Supporting Information

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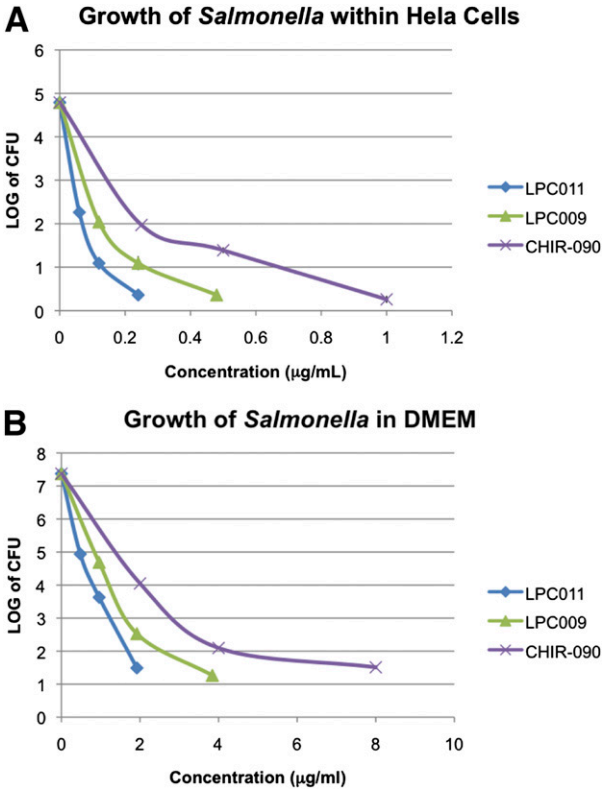


Fig. S1. LpxC inhibitors restrict the growth of intracellular bacteria. (*A*) HeLa cells were infected with *S.* Typhimurium SMO22 and incubated in media containing increasing concentrations of LpxC inhibitors and 25 μg/mL of gentamicin to inhibit growth of extracellular bacteria. Bacteria were harvested from infected cells, and the number of CFUs present determined on agar plates. To directly compare the sensitivity of extracellular *S.* Typhimurium, bacteria were incubated in DMEM at 37 °C and 5% CO_2 with or without LpxC inhibitors (*B*). The number of surviving CFUs was determined on LB agar plates at appropriate dilutions. All experiments were performed in triplicate. Methods: SMO22 was grown in LB broth without agitation at 37 °C to an OD_{600} of 0.5. Bacteria were washed in PBS and diluted in DMEM/10% FBS. Confluent monolayers of HeLa cells were infected with SMO22 at a multiplicity of infection (MOI) of ∼20 followed by incubation at 37 °C for 10 min. After extensive washes with PBS, gentamicin (100 μg/mL) was added for 2 h to kill extracellular bacteria. Media supplemented with 25 μg/mL gentamicin and the indicated LpxC inhibitors was then added, and the infected cells were incubated for 24 h in a 37 °C/5% CO_2-humidified incubator. Intracellular bacteria were lysed with 1% Triton X-100/PBS, and the number of CFUs was determined on LB-agar plates. In parallel, the minimal inhibitory concentrations (MIC) for SMO22 were determined by treatment with LpxC inhibitors diluted in DMEM/10% FBS for 24 h at 37 °C and 5% CO_2. All assays were completed in triplicate.

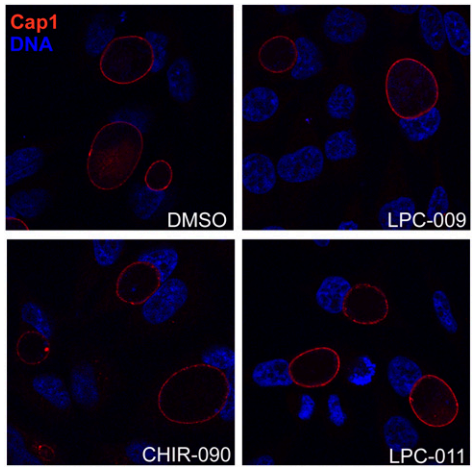


Fig. S2. LpxC inhibitors do not disrupt inclusion membrane integrity. HeLa cells were infected with *C. trachomatis* at MOI of 1 in media containing DMSO only, 10 μg/mL of ampicillin, 16 μg/mL of CHIR-090, 1.92 μg/mL of LPC-009, or 1.92 μg/mL of LPC-011. At 36 hpi, cells were fixed, permeabilized in 0.1% Triton X-100, and immunolabeled with anti-Cap1 (Alexa 555) and anti-MOMP (Alexa 488) antibodies.

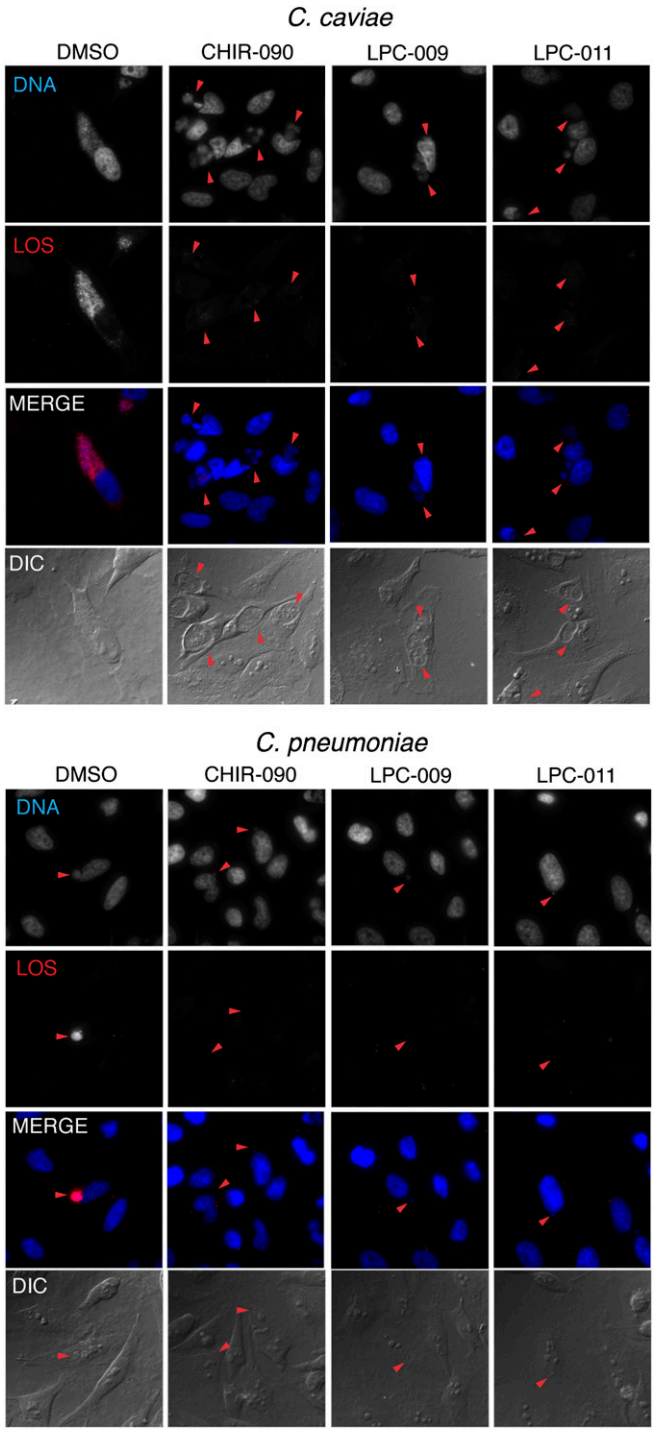


Fig. S3. LpxC inhibitors block LOS synthesis in other members of the *Chlamydiales.* HeLa cells were infected with *C. caviae* and *C. pneumoniae* in the presence of LpxC inhibitors and fixed at 48 hpi and 60 hpi, respectively. Following fixation, cells were permeabilized with 0.1% Triton X-100 and immunolabeled with anti-LOS antibodies (Alexa 555). Inclusions were detected by Hoechst staining of bacterial DNA.

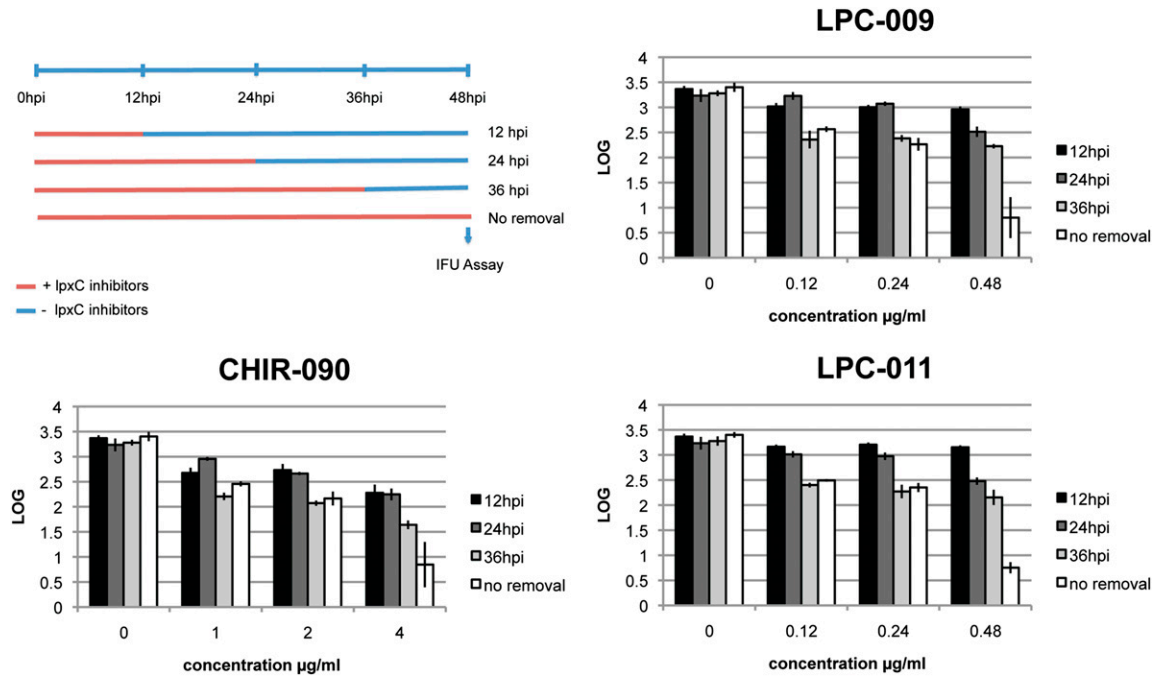
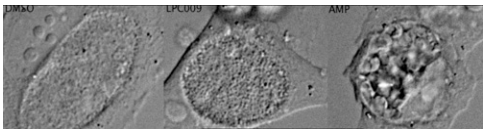


Fig. S4. The effects of LpxC inhibitors on IFU production is reversible. *C. trachomatis*-infected HeLa cells were incubated in media containing indicated concentrations of inhibitors. At 12 hpi, 24 hpi, and 36 hpi, the inhibitors were removed, followed by two washes and incubation in inhibitor-free media. Cells were lysed at 48 hpi for IFU determination.



Movie S1. *C. trachomatis* displays robust replication and inclusion expansion in thepresence of LpxC inhibitors. HeLa cells infected with *C. trachomatis* for 32 h were imaged over a 20-h period in the presence of LpxC inhibitors (16.0 μg/mL CHIR-090, 1.92 μg/mL LPC-011, or 1.92 μg/mL LPC-009), DMSO (untreated control), and ampicillin (2 μg/mL; added at 32 hpi). Note that ampicillin-treated chlamydiae form distinctive, large aberrant RBs that were absent in inhibitor-treated and untreated cells. Also note that the intrainclusion contents appear darker in inhibitor-treated cells, causing RBs to appear more distinctive, compared with those of the DMSO controls. The differences are due to the lack of EBs in treated cells. Methods: HeLa cells were seeded on glass-bottom six-well plates (In Vitro Scientific) and infected with *C. trachomatis* LGV-L2 without centrifugation at MOI of 5 in the presence of indicated concentrations of inhibitors. Before imaging, the media was removed and replaced with fresh Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] (Gibco) buffered media with or without inhibitor. Infected cells were placed on an atmospherically controlled stage (37 °C, 5% CO_2) and imaged live at 4-min intervals for 20 h on the Inverted Axio Observer microscope (Zeiss). Images were processed using Metamorph software (Molecular Devices).

Movie S1