized with 90% methanol. The samples were stained for major outer membrane protein (MOMP; red) and GFP (green). Images were acquired on a Zeiss LSM 800 confocal microscope. Scale bar $= 1 \mu m$

Supplementary Figure 2. Protein sequence alignment of the N-terminus of MreB from diverse *Chlamydia* **phylum members.** The extended N-terminus of chlamydial MreB is conserved across *Chlamydia*. (A) The alignment was performed with Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) and represented with ESPript 3.0 (http://espript.ibcp.fr). (B) The AMPHIPASEEK prediction of amphipathicity for the *Waddlia* MreB ortholog. The blue and red residues represent the extended N-terminus and predicted amphipathic helix, respectively.

Supplementary Figure 3. Test of complementation of chlamydial MreB in *E. coli* **and interaction between chlamydial and** *E. coli* **MreBs by BACTH.** An *E. coli mreB*-deficient mutant (P2733) strain was transformed with an empty arabinose-inducible vector (A) or vectors encoding *E. coli* MreB (B), chlamydial MreB (C), or truncated chlamydial MreB lacking the extended N-terminal region (D). Stationary phase cultures were diluted to 1:50 in LB media containing 50 µg/mL spectinomycin, 25 µg/mL tetracycline, and 34 µg/mL chloramphenicol and cultured at 37℃ with 225 rpm shaking for 2 h. The cells were then induced or not with 0.01% (w/v) arabinose. After induction, 4 μ L of each culture at 2 h and 6 h were spotted under a 1% LB agar pad and covered with a coverslip. Images were acquired on a Zeiss Imager.Z2 equipped with an Apotome2 using a 100X objective. The arrows indicate the cells complemented by the induction of *E. coli* MreB. Scale bar $= 2 \mu m$. (E) BACTH assays were carried out to test interactions between chlamydial MreB and *E. coli* MreB. DHT1 *E. coli* were co-transformed with plasmids encoding the indicated fusion proteins and plated on M63 minimal medium containing 50 μ g/mL ampicillin, 25 μ g/mL kanamycin, 0.5 mM IPTG, 40 µg/mL X-gal, 0.04% casamino acid, and 0.2% maltose. The plates were incubated at 30℃ for 5-7 days. A positive control is the interaction between T25-zip and T18-zip. A negative control is the lack of interaction between T25 and T18-chlamydial MreB and T18-*E. coli* MreB. These tests were performed a minimum of two times. (F) Western blotting was performed to test the expression of chlamydial MreB in strains used in the complementation assay depicted in (C&D). Whole cell lysates from cultures tested in the complementation assay were separated by SDS-PAGE and transferred to a PVDF membrane. The chlamydial MreB was detected with rabbit anti-MreB primary antibody and IRDye goat anti-rabbit 800CW (LI-COR, Lincoln, NE).

Supplementary Figure 4. Localization of chlamydial N-terminal MreB-GFP fusion proteins in an *E. coli* **Δ***mreB* **mutant strain (P2733).** The *E. coli* Δ*mreB* mutant (P2733) was transformed with the arabinose-inducible vectors encoding GFP fused with diverse N-terminal regions of chlamydial MreB. Samples were prepared as described in the legend to Figure 4 with the membrane labeled with FM4-64. Images were acquired on a Zeiss Imager. Z2 equipped with an Apotome2 using a 100X objective. Scale bar = 2μ m.

Supplementary Figure 5. Localization of the N-terminus of *C. suis* **MreB-GFP fusion peptides in** *C. trachomatis* **and** *E. coli.* (A) The predicted amphipathicity of the N-terminus of *C. suis* (Cs) MreB. (B) A helical wheel prediction is shown. (C) The CsMreB_{1-23aa}-GFP peptide is localized in the cytosol in E . *coli*. In contrast, the CsMreB_{1-28aa}-GFP peptide is localized at the membrane at the poles of *E. coli*. These patterns are the same as those of *C. trachomatis* (see Figure 3). (D, E) HeLa cells were infected with *C. trachomatis* transformants containing aTc-inducible vectors encoding CsMreB1-23aa-GFP or CsMreB1-28aa-GFP fusion proteins. Expression of these fusion proteins was induced at 6 hpi or 16 hpi with 10 nM aTc. At 10.5 hpi or 20 hpi, the samples were fixed (3.2% Formaldehyde, 0.022% Glutaraldehyde in 1X PBS) for 2 min and permeabilized with 90% methanol (MeOH) for 1 min. These samples were stained for major outer membrane protein (MOMP; red) with GFP imaged in green. The arrowheads indicate the CsMreB1-23aa-GFP localized at the membrane (see also Figure 4). Images were acquired on a Zeiss LSM 800 confocal microscope with 63X objective. Scale bar $= 0.5 \mu m$ (10.5 hpi) or 1 μm (20 hpi).

Supplementary Figure 6. The localization of various truncated chlamydial MreBs in *C. trachomatis* **L2.** (A) Representation of the various truncated chlamydial MreBs tested. The blue and red residues represent the extended N-terminus and predicted amphipathic helix, respectively. (B) *C. trachomatis* serovar L2 transformants containing aTc-inducible vectors encoding the truncated MreBs were used to infect HeLa cells. At 16 hpi, expression of the MreB 6xH constructs was induced with 10 nM aTc, and these samples were fixed $(3.2\%$ Formaldehyde, 0.022% Glutaraldehyde in 1X DPBS) at 20 hpi for 2 min and permeabilized with 90% methanol. The samples were stained for major outer membrane protein (MOMP; red) and six histidine tag (green). Images were acquired on a Zeiss Imager.Z2 equipped with an Apotome2 using a 100X objective. The white box represents the cells which are zoomed in at the upper right. Scale bar $= 2 \mu m$.

Construct Plasmid	Relevant genotype	Ori	Source of Reference
pASK-GFP- mKate-L2	bla Ptet:: gfp	ColE1	(1)
(pTLR2)			
$pTLR2-$ $mreB$ $6xH$	bla Ptet::Ctr mreB 6xH	ColE1	This study
$pTLR2-$ $mreB$ 67 6xH	bla Ptet:: $\Delta N66$ nt Ctr mreB 6xH	Col _{E1}	This study

Supplementary Table 1. List of Plasmids, Strains, and Primers Used in the Study

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- 8. Bendezu FO, de Boer PA. 2008. Conditional lethality, division defects, membrane involution, and endocytosis in mre and mrd shape mutants of Escherichia coli. J Bacteriol 190:1792-811.
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 (B)

 10 20 30 Amino acid MNKKTETGLRESMNKMRTSLGNFKNFRGVFSNDIGIDL... 2nd structure cccccccchhhhhhhhhhhhhhhhhhhhhcccceeeec... Amphipathic(A) score 32224434333444433333432233333222112222... Extra N-terminal region in Waddlia **Predicted Amphipathic helix residues**

Ec_MreB

AN22 Ctr_MreB

Supplemental Figure 5

 (A) $\begin{tabular}{ll} \bf 10 & 20 & 30 \\ \bf Amino acid MSPYRSLYKIKKHLSNRLYNKALGRFDRVFNFFSGNVGIDL... \\ \bf 2^{nd} structure & \texttt{cccccchhhhhhhhhhhhhhhhhhhhhhhchhh0ccccceec...} \end{tabular}$ Amphipathic(A) score 2334434434344443434455555454444321122222... MreB₁ AN22 MreB ⊦ AN28 MreB ⊦ AN32 MreB ⊦

 $\qquad \qquad \textbf{(B)}$

Extra N-terminal region in C.trachomatis
Predicted Amphipathic helix residues

