

Proinflammatory cytokines promote tau aggregation by inducing cleavage in human Alzheimer's disease

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Proinflammatory cytokines promote tau aggregation by inducing cleavage in human Alzheimer's disease.

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SUMMARY

Neuroinflammation^{1,2} and tau aggregation^{3,4} are hallmarks of Alzheimer's disease (AD)⁵, yet the mechanistic link between them remains unknown. Analyzing a cohort of 46 AD and 40 control subjects using mass spectrometry-based proteomics, we identified a strong correlation between upregulation of the cytokine-induced antigen-presentation pathway and site-specific tau cleavage. This cleavage enriches the microtubule-binding repeat (MTBR) domain in AD tau aggregates; furthermore, synthetic MTBR fragments recapitulate tau aggregation both *in vitro* and *in vivo*. Our data show that the immunoproteasome (IP) colocalizes with tau aggregates in human AD neurons and drives the observed cleavage pattern. Proinflammatory cytokines reproduced AD-like tau cleavage and puncta formation in HEK293T cells expressing 0N4R tau, the hTau knock-in mouse, and human iPSC-derived neurons (iNeurons). IP knockdown via siRNA reduced tau-positive inclusion and upstream JAK/STAT pathway inhibition across model systems, including iNeurons, effectively abrogated cleavage and tau aggregation. These findings identify an inflammation-responsive pathway that promotes tau pathology and provide a mechanistic framework and potential therapeutic entry point for AD and potentially other tauopathies.

INTRODUCTION

Over 75% of neurodegenerative disorders display neuropathological aggregates of the microtubule-associated protein tau (MAPT)^{3,4}, including the most common form of dementia, Alzheimer's disease (AD)⁵. Despite significant progress in understanding the biochemical signatures⁶⁻⁹ and structural properties¹⁰⁻¹⁶, the biological drivers that initiate tau aggregation in AD remain unknown, limiting the development of potential diagnostics and clinical therapeutics to regulate tau aggregation.

Tau protein fragments encompassing the microtubule-binding repeat (MTBR) domain are prone to aggregate^{17,18,19}, and tau containing the MTBR has been identified in extracellular vesicles capable of cell-to-cell transmission and seeding of further pathology²⁰. Cleavage of tau at the MTBR²¹, N-terminus²², and C-terminus^{21,23,24} has been documented in AD patients, yet a systematic, quantitative atlas of tau cleavage sites across a sizeable human cohort has not been achieved, and the mechanism initiating this cleavage in AD pathology remains unknown.

This manuscript describes a systematic mass spectrometry (MS)-based proteomics study of brain tissue from 46 patients with AD and 40 asymptomatic control subjects (CTR), providing a detailed map of tau cleavage sites and quantified tau domains in pathological aggregates. These analyses identified extensive tau cleavage in AD patients compared with control subjects, resulting in MTBR fragments enriched in AD tau aggregates. Interestingly, synthetic tau peptides mimicking AD tau fragments display increased fibrillization competence and promote tau assembly *in vitro* and *in vivo*, suggesting that tau cleavage can promote fibril formation. Pathway analysis of the collected proteomics data was performed to identify potential upstream effectors and mechanisms underlying MTBR cleavage and enrichment in pathological aggregates. This identified an inflammatory pathway that upregulates the 20S immunoproteasome (IP) subunits PSMB8, PSMB9, and PSMB10, as well as other proteases in the antigen presentation pathway, which strongly correlate with key cleavage sites in AD patients. Immunostaining of human tissues showed that tau aggregates co-localize with IP subunits and MHC-I only in AD neurons. Remarkably, across multiple systems, including HEK293T cells overexpressing full-length 0N4R tau, human tau knock-in mice (hTau), and human iPSC-derived neurons (iNeurons), proinflammatory cytokines consistently increased IP subunit levels, tau cleavage, and spontaneous tau aggregation without the presence of human-derived AD tau seeds. Given that IP is the most

upstream protease in the antigen presentation pathway, IP activity was targeted either by siRNA knockdown of IP subunits or by inhibiting Janus Kinase (JAK) using Baricitinib²⁵. Baricitinib is a small-molecule drug that inhibits JAK and thereby the entire JAK/STAT pathway. Both the targeted reduction of IP subunits using siRNA and a global inhibition of all antigen-presenting proteins lowered tau cleavage and puncta formation in HEK293T cells. In human iNeurons, Baricitinib reversed cytokine-induced tau cleavage and tau antibody-positive inclusions, demonstrating that inflammation drives tau pathology through IP-mediated processing. These findings provide a mechanistic link between neuroinflammation, impaired proteostasis, and tau aggregation in AD. Although inflammaging²⁶ and proteostasis impairment²⁷⁻²⁹ are well-documented features of aging, their direct connection to tau processing and aggregation has not previously been explored. Our data reveal that inflammation drives a switch in proteasome composition toward the IP, generating MTBR-enriched tau fragments that seed aggregation and further compromise proteostatic clearance of aberrant tau species. Together, these results establish inflammation as a causal upstream driver of tau pathology and identify the JAK/STAT–immunoproteasome–antigen presentation axis as a tractable therapeutic target for AD and potentially other tauopathies.

RESULTS

AD tau displays extensive cleavage flanking MTBR domain

To investigate tau cleavage in aggregates, protein lysates from brain tissues (BA39) were prepared from 46 AD patients and 40 controls stratified using clinical and pathological diagnosis (Extended Data Table 1) and subjected to sarkosyl detergent fractionation, producing the sarkosyl insoluble and soluble fractions (SIF and SSF, respectively) for each sample. The SIF comprises oligomeric and fibrilized tau species that induce tau aggregation in cell lines and mouse models (seed-

competent). The SSF primarily contains monomeric tau species, which are largely inactive in cell-based tau seeding assays (seed-inert)³⁰⁻³². All fractions were analyzed with the targeted MS method, FLEXITau (Full-Length Expressed Stable Isotope-labeled Tau)^{33,34}, to quantify tau peptide abundance, and label-free proteomics to identify specific cleavage sites across the tau sequence (Fig. 1A).

We observed extensive tau cleavage in the SIF, and major differences between AD and CTR groups were documented (Fig. 1B). While some tau cleavages were detected in the SSF, the sites and identification frequencies (Extended Data Fig. 1A) were similar for both AD and CTR, showing no evidence of altered tau processing in the SSF. Using FLEXITau, significant tau abundance differences were observed in SIF between the AD and CTR groups. While both CTR and AD subjects display tau cleavage at both N- and C-terminus (Fig. 1B), there are many more cleavage sites in AD, with 50% higher abundance in the AD, resulting in an enrichment of peptides within the MTBR (a.a. 242-372, Fig. 1C, and Extended Data Fig. 1B), consistent with previous studies^{6,18,35}. Importantly, cleavage frequency in the AD group increases with disease progression, and an inflection point between stages III and IV suggests a critical change at this point (Extended Data Fig. 1D). Binary hierarchical clustering shows a clear separation between AD and CTR cohorts based on the number of detected cleavage sites (Extended Data Fig. 1E). A correlation plot of cleavage and tau aggregate abundance further suggests that increased cleavage promotes pathological tau aggregation (Fig. 1D). 44 cleavage sites around the core domain (a.a. 306-378 in AD tau filament) were detected in the AD group. Only 4 nontryptic proteolytic cleavages were observed in the CTR group (Extended Data Fig. 2D, Extended Data Table 2). The multiple cleavages result in variable fragment lengths around the MTBR (Extended Data Fig. 1D) and likely explain the ‘fuzzy coat’ observed by cryo-EM¹⁵ (Fig. 1E).

119 CTR insoluble tau is 100-fold less abundant than AD insoluble tau and shows no significant
120 cleavage flanking MTBR⁶, further supporting the hypothesis that proteolysis and removal of the
121 N- and C-terminal regions from the core domain are critical to pathological tau aggregation in AD.
122 In all subjects, the majority of full-length tau identified in the SIF is the 0N4R isoform, as described
123 in our previous study⁶, suggesting that the presence of the N1 and N2 and C-terminal domains
124 promotes tau solubility and prevents aggregation. Higher amounts of N- and C-terminal peptides
125 in the SSF in AD, compared to the SIF (Extended Data Fig. 1B), also support the notion that these
126 terminal regions of tau are soluble and inhibit aggregation.

127 To ensure that the cleavage is not an artifact due to the sarkosyl preparation, seed-competent tau
128 species were alternatively purified by two other methods: immuno-purification using the MC1
129 antibody³⁶ or size exclusion chromatography (SEC) to obtain the high molecular weight (HMW)
130 fraction³⁷. AD-specific cleavage sites were observed in both preparations of these seeding positive
131 samples; however, they were not detected in non-seeding low molecular weight (LMW) AD tau
132 from the SEC (Extended Data Fig.2 A-D), indicating that tau cleavage is critical to seeding activity.
133 These results further support the hypothesis that cleavage promotes tau aggregation in AD.

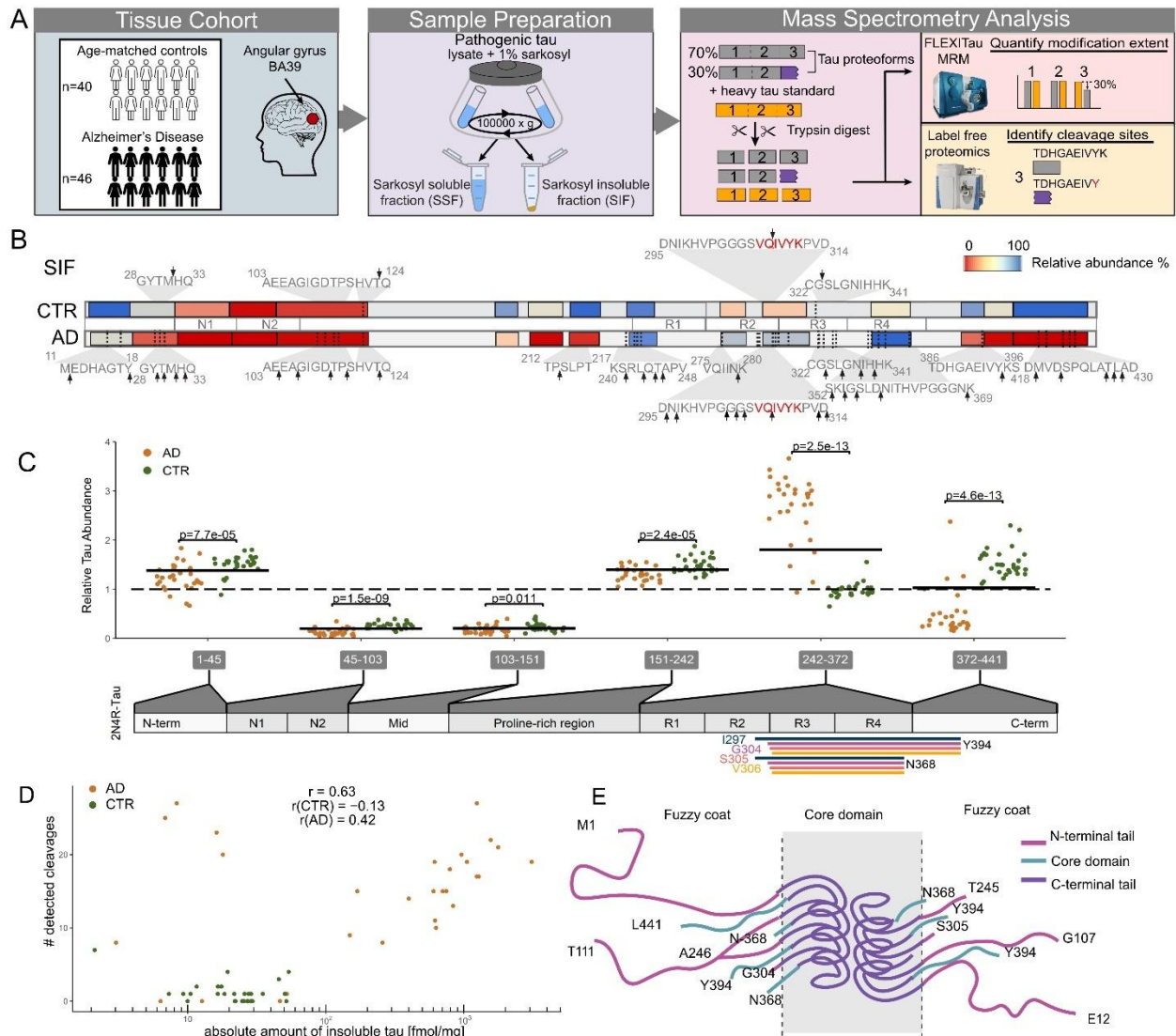


Fig. 1 Pathological AD tau exhibits extensive cleavage, flanking the aggregation-prone MTBR core domain. (A) Proteomics workflow used to study tau cleavage sites in the sarkosyl-insoluble fractions (SIF, aggregated tau) and sarkosyl-soluble fractions (SSF, soluble tau). (B) A quantitative cleavage map of SIF tau aggregates shows that AD tau is extensively cleaved relative to CTR in human brain tissue. (C) A FLEXITau plot displaying the relative abundance of unmodified peptides in AD and CTR subjects shows that the tau core domain is enriched relative to the N-terminal and the C-terminal regions. These data show that tau fragments are cleaved at both termini, and the aggregating peptides are from the core region between a.a. 243-369. Colored lines below the tau sequence represent enriched peptide fragments synthesized for the studies in Fig. 2A. (D) A correlation plot of the absolute amount of aggregated tau relative to the abundance of tau cleavages indicates that increased tau cleavage is associated with increased insoluble tau. The absolute tau abundance is measured using FLEXITau and is reported as fmol/mg wet weight of tissue. (E) Multiple cleavage sites were identified at the N- and C-termini. Hence, the tau

148 fragments containing the core domain are heterogeneous in length. The diagram illustrates the
149 heterogeneity in the length of N- and C-terminal tails extending from the core domain.
150

Tau fragments identified in AD subjects form fibrils *in vitro* and seed aggregation *in vivo*

We hypothesize that tau cleavage reduces tau's degrees of freedom, thereby reducing entropy³⁸ and enabling the core domain to form fibrils. To determine if the *in vivo* cleavage of full-length tau in the human brain promotes tau fibrillization, we developed a scoring scheme to select the representative AD-specific tau peptides observed in AD subjects for further study. Cleavage sites were scored based on patient frequency, specificity in AD subjects, identification in HMW tau (seeding fraction), and the presence of the hexapeptide ₃₀₆VQIVYK₃₁₁ known to promote self-assembly.³⁹ (Extended Data Table 3). Based on these scores, cleavage sites and the resulting fragment tau sequences were selected and tested for aggregation and seeding propensity. The top 4 ranked N-terminal cleavage sites flanking the core were located immediately before the ₃₀₆VQIVYK₃₁₁ amyloid motif in the R3 domain. Two of the most prominent C-terminal cleavage sites were also prioritized, and the observed core domain fragments were synthesized. We assign the following nomenclature for clarity: N-terminal side cleavages (1) N296-I297 (2) G303-G304, (3) G304-S305, (4) S305-V306, and C-terminal side cleavages (a) N368-K369 and (b) Y394-K395. Combinations of the prioritized cleavage sites resulted in 8 unique tau fragments **1a-4a** and **1b-4b** (Fig. 2A). These coincidentally span the AD tau filament core (a.a. 306-378) resolved by cryo-EM¹⁵. The **a**-series lacks ten residues at the C-terminal end of the core structure.

We produced recombinant peptides representing tau fragments **1a-4a** and **1b-4b** for characterization of fibrillization and seeding properties, with Tau187 (a.a. 255-441) as a positive control because it includes the core identified by cryo-EM in AD and other tauopathies^{11,12,15,40}. These fragments were first tested for their ability to form fibrils *in vitro* in the presence of heparin (a potent cofactor for fibrillization⁴¹), evaluated by the Thioflavin T (ThT) fluorescence assay. All fragments could form fibrils, with the longer **b**-series fragments all showing a higher fibrillization

propensity than any of the **a**-series fragments. N-terminal cleavages closer to the ³⁰⁶VQIVYK₃₁₁ motif increased fibrillization for the **b**-series peptides, with a rank order of **1b** = **2b** < **3b** < **4b** (Fig. 2B). Transmission electron microscopy (TEM) with negative staining showed that all fibrils formed by these fragment peptides display characteristic paired helical filaments (PHF)-like structures (Extended Data Fig. 3A).

Next, we tested the ability of the fragment fibrils to seed aggregation in mCerulean-TauRD cells⁴¹. After the transfection of different tau fragments, the ranked seeding activity of peptide fibrils showed excellent agreement with the *in vitro* aggregation propensity: fibrils formed by **b**-peptides with the longer C-terminus were more effective at seeding (Fig. 2C).

With the 1st generation of seeds in hand (Fig. 2D), we then tested whether the fibrilized tau fragments could seed naïve tau without heparin *in vitro*, using the longest **2b** as a representative peptide to include the effect of residues 304-305, which are very close to the PHF6 and might be critical in catalyzing the formation of oligomeric tau⁴². ThT assays and TEM analysis indicated that a catalytic amount (1:40 molar ratio) of **2b** fibril could directly induce the formation of 2nd generation fibrils of **2b** (Fig. 2E, F). Surprisingly, **2b** fibril could also seed monomeric Tau187. However, the Tau187 fibril seed could not produce appreciable 2nd generation fibrils. When normalized by the total mass of the seed (or heparin cofactor), **2b** seeds were the most effective at generating fibrils among other tau species (Fig. 2E). These data demonstrate that specific tau fragments such as **2b** can self-aggregate, as well as recruit longer, naïve, monomeric tau in the seeding process.

To examine if the synthetic tau fragments could also induce aggregation *in vivo*, we injected **2b** into the hippocampus of a PS19 mouse model expressing tau (P301S). Thioflavin-S and tau AT8 antibody staining showed that tau inclusions were successfully initiated by **2b**. In addition, an

197 increase of microglia (stained by Iba1 antibody) was observed in the **2b** injection area, but not in
198 the control PBS-injected area (Extended Data Fig. 3B).

199

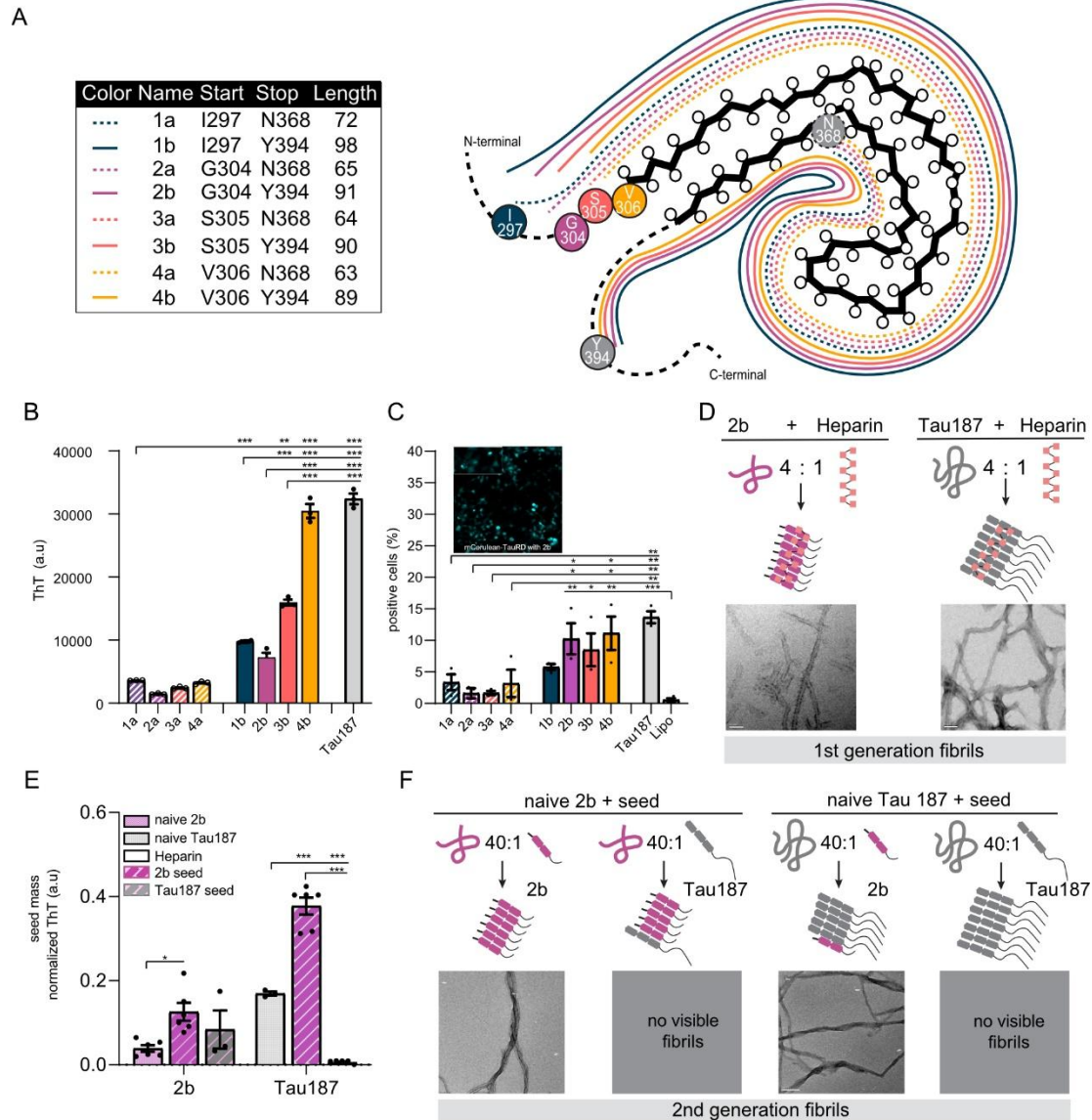


Fig. 2 Recombinant tau fragments defined by cleavages in post-mortem AD tissue fibrilize tau *in vitro* and *in vivo*. (A) Identified cleavage fragments map to the cryo-EM structure of the protofilament. (B) *In vitro* ThT aggregation assay of fragment peptides. Aggregation is indicated by the maximum ThT after 15 hours. TEM confirmed the fibril formation of the tau fragments. Mean $\pm$ SEM. One-way ANOVA ($F(8, 17) = 428.0, p < 0.001$); stars indicate Tukey post-hoc test p-values ** $p < 0.01$, *** $p < 0.001$. (C) Preformed fibrils of tau fragments triggered aggregation in HEK293T cells expressing mCerulean-TauRD. Each unique tau fragment shows varying seeding efficacy in the seeding assays (data shown as % seeded cells). Mean $\pm$ SEM. One-way ANOVA ($F(9, 13) = 9.765, p < 0.001$); stars indicate Tukey post-hoc test p-values * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (D) Schematic of *in vitro* seeding tests showing 1st generation fibrils were formed by adding heparin to cause aggregation. (E) Second-generation seeding of naïve tau fragment **2b** or Tau187 with **2b** or Tau187 seeds (40:1 tau to seed), compared to seeding with heparin (4:1 tau to heparin). 1st generation **2b** fibrils can induce 2nd generation fibrils whereas 1st generation Tau187

214 cannot. The ThT fluorescence values were normalized by the mass of the cofactor and protein
215 used. Mean $\pm$ SEM. One-way ANOVA (**2b** group - $F(2, 12) = 4.954$, $p = 0.0270$, Tau187 group
216 $F(2, 12) = 199.9$, $p < 0.001$); stars indicate Tukey post-hoc test p-values * $p < 0.05$, *** $p < 0.001$.
217 (F) Schematic of 2nd generation fibril seeding by 1st generation fibrils, pictured with TEM images
218 of the 2nd generation fibrils.

IP and inflammation-related pathways are associated with tau cleavage in AD

To identify AD-specific protease activity that causes the increase in AD MTBR region fragments, we analyzed differential protein abundance between AD and CTR subjects from SIF MS data (Fig.3A). Seven proteases were enriched, including Cathepsin B (CTSB), Aminopeptidase-like 1 (NPEPL1), Leucine aminopeptidase (LAP3), and Calpain 1 (CAPN1), with known tau cleavage activities¹⁹. The three IP subunits (PSMB8, 9, and 10) with protease activity were identified for the first time. The resulting proteases were then cross-referenced with the MEROPS peptidase database and ranked by significance scores (Fig. 3B).⁴³

In contrast, subunits of the constitutive proteasome (CP) were not significantly changed in both SIF and SSF from all subjects (Fig. 3A and Extended Data Fig. 4A). Of note, 3 deubiquitinating enzymes (USP14, UCHL1, and OTUB1), which may regulate the abundance of tau and could be potential drug targets in neurodegeneration⁴⁴, were decreased in both fractions of AD (Fig. 3A and Extended Data Fig. 4A). This analysis also identified AD-risk proteins, including GFAP (upregulated in both SIF and SSF of AD), and APOE (significantly enriched in SIF) (Fig. 3A and Extended Data Fig. 4A). No significant correlation was observed between amyloid-beta precursor protein (APP) burden and tau aggregation for each patient in the insoluble fraction, suggesting a non-synergistic role in amyloid deposition and tau inclusion (Extended Data Fig. 4B).

To rank the contribution of proteases associated with AD-specific tau cleavage, we evaluated each protease by its relative abundance across tau cleavage intensities in each patient (Fig. 3C, Extended Data Fig. 4C). The N- and C-termini observed for AD-specific tau MTBR fragments are primarily attributable to IP activity, as their abundance correlates more strongly with IP abundance and specificity (Fig. 3B-C), suggesting a significant role for the IP in pathological tau aggregation. In addition, some tau fragments display termini attributed to post-proteasomal trimming by CTSB,

LAP3, and NPEPL1 activities⁴⁵. Other cleavage sites might be attributed to lysosomal degradation⁴⁶ and to caspases involved in apoptosis⁴⁷⁻⁵⁰. However, we did not observe changes in caspases or apoptotic proteins in our MS data from the given AD brain samples.

A pathway enrichment analysis was performed on proteins identified in SIF to identify the dominant pathways involved in IP regulation in AD subjects. The top enriched Reactome pathways in AD included ‘immune system’ and ‘cellular response-to-stress’, which contain PSMB8/9/10 (Fig. 3D), suggesting that IP abundance in AD may be explained by stress or inflammation. Further, Ingenuity Pathway Analysis⁵¹ classified inflammation-related cytokine signatures, including IL6, OSM (IL6 family), IL4, IL1B, IL15, CD40LG, IFN- γ (interferon- γ), and TNF(tumor necrosis factor), as being upregulated in AD subjects (Fig. 3E), which is consistent with the well-established role of the IP of proteolysis in antigen presentation pathways under inflammatory or stress conditions unrelated to AD^{52,53}.

The immunoproteasome colocalizes with tau aggregates in neurons

If the IP contributes to tau aggregation in neurons, those that contain tau fibrils will express IP subunits. To determine if the IP is observed in neurons that contain tau aggregates in AD brain tissue, multiplexed immunostaining was performed to visualize the localization of IP-specific subunits PSMB8/9/10, in relation to two established pathological tau epitopes (pT231 in Fig. 3F and Extended Data Fig. 5A-D; AT8 in Extended Data Fig. 6) and the neuronal marker NeuN. Strikingly, in AD brain tissue sections, PSMB8 and PSMB9 only co-occurred in neurons that stain for pathological tau inclusions, including neurofibrillary tangles and neuropil threads. Pathological tau and neuronal IP co-expression were not observed in CTR brain tissue sections. No cross-reactivity was observed between the antibodies towards pan-Tau and IP (Extended Data Fig. 5E).

264 Remarkably, major histocompatibility complex-I (MHC-I) proteins, which are signatures of the
265 antigen display pathway driven by proinflammatory cytokines⁵⁴, are also upregulated in the AD
266 samples and in neurons containing tau aggregates (AT8) (Extended Data Fig. 7). The antigen
267 presentation pathway and other proteases are downstream of the IP, which initiates antigen peptide
268 processing. The observation that IP and tau colocalize in the brains of AD subjects supports our
269 hypothesis that the antigen presentation pathway initiated by IP-mediated proteolysis promotes tau
270 aggregation.

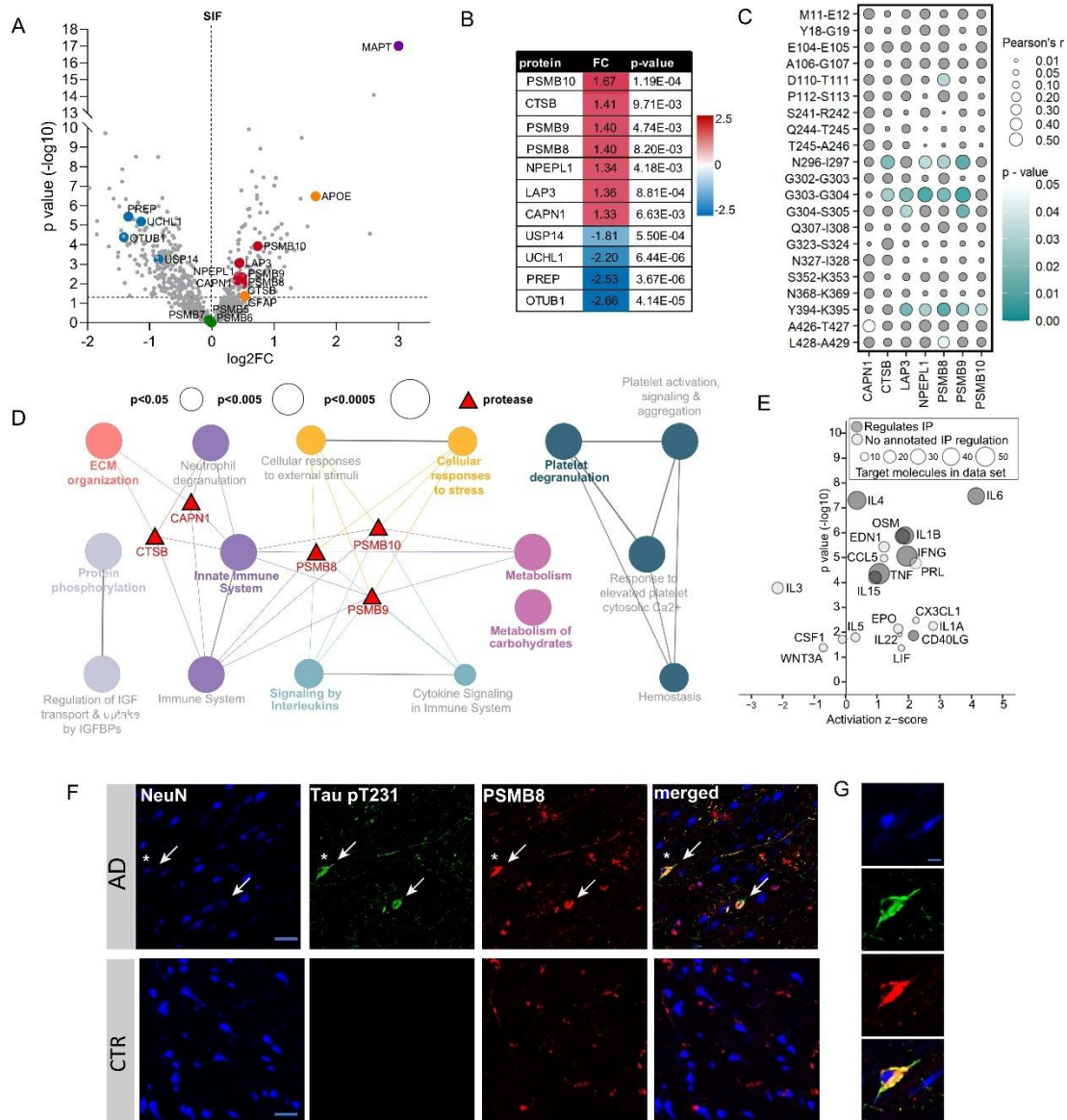


Fig. 3 IP and inflammatory pathways are associated with pathological AD-specific tau cleavage. (A) A volcano plot revealing differential protein expression in the insoluble fraction of AD vs CTR brain. Proteases from 20S CP are labeled green. AD-related factors are labeled orange. Tau is labeled purple. (B) Significantly changed proteins were cross-referenced with the MEROPS peptidase database to identify upregulated (red) or downregulated (blue) proteases. (C) Correlation plot of the abundance of upregulated proteases and the intensity of cleavage site-specific peptides in AD. (D) The Reactome pathway analysis of upregulated proteins in the SIF identified an overrepresentation of immune and cell stress pathways. (E) Analysis of protein abundance changes using Ingenuity Pathway Analysis reveals increased proinflammatory cytokine regulators, including IFN- γ and TNF. (F) 3-plex immunostaining of the middle frontal gyrus shows PSMB8 expression in neurons in AD brain tissue, but not in CTR brain tissue. PSMB8 immunoreactivity overlaps with the pathological tau marker Thr231 pTau in neurites and neurofibrillary tangles. Arrows represent neurofibrillary tangles with colocalized pTau and PSMB8 immunoreactivity.

285 Stars represent neuronal cells at a magnification of F. *Scale bar = 10 μm .* (G) A higher
286 magnification view of neurofibrillary tangle from E. *Scale bar = 2 μm .*

Inflammation factors induce IP-dependent tau cleavage and aggregation *in vitro* and *in vivo*

The identification of the cytokine-induced antigen presentation pathway, including IP expression and tau cleavage in AD patient neurons, prompted further experiments to understand the causal relationship between these factors and tau aggregation. To test if tau cleavage is associated with IP induction and protease activity in the antigen presentation pathways, a biosensor HEK293T cell line stably expressing a Full-Length (FL) human tau isoform (0N4R: P301S) with a C-terminal-EYFP was developed⁵⁵ and treated with IFN- γ /TNFs (Extended Data Fig. 8A).

Fluorescence imaging shows that EYFP-fused tau is diffusely expressed throughout the cytoplasm without accumulation after treatment (Fig. 4A), indicating that no significant aggregation occurred when tau has an intact C-terminus. However, tau aggregates were observed throughout the course of IFN- γ /TNF treatment (Fig. 4A) when the anti-tau RD4 antibody recognizing the R1-R2 inter-region (a.a. 275-291)⁵⁶ was used to visualize tau aggregates, including the ²⁷⁵VQIINKK²⁸¹ motif that contributes to tau aggregation.⁴² This supports the hypothesis that IFN- γ /TNF treatment induces the formation of tau aggregates from tau fragments after the C-terminus is cleaved (removing C-terminal a.a. and YFP). Moreover, western blotting further validates this conclusion and shows signals for C-terminal YFP cleavage after IFN- γ /TNF treatment (Fig. 4B, Extended Data Fig. 8B). This is likely a result of proteases, including the IP, in the antigen presentation pathway⁵⁴. Staining using an N-terminus tau antibody (Tau12) or the proline-rich region (PRR) tau antibody (HT7) shows additional inclusions contain the N-terminus or PRR, suggesting that while the cleaved tau fragments favor tau aggregation without N- and C-terminus, the aggregates can incorporate full length and longer tau fragments and are heterogeneous in length (Extended Data Fig. 8C), as observed in the human MS data.

To identify the proteins and pathways are induced by IFN- γ /TNF treatment in biosensor cells, cell lysates treated by IFN- γ /TNFs for 7 days were analyzed using MS. Pathway enrichment analysis confirmed activation of antigen presentation pathways (Fig. 4C)⁵⁴. The PSMB9/10 subunits and the IFN-inducible proteasome activator PA28 (PSME1/2)⁵³, which is were significantly upregulated by IFN- γ /TNFs (Fig. 4D). While MS did not detect PSMB8, it increased compared to basal levels when measured by western blot (Extended Data Fig. 8B). In contrast, the CP subunits PSMB6/7 were downregulated (Fig. 4D), consistent with established observations⁵⁷. Not every protease upregulated in the AD SIF fraction was observed in the IFN- γ /TNF -treated cell model, suggesting a role for additional pathways.

To examine if IFN- γ /TNF-treated biosensor cells exhibited a tau cleavage pattern detected in human AD, an in-depth proteomic analysis of tau cleavages was performed on the cell lysate treated by IFN- γ /TNF (Fig. 4E). The data indicated that the increasing cytokine concentrations exacerbated tau cleavage around the tau core domain, similar to that observed in AD patients.

Next, to determine whether the IP directly cleaves tau, we incubated recombinant 0N4R tau with purified IP or CP and mapped cleavage sites by mass spectrometry (Extended Data Fig. 9). Although many cleavage sites were shared, IP-specific cleavage was enriched within the MTBR, particularly across R2–R4, whereas CP-specific cleavage occurred predominantly at the N- and C-termini. Several IP-generated cleavage sites localized near the R2–R3 and R4–C-terminal boundaries, surrounding the aggregation-prone MTBR core. Notably, the *in vitro* profile did not fully recapitulate that observed in AD brain, likely reflecting contributions from additional proteolytic pathways *in vivo*. Together, these results show that IP alters tau proteolysis toward the MTBR, providing a direct biochemical link between inflammation-induced IP activity and the generation of aggregation-prone tau species.

To demonstrate that inflammation can also induce tau aggregation in a sporadic AD model beyond tau mutations identified in familial tauopathy, we established a stable HEK293T cell line expressing wild-type (WT) 0N4R tau fused to the C-terminal EYFP. IFN- γ /TNF treatment induced RD4-positive tau aggregates in WT tau biosensor cells, albeit with slower kinetics than those of mutant tau, paralleling the delayed onset observed in sporadic versus familial AD. These findings confirm that inflammation-driven tau aggregation occurs independently of pathogenic mutations and can also affect WT tau, contributing to sporadic AD (Extended Data Fig. 10).

To study the effect of chronic, systemic inflammation, we selected the hTau mouse⁵⁸ because it more closely reflects sporadic AD without tau mutations, which affect tau modifications and cleavage⁵⁹. Chronic administration of lipopolysaccharide (LPS) induces robust neuroinflammation in mice, marked by elevated pro-inflammatory cytokine levels and the accumulation of hyperphosphorylated tau and amyloid tangles.⁶⁰ To investigate whether chronic systemic inflammation promotes tau cleavage and aggregation through activation of IP subunits *in vivo*, we treated hTau mice with LPS daily for 7 days (Extended Data Fig. 11A). Global proteomic profiling of brain tissues (cortex, hippocampus and striatal regions) confirmed the strong induction of innate immune pathways including and not limited to the antigen presentation proteins PSMB8, PSME1 and STAT, as well as the upregulation of complement components (C4b, Q1qc) and the cytokine receptor IL6ST (Extended Data Fig. 11B-C). Even after a 7-day chronic LPS treatment, pronounced tau aggregation in the cortex and hippocampus was observed, as detected by the phospho-tau antibody pT217 (Extended Data Fig. 11D-E). Extensive tau cleavage was observed in LPS-treated brains, particularly at hotspots within or adjacent to the MTBR, including 306V–307Q, 325L–326G, and 368N–369K (Extended Data Fig. 11F). Together, these results demonstrate that neuroinflammation drives tau cleavage flanking the MTBR core and promotes

aggregation both *in vitro* and *in vivo*, supporting our hypothesis that cytokine-induced IP activation mediates tau proteolysis in the human brain.

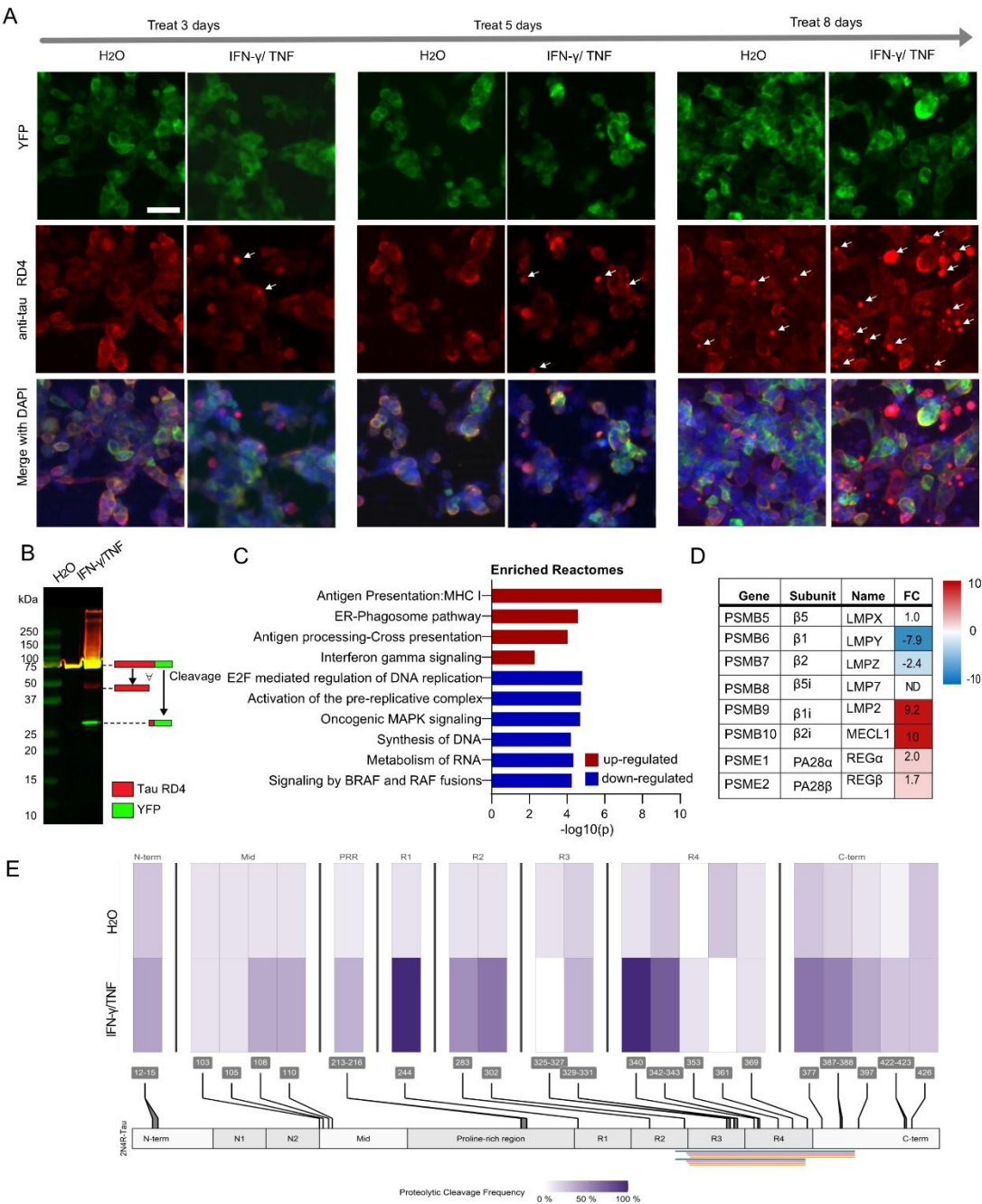


Fig. 4 IFN-γ/TNF induces tau cleavage and inclusion formation in HEK293T tau biosensor cells. (A) Immunofluorescence shows IFN-γ/TNF-induced tau inclusions that are RD4-positive but EYFP-negative, indicating C-terminal tau cleavage. Scale bar = 50 μm. (B) Immunoblotting confirms tau cleavage following IFN-γ/TNF treatment at D8. (C) Enriched pathways identified in

362 day 8 cell lysate proteomics data include IFN- γ signaling. (D) Fold changes of proteasome
363 subunits in IFN- γ /TNF treated cells compared to control IP subunits in label-free proteomics data
364 of day 8 cell lysate show a significant increase in the IP subunits. (E) Tau endogenous cleavage
365 frequencies observed in MS analysis of IFN- γ /TNF-treated/IP-induced biosensor cells (n = 8). Tau
366 cleavage increases at the sites near the R3 and R4 regions of the MTBR, generating core tau
367 fragments like those found in AD. The colored lines below the tau protein scheme correspond to
368 the synthetic peptide fragments in Fig. 2A.

Inhibition of IP subunits reduces inflammation-induced tau aggregation

To investigate the causal relationship between inflammation and tau pathology, we used Baricitinib, a JAK/STAT pathway inhibitor identified in a high-throughput small molecule screen to identify tau aggregation inhibitors. Baricitinib suppresses IFN- γ /TNF signaling and is FDA-approved for the treatment of rheumatoid arthritis and severe COVID-19. While Baricitinib is in ongoing clinical trials for AD and amyotrophic lateral sclerosis (ALS), the mechanism of action (MoA)²⁵ is not fully understood. Pre-treatment with Baricitinib significantly reduced IFN- γ /TNF-induced tau aggregation compared to vehicle control in FL tau biosensor (Fig. 5A). Critically, Baricitinib reversed the expression of the MHC-1-mediated antigen presentation pathway, which is most enriched in human brain data. PSMB9, PSMB10, and PSME1/2 expression—the proteins most significantly upregulated by IFN- γ /TNF treatment were suppressed (Fig. 5B-C). These results demonstrate that pharmacological intervention can counteract the inflammatory molecular signatures associated with tau pathology.

Another test of the causal role of this pathway was to reduce the most upstream protease in the antigen presentation pathway, the IP subunits. To determine whether this could lower tau aggregates in biosensor cells after IFN- γ /TNF treatment, a knockdown experiment targeting the IP was performed. Experiments using small interfering RNA (siRNA) targeting PSMB9 (Fig. 5D-F) and PSMB10 (Fig. 5G-H) were performed by transfecting the cells one day before IFN- γ /TNF treatment. IFN- γ /TNF-induced tau aggregates were significantly reduced by PSMB9 or PSMB10 knockdown, supporting the premise that the tau aggregates observed in the IFN- γ /TNF treatments are driven by cytokine-induced IP expression.

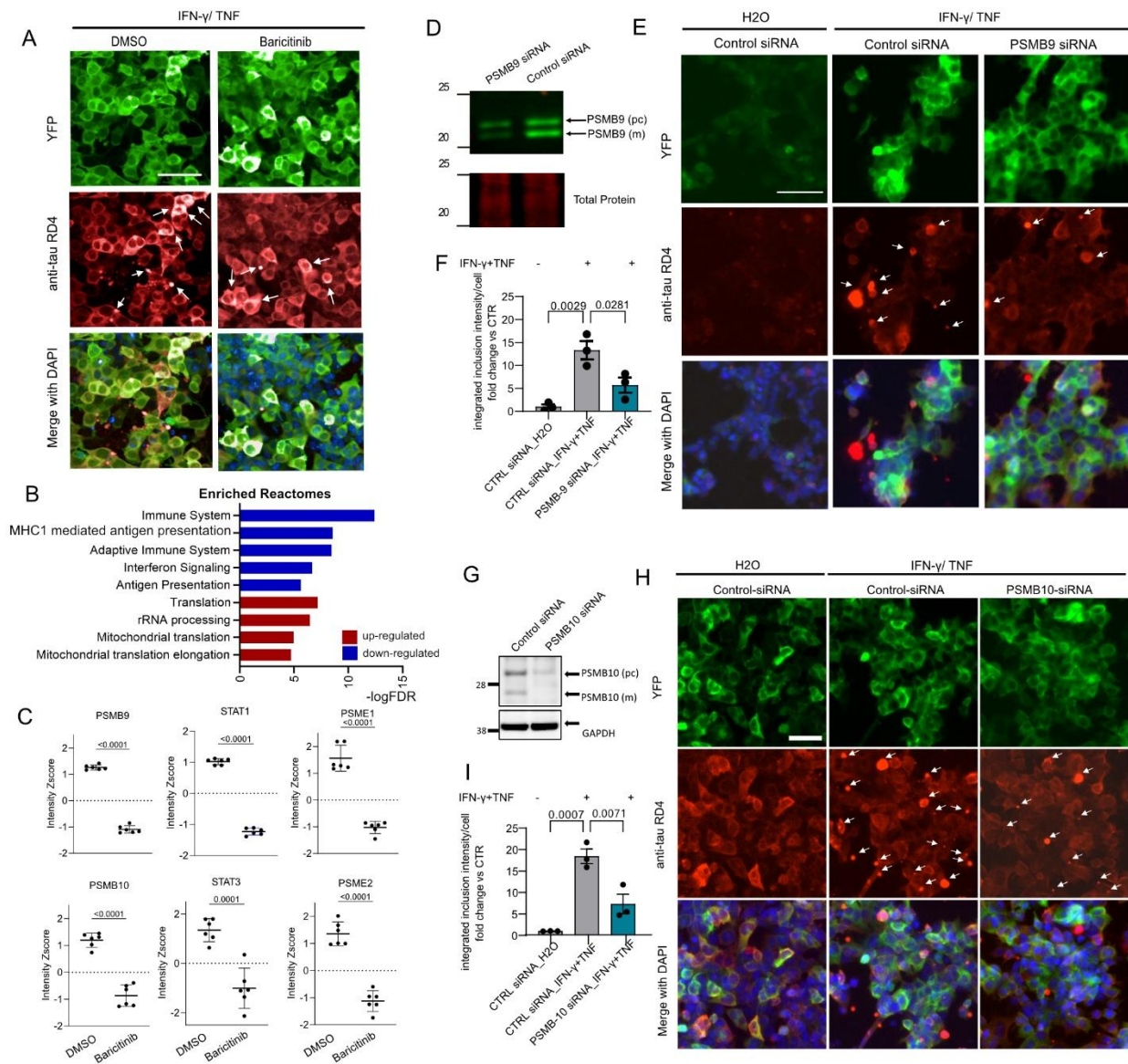


Fig. 5 Reduction in IP expression using small molecule and genetic approaches attenuates IFN- γ /TNF-induced tau aggregation in HEK293T cells. (A) Immunofluorescence after IFN- γ /TNF treatment with either DMSO or Baricitinib shows reduced tau puncta in Baricitinib-treated cells (white arrows). Scale bar = 50 μ m. (B) Top enriched Reactome pathways following Baricitinib treatment. (C) Baricitinib reduced the abundance of IP subunits and key components of the MHC-I antigen presentation pathway. (D) Western blotting of cells transfected with control or PSMB9 siRNA prior to IFN- γ /TNF treatment. Both precursor (pc) and mature (m) PSMB9 forms were reduced by 90% upon siRNA knockdown, as shown by western blotting. (E) Immunofluorescence of cells transfected with control or PSMB9 siRNA after vehicle or IFN- γ /TNF treatment. Tau aggregates were reduced in PSMB9 knockdown cells (white arrows). Scale bar = 50 μ m. (F) Quantification of integrated RD4-positive tau aggregation from (E) (n = 3). Mean $\pm$ s.e.m. One-way ANOVA. (G) Immunoblot of control- or PSMB10 siRNA-transfected cells showing reduced PSMB10 (pc and m forms) upon knockdown. (H) Immunostaining of cells

transfected with control or PSMB10 siRNAs after treatment with vehicle or IFN- γ /TNF(100 ng/mL each). Knockdown of PSMB10 reduced tau aggregates (white arrows). Scale bar = 50 μ m. (I) Quantification of integrated RD4-positive tau aggregation in (H). Knockdown of PSMB9 or PSMB10 significantly suppressed IFN- γ /TNF-induced tau aggregation (n = 3 per group). Mean $\pm$ s.e.m. One-way ANOVA.

Baricitinib attenuates inflammation-induced tau cleavage and aggregation in human iPSC-derived neurons.

To extend our mechanistic findings to a human neuronal context, iPSC-derived neurons expressing 4R-P301S tau⁶¹ were subjected to chronic IFN- γ /TNF exposure for 6 days (Extended Data Fig. 12A). Consistent with observations from the HEK biosensor and hTau mouse models, IFN- γ /TNF-treated iNeurons developed significant tau pathology, characterized by elevated RD4- and pT217-positive aggregates (Fig. 6A-B). Interestingly, many aggregates were positive for both markers, while others were detected exclusively by RD4 or pT217, indicating molecular heterogeneity in aggregate composition. Notably, this aggregation occurred without exogenous tau seeds or glial co-culture and, to our knowledge, represents the first demonstration that inflammatory signaling alone is sufficient to initiate tau pathology in human neurons.

Quantitative proteomic profiling revealed a coordinated remodeling of the neuronal proteasome, characterized by significant upregulation of the IP subunits PSMB8, PSMB9, and PSMB10, alongside a reduction in the constitutive subunits PSMB5 and PSMB7 (Fig. 6C-E). This IP shift closely mirrors proteomic alterations observed in postmortem human AD brain tissue, supporting its relevance to the disease. Tau peptide analysis further revealed an overall increase in tau cleavage surrounding the MTBR domain, including at sites 323G-324S, 354I-355G, 355G-356S, and 368N-369K (Fig. 6F). These cleavage sites flank or reside within aggregation-prone tau

domains and are predicted to generate core tau fragments like those detected in insoluble tau from AD brains.

Critically, pharmacological inhibition of JAK/STAT signaling with Baricitinib markedly reduced IFN- γ /TNF-induced tau aggregation and attenuated the accumulation of these MTBR-associated cleavage fragments (Fig. 6A, F), as well as inhibiting the MHC-1 antigen presentation pathway (Extended Data Fig. 12 B-C). Baricitinib partially restored the balance between immunoproteasome and constitutive proteasome subunits, concomitant with reduced tau cleavage and aggregation. Although changes in PSMB5, PSMB7, and PSMB9 did not reach statistical significance, they followed a consistent corrective trend. Importantly, the convergence of reduced tau cleavage, attenuated aggregation, and normalized proteasome composition under a single JAK/STAT inhibitor suggests that these events are mechanistically linked and amenable to therapeutic intervention at the level of upstream inflammatory signaling. The ability of Baricitinib to simultaneously suppress pathological tau processing and restore proteostasis balance positions it as a particularly compelling candidate, as it targets an early node in the inflammation-to-tauopathy cascade rather than downstream aggregation products alone.

To assess whether inflammation-driven tau pathology extends beyond the tau mutation, P301S, we performed parallel IFN- γ /TNF and Baricitinib treatments in iNeurons expressing WT 4R tau⁶¹. Cytokine exposure induced visible tau puncta in WT neurons, and Baricitinib partially reduced this aggregation, though the response was more modest than in P301S-expressing cells (Extended Data Fig. 12D). These findings indicate that inflammation-driven tau pathology is not restricted to mutant tau, and that JAK/STAT inhibition may therefore offer therapeutic relevance across both familial and sporadic forms of tauopathy, including FTD and AD.

450 Together, these results establish an inflammation-driven mechanism of tau cleavage and
451 aggregation in human iNeurons, consistent with findings from the HEK biosensor and hTau mouse
452 models, which implicate the MHC-I antigen presentation pathway in this process. The most
453 upstream JAK/STAT inhibition and rescue is achieved by Baricitinib, thereby reducing MHC-1
454 antigen presentation pathways, including immunoproteasome expression, which results in the
455 abrogation of proteolytic cleavage flanking the MTBR and, hence, tau aggregation. This
456 experimental data provides a strong MOA and supports the rationale for clinical investigation of
457 Baricitinib and other brain-penetrant, brain-specific JAK inhibitors as disease-modifying therapies
458 for AD (Fig. 6G).

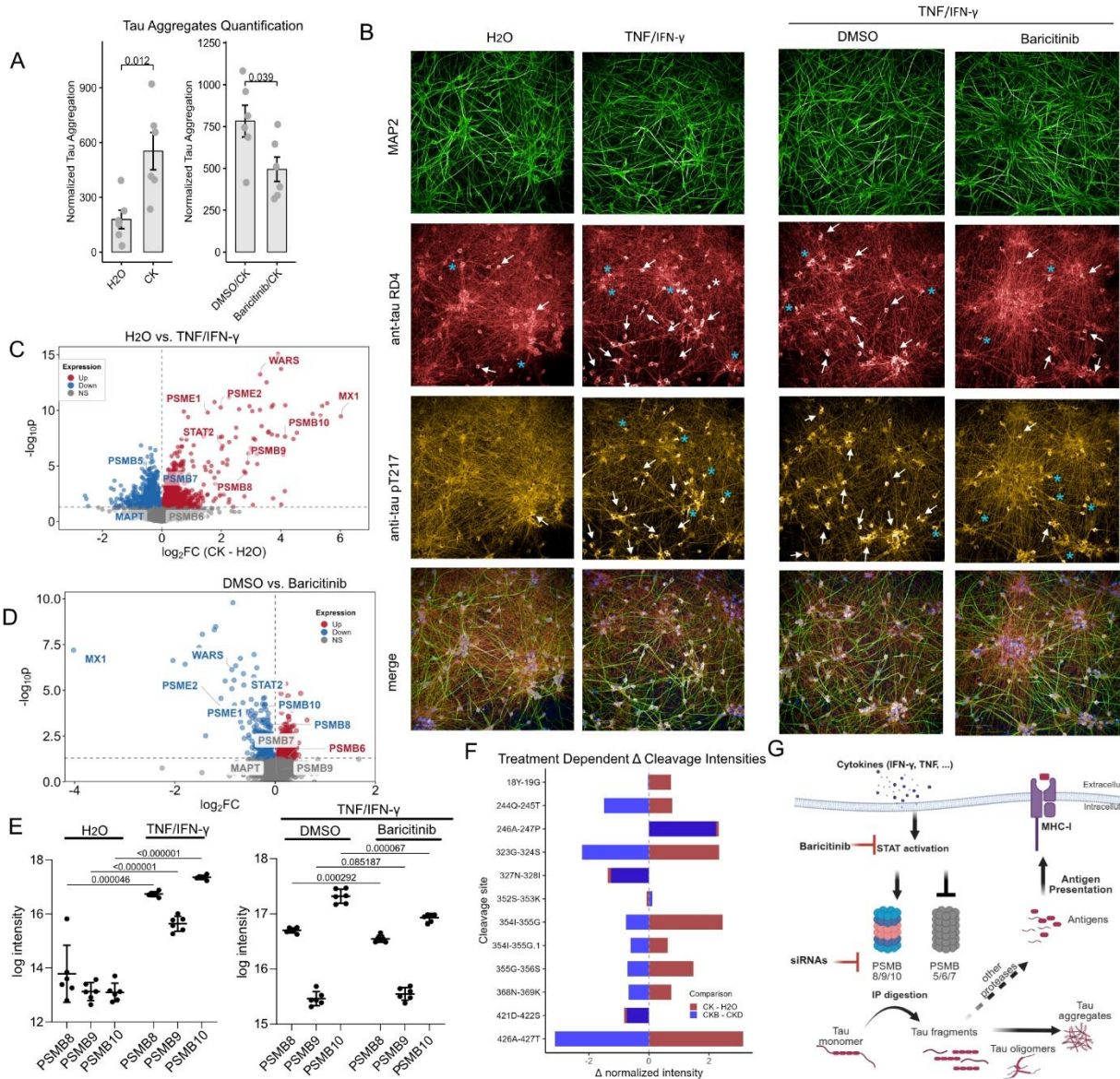


Fig. 6 Inflammation induces tau aggregation in human iPSC-derived neurons and is decreased by Baricitinib. (A) Representative images showing IFN-γ/TNF-induced tau-positive inclusions and their reduction by Baricitinib in iNeurons. White arrows indicate tau inclusions positive for both antibodies; cyan asterisks indicate RD4- or pT217-specific inclusions. (B) Quantification of RD4-positive tau inclusions following IFN-γ/TNF stimulation, with or without Baricitinib treatment (DMSO as control). Neurons were pre-treated with 500 nM Baricitinib or DMSO for 24 h, followed by exposure to 200 ng/mL TNF and IFN-γ for 6 days (n=6 well replicates, n=3 separate differentiations). (C–D) Global proteomic profiling demonstrates that IFN-γ/TNF treatment induces a robust inflammatory response in iPSC-derived neurons, which is attenuated by Baricitinib. (E) Quantification of 20S IP subunits. IFN-γ/TNF (CK) treatment upregulated PSMB8/9/10, whereas Baricitinib partially restored their expression. (F) Bar plot showing the intensity changes (Δ) in tau cleavage sites. Δ values represent the difference between IFN-γ/TNF-treated and control neurons (CK-H₂O, red) or between Baricitinib-treated and DMSO control (CKB-CKD, blue). Baricitinib markedly reduced IFN-γ/TNF-induced cleavage at the C-

terminus, proline-rich region, and within the MTBR. (G) Illustration of the proposed mechanism in which inflammatory cytokines activate the IP, increasing tau cleavage and promoting tau aggregation. The resulting fragments may also serve as MHC-I peptides.

DISCUSSION

This study establishes one mechanism where neuroinflammation is a direct initiator rather than a consequence of tau aggregation in sporadic Alzheimer's disease and related disorders. We demonstrate a mechanistic cascade: proinflammatory cytokines induce neuronal immunoproteasome expression, which cleaves tau into aggregation-prone MTBR-enriched fragments that spontaneously form pathological fibrils — independently of pre-existing seeds, suggesting inflammation triggers *de novo* tau pathology.

Three lines of evidence support inflammation as a primary driver. First, IFN- γ /TNF treatment recapitulates the identical tau cleavage patterns observed in human AD tissue across HEK293T cells, humanized tau knock-in mice, and iPSC-derived neurons. Second, synthetic tau fragments that match human AD cleavage sites autonomously form fibrils and recruit full-length tau without cofactors. Third, blocking the cascade through immunoproteasome knockdown or JAK/STAT inhibition with Baricitinib prevents both cleavage and tau inclusion formation, thereby establishing causality.

Our identification of the immunoproteasome as the key tau-cleaving enzyme resolves a longstanding paradox. While 26S proteasome activity is known to decline with aging and AD progression, the reason was unclear. We show that inflammatory stress simultaneously upregulates the 20S immunoproteasome, with subunit switching from PSMB5/6/7 to PSMB8/9/10, thereby fundamentally altering specificity and generating the precise cleavage patterns that enrich MTBR fragments in AD aggregates. Colocalization of immunoproteasome subunits with pathological tau is observed exclusively by immunohistochemistry in neurons of AD postmortem brain tissue, but

not in CTR, providing orthogonal and direct evidence in humans. Beyond the immunoproteasome, the enrichment of downstream proteases, including aminopeptidases^{19,43,45} in the antigen presentation pathway also contributes to further processing of the tau fragments, thereby producing the heterogeneous fragment termini observed in human tissue. The IP-mediated cleavage is the initial protease step, which may act as a catalytic seed leading to the subsequent cleavage cascade in the MHC-I antigen presentation pathway⁵¹. (Extended Data Fig. 13)

The immunoproteasome-generated fragments serve a dual pathogenic role. The removal of N- and C-terminal domains at multiple cleavage sites around the MTBR reduces conformational entropy, favoring protein aggregation and producing heterogeneous tau fragments flanking the R2-R4 region. Another less abundant population of fragments is produced by cleavage around the R2-R4 region, with VQIVYK³⁹ and VQIINK⁴² aggregation motifs that accelerate tau aggregation, thereby explaining their preferential incorporation into pathological aggregates. Simultaneously, these fragments may function as antigenic peptides: upregulated MHC-I components colocalizing with immunoproteasome and pathological tau suggest neuronal surface display, potentially triggering adaptive immune responses and creating a self-amplifying T-cell-mediated inflammatory cycle.

Critically, this pathway operates in both mutant and wild-type tau systems, suggesting sporadic and familial tauopathies differ in aggregation threshold rather than mechanism. This reconciles why mouse models without sustained inflammatory stress fail to fully recapitulate neurodegeneration: inflammation is a required cofactor, not merely a bystander.

Our findings reveal a previously unrecognized mechanism of action for Baricitinib. While currently in AD trials on general anti-inflammatory grounds, Baricitinib specifically blocks the proximal step in tau pathogenesis — IFN- γ /TNF-induced immunoproteasome expression — offering a mechanism-based rationale that strengthens its clinical promise. Beyond Baricitinib, the

pathway offers multiple intervention points: direct immunoproteasome inhibitors, antibodies targeting cleavage-site neoepitopes, and agents disrupting the MHC-I amplification loop. The specific MTBR-containing fragments identified here also represent promising biomarkers that directly reflect immunoproteasome activity and may enable risk stratification prior to irreversible neuronal loss. Targeting neuroinflammation before irreversible tau aggregation may represent a tractable route to disease modification.

Study limitations include possible MS acquisition bias toward dominant proteolytic pathways, given the high patient frequency in AD, which may omit less frequent patient-specific pathways, such as autophagy, that would require larger cohorts. While our tau-inflammation models replicate the initiation of tau aggregation in sporadic AD, the tau inclusions or aggregates formed are likely premature compared with the mature paired helical filaments found in the aging human brain, which take decades to fibrilize. While other mechanisms may contribute to tau aggregation, including PTMs that modify tau chemistry, this research confirms inflammation-driven proteolysis as a causative initiating pathway, linking two key features of aging and Alzheimer's disease via the immunoproteasome. Future studies using immunoproteasome-deficient neuronal or *in vivo* models will be important to further investigate the contribution of immunoproteasome activity to tau processing, assembly, and disease progression.

REFERENCES

- 1 Heneka, M. T. *et al.* Neuroinflammation in Alzheimer disease. *Nat Rev Immunol* **25**, 321-352 (2025). <https://doi.org/10.1038/s41577-024-01104-7>
- 2 Wilson, D. M., 3rd *et al.* Hallmarks of neurodegenerative diseases. *Cell* **186**, 693-714 (2023). <https://doi.org/10.1016/j.cell.2022.12.032>
- 3 Limorenko, G. & Lashuel, H. A. Revisiting the grammar of Tau aggregation and pathology formation: how new insights from brain pathology are shaping how we study and target Tauopathies. *Chem Soc Rev* **51**, 513-565 (2022). <https://doi.org/10.1039/d1cs00127b>
- 4 Chang, C. W., Shao, E. & Mucke, L. Tau: Enabler of diverse brain disorders and target of rapidly evolving therapeutic strategies. *Science* **371** (2021). <https://doi.org/10.1126/science.abb8255>

- 5 Knopman, D. S. *et al.* Alzheimer disease. *Nature Reviews Disease Primers* **7** (2021).
<https://doi.org/10.1038/s41572-021-00269-y>
- 6 Wesseling, H. *et al.* Tau PTM Profiles Identify Patient Heterogeneity and Stages of Alzheimer's Disease. *Cell* **183**, 1699-1713 e1613 (2020). <https://doi.org/10.1016/j.cell.2020.10.029>
- 7 Arakhamia, T. *et al.* Posttranslational Modifications Mediate the Structural Diversity of Tauopathy Strains. *Cell* **180**, 633-644 e612 (2020). <https://doi.org/10.1016/j.cell.2020.01.027>
- 8 Dujardin, S. *et al.* Tau molecular diversity contributes to clinical heterogeneity in Alzheimer's disease. *Nat Med* **26**, 1256-1263 (2020). <https://doi.org/10.1038/s41591-020-0938-9>
- 9 Kumar, M. *et al.* Molecular features of human pathological tau distinguish tauopathy-associated dementias. *Cell* **189**, 956-968 e913 (2026). <https://doi.org/10.1016/j.cell.2025.12.036>
- 10 Scheres, S. H. W., Ryskeldi-Falcon, B. & Goedert, M. Molecular pathology of neurodegenerative diseases by cryo-EM of amyloids. *Nature* **621**, 701-710 (2023). <https://doi.org/10.1038/s41586-023-06437-2>
- 11 Shi, Y. *et al.* Structure-based classification of tauopathies. *Nature* **598**, 359-363 (2021).
<https://doi.org/10.1038/s41586-021-03911-7>
- 12 Zhang, W. *et al.* Novel tau filament fold in corticobasal degeneration. *Nature* **580**, 283-287 (2020). <https://doi.org/10.1038/s41586-020-2043-0>
- 13 Falcon, B. *et al.* Novel tau filament fold in chronic traumatic encephalopathy encloses hydrophobic molecules. *Nature* **568**, 420-423 (2019). <https://doi.org/10.1038/s41586-019-1026-5>
- 14 Falcon, B. *et al.* Structures of filaments from Pick's disease reveal a novel tau protein fold. *Nature* **561**, 137-140 (2018). <https://doi.org/10.1038/s41586-018-0454-y>
- 15 Fitzpatrick, A. W. P. *et al.* Cryo-EM structures of tau filaments from Alzheimer's disease. *Nature* **547**, 185-190 (2017). <https://doi.org/10.1038/nature23002>
- 16 Lovestam, S. *et al.* Disease-specific tau filaments assemble via polymorphic intermediates. *Nature* (2023). <https://doi.org/10.1038/s41586-023-06788-w>
- 17 Wille, H., Drewes, G., Biernat, J., Mandelkow, E. M. & Mandelkow, E. Alzheimer-like paired helical filaments and antiparallel dimers formed from microtubule-associated protein tau in vitro. *J Cell Biol* **118**, 573-584 (1992). <https://doi.org/10.1083/jcb.118.3.573>
- 18 Goedert, M., Spillantini, M. G., Cairns, N. J. & Crowther, R. A. Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. *Neuron* **8**, 159-168 (1992).
[https://doi.org/10.1016/0896-6273\(92\)90117-v](https://doi.org/10.1016/0896-6273(92)90117-v)
- 19 Quinn, J. P., Corbett, N. J., Kellett, K. A. B. & Hooper, N. M. Tau Proteolysis in the Pathogenesis of Tauopathies: Neurotoxic Fragments and Novel Biomarkers. *J Alzheimers Dis* **63**, 13-33 (2018).
<https://doi.org/10.3233/JAD-170959>
- 20 Fowler, S. L. *et al.* Tau filaments are tethered within brain extracellular vesicles in Alzheimer's disease. *Nat Neurosci* **28**, 40-48 (2025). <https://doi.org/10.1038/s41593-024-01801-5>
- 21 Zhang, Z. *et al.* Cleavage of tau by asparagine endopeptidase mediates the neurofibrillary pathology in Alzheimer's disease. *Nat Med* **20**, 1254-1262 (2014).
<https://doi.org/10.1038/nm.3700>
- 22 Derisbourg, M. *et al.* Role of the Tau N-terminal region in microtubule stabilization revealed by new endogenous truncated forms. *Sci Rep* **5**, 9659 (2015). <https://doi.org/10.1038/srep09659>
- 23 Kang, S. S., Ahn, E. H. & Ye, K. Delta-secretase cleavage of Tau mediates its pathology and propagation in Alzheimer's disease. *Exp Mol Med* **52**, 1275-1287 (2020).
<https://doi.org/10.1038/s12276-020-00494-7>
- 24 Gamblin, T. C. *et al.* Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease. *Proc Natl Acad Sci U S A* **100**, 10032-10037 (2003).
<https://doi.org/10.1073/pnas.1630428100>

- 25 Faquetti, M. L. *et al.* Baricitinib and tofacitinib off-target profile, with a focus on Alzheimer's disease. *Alzheimers Dement (N Y)* **10**, e12445 (2024). <https://doi.org/10.1002/trc2.12445>
- 26 Franceschi, C., Garagnani, P., Parini, P., Giuliani, C. & Santoro, A. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat Rev Endocrinol* **14**, 576-590 (2018). <https://doi.org/10.1038/s41574-018-0059-4>
- 27 Upadhyay, A. & Joshi, V. Proteasome Activators and Ageing: Restoring Proteostasis Using Small Molecules. *Subcell Biochem* **107**, 21-41 (2024). https://doi.org/10.1007/978-3-031-66768-8_2
- 28 Hegde, A. N., Duke, L. M., Timm, L. E. & Nobles, H. The Proteasome and Ageing. *Subcell Biochem* **102**, 99-112 (2023). https://doi.org/10.1007/978-3-031-21410-3_5
- 29 Hsieh, Y. C. *et al.* Person-specific differences in ubiquitin-proteasome mediated proteostasis in human neurons. *Alzheimers Dement* **20**, 2952-2967 (2024). <https://doi.org/10.1002/alz.13680>
- 30 Lasagna-Reeves, C. A. *et al.* Identification of oligomers at early stages of tau aggregation in Alzheimer's disease. *FASEB J* **26**, 1946-1959 (2012). <https://doi.org/10.1096/fj.11-199851>
- 31 Narasimhan, S. *et al.* Pathological Tau Strains from Human Brains Recapitulate the Diversity of Tauopathies in Nontransgenic Mouse Brain. *J Neurosci* **37**, 11406-11423 (2017). <https://doi.org/10.1523/JNEUROSCI.1230-17.2017>
- 32 Kumar, M. *et al.* Alzheimer proteopathic tau seeds are biochemically a forme fruste of mature paired helical filaments. *Brain* **147**, 637-648 (2024). <https://doi.org/10.1093/brain/awad378>
- 33 Mair, W. *et al.* FLEXITau: Quantifying Post-translational Modifications of Tau Protein in Vitro and in Human Disease. *Anal Chem* **88**, 3704-3714 (2016). <https://doi.org/10.1021/acs.analchem.5b04509>
- 34 Wenger, K., Viode, A., Kumar, M., Steen, H. & Steen, J. A. Quantitative profiling of posttranslational modifications of pathological tau via sarkosyl fractionation and mass spectrometry. *Nat Protoc* **19**, 1235-1251 (2024). <https://doi.org/10.1038/s41596-023-00939-z>
- 35 Horie, K., Barthelemy, N. R., Sato, C. & Bateman, R. J. CSF tau microtubule binding region identifies tau tangle and clinical stages of Alzheimer's disease. *Brain* **144**, 515-527 (2021). <https://doi.org/10.1093/brain/awaa373>
- 36 Jicha, G. A., Bowser, R., Kazam, I. G. & Davies, P. Alz-50 and MC-1, a new monoclonal antibody raised to paired helical filaments, recognize conformational epitopes on recombinant tau. *J Neurosci Res* **48**, 128-132 (1997). [https://doi.org/10.1002/\(sici\)1097-4547\(19970415\)48:2<128::aid-jnr5>3.0.co;2-e](https://doi.org/10.1002/(sici)1097-4547(19970415)48:2<128::aid-jnr5>3.0.co;2-e)
- 37 Takeda, S. *et al.* Neuronal uptake and propagation of a rare phosphorylated high-molecular-weight tau derived from Alzheimer's disease brain. *Nat Commun* **6**, 8490 (2015). <https://doi.org/10.1038/ncomms9490>
- 38 Louros, N. *et al.* Local structural preferences in shaping tau amyloid polymorphism. *Nat Commun* **15**, 1028 (2024). <https://doi.org/10.1038/s41467-024-45429-2>
- 39 Chen, D. *et al.* Tau local structure shields an amyloid-forming motif and controls aggregation propensity. *Nat Commun* **10**, 2493 (2019). <https://doi.org/10.1038/s41467-019-10355-1>
- 40 Fichou, Y. *et al.* Tau-Cofactor Complexes as Building Blocks of Tau Fibrils. *Front Neurosci* **13**, 1339 (2019). <https://doi.org/10.3389/fnins.2019.01339>
- 41 Fichou, Y. *et al.* Cofactors are essential constituents of stable and seeding-active tau fibrils. *Proc Natl Acad Sci U S A* **115**, 13234-13239 (2018). <https://doi.org/10.1073/pnas.1810058115>
- 42 Seidler, P. M. *et al.* Structure-based inhibitors of tau aggregation. *Nat Chem* **10**, 170-176 (2018). <https://doi.org/10.1038/nchem.2889>
- 43 Rawlings, N. D. *et al.* The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. *Nucleic Acids Res* **46**, D624-D632 (2018). <https://doi.org/10.1093/nar/gkx1134>

- 44 Liu, B. *et al.* Deubiquitinating enzymes (DUBs): decipher underlying basis of neurodegenerative diseases. *Mol Psychiatry* **27**, 259-268 (2022). <https://doi.org/10.1038/s41380-021-01233-8>
- 45 Beninga, J., Rock, K. L. & Goldberg, A. L. Interferon-gamma can stimulate post-proteasomal trimming of the N terminus of an antigenic peptide by inducing leucine aminopeptidase. *J Biol Chem* **273**, 18734-18742 (1998). <https://doi.org/10.1074/jbc.273.30.18734>
- 46 Sampognaro, P. J. *et al.* Mutations in alpha-synuclein, TDP-43 and tau prolong protein half-life through diminished degradation by lysosomal proteases. *Mol Neurodegener* **18**, 29 (2023). <https://doi.org/10.1186/s13024-023-00621-8>
- 47 Stancu, I. C. *et al.* Aggregated Tau activates NLRP3-ASC inflammasome exacerbating exogenously seeded and non-exogenously seeded Tau pathology in vivo. *Acta Neuropathol* **137**, 599-617 (2019). <https://doi.org/10.1007/s00401-018-01957-y>
- 48 Ising, C. *et al.* NLRP3 inflammasome activation drives tau pathology. *Nature* **575**, 669-673 (2019). <https://doi.org/10.1038/s41586-019-1769-z>
- 49 Lin, W. L., Dickson, D. W. & Sahara, N. Immunoelectron microscopic and biochemical studies of caspase-cleaved tau in a mouse model of tauopathy. *J Neuropathol Exp Neurol* **70**, 779-787 (2011). <https://doi.org/10.1097/NEN.0b013e31822ac9c9>
- 50 de Calignon, A. *et al.* Caspase activation precedes and leads to tangles. *Nature* **464**, 1201-1204 (2010). <https://doi.org/10.1038/nature08890>
- 51 Kramer, A., Green, J., Pollard, J., Jr. & Tugendreich, S. Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics* **30**, 523-530 (2014). <https://doi.org/10.1093/bioinformatics/btt703>
- 52 Seifert, U. *et al.* Immunoproteasomes Preserve Protein Homeostasis upon Interferon-Induced Oxidative Stress. *Cell* **142**, 613-624 (2010). <https://doi.org/10.1016/j.cell.2010.07.036>
- 53 Murata, S., Takahama, Y., Kasahara, M. & Tanaka, K. The immunoproteasome and thymoproteasome: functions, evolution and human disease. *Nat Immunol* **19**, 923-931 (2018). <https://doi.org/10.1038/s41590-018-0186-z>
- 54 Pishesha, N., Harmand, T. J. & Ploegh, H. L. A guide to antigen processing and presentation. *Nat Rev Immunol* **22**, 751-764 (2022). <https://doi.org/10.1038/s41577-022-00707-2>
- 55 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). <https://doi.org/10.1074/jbc.M808759200>
- 56 de Silva, R. *et al.* Pathological inclusion bodies in tauopathies contain distinct complements of tau with three or four microtubule-binding repeat domains as demonstrated by new specific monoclonal antibodies. *Neuropathol Appl Neurobiol* **29**, 288-302 (2003). <https://doi.org/10.1046/j.1365-2990.2003.00463.x>
- 57 Akiyama, K. *et al.* cDNA cloning and interferon gamma down-regulation of proteasomal subunits X and Y. *Science* **265**, 1231-1234 (1994). <https://doi.org/10.1126/science.8066462>
- 58 Andorfer, C. *et al.* Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. *J Neurochem* **86**, 582-590 (2003). <https://doi.org/10.1046/j.1471-4159.2003.01879.x>
- 59 Wenger, K. *et al.* Common mouse models of tauopathy reflect early but not late human disease. *Mol Neurodegener* **18**, 10 (2023). <https://doi.org/10.1186/s13024-023-00601-y>
- 60 Bhaskar, K. *et al.* Regulation of tau pathology by the microglial fractalkine receptor. *Neuron* **68**, 19-31 (2010). <https://doi.org/10.1016/j.neuron.2010.08.023>
- 61 Parra Bravo, C. *et al.* Human iPSC 4R tauopathy model uncovers modifiers of tau propagation. *Cell* **187**, 2446-2464 e2422 (2024). <https://doi.org/10.1016/j.cell.2024.03.015>
- 62 Wisniewski, J. R., Zougman, A., Nagaraj, N. & Mann, M. Universal sample preparation method for proteome analysis. *Nat Methods* **6**, 359-362 (2009). <https://doi.org/10.1038/nmeth.1322>

- 63 Jicha, G. A., O'Donnell, A., Weaver, C., Angeletti, R. & Davies, P. Hierarchical phosphorylation of
691 recombinant tau by the paired-helical filament-associated protein kinase is dependent on cyclic
692 AMP-dependent protein kinase. *J Neurochem* **72**, 214-224 (1999).
693 <https://doi.org/10.1046/j.1471-4159.1999.0720214.x>
- 64 MacLean, B. *et al.* Skyline: an open source document editor for creating and analyzing targeted
695 proteomics experiments. *Bioinformatics* **26**, 966-968 (2010).
696 <https://doi.org/10.1093/bioinformatics/btq054>
- 65 Ehrenberg, A. J. *et al.* A manual multiplex immunofluorescence method for investigating
698 neurodegenerative diseases. *J Neurosci Methods* **339**, 108708 (2020).
699 <https://doi.org/10.1016/j.jneumeth.2020.108708>
- 700 66 Lagomarsino, V. N. *et al.* Stem cell-derived neurons reflect features of protein networks,
701 neuropathology, and cognitive outcome of their aged human donors. *Neuron* **109**, 3402-3420
702 e3409 (2021). <https://doi.org/10.1016/j.neuron.2021.08.003>

METHODS

Sarkosyl fractionation of post-mortem tissue

To identify endogenous proteolytic cleavage sites, data for semi-tryptic peptide analyses were studied from a recently published set of MS data⁶. For this data set, human post-mortem parietal cortex (Brodmann area, BA39 – angular gyrus) specimens from AD patients and non-demented age-matched CTR subjects were obtained from 5 different brain banks: 1) the Neurodegenerative Disease Brain Bank (NDBB), Memory and Aging Center, University of California, San Francisco (UCSF), CA; 2) the University of Maryland Brain & Tissue Bank at the University of Maryland School of Medicine, Baltimore, MD; 3) the Harvard Brain Tissue Resource Center, McLean Hospital, Harvard Medical School, Belmont, MA; 4) the University of Miami (UM) Brain Endowment Bank, Miller School of Medicine, Miami, MD; 5) the Human Brain and Spinal Fluid Resource Center (HBSFRC), VA West Los Angeles Healthcare Center, Los Angeles, CA. Tissues from brain banks 2) to 5) were acquired through the NIH NeuroBioBank (U.S. Department of Health and Human Services, National Institutes of Health). CTR subjects had no clinical diagnosis of dementia, and all the AD subjects fulfilled the NIA-Reagan criteria for a high likelihood of AD. Neuropathological details for subjects are summarized in Table 1. Pathological and clinical information, if available, were de-identified.

To isolate insoluble tau, 0.25-0.35 g sections of cortical brain specimens were homogenized in 5 volumes of lysis buffer (25 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl, 10 mM EDTA, 10 mM EGTA, 1 mM DTT, 10 mM nicotinamide, 2 μ M trichostatin A, phosphatase inhibitor cocktail (Sigma), and protease inhibitor cocktail (Roche)), using a Precellys® bead beater and clarified by centrifugation at 11,000 x g for 30 min at 4°C. Lysates were extracted with 1% sarkosyl for 60 min at 4°C and ultra-centrifuged at 100,000 x g for 2 h at 4°C. The soluble supernatant was

removed, and the SIF was solubilized in 1% SDS. SSF and SIF tau fractions were diluted with 8 M urea and processed separately using filter-aided sample preparation (FASP)⁶². Protein mixtures were digested with 12.5 ng/ μ L trypsin (sequencing grade modified trypsin, Promega, Madison, WI) overnight at 37°C. Acidified peptides were desalted using C18 extraction plates (Waters). Vacuum-dried peptides were reconstituted in sample buffer (5% formic acid, 5% acetonitrile (ACN)) containing indexed retention time (iRT) peptides (Biognosys).

Preparation of LMW/HMW tau

Analysis of cleavage sites in HMW and LMW tau was performed on previously published raw MS data⁶. HMW and LMW were prepared as described³⁷. Briefly, 300-500 mg frozen brain tissue from the frontal cortex of four patients with AD and four CTR subjects were homogenized in 5x volumes of cold PBS (+ protease inhibitor), which was centrifuged at 10,000X g for 10 min at 4°C. The supernatants (10,000 g extract) were fractionated by molecular weight using SEC with an AKTA purifier 10 (GE Healthcare). Fractions were collected every 1 min (0.5 ml/fraction) from 5.5 mL elution volume (Fraction 2) to 16.5 ML (Fraction 20). For MS analysis, fractions 3 and 4 were processed for HMW tau and fractions 14, 16, and 18 for LMW tau. Four volumes (vol/vol) of cold acetone (-20°C) were added to each fraction to precipitate the protein, followed by vigorous vortex and incubation for 90 min at -20°C. Pelleted protein was collected by centrifugation at 13,000X g for 10 min at 4°C, aspiration of the supernatant, and resuspension of the pellets in 105 μ L of PBS. Samples were digested with trypsin using the FASP protocol after reduction with TCEP and alkylation using iodoacetamide. Peptide eluates were acidified and desalted using reversed-phase C-18 microspin columns (SEMSS18R, Nest Group), vacuum dried and frozen at -20°C prior to LC-MS/MS analysis.

Preparation of soluble MC1-isolated tau filaments

MS analysis on MC1-isolated tau was performed on previously published data⁶. Briefly, MC1-isolated tau was obtained from Peter Davies (Feinstein Institutes for Medical Research, Manhasset, NY 11030, USA), purified using MC-1 antibody immunoaffinity columns from 3 separate AD patients as previously described⁶³. Purified MC-1 isolated tau was digested with trypsin using the FASP method as described above after reduction with DTT and alkylation with 1% acrylamide. Heavy tau standard peptide was spiked in for FLEXITau experiments. Peptide eluates were acidified and desalted using reversed-phase C-18 microspin columns (SEMSS18R, Nest Group), vacuum dried and frozen at -20°C before LC-MS/MS analysis.

LC-MS/MS analysis of AD tau filaments

SIF AD (n = 46) patients and 40 matched CTR individuals were analyzed using a Q Exactive HF and QE MS (Thermo Fisher Scientific, Bremen) coupled to a micro-autosampler AS2 and a nanoflow HPLC pump (Eksigent, Dublin, CA). Peptides were separated using an in-house packed C18 analytical column (Magic C18 particles, 3 μ m, 300 Å, Michrom Bioresource) or a PicoChip column (150 μ m x 10 cm Acquity BEH C18 1.7 μ m 130 Å, New Objective, Woburn, MA) column over a linear 120 min gradient starting from 95% buffer A (0.1% (v/v) formic acid in HPLC-H₂O) and 5% buffer B (0.2% (v/v) formic acid in acetonitrile) to 35% buffer B. The MS was operated in positive DDA top 20 mode with the following MS1 scan settings: mass-to-charge (m/z) range 300-1650, resolution 60,000@ m/z 400, AGC target 3e6, max IT 20ms. MS2 scan settings: resolution 30000 @ m/z 400, AGC target 1e5, max IT 25ms, isolation window m/z 1.4, NCE 27, charge state exclusion unassigned, 1, >8, peptide match preferred, exclude isotopes on and dynamic exclusion of 20s.

For LMW/HMW tau, randomized samples (SEC fractions 2,3,4 and 14,16,18 for 4 AD and 4 CTR human brain samples) were analyzed in duplicates using a Q Exactive™ MS (Thermo) coupled to a micro-autosampler AS2 and a nanoflow HPLC pump (Eksigent). For DDA experiments LC-MS/MS analysis, peptides were loaded on a capflow PicoChip column (150 µm x 10 cm Acquity BEH C18 1.7 µm 130 Å, New Objective, Woburn, MA) with 2 µL/min solvent A. The MC1-immunopurified PHF tau samples were run using the same parameters as the LMW/HMW tau on the Q Exactive MS.

For each run, the column was first equilibrated with 4 column volumes of 100% mobile phase A and then the sample was loaded; both steps were performed at 900 bars. After the gradient was completed, the concentration of mobile phase B was ramped up to 80% over the course of 10 minutes and then kept constant at 80% for 10 minutes.

FLEXITau analysis

FLEXITau is an MS-based strategy that is based on the addition of a heavy full-length 2N4R tau protein to each sample prior to digestion. Because all heavy tau peptides are present at equimolar amounts, the ratio of light to heavy peptide can be used to infer the extent of modification/enrichment for each peptide. In brief, the heavy tau standard was expressed in a cell-free wheat germ expression system containing a full complement of amino acids, with heavy isotopes (i.e. ¹³C and ¹⁵N) labeled lysine, arginine, and aspartate. The standard was purified using Ni-Sepharose beads (Ni-Sepharose High-Performance resin, GE Healthcare). Heavy tau standard was digested along with samples and spiked in to reach an L/H ratio of approximately 1:1.

LC-SRM measurements of tau L/H peptide ratios were performed as described previously³³. The FLEXITau SRM assay quantified an in-house list of validated transitions for unmodified tau peptides that are highly reproducible with low CV. Peptide mixtures were analyzed on a triple

quadrupole MS (5500 QTRAP, Sciex) using a micro-autosampler AS3 and a nanoflow UPLC pump (both Eksigent/Sciex), using the trap-elute chip system (cHiPLC nanoflex, Eksigent). Briefly, peptides were first loaded onto the trap-chip (200 μm x 75 μm , ChromXP C18-CL 3 μm 120 Å, Nano cHiPLC Eksigent) and then separated using a 120 min gradient from 95% buffer A (0.1% (v/v) formic acid in HPLC- H_2O) and 5% buffer B (0.2% (v/v) formic acid in ACN) to 35% buffer B on the analytical column-chip (75 μm x 15 cm, ChromXP C18-CL 3 μm 120 Å, Nano cHiPLC Eksigent). The retention time window was set to 5 min and the total scan time to 1.2 s, which ensured a dwell time of over 20 ms per transition. To avoid sample carryover, blanks were analyzed between every SRM run. Samples were run in randomized order, with 3 technical replicates per sample. SRM data were analyzed and validated in Skyline (version 2.6, MacCoss Lab Software, University of Washington, Seattle, WA)⁶⁴. Only peptides quantified in every sample were included in downstream analysis yielding a final peptide list consisting of 17 tau peptides. To calculate the relative abundance of peptides, the L/H ratio of peak areas for each peptide was divided by the average of the three tau peptides with the highest ratio, representing the unmodified peptides with the highest relative abundance.

Identification of endogenous tau cleavage sites and analysis of differential expression from human tissue

Raw data from human brain derived from the Q Exactive/Q Exactive HF or the timsTOF Pro were analyzed with MaxQuant Version 1.6.6.0 or 1.6.10.43, respectively, using an in-house database for SSF and SIF datasets, and the Uniprot database including isoforms for MC1-isolated tau, LMW/HMW tau, cellular, and the *in vitro* tau degradation assays. For the FL tau biosensor experiments, the data were acquired from the timsTOF pro2 mass spectrometer and analyzed with Fragpipe version 18.0. The UniProt database, which includes various tau isoforms, was modified

817 to add entries for the mutant and WT fusion protein constructs. For all differential expression
818 analyses, databases containing only reviewed entries and no isoforms were used for database
819 searches. For data acquired on the Orbitrap systems, mass tolerances for the first search were set
820 to 20 ppm and for the second search to 4.5 ppm, while for the TIMS-DDA, first and main search
821 peptide tolerance were decreased to 30 ppm; other “Type”-specific settings were kept at default.
822 To identify endogenous proteolytic cleavage sites, we included semi-tryptic peptide database
823 searches within the analysis workflow. The digestion mode was set to specific trypsin (one tryptic
824 site) with up to two missed cleavages for the differential expression analysis, whereas for the semi-
825 tryptic searches, the digestion mode was switched to semi-specific (one tryptic site).
826 Carbamidomethylation on cysteine (57.021464) was set as a fixed modification for LMW/HMW
827 tau searches, and propionamide on cysteine (+71.0371137878 Da) was set for all searches.
828 Oxidation of methionine (+15.9949146221 Da) and acetylation of the N-terminus
829 (+42.0105646863 Da) were included as variable modifications. For all other search parameters,
830 the default settings were used. Identified peptides with a prior amino acid that was not K or R were
831 designated as N-terminal semi-tryptic peptides, and peptides not ending in K or R were designated
832 as C-terminal semi-tryptic peptides. Correct identification was manually validated, and only
833 samples in which peptides were identified by MS/MS were used for postmortem tissue population
834 frequency counts and tau degradation assay identifications. All peptides found by MaxQuant were
835 exported as .txt files, normalized, and filtered in Excel. For the SIF/SSF analysis, sites identified
836 in fewer than 15% of cases were filtered out, with the cut-off determined by the distribution of
837 identification frequencies.

Tau fragment expression and purification

The 8 fragments defined by combinations of the N-terminal and C-terminal cleavage sites were produced by recombinant expression in *E. coli*. cDNA for each tau fragment was inserted into Addgene plasmid #29663 with HiFi assembly. The resulting vector encodes a fusion protein consisting of a hexahistidine tag followed by eGFP and a tobacco etch virus (TEV) enzymatic cleavage site attached to the desired fragment of tau. The plasmid was transformed into DH5 α and BL21(DE3) cells. Each fragment was named with a number followed by a letter, with increasing numbers (1-4) corresponding to a shorter N-terminus, and a/b corresponding to the N368-K369 and Y394-K395 cleavages, respectively. *E. coli* BL21 (DE3) cells were transfected with constructed DNA variants and stored as frozen glycerol stock at -80°C . Cells from glycerol stock were grown in 10 mL luria broth (LB, Sigma Aldrich, L3022) overnight and then used to inoculate 1 L of fresh LB. Growth of cells was performed at 37°C , 200 rpm with the addition of 10 $\mu\text{g/mL}$ kanamycin (Fisher Scientific, BP906) until the optical density at $\lambda = 600\text{nm}$ reached 0.6-0.8. Expression was induced by incubation with 1 mM isopropyl- β -D-thiogalactoside (Fisher Bioreagents, BP175510) for 3 hr. Cells were harvested with centrifugation at 5000 g for 30 min. Harvested cells were resuspended in lysis buffer (Tris-HCl, pH = 7.4, 100 mM NaCl, 0.5 mM DTT, 0.1 mM EDTA) added with 1 Pierce protease inhibitor tablet (Thermo Scientific, A32965), 1 mM PMSF, 2 mg/mL lysozyme, 20 $\mu\text{g/mL}$ DNase (Sigma, DN25) and 10 mM MgCl_2 (10 mM), and incubated on ice for 30 min. Samples were incubated at 30°C for 20 minutes, then centrifuged at 10,000 rpm for 10 min to remove cell debris. 1 mM PMSF was added again, and the resulting supernatant was incubated for at least 4 h with Ni-NTA resins pre-equilibrated in buffer A (20 mM sodium phosphate, pH = 7.0, 500 mM NaCl, 10 mM imidazole, 100 μM EDTA).

860 The resin was loaded onto a column and washed with 20 mL of buffer A and 25 mL of buffer B
861 (20 mM sodium phosphate, pH = 7.0, 1 M NaCl, 20 mM imidazole, 0.5 mM DTT, 100 μ M EDTA).
862 Purified protein was eluted with 15 mL of buffer C (20 mM sodium phosphate, pH = 7.0, 0.5 mM
863 DTT, 100 mM NaCl, 300 mM imidazole), or until the resin no longer contained traces of GFP
864 visible. The protein was concentrated to a volume of 2.5mL and was buffer exchanged into TEV
865 buffer (50mM Tris buffer, pH7, 100mM NaCl, 50mM CaCl₂) to remove the imidazole and prepare
866 the protein for TEV cleavage. 35 μ L of TEV (~1mg/mL stock) prepared in-house was added to the
867 solution and incubated at 4°C overnight with gentle rotation.

868 To separate each fragment from GFP and TEV peptide, a cation exchange (GE CMFF sepharose
869 column) separation was followed by size exclusion chromatography using a Biorad superdex70
870 column. Cation exchange was conducted at pH 8 with a gradient of 0-1 M NaCl elution profile.
871 All peaks showing a UV-Vis signal were collected and were concentrated to 250 μ L. The SEC
872 column was pre-equilibrated with 2 column volumes of working buffer, and the protein sample
873 was loaded onto the sample loop. 0.5mL fractions were collected and run on an SDS page gel
874 stained with Coomassie blue to determine the fractions containing tau. All fractions containing tau
875 were pooled and concentrated to approximately 200 μ M as determined by the UV-Vis absorption
876 at 274 nm.

877 **Recombinant tau fibrillization**

878 Fibrils of recombinant tau fragments used in ThT and TEM assays were generated with 50 μ M
879 total protein concentration. 12.5 μ M heparin (Galen laboratory supplies, HEP001) was used for a
880 4:1 molar ratio of tau:heparin. Samples were incubated at 37°C in 384-well Corning plates. Fibrils
881 used in HEK293T seeding assays were formed at 120 μ M protein concentration with 30 μ M
882 heparin.

***In vitro* seeding and Thioflavin T experiments**

Seeds for use in 2nd generation seeding studies were generated following the procedure described above at 50 μ M protein concentration. The sample was vigorously pipetted to evenly disperse fibrils and was added at a 2.5mol% (1.25 μ M protein concentration) to a 50 μ M sample of new protein. Aggregation was followed with ThioflavinT assays. Thioflavin T (ThT) assays for Beta-sheet content were conducted with 50 μ M protein content, and 20mM ThT in a 384-well Corning plate. A BioTek synergy2 plate reader was used with temperature control set at 37°C.

Transmission Electron Microscopy (TEM)

TEM imaging was conducted with a 200 kV FEI Tecnai G2 Sphera Microscope. Samples were blotted onto 200-mesh Formvar copper grids and were negative-stained with uranyl acetate.

Cellular seeding assays

For recombinant peptide seeding assays, HEK293T cells stably expressing mCerulean-K18 (P301L/V337M) or mCerulean-Tau187 (P301L/V337M) were cultured in DMEM supplemented with 10% FBS, 100 μ g/ml penicillin/streptomycin. Cultures were maintained in a humidified atmosphere of 5% CO₂ at 37°C. Cells were plated in a 96-well plate and the following day tau species were sonicated for 30 s with the micro tip of a Qsonica sonicator and transfected (50 ng/ μ L final) using Lipofectamine 2000 (ThermoFisher Scientific). For the quantification, the number of cells containing one or several puncta was counted and divided by the total number of cells in the well. Each well on a cultured plate provides one point. Quantification results originate from three independent cultures.

***In vivo* seeding assay in tau transgenic mice**

Three-month-old male PS19 tau transgenic mice (B6;C3-Tg(Prnp-MAPT*P301S)PS19Vle/J, Jackson Laboratory) overexpressing the human 1N4R isoform of tau with a P301S mutation were

used (n = 5/group). Following anesthesia with 1.5% (vol/vol) isoflurane, mice were placed onto a stereotaxic frame, ophthalmic ointment was placed on the eyes, and lidocaine (lidocaine hydrochloride, 2 mg/kg) was subcutaneously administered under the scalp skin before the beginning of surgery. Mice were intracerebrally injected into the CA1 of the hippocampus (anteroposterior -2.4 mm from bregma, mediolateral +/- 1.5 mm from midline, and dorsoventral -1.6 mm from the skull surface) using a 33-gauge blunt tip needle linked to a Hamilton syringe. For each mouse, the right hemisphere was injected with 1.2 µg/µl Tau2b peptide in PBS (2.5 µl/site) and the contralateral left hemisphere was injected with 2.5 µl PBS at a flow rate of 0.2 µl/min. After surgery, the skin was sutured, and buprenorphine (buprenorphine hydrochloride 0.05 mg/ml) was subcutaneously administered every 12 hours for 72 hours. Post-surgery analgesia was further ensured by the administration of acetaminophen (300 mg/kg) in drinking water for 72 hours.

Three months after injection mice were euthanized with CO₂ and transcardially perfused with ice-cold PBS followed by ice-cold 4% PFA for 5 min at 20 ml/min. The brains were removed, post-fixed for 24 hours in 4% PFA and cryoprotected in 30% sucrose for 72 hours before sectioning. Coronal sections (40 µm-thick) were sliced using a freezing microtome and were collected at 400 µm intervals.

All animal care, housing, and experiments were performed in compliance with guidelines established by the Massachusetts General Hospital institutional animal care and use committee and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Thioflavin-S staining and immunofluorescence imaging

For Thioflavin-S staining, free-floating 40 µm-thick coronal sections were washed in PBS, mounted on Superfrost Plus slides (Fisher Scientific), and let dry overnight. The following day,

sections were rinsed in PBS before incubation in 0.05% Thioflavin-S (T1892-25G, Sigma-Aldrich) in 50% ethanol for 8 min in the dark. Sections were then briefly rinsed in 80% ethanol and transferred to dH₂O before placing the coverslip. Images were acquired at 20X using a VS120 (Olympus) slide scanner. Manual counting of Thioflavin-S-positive somas in the pyramidal layer of the hippocampus was performed blinded using QuPath software on 3 sections adjacent to the injection site.

For Iba1 and anti-Tau AT8 co-staining, free-floating 40 µm-thick coronal sections were washed in PBS, blocked with 4.5% normal goat serum (Vector Laboratories) in 0.2% Triton X-100 PBS for 1 hour then incubated in primary antibody solution (biotinylated mouse anti-Tau AT8, 1: 500, MN1020B Thermofisher; rabbit anti-Iba1, 1:1000, 019-19741 WAKO) in blocking solution at 4°C overnight. The next day, sections were washed in PBS and incubated in secondary antibody solution (anti-mouse Alexa Fluor 488, anti-rabbit Alexa Fluor 647, Invitrogen) in blocking solution for 1 hour before being mounted on Superfrost Plus slides and coverslipped with DAPI-containing Fluoromount-G (0100-20, Southern Biotech). Images were acquired at 20X using a NanoZoomer (Hamamatsu). Manual segmentation of the CA1-2-3 of the hippocampus and thresholding of each fluorescent channel was then performed on 3 sections adjacent to the injection site using QuPath software.

Triple-labelled immunofluorescence in human brain tissue

Immunofluorescence (IF) assays are prone to batch variations. To minimize this risk, we created tissue microarrays (TMAs) by using a 1 cm biopsy punch to sample the middle frontal gyrus (MFG) at the level of the optical chiasm in two AD (A3B3C3) and two healthy controls (A0B1C0) cases. An 8-µm thick section of the TMA block was mounted on histological slides before undergoing multiplex IF for detecting neurons (NeuN, 266004, 1:300, guinea pig, Synaptic

System), p-Tau (pT231, EPR2488, 1:8000, rabbit, Abcam), TauAT8 (pS202/pT205, MN1020, 1:4000 mouse, Thermo Fisher), Beta-2M (Beta-2M, sc13565, 1:25, mouse, Santa Cruz), MHC/F3 (MHC/F3, sc55582, 1:100, mouse, Santa Cruz), and either PSMB8 (PSMB8, GTX55763, 1:250, rabbit, Genetex), PSMB9 (LMP2, ab3328, 1:250, rabbit, Abcam), or PSMB10 (PSMB10, GTX32818, 1:250, rabbit, Genetex). The general procedure and quality control steps of our IF protocol have been described in detail elsewhere⁶⁵.

Western blotting of AD brain tissue

A western blot analysis was performed on the SIF from 3 CTR and 3 AD subjects, loading 10 µg of protein per sample. To visualize C-terminal tau fragments, blots were probed using the MC1 antibody (from Peter Davies, 1:1000), which recognizes the C-terminal 396 and 404 phosphorylation sites of tau, followed by IRDye® 680RD Goat anti-Mouse antibody (Licor, 1:5000).

As for the cross-reactivity test of PSMB8 and Tau12 antibodies, SDS PAGE was performed for AD and CTR sarkosyl insoluble fractions (n = 5 AD and n = 5 CTR human subjects were pooled per lane). 10 µg proteins of each sample were denatured and run on a BOLT 4-12% Bis-Tris gel. Then the proteins were transferred to a PVDF membrane and stained with LICOR REVERT 700 total protein stain (Licor). The membrane was imaged on a Licor Odyssey CLx imaging system and then destained with Revert 700 Wash Solution (P/N 926-11012), followed by blocking with Intercept® Blocking Buffer. The membrane was then probed with PSMB8 (GTX55763, 1:250, rabbit, Genetex) and Tau12 (MAB2241, 1:2000, mouse, Sigma) antibodies followed by IRDye® 800CW Goat anti-Rabbit and anti-Mouse antibody (Licor). The membrane was subsequently scanned on the Odyssey CLx imaging system.

Lentivirus production

The lentiviral plasmid constructs were made by Genecopoeia, Inc. In brief, the open-reading frame of gene coding for human tau isoform 0N4R (NCBI accession No. 016834.4) with or without P301S mutation was subcloned into pReceiver-Lv vector with CMV promotor and C-terminus fused to EYFP. The lentiviruses were made by viral core at Boston Children's Hospital. In brief, each transfer plasmid was co-transfected with lentiviral packaging plasmids into HEK293T cells using PEI max. 3 days after transfection, 72 ml virus-containing culture medium was cleared by centrifugation at 1500 rpm for 5 min and then passed through a 0.45 μ m filter. The concentration of lentivirus using ultracentrifugation was performed with an XE90 Beckman Coulter centrifuge using a SW32Ti rotor. Centrifugation was performed for 2 h at 18000 rpm. The supernatant was completely removed, and virus pellets were resuspended gently in 150 μ L DPBS with 0.001% F68. 10 μ L aliquots were made and stored at -80°C until use.

Cell culture, lentiviral transduction, FACS sorting, and stable cell line generation

To generate the FL tau biosensor cell lines with or without P301S mutation, HEK293T cells (ATCC) were cultured in DMEM (ThermoFisher Scientific) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (ThermoFisher Scientific). The cells were maintained in a humidified incubator at 37°C supplied with 5% CO₂ (v/v) and passaged by 0.25% trypsin/EDTA digestion and dissociation every other day, and culture mediums were tested for myoblast contamination. To generate a stable cell line, HEK293T cells were plated on a 6-well plate and infected the next day with lentivirus expressing human Tau-0N4R(P301S) or Tau-0N4R with C-terminus fused EYFP. The medium was changed 1 day after infection, and transduced cells were passed to a 10 cm culture plate. Once the YFP signal can be seen in 80~90% of total cells under a fluorescent microscope, the cells were digested and dissociated as single cells by 0.25%

trypsin/EDTA, pelleted by centrifugation at 1200 rpm for 3 min, and resuspended in DMEM medium supplemented with 2% fetal bovine serum and 1% penicillin/streptomycin. Then, 1 ml cell suspension containing $\sim 8 \times 10^6$ cell was subjected to FACS sorting (FACS Aria II flow cytometer, flow cytometry research facility at Boston Children's Hospital), and a population of single live cells with YFP signal was collected and plated on a 6-well plate. Two days later, the cells were dissociated as single cells as described above and plated on a 10 cm culture plate at a density as low as ~ 120 cells in total. After 10 days single cells expanded to mono-clones so that multiple clones could be isolated under a microscope and transferred separately to a 96-well plate. Finally, multiple mono-clonal stable cell lines with homogeneous and stable expression of human Tau-0N4R(P301S)-EYFP or Tau-0N4R-EYFP were selected, expanded, and cryopreserved in liquid nitrogen for future study.

IFN- γ /TNF treatment and siRNA transfection to cell culture, baricitnib co-treatment with IFN- γ /TNFs, immunofluorescence cell staining, and microscopy imaging

The IFN- γ /TNF treatment experiments were performed in the monoclonal cell line stably expressing human Tau-0N4R (P301S)-YFP or Tau-0N4R (WT)-YFP plated in a 96-well plate. Both TNF and IFN- γ (Pepro tech) were applied to the culture medium at a final concentration of 50~200 ng/ml for each factor, and H₂O was used as a control to IFN- γ /TNFs treatment. Cells were continuously maintained in IFN γ /TNF-containing medium throughout the entire experiment unless otherwise stated.

LDH assay was conducted by protocol from a standard assay kit (Sigma). Take culture media of each experimental condition (3 repeats for each condition) to a new 96-well plate, and pre-aspirate a small volume before moving 50 μ L/well over to a new 96-well plate for LDH assay. Then make

1019 an LDH solution (48 μ L LDH buffer + 2 μ L LDH substrate). Add 50 μ L LDH solution to each
1020 well and cover the plate with foil before running plates on the LDH scanner.

1021 For knock-down experiments, the siRNA targeting PSMB9 and 10 were ordered from Millipore
1022 Sigma. Two different sequences were tested for PSMB10 target, four different sequences were
1023 tested for PSMB9, and then one sequence was chosen for final experiments. The
1024 MISSION[®] siRNA Universal Negative Control #1(SIC001) from Millipore Sigma was used as a
1025 negative control to specific targeting siRNAs. For PSMB10 knockdown experiment, specific
1026 siRNA or control siRNA were transfected by Lipofectamine 2000 (ThermoFisher Scientific) at a
1027 final concentration of 200 nM. For PSMB9 knockdown experiment, specific or control siRNA
1028 were transfected by DharmaFECT 1 Transfection Reagent (T-2005-01, Horizon) at final volume
1029 of 6ul/well with 200nM siRNA each. The IFN- γ /TNFs were added to the culture medium the next
1030 day after siRNA transfection.

1031 For the Baricitinib experiment, cells were plated on a 96-well plate in triplicate. After 24 hours,
1032 cells were treated with 100 nM Baricitinib (in DMSO) or DMSO as control. Then the cells were
1033 treated with IFN- γ /TNFs at a final concentration of 200ng/ml, and Baricitinib (or DMSO) was
1034 supplemented to keep the final concentration at 100nM. Cells were treated with IFN- γ /TNFs and
1035 Baricitinib or DMSO for 3 days. 3~8 days after IFN- γ /TNF treatment, the medium was removed,
1036 and the cells were fixed by 8% paraformaldehyde in 0.01 M PBS for 15 minutes. Following three
1037 washes with 0.01 M PBS, cells were permeabilized with 0.15 % Triton-X100/0.01 M PBS for 10
1038 minutes, incubated in blocking buffer (8% normal goat serum, 1% BSA, 0.15 % Triton-X100 in
1039 0.01 M PBS) for 1 hour at room temperature and followed by incubation with primary antibodies
1040 mouse Anti-Tau-RD4 (4-repeat isoform RD, clone 1E1/A609) (1:200, EMD Millipore) diluted in
1041 blocking buffer. Following 5 washes with 0.01 M PBS, cells were incubated with Alexa Fluor 568

conjugated goat anti-mouse IgG secondary antibodies or Alexa Fluor 647 conjugated goat anti-mouse IgG secondary antibodies (both 1:700, Invitrogen, Carlsbad, California) for 1 hour at room temperature . After 5 washes with 0.01M PBS, cells were counterstained nuclei with DAPI and subjected to imaging.

Co-staining experiments were carried out as below. 7 days after IFN- γ /TNF treatment, the medium was removed, and the cells were fixed with 8% paraformaldehyde in 0.01 M PBS for 15 minutes. Following three washes with 0.01M PBS, cells were permeabilized with 0.15 % Triton-X100/0.01 M PBS for 10 minutes, incubated in blocking buffer (8% normal goat serum, 1% BSA, 0.15 % Triton-X100 in 0.01 M PBS) for 1 hour at room temperature and followed by incubation with primary antibodies overnight at 4°C. (Mouse Anti-Tau-RD4 was conjugated with Alexa Fluor647 using the Alexa Fluor® 647 Conjugation Kit (Fast) - Lightning-Link® (ab269823). Mouse Anti-Tau-HT7 (MN1000, Thermo Fisher) and Mouse Anti-Tau12 were conjugated with Alexa Fluor568 using the Alexa Fluor® 568 Conjugation Kit (Fast) - Lightning-Link® (ab269821). Then the conjugated antibodies were diluted (1:200) in blocking buffer). After 5 washes with 0.01 M PBS, cells were counterstained nuclei with DAPI and subjected to imaging.

Cell immunostaining in a 96-well plate was imaged by a Nikon Ti Eclipse inverted microscope with a Coolsnap HQ2 camera (Photometrics, Tucson, Arizona) and Nikon Elements software (Nikon, Melville, New York). Images were acquired as large images stitched with 6x6 fields covering the whole area of a well using a 10X Plan Apo objective. Data were analyzed using Fuji software (National Institutes of Health) and cropped images were used as representative. The measurement and quantification of the assay were processed as described below using Fuji software (National Institutes of Health). A rectangular area covering > 50% total area of a whole well from the center (96-well plate) was selected. The optimized threshold for Tau-RD4 positive

aggregation was consistently applied to each image of the selected area. The total integrated intensity of Tau-RD4 positive aggregation was measured and normalized by the total number of cells in the same selected area. Each selected area of a well provided one repeat and quantification results originate from three independent wells for each individual condition. The statistical significance of differences between control and treatments was determined by one-way ANOVA with Tukey post-hoc test for multiple comparisons.

For images in Extended Data Fig. 9 and Baricitinib experiment staining, we used the Opera Phenix imaging system with following parameters: Autofocus was set to two-peak (default); Objective was 20X water, NA 1.0; Opt. Mode was confocal; Camera ROI was 2260*2160 pixels, and images were acquired with 2x2 binning. Exposure parameters were set as follows: DAPI for 80 ms, Cy5 for 100 ms, and Alexa 488 for 120 ms. Nine fields per well were imaged and analyzed for DAPI counting and RD4 inclusions intensities normalized to DAPI number, with Harmony software version 5.2.

Cell lysates preparation, SDS-PAGE, and Immunoblotting

HEK293T cells were harvested in cold 0.01 M PBS and lysed in cold lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% NP40, pH 6.8) supplemented with protease and phosphatase inhibitor. After incubation on ice for 30 mins, cell lysates were clarified by centrifugation at 12,000 rpm for 15 mins. Clarified cell lysates were denatured and were run on BOLT 4-12% Bis-Tris gels in duplicate. One gel was stained with SimplyBlue Safestain and one gel was transferred to a PVDF membrane. The PVDF membrane was stained with LICOR REVERT 700 total protein stain (Licor) and imaged on a Licor Odyssey CLx imaging system. Membranes were then probed with either GFP antibody (B-2) Alexa Fluor® 680 (Santa Cruz Biotechnology), Tau-RD4 antibody (Millipore Sigma), PSMB8 antibody (Genetex), and Tau12

antibody (Sigma), followed by incubation with corresponding secondary antibodies if needed. Membranes were subsequently scanned on the Odyssey CLx imaging system. For IP knockdown experiments, proteins were instead transferred to nitrocellulose membranes (Invitrogen, Carlsbad, California) and membranes were blocked with 5% non-fat-milk in 0.01 M PBST (0.1% Tween20) at RT for 1 hr. Membranes were then incubated overnight at 4°C with primary antibodies diluted in 0.01 M PBST as follows: rabbit anti-PSMB10 (1:2000, GeneTex), Rabbit anti-PSMB9 (1:1000, GeneTex), Rabbit anti-PSMB8 (1:1000, Abcam), Rabbit-anti GAPDH (1:5000, Cell Signaling). Following several washes with PBST, membranes were incubated with goat anti-rabbit (1:5000, Calbiochem) at RT for 1 hr. Following several washes with PBST, Chemiluminescent substrate (Amersham ECL Plus, GE Healthcare Life Sciences) was added, and images were captured in real-time at 10-20 sec intervals using a FujiFilm LAS-3000 with a CCD camera (GE Healthcare Life Sciences).

***In vivo* validation using the hTau mouse**

B6.Cg-Tg(MAPT)8cPdav Mapttm1(EGFP)Klt/J (hTau) mice were obtained from The Jackson Laboratory and aged to 6–7 months. All procedures were approved by the Institutional Animal Care and Use Committee. Mice received intraperitoneal injections of lipopolysaccharide (LPS; 0.2mg kg⁻¹) or PBS for 7 consecutive days. On Day 8, mice were anesthetized with isoflurane and transcardially perfused with PBS. The right brain hemisphere was fixed in 4% paraformaldehyde for immunohistochemistry, and the left hemisphere was flash-frozen for mass spectrometry. Fixed tissue was cryoprotected in 30% sucrose, embedded in OCT, and stored at -80 °C prior to sectioning.

OCT-embedded tissue was sectioned at 10 µm and then blocked with 5% BSA containing Triton X-100. Sections were incubated with primary antibodies anti-tau pT217 antibody (CST, Catalog

1111 # 51625) for 24 h at 4 °C and with secondary antibodies for 2 h at room temperature. After washing,
1112 sections were mounted and stored at 4 °C until imaging.

1113 **Generation of engineered MAPT iPSC lines**

1114 Engineered MAPT iPSC lines (4R, 4R-P301S) were generated previously in the laboratory of Li
1115 Gan and were kindly provided to our group. The full engineering procedures are described in Bravo
1116 et al. (2024). In brief, human iPSCs were engineered for inducible expression of a Neurogenin 2
1117 (NGN2) transgene integrated into the AAVS1 locus of WTC11 cells with a wild-type genetic
1118 background. To generate the 4R-P301S line, Bravo et al. introduced the MAPT P301S (C>T)
1119 substitution via homology-directed repair using ssDNA donors and Cas9 RNPs. Correctly edited
1120 clones were identified by PCR spanning the homology arms and confirmed by Sanger sequencing.
1121 All cells used in this study were validated by the originating laboratory and gifted to us for
1122 downstream experiments. All cell lines were routinely tested for mycoplasma using a PCR-based
1123 kit (MP0035-1KT) and authenticated by STR profiling to prevent potential contamination or
1124 genetic drift.

1125 **Differentiation of iPSCs to iNeurons and treatment**

1126 iNeurons were differentiated as described previously⁶⁶. NGN2-expressing WT, 4R, or 4R-P301S
1127 iPSCs were plated at 200,000 cells/cm² in StemFlex Medium (Life Technologies, Cat# A3349401)
1128 supplemented with 10 µM Y-27632 (ROCK inhibitor; STEMCELL Technologies, Cat# 72304)
1129 (day 0). Medium was changed to KSR medium on day 1, a 1:1 mixture of KSR and N2B medium
1130 on day 2, and N2B medium on day 3. Doxycycline (2 µg/ml; Sigma, Cat# D9891-5G) was added
1131 from day 1 and maintained throughout differentiation. On day 3, B27 supplement (1:100; Life
1132 Technologies, Cat# 17504-044) was added. On day 4, cells were dissociated using Accutase

1133 (diluted 1:3 in PBS; Life Technologies, Cat# A1110501) and cryopreserved in a 1:1 mixture of
1134 20% DMSO in FBS and iN day-4 medium (NBM medium + 1:50 B27 + 10 ng/ml each BDNF,
1135 GDNF, and CNTF) supplemented with 10 μ M Y-27632 and 2 μ g/ml doxycycline. One day prior
1136 to thawing, plates were coated overnight at 37 °C with poly-L-ornithine (Sigma-Aldrich, Cat#
1137 P3655) and laminin (Gibco, Cat# 23017015). The next day, plates were washed with PBS and
1138 further coated with Matrigel (Corning, Cat# 356234). Cryopreserved day-4 iNs were thawed into
1139 iN medium containing 10 μ M Y-27632 and 2 μ g/ml doxycycline and plated at 62,500 cells/cm².
1140 From day 4 to the end of maturation (treatment day 0, corresponding to day 30 of differentiation),
1141 cells were cultured in iN medium and fed twice weekly. On day 9, cells are treated with 1:600
1142 ARA-C (final dilution 1:1200; Sigma-Aldrich Cat# C1768-100MG) to reduce any proliferative
1143 cells.

1144 On day 30 (treatment day 0), iNs received a half-medium change with iN medium and one of the
1145 following treatment conditions: (1) H₂O vehicle (equivalent volume to IFN- γ /TNFs), (2) IFN- γ +
1146 TNFin H₂O carrier (final concentration 200 ng/ml each), (3) DMSO vehicle (equivalent volume to
1147 Baricitinib), or (4) in DMSO carrier (final concentration 500 nM) plus IFN- γ + TNFin H₂O carrier
1148 (200 ng/ml each). All cells received a half-medium change on day 3 with replenishment of the
1149 assigned treatments and were harvested on day 6 for mass spectrometry or fixed for
1150 immunocytochemical analysis.

1151 **Data-dependent acquisition analysis of cell lysates**

1152 For the MS analysis of the peptides from cell lysates, the timsTOF Pro2 was operated in PASEF
1153 mode and the TIMS enabled using Bruker's default method "DDA PASEF-
1154 low_sample_amount_1.9sec_cycletime". Briefly, the parameters were as follows: mass range 100-
1155 1700 m/z and mobility range 1/K₀ 0.6-1.6 V·s/cm² with a corresponding ramp time of 166 ms and

lock duty cycle to 100%. The source was put to CaptiveSpray with the capillary voltage set to 1500 V, the dry gas to 3.0 l/min, and the dry temperature to 180°C. The parameters for PASEF were set to 10 MS/MS scans with a total cycle time of 1.88 s, charge range 0-5, active exclusion for 0.4 minutes (precursors were reconsidered within that time frame if the current intensity was at least 4-fold of the previous one), target intensity of 20,000 and intensity threshold of 1,000. The collision energy was adjusted to the mobility of the ions ranging from 20 eV for $1/K_0$ 0.6 V·s/cm² up to 59 eV for $1/K_0$ 1.6 V·s/cm².

Spectral library generation for FL biosensor with/without IFN- γ /TNFs

Tau-0N4R(P301S)-EYFP cells were plated in a 6-well plate. Next day, cells were treated with 200ng/ml TNF/IFN- γ or H₂O. After 7 days of treatment, cells were washed with 0.01M PBS, then harvested in cold PBS and lysed in Triton-X100 lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, and 5 mM EDTA, pH 7.4) supplemented with protease and phosphatase inhibitors. After incubation on ice for 30 mins, cell lysates were clarified by centrifugation at 12,000 rpm for 15 mins. Total protein concentration from clarified cell lysate was determined by bicinchoninic acid assay (Pierce™ BCA Protein Assay Kit, ThermoFisher Scientific). 100 μ g of protein was digested using SP3 as described above. Peptides were then fractionated with the Pierce™ High pH Reversed-Phase Peptide Fractionation Kit (Cat # 84868) into 23 fractions of IFN- γ /TNF-treated and control samples. ACN composition of each elute buffer is 5%, 6.25%, 6.85%, 7.5%, 8.1%, 8.75%, 9.35%, 10%, 10.6%, 11.25%, 11.85%, 12.5%, 13.1%, 13.75%, 14.35%, 15%, 15.6%, 16.25%, 17.5%, 18.75%, 20%, 35%, and 50%. Eluted peptides were vacuum-dried and reconstituted in 20 μ l sample buffer, and 1 μ l was injected and analyzed by the timsTOF pro2 mass spectrometer (Bruker) in DDA-PASEF mode as described above.

DDA .d files from fractionations were searched with PaSER (2023b version) with the search engine Prolucid against a Homo sapiens uniprot protein database containing 40474 entries, supplemented with FL tau biosensor sequence. In brief, precursor tolerance was set to 20 ppm and fragment tolerance was 30 ppm, trypsin digestion on both ends at residue KR, with 2 max missed cleavages, fixed modification Carboxyamidomethylated cysteine, with mass shift of + 57.021464 Da. Variable modifications include phosphorylation on STY with mass shift + 79.966331 Da, acetylation on K + 42.010565, ubiquitination on K + 114.042927 Da, and citrullination on R + 0.984016 Da. FDR on protein level was set to 0.01. Then, the DDA files were searched again using Fraggpipe version 18.0 with the following parameters: precursor and fragment mass tolerances set to 20 ppm. Protein Digestion Cleavage settings were set to SEMI, with fixed modification Carboxyamidomethylated cysteine (+ 57.021464 Da) and variable modification of Oxidation on M + 15.9949 Da. The semi-tryptic tau peptides were filtered and merged with the spectral library, enabling analysis of both tryptic and semi-tryptic tau peptides.

Data independent acquisition (DIA) parameters for Baricitinib experiment

Approximately 500 ng of tryptic peptides was loaded onto Evotip and analyzed using an Evosep One LC (EVOSEP) connected to a timsTOF HT (Bruker). The Evosep One method was 60 SPD (21-min gradient, cycle time of 24 min) and the mass spectrometer was operated in DIA-PASEF mode. The DIA-PASEF method consisted of eight 100 m/z windows. Two windows were acquired per 100-ms scan leading to a cycle time of 1.37 s. The other mass spectrometer parameters were set as follows: m/z range, 300 to 1400, the mobility ($1/K_0$) range was set to 0.6 to 1.6 V·s/cm², and the accumulation and ramp time were 100 ms, and ramp rate was 9.52 Hz.

1199 **Data analysis of the Baricitinib DIA experiment**

1200 Acquired DIA data was searched in DIANN 1.9 with the spectral library and human proteome
1201 fasta with the 0N4R isoform of tau (P301S). N-terminal methionine excision was enabled, in silico
1202 digest will involve cuts at K and R, maximum number of missed cleavages was set to 2, cysteine
1203 Carbamidomethylation was enabled as a fixed modification, heuristic protein grouping was used,
1204 the spectral library retained the original spectra but included empirically aligned RTs. Output was
1205 filtered at 0.01 FDR, and the cross-run normalization was set to RT-dependent. Proteins identified
1206 in less than 30% of the DMSO or Baricitinib group were filtered out, based on identification
1207 frequency. Protein differential expression was analyzed using in-house R code, with reference to
1208 Reactome pathways. For tau semi-tryptic analysis, the identified sites were manually verified and
1209 plotted as a function of the difference in semi-tryptic peptide intensities.

1210

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1222 supports BTH.

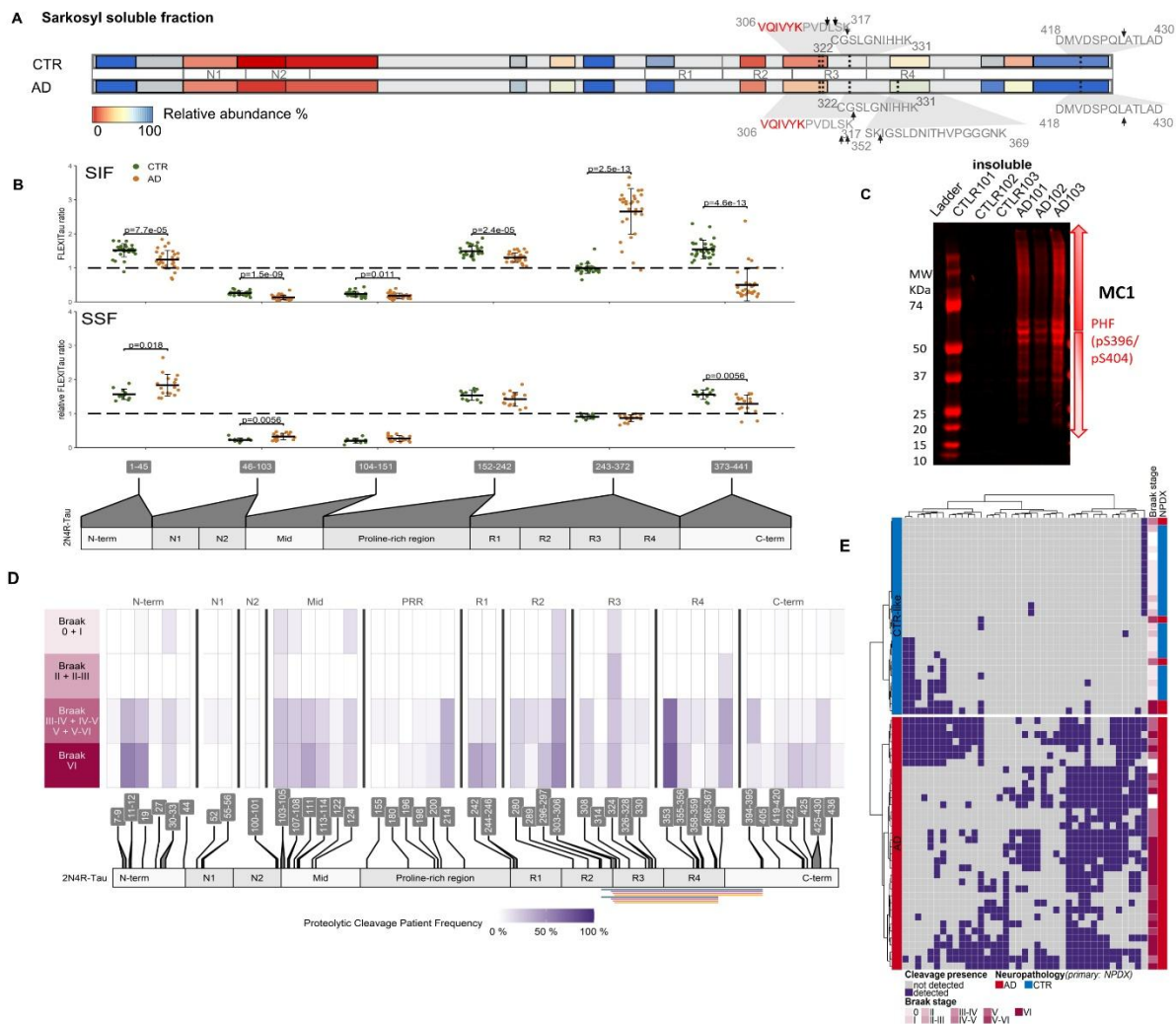
1223 **AUTHOR CONTRIBUTIONS**

1224 Method development, data collection, and analysis, JX, PB, LC, TW, CNS, AJC, SG, MV, JNR,
1225 CL, AMG, HW, WM, EMS, AJG, AM, ST, BTH, ST, HS, KSK, TYP, LTG, SH, JAS; Data
1226 Curation, JX, PB, HW, WM, CNS, JAS; Writing - Original Draft, PB, LC, MV, SH, JAS; Writing
1227 – Review & Editing, JX, PB, LC, TW, CNS, AJC, SG, MV, JNR, CL, AMG, HW, WM, EMS,
1228 AJG, AM, ST, BTH, ST, HS, KSK, TYP, LTG, SH, JAS; Conceptualization JAS, JX, PB and LC.

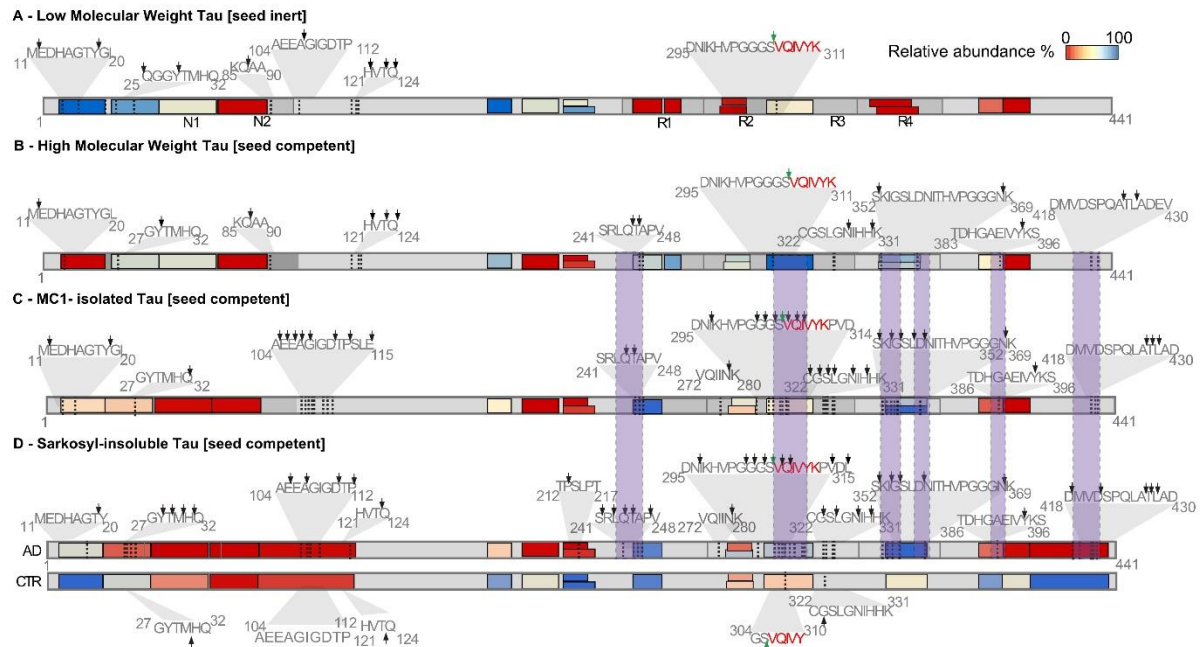
1229 **DECLARATION OF INTERESTS**

1230 Dr. Bradley T. Hyman is a member of the SAB and owns shares in Dewpoint. He also serves on
1231 an advisory panel for Biogen, and his laboratory has current research funding from AbbVie. Dr.

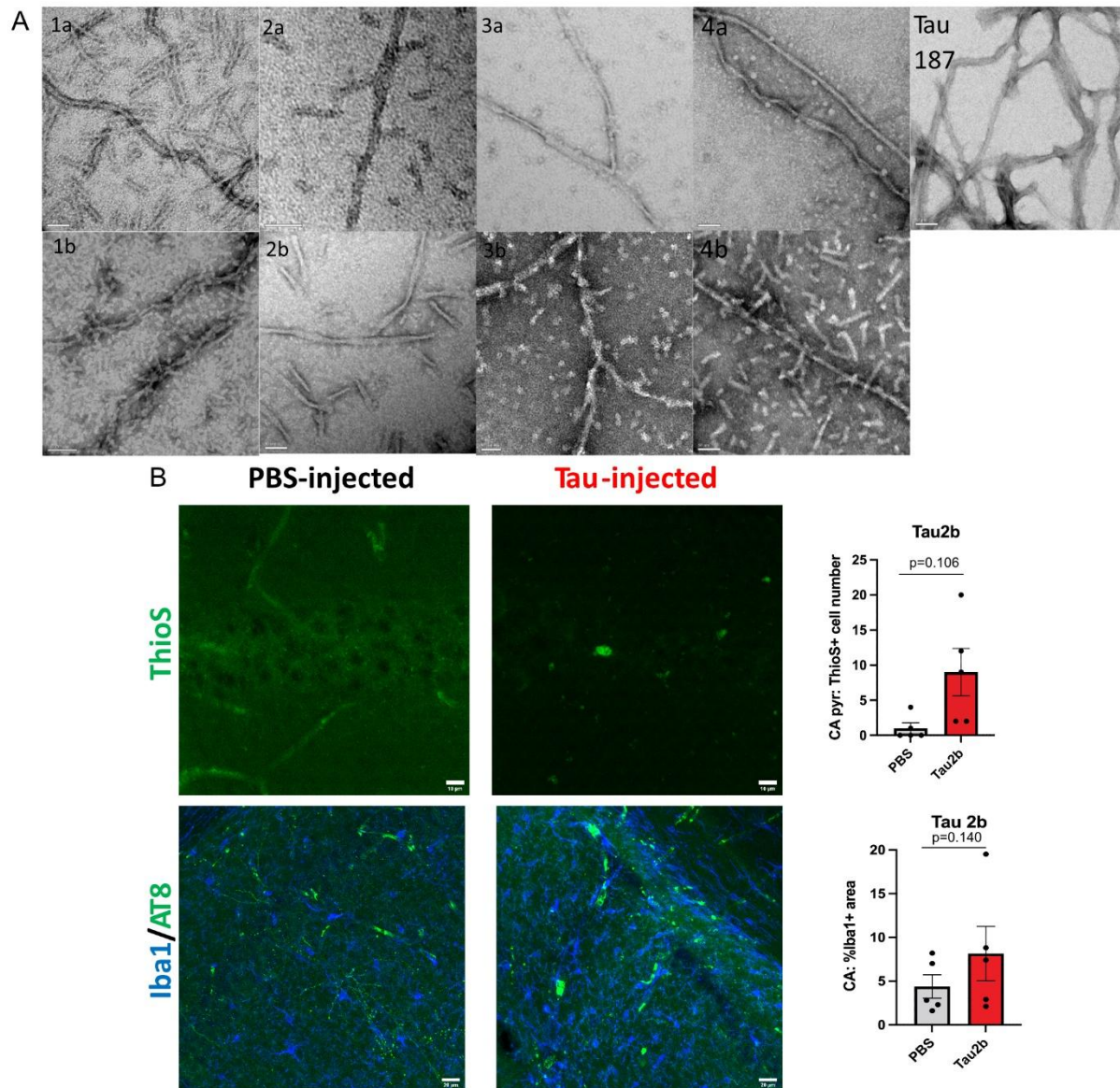
1232 Hyman's wife is an employee and shareholder of Novartis. All the other authors declare no
1233 conflicts of interest.



Extended Data Fig. 1 Tau cleavage is largely observed in SIF of AD but not in other samples or fractions. (A) SSF of AD and CTR cleavage map. (B) Quantitative tau peptide measurements show significant enrichment of the MTBR (242-372) in the AD SIF fraction relative to the CTR. FLEXITau peptide measurements were used to calculate the relative ratios for domains from N- to C-terminus of AD (red) and CTR (blue) in the SIF and SSF. This enrichment of the core domain is not observed in the SSF. (C) Fragments of tau are observed with a wide range of molecular sizes in the AD insoluble fraction. Western blot analyses with a C-terminal phosphorylation site antibody (PHF) identify fragments below 50 kDa, showing that the intact C-terminus can be present when the N-terminus is cleaved. (D) A plot of proteolytic cleavage frequencies measured by MS for tau in SIF across human subjects at different Braak stages shows that cleavage increases between stages III and IV. Cleavage sites locate after the numbers pointed out. Colored lines below the tau sequence scheme refer to the peptides in Fig. 2A. (E) Hierarchical clustering analysis of the tau cleavage site data in SIF clearly separates AD and CTR subjects. Each row shows data for a single patient, and each column represents a cleavage site. The pathological diagnosis and the Braak stage are shown to the left of the matrix. The data is clustered based on the presence or absence of cleavage sites (23 CTR and 41 AD).

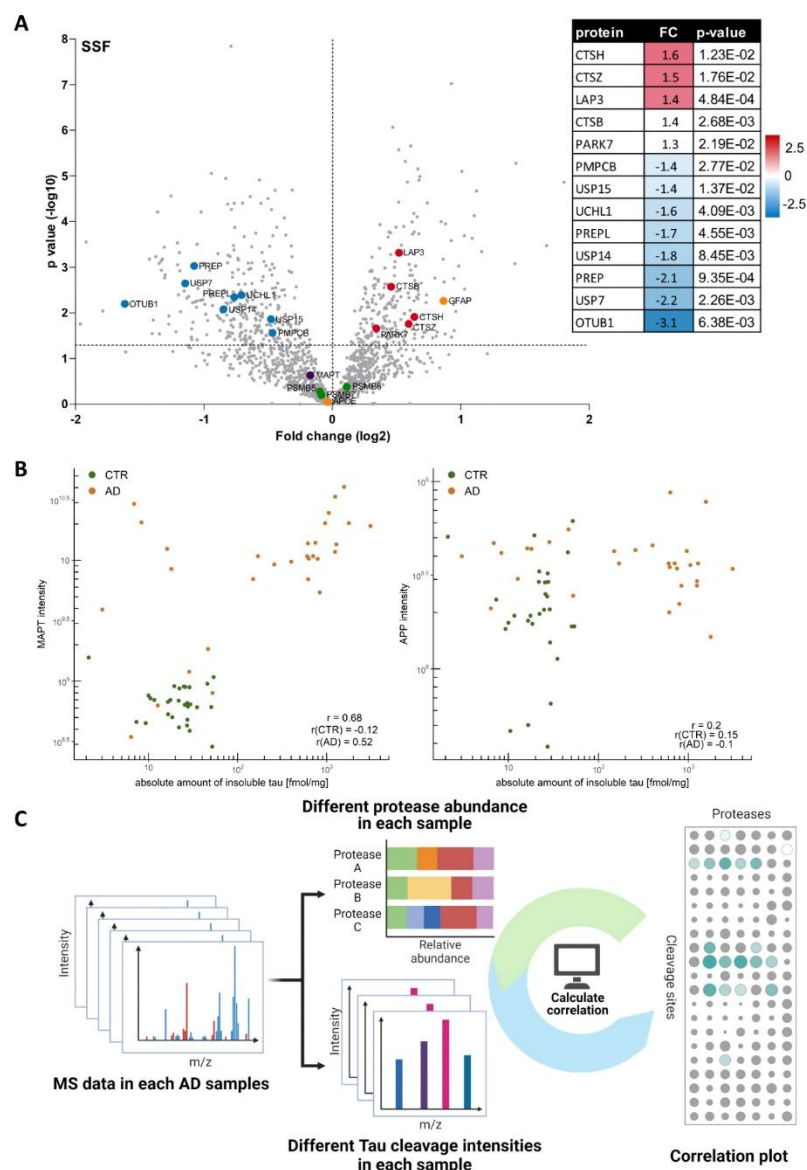


Extended Data Fig. 2 Seeding competent fractions show extensive cleavage that results in the enrichment of the core domain observed by cryo-EM studies. Tau cleavages are identified in seeding competent and seeding incompetent tau preparations from human postmortem tissue. (A) LMW tau. (B) HMW tau. (C) MC1-purified tau. (D) SIF tau. Cleavage ‘hotspots’ are highlighted in purple.



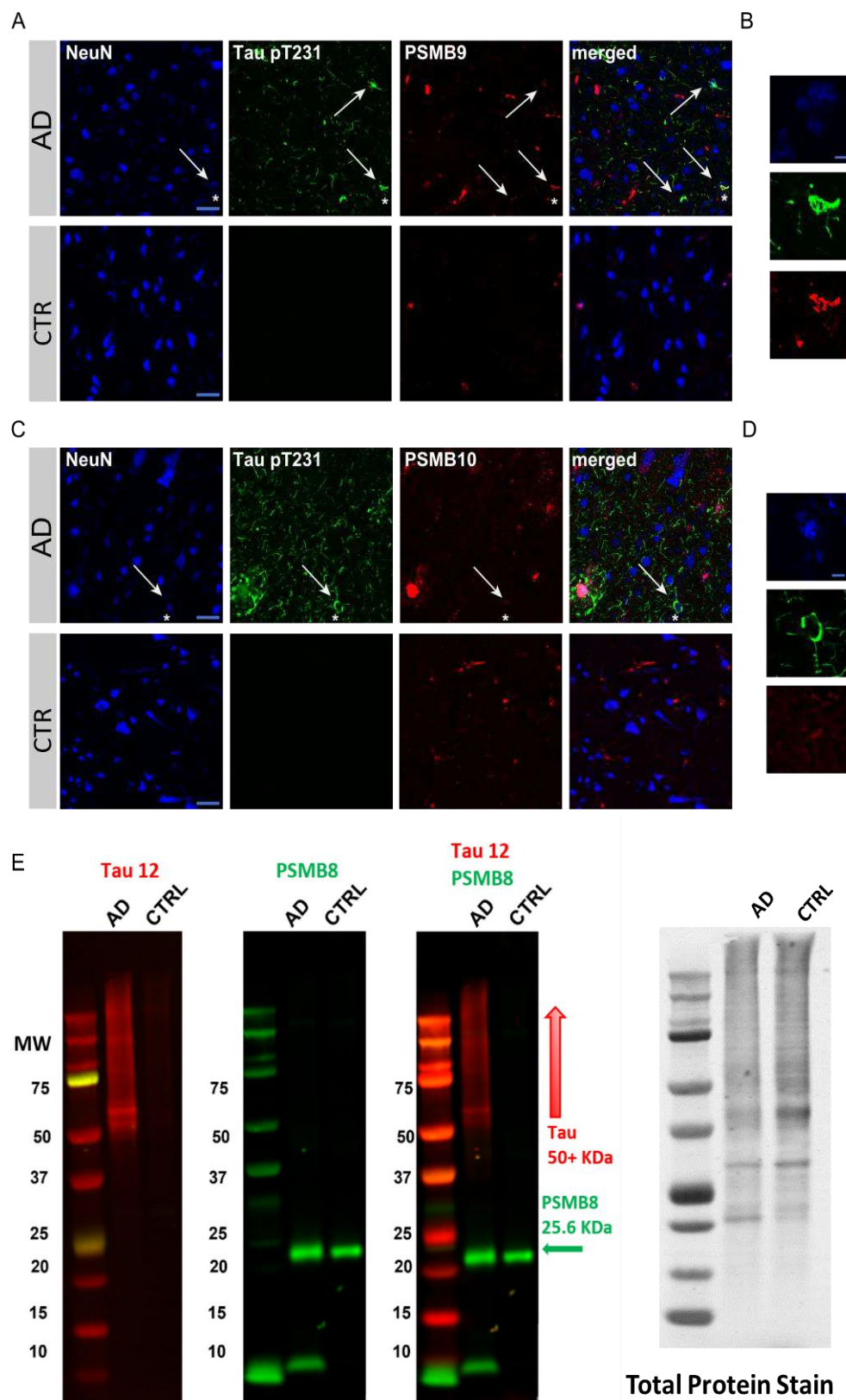
1259

1260 **Extended Data Fig. 3 AD-specific tau fragments can form tau fibers *in vitro* and induce**
 1261 **aggregation in mouse brain.** (A) TEM images of tau fragment fibrils. Scale bar = 50 nM. (B)
 1262 Bilateral injection of **2b** tau peptide into the CA1 of dorsal hippocampus of PS19 mice
 1263 overexpressing the P301S tau protein. ThioS+ cell somas in the pyramidal layer of the
 1264 hippocampus (CA1-2-3) on 3 sections around the injection site were counted. An increase in
 1265 ThioS+ cell number was observed in **2b**-injected mice compared with PBS controls, although the
 1266 difference was not statistically significant (two-tailed paired t-test, $p = 0.106$). Iba1 staining
 1267 showed an increase in microglia around the injection site, although the difference in Iba1+ area
 1268 was not statistically significant (two-tailed paired t-test, $p = 0.140$). AT8 staining shows tau
 1269 aggregates in the region.

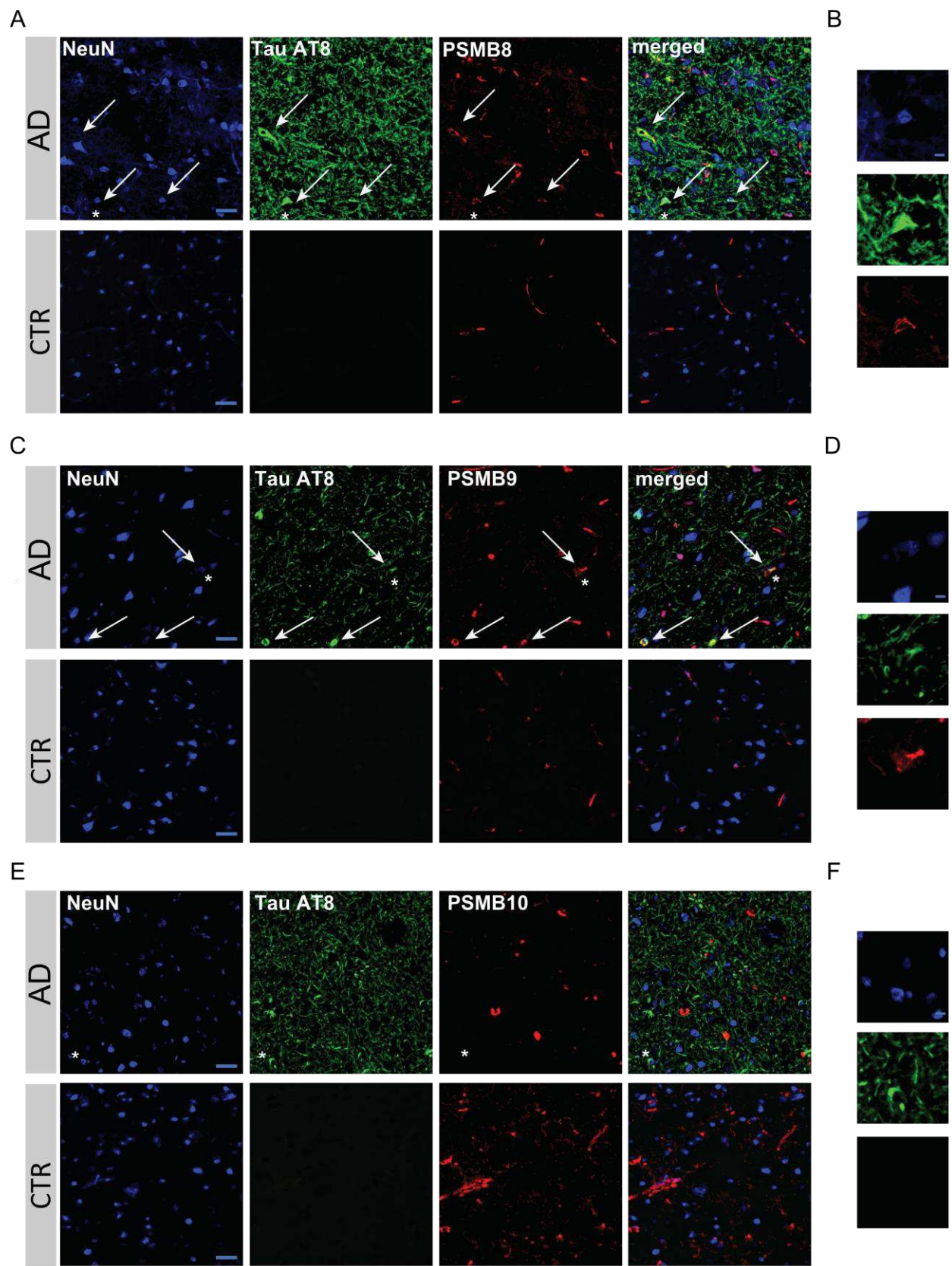


Extended Data Fig. 4 Analysis of proteins in SSF and SIF of AD and CTR. (A) Volcano plot showing differential protein expression of soluble fraction in AD vs CTR. Significantly changed proteins were cross-referenced with the MEROPS peptidase database to identify proteases that were upregulated (red) or downregulated (blue). Proteases from 20S CP are labeled green. AD-related factors are labeled orange. Tau is labeled purple. (B) Plots regarding the correlations between absolute insoluble tau amount (FLEXITau total abundance, fmol/mg wet tissue) and MAPT intensity (left) and between absolute insoluble tau amount (FLEXITau total abundance, fmol/mg wet tissue) and APP intensity (right) in AD and CTR. (C) Schematic to identify proteases and cross-referencing with tau cleavage. Differentially expressed proteins were cross-referenced against the MEROPS peptidase database to identify proteins with peptidase activity that could act as tau proteases. Pearson's correlation between the abundance of each protease and the intensity of the semi-tryptic tau peptides was calculated across all AD samples. The data were visualized in

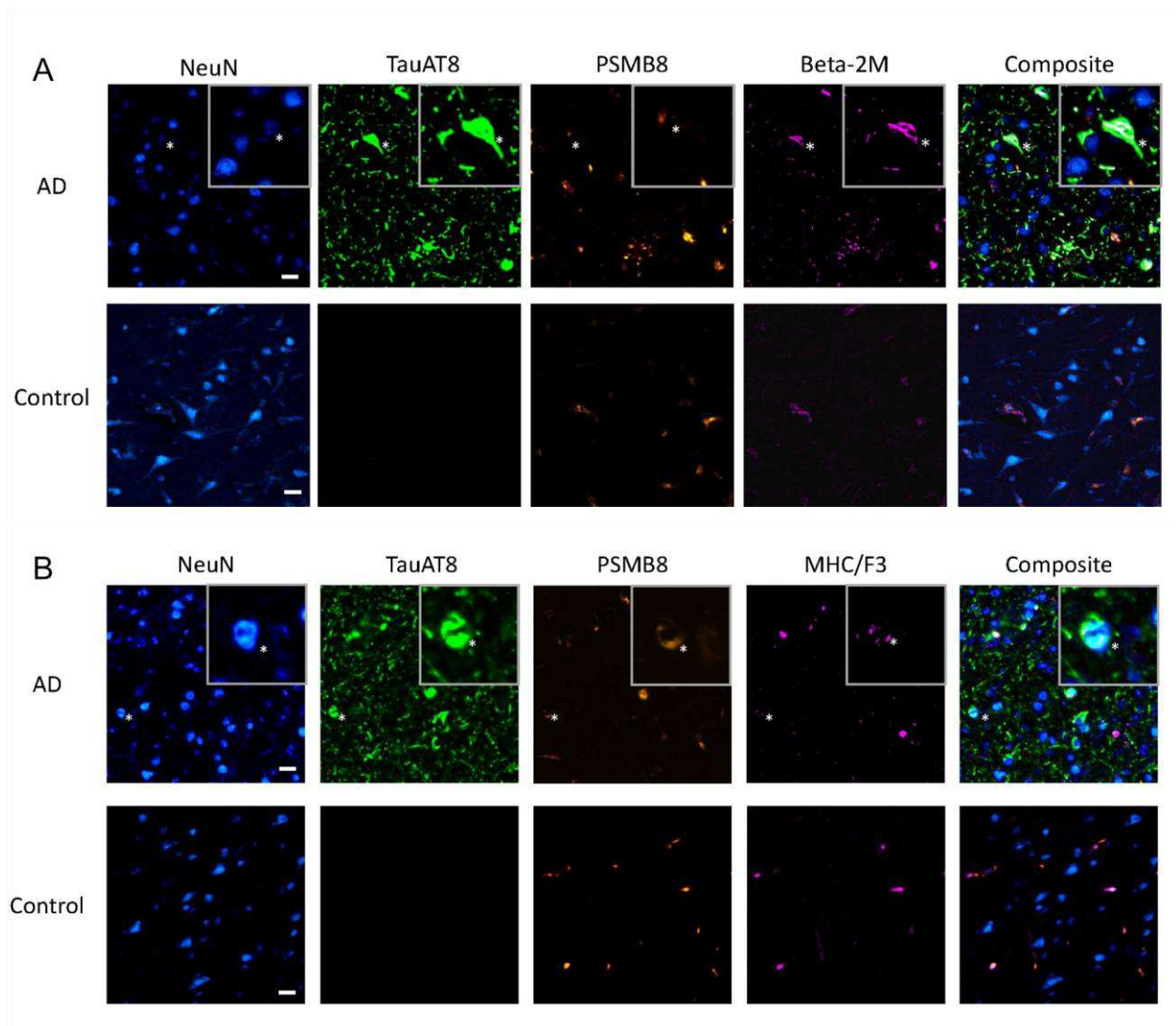
1283 a bubble plot, with Pearson's r values encoded as bubble size, and p-values for the correlations
1284 encoded by color.



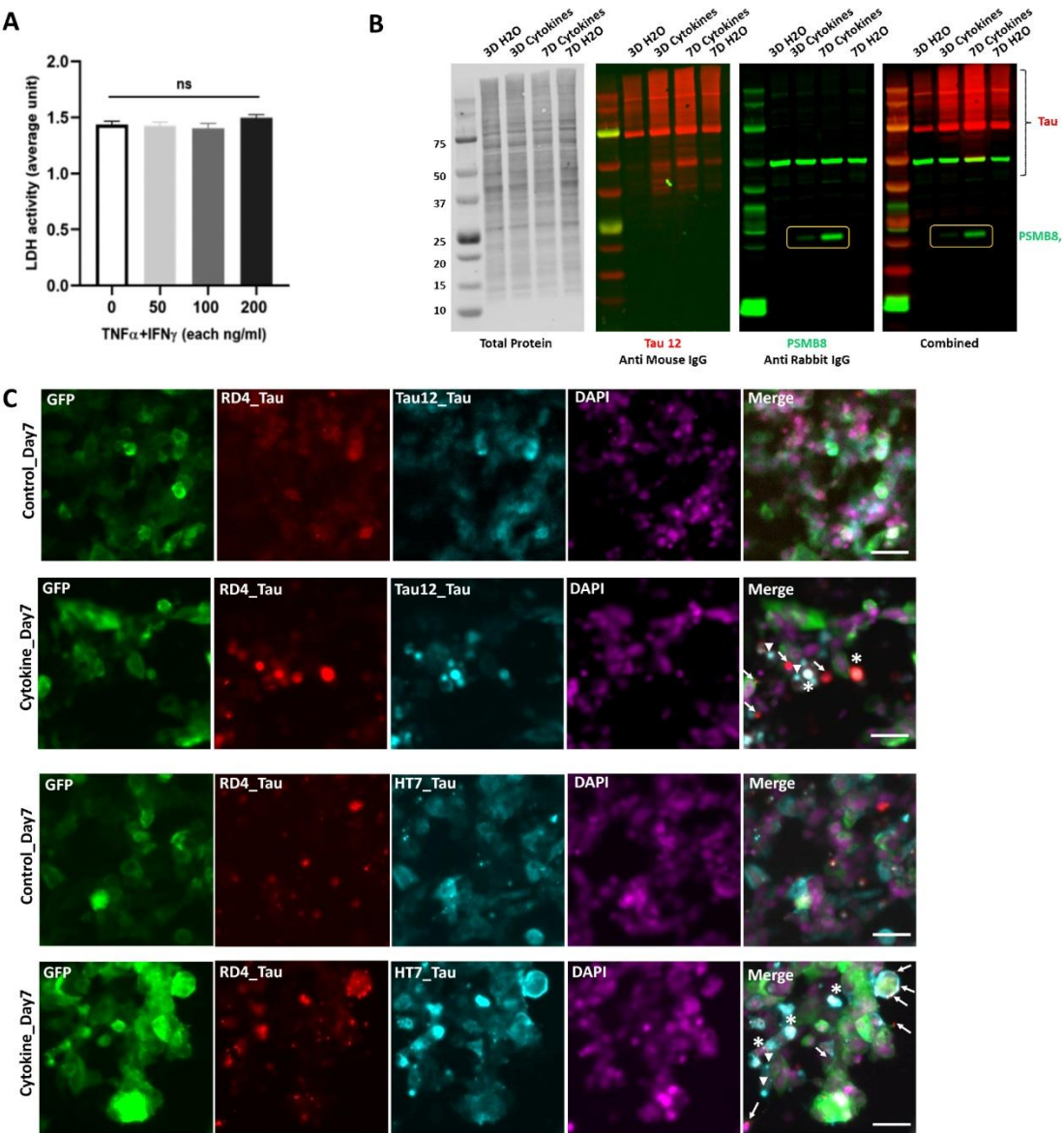
Extended Data Fig. 5 Neuronal expression of IP subunits overlaps with tau aggregation.
Triple-plex immunostaining of middle frontal gyrus shows the expression of pT231 tau in the same neurons as (A) PSMB9 in AD, but not CTR brain tissue. (C) PSMB10 expression does not overlap with Thr231 pTau positive neurofibrillary tangles. Arrows -neurofibrillary tangles with colocalized pTau and IP subunit immunoreactivity Star- neuron showed in magnification on the right. Scale bar = 10 μ m. (B) & (D) Closeups of neurofibrillary tangles from (A) & (C) respectively. Scale bar = 2 μ m. (E) Western Blots of SIF from AD and control subjects show no cross-reactivity between PSMB8 and Tau12 antibodies in sarkosyl insoluble tau. SDS PAGE was performed for AD and CTR sarkosyl insoluble fractions (n= 5 AD and n= 5 CTR human subjects were pooled per lane). The proteins were transferred to membranes and were probed with the PSMB8 and Tau12 antibody and visualized using fluorophores.



Extended Data Fig. 6 Neuronal expression of IP subunits overlaps with tau aggregation using tau AT8 antibody. Triple-plex immunostaining of the middle frontal gyrus shows expression AT8 pTau 5n the same neurons as (A) PSMB8, (C) PSMB9 in AD, but not CTR brain tissue. (E) PSMB10 expression does not overlap with AT8 pTau positive neurofibrillary tangles. Arrows show neurofibrillary tangles with colocalized pTau and IP subunit immunoreactivity. Star indicates neuron shown in magnification on the right. Scale bar = 10 μ m. (B), (D), & (F) Closeups of neurofibrillary tangles from (A), (C), & (E) respectively. Scale bar = 2 μ m.



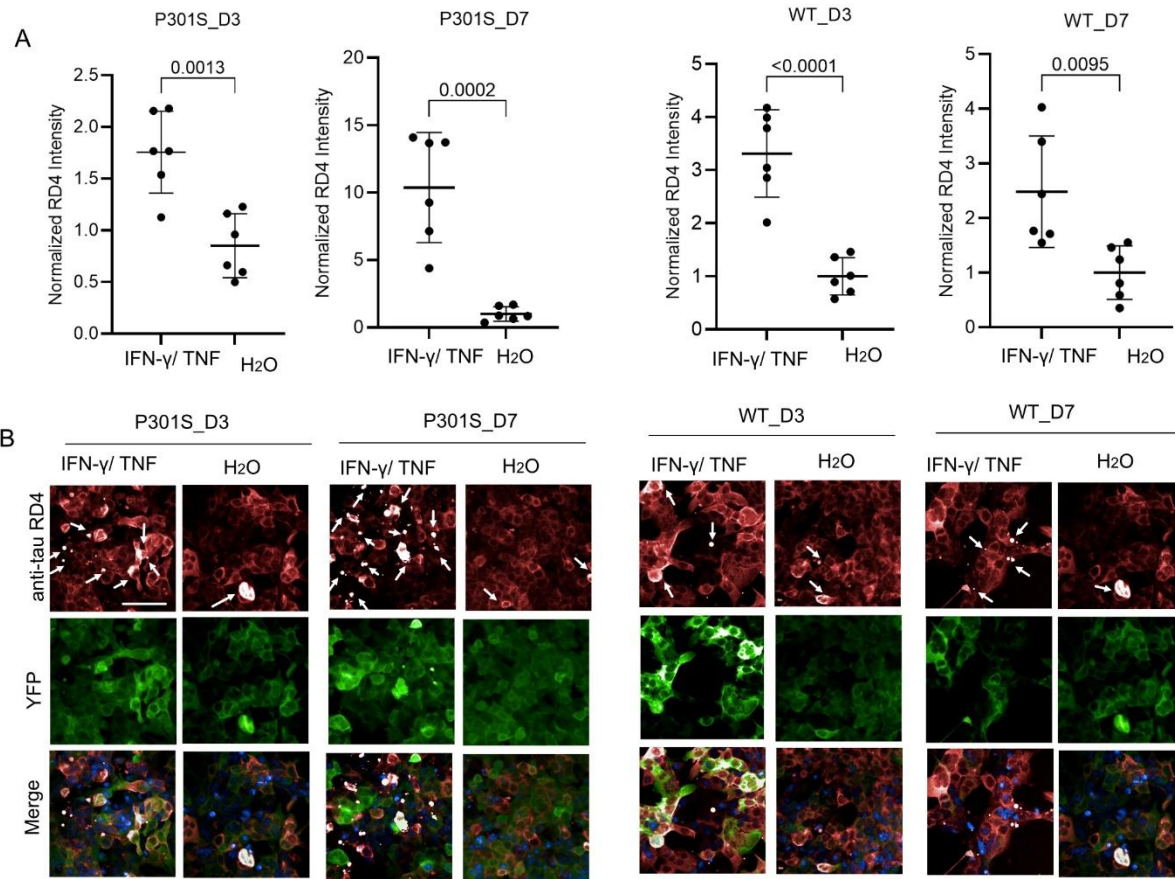
Extended Data Fig. 7 MHC1 proteins colocalize IP in neurons containing tau neurofibrillary tangles. Immunohistochemical staining of tau aggregation (AT8 antibody in green), PSMB8 (in yellow), and MHC-I components (A) Beta-2M and (B) F3 (both in purple). Star indicates the colocalization region. Scale bar = 2 μ m.



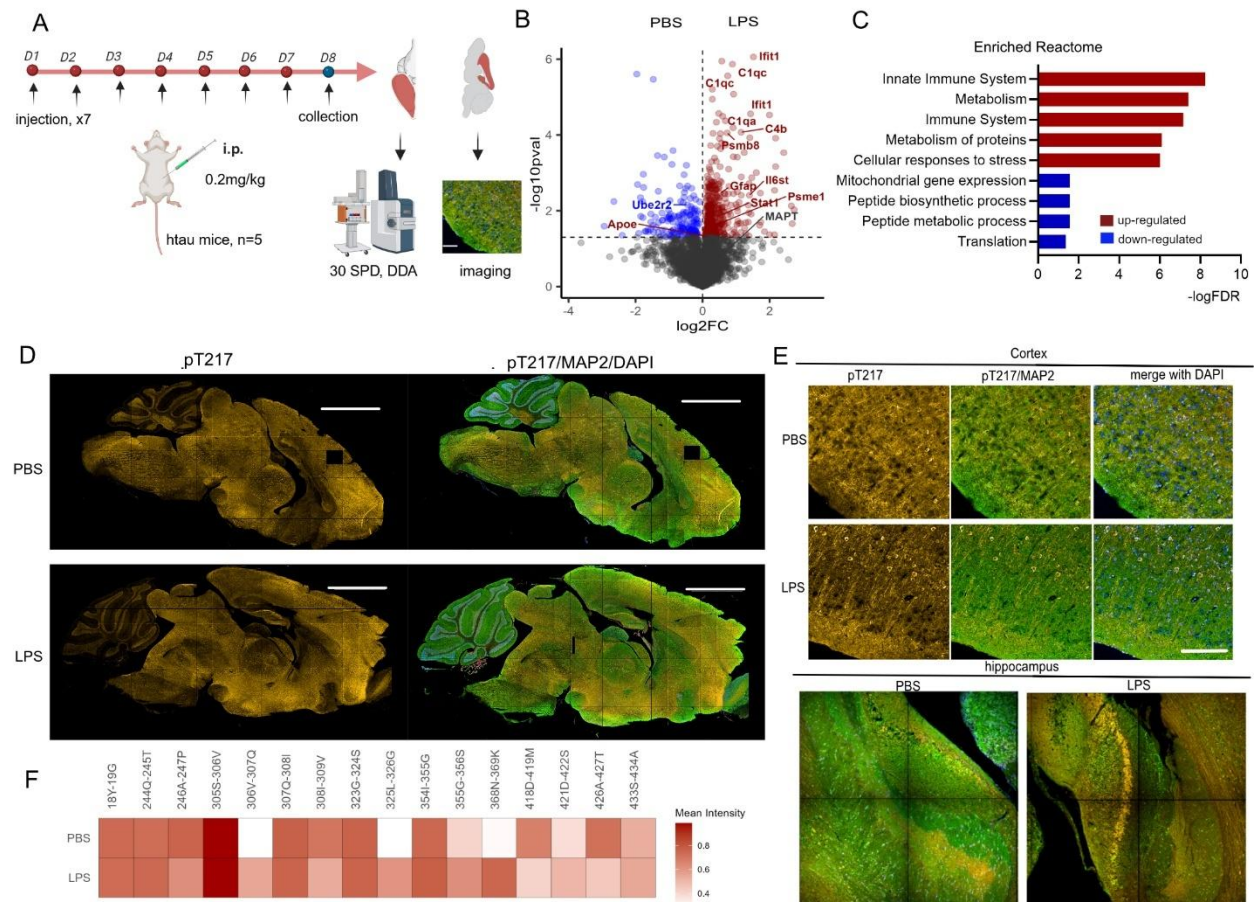
1312 **Extended Data Fig. 8 Cytokine(IFN- γ /TNF)-treated biosensor cells show induction of**
1313 **PSMB8 and tau cleavage.** (A) LDH assays are used to test differences in apoptosis across
1314 biosensor tau cell experiments under the conditions used. No significant difference in cell viability
1315 is observed between the IFN- γ /TNF-treated cells and controls. (B) Biosensor cells overexpressing
1316 tau show increased tau inclusions upon IFN- γ /TNF treatment, displaying heterogeneous
1317 molecular-weight fragments, as observed in human AD tissue. The total protein stain of the
1318 membrane shows that all lanes contain equal amounts of protein. Western blot analysis of whole
1319 cell lysate from the Full-Length YFP labeled Tau (75kDa) biosensor after treatment with IFN-
1320 γ /TNFs using the Tau12 (N-terminal) antibody shows that tau cleavage fragments are observed
1321 below the 75kDa FL-YFP-Tau at 3 and 7 days after IFN- γ /TNF treatment in the 2nd and 3rd lanes.
1322 PSMB8 staining shows that this immunoproteasome subunit is observed in IFN- γ /TNF-treated
1323 cells at days 3 and 7. (C) Immunostaining of IFN- γ /TNF-treated and control full-length Tau

1325 biosensor cell line. The YFP (green), RD4 antibody (red), the N-terminal Tau12 antibody (cyan in
1326 the 2 upper lanes), and the Mid domain HT7 antibody (cyan in the 2 bottom lanes) show some
1327 overlap between the N-terminal and C-terminal GFP signals with the RD4 antibodies. This
1328 suggests tau aggregates contain some full-length tau. The strongest signal is from the RD4
1329 antibody, suggesting MTBR enrichment, as observed in the mass spectrometry data. The white
1330 line depicts a 50 μm scale bar.

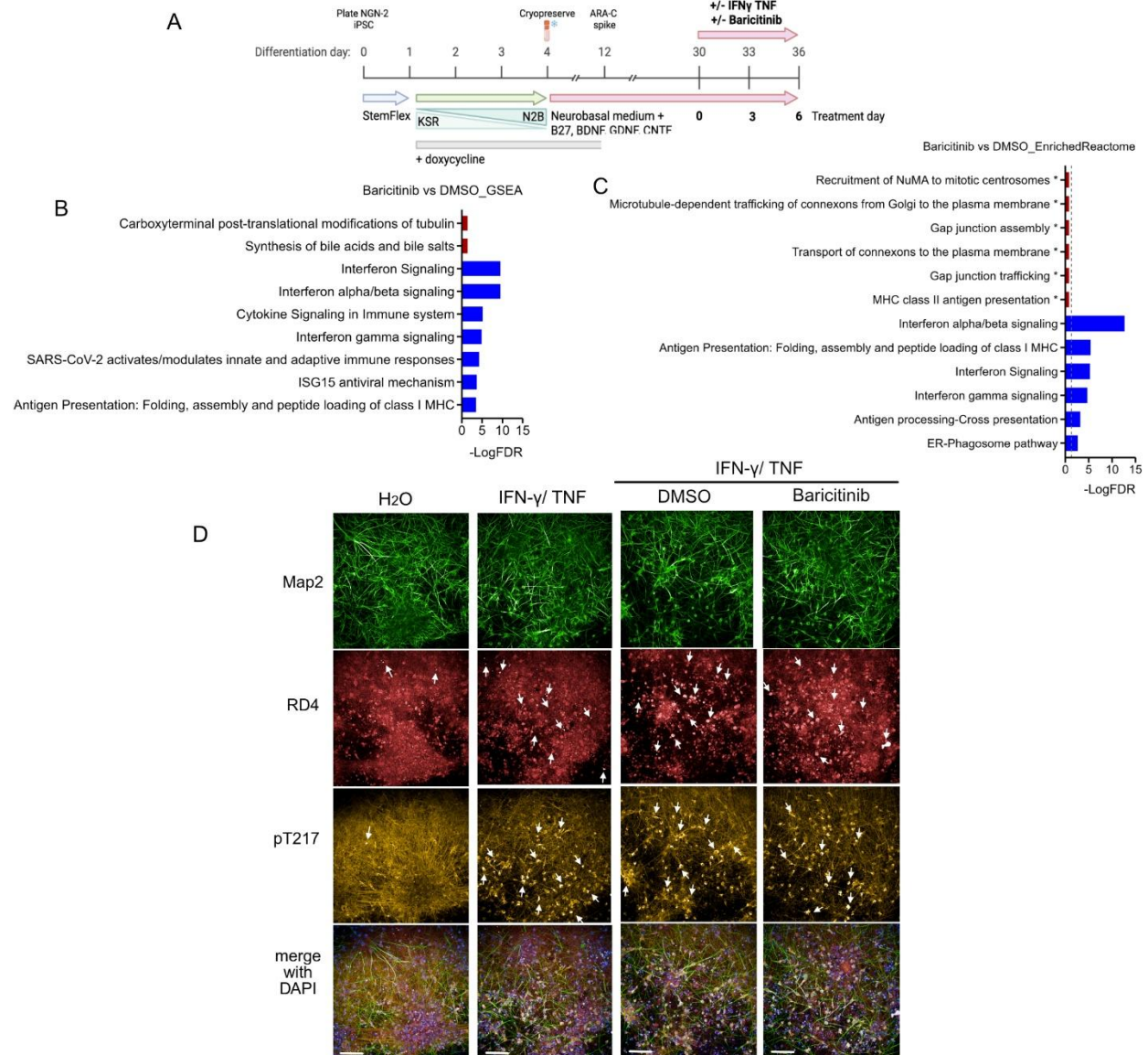
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Extended Data Fig. 10 Biosensor cells expressing WT tau form spontaneous tau puncta with IFN-γ/TNF. (A) Both biosensor cells, HEK293T-0N4R(P301S)-EYFP and HEK293T-0N4R-EYFP, were treated with 200 ng/ml IFN-γ/TNF for 3 days and 7 days. Both biosensors formed spontaneous tau puncta in a time-dependent manner, albeit the WT tau biosensor forms puncta more slowly. (B) Representative images showing tau puncta in both tau biosensors. This shows that IFN-γ/TNF-dependent tau aggregation is not P301S dependent. Scale bar = 50μm.

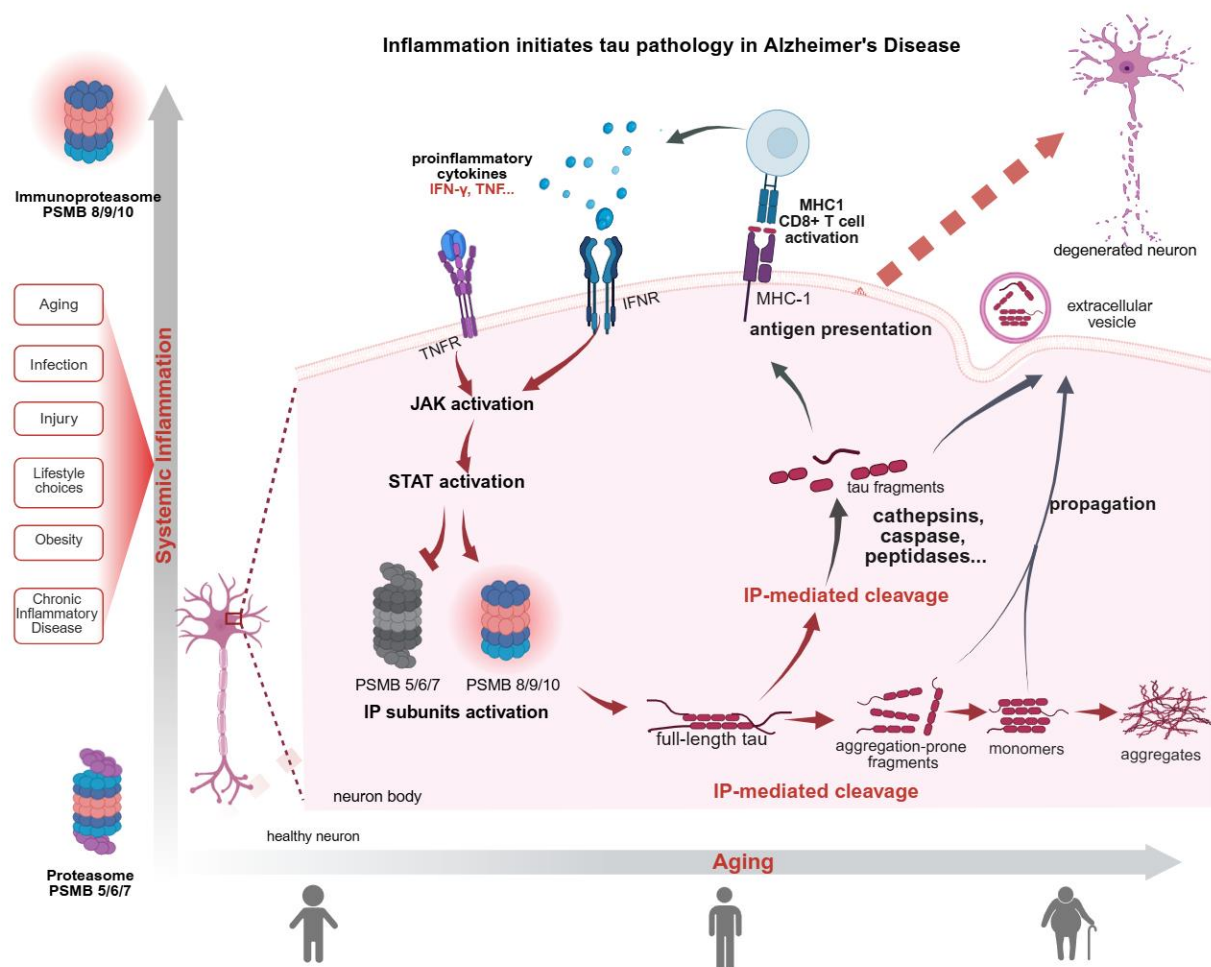


Extended Data Fig. 11 LPS induces tau aggregation *in vivo*. (A) Experimental workflow of chronic LPS administration in hTau mice. 6.5-month-old hTau mice were intraperitoneally (i.p.) injected with 0.2 mg/kg LPS (n=5) or PBS (control; n=5) daily for seven days, and brain tissues were collected on day 8 for mass spectrometry and immunofluorescence analysis. (B) Global proteomics revealed robust induction of inflammatory pathways, including complement components, PSME subunits, STAT1, and PSMB8. (C) Reactome enrichment analysis showed enhanced immune-related pathways and decreased mitochondrial and peptide-processing functions in LPS-treated brains. (D) Immunofluorescence of whole-brain sections demonstrated increased pT217-positive tau in cortical, hippocampal, and caudate regions following LPS treatment. Scale bar = 5mm. (E) Tau aggregates in the cortex and hippocampus of LPS-treated hTau mice. Scale bar = 100 μm. (F) Quantification of non-tryptic proteolytic cleavage site peptide intensities in LPS-treated mice compared with PBS controls.



Extended Data Fig. 12 IFN- γ /TNF activates neuronal antigen presentation and promotes tau pathology in 4R-WT iNeuron. A) Schematic showing the workflow for iNeuron differentiation, maturation, and treatment. Briefly, iPSCs expressing NGN2 (4R-WT or 4R-P301S) were differentiated into excitatory neurons using a staged medium-switch protocol. Cells were dissociated on day 4, cryopreserved, and later replated into 96-well plates. Replated iNeurons were matured in iNeuron medium until day 30. On differentiation day 30 (treatment day 0), the iNeurons received one of four conditions: (1) H₂O vehicle; (2) IFN- γ + TNF (200 ng/mL each); (3) DMSO vehicle; or (4) Baricitinib (500 nM) plus IFN- γ + TNF. Treatments were refreshed with a half-medium change on day 3, and cells were collected on day 6 for mass spectrometry or fixed for immunocytochemistry. B-C) Reactome pathway analysis of Baricitinib vs DMSO in IFN- γ /TNF-treated neurons. Proteins were compared by Welch's t-test ($n = 6$ per group, treatment day 6) and pathways tested with ReactomePA (Benjamini-Hochberg correction). Bars show $-\log_{10}(\text{adjusted } p)$; red, higher with baricitinib; blue, lower. (B) GSEA of all quantified proteins ranked by

1376 $\text{sign}(\log_2\text{FC}) \times -\log_{10}(p)$. C) Over-representation analysis of proteins changed at $p < 0.05$; dashed
1377 line, FDR = 0.05; asterisks, terms significant only before correction. D) Representative images of
1378 the 4R-WT iNeurons under the indicated treatment conditions. IFN- γ /TNF exposure induced
1379 distinct pT217-positive inclusions, identified by white arrows. RD4 staining in the WT tau
1380 iNeurons is less sharply defined than in the 4R-P301S neurons, but also increased following IFN-
1381 γ /TNF treatment. Baricitinib reduced the RD4-positive inclusions and produced a modest decrease
1382 in pT217-positive inclusions. These findings are consistent with our HEK293T assays, in which
1383 the P301S mutation drives the faster formation of RD4-positive inclusions. Scale bar 100 μm .



Extended Data Fig. 13 Neuroinflammation initiates tau pathology in Alzheimer's disease. Insult- and disease-associated inflammatory stimuli, including infection, injury, and comorbidities, elevate pro-inflammatory cytokines, such as IFN- γ and TNF, systemically and in the brain. These cytokines activate the JAK/STAT signaling pathway in neurons, leading to the induction of IP subunits (PSMB8/9/10) and a shift from constitutive proteasome to IP activity. IP activation promotes tau proteolytic cleavage, generating aggregation-prone tau fragments that form aggregates flanking the MTBR core. These fragments can be further processed by proteases (e.g., cathepsins and peptidases), producing shorter tau species that can be presented by MHC-1 as antigens, linking immune activation by T-cells to tau processing pathways. As shown by others, T-cell recognition of neurons presenting neuronal antigens results in neuronal permeation and subsequent release of cellular contents, including vesicles. The recognition of neuronal antigens by T-cells will result in the propagation of neuron-recognizing T-cells, thereby amplifying neuroinflammation in subsequent acute inflammation events. Over a lifetime, these events will lead to chronic inflammation. Together, these models illustrate how acute neuroinflammation

1399 drives the initiation, amplification, and spread of tau aggregation in Alzheimer's disease. Red
1400 arrows indicate mechanisms investigated in this study, and gray arrows represent speculative or
1401 reported pathways based on our observations and published literature.

1402

1403 **Extended Data Table 1. Neuropathological details of subjects**

PaperID	BioBank	Pathological Diagnosis	PMI	Age	Sex	ABC (A) Thal score	ABC (B) Braak Score	ABC (C) Cerad score	Cerad criteria	AD Braak Stage	CDR	Amyloid (Severe, Yes, Mild, Rare, NA)	Clinical Diagnosis
AD01	Harvard/McLean Hospital	AD	12.08	76	Female					VI		Y	
AD02	Harvard/McLean Hospital	AD	8.3	80	Female					VI		Y	
AD03	Harvard/McLean Hospital	AD	13.28	74	Female					V		Y	
AD04	Harvard/McLean Hospital	AD	15	90	Female					VI		Y	
AD05	Harvard/McLean Hospital	AD	17.42	64	Female					VI		MILD	
AD06	Harvard/McLean Hospital	AD	21.08	83	Female					VI		Y	
AD07	Harvard/McLean Hospital	AD	9.05	89	Male					V		Y	
AD08	Harvard/McLean Hospital	AD	13.5	62	Male					VI		Y	
AD09	Harvard/McLean Hospital	AD	10.08	70	Male					V		Y	
AD10	Harvard/McLean Hospital	AD	4.93	77	Male					V		Y	
AD12	Harvard/McLean Hospital	AD	24.75	80	Male					V		Y	
AD13	Harvard/McLean Hospital	AD	20.58	80	Male					V		Y	
AD14	Miami	AD	22.10	80	Male	3	3	3		Stage VI	3	YES	AD, FLD
AD15	Miami	AD	18.00	77	Male	3	3	3		Stage V-VI	NA	YES	AD
AD16	Miami	AD	22.00	69	Female	3	3	3		Stage V-VI	3	YES	AD, LBD
AD17	Miami	AD	7.90	60	Female	2	3	3		Stage V-VI	3	YES	Dementia
AD18	Miami	AD	23.80	78	Male	3	3	3		Stage V-VI	3	YES	AD
AD19	Miami	AD	6.50	80	Female	3	3	3		Stage VI	3	YES	AD
AD20	UCLA	AD	12.3	78	Female				AD	NA			AD
AD21	UCLA	AD	12	78	Male				AD definite	IV-V		YES	AD
AD22	UCLA	AD	10.5	74	Female				AD definite	NA		YES	AD/PD
AD23	UCLA	AD	18.4	89	Female				AD definite	NA			AD

AD24	UCLA	AD	11.3	81	Female				AD definite	NA			Dementia
AD25	UCLA	AD	23	41	Female				AD definite	NA			AD
AD26	UCLA	AD	23	77	Female				AD definite	V		YES	AD
AD27	UCLA	AD	13	77	Female				AD	V		YES	AD
AD29	UCLA	AD	19.5	86	Male				AD probable	NA			AD
AD30	UCLA	AD	19.8	75	Female				AD definite	V		YES	AD
AD31	UCLA	AD	17	86	Male				AD definite	V		YES	AD
AD32	UCLA	AD	11	78	Female				Alzheimer probable	NA			CTR
AD33	UCLA	AD	13.8	76	Male				AD definite	VI		YES	AD
AD34	UCLA	AD	14	84	Female				AD definite	V		YES	AD
AD35	UCLA	AD	18.4	87	Female				AD definite	V-VI		YES	AD
AD36	UCLA	AD	20	88	Male				AD definite	III-IV		YES	Dementia
AD37	UCLA	AD	6.7	81	Female				AD definite	V		YES	AD
AD38	UCSF	AD	8.2	84	Male	3	3	3		VI			
AD39	UCSF	AD	12.9	69	Female	3	3	3		VI			
AD40	UCSF	AD	9.0	70	Male	3	3	3		VI			
AD42	UCSF	AD	6.9	72	Male	3	3	3		VI			
AD43	UCSF	AD	8.2	64	Female	3	3	3		VI			
AD44	UCSF	AD	8.8	75	Female	3	3	3		VI			
AD45	UCSF	AD	7.9	72	Male	3	3	3		VI			
AD46	UCSF	AD	6.5	80	Male	3	3	3		VI			
AD47	UCSF	AD	8.5	61	Male	3	3	3		VI			
AD48	UCSF	AD	11.9	73	Female	3	3	3		VI			
AD49	Miami	AD	17.70	66	Female	3	3	3		Stage V-VI	3	YES	FTD
CTR01	Harvard/McLean Hospital	CTRL	15.7	82	Female					I			
CTR02	Harvard/McLean Hospital	CTRL	20.83	85	Male					II-III			
CTR03	Harvard/McLean Hospital	CTRL	20.6	79	Female					II			
CTR04	Harvard/McLean Hospital	CTRL	17	97	Male					II			
CTR05	Harvard/McLean Hospital	CTRL	8.08	76	Female					I			
CTR06	Harvard/McLean Hospital	CTRL	16.8	61	Female					0			
CTR07	Harvard/McLean Hospital	CTRL	21.33	70	Female					I			

CTR08	Harvard/ McLean Hospital	CTRL	18.02	73	Male					I			
CTR10	Harvard/ McLean Hospital	CTRL	14.33	64	Male					I		rare sparse	
CTR11	Miami	CTRL	23.90	71	Male	NA	NA	NA		NA	N A	NA	CTR
CTR12	Miami	CTRL	19.10	68	Female	NA	NA	NA		NA	N A	NA	CTR
CTR13	Miami	CTRL	14.20	75	Male	NA	1	NA		Stage I	N A	NA	CTR
CTR14	Miami	CTRL	20.50	60	Female	NA	NA	NA		NA	N A	MILD	CTR
CTR15	Miami	CTRL	17.80	79	Female	NA	NA	NA		NA	N A	NA	CTR
CTR17	Miami	CTRL	14.70	77	Male	NA	1	1		Stage II	N A	RARE	CTR
CTR19	UCLA	CTRL	17.75	90	Male				CTR	NA			CTR
CTR20	UCLA	CTRL	20	79	Male				CTR	NA			CTR
CTR21	UCLA	CTRL	11.8	90	Female				Essenti al normal brain	0			CTR
CTR22	UCLA	CTRL	15.5	77	Male				Essenti al normal brain	0			CTR
CTR23	UCLA	CTRL	12.9	82	Female				Essenti al normal brain	0			CTR
CTR24	UCLA	CTRL	14.5	86	Male				Essenti al normal brain	0			CTR
CTR25	UCLA	CTRL	16	52	Male				Essenti al normal brain	0			CTR
CTR26	UCLA	CTRL	14	82	Male				Minor aging change s	0			CTR
CTR27	UCLA	CTRL	12.2	72	Male				Inciden tal change s consist ent with aging	NA			CTR
CTR28	UCLA	CTRL	9	58	Male				Essenti al normal brain	0			CTR
CTR29	UCLA	CTRL	15	92	Male				Control Hypoxs ia	NA			AD
CTR30	UCLA	CTRL	10.5	68	Male				Essenti al normal brain	0			CTR
CTR31	UCLA	CTRL	11.5	75	Male				Essenti al normal brain	0			CTR

CTR32	UCLA	CTRL	15.4	75	Female				Essential normal brain	0			CTR
CTR33	UCLA	CTRL	22	88	Female				Essential normal brain with age changes	0			CTR
CTR34	UCLA	CTRL	12.6	57	Male				Essential normal brain	0			CTR
CTR35	UCLA	CTRL	12	80	Male				Hypoxia	0			CTR
CTR36	UCLA	CTRL	18.3	45	Female				Essential normal brain	0			CTR
CTR37	UCLA	CTRL	22.2	76	Male				Essential normal brain	0			Dementia
CTR38	UCLA	CTRL	17.6	83	Female				Essential normal brain	0			CTR
CTR39	UCLA	CTRL	20.2	59	Male				Essential normal brain	0			CTR
CTR40	UCSF	CTRL	30.3	81	Female	0	1	0		II			
CTR41	UCSF	CTRL	7.8	86	Female	1	1	2		II			
CTR42	UCSF	CTRL	8.2	76	Male	1	1	1		II			
CTR43	Miami	CTRL	24.20	64	Male	NA	1	1		Stage I	0	NA	CTR

1404

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1406 **Extended Data Table 2. Known tau cleavage sites**

Cleavage site	Reported protease	CT R	A D	Reference	Current study
M1-A2	20s CP	V	V	Derisbourg et al., 2015; Ukmar-Godec et al., 2020	X
A2-E3	Calpain-2	-	-	Garg et al., 2011	X
V10-M11	-	V	V	Derisbourg et al., 2015	X
M11-E12	20s CP	V	V	Derisbourg et al., 2015; Ukmar-Godec et al., 2020; Behrendt et al., 2019	V
D13-H14	Caspase-6, 20s Proteasome	-	-	Horowitz et al., 2004, Ukmar-Godec et al., 2020	X
Y18-G19	-	V	V	Current study	V
D25-Q26	Caspases	V	↑	Corsetti et al., 2008, Amadoro et al., 2012	X
Y29-T30	-	X	V	Current study	V
T30-M31	-	X	V	Current study	V
M31-H32	-	V	V	Current study	V
H32-Q33	-	X	V	Current study	V
A66-K67	20s CP	-	-	Ukmar-Godec et al., 2020	X
G83-A84	20s CP	-	-	Ukmar-Godec et al., 2020	X
A89-A90	20s CP	-	-	Ukmar-Godec et al., 2020	X
A90-A91	20s CP	-	-	Ukmar-Godec et al., 2020	X
T102-A103	-	V	V	Derisbourg et al., 2015	X
A103-E104	20s CP	-	-	Ukmar-Godec et al., 2020	X
E104-E105	IP	X	V	Current study	V
E105-A106	20s CP	-	-	Ukmar-Godec et al., 2020	X
A106-G107	IP	X	V	Current study	V
D110-T111	IP	X	V	Current study	V
P112-S113	IP	X	V	Current study	V
A118-A119	20s CP	-	-	Ukmar-Godec et al., 2020	X
T123-Q124	IP	V	V	Derisbourg et al., 2015	V
Q124-A125	Calpain-2	V	V	Garg et al., 2011	X
A125-R126	20s CP	-	-	Ukmar-Godec et al., 2020	X
R126-M127	-	V	V	Derisbourg et al., 2015	tryptic
A152-T153	ADAM10	-	V	Henriksen et al., 2013	X
R155-G156	Thrombin	V	V	Arai et al., 2005, Behrendt et al., 2019	tryptic
A166-N167	20s CP	-	-	Ukmar-Godec et al., 2020	X
N167-A168	AEP	V	V	Behrendt et al., 2019	X
A168-T169	20s CP	-	-	Ukmar-Godec et al., 2020	X
T169-R170		V	V	Behrendt et al., 2019	X
I171-P172	20s CP	V	V	Derisbourg et al., 2015; Ukmar-Godec et al.	X

P172-A173	-	V	V	Behrendt et al., 2019	X
A173-K174	-	V	V	Derisbourg et al., 2015	X
T175-P176	20s CP	-	-	Ukmar-Godec et al., 2020	X
G192-D193	20s CP	-	-	Ukmar-Godec et al., 2020	X
G196-Y197	Calpain-1	X	V	Chen et al	X
R209-S210	Thrombin	-	-	Arai et al., 2005,	tryptic
S210-R211	20s CP	-	-	Ukmar-Godec et al., 2020	X
T212-P213	20s CP	-	-	Ukmar-Godec et al., 2020	X
E222-P223	20s CP	-	-	Ukmar-Godec et al., 2020	X
P223-K224	-	V	V	Derisbourg et al., 2015	X
V226-A227	20s CP	-	-	Ukmar-Godec et al., 2020	X
A227-V228	20s CP	-	-	Ukmar-Godec et al., 2020	X
R230-T231	Thrombin, Calpain-1&2	V	V	Arai et al., 2005; Park et al., 2005; Garg et al., 2011; Ferreira et al., 2011; Gu et al., 2020	tryptic
S237-S238	-	V	V	Derisbourg et al., 2015	X
A239-K240	-	V	V	Derisbourg et al., 2015	X
S241-R242	-	X	V	Current study	V
R242-L243	Calpain-1	V	V	Matsumoto et al., 2015; Behrendt et al., 2019	tryptic
L243-Q244	20s CP, IP	X	V	Chen et al., 2018; Ukmar-Godec et al., 2020	V
Q244-T245	20s CP, Calpain-1	X	V	Chen et al., 2018; Ukmar-Godec et al., 2020	V
T245-A246	20s CP, IP	-	-	Ukmar-Godec et al., 2020	V
A246-P247	20s CP	-	-	Ukmar-Godec et al., 2020	V
L253-K254	20s CP	-	-	Ukmar-Godec et al., 2020	X
N255-V256	AEP, 20s CP			Zhang et al. 2014; Ukmar-Godec et al., 2020	X
V256-K257	20s CP	-	-	Ukmar-Godec et al., 2020	X
K257-S258	Thrombin	-	-	Arai et al., 2005,	tryptic
S258-K259	-	V	V	Derisbourg et al., 2015	X
I260-G261	-	V	V	Derisbourg et al., 2015	X
G272-G273	-	V	V	Behrendt et al., 2019	X
Q276-I277	-	V	V	Behrendt et al., 2019	X
N279-K280	-	V	V	Derisbourg et al., 2015	V
N296-I297	-	X	V	Current study	V
G302-G303	IP	X	V	Current study	V
G303-G304	IP	X	V	Current study	V
G304-S305	IP	X	V	Chen et al., 2018	V

S305-V306	IP	V	V	Derisbourg et al., 2015	tryptic
Q307-I308	20s CP, IP	V	V	Derisbourg et al., 2015; Ukmar-Godec et al., 2020	V
I308-V309	20s CP	V	V	Derisbourg et al., 2015; Ukmar-Godec et al., 2020	X
Y310-K311	20s CP	V	V	Derisbourg et al., 2015; Ukmar-Godec et al., 2020	X
V313-D314	-			Current study	V
D314-L315,	Caspase-2	V	↑	Zhao et al., 2016	V
L315-S316	20s CP, IP	-	-	Ukmar-Godec et al., 2020	V
S316-K317	20s CP	-	-	Ukmar-Godec et al., 2020	X
V318-T319	20s CP	-	-	Ukmar-Godec et al., 2020	X
T319-S320	20s CP	-	-	Ukmar-Godec et al., 2020	X
S320-K321	20s CP	-	-	Ukmar-Godec et al., 2020	X
G323-S324	Calpain-1, IP	V	V	Chen et al., 2018	V
L325-G326	-			Current study	V
G326-N327	Calpain-1	V	V	Chen et al., 2018	X
N327-I328	-			Current study	V
H329-H330	-			Current study	V
H330-K331	-	V	V	Derisbourg et al., 2015	X
K340-S341	Thrombin	-	-	Arai et al., 2005	tryptic
S352-K353	IP			Current study	V
I354-G355	20s CP, IP	-	-	Ukmar-Godec et al., 2020	V
G355-S356	20s CP	-	-	Ukmar-Godec et al., 2020	V
S356-L357	20s CP	-	-	Ukmar-Godec et al., 2020	X
L357-D358	20s CP	-	-	Ukmar-Godec et al., 2020	X
D358-N359	IP			Current study	V
V363-P363	-	V	V	Behrendt et al., 2019	X
G366-G367	20s CP	V	V	Ukmar-Godec et al., 2020; Behrendt et al., 2019	X
G367-N368	20s CP	-	-	Ukmar-Godec et al., 2020	X
N368-K369	AEP, 20s CP, IP	X/ V	V	Zhang et al., 2014; ,Ukmar-Godec et al.,2020; Behrendt et al., 2019	V
I371-E372	20s CP	-	-	Ukmar-Godec et al., 2020	X
F378-R379	20s CP	-	-	Ukmar-Godec et al., 2020	X
R379-E380		V	V	Behrendt et al., 2019	tryptic
A384-K385	20s CP	-	-	Ukmar-Godec et al., 2020	X
D387-H388	20s CP	-	-	Ukmar-Godec et al., 2020	X
G389-A390	20s CP	-	-	Ukmar-Godec et al., 2020	X
A390-E391	20s CP	-	-	Ukmar-Godec et al., 2020	X

E391-I392	-	V	V	Derisbourg et al., 2015; Novak et al., 1993; Behrendt et al., 2019	X
I392-V393	20s CP	-	-	Ukmar-Godec et al., 2020	X
Y394-K395	-	X	V	Derisbourg et al., 2015; Behrendt et al., 2019	V
V399-S400	20s CP	-	-	Ukmar-Godec et al., 2020	X
S400-G401	-	V	V	Behrendt et al., 2019	X
G401-D402	20s CP	-	-	Ukmar-Godec et al., 2020	X
D402-T403	Caspase-6		V	Albrecht et al. 2007; Foveau et al., 2016; Guo et al., 2004	X
T403-S404	-	V	V	Behrendt et al., 2019	X
V411-S412	20s CP	-	-	Ukmar-Godec et al., 2020	X
S412-S413	20s CP	-	-	Ukmar-Godec et al., 2020	X
S413-T414	20s CP	-	-	Ukmar-Godec et al., 2020	X
T414-G415	20s CP	-	-	Ukmar-Godec et al., 2020	X
G415-S416	20s CP	-	-	Ukmar-Godec et al., 2020	X
S416-I417	20s CP	-	-	Ukmar-Godec et al., 2020	X
I417-D418	20s CP	-	-	Ukmar-Godec et al., 2020	X
D418-M419	IP	V	V	Behrendt et al., 2019, Current study	V
D421-S422	Caspase-1, 3, 6, 7, & 8, 20-S CP	X/ V	V	Gamblin et al., 2003; Ukmar-Godec et al., 2020; Behrendt et al., 2019	V
L425-A426	20s CP	-	-	Ukmar-Godec et al., 2020	X
A426-T427	20s CP, IP	-	-	Ukmar-Godec et al., 2020	V
T427-L428	-	X	V	Current study	V
L428-A429	20s CP, IP	-	-	Ukmar-Godec et al., 2020	V
S433-A434	-	V	V	Behrendt et al., 2019	X
A434-S435	20s CP	-	-	Ukmar-Godec et al., 2020	X

Extended Data Table 3. Scoring of cleavage sites

Site	CTR	AD	Patient Freq.	AD specificity	HMW/PHF	Hexapeptide	Score	Selection
N-term (peptide before the site is cleaved off from tau)								
M11-E12	7.32	43.48	1	1	2	0	4	
Y29-T30	0.00	15.22	0	1	1	0	2	
T30-M31	7.32	17.39	0	1	0	0	1	
M31-H32	19.51	23.91	0	0	0	0	0	
H32-Q33	0.00	23.91	0	1	0	0	1	
E104-E105	0.00	43.48	1	1	1	0	3	
A106-G107	9.76	45.65	2	1	2	0	5	
D110-T111	2.44	36.96	1	1	1	0	3	
P112-S113	2.44	50.00	2	1	1	0	4	
P213-S214	2.44	19.57	0	1	0	0	1	
S241-R242	2.44	32.61	1	1	1	0	3	
L243-Q244	2.44	15.22	0	1	1	0	2	
Q244-T245	2.44	71.74	2	1	2	0	5	
T245-A246	2.44	65.22	2	1	2	0	5	
A246-P247	0.00	21.74	0	1	0	0	1	
N279-K280	0.00	23.91	0	1	1	1	3	
K295-N296	0.00	15.22	0	1	0	1	2	
N296-I297	0.00	36.96	1	1	1	1	4	1
G302-G303	2.44	63.04	2	1	1	1	5	
G303-G304	0.00	45.65	2	1	2	1	6	2
G304-S305	0.00	56.52	2	1	1	1	5	3
S305-V306		Tryptic*	2	2	2	1	7	4
Q307-I308	12.20	32.61	1	0	2	0	3	
G323-S324	31.71	45.65	2	0	2	0	4	
L325-G326	0.00	17.39	0	1	0	0	1	
N327-I328	0.00	28.26	1	1	2	0	4	
H329-H330	0.00	19.57	0	1	1	0	2	
S352-K353	2.44	76.09	2	1	2	0	5	
I354-G355	0.00	15.22	0	1	1	0	2	
G355-S356	0.00	17.39	0	1	1	0	2	
D358-N359	0.00	19.57	0	1	0	0	1	
D418-M419	2.44	15.22	0	1	1	0	2	
D421-S422	0.00	15.22	0	1	0	0	1	
A426-T427	0.00	28.26	0	1	1	0	2	
T427-L428	0.00	23.91	0	1	1	0	2	
L428-A429	0.00	34.78	1	1	1	0	3	
C-term (peptide after the site is cleaved off from tau)								
Y18-G19	2.44	36.17	1	1	2	0	4	
T123-Q124	19.51	23.91	0	0	1	0	1	
Q307-I308	7.32	19.57	0	1	0	0	1	
V313-D314	2.44	17.39	0	1	0	1	2	
D314-L315	0.00	13.04	0	1	0	1	2	
L315-S316	4.88	26.09	0	1	1	1	3	
N368-K369	2.44	47.83	2	1	2	1	6	a
Y394-K395	0.00	54.35	2	1	1	1	5	b
A426-T427	4.88	8.70	0	0	1	1	2	
Scores assigned as follows: <u>Patient freq.</u> - top 10 ranked frequency received 2 pts, top 20 ranked frequency received 1 pt. <u>AD specificity</u> - sites that were identified in 3 or fewer CTR subjects received 1 pt. <u>HMW/PHF</u> - Identification for each preparation received 1 pt. <u>Hexapeptide</u> - cleavage between first (275 VQIINK 280) and second (306 VQIVYK 311) hexapeptide (N-term)								

amyloid motifs, or after the VQIVYK hexapeptide (C-term) received 1 pt. Colors indicate the higher (red) and lower (green) scores assigned.

*3R isoform of tau without R2 domain will display this cleavage site as K-V, which is tryptic and not able to be evaluated the frequency when mixed with 4R isoform.

1409