

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 PIP₂ 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•GTP-γS	
180	190					
200	210					
220	230					
240	250					
260	270					
NI SH (TEKKKKGAKKKRLT)ADI GTPENFQHTGHVGMPTGFDLNNLDPELKNLPDMGCI SEAGLKDRETSXYI YDFT ERTGGVEAVKNELRRGAP						
Basic		GBD				
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		-		-

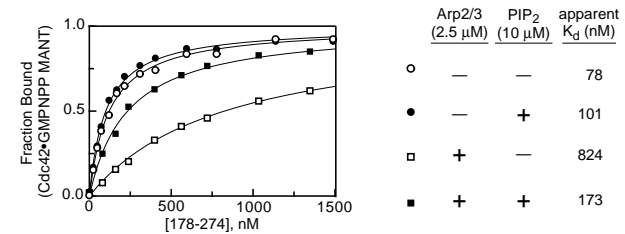
ii. VCA Domain (Output Region):

		Binding	
		Arp2/3	GBD
<div><div>400410420430440450460470480490500</div><div>DHIQVPASSGSAALLDQIREGAQLKKYQNSRPVSCGRDALLDQIREGILQLKNSVSDGQESTPTTPAITSGLVCAIMVYMQRKSKALNSSEDEDDDDDEDFDDDDWED</div><div>Verprolin HomologyCofilin HomologyAcidic</div></div>			
392	501	+	+
432	501	+	+
452	501	+	+
	490 501	+	
392	481		-
392	486		+

Supplemental Data B:

Cdc42 and PIP₂ 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 PIP₂) 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 PIP₂s restores the higher affinity, indicating that PIP₂ cooperates with Cdc42 to oppose Arp2/3 binding. PIP₂ 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•GTPγS and PIP₂ vesicles

Methods: Actin polymerization by 50 nM Arp2/3, 50 nM mini-N-WASP was measured as a function of both PIP₂ and/or CDC42. The elongation rate from titrations of CDC42 and/or PIP₂ were fit to binding isotherms to give K_{act} : the concentration of activator required for half maximal activation.

