Article

Orthologs of NOX5 and EC-SOD/SOD3: dNox and dSod3 impact egg hardening process and egg laying in reproductive function of *Drosophila melanogaster*

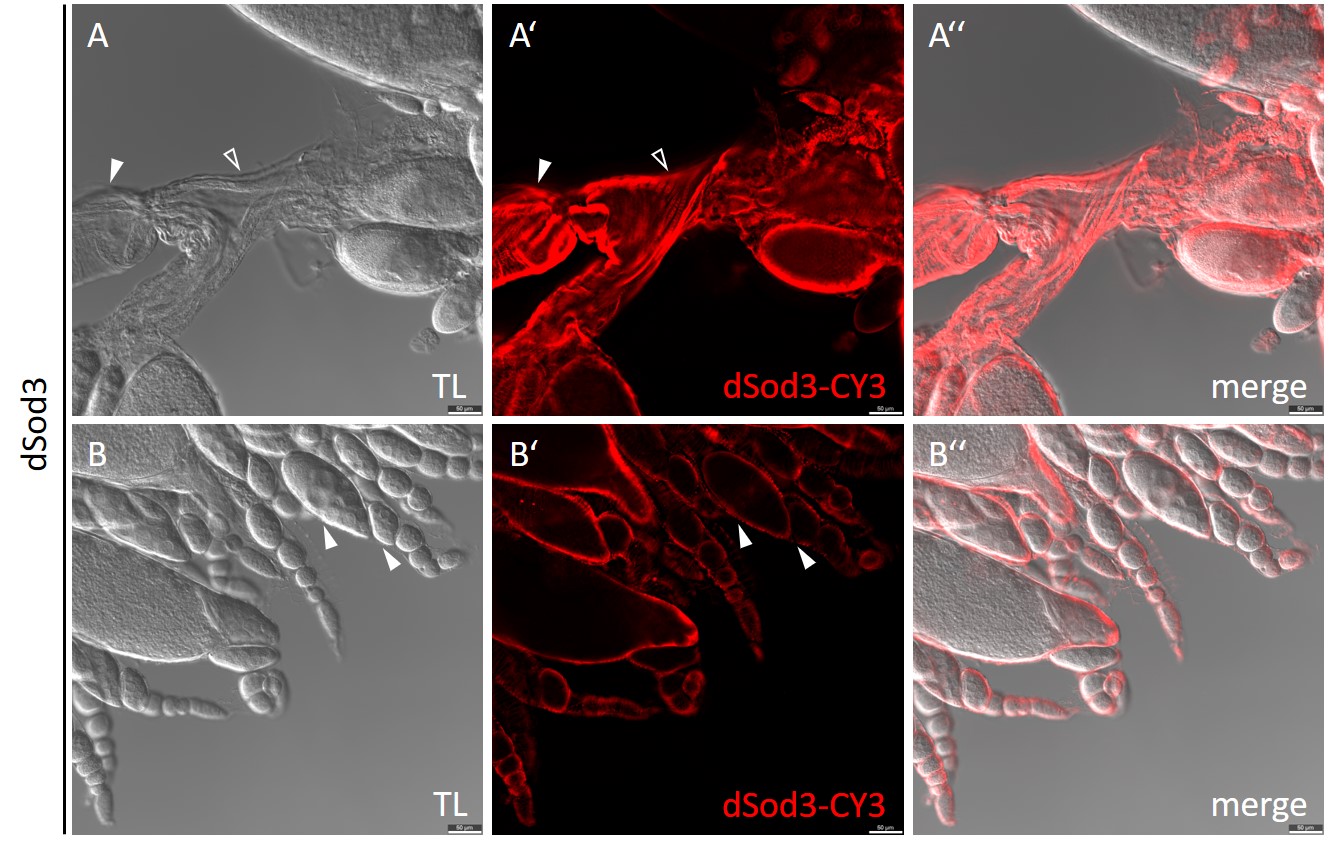
Eva Louise Steinmetz 1,\*, Annika Scherer 1, Célestine Calvet 1 and Uli Müller 1

|  |
| --- |
| **Citation:** To be added by editorial staff during production.  Academic Editor: Firstname Lastname  Received: date  Revised: date  Accepted: date  Published: date    **Copyright:** © 2024 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/). |

1 Zoology & Physiology, ZHMB (Center of Human and Molecular Biology), Saarland University, Building B2.1, D-66123 Saarbrücken, Germany; eva.steinmetz@uni-saarland.de

**\*** Correspondence eva.steinmetz@uni-saarland.de; Tel.: +49-681-302-6654 (E.S.)

**Supplementary figures**



**Fig.S1: dSod3 localization in oviduct and ovariole muscle sheath**

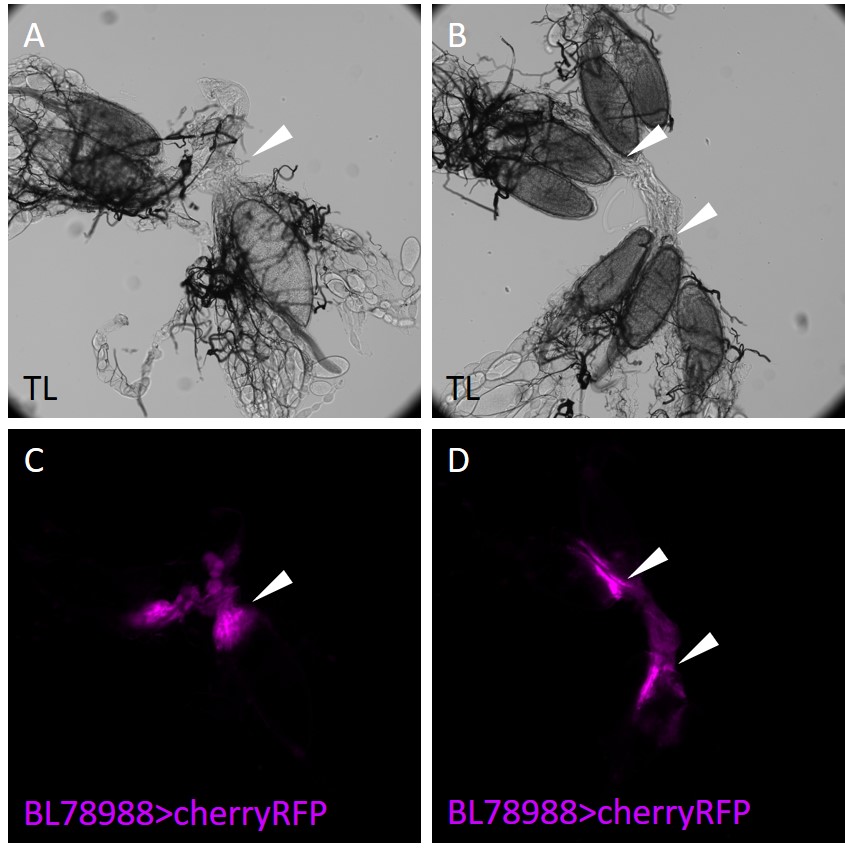
**A-A’’** lateral (white arrowhead) and common (unfilled white arrowhead) oviduct, **B-B’’** ovarioles with early-stage egg chambers, surrounded by epithelial muscle sheath (white arrowheads). Fluorescent images are accompanied by the respective transmitted light (TL) images for a better identification of all structures. Indirect immunostaining was done using anti-dSod3 (1:250; #PA5-102904 Invitrogen) antibody combined with fluorophore coupled (Cy3) secondary antibodies on fixed ovaries. Images were taken with Thunder imaging system (Leica).

Ein Bild, das Screenshot enthält.

Automatisch generierte Beschreibung

**Fig.S2:dNox localization in corpus luteum (CL) and follicle cells of mid-stage egg chamber**

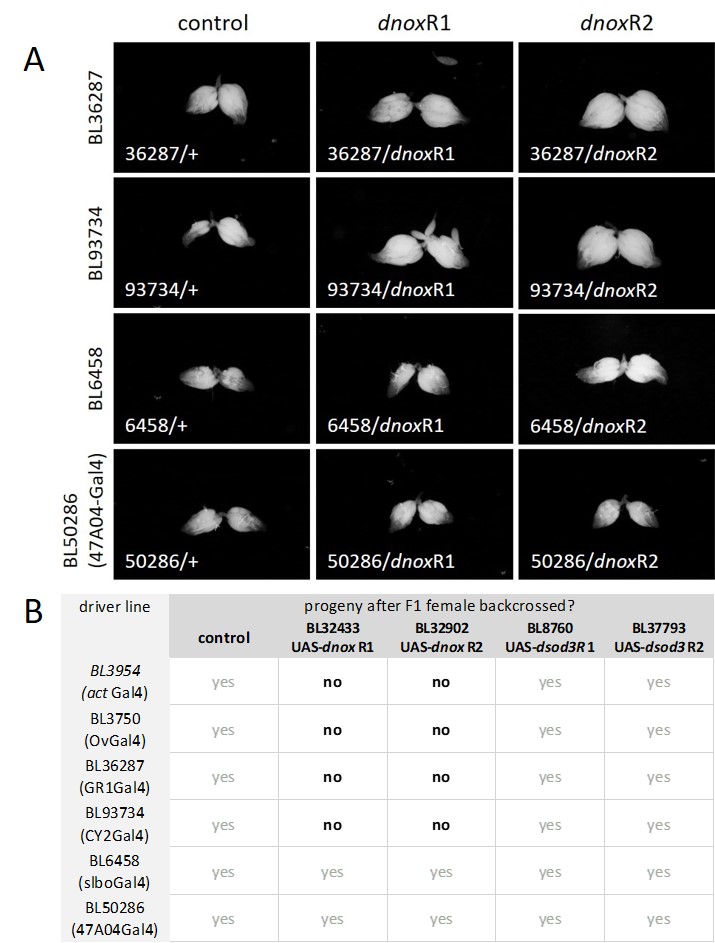
**A-A’’** mid-stage egg chambers and corpus luteum (CL; white asterisk) **B, B’** stage 10 egg chamber, in B focus was set to the follicle cell epithel that surrounds the growing oocyte, in B’ focus was set to the cross-section level of the egg chamber for a better view at the follicle cell epithel surrounding the oocyte. Scale bars indicate 50 µm. Fluorescent images are accompanied by the respective transmitted light (TL) images for a better identification of all structures. Indirect immunostaining was done using anti-dNox (1:250; this work) antibody combined with fluorophore coupled (Cy3) secondary antibodies on fixed ovaries. Images were taken with Thunder imaging system (Leica).

****

**Fig.S3: *dnox* expression in lateral and common oviduct of *Drosophila* ovaries**

**A-D** ovaries expressing reporter gene (mCD8-cherryRFP) under the control of a *dnox* gene trap driver line (BL78988)

Using UAS-mCD8-cherryRFP (BL27392) reporter gene expression driven by a Gal4 driver line (BL78988) from the T2A-Gal4 library [71], we visualized *dnox* expression in lateral and common oviduct.



**Fig.S4: *dnox* RNAi knockdown with alternative driver lines A** ovary morphology after *dnox* RNAi knockdown; driver lines (left panel) were crossed to control line (w1118) or UAS-RNAi lines*dnox*R1 or *dnox*R2(upper panel); ovaries of few days old resulting F1 females were dissectedand morphologically compared **B** fecundity test of F1-females; driver lines (left panel) were crossed to control line (w1118) or UAS-RNAi lines*dnox*R1, *dnox*R2, *dsod3*R1 or *dsod3*R2(upper panel); resulting F1 females were backcrossed with control line males and checked if they deliver F2 individuals or not (“yes” or “no”)

Ein Bild, das Screenshot enthält.

Automatisch generierte Beschreibung

**Fig.S5: *dnox* RNAi knockdown with alternative effector lines *dnox*R1 (BL32433) or *dsod3*R1 (BL8760)** (Extension to Fig.4 and Fig.5 in the main document; explanation is given there)

**Ein Bild, das Screenshot, Farbigkeit, Grün enthält.

Automatisch generierte Beschreibung**

**Fig.S6: Chorion dysmorphology in dnox downregulated ovaries using alternative effector lines**

**A-C** driver line BL36287 (GR1-Gal4) **B-F** driver line BL50286 (47A04Gal4). White arrowheads point to dorsal appendages of the chorion, unfilled white arrowheads point to the impacted yolk structure of mature egg chambers after dnox knockdown. Scale bars 100 µm. Excitation at 470 nm, GFP-specific emission filter.

We used alternative ovarian specific driver lines to test if they induce chorionic dysmorphologies. dnox knockdown by BL36287 (GR1-Gal4) (Fig.S6, B, C) revealed differences in intrinsic fluorescence compared to control (without RNAi induction) (Fig.S6, A). Furthermore, structural differences of the yolk can be seen. In contrast, dnox knockdown using driver line BL50286 (47A04Gal4) (Fig.S6, E, F), which was used in previous studies of [28], showed no differences of the intrinsic fluorescence or the yolk structure compared to control (Fig.S6, D).