Supplemental Materials

Molecular Biology of the Cell

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Supplementary Figure Legends

Supplementary Figure S1 (A) Single confocal section of purple urchin embryo expressing wt 3xGFP SpEct2 (white) and H2B-mCh (red). (A′) Arrowheads indicate autofluorescent granules, which were subtracted to obtain sequence series (A). Times are min:sec after filming began. (B) Single section of starfish blastula expressing wt 3xGFP SpEct2 showing Ect2 localization at all stage of mitosis. (B′) Time lapse series, region from B, of one cell going through mitosis. (C) Time lapse series of dividing HeLa cell stably expressing AcGFP-hEct2. Times are min:sec after anaphase onset. (D) Projection of 4 1-µm sections of a sand dollar embryo injected with mRNA encoding 3xGFP SpEct2 GEF4A. Numbered regions (dotted outlines) were used to generate kymographs D1-3.

Supplementary Figure S2 (A) Single confocal section of 32-cell purple urchin embryo expressing wt 3xGFP SpEct2 (white) and H2B-mCh (red). To clearly show the Ect2 rind, autofluorescent granules were subtracted as in Supplementary Figure S1, A. Times are min:sec after filming began; numbers indicate cells used for measurements. At the start of filming, one cell (lower left of center) had started to cleave, and displayed a clear cortical Ect2 accumulation; other cells did not, but acquired an Ect2 rind shortly before furrowing. (B) All measurements of time between development of Ect2 rind and furrow initiation, versus cell diameter (measured pole-to-pole prior to elongation), in purple urchins (purple circles), sand dollars (green triangles), and starfish (orange diamonds). Shaded points are from swimming-stage blastulas; bold points are the cells shown in (A). Only cells that cleaved without overt abnormalities were included in this analysis. (C) Sample kymograph illustrating the measurement of ladder extension, from the cell indicated in the inset (C′; one cell in a 32-cell sand dollar embryo expressing 3xGFP Ect2 GEF4A): red lines extrapolate the pre-furrow cell diameter, the extension of the ladder from spindle midzone, and furrow progression; the relative intersection of these lines gives rise to the data points in (D). This particular case shows that the ladder reaches the cortex on the left side two time points (48 sec.) before furrow ingress begins, but one time point after on the right side. (D) All measured times between full ladder extension and furrow onset: the overwhelming majority of data points are to the left of the furrow initiation time; all of the points to the right (8 cases) represent instances like (C) in which the ladder reaches one side of the cell after furrowing begins, but in which the ladder was complete on the other side at the time of furrowing. Only cells that cleaved completely and in a timely fashion were used for this analysis.
Video legends

**Video 1.** Wild-type 3xGFP SpEct2 expressed in 32-cell sand dollar embryo, along with mCh-Histone H2B. This embryo has cleaved successfully and displays no overt errors, but exhibits more blebbing and surface contractility than normal. Corresponds to Figure 1, B. Single optical section, 8 sec/frame, 15 fps.

**Video 2.** 3xGFP SpEct2 GEF4A expressed in 16-cell sand dollar embryo, along with 2xmCh EMTB. At this level of expression no delays or overt defects in cell division are apparent. Corresponds to Figure 1, C. Single optical section, 6 sec/frame, 30 fps.

**Video 3.** 3xGFP SpEct2 GEF4A expressed in sand dollar embryos: projections from 16-cell (segment 1; 6 1 µm sections), 32-cell (segment 2; 7 1 µm sections) and ~64-cell (segment 3; 4 1 µm sections) embryos. Segment 3 corresponds to Supplementary Figure S1, D. These show no signs of cleavage abnormalities. Z projections emphasize the spread of Ect2 from midzone to astral microtubules; segment 2 includes two axial views, showing how Ect2 on astral microtubules apparently sweeps into the midbody. All segments recorded at 12 sec/frame, played back at 15 fps.

**Video 4.** Wild-type 3xGFP SpEct2 expressed in 16-cell sand dollar embryo, along with mCh-Histone H2B. This embryo is cleaving successfully, but most cells are abnormal, in that they display episodic ectopic contraction outside the furrow region, blebbing, and large-scale shifts by the spindle. Corresponds to Figure 2, A. Single optical section, 8 sec/frame, 15 fps.

**Video 5.** 3xGFP SpEct2 GEF4A expressed in sand dollar embryos at levels which inhibit cytokinetic furrowing. Segment 1 is one binucleate cell within a four-cell embryo (projection of 16 1 µm sections; corresponds to Figure 3, A). One spindle is almost parallel to the focal plane, the other (left) is nearly perpendicular. Segment 2 shows an embryo that failed second cleavage; in the two binucleate cells, both spindles are parallel to the focal plane. In the next division attempt, all four spindles in both cells are perpendicular to the focal planes, and the Z-series contains all spindle midzones. In both sequences, Ect2 accumulates on the midzone first, then appears to stream along astral rays toward the cell surface, accumulating at points where asters intersect.

**Video 6.** Wild-type 3xGFP SpEct2 expressed in starfish embryos, one of eight cells, along with 2xmCh EMTB. This sequence highlights the progressive accumulation of Ect2 on astral microtubules in the cytoplasmic disc that unites midzone with equatorial surface. Although these cells successfully cleave at this level of excess Ect2, they exhibit excessive surface motility during cleavage. Corresponds to Figure 4, A. Single optical section, 6 sec/frame, 20 fps.

**Video 7.** 3xGFP SpCyk4 expressed in starfish embryos. Both segments show one of 16 cells. Segment 1 is a single optical section and highlights the appearance of Cyk4 on individual equatorially-directed astral rays as cleavage commences. Segment 2 is a projection of 4 1.5 µm sections, alongside a surface plot made using Kai Uwe Barthel's
ImageJ plug-ins, to highlight the bulk traffic of Cyk4: at the beginning of anaphase, Cyk4 is uniformly distributed in the cytoplasm; as anaphase proceeds, Cyk4 not only accumulates on the midzone but also in a peripheral rim as it vacates the asters; then Cyk4 accumulates equatorially, both in prominent peaks that correspond to an equatorial annulus, and also more faintly in the cytoplasmic disc between midzone and cell surface. Corresponds to Figure 4, C. Collected at 7 and 15 sec/frame, respectively, and played back at 15 fps.

**Video 8.** Rho zones in normal versus Ect2-expressing sand dollar embryos. Segments 1 and 2 are normal 8-cell and 16-cell embryos; segment 3 is an embryo that is expressing 3xGFP wt SpEct2 and cleaving normally but with broader and brighter Rho zones than normal (segments 2 and 3 correspond to Figure 5, A); segment 4 is a similar embryo which exhibits dramatic cortical hypercontractility in response to excess Ect2 (corresponds to Figure 3, B). Projections of 15-20 1 µm sections at time intervals indicated, played back at 15 fps.

**Video 9.** Ect2 accumulation in secondary furrows. Starfish zygote (segment 1) or one of four cells (segment 2) perforated with a ~50 µm glass bead, then filmed through two mitoses. Corresponds to Figure 7, A and B; gold=3xGFP wt SpEct2, cyan=2xmCh EMTB. Segment 1: single section, 12 sec/frame, 30 fps; segment 2: projection of 4 sections, 30 sec/frame, 15 fps.

**Video 10.** Cyk4 accumulation in secondary furrows in toroidal cells. Starfish blastomeres expressing 3xGFP SpCyk4 were perforated with a ~50 µm glass bead, then filmed through two mitoses. Segment 1 (one of two cells) corresponds to Figure 7, C, and is a projection of 6 1.5 µm sections recorded at 30 sec/frame. Segment 2 (one of four cells) is a single optical section recorded at 12 sec/frame. Segment 3 (one of eight cells) is a projection of 5 1.5 µm sections recorded at 20 sec/frame. All are played back at 20 fps.

**Video 11.** Cyk4 accumulation in the cleavage furrows of anucleate cytoplasts. Both segments show the first cleavage attempt of a cytoplast that resulted from bisection of a starfish zygote expressing 3xGFP SpCyk4; correspond to Figures 8, A and B. Segment 1 is a projection of 6 1.5 µm sections, and emphasizes the recruitment of Cyk4 to astral microtubules throughout the cell, migration toward the periphery, followed by accumulation in an equatorial annulus, just as in an intact cell. Segment 2 is a single section and emphasizes the persistence of individual Cyk4-decorated astral microtubules in association with the cleavage furrow and forming midbody. 20 fps.
Su et al. Supplemental Figure 1
