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Supplementary Information for

Metformin rescues Parkinson's disease phenotypes caused by hyperactive mitochondria

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Datasets S1 to S2

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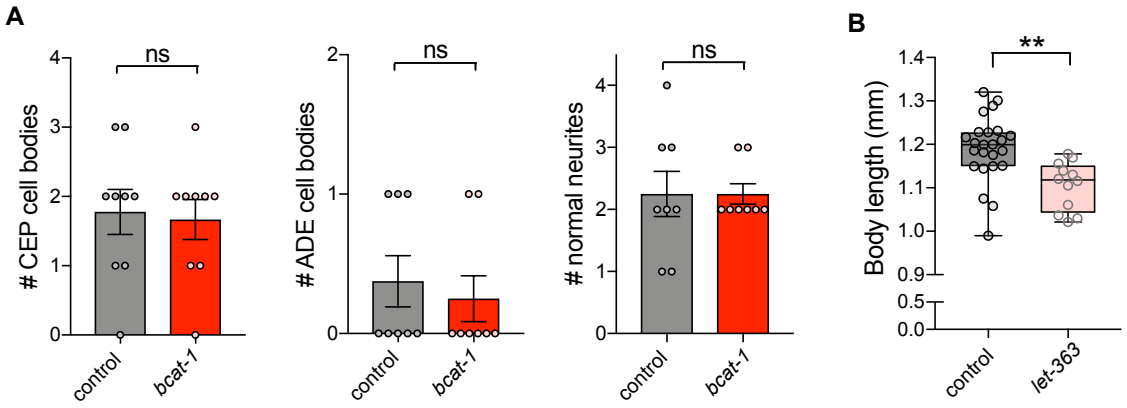

Fig. S1. A, α-synuclein-expressing dopaminergic neurons with or without *bcat-1* knockdown are largely degenerated by day 8. n=9 each for control and *bcat-1* CEP*,* 8 each for control and *bcat-1* ADE and neurites. Data are mean±s.e.m. **B**, Whole-life *let-363* RNAi causes a growth defect in TU3311 worms, measured on day 1 of adulthood. n=24 for control, 12 for *let-363*. Two-tailed *t*-tests. ns, not significant. ***P*<0.01. Box-plots show minimum, 25th percentile, median, 75th percentile, maximum.

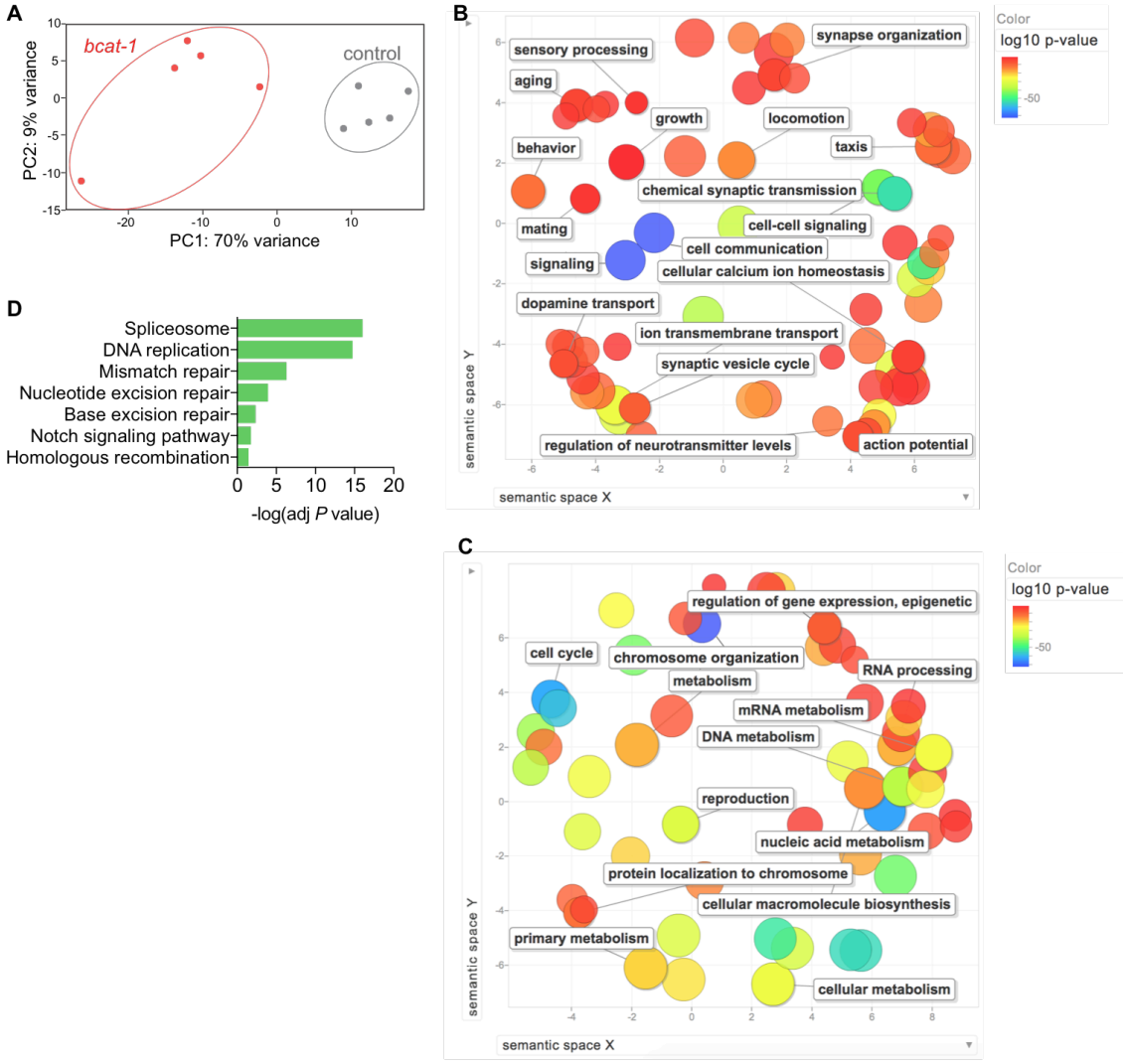

Fig. S2. A, PCA plot of neuronal transcriptomes from worms with *bcat-1* knockdown and controls on day 5. **B-C**, Gene Ontology analysis of significantly upregulated (**B**) and downregulated (**C**) genes in *bcat-1(RNAi)* neurons. **D**, KEGG pathway analysis of significantly downregulated genes in *bcat-1(RNAi)* neurons. FDR<0.05. n=5 independent collections/group.

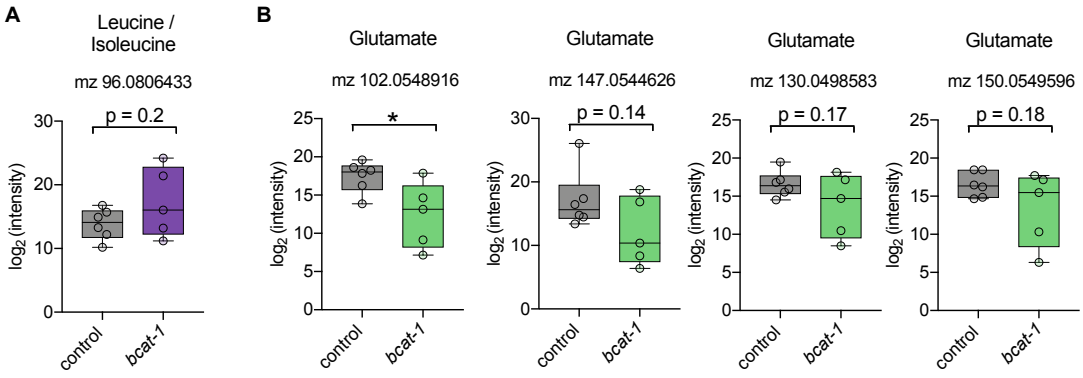

Fig. S3. A-B, Levels of additional features putatively annotated by mummichog as leucine/isoleucine (**A**) or glutamate (**B**) in *bcat-1(RNAi)* worms and controls. n=6 independent collections for control, 5 for *bcat-1.* Two-tailed *t*-tests. **P*<0.05. Box-plots show minimum, 25th percentile, median, 75th percentile, maximum.

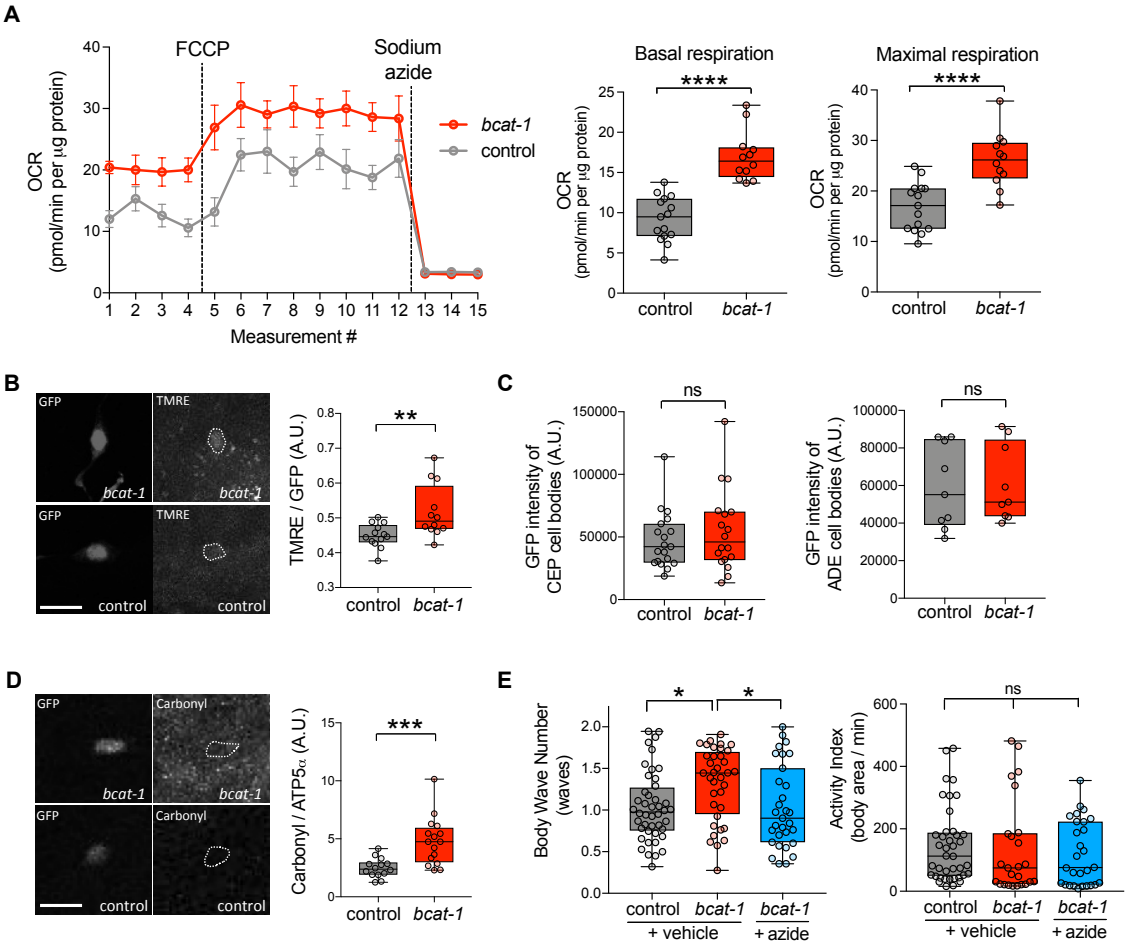

Fig. S4. A, Mitochondrial respiration was increased on day 5 in N2 worms (which are refractory to RNAi in neurons) fed *bcat-1* RNAi. n=15 wells totaling 152 worms for control, 12 wells totaling 111 worms for *bcat-1*. Two-tailed *t*-tests. **B**, Mitochondrial activity was increased on day 5 in ADE α-synuclein-expressing dopaminergic neurons with *bcat-1* knockdown. Scale bar, 10µm. n=13 worms totaling 13 ADEs for control, 11 worms totaling 12 ADEs for *bcat-1*. Two-tailed *t*-test. **C**, GFP intensity (used for normalization in each TMRE experiment) was unchanged by *bcat-1* knockdown in α-synuclein-expressing dopaminergic neurons, as measured on day 5. n=9 worms totaling 19 CEPs for control, 10 worms totaling 18 CEPs for *bcat-1*, and 9 worms totaling 9 ADEs each for control and *bcat-1*. Two-tailed *t*-tests. **D**, Protein carbonylation was increased on day 8 in ADE α-synuclein-expressing dopaminergic neurons with *bcat-1* knockdown. Scale bar, 10µm. n=10 worms totaling 14 ADEs for control, 10 worms totaling 16 ADEs for *bcat-1*. Two-tailed *t*-test.

E, *bcat-1(RNAi)* worms (strain CF512) treated with 100 μM sodium azide starting on day 4 had improved body wave number on day 8, while total activity levels remained unchanged. n=28 worms for control, 41 for *bcat-1*+vehicle, 30 for *bcat-1*+azide. One-way ANOVA with Tukey's post-hoc. A.U., arbitrary units. ns, not significant. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*< 0.0001. Box-plots show minimum, 25th percentile, median, 75th percentile, maximum.

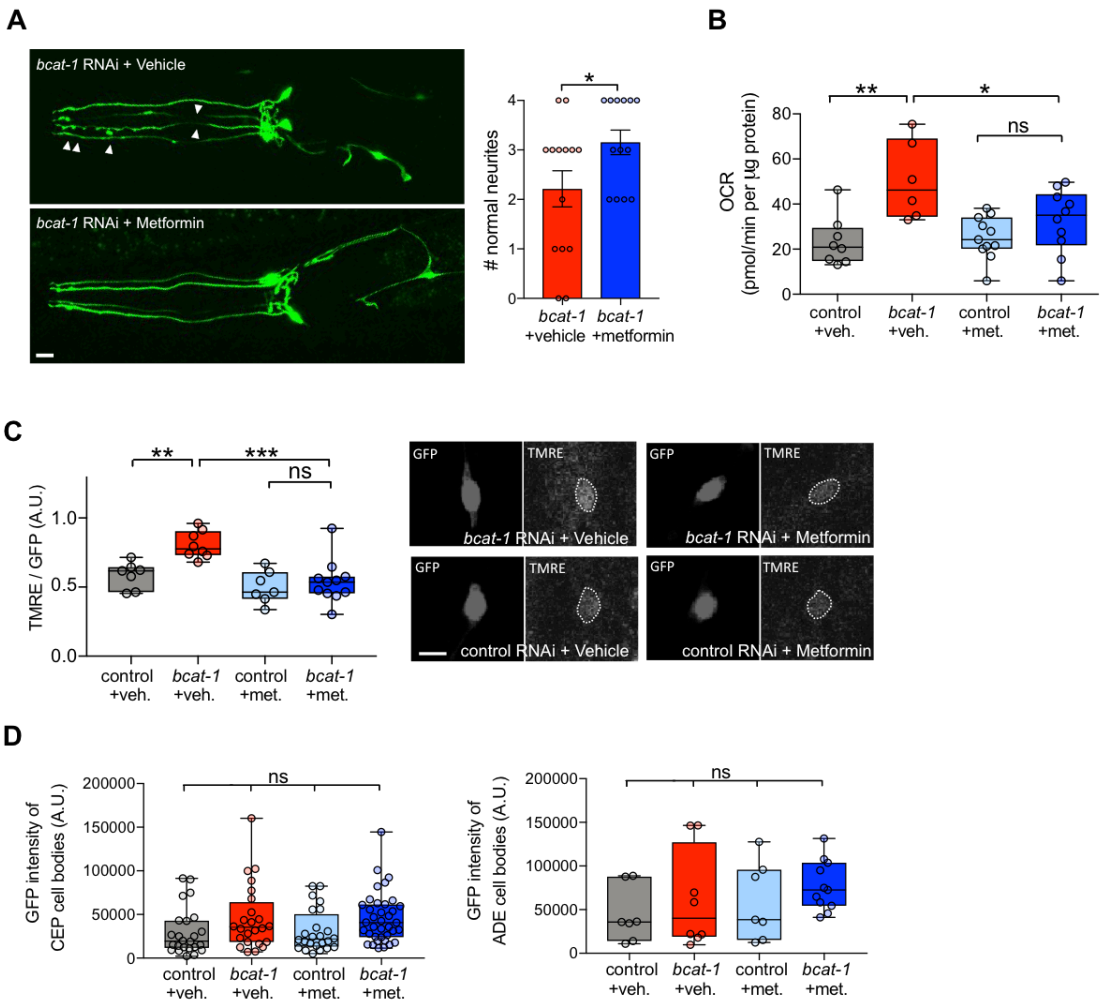

Fig. S5. A, Metformin reduced degenerated morphologies (blebbing and fragmentation, arrowheads) in α-synuclein-expressing dopaminergic neurites with *bcat-1* knockdown, as measured on day 6, following only two days of treatment. Scale bar, 10µm. n=14 for vehicle, 13 for metformin. Two-tailed *t*-test. Data are mean±s.e.m. **B**, Metformin reduced maximal mitochondrial respiration in *bcat-1(RNAi)* worms (strain CF512) on day 8. n=8 wells totaling 119 worms for control vehicle, 6 wells totaling 50 worms for *bcat-1* vehicle, 11 wells totaling 139 worms for control metformin, 10 wells totaling 123 worms for *bcat-1* metformin. Two-way ANOVA with Tukey's post-hoc. **C**, Metformin reduced mitochondrial activity in α-synuclein-expressing ADE dopaminergic neurons with *bcat-1* knockdown, as measured on day 6. Scale bar, 5µm. n=7 worms totaling 7 ADEs for control vehicle, 7 worms totaling 8 ADEs for *bcat-1* vehicle, 7 worms

totaling 7 ADEs for control metformin, 10 worms totaling 11 ADEs for *bcat-1* metformin. Two-way ANOVA with Tukey's post-hoc. **D**, GFP intensity (used for normalization in each TMRE experiment) was unchanged by either *bcat-1* knockdown or metformin treatment in α-synuclein-expressing dopaminergic neurons, as measured on day 6. n=8 worms totaling 24 CEPs for control vehicle, 7 worms totaling 24 CEPs for *bcat-1* vehicle, 9 worms totaling 24 CEPs for control metformin, 10 worms totaling 37 CEPs for *bcat-1* metformin, 7 worms totaling 7 ADEs for control vehicle, 7 worms totaling 8 ADEs for *bcat-1* vehicle, 7 worms totaling 7 ADEs for control metformin, 10 worms totaling 11 ADEs for *bcat-1* metformin. Two-way ANOVAs. A.U., arbitrary units. met., metformin. ns, not significant. veh., vehicle. **P*<0.05, ***P*<0.01, ****P*<0.001. Box-plots show minimum, 25th percentile, median, 75th percentile, maximum.

Dataset S1 (separate file). Differentially-expressed genes in *bcat-1(RNAi)* neurons. Lists of all genes significantly up- or downregulated in *bcat-1(RNAi)* neurons (FDR<0.05), as well as subsets of each that were previously identified as neuronally-expressed (1).

Dataset S2 (separate file). Annotated features significant at p<0.05 from metabolomics analysis of *bcat-1(RNAi)* worms. m.z, mass-to-charge ratio; retention time, retention time off of the column; KEGG.ID, KEGG ID determined by mummichog hosted on metaboanalyst; Annotation, Compound name from KEGG. Level 5 confidence in annotation (2). Adduct, Metabolite adduct annotated; Mass.Diff, Difference in mass of adduct detected and true mass of adduct.

SI References

1. R. Kaletsky *et al.*, Transcriptome Analysis of Adult Caenorhabditis elegans Cells Reveals Tissue-specific Gene and Isoform Expression. *PLoS Genet.* **14**, e1007559 (2018).
2. E. L. Schymanski *et al.*, Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ. Sci. Technol.* **48**, 2097–8 (2014).