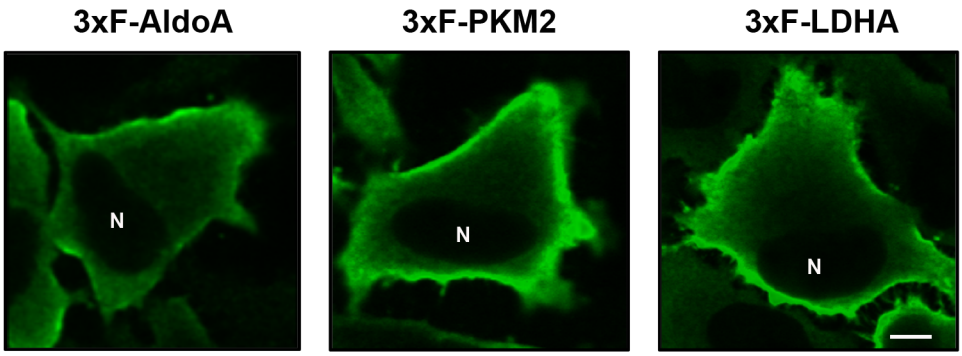

Figure S1. Expression of tagged host glycolytic enzymes in uninfected cells. Confocal micrographs of HeLa cells expressing 3xFLAG-tagged Aldolase A (AldoA), Pyruvate Kinase (PKM2), or Lactate Dehydrogenase (LDHA) (green). The cells were fixed at 42 h post transfection and stained with anti-FLAG antibodies. N: Nucleus. Scale bar: 10μm.

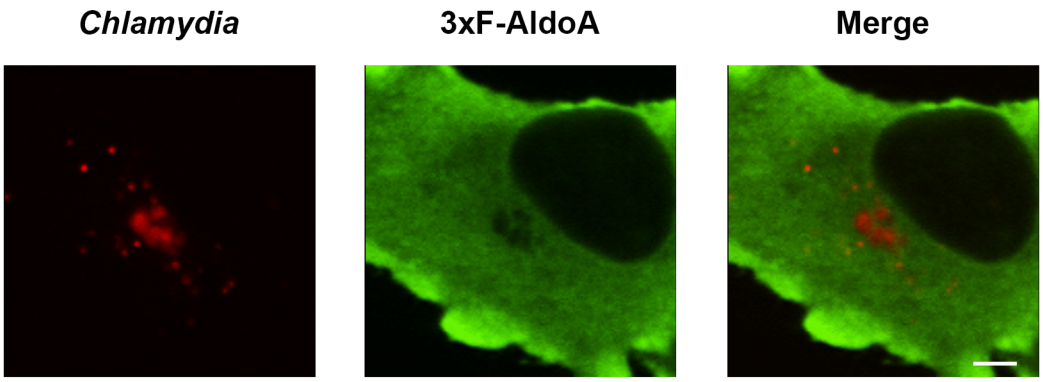

Figure S2. Inclusions are negative for Aldolase A at 8 h post infection. Confocal micrographs of HeLa cells expressing 3xFLAG-tagged Aldolase A (AldoA), infected for 8 h with the mCherry CtL2 strain (red), and immunostained with anti-FLAG antibodies (green). The merge is shown on the right. Scale bar: 10μm.

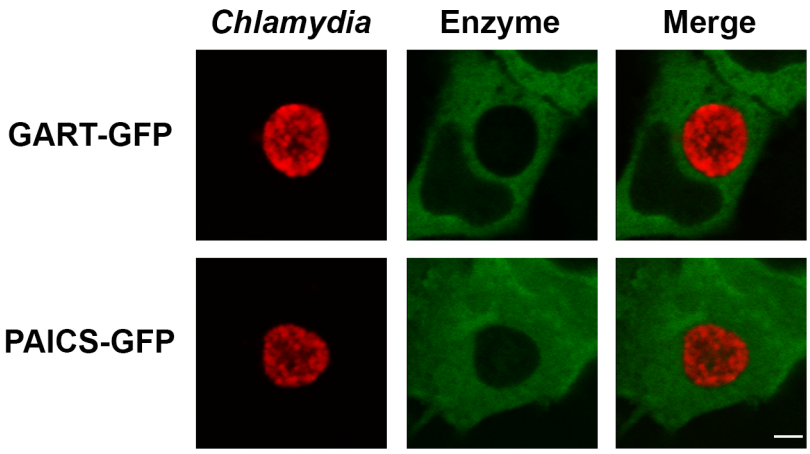

Figure S3. GFP-tagged purine biosynthesis enzymes do not localize at the inclusion membrane. Confocal micrographs of HeLa cells expressing GFP-tagged Glycinamide ribonucleotide transformylase (GART) or Phosphoribosylaminoimidazole carboxylase (PAICS) (green) and infected for 24 h with the mCherry CtL2 strain (red). The merge is shown on the right. Scale bar: 10μm.

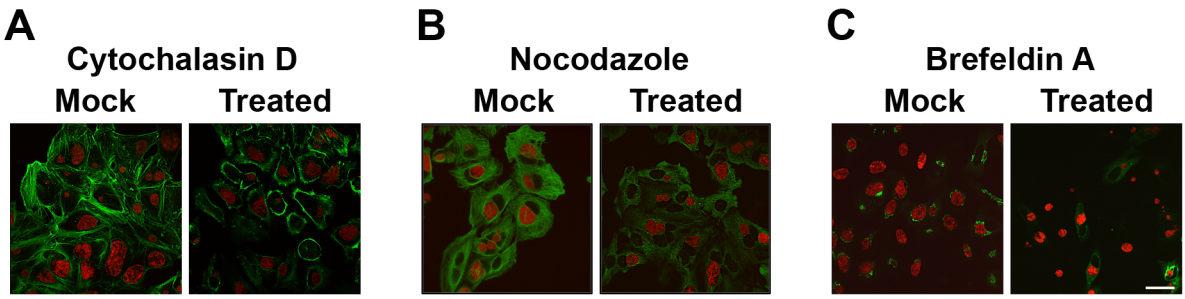

Figure S4. Validation of Cytochalasin D, Brefeldin A, and Nocodazole drug treatments. (A) Confocal micrographs of HeLa cells infected with the mCherry CtL2 strain (red) and fixed at 24 h post infection following the absence (Mock, left panel) or presence of 1µM Cytochalasin D at 23.5 h post infection (Treated, right panel) and immunostained with Phalloidin (green). (B) Confocal micrographs of HeLa cells infected with the mCherry CtL2 strain (red) and fixed at 24 h post infection following the absence (Mock, left panel) or presence of 33µM Nocodazole at 23.5 h post infection (Treated, right panel) and immunostained with anti-α-tubulin antibodies (green). (C) Confocal micrographs of HeLa cells expressing YFP-Golgi (green), infected with the mCherry CtL2 strain (red), and fixed at 24 h post infection following the absence (Mock, left panel) or presence of 1µg/mL Brefeldin A at 6 h post infection (Treated, right panel). Scale bar: 20µm.

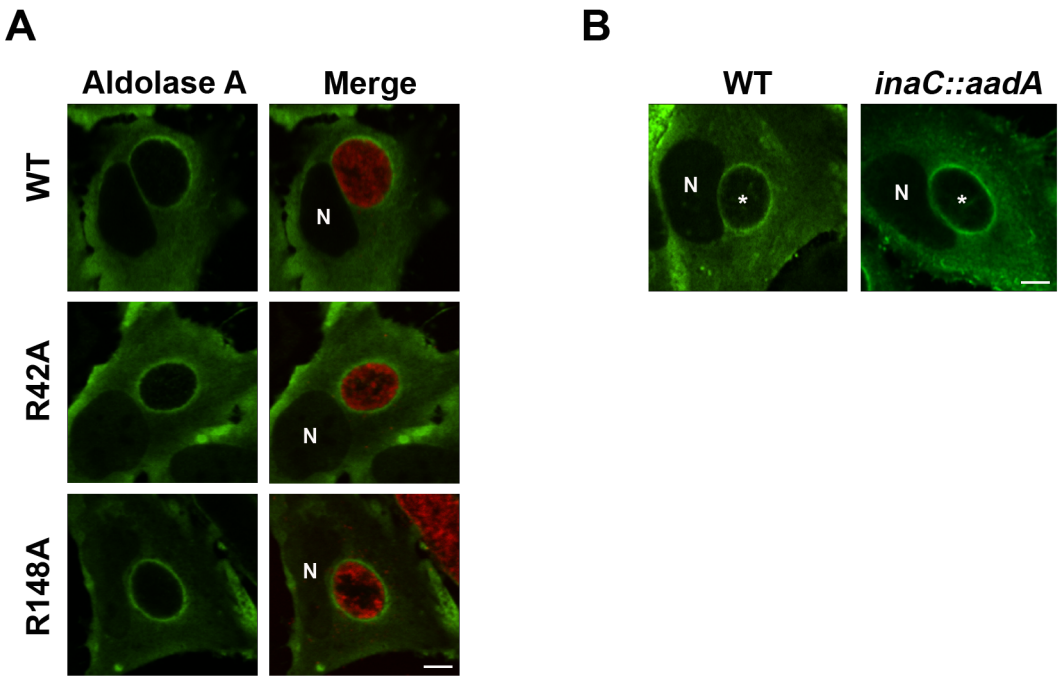

Figure S5. Host actin does not affect localization of Aldolase A at the inclusion membrane. (A) Confocal micrographs of HeLa cells expressing wild-type 3xFLAG-AldoA (WT, top panels), or point mutants no longer able to bind actin 3xFLAG-R42A AldoA (R42A, middle panels) and 3xFLAG-R148A AldoA (R148A, bottom panels) (green), infected with the mCherry CtL2 strain (red) and fixed at 24 h post infection. (B) Confocal micrographs of HeLa cells expressing 3xFLAG-AldoA (green) and infected with wild-type *C. trachomatis* (WT, left panel) or an *inaC* mutant strain (*inaC::aadA*, right panel) that were fixed at 24 h post infection. The asterisk indicates the inclusion. N: Nucleus. Scale bar: 10μm.

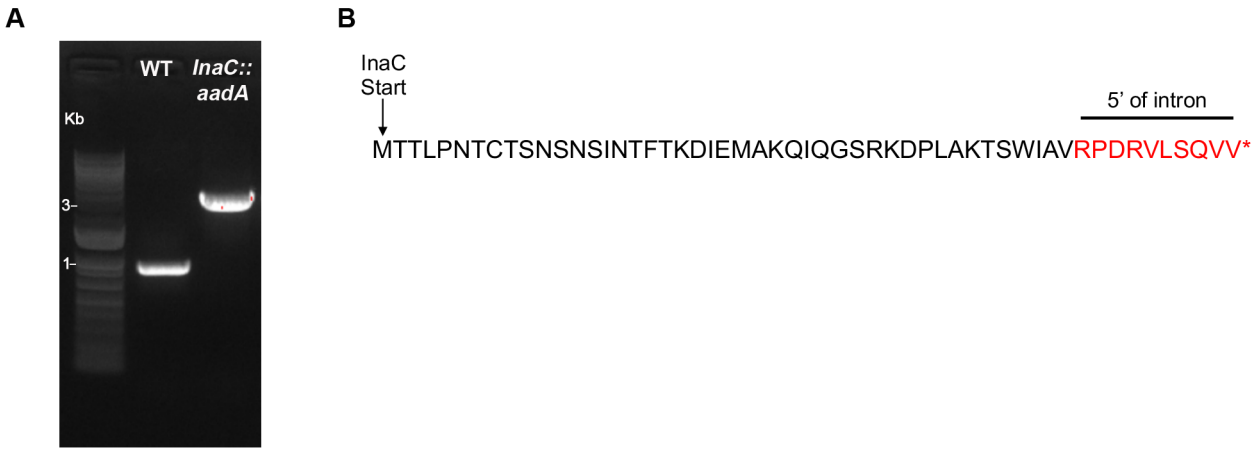

Figure S6. Validation of group II intron insertion in the *C. trachomatis inaC::aadA* mutant. (A) PCR analysis of the *inaC::aadA* mutant. The *inaC* ORF was amplified from genomic DNA extracted from WT *C. trachomatis* (WT) or the *inaC::aadA* mutant and the corresponding PCR products were resolved on a 1% DNA agarose gel stained with ethidium bromide. Lane 1: molecular weight marker; lane 2: WT; lane 3: *inaC::aadA* mutant. The marker sizes are listed in kilo base pairs to the left. (B) The site of insertion of the group II intron was determined by Sanger sequencing. A translation of the resulting InaC truncated peptide is presented. The asterisk denotes the early stop codon introduced by insertion of the group II intron (black: InaC, red: group II intron).

Table S1: Primers used in this study

Primer Name	Primer Sequence
AldoA Fwd BamHI	GGAGGATCCATGCCCTACCAATATCCAGCACTG
AldoA Rev XhoI	CTCCTCGAGTTAATAGGCGTGGTTAGAGAC
PKM2 Fwd BamHI	GGAGGATCCATGTCGAAGCCCCATAGTGAAGC
PKM2 Rev XhoI	CTCCTCGAGTCACGGCACAGGAACAACACG
LDHA Fwd EcoRI	GAAGAATTCTATGGCAACTCTAAAGGATCAG
LDHA Rev XhoI	CTCCTCGAGTTAAAATTGCAGCTCCTTTTG
HAAldoA 5 KpnI	GGTGGTACCATGTACCCTTACGATGTACCGGATTACGCAATGCCCTACCAATATCC
AldoA 3 NotI	GCGGCGGCCGCTTAATAGGCGTGGTTAGAGACG
R42A AldoA Fwd	GAGCATTGCCAAGGCACTGCAGTCCATTGG
R42A AldoA Rev	CCAATGGACTGCAGTGCCTTGGCAATGCTC
R148 AldoA Fwd	CTTCGCCAAGTGGGCATGTGTGCTGAAG
R148 AldoA Rev	CTTCAGCACACATGCCCACTTGGCGAAG
CTL0184 129 130 IBS1/2	AAAAAAGCTTATAATTATCCTTATCATGCATCGCAGTGCGCCCAGATAGGGTG
CTL0184 129 130 EBS1/delta	CAGATTGTACAAATGTGGTGATAACAGATAAGTCATCGCAGGTAACTTACCTTTCTTTGT
CTL0184 129 130 EBS2	TGAACGCAAGTTTCTAATTTCGATTCATGATCGATAGAGGAAAGTGTCT
CTL0184 Up	GCAGAAATAGGTCTGAGGCTG
CTL0184 Dwn	CAACGAATTAGACATTCGTC

Table S2: Primers and probes used for quantitative PCR analysis

Gene	Probe	Primer	Sequence
16S rRNA	#105	Ct 16s Fwd	gccgctaataccgaatgtg
		Ct 16s Rev	aaggtcctaagatccccttcttt
omcA	#33	CTL0703 Fwd	cggtggttgcaactcttgta
		CTL0703 Rev	tcagcatcttggtgcgtatc
pgi	#69	CTL0633 Fwd	gctgctgcgatagcaagac
		CTL0633 Rev	atacagaaactctgcaagacgaag
dhnA	#24	CTL0467 Fwd	ttcagtcaagttgaagatgccta
		CTL0467 Rev	agtaaatcgttgctccgacag
pykF	#159	CTL0586 Fwd	gataggccctgcaacgaata
		CTL0586 Rev	cattcatccctgcatcgag