

# LKB1 and AMPK maintain epithelial cell polarity under energetic stress

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LKB1 is mutated in both familial and spontaneous tumors, and acts as a master kinase that activates the PAR-1 polarity kinase and the adenosine 5' monophosphate-activated kinase (AMPK). This has led to the hypothesis that LKB1 acts as a tumor suppressor because it is required to maintain cell polarity and growth control through PAR-1 and AMPK, respectively. However, the genetic analysis of LKB1-AMPK signaling in vertebrates has been complicated by the existence of multiple redundant AMPK subunits. We describe the identification of mutations in the single *Drosophila melanogaster* AMPK

catalytic subunit AMPK $\alpha$ . Surprisingly, *ampk $\alpha$*  mutant epithelial cells lose their polarity and overproliferate under energetic stress. LKB1 is required in vivo for AMPK activation, and *lkb1* mutations cause similar energetic stress-dependent phenotypes to *ampk $\alpha$*  mutations. Furthermore, *lkb1* phenotypes are rescued by a phosphomimetic version of AMPK $\alpha$ . Thus, LKB1 signals through AMPK to coordinate epithelial polarity and proliferation with cellular energy status, and this might underlie the tumor suppressor function of LKB1.

## Introduction

*LKB1* is a serine/threonine kinase that is mutated in most cases of Peutz-Jeghers syndrome, which is an autosomal dominant disorder in which patients develop benign hamartomas and a high frequency of malignant tumors (Alessi et al., 2006). Furthermore, *LKB1* is also mutated in some sporadic cancers, such as 30% of lung adenocarcinomas, and the expression of the kinase is also down-regulated in a substantial portion of breast cancers (Sanchez-Cespedes et al., 2002). In both cases, tumors associated with *LKB1* mutations usually derive from epithelial tissue. *LKB1* is a master kinase that can potentially activate several downstream kinases by phosphorylating a conserved threonine in their activation loops (Lizcano et al., 2004). Two of these kinases have been extensively characterized: PAR-1/microtubule affinity-regulating kinases (MARKs) and AMPK (AMP-activated protein kinase). PAR-1 regulates cell polarity in numerous cell types and organisms (Kemphues et al., 1988; Bohm et al., 1997; Shulman et al., 2000; Benton and St Johnston, 2003; Cohen et al., 2004). AMPK acts as a cellular energy sensor because it is activated by AMP, which accumulates when ATP levels are low (Kahn et al., 2005). AMPK then mediates the cellular response to energetic stress by activating energy-

limiting activities, while inhibiting energy-consuming ones, such as translation and proliferation. *LKB1* regulates both cell polarity and cell growth and division in cell culture and in vivo (Kemphues et al., 1988; Tiainen et al., 1999; Martin and St Johnston, 2003; Baas et al., 2004; Narbonne and Roy, 2006). One hypothesis envisions *LKB1* signaling mediated by PAR-1 regulating cell polarity, whereas *LKB1* signaling through AMPK could control cell growth and proliferation. However, recent cell culture experiments suggest that AMPK also plays a role in the polarization of MDCK cells by promoting tight junction assembly (Zhang et al., 2006; Zheng and Cantley, 2007). We show that *LKB1* and AMPK are required to maintain epithelial polarity and integrity under energy-limiting conditions in *Drosophila melanogaster*. Therefore, these results provide a potential mechanism to coordinate the regulation of cell polarity and proliferation with energy conditions within a multicellular animal.

## Results and discussion

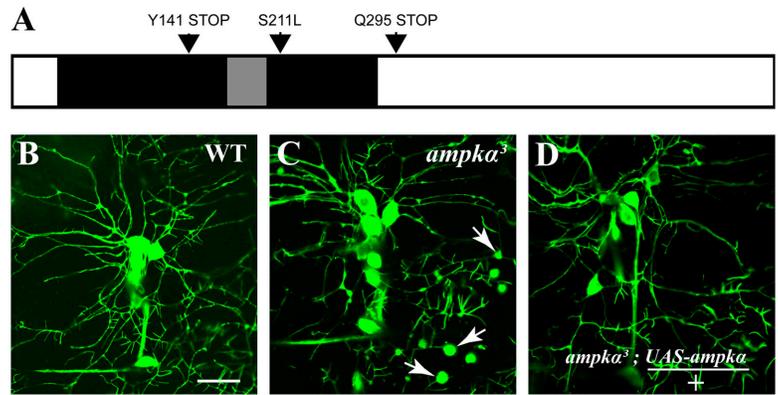
AMPK contains three protein subunits,  $\alpha$ ,  $\beta$ , and  $\gamma$ , which form a heterotrimer. The  $\alpha$  subunit (AMPK $\alpha$ ) encodes a highly conserved serine/threonine kinase, and the other subunits are regulatory. From a *D. melanogaster* forward genetic screen for mutants affecting larval neuronal dendrite development (Medina et al., 2006), we identified several lethal mutations in AMPK $\alpha$ . The ethylmethanesulfonate mutants, *ampk $\alpha$ <sup>1</sup>* and *ampk $\alpha$ <sup>2</sup>*, contain a

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Abbreviations used in this paper: AMPK, AMP-activated kinase; aPKC, atypical PKC; MARK, microtubule affinity-regulating kinase.

Figure 1. **Identification of mutations within the single *D. melanogaster ampk $\alpha$*  gene.** (A) Schematic domain representation of AMPK $\alpha$  and corresponding genetic lesions in mutants. The serine/threonine kinase domain (black, aa 39–280) and T-Loop (gray, aa 167–194) are shown with the sites of mutations, S211L, Q295STOP, and Y141STOP, for *ampk $\alpha$ <sup>1</sup>*, *ampk $\alpha$ <sup>2</sup>*, and *ampk $\alpha$ <sup>3</sup>*, respectively. (B) Representative image of wild-type da neurons expressing an *Actin::GFP* fusion transgene in a second instar larva. (C) *ampk $\alpha$*  mutants display enlarged plasma membrane domains (arrows) in sensory neuron dendrites, but not axons. (D) A wild-type *ampk $\alpha$*  transgene expressed autonomously within da neurons completely rescues the dendrite phenotype. (B–D) Background genotypes are *w*; *Gal4109[2]80*, *UAS-actin::GFP*. Anterior at left and dorsal at top. Bars, 20  $\mu$ m.



single amino acid change (S211L, completely conserved) and a premature stop codon (Q295 STOP), respectively, whereas *ampk $\alpha$ <sup>3</sup>* has a 16-bp deletion creating a stop codon (Y141 STOP; Fig. 1 A). All *ampk $\alpha$*  mutants, whether homozygous or in trans with a deletion covering the locus, displayed a completely penetrant and nearly identical phenotype, with greatly enlarged plasma membrane domains in dendrites, but not in axonal compartments (Fig. 1 C; unpublished data). In addition, *ampk $\alpha$ <sup>1</sup>* and *ampk $\alpha$ <sup>3</sup>* could be rescued to viability with either a chromosomal duplication carrying a wild-type *ampk $\alpha$*  gene, a wild-type AMPK $\alpha$  transgene, or a transgene that is tagged with the red fluorescent protein mCherry (Fig. 1 D; see Materials and methods). The requirement for *ampk $\alpha$*  is cell autonomous because transgene expression within only neurons rescues the phenotype (Fig. 1 D). Therefore, these mutations represent the first knockouts of the single AMPK catalytic subunit in the *D. melanogaster* genome and allow the genetic analysis of AMPK function in vivo.

Although *ampk $\alpha$*  mutants display a strong phenotype in larval neuronal dendrites, no phenotype was observed in early larval *lkb1* mutants (unpublished data), probably because of the large maternal contribution of this protein. To explore the relationship between AMPK $\alpha$  and LKB1 function without the confounding issues caused by the differing maternal contributions of each protein, we chose to examine follicle cells of the *D. melanogaster* ovary. The follicle cells that surround the oocyte have a typical epithelial architecture with a highly polarized actin cytoskeleton in which the apical surface is marked by dense actin bundles in the apical microvilli, the lateral cortex is covered by a thin actin mesh, and the basal side contains a prominent network of parallel actin stress fibers. This polarized organization of actin typifies many epithelia, including the main mammalian tissue culture model for polarized epithelial cells, MDCK cells (Fievet et al., 2004). We did not observe any actin phenotypes in *ampk $\alpha$ <sup>3</sup>* mutant follicle cells using standard detection procedures (Fig. 2 A). Because AMPK is maximally activated under low cellular energy levels, we also tested the influence of energy stress by strongly reducing the availability of sugar in the *D. melanogaster* culture medium. Under these conditions, *ampk $\alpha$ <sup>3</sup>* mutant cells display a strong actin phenotype (Fig. 2 A). The density of basal stress fibers is strongly reduced, whereas the amount of apical F-actin increases. This phenotype is highly penetrant under these starvation

conditions (98%;  $n = 49$ ) and is also observed with the two other alleles of *ampk $\alpha$* .

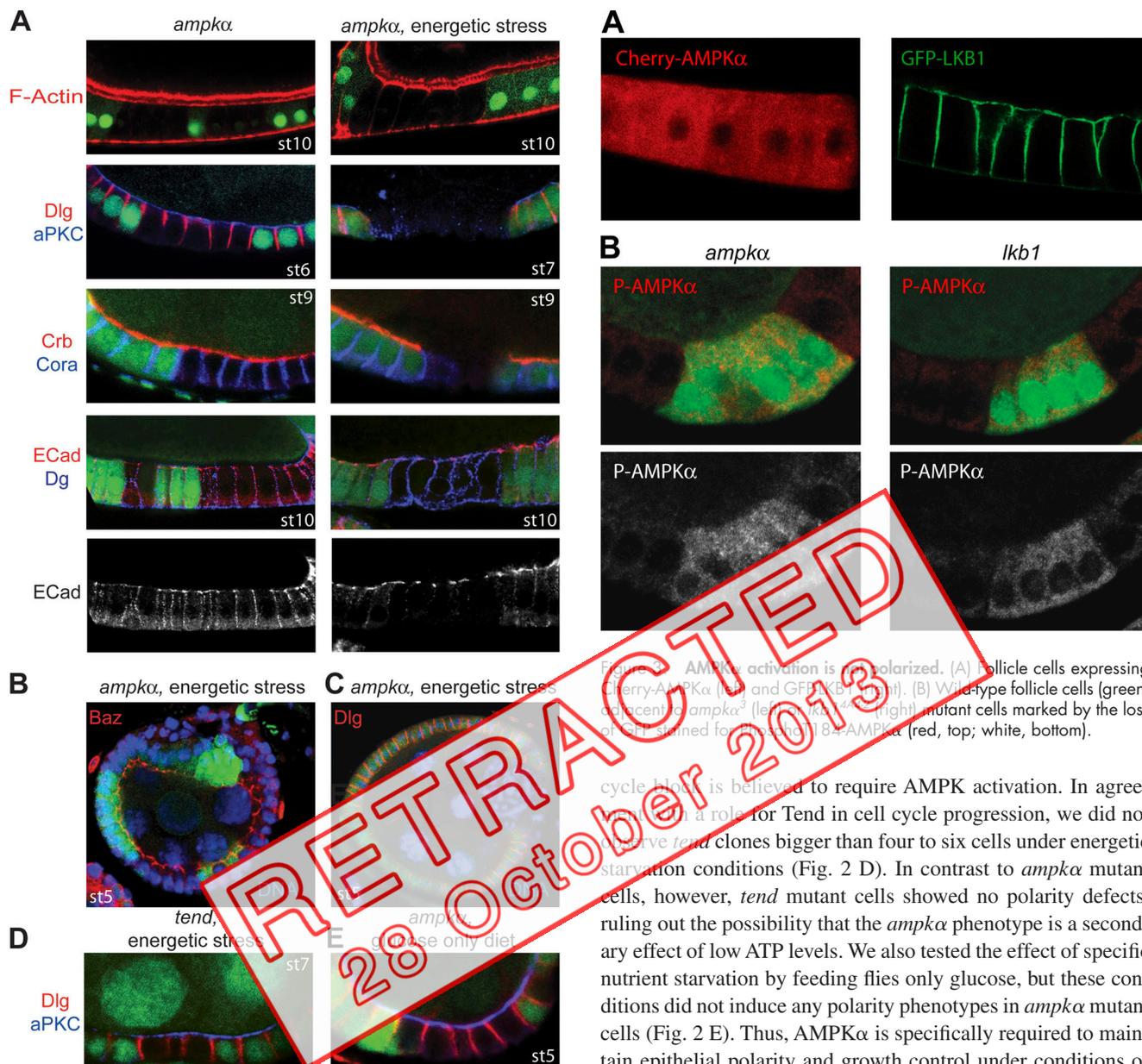
Because this phenotype reflects a disruption of the apical–basal polarity of the actin cytoskeleton, we examined other polarity markers within these cells. *ampk $\alpha$*  mutant clones induced in adult flies fed with high sugar diets did not show any polarity phenotypes, which is consistent with the absence of an actin phenotype under these conditions (Fig. 2 A). Under energetic starvation conditions, however, *ampk $\alpha$*  mutant cells show a fully penetrant loss of polarity. Apical markers, such as atypical PKC (aPKC) and Crumbs (Crb) lose their cortical localization completely and are found down-regulated, as do the lateral markers Disc-large (Dlg) and Coracle (Cora; Fig. 2 A). In contrast, Dystroglycan (Dg), which is normally enriched at the basal cortex, extends into the lateral domain, and occasionally reaches the apical membrane (Fig. 2 A). This suggests that the phenotype represents an expansion of the basal domain at the expense of the lateral and apical domains.

Although most aspects of apical–basal polarity are completely disrupted in *ampk $\alpha$*  mutant clones under energetic stress, E-cadherin (ECad) is usually still enriched at the adherens junctions, suggesting that the altered polarity is not a secondary consequence of a loss of intercellular adhesion. The subapical localization of Bazooka (Baz) with cadherin is also maintained in most cases (Fig. 2 B). This indicates that Baz is not in a complex with aPKC in columnar follicle cells, but is instead associated with the adherens junctions, as has recently been described in the *D. melanogaster* embryo and in neuroepithelial cells of the Zebrafish neural tube (Harris and Peifer, 2005; Afonso and Henrique, 2006).

A considerable proportion of *ampk $\alpha$*  mutant clones show a more severe phenotype, in which the cells round up and lose their epithelial organization to form multiple layers of cells (Fig. 2 B). In these cases, Baz is now also absent from the cell cortex. Finally, larger mutant clones, particularly at the anterior or the posterior of the egg chamber, show a complete loss of epithelial organization and overproliferate to form small, tumor-like growths (Fig. 2 C).

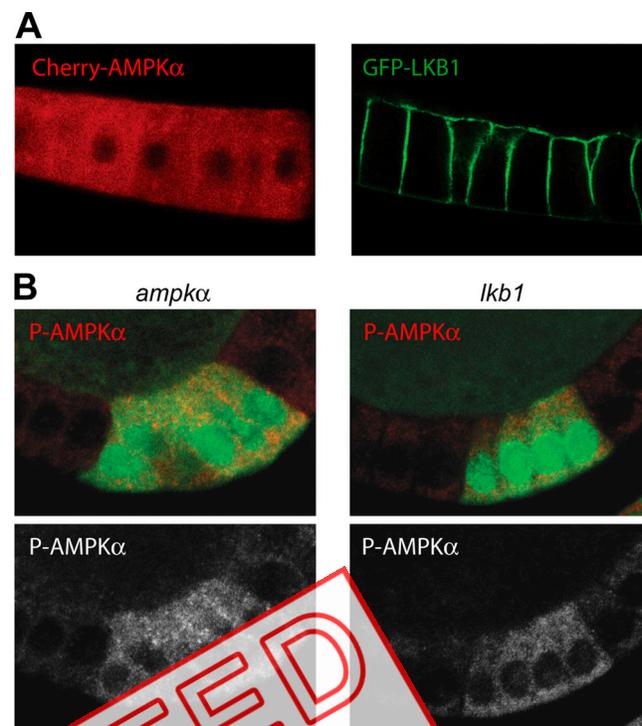
As one proposed function for AMPK is to sense and maintain cellular ATP levels, the polarity phenotype observed under starvation conditions could be caused by low cellular ATP concentrations. To test this hypothesis, we examined cells that were mutant for *tenured* (*tend*). *Tend* encodes a mitochondrial cytochrome

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**Figure 2. *ampkα* is required to maintain epithelial polarity under energetic stress.** (A) *ampkα*<sup>3</sup> mutant follicle cell clones under normal (left) or energetic stress conditions (right). Mutant cells are marked by the absence of GFP (green). Markers are indicated to the left, as follows: F-actin, red; Dlg, red; aPKC, blue; Crb, red; Cora, blue; DECad, red; and Dg, blue. The apical domain is at the top and faces the oocyte, which also contains a layer of cortical actin (top). (B) *ampkα*<sup>3</sup> clone stained for DNA (blue) and Baz (red). Baz is usually present at the apical domain of mutant cells, but is lost in the most severely affected clones that lose their epithelial organization and form multiple layers. (C) *ampkα*<sup>3</sup> clone stained for DNA (blue) and Dlg (red). Large *ampkα*<sup>3</sup> clones at the anterior or posterior poles of the egg chamber overproliferate to form tumorlike structures composed of unpolarized cells. (D) *tend* mutant cells stained for aPKC (blue) and Dlg (red). Cells maintain normal epithelial polarity. (E) *ampkα*<sup>3</sup> clone from a female fed on a glucose-only diet stained for aPKC (blue) and Dlg (red). These conditions of protein and lipid starvation do not affect the polarity of *ampkα*<sup>3</sup> mutant cells. The stage (st) of the egg chamber is indicated on each picture.

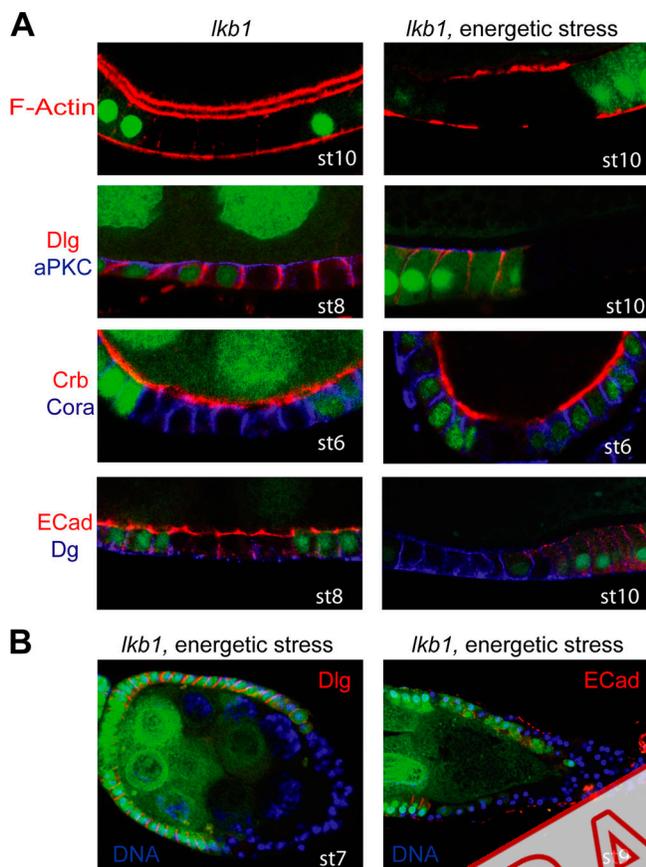
oxidase subunit; therefore, mutants have reduced intracellular ATP concentrations to levels sufficient to maintain cell survival and growth, but not cell division (Mandal et al., 2005). This cell



**Figure 3. AMPKα activation is polarized.** (A) follicle cells expressing Cherry-AMPKα (top) and GFP-LKB1 (bottom). (B) Wild-type follicle cells (green) adjacent to *ampkα*<sup>3</sup> (left) or *lkb1*<sup>3</sup> (right) mutant cells marked by the loss of GFP stained for phospho-184-AMPKα (red, top; white, bottom).

cycle block is believed to require AMPK activation. In agreement with a role for *Tend* in cell cycle progression, we did not observe *tend* clones bigger than four to six cells under energetic starvation conditions (Fig. 2 D). In contrast to *ampkα* mutant cells, however, *tend* mutant cells showed no polarity defects, ruling out the possibility that the *ampkα* phenotype is a secondary effect of low ATP levels. We also tested the effect of specific nutrient starvation by feeding flies only glucose, but these conditions did not induce any polarity phenotypes in *ampkα* mutant cells (Fig. 2 E). Thus, AMPKα is specifically required to maintain epithelial polarity and growth control under conditions of energetic stress.

Because our results indicate that *ampkα* plays a role in epithelial polarity, we assessed whether the localization of the protein itself is polarized. We also examined LKB1 localization, as it is a potential regulator of AMPK. Transgenic wild-type fusion proteins for both AMPKα and LKB1 rescue lethal null mutants to viability, and should therefore mimic the localizations of the endogenous proteins. LKB1-GFP is mainly found at the apical and lateral cortex of the follicle cells, and is absent from the basal domain (Fig. 3 A). This basal exclusion is surprising, as cortical localization of LKB1 requires its membrane targeting by prenylation of a conserved CAAX motif (Martin and St Johnston, 2003). This suggests that the lipid composition of the basal domain is different from the rest of the plasma membrane and/or that LKB1 posttranslational modifications are asymmetrically controlled. In contrast, mCherry-AMPKα does not show any enrichment or asymmetric localization at the plasma membrane, and it is found distributed throughout the



**Figure 4. LKB1 is required to maintain epithelial polarity under energetic starvation conditions.** (A) *lkb1*<sup>4A</sup> mutant follicle cell clones under normal (left) or energetic stress conditions (right). Mutant cells are marked by the absence of GFP (green) and visualized with the same markers (as listed as described in Fig. 2 A). (B) *lkb1* clone stained for DNA (blue) and Dlg (red, left) or ECad (red, right). Large mutant clones at the anterior/posterior poles of the egg chamber overproliferate to form tumorlike aggregates of unpolarized cells.

cytoplasm, but absent from the nucleus (Fig. 3 A). The localization of LKB1 suggests that AMPK could be activated specifically at the apical and lateral cortices of the cells. To test this hypothesis, we used an antibody against the LKB1 phosphorylation site of AMPK (phospho-T184). The immunostaining is reduced to background levels in both *ampkα* and *lkb1* mutant clones. This confirms the specificity of the antibody and indicates that LKB1 is the principle AMPK kinase in these cells (Fig. 3 B). In wild-type cells, PhosphoT184-AMPK is found diffusely in the cytoplasm (Fig. 3 B). The effect of AMPK on apical–basal polarity is therefore not related to a polarized distribution of the kinase or its localized activation by LKB1.

Because LKB1 activates AMPK, we wondered if similar phenotypes could be observed in *lkb1* mutant cells. *lkb1* clones can lead to severe polarity defects in follicle cells in normally fed flies (Martin and St Johnston, 2003). However, these defects are observed only in large clones that are induced in the stem cells that give rise to the follicular epithelium, whereas small *lkb1* mutant clones, which are induced after the formation of the epithelium, have no effect on follicle cell polarity or the organization of the actin cytoskeleton ( $n = 24$ ; Fig. 4 A). This suggests

that LKB1 is required for the establishment of epithelial polarity in well-fed flies, but not for its maintenance, as is the case for PAR-1 (Doerflinger et al., 2003). In contrast, under conditions of glucose starvation, small *lkb1* clones that were induced after the formation of the follicular epithelium show a fully penetrant polarity phenotype (100%;  $n = 21$ ). Under these conditions, we observed a loss of the polarized localization of Dlg, aPKC, Crb, and Cora (Fig. 4 A). However, Baz distribution is usually not affected by *lkb1* loss of function (unpublished data). Dg extends laterally and occasionally localizes to the apical domain (Fig. 4 A). The actin cytoskeleton is also disturbed, with more F-actin apically and a decreased density of stress fibers on the basal side. Finally, large *lkb1* clones lose their epithelial organization completely and overproliferate to form small neoplasms (Fig. 4, B and C). Thus, *lkb1* mutant cells exhibit identical phenotypes to *ampkα* mutant cells under low-energy conditions.

Because *lkb1* and *ampkα* mutant clones lead to very similar polarity defects and LKB1 phosphorylates AMPK $\alpha$ , we wondered if a constitutively active form of AMPK $\alpha$  could rescue the *lkb1* phenotype. Therefore, we generated transgenic lines carrying a *UAS-AMPK* construct, in which Threonine184 is replaced by an aspartate, which should mimic the activating phosphorylation of this site by LKB1 (Lizcano et al., 2004). The expression of the AMPK $\alpha$ -T184D transgene in *lkb1* mutant clones fully rescues the starvation-dependent polarity and overproliferation phenotype ( $n = 37$ ), whereas the Gal4 driver alone has no effect (Fig. 5). Furthermore, AMPK $\alpha$ -T184D-expressing mutant clones also have a normal actin cytoskeleton (100%;  $n = 13$ ; Fig. 5). Thus, the phosphomimetic version of AMPK $\alpha$  completely rescues the *lkb1* mutant phenotype under conditions of energetic stress.

The recovery of null mutations in *ampkα* has allowed the first in vivo analysis of AMPK function in a multicellular organism, which has revealed an unexpected role for the kinase in the maintenance of epithelial polarity, but only under conditions of energetic stress. This implies that at least one of the pathways that normally maintain cell polarity cannot function when cellular energy levels are too low, and that AMPK activation compensates for this defect.

A surprising feature of the *ampkα* polarity phenotype is that it has opposite effects on the actin cytoskeleton and the cortical polarity cues. In mildly affected clones, basal actin is strongly reduced, with a corresponding increase in the amount of apical actin. In contrast, mutant clones show an expansion of the basal markers into the lateral and apical regions, as well as a loss of lateral and apical markers. Thus, the effects on actin may be independent of other polarity defects, suggesting that AMPK acts through different pathways to regulate actin and cortical polarity in opposite ways.

It is unclear how AMPK regulates the actin cytoskeleton, but it is possible that it acts on only one side of the cell and that the reciprocal changes on the other are caused by a change in the concentration of free G-actin or an actin nucleator, as has been shown for *abl* mutants during cellularization (Grevenengo et al., 2003). For example, loss of AMPK could increase actin polymerization apically, thereby depleting the pool of free actin that can polymerize basally. Alternatively, *ampkα* mutants may

*lkb1*, AMPK $\alpha$ -T184D,  
energetic stress

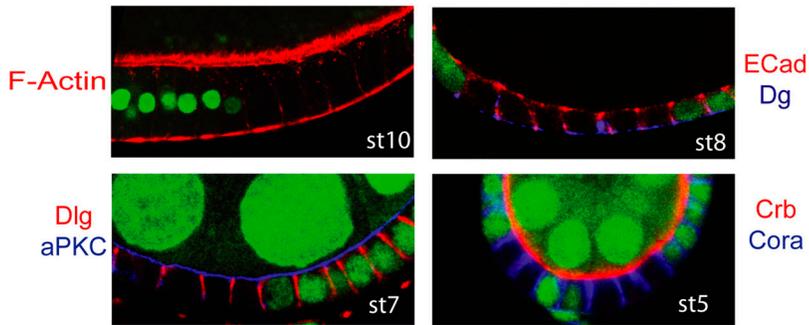


Figure 5. **The AMPK $\alpha$ -T184D phosphomimetic transgene rescues the starvation-dependent *lkb1* phenotypes.** *lkb1* mutant cells marked by the absence of GFP (green) expressing the UAS-AMPK $\alpha$ -T184D transgene. The expression of the phosphomimetic AMPK $\alpha$  transgene (T184D) rescues the *lkb1* energetic stress-dependent phenotypes, as indicated by the normal distribution of various polarity markers (indicated to the left or right of the images). (top) F-actin and DECaD, red; DG, blue. (bottom) Dlg and Crb, red; aPKC and Cora, blue.

prevent the formation of basal actin stress fibers, and thus increase the concentration of free actin, which enhances apical actin polymerization.

The cortical polarity defects of *ampk $\alpha$*  mutant clones also suggest a reciprocal relationship between the basal and apical/lateral membrane domains because the basal domain, marked by Dg, is dramatically expanded, whereas the determinants for the lateral domain (Dlg) and the apical domain (aPKC and Crb) disappear from the cortex. This suggests that there is some form of mutual antagonism between the basal and lateral domains that maintains a sharp boundary between them, as has been described for apical and lateral domains through the inhibition of phosphorylation of Baz (PAR-3) by lateral PAR-1, and of PAR-1 by apical aPKC (Benton and St Johnston, 2003; Suzuki et al., 2004). If this model is correct, AMPK could be required to restrict the extent of the basal domain, with the expansion of this domain in *ampk $\alpha$*  mutants leading to the exclusion of lateral and apical markers. Indeed, the overexpression of Dg has been found to cause a similar loss of apical and lateral markers as that seen in *ampk $\alpha$*  clones (Deng et al., 2003). Alternatively, AMPK could be necessary to maintain the localization of the apical and lateral determinants, which in turn prevent the basal domain from extending into these regions.

Mutations in AMPK not only disrupt the polarity of the follicle cell epithelium, but also cause the cells to overproliferate, giving rise to a tumorous phenotype. One possible explanation for this phenotype is that it is caused by the mislocalization and down-regulation of Dlg. Dlg is a member of a class of tumor suppressors in *D. melanogaster* that also includes Lgl and Scribble, and follicle cell clones mutant for any of these genes overproliferate to form invasive tumors that are similar to those formed by *ampk $\alpha$*  and *lkb1* clones under low-energy conditions (Bilder and Perrimon, 2000; Goode et al., 2005; Hariharan and Bilder, 2006). Furthermore, the tumor suppressor function of these proteins is probably conserved in humans because Scribble restricts proliferation by repressing the G1/S transition, and is a target of the papilloma virus E6 oncoprotein (Nagasaka et al., 2006; Takizawa et al., 2006). This may account for the observation that AMPK is required to trigger the G1/S checkpoint under conditions of energetic stress (Mandal et al., 2005). However, it has also been shown in mammals that AMPK activates TSC2 to repress the insulin-TOR pathway, and thus it functions as a tumor suppressor that inhibits cell growth and division (Inoki et al.,

2003, 2005). Loss of this repression might provide an alternative explanation for the overgrowth of *ampk $\alpha$*  mutant clones.

Although the molecular pathways involved remain to be elucidated, our results demonstrate that *ampk $\alpha$*  mutant cells lose their polarity under low energy conditions and overproliferate to give rise to tumorlike growths. The activation of AMPK depends on its phosphorylation by LKB1, and loss of LKB1 produces an identical tumorous phenotype. Thus, the novel functions of AMPK reported in this work may provide a basis for the tumor suppressor function of LKB1.

## Materials and methods

### Mutant characterization

An ethylmethanesulfonate mutagenesis screen on the X chromosome was performed as previously described (Medina et al., 2006). Early second instar clones were visually screened for dendritic defects using fluorescent microscopy. The *ampk $\alpha$*  mutants, lethal at late second instar stages, were mapped to ~150 kb on the X chromosome using a molecularly defined deficiency (*Df[1]Exel6227*), an undefined deficiency (*Df[1]AD11*), and a duplication of the Y chromosome (*Dp[1;Y]/Df[1]svr*). Predicted coding regions for genes in the region were sequenced using PCR amplicons made from mutant genomic DNA, and one gene (AMPK $\alpha$ ; CG3051; NM\_057965) was discovered that had mutations in all three alleles.

### Construction of AMPK $\alpha$ transgenes

The wild-type AMPK $\alpha$  transgene was cloned into the *pUAST* vector (Brand and Perrimon, 1993) as an EcoRI-BglIII fragment of an EST, corresponding to an AMPK $\alpha$ -RA transcript (www.flybase.org). The mCherry-AMPK $\alpha$  fusion protein was made using a mCherry construct (provided by R. Tsien, University of California, San Diego, San Diego, CA) at the N terminus fused in-frame to AMPK $\alpha$  into the *pUAST* vector. The UAS-mCherry-AMPK $\alpha$  transgene rescues viability and fertility when expressed by *Ubiquitin-Gal4* in either *ampk $\alpha$ <sup>1</sup>* or *ampk $\alpha$ <sup>3</sup>* mutants. The phosphomimetic activated form of AMPK $\alpha$  (AMPK $\alpha$  T184D) was made by PCR-based, site-directed mutagenesis converting base C549 to G549. The transgenes were introduced into a *w<sup>1118</sup>* stock by *P* element-mediated transformation.

### Fly stocks and crosses

AMPK $\alpha$  alleles were recombined with *FRT101* for mitotic recombination. Other mutant stocks used were *FRT82B*, *lkb<sup>4A4.2</sup>* and *FRT82B*, *tend*. UAS:Cherry-AMPK $\alpha$  and UAS:GFP-LKB1 were expressed in follicle cells using the *Cy2-Gal4* driver. Flip-out experiments were performed by crossing UAS:Cherry-AMPK $\alpha$  and UAS:AMPK $\alpha$ -T184D to *y, w, hs:Flp; tub-FRT-cc-FRT-Gal4*, UAS:GFP and heat-shocking pupae. For rescue experiments, two independent stocks were established and crossed together: *w; UAS:AMPK $\alpha$ -T184D/CyO; FRT82B*, *Ubi:nlsGFP* and *y, w, hs:flp; da:Gal4, FRT82B, lkb<sup>14A4.2</sup>/TM3, Sb*.

### Starvation conditions and clone induction

Adult flies were placed in vials containing "normal" *D. melanogaster* food media (5% glucose, 5% yeast extract, 3.5% wheat flour, and agar 0.8%), energetic starvation medium (1% yeast extract, 3.5% wheat flour, and agar 0.8%),

or specific nutrient-starvation medium [5% glucose and agar 0.8%]. Clones were induced by heat-shocking adult females at 37°C for 2 h on two consecutive days. Females were dissected 2 d after the last heat shock.

#### Staining and imaging procedures

Immunofluorescence on ovaries was performed using standard procedures. Primary antibodies were used as follows: rat anti-DECad (1:1,000; Oda et al., 1994); mouse anti-Crb (cq4; 1:50; Developmental Studies Hybridoma Bank); Guinea pig anti-Cora (1:2,000; Fehon et al., 1994); rabbit anti-aPKC (1:500; Sigma-Aldrich); rat anti-Baz (1:500; Wodarz et al., 1999); mouse anti-Dlg (1:50; Developmental Studies Hybridoma Bank); rabbit anti-Dg (1:1,000; Deng et al., 2003); and rabbit anti-phosphoT385-AMPK (1:100; Cell Signaling Technology). Actin staining was performed with rhodamine-conjugated phalloidin (Invitrogen). Second instar larvae were dissected in 4% paraformaldehyde, as previously described (Medina et al., 2006). Secondary antibodies coupled with Cy5 (anti-rabbit and anti-guinea pig) or Texas red (anti-mouse and anti-rabbit; Jackson Immuno-Research Laboratories; 1:500) were used. Images of follicle cells were collected on a confocal microscope (Radiance 2000; Bio-Rad Laboratories) with a 40×/1.3 NA objective (Plan Fluor; Nikon) using LaserSharp software. Live images of dendrite morphology were acquired using a confocal microscope (LSM 510; Carl Zeiss MicroImaging, Inc.) by using the 488-nm argon line to excite GFP. Larvae were covered in a glycerol solution at 22°C and gently covered with a coverslip (22 × 50 mm; Fisher Scientific) to restrict movement, but not cause bursting of the body wall. Images were taken using a Pan-Neofluar 40×/1.3 NA oil immersion lens with a 2-μm optical slice and LSM Imaging software (Carl Zeiss MicroImaging, Inc.). Images were resized and cropped with Photoshop (Adobe), and imported into Illustrator (Adobe) for labels and arrangement.

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