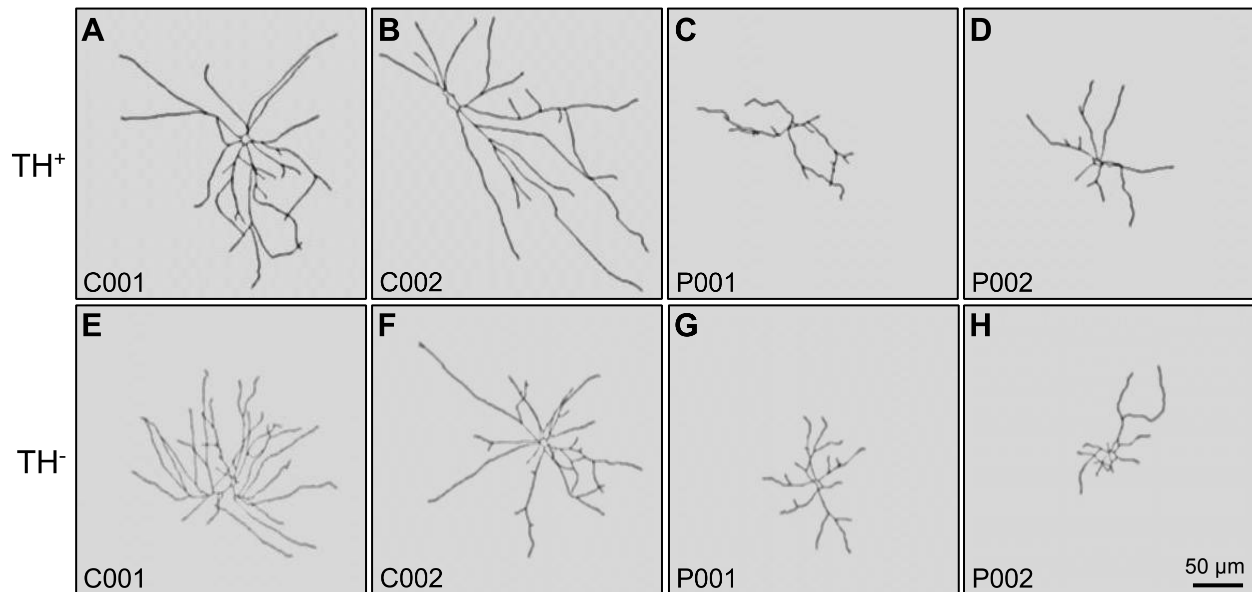

Figure S1. Tracing of TH$^+$ and TH$^-$ neurons in Fig. 1. **(A-D)** Tracing of TH$^+$ neurons differentiated from iPSCs from two normal subjects C001 (A) and C002 (B), as well as two PD patients with parkin mutations P001 (C) and P002 (D). **(E-H)** Tracing of TH$^-$ neurons (as identified by infection of low titer palGFP sindbis virus) from C001 (E), C002 (F), P001 (G) and P002 (H).

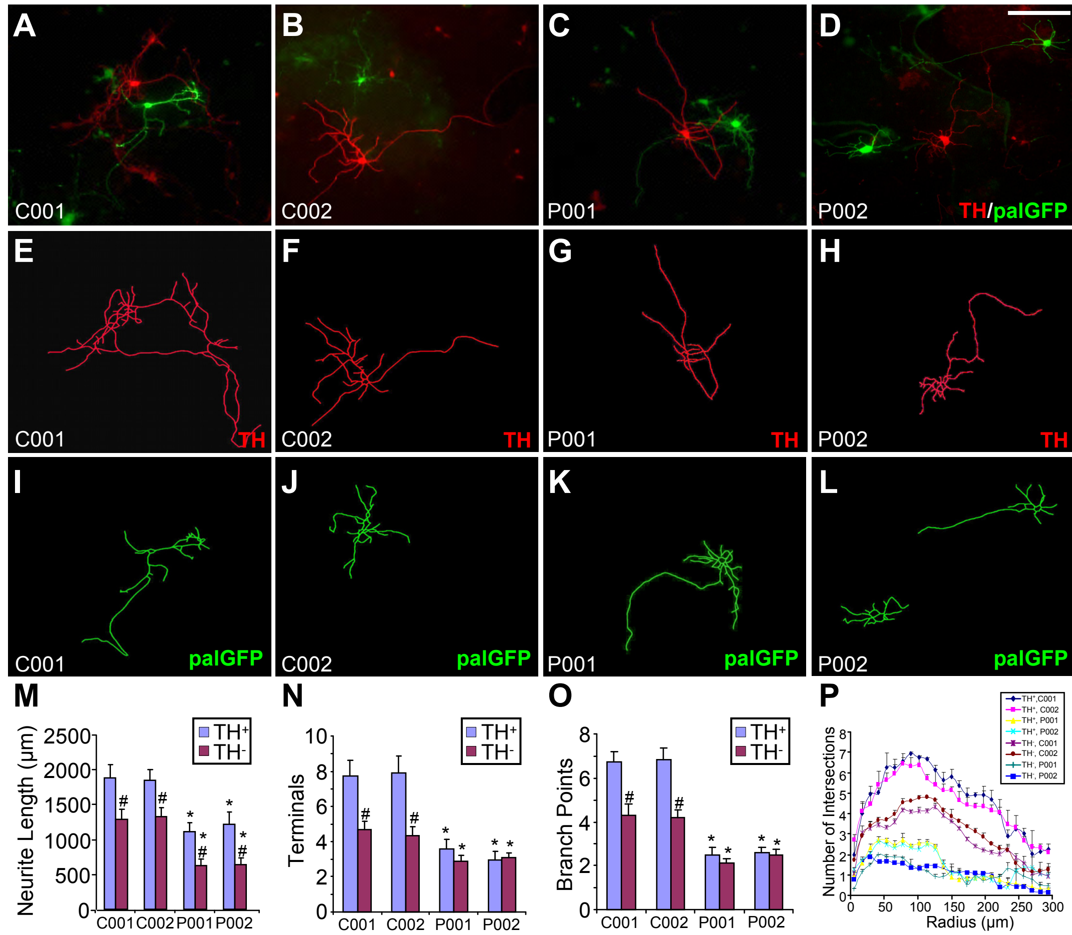

Figure S2. Comparison of iPSC-derived TH$^+$ and TH$^-$ neurons. Cultures around 76 days were infected with low titer sindbis virus for palGFP. Around 90 days, the cultures were fixed and stained for tyrosine hydroxylase (TH). Total neurite length, number of terminals and number of branch points from randomly selected TH$^+$ and TH$^-$ neurons were analyzed. **(A-D)** Representative fluorescence images of the indicated neuronal cultures showing the expression of palGFP (green) and TH (red). **(E-H)** Contour tracing of TH$^+$ neurons from the indicated cultures. **(I-L)** Contour tracing of TH$^-$/palGFP$^+$ neurons from the indicated cultures. **(M)** Average total neurite length (μm) per neuron was measured in each cell lines. **(N)** Average number of processes per neuron was quantified for each line. **(O)** Average number of branch points per neuron was quantified for each line. **(P)** Sholl analysis of each line. *, $p < 0.001$, vs. normal subjects for TH$^+$ or TH$^-$ neurons, respectively, #, $p < 0.01$, vs. TH$^+$ neurons of the same subject, n = 58 TH$^+$ neurons and n = 62 TH$^-$ neurons for each line of iPSC, all from six independent experiments. Bar 50 μm.

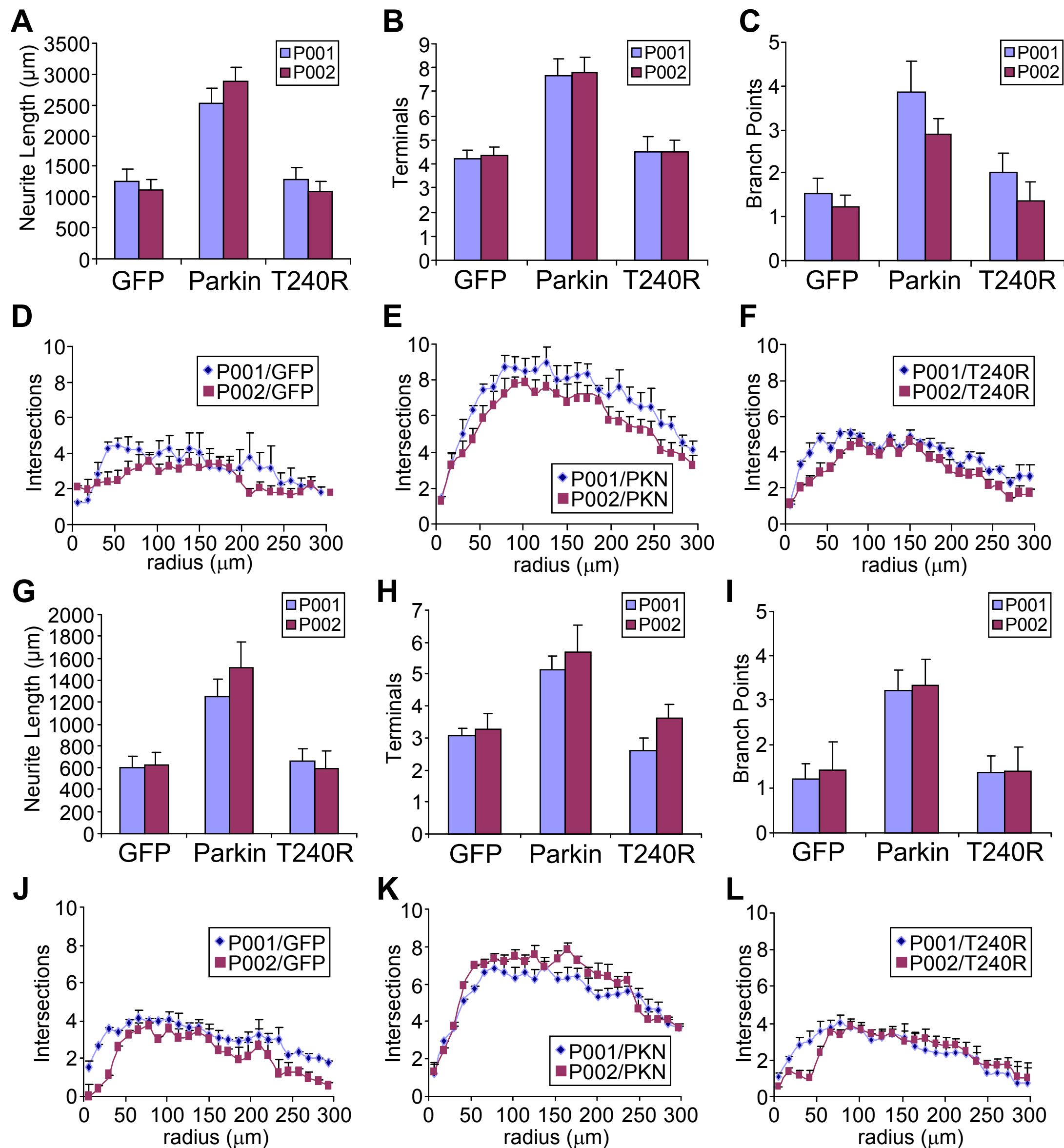

Figure S3. Comparison of TH$^+$ and TH$^-$ neurons from P001 and P002 midbrain neuronal cultures transduced with GFP, parkin or T240R. **(A-F)** Total neurite length (A), the number of terminals (B), the number of branch points (C), and Sholl analysis (D-F) were quantified for TH$^+$ neurons in P001 or P002 neuronal cultures infected with lentivirus expressing GFP (D), parkin (E) or T240R mutant parkin (F). $p > 0.05$, P001 vs. P002 for all parameters, n = 45 TH$^+$ neurons for each derivative line of P001 neurons and n = 47 TH$^+$ neurons for each derivative line of P002 neurons, all from three independent experiments. **(G-L)** Total neurite length (G), the number of terminals (H), the number of branch points (I), and Sholl analysis **(J-L)** were quantified for TH$^-$ neurons in P001 or P002 neuronal cultures infected with lentivirus expressing GFP (J), parkin (K) or T240R mutant parkin (L). $p > 0.05$, P001 vs. P002 for all parameters, n = 46 TH$^-$ neurons for each derivative line of P001 neurons and n = 47 TH$^-$ neurons for each derivative line of P002, all from three independent experiments.

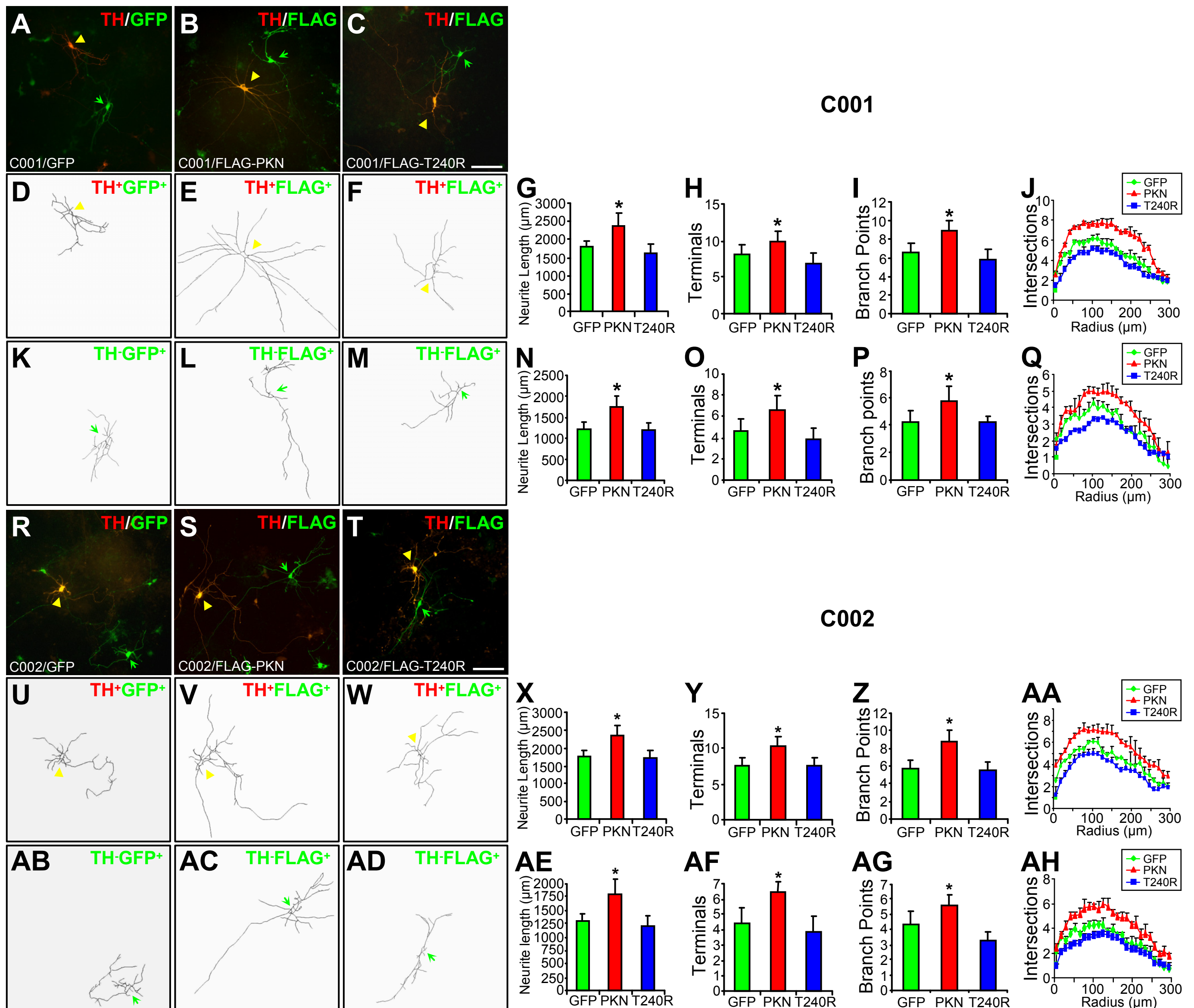

Figure S4. The effect of GFP, wildtype or mutant parkin on C001 and C002 iPSC-derived neurons. **(A-Q)** C001 iPSC-derived neuronal cultures were infected with lentivirus expressing GFP (A, D, K), FLAG-parkin (PKN) (B, E, L) or FLAG-T240R mutant parkin (C, F, M). Cells were stained for TH and GFP (A) or TH and FLAG (B, C). TH^+ neurons (D-F) or TH^- neurons (K-M) infected with GFP (D, K), FLAG-PKN (E, L) or FLAG-T240R (F, M) were traced and analyzed for total neurite length (G, N), number of terminals (H, O), number of branch points (**I**, P) and Sholl analysis (J, Q). **(R-AH)** The same experiments were done on C002 iPSC-derived neurons. Yellow arrowheads, infected TH^+ neurons; green arrows, infected TH^- neurons. Bar, 50 mm. *, p<0.05 vs. GFP or T240R, n = 64 TH^+ neurons and n = 63 TH^- neurons for each derivative line of C001 neurons, all from 3 independent experiments; n = 62 TH^+ neurons and n = 64 TH^- neurons for each derivative line of C002 neurons, all from 3 independent experiments.

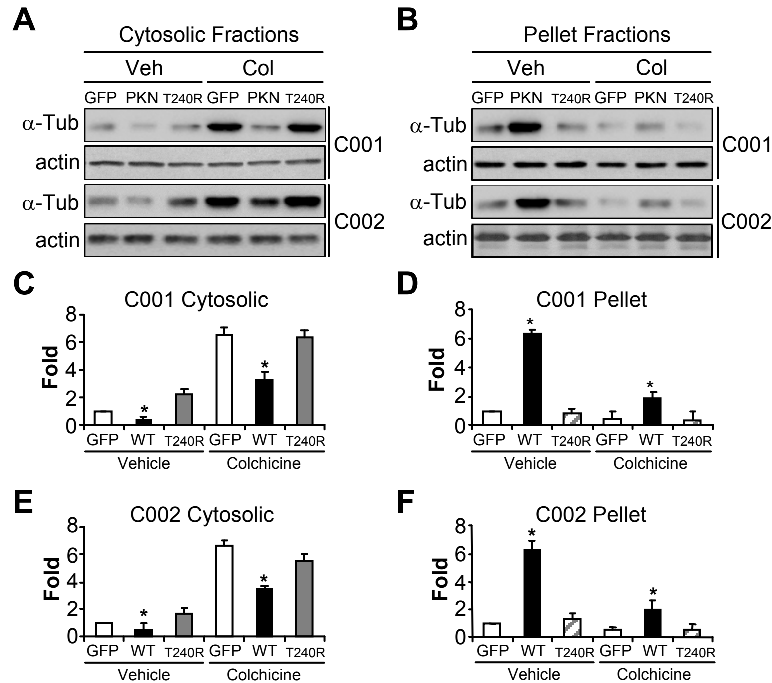

Figure S5. Microtubule stability in normal neurons infected with lentivirus expressing GFP, wild-type or mutant parkin. (**A-F**) iPSC-derived neuronal cultures from two normal subjects (C001 and C002) were infected with lentiviruses expressing GFP, wild-type parkin (PKN) or its PD-linked T240R mutant (T240R). After colchicine (Col) treatment (10 μM for 30 min), cytosolic fractions (A) and pellet fractions (B) were blotted with antibodies against α-tubulin or actin. The amount of free tubulin in cytosolic fractions (C, E) and polymerized tubulin in pellet fractions (D, F) were quantified by normalizing against the amount of α-tubulin in the first lane of C001 (C, D) or C002 (E, F), respectively. *, $p < 0.01$, vs. the preceding bar, n = 3 independent experiments.