Supplementary Materials and Methods

Mice

CRISPR/Cas9-mediated knock-in of the common variant (CV) or R47H human *TREM2* cDNA in place of *mTrem2* was done by injecting embryos with Cas9, short-guide RNA (sgRNA), and donor vectors (generated by PNA Bio). The human *TREM2* cDNA sequence was flanked on each side by 1-kb homology arms for the *mTrem2*. The sequences are as follows: *Trem2* targeted region 5′CCTGCTGCTGATCACAGGTGGGA and sgRNA sequence (antisense) 5′TCCCACCTGTGATCAGCAGCAGG. Potential off-target genes were identified with CRISPR off-target prediction software (http://www.crispor.tefor.net). There were no predicted off-targets for 1- or 2-basepair mismatches. CV hTREM2 and R47H hTREM2 lines were maintained independently and backcrossed to nontransgenic C57BL/6 mice for two to three generations, then crossed to *Cx3cr1*$^{GFP/GFP}$ or P301S mice. *Cx3cr1*$^{GFP/GFP}$ (https://www.jax.org/strain/005582) were crossed with CV or R47H hTREM2 knock-in lines to obtain *Cx3cr1*$^{GFP/+}$*hTREM*$^{R47H/+}$, *Cx3cr1*$^{GFP/+}$*hTREM2*$^{CV/+}$, and *Cx3cr1*$^{GFP/+}$*mTrem2*$^{+/+}$ littermates for both lines. P301S transgenic mice (https://www.jax.org/strain/008169) were crossed with CV or R47H hTREM2 knock-in mice to generate P301S *hTREM2*$^{R47H/+}$ and littermate P301S *mTrem2*$^{+/+}$ mice, as well as P301S *hTREM2*$^{CV/+}$ and littermate P301S *mTrem2*$^{+/+}$ mice. Mice of both sexes were used, and analyses based on sex are included in the main and supplementary figures. Mice underwent behavioral testing at 7 to 9 months of age and had not been used for any other experiments. At 8 to 9 months of age, the same mice were used for pathology and RNA-seq studies after completion of behavioral tests. *Cx3cr1*$^{GFP/+}$ mice for in vivo imaging were studied at 12 to 17 months of age. For MK-2206 in vivo treatment, *mTrem*$^{R47H//+}$ and P301S *mTrem*$^{R47H//+}$ female mice at 7-8 months were used. All

mouse protocols were approved by the Institutional Animal Care and Use Committee, University of California, San Francisco and Weill Cornell Medicine.

Human Postmortem Samples

Tissues from the mid-frontal cortices from brains of patients with AD carrying the R47H mutation (n=24, 13 females and 11 males) or the common variant (CV) (n = 22; 11 females and 11 males) were used for snRNA-sequencing, for a total of 46 samples. Samples were matched in age, TAU and amyloid pathology burden, and Clinical Dementia Ratings. Samples were obtained from the University of Pennsylvania brain bank and the Mayo Clinic brain bank and derived from several different studies: State of Florida Alzheimer's Disease Initiative (ADI), Alzheimer's cases derived from the Mayo Clinic (ADC), and cases obtained from outside sources, usually because of atypical clinical syndromes (such as corticobasal syndrome, frontal lobe dementia or progressive aphasia), but AD as the underlying pathology (Consult). Mayo Clinic brain bank operates under procedures approved by the Mayo Clinic institutional review board (IRB# is 15-009452). Brain tissues of University of Pennsylvania brain bank used in this study are not considered identified "human subjects" and not subjected to IRB oversight. All brains were donated after consent from the next-of-kin or an individual with legal authority to grant such permission. The institutional review board has determined that clinicopathologic studies on de-identified postmortem tissue samples are exempt from human subject research according to Exemption 45 CFR 46.104(d)(2).
Additional information about the donors can be found in table S1.

Isolation of Nuclei from Frozen Postmortem Human Brain Tissue

The protocol for isolating nuclei from frozen postmortem human brain tissue was adapted from a previous study with modifications (*27, 28*). All procedures were done on ice or at 4°C. In brief, postmortem brain tissue was placed in 1500 µl of Sigma nuclei PURE lysis buffer (Sigma,

NUC201-1KT) and homogenized with a Dounce tissue grinder (Sigma, D8938-1SET) with 20 strokes with pestle A and 15 strokes with pestle B. The homogenized tissue was filtered through a 35-μm cell strainer, and were centrifuged at 600 *g* for 5 min at 4°C and washed three times with 1 ml of PBS containing 1% BSA, 20 mM DTT and 0.2 U μl^{-1} recombinant RNase inhibitor. Then the nuclei were centrifuged at 600 *g* for 5 min at 4°C and re-suspended in 800 μl of PBS containing 0.04% BSA and 1x DAPI, followed by FACS sorting to remove cell debris. The FACS-sorted suspension of DAPI-stained nuclei were counted and diluted to a concentration of 1000 nuclei per microliter in PBS containing 0.04% BSA.

Droplet-based snRNA-seq of Human Brain Tissue

For droplet-based snRNA-seq, libraries were prepared with Chromium Single Cell 3' Reagent Kits v3 (10x Genomics, PN-1000075) according to the manufacturer's protocol. The snRNA-seq libraries were sequenced on the NovaSeq 6000 sequencer (Illumina) with 100 cycles.

Analysis of Droplet-Based snRNA-seq Data from Human Brain Tissue

Gene counts were obtained by aligning reads to the hg38 genome with Cell Ranger software (v.3.1.0) (10x Genomics). To account for unspliced nuclear transcripts, reads mapping to pre-mRNA were counted. Cell Ranger 3.1.0 default parameters were used to call cell barcodes. We further removed genes expressed in no more than 3 cells, cells with unique gene counts over 9,000 or less than 300, cells with unique molecular identifiers (UMI) count over than 50,000, and cells with high fraction of mitochondrial reads (> 5%). Potential doublet cells were predicted using DoubletFinder (*29*) for each sample separately with high confidence doublets removed. Normalization and clustering were done with the Seurat package v3.0.1 (*61*). In brief, counts for all nuclei were scaled by the total library size multiplied by a scale factor (10,000), and transformed to log space. A set of 2000 highly variable genes were identified with FindVariableFeatures

function in Seurat package with the default "vst" method. This returned a corrected UMI count matrix, a log-transformed data matrix, and Pearson residuals from the regularized negative binomial regression model. Principal component analysis (PCA) was done on all genes, and *t*-SNE was run on the top 15 PCs. Cell clusters were identified with the Seurat functions FindNeighbors (using the top 15 PCs) and FindClusters (resolution = 0.1). In this analysis, the neighborhood size parameter pK was estimated using the mean-variance normalized bimodality coefficient (BCmvn) approach, with 15 PCs used and pN set as 0.25 by default. For each cluster, we assigned a cell-type label using statistical enrichment for sets of marker genes (*30, 31*) and manual evaluation of gene expression for small sets of known marker genes. Differential gene expression analysis was done using the FindMarkers function and MAST (*62*). To identify gene ontology and pathways enriched in the differentially expressed genes (DEGs), we tested enrichment of gene annotation collections from the Molecular Signatures Database (MSigDB) gene annotation database v6.1 (*63, 64*). For convenience, the MSigDB gene set collections have been assembled into an R package called "msigdb" which is publicly available from https://github.com/mw201608/msigdb. Enrichment analysis P values were calculated using the hypergeometric test (equivalent to the Fisher's exact test, FET) in R language. To control for multiple testing, we employed the Benjamini-Hochberg (BH) approach (*65*) to constrain the false discovery rate (FDR).

Microglia Subclustering and downstream analysis. The subset() function from Seurat was used to subset microglia-only cells and integration of each individual sample was performed by first finding the most variable features and using three non-R47H samples as references for integration anchor identification. The integrated object was then scaled and dimensionality reduction was performed. Cell clusters were identified with the Seurat functions FindNeighbors (using the top 30

PCs) and FindClusters (resolution = 0.4). Subcluster markers were identified using the FindAllMarkers function. Differential subcluster abundance between R47H vs CV samples was done using a negative binomial generalized linear model (NB GLM) using the glmQLFTest function in EdgeR, a method adopted from mass cytometry data analysis (*66, 67*). The model was adjusted for the following covariates: brain bank location, sex, age, and APOE genotype. Geneset Enrichment Analysis (GSEA) was performed using the GSEA software (*63*). Genes were pre-ranked based on the sign of the log-fold-change value multiplied by the inverse of the FDR. Reference gene datasets were pulled from various studies of microglial gene signatures (*14, 21, 24, 32-34*).

Quantitative Reverse-Transcription PCR

Snap-frozen cortices were thawed and homogenized with a 21G needle in RLT buffer with 1% β-mercaptoethanol. RNA was isolated with the RNeasy Mini-Kit (Qiagen), and the remaining DNA was removed by incubation with RNase-free DNase. Purified mRNA was then converted to cDNA with the iScript cDNA Synthesis Kit (Bio Rad). Quantitative RT-PCR was performed on the ABI 7900 HT sequence detector (Applied Biosystems) with PowerUp SYBR Green master mix (ThermoFisher Scientific). The average value of three replicates for each sample was expressed as a threshold cycle (C_t), the point at which the fluorescence signal starts to increase rapidly. Then, the difference (ΔC_t) between C_t values for the transcript of interest and for mouse *Gapdh* was calculated for each sample. The gene expression for each sample was calculated using the $2^{-\Delta\Delta Ct}$ method and reported as fold change relative to the average of the $hTREM2^{CV/+}$ sample. The following primers were used:

Primer: Human TREM2 Fwd: CCGGCTGCTCATCTTACTCT

Primer: Human TREM2 Rev: GGAGTCATAGGGGCAAGACA

Primer: Mouse GAPDH Fwd: TGGCCTTCCGTGTTCCTAC

Primer: Mouse GAPDH Rev: GAGTTGCTGTTGAAGTCGCA

Western Blot

Mouse brains were homogenized in RIPA buffer containing 50 mM Tris, pH 7.5, 150 mM NaCl, 0.5% Nonidet P-40, 1 mM EDTA (ThermoFisher Scientific), 1 mM phenylmethyl sulfonyl fluoride, protease inhibitor cocktail (Millipore Sigma) and phosphatase inhibitor cocktail (Millipore Sigma). After sonication, brain lysates were centrifuged at 18,000 *g* at 4°C for 30 min. Supernatants were collected and protein concentrations were measured with the Pierce BCA Protein Assay Kit (ThermoFisher Scientific). The same amount of protein was loaded onto a 4–12% SDS-PAGE gel (Invitrogen), transferred to nitrocellulose membranes (GE Healthcare), blocked with 5% milk, and immunoblotted in 2% milk. Bands in immunoblots were visualized by enhanced chemiluminescence (ThermoFisher Scientific) and quantified by densitometry with ImageJ (NIH). The antibodies used for western blot were anti-TREM2 (1:500, Cell Signaling, #91068S), anti-synaptophysin (1:1000, Abcam, #ab23754), anti-pAKT Ser473 (1:1000, Cell Signaling, #4060S), a-tubulin (GeneTex, dilution at 1:20000). Immunoreactivity was detected with goat anti-rabbit HRP (1:2000, Millipore Sigma).

Isolation of Adult Microglia

Adult microglia were isolated from 3- to 4-month-old *mTrem2*$^{+/+}$, *hTREM2*$^{R47H/+}$, and *hTREM2*$^{R47H/R47H}$ mice as described (*16*). Briefly, after perfusion, brains were chopped with a razor blade, incubated with 3% collagenase type 3 (Worthington), 3 U/ml dispase (Worthington) and DNase (Millipore Sigma) at 37°C, inactivated with 2.5 mM EDTA (ThermoFisher Scientific) and 1% fetal bovine serum (FBS) (Invitrogen), filtered through a 70-µm filter, centrifuged at 300 *g* for 5 min at 18°C and resuspended in fluorescence-activated cell sorting (FACS) buffer. Samples were

incubated with myelin-removal beads (Miltenyi Biotec) for 15 min at 4°C, passed through a magnetic LD column (Miltenyi Biotec), centrifuged at 300 *g* for 10 min, and resuspended in FACS buffer. Cells were magnetically separated and sorted with CD11b beads (Miltenyi Biotec) and a magnetic MS column (Miltenyi Biotec). CD11b-positive cells were centrifuged at 300 *g* for 10 min, and RNA was extracted for RNA-seq.

Behavioral Tests

In all behavioral tests, *hTREM2*$^{CV/+}$ and *hTREM2*$^{R47H/+}$ mice were compared to their respective nontransgenic or P301S littermates. Experimenters were blinded to mouse genotypes throughout the experiments. Male and female mice were tested on separated days.

Morris Water Maze Test. The water maze consists of a pool (122 cm in diameter) containing opaque water (20 ± 1°C) and a platform (10 cm in diameter) 1.5 cm below the surface. Three different images were posted on the walls of the room as spatial cues. Hidden platform training (days 1–7) consisted of 14 sessions (two per day, 2 hrs apart), each with two trials. The mouse was placed into the pool at alternating quadrants for each trial. A trial ended when the mouse located the platform or after 60 sec had elapsed. At 24 and 72 hrs after training, the mice were tested in probe trials, in which the hidden platform was removed and mice were allowed to swim for 60 sec. Mice received 7 days of hidden platform training before the 24-hr and 72-hr probe trials. Visible platform testing was done 24 hrs after the last probe trial. Performance was measured with an EthoVision video tracking system (Noldus Information Technology).

Elevated Plus Maze Test. The maze consists of two 15 x 2-inch open arms without walls and two closed arms with walls 6.5 inches tall and is 30.5 inches above the ground. Mice were moved to the testing room 1 hr before testing to acclimate to the dim lighting. Mice were individually placed

in the maze at the intersection of the open and closed arms and allowed to explore the maze for 10 min.

Open Field Test. Mice were individually placed into brightly lit automated activity chambers equipped with rows of infrared photocells connected to a computer (San Diego Instruments). Open field activity was recorded for 5 min. Recorded beam breaks were used to calculate total time of activity.

Immunohistochemistry and Image Analysis

Mice were transcardially perfused with phosphate-buffered saline (PBS). The brains were cut vertically into hemibrains. Half of each brain was flash frozen at –80°C for RNA-seq analyses; the other half was placed first in 4% paraformaldehyde for 48 hr and then in a 30% sucrose solution for 48 hr at 4°C and cut into 30-µm-thick sections with a freezing microtome (Leica). For immunostaining, free-floating sections (8–10 per mouse) were washed in PBS, placed in sodium citrate buffer for 30 min at 90°C for antigen retrieval, permeabilized with PBS containing 0.5% Triton X-100 for 10 min, and blocked in PBS containing 10% normal goat serum for 1 hr. Sections were then placed in PBS with 5% normal goat serum and primary antibodies overnight at 4°C. The next day, sections were washed in PBS containing 0.1% Triton X-100, incubated with Alexa-conjugated secondary antibodies in PBS with 5% normal goat serum and washed in PBS containing 0.1% Triton X-100. Images were acquired with a Keyence BZ-X700 microscope and a 10x objective. Immunoreactivity was quantified with ImageJ software (NIH). Antibodies used for staining were anti-MC1 (1:500, kind gift from Dr. Peter Davies) and anti-Iba1 (1:500, Wako). Secondary antibodies used for staining were donkey anti-mouse 488 and donkey anti-rabbit 546 (both 1:500, ThermoFisher Scientific). For synaptophysin immunoreactivity quantification, free-floating sections (4 per mouse) were permeabilized in 0.5% Triton X/TBST (30min). Sections then

underwent antigen retrieval (95°C 13min total). Following blocking with 10% BSA/TBST, sections were immediately incubated with primary antibody anti-synaptophysin (1:100, Cell Signaling, #5461) in 0.5% BSA/TBST overnight at 4°C. Sections were then incubated with donkey anti-rabbit IgG secondary antibody conjugated with Alexa Fluor 568 (1:500, ThermoFisher Scientific, #A10042) for 1 hour at room temperature. Sections were washed with TBST between each step and mounted with Vectashield with DAPI (Vector Laboratories, #H-1200). Maximum projection 40x 2x2 Z-Stack images were acquired using a confocal microscope (Zeiss LSM880). All images were processed in FIJI, undergoing a standardized background subtraction and thresholding. An ROI of the CA3 stratum lucidum and stratum radiatum and mean integrated density/area was collected in FIJI. To correct for background signal, average signal in negative control sections was subtracted by genotype for all sections. Data were analyzed in RStudio using linear mixed models fit with fixed effects of genotype and treatment and a random effect to account for variation amongst sections from the same animal. Outliers were calculated using the 1.5xInter-Quartile Range rule: any datapoints more than 1.5xIQR below Q1 or above Q3 were considered outliers and removed. This resulted in the exclusion of all 4 datapoints from 1 mouse in the vehicle-treated P301S $hTREM2^{R47H/+}$ group.

RNA-Seq and Analysis of Adult Microglia

RNA from adult microglia was extracted from $mTrem2^{+/+}$ (n = 7), $hTREM2^{R47H/+}$ (n = 6), and $hTREM2^{R47H/R47H}$ (n = 7) mice with the Qiagen RNeasy Mini Kit. RNA concentration was determined with a NanoDrop, and RNA quality was measured with a Bioanalyzer and Agilent RNA Pico Chip. Samples with an RNA integrity number >7 were considered of good quality and used for subsequent steps. Libraries were then prepared with the QuantSeq 3′ mRNA-Seq Library Prep Kit FWD for Illumina. Library quality was assessed with a Bioanalyzer and the Agilent High

Sensitive DNA Chip. Individual library concentrations were measured with the Qubit dsDNA HS Assay Kit and submitted for SE50 sequencing on an Illumina HiSeq 4000. Quality control was done on base qualities and nucleotide composition of sequences. Alignment to the GRCm38.84 *Mus musculus* (mm10) refSeq (refFlat) reference gene annotation was done with the STAR spliced read aligner and default parameters. Counts were normalized with the median of ratios method.

RNA-Seq and Analysis of Bulk Hippocampal Tissue

Hippocampal RNA was isolated with the Qiagen RNeasy Mini Kit. After quality control analysis with a Bioanalyzer, the RNA was sent to Novogene for library preparation and PE150 sequencing with an Illumina HiSeq 4000 instrument. 48 samples were sent in for sequencing: 6 samples each from male and female P301S CV and R47H hTREM2 knock-in mice, and 3 samples each for male and female P301S $mTrem2^{+/+}$ littermates of the $hTREM2^{CV/+}$ line and male and female P301S $mTrem2^{+/+}$ littermates of the $hTREM2^{R47H/+}$ line.

Quality Control. We used multiple clustering methods to examine the quality of replicates and to identify possible outlier samples for exclusion if necessary. For the hTREM2 R47H line, three samples were excluded from further analysis based on clustering. Clustering techniques were applied to variance stabilizing transformed expression values, fragments per kilobase of transcript per million mapped reads, and values of log counts per million. The Pearson correlation coefficient was first used as a distance metric between samples. Hierarchical cluster analysis was then applied to measure similarity between the Pearson correlations. The hierarchical clustering algorithm was an iterative process. Each iteration joined the two most similar clusters (based on Pearson correlation) and computed the distance between remaining clusters, continuing until there is just a single cluster. The distances between clusters were computed at each stage using the complete linkage clustering method (see manual for R hclust function).

Differential Expression Analysis. Differential gene expression was calculated with the R package DESeq2 (*68*). Counts were normalized with the median of ratios. Genes with <15 counts across all samples were excluded from analysis. The false discovery rate (FDR) was calculated with the Benjamini-Hochberg method (*65*).

Weighted Gene Co-expression Network Analysis. Weighted gene co-expression network analysis (WGCNA) was done on normalized expression data with the R package WGCNA v1.51 (*69*). The top 5000 most variable genes were used to create modules, and the soft-thresholding power parameter was set to 14. The minimum module size was 30 genes, and modules with a module eigengene dissimilarity below 0.2 were merged, creating 11 modules of 43–1361 genes each after removal of the module (gray) that contain genes that do not belong to any other module. KEGG and GO biological processes were used for pathway analysis as described below.

Pathway Enrichment Analysis. Gene network analyses of RNA-seq data were done with gene set enrichment analysis (GSEA) (*63*); cell types were defined by the top 100 genes expressed by each CNS cell type (*70*). Pathway analysis was done with Gene Ontology (GO) biological processes (*71, 72*) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) (*73*).

Network Visualization. Networks were visualized with Cytoscape (version 3.6.1) (*74*), the STRING database (*75*), and perfuse force directed layout.

Mouse Hippocampus scRNA-seq

Preparation of samples. Brain tissue was prepared using a previously published protocol (*39*). Briefly, 8-month-old female mice were anesthetized with avertin and transcardially perfused with phosphate-buffered saline (PBS). The brain without the cerebellum was harvested and collected into cold media with 15mM HEPES, 0.5% glucose in 1X Hanks' Balanced Salt Solution without

phenol red (ThermoFisher Scientific 13185-052) on ice. The entire procedure was done on ice. The hippocampus was extracted and chopped into pieces using a razor blade then further homogenized using a 2ml douncer containing 2ml medium A with 80uL DNase (12500 units/mL) and 5ul recombinant RNase inhibitor (Takara Bio 2313B). Homogenized tissue was filtered through a 70um strainers to obtain a single cell suspension. Cells were washed with medium A and resuspended in 850uL MACS buffer with 1.8uL RNase inhibitor (sterile-filtered 0.5% BSA, 2mM EDTA in 1 X PBS). Cells were incubated with 100uL myelin removal beads (MACS Miltenyl Biotec) for 10 min, and loaded onto LD columns (Miltenyi Biotec). Cells were collected and washed for FACS staining.

Single Cell Sorting for scRNA-seq. Cells were blocked in 5uL mouse Fc block for 5 min on ice then incubated with primary antibodies for 10 min then washed with FACS buffer (sterile-filtered 1% FCS, 2mM EDTA, 25mM HEPES in 1XPBS). Cells were incubated with secondary antibodies for 10min then washed with FACS buffer. Cells were resuspended in 500uL FACS buffer with RNase inhibitor (Takara Bio 2313B, 1:500) and 0.5ul Propidium Iodide (ThermoFisher Scientific P3566, 1:1000) for single cell index sorting. Cell sorting/flow cytometry analysis was done on the cell sorter (BD InFlux) at the Stanford FACS Facility. 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) $CD45^{int}CD11b^{+}$ and $CD45^{hi}CD11b^{+}$. Single cells were sorted into 96-well plates containing 4uL lysis buffer containing 4U Recombinant RNase Inhibitor (Takara Bio 2313B), 0.05% Triton X-100, 2.5mM dNTP mix (ThermoFisher Scientific R0192), 2.5uM Oligo-dT30VN (50-AAGCAGTGGTATCAACGCAGAGTACT30VN-30), and ERCC Spike-ins (ThermoFisher Scientific 4456740) diluted at $1:2.4x10^{7}$. Plates were vortexed, spun down and frozen on dry ice, and plates were stored at −80°C freezer. Antibodies used for FACS:

rabbit anti-mouse Tmem119 (Abcam ab210405, ~200ug/ul, 1:400 dilution), CD45-PE-Cy7 (ThermoFisher Scientific 25-0451-82, 1:300), CD11b-BV421 (BioLegend 101236, 1:300), goat anti-rabbit Alexa 488 (ThermoFisher Scientific 11034, 1:300).

scRNA-seq library preparation. Sequencing libraries were prepared following the Smart-seq2 published protocol(*76*). Briefly, plates were thawed and incubated at 72°C for 3 min in order to anneal RNAs to the Oligo-dT30VN primer. The 6uL of the reverse transcription mixture was added to each well: 95U SMARTScribe Reverse Transcriptase (100U/µl, Clontech 639538), 10U RNase inhibitor (40U/µl), 1XFirst-Strand buffer, 5mM DTT (Promega P11871), 1M Betaine (Sigma-Aldrich B0300-5VL), 6mM MgCl2, 1µM TSO (Exiqon, Rnase free HPLC purified). RT was performed at 42°C for 90 min, followed by 70°C, 5 min. 15ul of PCR amplification mix containing the following reagents was added to each well: 1X KAPA HIFI Hotstart Master Mix (Kapa Biosciences KK2602), 0.1uM ISPCR Oligo (AAGCAGTGGTAT CAACGCAGAGT), 0.56U Lambda Exonuclease (5U/ul, New England BioLabs M0262S). cDNA was amplified using the following PCR program: (1) 37°C 30 min; (2) 95°C 3 min; (3) 23 cycles of 98°C 20 s, 67°C 15 s, 72°C 4 min; (4) 72°C 5 min. cDNA samples were purified using PCRClean DX beads (0.7:1 ratio, Aline C-1003-50), and resuspended in 20ul EB buffer. cDNA quality was examined with a Fragment Analyzer (AATI, High Sensitivity NGS Fragment Analysis Kit:1 bp - 6000 bp, Advanced Analytical DNF-474-1000). To make libraries, all samples were diluted down to 0.15ng/ul in 384-well plates using Mantis Liquid Handler (Formulatrix) and Mosquito X1 (TTP Labtech) with customized scripts. Nextera XT DNA Sample Prep Kit (Illumina FC-131-1096) was used at 1/10 of recommendation volume, with the help of a Mosquito HTS robot for liquid transfer. Tagmentation was done in 1.6ul (1.2ul Tagment enzyme mix, 0.4ul diluted cDNA) at 55°C, 10 min. 0.4ul Neutralization buffer was added to each well and incubated at room temperature for 5

min. 0.8ul Illumina Nextera XT 384 Indexes (0.4ul each, 5uM from 4 sets of 96 indexes) and 1.2ul PCR master mix were added to amplify whole transcriptomes using the following PCR program: (1) 72°C 3 min; (2) 95°C 30 s; (3) 10 cycles of 95°C 10 s, 55°C 30 s, 72°C 1 min; (4) 72°C 5 min. Libraries from one 384 plate were pooled into an Eppendorf tube and purified twice using PCRClean DX beads. Quality and concentrations of the final libraries were measured with Bioanalyzer and Qubit, respectively. Libraries were sequences on the Illumina HiSeq 4000 at the Weill Cornell Medicine Genomics and Epigenomics Core Facility.

Processing of scRNA-seq Raw Data. Prinseq (*77*) v0.20.4 was first used to filter sequencing reads shorter than 30 bp (-min_len 30), to trim the first 10 bp at the 50 end (-trim_left 10) of the reads, to trim reads with low quality from the 30 end (-trim_qual_right 25) and to remove low complexity reads (-lc_method entropy, -lc_threshold 65). Then, Trim Galore v0.4.3 (https://github.com/FelixKrueger/TrimGalore) was applied to trim the Nextera adapters (–stringency 1). The remaining reads were aligned to the mm10 genome by calling STAR (*78*) v2.5.3a with the following options: –outFilterType BySJout,–outFilterMultimapNmax 20,–alignSJoverhangMin 8,–alignSJDBoverhangMin 1,– outFilterMismatchNmax 999,–outFilterMismatchNoverLmax 0.04,–alignIntronMin 20,–alignIntronMax 1000000,–alignMatesGapMax 1000000,–outSAMstrandField intronMotif. Picard was then used to remove the duplicate reads (VALIDATION_ STRINGENCY = LENIENT, REMOVE_DUPLICATES = true). Finally, the aligned reads were converted to counts for each gene by using HTSeq (-m intersection-nonempty, -s no) (*79*).

Quality Control for scRNA-seq Data. We used the following criteria to filter out cells with low sequencing quality. 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 as

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 where 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. 1424 cells were retained for downstream analysis after filtering from 1480 cells.

Clustering Analysis of scRNA-seq Data. The Seurat R package v3.0.1 was used to perform unsupervised clustering analysis on the filtered scRNA-seq data (*61, 80*). 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 metric and generate cell clusters. Non-linear dimensional reduction (t-SNE) was used to visualize clustering results. Differentially expressed genes were found using the FindAllMarkers function that ran Wilcoxon rank sum tests.

Ingenuity Pathway Analysis. DEGs and their log2-fold change expression values were inputted into IPA (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity- pathway-analysis). For Trem2-signaling pathway predictions, previously published molecules involved in Trem2-signaling were manually curated and highlighted through network construction.

RNAscope

Mouse brains were harvested and snap-frozen on dry ice followed by OCT embedment. Frozen brains were cut into 20 μm sections using a cryostat and mounted onto superfrost plus slides. The RNAscope assay was carried out with RNAscope Fluorescent Multiplex Assay (ACD, 320513).

Briefly, after dehydration with ethanol, sections were permeabilized with Protease IV. Then samples were hybridized with RNA probes C1qa (ACD, 441221-C2) for activated microglia and Apoe (ACD, 313271-C3) at 40℃ for 2 h. Signal was amplified according to the instructions. After counterstain with DAPI, sections were mounted by Prolong Gold (Thermo Fisher, P36930). Line-scanning confocal images of hippocampal dentate gyrus region were obtained with a confocal microscope (LSM880, Carl Zeiss Microscopy, Thornwood, NY) and a 40x objective (1-μm focal plane intervals, one field of view per brain section) within a day post-mounting of sections. Images were examined by maximum intensity Z-projection and analysis was done using ImageJ v2.0.0 (NIH). All analysis and imaging were done blinded to the genotype.

Isolating Primary Microglia: in vitro TAU Fibril Stimulation Assay and MK-2206 Treatment

Cortices was harvested from postnatal day 3 pups. The meninges were removed, and the cortical tissue was finely chopped with a razor blade and digested in 0.25% trypsin with DNAse (Millipore Sigma) at 37°C for 25 min. Digestion was stopped with DMEM containing 10% FBS. The tissue was then triturated and spun at 200 *g* for 5 min, and the pellet was resuspended in DMEM and 10% FBS and plated in T75 flasks that had been pre-coated with 5 μg/ml poly-D-lysine (Millipore Sigma) and rinsed with water. Mixed cultures were maintained in DMEM/10% FBS supplemented with 5ng/ml GM-CSF in flasks for 10–11 days. The flasks were then shaken for 2 hr, spun at 200 *g* for 15 min, and the cells were plated at a density of 400,000/well in a 24-well plate in DMEM and 10% FBS. After 24 hours, cells were treated with either 1 μm MK-2206 (MedChem Express) suspended in DMSO or no drug with DMSO as a control for 6 hours of incubation. Then, cells were treated with 1 ug/ml of 0N4R TAU fibril (from Dr. Jason Gestwicki) per well for 18 hours. The conditioned media were collected and debris was removed by spinning at 4°C for 10 minutes

at 3,000 *g*. The concentrations of 32 cytokine/chemokine in the conditioned media were measured by a Multiplex mouse cytokine/chemokine 32-plex kit (Millipore #MCYTMAG-70K-PX32) with the Luminex MAGPIX CCD Imager. Cells were lysed for RNA isolation.

RNA-Seq and Analysis of Primary Microglia

RNA was isolated using the *Quick*-RNA MiniPrep kit (Zymo Research). For experiments with MK-2206 drug treatment, RNA was sent to Novagene for library preparation and sequencing. For experiments without drug treatment, RNA concentration was determined with a NanoDrop, and RNA quality was measured with a Bioanalyzer and Agilent RNA Pico Chip. Samples with an RNA integrity number >7 were considered of good quality and used for subsequent steps. Libraries were then prepared with the QuantSeq 3´ mRNA-Seq Library Prep Kit FWD for Illumina. Library quality was assessed with a Bioanalyzer and the Agilent High Sensitive DNA Chip. Individual library concentrations were measured with the Qubit dsDNA HS Assay Kit and submitted for SE50 sequencing on an Illumina HiSeq 4000. Quality control was done on base qualities and nucleotide composition of sequences. Alignment to the GRCm38.84 *Mus musculus* (mm10) refSeq (refFlat) reference gene annotation was done with the STAR spliced read aligner and default parameters. Differential gene expression analysis was done with the R package DESeq2. Counts were normalized with the median of ratios method. Genes with <15 counts across all samples were excluded from analysis. The FDR was calculated with the Benjamini-Hochberg method. Pathway analysis was done using Gene Set Enrichment Analysis referencing Gene Ontology Biological Processes dataset. Predicted upstream activators of the transcriptome were determined using Ingenuity Pathway Analysis software (Qiagen).

MK-2206 in vivo treatment

mTrem$^{R47H//+}$ and P301S *mTrem*$^{R47H//+}$ female mice at 7-8 months were treated with 100mg/kg MK-2206 (Medkoo Biosciences, 1:10 DMSO:saline) via oral gavage, three times a week for a total of 4 weeks (western blot experiments) or 9 weeks (single nuclei-RNAseq and immunohistochemistry experiments). The body weights of mice were recorded at each feeding. At post 24 hours of last feeding, the mice were transcardially perfused with cold PBS. One hemi-brain was drop fixed with 4% paraformaldehyde for immunohistochemistry analysis. The other hemi-brain was snap-frozen at -80C for biochemical analyses, including single nuclei-RNAseq and western blot.

In Vivo Imaging

For intravital imaging with two-photon microscopy, thinned-skull windows were made in 12–17-month-old *Cx3cr1*$^{GFP/+}$ *hTREM*$^{R47H/+}$, *Cx3cr1*$^{GFP/+}$ *hTREM2*$^{CV/+}$, and *Cx3cr1*$^{GFP/+}$ *mTrem2*$^{+/+}$ as described previously (*16*). Briefly, mice were anesthetized, the skull was exposed, and a small area over the cortex was thinned manually and with a high-speed drill (K.1070 High Speed Rotary Micromotor drill; Foredom). Mice were fixed onto a custom-made head plate and imaged with an Ultima IV multiphoton microscope (Bruker) equipped with MaiTai DeepSee-eHP lasers (Spectra Physics) tuned to 920 nm for imaging and InSight X3 lasers (Spectra Physics) tuned to 880 nm for ablation. Z-stacks of images were acquired every 3 min in 1-μm steps with a 25x water-immersion objective at 2.4 optical zoom. Extensions and retractions of processes during baseline recordings were manually traced with the mTrackJ plugin. The movement of microglial cells toward a laser ablation site was analyzed by normalizing the number of processes near the injury site at each time point to the overall microglial density at that time point.

Primary Microglia Phagocytosis Experiment

Experimental and Imaging Set-up: Primary microglia were prepared as described above. Microglia were seeded at 30,000 cells / well in a 96-well plate. After 24 hours, media was replaced with FluoroBrite DMEM media (ThermoFisher) with 10% FBS and 0.015 mg pHrodo *E. coli* (ThermoFisher; P35366) was added to each well. 4 fields of view were taken for each well every 30 minutes using Incucyte (Essen BioScience). Background fluorescence was subtracted and population-level fluorescence information was determined through the Incucyte analysis software using Top-Hat and threshold based on negative control well with no pHrodo *E. coli*.

Single-cell image tracking. Model Training: The single-cell analysis pipeline was adapted from previous work, using the Usiigaci framework (*81*). A custom FIJI/ImageJ macro was written to manually annotate phase contrast images, such that a unique RGB value corresponded to an individual cell mask (*82*). A preprocessing step converted the 8-bit RGB image into a 16-bit grayscale mask image (8-bit would yield max of 255 unique cells per image), such that each unique RGB cell mask was a unique grayscale value. A Mask R-CNN model implemented in Tensorflow and Keras (open-source Matterport Inc. – MIT License), was trained to segment cells in phase contrast microscopy images (*83-86*). The model had a learning rate of 1x10-3 for training and used a Resnet-101 backbone (*81, 85*). The network heads were trained first for 50 epochs and then the full network was trained for another 100 epochs. All model training was performed using a GPU on Google Colab. The imgaug package was used for data augmentation during training, incorporating image rotations and flips, along with brightness augmentations and gaussian blur augmentations stochastically. Transfer learning was performed based on final weights from Usiigaci model. The average of two different models (epochs 130 and 134) were used for instance segmentation during this study. These models were chosen based on lowest validation loss during

training. The model was trained on 20 images with ~50 cells per image to assess accuracy of the model on data that were not used during model training.

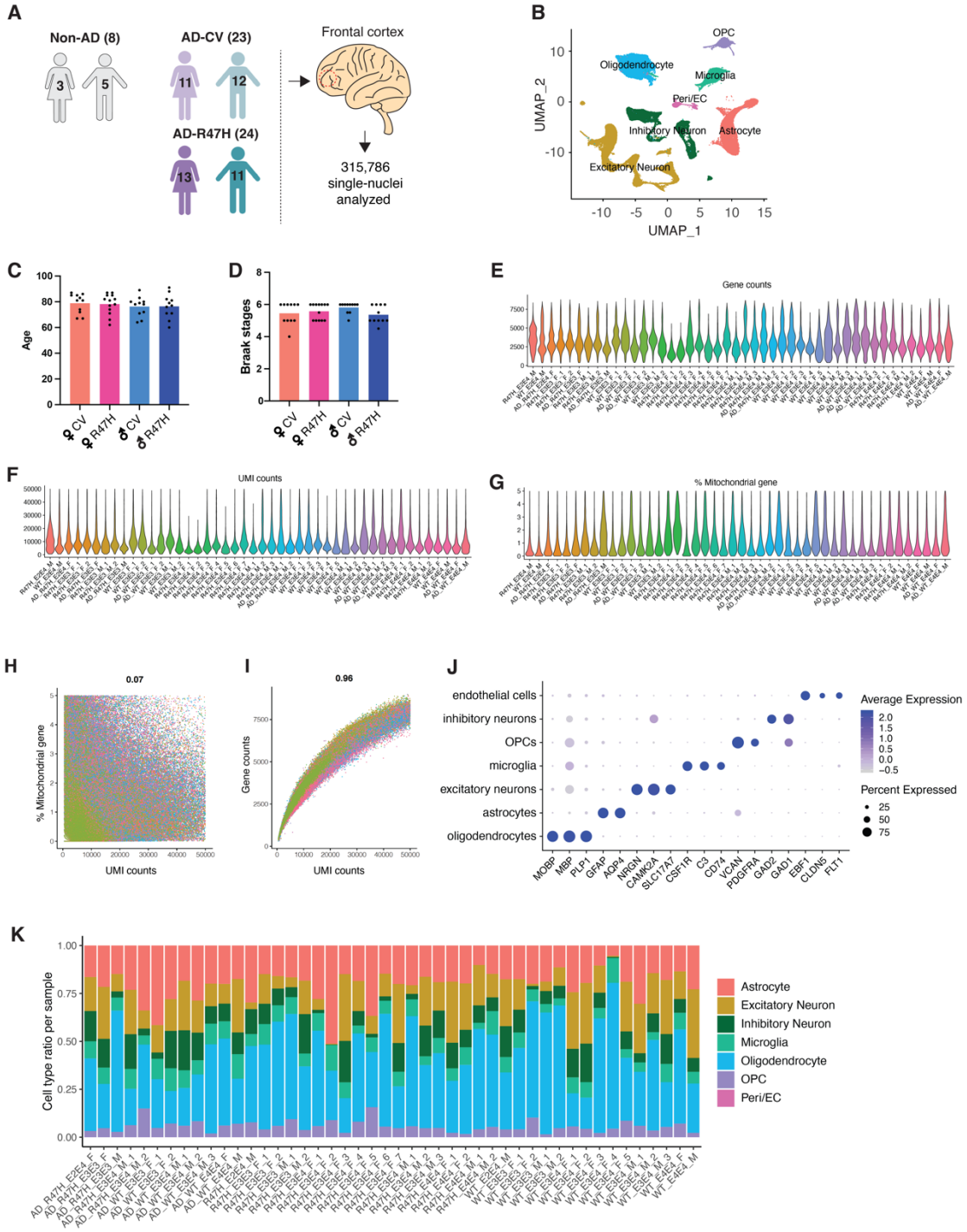
Analysis of Phase Contrast Microscopy Images: Phase contrast and fluorescent image were exported as TIFF images from the Incucyte system. Top-hat background subtraction was performed on the fluorescence image to remove autofluorescence. Time-lapse images were registered using a manual drift correction FIJI plug-in (*87*). The output from the Mask R-CNN model was fed into a K-D tree-based cell tracking pipeline based on trackpy, from the Usiigaci pipeline. Once cells from each frame were annotated, a python script was used to measure fluorescence of each cell over the entire time-lapse. A threshold was manually set for each individual experiment. Average intensity measurements above the threshold were measured for each individual cell by taking the total intensity within a cell above the threshold divided by the area of the cell above the set threshold. Cells with more than 30 missing time points and cells with more than 5 missing time points within the first 10 frames were excluded from the analysis and a linear regression was used to impute missing data from the time-lapse. Cells that contained enough datapoints to be included in the cut-off but missing datapoints at the beginning and end of the time-lapse were filled with zeros.

snRNA-seq of Mice Treated with MK-2206

Isolation of nuclei from frozen mouse hippocampi and Droplet-based snRNA-seq library preparation were performed using the same method as for the human brain tissues. Gene counts were obtained by aligning reads to the mm10 genome with Cell Ranger software (v.3.1.0) (10x Genomics). To account for unspliced nuclear transcripts, reads mapping to pre-mRNA were counted. The R package SoupX (*88*) was used to identify and remove ambient RNA from the dataset. Potential doublet cells were predicted using DoubletFinder (*29*) for each sample separately

with high confidence doublets removed. Normalization and clustering were done with the Seurat package v3.0.1 (*61*). We assigned a cell-type identity to each cell cluster according to the expression of known cell-type markers. Microglia cluster was identified by the expression of *Cx3cr1*, *P2ry12* and *Csf1r*. The subset() function from Seurat was used to subset microglia-only cells and the standard workflow for visualization and clustering was performed with default assay as "integrated" with 12 PCs used and resolution = 0.1. Subcluster markers were identified using the FindAllMarkers function with GLM-framework MAST used (*62*). Geneset Enrichment Analysis (GSEA) was performed using the GSEA software (*63*).

Fig. S1. Quality Control Assessment of snRNA-Seq of Brain Tissues from Patients with AD (refer to Fig. 1)



(A) Schematic showing the sex and genotypes of age-matched human donors used for snRNA-seq. n = 315,786 nuclei were isolated from the mid-frontal cortex of patients without AD (n=8) and with AD carrying the CV-TREM2 variant (n=23) or the R47H-TREM2 variant (n=24).

(B) UMAP plots of all single nuclei and their annotated cell types. Peri/EC = pericyte/endothelial cells, OPC = Oligodendrocyte precursor cells. We focused on samples from patients with AD in this study (8 non-AD samples were excluded).

(C-D) Bar plots of age (C), and Braak stage (D) of the patient tissues.

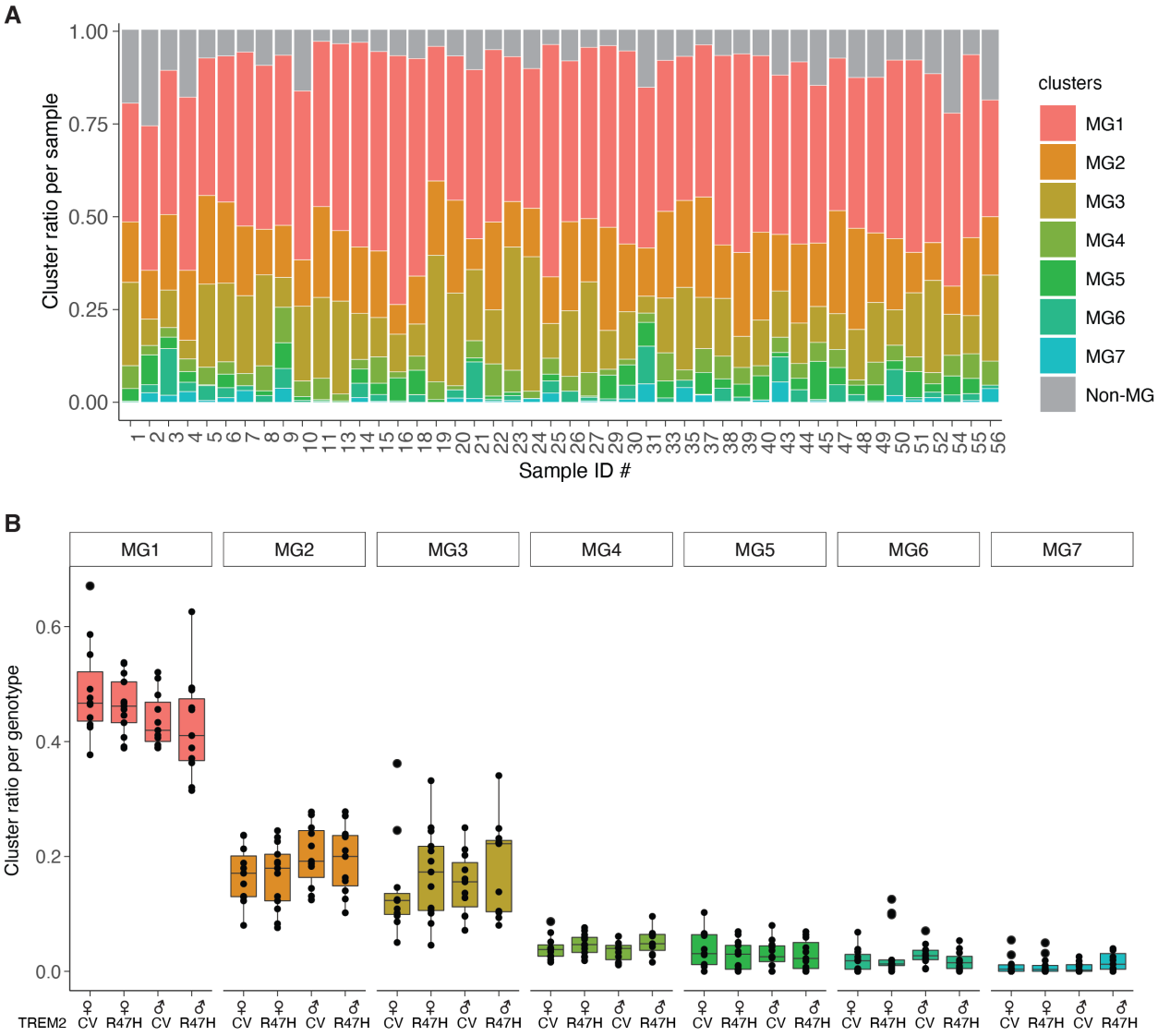
(E-G) Violin plots showing spread of total genes (E), total UMIs (F), and percent of mitochondrial genes detected (G) per nuclei for each individual sample.

(**H and I**) Correlation between UMI counts and percentage of mitochondrial genes per nuclei (H) and total genes detected (I) for all samples.

(J) Dot plot showing expression of cell type marker genes for each cell type.

(K) Proportion of different cell types captured per sample.

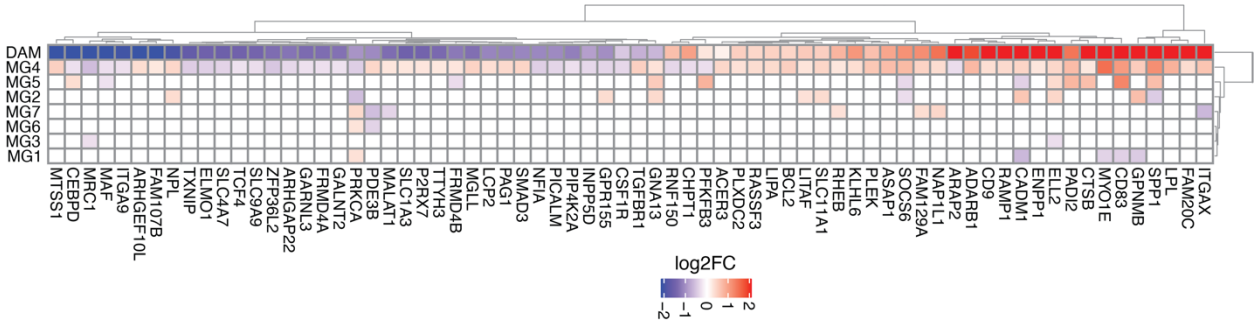
Fig. S2. Differential Microglial States Identified in Brain Tissues from Patients with AD (refer to Fig. 2)



(A) Microglia subcluster ratio per individual sample.

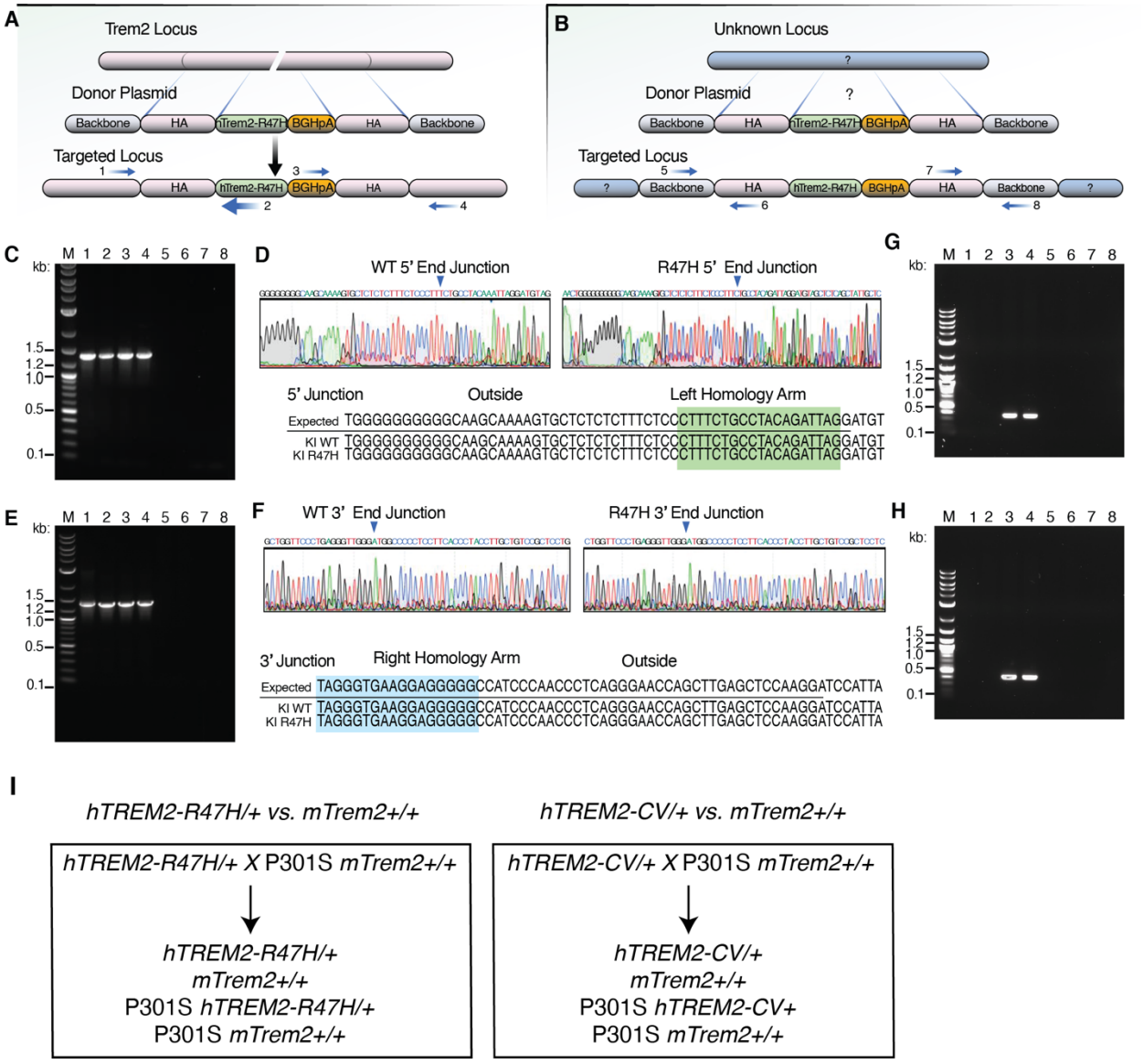
(B) Boxplot of cluster ratio per genotype.

Fig. S3. R47H-associated Microglia Signature Overlaps with Disease-associated Microglia Signatures (refer to Fig. 2)



Heatmap showing Log2-fold change values for all microglial subclusters for genes overlapping between MG4 markers and the DAM signature (*34*).

Fig. S4. Characterization of CV-*hTREM2* and R47H-*hTREM2* Knock-in Mouse Lines (refer to Fig. 3)



(A) Diagram of the strategy to detect the specific targeting of the R47H-*hTREM2* (or CV-*hTREM2*) into the mouse *Trem2* genomic locus and primer locations for detecting integration. PCR primers labeled as arrows and are located as follows: Primer 1 is located outside of the left homology arm, Primer 2 is on 5' end of *hTREM2* cDNA, Primer 3 is on BGHpA, Primer 4 is located outside of the right homology arm.

(**B)** Diagram of the strategy to detect non-specific integration of the R47H-*hTREM2* (or CV-*hTREM2*) cDNA in the mouse genome. PCR primers labeled as arrows and are located as follows: Primer 5 is on the vector, Primer 6 is on the left arm, Primer 7 is on the right arm, Primer 8 is on the vector.

(C) PCR screening for the 5' end specific homologous recombination of CV-*hTREM2* and R47H-*hTREM2* mice using Primers 1 and 2. Lane M = 1kb plus DNA ladder, Lanes 1-2 = R47H-*hTREM2*, Lanes 3-4 = CV-*hTREM2*, Lanes 5-6 = C57BL/6 control, Lanes 7-8 = H_2O control. The size of PCR product is about 1345 bp.

(D and F) Sanger sequencing chromatograms for the junctions. Sequences of the intended and obtained knock-in junctions in the mouse lines show correct homologous recombination. 5' end of left homology arm is highlighted in green (D) and the 3'end of right homology arm is highlighted in blue (F).

(E) PCR screening for the 3' end specific homologous recombination of CV-*hTREM2* and R47H-*hTREM2* mice using Primers 3 and 4. Lane M = 1kb plus DNA ladder, Lanes 1-2 = R47H-*hTREM2*, Lanes 3-4 = CV-*hTREM2*, Lanes 5-6 = C57BL/6 control, Lanes 7-8 = H_2O control. The size of PCR product is about 1339 bp.

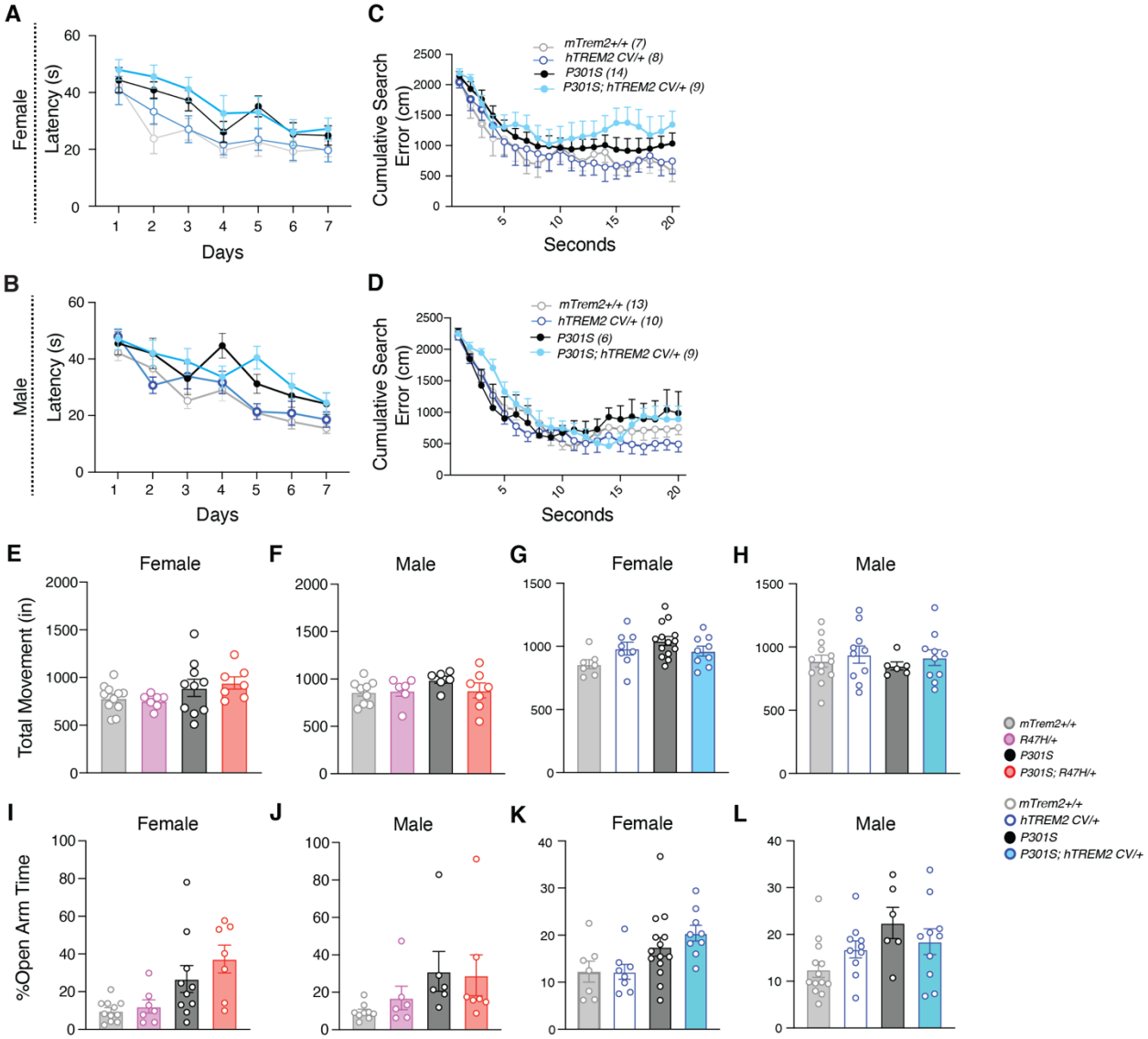
(G) PCR detection of non-specific integration on 5' end of the CV-*hTREM2* and R47H-*hTREM2* cDNA using Primers 5 and 6. Lane M = 1kb plus DNA ladder, Lanes 1-2 = R47H-*hTREM2*, Lanes 3-4 = CV-*hTREM2*, Lanes 5-6 = C57BL/6 control, Lanes 7-8 = H_2O control. The PCR product size is 347 bp.

(H) PCR detection of non-specific integration on 3' end of the CV-*hTREM2* and R47H-*hTREM2* cDNA using Primers 7 and 8. Lane M = 1kb plus DNA ladder, Lanes 1-2 = R47H-*hTREM2*, Lanes

3-4 = CV-*hTREM2*, Lanes 5-6 = C57BL/6 control, Lanes 7-8 = H_2O control. The PCR product size is 398 bp.

(I) Mouse cross diagram detailing the generation of the two separate mouse lines and their littermate controls.

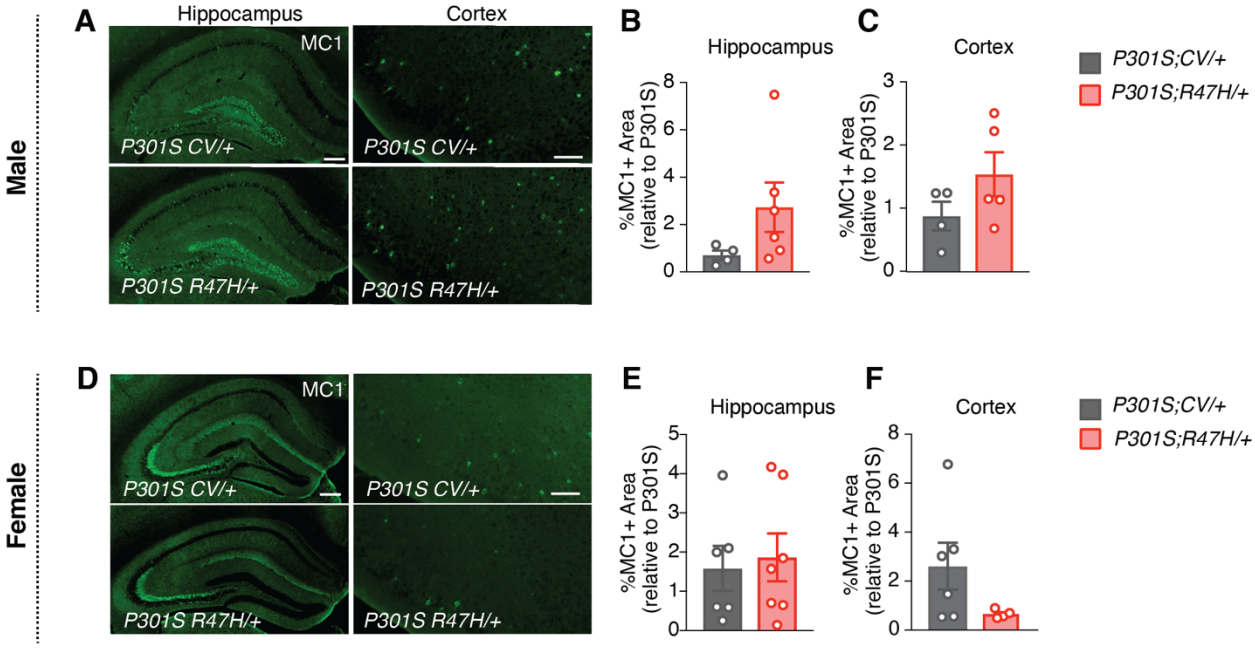
Fig. S5. WT-*hTREM2* Tauopathy Mice Behave Similar to *mTrem2*$^{+/+}$ Tauopathy Mice (refer to Fig. 3)



(A-D) Latency to reach the platform during hidden trials (d1-d7) and cumulative search errors during the 72-hr probe trial for female (A and C) and male (B and D) *mTrem2*$^{+/+}$, *hTREM2*$^{CV/+}$, P301S, and P301S *hTREM2*$^{CV/+}$ mice. STATA mixed-effects modeling for (A and B). Two-tailed Mann-Whitney U-test of area under the curve for (C and D).

(E-H) Open-field assessment of overall activity of 7–9-month-old *hTREM2*$^{R47H/+}$ and P301S *hTREM2*$^{R47H/+}$ female (E) and male (F) and their respective littermate *mTrem2*$^{+/+}$ controls, and *hTREM2*$^{CV/+}$ and P301S *hTREM2*$^{CV/+}$ female (G) and male (H) and their respective littermate *mTrem2*$^{+/+}$ controls. Two-tailed Student's t-test for n>8, two-tailed Mann-Whitney U-test for n<8. **(I-L)** Elevated plus maze assessment of anxiety levels in 7–9-month-old *hTREM2*$^{R47H/+}$ and P301S *hTREM2*$^{R47H/+}$ female (I) and male (J) and their respective littermate *mTrem2*$^{+/+}$ controls, and *hTREM2*$^{CV/+}$ and P301S *hTREM2*$^{CV/+}$ female (K) and male (L) and their respective littermate *mTrem2*$^{+/+}$ controls. Two-tailed Student's t-test for n>8, two-tailed Mann-Whitney U-test for n<8. Values are mean ± SEM.

Fig. S6. R47H-*hTREM2* Does not Alter TAU Pathology Load (refer to Fig. 3)



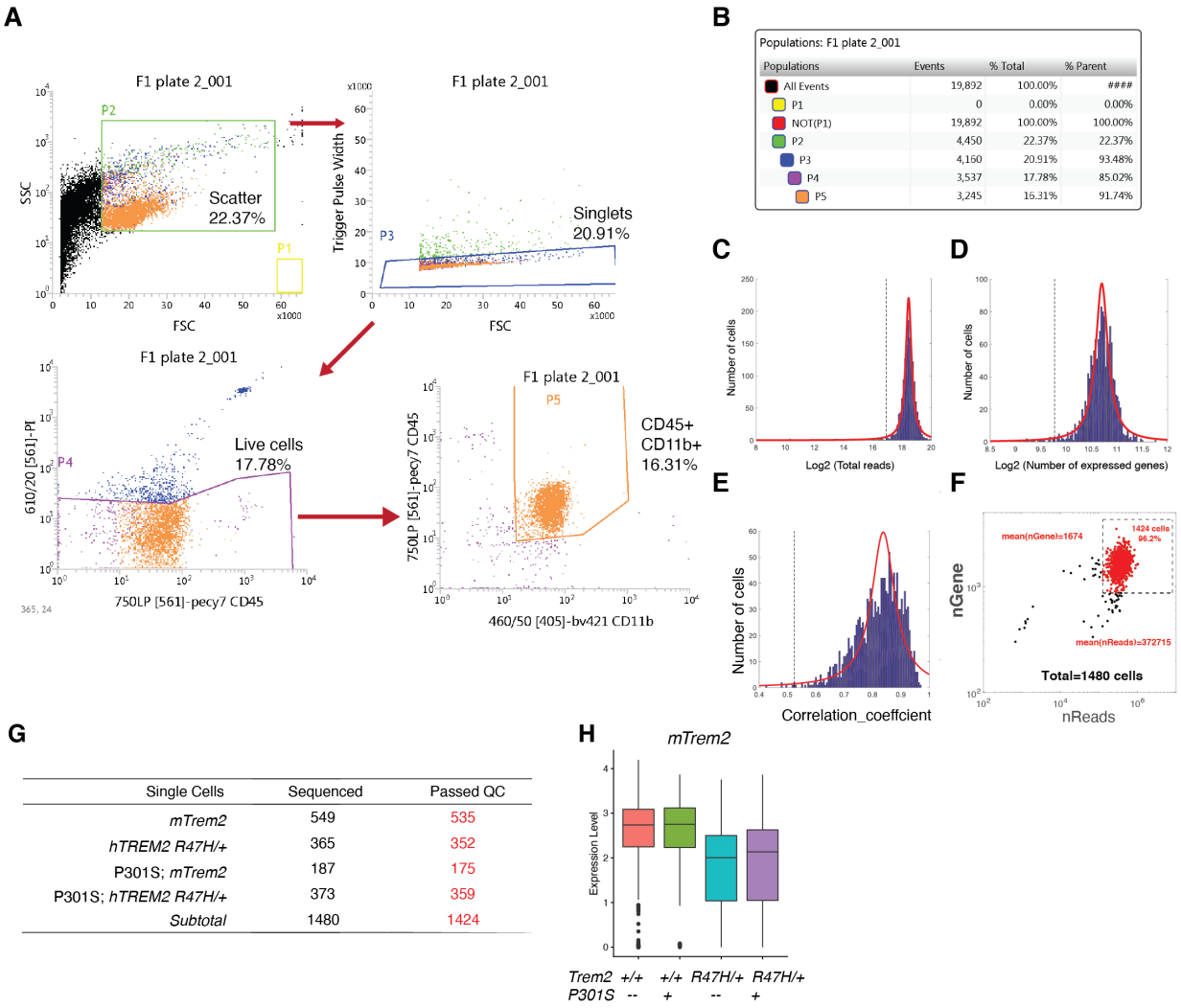
IHC analysis of 8–9-month-old mice. Each circle in bar graphs represents the average of 8–10 sections per mouse. Values are normalized to line-specific, sex-matched P301S *mTrem2*$^{+/+}$ littermate controls.

(A and D) Representative images of MC1 immunostaining in the hippocampus and entorhinal cortex of male (A) and female (D) mice.

(B, C, E and F) Percentage of MC1+ area in male hippocampus (B) and cortex (C) and female hippocampus (E) and cortex (F) of P301S *hTREM2*$^{CV/+}$ and P301S *hTREM2*$^{R47H/+}$ mice. Entire hippocampus and entire cortex were quantified. Two-tailed Mann-Whitney U-test.

Values are mean ± SEM.

Fig. S7. scRNA-Seq of Brain CD45^{+};CD11b^{+} Cells (refer to Fig. 4)



Single Cells	Sequenced	Passed QC
mTrem2	549	535
hTREM2 R47H/+	365	352
P301S; *mTrem2*	187	175
P301S; *hTREM2 R47H/+*	373	359
Subtotal	1480	1424

(A) Representative FACS plots showing gating strategies and cells sequenced.

(B) Number of cells and proportions based on FACS from (A).

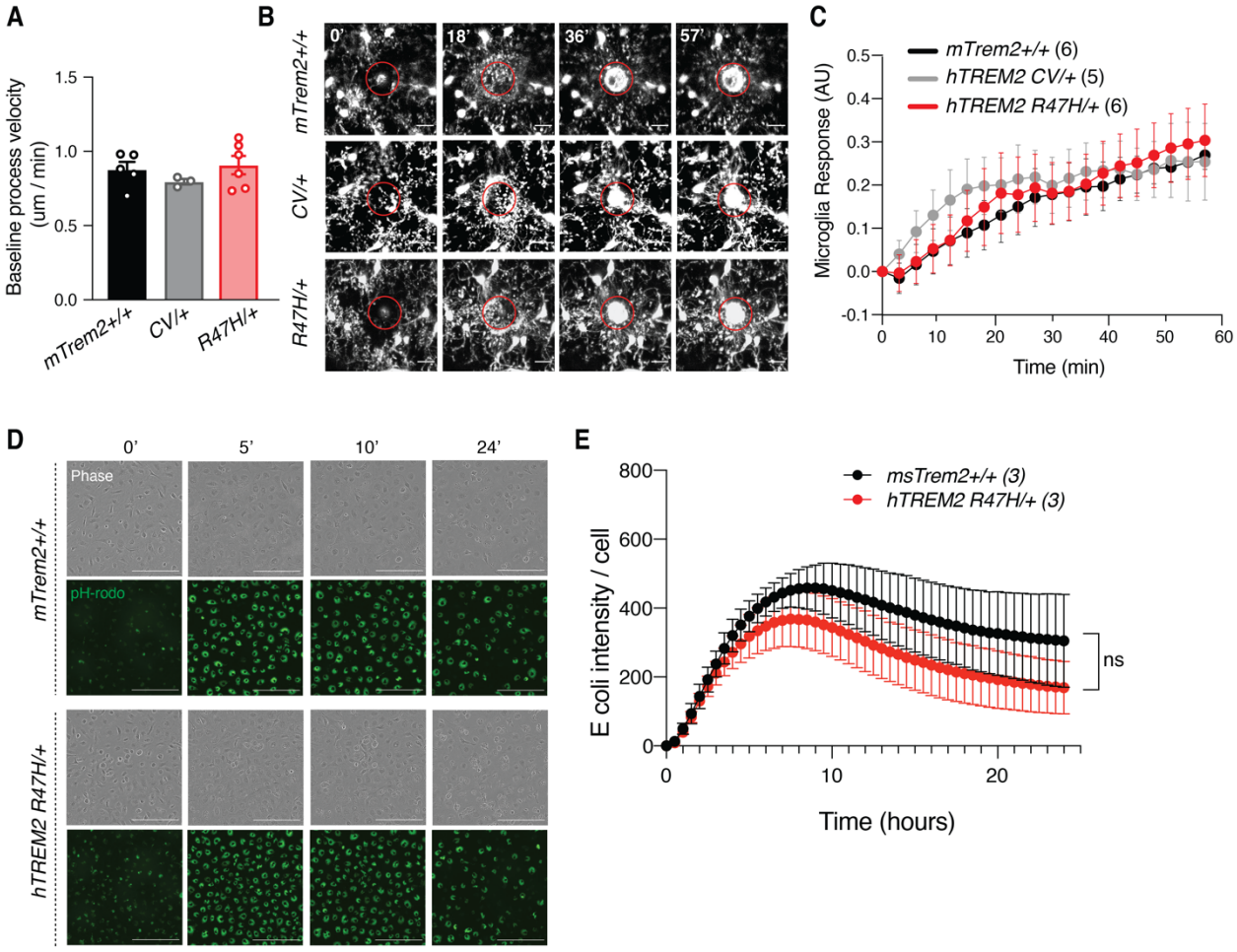
(C-E) Quality control criteria for the single-cell sequencing data. Fitted curves for histograms of mapped reads (C), number of detected genes (D) and ERCC correlation coefficient (E) are labeled in red. Dashed lines are statistical cutoffs. Cells that passed all three criteria were retained for analysis.

(F) Scatter plot highlighting cells that passed QC (red) among all cells sequenced. Each dot is a cell.

(G) Summary table for the numbers of cells sequenced and cells that passed QC (red). 1424 cells were retained for analysis.

(H) Normalized *mTrem2* expression level. Boxplot elements: center line, median; box limits, upper and lower quartiles; whiskers, 1.5x interquartile range; dots, outliers.

Fig. S8. R47H-*hTREM2* Does not Affect Microglial Injury Response or Phagocytosis (refer to Fig. 4)



(A) Quantification of microglial process velocity in 12-17-month-old mice during 10 min of baseline recordings (n = 5 *mTrem2*$^{+/+}$, n = 3 *hTREM2*$^{CV/+}$, n = 6 *hTREM2*$^{R47H/+}$ mice, ~25 processes/mouse). One-way ANOVA with Tukey's multiple comparisons correction.

(B) Representative images at 0, 18, 36, and 57 minutes after laser-induced injury. Red circle indicates region of injury.

(C) Quantification of the normalized microglial response to focal laser-induced tissue injury for 60 min after injury. (n = 6 *mTrem2*$^{+/+}$, n = 5 *hTREM2*$^{CV/+}$, n = 6 *hTREM2*$^{R47H/+}$ mice). STATA

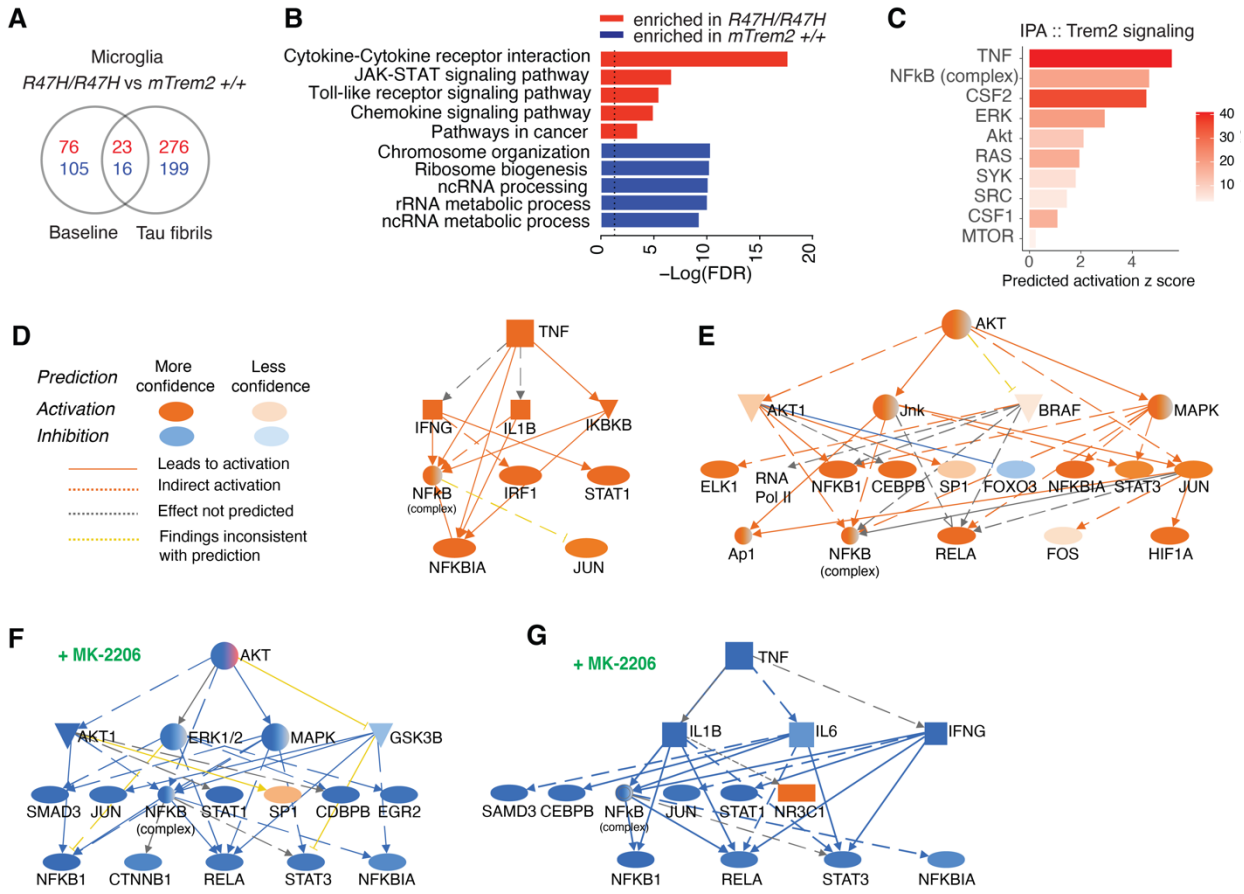
mixed-effects modeling. Scale bars: 15 μm. Values are mean ± SEM, one mouse was imaged per imaging session.

(D) Representative images of primary microglia at 0, 5, 10, and 24 hours after incubation with pH-rodo conjugated *E. coli* substrates. Scale bar: 200 μm.

(E) Bulk fluorescence intensity quantification of field of view normalized by cell number. (n = 3 $mTrem2^{+/+}$, n = 3 $hTREM2^{R47H/+}$ biologically independent experiments). Student's two-tailed t-test. Values are mean ± SD.

See also movie S1.

Fig. S9. MK-2206 Reverses R47H-induced Increase in AKT and TNF Signaling (refer to Fig. 5)



(A) Venn diagram of differentially expressed genes between *mTrem2*$^{+/+}$ and *hTREM2*$^{R47H/R47H}$ primary microglia with or without TAU fibril stimulation. Red and blue numbers denote upregulated and downregulated genes, respectively. (n = 3 biological replicates for all conditions).

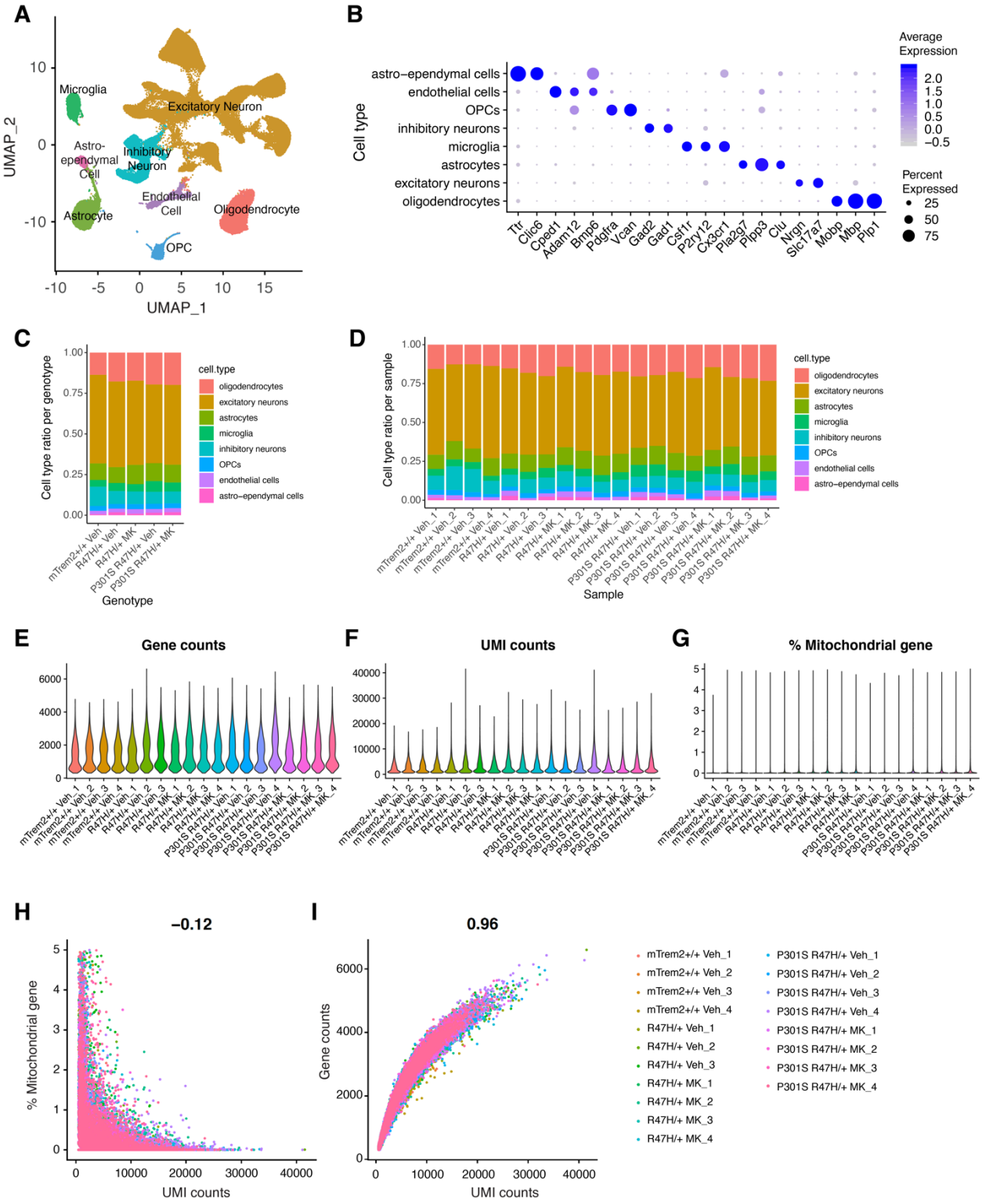
(B) KEGG pathway enrichment analysis of the genes from (A) that were uniquely changed in *hTREM2*$^{R47H/R47H}$ microglia under TAU fibril stimulation conditions. Dashed line indicates the threshold of significant enrichment for the pathway analysis (-Log10(FDR) ≥ 1.3).

(C) Heatmap comparing the IPA predicted activation z score of TREM2 signaling molecules.

(D and E) IPA TNF (D) and AKT (E) activated networks from (Fig. 5D) and their downstream predicted targets.

(F and G) IPA AKT (F) and TNF (G) network and predicted inhibition states after MK-2206 (AKT-inhibitor) vs. vehicle treatment of *hTREM2*$^{R47H/+}$ microglia with TAU fibril stimulation.

Fig. S10. Quality Control Assessment of snRNA-Seq of Mouse MK-2206 Cohort (refer to Fig. 6)



(A) UMAP of plots of 198,741 single nuclei and their annotated cell types. OPC = Oligodendrocyte precursor cells.

(B) Dot plots showing expression of cell type marker genes for each cell type.

(C) Proportion of different cell types in each of genotype and condition.

(D) Proportion of different cell types per individual sample.

(E-G) Violin plots showing spread of total genes (E), total UMIs (F), and percent of mitochondrial genes detected (G) per nuclei for each individual sample.

(**H and I**) Correlation between UMI counts and percentage of mitochondrial genes per nuclei (H) and total genes detected (I) for all samples.

Table S1: Patient Clinical Information (refer to Fig. 1)

Table of characteristics pertaining to the 46 donors whose brain tissues were used for snRNA-sequencing.

Table S2: Sequencing Information and Quality Control Metric for All AD Tissue Samples Sequenced (refer to Fig. 1)

Table of sequencing metrics for all 46 samples used for single-nuclei RNA-sequencing.

Table S3: DEGs Induced by the R47H Mutation in Each Cell Type and Sex in Brains from Patients with AD (refer to Fig. 1)

Table summarizing differential expression analysis between R47H-AD cells vs CV-AD cells for each brain cell type and each patient sex.

Table S4: Markers for the Microglia Subpopulations Isolated from AD Tissues (refer to Fig. 2)

Table summarizing microglial subpopulation marker genes.

Table S5. DEGs Induced by R47H-hTREM2 in Female Tauopathy Mice (refer to Fig. 3)

Table of differential expression analysis between female P301S *hTREM2*$^{R47H/+}$ mice and line-specific female P301S controls.

Table S6: WGCNA Brown and Cyan Module Genes (refer to Fig. 3)

Table of genes in the brown and cyan module of the weighted gene co-expression network analysis of bulk RNA-sequencing data from female P301S *hTREM2*$^{WT/+}$, P301S *hTREM2*$^{R47H/+}$ mice and line-specific female P301S controls.

Table S7: Marker Genes for Cluster 2 of Microglia from scRNA-Seq of Female Mice (refer to Fig. 4)

Table of differential expression analysis between microglia cluster 2 vs cluster 1 from scRNA-seq of 8-month-old female *hTREM2*$^{R47H/+}$, P301S *hTREM2*$^{R47H/+}$, and their line-specific female controls.

Table S8: DEGs Induced in R47H/+ Microglia in Response to TAU Fibrils (refer to Fig. 5).

Table of differential expression analysis between *hTREM2*$^{R47H/+}$ vs. *mTrem2*$^{+/+}$ primary microglia treated with TAU fibrils.

Table S9: DEGs between R47H/+ Microglia Treated with MK-2206 versus Vehicle in Response to TAU Fibrils (refer to Fig. 5).

Table of differential expression analysis between *hTREM2*$^{R47H/+}$ primary microglia treated with AKT-inhibitor MK-2206 vs. *hTREM2*$^{R47H/+}$ primary microglia treated with vehicle, both in the presence of TAU fibrils.

Table S10: Markers for the Microglia Subpopulations Isolated from Mice Treated with MK-2206 vs Vehicle (refer to Fig. 6)

Table summarizing microglial subpopulation marker genes from Fig. 6G.

Data file S1. Western blot of hTREM2 (refer to Fig. 3C)

Full western blots accompanying representative western blots from Fig. 3C.

Data file S2. Western blot of hTREM2 (refer to Fig. 6, B-D)

Full western blots accompanying representative western blots from Fig. 6, B-D.

Data file S3. Female Mice Latency to Reach the Platform (refer to Fig. 3K)

Individual data values from Fig. 3K for latency to reach the platform during hidden trials for female P301S *hTREM2*$^{R47H/+}$ mice and littermate controls.

Data file S4. Female Mice Cumulative Search Error (refer to Fig. 3L)

Individual data values from Fig. 3L for cumulative search error for female P301S *hTREM2*$^{R47H/+}$ mice and littermate controls.

Data file S5. Male Mice Latency to Reach the Platform (refer to Fig. 3M)

Individual data values from Fig. 3M for latency to reach the platform during hidden trials for male P301S *hTREM2*$^{R47H/+}$ mice and littermate controls.

Data file S6. Male Mice Cumulative Search Error (refer to Fig. 3N)

Individual data values from Fig. 3N for cumulative search error for male P301S *hTREM2*$^{R47H/+}$ mice and littermate controls.

Data file S7. Female Mice Latency to Reach the Platform (refer to fig. S5A)

Individual data values from fig. S5A for latency to reach the platform during hidden trials for female P301S *hTREM2*$^{CV/+}$ mice and littermate controls.

Data file S8. Male Mice Latency to Reach the Platform (refer to fig. S5B)

Individual data values from fig. S5B for latency to reach the platform during hidden trials for male P301S *hTREM2*$^{CV/+}$ mice and littermate controls.

Data file S9. Female Mice Cumulative Search Error (refer to fig. S5C)

Individual data values from fig. S5C for cumulative search error for female P301S *hTREM2*$^{CV/+}$ mice and littermate controls.

Data file S10. Male Mice Cumulative Search Error (refer to fig. S5D)

Individual data values from fig. S5D for cumulative search error for male P301S *hTREM2*$^{CV/+}$ mice and littermate controls.

Data file S11. MK-2206 in vivo Concentration (refer to Fig. 6A)

Individual data values from Fig. 6A for MK-2206 plasma and brain concentrations.

Data file S12. Synaptophysin IHC Quantification (refer to Fig. 6F)

Individual data values from Fig. 6F for synaptophysin IHC quantification with and without MK-2206 treatment.

Movie S1. R47H-*hTREM2* Does Not Affect Microglial Response to Injury (refer to fig. S8)

Video file of microglial responses of 12-17-month-old *Cx3cr1*$^{GFP/+}$ *mTrem2*$^{+/+}$, *hTREM2*$^{CV/+}$, and *hTREM2*$^{R47H/+}$ mice to an acute laser-induced injury in vivo over a 60-min time period, played one after the other. Scale bar: 15 μm.