Supporting Information

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SI Materials and Methods

Cloning. Expression constructs for p10′, p25, p35, and CDK5 are described in Fig. S1 legend.

Western Blotting. Cells were lysed in lysis buffer (50 mM Tris·HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100) and whole cell lysates resolved on SDS/PAGE. Western blotting was performed following standard protocols. Detection was by HRP-catalyzed enhanced chemiluminescence.

Cell Culture. All cells were cultured in DMEM supplemented with 10% FBS, 1% L-glutamine, 1% penicillin/streptomycin. Culture dishes were kept in a humidified incubator at 37 °C under 95% air and 5% carbon dioxide gas.

Plasmid Transfection and Stable Cell Line Generation. Plasmid transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol.

COS7 cells or SH-SY5Y cells were transiently transfected with pCMV3B-p10. Forty-eight hours posttransfection, COS7 cells and SH-SY5Y cells were treated with 200 μg/mL or 50 μg/mL G148 (Invitrogen), respectively. After 3 wk, positive colonies were isolated and Western blot analysis was performed to verify protein expression.

Lentivirus Production and Transduction. Lentiviral constructs were cotransfected into HEK293T cells with three other packaging plasmids: pMDL, VSV-G, and Rev at a ratio of 5:5:3:2. Forty-eight hours after transfection, cultured medium was collected and filtered through a 0.45-μm filter. Viruses were either frozen at −70 °C for future use, or concentrated by centrifuging at 120,000 × *g* (Beckman; SW-28) for 2 h at 4 °C. After centrifugation, the supernatant was discarded and virus was resuspended in the desired volume of PBS/1% BSA, aliquoted, and stored at −70 °C.

Lentiviral titers were determined by seeding HEK293T cells in six-well plates at 5×10^5 cells per well the day before infection with serial dilutions of the concentrated viral stock. After a 4-h incubation, the culture medium was changed and the cells were incubated for 2 d. Cells expressing EGFP were identified using a fluorescence-activated cell sorter (FACS). Titers ranged from 5 to 10×10^7 infectious units per milliliter (IU/mL). Neurons were cultured for 4–7 d before virus transduction. Half of the culture medium was removed and lentivirus (MOI = 10) was added to the neuronal culture. The virus-containing medium was removed 4–6 h later and replaced with medium mixture (half of fresh neurobasal medium and half of used medium). Neurons were cultured for an additional 48–72 h before treatment or analysis.

Protein Immunoprecipitation and Immunoblotting. Cell lysates were resolved on 4–12% Bis-Tris NuPAGE gels (Invitrogen) and proteins transferred onto nitrocellulose membranes (Bio-Rad) by semidry transfer. Western blotting was performed following standard protocols. Membranes were incubated with primary antibody for 1 h (C-19 or N-20 1:500; anti-MAP2, anti–Tuj-1, anti-GFP, anti-myc, and anti-γH2AX 1:1,000; anti-Prx2 1:5,000; and anti-pPrx2 1:100), followed by five times wash with Tris-buffered saline with Tween (TBST) buffer. Corresponding secondary antibodies conjugated to HRP (Jackson Immunoresearch Laboratories) were then added for 1 h before washing with TBST buffer five times. Image development was by enhanced chemiluminescent detection (Promega).

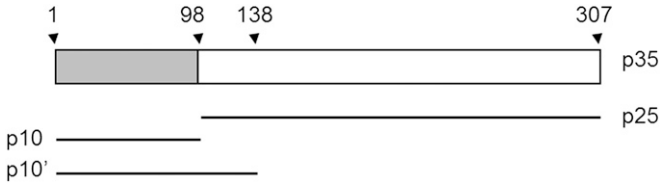

Fig. S1. Schematic of p10′, p25, and p35. p10′, p25, and p35 were PCR amplified and cloned into pCMV3B (Stratagene) at BamH1 and EcoR1 sites. A similar strategy was used to clone full-length CDK5 into pEGFPC1 vector (Clontech). All constructs were then subcloned into pBOBI-CMV lentiviral vectors (from Eng Tan, The Scripps Research Institute, La Jolla, CA) at BamH1 and Xho1 digestion sites. All constructs were verified by sequencing.

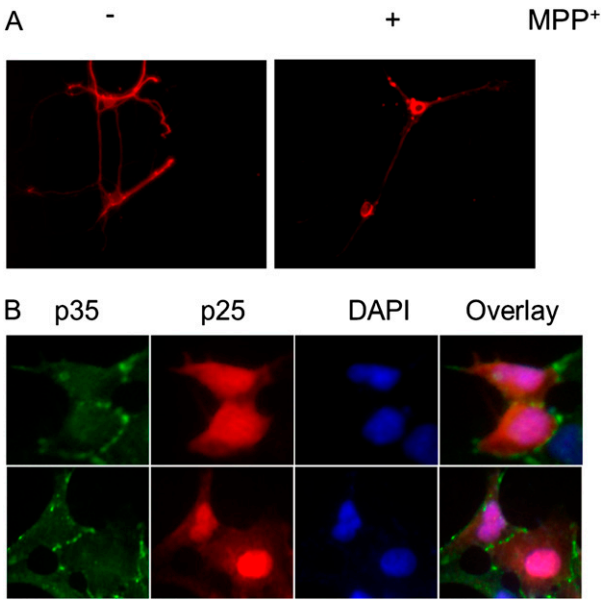

Fig. S2. MPP$^+$ causes p25-like subcellular distribution. (*A*) Neurons were treated without (–) or with (+) MPP$^+$ (50 μM) and then stained with anti-p25 (C-19) and visualized with Cy3-conjugated second antibody. (*B*) COS7 cells were transfected with RFP-p25, or p35 stained with C-19 and visualized with Alexa Fluor 488-conjugated second antibody. Nuclei are stained with DAPI.

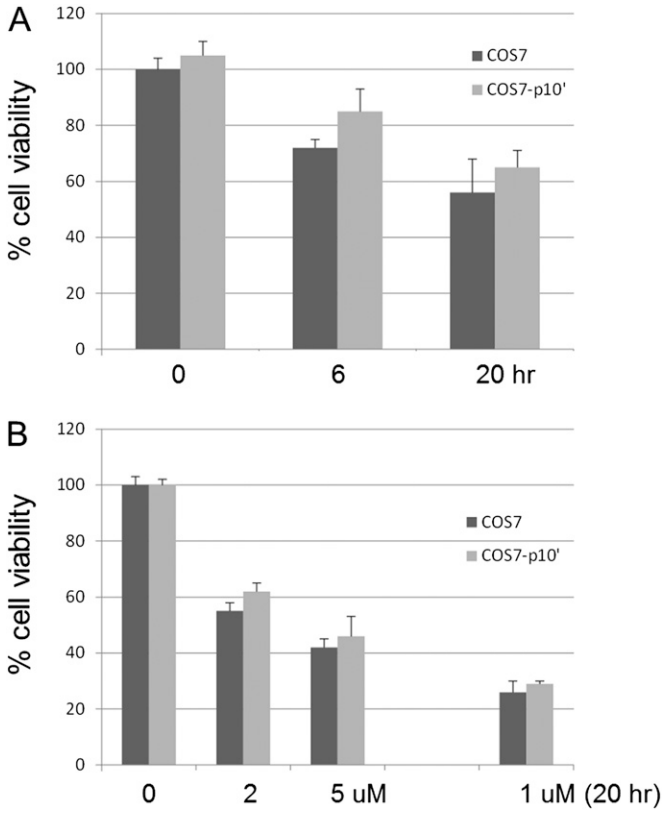

Fig. S3. p10′ does not inhibit toxicity from staurosporin. COS7 cells or COS7 cells stably expressing p10′ were treated with (*A*) 1 μM staurosporin for 0, 6, and 20 h. (*B*) Cells were treated with 0, 2, or 5 μM staurosporin for 6 h. Cell viability was determined by MTT assay. Cell viability in response to 1 μM staurosporin for 20 h was used as a control. Values are mean ± SD; $n = 3$; $P > 0.05$ (between COS7 and COS7-p10′) for each condition.

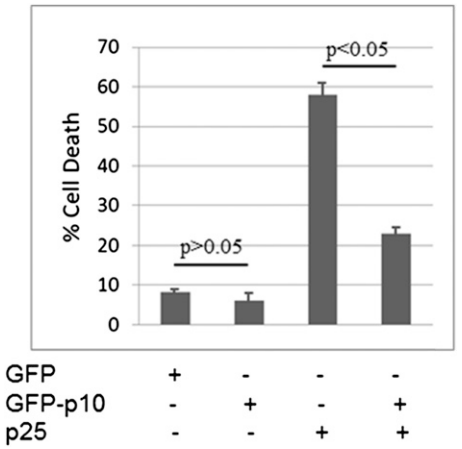

Fig. S4. GFP-p10 is nontoxic. SH-SY5Y cells were transfected with GFP, GFP-p10$^{1-98}$ obtained from Chew et al. (1) or with p25. After 48 h, percent cell death was determined as described in *Materials and Methods*. Three hundred cells were counted per experimental condition (n = 3).

1. Chew J, et al. (2010) Identification of p10 as a neurotoxic product generated from the proteolytic cleavage of the neuronal Cdk5 activator. *J Cell Biochem* 111(5):1359–1366.

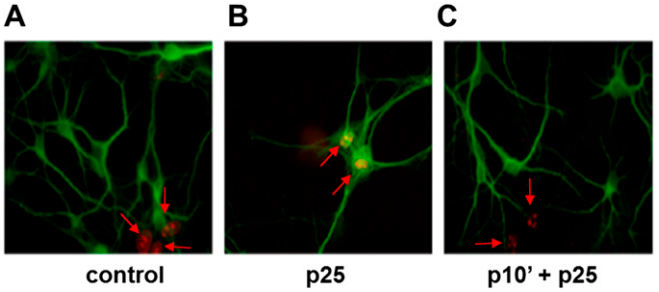

Fig. S5. Identification of Ki67-expressing cells for cell counting. (*A*) Uninfected cells were probed for Ki67 (anti-Ki67/Cy3) (red); neurons were identified by anti-MAP2/FITC (green). Ki67 (arrows) was typically non-neuronal. (*B*) In the presence of p25, Ki67 typically accumulated in neurons (arrows). (*C*) In the presence of p25 + p10′, Ki67 (arrows) was typically non-neuronal. Individual p10′- and p25-expressing neurons could not be identified because anti-MAP2, anti-p10, and anti-p25 were all rabbit polyclonal. Western blot analysis (Fig. 5*B*) confirmed that p25 and p10′ were expressed as indicated. Neurons represented >90% of the cell population.