SUPPLEMENTARY INFORMATION

The Adherens Junction: A Mosaic of Cadherin and Nectin Clusters Bundled by Actin Filaments

Indrajyoti Indra, Soonjin Hong, Regina Troyanovsky, Bernadett Kormos, Sergey Troyanovsky

Department of Dermatology, Northwestern University, The Feinberg School of Medicine, Chicago, IL 60611

Contact: s-troyanovsky@northwestern.edu

Contents:

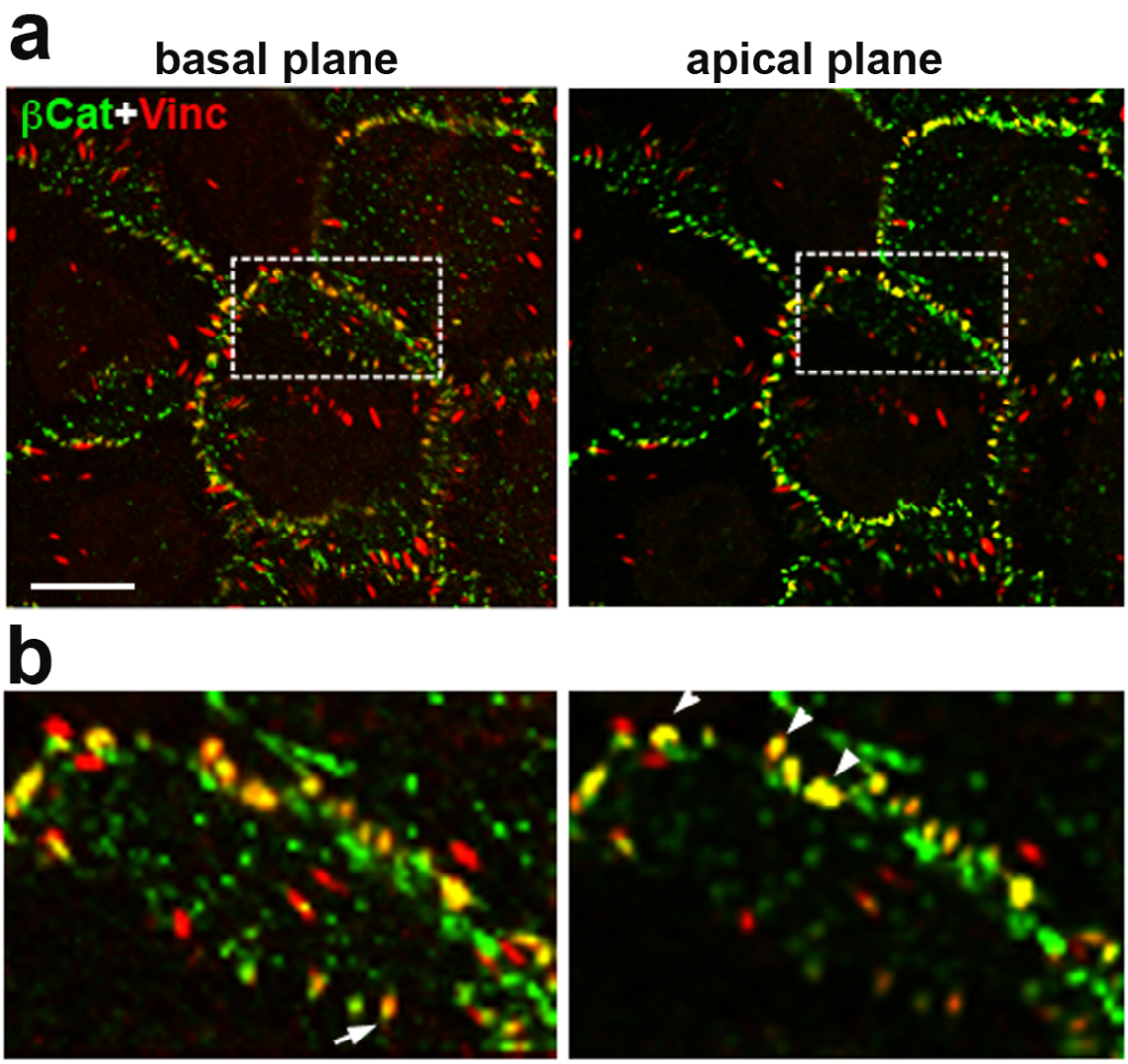

Figure S1: Heterogeneity of adherens junctions in A431 cells. Double immunofluorescence microscopy of A431 cells using rabbit anti-β-catenin (green) in comparison with mouse anti-vinculin (red). The images were taken in two, apical and basal, focus planes using confocal microscope Nikon A1R. Low magnification images (upper row) show general distribution of the vinculin-positive and cadherin/catenin-positive structures. Note that only a fraction of these structures, predominantly located at the apical level, contains both proteins. Bar, 20 μm. (b) The higher magnification of the cell-cell contact denoted by broken line. Note that majority of apical junctions (some of them are marked by arrowheads in the apical image) are positive for both vinculin and β-catenin. Some of the lateral junctions (marked by an arrowhead on the basal image) also exhibit weak vinculin staining. Most of the lateral junctions, however, lack vinculin staining.

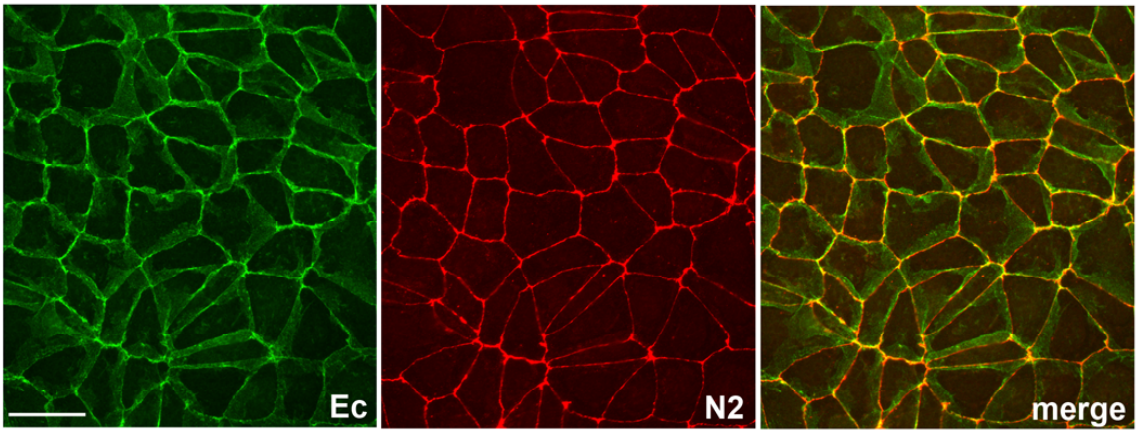

Figure S2: **Nectin and cadherin localization in highly polarized colon carcinoma DLD1 cells.** Double-label immunofluorescence microscopy of DLD1 cells for E-cadherin (Ec, green) and for nectin-2 (N2, red). Note that the majority of nectin-2 is concentrated in the apical junctional complex, where it is co-localized with E-cadherin. Super-resolution microscopy (see Fig. 4b) shows, however, that these two proteins are present in distinct clusters. Bar, 40 μm.

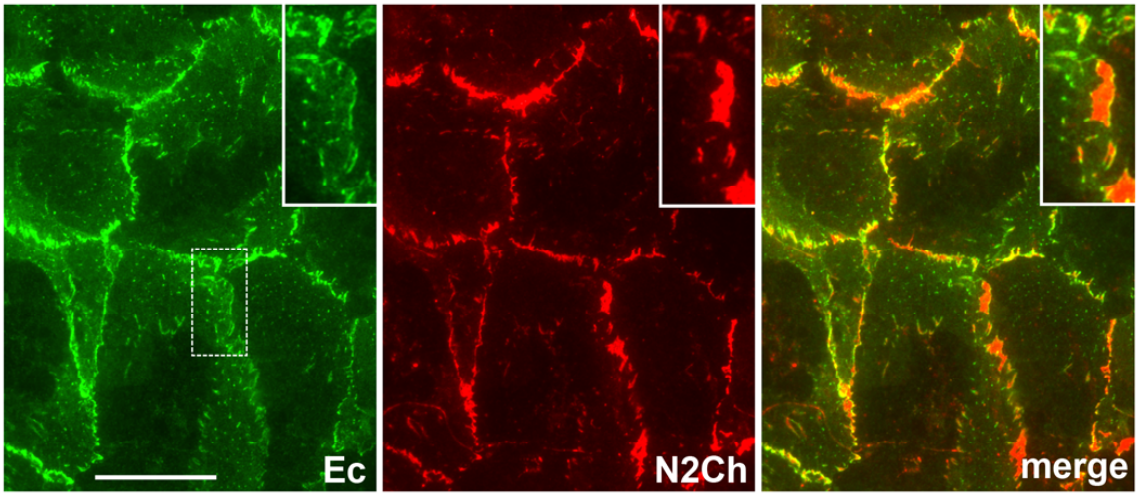

Figure S3: **Cadherin junctions are nectin-independent.** Double-label immunofluorescence microscopy of A431cells overexpressing recombinant mCherry-tagged nectin-2. Cells were double stained for E-cadherin (Ec, green) and for mCherry (N2Ch, red). Higher magnifications of the boxed region is shown in the insets. Note that E-cadherin is not present in the giant clusters formed by overexpressed nectin-2. Instead, it tends to be concentrated on the periphery of these giant clusters. Bar, 20 μm.

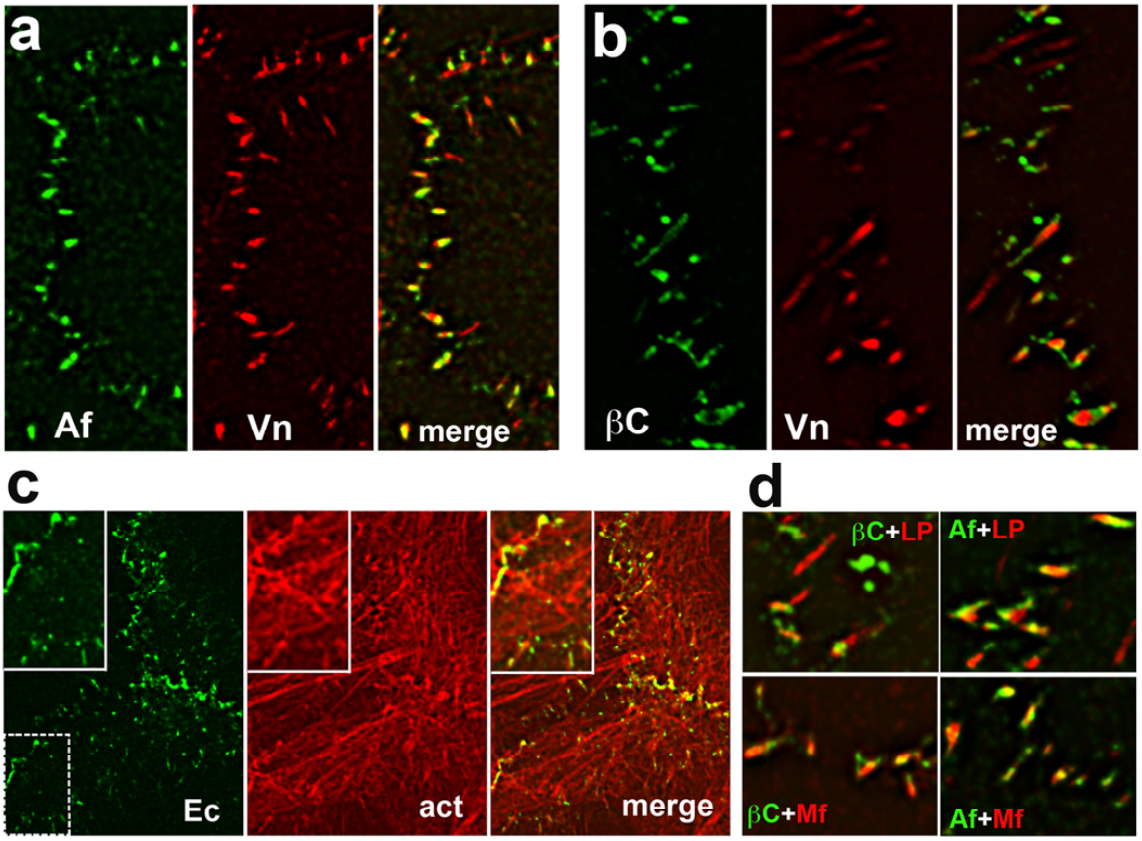

Figure S4: Characterization of the actin cytoskeleton associated with adherens junctions. (a and b) N-SIM-generated images of A431 cells stained for afadin (Af, green) and vinculin (Vn, red) and β-catenin (βC) and vinculin (Vn). (c) N-SIM-generated images of cells stained for E-cadherin (Ec, green) and F-actin (act, red) using phalloidin. (d) N-SIM-generated images of adherens junctions stained either for β-catenin (βC, green) or afadin (Af, green) and either LPP (LP, red) or migfilin (Mf, red).