Supplemental Material

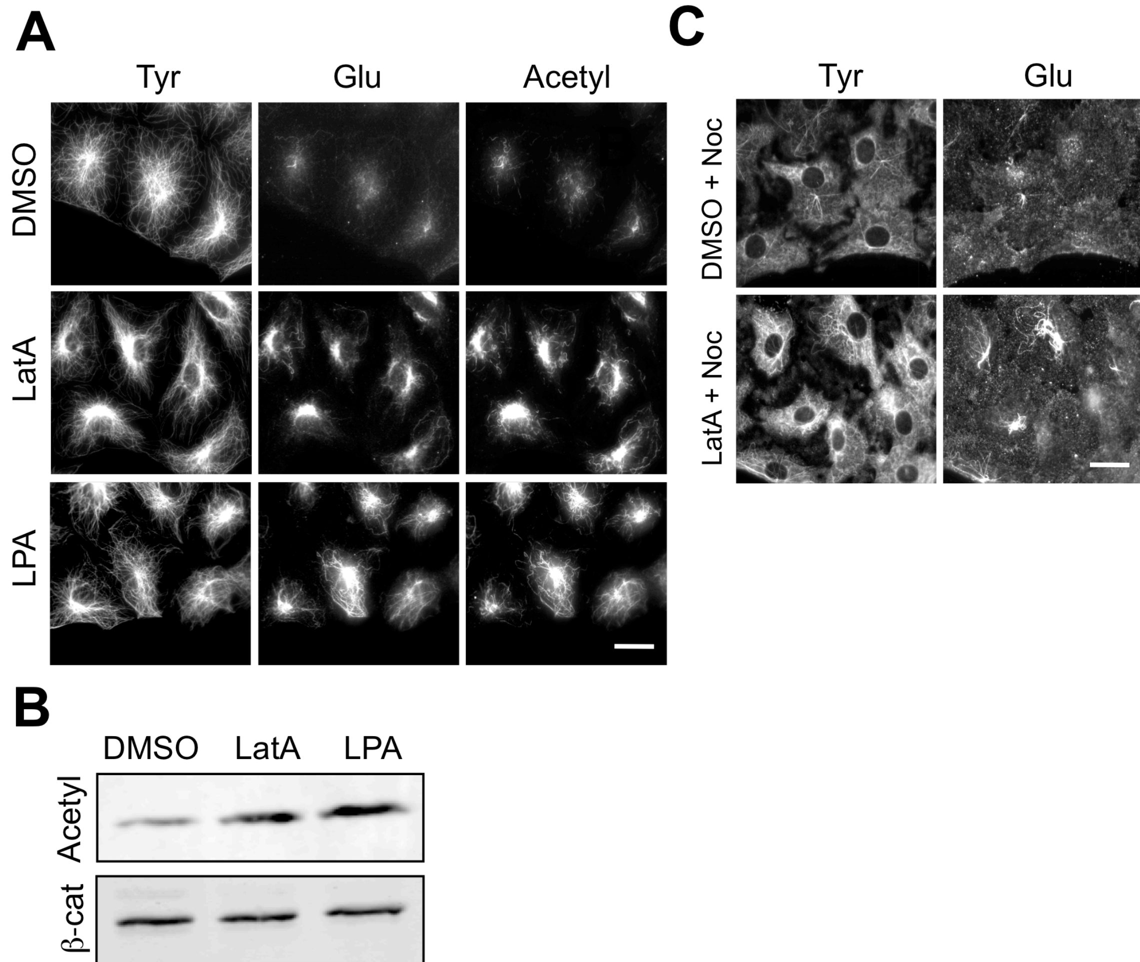
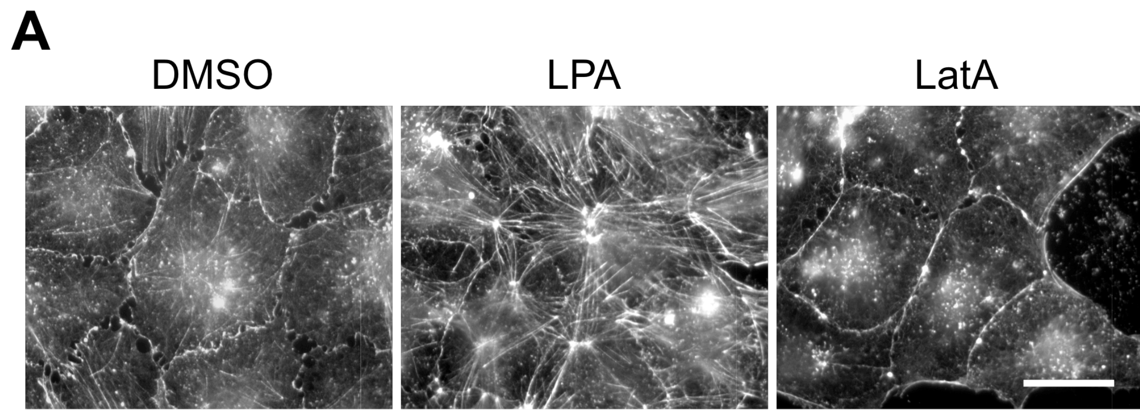


Figure S1. Glu MTs induced by LatA are stable. (A) Glu, Tyr and acetylated (Acetyl) tubulin immunostaining of serum-starved NIH3T3 cells incubated with DMSO, 0.1 μM LatA or 5 μM LPA 1 h before fixation. (B) Western blot analysis of whole cell lysates from serum-starved cells treated as in (A). Proteins were resolved by SDS-PAGE and acetylated tubulin (Acetyl) or β-catenin (β-cat) (used as a loading control) were detected using specific antibodies. (**C**) Tyr and Glu tubulin immunostaining of serum-starved NIH3T3 cells treated with DMSO or 0.1 μM LatA followed by incubation with nocodazole (Noc) (2 μM for 30 min) to depolymerize MTs. Bars, A and C: 20 μm.



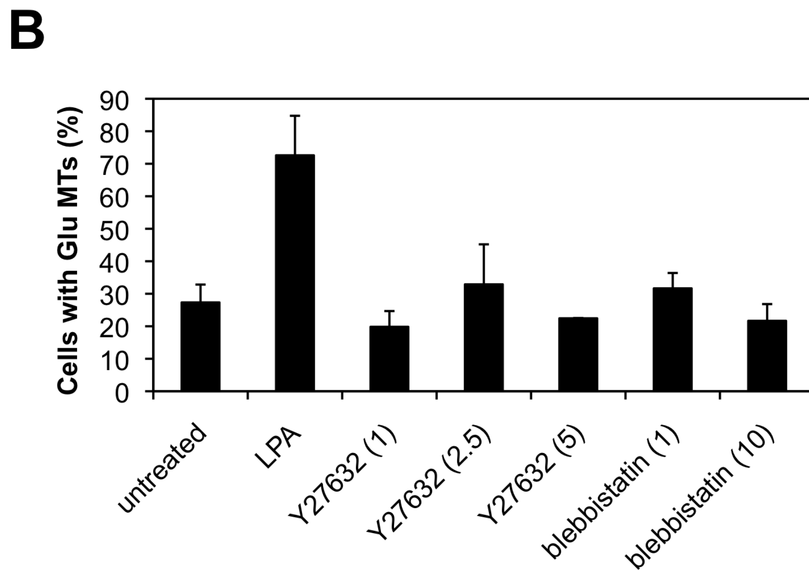


Figure S2. Inhibitors of stress fibers or acto-myosin contractility do not induce stable Glu MTs. (A) Phalloidin staining of F-actin in serum-starved NIH3T3 cells treated with DMSO, 5 μM LPA or 0.1 μM LatA for 1 h before fixation. Bar: 20 μm. **(**B) Quantification of serum-starved cells that exhibited >10 Glu MTs after treatment with the indicated drugs for 1 h. Numbers in parentheses refer to μM concentrations. Data are mean $\pm$ s.d. of three independent experiments (n >100 cells per experiment).

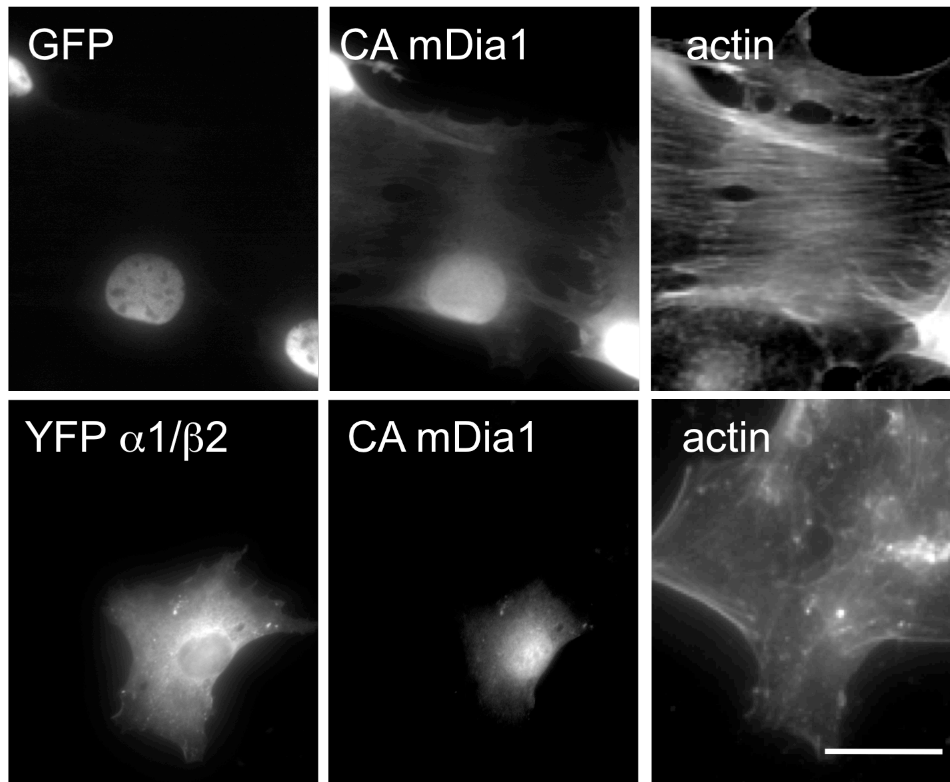


Figure S3. Capping protein inhibits formation of actin stress fiber by mDia1. Cy-5 phalloidin staining of F-actin in serum starved NIH3T3 cells that were injected with constitutively active Cherry-mDia1 (CA mDia1) and either YFP- $\alpha1/\beta2$ capping protein subunits or GFP vector. Bar, 20 μm.

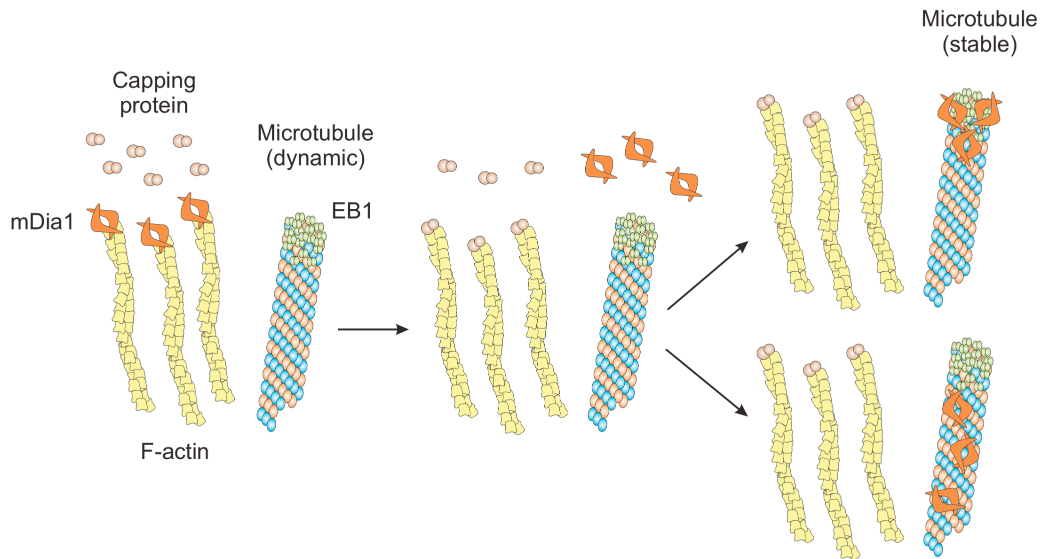


Figure S4. Model for the sequential action of mDia1 on actin filaments and MTs. mDia1 activated by upstream factors functions at the barbed ends of actin filaments. Capping protein competes with mDia for actin barbed ends and releases active mDia1 that then acts on MTs to stabilize them. Shown are two possibilities for mDia association with MTs: EB1-mediated (Wen et al., 2004) and direct association with the MT lattice (Bartolini et al., 2008). Both modes of association may contribute to MT stabilization. Note that the stoichiometry of mDia1 to MT necessary for stabilization is unknown and may require more molecules of mDia1 than depicted.

Movie 1. TIRF microscopy of GFP-FH1FH2mDia2 puncta in a control NIH3T3 fibroblast (injected with rhodamine-dextran alone). The majority of GFP-FH1FH2mDia2 puncta move linearly and accumulate at the tips of filopodia-like protrusions. Movie represents 2 min at 1 s intervals. Acquisition frame rate, 5 fps. Bar, 10 μm.

Movie 2. TIRF microscopy of GFP-FH1FH2mDia2 puncta in NIH3T3 fibroblast injected with capping protein and rhodamine-dextran. GFP-FH1FH2mDia2 puncta do not move directionally, but instead exhibit sporadic non-directional movement. Movie represents 2 min at 1 s intervals. Acquisition frame rate, 5 fps. Bar, 10 μm.

References for Supplemental Material

Bartolini, F., Moseley, J.B., Schmoranzer, J., Cassimeris, L., Goode, B.L., and Gundersen, G.G. (2008). The formin mDia2 stabilizes microtubules independently of its actin nucleation activity. J Cell Biol *181*, 523-536.

Wen, Y., Eng, C.H., Schmoranzer, J., Cabrera-Poch, N., Morris, E.J., Chen, M., Wallar, B.J., Alberts, A.S., and Gundersen, G.G. (2004). EB1 and APC bind to mDia to stabilize microtubules downstream of Rho and promote cell migration. Nat Cell Biol *6*, 820-830.