Supplementary data

UVRAG is required for organ rotation by regulating Notch endocytosis in *Drosophila*

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Supplemental Figure legends

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Supplemental Figure legends

Fig. S1. Comparison of amino acid sequences among human, mouse, *Xenopus*, zebrafish and *Drosophila* UVRAG homologs.

Fig. S2. *UVRAG* null clones show cell proliferation in ovary.
Adult ovary containing *UVRAG^{B21}* clones was stained with TRITC-phalloidin (F-actin) and Hoechst 33258 (blue). *UVRAG^{B21}* clones are GFP-negative cells. Scale bar, 10 μm.
Genotype: *hsFLP/+; UVRAG^{B21} FRT40A/FRT40A UbiGFP*.

Fig. S3. Expression of transgenic *UVRAG* in flies and developmental expression profile of *UVRAG*.
(A) Immunoblot analysis was performed to confirm transgenic expression of *Flag-UVRAG* using *heat-shock* (*hs*)-*Gal4*. Tubulin was used as a loading control.
(B) Quantitative RT-PCR was performed to examine *UVRAG* gene expression levels in embryo, larva, pupa, male and female flies. Error bars indicate the s.e.m. *rp49* was used for internal control.

Fig. S4. Autophagy may not be involved in the organ rotation process.
(A) Left panels: Larval fat bodies containing *UVRAG^{B21}* clones were fed (left) or starved (middle and right), and stained with LysoTracker (red in left and middle panels) and Hoechst 33258 (blue in left and middle panels) or mCherry-Atg8 (right). Right panels: Larval wing (upper) or eye (lower) imaginal discs containing *UVRAG^{B21}* clones were stained with mCherry-Atg8. Absence of GFP indicated by dotted lines marks the *UVRAG^{B21}* clones.
(B) Left panels: Larval genital discs expressing GFP-Atg8 (green and white in left and right panels, respectively) under *AbdB-Gal4* driver were stained with TRITC-phalloidin (F-actin). Right panels: Immunoblot analyses were performed in wandering larva fat body (left) or male pupa genital disc (right) from control or *UVRAG* mutant expressing GFP-mAtg8 (Mammalian Atg8 homolog, LC3) using anti-GFP antibody. Wandering larva fat body shows both full-length (~43 kDa) and cleaved (~36 kDa) GFP-mAtg8 while pupa genitalia show only full-length.
(C) Ventral side views of adult male genitalia from the indicated genotypes.

Scale bars, yellow, 10 μm; blue, 50 μm.

Genotypes: (A) Left panels: left four panels (*hsFLP/+; UVRAG^{B21} FRT40A/UAS-2XeGFP FRT40A fb-Gal4*), right two panels (*hsFLP/+; UVRAG^{B21} FRT40A/UAS-2XeGFP FRT40A fb-Gal4; UAS-mCherryAtg8*). Right panels: upper panels (*hsFLP/+; UVRAG^{B21} FRT40A/FRT40A UbiGFP; da-Gal4 UAS-mCherryAtg8*), lower panels (*eyFLP/+; UVRAG^{B21} FRT40A/FRT40A UbiGFP; da-Gal4 UAS-mCherryAtg8*).

Fig. S5. RT-PCR analyses to validate transgenic RNAi alleles.
RT-PCR analyses of the indicated genes were performed in adult flies from the indicated genotypes. *Actin* was used as a loading control.

Fig. S6. Genetic interaction between UVRAG and signaling receptors.
(A) Left: Microscopic images of the adult flies from the indicated genotypes. Right: Comparison of the adult viability among the indicated genotypes. N>50 for each genotype. Error bars denote the s.e.m. **P*<4.5x10^{-5}, #*P*<3.88x10^{-4}, *#*P*<7.1x10^{-5}.
(B) Left: Microscopic images of the wing preparations from the indicated genotypes. Lower panels show the magnified images of the upper panels. Right: Quantification of the total wing hair cell numbers in the indicated genotypes. Total wing hair cell number = Wing hair cell number per unit area X Whole wing area. N>10 for each genotype. Error bars denote the s.e.m. **P*<9.06X10^{-6}, #*P*<5.52X10^{-4}.
(C) Larval wing discs expressing *UVRAG^{RNAi}* with or without transgenic Notch. UAS-myrRFP was used to mark *engrailed (en)-Gal4*-specific region. *Notch response element* (*NRE*)*-EGFP* was used to show Notch transcriptional activity.
Scale bars: black, 0.5 mm; red, 100 μm; yellow, 10 μm.

Fig. S7. *UVRAG* null clones show increased hair cell number in adult wings.
Microscopic images of the wing preparations containing control clones (upper in left and right panels), *UVRAG^{B21}* clones (middle and lower in left and right panels, respectively) or Notch overexpression clones (lower left panel). The short and crinkled hair marker (homozygous *ck^{13}* cells) was used to find the clone-generated region. *UVRAG^{B21}* clones (marked by *-/-*) were found by thick vein phenotypes which are not observed in control clones (upper left panel) but found in *UVRAG^{B21}* and Notch over clones (arrows in the middle and lower left panels).

Scale bars: red, 100 μm; yellow, 10 μm.

Genotypes: Control clone, *hsFLP/+; FRT40A/FRT40A ck^{13}*; *UVRAG* null clone, *hsFLP/+; UVRAG^{B21} FRT40A/FRT40A ck^{13}*; Notch over clone, *hsFLP/+; UAS-Notch/+; Act>CD2>Gal4 UAS-GFPnls/+*.
Genotypes of the clones in the right panels: Upper panels (+/+, *FRT40A/FRT40A ck^{13}* or *FRT40A/FRT40A*; +/+(*ck^{13}*), *ck^{13} FRT40A/FRT40A ck^{13}*), Lower panels (+/-, *ck^{13} FRT40A/FRT40A UVRAG^{B21}*; +/+(*ck^{13}*), *ck^{13} FRT40A/FRT40A ck^{13}*; -/-, *UVRAG^{B21} FRT40A/FRT40A UVRAG^{B21}*).

Fig. S8. *UVRAG* null clone phenotypes are rescued by *Notch* knockdown.
(A) Upper panels: Adult ovaries were stained with Hoechst 33258 (blue). GFP-negative cells are clones in the left six panels. GFP-positive cells are overexpression clones in the right two panels. Lower panel: Quantification of the ovary phenotypes from the indicated flies. N>50 for each genotype.
(B) Upper panels: Larval eye discs were immunostained using anti-Notch antibody (red and white in upper and lower panels, respectively). Absence of GFP marks clones. Lower panels: Adult eye images were obtained by light microscopy (upper) or scanning electron microscopy (lower). White ommatidia marks clones. Flies in the right panel contain clones with red ommatidia due to the *white* gene expression (w^+) in the whole fly body from the *da-Gal4* and *UAS-Notch^{RNAi}* transgenes.

Scale bars: yellow, 10 μm; red, 100 μm.
Genotypes: (A) Control clone, *hsFLP/+; FRT40A/FRT40A UbiGFP*; *UVRAG* null clone, *hsFLP/+; UVRAG^{B21} FRT40A/FRT40A UbiGFP*; *UVRAG* null clones with *Notch* RNAi, *hsFLP/+; UVRAG^{B21} FRT40A/FRT40A UbiGFP; da-Gal4 UAS-Notch^{RNAi}*; Notch over clone, *hsFLP/+; UAS-Notch^{ICD}/+; Act>CD2>Gal4 UAS-GFPnls*. (B) Control clone, *eyFLP/+; FRT40A/FRT40A UbiGFP*; *UVRAG* null clone, *eyFLP/+; UVRAG^{B21} FRT40A/FRT40A UbiGFP*; *UVRAG* null clones with *Notch* RNAi, *eyFLP/+; UVRAG^{B21} FRT40A/FRT40A UbiGFP; da-Gal4 UAS-Notch^{RNAi}*.

Fig. S9. Tissue enlargement may not account for the genitalia rotation defect.
Lateral side views of adult male abdomen from the indicated genotypes.
Scale bar: 50 μm.

Fig. S10. Expression levels of rotation-related genes in *UVRAG* and *Notch* mutants.

Quantitative RT-PCR was performed to examine the transcription levels of rotation-related genes from the male pupa genitalia. Error bars indicate the s.e.m. *rp49* was used for internal control. The change of gene expression levels in mutants is not significant ($P>0.05$).

Table S1. Genitalia rotation phenotypes of male flies.
The percentage of male flies showing each genitalia rotation phenotype. Normal: =360°, Defective: <360°. N>50 for each genotype.

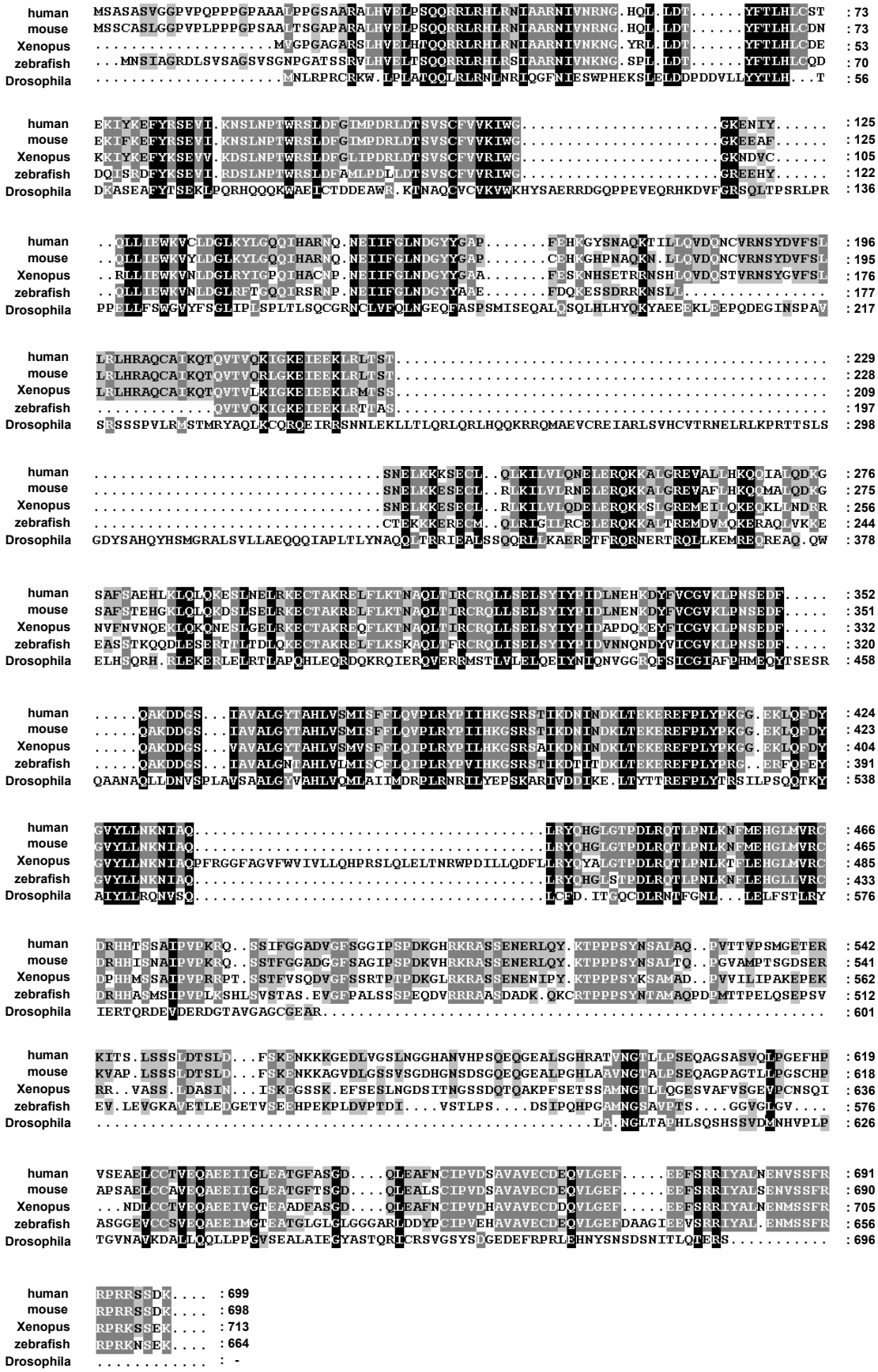


Fig. S1. Comparison of amino acid sequences among human, mouse, *Xenopus*, zebrafish and *Drosophila* UVRAG homologs.

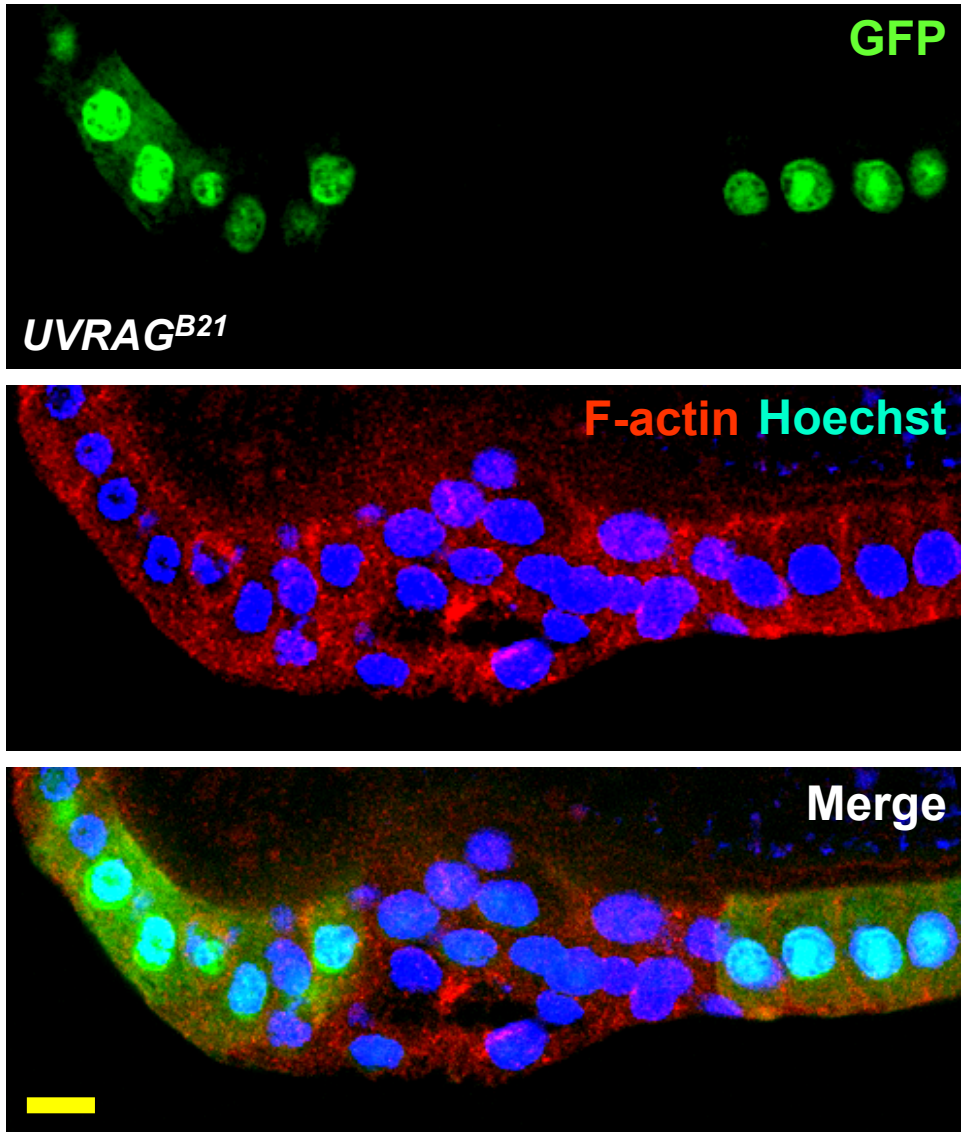


Fig. S2. *UVRAG* null clones show cell proliferation in ovary.

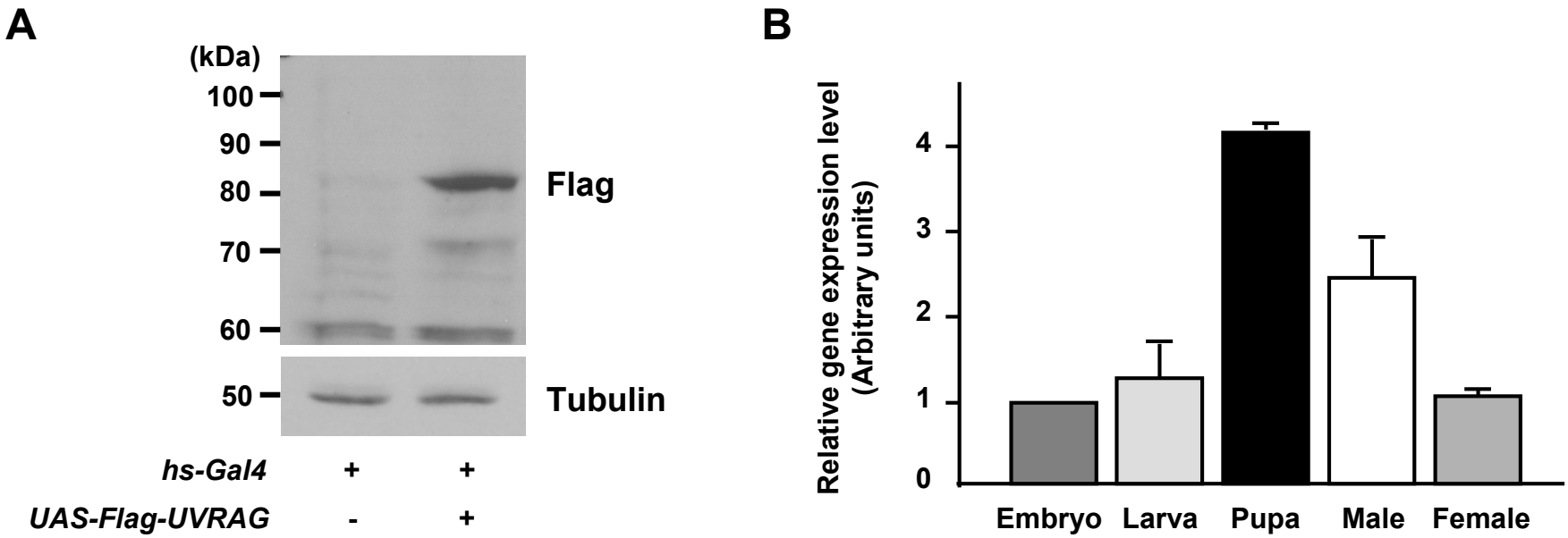


Fig. S3. Expression of transgenic *UVRAG* in flies and developmental expression profile of *UVRAG*.

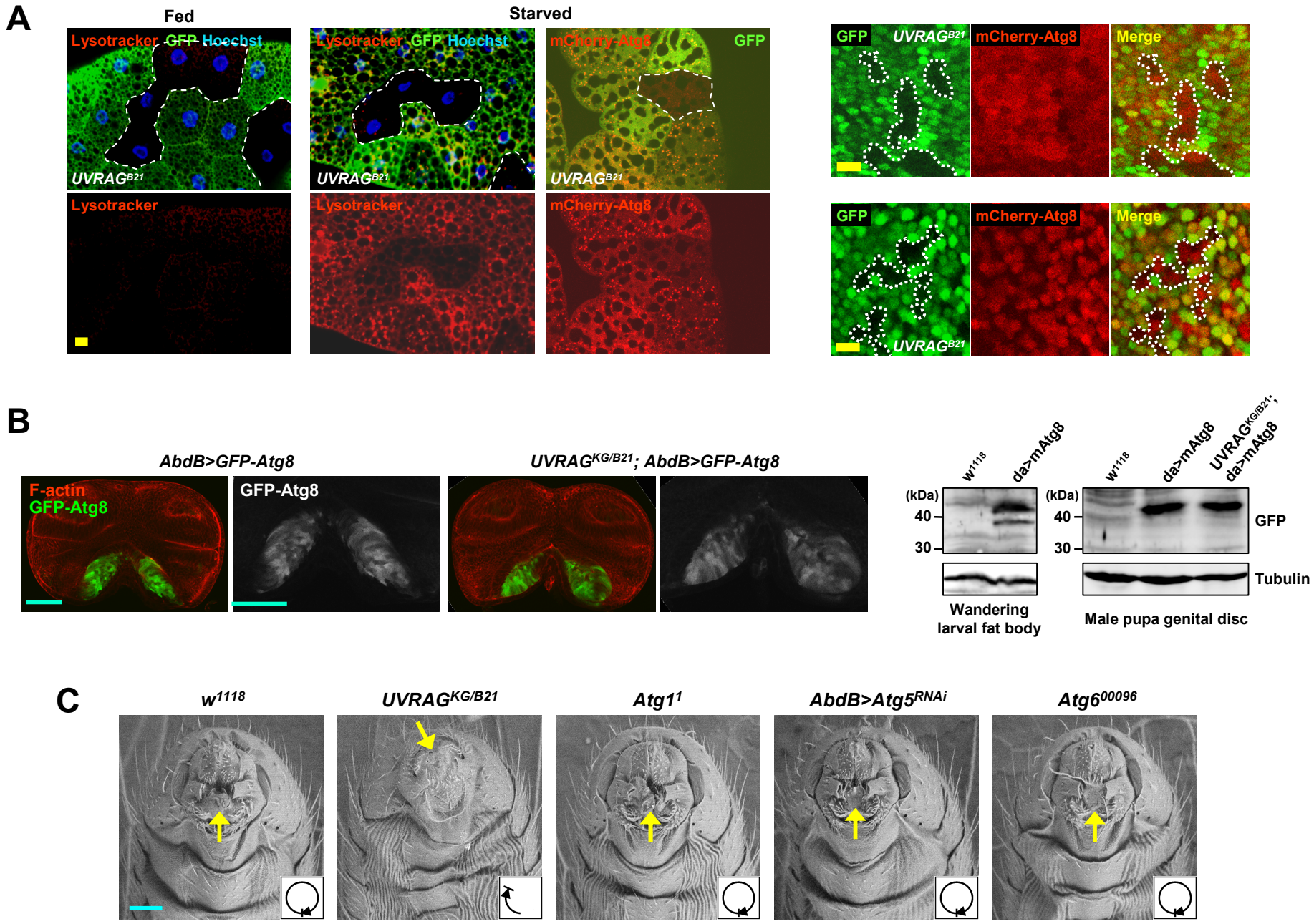


Fig. S4. Autophagy may not be involved in the organ rotation process.

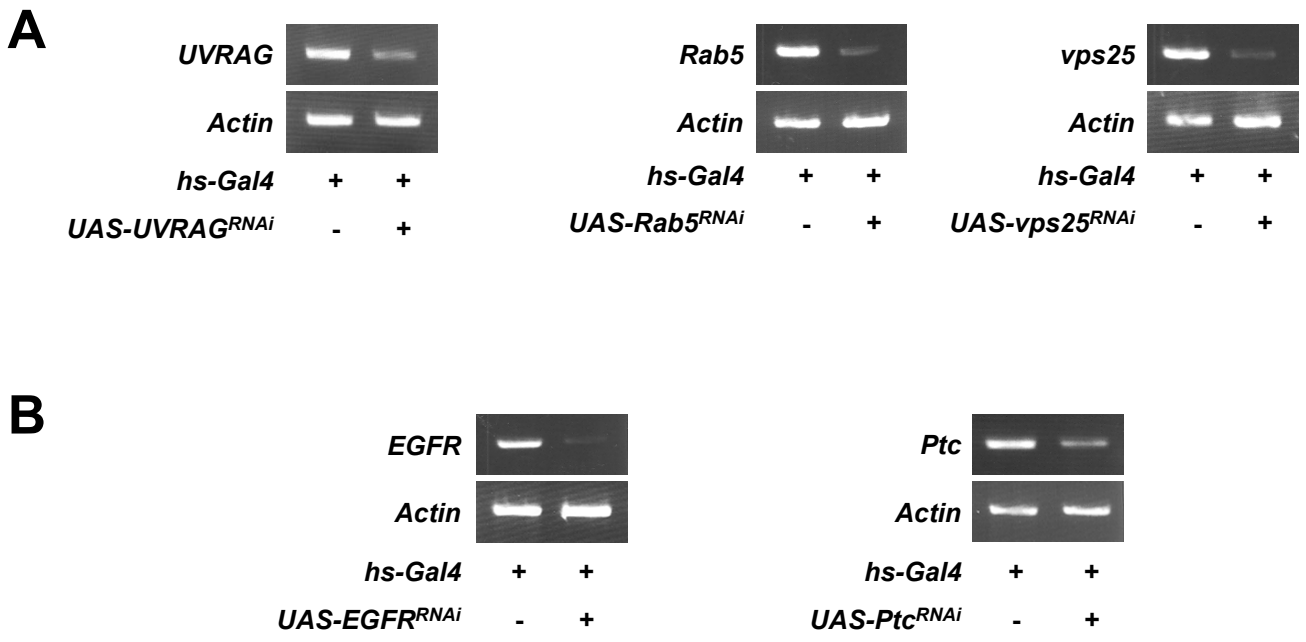
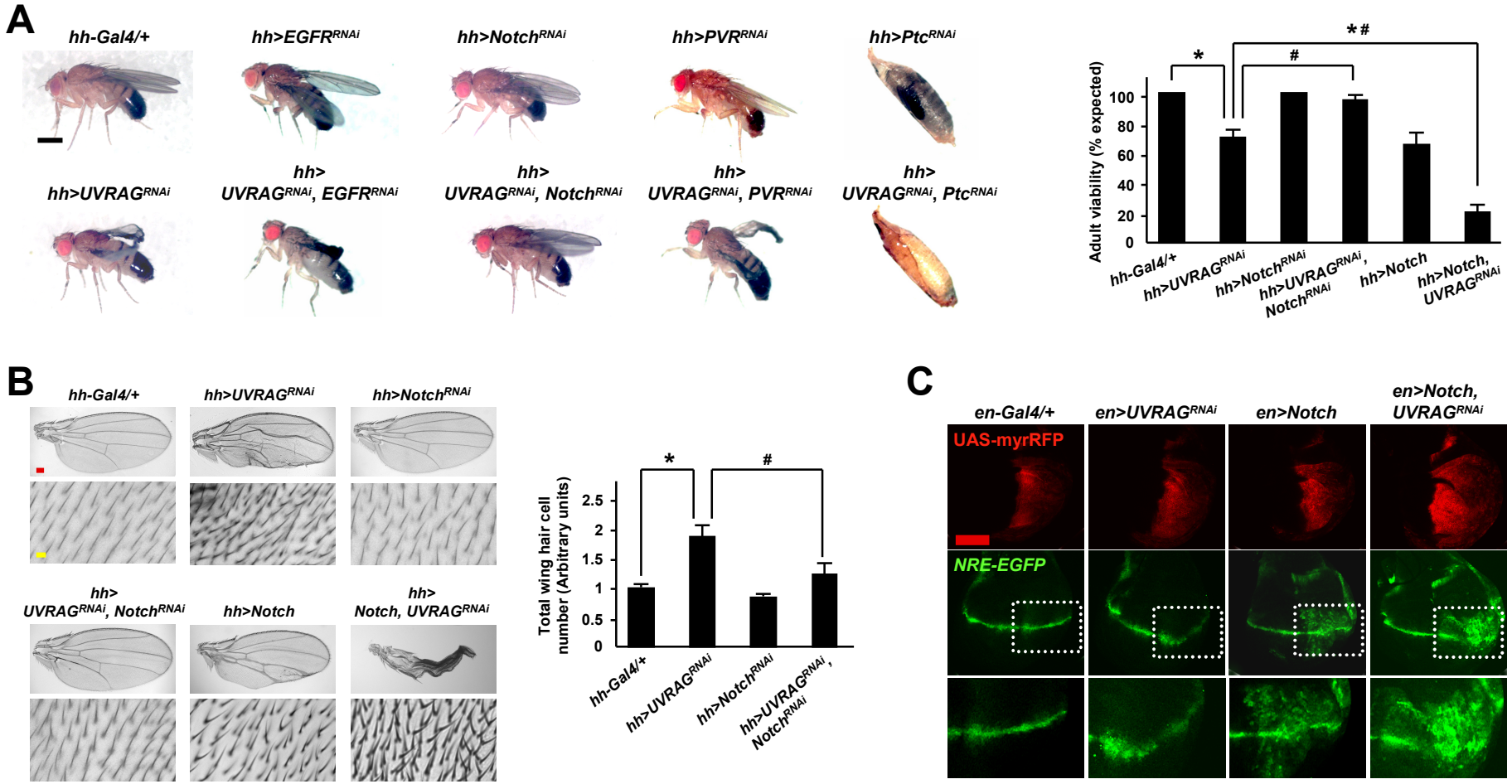


Fig. S5. RT-PCR analyses to validate transgenic RNAi alleles.



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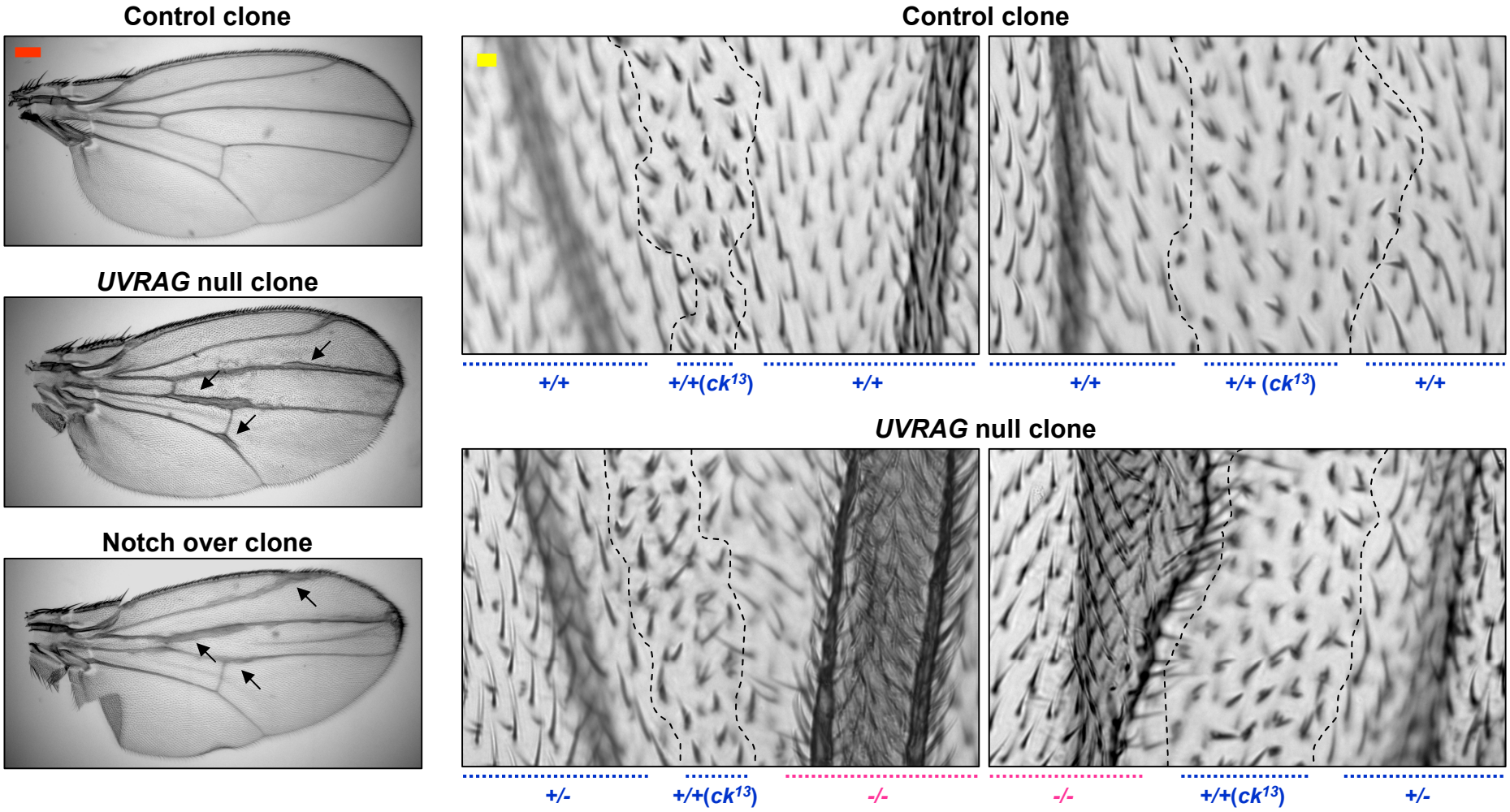

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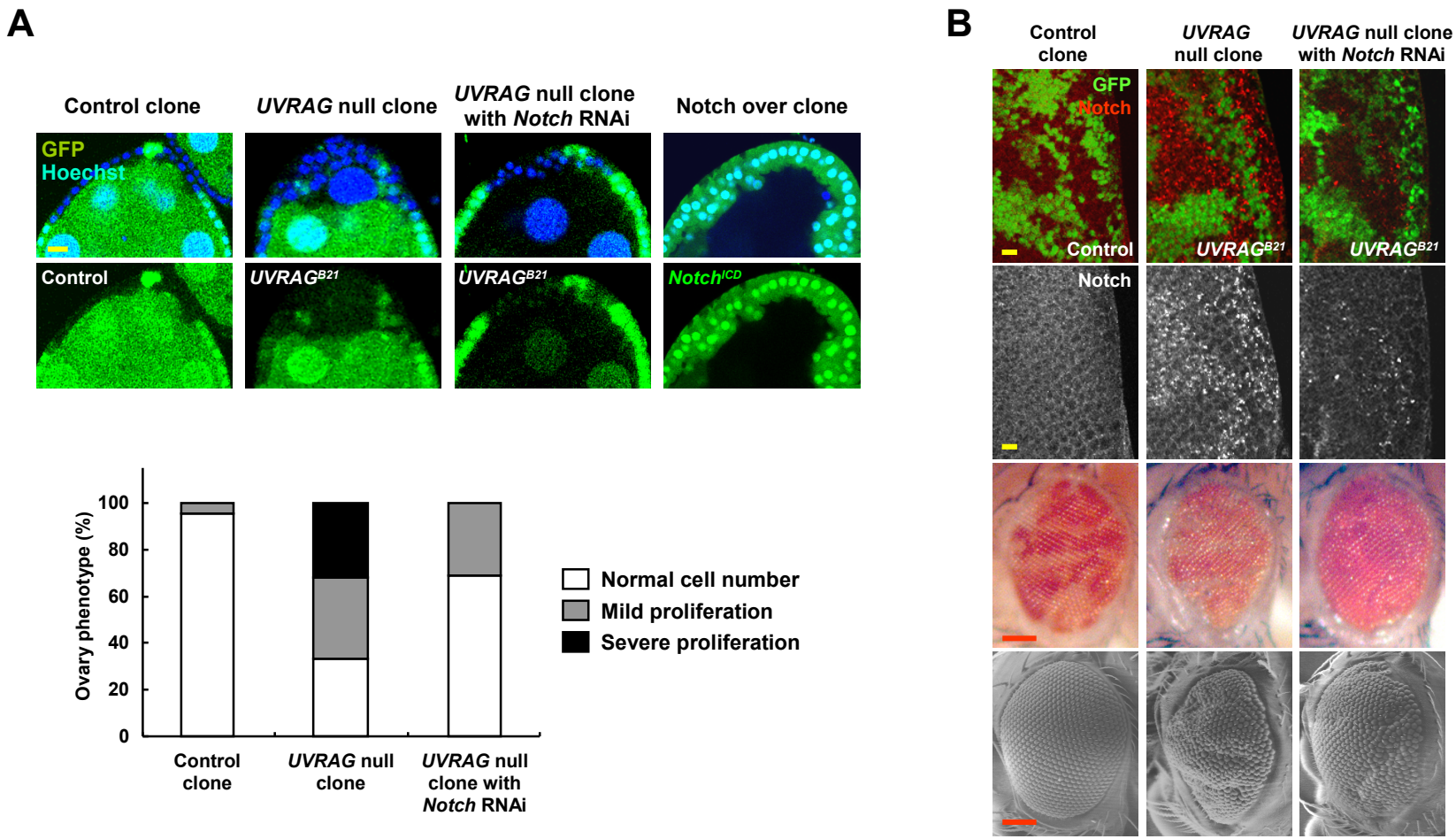

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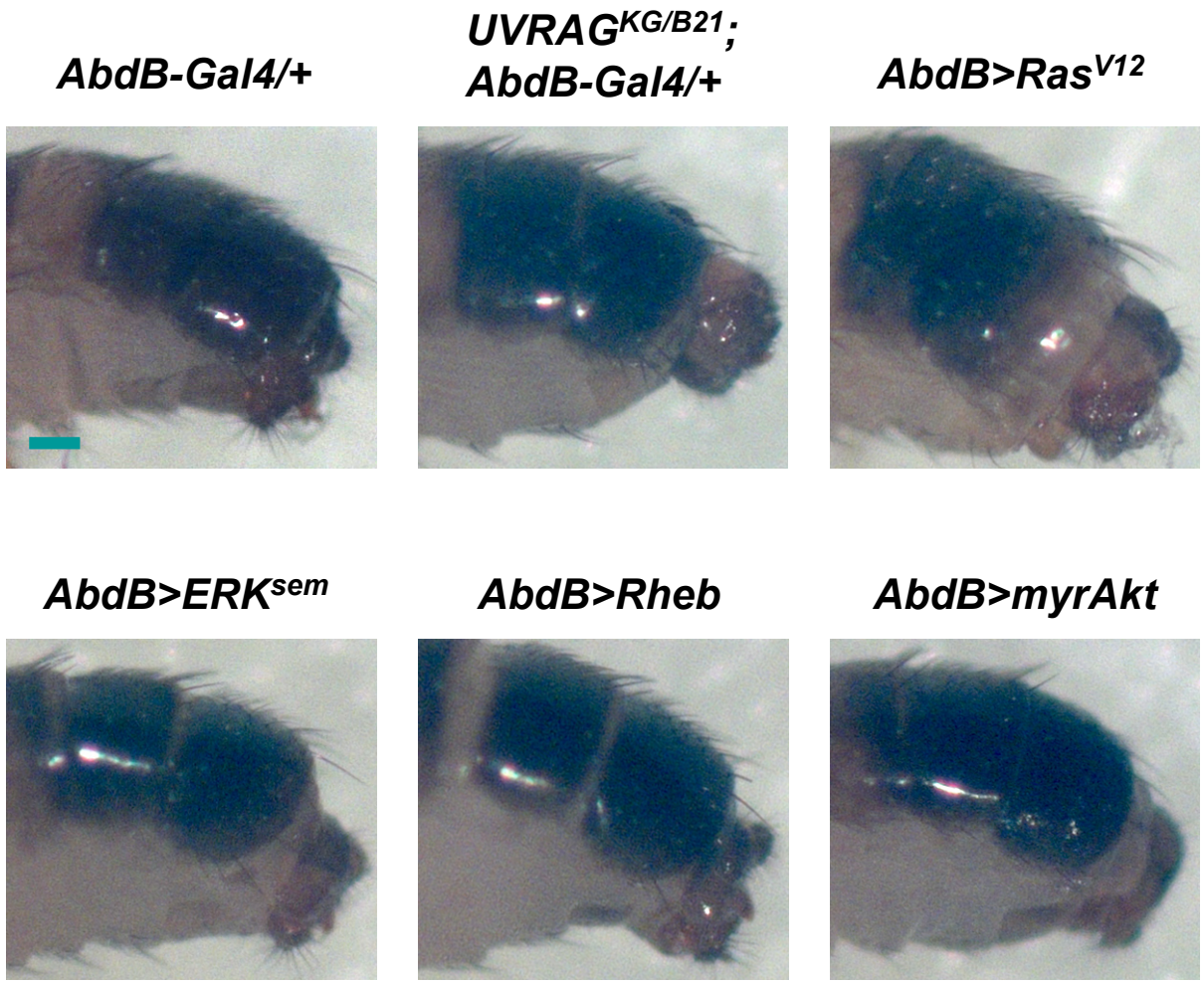

Fig. S9. Tissue enlargement may not account for the genitalia rotation defect.

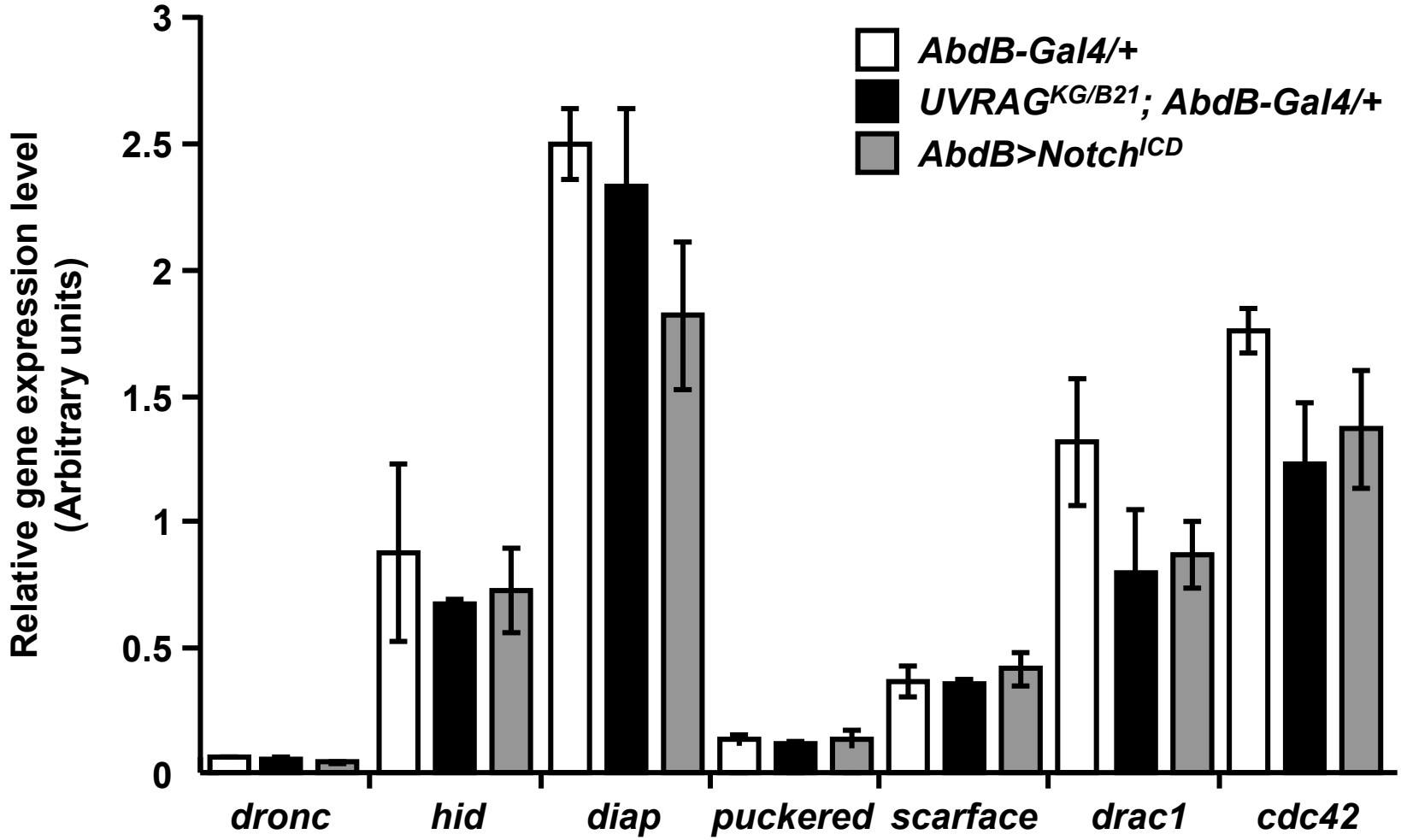

Fig. S10. Expression levels of rotation-related genes in *UVRAG* and *Notch* mutants.

Genotype	360° dextral genitalia rotation (% of flies showing each phenotype)
w^{1118}	Normal (100%)
$UVRAG^{KG/B21}$	Defective (74%)
$AbdB>UVRAG^{RNAi}$	Defective (60%)
$Atg1^{1}$	Normal (100%)
$AbdB>Atg5^{RNAi}$	Normal (100%)
$Atg6^{00096}$	Normal (100%)
$Atg7^{d14/d77}$	Normal (100%)
$AbdB>Rab5^{RNAi}$	Defective (70%)
$AbdB>vps25^{RNAi}$	Defective (30%)
$AbdB>Ras^{V12}$	Normal (90%)
$AbdB>ERK^{sem}$	Normal (100%)
AbdB>Rheb	Normal (100%)
AbdB>myrAkt	Normal (100%)

Table S1. Genitalia rotation phenotypes of male flies.