Abnormalities of the Duo/Rac-1/PAK1 Pathway Drive Myosin Light Chain Phosphorylation in Frontal Cortex in Schizophrenia

Supplemental Information

Table S1. Assays used for Western blots analyses.

Target protein	Host	Vendor (cat. #) location	Buffer	Final Dilution	CV (%)
Duo	Goat	Abcam (ab52012) Cambridge, MA	Li-Cor	1:1000	15
Cdc42	Rabbit	Millipore (07-1466) Temecula, CA	Li-Cor	1:2000	21
PAK1	Rabbit	Cell Signaling (2602) Danvers, MA	Li-Cor	1:10000	8.0
$pPAK^{T423}$	Rabbit	Cell Signaling (2601) Danvers, MA	1% BSA	1:500	17
$pPAK^{S141}$	Rabbit	Invitrogen (44940G) Camarillo, CA	1% BSA	1:500	26
LIMK1	Mouse	Novus Biologicals (H00003984-M01A) Littleton, CO	Li-Cor	1:1000	19
$pLIMK1^{T508}$	Rabbit	Abcam (ab38508) Cambridge, MA	1% BSA	1:500	26
Cofilin	Rabbit	Cell Signaling (5175) Danvers, MA	Li-Cor	1:10000	16
$pCofilin^{S3}$	Rabbit	Cell Signaling (3313) Danvers, MA	1% BSA	1:1000	9.5
$pMLC^{S19}$	Rabbit	Cell Signaling (3671) Danvers, MA	1% BSA	1:250	6.5
MLC	Mouse	Novus Biologicals (NBP1-30249) Littleton, CO	1% BSA	1:500	13
VCP	Mouse	Abcam (ab11433) Cambridge, MA	Li-Cor	1:20000	
β-Tubulin	Mouse	Upstate (05-661) Temecula, CA	Li-Cor	1:20000	

BSA, bovine serum albumin; CV, coefficient of variation.

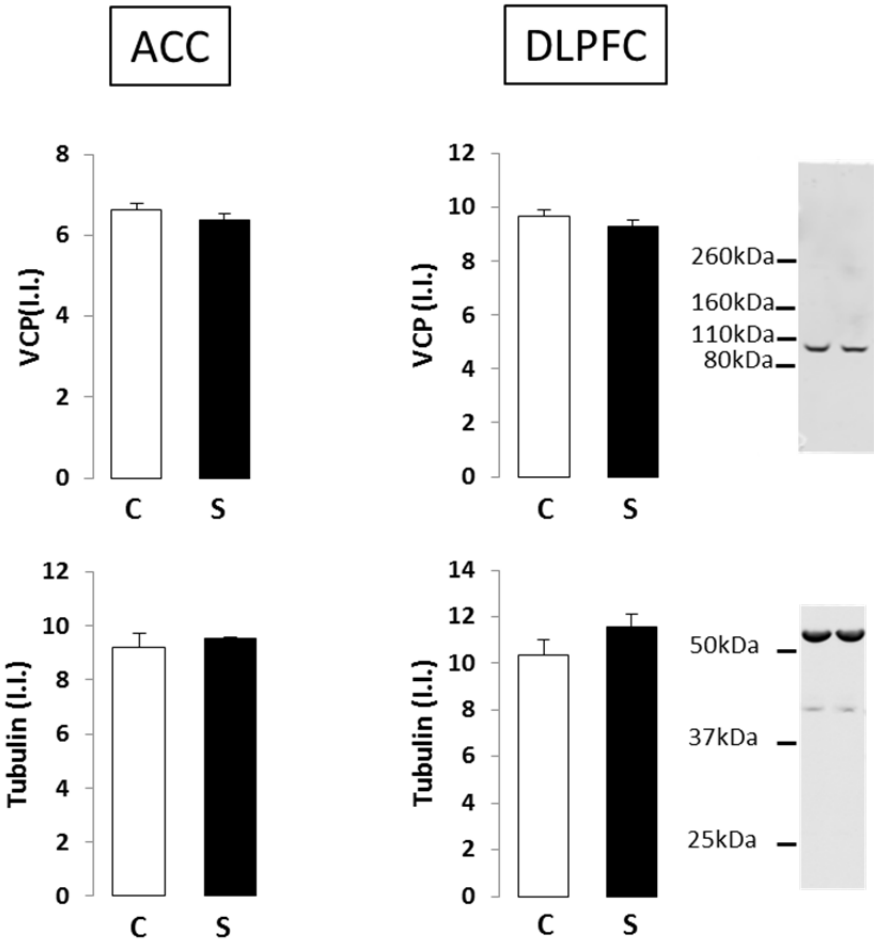

Figure S1. Quantification and blots of proteins used as loading controls in anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (DLPFC). Both VCP and β-tubulin were used as loading controls depending of the molecular weight of the protein under study. Neither VCP nor β-tubulin were significantly different between groups in either region. Data are expressed as integrated intensity (I.I.) of the optical density value of the β-tubulin or VCP band from each subject and displayed as mean ± SEM.

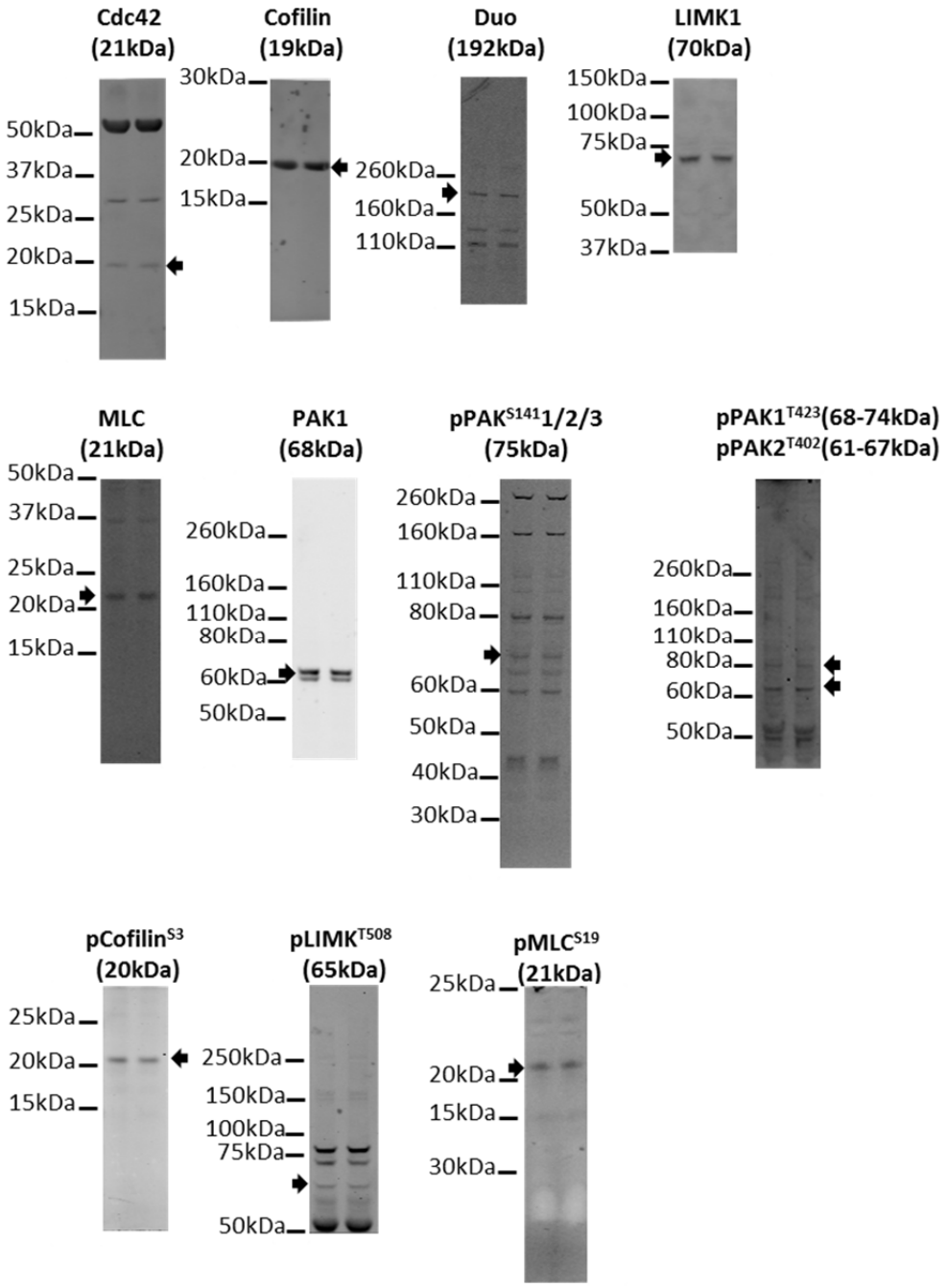

Figure S2. Representative full blot images of all total and phospho-proteins studied. Arrows represent bands at predicted molecular weight that were analyzed for each specific antibody.

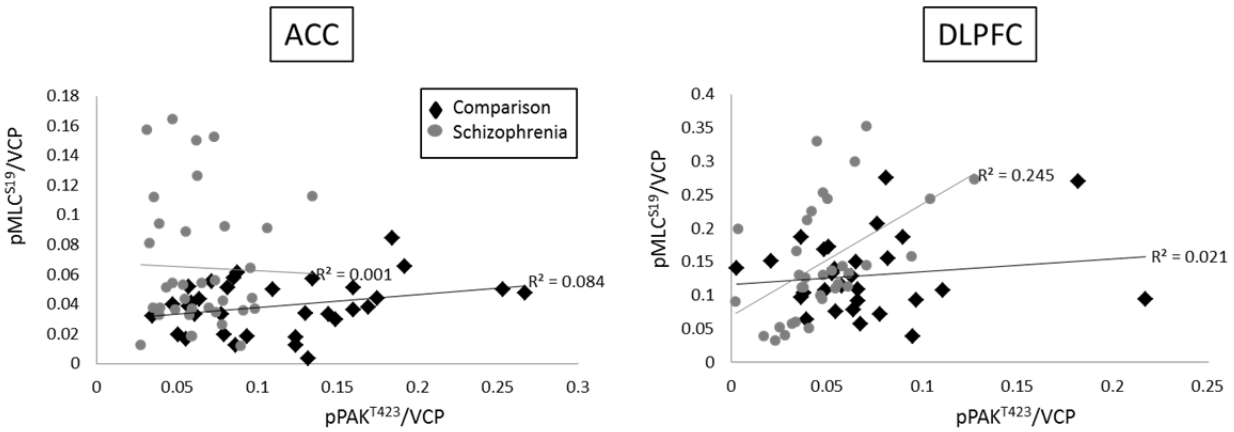

Figure S3. Correlations between $pPAK^{T423}$ and $pMLC^{S19}$ expression in anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (DLPFC) in schizophrenia and comparison subjects. $pMLC^{S19}$ expression was plotted as a function of $pPAK^{T423}$ expression, and r and R^2 values for schizophrenia and comparison groups were obtained for each brain area. Each data point corresponds to the ratio of the optical density value for the protein of interest to the optical density of the β-tubulin or VCP band from the same subject. No significant correlations between these phospho-proteins were found in either area studied.

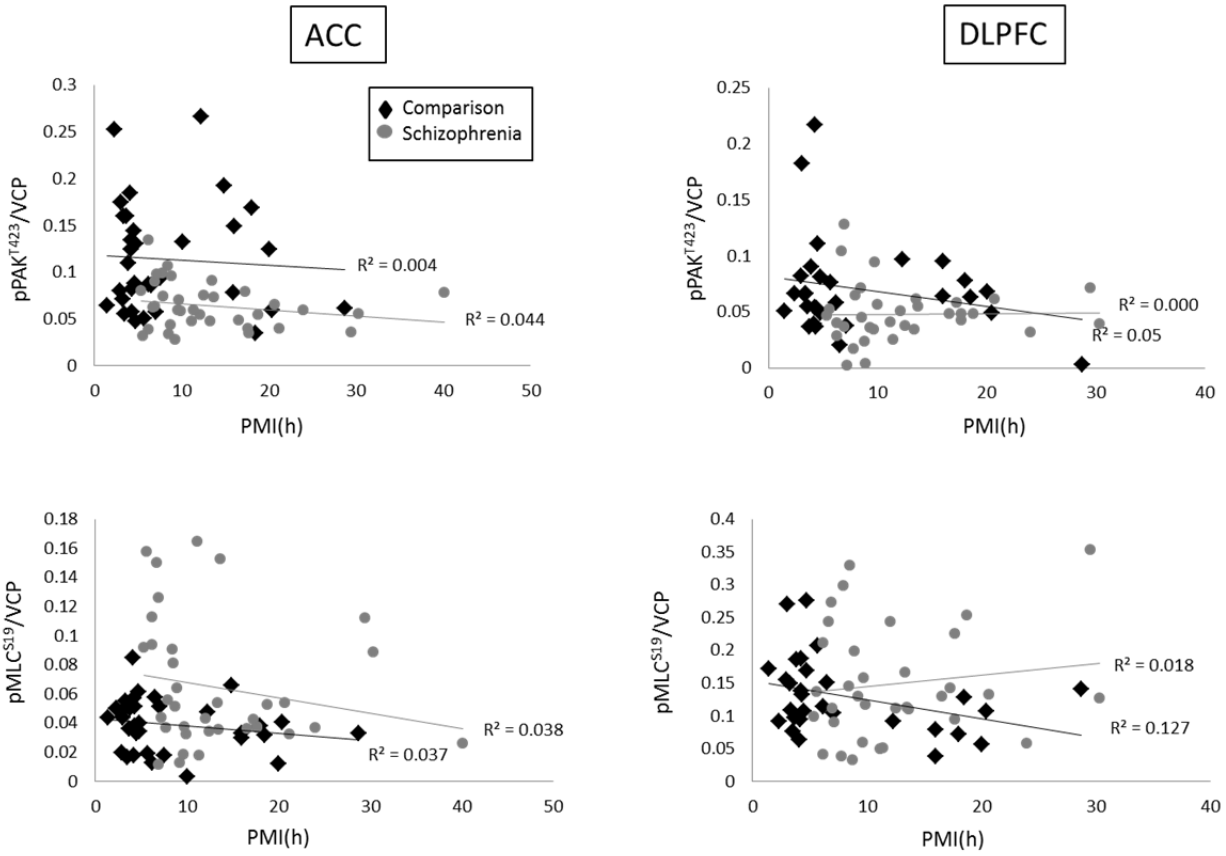

Figure S4. Correlations between PMI (postmortem interval, expressed in hours) and $pPAK^{T423}$ or $pMLC^{S19}$ expression in anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (DLPFC) in schizophrenia and comparison subjects. Each phospho-protein was plotted as a function of PMI and the values for r and R^2 were obtained for each group. Each data point corresponds to the ratio of the optical density value for the protein of interest to the optical density of the β-tubulin or VCP band from the same subject. No significant correlations between the expression of these phospho-proteins and PMI were found in the brain areas studied for either group.

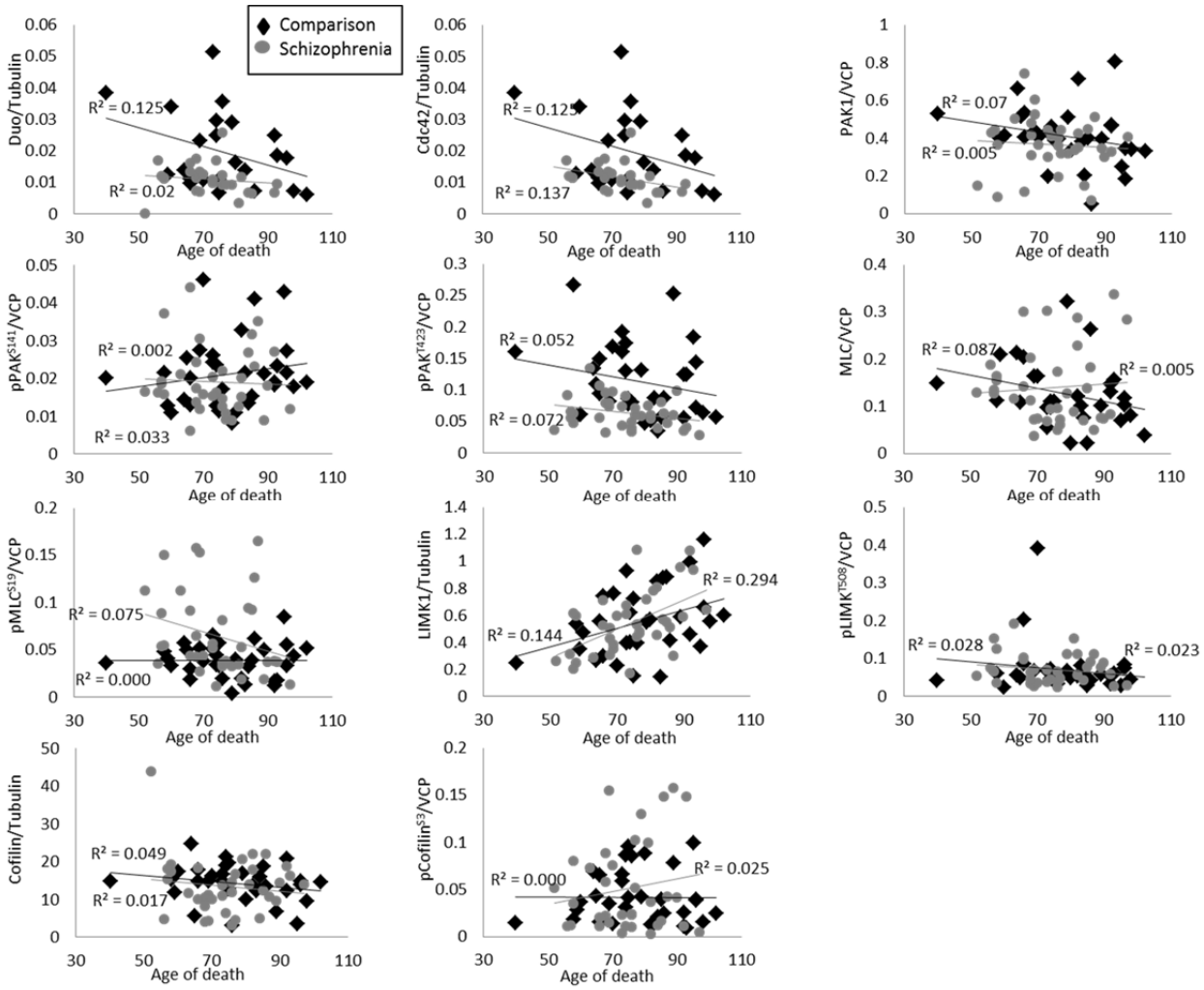

Figure S5. Correlations between age at time of death and protein expression in anterior cingulate cortex (ACC) of schizophrenia and comparison subjects. Each protein was plotted as a function of age at time of death and the values of r and R^2 were obtained for each group. Each data point corresponds to the ratio of the optical density value for the protein of interest to the optical density of the β-tubulin or VCP band from the same subject. No significant correlations between the expression of these proteins and age at time of death were found in the ACC for either diagnostic group.

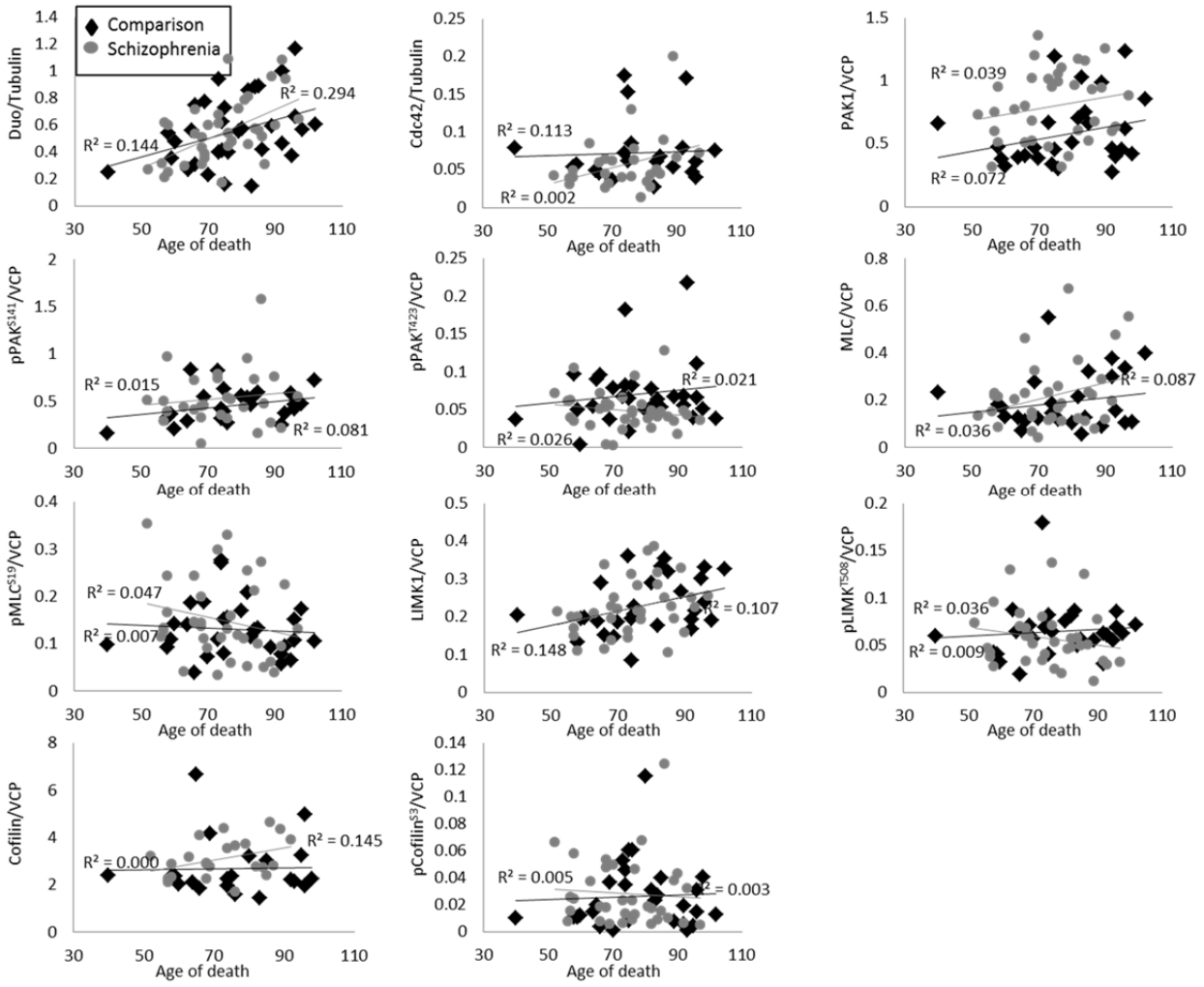

Figure S6. Correlations between age at time of death and protein expression in dorsolateral prefrontal cortex (DLPFC) of schizophrenia and comparison subjects. Each protein was plotted as a function of age at time of death and the values of r and R^2 were obtained for each group. Each data point corresponds to the ratio of the optical density value for the protein of interest to the optical density of the β-tubulin or VCP band from the same subject. No significant correlations between the expression of these proteins and age at time of death were found in DLPFC for either diagnostic group.

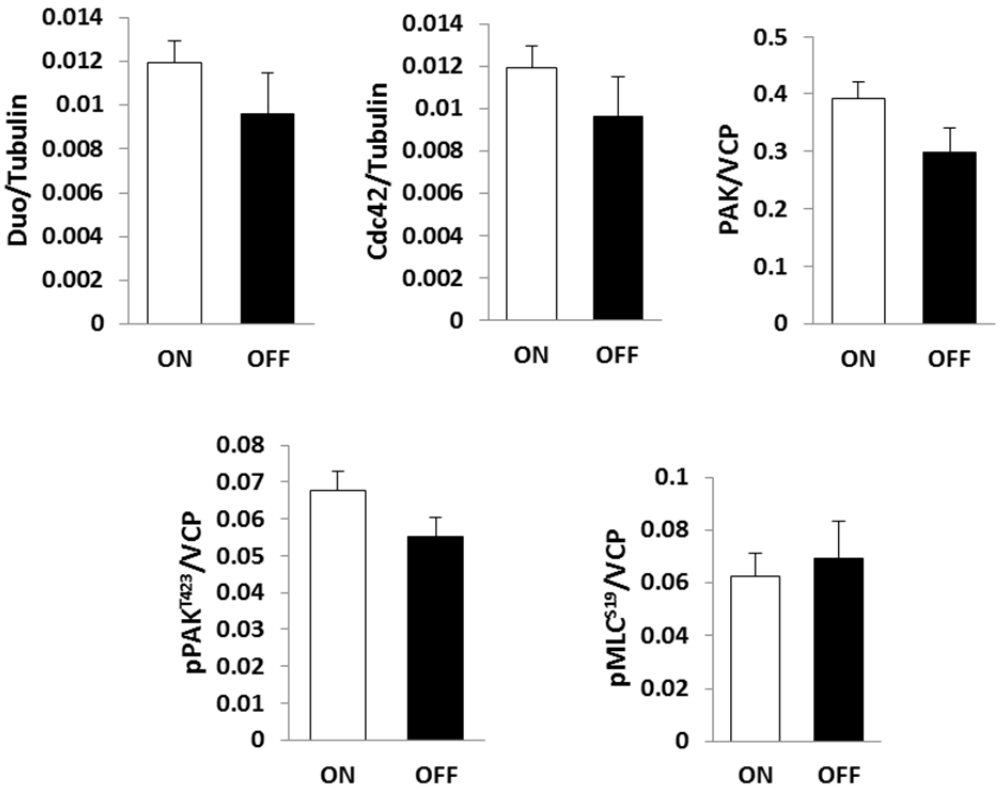

Figure S7. Protein expression in schizophrenia subjects on and off antipsychotic medications for more than 6 weeks at the time of death in anterior cingulate cortex (ACC). No significant differences were found between these subgroups for any of the proteins studied. Data are expressed as means ± SEM of the ratio of the optical density value for the protein of interest to the optical density of the β-tubulin or VCP band from the same subject.

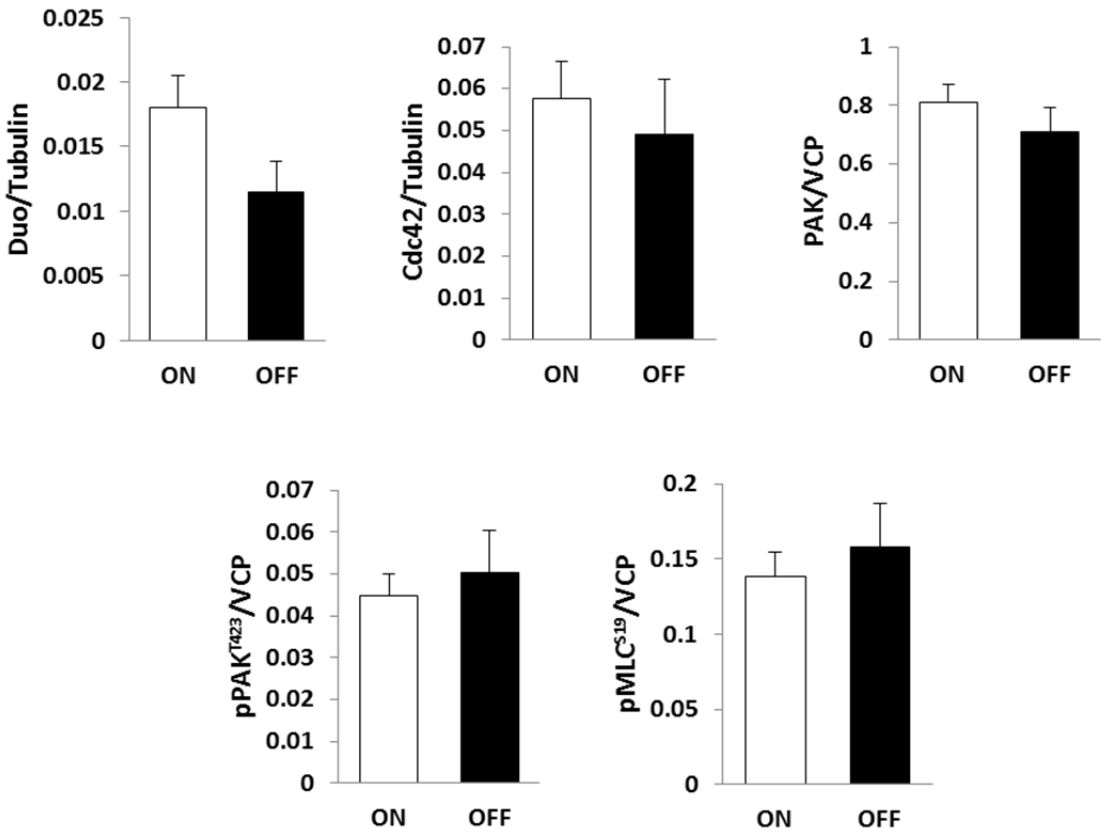

Figure S8. Protein expression in schizophrenia subjects on and off antipsychotic medications for more than 6 weeks at the time of death in dorsolateral prefrontal cortex (DLPFC). No significant differences were found between these subgroups for either of the proteins studied. Data are expressed as means ± SEM of the ratio of the optical density value for the protein of interest to the optical density of the β-tubulin or VCP band from the same subject.