Supplemental Data

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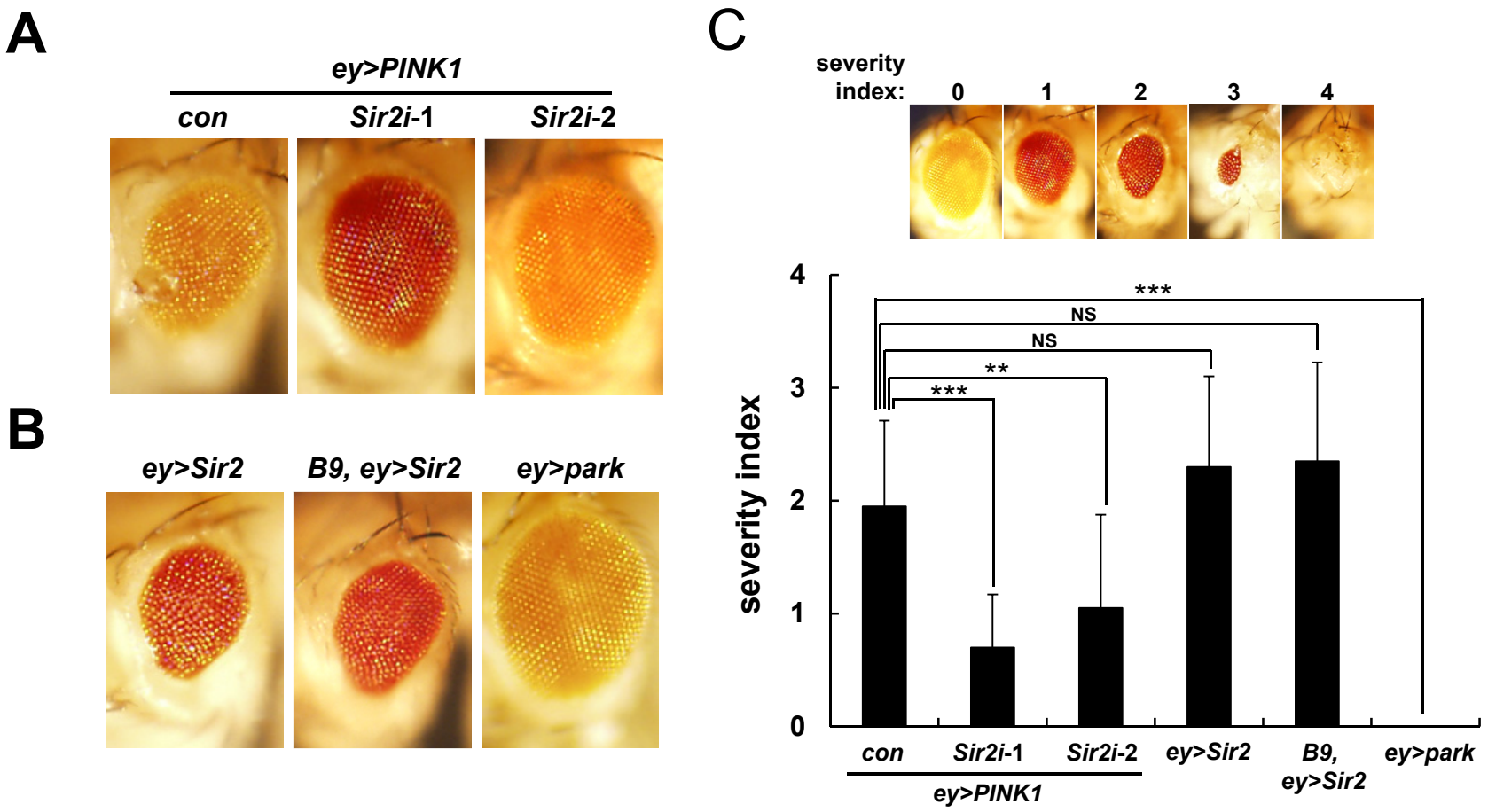
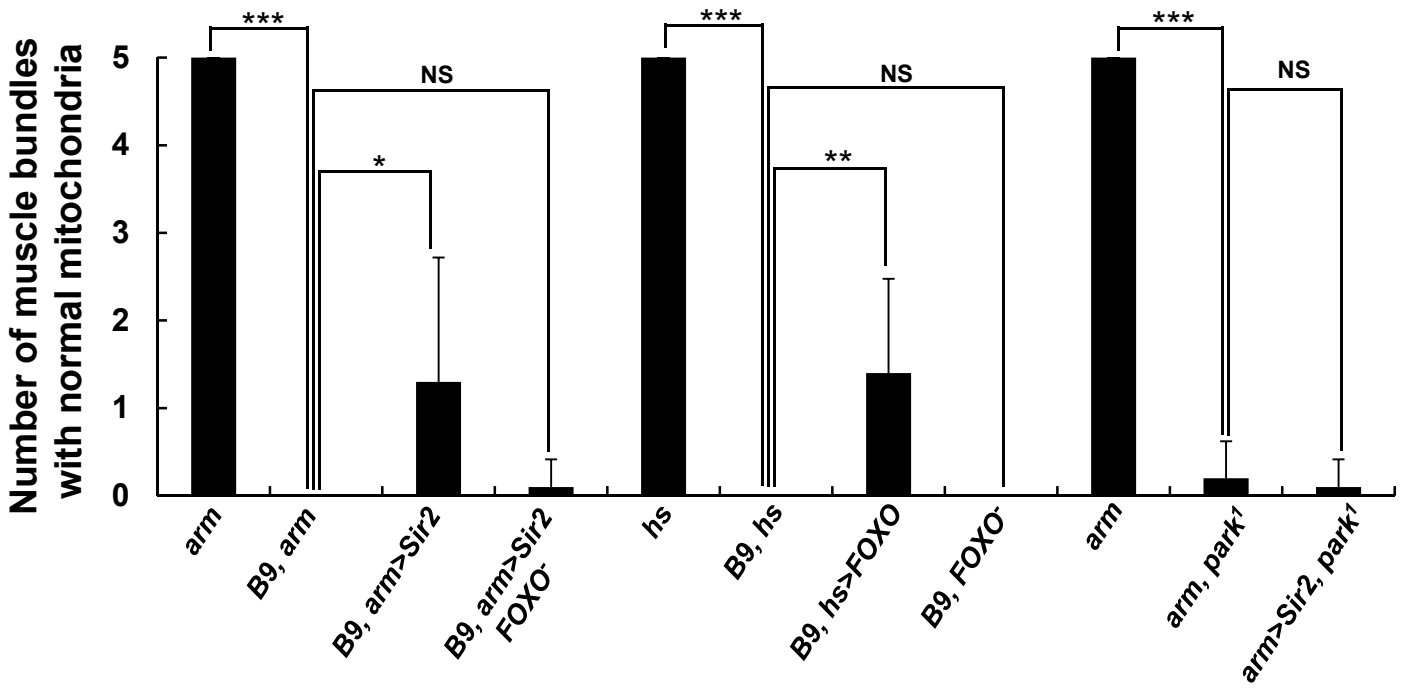
This section contains:

1) **Supplemental Figures (1-4)**
2) **Supplemental Figure Legends**
3) **Supplemental Experimental Procedures**

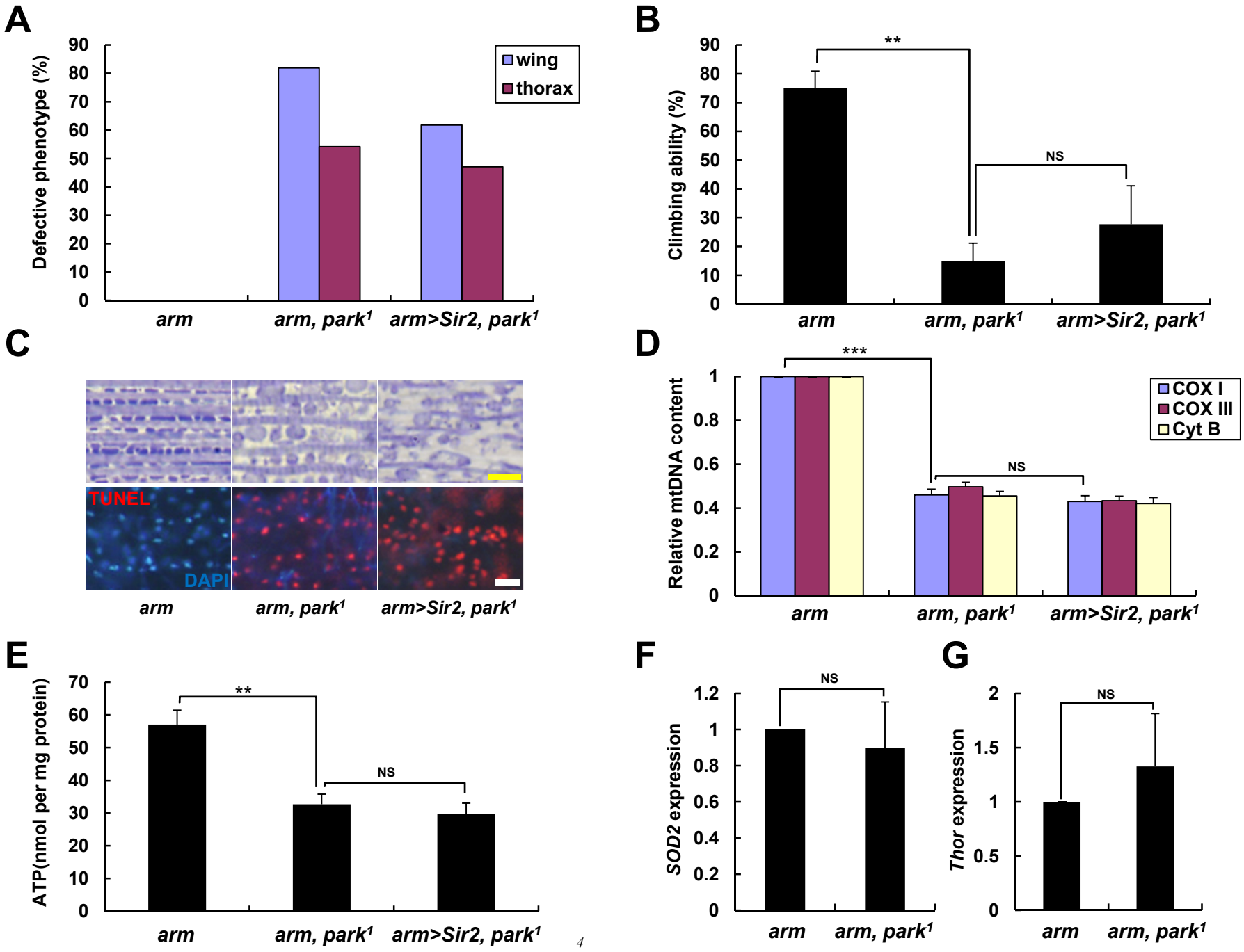
1) Supplemental Figures


A
ey>PINK1
con
Sir2i-1
Sir2i-2
B
ey>Sir2
B9, ey>Sir2
ey>park
C
severity index:
0
1
2
3
4
severity index
***
NS
NS
**
***
con
Sir2i-1
Sir2i-2
ey>Sir2
B9, ey>Sir2
ey>park
ey>PINK1

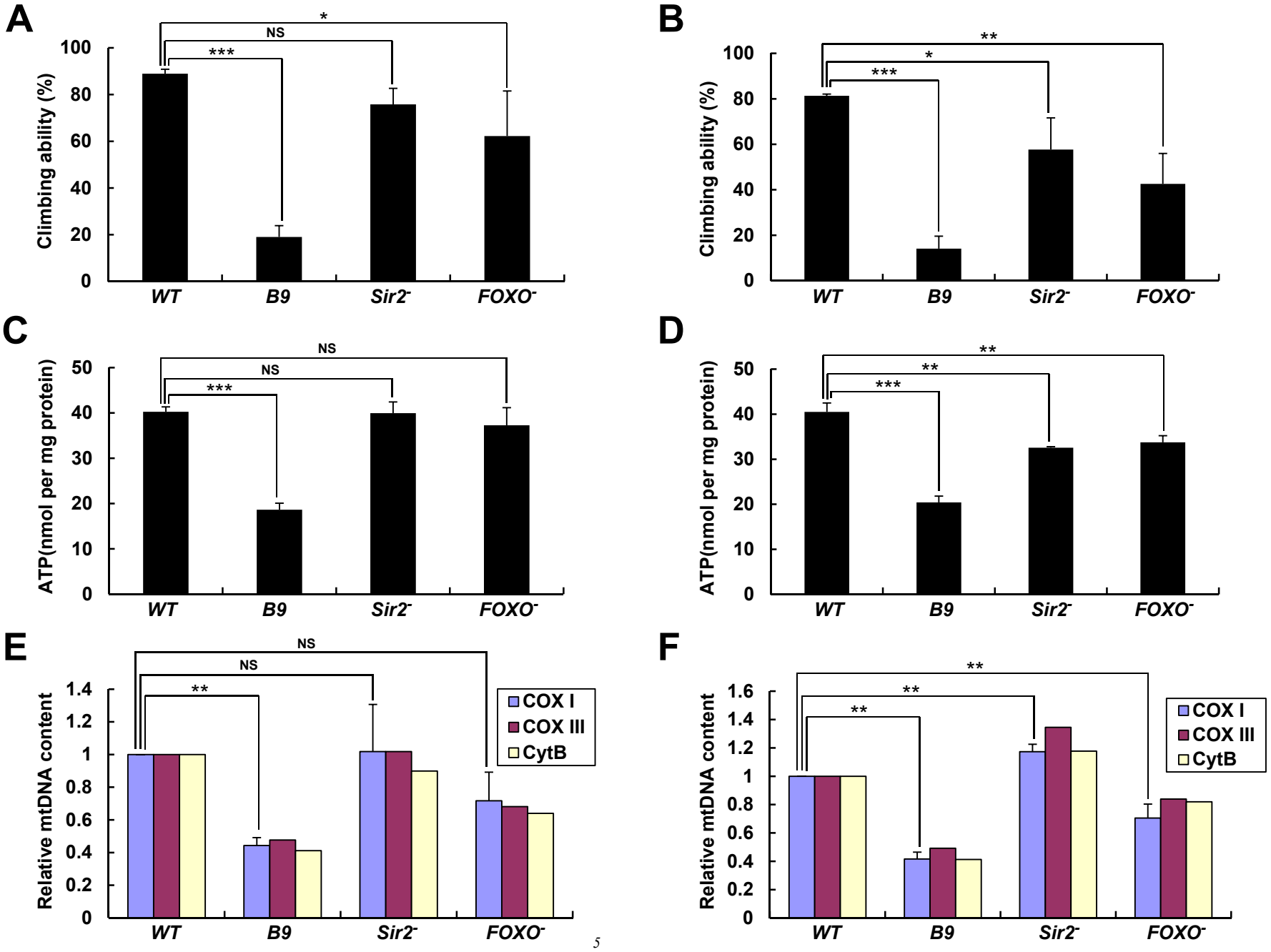
Supplemental **Fig. 2**



Supplemental **Fig. 3**



Supplemental **Fig. 4**



2) Supplemental Figure Legends

Supplemental Figure 1. Genetic interaction between *PINK1* and *Sir2*. A, Light stereo-micrographs of PINK1-expressing [*ey* (*ey*-GAL4)>*PINK1*] control (c*on*), and *Sir2* RNAi (*Sir2i*) knock-down (*Sir2i*-1 or *Sir2i*-2) fly eyes. B, Light stereo-micrographs of Sir2-expressing (*ey>Sir2*), Sir2-expressing *PINK1* null (*B9, ey>Sir2*), and Parkin-expressing (*ey>park*) adult *Drosophila* eyes. C, Comparison of the severity of eye defect. For each genotype, 20 eyes were examined using severity indexes from 0 (no defect) to 4 (no eye) (n=20). Significance was determined by one-way ANOVA with Bonferroni correction (**, $P<0.01$; ***, $P<0.001$; NS, not significant). Error bars indicate mean ± SD.

Supplemental Figure 2. The number of muscle bundles with normal mitochondria in thoraces. Thorax sections from 10 flies were examined for each genotype (n=10). Significance was determined by one-way ANOVA with Bonferroni correction (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; NS, not significant). Error bars indicate mean ± SD.

Supplemental Figure 3. Expression of Sir2 fails to rescue *parkin* mutant phenotypes. A, Percentage of defective thorax and wing phenotypes in *parkin* mutants (*arm, park*^{1}) and Sir2-expressing *parkin* mutants (*arm>Sir2, park*^{1}). *arm*-GAL4/+ (*arm)* flies were used as wild type controls. B, Comparison of climbing ability (n=4). C, Toluidine blue stained longitudinal sections (top panels) and merged images of TUNEL (red) and DAPI (blue) staining (bottom panels) of indirect flight muscle in the thoraces. D, Quantification of the mtDNA of thoraces (n=3). E, Comparison of the ATP content of thoraces (n=3). F, Comparison of *SOD2* mRNA level in the thoraces (n=3, Student's *t* test). G, Comparison of *Thor* mRNA level in the thoraces (n=3; NS, not significant, Student's *t* test). If not indicated, significance was determined by one-way ANOVA with Bonferroni correction (**, $P<0.01$; ***, $P<0.001$; NS, not significant). Error bars indicate mean ± SD. Scale bars: yellow, 5 μm; white, 10 μm.

Supplemental Figure 4. *Sir2* and *FOXO* are necessary to maintain mitochondrial functions. 3-day-old (A, C, and E) or 15-day-old (B, D, and F) wild type controls (*WT*), *PINK1* null mutants (*B9*), *Sir2* mutant (*Sir2*$^{-}$), and *FOXO* mutants (*FOXO*$^{-}$) were examined in following analyses. A and B, Comparison of climbing ability (n=4). C and D, Comparison of the ATP content of thoraces (n=3). E and F, Quantification of the mtDNA of thoraces (n=3). Significance was determined by one-way ANOVA with Bonferroni correction (*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; NS, not significant). Error bars indicate mean ± SD.

3) Supplemental Experimental Procedures

Quantitative RT-PCR-Total RNA from five thoraces of 3-day-old flies was extracted with Easy-Blue™ (Intron), and reversely transcribed using *Maxime* RT premix kit (Intron). Then, quantitative real-time PCR was performed using SYBR Premix Ex Taq (Takara) on Prism 7000 Real-Time PCR System (ABI). *rp49* revels were measured for internal control. Results are expressed as fold change compared relative to the control. Average ± SD is from three experiments. For primer pairs, we used *rp49-F* (GCT TCA AGA TGA CCA TCC GCC C) and *rp49-R* (GGT GCG CTT GTT CGA TCC GTA AC), *SOD2-F* (CGC AGA TAT GTT CGT GGC CCG) and *SOD2-R* (GGG CGA GAG GTT CTG CCA G), *Thor-F* (GAA GGT TGT CAT CTC GGA TCC) and *Thor-R* (CAT GAA AGC CCG CTC GTA G).

Genotypes-ey>PINK1, con (ey-GAL4, UAS-*PINK1*/+; *white*^{GD30033}/+); *ey>PINK1, Sir2i*-1 *(ey*-GAL4, UAS-*PINK1*/*Sir2*^{KK105502}); *ey>PINK1, Sir2i*-2 *(ey*-GAL4, UAS-*PINK1*/+; *Sir2*^{GD23201}/+); *ey>Sir2* (*ey*-GAL4/UAS-*Sir2*); *B9*, *ey>Sir2* (*PINK1*^{B9}/Y; *ey*-GAL4/UAS-*Sir2*); *ey>park* (*ey*-GAL4/UAS-*parkin*); *arm*

(+/Y; *arm*-GAL4/+); *B9*, *arm* (*PINK1*^{B9}/Y; *arm*-GAL4/+); *B9*, *arm>Sir2* (*PINK1*^{B9}/Y; *arm*-GAL4/UAS-*Sir2*); *B9*, *arm>Sir2, FOXO*$^{-}$ (*PINK1*^{B9}/Y; *arm*-GAL4/UAS-*Sir2*; *FOXO*^{21}/*FOXO*^{25}); *arm*, *park*^{1} (+/Y; *arm*-GAL4/+; *park*^{1}/*park*^{1}); *arm>Sir2*, *park*^{1} (+/Y; *arm*-GAL4/UAS-*Sir2*; *park*^{1}/*park*^{1}); *hs* (+/Y; *hs*-GAL4/+); *B9*, *hs* (*PINK1*^{B9}/Y; *hs*-GAL4/+); *B9*, *hs>FOXO* (*PINK1*^{B9}/Y; *hs*-GAL4/UAS-*FOXO*); *B9*, *FOXO*$^{-}$ (*PINK1*^{B9}/Y;; *FOXO*^{21}/*FOXO*^{25}); *B9*, *hs>SOD2* (*PINK1*^{B9}/Y; *hs*-GAL4/UAS-*SOD2*); *B9*, *hs>Thor* (*PINK1*^{B9}/Y; *hs*-GAL4/UAS-*Thor*); WT, *TH>mitoGFP* (+/Y;; *TH*-GAL4, UAS-*mitoGFP*/+); *B9*, *TH>mitoGFP* (*PINK1*^{B9}/Y;; *TH*-GAL4, UAS-*mitoGFP*/+); *B9*, *TH>Sir2*, *TH>mitoGFP* (*PINK1*^{B9}/Y; *Sir2*^{EP2300}/+; *TH*-GAL4, UAS-*mitoGFP*/+); *B9*, *TH>Sir2*, *FOXO*$^{-/+}$, *TH>mitoGFP* (*PINK1*^{B9}/Y; *Sir2*^{EP2300}/+; *TH*-GAL4, UAS-*mitoGFP/FOXO*^{21}); *B9*, *TH>SOD2*, *TH>mitoGFP* (*PINK1*^{B9}/Y; UAS-*SOD2*/+; *TH*-GAL4, UAS-*mitoGFP*/+); *B9*, *TH>Thor*, *TH>mitoGFP* (*PINK1*^{B9}/Y; UAS-*Thor*/+; *TH*-GAL4, UAS-*mitoGFP*/+); *TH* (+/Y;; *TH*-GAL4/+); *B9*, *TH* (*PINK1*^{B9}/Y;; *TH*-GAL4/+); *B9*, *TH>Sir2* (*PINK1*^{B9}/Y; *Sir2*^{EP2300}/+; *TH*-GAL4/+); *B9*, *TH>Sir2*, *FOXO*$^{-/+}$ (*PINK1*^{B9}/Y; *Sir2*^{EP2300}/+; *TH*-GAL4/*FOXO*^{21}); *B9*, *TH>SOD2* (*PINK1*^{B9}/Y; UAS-*SOD2*/+; *TH*-GAL4/+); *B9*, *TH>Thor* (*PINK1*^{B9}/Y; UAS-*Thor*/+; *TH*-GAL4/+); WT (+/Y); *FOXO*$^{-}$ (*FOXO*^{21}/*FOXO*^{25}); *Sir2*$^{-}$ (*Sir2*$^{2A\text{-}7\text{-}11}$/*Sir2*$^{2A\text{-}7\text{-}11}$); *B9* (*PINK1*^{B9}/Y); *B9*, *Sir2*$^{-}$ (*PINK1*^{B9}/Y; *Sir2*$^{2A\text{-}7\text{-}11}$/*Sir2*$^{2A\text{-}7\text{-}11}$).