SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Vinculin tail inhibits barbed end elongation by acting on the actin filament barbed ends rather than on the actin monomers. (A) The inhibition of barbed end elongation is independent of the concentration of monomeric actin. Barbed end elongation was measured in the presence of 100 pM of spectrin actin seeds. The rate of barbed end growth at 25 % polymerisation was plotted as a function of actin concentration in the absence or presence of Vt (0.5 µM). **(B)** Vt inhibits actin depolymerisation induced by dilution. After dilution, the depolymerization of uncapped (0.05 µM) or gelsolin-capped (0.25 µM F-actin containing 1 nM gelsolin) actin filaments (50% pyrenyl-labeled) was measured at the indicated concentrations of Vt. Depolymerisation rates were plotted versus the concentration of Vt.

Figure S2. Vinculin tail generates actin filament bundles.
(A) Actin filaments (2 µM) were incubated with increasing concentrations of Vt. Actin filament bundles were pelleted by centrifugation at 10,000 g during 30 min. Pellet fractions and total input (T) were analysed by PAGE-SDS followed by Coomassie blue staining. **(B)** The gel was quantified by using the ImageJ software and the fraction of pelleted actin was plotted versus the concentration of vinculin tail. **(C)** Actin filaments (0.6 µM MgATP-actin, 10 % alexa488-labelled) were incubated with increasing concentrations of Vt. The reaction is placed in a flow chamber coated with NEM-myosin in 5mM Tris pH 7.8, 100 mM KCl, 0.2 mM ATP, 0.1 mM CaCl2, 1 mM MgCl2, 10 mM DTT, 0.2 mM DABCO, 0.2 % methyl cellulose and observed by TIRF microscopy. Arrows indicate bundles. Scale bar, 10 µm.

Figure S3. Direct real-time observation of actin filament barbed-end capping by vinculin tail immobilised at the surface of a coverslip. Condtions: 0.6 µM MgATP-G-actin, 10 % alexa488-labelled, is polymerised in a flow chamber coated with 0.5 µM Vt in 5mM Tris pH 7.8, 100 mM KCl, 0.2 mM ATP, 0.1 mM CaCl2, 1 mM MgCl2, 10 mM DTT, 0.2 mM DABCO, 0.2 % methyl cellulose. **(A)** Time-lapse of the elongation of single actin filaments. **(B)** Kymograph corresponding to the growing filament shown in (A). **(C)** Two pictures of the filament shown in (A) are extracted at different times (t = 865 s in green and t = 1005 s in red) during the growth arrest and are superimposed to show that the barbed end of this filament is anchored to the vinculin-coated surface while the filament remains flexible. **(A, C)** Scale bar, 5 µm.

SUPPLEMENTAL MOVIE LEGENDS

Supplemental movie 1. Direct real-time observation of actin filament elongation
10 % Alexa 488-labelled MgATP-G-actin (0.6 µM) is polymerised in a flow chamber coated with NEM-myosin in a buffer containing 5mM Tris pH 7.8, 100 mM KCl, 0.2 mM ATP, 0.1 mM $CaCl_2$, 1mM $MgCl_2$, 10 mM DTT, 0.2 mM DABCO, 0.2 % methyl cellulose. The filament grows at a constant rate.

Supplemental movie 2. Direct real-time observation of actin filament barbed-end capping by vinculin tail.
10 % Alexa 488-labelled MgATP-G-actin (0.6 µM) is polymerised in the presence of 0.5 µM of Vt in a flow chamber coated with NEM-myosin in 5mM Tris pH 7.8, 100 mM KCl, 0.2 mM ATP, 0.1 mM $CaCl_2$, 1mM $MgCl_2$, 10 mM DTT, 0.2 mM DABCO, 0.2 % methyl cellulose. One filament shows a phase of continuous growth followed by a pause. The

transition corresponds to a barbed-end capping event. The second filament, that is rapidly capped, shows a pause followed by a period of continuous growth. The transition corresponds to the dissociation of vinculin from the barbed end.

Supplemental movie 3. Direct real-time observation of actin filament barbed-end capping at the surface of coverslip coated with vinculin tail.
10 % Alexa 488-labelled MgATP-G-actin (0.6 μM) is polymerised in a flow chamber coated with 0.5 μM Vt in 5mM Tris pH 7.8, 100 mM KCl, 0.2 mM ATP, 0.1 mM $CaCl_2$, 1mM $MgCl_2$, 10 mM DTT, 0.2 mM DABCO, 0.2 % methyl cellulose.

Supplemental movie 4. Direct real-time observation of actin filament elongation in the presence of profilin.
10 % Alexa 488-labelled MgATP-G-actin (0.6 μM) is polymerised in the presence of 6 μM profilin in a flow chamber coated with NEM-myosin in 5mM Tris pH 7.8, 100 mM KCl, 0.2 mM ATP, 0.1 mM $CaCl_2$, 1mM $MgCl_2$, 10 mM DTT, 0.2 mM DABCO, 0.2 % methyl cellulose.

Supplemental movie 5. Direct real-time observation of actin filament barbed-end capping by vinculin tail in the presence of profilin.
10 % Alexa 488-labelled MgATP-G-actin (0.6 μM) is polymerised in the presence of 6 μM profilin and 1 μM Vt in a flow chamber coated with NEM-myosin in 5mM Tris pH 7.8, 100 mM KCl, 0.2 mM ATP, 0.1 mM $CaCl_2$, 1mM $MgCl_2$, 10 mM DTT, 0.2 mM DABCO, 0.2 % methyl cellulose.

Supplemental movie 6. Direct real-time observation of actin filament elongation in the presence of mDia1 and profilin.
10 % Alexa 488-labelled MgATP-G-actin (0.6 μM) is polymerised in the presence of 20 nM mDia1 and 6 μM profilin in a flow chamber coated with NEM-myosin in 5mM Tris pH 7.8, 100 mM KCl, 0.2 mM ATP, 0.1 mM $CaCl_2$, 1mM $MgCl_2$, 10 mM DTT, 0.2 mM DABCO, 0.2 % methyl cellulose.

Supplemental movie 7. Direct real-time observation of actin filament elongation in the presence of mDia1, profilin and vinculin tail.
10 % Alexa 488-labelled MgATP-G-actin (0.6 μM) is polymerised in the presence of 20 nM mDia1, 1 μM Vt and 6 μM profilin in a flow chamber coated with NEM-myosin in 5mM Tris pH 7.8, 100 mM KCl, 0.2 mM ATP, 0.1 mM $CaCl_2$, 1mM $MgCl_2$, 10 mM DTT, 0.2 mM DABCO, 0.2 % methyl cellulose.