

Supplementary data

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

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Supplemental Figure legends

Supplemental Figures S1-S10

Supplemental Table S1

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 μ m.

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 μ m; blue, 50 μ m.

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. * $P < 4.5 \times 10^{-5}$, # $P < 3.88 \times 10^{-4}$, *# $P < 7.1 \times 10^{-5}$.

(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. * $P < 9.06 \times 10^{-6}$, # $P < 5.52 \times 10^{-4}$.

(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 μ m; yellow, 10 μ m.

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 *ck¹³* 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 μ m; yellow, 10 μ m.

Genotypes: Control clone, *hsFLP/+; FRT40A/FRT40A ck¹³*; *UVRAG* null clone, *hsFLP/+; UVRAG^{B21} FRT40A/FRT40A ck¹³*; Notch over clone, *hsFLP/+; UAS-Notch/+; Act>CD2>Gal4 UAS-GFPnls/+*.

Genotypes of the clones in the right panels: Upper panels (+/+, *FRT40A/FRT40A ck¹³* or *FRT40A/FRT40A; +/+(ck¹³)*, *ck¹³ FRT40A/FRT40A ck¹³*), Lower panels (+/-, *ck¹³ FRT40A/FRT40A UVRAG^{B21}*; +/+(*ck¹³*), *ck¹³ FRT40A/FRT40A ck¹³*; -/-, *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 μ m; red, 100 μ m.

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 μ m.

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.

human	MSASASVGGPPVPPPPGPAALPPGSAARALHVELPQQRRRLRLRNIAARNIVNRNG.HQL.LDT.....YFTLHLQST	: 73
mouse	MSSCASLGGPPVLPPLPPGSAALTSQAPARALHVELPQQRRRLRLRNIAARNIVNRNG.HQL.LDT.....YFTLHLQDN	: 73
XenopusMGGPGAGARSLHVELPQQRRRLRLRNIAARNIVNKNK.YRL.LDT.....YFTLHLQDE	: 53
zebrafish	...MNSIAGRDLVSAGSVSGNPGATSSRVLHVELTQQRRRLRLRSLAARNIVNKNK.SPL.LDT.....YFTLHLQD	: 70
DrosophilaMNLPRCRKW.LPLAPQQLRLRLNRIOGFNIESWPHKESLELDPPDDVLLYTLH...T	: 56
human	EKLYKEFYRSEVI.KNSLNPTWRSLDFGIMPDRDLTSSVCFVVKIWG.....GKENIY.....	: 125
mouse	EKLYKEFYRSEVI.KNSLNPTWRSLDFGIMPDRDLTSSVCFVVKIWG.....GKEEAF.....	: 125
Xenopus	KKLYKEFYRSEVI.KDSLNPWRSLDFGLIPDRDLTSSVCFVVRIWG.....GKNDVC.....	: 105
zebrafish	DQISLDFYKSEVI.RDSLNPWRSLDFGAMPLDLDLTSVCFVVRIWG.....GREEHY.....	: 122
Drosophila	DKASEAFYRSEKLPQRHQQKFAELCTDDEAWR.KTNAQCVCVVKVVKHYSAEERRDQPPPEVEQRHKDVFGRSQTPTSRRLP	: 136
human	...QLLIEWKVCIDGLKYLGOQHARNO.NEIIIFGLNDGYGAP.....FEHKGYSNAQKTLLOYDNCVVRNSYDVFSI	: 196
mouse	...QLLIEWKVCIDGLKYLGOQHARNO.NEIIIFGLNDGYGAP.....CEHKGHPNAQKN.LLOYDNCVVRNSYDVFSI	: 195
Xenopus	...RLLIEWKVNLDGLRYIGQIHACNP.NEIIIFGLNDGYGAA.....FESKNHSETRRNSHLQVDSTVVRNSYGVFSI	: 176
zebrafish	...QLLIEWKVNLDGLRFTGQIRSRNP.NEIIIFGLNDGYGAAE.....FDQESSDRRKNLSL	: 177
Drosophila	PPLELFSWGVYFSGLIPLSPLTLLSQGRNCLVFLNNGEQFASPSMISEQALSQLHLHYQKYAEKLEPQDEGINSPA	: 217
human	LRLHRAQCAIKQTQVTVOKIGKEIEEKLRLTST.....	: 229
mouse	LRLHRAQCAIKQTQVTVORLGKEIEEKLRLTST.....	: 228
Xenopus	LRLHRAQCAIKQTQVTVOKIGKEIEEKLRLTSS.....	: 209
zebrafishQVTVOKIGKEIEEKLRLTAS.....	: 197
Drosophila	SSSSPVLRMSTMRYAQLKCRQETIRSNLEKLLTLQRLQRLHQKRRQMAEVCREIARLSVHCVTRNELRLKPRTTSL	: 298
humanSNELKKKSECL...RLKILVLRNELEKQKALGREVALHKKOALADQYG	: 276
mouseSNELKKKSECL...RLKILVLRNELEKQKALGREVALHKKOALADQYG	: 275
XenopusSNELKKKSECL...RLKILVLRNELEKQKALGREVALHKKOALADQYG	: 256
zebrafishCTEKKKKRECM...QIRIGILRCELEKQKALGREVALHKKOALADQYG	: 244
Drosophila	GDYSAHQYHSMGRALSVLLAEQQQIAPLFLYNAQQLTRRLEALSSQQLKAEERTFRORNERTQLLKKEMREOREAQ.QW	: 378
human	SAFSAEHLKLOLQKESLNELEKREKTAKEFLFKTNAQLTTRCROLLSELSYIYPIDLNEHKDYFVCGVKLPNSED	: 352
mouse	SAFSAEHLKLOLQKESLNELEKREKTAKEFLFKTNAQLTTRCROLLSELSYIYPIDLNEHKDYFVCGVKLPNSED	: 351
Xenopus	NVENNQEKLOQONESLGELEKREKTAKEFLFKTNAQLTTRCROLLSELSYIYPIDAPDQKDYFVCGVKLPNSED	: 332
zebrafish	EASSFKQDLSEKERTLTLQKESLNELEKREKTAKEFLFKTNAQLTTRCROLLSELSYIYPIDVNNQNDYFVCGVKLPNSED	: 320
Drosophila	ELHSQRH.RLEKRELELRATLAPQHLEQDQKQIETQVERMSTLVLELOEYNTLQNVGGQFSSICGTAFFHMEQYTSER	: 458
humanQAKDDGS...IAVALGYTAHLVSMISFELQVPLRYPILHKGSRSTIKDNINDKLTEKEREFPFLYKGC.EKIQFDY	: 424
mouseQAKDDGS...IAVALGYTAHLVSMISFELQVPLRYPILHKGSRSTIKDNINDKLTEKEREFPFLYKGC.EKIQFDY	: 423
XenopusQAKDDGS...IAVALGYTAHLVSMISFELQVPLRYPILHKGSRSTIKDNINDKLTEKEREFPFLYKGC.EKIQFDY	: 404
zebrafishQAKDDGS...IAVALGNIAHLVSMISFELQVPLRYPVILHKGSRSTIKDITINDKLTEKEREFPFLYKGC.EKIQFDY	: 391
Drosophila	QAANAQLDNDVSPPLAVSALGVVAHLVQMLATMDRPLRNLILYEPSKARLVDDIKEALTYTTRFPFLYKRSILPSQQT	: 538
human	GVYLLNKNIAQ.....LRYQHGLGTPDLROTLPNLKNEHEHGLMVR	: 466
mouse	GVYLLNKNIAQ.....LRYQHGLGTPDLROTLPNLKNEHEHGLMVR	: 465
Xenopus	GVYLLNKNIAQPFRRGFAGVFWIVLLOHPRSLOLELTNRWPDILLQDFLRYQYALGTPDLROTLPNLKNEHEHGLMVR	: 485
zebrafish	GVYLLNKNIAQ.....LRYQHGLGTPDLROTLPNLKNEHEHGLMVR	: 433
Drosophila	AIYLLRQVNSQ.....LQFD.ITQCDLRNIFGNL...LELFTSLRY	: 576
human	DRHHTSSAIPVVKRQ...SSIFGGADVGFSGGIPSPDKGHRKRASSENERLOY.KTPPPSYNSALAQ...PVTTVPSMGETER	: 542
mouse	DRHHTSSAIPVVKRQ...SSTFGGADGFSAGIPSPDKVHRKRASSENERLOY.KTPPPSYNSALAQ...PGVAMPTSGDSE	: 541
Xenopus	DRHHTSSAIPVVKRPT...SSTFVSQDVGFSSRTPPDKGLKRASSENERIPY.KTPPPSYNSAMAD...PVVILIPAKEPEK	: 562
zebrafish	DRHHTSSAIPVVKRSHLSVSTAS.EVGFPAALSSPEQDVRRAASDADK.QKCRTPPPSYNTAMAPDPMTPPELQSEPSV	: 512
Drosophila	IERTQRDEVDERDGTAVGAGCGEAR.....	: 601
human	KITS.LSSSLDTSLD...FSKENKKKGEDLVGSLNGGHANVHPSQEQEALSGHRATVNGDLPSEQAGSASVQLPGFHP	: 619
mouse	KVAP.LSSSLDTSLD...FSKENKKAGVDLGSVSGDHGNSDQEQEALPGHLAAVNGTALPSEQAGPAGTLLPGSCHP	: 618
Xenopus	RR.VASSLDASIN...ISKEGSSK.EFSESLNGDSITNGSSDQTAQPFSETSSAMNGTLLQGESVAVFVSGEVPQNSQI	: 636
zebrafish	EV.LEVGVKAVETLEGETVSEEHPEKPLDVPDID...VSTLPS...DSLPHPGAMNGSAVPTS...GGVGLGV...	: 576
DrosophilaLA.NGLTAPHLSQSHSSVDMNHVPLE	: 626
human	VSEAEIACCIVQAEELIGLEATGFASCD...OLEAFNCIPVDSAVAVECDQVLGEF...EEFSRRIYALNENYSSFR	: 691
mouse	APSAEIACCIVQAEELIGLEATGFASCD...OLEAFNCIPVDSAVAVECDQVLGEF...EEFSRRIYALNENYSSFR	: 690
Xenopus	...NDLCCIVQAEELVGTFAADFASCD...OLEAFNCIPVDHAVAVECDQVLGEF...EEFSRRIYALNENYSSFR	: 705
zebrafish	ASGGEVCCSVEQAEELMGTFAATGLGLGLGGARDDYPCIPVEHAVAVECDQVLGEFDAAGTEEVSRIYALNENYSSFR	: 656
Drosophila	TGVNAVKDALLOQLLPPGVSEALAEYASTQRICRSVGSYSYDGEDEFPRLEHNYNSDSNITLQERS.....	: 696
human	RPRRSDDK... : 699	
mouse	RPRRSDDK... : 698	
Xenopus	RPRRSSEK... : 713	
zebrafish	RPRRSSEK... : 664	
Drosophila : -	

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

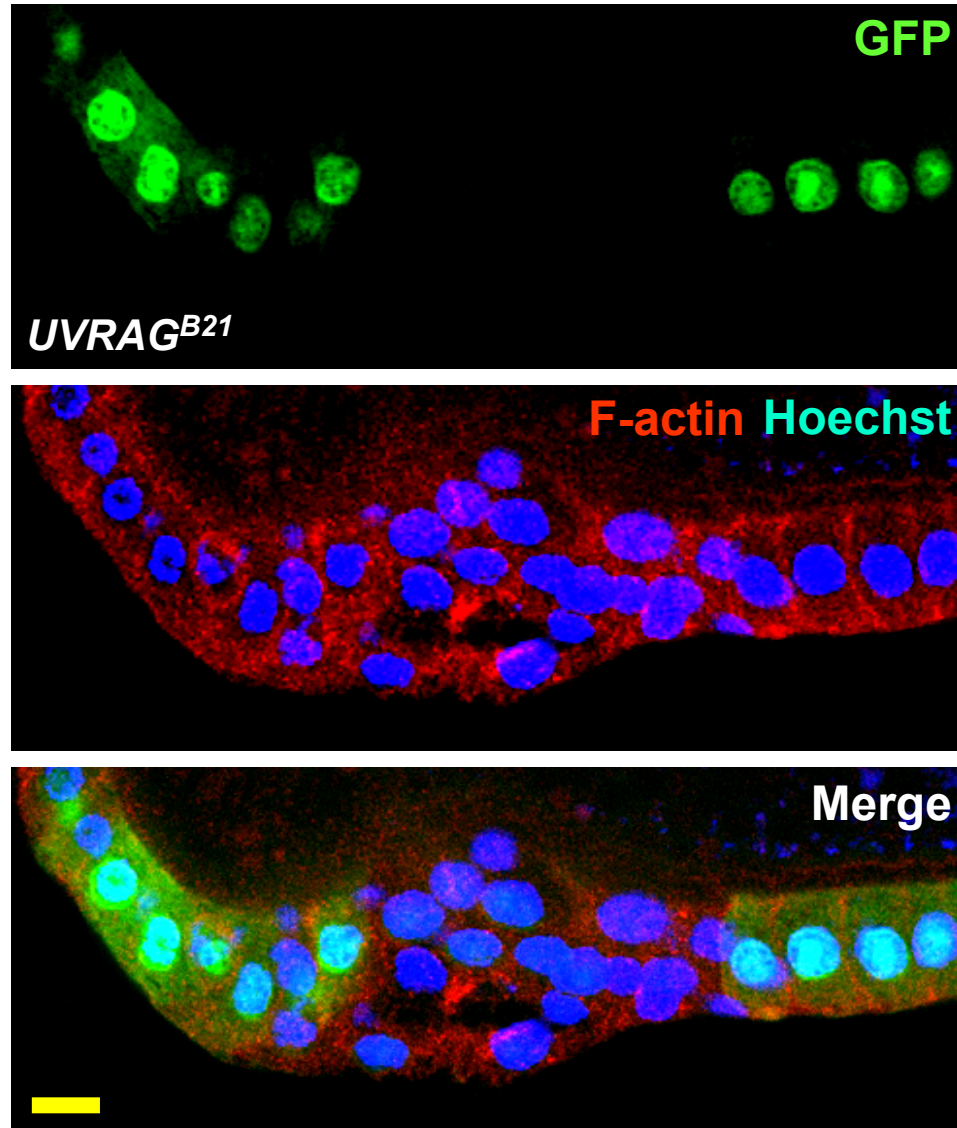


Fig. S2. *UVRAG* null clones show cell proliferation in ovary.

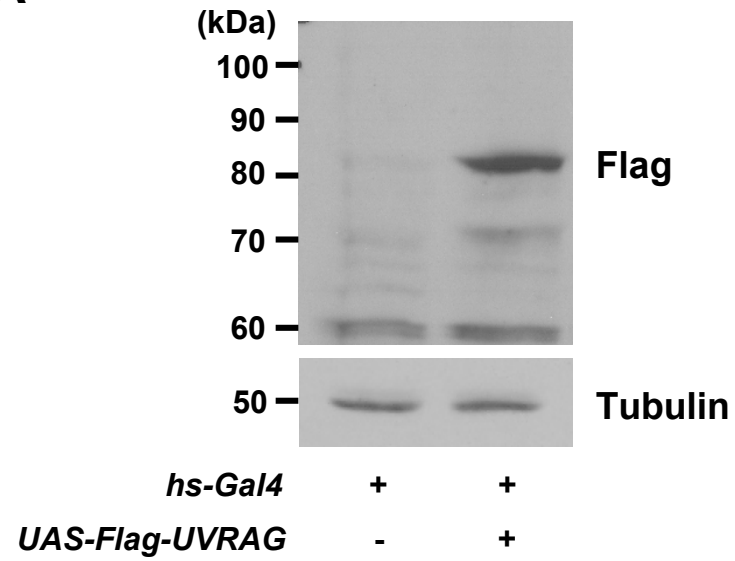
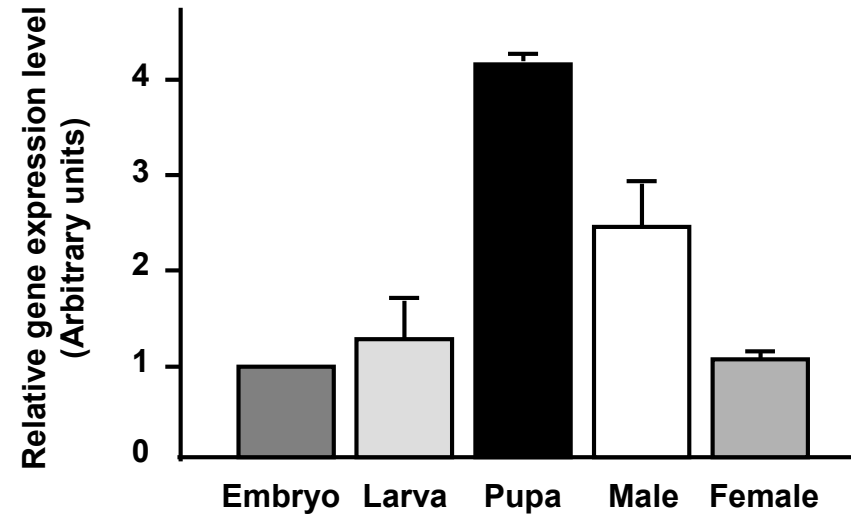
A**B**

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

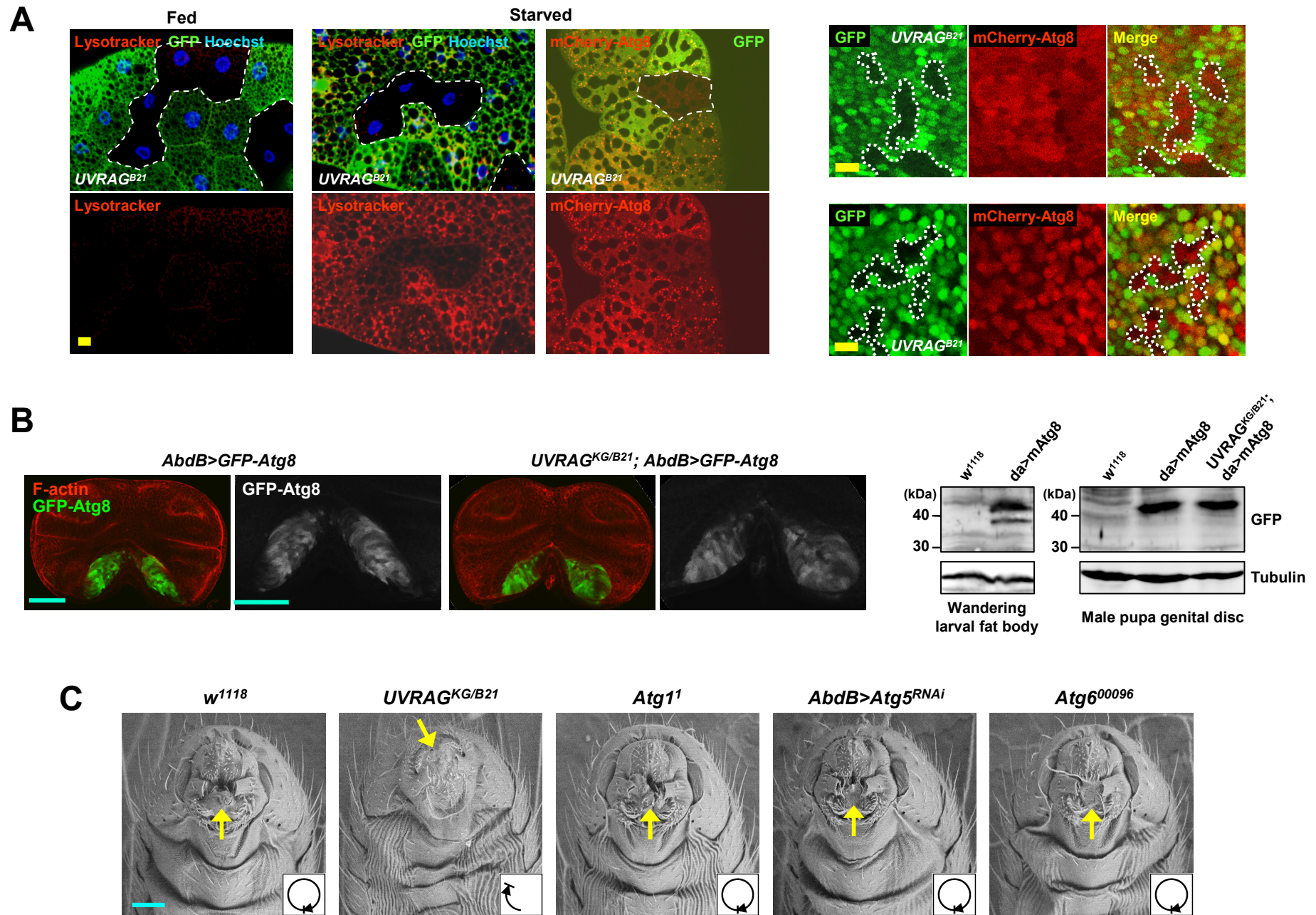


Fig. S4. Autophagy may not be involved in the organ rotation process.

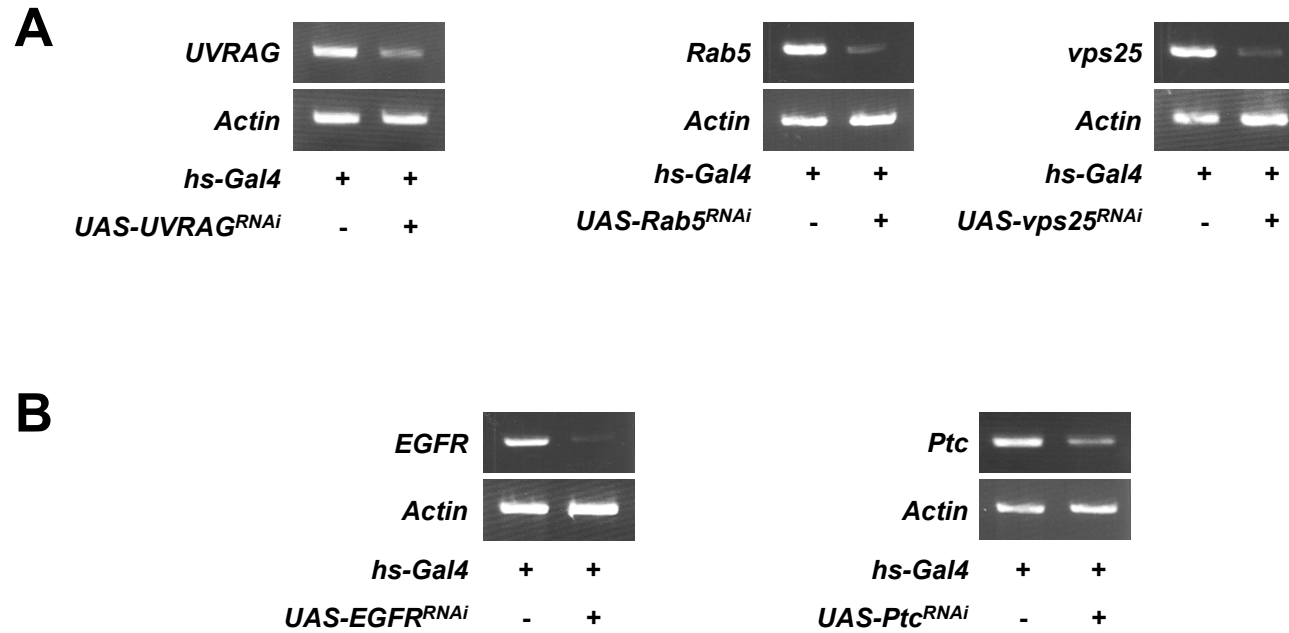


Fig. S5. RT-PCR analyses to validate transgenic RNAi alleles.

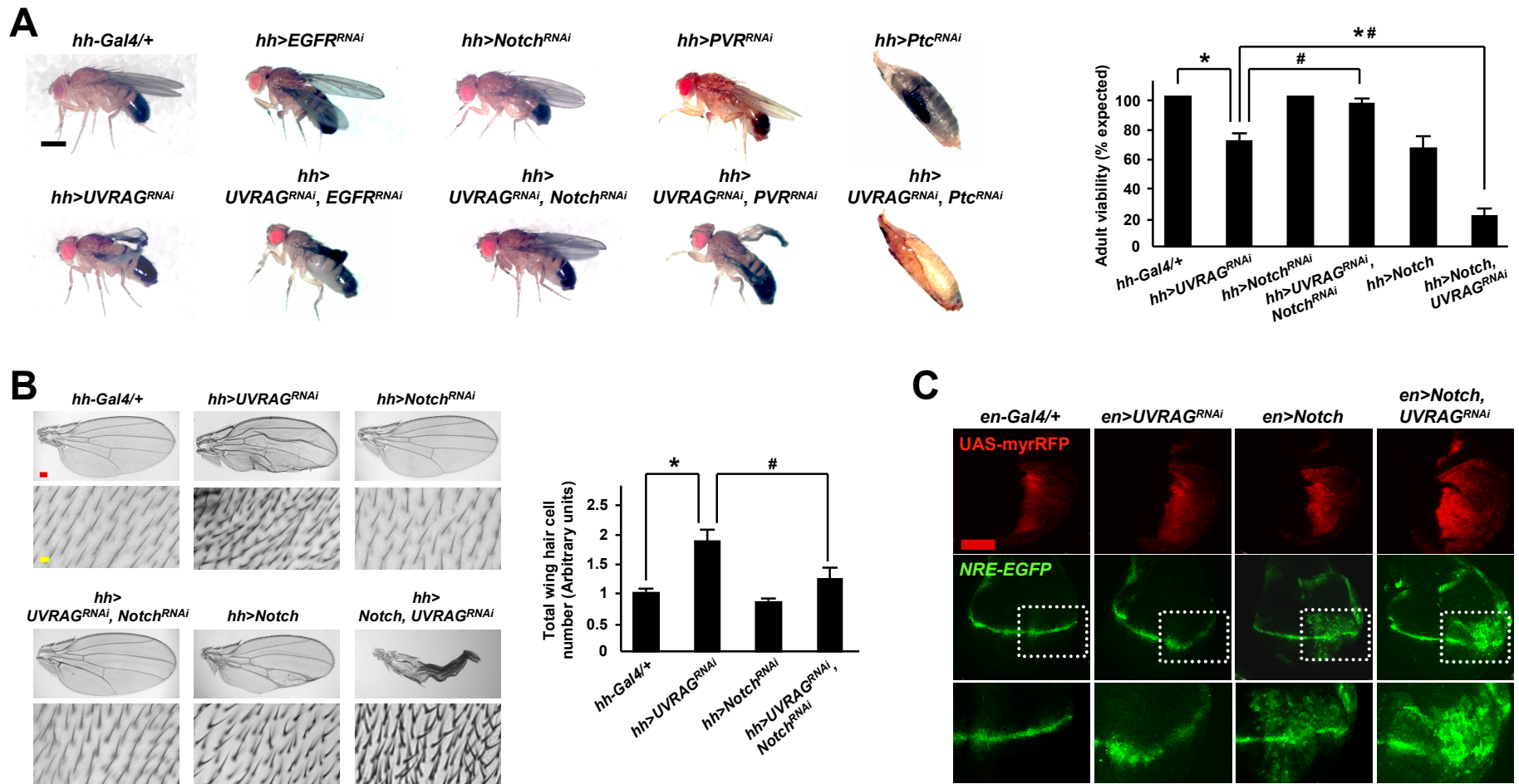


Fig. S6. Genetic interaction between UVRAG and signaling receptors.

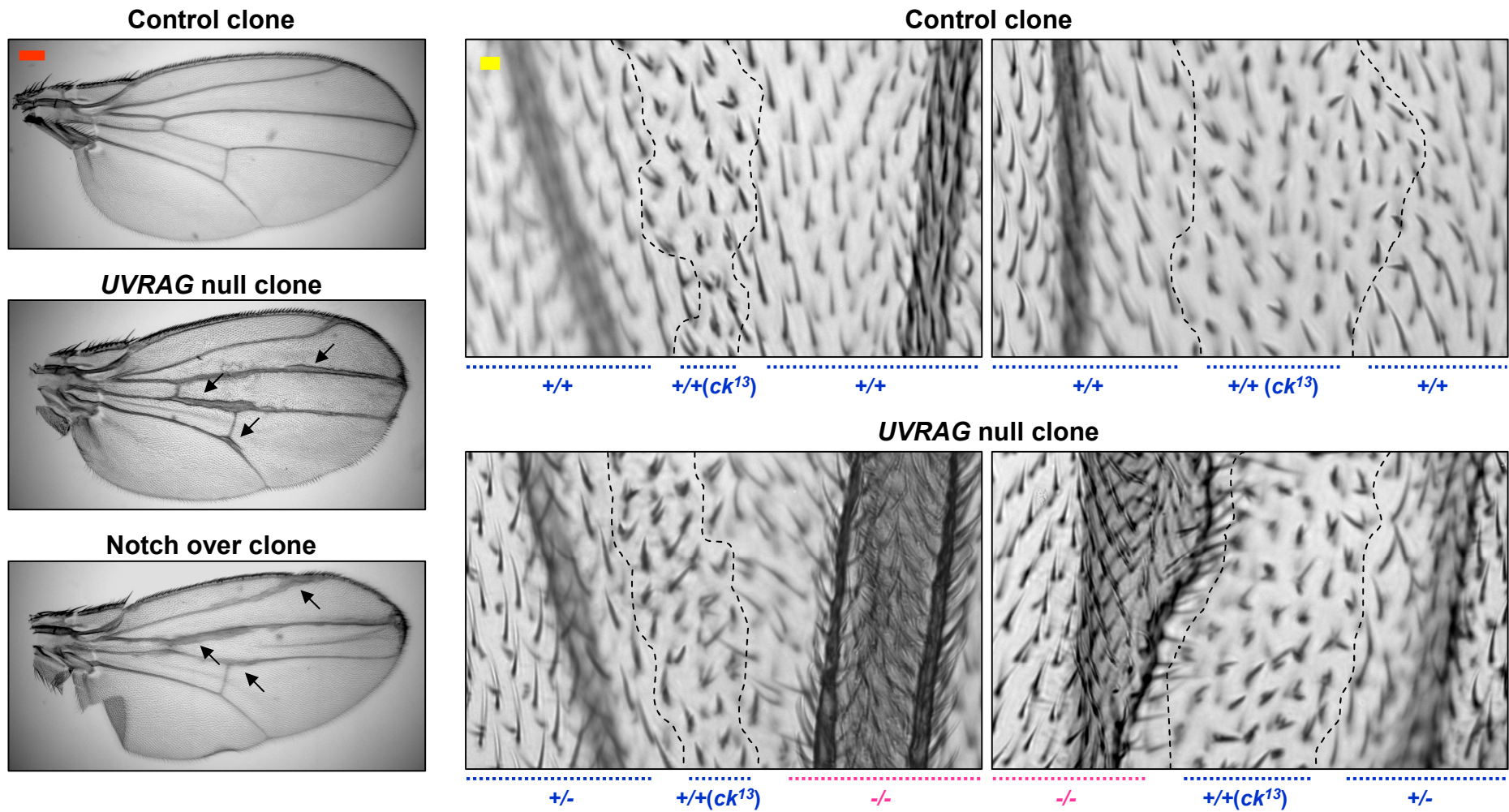


Fig. S7. UVRAG null clones show increased hair cell number in adult wings.

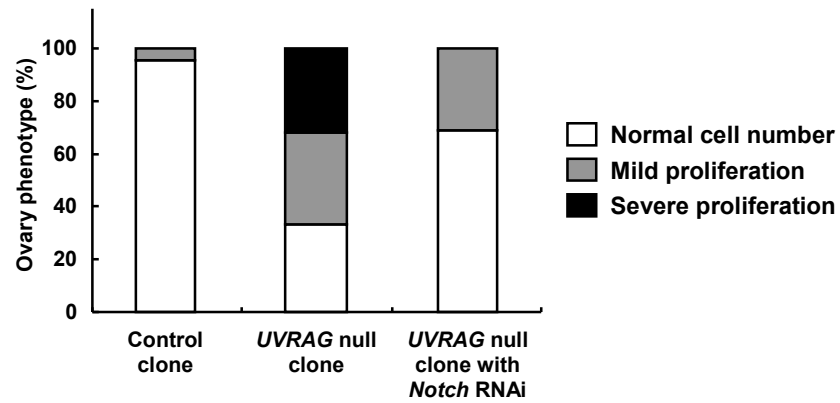
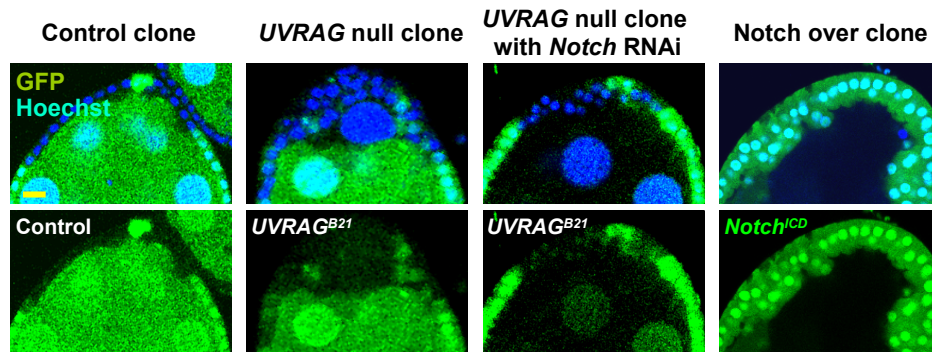
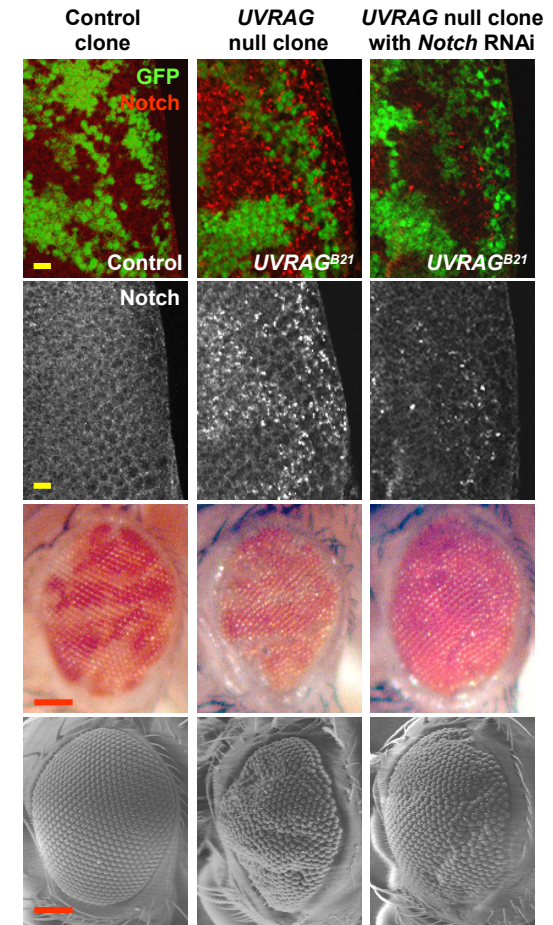
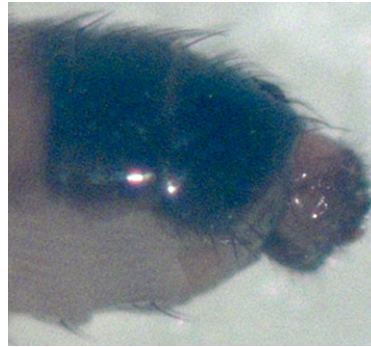
A**B**

Fig. S8. *UVRAG* null clone phenotypes are rescued by *Notch* knockdown.

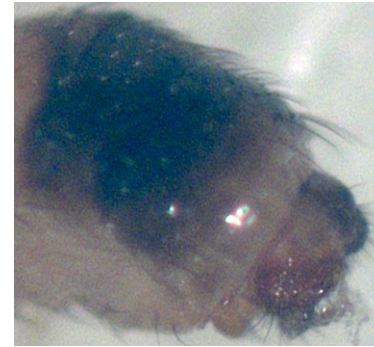
AbdB-Gal4/+



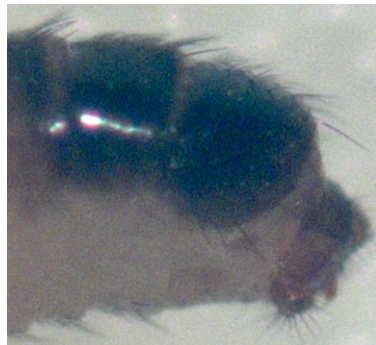
*UVRAG^{KG/B21};
AbdB-Gal4/+*



AbdB>Ras^{V12}



AbdB>ERK^{sem}



AbdB>Rheb



AbdB>myrAkt

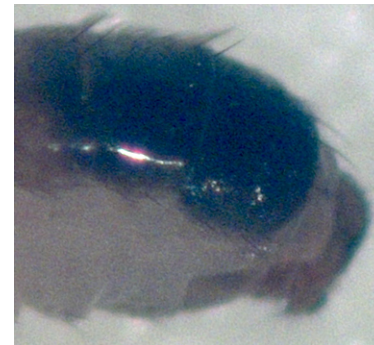


Fig. S9. Tissue enlargement may not account for the genitalia rotation defect.

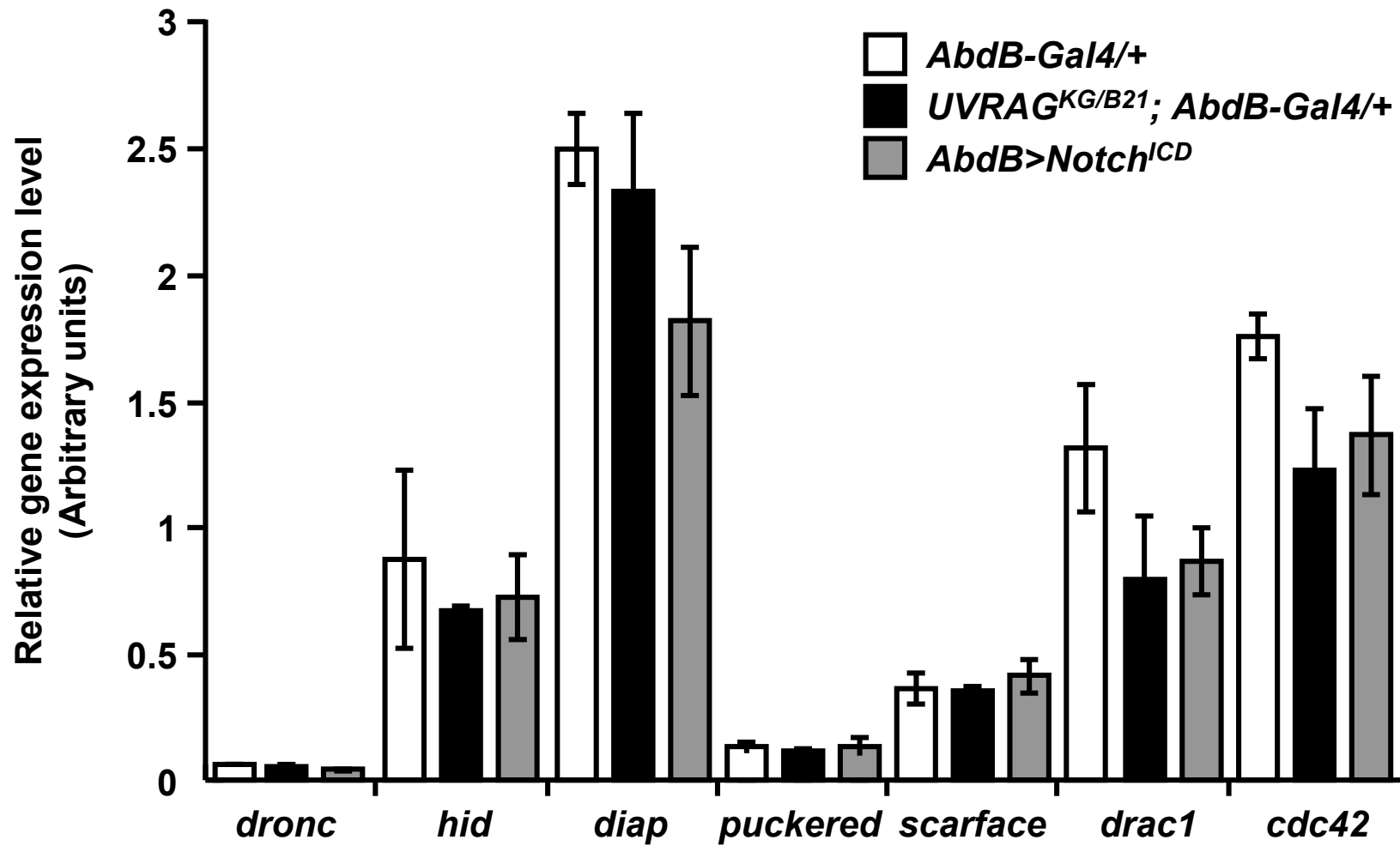


Fig. S10. Expression levels of rotation-related genes in *UVRAG* and *Notch* mutants.

Genotype	360° dextral genitalia rotation (% of flies showing each phenotype)
<i>w¹¹¹⁸</i>	Normal (100%)
<i>UVRAG^{KG/B21}</i>	Defective (74%)
<i>AbdB>UVRAG^{RNAi}</i>	Defective (60%)
<i>Atg1¹</i>	Normal (100%)
<i>AbdB>Atg5^{RNAi}</i>	Normal (100%)
<i>Atg6⁰⁰⁰⁹⁶</i>	Normal (100%)
<i>Atg7^{d14/d77}</i>	Normal (100%)
<i>AbdB>Rab5^{RNAi}</i>	Defective (70%)
<i>AbdB>vps25^{RNAi}</i>	Defective (30%)
<i>AbdB>Ras^{V12}</i>	Normal (90%)
<i>AbdB>ERK^{sem}</i>	Normal (100%)
<i>AbdB>Rheb</i>	Normal (100%)
<i>AbdB>myrAkt</i>	Normal (100%)

Table S1. Genitalia rotation phenotypes of male flies.