

**FILE S1****Supplemental Experimental Procedures***Bloomington deficiency screening*

We stained collections of Bloomington embryos with rabbit anti-Miranda (gift of Fumio Matsuzaki) and identified stocks with Mendelian segregation of delocalized Miranda in neuroblasts. We subsequently tested the entire current 2nd chromosome collection (DK2) for failure to complement *lgl[4]*.

*Bruinfly FRT-P lethal screening*

We induced germline clones in *hs-FLP/+; P lethal FRT40A/ovo[D], FRT40A* females by heat-shocking for 1 hr at 38°C during the first instar. We mated these to sibling males and prepared cuticles from 16-24 hr embryos, which were scored for the presence of epithelial patterning defects. We subsequently tested 4-10 individual flies from 24 randomly selected Bruinfly stocks for failure to complement *lgl[4]*.

*Analysis of insensitive*

We analyzed thorax clones in *Ubx-FLP/+; insv [23B]* (or *insv[23L]*), *FRT40A/ubi-GFP, FRT40A* animals. We dissected 24 hr APF pupae and stained these with rabbit anti-GFP (Molecular Probes), rat anti-Elav Developmental Studies Hybridoma Bank, DSHB), mouse anti-Prospero (DSHB), rat anti-Suppressor of Hairless (gift of Francois Schweisguth), rabbit anti-Numb (gift of Yuh Nung Jan), rabbit anti-Lgl (gift of Scott Goode), followed by Alexa-coupled secondary antibodies (Molecular Probes). Following the determination that *insv* chromosomes harbored *lgl*, we performed rescue tests in *Ubx-FLP/+; tub-Gal80, FRT40A/lgl, insv [23B], FRT40A; neur-Gal4, UAS-lgl-GFP* (or *UAS-Histone2B-RFP*) animals.