Koto et al., http://www.jcb.org/cgi/content/full/jcb.200905110/DC1

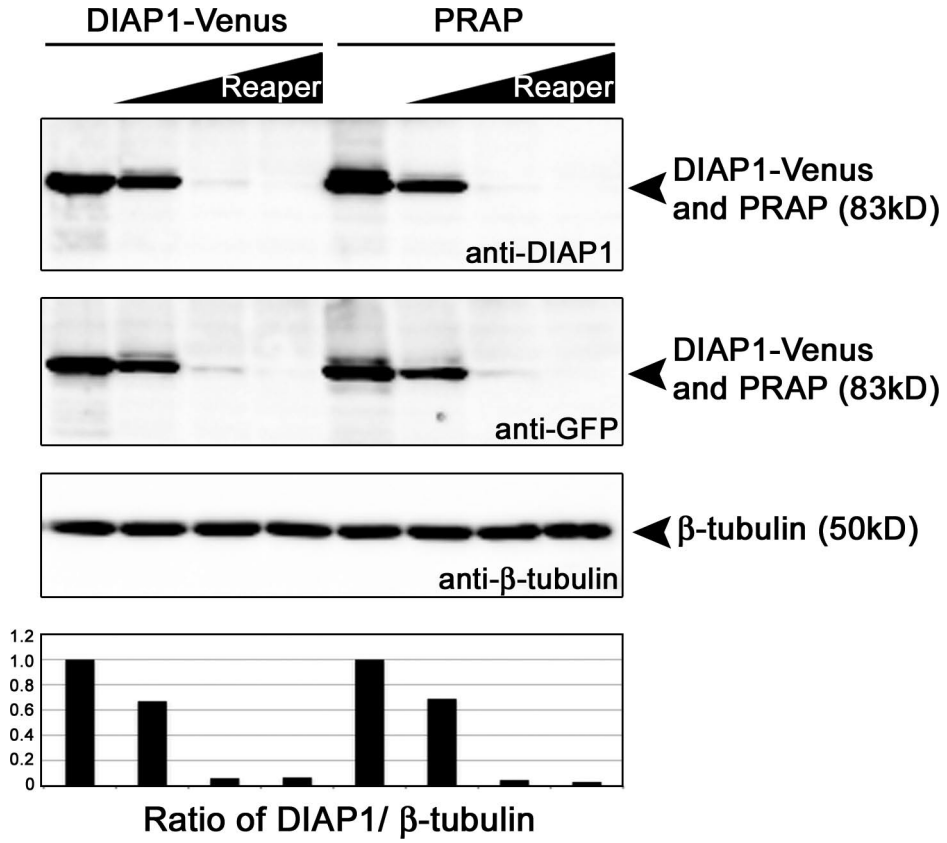


Figure S1. **PRAP could be degraded by Reaper expression.** PRAP and DIAP1-Venus could be degraded by Reaper expression in a dose-dependent manner. S2 cells were transfected with 0, 100, 200, or 400 ng of *pUAST-Reaper*, *pUAST-p35*, *pWAGAL4*, and each indicator. The experiment was repeated three times, and one representative is shown. The expression levels of PRAP and DIAP1-Venus were detected in whole-cell lysates by immunoblotting with anti-DIAP1 and anti-GFP antibodies.

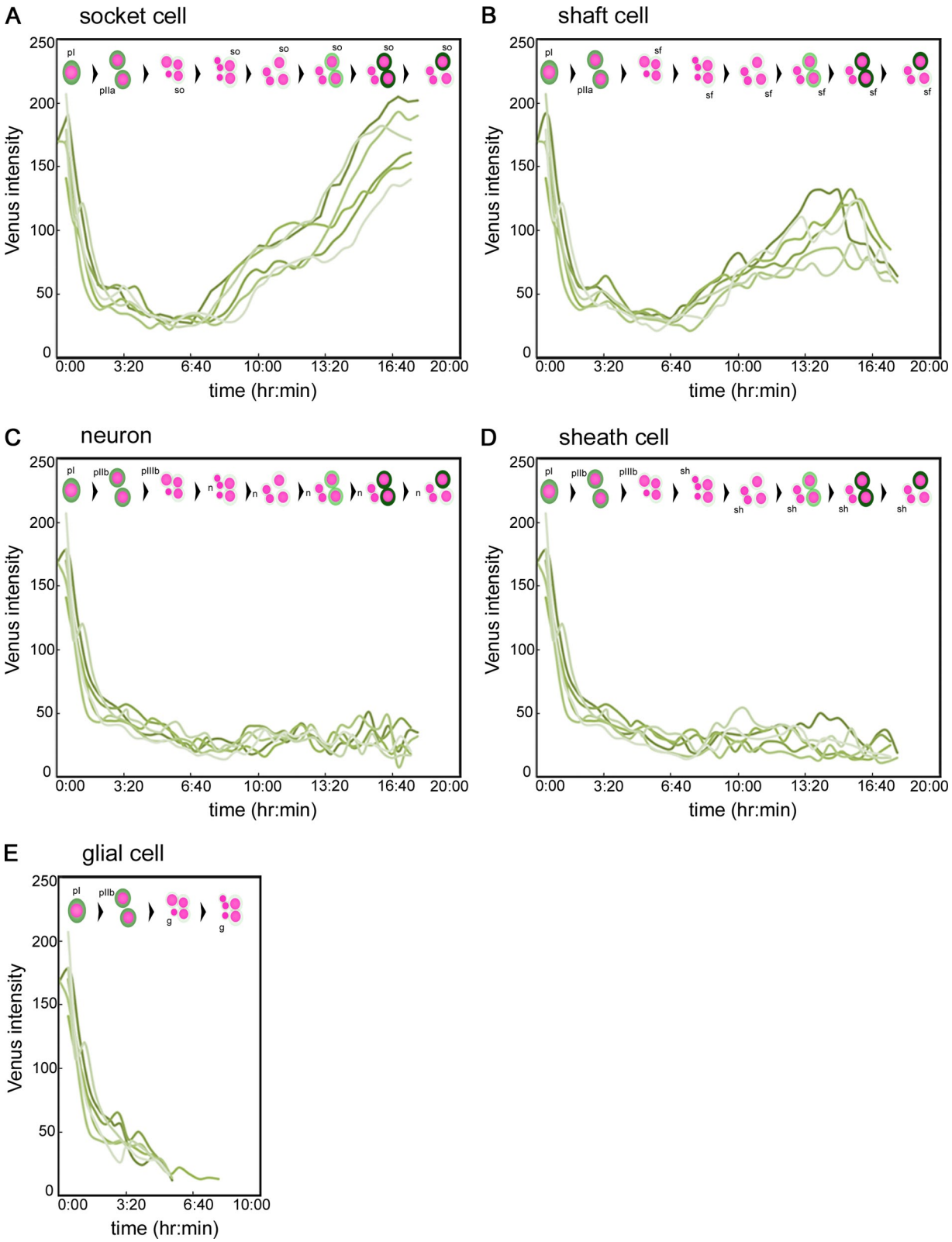


Figure S2. **PRAP pattern in each cell type.** (A–E) Sequential variation of Venus intensity in the socket cell (A), shaft cell (B), neuron (C), sheath cell (D), and glial cell (E). Six lineages were analyzed for each developmental stage. Each line shows the Venus intensity in independent lineages. The genotype was *neu-GAL4 UAS-Histone2B-ECFP/UAS-PRAP39*.

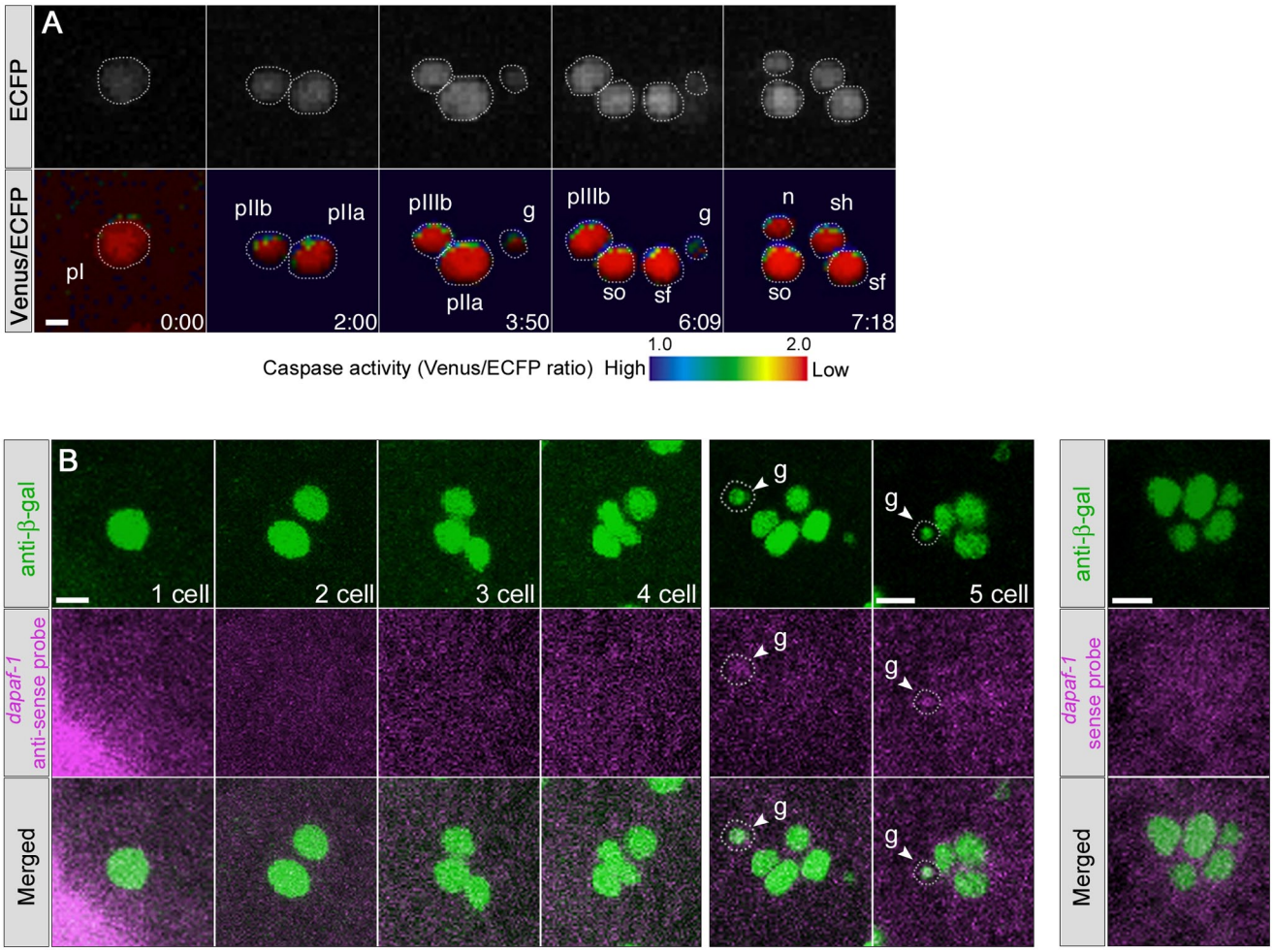


Figure S3. **PRAP degradation does not induce caspase activation in the SOP lineage.** (A) Caspase activation was detected only in the glial cell before it died, but not in other living cells. Ratio images of nls-SCAT3–expressing cells are shown. Bar, 5 μm. (B) *dapaf-1* mRNA is detected in the glial cell when it dies at the five-cell stage. *dapaf-1* mRNA (magenta) was detected by in situ hybridization using a *dapaf-1* anti-sense probe. No signal was detected by the *dapaf-1* sense probe. Each SOP nucleus is marked by an anti–β-gal antibody (green). The genotypes were (A) *UAS-nls-SCAT3/CyO; neu-GAL4/MKRS;* and (B) *A101/TM3*.

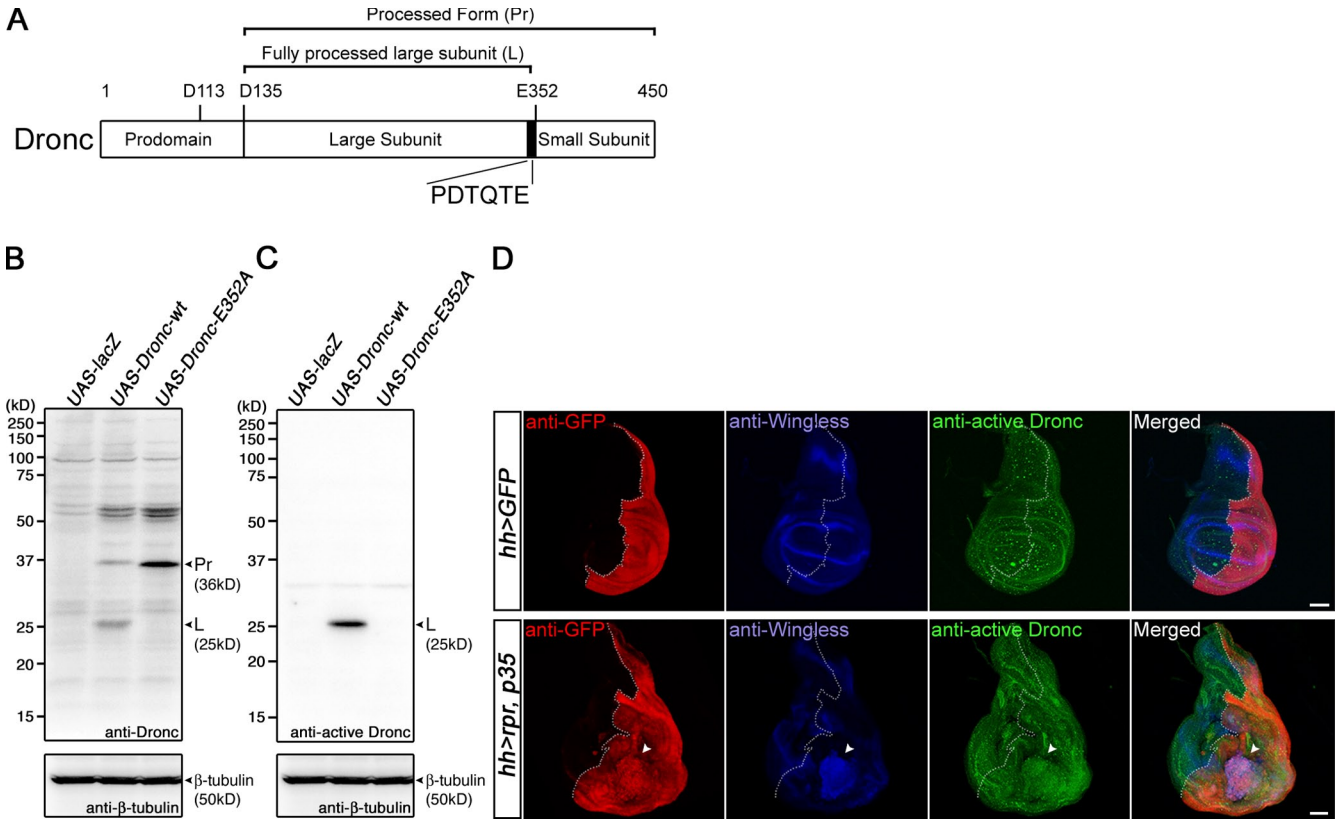


Figure S4. **Generation of active Dronc antibody.** (A) The scheme for the cleavage sites of Dronc at Glu^{352}. (B) When Dronc-wild type (Dronc-wt) was overexpressed in S2 cells, full-length, processed form (Pr; 36 kD), and fully processed large subunit (L; 25 kD) were detected using anti-Dronc antibody (Quinn et al., 2000) (lane 2). When Dronc-E352A was overexpressed, processing at 352 never occured, and fully processed large subunit (L) was not produced. Alternatively, the amount of processed form (Pr) increased (lane 3). (C) By using anti-active Dronc antibody, only fully processed large subunit (L) was detected when Dronc-wt was overexpressed in S2 cells (lane 2). However, this band was diminished when Dronc-E352A was overexpressed (lane 3). S2 cells were transfected with *pUAST-lacZ* (100 ng), *pUAST-Dronc-wt* (100 ng), or *pUAST-Dronc-E352A* (100 ng) with *pWAGAL4* (5 ng). (D) Dronc activation is observed in the wing discs expressing Reaper and p35. The active Dronc signal is strong in Wingless-positive cells (white arrowhead). Co-expression of Reaper and p35 in posterior compartment induces dramatic tissue overgrowth, which is accompanied by ectopic Wingless expression. This abnormal tissue overgrowth requires Dronc (Huh et al., 2004; Kondo et al., 2006). Bar, 50 μm. The genotypes were *UAS-GFP/+; hh-GAL4/+* (*hh>GFP*), *UAS-GFP/UAS-Reaper, UAS-p35; hh-GAL4/+* (*hh>rpr, p35*).

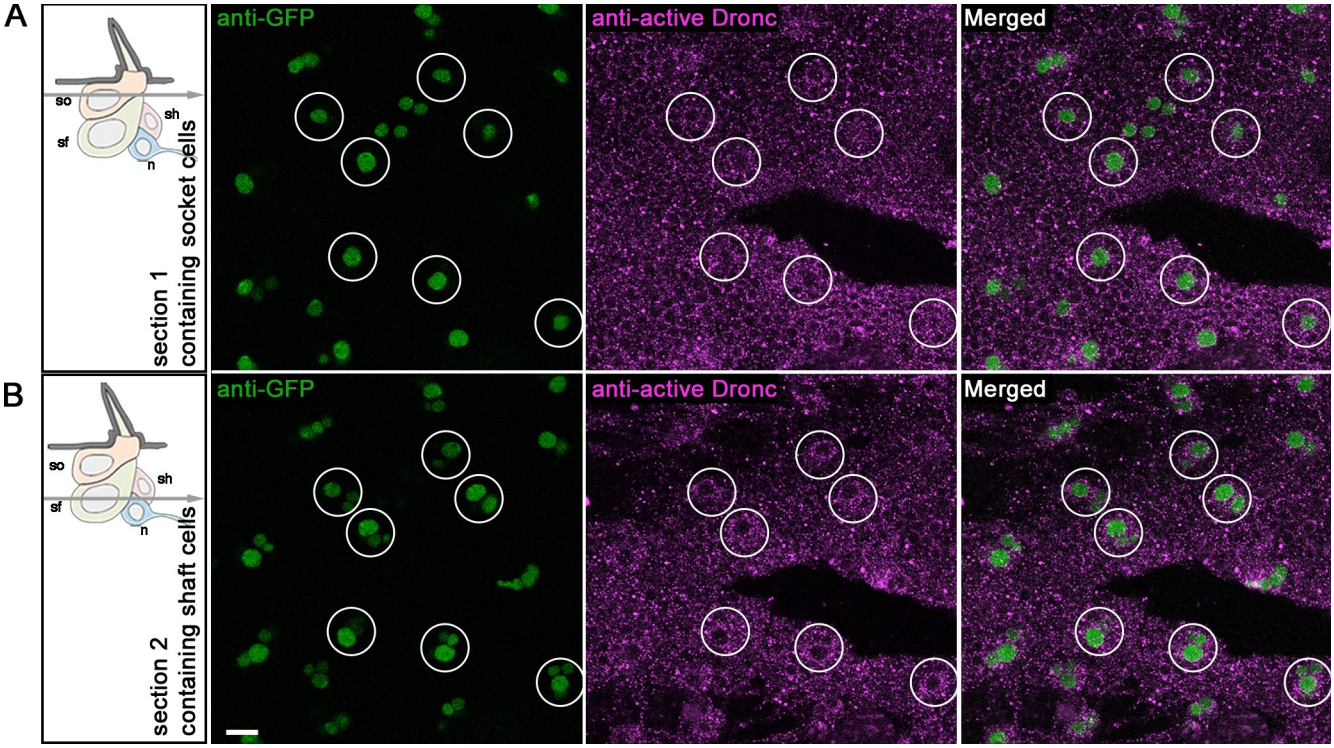
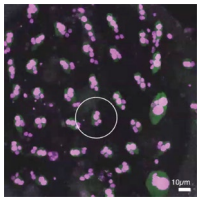


Figure S5. **Dronc activation in the shaft cell during bristle elongation.** The active Dronc signal (magenta) was observed in the cytoplasmic region of the shaft cell (B), which seemed to be stronger compared with that in the socket cell (A). The positions of SOP lineage are marked by a white circle. Each nucleus is marked by Histone2B-ECFP (green). Bar, 10 µm. Genotype was *neu-GAL4 UAS-Histone2B-ECFP/TM6B*.



Video 1. **PRAP pattern in the SOP lineage.** Time-lapse movie of PRAP during sensory organ development. PRAP is shown in green and Histone2B-ECFP in magenta, as in Fig. 2. The genotype was *neu-GAL4 UAS-Histone2B-ECFP/ UAS-PRAP39*.

References

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