Developmental Cell

Supplemental Information

Square Cell Packing in the *Drosophila* Embryo through Spatiotemporally Regulated EGF Receptor Signaling

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Supplemental Figures and legends

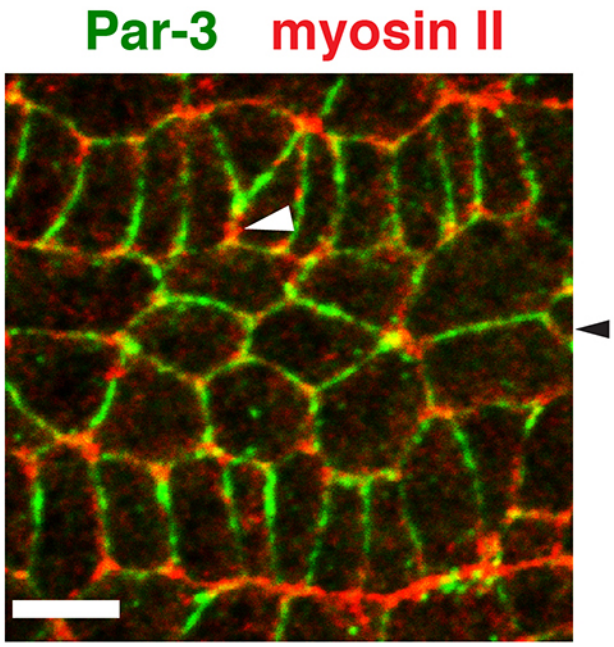

Figure S1 (related to Figure 2). Localization of Par-3 and myosin II in Phase I.
Par-3 (green) is enriched at cell interfaces oriented perpendicular to the ventral midline in Phase I and myosin II (red) is enriched at interfaces oriented parallel to the midline (stage 11). White arrowhead, myosin II accumulation at a contracting cell interface. Black arrowhead, ventral midline. Bar, 5 μm.

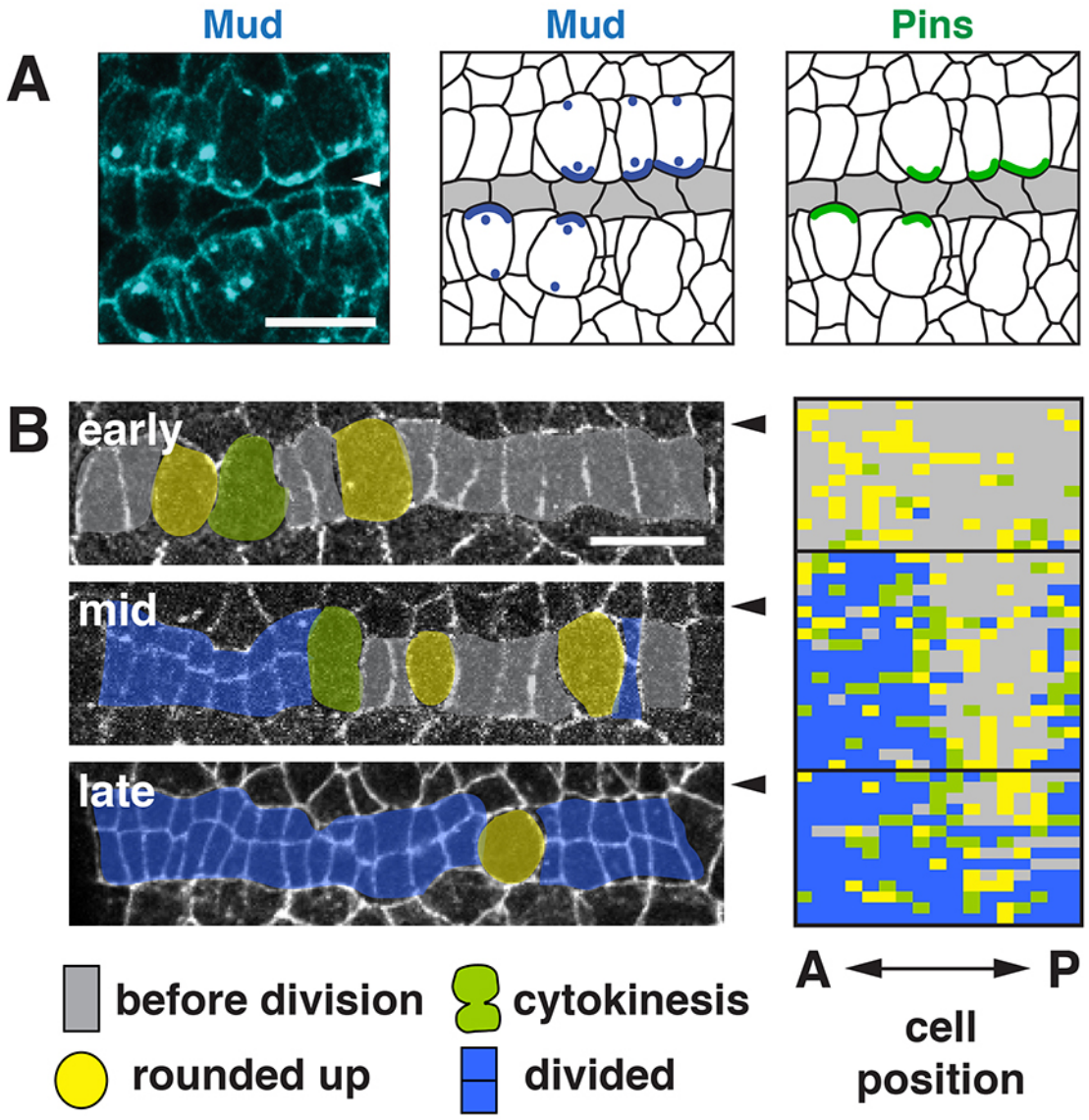

Figure S2 (related to Figure 3). Spatiotemporal regulation of cell division during square grid formation. (A) Mud/NuMA accumulates at cell interfaces contacting midline cells and was also present at centrosomes (stage 12) (left). Pins (right) and Mud (middle) asymmetrically accumulate at cell interfaces contacting midline cells within dividing cells. (B) Distribution of mitoses in fixed embryos. Left: Embryos at early stage 12, mid-stage 12, and late stage 12. Right: Each rectangle represents one cell colored according to its division status, each row shows 17 cells on one side of the midline in a single embryo (n = 48 rows from 24 Phase II embryos). Cell divisions initiate in an anterior region and proceed toward the posterior and extreme anterior regions. Arrowheads, ventral midline. Bars, 10 μm.

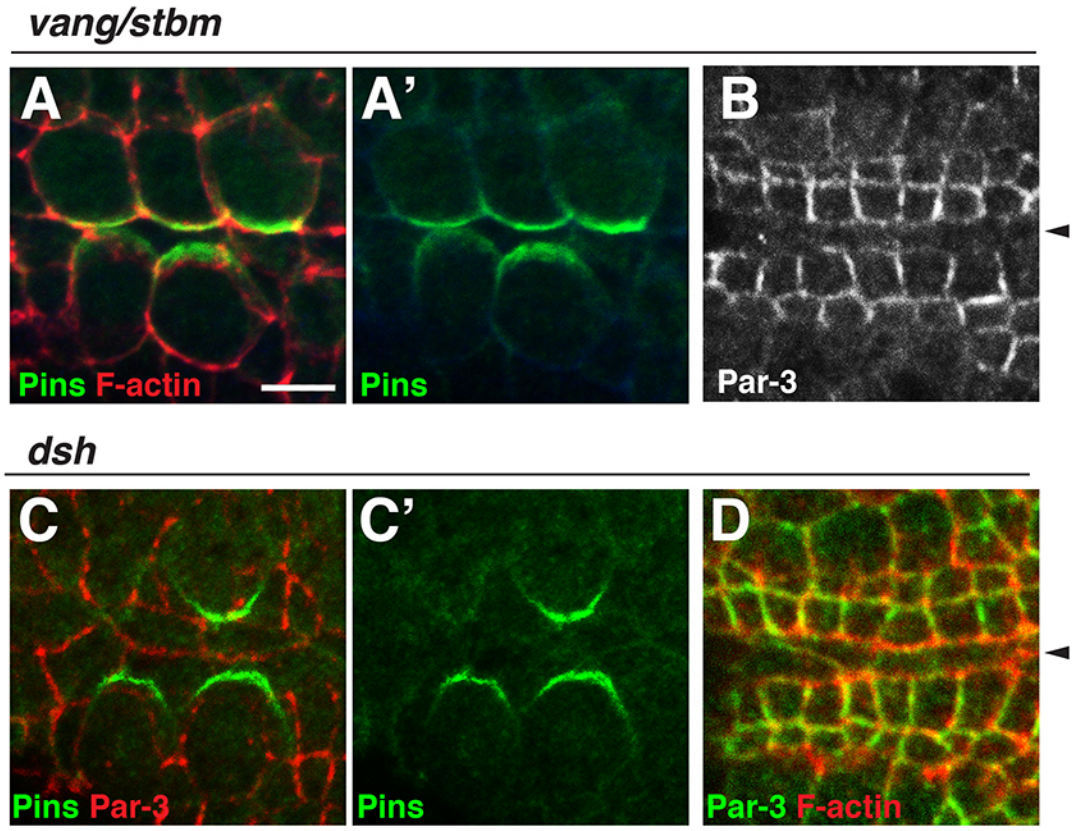

Figure S3 (related to Figure 4). Pins asymmetry and square cell grid formation are independent of the core planar cell polarity (PCP) pathway. (A and C) Asymmetric accumulation of Pins/LGN was detected at interfaces contacting midline cells in *strabismus* (green in A and A') and *dishevelled* mutants (green in C and C') (stage 12). (B and D) The square cell grid formed correctly in *strabismus* (B) and *dishevelled* mutants (D) (stage 13). Bar, 5 μm.

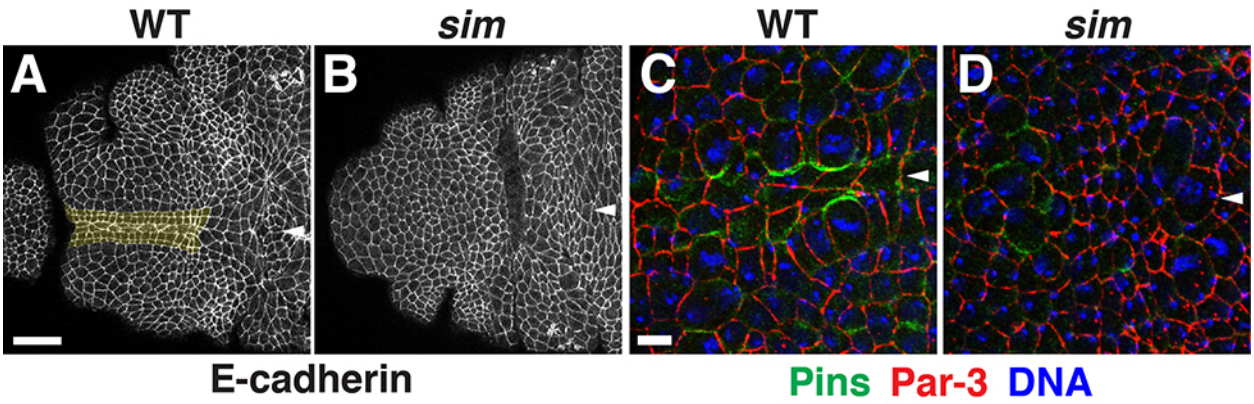

Figure S4 (related to Figure 5). A midline signal is required for Pins asymmetry and square grid formation. (A,B) The square cell grid does not form in the absence of midline cells in *sim* mutants (stage 13). (C,D) Pins asymmetry was not observed in anterior ventral cells in *sim* mutants (stage 12). Arrowheads, ventral midline. Bars, 20 μm (A,B), 5 μm (C,D).

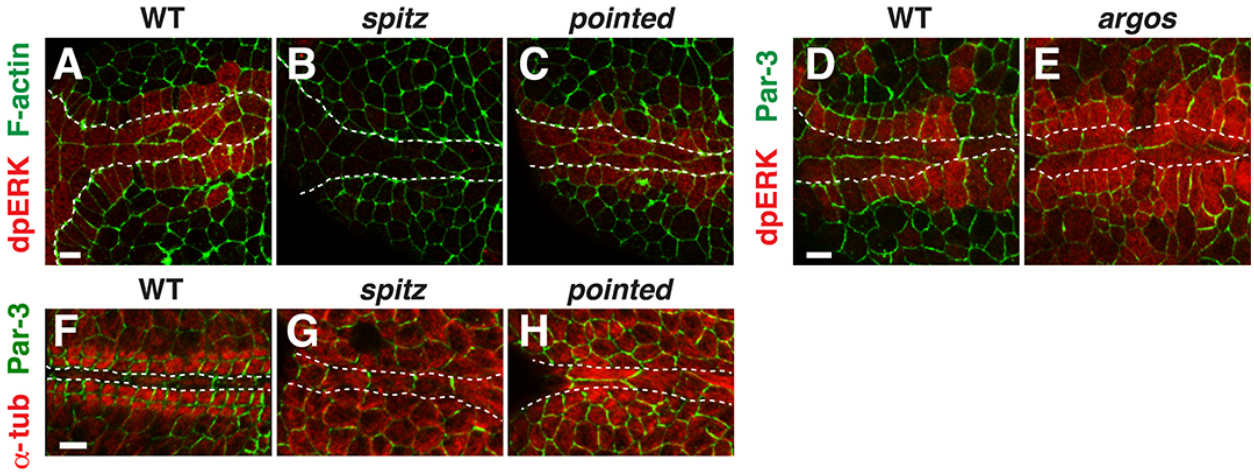

Figure S5 (related to Figure 5). EGFR signaling in the square cell grid. (A-C) dpERK signal, an indicator of MAP kinase activity, is increased in cells adjacent to the ventral midline in WT and *pointed* mutants and is absent in *spitz* mutants (stage 11-12). (D,E) dpERK signal expanded to lateral cells in *argos* mutants (stage 12). (F-H) Microtubule reorganization in the square cell grid failed to occur in *spitz* and *pointed* mutants (stage 13). Dashed lines show boundaries between midline cells and square grid cells. Bars, 5 μm.

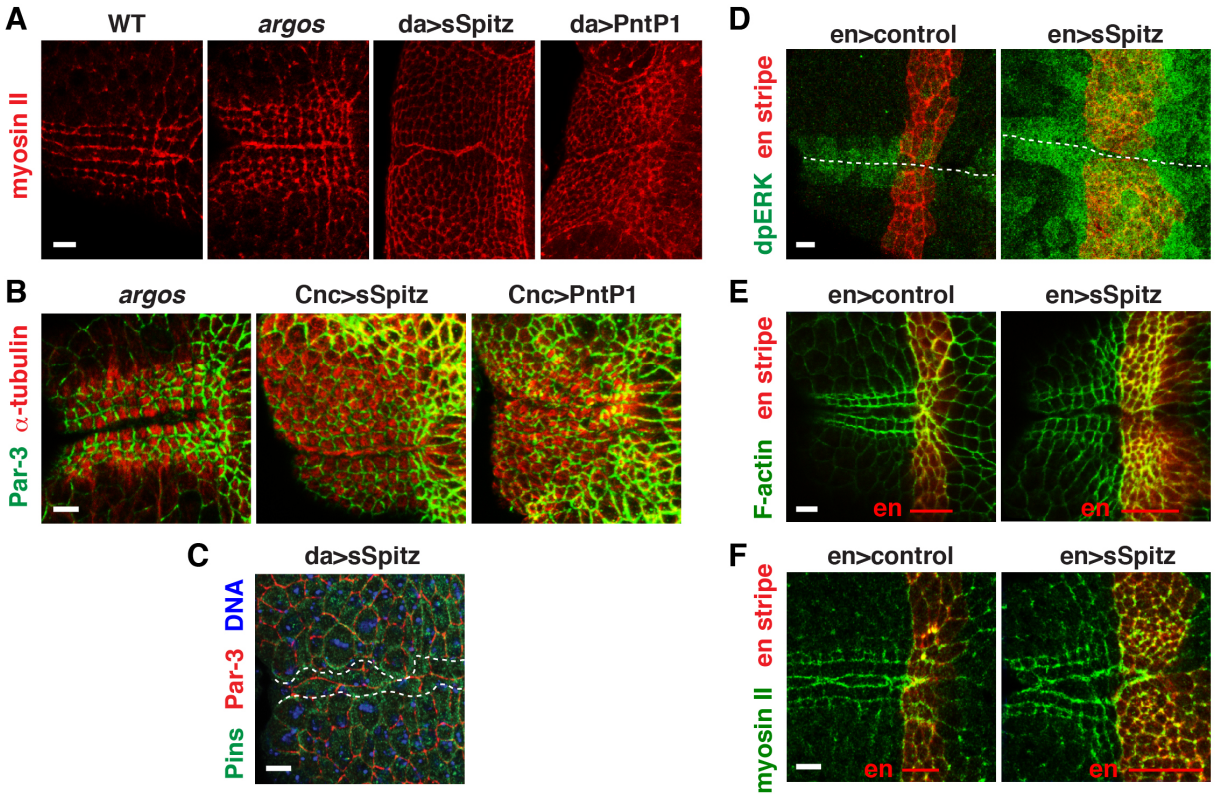

Figure S6 (related to Figure 7). Ectopic EGFR signaling alters cell shape and polarity. (A) Expanded EGFR signaling induces a broader region of square-like cells in *argos* mutants and in embryos that ubiquitously express secreted Spitz (sSpitz) or constitutively active Pointed P1 (PntP1) with the da-Gal4 driver. (B) Ectopic EGFR signaling induces apicobasal microtubule reorganization in *argos* mutants and in embryos expressing sSpitz or PntP1 with the Cnc-Gal4 driver. Similar results were obtained with da-Gal4 (data not shown). (C) Pins localized weakly and uniformly to the cortex of dividing cells in embryos expressing sSpitz with the da-Gal4 driver. (D) dpERK signal in a control (en>mCherry:Moesin) embryo (left) and in an embryo expressing sSpitz (right) in an ectopic stripe (red) with the engrailed-Gal4 (en-Gal4) driver. dpERK is high within and adjacent to the ectopic stripe of sSpitz expression. (E,F) F-actin (E) and myosin II (F) localization were increased at cell interfaces in 2-3 rows of cells anterior to the anteriormost engrailed stripe in embryos expressing sSpitz with the en-Gal4 driver. Stage 13 embryos shown in D-F. Engrailed stripes were visualized by coexpression of mCherry:Moesin (red). Dashed lines in C and D indicate boundaries between midline cells and square grid cells. Bars, 5 μm.

Supplemental Movie legends

Movie S1, related to Figure 1. Time-lapse confocal movie of Phase I in a wild-type embryo expressing β-catenin:GFP. Images were acquired at 1 min intervals and apical junctional planes were projected for analysis. Ventral view, anterior left.

Movie S2, related to Figure 1. Time-lapse confocal movie of Phases II and III in a wild-type embryo expressing β-catenin:GFP. Images were acquired at 1 min intervals and apical junctional planes were projected for analysis. Ventral view, anterior left.