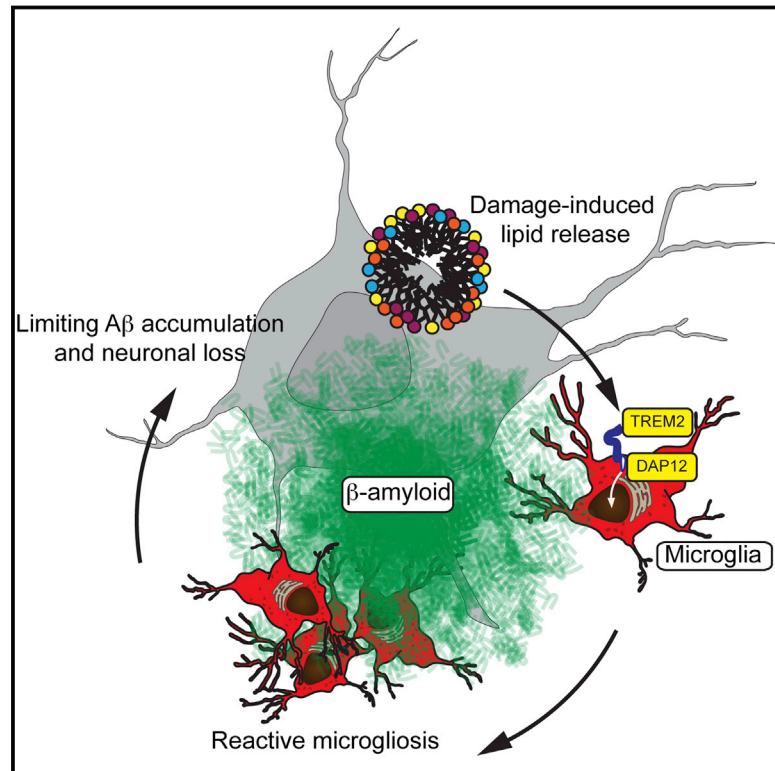


TREM2 Lipid Sensing Sustains the Microglial Response in an Alzheimer's Disease Model

Graphical Abstract



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In Brief

TREM2 acts in microglia as a sensor for a wide array of lipids that are associated with β -amyloid accumulation and neuronal loss. The TREM2 mutation that has recently been identified as a risk factor for Alzheimer's disease attenuates microglial detection of damage-associated lipids, providing a mechanistic basis for the genetic association.

Highlights

- TREM2 deficiency augments $A\beta$ accumulation and neuronal loss in a mouse model of AD
- TREM2 sustains the microglial response to $A\beta$ plaques by promoting microglial survival
- TREM2 senses anionic lipids that have been found to interact with fibrillar $A\beta$
- TREM2 R47H mutation linked to Alzheimer's disease impairs lipid recognition

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TREM2 Lipid Sensing Sustains the Microglial Response in an Alzheimer's Disease Model

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SUMMARY

Triggering receptor expressed on myeloid cells 2 (TREM2) is a microglial surface receptor that triggers intracellular protein tyrosine phosphorylation. Recent genome-wide association studies have shown that a rare R47H mutation of TREM2 correlates with a substantial increase in the risk of developing Alzheimer's disease (AD). To address the basis for this genetic association, we studied TREM2 deficiency in the 5XFAD mouse model of AD. We found that TREM2 deficiency and haploinsufficiency augment β -amyloid (A β) accumulation due to a dysfunctional response of microglia, which fail to cluster around A β plaques and become apoptotic. We further demonstrate that TREM2 senses a broad array of anionic and zwitterionic lipids known to associate with fibrillar A β in lipid membranes and to be exposed on the surface of damaged neurons. Remarkably, the R47H mutation impairs TREM2 detection of lipid ligands. Thus, TREM2 detects damage-associated lipid patterns associated with neurodegeneration, sustaining the microglial response to A β accumulation.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with histopathological hallmarks of β -amyloid (A β) plaques and neurofibrillary tangles in the brain (Huang and Mucke, 2012; Tanzi, 2013). Although disease etiology is incompletely understood, families with inherited early-onset AD have mutations in three proteins directly involved in the A β processing pathway, suggesting a key role for A β in disease pathogenesis. Early studies have shown that brain microglia accumulate around A β plaques and occasionally contain A β in both AD patients (D'Andrea et al., 2004; McGeer et al., 1987; Perlmutter et al., 1990) and transgenic mouse models of AD (Dickson, 1999; Frautschy et al., 1998; Stalder et al., 1999). Microglia contribute to A β clearance, at least in the early phases of neurodegeneration (El Khoury et al.,

2007); however, the ability of microglia to clear A β may wane with age (Streit et al., 2004; Streit and Xue, 2009). At late stages of AD, microglia may paradoxically contribute to the disease by releasing pro-inflammatory cytokines in response to A β deposition (El Khoury et al., 2007; Hickman et al., 2008).

Recent genome-wide association studies (GWASs) have shown that a rare Arginine-47-Histidine (R47H) mutation of the triggering receptor expressed on myeloid cells 2 (TREM2) is associated with a substantial increase in the risk of developing AD (Guerreiro et al., 2013b; Jonsson et al., 2013). TREM2 is a cell-surface receptor of the Ig-superfamily that is expressed by microglia and osteoclasts in vivo (Kialainen et al., 2005; Paloneva et al., 2002; Schmid et al., 2002; Thrash et al., 2009) as well as monocyte-derived DCs, bone marrow-derived macrophages, and macrophage cell lines in vitro (Bouchon et al., 2001; Daws et al., 2001). Although TREM2 was detected in other cells of the CNS (Guerreiro et al., 2013b; Sessa et al., 2004), these observations have not been confirmed (Jiang et al., 2014). TREM2 binds anionic carbohydrates, anionic bacterial products, and various phospholipids (Cannon et al., 2012; Daws et al., 2003). It transmits intracellular signals through the associated transmembrane adaptor DAP12, which recruits the protein tyrosine kinase Syk, leading to phosphorylation of many downstream mediators, such as PLC- γ , PI-3K, and Vav2/3 (Ford and McVicar, 2009; Peng et al., 2010). Individuals homozygous for rare mutations that impair expression of either TREM2 or DAP12 develop lethal forms of progressive dementias such as Nasu-Hakola disease (NHD) and frontotemporal dementia (FTD) (Guerreiro et al., 2013a, 2013c; Kleinberger et al., 2014; Paloneva et al., 2002).

The association between the R47H mutation of TREM2 and the increased risk for late-onset AD suggests that microglia may require TREM2 to respond to A β deposition and to limit neuronal degeneration. Consistent with this hypothesis, we recently showed that APPS1-21 transgenic mice, an AD model with rapid deposition of A β , have a marked decrease in the number and size of A β -associated microglia when they lack one copy of the *Trem2* gene, although this defect did not increase A β accumulation (Ulrich et al., 2014). The mechanisms underlying this altered microglial response and its impact on A β deposition have not been delineated. To address these questions, we studied TREM2 deficiency in the 5XFAD mouse model of AD, in which

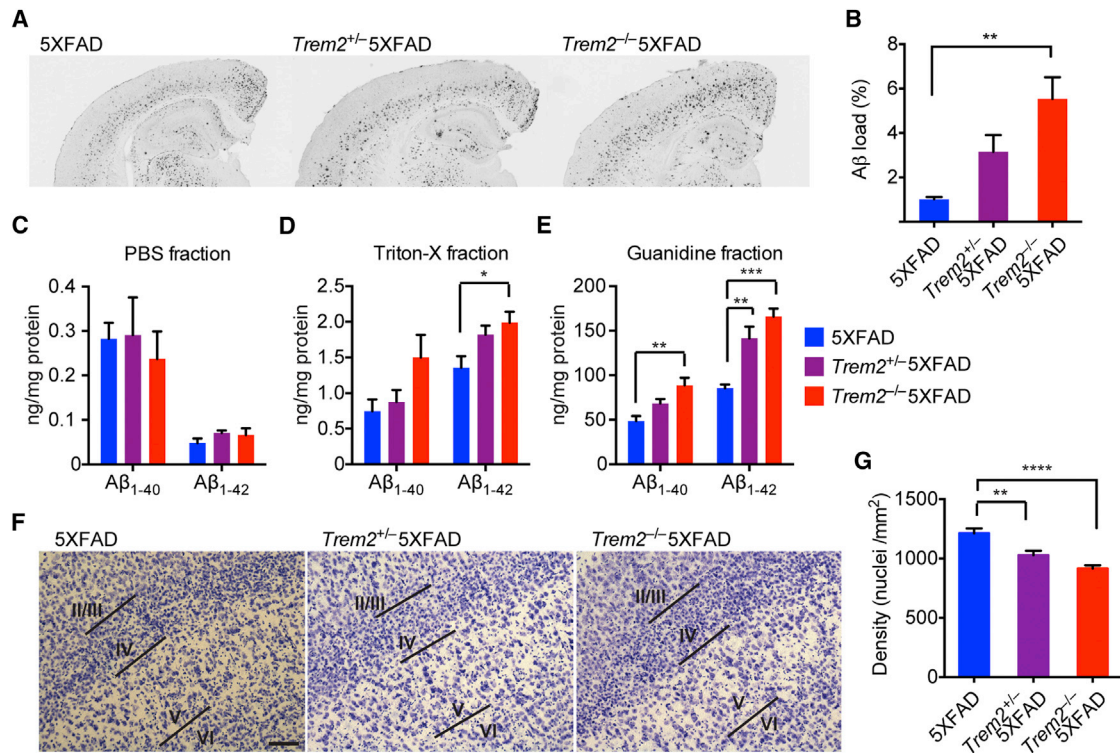


Figure 1. TREM2-Deficient 5XFAD Mice Have Increased Hippocampal A β Burden and Accelerated Loss of Layer V Cortical Neurons

A β burden in 8.5-month-old *Trem2*^{-/-}5XFAD, *Trem2*^{+/+}5XFAD, and 5XFAD mice.

(A) Matching coronal hippocampus and cortex sections were stained with an A β -specific antibody mHJ3.4.

(B) Amounts of A β loads in hippocampi.

(C–E) Soluble and insoluble A β ₁₋₄₀ and A β ₁₋₄₂ levels in hippocampi as detected by ELISA.

(F and G) Densities of layer V neurons in 8.5-month-old *Trem2*^{-/-}5XFAD, *Trem2*^{+/+}5XFAD, and 5XFAD mice. (F) Matching coronal sections stained with cresyl violet. (G) Summary of densities of layer V neurons.

Original magnification: 10 \times ; scale bar, 100 μ m. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001, one-way ANOVA. Data represent analyses total of eight to ten 5XFAD mice, eight to 12 *Trem2*^{+/+}5XFAD mice, and eight to 16 *Trem2*^{-/-}5XFAD mice (B, C–E, and G). Bars represent mean \pm SEM. See also Figure S1.

A β deposition develops less rapidly than in APPS1-21 mice (Oakley et al., 2006). We find that both TREM2 deficiency and haploinsufficiency augment A β accumulation due to a dysfunctional response of microglia, which become apoptotic rather than undergoing activation and proliferation. We further show that TREM2 sustains microglial survival by synergizing with colony stimulating factor-1 receptor (CSF-1R) signaling. Finally, we demonstrate that TREM2 binds to a broad array of anionic lipids, which were found in association with fibrillar A β and are also exposed during neuronal and glial cell death. Remarkably, the R47H mutation impairs TREM2 binding to anionic lipids. We conclude that TREM2 is a receptor that detects damage-associated lipids, thereby enabling microglia to sense A β accumulation and cell damage, as well as supporting microglial survival and A β reactive microgliosis.

RESULTS

TREM2 Modulates A β Accumulation

We examined the deposition of A β aggregates in *Trem2*^{-/-} mice bred to 5XFAD transgenic mice (APPSwFILon, PSEN1*M146L*L286V), an accelerated mouse model of AD (Oakley et al.,

2006). Staining of matched coronal brain sections from *Trem2*^{-/-}5XFAD mice and control 5XFAD mice at 8.5 months of age with a monoclonal antibody (mAb) against A β revealed significantly increased A β accumulation in the hippocampus but not cortical regions of *Trem2*^{-/-}5XFAD mice (Figures 1A, 1B, and S1A). *Trem2*^{+/+}5XFAD mice had an intermediate phenotype, although it was not statistically significant (p = 0.104). We also determined levels of A β ₄₀ and A β ₄₂ in the hippocampus and cortex of these mice by ELISA. While levels of soluble A β ₄₀ and A β ₄₂ were similar (Figures 1C and S1B), we detected a significant increase in insoluble, guanidine-extracted A β ₄₀ and A β ₄₂ in the hippocampal regions of *Trem2*^{-/-}5XFAD mice compared to 5XFAD mice (Figures 1D and 1E). Moreover, there was a significant effect of *Trem2* gene copy number on insoluble A β protein levels in the hippocampi, whereas levels of insoluble A β ₄₀ and A β ₄₂ in the cortex were equivalent across all three genotypes (Figures S1C and S1D). We also found that the loss of layer V neurons, a feature of 5XFAD mice (Eimer and Vassar, 2013; Oakley et al., 2006), was more prominent in *Trem2*^{-/-}5XFAD mice (Figures 1F and 1G). *Trem2*^{+/+}5XFAD mice presented an intermediate phenotype. Collectively, these data suggest that TREM2 modulates A β accumulation, limiting neuronal

loss. The lack of a significant difference in A β accumulation in the cortices of *Trem2*^{-/-}5XFAD mice and 5XFAD mice may be the result of the fast kinetics of A β deposition in 5XFAD mice, such that the potential cortical differences are no longer detectable at 8.5 month of age.

TREM2 Is Required for Reactive Microgliosis

How does lack of TREM2 impact A β accumulation? Although TREM2 expression has been reported in CNS cells other than microglia (Guerreiro et al., 2013b; Sessa et al., 2004), this finding is controversial (Jiang et al., 2014). Indeed, a recently published RNA sequencing (RNA-seq) data set demonstrated that *Trem2* is specifically expressed in microglia, but not other cells in the CNS under steady-state conditions (Butovsky et al., 2014). We also found that *Trem2* expression is further upregulated in microglia isolated from 5XFAD mice during A β deposition (Figures S2A and S2B). Thus, we focused our studies on microglia. One of the many effects of A β deposition is the induction of reactive microgliosis, which involves the expansion of microglia and conversion to an activated state (Ransohoff and Cardona, 2010). Microgliosis predominantly involves the proliferation of brain-resident microglia, with some contribution from blood-borne monocytes and microglia migrating from adjacent non-damaged brain areas (El Khoury et al., 2007; Grathwohl et al., 2009; Malm et al., 2005; Mildner et al., 2011; Simard et al., 2006; Stalder et al., 2005). To evaluate the impact of TREM2 deficiency on A β -induced microglial responses in 5XFAD mice, we examined transcriptional profiles of microglia purified from 5XFAD and *Trem2*^{-/-}5XFAD mice as well as transgene negative wild-type (WT) and *Trem2*^{-/-} littermates (Figure S2A). To evaluate changes in global transcripts, we first performed principle component analysis (PCA) of the top 15% most variable transcripts. We noticed that WT and *Trem2*^{-/-} replicates clustered closely, suggesting a limited impact of TREM2 deficiency in the steady state, which was confirmed by a volcano plot comparing the two groups (Figures 2A and 2B). In contrast, 5XFAD microglial replicates were dramatically different from WT replicates (Figure 2A), and a volcano plot revealed that 5XFAD microglia expressed many more transcripts including those associated with microglial activation (MHC-II, CD11c), production of inflammatory cytokines (interleukin-1 β [IL-1 β], tumor necrosis factor- α [TNF- α], IL-12, and SPP1), and neurotrophic factors (insulin growth factor 1 [IGF-1] and VEGFA) (Figure 2C). *Trem2*^{-/-}5XFAD microglia had an intermediate behavior in the principle component analysis compared to 5XFAD and WT microglia. To further interrogate how TREM2 deficiency affected the microglial response to A β deposition, we selected the transcripts upregulated 2-fold between 5XFAD and WT microglia (Figure 2C) and compared the expression of these transcripts among the entire data set. We found that *Trem2*^{-/-}5XFAD microglia failed to upregulate these transcripts and behaved more similarly to WT microglia, as shown by hierarchical clustering and expression-by-expression plots (Figures 2D and 2E). Flow cytometric analysis of isolated microglia confirmed phenotypic changes in 5XFAD microglia consistent with increased activation, including a marked increase in cell size and strong upregulation of MHC-II, CD11c, and CD11b (Figures S2C–S2G). We also confirmed

increased expression of inflammatory cytokine transcripts by qPCR in whole-brain lysates of 5XFAD mice (Figures S2I–S2L). However, in *Trem2*^{-/-}5XFAD mice, these changes were markedly attenuated (Figures 2D, 2E, and S2C–S2L). In fact, *Trem2*^{-/-}5XFAD microglia were phenotypically more similar to WT microglia in steady state than 5XFAD microglia. Overall, these results implied that TREM2 is required for reactive microgliosis.

Microglia Fail to Colocalize with A β Plaques in *Trem2*^{-/-} Mice

Initial staining of microglia in coronal brain sections with Iba-1 revealed very similar distribution of microglia in *Trem2*^{-/-}, *Trem2*^{+/-} and WT adult mice (Figures S3A–S3C). However, co-staining of coronal brain sections from *Trem2*^{-/-}5XFAD and 5XFAD mice with Iba-1 and X-34, to visualize microglia and A β plaques, respectively, showed remarkable differences. We found that *Trem2*^{-/-}5XFAD mice had reduced Iba-1 reactivity both in the hippocampi and cortices compared to 5XFAD mice (Figures 3A–3D). This was particularly evident in the areas surrounding A β plaques (Figures 3E and 3F; Movies S1, S2, and S3), suggesting a preferential reduction of microgliosis near amyloid deposits. *Trem2*^{+/-}5XFAD mice also had a partial reduction of amyloid-associated Iba-1 reactivity.

Examination of a second model of AD, APPPS1-21 mice that have been bred to *Cx3cr1*^{GFP/+} mice in order to visualize endogenous microglia, confirmed that complete TREM2 deficiency results in a marked reduction of GFP⁺ microglial clusters around A β plaques (Figures S3D–S3F). This corroborates our previous observation that TREM2 haploinsufficiency correlates with fewer amyloid-associated microglia in APPPS1-21x*Cx3cr1*^{GFP/+} mice (Ulrich et al., 2014). Moreover, since CX3CR1 marks brain-resident microglia (Ransohoff and Cardona, 2010), these results also suggest that TREM2 deficiency primarily affects the response of brain-resident microglia to A β .

To further quantify the number of microglia around A β plaques, we recorded the coordinates (x, y, and z) of all visible microglial cell bodies and the location of A β plaques in each z stack confocal image and calculated the number of microglia within 30 μ m radius of the plaques (defined as plaque-associated microglia) and non-plaque-associated microglia. While no statistically significant difference was observed among non-plaque-associated microglia (Figure S4A), we noted a high degree of microglial clustering around amyloid plaques in 5XFAD mice (average 4.28 microglia per plaque), which gradually decreased in *Trem2*^{+/-}5XFAD mice (average 3.42 microglia per plaque) and *Trem2*^{-/-}5XFAD mice (average 2.36 microglia per plaque) (Figures 4A and 4B). To confirm the “negligence” of microglial responses to A β in the absence of TREM2, we compared the actual frequency of microglia per plaque to that obtained by Monte Carlo simulations where the same numbers of microglia and plaques observed in z stack images were positioned by chance in each genotype (Figure S4B). The probability that observed microglial frequencies per plaque fell outside of simulated random frequencies was inversely proportional to *Trem2* gene copy number (Figure 4C). Moreover, while 27.9% of microglial distribution in 5XFAD mice with respect to A β plaques was

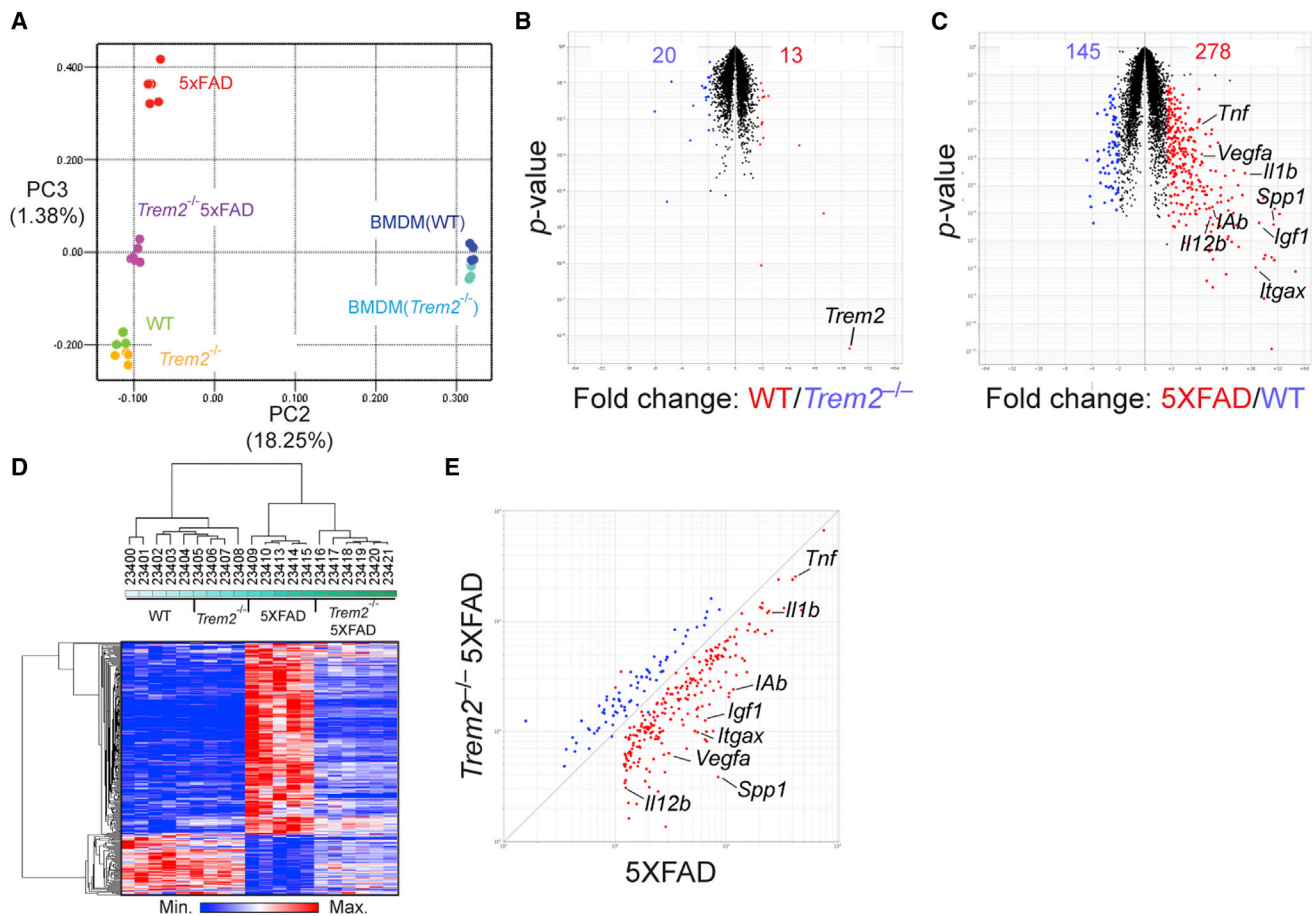


Figure 2. TREM2 Deficiency Impairs A β -Induced Transcriptional Program in Microglia

Transcriptional analysis of microglia isolated from hippocampi and cortices of 8.5-month-old *Trem2*^{-/-}5XFAD, 5XFAD, *Trem2*^{-/-}, and WT mice.

(A) Top 15% most variable transcripts were subjected to principle component analysis (PCA). Plot shows two-dimensional (PC2 versus PC3) comparison of transcriptional changes in all classes analyzed. WT and *Trem2*^{-/-} bone marrow-derived macrophages were used as references.

(B) Volcano plot comparing microglial transcripts in *Trem2*^{-/-} and WT mice. *Trem2* transcript is indicated.

(C) Volcano plot comparing microglial transcripts in 5XFAD and WT mice. Numbers in plots (B) and (C) indicate probes that are significantly upregulated or downregulated (± 2 -fold, $p < 0.05$, Student's *t* test). Representative transcripts are indicated.

(D and E) Visualization of A β -induced changes in microglial transcripts from (C). (D) A heatmap displays hierarchical clustering of all samples analyzed. (E) A scatterplot compares these transcriptional changes in *Trem2*^{-/-}5XFAD and 5XFAD microglia. Representative transcripts are shown.

See also Figure S2.

not explained statistically by chance, the frequency of non-random microglial distribution was reduced to 9.5% in *Trem2*^{-/-} 5XFAD mice (Figure 4D).

Another feature of reactive microgliosis is morphological transformation. In 5XFAD mice, plaque-associated microglia showed morphological changes associated with microglial activation, including a partial retraction and a slight hypertrophy of the microglial cell processes as well as an increase in size (Figures 4E–4G). These changes in microglial morphology were significantly attenuated in *Trem2*^{+/-}5XFAD and *Trem2*^{-/-}5XFAD mice (Figures 4E–4G) and were paralleled by an increased distance between microglia and the center of their associated plaques (Figure 4H). Collectively, these data indicate that TREM2 is essential for the microglial response to A β plaques.

TREM2 Deficiency Affects Microglial Survival in 5XFAD Mice

Why is TREM2 required for A β reactive microgliosis? We first hypothesized that TREM2 may be necessary for A β uptake and microglial activation. We initially investigated the impact of TREM2 deficiency on microglial activation in vitro. For this analysis, we used primary microglia isolated from adult mice and expanded in the presence of optimal amounts of CSF-1 and TGF- β (Figure S5A), as they closely resemble microglia in vivo (Butovsky et al., 2014). TREM2 deficiency did not affect microglial expansion, migration, or TNF- α secretion in response to A β (Figures S5B–S5D). In contrast, *Trem2*^{-/-} microglia produced significantly more TNF- α than WT microglia in response to lipopolysaccharide (LPS), consistent with previous demonstrations that TREM2 attenuates cytokine responses to certain TLR ligands

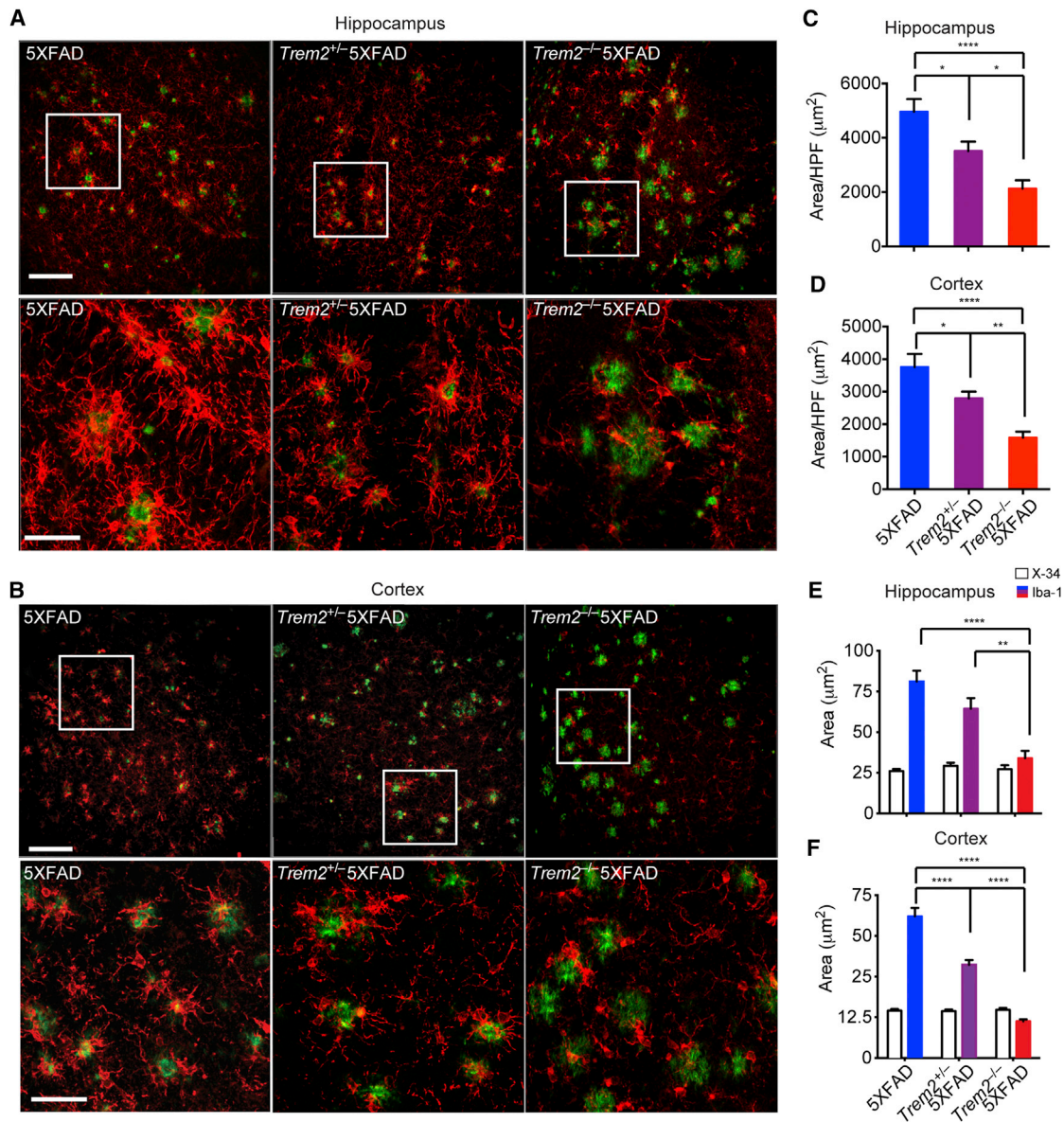


Figure 3. TREM2 Deficiency Leads to Reduced Microgliosis in 5XFAD Mice

Microgliosis in 8.5-month-old *Trem2*^{-/-}5XFAD, *Trem2*^{+/-}5XFAD, and 5XFAD mice.

(A and B) Matching coronal sections were stained with Iba-1 (red) for microglia and X-34 (green) for amyloid plaques. Representative z stack images with maximum projection are shown.

(C and D) Quantification of total Iba-1 reactivity per high-power field (HPF) in hippocampi and cortices.

(E and F) Quantification of microgliosis associated with plaques of similar sizes in hippocampi and cortices.

Original magnification 20 \times (A, B, upper panels), 40 \times (A, B, lower panels); scale bar, 10 μm (A, B, upper panels), 50 μm (A, B, lower panels). * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, one-way ANOVA. Data represent analyses of a total of eight of ten 5XFAD, eight of 12 *Trem2*^{+/-}5XFAD mice, and eight of 16 *Trem2*^{-/-}5XFAD mice. Bars represent mean \pm SEM. See also [Figure S3](#) and [Movies S1](#), [S2](#), and [S3](#).

([Hamerman et al., 2006](#); [Turnbull et al., 2006](#)). Moreover, TREM2 deficiency had very little impact on microglial uptake of A β aggregates ([Figure S5E](#); [Movie S4](#)) or their subsequent proteolytic processing, as demonstrated by similar degradation of the intracellular concentration of A β after initial loading ([Figure S5F](#)). Thus, TREM2 deficiency does not engender a direct defect in phagocytosis of A β .

Previous studies have suggested that the CSF-1-CSF-1R pathway promotes reactive microgliosis ([Chitu and Stanley, 2006](#)) and A β clearance ([Mitrassinovic et al., 2003](#)); consistent with this, CSF1-deficient osteopetrotic (*op/op*) mice are characterized by increased deposition of A β , scarcity of microgliosis and neuronal loss ([Kaku et al., 2003](#)). We had previously demonstrated that TREM2 signaling via its associated adaptor DAP12

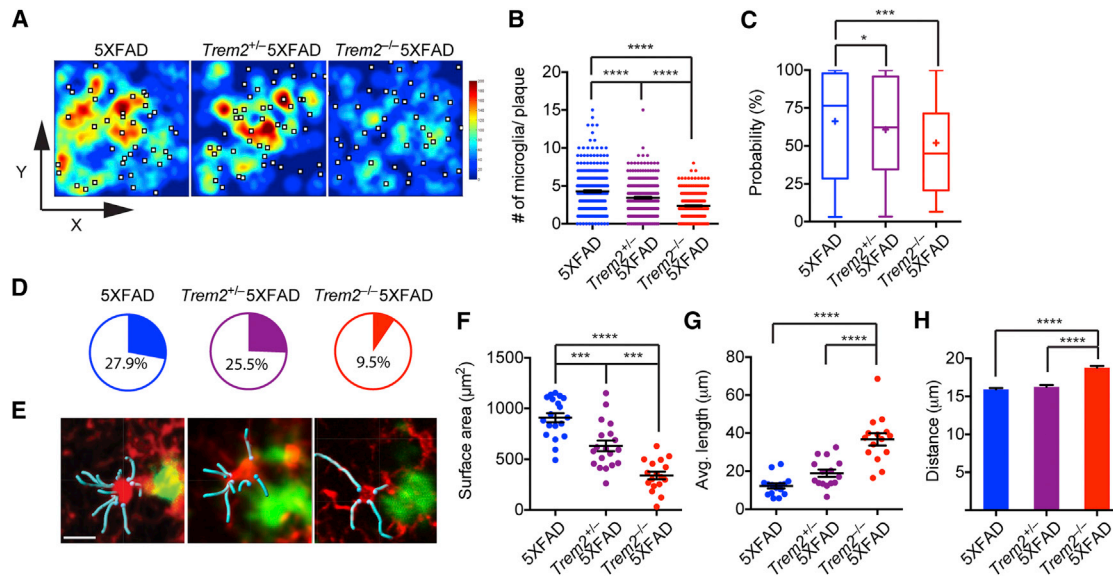


Figure 4. TREM2 Deficiency Diminishes the Capacity of Microglia to Cluster around A β Plaques

Frequencies of plaque-associated microglia in 8.5-month-old *Trem2*^{-/-}5XFAD, *Trem2*^{+/-}5XFAD, and 5XFAD mice were determined.

(A) Heatmap shows frequencies of microglia in relation to A β plaques shown as white squares.

(B) Summary of frequencies of plaque-associated microglia in all analyzed genotypes.

(C and D) Microglial clustering around plaques in 5XFAD, *Trem2*^{+/-}5XFAD, and *Trem2*^{-/-}5XFAD mice were compared to Monte Carlo simulations that assume total randomness between plaques and microglia. Probabilities that any given microglia-plaque cluster are non-random are shown in (C). Pie charts show frequencies of microglia-plaque clusters that cannot be statistically explained as random ($p < 0.05$) (D).

(E) Morphology of plaque-associated microglia highlighting the shape of cell bodies (red) and primary processes (cyan).

(F–H) Plaque-associated microglia are analyzed for their surface area (cell body only), average length of primary processes, and distance from the center of adjacent A β plaque.

Original magnification: 20 \times ; scale bar, 15 μ m. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$, one-way ANOVA. Data represent analyses of a total seven mice per group (A–D) and a total of five mice per group (E–G). Bars represent mean \pm SEM. See also Figure S4.

synergizes with CSF-1R signaling to promote survival of macrophages (Otero et al., 2009, 2012). Specifically, TREM2/DAP12 were required to induce activation of the Syk tyrosine kinase pathway downstream of CSF-1R (Otero et al., 2009; Zou et al., 2008). Thus, we hypothesized that TREM2 may synergize with CSF-1-CSF-1R signaling to sustain reactive microgliosis during A β deposition. We initially tested this hypothesis in vitro by measuring the survival of adult primary microglial cultures from WT and *Trem2*^{-/-} mice in the presence of graded concentrations of CSF-1 (10%, 1%, and 0.1% L-cell conditioned medium [LCM]). While TREM2 deficiency did not affect viability at high concentrations of CSF-1 (10% and 1%), *Trem2*^{-/-} microglia were markedly less viable than WT microglia in 0.1% CSF-1 (Figures 5A–5C). We next purified microglia from *Trem2*^{-/-}5XFAD and 5XFAD mice and cultured them in medium containing low levels of CSF-1 (0.1% LCM) for 5 days. *Trem2*^{-/-}5XFAD microglia were significantly less viable than 5XFAD microglia (Figure 5D). Since CSF-1R captures CSF-1 and targets it for degradation (Stanley and Chitu, 2014), the reduced survival of *Trem2*^{-/-} microglia at low CSF-1 concentrations may reflect a marked susceptibility of these cells to CSF-1 deprivation that occurs when microglia consume a limited supply of CSF-1. Indeed, CSF-1R blockade reduced viability of 5XFAD microglia, confirming that the pro-survival effect of TREM2 cannot replace that of CSF-1R, but only synergize with it (Figure 5D).

To evaluate the impact of TREM2 deficiency on microglia apoptosis in vivo, we analyzed coronal sections of *Trem2*^{-/-}5XFAD and 5XFAD mice by TUNEL staining. Markedly more TUNEL⁺ microglia were evident in *Trem2*^{-/-}5XFAD mice than the very few observed in control 5XFAD mice (Figures 5E and 5F), corroborating a role for TREM2 in maintaining microglial survival during reactive microgliosis. Consistent with this, significantly fewer microglia were recovered from the cortices and hippocampi of *Trem2*^{-/-}5XFAD mice than from 5XFAD mice (Figure 5G). We postulate that reactive microgliosis is associated with increased CSF-1 uptake by CSF-1R and degradation restricting CSF-1 range of action, such that microglia in close proximity must compete for CSF-1. Because of their inability to survive CSF-1 limitation, TREM2-deficient microglia are incapable of sustaining reactive microgliosis and undergo apoptosis rather than becoming activated and expanding.

TREM2 Is a Sensor for Anionic and Zwitterionic Lipids that Accumulate in the CNS during A β Deposition

We next sought to identify the ligand(s) that trigger TREM2 signaling during A β deposition. Since TREM2 binds anionic carbohydrates, anionic bacterial products, and phospholipids (Cannon et al., 2012; Daws et al., 2003), we focused on lipids that have been shown to accumulate during A β deposition and

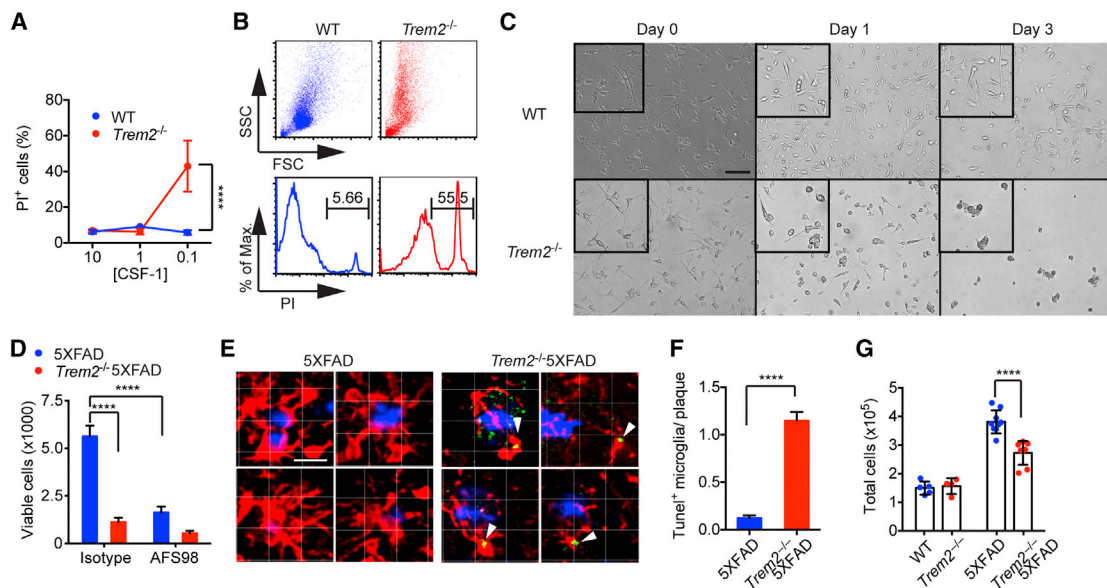


Figure 5. TREM2 Promotes Microglial Survival Ex Vivo and In Vivo

(A–C) Adult primary microglia were cultured with various concentration of CSF-1-containing L-cell medium (LCM). Viability of microglia by PI staining (A and B) and morphology (C) were assessed on day 3. Original magnification: 20 \times (main images) and 40 \times (insets); scale bar, 10 μ m.

(D) Microglia were purified ex vivo from 5XFAD mice and cultured in 0.1% LCM with or without CSF-1R blocking antibody AFS98. Viability was determined on day 5.

(E and F) Apoptosis of plaque-associated microglia (Iba-1, red) in 5XFAD and *Trem2*^{-/-}5XFAD mice was determined by TUNEL staining (green). Plaques were identified by X-34 (blue). Representative single-stack images of 5XFAD and *Trem2*^{-/-}5XFAD microglia (E) and summary of frequencies of TUNEL⁺ microglia associated with plaques (F) are shown. Original magnification: 20 \times ; scale bar, 15 μ m (E).

(G) Total numbers of live microglia in cortices and hippocampi of 5XFAD, *Trem2*^{-/-}5XFAD, *Trem2*^{-/-}, and WT mice.

**** p < 0.0001, two-way ANOVA (A, D, and G), Student's t test (F). Data represent a total of three independent experiments (A–D) and a total of five to eight mice per group (E–G). Bars represent mean \pm SEM. See also Figure S5 and Movie S4.

might stimulate microglia. These included negatively charged phospholipids, which have been shown to associate with A β in lipid membranes (Ahyyauch et al., 2012; Nagarathinam et al., 2013); membrane phospholipids, such as phosphatidylserine, which are exposed by damaged neurons and glial cells; and anionic and zwitterionic non-phosphate lipids, such as sulfatides and sphingomyelin, which are released by damaged myelin. We transfected human TREM2 in reporter cells that express GFP under the control of NFAT, such that Ca²⁺ mobilization turns on GFP expression when TREM2 is engaged. Incubation of TREM2 reporter cells with many of these lipids activated reporter activity, although to differing extents, with phosphatidylcholine (PC) and sphingomyelin (SM) performing best in these assays (Figures 6A and 6B). Similar results were obtained with a mouse TREM2 reporter (data not shown). Addition of a blocking TREM2 antibody abolished reporter activation by all ligands, demonstrating specificity (Figure 6B). Interestingly, other potential candidates, such as cardiolipin, which is released by damaged mitochondria, did not significantly activate the TREM2 reporter despite its phospholipid structure. This suggests that the ability to engage TREM2 may only partially depend on the presence of negatively charged moieties like phosphoric acid (Figures 6A and 6B). Furthermore, TREM2 reporter activation was not detected with plate-bound synthetic or extracted A β (data not shown). In agreement with the ability of phosphatidylserine (PS) to activate

TREM2 reporter cells, apoptotic cells, which expose PS on the cell surface, also activated TREM2 reporter cells (Figure 6C). However, microglia isolated from *Trem2*^{-/-}5XFAD and 5XFAD mice engulfed apoptotic cells equally well (Figures 6D and 6E). Thus, TREM2 is not directly involved in phagocytosis of apoptotic cells. We conclude that TREM2 is a sensor for several anionic and zwitterionic lipids that are exposed during A β deposition as well as during neuronal and glial cell death.

R47H Mutation Impairs TREM2 Recognition of Lipid Ligands

What is the impact of the R47H mutation on TREM2 ligand recognition? We generated TREM2 R47H reporter cells and compared their response to identified ligands to that of TREM2 reporter cells. The R47H mutation considerably reduced reporter activation in response to many ligands, including phosphatidic acid (PA), phosphatidylglycerol (PG), PS, phosphatidylinositol (PI), and sulfatides (Figures 7A–7G). The R47H mutation had less impact on SM recognition and very little influence on PC-mediated activation. Importantly, the R47H mutation did not detectably affect cell-surface expression or signaling of TREM2, as assessed by stimulating the R47H reporter cells with a plate-bound anti-TREM2 antibody (Figure 7H). Thus, these data suggest that the R47H reduces the overall capacity of TREM2 to bind anionic ligands.

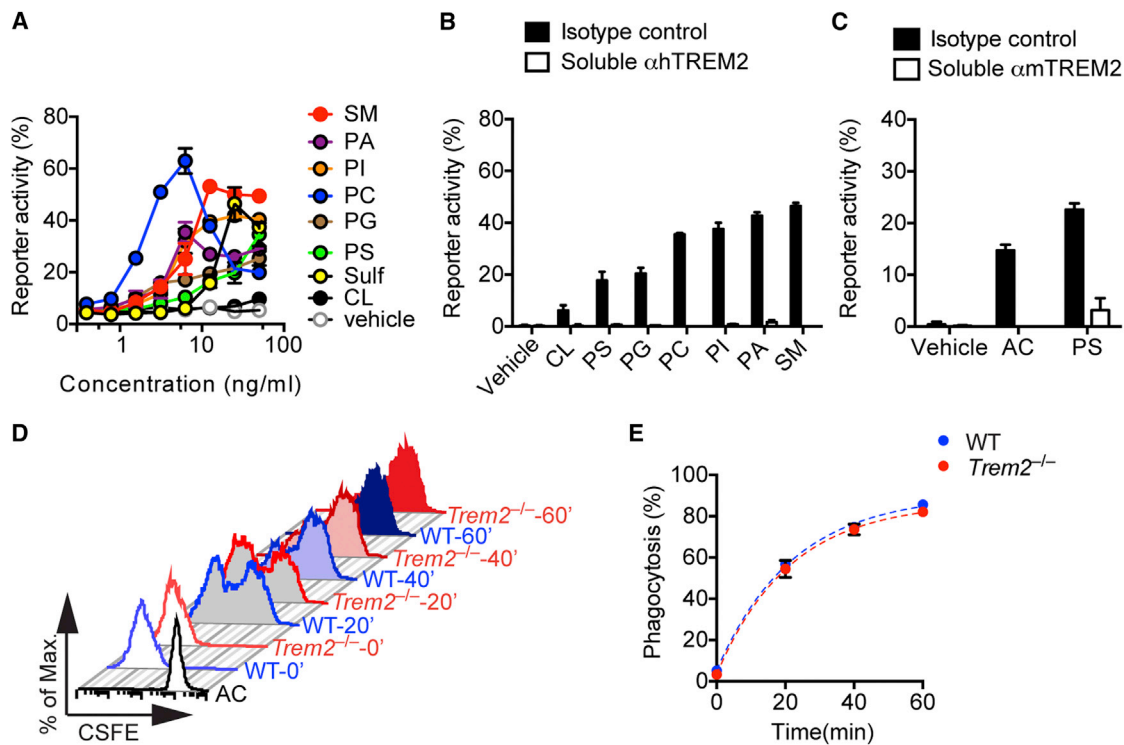


Figure 6. TREM2 Is a Receptor for Lipid Patterns Associated with A β

(A and B) Human TREM2 reporter cells were stimulated with various phospholipids and anionic and zwitterionic lipids at the indicated concentrations. Reporter activation (GFP expression) was assessed after overnight incubation by flow cytometry. TREM2 reporter cells responding to lipids at various concentrations are shown in (A). Blockade of reporter activation by a soluble anti-hTREM2 mAb is shown in (B). SM, sphingomyelin; PA, phosphatidic acid; PI, phosphatidylinositol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; Sulf, sulfatide; and CL, cardiolipin.

(C) mTREM2 reporter cells were cultured with either apoptotic cells (AC) or phosphatidylserine (PS) in the presence of soluble anti-TREM2 mAb or isotype control.

(D and E) Adult primary microglia from *Trem2*^{-/-}5XFAD and 5XFAD mice were pulsed with CFSE-labeled AC. (D) Phagocytosis of AC was determined 20, 40, and 60 min post co-culturing by flow cytometry. (E) Summary of AC uptake by WT and *Trem2*^{-/-} microglia.

Data represent a total of three (A–C) and two (D and E) independent experiments.

DISCUSSION

This study showed that TREM2 modulates A β accumulation in the 5XFAD mouse model of AD, thereby reducing neuronal damage. The importance of TREM2 in A β clearance is underscored by the fact that even the loss of one copy of *Trem2* gene is sufficient to increase A β accumulation. TREM2 acts in microglia by supporting A β -reactive microgliosis, a process of expansion and activation that leads to microglial clustering around A β plaques and subsequent A β removal (Ransohoff and Cardona, 2010). In the absence of TREM2, this microgliosis is impaired. In fact, microglia from *Trem2*^{-/-}5XFAD mice are unable to survive, as evidenced by the accumulation of apoptotic microglia around A β plaques. Cells involved in TREM2-dependent microgliosis had phenotypic features of brain resident microglia, such as expression of CX3CR1. However, it is possible that monocytes from peripheral blood contribute to microgliosis and that TREM2 supports their survival as well.

Previous studies have shown that CSF-1-CSF-1R signaling is essential for microgliosis in response to A β (Chitu and Stanley, 2006; Kaku et al., 2003; Mitrasinovic et al., 2003). Since CSF-1 is rapidly consumed during this process (Stanley and Chitu, 2014), there is probably a limited supply of CSF-1 surrounding

the A β plaques. Our results demonstrate that TREM2 provides a signal that is necessary for survival of microglia at low CSF-1 concentrations. We postulate that TREM2 acts as a costimulatory molecule that sustains survival of microglia, which are activated and proliferate in the presence of A β . Previous studies of cultured myeloid cells indicate that TREM2 may synergize with CSF-1-CSF-1R signaling to activate the protein tyrosine kinase Syk, which, in turn, activates multiple downstream mediators, such as ERK, PI-3K, and Akt (Zou et al., 2008). In addition, TREM2 may provide survival signals through activation of anti-apoptotic mediators such as β -catenin (Otero et al., 2009) and Mcl-1 (Peng et al., 2010). It is also possible that TREM2 is necessary to support increased microglial metabolism during activation.

Why is TREM2 activated during A β accumulation? Previous studies have indicated that TREM2 binds phospholipids, such as PS, and acts as a scavenger receptor for apoptotic cells that might be generated during neuronal damage (Hsieh et al., 2009; Takahashi et al., 2005, 2007). In our study, we demonstrate that TREM2 is a sensor for a broad array of acidic and zwitterionic lipids, which may or may not contain a phosphoric acid moiety. Membranes containing these lipids strongly interact with A β , facilitating the formation of fibrillar A β

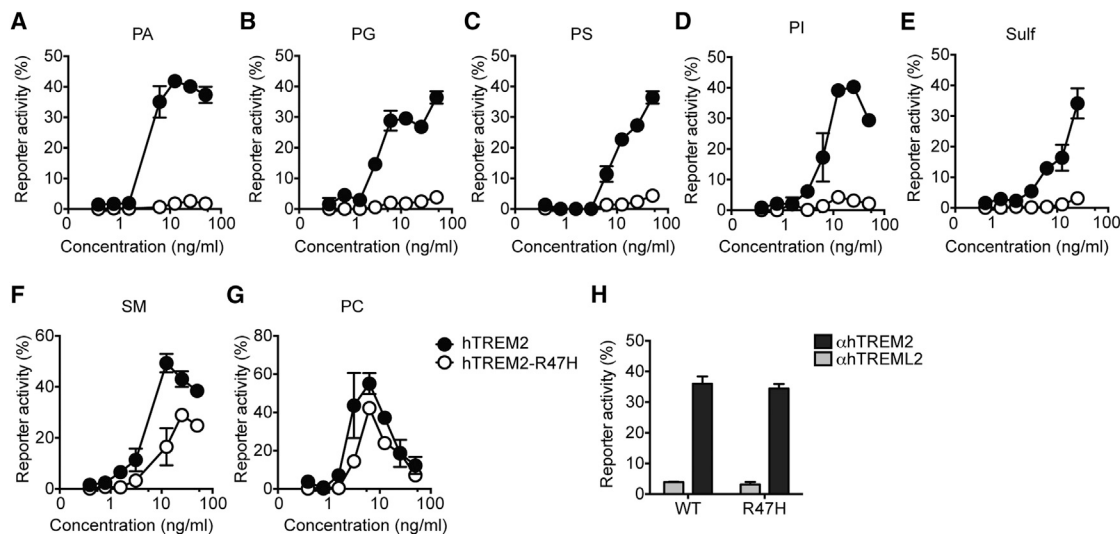


Figure 7. R47H Mutation Attenuates TREM2 Recognition of Lipids

(A–H) Reporter cells expressing either common allele or R47H variant of human TREM2 were stimulated with various species of lipids or plate-bound anti-hTREM2 mAb. A plate-bound control antibody (anti-hTREM2L2) was used as a negative control. Data represent a total of two independent experiments. Bars represent mean \pm SEM.

(Ahyayauch et al., 2012; Del Mar Martínez-Senac et al., 1999; Nagarathinam et al., 2013). Moreover, some TREM2 lipidic ligands accumulate on the cell surface of neurons and glial cells damaged by A β accumulation, such as PS (Eckert et al., 2005; McLaurin and Chakrabarty, 1996), or are released by damaged myelin, such as SM and sulfatides. In contrast, TREM2 did not directly bind A β . Consistent with its ability to bind anionic lipids, the TREM2 extracellular domain is rich in arginine residues that may form salt bridges with polyanions. Remarkably, we found that the R47H mutation associated with AD affected the binding of multiple lipid ligands, although to differing extents. Most likely, the R47H mutation is sufficient to considerably reduce the binding affinity of TREM2 extracellular domain for most anionic lipids. Structural studies will be essential to validate this model.

Our findings demonstrated that TREM2 functions as a microglial sensor that is alerted by damage-induced molecules that share a common lipidic backbone and an anionic group. In contrast with previous reports (Hsieh et al., 2009; Takahashi et al., 2005, 2007), we found that the engagement of TREM2 does not directly mediate phagocytosis of apoptotic cells. However, TREM2 signaling may indirectly support phagocytosis by promoting survival of activated microglia. It has been shown that individuals homozygous for rare mutations that impair expression of either TREM2 or DAP12 develop lethal forms of progressive, early-onset dementia such as Nasu-Hakola disease (NHD) and frontotemporal dementia (Guerreiro et al., 2013a, 2013c; Kleinberger et al., 2014; Paloneva et al., 2002). Although the pathology of these forms of dementia differs from that of AD and often involves demyelination, our study suggests that TREM2 may be required for microglia to sense glycolipids such as SM and sulfatides that are exposed on damaged myelin sheaths; thus, TREM2 binding to these glycolipids may trigger the microglial response to damaged myelin, which is necessary to clear myelin residues and produce trophic factors that induce

repair and remyelination. While the R47H mutation associated with AD did not entirely abolish ligand binding, mutations associated with Nasu-Hakola disease result in a complete lack of TREM2 expression (Kleinberger et al., 2014), which may explain the distinct pathology and more dramatic clinical course of this disease.

EXPERIMENTAL PROCEDURES

Mice

Trem2^{-/-} mice were generated as previously described. 5XFAD mice were purchased from the Jackson Laboratory (MMRRC) and crossed to *Trem2*^{-/-} mice to generate *Trem2*^{+/-}5XFAD and *Trem2*^{-/-}5XFAD mice. All mice were bred and housed in the same animal facility. *Trem2*^{-/-}*Cx3cr1*^{+/-}GFP^{APPPS1-21} mice were generated in a similar manner, as previously described (Ulrich et al., 2014). All animal studies were approved by the Washington University Animal Studies Committee.

Preparation of Brain Samples

For histological analysis 5XFAD mice, APPPS1-21 and transgene negative controls were anesthetized with ketamine and perfused with ice-cold PBS. Right-brain hemispheres were fixed in 4% PFA overnight and placed in 30% sucrose before freezing and cutting on a freezing sliding microtome. Serial 40- μ m coronal sections of the brain were collected from the rostral anterior commissure to caudal hippocampus as landmarks. For biochemical and mRNA expression analysis, cortices and hippocampi of the left-brain hemispheres were carefully dissected out and flash frozen in liquid nitrogen.

Immunohistochemistry and Microscopy

For detailed procedures, see [Extended Experimental Procedures](#).

Gene Expression Analysis

For frozen brain tissues, RNA was extracted using a RNeasy mini kit according to manufacture protocol (QIAGEN). Microglia were fluorescence-activated cell-sorted (FACS) directly into RLT-plus lysis buffer, and RNA extraction was performed using a RNeasy micro kit according to manufacture protocol (QIAGEN). Primers for qPCR analysis are provided in [Table S1](#). For detailed procedure on microarray analysis, see [Extended Experimental Procedures](#).

ELISA

A β levels were assessed using sandwich ELISAs as described (Kim et al., 2009). For detailed procedure, see [Extended Experimental Procedures](#).

Ex Vivo Microglia Cultures

Primary adult microglia culture was generated as previously described (Butovsky et al., 2014). Briefly, purified adult microglia were cultured in the presence of 15% LCM media (Otero et al., 2009) and 10 ng/ml human TGF- β 1 (Pepro-Tech) for 7 days before experiments. For details on in vitro assays performed, see [Extended Experimental Procedures](#).

Reporter Assay

2B4 GFP-NFAT reporter T cells were stably transfected with murine or human TREM2 cDNAs. Cells were cultured with apoptotic thymocytes in round-bottom 96-well plates or plated onto high-absorbance flat-bottom plate coated with various lipids at indicated concentration. Reporter cells were assessed after overnight incubation. Reporter activity (%) is defined as %GFP⁺ cells subtracted from background (vehicle controls).

Statistics

Data in figures are presented as mean \pm SEM. All statistical analysis was performed using Prism (GraphPad). Statistical analysis to compare the mean values for multiple groups was performed using a one-way or two-way ANOVA with correction for multiple comparisons. Comparison of two groups was performed using a two-tailed unpaired t test (Mann-Whitney). Values were accepted as significant if $p \leq 0.05$.

ACCESSION NUMBERS

All data have been deposited at GEO (GSE65067).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, five figures, one table, and four movies and can be found with this article online at <http://dx.doi.org/10.1016/j.cell.2015.01.049>.

AUTHOR CONTRIBUTIONS

Y.W., J.D.U., M.L.R., S.S., and B.H.Z. performed two-photon and confocal imaging analyses; Y.W., B.H.Z., and G.M.K. performed computational analyses; K.M., K.L.Y., and J.R.C. analyzed A β deposition; Y.W. performed ex vivo cell-culture experiments; M. Cella and Y.W. generated TREM2 reporter cells and performed reporter assays; and S.G. generated and maintained *Trem2*^{+/-} and *Trem2*^{-/-}5XFAD mice. D.M.H. supervised research on APPPS1-21 mice and provided critical comments. M. Colonna supervised the entire project; M. Colonna and Y.W. wrote the manuscript.

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