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Supplemental Information

A Role for the Centrosome and PAR-3 in the Hand-Off of MTOC Function during Epithelial Polarization

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Supplemental Inventory

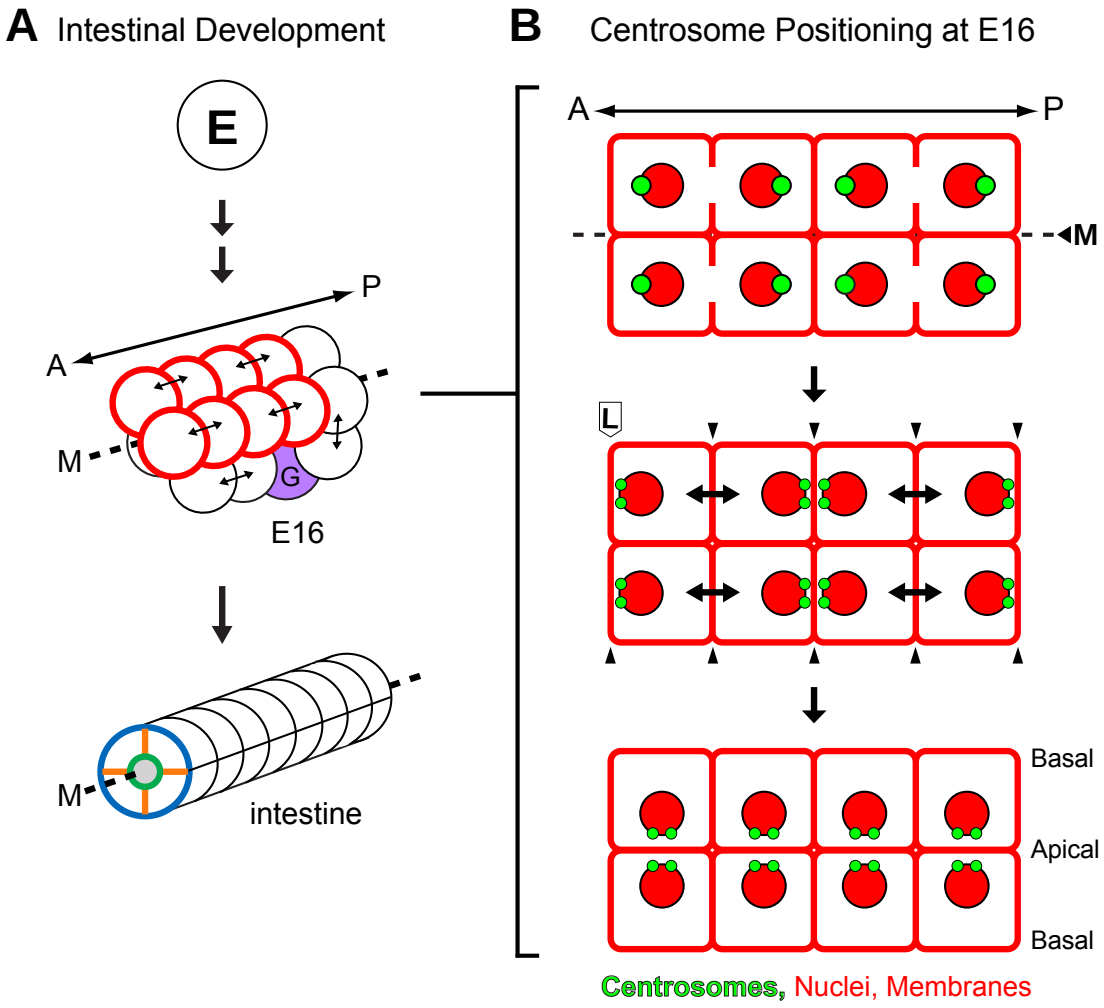

Figure S1. Cartoon of Intestinal Development and Centrosome Positioning, Related to Figure 1

(A) Cartoon of the E16 primordium, including the position of one of two germ cells (G) that insert between specific intestinal cells; during subsequent morphogenesis. Cells in the primordium divide, intercalate and change shape to form an elongated intestine; the most anterior and posterior cells undergo one additional division to generate the 20-cell intestine. In cross section, each of the intestinal cells appears wedge-shaped with a small, midline-facing surface (green), a broad peripheral surface (blue), and two lateral surfaces (orange). The midline-facing surfaces differentiate into apical membranes with microvilli and adherens junctions, and the peripheral surfaces differentiate into basal membranes associated with a basal lamina.

(B) The diagram at right summarizes polarization events in eight of the dorsal (top) E16 cells (red outlines), beginning with their birth at the E8 to E16 division; a similar orientation was used for most images in the main text. Centrosomes are shown in green and nuclei in red. Lateral membranes (“L”) are indicated by triangles.

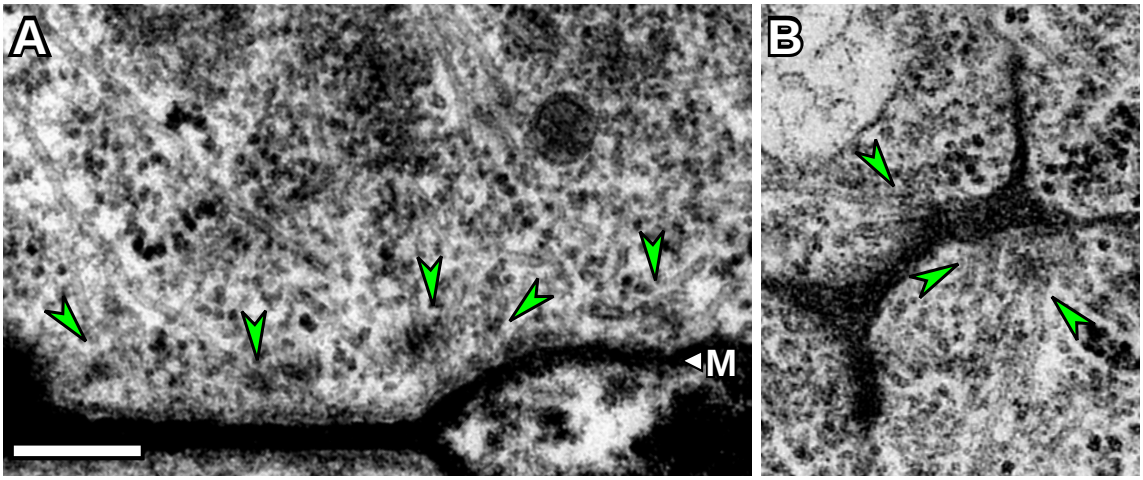

Figure S2. Association of Microtubules with the Apical Surface, Related to Figure 2

(A and B) Electron micrographs of the midline-facing, future apical membranes of wild-type E16 cells. Note that the ends of numerous microtubules (green arrowheads) approach or contact electron dense material near the apical membrane (M). At this stage, the paired centrosomes have migrated near the apical membrane, but show little or no association with microtubules. Scale bar = 200 nm.

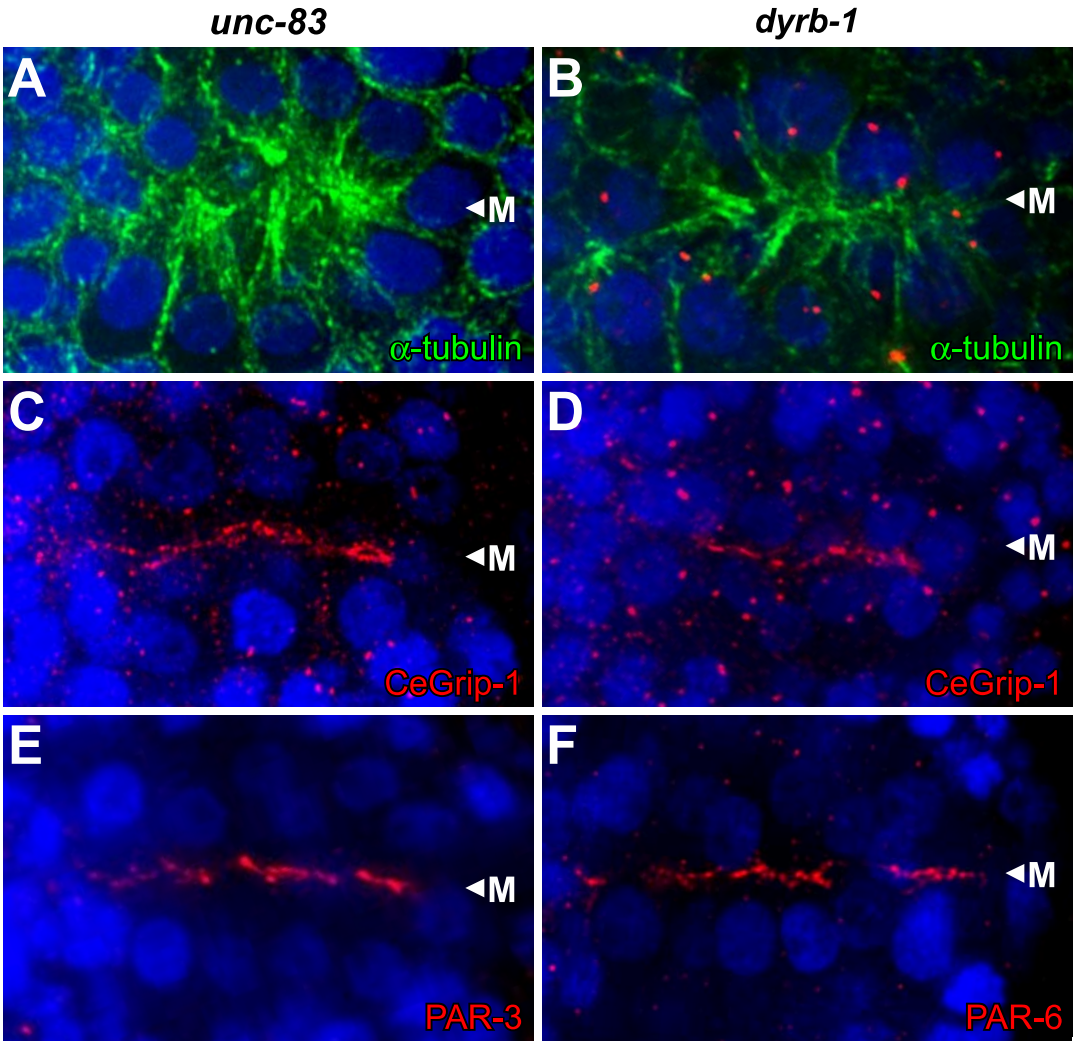

Figure S3. Apical Differentiation in *unc-83* and *dyrb-1* Mutants, Related to Figure 2 and 3

Each panel shows an optical section through the midline of the polarized E16 intestinal primordium.

(A and B) *unc-83* and *dyrb-1* embryos fixed and stained for microtubules (green, α-tubulin), centrosomes (red in panel B, IFA1), and nuclei (blue, DAPI). Note distance of the centrosomes/nuclei from an apical surface that is highly enriched in α-tubulin.

(C-F) *unc-83* (C and E) and *dyrb-1* (D and F) embryos fixed and stained for nuclei (blue, DAPI) and for either CeGrip-1, PAR-3, or PAR-6 (red, as indicated). The indicated proteins show proper apical localization, although the nuclei and centrosomes fail to localize apically.

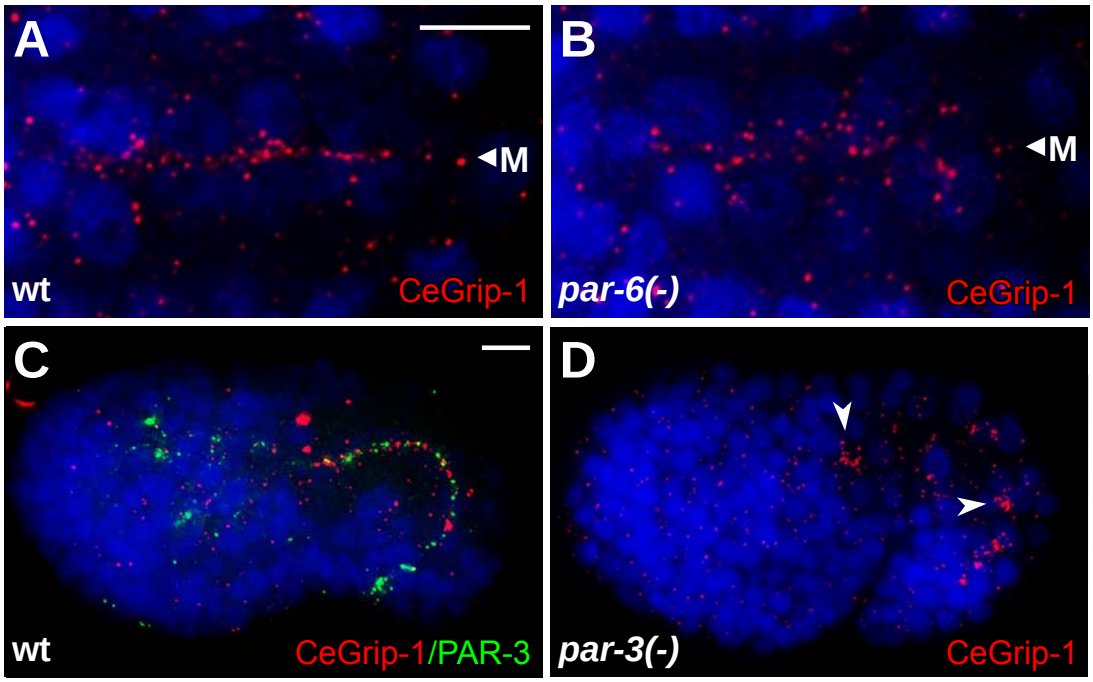

Figure S4. PAR-3, But Not PAR-6, Is Required for Apical CeGrip-1 Localization, Related to Figure 4

Panels are optical sections through the E16 primordium (A-B) and through entire embryos at later morphogenesis (C-D). Embryos are stained as indicated for CeGrip-1 (red) and PAR-3 (green). Panels A and C are of wild-type (wt) embryos, and panels B and D are embryos with stage-specific depletion of PAR-6 or PAR-3 (see Experimental Procedures). For the experiments represented by panels A and B, it was necessary to infer the genotype of the depleted embryos indirectly (see Experimental Procedures). 220/220 embryos scored had apical localization of CeGrip-1, but a subset appeared to have a diffuse distribution of apical CeGrip-1 (B).

(D) Note aberrant foci of CeGrip-1 in the PAR-3-depleted embryo (arrowheads).

Scale bar = 5 μm

Supplemental Experimental Procedures

Immunofluorescence

Embryos were fixed and stained as previously described [17]. Briefly, embryos of the appropriate stage were collected and adhered to a poly-lysine coated slide with a Teflon spacer and covered with a coverslip. Embryos were fixed by freeze-crack followed by 100% MeOH for 5 to 10 minutes. Embryos were rehydrated in PBS and incubated with primary antibody overnight at 4°C. The primary antibodies used in this study were α-GFP (Abcam), α-CeGrip-1 (provided by A.H. [1]), α-α-tubulin (Harlan Laboratories), α-IFA [S1], α-PAR-6 [S2] (provided by A.H.), α-ZYG-9 [15] (provided by P.G.), α-TAC-1 [15] (provided by P.G.), α-AIR-1 [14] (provided by K.O.), α-phospho-AIR-1 [30] (provided by A.S.), α-SPD-5 [16] (provided by B.B.), and α-PAR-3 [24]. Embryos were washed in PBT and incubated with the appropriate Alexa 488-, FITC-, CY3-, and/or Cy5-conjugated secondary antibodies (Invitrogen and Jackson Immunoresearch Laboratories). Embryos were incubated with 100 ng/mL DAPI (Sigma) and mounted under coverslips using DABCO (Sigma). Unless indicated, at least 25 mutant embryos were scored for analysis of mutant phenotypes.

Microscopy

Fixed images were obtained using a 100x objective (N.A.= 1.4) on a Deltavision microscope and processed using Softworx (Applied Precision). For live imaging, samples were mounted on a pad made of 3% agarose dissolved in M9. Live imaging was performed on a Nikon TE2000-E (Nikon Instruments), using a 60x objective (NA=1.4) and controlled by Volocity software (Improvision). Images were acquired with a Hamamatsu C9100-13 EM CCD camera using 491 nm or 561 nm lasers and a Yokogawa CSU-10 confocal spinning disc head equipped with a 1.5x magnifying lens. For live imaging, 10 μm stacks of images were taken at a sampling rate of 0.5 μm. Images were processed in Volocity, ImageJ, or Adobe Photoshop. Fluorescence intensities were measured using ImageJ.

Electron Microscopy

Embryos were prepared for electron microscopy as previously described [S3]. Embryos of mixed stages were fixed together and E8 to E16 embryos were selected under the dissecting microscope and processed for further analysis.

Centrosome Ablation (continued)

Ablation depletes the centrosomal γ-tubulin:GFP, and should non-specifically deplete some fraction of the non-centrosomal pool. CT cells were selected for analysis that showed minimal non-specific photobleaching, determined as follows. Integrated density measurements were made of cytoplasmic γ-tubulin:GFP levels using 5x5 pixel boxes. Twenty boxes were measured throughout the intestine at t=0 to establish variation in baseline values. The standard deviation of these measurements was calculated as percent difference from the mean (ranging between 1.3 to 4.4%). Measurements were made in the CT cell, its sister and/or an adjacent non-CT cells at t=0, directly following ablation, and again after γ-tubulin appeared at the midline of non-CT cells. CT cells

were not included for further analysis if photobleaching reduced their level of cytoplasmic γ-tubulin:GFP two standard deviations or more from that of control cells. Apical γ-tubulin enrichment was determined by measuring the integrated density of 5x2 pixel boxes near the apical membrane and near the basal membrane. Comparison of means was performed using a two-tailed Student's t-test in Excel. Error is shown as the standard deviation of the mean.

Supplemental References

S1. Pruss R.M., et al. (1981). All classes of intermediate filaments share a common antigenic determinant defined by a monoclonal antibody. Cell. 27, 419-2

S2. Schonegg S, Hyman AA. (2006). CDC-42 and RHO-1 coordinate acto-myosin contractility and PAR protein localization during polarity establishment in C. elegans embryos. Development.133, 3507-16.

S3. Pitt, J.N., Schisa, J.A., Priess, J.R. (2000). P granules in the germ cells of Caenorhabditis elegans adults are associated with clusters of nuclear pores and contain RNA. Dev Biol. 219, 315-33.