**Figure S1. Mid-cycle localization of cytoskeletal elements in relation to the C. trachomatis inclusion.** (A) *Chlamydia* inclusions at 48 h post-infection. Widefield microscopy images of EMO infected with mCherry-expressing Ct-L2. Scale bar: 100 µm. (B) β-catenin localization in relation to mature *C. trachomatis* inclusions. Confocal microscopy images of an EMO infected with mCherry-expressing *Ct*-L2 for 48 h and stained for β-catenin. Scale bar: 10 µm. (C-D) Septins localize to the *C. trachomatis* inclusion. Confocal microscopy images of an uninfected (C) and infected (D) EMO stained for Sept2 and F-actin. Scale bars: 10 µm. (E-F) Cytokeratins localize to the *C. trachomatis* inclusion. Confocal microscopy of an uninfected (E) and infected (F) EMO stained for cytokeratins and F-actin. Scale bars: 20 µm (uninfected) and 10 µm (infected). (G-H) Vimentin is not expressed in EMO epithelia. (G) Widefield deconvolution image of an endometrial organoid expressing tdTomato and stained for vimentin. Scale bar: 20 µm. (H) EMO infected with *C. muridarum* for 24 h and stained for vimentin. Scale bar: 20 µm (left) and 10 µm (right). (I) Vimentin localizes to the *Chlamydia* (*Ct*-L2 or *C. mu*) inclusion in primary stromal fibroblasts. Widefield deconvolution images of primary stromal fibroblasts uninfected or infected with *C. trachomatis* or *C. muridarum* (arrow denotes inclusion) for 24 h and stained for vimentin. Scale bars: 10 µm. For all images DNA stains used 2 µg/mL Hoechst (blue).
Figure S2. Validation of the *tepP* mutant and complementation. (A) Lysates from infected cells with wild type, *tepP* mutants or *tepP* mutants complemented with *tepP* on a plasmid were characterized by western blot analysis. The *Chlamydia* chaperone S1c1 is shown as a loading control.
Movie 1. *C. trachomatis* inclusions are fusogenic. Time-lapse spinning disk confocal microscopy of Ct L2-GFP inclusions in the process of fusion. Images were collected every 10 min for ~ 14 hours. Time display (h:m)

Movie 2. *C. muridarum* inclusions extrude into the lumen of EMOs. Time-lapse spinning disk microscopy *C.mu* – GFP inclusions extruding into the lumen of an EMO expressing membrane-bound tdTomato (magenta). Images were collected every 10 min for ~ 9 hours. Time display (h:m)
Movie 3. Lysis of *C. muridarum* inclusion and host cell. Time-lapse spinning disk confocal microscopy of *C. mu* – GFP inclusion extruding/lysing into the lumen of an EMO expressing membrane-bound tdTomato (magenta). Images were collected every 10 min for ~18 hours. Time display (h:m)

Movie 4. *C. trachomatis* L2 inclusions extruding basolaterally. Time-lapse spinning disk confocal microscopy of *Ct* L2-GFP inclusions extruding into the extracellular matrix. Images were collected every 10 min for 18 hours. Time display (h:m)
**Movie 5. CpoS-deficient inclusion and host cell lysis.** Time-lapse spinning disk confocal microscopy of a GFP-expressing cpoS mutant inclusion and host cell lysing and producing propidium iodide puncta. Images were collected every 10 min for 16 hours. Time display (h:m).

**Movie 6. CpoS-deficient Ct L2 inclusion becoming PI-positive.** Time-lapse spinning disk confocal microscopy of a GFP-expressing cpoS mutant inclusion becoming labeled with propidium iodide. Images were collected every 10 min for 16 hours. Time display (h:m).
Movie 7. Neutrophil recruitment towards an EMO infected with C. trachomatis L2. Time-lapse spinning disk confocal microscopy of an EMO (brightfield) infected with Ct L2-GFP and co-cultured with a tdTomato-expressing PMN (magenta). Images were collected every 3 min for 3 hours. Time display (h:m).

Movie 8. Neutrophil recruitment towards an EMO infected with CPAF-deficient Chlamydia. Time-lapse spinning disk confocal microscopy of an EMO (brightfield) infected with mCherry-expressing cpaf mutants (green) and co-cultured with PMN (magenta). Images were collected every 6 min for ~ 4 hours. Time display (h:m).
Movie 9. Neutrophil recruitment and transmigration to EMOs infected with TepP-deficient *Chlamydia*. Time-lapse spinning disk confocal microscopy of an EMO (brightfield) infected with GFP-expressing *tepP* and co-cultured with a tdTomato-expressing PMN. Images were collected every 10 min for 16.5 hours. Time display (h:m).

Movie 10. Neutrophil interacts with a budding inclusion. Time-lapse spinning disk confocal microscopy of an EMO (brightfield) infected with GFP-expressing *tepP* mutants and co-cultured with a tdTomato-expressing PMN (green). Images were collected every 10 min for 16.5 hours. Time display (h:m).