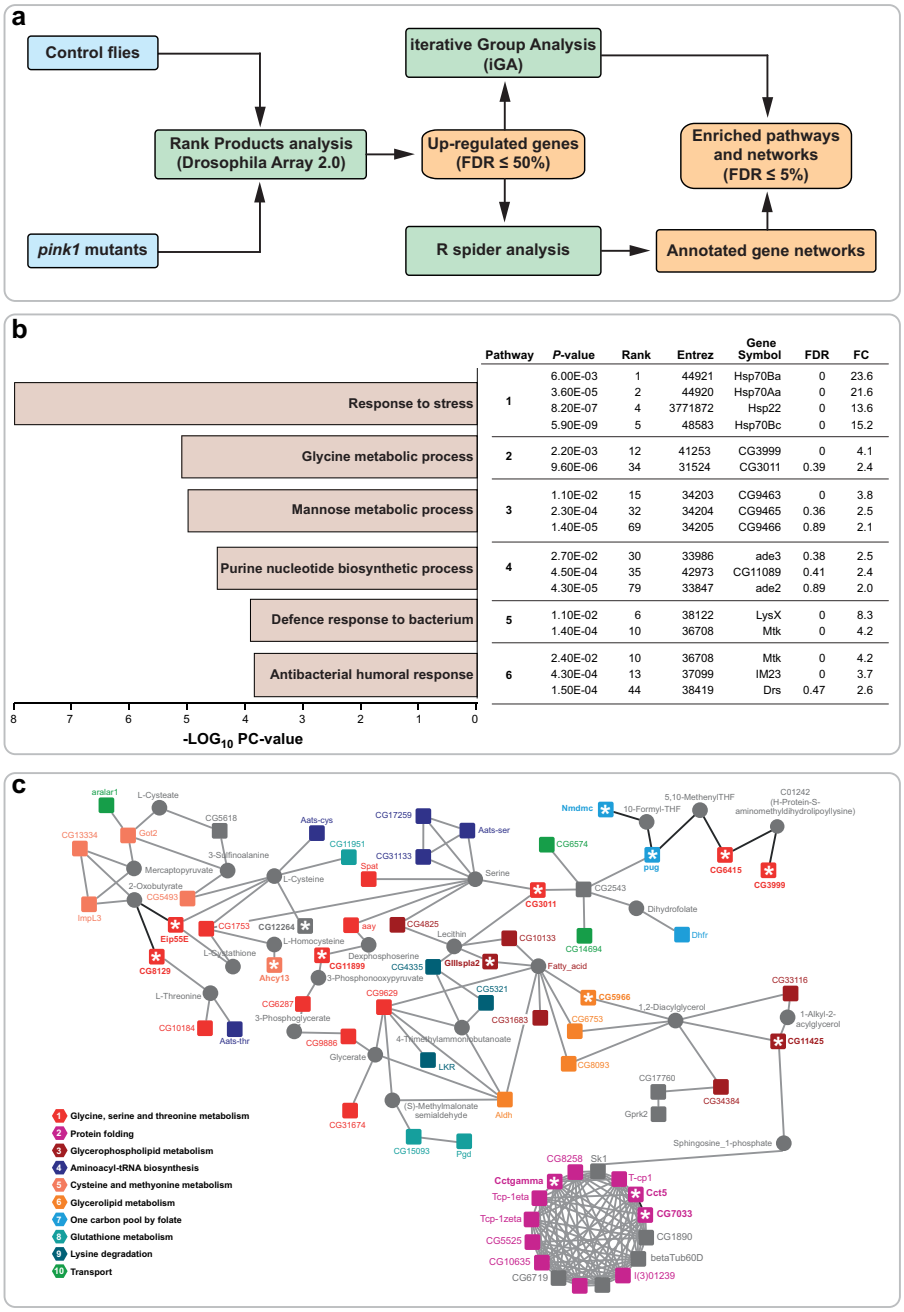
DOI: 10.1038/ncb2901

nature cell biology



Supplementary Figure 1 Analysis of gene networks induced upon loss of pink1**.** Related to Fig.1. **(a)** Workflow employed for the identification of specific gene networks induced by loss of *pink1* function. Inputs are depicted in cyan, outputs in orange and processing steps in green. Arrows illustrate the flow of information. **(b)** Groups of functionally related genes upregulated in *pink1* mutant flies. Groups were identified by iGA. Gene ontology classes are ranked by PC-value. *P*-values were calculated using the hypergeometric distribution; rank of a particular gene in the list of differentially expressed genes is created from the RP list by exclusion of genes that are not assigned to the gene ontology classes; FDR, false discovery rate; FC, fold-change calculated as an antilog of a mean log-fold-change over all possible between-chip comparisons contributing to a given between-group comparison. See also Supplementary Table 2. **(c)** Network organization of genes upregulated in *pink1* mutant flies. A D1 network model was returned by *R* spider on submission of 1693 candidate genes found to be upregulated in *pink1* mutant flies (p<0.005, computed according to Antonov et al [23]). Boxes represent input genes, circles represent compounds which are common substrates or products for connected genes. Hexagons are used to specify the colour of canonical Reactome or KEGG pathway. Asterisks correspond to network components below 5% FDR threshold and thick connectors link components above such threshold. See also Supplementary Tables 3 and 4.

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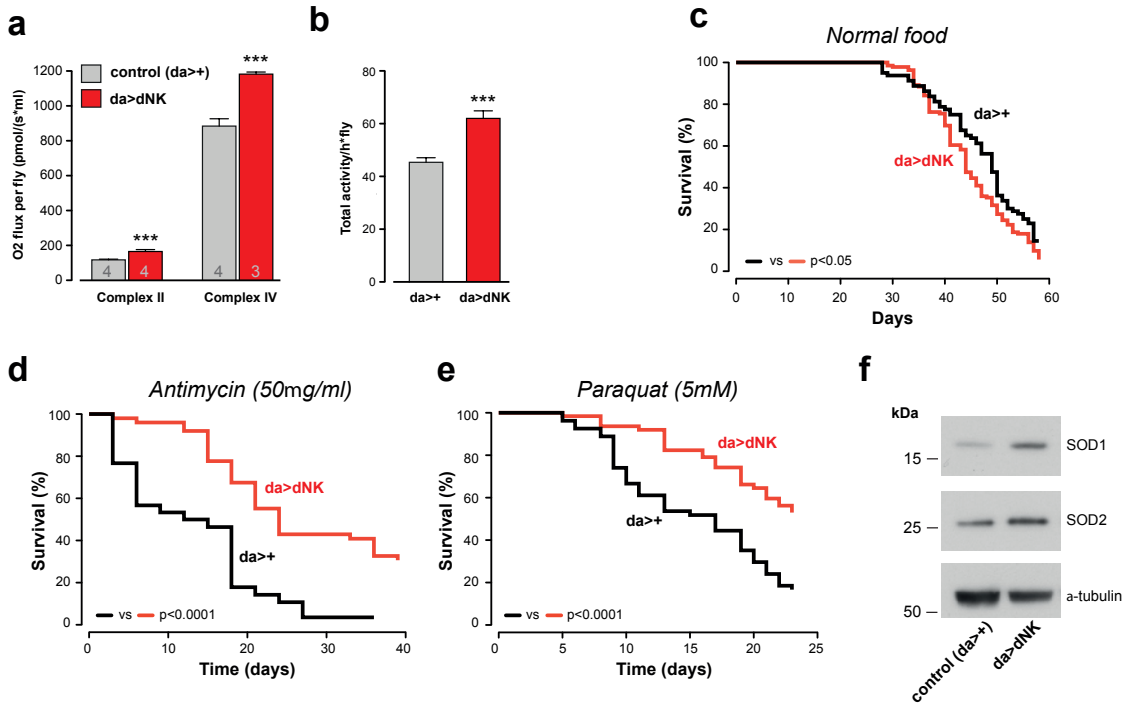

Supplementary Figure 2 Analysis of dNK expressing flies. Related to Fig. 2. (**a**) Enhanced respiration in *dNK* expressing flies. Activity of the indicated complexes in uncoupled mitochondria was measured by high-resolution respirometry. Data are shown as the mean ± SD (n values are indicated in the bars). Statistically significant values relative to control (da > +) are indicated by the asterisks (two-tailed unpaired *t* test) See Supplementary Table 9 for statistic source data. (**b**) *dNK* expression enhanced locomotor activity. Quantification of locomotor activity (counted as number of midline crossings per hr) was recorded for control (da > +) and *dNK* expressing flies for a period of 260 hr, n = 16 for each genotype. The *p* value (***, p<0.0001) was calculated by two-tailed unpaired t-test. (**c**) *dNK* expressing flies (red) show a reduction in total lifespan, compared to controls (black). Fly viability was scored over a period of 60 days, n = 80 for da>+ and n = 138 for da>dNK. Statistical significance is indicated (log-rank, Mantel-Cox test). (**d**) *dNK* expressing flies (red) show enhanced resistance to antimycin A toxicity, compared to controls (black). Fly viability was scored over a period of 40 days, n = 30 for da>+ and n = 31 for da>dNK. Statistical significance is indicated (log-rank, Mantel-Cox test). (**e**) *dNK* expressing flies (red) show enhanced resistance to paraquat toxicity, compared to controls (black). Fly viability was scored over a period of 25 days, n = 54 for da>+ and n = 62 for da>dNK. Statistical significance is indicated (log-rank, Mantel-Cox test). (**f**) *dNK* expressing flies show enhanced levels of mitochondrial ROS detoxification components, superoxide dismutases 1 and 2 (SOD1 and SOD2). Whole-fly lysates were analysed by western blot analysis using the indicated antibodies.

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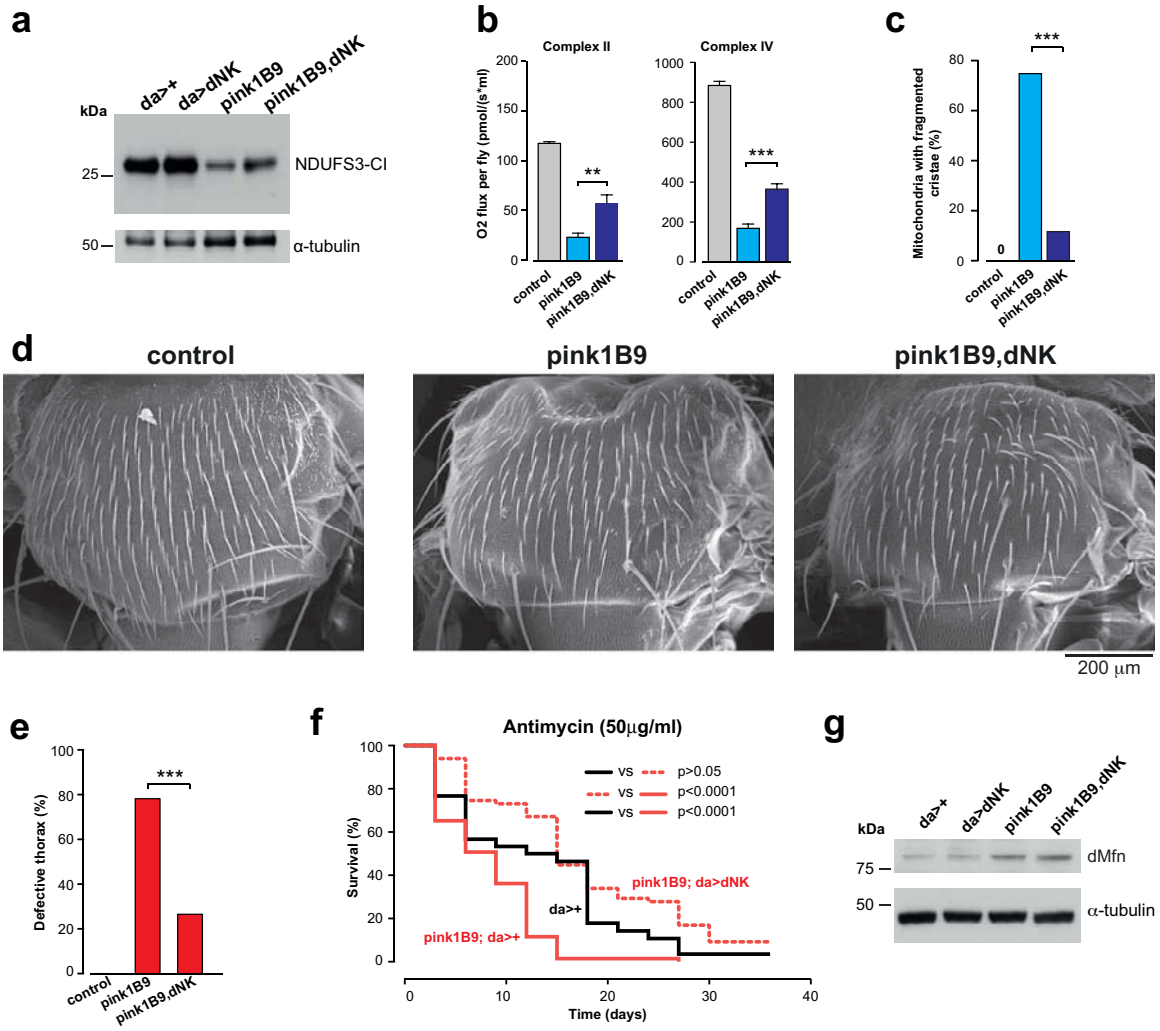

Supplementary Figure 3 Mitochondrial dysfunction in pink1 mutants is complemented by dNK. Related to Fig. 3. (**a**) Expression of restores levels of complex I subunit NDUFS3 in mutants. Whole fly lysates were analysed using the indicated antibodies. (**b**) expression enhances respiration in mutants. Activity of the indicated complexes in the uncoupled mitochondria was measured by high-resolution respirometry. Data are shown as the mean ± SEM (n = 6 in each genotype). Statistically significant values are indicated by asterisks (one-way ANOVA with Bonferroni's multiple comparison test). (**c**) Percentages of indirect flight muscle mitochondria exhibiting fragmented cristae are presented for the indicated genotypes (n = 273 for control, n = 240 for pink1B9, n = 340 for pink1B9,dNK). Asterisks indicate statistical significance (chi-square, two-tailed, 95% confidence intervals) (**d**) Expression of rescues the thoracic defects of mutants. SEM micrographs of thoraces from flies with the indicated genotypes show that the collapsed-thorax phenotype (middle) of mutants is suppressed by expression (right). A control thorax (from da > +) is also shown (left). (**e**) Percentages of flies exhibiting defective thorax after eclosion are presented for the indicated genotypes (n = 400 per genotype). Asterisks indicate statistical significance relative to pink1B9 (chi-square, two-tailed, 95% confidence intervals). (**f**) expression enhances the resistance of mutant flies to antimycin (50μg/ml) toxicity. Fly viability was scored over a period of 40 days, n = 60 for da>+, n = 68 for pink1B9,da>+ and n = 66 for pink1B9,da>dNK. Statistical significance is indicated (log-rank, Mantel-Cox test). (**g**) Expression of does not alter dMfn levels. Whole fly lysates were analysed using the indicated antibodies.

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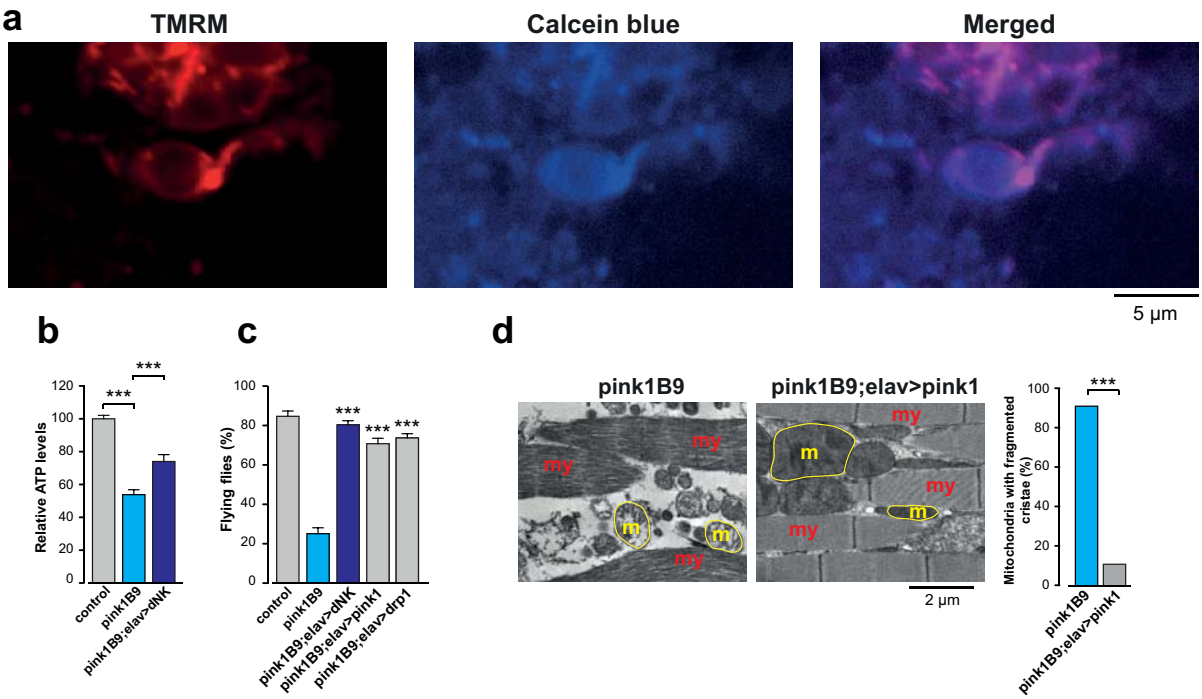

Supplementary Figure 4 Targeted neuronal expression of dNK rescues mitochondrial dysfunction in pink1 mutant. Related to Fig. 4. (**a**) Confocal image taken from a whole mounted *Drosophila* brain showing a neuron loaded with TMRM and calcein blue. (**b**) Neuronal expression of *dNK* enhances ATP levels in *pink1* mutants. ATP levels were measured using a bioluminescence assay. Data are shown as the mean ± SD (n = 9 in each group). Statistical significance is indicated (one-way ANOVA with Bonferroni's multiple comparison test). (**c**) Neuronal expression of *dNK, pink1 and drp1* rescues the flying ability of *pink1* mutants. (mean ± SEM, n = 150 flies per genotype). Statistically significant values relative to pink1B9 are indicated by asterisks (one-way ANOVA with Bonferroni's multiple comparison test). Datasets labelled "control" and "pink1B9" are also used in Fig. 7c. (**d**) *pink1* expression suppresses flight muscle defects observed in *pink1* mutants. Ultrastructural analysis of the indirect flight muscles showed that *pink1* expression rescues mitochondrial defects in *pink1B9* mutants (my, myofibrils; m, mitochondria; yellow outlines, mitochondria). Percentages of indirect flight muscle mitochondria exhibiting fragmented cristae are presented for the indicated genotypes (n = 185 for pink1B9, n = 340 for pink1B9;elav>pink1). Asterisks indicate statistical significance (chi-square, two-tailed, 95% confidence intervals).

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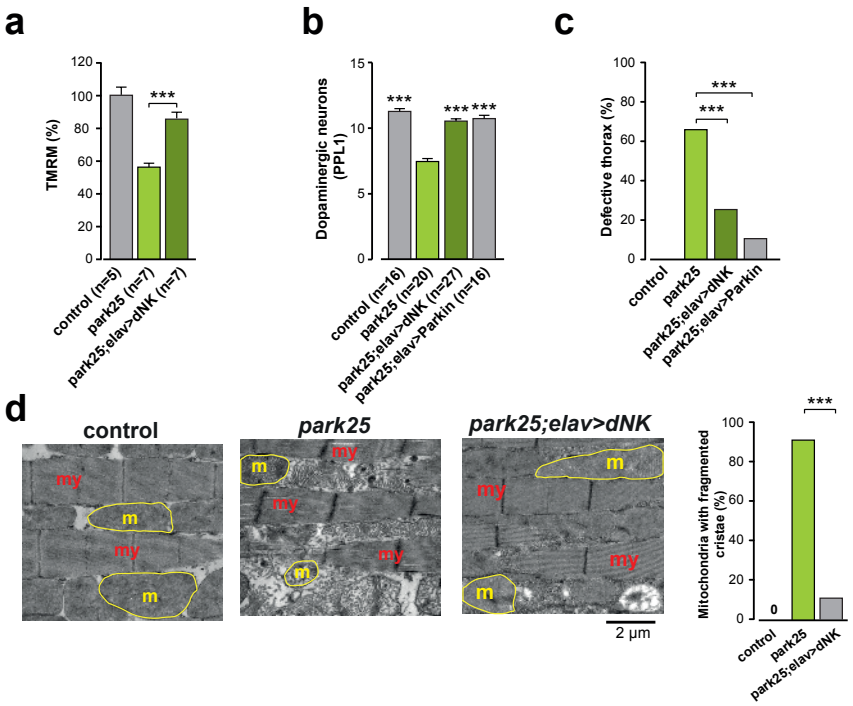

Supplementary Figure 5 Targeted neuronal expression of dNK rescues mitochondrial dysfunction in parkin mutant. Related to Fig. 5. (**a**) Neuronal expression of *dNK* reverses the loss of Δψm in *parkin* mutants. The Δψm is represented as percentage of control. Data are shown as the mean ± SEM (n values are indicated). Statistical significance is indicated by asterisks (two-tailed paired *t* test). Datasets labelled "control" and "park25" are also used in Supplementary Fig. 7c. (**b**) Neuronal expression of *dNK* rescues the loss of dopaminergic neurons in the PPL1 cluster of *parkin* mutant flies. Data are shown as the mean ± SEM (n values are indicated), and the asterisks indicate statistically significant values (one-way ANOVA with Bonferroni's multiple comparison test) relative to control. Datasets labelled "control" and "park25" are also used in Supplementary Fig. 7d. (**c**) Neuronal expression of *dNK* rescues the thoracic defects of *parkin* mutants. Asterisk(s) indicate statistical significance (chi-square two-tailed, 95% confidence intervals), n = 344 for park25, n = 1058 for park25;elav>dNK and n = 131 for park25;elav>Parkin. (**d**) *dNK* expression suppresses flight muscle defects observed in *parkin* mutants. Ultrastructural analysis of the indirect flight muscles showed that *dNK* expression rescues mitochondrial defects in *park25* mutants (my, myofibrils; m, mitochondria; yellow outlines, mitochondria). Percentages of indirect flight muscle mitochondria exhibiting fragmented cristae are presented for the indicated genotypes (n = 140 for control, n = 174 for park25, n = 130 for park25; elav>dNK). Asterisks indicate statistical significance (chi-square, two-tailed, 95% confidence intervals).

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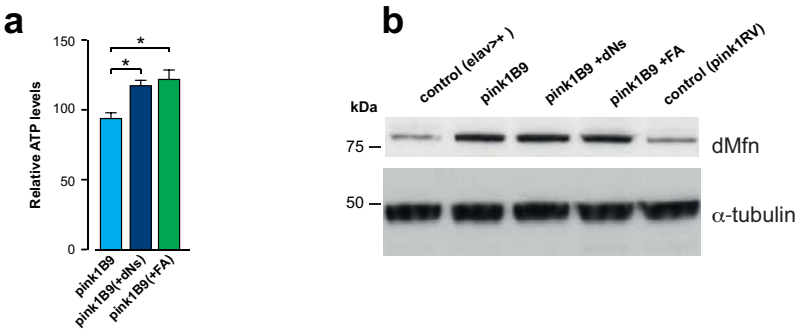

Supplementary Figure 6 Effects of dietary supplementation with dNs or FA in pink1 mutants. Related to Fig. 6. (**a**) dNs or FA enhance ATP levels in *pink1* mutants. ATP levels were measured using a bioluminescence assay. Data are shown as the mean ± SD (n = 3 in each group). Statistical significance is indicated (one-way ANOVA with Bonferroni's multiple comparison test). See Supplementary Table 9 for statistics source data. (**b**) dNs or FA do not affect dMfn levels. Whole fly lysates were analysed using the indicated antibodies.

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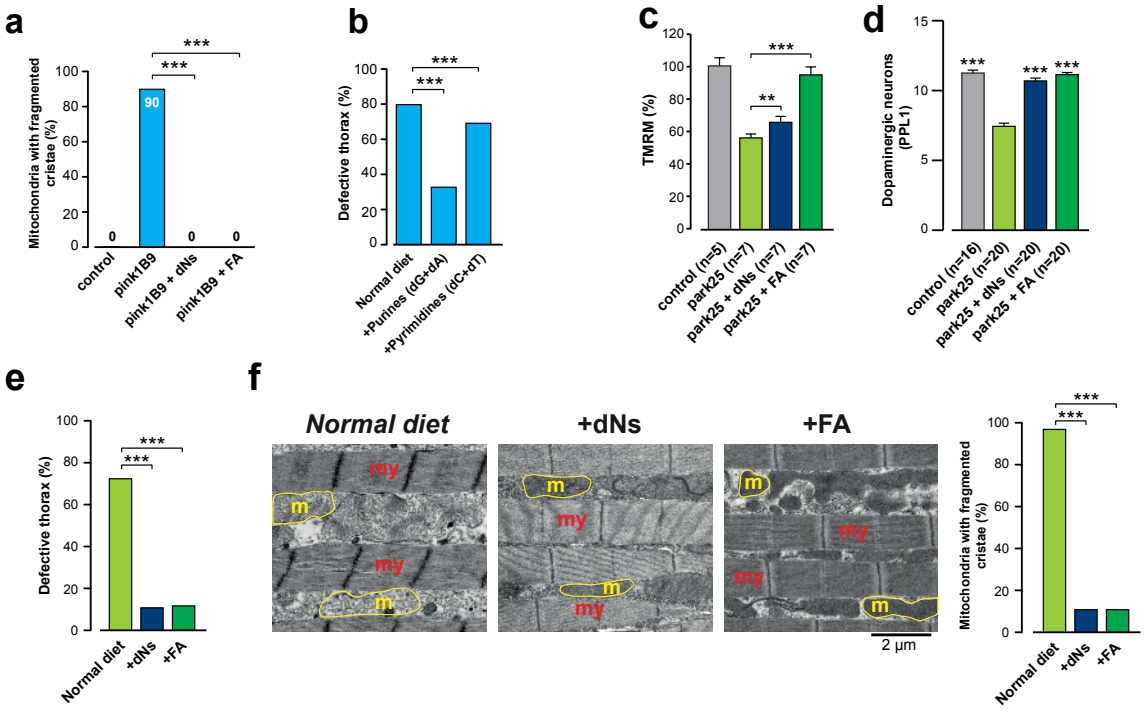

Supplementary Figure 7 Effects of dietary supplementation with dNs or FA in pink1 and parkin mutants. Related to Fig. 7. (**a**) Percentages of indirect flight muscle mitochondria exhibiting fragmented cristae (numbers on the bar) are presented for the indicated genotypes. Asterisks indicate statistical significance (chi-square, two-tailed, 95% confidence intervals), n = 140 for control, n = 469 for pink1B9, n = 226 for pink1B9+dNs, n = 118 for pink1B9+FA. (**b**) Dietary supplementation with purine rescues the thoracic defects of *pink1* mutants more effectively than those with pyrimidines. *pink1* mutants were exposed to a purine (dG+dA) or pyrimidine (dC+dT)-supplemented diet after egg laying. *P*-values are indicated (chi-square, two-tailed, 95% confidence intervals), n = 129 for normal diet, n = 194 for purines, n = 404 for pyrimidines. (**c**) dNs or FA reverse the loss of $\Delta\psi$m in *parkin* mutants. The $\Delta\psi$m is represented as percentage of control. The error bars represent the mean ± SEM (n values are indicated). Statistical significance is indicated by asterisks (two-tailed paired *t* test). Datasets labelled "control" and "park25" are also used in Supplementary Fig. 5a (**d**) dNs or FA reverse the loss of dopaminergic neurons in the PPL1 cluster of *parkin* mutant flies. Data are shown as the mean ± SEM (n values are indicated), and the asterisks indicate statistically significant values (one-way ANOVA with Bonferroni's multiple comparison test). Datasets labelled "control" and "park25" are also used in Supplementary Fig. 5b (**e**) Dietary supplementation with dNs or FA rescues the thoracic defects of *parkin* mutants. *parkin* mutants were exposed to a dNs or FA-supplemented diet after egg laying (n = 355 for normal diet, n = 96 for +dNs and n = 66 for +FA). *P*-values are indicated (chi-square two-tailed, 95% confidence intervals). (**f**) Dietary supplementation with dNs or FA rescues the flight muscle defects observed in *parkin* mutants. Ultrastructural analysis of the indirect flight muscles showed that *dNK* expression rescues mitochondrial defects in *park25* mutants (my, myofibrils; m, mitochondria; yellow outlines, mitochondria). Percentages of indirect flight muscle mitochondria exhibiting fragmented cristae are presented for the indicated diets (n = 174 for normal diet, n = 130 for +dNs, n = 278 for +FA). Asterisks indicate statistical significance (chi-square, two-tailed, 95% confidence intervals).

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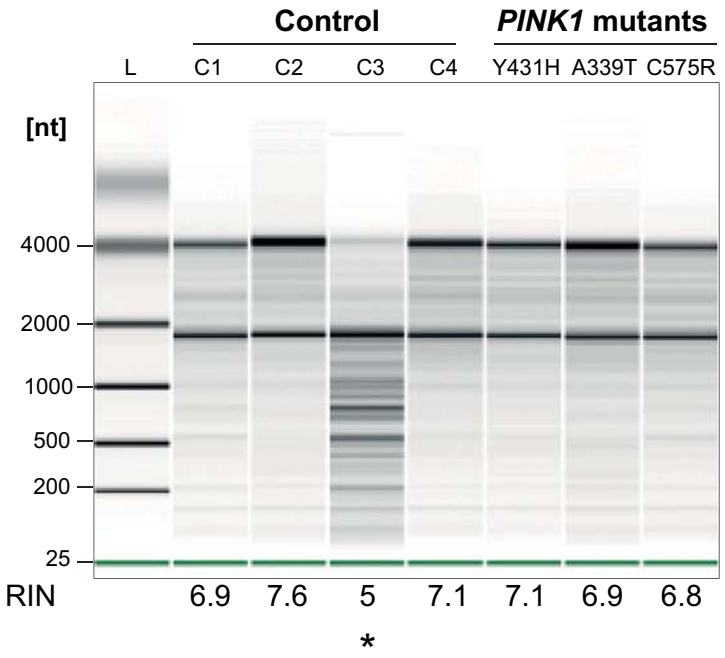

Supplementary Figure 8. Analysis of RNA quality in human post-mortem brain samples. Related to Fig. 8. Agilent 2100 Bioanalyzer digital gel of total RNA from human brains. A high-quality sample appears as two distinct bands corresponding to the 18S and 28S ribosomal RNAs. Smearing of these bands is indicative of degradation. Lanes: L, ladder; C1-C4 control brains; Y431H, A339T, C575R, *PINK1* brains carrying mutant *PINK1*. Between 300 and 350 nanograms of RNA were applied to each non-ladder lane. The asterisk indicates the sample excluded from qRT-PCR analysis due to excessive RNA degradation (low RIN score).

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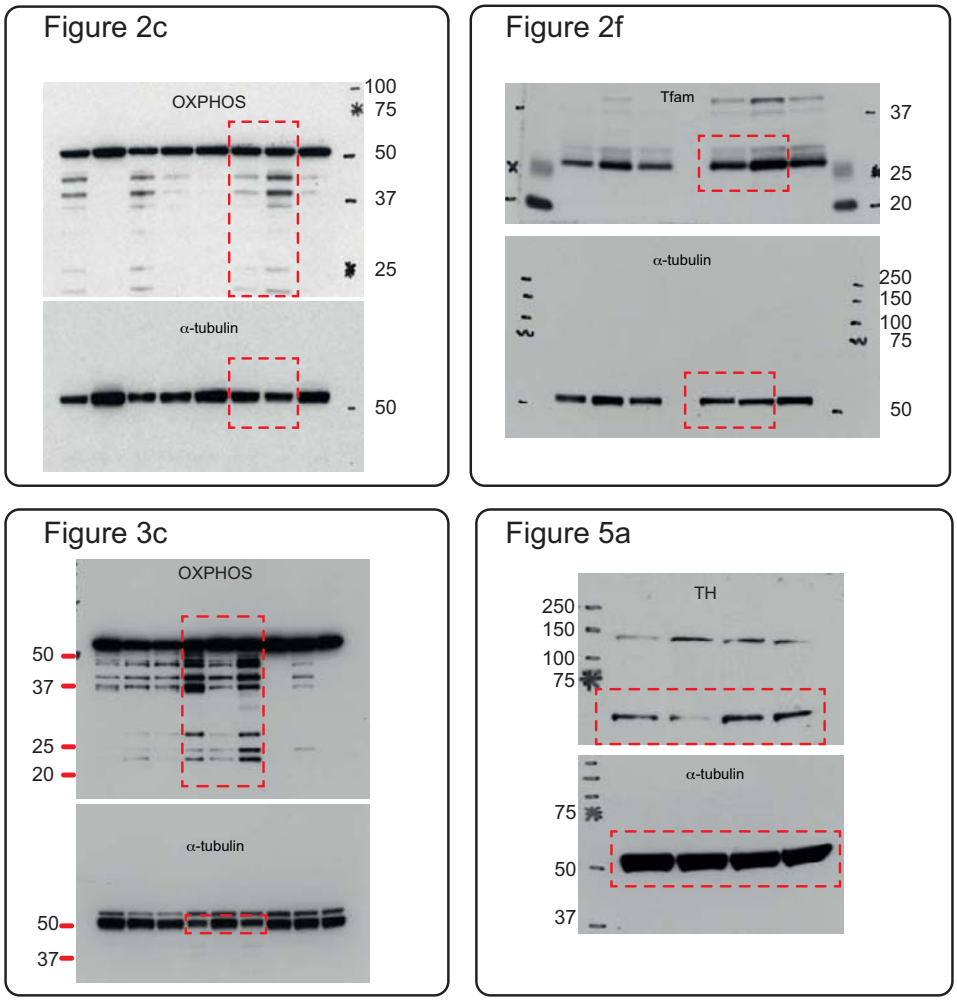

Supplementary Figure 9. Full scans of immunoblots shown in the main figures Dashed boxes correspond to the cropped areas used in the corresponding main figure.

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