The tumor suppressor adenomatous polyposis coli controls the direction in which a cell extrudes from an epithelium

Thomas W. Marshall[a], Isaac E. Lloyd[a], Jean Marie Delalande[a,*], Inke Näthke[b], and Jody Rosenblatt[a]

[a]Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84112; [b]Cell and Developmental Biology, University of Dundee, Dundee DD1 5EH, Scotland, United Kingdom

ABSTRACT **Despite high rates of cell death, epithelia maintain intact barriers by squeezing dying cells out using a process termed cell extrusion. Cells can extrude apically into the lumen or basally into the tissue the epithelium encases, depending on whether actin and myosin contract at the cell base or apex, respectively. We previously found that microtubules in cells surrounding a dying cell target p115 RhoGEF to the actin cortex to control where contraction occurs. However, what controls microtubule targeting to the cortex and whether the dying cell also controls the extrusion direction were unclear. Here we find that the tumor suppressor adenomatous polyposis coli (APC) controls microtubule targeting to the cell base to drive apical extrusion. Whereas wild-type cells preferentially extrude apically, cells lacking APC or expressing an oncogenic APC mutation extrude predominantly basally in cultured monolayers and zebrafish epidermis. Thus APC is essential for driving extrusion apically. Surprisingly, although APC controls microtubule reorientation and attachment to the actin cortex in cells surrounding the dying cell, it does so by controlling actin and microtubules within the dying cell. APC disruptions that are common in colon and breast cancer may promote basal extrusion of tumor cells, which could enable their exit and subsequent migration.**

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INTRODUCTION

Epithelia provide a protective coat for the organs that they encase; yet cell division and death occur continuously and could impair this barrier. To preserve the barrier function when epithelial cells die, the surrounding cells squeeze the dying cell out by a process termed epithelial cell extrusion. To extrude, a dying cell signals its live, neighboring cells to form and contract an actin and myosin ring that squeezes it out of the epithelium while simultaneously closing any gaps that might have formed by the dying cell's exit (Rosenblatt *et al.*, 2001; Gu *et al.*, 2011). Extrusion typically occurs apically but can also occur basally, depending on whether the ring contracts at the base or apex of the cell, respectively. Apically extruded cells are eliminated into the lumen of an organ, whereas basally extruded cells are pushed into the underlying tissue (Slattum *et al.* 2009). Although cells targeted for apoptosis extrude from epithelia, live cells can also be extruded (Gibson and Perrimon 2005; Shen and Dahmann 2005; Monks *et al.* 2008). The direction that a live cell extrudes has an even greater impact on its subsequent fate. For example, neuroblasts delaminate from the neuroepithelium in *Drosophila* embryos by a process that appears to be similar to basal extrusion (Hartenstein *et al.* 1994). Cancer cells that bypass apoptotic signals by up-regulating inhibitors of apoptosis or survival signaling or by down-regulating proapoptotic signals (Hanahan and Weinberg, 2011) may still be eliminated if they extrude apically. However, basal extrusion could enable their exit from the epithelium into the underlying tissue and allow these cells to migrate to other parts of the body. Therefore understanding what regulates the direction in which a cell extrudes may be important for developmental differentiation or the potential for a cancer cell to invade.

Our previous studies showed that microtubule reorientation in the cells neighboring a dying cell is important for controlling the direction in which a cell extrudes (Slattum *et al.* 2009). Microtubules target p115 RhoGEF to activate actomyosin contraction near the

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*Present address: Institute of Child Health, University College London, London WC1N 1EH, United Kingdom.

Address correspondence to: Jody Rosenblatt (jody.rosenblatt@hci.utah.edu).

Abbreviations used: APC, adenomatous polyposis coli; HBE, human bronchial epithelial cells, 16HBE14; shNS, nonspecific short hairpin RNA.

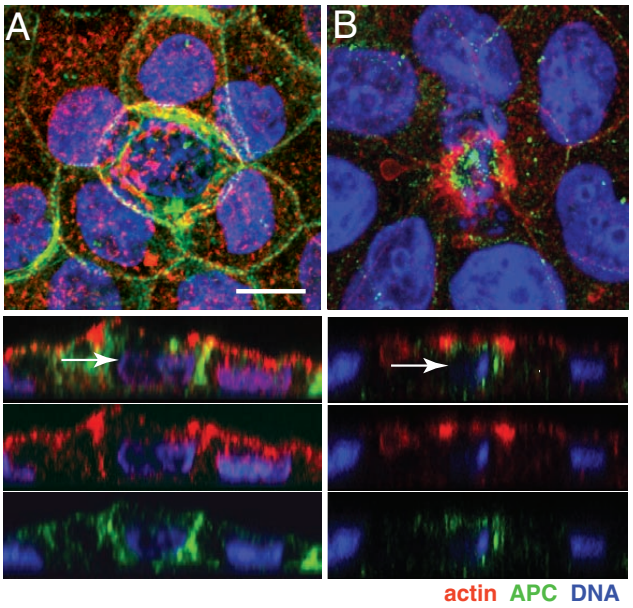
base of the cell to extrude it apically. Disrupting microtubules alters actomyosin localization, increasing the frequency of basal extrusion events. Thus proteins that coordinate microtubules must be involved in these processes. Of importance, microtubule disruption did not completely reverse the direction of extrusion, suggesting that other factors are important for controlling extrusion polarity.

A good candidate for controlling both actin and microtubules during extrusion is adenomatous polyposis coli (APC), a 312-kDa tumor suppressor protein that acts as a scaffold for F-actin, microtubules, microtubule end–binding protein-1 (EB1), β-catenin, and other proteins. APC is truncated in most familial adenomatous polyposis and >80% of spontaneous colorectal cancer cases (Näthke, 2004; Aoki and Taketo, 2007). Although many studies suggest that APC truncation promotes colorectal oncogenesis by activating Wnt signaling via β-catenin misregulation or genetic instability, it is important to note that APC truncation also eliminates the basic, EB1, and PDZ-binding domains, which can lead to cellular defects that could promote colorectal cancer progression (Fodde *et al.*, 2001; Zumbrunn *et al.*, 2001; Aoki and Taketo, 2007; McCartney and Näthke, 2008). Although the effects of APC on the cytoskeleton have been examined, little is known of the function of APC in maintaining the integrity of the epithelium or tissue homeostasis. Because APC can link actin with microtubules, we sought to determine whether APC plays an important role in controlling the direction in which cells extrude from epithelia.

RESULTS

To determine whether APC plays a role in cell extrusion, we examined APC localization during apical and basal extrusion of apoptotic cells from a cultured human bronchial epithelial monolayer, 16-HBE-14o(HBE) (Cozens et al., 1994), induced to extrude with UV C apoptotic stimulus (Rosenblatt et al., 2001). APC typically localizes near the apex of HBE cells in a nonextruding epithelial monolayer (Figure 1D), as seen previously in other epithelial cell lines and colon sections from mice (Reinacher-Schick and Gumbiner, 2001). During apical extrusion, APC shifts to the base of the cell near sites where actomyosin contracts (Figure 1A, quantified in C). On the other hand, when cells extrude basally, APC remains near the apex, where the actin ring forms and contracts (Figure 1B, quantified in C). Thus APC localizes near sites of actomyosin contraction during both apical and basal extrusion.

To test the function of APC during extrusion, we used lentiviral-based short hairpin RNA (shRNA) constructs to knock down APC in HBE cells. On induction of extrusion, control HBE cells extrude apically ~76% of the time, whereas the remainder extrude basally (Figure 2A). Depletion of APC (Figure 2B) significantly shifted cells from extruding predominantly apically to basally (from ~24% to ~64% basal extrusion), whereas expression of a nonspecific short hairpin RNA (shNS) had no affect on the extrusion direction (Figure 2A). Depletion of APC also decreased the rate of UV-induced death rates by ~20% (Figure 2C). Similar reduced death rates were noted in other cell lines depleted of APC (Dikovskaya et al., 2007*).* Small interfering RNA–mediated knockdown of APC with a different sequence gave similar results (57% basal extrusion), suggesting that the shift in extrusion direction was not due to off-target effects. To rule out any other inhibitory effects that might be caused by UV irradiation, we also tested the effects of APC knockdown after inducing apoptosis with etoposide, a topoisomerase II inhibitor that induces DNA strand breaks. Similarly, 75% of control knockdown cells and 51% of the shAPC cells extrude apically following etoposide treatment (Figure 2D). Thus APC function is critical for driving extrusion apically.



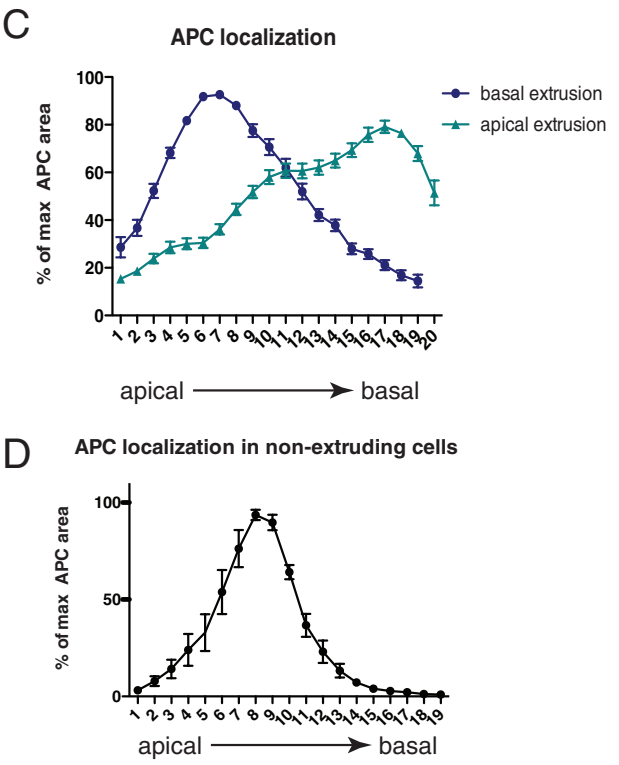


FIGURE 1: APC localization during apical (A) and basal (B) extrusion. Confocal projections and XZ sections below indicate that APC (green) localizes to the basolateral surface during apical extrusion and to the apical surface during basal extrusion. (C) Quantification. Red, actin; blue, DNA; white arrows, extruding cell. APC localization in nonextruding cells is predominantly apical (D). Scale bar, 10 μm.

Although total APC protein is required for apical extrusion, we wondered whether epithelia expressing APC C-terminal truncations typically found in most colorectal cancers would affect the extrusion direction. Most APC mutations occur in the mutation cluster region and delete the basic, EB1, and PDZ domains, as depicted in Figure 3A (Zumbrunn *et al.*, 2001; Näthke, 2004; Aoki and Taketo, 2007). We found that DLD-1 cells, a colorectal carcinoma cell line expressing truncated APC, predominantly extrude basally (~77%;

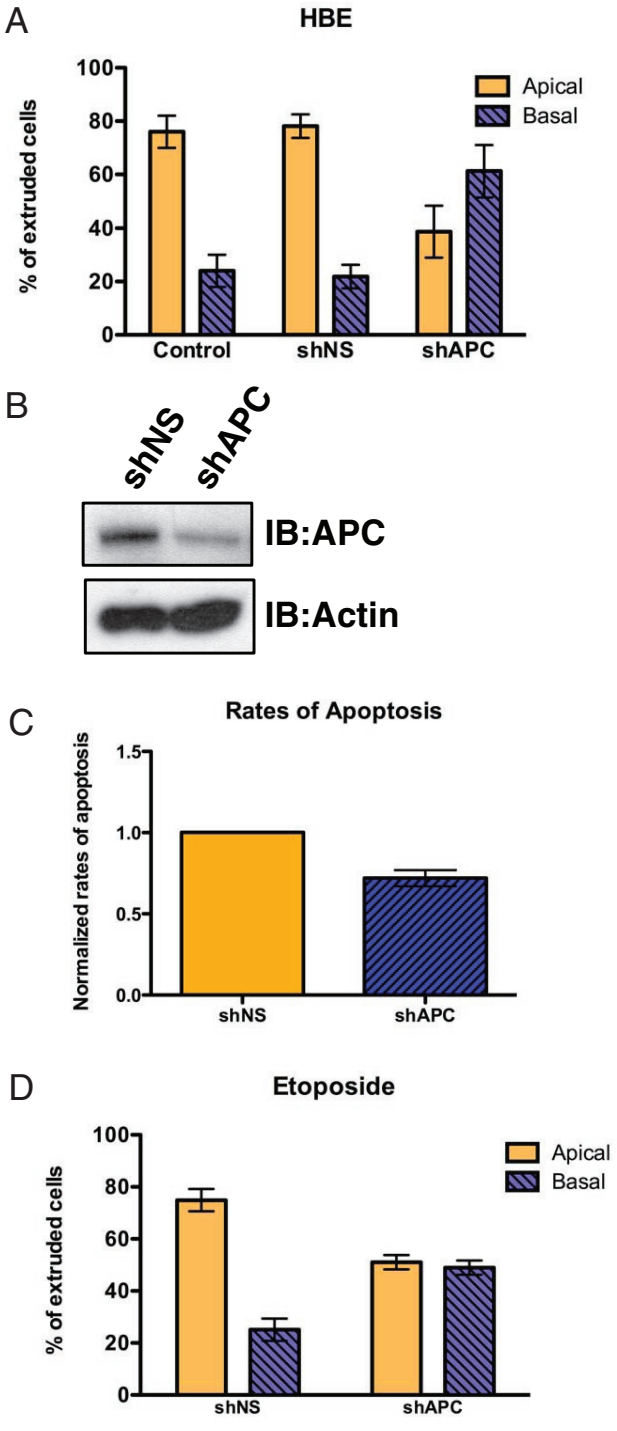


FIGURE 2: Depletion of APC biases cells to extrude basally. (A) Quantification of cell extrusion events of HBE cells or HBE cells that express either a nonspecific shRNA (shNS) or an shRNA that targets APC transcripts (shAPC). $p < 0.001$ by Student's *t* test comparing control or shNS to shAPC. (B) Western blots of HBE shNS or shAPC cell lysates for APC or actin (control). (C) Monolayers of HBE cells expressing either shNS or shAPC were exposed to UV irradiation. After 2 h, cells were fixed and immunostained for active caspase-3. The number of apoptotic cells from five representative fields was counted. N = 3. $p = 0.014$ by Student's *t* test. (D) Quantification of cell extrusion events of HBE cells expressing either shNS or shAPC, where apoptosis is induced by exposure to 10 μM etoposide for 6 h. $p < 0.001$ by Student's *t* test.

Figure 3B, control). Because attachment of epithelial cells to a glass coverslip might alter cell adhesion and artificially affect extrusion directionality, we tested whether APC is also important for controlling the direction of extrusion in vivo. We analyzed the frequency of apical and basal extrusions from $APC^{mcr/mcr}$ zebrafish epidermis expressing a similar APC truncation (Hurlstone *et al.*, 2003) compared with wild-type zebrafish epidermis. We found that 66% of cells from $APC^{mcr/mcr}$ fish epidermis extruded basally, whereas 100% of the cells from the wild-type zebrafish extruded apically (Figure 3C, D). These data indicate that apical extrusion requires the APC C-terminus.

To identify the APC domains required for apical extrusion, we next tested the ability of various domains of the APC C-terminus to rescue apical extrusion in DLD-1 cells (depicted in Figure 3A). Expression of the entire C-terminal fragment missing in DLD-1 cells except for the β-catenin–binding domains (CT) or the same fragment lacking the PDZ-binding domain (CTΔPDZ) were able to shift most DLD-1 cells to extrude apically (Figure 3B), suggesting that interactions with β-catenin or proteins that interact with the PDZ-binding domain (Aoki and Taketo, 2007) are dispensable for apical extrusion. The remaining part of the APC C-terminus is composed of the basic domain, which binds microtubules and enhances formin-mediated actin polymerization (Okada et al., 2010), and the EB1-binding domain, which promotes microtubule stability and polymerization. We found that expression of either the basic or EB1-binding domain alone is sufficient to induce apical extrusion (Figure 3B). Because both domains can directly or indirectly bind microtubules, we reasoned that the microtubule-binding function is critical for APC to control apical extrusion.

Because our previous data suggested that microtubules were required in cells surrounding an extruding cell (Slattum *et al.*, 2009), we hypothesized that APC also functions in these cells to elicit apical extrusion. To test which cells require APC, we assayed extrusion in monolayers mosaically depleted of APC by mixing knockdown cells, marked by green fluorescent protein (GFP) coexpression, with wild-type cells at a 1:10 ratio. We then quantified extrusion when APC was depleted only in the dying cell but not in the surrounding cells. Surprisingly, we found that depleting APC in the dying cell alone caused extrusion to occur predominantly basally, suggesting that APC controls extrusion in a cell-autonomous manner (Figure 3E). If loss of APC in dying cells drives basal extrusion, we wondered whether expressing the APC C-terminus in DLD-1 cells was sufficient to induce apical extrusion cell autonomously. To test this, we mosaically expressed CTΔPDZ in DLD-1 cells and found that only when the dying cell expressed the APC C-terminus did extrusion shift from predominantly basal to apical (Figure 3F). Together, these data show that APC functions cell autonomously to control apical extrusion.

We next determined the mechanism by which APC cell autonomously controls apical extrusion. Because APC binds microtubule ends and only its microtubule-binding domains are required to rescue apical extrusion in APC C-terminal mutant cells, we next tested how APC truncation affects microtubule localization during extrusion. Microtubules are highly disorganized in DLD-1 cells surrounding a dying cell compared with those in wild-type HBE monolayers, which point toward the extruding cell (Figure 4A). Similarly, microtubules reorient toward the extruding cell in wild-type zebrafish epidermis but are greatly reduced and disorganized in APC mutant fish epidermal cells surrounding an extruding cell (Figure 4B), suggesting that APC controls microtubule dynamics and reorientation during extrusion. Furthermore, by cell fractionation, we found that HBE cells expressing shAPC contained significantly more soluble tubulin protein than did shNS-expressing cells (Supplemental Figure 1). To

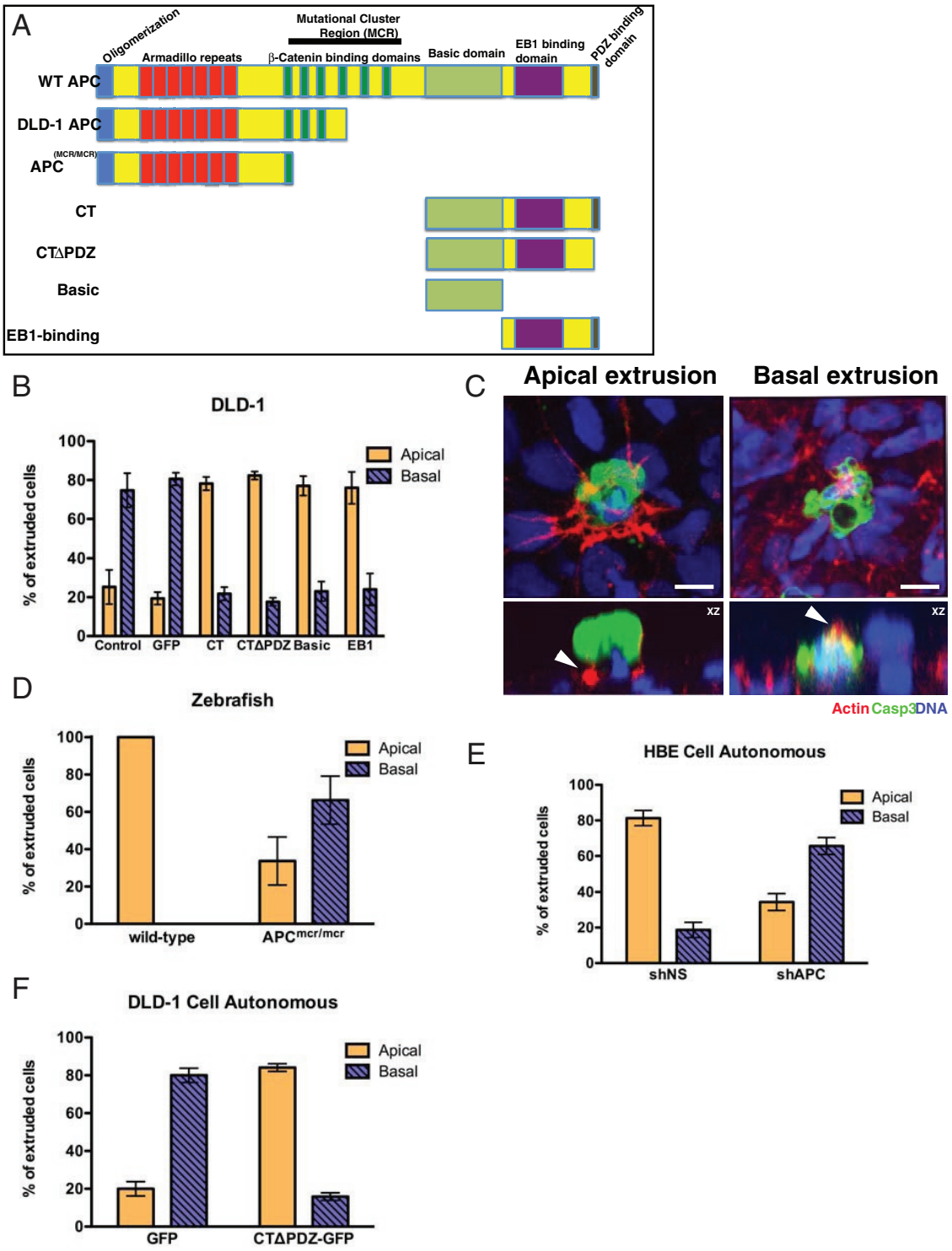


FIGURE 3: Cells expressing an oncogenic, truncated form of APC extrude basally in a cell autonomous manner. (A) Depiction of APC domains. (B) Quantification of cell extrusion events for DLD-1 cells or DLD-1 cells expressing GFP, CT, CTΔPDZ, basic, or EB1-binding domains. $p < 0.001$ by Student's *t* test comparing GFP to CT, CTΔPDZ, basic, or EB1-binding domains. (C) Representative images of either apical or basal extrusions from wild-type or $APC^{mcr/mcr}$ zebrafish epidermis (bars, 10 μm). (D) Quantification, where $p < 0.001$ by Student's *t* test comparing wild-type to $APC^{mcr/mcr}$. (E) Quantification of cell autonomous extrusion events of HBE shNS or shAPC cells. $p < 0.001$ by Student's *t* test comparing shNS to shAPC. (F) Quantification of cell autonomous extrusion events of DLD-1 GFP or CTΔPDZ cells. $p < 0.001$ by Student's *t* test comparing GFP to CTΔPDZ. Error bar, SEM.

test whether microtubules are, in fact, critical for APC function in controlling the direction of extrusion, we depolymerized microtubules in CT-rescued DLD-1 cells with nocodazole. Depolymerizing microtubules interfered with the ability of CT to rescue DLD-1 cells, similar to that seen with nocodazole treatment of wild-type monolayers (50 ± 5%; Slattum *et al.*, 2009). As expected, it had no effect

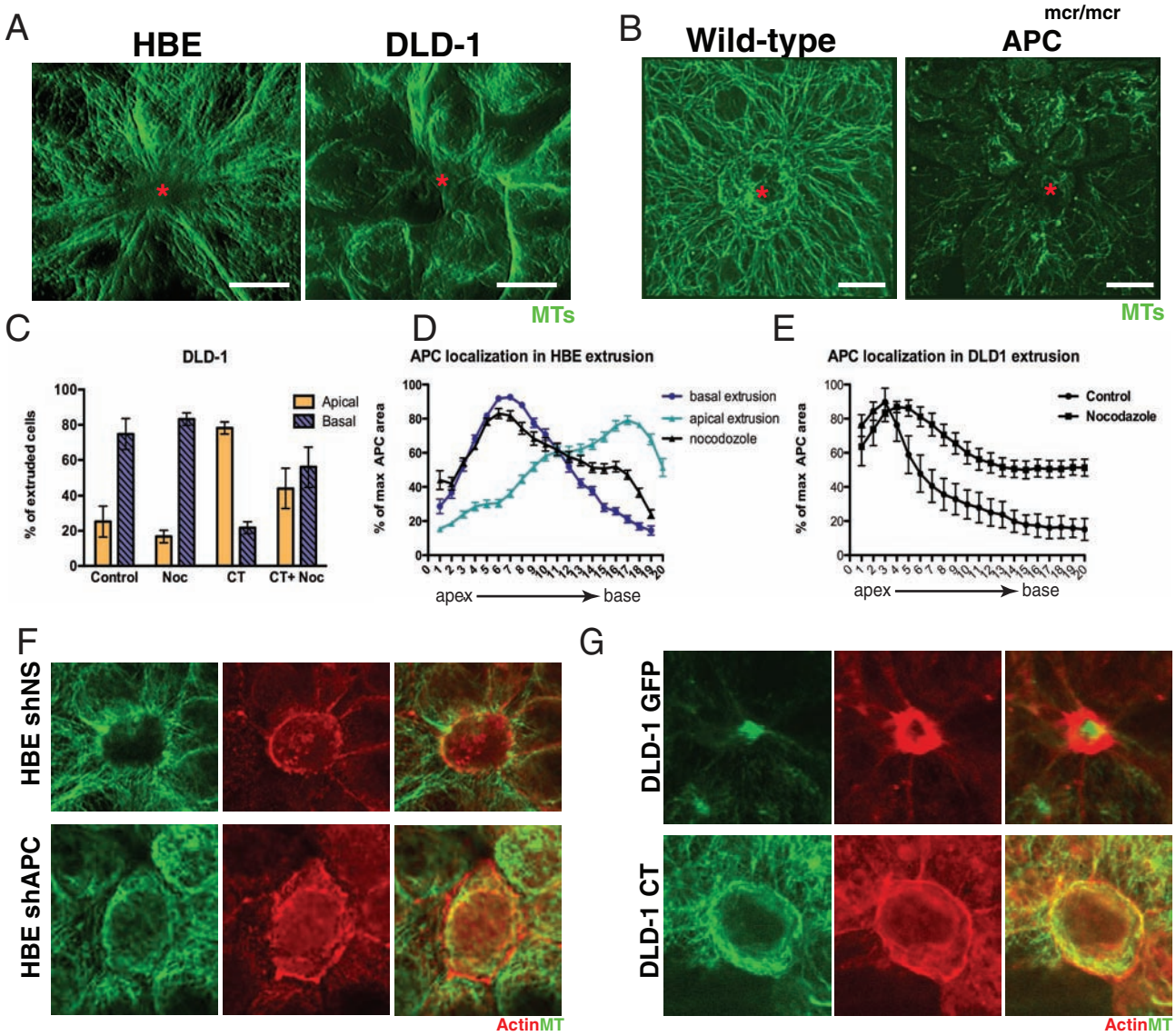


FIGURE 4: APC and microtubules reciprocally regulate each other during extrusion. (A) Deconvolved images of microtubules (MT) in either HBE or DLD-1 cells surrounding an extruding cell (asterisk). (B) Confocal images of microtubules in either wild-type or APC$^{mcr/mcr}$ zebrafish epidermal cells surrounding an apically and basally extruding cell (asterisk), respectively. (C) Quantification of extrusion frequency of DLD-1 cells or cells expressing CT treated with DMSO (control) or 10 μM nocodazole. There is no significant difference between DLD-1 control and nocodazole-treated cells. $p < 0.001$ by Student's *t* test comparing CT to CT + nocodazole. (D) Localization of APC in HBE cells during apical and basal extrusion and around dying cells when microtubules are blocked with nocodazole. N = 10 for each condition. (E) APC localization in DLD-1 cells during basal extrusions with and without nocodazole. N = 9 for each condition. Error bars, SEM (F) Confocal image of F-actin (red) and microtubules (MT) (green) during extrusion of HBE shNS- or shAPC-expressing cells. (G) Confocal image of F-actin (red) and microtubules (MT) (green) during extrusion of DLD-1 GFP-expressing or CT-GFP–expressing cells. (F) Confocal image of F-actin (red) and microtubule (green) during extrusion of HBE shNS- or shAPC-expressing cells. (G) Confocal image of F-actin (red) and microtubules (MT) (green) during extrusion of DLD-1 GFP-expressing or CT-GFP–expressing cells.

on the basal extrusion frequency of control DLD-1 cells, as microtubules were already greatly disorganized, and APC is not able to interact with microtubules (Figure 4C). Thus microtubules are critical for APC to control the direction in which a cell extrudes.

Given that APC localizes to microtubule plus ends and is critical for microtubule reorientation during extrusion, we tested whether microtubules are reciprocally important for APC localization during extrusion. Although APC typically localizes at the base of the extruding ring during apical extrusions, disrupting microtubules with nocodazole prevented APC from relocalizing to the base of the cell (Figures 4D and 1C). Instead, most APC is retained near the apex, similar to that seen during basal extrusion and in nonextruding, background epithelia (Figure 4D). Nocodazole treatment of DLD-1 cells did not significantly shift APC localization from the apex of the cell, suggesting that other factors tether APC to apex of C-terminal–truncated cells (Figure 4E). Together, these data show that APC and microtubules are dependent on each other: microtubules control APC relocalization toward the base of an extruding cell during apical

extrusion, and APC controls microtubule stability in the extruding cell.

Although APC controls microtubule orientation in cells surrounding an extruding cell, we were puzzled by the fact that it is required only in dying, extruding cells. Therefore we decided to investigate whether the presence of APC only in the extruding cell could control microtubule reorganization in the cells surrounding it. To do so, we mixed APC knockdown cells 1:10 with wild-type HBE cells and investigated whether the wild-type cells surrounding an extruding cell depleted of APC could still reorient microtubules toward this extruding cell. Whereas HBE cells surrounding an extruding shNS-expressing cell had enriched microtubule staining at the contractile ring (Figure 4F), those surrounding an extruding cell expressing shAPC had greatly reduced microtubule staining (Figure 4F), indicating that APC is necessary for microtubule reorientation. Furthermore, whereas DLD-1 cells display no significant reorientation of microtubules toward the contractile ring, those surrounding a CT-expressing cell have increased microtubule staining at the ring (Figure 4G), indicating that expression of CT is sufficient to induce microtubule reorientation in neighboring APC mutant cells.

How can APC in the dying cell control microtubule reorientation in the neighboring cells that extrude it? Because the C-terminus of APC could induce myosin contraction via the basic or EB1-binding domains, we wondered whether myosin contraction within the dying cell could induce microtubule reorientation in the contacted surrounding cells. To test whether myosin contraction is required for microtubule reorientation, we blocked myosin contraction with either Y-27632 or ML-9 to inhibit Rho kinase or myosin light chain kinase, respectively, and immunostained for EB1. Either treatment blocked EB1 accumulation in live cells that bordered the apoptotic cell compared with control, dimethyl sulfoxide (DMSO)–treated cells (Supplemental Figure 2), suggesting that microtubule reorientation toward the dying cell requires myosin contraction.

Our data suggest that wild-type APC controls microtubule dynamics and actomyosin contraction, but it was not clear whether wild-type APC could coordinate both to control where contraction occurs during extrusion. Using a colocalization program, we found that APC clearly colocalizes with both microtubules and F-actin at sites of contraction during apical extrusion (Figure 5, A, A′, and C). In Figure 4, we showed that the microtubules and APC localization are clearly disrupted in cells containing the truncated APC mutant. However, we wondered whether APC localization with F-actin and microtubules was also aberrant in *randomly* occurring basal extrusions in *wild-type* epithelia. We found that in wild-type cells, APC colocalizes with actin at the contractile ring during basal extrusion but is uncoupled from microtubules at sites of contraction (Figure 5B). The difference between APC/microtubule/F-actin colocalization in apical versus basal extrusions is illustrated in Figure 5, A′ and B′, and quantified in Figure 5C. These data indicate that APC interacts only with actin during basal extrusion but interacts with both microtubules and actin during apical extrusion. Together, our data show that interactions between the APC C-terminus and microtubules are required for apical but not basal extrusion.

DISCUSSION

Here we show that APC is critical for dictating the direction in which a cell extrudes. Cells lacking APC or harboring a truncating APC mutation commonly found in colorectal cancer extrude predominantly basally in both cell culture and zebrafish epidermis in vivo. Of importance, either the C-terminal basic or the EB1-binding domain of APC is sufficient to induce apical extrusion in DLD-1 cells by regulating microtubule interactions with APC. Although our previous work suggested that contraction of cells surrounding a dying cell control the direction of extrusion (Slattum *et al*., 2009), here we find that APC functions in the dying cell to control where contraction occurs to drive apical extrusion.

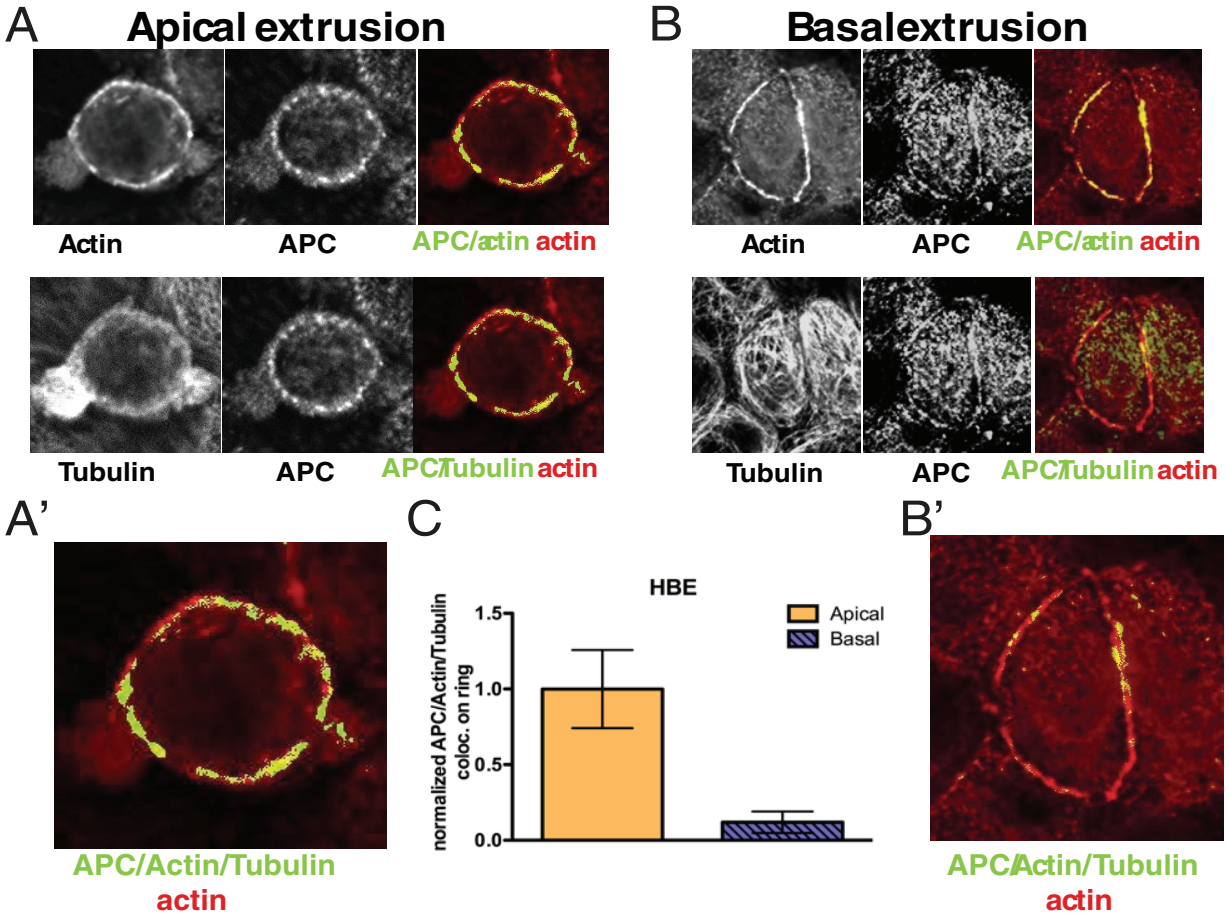
On the basis of our data, we propose a model for how APC functions in the dying cell to coordinate microtubules and actomyosin contraction in both the dying and neighboring cells during apical extrusion (Figure 5D). Microtubules and APC are interdependent: microtubules localize APC, and APC stabilizes and coordinates microtubules to target the cell cortex. In wild-type epithelia, APC and microtubules relocalize to the base of the dying cell to activate contraction at these sites (Figure 5D, top). Contraction of the dying cell at the base results in reorientation of microtubules in neighboring cells toward the dying cell, which helps to reinforce contraction in the surrounding cells.

Although it is not clear how microtubules and APC activate contraction, we favor a model in which microtubules promote RhoA-dependent contraction by targeting a Rho guanine nucleotide-exchange factor (GEF), such as p115 RhoGEF, that colocalizes with microtubules (Slattum *et al.*, 2009). Although APC can promote formin-mediated actin polymerization via the basic domain (Moseley *et al*., 2007; Okada *et al.*, 2010), the fact that the EB1-binding domain alone can cause apical extrusion of DLD-1 cells suggests that only APC interactions with microtubules are required to control where contraction occurs. Although contraction in the dying cell controls microtubule reorientation in the neighboring cells, it is not clear how it does so. One possibility is that contraction inside the dying cell pulls the membrane of the adherent surrounding cells and this force, in turn, reorients apically localized microtubules toward sites of contraction at the base of the extruding cell.

During basal extrusion, APC remains at the cell apex, likely because it is unable to interact with microtubules that would reorient it toward the base. This induces contraction near the apex of the cell resulting in basal extrusion (Figure 5D, bottom). Because APC, actin, myosin, and microtubules typically localize to the apex of a cell, we predict that any contraction activated in the cell during apoptosis would likely occur at the apex. Furthermore, contraction at the apex of the dying cell is not sufficient to recruit microtubules in the surrounding cells to the base of live cell/dead cell interface required for apical extrusion, and cells instead extrude basally.

The direction of extrusion is important for the fate of cells, especially when live cells extrude (Gibson and Perrimon, 2005; Shen and Dahmann, 2005; Monks *et al*., 2008). Apical extrusion removes cells into the lumen of an organ, whereas basal extrusion would force cells back into the tissue the epithelium encases. In development, stem cells delaminate, or extrude basally, from *Drosophila* neuroepithelium to differentiate into neurons (Hartenstein *et al*., 1994). If they extruded apically, they would be lost. Of note, *Drosophila* epithelia extrude cells basally (Gibson and Perrimon, 2005). Of interest, *Drosophila* APC basic domain shows little homology to mammalian APCs and does not appear to interact with microtubules (Bienz and Clevers, 2000). This might explain why *Drosophila* epithelia extrude cells basally.

The direction in which a cell extrudes might also be important for cancer progression. If cancer cells extrude apically, extrusion could act as a tumor suppressor by removing tumor cells into the lumen of the organ. For instance, ductal carcinoma in situ breast tumors are trapped within the lumen and may be caused by the apical extrusion of live cells. In cases such as colon cancer, in which the lumen is open to the digestive tract, apically eliminated cells may never be apparent. However, if tumor cells extrude basally, extrusion could enable their invasion if they can also cut through the underlying matrix. Although these cells would typically die, other alterations



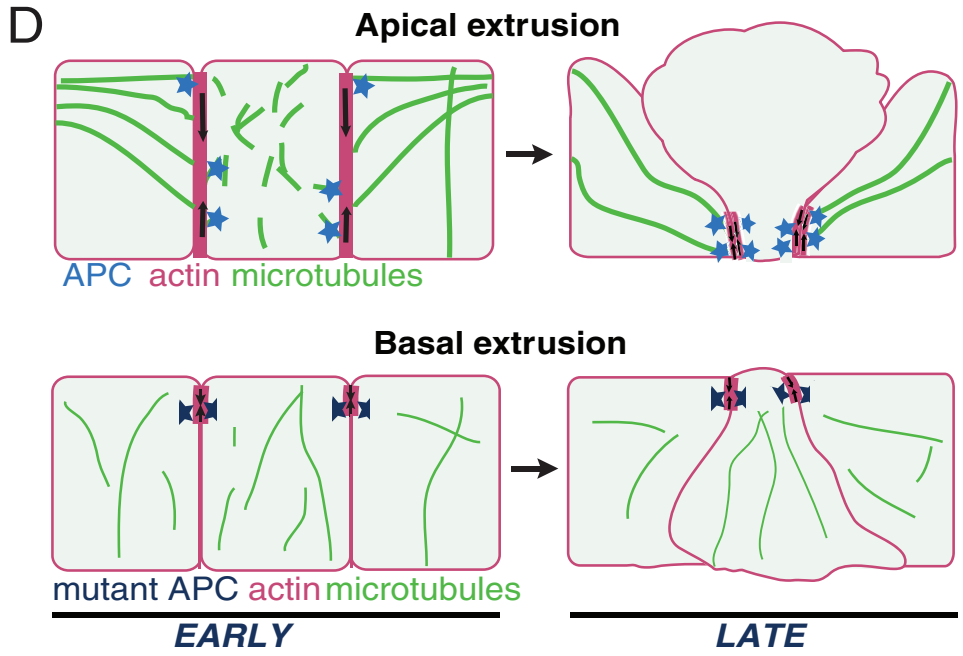


FIGURE 5: APC colocalizes with both F-actin and microtubules at sites of contraction only during apical extrusion. Immunofluorescence of F-actin (top) or tubulin (middle) with APC during apical (A) or basal extrusion (B). Colocalization of APC/F-actin/tubulin (green) at sites of contraction (red) (bottom, A' and B'). (C) Quantification of the colocalization of APC/F-actin/tubulin at the contractile ring. $p < 0.01$ by Student's *t* test comparing colocalization of these proteins during apical and basal extrusion. N = 8 for each condition. (D) Model of extrusion in wild-type APC (left) or truncated APC epithelial monolayers (right). In wild-type cells, APC controls microtubule dynamics, which in turn targets APC basolaterally, where it promotes actomyosin contraction. This contraction causes microtubules in the neighboring cells to target the basolateral surface and activate contraction, reinforcing apical extrusion. In APC-mutant cells, microtubules are less stable and cannot target APC to the base of the cell. As a result, apical contraction occurs, leading to basal extrusion.

frequently associated with carcinogenesis, such as increased polyploidy (associated with APC truncation), activation of KRas, and/or loss of p53 activity could increase survival of basally extruded cells, promoting their ability to invade the underlying tissue (Phelps *et al.*, 2009; Hanahan and Weinberg, 2011). Thus, we believe that our work suggests a new, added role for how APC functions as a tumor suppressor by eliminating transformed cells through apical extrusion and preventing their invasion.

MATERIALS AND METHODS

Cell culture

DLD-1 cells (American Type Culture Collection, Manassas, VA) were cultured in DMEM with high glucose and 5% fetal bovine serum (FBS), 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, MEM nonessential amino acids, and 100 µg/ml penicillin/streptomycin (all from Invitrogen, Carlsbad, CA) at 5% CO_2, 37°C. 16-HBE-14o (provided by D. Gruenert, California Pacific Medical Center, San Francisco, CA) and J. Porter (University College London, London, United Kingdom) were cultured in MEM containing Earle's salts, 1 g/l glucose, 2.2 g/l $NaHCO_3$, L-glutamine, 10% FBS, and 100 µg/ml penicillin-streptomycin (Invitrogen) in a flask coated with human fibronectin type I (BD Biosciences, San Diego, CA), bovine collagen I (PureCol; Inamed, Advanced BioMatrix, San Diego, CA), and BSA (Invitrogen) at 5% CO_2, 37°C. 293FT cells were cultured in DMEM with high glucose containing 10% FBS and 100 µg/ml penicillin/streptomycin at 5% CO_2, 37°C. Nocodazole and Taxol treatments and exposure to UV irradiation were performed as in Slattum *et al.* (2009). Cells were treated with etoposide (10 µM) for 6 h to induce apoptotic extrusion.

Molecular cloning

shRNA sequences were designed to target either human APC or a nonspecific hairpin that does not target any sequence in the human genome (Cai *et al.*, 2007). shAPC and siAPC targeting sequences used were, respectively, 5′-gagaacaactgcctgcaag-3′ (1972–1990 base pairs of human cDNA sequence) and 5′-aggggcagcaactgatgaaaa-3′. Oligonucleotides encoding the shRNA sequences were produced at the University of Utah Oligonucleotide Core Facility. pLL5.0 is a lentiviral expression plasmid containing a U6 promoter to drive expression of the shRNA sequence and a 5′-long terminal repeat to drive the expression of GFP (Cai *et al.*, 2007). All cells that are GFP positive also express the shRNA. Oligonucleotides were annealed and ligated into the *Hpa*I and *Xho*I sites in pLL5.0. The C-terminus of APC (CT-GFP) (amino acids 2039–2843) or CT-ΔTSV (amino acids 2039–2840) were PCR amplified from pEGFP-APC (Kroboth *et al.* 2007) and ligated into the *Sac*II/*Bam*HI sites of pLL5.0.

Lentiviral production and transduction

Lentiviral production and transduction were performed as previously described (Cai *et al.*, 2007). 16-HBE-14o cells were transduced with *Lentivirus* for 16 h and then the medium was changed. Effects of lentiviral transductions were examined by Western blot at 4–5 d posttransduction.

Immunofluorescence

HBE cells were plated on fibronectin/collagen I–coated coverslips and grown to confluency. DLD-1 cells were plated on untreated coverslips. HBE or DLD-1 cells were exposed to 1200 or 1800 µJ/cm^2 UV^{254}, respectively, and incubated for 2 h or 10 µM etoposide for 6 h. Cells were either fixed with ice-cold methanol for 2 min or 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min. Cells were then permeabilized with 0.5% Triton X-100 in PBS (PBS-T) for 5 min. Coverslips were blocked by incubating with 1% normal goat serum/5% fatty acid–free BSA in PBS for 15 min. Primary antibodies (APC 1:2000 [Näthke *et al.*, 1996], β-actin 1:500 [clone AC-74; Sigma-Aldrich, St. Louis, MO], α-tubulin 1:250 [Sigma-Aldrich], EB-1 1:50 [BD Biosciences], and Alexa Fluor–conjugated phalloidin [1:500, Invitrogen]) were applied in 1% BSA in PBS for 1 h. After washing coverslips three times with PBS-T, secondary antibodies (Alexa Fluor-conjugated, Molecular Probes, Invitrogen) and 4′,6-diamidino-2-phenylindole and/or 5 µM DRAQ5 (Axxora, San Diego, CA) were applied for 45 min. Coverslips were mounted onto slides with Prolong Gold mounting media (Molecular Probes, Invitrogen). Colocalization of APC/F-actin/tubulin was done with ImageJ Colocalize RGB plugin (National Institutes of Health, Bethesda, MD). To do so, images were threshholded, and measurements of colocalization were quantified only on the extrusion ring.

Zebrafish procedures

Fish were raised and kept under standard laboratory conditions at 28.5°C. Cell death in the epidermis was triggered using a cold shock: Briefly, 60 h–postfertilization AB wild-type or $APC^{-/-}$ (Hurlstone *et al.*, 2003) zebrafish were placed on ice for 30 min in E3 (0.5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$) + 20% DMSO and allowed to recover for 30 min at 20°C. Fish larvae were processed for immunohistochemistry as previously described (Westerfield, 1993).

Western blotting

HBE cells transduced to express shNS or shAPC were lysed with 1% Triton X-100 in PBS. Proteins in lysate were separated by SDS–PAGE, transferred to polyvinylidene fluoride (PVDF) membrane, and immunoblotted for APC or tubulin.

Microtubule fractionation

HBE cells expressing either shNS or shAPC were grown into monolayers. Cells were lysed with 1% Triton X-100 in PBS containing 1× protease inhibitor cocktail, phenylmethylsulfonyl fluoride, and 100 µM Taxol. Cytoskeletal (pellet) and supernatant (sup) fractions were separated by centrifugation. Equal amounts of protein were run by SDS–PAGE and transferred to PVDF membrane and immunoblotted for tubulin using standard techniques.

Statistical analysis

For all statistical analyses, experimental conditions were compared with controls (shNS or GFP) by individual Student's *t* tests. For extrusion assays, at least 300 cells were counted per experiment. For each result N = 3–5, where error bars indicate SDs.

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