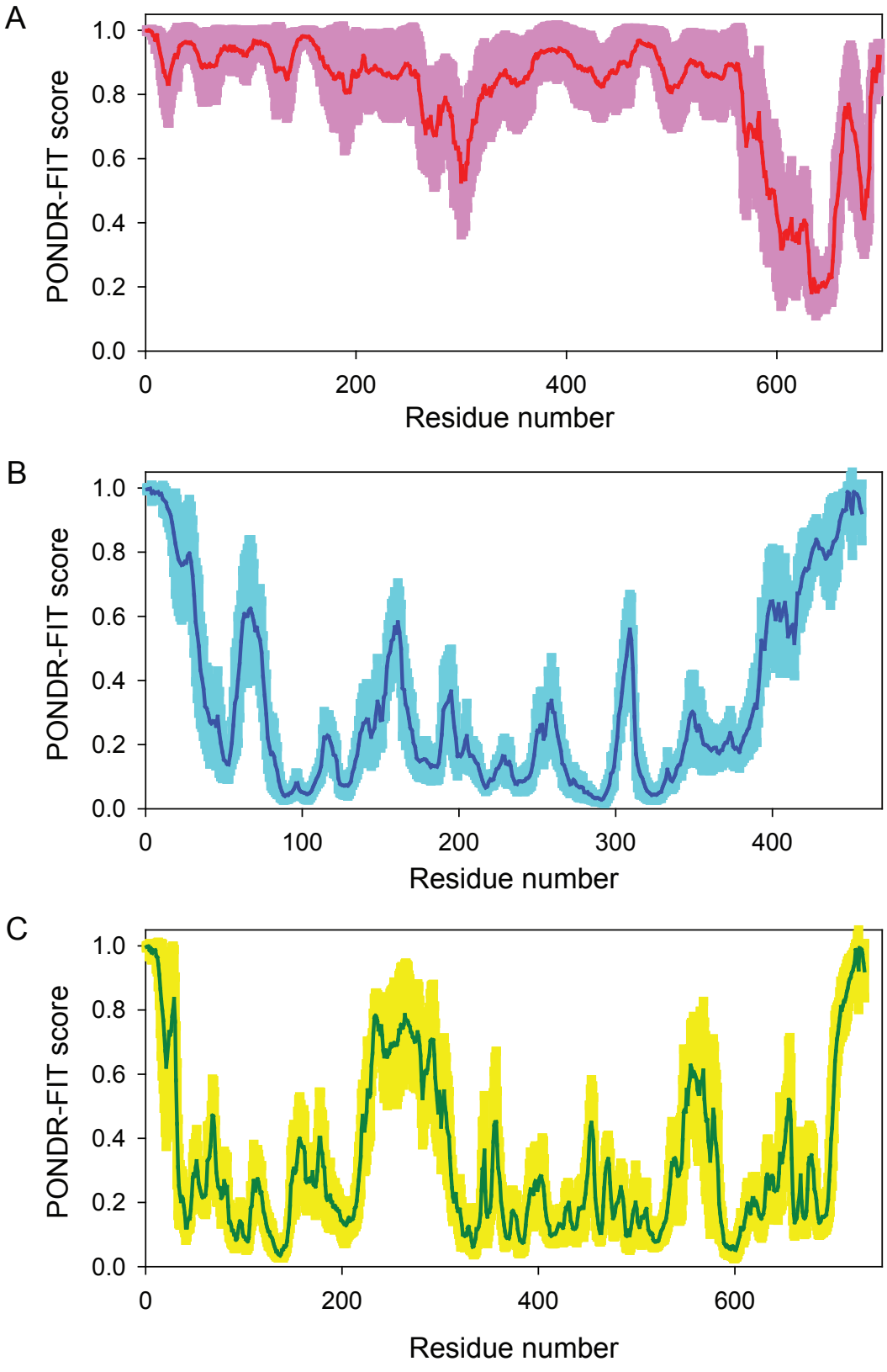


Figure S1. Evaluation of intrinsic disorder propensity of human tau protein. Tau (A), FKPB51 (B), and Hsp90 (C) by disorder predictor PONDR-FIT. The light pink, cyan and yellow shadows around the corresponding PONDR-FIT curves show the statistical error of PONDR-FIT predictions.

Supplement 2

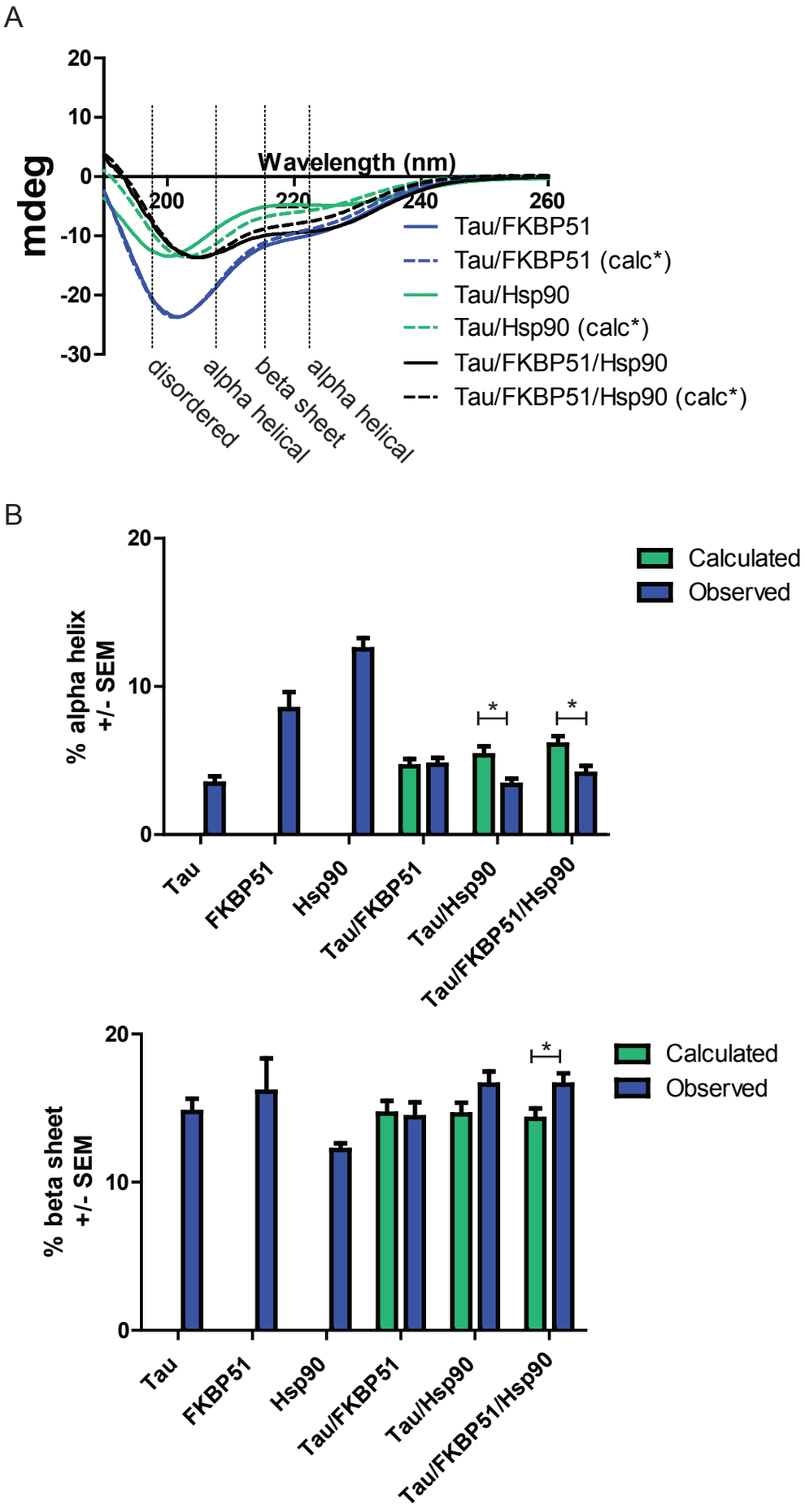


Figure S2. Hsp90 and FKBP51 cause significant tau secondary structural changes (A) CD spectra readout of tau incubated with FKBP51, Hsp90, or both FKBP51 and Hsp90 (solid lines). Calculated and scaled (calc*) CD spectra (dashed lines) from the addition of each protein's spectra shows the calculated readout if there were not protein interactions scaled to the minimum peak. (B) Average deconvolution of CD spectra by Contin and CDSSTR. The green bar represents the calculated predicted result based on the addition of the individual Tau alpha helical structure is significantly changed when incubate with Hsp90 (*p=0.0102) and Hsp90/FKBP51 combined (*p=0.0156). Tau beta sheet structure is significantly changed in the presence of Hsp90 and FKBP51 together (*p=0.0298). Beta sheet structure is altered in the presence of Hsp90 alone, however this change was not found to be significant by the deconvolution results (p=0.092). Tau unordered and turn structure was unchanged in all combinations (data not shown).

Supplement 3

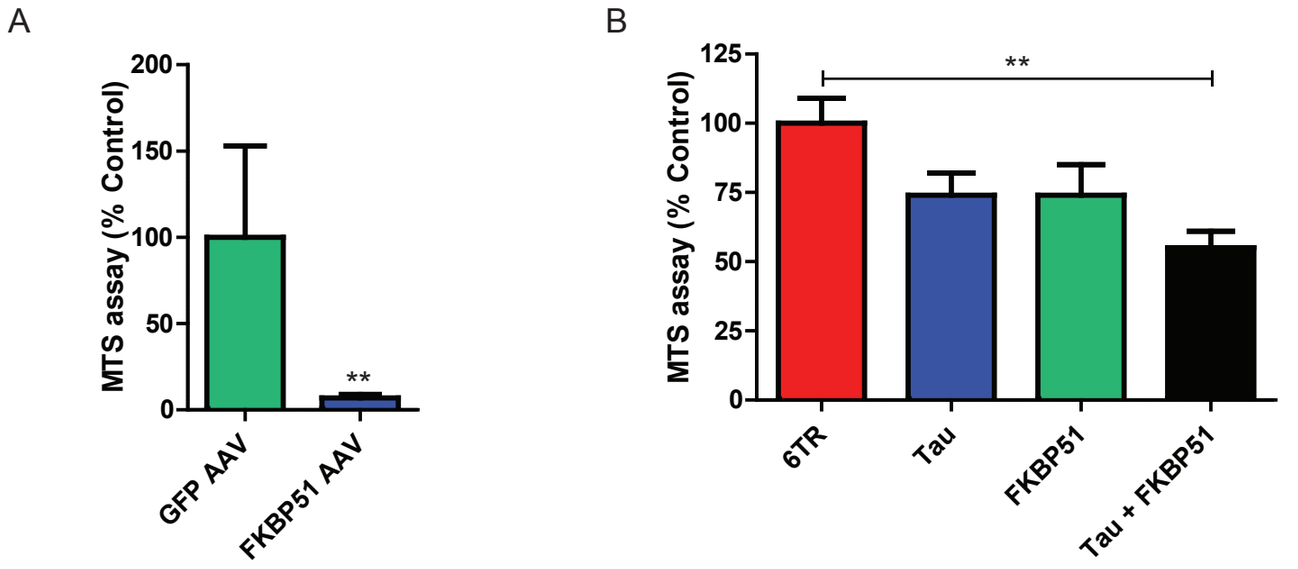


Figure S3. FKBP51 is neurotoxic. (A) Murine primary neurons from tau mice were transduced with FKBP51 or GFP AAV9. **p=0.0041 (B) Neuro-2A cells were transfected with 6TR, P301L tau, FKBP51, or both P301L and FKBP51. **p=0.0064 Toxicity was measured colormetrically by MTS assay.

Supplement 4

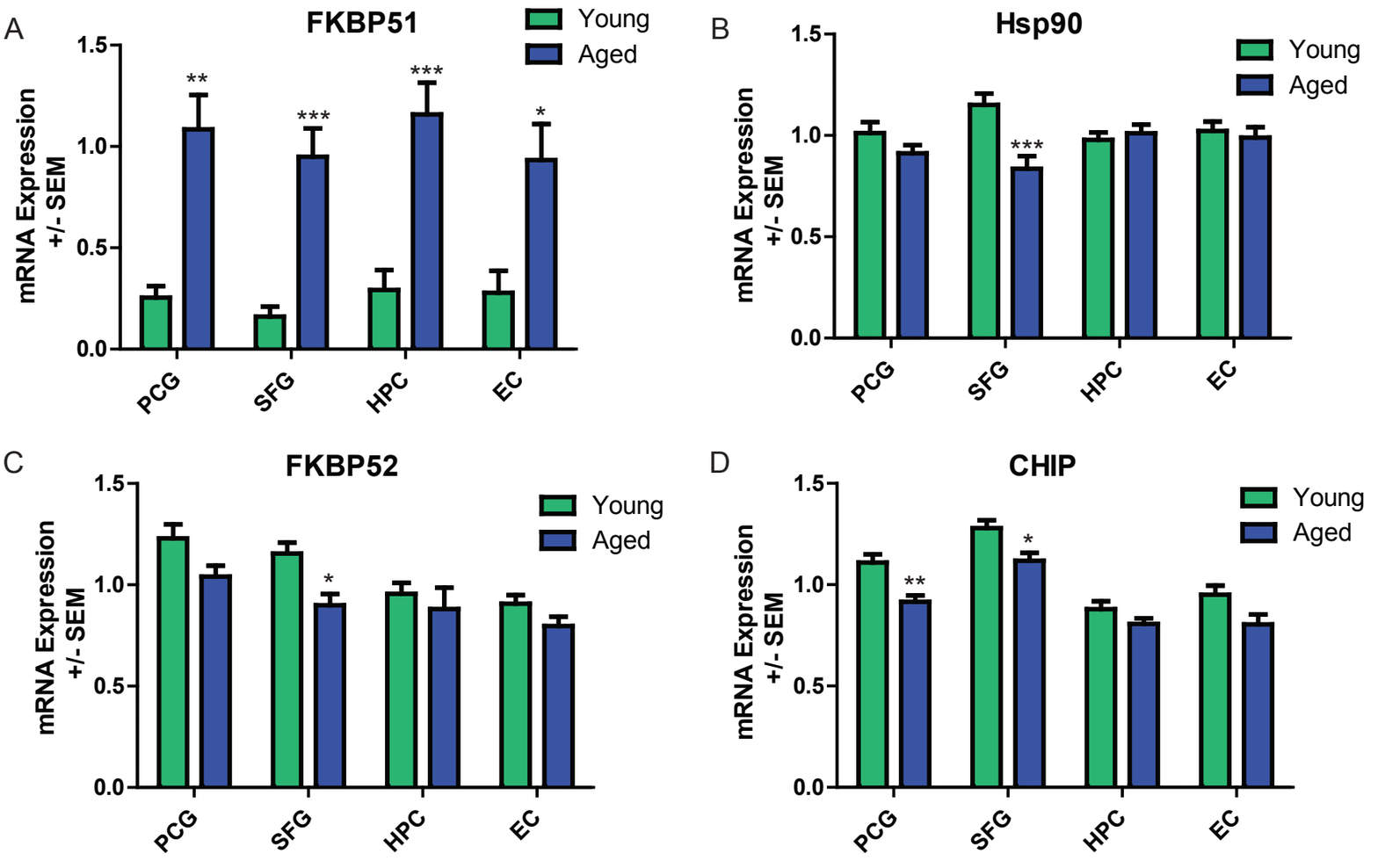


Figure S4. FKBP51 mRNA expression increase with age in many brain regions. (A) FKBP51, (B) Hsp90, (C) FKBP52, and (D) CHIP mRNA expression from young (20-59) and aged (69-99) samples from the post central gyrus (PCG), superior frontal gyrus (SFG), hippocampus (HPC), and entorhinal cortex (EC). *p<0.05, **p<0.01, ***p<0.001

Supplement 5

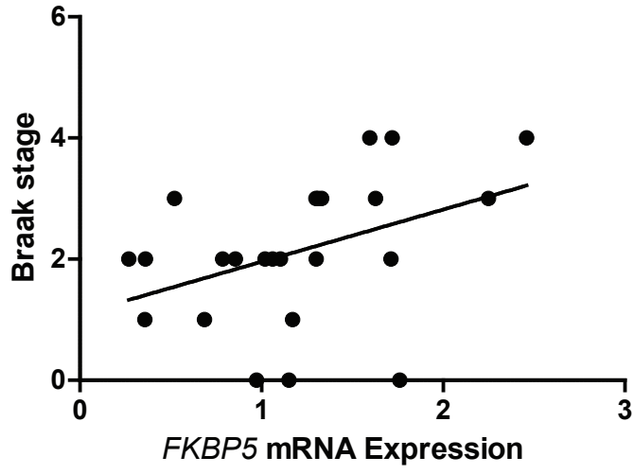


Figure S5. Braak staging correlates with FKBP5 expression. Graph showing the linear regression of FKBP51 levels with Braak staging. The average mRNA expression from all 4 regions for each sample was plotted against Braak stage from all non-demented human samples between 69 and 99 years of age. **p=0.0474; R=0.1670

Supplement 6

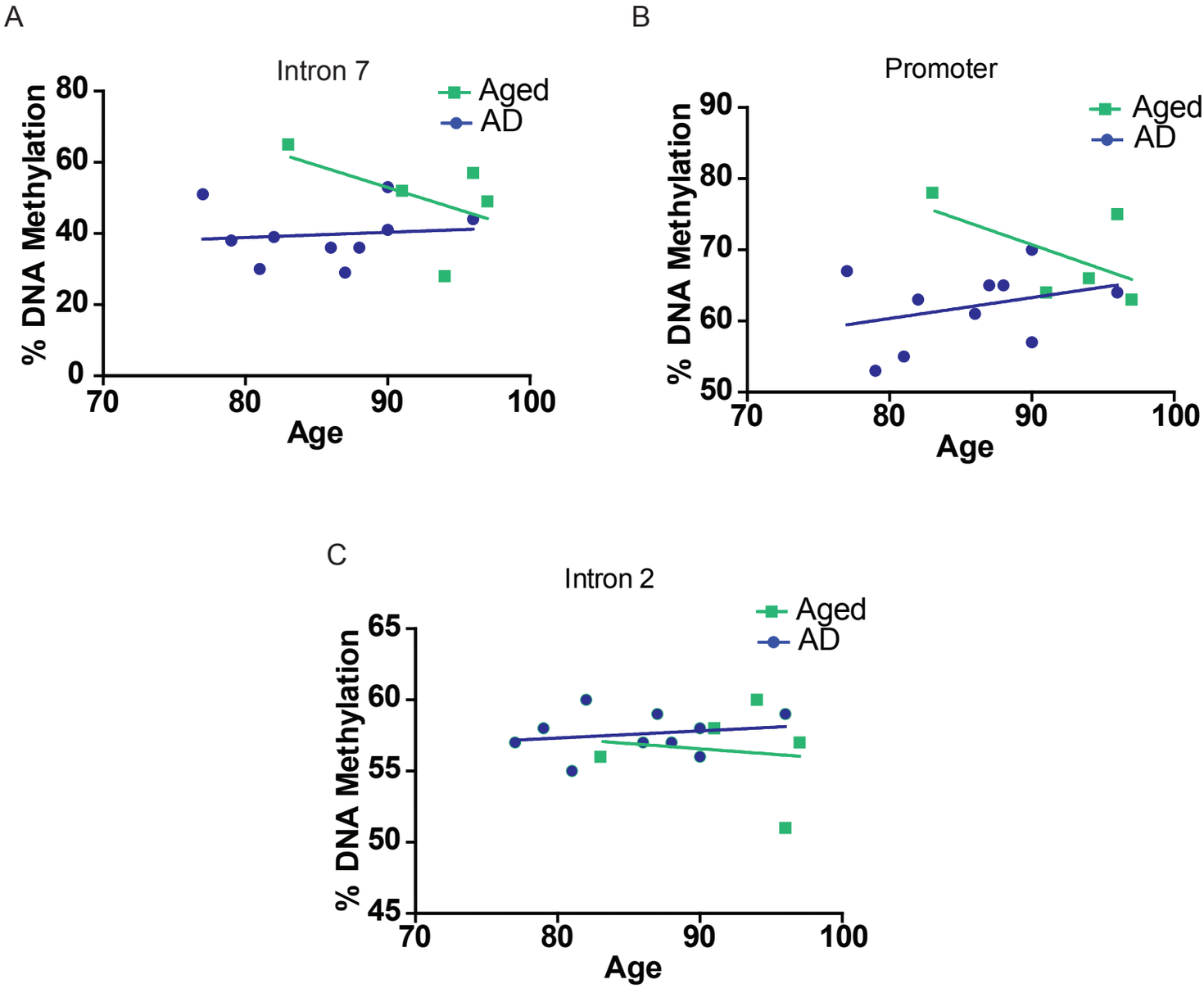


Figure S6. Methylation analysis of an aging AD population. Average Methylation analysis of human DNA form the MTG of AD and aged matched samples from multiple CpG sites from (A) Intron 7, (B) Intron 2, and the (C) Promoter of FKBP5 by age.

Materials and Methods

Antibodies: pT231 (Anaspec), Tau V-20 (Santa Cruz Biotechnology), H150 tau (Santa Cruz Biotechnology), PHF1 (pS396/404; gift from Dr. Peter Davies, The Feinstein Institute for Medical Research, Manhasset, NY), CP13 (pS202/t205; gift from Dr. Peter Davies), Tau12 (N-Terminal 2-18; gift from Dr. Lester Binder, Northwestern University, Chicago, IL), pT231 (Abcam), NeuN (Millipore), Neurotrace (Invitrogen), H150 (Santa Cruz), GFP (Invitrogen), T22 (Dr. Rakez Kayed, University of Texas Medical Branch, Galveston, TX), Hsp90 (BD Biosciences), GAPDH (Meridian Life Science), FKBP51 (gift from Marc Cox), CHIP (Cell Signaling), FKBP51 (Cell Signaling), and FKBP52 (gift from Marc Cox, University of Texas at El Paso, El Paso, TX), Actin (Sigma).

Plasmids: Hsp90α, CHIP, tau, FKBP51 were generated in our lab using the pcDNA3.1 and pCMV6 backbones.

Viruses: Adeno-associated virus serotype (AAV) 9 *FKBP5* (Dr. Todd Golde, University of Florida, Gainesville, FL) and AAV9 EGFP (gift from Dr. Kevin Nash, University of South Florida, Tampa, FL) were obtained for murine gene therapy studies.

Human Tissue: Human tissue was obtained from the University of California at Irvine MIND Alzheimer ’s Disease Research Center (Irvine, California). See Tables 1 and 2 for specific details.

Cell culture and transfection

HEK293 cells were cultured in Opti-Mem plus media supplemented with 10% FBS and 1% Penicillin-Streptomycin (Invitrogen). DNA transfection and lysis procedures were followed as described earlier (17). In brief, transfections were performed with 2.5

µl Lipofectamine 2000 (Invitrogen) per 1ug DNA was added to serum-free Opti-MEM for 4 hrs and replaced with serum-containing Opti-MEM for another 44 hrs. For co-IP from cellular lysates, cells were harvested in co-IP buffer and pre-cleared with protein G (Pierce) and anti-IgG (species) antibody for 1 hr. After passing through a spin column, lysates were incubated with Hsp90 antibody for 3 hrs. Protein G was added to the mixture and incubated overnight on a shaker at 4ºC. The beads were spun down and washed five times. The precipitate was used for Western blots. N2A cells were cultured in Opti-Mem plus media supplemented with 10% FBS and 1% Penicillin-Streptomycin (Invitrogen).

Primary neurons were harvested from P0 pups as previously described (74). Briefly, newborn pups were immersed in 70% EtOH and rapidly decapitated. Intact brains were removed and stored in dissociation buffer containing HBSS supplemented with 1% sodium pyruvate, 0.1% glucose, and 10mM HEPES (pH 7.3). Following removal of the meninges, brains were cut into small pieces with a razor blade. Neurons were trypsinized and suspended in a single cell solution in MEM media supplemented with 10% FBS, 0.45% glucose, 1% 100mM sodium pyruvate, 1% 200 mM glutamax, and 1% Penicillin/Streptomycin. Cells were plated on a 96 well plate and grown for two weeks in Neurobasal media supplemented with 1% B-27, 1% 200mM glutamax, and 1% Penicillin/streptomycin.

MTS Assay

Primary neurons in 96 well plates were transduced for 14 days. N2A cells were plated in 96 well plates and transfected for 48 hours. At the end of the experiments MTS assays (Promega) were performed. Replicate wells of no less than 8 replicates for each treatment were analyzed.

Western blot and dot blot analysis

Samples extracted from cellular lysates and human brain tissues were analyzed by Western blotting using 10% acrylamide gels (74). Antibody dilutions were 1:1000 unless otherwise stated. All secondary antibodies were used at 1:1000 (Southern Biotech). Blots were developed using ECL (Pierce) on a LAS-4000 mini imager (GE Healthcare). For dot blots, proteins were applied onto a wet nitrocellulose membrane and dried by vacuum. Once dry, membrane was blocked and developed as described above. For T22 dot blots with recombinant protein, 50ng of tau was the minimum required for T22 detection.

Protein purification

Production and purification of recombinant proteins was based on protocols described previously (75). Briefly, WT 0N4R tau, FKBP51, Hsp90α was cloned into pET28 vectors containing an N-terminal polyhistidine tag. Proteins were expressed in E. coli, and purified via Ni–NTA–Sepharose chromatography. With the exception of FKBP51, all proteins were subsequently purified by size-exclusion chromatography with a Superdex 200 column. Verification by Coomassie Brilliant Blue-stained SDS polyacrylamide gels was used to deptermine the purity of all proteins.

20S Proteasome Assay

6µg 20S proteasome (ENZO Life Sciences) was incubated with 1µM ON4R-Tau (final volume 10µl) for the 5 minutes in reaction buffer at 37°C (50mM Tris pH 7.5, 50mM KCl, and 0.2mM DTT). FKBP51 and HSP90 (concentrations are indicated in figure) were added in lanes indicated. FKBP51 and HSP90 were pre-incubated with tau for 5 minutes prior to addition of 20S proteasome. Reactions were stopped by adding laemmli buffer and boiling 4 minutes. Samples were then resolved on a 15% SDS-PAGE gel and analyzed by Western blot. To determine if FKBP51 inhibited 20S activity 100µg Suc-LLVY-AMC was incubated with 6µg of 20S proteasome for 5 minutes at 37°C. Readings were then taken with an excitation wavelength of 380nm and an emission wavelength of 460nm.

Circular dichroism spectroscopy

Far- UV circular dichroism (CD) measurements were performed on a JASCO J-815 spectropolarimeter. The proteins (WT 0N4R tau, FKBP51, Hsp90) were dialyzed overnight in 10mM sodium phosphate buffer, pH 7.5 and the concentration was determined using a commercially available BCA kit (Pierce). The proteins were mixed prior to transfer to a 1mm quartz cuvette and measured at 25^{o}C. Each spectrum is the average of 3 scans from 190-260nm at a rate of 50nm/min. Control curves from buffer alone were subtracted for each experiment. The calculated spectrums were determined by simple addition of the indicated individual protein spectrums. All CD spectra were submitted to two different algorithms (CONTINLL and CDSSTR) each using three

different reference sets. All results collected were combined and averaged for each secondary structural component and used in the t-test to determine significance.

Tau aggregation measured by dynamic light scattering and Thioflavin T fluorescence

All proteins were dialyzed overnight into buffer containing 20 mM Tris-HCl and 100 mM NaCl at pH 7.4. The concentrations of purified tau, FKBP51, Hsp90α were determined using the UV extinction coefficients at 280nm of 0.155, 0.788, 0.700, and 0.694 M^{-1} cm^{-1} respectively (76).

Recombinant P301L tau (300 μM) was combined with FKBP51 and/or Hsp90 at a stoichiometric ratio of 50 parts tau to 1 part FKBP51 and/or 1 part Hsp90. All proteins were filtered through a 0.2 μm syringe filter (Anotop) and a 100 kDa molecular cutoff filter (Nanosep). Hsp90 and FKBP51 required an additional filtration through a 0.02 μm filter (Anotop). Heparin sodium salt (MP Biomedicals LLC cat#194114) was filtered through a 0.2 μm and 0.02 μm syringe filter and added at 75 μM to initiate aggregation. Samples were placed in a Greiner Bio-One 384 well glass-bottom plate (cat# 781892), incubated at 37°C, and aggregation kinetics in each well were monitored for 3 min every 2 hours using a Wyatt DynaPro DLS Plate Reader Plus. The low molar ratios of FKBP51 or Hsp90 to tau ensured that the DLS correlation function was dominated by the scattering of tau. Mean aggregate radius were plotted as a function of time using Igor Pro and fitted using the sigmoidal curve described by the following equation.

$$R_{□} = R_{□(min)} + \frac{R_{□(max)}}{1 + \exp([t_{□alf} - t]/\tau)}$$

where t_{half} is the time in days to reach half the maximum aggregate radius and the apparent rate constant, k_{app} is equal to $1/\tau$. Thus, a larger apparent rate constant indicates a faster increase in aggregation (77).

After incubation at 37^{o}C for 6 days, Thioflavin T (ThT) fluorescence was measured by removing the sample and diluting 30 fold into 10 μM ThT. The maximum fluorescence intensity was measured at 485 nm with an excitation of 445 nm using a FluoroMax-4 Spectrofluorometer (Horiba Jobin Yvon, Edison, NJ). Data from 3 dilutions of each sample was averaged and normalized to a tau only control that was set at 100%.

AFM Characterization of Tau/Hsp90/FKBP51 Aggregate Morphology

Samples from the day 6 time point of the DLS measurements were diluted 1:10 in polymerization buffer, deposited onto freshly cleaved mica for 15 min, rinsed with deionized water, and dried with dry nitrogen. Mounted samples were imaged in air with an MFP-3D atomic-force microscope (Asylum Research) using PPP-FMR silicon tips (Nanosensor) with a nominal tip radii of 7 nm. The cantilever was driven at 60–70 kHz in alternating current mode at a scan rate of 0.5 Hz. Images were acquired at 1024x1024 pixel resolution.

Animals

The *FKBP5* -/- colony was bred and genotyped as previously described (30). The rTg4510 colony was bred and genotyped as previously described (5). Four month old rTg4510 (n=5) and wildtype littermates (n=5) were stereotaxically injected bilaterally into

the hippocampus at X= ±2.7 Y= -2.7 Z= +3.0. Each injection delivered 2 µL of adeno-associated viral particles serotype 9 tagged with either green fluorescent protein (GFP) or FKBP51. After 2 months of expression, mice were perfused with 0.9% saline. Brains were immediately fixed with 4% paraformaldehyde overnight. Following sucrose gradients up to 30%, tissue was sectioned using a sliding microtome into 0.2% Sodium Azide in PBS. Sections for general histochemical staining were sectioned at 25 µm. Additionally, 50 µm sections were taken for stereology.

Immunohistochemistry

Free floating staining was performed as previously described (47). Briefly, sections were incubated in PBS supplemented with 10% MeOH/3% H_20_2 to block endogenous peroxidases. Following PBS washes, tissue was permeabilized by 0.2% Triton X-100 with 1.83% lysine and 4% serum in PBS, after 30 minutes, tissue was incubated at room temperature in primary antibody. Tissue was incubated overnight with either anti-pT231 tau (1:300), anti-FKBP51 (1:1000), or T22 (1:700). Following three washes, biotinated goat anti-rabbit (Southern Biotech) secondary (1:10000) was added for 2 hours. Prior to peroxidase development, an ABC kit (Vectastain) was used to increase visibility. Following washes, tissue was incubated with 0.05% diaminobenzidine plus 0.5% nickel and developed with 0.03% H_20_2. Sections mounted, allowed to dry overnight and dehydrated in alcohol gradients. Slides were coverslipped with DPX following clearing by Histoclear. Sections stained with cresyl violet (nissl) were incubated with 0.05% cresyl violet briefly and quickly destained with 0.3% acetic acid in water prior to dehydration. One series of sections was stained by immunofluoresence.

This tissue was permebalized as described above and incubated overnight with anti-Tau V-20 (1:50), anti-PHF1 (1:1000), anti-Biotynalated NeuN (1:100), and anti-FKBP51 (1:300) or anti-GFP (1:300) respective to the injected virus. Following washes, sections were incubated for 2 hours with goat anti-rabbit (1:3000), donkey anti-goat (1:1000), and strepavidin (1:1000) AlexaFluor tagged secondary antibodies. Sections stained with Neurotrace (1:25) (Invitrogen) were incubated following secondary for 20 minutes.

Human tissue was incubated in 0.1% Sudan Black B in 70% EtOH (Sigma) to reduce autofluoresence for 20 minutes and then washed three times with 0.2% Tween in PBS following fluorescent secondary. Tissue was mounted after three washes and coverslipped with ProLong Gold antifade (Invitrogen) reagent. Gallyas silver stain was performed as previously described for non-parafin imbedded tissue (78).

Sections stained for stereology were blocked and permeabilized as described above and incubated overnight with biotynylated NeuN (1:3000) at room temperature. Following washes, ABC conjugation, and peroxidase development tissue was mounted on glass slides and dried overnight. A cresyl violet counterstain was applied to the slides as described before coverslipping.

Microscopy

A slide scanning microscope (Zeiss) was used to image all brightfield stained tissue. Fluorescent microscopy was performed using a Leica TCS SP2 laser scanning confocal microscope. Fields of view were selected in the hippocampus based on the spread of the virus. Using a 63x/1.40-0.60 PLAN APO Oil objective, a minimum of 15 1 µm Z-stacked images was collected from sections taken from each animal with Argon and HeNe lasers.

Imaging analysis

Bright field analysis was performed using Zeiss Neuroquant IAE analysis software. We used this program to outline regions of interest from the entire slide. Thresholds were then manually set until all of the positive cells, as determined by the analyzer, were selected while as little non-specific areas were selected as possible. Using the batch process option, each stained group was automatically calculated for the Area Ratio of positive cells within the determined regions of interest.

Fluorescent image analysis was performed using ImageJ as previously described with minor modifications. (44) Briefly, images were opened and channels separated (Red=Tau, Green=FKBP51, and Blue=NeuN) using the Bio-Formats plug-in. Background was subtracted from the Green and Red channels using the Gaussian Blur tool (Radius = 50um) of the image and subtracting this new image from the original image. Both the Green and Red channels were despeckled and a binary mask was made of the Blue channel. Using the image calculator, we created a new image that represented all neurons (Blue) and also FKBP51/GFP expressing (Green). Next, using Analyze>Set measurements: we redirected this new image of only infected neurons to the red (tau) channel. Individual cells were accounted for by using Analyze> Analyze Particles and a radius of 50 to infinity. We then used the mean of the area and the intensity density to determine the amount of tau (red) in those cells.

To calculate the effect of the virus on all surrounding neurons, the same images were used. We cleaned up the red channel by using background subtraction and despeckling the images. A mask was made of all the neurons shown by the blue

channel. This mask was redirected to the red channel and the particles were analyzed (100-infinity) to measure the area fraction of the red within the blue. This would tell us how much tau was in each neuron in images taken that had virus expression in at least one cell. Comparisons were made of the total area fraction between the FKBP51 and GFP injected.

Stereology

Neurons were defined by NeuN and cresyl violet staining, those positive for both were counted in the CA3. Using a computerized stereological system, connected to an Leica DM4000B microscope with a Prior motorized stage, the area was outlined using distinct landmarks in the brain using 4x magnification (79, 80). Neurons were counted in this region by randomly designated areas in the computer generated grid using a 100x oil immersion lens. Neurons, as defined by NeuN and nissl staining, were counted when they were randomly located within the three-dimentional dissectors or touching the inclusion lines. The top and bottom 1 μM of tissue was excluded. The tissue thickness was determined to be 10.48 ± .028 μM for all sections analyzed.

Microarray

Postmortem tissue for histochemical and biochemical analysis was obtained from 7 different ADR brain banks by UC Irvine Brain Bank. Microarray analysis was undertaken on 4 brain regions (PCG, SFG, HPC and EC) from AD cases (n=26, age 74-95, mean age 85.7 ± 6.5 yrs), age-matched controls (n=33, age 69-99 yrs, average age 84.2 ± 6.5 yrs) and young normal cases (ages 20 to 59; n=22, mean 35.4 ± 10.5 years)

using Affymetrix arrays 9HgU133 plus 2.0 as described previously (51, 81) All subjects were evaluated to be cognitively normal or AD based on diagnoses from medical records. mRNA expression of each sample was evaluated by gene-chip hybridization as described by Cribbs et al (51). These data are deposited on the MIAME-compliant GEO database (Accession number GSE11882).

Pyrosequencing for DNA methylation status

Tissue from young, aged, and AD samples were homogenized as described above. DNA was extracted using Puregene (Quiagen) chemistry and DNA concentration was measured using Qubit 1.0 Fluorometer (Invitrogen). Methylation analysis by bisulfite pyrosequencing was performed as previously described. (54, 82). Intron 7 was evaluated at six CpG sites: P1_S1_Pos1, P1_S1_Pos2, P1_S2_Pos1, P1_S2_Pos2, P1_S2_Pos3, and P1_S3_Pos1. Intron 2 was analyzed at four CpG sites: P4_S1_CG1, P4_S1_CG2, P4_S2_CG1, and P4_S2_CG2. Methylation in the promoter region was analyzed for 6 CpG sites: GRE_S1_CG1, GRE_S1_CG2, GRE_S1_CG3, GRE_S2_CG1, GRE_S2_CG2, and GRE_S2_CG3.

Age	Sex	ApoE
21	M	2-3
36	F	3-4
42	M	3-4
74	F	2-3
85	M	3-3
95	M	2-3

Table 1. Human sample data for tissue used in Figure 7. ApoE indicates allelic variant.

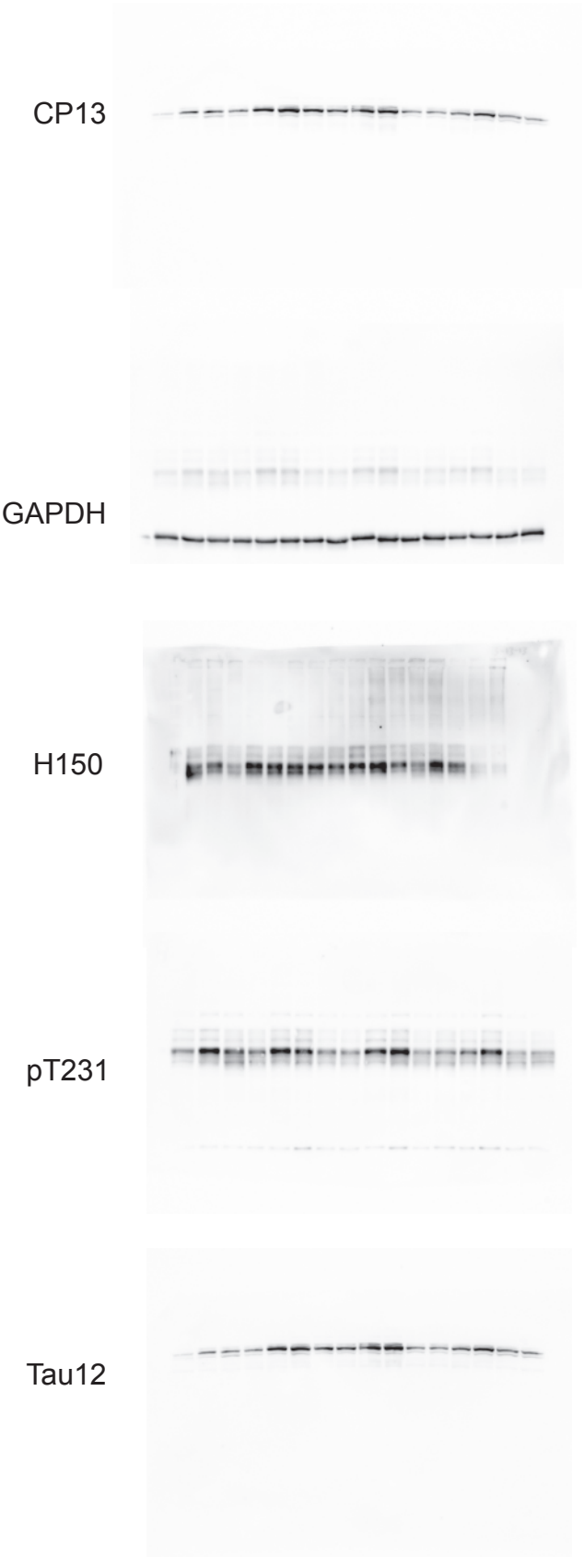
Age	Sex	PMI	ApoE	NPDx1	Tangle Stage	Plaque Stage
81	M	2.87	n/a	Alzheimer's disease	Stage 6	Stage B
90	F	6.58	3/3	Alzheimer's disease	Stage 6	Stage C
90	M	4.17	3/3	Alzheimer's disease	Stage 6	Stage C
88	F	4.5	3/3	Alzheimer's disease	Stage 5	Stage C
77	M	5.67	2/4	Alzheimer's disease	Stage 6	Stage C
82	F	4.58	3/4	Alzheimer's disease	Stage 6	Stage C
87	M	6.17	3/4	Alzheimer's disease	Stage 5	Stage A
96	F	4.5	3/3	Alzheimer's disease	Stage 6	Stage C
86	M	4.2	3/4	Alzheimer's disease	Stage 5	Stage C
79	F	5	3/3	Alzheimer's disease	Stage 6	Stage C
94	M	3.87	3/3	Normal (Mild Braak Changes)	Stage 1	None
97	M	4	3/3	Normal (Mild Braak Changes)	Stage 2	Stage B
91	F	3.8	3/3	Normal (Mild Braak Changes)	Stage 2	Stage A
96	M	3.58	2/3	Normal (Mild Braak Changes)	Stage 2	None
83	M	1.8	3/3	Normal (Mild Braak Changes)	Stage 2	None

Table 2. Human Sample data for tissue used in Figure 9. PMI indicates post mortem interval. ApoE indicates allelic variant. NPD indicates neuropathological determination. Tangle stage is used for Braak staging.

Full uncut blots for Figure 1D

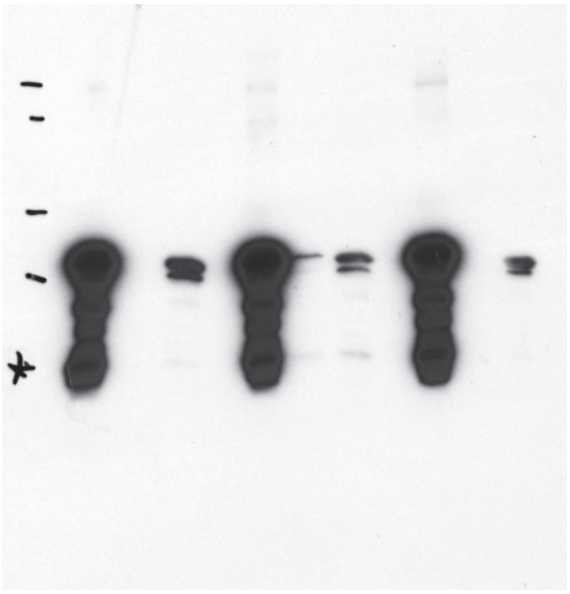
Middle 8 lanes used for 1D

WT KO WT KO WT KO WT KO

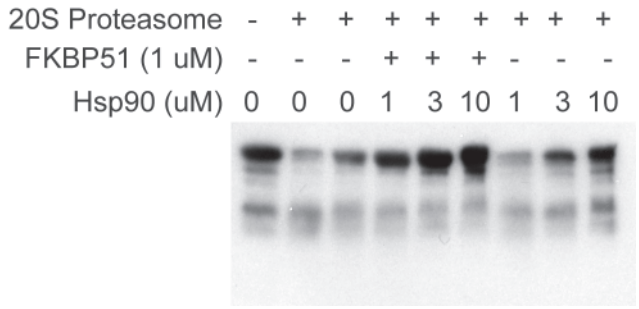


Full Uncut blots for Figure 2

2A

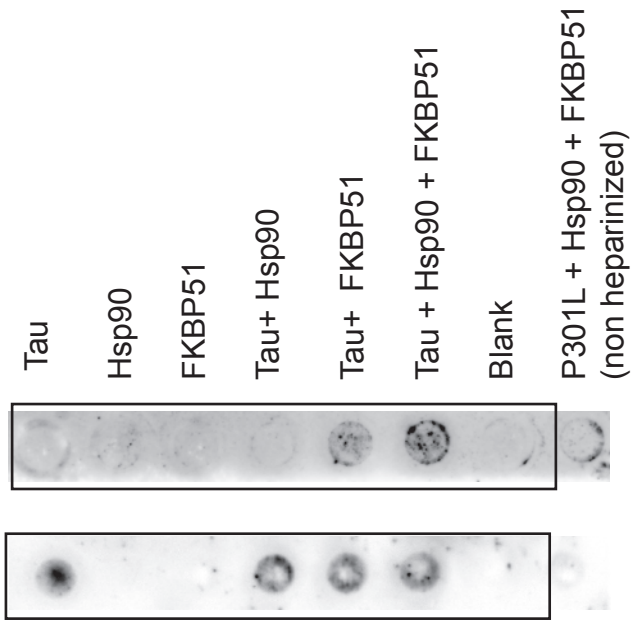


2B



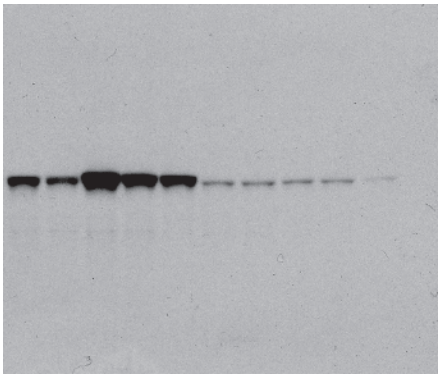
Uncut blots for Figure 3F

Ran as dot blot strips
Used top 7 lanes, did not include non heparinized in final figure.

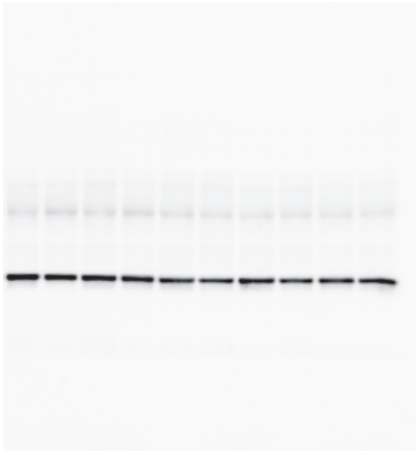


Full uncut blots for Figure 4F

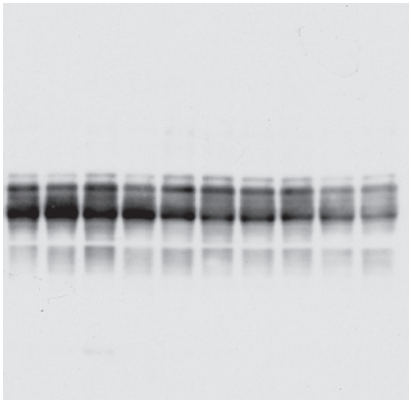
FKBP51



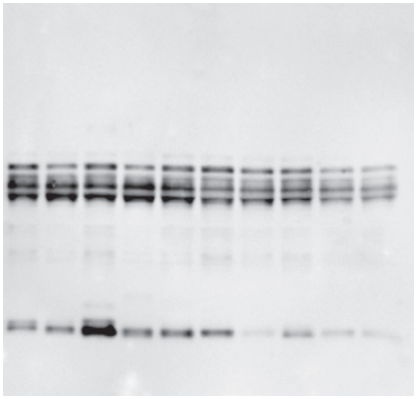
GAPDH



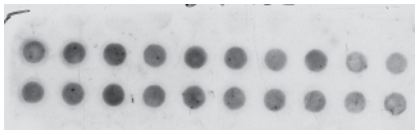
H150



pT231

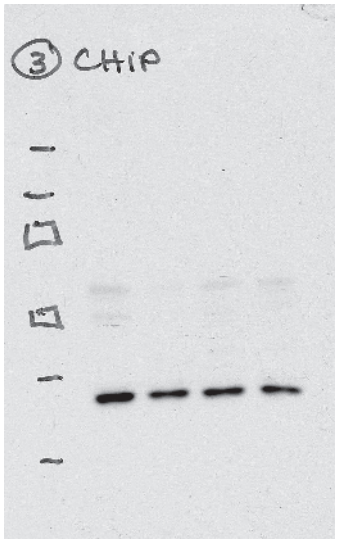


T22

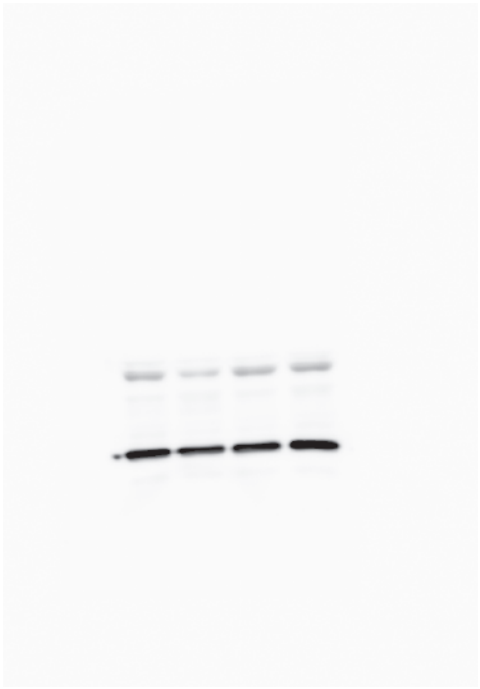


Full uncut blots for Figure 6C

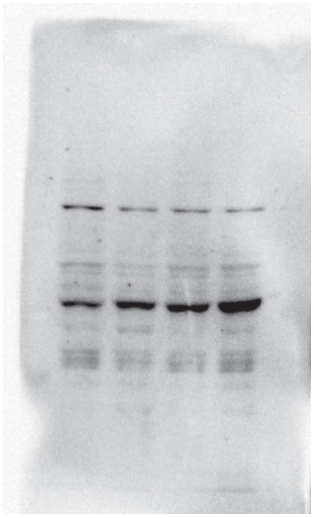
CHIP



GAPDH



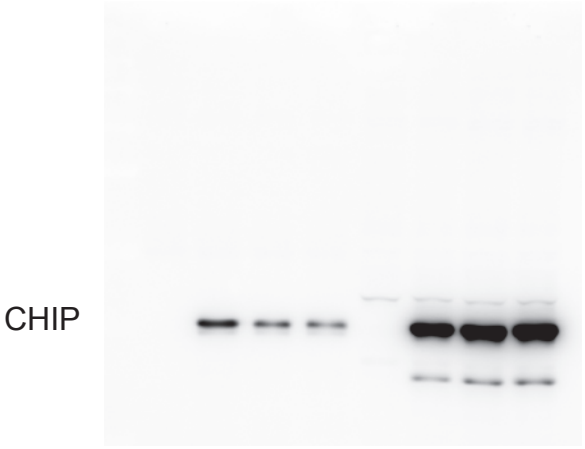
FKBP51

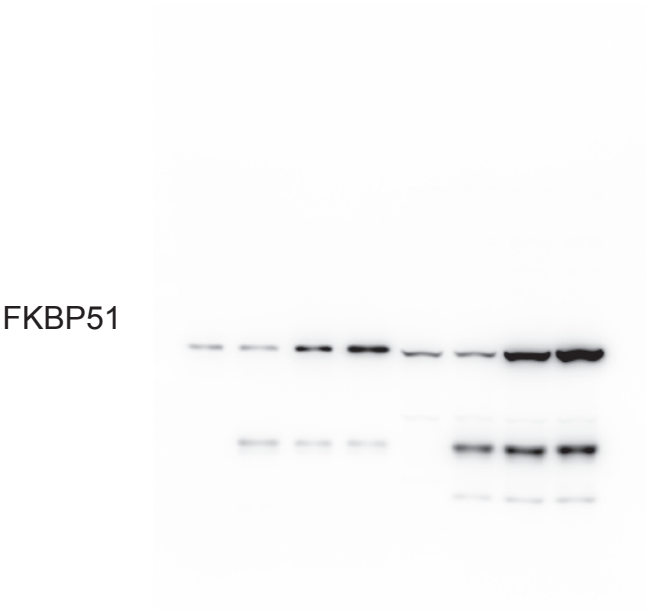


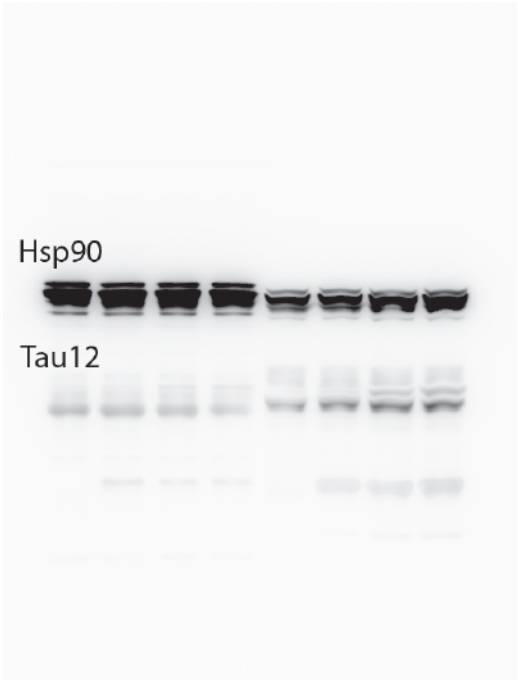
FKBP52

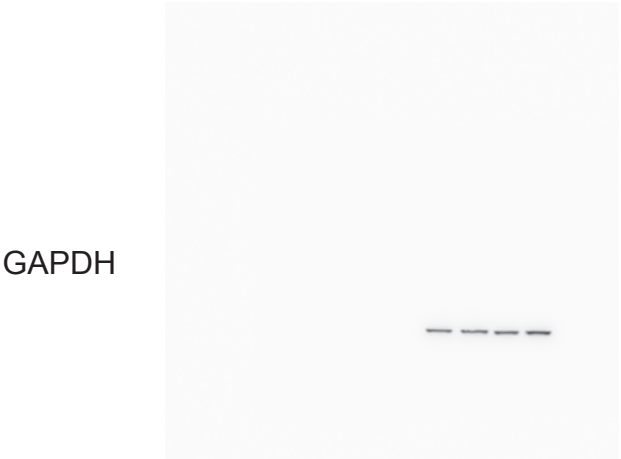
Hsp90

Full uncut blots for Figure 6E



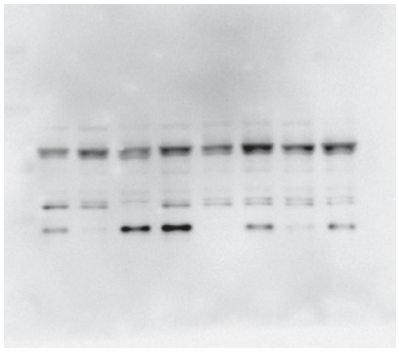




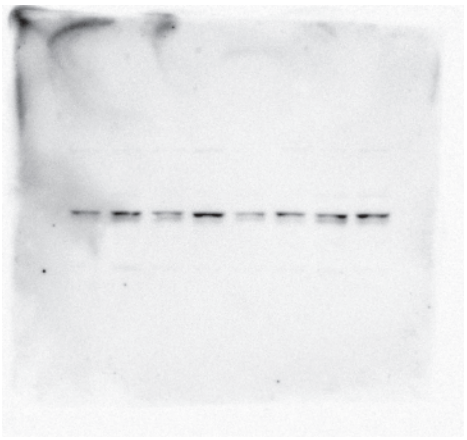


Full uncut blots for Figure 8C

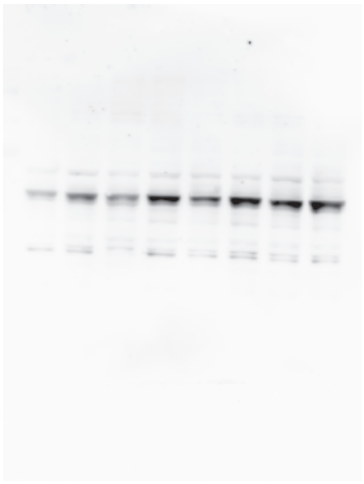
CHIP



FKBP52

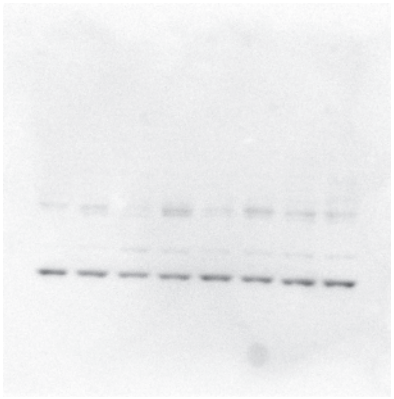


FKBP51



H150

Actin



pT231

Hsp90

T22