Supplemental methods:

Tau protein isolation: Tissue was homogenized in 1 ml of MES buffer (pH 6.5) and centrifuged at 27,000 x g for 60 minutes at 4˚C. The supernatant was then removed and centrifuged at 95,000 x g for 60 minutes at 4˚C. The supernatant containing the soluble tau fraction was saved and stored at -80˚C. The pellet containing the insoluble tau fraction was solubilized in 150 µl 4 M guanidine HCl for 1 hour at room temperature with a brief sonication and then dialyzed against 50 mM Tris-HCl, (pH 7.5, 1 mg/ml PMSF) overnight at 4ºC. The following day the dialysate was centrifuged at 15,000 x g for 60 minutes at 4ºC. The supernatant containing the insoluble tau was boiled at 100ºC for 10 minutes and then centrifuged at 15,000 x g for 30 minutes at 4ºC. Supernatant volume was then brought to approximately 3.0 ml in 50 mM Tris-HCl, 1.35 g ammonium sulphate added, and then cooled on ice for 15 minutes. Precipitated proteins were collected after centrifugation at 15,000 x g for 30 minutes at 4˚C and resuspended in 150 µl 50 mM Tris-HCl (pH 7.5). The suspension was dialyzed against 50 mM Tris-HCl (pH 7.5, 1 mg/ml PMSF) overnight at 4ºC and the dialysate clarified by centrifugation at 15,000 x g for 30 minutes at 4ºC.

Tau protein dephosphorylation: Dephosphorylation of tau protein was conducted on aliquots of the soluble or insoluble tau in 50 mM Tris-HCl (pH 7.5) were incubated with lambda alkaline phosphatase (20 U/µl, Sigma, Oakville ON, Canada) for 6 hours at 30ºC (16). Reactions were stopped by the addition of 2x electrophoresis buffer (100 mM Tris-HCl, 4% SDS, 0.02% bromophenol blue, 20% glycerol, 200 mM DTT).

Western blot: After fractionation and dephosphorylation, equal aliquots of supernatant from dephosphorylated and non-dephosphorylated samples were run on 7.5% SDS-PAGE gels and

electrophoretically transferred to a nitrocellulose membrane. Gels were probed for total tau with rabbit anti tau T14/T46 antibodies (1:1000 and 1:3000 titres, respectively; Thermo-Fischer, Burlington, Canada). After blocking with 10% bovine serum albumin (BSA) in tris-buffered saline with 0.2% tween (TBS-T) for 1 hour at room temperature (RT), nitrocellulose membranes were probed with primary antibody overnight at 4˚C. Blots were then washed in TBS-T before probing with horseradish peroxidase tagged secondary antibody (Goat anti-Mouse IgG (1:5000 titer; Bio-Rad, Herculese, USA). Blots were visualized using enhanced chemiluminescence (Perkin Elmer, Waltham, USA).

Western blots from moderate TBI tissues: Immunoblots were also performed using isolates from 6 moderate TBI rats and 4 age matched controls. Brain tissue was homogenized in RIPA buffer (1% NP40, 10% glycerol, 137 mM NaCl, 2mM EDTA) containing protease inhibitors (cOmplete; Roche Diagnostics, Indianapolis, USA) and phosphatase inhibitors (Phosstop, Roche Diagnostics) using a Brinkmann Polytron PT 3000 (Kinetamica, Bohemia, NY, USA). Protein concentration was determined by modified Bradford assay (BioRad).

Immunoprecipitations (IP) were performed on 1 mg of brain lysate protein using mouse anti-total tau (T46) in order to isolate all tau isoforms from the rat brain homogenates. The entire IP yield was then run on a 10% SDS-PAGE gel and probed with rabbit anti $pThr^{175}$ tau (1:1000). Gels were stripped (2% SDS, 1M Tris, 7 µl/ml β-mercaproethanol) for 30 min at 50˚C and reprobed with rabbit anti-total tau (1:5000, Abcam ab24230). pGSK3β studies were performed on total brain lysate with mouse anti GSK3β $pTyr^{216}$ (1:10,000) followed by reprobing with mouse anti total GSK3β (1:10,000, BD Biosciences). Blots were visualized by enhanced

chemiluminescence (Perkin Elmer) (BioRad Chemidoc MP imaging system and acquired with ImageLab 5.2.1 software). Densitometry was conducted in imageJ.