

# Supplemental Materials

*Molecular Biology of the Cell*

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## Supplemental Figure Legends

### Supplemental Figure 1. $\alpha$ E-catenin binds cooperatively to filamentous actin

**(A)** The  $\alpha$ E-catenin ABD is a monomer in solution. The molecular mass of  $\alpha$ E-catenin ABD was determined by small-angle x-ray scattering (SAXS). The calculated molecular weight of the construct is 26.5 kDa. A small amount of aggregation was apparent at the higher concentrations, but analysis of the data either assuming some aggregation or assuming no aggregation clearly showed that the ABD is a monomer at all concentrations tested.

**(B)** Representative images of GFP  $\alpha$ E-catenin ABD binding to filamentous actin *in vitro*. Localization of increasing concentrations of  $\alpha$ E-catenin ABD (25 nM to 4  $\mu$ M GFP) bound to phalloidin-stabilized F-actin (20% Cy3 labeled, also see Methods).

**(C-D)** Bulk actin filament pelleting assays with  $\alpha$ E-catenin ABD **(C)** and full-length  $\alpha$ E-catenin dimer **(D)**. Bound  $\alpha$ E-catenin ( $\mu$ M/ $\mu$ M actin) was plotted against free  $\alpha$ E-catenin ( $\mu$ M), and data were fit to a Hill equation (red line;  $K_d$  and Hill coefficient listed in red) or hyperbolic function (green line;  $K_d$  listed in green). Data shown represent one example of  $\geq 3$  experiments for  $\alpha$ E-catenin ABD and dimer. The maximal number of  $\alpha$ -catenin molecules bound per actin monomer ( $B_{max}$ ) varied between different experiments and values between 0.6-1.4 were obtained for the ABD (curve fit assuming cooperativity). For full-length  $\alpha$ E-catenin, values ranged from 0.6-1.8 (curve fit assuming no cooperativity).

### Supplemental Figure 2. $\alpha$ E-catenin ABD reduces barbed end polymerization

**(A)** Average barbed end polymerization rate in 2  $\mu$ M Mg-ATP-actin (10% Cy3 labeled) was measured in the presence of increasing concentrations of GFP  $\alpha$ E-catenin ABD. Error bars indicate SD ( $n \geq 30$  actin filaments from  $\geq 2$  experiments).

**(B)** Kymographs of Cy3-labeled actin filaments growing in the absence or presence of 2  $\mu$ M GFP  $\alpha$ E-catenin ABD. Arrowheads mark pauses in barbed end elongation (frequency was  $0.087 \text{ min}^{-1}$ , average lifetime was  $62 \pm 36$  seconds,  $n=32$  pauses).

**(C)** Montage of 2  $\mu$ M Mg-ATP-Actin (10% Cy3 labeled) polymerizing and bundling in the presence of 0.25  $\mu$ M GFP  $\alpha$ E-catenin ABD. We observed a 2.9-fold increase in GFP  $\alpha$ E-catenin ABD fluorescence per actin filament at sites of bundling. Scale bar is 10  $\mu$ m.

**(D)** Kymograph of actin filaments bundling in the presence of 2 $\mu$ M Mg-ATP-Actin (10% Cy3 labeled) and 0.25  $\mu$ M GFP  $\alpha$ E-catenin ABD. Note the change in BE growth during filament bundling. Vertical scale is 1 minute. Horizontal scale bar 5 $\mu$ m.

## Movie Legends

### Movie 1

Dynamic localization of 2 nM GFP  $\alpha$ E-catenin ABD (green) binding to phalloidin-stabilized F-actin (20% Cy3-labeled, red). Images were acquired at 50 ms intervals. Movie plays at 20 frames per second. Scale bar 1  $\mu$ m.

### Movie 2

Dynamic localization of 2 nM GFP  $\alpha$ E-catenin ABD (green) binding to phalloidin-stabilized F-actin (20% Cy3-labeled, red) in the presence of 0.5  $\mu$ M dark  $\alpha$ E-catenin ABD. Images were acquired every 50 ms during data acquisition. Movie plays at 20 frames per second. Scale bar 1  $\mu$ m.

### Movie 3

Inhibition of Arp2/3 complex-mediated filament branching in the presence of GFP  $\alpha$ E-catenin ABD. Actin filaments were polymerized and nucleated in the presence of 1  $\mu$ M Mg-ATP-actin (10% Cy3), 50 nM Arp2/3, 100 nM SCAR<sup>VCA</sup>, and 0-1  $\mu$ M GFP  $\alpha$ E-catenin ABD. Images were acquired every 10 sec during data acquisition. Movie plays at 15 frames per second. Scale bar 10  $\mu$ m.

### Movie 4

Inhibition of Arp2/3 complex-mediated filament branching in the presence of full length GFP  $\alpha$ E-catenin. Actin filaments were polymerized and nucleated in the presence of 1  $\mu$ M Mg-ATP-actin (10% Cy3 labeled), 50 nM Arp2/3 and 100 nM SCAR<sup>VCA</sup> in the absence (right panel) or presence (left panel) of 2  $\mu$ M full length GFP  $\alpha$ E-catenin. Images were acquired every 10 sec during data acquisition. Movie plays at 15 frames per second. Scale bar 10  $\mu$ m.

### Movie 5

Inhibition of cofilin severing in the presence of  $\alpha$ E-catenin ABD. ADP-actin filaments (20% Cy3, red) were immobilized and disassembled by 75 nM ybbr-Atto488-hCofilin (green) in the absence (right panel) or presence (left panel) of 2  $\mu$ M  $\alpha$ E-catenin ABD. Images were acquired every 15 sec during data acquisition. Movie plays at 15 frames per second. Scale bar 5  $\mu$ m.

**A**

Protein Concentration (mg/ml)	Rg(Å)		MW(kDa)	
	Fit assuming no aggregation	Fit assuming aggregation	Fit assuming no aggregation	Fit assuming aggregation
5.0	25.2	27.4	25.9	27.6
2.5	26.0	27.1	28.0	28.9
1.0	27.3		25.2	
0.5	28.2		21.2	



