Supplementary information:

Methods

Drosophila stocks:

We used *5015-Gal4, btl-Gal4* (Ohshiro & Saigo, 1997), *Lsp2-Gal4* (Cherbas et al, 2003), *A58-Gal4* (Galko & Krasnow, 2004), *Dilp2-Gal4* (Broughton et al, 2010), *Antp-Gal4* (Mandal et al, 2007), *Ser-Gal4*, *hand-Gal4* (Han et al, 2006), *c127-Gal4* (Hewes et al, 2003), *ap-Gal4* (Rincon-Limas et al, 1999), *Hml$^{\Delta}$-Gal4* (Sinenko & Mathey-Prevot, 2004), *dome-Gal4* (Ghiglione et al, 2002), *Dot-Gal4* (Kimbrell et al, 2002) to express target genes in corresponding larval tissue. *UAS-RNAi* lines were obtained from the Vienna *Drosophila* RNAi Center (Austria) and the *Drosophila* Genetic Resource Center (Japan). *UAS-s.spi* and *UAS-vn* were gifts from A. Simcox. *UAS-Egfr^{DN}, UAS-Cat, UAS-SOD2, UAS-Foxo, S^{1}* mutant stocks were obtained from Bloomington Drosophila Stock Center. *UAS-Egfr$^{\lambda TOP}$* was a gift from T. Schupbach (Queenan et al, 1997). The recombineered gene line (*gRho1-YGP*) was kindly provided by B.-Z. Shilo (Yogev et al, 2010).

Immunocytochemistry and cell counts:

Antibodies against Antennapedia (Antp, 4C3), L1(Honti et al, 2010) and Prophenoloxidase (PPO)(Muller et al, 1999) were used to identify corresponding cell lineages. Nuclear DNA was visualized with the ToPro-3 dye (Invitrogen). EGFR activation was monitored with the use of anti-diphosphorylated Erk kinase (dpERK) antibody (Sigma-Aldrich, Inc). Cy3-labeled anti-mouse and -rabbit secondary antibodies were obtained from Jackson ImmunoResearch Laboratories, Inc. Hemocyte immunostaining was performed as previously described, with the use of Cy3-labeled secondary antibodies (Jackson ImmunoResearch Laboratories) (Sinenko et al, 2004). Briefly, hemocytes from single larvae were isolated in 30µl of Schneider's medium into

14-well glass slides (Fisher Scientific Inc, USA) and incubated for 30 min and fixed with 4% formaldehyde (Polysciences, Inc) for 10 min. Glass wells were washed once and processed for immunostaining. After immunostaining absolute numbers of lamellocytes or crystal cells from different mutant backgrounds were counted for each sample with use of a Zeiss Axioskop 2 plus Fluorescence Stereomicroscope (20X lenses). Larval lymph gland immunostaining and ROS staining was performed as previously described (Jung et al, 2005; Owusu-Ansah & Banerjee, 2009; Owusu-Ansah et al, 2008). For determination of reactive oxygen species we used the superoxide specific dye dihydroethdium (DHE, Invitrogen) and the H_2O_2 sensitive dye 2,3,4,5,6 pentafluorodihydrotetramethylrosamine (Redox Sensor red CC-1, Invitrogen)(Kang et al, 2003). Cell and tissue samples were imaged using a BioRad Radiance 2000 and Zeiss LSM700 confocal microscopes.

Supplementary Fig 1. ETC-mediated oxidative stress in the PSC induces differentiation of lamellocytes

A) Oxidative stress induced by inactivation of *ND75 (ND75^{RNAi})* in hematopoietic niche PSC cells (*Antp-Gal4*) causes robust increase in differentiation of lamellocytes in circulation, n = 14. However oxidative stress induced in cells of MZ (*dome-Gal4*), dorsal vessel (*hand-Gal4*), neuronal (*c127-Gal4*), endocrinal (*5015-Gal4*), wing imaginal (*ap-Gal4*), tracheal (*btl-Gal4*) epidermal (*A58-Gal4*) or fat body tissue (*LSP2-Gal4*) has no effect on the differentiation of circulating lamellocytes, n = 10 for each experiment. Corresponding genotypes are indicated under X-axis. All data represent the mean ±s.d.

B) Mitochondria mediated oxidative stress caused by impaired function of components of electron transport chain in the PSC induces differentiation of lamellocytes.

In wild-type (+) larvae lamellocytes do not develop, n = 14. Oxidative stress induced by inactivation of *ND75* (n = 14), *PDSW* (n = 14), *CoVa* (n = 14) and *Marf* (n = 11) with corresponding *RNAi* constructs in the PSC causes lamellocyte differentiation in circulation.

C) Oxidative stress in the PSC cells (*Antp-Gal4, UAS-ND75^{RNAi}*) has no significant effect on differentiation of crystal cells in the larval circulation ($p = 0.36$, $n \geq 10$).

D) Oxidative stress in the PSC cells (*HHLT-Gal4, UAS-ND75^{RNAi}*) has no significant effect on total hemocytes number in larval circulation ($p > 0.42$, $n \geq 10$, vast majority of the circulating hemocytes (~95%) are plasmatocytes).

E) Induction of apoptosis in PSC cells by overexpression of the Hid and Reaper (Rpr) pro-apoptotic proteins does not affect lamellocyte differentiation in larval circulation (*Antp-Gal4, UAS-Hid, Rpr*, n =14) compared with oxidatively challenged PSC (*Antp-Gal4, UAS-ND75^{RNAi}*, n =14).

Supplementary Fig 2. ETC-mediated oxidative stress induces activation of FoxO in the PSC cells.

(A) Inactivation of *ND75* in PSC (+) causes significant increase in lamellocyte numbers, n =14. Inactivation of JNK pathway by overexpression of dominant negative form of *basket* (*bsk^{DN}*) or activation of the pathway by overexpression of *misshapen* (*msn*) in the *ND75* deficient PSC does not significantly affect the lamellocyte response ($p = 0.14$, n =10 and $p = 0.18$, n =17, respectively). All error bars shown represent standard deviation from the mean.

(B-B') In wild type, *4E-BP-LacZ* (*Antp-Gal4, UAS-GFP; 4E-BP-lacZ, red*) is not active in PSC cells.

(C-C') Inactivation of *ND75* (*Antp-Gal4, UAS-GFP, UAS-ND75^{RNAi}*; *4E-BP-LacZ*,) in PSC cells causes robust up-regulation of *4E-BP* expression (red).

D) Non-stressed conditions do not cause lamellocyte response by further ROS reduction in the PSC.

In wild type (*Antp-Gal4*/+), lamellocytes are not seen in circulation, n = 14.

Inactivation of *ND75* in the PSC (*Antp-Gal4, UAS-ND75*^{ND75i}), which would raise ROS causes significant increase in lamellocyte numbers, n = 14.

However, overexpression of SOD2 (n = 10), Foxo (n = 14), *Foxo*$^{RNA\ i}$(n = 11), Akt1(n = 12) or *Akt1*^{RNAi} (n = 10) in the PSC has no significant effect on lamellocyte differentiation in circulation ($p > 0.5$) as ROS in the PSC remains low.

Supplementary Fig 3. JAK/STAT ligands, *upd*, *upd2*, *upd3*, and JNK ligand, *egr* are not required in the PSC for the lamellocyte response.

A) Inactivation of *ND75* in PSC (+) causes significant increase in lamellocyte numbers. Overexpression of *RNAi* of *upd3* (n = 14), *upd* (n = 10), *upd2* (n = 12) or *egr* (n = 9) in the *ND75* deficient PSC does not significantly suppress the lamellocyte differentiation in the circulation ($p > 0.4$). All error bars shown represent standard deviation from the mean.

(B,C) Lymph gland is normal in *Egfr*^{TS}/*Egfr*^{18} mutants.

B) In wild-type 3rd instar larval lymph glands there are distinct cellular zones; the medullary zone populated with prohemocytes (blue) and the cortical zone where differentiated hemocytes, expressing Pxn (red) and PPO (green) reside.

C) Lymph glands are unaffected in *Egfr*^{TS}/*Egfr*^{18} mutants raised at nonpermissive temperature (29ºC) with proper zonation as revealed by cortical zone markers Pxn (red) and PPO (green).

Supplementary Fig 4. Wasp triggered immune challenge induces high levels of ROS in the PSC.

(A-A') Under normal growth conditions PSC cells (marked by *Antp-Gal4, UAS-GFP*, green in A') are largely negative for hydrogen peroxide sensitive dye (RedoxSensor, red in A).

(B-B') Hydrogen peroxide levels (red in B) are significantly increased in the PSC cells (green in B') after 12 hours of wasp infestation. The increase in peroxide levels

(C) Lamellocytes rarely develop in wild-type (*Antp*/+, n = 12), or when SOD2 (n = 10), Catalase (n = 10) or GPx (n = 10) are overexpressed in the PSC using *Antp-Gal4*.

Large numbers of lamellocytes develop in wild-type larvae infested by wasp (*Antp/+ (wasp)*, n = 12). Overexpression of SOD2 (*Antp-Gal4, UAS-SOD2 (wasp)*, n = 15) or Cat (*Antp-Gal4, UAS-Cat (wasp)*, n = 19) in the PSC results in a suppression of lamellocyte production in the infested mutant larvae ($p < 0.0001$). However overexpression GPx (*Antp-Gal4, UAS-GPx (wasp)*) in the PSC does not significantly suppress the lamellocyte production in the infected mutant larvae ($p > 0.17$, n = 10).

(D-D') Under normal growth conditions PSC cells (marked by *Antp-Gal4, UAS-GFP*, green outlined) are largely negative for ROS (red).

(E-E') ROS levels (red) are significantly increased in the PSC cells after wasp infestation.

(F-F') Inactivation of *spi* in the PSC (*Antp-Gal4, UAS-spi*^{RNAi}) does not affect ROS levels (red) in the Antp^{+} PSC cells (green) upon wasp infestation.

(G) In wild-type larvae (WT) 80% of L1^{+} cells are *Hml*$^{+}$ (light blue) and about 20% are *Hml*$^{-}$ (dark blue), n = 10. When Egfr^{DN} is expressed using lineage traced *Hml*$^{\Delta}$*-Gal4* as a driver (*HLT Gal4/UAS-Egfr*^{DN}), the *Hml*$^{+}$ L1^{+} population (light blue) is essentially eliminated, n = 11.

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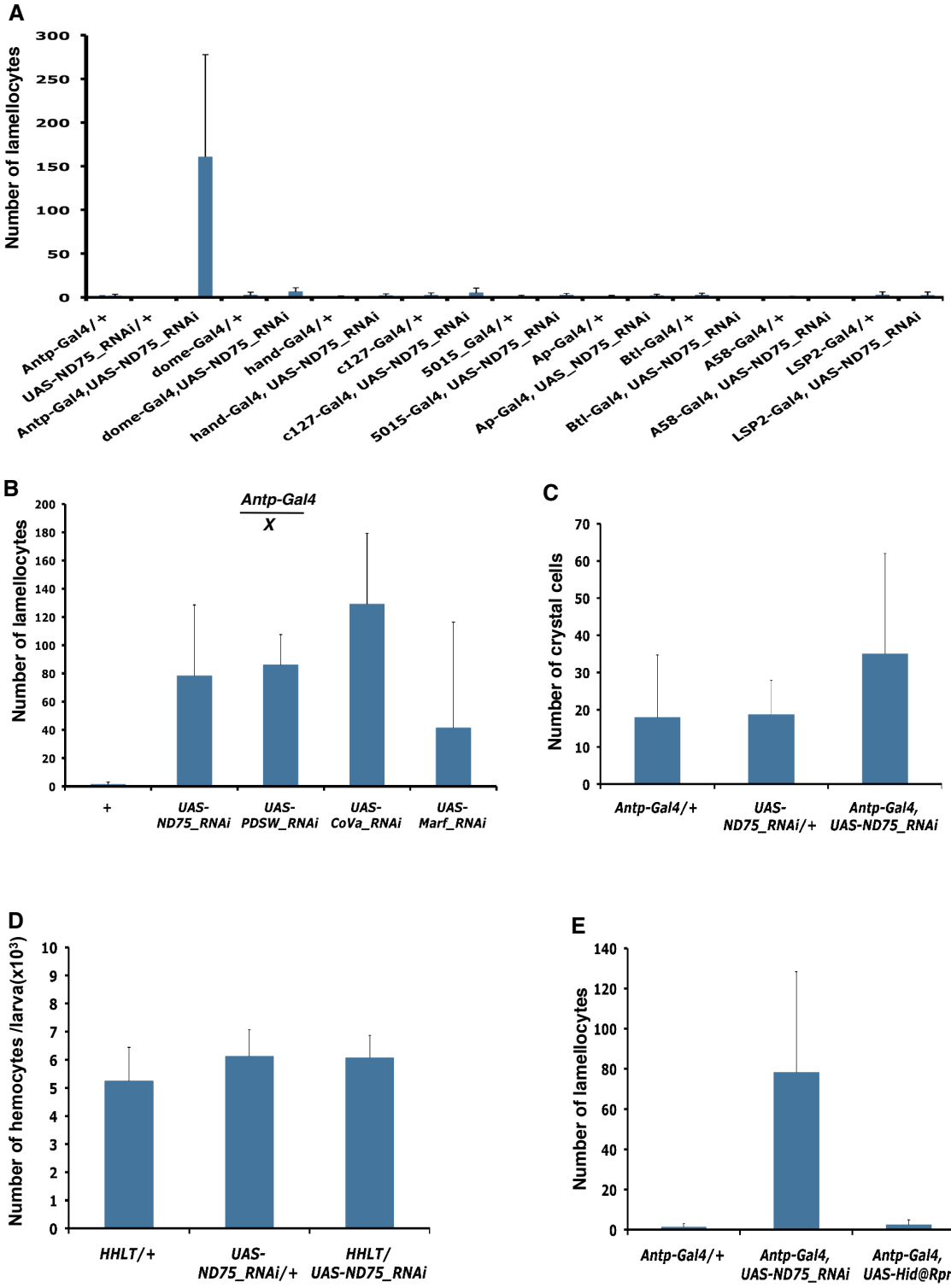
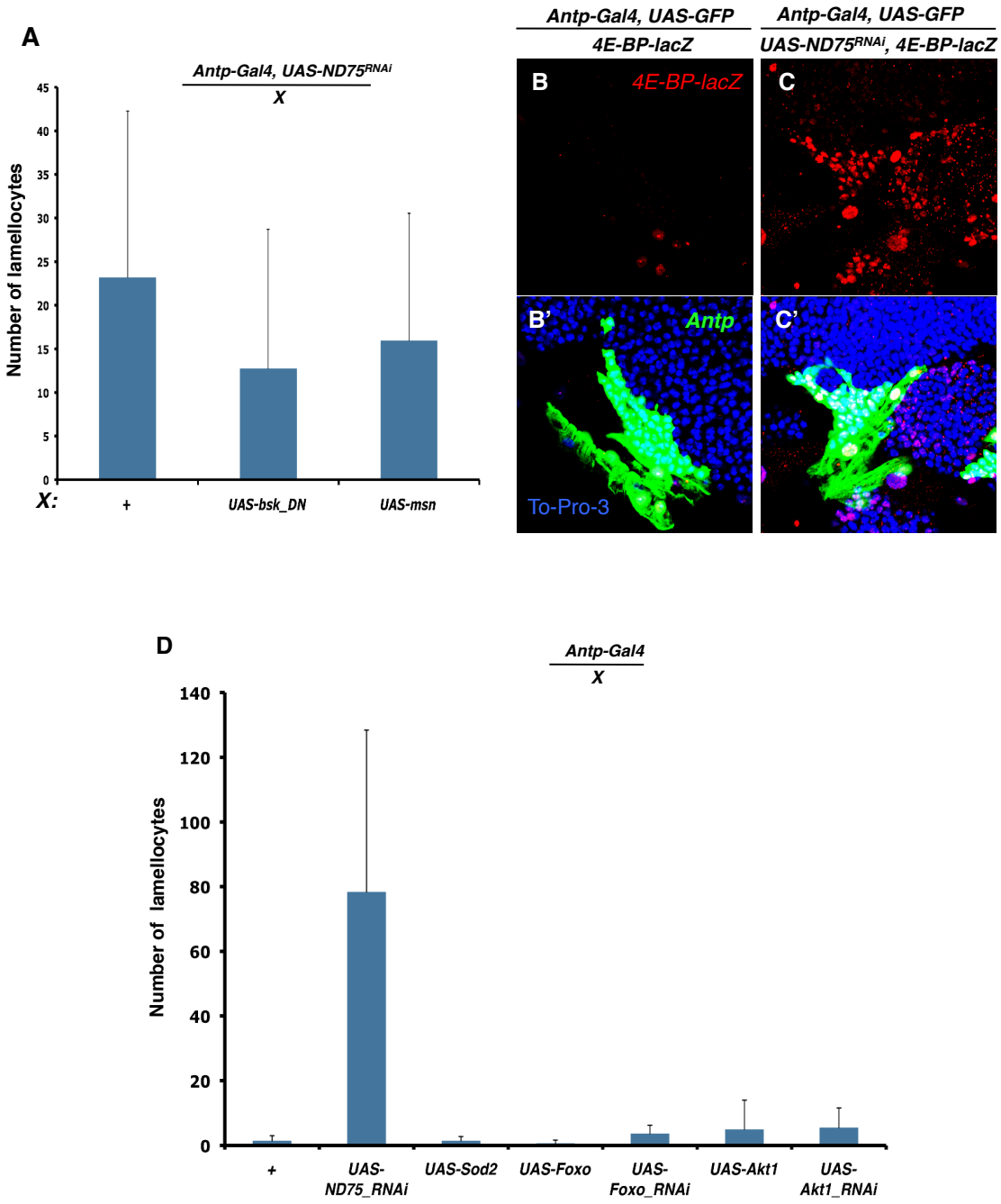


Supplementary Figure 1



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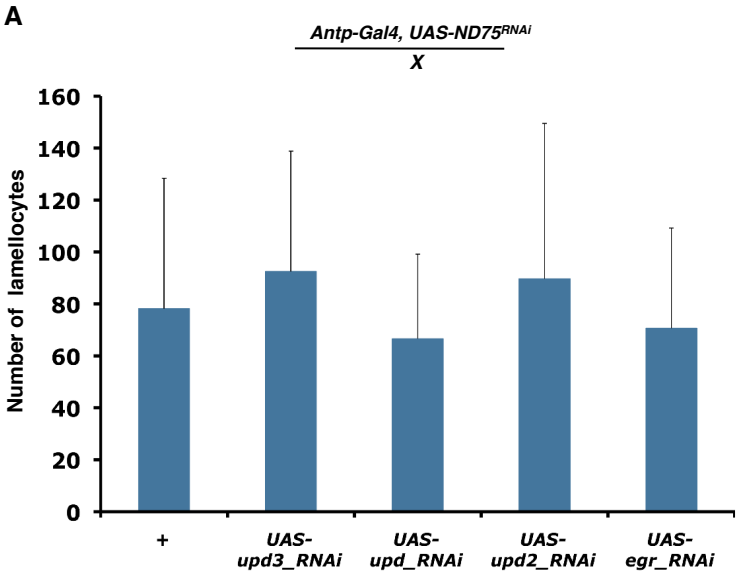
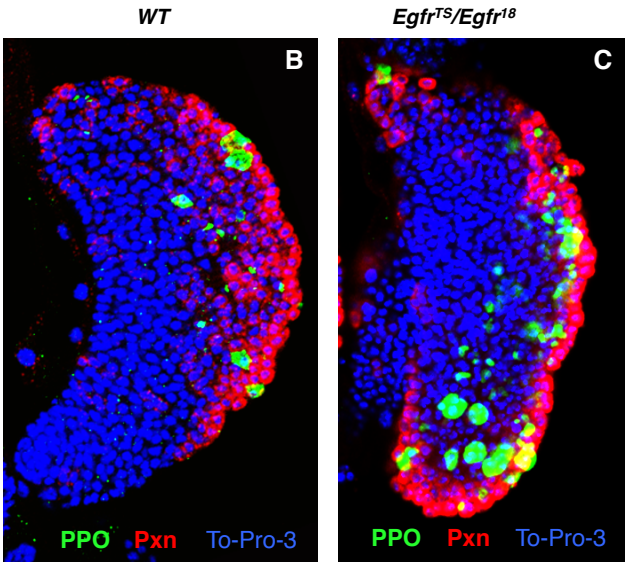
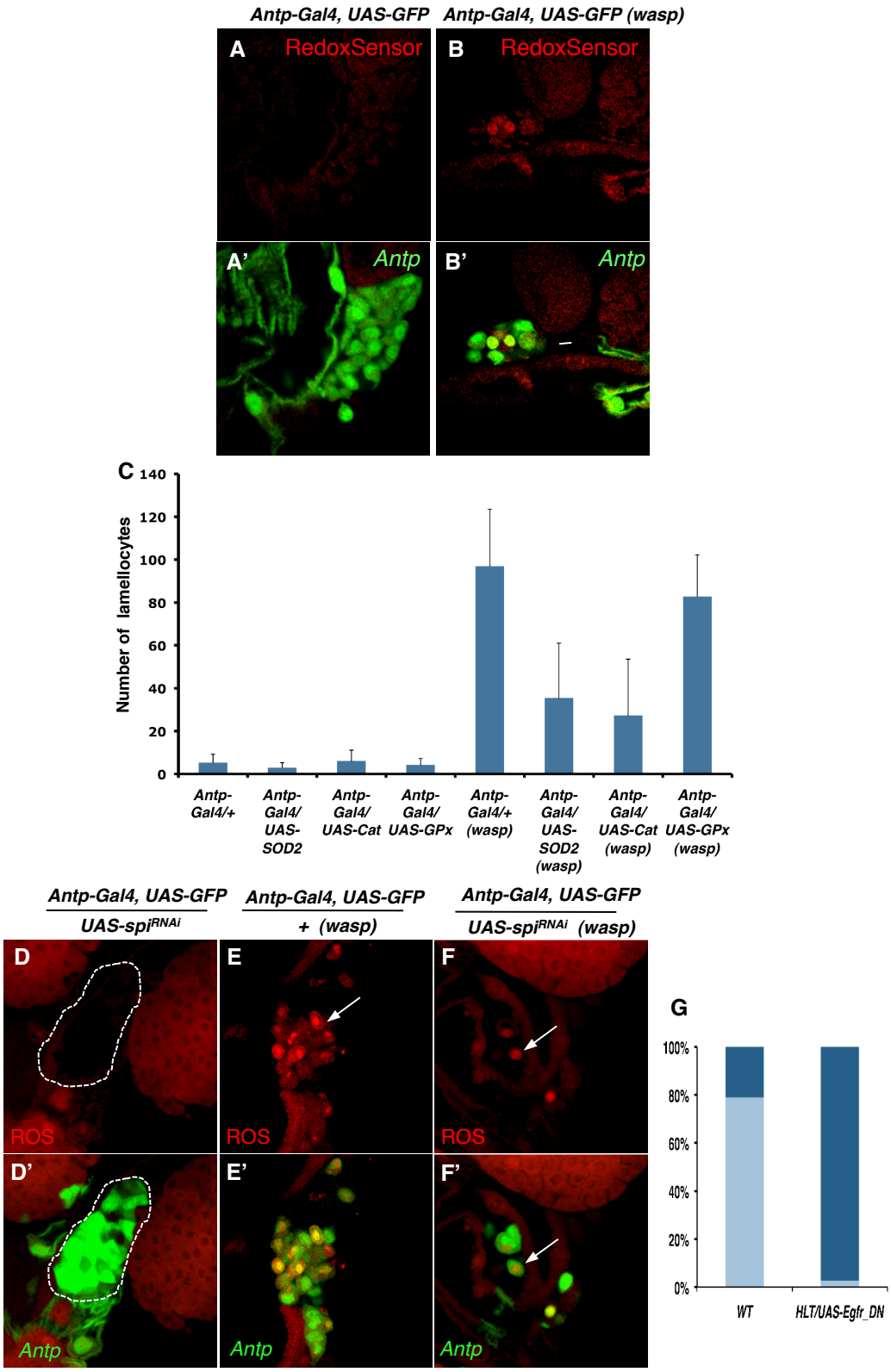




Supplementary Figure 3



Supplementary Figure 4