NEURODEGENERATIVE DISEASES

Nasal tau immunotherapy clears intracellular tau pathology and improves cognitive functions in aged tauopathy mice

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Pathological tau aggregates cause cognitive decline in neurodegenerative tauopathies, including Alzheimer's disease (AD). These aggregates are prevalent within intracellular compartments. Current tau immunotherapies have shown limited efficacy in clearing intracellular tau aggregates and improving cognition in clinical trials. In this study, we developed toxic tau conformation–specific monoclonal antibody-2 (TTCM2), which selectively recognized pathological tau aggregates in brain tissues from patients with AD, dementia with Lewy bodies (DLB), and progressive supranuclear palsy (PSP). TTCM2 potently inhibited tau-seeding activity, an essential mechanism underlying tauopathy progression. To effectively target intracellular tau aggregates and ensure rapid delivery to the brain, TTCM2 was loaded in micelles (TTCM2-ms) and administered through the intranasal route. We found that intranasally administered TTCM2-ms efficiently entered the brain in hTau-tauopathy mice, targeting pathological tau in intracellular compartments. Moreover, a single intranasal dose of TTCM2-ms effectively cleared pathological tau, elevated synaptic proteins, and improved cognitive functions in aged tauopathy mice. Mechanistic studies revealed that TTCM2-ms cleared intracellular, synaptic, and seed-competent tau aggregates through tripartite motif-containing 21 (TRIM21), an intracellular antibody receptor and E3 ubiquitin ligase known to facilitate proteasomal degradation of cytosolic antibody-bound proteins. TRIM21 was found to be essential for TTCM2-ms–mediated clearance of tau pathology. Our study collectively provides evidence of the effectiveness of nasal tau immunotherapy in targeting and clearing intracellular tau pathology through TRIM21 and enhancing cognition in aged tauopathy mice. This study could be valuable in designing effective tau immunotherapies for AD and other tauopathies.

INTRODUCTION

Intraneuronal accumulation of pathological tau aggregates in the brain is a hallmark of Alzheimer's disease (AD) and related dementias, including Pick's disease (PiD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and frontotemporal dementia (FTD), collectively known as tauopathies. Tau pathology is common in dementia with Lewy bodies (DLB). These tau aggregates are typically found within neuronal cell bodies and dendrites in the brains of patients with tauopathy, where they are referred to as neurofibrillary tangles (NFTs) and neuropil threads (*1*). Studies show that clinical severity and cognitive decline in AD strongly correlate with the deposition of pathological tau aggregates (*1*–*4*). Deposition of pathological tau is found in numerous neurodegenerative diseases, emphasizing the central role of tau aggregates in driving neurodegeneration and cognitive decline and highlighting the potential importance of tau-targeted therapy as a disease-modifying treatment. One promising approach to specifically target and remove pathological tau aggregates from the brain is immunotherapy. However, therapeutic antibodies face challenges in reaching the brain because of the blood-brain barrier (BBB) and poor internalization by neurons. Given that pathological tau aggregates are prevalent within intracellular compartments of neurons, the ability of antibodies to effectively target and eliminate pathological tau aggregates is impeded (*5*, *6*). These limitations present an important barrier to advancing effective therapies for neurodegenerative tauopathies (*5*, *6*).

Pathological deposition of tau is initiated by transition of monomeric tau, an intrinsically disordered protein, into misfolded conformations that form toxic tau aggregates (*7*). These self-propagating misfolded toxic tau seeds are commonly found in the early stages of AD, PiD, PSP, CBD, FTD, and DLB and are implicated in disease progression. Tau-seeding activity is positively associated with pathological tau loads and negatively correlated with cognitive function across multiple neurodegenerative diseases (*8*, *9*). Neurons further release pathological tau seeds into the extracellular environment, facilitating the trans-cellular propagation of tau pathology in a specific stereotypical spatiotemporal pattern. This process leads to neuronal loss and cognitive decline, underscoring the key role of seed-competent toxic tau conformations in disease progression (*10*–*13*).

Previous studies using tau immunotherapy in AD mouse models demonstrated the ability of anti-tau monoclonal antibodies (mAbs) to halt tauopathy progression and even improve cognitive and motor functions in some cases (*14*–*16*). However, conventional mAbs primarily target extracellular tau. As a result, intracellular pathological tau aggregates remain largely unaffected upon immunotherapy. Despite this limitation, several clinical trials were launched to test the efficacy of anti-tau mAbs on the basis of promising results in mice (*17*, *18*). Disappointingly, trials with the mAbs tilavonemab and gosuranemab failed to demonstrate improvements in patients with tauopathies (*19*). suggesting that solely targeting extracellular tau may not be efficacious for treating patients with tauopathies (*5*). Consistent with these observations, recent studies suggest that intracellular tau aggregates and synaptic tau seeds play a more crucial

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role in the disease than their extracellular counterparts (*20*, *21*). Tau pathology can propagate through exosomes or nanotubes, protecting tau seeds from therapeutic antibodies (*22*). Thus, to effectively attenuate tauopathy progression and cognitive decline, a strong rationale exists for developing immunotherapies that target intracellular tau and block seeding activity.

Several challenges exist to develop safe and effective tau immunotherapies, including (i) identifying anti-tau mAbs that selectively recognize pathological, misfolded, and toxic tau conformations rather than the more abundant physiological tau; (ii) efficiently reaching intracellular compartments in the brain to remove pathological tau; (iii) effectively neutralizing or clearing pathological tau seeds; and (iv) removing pathological tau without triggering deleterious inflammatory responses.

With these considerations in mind, we have developed a toxic tau conformation–specific mAb (TTCM2). TTCM2 specifically detected disease-relevant tau aggregates in postmortem brain tissues from patients with AD, DLB, and PSP and effectively neutralized the tau-seeding activity of AD brain-derived tau oligomers (AD-BDTOs). TTCM2 was loaded in micelles (TTCM2-ms) for improved intracellular delivery in the brain upon intranasal delivery in tauopathy mice. We chose the intranasal route because it is recognized as a viable, noninvasive, safe approach for effective drug delivery to the brain through a direct nose-to-brain anatomic pathway (*23*). We show that TTCM2-ms reached the brain rapidly and efficiently, eliminated intracellular and seed-competent tau aggregates from the brain, and improved cognition in a mouse model of tauopathy.

RESULTS

TTCM2 recognizes disease-relevant tau aggregates in brain tissue from patients with tauopathies and inhibits tau-seeding activity of AD-BDTOs in vitro

To determine whether TTCM2 specifically recognizes disease-relevant pathological tau, we performed double-label immunostaining using TTCM2 and anti-phospho-tau monoclonal antibody (AT8) antibodies on brain tissue from patients with AD ($n = 8$), DLB ($n = 6$), and PSP ($n = 6$) and age-matched nondemented controls (NDCs; $n = 8$) (*12*, *24*, *25*). The AT8 antibody recognizes tau phosphorylation at Ser^{202}/Thr^{205} and is widely regarded as a reliable marker to detect pathological NFTs. AT8 immunoreactivity is primarily found in intraneuronal NFTs (iNFTs), extraneuronal NFTs, and pretangle phospho-tau aggregates (pre-NFTs) (*26*). Our results revealed strong TTCM2 (red) immunoreactivity in brain sections from patients with AD, DLB, and PSP and relatively sparse staining in NDCs (Fig. 1, A and B). Correlation analysis of immunostaining for AT8 (green) and TTCM2 (red) demonstrated colocalization between TTCM2 and AT8 in all human tauopathy brain tissues examined (Fig. 1C). We observed that TTCM2 primarily stained iNFTs and pre-NFTs (perinuclear staining) (Fig. 1, A to C). In addition, immunohistochemistry staining revealed that TTCM2 exhibited strong immunoreactivity to pathological tau present in neuropil threads, iNFTs and pre-NFTs in AD, DLB, and PSP cases (fig. S1A); and little or no immunoreactivity in brain sections from NDC cases (fig. S1A).

To further validate these data, we performed enzyme-linked immunosorbent assays (ELISAs) using brain homogenates from human and murine tauopathy brain tissues, as well as different forms of tau, including tau monomers (TauM), tau oligomers (TauO), and tau fibrils (TauF) (*27*, *28*). The results confirmed strong TTCM2 immunoreactivity in brain homogenates from patients with AD, DLB, and PSP compared with NDCs (Fig. 1D). Moreover, we observed enhanced TTCM2 immunoreactivity in 18-month-old hTau mice relative to 3-month-old hTau mice, with no reactivity detected in tau-knockout mice (Fig. 1D). In addition, we found that TTCM2 exhibited the highest reactivity to TauO relative to TauF and TauM (Fig. 1D).

To assess the binding specificity of TTCM2 with TauO, we performed dot blot assays with TTCM2 and Tau5 antibodies and various tau aggregates, including T22 affinity–purified brain-derived tau oligomers (BDTOs) from patients with AD, DLB, and PSP and recombinant Aβ oligomers, α-synuclein oligomers, and TauO. Results indicated strong immunoreactivity for TTCM2 against BDTOs from patients with AD, DLB, and PSP and recombinant TauO. However, TTCM2 did not detect Aβ oligomers or α-synuclein oligomers (fig. S1B), suggesting that TTCM2 specifically detects TauO. We and others have demonstrated that TauO are highly toxic in vitro and in vivo (*27*, *29*–*31*). We found that TTCM2 effectively neutralized TauO toxicity in SH-SY5Y neuroblastoma cells in a dose-dependent manner (fig. S1C). Given that TTCM2 detected abnormal TauM, it is worth noting that although TauO and TauF are well-established pathological forms of tau in neurodegenerative diseases (*24*, *32*–*38*), recent studies indicated that TauM can also exist in abnormal or pathological forms that initiate seeding and spreading of tau pathology (*39*–*42*). The ability to detect the monomeric form of tau is a characteristic feature of several tau conformational antibodies (*40*–*45*).

Next, to examine the tau conformational specificity of TTCM2, we used dot blot assays to measure its immunoreactivity in different conformations of tau protein, including nondenatured (ND) and denatured (D) states, by chemical and thermal treatment of TauM, TauO, and TauF. We found that TTCM2 shows stronger reactivity with ND forms of tau, and when tau loses its natural structure (upon chemical and thermal denaturation), TTCM2's reactivity with TauM and TauO decreased (fig. S1, D and E). TTCM2 demonstrated greatest differences in reactivity with TauO compared with TauM or TauF (fig. S1, D and E). Conversely, the tau 13 antibody, which detects total tau, showed no differences in immunoreactivities between ND and D tau forms (fig. S1, F and G). These data indicate that TTCM2 is a tau conformational antibody with strong immunoreactivity to pathological and toxic TauO (Fig. 1D and fig. S1, A to G).

An important aspect of tauopathy is the presence of seed-competent toxic tau conformations in the brain at early stages of disease (*9*, *46*). These pathological tau conformations act as seeds that recruit and misfold endogenous naïve TauM and propagate tau pathology across the brain (*47*). Tau-seeding activity is clinically relevant, correlating with disease progression and cognitive decline in patients with AD (*9*, *48*). Several studies indicate that TauO isolated from human tauopathies exhibit potent tau-seeding activities (*20*, *48*–*51*). We previously demonstrated that BDTOs from patients with AD are highly neurotoxic, propagate tau pathology across brain regions, and induce cognitive dysfunction in mouse models (*27*, *52*). These observations suggest that blocking tau-seeding activity through immunotherapy may be beneficial for halting disease progression.

Here, we used a biosensor cell line that constitutively expresses human tau repeat domain (TauRD) with P301S mutation, which is commonly used to quantify tau seeding activity (*11*, *48*). These cells form intracellular aggregates when exposed to bioactive tau seeds. We investigated whether TTCM2 treatment inhibits seeding activity

of AD-BDTOs in tau biosensor cells. Lipofectamine 2000 transfection reagent was used to deliver the AD-BDTOs:TTCM2 immune complexes to the cells (Fig. 1E). Results show that at nanomolar concentrations, AD-BDTOs profoundly induced tau aggregation in biosensor cells, and this seeding activity was substantially inhibited by TTCM2 treatment (Fig. 1, F and G). The optimal ratio of tau to TTCM2 for inhibiting seeding activity was 1:4, which reduced the seeding activity by 70% compared with AD-BDTO alone. Higher ratios of tau to TTCM2 (such as 1:8) did not show further improvement over 1:4 ratio, suggesting a plateau in the inhibition of seeding activity and that a 1:4 ratio is the optimal stoichiometry of AD-BDTOs to TTCM2 for neutralizing tau seeding. Together, these findings demonstrate that TTCM2 is a tau conformation–specific antibody capable of recognizing disease-relevant tau aggregates in brain tissue from patients with AD, DLB, and PSP. Moreover, TTCM2 effectively neutralized the toxicity of TauO and blocked tau-seeding activity of AD-BDTOs.

Intranasally administered TTCM2-ms rapidly distributed to various brain regions and engaged with pathological tau in aged hTau tauopathy mice

The BBB prevents efficient transport of therapeutic tau mAbs from blood circulation to the brain. The intranasal drug delivery approach

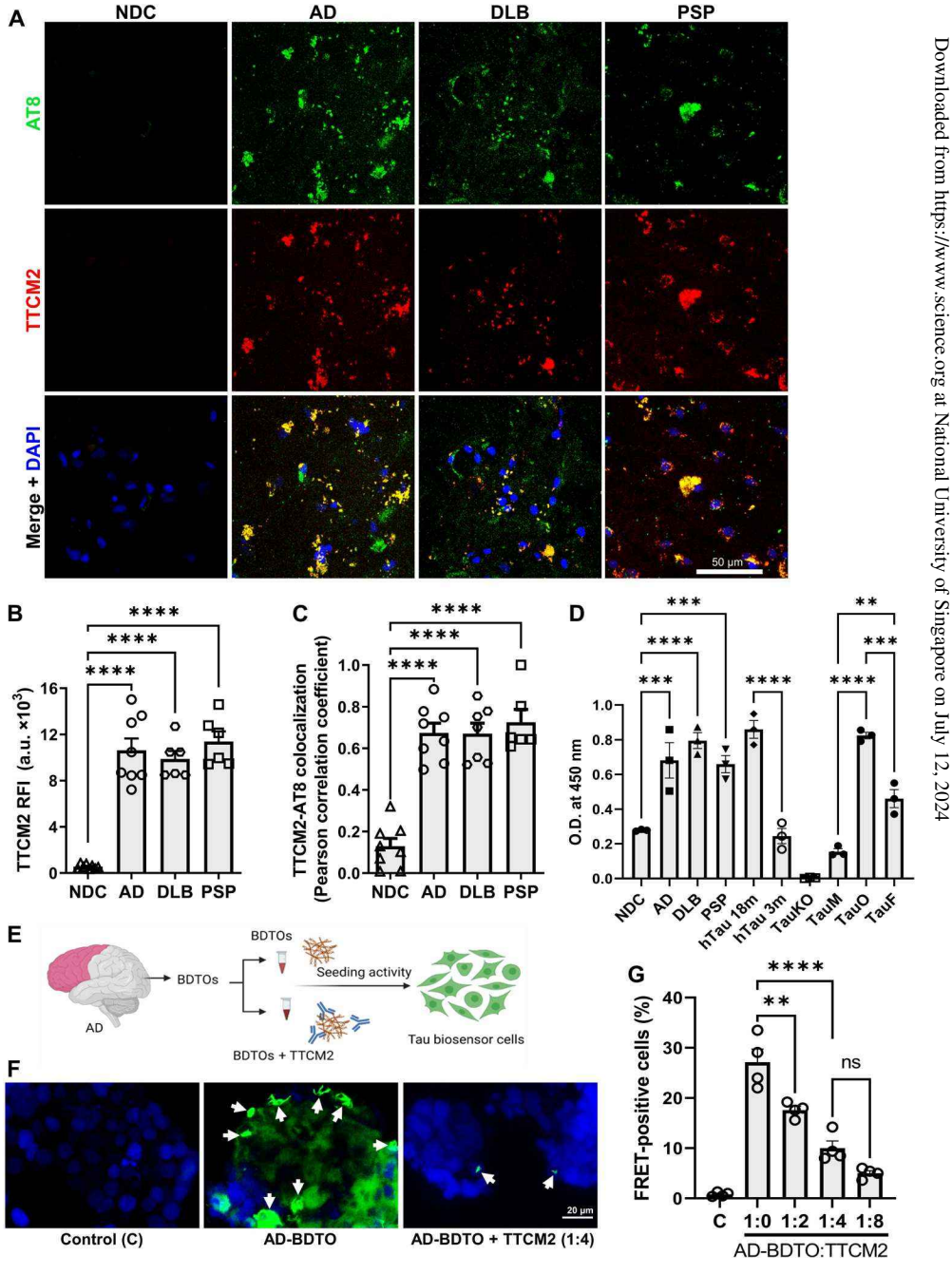

Fig. 1. TTCM2 recognizes pathological tau in brain tissue from patients with AD, DLB, and PSP, but not NDCs, and inhibits tau-seeding activity of AD-BDTOs. (**A**) Representative immunofluorescence staining with AT8 antibody (green), TTCM2 antibody (red), and merge + 4′,6-diamidino-2-phenylindole (DAPI) (blue) in frontal cortex from patients with AD, DLB, and PSP and NDCs. (**B**) Mean relative fluorescence intensities (RFI) of TTCM2 staining in brain tissue from AD ($n = 8$), DLB ($n = 6$), and PSP ($n = 6$) and NDCs ($n = 8$). (**C**) Pearson's correlation coefficient analysis assessing colocalization between TTCM2 and AT8. (**D**) ELISA was used to measure immunoreactivity of TTCM2 against brain homogenates from patients with AD, DLB, and PSP and tauopathy mouse model, including 3- and 18-month-old hTau mice (n = 3 per group). Brain homogenates from Tau-knockout mice were used as a control ($n = 3$ mice per group). Recombinant TauM, TauO, and TauF were also assessed for immunoreactivity with TTCM2. The graph shows the mean ± SEM of optical density (O.D.) at 450 nm. (**E**) Illustration of experiments to test seeding activity of AD-BDTOs isolated from PBS-soluble fractions by immunoprecipitation with T22 antibody analyzed using tau biosensor cells ± TTCM2. (**F**) Representative images showing seeding activity of AD-BDTOs (green) and its inhibition by TTCM2; the untreated control is shown at the left. DAPI (blue) counterstain was used to detect total nuclei. Scale bar, 20 μm. (**G**) Graph showing dose-dependent effect of TTCM2 on AD-BDTO seeding activity in biosensor cells. Percentage of fluorescence resonance energy transfer (FRET)–positive cells was determined by dividing the number of FRET-positive cells by total DAPI-positive cells representative of $n = 4$ experiments. Significance was determined by one-way ANOVA followed by Tukey's test. Graphs show the mean ± SEM; $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. ns, nonsignificant; a.u., arbitrary units.

can bypass the BBB and thus is amenable for direct delivery of brain-targeted therapeutic drugs (*23*, *53*). However, despite efficient delivery to the brain, intranasally delivered tau mAbs are poorly internalized by neurons, resulting in intact intracellular tau and immunotherapy failure (*5*). Here, to investigate their therapeutic potential, we assessed the brain and cellular distribution of TTCM2-ms after intranasal administration to hTau mice—a transgenic mouse model of human tauopathy, which expresses all six isoforms of human tau protein and exhibits cognitive dysfunction and tau pathology in an age-dependent manner (*54*). Aged hTau and wild-type (WT) mice were intranasally treated with Alexa Fluor (AF) 568–labeled TTCM2-ms or a nonspecific immunoglobulin G (IgG) control antibody (anti-rhodamine antibody) and euthanized 3 hours after treatment. After perfusion, brain tissues were extracted, and unfixed tissues were immediately analyzed by ex vivo fluorescence imaging and immunofluorescence microscopy (Fig. 2A). Imaging results indicate that TTCM2-ms readily reached the brain, exhibiting a radiant efficiency that is significantly increased ($P < 0.001$) in hTau mice relative to WT control mice (Fig. 2B). In contrast, radiant efficiency of IgG-ms retention was similar in hTau mice and WT mice (Fig. 2B), suggesting that TTCM2-ms antibody has higher affinity and specificity to recognize and target the pathological tau present in the brains of tauopathy mice. It is important to note that the intranasal delivery approach bypasses the BBB and delivers drugs to the brain within minutes (*55*, *56*). Thus, regardless of the BBB integrity, intranasally administered TTCM2-ms and IgG-ms are efficiently delivered in the brains of hTau mice. Given the higher pathological tau loads in the aged hTau tauopathy mice relative to WT controls (*54*, *57*, *58*), we observed an increased retention of TTCM2-ms in tauopathy mice (Fig. 2B).

We then evaluated TTCM2-ms distribution in hTau mouse brain sections using the neuronal postsynaptic marker postsynaptic density protein 95 (PSD95) (Fig. 2C). We found that 3 hours after intranasal administration, TTCM2-ms (red) followed the nose-to-brain

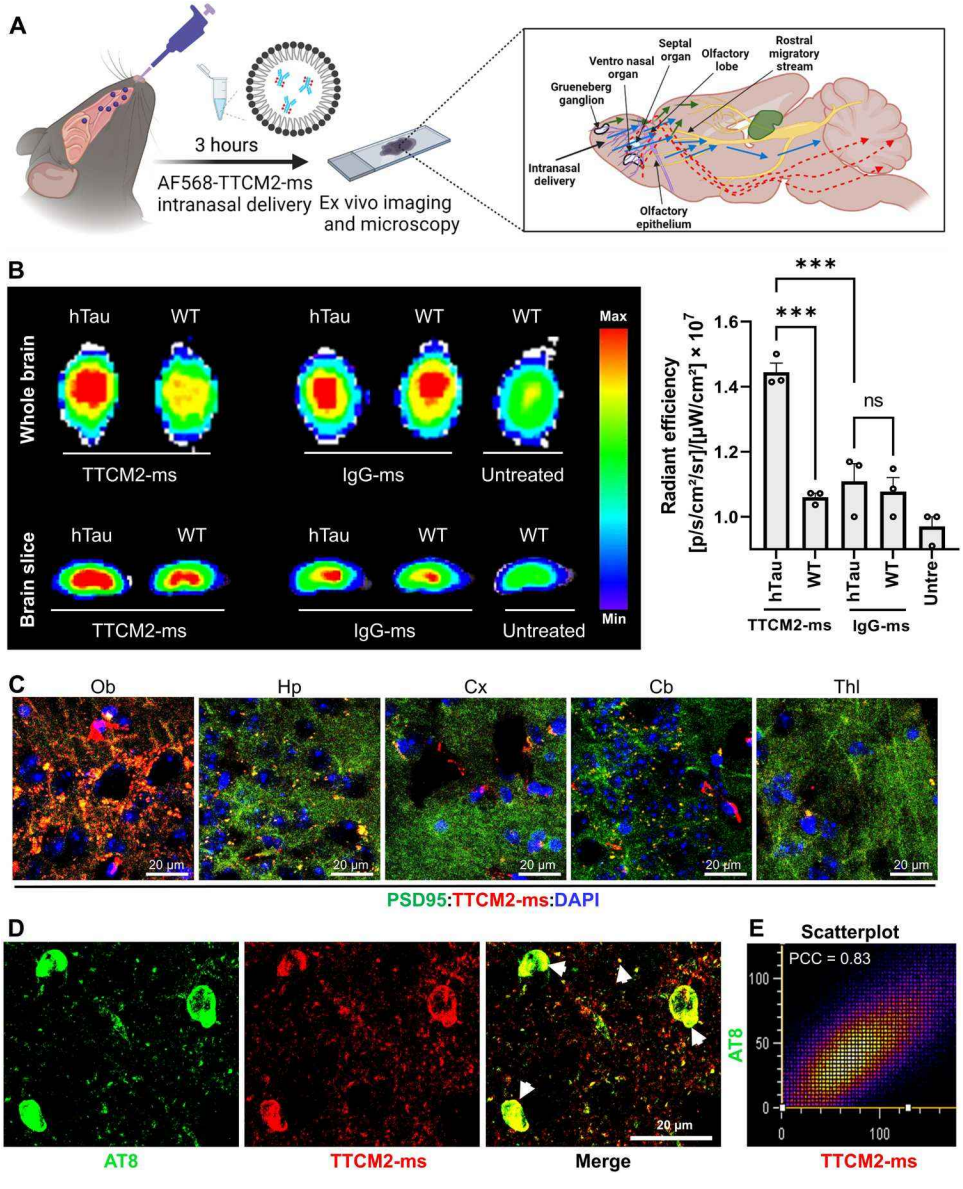

Fig. 2. Intranasally administered TTCM2-ms rapidly distribute in the brains of tauopathy mice. (**A**) Schematic diagram showing the experiment design and nose-to-brain drug delivery pathway, which circumvents the BBB and efficiently delivers drugs to various brain regions. Intranasal administration of AF568-labeled TTCM2-ms (15 μl per nostril; 30 μl per mouse) was performed on lightly anesthetized animals, and mice were euthanized 3 hours after treatment. After perfusion, brain tissues were extracted, and unfixed tissues were immediately analyzed for ex vivo fluorescence imaging and immunofluorescence microscopy. (**B**) Left: Representative heatmaps of high radiant efficiency in ex vivo whole brain (top) and brain slices (bottom) of hTau and WT mice intranasally treated with TTCM2-ms or IgG-ms. The scale indicates the minimum (blue) and maximum (red) radiant efficiency in the brain tissues. Untreated WT mice were used as a baseline. Right: Average radiant efficiency from three mice per treatment group. Graphs show the mean ± SEM. Significance was determined by one-way ANOVA followed by Tukey's test. ***$P < 0.001$. (**C**) Representative immunofluorescence images depicting the neuronal postsynaptic marker, PSD95 (green), TTCM2-ms (red), and DAPI (blue) in the olfactory bulb (Ob), hippocampus (Hp), cortex (Cx), cerebellum (Cb), and thalamus (Thl). Scale bars, 20 μM. The data are representative of three mice. (**D**) Representative immunofluorescence images showing TTCM2 in the hippocampi of hTau tauopathy mice after intranasal delivery. TTCM2-ms (red) colocalized with AT8-positive tau aggregates (green). Arrows highlight areas of colocalization between AT8 and TTCM2-ms. (**E**) The scatterplot illustrates significant colocalization between TTCM2-ms and AT8, as indicated by Pearson's correlation coefficient (PCC) of 0.83.

drug delivery route and were distributed in various brain regions, including the olfactory bulb, hippocampus, cortex, cerebellum, and thalamus (Fig. 2C). In addition, we observed that TTCM2-ms can reach into neuronal intracellular compartments as shown by colocalization between TTCM2 (red) with PSD95 (green) (Fig. 2C). Subsequently, we explored the target engagement of intranasally delivered TTCM2-ms in the brains of hTau mice by staining brain tissues with AT8 antibody (green) and determined its colocalization with TTCM2-ms (red). Immunofluorescence microscopy revealed a colocalization between TTCM2-ms and AT8-positive pathological tau aggregates (Fig. 2D). Scatterplot demonstrated a significant colocalization between TTCM2 and AT8, as indicated by a Pearson's correlation coefficient of 0.83 (Fig. 2E). Therefore, our findings indicate that intranasally administered TTCM2-ms rapidly distributed across the brains of hTau mice, entered the intracellular compartments of neuronal cells, and engaged with pathological tau in vivo.

A single intranasal TTCM2-ms treatment ameliorates tau pathology and cognitive decline in aged tauopathy mice

We next determined whether intranasal administration of TTCM2-ms ameliorates tau pathology and cognitive decline in aged hTau mice. Beginning at 15 months of age, the time when these animals show advanced-stage tau pathology and cognitive impairment (*24*, *54*), both male and female hTau mice were treated with a single dose of intranasal TTCM2-ms or IgG-ms. Hippocampus-dependent spatial memory and exploration potential of aged hTau mice were measured using the Y-maze test immediately before and at 2 weeks after intranasal administration. Mice were also subjected to the novel object recognition (NOR) test at 2 weeks post-intranasal TTCM2-ms or IgG-ms treatment. After behavior testing, mice were euthanized, and brains were processed for biochemical and pathological analyses (Fig. 3A). Compared with IgG-ms–treated hTau mice, TTCM2-ms–treated hTau mice exhibited a significantly ($P < 0.01$) enhanced discrimination index, indicative of a greater tendency to explore a novel object than a familiar one (Fig. 3, B and C). Moreover, TTCM2-ms–treated hTau mice performed markedly better in the Y-maze test, suggesting that TTCM2-ms treatment alleviated short-term memory loss in mice with established tauopathy (Fig. 3D). Consistent with these findings, compared with IgG-ms–treated hTau mice, TTCM2-ms–treated hTau mice showed increased protein levels of neuronal markers, including PSD95, synaptophysin, and neuronal nuclear protein (NeuN) in the hippocampus—the region associated with memory formation and cognitive functions (Fig. 3, E to H). Immunofluorescence microscopy also showed an increase in the number of NeuN-positive cells in the hippocampi of TTCM2-ms–treated hTau mice (fig. S2, A and B).

A distinctive characteristic of hTau mice is the development of disease-relevant tau pathology, including the appearance of NFTs and phosphorylated tau aggregates by 12 months of age (*24*, *54*). To determine whether these pathologic features are affected by TTCM2-ms treatment, we assessed NFTs using thioflavin-S staining and measured phosphorylated tau by immunofluorescence staining with pathological tau-specific antibodies, including AT8 (anti-pTau S202 and T205), AT100 (anti-pTau T212 and S214), and AT180 (anti-pTau T231) (*24*, *59*), in brain sections from IgG-ms– and TTCM2-ms–treated hTau mice (Fig. 4). Results show that NFT deposition was significantly ($P < 0.01$) reduced after intranasal TTCM2-ms treatment (Fig. 4, A and C). Consistent with this finding, intranasal TTCM2-ms treatment reduced AT8-, AT100-, and AT180-positive pathological tau species (Fig. 4, B and D to L, and fig. S2, C and D). Immunoblot analyses revealed reduced protein levels of high–molecular weight tau aggregates (detected by Tau13), oligomeric tau (detected by T22), phosphorylated tau (detected by AT8, AT100, and AT180), and misfolded tau conformations (detected by TTC18) in brains of TTCM2-ms-treated relative to IgG-ms–treated mice (fig. S3). Conversely, monomeric tau (approximately 50 kDa; detected by Tau 13) was unaltered by TTCM2-ms treatment (fig. S3). Together, these results suggest that intranasal TTCM2-ms treatment removes pathological tau aggregates in the brain and ameliorates cognitive impairments in hTau mice.

TTCM2-ms clear intracellular tau aggregates through cytosolic Fc receptor TRIM21

TRIM21 is a cytosolic Fc receptor and E3 ubiquitin ligase that recognizes intracellular antibody-bound proteins (for example, antigens and pathogens) and triggers their clearance by the ubiquitin-proteasome degradation pathway (*60*). During this process, TRIM21 and the antibody are also degraded (*60*). TRIM21 is widely expressed and active in different cell types, including neurons (*61*, *62*). TRIM21 exhibits broad antibody isotype specificity, with the ability to bind IgG, IgM, and IgA (*63*); thereby, TRIM21 promotes degradation of antibody-bound pathological tau aggregates (*64*). Therefore, we assessed whether TRIM21 is involved in the clearance of intracellular pathological tau from neurons by TTCM2-ms. We established primary neuronal cultures from hTau mice and exposed them to tau seeds (AD-BDTOs) for 24 hours to induce intracellular tau pathology. After the seeding period, the cultures were treated with either TTCM2-ms or IgG-ms for varying time intervals. We observed recruitment of bright TRIM21 foci to the TTCM2-ms–tau complex 30 min after TTCM2-ms treatment. Both TRIM21 and the TTCM2-ms–tau complex were cleared after 1 hour of TTCM2-ms treatment, as evidenced by the decreased fluorescence intensity of total tau (Tau5; green) (Fig. 5, A and B) and TauO (T22; red) (Fig. 5C), as well as the reduced size of tau aggregates (Fig. 5D) and number of TRIM21 puncta (TRIM21; magenta) (Fig. 5E). These data suggest that the TTCM2-ms–tau aggregate complex rapidly promotes recruitment of TRIM21 and clears antibody-bound tau aggregates. The number of TRIM21 puncta per cell in IgG-ms– and TTCM2-ms–treated cells was similar at 0 min (mean IgG = 154 versus TTCM2 = 147) but increased significantly by TTCM2-ms treatment at 30 min (mean IgG-ms = 193 versus TTCM2-ms = 267; $P < 0.0001$) and decreased significantly at 60 min (mean IgG-ms = 172 versus TTCM2-ms = 38; $P < 0.0001$), indicating clearance of TRIM21 and antibody-bound tau aggregates upon TTCM2-ms treatment.

To further validate and extend these findings, we investigated the ability of TTCM2-ms to induce intracellular tau-positive TRIM21 puncta and their subsequent clearance in vivo after intranasal administration. To this end, aged hTau mice received intranasal treatment with AF568-labeled TTCM2-ms and were euthanized at 3 and 24 hours posttreatment. Brain tissues were then examined using immunofluorescence microscopy. The analyses revealed that at 3 hours posttreatment, TTCM2-ms readily colocalized with intracellular TRIM21 and tau aggregates (fig. S4A). In addition, the presence of TTCM2-ms led to the increased number of puncta for intracellular TRIM21-positive tau aggregates, indicating that, upon intranasal delivery, TTCM2-ms can reach the intracellular compartment and form a complex with intracellular tau aggregates and TRIM21

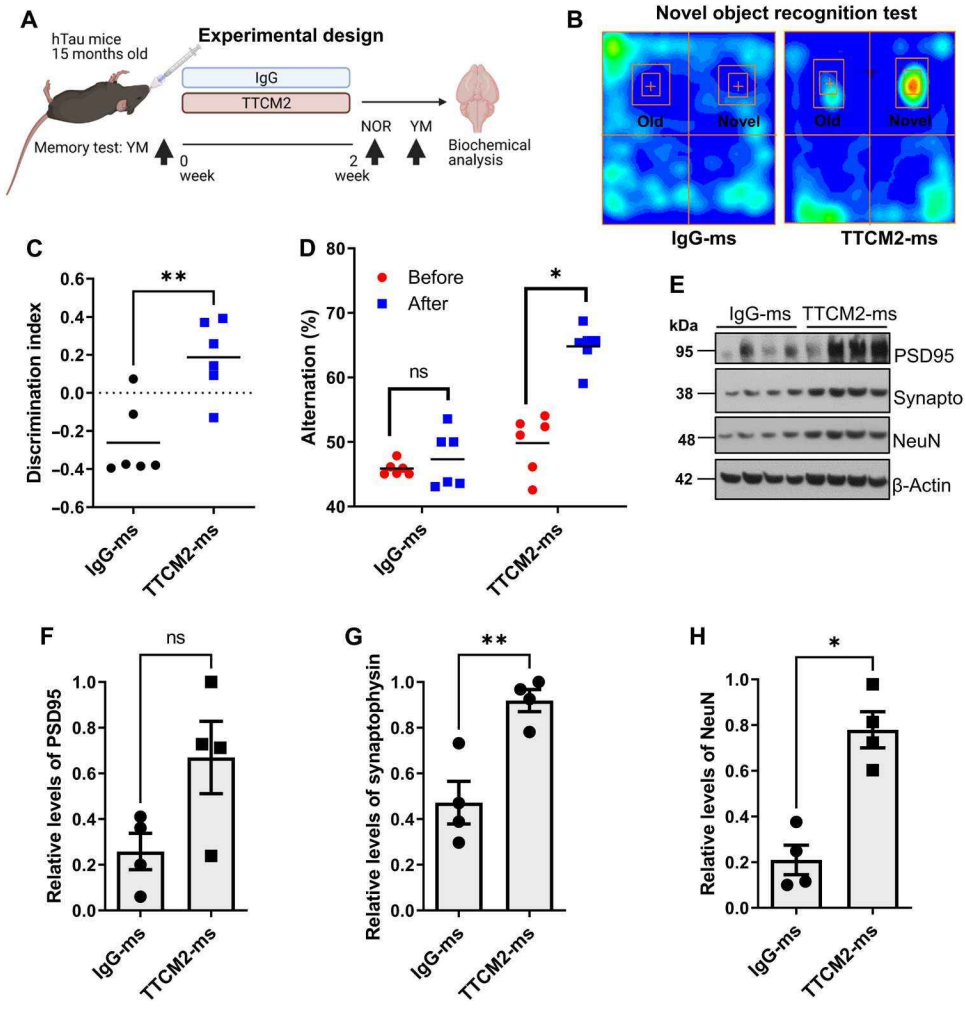

Fig. 3. Intranasal TTCM2-ms treatment ameliorates cognitive impairments and enhances presynaptic and synaptic markers in the brains of hTau mice. (**A**) Experimental design: Fifteen-month-old hTau mice were intranasally treated with a single dose of TTCM2-ms or IgG-ms. After 2 weeks, animals were subjected to the NOR and Y-maze (YM) test, followed by brain pathology analysis. (**B** and **C**) NOR test measuring the impact of intranasal TTCM2-ms or IgG-ms treatment on memory in hTau mice. Representative heatmap (B) and discrimination index (C), indicating time spent exploring old or novel objects for representative TTCM2-ms– and IgG-ms–treated mice ($n = 6$ per group). Significance was determined by unpaired, two-tailed Student's t test. (**D**) Y-maze spontaneous alternation test: The percentage of spontaneous alternations was measured before and 2 weeks after TTCM2-ms or IgG treatment ($n = 6$ per group). Graph show the mean ± SEM; significance was determined by unpaired, two-tailed Student's t test. (**E** to **H**) Immunoblot analysis showing the impact of intranasal TTCM2-ms or IgG on the levels of PSD95 (F), synaptophysin (G), and NeuN (H) in the hippocampi of aged hTau mice ($n = 6$ per group). β-Actin is the loading control. The graphs show the mean ± SEM, and significance was determined using an unpaired, two-tailed Student's t test. $^*P < 0.05$ and $^{**}P < 0.01$.

(fig. S4B). Furthermore, we observed that TTCM2-ms reduced pathological tau aggregates detected by AT8 at 24 hours but not at 3 hours posttreatment (fig.S4, C and D). Tau-positive TRIM21 puncta were increased at 3 hours and decreased at 24 hours after TTCM2-ms treatment (fig. S4, B and E). No changes were observed in IgG-ms–treated mice (fig. S4, C to F). TTCM2-ms also reduced sarcosyl-insoluble $HT7^+$ human tau protein and $AT8^+$ pathologically relevant phosphorylated tau aggregates (fig. S5, A to C). These observations indicate that upon intranasal delivery, TTCM2-ms readily colocalized with intracellular antibody receptor TRIM21 and tau aggregates, resulting in the clearance of pathological tau aggregates from the brains of aged hTau mice.

To examine the role of TRIM21 in TTCM2-ms–mediated clearance of pathological tau in tau biosensor cells, we conducted TRIM21 silencing using small-interfering RNA (siRNA). To this end, cells were first treated with TRIM21 or control siRNAs for 48 hours. Then, the cells were exposed to preincubated AD-BDTO and TTCM2-ms complexes (at a 1:4 ratio). After 3 hours, tau aggregate clearance was examined by immunoblots and immunofluorescence microscopy. Immunoblot analyses showed that TRIM21 siRNA reduced TRIM21 protein levels compared with control siRNA (Fig. 5, F and G). In control siRNA-treated cells, TTCM2-ms effectively cleared tau aggregates (Fig. 5, F and H), whereas tau clearance was impaired in TRIM21-siRNA–treated cells (Fig. 5, F and H). In addition, immunofluorescence analysis indicated that control siRNA-treated cells markedly cleared intracellular tau aggregates compared with TRIM21-siRNA–treated cells (Fig. 5, I to K). These data indicate that TRIM21 is essential for TTCM2-ms–mediated clearance of intracellular tau aggregates. TauRD, expressed by tau biosensor cells, contains aggregation-competent residues that form pathological tau aggregates (*13*, *65*–*70*).

To understand the nature of TTCM2's immunoreactivity with TauRD, specifically whether it is conformation dependent, like full-length tau, we conducted immunoblot assays using cell lysates from tau biosensor cells and control untransfected human embryonic kidney (HEK) cells. These cell lysates were subjected to ND or D conditions by urea and heat treatment, followed by immunoblotting with TTCM2. We observed that TTCM2 exhibited strong immunoreactivity with TauRD aggregates in ND conformations but showed weak immunoreactivity after denaturation (fig. S5E). No immunoreactivity was observed in control HEK cells (fig. S5E), indicating that TTCM2's immunoreactivity with TauRD is both conformation dependent and specific. Collectively, our data suggest that upon intranasal administration, TTCM2-ms formed complexes with the cytosolic Fc receptor TRIM21 and tau aggregates, thereby promoting clearance of tau pathology. Our findings align with previous studies that have established the crucial role of TRIM21 in effective tau immunotherapy (*64*, *71*).

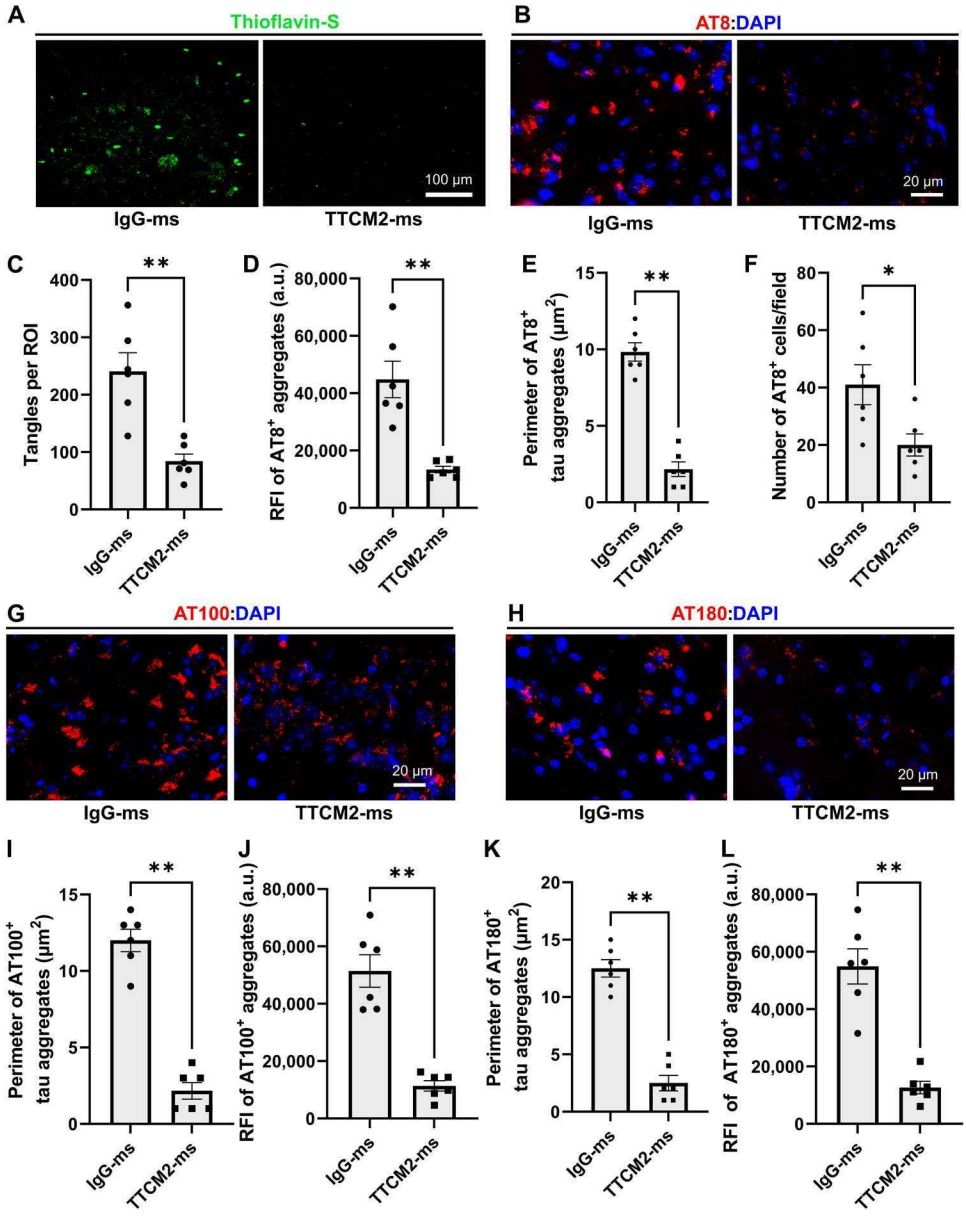

Fig. 4. Intranasal TTCM2-ms treatment reduces tau pathology in the brains of hTau mice. Representative images of thioflavin-S staining (**A**) and quantification of NFTs (**C**) per region of interest (ROI) in brains from 15-month-old hTau mice intranasally treated with IgG-ms or TTCM2-ms. Scale bar, 100 μm. The graph shows the mean ± SEM number of tangles ($n = 6$ per group). Significance was determined by unpaired, two-tailed Student's t test. (**B**) Representative immunofluorescence images of phosphorylated tau detected by AT8 (B) and its quantification (**D** to **F**) in brains from 15-month-old hTau mice intranasally treated with IgG-ms or TTCM2-ms. Scale bar, 20 μm. The graphs show RFI of $AT8^+$ tau aggregates (D), perimeter of AT8-positive tau aggregates (E), and number of AT8-positive cells (F) ($n = 6$ mice per group). Representative immunofluorescence images of phosphorylated tau detected by AT100 (**G**) and its quantification (**I** and **J**) in brains from 15-month-old hTau mice intranasally treated with IgG-ms or TTCM2-ms. Scale bar, 20 μm. The graph shows the mean ± SEM perimeter of $AT100^+$ tau aggregates (I) and RFI of $AT100^+$ tau aggregates (J) ($n = 6$ per group). Significance was determined by unpaired, two-tailed Student's t test. Representative immunofluorescence images of phosphorylated tau detected by AT180 (**H**) and its quantification (**K** and **L**) in brains from 15-month-old hTau mice intranasally treated with IgG-ms or TTCM2-ms. Scale bar, 20 μm. The graph shows the mean ± SEM perimeter of $AT180^+$ tau aggregates (K) and RFI of $AT180^+$ tau aggregates (L) ($n = 6$ per group). Significance was determined by unpaired, two-tailed Student's t test (*$P < 0.05$ and **$P < 0.001$).

TTCM2-ms efficiently internalize into intracellular compartments and clear seed-competent, intracellular tau from the brains of aged hTau mice

Given that pathological tau aggregates are primarily intraneuronal in AD and other tauopathies, therapeutic tau antibodies should be able to efficiently reach the intracellular compartments (*72*). We therefore measured the internalization efficiency of TTCM2 by treating hTau-expressing cells (green) with AF568-labeled TTCM2 (red) with or without ms at 4° or 37°C and analyzed by fluorescence microscopy. We observed a substantial increase in binding and internalization of TTCM2-ms relative to TTCM2 in phosphate-buffered saline (PBS), which displayed only marginal binding and internalization (Fig. 6, A to D).

To achieve clinical relevance, tau antibodies must not only neutralize tau seeding activities but also clear preexisting intracellular tau pathology (*5*, *20*, *21*). To determine whether TTCM2-ms treatment in biosensor cells can clear preexisting intracellular tau aggregates (*11*), cells were treated with AD-BDTO seeds for 24 hours to establish intracellular tau aggregates and then incubated with or without TTCM2-ms for 3 hours. Fluorescence microscopy was used to assess tau clearance (Fig. 6E). Results indicated that intracellular tau aggregates were formed in AD-BDTO–treated cells. TTCM2-ms treatment reduced the number of tau aggregate–positive cells and decreased the size of the intracellular tau aggregates (Fig. 6, F to H), suggesting clearance of preexisting intracellular tau aggregates. We further hypothesized that TTCM2-ms–mediated removal of seed-competent tau conformers may be associated with reduced tau pathology in the brains of aged hTau mice. To test this possibility, we measured seeding activities of brain homogenates from IgG-ms– and TTCM2-ms–treated hTau mice (Fig. 6I). We found that brain homogenates from IgG-ms–treated control mice exhibited enhanced seeding activity, as evidenced by the formation of tau aggregates in tau biosensor cells. Conversely, brain homogenates from TTCM2-ms–treated mice displayed a marked reduction

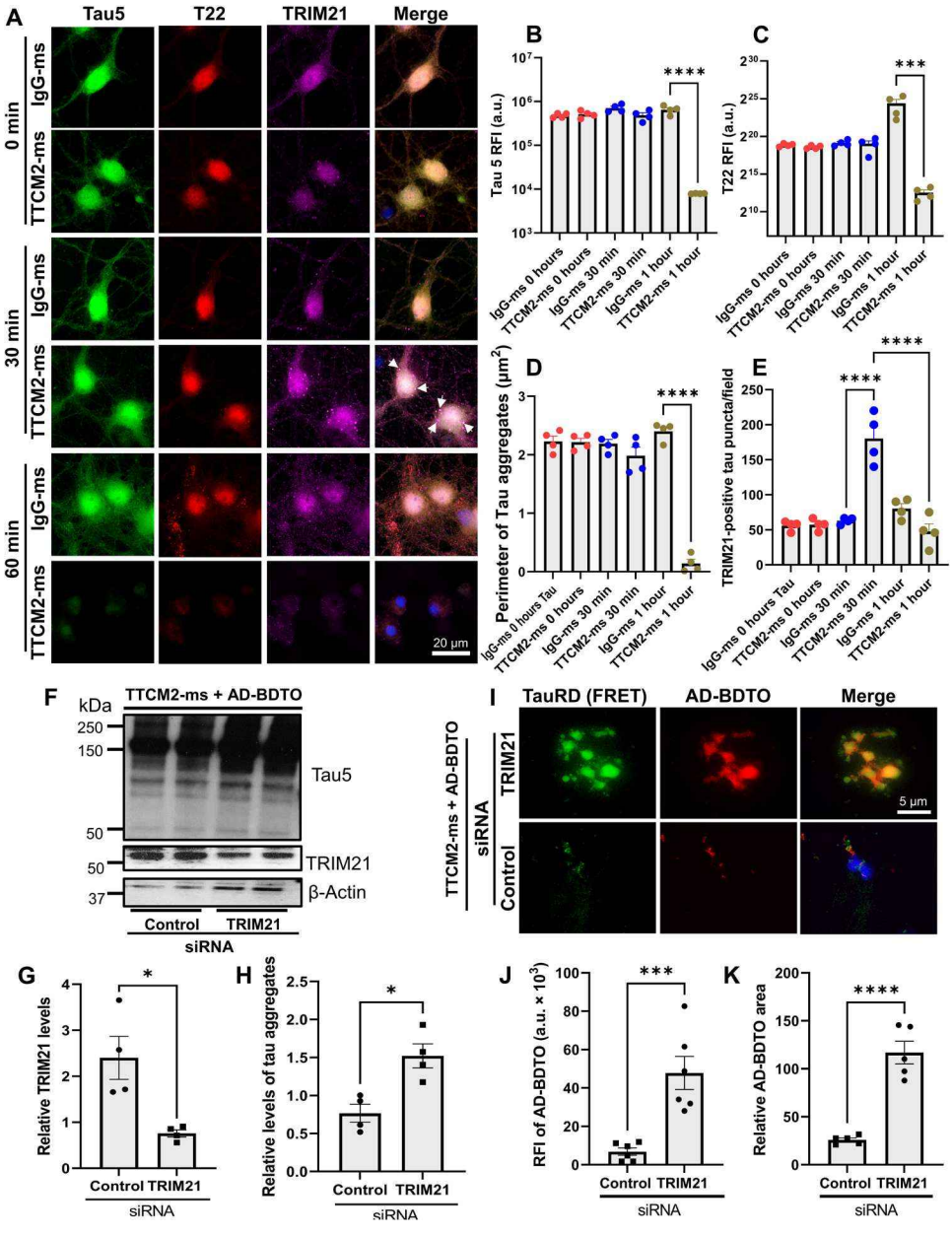

Fig. 5. Cytosolic Fc receptor TRIM21 is linked to TTCM2-ms antibody–mediated clearance of intracellular tau aggregates. (**A**) Immunofluorescence analysis of primary cortical neurons treated with AD-BDTOs for 24 hours to establish intracellular tau aggregates. Subsequently, the cells were washed and treated with IgG-ms or TTCM2-ms for 0 to 60 min. Intracellular TRIM21-tau puncta are indicated by arrows. (**B** to **E**) Quantification of total tau (tau5; green) (B), TauO (T22, red) (C), size of the tau aggregates (D), and number of TRIM21-positive tau puncta (E) at 0, 30, and 60 min. Graphs represent the mean ± SEM from four experiments. Significance was determined by one-way ANOVA followed by Tukey's test. (**F** to **H**) Immunoblot analysis and quantification of TRIM21 and tau aggregates in cell lysates from the cells treated with control or TRIM21 siRNAs followed by exposure to AD-BDTOs:TTCM2 at a 1:4 ratio. β-Actin is the loading control. (**I**) Immunofluorescence analysis to assess the clearance of AD-BDTOs in cells treated with TRIM21-siRNA or control siRNA by TTCM2, using AD-BDTOs:TTCM2 at a 1:4 ratio. (**J** to **K**) The graphs showing the mean ± SEM RFI of AD-BDTOs (J) and relative AD-BDTO area (K) from four experiments in (I). Significance was determined by unpaired, two-tailed Student's *t* test. $^{*}P < 0.05$, $^{***}P < 0.001$, and $^{****}P < 0.0001$.

in tau-seeding activity (Fig. 6, J and K), suggesting that TTCM2-ms treatment effectively removed seed-competent tau aggregates from the brains of hTau mice with tauopathy.

TTCM2-ms treatment clears intracellular and synaptic tau aggregates from the brains of tauopathy mice

Tau aggregates isolated from synaptic compartments of mouse and human AD brains exhibit tau-seeding activity, which promotes tauopathy progression and cognitive deficits (*46*, *73*, *74*). An absence of TauO in synapses is linked to better cognitive function in individuals with high AD neuropathology (*75*, *76*), suggesting that pathological tau in synapses may preferentially cause synaptic loss and cognitive decline. Therefore, we examined whether TTCM2-ms can remove synaptic tau aggregates from the brains of aged tauopathy mice. To this end, brain sections from IgG-ms– and TTCM2-ms–treated hTau mice were stained with anti-tau antibody (Tau HT7) and a synaptic marker (anti-PSD95 antibody), followed by microscopic analysis. As evidenced by HT7 tau and PSD95 colocalization, TTCM2-ms–treated mice exhibited decreased protein levels of total tau aggregates (Tau HT7, red) and within PSD95-positive synaptic compartments (PSD95, green) in the brains of aged hTau mice compared with IgG-ms–treated mice (Fig. 7, A to D). We also found that the number of PSD95 puncta was significantly ($P < 0.01$) increased in the brains of TTCM2-ms–treated mice compared with IgG-ms–treated mice (Fig. 7, A and E). In addition, we found reduced T22 antibody–positive TauO (green) in synaptophysin 1–positive synaptic compartments (synaptophysin 1; red) in TTCM2-ms–treated hTau mice (fig. S6, A to D). Furthermore, the number of synaptophysin 1 puncta was significantly ($P < 0.01$) increased in the brains of TTCM2-ms–treated mice compared with IgG-ms–treated mice (fig. S6, A to D). These findings indicate that TTCM2-ms effectively clear pathological tau aggregates from intracellular and synaptic compartments of neurons in the brains of hTau mice.

Cryo–electron microscopy studies have shown that tau forms distinct pathological tau structures known as tau strains/polymorphs (*77*, *78*). This diversity of tau strains/polymorphs drives specific neuropathological features in AD, PiD, PSP, and CBD (*77*, *78*). Tau aggregates exhibit protease K (PK)–resistant cores,

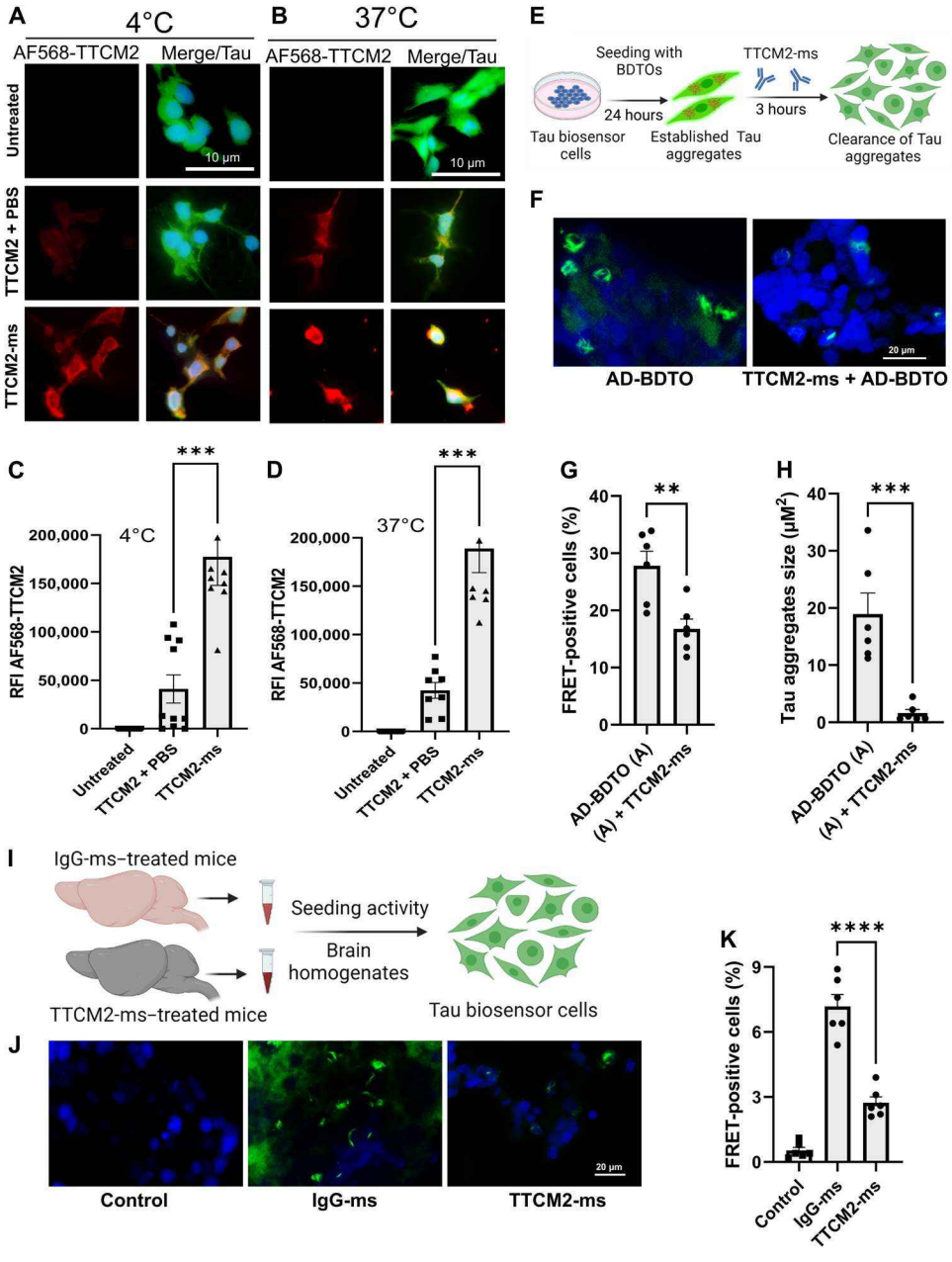

Fig. 6. TTCM2-ms are efficiently internalized and clear seed-competent tau from the brains of aged hTau mice. (**A** and **B**) Representative images showing the internalization of TTCM2-ms at 4°C (A) and 37°C (B). Cells expressing hTau (green) were exposed to AF568-labeled TTCM2 or TTCM2-ms for 30 min, followed by washing to remove unbound/extracellular antibodies. (**C** and **D**) Quantification of RFIs of AF568-labeled TTCM2 measured at 4°C (C) and 37°C (D) to detect binding and internalization, respectively. The graphs show the mean ± SEM; $n = 8$ to 10 ROIs from three experiments. Significance was determined by unpaired, two-tailed Student's t test. (**E**) Experimental design to assess clearance of intracellular tau aggregates. Tau biosensor cells incubated with AD-BDTOs for 24 hours to establish intracellular tau aggregates were treated with or without TTCM2-ms antibody for 3 hours. (**F**) Representative images showing size of tau aggregates (green) in AD-BDTO–treated cells ± TTCM2-ms. (**G**) Percentage of FRET-positive cells and (**H**) size of tau aggregates in AD-BDTO–treated cells ± TTCM2-ms. Significance was determined by unpaired, two-tailed Student's t test. Graphs represent the mean ± SEM from four experiments. (**I**) Schematic showing experimental strategy for detecting tau-seeding activity in brain homogenates of tauopathy mice intranasally treated with IgG-ms or TTCM2-ms. (**J**) Representative images showing tau-seeding activity in IgG-ms– and TTCM2-ms–treated mice; untreated control is shown at left. DAPI (blue) counterstain was used to detect total nuclei. Scale bar, 20 μm. (**K**) Percentage of FRET-positive cells in TTCM2-ms– versus IgG-ms–treated mice ($n = 4$ brain homogenates per group). Significance was determined by one-way ANOVA followed by Tukey's test. Graph represents the mean ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$.

and their distinct strains or polymorphs can be distinguished by their PK-resistant patterns (*13*, *79*–*81*). We used PK digestion analysis to investigate structural differences of tau aggregates within brain homogenates of IgG-ms– and TTCM2-ms–treated mice. Homogenates were treated with PK at various concentrations (0 to 20 μg/ml) for 1 hour, and digestion patterns were evaluated by immunoblot using different sequence-specific tau antibodies, including Tau5 (recognizes the middle region), RD4 (recognizes the four-repeat isoform), and Tau46 (recognizes the C-terminal sequence). We found that tau aggregates in brain homogenates from IgG-ms–treated mice were resistant to PK digestion, an indicator of compacted tau structures (*82*), whereas tau aggregates in brain homogenates from TTCM2-ms–treated mice were sensitive to PK digestion, an indicator of relaxed tau structure (fig. S7, A to C) (*82*). Immunofluorescence staining analyses further validated the differential PK sensitivity of tau in IgG-ms– versus TTCM2-ms–treated mice (fig. S8, A and B).

DISCUSSION

The primary pathological hallmark of neurodegenerative tauopathies is the formation of intracellular tau aggregates. In the brains of patients afflicted with tauopathies, an abundant proportion of pathological tau localizes within the intracellular and synaptic compartments of neurons (*5*, *83*, *84*). These neurons release tau seeds into the extracellular space, where they enter neighboring cells and facilitate tau aggregation, thereby promoting tauopathy progression (*10*, *84*–*86*). Distribution of pathological tau strongly correlates with clinical severity and neurodegeneration in patients with AD (*2*, *3*). However, development of therapeutic antibodies against tau faces challenges related to poor bioavailability, limited brain uptake, and inadequate internalization by neurons. As a result, therapeutic antibodies are unable to effectively clear intracellular tau pathology, leading to failure in improving cognition in clinical trials. These

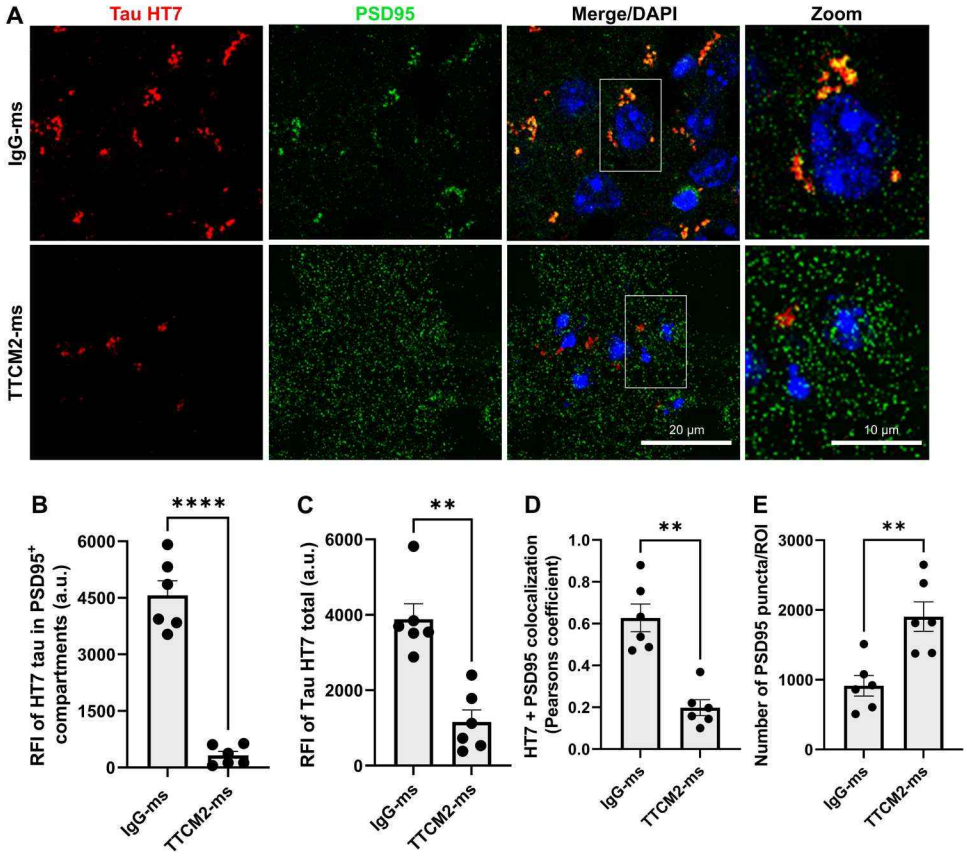

Fig. 7. TTCM2-ms treatment promotes the clearance of tau aggregates from synaptic compartments in brains of hTau mice. (**A**) Immunostaining for PSD95 (red) and Tau HT7 (green) and (**B**) quantification of RFI of tau in PSD95-positive compartments in brain tissue from 15-month-old hTau mice intranasally treated with TTCM2-ms or IgG-ms ($n = 6$ mice per group). Scale bars, 20 μm. (**C**) RFI of total tau (Tau HT7, green). (**D**) Pearson correlation coefficient analysis assessing colocalization between HT7 tau and PSD95. (**E**) Number of PSD95 puncta/ROIs in the brains of 15-month-old hTau mice intranasally treated with TTCM2-ms or IgG-ms control. Graphs show the mean ± SEM, $n = 6$ mice per group. Significance was determined by unpaired, two-tailed Student's t test (**$P <$ 0.01 and ****$P < 0.0001$).

challenges provide the impetus for advancements in tau immunotherapy (*5*, *6*).

Here, we report that, (i) in brain tissues from patients with neurodegenerative tauopathies, including AD, DLB, and PSP, TTCM2 specifically recognized pathological tau and effectively neutralized the seeding activity of AD-BDTOs. (ii) Upon intranasal administration, TTCM2-ms rapidly and efficiently distributed across various brain regions in hTau mice and engaged with pathological tau in intracellular compartments of neurons in vivo. (iii) TTCM2-ms treatment promoted clearance of pathological tau aggregates, including NFTs and phosphorylated and oligomeric tau from brains of aged hTau mice, leading to improved cognitive functions. (iv) TTCM2-ms readily colocalized with TRIM21 and pathological tau complexes and facilitated clearance of intracellular tau aggregates. We found that TRIM21 was essential for TTCM2-ms–mediated clearance of intracellular tau aggregates. (v) TTCM2-ms effectively cleared seed-competent, intracellular, and synaptic tau from the brains of aged hTau mice. Together, these findings highlight the crucial role of seed-competent pathological tau conformations in disease progression and cognitive decline, as well as the potential of intranasal administration of TTCM2-ms as a promising strategy for rapidly and effectively removing intracellular pathological tau from the brain. Thus, this work advances our understanding of tau pathology progression and provides key insights that may lead to improved immunotherapies for halting tauopathy progression and improving cognitive functions.

In the past decade, tau immunotherapy has progressed from proof-of-concept experiments to several clinical trials (*19*, *87*). However, mAbs that exclusively target extracellular tau have failed clinical testing. This outcome is expected, considering that the majority of pathological tau resides within the intracellular and synaptic compartments of neurons, with only a small fraction found extracellularly (*6*, *88*). Thus, effective clearance of intracellular tau aggregates is necessary for achieving clinical benefits. Studies in mouse models have revealed that conventionally delivered tau immunotherapy primarily focuses on reducing the deposition and spread of tau pathology in young to middle-aged tauopathy mice. However, its effectiveness diminishes in aged animals with well-established tau pathology (*89*). Moreover, because of the BBB, only a minimal fraction (0.1 to 1%) of therapeutic mAbs delivered through conventional routes reach the brain from the circulation (*90*, *91*). Here, we used intranasal delivery, which allows direct delivery of antibodies into the brain through the olfactory and trigeminal nerves, bypassing the BBB (*92*). We observed that upon intranasal treatment, TTCM2-ms efficiently reached various regions of the brain, including the olfactory bulb, cortex, hippocampus, cerebellum, and thalamus, as indicated by ex vivo fluorescence imaging, demonstrating successful delivery to the brain. We also found that intranasally administered TTCM2-ms colocalized with the synaptic compartments. In some instances, whole cellular bodies were stained by TTCM2-ms, indicating delivery to the intracellular and synaptic compartments. These observations align with numerous studies consistently showing the presence of pathological tau in various cellular regions, including the dendritic, somatic, and axonal compartments of neuronal cells (*25*, *93*–*95*). Clinical and preclinical studies have shown that intranasal drug delivery can effectively and safely treat brain pathologies (*96*–*100*). This evidence supports the feasibility of direct delivery of tau mAbs through the nose-to-brain pathway in patients with tauopathies, potentially for diagnostic and therapeutic purposes. Our results are particularly noteworthy because TTCM2-ms demonstrate rapid and efficient distribution in various brain regions upon intranasal administration. Furthermore, TTCM2-ms internalized into intracellular compartments, effectively clearing intracellular tau pathology in older

tauopathy mice with established disease (*54*, *101*). In addition, TTCM2 specifically detects pathological tau aggregates in brain tissue from patients with AD, DLB, and PSP.

Synaptic accumulation of tau aggregates leads to cognitive decline in tauopathies (*76*). These aggregates serve as seeds, promoting further aggregation and the spread of tau pathology (*46*, *73*). Thus, effective clearance of intracellular and synaptic tau aggregates is crucial for improving cognitive function. We investigated the impact of intranasal TTCM2-ms treatment on hippocampus-dependent learning and memory in 15-month-old hTau mice using the Y-maze and NOR tests. A single dose of intranasal TTCM2-ms improved cognitive functions in aged tauopathy mice. The beneficial effects of TTCM2-ms treatment included reductions in NFT deposition, phosphorylated tau, and oligomeric tau aggregates, whereas monomeric tau protein levels remained unaltered. Our findings are consistent with previous studies showing that inhibiting tau-seeding activity and clearing synaptic tau aggregates resulted in reduced tau pathology progression and improved cognitive functions in various mouse models of AD (*16*, *73*, *83*, *102*–*105*). Moreover, our observations are supported by prior studies showing that immunotherapy using conformation-specific tau mAbs efficiently reduces tau pathology, neuronal loss, and cognitive impairments in different AD animal models (*15*, *17*, *18*, *104*, *106*–*108*). Thus, targeting seed-competent, toxic tau conformations through intranasal delivery of TTCM2-ms may represent a promising therapeutic strategy for tauopathies.

The mechanisms underlying antibody-mediated clearance of tau aggregates are not yet fully elucidated, and several possible pathways have been proposed. These include blocking neuronal tau uptake (*16*), clearing antibody-bound tau by the TRIM21 pathway (*109*), and FcγRII/III-dependent endolysosomal degradation (*110*). Recent evidence from Lee *et al.* (*89*) demonstrated that FcγR and microglia are not necessarily involved in antibody-mediated clearance of tau aggregates. Our observation of the essential role of TRIM21 in the clearance of tau aggregates is in line with previous reports (*64*, *109*), emphasizing the critical role of TRIM21 in effective tau immunotherapy. TRIM21 is endogenously expressed in neurons and human brain (*62*), and its basal protein levels are sufficient for antibody-mediated protein degradation through the ubiquitin-proteasome pathway (*111*). In a recent high-profile study, Mukadam *et al.* (*71*) demonstrated that 17 weeks of weekly intraperitoneal administration of the tau-mAb AP422 reduced tau pathology and seed-competent tau in the brains of PS19 mice, a model of tauopathy. TRIM21-deficient mice failed to exhibit the protective effects of tau-mAb AP422 against tau pathology, highlighting the essential role of TRIM21 in tau immunotherapy in vivo (*71*). Mukadam *et al.* (*71*) used a long-term immunotherapy approach, administering anti-tau antibodies (30 mg/kg per dose) lasting 8 to 16 weeks to achieve effective clearance of intracellular tau pathology by the TRIM21 pathway. In contrast, with just a single intranasal dose of TTCM2-ms (1 mg/kg), we observed effective and rapid clearance of intracellular tau pathology. This demonstrates that a single dose of antibody delivered through noninvasive intranasal administration is sufficient to achieve beneficial effects. Further investigation is warranted, specifically regarding its long-term effects, to fully understand its efficacy and potential clinical implications. In this context, our previous studies indicated that a single dose of anti-tau antibody provides protection for a minimum of 2 months (*112*).

Data from various laboratories demonstrate that different tauopathies are characterized by unique tau strains or polymorphs. These tau strains or polymorphs indefinitely propagate tau pathology both in vitro and in vivo, contributing to the development of unique neuropathological patterns (*13*, *79*–*81*). PK digestion results revealed that brain homogenates from IgG-ms–treated mice showed PK-resistant tau aggregates, indicating compacted tau structures. Brain homogenates from TTCM2-ms–treated mice exhibited PK-sensitive tau aggregates, suggesting relaxed tau structure. These findings indicate that aged hTau tauopathy mice treated with IgG exhibit different PK-resistant tau strains or polymorphs compared with TTCM2-treated mice, and TTCM2-ms effectively cleared PK-resistant tau aggregates. The distinct pattern of PK digestion observed in this study may indicate the existence of different tau strains or polymorphs in the brains of aged hTau tauopathy mice. This finding is consistent with previous studies that have reported the presence of diverse tau strains/polymorphs in both mouse and human tauopathies (*13*, *48*, *81*, *101*, *113*). Thus, understanding and targeting these tau strains/polymorphs could contribute to the development of effective immunotherapy strategies. Further advancements in methods and reagents are needed to accurately classify tau strains/polymorphs in patients with tauopathy. This would enable precise diagnoses and the development of effective immunotherapy strategies.

Limitations of this study are as follows: (i) Although our in vitro and in vivo studies demonstrated that TRIM21 is involved in TTCM2-ms–mediated clearance of intracellular pathological tau aggregates, in future studies, it is important to use the TRIM21 knockout mice to better understand the role of TRIM21 in TTCM2-ms–mediated clearance in tauopathy models. (ii) It remains unclear whether TTCM2-ms directly clear intracellular tau pathology in glial cells by TRIM21. (iii) Further investigation is needed to determine whether TRIM21 participates in the clearance of other intracellular pathological proteins such as α-synuclein and TAR DNA binding protein (TDP43), which play crucial roles in the pathophysiology of neurodegenerative diseases.

In summary, our study revealed that a single intranasal dose of TTCM2-ms efficiently entered the brain and distributed across various brain regions and targeted pathological tau in cytosolic and synaptic compartments. TRIM21 recognized the complexes formed by TTCM2-ms and pathological tau in the cytosol. This distinctive property facilitated clearance of intracellular tau aggregates from the brain, leading to improved cognitive functions in aged tauopathy mice. Our work establishes a rapid and efficient approach for clearance of intracellular tau pathology—a major challenge in the field of tau immunotherapy. Our findings are consistent with a recent study demonstrating that TRIM21 is required for effective tau immunotherapy and clearance of tau pathology in mouse models (*71*). Overall, our study provides important mechanistic insights and strategies for developing effective tau immunotherapy approaches in neurodegenerative tauopathies.

MATERIALS AND METHODS

Study design

The study aimed to develop TTCM2-ms for rapid clearance of toxic tau conformations from the brains of aged hTau mice and to evaluate whether intranasal treatment with TTCM2-ms effectively clears intracellular, synaptic, and seed-competent tau, thereby reducing the impact on cognitive impairments. This study used rigorous

scientific methods, statistical analyses, and a focus on reproducibility to ensure the validity and reliability of the findings. No power analysis was performed to determine the sample size. Sample sizes were chosen on the basis of previous experience with tau immunotherapeutic studies using the same hTau mouse line and human postmortem brain tissues. A randomized experimental design was used, where aged hTau mice were randomly assigned to different experimental groups. No samples or data points were excluded from the analyses. Blinded investigators conducted most assays, including animal treatments, behavioral testing, tau seeding activities, quantification of immunofluorescence images, and immunoblot analyses. Using primary neuronal cultures from hTau mice and Tau biosensor cells, we tested the effects of TTCM2-ms on intracellular tau pathology. We determined the mechanism underlying rapid clearance of intracellular tau aggregates by TTCM2-ms. All animal experiments were conducted following the guidelines established by the National Institutes of Health (NIH) and were approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch, Galveston, Texas. Detailed methodologies for both in vitro and in vivo experiments can be found in the Supplementary Materials and Methods.

Statistical analysis

All in vitro experiments were performed at least three times. Data shown represent the means ± SEM, analyzed using GraphPad Prism 9. Statistical analyses included the unpaired, two-tailed Student's *t* test or one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were considered statistically significant if $P < 0.05$. Details of statistical analyses performed for each experiment are described in the figure legends. Individual-level data can be found in data file S1.

Supplementary Materials

This PDF file includes:
Materials and Methods
Figs. S1 to S11
Table S1
References (*114*–*124*)

Other Supplementary Material for this manuscript includes the following:
Data file S1
MDAR Reproducibility Checklist

REFERENCES AND NOTES

1. K. R. Brunden, J. Q. Trojanowski, V. M. Lee, Advances in tau-focused drug discovery for Alzheimer's disease and related tauopathies. *Nat. Rev. Drug Discov.* **8**, 783–793 (2009).
2. P. V. Arriagada, J. H. Growdon, E. T. Hedley-Whyte, B. T. Hyman, Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* **42**, 631–639 (1992).
3. M. R. Brier, B. Gordon, K. Friedrichsen, J. McCarthy, A. Stern, J. Christensen, C. Owen, P. Aldea, Y. Su, J. Hassenstab, N. J. Cairns, D. M. Holtzman, A. M. Fagan, J. C. Morris, T. L. Benzinger, B. M. Ances, Tau and Aβ imaging, CSF measures, and cognition in Alzheimer's disease. *Sci. Transl. Med.* **8**, 338ra366 (2016).
4. A. J. Schwarz, P. Yu, B. B. Miller, S. Shcherbinin, J. Dickson, M. Navitsky, A. D. Joshi, M. D. Devous Sr., M. S. Mintun, Regional profiles of the candidate tau PET ligand 18F-AV-1451 recapitulate key features of Braak histopathological stages. *Brain* **139**, 1539–1550 (2016).
5. E. E. Congdon, Y. Jiang, E. M. Sigurdsson, Targeting tau only extracellularly is likely to be less efficacious than targeting it both intra- and extracellularly. *Semin. Cell Dev. Biol.* **126**, 125–137 (2022).
6. P. Han, G. Serrano, T. G. Beach, R. J. Caselli, J. Yin, N. Zhuang, J. Shi, A quantitative analysis of brain soluble tau and the tau secretion factor. *J. Neuropathol. Exp. Neurol.* **76**, 44–51 (2017).
7. G. Limorenko, H. A. Lashuel, 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).
8. M. Manca, H. G. Standke, D. F. Browne, M. L. Huntley, O. R. Thomas, C. D. Orru, A. G. Hughson, Y. Kim, J. Zhang, C. Tatsuoka, X. Zhu, A. Hiniker, D. G. Coughlin, D. Galasko, A. Kraus, Tau seeds occur before earliest Alzheimer's changes and are prevalent across neurodegenerative diseases. *Acta Neuropathol.* **146**, 31–50 (2023).
9. J. L. Furman, J. Vaquer-Alicea, C. L. White III, N. J. Cairns, P. T. Nelson, M. I. Diamond, Widespread tau seeding activity at early Braak stages. *Acta Neuropathol.* **133**, 91–100 (2017).
10. J. L. Guo, V. M. Lee, Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. *Nat. Med.* **20**, 130–138 (2014).
11. B. B. Holmes, J. L. Furman, T. E. Mahan, T. R. Yamasaki, H. Mirbaha, W. C. Eades, L. Belaygorod, N. J. Cairns, D. M. Holtzman, M. I. Diamond, Proteopathic tau seeding predicts tauopathy in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **111**, E4376–E4385 (2014).
12. N. Puangmalai, N. Bhatt, M. Montalbano, U. Sengupta, S. Gaikwad, F. Ventura, S. McAllen, A. Ellsworth, S. Garcia, R. Kayed, Internalization mechanisms of brain-derived tau oligomers from patients with Alzheimer's disease, progressive supranuclear palsy and dementia with Lewy bodies. *Cell Death Dis.* **11**, 314 (2020).
13. D. W. Sanders, S. K. Kaufman, S. L. DeVos, A. M. Sharma, H. Mirbaha, A. Li, S. J. Barker, A. C. Foley, J. R. Thorpe, L. C. Serpell, T. M. Miller, L. T. Grinberg, W. W. Seeley, M. I. Diamond, Distinct tau prion strains propagate in cells and mice and define different tauopathies. *Neuron* **82**, 1271–1288 (2014).
14. A. Boutajangout, J. Ingadottir, P. Davies, E. M. Sigurdsson, Passive immunization targeting pathological phospho-tau protein in a mouse model reduces functional decline and clears tau aggregates from the brain. *J. Neurochem.* **118**, 658–667 (2011).
15. X. Chai, S. Wu, T. K. Murray, R. Kinley, C. V. Cella, H. Sims, N. Buckner, J. Hanmer, P. Davies, M. J. O'Neill, M. L. Hutton, M. Citron, Passive immunization with anti-tau antibodies in two transgenic models: Reduction of tau pathology and delay of disease progression. *J. Biol. Chem.* **286**, 34457–34467 (2011).
16. K. Yanamandra, N. Kfoury, H. Jiang, T. E. Mahan, S. Ma, S. E. Maloney, D. F. Wozniak, M. I. Diamond, D. M. Holtzman, Anti-tau antibodies that block tau aggregate seeding in vitro markedly decrease pathology and improve cognition in vivo. *Neuron* **80**, 402–414 (2013).
17. E. E. Congdon, E. M. Sigurdsson, Tau-targeting therapies for Alzheimer disease. *Nat. Rev. Neurol.* **14**, 399–415 (2018).
18. S. Jadhav, J. Avila, M. Scholl, G. G. Kovacs, E. Kovari, R. Skrabana, L. D. Evans, E. Kontsekova, B. Malawska, R. de Silva, L. Buee, N. Zilka, A walk through tau therapeutic strategies. *Acta Neuropathol. Commun.* **7**, 22 (2019).
19. C. Ji, E. M. Sigurdsson, Current status of clinical trials on tau immunotherapies. *Drugs* **81**, 1135–1152 (2021).
20. M. Colom-Cadena, C. Davies, S. Sirisi, J. E. Lee, E. M. Simzer, M. Tzioras, M. Querol-Vilaseca, E. Sanchez-Aced, Y. Y. Chang, K. Holt, R. I. McGeachan, J. Rose, J. Tulloch, L. Wilkins, C. Smith, T. Andrian, O. Belbin, S. Pujals, M. H. Horrocks, A. Lleo, T. L. Spires-Jones, Synaptic oligomeric tau in Alzheimer's disease - A potential culprit in the spread of tau pathology through the brain. *Neuron* **111**, 2170–2183.e6 (2023).
21. G. Meisl, E. Hidari, K. Allinson, T. Rittman, S. L. DeVos, J. S. Sanchez, C. K. Xu, K. E. Duff, K. A. Johnson, J. B. Rowe, B. T. Hyman, T. P. J. Knowles, D. Klenerman, In vivo rate-determining steps of tau seed accumulation in Alzheimer's disease. *Sci. Adv.* **7**, eabh1448 (2021).
22. E. Leroux, R. Perbet, R. Caillierez, K. Richetin, S. Lieger, J. Espourteille, T. Bouillet, S. Begard, C. Danis, A. Loyens, N. Toni, N. Deglon, V. Deramecourt, S. Schraen-Maschke, L. Buee, M. Colin, Extracellular vesicles: Major actors of heterogeneity in tau spreading among human tauopathies. *Mol. Ther.* **30**, 782–797 (2022).
23. Z. Wang, G. Xiong, W. C. Tsang, A. G. Schatzlein, I. F. Uchegbu, Nose-to-brain delivery. *J. Pharmacol. Exp. Ther.* **370**, 593–601 (2019).
24. S. Gaikwad, N. Puangmalai, A. Bittar, M. Montalbano, S. Garcia, S. McAllen, N. Bhatt, M. Sonawane, U. Sengupta, R. Kayed, Tau oligomer induced HMGB1 release contributes to cellular senescence and neuropathology linked to Alzheimer's disease and frontotemporal dementia. *Cell Rep.* **36**, 109419 (2021).
25. M. Montalbano, L. Majmundar, U. Sengupta, L. Fung, R. Kayed, Pathological tau signatures and nuclear alterations in neurons, astrocytes and microglia in Alzheimer's disease, progressive supranuclear palsy, and dementia with Lewy bodies. *Brain Pathol.* **33**, e13112 (2023).
26. J. C. Augustinack, A. Schneider, E. M. Mandelkow, B. T. Hyman, Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta Neuropathol.* **103**, 26–35 (2002).
27. C. A. Lasagna-Reeves, D. L. Castillo-Carranza, U. Sengupta, A. L. Clos, G. R. Jackson, R. Kayed, Tau oligomers impair memory and induce synaptic and mitochondrial dysfunction in wild-type mice. *Mol. Neurodegener.* **6**, 39 (2011).
28. C. A. Lasagna-Reeves, D. L. Castillo-Carranza, U. Sengupta, M. J. Guerrero-Munoz, T. Kiritoshi, V. Neugebauer, G. R. Jackson, R. Kayed, Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau. *Sci. Rep.* **2**, 700 (2012).

29. C. A. Lasagna-Reeves, D. L. Castillo-Carranza, M. J. Guerrero-Muñoz, G. R. Jackson, R. Kayed, Preparation and characterization of neurotoxic tau oligomers. *Biochemistry* **49**, 10039–10041 (2010).
30. G. Niewiadomska, W. Niewiadomski, M. Steczkowska, A. Gasiorowska, Tau oligomers neurotoxicity. *Life* **11**, 28 (2021).
31. S. S. Shafiei, M. J. Guerrero-Munoz, D. L. Castillo-Carranza, Tau oligomers: Cytotoxicity, propagation, and mitochondrial damage. *Front. Aging Neurosci.* **9**, 83 (2017).
32. A. Bittar, N. Bhatt, T. F. Hasan, M. Montalbano, N. Puangmalai, S. McAllen, A. Ellsworth, M. Carretero Murillo, G. Taglialatela, B. Lucke-Wold, A. Logsdon, C. Rosen, R. C. Turner, R. Kayed, Neurotoxic tau oligomers after single versus repetitive mild traumatic brain injury. *Brain Commun.* **1**, fcz004 (2019).
33. M. Goedert, D. S. Eisenberg, R. A. Crowther, Propagation of tau aggregates and neurodegeneration. *Annu. Rev. Neurosci.* **40**, 189–210 (2017).
34. R. Kayed, Anti-tau oligomers passive vaccination for the treatment of Alzheimer disease. *Hum. Vaccin.* **6**, 931–935 (2010).
35. D. Lamontagne-Kam, A. K. Ulfat, V. Herve, T. M. Vu, J. Brouillette, Implication of tau propagation on neurodegeneration in Alzheimer's disease. *Front. Neurosci.* **17**, 1219299 (2023).
36. G. Lippens, B. Gigant, Elucidating tau function and dysfunction in the era of cryo-EM. *J. Biol. Chem.* **294**, 9316–9325 (2019).
37. S. Maeda, A. Takashima, Tau oligomers. *Adv. Exp. Med. Biol.* **1184**, 373–380 (2019).
38. H. Xu, M. O'Reilly, G. S. Gibbons, L. Changolkar, J. D. McBride, D. M. Riddle, B. Zhang, A. Stieber, J. Nirschl, S. J. Kim, K. H. Hoxha, K. R. Brunden, G. D. Schellenberg, J. Q. Trojanowski, V. M. Lee, In vitro amplification of pathogenic tau conserves disease-specific bioactive characteristics. *Acta Neuropathol.* **141**, 193–215 (2021).
39. M. Goedert, The ordered assembly of tau is the gain-of-toxic function that causes human tauopathies. *Alzheimers Dement.* **12**, 1040–1050 (2016).
40. H. Mirbaha, D. Chen, O. A. Morazova, K. M. Ruff, A. M. Sharma, X. Liu, M. Goodarzi, R. V. Pappu, D. W. Colby, H. Mirzaei, L. A. Joachimiak, M. I. Diamond, Inert and seed-competent tau monomers suggest structural origins of aggregation. *eLife* **7**, e36584 (2018).
41. H. Mirbaha, D. Chen, V. Mullapudi, S. J. Terpack, C. L. White III, L. A. Joachimiak, M. I. Diamond, Seed-competent tau monomer initiates pathology in a tauopathy mouse model. *J. Biol. Chem.* **298**, 102163 (2022).
42. A. M. Sharma, T. L. Thomas, D. R. Woodard, O. M. Kashmer, M. I. Diamond, Tau monomer encodes strains. *eLife* **7**, e37813 (2018).
43. G. I. Hallinan, M. Vargas-Caballero, J. West, K. Deinhardt, Tau misfolding efficiently propagates between individual intact hippocampal neurons. *J. Neurosci.* **39**, 9623–9632 (2019).
44. B. D. Hitt, A. Gupta, R. Singh, T. Yang, J. D. Beaver, P. Shang, C. L. White III, L. A. Joachimiak, M. I. Diamond, Anti-tau antibodies targeting a conformation-dependent epitope selectively bind seeds. *J. Biol. Chem.* **299**, 105252 (2023).
45. C. H. Michel, S. Kumar, D. Pinotsi, A. Tunnacliffe, P. St. George-Hyslop, E. Mandelkow, E. M. Mandelkow, C. F. Kaminski, G. S. Kaminski Schierle, Extracellular monomeric tau protein is sufficient to initiate the spread of tau protein pathology. *J. Biol. Chem.* **289**, 956–967 (2014).
46. S. L. DeVos, B. T. Corjuc, D. H. Oakley, C. K. Nobuhara, R. N. Bannon, A. Chase, C. Commins, J. A. Gonzalez, P. M. Dooley, M. P. Frosch, B. T. Hyman, Synaptic tau seeding precedes tau pathology in human Alzheimer's disease brain. *Front. Neurosci.* **12**, 267 (2018).
47. I. Ferrer, M. V. Zelaya, M. Aguilo Garcia, M. Carmona, I. Lopez-Gonzalez, P. Andres-Benito, L. Lidon, R. Gavin, P. Garcia-Esparcia, J. A. Del Rio, Relevance of host tau in tau seeding and spreading in tauopathies. *Brain Pathol.* **30**, 298–318 (2020).
48. S. Dujardin, C. Commins, A. Lathuiliere, P. Beerepoot, A. R. Fernandes, T. V. Kamath, M. B. De Los Santos, N. Klickstein, D. L. Corjuc, B. T. Corjuc, P. M. Dooley, A. Viode, D. H. Oakley, B. D. Moore, K. Mullin, D. Jean-Gilles, R. Clark, K. Atchison, R. Moore, L. B. Chibnik, R. E. Tanzi, M. P. Frosch, A. Serrano-Pozo, F. Elwood, J. A. Steen, M. E. Kennedy, B. T. Hyman, Tau molecular diversity contributes to clinical heterogeneity in Alzheimer's disease. *Nat. Med.* **26**, 1256–1263 (2020).
49. E. Miyoshi, T. Bilousova, M. Melnik, D. Fakhrutdinov, W. W. Poon, H. V. Vinters, C. A. Miller, M. Corrada, C. Kawas, R. Bohannan, C. Caraway, C. Elias, K. N. Maina, J. J. Campagna, V. John, K. H. Gylys, Exosomal tau with seeding activity is released from Alzheimer's disease synapses, and seeding potential is associated with amyloid beta. *Lab. Invest.* **101**, 1605–1617 (2021).
50. N. Puangmalai, U. Sengupta, N. Bhatt, S. Gaikwad, M. Montalbano, A. Bhuyan, S. Garcia, S. McAllen, M. Sonawane, C. Jerez, Y. Zhao, R. Kayed, Lysine 63-linked ubiquitination of tau oligomers contributes to the pathogenesis of Alzheimer's disease. *J. Biol. Chem.* **298**, 101766 (2022).
51. M. Usenovic, S. Niroomand, R. E. Drolet, L. Yao, R. C. Gaspar, N. G. Hatcher, J. Schachter, J. J. Renger, S. Parmentier-Batteur, Internalized tau oligomers cause neurodegeneration by inducing accumulation of pathogenic tau in human neurons derived from induced pluripotent stem cells. *J. Neurosci.* **35**, 14234–14250 (2015).
52. J. E. Gerson, R. Kayed, Formation and propagation of tau oligomeric seeds. *Front. Neurol.* **4**, 93 (2013).
53. F. Jiang, L. Lilge, B. Logie, Y. Li, M. Chopp, Photodynamic therapy of 9L gliosarcoma with liposome-delivered photofrin. *Photochem. Photobiol.* **65**, 701–706 (1997).
54. M. Polydoro, C. M. Acker, K. Duff, P. E. Castillo, P. Davies, Age-dependent impairment of cognitive and synaptic function in the htau mouse model of tau pathology. *J. Neurosci.* **29**, 10741–10749 (2009).
55. R. De Rosa, A. A. Garcia, C. Braschi, S. Capsoni, L. Maffei, N. Berardi, A. Cattaneo, Intranasal administration of nerve growth factor (NGF) rescues recognition memory deficits in AD11 anti-NGF transgenic mice. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 3811–3816 (2005).
56. L. R. Hanson, W. H. Freyll, Intranasal delivery bypasses the blood-brain barrier to target therapeutic agents to the central nervous system and treat neurodegenerative disease. *BMC Neurosci.* **9**, S5 (2008).
57. S. Gaikwad, S. Senapati, M. A. Haque, R. Kayed, Senescence, brain inflammation, and oligomeric tau drive cognitive decline in Alzheimer's disease: Evidence from clinical and preclinical studies. *Alzheimers Dement.* **20**, 709–727 (2024).
58. A. N. Nilson, K. C. English, J. E. Gerson, T. Barton Whittle, C. Nicolas Crain, J. Xue, U. Sengupta, D. L. Castillo-Carranza, W. Zhang, P. Gupta, R. Kayed, Tau oligomers associate with inflammation in the brain and retina of tauopathy mice and in neurodegenerative diseases. *J. Alzheimers Dis.* **55**, 1083–1099 (2017).
59. E. D. Smith, Q. Vo, B. I. Giasson, D. R. Borchelt, S. Prokop, P. Chakrabarty, Human tauopathy strains defined by phosphorylation in R1-R2 repeat domains of tau. *Acta Neuropathol. Commun.* **11**, 172 (2023).
60. A. J. Fletcher, D. L. Mallery, R. E. Watkinson, C. F. Dickson, L. C. James, Sequential ubiquitination and deubiquitination enzymes synchronize the dual sensor and effector functions of TRIM21. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 10014–10019 (2015).
61. Y. Zhang, K. Chen, S. A. Sloan, M. L. Bennett, A. R. Scholze, S. O'Keeffe, H. P. Phatnani, P. Guarnieri, C. Caneda, N. Ruderisch, S. Deng, S. A. Liddelow, C. Zhang, R. Daneman, T. Maniatis, B. A. Barres, J. Q. Wu, An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J. Neurosci.* **34**, 11929–11947 (2014).
62. M. Uhlén, L. Fagerberg, B. M. Hallström, C. Lindskog, P. Oksvold, A. Mardinoglu, A. Sivertsson, C. Kampf, E. Sjöstedt, A. Asplund, I. Olsson, K. Edlund, E. Lundberg, S. Navani, C. A. Szigyarto, J. Odeberg, D. Djureinovic, J. O. Takanen, S. Hober, T. Alm, P. H. Edqvist, H. Berling, H. Tegel, J. Mulder, J. Rockberg, P. Nilsson, J. M. Schwenk, M. Hamsten, K. von Feilitzen, M. Forsberg, L. Persson, F. Johansson, M. Zwahlen, G. von Heijne, J. Nielsen, F. Pontén, Proteomics. Tissue-based map of the human proteome. *Science* **347**, 1260419 (2015).
63. S. Foss, R. Watkinson, I. Sandlie, L. C. James, J. T. Andersen, TRIM21: A cytosolic Fc receptor with broad antibody isotype specificity. *Immunol. Rev.* **268**, 328–339 (2015).
64. W. A. McEwan, B. Falcon, M. Vaysburd, D. Clift, A. L. Oblak, B. Ghetti, M. Goedert, L. C. James, Cytosolic Fc receptor TRIM21 inhibits seeded tau aggregation. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 574–579 (2017).
65. C. Ballatore, V. M. Lee, J. Q. Trojanowski, Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat. Rev. Neurosci.* **8**, 663–672 (2007).
66. V. M. Lee, M. Goedert, J. Q. Trojanowski, Neurodegenerative tauopathies. *Annu. Rev. Neurosci.* **24**, 1121–1159 (2001).
67. M. M. Mocanu, A. Nissen, K. Eckermann, I. Khlistunova, J. Biernat, D. Drexler, O. Petrova, K. Schonig, H. Bujard, E. Mandelkow, L. Zhou, G. Rune, E. M. Mandelkow, The potential for beta-structure in the repeat domain of tau protein determines aggregation, synaptic decay, neuronal loss, and coassembly with endogenous tau in inducible mouse models of tauopathy. *J. Neurosci.* **28**, 737–748 (2008).
68. J. Stohr, H. Wu, M. Nick, Y. Wu, M. Bhate, C. Condello, N. Johnson, J. Rodgers, T. Lemmin, S. Acharya, J. Becker, K. Robinson, M. J. S. Kelly, F. Gai, G. Stubbs, S. B. Prusiner, W. F. DeGrado, A 31-residue peptide induces aggregation of tau's microtubule-binding region in cells. *Nat. Chem.* **9**, 874–881 (2017).
69. C. M. Wischik, M. Novak, H. C. Thogersen, P. C. Edwards, M. J. Runswick, R. Jakes, J. E. Walker, C. Milstein, M. Roth, A. Klug, Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4506–4510 (1988).
70. M. Roberts, I. Sevastou, Y. Imaizumi, K. Mistry, S. Talma, M. Dey, J. Gartlon, H. Ochiai, Z. Zhou, S. Akasofu, N. Tokuhara, M. Ogo, M. Aoyama, H. Aoyagi, K. Strand, E. Sajedi, K. L. Agarwala, J. Spidel, E. Albone, K. Horie, J. M. Staddon, R. de Silva, Pre-clinical characterisation of E2814, a high-affinity antibody targeting the microtubule-binding repeat domain of tau for passive immunotherapy in Alzheimer's disease. *Acta Neuropathol. Commun.* **8**, 13 (2020).
71. A. S. Mukadam, L. V. C. Miller, A. E. Smith, M. Vaysburd, S. A. Sakya, S. Sanford, S. Keeling, B. J. Tuck, T. Katsinelos, C. Green, L. Skov, S. S. Kaalund, S. Foss, K. Mayes, K. O'Connell, M. Wing, C. Knox, J. Banbury, E. Avezov, J. B. Rowe, M. Goedert, J. T. Andersen, L. C. James, W. A. McEwan, Cytosolic antibody receptor TRIM21 is required for effective tau immunotherapy in mouse models. *Science* **379**, 1336–1341 (2023).

72. E. E. Congdon, J. E. Chukwu, D. B. Shamir, J. Deng, D. Ujla, H. B. R. Sait, T. A. Neubert, X. P. Kong, E. M. Sigurdsson, Tau antibody chimerization alters its charge and binding, thereby reducing its cellular uptake and efficacy. *EBioMedicine* **42**, 157–173 (2019).
73. A. W. Schaler, A. M. Runyan, C. L. Clelland, E. J. Sydney, S. L. Fowler, H. Y. Figueroa, S. Shioda, I. Santa-Maria, K. E. Duff, N. Myeku, PAC1 receptor-mediated clearance of tau in postsynaptic compartments attenuates tau pathology in mouse brain. *Sci. Transl. Med.* **13**, eaba7394 (2021).
74. A. Bejanin, D. R. Schonhaut, R. La Joie, J. H. Kramer, S. L. Baker, N. Sosa, N. Ayakta, A. Cantwell, M. Janabi, M. Lauriola, J. P. O'Neil, M. L. Gorno-Tempini, Z. A. Miller, H. J. Rosen, B. L. Miller, W. J. Jagust, G. D. Rabinovici, Tau pathology and neurodegeneration contribute to cognitive impairment in Alzheimer's disease. *Brain* **140**, 3286–3300 (2017).
75. B. G. Perez-Nievas, T. D. Stein, H. C. Tai, O. Dols-Icardo, T. C. Scotton, I. Barroeta-Espar, L. Fernandez-Carballo, E. L. de Munain, J. Perez, M. Marquie, A. Serrano-Pozo, M. P. Frosch, V. Lowe, J. E. Parisi, R. C. Petersen, M. D. Ikonomovic, O. L. Lopez, W. Klunk, B. T. Hyman, T. Gomez-Isla, Dissecting phenotypic traits linked to human resilience to Alzheimer's pathology. *Brain* **136**, 2510–2526 (2013).
76. A. Singh, D. Allen, A. Fracassi, B. Tumurbaatar, C. Natarajan, P. Scaduto, R. Woltjer, R. Kayed, A. Limon, B. Krishnan, G. Taglialatela, Functional integrity of synapses in the central nervous system of cognitively intact individuals with high Alzheimer's disease neuropathology is associated with absence of synaptic tau oligomers. *J. Alzheimers Dis.* **78**, 1661–1678 (2020).
77. T. Arakhamia, C. E. Lee, Y. Carlomagno, M. Kumar, D. M. Duong, H. Wesseling, S. R. Kundinger, K. Wang, D. Williams, M. DeTure, D. W. Dickson, C. N. Cook, N. T. Seyfried, L. Petrucelli, J. A. Steen, A. W. P. Fitzpatrick, Posttranslational modifications mediate the structural diversity of tauopathy strains. *Cell* **180**, 633–644.e12 (2020).
78. Y. Shi, W. Zhang, Y. Yang, A. G. Murzin, B. Falcon, A. Kotecha, M. van Beers, A. Tarutani, F. Kametani, H. J. Garringer, R. Vidal, G. I. Hallinan, T. Lashley, Y. Saito, S. Murayama, M. Yoshida, H. Tanaka, A. Kakita, T. Ikeuchi, A. C. Robinson, D. M. A. Mann, G. G. Kovacs, T. Revesz, B. Ghetti, M. Hasegawa, M. Goedert, S. H. W. Scheres, Structure-based classification of tauopathies. *Nature* **598**, 359–363 (2021).
79. G. Legname, H. O. Nguyen, I. V. Baskakov, F. E. Cohen, S. J. Dearmond, S. B. Prusiner, Strain-specified characteristics of mouse synthetic prions. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 2168–2173 (2005).
80. S. Narasimhan, J. L. Guo, L. Changolkar, A. Stieber, J. D. McBride, L. V. Silva, Z. He, B. Zhang, R. J. Gathagan, J. Q. Trojanowski, V. M. Y. Lee, Pathological tau strains from human brains recapitulate the diversity of tauopathies in nontransgenic mouse brain. *J. Neurosci.* **37**, 11406–11423 (2017).
81. J. Vaquer-Alicea, M. I. Diamond, L. A. Joachimiak, Tau strains shape disease. *Acta Neuropathol.* **142**, 57–71 (2021).
82. P. Martinez, H. Patel, Y. You, N. Jury, A. Perkins, A. Lee-Gosselin, X. Taylor, Y. You, G. Viana Di Prisco, X. Huang, S. Dutta, A. B. Wijeratne, J. Redding-Ochoa, S. S. Shahid, J. F. Codocedo, S. Min, G. E. Landreth, A. L. Mosley, Y. C. Wu, D. L. McKinzie, J. C. Rochet, J. Zhang, B. K. Atwood, J. Troncoso, C. A. Lasagna-Reeves, Bassoon contributes to tau-seed propagation and neurotoxicity. *Nat. Neurosci.* **25**, 1597–1607 (2022).
83. M. Albert, G. Mairet-Coello, C. Danis, S. Lieger, R. Caillierez, S. Carrier, E. Skrobala, I. Landrieu, A. Michel, M. Schmitt, M. Citron, P. Downey, J. P. Courade, L. Buee, M. Colin, Prevention of tau seeding and propagation by immunotherapy with a central tau epitope antibody. *Brain* **142**, 1736–1750 (2019).
84. H. Braak, E. Braak, Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiol. Aging* **16**, 271–278 (1995).
85. B. Frost, R. L. Jacks, M. I. Diamond, Propagation of tau misfolding from the outside to the inside of a cell. *J. Biol. Chem.* **284**, 12845–12852 (2009).
86. A. M. Pooler, E. C. Phillips, D. H. W. Lau, W. Noble, D. P. Hanger, Physiological release of endogenous tau is stimulated by neuronal activity. *EMBO Rep.* **14**, 389–394 (2013).
87. A. Bittar, N. Bhatt, R. Kayed, Advances and considerations in AD tau-targeted immunotherapy. *Neurobiol. Dis.* **134**, 104707 (2020).
88. E. M. Sigurdsson, Alzheimer's therapy development: A few points to consider. *Prog. Mol. Biol. Transl. Sci.* **168**, 205–217 (2019).
89. S. H. Lee, C. E. Le Pichon, O. Adolfsson, V. Gafner, M. Pihlgren, H. Lin, H. Solanoy, R. Brendza, H. Ngu, O. Foreman, R. Chan, J. A. Ernst, D. DiCara, I. Hotzel, K. Srinivasan, D. V. Hansen, J. Atwal, Y. Lu, D. Bumbaca, A. Pfeifer, R. J. Watts, A. Muhs, K. Scearce-Levie, G. Ayalon, Antibody-mediated targeting of tau in vivo does not require effector function and microglial engagement. *Cell Rep.* **16**, 1690–1700 (2016).
90. W. Wang, E. Q. Wang, J. P. Balthasar, Monoclonal antibody pharmacokinetics and pharmacodynamics. *Clin. Pharmacol. Ther.* **84**, 548–558 (2008).
91. D. K. Shah, A. M. Betts, Antibody biodistribution coefficients: Inferring tissue concentrations of monoclonal antibodies based on the plasma concentrations in several preclinical species and human. *MAbs* **5**, 297–305 (2013).
92. J. J. Lochhead, R. G. Thorne, Intranasal delivery of biologics to the central nervous system. *Adv. Drug Deliv. Rev.* **64**, 614–628 (2012).
93. H. Braak, K. Del Tredici, Spreading of tau pathology in sporadic Alzheimer's disease along cortico-cortical top-down connections. *Cereb. Cortex* **28**, 3372–3384 (2018).
94. R. M. Brady, R. P. Zinkowski, L. I. Binder, Presence of tau in isolated nuclei from human brain. *Neurobiol. Aging* **16**, 479–486 (1995).
95. H. Zempel, E. M. Mandelkow, Tau missorting and spastin-induced microtubule disruption in neurodegeneration: Alzheimer disease and hereditary spastic paraplegia. *Mol. Neurodegener.* **10**, 68 (2015).
96. S. Craft, R. Raman, T. W. Chow, M. S. Rafii, C. K. Sun, R. A. Rissman, M. C. Donohue, J. B. Brewer, C. Jenkins, K. Harless, D. Gessert, P. S. Aisen, Safety, efficacy, and feasibility of intranasal insulin for the treatment of mild cognitive impairment and Alzheimer disease dementia: A randomized clinical trial. *JAMA Neurol.* **77**, 1099–1109 (2020).
97. C. Benedict, M. Hallschmid, A. Hatke, B. Schultes, H. L. Fehm, J. Born, W. Kern, Intranasal insulin improves memory in humans. *Psychoneuroendocrinology* **29**, 1326–1334 (2004).
98. M. S. Dhamoon, J. M. Noble, S. Craft, Intranasal insulin improves cognition and modulates β-amyloid in early AD. *Neurology* **72**, 292–294 (2009).
99. M. Hallschmid, Intranasal insulin for Alzheimer's disease. *CNS Drugs* **35**, 21–37 (2021).
100. R. Pietrowsky, C. Struben, M. Molle, H. L. Fehm, J. Born, Brain potential changes after intranasal vs. intravenous administration of vasopressin: Evidence for a direct nose-brain pathway for peptide effects in humans. *Biol. Psychiatry* **39**, 332–340 (1996).
101. A. Bittar, R. Al-Lahham, N. Bhatt, K. Moore, M. Montalbano, C. Jerez, L. Fung, S. McAllen, A. Ellsworth, R. Kayed, Passive immunotherapy targeting tau oligomeric strains reverses tauopathy phenotypes in aged human-tau mice in a mouse model-specific manner. *J. Alzheimers Dis.* **90**, 1103–1122 (2022).
102. K. Yanamandra, H. Jiang, T. E. Mahan, S. E. Maloney, D. F. Wozniak, M. I. Diamond, D. M. Holtzman, Anti-tau antibody reduces insoluble tau and decreases brain atrophy. *Ann. Clin. Transl. Neurol.* **2**, 278–288 (2015).
103. K. Herline, F. Prelli, P. Mehta, C. MacMurray, F. Goni, T. Wisniewski, Immunotherapy to improve cognition and reduce pathological species in an Alzheimer's disease mouse model. *Alzheimers Res. Ther.* **10**, 54 (2018).
104. D. L. Castillo-Carranza, M. J. Guerrero-Muñoz, U. Sengupta, C. Hernandez, A. D. Barrett, K. Dineley, R. Kayed, Tau immunotherapy modulates both pathological tau and upstream amyloid pathology in an Alzheimer's disease mouse model. *J. Neurosci.* **35**, 4857–4868 (2015).
105. A. Boutajangout, D. Quartermain, E. M. Sigurdsson, Immunotherapy targeting pathological tau prevents cognitive decline in a new tangle mouse model. *J. Neurosci.* **30**, 16559–16566 (2010).
106. G. S. Gibbons, S. J. Kim, Q. Wu, D. M. Riddle, S. N. Leight, L. Changolkar, H. Xu, E. S. Meymand, M. O'Reilly, B. Zhang, K. R. Brunden, J. Q. Trojanowski, V. M. Y. Lee, Conformation-selective tau monoclonal antibodies inhibit tau pathology in primary neurons and a mouse model of Alzheimer's disease. *Mol. Neurodegener.* **15**, 64 (2020).
107. G. A. Jicha, R. Bowser, I. G. Kazam, P. Davies, 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).
108. S. Schroeder, A. Joly-Amado, A. Soliman, U. Sengupta, R. Kayed, M. N. Gordon, D. Morgan, Oligomeric tau-targeted immunotherapy in Tg4510 mice. *Alzheimers Res. Ther.* **9**, 46 (2017).
109. A. Kondo, K. Shahpasand, R. Mannix, J. Qiu, J. Moncaster, C. H. Chen, Y. Yao, Y. M. Lin, J. A. Driver, Y. Sun, S. Wei, M. L. Luo, O. Albayram, P. Huang, A. Rotenberg, A. Ryo, L. E. Goldstein, A. Pascual-Leone, A. C. McKee, W. Meehan, X. Z. Zhou, K. P. Lu, Antibody against early driver of neurodegeneration cis P-tau blocks brain injury and tauopathy. *Nature* **523**, 431–436 (2015).
110. E. E. Congdon, J. Gu, H. B. Sait, E. M. Sigurdsson, Antibody uptake into neurons occurs primarily via clathrin-dependent Fcγ receptor endocytosis and is a prerequisite for acute tau protein clearance. *J. Biol. Chem.* **288**, 35452–35465 (2013).
111. D. Clift, W. A. McEwan, L. I. Labzin, V. Konieczny, B. Mogessie, L. C. James, M. Schuh, A method for the acute and rapid degradation of endogenous proteins. *Cell* **171**, 1692–1706.e18 (2017).
112. D. L. Castillo-Carranza, U. Sengupta, M. J. Guerrero-Muñoz, C. A. Lasagna-Reeves, J. E. Gerson, G. Singh, D. M. Estes, A. D. Barrett, K. T. Dineley, G. R. Jackson, R. Kayed, Passive immunization with Tau oligomer monoclonal antibody reverses tauopathy phenotypes without affecting hyperphosphorylated neurofibrillary tangles. *J. Neurosci.* **34**, 4260–4272 (2014).
113. A. Bittar, U. Sengupta, R. Kayed, Prospects for strain-specific immunotherapy in Alzheimer's disease and tauopathies. *NPJ Vaccines* **3**, 9 (2018).
114. U. Sengupta, M. Carretero-Murillo, R. Kayed, Preparation and characterization of tau oligomer strains. *Methods Mol. Biol.* **1779**, 113–146 (2018).
115. S. E. Fischer, L. Mi, H. Q. Mao, J. L. Harden, Biofunctional coatings via targeted covalent cross-linking of associating triblock proteins. *Biomacromolecules* **10**, 2408–2417 (2009).
116. L. Liu, K. T. Yong, I. Roy, W. C. Law, L. Ye, J. Liu, J. Liu, R. Kumar, X. Zhang, P. N. Prasad, Bioconjugated pluronic triblock-copolymer micelle-encapsulated quantum dots for

targeted imaging of cancer: In vitro and in vivo studies. *Theranostics* **2**, 705–713 (2012).
117. A. Sahu, N. Kasoju, P. Goswami, U. Bora, Encapsulation of curcumin in Pluronic block copolymer micelles for drug delivery applications. *J. Biomater. Appl.* **25**, 619–639 (2011).
118. H. Song, R. He, K. Wang, J. Ruan, C. Bao, N. Li, J. Ji, D. Cui, Anti-HIF-1α antibody-conjugated pluronic triblock copolymers encapsulated with paclitaxel for tumor targeting therapy. *Biomaterials* **31**, 2302–2312 (2010).
119. L. R. Hanson, J. M. Fine, A. L. Svitak, K. A. Faltesek, Intranasal administration of CNS therapeutics to awake mice. *J. Vis. Exp.* **8**, 4440 (2013).
120. M. Rodriguez, J. Lapierre, C. R. Ojha, A. Kaushik, E. Batrakova, F. Kashanchi, S. M. Dever, M. Nair, N. El-Hage, Intranasal drug delivery of small interfering RNA targeting Beclin1 encapsulated with polyethylenimine (PEI) in mouse brain to achieve HIV attenuation. *Sci. Rep.* **7**, 1862 (2017).
121. J. Gerson, D. L. Castillo-Carranza, U. Sengupta, R. Bodani, D. S. Prough, D. S. DeWitt, B. E. Hawkins, R. Kayed, Tau oligomers derived from traumatic brain injury cause cognitive impairment and accelerate onset of pathology in Htau mice. *J. Neurotrauma* **33**, 2034–2043 (2016).
122. F. Yang, G. P. Lim, A. N. Begum, O. J. Ubeda, M. R. Simmons, S. S. Ambegaokar, P. P. Chen, R. Kayed, C. G. Glabe, S. A. Frautschy, G. M. Cole, Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo. *J. Biol. Chem.* **280**, 5892–5901 (2005).
123. F. Lo Cascio, N. Puangmalai, A. Ellsworth, F. Bucchieri, A. Pace, A. Palumbo Piccionello, R. Kayed, Toxic tau oligomers modulated by novel curcumin derivatives. *Sci. Rep.* **9**, 19011 (2019).
124. U. Sengupta, N. Puangmalai, N. Bhatt, S. Garcia, Y. Zhao, R. Kayed, Polymorphic α-synuclein strains modified by dopamine and docosahexaenoic acid interact differentially with tau protein. *Mol. Neurobiol.* **57**, 2741–2765 (2020).

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