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TREM2 limits progression of deficits and spreading of tau pathology in mice

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Abstract

Background. Amyloid- β (A β) and tau form pathogenic lesions in Alzheimer's disease (AD) brains. As AD clinically progresses, tau pathology propagates in a very distinct pattern between connected brain areas. The molecular mechanisms underlying this tau pathology spread remain largely unknown. Genome-wide association studies have identified polymorphisms in *triggering receptor expressed on myeloid cells 2 (TREM2)* as genetic risk factors for AD and regulators of A β pathology-dependent tau propagation. Whether TREM2 contributes to neuron-to-neuron spreading of pathological tau remains unknown.

Methods. Here, we crossed *Trem2*-deficient mice with P301S tau transgenic TAU58 mice and subjected the mice to behavioral testing and assessed neuropathology. Microglial activation states were determined using cytometry by of flight (CyTOF) and quantitative PCR. Tau spreading was assessed *in vivo* using tracing of focal tau expression.

Results. *Trem2* depletion significantly aggravated tau-induced early-onset motor and behavioural deficits. Neuropathologically, *Trem2* reduction increased the number of hyperphosphorylated tau lesions in young TAU58 brains and reduced disease-associated microglia. Direct assessment of inter-neuronal spread of tau *in vivo* revealed significantly enhanced propagation of tau in the absence of *Trem2*, suggesting that microglial TREM2 limits the progression of tau pathology in disease.

Conclusion. Taken together, our data suggests that reduced TREM2 function accelerates the onset and progression of functional deficits and tau neuropathology in tau transgenic mice, which is - at least in part - due to increased tau spreading. Therefore, reduced TREM2 function may contribute to early AD by augmenting tau toxicity and its inter-neuronal propagation.

Keywords

TREM2; tau; spreading; dementia; Alzheimer's disease; microglia signature; microglia activation; neuroinflammation

Background

Alzheimer's Disease (AD) is the most prevalent form of dementia (1,2). Cognitive decline is often accompanied by behavioural changes, anxiety, depression or sleep disturbances, together leading to full-time care dependency and ultimately death (3,4). AD brains are characterised by two pathological hallmark changes, the extracellular deposition of amyloid- β (A β) in plaques and the formation of intracellular neurofibrillary tangles (NFT), composed of hyper phosphorylated microtubule-associated protein tau (5). Fibrillary tau pathology (without A β plaques) is furthermore characterizing brains in different forms of frontotemporal dementia (FTD), the second most common form of dementia in individuals age 65 or younger (6) and coining the term 'tauopathies'. Studies into familial FTD identified pathogenic mutations in the tau encoding *MAPT* gene, highlighting its role in

dementia (7,8), and have been instrumental for developing mouse models with AD/FTD-like NFT pathology (9).

Over 25 years ago, Braak & Braak showed that NFT deposition in AD brains occurs in a highly conserved pattern that correlates with disease progression (10). Accordingly, tau pathology spreads in a stereotypical fashion, occurring first in entorhinal areas before affecting connected limbic and cortical brain areas (10). The molecular and cellular mechanisms that drive tau propagation remain largely unknown (11). Mouse models recapitulating the distribution pattern have been used to show neuron-to-neuron spread of tau, most likely via synaptic connections (12), inducing recipient neuron tau to aggregate (11). Further, it has been shown that extracellular tau aggregates can be taken up by cells and induce tau aggregation in culture (13). More recently, microglial involvement in tau propagation has emerged (14–16), however the exact mechanisms remain to be defined. It has previously been shown that microglia can internalize tau *in vitro* and *in vivo* (17) and degrade hyperphosphorylated tau (18).

Interestingly, several polymorphisms of microglial genes have been linked to increased AD risk over the past years (reviewed in (19,20)). Accordingly, genome wide association studies identified polymorphisms of the gene *triggering receptor expressed on myeloid cells 2* (*TREM2*) linked to a higher risk to develop AD (21). The most common variant, R47H, conveys a 3–4 fold risk increase for late onset AD (LOAD) (21,22). While it is not fully understood how *TREM2* mutations increase the risk for AD, loss of function rather than a toxic gain of function has been proposed (21). Subsequent studies found additional polymorphisms and an association with an increased risk for FTD (23,24) and other neurodegenerative diseases like amyotrophic lateral sclerosis (ALS) (25,26) or Parkinson's disease (27,28). *TREM2* is a transmembrane receptor of the immunoglobulin superfamily and predominately expressed by microglia in the brain. *TREM2* signalling has been linked to different cellular processes of innate immunity including phagocytosis (29,30), proliferation (31), promotion of survival (32,33) as well as the secretion of cytokines and chemokines (34). Activity of *TREM2* has been reported to be involved in the clearance of apoptotic neurons (29). Further, *TREM2* is a receptor for lipoproteins such as APOE (35) and can bind A β (36,37). Consequently, early studies have focused on *TREM2* and A β (38–45), while its link to tau was only more recently investigated (46–50). Apparently contradicting findings with *TREM2* and A β (38–44), were likely due to differential roles of *TREM2* during disease stages (45). Similarly, early *TREM2*–tau literature was somewhat contradictory; while some reported that *Trem2* depletion attenuated neurodegeneration in tau transgenic mice (47,48), others found detrimental effects of *Trem2* loss (46,49,50) which now is the emerging consensus. Augmented spatial learning deficits in tau transgenic mice upon hippocampal shRNA-mediated

TREM2 reduction has been observed (49), however, a detailed functional analysis of *Trem2*-deficient tau transgenic mice has not been done to date. Furthermore, the effects of reduced TREM2 function on tau propagation has not been assessed.

In the present study, we crossed the established tau P301S transgenic line TAU58 (51–54) with *Trem2* knockout mice, generated by CRISPR/Cas9-mediated gene targeting. We found that TREM2 reduction worsened motor deficits of TAU58 mice and aggravated behavioural deficits together with accelerated tau neuropathology in young but not older mice. Interestingly, numbers of active, disease-associated (vs. resting/homeostatic) microglia were reduced early-on in TREM2-deficient TAU58 mice. Injecting mice with an adeno-associated virus (AAV) to visualize tau protein spread between neurons (55,56) revealed increased tau propagation throughout the brains of TREM2 depleted mice. Our data supports a disease-limiting role of TREM2 in onset and propagation of tau pathology.

Methods

Mice All animal experiments were approved by the Animal Ethics Committees of Macquarie University. Mice were group housed on a normal 12h light/dark cycle and had access to food and water *ad libitum*. TAU58 (TAU58/2 line) transgenic mice express the human P301S mutation on the 0N4R tau isoform under the control of the murine Thy1.2 neuronal promoter on a C57Bl/6 background and have been described previously (51–53). The first exon of the murine *Trem2* gene (ENSEMBL ENSMUSG00000023992) was targeted to remove the endogenous start codon (**Figure S1A**) by CRISPR-mediated gene targeting using two guides (Guide 1: 5'-TGCACAAGGTCCCCTCCGGC-3'; Guide 6: 5'-CTGAAGGTAGGTCCCTCCTG-3'). These single-guide RNAs (sgRNAs) were rationally designed using a computational tool to minimize off-targets (<http://crispr.mit.edu>) and were produced using a non-cloning method whereby a T7-conjugated forward primer generates a linear template by PCR, as previously described (57). Briefly, the sgRNA scaffold of the pX330 (Addgene #42230, gift from Dr. Feng Zhang) was used to synthesize a small linear DNA template. Next, this template was *in-vitro* transcribed using a T7 Quick High Yield RNA synthesis kit (NEB #E2050S) following the manufacturer's instructions. The resulting sgRNAs were purified using NucAway Spin columns (ThermoFisher #AM10070) and incubated with S.p.Cas9 protein (NEB #M0646T) to form ribonucleoprotein complexes (RNPs). RNPs were electroporated (NEPA21, Nepagene) into fertilised C57Bl/6 zygotes with the respective concentrations: 200ng/ul Cas9, 300ng/ul Guide 1 and 150ng/ul Guide 6. Nineteen live pups were produced, nine

of which displayed a deletion at the endogenous *Trem2* locus by PCR genotyping (Primer sequences: Fwd 5'-TAAGAAGCCACTGTCCTCCAATCT-3'; Rev 5'-AGAAAAGCAGAGTCAAAGCAGGAC-3'). One founder was selected to establish the colony based on Sanger sequencing (Macrogen, South Korea) of the edited allele (**Figure S1B**), which revealed a 92bp deletion including the start codon. Homozygous (Δ/Δ) and heterozygous ($\Delta/+$) *Trem2* KO mice were identified via PCR using the above genotyping primers (**Figure S1C**). *Trem2* KO mice were maintained on a C57Bl/6 background and crossed with TAU58 transgenic mice to generate *Trem2* heterozygous TAU58 transgenic (TAU58/*Trem2* ^{$\Delta/+$}) and *Trem2* heterozygous (*Trem2* ^{$\Delta/+$}) mice. These mice were used as breeders to create the experimental mice, including all littermate controls (**Figure 1B**). Animals were subjected to behavioural test battery at 1-, 2-, 3- and 6 months of age. Both genders were used, and different animals were used at each time point, to avoid potential artefacts induced by re-testing mice on the same test. Group sizes for each test are provided in **Table S1**.

RotaRod To assess motor function mice were placed on the RotaRod (UgoBasile, Gemonio VA, Italy) as described elsewhere (58). Briefly, mice were placed on a rotating rod with accelerating speed (acceleration mode 5-60rpm over 120 seconds). The latency to fall or until mice did one full revolution was recorded. Mice were trained for 3 consecutive days, 5 trials each day and the best trial of the last day was used for analysis.

Beam To further assess the fine-motor performance, mice were subjected to the Beam traversing challenge (51). For this challenge mice need to cross a thin elevated wooden beam. The house of their home cage was placed at the end of the beam, on a platform the same height as the beam. Mice were trained in the morning and tested in the afternoon of the same day. The testing session was recorded, and mice performed two consecutive trials. The videos were analysed manually, by an experimenter blinded to the experimental groups. The time to cross as well as the number of foot slips was counted. The two trials were averaged for analysis. If mice fell off the beam in one trial, the successful trial was used for analysis. Mice that fell in both trials were excluded from the analysis.

Morris Water Maze To assess spatial memory, mice were subjected to Morris Water Maze (MWM) testing (59). The test apparatus consisted of a large tank (1.4 m diameter), a Perspex platform (10cm diameter, 40cm high) and four different visual cues. Each visual cue was placed equidistant from one another around the tank. The platform was positioned roughly 20 cm from the inside wall of the tank. The tank was filled with water to a height of approximately 0.5 - 1 cm above the platform with white non-toxic acrylic-based paint diluted in the water to conceal the platform. Additionally, the lights in the testing room were dimmed. Mice were acclimatised

in the room for one hour prior to testing. On days 1-5, mice were trained to find the hidden platform. To achieve this, mice had 4 swims per day, each trial starting from a different position. This was done to ensure mice learned the location of the platform rather than the swimming trajectory. Additionally, the order of the positions was changed every day. Mice were given 1 minute to locate the platform and then had to sit on the platform for one additional minute once they found it. When mice did not find the platform within 1 minute they were guided to it and had to stay on the platform for a minute. Two to three mice were alternated per round, to avoid fatigue of the mice. The order of the mice and the number per round was kept consistent for the whole duration of the test. Each trial was recorded and the latency to find the platform was noted. On day 6 mice were subjected to the probe trial for 30 seconds. The hidden platform was removed from the maze and mice were placed into the tank from position 1 and recorded. The video was analysed using AnyMAZE software (Stoelting). For the analysis, the tank was divided into four quadrants (the target quadrant, the right quadrant, the left quadrant and the opposite quadrant) and the platform zone (a circle corresponding to the size and position of the platform). The software counted the number of entries into the platform zone, the latency for the first entry into the platform zone, the time spent in the platform zone as well as the time spend in each of the quadrants and the overall swim speed and distance.

A visual cue test was performed after the probe trial to ensure all mice used have sensorimotor abilities and motivation. The platform was placed back into the same position as before, with a flag placed on top of it. Mice had four swims from positions 1 through 4. The latency to reach the platform/ flag was noted and mice were taken out of the maze as soon as they reached the platform. The four trials were averaged and an exclusion criteria of an average time greater than 20 seconds was applied.

Elevated Plus Maze To test for disinhibition behaviour mice were subjected to Elevated Plus Maze (UgoBasile, Gemonio VA, Italy; EPM) testing at all ages. The EPM consist of two open and two closed arms (35cm x 5.5cm each) and an inner area where all four arms meet (5.5cm x 5.5cm) and is elevated 60cm off the ground. Mice were acclimatised in the room for one hour prior to testing. At the start of the test mice were placed in the inner area, facing the open arm, and allowed to explore for 5min while being recorded. The time mice spend in the different areas was analysed using the AnyMAZE software (Stoelting).

Open Field Activity and exploration behaviour was assessed using the Open Field test (OF), as described previously (54). Briefly, the OF consists of an open arena (40cm x 40cm) placed in an enclosed space containing a camera as well as a ceiling-mounted fan. Mice were acclimatised in the testing space for an hour prior to the test. At the start of the test, mice were placed in the top right corner of the arena and were allowed to

explore it freely for 10 minutes. Mice were video recorded while performing the test and the videos were later analysed using AnyMAZE software (Stoelting). For the analysis, the arena was divided into an inner zone (17.5 cm x 17.5 cm square in the middle) and an outer zone. The software tracked how much time animals spent in each respective zone as well as the total distance travelled.

AAV production Packaging of recombinant AAVs with AAV1 capsid (Penn Vector Core – Gene Therapy Program, University of Pennsylvania) was performed as previously described (60). AAV1-eGFP-2a-P301L-tau plasmid was a gift from Bradley Hyman (55,56). Titres of AAVs were determined by quantitative polymerase chain reaction (qPCR) and liquid droplet PCR using the following primers (WPRE_F: ggctgttgggcactgacaaat and WPRE_R: ccgaaggacgtagcagaag).

Stereotaxic Injections 4-week-old mice were anaesthetised with 7.2µl/g of Ketamine/Xylazine. Their heads were fixed in a stereotaxic frame (Kopf instruments). A small midline incision was made and bregma located. A small burr hole was made above the entorhinal cortex (Coordinates: A/P: 3; M/L: -3 both from bregma and D/V: -3 from skull surface) using a bone micro-drill (Fine Science Tools). 1µl of AAV (titre: 1.64×10^{13} viral genomes) was injected at a rate of 300µl/minute. The needle was left in place for 5 minutes to reduce backflow after injection.

Histological Analysis Mice were anaesthetised and transcardially perfused with ice-cold phosphate buffered saline (p.H 7.4). Brains were carefully removed and separated into hemispheres. The right hemisphere was further sub-dissected into hippocampus and cortex, snap frozen in liquid nitrogen and stored at -80°C for biochemical analysis. The left hemisphere was used for immunohistochemical analysis which was carried out as described previously (61). Mice that had undergone stereotaxic surgery were additionally perfused with 4% paraformaldehyde prior to removal of the brain and the whole brain was used for immunohistochemistry. Briefly, the brain was post-fixed in cold 4% paraformaldehyde overnight and changed to 70% ethanol the next day. Tissue was placed in histology cassettes and processed overnight in the Excelsior tissue processor (ThermoFisher, Waltham, MA, USA). Processed tissue was embedded in paraffin and sectioned either coronally at 3µm or horizontally at 5µm (whole brain). Sections were allowed to air dry over night before being baked at 65°C for two hours. To remove any residual paraffin, slides were placed in xylene for 20 minutes and then re-hydrated by immersing the slides in decreasing concentrations of ethanol. Heat-mediated antigen retrieval was performed in a Milestone Histology microwave (Milestone, Sorisole, Italy) using citrate buffer. Sections were stained using sequenza racks (ThermoFisher, Waltham, MA, USA). Slides were blocked in 100µl blocking

buffer (BB, containing 3% heat inactivated Goat Serum 2% Bovine Serum Albumin in PBS) for one hour at room temperature. 100µl primary antibody diluted in BB was added and slides were incubated overnight at 4°C (Table S2). The next day slides were washed with PBS. AlexaFluor (Invitrogen, Carlsbad, California, United States) conjugated secondary antibody (Table S2) and DAPI (1:1000, Molecular Probes, Carlsbad, Ca, USA) were diluted in BB, and 100µl were added on to the slides and incubated for one hour at room temperature. Slides were washed and mounted with Fluoromount-G (SouthernBiotech, Birmingham, Alabama, United States) mounting media. Slides were scanned using an Axioscan Z.1 slide scanner (Zeiss, Oberkochen, Germany). Quantification of images was performed using either the Zeiss ZEN 2.6 blue edition software or ImageJ. Inkscape was used to create the figures and construct the heat maps. For the heat maps counts were divided into 14 different areas and averaged across mice per genotype, per depth. Depth is given relative to bregma.

RNA Extraction. Mice for RNA extraction were sacrificed, brains were removed and sub-dissected into hippocampus and cortex. Tissues were snap frozen in liquid nitrogen. RNA was extracted according to manufacturer's protocol for TRIzol (ThermoFisher Scientific). Briefly, tissue was homogenised in TRIzol using a plastic pestle. Lysates were centrifuged briefly to remove debris. Chloroform was added to the supernatant to precipitate proteins. After a short incubation period, tubes were centrifuged to separate the aqueous from the phenol phase. The aqueous phase was transferred to a new tube and 70% ethanol added. After gentle mixing, RNA purification was performed by using the Qiagen RNeasy Kit according to manufacturer's instructions, including the optional DNase treatment. RNA concentrations were measured using a Nanodrop (ThermoFisher Scientific).

qRT-PCR First-strand cDNA was synthesised from the extracted RNA by using the SuperScript VILO MasterMix (ThermoFisher Scientific) according to manufacturer's instructions. 1µg of RNA was used in the cDNA reaction and resulting cDNA was diluted 1:10 for qRT-PCR. Standards were made from pooled samples. A master mix consisting of 2x Power SYBR Green (ThermoFisher Scientific), DEPC treated water and the respective primers was made for each gene of interest for qRT-PCR analysis. Primers were designed specifically for qRT-PCR and ordered from Macrogen (South Korea) (Primer list Table S3). qRT-PCR was run on a ViiA 7 Real-Time PCR system (ThermoFisher Scientific).

Brain single cell suspensions. Following perfusion with 1X PBS, brains from adult mice were collected and placed in ice-cold Hibernate A medium (A1247501, Thermo Fisher Scientific). The brain was transferred to a

petri dish on ice without media and cut into 2mm² pieces with a scalpel blade. Tissue dissociation was performed with Digestion Media containing 1 mg/mL Collagenase D (Sigma) dissolved in Hibernate A Medium in a GentleMACS C tube (Miltenyi Biotec). The tissue was incubated at 37°C for 30 min with stirring using program 37C_ABDK_01 on the GentleMACS Octo Dissociator (Miltenyi Biotec). Tissue suspensions were further mechanically dissociated by gentle trituration using a 5 mL serological pipette for twenty strokes. The cell suspension was then passed through a pre-wetted 70 µm cell strainer with a syringe plunger and washed with ice-cold 1X PBS without calcium and magnesium. After centrifugation at 600 x g for 4 min at 4°C, the supernatant was discarded and the cell pellet resuspended in 10 mL of 1X PBS. The cell suspension was transferred to a 15 mL conical tube and mixed with 5 mL of isotonic Percoll (9 parts Percoll: 1 part 10X PBS) for a final 30% (vol/vol) Percoll gradient. This was centrifuged at 1500 x g for 20min at 4°C with maximum acceleration and no brakes during deceleration. The cloudy white myelin overlay was removed by aspiration, and the remaining supernatant and cell pellet were resuspended and transferred to a new 15 mL conical tube. The cells were centrifuged at 600 x g for 4 min at 4°C, the supernatant was discarded and the cell pellet resuspended in 200 µL FACS Buffer (2% FBS, 2mM EDTA, 1X PBS). A hemocytometer was used to confirm single cell dissociation and viability before proceeding with cell barcoding and staining.

Mass cytometry. Single cell suspensions from mouse brains were Live/Dead stained with Cisplatin-198 for three minutes at room temperature. To achieve increased throughput and homogenous staining, metal cell barcoding against mouse CD45 positive cells was used (62–64). The metal isotopes 106Cd, 110Cd, 111Cd, 112Cd, 113Cd, 114Cd, and 116Cd were conjugated to anti-mouse CD45.2 antibodies according to manufacturer's instruction using the MCP9 Antibody Labeling kit (Fluidigm). A seven choose three barcoding matrix was generated and barcoding reagents were titrated to achieve optimal labelling. Cells were stained in 96-well plates. To each sample a unique combination of exactly three metal cell barcoding reagents diluted in 50µL PBS, 0.5% BSA was added and then incubated for 20 min at room temperature. Cells were washed with 150µL FACS buffer at 4°C. Barcoded cells were then combined in a single 1.5ml tube and washed with FACS buffer. Surface staining mix was prepared by diluting all primary antibodies in FACS buffer. Cell suspension was centrifuged at 600g for four minutes at 8°C and the supernatant was removed. Cells were re-suspended in surface stain mix (Table S4) and incubated at room temperature for 20 minutes. Cells were washed in FACS buffer and staining was repeated with secondary antibodies at room temperature for 20 minutes. Cells were washed in FACS buffer and incubated over night with 250nM iridium intercalator (Fluidigm) in Maxpar cell fix./perm. buffer (Fluidigm) to label cellular DNA. Subsequently, cells were washed with PBS followed by

distilled water and resuspended in 10% EQ beads (Fluidigm) in distilled water. Mass cytometry acquisition was performed on a CyTOF2.1 (Helios) mass cytometer (Fluidigm). After acquisition, data was normalized by using the bead standard and the executable MATLAB normalizer application, dead cells and beads removed and debarcoded using Boolean gating using FlowJo software (BD) (65). All analyses on CyTOF data were performed after arcsinh (with cofactor equal to 5) transformation of marker expression. Data was analyzed in R using our published bioinformatic workflow (66).

Statistical analysis Statistical analysis was performed using GraphPad Prism software (GraphPad, La Jolla, CA, USA). A value of $p=0.05$ was considered significant. One-way analysis of variance (ANOVA) tests was used to compare more than two groups and two-way ANOVA's were used to compare groups across time. Students t-test was performed to compare two groups.

Results

Accelerated deficits in P301S tau transgenic mice upon *Trem2* reduction. To study the role of *Trem2* in tau pathology *in vivo*, we generated a new *Trem2*-deficient mouse line, *Trem2*^{Δ/Δ}, by introducing a 92 base pair deletion in exon 1 of *Trem2* comprising its translational start codon using CRISPR/Cas9-mediated genome editing in C57Bl/6 mice (**Figure S1A**). Quantitative PCR revealed half the *Trem2* mRNA levels of wild-type C57Bl/6 mice in the brains of heterozygous *Trem2*^{Δ/+} mice, while no *Trem2* mRNA was detectable in homozygous *Trem2*^{Δ/Δ} mice (**Figure 1A**). To determine whether *Trem2* modulates functional deficits of tau transgenic mice, we crossed TAU58 mice expressing neuronal human P301S mutant tau with the *Trem2*^{Δ/Δ} strain to obtain TAU58/*Trem2*^{Δ/Δ}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{+/+} mice (**Figure 1B**) and subjected them to functional testing at different ages. Unless otherwise specified, littermate controls refer to *Trem2*^{Δ/Δ}, *Trem2*^{Δ/+} and *Trem2*^{+/+} mice that do not carry the P301S tau transgene. We have previously reported learning deficits in 4-month-old TAU58 mice when subjected to Morris water maze (MWM) testing for spatial memory formation (53). To study mice at pre-symptomatic stage, we tested young 3-month-old mice and found no learning performance deficits in TAU58/*Trem2*^{+/+} and TAU58/*Trem2*^{Δ/+} compared to *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} littermate controls (**Figure 1C**). In contrast, 3-month-old TAU58/*Trem2*^{Δ/Δ} mice displayed significantly delayed learning in the MWM acquisition phase while the probe trial was unaffected. We have furthermore previously reported motor deficits in 3-month-old TAU58 mice, which were phenotypically still normal at 2 months of age (51,52). Accordingly, TAU58/*Trem2*^{+/+} mice performed like littermate controls during Rotarod testing at 1 and

2 months of age (**Figure 1D**). While TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice performed normally at 1 month of age, they presented significant deficits as compared with littermate controls at 2 months of age, with TAU58/*Trem2*^{Δ/Δ} mice significantly impaired compared to TAU58/*Trem2*^{+/+} mice. At 3 months of age, TAU58/*Trem2*^{+/+} have deteriorated and presented similar deficits as TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice compared to littermates. These deficits progressed further by 6 months of age. We have previously shown that TAU58 mice present with increased foot slips while crossing a narrow beam at 3 months of age (51). In the present cohort, significant differences in the number of foot slips began in 1 month-old TAU58/*Trem2*^{Δ/+} mice, which progressed to include TAU58/*Trem2*^{Δ/Δ} mice by 2 and 3 months of age (**Figure 1E**). No significant differences in the number of foot slips between the three genotypes was detected in the 6 months cohort. Analysing the time taken to cross the narrow beam, we found significant differences only in 1 month-old TAU58/*Trem2*^{Δ/+} mice compared to TAU58/*Trem2*^{+/+} mice, with TAU58/*Trem2*^{Δ/+} mice taking longer to cross the beam (**Figure 1E**). The analysis of the 3- and 6-month-old cohorts remained incomplete as a large number of transgenic mice fell off the beam during testing. Notably, 16% of 3-month-old TAU58/*Trem2*^{Δ/Δ} and TAU58/*Trem2*^{Δ/+} mice fell off the beam during both trials, while all TAU58/*Trem2*^{+/+} mice completed at least one trial successfully. 40% of 6 months-old TAU58/*Trem2*^{Δ/Δ} mice fell off the beam during both trials and an additional 20% fell during one of the trials, while 66% of TAU58/*Trem2*^{Δ/+} mice fell during both and 22% during one of the trials compared to 30% (both trials) and 20% (one trial) of TAU58/*Trem2*^{+/+} mice (**Figure 1F**). In addition to motor deficits, TAU58 mice present with an early-onset and progressive disinhibition behaviour that presents as increased time mice spent in the open arms of the Elevated Plus Maze (EPM) (52,67). TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice showed trends toward increased open arm times compared to littermate controls at 1 month of age (**Figure 1G**). By 2-month-old, TAU58/*Trem2*^{Δ/Δ} mice progressed to significant deficits as compared with TAU58/*Trem2*^{+/+} littermates. Interestingly, 6-month-old *Trem2*^{Δ/Δ} mice displayed significantly increased open arm time and activity compared to *Trem2*^{Δ/+} or *Trem2*^{+/+} mice. Overall locomotor activity was comparable in EPM (**Figure S2A**), as well as in the open field for all groups and ages tested (**Figure S2B**). Taken together, *Trem2* reduction in TAU58 mice resulted in earlier onset of learning, motor and behavioural deficits. Interestingly, *Trem2* knockout appeared to have disinhibitory effects independent of transgenic tau expression as mice aged.

Increased tau phosphorylation upon *Trem2* loss in young TAU58 mice. TAU58 mice are characterized by progressive tau hyperphosphorylation with brain region-specific differences in onset and progression of

pathology (51–53). Phosphorylation of tau at serine 214 (pS214) is known to be an early marker in the disease process, while phosphorylation at serine 422 (pS422) is described to be present later in disease course (68). We have previously reported the presence of tau pS214 in TAU58 amygdala as young as 3 months of age with the late-stage marker tau pS422 appearing from 6 months of age (52). Here, we found that pS214 occurred in the amygdala as early as 1 month of age (**Figure 2A**). By immunostaining of brain sections, there was no notable difference in levels of pS214 between TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice. For comparison, pS214 showed a similar pattern in the motor cortex (**Figure S3A**). pS422 labelling was sparse at 1 and 2 months of age with no differences between TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice (**Figure 2B**). However, at 3 months of age, TAU58/*Trem2*^{Δ/Δ} mice showed significantly more pS422 positive cells in the amygdala as compared with TAU58/*Trem2*^{+/+} and TAU58/*Trem2*^{Δ/+} mice. We observed a similar trend towards increased numbers of pS422-positive cells in the motor cortex of 2-months-old TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice (**Figure S3B**). At 6 months of age, levels of pS422 were comparably high in TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice, consistent with our previously reported numbers (52). Taken together, Trem2 depletion in TAU58 mice resulted in accelerated tau phosphorylation at the late-stage disease site pS422.

Trem2 reduction reduces tau-dependent microglial activation. Given that microglial activation associated with neurodegenerative processes is regulated by *Trem2* (69), we next determined microglial homeostasis in TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice. Staining of brain sections with the microglial marker Iba1 revealed no overt differences in distribution and morphology of microglia between *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} mice, or between TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice (**Figure S3C**). In all TAU58 lines, microglia were frequently observed in the proximity of neurons with transgenic P301S tau expression (**Figure 3A**), consistent with our previous reports of microglial engulfment of neurons harbouring tau pathology (51). To obtain quantitative data on microglial populations in TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice and the respective littermate controls, we obtained single cells from brains of TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice and subjected them to cytometry by time of flight (CyTOF) analysis with a panel of microglial markers (**Table S4**). This panel includes markers to differentiate between distinct populations of resting state and activated microglia in all genotypes (**Figure 3B** and **3C**). Elevated numbers of activated microglia were observed in TAU58/*Trem2*^{+/+} and TAU58/*Trem2*^{Δ/+} mice with a concomitant reduction of resting state microglia (**Figure**

3D). In contrast, TAU58/*Trem2*^{Δ/Δ} exhibited numbers of resting state and activated microglia comparable to *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} mice, suggesting the absence of overt microglia activation. Supporting these findings, quantitative PCR showed increased mRNA levels of selected microglial activation markers (CD16, APOE) and a converse reduction of homeostatic marker genes (Siglech) in TAU58/*Trem2*^{+/+} brains compared to TAU58/*Trem2*^{Δ/+}, and to an even higher degree compared to TAU58/*Trem2*^{Δ/Δ} littermates (Figure 3E). For example, CD16, a surface marker of M1 activated microglia (70), showed a gene dosage-dependent reduction in TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} brains. Conversely, the homeostatic marker Siglech was increased in TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} brains compared to TAU58/*Trem2*^{+/+} mice. Complement factor C4, which was significantly reduced in TAU58/*Trem2*^{+/+} brains compared to *Trem2*^{+/+} controls, reverted to control-like levels in TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice. ApoE, a marker of disease-associated microglia (DAM), which was increased in TAU58/*Trem2*^{+/+} mice, was reduced to control-like levels in TAU58/*Trem2*^{Δ/Δ} brains. Taken together, *Trem2* depletion in TAU58 mice (i.e. TAU58/*Trem2*^{Δ/Δ}) retained microglia in the resting state, while reduction of *Trem2* (i.e. TAU58/*Trem2*^{Δ/+}) showed intermediate microglial gene activation compared to TAU58/*Trem2*^{+/+} littermates.

***Trem2* reduction results in increased tau spreading.** Microglia have been previously shown to contribute to propagation of tau pathology (14–16). To understand the contribution of tau spreading towards the augmentation of behavioural and neuropathology in TAU58/*Trem2*^{Δ/Δ}, we used a previously established adeno-associated virus (AAV) model of *in vivo* tau propagation, AAV1-eGFP-2a-P301L-tau (55,56). Briefly, neurons express eGFP and P301L tau as individual proteins after co-translational cleavage of the peptide chain induced by a viral 2a peptide sequence (donor neurons; GFP⁺/tau⁺). When tau spreading occurs, donor neurons release P301L tau that is then taken up by recipient neurons (GFP⁻/tau⁺), whereas donor neurons retain eGFP (Figure 4A). *Trem2*^{Δ/Δ} and *Trem2*^{+/+} mice were injected with AAV1-eGFP-2a-P301L-tau at 4 weeks of age and analysed 8 weeks post injection. The number of recipient neurons positive for human tau and negative for GFP (GFP⁻/tau⁺) was determined in horizontal brain sections throughout the ipsilateral and contralateral hippocampus, entorhinal cortex and adjacent cortical areas, at 4 different positions along the dorsal-ventral axis of the brain. We found a significant increase in the overall number of GFP⁻/tau⁺ cells averaged across all dorsal-ventral positions for ipsilateral, contralateral and the total brain counts in *Trem2*^{Δ/Δ} mice compared to *Trem2*^{+/+} controls (Figure 4B and 4C, individual dorsal-ventral positions in Figure S4). The number and distribution heatmaps of GFP⁻/tau⁺ neurons illustrate the increased and more widespread spreading of tau in *Trem2*^{Δ/Δ} mice than *Trem2*^{+/+} controls

(Figure 4D). Taken together, the depletion of *Trem2* appears to enhance tau pathology due to increased tau spreading in the brain.

Discussion

In the present study, we show that reducing *Trem2* accelerates phenotypes of P301S tau transgenic TAU58 mice in early disease stages. These deficits were accompanied by increased levels of tau phosphorylated at Serine 422, a late stage pathology marker (71), in young TAU58/*Trem2*^{4/4} mice, as well as by reduced microglial activation compared to TAU58 mice. Using an independent *in vivo* model for tau spreading, we showed that loss of *Trem2* leads to an increased inter-neuronal propagation of pathological tau.

TREM2 limits tau pathology and associated functional deficits. Our study supports the emerging consensus that reduced TREM2 function/levels are associated with increased tau pathology in transgenic mice (46,49,50). Experimental differences (e.g. mouse lines, genetic backgrounds and ages used) may explain the deviating findings of heterozygous *Trem2*-deficient mice augmenting tau phosphorylation in tau transgenic mice in one study, although even in these mice brains were protected from atrophy (48). Recent studies have connected the role of TREM2 in tau pathology to A β , reporting increased tau pathology in the vicinity and/or presence of A β pathology (72,73). Mechanistically, increased tau phosphorylation upon *Trem2* reduction/depletion in tau transgenic models has been linked to increased kinase activity (46). Reports of functional changes induced by *Trem2* reduction were limited to studies using virus-mediated localized knockdown of *Trem2* that exacerbated spatial learning deficits of aged P301S tau transgenic mice (49), while overexpression of TREM2 rescued these deficits (38). Here, we show that *Trem2* reduction accelerated onset of behavioural and motor deficits of TAU58 mice in a gene dosage-dependent manner in early disease stages which tapered out in aged animals.

TREM2 confers disease-related microglial profiles in tau pathology. TREM2 has been implicated in the activation of DAM in mouse models of AD and human disease associated with A β pathology (74). DAM in AD and other disease mouse models have been identified by single-cell mRNA profiling, revealing activation-associated gene signatures distinct from resting/homeostatic microglia (74,75). Here we used CyTOF to profile isolated microglia based on differential surface marker expression (64,66) as a novel and alternative approach to determine cell signatures associated with tau pathology in mice. Since this method has not been applied in tau transgenic mice (or any other AD mouse model), direct comparison to previous reports and mRNA signatures is difficult. However, the DAM marker CD11c (=Itgax), that is upregulated in mRNA signature profiling of A β -forming AD mouse brains (73–75), identified a distinct TREM2-dependent population that is increased in

TAU58 mice. Conversely, P2ry12, which distinguishes resting/homeostatic microglia from other populations (and in particular from DAM) in mRNA signature profiles in tau and APP transgenic mice (73), also distinguished resting/homeostatic from activated/DAM microglia by CyTOF in TAU58 mice. P2ry12 and Cx3cr1 are part of the microglial homeostatic mRNA signature reported to be increased in *Trem2*^{-/-} mice and increased in aging Aβ-forming AD mice (74–76) and are lead markers of resting/homeostatic microglia by CyTOF in our experiments in TAU58 and *Trem2*^{Δ/Δ} mice. While DAM in TAU58/*Trem2*^{Δ/Δ} brains were reduced to the level of littermate controls, DAMs remained high in TAU58/*Trem2*^{Δ/+} brains. In contrast, mRNA quantification showed that microglial marker gene expression in TAU58/*Trem2*^{Δ/+} brains aligned with TAU58/*Trem2*^{Δ/Δ} brains, rather than that of TAU58 mice. This suggests that DAM in TAU58/*Trem2*^{Δ/+} brains may have adopted an intermediate activation state that is not captured by the current microglial CyTOF panel. Nevertheless, we identified *Trem2*-dependent DAM in TAU58 mice using CyTOF.

TREM2 limits the spreading of tau pathology. Recent evidence suggested that TREM2 limits tau pathology progression in an Aβ-dependent context in tau transgenic pR5 mice (73). In fact, Aβ has been shown to enhance tau pathology progression also in other models (77,78). This may explain why Aβ was necessary for Lee et al. (73) to detect tau pathology progression in pR5 mice, which have a low and localized expression of transgenic tau, and show *per se* only slow progression of tau pathology, as shown by us previously (71). Here, using a previously established model for tracing locally expressed human P301L mutant tau in mouse brains (55,56), we show for the first time that TREM2 limits tau protein spreading, notably, independent of Aβ. AAV-mediated human tau expression is expected to be comparable or higher than transgenic human tau expression in TAU58 mice (51,55,56). This suggests that a certain threshold concentration of tau in donor neurons may be required for tau spreading between neurons. During cell-to-cell spreading, tau is released by donor neurons and then taken-up by recipient neurons. Here, increased spreading of tau upon reduction of *Trem2* indicated that *Trem2* itself, or the associated microglia activation state (DAM: TAU58/*Trem2*^{+/+} > TAU58/*Trem2*^{Δ/Δ}) may be needed for efficient clearance of released tau from the extracellular space. Alternatively, the uptake of tau by recipient neurons - and hence spreading - may be enhanced in *Trem2*^{Δ/Δ} mice, for example through a mechanism involving DAM activation as an uptake inhibitor signal for neurons. Interestingly and in line with our findings, tau spreading was increased by the depletion of the TREM2 downstream effector TYROBP/DAP12 in mice (79).

Conclusion

In summary, our data support a disease-limiting function of TREM2 on tau pathology due to directly limiting neuron-to-neuron transmission of tau proteins, decreasing pathological tau phosphorylation, and, therefore, slowing disease progression. These findings support the role of microglia in the propagation of tau pathology (15). Together with TREM2 polymorphisms increasing the risk of frontotemporal dementia with tau pathology (80), our study provides important tau focused support for the potential therapeutic benefit of agonistic TREM2 therapies (81,82) in AD and tau-driven neurodegenerative diseases.

List of abbreviations

A β , Amyloid- β ; AD, Alzheimer's disease; TREM2, triggering receptor expressed on myeloid cells 2; NFT, neurofibrillary tangles; FTD, frontotemporal dementia; LOAD, late onset Alzheimer's disease; ALS, amyotrophic lateral sclerosis; AAV, adeno-associated virus; sgRNAs, single-guide RNAs; KO, knock out; OF, Open Field test; MWM, Morris Water Maze; EPM, Elevated Plus Maze; BB, Blocking buffer; CyTOF, cytometry by time of flight.

Declarations

Ethics approval and consent to participate

All animal experiments were approved by the Animal Ethics Committees of Macquarie University.

Consent for publication

Not applicable.

Availability of data and materials

All data generated and analysed during this study are included in this published article and its supplementary files. The detailed datasets generated during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests related to this study.

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Author's contributions

AFF, CK, YDK and LMI designed experiments. AFF, CA, AT, SG, CK, AvH, JvdH, YD, MP, TB, FB and FD performed experiments. SW and BTH provided essential reagents. CK, YDK and LMI supervised the study. YDK and LMI have obtained funding. AFF, YDK and LMI wrote the first manuscript draft. All authors edited the manuscript.

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Figure Legends

Figure 1 | Reducing *Trem2* accelerated functional deficits in P301S tau transgenic TAU58 mice.

(A) Significant reduction and loss of *Trem2* mRNA expression in brains of respective *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} mice as compared to *Trem2*^{+/+} littermates (n=4; ***, p<0.001; ****, p<0.0001 [ANOVA, Student's t-test]). (B) Schematic of breeding strategy to obtain littermates with different genotypes for this study (colour coding of genotypes apply to all figures and panels). (C) Latency to find the hidden platform during consecutive daily Morris water maze (MWM) acquisition trials (left) and linear regression slopes (middle) in TAU58/*Trem2*^{Δ/Δ} mice compared to all other genotypes (n=9-12; *, p<0.05 [ANOVA, Student's t-test]). Comparable times spent in the target quadrant (Q1) as opposed to other quadrants (Q2-Q4) during MWM probe trials for all genotypes tested. (D) Latency to fall off an accelerating rotating rod for TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice and *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} littermate controls at indicated ages (n=10-29; *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001 [ANOVA, Student's t-test]) (E) Time to cross a narrow beam and number of foot slips during the crossing for TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice and *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} littermate controls at indicated ages (1 and 2 months top row, 3 and 6 months bottom row) (1 and 2 months n=10-14, 3 and 6 months n=3-19; *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001 [ANOVA, Student's t-test]). (F) Proportion of mice that fail to cross the challenging beam during one or both test trials at 3 and 6 months of age comparing TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice. (G) Time spent in the open arms of the elevated plus maze for TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice and *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} littermate controls at indicated ages (1 and 2 months n=9-14, 3 and 6 months n=5-20; *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001 [ANOVA, Student's t-test]).

Figure 2 | Augmented late-stage tau phosphorylation upon *Trem2* reduction in P301S tau transgenic TAU58 mice.

(A) Representative immunofluorescence staining with an antibody specific for phosphorylated Serine 214 (pS214) of tau in TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} brains and *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} littermate controls at indicated ages. Nuclei are stained with DAPI (blue). Graphs show stereological quantification of pS214-stained brain sections in these brains at indicated ages (n=4-6; *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001 [ANOVA]). (B) Representative immunofluorescence staining with an antibody specific for late-stage phosphorylated Serine 422 (pS422) of tau in TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and

TAU58/*Trem2*^{Δ/Δ} brains at indicated ages. Nuclei are stained with DAPI (blue). Graphs show stereological quantification of pS422-stained brain sections in these brains at indicated ages (n=4-6; *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001 [ANOVA]). No pS422 staining was found in *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} littermate controls at all ages. Scale bars, 100μm.

Figure 3 | Trem2 reduction diminished microglia activation in P301S tau transgenic TAU58 mice.

(A) Representative immunofluorescence images of hippocampus and motor cortex from TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice co-stained with antibodies to microglial Iba1 (green) and pS396/pS401 tau (PHF-1, red). Arrowheads indicate Iba1-positive cells in the proximity of pS396/pS401-stained neurons. Scale bar, 20μm. Graphs show quantification of Iba1+ microglia in the proximity of pS396/pS401 tau+ neurons (n=6; *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001 [ANOVA]). (B) UMAP-graph of distinct clusters of homeostatic/resting and activated/DAM microglia by CyTOF of single-cell extracts from brains of TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice and *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} littermate controls. (C) Corresponding heat map of marker expression showed clustering of homeostatic/resting and activated/DAM microglia (D) Comparison of relative abundance of homeostatic/resting and activated/DAM microglia in TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice and *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} littermate controls (n=3-5; *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001 [ANOVA]). (E) Quantitative real-time PCR of *SiglecH*, *CD16*, *C4* and *ApoE* mRNA in hippocampal extracts from TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice and *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} littermate controls (n=2-4; *, p<0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001 [ANOVA, Student's *t*-test]).

Figure 4 | Trem2 depletion increased spreading of pathological tau.

(A) Schematic of tau spreading mouse model; Unilateral stereotaxic delivery of adeno-associated virus (AAV) for neuronal co-expression of green fluorescence protein (GFP) and human P301L mutant tau into the entorhinal cortex of 4 week-old *Trem2*^{+/+} and *Trem2*^{Δ/Δ} mice. When spreading occurs, GFP⁺/tau⁺ donor neurons release tau which is taken up by GFP⁻/tau⁺ recipient neurons. (B) Representative immunofluorescent staining for GFP (green) and human tau (red) in ipsilateral and contralateral hippocampi of *Trem2*^{+/+} and *Trem2*^{Δ/Δ} mice. Arrowheads indicate recipient neurons (GFP⁻/tau⁺) in close-up images of the area delineated by broken boxes in hippocampus overview images. Scale bars, 200μm and 20μm respectively. (C) Stereological quantification of recipient neurons (GFP⁻/tau⁺) in serial sections of *Trem2*^{+/+} and *Trem2*^{Δ/Δ} mice,

for ipsilateral, contralateral and total brain (n=9-10; *, p<0.05; **, p<0.01; [Student's *t*-test]). (D) Heat maps of tau spreading at distinct levels in the brains of *Trem2*^{+/+} and *Trem2*^{Δ/Δ} mice (n=9-10). The asterisk indicates the injection site.

Supplementary Figure Legends

Figure S1 | Generation of the *Trem2*^{Δ/Δ} mouse line.

(A) CRISPR targeting strategy to introduce a deletion (Δ) into exon 1 of the murine *Trem2* locus, comprising the transcriptional start codon. Arrows indicate position of genotyping primers for identification of mutant alleles. (B) Genome sequencing confirmed a 92bp deletion in *Trem2*^{Δ/Δ} mice. Boxes outline position of guide RNAs used for CRISPR/Cas9 genome editing projected on the unmodified *Trem2* sequence, highlighting the transcriptional start codon. (C) Example of genotyping PCR showing the mutant band at around 500bp and wild-type band at 600bp in *Trem2*^{Δ/Δ} (lane 1), *Trem2*^{Δ/+} (lane 2) and *Trem2*^{+/+} (lane 3) mice.

Figure S2 | Unaffected locomotor activity in TAU58/*Trem2*^{Δ/Δ} mice.

(A) Distance travelled during elevated plus maze (EPM) for TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice and *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} littermate controls. (B) Time in inner zone and distance travelled during open field testing for TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} mice and *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} littermate controls.

Figure S3 | Tau phosphorylation and microglia distribution in TAU58/*Trem2*^{Δ/Δ} mice

(A) Representative immunofluorescence staining with an antibody specific for phosphorylated Serine 214 (pS214; green) of tau in the cortex of TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} brains and *Trem2*^{+/+}, *Trem2*^{Δ/+} and *Trem2*^{Δ/Δ} littermate controls at 2 months of age. Nuclei are stained with DAPI (blue). Graph shows stereological quantification of pS214-stained brain sections in these brains (n=4-6; ***, p<0.001; ****, p<0.0001 [ANOVA]). (B) Representative immunofluorescence staining with an antibody specific for phosphorylated Serine 422 (pS422; green) of tau in the cortex of TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+} and TAU58/*Trem2*^{Δ/Δ} brains at 2 months of age. Nuclei are stained with DAPI (blue). Graph shows stereological quantification of pS214-stained brain sections in these brains (n=4-6; ***, p<0.001; ****, p<0.0001 [ANOVA]). (C) Representative immunofluorescence staining with an antibody specific for microglia Iba1 (green) and human tau (red) co-stained for nuclear DAPI (blue) of tau in TAU58/*Trem2*^{+/+}, TAU58/*Trem2*^{Δ/+}

and TAU58/*Trem2^{Δ/Δ}* brains and *Trem2^{+/+}*, *Trem2^{Δ/+}* and *Trem2^{Δ/Δ}* littermate controls at indicated ages. Graphs show stereological quantification of microglial density in brain sections at indicated ages (n=4-6; ns, not significant [ANOVA]).

Figure S4 | *Trem2* depletion increased spreading of pathological tau

Representative immunofluorescent staining for GFP (green) and human tau (red) in ipsilateral and contralateral hippocampi of *Trem2^{+/+}* and *Trem2^{Δ/Δ}* mice at different depths relative to the Bregma covering the ventral, medial and dorsal hippocampus. Arrowheads indicate recipient neurons (GFP⁻/tau⁺) in close-up images of the area delineated by broken boxes in hippocampus overview images. Scale bars, 200μm and 20μm respectively.

Figure 1

Figure 2

A **B** **C** **D**

Figure 4

A **B** **C**

Supplementary Figure 1

A **B**

Supplementary Figure 2

Supplementary
Figure 3

Supplementary Figure 4

Table S1

Animals used in behaviour tests

Genotype	Test		RR				Beam				EPM				OF				MWM
	N	Age (months)	1	2	3	6	1	2	3	6	1	2	3	6	1	2	3	6	3
TAU58/ <i>Trem2</i> ^{Δ/Δ}	total		10	12	10	11	10	12	9	6	9	10	7	8	10	10	10	11	10
	M		5	6	5	9	5	6	4	4	5	7	4	2	5	6	5	9	5
	F		5	6	5	2	5	6	5	2	4	3	3	6	5	4	5	2	5
<i>Trem2</i> ^{Δ/Δ}	total		11	10	11	14	11	10	11	8	11	10	11	10	11	10	10	14	10
	M		4	4	5	9	4	4	5	5	4	4	5	7	4	4	5	9	5
	F		7	6	6	5	7	6	6	3	7	6	6	3	7	6	5	5	5
TAU58/ <i>Trem2</i> ^{Δ/+}	total		14	13	13	12	14	12	10	3	14	12	10	7	14	9	13	12	11
	M		8	11	7	8	8	10	4	1	8	10	4	5	8	8	7	8	6
	F		6	2	6	4	6	2	6	2	6	2	6	2	6	1	6	4	5
<i>Trem2</i> ^{Δ/+}	total		13	13	10	29	13	13	10	16	12	12	8	20	13	12	10	29	8
	M		6	4	4	20	6	4	4	14	6	3	3	16	6	3	4	20	2
	F		7	9	6	9	7	9	6	2	6	9	5	4	7	9	6	9	6
TAU58/ <i>Trem2</i> ^{+/+}	total		10	10	11	12	10	10	11	7	10	9	10	9	10	9	11	12	10
	M		2	4	3	5	2	4	3	1	2	4	2	4	2	4	3	5	5
	F		8	6	8	7	8	6	8	6	8	5	8	5	8	5	8	7	5
<i>Trem2</i> ^{+/+}	total		11	11	19	16	11	11	19	7	11	11	15	13	11	11	19	15	14
	M		4	5	8	9	4	5	8	3	4	5	5	7	4	5	8	9	6
	F		7	6	11	7	7	6	11	7	7	6	10	6	7	6	11	6	8

Table S2

Antibodies used for histology

Antibody	Company	Dilution
pS214	Abcam	1/1000
pS422	Abcam	1/500
Gfap	Sigma	1/200
Iba-1	Wako	1/250
Tau13	Abcam	1/500
PHF-1	gift from P. Davies	1/250
GFP rbt	Abcam	1/100
GFP goat	Abcam	1/200
DAPI	Molecular Probes	1/1000
AlexaFluor mse 555	Invitrogen	1/250
AlexaFluor rbt 488	Invitrogen	1/250
AlexaFluor rbt 647	Invitrogen	1/250

Table S3

Primers list

Gene name	Forward	Reverse
Siglech	AAGAGTTGGTCCAGGGAAACC	GCCTGATCCATGTGTGTTGC
CD16	CCCTGATATCAAGTCGCGCA	TTCTCAAGCAGCGAGACAGG
C4	CAGGACAGAACAGTGGAGCA	ATCTTCAGCACGAAGGCAGT
ApoE	CTCCCAAGTCACACAAGAAGTCTG	CCAGCTCCTTTTTGTAAGCCTTT
Trem2	ACAGCACCTCCAGGAATCAAG	ACTTGCTCAGGAGAACGCAG

Table S4

Antibody panel CyTOF

Antigen	Metals	Clone	Vendor	Cell type	Description
Lineage markers					
B220	Nd144	RA3.3A1	Fluidigm	B cells, pDC	lineage/ signal transduction in hematopoiesis
Ly6G	Pr141	1A8	Fluidigm	neutrophils, eosinophil subset	migration
Siglec-H	Gd158	551	BioLegend	microglia, pDC	reduces type I IFN response
CD45.2	Sm147	30-F11	Fluidigm	pan-leukocytes	TCR and BCR signal transduction complex
CD11b	Nd148	M1/70	Fluidigm	myeloid cells, NK cells	complement receptor 3
Ly6C	Nd150	HK1.4	Fluidigm	myeloid cells	urokinase-type plasminogen activator receptor (uPAR)
CD3	Sm152	145-2C11	Fluidigm	T cells	T cell co-receptor
Ter119	Sm154	TER-119	Fluidigm	erythrocytes	glcophorin-A associated protein
Siglec-F	Nd145	E50-2440	BD	eosinophiles, alveolar macrophages	cell adhesion
CD64	Eu151	X54-5/7.1	Fluidigm	macrophages, monocytes	Igh affinity IgG receptor
NK1.1	Ho165	PK136	Fluidigm	NK cells	CD161, killer cell lectin-like receptor
CD11c	Bi209	N418	Fluidigm	DC	cell - cell contact
F4/80	Tb159	BM8	Fluidigm	macrophages, microglia	cell adhesion, cell activation
CD117	Er166	2B8	Fluidigm	stem cells, lymphocytes	c-kit
Immune modulation					
CD88	Gd160	20/70	BioLegend	granulocytes, monocytes, macrophages	complement receptor 5
CD14	Gd156	Sa14-2	Fluidigm	macrophages	LPS co-receptor
Sca-1	Tm169	D7	Fluidigm	stem cells, lymphocytes	stem cell antigen 1
CD206	Er168	CO8C2	BioLegend	macrophages, DC	PAM
C3aR	Dy161	14D4	HyCult	myeloid and microglia	complement receptor
CD36	Nd146	HM36	BioLegend	monocytes	scavenger receptor
Migration & Differentiation					
CCR2	Dy163	475301	R&D Systems	monocytes, macrophages, immature B cells	CCL2 receptor, anti-APC
CX3CR1	Dy164	SA011F11	Fluidigm	macrophages, monocytes	migration
CD16/32	Er167	93	BioLegend	myeloid cells, Nk cells	low affinity IgG receptor
PD-L1	Eu153	10F.9G2	BioXcell	multiple cells	co-inhibitor, activated cells
CD44	Yb171	IM7	Fluidigm	most cell types, memory and activated T cells	homing, adhesion
CD86	Yb172	GL-1	Fluidigm	antigen presenting cells	co-stimulation ligand
CD172	Yb173	P84	BioLegend	myeloid cells, stem cells, neurons	SIRP1a

MHC2	Yb174	M5/114.15.2	Fluidigm	antigen presenting cells	antigen presentation
CD38	Lu175	90	Fluidigm	macrophages, B cells, monocytes, lymphocytes, neurons	metabolism, differentiation
FcER1a	Yb176	Mar-01	Fluidigm	mast cells, basophils, monocytes, DC	High affinity IgE receptor
CD163	Er170	S150491	BioLegend	myeloid cells, microglia	scavenger receptor
Microglia					
MerTK	Nd143	polyclonal	R&D Systems	macrophages	multifunctional, anti-biotin
P2X7R	Nd142	1F11	BioLegend	macrophages, microglia	binding of extracellular adenosine 5'-triphosphate (ATP)
P2Y12R	Dy162	S16007D	BioLegend	macrophages, microglia	binding of extracellular adenosine 5'-triphosphate (ATP)

Other reagents CyTOF

Term	Metals	Target	Vendor	Cell type	Description
BC106	Cd106	CD45.2 (104.2)	BioXcell	all immune cells and microglia	technical-barcoding reagents
BC110	Cd110	CD45.2 (104.2)	BioXcell	all immune cells and microglia	technical-barcoding reagents
BC111	Cd111	CD45.2 (104.2)	BioXcell	all immune cells and microglia	technical-barcoding reagents
BC112	Cd112	CD45.2 (104.2)	BioXcell	all immune cells and microglia	technical-barcoding reagents
BC113	Cd113	CD45.2 (104.2)	BioXcell	all immune cells and microglia	technical-barcoding reagents
BC114	Cd114	CD45.2 (104.2)	BioXcell	all immune cells and microglia	technical-barcoding reagents
BC116	Cd116	CD45.2 (104.2)	BioXcell	all immune cells and microglia	technical-barcoding reagents
dead	Rh103	DNA, extracellular	Fluidigm	all cells	technical-live/dead stain
DNA1	Ir191	DNA, intracellular	Fluidigm	all cells	technical-cellular marker
DNA2	Ir193	DNA, intracellular	Fluidigm	all cells	technical-cellular marker