Microglia control small vessel calcification via TREM2

Yvette Zarb  
*University of Zurich and ETH Zurich*

Sucheta Sridhar  
*University of Zurich and ETH Zurich*

Sina Nassiri  
*Swiss Institute of Bioinformatics*

Sebastian Guido Utz  
*University of Zurich*

Johanna Schaffenrath  
*University of Zurich and ETH Zurich*

*See next page for additional authors*

Follow this and additional works at: [https://digitalcommons.wustl.edu/open_access_pubs](https://digitalcommons.wustl.edu/open_access_pubs)  
**Please let us know how this document benefits you.**

**Recommended Citation**
Zarb, Yvette; Sridhar, Sucheta; Nassiri, Sina; Utz, Sebastian Guido; Schaffenrath, Johanna; Maheshwari, Upasana; Rushing, Elisabeth J; Nilsson, K Peter R; Delorenzi, Mauro; Colonna, Marco; Greter, Melanie; and Keller, Annika, "Microglia control small vessel calcification via TREM2." Science Advances. 7, 9. (2021). [https://digitalcommons.wustl.edu/open_access_pubs/10184](https://digitalcommons.wustl.edu/open_access_pubs/10184)

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact vanam@wustl.edu.
Authors
Yvette Zarb, Sucheta Sridhar, Sina Nassiri, Sebastian Guido Utz, Johanna Schaffenhath, Upasana Maheshwari, Elisabeth J Rushing, K Peter R Nilsson, Mauro Delorenzi, Marco Colonna, Melanie Greter, and Annika Keller

This open access publication is available at Digital Commons@Becker: https://digitalcommons.wustl.edu/open_access_pubs/10184
Microglia control small vessel calcification via TREM2

Yvette Zarb1,2*, Sucheta Sridhar1,2†, Sina Nassin3*, Sebastian Guido Utz4, Johanna Schaffenrath1,2, Upasana Maheshwari1,2, Elisabeth J. Rushing5, K. Peter R. Nilsson6, Mauro Delorenzi3,7, Marco Colonna8, Melanie Greter9, Annika Keller1,2‡

Microglia participate in central nervous system (CNS) development and homeostasis and are often implicated in modulating disease processes. However, less is known about the role of microglia in the biology of the neurovascular unit (NVU). In particular, data are scant on whether microglia are involved in CNS vascular pathology. In this study, we use a mouse model of primary familial brain calcification, Pdgfb+/-mice, to investigate the role of microglia in calcification of the NVU. We report that microglia engulfing vessel calcifications, coined calcification-associate microglia, display a distinct activation phenotype. Pharmacological ablation of microglia with the CSF1R inhibitor PLX5622 leads to aggravated vessel calcification. Mechanistically, we show that microglia require functional TREM2 for controlling vascular calcification. Our results demonstrate that microglial activity in the setting of pathological vascular calcification is beneficial. In addition, we identify a previously unrecognized function of microglia in halting the expansion of vascular calcification.

INTRODUCTION

Microglia participate in central nervous system (CNS) development and homeostasis by regulating neural cell numbers, migration of interneurons, as well as promoting connectivity, synapse formation, and pruning (1). Microglia are considered the first line of immune defense in the brain by monitoring the brain parenchyma under homeostatic conditions and resolving cerebral insults (2). However, less is known about the function of microglia at the neurovascular unit (NVU), which is composed of vascular cells (endothelial and mural cells, as well as perivascular fibroblasts and macrophages), associated neurons, and glia (3). Microglia have been implicated in shaping brain vasculature during development (4). In adults, microglial processes directly contact the endothelial basement membrane at the NVU (5), and microglia has been shown to maintain vascular integrity during various insults such as mechanical injury or systemic inflammation (6, 7). Disruption of the NVU is common to many neurodegenerative diseases (3); however, studies on microglial function have mainly focused on brain parenchyma and not the NVU.

Optimal functioning of the NVU, which mediates functional hyperemia, is crucial for cerebral perfusion (3). Blood vessels play an integral role in brain development and provide a niche for brain stem cells. In addition, cerebral vasculature senses the environment and communicates changes to neural tissue, participates in lymphatic clearance, and controls immune quiescence in the CNS (8, 9). Accordingly, dysfunction of the NVU accompanies or may even represent a primary cause of many neurodegenerative diseases (3). In the case of primary familial brain calcification (PFB), bilateral basal ganglia calcification of blood vessels is a key diagnostic criterion. The pathogenetic mechanism points to a compromised NVU (10). PFB is a clinically and genetically heterogeneous neuropsychiatric disorder (composed of psychiatric signs—anxiety and psychosis, cognitive impairment, and movement disorders—ataxia, dystonia, and parkinsonism) caused by mutations in at least six genes, JAM2, MYORG, PDGFB, PDGFRB, SLC20A2, and XPR1 (10, 11). Notably, recent studies have estimated the minimal prevalence of PFB ranges from 4 to 6 per 10,000, depending on the causative gene mutation, thus suggesting that PFB is not a rare disorder and is likely underdiagnosed (12). In addition, basal ganglia calcification is a common radiological finding, estimated in up to 20% of patients undergoing computed tomography (CT) imaging (13). Although the effect of cerebral calcification on the NVU and brain parenchyma is unknown, peripheral vascular calcification can lead to cardiovascular morbidity and mortality (14). Recent studies have suggested the involvement of glia in PFB pathophysiology (15–17); however, functional studies demonstrating the role of glia in cerebral vessel calcification pathology are absent.

In this study, we investigated the role of microglia in vascular calcification using a mouse model of PFB. Previously, we described that mouse platelet-derived growth factor B (Pdgfb) hypomorphs (Pdgfb+/-) develop brain vessel-associated calcifications similar to human PFB (18). Vascular calcifications in human PFB and mouse models of PFB were conspicuously encircled by activated microglia (15, 17, 19). In addition, an osteogenic environment with the surrounding cells expressing osteoclast markers was recently reported (17), although the cellular origin of these cells remains unknown.

Here, we characterize a distinct activation phenotype of microglia in vivo in calcification-associate microglia (CAM). CAMs exhibit a distinct morphology and expression profile, suggesting a specialized role in calcification pathology.
vascular calcification. In conclusion, our study shows that microglia play an important role in modifying vascular calcification in the brain and identifies microglia as a potential therapeutic target in PFBC.

RESULTS

Brain vessel–associated calcifications trigger an inflammatory environment

Vascular calcification in PFBC elicits a conspicuous glial reaction (17–19). To gain insights into inflammatory changes accompanying vessel calcifications, we performed transcriptome analysis using RNA sequencing (RNA-seq) on tissue enriched with vessel-associated calcifications isolated from brains of Pdgfb"/" mice, a mouse model of PFBC. Brain calcifications are autofluorescent (18), which we exploited to manually isolate calcifications from the brains of Pdgfb"/" animals under a fluorescent stereomicroscope. Noncalcified tissue from the same anatomical region (thalamus/midbrain) was also collected from control animals (Pdgfb"/"/"). This brain region is referred to as a “calcification-prone region” (Fig. 1A). We also isolated tissue from the cortex (referred to as a “non-calcification-prone region”) of both Pdgfb"/" and control animals (Fig. 1A). Principal components analysis (PCA) of transcriptomic data showed that the first PC accounts for variability due to anatomical differences (thalamus/midbrain versus cortex), while the second PC accounts for differences between genotypes (fig. S1A). When comparing calcification- and non-calcification-prone brain regions of Pdgfb"/" and control animals, we detected 92 and 94 deregulated genes, respectively [false discovery rate (FDR) < 0.05 and fold change > 2; Fig. 1B, fig. S1B, and tables S3 and S4]. Contrasting differentially expressed genes in non-calcification-prone and calcification-prone regions between Pdgfb"/" and control animals showed that 74 genes were deregulated only in the calcification-prone region (thalamus/midbrain) and that 60 genes were deregulated only in a non-calcification-prone region (cortex) (Fig. 1C and D, and table S5). The remaining 26 deregulated genes detected in both regions (Fig. 1C and D, and table S5) concur with previously reported vascular transcriptional alterations due to reduced pericyte number in Pdgfb"/" mice (20). Enrichment analysis of hallmark pathways showed that several inflammatory pathways, such as interferon-γ and α pathways, reactive oxygen species pathway, and inflammatory response pathway, are enriched in Pdgfb"/" mice compared to controls (Fig. 1E). Several pathways were enriched only in calcification-prone regions, such as interleukin-2–signal transducer and activator of transcription 5 signaling, unfolded protein response, and tumor necrosis factor–α signaling via nuclear factor κB (Fig. S1C). Various significantly deregulated hallmark pathways (Fig. 1E and fig. S1C) have been associated with activated microglia (e.g., interferon related and complement related) or microglia quiescence (e.g., transforming growth factor–β related) (21, 22). Notably, Csf7, which encodes a cysteine protease inhibitor (23), was up-regulated in Pdgfb"/" mice (Fig. 1F). To summarize the node profiles of the M4 module, we examined its intramodular connectivity to identify the most highly connected intramodular hub genes (see Materials and Methods for details). Intuitively, hub genes can be viewed as module representatives that are centrally located in a network representation of correlated genes. Among the hub genes within the M4 module, we identified Csf7 and Ijgax (Fig. 1G), genes induced in microglia in neurodegenerative diseases, termed DAM (disease-associated microglia), and in aging (23). Furthermore, in addition to Csf7 and Ijgax, several other genes associated to reactive microglia or the DAM signature (e.g., Cad68, Clec7a, and Lpl) were significantly up-regulated in the calcified brain regions of Pdgfb"/" mice (Figs. 1B and 2A). However, when comparing up-regulated genes in calcification-prone regions in Pdgfb"/" mice using stringent criteria (FDR < 0.05 and fold change > 2) with the DAM signature, we found an overlap of only six genes (Lpl, Cad68, Tyrobp, Ijgax, Csf7, and Clec7a) (fig. S1G). The DAM signature was recently reported to overlap with the “proliferative region–associated microglia” (PAM) signature that defines a subset of microglia found in the white matter during development (24). By comparing the PAM signature with our dataset using the same criteria as for the DAM genes, an overlap of seven genes (Itm1x1, Lpl, Cad68, Slc16a3, Lgr2, Tyrobp, and Clec7a) was found (fig. S1H). We validated the expression of DAM/PAM signature genes Timp2 (Fig. 2B), Cd68 (Fig. 2C), Clec7a (Fig. 2, D and E), and Itgax (Fig. 2F) and Csf7 (Fig. 2G) in microglia surrounding vessel calcifications with immunohistochemistry. Cd68 was expressed by microglia and perivascular macrophages (PVM) throughout the brain (fig. S2A), but its expression was up-regulated in CAM (Fig. 2C). Conspecific Clec7a positivity was seen in CAM (Fig. 2D), which showed down-regulation of P2yr12, a homeostatic microglia marker, and up-regulation of Iba1 (Fig. 2E). Clec7a expression was also seen in a subpopulation of white matter microglial cells (fig. S2B), in agreement with reported expression of Clec7a in white matter microglia (25). In addition, single microglial cells with normal morphology in the cortex of Pdgfb"/" mice showed Clec7a expression (fig. S2C). Itgax and Csf7 were expressed only in CAM and not in microglia in other brain regions or in other cell types. Similarly, Timp2 was expressed only by microglia surrounding calcifications and in neurons (fig. S2D), as previously reported (26). Thus, CAM exhibited an activation profile overlapping with microglia during aging, neurodegenerative proteinopathies, and development.

Brain vessel–associated calcifications contain protein aggregates

Because the CAM protein expression profile resembles the DAM signature reported in β-amyloid plaque–associated microglia in Alzheimer’s disease (AD) (2) (23) and vascular calcifications contain protein deposits such as amyloid β precursor protein (APP) and amyloid precursor–like protein 2 (27), we next asked whether vascular calcifications in CAM exhibit a similar profile of protein deposits and analyzed the coregulation of protein expression and gene expression in the PCA of microglial brain calcifications. To distinguish possible changes in protein expression from changes in gene expression, we performed PCA on the microglial transcriptomic data, and the two sets of data were analyzed together. The results of these analyses revealed a general enrichment of protein expression and gene expression in the brain regions that were enriched in the DAM signature (23). In addition, we identified a number of protein expression and gene expression profiles that were not enriched in the DAM signature, but which were enriched in the PAM signature (24). These findings suggest that the DAM signature may be specific to the white matter and that other signatures may be present in other brain regions. Overall, these results suggest that vascular calcifications in CAM exhibit a protein expression profile similar to that of β-amyloid plaque–associated microglia in AD.
lack the β-pleated sheet conformation and structural regularity recognized by thioflavin T or Congo red.

**CAM exhibit a distinct phenotype from DAM**
Osteopontin (OPN, Spp1), one of the signature genes defining PAM and DAM (23–29), exhibits high expression in both PBCs of ret/ret, and DAM (ret/+) mice. Hydroxyapatite, found in vessel-associated calcifications (17), and β-amyloid plaques induce formation of the inflammasome in macrophages and microglia, respectively (fig. S1D) (28, 29). We therefore investigated whether microglia around calcifications express ASC, an adaptor protein for inflammasome-mediated caspase-1 activation.

Fig. 1. An inflammatory microenvironment surrounds brain calcifications. (A) Samples collected for transcriptome analysis. RNA-seq was performed from tissue isolated from two anatomical regions of Pdgfb<sup>ret/ret</sup> and control animals (n = 4). Tissue enriched with brain calcifications was isolated from the thalamus/midbrain region labeled as calcification-prone region. Tissue isolated from the cortex is labeled as non–calcification-prone region. (B) Volcano plot showing deregulated genes in calcification-prone regions in Pdgfb<sup>ret/ret</sup> animals compared to control animals. (C and D) Venn diagrams showing up-regulated (C) and down-regulated (D) genes in calcification-prone and non–calcification-prone regions. (E) Significantly up-regulated (in red) and down-regulated (in blue) pathways identified by gene set enrichment analysis (GSEA) (P < 0.05) in calcification-prone regions in Pdgfb<sup>ret/ret</sup> animals compared to controls. (F) For each module identified by the network analysis, the module eigengene was calculated, which summarizes the expression profile of the module. The M4 module is associated with calcification-prone brain regions in Pdgfb<sup>ret/ret</sup> mice. Heatmap shows column-wise standardized (z score) module eigengene values. CPR, calcification-prone region; NCPR, non–calcification-prone region; TOM, topological overlap matrix. (G) Graphical representation of representative hub genes with a high gene significance and module membership in the M4 module.
multicolor flow cytometry panel to distinguish immune cells and activated microglia from normal microglia. We detected eight immune cell clusters both in control and Pdgfb\textsuperscript{ret/ret} mice, as visualized by a dimension reduction algorithm uniform manifold approximation and projection (UMAP) (Fig. 3A and fig. S5A) (30). Within the microglial cluster (fig. S5B), we defined three subclusters (homeostatic microglia, phagocytic microglia, and Ki67\textsuperscript{+} microglia) of microglia on the basis of median marker expression of seven markers (CD11b, Ki67, F4/80, CD64, CX3CR1, CCR5, and MerTK) (Fig. 3, B and C). Overall changes in Pdgfb\textsuperscript{ret/ret} microglia compared to controls included increased expression of CX3CR1 and reduced CCR5 expression (fig. S5C). We also detected an increase in Ki67\textsuperscript{+} microglia and the emergence of CX3CR1\textsuperscript{hi} MerTK\textsuperscript{hi} microglia (phagocytic microglia) in Pdgfb\textsuperscript{ret/ret} animals (Fig. 3, A to C). Using immunohistochemical staining for the proliferation marker Ki67, we confirmed the presence of proliferating microglia (Fig. 3D). Also in vivo labeling of proliferating cells using 5-ethyl-2\textsuperscript{-}deoxyuridine (EdU) in control and Pdgfb\textsuperscript{ret/ret} mice showed EdU-positive microglial cells around calcifications (Fig. 3E). In addition, we detected proliferating CD45\textsuperscript{hi} cells with round morphology surrounding calcifications.
(fig. S6A), while other cell types of the NVU (endothelial cells, pericytes, and astrocytes) were K167 negative (fig. S6, B to D). Brain vasculature in \textit{Pdgfb}\textsuperscript{ret/ret} mice is permissive for leukocyte entry (31); therefore, we investigated whether CD45\textsuperscript{high} cells with a round morphology (fig. S6A) surrounding calcifications could be T cells. Using immunohistochemistry, CD3\textsuperscript{+} T cells were detected in the brain parenchyma surrounding calcifications (fig. S6E) and in brain regions not prone to vascular calcification, e.g., in corpus callosum (fig. S6F). Some of these T cells were K167 positive (fig. S6, E and F, yellow arrowheads). To assess whether T cells trigger the formation of brain calcification, we crossed \textit{Pdgfb}\textsuperscript{ret/ret} mice with \textit{Rag1}\textsuperscript{-/-} mice, which lack mature T cells. The absence of T cells did not prevent the formation of brain calcifications in \textit{Pdgfb}\textsuperscript{ret/ret}, \textit{Rag1}\textsuperscript{-/-} mice (fig. S7), indicating that T cells, although present around brain calcifications, do not induce vessel calcification.

**Microglia give rise to cathepsin K–expressing cells around vessel calcifications**

We have reported previously that a subset of cells surrounding calcifications expresses osteoclast–associated markers receptor activator of nuclear factor \textit{kB} (RANK) and cathepsin K (17). In addition, cathepsin K, which is a principal collagen I–degrading protease in bone, is deposited in brain calcifications (17). Here, we show that RANK-expressing cells around calcifications are positive for the microglial marker Iba1 (Fig. 4A). To investigate whether resident microglia give rise to osteoclast-like cells around vessel-associated brain calcifications in \textit{Pdgfb}\textsuperscript{ret/ret} mice, we crossed two inducible Cre lines (\textit{Sall1-CreER}\textsuperscript{T2} and \textit{Cx3cr1-CreER}\textsuperscript{T2}) and a reporter line expressing tdTomato under the Rosa26 promoter (Ai14) with \textit{Pdgfb}\textsuperscript{ret/ret} animals to genetically label microglia. The \textit{Cx3cr1-CreER}\textsuperscript{T2} line targets CX3CR1+ macrophages including microglia and “border–associated” macrophages in the CNS (32), whereas the \textit{Sall1-CreER}\textsuperscript{T2} line targets resident microglia but not infiltrating immune cells or brain PVM (33). Mice were treated with tamoxifen at 1 month of age to induce tdTomato expression and euthanized at 4 months of age (Fig. 4B). Immunostaining with cathepsin K and Iba1 showed a colocalization with tdTomato both when using the \textit{Cx3cr1-CreER}\textsuperscript{T2} (Fig. 4C) or \textit{Sall1-CreER}\textsuperscript{T2} (Fig. 4D) line, indicating that resident microglia respond to calcifications by expressing and deposing cathepsin K into vascular calcifications.

**Pharmacological ablation of microglia intensifies vessel calcification**

After establishing that cathepsin K–expressing cells surrounding vascular calcifications are derived from resident microglia (Fig. 4), we asked whether microglia actively participate in calcification–associated pathology. To this end, we depleted microglia in \textit{Pdgfb}\textsuperscript{ret/ret} and control animals for 2 months by using the colony-stimulating factor 1 receptor (CSF1R) inhibitor PLX5622 (Fig. 5A). In contrast to mice fed with control chow, mice fed with chow containing PLX5622 showed a reduction in the number of microglia (fig. S8, A and B). Compared to control chow–fed \textit{Pdgfb}\textsuperscript{ret/ret} mice (Fig. 5B), in microglia-depleted \textit{Pdgfb}\textsuperscript{ret/ret} mice, the staining pattern of APP and osteocalcin was altered. APP and osteocalcin immunostainings were stronger along the periphery and weaker within calcifications. Some calcifications only stained for APP, whereas others immunolabeled only with osteocalcin (Fig. 5B, arrowheads). However, the ratio of APP- and osteocalcin-positive calcifications between PLX5622-treated and control chow–treated \textit{Pdgfb}\textsuperscript{ret/ret} mice was not statistically significant (Fig. 5D). Depletion of microglia did not alter vascular density (Fig. 5E) or modify already impaired vascular permeability to plasma basic (Fig. 5F).
Fig. 5. Pharmacological ablation of microglia aggravates vascular calcification. (A) Experimental setup of the pharmacological ablation of microglia. One-month-old mice (Pdgfb<sup>+/−</sup>, Pdgfb<sup>+/−</sup> or C57BL/6) were fed PLX5622 or control chow for 2 months. Mice were euthanized at 3 months of age. (B) Vessel-associated calcifications, visualized by osteocalcin (in red) and APP staining (in cyan), are increased in Pdgfb<sup>+/−</sup> compared to control chow-fed Pdgfb<sup>+/−</sup> animals. Blood vessels are visualized using CD31 staining (in white). Note that some calcifications in PLX5622-treated Pdgfb<sup>+/−</sup> mice are only positive for APP (yellow arrowheads), whereas others are positive only for osteocalcin (magenta arrowheads). (C) Quantification of calcification number in Pdgfb<sup>+/−</sup> mice administered PLX5622 or control chow (unpaired two-tailed t test; **P = 0.0087). (D) Ratio between APP- and osteocalcin-positive calcifications after PLX5622 and control chow treatment. (E) Quantification of vessel density in Pdgfb<sup>+/−</sup> mice with administered PLX5622 or control chow (unpaired two-tailed t test; P = 0.8782). (F) Cathepsin K (in white) deposition in calcifications (in red) in Pdgfb<sup>+/−</sup> mice is reduced after PLX5622 treatment. (G) Quantification of cathepsin K intensity from immunohistochemical stains in (F) (unpaired two-tailed t test; *P = 0.0276). Control chow, n = 4; and PLX5622 chow, n = 3. Scale bars, 100 μm (B and F). ns, not significant. All data are presented as means ± SEM.
Fig. 6. Microglia ablation leads to bone protein–containing axonal spheroids in white matter. (A) Coronal sections of Pdgfb<sup>ret/ret</sup> mouse brains fed with control chow or PLX5622 chow. (B) Immunofluorescence images of C57BL6 mouse brains with control (left) and PLX5622 chow (right) treatment. (C) Bielschowsky PAS staining of control and PLX5622 chow. (D) Osteopontin staining of control and PLX5622 chow.
Accordingly, spheroidal deposits on vessels in the thalamus were Alizarin red negative (fig. 5G, mid blue panel, arrowheads), whereas occasional spheroids in the midbrain showed Alizarin red positivity (fig. 5G, bottom orange panel). We further characterized deposits in the white matter that appeared after microglial depletion using classical histochemical stains. Deposits were periodic acid–Schiff (PAS) positive, indicating the presence of glycoproteins (fig. 6C, black arrowheads). The Bielschowsky silver stain revealed the presence of brown to black spheroids in the white matter, indicative of degenerating neurites (fig. 6D, white arrowheads). In addition, staining of osteocalcin with APP, a marker for neuronal injury, and with myelin basic protein (MBP) showed colocalization within axonal spheroids (fig. 6, E and F). Furthermore, APP-positive spheroids in the white matter were also positive for another bone protein, osteopontin (fig. 6G). Thus, prolonged microglial depletion using the CSF1R inhibitor PLX5622 in adult mice leads to white matter injury, characterized by the formation of axonal spheroids, which immunolabel with APP and MBP as well as osteopontin, indicating the accumulation of bone proteins.

Microglia control vascular calcification in a TREM2-dependent manner

In addition to microglial depletion using a pharmacological CSF1R inhibitor, we used a genetic approach to impair microglial function in Pdgfrα−/− mice by crossing Pdgfrα−/− with Trem2−/− mice. Deletion of TREM2 leads to reduced microglial functionality (35). Furthermore, TREM2 expression is necessary for microglia to achieve the full DAM signature in a mouse model of AD (23). However, Trem2 is expressed in two brain-resident myeloid populations, microglia and PVM (36). We performed immunohistochemical detection of CD206+ PVM and observed that PVM are located around arteries and veins on the vascular tree and are not present at the capillary bed (fig. 5A), which is the calcified vascular bed in PFFC. Thus, we conclude that PVM are unlikely to play a role in a calcification process at the capillaries. We noticed an altered pattern of calcification in Pdgfrα−/−; Trem2−/− mice with vessels frequently encrusted with multiple osteocalcin-positive nodules (fig. 7A, bottom), yielding the "pears-on-a-string" phenotype reminiscent of human PFFC (19). This pattern was particularly evident in rostral thalamic regions that show less calcification than caudal thalamus and midbrain. Calcifications in Pdgfrα−/−; Trem2−/− animals appeared as single nodules (fig. 7A, bottom) in accordance with the reported phenotype of calcifications in Pdgfrα−/− mice (15, 17, 18). In addition to the altered pattern, vascular calcification was markedly aggravated in Pdgfrα−/−; Trem2−/− and Pdgfrα−/−; Trem2−/− mice (fig. 7B). Similar to the microglia depletion experiments, we observed altered APP and osteocalcin immunostaining of calcifications in Pdgfrα−/− mice in the absence of one or two Trem2 alleles (fig. 5B). In Pdgfrα−/−; Trem2−/− mice, calcifications stain uniformly with both antibodies used to visualize calcifications (osteocalcin and APP), whereas in Pdgfrα−/−; Trem2−/−, APP and osteocalcin staining is reduced (fig. 5B). Thus, functional TREM2 in microglia is necessary to limit the formation of vessel calcifications in Pdgfrα−/− mice. In addition, we observed that microglia surrounding calcifications express CLEC7A, a DAM-associated protein dependent on Trem2, in Pdgfrα−/−; Trem2−/− animals (fig. 5G). Cathepsin K deposition into calcifications is TREM2 dependent; Pdgfrα−/−; Trem2−/− and Pdgfrα−/−; Trem2−/− mice displayed a strongly reduced cathepsin K deposition into calcifications (fig. 7, D and E). In summary, these data show that cathepsin K expression by CAM is Trem2 dependent and further corroborate that microglia control the growth of vascular calcifications in the brain.

Microglia depletion or functional modulation alters astrocyte reactivity but not the neurotoxic-like profile around calcifications

We next explored whether microglia also modify the strong astrocyte reactivity around calcifications (17, 18). We had shown previously that astrocytes encircling brain calcifications exhibit a neurotoxic response [e.g., C3 and lipocalin-2 (LCN2) expression] and an unusual reactive phenotype [e.g., podoplanin expression] (17). We investigated whether astrocyte reactivity and the expression of neurotoxic markers are altered after modifying the number and function of microglia in Pdgfrα−/− mice. Microglia ablation using PLX5622 or compromised function (Trem2 genotype) in Pdgfrα−/− mice resulted in an altered staining pattern of GFAP and podoplanin, proteins expressed by reactive astrocytes surrounding calcifications (fig. S10, A and B). In Pdgfrα−/− mice treated with PLX5622, GFAP reactivity showed a diffuse pattern, most likely because of an increased density of calcifications, compared to mice that received control chow (fig. S10, A, E, and G). Furthermore, similar alterations in GFAP staining were observed in Pdgfrα−/− mice with impaired microglial function: Pdgfrα−/−; Trem2−/− and Pdgfrα−/−; Trem2−/− (fig. S10B). Podoplanin staining in reactive astrocytes surrounding calcifications was markedly reduced (fig. S10, A and B). We quantified podoplanin and GFAP staining intensity and calculated the staining intensity ratio, which showed a trend toward a reduction in Pdgfrα−/− mice treated with PLX5622 (fig. S10C) and a significant reduction in Pdgfrα−/−; Trem2−/− and Pdgfrα−/−; Trem2−/− mice (fig. S10D) compared to Pdgfrα−/− mice. Notably, we did not detect a reduction in the expression of neurotoxic signature markers LCN2 and C3 by reactive astrocytes surrounding calcifications after microglia depletion in Pdgfrα−/− mice (fig. S10, E and F). We quantified LCN2 and C3 and GFAP staining intensity and calculated the staining intensity ratio, which showed no significant difference between PLX5622-treated and nontreated Pdgfrα−/− mice (fig. S10, G and H). Together, these results indicate that microglia modulate astrocyte reactivity but are not required to evoke a neurotoxic-like astrocyte phenotype in response to vessel calcification.

DISCUSSION

Our findings demonstrate that in addition to vascular calcifications, microglia play a role in the regulation of astrocyte reactivity and calcification. Furthermore, microglia depletion or functional modulation alters astrocyte reactivity but not the neurotoxic-like profile around calcifications. These results provide insights into the complex interactions between microglia and astrocytes in the context of vascular calcifications.
Unexpectedly, studies investigating vascular calcifications in the brain are rare (38). Therefore, the consequences of calcification on vascular function at the NVU, including neurovascular coupling, have not been studied. As noted, brain calcification is a common incidental CT finding (13). It is plausible that under homeostatic conditions, i.e., without underlying vascular pathology, microglia limit vascular calcification during aging, which should be addressed by future studies.

Vascular calcification is commonly observed in neurodegenerative diseases such as AD (39), which warrants further investigation to determine whether microglial dysfunction contributes to vascular pathology in AD. Thus, in addition to parenchymal surveillance and the removal of various injurious stimuli, microglia could remove calcium phosphate precipitates and halt calcification of the NVU (fig. S11).

Microglial activation is insult dependent (22); however, some microglial activation gene signatures are shared (e.g., Spp1 and Clec7a) but not all (e.g., Osteopontin and CD31) (23). We detected a strong osteopontin expression in a subset of GFAP-positive astrocytes surrounding calcifications (fig. S4C), similar to osteopontin expression in reactive astrocytes after a stab wound injury (40). Osteopontin is highly expressed in injured tissues, including brain diseases (e.g., Parkinson disease and multiple sclerosis). One of the biological functions of osteopontin is to act as a hydroxyapatite binding mineral chaperone to inhibit the formation of hydroxyapatite crystals (41). The induction of osteopontin by different cells (e.g., microglia, astrocytes, and neurons) in response to various brain insults could prevent injury and ectopic calcification in the parenchyma or at the NVU. Ectopic soft-tissue calcifications in the periphery and in the brain, which are induced by excitotoxic insults, have a similar lamellar structure with darker–appearing lamellae rich in osteopontin (42). Notably, Spp1−/− mice develop severe secondary neurodegeneration accompanied by increased brain calcification in response to excitotoxic insults (43).
for calcium phosphate precipitation. Microglia-specific modification of cathepsin K expression should clarify the role of cathepsin K in vascular calcification. Microglial activity that impedes vessel calcification could trigger a coordinated activation of several pathways in protein and hydroxyapatite degradation. However, it is currently unclear whether microglia remove hydroxyapatite present in vascular calcifications (17). Macrophages have been shown to remove various crystals, including hydroxyapatite, which leads to activation of the NLRP3 inflammasome pathway (29). Our studies show that although vessel calcifications elicit strong microglial reactivity in vivo, they do not activate the inflammasome (Fig. S4E). Further studies are needed to understand how microglia sense and remove vascular calcification and whether this activates intracellular pathways as those evoked by self-derived damage-associated molecular patterns via pattern recognition receptors. In addition to altered cathepsin K deposition, we observed an altered deposition of APP and osteocalcin into calcifications in Pdgfb<sup>−/−</sup> crossed with Treg<sup>−/−</sup> or Treg<sup>−/−</sup> or after microglia depletion (Figs. 5B and 7B). This finding indicates that microglial activity not only impedes the growth of calcifications but also modifies matrix composition of calcifications.

In this study, we observed that functional TREM2 is required to halt vessel calcification in a mouse model of PFBC (Fig. 7). TREM2 deficiency also leads to an altered vessel calcification pattern in Pdgfb<sup>−/−</sup> mice (Fig. 7A), similar to that described in human autopsy cases. Vascular calcification in PFBC is sometimes described as pearls on a string because of numerous, tiny spherical calcifications that encrust almost the entire abluminal side of capillaries (18, 47). Calcifications appear as single nodules (15–18, 47) in mouse models of PFBC. Variants in a microglia-specific gene, TREM2, have been associated with an increased risk for AD (2). Studies in mice models of AD have linked functional TREM2 to the development of a specific microglia activation state, DAM (23, 48), and to maintaining microglial metabolic fitness as well as a sustained microglial response to Aβ plaque–induced pathology (49). It has been proposed that although microglia are efficient in phagocytosing protein aggregates in the early stages of AD, over time, they lose their efficiency (2). Therefore, it is plausible that crossing Pdgfb<sup>−/−</sup> with Treg<sup>−/−</sup>
or Treg<sup>−/−</sup>, vascular calcification is accelerated because of microglial exhaustion (2). It is noteworthy that microglia encircling calcifications contain numerous phagocytic vesicles (15), and also, our study identifies a population of phagocytic microglia in the calcified brain regions of Pdgfb<sup>−/−</sup> mice (Fig. 3, B to E). Further studies are needed to understand whether TREM2-driven microglial function is initiated because of altered proteostasis and calcification at the NVU or neuronal death occurring because of altered vessel function. These processes, however, are not necessarily mutually exclusive.

Several lines of evidence indicate that cell-autonomous defects in microglia lead to brain disease and cerebral calcification (50). Microglia have also emerged as a disease modifier in a wide range of neurodegenerative diseases (e.g., AD and frontotemporal demen
tias) (51, 52). Deregulated microglia have been implicated in the pathogenesis of tissue-resident macrophages and microglia (51). In mice, the full knockout of Xpr1 is embryonic lethal (52), but whether the absence of Xpr1 affects microglia development has not been assessed. Thus, further studies are needed to dissect the role of PFBC genes in microglial function.

In this study, on the basis of positivity for osteocalcin, we found by serendipity that microglial ablation by chronic CSF1R inhibition using PLX5622 for 2 months resulted in localized axonal damage to fiber tracts of the internal capsule and adjacent thalamic and striatal areas (Fig. 6). Positive staining for osteocalcin and osteopontin coincided with the presence of dystrophic neurites exhibiting spheroid formation (Fig. 6, E to G), similar to the pathology described in patients with leukoencephalopathy caused by CSF1R mutations (50). These patients exhibit brain calcification in white matter regions and a reduction in microglia in affected regions. It is plausible that axonal spheroids become calcified during the course of the disease. Further studies are needed to understand the relationship between the appearance of axonal spheroids positive for bone proteins and white matter calcification. We observed that inclusions within dystrophic neurites stain positive for MBP (Fig. 6G), a protein secreted by oligodendrocytes, indicating a disrupted homeostasis in oligodendrocytes. Previous studies in white matter microglia have shown that microglia promote myelogenesis during early development, providing evidence for a role in optimizing oligodendrocyte function (53). Thus, further studies are needed to ascertain whether axonal damage is directly caused by a reduction in microglia or the absence of microglia has an effect on another cell type (e.g., oligodendrocytes), which may enhance spheroid formation in neurites.

In conclusion, we describe an unrecognized role of microglia in brain vascular calcification. In addition, we show that functional microglia are important to prevent calcification of the NVU in neurodegenerative diseases with a compromised NVU. Proposed mechanisms by which microglia control vascular calcification include the removal of apoptotic cells and spontaneous calcium phosphate precipitates as well as the prevention of nucleation of hydroxyapatite by controlling proteostasis of the ECM and/or the secretion of anti-calci
cifying proteins or molecules.

MATERIALS AND METHODS

Mice
Mice used in this study were 1 to 5 months old. Both sexes were used for experiments. The following mouse strains were used: C57BL/6J (Charles River Laboratories), B6.Pdgfb<sup>tm3Cret</sup> (Pdgfb<sup>−/−</sup>) (18, 54), Cx3cr1<sup>GFP</sup> (55), Sall1<sup>−/−</sup>Cxcr4<sup>−/−</sup> (53), B6.Cg-Gt(Rosa26Sor<sup>Sor<sup>tm14(CAG-Mylp1-Myr-GFP)Eom</sup></sup>)<sup>−/−</sup> (A14) (Jackson Laboratory, stock no. 007914), APP/PS1 (56), Rag1<sup>−/−</sup> (57), and Treg<sup>−/−</sup> (58). This study was carried out in accordance with study protocols approved by the Cantonal Veterinary Office Zurich (permit numbers ZH196/2014 and ZH151/2017).
Pharmacological ablation of microglia

Oral CSF1R inhibitor, PLX5622 (Plexikon Inc.), was formulated in AIN-76A standard chow by Research Diets (New Brunswick, NJ) at 1200 parts per million. Control mice received AIN-76A chow without PLX5622. PLX5622 chow and control chow were provided by Plexikon Inc. under a material transfer agreement. One-month-old mice were fed with chow containing PLX5622 or control chow for 2 months and euthanized at the age of 3 months.

EdU treatment

Three-month-old mice were injected with EdU (50 mg/kg; Sigma-Aldrich, catalog no. 900584) intraperitoneally for three consecutive days and euthanized on the following day.

Antibodies

Primary antibodies used for immunofluorescence staining are listed in table S1. All secondary antibodies (suitable for multiple labeling) labeled with various fluorophores (Alexa Fluor 488, Cy3, and DyLight 649) made in donkey (anti-rabbit, anti-rat, and anti-goat) or in goat (anti-chicken Cy3) were purchased from Jackson ImmunoResearch. Antibodies used for flow cytometry analysis are listed in table S2.

Histochemistry and immunohistochemistry

Immunohistochemistry was performed according to methods described previously (17). For EdU detection, the Click-iT EdU Alexa Fluor 555 Imaging Kit (Thermo Fisher Scientific) was used, and slices were treated according to the manufacturer’s instructions. Immunohistochemical stainings were imaged with a confocal microscope [Leica SP5 or SP8, 20× numerical aperture (NA), 0.7; 40× NA, 1.25; and 63× NA, 1.4] or stereomicroscope (Zeiss Axio Zoom.V16; 1× NA, 0.25). For stains that exhibited salt-and-pepper noise, a median filter of 5 × 5 × 5 was applied to eliminate noise. Images were analyzed using the image processing software Imaris 9.2.0 (Bitplane) and Adobe Illustrator CS6.

For histochemistry, mouse brains were collected and embedded in paraffin. Two-micrometer-thick tissue sections were stained with PAS, hematoxylin and eosin, or the Bielschowsky silver stain using standard protocols. For Alizarin red staining, sections were deparaffinized and rehydrated, as well as incubated for 1 hour in 1% Alizarin red solution (pH 9.0) followed by 1 hour in 1% Alizarin red solution (pH 6.4) at room temperature. Stained paraffin sections were scanned with NanoZoomer HT (Hamamatsu Photonics), equipped with a 20× objective (UPlanSApo; NA, 0.75; Olympus). Images were analyzed using Digital Image Hub software (SlidePath) and Adobe Illustrator CS6.

Detection of aggregated proteins

Paraffin-embedded 6-μm-thick brain sections were used from Pdlgn−/− mice and controls of 1 year of age or older. As a positive control, paraffin-embedded sections from APP/PS1 mice (56) were used. Sections were deparaffinized and hydrated, followed by a 30-min incubation with LCO h-HTAA (27) at room temperature. For thioflavin T (Sigma-Aldrich, T3516) staining, deparaffinized sections were processed as the same for paraffin. Sections were counterstained with Hoechst 33342 and mounted in antifade reagent (FDHC) and analyzed with a 20× objective (NA, 0.7; Leica SP5) 42 z-stacks with a 1.48-μm step and 512 × 512 pixel resolution. For quantification of total brain calcification, z-stacks of APP staining and osteocalcin were summed in Fiji (ImageJ). Imaris software was used to quantify calcification using the function “surfaces.” Quantification of calcifications was performed in mid-midbrain, which shows the smallest interindividual variation in calcification load (17). Cathepsin K intensity was calculated using Fiji (ImageJ) software. Vessel density was quantified using CD31 staining. The analysis was performed with a vessel density plugin (version 1.1, with minor modifications) in Fiji (ImageJ) software. Astrocyte reactivity was quantified using GFAP, podoplanin, LCN2, and C3 staining in Fiji (ImageJ). The LCN2 and C3 signal intensity was first masked to GFAP staining to eliminate nonastrocytic expression or deposition. Signal intensity was then normalized to the GFAP signal. Two technical replicates were quantified for each animal.

Images used for quantification of extravasated fibrinogen were acquired using a 20× objective (NA, 0.75; Leica SP8), seven z-stacks with a 1.04-μm step, and 1024 × 1024 pixel resolution. CD31 staining marking blood vessels was thresholded and de-speckled, afterward subtracted from the thresholded fibrinogen staining. Image subtraction was performed to eliminate any signal inside the vessel lumen. Quantification of extravasated fibrinogen was performed using Fiji (ImageJ) software.

Microglia were quantified on images acquired using a 20× objective (NA, 0.7; Leica SP5), 41 z-stacks with a 0.64-μm step, and 1024 × 1024 pixel resolution. Quantification of microglia was performed using the morphoJ package in Fiji.

Flow cytometry analysis

Mice were deeply anesthetized using a mixture of ketamine and xylazine and perfused transcardially using ice-cold phosphate-buffered saline (PBS). Subsequently, mouse brains were dissected into non-calcification-prone brain regions, i.e., cortex, hippocampus, and cerebellum, and calcification-prone regions, i.e., thalamus, midbrain, and pons. Brain cell suspensions were prepared by cutting the tissue into small pieces, followed by collagenase type IV treatment. Dissociated tissue was passed through an 18-gauge syringe to obtain a homogeneous cell suspension and further enriched with a Percoll gradient. Samples were then passed through a 70-μm filter, followed by red blood cell lysis and antibody staining. Flow cytometric analysis was carried using MACSQuant (BD Biosciences) and analyzed with FlowJo and R software.

High-dimensional analysis

Raw data were preanalyzed with FlowJo, subsequently transformed in MATLAB using cyto3 and percentile-normalized in R. Dimensionality reduction was achieved by UMAP. FlowSOM was used for automated and expert-guided cell clustering. Median marker expression was projected onto UMAP to generate a heatmap of median expression values.
surrounding tissue. Cortical sections were also removed as examples of non-calcification-prone regions. RNA was isolated with a micro RNA kit (Qiagen) according to the manufacturer’s instructions. The concentration of RNA and sample purity were assessed using a 2100 Bioanalyzer (Agilent) and RNA 6000 Pico Kit (Agilent). RNA samples were poly(A)-enriched, and libraries were prepared using the Illumina TruSeq Stranded RNA kit. RNA was sequenced on an Illumina platform HiSeq 4000 at the Functional Genomic Center Zurich (UZH, ETH). The Illumina single-read approach (1 × 125 base pair) was used to generate raw sequencing reads with a depth of 20 million to 30 million reads per sample.

Bioinformatics analysis
Quantification of RNA-seq data was performed using kallisto. Briefly, target transcript sequences were obtained from ENSEMBL (GRCh38.p6), and the abundance of transcripts was quantified using kallisto 0.44.0 with sequence-based bias correction. All other parameters were set to default when running kallisto. Kallisto’s transcript-level estimates were further summarized at the gene level using tximport 1.8.0 from Bioconductor.

For downstream analysis, genes of low abundance were filtered out, and unwanted variation was estimated using the RUVr functionality from the RUVseq 1.16.0 package within Bioconductor. The number of factors of unwanted variation estimated from the data was set to 3, and the genes by samples matrix of residuals was obtained from a first-pass quasi-likelihood negative binomial generalized log-linear regression of the counts on biological covariates using the edgeR package from Bioconductor.

Differential expression analysis was performed using DESeq2 1.22.0 from Bioconductor, with estimated factors of unwanted variation included as additional covariates in the design formula. Significant genes were identified using FDR < 0.05 and fold change > 2.

Coexpression network analysis was performed on the combination of 16 samples (4 groups × 4 replicates) using weighted correlation network analysis (WGCNA) R package. Briefly, normalized count data obtained from RUVseq were first adjusted for mean-variance trend using the regularized log transformation of DESeq2 1.22.0 from Bioconductor. To exclude uninformative genes from coexpression analysis in an unbiased manner, 867 highly variable genes were selected on the basis of the overall distribution of coefficients of variation. Subsequently, a signed weighted network of highly variable genes was constructed using bi-weight midcorrelation with beta = 23 as soft thresholding power. Hierarchical clustering of the topologically overlapped module dissimilarity further revealed six modules of positively correlated genes. Module eigengene was defined as the first PC of the expression matrix of the corresponding module. Module membership was defined per gene as the Pearson correlation of gene expression and module eigengene. In the context of module membership, gene significance was defined as the geometric mean of the absolute value of pairwise Welch’s t-statistics comparing Pdgfrα+ CPR (calcification-prone region) to Pdgfrα− NCPR (non-calcification-prone region) with 1000 permutations. When the Pdgfrα mouse gene, then the mean gene with the maximum mean expression was selected using the collapseRows functionality within the WGCNA R package. Signaling pathways analyzed by GSEA were obtained from the Hallmark gene sets of the MSigDB. Gene signature of DAM and PAM were obtained from literature (23, 24). Heatmaps were generated using the heatmap R package, with clustering distance and method set to Euclidean and ward.D2, respectively.

Statistical analysis
Quantified values are represented as means ± SEM. The following statistical tests were performed with Prism8 software (GraphPad). Normality was assessed using a Shapiro–Wilks test. The following tests were used to calculate statistical significance: Student’s t test (unpaired and two-tailed), one-way analysis of variance (ANOVA) with Dunnett’s multiple comparison, or Mann-Whitney two-tailed test. P values < 0.05 were considered significant.

SUPPLEMENTARY MATERIALS
Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/9/eabc6896/DC1

REFERENCES AND NOTES
1. M. S. Thior, F. Gineaux, S. Garel, Microglia and early brain development: An intimate journey. Science 362, 185–189 (2018).
2. S. Hickman, S. Izzy, P. Sen, L. Morisset, J. El Khoury, Microglia in neurodegeneration. Nat. Neurosci. 21, 1359–1369 (2018).
3. C. Iadeola, The neurovascular unit coming of age: A journey through neurovascular coupling in health and disease. Neuron 96, 17–42 (2017).
4. A. Fantin, J. M. Vieira, G. Giesiti, L. Denti, Q. Schwarz, S. Pykhovkhij, F. Peri, S. W. Wilson, C. Ruhling, Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. Blood 116, 829–840 (2010).
5. T. M. Mathiesen, K. P. Lehe, N. C. Danbolt, O. P. Ottersen, The perivascular astroglial sheath provides a complete covering of the brain microvessels: An electron microscopic 3D reconstruction. Glia 58, 1094–1103 (2010).
6. K. Haruwaka, A. Bogani, T. Tachibana, N. Ohno, H. Kaniishi, A. Hashimoto, M. Matsumoto, D. Kato, R. Oto, H. Kiyama, A. J. Moorhouse, J. Nabekura, H. Wake, Dual microglia effects on blood brain barrier permeability induced by systemic inflammation. Nat. Commun. 10, 5816 (2019).
7. A. Nimmberger, F. Kirchhoff, F. Helmchen, Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308, 1314–1318 (2005).
8. R. Ransohoff, B. Engelhardt, The anatomical and cellular basis of immune surveillance in the central nervous system. Nat. Rev. Immunol. 12, 623–635 (2012).
9. S. Raff, J. M. Butler, B. S. Ding, Angiogenic functions of organ-specific endothelial cells. Nature 529, 316–325 (2016).
10. A. Westenberger, A. Balck, C. Klein, Primary familial brain calcifications: Genetic and clinical update. Curr. Opin. Neurol. 32, 571–578 (2019).
11. Z. Cen, Y. Chen, S. Chen, H. Wang, D. Yang, H. Zhang, H. Wu, L. Wang, S. Tang, J. Ye, J. Shen, H. Wang, F. Liu, X. Chen, F. Xie, P. Liu, K. Xu, J. Cao, P. Cai, Q. Pan, J. Li, W. Yang, P. F. Shan, Y. Li, J. J. Liu, B. Zhang, W. Luo, Biallelic loss-of-function mutations in JAM2 cause primary familial brain calcification. Brain 143, 491–502 (2020).
12. G. Nicola, C. Charbonnier, D. Campanse, J. A. Veltman, Estimation of minimal disease prevalence from population genomic data: Application to primary familial brain calcification. Am. J. Med. Genet. B Neuropsychiatr. Genet. 177, 68–74 (2018).
13. H. Deng, W. Zheng, J. Kuncic, Genetics and molecular biology of brain calcification. Aging Dis. 7, 220–237 (2016).
14. X. Chen, S. M. Moe, Vascular calcification: Pathophysiology and risk factors. Curr. Hypertens. Rep. 14, 220–237 (2012).
M. Jucker, Abeta42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. EMBO Rep. 7, 940–946 (2006).
57. P. Mombaerts, J. Iacomini, R. S. Johnson, K. Herrup, S. Tonegawa, V. E. Papassouliou, RAG-1-deficient mice have no mature B and T lymphocytes. Cell 68, 869–877 (1992).

58. J. R. Turnbull, S. Gillilan, M. Cella, T. Aoshi, M. Miller, L. Piccio, M. Hernandez, M. Colonna, Cutting edge: TREM-2 attenuates macrophage activation. J. Immunol. 177, 3520–3524 (2006).

59. A. Subramanian, P. Tamayo, V. K. Mootha, S. Mukherjee, B. L. Ebert, M. A. Gillette, A. Paulovich, S. L. Pomeroy, T. R. Golub, E. S. Lander, J. P. Mesirov, Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. USA. 106, 15440–15445 (2009).

Acknowledgments: Imaging was performed with equipment maintained by the Center for Microscopy and Image Analysis, University of Zurich, and RNA-seq was performed at the Functional Genomics Center Zurich, University of Zurich and ETH. Flow cytometry analysis was carried out at the Cytometry Facility at the University of Zurich. We thank Plexikon for providing PLX5622, F. Franzova for technical help, and S. Homemann for discussions.

Funding: This work was supported by the Swiss National Science Foundation (grants 31003A_159514 and 310030_184952 to A.K. as well as PPO0P3_170626 and BSGB_155832 to M.G.), the European Research Council under the European Union’s Horizon 2020 research and innovation program (grant agreement no. 819229) to M.G., and the Synapse Foundation and the Chouette Foundation (grant 2019-P102), Fonds zur Förderung des akademischen Nachwuchses (Zurich University), the Leducq Foundation (grant 14002002), the Swiss Heart Foundation, the Swiss Cancer League (grant KL-3848-02-2016) to A.K., and Forschungskredit und Stiftung für Forschung an der Medizinischen Fakultät der Universität Zürich (grant FK-16-034) to Y.Z. Author contributions: Y.Z. and A.K. conceived the study, designed the experiments, and wrote the manuscript. Y.Z., A.K., S.S., and S.G.U. performed the experiments and analyzed and interpreted the data. S.N. and M.D. analyzed and interpreted the bioinformatics data. J.S. and U.M. performed the experiments. K.P.R.N. and M.C. contributed with reagents. E.J.R. and M.G. analyzed and interpreted the data. A.K. supervised the study.

Competing interests: M.C. received research support from Alector, Argenx, Ono, and Pfizer for activities not related to the findings described in this publication. M.C. is a scientific advisory board member of Vigil Cell Signaling Technology, and Bluefin and has a patent to TREM2 pending. M.C. is an inventor of an unpublished patent application to this work filed by Washington University no. 62/891,827 filed on February 26, 2020. All other authors declare that they have no competing interests.

Data and materials availability: RNA-seq data, both raw data and gene-by-sample matrix of estimated counts, were deposited in Gene Expression Omnibus (GEO) under accession number GSE135449. All other data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Submitted 27 April 2020
Accepted 15 January 2021
Published 26 February 2021
10.1126/sciadv.abc4898

Citation: Y. Zabar, S. Nirdhar, S. Nasser, S. G. Uta, J. Schaffernoth, U. Maheshwar, E. J. Rushing, K. P. R. Nilson, M. Delorenzi, M. Colonna, M. Greter, A. Keller, Microglia control small vessel calcification via TREM2. Sci. Adv. 7, eabc4898 (2021).
Microglia control small vessel calcification via TREM2
Yvette Zarb, Sucheta Sridhar, Sina Nassiri, Sebastian Guido Utz, Johanna Schaffernath, Upasana Maheshwari, Elisabeth J. Rushing, K. Peter R. Nilsson, Mauro Deloreni, Marco Colonna, Melanie Greter and Annika Keller

Sci Adv 7 (9), eabc4898.
DOI: 10.1126/sciadv.eabc4898

ARTICLE TOOLS
http://advances.sciencemag.org/content/7/9/eabc4898

SUPPLEMENTARY MATERIALS
http://advances.sciencemag.org/content/suppl/2021/02/22/7.9.eabc4898.DC1

REFERENCES
This article cites 57 articles, 10 of which you can access for free
http://advances.sciencemag.org/content/7/9/eabc4898#BIBL

PERMISSIONS
http://www.sciencemag.org/help/reprints-and-permissions
