Strale et al., http://www.jcb.org/cgi/content/full/jcb.201410111/DC1

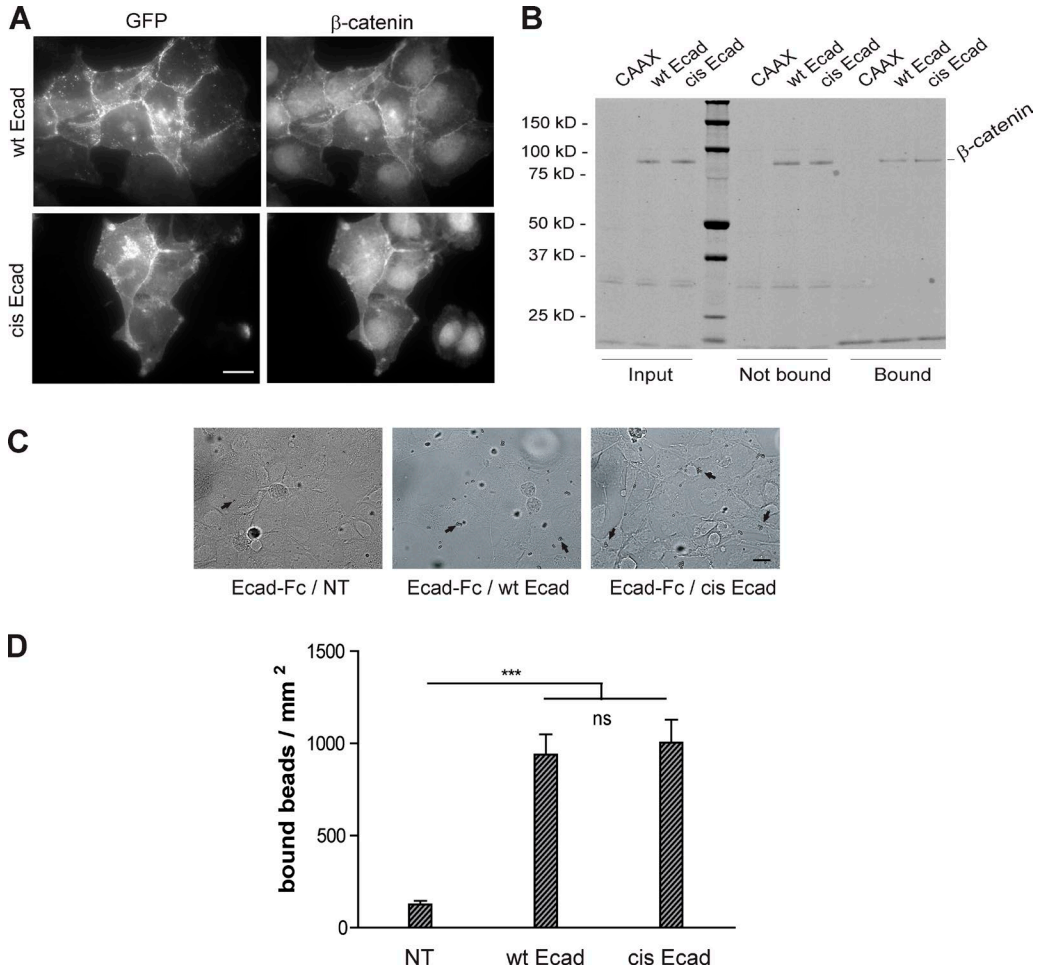
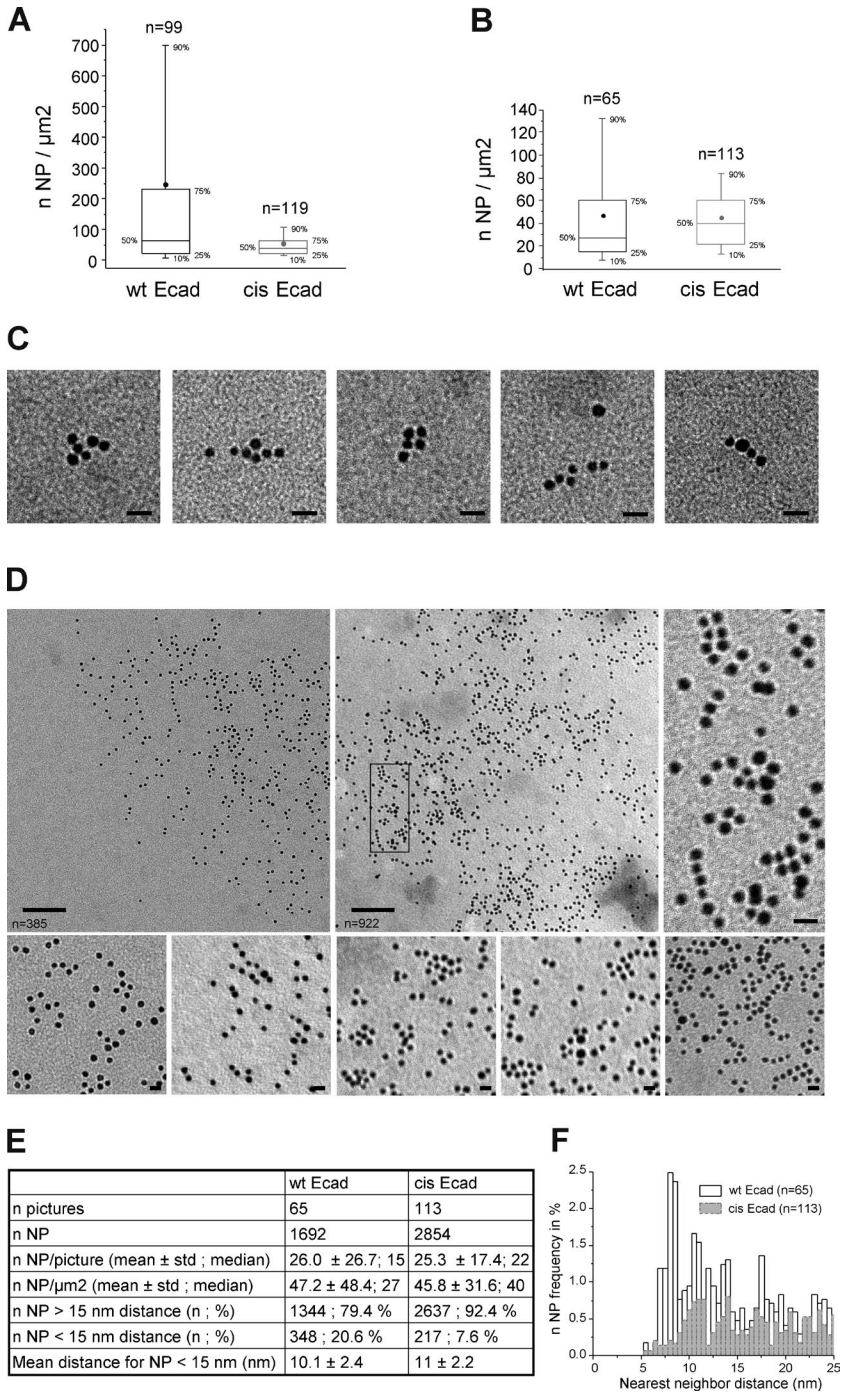


Figure S1. **Recruitment of β-catenin at cell–cell contacts and binding of Ecad-Fc beads is independent of Ecad cis-oligomerization.** (A) Immunocytochemistry experiments reveal that β-catenin colocalizes with wt Ecad-GFP (wt Ecad) or cis-Ecad-GFP (cis Ecad) at the cell–cell contacts of transfected A431D cells. Bar, 20 µm. (B) A431D cells were transfected with wt Ecad-GFP (wt Ecad), cis-Ecad-GFP (cis Ecad), or CAAX-GFP (CAAX) as a control. After 36 h, GFP-tagged proteins were immunoprecipitated from soluble cell lysates using GFP-Trap and Input, Not bound, and Bound fractions were subjected to Western blot analysis to detect β-catenin. β-Catenin was coimmunoprecipitated similarly with wt Ecad-GFP and cis-Ecad-GFP proteins. (C) Representative pictures of nontransfected (NT), wt Ecad, or cis-Ecad expressing A431D cells incubated for 1 h with Ecad-Fc–coated beads. Bar, 20 µm. (D) Bar graph showing the density of Ecad-Fc–coated beads bound on nontransfected cells and wt Ecad and cis-Ecad expressing cells. Histograms represent the mean ± SEM out of beads counted over at least 18 fields per condition and repeated in two independent experiments. The binding of Ecad-Fc–coated beads was specific as demonstrated by the low binding on NT cells. The oligomerization status of Ecad did not significantly affect Ecad-Fc bead binding.



	wt Ecad	cis Ecad
n pictures	65	113
n NP	1692	2854
n NP/picture (mean ± std ; median)	26.0 ± 26.7; 15	25.3 ± 17.4; 22
n NP/µm2 (mean ± std ; median)	47.2 ± 48.4; 27	45.8 ± 31.6; 40
n NP > 15 nm distance (n ; %)	1344 ; 79.4 %	2637 ; 92.4 %
n NP < 15 nm distance (n ; %)	348 ; 20.6 %	217 ; 7.6 %
Mean distance for NP < 15 nm (nm)	10.1 ± 2.4	11 ± 2.2

Figure S2. **Nanometric organization of wt and cis-Ecad-GFP at the cell membrane.** (A) Whisker plot showing the mean number of NPs per squared micrometer for all images acquired for wt and cis-Ecad expressing cells. Number of images (*n*) is given in the top of each chart box. (B) Similar representation showing the mean number of NPs per squared micrometer for the images used in the analysis shown in Fig. 2 (all images have from 2 to 100 NPs per picture; 4–180 NP/µm^2). A mean of 47.2 and 45.8 NP/µm^2 was found for wt and cis-Ecad expressing cells, respectively. (C) An additional example of clusters seen on the field analyzed for wt Ecad as in Fig. 2 (4 to 180 NP/µm^2). Images are 100 × 100 nm. Bars, 15 nm. (D) TEM visualization of high density labeling for plasma membrane sheets of wt Ecad-GFP. Large images are 742.7 × 742.7 nm; Bars, 100 nm. Number of NPs in the picture is given at the bottom left. (top right) Zoom-in of the previous large-scale image at 100 × 200 nm. Bar, 15 nm. (bottom) Insets at 200 × 200 nm. Bars, 15 nm. Such dense labeling was never observed for cis-Ecad expressing cells (up to 279 NP/µm^2). (E) Overview table of the TEM data obtained for wt Ecad and cis-Ecad images with 4 to 180 NP/µm^2. *n* pictures, number of pictures analyzed; *n* NP, overall number of NPs for all pictures analyzed; *n* NP > 15 nm, total number of NPs at a distance of 15 nm or more center to cente (number; percent); *n* NP < 15 nm: total number of NPs at a distance smaller than 15 nm (number; percent). Mean distance for NPs at <15-nm distance is given as mean ± SD. (F) Distribution of the center to center distance between each NP and its nearest neighbor for the same datasets presented in Fig. 2 but with a binning of 0.5 nm and restricted to values of distance (x axis) from 0 to 25 nm. *n* is the number of images analyzed per condition.

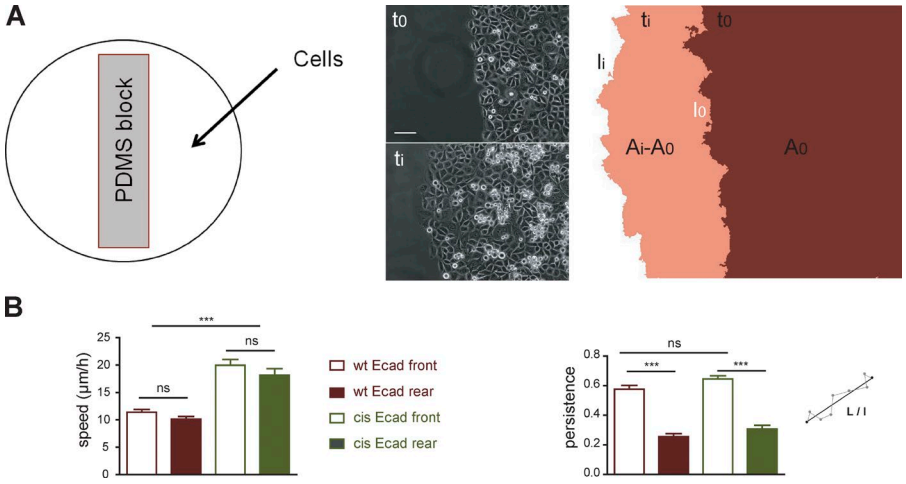


Figure S3. **Quantitative analysis of cell layer expansion.** Transfected A431D cells were grown in a dish where part of the culture area was initially masked by an inert PDMS block. When confluence was reached the block was removed, freeing new space for cell layer expansion. (A) After removal of the PDMS block, the free edge of the monolayer was imaged over time. The area covered by the monolayer A_i and length of the expansion front l_i at time t_i were extracted. The front displacement at a given time was obtained by the square root of $(A_i - A_0)$ and the roughness of the migration front by dividing l_i by l_0. (B) Bar graphs showing the mean migration speed (spead in µm ± SEM) and directionality (persistence; mean ± SEM) of front and rear cells for conditions presented. ***, $P < 0.05$, one-way analysis of variance plus Bonferroni's multiple comparison test. Cell migration speed was strongly affected in cis-Ecad expressing cells both at the front and rear of the migrating sheet. The persistence of migration was not significantly affected.

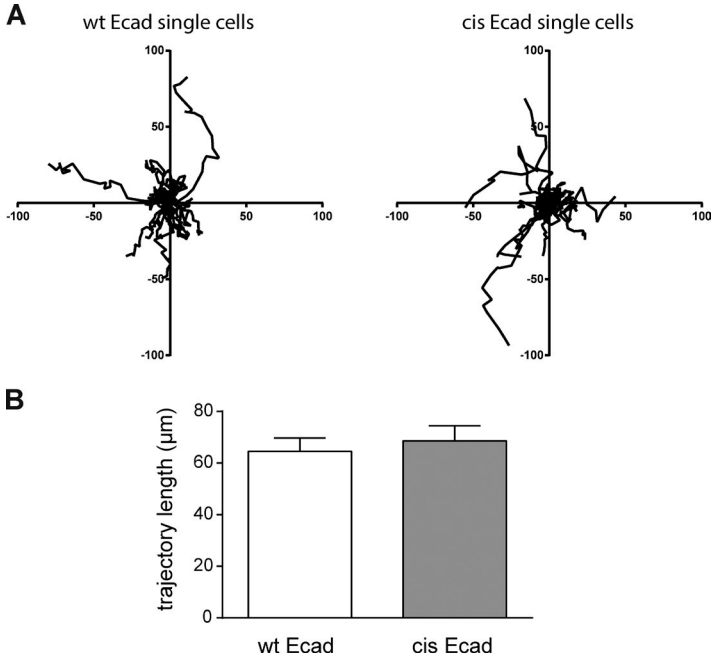


Figure S4. **Single cell migration of A431D-transfected cells.** wt Ecad and cis-Ecad expressing cells were seeded at low density on fibronectin-coated surfaces, imaged, and individually tracked over time. (A) 120-min trajectories of single wt Ecad (left) and cis-Ecad (right) expressing cells migrating on fibronectin ($n \geq 22$ cells). (B) Mean length (± SEM) of the trajectories.

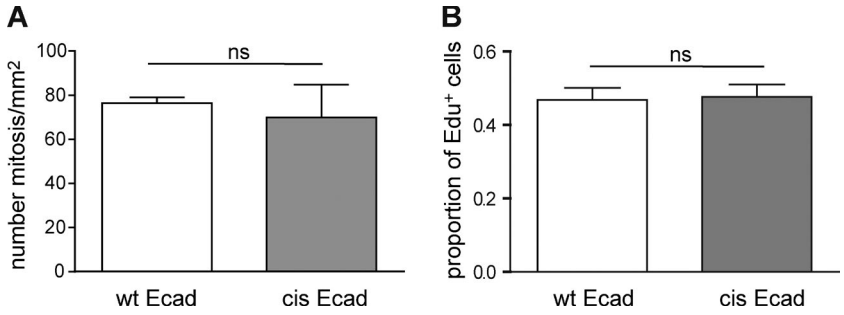
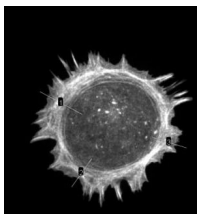
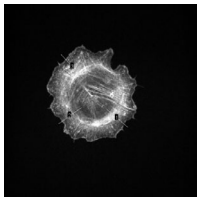


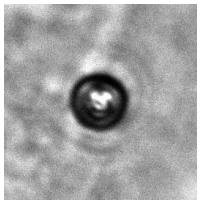
Figure S5. **Division rate of A431D-transfected cells.** (A) Bar graph showing the number of mitotic figures counted on phase-contrast images of wt Ecad and cis-Ecad monolayers during 6 h (six videos analyzed for each condition). (B) Subconcluent wt Ecad and cis-Ecad cell cultures were subject to a 1-h 5-ethynyl-2-deoxyuridine (Edu) pulse and the proportion of Edu^+ wt Ecad and cis-Ecad cells evaluated. Results are given as mean ± SEM.



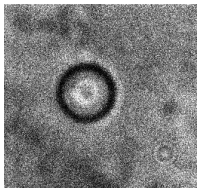
Video 1. **Actin retrograde flow in a wt Ecad-GFP expressing cell spread on Ecad-Fc.** A431D cells expressing Life-Act Ruby and wt Ecad-GFP were allowed to spread on Ecad-Fc substrate for 2 h. Images were analyzed by a time-lapse confocal microscope equipped with a Nipkov disk. Time-lapse images were acquired using an inverted microscope (DMI4000; Leica; equipped with a 63× objective) coupled to a spinning-disk confocal module (CS20 head; Yokogawa Electric Corporation) and an intensified CCD camera (Quantem S12SC; Roper Scientific), driven by MetaMorph software. Frames were taken every 500 ms for 3 min. The total duration of the movie is 180 s.



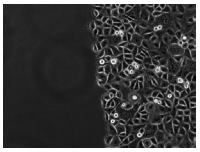
Video 2. **Actin retrograde flow in a cis-Ecad-GFP expressing cell spread on Ecad-Fc.** A431D cells expressing Life-Act Ruby and cis-Ecad-GFP were allowed to spread on Ecad-Fc substrate for 2 h. Images were analyzed by time-lapse confocal microscope equipped with a Nipkov disk. Time-lapse images were acquired using an inverted microscope (DMI4000; Leica; equipped with a 63× objective) coupled to a spinning-disk confocal module (CS20 head; Yokogawa Electric Corporation) and an intensified CCD camera (Quantem S12SC; Roper Scientific), driven by MetaMorph software. Frames were taken every 500 ms for 3 min. The total duration of the movie is 180 s.



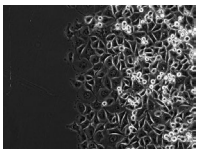
Video 3. **Ecad-Fc magnetic bead displacement under force (wt Ecad-GFP expressing cells).** Ecad-Fc–coated beads were allowed to bind to wt Ecad-GFP expressing cell for 1 h. Force was applied from the sixth frame and maintained until the end of the movie, from left to right. Images were acquired on a microscope (DMIRB; Leica; 100× oil objective) equipped with a CCD camera (Coolsnap HQ2; Roper Scientific). Frames were taken in the burst mode (~13 frames/s) for 170 s.



Video 4. **Ecad-Fc magnetic bead displacement under force (cis-Ecad-GFP expressing cells).** Ecad-Fc–coated beads were allowed to bind to cis-Ecad-GFP expressing cell for 1 h. Force was applied from the sixth frame and maintained until the end of the movie, from left to right. Images were acquired on a microscope (DMIRB; Leica; 100× oil objective) equipped with a CCD camera (Coolsnap HQ2; Roper Scientific). Frames were taken in the burst mode (~13 frames/s) for 170 s.



Video 5. **Collective cell migration of wt Ecad-GFP expressing cells.** wt Ecad-GFP expressing cells were plated at high density in 3.5-cm Petri dishes where a PDMS block was previously deposited. When cells reached confluence, the PDMS block was removed. Images were then acquired every 5 min during 24 h under a controlled temperature and CO_2 environment (37°C, 5% CO_2; 10× objective; BioStation; Nikon). Frame rate: 15 frames/s.



Video 6. **Collective cell migration of cis-Ecad-GFP expressing cells.** cis-Ecad-GFP expressing cells were plated at high density in 3.5-cm Petri dishes where a PDMS block was previously deposited. When cells reached confluence, the PDMS block was removed. Images were then acquired every 5 min during 24 h under a controlled temperature and CO_2 environment (37°C, 5% CO_2; 10× objective; BioStation; Nikon). Frame rate: 15 frames/s.