le Duc et al., http://www.jcb.org/cgi/content/full/jcb.201001149/DC1

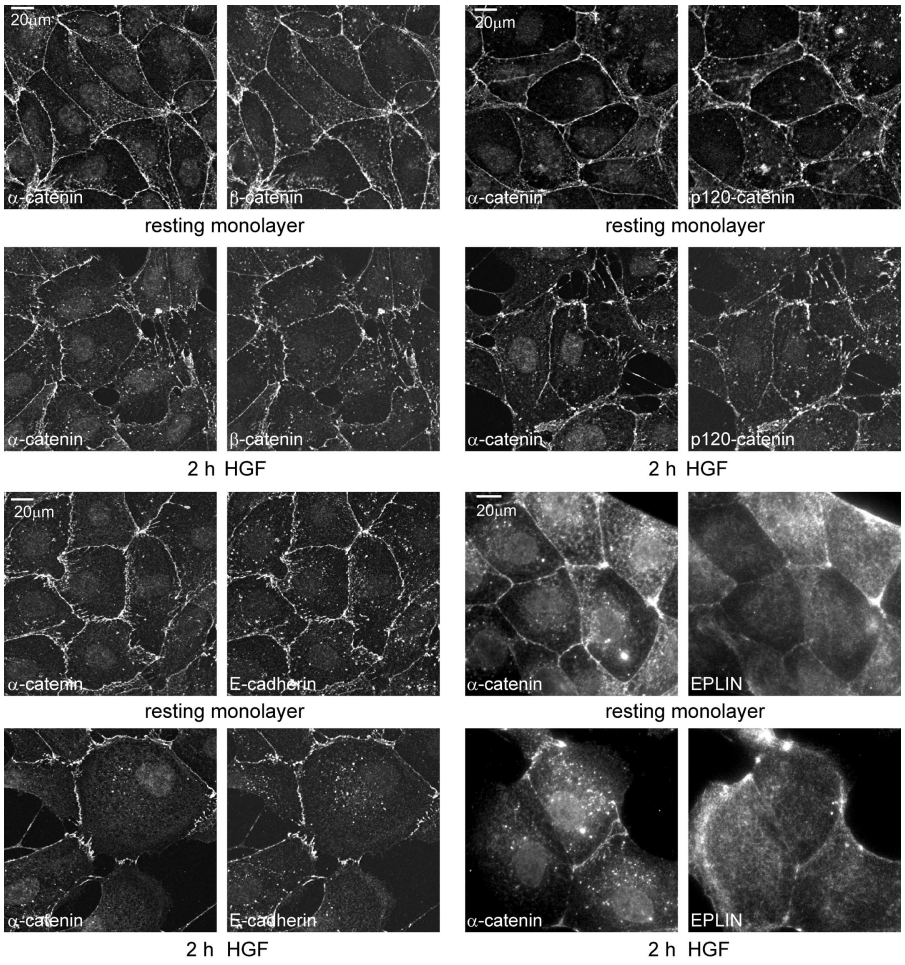


Figure S1. **Localization of E-cadherin complex proteins after HGF stimulation.** Cells were grown for 20 h and treated with HGF for 2 h. Cells were washed two times in CSK buffer before fixation to remove cytosolic proteins and stained as indicated.

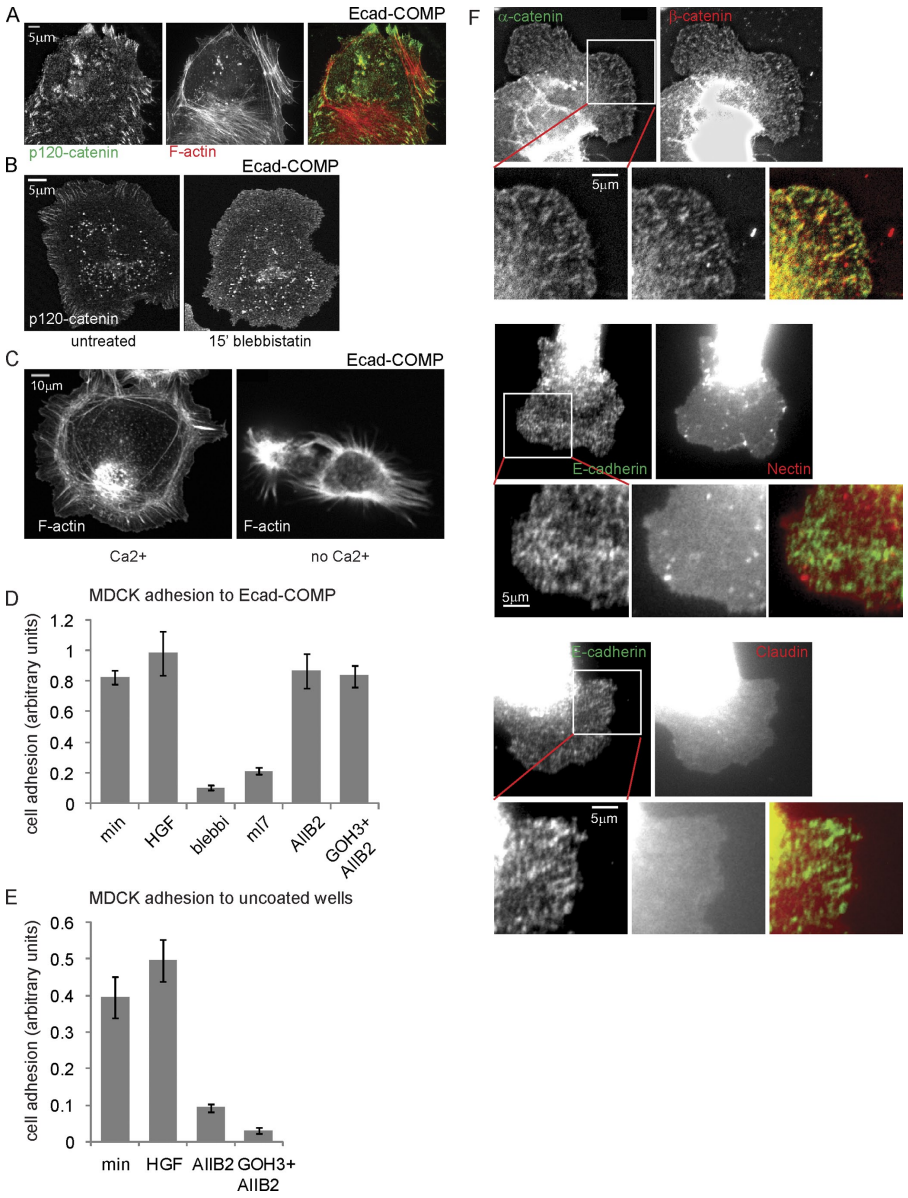


Figure S2. **E-cadherin–COMP adhesions are cadherin and myosin II–dependent structures that contain the core E-cadherin complex.** (A) p120-catenin staining of cells on E-cadherin–COMP-coated coverslips reveals elongated adhesion structures containing the E-cadherin complex. Phalloidin-stained F-actin fibers terminate at these adhesions. (B) Treatment with blebbistatin for 15 min after cell spreading abrogates the elongated E-cadherin adhesions, whereas prolonged treatment results in cell rounding and detachment (not depicted). (C) Washing MDCK cells adhering to E-cadherin–COMP in PBS without calcium results in loss of adhesion, as shown by phalloidin staining of the few remaining cells, whereas cells on collagen or uncoated coverslips are completely insensitive to the absence of calcium (not depicted). (D) Adhesion of MDCK cells to E-cadherin–COMP depends on myosin II activity and does not involve α6- or β1-integrins. MDCK cells were allowed to adhere for 45 min in the presence of inhibitors of myosin II activity or ∼20 μg/ml integrin-blocking antibodies (β1-integrins, AIIB2; α6-integrins, GOH3) followed by rigorous washing. Quantification was performed by acid phosphatase activity. (E) MDCK cells spontaneously adhere mainly through β1- and α6-integrins, as indicated by the complete loss of adhesion in the presence of ∼20 μg/ml AIIB2 and ∼20 μg/ml GOH3. MDCK cells were allowed to adhere for 3 h to uncoated, BSA-blocked wells in the presence of the indicated antibodies. Treatment and quantification were performed as described in D. (F) α-Catenin and β-catenin colocalize to E-cadherin–COMP adhesions, whereas GFP-nectin1 (stably expressed) and GFP-claudin3 (stably expressed) do not localize to E-cadherin–COMP adhesions, as shown by GFP imaging and IF staining in MDCK cells adhering to E-cadherin–COMP. GFP-nectin1 and GFP-claudin3 do display membrane targeting and localize to junctions between neighboring cells (not depicted), showing that these fusion proteins do behave as their wt counterparts and are properly used in this study to conclude that nectin-based junctions and tight junctions are not recruited to E-cadherin–COMP adhesions. Error bars represent SD in triplicate samples.

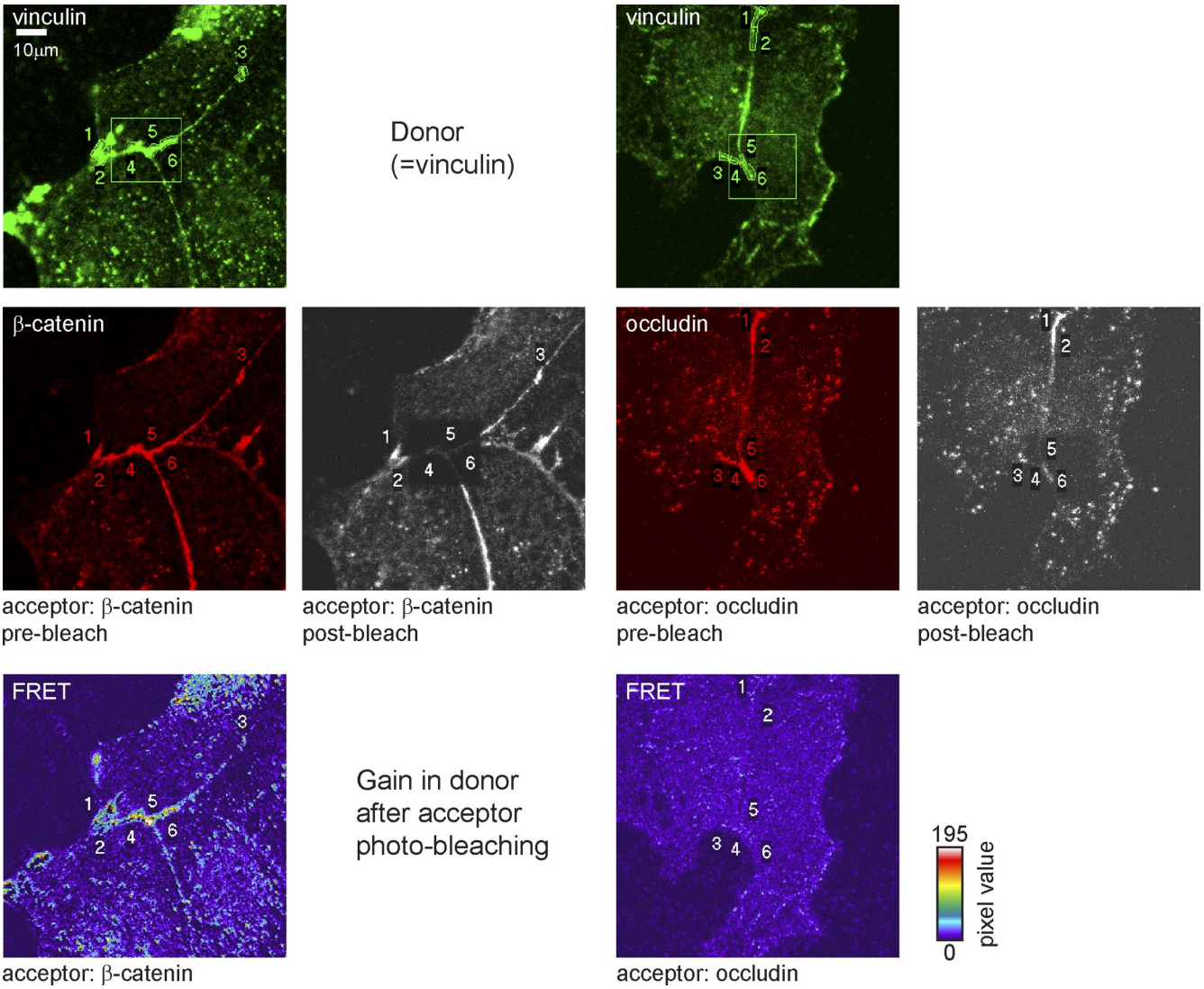
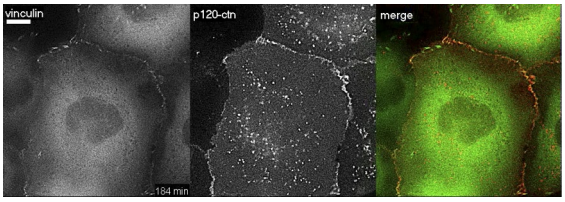
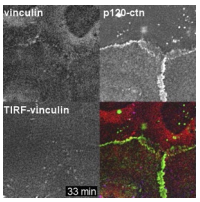


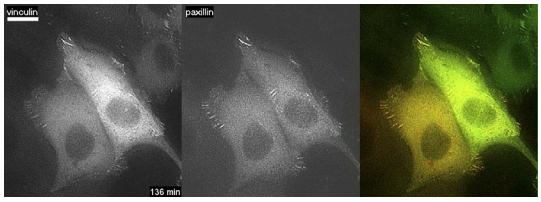
Figure S3. **FRET in cell–cell junctions between Alexa Fluor 488– and rhodamine-labeled proteins.** To measure FRET, GFP-vinculin–expressing MDCK cells were CSK extracted, paraformaldehyde fixed, and stained with polyclonal anti-GFP followed by Alexa Fluor 488–labeled secondary antibodies as the donor. To function as the acceptor, β-catenin or occludin was detected with monoclonal antibodies followed by a rhodamine-labeled secondary antibody. Donor and acceptor images were taken before and after bleaching a central area (boxed regions) of the acceptor using high laser power. ROIs were drawn around vinculin-containing areas, as outlined and numbered in the top panels, of cell–cell junctions both outside and inside the bleach area to calculate bleaching caused by imaging and the percentage of acceptor bleaching. Gain in donor fluorescence as a result of acceptor photobleaching was calculated by correcting the postbleach donor image for imaging bleaching and subtracting, from the photobleach area of this image, the photoconversion fraction of the prebleach acceptor image (1.12%). This is followed by subtraction of the prebleach donor image, and the resulting gain in donor image is displayed in pseudocolor (color scale). The ROIs inside the bleach area were used to calculate the FRET values explained and displayed in Fig. 3.



Video 1. **Vinculin is recruited to p120-catenin–containing cell–cell junctions upon HGF stimulation.** Cells stably expressing GFP-vinculin were transiently transfected with mCherry–p120-catenin, grown for 20 h in a collagen-coated glass-bottom chamber, transferred to an epifluorescence microscope, and imaged at 8 min/frame. At 32 min after the start of imaging, 5 ng/ml HGF was added to the medium. At each time point, four consecutive images at increasing z steps were taken (200-nm step size). These images were deconvoluted using Huygens software (Scientific Volume Imaging), and the plane displayed is focused ∼400 nm above the basal cell surface. Bar, 5 µm.



Video 2. **Vinculin presence at FAs as well as suprabasal cell–cell junctions depends on actomyosin contractility.** Cells stably expressing GFP-vinculin and transiently expressing mCherry–p120-catenin were grown for 20 h in a collagen-coated glass-bottom chamber, stimulated with HGF for 1 h, transferred to an epifluorescence microscope, and imaged at 90 s/frame. At 15 min after the start of imaging, 3 µM ML-7 and 10 µM Y27632 were added to inhibit all actomyosin contractility. 10 min after their addition, the inhibitors were removed, and fresh medium was added. At each time point, widefield images and TIRF images were taken (using a steep angle; ∼100 nm was illuminated).



Video 3. **Vinculin is present in paxillin-marked FA and appears in paxillin-devoid cell–cell adhesions upon HGF stimulation.** Cells stably expressing GFP-vinculin were transiently transfected with mCherry-paxillin, grown for 20 h in a collagen-coated glass-bottom chamber, transferred to an epifluorescence microscope, and imaged at 8 min/frame. At 32 min after the start of imaging, 5 ng/ml HGF was added to the medium. At each time point, three consecutive images at increasing z steps were taken (200-nm step size). These images were deconvoluted using Huygens software (Scientific Volume Imaging), and the plane displayed is focused ∼400 nm above the basal cell surface. Bar, 10 µm.