Tau modulates mRNA transcription, alternative polyadenylation profiles of hnRNPs, chromatin remodeling and spliceosome complexes

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Abbreviations: AD, Alzheimer's disease; APA, alternative polyadenylation; DDR, DNA damage response; FTD, frontal temporal dementia; GO, Gene Ontology; GSEA, gene set enrichment analysis; GWAS, genome wide association study; MTs, microtubules; PAC, poly(A) cluster; PAC-Seq; Poly(A)-ClickSeq, PAP, poly-A polymerase; PAS, poly(A) site; RNA, ribonucleotide acid; Tet, Tetracycline; 3'UTR, 3' untranslated region; iHEK, inducible human embryonic kidney cells; ER, endoplasmic reticulum;

Author summary

While tau biology has been extensively studied and closely linked to several neurodegenerative diseases, our current understanding of tau's functions in the nucleus is limited. Given the role of tau in disease progression and pathogenesis, elucidating the function of tau activity in transcription and its nuclear accumulation may reveal novel therapeutic targets; therefore, helping identify new upstream pathways that have yet to be investigated. In this study, we used tau-inducible cell lines to uncover new molecular mechanisms by which tau functions in the nucleus. This study systematically investigates the changes in transcriptomic and alternative polyadenylation profiles modulated by WT and mutant P301L tau protein. In this manuscript, we report following new findings (i) tau modulates gene expression of transcripts associated with chromatin remodeling and splicing complexes; (ii) WT and mutant P301L tau regulate, differentially, transcription and alternative polyadenylation (APA) profiles; and (iii) P301L mutation affects the transcription mediated by tau protein. The potential role of tau in mediating transcription and alternative polyadenylation processes is not well studied, representing a novelty in the field. Therefore, this research establishes a new direction for investigating tau nuclear function in both human and mouse brains.

Abstract

Tau protein is a known contributor in several neurodegenerative diseases, including Alzheimer's disease (AD) and frontotemporal dementia (FTD). It is well-established that tau forms pathological aggregates and fibrils in these diseases. Tau has been observed within the nuclei of neurons, but there is a gap in understanding regarding the mechanism by which tau modulates transcription. We are interested in the P301L mutation of tau, which has been associated with FTD and increased tau aggregation. Our study utilized tau-inducible HEK (iHEK) cells to reveal that WT and P301L tau distinctively alter the transcription and alternative polyadenylation (APA) profiles of numerous nuclear precursors mRNAs, which then translate to form proteins involved in chromatin remodeling and splicing. We isolated total mRNA before and after over-expressing tau and then performed Poly(A)-ClickSeq (PAC-Seq) to characterize mRNA expression and APA profiles. We characterized changes in Gene Ontology (GO) pathways using EnrichR and Gene Set Enrichment Analysis (GSEA). We observed that P301L tau up-regulates genes associated with reactive oxygen species responsiveness as well as genes involved in dendrite, microtubule, and nuclear body/speckle formation. The number of genes regulated by WT tau is greater than the mutant form, which indicates that the P301L mutation causes loss-of-function at the transcriptional level. WT tau up-regulates genes contributing to cytoskeleton-dependent intracellular transport, microglial activation, microtubule and nuclear chromatin organization, formation of nuclear bodies and speckles. Interestingly, both WT and P301L tau commonly down-regulate genes responsible for ubiquitin-proteasome system. In addition, WT tau significantly down-regulates several genes implicated in chromatin remodeling and nucleosome organization. Although there are limitations inherent to the model systems used, this study will improve understanding regarding the nuclear impact of tau at the transcriptional and post-transcriptional level. This study also illustrates the potential impact of P301L tau on the human brain genome during early phases of pathogenesis.

Introduction

Tau is a neuronal protein found both inside and outside of the nucleus that contributes to the pathology of neurodegenerative diseases such as frontotemporal dementia (FTD) and Alzheimer's disease (AD)¹. It is primarily described as a microtubule-associated protein². Nuclear tau has been found to 'protect' DNA¹⁻³ during reactive oxygen species (ROS)-induced heat stress. However, nuclear and cytosolic tau interact with RNA to form droplets⁴ and aggregates⁵. Tau has also been observed altering nuclear structure^{6,7} in the human nuclei of neuroblastoma^{8,9} and in HEK-293 cells. More specifically, phosphorylation of nuclear tau negatively regulates its nuclear function in pluripotent neuronal cells and neuroblastoma cells¹⁰. Previous studies have revealed that nuclear tau plays a role in the DNA damage response (DDR) through deadenylation, which triggers major mRNA decay pathways^{11,12}. Most recently, we found that oligomeric assemblies of tau containing RNA-binding proteins impair chromatin remodeling and nuclear lamina formation through associations with histones and chromatin components in the nuclear compartment¹³.

Despite the well-established importance of tau in the cytoskeleton of neurons¹⁴, there is growing evidence that tau is notably involved in nucleolar transcription and cellular stress responses^{15,16}. Recently, it was shown that mutations and/or the phosphorylation of tau results in the deformation of the neuronal nuclear membrane and can disrupt nucleocytoplasmic transport¹⁷ in FTD^{7,18} and AD^{19,20}. Related studies analyzed the direct impact in transcriptional activity due to tau and found that nuclear tau regulates the expression of VGluT1, a gene that controls glutamatergic synaptic transmission, and that tau displacement from microtubules (MTs) increases nuclear accumulation of tau²¹. Furthermore, tau modifies histone acetylation and was shown to have a broad epigenomic impact in the aging and pathology of AD human brains²². It has also been observed that tau interacts with neuronal pericentromeric DNA regions, particularly in association with HP1 and H3K9me3²³, this observation spots tau protein as potential chromatin remodeling factor. Lastly, tau exhibits binding interactions with genic and intergenic DNA

sequences of primary cultured neurons, especially in positions ± 5000 bp away from the start site of transcription²⁴.

In eukaryotic cells, the maturation of 3' ends in mRNA involves endonucleolytic cleavage of the nascent RNA followed by the synthesis of a poly(A) tail on the 3' terminus of the cleaved product by a poly(A) polymerase (PAP)²⁵. This reaction is called polyadenylation and is fundamentally linked to transcription termination. The sequences for the mRNA precursors and the proteins required for polyadenylation are well understood. It has been clearly elucidated that a single gene can give rise to many possible transcripts, each with different polyadenylation sites (poly(A)-sites, or PASs), and that differential usage of these sites can lead to the formation of mRNA isoforms. This phenomenon is called alternative polyadenylation (APA)²⁶ and is a common event in eukaryotic cells. In fact, researchers have determined that 50% of mammalian mRNA-encoding genes express APA isoforms^{27,28}. Considering this information, we used tau inducible HEK (iHEK) cell lines to obtain and analyze transcriptomic and APA profiles in the presence of WT and P301L tau. To characterize transcriptional and post-transcriptional profiles modified by WT and P301L, we utilized Poly(A)-ClickSeq (PAC-Seq) to measure changes in the expression of the host mRNA transcript whilst simultaneously characterizing changes in the PAS usage or creation of mRNA isoforms. In addition, we employed Gene Set Enrichment Analysis (GSEA) and Gene Ontology (GO) to study the main gene domains modulated by tau.

Materials and Methods

Cell Culture and Tau Expression. In this study we used two different versions of tau inducible HEK (iHEK) cells: iHEK overexpressing WT tau and iHEK overexpressing mutated P301L tau. They were maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C in 5% CO₂. To induce WT and mutant tau overexpression, iHEK cells were treated with 1µg/mL of Tetracycline (Tet) for 24 hours in FBS-depleted DMEM (Gibco™ LS11965118, Fisher Scientific). iHEK cells not treated with Tet were named control (Ctr). After 24 hours, two washes with medium were done to remove excess Tet. Immediately after the washes, the cells were stained and collected. Detachment of cells was completed with Trypsin (Gibco™ Trypsin-EDTA, 0.25% Phenol red, LS25200114 Fisher Scientific), and the cells warmed for 3 minutes in the incubator following the addition of Trypsin. The cells were then centrifuged at 1000 rpm for 5 minutes. Lastly, cell pellets were harvested and used for protein fractionation, and mRNA extraction.

RNA Extraction. Total mRNA was collected by using TRIzol extraction reagent according to established protocol²⁹. RNA samples for Real Time Analysis (RT-PCR) were quantified using a Nanodrop Spectrophotometer (Nanodrop Technologies), followed by analysis on an RNA Nano chip using the Agilent 2100 Bioanalyzer (Agilent Technologies). Only samples with high quality total RNA were used (RIN: 7.5-10.0) for the study. Synthesis of cDNA was performed with either 0.5µg or 1µg of total RNA in a 20µl reaction using the reagents available within the Taqman Reverse Transcription Reagents Kit from Life Technologies (#N8080234). Q-PCR amplifications (performed in duplicate or triplicate) were done using 1µl of cDNA in a total volume of 20µl using the iTaq Universal SYBR Green Supermix (Bio-Rad #1725125). The final concentrations of the primers were 300nM. Relative RT-QPCR assays are performed with either 18S RNA gene as a normalizer. Absolute RNA quantification analysis was performed using known amounts of a synthetic transcript created from the gene of interest.

Library Preparation Protocol. Protocols for Poly(A)-ClickSeq (PAC-Seq) have been described in detail by Jaworski et al. 2018^{30,31}. Approximately 1µg of total cellular RNA per sample was used as a template in reverse-transcription reactions supplemented with 40uM Azido-VTPs and primed using an oligo-dT primer containing a partial Illumina i7 indexing adaptor. Azido-terminated cDNA fragments were 'click-ligated' to hexynyl-functionalized click-adaptors containing the Illumina i5 universal sequencing adaptor. Single-stranded cDNA libraries were indexed in a final PCR reaction for 15-18 PCR cycles. Final libraries were size extracted by gel-electrophoresis and submitted for sequencing using an Illumina NextSeq550 to prepare 1x150 SE reads. RNAseq datasets is uploaded to NCBI SRA, reference number: [PRJNA744518](https://www.ncbi.nlm.nih.gov/sra/PRJNA744518).

Poly(A)-ClickSeq. PAC-Seq data were analyzed using the Differential Poly-A Clustering (*DPAC*) program, which ran with default settings as previously described³². *DPAC* trims and quality-filters raw FASTQ data and therefore requires each read to have at least 25 'As' at the 3' end of the read. These reads are then trimmed using *cutadapt*. Trimmed reads are mapped to the reference human genome (hg19) using *HISAT2*³³. The 3'end of mapped reads are thus used to annotate poly(A)-sites and annotated based upon overlaps with gene annotations obtained from UCSC genome browser. Gene counts were extracted and *DESeq2* was used to calculated changes in gene expression as well as relative changes in expression in individual poly(A)-sites found within single genes. Differential gene expression was assigned when a gene had a fold change greater than +/- 1.5-fold with a p-adj value less than 0.1. Alternative polyadenylation is assigned when a single gene has two or more clustered poly(A)-sites wherein at least one of these sites has a differential usage greater than a +/- 1.5-fold, a p-adj value less than 0.1, and a change of the relative usage of a poly(A)-cluster within the gene of greater than 10%.

Western Blotting and Cell Fractioning. Immunoblot (IB) analyses were performed with iHEK cell fraction samples as previously described¹³. Approximately 10 µg of protein preparations were loaded onto precast NuPAGE 4-12% Bis-Tris gels (NP0335BOX, Invitrogen) for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analyses. Gels were subsequently transferred onto nitrocellulose membranes and blocked overnight at 4°C with 10% nonfat dry milk. Membranes were then probed for 1 hour at room temperature with Pan-Tau (Tau13, 1:10,000, MMS-520R Covance), (GAPDH, 1:1000, ab9485 Abcam), Histone3 (1:1000, ab201456 Abcam), RCC1 (1:100, Clone E-6 sc-55559 Santa Cruz Tech.), DNAJC2 (1:5000, ab134572 Abcam), Histone 1.2 (1:500, ab4086 Abcam), HMGB1 (1:500, ab18256 Abcam), SMARCA5 (1:10000, #PA5-78253, Invitrogen), SMARCC1 (0.4µg/mL, #PA5-55058, Invitrogen), and β-Actin (1:5000, #A1978, Sigma Aldrich). Antibodies were diluted in 5% nonfat dry milk. Immunoreactivity was detected using a horseradish peroxidase (HRP)-conjugated anti-rabbit immunoglobulin G (IgG, 1:10,000, NA934 GE Healthcare). Tau13 and Tau5 immunoreactivity were detected using an anti-mouse IgG (1:10,000, NA931 GE Healthcare) diluted in 5% milk. ECL Plus (K-12045-D50, GE Healthcare) was used to visualize protein bands. LaminB1/Histone3 and GAPDH were used to normalize and quantify nuclear and cytoplasmic proteins, respectively. The compartment extraction was conducted with Qproteome Cell Compartment Kits (Qiagen, #37502); nuclear, cell membrane, and cytoplasmic proteins were isolated and preserved for IB analysis.

Immunofluorescence of Fixed Cells and Fluorescence Microscopy. Cells on a 24-well coverslip were fixed with 0.5 ml of 4% PFA/PBS for 15 min. The cells were then washed 3 times in phosphate buffered saline (PBS), for 5 min for each wash. The cells were permeabilized in 0.5ml PBS and 0.2% Triton X-100 in phosphate buffered saline containing 0.5% Tween (PBST) for 5 min. Blocking was done in 0.5 ml of 5% normal goat serum (NGS) in PBST for 1 hour. Primary antibody was diluted in 5% NGS/PBST overnight at 4°C for incubation, and then washed 3 times in PBST, for 10 min each. Secondary antibody diluted in 5% NGS/PBST was incubated

for 2 hours at room temperature. All the secondary antibodies were purchased from Thermo Fisher Scientific and used at a 1:800 dilution for staining. After applying secondary antibodies, cells were incubated in DAPI (nuclei staining) diluted 1:10,000 in PBST (5 mg/ml stock solution) for 5 min after the first wash. The cells were then washed 2 times with PBST, and once with PBS (10 min each) prior to mounting coverslips. Coverslips were mounted on glass microscope slides using 8-10 μ l of Prolong Gold Antifade mounting media with DAPI (Invitrogen, P36941) per coverslip. Slides were air-dried in fume hood or stored at 4°C until ready to be dried in the fume hood. The primary antibodies used in this study for immunocytochemistry (ICC) are as follows: Histone 1.2 (Abcam ab4086 - 1 μ g/ml), Ki-67 (Abcam ab92742 - 1 μ g/ml), SMARCC1 (Invitrogen PA5-55058 - 0.25 μ g/ml, SMARCA5 (Invitrogen PA5-78253 - 1 μ g/ml, MCM2 (Abcam ab108935 - 1/1000), RCC1 (Santa Cruz, INC. sc-55559 - 1:50), and Tau13 (Bio Legend MMS-520R - 1/200). After three washes with PBS, cells were probed with mouse and rabbit-specific fluorescent-labeled secondary antibodies (1:200, Alexa Fluor 488 and 633, Life Technologies). Single frame images were collected using the Keyence BZ-X 710 Microscope. Images for quantification of area and integrated density were taken in nuclear target areas guided by the DAPI fluorescence. We then performed single extraction analysis using BZ-X Analyzer software (Keyence). We used 200 nuclei per target area and used the Nikon 20X objective for imaging and quantification analysis.

Statistical Analysis. All in-vitro experiments were performed in at least three biological replicates. All data are presented as means $\pm$ SD and were analyzed using GraphPad Prism Software 6.0. Statistical analyses included the Student-t Test or one-way ANOVA followed by Tukey's Multiple Comparisons Test. Column means were compared using one-way ANOVA with treatment as the independent variable. In addition, group means were compared using two-way ANOVA considering factors for each treatment respectively. When ANOVA showed a significant difference, pair-wise comparisons between group means were examined by the Tukey and Dunnett Multiple Comparison Test.

Results

WT tau up-regulates genes associated with cytoskeleton organization and nuclear speckles/bodies. Firstly, we evaluated changes in gene expression profiles upon expression of WT and P301L tau in iHEK cells that were induced with tetracycline (Tet). After 24h of Tet induction, we confirmed tau expression in the cytoplasm and nuclei of iHEK cells (Fig S1A). Total cellular RNA from WT and P301L tau (untreated (Control) and treated (+Tet)) study groups was extracted using TRIzol reagent and by following established protocol^{7,13}. RNA was sequenced using Poly(A)-ClickSeq (PAC-Seq) to measure changes in gene expression and poly(A)-site usage³¹. A schematic of the experimental design is provided in Fig 1A. Volcano scatterplots from WT and P301L tau iHEK (Fig 1B and 1C, respectively) demonstrate a substantial difference in the number of genes regulated by WT tau and P301L tau. After Tet induction in the WT tau iHEK cell system, we observed up-regulation of 88 genes and down-regulation of 30 genes (gene names listed in Fig 1D). In the P301L tau iHEK cell system, these numbers dropped to 10 up-regulated genes and only 1 down-regulated (gene names listed in Fig 1E).

Fig S1B displays the scatterplots of WT and P301L tau gene expression, while Fig S1C reports the Principal Component Analysis (PCA). PCA demonstrates significant variation among the study groups. More specifically, the analysis suggests a significant difference in transcriptional activity of WT tau due to the higher number of genes modulated in comparison to the mutant P301L tau form. Using EnrichR³⁴, we established Gene Ontology (GO) of the biological processes, molecular functions, and cellular components altered by both the up-regulated and the down-regulated sets of genes. WT tau GO is summarized in Fig 2. WT tau up-regulated genes belonging mainly to classes of cytoskeleton-dependent intracellular transport genes (GO: 0030705, TUBA1A, TUBB2B TUBA1B, TUBB2A and HOOK3) and genes responsible for the regulation of cytoskeleton organization (GO: 0051493). Imbalanced expression of tubulin and tau induces

neuronal dysfunction in *C. elegans*,³⁵ indicating that tau itself can disturb tubulin gene expression. The reason behind this pronounced involvement of TUBB genes could be due to the fact that *TUBB1B*, *TUBB2B*, *TUBA1A* and *TUBB2A* are clustered together within the genome³⁶.

It is important to note that biological process such as microglial cell activation (GO: 0001774) and macrophage activation (GO: 0042116) were also observed as being up-regulated, which confirms known effects of tau on the neuro-inflammatory response commonly observed in neurodegenerative diseases³⁷. Neuro-immunomodulation can also effect cytoskeleton reorganization³⁸. Our GO analysis revealed up-regulated genes involved in mitochondrion distribution (GO: 0048311, *MAPT* and *MEF2A* genes), morphogenesis (GO: 0070584, *SUPV3L1* p=0.03892), neurogenesis (GO 0022008, *NOM1*, *MAPT* and *DAGLB* genes), and positive regulation of cell death (GO 0010942, *SAP30BP*, *MAPT* and *CLU* genes). The increase of *CLU* expression was a particularly interesting observation. Clusterin is a multifunctional, secreted chaperone involved in several basic biological events, including cell death, tumor progression and neurodegeneration. The *CLU* gene is notably associated with an increased AD risk³⁹. In terms of molecular functions, the up-regulated genes we observed have several enriched pathways, including RNA binding (GO: 0003723, *USP36*, *NOM1*, *TFRC*, *BAZ2A*, *SUPTSH*, *PHF6*, *FTSJ3*, *SUPV3L1*, *TUBA1B*, *RBM20*, *MAPT*, *RBM33*, *PELP1*, *HIST1H1C*, *CPEB4*) and several nuclear functions, such as histone deacetylase binding (GO: 0042826, *MEF2A*, *SUDS3* and *PHF6*) and sequence-specific double stranded DNA binding (GO: 1990837, *MEF2A*, *KAT7* and *MAPT*).

Transcriptional products of up-regulated genes are mostly localized in the cytoplasm and nuclear compartments. We detected transcripts associated with nuclear chromatin (GO: 0000790), such as *MEF2A*, *ZEB2*, *ANP32E*, *SUDS3*, *HIST2H2AC*, and *HIST1H1C*. We also examined nuclear speck transcripts (GO: 0016607), such as *CARMIL1*, *USP36*, *GTF2H2C*, *BAZ2A*, and *MAPT* genes, which are also included in nuclear body components (GO: 0016604), along with *SUDS3* and *SENP2*. The other cell compartment well represented in our GO analysis

is the cytoplasm. In particular, the microtubule cytoskeleton (GO: 0015630) contained the following up-regulated genes: *TUBB2B*, *SAP30BP*, *TUBA1B*, *TUBA1A*, *TMOD3*, *MAP7*, *TARS*, *TACC1*, *MAPT*, *CLU*, and *RHOQ*. A complete Enrich-GO list of significant up-regulated genes observed in WT tau is presented in Supplemental Table 1.

WT tau down-regulates genes involved in ubiquitin-related processes as well as genes associated with Golgi and mitochondrial components. Overall, thirty genes were significantly downregulated by WT tau protein. The main biological process affected was the regulation of cellular component organization (GO: 0051128) as it relates to cytoskeleton organization and structure morphogenesis. Molecular functions associated with the aforementioned genes are closely related to ubiquitin protein ligase binding (GO: 0031625) and ubiquitin-like protein ligase binding (GO: 0044389). Genes important to neuronal components included genes essential to the structure of initial axonal segments, nodes of Ranvier, and main axons. These three groups typically involve the gene *KCNQ2*. This gene encodes for Potassium voltage-gated channel subfamily KQT member 2, which plays a critical role in determining the subthreshold electrical excitability of neurons as well as the responsiveness of neurons to synaptic inputs. Therefore, *KCNQ2* is important in the regulation of neuronal excitability and the loss-of-function or gain-of function of this gene can lead to various forms of neonatal epilepsy⁴⁰.

Furthermore, Cullin-RING E3 ubiquitin-ligase complex component *KLHL11* is down-regulated, as well as the *STX6* gene. *STX6* encodes for Syntaxin-6, which is involved in intracellular vesicle trafficking and is integrally associated with the Golgi apparatus. Another Golgi protein that is down-regulated is Golgin-45 (*BLZF1*). It is required for normal Golgi structure and for protein transport from the Endoplasmic Reticulum (ER) through the Golgi apparatus to the cell surface⁴¹. Lastly, the ER gene *STC2* is downregulated and encodes for Stanniocalcin-2. This glycoprotein has an anti-hypocalcemic action on calcium and phosphate homeostasis⁴².

We also detected two nucleolus-localized genes among the down-regulated group: UBE2T (ubiquitin-conjugating enzyme with E2 T) and UPF3A, (a regulator of nonsense transcript 3A). The mitochondrial genes that were down-regulated included OXCT1 (Succinyl-CoA: 3-ketoacid coenzyme A transferase 1, mitochondrial enzyme), TRUB1 and PFDN2 (Prefoldin subunit 2). An Enrich-GO list of downregulated genes present in WT tau is depicted in Supplemental Table 2.

Although there are limitations inherent with the model used, these data suggest that WT tau intrinsically and significantly impacts the cell at a transcriptional level. More specifically, a higher number of genes are up-regulated and down-regulated by WT tau when compared to P301L tau. This suggests that the P301L mutation leads to a loss-of-function (LOF) of tau at the transcriptional level. This sort of loss could have detrimental effects on cell structure and organization.

P301L tau up-regulates gene expression of components related to axonal microtubule skeleton, nuclear speckles, and ribonucleoprotein. The GO pathways and cellular compartments upregulated and downregulated by P301L tau are listed In Supplemental Tables 3 and 4 respectively. As observed in WT tau iHEK cells, the *MAPT* gene is on the upregulated gene list for P301L tau, as expected after Tet induction of the iHEK cells. Within the group of axonal and cytoskeleton genes, we noticed up-regulation of NLGN1, a gene that encodes for Neuroligin-1. Neuroligin is a postsynaptic neuronal surface protein involved in cell-to-cell interactions via its interactions with neurexin family members⁴³. It has been established that the NLGN1 gene is associated with amyloid- β oligomers (A β Os) in AD-causing synaptic impairment⁴⁴. In addition, NLGN1 is typically altered in AD hippocampi and also modulates amyloid-beta oligomer toxicity⁴⁵. Neuroligin-1 plays an influential role in synaptic function and synaptic signal transmission, most likely through its ability to recruit and cluster together other synaptic proteins⁴³.

For instance, neuroligin-1 may promote the initial formation of synapses⁴⁶, but is not essential for the complete formation of synapses. *In vitro*, Neuroligin-1 triggers the *de novo* formation of presynaptic structures. NLGN1 may also be involved in specification of excitatory synapses⁴³. For example, NLGN1 functions to maintain wakefulness quality and normal synchrony of cerebral cortex activity during wakefulness and sleep⁴⁷. Neuroligin-1 is predominantly located in synaptic cleft of the cell membrane⁴⁸.

When we analyzed upregulated genes, we detected a considerable number of genes related to nuclear body (GO: 0016604) and nuclear speck (GO:0016607) domains including the genes *ITPKC* and *MAPT*. Interestingly, it has been observed that the *FER* gene participates in several different cytoplasmic and nuclear functions. For example, *FER* is associated with nuclear chromatin (GO:0000790) and the microtubule skeleton (GO:0015630). The *FER* gene also encodes for a tyrosine-protein kinase that plays a role in synapse organization, trafficking of synaptic vesicles, the generation of excitatory post-synaptic currents, and neuron-to-neuron synaptic transmission⁴⁹. Lastly, *FER* plays a role in neuronal cell death after brain damage⁴⁹. The only gene down-regulated by P301L tau is *DCAF12*, which is a component of the Cullin-RING ubiquitin ligase complex⁵⁰. This gene is also down-regulated by WT tau and belongs to genes associated with ubiquitination processes. The failure of ubiquitination pathways is known to have a strong connection to neurodegenerative diseases⁵¹. Supplemental Figure 2 summarizes upregulated and downregulated genes in P301L tau, subcategorized by biological process and molecular function.

In summary, the P301L mutation upregulates genes involved in positive regulation of neuronal death and responsiveness to reactive oxygen species (ROS) production. This is in contrast to the genes altered by WT tau that have a greater effect on cell structural processes. The most important molecular function altered by such genes would be sequence-specific double-stranded DNA binding, transcriptional expression, and chromatin remodelling. Overall, our GO data

suggests the presence of both loss-of-function (LOF) and gain-of-function (GOF) events in mutated P301L tau that may relate to pathology. Modulating genes known to be associated with neurodegenerative disease suggests that mutated tau engenders harmful transcription patterns that contribute to the well-established effects of tau proteinaceous-aggregation toxicity.

WT tau modulates gene expression of chromatin organization and remodeling factors.

Gene Set Enrichment Analysis (GSEA) offers an opportunity to evaluate and identify classes of genes or proteins that are over-represented in a large set of genes or proteins and may have an association with disease phenotypes. Due to the differences in gene numbers modulated by WT tau versus P301L tau, we performed GSEA. This analysis compared models with and without WT tau. We observed that WT tau down-regulates the expression of numerous genes linked to chromatin organization (Fig 3A) and chromatin remodeling (Fig 3B) domains. By looking at the chromatin organization and remodeling gene clusters, we identified that several high-mobility group box proteins (HMG) *HMGN5*, *HMGB2* and *HMGA1* are up-regulated while *HMGB1* and *HMGN1* are down-regulated. It is important to note that HMGB1 is an activator of neuro-inflammatory responses and has been implicated in AD⁵². In addition, several components of the SWI/SNF chromatin remodeling complex are downregulated. The identification of genes *SMARCE1*, *SMARCA5* and *SMARCC1*, imply that tau has a substantial impact on chromatin remodeling in the cells. The heterogeneous nuclear ribonucleoproteins (hnRNPs), *HNRNPU* and *HNRNPC*, were also found to be downregulated in WT tau. Down-regulation of several factors implicated in DNA replication and repair processes, indicates that WT tau also significantly affects the nuclear compartment of cells in terms of structure and content. Several of these genes are clustered as covalent chromatin modification in GO (Fig 3C).

To validate gene expression changes observed in GSEA analysis, we verified multiple proteins via western blot by using the up-regulated and down-regulated lists generated from Histone Binding GO. We verified up-regulation of RCC1, DnaJC2 and Histone1.2 proteins in the

cytoplasm and in nuclear fractions of WT and P301L tau iHEK cells (Fig 3D). We also confirmed RCC1 expression and noticed its accumulation in the cytoplasm for both cell lines. Interestingly, we discerned that RCC1 is not imported into the nuclei where it should function as a regulator of chromatin condensation. Instead, DnaJC2 in P301L tau iHEK cells appear to be downregulated. However, Histone 1.2 is upregulated in both cell lines. We did not observe down-regulation of the chromatin remodeling complex factors SMARCC1 and SMARCA5. Instead, we detected their accumulation in the cytoplasmic fractions while in the presence of tau, which suggests a deficit in these factors in the nuclei, as observed in our western blots. Lastly, HMGB1 and β -Actin are down-regulated, but HMGB1 is not detected in the nuclei when in the presence of tau. Histone 3 was used as a nuclear loading control.

To verify gene expression results, alongside western blots, we performed co-immunofluorescence in WT tau iHEK cells. We evaluated integrated density of Histone 1.2 (Fig 3E and 3F), Ki67 (Fig 3G and 3H), SMARCC1 (Fig 3I and 3J), and SMARCA5 (Fig 3K and 3L). Analysis was performed by considering nuclear integrated density of “–” and “+” tau WT iHEK proteins. To detect and confirm tau expression, we used the Tau13 antibody. MCM2 and RCC1 images and their relative integrated density quantifications are presented in Supplemental Fig. 4.

GSEA analysis for WT tau revealed significant down-regulation in the pathways for histone-binding (Fig 4A) and nucleosome organization clusters (Fig 4B). Several genes were detected in the histone and nucleosome domains, which were recurring and can be viewed in the chromatin gene list showed in Fig 3. In addition, we observed an up-regulation of *RCC1* (a regulator of chromosome condensation), *CTSL* (Cathepsin L), *MCM2* (Minichromosome maintenance complex component 2), and *DNAJC2* (DnaJ heat shock protein member C2). In Nucleosome GO, we observed up-regulated *HMGB2* and *HMGA1* (high mobility group box B2 and A1). On the contrary, several Lysine acetylation regulators were downregulated: *BRD3* and *BRD9* (from BRD family), *HDAC2*, *KDM5B*, *KAT7*, and *SFTD2*.

We also used western blotting to verify tau levels in cytoplasm and nuclear fractions of WT and P301L tau iHEK cells (Fig 4C and 4D, respectively). We found that upon Tet induction in both compartments, tau was detected, which was previously observed⁷ and expected. Western blot analysis demonstrated that tau is represented mainly in its monomeric form (mTau_N) when probing the nucleus. We compared the level of mTau_N in both cell lines and we determined that mTau_N increased in both cell lines after Tet induction. However, the WT mTau_N was present in a significantly higher level when compared to the P301L mTau_N (Fig 4E). This difference is due to the higher *MAPT* transgene expression efficiency in WT tau iHEK cell lines as was confirmed by RT-qPCR in a previous study⁷. These observations suggest that the monomeric form of tau protein predominantly carries out transcriptional activity and that the P301L mutation did not affect the nuclear import of tau, but instead modulated transcriptional activity. Cytoplasmic mTau was quantified as well (Supplemental Figure 4). In general, we propose that WT and P301L tau both shuttle into the nuclei but then modulate transcription differently. The schematic model for this idea is represented in Fig 4F. In summary, many nuclear factor genes involved in several nuclear activities, including chromatin condensation, are downregulated in WT tau, which indicates a potential role of WT and P301L tau in the control of chromatin factors, expression and subsequent cellular localization.

RNA metabolism, chromatin organization and *HNRNPs* precursor's display shortened APAs in the presence of WT tau. From PAC-seq analysis, we identified 110 genes with shortened 3'UTRs. The majority of these shortened genes belong to significant pathways associated with mRNA processing (GO: 0006397), RNA Splicing (GO: 0000377, GO: 0000398), and RNA metabolic processes (GO: 0016070) (Fig 5A). These domains share several genes: *HNRNPA3*, *SRRT*, *PRPF4B*, *CCAR1*, *LSM8*, *SNRNP40*, *HNRNPK*, *ZMAT2*, *ZC3H11A*, *HNRNPF*, *PCBP2*, *SNRPE*, and *HNRNPC*. The regulation of responses to DNA damage (GO:

2001020) comprise the following genes: *BCLAF1*, *FMR1*, *USP1* and *HMGA2* (others listed in Fig 5B).

Within the shortened APA precursors, various genes are related to nuclear function, such as the chromosome related genes (GO: 0005694) *IK*, *FMR1*, *HMGA2*, *SMC4*, *SMC3*, *SMC2* and *SMC6*. Structural maintenance of chromosome (SMC) proteins are ATPases that are essential to chromosomal condensation, sister-chromatid cohesion, recombination, DNA repair, and epigenetic silencing of gene expression⁵³. Eukaryotes have at least six genes encoding SMCs (SMC1-SMC6)⁵⁴. They inherently work as heterodimers: SMC1/SMC3 (Cohesin Complex), SMC2/SMC4 (Condensin Complex) and SMC5/SMC6⁵⁴.

Several nucleolar (GO: 0005730) genes have altered poly(A) site usage by WT tau including: *PARP1*, *FMR1*, *CHD7*, *DDX21*, *PWP1*, *PPM1E*, *SMC2*, *RSL1D1*, *ILF3*, *NCL*, *S100A13*, *KIF20B*, *RAN*, and *GET4*. As we saw in the shortened 3'UTRs, the most affected genes for lengthened 3' UTRs lie within the RNA binding function domain (GO: 0003723). mRNA processing, RNA splicing, and nucleic acid metabolic processes received the top scores, indicating a strong impact of WT tau in the regulation of mRNA isoforms at different levels. All significant enrichment terms are clustered and represented in a scatterplot in Fig 5C. In the mRNA processing domain (GO: 0006397) we identified several heterogeneous nuclear ribonucleoproteins (hnRNPs) genes (*HNRNPA3*, *HNRNPK*, *HNRNPF*, *HNRNPC*, *HNRNPDL*). HnRNPs are involved in alternative splicing, transcriptional and translational regulation, stress granules formation, cell cycle regulation, and axonal transport⁵⁵. Their dysfunction has been shown have neurological implications, but their roles have not been comprehensively investigated. Several neurodegenerative diseases, including AD, FTD, and amyotrophic lateral sclerosis (ALS) have been associated with hnRNPs when it comes to the progression of these pathologies⁵⁶. More specifically, hnRNPK has been linked to the transcripts of several cytoskeletal genes, including *MAPT*, which is needed for axonogenesis⁵⁷.

In Alzheimer's disease, hnRNPC promotes APP translation⁵⁸ and stabilizes the APP precursors mRNA, which could suggest that increasing hnRNPC levels may promote A β secretion⁵⁹. Within the hnRNPs group, hnRNPA3, hnRNPF and hnRNPD are all detected in pathological inclusions of ALS and FTD brains^{56,60,61}. Moreover, hnRNPK is a regulator of p53⁶², which we and others recently discovered was present in elevated amounts in AD cortices^{11,12}. It has been also determined that hnRNPK sumoylation mediates p53 activity⁶³. All this evidence places hnRNPs in a central position for further experimental analysis in human brain tissues to elucidate more valuable information about the localization and function of this large family of ribonucleoproteins.

HnRNPA3 has been identified in neuronal cytoplasmic and intranuclear inclusions in patients with GGGGCC expansion repeats⁶¹ and hnRNP F were also found to co-localize with GGGGCC expansion foci in immunoprecipitation studies⁶⁴. In addition, western blot analyses imply that hnRNP may be in part responsible for the toxicity incurring by C9orf72 mutations, considering important RNA processes such as splicing are compromised. hnRNP A3 and K have been found associated with TDP-43⁶⁵. Implications of tau-mediated APAs in hnRNPs open new venues for investigators to study new mechanistic insights of these proteins in several proteinopathies. Within RBPs group, we also observed the *MATR3* gene. This gene encodes for Matrin3, a DNA/RNA-binding protein. Mutations in this gene cause familial ALS/FTD, and MATR3 pathology is a feature of sporadic disease, suggesting that its dysfunction is inherently linked to ALS pathogenesis⁶⁶.

Shorter 3'UTR are generally associated with enhanced translation of the mRNA APA in the presence of WT tau, which supports the finding that high-levels of hnRNPs sustain dysfunction of stress granules in ALS and FTD. Recent proteomic analysis in AD human Neurofibrillary Tangles (NFTs) showed that phospho-tau in NFTs is associated with more than 500 proteins⁶⁷. We observed several of these proteins in the APAs shortened WT tau, such as HNRNPK, ILF3,

AP2B1, RAN, RAB11A, HSP90B1, PARP1, MATR3, PPIA, NCL, HNRNPA3, HSP90AA1, and HNRNPC. It is intriguing that the presence of chaperone Hsp90, a tau-regulated gene, plays a crucial role in neurodegenerative pathologies and has been studied in AD for a long time⁶⁸.

These observations suggest that tau has early effects on gene expression that results in later stages of toxic associations commonly found in neurodegeneration. Enrich-GO (Cellular Function) of shortened-APAs genes by WT tau is provided in the supplemental information section. GO-Cellular Process, Molecular Process and Cellular Components bar charts of shortened APAs are shown in Fig S4.

SWI/SFN, THO complexes, and several RNA-Binding protein precursors display lengthened 3'UTRs in presence of WT tau. Further analysis revealed 173 genes with lengthened APAs. The complete list of the 173 genes with lengthened APAs is reported in the supplemental information section. Among these genes, we found that many of them are related to three major biological processes: chromatin remodeling (GO:0006338), negative regulation of gene expression (GO: 0010629), and mRNA processing (GO:0006397) (Fig 6A). To be more specific, we noticed several genes belonging to the ATP-dependent chromatin remodeling complex npBAF (mammalian SWI/SFN, GO: 0071564): *SMARCC2*, *ARID1A*, *SMARCA2* and *SMARCA4*. This complex is found in neuronal progenitor's cells and post-mitotic neurons, and it is essential for the maturation of the post-mitotic neuronal phenotype as well as long-term memory formation⁶⁹. Along with the chromatin remodeling complex, other genes contained altered APAs, including pericentric chromatin components (GO: 0005721, *HELLS* and *CBX3*), and nuclear chromatin factors (GO:0000790, *SMARCC2*, *CBX3*, *H3F3A*, *NUCKS1*, *ARID1A*, *SMARCA2*, *SMARCA4*, *HIST2H2AC*, *RAD50*, *NASP*, *MYC*, *NSMF*, *TCF3*) (Fig 6B).

Several nuclear speck (GO: 0016607) genes were also identified: *BASP1*, *POM1*, *ERBI*, *YLPM1*, *HNRNPU*, *LUC7L3*, *CDC5L*, *TCF3*, *SRSF6*, and *KIF20B*. Cytoplasmic ribonucleoprotein

granule (GO: 0036464) and cytoplasmic stress granules (GO: 0010494) genes were delineated as *MBNL1*, *CARHSP1*, *NCL*, *HNRNPU*, *IQGAP1*, *YBX1*, *RAC1*, *PABPC1*, *CNOT9*. Within the domain of RNA processing, two genes *THOC2* and *THOC3* were also identified. They are components of the THO complex (GO: 0000445) involved in efficient export of poly-adenylated RNA and spliced RNAs²⁵.

The THO complex appears to coordinate transcripts for synapses development and dopamine neuron survival⁷⁰. Recently, it has been found to interact with *ZC3H14*, which regulates the processing of neuronal transcripts⁷¹, so it is not surprising to find in our dataset another polyadenosine RNA-binding protein *ZC3H15* on the list of lengthened APAs. These observations indicate that export complex RNA precursors are meaningfully affected by WT tau.

Not surprisingly, many translation initiation factors (GO: 0003743) were also discovered in our analysis including *EIF2S3*, *EIF3E*, *EIF3A*, *EIF1*, and *EIF4G1*. It is important to note that many APA-lengthened proteins in our study are RNA-Binding Proteins (RBPs). In fact, 46/173, or ~27% of the total were. RBPs are implicated in the pathogenesis and progression of numerous neurodegenerative diseases, and they are linked to toxic interactions and aggregations in amyloidogenic proteins such Amyloid-beta and tau. The subsequent dysfunction of RBPs is closely related to distinct pathways that are altered in proteinopathies⁷².

Considering the above, we also studied the presence of lengthened APAs of *ELAVL1*. This gene encodes for HuR (RBPs), which is a neuroprotective protein. This protein has been demonstrated in the regulation of oxidative metabolism in neurons as a way to protect from neurodegeneration⁷³.

Apical dendrites (GO: 0097440) (*MAP1B*, *NSMF* and *CLU*) and other cytoskeletal genes (*ACTR2*, *LIMA1*, *TPM4*, *PPP2R1A*, *BASP1*, *TARS*, *PHIP*, *NSMF*, *IQGAP1*, *RAC1*, *CLU*, and *SMARCA2*) display lengthened poly-A tails as well. Enrich-GO (Cellular Function) of lengthened-

APAs genes in WT tau is provided in the supplemental information section. GO-Cellular Process, Molecular Process and Cellular Components bar charts of lengthened-APAs are shown in Fig S4.

P301L tau modulates 3'UTRs of RNA export complex THOC and splicing precursors SNRPE. In P301L tau precursor APAs, we detected 23 lengthened genes in total. More specifically, the THOC2 gene, which is a component of the THO complex (GO: 0000445) was lengthened in WT tau. Another gene of the small nuclear ribonucleoprotein complex (SNRPE) was detected. *SNRPE* is also a gene for the spliceosome complex (GO: 0005681) (Fig 7A). Lastly, the nuclear replication fork (GO: 0043596) gene *BAZ1B* was also observed.

In contrast to WT tau, P301L tau induces lengthening of the *HNRNPF* gene. HnRNPs represent a large RNA-Binding protein family that contributes to many aspects of nucleic acid metabolism, including alternative splicing, mRNA stabilization, transcriptional, and translational regulation⁵⁵. Dysregulation of RNA metabolism is crucial in the pathogenesis of several neurodegenerative diseases as Parkinson's⁷⁴, FTD and overlaps with aspects of ALS. Some studies revealed possible involvement of hnRNPs in the pathogenesis and progression of these diseases⁷⁵. Furthermore, hnRNP F has been uncovered in RNA foci in human brain tissue of FTD-ALS patients⁵⁶. Affinity pull-down assays and genome-wide analysis also revealed a hnRNP F-bound splicing complex that regulates neuronal and oligodendroglial differentiation pathways in the developing brain⁶⁴. As observed for WT tau, the mutant P301L form also modulates several RNA-Binding Proteins (GO: 0003723): *SLFN11*, *HNRNPF*, *FASN*, *HUWE1*, *PRRC2C*, *THOC2*, *HMG2*, *SRSF7*, and *GIGYF7*. We found 34 genes in total with evidence of APA and shortened 3'UTRs (Fig 7B). The three top-scored cellular components were nuclear speck (GO: 0016607), nuclear body (GO: 0016604) with *RBM39* (ALS associated gene⁷⁶) and Nuclear heterochromatin genes (GO: 0005720). Nuclear speck and body genes consisted of *LUC7L3*, *SRSF4*, *NSRP1* and *SRSF11*. Nuclear heterochromatin genes detected were *H2AFY* and *HIST1H1E*. *H2AFY* encodes

for a variant of the H2A histone that is present in a subset of nucleosomes where its role is to represses transcription⁷⁷.

The Cellular Components scatterplot of lengthened APAs in WT Tau is presented in Fig 6C and GO Cellular component bar charts in Fig 6D.

These data suggest that the mutant P301L form of tau reduces activity in transcription and alternative poly(A) tails processes due to loss-of-function. However, P301L tau does generate different mRNA isoforms of transcripts mainly translated in splicing factors, nuclear speckle/body structures and chromatin remodeling proteins. Enrich-GO (Cellular Function) of shortened and lengthened-APAs by P301L tau is provided in the supplemental information section. GO-Cellular Process, Molecular Process and Cellular Components bar charts of shortened and lengthened-APAs are shown in Fig S4.

Discussion

In this study, we revealed new mechanistic insights into non-canonical tau functions. In particular, we showed novel tau activities in transcription and alternative poly-adenylation (APA) pathways. APA is a widespread mechanism of gene regulation that generates 3' ends in transcripts made by RNA polymerase II⁷⁸. APA is regulated in cell proliferation, differentiation and extracellular cues. It occurs in the 3'UTR and leads to the production of mRNA isoforms, followed by splicing which leads to the production of distinct protein isoforms⁷⁸. Tau is typically described as an abundant neuronal microtubule-binding protein. Recently, we observed its presence within non-neuronal human cell lines and neuronal nuclei in AD brains ^{7,13} alongside other study². We were particularly interested in the possibility of non-canonical tau functions. We hypothesized that nuclear tau acts as a transcriptional regulator. To test our hypothesis, we used the tau inducible HEK system, which is a well-established cell line capable of studying mechanisms related to the tau aggregation process within a controlled system of MAPT gene expression⁷⁹. Our study employed new technologies such as Poly(A)-ClickSeq to resolve whether genes were upregulated or downregulated by WT and P301L tau in an *in-vitro* model. Furthermore, we analyzed alternative polyadenylation (APA) profiles under the presence of WT and P301L tau⁷⁶.

Our results suggest that both WT and P301L tau are able to shuttle into the nuclei (Fig 4). This observation confirmed our previous observations ⁷. We did not investigate the effect of the P301L mutation on nuclei-cytoplasm shuttling in this report. The decreased number of genes expressed in P301L cells suggests that this particular mutation of tau impairs transcriptional activity. We did not investigate the LOF consequences of P301L tau in great detail, but our observations suggest new mechanistic insights linked to alternative nuclear tau function.

One APA transcript of significance is the *SFPQ* gene, which we identified in WT tau expression as having a lengthened 3'UTR. *SFPQ* has been associated with tau as a critical factor for rapid progression of AD, and it has been observed as downregulated in post-mortem brain

tissue of rapidly progressive AD patients⁸⁰. Therefore, the lengthened APAs in this gene could explain the down-regulation in the presence of a high level of tau, which mimics late-stage AD. In-vitro data of SFPQ down-regulation due to human tau suggest a causal role of tau, possibly through the alternative poly-adenylation of *SFPQ* transcripts.

Further analysis comparing 3'UTRs lengthened between WT and P301L tau revealed that a significant number of RBPs showed lengthened 3'UTRs in P301L compared to WT tau. For example, we detected 72 RBPs including *FUS* (found in the supplemental information section). These data suggest a significant difference in RNA isoforms based on genetic tau background, which then subsequently modulates different aspects of RNA metabolism in neurons.

Using the same cellular models, we determined that the prominent form of nuclear tau is monomeric, but Tet induction causes tau oligomerization within the nuclei⁷. The formation of large and nuclear oligomeric forms is another possible explanation for LOF observed as a consequence of mutated tau. Mutant P301L tau shows a distinct aggregation mechanism compared to WT⁸¹ and aggregates faster than WT^{82,83}. For example, monomeric tau in the cytoplasm of cells producing (WT or P301L) tau aggregate and subsequently avoid nuclear translocation. In addition, aggregation in the cytoplasm and within the nuclei of tau reduces the pool of monomeric nuclear tau. This pathological mechanism can compete with functional monomeric and oligomeric tau, which then alters tau transcriptional activity. This phenomenon should be investigated in the near future using neuronal models. Another function of tau is binding DNA in-vitro. Overall, the multifunctional nature of nuclear tau should be thoroughly scrutinized in order to identify unrevealed functions connected to DNA expression and RNA processing. We suggest that the nature of nuclear tau as a transcriptional factor, chromatin remodeler and/or transcriptional co-factor must be elucidated using proper models such as induced pluripotent stem cells or mouse primary neurons carrying mutation on P301 site. At this stage, we can only hypothesize the direct and indirect effects of tau during transcription.

This study utilized PAC-ClickSeq technology to identify the APA modulated by P301L and WT tau. Alternative Poly-A (APA) sites in human genome have been identify mainly in 3'UTRs (UTR-APA) sites, which harbor diverse regulatory sequences. This type of APA can change the length and composition of 3'UTR, which subsequently affects the binding of miRNAs and/or RBPs. This post-transcriptional modification leads to differences in mRNA stability, export, localization, translational efficiency²⁶. Although the currently accepted theory is that genes with longer 3'UTR tend to show decreased expression levels, this does not necessarily mean that every single gene with a longer 3'UTR is less stable those with a shorter one.

We plan to investigate these findings using primary neurons and in-vivo models in the near future. We are choosing these alternative models because the iHEK cell model have inherent limitations in terms of reliability as a neuronal system. However, the iHEK cells used in this study are an established model used by many researchers to study the mechanistic insights of tau aggregation and toxicity. The results presented in this study support non-canonical functions of tau. Therefore, we report broad tau-driven, post-transcriptional regulation in APAs by both WT and P301L tau considering both cell lines produced high levels of monomeric and aggregated tau. In this study, we did not investigate which tau isoform regulates APA in cells and by what method tau regulates APAs, but we established a new category of interest in post-translational modification. We hope further studies of nuclear tau and its relation to DNA and RNA processing will identify new targets in tauopathies and eventually find new therapeutic targets.

Limitations of the study. As mentioned in the discussion, the main limitation of this study is the nature of tau inducible HEK cells. We are aware that further study on neuronal cells is necessary. However, iHEK models are commonly used to study mechanisms that are tau-dependent and several of them have been translated into neurons models. All relevant datasets used and/or analyzed in this current study are available upon request from the corresponding author.

Supplemental Information. The source data underlying all main and supplementary figures are provided as a Source Data file. RNAseq datasets is uploaded to NCBI SRA, reference number: [PRJNA744518](https://www.ncbi.nlm.nih.gov/sra/PRJNA744518). Figure 1A, 4F and 7C were generated using BioRender Software (<https://biorender.com>).

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Declaration of Interests

The authors declare no competing interests.

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Figure Legends

Fig 1. Tau-dependent Gene expression. (A) Schematic representation of experimental plan, from Tet induction in WT and P301L Tau iHEK to RNA isolation, sequencing to gene expression analysis. (B) Volcano Plot for Down- and Up-regulated gene in WT Tau iHEK. (C) Volcano Plot for Down- and Up-regulated gene in P301L Tau iHEK. (D) Gene Lists of Down-Regulated (Red Boxes) and Up-Regulated (Green Boxes) Genes in WT Tau iHEK. (E) Gene Lists of Down-Regulated (Red Boxes) and Up-Regulated (Green Boxes) Genes in P301L Tau iHEK.

Fig 2. Up- and Down regulated genes in WT Tau iHEK Gene Ontology. Left Column (Green) Up-regulated genes analyzed by Enrich GO and divided by Biological Process, Molecular Function and Cellular Component. Right Column (Blue) Down-regulated genes analyzed by Enrich GO and divided by Biological Process, Molecular Function and Cellular Component. Grey bars represent not significant correlation.

Fig 3. WT tau modulates gene expression of chromatin organization and remodeling factors. (A) Enrichment plot for GO Chromatin organization. (B) Enrichment plot for GO Chromatin remodeling. (C) Enrichment plot for GO-Covalent Chromatin modification. (D) IB of Up-regulated genes: RCC1, DNAJC2 and Histone 1.2 (red box) and Down-regulated genes: SMARCC1, SMARCA5 and HMGB1 (blue box) in cytoplasm and nuclear fractions from WT and P301L Tau iHEK. Histone 3 and β -Actin has been used as loading control for nuclear and cytoplasmic fractions, respectively. (E) representative Tau 13 (magenta) and Histone 1.2 (green) Co-IF of control (-Tet) and treated WT Tau iHEK. (F) Histone 1.2 integrated density quantification in control and +Tet cells (Unpaired t-test, $p < 0.0001$, ****). (G) representative Tau 13 (magenta) and Ki67 (green) Co-IF of control (-Tet) and treated WT Tau iHEK. (H) Ki67 integrated density quantification in control and +Tet cells (Unpaired t-test, $p < 0.0001$, ****). (I) representative Tau 13 (magenta) and SMARCC1 (green) Co-IF of control (-Tet) and treated WT Tau iHEK. (J) SMARCC1 integrated density quantification in control and +Tet cells (Unpaired t-test, $p < 0.0001$, ****). (K) representative Tau 13 (magenta) and SMARCA5 (green) Co-IF of control (-Tet) and treated WT Tau iHEK. (L) SMARCA5 integrated density quantification in control and +Tet cells (Unpaired t-test, $p < 0.0001$, ****).

Fig 4. Tau nuclear shuttling. (A) GWAS GO-Histone Binding heat map in WT Tau. (B) GWAS GO-Nucleosome organization heat map in WT Tau. (C) Enrichment plot for GO Histone Binding. (D) Enrichment plot for GO Nucleosome Organization. (E) Immunoblot (IB) with Tau13 (1:1000) and β -Actin () of cytoplasm and nuclear fraction from WT (left panel) and P301L (right panel) Tau induced with Tet. (F) Relative density of nuclear monomeric Tau (mTau_N, normalized with Histone3). Unpaired *t*-test has been performed to compare column means ((-) WT Tau vs WT Tau ***, $p = 0.0009$, (-) p301l Tau vs P301L Tau *, $p = 0.0169$, WT Tau vs P301L Tau **, $p = 0.0065$). (G) Schematic model on Tau nuclear import in the two iHEK cell lines.

Fig 5. RNA metabolism, chromatin organization and HNRNPs precursor's display shortened APAs in the presence of WT tau. (A) Enrich-GO Biological Process of WT Tau shortened APAs (p-value reported). (B) Partial list of biological process genes with shortened APAs upon presence of WT Tau (mRNA processing, RNA splicing, chromatin remodeling and regulation of response to DNA damage). (C) The Cellular Components scatterplot is organized so that similar gene sets are clustered together. The larger blue points represent significantly enriched terms - the darker the blue, the more significant the term and the smaller the p-value. The gray points are not significant. Plots has been generated and downloaded using scatter plot visualization Appyter. (D) Enrich-GO Cellular Component of WT Tau shortened APAs (p-value reported).

Fig 6. RNA processing and splicing precursor's display lengthened APAs in presence of WT tau.

(A) Enrich-GO Biological Process of WT Tau lengthened APAs (p-value reported). (B) Partial list of biological process genes with lengthened APAs upon presence of WT Tau negative control of gene expression, chromatin remodeling, nucleosome organization, mRNA processing and regulation of transcription). (C) The Cellular Components scatterplot of lengthened APAs in WT Tau is organized so that similar gene sets are clustered together. The larger blue points represent significantly enriched terms - the darker the blue, the more significant the term and the smaller the p-value. The gray points are not significant. Plots has been generated and downloaded using scatter plot visualization Appyter. (d) Enrich-GO Cellular Component of WT Tau lengthened APAs (p-value reported).

Fig 7. Mutant P301L Tau modulates APAs associated with spliceosome and nuclear chromatin. (A)

Scatterplot of gene clusters from lengthened mRNA precursors upon P301L Tau expression. (B) Scatterplot of gene clusters from shortened mRNA precursors upon P301L Tau expression. (C) Model for nuclear Tau activity to transcriptional and post-transcriptional levels.

A

B

C

D

E

F

G

H

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K

L

A **B**

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C **D** **E** **F**

A

Scatterplot of gene clusters from lengthened mRNA precursors

Lengthened APAs Genes

WDR60
SRSF7
DEGS1
THOC2
ARID4B
HMG2
HUWE1
HNRNPF
FASN
JPT1
EGR1
SNRPE
SLFN11
GIGYF2
TMA7
PDIA6
BAZ1B
PRRC2C
NASP

SLC16A1
TET1

B

Scatterplot of gene clusters from shortened mRNA precursors

Shortened APAs Genes

RAB13
NEFL
EIF3A
UBE2G2
DBI
HIST1H1E
PABPC1
WDR5
ACAT2
SHMT2
SRSF4
RPL22
GUSBP11
CLK3
CALM3
MYO6
IRS4
SEN3-EIF4A1
TOMM22
RBM39
ENTR1

SMC1A
PMAIP1
H2AFY
ID4
ARGLU1
NSRP1
DYNLL1
KTN1
SRSF11
DSP
MARCKSL
PSIP1
LUC7L3

C

Nuclear Tau

Mechanism of action

Wild type Tau

Cytoplasm

Nucleus

- Cytoskeleton organization/intacellular transport genes
- Microglia and macrophages activation genes
- Nuclear chromatin, nuclear body and specks genes
- RNA Binding Proteins associated genes
- Histone deacetylase binding
- Sequence-specific dsDNA binding genes

- Ubiquitin protein ligase binding
- Mitochondrial and Golgi apparatus genes

P301L Tau

FTDP-17 mutation

- Positive regulation of neuron death genes
- Response to reactive oxygen species genes
- Sequence-specific dsDNA binding genes
- Microtubule cytoskeleton genes
- Nuclear body and speck genes

- Ubiquitin ligase complex

A

B

Genes with lengthened APAs

Negative control of gene expression: SMARCC2;POU2F1;PRMT2;CBX3;NONO;H3F3A; SMARCA2;POU3F3;SMARCA4;ILF3;SFPQ;BASP1; KAT6B;NCL;STC2;ID4;ANXA7;BIRC5;ZBTB7A;CNOT9

Chromatin remodelling: SMARCC2;CBX3;MYC;CHD1L;ANP32B;HMGB1;ARID1A;SMARCA2;SMARCA4

Nucleosome organization: SMARCC2;NASP;KAT6B;H3F3A;ANP32B;ARID1A;SMARCA4

mRNA processing: SFPQ;NONO;HNRNPU;CDC5L;THOC3;THOC2;YBX1;PABPC1;SRSF6;ELAVL1;CTNNBL1

Regulation of Transcription: ARID4B;NUCKS1;YBX1;HMGB1;PPP2R1A;BASP1;MYC;ZNF227;ZBTB7A;TRIM44;TCEAL9;SMARCC2;POU2F1;PRMT2;CDX2;CBX3;NONO;CDC5L;ARID1A;SMARCA2;POU3F3;SMARCA4;ILF3;SFPQ;KAT6B;ID4;BIRC5;ZNF711;PHIP;TCF3

C

D

A

B

mRNA Processing: HNRNPA3;SRRT;PRPF4B;CCAR1;LSM8;SNRNP40;HNRNPK;ZMAT2;ZC3H11A;HNRNPF;PCBP2;SNRPE;HNRNPC

RNA Splicing: LSM8;HNRNPA3;SNRNP40;HNRNPK;ZMAT2;HNRNPF;PCBP2;SRRT;PRPF4B;SNRPE;HNRNPC;CCAR1

Chromatin remodelling: HDAC1;ATRX;ANP32E;HMGA2;HMGB3;HNRNPC;NUDT5

Regulation of response to DNA damage: BCLAF1;FMR1;USP1;HMGA2

C

D

A

B

C

D

Gene List **Down-** and **Up-**Regulated by WT Tau

Down (30 Genes)

DDIT4
NPFB11
PFDN2
TRIM33
STRIP1
MEGF9
KLHL11
MIRS48N
LYPD6
ORMDL1
UPF3A
OXCT1
STX6
ICAM5
IFRD1
STC2
ZMYM3
SNHG1
NCK1
SLC26A2
TRUB1
KCNQ2
KHDC4

Up (88 Genes)

DNAH14
C21orf59-TCP10L
SFSWAP
NRG3
ARHGAP11B
GTF2H2B
STRP2
RBM33
CPEB4
OTUD6B
KAT7
EPC2
ID4
GALNT6
ANP32E
MEF2A
MIRS48AC
CLCN3
PHF6
ZNF234
ZEB2
TARS
CDC73
RBM20

TACCL1
IRS4
NBPFL1
NRGN
TNFRSF12A
PELP1
TUBA1A
SNAR-A11
SAP30BP
SMAAD5
TUBA1B
TUBB2A
BEX3
PRKG1
NOM1
GTF2H2C
SNAR-A2
PEA15
BMS1P20
NKAPD1
SUPV3L1
LONRF3
TMOD3

SUPTSH
GIGYF1
FTSJ3
UBIAD1
RRP15
TRIM44
SNAR-A12
SENP2
UBE2Q2P2
BAZ2A
ARIHGEF5
HIST1H1C
ARIHGEF35
MAPT
SRRT
SDK1
TCHP
POLE3
DAAM1
UBE2E2
TRAF3IP1
N4BP2L2
DSE

MAP7
SUD53
CERK
TFRC
CARMIL1
CLU
HOOK3
ATP6V1D
HIST2H2AC
USP36
DAGLB
HOXA6
TUBB2B
RHOQ
PHACTR1
TMEM178B
PANK4
ANKHD1-EIF4EBP3

E

Gene List **Down-** and **Up-**Regulated by P301L Tau

Down (1 Gene)

DCAF12

Up (10 Genes)

NLGN1
FER
PLCB4
FOS
DDIT4
EGR1
RNAS-
BSN5
ITPKC
TSPYL2
MAPT

Up-regulated Gene Ontology

Biological Process

Molecular Function

Cellular Component

Down-regulated Gene Ontology

Biological Process

Molecular Function

Cellular Component

Supplemental Table 1. Up-Regulated Genes by WT Tau

Term	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes
cytoskeleton (GO:0005856)	1.73E-05	0.007735674	4.807692308	52.70303651	TUBA1B;SAP30BP;TUBB2B;TUBA1A;TMOD3;MAP7;TARS;TACC1;MAPT;CLU;RHOQ
polymeric cytoskeletal fiber (GO:0099513)	4.34E-04	0.096694562	6.170300288	47.77891745	TUBA1B;TUBB2B;TUBA1A;TUBB2A;MAPT;RHOQ
nuclear chromatin (GO:0000790)	8.81E-04	0.130903368	5.38986705	37.91772839	MEF2A;ZEB2;ANP32E;SUDS3;HIST2H2AC;HIST1H1C
microtubule cytoskeleton (GO:0015630)	0.001616162	0.180202106	4.100281162	26.35538067	TUBA1B;TUBB2B;TUBA1A;TUBB2A;MAP7;TACC1;MAPT
nuclear chromosome part (GO:0044454)	0.001713127	0.152810938	4.058441558	25.84997914	MEF2A;ZEB2;POLE3;ANP32E;SUDS3;CDC73;HIST2H2AC
microtubule (GO:0005874)	0.0035899	0.1749798	5.41125	32.71698914	TUBA1B;TUBB2B;TUBA1A;TUBB2A;MAPT
preribosome, large subunit precursor (GO:0030687)	0.00580801	0.370053218	17.48251748	90.00904291	RRP15;FTSJ3
nuclear speck (GO:0016607)	0.009908573	0.552402927	3.839066339	17.71481484	USP36;CARMIL1;GTF2H2C;BAZ2A;MAPT
cytoplasmic vesicle (GO:0031410)	0.015007567	0.743708322	4.22832981	17.75560565	TFRC;ANP32E;CLCN3;RHOQ
nuclear body (GO:0016604)	0.019146203	0.853920672	2.574286555	10.18297877	USP36;CARMIL1;GTF2H2C;BAZ2A;MAPT;SUDS3;SEN2
axolemma (GO:0030673)	0.026114353	1	37.87878788	138.0784165	MAPT
nucleolus (GO:0005730)	0.029395392	1	2.353415815	8.300303054	USP36;NOM1;ZEB2;BAZ2A;PHF6;PELP1;FTSJ3
messenger ribonucleoprotein complex (GO:1990124)	0.030400721	1	32.46753247	113.4184725	CPEB4
spherical high-density lipoprotein particle (GO:0034366)	0.034668438	1	28.40909091	95.50924912	CLU
HFE-transferrin receptor complex (GO:1990712)	0.034668438	1	28.40909091	95.50924912	TFRC
Cdc73/Paf1 complex (GO:0016593)	0.034668438	0.966382714	28.40909091	95.50924912	CDC73
apical dendrite (GO:0097440)	0.034668438	0.909536672	28.40909091	95.50924912	CLU
chromatin silencing complex (GO:0005677)	0.034668438	0.859006857	28.40909091	95.50924912	BAZ2A
Swr1 complex (GO:0000812)	0.038917584	0.913539084	25.25252525	81.97750232	ANP32E
chromatin (GO:0000785)	0.041696789	0.929838401	3.071253071	9.758388048	MEF2A;ZEB2;HIST2H2AC;PELP1
preribosome (GO:0030684)	0.043179537	0.917051121	6.060606061	19.04477924	RRP15;FTSJ3
proton-transporting V-type ATPase complex (GO:0033176)	0.047360482	0.960126139	20.66115702	63.01584932	ATP6V1D
ribonucleoprotein granule (GO:0035770)	0.04848675	0.940221322	5.681818182	17.19582226	TUBA1A;MAPT

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holo TFIIF complex (GO:0005675)	0.051554392	0.95805246	18.93939394	56.1575353	GTF2H2C
Sin3 complex (GO:0016580)	0.055730049	0.994224071	17.48251748	50.47615038	SUDS3
DNA-directed RNA polymerase II, holoenzyme (GO:0016591)	0.059760267	1	5.050505051	14.22936503	GTF2H2C;CDC73
cytoplasmic exosome (RNase complex) (GO:0000177)	0.064026913	1	15.15151515	41.64320855	SUPV3L1
keratin filament (GO:0045095)	0.064026913	1	15.15151515	41.64320855	TCHP
pericentriolar material (GO:0000242)	0.068148277	1	14.20454545	38.15439487	HOOK3
nuclear replisome (GO:0000001)	0.068148277	1	14.20454545	38.15439487	POLE3
Sin3-type complex (GO:0070822)	0.068148277	0.980455861	14.20454545	38.15439487	SUDS3
dendrite (GO:0030425)	0.06924185	0.965058279	3.171247357	8.46770561	MAPT;CLU;CPEB4
nuclear euchromatin (GO:0005719)	0.076337257	1	12.62626263	32.48224959	HIST1H1C
striated muscle thin filament (GO:0005865)	0.080405026	1	11.96172249	30.15165779	TMOD3
euchromatin (GO:0000791)	0.088487507	1	10.82251082	26.2434405	HIST1H1C
transcriptionally active chromatin (GO:0035327)	0.09250237	1	10.33057851	24.59215918	PELP1
histone acetyltransferase complex (GO:0000123)	0.09250237	1	10.33057851	24.59215918	KAT7
INO80-type complex (GO:0097346)	0.09250237	1	10.33057851	24.59215918	ANP32E
recycling endosome (GO:0055037)	0.09666912	1	3.819709702	8.924603778	TUBA1A;TFRC
contractile fiber (GO:0043292)	0.108387738	1	8.741258741	19.42342931	TMOD3
MLL1 complex (GO:0071339)	0.112315932	1	8.417508418	18.40437335	PELP1
myofibril (GO:0030016)	0.112315932	1	8.417508418	18.40437335	TMOD3
MLL1/2 complex (GO:0044665)	0.112315932	1	8.417508418	18.40437335	PELP1
main axon (GO:0044304)	0.135528342	1	6.887052342	13.76428715	MAPT
actin cytoskeleton (GO:0015629)	0.139839351	1	2.319109462	4.562293623	TMOD3;TARS;RHOQ
centrosome (GO:0005813)	0.145540984	1	1.971997634	3.800626224	TCHP;TRAF3IP1;HOOK3;ATP6V1D
specific granule (GO:0042581)	0.1565438	1	2.840909091	5.268237029	CLCN3;ATP6V1D
contractile actin filament bundle (GO:0097517)	0.161851895	1	5.681818182	10.34700903	DAAM1

integral component of Golgi membrane (GO:0030173)	0.161851895	1	5.681818182	10.34700903	UBIAD1
stress fiber (GO:0001725)	0.161851895	1	5.681818182	10.34700903	DAAM1
heterochromatin (GO:0000792)	0.165547132	1	5.543237251	9.969508533	BAZ2A
cytoplasmic ribonucleoprotein granule (GO:0036464)	0.172060346	1	2.673796791	4.705641744	TUBA1A;MAPT
intermediate filament (GO:0005882)	0.183783091	1	4.940711462	8.369560635	TCHP
microtubule organizing center (GO:0005815)	0.184397492	1	1.793078716	3.031489277	TCHP;TRAF3IP1;HOOK3;ATP6V1D
cis-Golgi network (GO:0005801)	0.187382719	1	4.835589942	8.097689209	HOOK3
actin filament (GO:0042641)	0.190988832	1	4.132238485	6.339818153	DAAM1
actin filament (GO:0005884)	0.215620364	1	4.132231405	6.339818153	RHOQ
perinuclear region of cytoplasm (GO:0048471)	0.232031019	1	1.803751804	2.635072538	GALNT6;TFRC;CLU
PML body (GO:0016605)	0.239531081	1	3.66568915	5.238534082	SENP2
platelet alpha granule lumen (GO:0031093)	0.256167561	1	3.392130258	4.619821964	CLU
nucleoplasm part (GO:0044451)	0.266189763	1	1.675228948	2.217242288	GTF2H2C;KAT7;SUDS3
intermediate filament cytoskeleton (GO:0045111)	0.269217242	1	3.201024328	4.200501392	SAP30BP
lytic vacuole membrane (GO:0098852)	0.273570882	1	1.950838861	2.528666646	DAGLB;ATP6V1D
nuclear periphery (GO:0034399)	0.291511782	1	2.913752914	3.591709949	MAPT
clathrin-coated vesicle membrane (GO:0030665)	0.300859484	1	2.805836139	3.370123323	TFRC
phagocytic vesicle (GO:0045335)	0.307023439	1	2.73822563	3.23338222	CLCN3
platelet alpha granule (GO:0031091)	0.328177166	1	2.525252525	2.813640594	CLU
specific granule membrane (GO:0035579)	0.331146544	1	2.497502498	2.76022545	ATP6V1D
clathrin-coated vesicle (GO:0030136)	0.357293708	1	2.272727273	2.33908437	TFRC
lysosomal membrane (GO:0005765)	0.367155241	1	1.562011871	1.565089849	DAGLB;ATP6V1D
nuclear chromosome, telomeric region (GO:0000784)	0.376929429	1	2.12404418	2.072424172	CDC73
endocytic vesicle (GO:0030139)	0.379685681	1	2.104377104	2.037903041	CLCN3
chromosome, telomeric region (GO:0000781)	0.422181171	1	1.832844575	1.580499895	CDC73

microtubule organizing center part (GO:0044450)	0.427286491	1	1.803751804	1.533731156	HOOK3
late endosome (GO:0005770)	0.461796269	1	1.623376623	1.254271854	CLCN3
axon (GO:0030424)	0.464181054	1	1.611863314	1.237073825	MAPT
RNA polymerase II transcription factor complex (GO:0090575)	0.478271942	1	1.546072975	1.140345997	GTF2H2C
lysosome (GO:0005764)	0.557038143	1	1.07712193	0.630247266	DAGLB;ATP6V1D
Golgi membrane (GO:0000139)	0.582354922	1	1.028383381	0.556021375	GALNT6;DSE
early endosome (GO:0005769)	0.626341943	1	1.023751024	0.478970947	CLCN3
early subcompartment (GO:0098791)	0.626341943	1	0.548946669	0.443898372	GALNT6;DSE
mitochondrion (GO:0005739)	0.667382777	1	0.886053518	0.358312528	TCHP;SUPV3L1;MAPT;CLU
mitochondrial matrix (GO:0005759)	0.745573163	1	0.737898465	0.216648473	SUPV3L1
secretory granule lumen (GO:0034774)	0.755626978	1	0.716948666	0.20089435	CLU
integral component of plasma membrane (GO:0005887)	0.96056947	1	0.466041136	0.018748356	TFRC;NRG3;CLCN3

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Supplemental Table 2 - Down-Regulated Genes by WT Tau

Term	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes
axon initial segment (GO:0043194)	0.013421829	1	74.07407407	319.3239135	KCNQ2
node of Ranvier (GO:0033268)	0.017856968	1	55.55555556	223.6311935	KCNQ2
main axon (GO:0044304)	0.048367756	1	20.2020202	61.19034096	KCNQ2
SCF ubiquitin ligase complex (GO:0019005)	0.077961385	1	12.34567901	31.50051407	KLHL11
coated vesicle (GO:0030135)	0.101264667	1	9.389671362	21.5025138	STX6
ribosome (GO:0005840)	0.108009525	1	8.771929825	19.52224439	NCK1
perinuclear region of cytoplasm (GO:0048471)	0.109697823	1	3.527336861	7.795505317	STC2;STX6
trans-Golgi network membrane (GO:0032588)	0.128027161	1	7.843137255	16.62774289	STX6
clathrin-coated vesicle (GO:0030136)	0.139709564	1	6.666666667	13.1212637	STX6
Golgi membrane (GO:0000139)	0.141642689	1	3.016591252	5.895769726	STX6;BLZF1
Golgi subcompartment (GO:0098791)	0.160952725	1	2.783576896	5.084605682	STX6;BLZF1
mitochondrion (GO:0005739)	0.197703841	1	1.949317739	3.159815052	TRUB1;OXCT1;PFDN2
cullin-RING ubiquitin ligase complex (GO:0031461)	0.238857152	1	3.683241252	5.273994836	KLHL11
trans-Golgi network (GO:0005802)	0.243455603	1	3.603603604	5.091245694	STX6
nucleolus (GO:0005730)	0.269438542	1	1.972386588	2.586617276	UBE2T;UPF3A
early endosome (GO:0005769)	0.284737465	1	3.003003003	3.772335434	STX6
endoplasmic reticulum lumen (GO:0005788)	0.335058182	1	2.469135802	2.699879222	STC2
mitochondrial matrix (GO:0005759)	0.37245128	1	2.164502165	2.13776849	OXCT1
integral component of plasma membrane (GO:0005887)	0.377736622	1	1.367053999	1.330906484	SLC26A2;KCNQ2;ICAM5

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Supplemental Table 3. Up-Regulated Genes by P301L Tau

Term	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes
axolemma (GO:0030673)	0.002996578	1	333.3333333	1936.761417	MAPT
dendrite (GO:0030425)	0.004890312	1	18.60465116	98.98603085	NLGN1;MAPT
filopodium tip (GO:0032433)	0.004989818	0.741819593	200	1060.071173	NLGN1
spanning component of membrane (GO:0089717)	0.005487568	0.611863795	181.8181818	946.412758	NLGN1
nuclear speck (GO:0016607)	0.009082553	0.810163748	13.51351351	63.53243152	ITPKC;MAPT
microtubule cytoskeleton (GO:0015630)	0.015239427	1	10.30927835	43.13267377	FER;MAPT
main axon (GO:0044304)	0.016381548	1	60.60606061	249.1878617	MAPT
cytoskeleton (GO:0005856)	0.026441482	1	7.692307692	27.94477859	FER;MAPT
axon (GO:0030424)	0.029604508	1	33.33333333	117.3275068	NLGN1
nuclear body (GO:0016604)	0.036393121	1	6.472491909	21.44579618	ITPKC;MAPT
nuclear periphery (GO:0034399)	0.038330884	1	25.64102564	83.62818815	MAPT
ribonucleoprotein granule (GO:0035770)	0.039296097	1	25	80.91575164	MAPT
axon (GO:0030424)	0.068319537	1	14.18439716	38.0646738	MAPT
RNA polymerase II transcription factor complex (GO:0090575)	0.07113123	1	13.60544218	35.9622965	FOS
cytoplasmic ribonucleoprotein granule (GO:0036464)	0.081838782	1	11.76470588	29.44710636	MAPT
microtubule (GO:0005874)	0.100196316	1	9.523809524	21.91070345	MAPT
polymeric cytoskeletal fiber (GO:0099513)	0.105186383	1	9.049773756	20.38028436	MAPT
nuclear chromatin (GO:0000790)	0.119561652	1	7.90513834	16.78990615	FER
actin cytoskeleton (GO:0015629)	0.137676093	1	6.802721088	13.48878577	FER
chromatin (GO:0000785)	0.138551081	1	6.756756757	13.35483925	FER
nuclear chromosome part (GO:0044454)	0.179622583	1	5.102040816	8.759680555	FER
nucleolus (GO:0005730)	0.291015997	1	2.958579882	3.652003078	TSPYL2
mitochondrion (GO:0005739)	0.409477333	1	1.949317739	1.740494599	MAPT
integral component of plasma membrane (GO:0005887)	0.532245452	1	1.367053999	0.862133316	NLGN1

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Supplemental Figure 4. Down-Regulated Genes by P301L Tau					
Term	P-value	Adjusted P-value	Odds Ratio	Combined Score	Genes
Cul4-RING E3 ubiquitin ligase complex (GO:0080008)	0.0018	0.80278471	555.5556	3511.104	DCAF12
cullin-RING ubiquitin ligase complex (GO:0031461)	0.00905	1	110.4972	519.8896	DCAF12
centrosome (GO:0005813)	0.02305	1	43.38395	163.5617	DCAF12
microtubule organizing center (GO:0005815)	0.02535	1	39.44773	144.9697	DCAF12