SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIGURE 1. pins tumors do not require lgl loss. (A) PCR analysis of genomic fragments corresponding to lgl exons I, VI and XII appear unchanged in the tumor line pins-TL2y compared to wild-type samples, hence discarding large deletions in these genomic regions. (B) Western blot from control larval brains, the GFP-pins-TL2y tumor line, and lgl^4 larval brain tumors. A protein band with the expected mobility of LGL, which is absent in lgl^4 larval brains, can be observed both in wild-type brains and in pins-TL2y. Staining with an antibody against α-TUBULIN was used as loading control.

SUPPLEMENTAL FIGURE 2. Overproliferation of MIRA-expressing cells in rapamycin-treated pins brains. (A) Optical sections from larval brains taken at ventral (+20 μm), middle (0 μm), and dorsal, (-20 μm) focal planes. The overall distribution of Mira expressing cells (white) remains unchanged upon loss of pins (pins SFF) or rapamycin treatment (wild type rapamycin). In contrast, in pins brains from rapamycin-treated larvae (pins rapamycin), Mira-expressing cells overproliferate in the dorsal side and invade deeper layers of the brain. Scale bar= 100 μm. (B) Most mitoses in pins brains exposed to rapamycin are symmetric (arrows). Scale bar= 10 μm.

SUPPLEMENTAL METHODS

Primers
Primer pairs GATCGCGACTTTGTGTGTGT / TTGCTCACCAACACGCATA, ACCCGGAGTTGAATTGTACG / AAGACCAACGCTCTGTCCAT, and ATAGGAACGCCCCAAAACAGC / TATTAAATGGGGACGGAACCA, were used to amplify genomic fragments from *lgl* exons I (233bp), VI (511bp), and XII (534bp), respectively.

**Western blots**

Larval brains and tumors were homogenized in NuPAGE® LDS sample buffer (Invitrogen) and incubated for 5 minutes at 95°. Samples were loaded on 10% NuPAGE® (Invitrogen), and transferred to iBlot® nitrocellulose membranes (Invitrogen) following the manufacturer’s instructions. Blocking and incubation with antibodies were carried out in 5% milk powder in PBT (Phosphate Buffered Saline, pH 7.5, 0.05% Tween20). The following antibodies were used: rabbit anti-LGL (provided by J. Knoblich) 1:100; mouse anti-PINS (provided by W. Chia) 1:200, mouse anti-a-TUBULIN (DM1A from Sigma) 1:500, HRP-labeled IgG antibodies (Jackson ImmunoResearch Laboratories), Alexa Fluor 600 and 800. Secondary antibody detection was carried out with the ECL Western blot detection system (Amersham) or using Odyssey® scanner (Li-cor bioscience).
FIGURE S1
Figure S2

Panel A: Images showing different genotypes and treatments: pins SFF, wild type rapamycin, and pins rapamycin at different locations: VENTRAL (+20μm), MIDDLE (0μm), and DORSAL (-20μm).

Panel B: Images showing DAPI staining, MIRA staining, and merged images. The merged image highlights specific regions with arrows indicating areas of interest.