Supplemental material

Supplemental movies

Supplemental movie 1

Phase contrast time lapse series of poorly coated N-WASP functionalised beads propelling in the biomimetic motility assay in the absence (left panel) or presence of 0.5 μM skTm (right panel). Conditions: 7 μM F-actin, 2.6 μM profilin, 6.5 μM ADF, 100 nM gelsolin, 100 nM Arp2/3 complex, d = 6 nm. Elapsed time in min : s, scale bar 20 μm.

Supplemental movie 2

Phase contrast time lapse series of highly coated N-WASP functionalised beads propelling in the biomimetic motility assay in the absence (left panel) or presence of 3 μM skTm (right panel). Conditions: 7 μM F-actin, 2.6 μM profilin, 5.2 μM ADF, 100 nM gelsolin, 50 nM Arp2/3 complex, d = 3 nm. Elapsed time in min : s, scale bar 20 μm.

Supplemental figures

Supplemental Figure 1

Tropomyosin does not affect Arp2/3 complex-independent actin assembly from actin or profilin actin

(A) Time courses of actin polymerisation (2.5 μM, 2% pyrenyl-labelled) monitored by the change in pyrene fluorescence in the absence (black line) and presence of increasing concentration of skTm, as indicated.

(B) Polymerisation kinetics of actin (1.5 μM, 2% pyrenyl-labelled) from spectrin-actin seeds (0.135 nM) in the presence of 4.8 μM profilin was monitored in the absence (black line) and in the presence of the indicated concentrations of skTm. Inset shows control curves: time course of actin assembly (1.5 μM) in the presence of 4.8 μM profilin and either in the absence (black curve) or in the presence (pink curve) of 0.135 nM spectrin-actin seeds. Note: actin nucleation is strongly inhibited by profilin reflected by the flat curve measured in the absence of spectrin-actin seeds, however profilin-actin effectively incorporates at the barbed-end (pink curve).

Supplemental Figure 2

The effects of Tropomyosin on bead propulsion when gelsolin is replaced by Capping Protein

(A) Time lapse phase contrast images of N-WASP functionalised beads propelling in the biomimetic motility assay in the absence or presence of skTm, as indicated. Upper panel: low N-WASP surface density beads (d = 6 nm), lower panel: high N-WASP surface density beads (d = 3 nm). Conditions: 7 μM F-actin, 2.6 μM profilin, 6.4 μM ADF, 105 nM capping protein, 70 nM (upper panel) or 93 nM (lower panel) Arp2/3 complex. Elapsed time in min : s, scale bar 20 μm. The initial position of the end of the actin tail is highlighted by white arrowheads on the subsequent images.

(B) skTm concentration dependence of the propulsion velocity of beads at low and high N-WASP surface density. Conditions as in panel (A). Results expressed as mean +/- SD, n = 15 - 35 beads from 3 - 4 different fields for each condition.

Supplemental Figure 3

Tropomyosin binds to the actin tail initiated by N-WASP-Arp2/3 complex

(A) N-WASP-coated beads were incubated with the motility medium in an eppendorf tube, to let beads form comets. Then skTm was added to the preformed actin tails at the indicated concentrations and the samples were processed for microscopy observation. Conditions: 7 μM F-actin, 2.6 μM profilin 6.5 μM ADF, 175 nM gelsolin, 50 nM Arp2/3 complex, d = 6 nm. Left panel: Gallery of phase contrast images of typical beads and their comets after addition of skTm at t = 0 s at the indicated concentrations. Scale bar 20 μm, elapsed time in min : s. The initial position of the end of the actin tail is highlighted by white arrowheads on the subsequent images. Note in the absence of skTm the end of the actin tail is following the bead as a consequence of the depolymerising effect of ADF, however upon addition of skTm this effect is abolished.

(B) Kymographs generated using the trajectory of each bead shown in panel (A). White line shows the position of the bead. White dotted line indicates the position of the end of the tail that is parallel to the bead trajectory in the absence of skTm but remains at the initial position in the presence of skTm. Note the slope of the white line, i.e. the velocity of the bead is lower after the addition of skTm.

Supplemental Figure 4

Tropomyosin and ADF bind to actin filaments in a competitive manner

2.5 μM F-actin (10 % pyrene-labelled) was incubated with 4.8 μM ADF then skTm was added to the samples at the indicated concentrations.

(A) The fluorescence intensity of the samples was measured before and after addition of skTm, indicated by an asterix. Before addition of skTm the fluorescence intensity is low since ADF quenches pyrenyl actin fluorescence (Carlier et al., 1997), addition of skTm was accompanied by a concentration dependent increase in pyrenyl fluorescence.

(B) To verify that the increase in the fluorescence signal upon skTm addition is due to an increased amount of polymerised actin, samples were removed from the pyrene polymerisation assay and ultracentrifuged. The protein content of supernatants and pellets was analysed by SDS-page analysis as described in Supplemental materials and methods. Coomassie stained gels of pellets and supernatants containing different amount of skTm ([skTm], μM: 0, 0.46, 1.4, 3.5, 7 from left to right) in the absence (lower panel) and presence of 4.8 μM ADF (upper panel).

(C) Percent of bound protein (ADF or skTm) to actin as a function of skTm concentration. The ratio of the intensity of ADF or skTm band to the intensity of actin band in the pellet was calculated at each concentration of skTm. The ADF : actin ratio was normalised by the ratio calculated in the absence of skTm, the skTm : actin ratio was normalised by the ratio calculated in the absence of ADF.

Supplemental Figure 5

Tropomyosin and ADF alter actin filament dynamics in the motility medium in the opposite manner

The steady state amount of polymerisable actin in the motility medium was measured by co-sedimentation assay as described in Supplemental materials and methods.

(A) Protein content of the supernatants in the absence or presence of the indicated concentrations of skTm, visualised by Coomassie staining. Conditions: 7 μM F-actin, 2.6 μM profilin, 105 nM gelsolin, [ADF], μM: 0, 0.49, 0.98, 3.27, 4.9, 0, 0.49, 0.98, 4.9, 0, 0.49, 0.98, 3.27, 4.9 from left to right, [skTm], μM: 0 in lane 1-5, 1.7 in lane 6 – 9 and 7 in lane 10 - 14.

(B) – (D) Quantification of the protein content of the supernatants. For (B): actin, (C): skTm and (D): ADF the ratio of the protein content of the supernatant to the total amount of protein was calculated at each ADF concentration.

Supplemental Figure 6

The binding of Tropomyosin is independent on the nucleotide state of actin filaments

The fraction of skTm bound to ADP-Pi-, or ADP-F-actin was calculated from the results of SDS-page analysis of a co-sedimentation assay as described in Supplemental materials and methods. The hyperbola fit to the data (shown by black and grey dashed lines for ADP-Pi-, and ADP-F-actin, respectively) gave the equilibrium dissociation constant of 0.644 ± 0.104 μM and 0.606 ± 0.224 μM for skTm to binding to ADP-Pi, or ADP actin filaments, respectively.

Supplemental Figure 7

(A) The time course of polymerisation of actin (2 μM, 2 % pyrenyl-labelled) in the presence of 312 nM His-VCA and 187 nM Arp2/3 complex was followed by monitoring the change in pyrenyl fluorescence. Aliquots were removed from the solution at different time points indicated by arrows (at 4 min, when filaments are branched and at 40 min, when debranching already took place) and phalloidin was added in a 1 : 1 molar ratio to actin. Then skTm was added at different concentrations, as indicated on panel (B). Samples were further incubated for 2.5 min and ultracentrifuged and processed for SDS-page analysis, as described in Supplemental materials and methods. Grey curve shows the time course of actin polymerisaiton in the presence of 312 nM His-VCA.

(B) The fraction of skTm bound to F-actin at each concentration of skTm was calculated from the results of SDS-page analysis as described in Supplemental materials and methods. The hyperbola fit to the data (shown by black and grey lines) gave the equilibrium dissociation constant of 0.590 ± 0.064 μM and 0.504 ± 0.103 μM for skTm to binding to actin filaments.

Supplemental materials and methods

Proteins

Mouse α1β2 capping protein was given by Pekka Lappalainen.

Co-sedimentation assay

To study the binding of skTm to F-actin in the presence of ADF, 2.5 μM F-actin was incubated with 4.8 μM ADF in F-buffer at RT, then skTm was added to the samples at different amounts (for exact concentrations see figure legend). After 1 h incubation the samples of 160 μl were ultracentrifuged (Beckman Optima MAX Benchtop ultracentrifuge, rotor: TLA120.1) at 400.000 g, 30 min, 20 ^{o}C. The supernatants and pellets were processed for SDS-PAGE (12.5 % acrylamide). The gels were stained with Coomassie Blue, and scanned. The protein content of the pellets and supernatants were quantified by measuring the corresponding band intensities using ImageJ. The effect of skTm on the steady state amount of polymerisable G-actin in the motility medium was measured in co-sedimentation assays. F-actin (7 μM) was incubated with gelsolin, profilin, Arp2/3 complex and ADF (for exact concentrations see figure legend) in F-buffer at RT for 40 min. Then skTm was added to the samples at different concentrations and the samples were further incubated for 40 min at RT. The samples of 100 μl were ultracentrifuged and processed for SDS-PAGE analysis as described above.

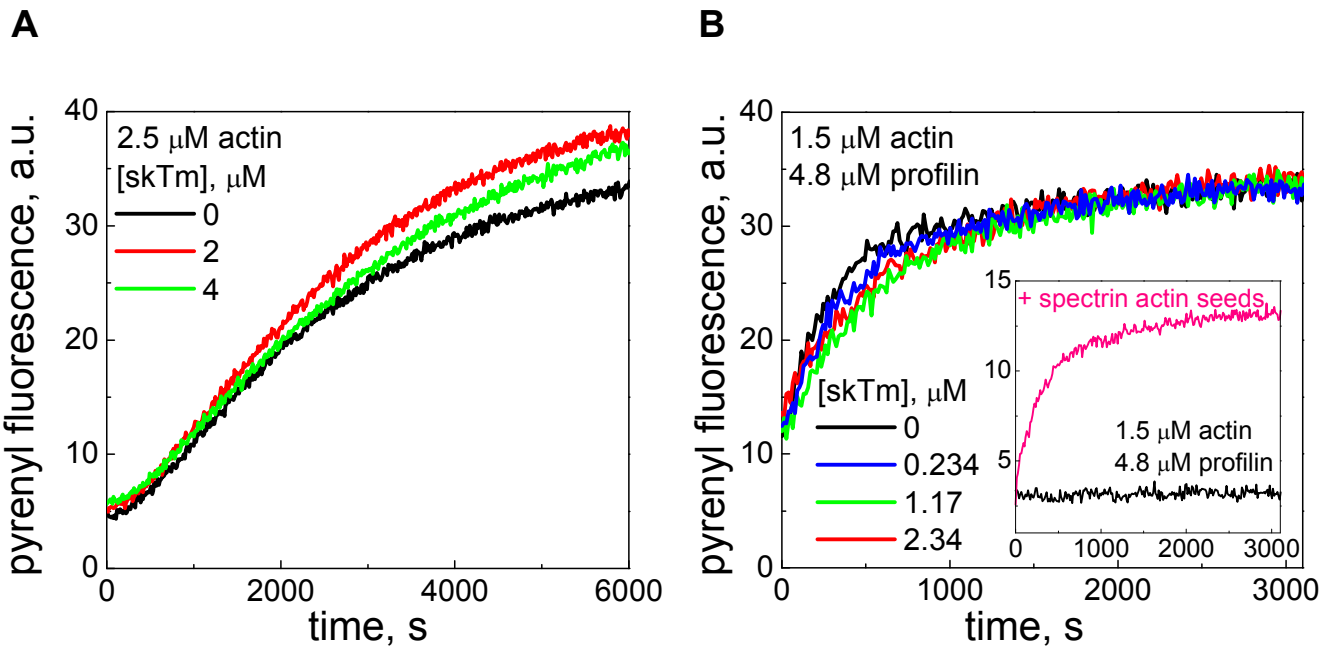
The binding of skTm to actin filaments in different nucleotide states was investigated. ADP-Pi-F-actin was prepared as follows, Ca-ATP-G-actin (50 μM) was polymerised by adding 100 mM KCl, 1 mM MgCl2 and 0.2 mM EGTA, for 30 min at RT then 0.5 mM $BeCl_2$ and 12.5 mM NaF were added to the F-actin solution (ADP-BeF-F-actin). For ADP-F-actin 12.5 mM

NaF was added since the binding of skTm is sensitive to the ionic strength. Samples of 100 μl were prepared by mixing ADP-BeF-F-actin or ADP-F-actin (2 μM) with different concentrations of skTm and incubated overnight at 4 ^{o}C. The samples were ultracentrifuged and processed for SDS-PAGE analysis as described above.

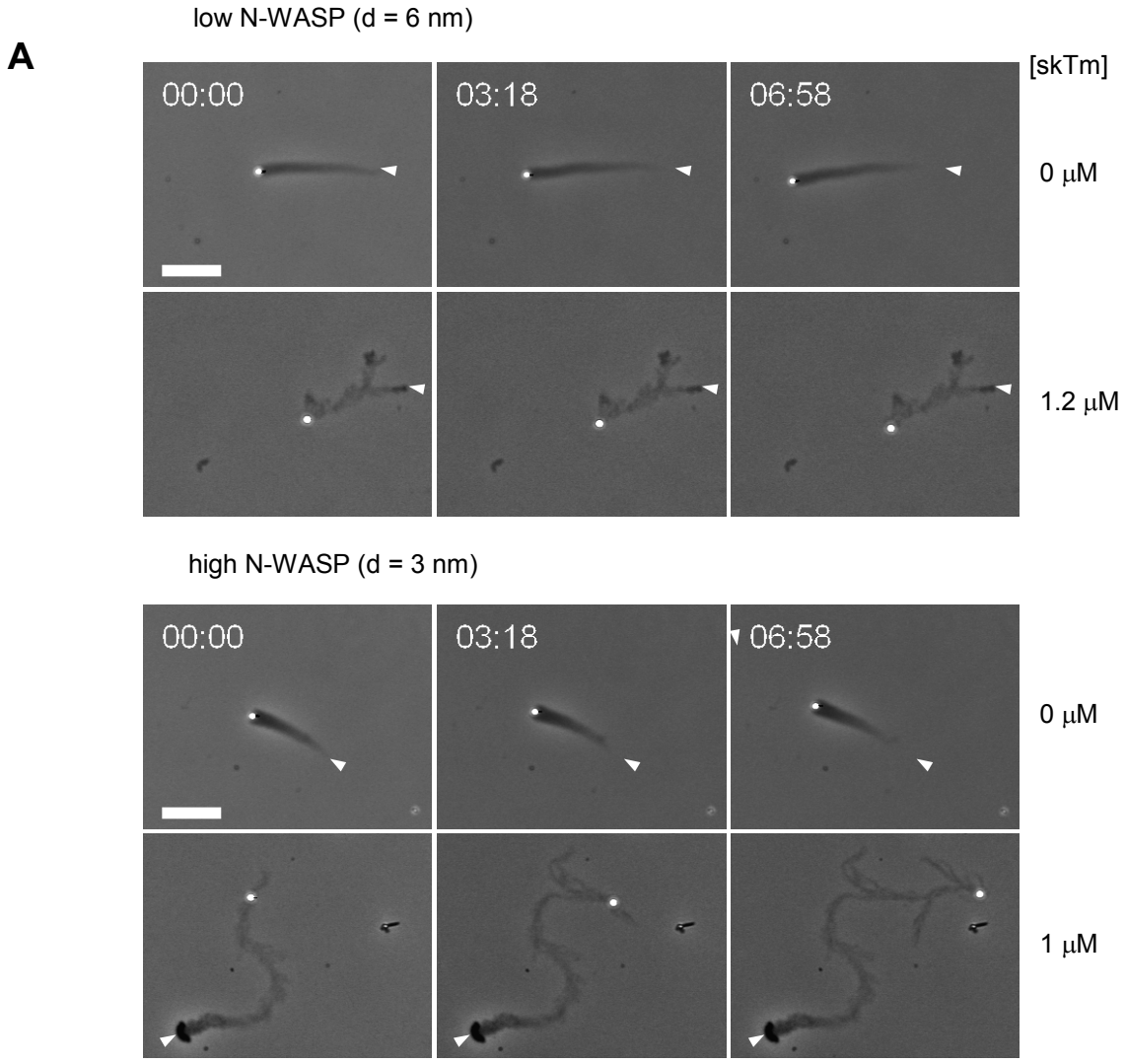
Supplemental references

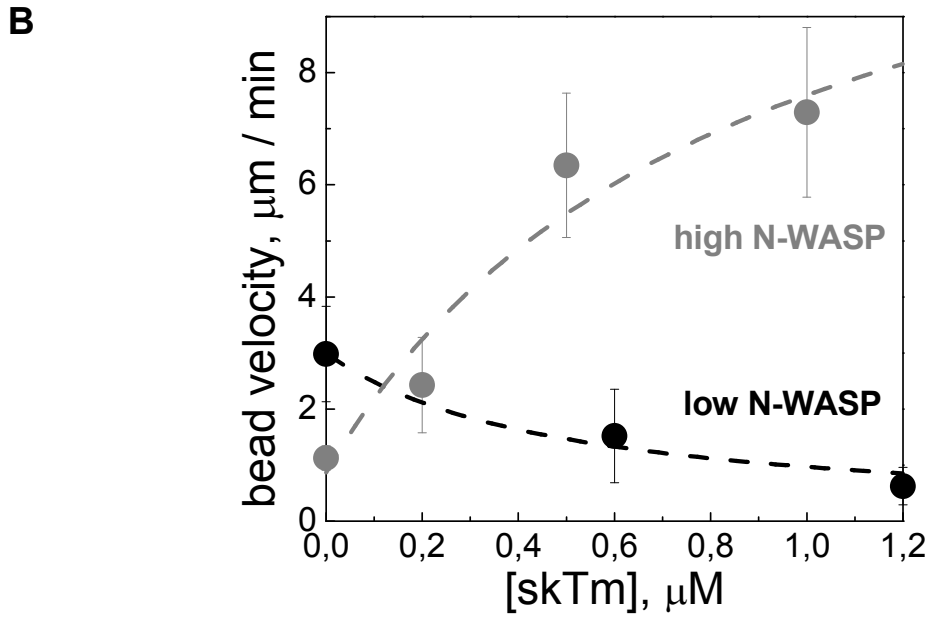
Carlier, M.F., V. Laurent, J. Santolini, R. Melki, D. Didry, G.X. Xia, Y. Hong, N.H. Chua, and D. Pantaloni. 1997. Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: implication in actin-based motility. *J Cell Biol*. 136:1307-22.

Supplemental Figure 1

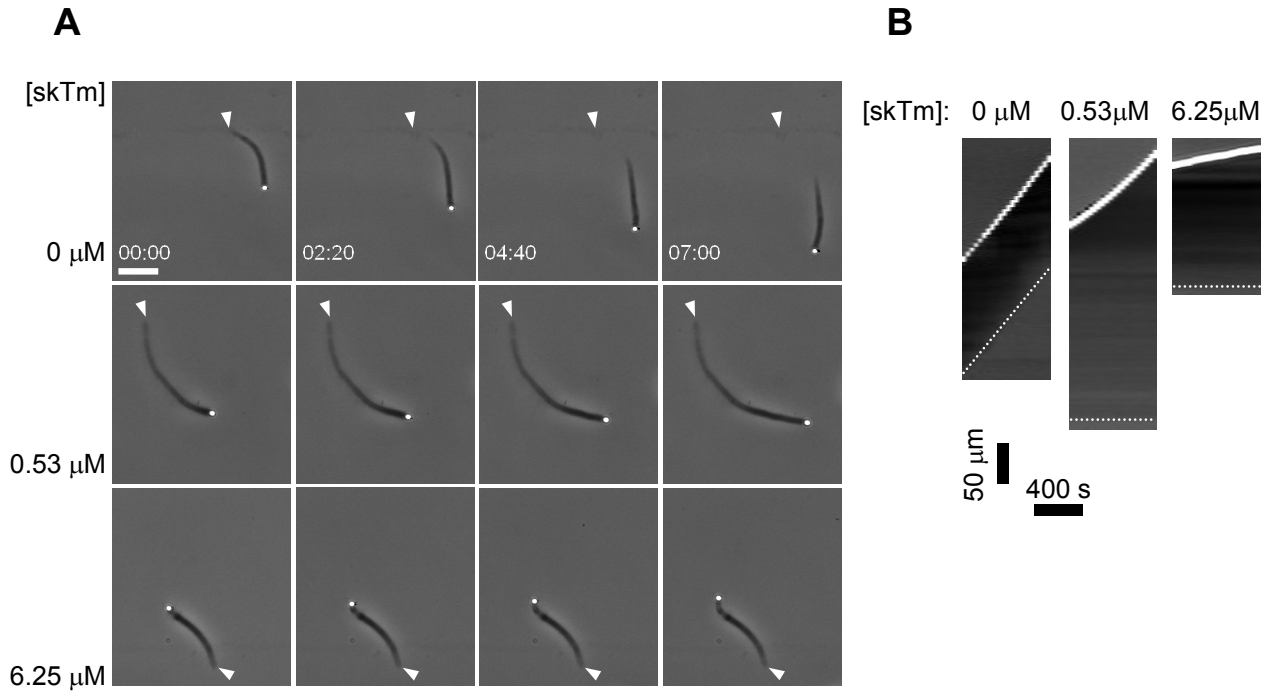


Supplemental Figure 2





Supplemental Figure 3



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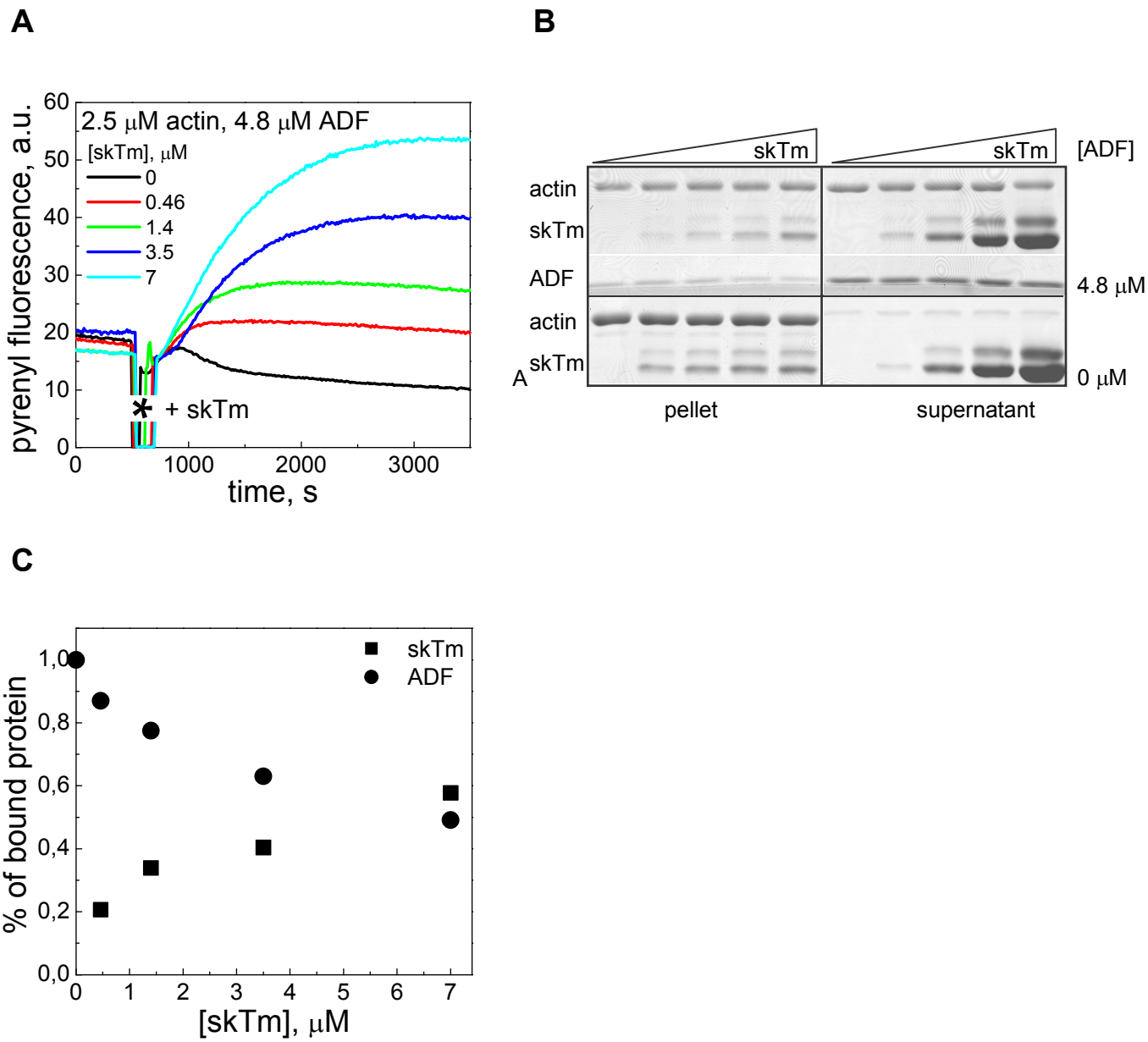

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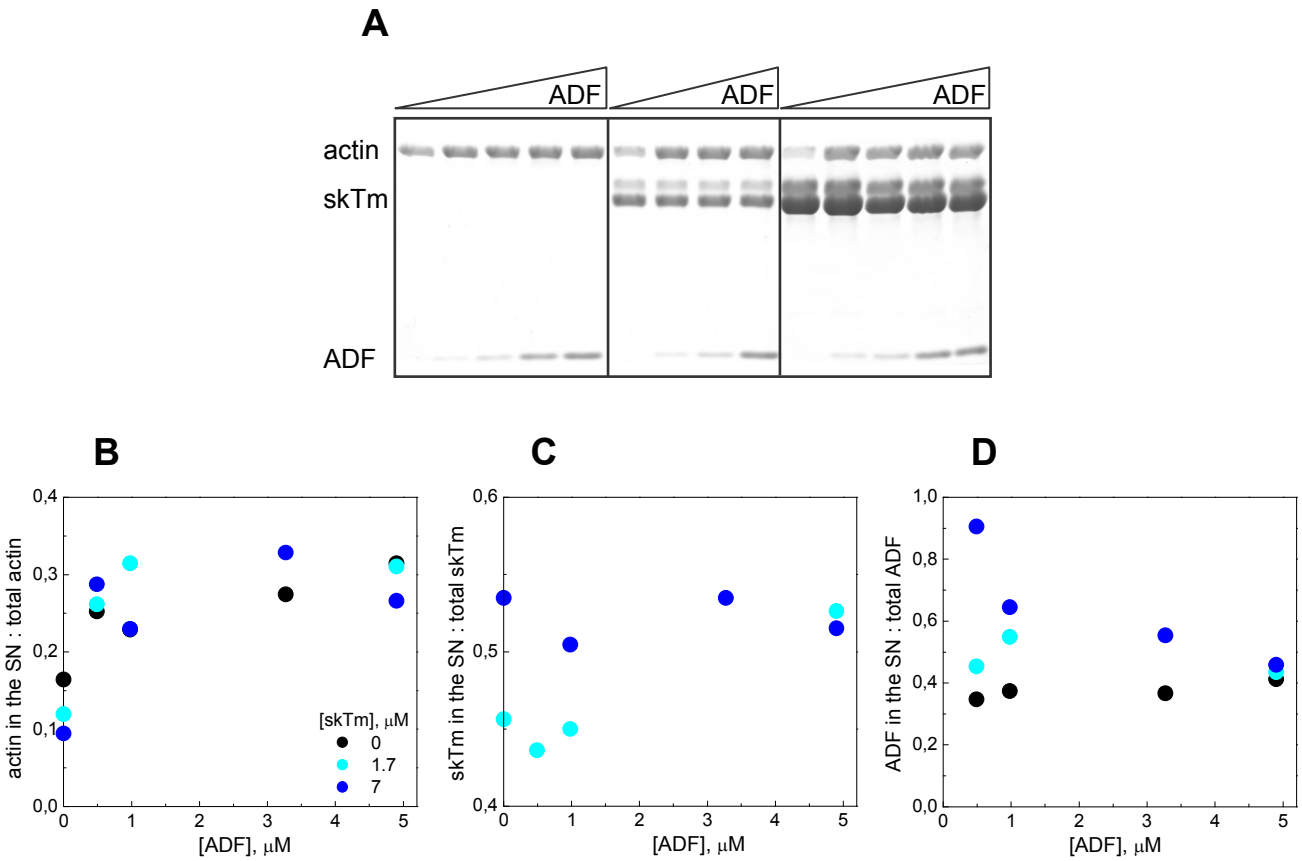

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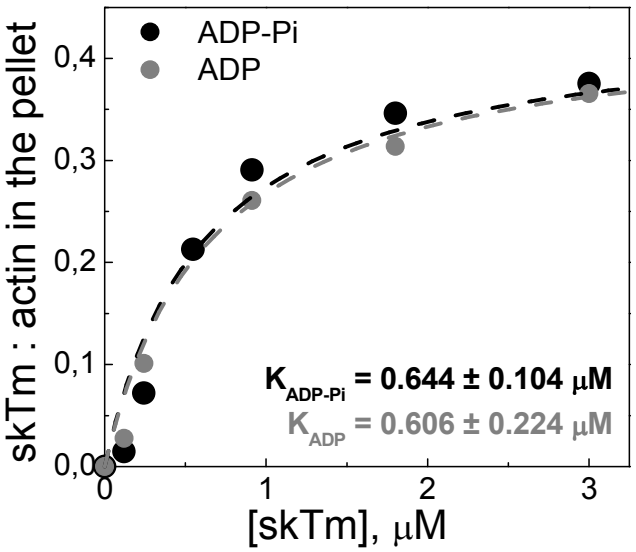

Supplemental Figure 7