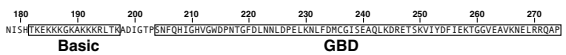
Prehoda et al. SUPPLEMENTAL DATA A

Supplemental Data A:

Interaction Mapping of N-WASP

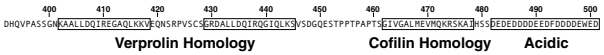
Methods: Control region interactions with Arp2/3, the VCA domain, PIP2, and CDC42•GTPγS were mapped using the fragments listed in panel i. For Arp2/3, VCA and CDC42•GTPγS experiments, fusions of the control region to GST were used in pull-down assays *(25)*. For PIP_2 binding, control region fragments 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:



	Binding				Repression of VCA (*in trans*)
	Arp2/3	VCA	PIP_2	Cdc42•GTPγS	
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):



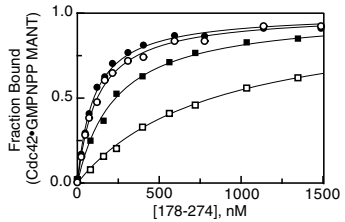
	Binding	
	Arp2/3	GBD
392–501	+	+
432–501	+	+
452–501	+	+
490–501	+	
392–481		-
392–486		+

Prehoda et al. SUPPLEMENTAL DATA B,C

Supplemental Data B:

Cdc42 and PIP_2 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_2) could then be detected by determining their effects on the apparent K_d. Addition of Arp2/3 weakens the apparent affinity, indicating that it competes against Cdc42 for binding to the control region. However, addition of PIP_2s restores the higher affinity, indicating that PIP_2 cooperates with Cdc42 to oppose Arp2/3 binding. PIP_2 alone has no effect on Cdc42 binding, indicating that these two ligands do not directly interact. Assays contain 50 nM CDC42•GMPPNP-mant.

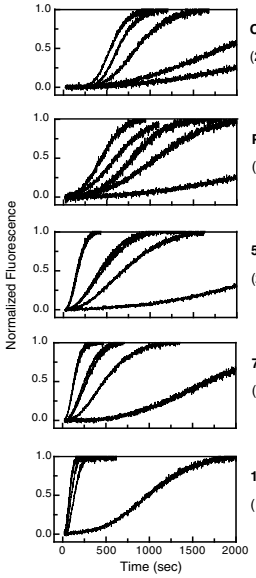


	Arp2/3 (2.5 μM)	PIP_2 (10 μM)	apparent K_d (nM)
○	—	—	78
●	—	+	101
□	+	—	824
■	+	+	173

Supplemental Data C:

Cooperativity of mini-N-WASP activation by CDC42•GTPγS and PIP_2 vesicles

Methods: Actin polymerization by 50 nM Arp2/3, 50 nM mini-N-WASP was measured ias a function of both PIP_2 and/or CDC42. The elongation rate from titrations of CDC42 and/or PIP_2were fit to binding isotherms to give K_{act}, the concentration of activator required for half maximal activation.



	Kact
Cdc42 only (21.0, 10.5, 2.1, 0.1, 0 μM)	3.0 μM Cdc42
PIP2 only (12.5, 6.25, 3.125, 2.34, 0 μM)	8.0 μM PIP_2
50 nM PIP2 + variable Cdc42 (2.0, 1.0, 0.5, 0.25, 0 μM)	1.1 μM Cdc42
75 nM PIP2 + variable Cdc42 (2.0, 1.0, 0.5, 0.25, 0 μM)	0.9 μM Cdc42
1 μM PIP2 + variable Cdc42 (10.5, 2.1, 0.1, 0.05, 0 μM)	0.1 μM Cdc42