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Chaperoning activity of the cyclophilin family prevents tau aggregation

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Abstract

Tauopathies, such as Alzheimer's disease, are characterized by the misfolding and progressive accumulation of the microtubule associated protein tau. Chaperones, tasked with maintaining protein homeostasis, can become imbalanced with age and contribute to the progression of neurodegenerative disease. Cyclophilins are a promising pool of underinvestigated chaperones with peptidyl-prolyl isomerase activity that may play protective roles in regulating tau aggregation. Using a Thioflavin T fluorescence-based assay to monitor *in vitro* tau aggregation, all eight cyclophilins, which include PPIA to PPIH prevent tau aggregation, with PPIB, PPIC, PPID, and PPIH showing the greatest inhibition. The low thermal stability of PPID and the strong heparin binding of PPIB undermines the simplistic interpretation of reduced tau aggregation. In a cellular model of tau accumulation, all cyclophilins, except PPID and PPIH, reduce insoluble tau. PPIB, PPIC, PPIE, and PPIF also reduce soluble tau levels with PPIC exclusively protecting cells from tau seeding. Overall, this study demonstrates cyclophilins prevent tau fibril formation and many reduce cellular insoluble tau accumulation with PPIC having the greatest potential as a molecular tool to mitigate tau seeding and accumulation.

KEYWORDS

cyclophilin, FRET biosensor cell, heparin binding, molecular chaperone, peptidyl-prolyl isomerase, PPIase, tau, tau seeding, thermal stability, Thioflavin T

1 | INTRODUCTION

The accumulation of misfolded protein is a characteristic of many neurodegenerative diseases with Alzheimer's disease (AD) being the most common.[1] The misfolding of the microtubule-associated protein tau (MAPT, tau) is responsible for a group of disorders called tauopathies, which includes AD, Pick's disease, and frontotemporal dementia with parkinsonism-17 (FTDP-17).[2,3] In AD, the accumulation of oligomeric and aggregated tau correlates with increased neuronal loss and disease progression by diagnostic Braak staging.[4] Previous work has shown that reducing tau levels, both soluble and insoluble, coincides with improved cognition.[5] Taken together, this suggests that regulating tau accumulation may have important therapeutic benefits for a variety of tauopathies.

Molecular chaperones, such as heat shock proteins (Hsps), are tasked with maintaining protein homeostasis and can either exacerbate or inhibit protein accumulation and toxicity.[6,7] Chaperones are not only limited to

[†]Deceased.

recognizing distinct misfolded clients but also different aggregate states, which can be on or off pathway toward fibril formation. In the case of productive chaperone-client interactions, chaperones either prevent, disaggregate, or neutralize toxic species.[8] Chaperones can be responsible for the folding and triage of misfolded proteins as part of the Hsp70/Hsp90 chaperone machinery,[9] or can act independently, primarily as holding chaperones.[10,11] A variety of chaperones have been shown to regulate tau biology,[12–14] suggesting chaperones are promising therapeutic targets for tauopathies. However, because of the complex and dynamic nature of the ubiquitous Hsp70/Hsp90 network,[15] chaperones with independent activity may be better suited to restore tau homeostasis with improved specificity.

Cyclophilins are a family of chaperones with peptidyl-prolyl isomerase (PPIase) activity that facilitate cis to trans isomerization and are characterized by their unique substrate specificity to the immunosuppressive drug, cyclosporin A (CsA).[16,17] Cyclophilins, consisting of PPIA to PPIH, PPIA-like (PPIAL3), and PPIase-like (PPIL1-PPIL6) genes,[18] have been shown to prevent aggregation and toxicity of a number of amyloid proteins, such as α-synuclein[19,20] and amyloid-β.[21–23] Only one cyclophilin, Cyp40 or PPID, has been studied in the context of aggregated tau. In this work, PPID was capable of dissolving tau aggregates *in vitro* and lowering silver-positive and oligomeric tau species in a mouse model of tau accumulation.[24] Taken together, cyclophilins are a promising pool of chaperones, which have not been systematically studied in the context of tau aggregation, and it is feasible that many cyclophilins, beyond PPID, play protective roles in regulating tau biology.[25]

Here, we sought to determine if members of the cyclophilin family could inhibit tau aggregation and prevent cellular tau accumulation. The ability to thwart the formation of new tau aggregates is a characteristic that is distinct from the previously described disaggregation activity of PPID. Using a Thioflavin T (ThT) fluorescence-based assay to monitor *in vitro* tau aggregation and cellular models to assess soluble and insoluble tau levels, eight cyclophilins, PPIA-PPIH, were investigated for their role in regulating tau aggregation, accumulation, and seeding. Additional biophysical properties of each cyclophilin, such as thermal stability and heparin binding, were also measured as these properties play important roles in understanding how tau aggregation is reduced in the presence of each cyclophilin.

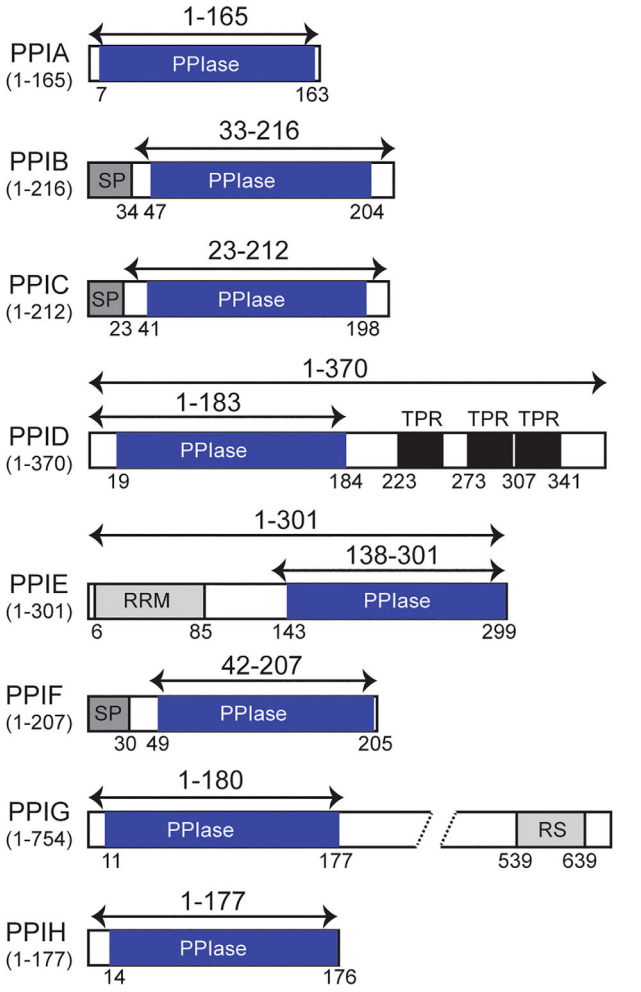

FIGURE 1 Cyclophilin family domain architecture. Arrows indicate constructs designed for expression in *E. coli*. PPIG is segmented to indicate amino acids are not drawn to scale. The start of an individual protein region is indicated by the amino acid residue designated according to UniProt. RRM, RNA recognition motif; RS, arginine-serine rich domain; SP, signal peptide sequence; TPR, tetratricopeptide repeat

2 | RESULTS

2.1 | Cyclophilins inhibit tau aggregation in a binary protein–protein environment

In order to evaluate how the cyclophilin family regulates tau aggregation *in vitro*, recombinant cyclophilins, PPIA-PPIH, along with tau 4R0N P301L were expressed in *E. coli* and purified using nickel affinity chromatography. For recombinant PPIB, PPIC and PPIF, the signal sequence was removed to promote proper folding in *E. coli*,[26] yielding only the PPIase domain and will be referred to as $PPIB_P$, $PPIC_P$, and $PPIF_P$. Recombinant constructs of PPID, PPIE, and PPIG were similarly designed to express only the PPIase domain, hereon referred to as $PPID_P$, $PPIE_P$, and $PPIG_P$, in addition to constructs of full-length PPID and PPIE (Figure 1). All cyclophilins expressed and purified at sufficient yield (8–60 mg per liter of culture) and purity (>90% pure by SDS-PAGE gel), except $PPID_P$, which had a lower yield (1 mg/L of culture) after purification optimization (Figure S1).

Cyclophilins, PPIA-PPIH, were incubated with tau P301L in a heparin-containing ThT fluorescence-based

assay at different molar ratios (1:10, 1:1, 10:1 and 25:1) of tau to cyclophilin over a 72-hr period (schematic shown in Figure 2a). At 10-fold molar excess of cyclophilin to tau, all cyclophilins significantly reduce final ThT fluorescence (Figure 2b) and kinetic measurements lack sigmoidal growth for all cyclophilins except PPIA, which shows a reduced aggregation rate as evidenced by an increase in the time to reach maximum signal (Figure S2a). Equal mixing of tau and cyclophilin was confirmed by SDS-PAGE gel prior to adding ThT (Figure S2b). To verify reduced tau aggregation, samples were separated by centrifugation after the ThT assay and run on SDS-PAGE gels to assess tau insolubility (Figure 2a). At 10-fold molar excess, all cyclophilins reduce insoluble tau by more than 10%, except for PPIA which showed the least ThT reduction (Figure S3a,b).

At an equal molar ratio of cyclophilin to tau, $PPIB_P$, $PPIC_P$, PPID, $PPID_P$, and PPIH significantly reduce ThT signal (Figure 2b) and increase the time to reach half maximum (Figure 2c), demonstrating reduced total aggregation and slower aggregation rate, respectively. Pellet analysis shows significant reduction in insoluble tau levels by $PPIB_P$ and $PPID_P$ with $PPIB_P$ also increasing soluble tau (Figures 2d and S3c). The pellet analysis agrees with the ThT results where $PPIB_P$ caused the most intense reduction in ThT signal, followed by PPID and $PPID_P$, then $PPIC_P$ and PPIH. At sub-stoichiometric ratios of cyclophilin to tau, $PPID_P$ and PPID are the only cyclophilins that reduce final ThT signal. $PPID_P$ shows significant reduction at 10:1, tau to cyclophilin, and PPID shows significant reduction at both 10:1 and 25:1, tau to cyclophilin (Figures 2b and S2).

2.2 | PPID is thermally unstable

During protein purification, PPID and $PPID_P$ appeared to be more susceptible to aggregate formation. This observation was also evident after incubation at 37°C in which extensive amounts of PPID and $PPID_P$ are detected in the pellet, even in the absence of tau (Figure S3b,c). Thermal stability of each cyclophilin was measured by differential scanning fluorimetry (DSF) using SYPRO Orange, a hydrophobic dye used to monitor protein unfolding during a temperature gradient. The melting temperature for all cyclophilins, except PPID and $PPID_P$, is between 46–56°C (Table 1 and Figure S4), consistent with the average melting temperature of ~52°C for all human proteins under physiological conditions.[27] In agreement with our hypothesis and previously published data,[28,29] the melting temperature of PPID and $PPID_P$ is lower than the other cyclophilins. At 35°C, half of the PPIase only construct ($PPID_P$) is in an unfolded state while the full-length PPID construct unfolds at a melting temperature of 40°C. The low thermal stability of PPID and $PPID_P$ corroborates the pellet analysis, which shows significant PPID aggregation at 37°C.

2.3 | Select cyclophilins are heparin-binding proteins

Most *in vitro* tau aggregation studies are limited by the addition of heparin or other negatively charged molecules, which promote facile fibril formation by inducing a tau conformational change from mostly random coil to β-sheet structure.[30] Although heparin-induced tau aggregation could be considered a proxy for negatively charged molecules inside the cell,[31,32] competition for heparin binding by cyclophilins could complicate the mechanism by which cyclophilins reduce tau aggregation *in vitro*. This could be especially true for PPIB in which three lysine residues at the N-terminus have been shown to be responsible for glycosaminoglycan binding.[33] Interestingly, tau aggregation is completely abolished at both 1:10 and 1:1, tau to $PPIB_P$ (Figures 2, S2 and S3), suggesting heparin competition could play a role.

Given heparin competition could be responsible for reduced tau aggregation, cyclophilin-heparin binding was determined experimentally by loading each cyclophilin on a heparin column and measuring the salt concentration needed to elute the protein. Tau P301L is a strong heparin binder, eluting from the column at 375 mM NaCl (Table 1 and Figure S5). This is consistent with previous surface plasmon resonance (SPR) experiments of the tau K18 isoform, which showed tau-heparin binding is abolished at 300 mM NaCl.[34] As expected, $PPIB_P$ is also a strong heparin binder, even stronger than tau, eluting from the heparin column at 600 mM NaCl. Other cyclophilins that moderately bind heparin ($PPIC_P$, PPID, $PPID_P$, PPIE, and $PPIF_P$) elute between 250 and 300 mM NaCl, while some show no heparin binding at all (PPIA, $PPIG_P$, and PPIH). Overall, cyclophilins PPIB, PPIC, PPID, PPIE, and PPIF are heparin-binding proteins that compete for heparin under physiological conditions and likely contribute to reduced tau aggregation in the ThT assay.

2.4 | All cyclophilins, except PPID and PPIH, reduce tau accumulation in cells

To determine whether cyclophilin overexpression can reduce tau aggregation in a cellular environment, HEK293T cells were transiently co-transfected with tau P301L/S320F and either empty vector or each cyclophilin

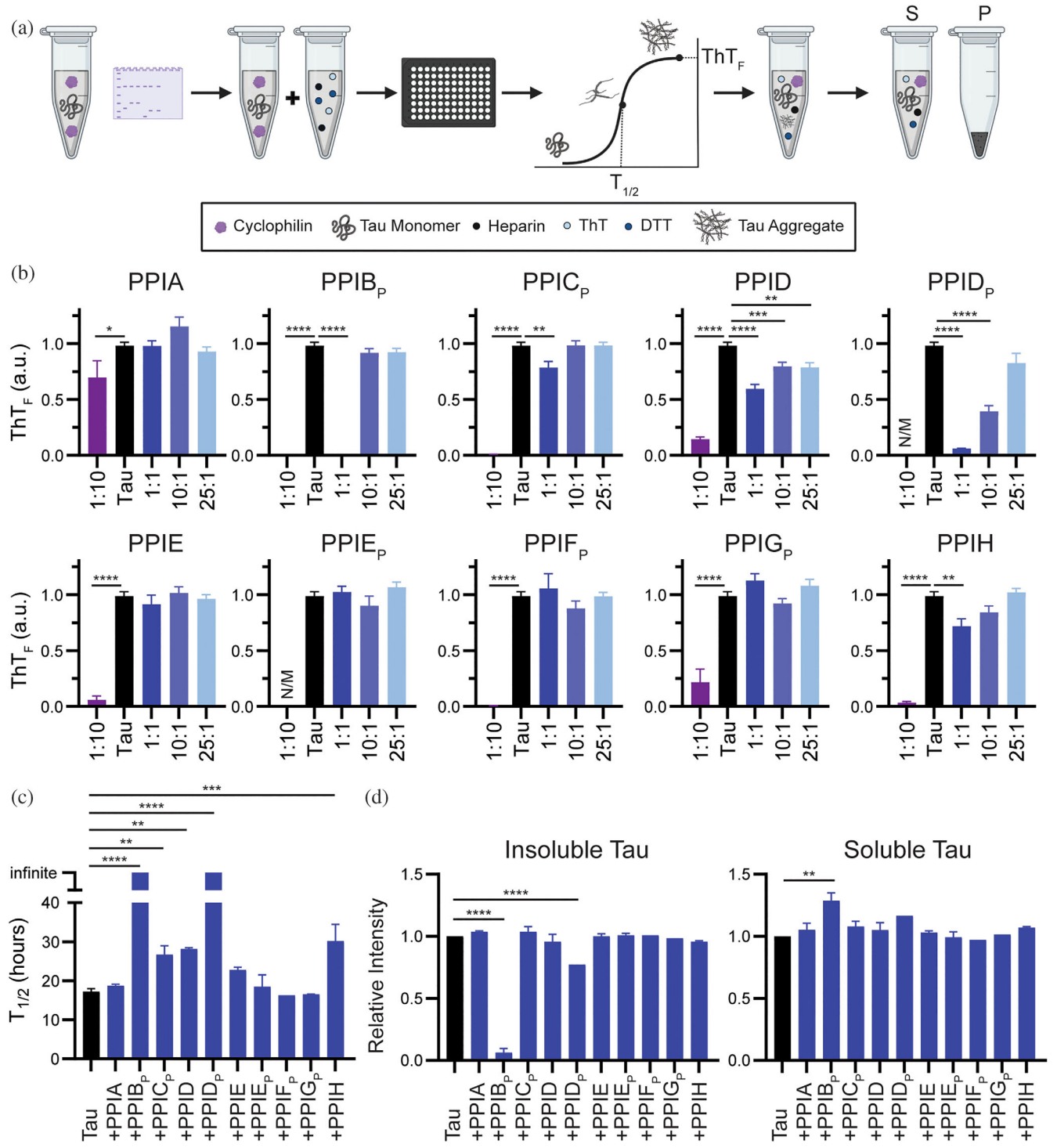

FIGURE 2 Cyclophilins inhibit tau aggregation in a concentration dependent manner. (a) Workflow schematic of ThT assay and pellet analysis. In a centrifuge tube, a master mix of tau and cyclophilin is prepared at twice the final concentration. A small aliquot is removed to evaluate protein purity by Coomassie stained SDS-PAGE gel. Next, a master mix of heparin, ThT, and DTT is prepared at twice the final concentration, then is equally combined with the tau plus cyclophilin master mix and aliquoted into a 96-well plate to measure ThT fluorescence. Output ThT kinetic curves are then used to calculate the time to reach half maximum ($T½$) and the final ThT fluorescence (ThT$_F$). At the completion of the ThT assay, replicate wells containing identical conditions are removed from the 96-well plate and combined in a single centrifuge tube, which is then centrifuged to yield a supernatant (S) and pellet (P) fraction. Figure created with biorender.com. (b) Average ThT$_F$ at 72 hr from at least two independent experiments (except 1:10, tau:cyclophilin, from one independent experiment), with at least four to six replicates per condition. N/M represents not measured. (c) $T½$ at 1:1, tau:cyclophilin. (d) Relative intensity of tau in the pellet (insoluble tau) and supernatant (soluble tau) at 1:1, tau:cyclophilin compared to tau alone. Error bars represent standard error of the mean (SEM). Data analyzed by one-way ANOVA with Dunnett's post hoc test, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. ThT, Thioflavin T

TABLE 1 Thermal stability and heparin binding

Protein	Stability (°C)	Heparin binding by NaCl concentration (mM)	
Tau	Disordered	Strong	375
PPIA	52.3 ± 0.1	No	0
$PPIB_P$	55.1 ± 0.1	Strong	600
$PPIC_P$	47.9 ± 0.1	Moderate	250
PPID	39.6 ± 0.2	Moderate	300
$PPID_P$	34.7 ± 0.1	Moderate	300
PPIE	49.2 ± 1.1	Moderate	275
$PPIE_P$	56.3 ± 0.1	Weak	<100
$PPIF_P$	49.8 ± 0.4	Moderate	300
$PPIG_P$	48.0 ± 0.1	No	0
PPIH	45.9 ± 0.4	No	0

Note: The intrinsic disorder of tau is not amenable to thermal stability measurements by DSF. Strong heparin binding is >300 mM NaCl, moderate heparin binding is 100–300 mM NaCl, weak heparin binding is <100 mM NaCl using a heparin column with a salt gradient from 0 M NaCl to 1 M NaCl.
Abbreviation: DSF, differential scanning fluorimetry.

at 1:10, tau to cyclophilin plasmid mass ratio. P301L/S320F, which combines two FTD-related mutants, was chosen because it is an aggressive cellular model of tau aggregation with detectable triton-insoluble tau aggregates.[35,36] All of the cyclophilins, except PPID and PPIH, significantly reduce triton-insoluble tau levels (Figures 3a,b). PPIB, PPIC, PPIE, and PPIF also significantly reduce soluble tau levels (Figures 3a,c). In order to begin to address whether this reduction in soluble protein was tau specific or more universal, HEK293T cells were transiently co-transfected with GFP and either empty vector or cyclophilin at 1:10, GFP to cyclophilin plasmid mass ratio. Only cyclophilins shown to reduce soluble tau were tested. None of the cyclophilins tested reduced GFP levels (Figures 3d,e), suggesting the effect is not due to widespread inhibition of protein synthesis or general enhancement of all protein degradation. After observing indications of PPIB cytotoxicity, such as inconsistent cell morphology and occasional reductions in extracted total soluble protein, cell toxicity was measured by lactate dehydrogenase (LDH) for each of the cyclophilins shown to reduce soluble tau levels (Figure 3f). Only PPIB is cytotoxic to the cells, suggesting the soluble tau reduction for PPIC, PPIE, and PPIF is not due to an increase in cell death causing tau release. It is feasible that cyclophilins may have a preference or greater chaperoning activity dependent on tau mutation, however, after replacing tau P301L/S320F with wild-type tau or tau P301L, similar reductions in soluble tau levels were measured for PPIC, PPIE, and PPIF (Figures 3g,h). Overall, cyclophilins, PPIC, PPIE, and PPIF reduce soluble and insoluble tau independent of tau mutation, while PPIA and PPIG solely reduce insoluble tau.

2.5 | PPIC inhibits tau seeding in FRET biosensor cells

To determine whether cyclophilins can slow tau aggregate seeding, HEK293T FRET biosensor cells stably co-expressing the repeat domain (RD) of tau with the disease-associated P301S mutation fused to either CFP or YFP (tau RD P301S-CFP and tau RD P301S-YFP) were co-transfected with each cyclophilin plus mKATE. The cells were seeded with sonicated recombinant tau P301L fibrils and FRET was monitored over 60 hr to measure tau aggregate formation (schematic shown in Figure 4a). The mKATE plasmid was used to identify successfully transfected cells and PPIB was excluded from the data due to cytotoxicity. PPIC significantly reduces tau seeding by approximately 15% (Figure 4b,c). Although the reduction in seeding is modest, previous work has demonstrated that altering FRET seeding by merely 10% *in vitro* correlates with altered tau accumulation *in vivo*.[37] Recombinant cyclophilin protein was then used to plot standard densitometry curves to quantify the amount of cyclophilin overexpressed when transfected. No significant difference in the expression of PPIC compared to other cyclophilins, such as PPIH, was observed (Figure S6). Since PPIE and PPIF reduce soluble tau levels but do not reduce tau seeding, this result suggests PPIC may act by an additional mechanism beyond the reduction of soluble tau.

3 | DISCUSSION

The chaperone network is a diverse group of proteins that regulate protein misfolding and tau aggregation.[6]

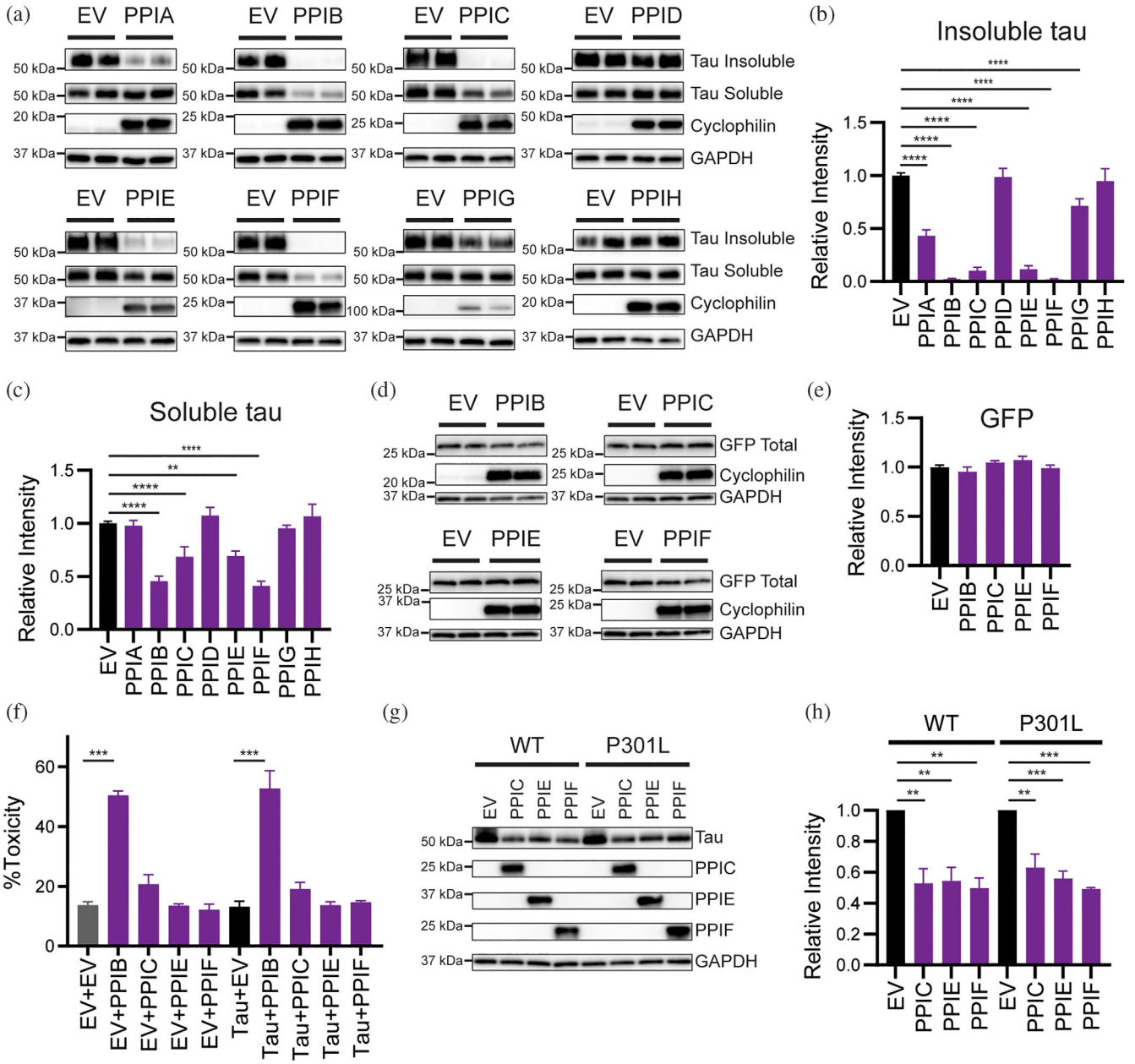

FIGURE 3 Multiple cyclophilins reduce soluble and insoluble tau levels. (a) Representative Western blot images of triton-soluble and triton-insoluble fractions from HEK293T cells co-transfected with tau P301L/S320F and either an empty vector (EV) plasmid or cyclophilin at a plasmid mass ratio of 1:10, tau:cyclophilin. (b) Quantification of relative insoluble tau levels compared to EV from at least two independent experiments with two replicates per condition. (c) Quantification of relative soluble tau levels compared to EV from at least two independent experiments with two replicates per condition. (d) Representative Western blot images of total GFP levels from HEK293T cells co-transfected with GFP and either an EV or cyclophilin at a plasmid mass ratio of 1:10, GFP:cyclophilin. (e) Quantification of relative GFP levels compared to EV from two replicates per condition. (f) Cell toxicity measured by LDH assay 48 hr post transfection of HEK293T cells co-transfected with either an EV plasmid or tau with each cyclophilin or EV control at a plasmid mass ratio of 1:10, tau:cyclophilin. (g) Representative Western blot images of total tau levels from HEK293T cells co-transfected with tau WT or tau P301L and either an EV or cyclophilin at a plasmid mass ratio of 1:10, tau:cyclophilin. (h) Quantification of relative tau, WT and P301L, levels compared to EV from three independent experiments. All intensities were normalized to GAPDH loading control prior to EV normalization. Error bars represent standard error of the mean (SEM). Data analyzed by one-way ANOVA with Dunnett's post hoc test, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. LDH, lactate dehydrogenase

Numerous studies continue to establish new chaperone-tau interactions and previous work has demonstrated genetic and chemical manipulation of chaperone activity is a viable strategy to influence tau biology.[13,14] In this study, cyclophilins, PPIA-PPIH, are systematically investigated for tau chaperoning activity. All cyclophilins

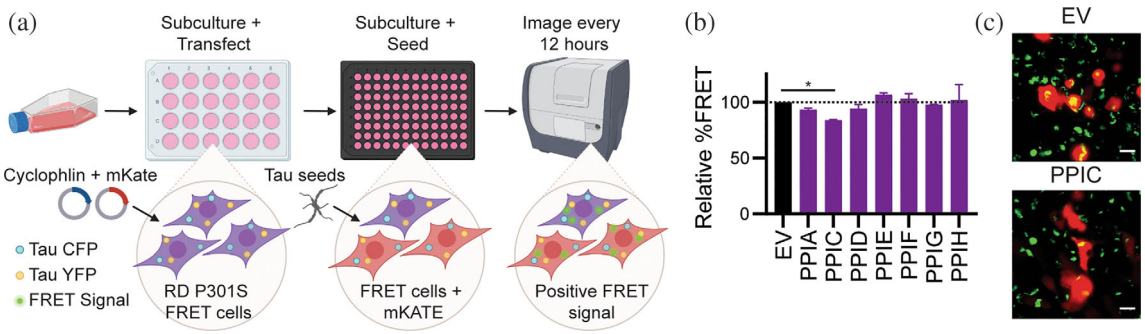

FIGURE 4 PPIC reduces tau seeding. (a) Workflow schematic of tau FRET biosensor cell assay. Tau RD P301S FRET HEK293T cells, subcultured into 24-well plates, are co-transfected with mKate and cyclophilin or empty vector (EV) plasmids at a 1:4, mKate:cyclophilin plasmid ratio. Twenty-four hours after transfection, cells are subcultured into 96-well plates, then allowed to recover for 24 hr before seeding with sonicated recombinant human tau 4R0N P301L fibrils. After the addition of seeds, cells are housed in a BioTek BioSpa and imaged by a Cytation 3 every 12 hr for 60 hr using FRET and Texas Red cubes. Figure created with BioRender.com. (b) Relative positive FRET signal of tau seeding in the presence of cyclophilin compared to EV at 60 hr after addition of sonicated recombinant tau P301L fibrils. (c) Representative images of EV and PPIC are shown. Green represents tau FRET signal and red represents transfected mKATE positive cells. Scale bar is 20 μm. Data analyzed by two-way ANOVA repeated measures with Dunnett's post hoc test, $*p < 0.05$ for PPIC at 60-hr time point. ANOVA, analysis of variance

prevent tau aggregation in a non-cellular environment with varying levels of inhibition due to distinct biophysical features. In the cell, many cyclophilins reduce insoluble tau and half of the cyclophilins tested reduce soluble tau.

PPIB and PPID are the most potent inhibitors of tau aggregation in a non-cellular environment. However, PPIB and PPID bind heparin, which most likely interferes with heparin-dependent tau aggregation. PPID has no effect on reducing soluble or insoluble tau levels, while for PPIB, the reduction in soluble and insoluble tau is partially driven by cell toxicity. For PPIA, PPIG, and PPIH, heparin binding is not a confounding issue as these cyclophilins show no heparin binding in the ThT assay buffer. PPIA, PPIG, and PPIH all reduce tau aggregation in the ThT assay with PPIA being the weakest inhibitor. Inside the cell, PPIA and PPIG reduce insoluble tau, while PPIH has no effect on cellular tau levels. PPIC, PPIE, and PPIF are the most promising candidates, as they reduce tau aggregation in a non-cellular environment, moderately bind heparin, and reduce soluble and insoluble tau inside the cell.

For PPIC, PPIE, and PPIF, the enhanced tau clearance is likely driven by altered proteasomal and/or autophagic degradation pathways. In relation to physiological tau, turnover is driven by ubiquitin-dependent proteasome degradation,[38] although ubiquitin-independent 20S proteasome degradation can also contribute.[13] Thus, it is possible PPIC, PPIE, and PPIF stabilize tau in a more disordered state enhancing effective proteolytic break down. PPIC, PPIE, and PPIF could also contribute to the clearance of soluble tau through autophagic pathways by altering tau conformation to expose chaperone mediated autophagic and endosomal microautophagic targeting motifs ^{336}QVEVK^{340} and ^{347}KDRVQ^{351} within the tau sequence.[39,40] In the case of aggregated tau, PPIA, PPIC, PPIE, PPIF, and PPIG may sequester tau into larger aggregates that are more likely to be engulfed by autophagosomes for clearance.[41] This could be especially true for PPIA and PPIG, which only reduce insoluble tau. How discrete cyclophilins differ or overlap in stimulating proteasomal or autophagic pathways is an important next step in understanding the mechanism by which cyclophilins reduce soluble and insoluble tau in the cell.

The ability of cyclophilins to bind heparin is also a likely contributor to regulating tau inside the cell, as heparin has many parallels to other negatively charged molecules.[42] PPIC, PPIE, and PPIF could potentially bind damage-associated molecular patterns (DAMPs) including ATP, DNA, RNA, uric acid, hyaluronan, and heparin sulfate, which are released by stressed cells undergoing autophagy.[43] The sequestering of DAMPs by PPIC, PPIE, and PPIF could prevent the autophagy system from being overloaded, which may synergize to prevent proteasomal overload.[44] Liquid–liquid phase separation could also play a role, as cyclophilin-heparin binding combined with tau-heparin binding could promote the formation of proteinaceous membraneless organelles containing tau, negatively charged molecules, and cyclophilins primed for clearance.[41]

PPIB and PPIC, which are residents of the endoplasmic reticulum (ER),[25,45] could play additional roles in regulating tau in the ER. PPIB could play a protective

role in preventing tau aggregation by regulating the unfolded protein response (UPR), because PPIB is known to have anti-apoptotic properties against ER stress-induced cell death.[46] PPIB has also been shown to interact with ER-resident chaperones, ERp72, GRP78, and GRP94 through basic lysine residues at the N-terminus of PPIB, which are highly conserved in PPIC.[47] Consequently, PPIB or PPIC could provide a scaffold for tau to interact with ER-resident chaperones that have been shown to resolve stress-induced protein aggregation[48] and control ER-associated degradation (ERAD).[49]

The mechanism by which PPIC prevents tau seeding is unknown. It is possible, since PPIC is also extracellular,[50] that extracellular PPIC contributes to the reduced uptake of tau seeds. However, in our assay uptake is lipofectamine-mediated, which would suggest PPIC reduces the uptake of cationic lipids. This scenario is unlikely as cyclophilins, such as PPIA, actually enhance lipid uptake in cells.[51] Thus, it is more likely that PPIC alters either monomeric tau or preformed tau fibrils into an incompatible state that prevents the templated addition of monomeric tau onto fibril seeds.

PPIases, consisting of FK506-binding proteins, parvulins, and cyclophilins, are known to be involved in regulating the aggregation of misfolded clients, but the mechanism of action is poorly understood.[52] Many PPIases, such as FKBP51 and FKBP52, have activity at low concentrations and counter to this study, enhance tau aggregation.[13,53] The low minimum PPIase concentration needed to impart activity has led to a number of studies proposing PPIase-dependent tau regulation. However, there are a few studies on diverse clients that support the PPIase-independent chaperone activity of cyclophilins, suggesting cyclophilins act as holdases.[54–56] In fact, PPIA has been shown to have a biphasic role in the aggregation of α-synuclein, another neurodegenerative disease-associated protein.[19] Similary, tau has been reported to be pushed toward or away from aggregation depending on the specific conditions of the experiment with molecular chaperones.[57] While we did not detect this in our studies, we did find differences in the ability of discrete chaperones to inhibit aggregation in cell-free systems compared to in cells, which suggests condition dependent effects on tau. Given many of the cyclophilins investigated here lacked activity at lower concentrations, it is likely cyclophilin chaperoning activity with respect to tau is not enzymatic but rather dependent on holdase activity. Understanding the differences in cyclophilin-tau interactions within the cyclophilin family and compared to other PPIase-tau interactions will hopefully begin to unravel the differences in beneficial (cyclophilin) versus detrimental (FKBP51 and FKBP52) effects on tau aggregation.

Many questions remain unanswered as to how cellular location and tissue specificity of cyclophilins with misfolded clients relates to disease. *In vivo*, knockout studies in mice show cyclophilins can regulate protein homeostasis and misfolding in neurodegenerative disease with both positive and negative outcomes. Knockout of PPIF in mice is beneficial,[58,59] as decreased levels of PPIF reduce mitochondrial permeability transition pore (mPTP) opening, a known contributors to cognitive decline in aging and AD. For PPIA and PPIB, *in vivo* knockout in mice is detrimental, as PPIA knockout induces transactive response DNA-binding protein 43 (TDP-43) proteinopathy[60] and PPIB knockout reduces functional presenilin 1.[61] Future *in vivo* studies, either by cyclophilins knockout or overexpression, will show the therapeutic potential of manipulating the cyclophilin family in respect to regulating tauopathies and neurodegenerative disease.

Overall cyclophilins reduce tau aggregation and likely alter the state of tau to promote degradation and restore the balance of proteasomal and autophagic clearance. Given many cyclophilins play protective roles in protein misfolding diseases, cyclophilins are well-suited for future *in vivo* studies to evaluate the role of the cyclophilin family in preventing neurotoxic tau accumulation.

4 | EXPERIMENTAL PROCEDURES

4.1 | Molecular cloning

Recombinant human tau 4R0N P301L, PPIA, PPID (amino acids 1–183), and PPIH were cloned into a pET-28a vector (MilliporeSigma #69864) with a TEV protease recognition sequence. Recombinant human PPIB (amino acids 33–216), PPIC (amino acids 23–212), $PPIE_P$ (amino acids 138–301), $PPIF_P$ (amino acids 42–207), and $PPIG_P$ (amino acids 1–180) were synthesized and cloned into a pET-28a(+)-TEV vector by Genscript USA. For mammalian tau expression constructs, human tau 4R0N WT, tau 4R0N P301L, and tau 4R0N P301L/S320F were cloned in a pRK5 vector backbone by removing GFP from the purchased constructs pRK5-EGFP-Tau (Addgene #46904) and pRK5-EGFP-Tau P301L (Addgene #46908), which were a kind gift from Karen Ashe,[62] and utilizing the Q5 high fidelity polymerase (NEB #M0491S) for site directed mutagenesis of serine 320 to phenylalanine. For mammalian cyclophilin constructs, N-FLAG PPIA, PPIB, N-FLAG PPIC, PPID, N-FLAG PPIE, N-FLAG PPIG, and N-FLAG PPIH were cloned into a pCMV6-XL6 vector backbone (Origene #PCMV6XL6). C-FLAG PPIF in a pCMV6-entry vector backbone was purchased from Origene (#RC223397) and pEGFP-N1 plasmid was

purchased from Clonetech (#6085-1). All plasmids were propagated in DH5α competent cells (Fisher Scientific #18265017) and verified by DNA sequencing (Eurofins Genomics).

4.2 | Protein expression and purification

For recombinant protein expression, plasmids were transformed into BL21 (DE3) One Shot Star competent cells (Fisher Scientific #C601003) and plated on kanamycin-agar plates for incubation overnight at 37°C. A single colony or glycerol stock was used to inoculate a 10 ml starter culture of LB broth supplemented with kanamycin for incubation overnight at 37°C. The next morning, 10 ml of starter culture was used to inoculate 1 L of LB-kanamycin broth and grown at 37°C. For tau P301L, cultures were grown to an OD_{600} of 0.7–0.8 then induced with a final concentration of 1 mM IPTG and grown for 3 hr before harvest. For cyclophilin expression, cultures were grown to an OD_{600} of 1.0, then the temperature was dropped to 16°C for 1–2 hr before cultures were induced with a final concentration of 1 mM IPTG and grown overnight at 16°C. Cells were then spun down at 3,500 × *g* for 20 min, supernatant discarded, and each pellet resuspended in 35 ml of running buffer (20 mM Tris–HCl pH 8.0, 500 mM NaCl, 10 mM Imidazole) supplemented with 1x EDTA-free protease inhibitor cocktail III (Bimake #B14002) and 1 mM PMSF (Sigma Aldrich #P7626). Resuspended pellets were frozen and stored at −80°C.

For protein purification, each pellet was thawed, sonicated on ice using a Sonic Dismembrator (Fisher Scientific Model 120) for 60 pulses at 85% amplitude for 5 seconds on/10 seconds off, and spun at 50,000 × *g* for 30 min at 4°C to isolate supernatant. The supernatant was loaded onto a standard gravity nickel column packed with Nickel Sepharose high performance resin (Cytiva #17-5268-02), washed with 35 ml of running buffer and eluted with 25 ml of elution buffer (20 mM Tris base pH 8.0, 500 mM NaCl, 250 mM Imidazole). TEV protease (~2–4 mg produced in-house) was added directly to the elution fraction, which was placed into a 3,500 MW cutoff dialysis bag for 4 hr at room temperature in a 1 L beaker of TEV cleavage buffer (50 mM Tris base pH 8.0, 250 mM NaCl), except for $PPID_P$, which due to instability was first separated by size exclusion chromatography using a HiLoad 16/600 Superdex 200 pg column (Cytiva #28989335) to isolate monomeric $PPID_P$ prior to TEV cleavage. Post cleavage, the solution was then dialyzed back into running buffer overnight at 4°C and another nickel column purification was performed to remove TEV protease and uncleaved protein. Finally, using a HiLoad 16/600 Superdex 200 pg column, proteins were separated by size exclusion chromatography in 20 mM Tris base pH 7.6, 500 mM NaCl, 0.5 mM EDTA, and 0.5 mM DTT to isolate monomeric protein. Protein purity was confirmed by Coomassie stained SDS-PAGE gel to be >90% pure and protein concentration was measured by BCA (Fisher Scientific #PI23225). Proteins were diluted to 2 mg/ml, aliquoted, and flash-frozen in liquid nitrogen for storage at −80°C.

4.3 | Thioflavin T fluorescence assay

To monitor tau aggregation, 10 μM recombinant human tau P301L was mixed with 2.5 μM low-molecular-weight heparin (MP Biomedicals #194114) and 2 mM DTT in the presence or absence of cyclophilin at molar ratios of 1:10, 1:1, 10:1, and 25:1, tau to cyclophilin, in 100 mM sodium acetate pH 7.0 buffer supplemented with 10 μM ThT. Four to six replicates of each condition, 200 μl for each replicate, were pipetted into a 96-well, black, clear-bottom nonbinding surface plate (Corning #3651), sealed, and incubated at 37°C without agitation for 3 days. ThT fluorescence, excitation at 440 nm and emission at 482 nm, was measured from the bottom of the plate every hour in a BioTek Synergy H1 multimode plate reader. At least two independent experiments were performed for each condition, except for 1 tau to 10 cyclophilin, which was from one independent experiment. ThT fluorescence data were blank-subtracted for buffer or cyclophilin only controls, normalized to the maximum signal for tau alone, and Boltzmann Sigmoid analysis was conducted using GraphPad Prism 9 to determine the time to reach half point maximum.

4.4 | Pellet analysis post ThT assay

Following each ThT assay, replicate wells of the same condition were pooled into microcentrifuge tubes and 600 μl volume of sample was subjected to centrifugation at 20,000 × *g* for 1 hr. The resulting supernatant was removed and the pellet was resuspended with 1 ml sodium acetate pH 7 buffer. After another centrifugation spin at 20,000 × *g*, the pellet was resuspended in 60 μl sodium acetate pH 7 buffer to make a 10x concentrated pellet sample. Final supernatant and pellet (10x concentrated) samples were mixed with 4× Laemmli (Bio-Rad #1610747) and analyzed by Coomassie stained Any kD SDS-PAGE gels (Bio-Rad #4569034).

4.5 | Differential scanning fluorimetry

To assess protein thermal stability, cyclophilins were diluted to a final concentration of 10 μM in 100 mM

sodium acetate buffer pH 7.0 containing 5× SYPRO Orange dye (Invitrogen #S6650). The 30 µl mixtures were dispensed into a Bio-Rad 96-well thin-wall PCR plate (Bio-Rad #HSP9601) and sealed with microplate adhesive film. SYPRO Orange fluorescence was monitored as a function of temperature in a Bio-Rad CFX96 Touch Real-Time PCR Detection System through use of the C1000 Touch Thermal Cycler and FRET channel. Thermal melts were conducted in triplicate for each condition by heating from 25 to 95°C in 1°C increments for 2 min while measuring fluorescence at each temperature step. Fluorescence data were blank-subtracted, normalized to the maximum signal for each individual melt, and Boltzmann Sigmoid analysis was conducted using GraphPad Prism 9 to determine the melting temperature (Tm) from two independent experiments.

4.6 | Heparin binding

Using a HiTrap Heparin HP column (Cytiva #17,040,701), heparin binding for each cyclophilin, ~1 mg of cyclophilin dialyzed overnight in 100 mM sodium acetate pH 7.0 buffer, was measured with an elution gradient of 0 M NaCl to 1 M NaCl. Peak absorbance values were verified to be protein by Coomassie stained SDS-PAGE gel. In the case of low absorbance or undetected protein, fractions were concentrated 10x using Amicon Ultra 0.5 ml 10 kDa MWCO filters (MilliporeSigma #UFC501096) prior to gel loading.

4.7 | HEK293T co-transfection and fractionation

HEK293T cells (ATCC #CRL-3216) were subcultured into 6-well or 12-well plates (Thermo Scientific #130184 and #130185) coated with poly-L-Lysine (Sigma-Aldrich #P4707) and co-transfected using Lipofectamine 2000 (5 µl for 6-well plates and 2.5 µl for 12-well plates) with tau and cyclophilin plasmids at a ratio of 1:10, tau:cyclophilin (0.2 µg tau: 2 µg cyclophilin for 6-well plates and 0.1 µg tau: 1 µg cyclophilin for 12-well plates). Forty-eight hours after transfection, cells were washed with PBS and harvested. For triton-soluble and triton-insoluble fractionation in 6-well plates, cells were lysed in 800 µl of Triton X-100 lysis buffer consisting of 50 mM Tris base pH 8.0, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100 (Research Products International #111036) supplemented with protease inhibitor cocktail, phosphatase inhibitor cocktails and PMSF (Sigma #P8340, #P5726, #P0044, and #P7626). After 10 min on ice, lysed cells were centrifuged at 3,000xg for 10 minutes to remove cell debris. The supernatants were then centrifuged at 21,000 × *g* for 30 min and the high-speed supernatant isolated as the triton-soluble fraction. The high-speed pellet was then resuspended with 1 ml of Triton X-100 lysis buffer and re-pelleted by centrifugation at 21,000 × *g* for 30 min. All centrifugation steps were performed at 4°C. The final pellet or triton-insoluble fraction was resuspended in a buffer containing 8 M urea, 50 mM Tris base pH 8.0, 2% SDS, 10 mM EDTA, and sonicated at room temperature for 3 pulses at 25% amplitude for 5 s on/10 s off. Total protein concentration from the initial supernatant was measured by BCA and used to load equal amounts of soluble and insoluble protein. For extraction of unfractionated or total cellular protein, cells were lysed from 12-well plates in 60 µl mammalian protein extraction reagent, M-PER (Fisher Scientific #78501), supplemented with protease inhibitor cocktail, phosphatase inhibitor cocktails, and PMSF and centrifuged at 10,000 × *g* for 10 min to isolate supernatant.

4.8 | Western blot

All samples, diluted into a final concentration of 1× SDS sample buffer (Bio-Rad #1610737) and heated at 100°C for 5 min, were loaded into Any kD SDS-PAGE gels (Bio-Rad #4569034) and transferred to PVDF membranes (MilliporeSigma #IPVH00010) followed by blocking for 1 hr in 7% milk. Rabbit anti-tau polyclonal antibody (Dako/Agilent #A0024) at 1:20,000 was used to detect total tau protein and mouse anti-GAPDH monoclonal antibody (Proteintech #1E6D9) was used at 1:1,000 to detect GAPDH loading control. Rabbit anti-PPIA polyclonal antibody (Abcam #ab3563), rabbit anti-PPIB monoclonal antibody (Cell Signaling #43603S), rabbit anti-PPIC monoclonal antibody (Abcam #ab184552), rabbit anti-PPID polyclonal antibody (Invitrogen #PA3-023), rabbit anti-PPIE polyclonal antibody (Invitrogen #PA5-41808), mouse anti-PPIF monoclonal antibody (Abcam #ab110324), and rabbit anti-PPIG polyclonal antibody (Cell Signaling #3803S) were used at 1:1,000 to detect each cyclophilin protein, as needed. For confirmation of PPIH expression, mouse anti-FLAG monoclonal antibody (Sigma Aldrich #F1804) or rabbit anti-PPIH polyclonal antibody (Sigma Aldrich #HPA059019) at 1:1,000 was used. HRP-conjugated secondary antibodies at 1:1,000 for mouse (SouthernBiotech #1030-05) and rabbit (SouthernBiotech #4010-05) were used for ECL visualization.

4.9 | Lactate dehydrogenase cytotoxicity

Cytotoxicity of HEK293T cells 48 hr after transfection in 12-well plates was measured by LDH release per the

manufacturer's directions (Promega #G1780). Four hours prior to LDH measurements, media were replaced. Each condition was measured in triplicate and a maximum LDH release was measured by lysing one well of cells 45 min prior to the assay.

4.10 | Tau FRET biosensor cells

HEK293T cells stably expressing tau RD P301S-CFP and tau RD P301S-YFP (ATCC #CRL-3275) were subcultured into 24-well poly-L-Lysine coated plates (0.5×10^6 cells per well) and co-transfected using 2.5 μl Lipofectamine 2000 per well (Invitrogen #11668019) with mKate (Evrogen #pmKate2-C) and cyclophilin plasmids at a 1:4 ratio of mKate to cyclophilin (0.2 μg mKate:0.8 μg cyclophilin). Twenty-four hours after transfection, cells were subcultured into 96-well, black, clear-bottom plates (Corning #3601) at a density of 0.4×10^5 cells per well and allowed to recover for 24 hr, reaching about 50–60% confluency, before seeding with a final concentration of 300 nM sonicated recombinant human tau 4R0N P301L fibrils per well. Cells were then housed in a Biospa 8 and imaged on a Cytation 3 using brightfield, FRET (excitation 445 nm/emission 542 nm—BioTek #1225110), and Texas Red (excitation 486 nm/ emission 647 nm—BioTek #1225102) cubes at 40x magnification (Plan Fluorite WD 2.7 NA 0.6) every 12 hr for 60 hr. Each condition was measured in triplicate, and 6-images per well were used for analysis with Gen5 Image Prime Software plus Spot Counting. Relative FRET positive intensity was calculated as the FRET positive aggregate area within total Texas Red area (mKate positive cells) and then normalized to the empty vector control.

4.11 | Statistical analysis

Significant differences relative to controls were conducted in GraphPad Prism 9 using a one-way analysis of variance (ANOVA) or two-way repeated measures ANOVA followed by Dunnett's post hoc test as indicated in the figure legends. *p*-values less than 0.05, 0.01, 0.001 or 0.0001 are marked by 1, 2, 3, or 4 asterisks (*), respectively. Error bars represent standard error of the mean (SEM).

AUTHOR CONTRIBUTIONS

Shannon E Hill: Conceptualization (equal); formal analysis (lead); investigation (lead); methodology (lead); validation (lead); writing – original draft (lead); writing – review and editing (lead). **Abigail R Esquivel:** Investigation (supporting); methodology (supporting); validation (supporting); writing – original draft (supporting); writing – review and editing (supporting). **Santiago Rodriguez Ospina:** Formal analysis (supporting); investigation (supporting); methodology (supporting); validation (supporting). **Lauren M Rahal:** Investigation (supporting). **Chad A Dickey:** Conceptualization (equal); funding acquisition (equal); methodology (supporting). **Laura J Blair:** Conceptualization (supporting); funding acquisition (equal); methodology (supporting); project administration (lead); resources (lead); supervision (lead); writing – original draft (supporting); writing – review and editing (supporting).

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest with the contents of this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Liyanage SI, Weaver DF. Misfolded proteins as a therapeutic target in Alzheimer's disease. Adv Protein Chem Struct Biol. 2019;118:371–411.
2. Medina M, Hernandez F, Avila J. New features about tau function and dysfunction. Biomolecules. 2016;6(2):21.
3. Josephs KA. Current understanding of neurodegenerative diseases associated with the protein tau. Mayo Clin Proc. 2017;92:1291–1303.

4. DeTure MA, Dickson DW. The neuropathological diagnosis of Alzheimer's disease. Mol Neurodegener. 2019;14:32.
5. Anglada-Huguet M, Rodrigues S, Hochgrafe K, Mandelkow E, Mandelkow EM. Inhibition of tau aggregation with BSc3094 reduces tau and decreases cognitive deficits in rTg4510 mice. Alzhimer Dement. 2021;7(1):e12170.
6. Lindberg I, Shorter J, Wiseman RL, Chiti F, Dickey CA, McLean PJ. Chaperones in neurodegeneration. J Neurosci. 2015;35:13853–13859.
7. Ciechanover A, Kwon YT. Protein quality control by molecular chaperones in neurodegeneration. Front Neurosci. 2017;11:185.
8. Wentink A, Nussbaum-Krammer C, Bukau B. Modulation of amyloid states by molecular chaperones. Cold Spring Harb Perspect Biol. 2019;11:11.
9. Lackie RE, Maciejewski A, Ostapchenko VG, et al. The Hsp70/Hsp90 chaperone machinery in neurodegenerative diseases. Front Neurosci. 2017;11:254.
10. Suss O, Reichmann D. Protein plasticity underlines activation and function of ATP-independent chaperones. Front Mol Biosci. 2015;2:43.
11. Webster JM, Darling AL, Uversky VN, Blair LJ. Small heat shock proteins, big impact on protein aggregation in neurodegenerative disease. Front Pharmacol. 2019;10:1047.
12. Abisambra JF, Blair LJ, Hill SE, et al. Phosphorylation dynamics regulate Hsp27-mediated rescue of neuronal plasticity deficits in tau transgenic mice. J Neurosci. 2010;30:15374–15382.
13. Blair LJ, Nordhues BA, Hill SE, et al. Accelerated neurodegeneration through chaperone-mediated oligomerization of tau. J Clin Invest. 2013;123:4158–4169.
14. Shelton LB, Baker JD, Zheng D, et al. Hsp90 activator Aha1 drives production of pathological tau aggregates. Proc Natl Acad Sci U S A. 2017;114:9707–9712.
15. Oroz J, Chang BJ, Wysoczanski P, et al. Structure and pro-toxic mechanism of the human Hsp90/PPIase/tau complex. Nat Commun. 2018;9:4532.
16. Schiene-Fischer C. Multidomain peptidyl prolyl cis/trans isomerases. Biochim Biophys Acta. 2015;1850:2005–2016.
17. Idris M, Idris M, Adeola F, Sedzro DM. Cyclophilins: The structure and functions of an important peptidyl-prolyl isomerase. Int J Biochem Biophys Mol Biol. 2019;4:1–6.
18. Nebert DW, Sophos NA, Vasiliou V, Nelson DR. Cyclophilin nomenclature problems, or, 'a visit from the sequence police'. Hum Genom. 2004;1:381–388.
19. Favretto F, Flores D, Baker JD, et al. Catalysis of proline isomerization and molecular chaperone activity in a tug-of-war. Nat Commun. 2020;11:11.
20. Torpey J, Madine J, Wood A, Lian LY. Cyclophilin D binds to the acidic C-terminus region of alpha-synuclein and affects its aggregation characteristics. Sci Rep. 2020;10:10159.
21. Ge YS, Teng WY, Zhang CD. Protective effect of cyclophilin A against Alzheimer's amyloid beta-peptide (25-35)-induced oxidative stress in PC12 cells. Chin Med J (Engl). 2009;122:716–724.
22. Oh Y, Kim EY, Kim Y, et al. Neuroprotective effects of overexpressed cyclophilin B against Abeta-induced neurotoxicity in PC12 cells. Free Radic Biol Med. 2011;51:905–920.
23. Villmow M, Baumann M, Malesevic M, et al. Inhibition of Abeta(1-40) fibril formation by cyclophilins. Biochem J. 2016; 473:1355–1368.
24. Baker JD, Shelton LB, Zheng D, et al. Human cyclophilin 40 unravels neurotoxic amyloids. PLoS Biol. 2017;15:e2001336.
25. Blair LJ, Baker JD, Sabbagh JJ, Dickey CA. The emerging role of peptidyl-prolyl isomerase chaperones in tau oligomerization, amyloid processing, and Alzheimer's disease. J Neurochem. 2015;133:1–13.
26. Gopal GJ, Kumar A. Strategies for the production of recombinant protein in Escherichia coli. Protein J. 2013;32:419–425.
27. Jarzab A, Kurzawa N, Hopf T, et al. Meltome atlas-thermal proteome stability across the tree of life. Nat Methods. 2020;17: 495–503.
28. Pirkl F, Buchner J. Functional analysis of the Hsp90-associated human peptidyl prolyl cis/trans isomerases FKBP51, FKBP52 and Cyp40. J Mol Biol. 2001;308:795–806.
29. Davis TL, Walker JR, Campagna-Slater V, et al. Structural and biochemical characterization of the human cyclophilin family of peptidyl-prolyl isomerases. PLoS Biol. 2010;8:e1000439.
30. von Bergen M, Barghorn S, Biernat J, Mandelkow EM, Mandelkow E. Tau aggregation is driven by a transition from random coil to beta sheet structure. BBA-Mol Basis Dis. 2005; 1739:158–166.
31. Weiss RJ, Esko JD, Tor Y. Targeting heparin and heparan sulfate protein interactions. Org Biomol Chem. 2017;15:5656–5668.
32. Lester E, Ooi FK, Bakkar N, et al. Tau aggregates are RNA-protein assemblies that mislocalize multiple nuclear speckle components. Neuron. 2021;109:1675–1691.
33. Allain F, Vanpouille C, Carpentier M, Slomianny MC, Durieux S, Spik G. Interaction with glycosaminoglycans is required for cyclophilin B to trigger integrin-mediated adhesion of peripheral blood T lymphocytes to extracellular matrix. Proc Natl Acad Sci USA. 2002;99:2714–2719.
34. Zhao J, Huvent I, Lippens G, et al. Glycan determinants of heparin-tau interaction. Biophys J. 2017;112:921–932.
35. Strang KH, Croft CL, Sorrentino ZA, Chakrabarty P, Golde TE, Giasson BI. Distinct differences in prion-like seeding and aggregation between tau protein variants provide mechanistic insights into tauopathies. J Biol Chem. 2018; 293:2408–2421.
36. Webster JM, Darling AL, Sanders TA, et al. Hsp22 with an N-terminal domain truncation mediates a reduction in tau protein levels. Int J Mol Sci. 2020;21(15):5442.
37. Darwich NF, Phan JM, Kim B, et al. Autosomal dominant VCP hypomorph mutation impairs disaggregation of PHF-tau. Science. 2020;370(6519):eaay8826.
38. Babu JR, Geetha T, Wooten MW. Sequestosome 1/p62 shuttles polyubiquitinated tau for proteasomal degradation. J Neurochem. 2005;94:192–203.
39. Wang YP, Martinez-Vicente M, Kruger U, et al. Tau fragmentation, aggregation and clearance: The dual role of lysosomal processing. Hum Mol Genet. 2009;18:4153–4170.
40. Jiang S, Bhaskar K. Degradation and transmission of tau by autophagic-endolysosomal networks and potential therapeutic targets for tauopathy. Front Mol Neurosci. 2020;13:586731.
41. Noda NN, Wang Z, Zhang H. Liquid-liquid phase separation in autophagy. J Cell Biol. 2020;219(8):e202004062.
42. Jones LS, Yazzie B, Middaugh CR. Polyanions and the proteome. Mol Cell Proteomics. 2004;3:746–769.

43. Zhang QH, Kang R, Zeh HJ, Lotze MT, Tang DL. DAMPs and autophagy cellular adaptation to injury and unscheduled cell death. Autophagy. 2013;9:451–458.
44. Chesser AS, Pritchard SM, Johnson GVW. Tau clearance mechanisms and their possible role in the pathogenesis of Alzheimer disease. Front Neurol. 2013;4:122.
45. Stocki P, Chapman DC, Beach LA, Williams DB. Depletion of cyclophilins B and C leads to dysregulation of endoplasmic reticulum redox homeostasis. J Biol Chem. 2014;289:23086–23096.
46. Kim J, Choi TG, Ding Y, et al. Overexpressed cyclophilin B suppresses apoptosis associated with ROS and Ca2+ homeostasis after ER stress. J Cell Sci. 2008;121:3636–3648.
47. Jansen G, Maattanen P, Denisov AY, et al. An interaction map of endoplasmic reticulum chaperones and foldases. Mol Cell Proteom. 2012;11:710–723.
48. Melo EP, Konno T, Farace I, et al. Stress-induced protein disaggregation in the endoplasmic reticulum catalysed by BiP. Nat Commun. 2022;13:2501.
49. Eletto D, Dersh D, Argon Y. GRP94 in ER quality control and stress responses. Semin Cell Dev Biol. 2010;21:479–485.
50. Bukrinsky M. Extracellular cyclophilins in health and disease. BBA-Gen Subjects. 2015;1850:2087–2095.
51. Ramachandran S, Vinitha A, Kartha CC. Cyclophilin A enhances macrophage differentiation and lipid uptake in high glucose conditions: A cellular mechanism for accelerated macro vascular disease in diabetes mellitus. Cardiovasc Diabetol. 2016;15:152.
52. Rein T. Peptidylprolylisomerases, protein folders, or scaffolders? The example of FKBP51 and FKBP52. Bioessays. 2020;42:1900250.
53. Criado-Marrero M, Gebru NT, Gould LA, et al. FKBP52 overexpression accelerates hippocampal-dependent memory impairments in a tau transgenic mouse model. NPJ Aging Mech Dis. 2021;7:9.
54. Chakraborty A, Sen B, Datta R, Datta AK. Isomerase-independent chaperone function of cyclophilin ensures aggregation prevention of adenosine kinase both in vitro and under in vivo conditions. Biochemistry. 2004;43:11862–11872.
55. Zhang XC, Wang WD, Wang JS, Pan JC. PPIase independent chaperone-like function of recombinant human cyclophilin A during arginine kinase refolding. FEBS Lett. 2013;587:666–672.
56. Keogh RA, Zapf RL, Wiemels RE, Wittekind MA, Carroll RK. The intracellular cyclophilin PpiB contributes to the virulence of Staphylococcus aureus independently of its peptidyl-prolyl cis/trans isomerase activity. Infect Immun. 2018;86(11):e00379-18.
57. Weickert S, Wawrzyniuk M, John LH, Rudiger SGD, Drescher M. The mechanism of Hsp90-induced oligomerizaton of tau. Sci Adv. 2020;6(11):eaax6999.
58. Du H, Guo L, Fang F, et al. Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. Nat Med. 2008;14:1097–1105.
59. Jara C, Cerpa W, Tapia-Rojas C, Quintanilla RA. Tau deletion prevents cognitive impairment and mitochondrial dysfunction age associated by a mechanism dependent on cyclophilin-D. Front Neurosci. 2020;14:586710.
60. Pasetto L, Grassano M, Pozzi S, et al. Defective cyclophilin a induces TDP-43 proteinopathy: Implications for amyotrophic lateral sclerosis and frontotemporal dementia. Brain. 2021;144:3710–3726.
61. Ben-Gedalya T, Moll L, Bejerano-Sagie M, et al. Alzheimer's disease-causing proline substitutions lead to presenilin 1 aggregation and malfunction. EMBO J. 2015;34:2820–2839.
62. Hoover BR, Reed MN, Su JJ, et al. Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. Neuron. 2010;68:1067–1081.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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