

Supplemental Material

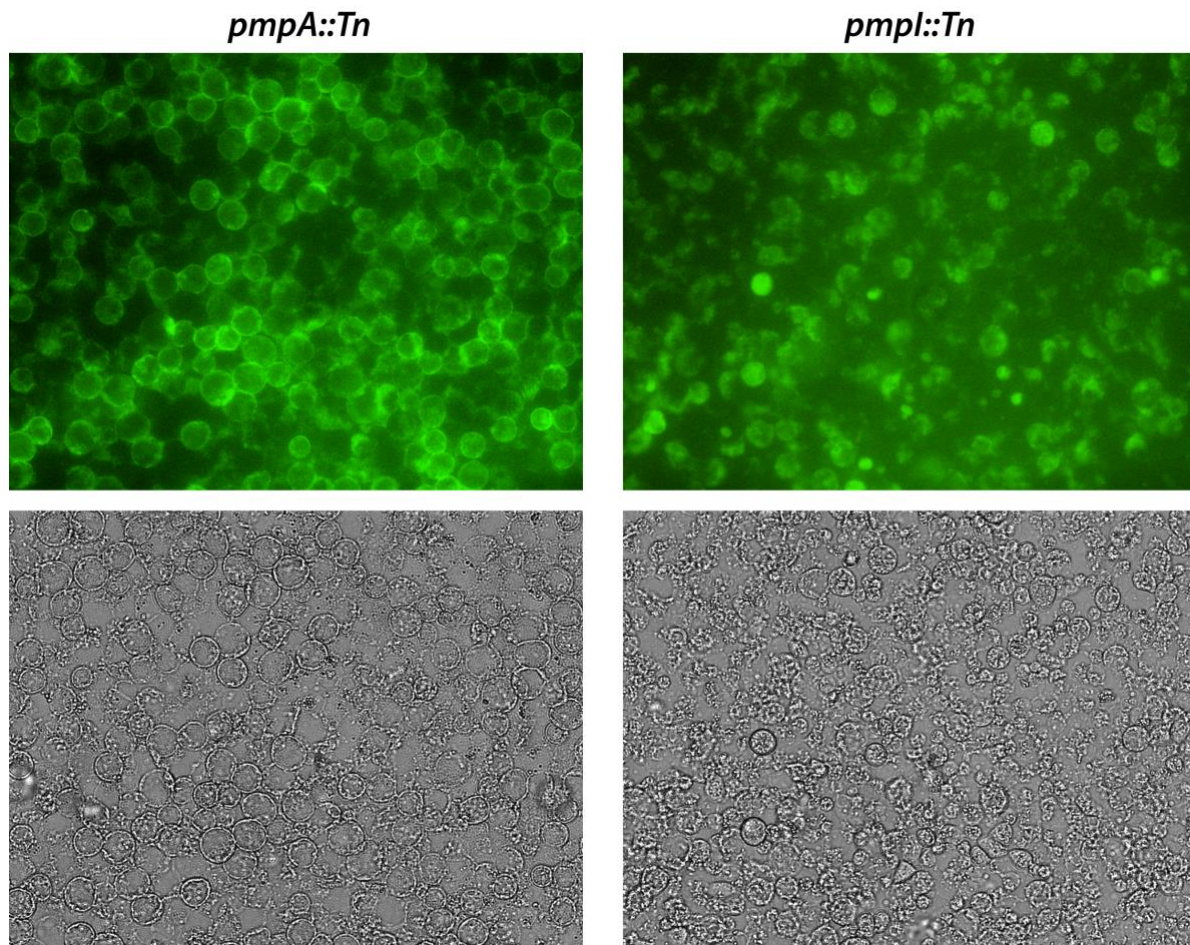
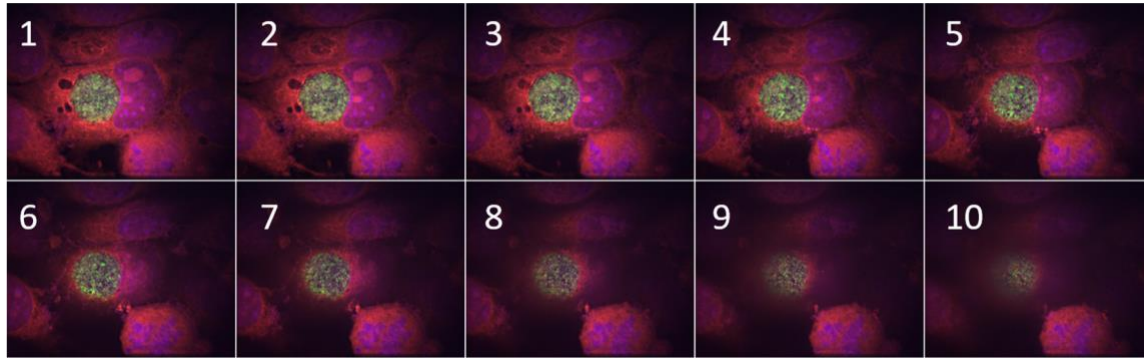


Fig S1 Accumulation of extrusions in *pmpA::Tn* infected monolayers. McCoy cells were infected with *pmpA::Tn* or *pmpl::Tn* and imaged at 46 hpi. Images were acquired by live fluorescence microscopy at 20× magnification, taken at focus planes above the monolayer of adherent cells. Upper panels: GFP-expressing *C. muridarum* mutants (green). Lower panels: brightfield images. We draw the reader's eye to the large number of circular, ring-like objects in the *pmpA::Tn* sample, which are strongly reminiscent of cell-free *Chlamydia* extrusions. These structures are largely absent in *pmpl::Tn* infected monolayers, and cells infected with WT *C. muridarum* (not shown).

WT *C. muridarum*



pmpl::Tn

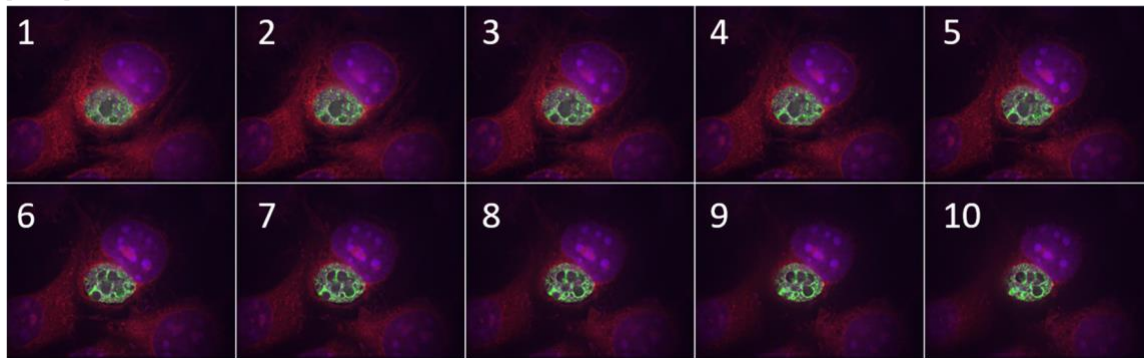


Fig S2 Confocal z-stack of WT and *pmpl::Tn* mutant. L929 cells were infected in 8-well microSlide dishes at an MOI of ~0.1 with WT *C. muridarum* or *pmpl::Tn*. Cells were fixed at 24 hours post infection, and stained with anti-LPS-GFP conjugated antibody (green), evans blue counterstain (red), and DAPI (blue). Images were taken using a 150X objective on an Olympus IX81/31 spinning disk confocal inverted microscope, and captured using an Andor Zyla 4.2 sCMOS camera. Images represent 10 z-stacks at 0.3 μm intervals of representative inclusions.

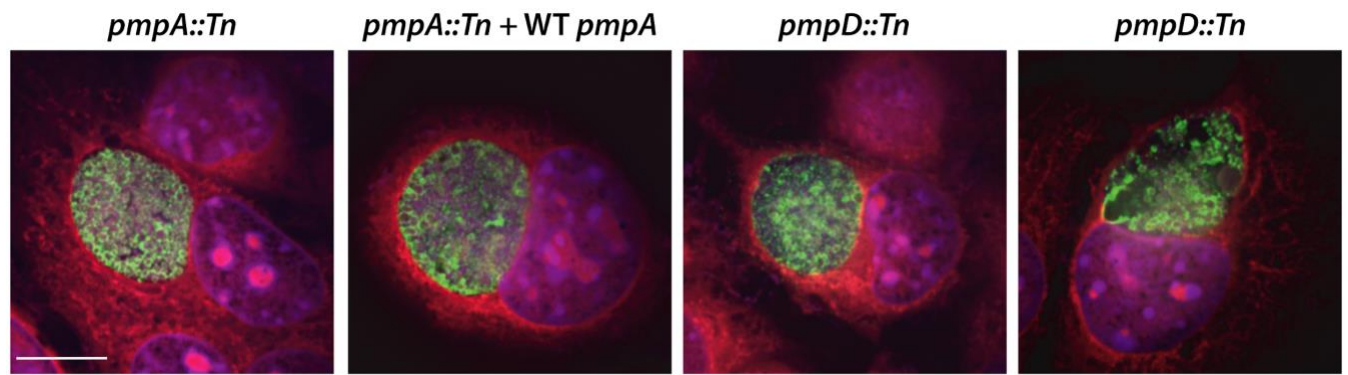


Fig S3. Morphologies of inclusions containing *pmpA::Tn*, *pmpA::Tn* complemented, and *pmpD::Tn* strains. McCoy cells were infected with indicated strains and analyzed by confocal immunofluorescence microscopy at 24 hpi. Anti-LPS, green; DAPI, blue, evans blue, red. Scale bar = 10 μ m.

Table S1 *C. muridarum* WT compared to *C. muridarum* Str Nigg (NC_002620.2)

Location (NCBI)	Gene	Change ^a	NC_002620	WT Cm	Search target (Base Change)
10505	TC0007	/	K	A	TCTAATGTAAAACGGCAAA (K→A)
10558	TC0007	/	W	A	AAGTGGCTAGGCTATTACGA (W→A)
10564	TC0007	/	Y	A	CTAGGCTATTACGAWTTTTA (Y→A)
32915	TC0027	/	M	G	ATCTGGTTTTTGACGGACCA (M→G)
58882	TC0052	/	M	T	TTGGGTTCCAAGTGGTCATT (M→T)
58904	TC0052	/	M	T	TAAGATAGAGGTCTCAAGCT (M→T)
59062	TC0052	DEL	GTT	-	TAGTATCTGTAGCGCTAACT (GTT→ -/-)
126383	IGR	/	Y	G	ACTCGACCAGCTTCATCCTA (Y→G)
126408	IGR	DEL	G	-	ATTTTTCGTTAGGGGCAAGG (G→ -)
126418	IGR	INS	-	A	AGGGGCAAGGGTCCTTGCAA (- →A)
126436	IGR	/	W	A	AAGATCTTTTCATTCTAAGT (W→A)
126479	IGR	DEL	A	-	TATTTAATCTATGGCTAAAA (A→ -)
136842	23S	/	A	R(A/G)	GGGCTAAGCATAAAAACCGAC (A→R)
141412	23S rlmD	INS	-	A	GTGCTCATGGCGTCTACAAA (- →A)
158800	23S	/	Y	R(A/G)	GGGCTAAGCATAAAAACCGAC (Y→R)
169452	TC0138	INS	T(3)	T(6)	ACTGGTGAAACCTCTTATTT (- →TTT)
187432	TC0255	H→Y	G	A	AGGAGCATGGTCTCTGTAAT (G→A)
200671	TC0168	T→R	G	C	CTCTAAATCCCACAGAATTT (G→C)
358414	TC0301	E→D	R	T	TTGCGTTCTCAAGATCCGGA (R→T)
401302	TC0338	/	Y	G	ATATGGGTGTATGGATTTTCG (Y→G)
403623	TC0341	L→Y	TA	AC	CTTTGTTTCGTTCTAGAAAAGT (TA→AC)
403652	TC0341	S→P	T	C	ATTTTGGGTCCAAAGCATTT (T→C)
403714	TC0341	/	M	G	TCTTGTTTCGATCATCAATG (M→G)
404473	TC0342	/	M	T	AGTATTTTTGGTTTTATTGA (M→T)
404885	TC0342	DEL	A	-	AGAAGGCATATTTTACTTCA (A→ -)
405819	TC0343	/	Y	G	GAATTTCTCCCTATTGATGA (Y→G)
419119	TC0359	/	R	C	TTCTAGAACTAAACAGCCTC (R→C)
468932	TC0408	P→Q	G	T	TACTTGAGGTTATTGCTGGT (G→T)
473705	TC0412	DEL	G	-	AAAGTATTATGGCCATTCAA (G→ -)
5000055	IGR	DEL	G(12)	G(11)	GTTGAAAGAGGTCTCTTTCCT (G12→G11)
505388	TC0436	INS	C(12)	C(13)	AAATAGTGATGAATAAACTG (C12→C13)
515204	TC0437	/	S	G	TGACCGCATCCTATTATCTA (S→G)
545710	TC0447	INS	G(13)	G(14)	TTACAAAGAGCGTTTCCTCT (G13→G14)
600435	TC0493	Silent	C	A	CGACCTCTTATTCTTGTTCC (C→A)
712587	TC0596	/	R	C	GAATGGTTTATGACGACCAC (R→C)
846475	IGR	DEL	G	-	TTGTCTTTGAAGATCCTTTG (G→ -)
866102	TC0727	Silent	G	T	TCAGCTAAGCTAATAACGTT (G→T)
1004257	TC0867	S→P	T	C	TCGGAGCTCTTACAGCCCAA (T→C)
1022529	TC0879	Silent	G	A	AGAAGATAGTAAATCTTCTA (G→A)
1022551	TC0879	/	N	A	TCACGAACAACAAGAGTGG (N→A)
1035913	TC0893	/	N	A	TTTTACAGAGGAAGTCATGT (N→A)

^a DEL denotes a deletion of 1 or more bases. INS denotes an insertion of 1 or more bases. / denotes ambiguous to confirmed base.

TABLE S2 Primers used for confirming transposon insertions

Primer name	Sequence	Primer location	Amplicon (without Tn)	Insertion confirmed
UWCM_001_F	AAGTGACTTAACGCAAATTCATAG	242,604-242,627	503 bp	<i>TC0206::Tn</i>
UWCM_001_R	TCTTTTCGAATTGCTGACGAG	243,106-243,087		
UWCM_007a_F	GTTACGTTTGCTATTGGGGTATC	301,330-301,352	517 bp	<i>glgB::Tn</i>
UWCM_007a_R	GCTACCATCATCATCCAGAG	301,846-301,827		
UWCM_007b_F	CATCAGCAACTAAAGAGACTCTG	498,197-498,219	483 bp	<i>macP::Tn</i>
UWCM_007b_R	AGATAATCTTTTCATTATGAGCTGTC	498,679-498,654		
UWCM_009_F	GTTAATCCTCCTGCAGACATAC	220,531-220,552	314 bp	<i>TC0189::Tn</i>
UWCM_009_R	TTGGGCTTTATTCTTGTTTTCTAC	220,844-220,821		
UWCM_010_F	TGTTACTTTTGAGAATGCCGAAG	362,995-363,016	271 bp	<i>TC0305::Tn</i>
UWCM_010_R	GCTCGACGACATTGCTTAAC	363,265-363,246		
UWCM_011_F	TCCGGCTTTAAATCCCAGATG	686,974-686,993	348 bp	<i>pknD::Tn</i>
UWCM_011_R	CTCTCGTAGGGTAGCTCTAAAG	687,321-687,300		
UWCM_012_F	AGGAATTGGAGCAGAAATGC	827,236-827,255	263 bp	<i>pmpA::Tn</i>
UWCM_012_R	CATTATGATCTAAGGCTGCAC	827,498-827,478		
UWCM_013_F	GTGTA AACCATCAACATCCTTATTAG	413,525-413,550	304 bp	<i>folD::Tn</i>
UWCM_013_R	CAAAGACAATATCCGATCCCAATC	413,828-413,805		
UWCM_014a_F	CTACTGAAGGACATGCGATTG	232,665-232,685	443 bp	<i>pmpD::Tn</i>
UWCM_014a_R	CTGAGCTTTAGAAGAAAATGATGC	233,107-233,084		
UWCM_014b_F	CGAGCTATTTTACATGCTGTTG	395,529-395,550	366 bp	<i>gnd::Tn</i>
UWCM_014b_R	AGGGTAAAAAACGAATGAAAGAG	395,894-395,872		
UWCM_019_F	CGAATAATTGTCCAGCAGCTAAG	319,401-319,423	291 bp	<i>pmpI::Tn</i>
UWCM_019_R	CTAATAGCTACATACCACACCCC	319,691-319,669		
UWCM_021_F	GTTTCAATTTCTCTCTAAATCTCTG	26,513-26,537	280 bp	<i>IGR::Tn</i>
UWCM_021_R	CATCATATTGCTCTGGAGTAGTC	26,792-26,770		
UWCM_022_F	CTAATAACGCTGTAGATAGGTGG	361,333-361,355	2506 bp	<i>TC0303::Tn^a</i>
UWCM_022R_R	CTATGCTCACCAACTACCTCC	363,838-363,818		
UWCM_023a_F	AGTTGAGTTCGATATTACTGTTCC	524,538-524,561	407 bp	<i>TC0438::Tn</i>
UWCM_023a_R	GCGTGGTAGATTTAGCTTCTTTC	524,944-524,922		
UWCM_023b_F	CAATTCCTTAACACAGAAGC	1,001,452-1,001,472	361 bp	<i>IGR::Tn</i>
UWCM_023b_R	TTCTACTAACAATATTAATGTGATTGG	1,001,812-1,001,785		
UWCM_024_F	GAGGAGAGTTCGATAAAGTGTG	429,694-429,715	304 bp	<i>ribF::Tn, truB::Tn</i>
UWCM_024_R	GTGGAGCTTATTTGGAAGAGC	429,997-429,977		
UWCM_025_F	GTTTTGCCAGCTCTTTGAC	725,811-725,830	356 bp	<i>TC0606::Tn</i>
UWCM_025_R	GATTATGATTGCTCCGTTGATC	726,166-726,144		
UWCM_026_F	CACGTTAGATTGCTCCTGCATAATC	251,137-251,160	303 bp	<i>rnuC::Tn</i>
UWCM_026_R	GTAGATGCTAATGATTCCTTCTCTC	251,439-251,415		

^a Insert occurred with a 2073 bp deletion beginning at 361,528