SI Materials and Methods

Nematode Strains. For neuroprotection analysis, three stable lines of either

UA53 [*baEx42*; P_{dat-1}::FLAG-C35D10.2, P_{unc-54}::DsRed2],

UA54 [*baEx43*; P_{dat-1}::FLAG-C54H2.5, P_{unc-54}::DsRed2],

UA55 [*baEx44*; P_{dat-1}::FLAG-F16A11.2, P_{unc-54}::DsRed2],

UA56 [*baEx45*; P_{dat-1}::FLAG-F32A6.3, P_{unc-54}::DsRed2],

UA57 [*baEx46*; P_{dat-1}::FLAG-F55A4.1, P_{unc-54}::DsRed2],

UA58 [*baEx47*; P_{dat-1}::FLAG-M7.5, P_{unc-54}::DsRed2], and

UA59 [*baEx48*; P_{dat-1}::FLAG-R05D11.6, P_{unc-54}::DsRed2] were crossed with integrated UA44 [*baInl1*; P_{dat-1}::α-syn high, P_{dat-1}::gfp] (1).

Male UA44 [*baInl1*; P_{dat-1}::α-syn high, P_{dat-1}::gfp] worms were generated by mating hermaphrodites with male N2 worms. GFP-positive males were crossed with hermaphrodites overexpressing candidate genes in DA neurons and DsRed2 in body wall muscle cells. The resulting GFP- and dsRed2-positive hermaphrodites were individually picked, and self-fertilized until all worms displayed GFP expression indicating homozygous expression of α-syn. These strains are designated as follows:

UA60 [*baEx49*; P_{dat-1}::FLAG-C35D10.2, P_{unc-54}::DsRed2, P_{dat-1}:: α-syn high, P_{dat-1}::gfp],

UA62 [*baEx51*; P_{dat-1}::FLAG-C54H2.5, P_{unc-54}::DsRed2, P_{dat-1}:: α-syn high, P_{dat-1}::gfp],

UA64 [*baEx53*; P_{dat-1}::FLAG-F16A11.2, P_{unc-54}::DsRed2, P_{dat-1}:: α-syn high, P_{dat-1}::gfp],

UA66 [*baEx55*; P_{dat-1}::FLAG-F32A6.3, P_{unc-54}::DsRed2, P_{dat-1}:: α-syn high, P_{dat-1}::gfp],

UA68 [*baEx57*; P_{dat-1}::FLAG-F55A4.1, P_{unc-54}::DsRed2, P_{dat-1}:: α-syn high, P_{dat-1}::gfp],

UA70 [*baEx59*; P_{dat-1}::FLAG-M7.5, P_{unc-54}::DsRed2, P_{dat-1}:: α-syn high, P_{dat-1}::gfp], and

UA72 [*baEx61*; P_{dat-1}::FLAG-R05D11.6, P_{unc-54}::DsRed2, P_{dat-1}:: α-syn high, P_{dat-1}::gfp]

Plasmid Constructs. Plasmids were constructed using Gateway Technology (Invitrogen). To generate α-syn::gfp, α-syn cDNA (a gift from Philipp Kahle, University of Tubingen, Germany) was cloned into a gfp-containing plasmid, pPD95.75 (Andy Fire, Stanford University) by double digestion using *XbaI* and *BamHI*. Gateway entry vectors were generated by cloning PCR-amplified α-syn::gfp and gfp as well as candidate cDNAs into pDONR201 or pDONR221 by BP reaction. The cDNAs encoding

C35D10.2, C54H2.5, F16A11.2, F55A4.1, M7.5, and R05D11.6 were obtained from Open Biosystems (Huntsville, AL) while F32A6.3 was isolated from our *C. elegans* cDNA library (Addgene). DsRed2 was obtained from Clontech (Mountain View, CA). An N-terminal FLAG tag sequence was added during the PCR amplification process.

The gene fusions were shuttled from entry vectors into the Gateway destination vector, pDEST-DAT-1 (2) or pDEST-UNC-54 via LR reaction. pDEST-UNC-54 was generated by converting a *unc-54* promoter containing plasmid, pPD30.38 (Andy Fire), using a Gateway Vector Conversion System (Invitrogen). The molecular cloning yielded expression plasmids, $P_{unc\text{-}54}$:: α-syn::gfp, $P_{unc\text{-}54}$::gfp, $P_{dat\text{-}1}$::FLAG-C35D10.2, $P_{dat\text{-}1}$::FLAG-C54H2.5, $P_{dat\text{-}1}$::FLAG-F16A11.2, $P_{dat\text{-}1}$::FLAG-F32A6.3, $P_{dat\text{-}1}$::FLAG-F55A4.1, $P_{dat\text{-}1}$::FLAG-M7.5, $P_{dat\text{-}1}$::FLAG-R05D11.6, and $P_{unc\text{-}54}$::DsRed2. The cDNAs were verified by DNA sequencing.

RNAi screen and analysis of α-syn misfolding or polyglutamine aggregation. RNAi feeding clones (Geneservice, Cambridge, UK) were grown for 14 h in LB culture with 100 mg/ml ampicillin and seeded onto NGM agar plates containing 1 mM isopropyl β-D-thiogalactoside. When the bacterial lawn was grown, five L4 hermaphrodites (strain UA52) were transferred onto the plates and incubated at 25°C for 44 h. The gravid adults were then placed onto the corresponding RNAi plates and allowed to lay eggs for 9 hours, and the resulting age-synchronized worms were analyzed at the indicated stage. For polyglutamine aggregation analysis, L3-staged 20 worms were transferred onto a 2% agarose pad and immobilized with 2 mM levamisole, and the quantity of aggregates was scored. The aggregation analysis was also conducted in duplicate.

Western blotting. For all worm strains, 30 μg/μl protein was loaded into 15% SDS PAGE gels (Bio-Rad) and detected by 1:2000 goat anti-α-syn primary antibody (Chemicon) and 1:10000 horseradish peroxidase-conjugated mouse anti-goat IgG secondary antibody (Pierce). For detection of actin, 1:8000 mouse anti-actin primary antibody (ICN Biochemicals) and 1:10000 horseradish peroxidase-conjugated sheep anti-mouse IgG secondary antibody (Amersham) was utilized.

RNA isolation and semi-quantitative RT-PCR. Total RNAs from 50 L4-staged worms were isolated using TRI Reagent (Molecular Research Center). Briefly, the worms were transferred into 10 μl 1:10-diluted Single Worm Lysis Buffer (10 mM Tris, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% NP-40, 0.45% Tween 20, 0.01% gelatin, and 60 μg proteinase K), mixed with 100 μl TRI Reagent, and incubated at RT for 10 min. The samples were freeze-thawed 5 times using liquid N_2, vortexed with 10 μl 1-bromo-3-chloropropane (Acros Organics) for 15 sec, incubated at RT for 10 min, and centrifuged at 14500 rpm at 4°C for 15 min. The supernatant was transferred to a new RNase-free tube, mixed with 2 μl glycoblue (Ambion) and 50 μl -20°C-chilled isopropanol, and incubated at -20°C overnight. After incubation, the supernatant was centrifuged at 14500 rpm at 4°C for 15 min, and discarded. The pellet was washed with 100 μl RNase-free 75% ethanol, and resuspended in 10 μl DEPC-treated water. For RT-PCR using SuperScript III RT (Invitrogen) with oligo dT primers, the total RNAs were treated with amplification grade RNase-free DNase I (Invitrogen) as well as RNase H (Invitrogen) following the manufacture's protocol. PCR was then performed using Phusion polymerase (Finnzymes). The PCR products were separated by 0.8% agarose gel electrophoresis and visualized by GelRed staining (Biotium). The following primers were designed for the PCR:

cdk-5	Primer 1:	5' ggg-gat-gat-gag-ggt-gtt-cca-agc 3'
	Primer 2:	5' ggc-gac-cgg-cat-ttg-aga-tct-ctg-c 3'
α-syn	Primer 1:	5' atg-gat-gta-ttc-atg-aaa-gga-ctt-tca-aag 3'
	Primer 2:	5' tta-ggc-ttc-agg-ttc-gta-gtc-ttg 3'

The FLAG-tagged genes were PCR amplified by using primer sequences specific to *FLAG* and each respective open reading frame.

FLAG	Primer 1:	5' gac-tac-aag-gac-gac-gat-gac 3'
C35D10.2	Primer 2:	5' gaa-tgt-ggg-cga-aga-gca-tat-c 3'
C54H2.5	Primer 2:	5' gtc-ctc-cac-caa-cgg-caa-tg 3'

F16A11.2	Primer 2:	5’ cca-gag-tga-ata-tct-gga-aga-cc 3’
F55A4.1	Primer 2:	5’ caa-att-cga-gga-aat-ggt-atg-gac 3’
F32A6.3	Primer 2:	5’ gag-cgg-aac-ctg-gtt-ctt-tat-g 3’
M7.5	Primer 2:	5’ ggc-tcc-gag-aga-tga-tag-tgg 3’
R05D11.6	Primer 2:	5’ cat-tgc-aag-aga-tgc-ctt-gag 3’

Imaging. All fluorescence microscopy was performed using a Nikon Eclipse E800 epifluorescence microscope equipped with Endow GFP HYQ filter cube (Chroma Technology). Images were captured with a Photometrics Cool Snap CCD camera driven by MetaMorph software (Universal Imaging).

Statistics. Statistical analysis for neuroprotection was performed using student’s t-test ($p<0.05$) to compare control worms with strains that overexpress candidate genes in DA neurons. The Fisher Exact Test was performed using the online program at http://home.clara.net/sisa/fisher.htm.

1. Cooper AA *et al*. (2006) Alpha-synuclein blocks ER-Golgi traffic and RAb1 rescues neuron loss in Parkinson’s models. *Science* 313:324-348.
2. Cao S, Gelwix CC, Caldwell KA, Caldwell GA (2005) Torsin-mediated neuroprotection from cellular stresses to dopaminergic neurons of *C. elegans*. *J Neurosc*i 25:3801-3812.