Supplemental material

Ko et al., https://doi.org/10.1083/jcb.201902011

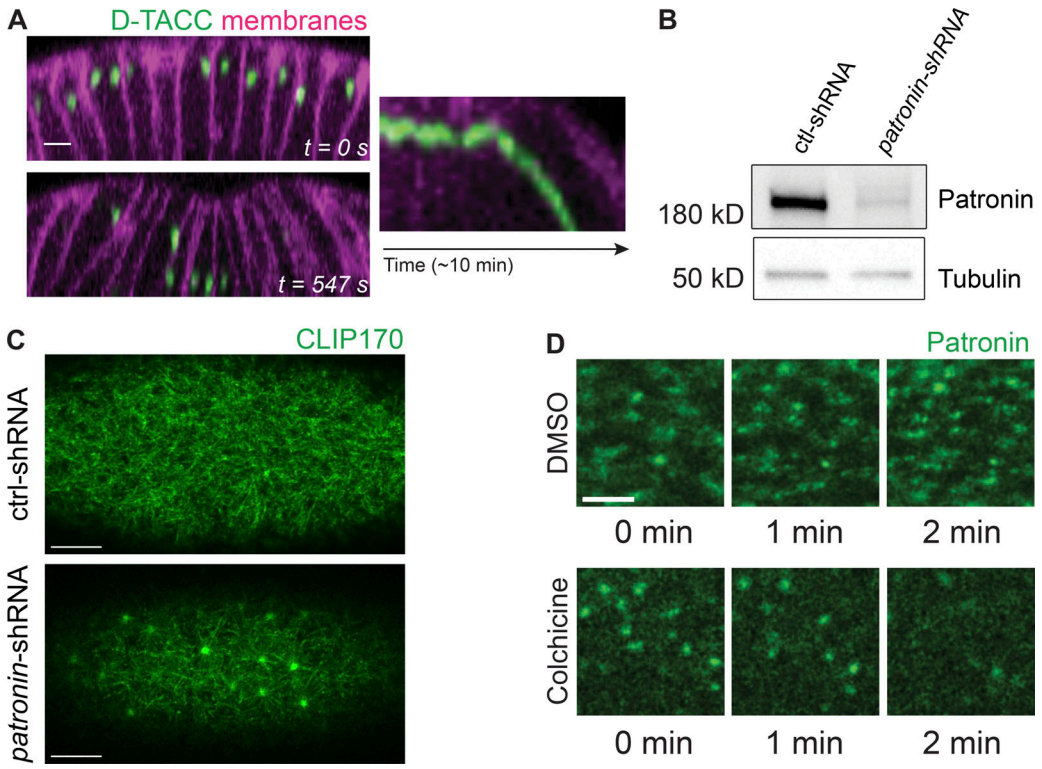


Figure S1. **Medioapical Patronin foci are not centrosomes. (A)** Time-lapse images showing an apical–basal cross section from a live embryo expressing D-TACC::GFP (green, a marker for centrosomes; Gergely et al., 2000) and Gap43::mCH (magenta). A kymograph using a line drawn along the apical–basal axis of a cell at the midline is shown on the right. **(B)** Lysates from control *rhodopsin-3*-shRNA and *patronin*-shRNA flies probed with Patronin antibody serum (gift from R. Vale). Anti-α-tubulin was used as a loading control. **(C)** Microtubule organization is disrupted after Patronin depletion. Images are single apical slices from a live movie of representative rhodopsin-3 control RNAi (top) and *patronin*-RNAi (bottom) embryos expressing GFP::CLIP170. **(D)** Patronin::GFP localization depends on an intact microtubule cytoskeleton. Images are a montage from a live movie of an embryo expressing Patronin::GFP and injected with either DMSO (top) or colchicine (5 mg/ml; bottom). Scale bars represent 15 µm (C) and 5 µm (A and D).

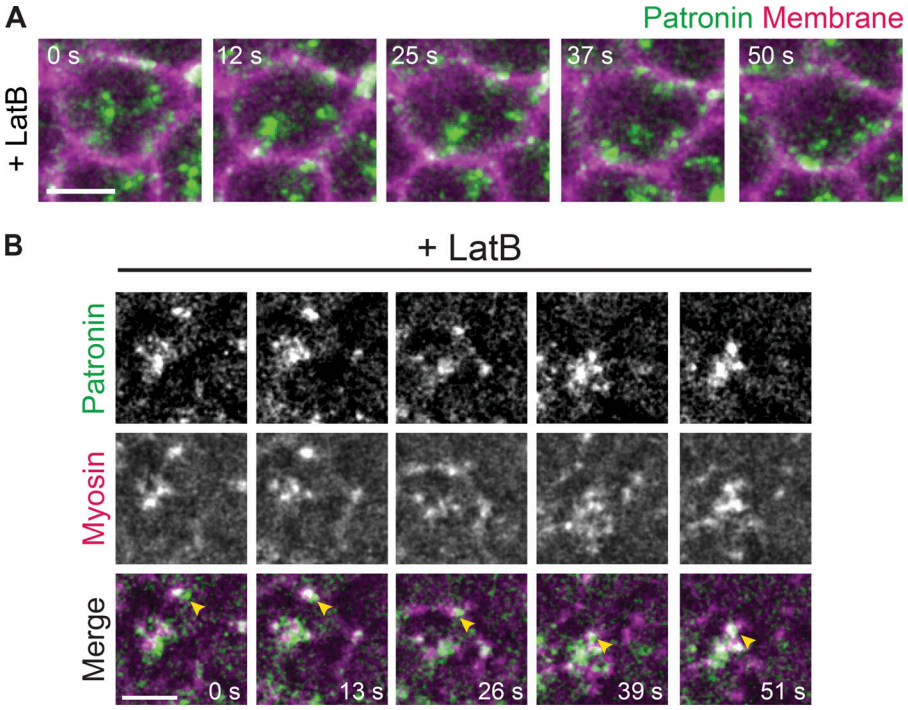


Figure S2. **CytoD and LatB affect the organization of apical microtubules. (A)** Patronin foci fragment into puncta after LatB injection. Time-lapse images are maximum-intensity Z-projections of a representative embryo expressing Patronin::GFP and Gap43:mCH injected with LatB (5 mg/ml in DMSO). **(B)** Patronin puncta colocalize with myosin puncta after F-actin network fragmentation with LatB (arrowheads). Time-lapse images are maximum-intensity Z-projections of a representative embryo expressing Patronin::GFP and Myo::mCH injected with LatB (5 mg/ml in DMSO). Scale bars represent 5 µm.

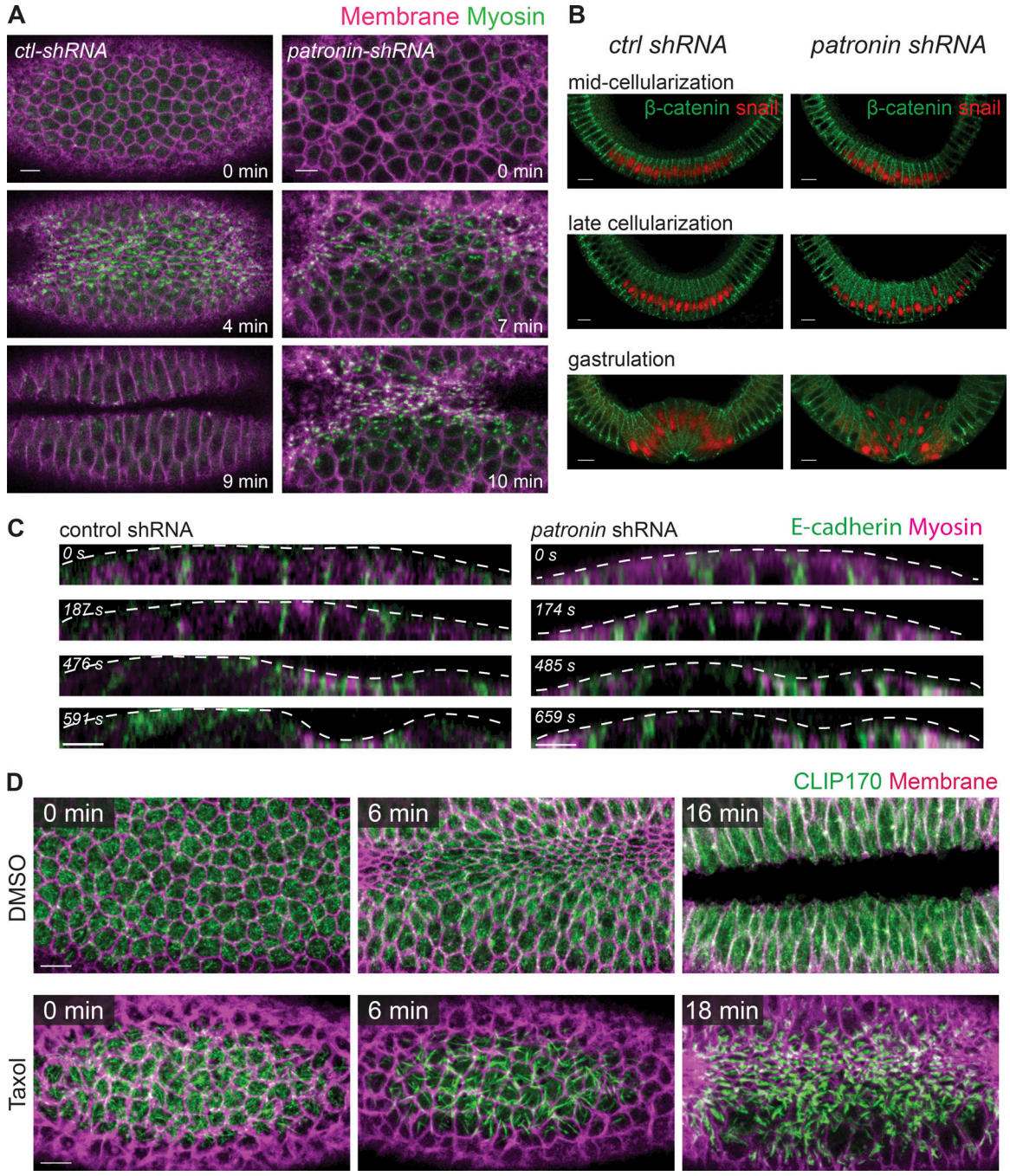


Figure S3. **Patronin depletion disrupts folding, but not apical adherens junctions. (A)** Depleting Patronin disrupts folding, despite apical myosin activation and apical constriction initiation. Time-lapse images are maximum-intensity Z-projections from live embryos expressing *control*-shRNA (left) or *patronin*-shRNA (right) and Myo::GFP (apical surface) and Gap43::mCH (subapical section). The phenotype was observed in 5 out of 12 embryos imaged from this cross. **(B)** In mesoderm cells, adherens junctions exhibit apical shift after Patronin depletion. Images are apical–basal cross sections from embryos fixed at different developmental stages stained for β-catenin (Armadillo) and Snail. **(C)** Apical adherens junctions are unaffected by Patronin depletion. Time-lapse images show apical–basal cross sections from representative live embryos expressing *control*-shRNA (left) or *patronin*-shRNA (right) and E-cadherin::GFP and Myo::mCH. 32 embryos were imaged in total. **(D)** Taxol injection causes dense microtubule bundles across the apical surface. Time-lapse images are maximum-intensity Z-projections from live embryos expressing GFP::CLIP170 (apical surface) and Gap43::mCH (subapical section) injected with either DMSO (top) or Taxol (5 mg/ml; bottom). Scale bars represent 10 µm (A, C, and D) and 5 µm (B).

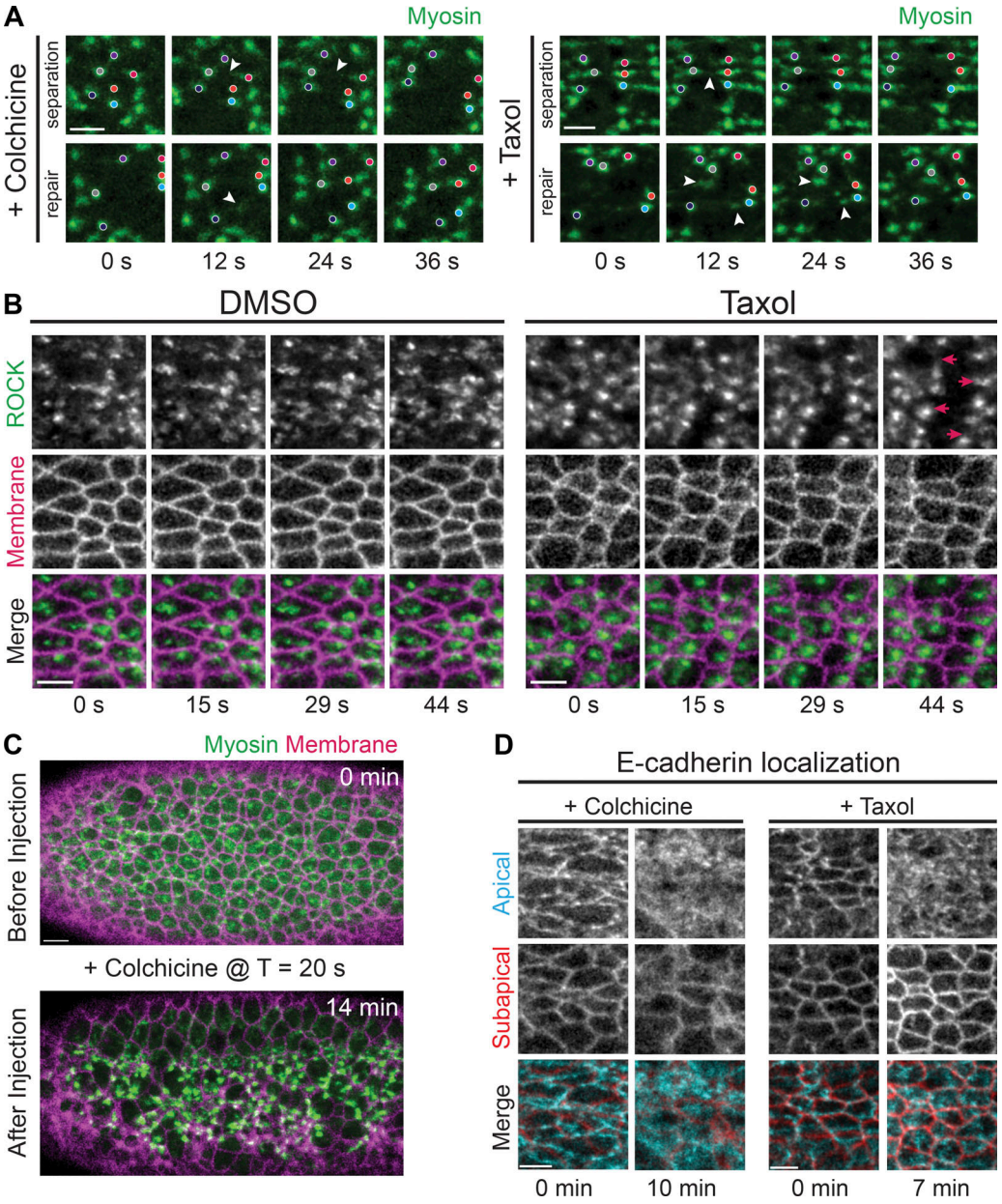
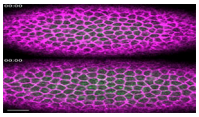
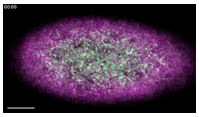


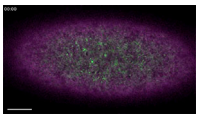
Figure S4. **Disrupting microtubules causes actomyosin network separations from adherens junctions. (A)** Myosin separation events are dynamic and attachments are reestablished following separation. Time-lapse images are maximum-intensity Z-projections of apical Myo::GFP in embryos injected with colchicine or Taxol. Individual myosin patches are labeled with colored dots. Fiber-like structures between myosin patches that either break or reform during repair are shown by arrowheads. **(B)** Taxol does not affect ROCK polarity but causes separation between ROCK foci and junctions. Time-lapse images are maximum-intensity Z-projections from live embryos expressing GFP::ROCK and Gap43::mCH injected with DMSO or Taxol (5 mg/ml). Arrows indicate the direction in which medioapical ROCK foci separate from cell junctions. **(C)** Microtubules were acutely inhibited by injecting embryo with colchicine ∼20 s after start of imaging. Images are maximum-intensity Z-projections from a live embryo expressing Myo::GFP and Gap43::mCH. **(D)** E-cadherin eventually loses junctional polarity over time. Time-lapse images showing a single apical (cyan) and subapical (red) slice of representative embryos expressing E-cadherin::GFP injected with colchicine (5 mg/ml; left) and Taxol (5 mg/ml; right). Scale bars represent 10 µm (C) and 5 µm (A, B, and D).



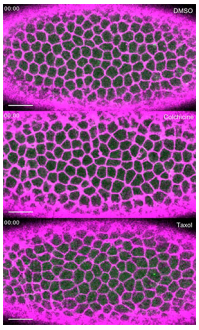
Video 1. **Patronin::GFP forms a medioapical focus in mesoderm cells.** Embryos expressing Patronin::GFP (green) and Gap43::mCH (magenta) shown with the midline of the mesoderm centered (top) or slightly turned (bottom) to show the ectoderm. Images were acquired every 44 s (top) or 40 s (bottom), and videos are displayed at 10 frames per second. Scale bars, 15 µm.



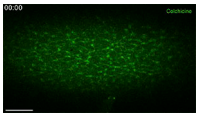
Video 2. **GFP::CLIP170 puncta colocalize with apical myosin.** Embryo expressing GFP::CLIP170 (green) and Myo::mCH (magenta). Images were acquired every 1.9 s, and the video is displayed at 15 frames per second. Scale bars, 15 µm.



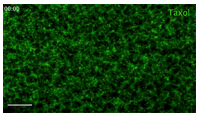
Video 3. **Patronin medioapical foci in mesoderm cells form by myosin contraction.** Embryo expressing Patronin::GFP (green) and Myo::mCH (magenta). Images were acquired every 1.9 s, and the video is displayed at 15 frames per second. Scale bars, 15 µm.



Video 4. **Colchicine and Taxol disrupt mesoderm invagination but does not interfere with apical constriction initiation.** Embryos expressing Myo::GFP (green) and Gap43::mCH (magenta) were injected with DMSO (top), colchicine (5 mg/ml; middle), or Taxol (5 mg/ml; bottom). Images were acquired every 6.4 s, and videos are displayed at 15 frames per second. Scale bars, 15 µm.



Video 5. **Microtubule disruption destabilizes intercellular actomyosin attachments.** Embryo expressing Myo::GFP was injected with colchicine (5 mg/ml). Images were acquired every 24 s, and the video is displayed at 15 frames per second. Scale bar, 15 µm.



Video 6. **The F-actin cortex exhibits longer-lived fractures and separations from junctions after microtubule disruption.** Embryo expressing Utr::GFP was injected with Taxol (5 mg/ml). Images were acquired every 19.6 s, and the video is displayed at 15 frames per second. Scale bar, 15 µm.

Provided online is Table S1, showing a list of genotypes of fly stocks used in this study as well as specific crosses that generated the data.

Reference

Gergely, F., D. Kidd, K. Jeffers, J.G. Wakefield, and J.W. Raff. 2000. D-TACC: A novel centrosomal protein required for normal spindle function in the early *Drosophila* embryo. *EMBO J.* 19:241–252. https://doi.org/10.1093/emboj/19.2.241