

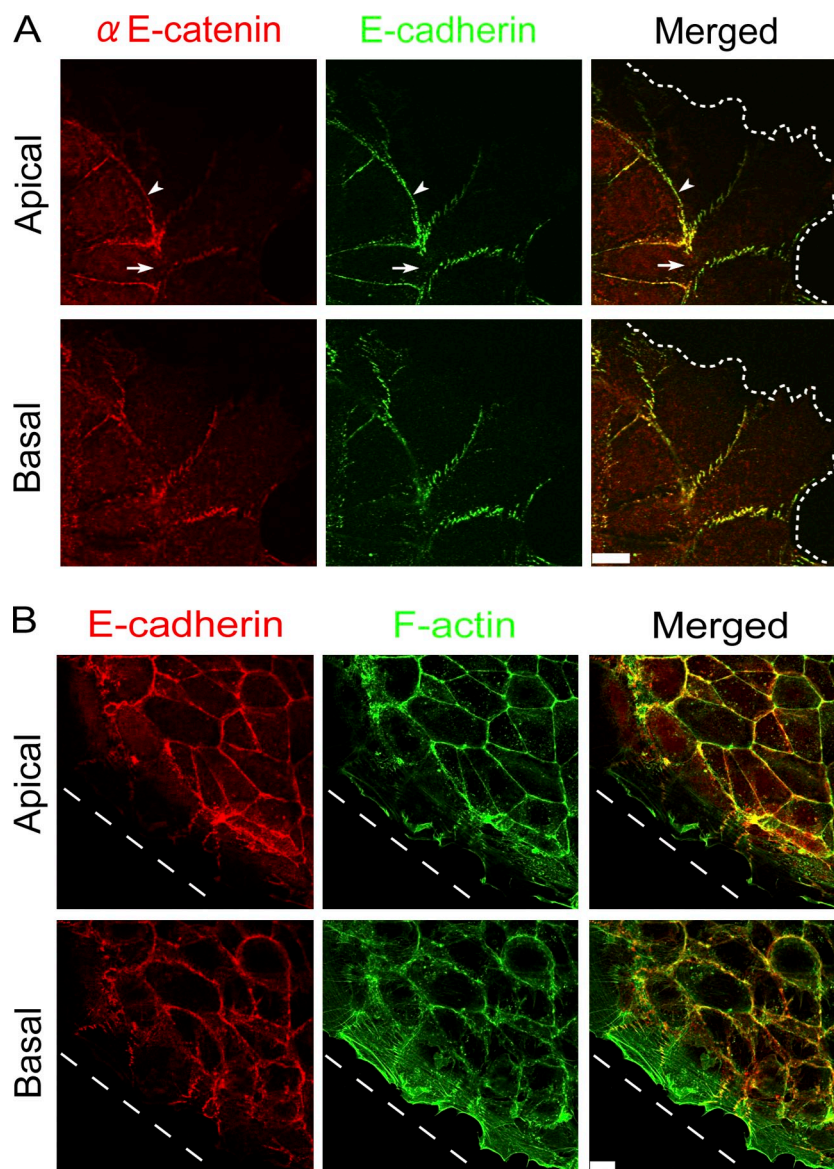
Taguchi et al., <http://www.jcb.org/cgi/content/full/jcb.201104124/DC1>

Figure S1. **Peripheral junction organization.** (A) Closed (arrowhead) or open (arrow) ZAs seen in peripheral cells of a DLD1 colony. Cells were double-immunostained for α E-catenin and E-cadherin. Dotted lines delineate the colony edges. (B) An edge of a DLD1 colony, created by cell scraping. Cells were double-immunostained for E-cadherin and F-actin at 3 h after the scraping. Broken lines indicate the initial position of cell removal. Note that the junctional organization in these cells is similar to that observed in natural DLD1 colonies. Images were focused on the apical and basal planes of the colony. Bars, 10 μ m.

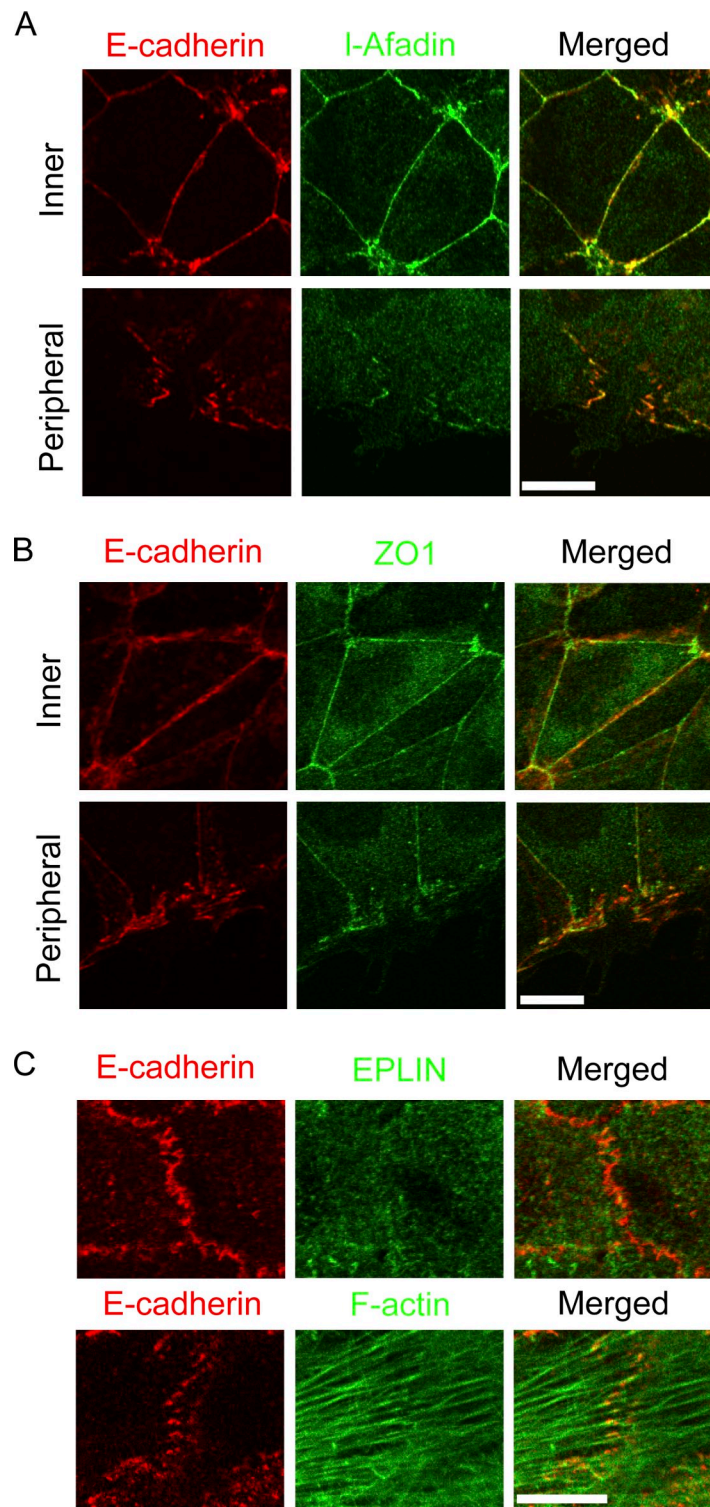


Figure S2. **Localization of various junctional proteins.** (A and B) Double-immunostaining for E-cadherin and I-Afadin or ZO-1 in DLD1 cells. Both proteins colocalize with E-cadherin in any forms of junction. (C) Double-immunostaining for E-cadherin and EPLIN in an inner portion of a MCF10A cell colony. In these cells, the entire junctions are identified as pAJs, in which EPLIN does not colocalize with E-cadherin. Bars, 10 μm .

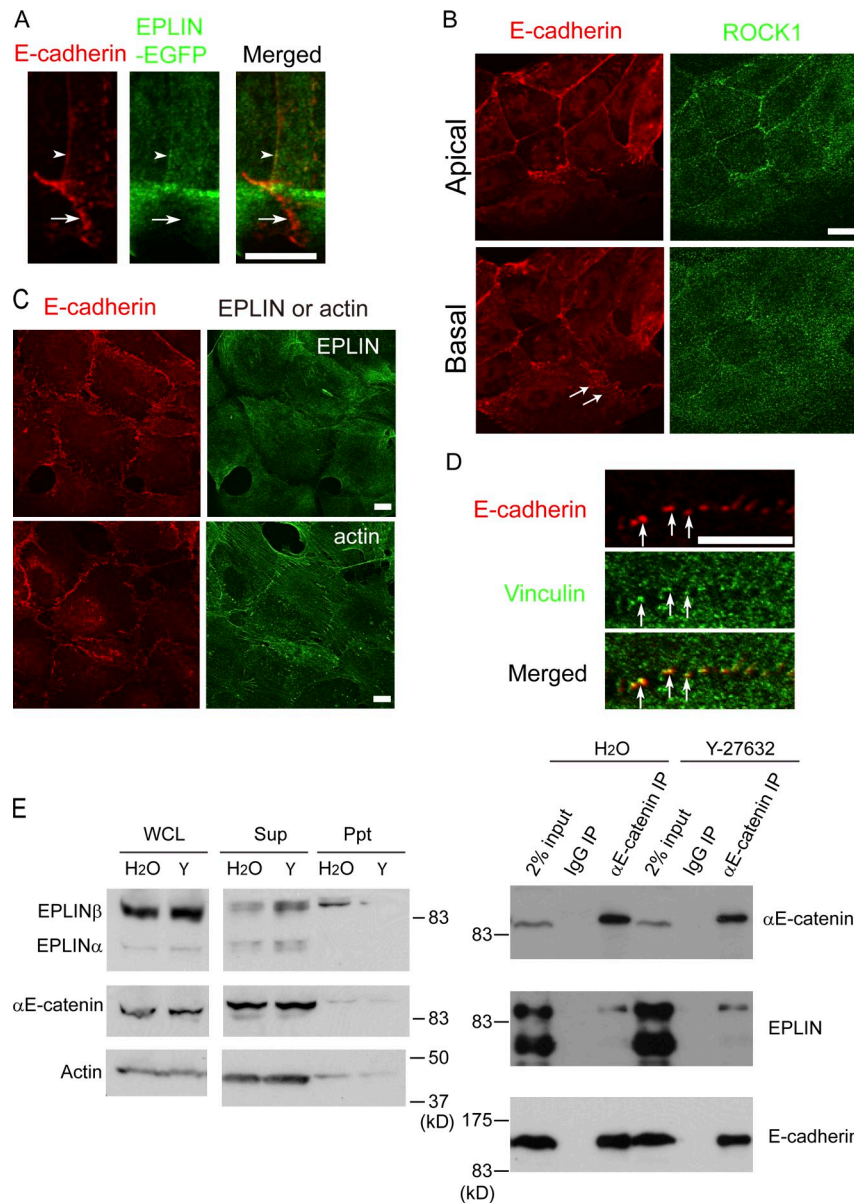


Figure S3. Analyses of EPLIN-AJ associations. (A) Exogenous EPLIN-EGFP is excluded from pAJs in DLD1 cells transfected with EPLIN-EGFP. EGFP signals are detected together with E-cadherin along IAJs, as indicated with arrowheads, but not at pAJs (arrows). (B) A DLD1 colony was double-immunostained for E-cadherin and ROCK1. Confocal images were collected at the apical and basal planes. ROCK1 is localized along the IAJs, but not at pAJs (arrows). (C) Cells were treated with 20 μ M of Y-27632 for 3 h, and double-immunostained for E-cadherin and EPLIN or F-actin. Junctional morphology became like the pAJs, and EPLIN disappeared from the junctions. (D) Vinculin localizes at cell junctions after Y-27632 treatment (examples are indicated with arrows). (E) Immunoprecipitation analysis of the interaction between α E-catenin and EPLIN before and after Y-27632 treatments. (E, left) The detergent solubility of EPLIN. Cells were cultured in the presence or absence of 20 μ M Y-27632 for 3 h, and their lysates, prepared with 1% NP-40, were centrifuged to obtain the supernatant (Sup) and pellet (Ppt) fractions. Densitometric analysis of the electrophoretic bands showed that the soluble fraction of EPLIN increased two-fold after Y-27632 treatment. WCL, whole cell lysate. (right) α E-catenin was immunoprecipitated from the 1% NP-40 lysates. EPLIN was then detected from the blots of the immunoprecipitates. A similar amount of EPLIN was coprecipitated with α E-catenin from the lysates of control and Y-27632-treated cells. Bars, 10 μ m.

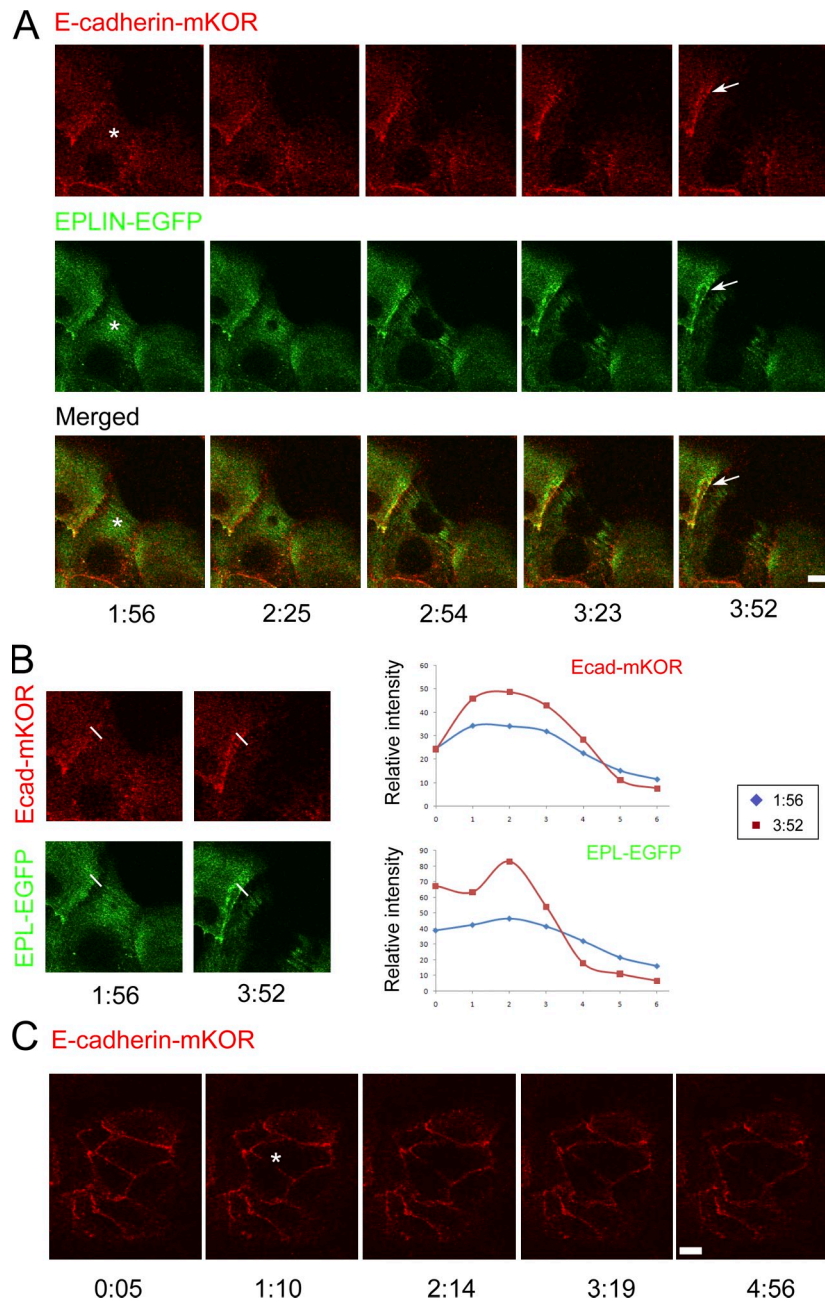


Figure S4. **Effects of laser ablation of peripheral actin fibers or ZA.** (A) Cells double-transected with E-cadherin-mKOR and EPLIN-EGFP were treated as described in Fig. 3 C. Time-lapse images are shown. See also Video 6. After laser ablation of peripheral actin fibers at the point shown by the asterisk, both EPLIN and E-cadherin became up-regulated at the cell-cell boundary (arrow). The cell-cell boundary at the opposite side of the cell is out of focus. (B) Choosing two time points in A, relative fluorescence intensity across the cell-cell boundaries marked with white lines was measured. EPL, EPLIN. (C) The ZA was cut at the position indicated by the asterisk in a cell colony expressing E-cadherin-mKOR. Scale bars, 10 μ m.

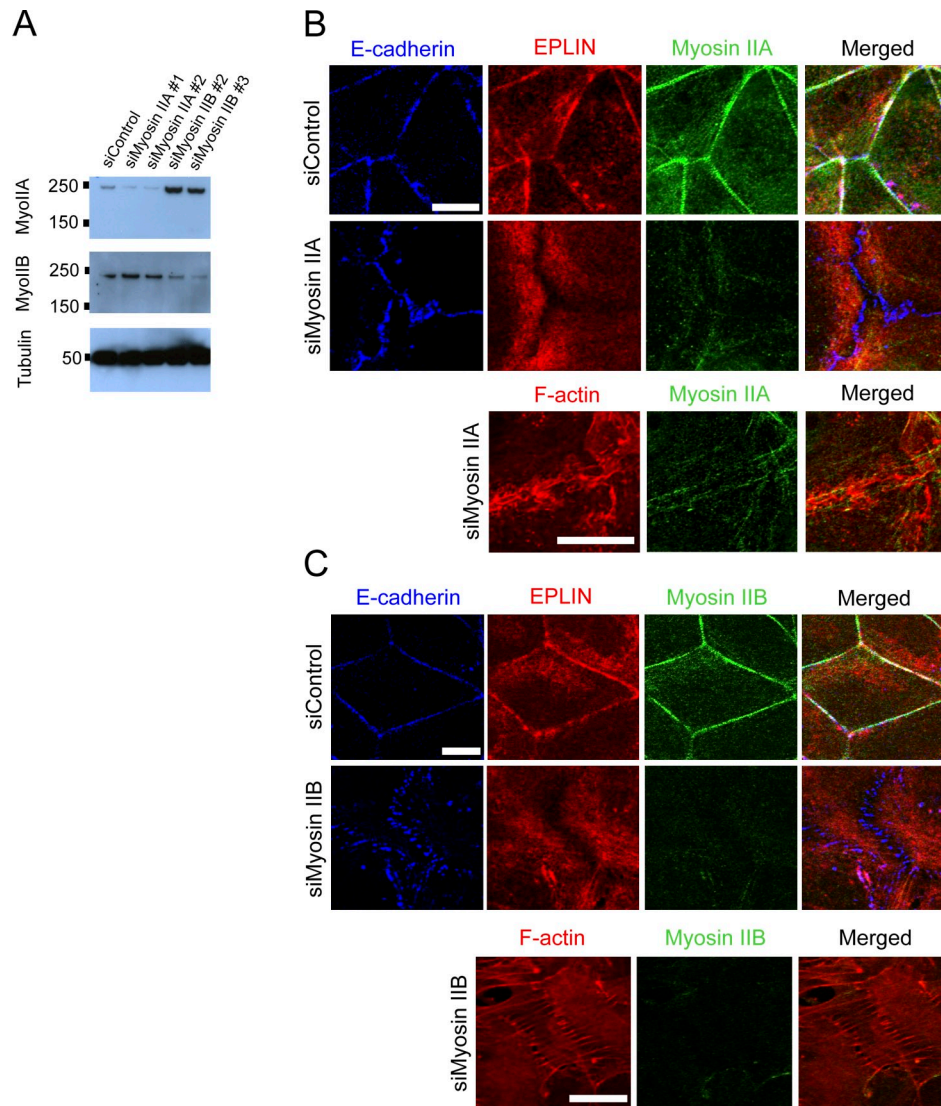
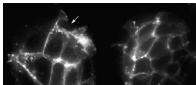
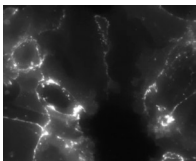


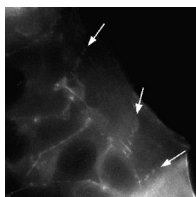
Figure S5. Effects of myosin IIA or IIB depletion on EPLIN localization. (A) Western blot confirmation of isoform-specific knockdown of myosin IIA or IIB. Molecular mass is indicated in kilodaltons next to the gel blot. (B and C) Cells treated with control siRNA, IIA-specific No. 2 siRNA (B), or IIB-specific No. 3 siRNA (C). These cells were costained for E-cadherin, EPLIN, and myosin II (A or B), or F-actin and myosin II (A or B). In both of IIA- and IIB-depleted cells, E-cadherin signals became punctate, and no longer colocalized with EPLIN. In the IIA-depleted cells, F-actin was irregularly arranged around the cell junctions, whereas, in the IIB-depleted cells, F-actin associated with E-cadherin signals in a pattern similar to that seen in EPLIN-depleted cells. Bars, 10 μ m.

Video 1. Time-lapse movie of E-cadherin-mKOR in DLD1 colonies. Two representative DLD1 colonies expressing E-cadherin-mKOR. In the left colony, pAJs are dynamic and pulled in parallel to the cellular edge, as indicated by the arrow, whereas IAJs are relatively stable. The arrowheads in the same colony indicate a temporary closure of ZA remnant. In the right colony, whose peripheral cells exhibit closed E-cadherin rings, pAJs flow into the ring structures, indicated by two arrowheads. Images were analyzed by time-lapse fluorescence microscopy using a DeltaVision microscope (Applied Precision). Frames were taken every 2 min for 2 h, and those from 37:58 to 1:59:59 (left) or 37:55 to 1:59:55 (right) are shown. Frame rate, 7.0 frames/s. Bar, 30 μ m.

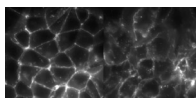


Video 2. Time-lapse movie of α E-catenin-EGFP during wound closure. A DLD1 culture expressing α E-catenin-EGFP was scratched to make an opening, and the process of wound closure was recorded. A peripheral cell initially has a closed α E-catenin ring (arrow), but this ring is soon disrupted. When this cell touches another cell coming from the opposite side, α E-catenin-EGFP signals with a pAJ-like distribution immediately accumulate at their contact sites (arrowhead). Images were analyzed by time-lapse fluorescence microscopy using a DeltaVision microscope (Applied Precision). Frames were taken every 1.5 min for 4 h, and those from 0:00:00 to 3:59:56 are shown. Frame rate, 7.0 frames/s. Bar, 32 μ m.

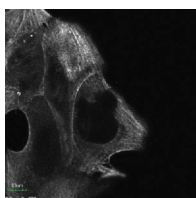




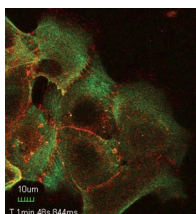
Video 3. **Time-lapse movie of actin-EGFP in DLD1 cells.** Cells were injected with actin-EGFP and recorded at a margin of their colony. Arrays of actin clusters (indicated by arrows), which are assumed to represent pAJs, move dynamically, and are stretched in parallel to the cellular edges. Some of these actin clusters move inward (e.g., see those indicated by the second arrow from the right). ZA-associated actin filaments are relatively more stable. Images were analyzed by time-lapse fluorescence microscopy using a DeltaVision microscope (Applied Precision). Frames were taken every 1.5 min for 3 h, and those from 0:00:00 to 2:14:54 are shown. Frame rate, 7.0 frames/s. Bar, 30 μ m.



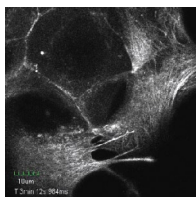
Video 4. **Time-lapse movies of E-cadherin-mKOR in a confluent culture of control or EPLIN-depleted DLD1 cells.** Cells transfected with E-cadherin-mKOR in a confluent culture were subjected to time-lapse imaging. Cells were treated with control (left) or EPLIN-specific siRNA (right). Images were analyzed by time-lapse fluorescence microscopy using a DeltaVision microscope (Applied Precision). Frames were taken every minute for 1 h, and those from 0:00 to 59:56 (left) or 0:00 to 59:55 (right) are shown. Frame rate, 7.0 frames/s. Bar, 30 μ m.



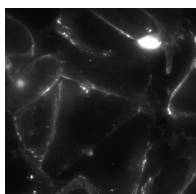
Video 5. **Time-lapse movie of EPLIN-EGFP after ablation of peripheral actin fibers.** The same movie as used in Fig. 3 B. Peripheral actin fibers were laser-ablated in a cell expressing EPLIN-EGFP. Frames were taken every 10.4 s with a FluoView FV1000 microscope (Olympus). 30 frames were obtained, and those from 0:00 to 5:11 are shown. Frame rate, 3.0 frames/s. Bar, 10 μ m.



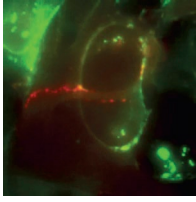
Video 6. **Time-lapse movie of EPLIN-EGFP and E-cadherin-mKOR after laser ablation of peripheral actin fibers.** DLD1 cells coexpressing EPLIN-EGFP and E-cadherin-mKOR were subjected to the same experiments as shown in Video 5. Both EPLIN and E-cadherin became concentrated at peripheral junctions after peripheral F-actin ablation. Frames were taken every 7.2 s with a FluoView FV1000 microscope (Olympus). 200 frames were obtained, and those from 1:12 to 3:52 are shown. Frame rate, 3.0 frames/s. Bar, 10 μ m.



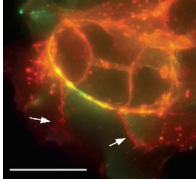
Video 7. **Time-lapse movie of EPLIN-EGFP after ablation of a part of the ZA.** The same movie as used in Fig. 3 D. ZA was laser-ablated in cells expressing EPLIN-GFP. The linear EPLIN signals are disorganized after laser treatment, although they reappear afterward. At junctions adjacent to the ablated junctions, EPLIN signals become transiently intensified. Frames were taken every 10.3 s with a FluoView FV1000 microscope (Olympus). 30 frames were obtained, and those from 0:00 to 5:10 are shown. Frame rate, 3.0 frames/s. Bar, 10 μ m.



Video 8. **Time-lapse movie of α E(1-508)EPLIN-EGFP introduced into R2/7 cells.** The fusion proteins are concentrated at cell-cell contact sites. However, these contacts appear unstable. Images were analyzed by time-lapse fluorescence microscopy using a DeltaVision microscope (Applied Precision). Frames were taken every 1 min for 3 h, and those from 0:00:00 to 2:59:58 are shown. Frame rate, 7.0 frames/s. Bar, 30 μ m.



Video 9. **Time-lapse movie of α E(1-508)-mKOR and α E(1-508)EPLIN-EGFP doubly introduced into R2/7 cells.** α E(1-508)-mKOR (red) localizes at all portions of cell-cell contacts, whereas α E(1-508)EPLIN-EGFP (green) mostly delineates the ZA-like ring structures. pAJ-like structures labeled with α E(1-508)-mKOR flow into the ring. Images were analyzed by time-lapse fluorescence microscopy using a DeltaVision microscope (Applied Precision). Frames were taken every 2 min for 3 h, and those from 1:39:28 to 2:59:04 are shown. Frame rate, 7.0 frames/s.



Video 10. **Time-lapse movie of α E(1-508)EPLIN-EGFP and E-cadherin-mKOR doubly introduced into DLD1 cells.** The same images as used for Fig. 5 C. α E(1-508)EPLIN-EGFP is localized along the ZA-like rings, and also diffusely in the cytoplasm at the marginal regions of the cells. E-cadherin-mKOR signals are initially detected at both pAJs and ZAs. However, the E-cadherin-mKOR signals forming pAJs are gradually absorbed into the α E(1-508)EPLIN-EGFP-positive rings, as indicated by the arrow. Also, portions of the ZAs are disrupted at later time points. Images were analyzed by time-lapse fluorescence microscopy using a DeltaVision microscope (Applied Precision). Frames were taken every 2 min for 3 h, and those from 0:17:56 to 2:19:56 are shown. Frame rate, 7.0 frames/s. Bar, 30 μ m.