Supplemental material

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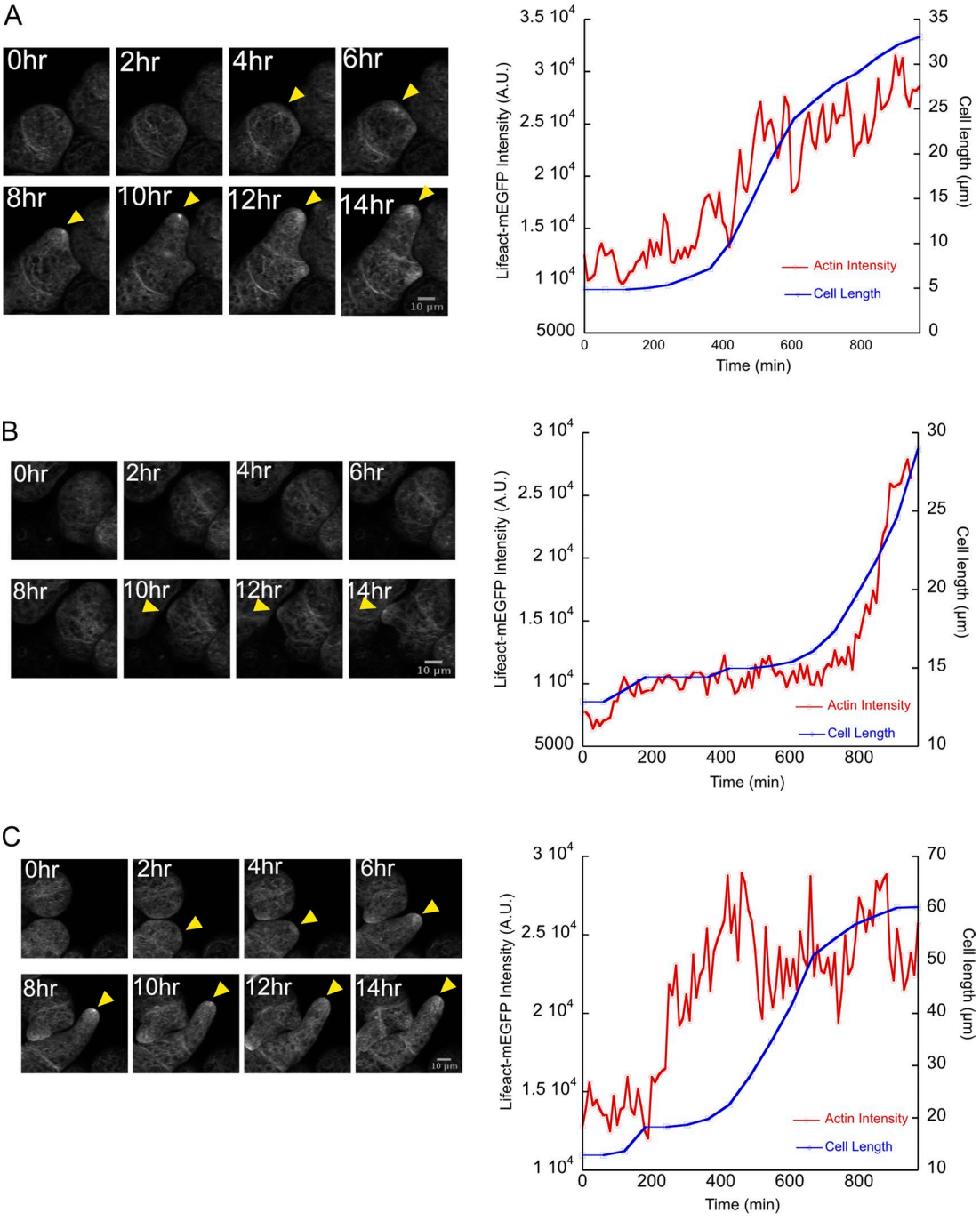


Figure S1. **Related to Fig. 1.** Three examples of tip cells emerging from protoplasts. Left, images of WT cells expressing Lifeact-mEGFP acquired on a scanning confocal microscope. Yellow arrowheads point to actin accumulation. Images are maximum projections of z-stacks acquired every 10 min. Bars, 10 µm. Right, mean intensity of Lifeact-mEGFP signal near the cell apex and cell length plotted against time. A.U., arbitrary units.

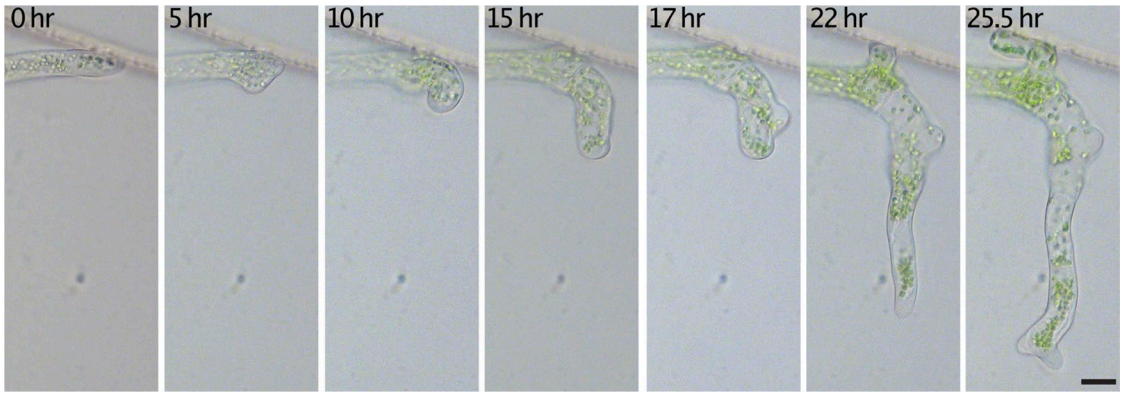


Figure S2. **Wild-type moss protonemal cells growing in the presence of 10 µM oryzalin imaged on a widefield microscope.** Bar, 20 µm.

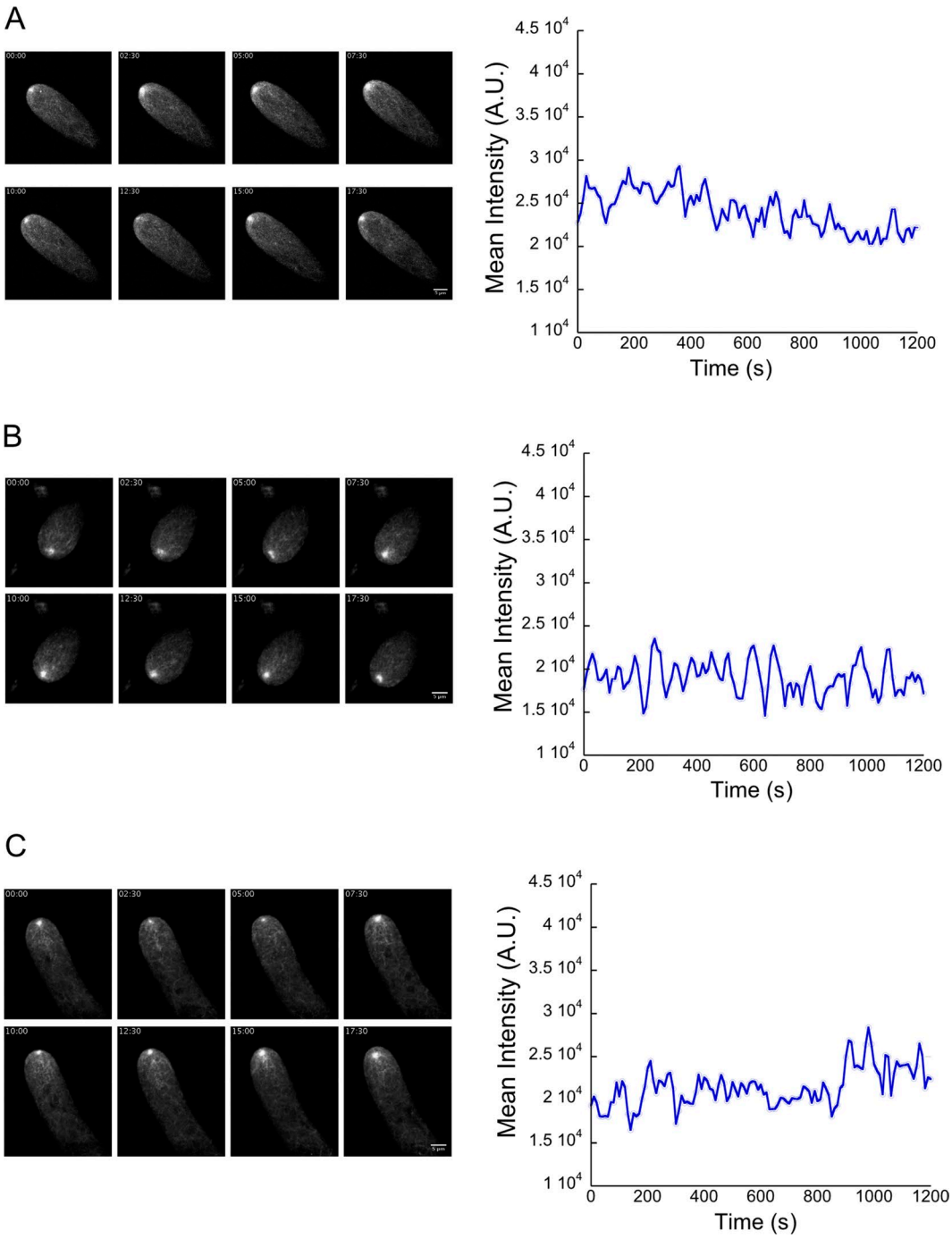


Figure S3. **Related to Fig. 7.** Three examples of WT cell expressing Lifeact-mEGFP. Left, actin accumulation near the cell apex is stable in size and shape over long periods of time. Images are maximum projections of z-stacks acquired every 10 s on a laser-scanning confocal micrscope. Bars, 5 µm. Time stamp, min:s. Right, a 7-µm diameter circle near the cell apex was tracked using TrackMate and the mean intensity of Lifeact-mEGFP signal was plotted over time. A.U., arbitrary units.

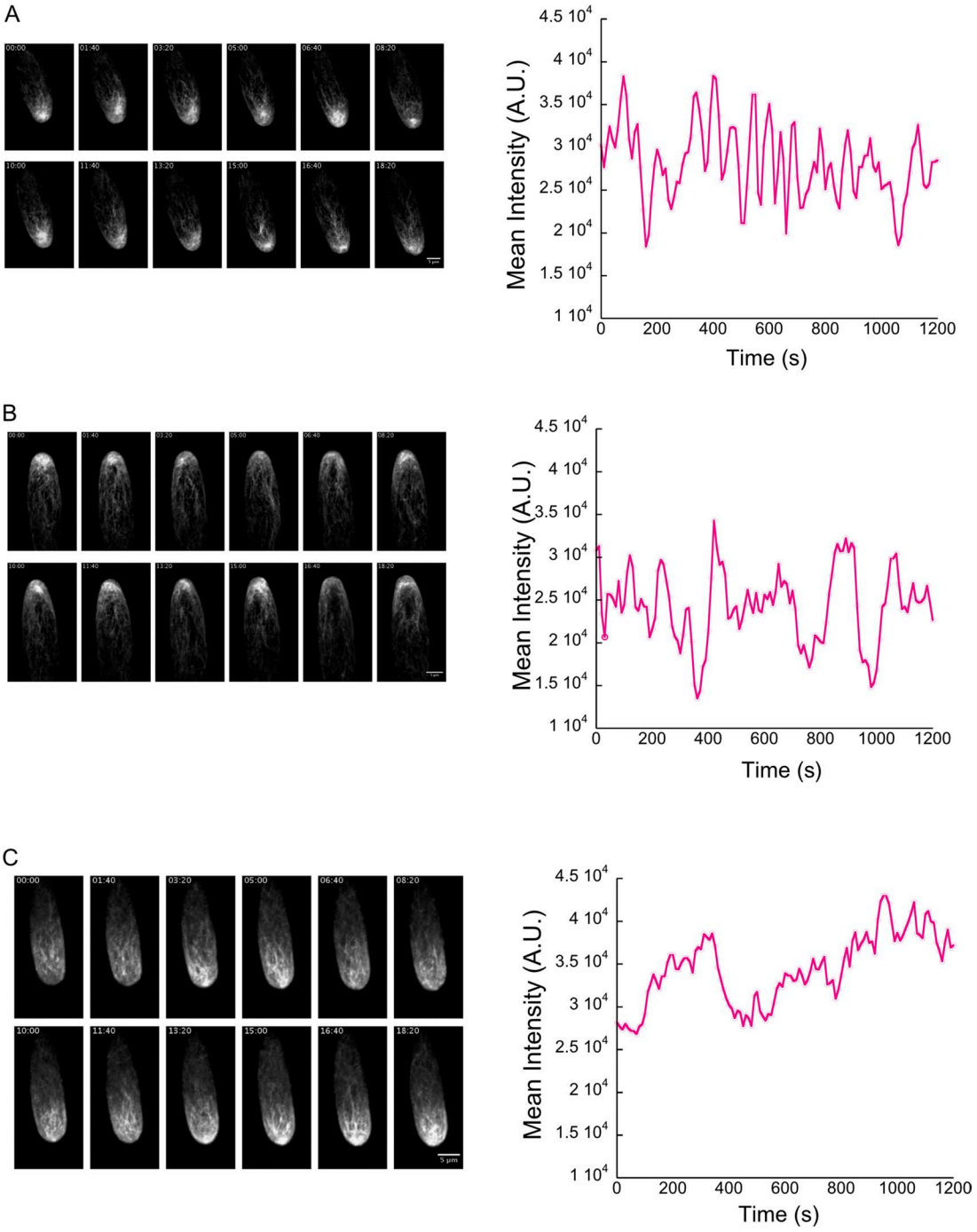


Figure S4. **Related to Fig. 7.** Three examples of Δmyo8 cell expressing Lifeact-mEGFP. Left, actin accumulation near the cell apex is more variable in size and shape compared with WT, and long filamentous actin structures were often observed emanating from the apical actin accumulation. Images are maximum projections of z-stacks acquired every 10 s on a laser-scanning confocal microscope. Bars, 5 µm. Time stamp, min:s. Right, a 7-µm diameter circle near the cell apex was tracked using TrackMate and the mean intensity of Lifeact-mEGFP signal was plotted over time. A.U., arbitrary units.

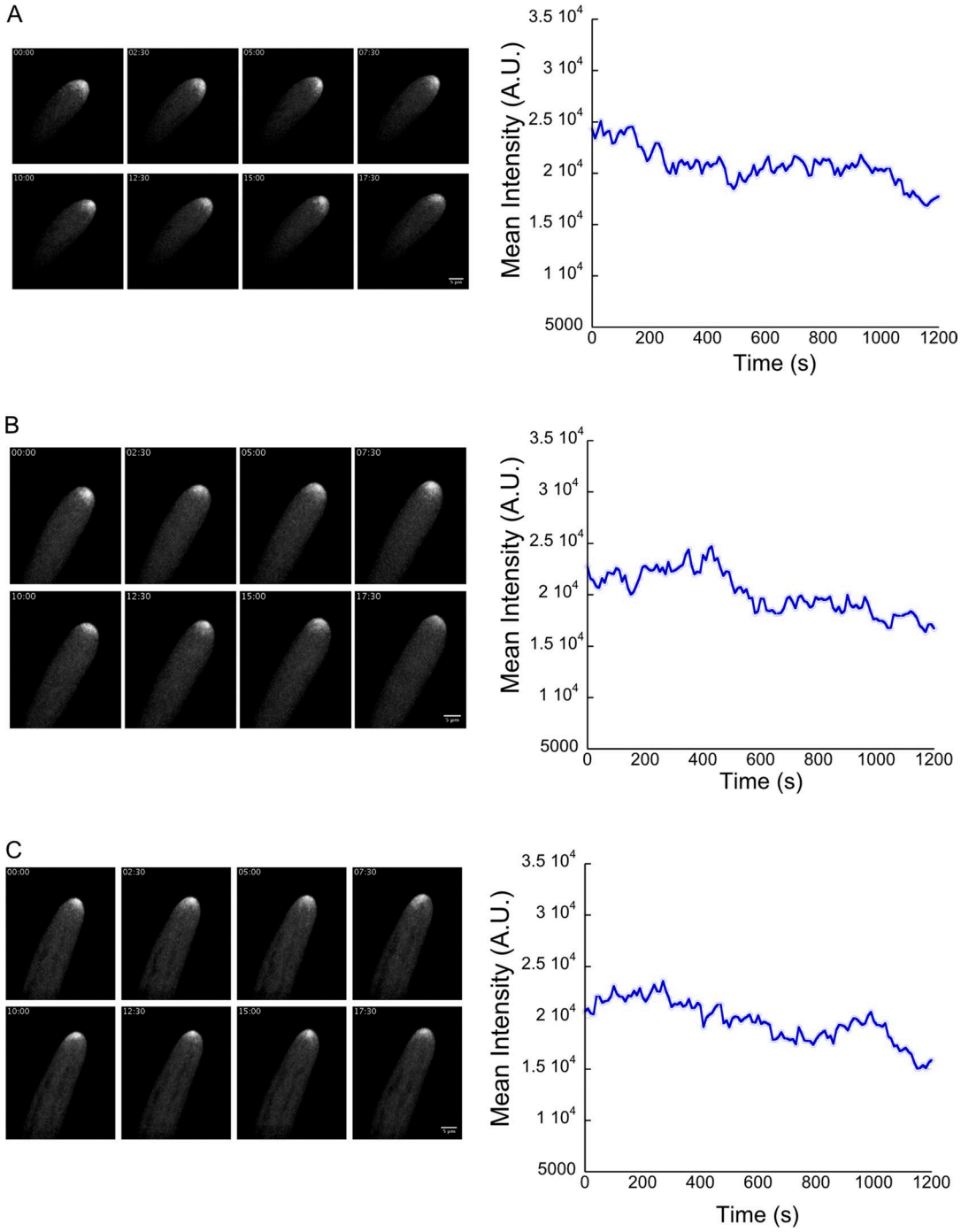


Figure S5. **Related to Fig. 8.** Three examples of WT cell expressing For2A-GFP. Left, For2A-GFP accumulation near the cell apex is stable over long periods of time. Images are maximum projections of z-stacks acquired every 10 s on a laser-scanning confocal microscope. Bars, 5 µm. Time stamp, min:s. Right, a 7-µm diameter circle near the cell apex was tracked using TrackMate and the mean intensity of For2A-GFP signal was plotted over time. A.U., arbitrary units.

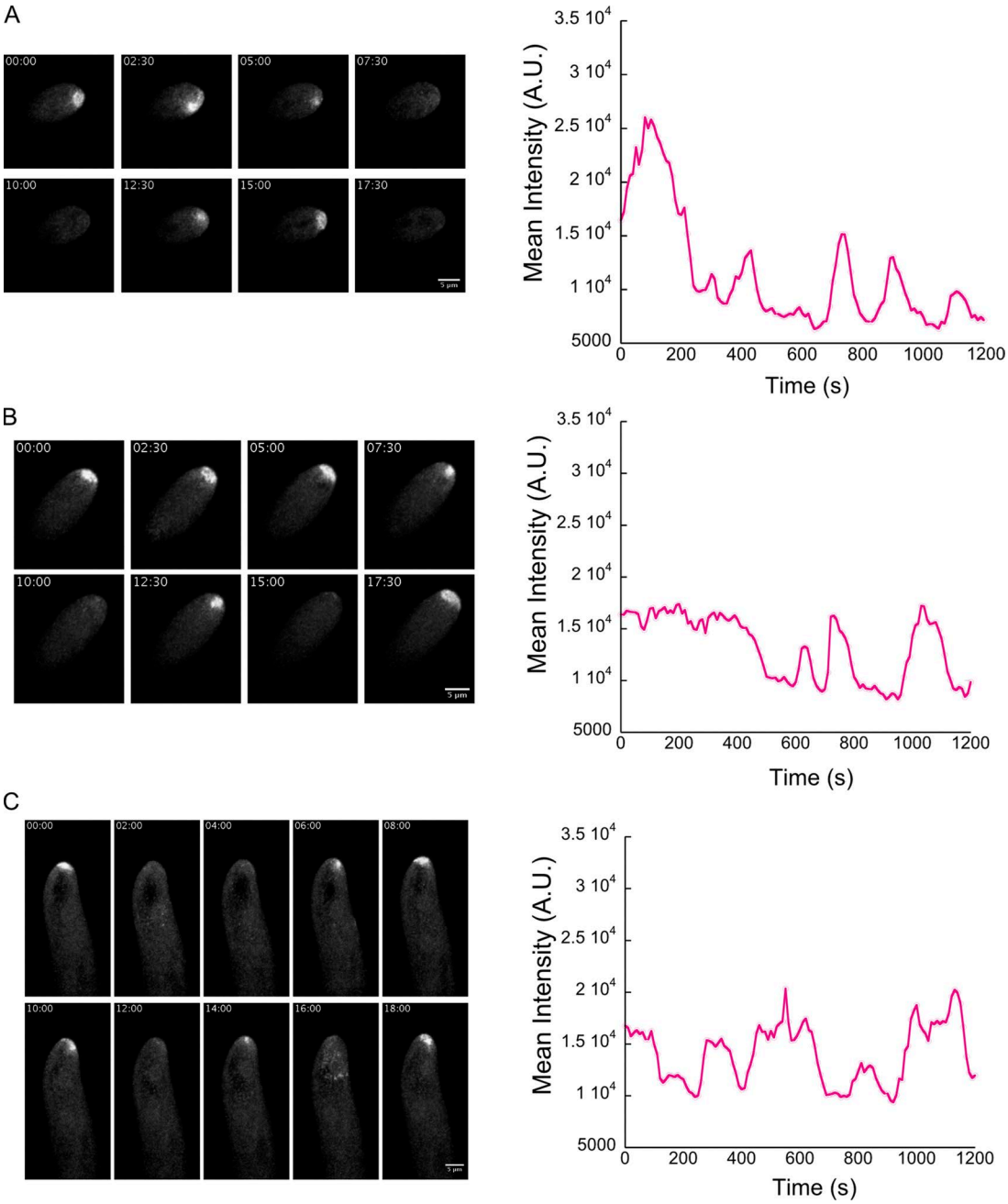


Figure S6. **Related to Fig. 8.** Three examples of Δmyo8 cell expressing For2A-GFP. Left, For2A-GFP accumulation near the cell apex is less stable compared to WT. The cells underwent period of times with very little For2A-GFP near the cell apex. Images are maximum projections of z-stacks acquired every 10 s on a laser-scanning confocal microscope. Bar, 5 µm. Time stamp, min:s. Right, a 7-µm diameter circle near the cell apex was tracked using TrackMate, and the mean intensity of For2A-GFP signal was plotted over time. A.U., arbitrary units.

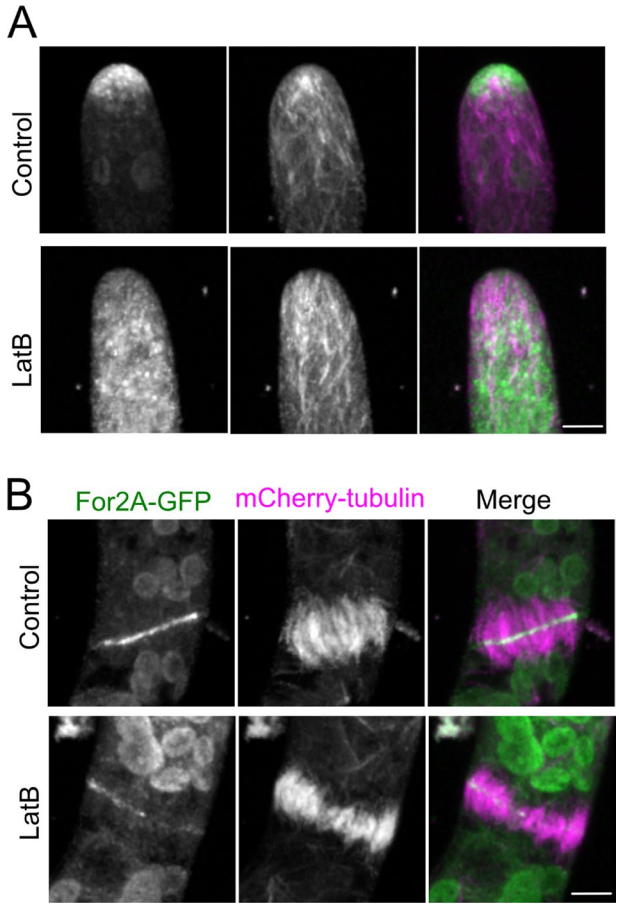
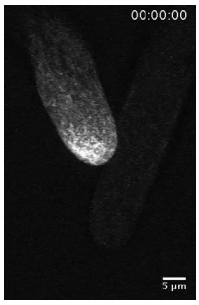
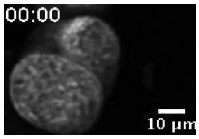


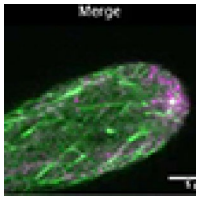
Figure S7. **Cells expressing For2A-GFP and mCherry-tubulin in the absence or presence of 25 µM latrunculin B (LatB). (A)** Cell apex. **(B)** Phragmoplast. Images are maximum projections of z-stacks acquired on a laser-scanning confocal microscope. Bars, 5 µm.



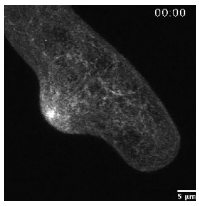
Video 1. **Apical actin cluster is closely correlated with the direction of growth.** Actin filaments labeled with Lifeact-mEGFP in a WT cell. Images are maximum projections of z-stacks acquired on a laser-scanning confocal microscope. Time interval, 20 s. Time stamp, h:min:s. Video is playing at 20 fps. See also Fig. 1 (A and B).



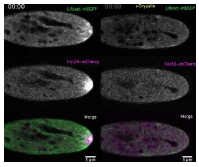
Video 2. **Apical actin cluster predicts the site of cell expansion.** Actin filaments labeled with Lifeact-mEGFP in a WT protoplast-regenerated cell. Images are maximum projection of z-stacks acquired on a laser-scanning confocal microscope. Time interval, 10 min. Time stamp, h:min. Video is playing at 10 fps. See also Fig. 1 (C and D).



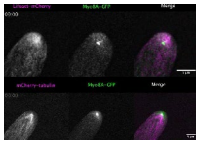
Video 3. **Microtubule convergence overlaps with apical actin cluster.** Actin labeled with Lifeact-mRuby2 (magenta) and microtubules labeled with mEGFP-tubulin (green) in a WT cell. Images are from single focal planes acquired on a laser-scanning confocal microscope. Time interval, 2 s. Video is playing at 15 fps. See also Fig. 2 A.



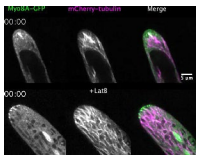
Video 4. **Microtubules are required for maintaining directional growth and the formation of the actin cluster near the cell apex.** A WT cell expressing Lifeact-mEGFP (green) treated with 10 µM oryzalin. Images are maximum projections of z-stacks acquired on a laser-scanning confocal microscope. Time interval, 30 s. Time stamp, min:s. Video is playing at 5 fps. See also Fig. 2 B.



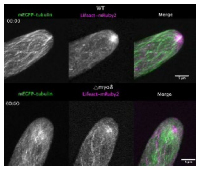
Video 5. **Microtubules confine class II formins and the actin cluster to the cell apex.** Left, a cell expressing Lifeact-mEGFP (green) and For2A-mCherry (magenta). Images are from single focal planes acquired on a laser-scanning confocal microscope. Time interval, 0.27 s. Time stamp, min:s. See also Fig. 3 A. Right, the same cell after 10 µM oryzalin was added to the microfluidic imaging device. Time interval, 1 s. Time stamp, min:s. Video is playing at 20 fps. See also Fig. 3 D.



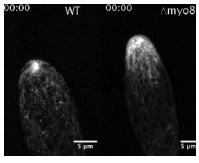
Video 6. **Myosin VIII accumulates near the cell tip overlapping with converging microtubules and the actin cluster.** Top, a cell expressing Myo8A-GFP (green) and Lifeact-mCherry (magenta). Bottom, A cell expressing Myo8A-GFP (green) and mCherry-tubulin (magenta). Images are maximum projections of z-stacks acquired on a laser-scanning confocal microscope. Time interval, 15 s. Time stamp, min:s. Video is playing at 5 fps. See also Fig. 4.



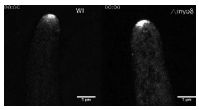
Video 7. **Actin is required to maintain the microtubule focus near the cell apex.** A cell expressing Myo8A-GFP (green) and mCherry-tubulin (magenta) ± 25 µm latrunculin B (LatB). Images are from single focal planes acquired on a spinning disk confocal microscope. Time interval, 2 s. Time stamp, min:s. Video is playing at 15 fps. See also Fig. 5.



Video 8. **Myosin VIII is required for maintaining both apical actin and microtubule structures.** WT (top) and Δmyo8 (bottom) cells expressing mEGFP-tubulin (green) and Lifeact-mRuby2 (magenta). Images are maximum projections of z-stacks acquired on a laser-scanning confocal microscope. Time interval, 15 s (WT) and 10 s (Δmyo8). Time stamp, min:s. Video is playing at 8 fps. See also Fig. 6.



Video 9. **Loss of myosin VIII enhances actin filament formation.** WT (left) and Δmyo8 (right) cells expressing Lifeact-mEGFP. Images are maximum projections of z-stacks acquired on a laser-scanning confocal microscope. Time interval, 20 s. Time stamp, min:s. Video is playing at 5 fps. See also Fig. 7.



Video 10. **Loss of myosin VIII affects For2A distribution.** WT (left) and Δmyo8 (right) cells expressing For2A-GFP. Images are maximum projections of z-stacks acquired on a laser-scanning confocal microscope. Time interval, 10 s. Time stamp, min:s. Video is playing at 5 fps. See also Fig. 8.