Supplemental methods

RT-PCR, Ret expression

3rd instar larvae, thoraces of pharate stage P11 pupae and 3 day old adult animals, or dissected IFMs of 3 day adult animals were homogenized in RLT buffer (Qiagen) using a rotor-stator homogenizer. Total RNA was prepared using the RNeasy mini kit and the QIAshredder column (Qiagen). RT-PCR analysis was performed using the OneStep RT-PCR kit (Qiagen) using 20 ng of template RNA and 40 cycles of PCR amplification. See below for list of primers.

Fly strains

UAS-Ret^{WT} was generated by reverse mutagenesis of a *UAS-Ret^{MEN2B}* expression construct (kindly provided by Ross Cagan), after which Ret^{WT} cDNA was subcloned into a pUAST_attB vector using Sph1 and Not1, and injected into the attP40 strain according to standard procedures (Genetic Services, Inc.).

Antibodies

For the western blot analysis, antibodies used were anti-NDUFS3 (Abcam #ab14711) and anti-Pyruvate dehydrogenase (Abcam #ab110334), anti-PINK1 (Novus Biologicals #BC100-494), anti-GAPDH (#Sigma-Aldrich G8795), panRet (provided by C. Ibanez) and alpha-Tubulin (clone DM1A, Sigma).

Cell Culture, treatments and RNA Interference

HeLa cells (ATCC number CCL-2) were cultured and transfected for RNAi as described previously (Exner et al). PINK1 (VHS50790, Invitrogen) or medGC control siRNA oligos (Invitrogen) were transfected using Lipofectamine RNAiMAX (Invitrogen). Wt-Ret9 (kindly provided by Marc Billaud) was overexpressed using Lipofectamine 2000. For stimulation of Ret, cells were incubated with hGDNF (50ng/ml) and hGFRα-1 (50ng/ml) for 24 hours.

Assessment of mitochondrial morphology

Mitochondrial morphology of Hela cells was assessed generally as described previously (Exner et al, 2007). Briefly, Mitotracker Green FM (200 nm) (Invitrogen) was added to the cells and incubated for 15 minutes to label mitochondria. Coverslips with live cells immersed in PBS supplemented with Ca^{2+} and Mg^{2+} were analyzed using a Zeiss Axioplan fluorescence microscope with a 63x objective. Random fields were selected and within each field, all cells were classified and counted by the morphology of the mitochondria as “tubular” or fragmented”.

List PCR primers

Primer	5’ to 3'
Drosophila genomic	
Pink1B9-F	CGACCAAATCGAATCGAAAC
Pink1B9-R	CCAGACTAACGCCAATCAG
Pink1-F	TCTGTGAGACTGCTGACCGT
Pink1-R	AAGATTCCACTGCTGCTGGT
Park1-F	GTTCCCGTTTGGGCGAGCGT
Park1-R	CACCCAAGGCTCTGCTCCCACAAT
Park25-F	GCAAATCGGCAGCCACGCAG
Park25-R	GCCTCATCCCATCGCGCCTC
Park-F	GCTGGTGTTTGGCAACGCGTAAG
Park-R	GGTCAGCGTTTTGCCCGTGT
RetcDNA-F	ATGCCAAGGCAGGGCAGCTG
RetcDNA-R	GTGGCAGTCGGGCCGAAACA
GAL80-F	GGCGGTTGGTACGGCTACGA
GAL80-R	GTCTTTGGGACTCGCTGGCCC
Drosophila RT-PCR	
CG10320_F	TAAGTCAACTTACAGCAATGGGC
CG10320_R	ATAGCGCCAGACTTCGTTCC
CG11423_F	TATACGGACGGCACGATTGG
CG11423_R	AGTACCGGGCTCTCCTTCAT
CG11455_F	CTTCCATGCAATGCGAAACGA
CG11455_R	GGGTCTCCGTCTAGTAGGCA
CG11752_F	ACCCAACACGTCCACTTTGT
CG11752_R	CACGCAATTGCTGGTCAGTC
CG11913_F	TCCGGTAATCATGTCTGGCG
CG11913_R	GGCTCTTCGGGGCAATCTTC
CG12079_F	GGATAAGCCCACTGTCCGC
CG12079_R	GTACCAGTTGGCCGCCTTAT
CG12203_F	AAGCCGATTTCTGCCGTGTA
CG12203_R	CTGGTACAGCTGCAGGGAGG
CG12400_F	GCTAACTGCGTTAGTTGCCC
CG12400_R	CTTCTGGATGCCGGAAAACAC
CG12859_F	ACCTGCCAGCTGTTGCTAAA
CG12859_R	AATCCCGCATCGAAAACGGT
CG13240_F	GGAGCTGAACAACCCCATCA
CG13240_R	CCTTCCGGGTCCAGTCATTC
CG15434_F	ACAGTTTGACAACCCTGCGT
CG15434_R	CCACGTATTCCCTGACTCCC
CG18624_F	AAAAACGACTCGACAGCCCT
CG18624_R	CCACAGTGCTCGCTTATCCA
CG1970_F	AGAACCATTATAATTCCGATTGCGG
CG1970_R	CGTCTGGATACATCACCGGG
CG2286_F	CGGTCGGTCTGTCAAAGCAA
CG2286_R	GATCTGAGCAGCAGCCTGG
CG3192_F	TGGCTGGCTGGAATAAGGAC
CG3192_R	CCGAGATGGGCTCGTGTTG
CG3214_F	ACTAAGGAGGCCTACTACCCC
CG3214_R	TGTTCTTCGAGTGCAGAGTCA
CG32230_F	TCAAATCCCGAACCATGGCA
CG32230_R	AGCTTCCACAAGCTTTCAAATCC
CG34063_F	TCTTGGTTAGGAGCTTGAATAGGT
CG34063_R	TCAAAAATGGAAAGGAGCGGC
CG34076_F	GCTTTAATCGACCGAGA
CG34076_R	TGGATCAAATCCACATTCAA
CG34085_F	TGAAGCTCCAGTTTCTGGGT
CG34085_R	TGAGCAACAGATGAATAAGCAA
CG34089_F	TCATCCATTAGCTTTAGGATTAACTTT
CG34089_R	TTTCATTAGAGGCTAAAGATGTTACG
CG34092_F	ATTCGTAAAGGTCCTAATAAAGTTG
CG34092_R	ATAATTTAATCGCATCACAAAAAGG
CG34439_F	CCCTACAAGATGAACGGTCTTGA
CG34439_R	GAGAGCTCCACTGGCGAAAT
CG3446_F	TTTACCCTGCCCCAAGTCAG
CG3446_R	TGGTGGTGAAGCCTGTGAAG
CG3621_F	CCTGGGTCGGGAGCACAAC
CG3621_R	ATCCCGCTGGCAGTAGTAGT
CG3683_F	AGCTGTTGTGTTGTTTTTGGTTG
CG3683_R	GCATTGCTTGCCCAAATGGA
CG3944_F	AGAACATGCCCTTCGTCGTT
CG3944_R	GGGACAGGCTTCCTGACAGA
CG40002_F	GAGGAGCTATGTGGTGGTGG
CG40002_R	TGTAACTACATGCCTACATATCGCA
CG40472_F	GGGAAATACCGCAGTAGGCA
CG40472_R	GGGGCCATTGCGGTAAGATA
CG5548_F	CAATTTACGATGGGCAACGC
CG5548_R	GACTCCATCTCCTCCTGGGT
CG5703_F	TGGAGACCTGCAAGAAGCAG
CG5703_R	TCCTTGGATGTCAGATCCTCATAG
CG6008_F	GCAGCTTCTACTGGGCCTAC
CG6008_R	CGCACTTCGCATTCGCATTA
CG6463_F	GTGGCCCAACACAAGGACAT
CG6463_R	CTCCATGATCTGGGCTGGTG
CG6485_F	ATGCAGTTCACGCTCAAGGA
CG6485_R	GGTGGTAAGTCCACCCTTGG
CG6914_F	AAGAAGATGCTCACCGCCAA
CG6914_R	ATGCACCTTTCCTGGAGTCG
CG7598_F	GGATGTGCTGGACAAGTGGA
CG7598_R	CGCTTAAAGGATTTCCGCACT
CG7712_F	GCTGGTCATCAAGGGCCAAA
CG7712_R	AAGGACACACGCGTTTCTGA
CG8680_F	AGAACTGGGGCATCAAGCTC
CG8680_R	TTTCCGGGTTTGTCCAGGTT
CG8844_F	ACGAACTGCGGTCCTACAAC
CG8844_R	CTCCACAATGCTCTCACGGA
CG9140_F	CAAAGCCGCAGATTGTTGCG
CG9140_R	TCATCGGCATTCACCACCAA
CG9160_F	CAGCCAGTTGAGCCAAGAGTG
CG9160_R	CGTGGTCCAAGGAATCCAGT
CG9172_F	GCGGTCACATCATACGCATC
CG9172_R	GCAGCATGTTGATCCTGCTAT
CG9306_F	ATATTTTCAGGCGCAGGGCT
CG9306_R	GTAGACATTGCGTCGGTCGT
CG9762_F	GTACGAGAAGCACCCCATCT
CG9762_R	TAAGATGATGTTGCGAAGTCGAT
GAPDH_F	CAAGCAAGCCGATAGATAAAC
GAPDH_R	CAAGTGAGTGGATGCCTTGT
RetcDNA-F	ATGCCAAGGCAGGGCAGCTG
RetcDNA-R	GTGGCAGTCGGGCCGAAACA
Human RT-PCR	
human PINK1_F	CGGTCTCGGCCTGTCAGGAG
human PINK1_R	GCCCTGCAAGCGTCTCGTGT
human Ret_F	CGGACATCAGCAAAGACC
human Ret_R	GCCGTCGTCATAAATCAGG
human β-actin_F	TGGACTTCGAGCAAGAGA
human β-actin_R	AGGAAGGAAGGCTGGAAGAG

References

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