Supplemental Figure Legends

Supplemental Figure 1.
***In vitro* AMPylation assay with Δ74 VopS and Δ74 VopS H348A mutant.** Reactions were incubated with 100 μM His_6-Cdc42-(1-179)-(Q61L) and ^{32}P-α-ATP at 25ºC alone or with Δ74 VopS or Δ74 VopS H348A mutant. The assay was stopped at 90 seconds with loading buffer.

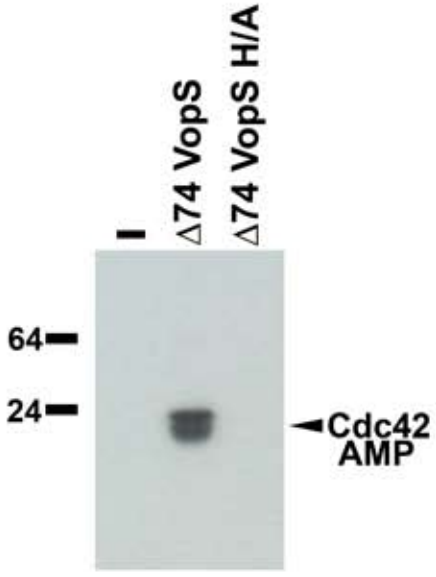
Supplemental Figure 2.
Schematic diagram of the secondary structure organization of known structures of Fic domain containing proteins. Shown are the VopS protein from *V. parahaemolyticus,* Fic protein from *Shewanella oneidensis* (PDB ID 3EQX), *Helicobacter pylori* (PDB ID 2F6S), *Bartonella henselae* (PDB ID 2JK8), and of Fido protein family member, AvrB, from *Pseudomonas syringae* (PDB ID 2NUN), and Doc domain family *Enterobacteria* phage P1 (PDB ID 3DD7). Highlighted in red and white are the structurally non-conserved N-terminus of VopS and the non-conserved secondary structures of Fido/Fic domain containing proteins, respectively. Shaded in green are the helices that form the conserved Fic domain. Purple arrowheads point to positions of the circularly permuted a' helix of Fic domains. The conserved histidine is shown for all proteins except AvrB, which lacks the conserved histidine and is thus highlighted with an asterisk.

Supplemental Figure 3.
Structural comparison of the permuted helix of VopS Fic domain. Colored in purple is the circularly permuted a' helix of the Fic protein family. Colored in red is the second permuted helix a7. Displayed in blue is the conserved H348 positioned near the C-terminus of the a4 helix.

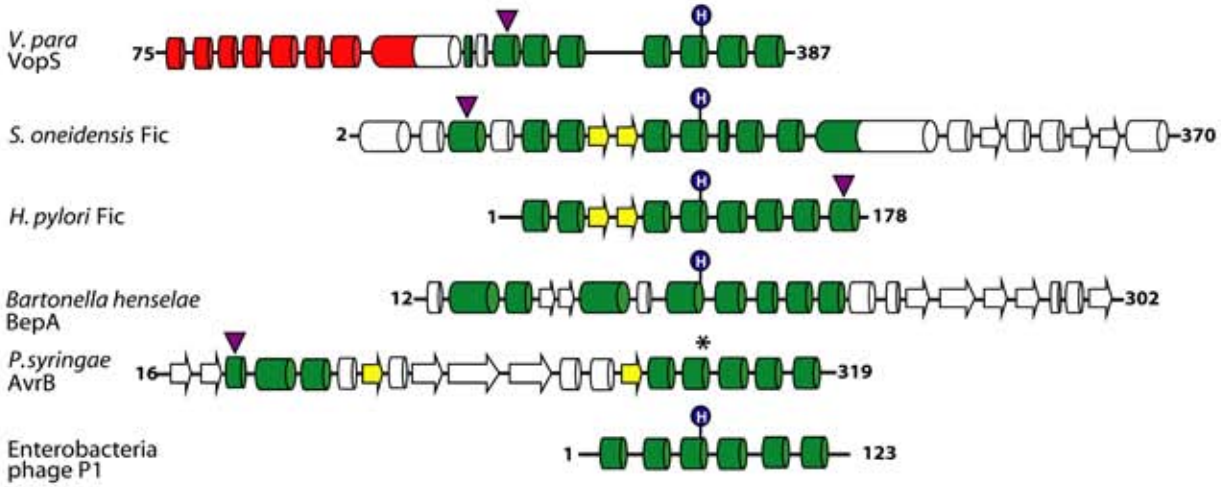
Supplemental Figure 4.
Global fit of bisubstrate kinetic data according to ordered rapid-equilibrium or ping-pong model. (A) Global fit of the data for ordered rapid-equilibrium mechanism. The square of the correlation coefficient (r^2) for the global fit data is 97.3. (B) Global fit of the data for the ping-pong mechanism. The square of the correlation coefficient (r^2) for the global fit data is 94.7. Global fits were performed with Sigma Plot.

Supplemental Figure 5.
pH Profile of VopS WT Activity. Assays were performed in triplicate at different pH ranges. Reactions were incubated with 5nM VopS (31-387), 100μM His6-Cdc42-(1-179)-(Q61L), and 200μM ^{32}P-α-ATP for 75 seconds and spotted onto P81 Whatman filters. Error bars represent standard error of the mean.

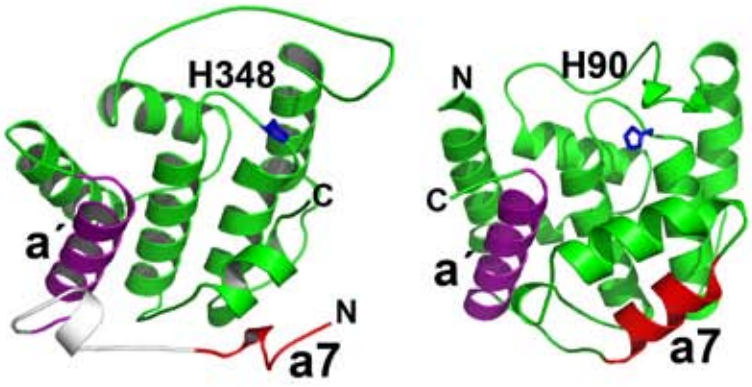
Supplemental Figure 1



Supplemental Figure 2

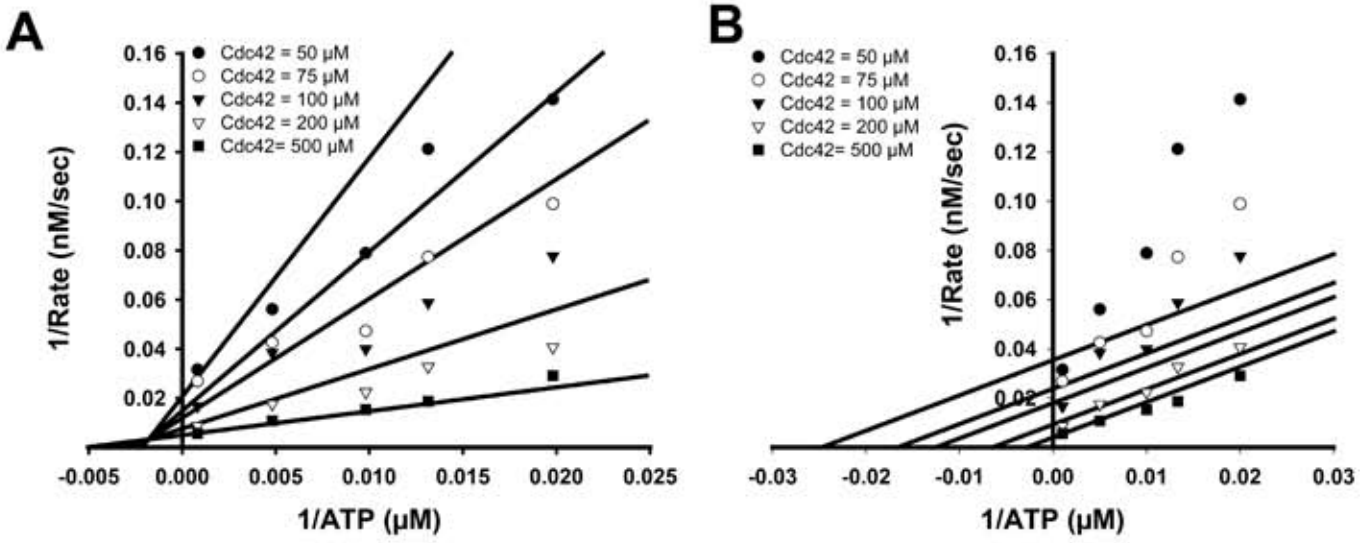


Supplemental Figure 3

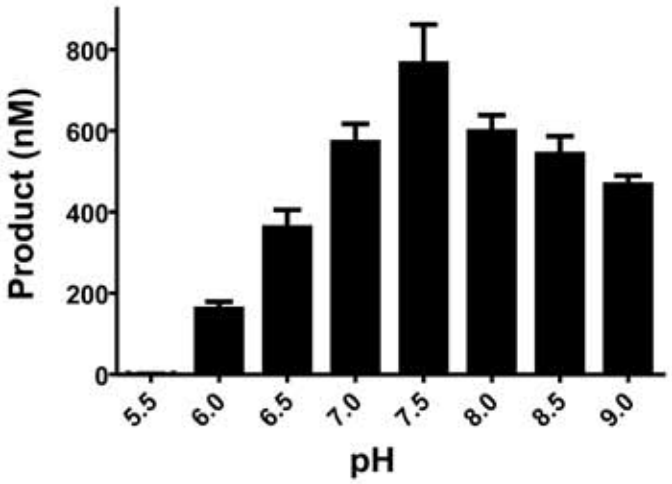


VopS *H. pylori* Fic

Supplemental Figure 4



Supplemental Figure 5



Supplemental Table 1. Data collection, phasing and refinement statistics for VopS Structure

Data collection		
Crystal	Native	SeMet[a]
Space group	$P2_1$	$P2_1$
Unit cell parameters (Å)	a = 66.67, b = 62.32, c = 75.76, b = 91.3°	a = 66.33, b = 61.96, c = 76.42, b = 91.1°
Energy (eV)	12,684	12,684
Resolution range (Å)	48.2 - 1.80 (1.87-1.80)	48.1 - 2.28 (2.36-2.28)
Unique reflections	57,468 (2,849)	24,947 (670)
Multiplicity	4.1 (3.6)	3.4 (2.0)
Data completeness (%)	99.9 (100.0)	89.9 (49.6)
R_{merge} (%)[b]	8.80 (84.0)	13.9 (57.3)
I/σ(I)	7.0 (1.7)	8.9 (2.2)
Wilson B-value (Å^2)	22.9	35.6
Phase determination		
Anomalous scatterers	21 out of 24 possible sites	
Figure of merit (30.0-2.28 Å)	0.17	
Refinement statistics		
Resolution range (Å)	48.2-1.80 (1.87-1.80)	
No. of reflections R_{work}/R_{free}	57,381/2,909 (5,363/263)	
Data completeness (%)	99.7 (99.0)	
Atoms (non-H protein/solvent)	5,178/514	
R_{work} (%)	17.2 (24.2)	
R_{free} (%)	22.4 (31.1)	
R.m.s.d. bond length (Å)	0.012	
R.m.s.d. bond angle (°)	1.089	
Mean B-value (Å^2) (protein/solvent)	33.2/36.8	
Ramachandran plot (%) (favored/disallowed)[c]	98/0.0	
Maximum likelihood coordinate error	0.28	
Missing residues	Chain A: 75-85, Chain B: 75-76, 387	
Alternate conformations	None	

Data for the outermost shell are given in parentheses.

[a]Bijvoet-pairs were kept separate for data processing

[b]$R_{merge} = 100\ \Sigma_h\Sigma_i|I_{h,\ i} - \langle I_h\rangle|/\Sigma_h\Sigma_i I_{h,\ i}$, where the outer sum (h) is over the unique reflections and the inner sum (i) is over the set of independent observations of each unique reflection.

[c]As defined by the validation suite MolProbity (Davis, I.W., Leaver-Fay, A., Chen, V.B., Block, J.N., Kapral, G.J., Wang, X., Murray, L.W., Arendall, W.B., Snoeyink, J., Richardson, J.S. and Richardson, D.C. (2007) MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. Nucleic Acids Res. **35**, W375-W383.).