Supplemental Information

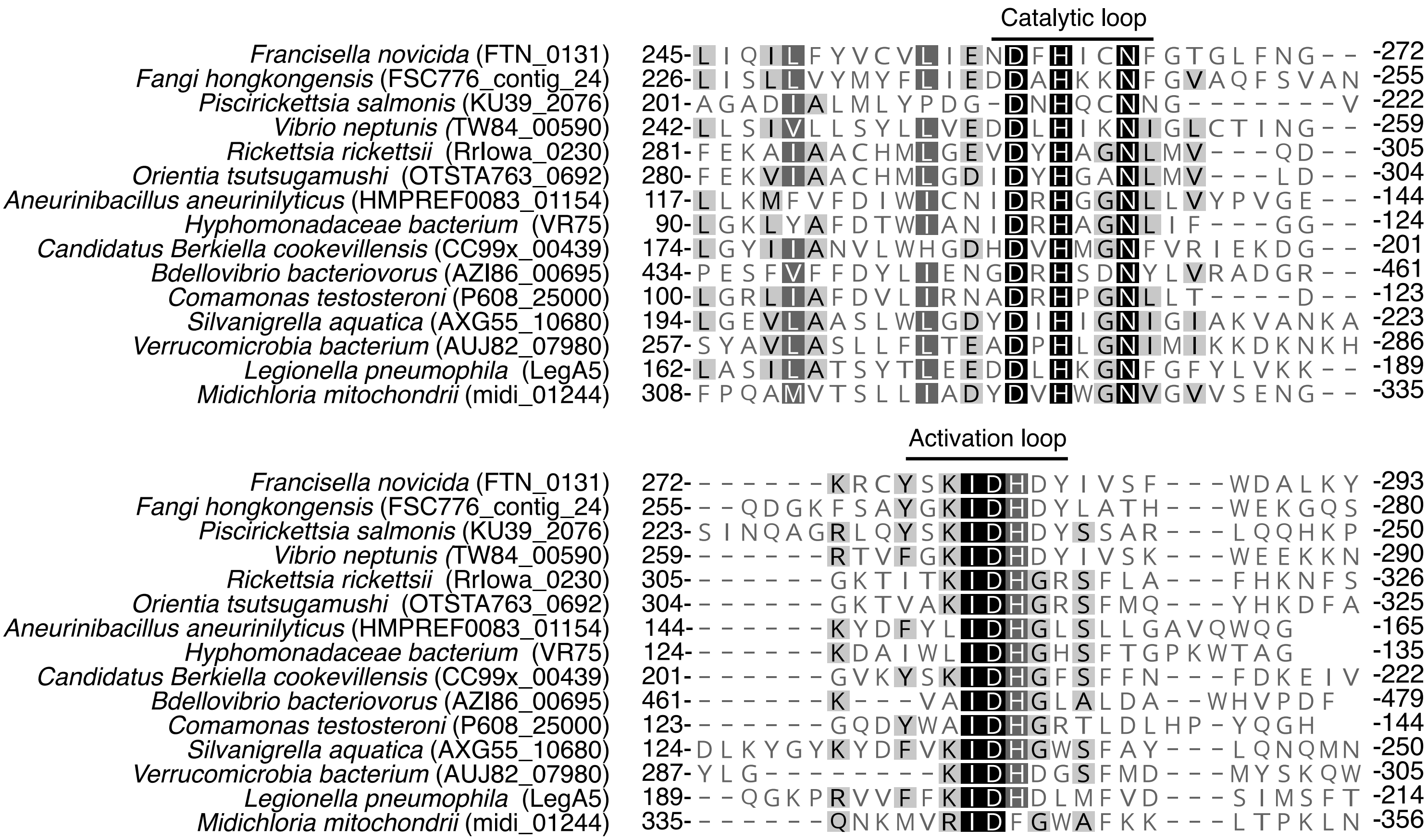

Figure S1. OFPs possess two conserved amino acid motifs. Related to Figure 1.
Partial alignment of OFP sequences from species representative of each OFP-containing genus, highlighting the two conserved motifs (perfectly conserved residues, black; highly conserved residues, dark grey; conserved residues, light grey).

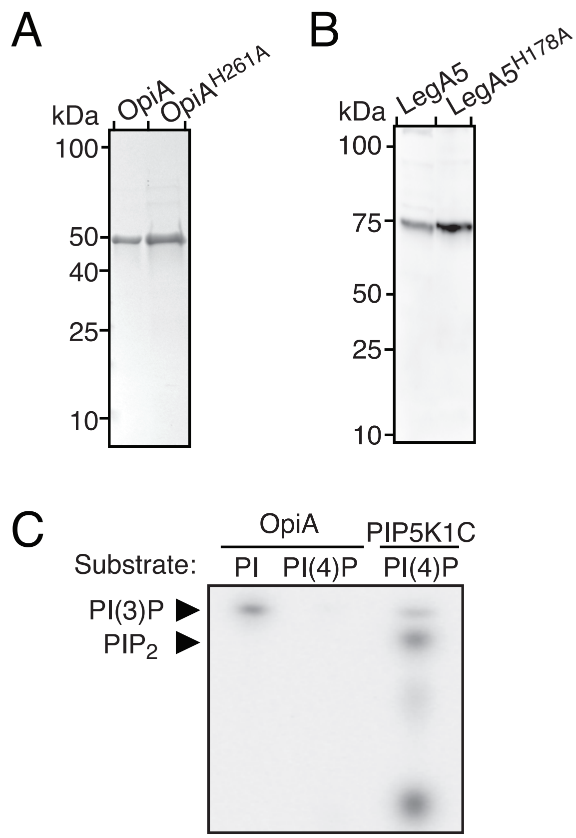

Figure S2. Amino acid substitutions in the catalytic motifs do not destabilize OpiA and LegA5 and OpiA does not phosphorylate a PI(4)P lipid substrate. Related to Figure 2.

(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.

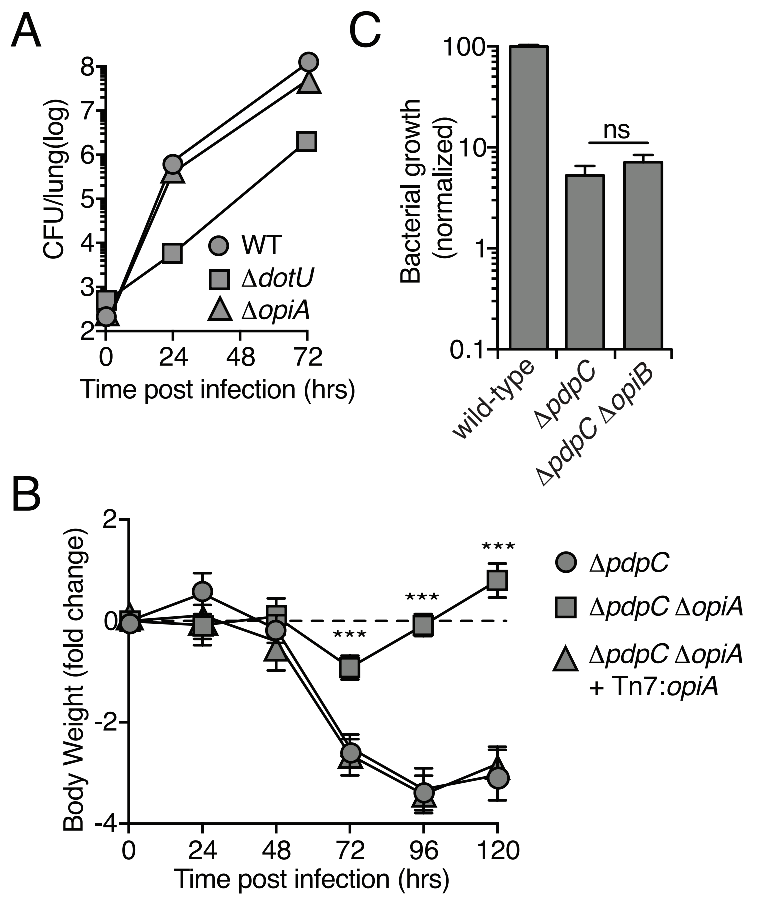

Figure S3. Phenotypic characterization of selected F. novicida mutants containing deletions of genes encoding secreted effectors. Related to Figure 3.

(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 ± 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%).

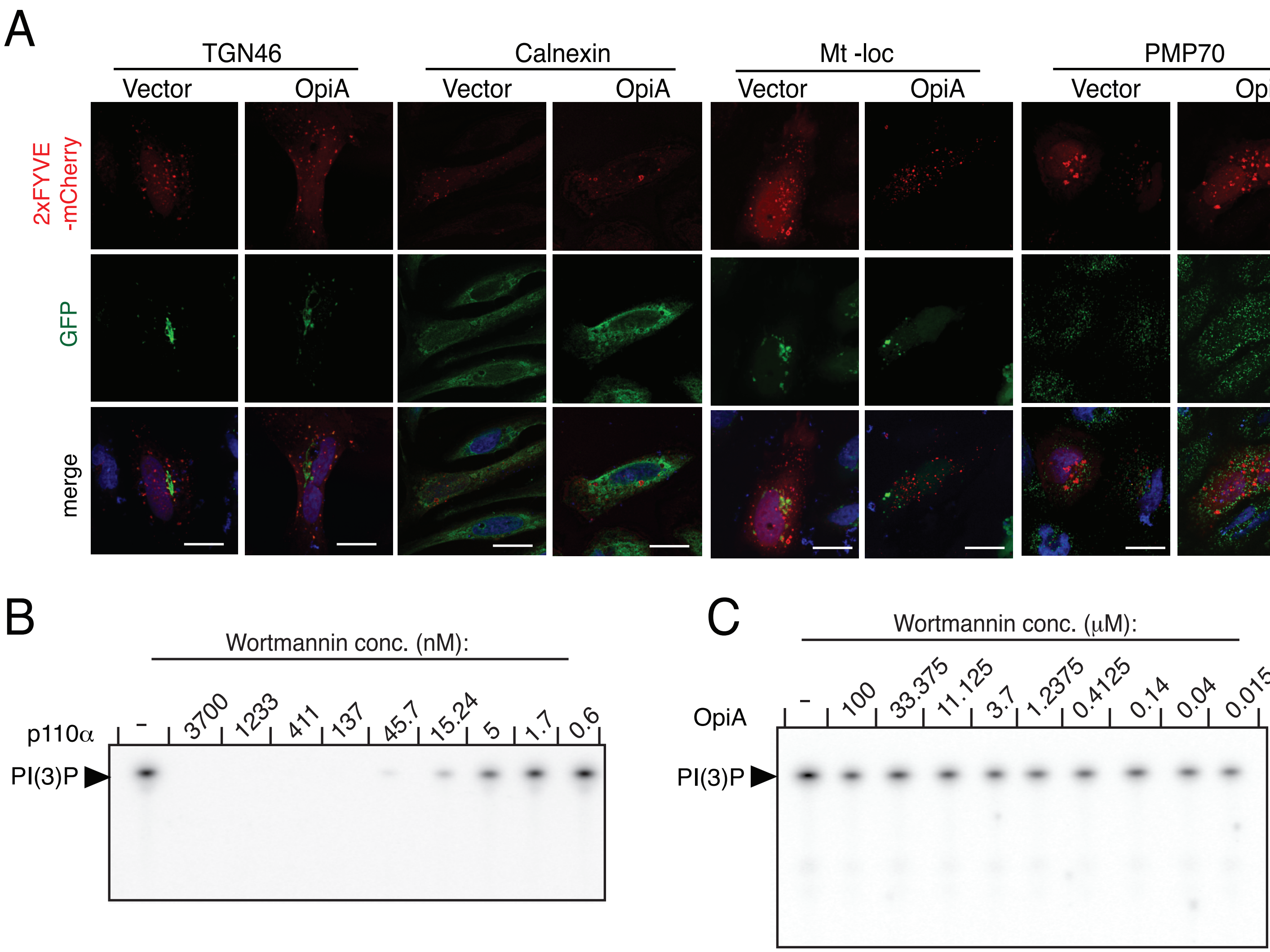

Figure S4. OpiA is a Wortmannin-insensitive PI3K that generates PI(3)P on endosomes. Related to Figure 4.

(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, immunofloursence; mitochondria, co-transfection to express GFP-labeled mitochondrial targeting sequence). Scale bar, 10 μM.

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

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’-TCAGTAACCCATGGGCAAAAATTTTGAAGTAATACGCAAAGATTTTC-3’	This paper	N/A
#6: 5’-TCAGAAATCTCGAGATTCAACAAATTACACAAATGAGAAAATC-3’	This paper	N/A
#7: 5’-GTACCAAAATTACAGATAGCAAAATCATTTTCTATTAACACACAAACATAAAATAAAATTTGTATTAGTC-3’	This paper	N/A
#8: 5’-GTTAATAGAAAATGATTTTGCTATCTGTAATTTTGGTACAGGACTATTTAATGGAAAG-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’-TCAGTAACGGATCCATGAAAAATTTTGAAGTAATACGCAAAGATTTTC-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