SUPPLEMENTAL MATERIAL

Detailed Methods:

Drosophila Heart Studies. Optical coherence tomography (OCT) was used to image heart tubes of one and four week adult flies on a Michelson Diagnostics (Maidstone, UK) EX 1301 OCT microscope as described previously (1, 2). Image J was used to analyze B-mode images to measure the internal chamber diameter at end-systole (ESD) and end diastole (EDD). % Fractional Shortening (FS) was calculated as (EDD-ESD/EDD).

Phasic calcium transients were measured by expressing UAS-GCaMP3.0 in fly cardiomyocytes and recording changes in the fluorescence intensities using Nikon AZ100 UV fluorescent microscope as described previously (3). One week old adult flies were dissected to expose the heart tube while still attached to the dorsal shell. This preparation was maintained in artificial haemolymph at 18°C during the recording. When measuring caffeine-induced Ca^{2+} release EGTA (8mM/L) was used to stop the heart immediately prior to addition of caffeine (10mM/L) (Sigma).

Confocal and Electron Microscopy. Fly heart tubes were dissected and mounted in haemolymph for live confocal imaging. A Nikon Eclipse Ti confocal system or Carl-Zeiss LSM510-Meta Laser Scanning confocal Microscope were used to image mitochondria in *Drosophila* cardiomyocytes expressing Tinc Δ4-Gal4 driven UAS-mitoGFP or mito-DSRed. Mitochondrial dimensions were measured using Image J.

UAS-GFP-cb5 and Tinc-UAS-DSRed were crossed to Park KO/+ to observe the sarcoplasmic reticulum structure (1).

Rhodamine 123 fluorescence was used to assess mitochondrial membrane potential. Heart tubes were dissected and incubated in Rhodamine 123 (25μM) (Sigma) for 20 min, and then washed for 10 min in phosphate-buffered saline (PBS) prior to confocal imaging.

To assess cardiomyocyte mitochondrial ROS production, dissected heart tubes were incubated in MitoSOX (2.5mM) (Life Technologies) in PBS for 20 minutes at 25°C, washed for 10 min with PBS, and visualized by confocal fluorescent microscopy.

For *Drosophila* heart tube anti-DNA immunostaining confocal fluorescence studies were performed on freshly isolated drosophila heart tubes. Briefly, fresh dissected heart tubes were fixed with 4%PFA for 5 minutes, and then permeabilized with 1% Triton X-100 for ten minutes. DNA was visualized using mouse monoclonal anti-dsDNA (1:200, Abcam) and Alexa Fluor 546-labeled goat anti-mouse IgG (1:500, Invitrogen).

For EM studies *Drosophila* heart tubes were fixed in a 2.5% solution of glutaraldehyde (4% PFA, 0.1M cacodylate, and 2.5% glutaraldehyde) (Electron Microscopy Sciences, Hatfield, CA), sequentially stained with osmium tetroxide and uranyl acetate, dehydrated, and embedded in Polybed 812. Tissue was thin sectioned on a Reichert-Jung Ultra-Cut (90nm thick), post stained in uranyl acetate and lead citrate, and viewed on a Jeol electron microscope (JEM-1400) at 5,000 direct magnifications (JEOL, Tokyo, Japan).

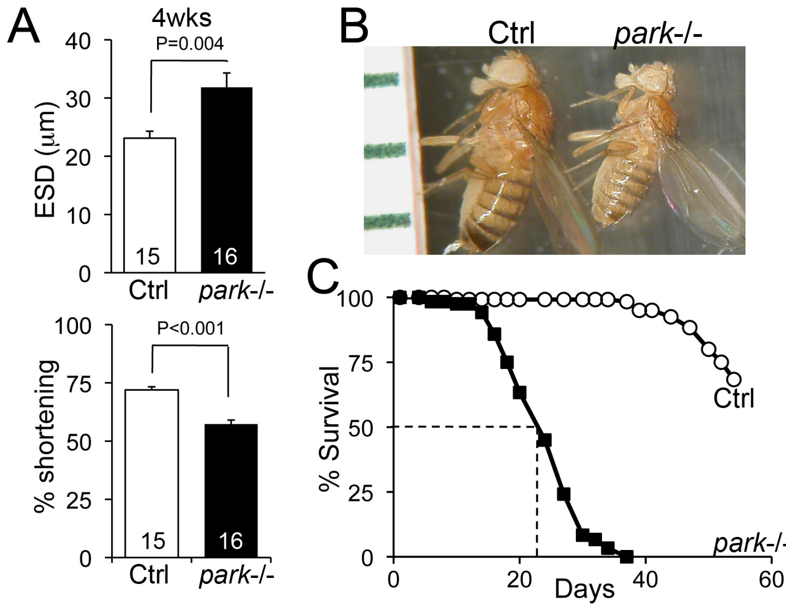
Longevity and Climbing Studies. For life span analysis, 150 flies of each genotype were collected within one day of hatching and placed in vials in groups of 25 each. Numbers of dead flies were counted every 2-3 days and surviving flies were flipped into new food vials. Survival curves were compared by Kaplan-Meier analysis and log–rank test.

For assessment of exercise capacity by negative geotaxis, freshly emerged 150 adult flies of each genotype were collected and maintained in 12” X 3.5” clear plastic containers. To examine the climbing ability the flies were displaced to the bottom of the container by quick firm tapping impact and the number of flies that had climbed greater than 10cm within 10 sec was determined daily (2). The climbing curves were compared by Kaplan-Meier analysis and log–rank test.

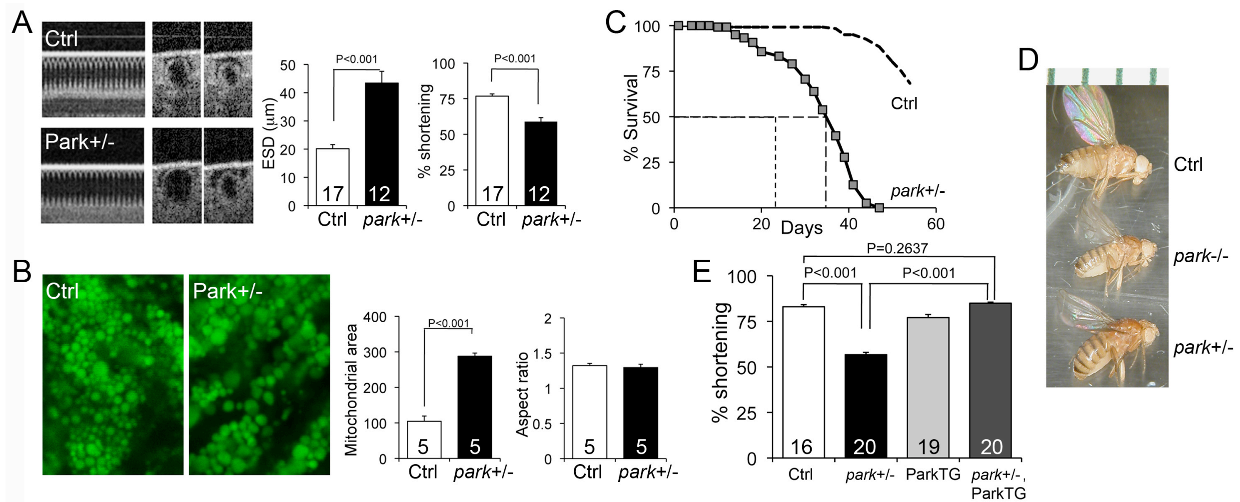
Molecular Biology. For RT-PCR, the total RNA samples were prepared from the whole fly homogenates of parkin -/-, parkin-/+, mef-Gal4>park KD2, mef>UAS park, mef1/+ strains using Trizol. 1 μg of RNA was used to prepare cDNA by random priming and reverse transcription. Primers for real-time qPCR of Parkin are 5'- TAAGCGATGCCACGACAATA and 3'- GCTAAGCGAAGGTTCCTCCT using SYBR green PCR Master Mix (Applied Biosystems).

Statistical methods. Microsoft Excel and Sigma Plot were used to analyze numerical data and generate graphs. Student's t-test (two groups), ANOVA (multiple groups), or log rank test (longitudinal data) were applied as appropriate. Data are presented as mean ± SEM. $P<0.05$ was considered significant.

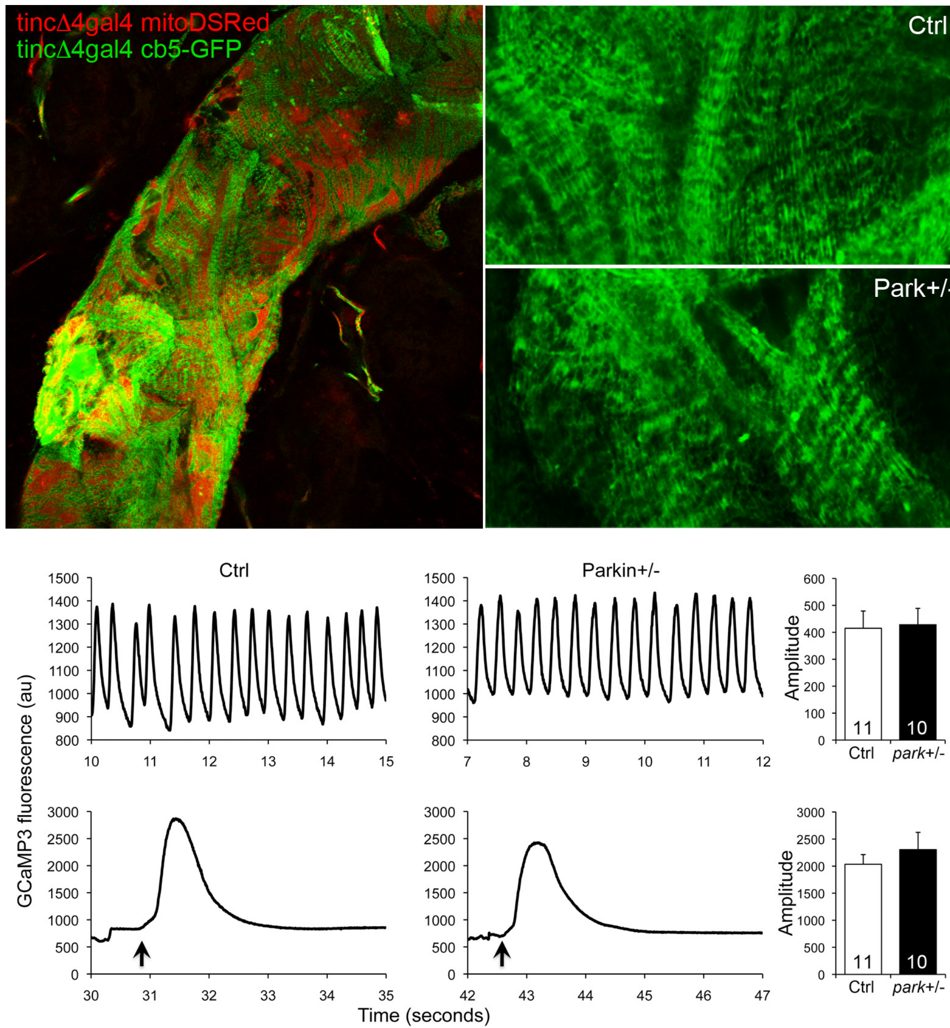
Online Figures:



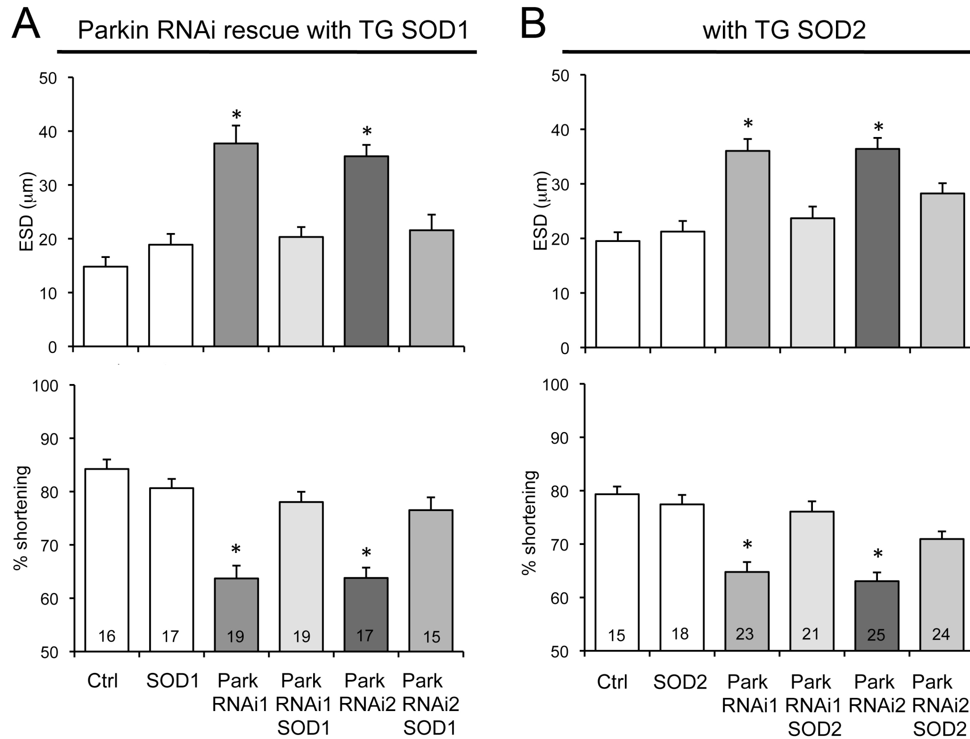
Online Figure I. *Cardiomyopathy in parkin -/- flies. Cardiomyopathy in parkin -/- flies.* **A.** Mean group results of heart tube OCT in TincΔ4-Gal4 (Ctrl) and parkin null (-/-) flies 28 days after eclosure showing end systolic dimension (ESD) and % fractional shortening. **B.** Photographs of Ctrl and parkin -/- flies 7 days after eclosure; scale is mm. **C.** Longevity analysis of n=150 flies per group.



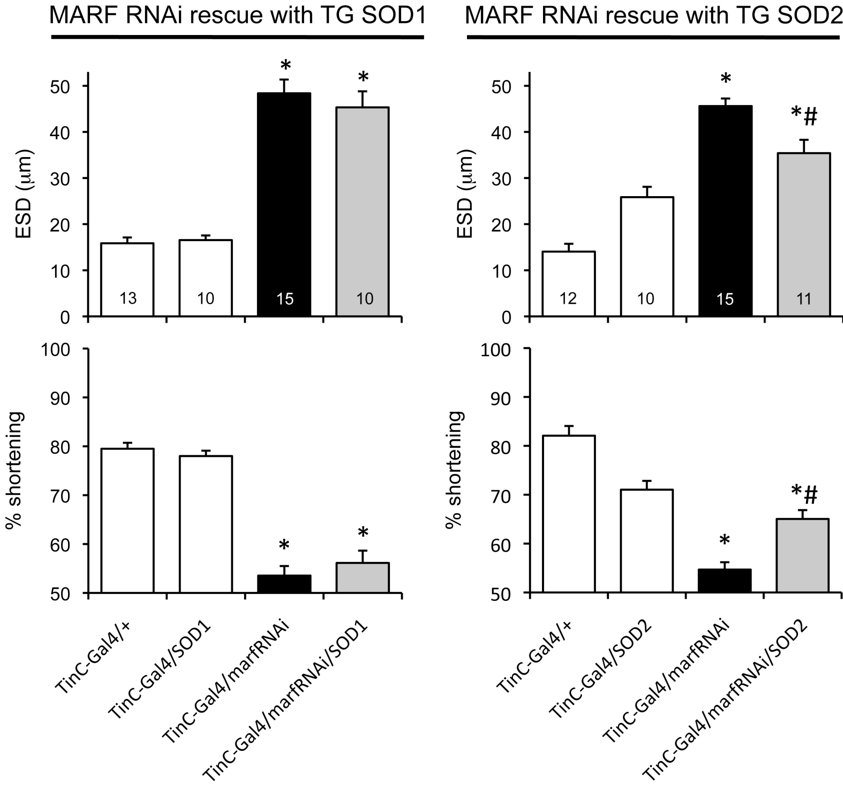
Online Figure II. *Parkin haploinsufficiency induces cardiomyopathy that is rescued by cardiomyocyte Parkin expression.* **A.** OCT studies of *parkin* +/- fly heart tubes 28 days after eclosure. **B**. Longevity analysis of *parkin* +/- fly lines (n=150 adult flies per line); control (dotted curve) and *parkin* -/- (50% survival) data from figure 1c are shown for comparison. **C.** Photographs of Ctrl, *parkin* -/-, and *parkin* +/- flies; scale is mm. **D**. Cardiomyocyte mitochondrial area assessed by confocal of TincΔ4-Gal4 pUAS mito-GFP; *parkin* +/- heart tubes. **E.** Heart tube fractional shortening (%FS) assessed by OCT in *parkin* +/- flies without and with the TincΔ4-Gal4 pUAS-Parkin transgene. Ctrl (+/+) and TincΔ4-Gal4 pUAS-Parkin (UAS-Park) are shown for comparison.



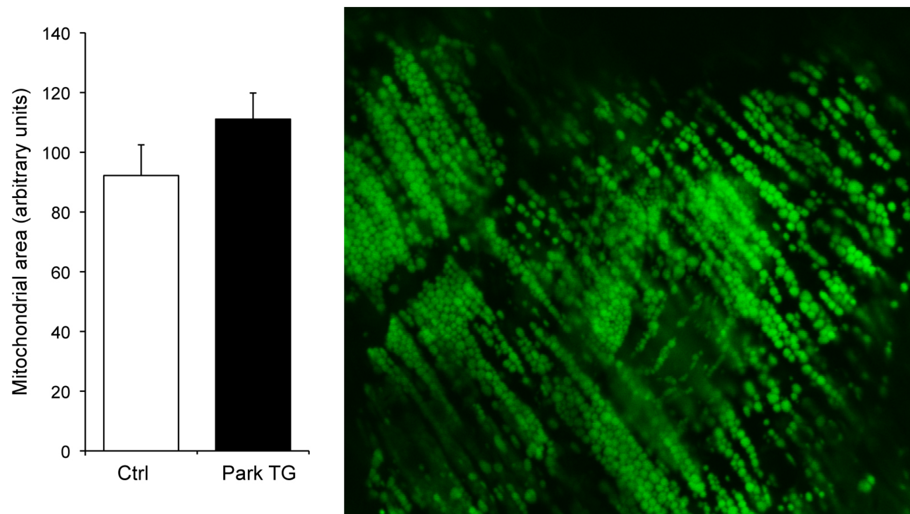
Online Figure III. *Sarcoplasmic reticulum (SR) calcium studies in Parkin deficient fly hearts.* **Top.** Confocal analysis of cardiomyocyte SR structure visualized in live heart tubes with TincΔ4-Gal4-cb5-GFP (green) and TincΔ4-Gal4 mitoDSRed (red). High magnification of SR is shown on the right. **Bottom.** Representative sarcoplasmic calcium transients in spontaneously contracting heart tubes (top) and caffeine-stimulated (arrow) quiescent heart tubes (bottom), measured by TincΔ4-Gal4-GCaMP3 fluorescence. Group mean data of calcium transient amplitude are shown to the right.



Online Figure IV. Comparative effects of SOD1 and SOD2 on two lines of cardiac Parkin RNAi *Drosophila*. **A and B.** Results of OCT studies of two lines of cardiomyocyte-specific Parkin RNAi flies without (dark grey) and with (lighter grey) concomitant cardiomyocyte-specific expression of SOD1 (A) or SOD2 (B). End-systolic dimension (ESD) is on top, % fractional shortening on bottom. Park RNAi1 data are the same as shown in Figure 5, for comparison with Park RNAi2. * = significantly different from Ctrl by ANOVA and Bonferroni test.



Online Figure V. *ROS scavenging has minimal effects on the cardiomyopathy of MARF suppression.* Results of OCT studies of cardiomyocyte-specific MARF suppression, without (black bars) and with (grey bars) concomitant cardiomyocyte-specific expression of SOD1 (left) or SOD2 (right). End-systolic dimension (ESD) is on the top and fractional shortening on the bottom. *=significantly different from TincΔ4-Gal4 control; #=significantly different from MARF RNAi by ANOVA and Bonferroni test.



Online Figure VI. *Cardiomyocyte mitochondrial morphology is not changed by overexpression of Parkin.* Quantitative assessment of mitochondrial size in control and cardiac Parkin transgenic (TG) fly cardiomyocytes using cardiomyocyte-specific expression of mitochondrial tagged GFP as in Figures 3c and 6f. Representative confocal image of Parkin TG cardiomyocyte mitochondria is on right, showing normal size distribution. Note: OCT of Parkin TG heart tube % shortening is shown in Supplemental Figure S2e.

Online Supplement References

1. Dorn GW II, Clark CF, Eschenbacher WH, Kang M-Y, Engelhard JT, Warner SJ, Matkovich SJ, Jowdy CC. (2011) MARF and Opa1 control mitochondrial and cardiac function in Drosophila. *Circ Res* 108:12-17.
2. Eschenbacher WH, Song M, Chen Y, Zhao P, Jowdy CC, Engelhard JT, Dorn GW II. (2012) Two rare human mitofusin 2 mutations alter mitochondrial dynamics and induce retinal and cardiac pathology in Drosophila. *PloS One* 7:e44296.
3. Chen Y, Csordas G, Jowdy C, Schneider TG, Wang W, Liu Y, Kohlhaas M, Meiser M, Bergem S, Nerbonne JM, Dorn GW II, Maack C. (2012) Mitofusin 2-containing mitochondrial-reticular microdomains direct rapid cardiomyocyte bioenergetic responses via interorganelle Ca2+ crosstalk. *Circ Res* 111:863-875.