SUPPLEMENTARY ONLINE MATERIAL

MATERIALS AND METHODS

Drosophila culture, crosses and mapping

Drosophila stocks were maintained using standard methods at 25°C. The original *tkv*^{xtr} mutant was generated by EMS mutagenesis and balanced over *CyO*. We narrowed the lesion in *EMS*^{4a21} to the interval between *dumpy* and *black* by recombination mapping against a multiply marked second chromosome and used a series of complementation tests with chromosomal deficiencies and *tkv* mutants to link EMS^{4a21} to the *tkv* locus at chromosomal interval 25D-25F. For rescue of *tkv*^{xtr} organismal lethality, we crossed *w; tkv*^{xtr}*, FRT40A,c,px,sp/ CyO; Tubulin-Gal4/TM6C* virgins to males of the genotype *w; tkv*^{xtr}*, FRT40A, c,px,sp/CyO; UAS-tkv* and scored for rescued *tkv*^{xtr}, *c, px, sp* homozygotes (non-TM6C and non-*CyO*). Mutant clones of *tkv*^{xtr}, *tkv*^{4} (also known as tkv^{a12}; *1*) and *mad*^{12} were induced by the directed mosaic method (*2*), using the epithelial driver *T155-Gal4* to express *UAS-flp*. The *T155-Gal4* driver exhibits patchy but ubiquitous expression throughout the wing disc. For rescue of *tkv*^{xtr} clones, virgin females of the genotype *w; Ub-GFP FRT40A; T155-Gal4, UAS-flp* were crossed to males of the genotype *w; tkv*^{xtr}*, FRT40A,c,px,sp/CyO; UAS-tkv*. In this and other crosses, *T155-Gal4* was employed to drive expression of UAS transgenes. To block apoptosis in *tkv*^{xtr} clones, we used a stock of the genotype *w; tkv*^{xtr} *FRT40A c,px,sp*/CyO; *UAS-p35*. For the *puc* experiments we analyzed clones in animals of the genotype *w; tkv*^{xtr}*, FRT40A,c,px,sp/Ub-GFP, FRT 40A; puc*^{E69LacZ}*/T155-Gal4, UAS-flp*, and for the piercing experiment we used

puc^{E69LacZ}/TM3Sb discs cultured *in vitro* in a 7:1 cocktail of Ringers:PBS supplemented with .0025M $CaCl_2$ and pH'd to 6.9.

Immunocytochemistry, confocal and electron microscopy

For immunocytochemistry, imaginal discs were fixed in 4% paraformaldehyde in PBS at room temperature for 30-40 minutes. All subsequent steps were performed according to standard protocols. Phalloidin-546 (Molecular Probes) was used at a dilution of 1:250, Mouse anti-Tubulin (Sigma) was used at a dilution of 1:2000, Rabbit anti-Phospho-Histone H3 at 1:2000, Rabbit anti-β-gal (Cappel) at 1: 500, and polyclonal Rabbit anti-Cleaved Caspase-3 (Cell Signalling) at 1:500. Secondary antibodies were Goat anti-Mouse Alexa 647 or Goat anti-Rabbit Alexa 647 (1:500; Molecular Probes). During mounting in 70% glycerol/PBS, double-stick tape was employed as a spacer to prevent the coverslip from compressing discs. This step is essential for optimal XZ image quality. Images were collected on a Leica TCS SP2 AOBS Confocal Microscope system and processed using Adobe Photoshop 7.0 and Metamorph software.

For electron microscopy, individual wing discs with visible extruded clones were selected under a dissecting scope and fixed in 1.25% formaldehyde, 2.5% glutaraldehyde and 0.03% picric acid in 100mM cacodylate buffer. Discs were then post-fixed with osmium tetroxide, embedded in Epon and sectioned using with a Reichert Ultracut. Sections were contrasted with uranyl acetate and lead citrate prior to image collection on a JEOL 1200EX Transmission Electron Microscope.

SUPPLEMENTAL FIGURES

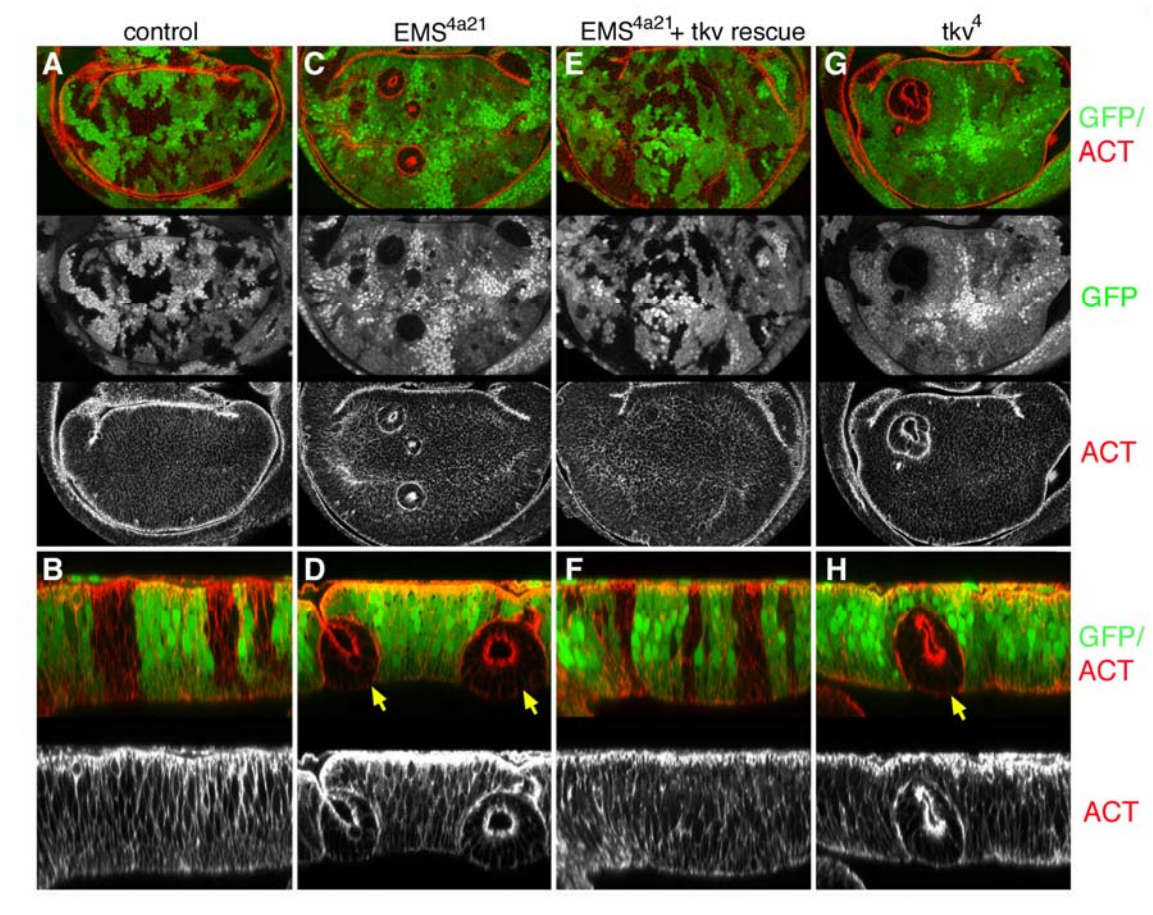

FIGURE S1. ***Expanded version of Fig. 1 showing individual channel images of GFP and ACT.*** Upper panels are standard confocal XY sections; lower panels are XZ optical cross sections. **A,B**) Control clones. **C,D**) *EMS*^{4a21} clones. **E,F**) *EMS*^{4a21} + *UAS-tkv* rescue. **G,H**) *tkv*^{4} clones.

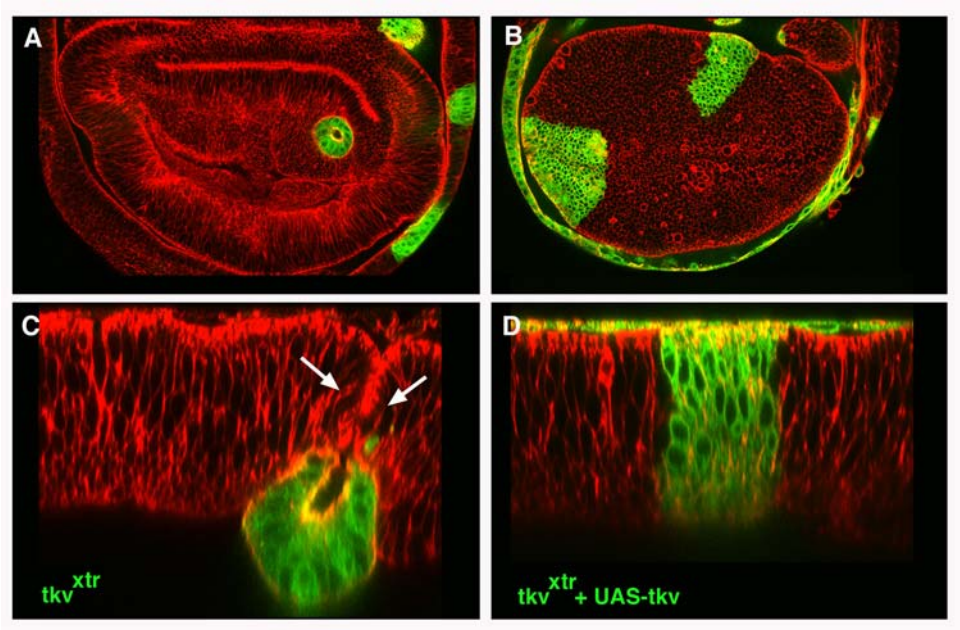

FIGURE S2. ***Autonomous rescue of extrusion using the MARCM technique (3).*** Discs are stained with phalloidin to label F-actin (*red*), and mutant cells are positively marked by expression of GFP (*green*). **A**) Basal XY section of a disc bearing a rare *hs>flp*-induced tkv^{xtr} clone, which has segregated from neighboring wild-type cells. **B**) Apical XY section of a disc bearing large tkv^{xtr} clones rescued by clone-autonomous expression of *UAS-tkv*. **C**) XZ section of the extruded clone from A. Note the deformation of the wild-type epithelial surface at the site of extrusion (*white arrows*), a point of potentially significant cytoskeletal stress. **D**) XZ section of the disc in B, illustrating normal integration of the rescued clone into the wild-type epithelium.

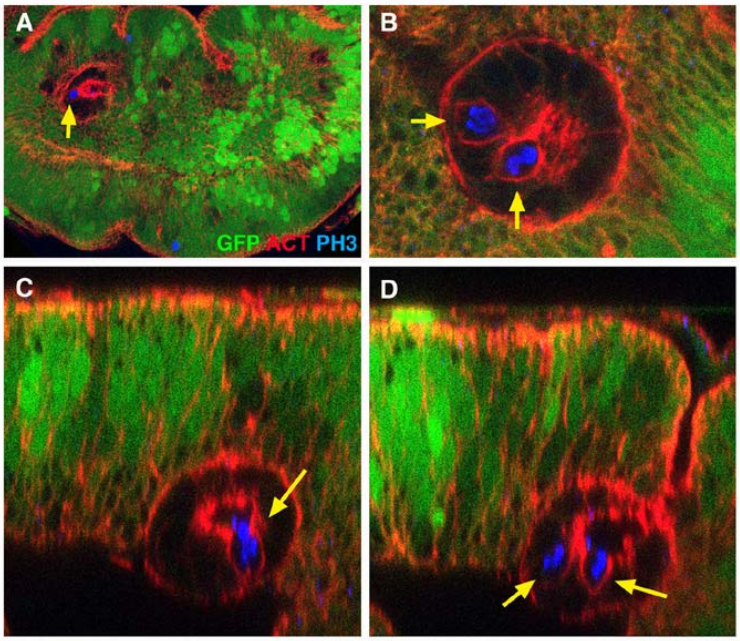

FIGURE S3. ***Cell proliferation in extruded tkv^4 clones induced by T155>flp***. Discs were stained with phalloidin to label F-actin (*red*) and anti-Phospho-Histone H3 (*blue*) to label mitotic cells (*yellow arrows*). **A**) XY section of an entire wing blade, showing an extruded clone containing a mitotic cell. **B**) Higher magnification XY section of an extruding clone with two mitotic cells. **C**) XZ section showing cell proliferation in an extruded clone. **D**) XZ section of the clone shown in B. Taken together, these results indicate that mutant cells continue to proliferate following extrusion, although whether they proliferate at the same rate as wild-type cells remains an important question.

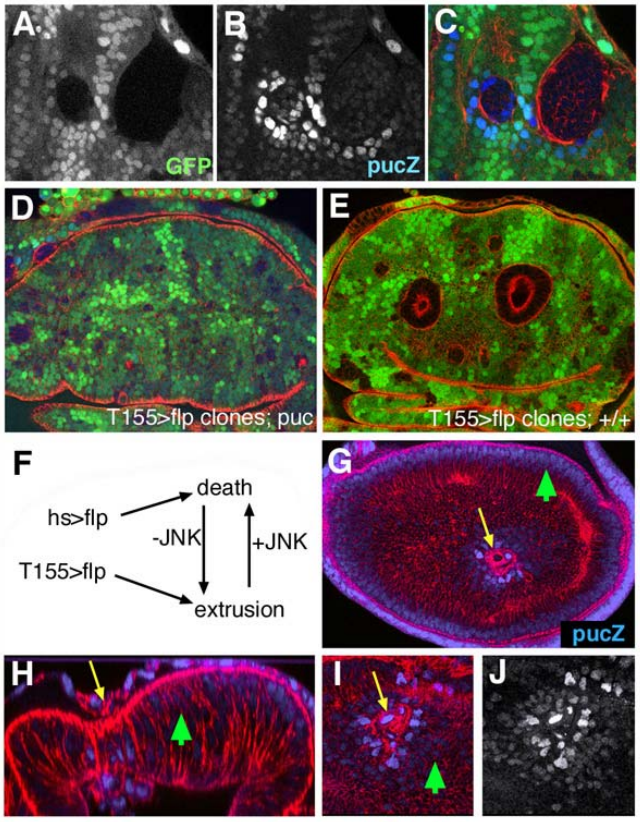

FIGURE S4. ***JNK activation and the elimination of extruded clones.*** JNK activity was monitored with *puc*^{E69LacZ}(*pucZ, blue*) in discs bearing *tkv*^{xtr} mutant clones marked by loss of GFP. **A-C**) *puc*^{E69LacZ} is detected within and around *T155>flp*-induced *tkv*^{xtr} clones in the presumptive notum, confirming that the JNK cascade is functional in this background. **D**) *tkv* clones are highly sensitive to levels of JNK activity. In the presence of a heterozygous copy of *puc*^{E69LacZ}, JNK activity is enhanced (after ref. *4*) and large *T155>flp*-induced clones were not recovered in the medial wing blade. **E**) "Control" disc bearing *T155>flp*-induced *tkv*^{xtr} clones, showing two large and several smaller extrusions. **F**) Schematized relationships between induction methods, background JNK levels, and phenotypic outcomes. *hs>flp*-induced *tkv* clones are normally killed, but when JNK activity is repressed such clones are often viable (*5*) and can actually be recovered as

extruding cysts (*6*). Conversely, *T155>flp*-induced *tkv* clones normally form viable extrusions, but when JNK activity is increased, these clones are killed. **G-J**) Discs pierced with a tungsten needle show elevated *puc*^{E69LacZ} reporter activity around the wound site. Discs are counterstained with phalloidin. Wound sites (*yellow arrows*) and selected control nuclei (*green arrows*) are indicated. **H**) XZ section through a pierced disc, note JNK activation in cells at the wound site as well as in cells forced basally out of the epithelium. **I**) Higher magnification XY section of a different wound site than shown in G, *puc* expression alone is shown in **J**.

References and Notes

1. The FlyBase Consortium. *N.A.R.* **31**,172 (2003). http://flybase.org/

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4. T. Adachi-Yamada, K. Fujimura-Kamada, Y. Nishida, K. Matsumoto, *Nature* **400**, 166 (1999).

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6. J. Shen and C. Dahmann, pers. comm.