Ditlev et al., http://www.jcb.org/cgi/content/full/jcb.201111113/DC1

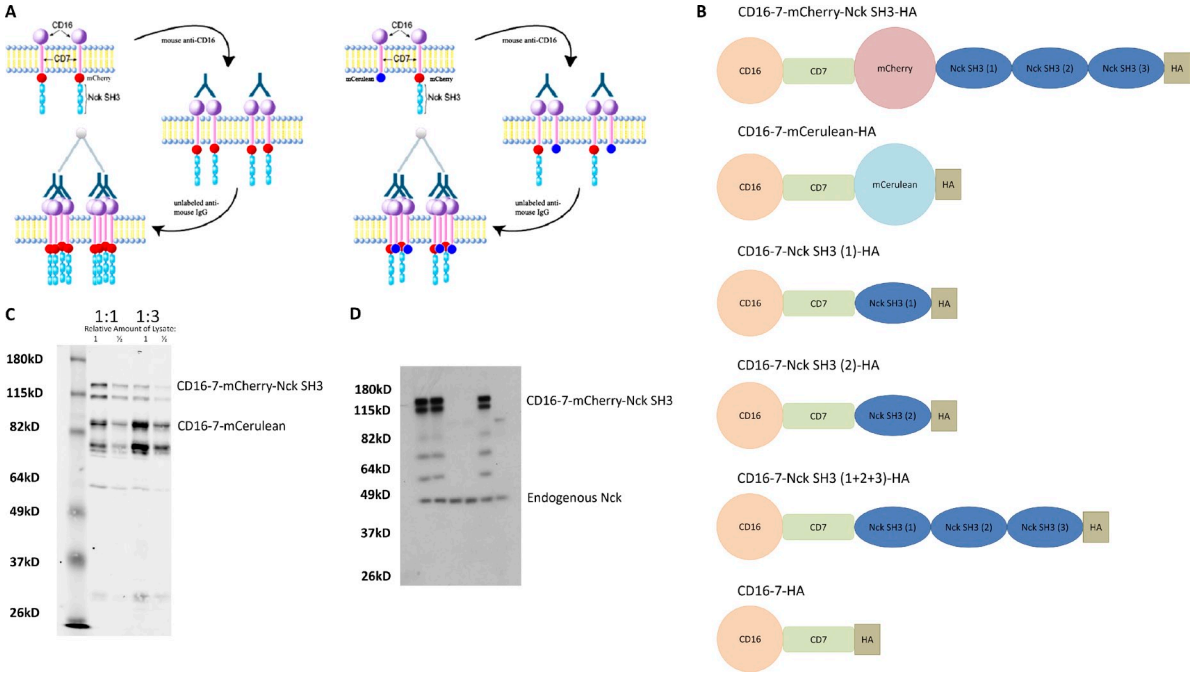
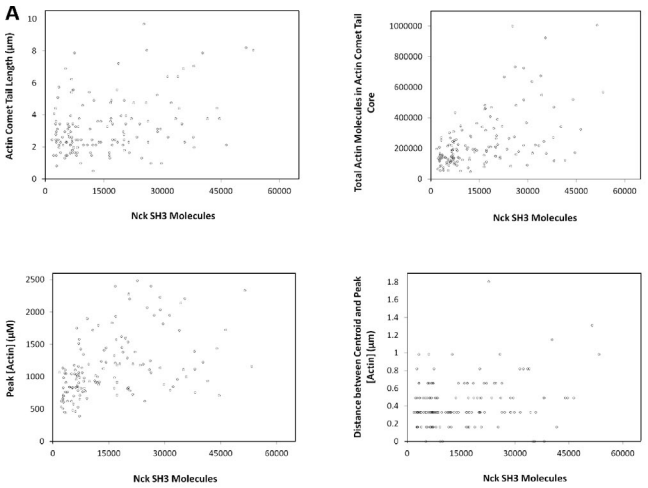
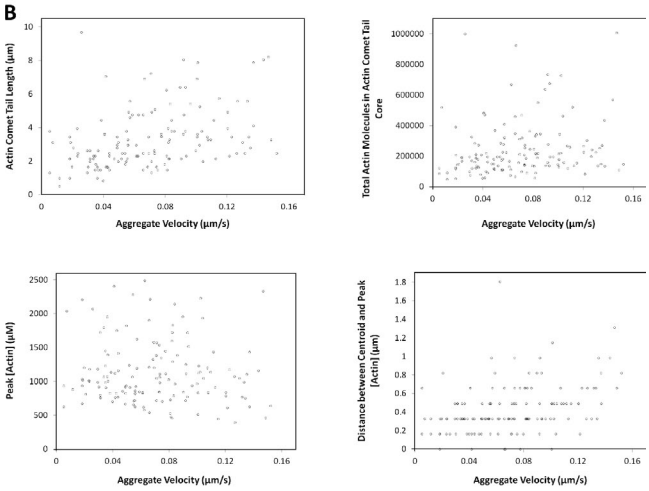


Figure S1. **Antibody-induced aggregation methodology, fusion protein design, and fusion protein expression in transfected cells.** (A) A schematic of antibody aggregation used in both 100% Nck SH3 density experiments (left) and variable Nck SH3 density experiments (right). (B) Diagrams of CD16-7 fusion proteins used in Nck density studies and mixed Nck aggregation studies. The top two fluorescently labeled constructs are used in actin comet tail and density studies in Figs. 1–5. The bottom four constructs are used in WIP KO or WT MEF and N-WASp KO or WT MEF studies and mixed Nck SH3 studies in Figs. 6 and S3. (C) Western blot–detected expression of two of the transfectants: 50% Nck SH3 and 50% dummy (1:1) and 25% Nck SH3 and 75% dummy (1:3). A double band is observed for each CD16-7 fusion protein: the top band is the full-length fusion protein, whereas the bottom band is a cleaved fusion protein lacking the CD16 domain. (D) A Western blot demonstrating CD16-7–mCherry–Nck SH3–HA versus endogenous Nck expression in transfected NIH-3T3 cells.



Aggregate Size vs. Actin Comet Tail Characteristics



Aggregate Velocity vs. Actin Comet Tail Characteristics

Figure S2. **Aggregate size and velocity are not well correlated with measured actin comet tail characteristics.** (A) Plots correlating the number of Nck SH3 molecules in aggregates with actin comet tail length (top left), total number of actin molecules in the comet tail core (top right), peak [actin] in the comet tail (bottom left), and distance between aggregate center and peak [actin] (bottom right). (B) Plots correlating the velocity of Nck SH3 aggregates with actin comet tail length (top left), total number of actin molecules in the comet tail core (top right), peak [actin] in the comet tail (bottom left), and distance between aggregate center and peak [actin] (bottom right).

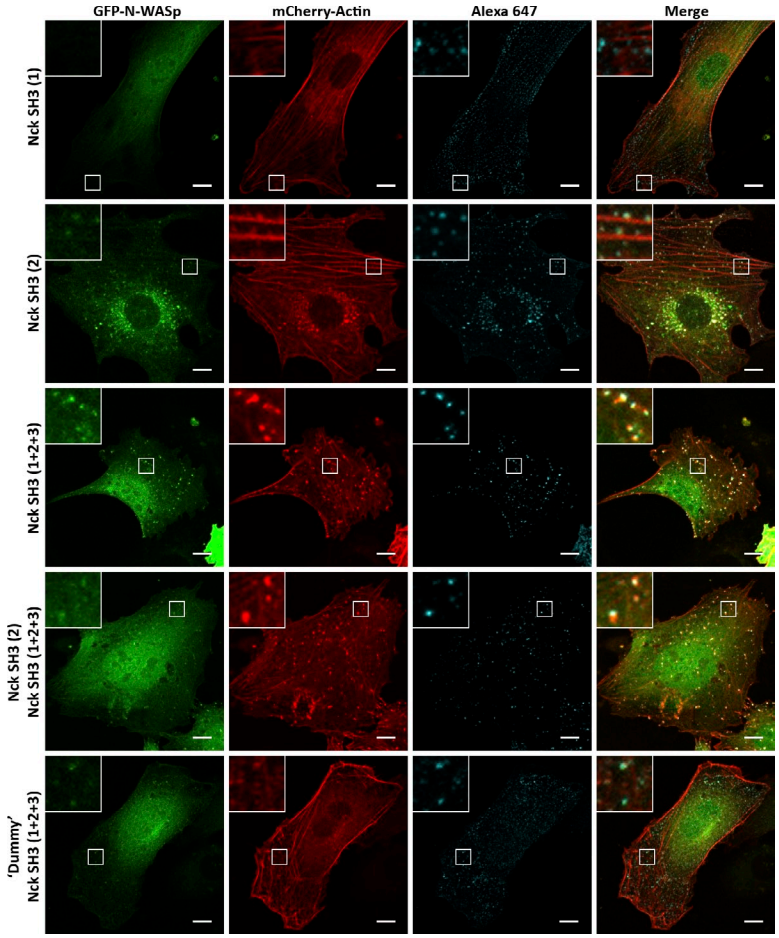


Figure S3. **Full activation of N-WASp requires both Nck SH3 (2) and Nck SH3 (1+2+3) in aggregates.** Confocal microscopy images of NIH-3T3 cells cotransfected with mCherry-actin, GFP–N-WASp, and CD16-7–Nck SH3 (1) or CD16-7–Nck SH3 (2), CD16-7–Nck SH3 (1+2+3), CD16-7–dummy, or a combination of CD16 fusion proteins. For all images, mCherry-actin is red, GFP–N-WASp is green, and CD16 aggregates are cyan. Nck SH3 (1) aggregates (top) did not recruit GFP–N-WASp or induce actin polymerization. Nck SH3 (2) aggregates (second from top) and Nck SH3 (1+2+3)/dummy aggregates (bottom) recruited GFP–N-WASp and induced actin polymerization in the form of actin spots. Both Nck SH3 (1+2+3) (middle) and Nck SH3 (2)/Nck SH3 (1+2+3) (second from bottom) aggregates induce actin comet tail–like structures. Higher magnifications of clusters are shown in the insets. Bars, 10 µm.

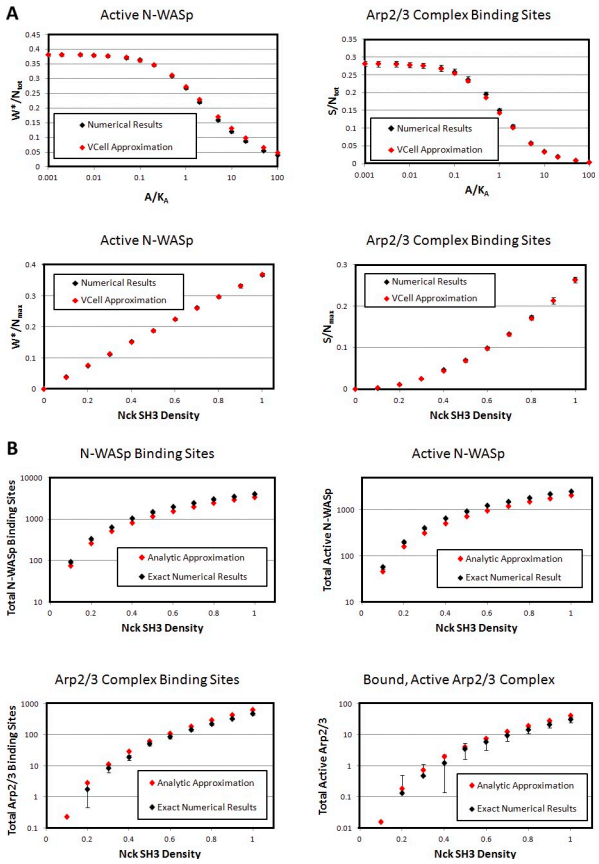
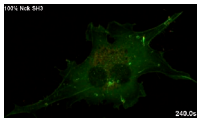
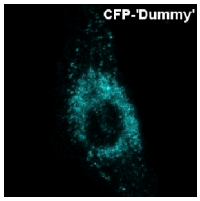


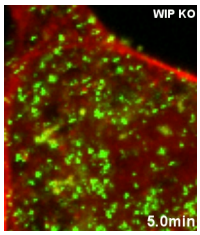
Figure S4. **Exact numerical results and analytical approximations used in the Virtual Cell are sufficiently similar to allow for the approximation to be used in the Virtual Cell model.** (A and B) Graphs displaying the similarity between the Virtual Cell approximations used to describe N-WASp and Arp2/3 complex binding and exact numerical calculations of the number of N-WASp and Arp2/3 complex molecules recruited to the Nck SH3 patch in both the 2:2:1 and 4:2:1 reaction schemes. Error bars are SDs. (A) Comparison of Virtual Cell predictions of N-WASp and Arp2/3 complex binding with exact numerical calculations using the 2:2:1 Nck/N-WASp/Arp2/3 reaction scheme in large 2D lattices. Top plots illustrate the equilibrium-activated N-WASp (left) and Arp2/3 complex binding sites (right) as a function of cytosolic Arp2/3 complex concentration when Nck density is 100% in both Virtual Cell (VCell) approximations and exact numerical calculations. Bottom plots illustrate activated N-WASp (left) and Arp2/3 complex (right) as functions of Nck density for the fixed value of $A/K_A = 0.07$, which is the steady-state ratio of bound and activated Arp2/3 complex to cytosolic Arp2/3 complex in our Virtual Cell simulations. (B) Comparison of Virtual Cell predictions of N-WASp and Arp2/3 complex binding with exact numerical calculations using the 4:2:1 Nck/N-WASp/Arp2/3 reaction scheme in a 30 × 30 square lattice at varying Nck densities. Top plots illustrate N-WASp binding sites (left) and active N-WASp molecules (right) in Virtual Cell predictions and exact numerical calculations. Bottom plots illustrate Arp2/3 complex binding sites (left) and bound and activated Arp2/3 complex (right) in Virtual Cell predictions and exact numerical calculations. As can be appreciated, although the Virtual Cell approximations overpredict the number of Arp2/3 complex binding sites and active Arp2/3 complexes across the range of Nck densities, the approximations capture the density-dependent variation in the curves and lie within the error bars of the exact numerical predictions, indicating that the Virtual Cell predictions are reliable for predicting actin polymerization downstream of the 4:2:1 Arp2/3 complex activation mechanism.



Video 1. **100% Nck SH3 aggregates induce actin comet tail formation.** NIH-3T3 fibroblasts were transfected with CD16-7–mCherry–Nck SH3–HA (red) and GFP-actin (green). After Nck SH3 domain aggregation, images were analyzed by time-lapse confocal microscopy using a spinning-disc confocal microscope (UltraView). Frames were obtained every 30 s for 5.5 min.



Video 2. **Increasing Nck SH3 density induces a nonlinear increase in actin polymerization.** NIH-3T3 fibroblasts were transfected with CD16-7–mCherry–Nck SH3–HA, YFP-actin, and/or CD16-7–dummy-HA. After Nck SH3 domain and/or dummy aggregation, images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 780). Frames were obtained every minute for 12 min.



Video 3. **WIP is necessary for Nck SH3–induced actin polymerization.** WIP WT or KO MEFs were transfected with CD16-7–Nck SH3–HA (green) and mCherry-actin (red); KO MEFs were rescued with transfected GFP-WIP (blue). After Nck SH3 domain aggregation, images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 780). Frames were obtained every minute for 9 min.

Table S1. **Fluorescence calculations to test for autoquenching of mCherry in aggregates**

	Total molecules (Western blot)	Fluorescent cells	Total fluorescence	Fluorescence/cell	Molecules/cell	Fluorescence/molecule
Control (no antibody-induced aggregation)	3.10506×10^{11}	88	13888914	157828.57	888557	0.177623461
Experiment (antibody-induced aggregation)	3.10506×10^{11}	35	5758937	164541.06	888557	0.185177833

Fluorescence/molecule calculations in control and experimental cells are 96% similar (0.178/0.185), indicating that fluorescence autoquenching has little, if any, effect on fluorescence per molecule calculations. Fluorescence autoquenching does not affect fluorescent quantification of CD16-7–mCherry–Nck SH3 aggregates. Fluorescence per molecule calculations in control (no CD16-7–mCherry–Nck SH3 aggregation) and experimental (CD16-7–mCherry–Nck SH3 aggregation) cells reveal that increased mCherry concentration caused by aggregation does not result in autoquenching of mCherry fluorescence.

Table S2. **Protein names and descriptions used in the Virtual Cell simplified actin dendritic nucleation model**

Protein name	Model name	Description
G-actin	Gt	ATP form of G-actin
	Gd	Sum of ADP-Pi and ADP forms of G-actin
F-actin	Fi	Sum of ATP and ADP-Pi forms of F-actin
	Fd	ADP form of F-actin
Barbed ends	Barb T	ATP barbed end
	Barb I	ADP-Pi barbed end
	Barb D	ADP barbed end
Pointed ends	Point T	ATP pointed end
	Point I	ADP-Pi pointed end
	Point D	ADP pointed end
β-thymosin	Bthy	
β-thymosin–bound G-actin	Bthy Gt	ATP form of G-actin bound to β-thymosin
	Bthy Gd	Sum of ADP-Pi and ADP forms of G-actin bound to β-thymosin
Profilin	Prof	
Profilin bound to G-actin	Prof Gt	ATP form of G-actin bound to profilin
	Prof Gd	Sum ADP-Pi and ADP forms of G-actin bound to profilin
Cofilin	Cof	
Cofilin bound to F-actin	Cof Fd	Cofilin bound on an ADP–F-actin subunit
	Cof Fd2	Two cofilins bound on adjacent ADP–F-actin subunits
Capping protein	Cap	
Capping protein bound to a barbed end	Cap Barb	A lumped variable representing capped barbed ends of the ATP, ADP-Pi, and ADP form
Arp2/3	Arp2/3	
Activated Arp2/3	Active Arp2/3	Membrane-bound species consisting of an N-WASp and an Arp2/3 that participates in branching reactions
Arp2/3 branch points	Branch I	A single species describing a pointed end in any form, an Arp2/3, and an F-actin subunit in either the ATP or ADP-Pi form
	Branch D	A single species describing a pointed end in any form, an Arp2/3, and an F-actin subunit in the ADP form
Activated N-WASp	Active N-WASp	Membrane-bound species defined as consisting of an Nck SH3 and an N-WASp that can bind Arp2/3
Nck SH3 domains	Nck SH3	Membrane-bound species that binds N-WASp
N-WASp	N-WASp	Freely diffusing N-WASp in the cytosol, which can bind Nck SH3 domains
WIP-bound N-WASp	WIP N-WASp	Freely diffusing WIP-bound N-WASp in the cytosol, which can bind two Nck SH3 domain molecules

Table S3. **Global parameters**

Parameter name	Unit	Functional form/value	Comment
ATP	μM	10,000	Typical cellular concentration of ATP, assumed buffered
ADP	μM	2,000	Typical cellular concentration of ADP, assumed buffered
Pi	μM	2,000	Typical cellular concentration of inorganic phosphate, assumed buffered
All F-actin	μM	Fi + Fd + CofFd + 2.0 × CofFd2 + PointT + PointI + PointD + BarbT + BarbI + BarbD + CapBarb + 2.0 × (BranchI_cyt + BranchD_cyt)	Sum of all forms of polymerized actin
All pointed ends	μM	PointT + PointI + PointD + BranchI + BranchD	Sum of free and Arp2/3 capped pointed ends
Pointed end total	μM	PointT + PointI + PointD	Sum of free pointed ends
Barbed end total	μM	BarbT + BarbI + BarbD	Sum of free barbed ends
BrF	1	(BranchI + BranchD)/AllPointedEnds	The fraction of pointed ends capped by Arp2/3. This is used as a measure of the fraction of F-actin bound in the network (as opposed to detached, freely diffusing filaments)
L	1	AllFactin/AllPointedEnds	The average length of a filament, given in number of subunits
Fi stability	1	((Fi/PointedEndTotal) + (Fi/PointedEndTotal)^3)/(1+ (Fi/PointedEndTotal) + (Fi/PointedEndTotal)^3)	Function that provides stability to certain reactions involving Fi, as described below
Fd stability	1	((Fd/PointedEndTotal) + (Fd/PointedEndTotal)^3)/(1+ (Fd/PointedEndTotal) + (Fd/PointedEndTotal)^3)	Function that provides stability to certain reactions involving Fd, as described below
barbK1	s^{-2}	koff_barbI × koff_barbD + koff_barbI × kbarb_ID × Pi + koff_barbD × kbarb_DI	Parameter required when lumping barbed-end reactions with G-actin
barbK2	s^{-2}	kG_DT × r_hydrolysis + kon_barbT × BarbedEndTotal × r_hydrolysis + kG_DT × koff_barbT	Parameter required when lumping barbed-end reactions with G-actin
barbprofK1	s^{-2}	koff_barb_profI_eff × Prof_cyt × koff_barb_profD_eff × Prof_cyt + koff_barb_profI_eff × Prof_cyt × kbarb_ID × Pi + koff_barb_profD_eff × Prof_cyt × kbarb_DI	Parameter required when lumping barbed-end reactions with profilin–G-actin
barbprofK2	s^{-2}	kG_DT × r_hydrolysis + kon_barb_profT_eff × BarbedEndTotal × r_hydrolysis + kG_DT × koff_barb_profT_eff × Prof_cyt	Parameter required when lumping barbed-end reactions with profilin–G-actin
barbprofQ DI	s^{-5}	kG_DI × barbprofK1 × barbprofK2 + koff_barb_profD_eff × Prof_cyt × kbarb_DI × kon_barb_profI_mod × BarbedEndTotal × barbprofK2	Parameter required when lumping barbed-end reactions with profilin–G-actin
barbprofQ ID	s^{-5}	kG_ID × Pi × barbprofK1 × barbprofK2 + koff_barb_profI_eff × Prof_cyt × kbarb_ID × Pi × kon_barb_profD_mod × BarbedEndTotal × barbprofK2	Parameter required when lumping barbed-end reactions with profilin–G-actin
barbQ DI	s^{-5}	kG_DI × barbK1 × barbK2 + koff_barbD × kbarb_DI × kon_barbI × BarbedEndTotal × barbK2	Parameter required when lumping barbed-end reactions with G-actin
barbQ ID	s^{-5}	kG_ID × Pi × barbK1 × barbK2+ koff_barbI × kbarb_ID × Pi × kon_barbD × BarbedEndTotal × barbK2	Parameter required when lumping barbed-end reactions with G-actin
cytJ DI	s^{-11}	((kon_bthyD × Bthy_cyt × cytQ_DI × (((koff_profD + kprof_DI) × cytK1) + (kprof_DT × kon_profT × Prof_cyt × kG_TD))) + (kprof_DI × ((cytK1 × kon_profI × Prof_cyt × cytQ_ID) + (kprof_DT × kon_profT × Prof_cyt × kG_TD × cytQ_DI))))	Parameter required when lumping cytosolic reactions
cytJ ID	s^{-11}	((kon_bthyD × Bthy_cyt × cytQ_ID × (((koff_profD + (kprof_ID × Pi)) × cytK1) + (kprof_TD × kG_DT × koff_profT))) + (kprof_ID × Pi × cytQ_DI × ((cytK1 × kon_profD × Prof_cyt) + (kprof_DT × kon_profT × Prof_cyt × kG_TD))))	Parameter required when lumping cytosolic reactions
cytK1	s^{-2}	((kprof_DT × kG_DT) + (kG_DT × koff_profT) + (kprof_DT × kon_profT × Prof_cyt))	Parameter required when lumping cytosolic reactions
cytK3	s^{-2}	((koff_profD × koff_profD) + (koff_profD × kprof_DI) + (koff_profD × kprof_ID × Pi))	Parameter required when lumping cytosolic reactions
cytK4	s^{-4}	((cytK1 × cytK3) + (kprof_TD × kG_DT × koff_profT × (koff_profD + kprof_DI)))	Parameter required when lumping cytosolic reactions

Table S3. **Global parameters** *(Continued)*

Parameter name	Unit	Functional form/value	Comment
cytQ DI	s^{-7}	((cytK4 × ((cytS1 × kG_DI) + (koff_bthyD × kbthy_DI × kon_bthyD × Bthy_cyt))) + (cytS1 × kprof_DI × kon_profD × Prof_cyt × ((cytK1 × koff_profD) + (kprof_TD × kG_DT × koff_profT))))	Parameter required when lumping cytosolic reactions
cytQ ID	s^{-7}	((cytK4 × ((cytS1 × kG_ID × Pi) + (koff_bthyD × kbthy_ID × Pi × kon_bthyD × Bthy_cyt))) + (cytS1 × koff_profD × kprof_ID × Pi × ((cytK1 × kon_profD × Prof_cyt) + (kprof_DT × kon_profT × Prof_cyt × kG_TD))))	Parameter required when lumping cytosolic reactions
cytS1	s^{-2}	((koff_bthyD × koff_bthyD) + (koff_bthyD × kbthy_ID × Pi) + (koff_bthyD × kbthy_DI))	Parameter required when lumping cytosolic reactions
point K1	s^{-2}	((koff_pointI × koff_pointD) + (koff_pointI × kpoint_ID × Pi) + (koff_pointD × kpoint_DI))	Parameter required when lumping pointed-end reactions with G-actin
point K2	s^{-2}	((kG_DT × r_hydrolysis) + (kon_pointT × PointedEndTotal × r_hydrolysis) + (kG_DT × koff_pointT))	Parameter required when lumping pointed-end reactions with G-actin
pointQ DI	s^{-5}	((kG_DI × pointK1 × pointK2) + (koff_pointD × kpoint_DI × kon_pointI × PointedEndTotal × pointK2))	Parameter required when lumping pointed-end reactions with G-actin
pointQ ID	s^{-5}	((kG_ID × Pi × pointK1 × pointK2) + (koff_pointI × kpoint_ID × Pi × kon_pointD × PointedEndTotal × pointK2))	Parameter required when lumping pointed-end reactions with G-actin

Table S4 is available online as a pdf file and contains information describing the reactants and reactions contained in the 1:1:1, 2:2:1, and 4:2:1 Virtual Cell models.

The models describing the 1:1:1, 2:2:1, and 4:2:1 Nck–N-WASp–Arp2/3 complex mechanisms are available online as XML files.