Microglial microRNAs mediate sex-specific responses to tau pathology

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Sex is a key modifier of neurological disease outcomes. Microglia are implicated in neurological diseases and modulated by microRNAs, but it is unknown whether microglial microRNAs have sex-specific influences on disease. We show in mice that microglial microRNA expression differs in males and females and that loss of microRNAs leads to sex-specific changes in the microglial transcriptome and tau pathology. These findings suggest that microglial microRNAs influence tau pathogenesis in a sex-specific manner.

Sex has heterogeneous effects on human disease pathogenesis, including in Alzheimer’s disease and other neurological diseases1–5. Dysfunction of microglia, the resident innate immune cells of the central nervous system, has been linked to many neurological diseases5–7. Sex differences in microglial gene expression and functions are seen in young adult mice5, and may be especially pronounced in the aging brain8. MicroRNAs (miRNAs) regulate immune networks in microglia9,10 and exhibit sex-specific expression in some cell types11. However, whether microglial miRNAs are expressed and function in a sex-specific manner is unknown. A better understanding of microglial miRNA function could identify previously unknown molecular networks that contribute to neurological diseases.

We performed miRNA sequencing (miRNA-seq) on microglia isolated from the brains of adult mice (B6C3F1/J). Unsupervised clustering grouped samples by sex (Fig. 1a). Sixty-one miRNAs were enriched in males and 26 in females (Fig. 1b and Supplementary Table 1); 13 of these (8 in males, 5 in females) were encoded by the X chromosome (Supplementary Table 2). Quantitative PCR (qPCR) analyses validated several differentially expressed miRNAs (Fig. 1c). Despite differing miRNA profiles, male and female cortical microglia had similar branching complexities (Fig. 1d–f).

To evaluate the transcriptional role of microglial miRNAs, we selectively depleted the miRNA-processing enzyme Dicer in adult microglia by crossing Cx3cr1CreERT2/loxP/+ with DicerfloxflxP/loxP mice (Dicer knockout (KO)). Dicer messenger RNA and Dicer protein levels were significantly reduced in CD11b+ cells, but not in CD11b− cells (Fig. 2a–d), accompanied by depletion of several Dicer-dependent miRNAs (Fig. 2e). Interestingly, loss of mature miRNAs resulted in far greater changes in the transcriptome of male than female microglia (Fig. 2f and Supplementary Tables 3 and 4). Pathway analysis of male differentially expressed genes (DEGs) showed enrichment in immune system pathways, including genes involved in tumor necrosis factor-α (TNF-α) signaling through NFκB activation, such as Il1b, Cdx69, Tr2 and Vegfa (Fig. 2g,h). Upstream activators of this expression pattern included TNF-α, NFκB, interleukin 2 (IL-2) and IL-1β (Fig. 2i), as predicted by Ingenuity Pathway Analysis, suggesting an overall enhanced inflammatory state. Thus, removing miRNAs alters the expression of immune-related genes to a greater extent in male compared to female microglia.

Microglia have been implicated in the spread of tau burden in the brain5,11, but whether male and female microglia respond to tau pathology differently is not known. To probe this, we used the P301S (PS19) tauopathy mouse model1 and labeled pathogenic forms of tau with the MCI antibody12. The densities of MCI+ tau inclusions were similar in male and female mice (Extended Data Fig. 1). However, the miRNA and mRNA profiles were altered by tau pathology to a far greater extent in male microglia (Extended Data Fig. 2a–c and Supplementary Tables 5–7). We then identified a subset of the differentially expressed miRNAs predicted to be targets of differentially expressed miRNAs in male microglia, focusing on mRNA and mRNA targets that have anticorrelated expression patterns (Extended Data Fig. 2d). Out of these, downregulation of miR-1249-5p explained the greatest number of upregulated targets in male PS19 microglia. These data suggest sex-specific microglial responses to similar levels of tau pathology.

Depleting Dicer in neurons results in tau hyperphosphorylation13, but how loss of microglial miRNAs alters tau pathology is unknown. We found that the density of MCI+ tau inclusions was higher in male PS19 Dicer KO mice than in females, reflecting differences in accumulation in the cortex, amygdala and piriform cortex (Fig. 3a–d). Male PS19 Dicer KO mice also had more amoeboid-like microglia, consistent with increased pathology (Fig. 3e–g). Bulk sequencing of isolated microglia from these mice showed that aside from differential expression of sex chromosome genes, such as Xist, Ddx3y, Uty and Eif2s3y, male microglia had enrichment of genes involved in inflammation and phagocytosis, including Spp1, Ccl6, Lpl, Il1b and Cst7 (Fig. 3h and Supplementary Table 8), characteristic of disease-associated microglia (DAMs)15,16. These differences may be exacerbated by age and tau pathology, since DAM genes were not upregulated in younger mice (Extended Data Fig. 3a). However, transcriptional sex differences were still...
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**Figure 1** | Adult microglia have sex-dependent miRNA expression. **a**, Heatmap of the top 50 most variable miRNAs measured by miRNA-seq of microglia isolated from 6-month-old mice. Counts were log-transformed, normalized and centered. \( n = 4 \) biologically independent samples per sex, 2 animals per sample. **b**, Volcano plot of data from **a**. Turquoise, enriched in males (61 miRNAs; Benjamini–Hochberg-corrected \( P \leq 0.05 \) and log2(fold change) \( \geq 1 \)); pink, enriched in females (26 miRNAs; \( P \leq 0.05 \) and log2(fold change) \( \leq -1 \)); gray, not significantly different. The top 10 miRNAs in each sex with the highest log2(fold change) values and normalized counts over 100 are labeled. The vertical dashed lines indicate a log2(fold change) \( \pm 1 \). The horizontal dashed line indicates \(-\log_{10}(0.05)\). The full list of differentially expressed miRNAs can be found in Supplementary Table 1. The Wald test was used. FDR, false discovery rate. **c**, qPCR validation of differentially expressed miRNAs from **b**. Each dot represents one mouse. The bar denotes the mean. \( n = 3 \) biologically independent samples per sex, 2 animals per sample. miR-200c-3p, *\( t = 2.784, \text{d.f.} = 4, P = 0.0496 \); miR-1298-5p, *\( t = 4.119, \text{d.f.} = 4, P = 0.0146 \); miR-365-3p, *\( t = 2.864, \text{d.f.} = 4, P = 0.05 \). Unpaired, two-tailed t-test. **d**, Representative three-dimensional (3D) Imaris morphology reconstruction (top) and corresponding Iba1 immunostaining (bottom) of cortical microglia from 9-month-old mice. Images were acquired and analysis repeated for every mouse quantified in **e,f**. Scale bars, 15 \( \mu \text{m} \). **e,f**, Number of branch points (**e**) and total length of processes (**f**) per cell, determined by Imaris-based automatic quantification. The error bars represent the s.e.m. The bar denotes the mean. Each dot represents one mouse. Approximately 60 cells per sex.

Observed in younger mice, highlighting the dynamic role of miRNAs in the sex-dependent adaptations to tau pathology at different stages of the disease.

To further probe these sex-specific effects in tauopathy, we carried out single-cell RNA sequencing (scRNA-seq) on PS19 Dicer KO microglia, gating on CD45+CD11b+ cells (Extended Data Fig. 3b–d). Of the 721 cells sequenced, 676 passed quality control and clustered into 5 distinct groups (Extended Data Fig. 3e–j). Female and male cells were differentially distributed, such that females had more cluster 1 and 2 cells while males had more cluster 3 cells (Fig. 3i,j). While the clusters were distinguishable by distinct markers (Extended Data Fig. 3k), the expression of resident microglial markers was relatively uniform, except in cluster 5, which probably consisted of CD45\(^+\) (Ptprc) peripheral macrophages (Extended Data Fig. 3l). Cluster 1 cells expressed higher levels of genes associated with microglia...
in human Alzheimer’s disease and experimental autoimmune encephalomyelitis mouse models\textsuperscript{19,20}, such as Apoe, Ms4a7, Kfra2, Clec12a and Mrc1, with a downregulation of homeostatic genes, such as Sall1, P2ry12 and Clec12a (Fig. 3l and Supplementary Table 11), consistent with the bulk RNA-seq (Fig. 3h). Immunohistochemistry confirmed upregulation of lipoprotein lipase (Lpl) in male microglia (Fig. 3n,o). This sex-dependent cluster enrichment supports the differential microglial response to tau pathology, where male microglia are more sensitive to tau, with a downregulation of homeostatic genes, such as Sall1, P2ry12 and Clec12a (Fig. 3l and Supplementary Table 11), consistent with the bulk RNA-seq (Fig. 3h). Immunohistochemistry confirmed upregulation of lipoprotein lipase (Lpl) in male microglia (Fig. 3n,o). This sex-dependent cluster enrichment supports the differential microglial response to tau pathology, where male microglia are more sensitive to tau.
Fig. 3 | Loss of microglial miRNAs increases DAMs and tau pathology in male PS19 mice. a–c, Representative images of MC1 immunostaining of hemibrains from 9-month-old Dicer KO PS19 female (a) and male (b) mice. Scale bars in a and b, 600 µm. The yellow dashed box is magnified in c. Two independent experimental cohorts were used. Scale bars in c, 300 µm. d, MC1 density of the hemibrains from Dicer KO nontransgenic (−) and transgenic (+) PS19 mice. n = 8 Dicer KO females, n = 6 Dicer KO males, n = 12 Dicer KO PS19 females and n = 10 Dicer KO PS19 males. *t = 2.578, d.f. = 20, P = 0.02. Unpaired, two-tailed t-test. Box plot elements: center line, median; box limits, upper and lower quartiles; whiskers, 1.5× interquartile range. e, Representative 3D Imaris morphology reconstruction of cortical microglia from 9-month-old Dicer KO PS19 male mice. Scale bars, 10 µm. Images acquired and analysis repeated for every mouse quantified in f and g, h. Number of branch points (f) and total length of processes (g) per cell in Dicer KO PS19 mice, determined by Imaris-based automatic quantification. The error bars represent the s.e.m. The bar denotes the mean. Each dot represents one mouse. n = 7 biologically independent animals per sex, approximately 70 cells per sex. *t = 2.200, d.f. = 12, P = 0.0481 (f), *t = 2.319, d.f. = 12, P = 0.0388 (g). Unpaired, two-tailed t-test. h, Volcano plot of RNA-seq data from Dicer KO microglia from 9-month-old male and female PS19 mice. Pink, enriched in females; turquoise, enriched in males; gray, not significantly different. The vertical dashed lines indicate log₂(fold change) ± 1. The horizontal dashed line indicates −log₂(0.05). n = 4 biologically independent samples per sex, 2 animals per sample. The Wald test was used. i, t-SNE plots of cells from female or male mice plotted based on the clustering from Supplementary Fig. 3h. Two biologically independent animals per sex. j, Percentage of cells in each microglial cluster for each sex from k. **P = 0.0088 (cluster 1), **P = 0.0021 (cluster 2). ****P < 0.0001 (cluster 3), not significant (NS) P = 0.6139 (cluster 4), NS P = 0.1145 (cluster 5). Two-sided Fisher’s exact test. k–m, Volcano plot of DEGs for clusters 1 (k), 2 (l) and 3 (m) as defined in l. Purple, upregulated genes; green, downregulated genes; gray, not significantly different in each cluster compared to all other clusters. A Wilcoxon rank-sum test was used. DEGs defined as Benjamini-Hochberg corrected P ≤ 0.05. The full list of DEGs can be found in Supplementary Tables 9–11. n, Representative images of Lpl immunostaining of 9-month-old PS19 female and male cortical Dicer KO microglia. Scale bars, 15 µm. Iba1+ cells are outlined. Images acquired and analysis repeated for every mouse quantified in o. o, Average Lpl fluorescence intensity per Iba1+ cell in each sex. n = 43 cells from 3 female mice, n = 78 cells from 4 male mice. ****t = 7.413, d.f. = 118, P = 2.04 × 10⁻¹⁰. Unpaired, two-tailed t-test. Box plot elements: center line, median; box limits, upper and lower quartiles; whiskers, 1.5× interquartile range. a.u., arbitrary units.
PS19 mice exhibited increased DAMs and decreased homeostatic microglia compared to females.

In summary, male and female microglia expressed different miRNAs, both at baseline and in tauopathy. Loss of these miRNAs had sex-dependent consequences on the microglial transcriptome and tau pathogenesis. Therefore, microglial miRNAs are key contributors to sex-specific phenotypes and should be further studied to understand microglial biology in the context of neurodegenerative diseases.

Online content
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Methods

Animals. For the RNA-seq and histological analyses, Cx3cr1CreERT2/mice (https://www.jax.org/strain/006366; The Jackson Laboratory) and P301S (P319) transgenic mice and their background control mice (B6C3F1/; https://www.jax.org/strain/100101, The Jackson Laboratory) were used. Mice of both sexes were studied. For the animal studies, sample size was determined by using power analysis with type 1 error = 5%, power = 80% and effect size and standard deviation based on previous studies using the P301S (P319) transgenic mouse line.

Group randomization was not relevant for this study since all animals in the study were treated similarly. Mice had free access to food and water and were housed in a pathogen-free barrier facility with a 12 h light–12 h dark cycle. All mouse experiments were approved by the Institutional Animal Care and Use Committee, University of California, San Francisco and complied with all relevant ethical regulations.

Drug administration. Tamoxifen (catalog no. T5648; Sigma-Aldrich) was prepared in corn oil at 20 mg ml−1 and given by intraperitoneal injection (2 mg d−1) for 10 consecutive days, starting when mice were 3–5 months of age.

Brain tissue collection. Mice were anesthetized with triphenylmethyl and transcardially perfused with PBS. Whole brains were fixed in 4% paraformaldehyde for 48 h and transferred to 30% sucrose for 48 h; 30–micron thick coronal sections were prepared on a sliding microtome (SM2010 R; Leica Microsystems), immersed in cryoprotectant and stored at −20 °C before staining.

Immunohistochemistry. Free-floating coronal sections were washed in PBS, permeabilized in PBS with Tween buffer (0.5% Triton X-100 diluted in PBS) and incubated in primary antibodies at 4 °C overnight and then with secondary antibodies at room temperature for 1 h. Tissues were mounted on glass slides with ProLong Gold Antifade Mountant with DAPI (catalog no. P36935; Thermo Fisher Scientific). The primary antibodies and dilutions used for staining were as follows: anti-Iba1 (1:300, catalog no. ab5076, Abcam and 1:50, catalog no. 019-19741, Fujifilm Wako); anti-Dicer (clone N167/7, 1:100, catalog no. ab167444, Abcam); anti-Ipl (clone LPL-A4, 1:50, catalog no. ab21356, Abcam); and anti-MC1 (1:500, a kind gift from P. Davies)11. Secondary antibodies used at a 1:500 dilution were: Cy3 AffiniPure Donkey Anti-Goat IgG (catalog no. 705-165-147, Jackson ImmunoResearch); Alexa Fluor 488 AffiniPure Donkey Anti-Mouse IgG (catalog no. 715-545-150; Jackson ImmunoResearch); and Alexa Fluor 488 Donkey Anti-Mouse IgG (catalog no. R37114, Thermo Fisher Scientific). Validation information for primary antibodies is included in the Nature Research Reporting Summary.

Epifluorescence fluorescence microscopy. Each coronal hemibrain slice was scanned at 10x magnification with an all-in-one fluorescence microscope (catalog no. B2Z-810, Keyence). The images were stitched together with the Keyence BX-Z Analyzer software v.1.15.0.3.

Confocal fluorescence microscopy. Images of hemibrain slices were acquired using an LSM 880 confocal microscope (ZEISS) at 1-μm focal plane intervals at ×40 magnification. Images were examined by maximum intensity Z-projection.

Image analyses. All images were analyzed with ImageJ (NIH) v.1.51 (ref. 25). For MC1 density quantification, TIFF images were processed with the auto local threshold Phansalkar plugin24; regions of interest were hand-traced and MC1 puncta were counted with the Analyze Particles function. For morphological analyses and trolleal images of the lower cortical region were analyzed with the Imaris software v.9.0.2 (Oxford Instruments) using the FilamentTracer function for macropipette processes. Investigators were blinded during image acquisition and analysis.

Adult microglia isolation. Adult microglia were isolated by magnetic-activated cell sorting as described previously19. Briefly, mice were anesthetized with triphenylmethyl and transcardially perfused with PBS to remove circulating blood cells in the central nervous system. Dissected brains were digested with 3% collagenase type 3 (catalog no. L050481R, Worthington) and 3 U ml−1 of dispase (catalog no. LS02104, Worthington) and incubated at 37 °C for 45 min. Digestion was stopped with inactivation buffer containing 2.5 mM of UltraPure EDTA (catalog no. 1557520, Thermo Fisher Scientific) and 1% fetal calf serum (catalog no. 10082147, Thermo Fisher Scientific). Tissue was then triturated with a serological pipette several times and passed through a 70-μm filter. Myelin in the homogenate was depleted with myelin removal beads (catalog no. 130-996-733, Miltenyi Biotec) and a magnetic LD column (catalog no. 130-042-901; Miltenyi Biotec). Microglia were isolated from the eluant with CD11b MicroReads (catalog no. 130-049-601; Miltenyi Biotec) and a magnetic MS column (catalog no. 130-042-201; Miltenyi Biotec).

High-throughput miRNA-seq. Freshly isolated microglia from two mice were pooled and the RNA enriched in miRNAs was extracted with the mirVana miRNA Isolation Kit with phenol (catalog no. AM1560; Invitrogen), as recommended by the manufacturer. Total RNA (100 ng) was used for adapter ligation with the Ion Total RNA-seq Kit v2 (catalog no. 4475936, Ion Torrent) and subsequently run on a 1% Tris-borate-EDTA acrylamide gel followed by gel extraction of small RNA (45–60 base pairs (bp)). The small RNA fraction was used for downstream reactions to build a small RNA library. Libraries were sequenced in multiplex on an Ion Proton System (catalog no. 4476610, Thermo Fisher Scientific) and reads were mapped to mature miRNA from mirBase with Bowtie 2 v2.3.4 (ref. 21).

miRNA-seq data analyses. Batch effects were removed with the R package RUVSeq v.1.16.1 and normalized in silico control genes. One male and one female samples were excluded from the miRNA-seq analysis based on principal component analysis. Differences in gene expression were calculated with the R package DESeq2 v1.22.2 (ref. 26). Counts were normalized with the trimmed mean normalization method27. Genes with <15 counts across all samples were excluded from the analysis. The false discovery rate (FDR) was calculated using the Benjamini–Hochberg method28. Predicted miRNA targeting of mRNA transcripts was carried out with the Ingenuity Pathway Analysis (https://www.ingenuity.com). Results were filtered for those with opposing log fold change values between mRNA and miRNAs and were either experimentally observed or highly predicted to be miRNA–mRNA target interactions.

High-throughput bulk RNA-seq. Freshly isolated microglia from two mice were pooled and total RNA was extracted with the RNeasy Mini Kit (catalog no. 74104, Qiagen). RNA quality was examined with a 2100 Bioanalyzer Instrument (Agilent Technologies). RNA samples with RNA integrity numbers >8 were used to construct a complementary DNA library. Oho(dT) beads were used to enrich for mRNA. cDNA library generation was done using the Ionxact 3′ mRNA-Seq Library Prep Kit FWD for Illumina (catalog no. 015.96, Lexogen). The quality of the cDNA library was assessed using a NanoDrop One/OneC Microvolume UV-Vis spectrophotometer (Thermo Fisher Scientific) to determine concentration and a 2100 Bioanalyzer Instrument to determine insert size. cDNA library samples were then sequenced with the HiSeq 4000 System (Illumina).

Data analyses of bulk RNA-seq. RNA-seq reads were mapped using the BlueRay genomics platform and the GENCODE GRCm38 mouse genome (Lexogen) in the R package DESeq2 v2.2.2 (ref. 29). Differential gene expression was calculated with the R package edgeR (ref. 30), using the Wald test. Counts were normalized with the trimmed mean normalization method31. Genes with <15 counts across all samples were excluded from the analysis. The FDR was calculated using the Benjamini–Hochberg method32.

qPCR with reverse transcription (RT–qPCR). For miRNA qPCR, 10 ng of total RNA was reverse-transcribed with the miRCURY LNA RT Kit (Qiagen). RT–qPCR was carried out with the PowerUp SYBR Green SYBR Green PCR Master Mix (Bio-Rad Laboratories). RT–qPCR was carried out with an ABI 7900HT sequence detector (Applied Biosystems) and SYBR Green PCR Master Mix (Applied Biosystems). Glyceraldehyde 3-phosphate dehydrogenase was used for normalization and the relative differences were calculated with the 2−ΔΔCt method. To validate Dicer mRNA reduction, RNA was converted to cDNA with the Script Reverse Transcription Superscript for RT–qPCR (Bio-Rad Laboratories). RT–qPCR was carried out with an ABI 7900HT sequence detector (Applied Biosystems) and SYBR Green PCR Master Mix (Applied Biosystems). Glyceraldehyde 3-phosphate dehydrogenase was used for normalization and the relative differences were calculated with the 2−ΔΔCt method. The following primers were used for RT–qPCR: Dicer (forward) CGACGCCACAGTTCTCTTCT; GAPDH (reverse) CGACCAGCACAGTCTTCTC; GAPDH (forward) GGGAGGCCCACACCATCCTT; (reverse) GCTCTCCTCATGTTGTTGAA.

Gene network and functional analyses. Gene network analyses of RNA-seq data were performed using gene set enrichment analysis33 and the Hallmark gene database34. Networks were visualized with Cytoscape v.3.6.1 (ref. 35), the STRING database36 and the perfuse force-directed layout.

Preparation of tissue for scRNA-seq. Brain tissue was prepared as described in Li et al.46. Briefly, 9-month-old mice were anesthetized with triphenylmethyl and transcardially perfused with PBS. The brain without the cerebellum was collected and placed into cold medium containing 15 mM of HEPES and 0.5% glucose in Hank's Balanced Salt Solution (Thermo Fisher Scientific), without phenol red; the entire procedure was done on ice. Brains were minced with a razor blade and homogenized with a 2 ml dounce containing 2 ml of medium A with 80 μl of DNase (12,500 units ml−1) and 5 μl of recombinant RNase inhibitor.
Single-cell sorting for scRNA-seq. Cells were blocked in 5 μl of mouse Fc block for 5 min on ice, then incubated with primary antibodies for 10 min and washed with FACS buffer (sterile-filtered 1% fetal calf serum, 2 mM of EDTA, 25 mM of HEPES in 1x PBS). Cells were incubated with secondary antibodies for 10 min then washed with FACS buffer. Cells were resuspended in 500 μl of FACS buffer with RNase inhibitor (1:500) and 0.5 μl of propidium iodide (1:1,000, catalog no. P3566; Thermo Fisher Scientific) for flow cytometry analysis. Cell sorting was performed using the Influx cell sorter (BD Influx) at the Stanford FACS Facility. The following gates were used to sort the microglia: (1) forward scatter area/side scatter area; (2) trigger pulse width/forward scatter; (3) LIVE/DEAD negative using propidium iodide; and (4) CD45<sup>low</sup>CD11b<sup>+</sup> and CD45<sup>low</sup>CD11b<sup>−</sup> gates were added to sort containing 4 μl of lysis buffer (recombinant RNase inhibitor), 0.05% Triton X-100, 2.5 mM of GNTP Mix (catalog no. R0192, Thermo Fisher Scientific) and 2.5 μM of oligo-dT30VN (50-ACACGTTGTACACAGCAGACTG30VN-30; ERCC: External RNA Controls Consortium) RNA Spike-IN Mix, catalog no. 4467470, Thermo Fisher Scientific). Plates were washed in cold PBS and air dried on ice plates were placed at −80°C in the freezer. The antibodies used for FACS were: rabbit anti-mouse TMEM119 (clone 106-6, approximately 200 ng/μl elution buffer). cDNA samples were purified using PCR Clean DX beads (0.7:1 ratio, catalog no. M0262S, New England Biolabs.). cDNA was amplified using the following PCR program: 37°C for 30 min; 95°C for 3 min; 23 cycles of 98°C for 20 s, 67°C for 15 s, 72°C for 4 min; and 72°C, for 5 min. cDNA samples were purified using PCRClean DX beads (0.7:1 ratio, catalog no. C-1003-50, Aline Biosciences) and reconstituted in 20 μl elution buffer. cDNA quality was examined with a Fragment Analyzer (High Sensitivity NGS Fragment Analysis Kit; 1–6,000 bp, Advanced Analytical Technologies). To make libraries, all samples were diluted to 0.15 ng/μl in 384-well plates using the Manuscript Liquid Handler (Formulatrix) and mosquito X1 (TTT Labtech) with customized scripts. The Nextera XT DNA Library Kit (catalog no. FC-131-1096; Illumina) was used at one-tenth of the recommended volume, with the help of a microscope HTS robot (TTT Labtech) for liquid transfer. Tagmentation was done in 1.6 μl (1.2 μl of Tagment Enzyme Mix from the library preparation kit (0.4 μl ampiclon tagment mix and 0.8 μl tagment DNA buffer), 0.4 μl of diluted cDNA) at 55°C for 10 min; 0.4 μl neutralization buffer was added to each well and incubated at room temperature for 5 min. Then, 0.8 μl of Illumina Nextera XT 384 Indexes (0.4 μl each, 5 μM from 4 sets of 96 indexes) and 1.2 μl of PCR master mix were added to amplify whole transcriptomes using the following PCR program: 72°C for 3 min; 95°C for 30 s; 10 cycles of 72°C for 30 s, 95°C for 30 s, 72°C for 30 s; and 72°C, for 5 min. The PCR product from one 384-well plate were pooled into an Eppendorf tube and purified twice using PCRClean DX beads. The quality and concentrations of the final libraries were measured with a Bioanalyzer assay and Qubit (qPCR and fluorometry), respectively. Libraries were sequenced on the Illumina HiSeq 4000 at the Weill Cornell Medicine Genomics and Epigenomics Core Facility.

Quality control for scRNA-seq data. The following criteria were used to filter out cells with low-quality sequencing. The distribution of total reads (in logarithmic scale) was fitted by a truncated Cauchy distribution and the data points in two tails of the estimated distribution were considered to be outliers and eliminated. Fitting and elimination were then applied to the remaining data. This process was run iteratively until the estimated distribution became stable. The threshold was set to the value at which the cumulative distribution function of the estimated distribution reached 0.05. Cells with small numbers of detected genes and poor correlation coefficients for ERCC spike-ins (low sequencing accuracy) were dropped; 676 cells were retained for downstream analysis after filtering from 721 cells.

Clustering analysis of scRNA-seq data. Seratav v.3.0.1 was used to perform unsupervised clustering analysis on the filtered scRNA-seq data<sup>1–19</sup>; Gene counts were normalized to the total expression and log-transformed. Principal component analysis was performed on the scaled data using highly variable genes as input. The JackStrawPlot function was used to determine the statistically significant principal components. These principal components were used to compute the distance matrix. The principal component index values and a t-distributed stochastic neighbor embedding (t-SNE) were used to visualize the clustering results. DEGs were found using the FindAllMarkers function, which ran the Wilcoxon rank-sum tests.

Statistical. All experiments were done with at least two biological replicates. The mean values from each mouse were used to compute the statistical difference. Data distribution was assumed to be normal but this was not formally tested. Individual data points are shown when possible. Statistical analyses were done with Prism 7.0e (GraphPad Software) and R v.3.5.1. Data were visualized with Prism or the R package ggplot2 v3.1.1.<sup>20</sup> Unpaired, two-tailed t-tests were used to compare two groups and two-sided Fisher’s exact tests were used to analyze the contingency tables. Differential expression analysis for bulk sequencing and single-cell sequencing was done using the Wald and Wilcoxon rank-sum tests, respectively, with Benjamini–Hochberg-corrected P values. P < 0.05 was considered statistically significant.

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

Data availability
The bulk RNA-seq and miRNA-seq data that support the findings of this study have been deposited with the Gene Expression Omnibus repository (https://www.ncbi.nlm.nih.gov/geo/). Bulk RNA-seq data have been deposited under accession number GSE122663 and scRNA-seq data have been deposited under accession number GSE135330.

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**Author contributions**

L.K. and L.G. conceived and designed the research. L.K., E.G., J.I.E., Y.L., Y.Z., F.A.S., Q.L., L.Zhan and D.L. performed the research. L.Zhan, F.A.S., Q.L., L.Zhou, Z.C., G.Y., J.C.U. and K.S.K. contributed new reagents and analytical tools. L.K., E.G., Q.L., Z.C., G.Y. and L.G. analyzed the data. L.K., C.D.C. and L.G. wrote the paper.

**Competing interests**

The authors declare no competing interests.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41593-019-0560-7. Supplementary information is available for this paper at https://doi.org/10.1038/s41593-019-0560-7.

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Extended Data Fig. 1 | Male and female PS19 mice have similar tau pathology. (a–c,e,f) Representative images of MC1 immunostaining of 9-month-old PS19 female (a) and male (b) hemibrains. Scale bar, 600 μm. Yellow dashed boxes magnified in (c). Scale bar, 300 μm. (e) Representative image of hippocampus. Scale bar, 600 μm. Yellow dashed boxes magnified in (f). Scale bar, 150 μm. 2 independent experimental cohorts were used. (d,g) MC1 density of entire hemibrain (d) and hippocampus (g) of nontransgenic (−) and transgenic (+) male and female mice. n = 11 nontransgenic females, 5 nontransgenic males, 10 PS19 females, and 9 PS19 males. Boxplot elements: center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range.
Extended Data Fig. 2 | Male and female microglia have differential transcriptional responses to tau pathology. (a,c) Venn diagram of differentially expressed (DE) miRNAs (a) and mRNAs (c) comparing microglia from PS19 vs nontransgenic control (Ctrl) mice. DE genes defined as those with log2FC ≥ 1 or ≤ -1 and FDR < 0.05. Purple numbers, up-regulated DE genes; green numbers, down-regulated DE genes. n = 4 Ctrl samples/sex, 5 PS19 samples/sex, 2 mice/sample (a). n = 5 male and 3 female Ctrl samples, 3 male and 4 female PS19 samples, 2 mice/sample (c). * P = 0.05 (a), * P = 0.0146 (c), two-sided Fisher’s exact test. Full list of DEGs in Supplementary Tables 5–7. (b) Volcano plot of male miRNA-seq data from (a). Purple and green dots represent miRNAs upregulated in PS19 samples (11 miRNAs; P ≤ 0.05 by Benjamini-Hochberg correction and log2FC ≥ 1) and downregulated in PS19 samples (43 miRNAs; P ≤ 0.05 and log2FC ≤ -1), respectively. Grey dots are miRNAs not significantly different. Dots with black circles represent those that were analyzed in (d). Vertical dashed lines indicate log2FC ± 1. Horizontal dashed line indicates -log10(0.05). Wald test was used. (d) Bar graph showing 9 Ingenuity Pathway Analysis predicted target coverage of DE mRNAs from male PS19 vs Ctrl microglia (c) by DE miRNAs from male PS19 vs Ctrl microglia (a). Results were filtered for those with opposing miRNA and mRNA log fold changes (i.e. focusing on miRNA and mRNA targets that have anti-correlated expression patterns) and were either experimentally observed or highly predicted to be miRNA-mRNA target interactions.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | RNA sequencing of Dicer KO microglia from PS19 mice. (a) Volcano plot of RNA-seq data from Dicer KO microglia from 3-month-old male and female PS19 mice. Pink, female-enriched; turquoise, male-enriched; grey, not significantly different. Vertical dashed lines indicate log2FC ± 1. Horizontal dashed line indicates -log10(0.05). n = 4 male samples, 2 female samples, 2 mice/sample. Wald test was used. (b) Schematic of the single-cell isolation method. Brains without the cerebellum were harvested from 9-month-old Dicer KO PS19 female and male mice and homogenized. After myelin depletion, cells were sorted using flow cytometry and gated by CD45⁺;CD11b⁺ expression. (c) Representative FACS plots showing gating strategy and the cells sequenced. Similar gating strategy was used for all samples sequenced (n = 2 biologically independent animals/sex). (d) Number of cells, proportion and statistics for FACS plots from (c). (e-g) Quality control criteria for the single-cell sequencing data. Fitted curves for histograms of mapped reads (e), numbers of detected genes (f) and ERCC correlation coefficient (g) are labeled in red. Dashed lines are statistical cutoffs. Cells that passed all three criteria were retained for analysis. (h) Scatter plot highlighting cells that passed QC (red) among all cells sequenced. Each dot is a cell. (i) Summary of the numbers of cells sequenced and cells that passed QC (red). (j) t-SNE plot of microglia clusters from 9-month-old Dicer KO PS19 female and male mice. n = 2 biologically independent animals/sex. (k) Heatmap of top genes defining each microglial cluster. (l) Ridge plots of microglial marker expression levels by each microglial cluster.
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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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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**  For IHC analysis, Keyence BZ-X Analyzer software v1.3.0.3 was used for stitching Keyence images. MC1 density data was collected through ImageJ 1.51. Tiff images were processed with the auto local threshold Phansalkar plugin, regions of interest were hand-traced, and MC1+ puncta were counted with the “Analyze Particles” function. Microglial morphology data was obtained using the Imaris software (v9.0.2, Bitplane). BD Influx at the Stanford Shared FACS facility was used for flow cytometry data collection.

**Data analysis**  For miRNA-seq, miRNA were mapped to mature miRNAs from mirBase with Bowtie2 v2.3.4 (Langmead, Salzberg, 2012) and batch effects were removed with the R package RUVSeq v1.16.1 (Risso et al. 2014). RNA-seq reads were mapped with the Bluebee Genomics Platform and using the GENCODE mouse genome GRCm38 (LexogenQuantSeq 2.2.3). For single-cell RNA-seq, Prinseq v0.20.4 (Schmieder & Edwards, 2011) was first used to filter sequencing reads. Reads were trimmed using Trim Galore v0.4.3 (https://github.com/FelixKrueger/TrimGalore) and mapped using the mm10 genome by calling STAR v2.5.3a (Dobin et al., 2013).

Differential gene expression for miRNA and RNA-seq were calculated with the R package DESeq2 v1.22.2 (Love et al. 2014). Single-cell RNA-seq was analyzed using the R package Seurat v3.0.1 (Butler et al. 2018, Stuart, Butler, et al. 2019).

Statistical analyses were done with Prism 7.0e (Graphpad, San Diego, CA) and R v3.5.1 (F Foundation for Statistical Computing, Vienna, Austria).

Networks were visualized with Cytoscape v3.6.1 and the STRING database. miRNA-target prediction was done using Ingenuity Pathway Analysis v1.14 (Qiagen).

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The bulk RNA-seq and miRNA-seq data that support the findings of this study were deposited in the Gene Expression Omnibus repository (https://www.ncbi.nlm.nih.gov/geo/). Bulk RNA seq was deposited under accession number GSE122663, and single-cell RNA-seq data was deposited under accession number GSE135330.

Field-specific reporting

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Life sciences study design

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

| Sample size | For animal studies, sample size was determined by using power analysis with type 1 error = 5%, power = 80%, and effect size and standard deviation based on previous studies using the P301S (PS19) transgenic mouse line. |
|-------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | 1 male and 1 female sample were excluded from the miRNA-seq analysis based on principle component analysis as these two samples did not cluster together with the other samples.  
For bulk-sequencing (RNA-seq and miRNA-seq), genes with <15 counts across all samples were excluded from analysis.  
For single-cell sequencing, the following criteria were used to filter out cells with low-quality sequencing. The distribution of total reads (in logarithmic scale) was fitted by a truncated Cauchy distribution, and data points in two tails of the estimated distribution were considered to be outliers and eliminated. Fitting and elimination were then applied to the remaining data. This process was run iteratively until the estimated distribution became stable. The threshold was set to the value at which the cumulative distribution function of the estimated distribution reaches 0.05. Cells with small numbers of detected genes and poor correlation coefficients for ERCC (low sequencing accuracy) were dropped. 676 cells were retained for downstream analysis after filtering from 721 cells. |
| Replication | All experiments were done with at least two biological replicates with all data points shown. MC1 and Iba1 IHC experiments were done with two independent experimental cohorts. All replicates showed similar trends. |
| Randomization | Randomization of groups is not relevant for this study as all animals in the study were treated similarly. |
| Blinding | Investigators were blinded during data acquisition (Immunohistochemistry, imaging, RNA collection, library preparation, sequencing) and data analysis (Image quantification and analysis). |

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 | Methods |
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| ☒ Antibodies                    | ☒ ChIP-seq |
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| ☒ Animals and other organisms   |         |
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| ☒ Clinical data                 |         |
Antibodies

Antibodies used

Antibodies for IHC: Anti-Iba1 (Abcam, ab5076, 1:500 and Wako, 019-19741, 1:500), anti-Dicer (Abcam, ab167444, N167/7, 1:100), anti-Lpl (Abcam, ab21356, LPL.A4, 1:50) and anti-MC1 (a kind gift from P. Davies, 1:500). Secondary antibodies used were the following at 1:500 dilution: Cy3 AffiniPure Donkey Anti-Goat (Jackson ImmunoResearch, 705-165-147), Alexa Fluor 488 Donkey Anti-Mouse (Jackson ImmunoResearch, 715-545-150), Alexa Fluor 488 Donkey Anti-Mouse (Thermo Fisher Scientific, R37114).

Antibodies used for FACS: rabbit anti-mouse Tmem119 (Abcam ab210405, ~200 μg/μl, 106-6, 1:400 dilution), CD45-PE-Cy7 (Thermo Fisher Scientific 25-0451-82, 30-F11, 1:300), CD11b-BV421 (BioLegend 101236, M1/70, 1:300), goat anti-rabbit Alexa 488 (Thermal Fisher Scientific 11034, 1:300).

Validation

1) MC1 antibody development and citation: Jicha, G. A., Bowser, R., Kazam, I. G. & Davies, P. Alz-50 and MC-1, a new monoclonal antibody raised to paired helical filaments, recognize conformational epitopes on recombinant tau. Journal of Neuroscience Research 48, 128-132 (1997).

2) Anti-Iba1 antibody has been referenced in 302 publications, according to Abcam’s website. The website, however, notes that "Although we have some very good Abreviews on mouse, some customers were receiving inconsistent results on mouse samples. We have therefore moved mouse to the predicted species and can no longer guarantee it." We did not notice any problems with the antibody for our studies.

3) Anti-Dicer antibody has been tested for application for IF/ICC, according to Abcam’s website.

4) Anti-LPL antibody has been tested for application for IF/ICC, IHC-P and has been referenced in 29 publications, according to Abcam’s website.

All FACS antibodies were validated by company and also used in the same experimental context by a previously published study: Li, Q. et al. Developmental Heterogeneity of Microglia and Brain Myeloid Cells Revealed by Deep Single-Cell RNA Sequencing. Neuron 101, 207-223.e210, doi:10.1016/j.neuron.2018.12.006 (2019).

Animals and other organisms

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

Laboratory animals

For miRNA-seq, female and male 6 month old P301S transgenic mice (https://www.jax.org/strain/008169) and their background control mice (https://www.jax.org/strain/100010) were used.

For RNA-seq, sc-RNA-seq and histological analysis, Cx3cr1CreERT2/+ (https://www.jax.org/strain/020940) were crossed with Dicerfl/fl (https://www.jax.org/strain/006366) and P301S transgenic mice (https://www.jax.org/strain/008169) mice to obtain the following mice as littermates:

1) Cx3cr1+/+;Dicerfl/fl
2) Cx3cr1+/+;Dicerfl/fl;P301S+
3) Cx3cr1CreERT2/+;Dicerfl/fl
4) Cx3cr1CreERT2/+;Dicerfl/fl;P301S+

Both male and female mice were used at 9 months of age.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight

All mouse experiments were approved by the Institutional Animal Care and Use Committee, University of California, San Francisco and complied with all relevant ethical regulations.

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).
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Methodology

Sample preparation

Detailed single cell isolation preparation method in methods section.

Instrument

Cell sorting/flow cytometry analysis was done on the cell sorter (BD InFlux) at the Stanford FACS Facility.

Software

BD Influx
For single-cell RNA-seq sorting, 721 microglia were isolated and sequenced.

Gating strategy

The following gates were used for sorting microglia: (1) forward scatter-area (FSC-A)/side scatter-area (SSC-A) (2) Trigger Pulse Width/FSC (3) Live-Dead negative using PI (4) CD45lowCD11b+ and CD45hiCD11b+.

The boundaries between positive and negative staining cell populations were set using a no-stain control for CD45 and CD11b, as well as a propidium iodide-only sample to sort for live/dead cells.

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