Wood et al., https://doi.org/10.1083/jcb.201612006.

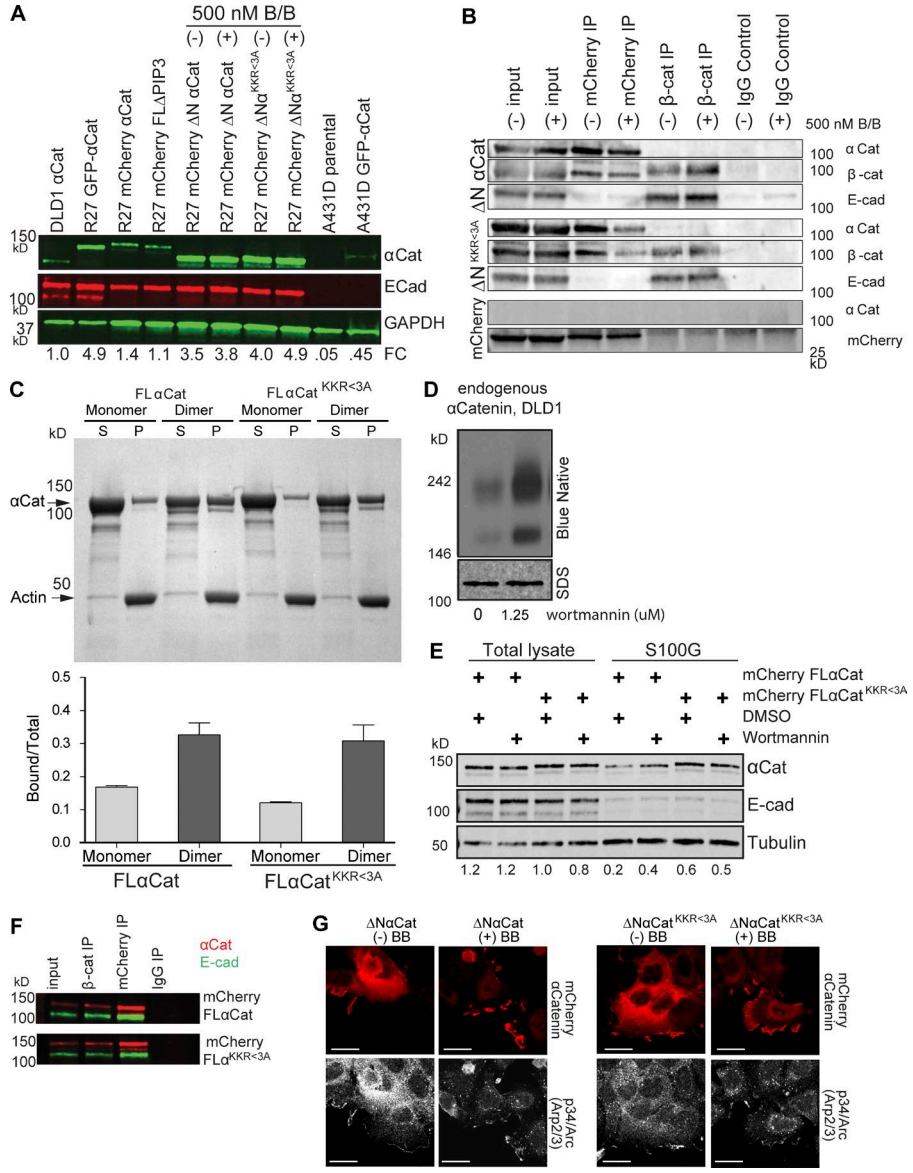
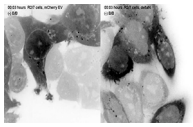
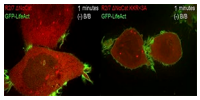


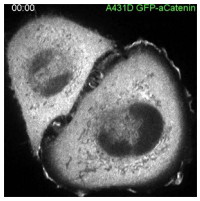
Figure S1. **Cell line characterization and controls.** (A) Immunoblot (LiCOR) analysis of all cell lines and constructs used in this study. αCat band intensities were normalized to GAPDH and expressed as a fold change (FC) of endogenous αCat in DLD1 cells. Note that fluorescently tagged FLαCat and FLαCat$^{KKR<3A}$ proteins are expressed similarly to endogenous αCat. ΔNαCat and ΔNαCat$^{KKR<3A}$ mutant proteins are overexpressed threefold compared with endogenous but, importantly, are similarly expressed to each other. (B) Immunoprecipitation of ΔNαCat and ΔNαCat$^{KKR<3A}$ cells (treated with [+] or without [–] the B/B homodimerizer) with antibodies to either mCherry or β-catenin and immunoblotted for E-cadherin to show that N-terminal ΔNαCat truncation prevents association with the cadherin–catenin complex. Although β-catenin coimmunoprecipitates with the ΔNαCat construct, concordant association with E-cadherin is lost, confirming that αCat incorporation within the cadherin–catenin complex is blocked. Because the inverse immunoprecipitation of β-catenin does not also pull down αCat, we reason that ΔNαCat coimmunoprecipitation is more efficient and likely binds β-catenin indirectly, perhaps via APC (Choi et al., 2013). (C) Monomeric and dimeric forms of FLαCat and FLαCat$^{KKR>3A}$ show similar actin binding activities. The actin-pelleting assay was performed with 5 µM of actin and 5 µM of αCat. Bottom: the actin-bound fraction of αCat was determined for each reaction by densitometry analysis of SDS-PAGE gels stained with Coomassie blue. Bar graph reflects mean of three independent assays with SD. WT versus mutant αCat homodimers show no significantly different binding to F-actin by *t* test. (D) PI3K signaling decreases solubility of αCat pool. BN-PAGE (top) analysis of a cytosolic fraction of endogenous αCat isolated by detergent-free, freeze-thaw lysis (see Materials and methods) from DLD1 cells (parent to the R2/7 αCat negative line). SDS gel (bottom) analysis of a total, Triton X-100–soluble fraction of αCat as a control. (E) Detergent-free lysis and cytosolic fractionation (S100G) of R2/7 FLαCat and FLαCat$^{KKR<3A}$ cells treated either with DMSO or 5 µM wortmannin. Quantification of αCat densitometry ratioed to tubulin blots below. (F) Immunoprecipitation of FLαCat and FLαCat$^{KKR<3A}$ constructs and blot back for components of the cadherin–catenin–complex. (G) Migrating cells in wound front (ΔNαCat/ΔNαCat$^{KKR<3A}$ in R2/7 cells) were costained for Arp2/3 to look for potential changes in colocalization. Bar, 20 µm. Although previous studies suggest αCat homodimerization inhibits Arp2/3 activity (Drees et al., 2005; Benjamin et al., 2010), and the slower wound closure of FLαCat relative to FLαCat$^{KKR<3A}$ cells in Fig. 7 support this concept, we find no obvious evidence of αCat competition of Arp3 from the leading edge.



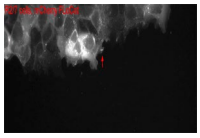
Video 1. **αCat forced dimerization promotes cortical recruitment and filopodia formation.** Part 1: live imaging of mCherry-iDimerize Empty-Vector (EV) and mCherry-iDimerize-ΔNαCat-expressing R2/7 DLD1 cells treated with (+) or without (–) bidentate (B/B "dimerizer") and monodentate washout (W/O) ligand in 6-h time course. Note that labels and time stamps are incorporated within corresponding image frames. mCherry-iDimerize-ΔNαCat is rapidly recruited to the periphery after addition of B/B ligand (within 10 s; red arrows). The mCherry-iDimerize EV (control construct) localizes to the nucleus and cytoplasm, where addition of the B/B ligand promotes nuclear exclusion rather than cortical recruitment. Part 2: live imaging of mCherry-iDimerize- WT-ΔNαCat– and ΔNαCat$^{KKR<3A}$ mutant–expressing cells (red) coinfected with GFP-LifeAct (green). Shown first: mCherry-iDimerize-WT-ΔNαCat before (left) and after (right) B/B treatment (40-s time course). Note formation of elongated filopodia within 10 s after B/B treatment. Shown second: mCherry-iDimerize- ΔNαCat$^{KKR<3A}$ mutant expressing cell before (left) and after (right) B/B treatment (60-s time course). Note no formation of elongated filopodia after B/B treatment.



Video 2. **αCat forced dimerization promotes protrusion-dependent cell–cell contact.** Split-screen video of mCherry-iDimerize-WT-ΔNαCat–expressing (left) and ΔNαCat$^{KKR<3A}$ mutant–expressing (right) cell couplets (red) coinfected with GFP-LifeAct (green). Labels and time stamps are incorporated within corresponding image frames. Note that addition of the B/B-dimerizer allows WT ΔNαCat-expressing couplets to close a gap between the two cells (yellow arrows) and remain in close proximity; this gap reappears upon addition of the wash-out (W/O) ligand. This activity is less prominent in an ΔNαCat$^{KKR<3A}$ mutant couplet.



Video 3. **EGF promotes αCat cortical recruitment independently of the cadherin–catenin complex.** Live imaging of GFP-full-length-αCat-expressing A431D cells (cadherin–catenin negative) and stimulation with EGF (CN02; Cytoskeleton). Labels and time stamps are incorporated within corresponding image frames. Note that GFP–αCat is rapidly recruited to the cortex upon EGF stimulation (within 1 s), with concomitant decrease in cytoplasmic staining, and subsequent appearance of filopodia (within 5 s, red arrows).



Video 4. **αCat basic patch contributes to epithelial cohesion and migration.** Live imaging of part 1: mCherry-full-length-αCat-restored R2/7 cells imaged for 12 h postwounding at 20×; WT, full-length αCat (FLαCat; shown first) and charge mutant αCat (FLαCat$^{KKR<3A}$; shown second) are compared. Images taken every 10 s during time course. Red arrows indicate αCat enrichments to the lamellipodial edge, which were quantified in Fig. 7 B. Part 2: Live imaging at 40× of mCherry-full-length-αCat and mutant αCat$^{KKR<3A}$-expressing cells (red) coinfected with GFP-LifeAct (green). Labels and time stamps are incorporated within corresponding image frames. Note colocalization of αCat with LifeAct (yellow arrowheads) and formation of radial protrusions (white arrows) during epithelial sheet wound closure.

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