

## **SUPPLEMENTAL INFORMATION**

### **Supplementary Methods**

#### **Fly Strains and Husbandry**

*ptc-GAL4* (II) (#2017), *UAS-GFP* (II) (#1521), *UAS-p35* (I) (#6298), *UAS-hid* (I), and *msn*<sup>06946</sup> (*msn-LacZ*, #11707) stocks were obtained from the Bloomington Stock Center; *UAS-Dronc*<sup>RNAi</sup> (II) (#8091R-1) and *UAS-Drice*<sup>RNAi</sup> (II) (7788R-1) were obtained from the National Institute of Genetics Fly Stock Center (Japan). *UAS-Dronc*<sup>Δ.3a</sup> (II) and *UAS-Dronc*<sup>Δ.2a</sup> (III) were a gift from A. Bergmann. *Drice*<sup>17</sup> and *drice*<sup>D1</sup> lines were a gift from H. Steller.

#### **Dissection and Immunostaining**

Ten larvae per genotype were dissected and serially placed into ice-cold phosphate buffered saline (PBS; 0.1M phosphate, 150mM NaCl). After all genotypes were dissected, the tissues were fixed in 4% Paraformaldehyde for 12 minutes, rinsed 3 x 10 minutes with PBS, incubated 3 x 10 minutes in PBST1 (PBS with 0.1% Triton X-100), then incubated in PAXDG (PBS, 1%BSA, 0.3% Triton X-100, 0.3% deoxycholate, 5% goat serum) overnight with the following antibodies and dilutions: anti-Mmp1 (mouse, 1:50, Developmental Studies Hybridoma Bank), anti-cleaved Caspase-3 (rabbit, 1:200, Cell Signaling Technology), anti-b-Galactosidase (mouse, 1:100, Developmental Studies Hybridoma Bank), and anti-phospho-SAPK/JNK (G9) (mouse, 1:100, Cell Signaling Technology).

Incubated tissues were washed with PBS-T for 3 x 10 minutes and then incubated with the either Alexa Fluor 568- or Cy5- conjugated secondary antibodies (Invitrogen; Jackson Immuno Labs) at a 1:1000 dilution in PAXDG overnight. They were then washed 3 x 10 minutes in PBS and incubated in 80% glycerol with 0.5% N-propyl gallate, followed by mounting with Vectashield (Vector Laboratories).

#### **TUNEL Assay**

Following fixation as above, tissues were washed three x 5 minutes in PBST1 on ice, washed 2 x 5 minutes in PBST5 (PBS with 0.5% Triton X-100) on ice, permeabilized in 100mM Citrate/0.1% Triton X-100 for 30 minutes at 65°C, washed 2 x 5 minutes in PBST5 on ice, and rinsed in the TUNEL Assay Buffer (In Situ Cell Death Detection Kit, TMR Red, Roche) for 10 minutes on ice. They were treated in a TUNEL reaction mixture consisting of 10μL of Enzyme Solution with 90μL of Label Solution at 37°C in the dark for 120 minutes. Tissues were then washed 2 x 5 minutes in PBST5 on ice and mounted.

#### **Imaging and Figure Preparation**

Imaging was performed on a Leica TCS SPE Confocal Microscope using an oil-immersion 63x lens objective. We chose laser intensity, gain, and offset settings to optimize imaging of cells in the posterior compartment. Confocal z-stacks were taken at a step rate of 0.6 microns and exported maximum projection images. Adobe Photoshop CS4 was used to adjust the orientation and levels and to assemble the images for figures. All images are uncropped.

### **Supplementary Figure Legends**

**Supplementary Figure 1: Characterization of caspase-directed invasion. (A)** Quantification of invasion into the posterior compartment. The y-axis shows distribution of observed invasion phenotypes. *p35-hid* wing discs showed a statistically significant increase in migration (asterisk). **(B,C)** Representative wing discs of *p35 hid* and *hid diap1* genotypes. Examples of migrating cells are shown in panel **B**. **(D,E)** Close ups of migrating cells from *p35-hid* wing discs showing co-localization of GFP and Mmp1 expression (arrows). **(D',E')** Mmp1 only channels of images shown in **D** and **E**.

**Supplementary Figure 2: Undead cells activate the Jnk pathway. (A,B)** MMP1 and cleaved caspase-3 staining in wing discs with indicated genotypes. **(C-F)** JNK reporter *msn-LacZ* activity as detected with an antibody against  $\beta$ -galactosidase in wing discs with indicated genotypes. **(C'-F')** *msn-LacZ* channel of images shown in **C-F**.

**Supplementary Figure 3: Precise effector caspase signaling is required for invasion. (A-B)** Representative images of wing discs with indicated genotypes. (arrow: migrating cell). **(C)** Quantification of invasion phenotypes. The y-axis shows the distribution of observed invasion phenotypes. Asterisk indicates bonferroni-corrected significance ( $\alpha=.05$ ) for *hid drice*<sup>RNAi</sup> vs. control.