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Figures and figure supplements

EGFR signaling promotes self-renewal through the establishment of cell polarity in *Drosophila* follicle stem cells

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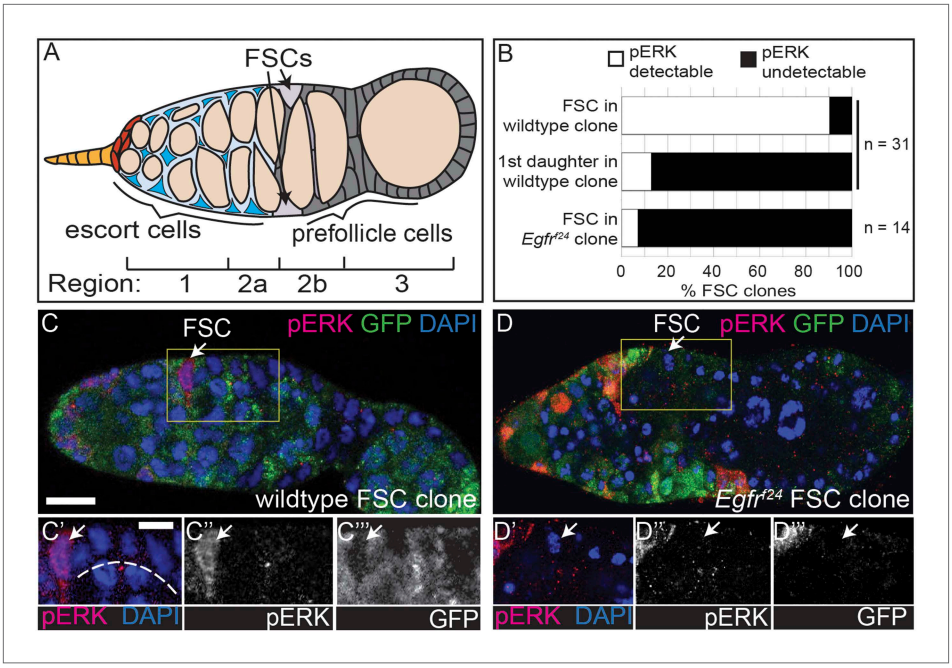

Figure 1. The EGFR pathway is upregulated specifically in FSCs. (**A**) Diagram of the germarium of the Drosophila ovary. The germarium is divided into four regions as indicated; anterior is to the left. Two follicle stem cells (FSCs, light grey) are maintained in the germarium at the Region 2a/2b border. Escort cells (blue) are anterior to the FSCs and support development of the early germline (orange). As they mature, germline cysts move posteriorly out of Region 2a and into the follicle epithelium. Each FSC divides once per incoming cyst, producing prefollicle cells (dark grey) that encapsulate the germline as it moves into Region 2b. (**B**) Quantification of pErk staining of FSCs and prefollicle cells just downstream from the niche within a wildtype or *Egfr*^{f24} FSC clone. (**C**–**D**) Wildtype (**C**) or *Egfr*^{f24} (**D**) FSC clones stained for pErk (red), GFP (green) and DAPI (blue). Boxed regions of **C**–**D** are magnified in **C**′–**C**‴ and **D**′–**D**‴. White arrows indicate the FSC, which is the anterior-most GFP$^{(-)}$ follicle cell in the clone. White dashed line in (**C**′) indicates prefollicle cells in which pErk is undetectable compared to the FSC. Scale bar represents 5 μm in **C**–**D** and 1 μm in magnified insets.

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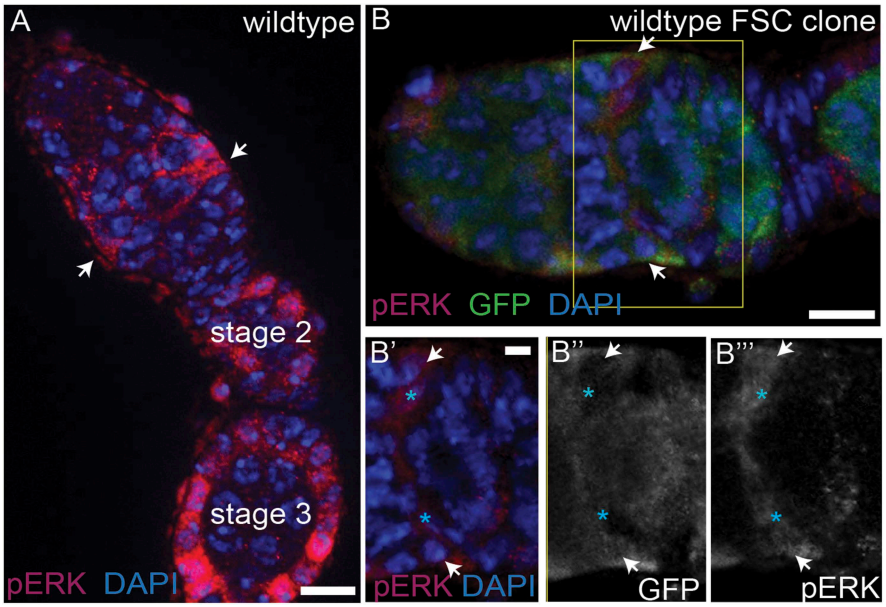

Figure 1—figure supplement 1. pErk staining in the ovariole **A**. (**A**) Wildtype ovariole with bright pErk staining (red) detectable in escort cells, early (stage 2 and 3) follicles, and at the position of the FSC niche (white arrows). (**B**) A wildtype GFP$^{(-)}$ FSC clone with bright pErk in FSCs (white arrows), and in prefollicle cells that recently divided from the FSC in the clone (blue asterisks in **B**′–**B**‴). All tissues stained with DAPI (blue). Anterior is to the left. Boxed region in B is magnified in **B**′–**B**‴. Scale bars represent 5 μm in **A**–**B** and 1 μm in magnified insets.

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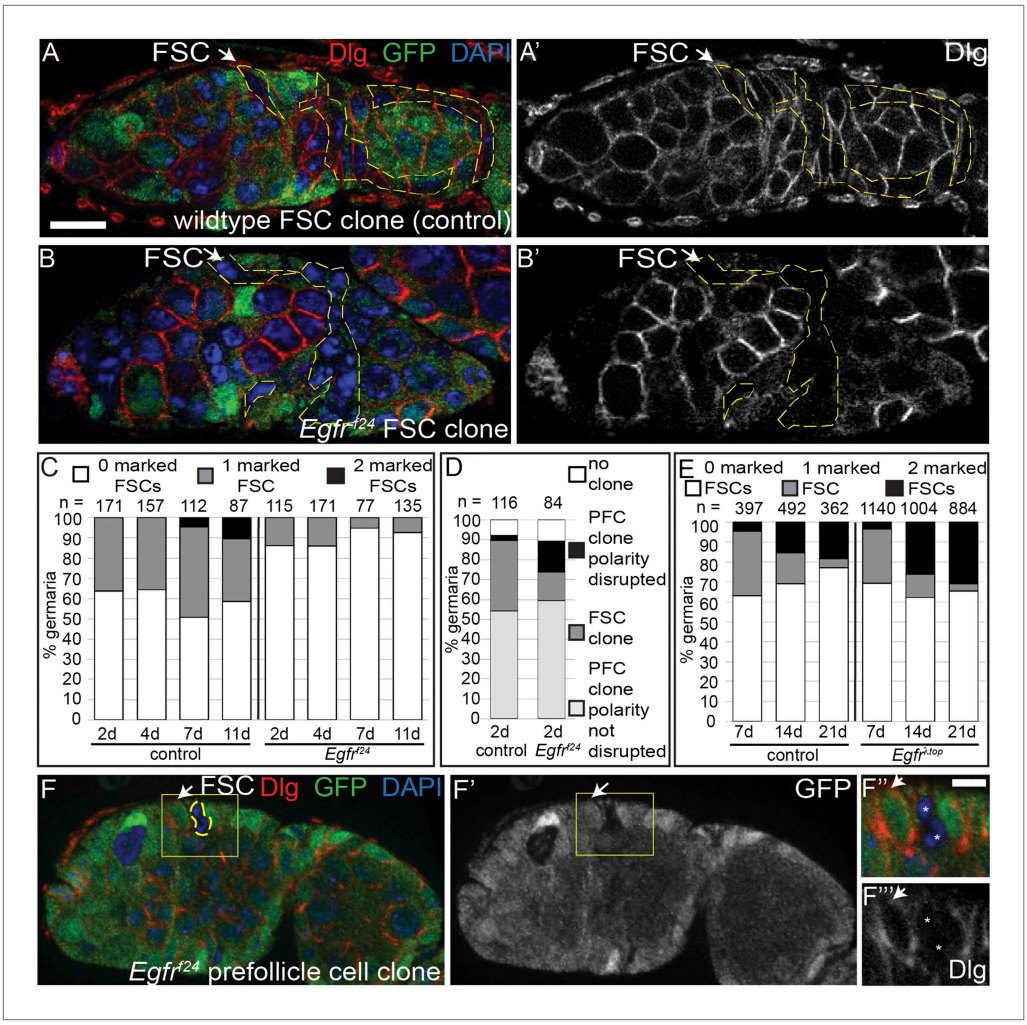

Figure 2. EGFR is required for FSC maintenance in the niche. (**A**–**B**) Germaria with a mature wildtype (**A**) or *Egfr*^{f24} (**B**) GFP$^{(-)}$ FSC clone stained for Dlg (red) and GFP (clone marker, green). (**C**–**E**) Graphs indicating the frequencies of the *Egfr*^{f24} or control FSC clones at 2, 4, 7, and 11 dpci (**C**); all *Egfr*^{f24} or control clones, including polarity-defective *Egfr*^{f24} prefollicle cell (PFC) clones, at 2 dpci (**D**); and the *Egfr*$^{\lambda top}$ or control FSC clones at 7, 14, and 21 dpci (**E**). (**F**) Polarity-defective *Egfr*^{f24} prefollicle cell clone at 2 dpci, stained for Dlg (red) and GFP (green); **F′** shows the GFP channel alone; boxed regions are magnified in **F″**–**F‴**. GFP$^{(-)}$ clones are indicated by dashed yellow lines, and by white asterisks in **F″**–**F‴**. White arrows indicate the position of the FSC niche. All tissues stained with DAPI (blue). Anterior is to the left. Scale bar represents 5 μm in **A**–**F** and 1 μm in magnified insets.

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	control FSC clones				*Egfr*^{f24} FSC clones			
	2 dpci	4 dpci	7 dpci	11 dpci	2 dpci	4 dpci	7 dpci	11 dpci
0 marked FSCs	64%	64%	51%	59%	86%	86%	95%	93%
1 marked FSC	36%	36%	45%	31%	14%	14%	5%	7%
2 marked FSCs	0%	0%	4%	10%	0%	0%	0%	0%

Figure 2—figure supplement 1. Quantification of marked control and *Egfr*^{f24} FSC clone frequencies at 2, 4, 7, and 11 dpci. Values reflect the percent of germaria that have the indicated the number of GFP$^{(-)}$ FSCs at the indicated timepoints. The GFP$^{(-)}$ cells are either wildtype (control column) or mutant for *Egfr* (*Egfr*^{f24} column).

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	control FSC clones			*Egfr*$^{\lambda top}$ FSC clones		
	2 dpci	4 dpci	7 dpci	2 dpci	4 dpci	7 dpci
0 marked FSCs	63%	69%	77%	69%	62%	66%
1 marked FSC	32%	15%	5%	27%	12%	4%
2 marked FSCs	5%	15%	18%	3%	26%	31%
p-values	Control versus *Egfr*$^{\lambda top}$					
0 marked FSCs	0.33	0.46	0.46			
1 marked FSC	0.09	0.26	0.08			
2 marked FSCs	0.02	0.19	0.01			

Figure 2—figure supplement 2. Quantification of marked control and *Egfr*$^{\lambda top}$ FSC clone frequencies at 7, 14, and 21 dpci. Values reflect the percent of germaria that have the indicated number of GFP$^{(-)}$ FSCs at the indicated timepoints. The GFP$^{(-)}$ cells are either wildtype (control column) or express *Egfr*$^{\lambda top}$ (*Egfr*$^{\lambda top}$ column). P-values were determined using a two-tailed t-test.

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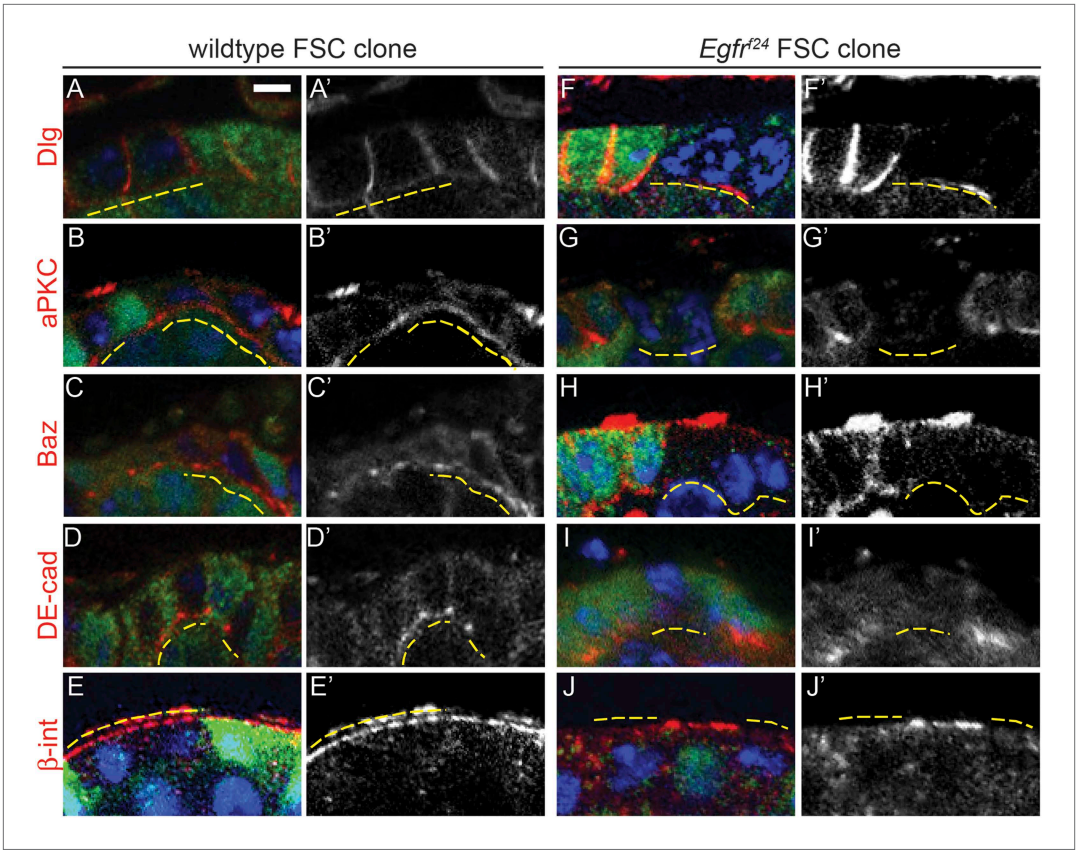

Figure 3. *Egfr*^{f24} FSC clones have epithelial polarity defects. (**A**–**J**) Wildtype (**A**–**E**) and *Egfr*^{f24} (**F**–**J**) FSC clones stained for polarity markers (red) Dlg (**A** and **F**), aPKC (**B** and **G**), Baz (**C** and **H**), DE-cad (**D** and **I**) and β-integrin (β-int) (**E** and **J**); GFP (green); and DAPI (blue). Panels **A**′–**J**′ show the red channel only. All polarity markers are able to localize properly in wildtype GFP$^{(-)}$ FSC clones and in the GFP$^{(+)}$ follicle cells within germaria that contain either wildtype or *Egfr*^{f24} FSC clones; all polarity markers are undetectable in GFP$^{(-)}$ *Egfr*^{f24} FSC clones. GFP$^{(-)}$ clones are indicated by dashed yellow lines. Images are oriented with the apical surface of the follicle cells on the bottom. Scale bar represents 1 μm.

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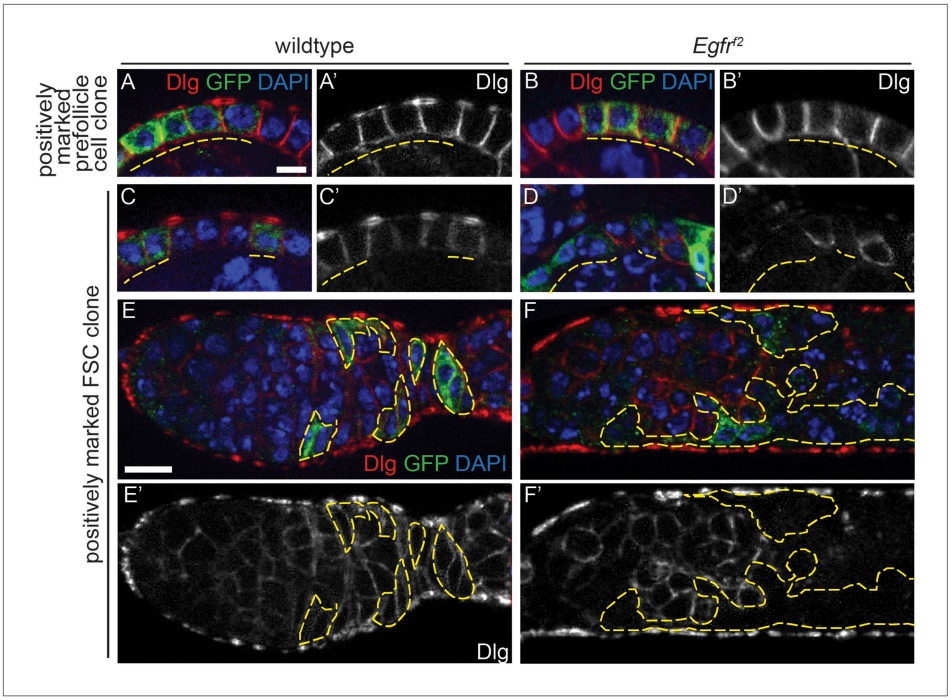

Figure 3—figure supplement 1. Positively marked *Egfr*^{f2} FSC clones have epithelial polarity defects. (**A**–**D**) Wildtype (**A**, **C**) and *Egfr*^{f2} (**B**, **D**) prefollicle cell clones (**A**–**B**) and FSC clones (**C**–**D**) marked by the presence of GFP and stained for polarity marker Dlg (red); GFP (green); and DAPI (blue). Dlg is able to localize properly in wildtype GFP$^{(+)}$ FSC clones, in the GFP$^{(-)}$ follicle cells within germaria that contain either wildtype or *Egfr*^{f2} FSC clones, and in *Egfr*^{f2} GFP$^{(+)}$ prefollicle cell clones; Dlg is undetectable in *Egfr*^{f2} GFP$^{(+)}$ FSC clones. (**E**–**F**) Germaria with wildtype (**E**) or *Egfr*^{f2} (**F**) GFP$^{(+)}$ FSC clones stained for Dlg (red). GFP$^{(+)}$ clones are indicated by dashed yellow lines. Images in (**A**–**D**) are oriented with the apical surface of the follicle cells on the bottom; in (**E**–**F**) anterior is to the left. Scale bar represents 1 μm in (**A**–**D**), and 5 μm in (**E**–**F**). Panels A′–F′ show the red channel only.

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	polarity not disrupted	polarity disrupted
Wildtype FSC clone	99%, 83/84	1%, 1/84
Egfr^{f2} prefollicle cell clone	95%, 121/127	5%, 6/127
Egfr^{f2} FSC clone	6%, 1/18	94%, 17/18

Figure 3—figure supplement 2. Quantification of the frequency of polarity phenotypes in positively marked control FSC clones, Egfrf2 FSC clones, and Egfrf2 prefollicle cell clones. Values reflect both the percent and fraction of each clone type in which polarity is disrupted or notdisrupted as indicated.

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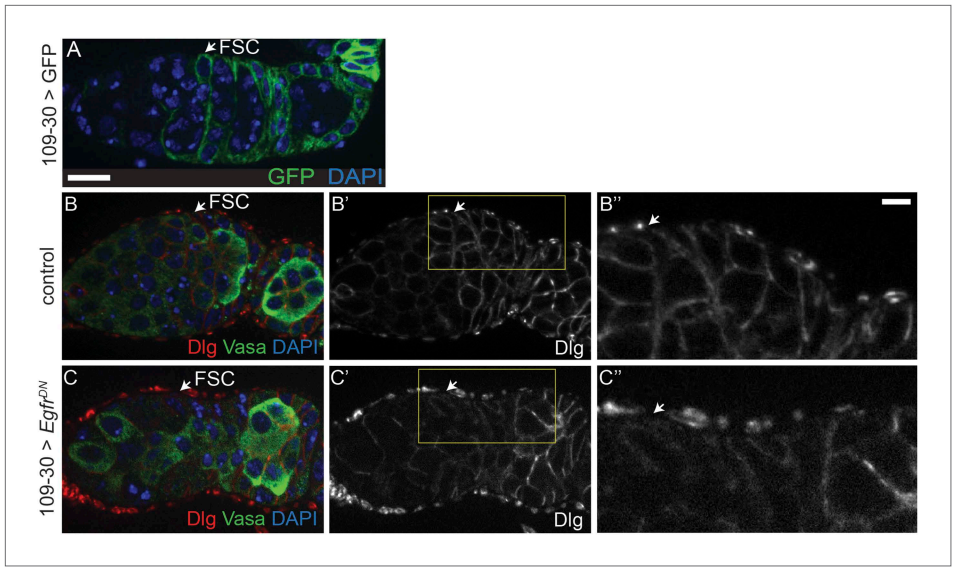

Figure 3—figure supplement 3. Polarity defects in *Egfr*^{DN} follicle cells. (**A**) Germarium in which GFP (green) is expressed using 109-30-Gal4 to indicate the expression pattern of the 109-30-Gal4 driver in follicle cells. (**B**–**C**) Germaria containing UAS-*Egfr*^{DN}/UAS-*Egfr*^{DN} but no Gal4 driver (**B**), or 109-30-Gal4 and UAS-*Egfr*^{DN}/UAS-*Egfr*^{DN} (**C**), stained for Dlg (red) and Vasa (green). Dlg localization is disrupted in the germarium overexpressing *Egfr*^{DN} (**C**), which phenocopies the polarity defects seen in *Egfr*^{f24} FSC clones. All tissues stained with DAPI (blue). White arrows indicate the position of the FSC niche. Anterior is to the left. Boxed regions of **B**′–**C**′ are magnified in **B**″–**C**″. Scale bar represents 5 μm in (**A**–**C**), and 1 μm in magnified insets.

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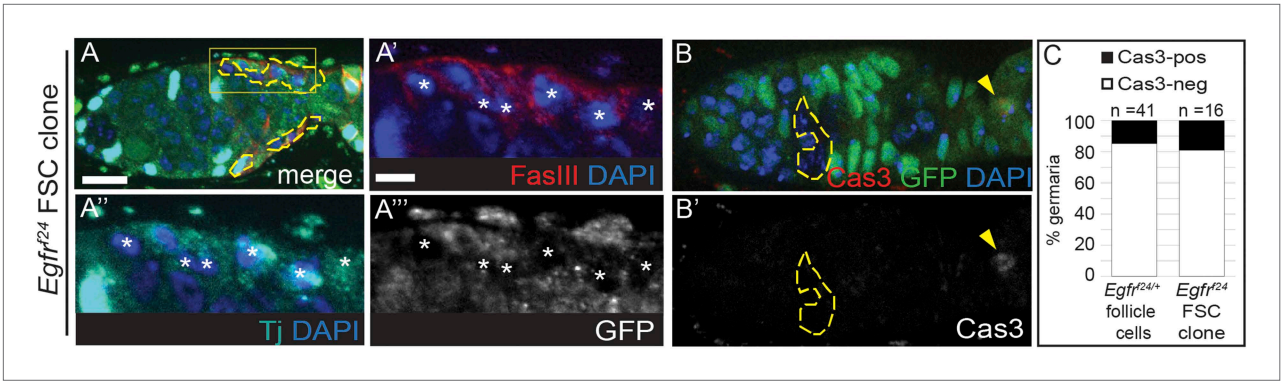

Figure 4. Loss of EGFR does not cause cell death or loss of follicle cell identity. (**A**) *Egfr*^{f24} FSC clone with normal FasIII (red) and Traffic jam (Tj) (cyan) in the clone, indicated by white asterisks in the magnified regions in (**A**′–**A**‴). (**B**) *Egfr*^{f24} FSC clone with a Cas3-positive cell (red, yellow arrowhead) in the polar region of a newly budded follicle, but not in the clone. Panel **B**′ shows the red channel only. (**C**) Graph indicating the frequency of Cas3-positive follicle cells in *Egfr*$^{f24/+}$ control germaria or in *Egfr*^{f24} FSC clones. GFP$^{(-)}$ clones are indicated by dashed yellow lines. All tissues stained with DAPI (blue). Anterior is to the left. Scale bar represents 5 μm in (**A**–**B**), and 1 μm in magnified insets.

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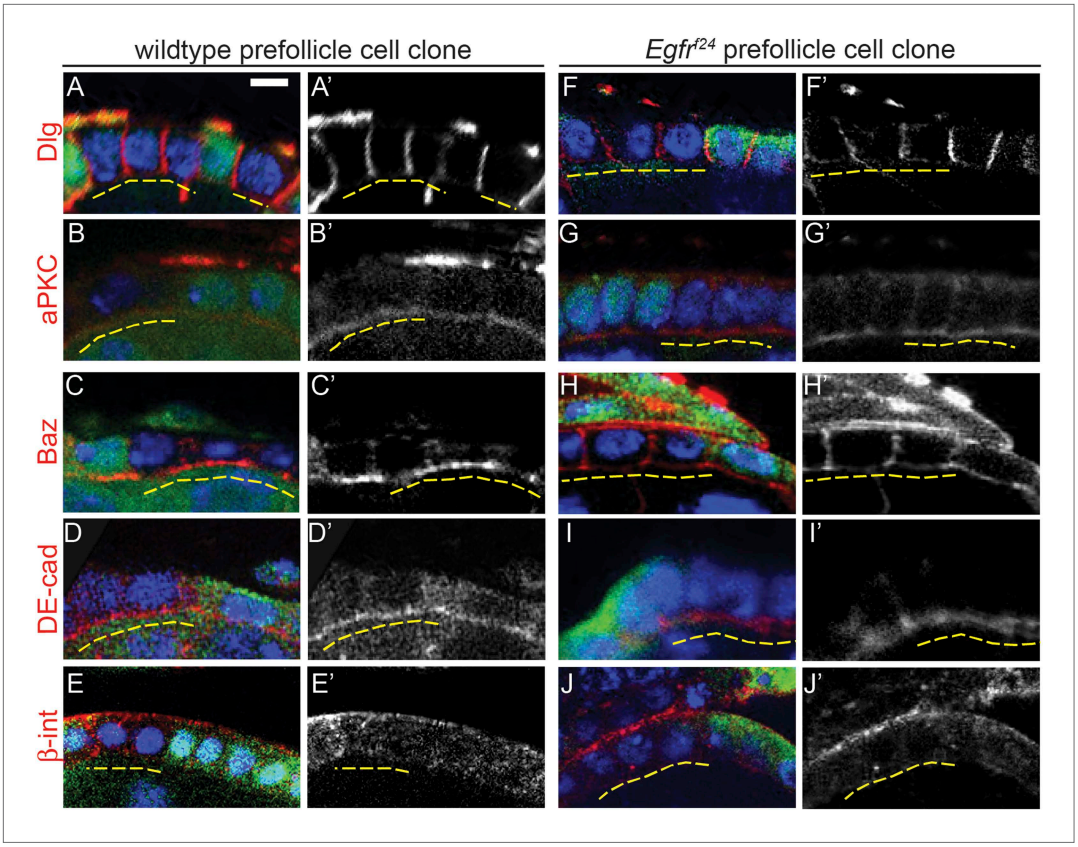

Figure 5. *Egfr*^{f24} prefollicle cell clones do not have epithelial polarity defects. (**A**–**J**) Wildtype (**A**–**E**) and *Egfr*^{f24} (**F**–**J**) prefollicle cell clones stained for polarity markers (red) Dlg (**A** and **F**), aPKC (**B** and **G**), Baz (**C** and **H**), DE-cad (**D** and **I**) and β-int (**E** and **J**); GFP (green); and DAPI (blue). Panels **A**′–**J**′ show the red channel only. All polarity markers are properly localized in both wildtype and *Egfr*^{f24} GFP$^{(-)}$ prefollicle cell clones. GFP$^{(-)}$ clones are indicated by dashed yellow lines. Images are oriented with the apical surface of the follicle cells on the bottom. Scale bar represents 1 μm.

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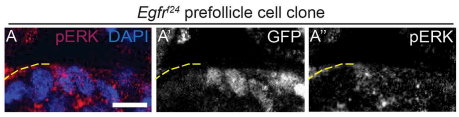

Figure 5—figure supplement 1. pErk is absent from *Egfr*^{f24} prefollicle cell clones. **A**. *Egfr*^{f24} prefollicle cell clone (indicated by dashed yellow line) that lacks pErk signal (red). Clone is GFP$^{(-)}$ and DAPI is in blue. The GFP channel alone is shown in **A**′ and the pERK channel alone is shown in **A**″. Images are oriented with the apical surface of the follicle cells on the bottom. Scale bar represents 1 μm.

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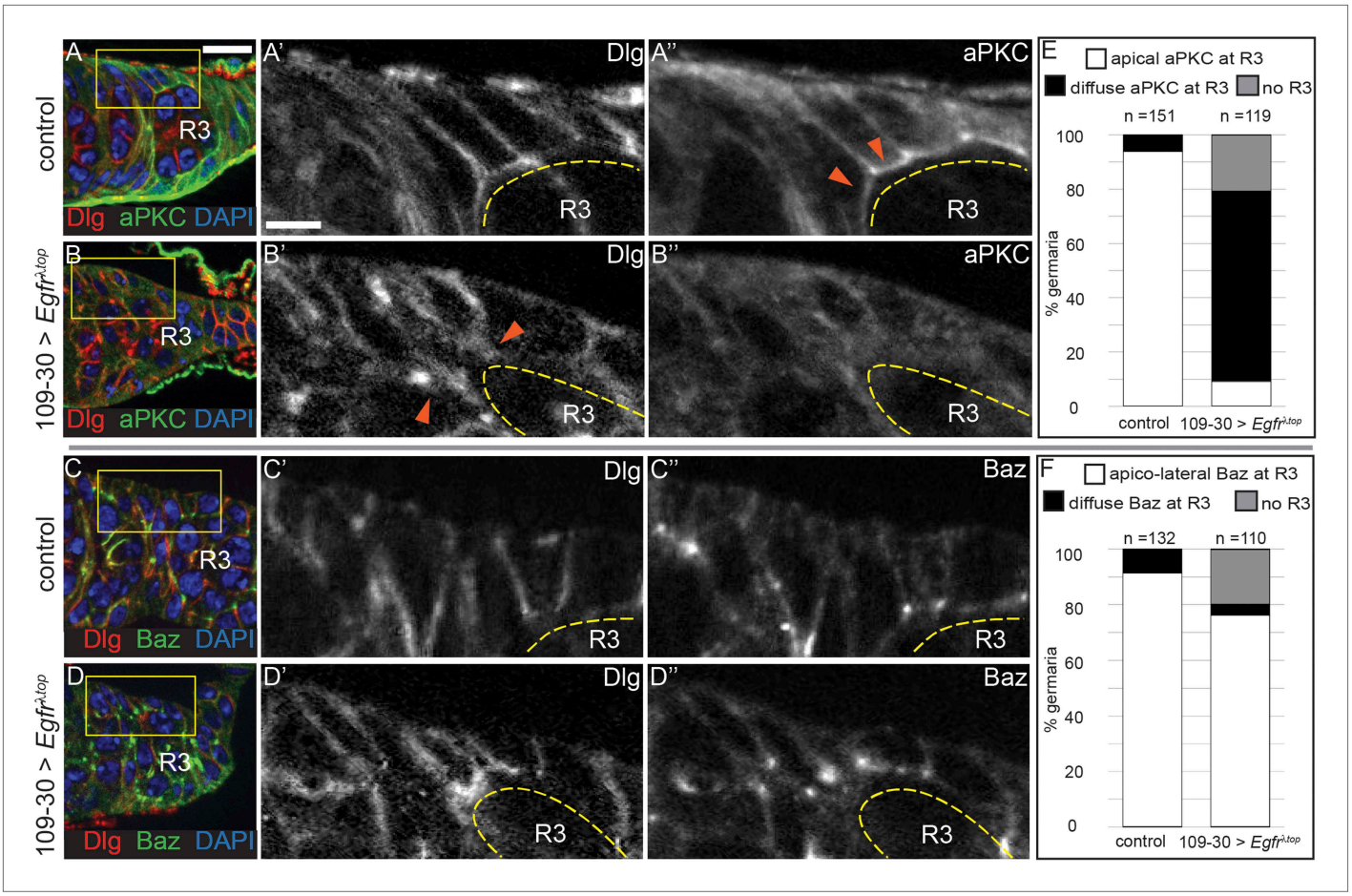

Figure 6. Constitutive activation of EGFR disrupts prefollicle cell apical polarity. (**A**–**D**) Control germaria containing UAS-*Egfr*$^{\lambda top}$ but no Gal4 driver (**A** and **C**) and experimental germaria in which *Egfr*$^{\lambda top}$ is expressed in follicle cells under the control of 109-30-Gal4 (**B** and **D**) and stained for Dlg (red), DAPI (blue), and either aPKC (green, **A**–**B**) or Baz (green, **C**–**D**). Follicle cells along the Region 3 cyst (R3, yellow dashed line) of control germaria have a cuboidal shape with a clear apical surface (**A**″, orange arrowheads); aPKC localizes to the apical surface (**A**″), Baz localizes to apical–lateral junctions (**C**″), and Dlg localizes to lateral surfaces (**A**′ and **C**′). In germaria expressing *Egfr*$^{\lambda top}$ in which the R3 cyst is present, cells have a pointed shape and form narrow contacts with the germline (**B**′, orange arrowheads). In addition, aPKC is delocalized from the cell surface of follicle cells (**B**″), but Dlg is detectable on the cell membrane (**B**′ and **D**′) and Baz localizes to apical–lateral junctions (**D**″). (**E**–**F**) Graphs indicating the frequencies of control or experimental germaria with no R3 cyst, or with localized or delocalized aPKC (**E**) or Baz (**F**) in follicle cells along the R3 cyst. Boxed regions of (**A**–**D**) are magnified in **A**′–**D**″. Anterior is to the left. Scale bar represents 5 μm in **A**–**D** an 1 μm in magnified insets.

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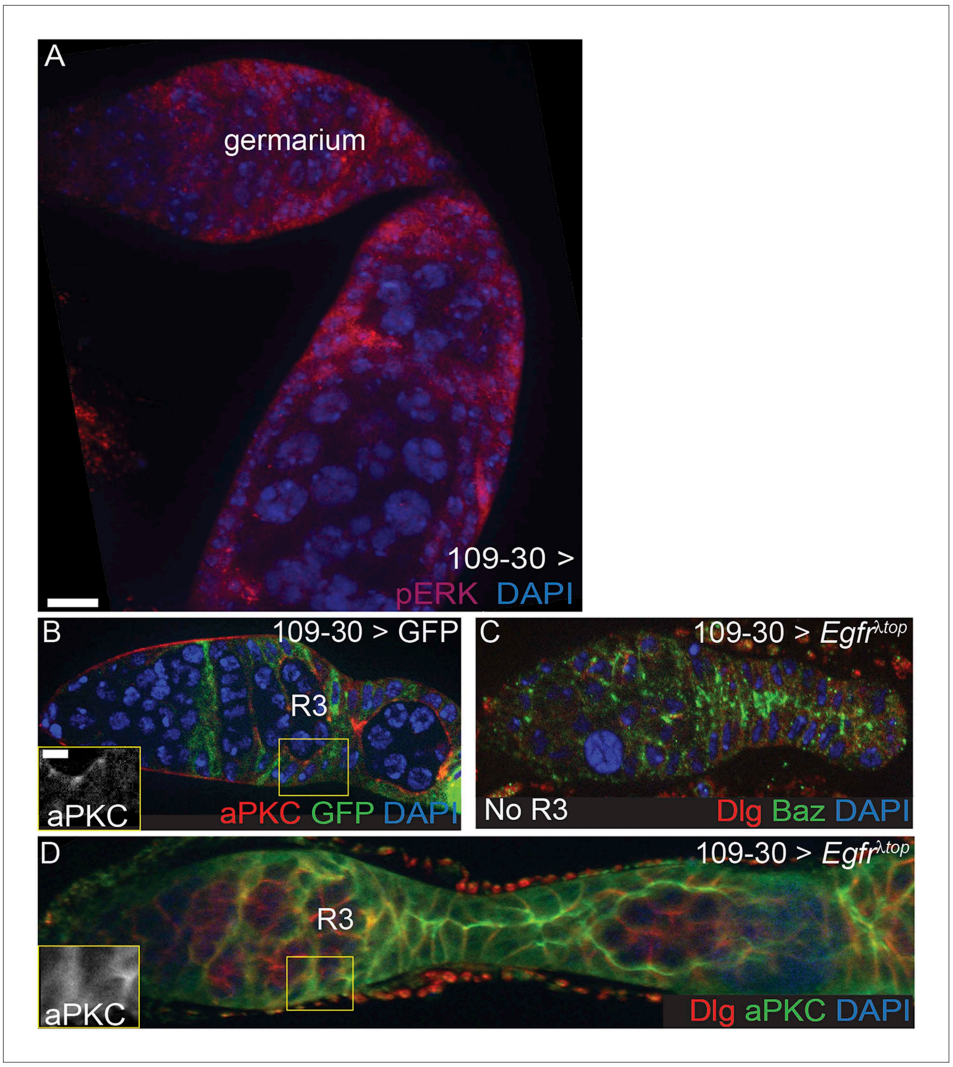

Figure 6—figure supplement 1. Expression of *Egfr*$^{\lambda top}$ in follicle cells. (**A**) Germarium in which *Egfr*$^{\lambda top}$ is expressed in follicle cells using 109-30-Gal4 with bright pErk (red) throughout the follicle epithelium. (**B**) Germarium stained for aPKC (red) in which GFP (green) is expressed using 109-30-Gal4 to indicate the expression pattern of the 109-30-Gal4 driver in follicle cells. (**C**–**D**) Germaria in which *Egfr*$^{\lambda top}$ is expressed in follicle cells using 109-30-Gal4 and stained for Dlg (red) and either Baz (green, **C**) or aPKC (green, **D**). Germarium in panel **C** has no observable R3 cyst. Boxed regions of **B** and **D** are magnified in insets. Anterior is to the left. All tissues stained with DAPI (blue). Scale bar represents 5 μm and 1 μm in magnified insets.

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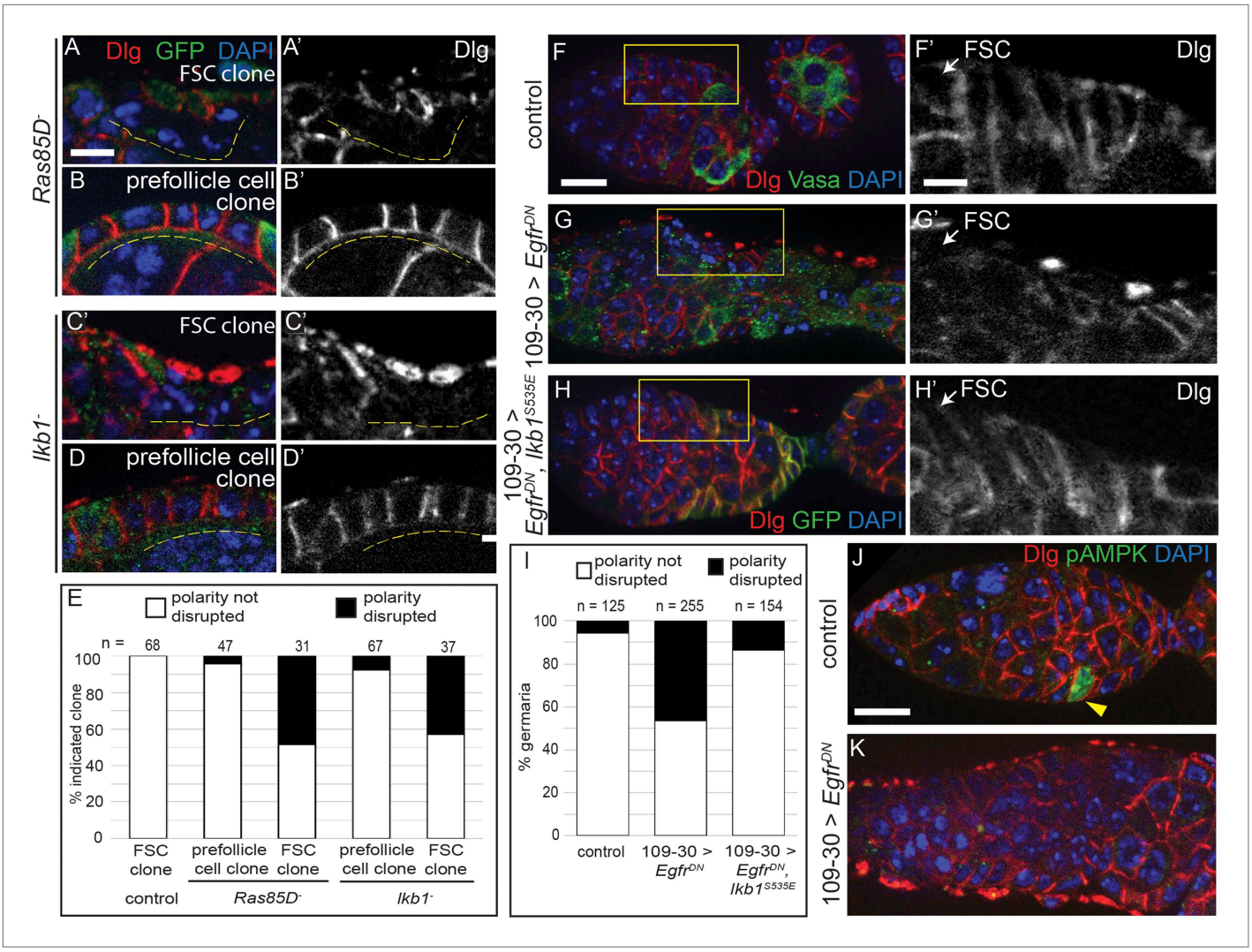

Figure 7. EGFR functions upstream of Ras and LKB1 to establish epithelial polarity. (**A**–**D**) GFP$^{(-)}$ *Ras85D*$^{-}$ (**A**) or *lkb1*$^{-}$ (**C**) FSC clones and *Ras85D*$^{-}$ (**B**) or *lkb1*$^{-}$ (**D**) prefollicle cell clones stained for Dlg (red) and GFP (green). (**E**) Graph indicating the frequencies of polarity phenotypes in wildtype, *Ras85D*$^{-}$, and *lkb1*$^{-}$ FSC clones and in *Ras85D*$^{-}$ and *lkb1*$^{-}$ prefollicle cell clones. (**F**–**H**) Germaria containing UAS-*Egfr*^{DN}/UAS-*Egfr*^{DN} but no Gal4 driver (**F**), 109-30-Gal4 and UAS-*Egfr*^{DN}/UAS-*Egfr*^{DN} (**G**), or 109-30-Gal4, UAS-*Egfr*^{DN}/UAS-*Egfr*^{DN} and UAS-GFP-*lkb1*^{S535E} (**H**) stained for Dlg (red), and either Vasa (green, **F**–**G**) or GFP (green, **H**). Dlg localization is disrupted in the germaria overexpressing *Egfr*^{DN} only (**G**), but it is restored in germaria overexpressing both *Egfr*^{DN} and *lkb1* ^{S535E} (**H**) (**I**) Graph indicating the frequencies of polarity phenotypes in control, *Egfr*^{DN} only, and *Egfr*^{DN} *and lkb1* ^{S535E} co-expressing germaria. (**J**–**K**) Germaria containing UAS-*Egfr*^{DN}/UAS-*Egfr*^{DN} but no Gal4 driver (**J**) or 109-30-Gal4 and UAS-*Egfr*^{DN}/UAS-*Egfr*^{DN} (**K**) stained for Dlg (red) and pAMPK (green), which is detectable in prefollicle cells of the control (yellow arrowhead, **J**) but not in germaria overexpressing *Egfr*^{DN} (**K**). Images in (**A**–**D**) are oriented with the apical surface of the follicle cells on the bottom, and GFP$^{(-)}$ clones are indicated by dashed yellow lines. Panels **A**′–**D**′ show the red channel only. Boxed regions of **F**–**H** are magnified in **F**′–**H**′, and white arrows indicate the position of the FSC niche. All tissues stained with DAPI (blue). Anterior is to the left in **F**–**K**. Scale bar represents 5 μm in **F**–**K** and 1 μm in **A**–**D** and in magnified insets.

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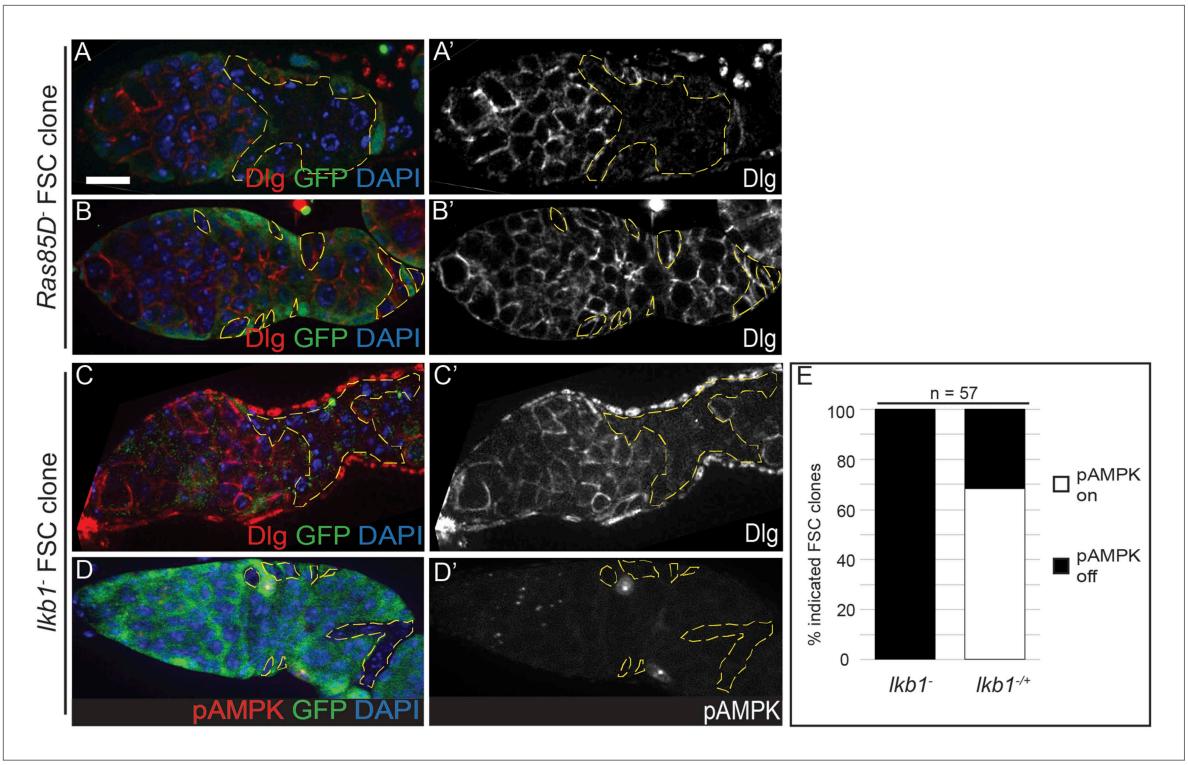

Figure 7—figure supplement 1. Polarity phenotypes of *Ras85D*− and *lkb1*− FSC clones. (**A**–**B**) Germaria with *Ras85D*− FSC clones stained for Dlg (red) and GFP (green). *Ras85D*− FSC clone in panel A has disrupted Dlg localization whereas in the *Ras85D*− FSC clone in panel **B** Dlg localization is not disrupted. (**C**) *lkb1*− FSC clone stained for Dlg (red) and GFP (green) with disrupted Dlg localization. (**D**) *lkb1*− FSC clone stained for pAMPK (red) and GFP (green) with no detectable pAMPK in the clone. Panels **A′**–**D′** show the red channel only. (**E**) Graph indicating the frequencies of detectable pAMPK in *lkb1*− FSC clones and in wildtype prefollicle cells of the same tissue (*lkb1*−/+). All tissues stained with DAPI (blue). GFP(−) clones are indicated by dashed yellow lines. Anterior is to the left. Scale bar represents 5 μm.

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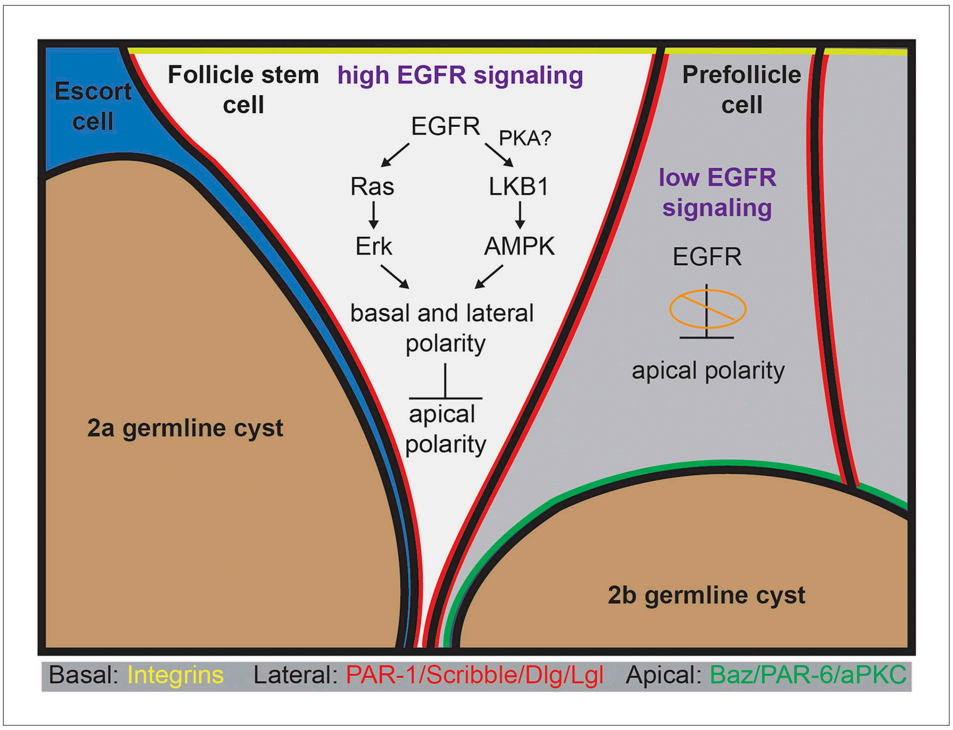

Figure 8. A model for the role of EGFR in the establishment of epithelial polarity. High levels of EGFR signaling in the FSC promote maintenance in the niche and the formation of basal and lateral domains while suppressing the formation of an apical domain. EGFR activates both the canonical Ras-mediated pathway leading to the phosphorylation of Erk, and the LKB1–AMPK pathway. Both Erk and AMPK are kinases that can regulate gene activity by activating transcription factors and phosphorylating proteins in the cytoplasm. AMPK directly promotes the lateral identity in polarized cells by activating lateral proteins. PKA is an upstream activator of LKB1 in follicle cells, and PKA can be activated by EGFR signaling, suggesting that EGFR signaling may activate LKB1 via PKA. EGFR signaling may suppress apical polarity either directly by regulating the transcription or activity of apical proteins, or indirectly by enhancing the activity of lateral proteins that suppress the localization of apical proteins. Low levels of EGFR signaling in prefollicle cells relieves this suppression, allowing apical domains to form and permitting differentiation away from the stem cell fate.

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