Materials and Methods:

Tau$^{-/-}$ mice: These tau knockout mice were originally generated as described by Dawson and colleagues on a B6.129X1 background (Dawson et al., 2001). Briefly, they created tau deficient mice by disrupting the MAPT gene. The engineered mice do not express the tau protein, appear physically normal and are able to reproduce. Embryonic hippocampal cultures from tau deficient mice show a significant delay in maturation as measured by axonal and neuritic extensions (Dawson et al., 2001). Both Tau-/- mice and control strain (B57Bl/6) breeders were obtained from Jackson Laboratory and bred in the Boston University Laboratory Animal Science Center.

TIA1$^{-/-}$ mice: These TIA1 knockout mice were originally generated as described by Piecyk et al. on a C57BL/6 background (Piecyk et al., 2000). These mice are viable, though they develop mild arthritis and are more susceptible to endotoxin shock. These mice also exhibit altered polysome disassembly. Primary neurons from these mice were obtained in Paul Anderson's lab at Brigham and Women's Hospital, Boston, MA.

rTg4510 mice: The rTg4510 line of tau transgenic mice were generated as described by Ashe and colleagues (Santacruz et al., 2005). The transgene is conditionally expressed in the forebrain and hippocampus under control of the Ca2+-calmodulin kinase II promoter. 8 month old mice exhibit severe pathology, mature NFTs and neuronal loss are reported (Santacruz et al., 2005).

HT22 Culture and Transfection: HT22 cells were provided by Carmela Abraham (Boston University School of Medicine), maintained in DMEM (4.5g glucose/L) media supplemented with 10% fetal bovine serum, 1x PenStrep (Invitrogen), 1x L-Glutamine (Invitrogen), and 1x non-essential amino acids. Authentication was done by immunoblotting for neuronal antigens; the cell lines were also tested for Mycoplasma contamination and found to be negative. Cells were passaged twice a week 1:5. Cells were seeded on 18mm poly-D-lysine coated coverslips in 12-well plates (75,000/coverslip) one day prior to transfection in serum-free media (DMEM, 10% FBS). Cells were transfected at 1:2 ratio DNA:Lipofectamine (Invitrogen), delivering 2 μg total DNA per well. Lipofectamine in OptiMEM (Invitrogen) is incubated for 5 min prior to the addition of the DNA/OptiMEM. The transfection mixture is then allowed to incubate for 20 min at room temperature prior to being added to the cells. 500μL of media is

removed and 500 μL of transfection mixture is added dropwise to each well. Cells were incubated 24 hours, after which the addition of 25μM salubrinal may be added to half of the plates and allowed to incubate for 24 hours. At 48 hours post-transfection, the cells were fixed in 4% PFA and stored in PBS.

shRNA Transfection: Transfection was done as stated above using Mission shRNA against TIA1 3'-UTR Clone 301302 (Sigma) or a scrambled shRNA control ± EGFP, WT Tau-EGFP, or P301L Tau-EGFP. Cells were either fixed in 4% PFA and stored in PBS or lysed in RIPA buffer for immunoblotting. All experiments were done in triplicate.

Primary Neuronal Culture: Primary hippocampal cultures were generated from P0 pups from C57Bl/6 WT, TIA1 -/-, or tau -/- mice. The hippocampus was dissected out in Hank's Balanced Salt Solution buffered with HEPES and PenStrep, and dissociated with 0.125% trypsin for 15 min at 37°C, followed by trituration. Dissociated cells were plated on plates of coverslips pre-coated with Poly-D lysine (100μg/mL, Sigma). Neurons were grown for 21 days in Neurobasal medium supplemented with B-27 (Invitrogen)

Overexpression Transduction: At DIV3 neurons were transduced with AAV9-TIA1-mRFP or AAV9-mRFP virus (MOI 20). At DIV7 neurons were transduced with AAV1-WT tau-V5 or AAV1-P301L tau-V5 virus (MOI 20). Neurons were fixed for immunolabeling and confocal microscopy in 4% PFA, or lysed in via sonication in PBS with protease and phosphatase inhibitors.

Knockdown Transduction: 150,000 P0 CD1 mouse hippocampal neurons were plated on 18mm glass coverslips previously coated with Poly-D-Lysine. Neurons were transduced on DIV2 with WT 0N4R Tau AAV (MOI of 200) and co-transduced on DIV5 with eGFP AAV containing either scrambled or TIA1 shRNAs (MOI of 200). 1/3 of the neuronal feeding media was replaced every 3-4 days until fixation on DIV21 with 4% paraformaldyhyde in PBS. Neurons were analyzed by immunocytochemistry as described above.

Tau kinase Inhibitor study: Human SH-SY5Y neuroblastoma cells were maintained in 50:50 DMEM/F-12 media supplemented with 10% FBS and 1% each of non-essential amino acids, L-glutamine, and penicillin/streptomycin. Cells were plated on 18 mm glass coverslips pre-coated with Poly-D Lysine and transfected 24 h later using Lipofectamine 2000 transfection reagent according to manufacturer's instructions (Life Technologies).

24 h after transfection, cells were treated with 10 μM Salubrinal (Santa Cruz Biotech, Cat#202332) or DMSO plus one of the following small molecule tau kinase inhibitors for 24 h: 5 μM GSK3β Inhibitor XXVI (EMD Millipore, Cat#361569), 5 μM Cdk 2/5 (EMD Millipore, Cat#219448), 250 nM p38 MAPK (Invitrogen, SB203580), 20 μM MARK/Par-1 (EMD Millipore, 39621), or 20 nM Fyn (Invitrogen, PP2, Cat#PHZ1223). Cells were fixed for 12 min in 4% paraformaldehyde and stored at 4°C in PBS. TIA1 and phosphorylated tau inclusions were labeled and analyzed as described in methods section for Immunocytochemistry (see below).

Cell Death studies: Caspase 3/7 cleavage was quantified with the Caspase-Glo 3/7 Assay kit, Promega. Apoptosis was analyzed by quantifying DNA fragmentation (TiterTACS Colorimetric Apoptosis Detection kit, Trevigen).

Immunocytochemistry: Coverslips were washed 3×5 min in PBS and washed with 0.1% Triton-X100 for 15 minutes. Coverslips were washed 3x 3minutes in PBS and blocked for 1 hr with 10% donkey serum in PBS. Incubation was performed with primary antibodies for tau and TIA1 overnight in 5% donkey serum/PBS. Primary antibodies used were used as follows: for tau: CP-13, PHF-1, MC1 (1:150, generously provided by Peter Davies, Albert Einstein College of Medicine), and Tau13 (1:5,000, generously provided by Skip Binder, Northwestern). For stress granules: TIA1 (1:400, Santa Cruz). For neuronal marker: MAP2 (1:1000, Avis).

Immunohistochemistry: 16 μm frozen brain sections (sagittal) were mounted onto microscope slides and rinsed 2x in TBS. Antigen retrieval was performed for 1 h in a 37°C water bath using citrate-based antigen unmasking solution (Vector, #H-3300). Slides were then washed 3x at room temperature (RT) 10 min each in TBS/0.25% Triton X-100 (TBS-T). Tissues were blocked for 2 h at RT in TBS-T supplemented with 5% BSA and 5% normal donkey serum and then incubated in primary antibodies diluted in blocking buffer for 36 h. The primary antibodies and dilutions used were as follows: synaptophysin, 1:500 (Abcam, #ab32127); NeuN, 1:1000 (EMD Millipore, #ABN78); TIA1, 1:200 (Abcam, #ab40693); PHF1, CP13, and MC1 anti-tau antibodies, all 1:100 (generously provided by Dr. Peter Davies). After primary antibody incubation, the slides were washed 5x in TBS-T prior to incubating in DyLight conjugated secondary antibodies (Jackson ImmunoResearch) diluted 1:500 in 5% BSA/TBS-T for 2 h at RT.

Slides were then washed 2x in TBS-T, 1x in TBS supplemented with 1:20,000 DAPI stain, and 3x TBS prior to mounting using ProLong Gold Antifade reagent (Life Technologies, #P36930).

Microscopy

Confocal Microscopy: Microscopy was performed using a Carl Zeiss LSM 510 META confocal laser scanning microscope, carrying lasers at 405, 488, 543 and 633 nm. Images were captured using a 63X oil objective. LSM proprietary software was used for digital image analysis. Images were combined into figures using Adobe Photoshop software.

Basic Microscopy: Quantitative and area analysis was done using Zeiss Axio Observer Z1 equipped with Colibri LED fluorescent illumination, digital camera (Orca R2) and analyzed with Zeiss Axiovision software.

Live Cell Microscopy: Live cell imaging was performed using an Olympus DSU spinning disk confocal capable carrying lasers at 405, 488, 543, and 633 nm, or a Zeiss Axio Observer Z1. Time course images were captured using a 63X oil objective, at 30s intervals up to 6 hours. Analysis was done using Imaris/Bitplane software.

Photo-Conversion**:** Photo-convertible WT human tau (PA-Tau) was generated by sub-cloning human 0N4R tau into the pPS-CFP2-C mammalian expression vector (Evrogen cat# FP801). This expression vector encodes a cyan-to-green fluorescent protein under control of the CMV promoter, and has been optimized for high expression in mammalian cells. Primary cortical cultures were generated from E16 embryos from C57Bl/6 WT mice, and cultured on MatTek glass-bottom dishes suitable for live-cell imaging. Neurons were transfected at DIV5 with PA-WT ± RFP-TIA1 or mCherry, using Lipofectamine-2000 (Invitrogen, per manufacturer's instructions), and aged to DIV18-23 prior to photo-conversion. Activation was optimized and performed using a diode 405nm laser on a Zeiss LSM-710 Duo Scan at 63X magnification, for efficient photo-conversion of tau with minimal photo-bleaching. Photo-conversion was performed using the 405nm laser powered at 20%. The samples were scanned first to make sure that no photo-conversion had occurred prior to laser treatment. To prevent photo-conversion during preliminary imaging, the laser was set with the 405nm laser powered at 9%, and imaged using a gain of 7-800. Next, the photo-conversion was accomplished with the 405nm laser powered at 20%, and samples scanned

with 20 iterations of treatment, after which the samples were imaged again to confirm photo-conversion.

Following laser-activation, neurons were imaged on a Zeiss Axio Observer Z1, at 63X magnification, at 20min intervals over 6 hours. Quantification of tau fluorescence at the neuron soma was performed using ImageJ, by manually outlining the neuronal soma at Time=0 and analyzing fluorescence intensity within that ROI at each subsequent time-point. All values are expressed as the percentage of initial fluorescence intensity for a given neuron (ie: Time=0 was set to 100% independently, for each neuron).

Image Analysis: *Granule count cells*: SG density was quantified using Image J software by calculating the number of puncta >1 mm^2 per 63x frame controlled for the number of cells. This was done by thresholding images, and using the analyze particle functionality. Quantifications of granule counts and area in primary neurons was done in ImageJ by using a free-hand tool to outline the neuron, thresholding the image, creating a mask over the nucleus, and using the particle analyzer tool. Samples were coded prior to analyses with the codes revealed only at completion of quantification.

Colocalization: To analyze the potential interaction between tau inclusions and stress granules, quantification of co-localization was done using the Carl Zeiss LSM software, and R-values for the degree of co-localization were recorded.

Granule count neurons: Quantifications of granule counts and area in primary neurons was done in ImageJ by using a free-hand tool to outline the neuron, thresholding the image, creating a mask over the nucleus, and using the particle analyzer tool.

Dendrite length: Measurements of dendritic processes were done using ImageJ plug-ins NeuronJ. Using the software to trace MAP2 positive processes of individual cells yields a pixel measurement that can then be back calculated to micrometers using the microscope scaling parameters.

Live cell granule trafficking: Measurements and analysis were done using Imaris Track on the Imaris/Bitplane software. Quantification of co-localization was done using the Carl Zeiss LSM software, and R-values for the degree of co-localization were recorded. Measurements of dendritic processes were done using ImageJ plug-ins NeuronJ, tracing MAP2 positive processes.

Biochemical Fractionation

Triton-X Fraction: HT22 cells were lysed in RIPA buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, 1% NP-40, 0.1% SDS, 0.5 mM sodium deoxycholate) with 1x Halt protease inhibitor cocktail (Thermo Scientific), 1x phosphatase inhibitor cocktail (PhosSTOP, Roche), and 1% Triton-X-100, and sonicated and spun down to collect the supernatant. Lysates were first centrifuged for 1 hour at 100,000×g 4°C, and the supernatants were collected as 1% Triton-X RIPA buffer soluble proteins. To prevent contamination caused by carrying over, the pellets were re-sonicated and re-centrifuged at 100,000×g for 30 min at 4°C. RIPA buffer-insoluble pellets were resuspended in sample buffer containing 100mM DTT. Soluble and insoluble proteins were analyzed by western blot.

Sarkosyl Insoluble and Soluble Tau Fraction: To produce a fraction enriched for tau, 10 µL of a 10% solution of sarkosyl detergent was added to 100 µg of supernatant. Additional RIPA was used to produce a final volume of 100 µL. This sample was rotated at room temperature for 1 hour prior to a 1-hour centrifugation at 100,000×g spin at room temperature. The supernatant was collected in a separate tube and the sarkosyl pellet was resuspended in sample buffer containing 100 mM DTT.

Immunoblotting: Immunoblots were preformed using gradient PAGE on a 15-well 4-12% Bis-Tris gel (Invitrogen). Blocking was done in 5% milk for 1hr at RT. Primary antibody incubation was at 4°C overnight with 1:7,500 mouse monoclonal Tau13, 1:500 mouse monoclonal PHF1, 1:500 goat polyclonal TIA1 (Santa Cruz), 1:500 mouse monoclonal synaptophysin (Santa Cruz), 1:1,000 mouse monoclonal PSD-95 (NeuroMab), 1:1,000 rabbit polyclonal caspase-3 or cleaved caspase 3 (Cell Signaling), or 1:10,000 mouse monoclonal actin (Millipore) in PBS. Secondary antibody was incubated in 5% milk for 1 hr at RT. Developing was done using SuperSignal West Pico Substrate (Thermo).

Immunoprecipitation: Equal amounts of lysate were pre-cleared by rec-Protein G-Sepharose 4B Conjugate beads (Invitrogen) for 1 hour at 4°C. Samples were spun down and lysates were removed and 0.5 µl of PHF-1 antibody, 1 µl of Tau-5 antibody (Abcam), 1µL of MC1 antibody, or 0.5µL of TIA1 antibody (Santa Cruz) was added to each cell lysate (100-300 µg) and the samples were incubated overnight at 4°C on a rotating wheel. 50 µl of protein G rec-Protein G-Sepharose 4B Conjugate beads was

added to the samples, then the samples were incubated for additional 1 hour at 4°C. The beads were spun down and washed three times in co-immunoprecipitation buffer. The beads were boiled at 95°C for 5 min in SDS-sample buffer. Proteins were then analyzed by western blot.

Immunoprecipitation with Fractionation: Equal amounts of lysate were pre-cleared by rec-Protein G-Sepharose 4B Conjugate beads (Invitrogen) for 1 hour at 4°C. Samples were spun down and lysates were removed and 0.5µL of TIA1 antibody (Santa Cruz) was added to each cell lysate (1000 µg) and the samples were incubated overnight at 4°C on a rotating wheel. 50 µl of protein G rec-Protein G-Sepharose 4B Conjugate beads was added to the samples, then the samples were incubated for additional 1 hour at 4°C. The beads were spun down and the non-bead bound portion was removed and sarkosyl stock buffer was added to form 1% and sonicated and centrifuged at 55,000g for 1 hour, the supernatant was taken and designated non-TIA1-bound soluble, and the pellet was washed once in RIPA buffer and centrifuged at 55,000g for 15 minutes. The pellet was resuspended in sample buffer with 100mM DTT and designated non-TIA1-bound insoluble. Concomitantly, the sepharose beads were washed three times in co-immunoprecipitation buffer. The beads were then rotated for 1 hour in 1% sarkosyl buffer and then spun down. The supernatant was taken as the TIA1-bound soluble fraction. The beads were boiled at 95°C for 5 min in SDS-sample buffer and this was designated the TIA1-bound insoluble fraction. Proteins were then analyzed by western blot.

Immunoprecipitation for Proteomics: 10 mo TIA1$^{-/-}$, Tau$^{-/-}$, and WT C57BL/6J (the genetic background) male mice were anesthetized in an isoflurane chamber and perfused with ice cold PBS. The brains were then dissected to separate the hindbrain, hippocampus, and cerebral cortex. Tissues were slowly frozen by submersion in methanol on dry ice and homogenized in RIPA lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, pH 6.8) supplemented with protease (Roche Cat#04693159001) and phosphatase inhibitor (Roche Cat#04906837001) cocktails. TIA-1 was immunoprecipitated from 2 mg cortex lysate using 10 µg goat anti-TIA-1 polyclonal antibody (Santa Cruz Biotech, Cat#sc-1751) immobilized on Pierce Direct IP columns according to manufacturer's instructions (Thermo Scientific, Cat#26148). The TIA-1 IP eluates were then separated on a Novex

4-12% Bis-Tris polyacrylamide gel (Life Technologies, Cat#NP0323) and stained with Simply Blue Coomassie G-250 SafeStain (Life Technologies, Cat#LC6060). Whole gel lanes were then excised and shipped to the UMass Mass Spectrometry and Proteomics facility for analysis by LC-MS/MS.

Proteomics, In Gel Digestion: Gel slices were cut into 1x1 mm pieces and placed in 1.5ml eppendorf tubes with 1ml of water for 30 min. The water was removed and 50ul of 250 mM ammonium bicarbonate was added. For reduction 20 µl of a 45 mM solution of 1, 4 dithiothreitol (DTT) was added and the samples were incubated at 50 C for 30 min. The samples were cooled to room temperature and then for alkylation 20 µl of a 100 mM iodoacetamide solution was added and allowed to react for 30 min. The gel slices were washed 2 X with 1 ml water aliquots. The water was removed and 1ml of 50:50 (50mM Ammonium Bicarbonate: Acetonitrile) was placed in each tube and samples were incubated at room temperature for 1hr. The solution was then removed and 200 ul of acetonitrile was added to each tube at which point the gels slices turned opaque white. The acetonitrile was removed and gel slices were further dried in a Speed Vac. Gel slices were rehydrated in 75 µl of 2ng/µl trypsin (Sigma) in 0.01% ProteaseMAX Surfactant (Promega): 50mM Ammonium Bicarbonate. Additional bicarbonate buffer was added to ensure complete submersion of the gel slices. Samples were incubated at 37C for 21hrs. The supernatant of each sample was then removed and placed in a separate 1.5 ml eppendorf tube. Gel slices were further dehydrated with 100 ul of 80:20 (Acetonitrile: 1% formic acid). The extract was combined with the supernatants of each sample. The samples were then dried down in a Speed Vac. Samples were dissolved in 25 µl of 5% Acetonitrile in 0.1% trifluroacetic acid prior to injection on LC/MS/MS.

LC/MS/MS on Q Exactive: A 3.0 µl aliquot was directly injected onto a custom packed 2cm x 100µm C18 Magic 5µm particle trap column. Labeled peptides were then eluted and sprayed from a custom packed emitter (75µm x 25cm C18 Magic 3µm particle) with a linear gradient from 95% solvent A (0.1% formic acid in water) to 35% solvent B (0.1% formic acid in Acetonitrile) in 90 minutes at a flow rate of 300 nanoliters per minute on a Waters Nano Acquity UPLC system. Data dependent acquisitions were performed on a Q Exactive mass spectrometer (Thermo Scientific) according to an experiment where full MS scans from 300-1750 m/z were acquired at a resolution of

70,000 followed by 10 MS/MS scans acquired under HCD fragmentation at a resolution of 17,500 with an isolation width of 1.6 Da. Raw data files were processed with Proteome Discoverer (version 1.4) prior to searching with Mascot Server (version 2.5) against the Uniprot database. Search parameters utilized were fully tryptic with 2 missed cleavages, parent mass tolerances of 10 ppm and fragment mass tolerances of 0.05 Da. A fixed modification of carbamidomethyl cysteine and variable modifications of acetyl (protein N-term), pyro glutamic for N-term glutamine, oxidation of methionine were considered. Search results were loaded into the Scaffold Viewer (Proteome Software, Inc.).

Proteomic Analysis: Quantitative proteomic analysis was performed using the total ion current (TIC) for proteins identified by LC-MS/MS normalized to the TIC level of TIA1 detected in each sample. Proteins identified in the $TIA1^{-/-}$ samples were considered to be nonspecific binding proteins to the IP antibody and excluded from all subsequent analyses. Gene lists of detected proteins were then uploaded into the Database for Annotation, Visualization and Integrated Discovery (DAVID) resource available via the NIH website. 3 different gene lists were uploaded: 1) the complete list of proteins detected to bind TIA1 in the WT mouse cortex; 2) the list of proteins whose association with TIA1 decreased by $\geq$ 2-fold in $tau^{-/-}$ compared to WT samples; and 3) the list of proteins whose association increased by $\geq$ 2-fold in $tau^{-/-}$ compared to WT samples. Functional annotation terms with False Discovery Ratio (FDR) values < 0.05 were considered to be statistically significant.

Network Visualization Analysis: The 163 proteins identified in the TIA1 binding proteome in WT C57BL/6J cortex were uploaded as a gene list and analyzed by the DAVID Functional Annotation Clustering tool (Huang et al, 2008; Huang et al, 2009). 12 resulting clusters with enrichment FDR < 0.05 were identified, and each of the 160 (163) proteins was associated to the cluster(s) based on its membership in the clustered gene sets. A network was created by adding a connection between protein pairs sharing annotation clusters. Edge weights were determined as the number of shared annotation clusters between protein pairs with thicker edges representing stronger functional associations between proteins (the smallest number of clusters shared between any two

proteins was 1, and the largest was 8). The resulting network was visualized using the software Gephi 0.8.2 and arranged using the Force Atlas 2 layout algorithm. The network was generated using the python programming language (Python Software Foundation), and the networkx (Hagberg, 2008), numpy, and pandas python packages (Jones, 2001).

Measurement of Protein Synthesis: The procedure followed the SUnSET protocol in which each group of cells were treated with puromycin for 30 min and immunoblotted with the 12D10 antibody (Schmidt et al., 2009).

Statistical Analyses: Experiments with 3 or more groups were analyzed using ANOVAs with Tukeys post-hoc testing; the sample sizes are presented with each experiment. Experiments with 2 groups were analyzed using an unpaired student's T-test. For studies of granule movement, a Mann-Whitney unpaired test with one-tailed p value was completed for comparing distances traveled, velocity analysis, and the directional percentages of granules within populations. Sample sizes are listed in the figure legends.

References:

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Supplemental Table 1, related to Figure 2: List of proteins identified by mass spectrometry through immunoprecipitation of TIA1 from C57BL/6J and tau$^{-/-}$ selected based on absence in immunoprecipitates from TIA1$^{-/-}$ mice. The identified proteins were analyzed using the NIH DAVID bioinformatics resource. The numerical values in the table list the total ion current for each protein normalized to the TIA1 signal set at TIA1 = 1000.

Supplemental Table 2, related to Figure 2: Functional categories of the TIA1 interactome. The complete gene list of proteins detected in the TIA1 binding proteome from WT mouse cortex was uploaded into the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Huang da et al., 2009) and analyzed for statistically significant annotation terms (False Discovery Rate < 0.05) using databases for protein domains (INTERPRO), functional categories (KEYWORDS), and gene ontology (GO) terms.

Supplemental Figures:

Supplemental Fig. S1: TIA1 is absent from axons, related to Figure 1. Hippocampal neurons labeled for MAP2 (blue, a dendritic marker), MC1 (green, detecting misfolded tau) or TIA1 (red). MC1 reactivity (green) can be seen in axons and dendrites. Axons are labeled with white arrows, and labeled with MC1 but not with MAP2; note that little or no TIA1 is apparent in the axons. Scale bar: 20 μm.

Supplemental Fig. S2: Tau promotes SG formation, related to Figure 2. **A)** Expanded data from Fig. 3a showing immunocytochemistry for TIA1, PABP and tau (Tau13 antibody) in HT22 immortalized hippocampal cells with tau positive granules (identified with the Tau13 antibody) formed by expressing WT or P301 tau followed by arsenite treatment (15 μM, 6 hrs) or arsenite + cycloheximide (10 μg/mL). Scale bar: 10 μm. **B)** Quantification of tau (Tau13), TIA1 and PABP positive inclusions in cells from "A". N=50 cells per condition from 5 mice per group. Scale bars, 10 μm. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Supplemental Fig. S4: Interaction of tau with TIA1, related to Figure 4**. A)** Tau immunoprecipitates with TIA1, and TIA1 is found bound to MC1^{+} misfolded tau in primary hippocampal Tau knockout neurons. **Left panel**: Immunoblots showing levels of total tau (Tau13), phosphorylated tau (PHF1), TIA1, and actin in lysates from primary hippocampal tau$^{-/-}$ neurons transduced AAV1-TIA1-RFP ± AAV9-WT or P301L Tau. **Middle panel:** Immunoprecipitation of TIA1, followed by immunoblotting with Tau13, PHF1, and TIA1. **Right panel:** Immunoprecipitation of MC1 tau, followed by immunoblotting with Tau13, PHF1, and TIA1. **B)** Immunoblots showing higher levels of total tau (Tau5 antibody) in primary cortical neurons from TIA1$^{-/-}$ vs C57Bl/6J (control)

mice. **C)** Immunoblots showing levels of total tau (Tau13), phosphorylated tau (PHF1), TIA1-RFP, endogenous TIA1 (eTIA1) and actin in lysates from HT22 cells transfected with β-gal, P301L Tau, P301L Tau-PMIM, or P301L Tau-NULL, then treated overnight with 5 μM MG132 or 50 μM chloroquine. **D)** Immunoprecipitation with HA-antibody in lysates from HEK293 cells transfected with TIA1-HA ± WT tau, showing loss of binding after treatment with RNaseA.

Supplemental Fig. S5: TIA1 promotes consolidation of misfolded tau into SGs, related to Figure 5. **A)** Immunoblots and immunoprecipitations demonstrating knockdown with shTIA1, and corresponding increased levels of total tau (Tau13 antibody) in lysates from HT22 cells transfected with EGFP, WT Tau, P301L Tau co-transfected with shControl or shTIA1. **B)** Immunoblots of total lysates from HT22 cells transfected with TIA1-RFP ± EGFP, WT Tau, WT Tau-PMIM, P301L Tau, or P301L Tau-PMIM. The WT Tau-PMIM construct has had 14 sites exhibiting increased phosphorylation in AD replaced with aspartate (Hoover et al., 2010). This was followed by TIA1 immunoprecipitation/immunoblot, followed by fractionation (1% sarkosyl) and immunoblotting of the TIA1-bound and non-TIA1 bound protein complexes. **c)** Quantification of the ratio of insoluble to soluble tau levels showing increased ratio of insoluble to soluble tau in the TIA1 bound fraction. Binding of P301L tau to TIA1 was not strongly affected by the PMIM modification, suggesting that the P301L mutation exerts effects on aggregation similar to that of phosphorylation, rendering the two changes non-additive (N=3).

Supplemental Fig. S6: Phospho-mimetic tau increases TIA1 granule formation, related to Figure 6. **C)** Phosphorylation of tau increases TIA1+ granules endogenously **(A,** images**; C,** quantification**)** and with over-expressed TIA1 **(B,** images; **C,** quantification) (N=50). Immunocytochemistry for endogenous TIA1 in HT22 cells transfected with EGFP, or Tau (P301L, P301L PMIM or P301L NULL) treated with vehicle (DMSO) or 25 μM salubrinal. The WT Tau-NULL construct has had 14 sites exhibiting increased phosphorylation in AD replaced with alanine (Hoover et al., 2010). The Tau NULL construct exhibited fewer cells with granules. Scale bar 10 μm. Inset = higher magnification, scale bar 2 μm. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Supplemental Fig. S7: Translational inhibitors modulate tau toxicity, related to Figure 7. **A)** Quantification of the % change in dendrite lengths in hippocampal neurons (primary culture, DIV21) from $tau^{-/-}$ mice transduced with AAV1-TIA1-mRFP or mRFP ± AAV9-WT or P301L tau and treated with translation inhibitors puromycin (SG-promoting) or cycloheximide (SG inhibiting). Comparison is to neurons from C57Bl/6J (control) mice. (N=30/condition). **B)** The same experiment as in D, but done in $TIA^{-/-}$ neurons (N=30/condition). Scale bar, 10 μm. *** $p<0.001$.

Supplemental Table 2

TIA1 interactome in WT C57BL/6J cortex

Protein domain terms

Category	Term	Count	%	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
INTERPRO	IPR000504:RNA recognition motif, RNP-1	12	7.94702	1.55E-06	U2AF2, HNRNPF,	148	212	17763	6.793600204	6.03E-04	6.03E-04	2.15E-03
INTERPRO	IPR012677:Nucleotide-binding, alpha-beta plait	12	7.94702	1.62E-06	U2AF2, HNRNPF,	148	213	17763	6.761705367	6.32E-04	3.16E-04	2.25E-03

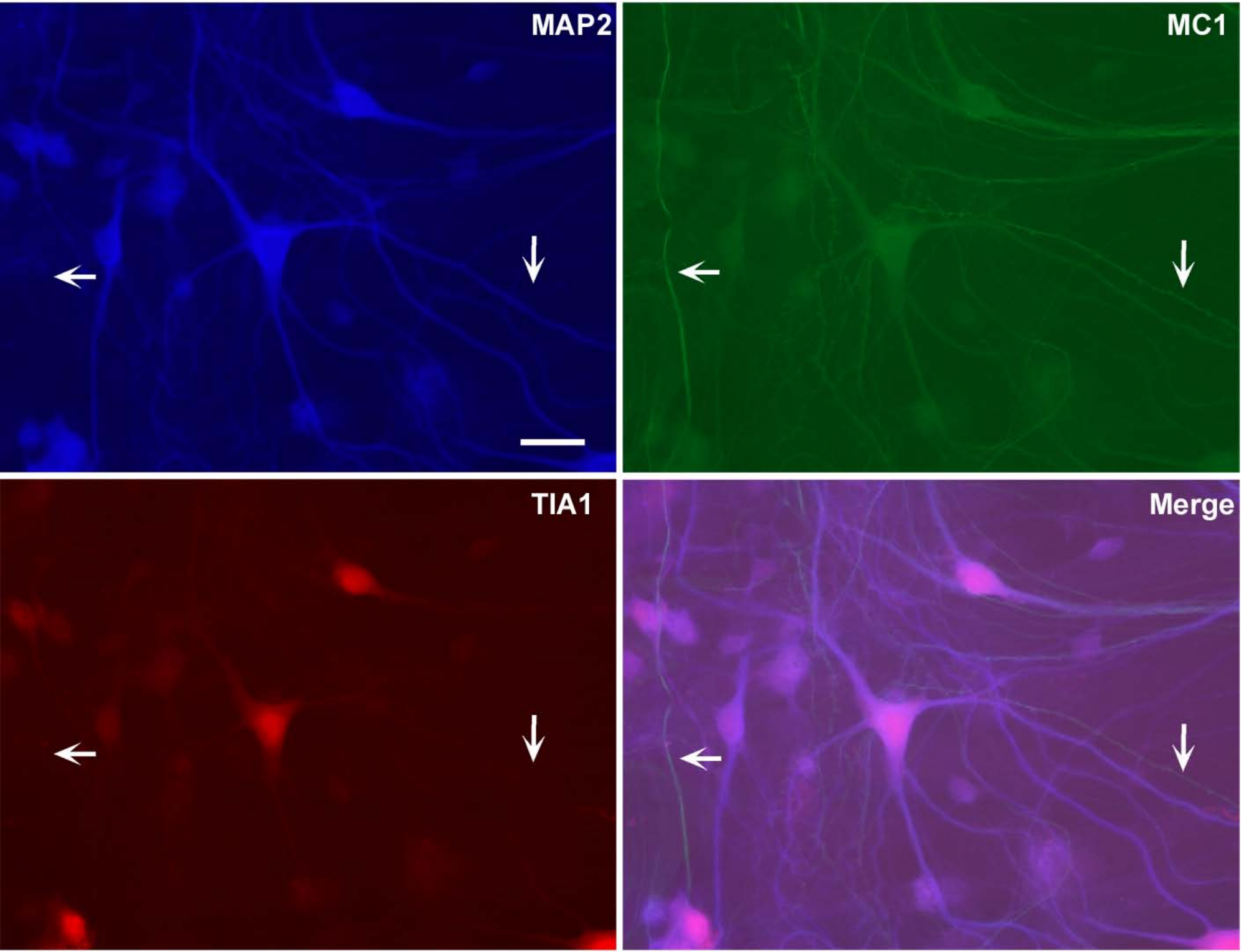
Functional annotation terms

Category	Term	Count	%	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
SP_PIR_KEYWORDS	acetylation	84	55.62914	2.75E-36	UQCRC1, RPL13,	147	2325	17854	4.388079877	5.99E-34	5.99E-34	3.49E-33
SP_PIR_KEYWORDS	phosphoprotein	108	71.52318	6.27E-21	SEPT5, SEPT4, RP	147	6311	17854	2.078470051	1.37E-18	6.83E-19	7.97E-18
SP_PIR_KEYWORDS	mitochondrion	29	19.2053	4.36E-11	SEPT4, UQCRC1,	147	790	17854	4.458503401	9.51E-09	3.17E-09	5.54E-08
SP_PIR_KEYWORDS	ribonucleoprotein	16	10.59603	4.96E-09	RPL13, U2AF2, SY	147	266	17854	7.305610966	1.08E-06	2.70E-07	6.30E-06
SP_PIR_KEYWORDS	transit peptide	20	13.24503	6.31E-09	GPD2, UQCRC1, A	147	457	17854	5.315351524	1.38E-06	2.75E-07	8.02E-06
SP_PIR_KEYWORDS	rna-binding	20	13.24503	1.65E-08	TRA2B, U2AF2, S	147	485	17854	5.008485869	3.59E-06	5.98E-07	2.09E-05
SP_PIR_KEYWORDS	coated pit	7	4.635762	1.36E-07	CLTA, AP2B1, AP2	147	30	17854	28.33968254	2.97E-05	4.24E-06	1.73E-04
SP_PIR_KEYWORDS	nad	12	7.94702	1.43E-07	CTBP1, GLUD1, N	147	169	17854	8.624079217	3.12E-05	3.90E-06	1.82E-04
SP_PIR_KEYWORDS	cytoplasm	51	33.77483	2.94E-07	SEPT5, SEPT4, SY	147	3029	17854	2.044980158	6.42E-05	7.13E-06	3.74E-04
SP_PIR_KEYWORDS	tricarboxylic acid cycle	6	3.97351	3.62E-07	ACO2, SUCLG1, S	147	19	17854	38.35445757	7.89E-05	7.89E-06	4.60E-04
SP_PIR_KEYWORDS	mitochondrion inner membrane	11	7.284768	2.66E-06	SLC25A12, GPD2	147	183	17854	7.300620795	5.79E-04	5.27E-05	3.38E-03
SP_PIR_KEYWORDS	nucleotide-binding	32	21.19205	6.78E-06	SEPT5, GNAZ, SEI	147	1631	17854	2.382946066	1.48E-03	1.23E-04	8.62E-03
SP_PIR_KEYWORDS	transport	31	20.5298	9.00E-06	UQCRC1, NACA, A	147	1571	17854	2.396644972	1.96E-03	1.51E-04	1.14E-02
SP_PIR_KEYWORDS	ATP	9	5.960265	3.41E-05	MAPK1, MAP2K1	147	151	17854	7.239086363	7.41E-03	5.31E-04	4.33E-02

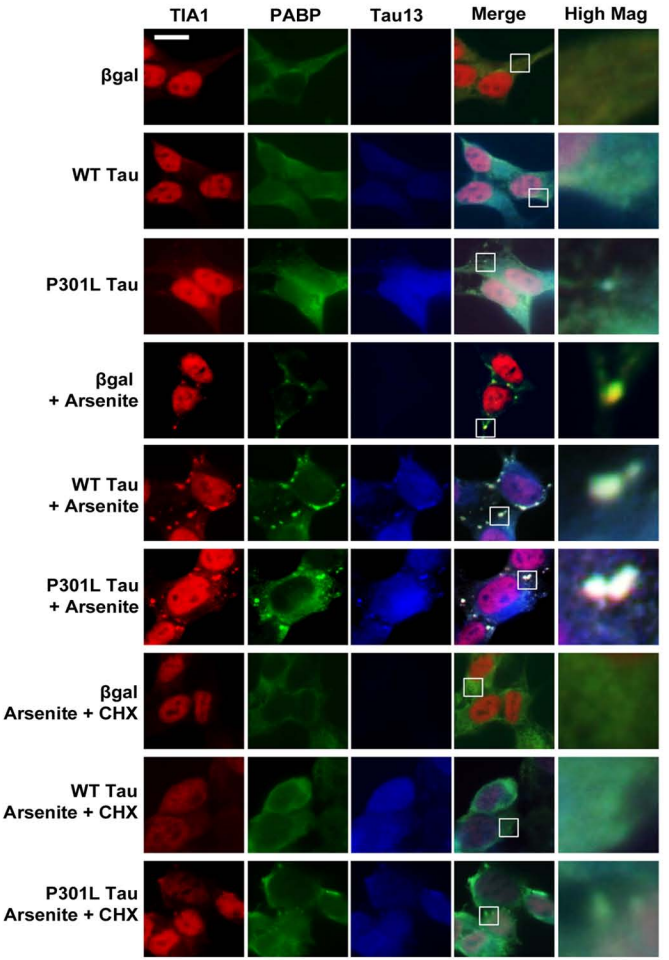
Gene ontology terms

Category	Term	Count	%	PValue	Genes	List Total	Pop Hits	Pop Total	Fold Enrichment	Bonferroni	Benjamini	FDR
GOTERM_BP_ALL	GO:0006091~generation of precursor metabolites and energy	24	15.89404	1.73E-16	UQCRC1, ACO2, A	132	261	14219	9.905259491	2.49E-13	2.49E-13	3.55E-13
GOTERM_BP_ALL	GO:0009987~cellular process	117	77.48344	6.78E-10	SEPT5, SEPT4, AT	132	9252	14219	1.362214067	7.61E-07	3.80E-07	1.09E-06
GOTERM_BP_ALL	GO:0015980~energy derivation by oxidation of organic compounds	12	7.94702	1.51E-09	SLC25A12, UQCR	132	98	14219	13.19016698	1.69E-06	5.64E-07	2.42E-06
GOTERM_BP_ALL	GO:0045333~cellular respiration	10	6.622517	3.09E-09	SLC25A12, UQCR	132	59	14219	18.25757576	3.46E-06	8.66E-07	4.95E-06
GOTERM_BP_ALL	GO:0006084~acetyl-CoA metabolic process	8	5.298013	1.05E-08	ACO2, SUCLG1, I	132	31	14219	27.79863148	1.18E-05	2.35E-06	1.68E-05
GOTERM_BP_ALL	GO:0006099~tricarboxylic acid cycle	7	4.635762	4.84E-08	ACO2, SUCLG1, I	132	23	14219	32.7842556	5.43E-05	9.05E-06	7.76E-05
GOTERM_BP_ALL	GO:0046356~acetyl-CoA catabolic process	7	4.635762	6.40E-08	ACO2, SUCLG1, I	132	24	14219	31.41824495	7.18E-05	1.03E-05	1.03E-04
GOTERM_BP_ALL	GO:0009060~aerobic respiration	7	4.635762	1.38E-07	ACO2, SUCLG1, I	132	27	14219	27.92732884	1.54E-04	1.93E-05	2.21E-04
GOTERM_BP_ALL	GO:0009109~coenzyme catabolic process	7	4.635762	2.18E-07	ACO2, SUCLG1, I	132	29	14219	26.00130617	2.44E-04	2.71E-05	3.49E-04
GOTERM_BP_ALL	GO:0051187~cofactor catabolic process	7	4.635762	4.06E-07	ACO2, SUCLG1, I	132	32	14219	23.56368371	4.55E-04	4.55E-05	6.51E-04
GOTERM_BP_ALL	GO:0006096~glycolysis	7	4.635762	2.89E-06	ALDOC, PFKP, PF	132	44	14219	17.13722452	3.23E-03	2.94E-04	4.63E-03
GOTERM_BP_ALL	GO:0044237~cellular metabolic process	81	53.64238	3.24E-06	UQCRC1, RPL13,	132	5857	14219	1.489720924	3.63E-03	3.03E-04	5.19E-03
GOTERM_BP_ALL	GO:0006007~glucose catabolic process	7	4.635762	7.84E-06	ALDOC, PFKP, PF	132	52	14219	14.50072844	8.76E-03	6.76E-04	1.26E-02
GOTERM_BP_ALL	GO:0019320~hexose catabolic process	7	4.635762	7.84E-06	ALDOC, PFKP, PF	132	52	14219	14.50072844	8.76E-03	6.76E-04	1.26E-02
GOTERM_BP_ALL	GO:0051234~establishment of localization	43	28.47682	9.09E-06	CLTA, NACA, CLT	132	2358	14219	1.964354101	1.02E-02	7.29E-04	1.46E-02
GOTERM_BP_ALL	GO:0046365~monosaccharide catabolic process	7	4.635762	9.80E-06	ALDOC, PFKP, PF	132	54	14219	13.96366442	1.09E-02	7.33E-04	1.57E-02
GOTERM_BP_ALL	GO:0008152~metabolic process	90	59.60265	1.21E-05	UQCRC1, RPL13,	132	7020	14219	1.381021756	1.35E-02	8.50E-04	1.94E-02
GOTERM_BP_ALL	GO:0044275~cellular carbohydrate catabolic process	7	4.635762	1.82E-05	ALDOC, PFKP, PF	132	60	14219	12.56729798	2.02E-02	1.20E-03	2.91E-02
GOTERM_BP_ALL	GO:0006810~transport	42	27.81457	1.87E-05	CLTA, NACA, CLT	132	2342	14219	1.931779365	2.08E-02	1.17E-03	3.00E-02
GOTERM_BP_ALL	GO:0046164~alcohol catabolic process	7	4.635762	2.88E-05	ALDOC, PFKP, PF	132	65	14219	11.60058275	3.18E-02	1.70E-03	4.62E-02

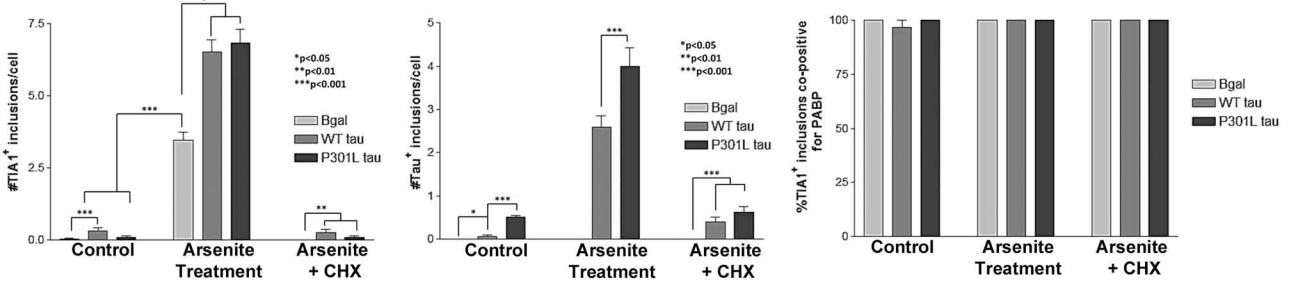
Supplemental Figure 1



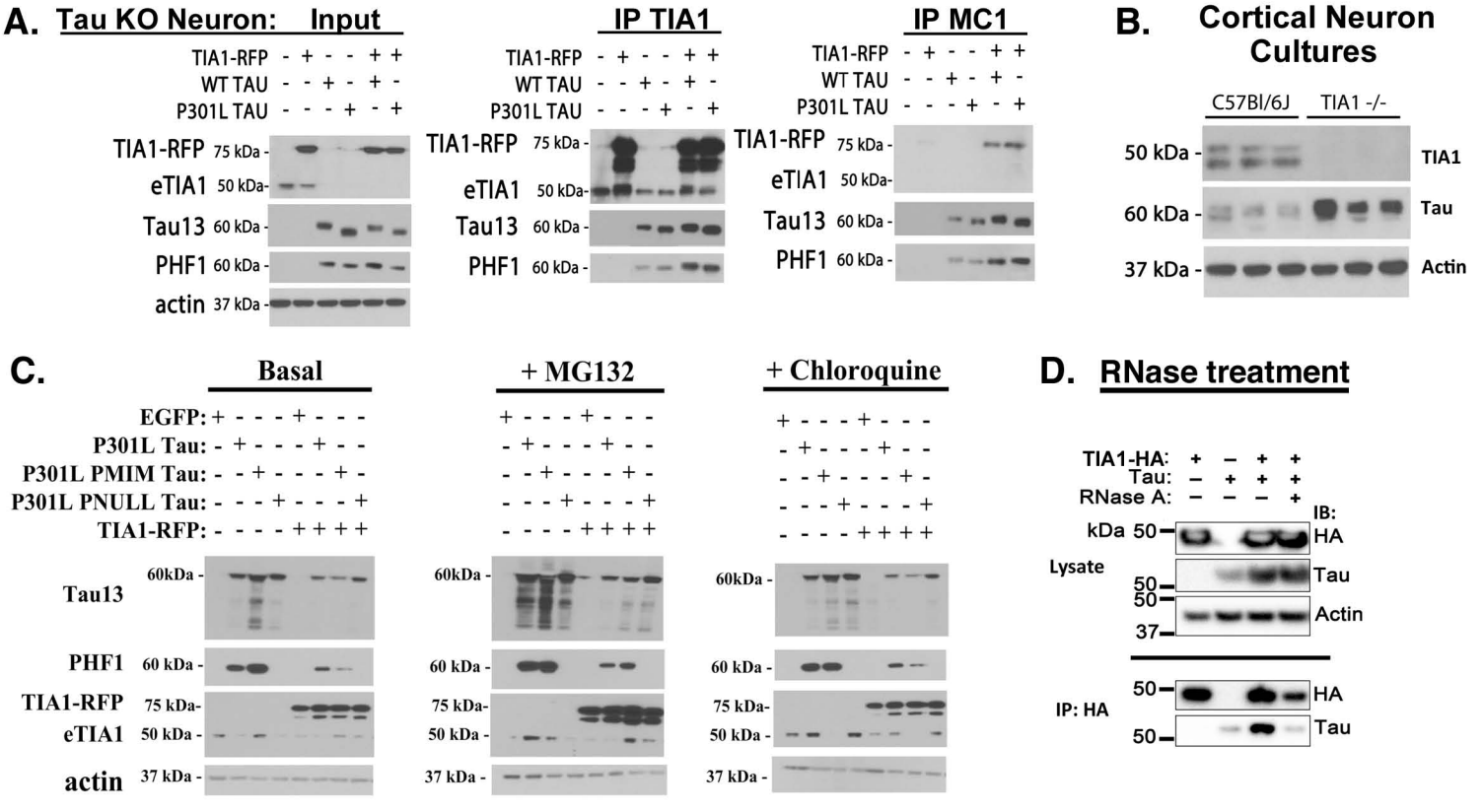
A. Supplemental Figure 2



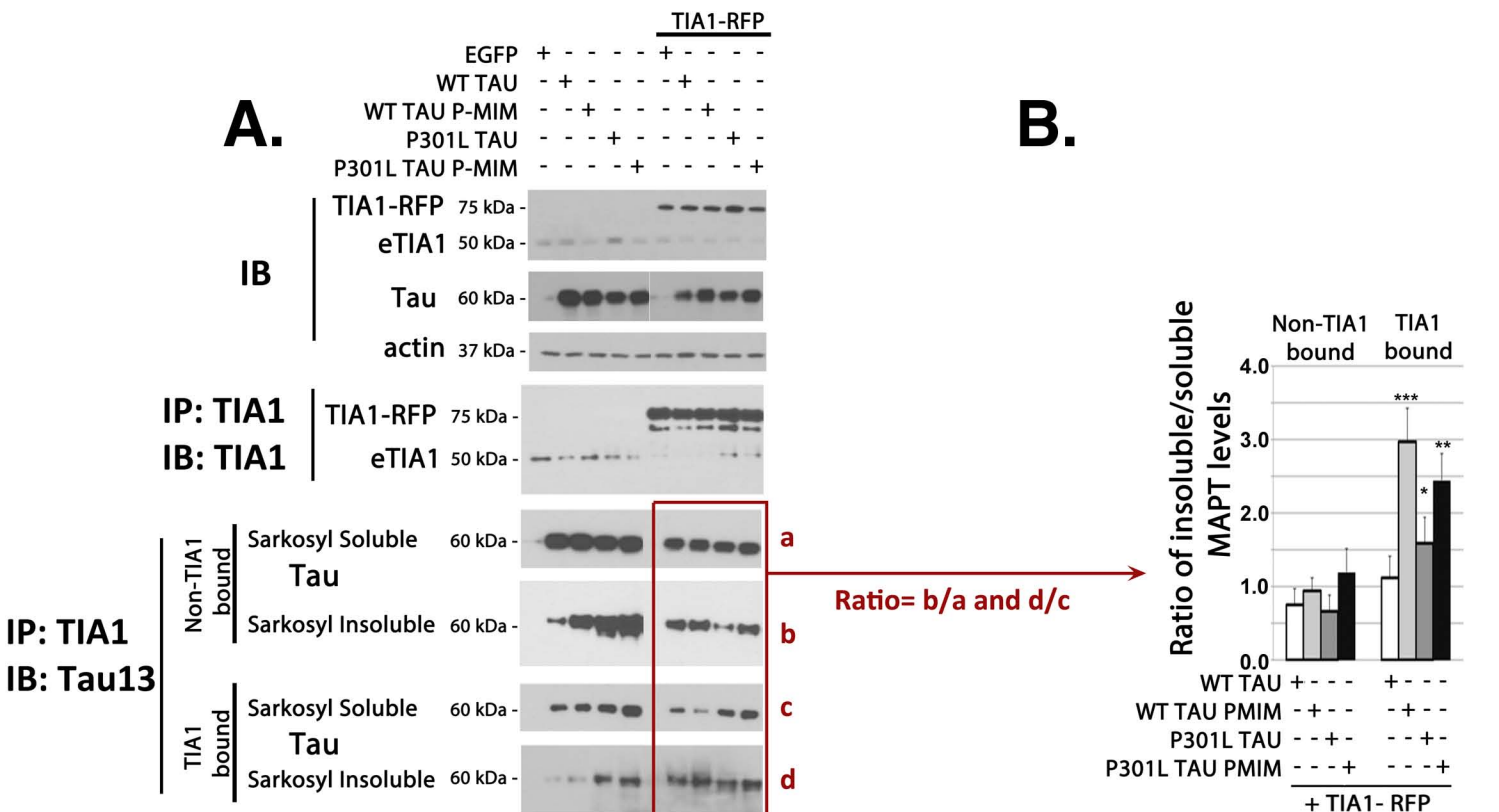
B.



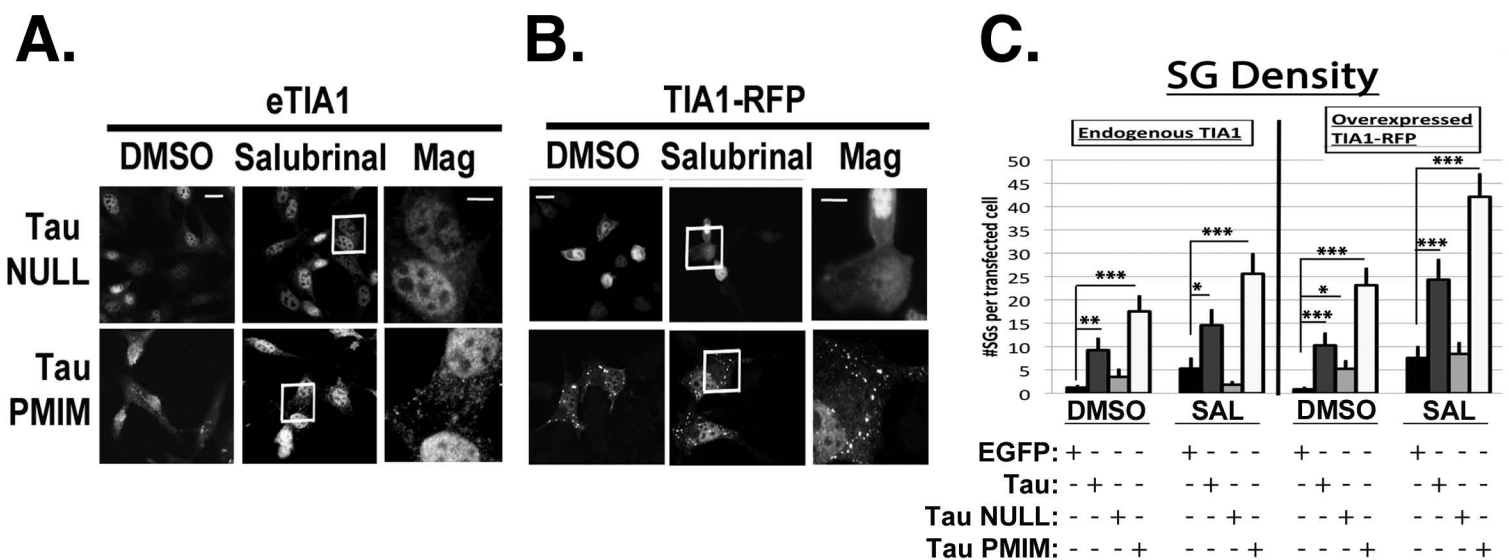
Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6



Supplemental Figure 7