Supplemental Data

Compensatory Proliferation in *Drosophila* Imaginal Discs Requires Dronc-Dependent p53 Activity

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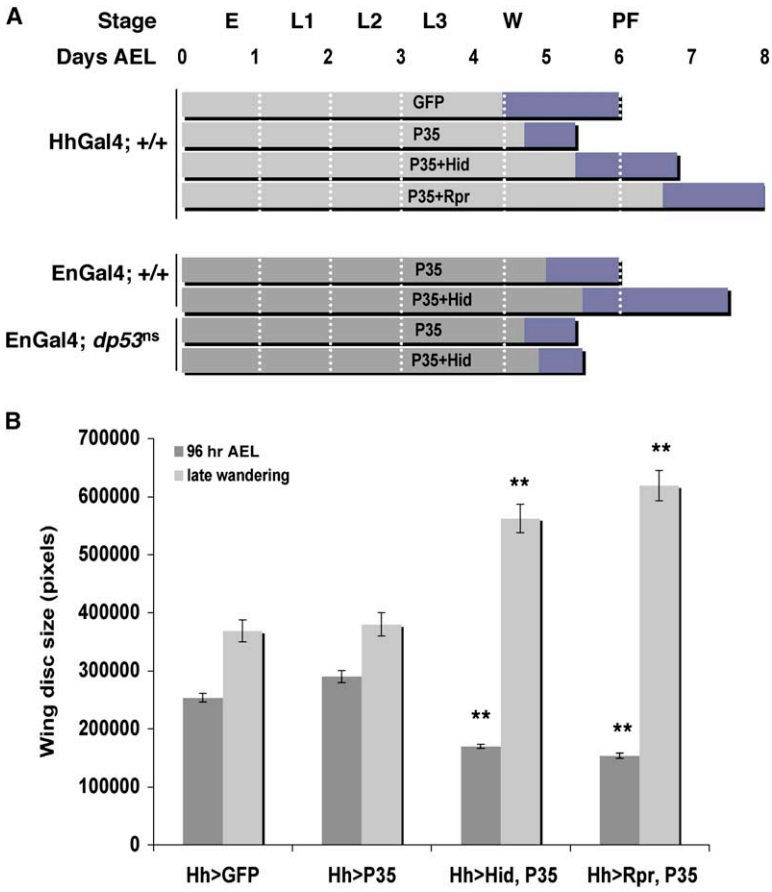

Figure S1. Developmental Timing and Quantification of Wing Size

(A) Developmental timing of larvae with or without posterior undead cells. Gray bars denote days AEL to wandering stage (W); blue bars denote days AEL to pupal formation (PF). Dashed white lines mark each developmental transition. Either the HhGal4 or the Engrailed (EnGal4) driver was used to express the UAS-transgenes indicated. In wild-type animals, undead cells created with either HhGal4 or EnGal4 cause significant delay to W and PF. In contrast, in *dp53* mutants, animals with undead cells developed with normal timing. However, more than 70% of *dp53* mutants with undead cells died before wandering stage. 83% of animals that wander survive to form adults. E, embryogenesis; L1, 2, and 3 mark first, second, and third larval instars, respectively.

(B) Quantification of wing disc size at 96 hr AEL (dark bars) and at late wandering stage (light bars). At 96 hr, wing imaginal discs with undead posterior cells are significantly smaller than controls ($p < 10^{-14}$). At late wandering stage, the discs with undead cells are significantly larger than controls ($p < 10^{-9}$). Error bars denote standard errors of the mean.

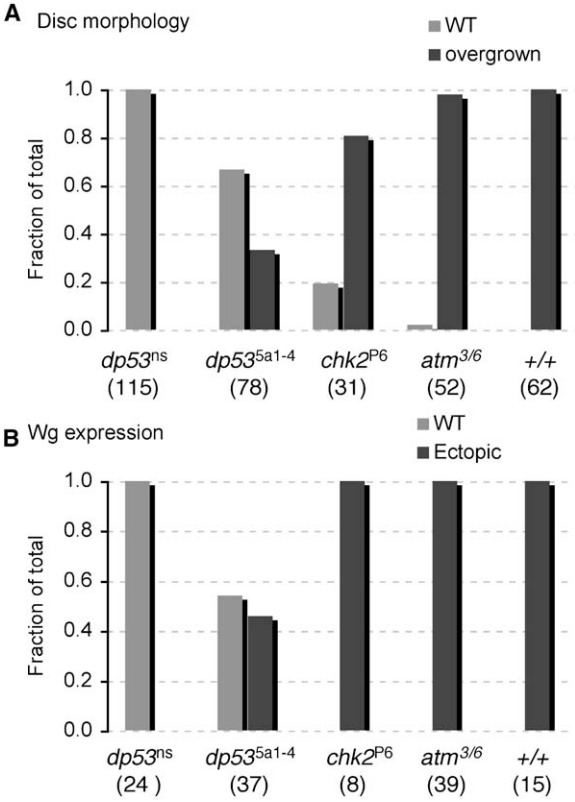

Figure S2. Phenotypic Analysis of Mutant Backgrounds

(A) Comparison of disc morphology in wing discs expressing Hid + P35. Shown is the fraction of wing discs examined that were overgrown at wandering stage. 100% of +/+ wing discs showed overgrowth, whereas wing discs from both *dp53* mutants had largely normal growth (wt). In contrast, the majority of *chk2* and *atm* mutant wing discs with undead cells overgrew. Number of wing discs analyzed is below each genotype in parentheses.

(B) Wg expression in discs with undead cells. Shown is the fraction of wing discs of each genotype with ectopic Wg expression. $dp53^{ns}$ mutants with undead cells expressed Wg normally (wt), i.e., they did not have any ectopic expression of Wg. In contrast, approximately 60% of $dp53^{5a-1-4}$ had normal (wt) Wg expression, but 40% expressed it ectopically. Based on the two phenotypes measured in the figure, $dp53^{5a-1-4}$ appears to be somewhat weaker than $dp53^{ns}$. 100% of *chk2* and *atm* mutants had ectopic Wg expression. Number of wing discs analyzed is below each genotype in parentheses.

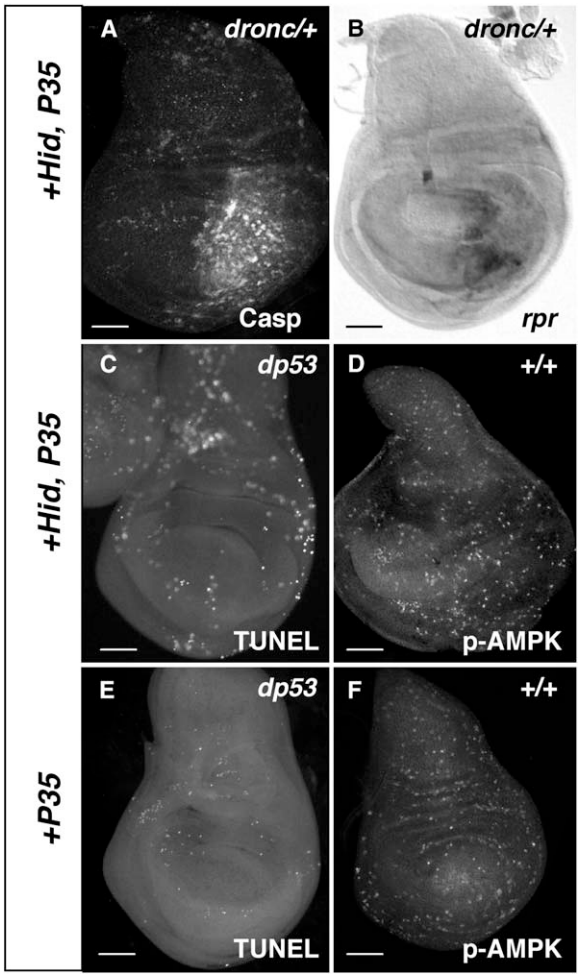

Figure S3. Compensatory Proliferation Is Sensitive to *dronc* Dosage

(A and B) Loss of one copy of *dronc* abolishes much of the growth effects of undead cells. In *dronc*/+ wing discs, overgrowth is reduced even though caspase-3 is highly active in undead cells (A), and *rpr* mRNA expression is decreased in undead cells relative to +/+ discs (B).

(C and E) *dp53* mutant discs with (C) or without (E) undead cells, stained for TUNEL activity. TUNEL-positive cells, despite inhibition of effector caspase activity by P35, are present in undead-containing *dp53* mutant discs.

(D and F) AMP-dependent kinase (AMPK) is not aberrantly activated in undead cells. Phospho-AMPK staining of a control disc (F) and disc with undead cells (D). Scale bars indicate relative sizes.

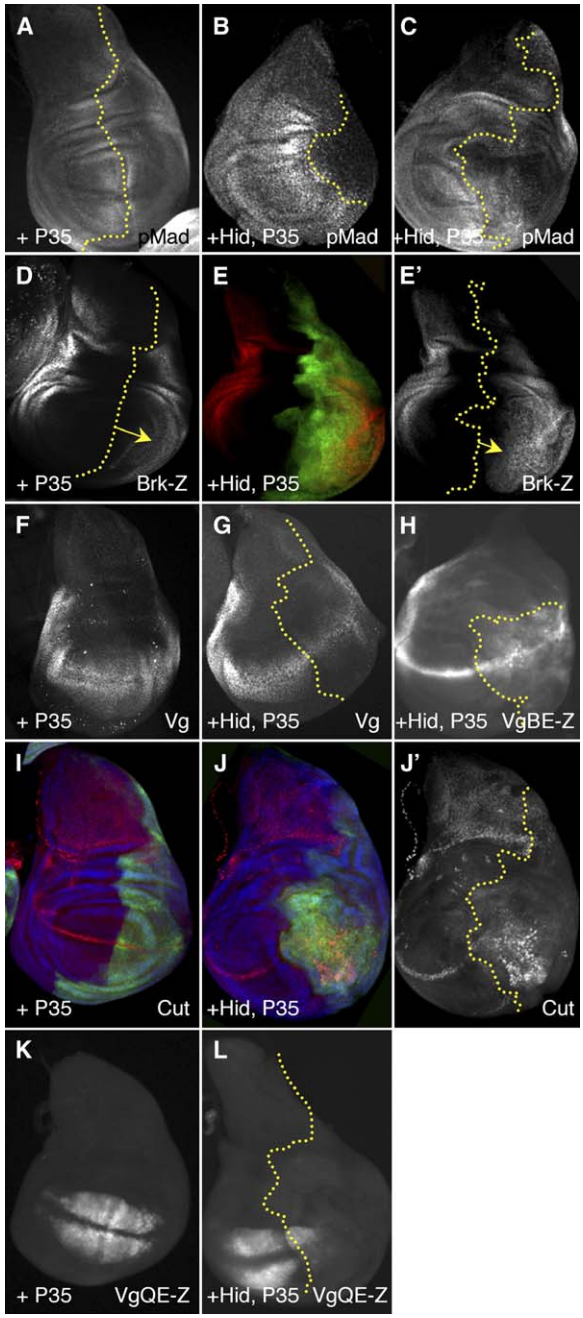

Figure S4. Signaling by Wg and Dpp Is Attenuated in Undead Cells

(A) Control LW wing disc stained with an antibody against phospho-Mad (pMad), an indicator of Dpp activity.

(B and C) Dpp activity (p-Mad staining) was suppressed in undead cells, particularly at early stages (B). Disc in (B) is 96 hr AEL, disc in (C) is LW (approximately 160 hr AEL).

(D–E′) *Brinker*-lacZ (*Brk-Z*) reports *Brk* expression, which is repressed by Dpp activity. *Brk*-Z is expanded in the undead cells. (D) *Brk*-lacZ expression in control discs. Dotted lines in (D) and (E′) show position of the A/P boundary. Arrows in (D) and (E′) denote distance from the A/P boundary to the posterior Brk expression domain. Brk-Z expression in undead cells (E′) is expanded and closer to the A/P boundary, indicating reduced Dpp activity in those cells.

(F and G) Expression of *vestigial* (*vg*), a target of both Wg and Dpp, is decreased in undead cells.

(F) Control Vg protein expression.

(G) Vg expression in wing discs with undead cells.

(H–J′) Notch activity is increased in undead cells.

(H) *vg* expression reported by the boundary enhancer-lacZ (VgBE-Z).

(I) Control disc.

(J and H) The Notch target gene Cut was ectopically expressed in undead cells (J and J′), and a Notch-responsive enhancer of the *vg* gene was also ectopically activated (H). Since Wg is also a target of Notch, its ectopic expression in undead cells could be due to enhanced Notch activity.

(K and L) Both Wg and Dpp activity are required for activation of the *vg* "quadrant" enhancer (VgQE-Z), but the activity of this enhancer is nearly silent in undead cells (L).