

Supplemental Material

Table S1: Bacterial strains used in this study.

Strains	Phenotype or Description	References
<i>C. burnetii</i>		
RSA439	Phase II, Clone 4	[57]
RSA439 MK1	CBU0012::Tn, Cm ^r	This study
RSA439 MK2	CBU0041::Tn, Cm ^r	This study
RSA439 MK3	CBU0344::Tn, Cm ^r	This study
RSA439 MK4	CBU0372::Tn, Cm ^r	This study
RSA439 MK5	CBU0388::Tn, Cm ^r	This study
RSA439 MK6	CBU0425::Tn, Cm ^r	This study
RSA439 MK8	CBU0937::Tn, Cm ^r	This study
RSA439 MK9	CBU1198::Tn, Cm ^r	This study
RSA439 MK10	CBU1457::Tn, Cm ^r	This study
RSA439 MK11	CBU1556::Tn, Cm ^r	This study
RSA439 MK12	CBU1569::Tn, Cm ^r	This study
RSA439 MK13	CBU1636::Tn, Cm ^r	This study
RSA439 MK14	CBU1639::Tn, Cm ^r	This study
RSA439 MK15	CBU1665::Tn, Cm ^r	This study
RSA439 MK16	CBU2007::Tn, Cm ^r	This study
RSA439 MK17	CBU2013::Tn, Cm ^r	This study
RSA439 MK18	CBU2016::Tn, Cm ^r	This study
RSA439 MK19	CBU2052::Tn, Cm ^r	This study
RSA439 MK20	CBU2059::Tn, Cm ^r	This study
RSA439 MK21	<i>icmX</i> ::Tn, Cm ^r	This study
<i>L. pneumophila</i>		
Lp02	Philadelphia-1 <i>rpsL hsdR thyA</i> mutant	[14]
Lp03	Lp02 Δ <i>dotA</i>	[14]

<i>E. coli</i>		
DH5α	<i>F'(Φ80dΔ(lacZ)M15), recA1, endA1, gyrA96, thi1, hsdR17 (rk-mk+), supE44, relA1, deoR, Δ(lacZYA-argF), U169</i>	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>	[58]

Table S2: Plasmids used in this study.

Plasmid	Description	References or Source
pKM225	pMW1650, <i>com1p</i> -TnA7, <i>groESp</i> -mCherry, <i>com1p</i> -cat, Cm ^R	Mertens et al. (Manuscript In Prep)
pKM244	pJB908a, <i>groESp</i> -mCherry, <i>com1p</i> -cat, Cm ^R , Amp ^R	Mertens et al. (Manuscript In Prep)
pCBTEM	pXDC61m, <i>groESp</i> -mCherry, <i>com1p</i> -cat, Cm ^R ,	[18]
pCBTEM-1	pCBTEM:: Cbu0069	This study
pCBTEM-2	pCBTEM:: Cbu0113	This study
pCBTEM-3	pCBTEM:: Cbu0344	This study
pCBTEM-4	pCBTEM:: Cbu0606	This study
pCBTEM-5	pCBTEM:: Cbu01576	This study
pCC108	pKM244, HSP10p-EGFP, <i>com1p</i> -Flag-TEM, Kan ^R	This study
pCC108-1	pCC108::CBU0080	This study
pCC108-2	pCC108::CBU0113	This study
pCC108-3	pCC108::CBU0388	This study
pCC108-4	pCC108::CBU0885	This study
pCC108-5	pCC108::CBU1665	This study
pCC108-6	pCC108::CBU1685	This study
pCC108-7	pCC108::CBU1754	This study
pEGFP-C1	C-terminal fusion to EGFP, kan ^r	Clontech
pEGFP35	pEGFP-C1::CBU0012	This Study

pEGFP36	pEGFP-C1::CBU0069	This study
pEGFP37	pEGFP-C1::CBU0080	This study
pEGFP38	pEGFP-C1::CBU0113	This study
pEGFP39	pEGFP-C1::CBU0183	This study
pEGFP40	pEGFP-C1::CBU0201	This study
pEGFP41	pEGFP-C1::CBU0212	This study
pEGFP42	pEGFP-C1::CBU0270	This study
pEGFP43	pEGFP-C1::CBU0295	This study
pEGFP44	pEGFP-C1::CBU0344	This study
pEGFP45	pEGFP-C1::CBU0372	This study
pEGFP46	pEGFP-C1::CBU0375	This study
pEGFP47	pEGFP-C1::CBU0376	This study
pEGFP48	pEGFP-C1::CBU0393	This study
pEGFP49	pEGFP-C1::CBU0425	This study
pEGFP50	pEGFP-C1::CBU0469	This study
pEGFP51	pEGFP-C1::CBU0513	This study
pEGFP52	pEGFP-C1::CBU0590	This study
pEGFP53	pEGFP-C1::CBU0606	This study
pEGFP54	pEGFP-C1::CBU0637	This study
pEGFP55	pEGFP-C1::CBU0665	This study
pEGFP55	pEGFP-C1::CBU0773	This study
pEGFP56	pEGFP-C1::CBU0885	This study
pEGFP57	pEGFP-C1::CBU1079	This study
pEGFP58	pEGFP-C1::CBU1102	This study
pEGFP59	pEGFP-C1::CBU1110	This study
pEGFP60	pEGFP-C1::CBU1150	This study
pEGFP62	pEGFP-C1::CBU1198	This study
pEGFP63	pEGFP-C1::CBU1434	This study
pEGFP64	pEGFP-C1::CBU1525	This study

pEGFP65	pEGFP-C1::CBU1566	This study
pEGFP66	pEGFP-C1::CBU1576	This study
pEGFP67	pEGFP-C1::CBU1594	This study
pEGFP68	pEGFP-C1::CBU1607	This study
pEGFP69	pEGFP-C1::CBU1620	This study
pEGFP71	pEGFP-C1::CBU1639	This study
pEGFP72	pEGFP-C1::CBU1665	This study
pEGFP73	pEGFP-C1::CBU1677	This study
pEGFP74	pEGFP-C1::CBU1685	This study
pEGFP75	pEGFP-C1::CBU1754	This study
pEGFP76	pEGFP-C1::CBU1789	This study
pEGFP77	pEGFP-C1::CBU1790	This study
pEGFP78	pEGFP-C1::CBU2007	This study
pEGFP79	pEGFP-C1::CBU2013	This study
pEGFP80	pEGFP-C1::CBU2016	This study
pEGFP81	pEGFP-C1::CBU2028	This study
pEGFP82	pEGFP-C1::CBU2059	This study
pEGFP83	pEGFP-C1::CBU2064	This study
pEGFP84	pEGFP-C1::CBU2076	This study
pEGFP85	pEGFP-C1::CBUA0015	This study
pEGFP86	pEGFP-C1::CBUA0019	This study
pEGFP87	pEGFP-C1::CBUA0020	This study

pYES

pYES1	pYES::CBU0041	This study
pYES2	pYES::CBU0129	This study
pYES3	pYES::CBU0175	This study
pYES4	pYES::CBU0410	This study
pYES5	pYES::CBU0414	This study
pYES6	pYES::CBU0794	This study

pYES7	pYES::CBU0801	This study
pYES8	pYES::CBU0814	This study
pYES9	pYES::CBU0881	This study
pYES10	pYES::CBU0937	This study
pYES11	pYES::CBU1045	This study
pYES12	pYES::CBU1217	This study
pYES13	pYES::CBU1314	This study
pYES14	pYES::CBU1379	This study
pYES15	pYES::CBU1406	This study
pYES16	pYES::CBU1425	This study
pYES17	pYES::CBU1457	This study
pYES18	pYES::CBU1460	This study
pYES19	pYES::CBU1461	This study
pYES20	pYES::CBU1524	This study
pYES21	pYES::CBU1543	This study
pYES22	pYES::CBU1556	This study
pYES23	pYES::CBU1569	This study
pYES24	pYES::CBU1599	This study
pYES25	pYES::CBU1636	This study
pYES26	pYES::CBU1751	This study
pYES27	pYES::CBU1769	This study
pYES28	pYES::CBU1823	This study
pYES29	pYES::CBU1825	This study
pYES30	pYES::CBU2052	This study
pYES31	pYES::CBU0012	This study
pYES32	pYES::CBU0080	This study
pYES33	pYES::CBU0113	This study
pYES34	pYES::CBU0183	This study
pYES35	pYES::CBU0201	This study

pYES36	pYES::CBU0212	This study
pYES37	pYES::CBU0295	This study
pYES38	pYES::CBU0344	This study
pYES39	pYES::CBU0372	This study
pYES40	pYES::CBU0375	This study
pYES41	pYES::CBU0376	This study
pYES42	pYES::CBU0393	This study
pYES43	pYES::CBU0425	This study
pYES44	pYES::CBU0469	This study
pYES45	pYES::CBU0513	This study
pYES47	pYES::CBU0590	This study
pYES48	pYES::CBU0606	This study
pYES49	pYES::CBU0637	This study
pYES50	pYES::CBU0665	This study
pYES51	pYES::CBU0773	This study
pYES52	pYES::CBU0885	This study
pYES53	pYES::CBU1079	This study
pYES54	pYES::CBU1102	This study
pYES55	pYES::CBU1150	This study
pYES57	pYES::CBU1198	This study
pYES58	pYES::CBU1434	This study
pYES59	pYES::CBU1525	This study
pYES60	pYES::CBU1566	This study
pYES61	pYES::CBU1576	This study
pYES62	pYES::CBU1594	This study
pYES63	pYES::CBU1607	This study
pYES64	pYES::CBU1620	This study
pYES66	pYES::CBU1639	This study
pYES67	pYES::CBU1665	This study

pYES68	pYES::CBU1677	This study
pYES69	pYES::CBU1685	This study
pYES70	pYES::CBU1754	This study
pYES71	pYES::CBU1789	This study
pYES72	pYES::CBU2007	This study
pYES73	pYES::CBU2013	This study
pYES74	pYES::CBU2016	This study
pYES75	pYES::CBU2028	This study
pYES76	pYES::CBU2059	This study
pYES77	pYES::CBU2064	This study
pYES78	pYES::CBU2076	This study
pYES79	pYES::CBUA0014	This study
pYES80	pYES::CBUA0015	This study
pYES81	pYES::CBUA0019	This study
pYES82	pYES::CBUA0020	This study

Table S3: Predicted PmrA regulated ORFs

ID	5' End	Putative PmrA binding site	3' End	Strand	Distance of predicted binding site to ATG
CBU0013	13016	TTAAGGTAACCTTAATATTTCTTGGTTAA--ACT	12985	-	29
CBU0021	18235	TTAATATTTTCCTTAACCTAATGAAGTTATT--CT	18266	+	71
CBU0023	22399	TTAATTTTATCTTAAGATAAGGAAATCTTT--ACT	22367	-	48
CBU0051	49435	CTCATACTTCCTTAAGGTTTTTTGGAGAT--ACT	49404	-	72
CBU0069	63320	CTAAGATTATCTTAATTTTAACTTGATAGC-A-T	63351	+	31
CBU0077	71460	TTAAAATTAATAATTA--TCAATTAATATTGACCT	71491	+	82
CBU0084	75607	TTAATATTATCTTAAGGTTATTGGGTTATT-C-T	75638	+	0
CBU0119	111571	TTAAGAAATCTTTAATCTCGCAGTGGTATG-C-T	111540	-	79
CBU0144	131010	TTAAGTTTGTTTTAATATCGATGTGTCAA--GAT	131041	+	38
CBU0183	172128	TTAAGAGTATTTTAAGTTTATGCAGATAT--AAT	172159	+	104

CBU0201	190143	TTAATATTATTTTAA-ATCTTACCTTTAAAAAAT	190111	-	70
CBU0213	197619	TTAAGATTGAATTAATTTTTGGATATTAC--TTT	197588	-	28
CBU0215	200426	TTAACAGAAAATTAA--TTTTAGGGATTTCACCT	200395	-	136
CBU0273	245187	TTAATATTATTTTAAACCTTTCGTCCCTAAA-A-T	245156	-	32
CBU0326	293301	TTAAGAAAATCTTAAATTTTATCGATGATT--CA	293270	-	52
CBU0343	310482	TTCATATTCACCTTAATCTTATTGTAGTAAC-T-T	310513	+	88
CBU0355	322015	CTAAGATTCTCTTAATCTCATTACAGAATGA--T	322046	+	31
CBU0361	327063	TTAAGAAAACTTAAGGAAGAATAGAAAAGT--TT	327032	-	106
CBU0388	348481	TTAATATTCCTTAAGCTTTATTCGTTACA-C-T	348512	+	72
CBU0393	357811	TTAATAAAGGTTAATGTTTTAGAGGTATT-C-T	357780	-	47
CBU0436	388876	TTAATATTTAATTAAGTTTATTTGTTTAGA-C-T	388845	-	94
CBU0505	445857	TTAATATTCCTTAAGTTTAAAGCTTTATA-A-T	445826	-	44
CBU0600	547351	TTAACTTTACCTTAACAAAACCTGATTAT--ACT	547382	+	35
CBU0619	565992	TTAATCTTTTCTTAAGGTTTTTGGCTTATA-C-T	565961	-	141
CBU0625	569123	TTAAGAATTCCTTAATAAATGAATGATAAG-A-T	569154	+	127
CBU0637	586648	TTAATTTTTACTTAATTTTT-TAATCGACT-TCT	586617	-	123
CBU0665	606974	TTAATATTTTCTTAATACCGCGATGGTATT-C-T	607005	+	13
CBU0690	636458	TGAACATGATTTTAA-ATCTTATGGTGTTATGCT	636490	+	82
CBU0790	737321	TTAATAATTCCTAATTTCTCAGTGTTATC-C-T	737352	+	83
CBU0860	816692	TTAATAAATCATTAATATTGTTGTTGGATA-A-T	816723	+	46
CBU0978	930285	TTAAGCTTTTTTTAATAATAAAAATGTTAG--ACT	930316	+	34
CBU0992	940945	TTAAGATTTTCCTAAGGGTTATGAAGTACT-A-T	940914	-	35
CBU1017	962048	TTAATTTTATTTTAA--TCTTATTGAT-TTTATT	962078	+	158
CBU1033	975307	TTAAGAGTTCTTTAAGGTTTCGCCCCTAC--ACT	975338	+	165
CBU1049	992640	TTCATATTTTCGTTAAGTTTGGAGTGCTAAT-A-T	992609	-	31
CBU1059	1005087	TTTAGATTATTTTAAATTTAAAAAGTTTAG--AGG	1005056	-	147
CBU1098	1047078	TTAATAAATTCTTAAGATTAAGCTGTTATT-C-T	1047047	-	39
CBU1103	1051114	TTAATATTCTCTTAAGGTTGATGAGGTAGA-T-T	1051083	-	30
CBU1110	1056911	TTAATGAAGCTTTAACAATAGAGCGATAT--ACT	1056880	-	23

CBU1114	1059926	TTCATACTTTTTTAAGCAAACGTTGATAT--CCT	1059895	-	37
CBU1167	1110630	TTAATATTCTCTTAATAGCTTCATCCAATAA--T	1110599	-	72
CBU1178	1119864	TTAAAATTTCTTTAATAAACTTCTACTAA--ACT	1119895	+	117
CBU1198	1142178	TTAAGGCTACGTTAAGTTGAAGGGAGTAA--GAT	1142209	+	44
CBU1213	1157000	TTAAGATTGATTTAATGTCTGGCGTTAG--AAT	1157031	+	36
CBU1216	1161120	TTAATTTTATTTTAATTTATTTTCGGATAC--ACT	1161089	-	33
CBU1226	1176853	TTAATTTTGCTTTAA--ATGCAAGCATTTTTATT	1176822	-	41
CBU1252	1204114	TTAACGGTAATTTAAGGTTTCTTTTCTAAA-A-T	1204083	-	21
CBU1253	1204021	TTCAGGTTCACTTAAGTTTAAAAGGTTAA--TTT	1204052	+	235
CBU1288	1242627	TTAACTTTCCTCTAAGAAAAGACTGTTAT--AGT	1242596	-	44
CBU1291	1245983	TTAATGGAAACTTAAGCTTTCCTTCATTACA-A-T	1246014	+	41
CBU1368	1318196	TTAATATTTTCTTAATCCAATTCAGTGTGC--A	1318165	-	94
CBU1369	1318222	TTAAGCGTAATTTAATATTATGCCCTTAT--AAT	1318253	+	17
CBU1370	1320557	TTAATTTTTTCATAATGTAGGAATTATAT--ACT	1320526	-	51
CBU1387	1336989	TTCATATAGCCTTAA--TATGGTTACTGTAAAAT	1337020	+	48
CBU1409	1359599	TTAAGGTTAACCTAAGCTTGAGTTGTTAGA-A-T	1359630	+	133
CBU1420	1369171	CTAATATTCCTTCAGTTTATATTGATAAC-A-T	1369202	+	17
CBU1523	1471228	TTAAGATTAATTTAC--TCTTGTTATTATTACA	1471259	+	88
CBU1530	1481188	TTAATATATTTTTAAGTTTCATACATTAAC-A-T	1481157	-	36
CBU1535	1484881	TTAAGATTTGTTAAGCAGGCTGCCCTAT--ACT	1484850	-	45
CBU1564	1512170	ATCAGATTCCGTTAATAATTCGGCAGTAT--ACT	1512139	-	43
CBU1576	1522991	TTCATATTTCTTTAATTTTTTCAGCGCTATC-A-T	1523022	+	43
CBU1611	1555499	TTAATATTTTATTAAGTTTATCGATTTATA-G-T	1555468	-	48
CBU1618	1557905	TTAATATTAATAATTAATTAAGAAAT-AGA-A-T	1557935	+	101
CBU1624	1563565	TTAATATTTTCCTTAAGGTAAGGAAGGTATA--GT	1563534	-	41
CBU1639	1579897	TTAATTTTGCTTTAAGTTTAACGCGCTAAC-A-T	1579866	-	58
CBU1649	1589543	TTAATACTGTCTTAAGGAAGTGGTGCTAGC-A-T	1589512	-	42
CBU1650	1589620	TTAATATTTTCGTTAAGGTTAGTGGGCTATT-C-T	1589651	+	48
CBU1685	1616231	TTAATTTTCTTTAA--TTAATTTTAATAATATT	1616262	+	18

CBU1701	1634450	CTAATATTCCTTAACTTCCTTCTGGTAGG-A-T	1634481	+	33
CBU1757	1688680	TCAAGACTTAATTAA--TTTATTTTATTTATATG	1688649	-	39
CBU1758	1689382	TTAACTTTTTCTTAACTTAAAAGTGGTAA--ACT	1689351	-	23
CBU1775	1705606	CTAAGGTTAGTTTAATCTTGCTAAGGTAA--TAT	1705637	+	65
CBU1780	1708193	TTCATTTTTCTTTAAGTTTTGATTGATAAC-A-T	1708224	+	129
CBU1794	1725403	TTAAGGTTTTCTTAATAAAGGCTGTTTAT--ACT	1725434	+	26
CBU1818	1748733	TTTATTTACATTTAA--TTAAAATCATTATTAT	1748764	+	51
CBU1822	1753234	TTAGGATTAATAATTAACATGCAGGTGTTAA--GAT	1753203	-	88
CBU1863	1793470	TTAATATTTTTTTAATATTTGGATTTTAAG-A-T	1793439	-	69
CBU2013	1920851	TTAATAATTTGTTAATATCGGTTTGGTAAT-A-T	1920820	-	20
CBU2051	1957518	TTCATATATTTTTAAGCATGGCTTGGTAC--ACT	1957487	-	36
CBUA0007	4919	TTAATTCTTCTTTAATAATACTGATAT—CCT	4888	-	34

Table S4: *C. burnetii* T4SS candidates possessing an E block motif

ID	E position
CBU0012	E_Position = [16,19,21]
CBU0050	E_Position = [3,6,9,28]
CBU0061	E_Position = [1,15,16,17,19]
CBU0067	E_Position = [18,22,26,28]
CBU0080	E_Position = [0,1,7,18,19,20]
CBU0092	E_Position = [5,20,21]
CBU0113	E_Position = [11,18,19,20,21]
CBU0115	E_Position = [3,7,12,13,23]
CBU0122	E_Position = [6,8,9,10,11,18,22]
CBU0134	E_Position = [14,16,25]
CBU0150	E_Position = [11,18,19]
CBU0211	E_Position = [5,8,10]
CBU0212	E_Position = [3,8,21,22,23]
CBU0270	E_Position = [9,13,15,26]

CBU0295	E_Position = [9,10,13,15,19]
CBU0328	E_Position = [1,15,18]
CBU0339	E_Position = [4,14,18,19]
CBU0340	E_Position = [3,8,15,24,25]
CBU0344	E_Position = [0,1,18,20]
CBU0360	E_Position = [6,9,11,15,19,23]
CBU0372	E_Position = [0,7,8,11,20,24]
CBU0375	E_Position = [6,8,27]
CBU0376	E_Position = [12,16,18,20,27]
CBU0377	E_Position = [3,13,16,20,21]
CBU0425	E_Position = [13,14,17]
CBU0469	E_Position = [2,5,10,26]
CBU0470	E_Position = [4,12,13,26]
CBU0498	E_Position = [2,13,16]
CBU0507	E_Position = [0,8,15,24,25]
CBU0510	E_Position = [5,12,16,17,24,25]
CBU0513	E_Position = [4,6,22,24]
CBU0562	E_Position = [1,3,4,7]
CBU0590	E_Position = [16,22,25]
CBU0606	E_Position = [1,10,11,13]
CBU0651	E_Position = [3,14,16,22,27]
CBU0654	E_Position = [0,13,14]
CBU0656	E_Position = [10,23,27,28]
CBU0658	E_Position = [4,5,6,9,12]
CBU0698	E_Position = [0,1,6,15]
CBU0699	E_Position = [0,2,9]
CBU0705	E_Position = [7,11,22,24]
CBU0706	E_Position = [3,13,14,15]
CBU0710	E_Position = [1,4,5]

CBU0719	E_Position = [2,17,18]
CBU0756	E_Position = [12,26,28]
CBU0773	E_Position = [11,16,17,24]
CBU0879	E_Position = [6,8,9,12,15,18,20]
CBU0885	E_Position = [10,12,14,16,18,20,21]
CBU0891	E_Position = [11,15,16,20,23]
CBU0910	E_Position = [7,9,18,19]
CBU0941	E_Position = [6,8,19,27]
CBU0957	E_Position = [0,1,10]
CBU0970	E_Position = [11,13,16]
CBU0972	E_Position = [4,7,26]
CBU0985	E_Position = [3,4,6,14,17,25]
CBU1044	E_Position = [3,4,13,20,24]
CBU1065	E_Position = [2,3,8]
CBU1069	E_Position = [12,15,27,28]
CBU1079	E_Position = [14,18,19,25]
CBU1088	E_Position = [7,8,11]
CBU1102	E_Position = [9,10,11,15,17]
CBU1107	E_Position = [7,11,15,17,20]
CBU1108	E_Position = [6,10,14,15,16,18,20,21]
CBU1124	E_Position = [13,16,23,26]
CBU1150	E_Position = [11,12,24,28]
CBU1164	E_Position = [3,4,5,21]
CBU1209	E_Position = [8,11,12,13,14,19]
CBU1215	E_Position = [14,15,25,27]
CBU1219	E_Position = [0,3,10,17]
CBU1224	E_Position = [2,5,27]
CBU1234	E_Position = [3,8,10,25]
CBU1295	E_Position = [0,10,11,16]

CBU1328	E_Position = [2,3,19]
CBU1354	E_Position = [2,4,6]
CBU1367	E_Position = [9,12,28]
CBU1377	E_Position = [10,11,17]
CBU1434	E_Position = [0,17,19,28]
CBU1463	E_Position = [2,4,6,19]
CBU1479	E_Position = [3,5,18]
CBU1496	E_Position = [5,6,19,21]
CBU1525	E_Position = [4,5,13,15,22,23]
CBU1533	E_Position = [1,5,19,22]
CBU1546	E_Position = [6,8,20,25]
CBU1566	E_Position = [0,7,10,26,27]
CBU1603	E_Position = [12,24,25]
CBU1607	E_Position = [11,15,17,20,23]
CBU1611	E_Position = [0,12,24,25,27]
CBU1616	E_Position = [7,10,12,22]
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CBU1659	E_Position = [7,20,21]
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CBU1662	E_Position = [5,7,16,26]
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CBU1673	E_Position = [5,8,9,22]
CBU1677	E_Position = [6,10,12,15,19,22]
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CBU1733	E_Position = [6,8,9,28]
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CBU1776	E_Position = [2,4,11,12,14,15,18]
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CBU1883	E_Position = [1,9,10,17,22]
CBU1954	E_Position = [7,25,28]
CBU1999	E_Position = [9,11,17]
CBU2007	E_Position = [7,9,11,19]
CBU2010	E_Position = [15,25,28]
CBU2016	E_Position = [2,7,10,16,19,21]
CBU2023	E_Position = [8,15,18,23,25]
CBU2028	E_Position = [4,5,6,10]
CBU2033	E_Position = [5,21,22]
CBU2034	E_Position = [4,11,12]
CBU2057	E_Position = [3,8,15,24,25]
CBU2059	E_Position = [8,10,16,20]
CBU2060	E_Position = [0,2,6,16]
CBU2064	E_Position = [3,4,6,11,14,18,20]
CBU2076	E_Position = [6,7,15,25]
CBUA0015	E_Position = [8,12,14,16,17,18]
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Table S5: *L. pneumophila* effectors used for the identification of homologs in *C. burnetii*

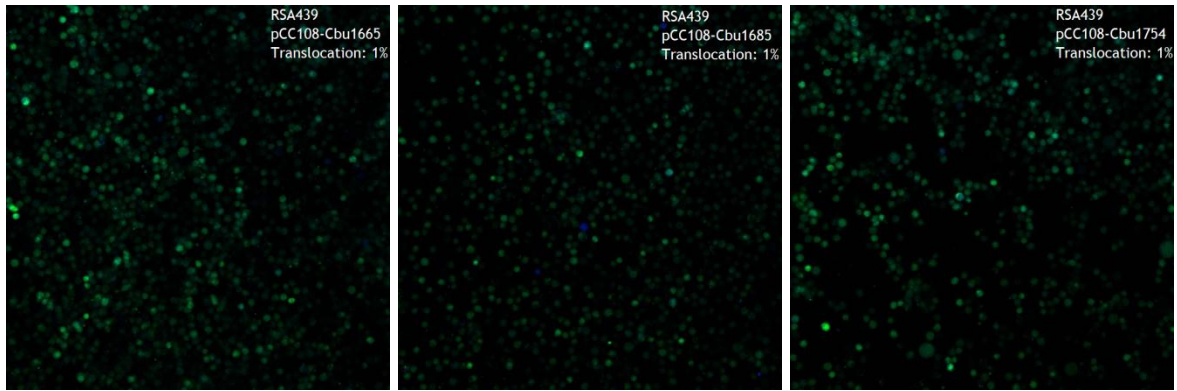
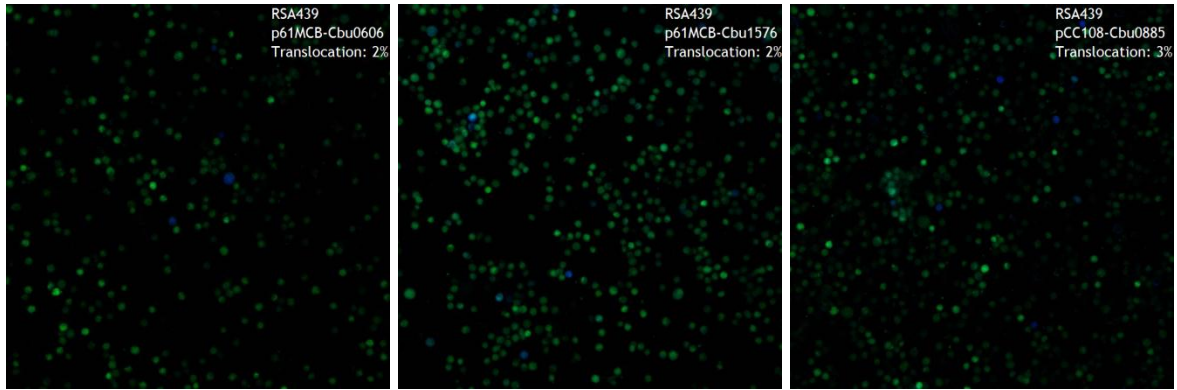
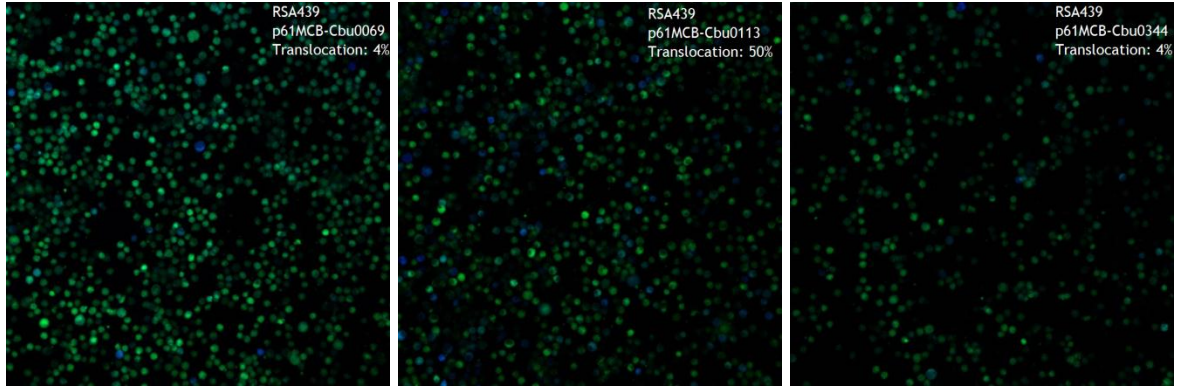
Hit	Query	Hit description	E value
CBU0001	lpg1924	chromosomal replication initiator protein DnaA (dnaA) {Coxiella burnetii RSA 493}	0.29
CBU0026	lpg1888	ribose 5-phosphate isomerase (rpiA) [5.3.1.6] {Coxiella burnetii RSA 493}	0.24
CBU0027	lpg1106	acyltransferase family protein {Coxiella burnetii RSA 493}	0.17
CBU0111	lpg1106	2-amino-3-ketobutyrate coenzyme A ligase (kbl) [2.3.1.2...	0.33
CBU0147	lpg1684	preprotein translocase, SecA subunit (secA) {Coxiella burnetii RSA 493}	0.32

CBU0235	lpg0021	translation elongation factor G (fusA) {Coxiella burnetii RSA 493}	0.56
CBU0530	lpg1716	TPR domain protein {Coxiella burnetii RSA 493}	0.16
CBU0676	lpg2539	NAD dependent epimerase/dehydratase {Coxiella burnetii RSA 493}	0.2
CBU0682	lpg0021	conserved domain protein {Coxiella burnetii RSA 493}	0.69
CBU0693	lpg1670	dehydrogenase, E1 component, alpha subunit [1.2.4.1] {Coxiella burnetii RSA 493}	0.52
CBU0753	lpg0260	transporter, AcrB/AcrD/AcrF family {Coxiella burnetii RSA 493}	0.49
CBU0845	lpg1692	UDP-glucose/GDP-mannose dehydrogenase family {Coxiella ...}	0.59
CBU0902	lpg1667	hypothetical protein {Coxiella burnetii RSA 493}	0.3
CBU1049	lpg1959	hypothetical protein {Coxiella burnetii RSA 493}	0.12
CBU1108	lpg0130	hypothetical protein {Coxiella burnetii RSA 493}	0.26
CBU1160	lpg2148	TPR domain protein {Coxiella burnetii RSA 493}	0.35
CBU1282	lpg2826	carbamoyl-phosphate synthase, small subunit (carA) [6.3...	0.15
CBU1292	lpg1083	ankyrin repeat domain protein {Coxiella burnetii RSA 493}	0.25
CBU1341	lpg2826	GMP synthase (guaA) [6.3.5.2] {Coxiella burnetii RSA 493}	0.5
CBU1363	lpg1171	exodeoxyribonuclease I, putative {Coxiella burnetii RSA 493}	0.15
CBU1370	lpg1639	hypothetical protein {Coxiella burnetii RSA 493}	0.95
CBU1387	lpg2546	hypothetical protein {Coxiella burnetii RSA 493}	0.054
CBU1494	lpg2555	pyridoxal phosphate biosynthetic protein PdxJ (pdxJ) {Coxiella burnetii RSA 493}	0.82
CBU1535	lpg0375	hypothetical protein {Coxiella burnetii RSA 493}	0.88
CBU1594	lpg2359	GatB/Yqey domain protein {Coxiella burnetii RSA 493}	4.00E-30
CBU1603	lpg2912	conserved hypothetical protein {Coxiella burnetii RSA 493}	0.13
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CBU2057	lpg2546	conserved hypothetical protein {Coxiella burnetii RSA 493}	0.33

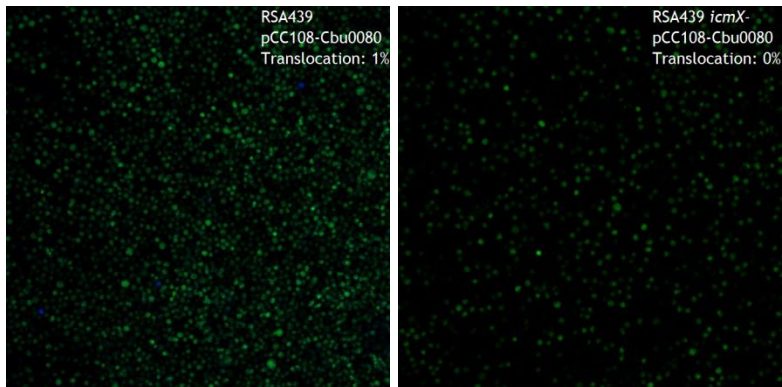
Table S6: Genome comparisons of T4SS substrates identified in this study. Brackets after each ORF indicate an alternative start (AS), an N-terminal (NT) and/or C-terminal (CT) truncation, or frame shift (FS) compared to corresponding NMI ORF. Numbers following abbreviation denote length of AS, amount truncated from N- or C- terminus, or site of frameshift.

Gene Locus in NMI	Alias in isolates			Domain
	K	G	Dugway	
Cbu0012	CbuK0201 (AS 242bp)	CbuG0281 (AS 242bp)	CbuD0136 (AS 242bp)	P-loop
Cbu0069	CbuK1982/CbuK1983 (NT 21bp, FS 137bp)		CbuD2035 (NT 29bp, FS 343bp)	HRDC-like
Cbu0080	CbuK1976 (AS 506bp,)	CbuG1937 (AS 506bp, CT 102bp)	CbuD2028	HLH DNA-binding domain
Cbu0113		CbuG1899	CbuD1994	PH-domain
Cbu0183	CbuK0377 (CT 7bp)	CbuG1822 (CT 86bp)	CbuD1910	
Cbu0201	CbuK0392 (NT 77bp)	CbuG1805 (NT 77bp)	CbuD1894 (NT 77bp)	Ankyrin repeat
Cbu0212	CbuK0401 (AS 1019bp)	CbuG1796 (AS 1019bp)	Cbu1884 (AS 1019bp)	
Cbu0270	CbuK0462	CbuG1738	CbuD1824	
Cbu0295	CbuK0492 (NT 272bp, CT 305bp)	CbuG1710 (NT 272bp)	CbuD1787 (NT 17bp)	SEL1
Cbu0344	CbuK0540 (AS 455bp)	CbuG1663 (NT 69bp)	CbuD1734 (AS 455bp)	Coiled-coil Fic, transmembrane domain
Cbu0372	CbuK0563 (FS 621bp)	CbuG1637 (FS 700bp)	CbuD1711	
Cbu0375	CbuK0569 (NT 209bp)	CbuG1634 (NT 209bp)	CbuD1704 (FS 468)	
Cbu0376	CbuK0569 (AS 27bp)	CbuG1634 (AS 27bp)	CbuD1704 (AS 515bp)	
Cbu0388	CbuK1672/CbuK1673/CbuK1674 (NT 11bp, FS 975bp)	CbuG1619 (NT 11bp, FS 2044bp)	CbuG1679/CbuG1680 (NT 11bp, FS 1588bp)	
Cbu0393	CbuK1668 (FS 287bp)	CbuG1615 (NT 142bp, FS 559bp)	CbuD1674 (FS 559bp)	RPT1 domain
Cbu0425 (<i>cirB</i>)	CbuK1643 (AS 116bp, CT 539bp)	CbuG1587 (AS 116bp)	CbuD1648 (AS 116bp, CT 59bp)	
Cbu0469	CbuK1391	CbuG1544	CbuD1609	
Cbu0513	CbuK1341 (NT 71bp, CT 54bp)	CbuG1498	CbuD1562	
Cbu0590	CbuK1248 (NT 47bp)	CbuG2044 (NT 47bp)	CbuD1474 (NT 47bp)	
Cbu0606	CbuK1444 (AS 434bp, FS 163bp)	CbuG1397 (AS 434bp, FS 296bp)	CbuD0618 (AS 434bp)	
Cbu0637	CbuK1619	CbuG1367	CbuD0648	
Cbu0665	CbuK1593 (NT 21bp)	CbuG1341 (NT 21bp, CT 85bp)	CbuD0675 (AS 92bp)	
Cbu0773	CbuK0643	CbuG1228	CbuD0821	
Cbu0885	CbuK0750	CbuG1117	CbuD0949 (NT 86bp)	HAD-like
Cbu1079	CbuK0945	CbuG0924	CbuD1181	
Cbu1102	CbuK0972 (AS 404bp, FS 1053)	CbuG0900 (CT 111bp)	CbuD1204 (AS 404bp)	
Cbu1110	CbuK0977 (FS 166bp)		CbuD1210 (NT 53bp, FS 166bp)	
Cbu1150	CbuK1017 (AS 57bp)	CbuG0859 (AS 476bp)	CbuD1247 (AS 476bp, CT 26bp)	Transmembrane helix hairpin
Cbu1198	CbuK1062 (NT 140bp)	CbuG0811 (NT 140bp)	CbuD1287	
Cbu1434	CbuK0601	CbuG0575	CbuD0561	
Cbu1525	CbuK1752 (AS 152bp)	CbuG0485 (NT 459bp, FS 924bp)	CbuD0461 (AS 1900bp)	YebC-like
Cbu1566	CbuK1793	CbuG0446	CbuD0422	
Cbu1576	CbuK1806 (NT 129bp)	CbuG0435/CbuG0436 (FS 902bp)	CbuD0410	GatB/YqeY motif
Cbu1594	CbuK1823		CbuD0395	Transmembrane helix
Cbu1607	CbuK1834 (NT 242bp)	CbuG0407 (NT 242bp, CT 13bp)	CbuD0383 (NT 242bp)	
Cbu1620	CbuK1842 (FS 240bp)	CbuG0403 (AS 171bp)	CbuD0378 (AS 296bp, FS 240bp)	
Cbu1639	CbuK1862	CbuG0383 (FS 309bp)	CbuD0358	
Cbu1665	CbuK0343 (FS 897bp)		CbuD0334	
Cbu1677	CbuK0333	CbuG0344	CbuD0334	
Cbu1685	CbuK0326 (FS 1060bp)	CbuG0337	CbuD0324	
Cbu1754	CbuK0252	CbuG0136	CbuD0317 (NT 35bp, FS 427bp)	
Cbu1789	CbuK0083	CbuG0170	CbuD0247	
Cbu1790	CbuK0084 (FS 924bp)	CbuG0171 (FS 1685bp)	CbuD0015	SGL-like
Cbu2007	CbuK2058	CbuG2016	CbuD0016 (FS 1263bp)	PRR domain
Cbu2013	CbuK2064 (NT 50bp)	CbuG2022 (NT 50bp)	CbuD2108	
Cbu2016	CbuK2067	CbuG2025	CbuD2113 (NT 50bp)	
Cbu2028	CbuK2080 (AS 68bp, FS 46bp)	CbuG2038 (AS 51bp, FS 361bp)	CbuD2117	
Cbu2059 (<i>cirE</i>)	CbuK2105 (AS 389bp, CT 615bp)	CbuG2063 (NT 182bp, FS 313bp)	CbuD2129 (AS 51bp)	WW domain- like
Cbu2064	CbuK2107 (AS 1233bp)	CbuG2065 (NT 22bp, CT 112bp)	CbuD2154 (AS 389bp)	
Cbu2076	CbuK2121	CbuG2077	CbuD2156 (NT 22bp)	
CbuA0015			Cbu2172	Coiled coil
CbuA0019				
CbuA0020		CbuG2038 (CT 447bp)	CbuDA0049 (CT 101bp)	

A.



B.



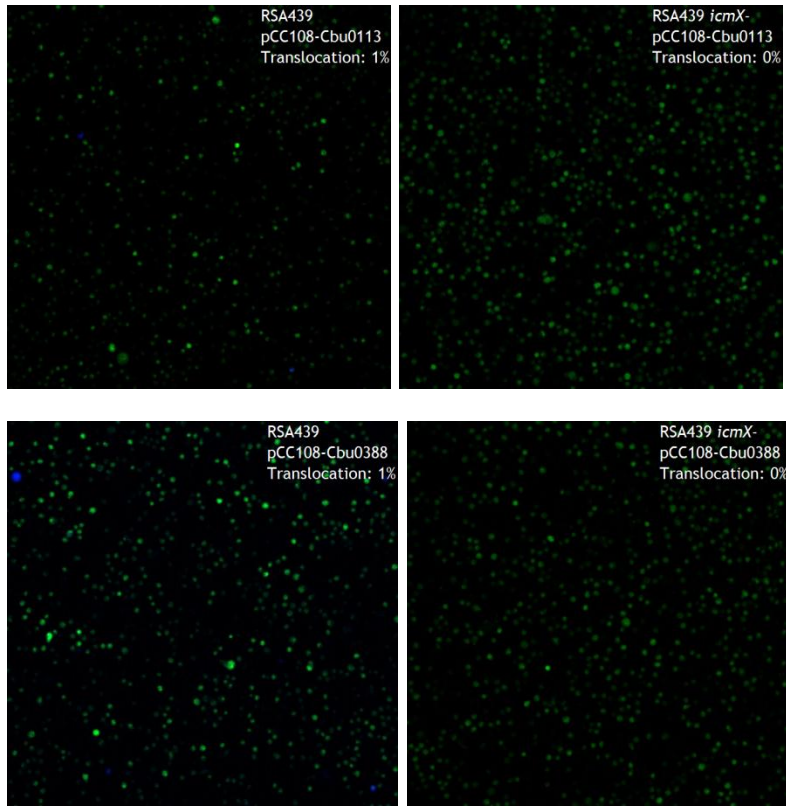
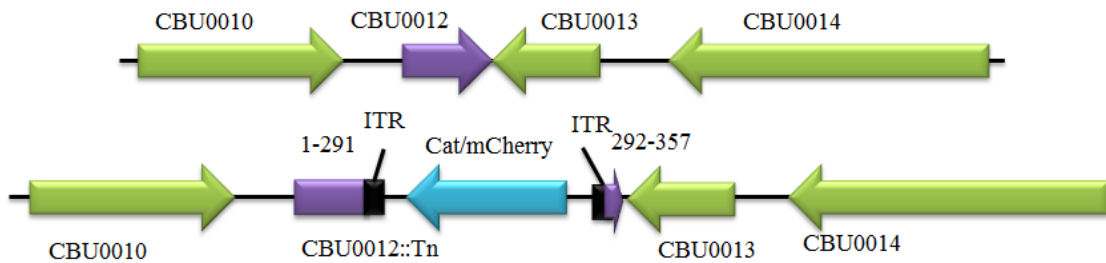
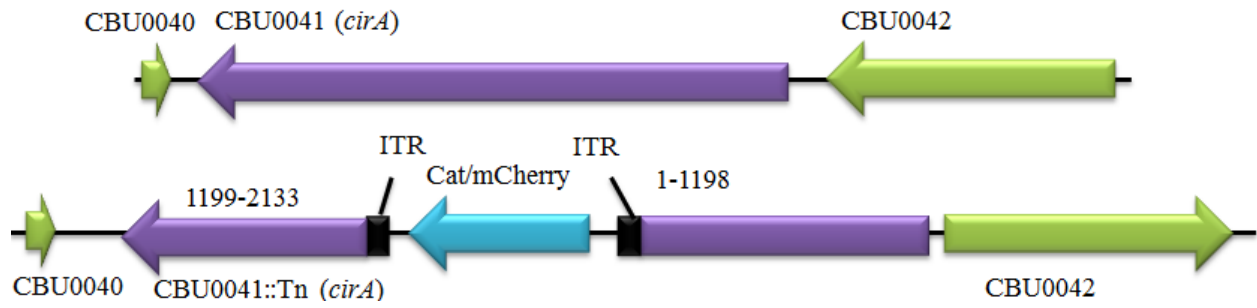


Figure S1: Newly identified secretion substrates are secreted by *C. burnetii*. THP-1 cells were infected for 48 h with *C. burnetii* expressing β -lactamase fusion constructs and subsequently loaded with CCF4/AM as described in materials and methods. (A) Confocal imaging of wild-type infected cells revealed the presence of blue cells (B) translocation of selected substrates was absent in cells infected with the *icmX*::Tn, indicating the identified substrates are translocated into host cells in a Dot/Icm-dependent manner.

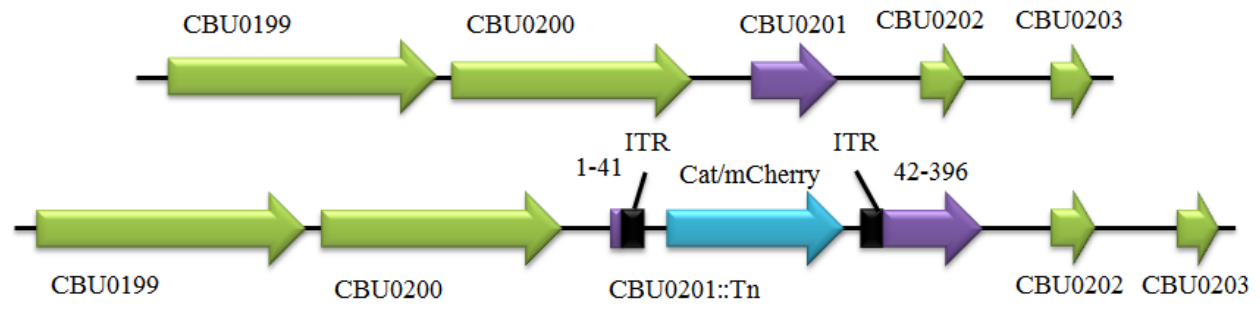
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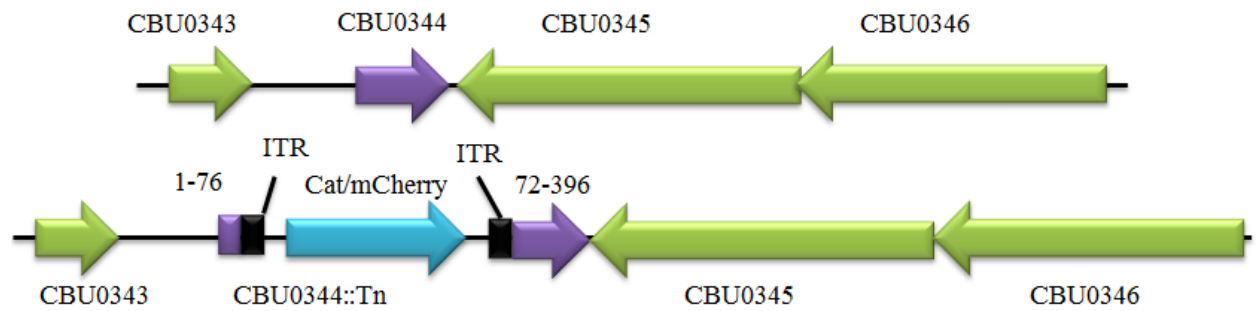
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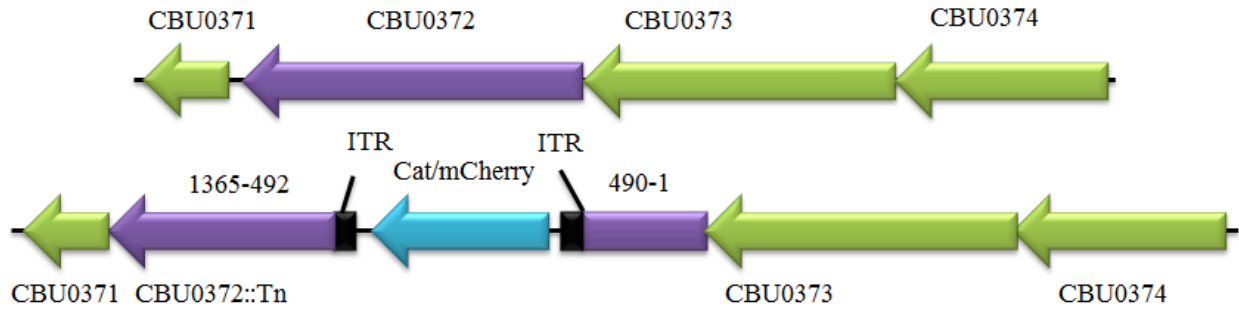
CBU0201



CBU0344



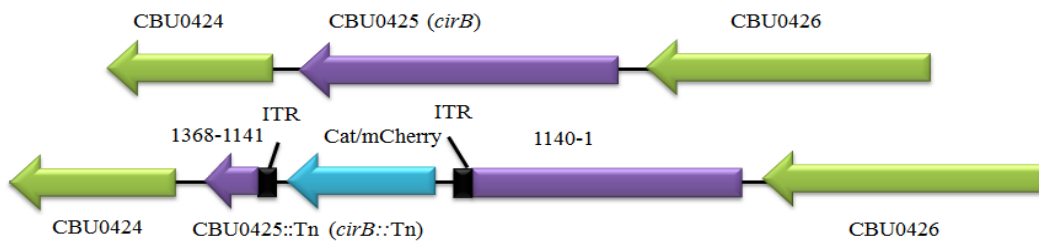
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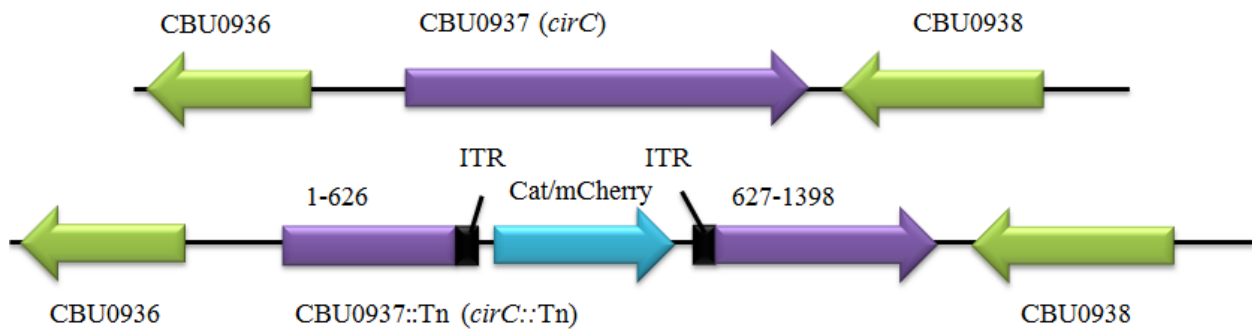
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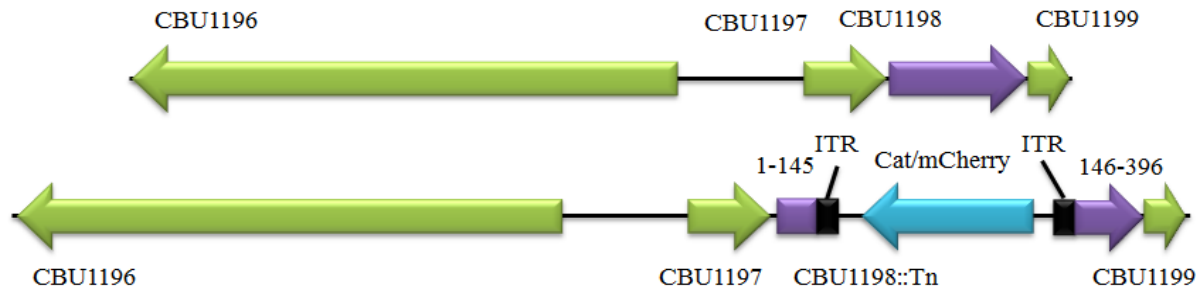
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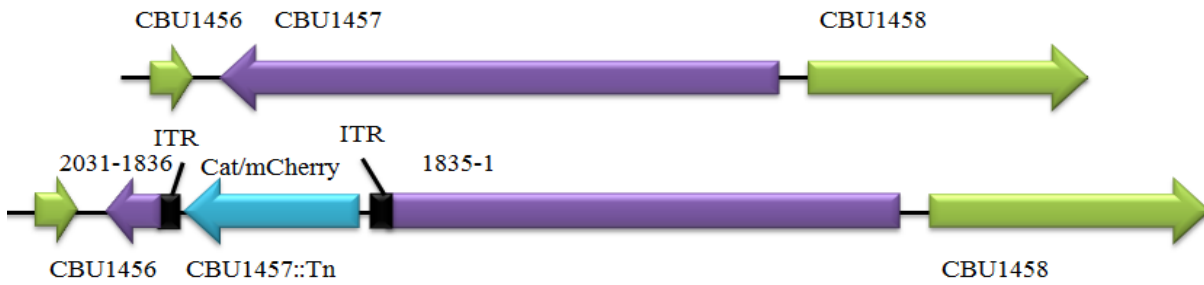
CBU0937 (*cirC*)



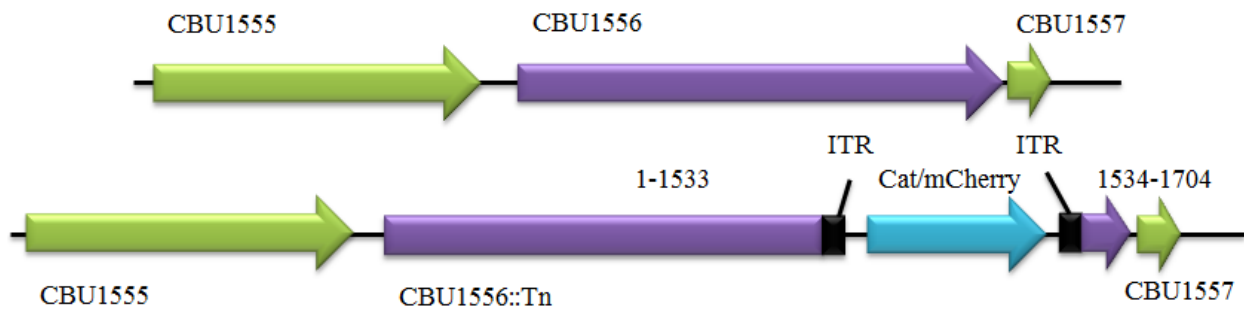
CBU1198



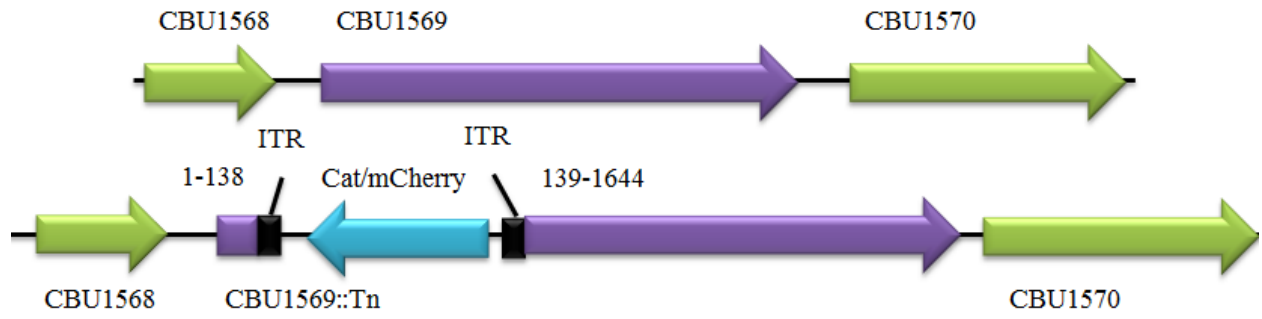
CBU1457



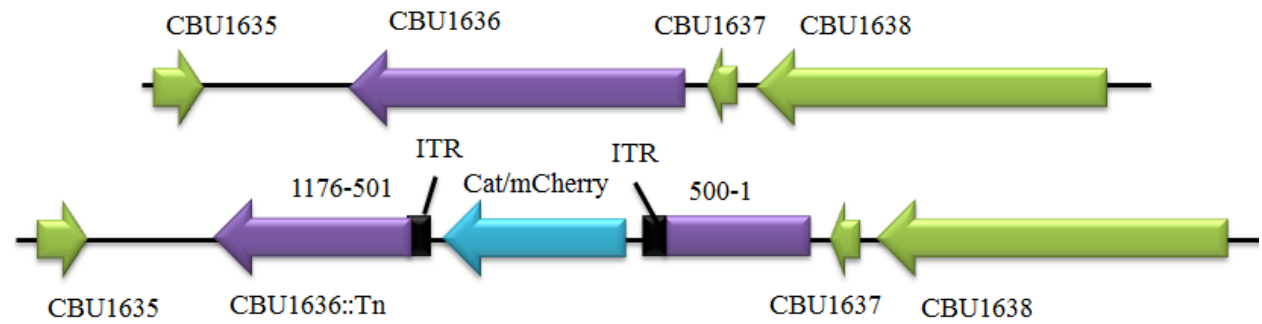
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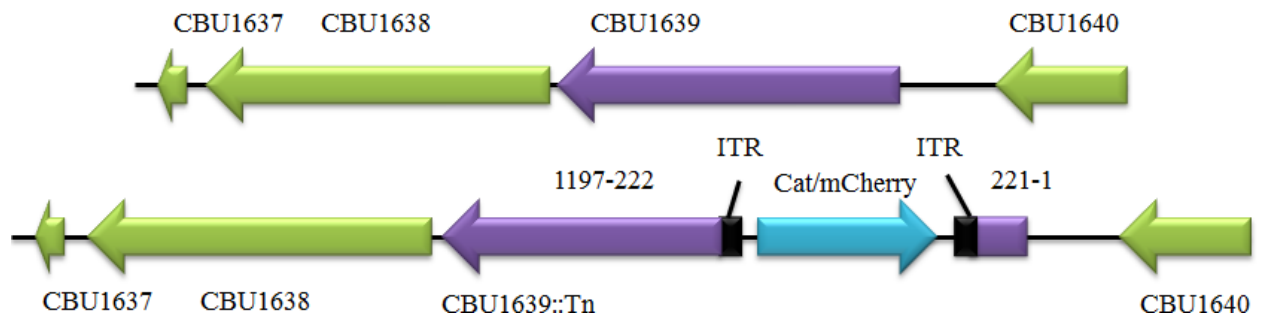
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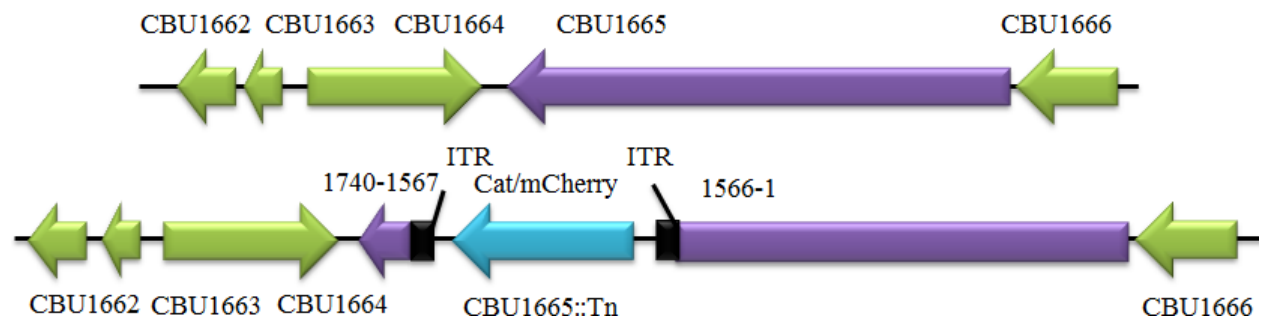
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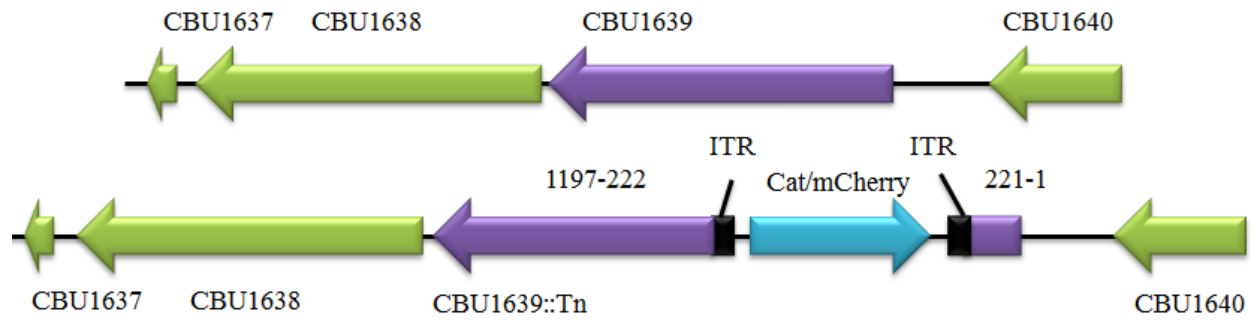
CBU1639



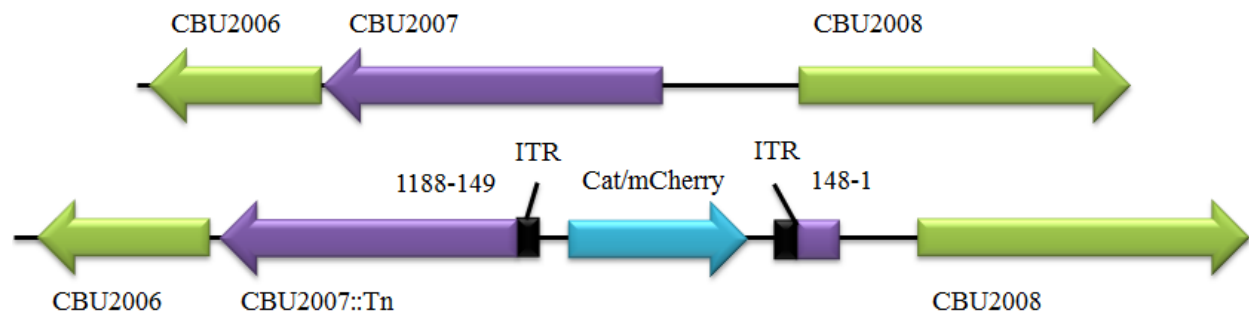
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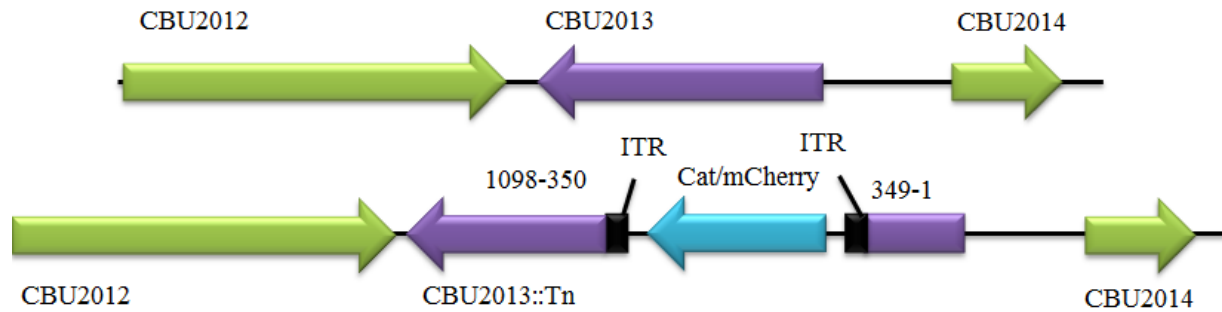
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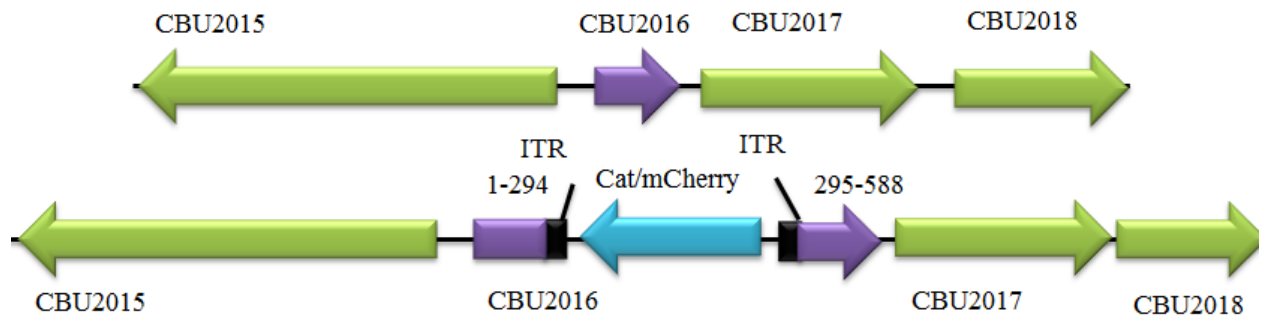
CBU2007



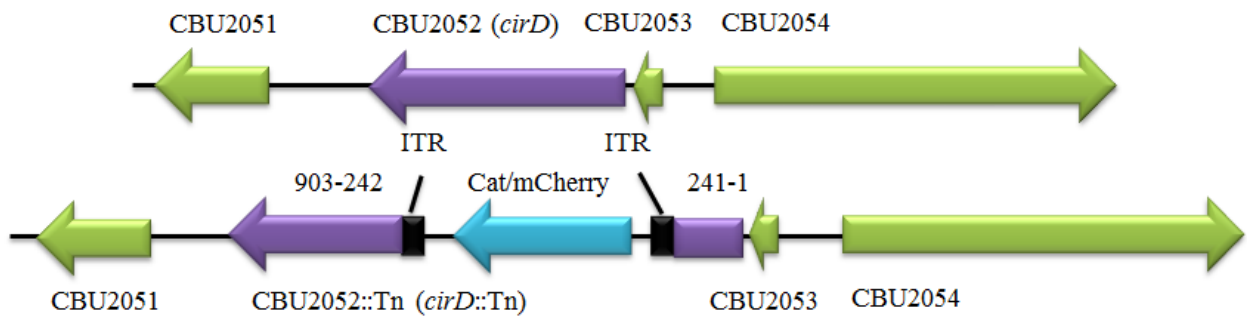
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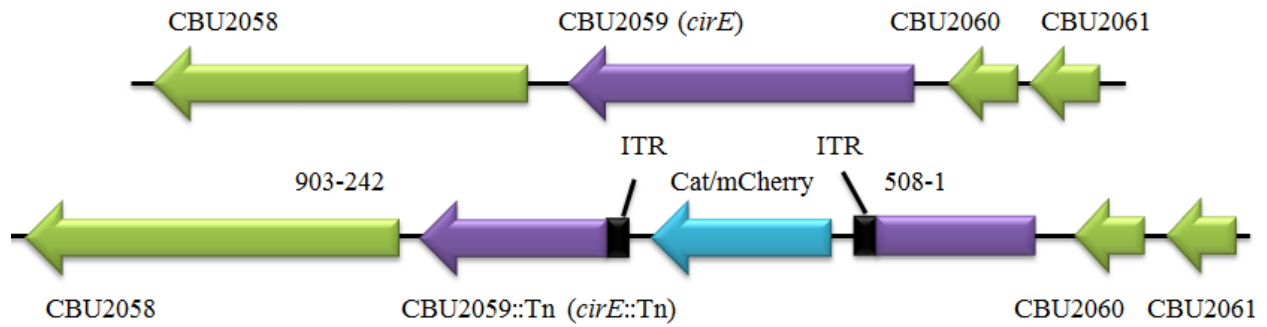
CBU2016



CBU2052 (*cirD*)



CBU2059 (*cirE*)



CBU1652 (*icmX*)

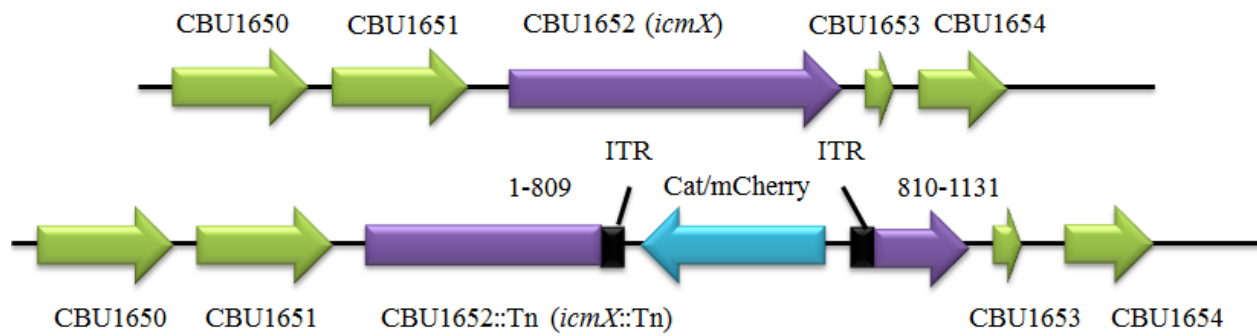
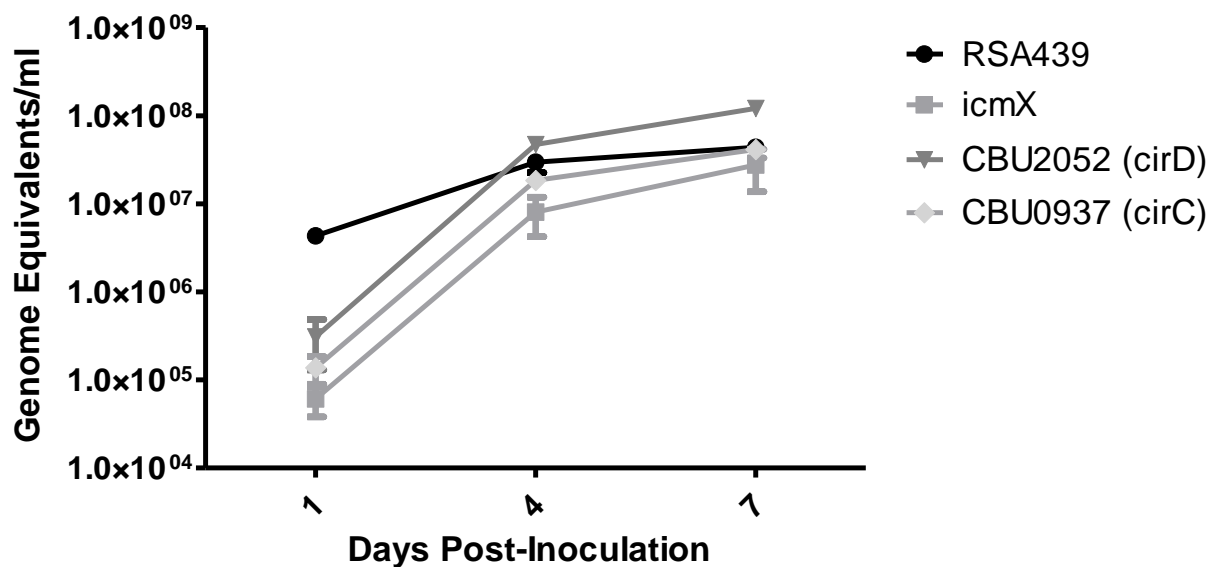
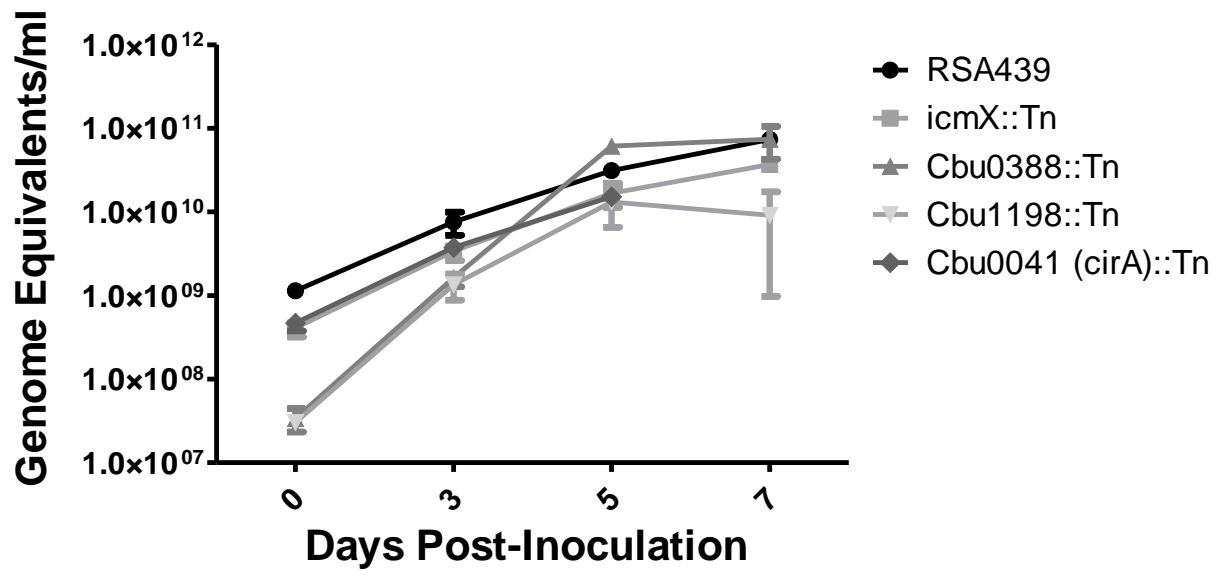


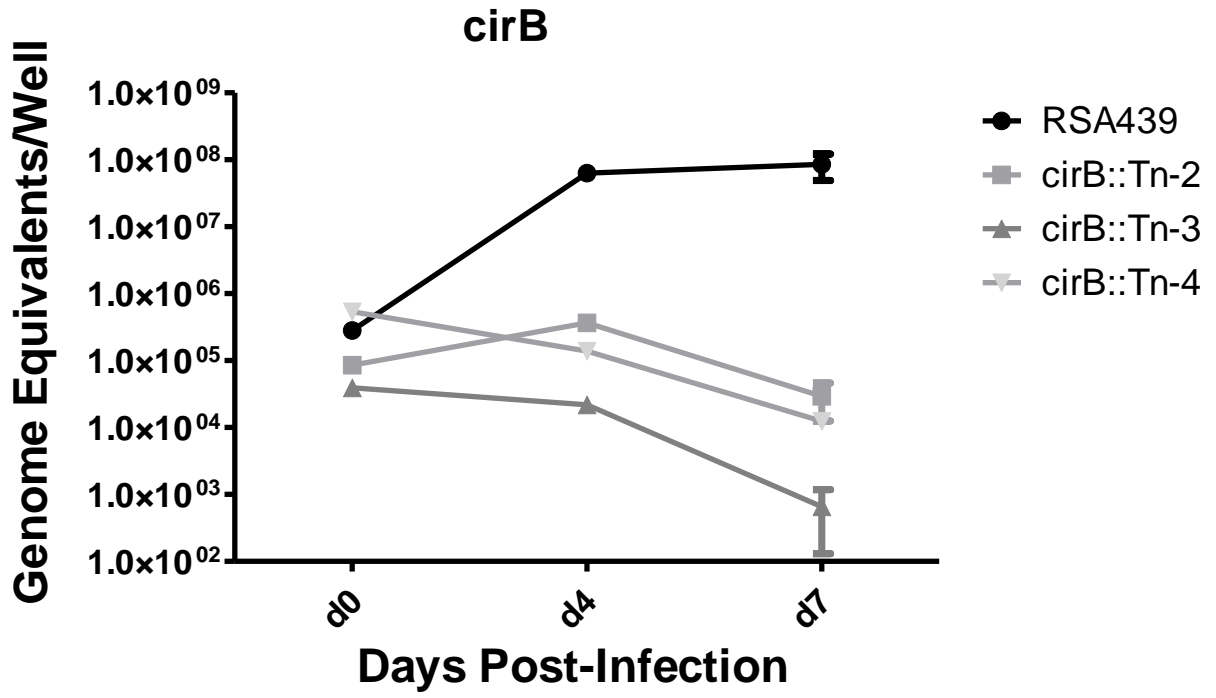
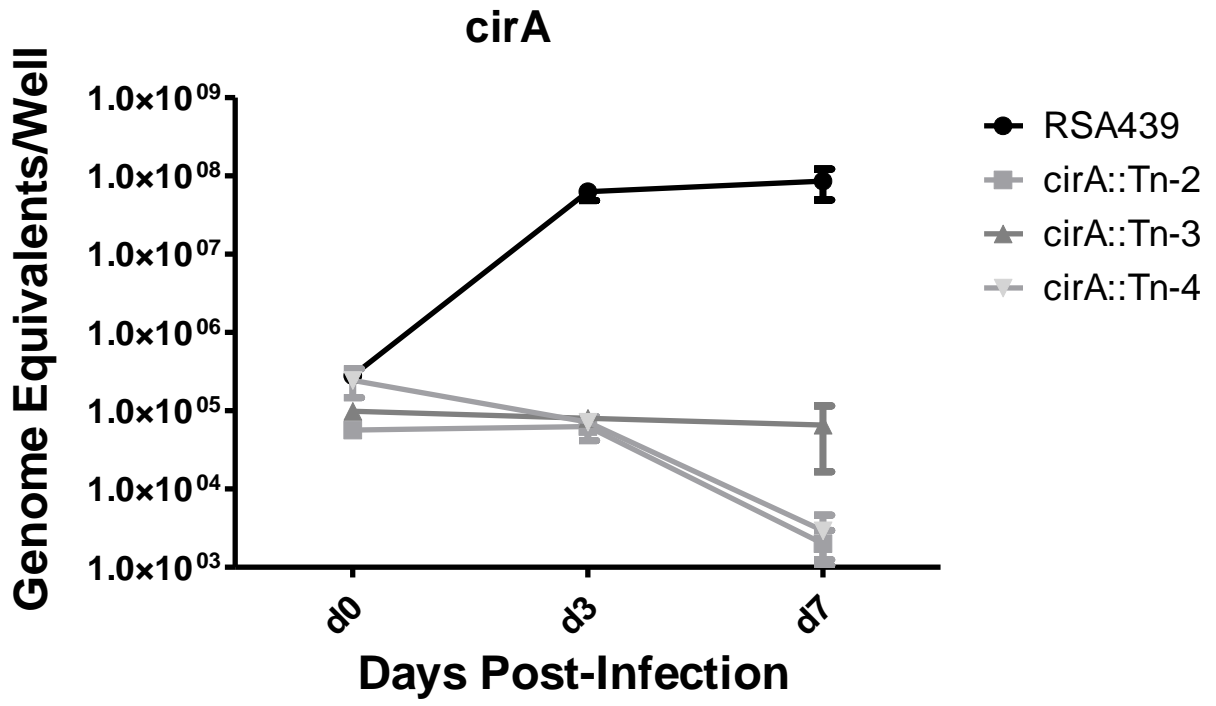
Figure S2: *Himar1* transposon insertion into *C. burnetii* T4SS substrate genes. To generate a large pool of *C. burnetii* Tn mutants, NMII was transformed with pKM225, and the resulting transformants were expanded and rescue cloned and three *E. coli* colonies were sequenced per transformation. The schematic shows the insertion of the *Himar1* transposon (blue) into the T4SS substrate genes (purple).

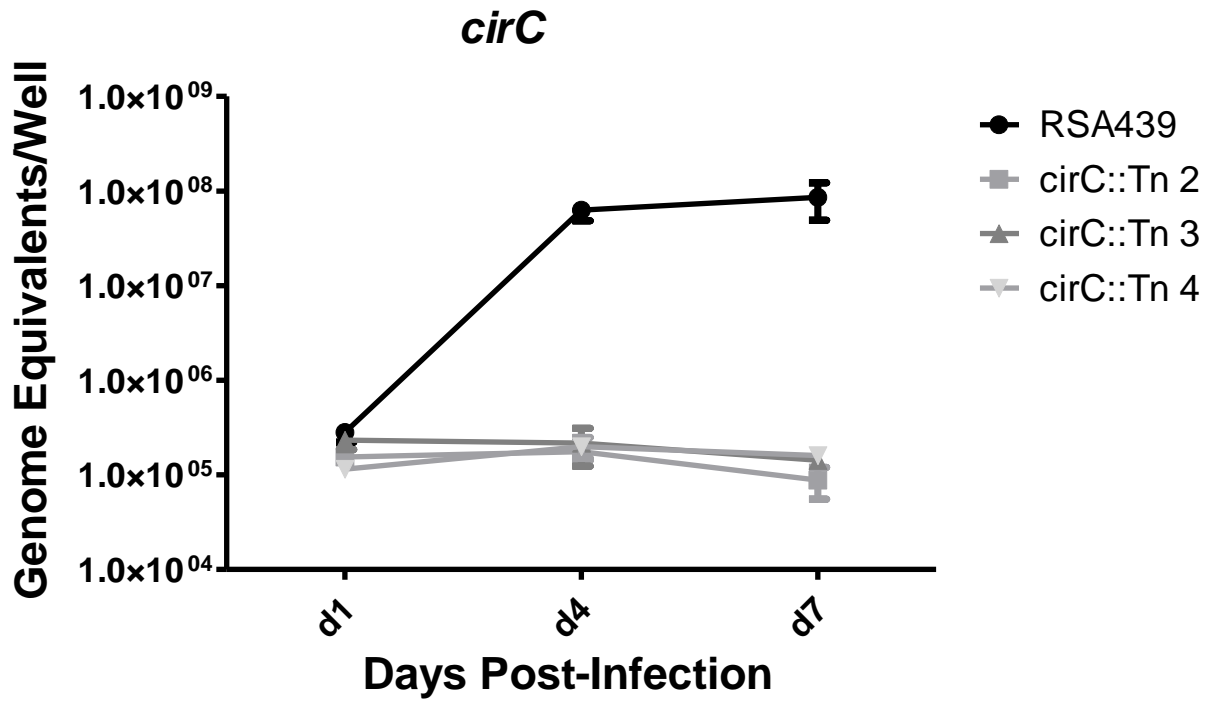
A





B





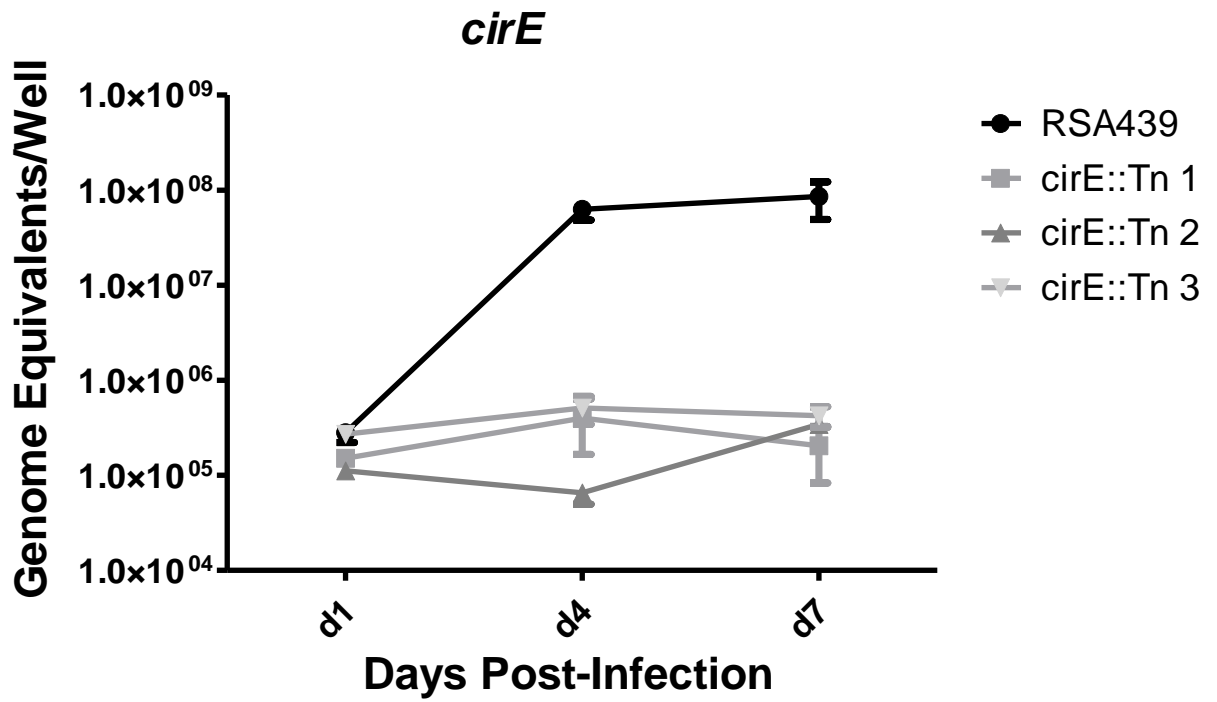
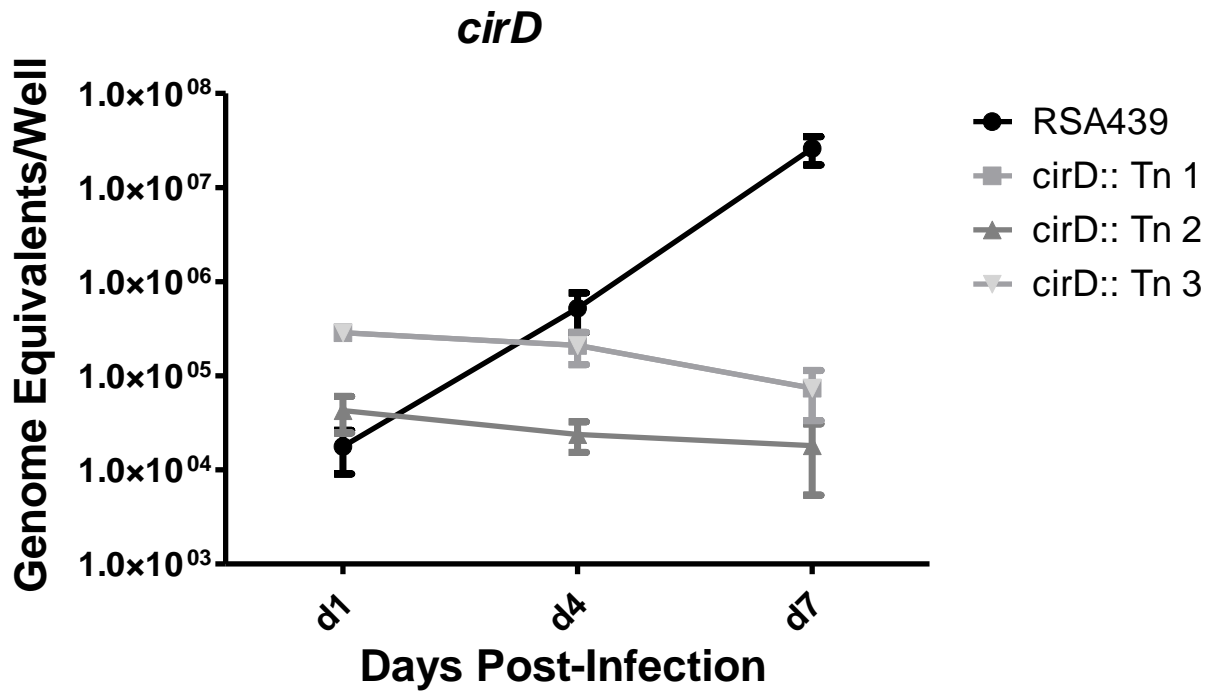


Figure S3: *C. burnetii* growth defects are due to the loss of individual effector proteins. (A) *C. burnetii* T4SS substrate mutants were used to inoculate ACCM-2 using 1.0×10^6 bacteria/ml. Loss of individual effectors had no effect on axenic culture. (B) To verify that the growth defect is due to loss of individual effector proteins, three additional mutants obtained from separate transformations were used to infect J774A.1 cells at an MOI of 100. Genome equivalents were calculated at d1, d4, and d7. Each isolated Tn mutant exhibited a similar phenotype, indicating the inability to replicate intracellularly is due to the loss of a specific individual effector and is not likely attributable to another mutation at a secondary site.