DnaJC7 specifically regulates tau seeding

Valerie A. Perez¹, David W. Sanders¹, Ayde Mendoza-Oliva¹, Barbara E. Stopschinski¹, Vishruth Mullapudi¹, Charles L White, III⁴, Lukasz A. Joachimiak¹,³, Marc I. Diamond¹,²

¹Center for Alzheimer’s and Neurodegenerative Diseases
²Department of Neurology
³Department of Biochemistry
⁴Department of Pathology

Peter O'Donnell Jr. Brain Institute
University of Texas Southwestern Medical Center, Dallas, TX

Corresponding Author: Marc Diamond, M.D.

NL10.120
6000 Harry Hines Blvd.
Dallas, TX 75390
Neurodegenerative tauopathies are caused by accumulation of toxic tau protein assemblies. This appears to involve template-based seeding events, whereby tau monomer changes conformation and is recruited to a growing aggregate. Several large families of chaperone proteins, including Hsp70s and J domain proteins (JDPs) cooperate to regulate the folding of intracellular proteins such as tau, but the factors that coordinate this activity are not well known. The JDP DnaJC7 binds tau and reduces its intracellular aggregation. However, it is unknown whether this is specific to DnaJC7 or if other JDPs might be similarly involved. We used proteomics within a cell model to determine that DnaJC7 co-purified with insoluble tau and colocalized with intracellular aggregates. We individually knocked out every possible JDP and tested the effect on intracellular aggregation and seeding. DnaJC7 knockout decreased aggregate clearance and increased intracellular tau seeding. This depended on the ability of the J domain (JD) of DnaJC7 to stimulate Hsp70 ATPase activity, as JD mutations that block this interaction abrogated the protective activity. Disease-associated mutations in the JD and substrate binding site of DnaJC7 also abolished its protective activity. DnaJC7 thus specifically regulates tau aggregation in cooperation with Hsp70.
**Introduction**

Neurodegenerative tauopathies are caused by neuronal and glial accumulation of tau protein in amyloid fibrils\(^1\). We previously reported that tau has properties of a prion, in which assemblies of defined structure enter a cell, serve as templates for their own replication, and lead to distinct patterns of neuropathology in a process termed "seeding"\(^2-5\). Recent cryo-electron microscopy studies have revealed distinct fibril morphologies (polymorphs) for several tauopathies, including Alzheimer’s Disease (AD), Progressive Supranuclear Palsy (PSP), and Corticobasal Degeneration (CBD)\(^6-8\). It has been known for decades that chaperones regulate intracellular protein aggregation. However, the factors that interact specifically with tau to regulate its assembly have not been comprehensively characterized.

Molecular chaperones regulate the folding, maturation, and degradation of proteins\(^9\). Heat shock proteins (HSPs) such as Hsp70 and Hsp90 have been reported to non-specifically regulate the folding of tau, alpha-synuclein, and other proteins implicated in neurodegenerative diseases\(^10\). The folding and refolding cycle of Hsp70 is regulated by an ATPase cycle, with rapid on/off rates and low substrate affinity in the ATP-bound state and a slow off rate and high substrate affinity in the ADP-bound state\(^11\). A separate group of chaperones, the J domain proteins (JDPs), bind myriad substrates and shuttle them to Hsp70\(^12,13\). JDPs are defined by a highly conserved ~70 amino acid J domain (JD) that enables interaction with Hsp70. The JD contains a conserved Histidine-Proline-Aspartic Acid (HPD) motif required to stimulate Hsp70 ATPase activity and thus enable Hsp70 substrate binding\(^12\). JDPs thus play a key role in the Hsp70 protein folding/refolding cycle by conferring substrate specificity to Hsp70.

JDPs have also been reported to directly modulate aggregation of tau and other neurodegenerative amyloid proteins\(^14,15\). We have previously determined the mode of tau:DnaJC7 interaction\(^16\). DnaJC7 binds tau with nanomolar affinity and sub-stoichiometrically reduces tau aggregation \textit{in vitro}. Additionally, DnaJC7 preferentially binds inert tau, a form that does not spontaneously aggregate. We have now tested the specificity of tau binding to DnaJC7 vs. other JDPs and the role of Hsp70 in DnaJC7-mediated regulation of intracellular tau aggregation.

**Results**

**Identification of proteins that copurify with tau aggregates**

Tau assemblies of distinct structure (“strains”) propagate indefinitely in clonal cultured cells that express the tau repeat domain (RD) containing two disease-associated mutations (P301L/V337M) fused to yellow fluorescent protein (YFP)\(^3,5\). To identify factors associated with tau, we studied the insoluble proteome of two clones, termed DS1 (which lacks inclusions) and DS10 (which propagates a unique strain). We first extracted DS1 and DS10 with sarkosyl to identify insoluble material. We boiled the insoluble fraction in SDS and resolved proteins by SDS-PAGE to isolate individual bands for extraction and analysis via mass spectrometry (Figure 1A, Figure 1 – Supplement 1). We identified 12 unique proteins significantly enriched in DS10.
compared to the DS1 control, and 49 proteins found only in the DS10 insoluble fraction (Figure 1B, C). These included VCP and Hsp70, which have previously been shown to modulate the tau aggregation process\textsuperscript{17,18}, and other factors associated with protein quality control, autophagy, and the ubiquitin-proteasome system. As expected, the DS10 insoluble fraction was significantly enriched in tau and YFP. In contrast, the insoluble fraction of DS1 consisted predominantly of RNA-binding proteins and was de-enriched for tau and YFP.

Figure 1. A proteomic approach to identify tau aggregate interactors. 

A) Tau aggresomes were partially purified from DS1 and DS10 HEK293 cells expressing tauRD-YFP. Detergent fractionation enabled generation of a sarkosyl-insoluble fraction containing tau aggresomes. Aggregates were recovered from the pellet by running protein on an SDS-PAGE gel and then extracting protein from individual lanes from the gel to be analyzed via LC-MS/MS. B) Volcano plot showing proteins enriched in the sarkosyl-insoluble fraction as a fold enrichment from cells expressing tauRD-YFP aggregates (DS10, dark blue dots) over cells expressing tauRD-YFP that does not form aggregates (DS1, gray dots). The red line indicates an FDR of 1.5%. GO term enrichment analyses of biological processes is also shown for select GO terms: orange dots, chaperone-mediated protein folding (chaperone folding); green dots, regulation of ubiquitination (Ub regulation); teal dots, ubiquitin-dependent protein catabolic process (Ub degradation). C) Spectral indices for a selection of the proteins identified only in the DS10 insoluble fraction. Viable knockouts are shown as green bars. Non-Viable knockouts are shown as black bars. Error bars represent the SEM of three extracted protein SDS-PAGE gel bands.
Figure 1 – Supplement 1. Partial purification of tau aggresomes.
Sarkosyl-soluble and sarkosyl-insoluble fractions were run on gels and proteins were stained with SimplyBlue protein stain. DS9 and DS10 featured significant enrichment of insoluble tau RD-YFP (arrow on gel). Whole lanes for pellet fractions were analyzed by mass spectrometry (biological triplicates). The DS9 cell line, which contains tau aggregates of a distinct strain, is included as a tau-aggregate containing control. Source data for this figure are provided in Figure 1 - Supplement 1 - Source Data 1.

DnaJC7 knockout reduces clearance and increases inclusion density
We first determined whether any of the top interactors from the proteomic screen would modulate tau aggregate clearance. We utilized a cell line that propagates the DS10 strain with tauRD-YFP expression regulated by a tetracycline-repressible (tet-off) promoter. These cells (henceforth termed OFF1::DS10) constitutively produce large juxtanuclear tau aggregates, whose clearance can be monitored by loss of YFP fluorescent puncta after addition of doxycycline to the cell media to shut off gene expression (Figure 2A).

We knocked out a selection of genes enriched in or found only in the DS10 insoluble fraction, plus control genes in the OFF1::DS10 cell lines and monitored tau clearance. Knockout of certain genes (e.g., VCP, SUMO2, black bars in Figure 1C) was lethal, and thus their effects could not be tested. We shut off tau expression for three or five days to identify cells that had fully cleared aggregates and manually counted cells to quantify the effects of the knockouts on tau aggregate clearance. OFF1::DS10 cells with DnaJC7 KO out had the greatest number of cells still containing tau aggregates after three days (~80% containing aggregates) or five days (~40% containing aggregates) of...
repression (Figure 2B). This contrasted sharply to the rest of the knockout cell lines generated, which all cleared most of the aggregates after five days of repression. Additionally, confocal microscopy revealed that DnaJC7 KO increased inclusion density and decreased the quantity of extra-aggresomal (diffuse) tau compared to the nontargeting control (Figure 2C). DnaJC7 knockout was confirmed by Western Blot (Figure 2 – Supplement 1).

To test for DnaJC7-mediated clearance of invisible seeds, we shut off tau expression for zero to five days and then restarted expression for two days. DnaJC7 KO decreased the rate of tau aggregate clearance, with ~40% of cells contained aggregates after five days of repression vs. ~0% of the nontargeting and untreated control cells (Figure 2D). The profound effects of DnaJC7 indicated that it likely played a key role in mediating seed clearance.
**Figure 2.** DnaJC7 KO uniquely extends tau seed lifespan in dividing cells.

**A)** Schematic showing the HEK293 OFF1::DS10 system. A selection of the hits from the proteomics screen were knocked out in these cells. The cells are then allowed to grow with tau expression turned OFF for 0-5 days before resuming tau expression. Error bars represent the SEM of six technical replicates. **B)** The persistence of tau aggregates in OFF1::DS10 cells with the indicated knockout was quantified following 3 (orange bars) or 5 (purple bars) days of repressed tau expression. Error bars represent the SEM of six technical replicates. **C)** Confocal microscopy images showing tau aggregate organization in the DnaJC7 KO and nontargeting control cells following 0 or 5 days of...
repression of tau expression. D) Extended time course for tau aggregate clearance in the OFF1::DS10 system with DnaJC7 KO (orange) and the nontargeting (purple) and untreated (green) controls. Error bars represent SEM of six technical replicates. * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.

![Image: Immunoblotting for DnaJC7 confirms that DnaJC7 is knocked out in the Tet-regulated tauRD-YFP aggregate line (OFF1::DS10). Source data for this figure are provided in Figure 2 - Supplement 1 - Source Data 1.]

**Figure 2 – Supplement 1.** DnaJC7 KO in (OFF1::DS10) cells. Immunoblotting for DnaJC7 confirms that DnaJC7 is knocked out in the Tet-regulated tauRD-YFP aggregate line (OFF1::DS10). Source data for this figure are provided in Figure 2 - Supplement 1 - Source Data 1.

DnaJC7 and DnaJB6 uniquely regulate tau aggregation

Given that DnaJC7 KO impeded the clearance of tau aggregates, we next tested whether this would modify seeded tau aggregation. We have previously developed a biosensor cell line that stably expresses tauRD (P301S) linked to the mClover3 or mCerulean3 fluorescent proteins. Application of exogenous aggregates induces intracellular tau aggregation that is quantified by fluorescence resonance energy transfer (FRET). Exogenous fibrils bind to heparan sulfate proteoglycans (HSPGs) on the cell surface and trigger their own uptake via macropinocytosis. Internalized tau seeds then escape endolysosomal trafficking, enter the cytoplasm, and trigger further intracellular aggregation. Alternatively, aggregates can be introduced directly into the cytoplasm by cationic lipids such as Lipofectamine 2000, which increases the induced seeding efficiency.

To test the specificity of DnaJC7, we used CRISPR/Cas9 to knock out all known JDPs in the biosensor line individually. We used gRNAs from the Brunello library in pools of four for each gene to produce 50 unique JDP knockout biosensor cell lines (Figure 3A). We exposed the knockout biosensors to naked seeds at various concentrations. After 48 h we analyzed seeding in the cells via flow cytometry. Only DnaJC7 and DnaJB6 KO significantly increased tau seeding relative to the nontargeting control (Figure 3B, full dose titrations shown in Figure 3 – Supplement 1A). Western blot analysis later revealed that DnaJC7 was only partially knocked out (Figure 3 – Supplement 1). However, even this partial knockout of DnaJC7 was sufficient to significantly increase tau seeding.
We also tested whether DnaJB6 and DnaJC7 KO changed the kinetics of intracellular seeding by evaluating seeding at 6, 12, 24, and 48 h timepoints. We observed no significant seeding in any cell lines at 6 and 12 h (Figure 3 – Supplement 1B). At 24 and 48 h, DnaJC7 KO enabled more intracellular aggregation than DnaJB6 KO at tau concentrations of 33.3 nM and 11.1 nM. Both knockout cell lines exhibited higher seeding than the nontargeting control cell line.
Figure 3. A targeted genetic screen for modifiers of transient tau seeding identifies specific JDPs.

A) Schematic showing the HEK293 tau biosensor system consisting of tauRD fused to either mCerulean3 or mClover3 fluorescent proteins. The base biosensor cells had each JDP individually knocked out to generate 50 distinct cell lines. Recombinant, sonicated tau fibrils (seeds) were added to the cells to induce seeding of the tauRD constructs, which was detected as a FRET signal via flow cytometry 48 hours after treatment. B) Representative data showing the effects of the individual knockouts of JDPs on tau seeding in biosensor cells, quantified as FRET signal via flow cytometry. Cells were seeded with a dose titration of sonicated tau fibrils. Knockout of DnaJC7 (ΔC7, orange) and DnaJB6 (ΔB6, green) are highlighted. The remaining JDP knockouts are denoted as ΔJDP (gray). Each batch of knockout cell lines was normalized to the DnaJC7 KO seeding signal and then all batches are plotted together. The seeding assay for the DnaJC7 KO and the nontargeting control (Non-Target, purple) were repeated ten times. C) Extended time course harvesting of the tau seeding assay for DnaJB6 KO, DnaJC7 KO, and nontargeting control cells harvested at 24 h (24H, dashed lines) and 48 h (48H, solid lines) timepoints. Coloring as in B). Error bars represent SEM of three technical replicates. * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001
**Figure 3 – Supplement 1. All individual groups of the JDP CRISPR screen.**

A) Full tau dose titrations for all batches of individual knockouts of JDPs on tau seeding in biosensor cells, quantified as FRET signal via flow cytometry. Cells were seeded with a dose titration of sonicated tau fibrils. Knockout of DnaJC7 (ΔC7, red) and the nontargeting control (NT, blue) are highlighted in each batch. B) Extended time course harvesting of the tau seeding assay for DnaJC7 KO (ΔC7, orange), DnaJB6 KO (ΔB6, green), and nontargeting control (Non-Target, purple) cells at 6 h and 12 h timepoints.
All error bars represent SEM of three technical replicates. C) Immunoblotting for DnaJC7 and DnaJB6 confirms partial and full knockout in their respective tau biosensor cell lines, ΔB6 and ΔC7. The nontargeting control cell line (NT) is also shown as a control. Source data for this figure are provided in Figure 3 - Supplement 1 - Source Data 1.

**DnaJC7 KO increases seeding from tauopathy brains**

To further characterize DnaJC7 and DnaJB6, we tested their effect on tau seeding with seeds derived from different sources. We treated DnaJC7 KO and DnaJB6 KO cell lines with either recombinant tau fibrils, DS tau strain cell lysates (DS1, 9, or 10)\(^3\), or brain lysates from subjects with Alzheimer’s Disease (AD), Progressive Supranuclear Palsy (PSP), or Corticobasal Degeneration (CBD). DS9 propagates a distinct strain of tau aggregates characterized by multiple nuclear inclusions. This contrasts DS10, which features a single large juxtanuclear aggregate. The KO tau biosensor lines were directly treated with 25 µL of brain lysates or transfected with 5 µL of brain lysates using Lipofectamine 2000. Additionally, biosensors were treated with 20 µg total cell lysate from DS1, 9, and 10 or transfected with 5 µg of DS cell lysates using Lipofectamine 2000.

Only DnaJC7 KO significantly increased the seeding of the brain homogenates (Figure 4A, B). DnaJC7 KO alone enhanced seeding from naked AD lysate, whereas control and DnaJB6 KO cell lines did not. Thus, the effects of DnaJC7 KO were not constrained by the specific conformation of tau seeds. We also observed that DnaJC7 and DnaJB6 KO induced differential effects on DS9 and DS10 cell lysate seeding in naked vs. Lipofectamine-mediated seeding experiments. When DS9 cell lysate was seeded directly on the cells, DnaJC7 and DnaJB6 KO both resulted in decreased seeding relative to the Non-Target control. By contrast, seeding with the DS10 cell lysate was decreased only in the DnaJB6 KO cell line. Conversely, when DS9 and DS10 cell lysates were transfected into the KO biosensor lines with Lipofectamine, DnaJC7 KO increased the seeding of DS9 lysate, and DnaJB6 KO decreased seeding for both DS9 and DS10 lysates.

**Figure 4. DnaJC7 knockout increases tau seeding across multiple seed sources.**
Intracellular A) naked or B) lipofectamine-mediated tau seeding in nontargeting control (Non-Target, purple), DnaJC7 KO (ΔC7, orange), and DnaJB6 KO (ΔB6, green) tau biosensor cells. Cells were seeded with sonicated recombinant tau fibrils (rTau); brain lysates from patients with Alzheimer’s Disease (AD), Progressive Supranuclear Palsy (PSP), or Corticobasal Degeneration (CBD); and cell lysates of DS1, DS9, or DS10 cells. 100 nM or 10 nM of recombinant tau was added for naked and lipofectamine seeding, respectively. 25 μL or 5 μL of patient brain or lysate or 20 μg or 5 μg cell lysate were added for naked and lipofectamine seeding, respectively. NT denotes no treatment with seeds. Error bars represent SEM of three technical replicates. * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001

DnaJC7 overexpression rescues knockout tau biosensors
To test for specificity of knockout of DnaJC7 in the biosensor cells, we transiently overexpressed DnaJC7 using a Ruby-DnaJC7 fusion with a coding sequence resistant to targeting by the gRNAs used to generate the original DnaJC7 KO cell line (Figure 5 – Supplement 1A). The DnaJC7 KO cell line was also remade and full knockout was confirmed by Western Blot (Figure 5 – Supplement 1B, vehicle control). Expression of the Ruby-DnaJC7 fusion constructs was confirmed by Western Blot (Figure 5 – Supplement 1B). Ruby-DnaJC7 overexpression in the DnaJC7 KO cells reduced tau seeding relative to Ruby alone and vehicle controls (Figure 5A). Overexpression of Ruby-DnaJC7 in the control tau biosensors without DnaJC7 KO modestly reduced tau seeding (Figure 5B).

To test the role of DnaJC7 binding to Hsp70, we introduced a point mutation into the Ruby-DnaJC7 fusion that inhibits Hsp70 substrate binding and handoff23,24. The aspartic acid in the histidine-proline-aspartic acid (HPD) motif was mutated to a glutamine (D411Q, henceforth termed HPQ mutant) to preclude DnaJC7’s ability to stimulate Hsp70 ATPase activity. Expression of Ruby-DnaJC7 (HPQ) in the DnaJC7 KO biosensors did not rescue the WT seeding phenotype and instead enhanced tau seeding (Figure 5A). Surprisingly, overexpression of DnaJC7 (HPQ) in the Non-targeted control tau biosensors also increased tau seeding, consistent with a dominant negative effect (Figure 5B).
Figure 5. DnaJC7 regulates tau seeding in multiple experimental approaches.

A) Rescue of DnaJC7 KO in tau biosensor cells with either wildtype (WT C7, green) or HPQ mutant (HPQ C7, dark blue) DnaJC7 constructs. The Ruby fluorophore alone (Ruby Control, orange) and a vehicle control (Vehicle Control, purple) were also added to the DnaJC7 KO cells.

B) Overexpression of DnaJC7 constructs in control tau biosensor cells. The cells were transfected with the same constructs as in (A).

C) Model of DnaJC7 with domains colored as follows: TPR1, green; TPR2A, teal; TPR2B, dark blue; JD, orange. Positions of ALS-associated mutations are shown as purple spheres.

D) Rescue of DnaJC7 KO in tau biosensor cells with ALS-associated mutants of DnaJC7 and WT control, sorted by domain location. Rescue with the WT DnaJC7 construct is shown in all domains as a grey, dashed line. Error bars represent SEM of three technical replicates. * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001
**Figure 5 – Supplement 1. Stability and expression of DnaJC7 mutants in tau biosensors.**

**A)** Schematic showing tau biosensor cells with or without DnaJC7 KO transiently overexpressing different Ruby-tagged gRNA-resistant DnaJC7 constructs. Cells were allowed to express the constructs for two days before being plated for the seeding assay. **B)** Immunoblotting for DnaJC7 confirmed expression of the Ruby-WT DnaJC7 and Ruby-DnaJC7 (HPQ) mutant constructs in tau biosensor cells without (Overexpression) and with endogenous DnaJC7 knocked out (Rescue). Ruby fusion constructs are highlighted by a red arrow. **C)** Immunoblotting for DnaJC7 confirmed expression of the Ruby-WT DnaJC7 and Ruby-DnaJC7 ALS mutant constructs in tau biosensor cells with endogenous DnaJC7 knocked out. Ruby fusion constructs are highlighted by a red arrow. **D)** Positive and negative controls utilized in the rescue of DnaJC7 KO in tau biosensor cells with ALS-associated mutants of DnaJC7, colored as follows: Vehicle control, purple; Ruby control, orange; WT DnaJC7, grey dashed; HPQ mutant, dark blue. Error bars represent SEM of three technical replicates. **E)** Rosetta-calculated mean Gibbs free energy shift (ddG) of the ALS-associated mutants of DnaJC7 vs. their rescue seeding with 33 nM of tau fibrils. Grey dashed line denotes a mean ddG total score of 0. Mutants are colored according to their domain localization: TPR1, green; TPR2A, teal; TPR2B, dark blue; JD, orange. **F)** Quantification of the Western Blot signal for the different ALS-associated mutants and controls in **C)**. All constructs were normalized to the band intensity of the WT construct. Domains are colored as in (C). **G)** Rosetta-calculated mean Gibbs free energy shift (ddG) of the ALS-associated mutants of DnaJC7 vs. their normalized Western Blot band intensity. Grey dashed line denotes a mean ddG total score of 0. Mutants are colored as in (C). All error bars represent SEM of three technical seeding replicates. * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001. Source data for this figure are provided in Figure 5 – Supplement 1 – Source Data 1.

**Disease-associated DnaJC7 mutations differentially modulate tau seeding.**

DnaJC7 mutations cause dominantly inherited Amyotrophic Lateral Sclerosis (ALS)\(^{25}\). 17 mutations have been identified that are distributed across all domains of DnaJC7 (Figure 5C). The TPR1 and TPR2A domains have been implicated in binding to EEVD motifs in Hsp70 and Hsp90, respectively\(^ {26}\). We have previously found that the TPR2B domain mediates DnaJC7 binding to tau\(^ {16}\). Additionally, DnaJC7 was recently shown to bind the prion-like domain of the ALS-associated protein TDP-43 and mitigate its ability to phase separate \textit{in vitro}\(^ {27}\). Although ALS is not traditionally known as a tauopathy, there is evidence of tau pathology in ALS case studies\(^ {28}\). To test whether these disease-associated mutations can also affect how DnaJC7 modulates tau seeding, we introduced each mutation individually into the Ruby-DnaJC7 construct. We then transiently transfected them into the DnaJC7 KO tau biosensor cell line and quantified changes in tau seeding capacity (Figure 5 – Supplement 1A).

Rescue of DnaJC7 KO with most of the mutants suppressed seeding similar to WT. Five mutants increased tau seeding vs. WT (Figure 5C). The five inhibitory mutations were in all four domains of DnaJC7, with two located in the J domain (R412W and R425K). Neither J domain mutant resembled the seeding phenotype of DnaJC7 (HPQ).
However, the T341P mutant recapitulated the effect of the DnaJC7 (HPQ) mutant, suggesting a toxic gain of function with effects similar to blocking DnaJC7 stimulation of Hsp70 ATPase activity. We ruled out mutant effects on DnaJC7 expression via Western Blot (Figure 5 – Supplement 1C). The expression of most of the constructs resembled WT, while those that increased tau seeding were slightly reduced in expression, consistent with a gain-of-function effect (Figure 5 – Supplement 1F). Thus, DnaJC7 may function within a more complex network of chaperones to control tau aggregation.

To further test the perturbations to the DnaJC7 structure afforded by the ALS-associated mutations, we used Rosetta to calculate the predicted change in the structural stability of the monomer in response to each mutation29. We found no significant correlation between the predicted energy perturbation of the mutations and the resulting seeding phenotype or mutant construct expression (Figure 5 – Supplement 1E,G).

**Discussion**

This study began with an unbiased proteomic screen to identify factors that co-purify with insoluble tau from cells stably propagating a tau strain (DS10). We confirmed the top hits and identified DnaJC7 as a unique regulator of tau aggregation and clearance. The chaperome network is thought to be functionally redundant30–33. However, when we tested the specificity of all members of the JPD family using a candidate CRISPR/Cas9 genetic knockdown approach, we identified DnaJC7 and to a lesser degree DnaJB6 as unique regulators of tau aggregation. Finally, we tested newly identified mutations in DnaJC7 which cause ALS and found that those in the J domain had dominant negative effects on tau aggregation. This is consistent with the idea that Hsp70-based coordination of DnaJC7 is central to its activity and is linked to protein aggregation in ALS (which usually doesn’t feature tauopathy) and to regulation of tau.

**Disease-associated mutations of DnaJC7 differentially affect tau seeding**

Pathogenic mutations in several JDPs have previously been linked to multiple heritable neurodegenerative diseases as well as diseases targeting other organ systems34. These mutations, ranging from missense to inhibitory, to gain-of-function mutants, reduce the ability of the JDPs to bind clients or transfer them to Hsp70, and produce different disease pathologies. Recently, a series of protein truncating variants and missense mutations to the DnaJC7 gene have been identified as causal for ALS25. The missense mutations were found across all domains of DnaJC7, with most localized to the helix-turn-helix loops on the TPR motifs.

DnaJC7 rescue experiments to test 17 known ALS-associated missense mutations revealed that the T341P mutant recapitulated the seeding profile of the DnaJC7 (HPQ) mutant incapable of stimulating Hsp70 ATPase activity. The T341P mutation is in the TPR2B domain, which we have previously found to be the main site of tau binding on DnaJC716. Additionally, rescue with the two J domain mutants (R412W and R425K) increased seeding relative to the WT DnaJC7 sequence, but both mutants still seeded lower than the T341P and HPQ mutants. Further, the S94T mutant, which may impede Hsp90 binding, also moderately increased tau seeding relative to WT. ALS-associated
mutations may inhibit DnaJC7 interaction with other chaperones (e.g. Hsp70 and Hsp90) and thus impair substrate (tau) handoff into the chaperone-mediated protein refolding cycle. This agrees with previous observations that DnaJC7 cooperates with other chaperones to mitigate FUS and TDP-43 toxicity in yeast and in vitro\textsuperscript{35,27}.

Additionally, Rosetta modeling found no significant correlation between the predicted energy perturbation of ALS-associated mutations and their resulting seeding phenotype (Figure 5 – Supplement 1E). Although the TPR2B mutations Y338N and Y344C were predicted to have the highest destabilizing effects on DnaJC7, they resembled WT in regulating seeding. In contrast, the two J domain mutants were predicted to stabilize the DnaJC7 structure but increased seeding. Instead of destabilizing DnaJC7, these mutations exhibited gain-of-function effects likely through dysregulation of interactions within the chaperone network. Disease-associated mutations occur in both DnaJC7 and DnaJB6\textsuperscript{25,36,37}. This suggests that DnaJB6, DnaJC7, and other JDPs are not functionally redundant.

**DnaJC7 cooperates with Hsp70 to regulate intracellular tau aggregation**

Chaperones function in networks to regulate the folding of myriad substrates. The Hsp70 chaperones need JDPs to provide client specificity and activate their ATPase activity. Additionally, co-chaperones such as the TPR-containing the Hsc70-Hsp90 organizing protein Hop bridge the activity of Hsp70, which functions on more nascent polypeptide clients, with Hsp90, which folds clients that are closer to their native conformation\textsuperscript{38}. Like Hop, DnaJC7 bridges Hsp70 and Hsp90. However, DnaJC7 also enables retrograde transfer of substrates from Hsp90 to Hsp70, allowing additional iterations of the Hsp70-Hsp90 cycle\textsuperscript{39}. DnaJC7 thus uniquely facilitates Hsp70/Hsp90-mediated folding and refolding cycles and could be involved in more complex chaperone networks than the canonical JDPs DnaJB1 or DnaJA2.

DnaJC7 complementation studies in the DnaJC7 KO cell lines indicated that expression of WT DnaJC7 in KO cells rescued the tau aggregation phenotype. In contrast, expression of the DnaJC7 (HPQ) mutant, which abolishes DnaJC7 stimulation of Hsp70 ATPase activity\textsuperscript{24}, increased seeding relative to all controls, indicating an Hsp70-dependent mechanism for DnaJC7 regulation of tau. By preventing substrate handoff to Hsp70, the DnaJC7 (HPQ) mutant proteins may become saturated with tau and unable to shuttle tau between Hsp70 and Hsp90, thus disrupting DnaJC7’s role in Hsp70-Hsp90 chaperoning activities and resulting in the apparent dominant negative effects we observed.

**DnaJB6 and DnaJC7 regulation of tau aggregation**

JDPs have previously been implicated in various neurodegenerative diseases, with prior work suggesting that DnaJA2, DnaJB1, and DnaJB4 suppress tau aggregation in vitro\textsuperscript{31,40}. The literature has also portrayed some JDPs as functionally redundant, as targeted genetic screens knocking out chaperones fail to find many hits in the JDP family that can affect the aggregation of different amyloidogenic proteins\textsuperscript{30,41}. We did not identify DnaJA2, DnaJB1, or DnaJB4 as tau aggregation modifiers. This may be because of the model cell line we used.
We also observed a striking difference in the effects of DnaJB6 and DnaJC7 KO in naked vs. Lipofectamine-mediated seeding of DS9 and DS10 cell lysates. Lipofectamine-mediated seeding allows us to bypass the cell's endogenous uptake pathways to transflect seeds into the cell directly. The differential effects of DnaJB6 vs. DnaJC7 KO in the seeding of the DS9 and DS10 cell lysates also hint at different mechanisms for these JDPs' modulation of tau seeding. DnaJB6 KO decreased DS9 and DS10 naked and Lipofectamine-mediated seeding, suggesting that the presence of DnaJB6 in cells may enhance tau seeding. In contrast, DnaJC7 KO decreased DS9 naked seeding and increased DS9 Lipofectamine-mediated seeding. This difference may be attributed to DnaJC7's decreased affinity for tauRD vs. full-length tau, as we have previously observed that DnaJC7 preferentially binds full-length vs. RD tau protein\textsuperscript{16}. The different effects of the DnaJB6 vs. DnaJC7 KO in naked vs. Lipofectamine seeding, therefore, hint at potential novel roles of these JDPs in the uptake and processing of certain tau seeds.

DnaJB6 oligomers have been reported to potently suppress polyQ, alpha-synuclein, amyloid beta, and TDP-43 aggregation\textsuperscript{36,42–45}. Yet DnaJB6 was not previously known to regulate tau aggregation. It remains unknown how DnaJB6 oligomerization impacts its activity, but experiments on DnaJB6 suppression of polyQ indicated that a serine/threonine-rich region may play a role in the recognition of substrates\textsuperscript{46}. DnaJB6 was not highly abundant in our proteomics screen for tau aggregate interactors, but its KO in tau biosensors increased tau aggregation second only to the DnaJC7 KO. DnaJB6 and its homolog, DnaJB8, have been observed to substoichiometrically inhibit substrate aggregation, suggesting a mechanism based on iterative binding/refolding to aggregation-prone seeds that prevents substrate aggregation\textsuperscript{43}.

We have previously reported that DnaJC7 preferentially bound natively folded tau monomer vs. seed-competent monomer or aggregation-prone mutants\textsuperscript{16}. In this study, DnaJC7 KO alone increased tau seeding for all seed sources tested, suggesting an interaction with endogenous tau in a mechanism independent of seed conformation. We hypothesize that DnaJC7 suppresses tau aggregation by binding to inert tau and preventing its templating by exogenous tau seeds. This aligns with our previous finding that DnaJC7 preferentially binds to WT tau, which exists in a more closed conformation, than to the P301L mutant tau, which exists in a more open conformation and more closely resembles tau seeds. In conclusion, we identified DnaJC7 as a specific regulator of tau aggregation. It binds tau via its TPR2B domain and engages Hsp70 to stabilize the inert monomer.
### Key Resources Table

| Reagent type (species) or resource | Designation | Source or reference | Identiﬁers | Additional information |
|-----------------------------------|-------------|---------------------|------------|------------------------|
| Cell line (human) | HEK293 tauRD P301S v2L FRET Biosensor | Produced by Diamond Lab | | The parent HEK293 cell line was obtained from ATCC and tested negative for mycoplasma. |
| Cell line (human) | HEK293 DS1 tauRD(P301L-V337M)-YFP | Produced by Diamond Lab | | Stably propagates diffuse tauRD monomers. |
| Cell line (human) | HEK293 DS10 tauRD(P301L-V337M)-YFP | Produced by Diamond Lab | | Stably propagates a unique tau strain. |
| Cell line (human) | HEK293 OFF1::DS10 tauRD(P301L-V337M)-YFP | Produced by Diamond Lab | | Stably propagates a unique tau strain under a tetracycline-repressible promoter. |
| biological sample (human) | Alzheimer’s Disease subject brain | UT Southwestern Alzheimer’s Disease Center | | |
| biological sample (human) | Progressive Supranuclear Palsy subject brain | UT Southwestern Alzheimer’s Disease Center | | |
| biological sample (human) | Corticobasal Degeneration subject brain | UT Southwestern Alzheimer’s Disease Center | | |
| antibody | rabbit polyclonal anti-DnaJC7 | Proteintech | 11090-1-AP | 1:2000 dilution |
| antibody          | rabbit polyclonal anti-DnaJB6 | Proteintech | 11707-1-AP | 1:2000 dilution |
|-------------------|--------------------------------|-------------|------------|----------------|
| antibody          | rabbit polyclonal anti-Beta-Tubulin | Proteintech | 10094-1-AP | 1:5000 dilution |
| antibody          | donkey-anti-rabbit HRP-linked F(ab')2 | Cytiva | NA9340-1ML | 1:5000 dilution (DnaJC7, Beta-Tubulin), 1:4000 dilution (DnaJB6) |
| antibody          | mouse monoclonal anti-GAPDH | Proteintech | 60004-1-Ig | 1:10000 |
| antibody          | mouse monoclonal anti-Beta-Actin | Proteintech | 66009-1-Ig | 1:5000 dilution |
| antibody          | goat-anti-mouse H&L (HRP) | Abcam | ab6789 | 1:10000 dilution (GAPDH), 1:5000 dilution (Beta-Actin) |
| peptide, recombinant protein | Human tau 2N4R (Full Length WT-tau) fibrils | Produced by Diamond Lab | | MAEPRQEFEV MEDHAGTYGL GDRKDAQGY TMHQDQEGD TDAGLKESPL QTPTEDGSEE PGSETSDAKS TPTAEDVTAP LVDEGAPGKQ AAAQPHTEIPE GTTAEAGIG DTPSLEDEAA GHVTQARMV SKSKDGTGSD DKKAKGADGK TKIATPRGAAP PGQKGQANA TRIPAKTPPAP KTPPSSGEPP KSGDRSGYSS PGSPGTPGSR SRTPSLPTTP TREPKKVAVV RTPPKSPSSA KSRL |
| recombinant DNA reagent | gRNA-resistant WT DnaJC7 cDNA sequence | gBlock from IDT, cloned by VAP | The sequence can be found in Materials and Methods: Design of a DnaJC7 construct resistant to targeting by used gRNA sequences |

**Identification of aggresome-associated proteins by mass spectrometry**

DS1 and DS10 cells were grown to confluency in two T300s per condition. Cells were harvested, pelleted, and washed prior to storage as 4x0.5 T300 pellets at -80°C. For each condition, three pellets were thawed on ice, and each was lysed by trituration in 1 mL ice-cold PBS with 0.25% Triton-X and containing cOmplete™ mini EDTA-free protease inhibitor cocktail tablet (Roche) at a concentration of 10% w/vol followed by a 15-minute incubation on ice. Aggresomes and nuclei were collected by centrifuging at 1000xg for 15 minutes followed by resuspension in 400 μL lysis buffer. An Omni-Ruptor 250 probe sonicator was then used at 30% power for thirty 3-second pulses to partially dissolve the pellets. Samples were centrifuged at 250xg for 5 minutes and the supernatant was set aside as Fraction B. Pellets were re-homogenized in an additional 400 μL lysis buffer and sonication and centrifugation was repeated. The final supernatant was added to the previous Fraction B (800 μL volume total). A Bradford assay (Bio-Rad) with BSA standard curve was performed and the protein concentrations were calculated for the nine fractions. Protein concentrations were normalized to 1.1 μg/μL. 72 μL of 10% sarkosyl was added to 650 μL of each sample in ultra-centrifuge tubes (Beckman Coulter) and samples were rotated end-over-end at room temperature for one hour. Samples were then spun at 186,000xg for 60 minutes, supernatant was set aside, and pellets were washed with 1 mL lysis buffer prior to an additional 30 minute 186,000xg spin. Final pellets were resuspended in 30 μL PBS containing 2% SDS and 2% BME by boiling and trituration. 5 μL of Fraction B supernatants and pellets were loaded onto NuPAGE 10% Bis-Tris gels (Life Technologies) and were run at 150 V for 60 minutes. Gels were washed 1x with water and were then stained with SimplyBlue SafeStain (Life Technologies). Images of gels were captured using a digital Syngene imager.

For LC-MS/MS-based detection of proteins, 20 μL re-suspended Fraction B pellets were run 1 cm onto an Any kD Mini-Protean TGX gel (Bio-Rad) followed by Coomassie Blue staining. Whole lanes were excised using ethanol-washed razor blades and gel samples were cut into 1 mm chunks. Gel pieces were reduced with DTT and alkylated with iodoacetamides (Sigma-Aldrich) and were then digested overnight with trypsin (Promega). Next, excised proteins were subjected to solid-phase extraction cleanup with Oasis HLB plates (Waters). The processed samples were then analyzed by LC-MS/MS using a Q Exactive mass spectrometer (Thermo Electron) coupled to an
Ultimate 3000 RSLC-Nano liquid chromatography system (Dionex). Samples were injected onto a 180 μm i.d., 15-cm long column packed with reverse-phase material ReproSil-Pur C18-AQ, 1.9 μm resin (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany). Peptides were eluted with a gradient from 1-28% buffer B (80% (v/v) ACN, 10% (v/v) trifluoroethanol, and 0.08% formic acid in water) over 60 minutes. The mass spectrometer could acquire up to 20 MS/MS spectra for each full spectrum obtained. Raw mass spectrometry data files were converted to a peak list format and analyzed using the central proteomics facilities pipeline (CPFP), version 2.0.3\textsuperscript{47,48}. Peptide identification was performed using the X!Tandem and open MS search algorithm (OMSSA) search engines against the human protein database from Uniprot, with common contaminants and reversed decoy sequences appended\textsuperscript{49,50}. Fragment and precursor tolerances of 20 ppm and 0.1 Da were specified, and three miscleavages were allowed. Carbamidomethylation of Cys was set as a fixed modification and oxidation of Met was set as a variable modification.

Label-free quantitation of proteins across samples was performed using SIRQ normalized spectral index software\textsuperscript{51}. Finally, spectral counts were added across triplicates. Proteins with a spectral count greater than 5 in DS10, but not identified in DS1 were reported. To calculate enrichment of proteins in the DS10 samples vs DS1 samples, the spectral counts were first negative log\textsubscript{10} transformed. False Discovery Rate (FDR) analysis was performed using the multiple unpaired t-tests analysis in Prism (GraphPad). The original FDR method of Benjamini and Hochberg was applied, with the desired FDR set to 1.5%. Differences are reported as DS10 spectral index – DS1 spectral index. The -log\textsubscript{10}(q-value) is reported. Gene ontology (GO) analysis was conducted via the Metascape gene annotation and analysis resource\textsuperscript{52}.

** Tau Aggregate Degradation Time Courses**

OFF1::DS10 cells were treated with two rounds of indicated pooled gRNA lentivirus and were maintained in 24-well plates to assess viability. Two weeks later, a time course examining the decay of tau aggregate seeding activity in the various non-lethal knockouts was performed as follows: Confluent 24-wells were resuspended in 1 mL media and 3.5 μL cells were re-plated into 200 μL total volume in 96-well plates. Tau RD-YFP expression was turned off using 30 ng/mL doxycycline for 1 day, 2 days, 3 days, 4 days, or 5 days. After five days, cells reached confluency and were passaged onto coverslips. 48 hours later, at which point tauRD-YFP expression reached its maximum, cells were fixed and cells containing or lacking inclusions were manually counted. Six replicates of 150+ cells were counted per condition, and averages were calculated. One-way analysis of variance with Bonferroni’s correction for multiple comparisons was used to assess statistical significance relative to Non-Target controls.

** CRISPR/Cas9 knockout of JDPs in tau biosensor cells**

Four human gRNA sequences per gene were selected from the Brunello library\textsuperscript{22}. A single non-targeting human gRNA sequence was used as a negative control. For all gRNA sequences not beginning with guanine, a single guanine nucleotide was added at the 5'-end of the sequence to enhance U6 promoter activity. DNA oligonucleotides were synthesized by IDT DNA and cloned into the lentiCRISPRv2 vector\textsuperscript{53} for lentivirus
production. The plasmids for the four gRNAs for each gene were pooled together to a final concentration of 40 ng/μL and used to generate lentivirus.

Lentivirus was produced as described previously\textsuperscript{54}. Briefly, HEK293T cells were plated at a concentration of 100,000 cells/well in a 24-well plate. 24 hours later, cells were transiently co-transfected with PSP helper plasmid (300 ng), VSV-G (100 ng), and gRNA plasmids (100 ng) using 1.875 μL of TransIT-293 (Mirus) transfection reagent in OptiMEM to a final volume of ~30 μL. 48 hours later, the conditioned medium was harvested and centrifuged at 1000 rpm for five minutes to remove dead cells and debris. For transduction, 30 μL of the virus suspension was added to HEK293T tau biosensor cells at a cell confluency of 60% in a 96-well plate. 48 hours post-transduction, infected cells were treated with 1 μg/ml puromycin (Life Technologies, Inc.) and maintained under puromycin selection for at least 10 days after the first lentiviral transduction to ensure knockout of the individual JDPs before conducting experiments.

**Recombinant tau seeding assay in JDP knockout tau biosensor cells**

The individual JDP knockout cell lines were tested in batches of ten knockout cell lines, including the DnaJC7 and nontargeting control cell lines for each batch. Each batch was assayed in biological duplicate. For all experiments, cells were plated in 96-well plates at 20,000 cells per well in 100 μL of media. 24 hours later, the cells were treated with 50 μL of a heparin-induced recombinant tau fibril dilution series in technical triplicates. Prior to cell treatment, the recombinant tau fibrils were sonicated for 30 seconds at an amplitude of 65 on a Q700 Sonicator (QSonica). A three-fold dilution series of the sonicated fibril concentrations ranging from 100 nM to ~15.2 pM and a media negative control was added to the cell media. 48 hours after treatment with tau, the cells were harvested by 0.05% trypsin digestion and then fixed in PBS with 2% PFA for ten minutes. The cells were then washed and resuspended in 150 μL of PBS.

**Flow cytometry of tau biosensor cells**

A BD LSRFortessa was used to perform FRET flow cytometry. To measure mCerulean3 and FRET signal, cells were excited with the 405 nm laser and fluorescence was captured with a 405/50 nm and 525/50 nm filter, respectively. To measure mClover3 signal, cells were excited with a 488 laser and fluorescence was captured with a 525/50 nm filter. To quantify FRET, we used a gating strategy where mCerulean3 bleed-through into the mClover3 and FRET channels was compensated using FlowJo analysis software, as described previously\textsuperscript{55}. FRET signal is defined as the percentage of FRET-positive cells in all analyses. For each experiment, 10,000 mClover3/mCerulean3 double-positive cells per replicate were analyzed and each condition was analyzed in triplicate. Data analysis was performed using FlowJo v10 software (Treestar). One-way analysis of variance with Dunnett’s correction for multiple comparisons was used to assess statistical significance relative to Non-Target controls in GraphPad Prism.

**Preparation of brain and DS cell lysates**

0.5 g frontal lobe sections from AD, PSP, and CBD patients were gently homogenized at 4°C in 5 mL of TBS buffer containing cOmplete™ mini EDTA-free protease inhibitor cocktail tablet (Roche) at a concentration of 20% w/vol using a Dounce homogenizer.
Samples were centrifuged at 21,000xg for 15 min at 4°C to remove cellular debris. Supernatant was partitioned into aliquots, snap frozen in liquid nitrogen, and stored at -80°C.

DS1, DS9, and DS10 cell pellets were lysed by resuspension in cold 0.05% Triton in PBS containing cOmplete™ mini EDTA-free protease inhibitor tablet (Roche) at a concentration of 10% w/vol followed by incubation in the lysis buffer for 20 minutes on ice. Homogenates were then clarified by centrifugation at 4°C at a speed of 500 RCF for 5 minutes followed by 1000 RCF for 5 minutes. The supernatant was then isolated, and total cell lysate protein concentrations were measured using the Pierce™ BCA Protein Assay Kit (ThermoFisher).

Biosensor seeding with brain and DS cell lysates

Seeding with the brain and DS cell lysates was conducted on the DnaJC7, DnaJB6, and nontargeting knockout tau biosensor cell lines. For all lipofectamine seeding experiments, cells were plated in 96-well plates at 20,000 cells per well in 130 µL of media. 24 hours later, the cells were treated with 20 µL of lipofectamine complexes. The complexes are generated by preparing a lipofectamine in OptiMEM (Gibco) master mix consisting of 1 µL Lipofectamine 2000 (Gibco) and 9 µL OptiMEM per well. The seeding material is prepared in a separate master mix for each lysate tested and consists of 5 µL of brain lysate and 5 µL of OptiMEM per well or 5 µg total protein of DS cell lysate and OptiMEM to 10 µL. The lipofectamine and lysate master mixes are combined in a 1:1 ratio and incubated for 30 minutes at room temperature. The final master mix is then distributed to triplicate wells for the three knockout cell lines, with each well receiving 20 µL of treatment. A lipofectamine control and OptiMEM-only negative control were also generated.

For all naked seeding experiments, cells were plated in 96-well plates at 10,000 cells per well in 100 µL of media. 24 hours later, the cells were treated with 50 µL of seeding complexes. The seeding material is prepared in a separate master mix for each lysate tested and consists of 25 µL of brain lysate and 25 µL of complete media per well or 20 µg total protein of DS cell lysate and media to 50 µL per well. The final master mix is then distributed to triplicate wells for the three knockout cell lines, with each well receiving 50 µL of treatment. A media-only negative control was also generated.

48 hours after treatment, the cells were harvested by 0.05% trypsin digestion and then fixed in PBS with 2% paraformaldehyde for flow cytometry. One-way analysis of variance with Dunnett’s correction for multiple comparisons was used to assess statistical significance relative to Non-Target controls in GraphPad Prism.

Design of a DnaJC7 construct resistant to targeting by used gRNA sequences

N-terminal Ruby fusion constructs of DnaJC7 were designed to be resistant to targeting by the four gRNAs used to generate the DnaJC7 KO biosensor line. Alternative codon sequences were used at the four gRNA sites to generate a distinct cDNA sequence that maintained the same amino acid sequence. The cDNA sequence is copied below, with alternative codon sequences underlined:
Transient overexpression of DnaJC7 constructs in tau biosensor cells

DnaJC7 KO or Non-Targeting control tau biosensor cells were plated at 500K cells/well in a six-well dish. 24 hours later, cells were transiently transfected with 1 ug of plasmid containing the WT, HPQ, or ALS-associated Ruby-tagged sequences of DnaJC7 using 5 µL of Lipofectamine 2000 (Gibco) transfection reagent in OptiMEM (Gibco) to a final volume of 125 µL. The Ruby control cells were transfected with 500 ng of plasmid expressing only Ruby using 5 µL of Lipofectamine 2000 transfection reagent in OptiMEM to a final volume of 125 µL. The vehicle control cells were treated with 5 µL of Lipofectamine 2000 transfection reagent in 120 µL OptiMEM. 48 hours after transfection, the cells were plated in 96-well plates at 20,000 cells per well in 100 µL of media for a standard naked seeding assay. Wells were run on the flow cytometer to completion to ensure collection of a sufficient number of cells with Ruby signal. One-way analysis of variance with Dunnett’s correction for multiple comparisons was used to assess statistical significance relative to Ruby-alone controls for overexpression and rescue experiments and relative to the WT C7 construct for rescue with ALS-associated mutants in GraphPad Prism.

Immunoblotting of DnaJC7 and DnaJB6 from cell lysates

HEK293 tauRD biosensor cell pellets were lysed by resuspension in cold 0.01% Triton in PBS containing cOmplete™ mini EDTA-free protease inhibitor tablet (Roche) at a concentration of 10% w/vol followed by incubation in the lysis buffer for 15 minutes on ice. Homogenates were then clarified by centrifugation at 4°C at a speed of 17,200 RCF for 15 minutes. The supernatant was then isolated, and total cell lysate protein...
concentrations were measured using the Pierce™ BCA Protein Assay Kit (ThermoFisher).

10 µg of total cell lysate protein was prepared in 1X (final) LDS Bolt™ buffer (Invitrogen) supplemented with 10% β-mercaptoethanol and heated for 5 minutes at 98°C. The proteins were resolved by SDS-PAGE using Novex NuPAGE pre-cast gradient Bis-Tris acrylamide gels (4–12%) (Invitrogen). After gel electrophoresis, resolved proteins were transferred onto Immobilon-P PVDF membranes (Millipore Sigma) using a Bio-Rad Trans-blot® semi-dry transfer cell. The membranes were then blocked in 1X TBST buffer (10 mM Tris, 150 mM NaCl, pH 7.4, 0.05% Tween-20) containing 5% non-fat milk powder (Bio-Rad). Membranes were then probed with antibody in TBST containing 5% milk powder.

The following antibodies were used for immunoblotting: rabbit polyclonal anti-DnaJC7 (Proteintech, 11090-1-AP) at a 1:2000 dilution; rabbit polyclonal anti-DnaJB6 (Proteintech, 11707-1-AP) at a 1:2000 dilution; rabbit polyclonal anti-BetaTubulin (Proteintech, 10094-1-AP) at a 1:5000 dilution; a secondary donkey-anti-rabbit HRP-linked F(ab')2 (Cytiva, NA9340-1ML) at a 1:5000 dilution when blotting for DnaJC7, a 1:4000 dilution when blotting for DnaJB6, and a 1:5000 dilution when blotting for Tubulin; mouse monoclonal anti-Beta-Actin (Proteintech, 66009-1-lg) at a 1:5000 dilution; mouse monoclonal anti-GAPDH (Proteintech, 60004-1-lg) at a 1:10000 dilution; and a secondary goat-anti-mouse H&L (HRP) (Abcam, ab6789) at a 1:5000 dilution when blotting for Beta-Actin and 1:10000 when blotting for Beta-Tubulin.

Normalized band intensities for the ALS-associated mutant constructs were calculated using Fiji image analysis software. Background signal was subtracted from each DnaJC7 and Beta-Actin control band. The DnaJC7 signal for each construct was then normalized to its corresponding Beta-Actin control band. Finally, the signals for all constructs were normalized to the signal of the WT DnaJC7 construct.

Calculation of ddG for ALS Mutants

Our structural model of DnaJC7 was built in ROSETTA using homology modeling using DnaJC3 (PDB ID: 3IEG) as a template and minimized using the relax protocol. A low scoring model was used to evaluate the energetics of ALS-associated mutations in DnaJC7. The 17 ALS-associated mutations were then individually introduced into the initial DnaJC7 model. For each mutation, 35 replicate simulations were run in parallel for WT and mutant DnaJC7 until convergence. To estimate the ddG of monomer, the mean free energy difference between the WT and mutant DnaJC7 structures was calculated. Simulations were performed on the BioHPC computing cluster at UT Southwestern Medical Center. The relax protocol and Flex ddG used Rosetta v3.13 and v3.12, respectively.

Acknowledgements

Work in the LAJ lab was supported by an NIH-NIA grant RF1AG078888. Work in the MID lab was supported by the following grants: NIH-NIA 3R01AG048678, NIH-NIA 1RF1AG059689, and NIH-NIA 1RF1AG065407. The CLW lab was supported by the
McCune Foundation and the Winspear Family Center for Research on the Neuropathology of Alzheimer's Disease. LAJ, CLW, and MID were supported by the Chan Zuckerberg Initiative 2018-191983 and Chan Zuckerberg Initiative 2021-237348. We would like to thank the UT Southwestern Alzheimer's Disease Center for providing pathological brain tissue samples. We also thank the Proteomics Core Facility and Moody Foundation Flow Cytometry Facility at the University of Texas Southwestern Medical Center.

Competing Interests
The authors declare that they have no competing interests.

Data Availability
Raw data for proteomic experiments is unavailable due to a data storage issue. Processed proteomic data and tau aggregate degradation time course data are available on Dryad at doi:10.5061/dryad.fj6q57402. Raw FCS files are deposited on the Cytobank digital repository at https://community.cytobank.org/cytobank/projects/1505.

References
1. Alicea, J. V., Diamond, M. I. & Joachimiak, L. A. Tau strains shape disease. *Acta Neuropathol. (Berl.)* **142**, 57–71 (2021).
2. Frost, B., Jacks, R. L. & Diamond, M. I. Propagation of Tau Misfolding from the Outside to the Inside of a Cell. *J. Biol. Chem.* **284**, 12845–12852 (2009).
3. Kaufman, S. K. *et al.* Tau Prion Strains Dictate Patterns of Cell Pathology, Progression Rate, and Regional Vulnerability In Vivo. *Neuron* **92**, 796–812 (2016).
4. Sanders, D. W., Kaufman, S. K., Holmes, B. B. & Diamond, M. I. Prions and Protein Assemblies that Convey Biological Information in Health and Disease. *Neuron* **89**, 433–448 (2016).
5. Sanders, D. W. *et al.* Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron* **82**, 1271–1288 (2014).
6. Fitzpatrick, A. W. P. *et al.* Cryo-EM structures of tau filaments from Alzheimer’s disease. *Nature* **547**, 185–190 (2017).
7. Shi, Y. *et al.* Structure-based classification of tauopathies. *Nature* **598**, 359–363 (2021).
8. Zhang, W. et al. Novel tau filament fold in corticobasal degeneration, a four-repeat tauopathy. http://biorxiv.org/lookup/doi/10.1101/811703 (2019) doi:10.1101/811703.

9. Hartl, F. U., Bracher, A. & Hayer-Hartl, M. Molecular chaperones in protein folding and proteostasis. *Nature* **475**, 324–332 (2011).

10. Lackie, R. E. et al. The Hsp70/Hsp90 chaperone machinery in neurodegenerative diseases. *Front. Neurosci.* **11**, 1–23 (2017).

11. Mayer, M. P. & Bukau, B. Hsp70 chaperones: Cellular functions and molecular mechanism. *Cell. Mol. Life Sci.* **62**, 670 (2005).

12. Kampinga, H. H. & Craig, E. A. The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat. Rev. Mol. Cell Biol.* **11**, 579–592 (2010).

13. Craig, E. A. & Marszalek, J. How Do J-Proteins Get Hsp70 to Do So Many Different Things? *Trends Biochem. Sci.* **42**, 355–368 (2017).

14. Ryder, B. D., Wydorski, P. M., Hou, Z. & Joachimiak, L. A. Trends in Biochemical Sciences Chaperoning shape-shifting tau in disease. *Trends Biochem. Sci.* **xx**, 1–13 (2021).

15. Mariscal Ayala, S. M. & Kirstein, J. J-domain proteins interaction with neurodegenerative disease-related proteins. *Exp. Cell Res.* **399**, 112491 (2021).

16. Hou, Z. et al. DnaJC7 binds natively folded structural elements in tau to inhibit amyloid formation. *Nat. Commun.* **12**, 5338 (2021).

17. Nachman, E. et al. Disassembly of Tau fibrils by the human Hsp70 disaggregation machinery generates small seeding-competent species. *J. Biol. Chem.* **295**, 9676–9690 (2020).

18. Saha, I. et al. The AAA+ chaperone VCP disaggregates Tau fibrils and generates aggregate seeds in a cellular system. *Nat. Commun.* **14**, 560 (2023).
19. Holmes, B. B. et al. Proteopathic tau seeding predicts tauopathy in vivo. *Proc. Natl. Acad. Sci.* **111**, E4376–E4385 (2014).

20. Hitt, B. D. et al. Ultrasensitive tau biosensor cells detect no seeding in Alzheimer’s disease CSF. *Acta Neuropathol. Commun.* **9**, 99 (2021).

21. Kolay, S. et al. The dual fates of exogenous tau seeds: Lysosomal clearance versus cytoplasmic amplification. *J. Biol. Chem.* **298**, 102014 (2022).

22. Doench, J. G. et al. Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nat. Biotechnol.* **34**, 184–191 (2016).

23. Kampinga, H. H. et al. Function, evolution, and structure of J-domain proteins. *Cell Stress Chaperones* **24**, 7–15 (2019).

24. Tsai, J. & Douglas, M. G. A Conserved HPD Sequence of the J-domain Is Necessary for YDJ1 Stimulation of Hsp70 ATPase Activity at a Site Distinct from Substrate Binding. *J. Biol. Chem.* **271**, 9347–9354 (1996).

25. Dilliott, A. A. et al. DnaJC7 in Amyotrophic Lateral Sclerosis. *Int. J. Mol. Sci.* **23**, 4076 (2022).

26. Assimon, V. A., Southworth, D. R. & Gestwicki, J. E. Specific Binding of Tetratricopeptide Repeat Proteins to Heat Shock Protein 70 (Hsp70) and Heat Shock Protein 90 (Hsp90) Is Regulated by Affinity and Phosphorylation. *Biochemistry* **54**, 7120–7131 (2015).

27. Carrasco, J. et al. Metamorphism in TDP-43 prion-like domain determines chaperone recognition. *Nat. Commun.* **14**, 466 (2023).

28. Moszczynski, A. J., Hintermayer, M. A. & Strong, M. J. Phosphorylation of Threonine 175 Tau in the Induction of Tau Pathology in Amyotrophic Lateral Sclerosis—Frontotemporal Spectrum Disorder (ALS-FTSD). A Review. *Front. Neurosci.* **12**, (2018).
29. Barlow, K. A. et al. Flex ddG: Rosetta Ensemble-Based Estimation of Changes in Protein–Protein Binding Affinity upon Mutation. *J. Phys. Chem. B* **122**, 5389–5399 (2018).

30. Mok, S.-A. et al. Mapping interactions with the chaperone network reveals factors that protect against tau aggregation. *Nat. Struct. Mol. Biol.* **25**, 384–393 (2018).

31. Nachman, E. et al. Disassembly of Tau fibrils by the human Hsp70 disaggregation machinery generates small seeding-competent species. *J. Biol. Chem.* **295**, 9676–9690 (2020).

32. Yan, P., Wang, T., Guzman, M. L., Peter, R. I. & Chiosis, G. Chaperome Networks – Redundancy and Implications for Cancer Treatment. in *HSF1 and Molecular Chaperones in Biology and Cancer* (eds. Mendillo, M. L., Pincus, D. & Scherz-Shouval, R.) 87–99 (Springer International Publishing, 2020). doi:10.1007/978-3-030-40204-4_6.

33. Gong, Y. et al. An atlas of chaperone–protein interactions in Saccharomyces cerevisiae: implications to protein folding pathways in the cell. *Mol. Syst. Biol.* **5**, 275 (2009).

34. Zarouchlioti, C., Parfitt, D. A., Li, W., Gittings, L. M. & Cheetham, M. E. DNAJ Proteins in neurodegeneration: essential and protective factors. *Philos. Trans. R. Soc. B Biol. Sci.* **373**, 20160534 (2018).

35. Stoltz, M. DNAJC7, a Molecular Chaperone Protein that Modulates Protein Misfolding in Amyotrophic Lateral Sclerosis (ALS). *Electron. Thesis Diss. Repos.* **7431**, (2020).

36. Ruggieri, A. et al. Complete loss of the DNAJB6 G/F domain and novel missense mutations cause distal-onset DNAJB6 myopathy. *Acta Neuropathol. Commun.* **3**, 44 (2015).

37. Sarparanta, J., Jonson, P. H., Kawan, S. & Udd, B. Neuromuscular Diseases Due to Chaperone Mutations: A Review and Some New Results. *Int. J. Mol. Sci.* **21**, 1409 (2020).
38. Daniel, S., Söti, C., Csermely, P., Bradley, G. & Blatch, G. L. Hop: An Hsp70/Hsp90 Co-Chaperone That Functions Within and Beyond Hsp70/Hsp90 Protein Folding Pathways. in Networking of Chaperones by Co-Chaperones (ed. Blatch, G. L.) 26–37 (Springer, 2007). doi:10.1007/978-0-387-49310-7_3.

39. Brychzy, A. et al. Cofactor Tpr2 combines two TPR domains and a J domain to regulate the Hsp70/Hsp90 chaperone system. EMBO J. 22, 3613–3623 (2003).

40. Irwin, R. et al. Hsp40s play complementary roles in the prevention of tau amyloid formation. eLife 10, e69601 (2021).

41. Kakkar, V., Kuiper, E. F. E., Pandey, A., Braakman, I. & Kampinga, H. H. Versatile members of the DNAJ family show Hsp70 dependent anti-aggregation activity on RING1 mutant parkin C289G. Sci. Rep. 6, 34830 (2016).

42. Aprile, F. A. et al. The molecular chaperones DNAJB6 and Hsp70 cooperate to suppress α-synuclein aggregation. Sci. Rep. 7, 9039 (2017).

43. Gillis, J. et al. The DNAJB6 and DNAJB8 protein chaperones prevent intracellular aggregation of polyglutamine peptides. J. Biol. Chem. 288, 17225–17237 (2013).

44. Hageman, J. et al. A DNAJB Chaperone Subfamily with HDAC-Dependent Activities Suppresses Toxic Protein Aggregation. Mol. Cell 37, 355–369 (2010).

45. Månsson, C. et al. Interaction of the molecular chaperone DNAJB6 with growing amyloid-beta 42 (Aβ42) aggregates leads to sub-stoichiometric inhibition of amyloid formation. J. Biol. Chem. 289, 31066–31076 (2014).
46. Månsson, C. et al. Conserved S/T Residues of the Human Chaperone DNAJB6 Are Required for Effective Inhibition of Aβ42 Amyloid Fibril Formation. *Biochemistry* **57**, 4891–4902 (2018).

47. Trudgian, D. C. & Mirzaei, H. Cloud CPFP: A Shotgun Proteomics Data Analysis Pipeline Using Cloud and High Performance Computing. *J. Proteome Res.* **11**, 6282–6290 (2012).

48. Trudgian, D. C. et al. CPFP: a central proteomics facilities pipeline. *Bioinformatics* **26**, 1131–1132 (2010).

49. Geer, L. Y. et al. Open Mass Spectrometry Search Algorithm. *J. Proteome Res.* **3**, 958–964 (2004).

50. Elias, J. E. & Gygi, S. P. Target-decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry. *Nat. Methods* **4**, 207–214 (2007).

51. Trudgian, D. C. et al. Comparative evaluation of label-free SINQ normalized spectral index quantitation in the central proteomics facilities pipeline. *PROTEOMICS* **11**, 2790–2797 (2011).

52. Zhou, Y. et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat. Commun.* **10**, 1523 (2019).

53. Sanjana, N. E., Shalem, O. & Zhang, F. Improved vectors and genome-wide libraries for CRISPR screening. *Nat. Methods* **11**, 783–784 (2014).

54. Stopschinski, B. E. et al. Specific glycosaminoglycan chain length and sulfation patterns are required for cell uptake of tau versus α-synuclein and β-amyloid aggregates. *J. Biol. Chem.* **293**, 10826–10840 (2018).
55. Furman, J. L., Holmes, B. B. & Diamond, M. I. Sensitive Detection of Proteopathic Seeding Activity with FRET Flow Cytometry. J. Vis. Exp. 53205 (2015) doi:10.3791/53205.

56. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682 (2012).

57. Song, Y. et al. High-Resolution Comparative Modeling with RosettaCM. Structure 21, 1735–1742 (2013).

58. Simons, K. T., Bonneau, R., Ruczinski, I. & Baker, D. Ab initio protein structure prediction of CASP III targets using ROSETTA. Proteins Struct. Funct. Bioinforma. 37, 171–176 (1999).

59. Wydorski, P. M. et al. Dual domain recognition determines SARS-CoV-2 PLpro selectivity for human ISG15 and K48-linked di-ubiquitin. 2021.09.15.460543 Preprint at https://doi.org/10.1101/2021.09.15.460543 (2023).