Supplemental Materials and Methods

Drosophila genetics

Fly culture and crosses were performed on standard fly food containing yeast, cornmeal and molasses and raised at 25°C unless otherwise indicated. For making UAS-dLRRK transgenic lines, dLRRK cDNA obtained by RT-PCR from adult Drosophila poly A+ RNA (Clontech) was subcloned into the pUAST vector. PD-associated point mutations of dLRRK were generated by site-directed mutagenesis. For making dLRRK RNAi lines, a genomic DNA/cDNA hybrid construct was generated as described (Kalidas & Smith, 2002). For making UAS-d4E-BP TA transgenic lines, the d4E-BP T37/46A mutant generated by site-directed mutagenesis was subcloned into the pUAST vector. Introduction of transgenes into Drosophila germ line and establishment of transgenic lines were performed in a w- background using standard methods. A chromosomal deficiency line covering dLRRK was obtained from the Bloomington Drosophila Stock center (Stock# 4962). The P-element insertion line for dLRRK mutant was a gift from Dr. Zhuohua Zhang at Burnham Institute (San Diego, CA, USA) and was backcrossed to w- for six generations to remove any background mutations. The appearance of dLRRK homozygous mutant from our fresh fly food is largely normal. However, flies with the malformed abdomen shown in Figure 1C often come up later from fly food exhausted by earlier cohort of larvae. The phenotype seems not all-or-none but rather graded. We collected only earlier eclosed flies (within ~7-days from the first eclosion of flies) with normal abdominal appearance for further analyses. All other general fly stocks and GAL4 lines were obtained from the Bloomington Drosophila stock center. The other flies were described before: Thor” (4E-BP null) and revertant (Bernal & Kimbrell, 2000); UAS-4E-BP and UAS-4E-BP(LL) (Miron et al, 2001); UAS-TSC1-TSC2 (Potter et al, 2001); UAS-Rheb (Saucedo et al, 2003); UAS-eIF4E (Sigrist et al, 2000); eIF4E07238 (eIF4E null) (Lachance et al, 2002).

Antibodies

Rabbit anti-dLRRK polyclonal antibody was raised against recombinant GST-N-terminal dLRRK (1 - 336 aa) produced in the E. coli strain, BL21(DE3)pLysS (Novagen). Anti-Drosophila β-Tubulin (E7) antibody was obtained from the Hybridoma Bank at University of Iowa. Anti-a-Tubulin (DM1A), anti-β-Tubulin
(Tub2.1) and anti-FLAG (M2) antibodies were purchased from Sigma. Anti-human eIF4E (#9742), anti-phospho-4E-BP (#9459, #9451, #9455) and anti-mTOR (#2971, #2972, #2974) antibodies were obtained from Cell Signaling. Anti-Actin (MAB1501) and anti-hLRRK2 (NB-300-268) were purchased from Chemicon and Novus, respectively. Anti-4-HNE (HNEJ-2) antibody was from Japan Institute for the Control of Aging (http://www.jaica.com) (Hung et al, 2001; Okada et al, 1999; Tam et al, 2006). *Drosophila* anti-4E-BP and anti-eIF4E antibodies were gifts from Drs. Nahum Sonenberg and Paul Lasko, respectively. Mouse anti-TH monoclonal antibody was purchased from ImmunoStar, and rabbit anti-*Drosophila* TH polyclonal antibody was described previously (Yang et al, 2006).

**RT-PCR, plasmids and siRNAs**

For RT-PCR analysis, a one-step RT-PCR kit from Clontech and total RNAs extracted from fly heads was used for PCR amplification. To generate 6xHis-tagged h4E-BP1, 6xHis-d4E-BP and GST-delIF4E for bacterial expression, we amplified the corresponding coding sequences from EST clones and cloned them into pET28a, pET45b or pGEX6P-1 vectors. Human *LRRK2* cDNA was purchased from Origene and the FLAG-tag sequence was then attached. *dLRRK* cDNA was also inserted into the pcDNA3-FLAG-N vector. Introduction of mutations was performed using QuikChange II XL Site-directed mutagenesis kit (Stratagene). Kinase-dead forms of *dLRRK/LRRK2* (3KD) were generated by the introduction of triple mutations (K1781M, D1882A and D1912A in *dLRRK*; K1906M, D1994A and D2017A in *hLRRK2*). All PCRs were performed with *Pfu* turbo DNA polymerase (Stratagene) and the accuracy of PCR product sequence confirmed by DNA sequencing. Human *LRRK2* and control non-targeting siRNA were purchased from Dharmacon.

**Cell culture**

Primary neuronal culture from *Drosophila* embryos was performed as described previously (Yang et al, 2003).

**ROS assay**

ROS assay with 2, 7-dichlorofluorescein diacetate (DCFH-DA) was performed as
described previously (Yang et al, 2005). For each genotype we dissected three fly brains per experiment and each ROS assay was repeated three times.

**Scanning electron microscopic (SEM) analysis**

Adult flies were directly soaked in 100% ethanol. After several changes of ethanol, the whole flies were processed following procedures described before (Nishimura et al, 2004). Area calculation of the eye surface and wing tissues was performed using Adobe Photoshop.

**Pseudopupil analysis**

Two-day-old flies were analyzed as described previously under LEICA DM LB microscope with x100 oil objective (Steffan et al, 2001). Thirty ommatidia in three flies were examined, and the number of visible rhabdomeres was counted for each.
References


Drosophila. *Neuron* **37**: 911-924