Research Articles: Neurobiology of Disease

Trem2 deletion reduces late-stage amyloid plaque accumulation, elevates the Aβ42:Aβ40 ratio, and exacerbates axonal dystrophy and dendritic spine loss in the PS2APP Alzheimer's mouse model

https://doi.org/10.1523/JNEUROSCI.1871-19.2019

Cite as: J. Neurosci 2020; 10.1523/JNEUROSCI.1871-19.2019
Received: 1 August 2019
Revised: 8 December 2019
Accepted: 23 December 2019
Title: Trem2 deletion reduces late-stage amyloid plaque accumulation, elevates the Aβ42:Aβ40 ratio, and exacerbates axonal dystrophy and dendritic spine loss in the PS2APP Alzheimer’s mouse model

Abbreviated Title: Trem2 mitigates Aβ neurotoxicity in PS2APP mice

Authors: William J. Meilandt1*, Hai Ngu2, Alvin Gogineni3, Guita Lalehzadeh1, Seung-Hye Lee1, Karpagam Srinivasan1, Jose Imperio1, Tiffany Wu1, Martin Weber1, Agatha J. Kruse3, Kimberly L. Stark1, Pamela Chan5, Mandy Kwong5, Zora Modrusan6, Brad A. Friedman4, Justin Elstrott3, Oded Foreman2, Amy Easton1, Morgan Sheng1, David V. Hansen1*

Departments of 1Neuroscience, 2Pathology, 3Biomedical Imaging, 4Bioinformatics, 5Biochemical and Cellular Pharmacology, and 6Molecular Biology, Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080 U.S.A.

* Correspondence: hansen.david@gene.com, meilandt.william@gene.com

Number of pages: 43
Number of figures: 13 (9 Main, 5 Extended Data)
Number of tables: 1 (Extended Data Figure 2-1)
Number of words (Abstract): 192
Number of words (Introduction): 618
Number of words (Discussion): 909

Conflict of interest statement: All authors are or were employees of Genentech, Inc., with interests in developing novel therapeutics for neurodegenerative diseases at the time of performing work for this manuscript.

Acknowledgments: We thank Melissa Gonzales Edick and Joanna Yung for imaging support; members of the Genentech animal care staff, FACS lab, and RNA sequencing lab for other research support; and Chris Bohlen for helpful critique of the manuscript. Karpagam Srinivasan’s current affiliation is Alector, South San Francisco, CA 94080. AJ Kruse’s current affiliation is Univ. Washington, Seattle, WA 98195. Morgan Sheng’s current affiliation is the Broad Institute of MIT and Harvard, Cambridge, MA 02142.
**ABSTRACT**

*TREM2* is an Alzheimer’s disease (AD) risk gene expressed in microglia. To study the role of *Trem2* in a mouse model of β-amyloidosis, we compared PS2APP transgenic mice versus PS2APP mice lacking *Trem2* (PS2APP;*Trem2*ko) at ages ranging from 4 to 22 months. Microgliosis was impaired in PS2APP;*Trem2*ko mice, with *Trem2*-deficient microglia showing compromised expression of proliferation/Wnt-related genes and marked accumulation of ApoE. Plaque abundance was elevated in PS2APP;*Trem2*ko females at 6-7 months, but by 12 or 19-22 months of age it was notably diminished in female and male PS2APP;*Trem2*ko mice, respectively. Across all ages, plaque morphology was more diffuse in PS2APP;*Trem2*ko brains, and the Aβ42:Aβ40 ratio was elevated. The amount of soluble, fibrillar Aβ oligomers also increased in PS2APP;*Trem2*ko hippocampi. Associated with these changes, axonal dystrophy was exacerbated from 6-7 months onward in PS2APP;*Trem2*ko mice, notwithstanding the reduced plaque load at later ages. PS2APP;*Trem2*ko mice also exhibited more dendritic spine loss around plaque and more neurofilament light chain in cerebrospinal fluid. Thus, aggravated neuritic dystrophy is a more consistent outcome of *Trem2* deficiency than amyloid plaque load, suggesting that the microglial packing of Aβ into dense plaque is an important neuroprotective activity.

**Significance statement**

Genetic studies indicate that *TREM2* gene mutations confer increased Alzheimer’s disease (AD) risk. We studied the effects of *Trem2* deletion in the PS2APP mouse AD model, in which overproduction of Aβ peptide leads to amyloid plaque formation and associated neuritic dystrophy. Interestingly, neuritic dystrophies were intensified in the brains of *Trem2*-deficient mice, despite these mice displaying reduced plaque accumulation at later ages (12-22 months). Microglial clustering around plaques was impaired, plaques were more diffuse, and the Aβ42:Aβ40 ratio and amount of soluble, fibrillar Aβ oligomers were elevated in *Trem2*-deficient brains. These results suggest that the *Trem2*-dependent compaction of Aβ into dense plaques is a protective microglial activity, limiting the exposure of neurons to toxic Aβ species.
INTRODUCTION

Since the discovery of TREM2 (triggering receptor expressed on myeloid cells 2) variants as genetic risk factors for Alzheimer’s disease (AD) (Guerreiro et al., 2013; Jonsson et al., 2013), TREM2 biology has become a focal point in research efforts to better understand how the innate immune system impacts Alzheimer’s and other neurodegenerative diseases (Jay et al., 2017b; Ulrich et al., 2017; Yeh et al., 2017). However, whether Trem2 exerts protective or detrimental functions in mouse models of AD-related neuropathology has been rather unclear (Gratuze et al., 2018; Hansen et al., 2018; Ulland and Colonna, 2018).

In transgenic models of cerebral β-amyloidosis, plaque load has been reportedly increased, decreased, or unchanged in mice that lack Trem2, depending on the model, age, and brain region being analyzed (Jay et al., 2017a; Jay et al., 2015; Parhizkar et al., 2019; Wang et al., 2015; Wang et al., 2016; Yuan et al., 2016). Trem2 deletion in amyloidosis models has also been reported to either increase or decrease phosphorylation of the endogenous tau protein (Jay et al., 2015; Wang et al., 2016). Similarly, studies in neurodegeneration models driven by transgenic expression of the human tau protein have suggested disparate roles of Trem2. In the hTau model (Andorfer et al., 2003), Trem2 deletion increased the amounts of tau phosphorylation and aggregation detected (Bemiller et al., 2017). In contrast, in the PS19 model (Yoshiyama et al., 2007) Trem2 deletion had a protective effect, preventing tau-driven synaptic loss and atrophy in the hippocampus and entorhinal cortex, respectively (Leyns et al., 2017).

Transcriptional profiling studies have defined a disease/damage-associated microglial (DAM) activation state that is commonly observed in the brains of neurodegeneration models (Deczkowska et al., 2018; Friedman et al., 2018). The acquisition of the DAM state is Trem2-dependent in mouse models of AD, amyotrophic lateral sclerosis, and demyelinating disease (Keren-Shaul et al., 2017; Krasemann et al., 2017; Poliani et al., 2015; Wang et al., 2015). Some have argued that this state of microglial activation or
alarm is fundamentally protective (Keren-Shaul et al., 2017), while others have argued that this state is damaging and that returning microglia to their normal "homeostatic" state would be beneficial (Krasemann et al., 2017). It is conceivable that microglial TREM2 activity may be either protective or detrimental, depending on the disease stage and types of pathology present.

To clarify the role of Trem2 in β-amyloid-driven AD models, we studied the effects of Trem2 deletion on microglial activation, plaque accumulation, and neuronal pathology in the PS2APP model across a wide range of ages and in both sexes. PS2APP mice develop amyloid plaque and attendant gliosis pathologies that increase with age, with female mice accumulating the pathology more rapidly than males (Ozmen et al., 2009). Here we report that the effects of Trem2 deficiency on plaque load varied with age and sex, but notably, plaque accumulation was reduced at older ages in both female and male Trem2 knockout (PS2APP;Trem2<sup>ko</sup>) mice compared to age-matched Trem2 wild type (PS2APP;Trem2<sup>wt</sup>) mice. We consistently observed that the Aβ<sub>42</sub>:Aβ<sub>40</sub> ratio was elevated, plaque morphology was more diffuse, and neuritic dystrophy histopathology was more marked in PS2APP;Trem2<sup>ko</sup> mice, even at older ages when total plaque was reduced. Additional analyses of PS2APP;Trem2<sup>ko</sup> mice at the 12-month age revealed ApoE-laden microglia, increased levels of soluble fibrillar oligomeric Aβ, and elevated neurofilament-L in the CSF. By RNAseq, we observed that proliferation-related transcripts in PS2APP;Trem2<sup>ko</sup> microglia were reduced, particularly those encoding certain components and regulators of Wnt-related signaling. Collectively, our data indicate that Trem2-dependent microglial proliferation and activation attenuate the toxic effects of Aβ toward neurons—i.e., the DAM state is mainly protective—and that measurements of neuronal pathology are more informative than plaque load as readouts of microglial modulation in models of β-amyloidosis.
MATERIALS AND METHODS

Animals. All animal care and handling procedures were reviewed and approved by the Genentech IACUC and were conducted in full compliance with regulatory statutes, IACUC policies and NIH guidelines. Animals were housed in SPF (specific pathogen-free) conditions with 12h light/12h dark/day and maintained on regular chow diets. The Trem2<sup>tm1(KOMP)Vlcg</sup> null allele (C57BL/6N background) was crossed into the PS2APP model (C57BL/6J background). To generate experimental cohorts, all breeding mice were homozygous carriers of the PS2APP transgene and heterozygous carriers of the Trem2-null allele to allow maximal use of littermates between Trem2 wild type (Trem2<sup>wt</sup>) and Trem2 knockout (Trem2<sup>ko</sup>) PS2APP groups. We designed our study to analyze female cohorts at early (4 months), intermediate (6-7 months), and late (12 months) stages of pathology. We also analyzed males at the age of 6-7 months—a common age for us to examine PS2APP histopathology—to check whether any observed effects of Trem2 deletion were sex-specific. The 4-month and 6-7 month cohorts were processed together; the 12 month and 19-22 month cohorts were separate batches. When we observed less plaque in 12mo PS2APP;Trem2<sup>ko</sup> females, we decided to also analyze their still aging male counterparts to learn whether that effect was reproducible. (By that time, the numbers of aged males were somewhat depleted so we included available Trem2<sup>het</sup> mice to round out the analysis.) For dendritic spine analysis, animals also carrying the Thy1:GFP-M transgene (Jackson stock 007788) were used.

Flow cytometry and fluorescence activated cell sorting (FACS). Animals were anesthetized with ketamine/xylazine and transcardially perfused with 30 ml of ice-cold PBS prior to dissection of cortex + hippocampus. Care was taken to remove the choroid plexus and as much of the meninges as possible prior to dissociation and sorting. Tissues were dissociated and cell suspensions prepared as described previously (Srinivasan et al., 2016). All steps were carried out on ice or at 4°C to prevent artifactual microglial activation. For quantifying CD45 immunoreactivity and collecting brain-resident myeloid cells by FACS, cell suspensions from seven PS2APP;Trem2<sup>wt</sup> (5 females, 2 males) and seven PS2APP;Trem2<sup>ko</sup> (6 females, 1 male) mice at 14-15 months of age were stained with the following antibodies in Hibernate-A medium for 20 minutes at 4°C on a rotator: APC-conjugated anti-CD11b (BD Biosciences 561690, 1:200), PE/Cy7-conjugated anti-CD45 (BD Biosciences 552848, 1:500), FITC-conjugated anti-Ly6g (Tonbo Biosciences 35-5931, 1:200), and PE-conjugated anti-Ccr2 (R&D Systems FAB5538P, 1:200). Samples were briefly washed and stained with DAPI prior to FACS sorting. Myeloid cells were selected by gating live (DAPI-negative) cells for CD11b and CD45 immunoreactivity. To avoid the presence of peripheral myeloid cells in the flow analysis and FACS collections, Ccr2<sup>+</sup> cells (peripheral monocytes/macrophages) and Ly6g<sup>+</sup> cells (neutrophils) were excluded. The total population of brain-resident myeloid cells (defined as CD11b<sup>+</sup>CD45<sup>+</sup>Ccr2<sup>−</sup>Ly6g<sup>−</sup>), consisting almost entirely of microglia but including CD45<sup>high</sup> perivascular macrophages (~2% of total), from each sample was collected in Hibernate-A. Collected cells were pelleted at 5,000 rcf for 8 minutes, and RNA was extracted from cell pellets using Qiagen RNeasy Micro kits. To measure CD45...
immunoreactivity of brain myeloid populations during FACS, CD45\textsuperscript{low} and CD45\textsuperscript{high} gates were defined using a non-transgenic animal as a control to identify microglia in their normal state (CD45\textsuperscript{low}). The same gates were transposed onto cells from PS2APP animals sorted on the same day in the same machine to ensure accuracy and consistency in determining percentages of CD45\textsuperscript{low} and CD45\textsuperscript{high} populations. Generally, animal pairs including one PS2APP;Trem2\textsuperscript{wt} and one PS2APP;Trem2\textsuperscript{ko} animal were processed together from perfusion through sorting. Data for non-transgenic animals in Fig. 1F includes the non-transgenic animals used to define the CD45 gates used during PS2APP cell sorting, as well as additional age-matched control animals from another cohort processed at another time (due to animal availability constraints).

For measuring microglial cells with \(\beta\)-amyloid content, animals at \(\sim\)12 months (n=3 PS2APP;Trem2\textsuperscript{wt} and 2 PS2APP;Trem2\textsuperscript{ko}) were intraperitoneally injected with methoxy-X04 (10 mg/kg) 24 hours before tissue collection. Animals were processed as described above and dissociated cells were stained for CD11b. Cells were also incubated with Calcein-AM (eBioscience 65-0853-39, 1:1000) just prior to flow cytometry to label live cells using the 488 nm excitation channel. Live CD11b\textsuperscript{+} cells were gated as X04\textsuperscript{+} or X04\textsuperscript{–} using the DAPI excitation channel to determine the percentage of microglia with ingested amyloid content. Transgenic animals injected with PBS were also used as negative controls (data not shown).

RNA sequencing, differential expression and gene set analysis.

RNA samples from sorted brain myeloid cells of seven PS2APP;Trem2\textsuperscript{wt} (5 females, 2 males) and six PS2APP;Trem2\textsuperscript{ko} (5 females, 1 male) mice (14-15 months old) were selected for sequencing. The concentration of RNA samples was determined using DS-11 spectrophotometer (DeNovix) and the integrity of RNA was determined by 2100 Bioanalyzer (Agilent Technologies). Approximately 1-5 ng of total RNA was used as an input material for the library generation using SMART-seq v4 Ultra Low Input RNA kit (Clontech). Size of the libraries was confirmed using 4200 TapeStation and High Sensitivity D1K screen tape (Agilent Technologies) and their concentration was determined by qPCR-based method using KAPA Library Quantification Kit. The libraries were multiplexed and then sequenced on HiSeq4000 (Illumina) to generate 30M of single-end 50 bp reads. Sorted cell RNA-Seq data were analyzed as described (Srinivasan et al., 2016). Briefly, Illumina adapters, low-quality sequences, and rRNA reads were first discarded. Remaining reads were aligned to the GRCm38 genome with GSNAP aligner (Wu et al., 2016) and reads overlapping each gene were quantified.

Normalization was based on the nRPKM method, which is proportional to size factor normalization of DESeq (Love et al., 2014). Differential expression was performed using voom+limma (Law et al., 2014). Raw RNA-Seq data have been deposited to NCBI GEO under accession number GSE140744.

Heat maps in Fig. 2A and Fig. 2-3 were generated using gene sets of interest to compare transcriptional responses in multiple datasets. Z-scores were calculated as follows. First, log2-scale expression matrices were calculated as \(\text{max}(\log2(\text{nRPKM}), -4)\). Then, each gene was centered and scaled to give Z scores: for a given gene/sample combination, the Z score represents distance of nRPKM value in standard deviations from the mean log2-scale expression value for that gene across all samples within a dataset. Rows (genes) were
organized hierarchically using the Euclidean distance function. Columns (sorted microglia samples) were organized by project and genotype.

Gene ontology (GO) query was submitted on the PANTHER Classification System version 14.1 (Mi et al., 2019) at the website (http://pantherdb.org/) using the following inputs: Gene list = genes differentially expressed between Trem2<sup>ko</sup> and Trem2<sup>wt</sup> PS2APP microglia (fold change ≥ 2, adj. p value ≤ 0.05); Organism = <i>Mus musculus</i>; Analysis = statistical overrepresentation test; Annotation set = GO biological process complete; Reference list = <i>Mus musculus</i> whole genome genes; Test type = Fisher’s exact; Correction = Calculate false discovery rate.

For gene set enrichment analyses in Fig. 2D and Fig. 2-2, each sample was assigned a gene set score using log<sub>2</sub> (nRPKM) values for each gene in the set. A sample’s gene set score reflected the average difference, for all genes in the set, between that sample’s measured log<sub>2</sub> (nRPKM) value for a given gene and the average log<sub>2</sub> (nRPKM) value for the same gene across all samples. In cases when one or more samples had no transcripts detected for a given gene, an imputed log<sub>2</sub> (nRPKM) value was assigned equal to one log<sub>2</sub> step below the lowest log<sub>2</sub> (nRPKM) value detected for that gene in that sample set. We compared gene set scores between genotype groups using two-tailed t tests assuming unequal variance between groups.

Sectioning, histological and immunological staining.

Single-sex cohorts of animals used for histological and biochemical analyses included 4-month (4mo) females (n=11 non-transgenic Trem2<sup>wt</sup>, 14 non-transgenic Trem2<sup>ko</sup>, 10 PS2APP;Trem2<sup>wt</sup>, and 12 PS2APP;Trem2<sup>ko</sup>), 6-7mo females (n=11 PS2APP;Trem2<sup>wt</sup> and 13 PS2APP;Trem2<sup>ko</sup>), 6-7mo males (n=16 PS2APP;Trem2<sup>wt</sup> and 14 PS2APP;Trem2<sup>ko</sup>), 12mo females (n=15 PS2APP;Trem2<sup>wt</sup> and 15 PS2APP;Trem2<sup>ko</sup>), and 19-22mo males (n=12 PS2APP;Trem2<sup>wt</sup>, 8 PS2APP;Trem2<sup>ko</sup> and 7 PS2APP;Trem2<sup>het</sup>). Animals were deeply anesthetized with 2.5% tribromoethanol (0.5 ml/25 g body weight) and transcardially perfused with PBS. One brain hemisphere was drop-fixed in 4% PFA for two days at 4ºC with agitation and then transferred to PBS for histopathological analyses. The other hemisphere was subdissected into cortical and hippocampal tissues that were frozen and stored at -80ºC for biochemical assays. Immersion-fixed hemi-brains were cryoprotected, embedded up to 40 per block in a solid matrix, and coronally sectioned at 35 μm (MultiBrain processing by Neuroscience Associates) as previously described (Kallop et al., 2014; Wang et al., 2011). Sheets of sections were stored in cryoprotectant (30% glycerol, 30% ethylene glycol in PBS) at -20ºC until use.

Immunohistochemical (IHC) stains for Iba1, CD68, and Gfap were performed at Neuroscience Associates as described previously (Wang et al., 2011), and CD68-stained sections were counterstained with Nissl (0.05% thionine/0.08 M acetate buffer, pH 4.5). Silver stains for amyloid plaque (Campbell-Switzer stain (Switzer et al., 1993)) and neuronal damage/degeneration (Amino Cupric Silver (AminoCuAg) or “Disintegrative Degeneration” stain (de Olmos et al., 1994)) were also performed at Neuroscience Associates. The bases for these silver stains are reviewed in (Switzer, 2000) and described on the Neuroscience Associates website (https://www.neuroscienceassociates.com/technologies/staining/). IHC and silver stains
spanned a broad rostral-caudal range including eight to eleven sections per animal. Stained slides were returned to Genentech for imaging and quantitation, and unused sections were also returned to Genentech for cryoprotected storage until used for additional stains.

For X-34 stains, sheets were mounted onto slides and completely dried. Slides were incubated with 10 μM X-34 in PBS containing 40% ethanol and 0.02 N NaOH for 10 min, followed by 3 quick washes in PBS, differentiation in 80% ethanol for 1 min, and additional 3 quick PBS washes. After applying ProLong Diamond Antifade Mountant (ThermoFisher P36961), slides were covered with no.1 coverslips. Two sections per animal were stained, with all cohorts stained and analyzed simultaneously.

For co-staining of plaque, microglia, and ApoE or dystrophic axons, sheets encompassing 2-3 sections per animal containing regions of the rostral and caudal hippocampus were washed in PBS and then PBS plus Triton X-100 (PBST, 0.1%) and then blocked in PBST (0.3%) with 5% bovine serum albumin (BSA) and 5% normal donkey serum, then incubated overnight with primary antibodies diluted in PBST (0.3%) plus 1% BSA at 4°C. Microglia were labeled with rabbit anti-Iba1 (Wako 019-19741, 1:1000) or goat anti-Iba1 (Abcam ab5076, 1:1000), ApoE with a rabbit monoclonal antibody (Abcam ab183597, 1:4000), and dystrophic neurites with rat anti-Lamp1 (Abcam ab25245, 1:2000). Primary antibody incubation was followed by three 10-minute washes in PBST, followed by incubation with secondary antibodies for 2 hours at room temperature. Donkey anti-rabbit IgG-Alexa555, anti-rat IgG-Alexa647, and anti-goat IgG-Alexa647 (Thermo Fisher, 1:500) were used as secondary detection reagents. Following the stain, tissue sheets went through three 10-minute washes in PBST (0.1%) and three quick washes in PBS. Sheets were mounted onto slides with 0.1% gelatin in PBS and allowed to dry and adhere to the slide at room temperature. To label plaque, slides were then incubated with 10 μM methoxy-X04 in 40% ethanol in PBS for 10 min, washed briefly in PBS, differentiated in 0.2% NaOH in 80% ethanol for 2 min, washed and then allowed to dry. Slides were coverslipped with added ProLong™ Gold Antifade Mountant (Thermo Fisher P36961). All cohorts were stained and analyzed simultaneously.

Imaging and quantitation of stained sections.

Brain tissue samples processed by NeuroScience Associates were imaged on the Leica SCN400 whole slide scanning system (Leica Microsystems, Buffalo Grove, IL) at 200x magnification. Matlab (Mathworks, Natick, MA) running on a high performance computing cluster was used for all whole slide image analysis performed in a blinded manner.

Quantification of CD68 or Iba1 staining and enlarged dark cluster areas was performed using morphometric-based methods as previously described (Kallop et al., 2014; Le Pichon et al., 2013). The large dark “cluster” of CD68 or Iba1+ cells coincided with the presence of amyloid plaques. Analysis of Amino Cupric staining was performed using color thresholds and morphological operations. Plaque area was analyzed from slides stained using the Campbell-Switzer method with plaques appearing with a black or amber hue. Multiple color classifiers spanning narrow ranges in RGB and HSV space were created for positive and negative features.

Plaques were segmented using these classifiers and applying adaptive thresholding, Euclidean distance transform, morphological operations, and reconstruction. The percentage plaque load,
Amino Cupric, Iba1, CD68, or Gfap positivity for the entire section was calculated by normalizing the positive pixel area to tissue section area and averaged from eight to eleven sections/animal. All images, segmentation overlays, and data were reviewed by a pathologist.

Image acquisition of immunofluorescent slides costained for plaque, microglia, and either ApoE or dystrophic neurites was performed at 200x magnification using the Nanozoomer S60 or XR (Hamamatsu Corp, San Jose, CA) digital whole slide scanner. Ideal exposure for each channel was determined based on samples with the brightest intensity and set for the whole set of slides to run as a batch. Total tissue area was detected by thresholding on the Iba1 signal and merging and processing of the binary masks by morphological operations. Methoxy-X04, Lamp1, ApoE and Iba1 staining was analyzed using a top-hat filter and local threshold followed by morphological opening and closing. For Lamp1 and methoxy-X04 staining, shape factor, roundness and solidity features were used to eliminate elongated objects. In addition, a minimum size of 34 \( \mu \text{m}^2 \) was applied to exclude small areas of staining. The detected plaques were used as markers in a marker-controlled watershed segmentation to create watershed lines of separation. The plaque mask was then dilated by 17 \( \mu \text{m} \) but constrained to be within watershed lines to prevent merging of plaques in close proximity during dilation. Total Lamp1-positive staining was normalized to the whole tissue area. Plaque-associated Lamp1 and Iba1 staining was constrained to be within the mask of dilated area around plaque and was normalized to the same area. Plaque-associated ApoE staining was constrained to be within the mask of plaque + dilated area and normalized to plaque area. Data was averaged from 2-3 sections per animal.

For X-34 stains, images were collected with a confocal laser scanning microscope LSM780 (Carl Zeiss, Inc.) using Zen 2.3 SP1 software (Carl Zeiss, Inc.). 11 z-stack images at 1 \( \mu \text{m} \) intervals were collected with Plan-Apochromat 20X/0.8 M27 and maximum intensity projection images were created using Zen software. Images were collected and processed blind to genotypes. Image analysis was performed using Matlab in a blinded fashion on the maximum intensity projection of the confocal z-stack. Control images that did not have X-34 positive staining were used to determine an initial threshold to exclude background. A threshold that is greater than 99.99% of all pixel intensities in the control images was applied to all images to determine an initial segmentation mask. The binary masks were then smoothed out using morphological opening and closing. A minimum size of 9 \( \mu \text{m}^2 \) was applied to exclude small areas of staining. A threshold corresponding to the 80th and 50th intensity percentile for the pixels within the segmentation mask of all positive images was applied to analyze compact and diffuse area, respectively. Post-threshold morphological operations and size exclusion was performed as described above. The plaque diffuseness index was calculated as 
\[
\frac{\text{Area}_{\text{diffuse+compact}} - \text{Area}_{\text{compact}}}{\text{Area}_{\text{diffuse+compact}}}
\]
For each animal, data was averaged from two sections per animal, with 3-4 images per section consisting of two fields from cortex, one field from dorsal subiculum and/or one field from dentate gyrus molecular layer.

For analysis of methoxy-X04 or ApoE colocalization within Iba1+ microglia, images were collected from the costained slides described above for the 12mo female cohort of animals using confocal laser scanning microscope LSM780 (Carl Zeiss, Inc.) with Zen 2.3 SP1 software (Carl Zeiss, Inc.). 11 z-stack images at 1 \( \mu \text{m} \) intervals were collected from the cortex with Plan-
Apochromat 20X/0.8 M27. To determine colocalization of ApoE and Iba1 staining, or methoxy-X04 and Iba1 staining, we calculated the Manders’ Colocalization Coefficients (MCC) using the ImageJ plugin JACoP, as described in (Dunn et al., 2011). The same thresholds were consistently used to identify the Iba1, ApoE, or methoxy-X04 channel across animal samples. Calculations were performed on the entire z-stack of images.

A-beta peptide measurements.

Frozen hippocampal tissues, described above, were homogenized in 10 volumes of TBS (50 mM Tris pH 7.5, 150 mM NaCl, including complete EDTA-free protease inhibitor cocktail (Roche) with aprotinin (20 µg/ml) and leupeptin (10 µg/ml)) in a Qiagen TissueLyser II (3 min at 30 Hz). Samples were then centrifuged at 20,000 x g for 20 min at 4°C. Supernatants were collected as the “TBS fraction” and stored at -80°C until analyzed. The pellet was then homogenized in 10 volumes of 5 M guanidine HCl using the TissueLyser II and then placed on a rotisserie for 3 hours at room temperature. Samples were diluted 1:10 in a casein buffer (0.25% casein/5 mM EDTA pH 8.0 in PBS, including aprotinin (20 µg/ml) and leupeptin (10 µg/ml)), vortexed and centrifuged at 20,000 x g for 20 min at 4°C. Supernatants were collected as “GuHCl fractions”. Aβ40 and Aβ42 concentrations in mouse hippocampal samples were measured using an ELISA. Briefly, rabbit polyclonal antibody specific for the C terminus of Aβ40 or Aβ42 (Milipore) was coated onto plates, and biotinylated monoclonal anti-Aβ1-16 (Covance, clone 6E10) was used for detection.

For dot blot analyses, approximately 10 µg in 1 µl of lysate (TBS soluble fraction of homogenized mouse hippocampus) was blotted onto nitrocellulose membranes (#LC2001, Invitrogen) and incubated for at least one hour at room temperature (RT) to ensure that the blots were dry. The membrane was blocked with Blocking Buffer (MB-070, Rockland Immunochemicals Inc.) with added 0.01% Tween-20, for 1 hour at RT. The membrane was incubated with Amyloid Fibrils OC (Millipore Sigma AB2286), Oligomer A11 (Thermo Fisher Scientific AHB0052), 4G8 (Biolegend 800703) or 6E10 (Biolegend 803015) primary antibody diluted 1:1000 in Blocking Buffer for 1 hr at RT. Total protein was normalized with mouse anti-β-actin (Cell Signaling 8H10D10, 1:10000) or rabbit anti-GAPDH (Novus Biological NB300-323, 1:10000). After primary antibody incubation, membranes were washed 3 times (10 minutes each) with TBST (50 mM Tris, 0.5 M NaCl, 0.01% Tween-20). The membrane was incubated with secondary antibodies in Blocking Buffer at 1:15,000 dilution (IRDye 800CW donkey anti-rabbit IgG and IRDye 680LT donkey anti-mouse IgG, LI-COR Biosciences 926-32213, 926-32212, 926-68022, and 926-68023) for 1 hour at RT. Membrane was washed 3 times (10 minutes each) in TBST on rocker. Blots were scanned on Odyssey/LICOR scanner for signals followed by image analysis in Image Studio (v5.2.5, LI-COR Biosciences).

CSF collection and neurofilament light chain (NfL) analysis.

A separate, mixed sex cohort of 12mo PS2APP;Trem2wt (n=8) and PS2APP;Trem2ko mice (n=8) were anesthetized and CSF was collected from the cisterna magna and placed on ice, then blood was collected from terminal cardiac puncture, placed into EDTA collection tubes and centrifuged at 20,000 x g for 2 minutes. (The two genotype groups in this analysis were not
littermates since we had not collected plasma or CSF from our original cohorts, and we
assembled this cohort just for NfL measurements due to a recommendation received during
peer review.) Plasma was collected into tubes and stored at -80ºC until transfer. CSF samples
were diluted 1:10 in 0.1% BSA in TBS and then stored at -80ºC until transfer. Plasma and CSF
samples were sent to Quanterix (Billerica, MA) for NfL measurements using the Simoa™ NF-
Light Advantage Kit (product 103186). The Simoa assay is a 2-step digital immunoassay, which
measures the quantity of NfL in samples using the Simoa HD-1 Analyzer and Single Molecule
Array (Simoa) technology.

Two-photon imaging of plaque and dendritic spine measurements.
The somatosensory cortex from PS2APP mice carrying the Thy1:GFP-M transgene and
different Trem2 genotypes was imaged ex vivo via 2-photon microscopy. Single-sex cohorts
used for this purpose included 6mo PS2APP females (n=7 for per Trem2 genotype, used for
both plaque counts and dendritic spine measurements) and 8mo PS2APP males (n=6 per
Trem2 genotype, used only for plaque counts). 24 hours before brain collection, animals
received intraperitoneal injections of methoxy-X04 (10 mg/kg) to label amyloid structures (Klunk
et al., 2002). Animals were anesthetized using isofluorane and transcardially perfused with 10
ml PBS followed by 10 ml of 4% PFA + 10% sucrose in PBS, and the collected brains were
fixed overnight in 4% PFA + 10% sucrose in PBS at 4°C. Postfixation, brains were mounted in
agarose and immersed in PBS. Imaging and analysis were performed under blinded conditions.
Apical dendrites and their spines in somatosensory cortex upper layers were imaged en
bloc via a two-photon laser-scanning microscope (Ultima In Vivo Multiphoton Microscopy
System; Prairie Technologies) using a Ti:sapphire laser (MaiTai DeepSee Spectra Physics;
Newport) tuned to 840 nm and a 60× numerical aperture 1.0 immersion objective lens
(Olympus) with pixel resolution of 0.1 µm/pixel across a 1024 × 1024 pixel field of view (FOV)
using 1.0 µm steps, with stack depth determined by the slant of the dendritic branch being
imaged. For comparison of spine density relative to plaques in PS2APP animals, a FOV
containing a dendrite and nearby plaque within 20 µm was considered “near plaque” and a FOV
containing only a dendrite with no visible plaque was considered “away from plaque.” To meet
the “away from plaque” criteria, we confirmed that no plaque was present in the FOV and at
least 100 µm outside of the containing FOV. From each brain, at least five dendrites per
condition (near plaque, away from plaque) were imaged. Dendritic spine density and size
measurements were generated using custom, semiautomated image analysis routines in
MATLAB (MathWorks). Spine density was estimated as the total number of visible dendritic
spines divided by the corresponding length of dendrite. Relative spine volumes were estimated
for each detected spine based on the number of corresponding GFP+ pixels in x, y, z
dimensions above a local threshold applied as part of an automated image segmentation
algorithm. For en bloc plaque measurements, larger volume stacks were collected using a 20×
immersion objective lens across a 1024 × 1024 pixel FOV with 2 µm steps (~200 µm depth).
Plaque density was quantified by a threshold-based MATLAB routine designed to automatically
identify methoxy-X04-labeled plaques.
All values are expressed as mean ± SEM. Statistical analysis was performed using the JMP (v14.2, SAS Institute) or Prism (v8.3.0 for Mac, GraphPad) software packages. To compare differences between PS2APP;Trem2<sup>wt</sup> and PS2APP;Trem2<sup>ko</sup> groups we performed unpaired t-tests. For comparisons of three or more groups we performed one-way ANOVA followed by Tukey’s multiple comparisons test. The 6-7mo cohort of male and female mice were purposely analyzed separately to determine the effects of Trem2 deficiency in each sex since female mice have accelerated amyloid pathology compared to males, and we did not want an analysis of interactions between sex and Trem2 genotype to be confounded by the age-dependent differences in pathology between males and females.

**RESULTS**

Plaque-associated microgliosis is impaired in PS2APP;Trem2<sup>ko</sup> microglia

PS2APP mice express transgenes encoding familial AD mutations in human presenilin 2 (PS2 N141I) and amyloid precursor protein (APP K670N/M671L). By 4 months of age the first deposits of β-amyloid plaque are detected, with age-dependent plaque accumulation occurring faster in females than in males (Kallop et al., 2014; Ozmen et al., 2009). To determine the role of Trem2 in the progression of amyloid disease pathology, we crossed PS2APP mice with Trem2-deficient mice and examined plaque-related phenotypes in single-sex groups at various stages of pathology. We found a stark reduction in Iba1<sup>+</sup> microglial clusters in PS2APP;Trem2<sup>ko</sup> mice at 4, 6-7, 12, and 19-22 months compared to PS2APP;Trem2<sup>wt</sup> (Fig. 1A,B). In addition, PS2APP;Trem2<sup>ko</sup> brains showed reduced staining for CD68 marking active microglial lysosomes by IHC (Fig. 1C,D), reduced percentage of CD45<sup>high</sup> (“activated”) microglia by flow cytometry (Fig. 1E,F), and reduced percentage of methoxy-X04<sup>+</sup> (amyloid-containing) microglia by flow cytometry (Fig. 1G,H). Analysis of confocal z-stack images of cortical tissue from 12-month (12mo) animals also found a significant reduction in the fraction of Iba1 and methoxy-X04 signals that colocalized with each other (Fig. 1-1). We also observed reductions in total Iba1 and Gfap staining at the later ages, indicative of reduced extents of microgliosis and astrogliosis, respectively, in PS2APP;Trem2<sup>ko</sup> mice (Fig. 1-1). These observations were consistent with reports of Trem2 deletion in other β-amyloidosis.
models (Jay et al., 2015; Parhizkar et al., 2019; Wang et al., 2015; Wang et al., 2016; Yuan et al., 2016) and suggested that Trem2 deficiency impairs the ability of microglia to engage plaques and phagocytose Aβ fibrils/aggregates.

Trem2-dependent induction of the Proliferation and Neurodegeneration-related gene expression modules

To further characterize the attenuated microglial response to β-amyloid pathology in PS2APP;Trem2^ko mice, we FACS-isolated the resident myeloid cell population from the cortex+hippocampus of 14-15mo PS2APP;Trem2^wt vs. PS2APP;Trem2^ko mice and compared their transcriptomic profiles by RNA sequencing (raw RNA-Seq data deposited in NCBI GEO under accession number GSE140744). Although not affording single cell resolution, our approach provided certain overall advantages—genome-wide analysis, robust detection of low-copy transcripts, and avoidance of artifactual gene expression that occurs during warm-temperature dissociations—compared to other approaches for transcription profiling of Trem2^ko microglia in β-amyloid models that used different cell isolation techniques and/or different RNA detection methods such as microarray, Nanostring, or single-cell RNAseq (Griciuc et al., 2019; Keren-Shaul et al., 2017; Krasemann et al., 2017; Wang et al., 2015).

Applying cutoffs of ≥ 2-fold change and adjusted p-value ≤ 0.05, we observed only 7 transcripts with increased abundance in PS2APP;Trem2^ko vs. PS2APP;Trem2^wt microgria (excluding Treml1, an artifact of the knockout cassette insertion (Kang et al., 2018)). In contrast, 144 transcripts (excluding Trem2) showed reduced abundance in PS2APP;Trem2^ko compared to PS2APP;Trem2^wt microgria using the same cutoffs (Fig. 2A; see Fig. 2-1 extended data table of genome-wide expression values for each sample and summary statistics for differential gene expression). The majority of these transcripts showed upregulation in microglial expression profiles from the PS2APP model (Friedman et al., 2018) and other models of β-amyloid pathology (Orre et al., 2014; Wang et al., 2015) compared to non-transgenic mice (Fig. 2A). Therefore, their reduced expression in PS2APP;Trem2^ko microgria is another manifestation of the
impaired microglial response to β-amyloid pathology. The dependence of these transcripts on Trem2 for their induction in PS2APP microglia was roughly concordant with published data from sorted microglial populations from the 5xFAD model (Wang et al., 2015) (Fig. 2A).

Four of the eight most starkly reduced transcripts (in terms of fold change) in PS2APP;Trem2\(^{−/+}\) microglia are regulators of canonical Wnt signaling or proliferation—Dkk2, Wif1, Ctnna3, and Asb11 (Fig. 2B). Dkk2, Wif1, and Ctnna3 can all negatively regulate Wnt activity (Busby et al., 2004; Gage et al., 2008; Hsieh et al., 1999), while Asb11 is important for maintaining progenitor cell activity in multiple cell types (Diks et al., 2006; Tee et al., 2012). Although the lack of induction for negative Wnt regulators might suggest that Wnt-related signaling was enhanced in Trem2\(^{−/+}\) microglia, another possibility is that Wnt-related signaling was impaired since Dkk2 is also a context-dependent activator of the pathway (Devotta et al., 2018; Mao and Niehrs, 2003; Wu et al., 2000) and since induction of regulators including Wif1 can occur downstream of active β-catenin as negative feedback (Boerboom et al., 2006; Diep et al., 2004).

Supporting this interpretation, the Gene Ontology (GO) knowledgebase identified “positive regulation of canonical Wnt signaling pathway” as a biological process overrepresented (fold-enrichment = 10.3, FDR = 0.026) among the 144 transcripts with ≥ 2-fold reduced abundance in PS2APP;Trem2\(^{−/+}\) microglia (Fig. 2-2A), with six positive factors in Wnt signaling showing reduced expression including Fzd9 (Karasawa et al., 2002), Sulf2 (Lai et al., 2010), Bambi (Lin et al., 2008), Ptk7 (Berger et al., 2017), and Aspm (Buchman et al., 2011) along with Dkk2 (Fig. 2C). We also analyzed recently published microglia RNA-Seq expression profiles from the 5xFAD model (Griciuc et al., 2019) and observed similar Trem2-dependent induction for the nine above-mentioned genes, with the exception of Ptk7 (Figure 2-2B). The reduced expression of Wnt-related signaling components and regulators in PS2APP;Trem2\(^{−/+}\) microglia may be consistent with previous reports of coordinated signaling between Trem2 and β-catenin pathways within microglia (Zheng et al., 2017; Zulfiqar and Tanriover, 2017).
Other GO biological processes implicated as being downregulated in PS2APP;Trem2<sup>ko</sup> microglia included positive regulation of bone resorption (fold-enrichment=24.6, FDR=0.030), protein kinase B signaling (fold-enrichment=16.2, FDR=0.047), negative regulation of tumor necrosis factor production (fold-enrichment=14.1, FDR=0.026), positive regulation of smooth muscle cell migration (fold-enrichment=13.9, FDR=0.025), transmembrane receptor protein tyrosine kinase signaling pathway (fold-enrichment=5.9, FDR=0.0037), and actin cytoskeleton reorganization (fold-enrichment=3.9, FDR=0.044) (Fig. 2-2A). The Trem2-dependent genes identified in these processes may underlie described roles for Trem2 in osteoclast function (Cella et al., 2003; Paloneva et al., 2003), AKT and mTOR signaling (Ulland et al., 2017), attenuation of pro-inflammatory macrophage activation (Turnbull et al., 2006), chemotaxis (Mazaheri et al., 2017), and DAP12 signaling (Bouchon et al., 2001).

We recently defined a number of gene expression modules that can be used to characterize the diverse ways that microglia respond to environmental and genetic perturbations (Friedman et al., 2018). In PS2APP compared to non-transgenic microglia, several of these gene sets were upregulated including the Neurodegeneration-related, Interferon-related, Proliferation, and LPS-related modules, while the Microglia and Brain Myeloid modules that typify microglia in their "homeostatic" or "resting" state were modestly but significantly downregulated (Fig. 2D, Fig. 2-3). (The entirety of these changes is roughly equivalent to the so-called DAM (Keren-Shaul et al., 2017) or MgND (Krasemann et al., 2017) microglial activation profiles.) These findings underscore the utility of these gene modules in characterizing microglial activation states. For instance, even though only 5 of 82 genes in the Proliferation module were upregulated strongly enough in PS2APP microglia to reach genome-wide significance (adj. p ≤ 0.05), the overall expression of the module was clearly enriched compared to microglia from non-transgenic mice (Fig. 2D, Fig. 2-3).

We next analyzed the degree to which these modular changes in microglial gene expression in PS2APP mice depended on Trem2. Again, despite only 2 of 82 genes (Ccna2 and Aspm) showing significant reduction in transcript abundance after
Correction for genome-wide analysis, the overall induction of the Proliferation module was compromised in PS2APP;Trem2\textsuperscript{ko} microglia (Fig. 2D, Fig. 2-3). We also observed less induction of the Proliferation module in Trem2\textsuperscript{ko} microglia expression profiles from the 5xFAD model (Griciuc et al., 2019) (Fig. 2-2C). The reduced expression of proliferation-related genes was consistent with the notion mentioned above that Wnt-related signaling was impaired and also corroborated reports from other β-amyloid models that proliferation markers such as Ki67 or BrdU were observed less frequently in Trem2-deficient microglia (Jay et al., 2017a; Wang et al., 2016).

As expected, the Neurodegeneration-related gene set—which overlaps with the so-called DAM (Keren-Shaul et al., 2017) and MGnD (Krasemann et al., 2017) genes but relates more specifically to neurodegenerative disease models—was notably impaired in PS2APP;Trem2\textsuperscript{ko} microglia (Fig. 2D, Fig. 2-3). Of the 134 genes in this set, 80 showed upregulation in PS2APP vs. normal microglia (Friedman et al., 2018), and roughly half of these showed impaired induction in PS2APP;Trem2\textsuperscript{ko} microglia (adjusted p-values ≤ 0.05) (Fig. 2E). Unlike the clear requirement of Trem2 for induction of many genes in the Neurodegeneration-related gene set, the downregulation of the Microglia and Brain Myeloid modules that normally occurs during any CNS challenge (Friedman et al., 2018) was not prevented by Trem2 deletion since expression of these modules was similar in PS2APP;Trem2\textsuperscript{wt} and PS2APP;Trem2\textsuperscript{ko} microglia (Fig. 2D, Fig. 2-3). No effect of Trem2 deletion on the induction of the LPS-related and Interferon-related modules was observed. Overall, our results are similar to previous analysis of the 5xFAD model, in which the microglial induction of many DAM genes showed substantial Trem2 dependence while the downregulation of so-called microglial “homeostatic” genes appeared largely Trem2-independent (Keren-Shaul et al., 2017).

Plaque load is reduced in aged PS2APP;Trem2\textsuperscript{ko} mice

In the 5xFAD mouse model, plaque load was reportedly unchanged in 4mo Trem2\textsuperscript{ko} mice but increased in 8mo Trem2\textsuperscript{ko} hippocampus (Wang et al., 2015; Wang et al., 2016; Yuan et al., 2016). In the APPPS1 model, plaque load was reduced in Trem2\textsuperscript{ko} brains at
2mo, reduced or unchanged at 4mo, and increased in the cortex at 8mo (Jay et al., 2017a; Jay et al., 2015).

The reductions in microglial clustering and amyloid engulfment in PS2APP;Trem2\(^{ko}\) mice (Fig. 1) suggested that more plaque might accumulate over time in these brains, relative to PS2APP;Trem2\(^{wt}\) mice. Indeed, using the Campbell-Switzer silver stain to label amyloid plaque (Campbell et al., 1987; Switzer, 2000), we observed that plaque burden was increased in 6-7mo PS2APP;Trem2\(^{ko}\) females, and trending upward but not reaching significance in 6-7mo PS2APP;Trem2\(^{ko}\) males (Fig. 3A,B). We observed similar results in distinct cohorts of 6mo females and 8mo males by in vivo labeling of plaque using methoxy-X04 injection, followed by fixation and two-photon imaging of intact somatosensory cortex (Fig. 3-1). We did not observe any effect of Tre2 deletion on the low levels of plaque deposition detected at the earliest stage examined (4mo).

We expected to see further exacerbation of amyloid plaque load in Trem2-deficient brains at more advanced ages, but, to our surprise, plaque load was reduced in 12mo PS2APP;Trem2\(^{ko}\) females and in 19-22mo PS2APP;Trem2\(^{ko}\) males compared to PS2APP;Trem2\(^{wt}\) mice (Fig. 3A,B). An intermediate reduction in plaque load was observed in Trem2 heterozygous (PS2APP;Trem2\(^{het}\)) mice at 19-22mo (the only age where heterozygous mice were analyzed) (Fig 3A). At this age, the Campbell-Switzer stain also revealed a distinctive pattern of “amber core” amyloid staining in PS2APP brains (Fig. 3C), thought to represent a mature form of highly condensed plaque. These amber cores were reduced in PS2APP;Trem2\(^{het}\) and nearly absent in PS2APP;Trem2\(^{ko}\) brains, indicating that Trem2-dependent microglial activity is essential for the formation of these particular amyloid structures.

Overall, our results are reminiscent of a recent study of APPPS1 mice which showed that Trem2 deletion produced increased seeding of amyloid plaques at early ages but slower rates of amyloid plaque accumulation at later ages (Parhizkar et al., 2019).
Reduced plaque consolidation, elevated neurotoxic Aβ species, and ApoE-laden microglia in Trem2-deficient brains

In contrast to the effects of Trem2 deletion on total plaque burden that varied with age or sex, we observed consistent changes in plaque compaction and composition across ages and sexes. We used the X-34 stain and confocal microscopy to visualize plaque morphology. Although we were blinded to Trem2 genotype, there was an obvious difference in plaque appearance between PS2APP;Trem2wt and PS2APP;Trem2ko brains, with X-34+ structures in PS2APP;Trem2ko brains looking more splayed and less compact (Fig. 4A), similar to descriptions of Trem2-dependent plaque alterations in other β-amyloid models (Wang et al., 2016; Yuan et al., 2016). Using an algorithm based on X-34 signal intensity to quantify the degree of plaque diffuseness, we observed that plaque morphologies were significantly more diffuse in PS2APP;Trem2ko brains in both sexes at all ages tested (Fig. 4B).

Because stains such as Campbell-Switzer, methoxy-X04, and X-34 only label fibrillar amyloid structures, we also measured the total abundance of Aβ40 and Aβ42 peptides in soluble (TBS) and insoluble (GuHCl) hippocampal fractions by ELISA. The abundance of Aβ peptides rose markedly with age while the Aβ42:Aβ40 ratio declined, particularly in the GuHCl fraction (Fig. 5). Notably, the Aβ42:Aβ40 ratio was higher in PS2APP;Trem2ko than in PS2APP;Trem2wt brains, in both TBS and GuHCl fractions across ages (Fig. 5A,B). The elevated Aβ42:Aβ40 ratio in PS2APP;Trem2ko brains resulted more from reduced abundance of Aβ40 than from increased abundance of Aβ42 (Fig. 5-1), although Aβ42 abundance was elevated in the 6-7mo females, coinciding with the increased plaque deposition we observed in that group (Fig. 3A).

Together with our observation that total amyloid plaque is reduced in PS2APP;Trem2ko brains at later ages (Fig. 3A), these results suggest that the elevated Aβ42:Aβ40 ratio in Trem2-deficient brains may increase plaque seeding at younger ages (since Aβ42 is more prone to aggregate and deposit than Aβ40 (Klein, 2002)) while reducing the incorporation and compaction of Aβ into existing plaques at older ages (since Aβ40
permeates dense core structures more readily than Aβ42 (Condello et al., 2015)). Thus, Trem2 may both restrict the initial seeding of plaques and promote sequestration and compaction of Aβ into existing plaques.

To determine if the elevated Aβ42:Aβ40 ratio was accompanied by altered abundance of soluble, fibrillar Aβ oligomers, we performed non-denaturing dot blots of hippocampal TBS homogenate supernatants from 12mo females. Using the conformation-specific OC antibody (Tomic et al., 2009), we detected significantly higher levels of soluble fibrillar Aβ oligomers in the soluble fraction from PS2APP;Trem2<sup>ko</sup> mice (Fig. 5C,D), and we observed a positive correlation between the amount of OC<sup>+</sup> fibrillar oligomers in this fraction and the Aβ42:Aβ40 ratio (Fig. 5E). In contrast, when we used the pan-reactive 6E10 Aβ antibody to detect total Aβ species in this fraction, the abundance trended slightly downward in PS2APP;Trem2<sup>ko</sup> mice and correlated negatively with Aβ42:Aβ40 ratio (Fig. 5F-H). Similar respective trends were also seen when staining with the prefibrillar Aβ oligomer antibody A11 and pan-reactive 4G8 Aβ antibody (data not shown). As a control, we spotted hippocampal TBS homogenate supernatant from a PS2APP;Bace1<sup>ko</sup> animal (Meilandt et al., 2019)—in which soluble APP is still produced by α-cleavage while β-cleavage and thus Aβ production are prevented—and demonstrated that the OC antibody had minimal detection whereas 6E10 still had substantial signal (compare # symbols in Fig. 5C,F and dotted lines in Fig. 5D,G from a PS2APP;Bace1<sup>ko</sup> mouse), consistent with the ability of 6E10 to detect both soluble APP and Aβ peptides. These results suggest that in the absence of Trem2 the increased Aβ42:Aβ40 ratio enhances the potential shift of soluble Aβ to a fibrillar oligomeric form. Alternatively, the increased abundance of fibrillar Aβ oligomers in the TBS-soluble fraction could result from reduced incorporation into highly condensed plaques in brains with Trem2-deficient microglia.

Apoe is one of the most highly induced genes in mouse microglia in response to neurodegenerative stimuli (Deczkowska et al., 2018). A recent report in the APPPS1 model found that plaque-associated ApoE was reduced in Trem2-deficient mice.
(Parhizkar et al., 2019). To learn whether Trem2 deletion altered ApoE localization in the PS2APP model and whether such alteration correlated with observed changes in plaque abundance, morphology, or composition, we co-stained for ApoE, plaques (methoxy-X04), and microglia (Iba1). In contrast to the findings of Parhizkar et al., quantification of plaque-associated ApoE in whole brain sections found a significant increase in female PS2APP;Trem2\textsuperscript{ko} mice at 6-7mo and 12mo ages, but no significant changes in male cohorts at any age tested (Fig. 6A). The ApoE immunostaining pattern was especially prominent in, but not limited to, the hippocampal subiculum where plaque tends to first deposit (Fig. 6B). While the incongruous results between studies may be explained by differences between mouse models, other variations in sampling and technical procedures could also account for the differences. Parhizkar et al. examined 4mo males; we only examined females at that age, and our findings of elevated plaque-associated ApoE in Trem2-deficient females were only observed at older ages. The studies used different ApoE monoclonal antibodies that likely interact with distinct epitopes or configurations of ApoE (Kim et al., 2012) and whose binding may be differentially impacted by variations in staining procedure. We immunostained for ApoE first, followed by plaque labeling which involves treatments with ethanol and NaOH. Parhizkar et al. did the plaque labeling first, followed by the ApoE immunostains, so the nature of the ApoE at the time of immunostaining was somewhat different between studies.

Parhizkar et al. also reported that colocalization of ApoE with Iba1\textsuperscript{+} microglia was reduced in Trem2-deficient mice. To assess this finding in the PS2APP model, we performed confocal imaging on the ApoE/Iba1/methoxy-X04 costains from the cortex of 12mo females and again observed a contrasting result. Instead of ApoE labeling being diminished, we observed microglia in PS2APP;Trem2\textsuperscript{ko} mice to be markedly laden with ApoE (Fig. 6C,D), suggesting that Trem2-deficient microglia exhibit deficits in lipid clearance. At the mRNA level, we did not detect a difference in Apoe expression between PS2APP;Trem2\textsuperscript{wt} and PS2APP;Trem2\textsuperscript{ko} microglia (Fig. 6E; see also Fig. 2E). Although two groups have reported reductions in microglial Apoe expression in the APPPS1 model when Trem2 is deleted (Krasemann et al., 2017; Parhizkar et al., 2019),
this does not appear to be a typical feature of Trem2 deficiency in β-amyloidosis models since previous analyses in the 5xFAD model observed Trem2-independent Apoe induction in either bulk microglia microarray (Fig. 6F) (Wang et al., 2015) or single cell microglia RNA-Seq profiles (Keren-Shaul et al., 2017). In a very recent dataset of bulk microglia RNA-Seq profiles from 5xFAD mice (Griciuc et al., 2019), we did see a 2-fold decrease in the extent of Apoe induction in Trem2-deficient microglia, but the gene was still highly induced relative to the expression level in microglia from non-transgenic mice (Fig. 6F). Altogether, our evidence indicates that microglial Apoe expression is induced by Aβ-driven neuropathology in a largely Trem2-independent manner, and that Trem2-deficient microglia accumulate disproportionately large amounts of ApoE compared to the smaller ApoE puncta observed in normal PS2APP microglia (Fig. 6C).

Axonal dystrophy, dendritic spine loss, and CSF NfL detection are exacerbated in PS2APP;Trem2ko mice

The elevated Aβ42:Aβ40 ratio and fibrillar Aβ oligomers in PS2APP;Trem2ko brains would seem to be detrimental for neuronal health since Aβ42 oligomers are commonly understood to be the more toxic form of Aβ (Haass and Selkoe, 2007; Klein, 2002). Alternatively, the reduced plaque load in aged Trem2ko brains suggested a possible benefit of Trem2 deficiency. Therefore, we turned to measures of neuronal dystrophy to better understand the potential consequences of loss of Trem2 function.

First, we looked at neuritic dystrophy around plaque (D’Amore et al., 2003) by fluorescent co-staining using methoxy-X04 to label plaque, anti-Iba1 to label microglia, and anti-Lamp1 to label dystrophic axons (Gowrishankar et al., 2015) (Fig. 7A). The methoxy-X04/Iba1 costain showed that microglial association with plaque was severely compromised in PS2APP;Trem2ko brains at all examined ages (Fig. 7A,B), corroborating our earlier Iba1 immunohistochemical stains that measured microglial clustering. The Lamp1 immunolabeling, which stains dilated dystrophic axons, revealed two important findings. First, on a per plaque basis, axonal dystrophy was exacerbated from 7 months onward in PS2APP;Trem2ko mice (Fig. 7C), similar to findings in other β-
amyloid models (Wang et al., 2016; Yuan et al., 2016). This is consistent with the idea that the diffuse plaque structures and elevated Aβ42:Aβ40 ratio in Trem2<sup>ko</sup> brains are more damaging to surrounding axons than the more compacted, Aβ40-enriched plaques in PS2APP;Trem2<sup>wt</sup> brains. Second, the total Lamp1<sup>+</sup> area was also increased from 7 months onward in PS2APP;Trem2<sup>ko</sup> brain sections (Fig. 7D), indicating that total axonal damage was exacerbated at later ages despite the reduced plaque burden. These data strongly suggest that the Trem2-dependent clustering of microglia and their functions around plaque serve to mitigate the neurotoxic effects of Aβ.

Another method we used to visualize neuronal pathology was an amino-cupric-silver stain or “disintegrative degeneration” stain that labels damaged or degenerating neurons (de Olmos et al., 1994; Switzer, 2000). Overall, the staining pattern appeared very similar to the Lamp1 stain, with “bouquets” of argyrophilic structures presumably surrounding plaques throughout the cortex and hippocampus. Staining was also observed in relevant white matter tracts such as the corpus callosum, perforant path and fornix, suggesting that degenerating axonal processes are not restricted to dystrophic neurites around plaques. Degenerating neurites detected by this stain were more abundant in PS2APP;Trem2<sup>ko</sup> brains of the 12mo female and 19-22mo male cohorts (Fig. 8A,B). Thus, again, axonal damage was exacerbated in Trem2-deficient mice from older ages despite the fact that plaque accumulation was reduced. The intermediate effect of Trem2 heterozygosity on microglial clustering around plaque (Figs. 1A and 7B) and Campbell-Switzer plaque staining (Fig. 3A) but not on plaque diffuseness (Fig. 4B), Aβ42:Aβ40 ratio (Fig. 5A), or neuritic dystrophy (Figs. 7C,D and 8A) suggested that the form rather than the amount of plaque correlates with neuronal damage, and that sufficient microgliosis occurs in PS2APP;Trem2<sup>het</sup> mice to enable plaque compaction and neuroprotection.

Neurofilament light chain (NFL) measured in the cerebrospinal fluid (CSF) or plasma has recently emerged as a potential biomarker of neurodegeneration in human patients and in mouse disease models (Bacioglu et al., 2016; Khalil et al., 2018). To determine whether Trem2 deficiency altered NFL levels, we collected plasma and CSF from a
Finally, we looked at whether another feature of neuronal pathology observed in AD tissues and β-amyloid mouse models—reduced synaptic density, particularly near plaque (Spires and Hyman, 2004; Spires et al., 2005; Tsai et al., 2004)—was altered in Trem2-deficient mice. In β-amyloid models, the reduction in synapse number requires the presence of microglia since depleting the microglial cell population largely prevents loss of synaptic density (Olmos-Alonso et al., 2016; Spangenberg et al., 2016). Therefore, we asked whether Trem2 deletion and the resulting lack of activated, plaque-associated microglia would prevent the dendritic spine loss from occurring or would worsen it. To answer this, we crossed the Thy1:GFP-M line, which labels a sparse population of excitatory neurons, into the PS2APP model and analyzed a cohort of 6mo female mice with different Trem2 genotypes. Spine density loss in the proximity of plaques was not rescued by Trem2 deletion, but was actually further exacerbated in PS2APP;Trem2ko mice compared to PS2APP;Trem2wt or PS2APP;Trem2het groups (Fig. 9). Thus, Trem2 is not required for microglia-mediated dendritic spine loss around plaque and in fact Trem2 seems to hedge against synapse loss. Overall, the exacerbated axonal dystrophy and dendritic spine loss observed around plaque in PS2APP;Trem2ko mice imply that Trem2-dependent microglial activity is fundamentally neuroprotective in β-amyloid-driven models of AD-like pathology.

DISCUSSION
In this study we examined the role of Trem2 in microglial activation, plaque accumulation, and neuronal dystrophy in the PS2APP model of β-amyloidosis. We observed both age- and sex-dependent effects of Trem2 deletion on plaque abundance assessed using the Campbell-Switzer silver stain, with slightly more plaque in PS2APP;Trem2\textsuperscript{ko} females at the 6-7mo age but markedly less plaque in both female and male PS2APP;Trem2\textsuperscript{ko} mice at later ages. Together with a recent similar report (Parhizkar et al., 2019), these results suggest that Trem2-dependent microglial activity may both restrain the formation/seeding of plaques at an early stage of pathology—conceivably through uptake and degradation of soluble A\textbeta\ species—but also enhance the sequestration of A\textbeta into existing plaque structures, particularly at later stages of pathology.

At all ages examined, and in both sexes, microglial clustering around plaque and other measurements of microglial activation were sharply reduced in PS2APP;Trem2\textsuperscript{ko} mice. Transcriptional induction of the Neurodegeneration-related modules, the Proliferation module, and certain genes related to Wnt regulation was impaired in PS2APP;Trem2\textsuperscript{ko} microglia. Presumably as a consequence of the impaired microglial response around plaque, plaques in PS2APP;Trem2\textsuperscript{ko} brains displayed a more diffuse morphology than in PS2APP;Trem2\textsuperscript{wt} brains. From 6-7mo onward, axonal injury was magnified in PS2APP;Trem2\textsuperscript{ko} mice, even at later ages when the abundance of argyrophilic amyloid plaques was diminished. Our finding that plaque-proximal dendritic spine loss was exacerbated in PS2APP;Trem2\textsuperscript{ko} mice further underscored that the Trem2-dependent microglial activation around plaque is protective for neurons and is distinct from microglia-mediated, complement-mediated activities that contribute to synapse loss in β-amyloid models (Fonseca et al., 2004; Hong et al., 2016; Shi et al., 2017; Wu et al., 2019). It will be important for future studies of microglial modulation in mouse models to analyze plaque-associated neuritic dystrophy and not assume that decreases (or increases) in amyloid plaque burden are evidence of beneficial (or detrimental) effects.

While the elevated A\textbeta42:A\textbeta40 ratio we observed in the soluble fraction of PS2APP;Trem2\textsuperscript{ko} brains could reflect direct deficits in the Trem2-mediated binding and
clearance of oligomeric Aβ42 by microglia (Lessard et al., 2018; Yeh et al., 2016; Zhao et al., 2018), it could also reflect enhanced clearance of Aβ40 through other means such as vascular efflux. The resulting elevation in soluble Aβ42:Aβ40 ratio may give rise to the increased plaque formation we observed in 6-7mo females, similar to the increased plaque seeding activity observed by others at a similar age in another model when Trem2 was deleted (Parhizkar et al., 2019). Given that Aβ40 is more easily incorporated into existing dense plaque structures than Aβ42 (Condello et al., 2015), our observation that the Aβ42:Aβ40 ratio declines as the mice age, particularly in the insoluble fraction, is consistent with the accumulation of compact plaques in size and number over time in the brains of PS2APP mice. The reduction of this plaque accumulation activity in PS2APP;Trem2^ko brains may underlie the elevated levels of fibrillar oligomeric Aβ we detected in the soluble fraction, which may in turn be a source of increased neuronal injury in these animals.

The weakness of correlation between plaque abundance and cognitive status in humans, along with several unsuccessful clinical trials aimed at preventing cognitive decline by reducing brain Aβ levels, has led some to question the relevance of Aβ in the etiology and progression of AD (Itzhaki et al., 2016; Krstic and Knuesel, 2013; Morris et al., 2014). Moreover, many have proposed that chronic microglial activation is a key damaging agent that contributes to the neurotoxic environment in AD (Heneka et al., 2015; Park et al., 2018). Our results suggest the opposite, since preventing the microglial response to Aβ pathology via Trem2 deletion reversed neither axonal dystrophy nor dendritic spine loss around plaque and in fact made both of these pathologies worse. Trem2 deletion also increased the amount of NFL detected in CSF. Evidence in humans and mice supports the mechanistic model that β-amyloid pathology accelerates the accumulation of tau pathology or enhances its spreading (He et al., 2018; Jack et al., 2018; Jacobs et al., 2018; Pooler et al., 2015), and a new report indicates that this effect is further magnified in Trem2-deficient mice (Leyns et al., 2019).

Altogether, these findings suggest that the form of microglial activation brought on by
Aβ-related pathologies (the DAM state, or at least the Trem2-dependent component of it) protects neurons by limiting Aβ-induced neuronal injury. Supporting the notion that microglial activation is primarily beneficial in the context of AD pathology, elevated PET signal for ligands of the “neuroinflammation marker” TSPO have predicted better cognitive measures and slower AD progression in mice with β-amyloid pathology and human patients, respectively (Focke et al., 2019; Hamelin et al., 2016). The fact that plaques in Trem2-deficient mice are more injurious to adjacent neurites but show weaker labeling with molecular probes (Thioflavin S, methoxy-X04 and X-34) related to those used in the clinic (11C-PiB, 18F-Florbetapir) helps explain why cognitive decline correlates better with tau pathology and synapse loss than with brain amyloid detection. The form of β-amyloid in the brain is more critical than the amount, and Trem2-mediated microgliosis facilitates the consolidation of β-amyloid into a highly compacted, less damaging form. Therefore, therapeutics that enhance this microglial activity may prevent AD or delay its progression while simultaneously (and perhaps counterintuitively) leading to increases in PET signals for amyloid content and microgliosis. Clinical biomarkers of neuronal degeneration and spreading AD pathology such as neurofilament-L and tau should be more informative indicators of whether a microglia-directed therapy is achieving efficacy.
Author contributions

WJM, AE, MS and DVH conceived the overall studies. WJM, JI, TW, MW, KLS, AE, and DVH managed the production of experimental animal cohorts, the collection of tissues, or the outsourcing of brain tissues for mounting, sectioning and histology. KS performed brain dissociations and immunolabeling, flow cytometry or FACS, and microglial RNA purification. BAF and DVH performed bioinformatic analyses of RNAseq data. S-HL performed the X-34 stains and confocal imaging and calculated plaque diffuseness indices. PC and MK coordinated and analyzed the ELISA measurements of Aβ40 and Aβ42 peptide abundance. GL performed the staining, imaging, and quantification of dot blots for soluble Aβ measurements. WJM, GL, KLS collected plasma and CSF for NfL measurements. GL and WJM performed the costaining and confocal imaging of plaque, microglia, and dystrophic axons or ApoE. HN and OF performed or managed all imaging and analysis of entire sections, and analysis of plaque diffuseness in confocal images with X-34 stains. AG, AJK and JE designed, conducted, or analyzed the two-photon imaging for dendritic spine analysis and volumetric plaque measurements. WJM performed all statistical analyses for stained sections and ELISAs. WJM and DVH wrote the manuscript with input from co-authors.
REFERENCES

Andorfer, C., Y. Kress, M. Espinoza, R. de Silva, K.L. Tucker, Y.A. Barde, K. Duff, and P. Davies. 2003. Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. *J Neurochem.* 86:582-590.

Bacioglu, M., L.F. Maia, O. Preische, J. Schelle, A. Apel, S.A. Kaeser, M. Schweighauser, T. Eninger, M. Lambert, A. Pilotto, D.R. Shimshek, U. Neumann, P.J. Kahle, M. Staufenbiel, M. Neumann, W. Maetzler, J. Kuhle, and M. Jucker. 2016. Neurofilament Light Chain in Blood and CSF as Marker of Disease Progression in Mouse Models and in Neurodegenerative Diseases. *Neuron.* 91:56-66.

Bemiller, S.M., T.J. McCray, K. Allan, S.V. Formica, G. Xu, G. Wilson, O.N. Kokiko-Coehran, S.D. Crish, C.A. Lasagna-Reeves, R.M. Ransohoff, G.E. Landreth, and B.T. Lamb. 2017. TREM2 deficiency exacerbates tau pathology through dysregulated kinase signaling in a mouse model of tauopathy. *Mol Neurodegener.* 12:74.

Berger, H., A. Wodarz, and A. Borchers. 2017. PTK7 Faces the Wnt in Development and Disease. *Front Cell Dev Biol.* 5:31.

Boerboom, D., L.D. White, S. Dalle, J. Courty, and J.S. Richards. 2006. Dominant-stable beta-catenin expression causes cell fate alterations and Wnt signaling antagonist expression in a murine granulosa cell tumor model. *Cancer Res.* 66:1964-1973.

Bouchon, A., C. Hernandez-Munain, M. Cella, and M. Colonna. 2001. A DAP12-mediated pathway regulates expression of CC chemokine receptor 7 and maturation of human dendritic cells. *J Exp Med.* 194:1111-1122.

Buchman, J.J., O. Durak, and L.H. Tsai. 2011. ASPM regulates Wnt signaling pathway activity in the developing brain. *Genes Dev.* 25:1909-1914.

Busby, V., S. Goossens, P. Nowotny, G. Hamilton, S. Smemo, D. Harold, D. Turic, L. Jehu, A. Myers, M. Womick, D. Woo, D. Compton, L.M. Doil, K.M. Tacey, S. Al-Saraj, R. Killick, S. Pickering-Brown, P. Moore, P. Hollingworth, N. Archer, C. Foy, S. Walter, C. Lendon, T. Iwatsubo, J.C. Morris, J. Norton, D. Mann, B. Janssens, J. Hardy, M. O’Donovan, L. Jones, J. Williams, P. Holmans, M.J. Owen, A. Grupe, J. Powell, J. van Hengel, A. Goate, F. Van Roy, and S. Lovestone. 2004. Alpha-T-catenin is expressed in human brain and interacts with the Wnt signaling pathway but is not responsible for linkage to chromosome 10 in Alzheimer’s disease. *Neuromolecular Med.* 5:133-146.

Campbell, S.K., R.C. Switzer, and T.L. Martin. 1987. Alzheimer’s plaques and tangles: a controlled and enhanced silver staining method. Soc. Neuroscience abstract, 13:678 (see also U.S. Patent 5192688).

Cella, M., C. Buonsanti, C. Strader, T. Kondo, A. Salmaggi, and M. Colonna. 2003. Impaired differentiation of osteoclasts in TREM-2-deficient individuals. *J Exp Med.* 198:645-651.

Condello, C., P. Yuan, A. Schain, and J. Grutzendler. 2015. Microglia constitute a barrier that prevents neurotoxic protofibrillar Abeta42 hotspots around plaques. *Nat Commun.* 6:6176.

D’Amore, J.D., S.T. Kajdasz, M.E. McLellan, B.J. Bacsukai, E.A. Stern, and B.T. Hyman. 2003. In vivo multiphoton imaging of a transgenic mouse model of Alzheimer disease reveals marked thioflavine-S-associated alterations in neurite trajectories. *J Neuropathol Exp Neurol.* 62:137-145.
de Olmos, J.S., C.A. Beltramino, and S. de Olmos de Lorenzo. 1994. Use of an amino-cupric-silver technique for the detection of early and semiacute neuronal degeneration caused by neurotoxicants, hypoxia, and physical trauma. *Neurotoxicol Teratol.* 16:545-561.

Deczkowska, A., H. Keren-Shaul, A. Weiner, M. Colonna, M. Schwartz, and I. Amit. 2018. Disease-Associated Microglia: A Universal Immune Sensor of Neurodegeneration. *Cell.* 173:1073-1081.

Devotta, A., C.S. Hong, and J.P. Saint-Jeannet. 2018. Dkk2 promotes neural crest specification by activating Wnt/beta-catenin signaling in a GSK3beta independent manner. *Elife.* 7.

Diep, D.B., N. Hoen, M. Backman, O. Machon, and S. Krauss. 2004. Characterisation of the Wnt antagonists and their response to conditionally activated Wnt signalling in the developing mouse forebrain. *Brain Res Dev Brain Res.* 153:261-270.

Diks, S.H., R.J. Bink, S. van de Water, J. Joore, C. van Rooijen, F.J. Verbeek, J. den Hertog, M.P. Peppelenbosch, and D. Zivkovic. 2006. The novel gene asb11: a regulator of the size of the neural progenitor compartment. *J Cell Biol.* 174:581-592.

Dunn, K.W., M.M. Kamocka, and J.H. McDonald. 2011. A practical guide to evaluating colocalization in biological microscopy. *Am J Physiol Cell Physiol.* 300:C723-742.

Focke, C., T. Blume, B. Zott, Y. Shi, M. Deussing, F. Peters, C. Schmidt, G. Kleinberger, S. Lindner, F.J. Gildehaus, L. Beyer, B. von Ungern-Sternberg, P. Bartenstein, L. Ozmen, K. Baumann, M.M. Dorostkar, C. Haass, H. Adelsberger, J. Herms, A. Rominger, and M. Brendel. 2019. Early and Longitudinal Microglial Activation but Not Amyloid Accumulation Predicts Cognitive Outcome in PS2APP Mice. *J Nucl Med.* 60:548-554.

Fonseca, M.I., J. Zhou, M. Botto, and A.J. Tenner. 2004. Absence of C1q leads to less neuropathy in transgenic mouse models of Alzheimer's disease. *J Neurosci.* 24:6457-6465.

Friedman, B.A., K. Srinivasan, G. Ayalon, W.J. Meilandt, H. Lin, M.A. Huntley, Y. Cao, S.H. Lee, P.C.G. Haddick, H. Ngu, Z. Modrusan, J.L. Larson, J.S. Kaminker, M.P. van der Brug, and D.V. Hansen. 2018. Diverse Brain Myeloid Expression Profiles Reveal Distinct Microglial Activation States and Aspects of Alzheimer's Disease Not Evident in Mouse Models. *Cell Rep.* 22:832-847.

Gage, P.J., M. Qian, D. Wu, and K.I. Rosenberg. 2008. The canonical Wnt signaling antagonist DKK2 is an essential effector of PITX2 function during normal eye development. *Dev Biol.* 317:310-324.

Gowrishankar, S., P. Yuan, Y. Wu, M. Schrag, S. Paradise, J. Grutzendler, P. De Camilli, and S.M. Ferguson. 2015. Massive accumulation of luminal protease-deficient axonal lysosomes at Alzheimer's disease amyloid plaques. *Proc Natl Acad Sci U S A.* 112:E3699-3708.

Gratuze, M., C.E.G. Leyns, and D.M. Holtzman. 2018. New insights into the role of TREM2 in Alzheimer's disease. *Mol Neurodegener.* 13:66.

Griciuc, A., S. Patel, A.N. Federico, S.H. Choi, B.J. Innes, M.K. Oram, G. Cereghetti, D. McGinty, A. Anselmo, R.I. Sadreyev, S.E. Hickman, J. El Khoury, M. Colonna, and R.E. Tanzi. 2019. TREM2 Acts Downstream of CD33 in Modulating Microglial Pathology in Alzheimer's Disease. *Neuron.* 103:820-835 e827.

Guerreiro, R., A. Wojtas, J. Bras, M. Carrasquillo, E. Rogaeva, E. Majounie, C. Cruchaga, C. Sassi, J.S. Kauwe, S. Younkin, L. Hazrati, J. Collinge, J. Pocock, T. Lashley, J. Williams,
J.C. Lambert, P. Amouyel, A. Goate, R. Rademakers, K. Morgan, J. Powell, P. St George-Hyslop, A. Singleton, J. Hardy, and G. Alzheimer Genetic Analysis. 2013. TREM2 variants in Alzheimer’s disease. *N Engl J Med*. 368:117-127.

Haass, C., and D.J. Selkoe. 2007. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer’s amyloid beta-peptide. *Nat Rev Mol Cell Biol*. 8:101-112.

Hamelin, L., J. Lagarde, G. Dorothée, C. Leroy, M. Labit, R.A. Comley, L.C. de Souza, H. Corne, L. Dauphinot, M. Bertoux, B. Dubois, P. Gervais, O. Colliot, M.C. Potier, M. Bottlaender, M. Sarazin, and I. Clinical. 2016. Early and protective microglial activation in Alzheimer’s disease: a prospective study using 18F-DPA-714 PET imaging. *Brain*. 139:1252-1264.

Hansen, D.V., J.E. Hanson, and M. Sheng. 2018. Microglia in Alzheimer’s disease. *J Cell Biol*. 217:459-472.

He, Z., J.L. Guo, J.D. McBride, S. Narasimhan, H. Kim, L. Changolkar, B. Zhang, R.J. Gathagan, C. Yue, C. Dengler, A. Stieber, M. Nitla, D.A. Coulter, T. Abel, K.R. Brunden, J.Q. Trojanowski, and V.M. Lee. 2018. Amyloid-beta plaques enhance Alzheimer’s brain tau-seeded pathologies by facilitating neuritic plaque tau aggregation. *Nat Med*. 24:29-38.

Heneka, M.T., M.J. Carson, J. El Khoury, G.E. Landreth, F. Brosseron, D.L. Feinstein, A.H. Jacobs, T. Wyss-Coray, J. Vitorica, R.M. Ransohooff, K. Herrup, S.A. Frautschy, B. Finsen, G.C. Brown, A. Verkhovsky, K. Yamanaka, J. Koistinaho, E. Latz, A. Halle, G.C. Petzold, T. Town, D. Morgan, M.L. Shihohara, V.H. Perry, C. Holmes, N.G. Bazan, D.J. Brookes, S. Hunot, B. Joseph, N. Deigendesch, O. Garaschuk, E. Boddeke, C.A. Dinarello, J.C. Breitner, G.M. Cole, D.T. Golenbock, and M.P. Kummer. 2015. Neuroinflammation in Alzheimer’s disease. *Lancet Neurol*. 14:388-405.

Hong, S., V.F. Beja-Glasser, B.M. Nfonoyim, A. Frouin, S. Li, S. Ramakrishnan, K.M. Merry, Q. Shi, A. Rosenthal, B.A. Barres, C.A. Lemere, D.J. Selkoe, and B. Stevens. 2016. Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science*. 352:712-716.

Hsieh, J.C., L. Kodjabachian, M.L. Rebert, A. Rattner, P.M. Smallwood, C.H. Samos, R. Nusse, I.B. Dawid, and J. Nathans. 1999. A new secreted protein that binds to Wnt proteins and inhibits their activities. *Nature*. 398:431-436.

Itzhaki, R.F., R. Lathe, B.J. Balin, M.J. Ball, E.L. Bearer, H. Braak, M.J. Bullido, C. Carter, M. Clerici, S.L. Cosby, K. Del Tredici, H. Field, T. Fufok, C. Grassi, W.S. Griffin, J. Haas, A.R. Kamer, D.B. Kell, F. Liciastro, L. Letenneur, H. Lovheim, R. Mancuso, J. Miklossy, C. Otth, A.T. Palamara, G. Perry, C. Preston, E. Pretorius, T. Strandberg, N. Tabet, S.D. Taylor-Robinson, and J.A. Whittum-Hudson. 2016. Microbes and Alzheimer’s Disease. *J Alzheimers Dis*. 51:979-984.

Jack, C.R., Jr., H.J. Wiste, C.G. Schwarz, V.J. Lowe, M.L. Senjem, P. Vemuri, S.D. Weigand, T.M. Jack, C.R., Jr., H.J. Wiste, C.G. Schwarz, V.J. Lowe, M.L. Senjem, P. Vemuri, S.D. Weigand, T.M. Therneau, D.S. Knopman, J.L. Gunter, D.T. Jones, J. Graff-Radford, K. Kantarcı, R.O. Roberts, M.M. Mielke, M.M. Machulda, and R.C. Petersen. 2018. Longitudinal tau PET in ageing and Alzheimer’s disease. *Brain*. 141:1517-1528.

Jacobs, H.I.L., T. Hedden, A.P. Schultz, J. Sepulcre, R.D. Perea, R.E. Amariglio, K.V. Papp, D.M. Rentz, R.A. Sperling, and K.A. Johnson. 2018. Structural tract alterations predict downstream tau accumulation in amyloid-positive older individuals. *Nat Neurosci*. 21:424-431.
Jay, T.R., A.M. Hirsch, M.L. Broihier, C.M. Miller, L.E. Neilson, R.M. Ransohoff, B.T. Lamb, and G.E. Landreth. 2017a. Disease Progression-Dependent Effects of TREM2 Deficiency in a Mouse Model of Alzheimer’s Disease. *J Neurosci*. 37:637-647.

Jay, T.R., C.M. Miller, P.J. Cheng, L.C. Graham, S. Bemiller, M.L. Broihier, G. Xu, D. Margovecious, J.C. Karlo, G.L. Sousa, A.C. Coteur, O. Butovsky, L. Bekris, S.M. Staugaitis, J.B. Leverenz, S.W. Pimplikar, G.E. Landreth, G.R. Howell, R.M. Ransohoff, and B.T. Lamb. 2015. TREM2 deficiency eliminates TREM2+ inflammatory macrophages and ameliorates pathology in Alzheimer’s disease mouse models. *J Exp Med*. 212:287-295.

Jay, T.R., V.E. von Saucken, and G.E. Landreth. 2017b. TREM2 in Neurodegenerative Diseases. *Mol Neurodegener*. 12:56.

Jonsson, T., H. Stefansson, S. Steinberg, I. Jonsdottir, P.V. Jonsson, J. Snaedal, S. Bjornsson, J. Huttenlocher, A.I. Levey, J.J. Lah, D. Rujescu, H. Hampel, I. Giegling, O.A. Andreassen, K. Engedal, I. Ulstein, S. Djurovic, C. Ibrahim-Verbaas, A. Hofman, M.A. Ikram, C.M. van Duijn, U. Thorsteinsdottir, A. Kong, and K. Stefansson. 2013. Variant of TREM2 associated with the risk of Alzheimer’s disease. *N Engl J Med*. 368:107-116.

Kallop, D.Y., W.J. Meilandt, A. Gogineni, C. Easley-Neal, T. Wu, A.M. Jubb, M. Yaylaoglu, M. Shamloo, M. Tessier-Lavigne, K. Scarcher-Levie, and R.M. Weimer. 2014. A death receptor 6-amyloid precursor protein pathway regulates synapse density in the mature CNS but does not contribute to Alzheimer’s disease-related pathophysiology in murine models. *J Neurosci*. 34:6425-6437.

Kang, S.S., A. Kurti, K.E. Baker, C.C. Liu, M. Colonna, J.D. Ulrich, D.M. Holtzman, G. Bu, and J.D. Fryer. 2018. Behavioral and transcriptomic analysis of Trem2-null mice: not all knockout mice are created equal. *Hum Mol Genet*. 27:211-223.

Karasawa, T., H. Yokokura, J. Kitajewski, and P.J. Lombroso. 2002. Frizzled-9 is activated by Wnt-2 and functions in Wnt/beta-catenin signaling. *J Biol Chem*. 277:37479-37486.

Keren-Shaul, H., A. Spinrad, A. Weiner, O. Matcovitch-Natan, R. Dvir-Szternfeld, T.K. Ulland, E. David, K. Baruch, D. Lara-Astaiso, B. Toth, S. Itzkovitz, M. Colonna, M. Schwartz, and I. Amit. 2017. A Unique Microglia Type Associated with Restricting Development of Alzheimer’s Disease. *Cell*. 169:1276-1290 e1217.

Khalil, M., C.E. Teunissen, M. Otto, F. Piehl, M.P. Sormani, T. Gattringer, C. Barro, L. Kappos, M. Comabella, F. Fazekas, A. Petzold, K. Blennow, H. Zetterberg, and J. Kuhle. 2018. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol*. 14:577-589.

Kim, J., A.E. Eltorai, H. Jiang, F. Liao, P.B. Verghese, J. Kim, F.R. Stewart, J.M. Basak, and D.M. Holtzman. 2012. Anti-apoE immunotherapy inhibits amyloid accumulation in a transgenic mouse model of Abeta amyloidosis. *J Exp Med*. 209:2149-2156.

Klein, W.L. 2002. Abeta toxicity in Alzheimer’s disease: globular oligomers (ADDLs) as new vaccine and drug targets. *Neurochem Int*. 41:345-352.

Kluck, W.E., B.J. Bacskaia, C.A. Mathis, S.T. Kajdasz, M.E. McLellan, M.P. Frosch, M.L. Debnath, D.P. Holt, Y. Wang, and B.T. Hyman. 2002. Imaging Abeta plaques in living transgenic mice with multiphoton microscopy and methoxy-X04, a systemically administered Congo red derivative. *J Neuropathol Exp Neurol*. 61:797-805.

Krasemann, S., C. Madore, R. Cialic, C. Baufeld, N. Calcagno, R. El Fatimy, L. Beckers, E. O’Loughlin, Y. Xu, Z. Fanek, D.J. Greco, S.T. Smith, G. Tweet, Z. Humulock, T. Zrzavy, P. Conde-Sanroman, M. Gacias, Z. Weng, H. Chen, E. Tjon, F. Mazaheri, K. Hartmann, A. ...
Madi, J.D. Ulrich, M. Glatzel, A. Worthmann, J. Heeren, B. Budnik, C. Lemere, T. Ikezu, F.L. Heppner, V. Litvak, D.M. Holtzman, H. Lassmann, H.L. Weiner, J. Ochando, C. Haass, and O. Butovsky. 2017. The TREM2-APOE Pathway Drives the Transcriptional Phenotype of Dysfunctional Microglia in Neurodegenerative Diseases. *Immunity*. 47:566-581 e569.

Krstic, D., and I. Knuesel. 2013. The airbag problem—a potential culprit for bench-to-bedside translational efforts: relevance for Alzheimer’s disease. *Acta Neuropathol Commun*. 1:62.

Lai, J.P., A.M. Oseini, C.D. Moser, C. Yu, S.F. Elsawa, C. Hu, I. Nakamura, T. Han, I. Aderca, H. Isomoto, M.M. Garrity-Park, A.M. Shire, J. Li, S.O. Sanderson, A.A. Adjei, M.E. Fernandez-Zapico, and L.R. Roberts. 2010. The oncogenic effect of sulfatase 2 in human hepatocellular carcinoma is mediated in part by glypican 3-dependent Wnt activation. *Hepatology*. 52:1680-1689.

Law, C.W., Y.S. Chen, W. Shi, and G.K. Smyth. 2014. voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biol*. 15.

Le Pichon, C.E., S.L. Dominguez, H. Solanoy, H. Ngu, N. Lewin-Koh, M. Chen, J. Eastham-Anderson, R. Watts, and K. Searce-Levie. 2013. EGFR inhibitor erlotinib delays disease progression but does not extend survival in the SOD1 mouse model of ALS. *PLoS One*. 8:e62342.

Lessard, C.B., S.L. Malnik, Y. Zhou, T.B. Ladd, P.E. Cruz, Y. Ran, T.E. Mahan, P. Chakrabaty, D.M. Holtzman, J.D. Ulrich, M. Colonna, and T.E. Golde. 2018. High-affinity interactions and signal transduction between Abeta oligomers and TREM2. *EMBO Mol Med*. 10.

Leyns, C.E.G., M. Gratuze, S. Narasimhan, N. Jain, L.J. Koscal, H. Jiang, M. Manis, M. Colonna, V.M.Y. Lee, J.D. Ulrich, and D.M. Holtzman. 2019. TREM2 function impedes tau seeding in neuritic plaques. *Nat Neurosci*.

Leyns, C.E.G., J.D. Ulrich, M.B. Finn, F.R. Stewart, L.J. Koscal, J. Remolina Serrano, G.O. Robinson, E. Anderson, M. Colonna, and D.M. Holtzman. 2017. TREM2 deficiency attenuates neuroinflammation and protects against neurodegeneration in a mouse model of tauopathy. *Proc Natl Acad Sci U S A*. 114:11524-11529.

Lin, Z., C. Gao, Y. Ning, X. He, W. Wu, and Y.G. Chen. 2008. The pseudoreceptor BMP and activin membrane-bound inhibitor positively modulates Wnt/beta-catenin signaling. *J Biol Chem*. 283:33053-33058.

Love, M.I., W. Huber, and S. Anders. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 15.

Mao, B., and C. Niehrs. 2003. Kremen2 modulates Dickkopf2 activity during Wnt/LRP6 signaling. *Gene*. 302:179-183.

Mazaheri, F., N. Snaidero, G. Kleinberger, C. Madore, A. Daria, G. Werner, S. Krasemann, A. Capell, D. Trumbach, W. Wurst, B. Brunner, S. Bultmann, S. Tahirovic, M. Kerschensteiner, T. Migsd, O. Butovsky, and C. Haass. 2017. TREM2 deficiency impairs chemotaxis and microglial responses to neuronal injury. *EMBO Rep*. 18:1186-1198.

Meilandt, W.J., J.A. Maloney, J. Imperio, G. Lalhezadeh, T. Earr, S. Crowell, T.W. Bainbridge, Y. Lu, J.A. Ernst, R.N. Fuji, and J. Atwal. 2019. Characterization of the selective in vitro and in vivo binding properties of crenezumab to oligomeric Aβ. *Alzheimers Res Ther*. In press.
Mi, H., A. Muruganujan, D. Ebert, X. Huang, and P.D. Thomas. 2019. PANTHER version 14: more genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. *Nucleic Acids Res.* 47:D419-D426.

Morris, G.P., I.A. Clark, and B. Vissel. 2014. Inconsistencies and controversies surrounding the amyloid hypothesis of Alzheimer’s disease. *Acta Neuropathol Commun.* 2:135.

Olmos-Alonso, A., S.T. Schetters, S. Sri, K. Askew, R. Mancuso, M. Vargas-Caballero, C. Holscher, V.H. Perry, and D. Gomez-Nicola. 2016. Pharmacological targeting of CSF1R inhibits microglial proliferation and prevents the progression of Alzheimer’s-like pathology. *Brain.* 139:891-907.

Orre, M., W. Kamphuis, L.M. Osborn, A.H.P. Jansen, L. Kooijman, K. Bossers, and E.M. Hol. 2014. Isolation of glia from Alzheimer’s mice reveals inflammation and dysfunction. *Neurobiol Aging.* 35:2746-2760.

Ozmen, L., A. Albientz, C. Czech, and H. Jacobsen. 2009. Expression of transgenic APP mRNA is the key determinant for beta-amyloid deposition in PS2APP transgenic mice. *Neurodegener Dis.* 6:29-36.

Paloneva, J., J. Mandelin, A. Kiallainen, T. Bohling, J. Prudlo, P. Hakola, M. Haltia, Y.T. Konttinen, and L. Peltonen. 2003. DAP12/TREM2 deficiency results in impaired osteoclast differentiation and osteoporotic features. *J Exp Med.* 198:669-675.

Parhizkar, S., T. Arzberger, M. Brendel, G. Kleinberger, M. Deussing, C. Focke, B. Nuscher, M. Xiong, A. Ghasemigharagoz, N. Katzmarski, S. Krasmann, S.F. Lichtenhaler, S.A. Muller, A. Colombo, L.S. Monasor, S. Tahirovic, J. Hermos, M. Willem, N. Pettkus, O. Butovsky, P. Bartenstein, A. Rominger, A. Erturk, S.A. Grathwohl, J.J. Neher, D.M. Holtzman, M. Meyer-Luehmann, and C. Haass. 2019. Loss of TREM2 function increases amyloid seeding but reduces plaque-associated ApoE. *Nat Neurosci.* 22:191-204.

Park, J., I. Wetzel, I. Marriott, D. Dreau, C. D’Avanzo, D.Y. Kim, R.E. Tanzi, and H. Cho. 2018. A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer’s disease. *Nat Neurosci.* 21:941-951.

Poliani, P.L., Y. Wang, E. Fontana, M.L. Robinette, Y. Yamanishi, S. Gilfillan, and M. Colonna. 2015. TREM2 sustains microglial expansion during aging and response to demyelination. *J Clin Invest.* 125:2161-2170.

Pooler, A.M., M. Polodyo, E.A. Maury, S.B. Nicholls, S.M. Reddy, S. Wegmann, C. William, L. Saqran, O. Cagsal-Getkin, R. Pitstick, D.R. Beier, G.A. Carlson, T.L. Spires-Jones, and B.T. Hyman. 2015. Amyloid accelerates tau propagation and toxicity in a model of early Alzheimer’s disease. *Acta Neuropathol Commun.* 3:14.

Shi, Q., S. Chowdhury, R. Ma, K.X. Le, S. Hong, B.J. Caldarone, B. Stevens, and C.A. Lemere. 2017. Complement C3 deficiency protects against neurodegeneration in aged plaque-rich APP/PS1 mice. *Sci Transl Med.* 9.

Spangenberg, E.E., R.J. Lee, A.R. Najafi, R.A. Rice, M.R. Elmore, M. Burton-Jones, B.L. West, and K.N. Green. 2016. Eliminating microglia in Alzheimer’s mice prevents neuronal loss without modulating amyloid-beta pathology. *Brain.* 139:1265-1281.

Spires, T.L., and B.T. Hyman. 2004. Neuronal structure is altered by amyloid plaques. *Rev Neurosci.* 15:267-278.

Spires, T.L., M. Meyer-Luehmann, E.A. Stern, P.J. McLean, J. Skoch, P.T. Nguyen, B.J. Bacskai, and B.T. Hyman. 2005. Dendritic spine abnormalities in amyloid precursor protein...
transgenic mice demonstrated by gene transfer and intravital multiphoton microscopy. J Neurosci. 25:7278-7287.

Srinivasan, K., B.A. Friedman, J.L. Larson, B.E. Lauffer, L.D. Goldstein, L.L. Appling, J. Borneo, C. Poon, T. Ho, F. Cai, P. Steiner, M.P. van der Brug, Z. Modrusan, J.S. Kaminker, and D.V. Hansen. 2016. Untangling the brain's inflammatory and neurodegenerative transcriptional responses. Nat Commun. 7:11295.

Switzer, R.C., 3rd. 2000. Application of silver degeneration stains for neurotoxicity testing. Toxicol Pathol. 28:70-83.

Switzer, R.C., S.K. Campbell, and T.M. Murdock. 1993. A histologic method for staining Alzheimer pathology. U.S. Patent 5192688.

Tee, J.M., M.A. Sartori da Silva, A.M. Rygiel, V. Muncan, R. Bink, G.R. van den Brink, P. van Tij, D. Zivkovic, L.L. Kodach, D. Guardavaccaro, S.H. Diks, and M.P. Peppelenbosch. 2012. asb11 is a regulator of embryonic and adult regenerative myogenesis. Stem Cells Dev. 21:3091-3103.

Tomic, J.L., A. Pensalfini, E. Head, and C.G. Glabe. 2009. Soluble fibrillar oligomer levels are elevated in Alzheimer's disease brain and correlate with cognitive dysfunction. Neurobiol Dis. 35:352-358.

Tsai, J., J. Grutzendler, K. Duff, and W.B. Gan. 2004. Fibrillar amyloid deposition leads to local synaptic abnormalities and breakage of neuronal branches. Nat Neurosci. 7:1181-1183.

Turnbull, I.R., S. Gilfillan, M. Cella, T. Aoshi, M. Miller, L. Piccio, M. Hernandez, and M. Colonna. 2006. Cutting edge: TREM-2 attenuates macrophage activation. J Immunol. 177:3520-3524.

Ulland, T.K., and M. Colonna. 2018. TREM2 - a key player in microglial biology and Alzheimer disease. Nat Rev Neurol. 14:667-675.

Ulland, T.K., W.M. Song, S.C. Huang, J.D. Ulrich, A. Sergushichev, W.L. Beatty, A.A. Loboda, Y. Zhou, N.J. Cairns, A. Kambal, E. Loginicheva, S. Gilfillan, M. Cella, H.W. Virgin, E.R. Unanue, Y. Wang, M.N. Artyomov, D.M. Holtzman, and M. Colonna. 2017. TREM2 Maintains Microglial Metabolic Fitness in Alzheimer's Disease. Cell. 170:649-663 e613.

Ulrich, J.D., T.K. Ulland, M. Colonna, and D.M. Holtzman. 2017. Elucidating the Role of TREM2 in Alzheimer's Disease. Neuron. 94:237-248.

Wang, A., P. Das, R.C. Switzer, 3rd, T.E. Golde, and J.L. Jankowsky. 2011. Robust amyloid clearance in a mouse model of Alzheimer's disease provides novel insights into the mechanism of amyloid-beta immunotherapy. J Neurosci. 31:4124-4136.

Wang, Y., M. Cella, K. Mallinson, J.D. Ulrich, K.L. Young, M.L. Robinette, S. Gilfillan, G.M. Krishnan, S. Sudhakar, B.H. Zinselmeyer, D.M. Holtzman, J.R. Cirrito, and M. Colonna. 2015. TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. Cell. 160:1061-1071.

Wang, Y., T.K. Ulland, J.D. Ulrich, W. Song, J.A. Tzaferis, J.T. Hole, P. Yuan, T.E. Mahan, Y. Shi, S. Gilfillan, M. Cella, J. Grutzendler, R.B. DeMattos, J.R. Cirrito, D.M. Holtzman, and M. Colonna. 2016. TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques. J Exp Med. 213:667-675.

Wu, T., B. Dejanovic, V.D. Gandham, A. Gogineni, R. Edmonds, S. Schauer, K. Srinivasan, M.A. Huntley, Y. Wang, T.M. Wang, M. Hedehus, K.H. Barck, M. Stark, H. Ngu, O. Foreman, W.J. Meilandt, J. Elstrott, M.C. Chang, D.V. Hansen, R.A.D. Carano, M. Sheng, and J.E.
Hanson. 2019. Complement C3 Is Activated in Human AD Brain and Is Required for Neurodegeneration in Mouse Models of Amyloidosis and Tauopathy. *Cell Rep.* 28:2111-2123 e2116.

Wu, T.D., J. Reeder, M. Lawrence, G. Becker, and M.J. Brauer. 2016. GMAP and GSNAp for Genomic Sequence Alignment: Enhancements to Speed, Accuracy, and Functionality. *Methods Mol Biol.* 1418:283-334.

Wu, W., A. Glinka, H. Delius, and C. Niehrs. 2000. Mutual antagonism between dickkopf1 and dickkopf2 regulates Wnt/beta-catenin signalling. *Curr Biol.* 10:1611-1614.

Yeh, F.L., D.V. Hansen, and M. Sheng. 2017. TREM2, Microglia, and Neurodegenerative Diseases. *Trends Mol Med.* 23:512-533.

Yeh, F.L., Y. Wang, I. Tom, L.C. Gonzalez, and M. Sheng. 2016. TREM2 Binds to Apolipoproteins, Including APOE and CLU/APOJ, and Thereby Facilitates Uptake of Amyloid-Beta by Microglia. *Neuron.* 91:328-340.

Yoshiyama, Y., M. Higuchi, B. Zhang, S.M. Huang, N. Iwata, T.C. Saito, J. Maeda, T. Suhara, J.Q. Trojanowski, and V.M. Lee. 2007. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron.* 53:337-351.

Yuan, P., C. Condello, C.D. Keene, Y. Wang, T.D. Bird, S.M. Paul, W. Luo, M. Colonna, D. Baddeley, and J. Grutzendler. 2016. TREM2 Haplodeficiency in Mice and Humans Impairs the Microglia Barrier Function Leading to Decreased Amyloid Compaction and Severe Axonal Dystrophy. *Neuron.* 92:252-264.

Zhao, Y., X. Wu, X. Li, L.L. Jiang, X. Gui, Y. Liu, Y. Sun, B. Zhu, J.C. Pina-Crespo, M. Zhang, N. Zhang, X. Chen, G. Bu, Z. An, T.Y. Huang, and H. Xu. 2018. TREM2 Is a Receptor for beta-Amyloid that Mediates Microglial Function. *Neuron.* 97:1023-1031 e1027.

Zheng, H., L. Jia, C.C. Liu, Z. Rong, L. Zhong, L. Yang, X.F. Chen, J.D. Fryer, X. Wang, Y.W. Zhang, H. Xu, and G. Bu. 2017. TREM2 Promotes Microglial Survival by Activating Wnt/beta-Catenin Pathway. *J Neurosci.* 37:1772-1784.

Zulfiqar, S., and G. Tanriover. 2017. beta-Catenin Pathway Is Involved in TREM2-Mediated Microglial Survival. *J Neurosci.* 37:7073-7075.
**FIGURE LEGENDS**

**Figure 1.** Trem2 deletion impairs microglial clustering, activation, and plaque uptake in PS2APP β-amyloidosis model. **A,** Immunohistochemical detection of microglial clustering in transgenic PS2APP mice or non-transgenic (Ntg) controls with either wild type (wt), heterozygous (het) or homozygous knockout (ko) Trem2 alleles. (See Figure 1-1 for analysis of total, rather than clustered, Iba1 signal.) Quantification of the percent area covered by clusters of Iba1+ microglia was measured from coronal sections of female mice at 4 months (mo), 6-7mo, and 12mo ages, and from male mice at 6-7mo and 19-22mo ages. Each data point represents the composite (average) histological score from several sections of an individual mouse. Bars and lines represent mean ± SEM. Significant differences between groups were determined by unpaired t-test for most cohorts, or by ANOVA followed by Tukey’s multiple comparison test for the 19-22mo cohort with three genotypes (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus PS2APP;Trem2wt or as indicated). **B,** Representative low (left, scale bar = 400 μm) and high (right, scale bar = 100 μm) magnification images of Iba1+ staining in 12mo female mice are shown. Iba1+ clusters (red arrowheads) showed reduced presence across Trem2ko mice at all ages. **C** and **D,** Like panels **A** and **B** except analyzing active microglial lysosomes as indicated by CD68 protein expression. **E,** Representative flow cytometry plots measuring CD45 immunoreactivity (low or high) of CD11b+ brain-resident myeloid cells from 14mo PS2APP mice with or without Trem2 and from Ntg mice. **F,** Percent of brain-resident myeloid cells with high CD45 expression measured from several mice of each genotype, with bars and lines representing mean ± SEM. **G** and **H,** Like panels **E** and **F** except analyzing plaque content in brain CD11b+ cells from ~12mo mice injected with methoxy-X04 dye to stain amyloid material. Ntg mice are not plotted in **H** since they have zero methoxy labeling. n=3 in Trem2wt, n=2 in Trem2ko. (Also see Figure 1-1 for histological analysis of methoxy-X04 and Iba1+ microglia colocalization in sections.)

**Figure 2.** Induction of Neurodegeneration-related and Proliferation gene modules and Wnt-related signaling components is impaired in Trem2ko microglia. **A,** Heat map of differentially expressed genes (DEGs) between Trem2wt and Trem2ko microglia from 14mo PS2APP mouse brains (fold change ≥ 2, adjusted p ≤ 0.05), cross-referenced against previously published datasets from the PS2APP (GSE89482), APPswe/PS1de9 (GSE74615), and 5xFAD (GSE65067) models. Each row is a DEG, and each column is a microglial sort from a different mouse. Z-score coloring represents a sample’s distance in standard deviations from the mean.
expression value for a given gene across samples within a dataset. Most of the downregulated
DEGs in Trem2\textsuperscript{ko} microglia were typically induced in models of \( \beta \)-amyloid pathology, and the
majority also showed impaired microglial induction in Trem2\textsuperscript{ko} 5xFAD mice. See Figure 2-1
Extended Data table for a complete list of genome-wide expression values in PS2APP;Trem2\textsuperscript{wt}
and PS2APP;Trem2\textsuperscript{ko} microglia. \( B \), Induction of the Wnt/proliferation regulators \textit{Dkk2}, \textit{Wif1},
\textit{Ctnna3}, and \textit{Asb11} were completely impaired in Trem2\textsuperscript{ko} microglia of PS2APP mice. \( C \), DEGs
identified by GO analysis as “Positive Regulators of Wnt Signaling” with reduced expression in
PS2APP;Trem2\textsuperscript{ko} microglia included \textit{Fzd9}, \textit{Sulf2}, \textit{Bambi}, \textit{Ptk7}, \textit{Aspm}, and \textit{Dkk2}. Bars and lines
in \( B \) and \( C \) represent mean ± SEM, with each data point representing microglial gene
expression level from a given mouse. For genes depicted in \( B \) and \( C \), see also Figure 2-2
showing microglial expression in the 5xFAD model with or without Trem2 (data from
independent investigators). \( D \), (top) Analysis of previously published expression profiles from
the PS2APP model (GSE89482) indicated that six gene expression modules for brain myeloid
cells (defined in Friedman et al., 2018) showed altered expression in microglia from brains with
\( \beta \)-amyloid pathology. (bottom) Analysis of Trem2\textsuperscript{ko} and Trem2\textsuperscript{wt} microglia expression profiles
from PS2APP mice showed that the Neurodegeneration-related and Proliferation gene sets
showed significant Trem2 dependence. (See also Figure 2-2 for data from independent
investigators showing enriched expression of Proliferation module in 5xFAD model microglia
and its dependence on Trem2 for full induction. See also Figure 2-3 for heat map displays of all
individual genes in each module.) Each data point represents a gene set expression score for
microglia isolated from a given mouse. Lines represent mean ± SEM. Asterisks represent \( p \)
values (*\( p < 0.05 \), **\( p < 0.01 \), ***\( p < 0.001 \), ****\( p < 0.0001 \)) from two-tailed t-tests assuming unequal
variance between groups. \( E \), 4-way plot of Neurodegeneration-related gene set, with each point
representing a gene’s fold change in expression between PS2APP vs. non-transgenic microglia
on the x-axis (red and blue points, adjusted \( p < 0.05 \)) and between PS2APP;Trem2\textsuperscript{ko} vs.
PS2APP;Trem2\textsuperscript{wt} microglia on the y-axis (green and blue points, adjusted \( p < 0.05 \)). Blue points
showed significant differential expression in both datasets. Tiny black points represent genes
not differentially expressed in either dataset.

Figure 3. \( \textit{Trem2} \) deletion shows age- and sex-dependent effects on amyloid plaque pathology,
with reduced plaque accumulation at later ages. \( A \) and \( B \), Amyloid plaque was visualized using
the Campbell-Switzer silver stain method in non-transgenic (Ntg) controls or transgenic
PS2APP mice with either wild type (wt), heterozygous (het) or homozygous knockout (ko)
\textit{Trem2} alleles. Quantification of the percent area covered by amyloid plaque was measured
from coronal sections of indicated sex and age. Representative low (left, scale bar = 400 μm) and high (right, scale bar = 100 μm) magnification images of amyloid stains are shown. In the absence of Trem2, plaque loads were elevated in 6-7mo females but reduced in 12mo females and in 19-22mo males. See also Figure 3-1 for two-photon imaging of methoxy-X04-labeled plaque in somatosensory cortex of 6mo females and 8mo males. C, The Campbell-Switzer silver stain turns highly mature plaque cores amber (red arrowheads). Quantification plot (left) and representative images (right, scale bar = 20 μm) of amber core frequency in 19-22mo male mice. Bars represent mean ± SEM. Significant differences between groups were determined by unpaired t-test or ANOVA followed by Tukey’s multiple comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus PS2APP;Trem2wt or as indicated).

**Figure 4.** Trem2 deletion impairs the consolidation of β-amyloid into dense plaque. A, β-amyloid plaques were visualized by staining X-34 staining and confocal z-stack imaging. Representative maximum projection images of X-34+ plaques from 12mo PS2APP females of different Trem2 genotypes are shown (top). High magnification images show a representative plaque from each genotype with the outlined masks used to delineate the compact core (green) and compact+diffuse (blue) areas of the plaque (bottom). Scale bars, 20 μm. B, The diffuseness index (see Methods) of the X-34+ plaques in cohorts of the indicated age and sex were quantified. Each data point represents one animal’s plaque diffuseness index averaged from 3-4 fields of view. Bars represent mean ± SEM. Significant differences between groups were determined by unpaired t-test or ANOVA followed by Tukey’s multiple comparison test (**p<0.01, ***p<0.001, ****p<0.0001 versus PS2APP;Trem2wt or as indicated).

**Figure 5.** Trem2 deletion increases the Aβ42:Aβ40 ratio and soluble fibrillar Aβ oligomers in PS2APP brains. A and B, Frozen hippocampal tissues were homogenized and processed for measuring the abundance of Aβ40 and Aβ42 peptides by ELISA in the soluble (TBS) and insoluble (GuHCl) fractions. The ratio of Aβ42:Aβ40 in the TBS (A) and GuHCl (B) fractions are shown. Each data point represents one animal’s Aβ42:Aβ40 ratio. For individual Aβ40 and Aβ42 peptide measurements, see Figure 5-1. C, Image of non-denaturing dot-blot of hippocampal soluble TBS homogenates from 12mo female animals immunostained with the fibrillar oligomeric Aβ antibody OC (green) and control β-actin antibody (red). Squares outline PS2APP;Trem2wt samples and circles outline PS2APP;Trem2ko samples. # identifies a control sample from a PS2APP;Bace1ko mouse. D, Signal intensity ratios of OC antibody to actin
antibody are plotted for the dot blot shown in C. The dotted line demarcates the OC:actin ratio for a control sample from a PS2APP;Bace1\(^{ko}\) mouse. E, The A\(\beta\)42:A\(\beta\)40 ratio (from A) and normalized OC\(^+\) dot blot signal (from D) in the TBS soluble fraction showed a significant positive correlation (linear regression; df(1,28) F=8.63, p<0.01). F, Same as C, except immunostained with pan-A\(\beta\) antibody 6E10 (green) and control GAPDH antibody (red). G, Signal intensity ratios of A\(\beta\) 6E10 antibody to control GAPDH antibody are plotted for the dot blot shown in F. The PS2APP;Bace1\(^{ko}\) control sample still has substantial 6E10 signal (see dotted line) since the N-terminal A\(\beta\) residues recognized by 6E10 are present in soluble APP when \(\alpha\)-secretase is the responsible enzyme. H, The A\(\beta\)42:A\(\beta\)40 ratio (from A) and normalized A\(\beta\) 6E10 dot blot signal (from G) in the TBS soluble fraction showed a significant negative correlation (linear regression; df(1,28) F=4.98, p<0.05). Bars represent mean ± SEM. Significant differences between groups were determined by unpaired t-test or ANOVA followed by Tukey’s multiple comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus PS2APP;Trem2\(^{wt}\) or as indicated).

Figure 6. ApoE markedly accumulates on plaques and in microglia in PS2APP;Trem2\(^{ko}\) females. A, Plaque-associated ApoE signal was quantified for each animal in each cohort using 2-3 sections per animal, with each section having a range of ~50-800 plaques contributing to the analysis. Significant increases were observed in 6-7mo and 12mo female (f) PS2APP;Trem2\(^{ko}\) mice but not in males (m). B, Representative low magnification images of the hippocampus (top row) and high magnification images of the subiculum (enlarged from the boxed regions) from 12mo female brain sections stained with methoxy-X04 to label plaque (blue), anti-Iba1 to label microglia (green), and anti-ApoE (red). Arrows point to examples of plaques with intense ApoE staining, which are atypical in Trem2\(^{wt}\) females but typical in Trem2\(^{ko}\) females at this age. C, Representative 20x maximum projection of confocal z-stacks imaged from cortex, highlighting ApoE (red) localization in microglia (Iba1, green) and plaque (blue). Microglia with small ApoE puncta (arrows) are typical in Trem2\(^{wt}\), while microglia with enlarged ApoE structures (arrowheads) are frequent in Trem2\(^{ko}\). Plaques with strong ApoE labeling are more common in Trem2\(^{ko}\) (asterisks). D, Analysis of the fraction of Iba1 signal that overlaps with ApoE staining (Manders colocalization coefficient) in 12mo PS2APP females revealed increased colocalization in Trem2\(^{ko}\) microglia. E, RNA-Seq profiles of microglia FACS-purified from brains of 14mo PS2APP mice showed no difference in Apoe expression between Trem2\(^{wt}\) and Trem2\(^{ko}\) microglia (MG). F, Expression profiles of microglia FACS-purified from brains of 8mo non-transgenic or 5xFAD mice revealed strong Apoe induction by \(\beta\)-amyloid pathology in both Trem2\(^{wt}\) and Trem2\(^{ko}\) microglia, with induction in 5xFAD;Trem2\(^{ko}\) relative to 5xFAD;Trem2\(^{wt}\)
microglia being 2-fold reduced in one dataset (GSE132508, RNA-Seq) and not significantly
different in another (GSE65067, microarray). Bars represent mean ± SEM. Significant
differences between groups were determined by unpaired t-test or ANOVA followed by Tukey’s
multiple comparison test (*p<0.05, **p<0.01, ****p<0.0001 versus PS2APP;Trem2wt or as
indicated).

Figure 7. Trem2 deletion exacerbates plaque-proximal axonal dystrophy. A, Representative
images from 12mo brain sections stained with methoxy-X04 to label plaque, anti-Iba1 to label
microglia, and anti-Lamp1 to label dystrophic axons around plaque. B, Microglial clustering
around plaque is impaired in Trem2-deficient mice. Plaque-associated Iba1 signal was
quantified for each animal in each cohort using 2-3 sections per animal, with each section
having hundreds or thousands of plaques contributing to the analysis. C, Axonal dystrophy per
plaque is exacerbated in Trem2-deficient mice. Plaque-associated Lamp1 signal was quantified
for each animal in a similar manner as Iba1 signal in panel B. D, Total axonal dystrophy is
exacerbated in Trem2-deficient mice. Total Lamp1 signal in each section was quantified,
and each data point represents the average score from 2-3 sections per animal. Bars represent
mean ± SEM. Significant differences between groups were determined by unpaired t-test or
ANOVA followed by Tukey’s multiple comparison test (**p<0.01, ***p<0.001, ****p<0.0001
versus PS2APP;Trem2wt or as indicated).

Figure 8. Disintegrative degeneration staining and CSF NfL measurements reveal exacerbated
neuronal damage in Trem2-deficient mice at later ages. A, Quantification of sections from non-
transgenic (Ntg) and PS2APP mice with indicated Trem2 genotypes stained using an amino-
cupric-silver staining method that labels degenerative neuronal processes. Each data point
represents the average %area covered/section for ~10 sections per animal. B, Representative
low (left, scale bar = 400 μm) and high (right, scale bar = 100 μm) magnification images from
the 12mo female and 19-22mo male cohorts are shown. Degenerative signal is apparent in
plaque-accompanying foci (red arrowheads) throughout the cortex and hippocampus and in
certain white matter tracts including the corpus callosum (cc). C, Plasma (left) and CSF (right)
NfL was measured from a separate, mixed sex cohort of 12mo PS2APP;Trem2wt and
PS2APP;Trem2ko mice. Bars represent mean ± SEM. Significant differences between groups
were determined by unpaired t-test or ANOVA followed by Tukey’s multiple comparison test
(**p<0.01, ****p<0.0001 versus PS2APP;Trem2wt or as indicated).
**Figure 9.** Trem2 deletion exacerbates dendritic spine loss near plaque.  

*A*, Intact brains from 6mo female PS2APP mice with wild type (wt), knockout (ko), or heterozygous (het) Trem2 genotypes and carrying the Thy1::GFP-M transgene, which sparsely labels excitatory cortical neurons, were imaged in the somatosensory cortex using two-photon microscopy. Dendritic shafts proximal to plaque (within 20 μm) or distally located (≥ 100 μm from any plaque) were imaged, with representative images shown.  

*B*, Each data point represents the average of five spine density measurements from one animal. Spine density was ~50% reduced on plaque-proximal dendrite segments relative to distal dendrite segments from the same animals. Spine density near plaque was lower in PS2APP;Trem2ko mice than in PS2APP;Trem2wt or PS2APP;Trem2het mice. Bars represent mean ± SEM. Significant differences between groups were determined by ANOVA (*p<0.05, **p<0.01). Spine volume was unaffected by either plaque proximity or Trem2 genotype (data not shown).

**EXTENDED DATA LEGENDS**

**Figure 1-1.** Gliosis and engagement/uptake of plaque by microglia are reduced in Trem2-deficient mice.  

*A*, Immunohistochemical stains for Iba1 demonstrated a significant reduction in Iba1+ area per section in Trem2-deficient mice. Representative Iba1 stains for the 12mo cohort are shown in Fig. 1B.  

*B*, (Top) Immunohistochemical stains for Gfap demonstrated a significant reduction in Gfap+ area per section, suggestive of reduced astrogliosis, in Trem2-deficient mice. (Bottom) Representative low (left, scale bar = 400 μm) and high (right, scale bar = 100 μm) magnification images of Gfap+ staining in the 12mo cohort are shown.  

*C*, Confocal z-stack images from 12mo female PS2APP cortical tissue were stained for microglia using anti-Iba1 and for plaque using methoxy-X04. Stark reductions in overlap were observed in Trem2-deficient mice using Manders’ colocalization coefficients, corroborating the finding in Fig. 1G,H that detection of methoxy-X04+ staining was reduced in PS2APP;Trem2ko compared to PS2APP;Trem2wt microglia dissociated from brains and measured by flow cytometry. Bars and lines represent mean ± SEM. Significant differences between groups were determined by unpaired t-test, or by ANOVA followed by Tukey’s multiple comparison test when more than two groups were compared (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus PS2APP;Trem2wt or as indicated).
**Figure 2-1.** Extended data table relating to Figure 2, with genome-wide RNA-Seq expression profiles and differential expression statistics for resident myeloid cells (Iba1+CD45+Ccr2-Ly6g−) FACS-purified from dissociated brains (cortex and hippocampus) of PS2APP;Trem2wt (n=7, 5 females and 2 males) and PS2APP;Trem2ko (n=6, 5 females and 1 male) at ~14 months of age. Raw RNA-Seq data files have been deposited to NCBI GEO under accession number GSE140744.

**Figure 2-2.** Induction of genes related to proliferation and Wnt-related signaling exhibit Trem2-dependence in multiple β-amyloidosis models. 

**A.** Gene ontology (GO) analysis implicated several biological processes whose induction was impaired in the absence of Trem2, based on overrepresentation of the genes listed for a given process (false discovery rate (FDR) ≤ 0.05) among the list of 144 transcripts with at least 2-fold reduced abundance (adjusted p ≤ 0.05) in PS2APP;Trem2ko vs. PS2APP;Trem2wt microglia. 

**B.** Nine genes featured in Fig. 2B,C that had shown reduced expression in PS2APP;Trem2ko microglia were analyzed in a separate dataset from independent researchers (GSE132508) for whether their expression was also reduced in Trem2ko relative to Trem2wt microglia, FACS-purified from 8-month 5xFAD mouse brains. Expression profiles for microglia from 8-month non-transgenic (Ntg) mice are also shown as a point of reference to visualize the extent of gene induction in 5xFAD;Trem2wt versus Ntg;Trem2wt microglia. Conservation of Trem2-dependence between models was observed for all except Ptk7. Bars and lines represent mean ± SEM. Significant differences between 5xFAD;Trem2ko and 5xFAD;Trem2wt microglia were determined by unpaired t-test (**p<0.01, ****p<0.0001). Note: These statistics are not from a genome-wide analysis of differential gene expression. 

**C.** The Proliferation microglial gene expression module (defined in Friedman et al., 2018) was analyzed for induction in 5xFAD;Trem2wt microglia relative to Ntg;Trem2wt microglia from non-transgenic mice and whether that induction was compromised in 5xFAD;Trem2ko microglia. This corroborated similar findings from Fig. 2D in the PS2APP model. Lines represent mean ± SEM. Significant differences between groups were determined by ANOVA followed by Tukey’s multiple comparison test (****p<0.0001 as indicated).

**Figure 2-3.** Gene expression modules change in PS2APP microglia, with Neurodegeneration-related and Proliferation gene sets showing Trem2-dependence (related to Fig. 2D). The left side of these heatmaps show how the expression of several microglial gene expression modules changes in microglia FACS-purified from PS2APP versus non-transgenic (Ntg) brains, using expression data from GSE89482. The right side of the heatmaps shows whether these
changes in gene expression were affected by Trem2 genotype in the current dataset. Induction of the Neurodegeneration-related and Proliferation modules was impaired in Trem2\textsuperscript{ko} microglia, while the other modules were expressed to similar extents in PS2APP;Trem2\textsuperscript{ko} and PS2APP;Trem2\textsuperscript{wt} microglia. Each row represents one gene in a module, each column is one animal's microglial expression profile, and coloring represents Z-score of a sample's nRPKM value for a given gene relative to average expression for that gene across all samples within a study (not across studies, since libraries were prepared using different methods and nRPKM values between studies are not directly comparable). Overall expression scores for each gene set in each sample are plotted in Fig. 2D.

Figure 3-1. Trem2 deletion increases plaque number in 6-month female somatosensory cortex. 6mo PS2APP females (A) or 8mo PS2APP males (B) with wild type (wt), knockout (ko), or heterozygous (het) Trem2 genotypes were i.p. injected with methoxy-X04 to label brain amyloid content one day before tissue collection. Brains were perfused, fixed, and embedded in agarose for en bloc two-photon imaging of intact somatosensory cortex (~200 µm depth) and quantitation of amyloid plaque content. Representative images are shown at left, with plots of plaque count per cubic millimeter shown to the right and each data point representing measurement from one animal. The brains used in A were the same brains used for dendritic spine measurements in Figure 8. Bars and lines represent mean ± SEM. Significant differences between groups were determined by ANOVA followed by Tukey’s multiple comparison test (**p<0.001, ****p<0.0001 versus PS2APP;Trem2\textsuperscript{wt} or as indicated).

Figure 5-1. Reduced abundance of A\textbeta\textsubscript{40} is more frequent than increased abundance of A\textbeta\textsubscript{42} in Trem2-deficient PS2APP mouse brains. Frozen hippocampal tissues from cohorts of PS2APP mice with indicated age, sex, and Trem2 genotype were homogenized in TBS, and the abundance of A\textbeta\textsubscript{peptides in the TBS-soluble (A) and guanidine HCl (GuHCl)-soluble (B) fractions was measured by ELISA assays specific for detecting A\textbeta\textsubscript{40} (top row) or A\textbeta\textsubscript{42} (bottom row). Bars and lines represent mean ± SEM. Significant differences between groups were determined by unpaired t-test or ANOVA followed by Tukey’s multiple comparison test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus PS2APP;Trem2\textsuperscript{wt} or as indicated).
FIGURE 3

A

|        | 4mo | 6-7mo | 12mo | 19-22mo |
|--------|-----|-------|------|---------|
| Plaque load (% Area) | | | | |

B

|        | PS2APP;Trem2<sup>wt</sup> | PS2APP;Trem2<sup>ko</sup> |
|--------|---------------------------|---------------------------|
| 6-7mo (f) | ![Image](image1) | ![Image](image2) |
| 12mo (f)  | ![Image](image3) | ![Image](image4) |
| 19-22mo (m) | ![Image](image5) | ![Image](image6) |

C

|        | PS2APP;Trem2<sup>wt</sup> | PS2APP;Trem2<sup>het</sup> | PS2APP;Trem2<sup>ko</sup> |
|--------|---------------------------|---------------------------|---------------------------|
| Amber plaque load (% Area) | ![Image](image7) | ![Image](image8) | ![Image](image9) |
FIGURE 4

A

12mo PS2APP;Trem2\textsuperscript{wt}  12mo PS2APP;Trem2\textsuperscript{ko}

B

\begin{tabular}{c|c|c|c|c}
 & \textbf{4mo} & \textbf{6-7mo} & \textbf{12mo} & \textbf{19-22mo} \\
\hline
\textbf{f} & \textbf{m} & \textbf{f} & \textbf{m} & \textbf{f} & \textbf{m} \\
\end{tabular}

Plaque diffuseness index

\textsuperscript{**} \textsuperscript{***} \textsuperscript{****}
Figure 5

A TBS fraction

- PS2APP;Trem2^{wt}
- PS2APP;Trem2^{ko}
- PS2APP;Trem2^{het}

4mo females
6-7mo males
12mo females
19-22mo males

B GuHCl fraction

C OC/actin

D 6E10/GAPDH
FIGURE 8

A

4mo (f) 6-7mo

12mo (f) 19-22mo (m)

B

12mo PS2APP (f)

Trem2wt

Trem2ko

19-22mo PS2APP (m)

Trem2wt

Trem2ko

C

12mo PS2APP

Plasma NFL (pg/mL)

CSF NFL (pg/mL)

***

****

**
FIGURE 9

A

6mo PS2APP fem., SS Cx

_Trem2_ plaque distal

|    | wt | het | ko |
|----|----|-----|----|
|    | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |

_Trem2_ plaque proximal

|    | wt | het | ko |
|----|----|-----|----|
|    | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |

_Thy1::GFP-M_ methoxy-X04

B

|    | distal | proximal |
|----|---------|----------|
|    | ![Graph](graph1.png) | ![Graph](graph2.png) |

Spine density (μm⁻¹)

_Trem2:_

|    | wt | het | ko |
|----|----|-----|----|
|    | ![Graph](graph1.png) | ![Graph](graph2.png) | ![Graph](graph3.png) |