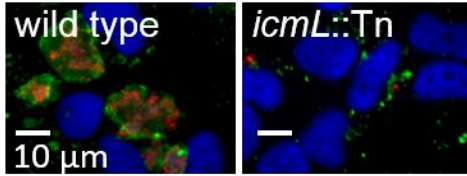


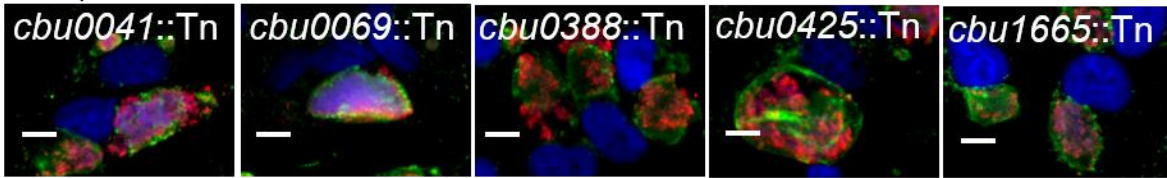
Supplemental Figure 1

A

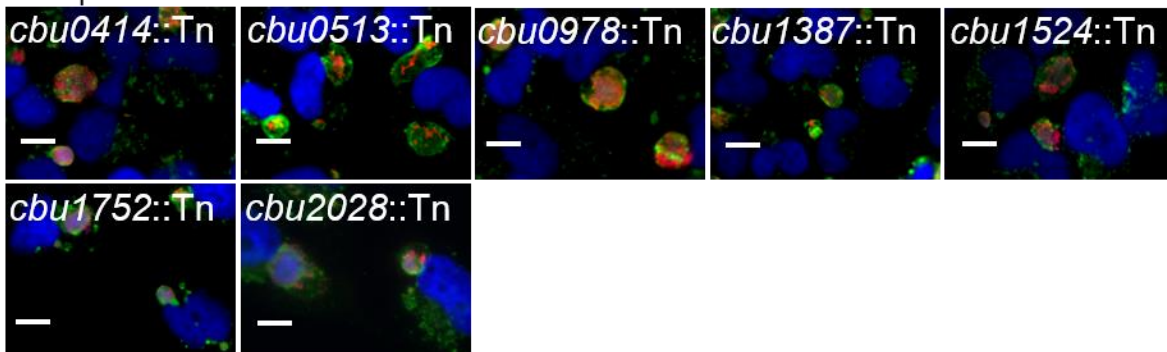
Control Strains



Group 1



Group 2



B

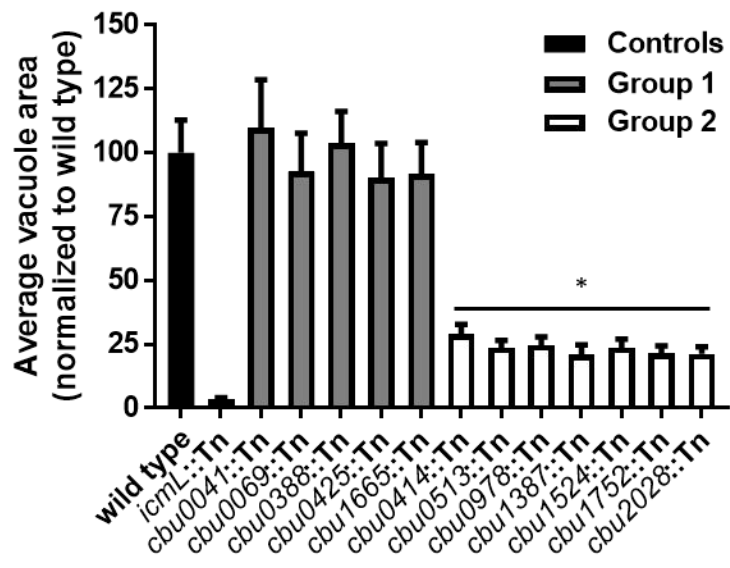


Fig. S1. CCV biogenesis phenotypes displayed after low MOI infection. HeLa cells were infected at an MOI of one and were analyzed five days post-infection. (A) The parental NMII strain (wild type) and a *Dot/Icm*-deficient *icmL::Tn* mutant were used as a positive and negative controls for CCV biogenesis, respectively. The previously described effector mutants in Group 1 displayed vacuoles that were equivalent in size to the vacuoles containing the NMII strain. The seven effector mutants described in this study were in Group 2, and the vacuoles displayed by these mutants were all reduced in size relative to the NMII-containing vacuoles, but larger than the vacuoles containing the *icmL::Tn* mutant. (B) Average vacuole area was determined for each mutant by averaging the size of all CCVs in the infected cells of eight random images acquired for each strain at 600X magnification. *, P-value < 0.001 indicate a significant difference in vacuole size compared to the size of vacuoles created by both the control NMII strain and the control *icmL::Tn* mutant strain as determined by Student's t-test.

Supplemental Figure 2

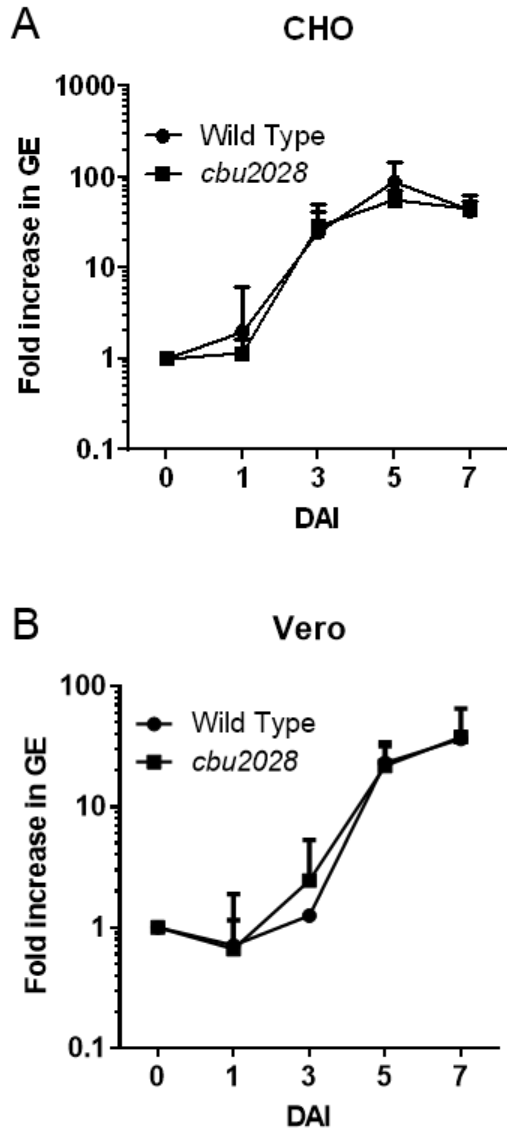


Fig. S2. Intracellular replication kinetics of *cbu2028*::Tn in CHO and Vero cells. Intracellular replication over a seven day time period was determined by fold-increase in genomic equivalents relative to the first day of infection (DAI=0). Intracellular replication for the parental NMII strain (Wild Type, circle), the *cbu2028*::Tn mutant (square), are shown for (A) CHO cells and (B) Vero cells.

Supplemental Table 1

Effector Gene	Gene Name	Genomic Insertion Site(s) (NC_002971.3)	CCV phenotype relative to wild type
<i>cbu0012</i>		12286	no difference
<i>cbu0021</i>	<i>cig2, cvpB</i>	19192; 19280	multi-vacuolar
<i>cbu0041</i>	<i>cirA, coxCC1</i>	38223; 39905	no difference*
<i>cbu0048</i>		45802; 45910	no difference
<i>cbu0049</i>		46672; 46994	no difference
<i>cbu0062</i>	<i>cem1</i>	56843	no difference
<i>cbu0069</i>	<i>ankP</i>	63560; 64162	no difference*
<i>cbu0072</i>	<i>ankA</i>	65711; 66529	no difference
<i>cbu0122</i>		111925	no difference
<i>cbu0128</i>		117057; 116556	no difference
<i>cbu0129</i>	<i>coxCC2</i>	116697; 116719	no difference
<i>cbu0144</i>	<i>ankB</i>	132025; 131885	no difference
<i>cbu0295</i>		262533; 262532	no difference
<i>cbu0344</i>		312064; 312062	no difference
<i>cbu0388</i>		352392	no difference*
<i>cbu0393</i>		357149	no difference
<i>cbu0414</i>	<i>coxH1</i>	369171	small
<i>cbu0425</i>	<i>cirB</i>	377489	no difference*
<i>cbu0447</i>	<i>ankF</i>	394134	no difference
<i>cbu0513</i>		453570	small
<i>cbu0534</i>		479403; 479397	no difference
<i>cbu0781</i>	<i>ankG</i>	722990	no difference
<i>cbu0885</i>	<i>cetCb4</i>	837619	no difference
<i>cbu0937</i>	<i>cirC, coxDFB1</i>	887774; 887017	small
<i>cbu0978</i>	<i>cem3</i>	930641	small
<i>cbu1043</i>	<i>gacA.4</i>	984953; 985088	no difference
<i>cbu1063</i>	<i>cem4</i>	1008750	no difference
<i>cbu1079</i>		1023981; 1023913	no difference
<i>cbu1110</i>		1056726	no difference
<i>cbu1198</i>		1142539	no difference
<i>cbu1217</i>	<i>coxU2</i>	1163535; 1162531	no difference
<i>cbu1251</i>		1202529	no difference
<i>cbu1292</i>	<i>ankK</i>	1246725; 1246917	no difference
<i>cbu1379</i>	<i>coxK2</i>	1328215	no difference
<i>cbu1387</i>	<i>cem6</i>	1337685	small
<i>cbu1406</i>	<i>coxDFB3</i>	1358424	no difference
<i>cbu1457</i>	<i>coxTPR1</i>	1410662	no difference

<i>cbu1461</i>	<i>coxCC8</i>	1417098; 1417177	small
<i>cbu1524</i>	<i>caeA</i>	1475299	no difference
<i>cbu1525</i>		1475299	no difference
<i>cbu1530</i>		1481087	no difference
<i>cbu1566</i>		1514458	no difference
<i>cbu1569</i>	<i>coxCC12</i>	1516828; 1517306	no difference
<i>cbu1576</i>		1523162; 1524222	no difference
<i>cbu1599</i>	<i>coxCC13</i>	1545143	no difference
<i>cbu1634</i>	<i>cem8</i>	1575307	no difference
<i>cbu1636</i>	<i>coxCC14, cig55</i>	1576055; 157917	no difference
<i>cbu1639</i>		1579591, 1579074	no difference
<i>cbu1665</i>		1601500	no difference*
<i>cbu1677</i>		1611159	no difference
<i>cbu1699</i>		1632356; 1632105	no difference
<i>cbu1724</i>		1658482	no difference
<i>cbu1751</i>	<i>cig57</i>	1682157; 1682180	small
<i>cbu1752</i>		1683656; 1683656	small
<i>cbu1754</i>		1684955; 1685031	small
<i>cbu1757</i>	<i>ankM, cig58</i>	1687615; 1688124	no difference
<i>cbu1769</i>	<i>coxH3</i>	1700167	no difference
<i>cbu1780</i>		1708652; 1709402	no difference
<i>cbu1789</i>		1721435; 1721437	no difference
<i>cbu1802</i>		1735650	no difference
<i>cbu2007</i>	<i>cem12</i>	1913579; 1913478	no difference
<i>cbu2013</i>	<i>cem13</i>	1920462	no difference
<i>cbu2028</i>		1935712; 1935580	small
<i>cbu2052</i>	<i>cirD</i>	1958215; 1958615	no difference
<i>cbu2056</i>		1962315; 1962702	no difference
<i>cbu2064</i>		1968966	no difference
<i>cbu2069</i>		1973680	no difference
<i>cbu2076</i>		1978466; 1978391	no difference
<i>cbu2078</i>	<i>coxFIC1</i>	1980054; 1979704	no difference

* Mutants with transposon insertions in these genes were reported previously (15, 17) to have severe intracellular replication defects that were not detectable in this screen with this mutant library.

Supplemental Table 2

Primer Name	Primer Sequence	Primer features
Partially complementary adapter 1	AGTTCTCCAGGTCTTGCGTTGCTCTTCCGATC*T	*Phosphothiorate linkage
Partially complementary adapter 2	GATCGGAAGAGCTCGTATGCCGTCTTCTGCTTG	5' phosphorylation
Universal adapter	CAAGCAGAAGACGGCATAACGAGCTCTTCCGATC*T	*Phosphothiorate linkage
Barcode primers	AATGATACGGCGACCACCGAGATCTACACTCTTTC CCTACACGACGCTCTTCCGATCTNNNNNNCGGGG ACTTATCAGCCAACC	NNNNNN = unique barcode
Barcode1	AAACAC	
Barcode2	TGAAGG	
Barcode3	AACATA	
Barcode4	CGCGTC	
Barcode5	GATACA	
Barcode6	GGTGTG	
Barcode7	TAAGAA	
Barcode8	AGCGAG	
Barcode9	CGGTTA	
Barcode10	AGCTTT	
Barcode11	TGGTCT	
Barcode12	TATCCC	
Barcode13	TGTCGT	
Barcode14	CCCCAC	
Barcode15	ATACGA	
Barcode16	CCCTTG	
Barcode17	ACCGGC	
Barcode18	TTACTG	
Barcode19	GGAACT	
Barcode20	GTTATT	
Barcode21	AAAAGT	
Barcode22	AAGGGA	
Barcode23	AAGTAT	
Barcode24	ACATCT	