SUPPLEMENTARY FILE

Materials and Methods

CVN-AD mice and surgery

All experiments were performed in strict compliance with animal protocols approved by the Institutional Animal Care and Use Committees (IACUC) of Duke University (A249-17-11). Breeding pairs of APPSwDI/mNos2$^{-/-}$ AD mice (CVN-AD) (kindly donated by Dr. Carol Colton) were bred in house and genotyped using standard PCR methods as previously described [1]. Age-match C57BL6J were purchased from the Jaxson Laboratory and housed under LC/DC 12-hr with free access to water and regular chow, same as CVN-AD mice. The orthopedic surgical model was performed with minor modifications as described in Xiong et al. [2] using 3 and 12 month of CVN-AD mice with isoflurane (Patterson Veterinary, Greeley, CO) anesthesia and analgesia (buprenorphine, 0.1 mg/kg subcutaneously; ZooPharm, Laramie, WY). All mice were included in the study.

Immunohistochemistry

One day after surgery mice were terminated under deep isoflurane anesthesia and perfused with 30 ml PBS. One hemisphere was dissected to separate the hippocampus and cortex, and snap frozen and stored at −80 °C. The other hemisphere was post-fixed in 4% PFA for 24 h at 4 °C, washed with PBS, and then transferred to 30% sucrose for OCT embedding. Serial sections were collected at 50 µm. Double- or triple-labeling immunofluorescence (IF) was performed using standard floating section protocols. Briefly, sections were washed with PBS three times for 5 min each, and incubated in PBST (PBS and 0.3% Triton) for 15 min. The sections were further blocked with 10% donkey serum in PBS for 1 h at RT, and subsequently incubated with primary antibodies of interest overnight at 4 °C. Sections were washed and incubated with Alexa-488- or 594- or 647-conjugated secondary antibodies (1:500; all from

Invitrogen, Carlsbad, CA). After washing in PBS, sections were coverslipped as described for dextran above. Images were acquired using either a LSM780 or 880 confocal laser microscope (Carl Zeiss). The following primary antibodies were used: mouse monoclonal anti-GFAP (G3893, 1:500; Sigma-Aldrich, St. Louis, MO), rabbit polyclonal anti-IBA1 (019-19741, 1:500; Wako Chemicals, Richmond, VA), goat polyclonal anti-AIF-1/IBA1 (NB100-1028, 1:500; Novus Biologicals, Centennial, CO), goat anti-m/rCD31 (AF3628, 1:200; R&D systems, Minneapolis, MN), rat anti-mouse CD68 (MCA1957, 1:500; Bio-Rad), rabbit anti-MAP2 (AB5622, 1:300; EMD Millipore, Burlington, MA), rabbit polyclonal anti-Aquaporin 4 (AB3594, 1:500; EMD Millipore, Burlington, MA), rabbit polyclonal anti-human fibrinogen (A0080, 1:1000; Dako, Santa Clara, CA). rabbit monoclonal anti-Aβ (D54D2, 1:500; Cell Signaling Technologies, Danvers, MA). For quantification of all the markers, the average signal density of two brain sections (30□m/each) was quantified using Image J software (Version: 1.51w). The images were subjected to threshold analysis at 20X magnification. Two to three fields were examined in the DG region, and sampling error was calculated using the SEM. All quantitative fluorescence was independently validated with a minimum of 3-6 biological replicates. The specific signal internalized by each cell was measured as percentage of area covered per cell. The same quantification strategy was applied to the MAP2+ cell counting. The MAP2+ cells were counted in the hilus region of the dentate gyrus using the cell counter plugin of ImageJ software. Counting was represented by mean counts per animal by an investigator blinded to the treatment conditions.

Dextran tracing

Mice were deeply anesthetized with isoflurane followed by transcardial injection of TMR (Tetramethyl Rhodamine) 70 kD dextran tracer (D1818; Thermo Fisher Scientific, Eugene, OR) diluted in sterile PBS into 2 mM stocks. After 5 min, mice were perfused with 20 ml PBS. Brains were harvested immediately and embed in OCT (Tissue-Tek, USA) on dry ice, then sliced into

10 µm sections with a cryostat (Microm HM550; Thermo Scientific, Waltham, MA) and stored at −80 °C. On the day of staining, slides were thawed at RT and fixed with 4% paraformaldehyde (PFA) (J531; VWR chemicals, Solon, OH) for 10 min at RT, followed by washing in PBS for 5 min. Sections were then blocked with 10% donkey serum in PBS (D9663; Sigma-Aldrich, St. Louis, MO) for 1 h at room temperature, and subsequently incubated with primary antibody, goat anti-m/rCD31 (AF3628, 1:200; R&D systems, Minneapolis, MN) overnight at 4 °C. Sections were washed and incubated with Alexa-633-conjugated secondary antibodies (Invitrogen, Carlsbad, CA) for 1 h at room temperature. Nuclei were counterstained with DAPI (4′,6-Diamidino-2-phenylindole dihydrochloride, 1:1000; Sigma-Aldrich, St. Louis, MO). After washing in PBS, sections were coverslipped with Fluoroshield histology mounting medium (F6182; Sigma-Aldrich, St. Louis, MO). Images were obtained using a LSM880 confocal laser microscope (Carl Zeiss). Z stack images were imported into Imaris software 9.21 (Bitplane AG, Zurich, Switzerland). After displaying adjustment, the DAPI filter was removed to view the images in blend mode with rendering quality set to 100%. A volume filter was applied to remove nonspecific staining and minimum thresholds were used for both control and surgery groups. To quantify dextran volume, a “Filament Trace” was created from a masked surface channel of CD31. Later, by removing the CD31 filter, dextran volume was quantified using the same threshold parameters for both control and surgery groups. To quantify extravasation of dextran another filament trace was created from the masked surface channel of dextran. By removing the intravascular dextran filter the volume of dextran outside the vessel was measured using similar threshold parameters in both groups.

CLARITY and 3D reconstructions

Mice were perfused transcardially with 30 ml PBS followed by 30 ml ice-cold 4% PFA whilst under deep isoflurane anesthesia. Brains were harvested and post-fixed in 4% PFA at 4°C for 24 h, and coronally sliced into 300 µm thickness with vibratome (PELCO easiSlicer; Ted Pella

Inc., Redding, CA). Slices were washed with PBS and incubated in 1 ml hydrogel solution (C1310X; Logos Biosystems, Annandale, VA) at 4°C for 24 h. Polymerization was performed using the X-CLARITY™ Polymerization System (Logos Biosystems) at 37°C for 3 h. Tissue clearing was performed via electrophoretic tissue clearing (ETC) solution (C13001; Logos Biosystems, Annandale, VA) with the X-CLARITY™ Tissue Clearing System (Logos Biosystems, Annandale, VA), and the following setting: 0.9 A, 37°C, for 3 h as described in [3]. Slices were washed and incubated in PBS overnight at RT with gentle shaking. Cleared slices were then incubated with primary antibody at 4°C for 3 days, and washed with PBST three times for 20 min each. Slices were incubated with secondary antibody at 4°C for 3 days, and washed with PBST three times for 20 min each. Nuclei were counterstained with DAPI and stored in PBS at 4°C before mounting. Images (x20 magnification; z stacked) were obtained using light-sheet fluorescence microscope (Carl Zeiss). 3D rendering of immunofluorescence was done using Imaris software 9.21 (Bitplane AG, Zurich, Switzerland) and the z-stacked images for each marker. Surface masks were used to form the base 3D model. Volume size of each microglia cell and Aβ plaque was calculated by the software. Signal intensity were determined by Imaris. All the adjustments during image processing were done under the same condition between surgical group and control group. Aβ engulfment analysis was performed by quantifying the Aβ positive volume present in the Iba1 channel using the mask function. The engulfed Aβ volume was then normalized to the Iba1 volume within the same acquisition frame. The following primary antibodies were used for IF analysis: rabbit monoclonal anti-Aβ (D54D2, 1:500; Cell Signaling Technologies, Danvers, MA), goat polyclonal anti-AIF-1/IBA1 (NB100-1028, 1:500; Novus Biologicals, Centennial, CO).

ELISA

Hippocampi were homogenized in RIPA buffer (50 mM Tris pH 8.0, 150 mM NaCl, 1% NP-40, 5 mM EDTA, 0.5% sodium deoxycholate, 0.1% SDS) supplemented with protease inhibitor

(78430; Thermo Scientific, Waltham, MA). Lysates were centrifuged at 21,100 × g for 15 min at 4 °C. The insoluble fractions were extracted in 70% Formic Acid buffer (27001; Sigma-Aldrich, St. Louis, MO) and centrifuged at 21,100×g for 15 min at RT. All soluble and insoluble fractions were subsequently analyzed by ELISA. Amyloid peptides were measured in lysates with the V-PLEX Aβ Peptide Panel 1 (4G8) Kit (K15199E; Meso Scale Diagnostics, Rockville, Maryland) according to the instructions in the manual. The assay was performed using MESO QuickPlex SQ 120 system. Amyloid peptides were quantified using Meso Scale Discovery Workbench software (Meso Scale Diagnostics, Rockville, Maryland). Plasma was collected via open thoracotomy as previously described [4] and analyzed by MILLIPLEX MAP Mouse Cytokine/Chemokine Magnetic Bead Panel (MCYTOMAG-70K-PMX; EMD Millipore, Burlington, MA) through the Duke biomarker profiling core. The full dataset is deposited at Discover Mendeley Data.

5-choice serial reaction time task

CVN-AD and age-matched C57BL6/J mice were trained to nose poke for a food reward [3]. During the entirety of testing the weight and food intake of the mice were monitored daily so as to maintain the mice at 90% of the free feeding weight for the duration of the study. This ensured proper motivation so the animals would learn the task. All testing was conducted in Med-Associates 5-CSRTT chambers for mice (24 × 18.5 cm; St. Albans, VT). Each chamber was equipped with five nose-poke apertures, illuminated with a single LED light. Infrared diodes detected nose pokes into each aperture. A food magazine with an LED light and infrared diodes was positioned opposite the nose poke apertures. The food magazine dispersed 20 mg chocolate flavored food pellets as rewards (BioServ, Flemington, NJ). Mice were trained and tested in the same chamber for the entire duration of the study and all testing was conducted between 0900 and 1400 hrs each day, with testing starting approximately 3 hrs following onset of the light cycle. Mice received 40 test trials per day during the entire duration of the study. All

trials were divided into several phases: illumination of the nose poke apertures in a darkened chamber, limited hold period for a specific time interval following illumination to allow the animal to respond, a consummatory phase when the mouse was allow to retrieve and eat the food reward earned, and a 20 sec inter-trial interval. If the mouse failed to nose poke for a food reward, an additional 10-sec time out was implemented along with the inter-trial interval. Mice were first trained to nose poke for a food reward by illuminating all apertures in the test chamber. Once a nose poke was registered, a food reward was dispersed and the food magazine illuminated. Once the mouse retrieved and ate the reward, the chamber was darkened and the inter-trial interval imposed before the start of the next trial. Mice were left in the chambers until 20 food rewards had been obtained or a maximum of 30 min. After the mice showed the ability to retrieve 20 food rewards in less than 30 min for 3 consecutive days the animals were trained to nose poke for food rewards with the same procedure, but only with a single nose poke aperture randomly illuminated. Criterion remained the same: ability to earn 20 food rewards in less than 30 min over 3 consecutive days. When this was achieved, the animal was graduated to nose poke training with a brief illumination of the aperture and stricter time limits imposed at each phase of the trail. Mice were given 40 trials each day, and up to 30 min to complete the 40 test trials. Testing began with a 5-sec illumination of a random nose poke aperture. Once the mouse selected the illuminated aperture, the nose poke light was extinguished and a food reward was delivered along with the simultaneous illumination of the food magazine for 5-sec and the trial was recorded as a successful response. If the nose poke extinguished before a response was made by the animal, an additional 5-sec limited hold period was given to allow the mouse to make a response. If the mouse made a correct response during the limited-hold, the food reward was delivered and the trial was also scored as correct. If the mouse failed to make a response during the illumination period or the limited hold, the trial ended, was scored as an omission, and a time-out period was imposed along with the inter-trial interval before the next trial began. If the mouse made an incorrect selection (non-illuminated

aperture) during either the illumination phase or the limited hold, the trial was ended and scored as an incorrect response with the time-out and inter-trial interval imposed before the start of the next trial. Once the animal had achieved at least an 80% success rate (40 daily trials) over three consecutive days, the nose poke illumination was shortened to 4-sec. Training remained the same until the animal achieved 80% success rate on 40 daily trails for 3 consecutive days, at which point the nose poke illumination was reduced to 2-sec. Once criterion of 80% success on 40 daily trials for 3 consecutive days, the nose poke illumination was decreased to 1.5-sec duration. In all cases the 5-sec limited hold period after the nose poke illumination and 5-sec to retrieve and consume a food reward remained stable, along with the 20-sec inter-trial interval. After mice exhibited at least three consecutive days of 80% success rate or higher at the 1.5 sec stimulus duration, the animal was scheduled for tibia fracture surgery. The three days of testing prior to fracture surgery were labelled as "baseline" performance for the animals. The test procedure and criterion for correct responses remained the same during this post-surgery period. Final assessment of the animals' performance in the three baseline days prior to surgery and in the 7 days following fracture were defined as the percent trials out of the daily 40 trials where the mouse registered a response (poked into any nose-poke aperture during the illumination or limited-hold phases of each trial). Out of the total number of trials the mouse responded, the percent of those trials with an incorrect response (selection of a non-illuminated aperture during the illumination or limited-hold phase) were also scored and expressed as the percent incorrect responses. The full dataset and statistical analyses are deposited at Discover Mendeley Data.

Statistics

Data are presented as mean ± SEM and analyzed using GraphPad Prism 7 (GraphPad Software, San Diego, CA) or IBM SPSS Statistics 25 (IBM, Chicago, IL) as described in the figure legends. Post-hoc analyses were performed using Bonferroni corrected pair-wise tests for

the behavioral study. In cases where a single direction in outcome was predicted *a priori* or only possible, 1-tail p-values were reported. In all cases, statistical significance was set to $P < 0.05$.

References

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