

Figure S1. **Depletion of Dlg-Scrib does not disrupt apical-basal polarity in nonproliferating epithelial cells.** (A and B) Confocal immunofluorescent localization of DE-cadherin (DE-Cad; A, A', B, and B') and Dlg (A, A'', B, and B'') in apical (A-A'') and lateral (B-B'') optical sections of *dlg*<sup>M52</sup> MARCM clones. The white line (A) identifies where the lateral section (B-B'') was taken. Yellow asterisks identify *dlg*<sup>M52</sup> MARCM clones, whereas white asterisks identify analogous nonclonal WT cells. Arrowheads identify AJs (A') and SJs (A'') around *dlg*<sup>M52</sup> clonal cells. (C and D) Confocal immunofluorescent localization of DE-cadherin (C, C', D, and D') and Dlg (C, C'', D, and D'') in *scrib*<sup>1</sup> MARCM clones (C-C'') or *scrib*<sup>1</sup> MARCM clones expressing Dlg-RNAi (D-D''). Arrowheads identify AJs (C' and D') and SJs (C'' and D'') around clonal cells. Bars, 10  $\mu$ m.



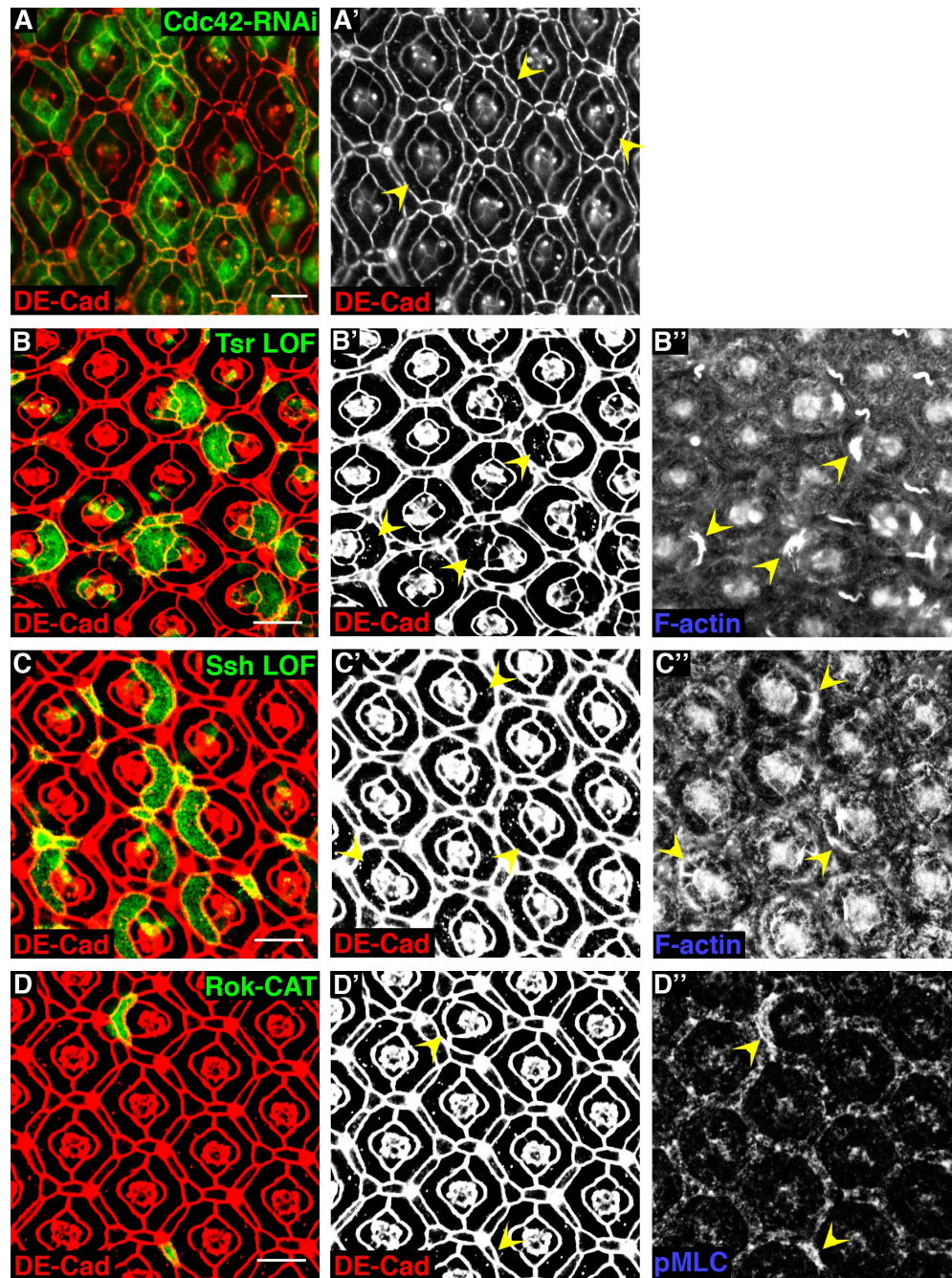


Figure S2. **Expression of Cdc42-RNAi decreases PEC apical area, and increases in F-actin or phospho-MLC at AJs are associated with increased apical tension.** (A) Immunofluorescent localization of DE-cadherin (DE-Cad) in Flp-out clones expressing Cdc42-RNAi. (B and C) Confocal immunofluorescent localization of DE-cadherin (B, B', C, and C') and F-actin (B'' and C'') in *tsr<sup>92E</sup>* MARCM clones (B-B'') and *ssh<sup>1-11</sup>* MARCM clones (C-C''). (D) Confocal immunofluorescent localization of DE-cadherin (D and D') and phospho-MLC (pMLC) in Flp-out clones expressing Rok-CAT. (A-D) Arrowheads identify clonal cells. Bars, 10 μm.



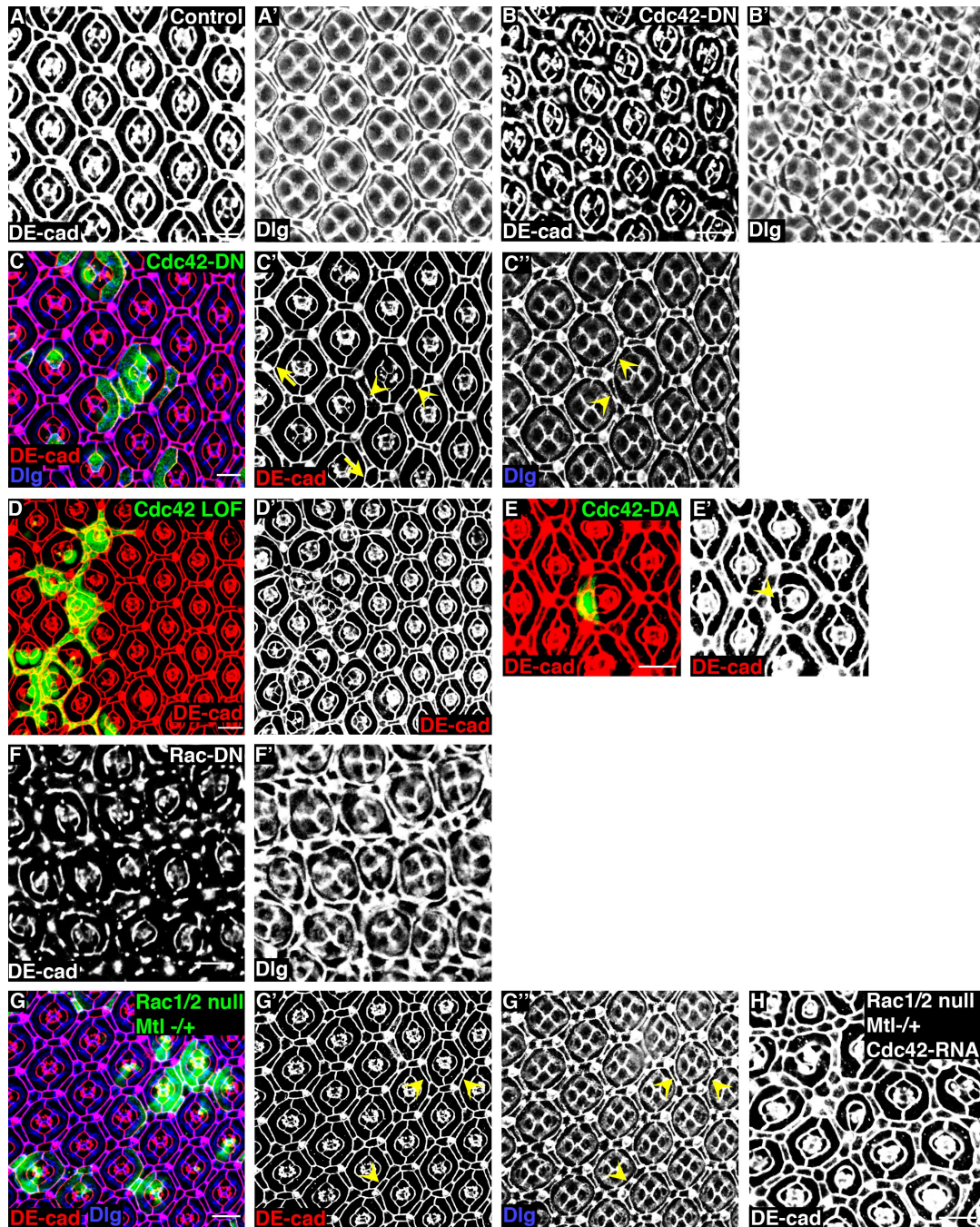
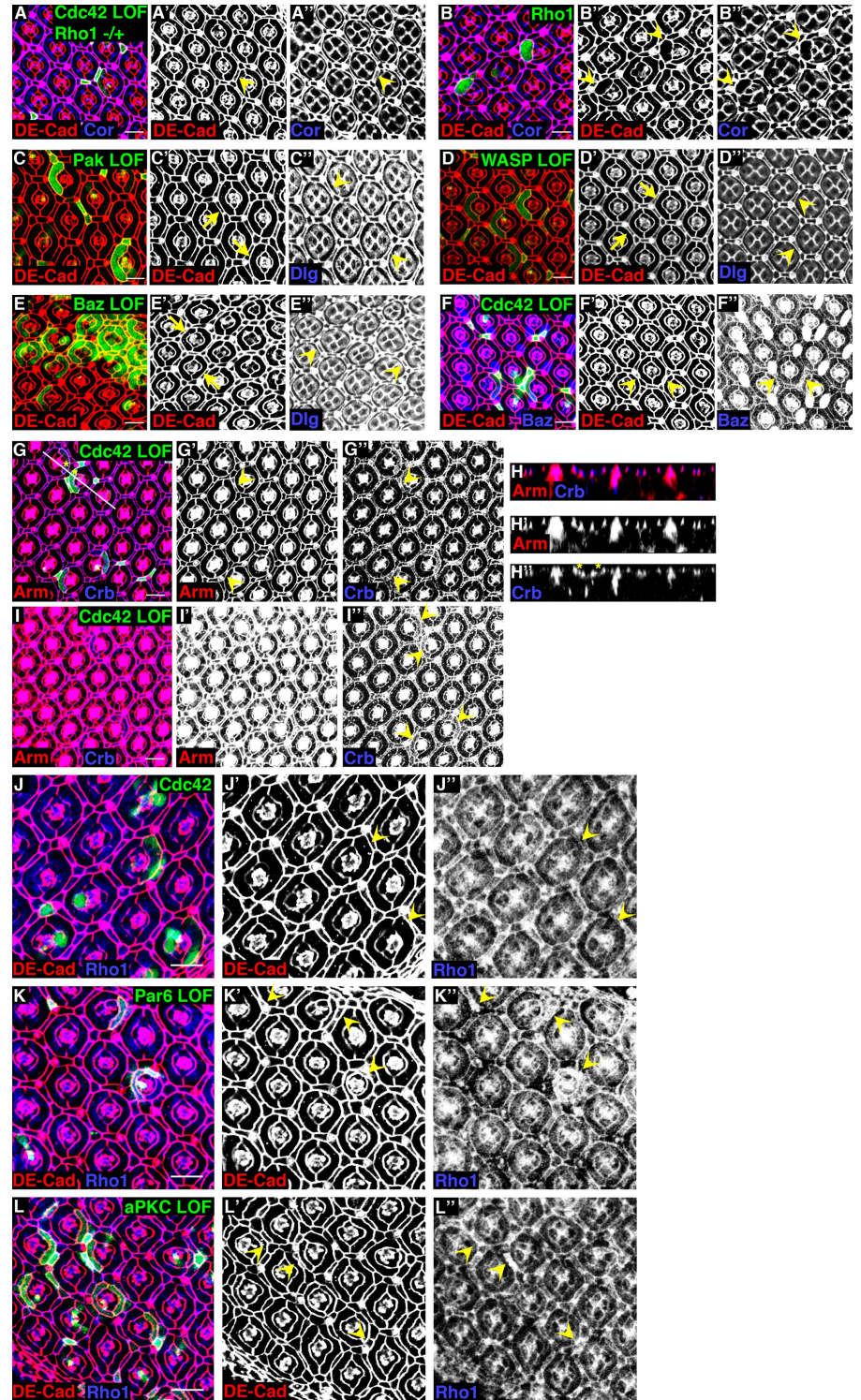


Figure S3. **Expression of Cdc42 and Rac dominant proteins results in nonspecific phenotypes.** (A and B) Confocal immunofluorescent localization of DE-cadherin (DE-cad; A and B) and Dlg (A' and B') in pupal eye expressing either *GMR-gal4* alone (A and A') or Cdc42-N17 with *GMR-gal4* (B and B'). (C) Confocal immunofluorescent localization of DE-cadherin (C and C') and Dlg (C and C'') in Flp-out clones expressing Cdc42-N17. Arrowheads identify AJs (C') and SJJs (C'') between clonal cells. Arrows identify clonal cells. (D) Confocal immunofluorescent localization of DE-cadherin in large *Cdc42*<sup>LOF</sup> MARCM clone. (E) Confocal immunofluorescent localization of DE-cadherin in Flp-out clone expressing Cdc42-V12. The arrowhead identifies a clonal cell. (F) Confocal immunofluorescent localization of DE-cadherin (F) and Dlg (F') in pupal eye expressing Rac-N17 with *GMR-gal4*. (G) Confocal immunofluorescent localization of DE-cadherin (G and G') and Dlg (G and G'') in MARCM clones of *rac1*<sup>111</sup>, *rac2*<sup>Δ</sup> in an *mtl*<sup>Δ</sup> heterozygote. Arrowheads identify AJs (G') and SJJs (G'') between clonal cells. (H) Confocal immunofluorescent localization of DE-cadherin in EGUF (*eyeless-gal4*, UAS-flippase) pupal eye homozygous for *rac1*<sup>111</sup>, *rac2*<sup>Δ</sup>, heterozygous for *mtl*<sup>Δ</sup>, and expressing Cdc42-RNAi. Bars, 10 μm.



Figure S4. **Rho1 does not regulate SJs; Pak, Wsp, and Baz LOF clones do not phenocopy Cdc42 LOF clones; Cdc42 depletion does not disrupt Baz or Crbs AJ localization; and Cdc42 overexpression decreases, whereas Par6 and aPKC depletion increases Rho1 localization at AJs.** (A and B) Confocal immunofluorescent localization of DE-cadherin (DE-Cad; A, A', B, and B') and Coracle (Cor; A, A', B, and B') in *Cdc42<sup>d</sup>* MARCM clones in a *Rho1<sup>72F</sup>* heterozygous background (A–A') and Flp-out clones overexpressing WT Rho1 (B–B'). Arrowheads identify AJs (A' and B') and SJs (A'' and B'') around clonal primary PECs. (C–E) Confocal immunofluorescent localization of DE-cadherin (C, C', D, D', E, and E') and Dlg (C'', D'', and E'') in *pak<sup>16</sup>* MARCM clones (C–C'), *wsp<sup>3</sup>* MARCM clones (D–D'), and *baz<sup>d</sup>* MARCM clones (E–E'). Arrows identify clonal cells (C', D', and E'), and arrowheads identify SJs around clonal primary PECs (C'', D'', and E''). (F–I) Confocal immunofluorescent localization of DE-cadherin (F and F'), Baz (F and F'), Armadillo (Arm; G, G', H, H', I, and I'), and Crbs (Crb; G, G', H, H', I, and I') in *Cdc42<sup>d</sup>* MARCM clones. Arrowheads identify AJs (F' and G'), Baz (F''), and Crbs (G'' and I'') between or within clonal cells. The white line (G) identifies where the lateral section (H–H'') was taken. Asterisks identify *Cdc42<sup>d</sup>* MARCM clones (G and H''). I is a maximum projection of a z-series stack. (J) Confocal immunofluorescent localization of DE-cadherin (J and J') and Rho1 (J and J') in Flp-out clones overexpressing WT Cdc42. (K and L) Confocal immunofluorescent localization of DE-cadherin (K, K', L, and L') and Rho1 (K, K', L, and L') in *par6<sup>Δ226</sup>* MARCM clones (K–K') and *aPKC<sup>K06403</sup>* MARCM clones (L–L'). (J–L) Arrowheads identify clonal cells. Bars, 10  $\mu$ m.





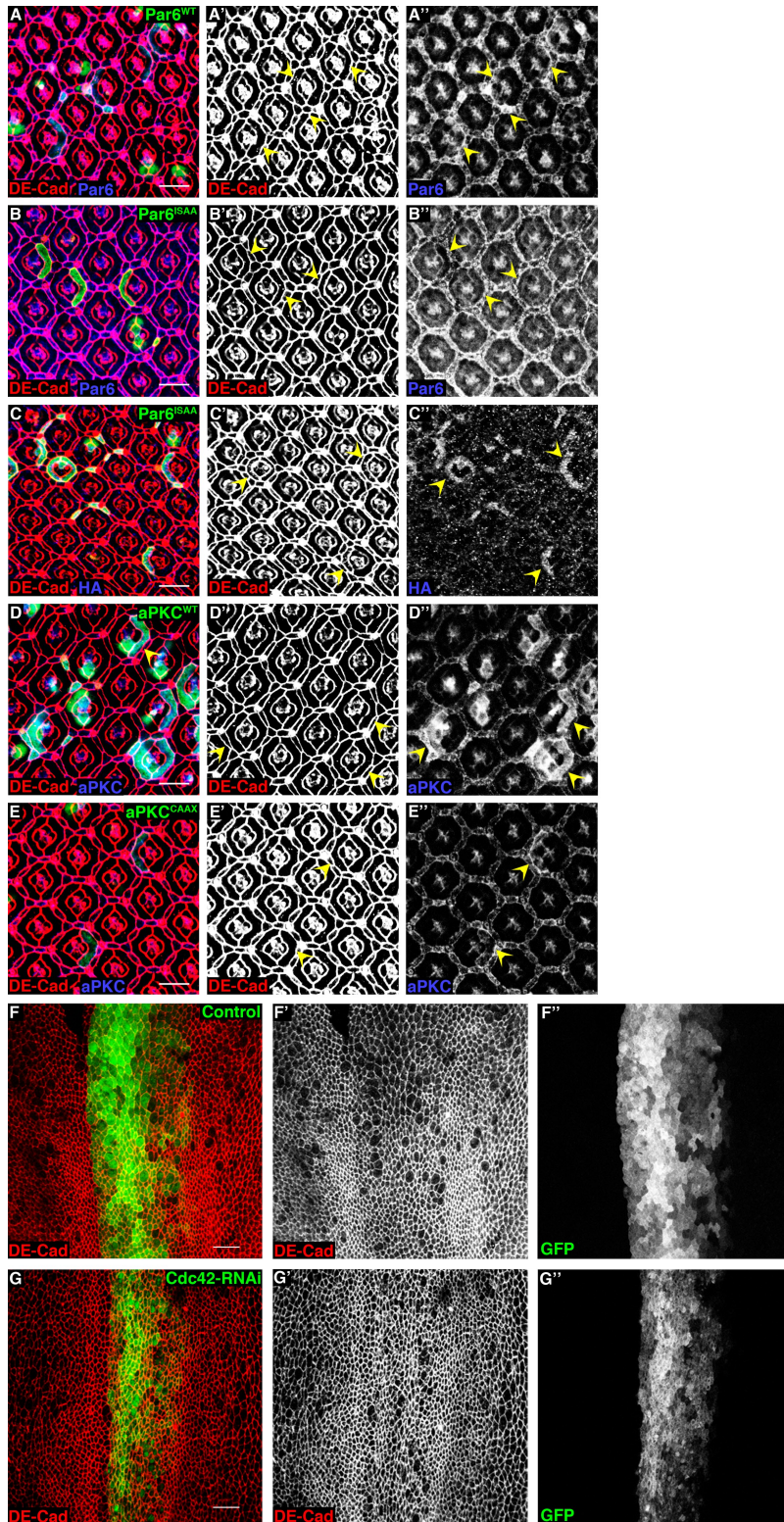


Figure S5. **Par6<sup>WT</sup> is expressed at equal or higher levels than Par6<sup>ISAA</sup>; aPKC<sup>WT</sup> localizes diffusely in the cell, whereas aPKC<sup>CAAX</sup> localizes to the cell membrane; and depletion of Cdc42 in the pupal wing decreases apical cell area.** (A–C) Confocal immunofluorescent localization of DE-cadherin (DE-Cad; A, A', B, B', C, and C'), Par6 (A, A', B, and B'), and HA (C and C') in Flp-out clones expressing WT Par6 (Par6<sup>WT</sup>; A–A') and Cdc42-binding mutant Par6 (Par6<sup>ISAA</sup>), which is tagged with HA (B–C'). (D and E) Confocal immunofluorescent localization of DE-cadherin (D, D', E, and E') and aPKC (D, D', E, and E') in Flp-out clones expressing WT aPKC (aPKC<sup>WT</sup>; D–D') and membrane-targeted aPKC (aPKC<sup>CAAX</sup>; E–E'). (A–E) Arrowheads identify clonal cells. (F) Confocal immunofluorescent localization of DE-cadherin (F and F') in pupal wing epithelial cells expressing GFP (F and F') using *patched-gal4*. (G) Confocal immunofluorescent localization of DE-cadherin (G and G') in pupal wing epithelial cells coexpressing GFP (G and G') and Cdc42-RNAi using *patched-gal4*. Bars, 10 μm.



Table S1. **SJ mislocalization quantification**

Genotype	Clonal primary PECs with SJ mislocalizations	<i>n</i>
	%	
<i>Cdc42<sup>d</sup></i>	86	83
<i>Cdc42<sup>d</sup></i> + <i>Cdc42</i>	0	15

Clonal primary PECs had mislocalized SJs if one or more of the surrounding SJs were mislocalized compared with SJs surrounding adjacent, nonclonal primary PECs.

Table S2. **Apical area index quantification**

Genotype	Apical area index mean	SD	<i>n</i>	P
WT	0.998	0.0286	25	NA
<i>Cdc42<sup>d</sup></i>	0.570	0.0393	105	0.0000033 (WT)
<i>Cdc42<sup>2</sup></i>	0.860	0.0843	55	0.041 (WT)
<i>Cdc42</i> -RNAi	0.790	0.0468	60	0.0053 (WT)
<i>Cdc42<sup>d</sup></i> + <i>Cdc42</i>	1.059	0.0395	30	0.000015 ( <i>Cdc42<sup>d</sup></i> )
<i>Cdc42</i>	1.245	0.0634	28	0.00032 (WT)
<i>Cdc42<sup>d</sup></i> , <i>Rho1<sup>72F</sup></i> +/–	0.736	0.0264	19	0.00096 ( <i>Cdc42<sup>d</sup></i> )
<i>Cdc42<sup>d</sup></i> + <i>Rho1</i> -RNAi	0.969	0.0519	38	0.0000066 ( <i>Cdc42<sup>d</sup></i> )
<i>Cdc42<sup>d</sup></i> + <i>aPKC<sup>WT</sup></i>	0.572	0.0179	20	0.78 ( <i>Cdc42<sup>d</sup></i> )
<i>Cdc42<sup>d</sup></i> + <i>aPKC<sup>CAAX</sup></i>	0.744	0.0317	52	0.000028 ( <i>Cdc42<sup>d</sup></i> )
<i>par6<sup>Δ226</sup></i>	0.542	0.0464	32	0.0000011 (WT)
<i>par6<sup>Δ226</sup></i> , <i>Rho1<sup>72F</sup></i> +/–	0.830	0.0742	31	0.0010 ( <i>par6<sup>Δ226</sup></i> )
<i>par6<sup>Δ226</sup></i> + <i>Rho1</i> -RNAi	0.977	0.0130	23	0.000015 ( <i>par6<sup>Δ226</sup></i> )
<i>aPKC<sup>K06403</sup></i>	0.624	0.0498	86	0.0000020 (WT)
<i>aPKC<sup>K06403</sup></i> + <i>Rho1</i> -RNAi	1.100	0.0343	30	0.0000037 ( <i>aPKC<sup>K06403</sup></i> )
Rok-CAT	0.449	0.0655	18	0.0000047 (WT)
<i>tsr<sup>99E</sup></i>	0.624	0.0590	24	0.00020 (WT)
<i>ssh<sup>1-11</sup></i>	0.808	0.0662	26	0.0060 (WT)
<i>Cdc42<sup>d</sup></i> (SJs)	1.0311	0.0760	43	0.00038 ( <i>Cdc42<sup>d</sup></i> , AJs)

NA, not applicable. Apical area index is the ratio of a clonal cell apical area divided by an analogous, neighboring nonclonal cell apical area at AJs (except for the last row, which was measured at SJs). Quantifications were performed using ImageJ version 1.38. P-values were calculated using an unpaired, two-sided Student's *t* test against the genotype indicated in parentheses.

Table S3. **AJ-associated F-actin index quantification**

Genotype	F-actin index mean	SD	<i>n</i>	P
WT	1.005	0.012	26	NA
<i>Cdc42<sup>d</sup></i>	1.737	0.136	29	0.011
<i>par6<sup>Δ226</sup></i>	1.842	0.228	16	0.023
<i>aPKC<sup>K06403</sup></i>	1.961	0.311	45	0.009
<i>tsr<sup>99E</sup></i>	2.001	0.187	19	0.010
<i>ssh<sup>1-11</sup></i>	1.704	0.329	26	0.024

NA, not applicable. AJ-associated F-actin index is the ratio of phalloidin staining pixel intensity at AJs in a clonal cell divided by an analogous, neighboring nonclonal cell. Quantifications were performed using ImageJ version 1.38. P-values were calculated using an unpaired, two-sided Student's *t* test against WT clones.



Table S4. **AJ-associated phospho-MLC index quantification**

Genotype	pMLC index mean	SD	n	P
WT	1.0694	0.0396	22	NA
<i>Cdc42</i> <sup>Δ</sup>	1.739	0.134	15	0.0075
<i>par6</i> <sup>Δ226</sup>	1.760	0.245	19	0.035
<i>aPKC</i> <sup>k06403</sup>	1.721	0.236	33	0.0098
Rok-CAT	2.353	0.717	13	0.037

NA, not applicable. AJ-associated phospho-MLC index is the ratio of phospho-MLC (pMLC) immunofluorescence pixel intensity at AJs in a clonal cell divided by an analogous, neighboring nonclonal cell. Quantifications were performed using ImageJ version 1.38. P-values were calculated using an unpaired, two-sided Student's *t* test against WT clones.

Table S5. **PKNG58AeGFP peak pixel intensity at AJs quantification**

Genotype	PKNG58AeGFP peak pixel intensity mean	SD	n	P
Control	149.816	25.727	165	NA
Cdc42-RNAi	212.958	19.723	162	0.0000974

NA, not applicable. PKNG58AeGFP peak pixel intensities at AJs were determined from plotting and listing pixel values across a line drawn through PEC AJs (as shown in Fig. 5, A and B). Quantifications were performed using ImageJ version 1.38. The p-value was calculated using an unpaired, two-sided Student's *t* test against the control.