

Prehoda et al. SUPPLEMENTAL DATA A

Supplemental Data A:

Interaction Mapping of N-WASP

**Methods:** Control region interactions with Arp2/3, the VCA domain, PIP<sub>2</sub>, and CDC42•GTP $\gamma$ S were mapped using the fragments listed in panel i. For Arp2/3, VCA and CDC42•GTP $\gamma$ S experiments, fusions of the control region to GST were used in pull-down assays (25). For PIP<sub>2</sub> binding, control region fragments were tested in vesicle spin-down assays [J. M. Kavran, et al., J Biol Chem 273, 30497-508 (1998)]. VCA domain interactions with Arp2/3 and the GBD were mapped using the fragments shown in panel ii.

i. Control Region:

Residues	Basic	GBD	Binding				Repression of VCA (in trans)
			Arp2/3	VCA	PIP <sub>2</sub>	Cdc42•GTP $\gamma$ S	
178-274			+	+	+	++	++
183-274			+/-	+		+	
190-274			+/-	+		+	-
196-274			-	+	-	+	-
204-274			-	+		+	
209-274			-	+		-	
219-274				+/-			
224-274				-			
178-204			-		+		
178-215			-			+	
178-224			+/-			+	
178-244			+		+	++	-
124-269				-			

ii. VCA Domain (Output Region):

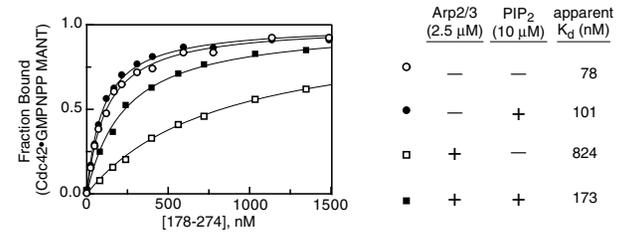
Residues	Verprolin Homology	Cofilin Homology	Acidic	Binding	
				Arp2/3	GBD
392-501				+	+
432-501				+	+
452-501				+	+
490-501				+	
392-481					-
392-486					+

Prehoda et al. SUPPLEMENTAL DATA B,C

Supplemental Data B:

Cdc42 and PIP<sub>2</sub> cooperatively compete against Arp2/3 for binding to the Control Region (residues 178-274)

**Methods:** Affinity of the control region for Cdc42 could be measured by loading Cdc42 with a fluorescent GTP analogue (GMPPNP-mant) and monitoring the change in fluorescence upon addition of the control region (29). Effects of additional factors (Arp2/3 and PIP<sub>2</sub>) could then be detected by determining their effects on the apparent K<sub>d</sub>. Addition of Arp2/3 weakens the apparent affinity, indicating that it competes against Cdc42 for binding to the control region. However, addition of PIP<sub>2</sub> restores the higher affinity, indicating that PIP<sub>2</sub> cooperates with Cdc42 to oppose Arp2/3 binding. PIP<sub>2</sub> alone has no effect on Cdc42 binding, indicating that these two ligands do not directly interact. Assays contain 50 nM CDC42•GMPPNP-mant.



Supplemental Data C:

Cooperativity of mini-N-WASP activation by CDC42•GTP $\gamma$ S and PIP<sub>2</sub> vesicles

**Methods:** Actin polymerization by 50 nM Arp2/3, 50 nM mini-N-WASP was measured as a function of both PIP<sub>2</sub> and/or CDC42. The elongation rate from titrations of CDC42 and/or PIP<sub>2</sub> were fit to binding isotherms to give K<sub>act</sub>: the concentration of activator required for half maximal activation.

