Tang and Brieher, http://www.jcb.org/cgi/content/full/jcb.201103116/DC1

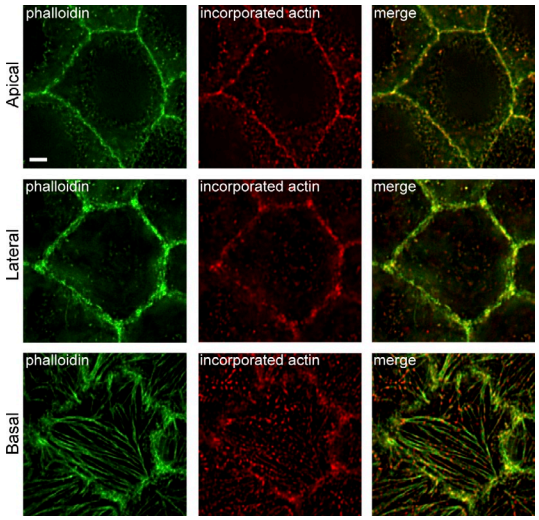

Figure S1. **Incorporation of fluorescently labeled actin at apical, lateral, and basal regions of saponin-permeabilized cells.** Bar, 2 µm.

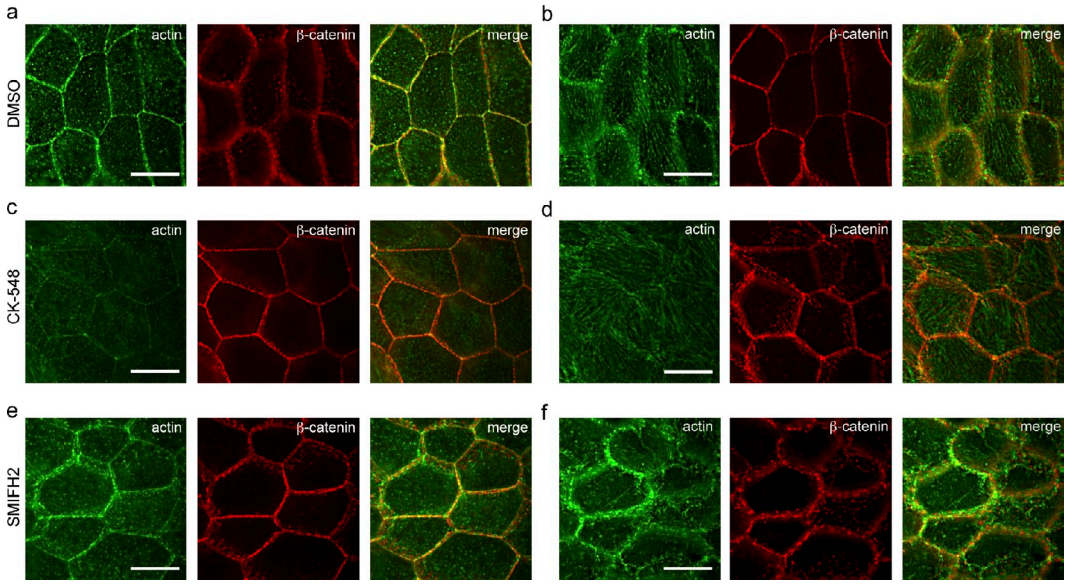

Figure S2. **Relative contribution of actin nucleators, Arp2/3, and formin-1 in junctional and basal actin structures in MDCK cells.** (a, c, and e) Projections of 10 optical z slices spanning the apical 2 µm of cells. Junctional actin is reduced in cells treated with Arp2/3 inhibitor CK-548 (c) but not formin inhibitor SMIFH2 (e) when compared with untreated (a) cells. (b, d, and f) Basal actin structures are disrupted in cells treated with SMIFH2 (f) but not CK-548 (d) when compared with untreated (b) cells. Bars, 10 µm.

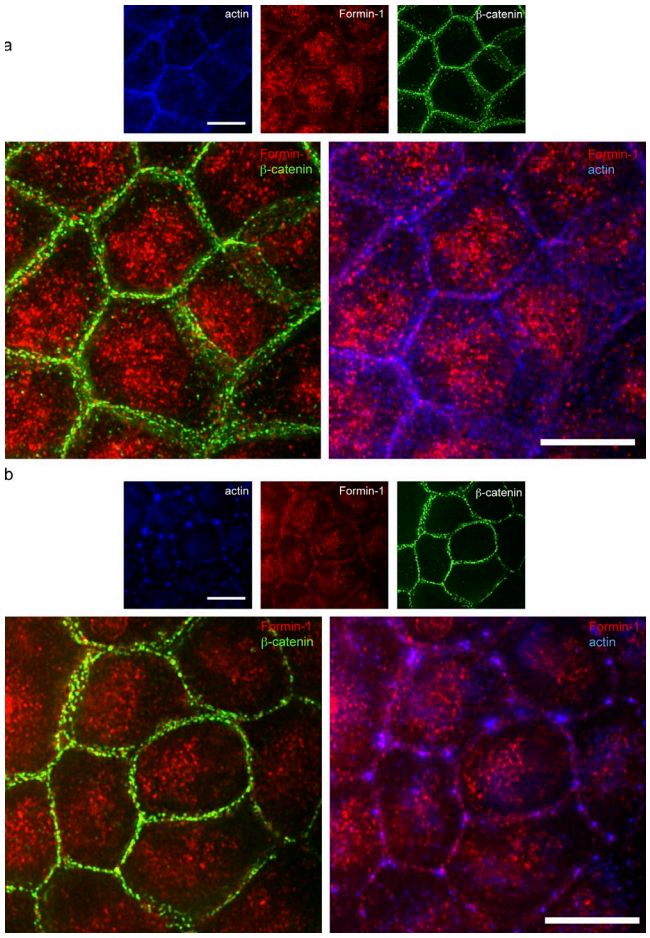

Figure S3. **Immunofluorescence staining showing a lack of strong colocalization between β-catenin and formin-1.** (a) Formin-1 does not accumulate at cadherin–catenin complexes but localize to lateral cell membranes. Projections of 10 deconvolved optical z slices spanning 4 µm of the apical–lateral region of cells are shown. (b) Formin-1 remains at the lateral membrane after latrunculin treatment. Projections of five deconvolved optical z slices spanning 2 µm from the apical region of cells are shown. Bars, 10 µm.

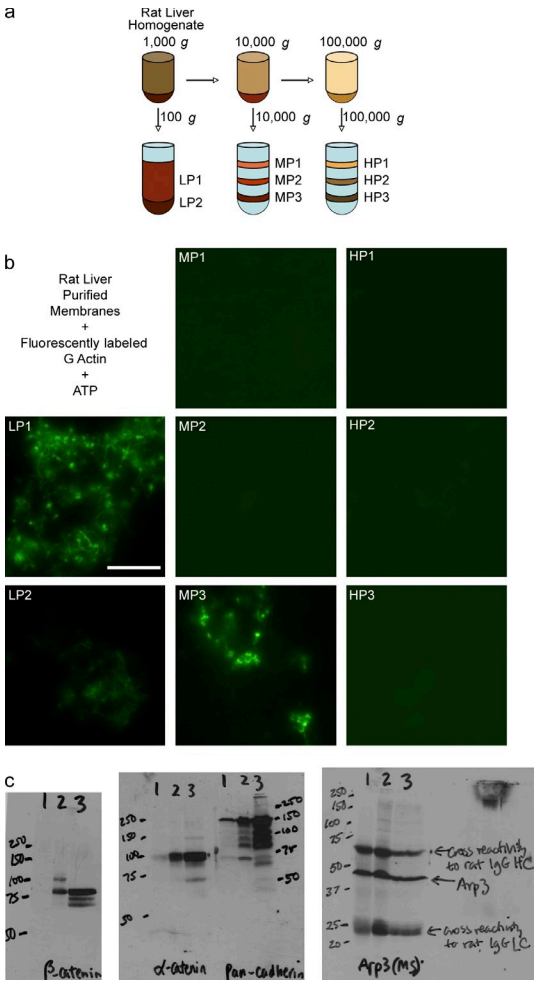

Figure S4. **Fractionation of liver homogenate recovers two membrane fractions that have actin assembly activities.** (a) The homogenate was subjected to differential centrifugation followed by sucrose density equilibrium density gradient. (b) Two membrane fractions showed actin assembly activity upon addition of monomeric actin and ATP. Bar, 10 µm. (c) Western blot of total liver homogenate (lane 1), purified membranes (lane 2), and stripped membranes (lane 3). Molecular mass is indicated in kilodaltons.

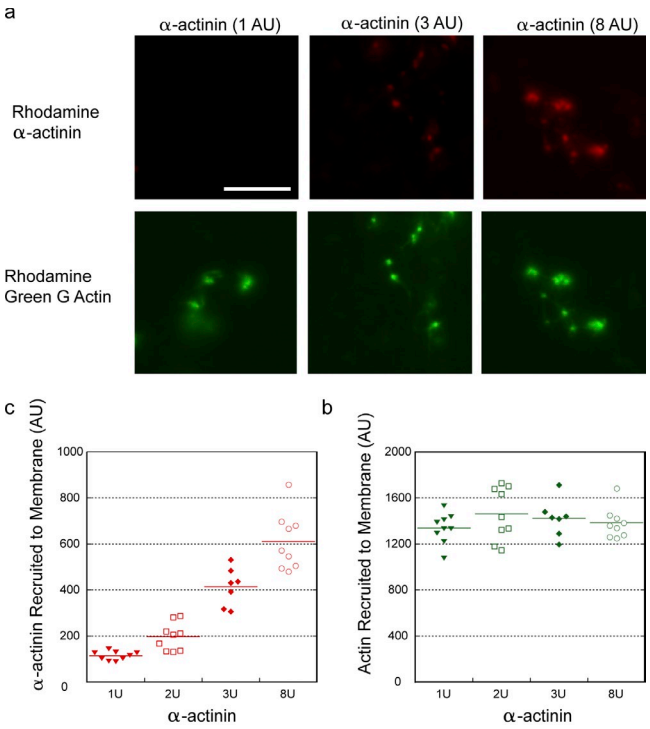

Figure S5. **Actin incorporation at the membrane is saturable.** (a) Increasing concentration of α-actinin did not increase actin assembly. One arbitrary unit (AU) of α-actinin is defined as the amount needed to restore actin incorporation of stripped membranes to the same level of unstripped membranes. Bar, 10 µm. (b) Quantitation of individual puncta revealed similar levels of rhodamine green actin across eight units of α-actinin. (c) Quantitation of individual puncta showing association of rhodamine–α-actinin increases with the amount added to the membranes, showing secondary recruitment. (b and c) Each line represents the mean value of all the individual data points in that group.