Inventory of Supporting Information

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- *Extended Data*
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1. Extended Data

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Figure #	**Figure title** One sentence only	**Filename** This should be the name the file is saved as when it is uploaded to our system. Please include the file extension. i.e.: *Smith_ED_Fig1.jpg*	**Figure Legend** If you are citing a reference for the first time in these legends, please include all new references in the main text Methods References section, and carry on the numbering from the main References section of the paper. If your paper does not have a Methods section, include all new references at the end of the main Reference list.
Extended Data Fig. 1	**Miro Binds to dMIC60 in a Redox-Dependent Manner.**	FigS1.jpg	(a) Two-day-old flies expressing Myc-tagged *UAS-dMIC60-WT* or *dMIC60-CS* driven by *Actin-GAL4* in *dMIC60* null background [13,28], were lysed and immunoblotted. Both anti-dMIC60 and anti-Myc recognize transgenic dMIC60 protein in *dMIC60* null background (no endogenous dMIC60) [28]. Anti-dMIC60 recognizes endogenous dMIC60 in flies with *Actin-GAL4* alone in wild-type background. (b) Whole-body lysates of wild-type flies (w^{1118}) were IPed with anti-DMiro or IgG, and immunoblotted (IB) as indicated. (c) Immunostaining of dMIC60 and ATP5β in third instar larval muscles. Scale bar: 5 μm. (d) Immunostaining of T7 in adult fly brains (day 7). Scale bar: 20 μm. (c-d) Confocal images were obtained using the same settings. (e) In Vitro GST pull-down using full-length GST-

			Miro1 or GST, together with recombinant dMIC60 (AAs 92-739). (f) In Vitro GST pull-down using full-length GST-Miro1, together with recombinant dMIC60 (AAs 92-739), either wild-type (WT) or mutant (CS). (g) Coomassie-stained gels. Arrowhead indicates the dMIC60 band. (h-i) In Vitro GST pull-down using full-length GST-Miro1 or GST, together with recombinant dMIC60 (AAs 92-739) (h) or Parkin (i). (a-i) Similar results were seen more than three times. (j) Lysates of wild-type flies fed with H_2O_2, paraquat, or vehicle (water) at day 7 for 24 hrs were immunoblotted as indicated. The band intensity of each marker is normalized to that of β–actin from the same blot and graphed as relative change compared to vehicle. n=4 independent experiments. Two-sided Student T Test. p=0.0336 (DMiro, H_2O_2), 0.0325 (dMIC60, H_2O_2), 0.0176 (DMiro, paraquat), 0.0383 (dMIC60, paraquat). Boxes show $25^{th}/75^{th}$ percentiles, whiskers are the minimum and maximum, and middle line is median.
Extended Data Fig. 2	**DMiro Protein Levels in *MICOS* Mutants.**	FigS2.jpg	(a) Lysates of 2^{nd} instar larvae (*dMIC19*^{null}), pupae (*dMIC60*^{null}), or 14-day-old adults (RNAi) were immunoblotted as indicated. The band intensity of each marker is normalized to that of ATP5β or β-actin from the same blot and graphed as relative change compared to control. *Wild-type*: *w*^{1118}. n=3 (Top panel: anti-DMiro for *WT*; and Middle panel) and 4 (the rest) independent experiments. Two-sided Mann-Whitney Test (Lower panel) and T Test with Welch's correction (Top and Middle). n.s.: not significant. p=0.0009, 0.0005, 0.0096 for *dMIC19* null, 0.0319, 0.0351, 0.0173 for *dMIC60* null. The rest of the precise p values are in Source Data. (b) Mitochondrial (Mito) and cytosolic (Cyto) fractions from 20-day-old flies were immunoblotted as indicated. The DMiro band intensity is normalized to that of VDAC from the same blot. n=3. Two-sided T Test with Welch's correction. p=0.0283, 0.0102. (c) Whole-body lysates of 20-day-old flies were immunoblotted

			as indicated. The band intensity is normalized to that of β-actin from the same blot and graphed as relative change compared to control (*Actin-GAL4*, gray bar) within the same experiment. n=5 flies per experiment, 4 independent experiments. p=0.0286. (d) The mRNA levels of *DMiro* were determined by qPCR in 20-day-old flies, normalized to *RP49* from the same experiment, and graphed as relative change compared to respective controls (white boxes) within the same experiment. n=4 independent experiments. p=0.0286. (c-d) Two-sided Mann-Whitney Test. (a, d) Boxes show $25^{th}/75^{th}$ percentiles, whiskers are the minimum and maximum, and middle line is median. (a, b, c) Data are presented as Mean±S.E.M. with dots.
Extended Data Fig. 3	**DMiro Protein Levels in Mitochondrial Mutants.**	FigS3.jpg	(a) The mRNA levels of indicated genes were determined by qPCR in 20-day-old flies, normalized to *RP49* from the same experiment, and graphed as relative change compared to respective controls (white boxes) within the same experiment. n=4 independent experiments. p=0.0286. (b) Whole-body lysates of 20-day-old flies were immunoblotted as indicated. The band intensity of DMiro is normalized to that of β-actin from the same blot and graphed as relative change compared to control (*Actin-GAL4*, gray bar). n=4 independent experiments. (c) Lysates of 2-day-old $CHCHD2^{null}$ and control (w^{1118}) were immunoblotted as indicated. The band intensity of DMiro is normalized to that of β-actin from the same blot and graphed as relative change compared to wild-type control (w^{1118}). n=4 independent experiments. (d) Lysates of 3^{rd} instar larvae were immunoblotted. The band intensity of each marker is normalized to that of ATP5β from the same blot and graphed as relative change compared to wild-type control (w^{1118}). n=7 larvae per experiment, 3 (anti-dMIC19 for *WT*) and 4 (the rest) independent experiments. p=0.0286. (e) qPCR results show no significant differences in *DMiro* mRNA

			expression normalized to *RP49* between young (day 3) and old (day 40) wild-type (w^{1118}) flies. n=4 replicates per genotype. (f) Lysates of 3rd instar larvae and 2-day-old adult flies were immunoblotted with antibodies against DMiro and the loading control β-actin. The band intensity of DMiro is normalized to that of β-actin from the same blot. n=4 independent experiments. Please note that DMiro is upregulated in 2-day-old flies compared to third instar larvae. p=0.0072, 0.0047. The rest of the precise p values are in Source Data. (a) One-sided Mann-Whitney Test (dMIC19). (a-e) Two-sided Mann-Whitney Test. (f) One-Way ANOVA Post Hoc Tukey Test. (a) Boxes show 25th/75th percentiles, whiskers are the minimum and maximum, and middle line is median. (b-f) Data are presented as Mean±S.E.M. with dots.
Extended Data Fig. 4	**Fig. 4. The Role of DMiro in Lifespan.**	FigS4.jpg	For all panels, "RU-" flies were fed with the same volume of the vehicle, ethanol. All flies were female. (a) Survival curves of wild-type flies (w^{1118}) fed with or without 200 μM RU. n=141 flies. p=0.46 by Log-Rank Test. (b) Later life-onset neuronal downregulation of *DMiro* using *Elav-GS-GAL4* induced in the presence of RU from day 30 (*Elav-GS>UAS-DMiro*^{RNAi}, RU+, blue line) extends lifespan, compared to un-induced controls (RU-, black line). n=106 (RU+) and 127 (RU-) flies. Median lifespan was increased by 10%, p=1.3×10^{-7} by Log-Rank Test. (c) Adult-onset downregulation of *DMiro* using the intestine enterocytes driver *5966-GS-GAL4* induced in the presence of RU (*5966-GS>UAS-DMiro*^{RNAi}, RU+, red line) shortens lifespan, compared to un-induced controls (RU-, black line). n=142 (RU+) and 151 (RU-) flies. p=0.0007 by Log-Rank Test. (d) Later life-onset downregulation of *DMiro* in intestine enterocytes using *5966-GS-GAL4* induced in the presence of RU from day 30 (*5966-GS>UAS-DMiro*^{RNAi}, RU+, red line) slightly extends lifespan, compared to un-induced controls (RU-, black line). n=135 (RU-) and 145 (RU+) flies.

			p=0.03 by Log-Rank Test. (e) Adult-onset ubiquitous knockdown of *DMiro* using *Da-GS-GAL4* in the presence of RU (*Da-GS>UAS-DMiro^{RNAi}*, RU+, red line) shortens lifespan, compared to un-induced controls (RU-, black line). n=143 (RU+) and 144 (RU-) flies. p=2.07×10^{-9} by Log-Rank Test. (f) Later life-onset ubiquitous knockdown of *DMiro* using *Da-GS-GAL4* in the presence of RU from day 30 (*Da-GS>UAS-DMiro^{RNAi}*, RU+, red line) does not alter lifespan, compared to un-induced controls (RU-, black line). n=141 (RU-) and 146 (RU+) flies. p=0.74 by Log-Rank Test. (g) Adult-onset downregulation of *DMiro* using the muscle driver *MHC-GS-GAL4* induced in the presence of RU (*MHC-GS>UAS-DMiro^{RNAi}*, RU+, red line) shortens lifespan, compared to un-induced controls (RU-, black line). n=146 (RU-) and 149 (RU+) flies. p=1.68×10^{-16} by Log-Rank Test. (h) Adult-onset neuronal downregulation of *white* using *Elav-GS-GAL4* induced in the presence of RU (*Elav-GS>UAS-white^{RNAi}*, RU+, red line) slightly shortens lifespan, compared to un-induced controls (RU-, black line). n=139 flies. p=0.01 by Log-Rank Test.
Extended Data Fig. 5	**Fig. 5. *dMIC60* or *dMIC19* RNAi Does not Affect Mitochondrial Structure.**	FigS5.jpg	(a) Representative TEM images of thin sections on body wall muscles of late third instar larvae. Scale bars: 500 nm. (b) Quantification of the crista junction (CJ) number per μm of mitochondrial circumference as described in [28]. n=30 mitochondria from 3 male larvae. (c) Quantification of the mitochondrial size as described in [28]. n=19 mitochondria from 3 male larvae. (d) Quantification of the aspect ratio as described in [28]. n=20 mitochondria from 3 male larvae. One-Way Anova Post Hoc Tukey Test.
Extended Data Fig. 6	**Fig. 6. The Role of MICOS in Lifespan.**	FigS6.jpg	(a) Later life-onset downregulation of *dMIC19* using the intestine enterocytes driver *5966-GS-GAL4* induced in the presence of RU from day 30 (*5966-GS>UAS-dMIC19^{RNAi}*, RU+, red line) does not alter lifespan, compared to un-induced controls (RU-, black line). n=143 (RU+) and 146 (RU-) flies.

			p=0.84 by Log-Rank Test. (b) Adult-onset downregulation of *dMIC19* in intestine enterocytes induced in the presence of RU (*5966-GS>UAS-dMIC19^{RNAi}*, RU+, red line) does not alter lifespan, compared to un-induced controls (RU-, black line). n=144 (RU+) and 150 (RU-) flies. p=0.59 by Log-Rank Test. (c) Later life-onset downregulation of *dMIC60* in intestine enterocytes induced in the presence of RU from day 30 (*5966-GS>UAS-dMIC60^{RNAi}*, RU+, red line) does not alter lifespan, compared to un-induced controls (RU-, black line). n=142 (RU+) and 143 (RU-) flies. p=0.99 by Log-Rank Test. (d) Adult-onset downregulation of *dMIC60* in intestine enterocytes induced in the presence of RU (*5966-GS>UAS-dMIC60^{RNAi}*, RU+, red line) does not alter lifespan, compared to un-induced controls (RU-, black line). n=140 (RU+) and 147 (RU-) flies. p=0.94 by Log-Rank Test. (e) The survival ability of female flies with a second *DMiro* RNAi line driven by *Elav-GS-GAL4* induced by RU since adulthood (*Elav-GS>UAS-DMiro$^{RNAi\ 2}$*, blue line, RU+) or of un-induced control flies (black line, RU-), in response to 20 mM paraquat (starting from day 7). n=14 (RU+) and 56 (RU-) flies. p=0.016 by Log-Rank Test. (f) Fly heads were lysed and blotted as indicated. The band intensity is normalized to that of α-tubulin from the same blot. n=4. One-Way Anova Post Hoc Tukey Test (p=0.6915 for ubiquitin, p=0.5884 for p62). Boxes show 25th/75th percentiles, whiskers are the minimum and maximum, and middle line is median.
Extended Data Fig. 7	**Fig. 7. α-Syn Neurotoxicity in Flies.**	FigS7.jpg	(a) Validation of an α-syn fly model where wild-type human α-syn transgene downstream of a modified UAS that significantly increases gene expression [59] is expressed using the inducible pan-neuronal driver *Elav-GS-GAL4* ("*Elav-GS>UAS-SNCA-WT*, RU+") through adulthood. Un-induced controls are "*Elav-GS>UAS-SNCA-WT*, RU-". Head lysates of induced and uninduced flies were immunoblotted as indicated. The band intensity of a-syn is normalized to that of

			β-actin. n=3 independent experiments. Two-sided T Test with Welch's correction. p=0.0238. (b) Climbing ability shown as Performance Index of flies with adult-onset induction and of un-induced controls at different ages. n=60 (RU-) and 61 (RU+) flies per genotype, 3 independent experiments. (c) The DA neuron number in the PPM1/2, PPL1, or PPL2 clusters per brain from flies as indicated at day 40 is counted and compared. n=6 fly brains per genotype. Two-sided Mann-Whitney Test. p=0.0022, 0.0065. The rest of the precise p values are in Source Data. (d) Head lysates of 40-day-old flies were immunoblotted with antibodies against DMiro and the loading control β-actin. The band intensity of DMiro is normalized to that of β-actin from the same blot. n=3 independent experiments. Two-sided Student T Test. p=0.0138. (e) Climbing time for 30-day-old flies. n=27, 26, 57, 26 flies (from left to right). One-Way Anova Post Hoc Tukey Test. p<0.0001. (f) Flying ability of 30-day-old flies. The percentage of flies scored as a "1" (able to fly) is shown in solid black bars, and the percentage of flies scored as a "0" (unable to fly) is shown in white bars. n=100. Chi-Square Test. p<0.0001. (e-f) Comparisons with "*TH-GAL4*". (g-h) HEK293T cells transfected as indicated were lysed and immunoblotted. One-Way Anova Post Hoc Tukey Test. (g) The band intensity of Miro1 is normalized to that of GAPDH from the same blot. Data from each genotype are compared to those of "Control RNAi, EGFP" (the left box) and asterisk is given directly on top of the box if it is significant. Data between "Control RNAi, EGFP-SNCA" and "MIC60 RNAi, EGFP-SNCA" (the right 2 boxes) are compared and asterisk is given on top of a line. n=5 transfections. p=0.0004, <0.0001. The rest of the precise p values are in Source Data. (h) The band intensity of Myc-Miro1 is normalized to that of β-actin from the same blot. Data from each genotype are compared to those of "Myc-Miro1, dMIC60-WT, EGFP" (the left box) and asterisk is given directly

			on top of the box if it is significant. Data between "Myc-Miro1, dMIC60-WT, EGFP-SNCA" and "Myc-Miro1, dMIC60-CS, EGFP-SNCA" (the right 2 boxes) are compared and asterisk is given on top of a line. n=5 transfections. p=0.013, 0.0006. The rest of the precise p values are in Source Data. (a, d) Data are presented as Mean±S.E.M. with dots. (c, e, g, h) Boxes show 25^{th}/75^{th} percentiles, whiskers are the minimum and maximum, and middle line is median.
Extended Data Fig. 8	**Fig. 8. Miro Reducers Benefit PD Models.**	FigS8.jpg	(a) Upper: Typical unfolding curves of human Miro1 protein in the absence (0 μM) and the presence of MR3 are shown. Lower: Quantification of melting temperatures of Miro1 protein in the absence (0 μM) and the presence of MR3 (100 μM). n=4 independent experiments. Two-sided Student T Test. p=0.0000424. (b) α-Syn-A53T-expressing flies (*Elav-GAL4>UAS-SNCA^{A53T}*) were fed with 10 μM MR5 for 15 days and then their heads were lysed for immunoblotting. The band intensity of DMiro is normalized to that of the mitochondrial loading control VDAC from the same blot. n=4 independent experiments. Two-sided Mann-Whitney Test. p=0.0286. (c) Both Antimycin A and MR5 were dissolved in ethanol. Neurons were pretreated with MR5 24 hrs before the application of Antimycin A for another 6 hrs. The same volume of ethanol was applied at the same time in negative controls. Confocal images overlay triple immunostainings of TH (DA neurons), TUNEL (indicator of cell death), and Dapi (nuclei). The percentage of TUNEL-positive cells out of total cells (Dapi-positive) is calculated in each condition under 20×. n=19 (PD, MR5) or 20 fields (the rest) from 4 independent experiments. p<0.0001. The rest of the precise p values are in Source Data. (d) From images such as in (c), the percentage of TH-positive neurons out of total cells (Dapi-positive) without Antimycin A treatment ranges from 17.08%-19.25%, and is not significantly different among all conditions (p=0.9532), consistent with

			previous studies from ours and others [20,67]. n=19 (PD, MR5) or 20 fields (the rest) from 4 independent experiments. (c-d) Comparisons with "*Wild-type*, no treatment" except otherwise indicated. One-Way Anova Post Hoc Tukey Test. Scale bar: (c) 100 µm. (a) Data are presented as Mean±S.D. with dots. (b-d) Boxes show 25th/75th percentiles, whiskers are the minimum and maximum, and middle line is median.
Extended Data Fig. 9			
Extended Data Fig. 10			

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2. Supplementary Information:

A. Flat Files

Complete the Inventory below for all additional textual information and any additional Supplementary Figures, which should be supplied in one combined PDF file.

- **Row 1:** A combined, flat PDF containing any Supplementary Text, Discussion, Notes, Additional Supplementary Figures, Supplementary Protocols, simple tables, and all associated legends. Only one such file is permitted.
- **Row 2:** Nature Research's Reporting Summary; if previously requested by the editor, please provide an updated Summary, fully completed, without any mark-ups or comments. **(Reporting Summaries are not required for all manuscripts.)**

Item	Present?	Filename	A brief, numerical description of file contents.

		This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. The extension must be .pdf	**i.e.: *Supplementary Figures 1-4, Supplementary Discussion, and Supplementary Tables 1-4.***
Supplementary Information	No		
Reporting Summary	Yes	Wang-reporting-summary	
Peer Review Information	Choose an item.	*OFFICE USE ONLY*	

B. Additional Supplementary Files

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  - Supplementary Data, for example: raw NMR Data, Cryo-EM Data, Computational Data, Crystallographic Data, etc.

Type	**Number** **If there are multiple files of the same type this should be the numerical indicator. i.e. "1" for Video 1, "2" for**	**Filename** **This should be the name the file is saved as when it is uploaded to our system, and should include the file**	**Legend or Descriptive Caption** **Describe the contents of the file**

	Video 2, etc.	extension. i.e.: *Smith_ Supplementary_Video_1.mov*	
Supplementary Table	1	TableS1	**Mass Spectrometry Data.**
Supplementary Video	1	Movie-S1	Supplementary Movie 1. **Locomotor Abilities of "*dMIC60-WT*" and "*dMIC60-CS*" Flies.** Left: *Actin-GAL4>dMIC60-WT; dMIC60 null*. Right: *Actin-GAL4>dMIC60-CS; dMIC60 null*.
Supplementary Video	1	Movie-S2	Supplementary Movie 2. **Flying Ability of a Fly with *TH-GAL4* Alone.** The fly is able to fly away immediately after being released.
Supplementary Video	1	Movie-S3	Supplementary Movie 3. **Flying Ability of a Fly with Expression of *SNCA-A53T* Driven by *TH-GAL4.*** The fly is not able to fly away even after 15 seconds of being released.
Supplementary Video	1	Movie-S4	Supplementary Movie 4. **Flying Ability of Flies with Expression of *SNCA-A53T* and *dMIC60 RNAi* Driven by *TH-GAL4*.** The flies are able to fly away either immediately or after several seconds of being released.
Choose an item.			

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3. Source Data

Complete the Inventory below for all Source Data files.

- Acceptable types of Source Data for Main Figures and Extended Data Figures are:
    - Statistical Source Data
        - Plain Text (ASCII, TXT) or Excel formats only
        - One file for each relevant Figure, containing all source data
    - Full-length, unprocessed Gels or Blots
        - JPG, TIF, or PDF formats only
        - One file for each relevant Figure, containing all supporting blots and/or gels
- 'Source Data' is only allowed for Main Figures and Extended Data Figures.
    - Include Unprocessed Gels or Blots for Supplementary Figures as additional Supplementary Figures.
    - Include Statistical Source Data for Supplementary Figures as 'Supplementary Data' files and list them in section 2B.
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Parent Figure or Table	**Filename** **This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. i.e.: *Smith_SourceData_Fig1.xls,* or *Smith_ Unmodified_Gels_Fig1.pdf***	**Data description** **i.e.: Unprocessed Western Blots and/or gels, Statistical Source Data, etc.**
Source Data Fig. 1	Fig1-Source-stats	Statistical source data
Source Data Fig. 1	Fig1-source-western	Unprocessed western blots
Source Data Fig. 2	Fig2-Source-stats	Statistical source data
Source Data Fig. 2	Fig2-source-western	Unprocessed western blots
Source Data Fig. 3	Fig3-Source-stats	Statistical source data
Source Data Fig. 4	Fig4-Source-stats	Statistical source data
Source Data Fig. 5	Fig5-Source-stats	Statistical source data
Source Data Fig. 6	Fig6-Source-stats	Statistical source data
Source Data Fig. 6	Fig6-source-western	Unprocessed western blots
Source Data Fig. 7	Fig7-Source-stats	Statistical source data
Source Data Fig. 8		

Source Data Extended Data Fig. 1	ExtFig1-Source-stats	Statistical source data
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Source Data Extended Data Fig. 2	ExtFig2-Source-stats	Statistical source data
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Source Data Extended Data Fig. 8	ExtFig8-Source-stats	Statistical source data
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