Supporting Information

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SI Materials and Methods

Mice. J20 $hAPP_{FAD}$ mice express hAPP harboring the Swedish (K670N,M671L) and Indiana (V717F) mutations under the control of the PDGF promoter (1). These mice were backcrossed with murine apoE knockout mice (mEKO) and subsequently cross-bred with NSE-apoE3, NSE-apoE4 (2), or low expresser Thy1-apoE4(Δ272–299) (3) mice on the mEKO background. $hAPP_{FAD}$ mice with mouse apoE ($hAPP_{FAD}$/mE) were used in some analyses as controls. Similar numbers of male and female mice were studied at 6–8 mo of age, except in behavioral experiments, in which female mice were studied at 5–9 mo of age. Brains were collected after deep anesthesia with avertin and transcardial perfusion with 0.9% saline. The left hemibrain was snap-frozen on dry ice; the right hemibrain was fixed in 4% paraformaldehyde for 48 h. After a 24-h rinse in PBS (PBS), hemibrains were immersed in 30% sucrose/PBS and cut into 30-μm-thick sections on a Leica sliding microtome mounted with a freezing stage (Physitemp).

Aβ ELISA. Total Aβ ($Aβ_{(1-x)}$) and $Aβ_{42}$ levels in the hippocampus were measured by ELISA as described (4). Briefly, snap-frozen hemibrains were thawed, and the hippocampi were dissected, weighed, and homogenized in 5 M guanidine buffer (10× volume/weight). Homogenates were spun at 14,000g for 20 min to pellet debris, serially diluted, and applied to plates coated with anti-$Aβ_{42}$ (clone 21F12) and anti-total-Aβ (clone m266) to detect Aβ peptides. The detection antibody was biotinylated anti-Aβ clone 3D6. All sample dilutions used to calculate Aβ levels were within the detection range based on a simultaneously generated standard curve.

ApoE and Aβ Binding Assay. Ninety-six well plates were coated with $Aβ_{40}$ or $Aβ_{42}$ (330 ng/well) (Biopeptides), washed several times with washing buffer (PBS containing 0.05% Tween-20), and blocked overnight at 4 °C with 4% BSA in PBS. Subsequent incubations were performed at room temperature. The highest concentration of apoE4 or apoE4(Δ272–299) was 62.5 ng (18.1 nM) diluted in 100 μL of blocking buffer with fourfold dilutions thereafter. The mixture was applied to the Aβ-coated plate for 1 h, the plate was washed, and bound apoE was detected with a polyclonal anti-apoE antibody (Calbiochem). Anti-goat IgG labeled with horseradish peroxidase and TMBE substrate (ThermoPierce) were sequentially applied, and the resulting signal was read at 450 nm on a SpectraMax M5 plate reader (Molecular Devices); data were collected with Softmax Pro. Each experiment was performed in duplicate and repeated three times. The detection of the apoE peptides by the polyclonal anti-apoE was determined to be comparable in a separate assay where apoE4 or apoE4(Δ272–299) was coated at 50 ng/well onto a 96-well ELISA plate. Polyclonal anti-apoE was diluted serially starting at 1:4,000 and fourfold thereafter and applied as the detection antibody using a similar protocol as above. Assay was performed in triplicates and repeated twice.

Detection of Aβ*56 Oligomer. Snap frozen hemibrains were slowly thawed on ice and dissected to isolate the cortex and hippocampus. A four step-fractionation protocol was used as described previously (5). Briefly, the tissue was homogenized in 500 microliters of Nonidet P-40 lysis buffer (0.05M Tris-HCl, 0.01% Nonidet P-40, 0.15M NaCl, 0.002M EDTA, 0.1% SDS) supplemented with PMSF, protease and phosphatase inhibitors (Sigma) and centrifuged at 3000g for 10 mins at 4C. The supernatant was collected and respun at 13000g for 90 mins at 4C to obtain an extracellularly enriched fraction. The resulting pellet received 500 microliters of TNT buffer (0.05M Tris-HCl, 0.15 NaCl, 0.1% Triton X-100) with inhibitors, was vortexed vigorously and pipetted repeatedly to resuspend the pellet, and spun at 13000g for 90 mins at 4C to obtain the intracellular fraction. The final remaining pellet was resuspended in RIPA buffer (0.05M Tris-HCl, 0.5% Triton X-100, 0.15 NaCl, 0.001 EDTA, 3% SDS, 1% sodium deoxycholate) with inhibitors and respun at 26000rpm for 90 mins at 4C. The resulting supernatant was considered the membrane-bound fraction, assayed for total protein content by the bicinchoninic acid assay (BCA kit, Pierce) and 500 μg of total protein was used for immunoprecipitation with 6E10 anti-Aβ (Signet). Lysates were eluted from the protein-G magnetic beads (Pierce), resolved by Western blot on 4–12% Bis/Tris Novex gels (Invitrogen), transferred to nitrocellulose membranes, and probed with biotinylated-6E10 (Signet, 1:2,000).

Immunohistochemistry, Image Collection, and Quantitative Analysis. Floating brain sections (30 μm) were immunostained with mouse anti-human Aβ (clone 3D6, Elan Pharmaceuticals), goat anti-apoE (Calbiochem), mouse anti-MAP2 (SIGMA), rabbit anti-calbindin (Swant), and rabbit anti-Fos (Chemicon). Fluorescence was detected with donkey anti-goat Alexa Fluor 488 and donkey anti-mouse Alexa Fluor 594 (Invitrogen). Diaminobenzidine (DAB) was detected with biotinylated goat anti-rabbit IgG (Vector) or biotinylated donkey anti-mouse IgG (Jackson Immunoresearch Labs) and the ABC Elite detection kit (Vector). DAB-immunostained sections were analyzed for percent area occupied by plaques using Image J software (NIH). The optical density of calbindin immunoreactivity and the number of Fos-positive granule cells were quantified as described, with minor modifications (4). To measure the levels of MAP2 immunoreactivity, images of four fluorescently immunostained sections (300 μm apart, starting at Bregma -1.6) from each mouse were collected with an epifluorescent upright microscope (Leica) using a 10× objective. Fluorescence intensities were quantified using Image J software (6).

Thioflavin S Staining and Quantification. Four serial sections (spaced 300 μm apart starting at Bregma –1.6) from each mouse were mounted onto Superfrost-plus slides (Fisher) and allowed to air-dry. After two rinses in PBS, the slides were incubated in 0.25% potassium permanganate for 5–10 min, washed 3 times in PBS, incubated in 2% $K_2O_5S_2$ and 1% oxalic acid for 5 min, washed 3 times in PBS, and stained with 0.015% Thioflavin S in 50% ethanol for 10 min. The slides were differentiated in 50% ethanol, rinsed with water followed by PBS, and coverslipped. Quantification of Thioflavin S-positive plaques was performed manually using a 20× objective by an investigator blinded to the mouse genotypes. Data represent the average number of plaques per section in the hippocampal subfield of four sections per animal from 4 to 7 mice per genotype.

Morris Water Maze. A water maze pool with a diameter of 122 cm containing opaque water at 22–23 °C was used with a platform 10 cm in diameter submerged 1.5 cm during hidden platform sessions (3, 7, 8). The platform base was switched to a black-and-white-striped mast (15 cm high) during cued training sessions. Mice were trained to locate the hidden platform for 5 d, in two daily sessions, each consisting of two 60-s trials 15 min apart. For the cued platform assessment, the mice were trained for 3 d in two daily sessions 3.5 h apart, each consisting of two 60-s trials with a 15-min intertrial interval. The platform location remained

constant during the hidden platform sessions and was changed for each cued platform session. Entry points were changed semi-randomly between trials. Twenty-four, 72, and 120 h after the last hidden platform training, a 60-s probe trial (platform removed) was performed. Performance was monitored with an EthoVision video-tracking system (Noldus Information Technology).

Elevated Plus Maze. The elevated plus maze assesses anxiety in naive rodents by challenging them to decide between exploring a novel environment and anxiogenic elements such as elevation and a brightly illuminated area or hiding in safe, enclosed narrow space (3, 8). The maze consists of two open arms and two closed arms equipped with rows of infrared photo-cells interfaced with a computer (Hamilton). Mice were placed individually into the center of the maze and allowed to explore for 10 min. The number of beam breaks was automatically recorded to calculate the amount of time spent, distance moved, and number of entries into the open or closed arms. Between tests, the maze was cleaned with 70% ethanol to standardize odors.

Statistical Analysis. Values are expressed as mean ± SEM or SD. The statistical significance of differences between means was assessed by *t* test or one-way ANOVA followed by Tukey–Kramer and Bonferroni post hoc tests (behavioral comparisons). The differences in mouse survival were assessed by Kaplan–Meier analysis.

1. Mucke L, et al. (2000) High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. *J Neurosci* 20:4050–4058.
2. Raber J, et al. (1998) Isoform-specific effects of human apolipoprotein E on brain function revealed in *ApoE* knockout mice: increased susceptibility of females. *Proc Natl Acad Sci USA* 95:10914–10919.
3. Andrews-Zwilling Y, et al. (2010) Apolipoprotein E4 causes age- and Tau-dependent impairment of GABAergic interneurons, leading to learning and memory deficits in mice. *J Neurosci* 30:13707–13717.
4. Palop JJ, et al. (2003) Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to Alzheimer's disease-related cognitive deficits. *Proc Natl Acad Sci USA* 100:9572–9577.
5. Lesné S, et al. (2006) A specific amyloid-beta protein assembly in the brain impairs memory. *Nature* 440:352–357.
6. Li G, et al. (2009) GABAergic interneuron dysfunction impairs hippocampal neurogenesis in adult apolipoprotein E4 knockin mice. *Cell Stem Cell* 5:634–645.
7. Harris FM, et al. (2003) Carboxyl-terminal-truncated apolipoprotein E4 causes Alzheimer's disease-like neurodegeneration and behavioral deficits in transgenic mice. *Proc Natl Acad Sci USA* 100:10966–10971.
8. Roberson ED, et al. (2007) Reducing endogenous tau ameliorates amyloid β-induced deficits in an Alzheimer's disease mouse model. *Science* 316:750–754.

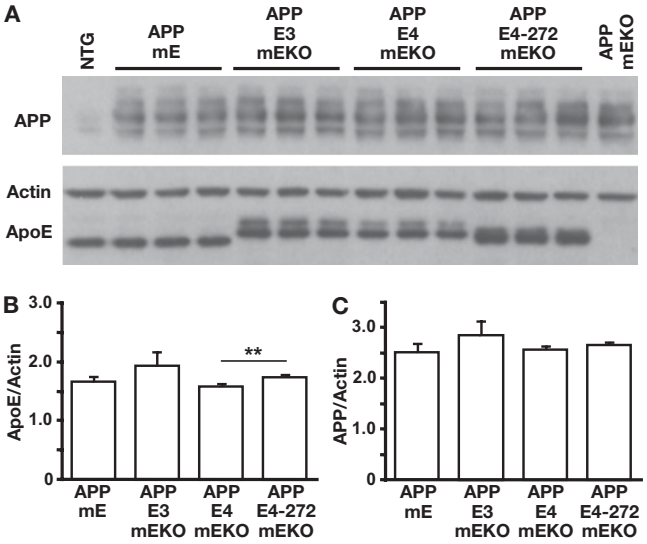

Fig. S1. Expression levels of $hAPP_{FAD}$ and apoE in the hippocampus of different transgenic mice. (*A*) Western blot analysis of $hAPP_{FAD}$, actin, and apoE in hippocampal lysates from different transgenic mice at 6–8 mo of age. (*B*) Quantification of the ratios of apoE to actin in hippocampal lysates from different transgenic mice. (*C*) Quantification of the ratios of $hAPP_{FAD}$ to actin in hippocampal lysates from different transgenic mice. Values are mean ± SEM $n = 3$ per genotype. $^{**}P < 0.01$. mEKO, mouse apoE knockout; E3, apoE3; E4, apoE4; E4-272, apoE4(Δ272–299).

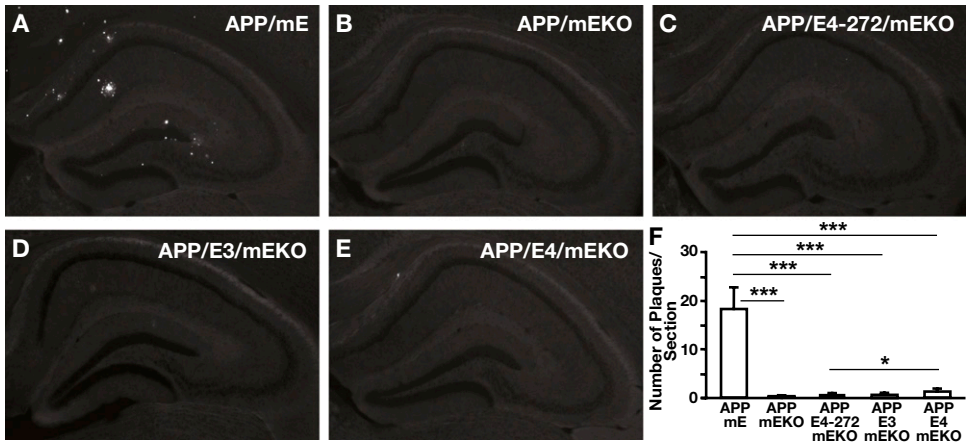

Fig. S2. Thioflavin S staining of hippocampal sections from different transgenic mice. (*A–E*) Serial sections from APP/mE (*A*), APP/mEKO (*B*), APP/E4-272/mEKO (*C*), APP/E3/mEKO (*D*), and APP/E4/mEKO (*E*) mice were stained with Thioflavin S. (*F*) Quantification of the average number of Thioflavin S-positive plaques per section. Values are mean ± SEM n = 4–9 per genotype. *$P < 0.05$, ***$P < 0.001$. mE, mouse apoE; mEKO, mouse apoE knockout; E3, apoE3; E4, apoE4; E4-272, apoE4(Δ272–299).

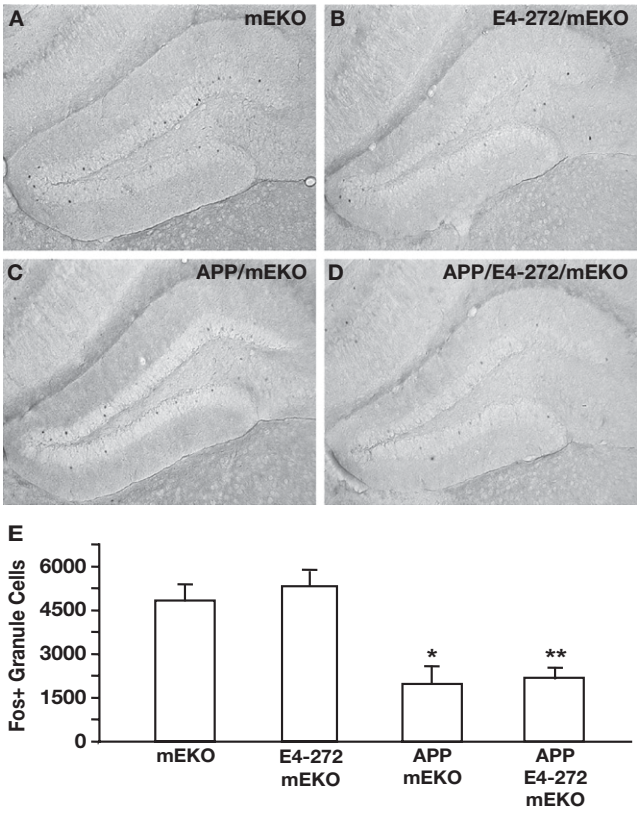

Fig. S3. Immunostaining of Fos in the dentate gyrus of different mice at 6–8 mo of age. (*A–D*) Representative anti-Fos images from mEKO (*A*), E4-272/mEKO (*B*), APP/mEKO (*C*), and APP/E4-272/mEKO (*D*) mice. (*E*) Quantification of Fos-positive granule cells in the dentate gyrus of different mice. Values are mean ± SEM n = 7–22 per genotype. *$P < 0.05$ versus mEKO mice, **$P < 0.01$ versus E4-272/mEKO mice. mEKO, mouse apoE knockout; E4-272, apoE4(Δ272–299).

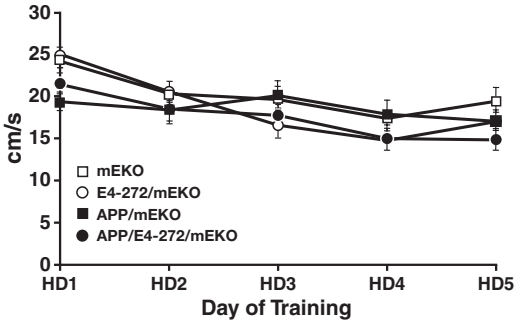

Fig. S4. There is no difference in swim speeds during the hidden platform trials in the Morris water maze among mEKO, E4-272/mEKO, APP/mEKO, and APP/E4-272/mEKO mice. Values are mean ± SEM n = 6–10 per genotype. mEKO, mouse apoE knockout; E4-272, apoE4(Δ272–299).

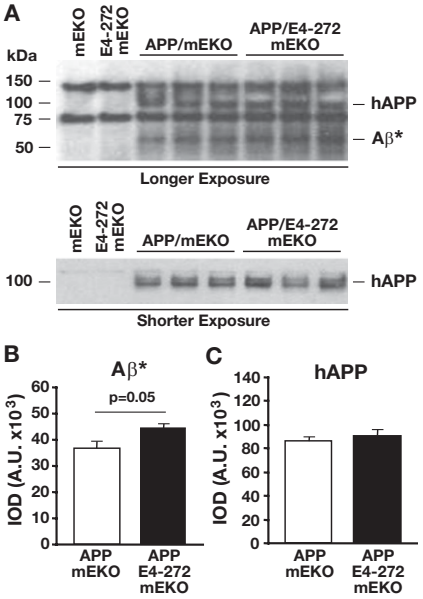

Fig. S5. Accumulation of pathogenic Aβ oligomers in the brains of $hAPP_{FAD}$/apoE4(Δ272–299)/mEKO mice with low levels of total Aβ. (*A*) Western blot analysis of Aβ*56 and hAPP. (*B*) Quantification of Aβ*56. (*C*) Quantification of hAPP. mEKO, mouse apoE knockout; E4-272, apoE4(Δ272–299); IOD, integrated optical density; A.U., arbitrary units.