Figure S1. Neurabin, but not spinophilin, undergoes a slight molecular weight shift in the presence of CDK5. HEK293 cells transfected with HA spinophilin or neurabin in the absence or presence of CDK5 were immunoprecipitated for HA tag and immunoblotted for HA. These samples are the same as those evaluated in figures 2 and 3. A. HA immunoprecipitates were immunoblotted for HA. Neurabin shows a modest positive molecular weight shift compared with spinophilin which does not appear to have differential migration in the presence of CDK5/p35. Due to lower expression of neurabin, 25% of the total spinophilin immunoprecitate and 75% of the total neurabin immunoprecipitate were loaded onto the SDS PAGE gels.

Figure S2. CDK5 substrate antibody and Thr320 PP1 immunoreactivity is increased in HEK293 cells transfected with CDK5/p35. A. Lysates from HEK293 cells transfected with HA spinophilin or neurabin in the absence or presence of CDK5+p35 were immunoblotted with the CDK5 substrate antibody. These samples are the same as those evaluated in figures 2 and 3. 1.5% of total input was loaded on the gels. B. Lysates from HEK293 cells transfected with WT or T320A (TA) myc-tagged PP1 $\alpha$  (N=2) or WT or T311A (TA) myc-tagged PP1 $\gamma$ 1 (N=1) in the absence or presence of CDK5+p35 were immunoblotted with the Thr320 PP1 antibody or a Myc antibody. 2% of the total input was loaded on the gels.

Figure S3. Linearity of ponceau stain and antibodies to endogenous proteins. Striatal lysates were diluted to different protein concentrations and separated by SDS-PAGE and transferred to nitrocellulose. Nitrocellulose membrane was stained with Ponceau S and then immunoblotted for spinophilin, PP1a, and PP1g1. Linear detection was observed for ponceau stain (630 ng - 78.3  $\mu$ g protein lysate) and all antibodies tested (130 ng - 78.3  $\mu$ g total protein lysate). Ponceau linearity plots are non-logarithmic. Plots for immunoblotting are on a logarithmic scale with lines shown plotted through 0,0.

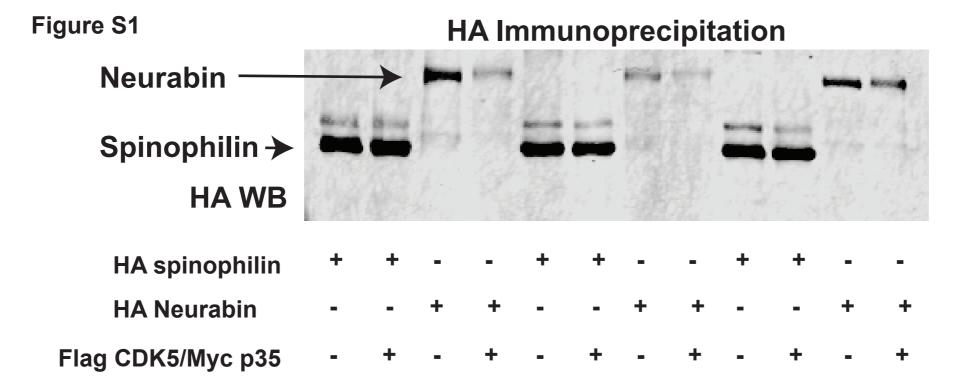
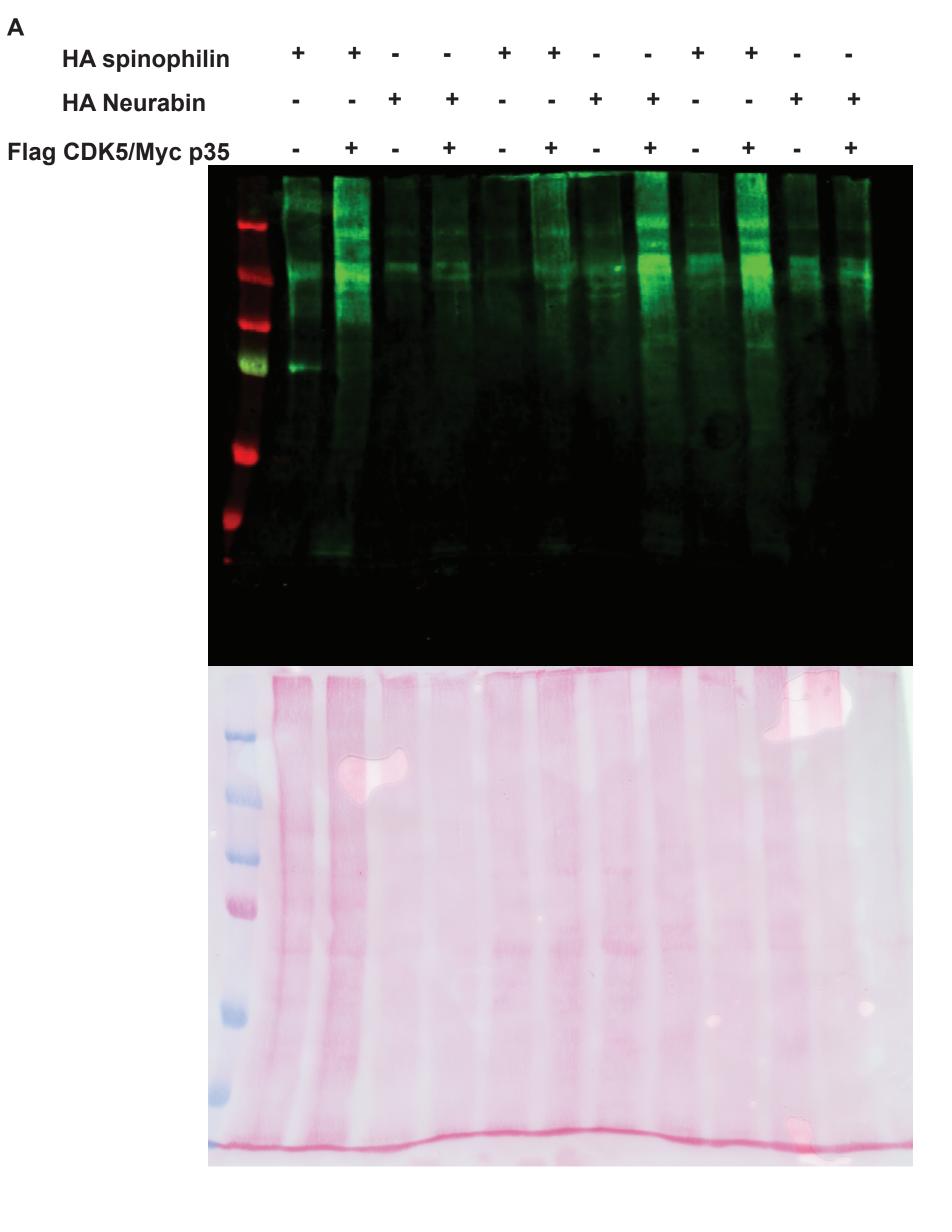
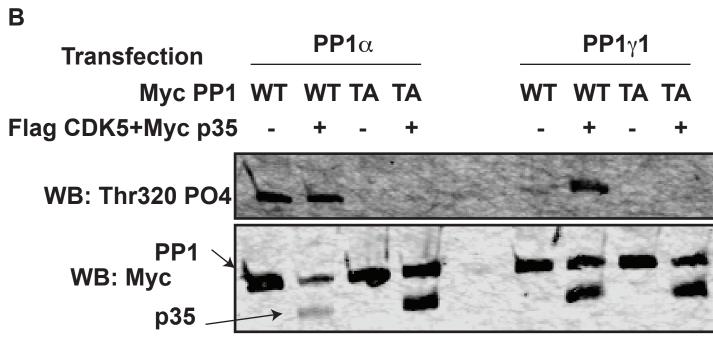


Figure S2

A





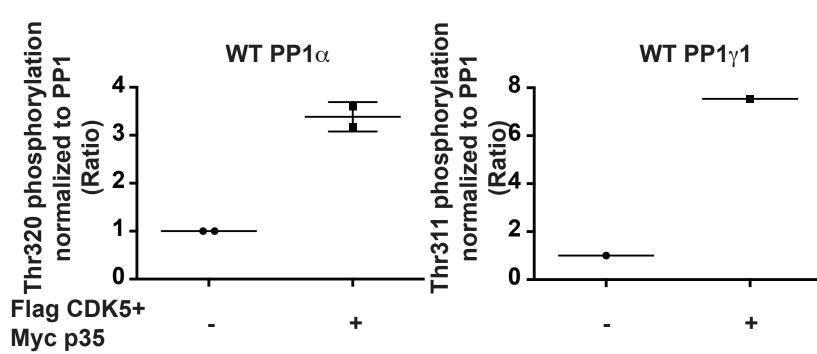


Figure S3 **Ponceau Stain PP1** $\alpha$ Fluorescence intensity 100000 R2=0.9914 (arbitrary units) 0000 100 101 101 **Dark adjustment** Light adjustment **Total Protein** 78.3 15.7 3.1 0.63 0.13 loaded (ug) 10 100 1000 **Total Protein Concentration (ug) ΡΡ1**γ1 R2=0.9952 100000 **Dark adjustment** 10000 1000 Light adjustment 100 **Total Protein** 78.3 15.7 3.1 0.63 0.13 loaded (ug) 10 0.1 10 100 1 **Spinophilin** 78.3 15.7 3.1 0.63 1000000 2500007 Integrated Intensity (Arbitrary Units) Dark adjustment 100000  $R^2 = 0.9963$ R<sup>2</sup>=0.9759 200000-10000 150000-1000 100000-Light adjustment 100 50000 **Total Protein** 10 78.3 15.7 3.1 0.63 0.13 loaded (ug) 0 0.1 10 100 80 20 40 60 100

**Total Protein Concentration (ug)**