

Supplemental Information

		Catalytic loop	
<i>Francisella novicida</i> (FTN_0131)	245-	L I Q I L F Y V C V L I E N D F H I C N F G T G L F N G --	-272
<i>Fangi hongkongensis</i> (FSC776_contig_24)	226-	L I S L L V Y M Y F L I E D D A H K K N F G V A Q F S V A N	-255
<i>Piscirickettsia salmonis</i> (KU39_2076)	201-	A G A D I A L M L Y P D G - D N H Q C N N G - - - - - V	-222
<i>Vibrio neptunus</i> (TW84_00590)	242-	L L S I V L L S Y L L V E D D L H I K N I G L C T I N G --	-259
<i>Rickettsia rickettsii</i> (Rrlowa_0230)	281-	F E K A I A A C H M L G E V D Y H A G N L M V - - - Q D --	-305
<i>Orientia tsutsugamushi</i> (OTSTA763_0692)	280-	F E K V I A A C H M L G D I D Y H G A N L M V - - - L D --	-304
<i>Aneurinibacillus aneurinilyticus</i> (HMPREF0083_01154)	117-	L L K M F V F D I W I C N I D R H G G N L L V Y P V G E --	-144
<i>Hyphomonadaceae bacterium</i> (VR75)	90-	L G K L Y A F D T W I A N I D R H A G N L I F - - - G G --	-124
<i>Candidatus Berkiella cookevillensis</i> (CC99x_00439)	174-	L G Y I I A N V L W H G D H D V H M G N F V R I E K D G --	-201
<i>Bdellovibrio bacteriovorus</i> (AZI86_00695)	434-	P E S F V F F D Y L I E N G D R H S D N Y L V R A D G R --	-461
<i>Comamonas testosteroni</i> (P608_25000)	100-	L G R L I A F D V L I R N A D R H P G N L L T - - - D - -	-123
<i>Silvanigrella aquatica</i> (AXG55_10680)	194-	L G E V L A A S L W L G D Y D I H I G N I G I A K V A N K A	-223
<i>Verrucomicrobia bacterium</i> (AUJ82_07980)	257-	S Y A V L A S L L F L T E A D P H L G N I M I K K D K N K H	-286
<i>Legionella pneumophila</i> (LegA5)	162-	L A S I L A T S Y T L E E D D L H K G N F G F Y L V K K --	-189
<i>Midichloria mitochondrii</i> (midi_01244)	308-	F P Q A M V T S L L I A D Y D V H W G N V G V V S E N G --	-335
		Activation loop	
<i>Francisella novicida</i> (FTN_0131)	272-	- - - - - K R C Y S K I D H D Y I V S F - - - W D A L K Y	-293
<i>Fangi hongkongensis</i> (FSC776_contig_24)	255-	- - Q D G K F S A Y G K I D H D Y L A T H - - - W E K G Q S	-280
<i>Piscirickettsia salmonis</i> (KU39_2076)	223-	S I N Q A G R L Q Y S K I D H D Y S S A R - - - L Q Q H K P	-250
<i>Vibrio neptunus</i> (TW84_00590)	259-	- - - - - R T V F G K I D H D Y I V S K - - - W E E K K N	-290
<i>Rickettsia rickettsii</i> (Rrlowa_0230)	305-	- - - - - G K T I T K I D H G R S F L A - - - F H K N F S	-326
<i>Orientia tsutsugamushi</i> (OTSTA763_0692)	304-	- - - - - G K T V A K I D H G R S F M Q - - - Y H K D F A	-325
<i>Aneurinibacillus aneurinilyticus</i> (HMPREF0083_01154)	144-	- - - - - K Y D F Y L I D H G L S L L G A V Q W Q G	-165
<i>Hyphomonadaceae bacterium</i> (VR75)	124-	- - - - - K D A I W L I D H G H S F T G P K W T A G	-135
<i>Candidatus Berkiella cookevillensis</i> (CC99x_00439)	201-	- - - - - G V K Y S K I D H G F S F F N - - - F D K E I V	-222
<i>Bdellovibrio bacteriovorus</i> (AZI86_00695)	461-	- - - - - K - - - V A I D H G L A L D A - - W H V P D F	-479
<i>Comamonas testosteroni</i> (P608_25000)	123-	- - - - - G Q D Y W A I D H G R T L D L H P - Y Q G H	-144
<i>Silvanigrella aquatica</i> (AXG55_10680)	124-	D L K Y G Y K Y D F V K I D H G W S F A Y - - - L Q N Q M N	-250
<i>Verrucomicrobia bacterium</i> (AUJ82_07980)	287-	Y L G - - - - - K I D H D G S F M D - - - M Y S K Q W	-305
<i>Legionella pneumophila</i> (LegA5)	189-	- - Q G K P R V V F F K I D H D L M F V D - - - S I M S F T	-214
<i>Midichloria mitochondrii</i> (midi_01244)	335-	- - - - - Q N K M V R I D F G W A F K K - - - L T P K L N	-356

Figure S1. OFPs possess two conserved amino acid motifs. Related to Figure 1.

Partial alignment of OFP sequences from species representative of each OFP-containing genus, highlighting the two conserved motifs (perfectly conserved residues, black; highly conserved residues, dark grey; conserved residues, light grey).

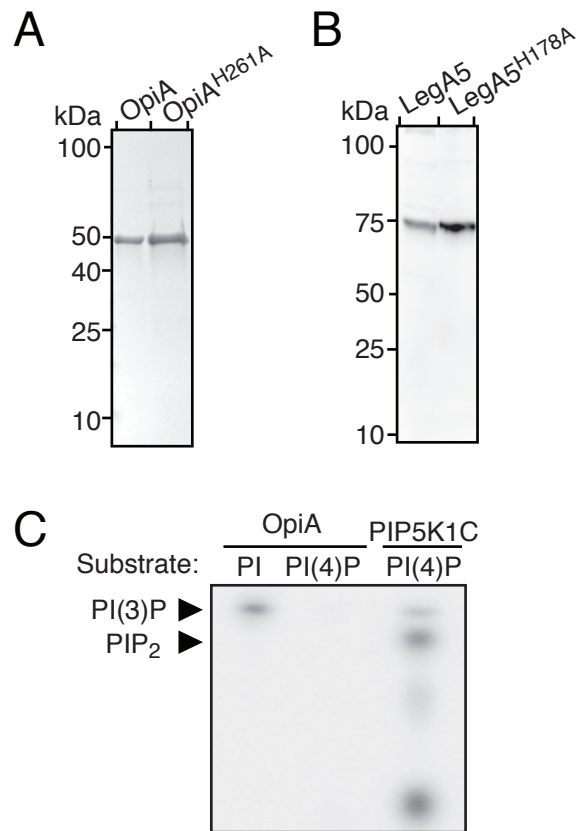


Figure S2. Amino acid substitutions in the catalytic motifs do not destabilize OpiA and LegA5 and OpiA does not phosphorylate a PI(4)P lipid substrate. Related to Figure 2.

(A) Coomassie brilliant blue staining of purified OpiA and OpiA^{H261A}.

(B) Western blot analysis of purified LegA5 and LegA5^{H178A}.

(C) Autoradiographs of thin layer chromatography (TLC)-separated lipid products produced by incubating the denoted proteins with [γ^{32} P]-ATP and the indicated lipid substrates.

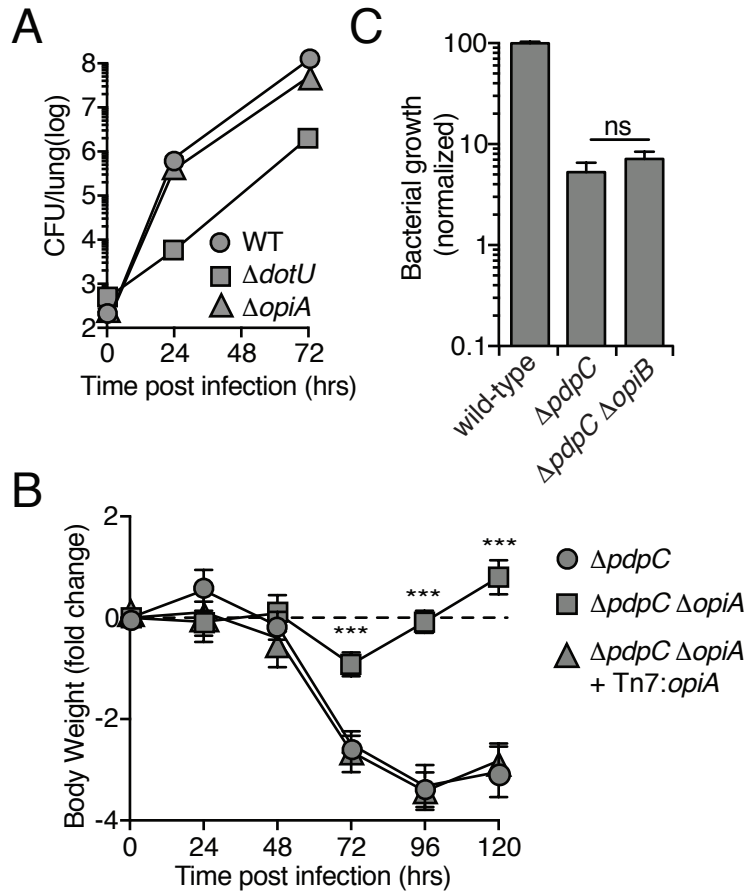


Figure S3. Phenotypic characterization of selected *F. novicida* mutants containing deletions of genes encoding secreted effectors. Related to Figure 3.

(A) Bacterial burden in the lungs of mice infected via the aerosol route with the indicated strains of *F. novicida*.

(B) Change in body weight over time of mice infected by the aerosol route with indicated strains of *F. novicida*. Data in A-C are shown as the mean \pm s.d. and asterisks represent statically significant differences (Student's t-test; *** $p < 0.0001$).

(C) Growth at 24hrs of the indicated strains of *F. novicida* in RAW 264.7 cells, normalized to level of growth by the wild-type strain (100%).

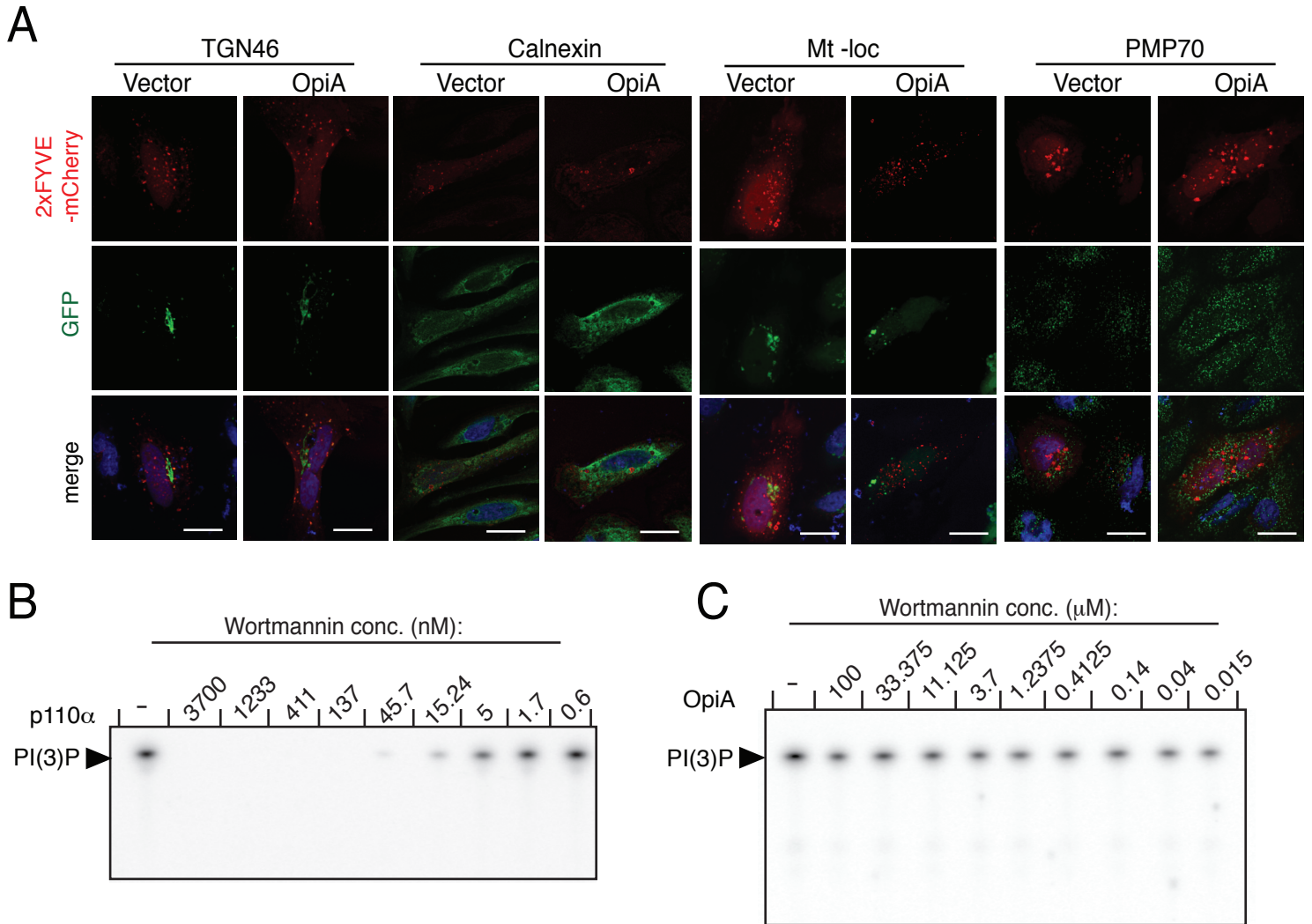


Figure S4. OpiA is a Wortmannin-insensitive PI3K that generates PI(3)P on endosomes. Related to Figure 4.

(A) Representative fluorescent images of HeLa cells expressing 2xFYVE-mCherry, co-transfected with vectors expressing the indicated proteins and stained (green) for the indicated markers (TGN46, Calnexin and PMP70, immunofluorescence; mitochondria, co-transfection to express GFP-labeled mitochondrial targeting sequence). Scale bar, 10 μ M.

(B and C) Autoradiographs of thin layer chromatography (TLC)-separated lipid products produced by incubating the indicated proteins with [γ ³²P]-ATP, PI-containing liposomes and wortmannin at the designated

Table S1: Oligonucleotides used in this study. Related to the STAR Methods section

Oligonucleotides		
#1: 5'-AGCATCACGTAAGCTTAGCTTAATTTTATAAGAATGTTTTCTGGATTTG-3'	This paper	N/A
#2: 5'-TCAGTAACGGATCCTCCTAGTGGTATGTCTTATAAATAAGATTATAG-3'	This paper	N/A
#3: 5'-GATAATAGTATTGTGCTAGAGTTAGATGCAGGAC-3'	This paper	N/A
#4: 5'CATAAGTCCACAAAGAACTTGGATGCTTATTTCC-3'	This paper	N/A
#5: 5'-TCAGTAACCCATGGGCAAAAATTTGAAGTAATACGCAAAGATTTTC-3'	This paper	N/A
#6: 5'-TCAGAAATCTCGAGATTCAACAAATTACACAAATGAGAAAATC-3'	This paper	N/A
#7: 5'-GTACCAAATTACAGATAGCAAATCATTCTATTAACACACAAACATAAAATAAAATTTGTATTAGTC-3'	This paper	N/A
#8: 5'-GTTAATAGAAAATGATTTTGCTATCTGTAATTTTGGTACAGGACTATTTAATGGAAG-3'	This paper	N/A
#9: 5' TCAAGTACTACATATGCCTAGAGTTTATAATCTTAAAGATATTTATCTGG-3'	This paper	N/A
#10: 5'-TCAGTAACCTCGAGTTAGATTTTATTCTTTGATAGTGATATATCCAAAAAGACATTATCC-3'	This paper	N/A
#11: 5'-TCCCTTTAGCCAAATCGTCTTCTTCCAAAG-3'	This paper	N/A
#12: 5'-AGACGATTTGGCTAAAGGGAATTTTGGTTTTTATTTG-3'	This paper	N/A
#13: 5'-TCAGTAACGGATCCATGAAAAATTTGAAGTAATACGCAAAGATTTTC-3'	This paper	N/A
#14: 5'-TCAGAAATGCGGCCGCTCAATTCAACAAATTACACAAATGAGAAAATC-3'	This paper	N/A
#15: 5'-AGAATCAGTGGATCCATGTCAGACATCGAAGAAGGTACGC-3'	This paper	N/A
#16: 5'-CTGAAAGATAGCGGCCGCTTAGTATTGGCCTTGTTGCGGTTC-3'	This paper	N/A
#17 5'-ACATGATGCCTCGAGGCCACCATGAAAAATTTTGAAGTAATACGCAAAGATTTTC-3'	This paper	N/A
#18: 5'CTACCCGGTAGAATTCTTAATTCAACAAATTACACAAATGAGAAAATC-3'	This paper	N/A