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Supplemental Data

Growth Arrest and Autophagy Are Required for Salivary Gland Cell Degradation in *Drosophila*

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SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Drosophila Strains

For ectopic expression studies, the following fly strains were used: UAS-Dp110 (Weinkove et al., 1999), UAS-Dp110 + UAS-TOR^{ted}, UAS-Dp110 + UAS-p35, UAS-Akt (Verdu et al., 1999), UAS-Ras^{V12} (Karim and Rubin, 1998), UAS-Ras^{V12} + UAS -TOR^{ted}, UAS-Myc (Johnston et al., 1999), UAS-CycD + UAS-Cdk4 (Datar et al., 2000), UAS-TOR^{ted} (Hennig and Neufeld, 2002), UAS-p35 (Hay et al., 1994), UAS-Atg1^{GS10797} (weak) (Kyoto Drosophila Genetic Resource Center), UAS-Atg1^{68} (strong), UAS-Atg1$^{KQ\#5B}$ (Scott et al., 2007), UAS-Atg1^{GS10797} + UAS-p35, UAS-Atg1^{GS10797} + UAS-Atg12-IR, and UAS-Atg1^{GS10797} + UAS-Dp110. For loss of function studies, *dronc* and *ark* mutants were as previously described (Srivastava et al., 2007; Xu et al., 2005) and the following trans-heterozygous combinations were analyzed: *dronc^{I24}/dronc^{I29}*, fkhGAL4; UAS-Dp110; *dronc^{I24}/dronc^{I29}, ark^{L46}/ark^{N28}*, *ark^{P46}/ark^{N28}* and *ark$^{P46/L46}$* . For *atg8a* loss of function studies, the *atg8a^{KG07569}* mutant and hsGFPdAtg8 strains were as previously described (Scott et al., 2007; Scott et al., 2004) and the following genotypes were analyzed: *y*, *atg8a^{KG07569}*/+ and *y*, *atg8a^{KG07569}*/+; hsGFPdAtg8/+ females as controls and *y*, *atg8a^{KG07569}* or *y*, *atg8a^{KG07569}*; hsGFPdAtg8/+ males as experimentals. To express GFPdAtg8, pupae with hsGFPdAtg8 were reared at 25^{o}C to 6 hours apf, heat shocked at 37^{o}C for 30 minutes, then recovered at 25^{o}C until 24 hours apf. For *atg18* loss of function studies, *atg18^{KG03090}* mutants were as previously described (Scott et al., 2004). Since *atg18^{KG03090}* homozygous mutants are larval lethal, the following genotypes were analyzed: *atg18^{KG03090}*/Canton-S and *atg18^{KG03090}*/*Df(3L)Exel6112* + UAS-p35/wildtype as controls, and *atg18^{KG03090}*/*Df(3L)Exel6112*, *atg18^{KG03090}*/*Df(3L)66C-G28*, and *atg18^{KG03090}*/*Df(3L)Exel6112* + UAS-p35/fkhGAL4 mutant pupae as experimentals. *atg2* and *atg3* mutants were as previously described (Scott et al., 2004) and *atg2ep3697*/*Df (3L) Exel6091* and *atg3* homozygous mutant pupae were analyzed. For RNAi studies, the following fly strains were used: UAS-*ark-IR* and UAS-*dronc-IR* (Leulier et al., 2006), UAS-*atg3-IR* (Juhasz et al., 2003), UAS-*atg6-IR* and UAS-*atg12-IR* (Pandey et al., 2007) and UAS-*atg7-IR* (Scott et al., 2004). The previously described tGPH strain was used as a sensor for PI3K activity (Britton et al., 2002) and the previously described UAS-LC3-UAS-GFP-LC3 strain was used as a marker for autophagy (Rusten et al., 2004).

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Supplemental Figure 1

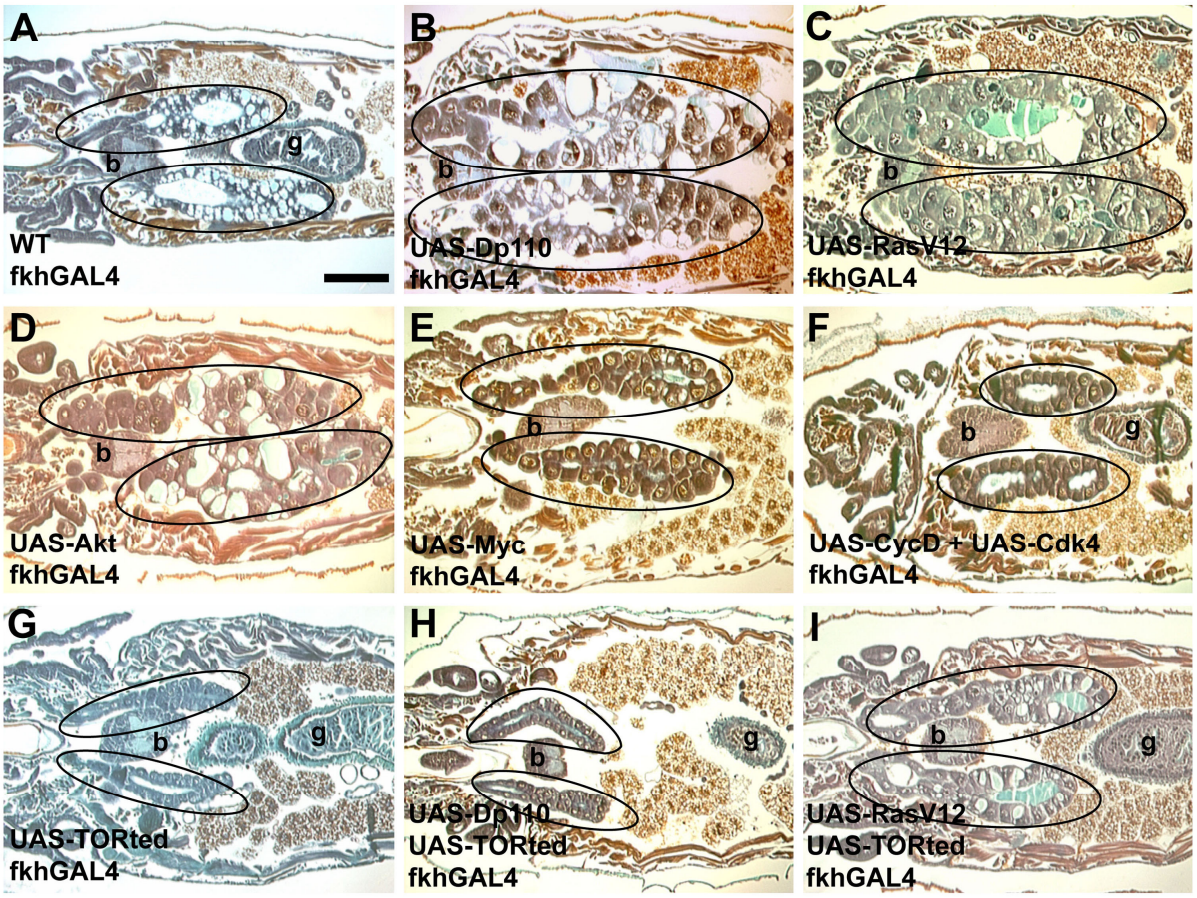

Figure S1. Expression of positive regulators of the PI3K pathway leads to overgrowth of salivary gland cells.

(A-I) Paraffin sections at 6 hours apf. (A) Canton-S/fkhGAL4 control pupae have wild-type normal-sized salivary glands. Expression of either (B) Dp110, (C) Akt, or (D) Ras^{V12} results in significant overgrowth of salivary glands. Expression of the cell cycle regulators (E) Myc or (F) Cdk4 + CycD increases nuclear size without a coincident increase in salivary gland size. (G) Expression of TOR^{ted} does not significantly affect normal salivary gland development. (H). Co-expression of Dp110 with TOR^{ted} prevents the overgrowth phenotype of Dp110. (I) Co-expression of Ras^{V12} with TOR^{ted} prevents the overgrowth phenotype of Ras^{V12} Scale bar in (A) is 200 μm and (A-I) are the same magnification. Circles delineate salivary glands. Symbols are (b) brain and (g) gut.

Supplemental Figure 2

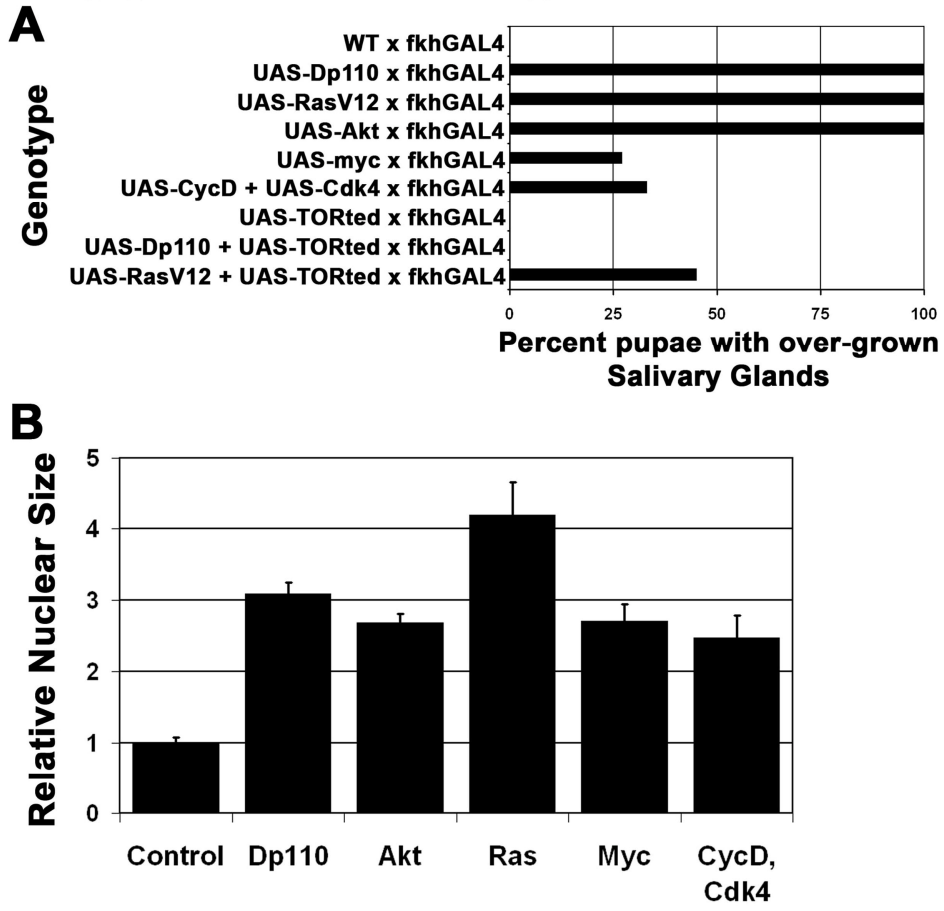

Figure S2. Expression of positive regulators of the PI3K pathway leads to overgrowth of salivary gland cells and nuclei.
(A) Percentage of pupae that had overgrown salivary glands for each genotype as determined by analysis of paraffin sections and summarizes the data shown in Figure S1 and additional data not shown. (B) Graph shows the relatively size of nuclei compared to wildtype Canton-S when the listed growth regulators are expressed in salivary glands. The data are represented as mean ± SE.

Supplemental Figure 3

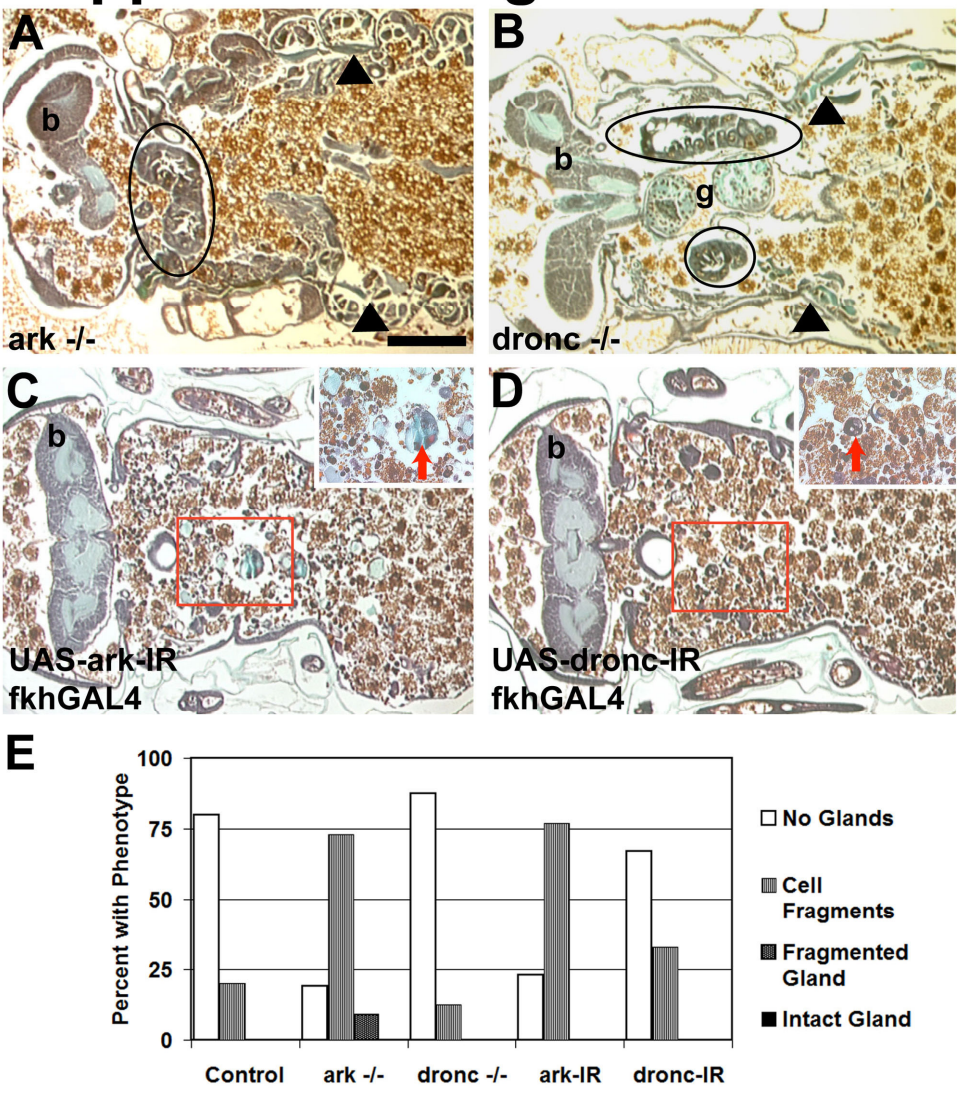

Figure S3. Salivary glands are degraded when *ark* or *dronc* function is reduced. (A-D) Paraffin sections at 24 hours apf. Salivary glands are only present in (A) *ark* or (B) *dronc* loss-of-function mutants that also have abnormal development indicative of a developmental delay. Note the inappropriate head and brain development and presence of larval muscle (arrowheads). When caspases are inhibited by expression of (C) *ark-IR* or (D) *dronc-IR*, all of the pupae develop normally and the salivary glands are predominantly or completely degraded. (E) Histological sections of the indicated genotypes were evaluated for the amount and type of salivary gland tissue that was present in pupae 24 hours apf. Greater than 20 pupae were analyzed per genotype and

the percentage of pupae with each phenotype is presented. For *ark* and *dronc* loss-of-function mutants, only normally developing pupae were included in this graph. Pupae with abnormal development such as those shown in (A and B) were excluded as we do not feel that animals that are arrested prior to the time of salivary gland degradation can be assessed for appropriate gland degradation. Scale bar in (A) is 200 μm and (A-D) are the same magnification. Red boxes indicate the region of higher magnification shown in the insets for each image. Red arrows point to salivary gland fragments. Black arrowheads point to fat body that is present in the area after the salivary gland has degraded. Symbols are (b) brain and (g) gut.

Supplemental Figure 4

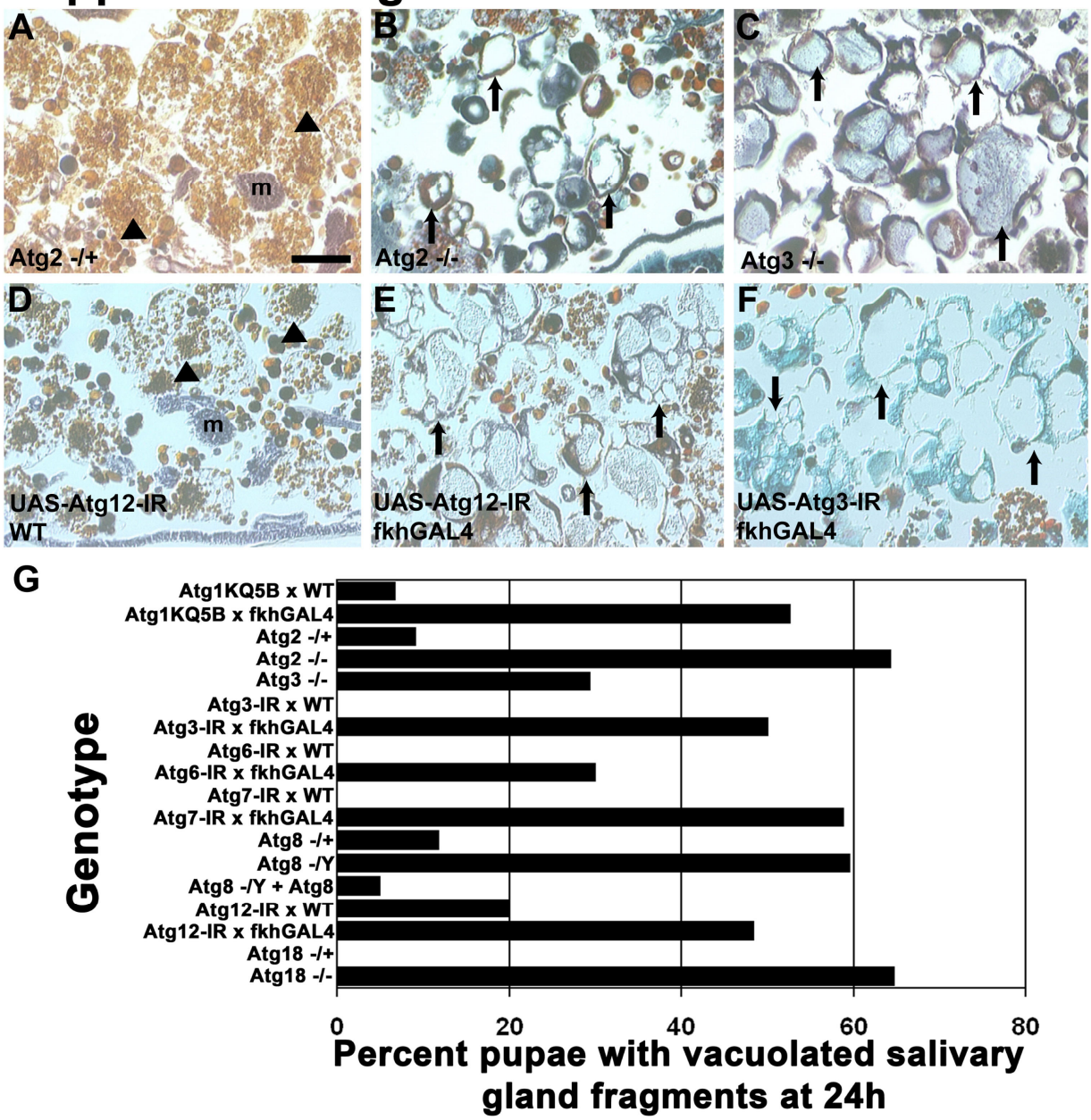

Figure S4. Reduced *atg* gene function inhibits salivary gland degradation. (A-F) Paraffin sections at 24 hours apf. Salivary glands are degraded in (A) *atg2ep3697*/Canton-S control pupae. Vacuolated salivary gland fragments persist at 24 hours apf in (B) *atg2ep3697*/*Df.(3L)Exel6091* and (C) *atg3* homozygous mutant pupae. Salivary glands are degraded in (D) UAS-*atg12-IR*/Canton-S control pupae. Vacuolated salivary gland fragments persist at 24 hours apf in (E) *atg12-IR*- and (F) *atg3-IR*–expressing pupae. (G) Summary of phenotypes shown in Figures 6 and S4 and additional data not shown. Scale bar in (A) is 50 μm and (A-F) are the same

magnification. Symbols are (m) muscle. Arrowheads point to fat body. Arrows point to vacuolated salivary gland fragments.

Supplemental Figure 5

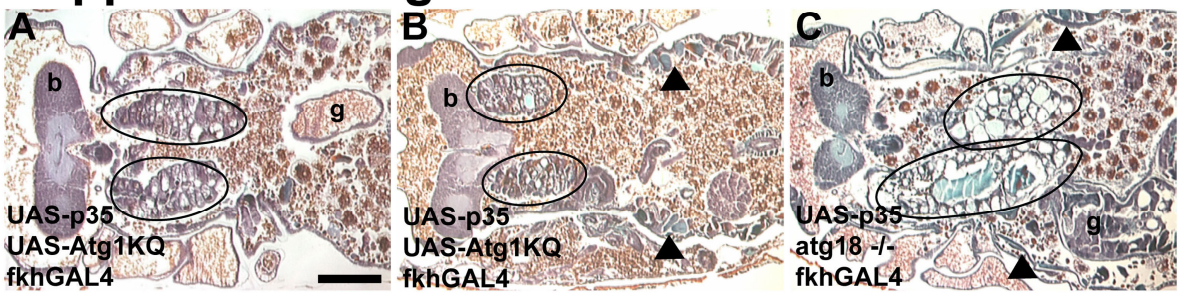

Figure S5. Combined inhibition of caspases and reduced *atg* gene function leads to an increase in persistence of salivary glands. (A-C) Paraffin sections at 24 hours apf. (A) 65% of the pupae with combined expression of p35 with $Atg1^{KQ\#5B}$ in the salivary gland that develop normally have increased persistence of salivary gland tissue compared with p35 or $Atg1^{KQ\#5B}$ expression alone. (B) A smaller percentage of pupae with combined expression of p35 with $Atg1^{KQ\#5B}$ in the salivary gland display developmental defects including disrupted head morphology, presence of larval muscle (arrowheads) and mid-gut. In all cases, intact salivary glands are present. (C) *atg18* loss-of-function mutants expressing p35 in their salivary gland often display similar developmental defects including disrupted head morphology, presence of larval muscle and mid-gut. In all cases, intact salivary glands are present. Scale bar in (A) is 200 μm and (A-C) are the same magnification. Symbols are (b) brain and (g) gut.