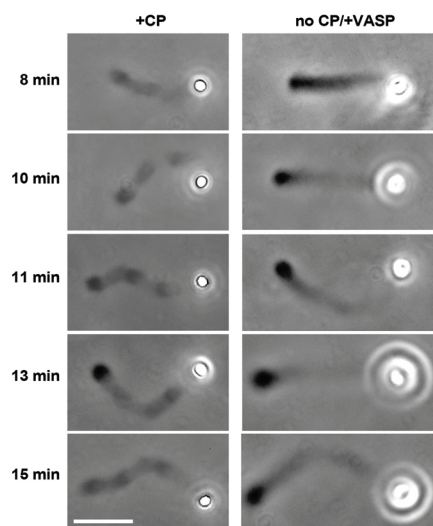
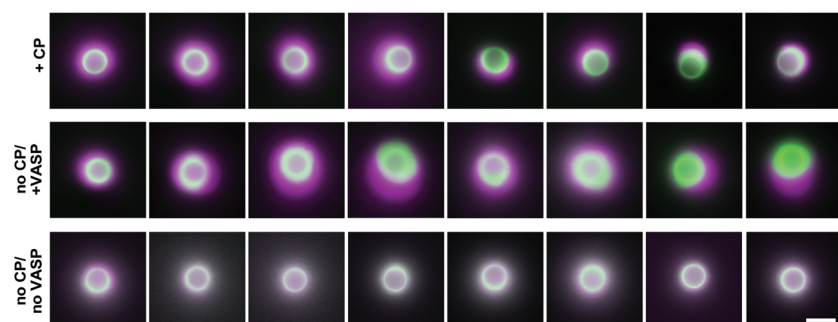


Supplementary Figure S1: TIRF microscopy analysis for determination of filament elongation rates in the presence of VASP. **A)** TIRF microscopy images over time of 1.5 μ M profilin-actin (15% Alexa-488 labeled) in the absence (top panels) and presence of 37 nM VASP. Filaments are globally longer over time with VASP, although present in about the same number as without VASP, due to VASP's lack of nucleation activity at the salt concentration used in this study. Bar 10 μ m. **B)** Quantification of filament elongation speeds in the absence and presence of VASP. The difference is significant ($p = 0.002$). $N \geq 16$.

Supplementary Figure S2 Abou-Ghali et al.

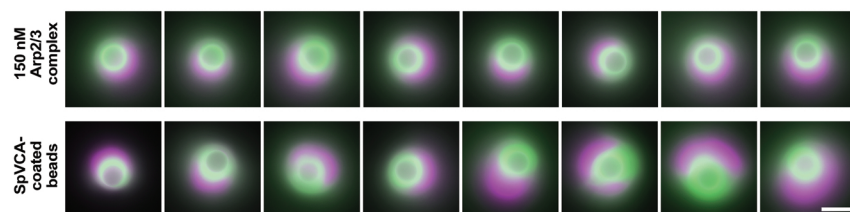


Supplementary Figure S2: Examples of actin comets in the presence of CP or without CP when VASP is added. 1 μ m diameter beads form actin comets and move in the presence of 25 nM CP or without CP but with 50 nM added VASP. Images are of five different beads for each condition at the times indicated. The concentration of the Arp2/3 complex is 50 nM. Phase contrast microscopy. Scale bar 5 μ m.



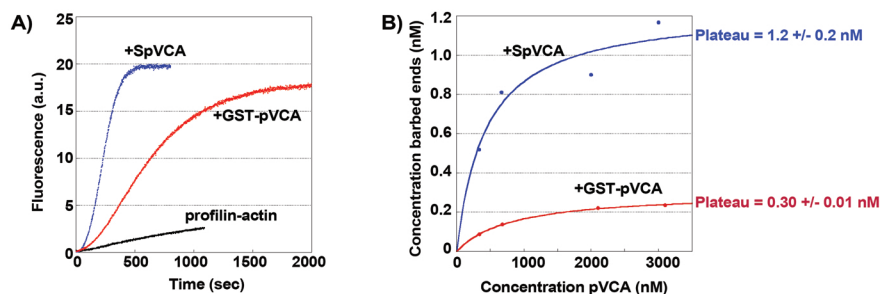
Supplementary Figure S3: Examples of actin clouds in the presence of CP or without CP, with and without added VASP. 4.5 μm diameter beads visualized using a two-color assay in the presence or absence of 25 nM CP, and in the absence of CP with 50 nM added VASP. Overlay images of eight different beads are shown for each condition. The first color is magenta (actin Alexa 594) and the second is green (actin Alexa 488); colocalization is white in the magenta/green overlays. The concentration of the Arp2/3 complex is 50 nM. Epifluorescence microscopy. Scale bar 5 μm .

Supplementary Figure S4 Abou-Ghali et al.



Supplementary Figure S4: Examples of actin clouds in the absence of CP, but either with an excess of Arp2/3 complex or with S-pVCA-coated beads. 4.5 μm diameter beads visualized using a two-color assay in the absence of CP. Top panels: actin growth in the presence of excess (150 nM) Arp2/3 complex in the absence of CP and VASP. Bottom panels: beads are coated with a tetrameric form of pVCA (S-pVCA). The polymerization mix contains no CP and no VASP, and 50 nM Arp2/3 complex. Overlay images of eight different beads are shown for each condition. The first color is magenta (actin Alexa 594) and the second is green (actin Alexa 488); colocalization is white in the magenta/green overlays. Epifluorescence microscopy. Scale bar 5 μm.

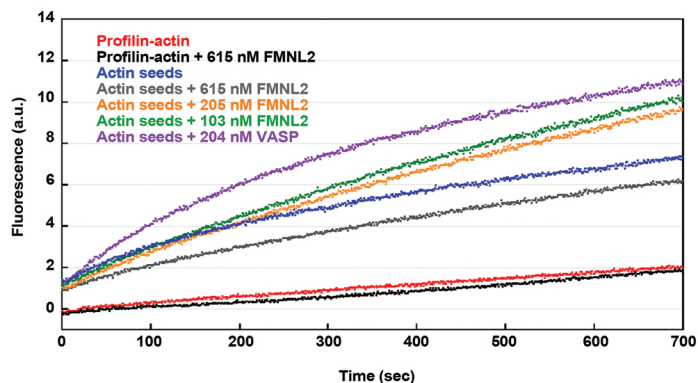
Supplementary Figure S5 Abou-Ghali et al.



Supplementary Figure S5: Comparison of the activity of S-pVCA and GST-pVCA to activate the Arp2/3 complex by pyrene assay.

A) Typical pyrene curves charting increase in fluorescence of pyrene actin over time. Profilin actin alone, and added Arp2/3 complex with either 2 μ M S-pVCA or 2 μ M GST-pVCA are shown. B) Using such curves, and varying the concentration of pVCA, allows for the calculation of the quantity of barbed ends formed as a function of pVCA concentration (see Experimental Procedures). The data is fit to a Michaelis-Menton curve to obtain the plateaus. Comparing plateaus indicates that S-pVCA produces four times more barbed ends than GST-pVCA under the same conditions.

Supplementary Figure S6 Abou-Ghali et al.



Supplementary Figure S6: Assessment of the filament elongating activity of FMNL2-8P by pyrene assay. Typical pyrene curves charting increase in fluorescence of pyrene actin over time. Profilin-actin (red curve) polymerizes slowly, and adding 615 nM FMNL2-8P (black curve) has little effect, consistent with FMNL2's weak nucleation activity. Reactions spiked with actin seeds polymerize less robustly in the presence of 615 nM FMNL2-8P (blue curve versus grey curve), perhaps due to profilin-actin sequestration by the binding activity of FMNL2-8P. Adding lower concentrations of FMNL2-8P (orange curve 205 nM and green curve 103 nM) enhance polymerization of actin seeds as compared to actin seeds alone, indicating elongation enhancement activity. 204 nM VASP is more efficient for elongation enhancement (purple curve) than a comparable amount of FMNL2-8P.