Figure S1. Characterization of the cadherin mutants and chimeric proteins used in the work. (a) General structures of catenin-uncoupled tailless cadherin mutant EcDnΔ and its derivatives (adapted from Hong et al., 2011). EcDnΔ mutant (initially described by Hong et al., 2010) consists of the extracellular cadherin-like repeats (1–5), the transmembrane domain (TM), the short, 17-aa-long fragment that is located between the transmembrane and the p120-binding domains in the intact E-cadherin (yellow box), and a Dendra2 tag (Dendra). To stabilize the mutant on the cell surface, two endocytic signals (K738 and LL motif) present in the 17-aa-long fragment are point inactivated (not depicted). Point mutations used in our study and their effects on cadherin dimerization are indicated. To couple the mutant with the actin cytoskeleton, the actin-binding domains of α-catenin (αABD, α-catenin residues 677–906) or human utrophin (UtrABD, residues 1–261) were placed at the Dendra C terminus. (b) Immunoblot analysis of cells used in the study: untransfected A431D cells (A431D), A431D cells stably expressing EcDnΔ (EcDnΔ), W2A-EcDnΔ (W2A-Δ), D1A-EcDnΔ (D1A-Δ), cis-EcDnΔ (cis-Δ), EcDnΔ-αABD (Δ-αABD), cis-EcDnΔ-αABD (cis-Δ-αABD), EcDnΔ-αABD-Δ42 (Δ-αABD-Δ42), EcDnΔ-HuABD (Δ-HuABD), EcDnΔ-UtrABD (Δ-UtrABD), EcDnΔ-UtrABD-Δ26 (Δ-UtrABD-Δ26), EcDn (EcD), and untransfected A431 cells (A431). Blots were stained for extracellular epitope of E-cadherin (to detect the transgenic proteins and endogenous E-cadherin in A431 cells, arrow), β-catenin (to verify the absence of any endogenous cadherin), or tubulin (as a loading control). Note that in all cases, the levels of recombinant proteins are similar to that of endogenous cadherin in A431 cells. Also note that all transfected A431D cells except those expressing full-size E-cadherin (EcD) or parental A431 cells exhibit extremely low levels of β-catenin. It indicates that these cells do not express any classical cadherins. Molecular mass markers are given on the left. (c) A431D cells expressing EcDnΔ versus adhesive-incompetent W2A-EcDnΔ. Cells were stained with the anti-Dendra antibody. Higher magnifications of the selected regions (indicated by arrows) are shown in the insets. Bars: (main images) 40 µm; (insets) 5 µm.
Figure S2. **Individual 1-s-long frames from time-lapse videos of A431D cells expressing different versions of the tailless mutant EcDnΔ.** (a) EcDnΔ: the individual clusters are not detectable at this microscope setting. (b) EcDnΔ-αABD: the cells exhibit clusters of different sizes at their lateral surface and significant concentration of the mutant at the apical area of cell–cell contact. (c) EcDnΔ-αABD-Δ42: note that the short C-terminal deletion of αABD that abolishes its binding to F-actin results in the disappearance of the mutant apical concentration as well as clear lateral clusters. (d) EcDnΔ-αABD, 5 min after low to high calcium switch performed in the presence of latrunculin A: clusters do not form. (e) Cis-EcDnΔ-αABD: similar to b, the cells exhibit both apical accumulation of the mutant and lateral clusters. Higher magnifications (the same in all cases) of the selected regions (indicated by dashed boxes) are shown in the insets. Arrows and arrowheads indicate the apical and basal areas of the boxed contacts, correspondingly. All images were taken using Plan Apochromat 60×/1.4 NA objective lens and were equally processed. Magnifications are the same in all images. Bars: (main images) 40 µm; (insets) 5 µm.

Figure S3. **Dendra photoconversion assay with EcDnΔ- and D1A-EcDnΔ-expressing cells.** The circular areas (diameter = 2.5 µm) in the selected cell–cell contacts were photoconverted from green to red fluorescence immediately after acquiring a green image (left), and then, cells were imaged in stream mode (100 µs/frame) for 2 s. Bars, 3 µm.
Figure S4. **Double-label immunofluorescence microscopy of A431D cells expressing EcDn-αABD.** (a–b’’) Cells were double stained for Dendra (a and b) and for ZO1 (a’) or Eplin (b’). In a’’ and b’’, the images are merged. Note that the majority of EcDn-αABD clusters are free of ZO1 and Eplin. Even in areas with high expression levels of ZO1, positive structures are not co-distributed. Higher magnifications of the selected regions (indicated by arrows) are shown in the insets. Bars: (main images) 40 µm; (insets) 5 µm.

Figure S5. **Immunofluorescence microscopy of A431D cells expressing EcDn-Δ-UtrABD and its EcDn-Δ-UtrABDΔ26 mutant in which the N-terminal unstructured 26-aa-long region of the UtrABD domain was deleted.** Cells were stained for Dendra. Note that the EcDn-Δ-UtrABD form large clusters extended along cell–cell contact regions. In contrast, the same chimera, but lacking the hinge, produced numerous small lateral clusters and multiple filopodia on the apical portions of cell–cell contacts. By these features, these cells are very similar to those expressing EcDn-αABD. Bars: (main images) 30 µm; (insets) 5 µm.
**Video 1.** The tailless cadherin clusters are unstable and move randomly. A431D cells expressing cadherin tailless mutant EcDnΔ were analyzed by time-lapse wild-field microscopy. Images were acquired in a stream mode at 100 ms/frame for 2 s. The central area of the video was used for Fig. 1 b.

**Video 2.** D1A mutation stabilizes the clusters. The clusters formed in A431D cells by the tailless cadherin mutant D1A-EcDnΔ were analyzed by time-lapse wild-field microscopy as indicated in Video 1. The central area of the video was used for Fig. 1 c. Note that the clusters are much more stable and immobile than in Video 1.

**Video 3.** αABD provides directionality to cluster movement. The clusters formed in A431D cells by the cadherin–α-catenin chimera EcDnΔ-αABD were analyzed by time-lapse wild-field microscopy. Frames were acquired at 30 s intervals for 10 min. Image acquisition time was 1 s. Selected frames from this video were used in Fig. 3 c. Note that the clusters form at the edge of the lamella contacting with another cell and then move in an apical direction.

**Video 4.** Clustering of the EcDnΔ-αABD chimera in the calcium-switch assay. Cells were preincubated with low calcium for 3 h. High calcium media were added at time 0. Images were acquired at 30 s intervals for 10 min (1 s/frame). This video was used in Fig. 4 a. Note that most clusters were assembled at the basal edge of cell–cell contacts immediately after calcium addition; once assembled, most of the clusters moved in an apical direction.

**Video 5.** Clustering of the D1A-EcDnΔ-αABD chimera in the calcium-switch assay. Experiment was performed exactly as Video 4. This video was used in Fig. 4 b. Note that the majority of the assembled clusters do not show directional movement.

**Video 6.** The cis-EcDnΔ-αABD mutant forms short-lived clusters. A431D cells expressing the cis-EcDnΔ-αABD chimera were analyzed by time-lapse microscopy. Images were acquired at 1 min intervals for 20 min (1 s/frame). Fragments of this video were used in Fig. 7 b. Note that similar to the cis-interface-intact version of this mutant, EcDnΔ-αABD, the clusters mainly form at the basal edge of cell–cell contacts. They are much more transient, whereas some of them move in an apical direction.

**References**
