

1 Table S1. Bacterial and yeast strains used in this study.

Strains	Phenotype or Description	References
<i>C. burnetii</i>		
RSA439	Phase II, Clone 4 (NMII)	(18)
RSA439 MK24-33	Cbu0041::Tn, Cm ^r	(18)
RSA439 MK25-95	<i>dotB</i> ::Tn, Cm ^r	This study
RSA439 MK21	<i>icmX</i> ::Tn, Cm ^r	(18)
RSA439 MK22	Intergenic Cbu0179-Cbu0180::Tn, Cm ^r	This study
RSA439 MK21-1	<i>enhC</i> ::Tn, Cm ^r	This study
RSA 439 MK2 EVS101-CirA	Cbu0041::Tn, Cm ^r , pEVS101-CirA	This study
<i>E. coli</i>		
DH5 α	<i>F</i> '(Φ 80d Δ (<i>lacZ</i>)M15), <i>recA1</i> , <i>endA1</i> , <i>gyrA96</i> , <i>thi1</i> , <i>hsdR17</i> (<i>rk-mk</i> +), <i>supE44</i> , <i>relA1</i> , <i>deoR</i> , Δ (<i>lacZYA-argF</i>), U169	Stratagene
<i>S. cerevisiae</i>		
W303	<i>MATa ura3-1 leu2-3,112 his3-11,15 trp1-1 ade2-1 cad-100 rad5-535</i>	(18)
W303 pYesNTA-CirA	W303, pYesNTA-CirA	(18)

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20 Table S2. Plasmids used in the study.

Plasmid	Description	References or Source
<i>C. burnetii</i> plasmids		
pKM225	pMW1650, <i>com1p</i> -TnA7, <i>groESp</i> -mCherry, <i>com1p</i> -cat, Cm ^R	(18)
pKM244	pJB908a, <i>groESp</i> -mCherry, <i>com1p</i> -cat, Cm ^R , Amp ^R	(18)
pEVS101	pJB908a, <i>groESp</i> -mCherry, <i>com1p</i> -cat, Cm ^R , Amp ^R , p1169-Kan ^R	This study
pEVS101-CirA	pEVS101-CirA	This study
Ectopic expression plasmids		
pRK5-myc-RhoA WT	pRK5-myc::RhoA, <i>carb</i> ^f	Addgene 12962
pRK5-myc-RhoA CA	pRK5-myc::RhoA Q63L, <i>carb</i> ^f	Addgene 12964
pRK5-myc-RhoA DN	pRK5-myc::RhoA T19N, <i>carb</i> ^f	Addgene 12963
pRK5-myc-Rac1 WT	pRK5-myc::Rac1, <i>carb</i> ^f	Addgene 12985
pEGFP-C1	C-terminal fusion to EGFP, <i>kan</i> ^f	Clontech
pEGFPC1-CirA	pEGFP-C1::Cbu0041	(18)
Yeast expression plasmids		
p415ADH	pGal, <i>carb</i> ^R , Leu2	(27)
P415ADH-Rho1	P415ADH:: <i>rho1</i>	This study
P415ADH-MRP2	P415ADH:: <i>mrp2</i>	This study
P415ADH-Met16	P415ADH:: <i>met16</i>	This study
P415ADH-Nut2	P415ADH:: <i>nut2</i>	This study
P415ADH-Jip5	P415ADH:: <i>jip5</i>	This study
pYESNTA	pGal, Carb ^R , Ura	(18)
pYESNTA-CirA	pYESNTA::CirA	(18)

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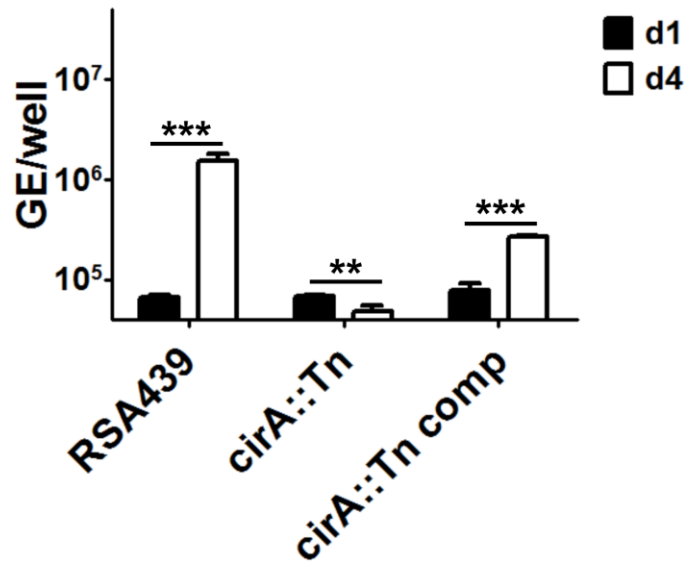
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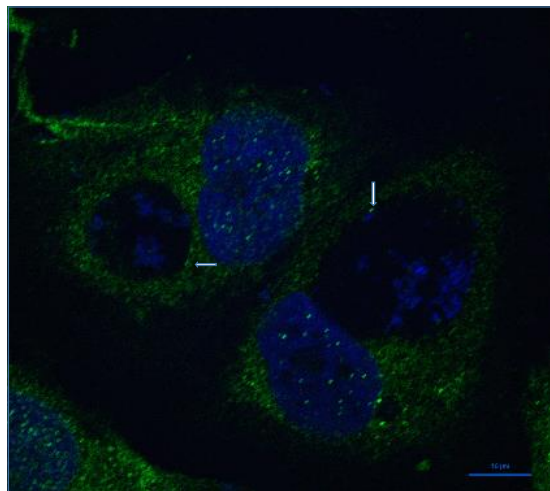
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38 **Figure S1.** Replication of wild-type RSA439, *cirA::Tn*, and the complemented mutant in J774A.1
 39 macrophages infected with an MOI of 50. Genome equivalents (GE) were determined at 1 d and
 40 4 d using quantitative PCR. Data are representative of two independent experiments.



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42 **Figure S2.** Figure A.2. Localization of endogenous RhoA during *C. burnetii* infection. Vero
 43 cells were infected at an MOI of 500 with RSA439 for 5 days and then seeded onto coverslips at
 44 a density of 1×10^5 /mL. 24 hours later coverslips were washed and fixed with 3% formaldehyde

45 for 10 minutes followed by aldehyde quenching using 50mM ammonium acetate for 10 minutes.
46 Blocking was performed using 0.02% Saponin plus 10% normal horse serum. Anti-RhoA
47 polyclonal rabbit antibody was diluted 1:50 (Pierce) and incubated on the coverslip for 1 hr.
48 After washing the coverslips were incubated with goat anti-rabbit Alexa Fluor 488 (Molecular
49 probe) diluted 1:150 for 1 hr. After washing the coverslips were stained with Hoechst 33258
50 (Invitrogen) for 10 minutes and then mounted using MOWIOL. Large green puncta localized
51 next to individual *Coxiella* were observed frequently (vertical arrow) and on CCV (horizontal
52 arrow).

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55 **Movie S1.** Time-lapse video of HeLa cells transiently transfected with EGFP-CirA. Images were
56 captured every 1 m for 30 m.

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58 **Movie S2.** Time-lapse video of HeLa cells transiently transfected with EGFP. Images were
59 captured every 1 m for 30 m.