SUPPLEMENTARY DATA

Supplementary Figure 1. Validation of hippocampal cell culture. Isolated hippocampal neurons were grown on glass cover slides for 10 days, and subsequently stained for cell markers indicated above each panel. All images represent overlays using cell type specific antibodies (GREEN) and nuclei (BLUE). No oligodendrocytes or microglia were detected, however, a small percentage (~5-7%) of cells were astrocytes. Hippocampal neurons represent the majority of cell types present in 10 D.I.V. cultures. The bottom two figures show two random fields overlaying astrocytes (RED) and neurons (GREEN) for comparison. More neuronal staining is evident compared to astrocyte staining.

Supplementary Figure 2. Cayman PHLPP1 antibody detects PHLPP1α. Blot showing that the PHLPP1 antibody, (Cayman Chemicals) used in all immunofluorescence experiments, also detects PHLPP1α by western analysis. The Cayman antibody detects PHLPP1α in HEK293-FT cells overexpressing human PHLPP1α but not in cells overexpressing GFP only. GAPDH (loading control) did not differ between PHLPP1α or GFP overexpressing cells.

Supplementary Figure 3. Enhanced image of Astrocyte & Neurons. From white box in **Figure 3 Panel I.** Image shows an astrocyte in green devoid of RED PHLPP1 staining. Astrocytic processes extend around several large neuronal nuclei that show perinuclear PHLPP1 (RED) staining.

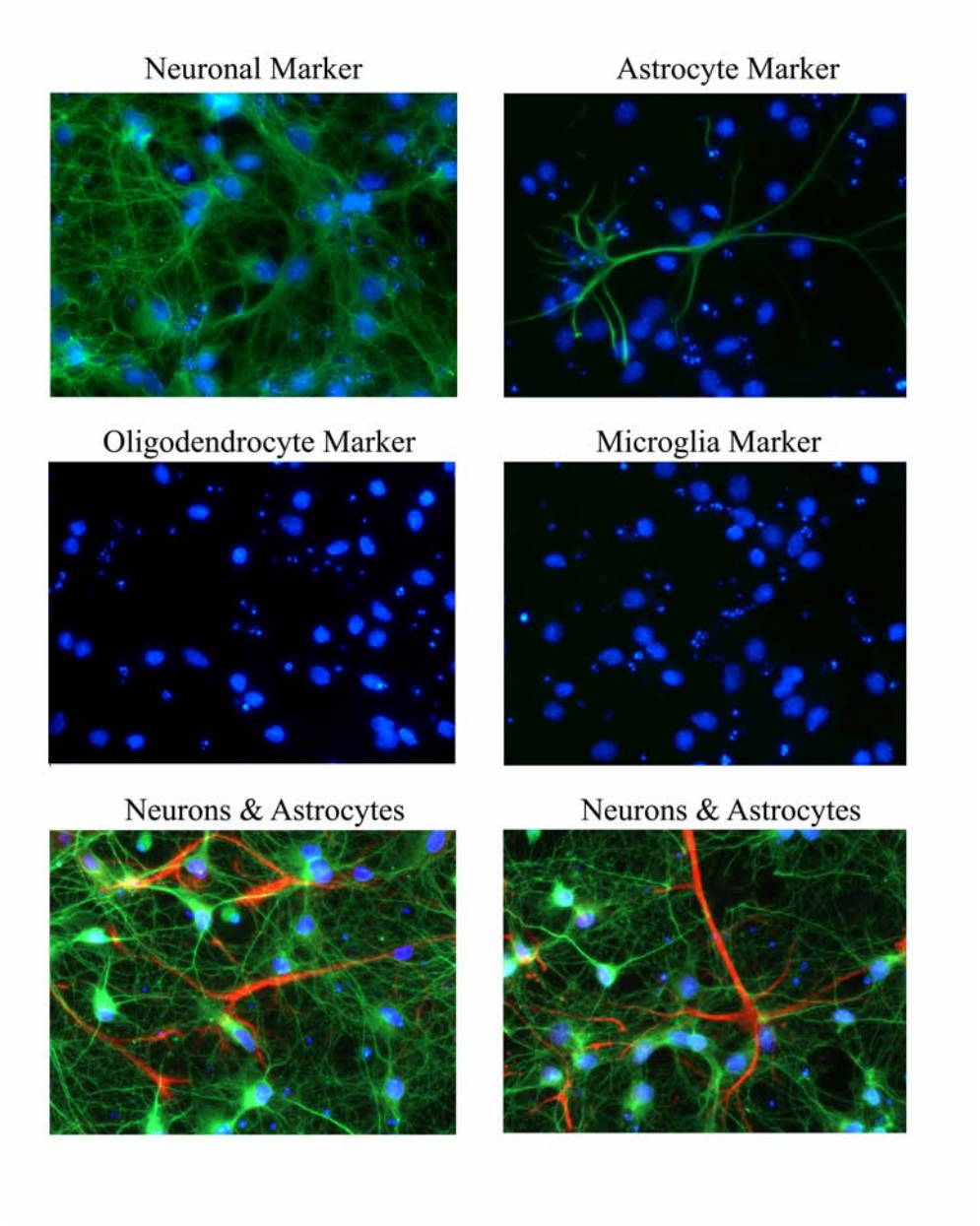
Supplementary Figure 4. PHLPP1 Brain Immunofluorescence, control and CA1 228ms exposure. (**A, B and C**) Control images showing little to no staining in brains incubated with 2ndary only. (**A**) red channel 228ms exposure and (**B**) green channel. (**D, E, and F**) The original exposure time (228ms exposure) of hippocampal sub-region CA1 in the adult rat brain showing intense PHLPP1 (RED) staining.

Supplementary Figure 5. Neuronal PHLPP1β knockdown alters basal activation of AKT, PKC, and ERK kinases. Hippocampal neurons were grown for 10 days in vitro (10 D.I.V.). At D.I.V. 0 and 7 neurons were transduced with either a non-targeting (NT) shRNA or selective PHLPP1β targeting shRNA. At D.I.V. 10, cells were serum starved for 2 hours, washed twice in ice cold PBS, and homogenized in RIPA buffer containing protease and phosphatase inhibitors. Activation of AKT, PKC, and ERK were analyzed by western analysis. (**A**) Representative blots (n=3) show **without** IGF-1 stimulation after a 2 hour starvation period, AKT and PKC phosphorylation is reduced and ERK activation is increased in PHLPP1β knockdown neurons. (**B-I**) Densitometry of proteins analyzed by western blot (n=5 for NT-Controls; n=4 for PHLPP1β knockdown neurons). The results indicate PHLPP1β knockdown alters the basal activation of AKT, PKC, and ERK kinases. Data is significant at $p<.05$ (*), $p<.01$(**), $p<.001$(***).

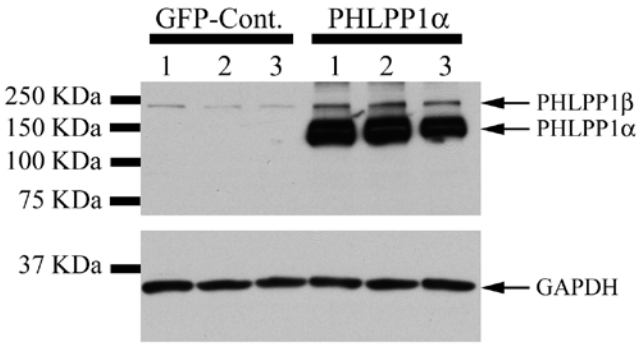
Supplementary Figure 6. Effect of PHLPP1 knockdown to alter AKT, PKC, and ERK activation in HEK293-FT cells. HEK293-FT cells were transduced with lentivirus delivering either a non-targeting (NT) shRNA or human specific PHLPP1 shRNA. (**A**) Western blot showing changes in phosphorylated/total levels of AKT, PKC, and ERK kinases after knockdown. (**B and C**) Densitometry of western blots in **panel A** (n=4) showing knockdown of PHLPP1 proteins induced a significant increase (p=.02211) in pPKCα657 levels but not in PKCα total levels. Only PKCα phosphorylation was altered by PHLPP1 knockdown, no significant change in AKT or ERK phosphorylation was observed.

Supplementary Figure 7. Effect of PHLPP1α overexpression to alter AKT, PKC, and ERK activation in HEK293-FT cells. HEK293-FT cells were transiently transfected with a plasmid containing either GFP only (i.e. control cells) or the human cDNA for PHLPP1α (**A**) Western blot showing changes in phosphorylated/total levels of AKT, PKC, and ERK kinases after overexpression of PHLPP1α. (**B**) Densitometry of western blots in **panel A** (n=3) showing pAKT473 levels had a tendency (p=.0776) to be higher in PHLPP1α overexpressing HEK293-FT cells. (**C**) pERK phosphorylation was significantly increased in PHLPP1α overexpressing cells (p=.0003).

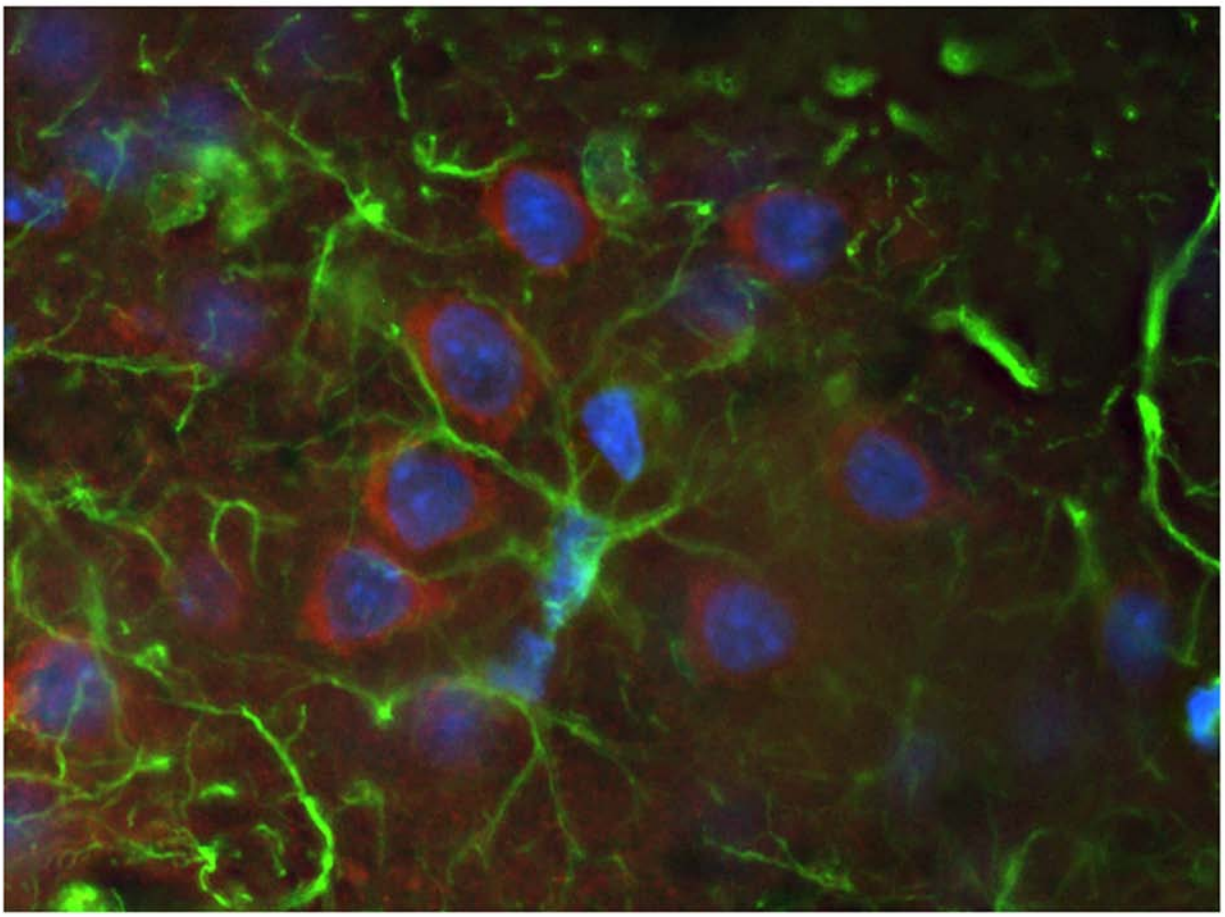
Supplementary Figure 1



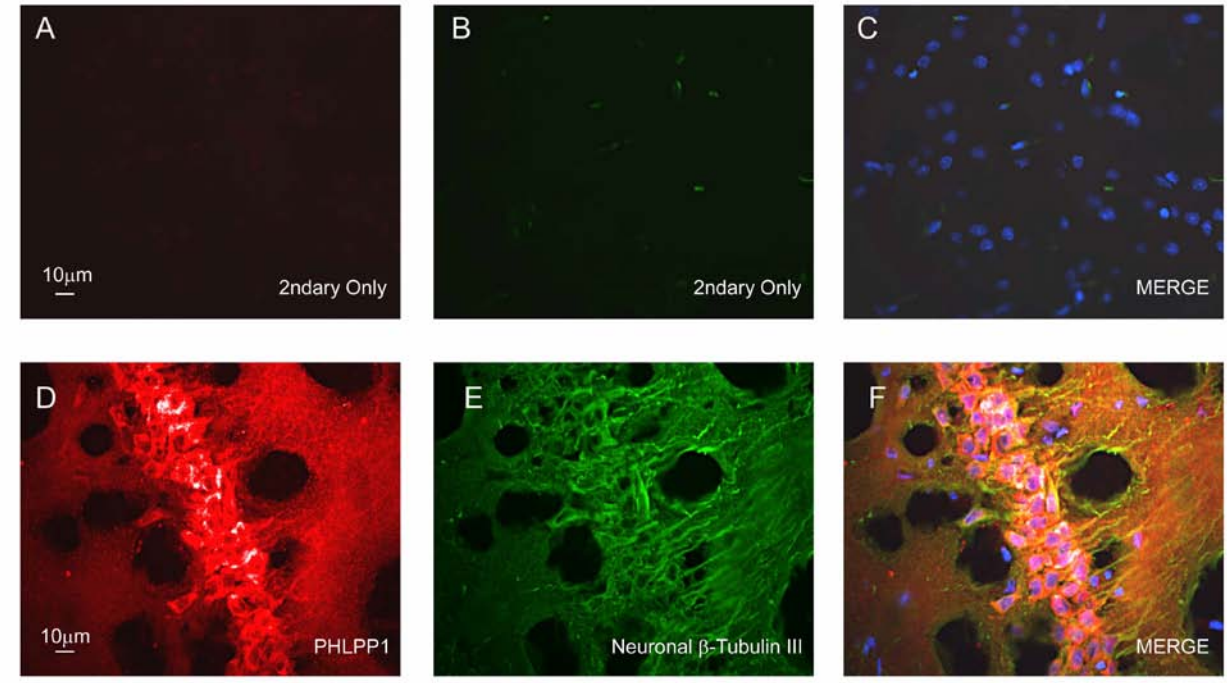
Supplementary Figure 2



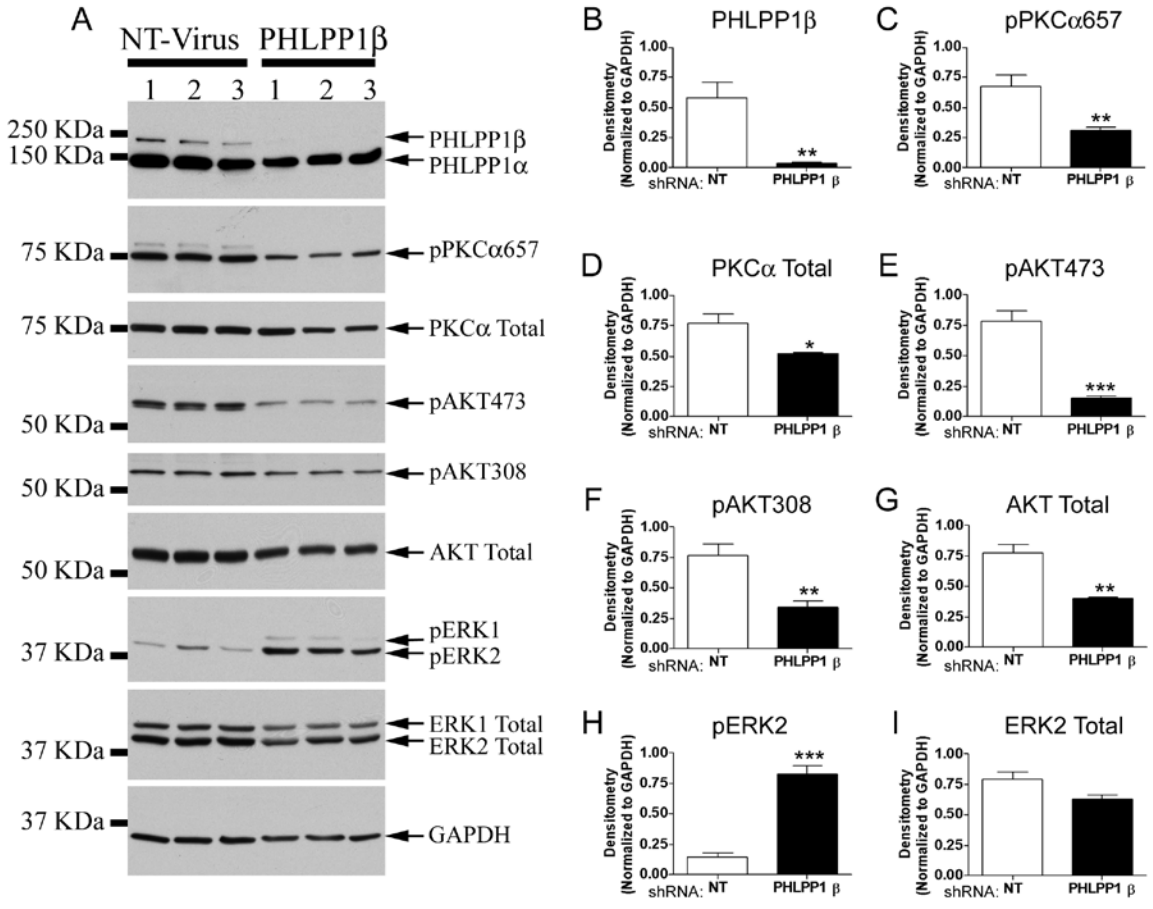
Supplementary Figure 3



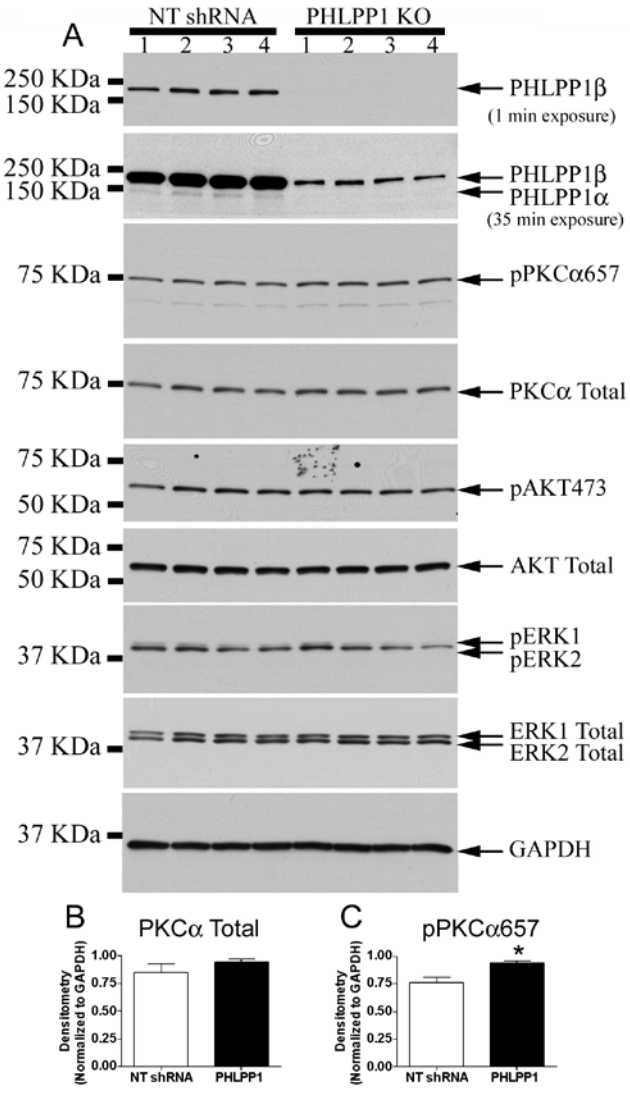
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7