Supporting Information

Bardet *et al.* 10.1073/pnas.0806983105

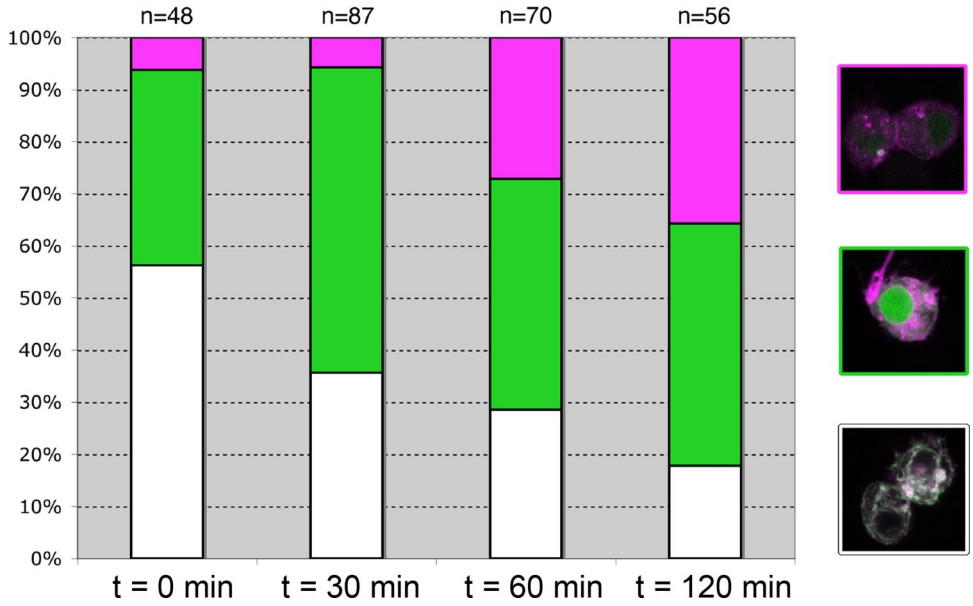


Fig. S1. Apoptosis in S2 cells induces Apoliner cleavage. S2 cells were transfected with Apoliner and then separated into four groups. One group was fixed 48 h after transfection (t = 0 min). The others were instead exposed to UV for 10 min and then fixed at various subsequent times, as indicated. Each group was then analyzed by confocal microscopy and cells were assigned to one of three categories according to the subcellular distribution of the two Apoliner fluorophores. In one group, GFP and RFP co-localize at membranes, indicating no cleavage (*Right Lower*). In the second group, RFP is at membranes and GFP in the nucleus, an indication of apoliner cleavage (*Right Middle*). In the last category, RFP is at membrane and GFP is barely detectable (*Right Upper*). This latter category becomes increasingly represented over time, suggesting that it represents a late stage of apoptosis. Presumably a late-staged nuclear GFP signal has been degraded by proteolytic activity. As expected, the number of cells in which RFP and GFP co-localize decreases after UV treatment.

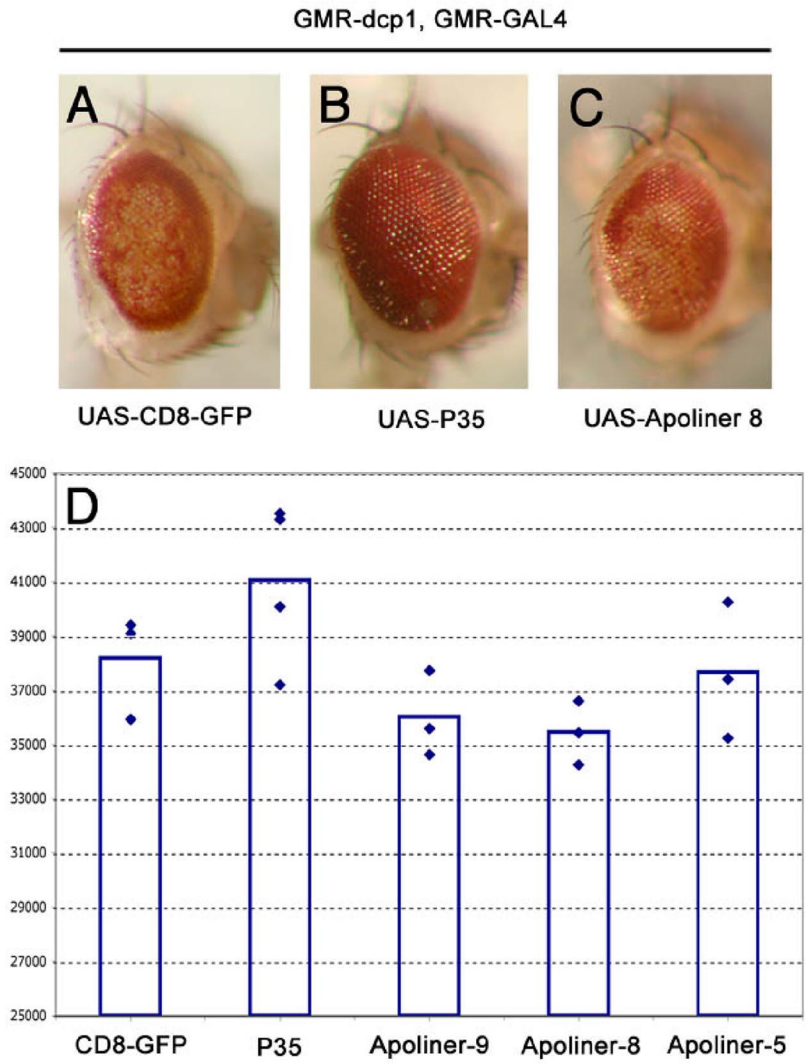
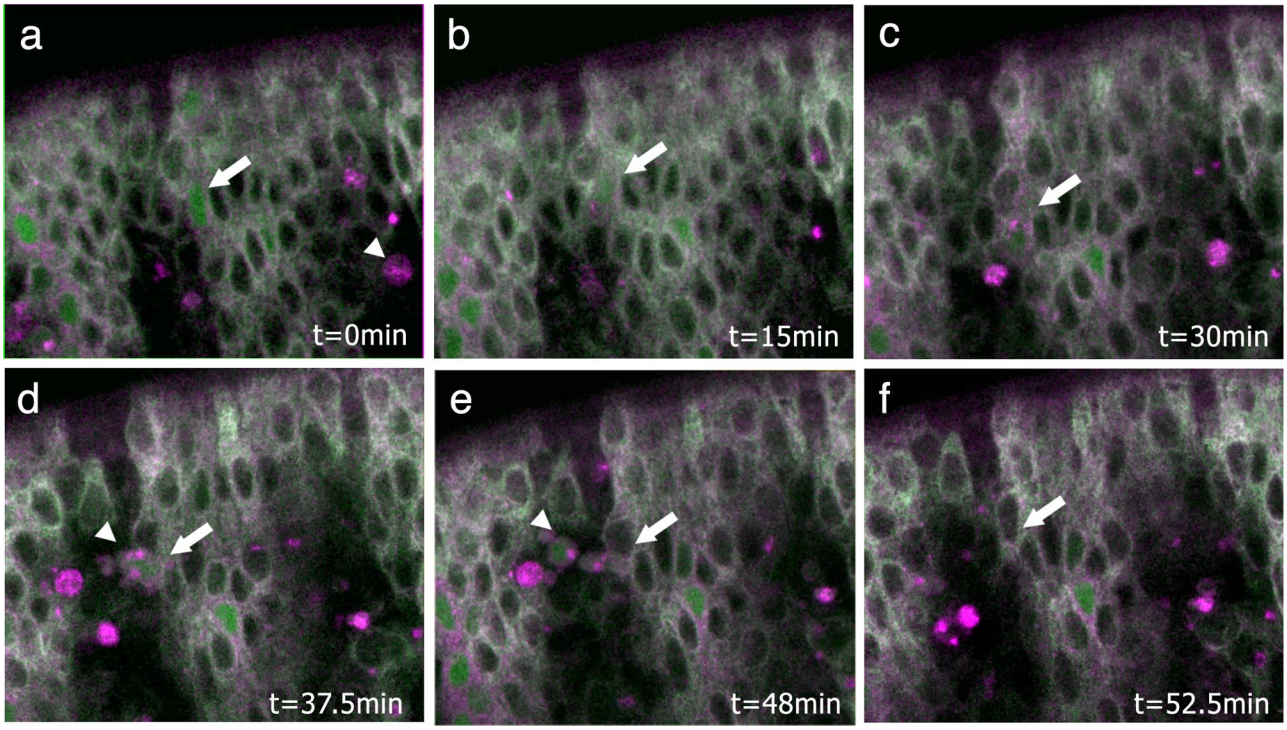


Fig. S2. Expression of Apoliner does not affect caspase-induced apoptosis in the adult eye. (*A–C*) Adult eyes expressing Dcp1, an effector caspase, under the control of *GMR-Gal4*. (*A*) Expression of Dcp1 alone (or with a 'neutral transgene' such as UAS-CD8-GFP, as shown) leads to increased cell death, the absence of some pigment cells and a rough eye phenotype. (*B*) Co-expression of p35, an inhibitor of apoptosis containing a BIR domain, completely suppresses Dcp1-induced cell death. (*C*) By contrast, co-expression of Apoliner does not affect the caspase activity induced by ectopic Dcp1: eye size is still reduced, indicating ectopic cell death (*A*). (*D*) Chart summarizing eye sizes as measured on electromicrographs. In all cases, Dcp1 is overexpressed under the control of *GMR-Gal4*. The following transgenes are co-expressed along with Dcp1, from left to right: CD8-GFP, P35, and Apoliner from three different insertions (UAS-Apoliner-9, -8, and -5, which have increasing strength). Bars represent the average, and individual eye sizes are represented by small squares. Size is given in square pixels, as evaluated with Adobe Photoshop.



Movie S1. Time-lapse imaging of Apoliner in a live embryo. Expression of Apoliner in the embryonic epidermis was driven with the 69B-Gal4 driver and the ventral side was imaged from stage 12 onward by time lapse confocal microscopy. For details on filming conditions, see Material and Methods. Note the fast-moving cells with strong red signal, corresponding to macrophages with residual mRFP from engulfed dead cells. Anterior to the left.

Movie S1 (MOV)