Genotype	<i>ptc</i> area/total area	Std dev	Ν	Р
ptc>P35	0.126	0.0194	10	< 0.000000001
<i>ptc</i> >Cdc42-RNAi + P35	0.304	0.0448	27	N/A
<i>ptc</i> >Cdc42-RNAi + P35 + Puc	0.205	0.0737	6	0.019
<i>ptc</i> >Cdc42-RNAi + P35 + JNK-			11	0.00000025
RNAi	0.202	0.0324		
<i>ptc</i> >Cdc42-RNAi + P35 + <i>Rho1</i> <sup>72F</sup>			9	0.0000000045
+/-	0.219	0.0194		
$ptc$ >Cdc42-RNAi + P35 + $Zip^{1}$ -/+	0.223	0.0482	9	0.00057
<i>ptc</i> >Cdc42-RNAi + P35 + Rpr	0.257	0.0491	5	0.093
<i>ptc</i> >Cdc42-RNAi + P35 + Diap1	0.329	0.0464	9	0.21
$ptc>Cdc42$ -RNAi + P35 + $Th^{1}$ -/+	0.305	0.0692	5	0.99

Supplemental Table 1. Hyperproliferation in *ptc* expression domain quantification

Quantification of hyperproliferation in *ptc* expression domain. The areas of GFP-positive cells in the *ptc* expression domain and the total wing disc areas were measured by outlining the respective regions and determining the number of pixels within the outline. Quantifications were performed using ImageJ v1.38. P-values were calculated using an unpaired, two-sided Student's t-test against *ptc*>Cdc42-RNAi + P35. N/A: not applicable.

## **Supplemental Figure Legends**

**Figure S1.** Discs large depletion induces apoptosis and activates JNK but does not regulate apoptosis-induced compensatory proliferation; Crb depletion does not induce apoptosis or activate JNK

Confocal immunofluorescent localization of DE-cadherin (A, A', B), Discs large (Dlg) (A, A''), and activated Caspase 3 (A, A''', B') in larval wing discs expressing Dlg-RNAi alone (A) and in a  $puc^{E69}$  heterozygous background (B) using *en-gal4*. Confocal immunofluorescent localization of DE-cadherin (C, D) and activated Caspase 3 (C', D') in larval wing discs expressing Cdc42-RNAi (C) and Dlg-RNAi (D) using *en-gal4* 3 hours after 40 Gy of irradiation exposure. Confocal immunofluorescent localization of DE-cadherin (E, E', F, G), Crumbs (Crb) (E, E'''), activated Caspase 3 (F'), and  $\beta$ -galactosidase (G') in larval wing discs expressing Crb-RNAi alone using *ptc-gal4* (E, F) and in a *puc*<sup>E69</sup> heterozygous background using *en-gal4* (G). Scale bars represent 100 µm (A-D, F, G) and 10 µm (E).

**Figure S2.** Scribble depletion induces apoptosis and activates JNK but does not regulate apoptosis-induced compensatory proliferation; and Rho1 depletion induces apoptosis but not compensatory proliferation

Confocal immunofluorescent localization of DE-cadherin (A, B), activated Caspase 3 (A'), and MMP1 (B') in larval eye discs with GFP-labeled *scrib*<sup>1</sup> clones alone (A) and GFP-labeled *scrib*<sup>1</sup> clones expressing P35 (B). Adult eyes resulting from generation of clones expressing P35 (C) and *scrib*<sup>1</sup> clones expressing P35 (D). Confocal

immunofluorescent localization of DE-cadherin (E, F) and activated Caspase 3 (E') in larval wing discs expressing Rho1-RNAi (E), and co-expressing Rho1-RNAi with P35 (F) using *ptc-gal4*. Scale bars represent 100 μm.

Figure S3. Cdc42/Par6/aPKC complex regulate JNK activity

Confocal immunofluorescent localization of DE-cadherin (A) and  $\beta$ -galactosidase (A') in larval eye disc with GFP-labeled *Cdc42<sup>4</sup>* clones in a heterozygous background of *puc<sup>E69</sup>* (*puc-lacZ*). Confocal immunofluorescent localization of DE-cadherin (B-E) and MMP1 (B'-E') in larval eye discs with GFP-labeled P35 expressing clones (B), *Cdc42<sup>4</sup>* clones expressing P35 (C), *par6*<sup> $\Delta$ 226</sup> clones expressing P35 (D), and *aPKC*<sup>K06430</sup> clones expressing P35 (E). Confocal immunofluorescent localization of DE-cadherin (F) and Wingless (Wg) (F'') in larval wing disc co-expressing Cdc42-RNAi and P35 with *engal4*. Confocal immunofluorescent localization of DE-cadherin (G, H) and phospho-MAD (pMAD) (G, G'', H, H'') in larval wing discs expressing P35 alone (G) and P35 and Cdc42-RNAi (H) with *ptc-gal4*. Scale bars represent 100 µm.

**Figure S4.** Par6<sup>ISAA</sup> expression mislocalizes aPKC and Cdc42 depletion does not mislocalize Baz

Confocal immunofluorescent localization of DE-cadherin (A-C, A'-C'), aPKC (A, A'', B, B''), and Bazooka (C, C'') in larval wing discs expressing wild type Par6 (A), Cdc42 binding mutant Par6 (B), and Cdc42-RNAi (C) with *ptc-gal4*. Scale bars represent 10 µm.

Figure S5. Rok regulates compensatory proliferation and activates JNK

Confocal immunofluorescent localization of DE-cadherin (A, B), activated Caspase 3 (A'), and MMP1 (B') in larval wing discs expressing Rok-catalytic domain (Rok-CAT) alone (A) and with P35 (B) using *ptc-gal4*. Scale bars represent 100 µm.

Figure S6. Disc Large depletion does not regulate phospho-MLC levels

Confocal immunofluorescent localization of DE-cadherin (A, A') and phospho-MLC (A''') in larval wing disc expressing Dlg-RNAi using *ptc-gal4*. Scale bar represent 40 µm.











