

Supplementary Figure 1. MA plot comparison of the Full PZ mutant gene expression to wild-type *C. trachomatis.* A) Only 10 genes were differentially regulated in the Full PZ mutant. These comparisons do not include the genes in the region of recombination with *C. muridarum.* Genes that are significantly up-regulated are highlighted in green; genes that are significantly down-regulated are highlighted in red. Significant regulation was determined by gene expression comparison ratios more than two standard deviations from the gene expression ratio average between two RNAseq runs.



Supplementary Figure 2. Additional mouse models to analyze the virulence and pathogenesis of the PZ chimeras. A) C3He/J mice were infected with 10⁵ IFU intravaginally and organs were harvested at day 7. Bacterial burdens were assessed using ddPCR to compare genome copies of *Chlamydia* to host at each tissue (n>3). ND means not detectable when compared to mock infection false positive levels. B) C57Bl/6 mice were infected with 10⁵ IFU intrarectally and rectal swabs were collected 7 days post-infection. The Right Partial PZ mutant was not able to be tested in these models.



Supplementary Figure 3. Schematic representations of the extended left end chimeras. Gene maps for the parental strains; wildtype *C. muridarum*, and wildtype *C. trachomatis* L2/tet, and the chimeras, RC#. Genes in blue are from *C. trachomatis* while orange genes represent those recombined from *C. muridarum*. Related plasticity zone genes share colors including: trp operon (dark blue), cytotoxins (pink), phospholipase D genes (green) and the gua operon (yellow). Genes with bold outlines correspond to the *CTL0402* or *TC0424* orthologs.

Apoptosis Videos

Supplementary Figure 4. Time lapse video microscopy of early lysis chimeras. Live-cell images captured every 20 minutes from 16 hpi to 28 hpi of cells infected with either (A) *C. muridarum*, (B) *C. trachomatis*, (C) RC826, or (D) RC1323. Infected cells were imaged by brightfield microscopy using a 40X objective while maintained under an environment of 5% CO2, 37°C, and humidity.