Supplementary Information for

Drosophila DUBA de-ubiquitylates the initiator caspase DRONC and is essential for spermatogenesis

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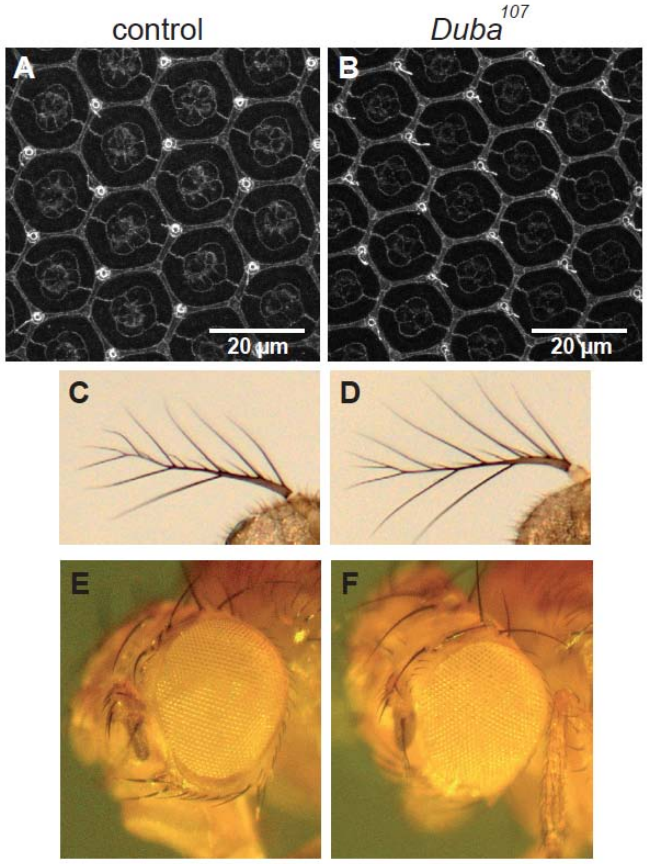
This file contains three supplementary figures together with additional methods description for the data shown in the supplement.

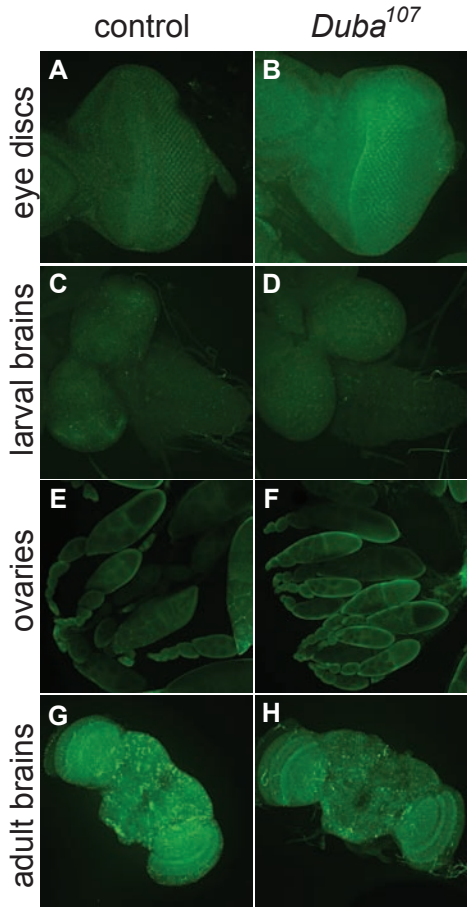
Supplementary Figure S1



Developmental cell death is not impaired in the *Duba*^{107} null mutant.

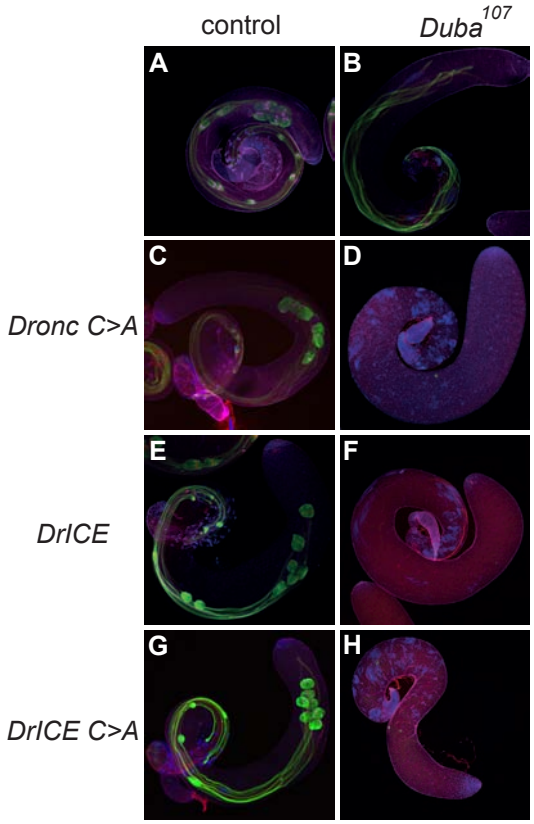
(A, B) Removal of interommatidial cells during eye development is not affected by loss of *Duba.* Pupal eye discs 42 h after pupariation (25 °C) were stained with α-E-Cadherin antibody to reveal plasma membranes. **(A)** Canton S **(B)** *Duba*^{107}. **(C, D)** Aristae of *Duba*^{107} do not display defects in apoptosis such as reduced or increased branching in comparison to controls (precise P-element excision) **(E, F)** Comparison of eye structure of homozygous *Duba*^{107} fly with control (precise P-element excision). No obvious differences were observed.

Supplementary Figure S2



Ubiquitin-conjugates accumulate in eye discs and ovaries of *Duba*^{107} null mutants. Tissues from control (precise P-element excision) and *Duba*^{107} animals were stained for conjugated ubiquitin. Maximum projections of confocal stacks are shown. **(A, B)** *Duba*^{107} eye discs of wandering third instar larvae show accumulation of poly-ubiquitylated proteins. **(C, D)** No accumulation is detectable in *Duba*^{107} brains from wandering third instar larvae. **(E, F)** Ovaries show a slight increase of ubiquitylated proteins in the *Duba*^{107} mutant. **(G, H)** Adult brains show no difference in poly-ubiquitin levels between control and *Duba*^{107}.

Supplementary Figure S3



Caspase expression leads to disruption of spermatogenesis in *Duba*^{107} flies. While caspase overexpression in wild type background allowed the formation of CBs, WBs and ICs (**C, E, G**), these individualisation structures and mature spermatids were completely missing when *Dronc*$^{C>A}$, *DrICE* or *DrICE*$^{C>A}$ were expressed in the *Duba*^{107} background (**D, F, H**). Testes from 0 to 2 day old adult males were prepared and stained against DAPI (blue), cleaved caspase (cDcp-1, green), Actin/Phalloidin (magenta) and Axo49 (red). Shown are maximum projections of confocal stacks. For comparison, testes from flies without exogenous caspase expression are shown (**A**: precise P-element excision, **B**: *Duba*^{107}). Genotypes: (C) *Hsp83-Gal4*/UAS-*Dronc*$^{C>A}$, (D) *Hsp83-Gal4*/UAS-*Dronc*$^{C>A}$; *Duba*^{107}, (E) *Hsp83-Gal4*/UAS-*DrICE-V5*, (F) *Hsp83-Gal4*/UAS-*DrICE-V5; Duba*^{107}, (G) *Hsp83-Gal4*/UAS-*DrICE*$^{C>A}$*-V5*, (H) *Hsp83-Gal4*/UAS-*DrICE*$^{C>A}$*-V5*; *Duba*^{107}.

Supplementary methods

Preparation and staining of pupal eye discs

Pupa were collected as white praepupa in 1.5 h intervals and aged at 25 °C until 42h after puparium formation (APF). Pupa were dissected in PBS and eye discs were fixed for 1h in 4 % PFA at room temperature. Eye discs were stained over night at 4 °C with anti-E-Cadherin antibody (dP-20, Santa Cruz sc-15751, 1:50). Samples were washed three times for 1 h each, incubated with secondary antibody (goat Cy5, 1:200) over night, washed three times and mounted in Vectashield. Eye discs were imaged with a Zeiss LSM780 with a 40x objective.

Staining of larval and adult tissues

Tissues were prepared in PBS. Larval eye discs were fixed for 30 minutes, ovaries for 20 minutes and larval and adult brains were fixed for 1 h at room temperature with 4 % PFA. Ovaries were blocked with 5 % horse serum for 20 minutes. Antibody staining was done as described for pupal eye discs with α-mono-polyubiquitin conj. FK2 (Enzo, 1:1000) and anti-mouse Alexa633 (Invitrogen, 1:200). Ovaries were incubated with secondary antibodies for 2 h at room temperature. Ovaries were imaged with a 10x, eye discs, larval and adult brains with a 25x objective.