PNAS
www.pnas.org

Supplementary Information for

Regulation of beta-amyloid production in neurons by astrocyte-derived cholesterol.

Hao Wang, Joshua A. Kulas, Chao Wang, David M. Holtzman, Heather A. Ferris, Scott B. Hansen

Scott B. Hansen and Heather A. Ferris
Email: shansen@scripps.edu, hf4f@virginia.edu

This PDF file includes:

Supplementary text
Figures S1 to S7
Extended Methods
SI References

Supplementary Text

Limitations of the Study. CTxB is a pentadentate toxin that can cause artificial clustering (1). Density-based spatial clustering of applications with noise (DBSCAN), a commonly used clustering analysis algorithm allows for an accurate comparison of relative size between apoE-treated and non-treated samples (Figure 1d), but does not necessarily correct artifacts in determining absolute size of the domain (Figure S5a) (2) (see also (3) for clustering artifacts that can affect determining absolute size). The changes in size are described as 'apparent' for this reason. Hence, the effects of a treatment are limited to an observed change in size due to a specific treatment or condition. None of the conclusions from pair correlations (Figures 2-3) are affected by clustering. In fact, artificial clustering may help pair correlations by tightening the boundary of GM1 domains and allowing a better determination of a protein that is in or out of the domain.

Our data largely support the reliability of DRMs as a first approximation of cluster affinity (4, 5). Both dSTORM and DRMs found β, and γ-secretases firmly anchored in GM1 domains and α-secretase unassociated. Some have argued DRMs are not always identical to GM1 domains (4, 6), and we did see some differences. Compared to DRMs, our data show APP associates more readily with GM1 domains. We suspect the addition of detergents in the preparation of DRMs causes some APP to leave GM1 clusters although we cannot rule out dSTORM introducing clustering artifacts that increase APP association with GM1 domains in dSTORM.

We used blood serum as a source of cholesterol for loading neurons. Serum has large lipoproteins like LDL and VLDL that are not present in the CNS. In the CNS, apoE is only present in HDL like lipoproteins whose main effect is more in line with reverse cholesterol transport as seen when we added lipid poor apoE to neurons.

Supplemental Figures
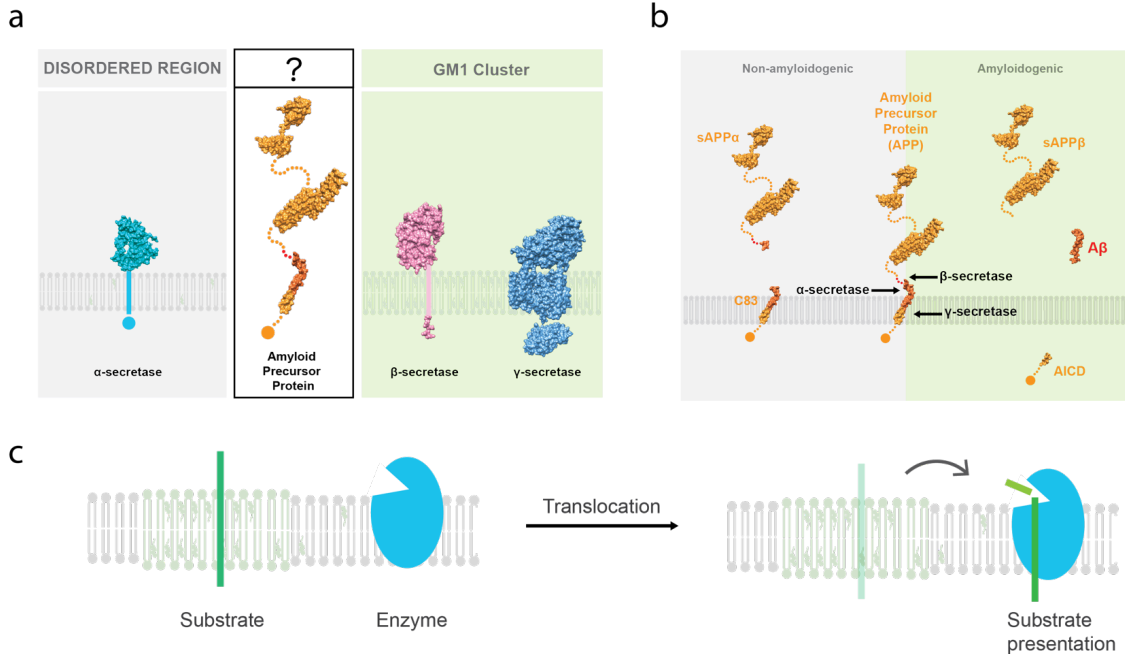
Fig. S1. Amyloid proteins and substrate presentation. (a) The atomic structures of amyloid proteins and their predicted sub-membrane localizations based on palmitate mediated localization. Amyloid precursor protein (APP) and β- and γ-secretases are palmitoylated on their N-terminus. The palmitoylation is known to cause partitioning into detergent resistant membrane and GM1 clusters (green shading). α-secretase is not palmitoylated and is expected to reside in the disordered regions (grey shading). (b) The known APP cleavage sites for APP-processing proteins. In general, the α-secretase or β-secretase cleaves the APP extracellular domain generating the soluble N-terminal fragments (e.g. sAPPα or sAPPβ) and membrane-associated C-terminal fragments (CTF). CTFβ is further cleaved by the γ-secretase within the transmembrane domain releasing p3 peptide or β-amyloid (Aβ) peptide (respectively). (c) Substrate presentation is a biological process that activates a protein. The protein is sequestered away from its substrate and then activated by release and exposure of the protein to its substrate.
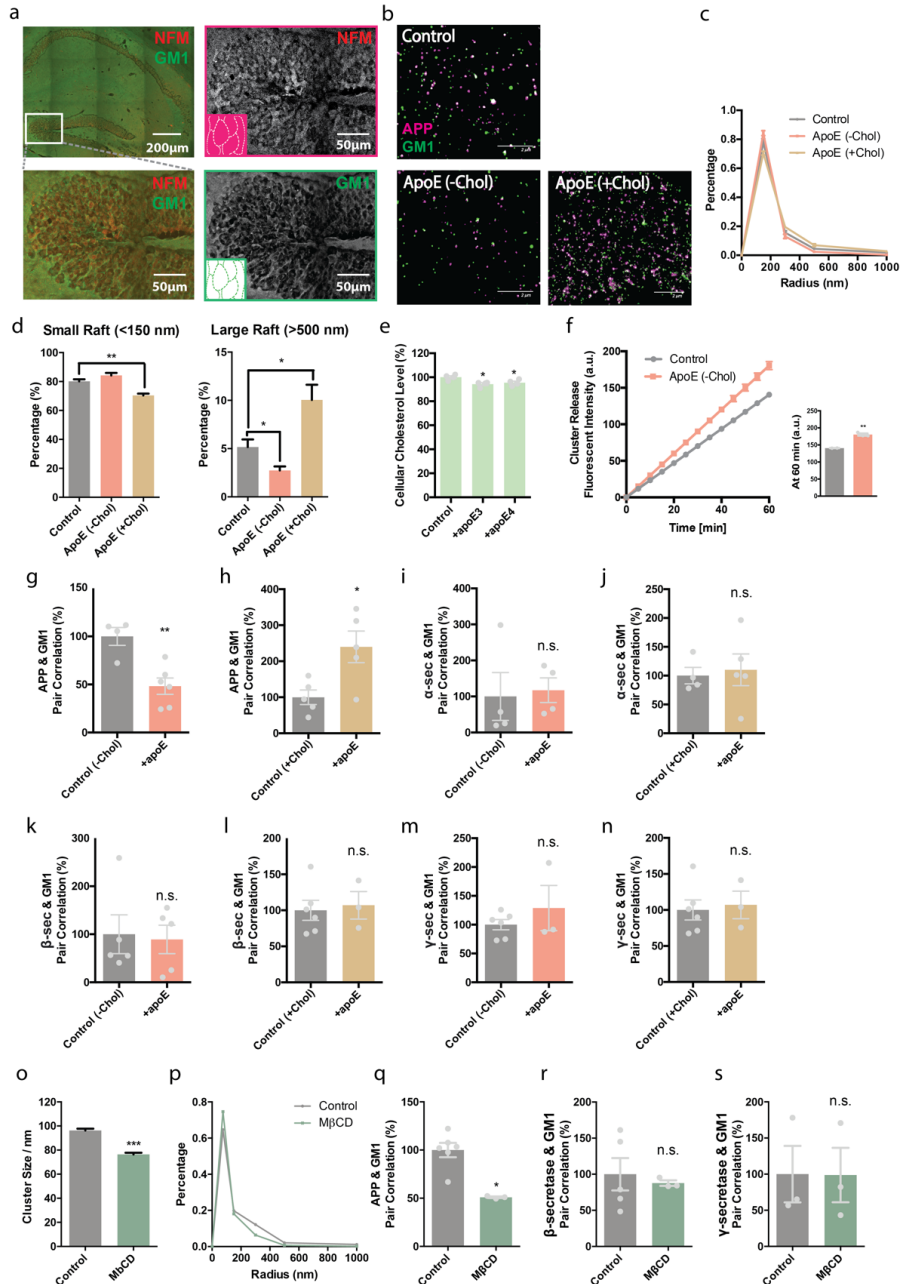
Fig. S2. Super resolution imaging of enzymes and substrate localization with apoE and MβCD. (a) Confocal imaging on mouse brain showing GM1 lipids in plasma membrane of neurons. Neurons are labelled with neurofilament medium chains (NFM) antibody (red) and GM1 lipids are labelled with fluorophore-conjugated cholera-toxin B subunit (CTxB, green). Representative images showing NFM filled the cell bodies of hippocampal neurons while GM1 lipids are in the plasma membrane. (b) dSTORM imaging shows the effect of apoE on membrane protein's cluster association (APP in magenta, GM1 in green) in N2a cells under low and high cholesterol conditions. Scale bar is 2 μm. (c-d) ApoE mediates a shift in distribution from small clusters (<150 nm) to larger clusters (>500 nm) under high cholesterol condition and opposite effect in low cholesterol condition; one-way ANOVA. (e) Cluster release assay showing treatment of N2a cells with apoE causes a palmitoyl reporter enzyme to exit the cluster and produce fluorescent product. Data are expressed as mean ± s.e.m., n=3-10, two-sided Student's t-test

*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. (f) Exposure of neuroblastoma 2a (N2a) cells to either apoE3 or apoE4 removes cholesterol from cellular membrane measured by a fluorescent based cholesterol assay. (g-n) Under low cholesterol conditions (-Chol), APP's cluster association decreases markedly after apoE treatment. Under high cholesterol conditions (+Chol), apoE-mediated APP co-localization with GM1 clusters increases (i.e., apoE induces APP to translocate into lipid clusters). α-, β- and γ-secretase localization do not respond to apoE-mediated cluster modulation. (o) Apparent cluster size of neuroblastoma 2a (N2a) cells before and after treatment with methyl-β-cyclodextrin (MβCD). (p) Cholesterol extraction by MβCD mediates a shift in cluster size distribution from large clusters (>500 nm) to smaller clusters (<150 nm). (q) Lipid cluster disruption by MβCD moves APP from lipid clusters into disordered region. (r-s) β- and γ-secretase's lipid cluster localization are insensitive to MβCD-mediated cluster disruption.
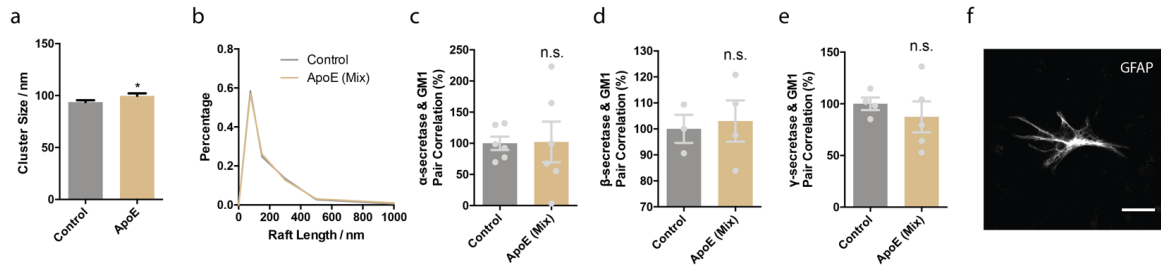
Fig. S3. ApoE's effect on secretases in cortical neurons (a) Apparent cluster size of primary cortical neurons before and after treatment with apoE in mixed culture with astrocytes. (b) Cluster size distribution analysis showing apoE mediates a shift in distribution from small clusters (<150 nm) to larger clusters (>500 nm) in cortical neurons when culturing with astrocytes. (c-e) α-, β- and γ-secretase lipid cluster localization in cortical neurons do not respond to apoE-mediated cluster modulation when culturing with astrocytes. Data are expressed as mean ± s.e.m., n=3-10, two-sided Student's t-test. (f) Astrocyte in mixed cortical culture is labelled with GFAP antibody.
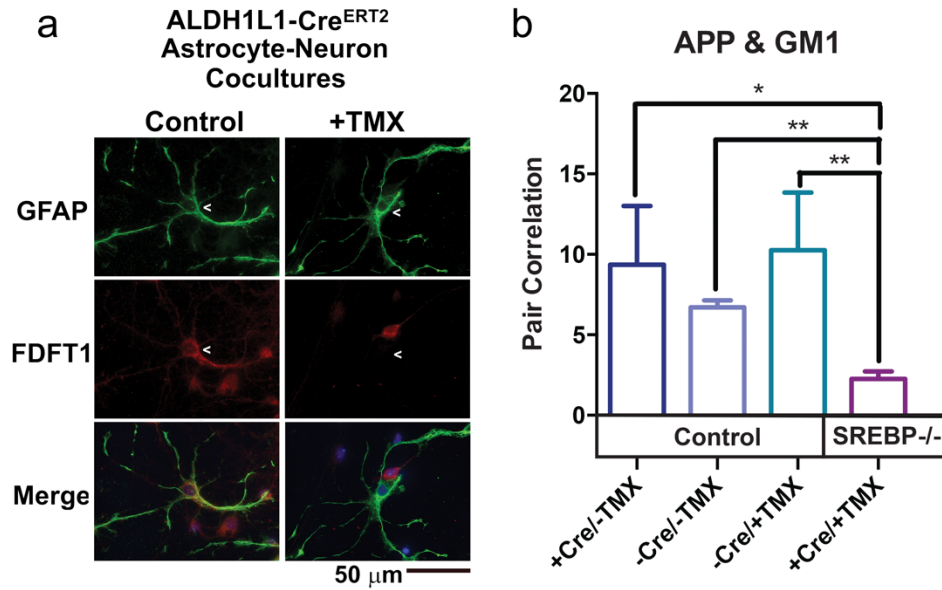
Fig. S4. **Induction of astrocyte SREBP2 gene ablation disrupts the cholesterol synthesis enzyme FDFT1 and abolishes APP and GM1 pair correlation.** (a) Astrocyte-neuron co-cultures were grown from SREBP2$^{flox/flox}$ Aldh1L1-Cre^{ERT2} embryos. Astrocyte SREBP2 knockout was induced by application of 4-hydroxytamoxifen (TMX) to cultures. Following knockout, cells were immunostained for the cholesterol synthesis enzyme FDFT1 and the astrocyte marker GFAP and imaged. (b) Mixed primary cultures of neurons and astrocytes derived from SREBP2$^{flox/flox}$ x Aldh1L1-Cre and Cre negative littermates were treated with 4-hydroxytomoxifen (TMX) to delete SREBP2. APP lipid cluster localization in cortical neurons is lost when SREBP2 is knocked out of astrocytes. TMX does not change APP localization in Cre negative cells. Data are expressed as mean ± s.e.m., n=3-10, two-sided Student's t-test *P<0.05, **P<0.01.
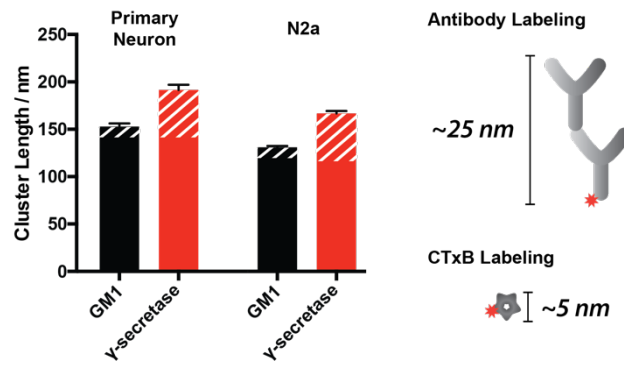
**Fig. S5. Analysis of cluster size.** Comparison of the size of CTxB labeled GM1 domains from primary neurons and N2a cells with antibody labeled γ-secretase. The potential added diameter from antibody or CTxB labeling is indicated by white stripes. Fixed γ-secretase within the GM1 lipids form clusters approximately the same size as CTxB labeled GM1 clusters, suggesting CTxB does not cluster unfixed GM1 lipids after treatment(3).
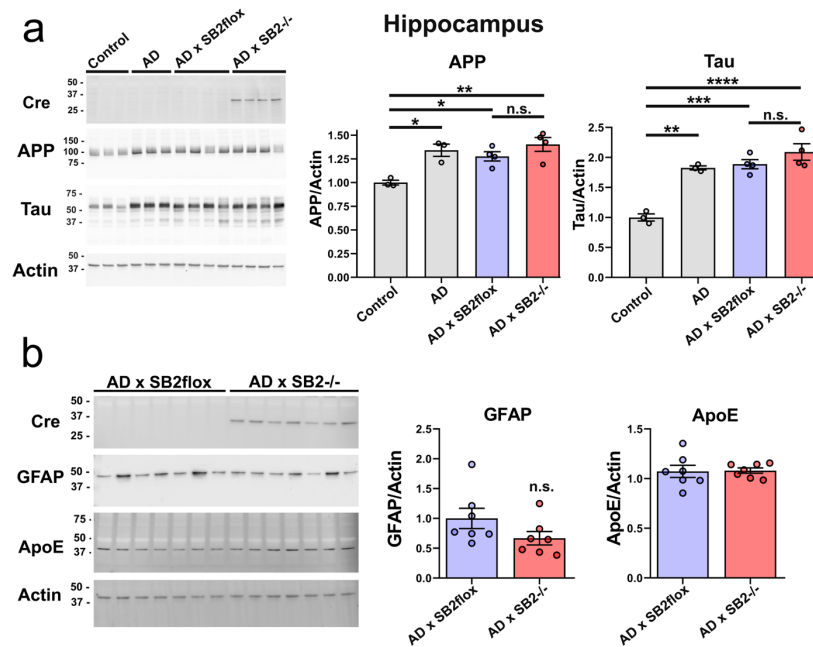
Fig. S6. **Loss of astrocyte SREBP2 in the 3xTg AD models does not impact total transgene protein levels or ApoE in the hippocampus.** 3xTg-AD (AD) mice express transgenes for mutant human amyloid precursor protein (APP) and tau. The AD mice were crossed to SREBP2$^{flox/flox}$GFAP-Cre$^{+/-}$ mice and littermate females with intact SREBP2 (AD x SB2flox) or deleted SREBP2 (AD x SB2$^{-/-}$) were compared. Control mice do not express the human transgenes. (a) Animals were aged to 40 weeks and hippocampus tissue was dissected and homogenized for western blot. Data are expressed as mean ± s.e.m., n=3-4 animals per genotype. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, one-way ANOVA with Tukey's post hoc analysis. (b) GFAP and ApoE were measured in 40-week-old female ADxSB2flox and ADxSB2-/- hippocampus lysate. Data are expressed as mean ± s.e.m., n=7 animals per genotype.

Astrocyte Conditioned Media
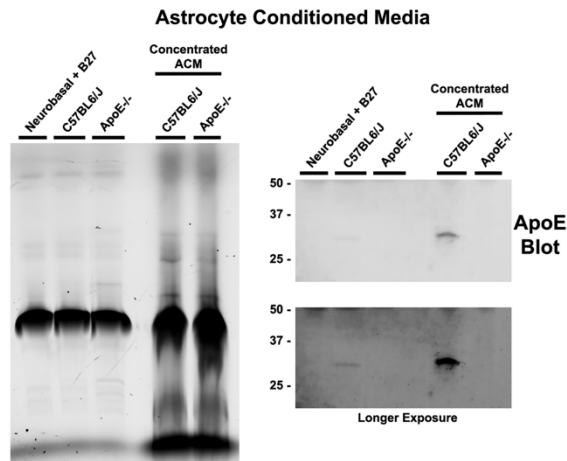
Fig. S7. Primary astrocyte cultures release apoE into cell culture media. C57BL6/J control and APOE-/- mouse astrocytes were maintained in neurobasal media supplemented with B27 for 72 hours. Media was collected and concentrated to test for the presence of apoE protein by western blot. Total protein imaged on stain-free gel (left panel) for unconcentrated and concentrated astrocyte conditioned media (ACM). Right panel, western blot demonstrates presence of ApoE in C57Bl6/J-derived ACM, but absence in media alone (neurobasal + B27) or ACM from ApoE-/- astrocytes.

Extended Methods

Animals

Housing, animal care and experimental procedures were consistent with the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the Scripps Research Institute and the University of Virginia. 3xTg-AD mice and B6129SF2/J controls were purchased from Jackson labs. The 3xTg-AD mice were maintained homozygous for all transgenes. SREBP2$^{flox/flox}$ mice (a generous gift of Dr. Jay Horton at UT Southwestern, now available through Jackson labs) were crossed to the hGFAP-Cre as previously described (7). These mice were in turn bred to the 3xTg-AD line and crossed back to homozygosity for both the 3xTg-AD transgenes and the SREBP2-flox. 3xTg-AD x SREBP2$^{flox/flox}$ (AD x SB2flox) and 3xTg-AD x SREBP2$^{flox/flox}$ x GFAP-Cre (AD x SB2$^{-/-}$) are littermates. Only female mice were used for the AD crosses as the male 3xTg-AD mice have a much milder phenotype.

Aldh1l1-Cre^{ERT2} mice were purchased from Jackson labs. These mice were crossed to the SREBP2$^{flox/flox}$ mice and the floxed allele was kept homozygous. Both male and female embryos were used from this mouse line. ApoE knockout mice utilized in astrocyte culture experiments were purchased from Jackson Labs.

To generate APPPS1-21/apoE3 or apoE4 mice (APP/PS1/E3F or APP/PS1/E4F, respectively), APPPS1-21 (APPPS1) transgenic mice were crossed with human APOE3 or APOE4 KI (E3F or E4F, respectively) mice (8) for several generations.

Cell culture

Neuroblastoma 2a (N2a) cells and human embryonic kidney 293T (HEK293T) cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The cells were changed to a serum-free media 24 hours prior to experimentation unless otherwise noted.

Neurons were isolated from the cortices of embryonic day 18-21 CD1 mice. Cells were dissociated by papain digestion and plated on poly-D-lysine-coated (0.01 mg/mL) 8-well Lab-Tek II chambered cover glass (Thermo Fisher Scientific, #Z734853). Specifically, dissociated cells were plated in Minimum Essential Medium (MEM) supplemented with 5% FBS and 0.6% glucose and grown in Neurobasal medium (Thermo Fisher Scientific) supplemented with 20% B27 (Invitrogen), 1% Glutamax and 1% penicillin/streptomycin at 37 °C in 5% CO_2. Cells were cultured *in vitro* for two days prior to experiment. Astrocytes were cultured in DMEM containing 10% FBS and 1% penicillin/streptomycin as described previously (9).

Inducible Astrocyte SREBP2 Knockout Cultures

Astrocyte SREBP2 knockout cells with WT neuron cocultures were grown by breeding ALDH1L1-Cre^{ERT2}xSREBP2$^{flox/flox}$ mice with SREBP2$^{flox/flox}$ mice. Embryonic day 17 brain cortices were harvested from embryos and cultured individually, while tails from each individual embryo were collected for determination of Cre genotype. Meninges-free embryo cortices were digested in papain supplemented with DNAse for 30 minutes then digestion was terminated by 4:1 addition of sterile HBSS + 20% fetal bovine serum. Following enzymatic dissociation, cells were centrifuged at 300 x g for 3 minutes. The resulting cell pellet was gently resuspended in plating media (MEM + 5% FBS + 0.6% glucose) using a 1mL pipette. Cells were plated on 0.01 mg/mL poly-d-lysine overnight coated Nunc™ Lab-Tek™ II 8-chambered cover glass or glass coverslips in plating media and placed in a 37-degree 5% CO2 cell culture incubator. After two hours, plating media was gently removed from the cells and replaced with neurobasal media supplemented with 20% B27 and 1% Glutamax. After 4 days in culture, astrocyte SREBP2 ablation was induced by addition of 100 nM 4-OH tamoxifen (Sigma-Aldrich H7904) to culture media for 48 hours. After 48

hours 4-OH tamoxifen was removed and media was replaced with fresh neurobasal media containing 20% B27 and 1% Glutamax. Cells were then maintained in culture for 4 days and fixed for imaging as described below.

3xTg Astrocyte SREBP2 Knockout Cultures

Mixed cultures of 3xTg neurons with WT or SREBP2 knockout astrocytes were generated from embryos by breeding 3xTg SREBP2$^{flox/flox}$ mice with 3xTg SREBP2$^{flox/flox}$ hGFAP-Cre mice. On embryonic day 17, the embryos were harvested and the cortices and tail of each embryo were collected for cell culture and PCR genotyping respectively. The mixed cultures were grown from individual embryonic cortices as performed in the inducible system described above, however cells were seeded at a high density (approximately 200,000 cells/well) to allow for a high density of neurons and astrocytes. After 3 days in culture, the cell culture media was exchanged for fresh supplemented neurobasal media. The culture media and cell lysate were then collected for ELISA and western blot experiments. Conditioned media was concentrated for western blot by addition of 250 μL trichloroacetic acid (1.42g/mL H2O) to 1 mL of conditioned media. Protein was precipitated for 20 minutes on ice and pelleted by centrifugation at 20,000 RCF at 4 degrees C. The pellet was then washed twice with ice cold acetone and resuspended in Laemmli buffer under reducing conditions.

Astrocyte conditioned media treatment of hGFAP-Cre neuron cultures

Primary astrocyte cultures from C57BL6/J and APOE-/- mice were grown from 2-day-old mouse pups using established methods. Brain cortices were harvested and meninges removed. Tissue was then digested in trypsin with DNAse for 30 minutes. Digestion was terminated using 10% FBS in DMEM/F12 and cells were centrifuged at 500 RCF for 10 minutes. The cell pellet was then suspended in astrocyte growth media (DMEM/F12 + 10% FBS + Penicillin streptomycin). The cells were plated into cell culture flasks (4 pup cortices/flask) with media refreshed after 24 hours. When cultures became confluent with astrocytes after 1 week, the culture flasks were shaken on an orbital shaker at 500 RPM for 6 hours to remove microglia. The astrocytes were then washed 2 times with sterile PBS. 15 mL of Neurobasal media supplemented with B27 and Penicillin/streptomycin was then added to the astrocyte cultures for conditioning. This media was conditioned with the astrocytes for 72 hours and then collected for subsequent experiments. For treatment of neurons, the astrocyte conditioned media (ACM) was collected from culture flasks and centrifuged for 10 minutes at 3000 RCF to remove cell debris. 0.5 mL of ACM was added to 1 mL of neuronal culture media and applied to primary embryonic hGFAP-Cre x SREBP2$^{flox/flox}$ neuron cultures grown on glass coverslips in 12 well plates. The neurons were in culture for 7 days at the time of ACM treatment. The ACM from control or APOE-/- astrocytes was applied to neurons for 24 hours. After treatment, neurons were washed with PBS and fixed for dSTORM analysis. ACM used in western blot experiments was concentrated using Millipore 10kD centrifugal filters (MRCPRT010) by centrifugation at 14,000 RCF for 20 minutes.

dSTORM Super-resolution imaging

Fixed cell preparation

N2a cells were grown to 60% confluence and then allowed to differentiate overnight in serum free media. Primary cortical cells were grown for two days *in vitro*. Cells were incubated with 4 μg/mL purified apoE protein for one hour in media with or without FBS supplementation. Cells were rinsed with PBS and then fixed with 3% paraformaldehyde and 0.1% glutaraldehyde for 15 min to fix both proteins and lipids. Fixative chemicals were reduced by incubating with 0.1% NaBH$_4$ for 7 min with shaking followed by three times 10 min washes with PBS. Cells were permeabilized with 0.2% Triton X-100 for 15 min and then blocked with a standard blocking buffer (10% bovine serum albumin (BSA) / 0.05% Triton in PBS) for 90 min at room temperature. For labelling, cells

were incubated with primary antibody (APP: Abcam, #ab15272; γ-secretase: Sigma, #MAB5232; β-secretase: Abcam, #ab10716; α-secretase: Santa Cruz, #sc-48400) for 60 min in 5% BSA / 0.05% Triton / PBS at room temperature followed by 5 washes with 1% BSA / 0.05% Triton / PBS for 15 min each. Secondary antibody was added in the same buffer as primary for 30 min at room temperature followed by 5 washes as stated above. Cells were then washed with PBS for 5 min. Cell labelling and washing steps were performed while shaking. Labeled cells were then post-fixed with fixing solution, as above, for 10 min without shaking followed by three 5 min washes with PBS and two 3 min washes with deionized distilled water.

The specificity of the APP antibody (ab15272) used for dSTORM staining in this study was validated by western blot with an expected MW of 87 kDa (see manufacture product page). A second APP antibody (ab32136), validated in KO animals, was used for the western blots. The y-secretase antibody (MAB5232) binds to proteolyzed PS1—mutation that blocks proteolysis and shifted all the major bands to full length demonstrating this antibody's specificity (10). Western blots of rat brain with α-secretase antibody (sc-48400) produces a single band of the appropriate MW (see product website). The BACE1 antibody from Abcam used in this study (product #ab10716) has potential cross contamination with a 42 kDa protein (see product website). We did not verify in our N2a cells if this cross contamination is present. However, we did not see a shift in BACE1 antibody localization with treatment, suggesting if there is cross reactivity, both BACE1 and the putative protein behave the same.

Brain slice preparation

Mouse brain slicing and staining were performed as previously described (11) with minor modifications. Mouse brains were fixed in 4% paraformaldehyde, incubated in a 20% sucrose/PBS solution at 4 °C for 3 days, and embedded in Tissue-Tek OCT compound (Sakura). Sagittal sections (50 µm) were collected and placed into 24-well plate wells containing PBS. Fixative chemicals were reduced by incubating with 0.1% $NaBH_4$ for 30 min while gently shaking at room temperature followed by three times 10 min washes with PBS. Samples were permeabilized with 0.2% Triton X-100 for 2 hours and then blocked with a standard blocking buffer (10% bovine serum albumin (BSA) / 0.05% Triton in PBS) for 6 hours at room temperature. For labelling, samples were incubated with primary antibody for 3 hours in 5% BSA / 0.05% Triton / PBS at room temperature then 3 days at 4 °C followed by 5 washes with 1% BSA / 0.05% Triton / PBS for 1 hour each. Secondary antibody was added in the same buffer as primary for 3 days at 4 °C followed by 5 washes as stated above. Sample labelling and washing steps were performed while shaking. Labeled brain tissues were then post-fixed with fixing solution, as above, for 1 hour without shaking followed by three 30 min washes with PBS and two 30 min washes with deionized distilled water. Brain slices were mounted onto the 35 mm glass bottom chamber (ibidi, #81158) and 2% agarose were pipetted onto the slice to form a permeable agarose pad and prevent sample movement during imaging.

dSTORM imaging

Images were recorded with a Zeiss ELYRA PS.1 microscope using TIRF mode equipped with a pil-immersion 63x objective. Andor iXon 897 EMCCD camera was used along with the Zen 10D software for image acquisition and processing. The TIRF mode in the dSTORM imaging provided low background high-resolution images of the membrane. A total of 10,000 frames with an exposure time of 18 ms were collected for each acquisition. Excitation of the Alexa Fluor 647 dye was achieved using 642 nm lasers and Cy3B was achieved using 561 nm lasers. Cells and brain tissues were imaged in a photo-switching buffer comprised of 1% β-mercaptoethanol (Sigma, #63689), oxygen scavengers (glucose oxidase (Sigma, #G2133) and catalase (Sigma, #C40)) in 50mM Tris (Affymetrix, #22638100) + 10mM NaCl (Sigma, #S7653) + 10% glucose (Sigma, #G8270) at pH 8.0. Sample drift during acquisition was corrected by an autocorrelative algorithm.

Images were constructed using the default modules in the Zen software. Each detected event was fitted to a 2D Gaussian distribution to determine the center of each point spread function plus the localization precision. The Zen software also has many rendering options including removing localization errors and outliers based on brightness and size of fluorescent signals. Pair correlation and cluster analysis was performed using the Statistical Analysis package in the Vutara SRX software. Pair Correlation analysis is a statistical method used to determine the strength of correlation between two objects by counting the number of points of probe 2 within a certain donut-radius of each point of probe 1. This allows for localization to be determined without overlapping pixels as done in traditional diffraction-limited microscopy. Cluster size estimation was calculated through cluster analysis by measuring the area of the clusters comprising of more than 10 particles with a maximum particle distance of 0.1 μm. Cluster size of brain slices was calculated from >3 fields of view from one animal.

Fluorescence activated cell sorting (FACS) of live cells

Live cell staining for FACS was performed as previously described (12). Immediately after dissection and dissociation, cortical cells from embryonic mice were labelled with Brilliant Violet 421™ anti-mouse CD90.2 (Thy1.2) antibody (BioLegend, #140327). Cells were incubated with antibody at 4 °C for 20 minutes followed by washing with wash buffer (Ca^{2+}/Mg^{2+} free PBS, 1mM EDTA, 25mM HEPES, 5% FBS, 10 units/mL DNase II). Cells were filtered through a 40-μm nylon filter (Corning, #431750) and sorted in a BD Biosciences FACSAria3 sorter. Thy1.2+ neurons were collected and cultured as described above.

Live cell cluster affinity assay

Modulation of cluster integrity was detected by a biochemical assay based on the activity of a palmitate mediated cluster localized enzyme phospholipase D (PLD), as described previously[18]. Briefly, N2a cells were seeded into 96-well flat culture plates with transparent-bottom (Corning™ Costar™, #3585) to reach confluency (~ 5 x 10^4 per well). Then the confluent cells were differentiated with serum-free DMEM for a day and washed with 200 μL of PBS. The cluster integrity assay reactions were promptly begun by adding 100 μL of working solution with or without apoE, (BioVision, #4696). The working solution contained 50 μM Amplex red, 1 U/mL horseradish peroxidase, 0.1 U/mL choline oxidase, and 30 μM dioctanoyl phosphatidylcholine (C8-PC). ApoE was directly dissolved into the working buffer from freshly made stocks before assay reagents were added. The PLD activity and the background (lacking cells) was determined in triplicate for each sample by measuring fluorescence activity with a fluorescence microplate reader (Tecan Infinite 200 PRO, reading from bottom) for 2 hours at 37°C with excitation wavelength of 530 nm and an emission wavelength of 585 nm. Subsequently, PLD activity was normalized by subtracting the background and to the control activity. Data were then graphed (Mean ± s.e.m.) and statistically analyzed (one-way ANOVA) with GraphPad Prism software (v6.0f).

Live cell cholesterol assay

To measure the relative changes in plasma membrane cholesterol after apoE treatment, we developed an Amplex Red-based cholesterol assay in live cells, modified from the cluster integrity assay described above. Briefly, N2a cells or astrocytes were seeded into 96-well flat culture plates with transparent-bottom to reach confluency (~ 5 x 10^4 per well). Then the confluent cells were differentiated with serum-free DMEM for a day. Cells were incubated with fresh DMEM for 1 hour followed by 1 hour of incubation in 100 μL of DMEM medium with or without treatment. After washing with 200 μL of PBS, cholesterol assay reactions were promptly begun by adding 100 μL of working solution containing 50 μM Amplex red, 1 U/mL horseradish peroxidase, 2 U/mL cholesterol oxidase and 2 U/mL cholesterol esterase in PBS. Relative cholesterol concentration and the background (lacking cells) was determined in triplicate for each sample by measuring fluorescence activity with a fluorescence microplate reader (Tecan Infinite 200 PRO, reading from

bottom) for 2 hours at 37°C with excitation wavelength of 530 nm and an emission wavelength of 585 nm. Subsequently, cholesterol level was normalized to the control activity after subtracting the background. End point cholesterol signals were then graphed (Mean ± s.e.m.) and statistically analyzed (one-way ANOVA) with GraphPad Prism software (v6.0f).

ELISA

To measure the relative changes in β- and α- cleavage products of APP, N2a cells were incubated with apoE with and without FBS supplementation for 1 hour, then washed with PBS once and incubated with PBS for 1 hour. Supernatants were harvested and analyzed with Aβ40 and sAPPα ELISAs.

Relative quantitation of secreted Aβ40 was performed with commercial human Aβ40 ELISA kit (Invitrogen, #KHB3481) following the manufacturer's instructions. For sAPPα ELISA, 5 µg/mL rabbit anti-APP antibody (abcam, #ab15272) was coated on immunoassay plates (Corning™ Costar™ Cell Culture 96 well plates, #3585) at 4 °C overnight. Then, the plates were washed with PBS three times. Next, 50 µL of supernatant was added and incubated at room temperature for 1 hour. After this, 50 µL of 2 µg/mL mouse anti-human sAPPα IgG monoclonal antibody (IBL, #11088) was added as primary detection antibody. After incubation for 3 hours at room temperature, the plates were washed with PBST buffer (PBS with 0.05% Tween-20) and then 100 µL of 100 ng/mL HRP-linked rabbit anti-mouse IgG secondary antibody (Invitrogen, #31450) was added and incubated for 1 hour. An HRP substrate, chromogen (Invitrogen, #KHB3481) was added and incubated at room temperature in the dark for 30 minutes. The substrate development was terminated by adding 100 µL of stop solution from ELISA kit (Invitrogen, #KHB3481). Relative sAPPα concentration was determined by measuring absorbance at 450 nm using a microplate reader (Tecan Infinite 200 PRO).

Amyloid and p-Tau ELISA from Mouse Hippocampus

Human amyloid beta 1-40 (R&D Systems DAB140B) and 1-42 (R&D Systems DAB142) was quantified in mouse hippocampus tissue using commercially available ELISA kits per manufacturer's instructions. 60-week-old female mouse whole hippocampus tissue was homogenized in RIPA buffer (Bioworld, 42020024-2) with protease inhibitors (Thermo Fisher Scientific, 78430) using a Bullet Blender® (Next-Advance) with zirconium oxide beads. The homogenate was centrifuged at 15,000 x g for 10 minutes, and the supernatant was collected to yield RIPA soluble amyloid. The remaining tissue pellet was then resuspended in 5 M guanidine-HCl diluted in 50 mM Tris, pH 8.0 with protease inhibitor and mechanically agitated at room temperature for 4 hours to extract the RIPA insoluble amyloid. Guanidine samples were diluted 1:10 in sterile PBS and centrifuged at 16,000 x G for 20 minutes. Supernatant was then collected to yield insoluble amyloid. Both soluble and insoluble amyloid supernatants were then assessed for total protein content using the Pierce 660 protein assay (Thermo Fisher Scientific, 22660). All samples were then diluted to equal concentrations of total protein. Prior to ELISA, samples were diluted 1:10 to prevent oversaturation of ELISA signal. Human phospho-tau [pT181] was measured using a commercially available ELISA per manufacturer's instructions (Thermo Fisher Scientific, KHO0631). Guanidine extracted supernatants of hippocampus homogenates described above were utilized for the pTau ELISA.

Confocal imaging on brain slices and primary cells

Mouse brains were fixed in 4% paraformaldehyde, incubated in a 20% sucrose/PBS solution at 4 °C for 3 days, and embedded in Tissue-Tek OCT compound (Sakura). Sagittal sections (50 µm) were collected on Superfrost/Plus slides and immunostained with mouse NF-M antibody (Santa Cruz, #sc-398532), Alexa Fluor-647-conjugated CTxB (Invitrogen, #C34778) and Cy3B-conjugated anti-mouse secondary antibody. Human amyloid beta was detected using a polyclonal

antibody from Cell Signaling (#8243) with Alexa Fluor 594 anti-rabbit secondary (Thermo Fisher Scientific, #A21207). Primary cortical cells were fixed and labelled with Alexa Fluor-488-conjugated mouse GFAP antibody (Biolegend, #644704), or rabbit GFAP antibody (Millipore, #MAB360) with Alexa Fluor 594 anti-rabbit secondary (Thermo Fisher Scientific, #A21207). SREBP2 knockout was confirmed by measuring the downstream enzyme FDFT1 (Abcam, #ab195046, Alexa Fluor 488 secondary Thermo Fisher Scientific #A32766) pTau was imaged in brain sections using the mouse monoclonal AT180 antibody from Thermo Fisher Scientific (#MN1040). Samples were imaged on Leica TCS SP8 confocal microscope using a 25x water emersion objective. ImageJ was used to analyze images.

Tissue Western Blots

40-week-old mouse hippocampus tissue was homogenized in RIPA buffer with protease inhibitors using a Bullet Blender®. The homogenate was centrifuged at 15,000 x g for 10 minutes, and the supernatant was collected. Total protein content was measure using the Pierce 660 absorbance assay. Sample protein content was diluted to equal concentrations, and Laemmli buffer (Biorad, 1610737) was added to each sample for SDS-Page. SDS-Page was performed to resolve protein by molecular weight using 10 micrograms of hippocampal lysate from each sample in 4-20% acrylamide pre-cast gels (Biorad, 4568094). Resolved protein was transferred to PVDF membrane and blocked with 5% BSA solution in PBST. Primary antibodies against target proteins were applied overnight at 4 C. Membranes were then washed and fluorescent secondary antibodies were applied for 2 hours at room temperature. Following 5 washes with TBS with 0.1% tween, membranes were imaged using the Bio Rad chemidoc MP fluorescent imaging system. Primary antibodies used in this study include: APP (Y188), Abcam, #ab32136; tau, Fisher Scientific, #AHB0042; Cre, Biolegend, #908001; Actin, Biorad, #12004163; sAPPβ, BioLegend, #813401; ApoE, Abcam, #ab183597.

Mass Spectrometry

Lipidomics profiling was performed using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MSMS).

Lipid extracts were prepared from cell preparations using modified Bligh and Dyer method, spiked with appropriate internal standards, and analyzed on a platform comprising Agilent 1260 Infinity HPLC integrated to Agilent 6490A QQQ mass spectrometer controlled by Masshunter v 7.0 (Agilent Technologies, Santa Clara, CA). Glycerophospholipids and sphingolipids were separated with normal-phase HPLC. An Agilent Zorbax Rx-Sil column (2.1 x 100 mm, 1.8 µm) maintained at 25°C was used under the following conditions: mobile phase A (chloroform: methanol: ammonium hydroxide, 89.9:10:0.1, v/v) and mobile phase B (chloroform: methanol: water: ammonium hydroxide, 55:39:5.9:0.1, v/v); 95% A for 2 min, decreased linearly to 30% A over 18 min and further decreased to 25% A over 3 min, before returning to 95% over 2 min and held for 6 min. Separation of sterols and glycerolipids was carried out on a reverse phase Agilent Zorbax Eclipse XDB-C18 column (4.6 x 100 mm, 3.5um) using an isocratic mobile phase, chloroform, methanol, 0.1 M ammonium acetate (25:25:1) at a flow rate of 300 µL/min.

Quantification of lipid species was accomplished using multiple reaction monitoring (MRM) transitions under both positive and negative ionization modes in conjunction with referencing of appropriate internal standards: PA 14:0/14:0, PC 14:0/14:0, PE 14:0/14:0, PG 15:0/15:0, PI 17:0/20:4, PS 14:0/14:0, BMP 14:0/14:0, APG 14:0/14:0, LPC 17:0, LPE 14:0, LPI 13:0, Cer d18:1/17:0, SM d18:1/12:0, dhSM d18:0/12:0, GalCer d18:1/12:0, GluCer d18:1/12:0, LacCer d18:1/12:0, D7-cholesterol, CE 17:0, MG 17:0, 4ME 16:0 diether DG, D5-TG 16:0/18:0/16:0 (Avanti Polar Lipids, Alabaster, AL). Lipid levels for each sample were calculated by summing up the total number of moles of all lipid species measured by all three LC-MS methodologies.

SI References

1. S. Moon, *et al.*, Spectrally Resolved, Functional Super-Resolution Microscopy Reveals Nanoscale Compositional Heterogeneity in Live-Cell Membranes. *J. Am. Chem. Soc.* **139**, 10944–10947 (2017).

2. S. L. Veatch, *et al.*, Correlation functions quantify super-resolution images and estimate apparent clustering due to over-counting. *PLoS One* **7** (2012).

3. E. N. Petersen, M. A. Pavel, H. Wang, S. B. Hansen, Disruption of palmitate-mediated localization; a shared pathway of force and anesthetic activation of TREK-1 channels. *Biochim. Biophys. Acta - Biomembr.* **1862**, 183091 (2020).

4. J. M. Cordy, N. M. Hooper, A. J. Turner, The involvement of lipid rafts in Alzheimer's disease (review). *Mol. Membr. Biol.* **23**, 111–122 (2006).

5. R. Ehehalt, P. Keller, C. Haass, C. Thiele, K. Simons, Amyloidogenic processing of the Alzheimer β-amyloid precursor protein depends on lipid rafts. *J. Cell Biol.* **160**, 113–123 (2003).

6. D. Lichtenberg, F. M. Goñi, H. Heerklotz, Detergent-resistant membranes should not be identified with membrane rafts. *Trends Biochem. Sci.* **30**, 430–436 (2005).

7. H. A. Ferris, *et al.*, Loss of astrocyte cholesterol synthesis disrupts neuronal function and alters whole-body metabolism. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 1189–1194 (2017).

8. T. P. V. Huynh, *et al.*, Lack of hepatic apoE does not influence early Aβ deposition: Observations from a new APOE knock-in model. *Mol. Neurodegener.* **14**, 1–23 (2019).

9. S. Schildge, C. Bohrer, K. Beck, C. Schachtrup, Isolation and culture of mouse cortical astrocytes. *J. Vis. Exp.* (2013) https:/doi.org/10.3791/50079.

10. M. Cacquevel, L. Aeschbach, J. Houacine, P. C. Fraering, Alzheimer's disease-linked mutations in presenilin-1 result in a drastic loss of activity in purified γ-secretase complexes. *PLoS One* **7**, 1–13 (2012).

11. C. L. German, M. V. Gudheti, A. E. Fleckenstein, E. M. Jorgensen, "Brain slice staining and preparation for three-dimensional super-resolution microscopy" in *Methods in Molecular Biology*, (2017) https:/doi.org/10.1007/978-1-4939-7265-4_13.

12. J. M. Schwarz, Using fluorescence activated cell sorting to examine cell-type-specific gene expression in rat brain tissue. *J. Vis. Exp.* (2015) https:/doi.org/10.3791/52537.