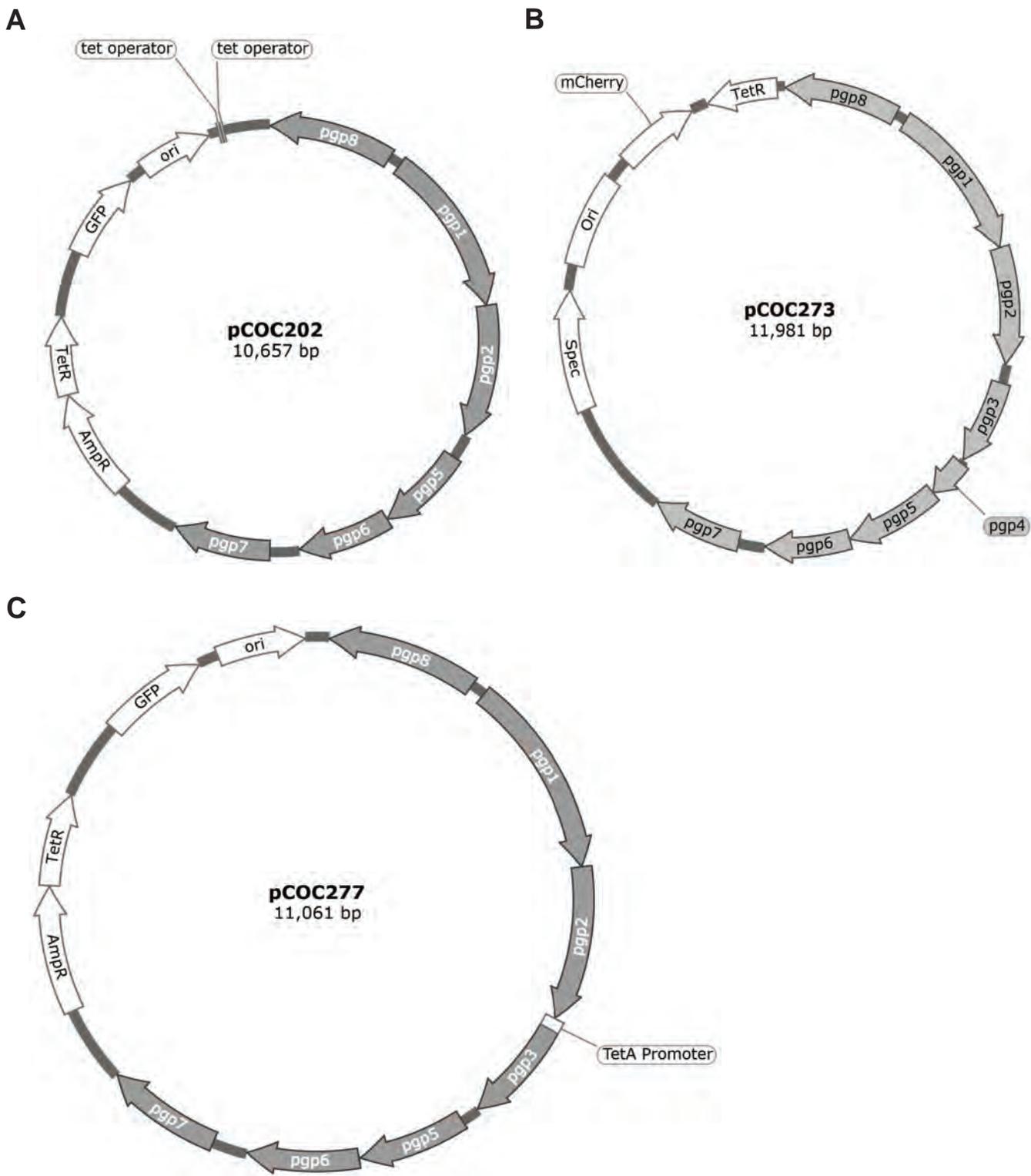
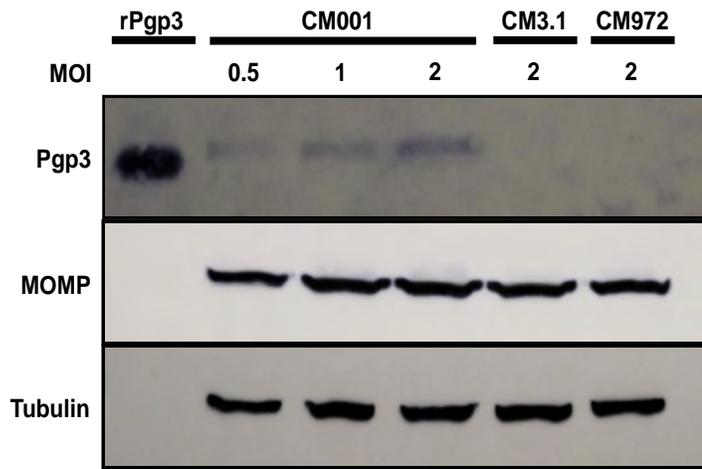
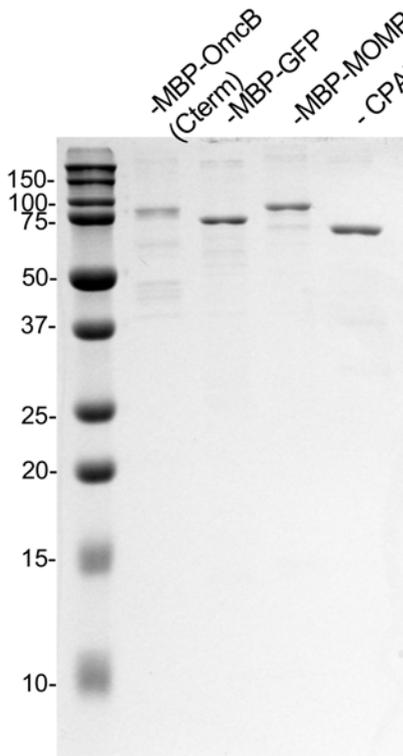
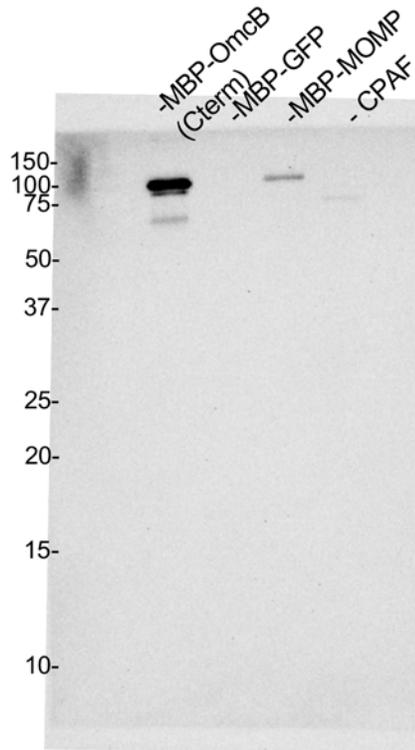


Supplemental table 1. Primers used in this study.

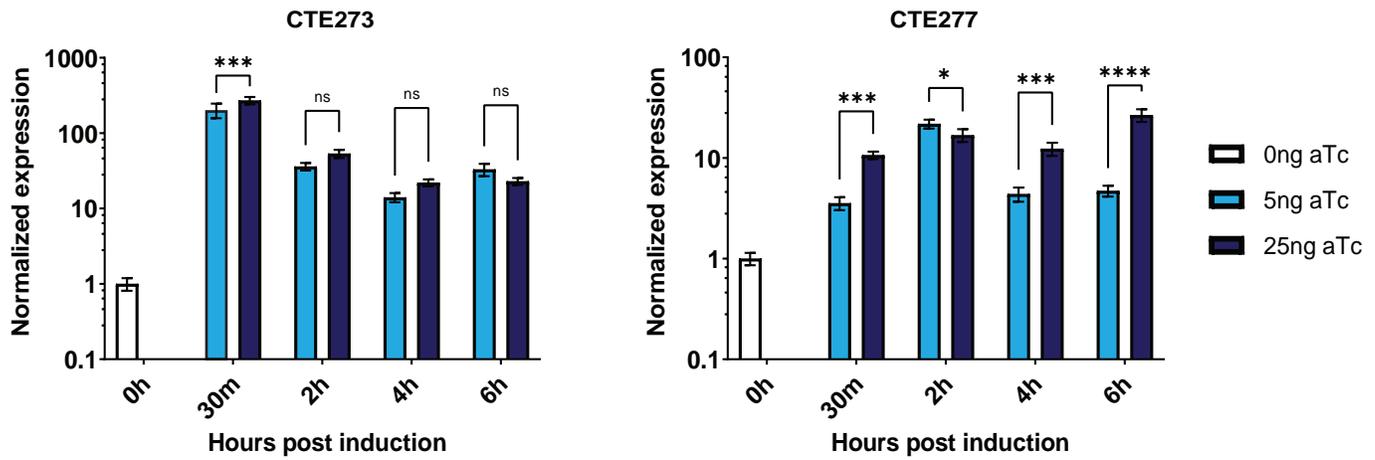
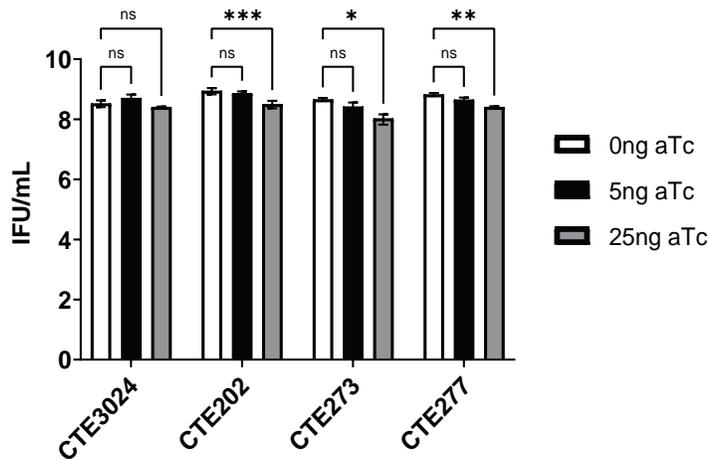
Primer	Sequence	Use (target, if applicable)
1f-tetAipgp3	ATGGAACAATTAGAACAAGAATCTATTAATCATTA ATTCCTAATTTTTGTTGAC	Cloning
1r-tetAipgp3	CATGTCATCCTTAAGCGTTTGTGGAGGTAT	
2f-term	AACAAACGCTTAAGGATGACATGTGATTCCG	
2r-term	AAGTTATCTATTCAGCCTTGGAAAAGTCTTAGGA GCTTTTTGC	
1r-5'pgp3	CGCCTGATGAGTATCCATAACTAATCGCGTAGG	
3f-3'pgp3	GTTATGGATACTCATCAGGCGTTCCTAAT	
3r-pgp4Pcil	ATCTATTCAGCCTTGGAAAAACATGTCTTTTCTAG ACAAGATAAGCATAA	
4f-pgp3-4	TGATTAATTAACCTTACTTCAATAATTTCAAACCTA GATCATGTATCACTAATGTGTAGCTACTTGCACAA TAGTAACCTTTGCAATCATGACACATGTCCA	pCOC202 random nucleotide linker
4r-pgp3-4	TGGACATGTGTCATGATTGCAAAGGTTACTATTGT GCAAGTAGCTACACATTAGTGATACATGATCTAG GTTTGAAATTATTGAAGTAAGTTTAATTAATCA	
omcA F1	CTGCTTTACTCGCTGCTTTATG	RT-qPCR (<i>omcA</i>)
omcA R1	GATAGGTGCGCATGGATCTT	
23S F1	GCTCACGTTTCGGAAAGGATAA	RT-qPCR (23S)
23S R1	GTGCTTACACCTCCAACCTATC	
pGP3 F1	TCACCTTCTCGTACCAAAGC	RT-qPCR (<i>pgp3</i>)
pGP3 R1	TCTGGGAGCATGTTCTTAGTC	
pGP4 F2	TATTCCCAGACTCTCCTGTTCT	RT-qPCR (<i>pgp4</i>)
pGP4 R2	CTAGACAAGATAAGCATAATCAAAGCC	



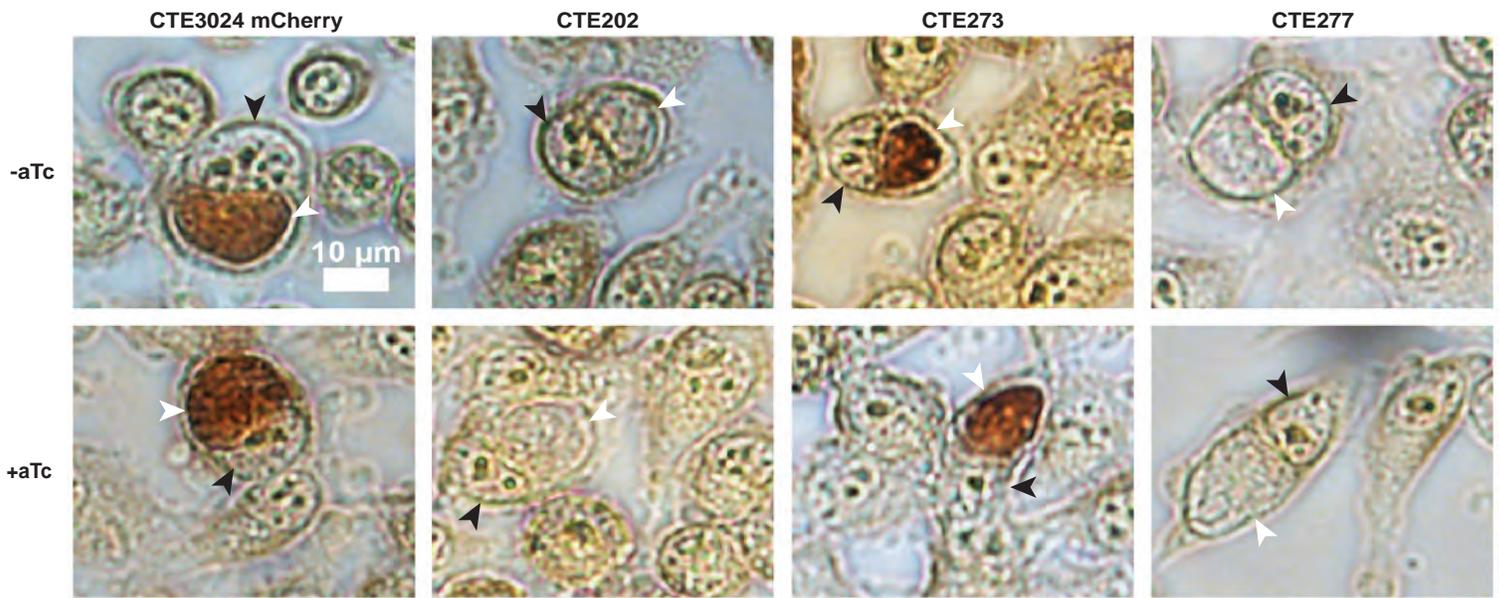
Supplemental figure 1. Shuttle vectors used in this study. (A) pCOC202, (B) pCOC273, and (C) pCOC277 were transformed into CTE3024 to create CTE202, CTE273, and CTE277 respectively. Plasmid maps were generated using SnapGene v. 6.1.

A**B****C**

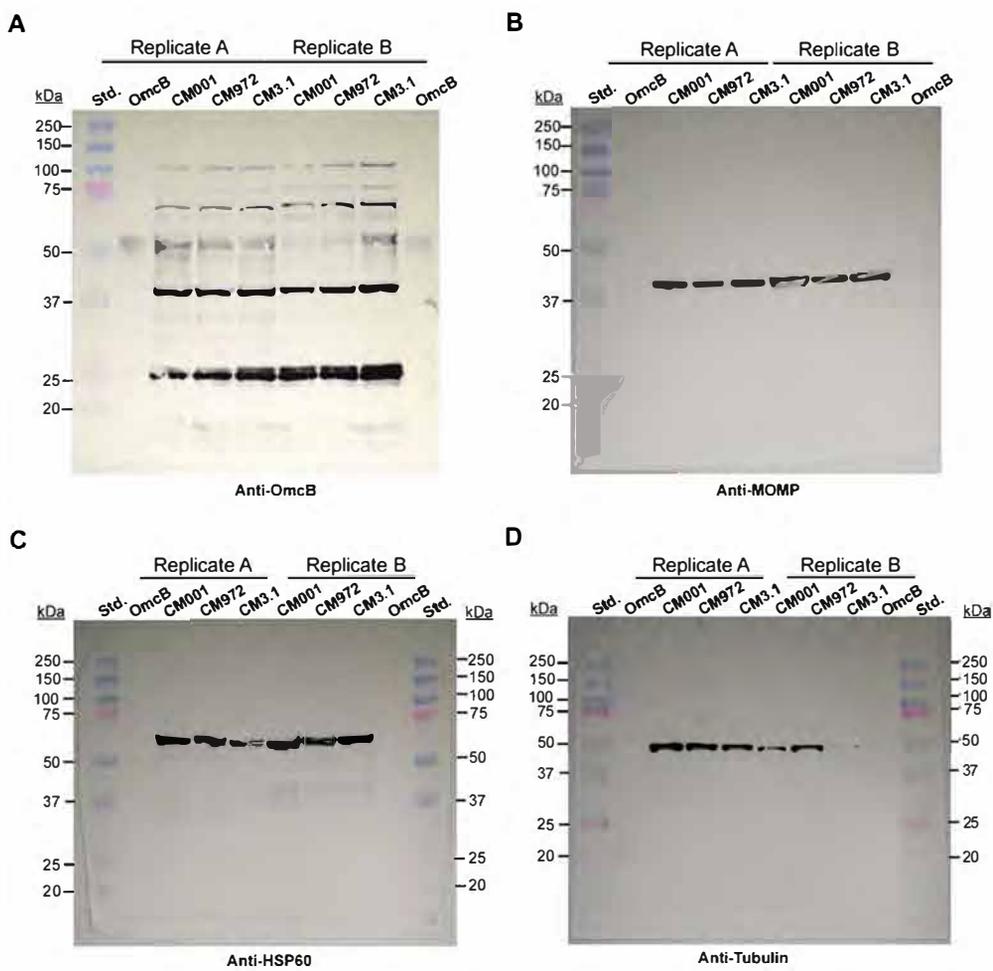
Supplemental figure 2. Validation of Pgp3 and OmcB antisera. (A) L929 cells infected with *C. muridarum* strains: CM001 (wild-type), CM3.1 (plasmid-deficient) or CM972 (plasmid-deficient) at an MOI=0.5, 1, and 2 were lysed in 1X RIPA buffer at 24 h p.i. Proteins from whole cell lysates were separated by SDS-PAGE and transferred to nitrocellulose membranes. 100 ng recombinant Pgp3 (rPgp3) was included as a positive control. Membranes were probed with rabbit anti-Pgp3 (1:1000), mouse anti-MOMP (1:500), and mouse anti-tubulin (1:500) antibodies. Images are representative of n=2 experiments. (B and C) Each lane was loaded with 250 ng of recombinant protein and stained with Coomassie blue (B). Approximately 25 ng protein was transferred per lane and probed with anti-OmcB serum diluted 1:500 (C).

A**B**

Supplemental figure 3. 5 ng/mL anhydrotetracycline induces *pgp3* expression without impacting the production of infectious progeny. (A) Transcription of *pgp3* measured by qRT-PCR from CTE273 and CTE277 following induction with 5 ng/mL or 25 ng/mL of aTc at 20 h p.i. Graphs show the average normalized expression of two biological replicates each assayed in triplicate. Gene expression was normalized to 23S and the uninduced control. (B) Infectious progeny quantified from monolayers treated as in (A). Chlamydiae were harvested 40 h p.i. and quantified as IFU/ml. Bars represent the average of two replicates. Data in (A) and (B) are representative of two experiments. (ns) not significant $P > 0.05$, (*) $P < 0.05$, (**) $P < 0.005$, (***) $P < 0.0005$, (****) $P < 0.001$ by two-way ANOVA with Šídák's multiple comparisons (A) or Tukey's multiple comparisons (B).



Supplemental figure 4. CTE273, but not CTE277 accumulate glycogen within their inclusions. Pgp4-expressing CTE3024 mCherry and CTE273 inclusions stained positively with iodine, while CTE202 and CTE277, which lack *pgp4*, stained negatively for glycogen accumulation. Infected L929 cells were treated with 5ng/ml aTc at 22 h p.i. or left untreated. Cells were fixed at 40 h p.i., stained with iodine, and imaged at 200X. Black arrows indicate nuclei and white arrows indicate chlamydial inclusions.



Supplemental figure 5. Immunoblots of whole cell lysates from CM001, CM972, and CM3.1 infected cells. Uncropped immunoblot images from Figure 6. Membranes were probed with (A) mouse OmcB antiserum or (B) mouse anti-MOMP (C) mouse anti-HSP60 and (D) rat anti-tubulin antibodies. (Std.) = protein standards.