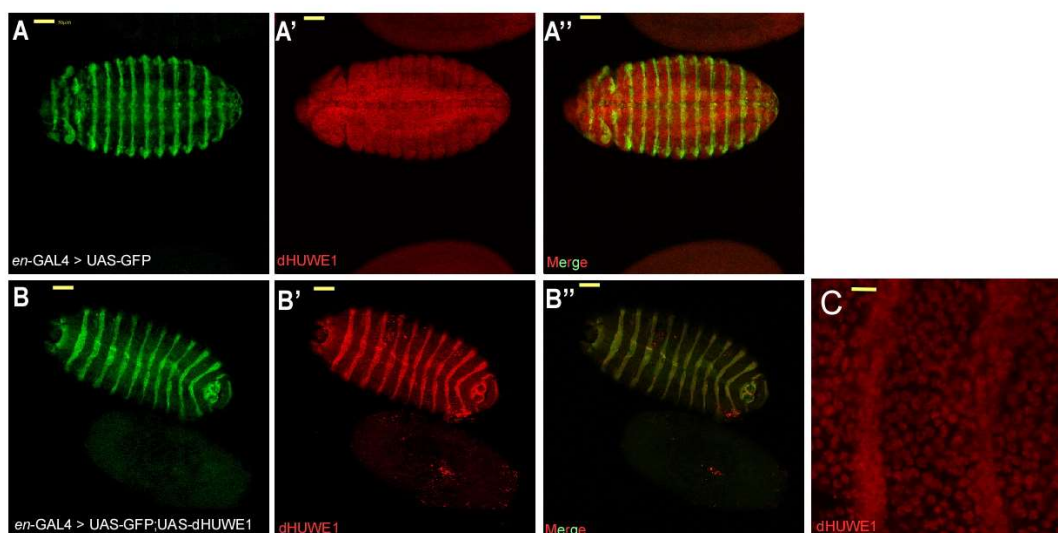


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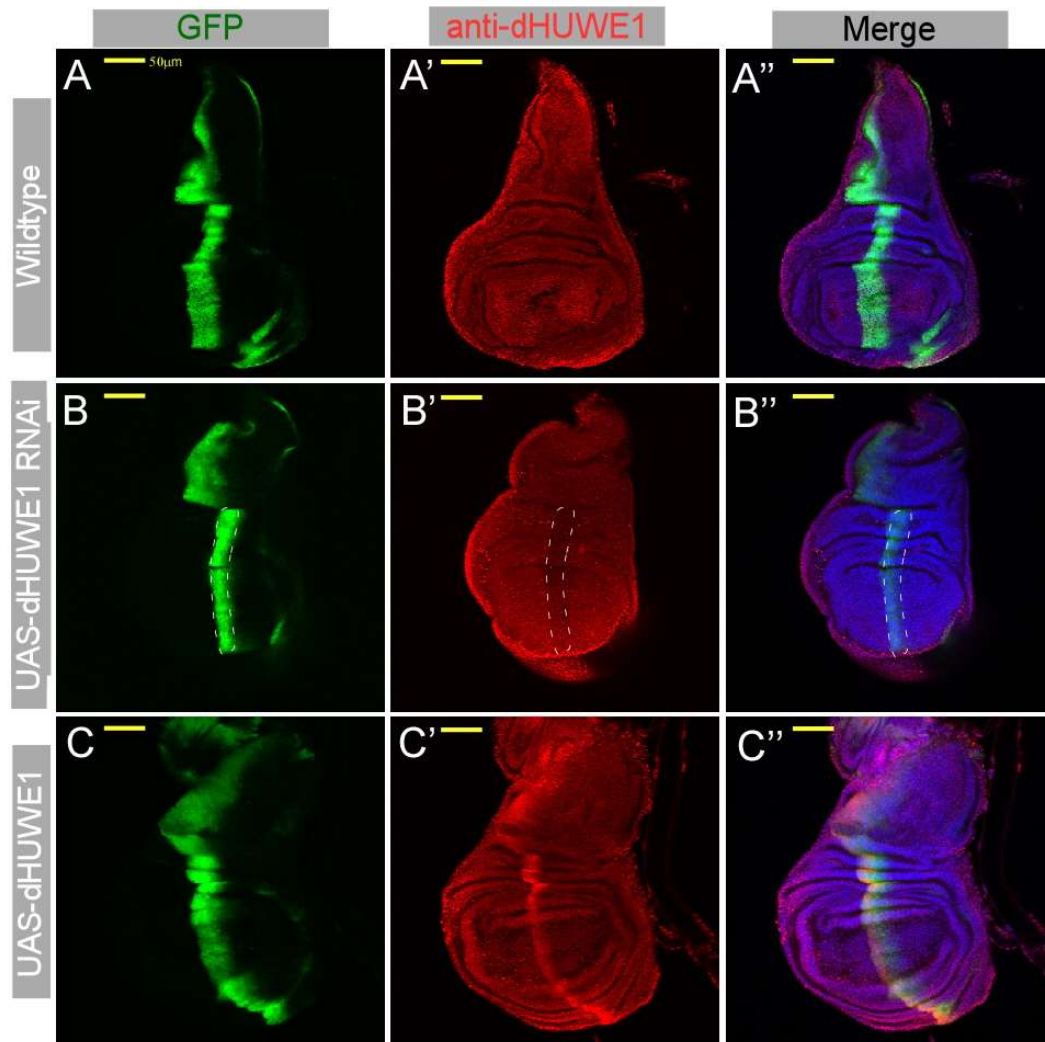
2 **Supplemental Figures 1-5**



3

4 **Figure S1. Expression pattern of endogenous and exogenous dHUWE1 . (A-C)** Indirect  
5 immunofluorescence confocal microscopy images of stage 12 embryo expressing UAS-dHUWE1  
6 and UAS-GFP under the control of *engrailed*-Gal4. Endogenous and ectopically expressed dHUWE1  
7 is identified using polyclonal anti-dHUWE1. scale bar is 50µm (C) Magnification of a section of a  
8 stage-12 embryo [x120].

9



10

11 **Figure S2. Expression and elimination of dHUWE1 protein in imaginal wing discs. (A-C)**

12 Expression of UAS-GFP, (A-A'') UAS-dHuwe1-RNAi, (B-B'') or UAS-dHUWE1 under the control of  
 13 the *ptc*-Gal4 driver. GFP marks the region of *ptc*-GAL4 activity (dashed line). dHUWE1 is identified  
 14 using anti-dHUWE1 (red). DRAQ5 marks DNA and scale bar is 50  $\mu$ m.

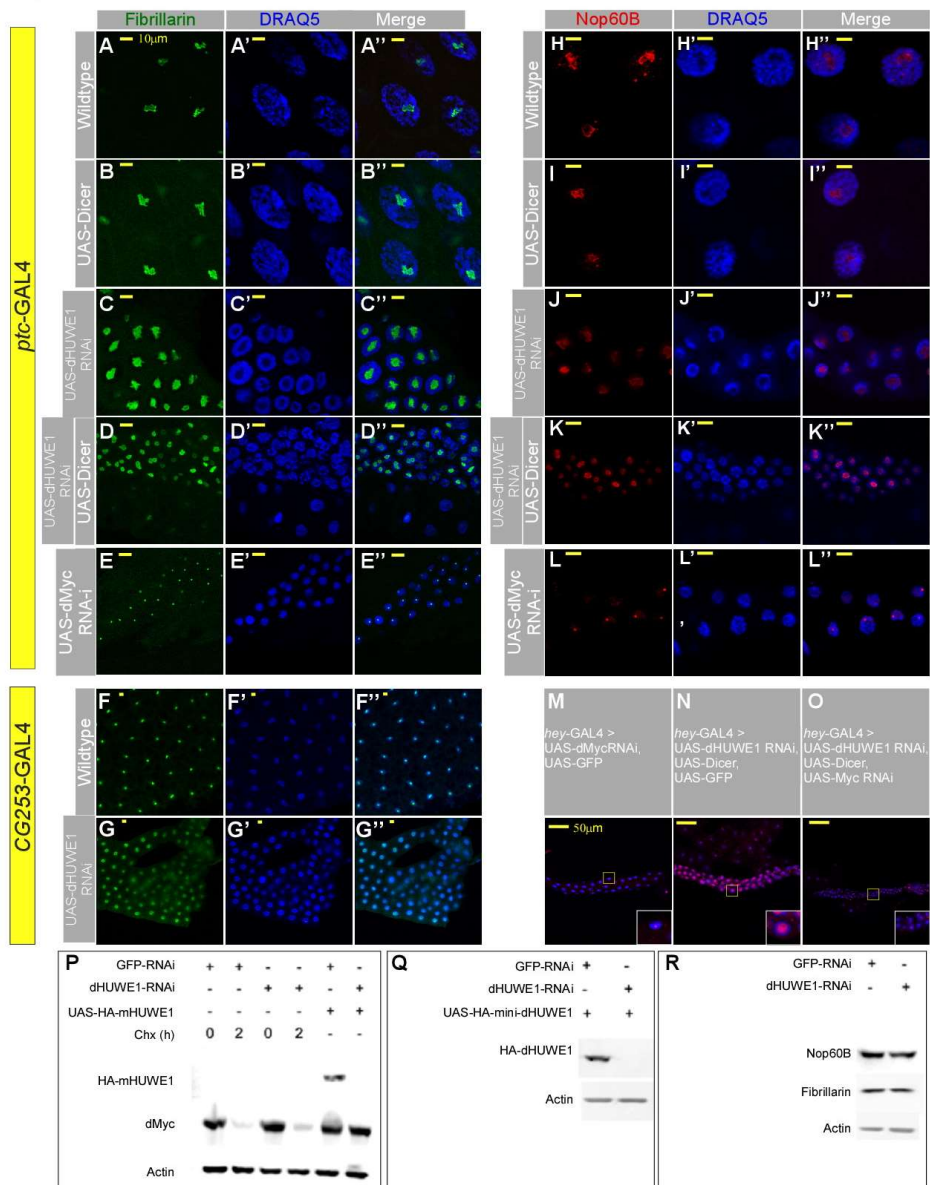
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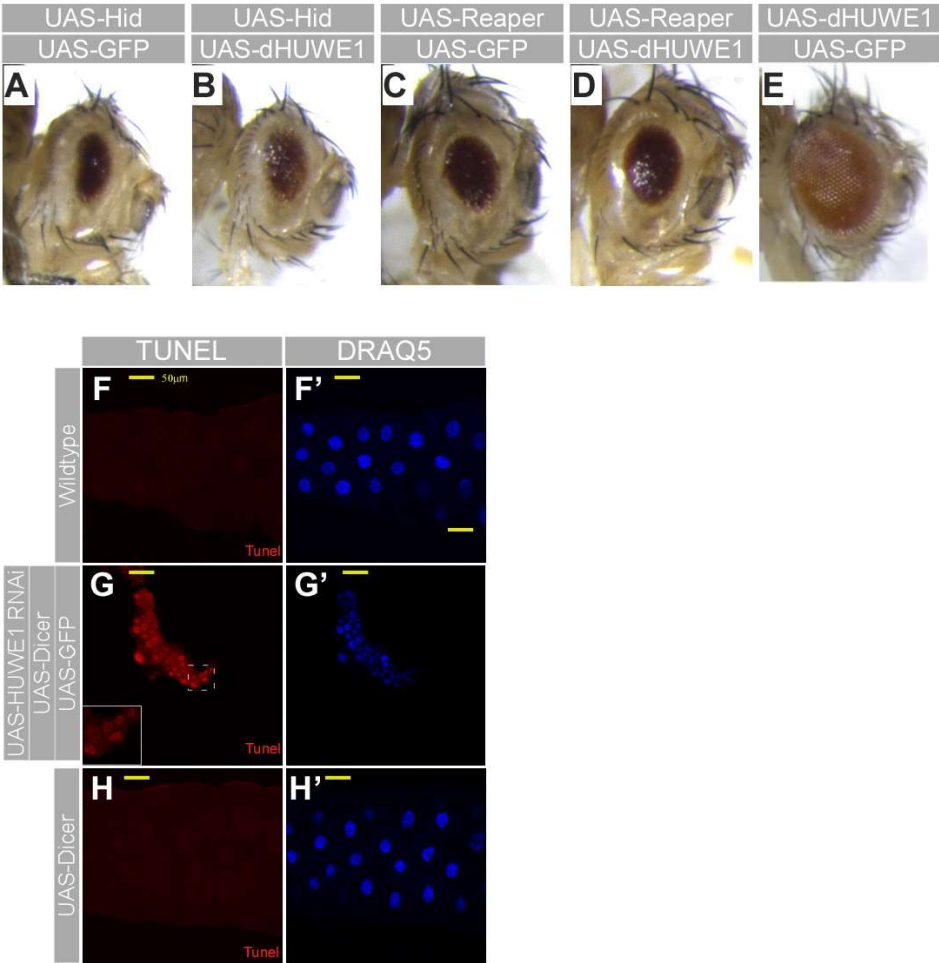
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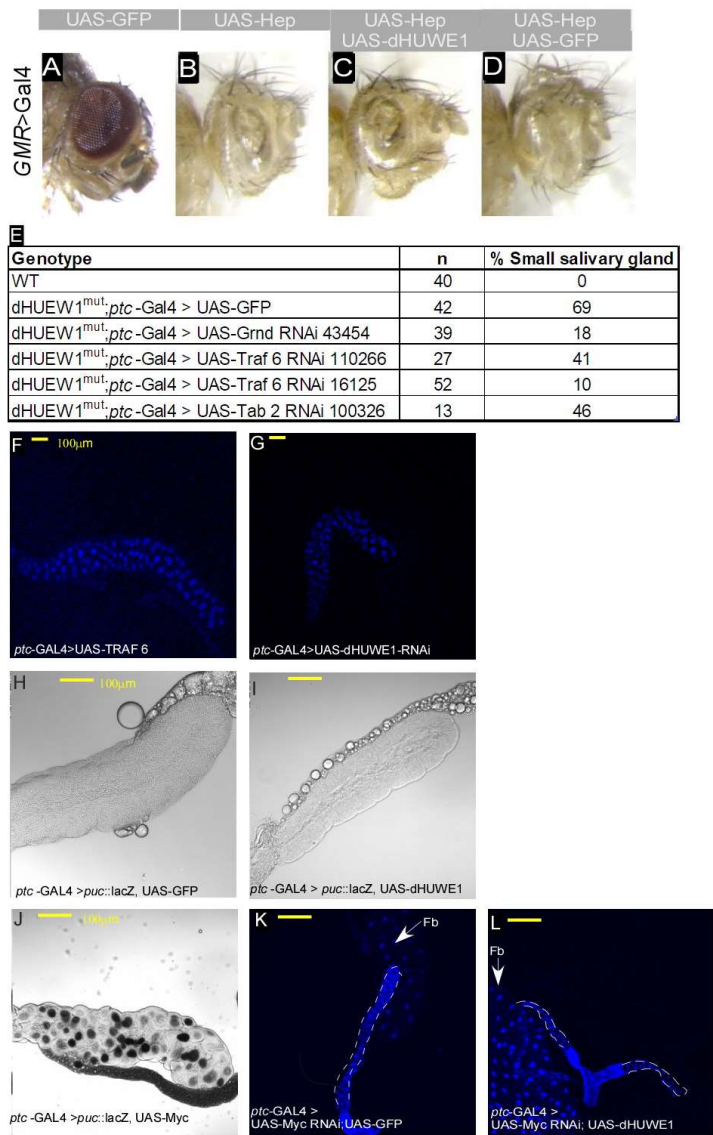
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**Figure S3. Expression of direct dMyc target genes is not affected by loss of dHUWE1. (A-J)** Indirect immunofluorescence confocal microscopy images of salivary glands (A-E''), and fat body tissue (F-G'') derived from wild-type, or the indicated transgenic lines under the regulation of *hey-Gal4* (A-E'') and *CG235-GAL4* (F-G''). (A-G'') expression of Fibrillarlin protein (green). (H-O) expression of Nop60B (red). DRAQ5 marks DNA and scale bar is 10  $\mu$ m. **(P)** Protein level of endogenous dMyc in dynamic cyclohexamide chase experiment in S2 *Drosophila* cells transfected with the indicated RNAi. HA-dHUWE1 codes for a short C-terminal fragment of dHUWE1 that indicates the effectiveness of dHUWE1 knockdown, and actin serves as a loading control. **(Q, R)** Western blot analysis of the indicated protein extracts derived from S2 *Drosophila* cells transfected with either GFP-RNAi or dHUWE1-RNAi. Actin serves as a loading control.



**Figure S4. Expression of dHUWE1 fails to suppress cell death induced by the expression of Hid, or Reaper, and its loss initiates cell death** (A-E) Expression of the indicated UAS-transgenic lines under the control of GMR>Gal4 driver. (A, C) Overexpression of either UAS-Hid (A) or UAS-Reaper (C), along with UAS-GFP control. Co-expression of UAS-dHUWE1 fails to suppress the small eye phenotype that results from overexpression of Hid (B) or Reaper (D). (F-H') Loss of dHUWE1 initiates cell death. TUNEL assay in salivary gland cells derived from the indicated transgenic lines. RNAi-mediated knockdown of dHUWE1 resulted in TUNEL positive cells. DRAQ5 marks DNA scale bar is 50µm.



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67

68 **Figure S5: dHUWE1 genetically interacts with component the JNK pathway** (A-D) dHUWE1

69 expression fails to suppress small eye phenotype induced by the expression of Hep. *ey-Gal4* was

70 used to express the indicated transgenes in the developing eyes. (E) Summary of epistatic analysis

71 testing for genetic interaction between *dHUWE1<sup>h</sup>* heterozygous mutant, and the indicated transgenic

72 RNAi lines under the control of *ptc-GAL4* scoring for salivary gland morphology and size. “n”

73 denotes number of salivary glands scored. (F, G) *ptc-GAL* dependent expression of UAS-TRAF6 (F)

74 or RNAi-mediated knockdown of dHUWE1 (G) resulted in a small salivary gland and failure to fully

75 endoreplicate. DAPI marks DNA and scale bar is 100µm. (H-J) Gross morphology and activation of

76 JNK pathway as indicated by the expression of the JNK reporter transgene *puc::LacZ* (black circles)

77 in salivary glands derived from the indicated transgenic lines. (K, L) Expression of either UAS-GFP

78 (K), or UAS-dHUWE1 (L) failed to suppress the underdeveloped salivary gland that resulted from

79 RNAi-dependent knockdown of dMyc.