

Supplementary materials

Figure S1. *C. burnetii* 2072::Tn replicates with similar dynamics to WT during axenic culture in ACCM-2. *C. burnetii* WT, 2072::Tn and 2072::Tn pFLAG-2072 were cultivated in ACCM-2, and samples were taken for quantification of GE/mL every 24 hours until 7 days post-inoculation. n = 3. Error bars denote standard deviation.

Figure S2. Functional EirA is predominantly retained within *C. burnetii* whole cell. Whole cell lysates (WCL) of *C. burnetii* *eirA*::Tn (A), *eirA*::Tn pEirA-FLAG (B), *eirA*::Tn pFLAG-EirA (C), and *eirA*::Tn pFLAG-EirA₂₄₋₁₆₅ (D) and TCA precipitated ACCM-2 media (supernatant) used to cultivate each respective strain were probed with anti-FLAG antibodies to determine EirA localization. RpoA was used as a cytoplasmic loading control to ensure ACCM-2 culture media did not contain any *C. burnetii* contamination. Immunoblots are representative of three independent biological replicates.

Figure S3. Western blot confirming specificity of anti-EirA antibody to EirA. Whole cell lysates of strains used for subcellular fractionations were probed with anti-EirA (A) or anti-FLAG (B). DotB was used as a cytoplasmic loading control. Blots were segmented to account for different protein expression levels requiring different exposure times in each strain.

Figure S1.

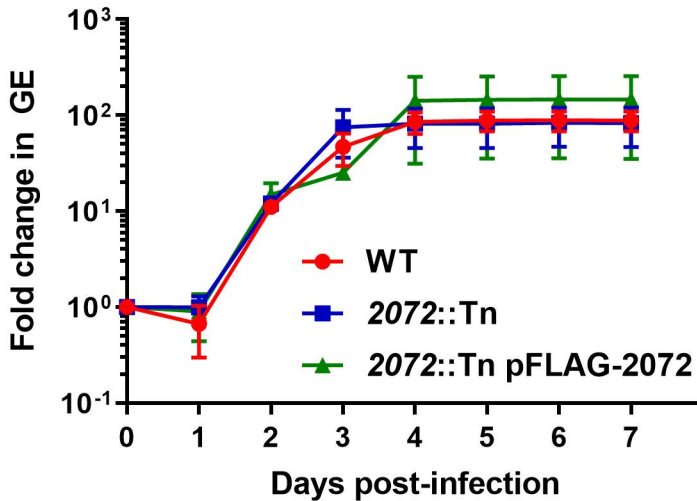


Figure S2.

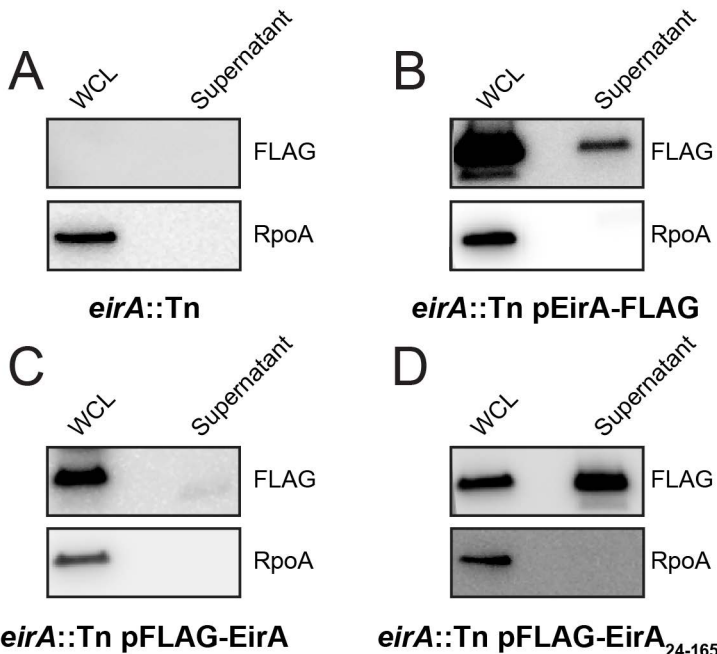


Figure S3.

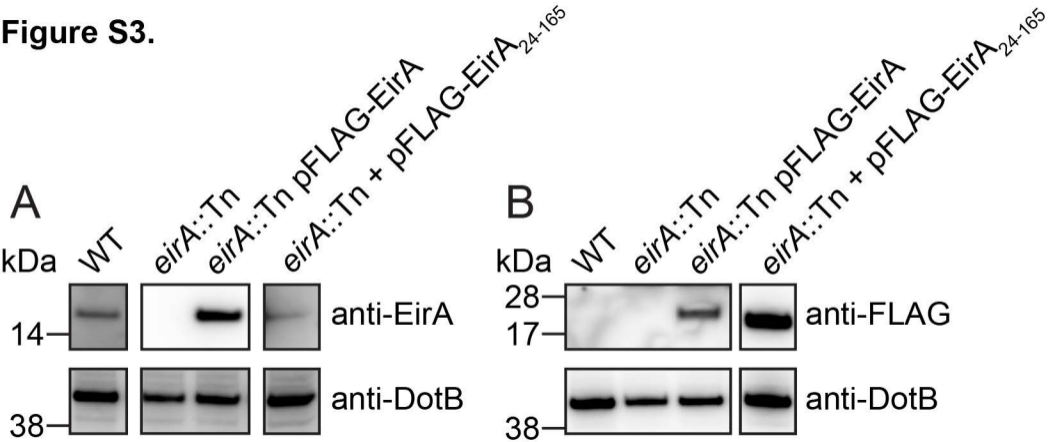


Table S1. Metabolite abbreviations.

Abbreviation	Metabolite
P	Phosphate
1P	1-phosphate
2P	2-phosphate
3P	3-phosphate
4P	4-phosphate
5P	5-phosphate
6P	6-phosphate
G6P	D-Glucose 6-phosphate
F6P	D-Fructose 6-phosphate
FBP	D-Fructose 1,6-bisphosphate
PEP	Phosphoenolpyruvate
R5P	D-Ribose 5-phosphate
UMP	Uridine 5'-monophosphate
UDP	Uridine 5'-diphosphate
dCMP	Deoxycytidine 5'-monophosphate
AMP	Adenosine 5'-monophosphate
ADP	Adenosine 5'-diphosphate
IMP	Inosine 5'-monophosphate
IDP	Inosine 5'-diphosphate
GMP	Guanosine 5'-monophosphate
dIMP	Deoxyinosine 5'-monophosphate
dGMP	Deoxyguanosine 5'-monophosphate
L-Ala	L-Alanine
L-Asp	L-Aspartate
L-Glu	L-Glutamine
L-Ile	L-Isoleucine
L-Leu	L-Leucine
L-Met	L-Methionine
L-Phe	L-Phenylalanine
L-Pro	L-Proline
L-Ser	L-Serine
L-Thr	L-Threonine
L-Trp	L-Tryptophan
L-Tyr	L-Tyrosine
L-Val	L-Valine
GSH	Glutathione
GlcNAc	N-Acetyl-D-glucosamine

Table S2. Metabolites identified as significantly different when comparing *eirA::Tn* pFLAG-EirA to WT.

Metabolite	Fold change ^A	BH-adjusted <i>p</i> -value ^B
D-Fructose	-0.56	9.02 x 10 ⁻³
D-Galactarate	-0.83	7.94 x 10 ⁻⁴
G6P	-1.63	5.55 x 10 ⁻³
F6P	-1.63	5.55 x 10 ⁻³
D-Gluconate	-0.46	1.63 x 10 ⁻³
R5P	-3.46	2.43 x 10 ⁻⁶
2-Deoxyribose-5P	-2.84	7.94 x 10 ⁻⁴
Orotate	-3.29	1.29 x 10 ⁻⁵
Xanthine	-1.29	2.43 x 10 ⁻⁶
Inosine	-3.09	9.18 x 10 ⁻⁶
IMP	-1.25	8.29 x 10 ⁻⁶
IDP	-0.54	2.30 x 10 ⁻³
dIMP	1.68	3.62 x 10 ⁻²
Adenine	-2.40	6.62 x 10 ⁻⁶
AMP	-0.85	2.43 x 10 ⁻⁶
ADP	-0.46	5.36 x 10 ⁻⁴
Uridine	-1.94	9.86 x 10 ⁻⁴
Uracil	-1.92	1.30 x 10 ⁻⁴
UMP	-1.96	7.24 x 10 ⁻⁵
dCMP	-1.11	6.81 x 10 ⁻³
dGMP	-0.85	2.43 x 10 ⁻⁶
L-Arg	1.40	1.73 x 10 ⁻³
L-Asp	1.22	4.02 x 10 ⁻⁵
L-Citrulline	0.62	3.68 x 10 ⁻²
L-Cystathione	0.24	2.87 x 10 ⁻³
L-Glu	1.08	2.43 x 10 ⁻⁶
L-Ser	1.08	5.97 x 10 ⁻⁶
L-Met	0.69	2.43 x 10 ⁻⁶
L-Val	-0.83	1.19 x 10 ⁻²
L-Ile	-0.61	5.55 x 10 ⁻³
L-Leu	0.78	2.88 x 10 ⁻⁴
L-Lys	1.50	1.43 x 10 ⁻⁴
L-Pro	-2.06	3.60 x 10 ⁻⁷
L-Ornithine	-0.77	2.09 x 10 ⁻²
L-Tyr	0.16	1.76 x 10 ⁻²
GSH	-0.59	5.78 x 10 ⁻⁶
3'-Methyladenine	0.87	9.59 x 10 ⁻⁴
2'-Methylcitrate	-3.28	5.36 x 10 ⁻⁴
Meso-2,6-Diaminopimelate	1.50	4.85 x 10 ⁻⁵
GDP-D-mannose	-1.14	5.63 x 10 ⁻⁶
UDP-GlcNAc	-1.20	2.50 x 10 ⁻⁵
D-Glucono-1,4-lactone	-1.08	9.68 x 10 ⁻³
D-Galactono-1,4-lactone	-1.61	1.90 x 10 ⁻²
Biotin	-2.29	2.61 x 10 ⁻³

Biopterin	-1.70	1.59×10^{-2}
NADH	0.45	3.78×10^{-3}
NADP	-0.46	5.55×10^{-3}

^APositive fold change value denotes metabolites with significantly high abundance, while negative fold change value denotes metabolites with significantly lower abundance in *C. burnetii eirA::Tn* pFLAG-EirA samples. ^BBenjamini-Hochberg (BH).

Table S3. Metabolites identified as significantly different when comparing *eirA::Tn* pFLAG-EirA to *eirA::Tn*.

Metabolite	Fold change	BH-adjusted <i>p</i> -value
D-Fructose	0.77	2.29 x 10 ⁻⁴
D-Galactarate	0.56	2.29 x 10 ⁻⁴
G6P	1.56	6.16 x 10 ⁻³
F6P	1.56	6.16 x 10 ⁻³
PEP	1.32	2.17 x 10 ⁻²
D-Gluconate	0.59	3.34 x 10 ⁻⁴
R5P	3.37	2.48 x 10 ⁻⁷
2-Deoxyribose-5P	2.68	5.69 x 10 ⁻⁴
Succinate	-0.28	1.48 x 10 ⁻⁴
Orotate	2.70	1.30 x 10 ⁻⁴
Inosine	3.22	2.48 x 10 ⁻⁷
IMP	1.02	1.48 x 10 ⁻⁴
IDP	0.38	7.75 x 10 ⁻³
dIMP	-1.43	6.16 x 10 ⁻³
Adenine	2.34	2.50 x 10 ⁻⁶
AMP	0.66	1.50 x 10 ⁻³
ADP	0.25	6.16 x 10 ⁻³
Uridine	2.35	2.35 x 10 ⁻⁴
Uracil	1.36	3.52 x 10 ⁻³
UMP	1.46	4.15 x 10 ⁻⁵
dCMP	1.32	2.50 x 10 ⁻⁶
GMP	-0.37	4.09 x 10 ⁻²
dGMP	0.64	2.49 x 10 ⁻³
L-Arg	-0.65	1.79 x 10 ⁻⁶
L-Asp	-0.60	1.31 x 10 ⁻⁶
L-Citrulline	-0.18	1.42 x 10 ⁻²
L-Cystathione	-0.18	3.70 x 10 ⁻³
L-Cysteate	-0.30	2.51 x 10 ⁻³
L-Glu	-0.39	3.18 x 10 ⁻⁵
L-Ser	-0.49	4.46 x 10 ⁻⁶
L-Met	-0.26	3.38 x 10 ⁻³
L-Val	0.51	5.69 x 10 ⁻⁴
L-Ile	0.28	8.54 x 10 ⁻³
L-Leu	-0.40	1.26 x 10 ⁻⁴
L-Lys	-0.46	4.83 x 10 ⁻³
L-Pro	1.92	2.48 x 10 ⁻⁷
L-Ornithine	0.67	2.11 x 10 ⁻⁴
L-Thr	-0.23	6.36 x 10 ⁻³
Homoserine	0.51	1.00 x 10 ⁻⁴
GSH	0.52	3.18 x 10 ⁻⁵
Methylmalonate	-0.28	1.48 x 10 ⁻⁴
N-Acetyl-L-Lysine	-0.29	1.27 x 10 ⁻³
3'-Methyladenine	-0.30	1.11 x 10 ⁻⁵
2'-Methylcitrate	2.78	1.48 x 10 ⁻⁴

Oxalate	-0.29	1.27×10^{-3}
Pthalate	-0.37	4.60×10^{-3}
Meso-2,6-Diaminopimelate	-0.64	9.55×10^{-7}
D-Mannitol	0.28	1.39×10^{-2}
D-Sorbitol	0.43	3.14×10^{-2}
GDP-D-mannose	0.81	3.05×10^{-5}
UDP-GlcNAc	0.83	1.19×10^{-4}
D-Glucono-1,4-lactone	0.85	1.40×10^{-3}
Biotin	2.66	2.04×10^{-3}
Biopterin	1.75	1.02×10^{-2}

^APositive fold change value denotes metabolites with significantly high abundance, while negative fold change value denotes metabolites with significantly lower abundance in *C. burnetii eirA::Tn* pFLAG-EirA samples. ^BBenjamini-Hochberg (BH).