Microglia use TAM receptors to detect and engulf amyloid β plaques

Youtong Huang¹,², Kaisa E. Happonen¹, Patrick G. Burrola¹, Carolyn O’Connor³, Nasun Hah⁴, Ling Huang⁵, Axel Nimmerjahn⁶ and Greg Lemke⁴,¹

Two microglial TAM receptor tyrosine kinases, Axl and Mer, have been linked to Alzheimer’s disease, but their roles in disease have not been tested experimentally. We find that in Alzheimer’s disease and its mouse models, induced expression of Axl and Mer in amyloid plaque–associated microglia was coupled to induced plaque decoration by the TAM ligand Gas6 and its co-ligand phosphatidylserine. In the APP/PS1 mouse model of Alzheimer’s disease, genetic ablation of Axl and Mer resulted in microglia that were unable to normally detect, respond to, or phagocytose amyloid-β plaques. These major deficits notwithstanding, TAM-deficient APP/PS1 mice developed fewer dense-core plaques than APP/PS1 mice with normal microglia. Our findings reveal that the TAM system is an essential mediator of microglial recognition and engulfment of amyloid plaques and that TAM-driven microglial phagocytosis does not inhibit, but rather promotes, dense-core plaque development.

The TAM receptor tyrosine kinases (RTKs) Axl and Mer (gene name Merk) exert two key functions in macrophages and other immune sentinel cells. First, they are required for the phagocytosis of apoptotic cells (ACs) and that TAM-driven microglial phagocytosis does not inhibit, but rather promotes, dense-core plaque development.

In the APP/PS1 mouse model of Alzheimer’s disease, genetic ablation of Axl and Mer resulted in microglia that were unable to normally detect, respond to, or phagocytose amyloid-β plaques. These major deficits notwithstanding, TAM-deficient APP/PS1 mice developed fewer dense-core plaques than APP/PS1 mice with normal microglia. Our findings reveal that the TAM system is an essential mediator of microglial recognition and engulfment of amyloid plaques and that TAM-driven microglial phagocytosis does not inhibit, but rather promotes, dense-core plaque development.

References:

¹Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA, USA. ²Division of Biological Sciences, University of California, San Diego, La Jolla, CA, USA. ³Flow Cytometry Core, The Salk Institute for Biological Studies, La Jolla, CA, USA. ⁴Chapman Foundations Genomic Sequencing Core, The Salk Institute for Biological Studies, La Jolla, CA, USA. ⁵Razavi Newman Integrative Genomics and Bioinformatics Core, The Salk Institute for Biological Studies, La Jolla, CA, USA. ⁶Waitt Advanced Biophotonics Center, The Salk Institute for Biological Studies, La Jolla, CA, USA. ⁷e-mail: lemke@salk.edu
calcium-binding adaptor molecule 1) or green fluorescent protein (GFP) in Cx3cr1-eGFP mice. Perivascular macrophages are also GFP+ in these mice, but are readily distinguished from microglia. Iba1 and Cx3cr1 are also expressed by peripheral myeloid cells that may enter the brain and assume microglia-like properties in neurodegenerative disease. Microglia near to but not touching plaques, identified with the 6E10 antibody to the amino terminus of human APP, were Axl− in aged APP/PS1 mice, whereas those contacting plaques were strongly Axl+ (Fig. 1a). Notably, Axl protein upregulation is also seen in plaque-associated microglia in human AD. Microglia that upregulate Axl (green) and Iba1 (white) protein expression in 9.5-mo APP/PS1 mice also upregulate expression of Trem2 (red). Bottom panels in a, c and d are enlargements of top panels. a, c and d contain representative images from N ≥ 3 sections per mouse from n = 3 mice from at least three independent replicates. Data in b were measured from N ≥ 3 images per mouse from n = 3 and 5 mice for 2.5-9.5-mo and 12- and 15-mo time points, respectively. All scale bars, 10 μm.

by immunohistochemistry in healthy wild-type (WT) microglia (Extended Data Fig. 1a). At 9.5 mo, Axl levels were ~25-fold higher in PAM versus NPAM (Fig. 1b). We detected a similar Axl upregulation in PAM in aged APP41 mice, in which Axl was also frequently concentrated at the center of plaques (Extended Data Fig. 1b). As Axl activation is always accompanied by cleavage of the Axl ectodomain, this plaque-deposited Axl is almost certainly sAxl. In contrast to Axl, Mer is abundant in all normal human and mouse microglia. Although modest reductions in MerTk mRNA have been reported for PAM in APP/PS1 mice, Mer protein expression was upregulated in PAM in aged APP/PS1 (Fig. 1c) and APP41 (Extended Data Fig. 1c) mice. In the 9.5 mo APP/PS1 brain, we quantified a 3.5-fold elevation in Mer in PAM versus NPAM (Extended Data Fig. 1d). Axl+Mer+ microglia were the same plaque-associated cells that also upregulated Trem2 (Fig. 1d and Extended Data Fig. 1e), mutations in which increase the risk of developing human AD. Although upregulated Axl, Mer and Trem2 were always present in the same cells, Trem2 and TAMs seemed to occupy distinct

Fig. 1 | TAM receptor upregulation is strictly plaque-associated. a, Axl protein (green) in 9.5-mo APP/PS1 mice is upregulated in microglia (Iba1, red) that are in contact with Aβ plaques (6E10, white), but not in microglia not contacting plaques (arrowheads in lower panels). Iba1 expression is also upregulated in PAM. Hoechst stains nuclei. b, Quantification of Axl mean fluorescence intensity (MFI) in cortical Iba1+ microglia in APP/PS1 mice over time reveals marked Axl upregulation coincident with the first deposition of plaques at ~5 mo, only in plaque-associated Iba1+ cells (green points). c, Expression of Mer protein (green) in 9.5-mo APP/PS1 mice is seen in all cortical microglia (Iba1, red), but is further upregulated in microglia that invest Aβ plaques (6E10, white). d, The same cortical microglia that upregulate Mer (green) and Iba1 (white) protein expression in 9.5-mo APP/PS1 mice also upregulate expression of Trem2 (red). Bottom panels in a, c and d are enlargements of top panels. a, c and d contain representative images from N ≥ 3 sections per mouse from n = 3 mice from at least three independent replicates. Data in b were measured from N ≥ 3 images per mouse from n = 3 and 5 mice for 2.5-9.5-mo and 12- and 15-mo time points, respectively. All scale bars, 10 μm.
that Gas6 protein is undetectable in nondiseased brains because astrocytes expressed no detectable Mer (Extended Data Fig. 1h and center of plaques were occasionally TMEM119+ mice was tied to Axl upregulation of plaque-associated Gas6 in which is already high in normal microglia9, does not change over Gas6 mouse AD models because microglial expression of 2a). This upregulation was missed in earlier scRNA-seq analyses of these tissues is Axl-dependent5. Gas6 expression is lost in been shown that most of the Axl on cells in WT mouse tissues is also marks plaques in human AD. Gas6 activates both Axl and Mer, but Mer can also be activated by the related ligand Protein S (Pros1)13. Although antibodies for high-resolution immunohistochemical detection of Pros1 in mouse are not currently available, microglia express abundant Pros1 mRNA and deep proteomic profiling of the 5xFAD hippocampus has revealed that elevated Pros1 is a new biomarker for disease severity in this model11. It is therefore very possible that plaques are also decorated with Pros1.

In addition to ligands and receptors, TAM signaling requires binding of the phospholipid phosphatidylserine (PtdSer) to the amino-terminal ‘Gla’ domains of Gas6 and Pros1 (refs. 13,19). For example, a ‘Gla-less’ truncation mutant of Gas6 binds Axl with the same affinity as the full-length ligand, but is incapable of activating the receptor13. With respect to TAM receptor interaction, PtdSer is generally externalized on the surface of a cell (often an AC) that is apposed to a TAM-expressing cell13. Although PtdSer is a component of every plasma membrane, in all normal cells it is enzymatically confined to the inner leaflet of the membrane bilayer1. Translocation of PtdSer to the plasma membrane surface is carried out by a set of PtdSer scramblases during apoptosis and in response to Ca2+ entry, viral infection and disease stressors1. During AC phagocytosis, Gas6 and/or Pros1 ‘bridge’ PtdSer that is externalized on the AC surface to TAM receptors expressed on the surface of microglia and other phagocytes1. To detect externalization of PtdSer in APP/PS1 mice, we stereotaxically injected their cortices with pSIVA, a ‘polarity-sensitive indicator for viability and apoptosis’ that fluoresces strongly only when bound to PtdSer25. The needle track of this injection (where cells were damaged) was strongly pSIVA+, but radiating out from the injection site we detected coincident pSIVA labeling of PtdSer on all 6E10+ Aβ plaques (Fig. 2c and Extended Data Fig. 2b). No pSIVA puncta were labeled outside the needle track after injection of WT brains (Extended Data Fig. 2c). Externalized plaque-associated PtdSer may mark the dystrophic neurites that are present in plaques and their immediate surroundings. Super-resolution microscopy suggested that Gas6 does indeed bridge PtdSer+ Aβ plaques to TAM+ microglia in the aged APP/PS1 brain (Extended Data Fig. 2d). Together, these results demonstrate that all of the essential components of the TAM system (the receptors, their ligand Gas6 and the essential co-factor PtdSer) are specifically upregulated on and around Aβ plaques in AD.
TAM regulation of the microglial transcriptome in AD. To obtain a global perspective on how TAM signaling influences gene expression in PAM and NPAM, we performed comparative scRNA-seq of CD45+ cells sorted (Extended Data Fig. 3a) from plaque-burdened cortices at ~18 mo, when plaques were abundant, using a 10X Genomics platform. Several previous RNA-seq analyses have been performed with human and mouse microglia in neurodegenerative settings12,13,14,15 and these provide data for comparison with our results. We performed high-throughput single-cell transcriptomics of a total of 14,628 CD45+ immune cells from APP/PS1 and APP/PS1Axl−/−Mertk−/− cortices and detected nine immune clusters by applying Seurat analyses16 (Extended Data Fig. 3b). Annotation using 18 marker genes (Extended Data Fig. 3c) allowed us to associate these clusters with specific cell types, of which microglia comprised the largest cluster. Also present in the CD45+ sort were natural killer and T cells, neutrophils, monocytes and nonmicroglial macrophages and smaller populations of B cells, oligodendrocytes, endothelial cells and proliferating cells of various lineages (Extended Data Fig. 3b).

In a second clustering round, we identified seven microglial clusters and numbered and staged these in the APP/PS1 and APP/PS1Axl−/−Mertk−/− replicas (Fig. 3a). Clusters were numbered based on transcriptomic state, with the largest cluster 0 (c0; Fig. 3a) representing the homeostatic microglial ground state. Mertk mRNA was distributed across all clusters, whereas Axl mRNA was largely restricted to clusters 4–6 (Extended Data Fig. 3d). Prominently expressed mRNAs in cluster 0 included Tnem119, Cx3cr1, P2ry12 and Maf (Fig. 3b and Extended Data Fig. 3e,f).

Cluster 1 microglia seem to represent a small one-way radiation from cluster 0, in which mRNAs indicative of immune stimulation, Cst7 cluster 5 cells included of cluster 5, in which microglia are primed for antigen presentation (Fig. 3e). The small cluster 6 seems to represent a further activation of Keren-Shaul et al.11 (Extended Data Fig. 3f). Similarly, cells in 5xFAD mice, which blocks in contrast to the mutation of Axl and Mertk (Fig. 3d,e and Extended Data Fig. 3g). This effect were seen for many DAM signature genes, including Spp1, Cd74 and Gpmb. It was also apparent for genes involved in lipid metabolism (Lpl, Apoc4, Ch25h, Apoe) and MHC class II antigen presentation (H2-Aa, H2-Ab1, H2-Eb1 and Cd74) and for the ‘plaque-forming’ gene Ccl6 (ref. 17). Although RNA for the phagocytosis regulator Sirpα was reduced in both transcriptomic states 5 and 0 in APP/PS1Axl−/−Mertk−/− microglia (Extended Data Fig. 3g), the principal phagocytic mediators affected by Axl and Mertk mutation were Axl and Mer themselves. (The Mertk mutation deletes an essential exon in the kinase domain and produces a protein null, but results in the synthesis of a nonfunctional mRNA that is detectable by PCR, RNase protection and RNA-seq18,19.) Conversely, we found that both the statistical significance (Fig. 3d) and magnitude (Fig. 3e and Extended Data Fig. 3g) of downregulation of many genes reduced in cluster 5 relative to cluster 0 was dampened in APP/PS1Axl−/−Mertk−/− relative to APP/PS1 microglia. These included the homeostatic genes Malat1 and Nfkbia (Fig. 3d,e and Extended Data Fig. 3g).

Finally, we did not observe large-scale upregulation of cytokine or chemokine mRNAs in state 5 versus state 0 microglia (Extended Data Fig. 3h) or in total mRNA purified from whole 12-mo cortex (Extended Data Fig. 3i), as a consequence of the combined loss of both microglial TAMs. Thus, it is unlikely that the phenotypes documented below result from major changes in APP/PS1 neuroinflammation consequent to Axl and Mertk mutation.

Microglial TAM activity at plaques. To determine whether the TAM system perturbations documented above are of significance to microglial interaction with plaques, we used live-two-photon imaging in APP/PS1 mice at 16 mo. We analyzed APP/PS1 hemizygotes that carried a single Cx3cr1GFP allele11 to label microglia and then crossed these mice with both WT and Axl−/−Mertk−/− mutants. We labeled Aβ plaques using intraperitoneal injection of methoxy-X04 (ref. 20) (MX04), a fluorescent amyloid dye and then live-imaged 4–7 plaque-containing volumes per mouse in layers 1/2 of somatosensory cortex for ~90 min, using modifications of two-photon methods described previously21,22. Representative time-lapse recordings of these analyses are shown in Supplementary Videos 1 and 2.

Quantification of microglial behavior allowed us to establish multiple differences in plaque recognition and interaction between WT and Axl−/−Mertk−/− microglia. First, we observed many fewer microglia encapsulating Aβ plaques in Cx3cr1GFP/APP/PS1Axl−/−Mertk−/− versus Cx3cr1GFP/APP/PS1 mice (Fig. 4a). Most plaques were enveloped by tightly bound and morphologically activated microglia in the latter (Fig. 4a), whereas plaques were often unattended by microglia in Cx3cr1GFP/APP/PS1Axl−/−Mertk−/− mice (Fig. 4a). Surface building reconstruction and quantification of imaging volumes (Fig. 4b) yielded a flat distribution of microglia cell body distances from plaques in Cx3cr1GFP/APP/PS1Axl−/−Mertk−/− mice, but showed a strong bias for microglia to be in contact with plaques in Cx3cr1GFP/APP/PS1 mice (Fig. 4c). This difference was independent of plaque size, although larger plaques were bound by more microglia in Cx3cr1GFP/APP/PS1 mice (Fig. 4c and Extended Data Fig. 4a).

Similarly, there were obvious differences in microglial morphology and motility (Fig. 4a,d–g). Cx3cr1GFP/APP/PS1Axl−/−Mertk−/− microglia often displayed a ramified ‘resting’ configuration that was similar to that seen in WT mice (Fig. 4a and Supplementary Video 1). In contrast, Cx3cr1GFP/APP/PS1 microglia displayed an activated
Fig. 3 | TAM regulation of the microglial transcriptome in disease. a. Seven microglial cell clusters (c) defining seven transcriptomic states in combined APP/PS1 and APP/PS1Axl−/−Mertk−/− microglia at 18 mo, displayed by uniform manifold approximation and projection (UMAP). c0 and c5 comprise major homeostatic (NPAM) and activated (PAM) transcriptomic states, respectively. b. Violin plots for expression distribution of the indicated genes for the combined APP/PS1, APP/PS1Axl−/−Mertk−/− (A/PS A/M−/−) microglia yields the same cluster number and mapping for all replicates. c. Scatter plot for signed log10-adjusted P value of shared differentially expressed (DE) genes in state 5 (blue) versus state 0 (red) in A/PS A/M−/− microglia, respectively, log10-adjusted P value was signed by the up/downregulation of the gene. Gray dot indicates where by = 0.05. d. UMAP for individual biological replicates of APP/PS1 (A/PS) and APP/PS1Axl−/−Mertk−/− (A/PS A/M−/−) microglia. e. Normalized mean microglial expression of the indicated genes in transcriptomic state 0 versus 5 in A/PS (black) versus A/PS A/M−/− (red) microglia. DE analysis was performed by Seurat function ‘FindAllMarkers’ and ‘FindMarkers’ with default Wilcoxon rank-sum test and log fold change (FC) > 0.25 on pooled biological replicates. Genes with Bonferroni-adjusted P value <0.05 were considered to be significant.

‘ameboid’ morphology, with larger cell bodies and fewer, shorter processes (Fig. 4a and Supplementary Video 1). Cx3cr1IgGAPP/PS1Axl−/−Mertk−/− PAM elaborated substantially more (Fig. 4d and Extended Data Fig. 4b) and longer (Fig. 4e and Extended Data Fig. 4c) primary processes than Cx3cr1IgGAPP/PS1 PAM.

Processes from microglia positioned >20μm from plaques (NPAM) were strongly oriented toward plaques in the Cx3cr1IgGAPP/PS1Axl−/−Mertk−/− cortex, but showed no orientation bias in the Cx3cr1IgGAPP/PS1Axl−/−Mertk−/− cortex (Fig. 4f and Extended Data Fig. 4d). In addition, microglial process motility, a reduction in which is indicative of microglial activation14, was markedly reduced in APP/PS1 mice relative to WT mice (Fig. 4g). In contrast, process motility was only modestly lower than WT for APP/PS1Axl−/−Mertk−/− PAM (microglia <5μm from the nearest plaque) (Fig. 4g) and was not statistically different from WT for APP/PS1Axl−/−Mertk−/− NPAM (Fig. 4g). Finally, AD microglia normally proliferate near plaques. Mutation of Trem2 in the 5xFAD mouse model blunts this microgliosis ∼twofold15,16 and we measured a similarly blunted proliferation of microglia, primarily those in close proximity to plaques, in the cortex of Cx3cr1IgGAPP/PS1Axl−/−Mertk−/− relative to Cx3cr1IgGAPP/PS1 mice (Extended Data Fig. 4e). Thus, by all of the above measures, TAM-deficient microglia were strikingly deficient in their ability to detect, bind and respond to amyloid plaques.

TAM-dependent phagocytosis and shaping of plaques. Microglia are vigorous phagocytes that exhibit an absolute TAM receptor requirement for AC engulfment6. As TAM-mediated phagocytosis of ACs is entirely dependent on PtdSer externalization on the AC surface17 and because all AP plaques are decorated with both PtdSer (Fig. 2c) and Gas6 (Fig. 2a,b), we used two-photon imaging to measure engulfment of MX04-labeled amyloid plaque material into GFP+ microglia that were either WT or Axl−/−/Mertk−/−. We
quantified internalized MX04 signals both per normalized volume and per GFP volume. These measurements revealed a clear deficit; we quantified ~tenfold lower levels of engulfed MX04-labeled plaque material inside of Axl<sup>−/−</sup>Merk<sup>−/−</sup> as compared to WT microglia by both measures (Fig. 5a,b). Note that internalized amyloid material, which may be routed to acidic lysosomes, occupies a remarkable 9% of WT microglial volume in APP/PS1 mice (Fig. 5b). These data indicate that microglia cannot effectively phagocytose amyloid material without TAM receptors.

Amyloid plaques are typically surrounded by a halo of dystrophic neuronal membranes<sup>17</sup>. This halo is shaped by microglial processes per nearest plaque for microglia<sup>−/−</sup> versus APP/PS1 and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> mice. Scale bars, 30 μm. c, Distribution of distances of microglial cell body centroids from the edge of Aβ plaques in APP/PS1 and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> cortex. A total of 22 and 37 plaques were investigated from three and four mice for APP/PS1 and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup>, respectively. d, Primary microglial processes per nearest plaque for microglia<sup>−/−</sup> <5 μm from plaques (PAM) in APP/PS1 (A/PS) and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> (A/PS A/M<sup>−/−</sup>) cortex. e, Summed length of primary microglial processes per nearest plaque for PAM in APP/PS1 and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> cortex. f, Process polarization to nearest plaque (ratio of summed length of primary processes oriented toward plaque to summed length of all primary processes) for NPA (microglia<sup>−/−</sup>) in APP/PS1 and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> cortex. g, Process motility for cortical PAM, NPA and non-diseased microglia in mice of the indicated genotypes. Data points are from 7–29 representative plaques from three mice from both genotypes (d–f) and 18–52 microglia from two (WT and Axl<sup>−/−</sup>Merk<sup>−/−</sup>) and three (APP/PS1 and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup>) mice (g). Data represented as mean ±1x s.d. Mann-Whitney U-test (d–f) and Kruskal-Wallis test followed by Dunn’s multiple comparisons test (g).

Fig. 4 | Microglia use Axl and Mer to detect, engage and react to Aβ plaques. a, Representative video stills from two-photon imaging of microglia (GFP signal, white) and amyloid plaques (MX04 signal, red) in 16-mo APP/PS1 and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> cortex. b, Imaris surface build of microglial volumes at 0–5 μm (white) and 5–20 μm (green) from the edge of nearest plaque (red) in APP/PS1 and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> cortex, representative of image volumes in cortices of n = 3 APP/PS1 and n = 5 APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> mice. Scale bars, 30 μm. c, Distribution of distances of microglial cell body centroids from the edge of Aβ plaques in APP/PS1 and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> cortex. A total of 22 and 37 plaques were investigated from three and four mice for APP/PS1 and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup>, respectively. d, Primary microglial processes per nearest plaque for microglia <5 μm from plaques (PAM) in APP/PS1 (A/PS) and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> (A/PS A/M<sup>−/−</sup>) cortex. e, Summed length of primary microglial processes per nearest plaque for PAM in APP/PS1 and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> cortex. f, Process polarization to nearest plaque (ratio of summed length of primary processes oriented toward plaque to summed length of all primary processes) for NPA (microglia<sup>−/−</sup>) in APP/PS1 and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> cortex. g, Process motility for cortical PAM, NPA and non-diseased microglia in mice of the indicated genotypes. Data points are from 7–29 representative plaques from three mice from both genotypes (d–f) and 18–52 microglia from two (WT and Axl<sup>−/−</sup>Merk<sup>−/−</sup>) and three (APP/PS1 and APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup>) mice (g). Data represented as mean ±1x s.d. Mann-Whitney U-test (d–f) and Kruskal-Wallis test followed by Dunn’s multiple comparisons test (g).

Dense-core plaque burden in the absence of TAM signaling. Microglial phagocytosis has heretofore been thought to inhibit the growth of plaques<sup>4</sup>. Thus, a prediction from the relative inability of APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> microglia to engulf Aβ material is that aged TAM-deficient AD mice should display a much higher plaque burden than AD mice with WT microglia. Our initial indication that this was not the case came from the APP/PS1Axl<sup>−/−</sup>Merk<sup>−/−</sup> two-photon volumes that we recorded, most of which contained fewer MX04-labeled plaques than APP/PS1 volumes with WT microglia (examples in Fig. 6a). To measure plaque burden in these populations, we used semi-automated quantification of thioflavin S (Thio S)-stained dense-core plaques in...
Fig. 5 | TAM-deficient microglia cannot neither phagocytose nor organize plaques. a,b, MX04-labeled Aβ plaque material engulfed within GFP+ microglia, imaged in vivo, in 16-mo APP/PS1 (A/PS) versus APP/PS1Axl−/−Mertk−/− (A/PS A/M−/−) cortex, normalized to imaging volume (a) and the volume of GFP+ cells (b). c, Representative images of the halo of LAMP1+ dystrophic membranes (green, bottom) that surround 6E10+ plaques in 12-mo APP/PS1 (A/PS; left) versus APP/PS1Axl−/−Mertk−/− (A/PS A/M−/−; right) cortex. Arrowheads mark weakly staining, diffuse 6E10+ plaques, which are more common in the APP/PS1Axl−/−Mertk−/− brain (see also Extended Data Fig. 5). Scale bar, 10 μm. d, Quantification of the ratio of LAMP1+ area to 6E10+ plaque area across all plaque sizes, both dense-core and diffuse. e, Quantification of the ratio of RTN3+ area to 6E10+ plaque area across all plaque sizes, both dense-core and diffuse. Data are 13–15 volumetric images from n = 3 and 4 mice for APP/PS1 and APP/PS1Axl−/−Mertk−/−, respectively (a,b). Data are 94–113 plaques (d) and 56–95 plaques (e) investigated from N ≥ 3 sections per mouse from n = 3 mice of each genotype. Mann-Whitney U-test (a,b,d,e). Data are represented as mean ± 1x.s.d.

Discussion

Our results lead to two principal conclusions. First, the TAM system is required for microglial recognition of, response to and phagocytosis of Aβ plaques. And second, TAM-mediated microglial phagocytosis of Aβ material does not inhibit, but rather promotes, the formation of dense-core plaques.

The first of these conclusions is consistent with previous knowledge as to TAM action in microglia and other macrophages. All Aβ plaques are decorated with externalized PtdSer, an essential ligand for TAM action in microglia and other macrophages. TAM ligands bridge macrophages and microglia to PtdSer-expressing ACs during engulfment. Our results, therefore, are consistent with previous knowledge. In the absence of Axl and Mer, microglia display a diminished transcriptomic response to plaques, are compromised in their attachment to plaques, do not re-orient their processes toward plaques and are blunted in their proliferative, process extension and process motility responses to plaques. Most notably, Axl−/−Mertk−/− microglia cannot effectively phagocytose Aβ plaque material.

There are some similarities between these phenotypes and those seen when Trem2−/− mice are crossed into amyloidogenic AD mouse models, but in general the TAM phenotypes are far stronger. The closest similarities are seen with respect to microglial proliferation and plaque binding. Microglial proliferation around plaques is reduced two- to fourfold in Trem2−/− compound mutants and also two- to fourfold for Axl−/−Mertk−/− compound mutants. Similarly, the number of microglia bound per plaque is reduced two- to fourfold in Trem2−/− compound mutants and ~ fivefold in the Axl−/−Mertk−/− compound mutants. Loss of Trem2 from the APP/PS1-21 line results in a ~3.5-fold increase in the volume of LAMP1+ membranes surrounding filamentous plaques and a ~1.5-fold increase for compact plaques in late-stage disease, whereas these numbers for the APP/PS1Axl−/−Mertk−/− mice are ~ eightfold and
~fourfold at a comparable stage of disease. As dystrophic neurites arise around plaques devoid of microgliar coverage,
the paucity of microgliar plaque association seen in APP/PS1Axl−/−Mertk−/− mice may result in collateral axonal damage.

There is no consensus among research groups as to the effect of Trem2 deletion on Aβ plaque burden in amyloidogenic AD models,
with different groups reporting either marginal increases or decreases or no changes, in plaque density (measured with Thio S, 6E10 or amyloid dyes) that vary with the model employed, age analyzed and brain region assessed. In contrast, APP/PS1Axl−/−Mertk−/− mice display a consistent reduction in dense-core plaque density, in both the cortex and the hippocampus, across the full course of disease in APP/PS1 mice. Most notably, Trem2−/− microglia exhibit a modest ~twofold reduction in their ability to phagocytose Thio S-labeled Aβ material and display normal phagocytosis of Aβ in vitro assays,
whereas Axl−/−Mertk−/− microglia exhibit a tenfold deficit in their ability to phagocytose Thio S-labeled Aβ material in vivo and are incapable of any Aβ phagocytosis in vitro. Together, these results argue that Mer and Axl are the main receptors that microglia use to detect, engage and engulf amyloid plaques. Indeed, it is possible that some of phenotypes observed in Trem2−/− mice are in part TAM-mediated, as scRNA-seq analyses have indicated that upregulation of Axl mRNA in the 5xFAD model is Trem2-dependent.

The second of our principal conclusions is contrary to expectation. How does a tenfold reduction in phagocytic capacity, coupled with a twofold reduction in microglial numbers and a fivefold reduction in plaque binding, result in 35% fewer dense-core plaques? This finding should be considered in light of two sets of previous findings: loosening of Aβ fibrils and that once routed to lysosomes, Aβ fibrils are compacted into dense-core material that is ‘indigestible’.

A second key observation is provided by studies in which microglia were killed by pharmacological inhibition of the CSF1 receptor. These studies demonstrated that plaques never appear in the 5xFAD brain when microglia are killed, except in the limited regions where microglia are spared from death. Together, these findings and our results suggest that dense-core Aβ plaques do not form spontaneously, but are instead constructed from loosely-organized Aβ material by phagocytic microglia and that TAM receptors are key components of the molecular machinery through which this is achieved. They are consistent with the hypothesis that dense-core plaques may represent a macrophage-mediated confinement mechanism, perhaps analogous to the granulomas of tuberculosis and other infections, which limits the dissemination of toxic pre-plaque Aβ oligomers throughout the brain. This may in part explain why agents that disaggregate dense-core plaques but do little to alter the production of Aβ peptides have largely failed as AD therapeutics.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41590-021-00913-5.

Received: 5 May 2020; Accepted: 5 March 2021;
Published online: 15 April 2021

References


**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature America, Inc. 2021
**Methods**

Mice. C57BL/6J WT mice were obtained from The Jackson Laboratory. Mice were typically group-housed at approximately 22 °C and provided with bedding and nesting material. All animals were maintained on a 12 h light–dark cycle and given ad libitum access to standard rodent chow and water. The Ab−/−, Merk−−, Axl−−, and Cx3cr1−− mice have been described previously.6 B6.Cg-Tg(APPsw-PSEN1dE9) hemizygous mice (APP/PS1) (JAX no. 005864) were crossed with Axl−− and/or Axl−− mice to generate APP/PS1Axl−−, APP/PS1Merk−− and APP/PS1Axl−− Mice. For two-photon microscopy, APP/PS1 mice were crossed with Cx3cr1GFP/GFP or Cx3cr1CreERT2Axl−− mice. Only female APP/PS1Cx3cr1GFP/GFP WT or Axl−− mice and healthy littermates were used in two-photon studies to avoid potential sex biases in pathology and microglial responses. APP/42 mice, which express a ‘Swedish’ + ‘London’ (V171I) mutant human APP under the Thy-1 promoter,32,33 were a kind gift of K.-F. Lee and were genotyped by the Salk Institute Animal Care and Use Committee (protocol no. 17-00009). Mice of both sexes were randomly allocated to experimental groups unless otherwise noted.

Reagents and antibodies. Antibodies used were as follows: anti-Aβ (R&D AF584, 1:50 dilution), anti-Mer (Ebioscience DSSMMER and R&D AF591, 1:200 dilution), anti-mouse Ga6 (R&D AF896, 1:50 dilution), anti-human Ga6 (R&D AF885, 1:25 dilution), anti-β-amyloid, clone 6E10 (BioLegend 803001, 1:500 dilution), anti-Iba1 (Wako 105-8942, 1:250 dilution), anti-CD11b (BioLegend 125-09C5, 1:200 dilution), anti-GEF (Dako z-334, 1:500 dilution), cleaved Casp3 (Cell Signaling 9661, 1:200 dilution), anti-CD68 (BioRad MCA1957, 1:200 dilution), anti-RTN3 (EMD Millipore ABN1723, 1:500 dilution), anti-Tmem119 (Abcam AB20964, 1:200 dilution), anti-LAMP1 (BD Biosciences, 553792, clone 1D4B, 1:200 dilution) and anti-Trem2 (R&D systems, AF1729 and BAF 1729, 1:200 and 1:50 dilution, respectively), anti-CD68 (BioRad MCA1957, 1:200 dilution), anti-laminin (Sigma L-9399, 1:500 dilution). For flow cytometry, anti-mouse CD16/32 (BioLegend 101301, 1:100 dilution), anti-CD45-PE, 2D1 (Invitrogen 12-4954-92, 1:25 dilution), anti-CD11b-FITC, M1/70 (Invitrogen 11-0112-85, 1:25 dilution), Anti-vGlut1 (Millipore, AB9505) and anti-PSD95 (Life Technologies, 51-6900) were kind gifts of the N. Allen laboratory. Secondary antibodies for immunohistochemistry were all used at 1:500 to 1:1000, and fluorescent-modified secondary antibodies were used for detection. In addition to primary antibodies, anti-CD11b-FITC (1:25 dilution, BioLegend) and Hoechst 33258 (Sigma) were used for cell and nuclear staining. Subsequent steps were carried out on ice, unless otherwise specified. Pooled cortical and hippocampal samples were snap-frozen in TBS tissue freezing medium. The left hemisphere was sagittally cut at a parallel plane to the midline, each spaced 0.15 mm apart. Plaque abundance was quantified by immunostaining with 6E10 antibodies. A serial set of 15-μm sections was used for both plaque number quantification and immunostaining with 6E10. A serial set of 15-μm sections was used for both plaque number quantification and immunostaining with 6E10. Whole brain sections were subjected to a second round of mechanical dissociation on ice using a 70-μm cell strainer.

**Isolement and purification of brain immune single cells for sequencing.** Mature APP/PS1 or APP/PS1Axl−−/− (18 mo) were transcardially perfused with ice-cold D-PBS containing Ca2+ and Mg2+ and brains were promptly dissected out and placed on pre-chilled Petri dishes on ice to extract cortices. Cortices from two mice were pooled for each sample and two biological samples per genotype were included in our study. All steps were carried out on ice, unless otherwise specified. Pooled cortices were minced with a razor blade, suspended in 5 ml D-PBS in 15-ml tubes for tissue chunks to settle before removal of supernatant. Single-cell suspensions were prepared following a modified version of the Neural Tissue Dissociation kit from Miltenyi Biotec (130-094-002). Briefly, for each sample, an enzyme mixture I containing 50 μl enzyme P in 1,910 μl buffer Z supplemented with 0.12 μg DNase (Sigma) and an enzyme mixture 2 containing 30 μl Buffer Y and 15 μl of enzyme A were prepared and these enzyme mixtures were kept on ice before use. The settled tissue pellet was resuspended in mixture 1 and horizontally agitated at room temperature for 15 min. Thirty microliters of enzyme mixture 2 was added before mechanical dissociation on ice using a fire-polished Pasteur pipette. The cell suspension was further incubated at room temperature with constant rotation for 10 min, after which the remaining mixture 2 was removed. Sample was collected from the second round of mechanical dissociation on ice with fire-polished Pasteur pipettes with decreasing diameter until no observable tissue pieces remained. The resulting cell suspensions were filtered using 70-μm cell strainers, which were subsequently washed with 10 ml D-PBS supplemented with 0.5% BSA. The cells were centrifuged at 300g for 5 min at +4 °C and pellets were suspended in 10 ml 30% isotonic Percoll (GE Healthcare 17-0891-01) diluted in 1X HBSS and then centrifuged again at 700g for 15 min at +4 °C with minimum acceleration and braking. The bottom 5 ml of the gradient containing microglia was collected, resuspended and passed through a 70-μm cell strainer. The cell suspension was washed in ice-cold 1X HBSS for a total volume of 40 ml and pelleted at 300g for 10 min at +4 °C.

**Flow cytometry and fluorescence activated cell sorting.** Dissociated cells were resuspended in 300 μl of FACS buffer (2% FBS and 1 mM EDTA in D-PBS, sterile). Fluorescence-labeling procedures were then carried out on ice. Fc-receptors were blocked by addition of anti-CD16/32 antibody (1:100 dilution, Biologend) for 15 min following the addition of labeled antibodies: anti-CD45-PE (1:25 dilution, Biologend), anti-CD11b-FITC (1:25 dilution, Biologend) and Hoechst 33258 (1:1000 dilution) for 1 h. Finally, samples were washed twice with D-PBS and immediately taken to the Salk Institute Flow Cytometry core facility (samples were kept on ice from this point onwards, or chilled at +4°C while undergoing FACS purification).

**FACS purification** was carried out on a BD FACs Aria Fusion sorter with 1X PBS for PBS wash buffer. Flow cytometry, concentrated cells suitable for downstream 10X Genomics analysis, an 85-μm nozzle was used with sheath pressure set to 45 PSI. Live cells were gated first (Hoechst dye negative), followed by exclusion of debris using forward and side-scatter pulse area parameters (FSC-A and SSC-A), exclusion of aggregates using forward and side-scatter pulse width parameters.
Articles

Analysis of scRNA-seq. Raw sequencing data were aligned to the reference built using Ensembl primary assembly and annotation (release-93) that included both protein coding genes and polymorphic pseudogenes by cell ranger pipeline (v.3.1.0). The result filtering gene expression matrix was further analyzed by Seurat (3.2.1.9002). Cells that had <10% of reads mapped to mitochondria genes and 2900–10,000 expressed genes were used in the analysis. Samples were integrated on an ice-cold platform and snap-frozen in liquid nitrogen. Tissues were lysed to analyze and UMAP analysis with default settings. After manually examining the principal component (PC) elbow plot, the top 25 PCs of the integrated data were used for clustering functions with default settings. The top 20 PCs were used for clustering and UMAP analysis. A cluster that coexpressed microglia to further reveal the relationship of cells within the population. The top examined to annotate the clusters. A second round of analysis was performed on large group of well-known immune markers (Extended Data Fig. 3b) were carefully

Quantitative PCR with reverse transcription. RNA from snap-frozen cortex or hippocampal tissue was isolated with TRIzol (Thermo Fisher Scientific, 15596026) according to the manufacturer’s instructions. The RT Transcriptor First Strand cDNA Synthesis kit (Roche) with anchored oligo(dT) primers (Roche) was used for reverse transcription. Quantitative PCR was run in a 384-well plate format on a QuantStudio Q5. Oligonucleotide primer sequences were ordered from Integrated DNA Technologies and reconstituted in house. Primer sequences used were: Gas6 forward primer 5′−3′ AACTGGTGAAGACGGGGAG; reverse primer 5′−3′ CCTCCAGGTTTTCTCCGT; TNC forward primer 5′−3′ GACACCACTGCTGCT and reverse primer 5′−3′ CGACTGCTCCTCCACCTGT. IfNα forward primer 5′−3′ CCAACAGCCAGAGAATGAC and reverse primer 5′−3′ GCCCTTCTGTCGCCGAGTT. IfNγ forward primer AGACAGAACGAGTAGCTCACA and reverse primer 5′−3′ GGAAGGCGCAAGTCACACGC. relative expression of genes was normalized to β-actin expression. The β-actin forward primer was 5′−3′ GGGTCCCTTTGATGGCAACAGCC CG. Relative expression of genes of interest was normalized to Gapdh (primer pairs: forward sequence 5′−3′ GGGTCCCTTTGATGGCAACAGCC reverse sequence 5′−3′ GGGTCCCTTTGATGGCAACAGCC CG). Extraction of soluble Ab and quantification of Ab(α) by ELISA. Briefly, whole cortices and hippocampi were swiftly dissected from freshly extracted brains on an ice-cold platform and snap-frozen in liquid nitrogen. Tissues were lysed on ice in RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% SDS, 1% Triton-X 100 and 0.5% deoxycholate) with protease and phosphatase inhibitor (Roche, Sigma) at 4°C. RIPA buffer volume was adjusted to 0.25 mL per well overnight at 4°C. The following day, plates were blocked at 150 μl per well with 1% casein blocking buffer for 2h at room temperature. Plates were washed five times with PBS + 0.05% Tween-20. JNF/AbN2 detection antibody to the N terminus of Ab(α) had been biotinylated and dialogized following manufacturer’s instructions. The 25 μl biotinylated detection antibody was diluted in blocking buffer and then mixed with either 25 μl of standards (synthetic human Ab(α) peptide or 25 μl extracellular medium before loading 50 μl per well and in duplicate. Following overnight incubation at 4°C, plates were rinsed and incubated in streptavidin–HRP at 0.1 μg/mL at 30°C for 1h. Plates were immediately measured on a TECAN Infinite 200 PRO reader at 450 nm.

Surgical and animal preparation for in vivo PtdSer labeling. Stereotactic injections were performed following previously published protocols. Briefly, thin-wall glass pipettes were pulled on a Sutter Flaming/Brown puller and cut using sterile techniques resulting in tip diameters of 10–15 μm. Adult APP/PS1 and WT control mice (15–16 mo) were anesthetized with isoflurane (4% for induction; 1–2% during surgery). Mice were head-fixed in a computer-assisted stereotaxic frame. The stereotactic coordinates were based on the stereotaxic atlas of Paxinos and Watson. Each mouse was stereotactically injected with 250 μL of pSIVA on the right hemisphere of each animal. Neocortical coordinates were (anterior–posterior (AP) +1.1 mm, medial–lateral (ML) +1.5 mm, dorsal–ventral (DV) +1 mm). AP (+0.0 mm, ML +1.5 mm, DV +1 mm) and AP (−1 mm, ML +1.5 mm, DV +1 mm) along the motor and somatosensory cortex around layer III/IV. First, craniotomy sites were marked and an electrical micro-drill with a fluted bit (0.5-mm tip diameter) was used to thin a 0.5–1-mm diameter part of the bone over the target injection site. Care was taken to ensure uninjured uplifting of the bone section. Next, undiluted pSIVA dye was loaded into the glass pipette with sufficient volume and was gently lowered to the desired depth (using the DV coordinate). A total of 500 μL of pSIVA per injection site was injected slowly at 1 nl/s over a period of 15–20 min and with a 5-min break halfway and at the end of each injection to avoid backflow before carefully retracting the injection pipette to the next target injection site (using AP and ML coordinates). At the end of the last injection, mice were sutured along the incision of the scalp and given subcutaneous Buprenex SR (0.5 mg kg−1). Each mouse was allowed to recover before placement in their home cage for 1.5–2h before undergoing routine perfusion and processing of both injected right hemisphere and control left hemisphere (see previous section).

Surgery and animal preparation for in vivo two-photon imaging. Surgical procedures closely followed established protocols. Briefly, mice were anesthetized with isoflurane (1–1.5% for induction), head-fixed with blunt ear bars and kept at 36−37°C on a custom surgical bed (Thorlabs). Eyes were protected with vet ophthalmic ointment (Puralube). Depilator cream (Nair) was used to remove hair on top of the mouse's head. The scalp was thoroughly cleansed and disinfected with a two-stage scrub of betadine and 70% ethanol. A scalp portion was surgically removed to expose frontal, parietal and interparietal skull segments. Scalp edges were attached to the lateral sides of the skull using tissue compatible adhesive (3M Vetbond). A custom-machined metal plate was affixed to the skull with dental cement (Coltene Whaledent, cat. no. H0305). Ear bars were removed and the head was stabilized by clamping the skull-attached plate with a custom holder. An approximately 3-mm diameter craniotomy was made over the target injection site. The craniotomy sites were marked and an electrical micro-drill with a fluted bit (0.5-mm tip diameter) was used to thin a 0.5–1-mm diameter part of the bone over the target injection site. Care was taken to ensure uninjured uplifting of the bone section. Next, undiluted pSIVA dye was loaded into the glass pipette with sufficient volume and was gently lowered to the desired depth (using the DV coordinate). A total of 500 μL of pSIVA per injection site was injected slowly at 1 nl/s over a period of 15–20 min and with a 5-min break halfway and at the end of each injection to avoid backflow before carefully retracting the injection pipette to the next target injection site (using AP and ML coordinates). At the end of the last injection, mice were sutured along the incision of the scalp and given subcutaneous Buprenex SR (0.5 mg kg−1). Each mouse was allowed to recover before placement in their home cage for 1.5–2h before undergoing routine perfusion and processing of both injected right hemisphere and control left hemisphere (see previous section).

Confocal microscopy. One-photon laser scanning confocal images were acquired with a Zeiss LSM 710 confocal microscope using Plan-apochromat x200 NA=1.40 air-matched or x63 1.4 NA oil objectives (laser lines, 405 nm, 488 nm, 594 nm and 633 nm). Image size was 1,024 × 1,024 pixels. Stack thickness was typically 1 μm for mouse brain sections and 5 μm for postmortem paraffin-embedded sections. For synaptic quantification specifically, CA1 apical dendrites were imaged at 3 μm thickness and z-stack image were obtained (optical slice 0.29 μm, 11 slices per...
Two-photon microscopy. Live animal imaging was performed as previously described35,36. Briefly, a Sutter Movable Objective Microscope equipped with a pulsed femtosecond Ti:Sapphire laser (Chameleon Ultra II, Coherent) and two fluorescence detection channels was used for imaging (dichroic beamsplitter, FF520-Di02 (Semrock); blue emission filter, FF01-452/45 (Semrock); green emission filter, ET525/70M (Chroma); photomultiplier tubes, IT422-40 GaAsP (Hamamatsu)). Laser excitation wavelength was set at 887 nm. Ablative laser power was <10–15 mW at the tissue surface and adjusted with depth as needed to compensate for signal loss due to scattering and absorption. An Olympus ×20 1.0 NA water immersion objective was used for light delivery and collection. Z-stacks included up to 350 images, acquired at 1-μm axial step size, and used a two-frame average, 512×512 pixel resolution and x2–0–10 zoom (corresponding to 350 μm × 350 μm × 72 μm × 72 μm fields of view). Time-lapse recordings typically included 60–70 images per stack, acquired at 1.0–1.2 μm axial step size, used a two-frame average, 60-stack repeat (corresponding to approximately 94 min total recording duration), 512×512 pixel resolution and x3.3–5 zoom (corresponding to 212 μm–142 μm fields of view). Up to 13 z-stacks and four to seven time-lapse recordings were acquired per animal in layers 1 and 2 of the somatosensory cortex.

Imaging data analysis. For fixed-brain thin sections, maximum-intensity projection images of 212 μm × 212 μm (1,024×1,024 pixel resolution) were analyzed in Fiji. Approximately 5–7 plagues per brain sections (3–5 sections per animal) were randomly chosen in the prefrontal cortex using the 6E10 channel. MFI for Mer, Axl and Gas6 expression analysis, was calculated as the quotient of integrated density divided by region of interest, for example, Iba1 area on ROI area, respectively, which were gated based on their intensity and applied for all images in each experiment. Phagosome-associated and nonphagosome-associated Iba1 areas were classified manually by investigator blind with respect to genotypes. For quantification of dystrophic neurite area using LAMP1 or RTN3, area above set fluorescent thresholds for either LAMP1 or RTN3 marked neuritic dystrophy and 6E10 immunopositive area were calculated and summed using the Imaris particle tool and the ratio of summed area (LAMP1 or RTN3 area/6E10 area) was calculated on per plaque basis. For LAMP1 analysis for binary categorization, dense-core plagues were defined as plagues that contain a single or solid (usually bright) 6E10 core area ≥100 μm² and diffuse plagues were defined as the complement of those dense-core plagues that are characterized as those devoid of compact 6E10+ area ≥100 μm². To assess excitatory synapse changes in the mouse hippocampus, colocalization of vGlut1 and PSD95 in the apical dendrite area of CA1 was analyzed using Imaris software (Bitplane). Three-dimensional (3D) z-stack images were background subtracted and positive puncta of vGlut1 and PSD95 were selected and built as spots by uniformly thresholding size and intensity across different experimental groups analyzed. Puncta were then transformed into a distance vector using the ‘distance transform’ function, which was followed by a calculation of distance between vGlut1 spots and PSD95 spots. Puncta were considered colocalized if the distance between vGlut1 and PSD95 was ≤9.7 μm. Number of colocalized puncta from each image was averaged for one section or for one animal and compared between experimental groups that contain three animals. Three or more images per section and ≥3–5 sections per animal were analyzed.

For analyses of two-photon image stacks, Imaris software (v.9.1.2; Bitplane) was used for 3D reconstruction of GFP microglia and MX04-labeled dense-core plagues and also for the analysis of (1) the distance of the centroid of GFP+ microglial cell bodies to the edge of a MX04+ mass; (2) microglial cell body volume and (3) intracellular Aβ, from 1-μm-step z-series stack images of both genotypes. Two types of ‘surfaces’, as digital representations that capture the volume of either a microglia cell body or a dense-core (not diffuse) plaque for the GFP channel and MX04 channel, respectively, were created under the same threshold for all stack images. For MX04 objects, a surface cutoff was set to be at the clear-cut border of the outlining of dense-core plagues, exclusive of surrounding cell bodies. Only dense-core plagues whose diameters fell between 10–30 μm were included in the following analysis. For GFP surfaces, filters for ‘surface grain size’, ‘diameter’ and ‘false contouring’ were set to 890 μm, 212 μm, and 65 μm, respectively, for capturing the structure of GFP microglial cell bodies in imaging volume of 142 μm × 142 μm × 70 μm for Fig. 4c and Extended Data Fig. 4a and a normalized imaging volume of 350 μm × 350 μm × 300 μm in somatosensory cortex (512×512 pixel resolution). The same thresholds were applied to all images of both genotypes. Structures on edge and/or captured incompletely were manually deleted from each object. These were then used to determine distance of the centroid of individual GFP surfaces to the closest edge of MX04 objects with the built-in Zen Blue editions. Images for cortical and hippocampal plaque quantification were acquired with Olympus VS-120 Virtual Slide Scanning Microscope using a ×10 objective.

For quantification of intracellular Aβ, a GFP ‘cell body only’ channel was built with GFP surfaces as mask borders using the ‘set outside voxel to 0’ mask. MX04 and the ‘cell body only’ GFP channel were then thresholded to capture the overlapping volume between MX04 and GFP channels using the ‘colocalization’ function in Imaris. The overlapping volume was then normalized to the total volume of GFP of the image as a representation of the percentage of GFP microglia cell body volume occupied by MX04 material. Primary process number, total length for PAM and process polarization ratio for NPM around dense-core plaques, whose diameters fell between 10–30 μm were analyzed with Fiji and were calculated on maximum-intensity projection stack images of 142 μm × 142 μm × 60 μm in volume. The following parameters were measured using volumetric analysis37,38. For primary process number and total length, PAM were defined as those whose cell body centroids were 0–5 μm away from the edge of the nearest MX04 edge39. On average 3–5 microglia per plaque that were unambiguously distinct, showed clear process visibility and of which no less than two were selected. Primary process number and length from PAM were measured using the ‘free-hand’ line tool on Fiji. Z-stacks of these projected images were gone through to ensure processes continuity from the same cell across layers. Mainly distal primary processes were analyzed in this group of cells due to the fact that majority of their somata were next to or in direct contact with a plaque surface. For polarization index, NPM were defined as those whose cell body centroids were 20–50 μm from a dense-core MX04 edge and whose processes were clearly visible. Cells equidistant between two plaques were not included in the analysis. Calculation of this index was performed38,39 using the following criteria: processes oriented toward plaques were classified as those on the plaque side of a line drawn through the microglia body perpendicular to a line drawn to the nearest plaque. The polarization index for one microglia cell is the ratio of the summed length of all ‘toward’ primary processes divided by the summed length of all processes.

For structural dynamics of individual microglia processes, process velocity was assessed as previously described35,36, by manually tracing process tips over time. The Manual Tracking plug-in on FIJI on maximum-intensity projected time-lapse videos, typically over 90–90 min time spans that cover a field of view of 212 μm × 212 μm × 70 μm in the somatosensory cortices of age-matched AD and healthy littermates of WT and Aβ+ ‘Merkit’ mice. As mentioned in the previous section, measurements were made for AD microglia whose cell body centroids were within 5 μm from the edge of dense-core MeX04 edge as measured on projected images (PAM) and for those whose centroids were over 20 μm from any plaque in the field of view (NPM). Resting microglia were microglia from non-AD age-matched littermates. At least 2–3 and 4–7 processes were quantified from PAM and NPM, respectively, to obtain an average process velocity per microglia.

For CAA quantification, CAA area percentage was defined as 6E10+ vessel area by calculating the ratio of 6E10+ area within CD31+ area over total CD31 area in either somatosensory or prefrontal cortex of mice of each genotype. Measurements were averaged from images of respective brain regions from 15 sections (spanning >1.8 mm³ of brain volume) per mouse.

Immuno blot analysis. Tissues were snap-frozen in liquid nitrogen. Frozen tissues were lysed using RIPA buffer and phosphatase protease inhibitor (Roche, Sigma) for 30 min on ice. Supernatants were stored at −80°C after samples were spun down at 12,000 rpm for 5 min. Immunoblotting was as previously published31. Briefly, equal amounts of proteins (10 μg) in Laemmli buffer containing 0.1 M dithiothreitol were separated by electrophoresis through 4–12% Bis-Tris polyacrylamide gels (Novex, Life Technologies) and were transferred to polyvinylidene difluoride membranes. Membranes were blocked with 1% casein block in PBS (BioRad 1610782), subsequently incubated with primary and secondary antibodies and washed with TBST (50 mM Tris-HCl, pH 7.5, 0.15 M NaCl and 0.25% Tween-20) between incubations. Blots were developed using an Odyssey Gel Imaging System (Li-Cor) and quantified using ImageStudio with GEPHAD as loading control.

Contextual fear-conditioning behavior test. Fear acquisition and contextual memory tests were adapted from an established fear-conditioning paradigm49. Briefly, mice were habituated for 5 min per animal for 5 consecutive days before the day of fear acquisition. One day before fear acquisition, mice were allowed to explore the conditioning chamber for 10 min. All testing was performed between 09:30 and 14:30 h and all mice were acclimated to the testing room for at least 1 h before the beginning of each behavioral assay. For fear acquisition (day 1), mice were evaluated for their abilities to learn the association between a context (conditional stimulus) and an aversive mild electrical stimuli (unconditional stimulus). After behavioral testing, resting blood pressure and heart rate were measured in p28 mice. A conscious mouse was briefly restrained in an auditory cue (30 s, 90 db, 2.5 kHz) and an electric shock (0.5 mA, 2 s) was delivered. The signal was considered as ‘freezing’ (the time the mouse was immobile during the time period in the presence of the paired context even when the aversive stimulus was absent). Freezing behavior was monitored and analyzed by an automated video-freeze software (Med Associates). Animals were returned to their home cage after the test. Conditioning using a contextual fear conditioning protocol was performed on day 2, 24 h after fear acquisition, freezing behavior was measured in the original conditioning context for 3 min to test the memory retention of the learned association between conditional stimulus and contextual cue.
unconditional stimulus. To avoid estrogen-related and other cognition-irrelevant variations, fear conditioning was performed only on cohorts of group-housed male mice of matched age.

**Statistical analyses and reproducibility.** Numerical data analysis and statistics were performed with Microsoft Excel (v.15.36) and GraphPad Prism (v.8.0) software, except for Fig. 3 and Extended Data Fig. 3. All data in all figure panels of the paper are represented as mean ±1×s.d. In most cases, n is used to denote the number of mice per experimental group and N denotes the number of brain sections/images/samples analyzed per mouse. Group sample sizes were chosen based on previous studies and/or power analysis. Experiments were performed with at least two independent replicates whenever applicable. No technical replicate samples across independent experiments were pooled in the datasets. Datasets displayed normal distribution and equal s.d. unless indicated by unequal variance test. Statistical tests were always performed two-sided. Bonferroni-adjusted P values were calculated for genes in Fig. 3 and Extended Data Fig. 3. P values were calculated using Mann–Whitney U-test in Figs. 4d–f, 5a,b, c,d,e and Extended Data Figs. 1d, 4b–d, 6a–e,g, 7a,c,d and 8c,d. A Student’s t-test was used in Fig. 6c,d and Extended Data Fig. 7b,e,f. For multiple group comparison, P values were calculated in Figs. 2b and 4g and Extended Data Fig. 8a using a Kruskal–Wallis test followed by Dunn’s multiple comparison test. P values were calculated in Fig. 4c and Extended Data Fig. 4a,e using two-way analysis of variance with Sidák’s multiple comparison test. NS (not significant) indicates P>0.05; significant P values are noted as exact values on graphs.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

We declare that the main data supporting the findings of this study are available within the article and its Extended Data information files. Sequencing data were deposited in the Gene Expression Omnibus with the accession number GSE166523. Additional supporting raw data are available from the authors upon request. Source data are provided with this paper.

**References**


**Acknowledgements**

We thank J. Hash for technical assistance, M. Mercken (Janssen Pharmaceuticals) for Aβ42 antibodies, R. Rissman and the ADRC at UCSD for AD brain sections, M. Shokhirev for advice on bioinformatic analyses, S. Parylak and F. Gage for advice on fear-conditioning assays and members of the Lemke laboratory and the Nomis Center for discussions. The research was supported by grants from the US National Institutes of Health (R01 AG060748 and RO1 AI101400 to G.L.; DP2 NS083038, RO1 NS108034 and U01 NS103522 to A.N.; P30 AG062429 to the UCSD ADRC; and P30 CA014195 and S10 OD023689 to the Salk Institute), the Cure Alzheimer’s Fund and the Coins for Alzheimer’s Research Trust (to G.L.) and the Leona M. and Harry B. Helmsley Charitable Trust (to the Salk Institute); by Goeddel’s Chancellor’s, Marguerite Vogt and H. A. and Mary K. Chapman Charitable Trust graduate fellowships (to Y.H.) and by Anderson, Nomis and Sweden—America Foundation postdoctoral fellowships (to K.E.H.).

**Author contributions**

Y.H. was an essential contributor to every aspect of the paper. K.E.H. made extensive contributions to experimental design and implementation. P.G.B. obtained and prepared tissue sections for immunostaining. C.O.C. contributed to the design and performance of flow cytometry. N.H. carried out scRNA-seq. L.H. performed clustering and bioinformatics for scRNA-seq analyses. A.N. designed and implemented methods for two-photon imaging and data analyses. G.L. conceived the project and designed experiments. All authors contributed to the writing of the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41590-021-00913-5. Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41590-021-00913-5.

Correspondence and requests for materials should be addressed to G.L.

Peer review information Nature Immunology thanks Cristofofo Comi and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. L. A. Dempsey was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

Reprints and permissions information is available at www.nature.com/reprints.
Extended Data Fig. 1 | Expression of Axl, Mer, TMEM119, Trem2, and GFAP in plaque-burdened AD brains. a, Axl protein (green, lower panels) is undetectable in WT (left) and APP/PS1 (right) microglia (Iba1, red, upper panels) in the 4mo cortex, prior to the appearance of plaques in APP/PS1 mice. n = 3 per genotype. b, Axl protein (green) in 15 mo APP41 mice (n = 3) is up-regulated in cortical microglia (Iba1, red) contacting Aβ plaques (6E10, white), and is also often concentrated in plaque centers, consistent with strong prior activation of Axl and subsequent cleavage of its ectodomain. (As is seen for other RTKs, robust activation of Axl results in nearly complete cleavage of the ectodomain from the cell surface.) Hoechst 33258 stains nuclei. c, Expression of Mer protein (green) in 15 mo APP41 mice is seen in all cortical microglia (Iba1, red), but is further up-regulated in microglia that invest Aβ plaques (6E10, white). d, Quantification of Mer up-regulation in Iba1+ plaque-associated microglia (PAM) versus non-plaque-associated microglia (NPAM) in APP/PS1 mice at 9.5 months. e, Axl (green) and Trem2 (red) are up-regulated in the same Iba1+ (white) microglia cell in the 9.5 mo APP/PS1 cortex. f, Expression of the homeostatic microglial marker TMEM119 (green) is lost in cortical microglia that surround plaques (6E10, white) in 15 mo APP41 mice, except for an occasional 1-2 cells at the center of plaques. g, This same TMEM119 down-regulation is seen in 15 mo APP/PS1 mice. TMEM119-microglia surrounding 6E10+ plaques are strongly Iba1+. h, The up-regulated Mer expression (green) seen in 15 mo APP41 mice is not in GFAP+ reactive astrocytes (red). (Activated S100b+ astrocytes are also negative for Mer expression by IHC.) Circles mark the position of Aβ plaques. Representative images obtained from immunostaining of N ≥ 3 sections from n = 3 mice of each genotype. Scale bars: 10 μm (a-c, e, h), 100 μm (f, g). Mann-Whitney test (d).
Extended Data Fig. 2 | Gas6 and PtdSer decoration of Aβ plaques. a, Gas6 protein (green) decorates 6E10⁺ plaques (white) on sections of postmortem cortex from human patients with advanced (BRAAK stage 6) AD (left panels), but is not evident in the plaque-free cortex of cognitively normal age-matched controls (right panels). Representative images obtained from immunostaining of >3 sections from 3 individuals of each condition. b, Visualization of externalized PtdSer in 15 mo APP/PS1 cortex following stereotaxic injection of pSIVA (green, left panel). pSIVA binds to externalized PtdSer in the needle track of the injection, where cells are damaged and undergoing apoptosis, and also to the PtdSer associated with all 6E10⁺ amyloid plaques adjacent to the injection site (white, right panel). (c) A similar pSIVA injection in 15 mo WT mice labels only the needle track of the injection, since there are no plaques in these mice. (d) Airyscan super-resolution image of the juxtaposition of plaque-associated microglia (Iba1, red), Gas6 (green), and Aβ plaque (6E10, white) in 12 mo APP/PS1 mice (n = 5). Scale bars: 100 μm (a), 50 μm (b), 200 μm (c), 10 μm (d). n = 3 and 2 for APP/PS1 and WT control, respectively (b, c) from two independent experiments.
Extended Data Fig. 3 | Transcriptomics of APP/PS1 and APP/PS1Axl−/−Mertk−/− microglia as quantified by single cell RNA-seq. a, Sorting scheme for isolation of CD45+ single cells. FSC and SSC, forward and side scatter, respectively. A, area; W, width. b, Uniform manifold approximation and projection (UMAP) clustering of CD45+ cells sorted from 18 mo APP/PS1 (A/PS) and APP/PS1Axl−/−Mertk−/− (A/PS A/M−/−) cortices (combined) and annotated using the 18 marker genes in c. c, Violin plots of population log-transformed normalized expression of the indicated genes in the indicated cell types. Cell type key applies to b and c. d, Log-transformed normalized expression of Axl (left) and Mertk (right) mRNA in APP/PS1 cells within the microglial clusters defined in Fig. 3a. e, Heat map of the scaled expression levels of the indicated genes in individual cells within transcriptomic state (cluster) 0 and state 5 microglia in the APP/PS1 cortex at 18 mo. f, Comparative composite heat maps for the indicated genes across stages and transcriptomic states in 5xFAD and APP/PS1 mice, as quantified in this study (top five rows) and in Keren-Shaul et al.11 (bottom three rows), respectively. Values shown as z-scaled log-transformed normalized average of each group. g, Violin plots of the log-transformed normalized expression distribution of the indicated genes at transcriptomic state 5 in A/PS (black) versus A/PS A/M−/− (red) microglia, as determined by single cell RNA-seq (scRNA-seq). Dotted lines indicate mean. h, Mean expression level of the indicated cytokine and chemokine genes in state 5 versus state 0 microglia in A/PS (black) versus A/PS A/M−/− (red) microglia at 18 mo, as determined by scRNA-seq. i, Relative expression level of the indicated inflammatory regulator mRNAs in RNA isolated from total cortex of mice of the indicated genotypes at 12 mo, as determined by qRT-PCR. n = 3-6. Kruskal-Wallis test with Dunn’s multiple comparison test. Data are represented as mean ± 1 STD.
Extended Data Fig. 4 | APP/PS1Axl<sup>−/−</sup>Mertk<sup>−/−</sup> microglia are unresponsive to Aβ plaques. **a**, Distribution of distance of microglial cell body centroids, in 2 μm bins, from the edge of MX04-labeled Aβ plaques with diameters of 10-15 μm in APP/PS1 (gray) and APP/PS1Axl<sup>−/−</sup>Mertk<sup>−/−</sup> (red) cortex. Values obtained for 56 and 23 plaques from 3 and 4 mice for APP/PS1 and APP/PS1Axl<sup>−/−</sup>Mertk<sup>−/−</sup>, respectively. **b**, Number of imaged GFP<sup>+</sup> primary processes per PAM (microglia < 5 μm from plaques) in APP/PS1 (gray, A/PS) and APP/PS1Axl<sup>−/−</sup>Mertk<sup>−/−</sup> (red, A/PS A/M<sup>−/−</sup>) cortex. **c**, Summed length of primary microglial processes per PAM in APP/PS1 (gray) and APP/PS1Axl<sup>−/−</sup>Mertk<sup>−/−</sup> (red) cortex. **d**, Process polarization ratio to nearest plaque per NPAM (microglia > 20 μm from plaques; see Materials and Methods) in APP/PS1 (gray) and APP/PS1Axl<sup>−/−</sup>Mertk<sup>−/−</sup> (red) cortex. **e**, Quantification of microglial cell density in the cortex of 16 mo APP/PS1 (gray) and APP/PS1Axl<sup>−/−</sup>Mertk<sup>−/−</sup> (red) mice for microglia 0-10 μm, 10-20 μm, and >20 μm from the edge of the nearest plaque. Data points are from 45-129 cells (PAM) investing 10-29 plaques (b, c), and 21-49 cells peripheral to 7-24 plaques (d) from n = 3 mice per genotype (b-d). Points in e represent 3-5 imaging volumes from 3 APP/PS and 4 APP/PS1Axl<sup>−/−</sup>Mertk<sup>−/−</sup> mice. Two-way ANOVA with Sidak’s multiple comparison test (a, e) and Mann-Whitney’s test (b-d), Data are represented as mean ±1 STD.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Expansive areas of plaque-associated dystrophic LAMP1+ membrane and poorly compacted plaques in the APP/PS1Axl−/−Mertk−/− brain. A montage of 24 paired sections in both APP/PS1 (top six rows) and APP/PS1Axl−/−Mertk−/− (bottom six rows) cortex, each stained with antibodies to both LAMP1 (green) and 6E10 (white). Each 6E10 image is paired with (from the same section as) the LAMP image immediately below. This montage, which is a subset of the images used to generate the data in Fig. 5d, is composed of images taken from three different mice of each genotype at 12 months. Note that: (a) 6E10+ Aβ plaques are in general more compact and brightly stained in APP/PS1 mice and more diffuse and weakly stained in APP/PS1Axl−/−Mertk−/− mice; and (b) the area occupied by LAMP1+ membrane is in general much larger in APP/PS1Axl−/−Mertk−/− mice. Scale bars: 10 μm.
Extended Data Fig. 6 | Accumulation of LAMP1+ dystrophic membrane and apoptotic cell debris in the APP/PS1Axl−/−Mertk−/− brain. a, Quantification of LAMP1/6E10 area ratio as in Fig. 5d, but only for dense-core plaques (plaques with solid 6E10+ cores with areas > 100 μm²). b, Quantification of LAMP1/6E10 area ratio as in Fig. 5d, but only for diffuse plaques (plaques without solid 6E10+ cores with areas > 100 μm²). c, Quantification of RTN3/6E10 area ratio as in Fig. 5e, but only for dense-core plaques. d, Quantification of RTN3/6E10 area ratio as in Fig. 5e, but only for diffuse plaques. e, Quantification of the density of diffuse plaques (defined as above) expressed as a fraction of total plaques in the cortex of mice of the indicated genotypes at 12 mo. Data represent diffuse plaques quantified from N = 4-5 sections from n = 6 mice per group. (f) Representative example of cerebral amyloid angiopathy (CAA) in the cortex of a 15 mo APP/PS1Axl−/−Mertk−/− mouse. 6E10+ Aβ material (white) is evident within laminin+ blood vessels (green). Asterisk marks an Aβ plaque in the parenchyma. (g) Quantification (see Methods) of CAA in the somatosensory cortex of 15 mo APP/PS1Axl−/−Mertk−/− mice (A/PS A/M−/−) relative to APP/PS1 mice (A/PS), n = 4/group and measurements were averaged from N > 15 sections (spanning > 1.8 mm³ of brain volume) per mouse. (h) cCasp3+ apoptotic debris (cyan, lower panels) accumulates around 6E10+ Aβ plaques (upper panels) in the APP/PS1Axl−/−Mertk−/− (right panels) but not the APP/PS1 (left panels) hippocampus at 12 mo. Images are representative of n = 3 mice per genotype from three independent experiments. Scale bars: 100 μm. Data are 18-47 (a), 67-78 (b), 30-43 (c) and 26-52 (d) plaques investigated from N ≥ 3 sections per mouse from n = 3 mice of each genotype from at least 3 independent replicates. Mann-Whitney test (a-e, g). Data are represented as mean ± 1 STD.
Extended Data Fig. 7 | TAM (Mer) signaling promotes dense-core Aβ plaque accumulation with functional consequences. a, Thio S plaque density in APP/PS1 (gray) versus APP/PS1Axl−/−Mertk−/− (red) cortex for plaques of the indicated size at 12 months. b, Thio S plaque density (all plaque sizes) in APP/PS1 (gray) versus APP/PS1Axl−/−Mertk−/− (red) hippocampus at 12 months. c, Soluble Aβ42 levels quantified in APP/PS1 (gray) versus APP/PS1Axl−/−Mertk−/− (red) cortex and hippocampus at 4 and 12 mo, as indicated. n = 5–6 per genotype. d, Quantitative LI-COR western blot measurement of APP protein levels in the 12 mo cortex of 3 cohorts of mice (4 genotypes each cohort) of the indicated genotypes demonstrates no change in APP expression in APP/PS1 mice upon mutation of Axl and Merk. Blots left and quantification right. e, ThioS plaque density (all plaque sizes) in APP/PS1 (gray) versus APP/PS1Mertk−/− (pink) cortex and hippocampus at 12 months. f, Thio S plaque density (all plaque sizes) in APP/PS1 (gray) versus APP/PS1Axl−/− (white) cortex and hippocampus at 12 months. Data points represent plaque density in n = 6–8 mice of the indicated genotypes averaged from N ≥ 5 cortical sections for each brain. Mann-Whitney test (a, c, d) and Student’s t-test (b, e, f). Data are represented as mean ± 1 STD. g, TAM-mediated microglial recognition, phagocytosis, and consolidation of Aβ plaques. Microglial Axl and Mer are bridged to the PtdSer-rich dystrophic membranes of plaques via TAM ligands, whose amino-terminal and carboxy-terminal domains bind PtdSer and Axl/Mer, respectively. Gas6 is shown, but a role for the Mer ligand Pros126 is not excluded. Engagement of the PtdSer-TAM ligand-TAM receptor complex activates the TAM tyrosine kinases (TK), which drives phagocytosis of forming plaque material. Internalized phagocytic cargo is eventually transferred to lysosomes, whose acidic interiors promote the aggregation of large, insoluble Aβ fibrils. Exocytosis or microglial death then delivers this aggregated material to growing dense-core plaques.
Extended Data Fig. 8 | Functional consequences of TAM deletion in APP/PS1 mice. a, Quantification of the 3D colocalization of the excitatory pre- and post-synaptic markers vGlut1 and PSD95 in the 15-mo hippocampus (CA1) (see Methods), as an index of synaptic connectivity. The previously documented decrease in co-localization of these markers in APP/PS1 mice is not altered by the combined mutation of Axl and Merk. Stack size is 85 x 85 x3 μm³ per image, averaged 3 images per CA1 section across 3-5 sections per mouse. Data points represent synaptic density index in n = 3 mice of the indicated genotypes. b, Acquisition of association between a 30 s auditory tone and a subsequent co-terminal 2 s 0.5 mA foot shock, expressed as percent time immobile (% freezing) during the indicated intervals, over three successive trial intervals in 15 mo mice of the indicated genotypes (see Methods). c, Data in b plotted for the indicated genotypes with the indicated statistical significance per interval. d, Contextual fear memory as assayed by percent of a 3 minute interval in which mice of the indicated genotypes were immobile (% freezing) when returned to the same testing cage 24 h after the fear acquisition trials of b (see Methods). A cohort of group-housed male mice (n = 12-20/group) were used in the behavioral assay. Data points in b represent the mean % freezing of each group in the interval duration immediately prior to the point. Each data point in d is the % freezing of one animal in the duration of the testing period. Kruskal Wallis test followed by Dunn’s multiple comparison test (a) and Mann-Whitney test (c, d). Data are represented as mean ±1 STD. Source data
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

*Our web collection on [statistics for biologists](#) contains articles on many of the points above.*

Software and code

Policy information about availability of computer code

**Data collection**

Zen Black and Zen Blue editions (ver 2.5) were used for collecting confocal images; Olympus VS-120 Virtual Slide Scanner and VS Desktop software (ver 2.8) was used for collecting thio-s labelled plaque burden data; QuantStudio Q5 was used for collecting qPCR data; TECAN Infinite® 200 PRO reader was used for collecting optical density reading for ELISA and BCA assay; Odyssey Gel Imaging System (LI-COR) was used for acquired western blots; Microsoft Excel (ver 15.36) was used for compiling collected data; Med Associate Video Freeze system (SOE-843) was used to record and analyze mouse freezing behavior in fear conditioning assays. Single cell RNA Sequencing was conducted on FACS sorted brain immune cells following the user guide on the Next Gem single cell v3.1 protocol. Briefly, single cell suspensions of approximately 14,000-16,000 cells from each sample was directly loaded onto microfluidic chip with barcoded beads to generate Gel Bead-in-Emulsions (GEMs) using 10X Genomics Chromium Single Cell Controller. Reverse transcription of GEMs for first strand cDNA synthesis and cDNA amplification were carried out according to 10X Next Gem single cell v3.1 protocol. Following indexed scRNA-Seq library construction, the final library size distribution was determined using TapeStation (Agilent) and the concentration was measured by a Qubit fluorometer (ThermoFisher). The libraries were pooled in equal molar ratio, quantified by qPCR, and sequenced on Illumina NextSeq500 at 28 cycles for Read 1, 8 cycles for i7 index and 91 cycles for Read 2 at an average sequencing read depth of 33k-41k reads per cell. For more details, see Methods section in manuscript.

**Data analysis**

Fiji (ver 1.0) was used for all confocal image analysis and for two-photon microscopy stack images and time-lapse recordings; Imaris (ver 9.1.2; Bitplane, Zurich, Switzerland) with build-in MATLAB function was used for two-photon microscopy stack images for microglia and plaques reconstruction, distance analysis as well as intracellular Abeta content; ImageStudio (ver 5.2.5) was used for quantifying western blot data; statistics except for single cell RNAseq data were performed with GraphPad Prism (version 8.0) software. Flowjo (v10) was used for FACS analysis and gating the sorted cell population. Raw sequencing data were aligned to the reference built using Ensembl primary assembly and annotation (release-93) that included both protein coding genes and polymorphic pseudogenes by cell ranger pipeline (v3.1.0). The resulting filtered gene expression matrix was further analyzed by "Seurat" (3.2.1.9002). Cells that had less than 10% of reads mapped to mitochondria genes and 200-10000 expressed genes were used in the analysis. Samples were integrated by Seurat standard workflow “FindIntegrationAnchors” and “IntegrateData” functions with default settings. After manually examining the Principle Component (PC) elbow plot, the top 25 PCs of the integrated data were used for clustering analysis and Uniform Manifold Approximation and Projection (UMAP)
analysis with default settings. Doublet scores were calculated by R package "scds" (v1.2.3) using the raw count matrix as input. The top 5% cells ranked by hybrid scores and clusters enriched (>50%) with doublets were removed from the downstream analysis. Data was re-scaled and re-clustered after doublet removal. The expression patterns of a large group of well-known immune markers (Supplementary Fig. 3b) were carefully examined to annotate the clusters. A second round of analysis was performed on microlaia to further reveal the relationship of cells within the population. The top 20 PCs were used for clustering and UMAP analysis. One cluster that co-expressed both microlaia markers (P2ry12 and Fcrl3) and T/NK markers (Cd3g and Nkg7) was regarded as doublets and was filtered out. Clustering resolution was set at 0.2 because the clusters highly correlated with the UMAP topologies. Differential Expression (DE) analysis was performed by Seurat function "FindAllMarkers" and "FindMarkers" with default: Wilcoxon Rank Sum test and logFC > 0.25 on pooled biological replicates. Genes with Bonferroni-adjusted p-value < 0.05 were considered to be significant. When plotted, the adjusted p-value was log10 transformed. Each adjusted p-value was added an extremely small number (1e-30) to avoid infinite values before transformation. Then the value was signed by the up/down-regulation of the gene. Supplemental Table 7 containing Trem2 positive expression from different stages of disease progression from Keren-Shaul et al. was used as validation dataset. Common symbols between this study and our top 10 DE genes ranked by logFC from each microlaia cluster were used for heatmap plotting. Average expression values from both datasets were offset by 1 and log2 transformed and z-scaled. For more details, see Methods section in manuscript.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. Github). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

We declare that the main data supporting the findings of this study are available within the article and its Supplementary information files. Sequencing data was deposited at Gene Expression Omnibus with the accession number GSE160523. Additional supporting raw data are available from the authors upon request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-list.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

In general, group sample sizes were chosen based on previous published studies from the Lemke lab and/or mice studies that used similar approaches and/or power analysis based on preliminary data. Biological sample number (denoted with ‘n’ in the manuscript) 3-8 per genotype per age group per condition was used in most studies. Whenever applicable, sex- and age-matched littermates mice were used as controls. For quantification of immunofluorescence imaging analysis (Mer, Axl, Gas6, LAMP1 and RTN3 mean fluorescence intensity), typically 3-5 field of view per section from 3-5 sections or whole brain tilename were collected for confocal imaging per experiment from two-three independent experiments as suggested by previous publications (Zagorska, Lemke, Nat Immunology 2014; Fourgeaud, Travas, Nimmerjahn, Lemke, Nature 2016; Blanco-Suarez Allen Neuron 2017; Wang, Colonna JEM 2016). Additionally, the cohort effect size is supported by preliminary quantification showed the significant differences with narrow variability among biological groups. For western blot and ELISA quantification, cohort sample size was determined by analysis from preliminary results and previous publications (Zagorska, Lemke, Nat Immunology 2014; Fourgeaud, Travas, Nimmerjahn, Lemke, Nature 2016; Zagorska, Lemke, Nat Immunology 2014; Fourgeaud, Travas, Nimmerjahn, Lemke, Nature 2016; Zagorska, Lemke, Nat Immunology 2014; Fourgeaud, Travas, Nimmerjahn, Lemke, Nature 2016; Zagorska, Lemke, Nat Immunology 2014; Fourgeaud, Travas, Nimmerjahn, Lemke, Nature 2016; Zagorska, Lemke, Nat Immunology 2014; Fourgeaud, Travas, Nimmerjahn, Lemke, Nature 2016; Zagorska, Lemke, Nat Immunology 2014; Fourgeaud, Travas, Nimmerjahn, Lemke, Nature 2016; Zagorska, Lemke, Nat Immunology 2014; Fourgeaud, Travas, Nimmerjahn, Lemke, Nature 2016). For two-photon imaging experiments, multiple recorded volumes (4-7 per animal) and (8-10 per animal) imaging stacks in order to gain representation within one biological sample and in biological sampling across 3-5 animals per genotype (Tufail, Nimmerjahn, Neuron 2017; Fourgeaud, Travas, Nimmerjahn, Lemke 2016 Nature; Belmont Cailhoun 2008 (Neurosci). 2 biological samples were chosen for non-transgenic WT and Axl/-/Mertk/- age-matched littermates mice for recording microlaia processes motility because 1) this has been extensive studied and published (Nimmerjahn Helmench 2005 Science; Fourgeaud, Travas, Nimmerjahn, Lemke, Nature 2016), 2) our results from recordings from these mice were consistent with previous publications and 3) averaged processes motility per microlaia basis was compared rather than per animal basis (Fig. 4g in the manuscript).

Data exclusions

No data exclusion

Replication

As stated above, wherever applicable, experiments have been repeated for at least two (mostly three) independent experiments with at least three technical replicates per experiments that represent at least 3 biological samples (except for two-photon imaging of only the non-transgenic groups and single cell RNAseq analysis with 2 biological replicates per group). All attempts of replication have been successful with a certain degree of variability.
Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

<table>
<thead>
<tr>
<th>n/a</th>
<th>Involved in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>☑</td>
<td>Antibodies</td>
</tr>
<tr>
<td>☑</td>
<td>Eukaryotic cell lines</td>
</tr>
<tr>
<td>☑</td>
<td>Palaeontology and archaeology</td>
</tr>
<tr>
<td></td>
<td>Animals and other organisms</td>
</tr>
<tr>
<td></td>
<td>Human research participants</td>
</tr>
<tr>
<td></td>
<td>Clinical data</td>
</tr>
<tr>
<td></td>
<td>Dual use research of concern</td>
</tr>
</tbody>
</table>

Methods

<table>
<thead>
<tr>
<th>n/a</th>
<th>Involved in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>☑</td>
<td>ChiP-seq</td>
</tr>
<tr>
<td>☑</td>
<td>Flow cytometry</td>
</tr>
<tr>
<td></td>
<td>MRI-based neuroimaging</td>
</tr>
</tbody>
</table>

Antibodies

Antibodies used were as follows:
- anti-Axl (R&D AF854 Lot CTC0214101, polyclonal goat)
- anti-Mer (Bioscience lot 4285684 clone: D55/MMER, monoclonal rat)
- anti-Mer (R&D AF591, Lot DGS0517061 and Lot DGS0213111, polyclonal goat)
- anti-mouse Gas6 (R&D AF986, lot: EJU031421, polyclonal goat)
- anti-human Gas6 (R&D AF885 lot GUS0218061 polyclonal goat)
- anti-human-beta-amyloid, 1-16 antibody (Biolegend 803001, clone 6E10)
- anti-Iba1 (Wako 019-19741, polyclonal rabbit)
- anti-Iba1 (Novus N8300-1028, polyclonal goat)
- anti-GFAP (Dako z-334, polyclonal rabbit)
- anti-cleaved Casp3 (Cell Signaling 9661, lot 45, polyclonal rabbit)
- anti-Tmem119 (Abcam AB290964, lot GR320057-1, monoclonal rabbit)
- anti-RTN3 (EMD Millipore ABN1723 lot 3109186 polyclonal rabbit)
- anti-LAMP1 (BD Biosciences, Cat# 553792, clone 1D4B, monoclonal rabbit)
- anti-Trem2 (R&D systems, AF1729 and BA9 1729, polyclonal sheep)
- anti-mouse CD16/32 (Biolegend 101301, rat IgG2a, lambda)
- anti-CD45-PE, 2D1 (Invitrogen 12-9459-42, mouse IgG1, kappa)
- anti-CD11b-FITC, M1/70 (Invitrogen 11-012-85, rat IgG2a, kappa)
- anti-CD31 (R&D, AF3628, polyclonal goat)
- anti-laminin (Sigma, L-9393, polyclonal rabbit)
- anti-VGlut1 (Millipore, AB5905, polyclonal guinea pig)
- anti-PSD95 (Life Technologies, 51-6900, polyclonal rabbit)
- anti-GAPDH (Millipore MAB374, clone 6G, monoclonal mouse)
- RFI A820/26 for Abeta42 capture antibody and detection antibody RFI/AbN/25, unlabeled (made in-house from Janssen Pharmaceuticals)

Secondary antibodies for immunohistochemistry were fluorophore-conjugated
- anti-rat (712-545-153 or 712-165-153 from Jackson ImmunoResearch),
- anti-goat (A-11056 from Life Technologies, or 705-166-147 from Jackson ImmunoResearch),
- anti-rabbit (A-11071 or A-21206 from Life Technologies),
- anti-sheep (A21098 from Thermo Fisher Scientific)
- anti-mouse (A-11029 from Life Technologies, 715-166-150 or 715-176-150 from Jackson ImmunoResearch)

IRDye 680RG IgG secondary antibodies were used for western blot li-cor detection.

Validation

For all primary antibodies below, WB = western blot, IHC = immunohistochemistry, ICC = Immunocytochemistry, FC = flow cytometry and IP = immunoprecipitation. Validation were summarized from manufacturer’s websites and from the usage in this manuscript.


anti-Mer (R&D AF591, lot DGS0517061 and lot DGS0213111, polyclonal goat; WB, FC on manufacturer’s website and used in IHC in manuscript). Antibody validated for IHC and WB by Lemke lab and others using Mer knockout animal tissue sections. Zagorska Lemke Nature Immunology 2014; Lew Lemke 2014 ELife). https://www.rndsystems.com/products/mouse-mer-antibody_af591


anti-human Gas6 (R&D AF885 lot GUS0218061 polyclonal goat; IHC, WB and direct ELISA on manufacturer’s website for human cancer tissues and samples and used in IHC in manuscript for detecting Gas6 in human AD postmortem samples). IHC studies performed in this manuscript were compared with age-matched controls and with no primary controls. Both of the negative controls showed no specific immunoreactivity whereas human AD samples show specific plaque-associated Gas6 expression. https://resources.rndsystems.com/pdfs/datasheets/a885.pdf?ev=20210303


anti-iba1 (Novus NB100-1028, polyclonal goat, WB, IHC, ICC on manufacturer’s website and used in IHC in manuscript). Previously validated in our lab for IHC (Fourgeaud, Traves, Lemke 2016 Nature) and by others (Imai, Y., & Kohsaka, S. 2002). Intracellular signaling in M-CSF-induced microglia activation: Role of Iba1. GLIA. https://doi.org/10.1002/glia.10149 https://www.novusbio.com/products/af-1-iba1-antibody_nb100-1028


anti-cleaved Casp3 (Cell Signaling 9661, lot 45, polyclonal rabbit, WB, IHC, ICC, FC on manufacturer’s website and used in IHC in manuscript), validated in lab for IHC comparing 12mo. AD TAM-deficient brains with age-matched WT brain sections [negative control] and TAM-deficient 1mo. thymus sections [positive control] and also from previous publication (Fourgeaud, Traves, Lemke 2016 Nature). https://www.cellsignal.com/products/primary-antibodies/cleaved-caspase-3-asp175-antibody/9661

anti-Tmem119 (Abcam AB209064, lot GR320057-1, monoclonal rabbit, IHC on manufacturer’s website and in manuscript). Validated from literature for IHC in mouse brain tissue sections [Bennett Barres 2016 FNAS] https://www.abcam.com/tmem119-antibody-28-3-microglial-marker-ab209064.html

anti-RTN3 (EMD Millipore ABN123 lot 3109186 polyclonal rabbit; WB, IHC, IP on manufacturer’s website and used in IHC in manuscript). Validation in AD mouse brain tissue sections from literature (Kraft, Lee FASEB 2013). https://www.emdmillipore.com/US/en/product/Anti-RTN3-R458,MM_ABN1273


anti-Trem2 (R&D systems, AF1729 and BAF1729, WB and ICC on manufacturer’s website and used in IHC in manuscript). Validation from C. Haass’s and M. Colonna labs’ publications and manufacturer’s website. https://www.rndsystems.com/products/mouse-trem2-antibody_af1729

JRF AS042/26 and JRF/ABN/25 (from Janssen Pharmaceuticals) for capture and detection of Abeta1-42 were described in numerous publications [Schmidt, S.D., Mazzella, M.J., Nason, R.A. & Mathews, P.M. Abeta measurement by enzyme-linked immunosorbent assay. Methods Mol Biol 849, 507-527 (2012)]. Human Abeta42 specificity was validated in our hands compared brain extracts from transgenic animals vs. from non-transgenic animals as well as from synthetic Abeta1-42 standards.
Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Mouse strains used in this study included wild-type and mutant mice. C57BL6j wild-type mice were obtained from The Jackson Laboratory. Mice were typically group-housed at approximately 22 degrees Celsius and provided with bedding and nesting material. All animals were maintained on a 12 hr light/dark cycle and given ad libitum access to standard rodent chow and water. Mouse facility is supplied with 100% fresh air with humidity equivalent to outside ambient air (30%–70%). The Axl/-, MerkK/-, Axl/-/MerkK/-, and Cx3cr1GFP+/- strains have been described previously (Lu, Q. et al., 2019, and Jung, S. et al., 2019). The fractalkine receptor CX3CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. Mol Cell Biol 20(11), 4106-4114 (2000). B6.Cg-TgAPPSwePSEN1dE9 (hemizygous mice (APP/PS1 JAX number: 005864) were crossed with Axl/-, MerkK-/- and/or Axl/-/MerkK-/- lines to generate APP/PS1 Axl/-, APP/PS1MerkK-/-, and APP/PS1Axl/-/MerkK-/- mice which were harvested and analyzed at 25mo, 4mo, 7mo, 9mo, 12mo, 15mo and 18mo of age. For two-photon microscopy, APP/PS1 mice were crossed with Cx3cr1GFP/GFP or Cx3cr1GFP/GFP Axl/-/MerkK-/- strains. Only 15-16mo female APP/PS1Cx3cr1GFP+/- Wt or Axl/-/MerkK-/- and healthy littermates were used in two-photon studies to avoid potential gender biases in pathology and microgliial responses. APP41 mice, which express a ‘Swedish’ and ‘London’ (V717I) mutant human APP under the Thy-1 promoter (Rockenstein, E., Mallory, M., Mante, M., Sisk, A., and Masliah, E., 2011) were used in this study to create an AD model. The samples were randomly assigned to the study by UCSD ADRC and delivered to the experimenters. Patient brain samples were matched with age (over 65 year olds), sex and postmortem hours. Both sexes were included in the postmortem study.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve field-collected samples.

Ethics oversight

All animal procedures were conducted according to protocols approved by the Salk Institute Animal Care and Use Committee (Protocol No. 17-00009). Mice of both genders were randomly allocated to experimental groups unless otherwise noted.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Paraffin-embedded brain sections from age-matched patients with clinical diagnosis of AD (BRAAK6) or with normal cognition were collected post-mortem and prepared by UCSD Alzheimer’s Research Center (ADRC) neuropathology core. The samples were randomly assigned to the study by UCSD ADRC and delivered to the experimenters. Patient brain samples were matched with age (over 65 year olds), sex and postmortem hours. Both sexes were included in the postmortem study.

Recruitment

The postmortem tissues used in this project were collected from the UCSD ADRC neuropathology core.

Ethics oversight

All participants consented to brain donation at the time of enrollment in the UCSD ADRC, as one of the 31 ADRC across the country.

Note that full information on the approval of the study protocol must also be provided in the manuscript.
Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
☒ All plots are contour plots with outliers or pseudocolor plots.
☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Dissociated cells were resuspended in 300 ul of FACS buffer (2% FBS and 1mM EDTA in D-PBS, sterile). Fluorescence labeling procedures were then carried out on ice. Fc-receptors were blocked by addition of anti-CD16/32 antibody (1:100 dilution, Biolegend) for 15 min followed by the addition of labelled antibodies: anti-CD45-PE (1:25, Biolegend), anti-CD11b-FITC (1:25 Biolegend) and Hoechst 33258 (1:1000) for 1 hour. Finally, samples were washed twice with FACS buffer and immediately taken to the Salk Institute Flow Cytometry core facility (samples were kept on ice from this point onwards, or chilled at 4°C while undergoing FACS purification).

Instrument

FACS purification was carried out on a BD FACS Aria Fusion sorter with 1x PBS for sheath fluid.

Software

FlowJo

Cell population abundance

Cells were purified using a 1-drop single cell sort mode (for counting accuracy). Approximately 20k cells were sorted per sample. These were directly deposited into a 1.5 ml Eppendorf tube without additional buffer to yield a sufficient concentration that permitted direct loading onto the 10x chip.

Gating strategy

FACS purification was carried out on a BD FACS Aria Fusion sorter with 1x PBS for sheath fluid. For high viability, concentrated cells suitable for downstream 10X Genomics analysis, an 85-um nozzle was used with sheath pressure set to 45PSI. Live cells were gated first (Hoechst dye negative), followed by exclusion of debris using forward and side scatter pulse area parameters (FSC-A and SSC-A), exclusion of aggregates using forward and side scatter pulse width parameters (FSC-W and SSC-W), before finally gating on CD45+ cells to be isolated. Cells were purified using a 1-drop single cell sort mode (for counting accuracy). These were directly deposited into a 1.5 ml Eppendorf tube without additional buffer to yield a sufficient concentration that permitted direct loading onto the 10x chip.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.