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Supplementary Data

Role of Mitochondrial Remodeling in Programmed Cell Death in *Drosophila melanogaster*

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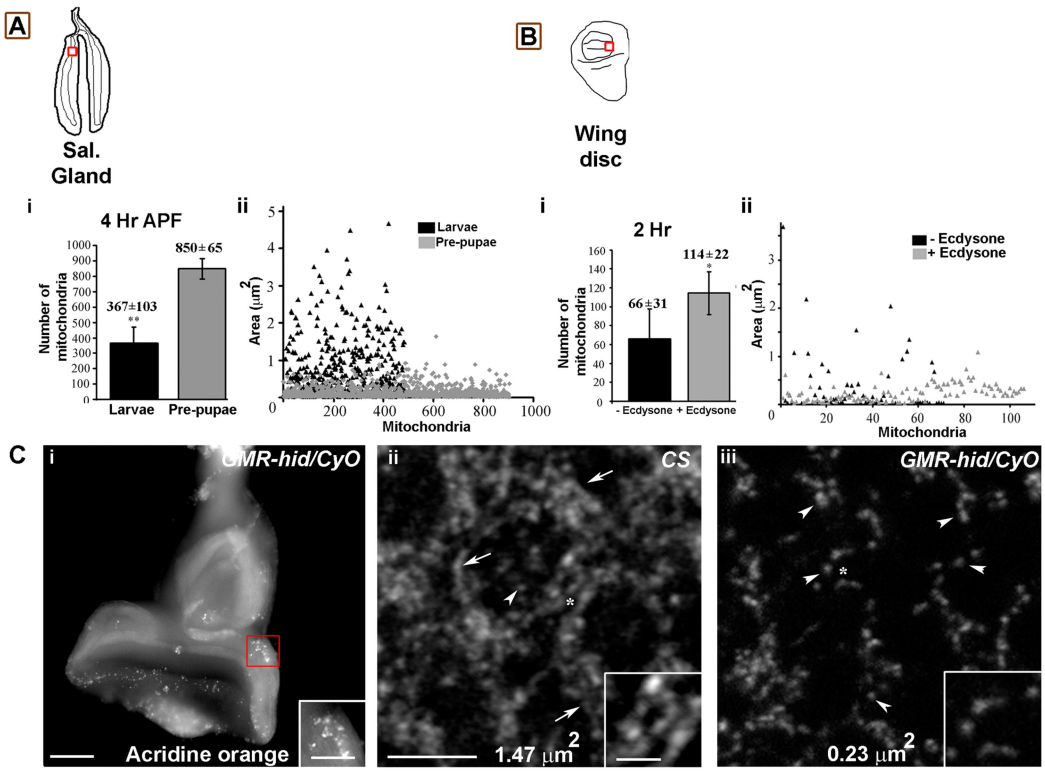
Supplementary Data for Goyal G et al. includes I) Supplementary Figures 1-8 and II) Supplementary movie 1.

I: Supplementary Figures

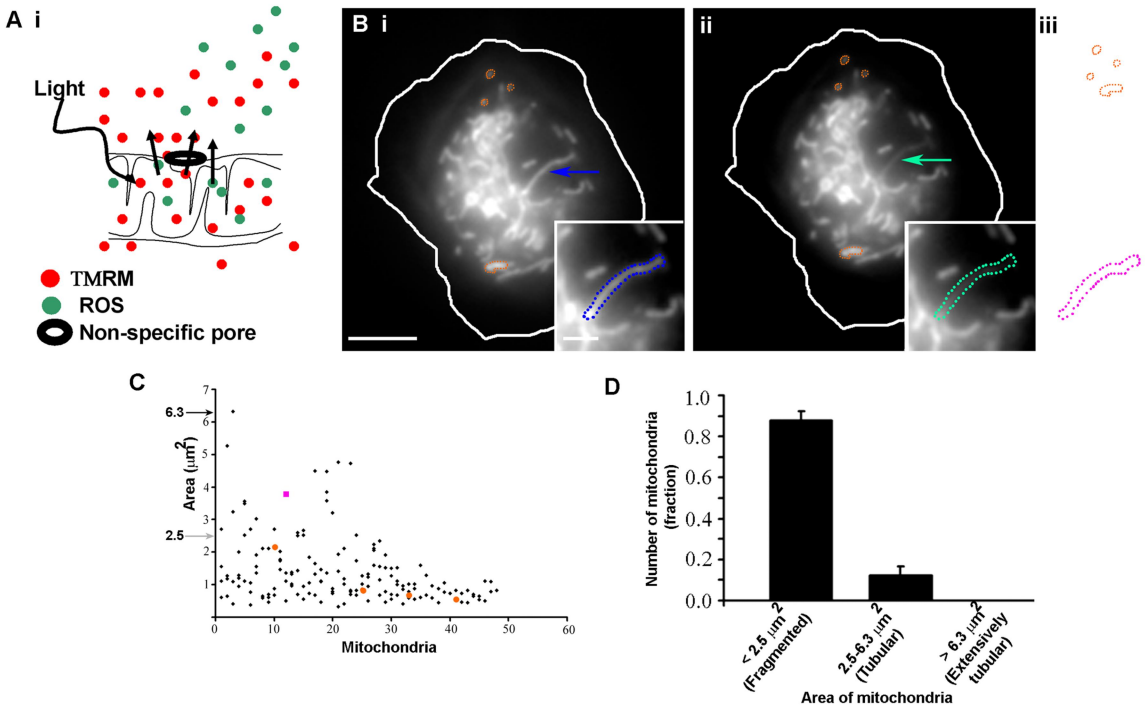


Sup. Fig 1

Mitochondria underwent fragmentation during PCD

A i) Histogram showing mitochondrial number per cell (mean ± SD, 5 cells) in larval (Black) or pre-pupal (4 Hr APF, Gray) salivary gland in a representative experiment (n=3). ii) Scatter plot showing the mitochondrial cross sectional area (CSA) in a representative 3rd instar larval (Black) or pre-pupal (4 Hr APF, Gray) salivary gland cell (n=5). ~1 % salivary gland mitochondria in pre-pupae had CSA >1 μm^2 as compared to ~20 % mitochondria in 3rd instar larvae. B i) Histogram showing number of mitochondria per cell (mean ± SD) in mock (Black) or ecdysone (Gray) treated wing disc cells (n=5) in a representative experiment (n=3). ii) Scatter plot showing mitochondrial CSA in a representative mock (Black) or ecdysone (Gray) treated wing disc cell (n=5). ~8 % mitochondria in ecdysone treated wing imaginal disc cells had CSA >0.5 μm^2 compared

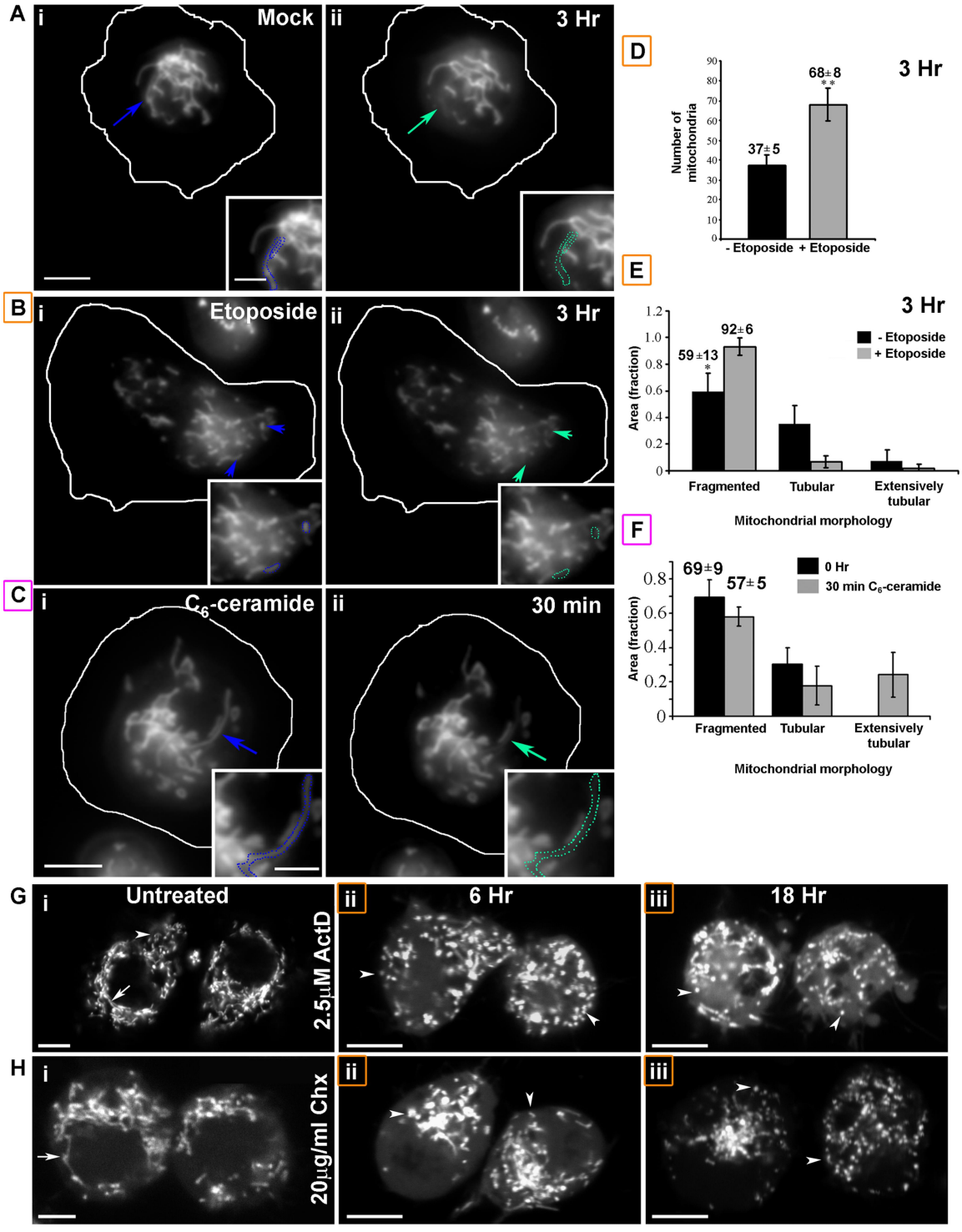
to ~21 % mitochondria in untreated controls. P values- * <0.05, ** <0.005. C i) Acridine orange positive cells in *GMR-hid/CyO* eye disc. The box indicates the region shown in ii and iii. Single plane reconstruction of the confocal images of mitochondria (anti-Biotin staining) in *GMR-hid/CyO* (iii) or *CS* (ii) eye disc cells. Mitochondria (anti-Biotin staining) in *GMR-hid/CyO* (iii) eye disc cells are fragmented as revealed by their highly reduced CSA (~0.2 μm^2) when compared with mitochondrial CSA (~1.5 μm^2) in cells of the same region in a control eye disc. The average mitochondrial CSA is indicated. Arrows and arrow-heads indicate tubular and fragmented mitochondria respectively. Bar-panels: i) 50 µm; inset: 25 µm ii-iii) 5 µm; insets: 2.5 µm.



Sup. Fig 2

Mitochondria are elongated or fragmented compartments in *Drosophila* hemocytes

A) Schematic showing excitation light mediated generation of reactive oxygen species (Green dots) by TMRM (Red dots) loaded in the mitochondrion. This results in opening of non specific pores in the mitochondrial membrane and release of TMRM (Collins et al., 2002). B i-ii) Sequential images from a time lapse movie (Sup. Movie 1) of hemocytes derived from wild-type larvae stained with TMRM dye showing release of TMRM from functionally individual mitochondria. Functionally isolated tubular mitochondria (blue arrow, B i) lost TMRM as observed in the subsequent image (green arrow, B ii). Insets show magnified views of the mitochondria (Blue dotted outline in B i) that lost fluorescence as observed in the subsequent image (Green dotted outline in B ii). Fragmented mitochondria are indicated by an orange dotted outline. B iii) Mitochondria categorized as tubular (pink) or fragmented (orange) as revealed by the dotted outline. The CSA of these mitochondria are indicated as pink or orange data points in C. C) Scatter plot showing the CSA of functionally isolated mitochondria in wild-type hemocytes in an experiment. Mitochondria of CSA less than 2.5μm^2 were categorized as fragmented while mitochondria of CSA between 2.5-6.3μm^2 were categorized as tubular. Mitochondria with CSA greater than 6.3 μm^2 was designated as extensively tubular. D) Histogram showing number of mitochondria that are fragmented, tubular or extensively tubular expressed as a fraction of total number of mitochondria in a cell (n=5). The results shown represent the mean ± SEM obtained from two experiments. Bar - panels: 5 μm; insets: 2.5 μm.

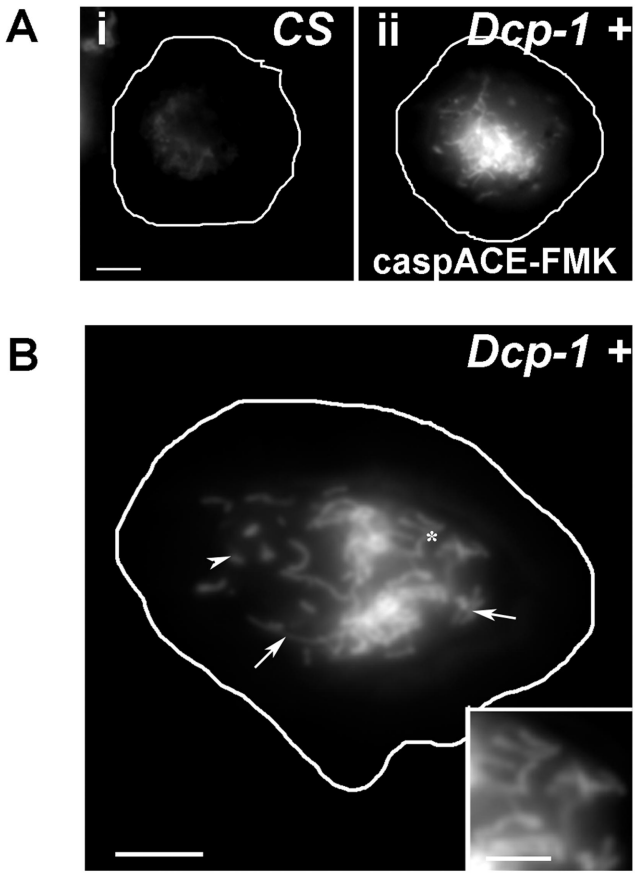


Sup. Fig 3

Mitochondria underwent fragmentation during genotoxic stress mediated cell death

A-C) Sequential images from a time lapse movie of wild-type hemocytes stained with

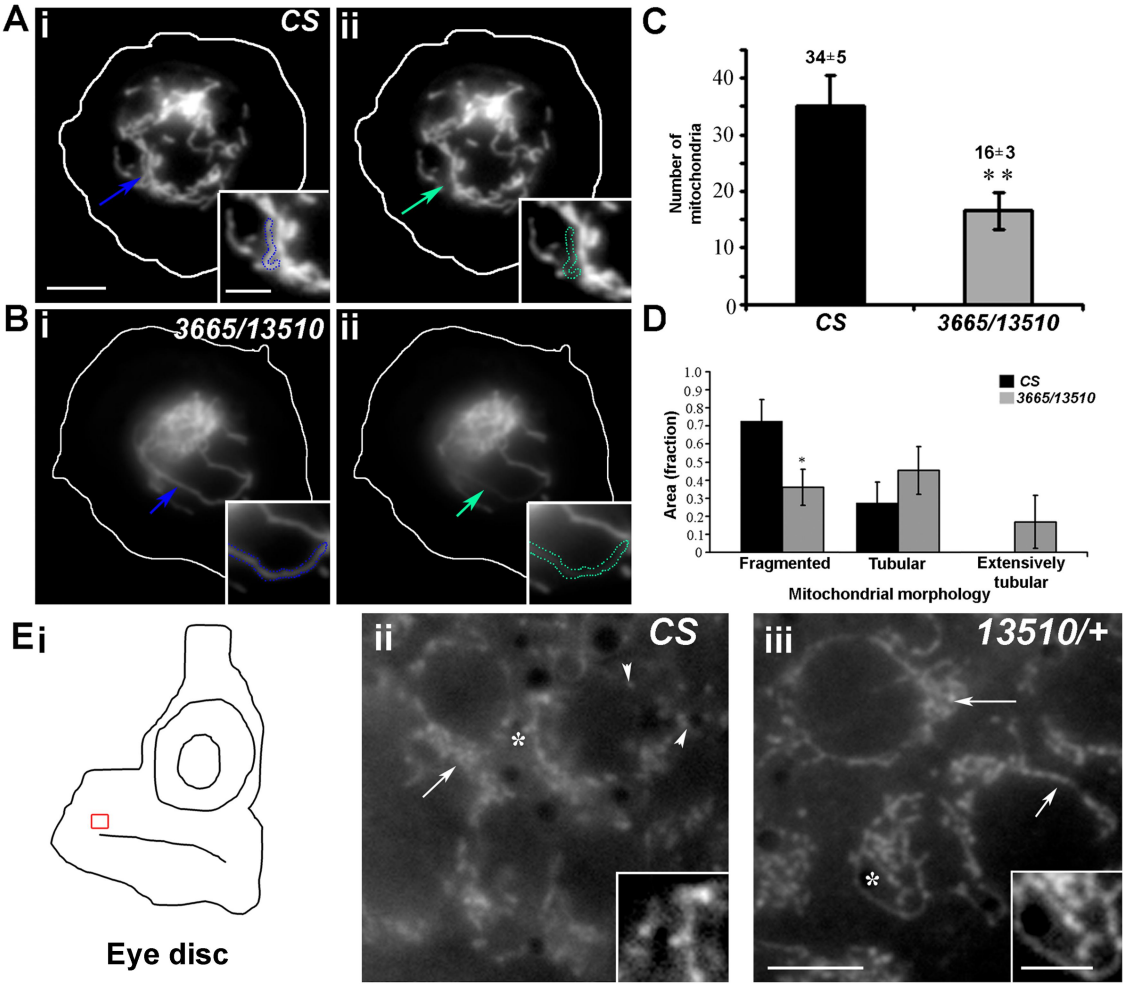
TMRM dye showing loss of TMRM from functionally individual mitochondria in mock (A), etoposide (B) or C_6-ceramide (C) treated cells. Functionally isolated mitochondria (blue arrows, i) lose TMRM as observed in the subsequent image (green arrows, ii). Insets show magnified regions of the mitochondria (blue dotted outline, i) that lose fluorescence as observed in the subsequent image (green dotted outline, ii). D) Histogram showing mitochondrial number in etoposide (Gray) or mock (Black) treated hemocytes at 3 Hr (mean ± SEM; n=2) estimated using TMRM assay (Supple. Fig 2). E, F) Histograms showing fraction of mitochondrial CSA in a mock (E, F, Black), etoposide (E, Gray) or C_6-ceramide (F, Gray) treated cell that is contributed by fragmented, tubular or extensively tubular mitochondria (mean ± SEM; n=2) at indicated times. P values- * <0.05, ** <0.005. There is a significant increase in contribution of fragmented mitochondria (~95 %; E) in etoposide treated cells when compared to mock (~60 %; E) treated cells. In cells incubated with C_6-ceramide for 30 min, there is a significant ($P<0.1$) increase in contribution of tubular or extensively tubular (~40 %; F) mitochondria compared with untreated cells [~25 %; (Black), F] that lack extensively tubular mitochondria. G-H) Mitochondria undergo fragmentation in Mito-YFP transfected S2R+ cells incubated with actinomycin D (G) or cycloheximide (H) for indicated time. Arrows and arrow-heads indicate tubular and fragmented mitochondria respectively. Bar- panels: 5 μm; insets: 2.5 μm.



Sup. Fig 4

Mitochondrial morphology is unaffected by over-expression of the effector caspase Dcp-1

Hemocytes derived from Dp (2;3)P [(Bloomington stock-676; *Dcp-1*+), Aii] or wild-type (Ai) larvae were incubated with the active caspase substrate caspACE-FMK to study the levels of basal caspase activation in these cells. *Dcp-1*+ hemocytes showed increased basal caspase activation (compare Aii with Ai) although the mitochondrial morphology (B) remained unaffected. Arrows and arrow-heads indicate tubular and fragmented mitochondria respectively. Bar- panels: 5 µm; insets: 2.5 µm.

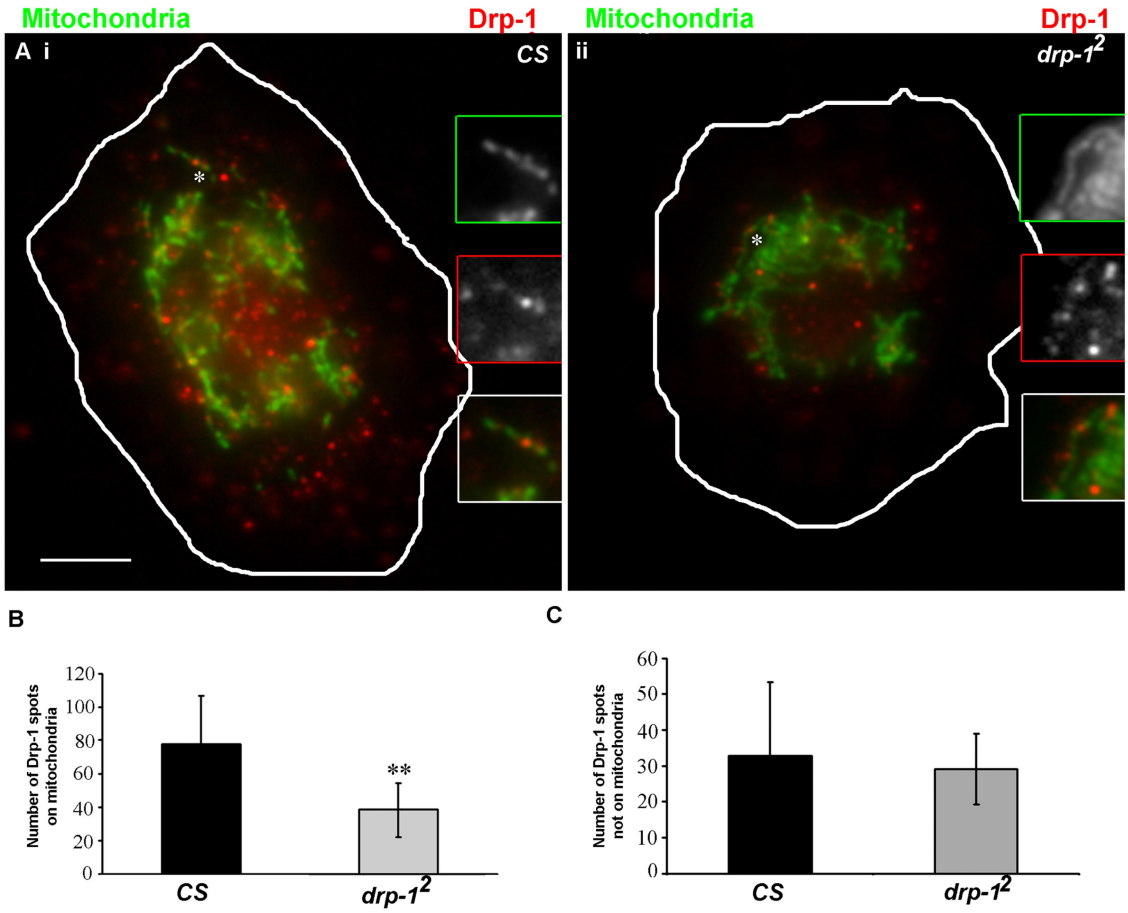


Sup. Fig 5

Mitochondria are extensively tubular in *drp-1* mutant hemocytes and eye disc

A, B) Sequential images from a time lapse movie of hemocytes derived from wild-type (A) and *3665/13510* (B) larvae stained with TMRM dye showing loss of TMRM from functionally individual mitochondria. Functionally isolated mitochondria (blue arrows, i) lose TMRM as observed in the subsequent image (green arrows, ii). Insets show magnified regions of the mitochondria (blue dotted outline, i) that lose fluorescence as observed in the subsequent image (green dotted outline, ii). C) Histogram showing the

mitochondrial number in *3665/13510* (Gray) and wild-type (Black) hemocytes (mean ± SEM; n=3) determined using the TMRM assay (A, B). D) Histogram showing fraction of mitochondrial CSA in a wild-type (Black) or *3665/13510* (Gray) hemocytes, contributed by fragmented, tubular or extensively tubular mitochondria (mean ± SEM; n=3). ~60 % mitochondrial CSA in *3665/13510* hemocytes is tubular or extensively tubular compared to ~25 % mitochondrial CSA in wild-type hemocytes. P values- * <0.05, ** <0.005. E i) Schematic of eye disc showing the area (marked with a red square) imaged in ii and iii. ii-iii) Mitochondrial morphology (anti-Biotin) in wild-type (ii) and *13510/+* (iii) eye disc cells show extensively tubular mitochondria in *13510/+* as compared to CS. Arrows and arrow-heads mark tubular and fragmented mitochondria respectively. Bar- panels: 5 μm; insets: 2.5 μm.

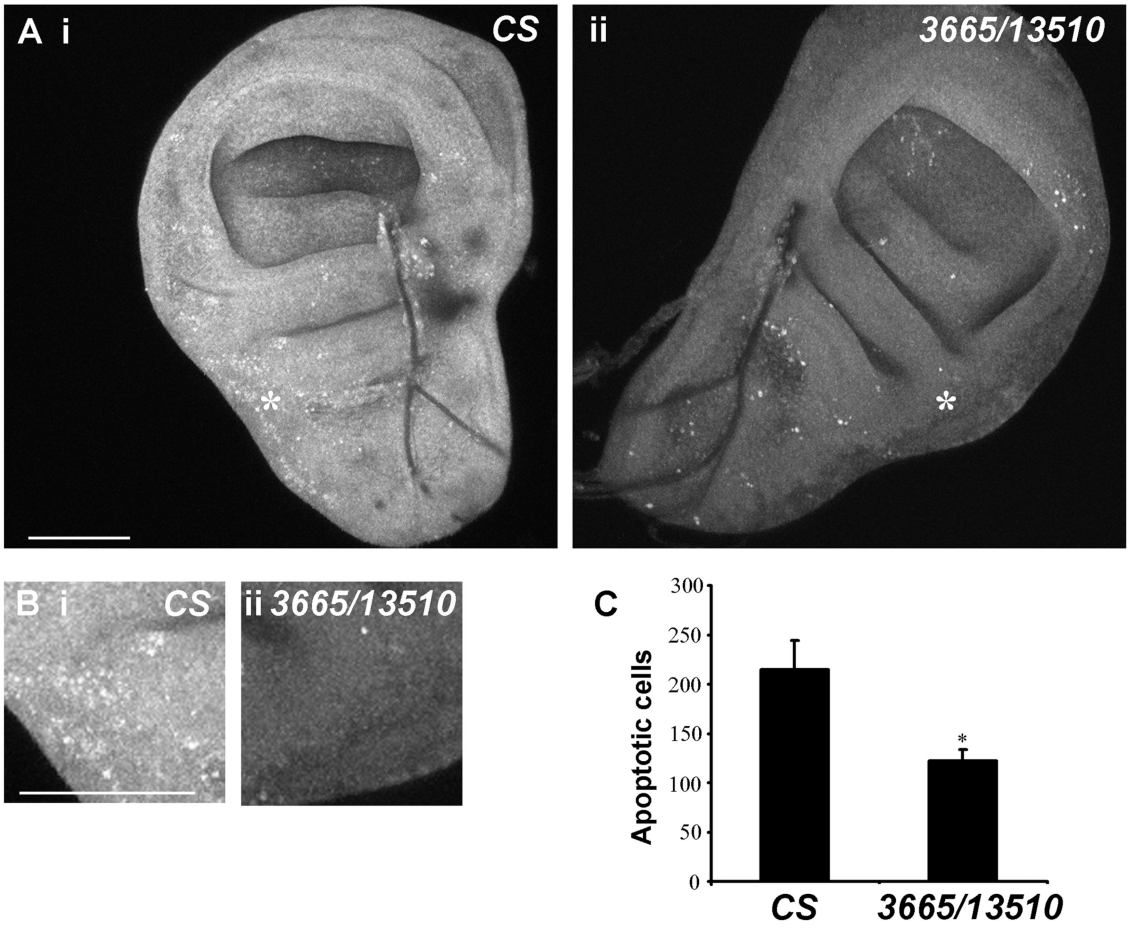


Sup. Fig 6

Mitochondrial localization of Drp-1 is reduced in *drp-1*^{2} hemocytes

A i-ii) Drp-1 localization on mitochondria (anti-Biotin; green) in wild-type (i) and *drp-1*^{2} (ii) hemocytes detected using an antibody generated against *Drosophila* Drp-1 (red). The number of Drp-1 (red) spots on mitochondria (green) was quantified. B) Histogram showing significantly reduced number of Drp-1 spots on mitochondria in *drp-1*^{2} (Gray) cells when compared with wild-type (Black) cells. C) Histogram showing the number of Drp-1 spots in wild-type (Black) and *drp-1*^{2} (Gray) hemocytes in regions excluding the mitochondria. Results shown represent the mean ± SD obtained from a representative

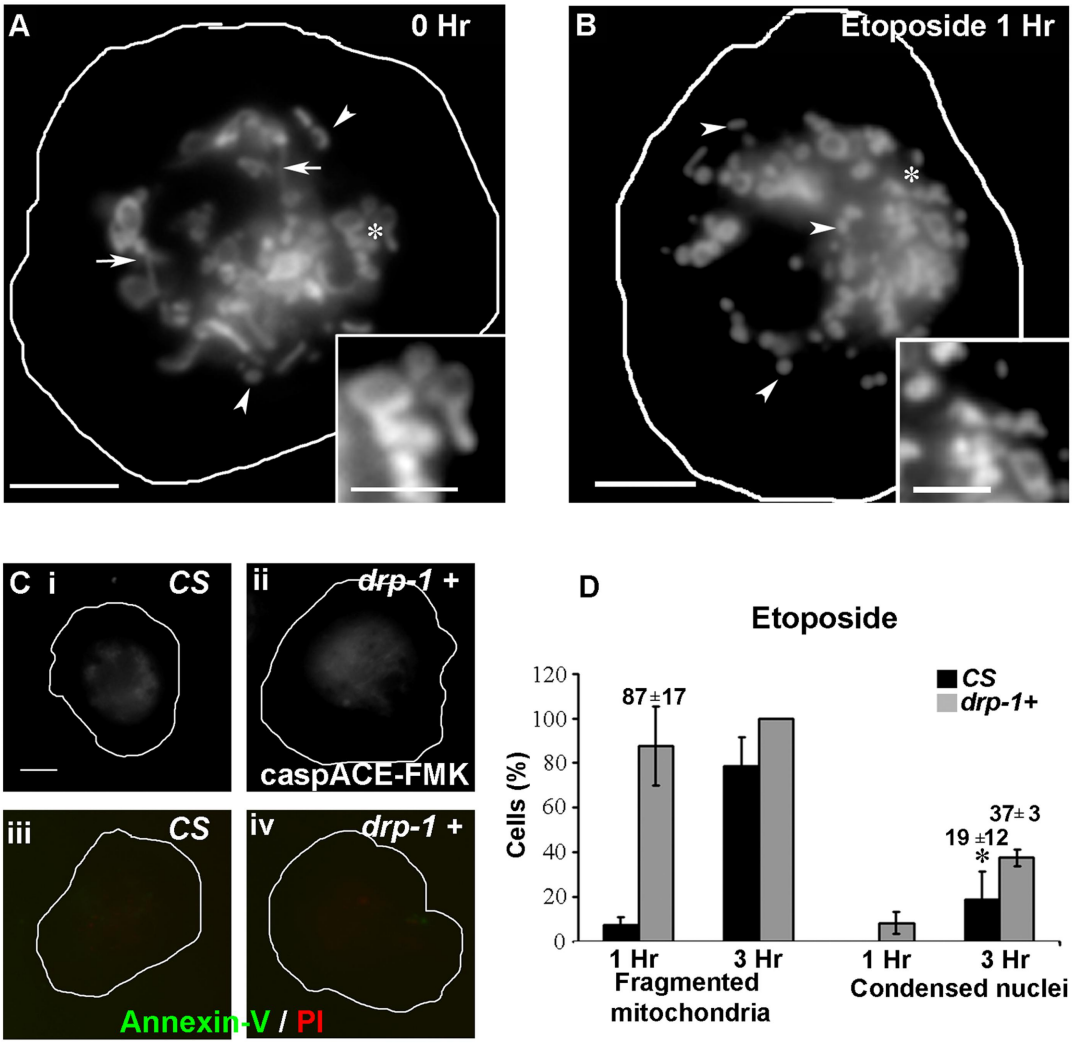
experiment (n=2). Bar- panels: 5 µm; insets: 2.5 µm. P value- ** <0.005.



Sup. Fig 7

***drp-1* mutants display protection to UV-B induced apoptosis**

A i-ii) Apoptotic cells [Acridine orange (3 µg/ml)] in UV-B exposed wild-type (i) or *3665/13510* (ii) wing discs. B) Magnified view of regions marked with an asterisk in A. Bar- 100 µm (A), 50 µm (B). C) Histogram showing two fold decrease in number of apoptotic cells per *3665/13510* wing disc when compared with wild-type disc (n=4; mean ± SD) in a representative experiment (n=5). P values- * <0.05.



Sup. Fig 8

Etoposide induced cell death is enhanced in hemocytes over-expressing Drp-1

A-B) Mitochondrial morphology (Mitotracker Green) in mock (A) or etoposide (B) treated Drp-1 over-expressing Dp (2;1) JS13 (*drp-1*+) hemocytes. Arrows and arrow-heads indicate tubular and fragmented mitochondria respectively. Insets show magnified view of region marked with an asterisk. C) Untreated wild-type (i, iii) or *drp-1*+ (ii, iv) hemocytes stained for active caspases (i, ii) or AnV (green) and PI (red) (iii, iv) show that

mitochondrial fragmentation in *drp-1*+ cells is not sufficient to induce cell death. D) Histogram showing fraction of etoposide treated wild-type (Black) or *drp-1*+ (Gray) hemocytes (n=100) with apoptotic nuclei or fragmented mitochondria at the indicated times (mean ± SD; n=2). P value- *<0.1. Bar- panels: 5 µm; insets: 2.5 µm.

II: Supplementary Movie legend

Sup. Movie 1

Spontaneous mitochondrial depolarization induced loss of TMRM from mitochondria in wild-type hemocytes

Spontaneous mitochondrial depolarization induced release of TMRM from mitochondria in wild type cells. Images were acquired every 1 sec as revealed by elapsed time that is indicated at top left corner of the movie played 6 fps. The dynamics of loss of TMRM from mitochondria is depicted 6 times faster than what was observed in the cell.