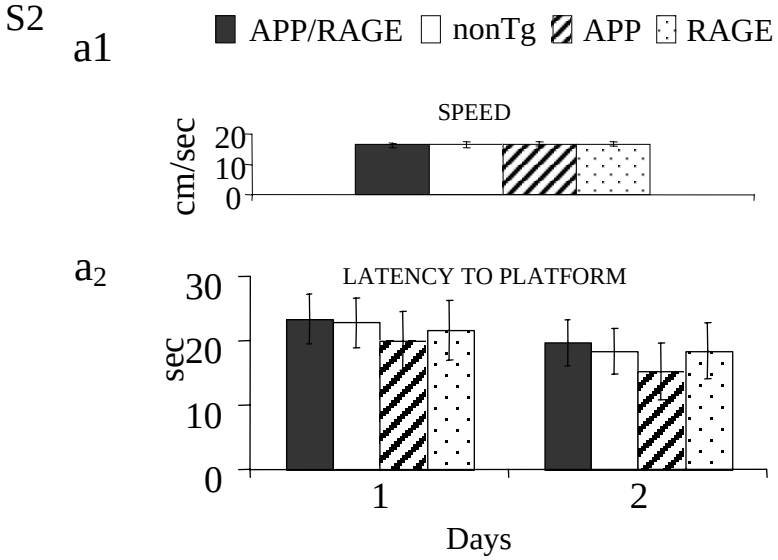
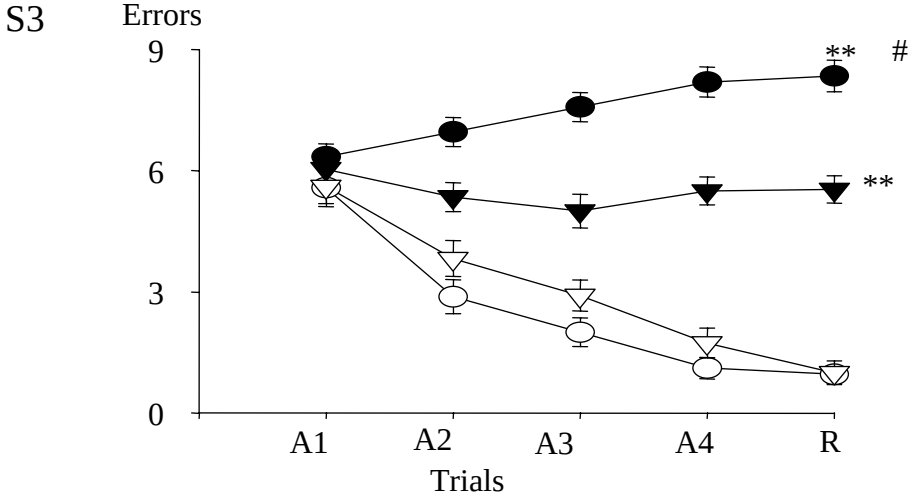
SUPPLEMENT: Arancio et al.

Supplementary Figure S1. Identification and characterization of transgenic RAGE mice. a. Southern analysis of tail DNA from two offspring of one founder for Tg RAGE mice (lanes 1-2) and the same for Tg DN-RAGE mice (lanes 3-4), and nonTg littermates (lanes 5-6): +, transgene present; -, transgene absent. *b.* Northern analysis of total RNA harvested from cerebral cortex (30 μg/lane) of mice hybridized with ^{32}P-labelled cDNA for human RAGE. Ethidium bromide staining of the same gel to display 28S RNA is shown below. *c.* Western blotting of cortical homogenates (100 μg/lane total protein) from Tg wt (wild-type)RAGE (lane 2) or Tg DN-RAGE mice (lane 3) subjected to SDS-PAGE (reduced; 10%) followed by immunoblotting with anti-RAGE IgG (6 μg/ml). Lane 1 demonstrates migration of endogenous murine RAGE from cortical homogenates in nonTg littermates. Migration of simultaneously run molecular weight standards is shown on the left. Densitometric analysis of gels is shown in the adjacent panel (Tg RAGE, n=5; nonTg, n=3; Tg DN-RAGE, n=4). *d.* Immunostaining (red) of neocortical sections from a Tg RAGE mouse, Tg DN-RAGE mouse or a nonTg littermate with anti-RAGE IgG. Sections were counterstained with hematoxylin. The above studies were performed on mice 3-4 months of age. Scale bar = 5 μm

Supplementary Figure S2. *a.* Groups of 3 to 4-month-old mice that had been studied in the radial arm water maze task (Tg mAPP/RAGE [APP/RAGE], nonTg, Tg mAPP [APP] and Tg RAGE [RAGE]) performed similarly during visible platform trials with respect to swim speed (*a1*) and time required to reach the platform (*a2*). Similar results were obtained with 5 to 6-month-old (b) and 12- to 13-month-old animals (not shown).



Supplementary Figure S3. Spatial learning and memory deteriorated in 12- to 13-month old Tg mAPP/RAGE mice compared with other genotypes (n=7-8 male/genotype). ** p<0.01 Tg mAPP/RAGE and mAPP mice compared with nonTg mice; # p<0.01 Tg mAPP/RAGE compared with Tg mAPP mice.



Supplementary Information related to the Methods Section.

Generation and characterization of transgenic mice. The human RAGE or DN-RAGE cDNA was subcloned into the 6apcI vector using the EcoRI cloning sites. For the production of Tg mice, transgenic cassettes were created by releasing the pvuI fragment, and these were injected into mouse B6CBAF$_1$/J oocytes. The oocytes were then implanted into pseudopregnant females which were subsequently mated with B6CBAF$_1$/J males resulting in the generation of three independent founders. These founders were bred into the C57BL/6 background eight times. Founders were identified by Southern analysis of tail DNA using a probe encoding the full-length RAGE cDNA (Yan et al., 1996; Nature 382:685-691). DNA was digested with EcoR I. Transgene expression was also studied by Western blotting with rabbit antibody produced to human RAGE (which is immunoreactive with murine RAGE) and by immunostaining with the same antibody (Yan et al., 1996). Tg mice overexpressing an alternatively spliced human (h)APP minigene that encodes hAPP695, hAPP751, and hAPP770 bearing mutations linked to familial AD (V717F, K670N/M671L) have been described (line J20) (Mucke et al., 2000; J. Neurosci 20:4050-4058). NonTg littermates matched for sex were used so that contributions of the different backgrounds would be comparable. Expression of human APP was studied by immunoblotting with 6E10 (mouse monoclonal antibody to human Aß[1-16], Signet Labs, Dedham, MA), and that of human and murine APP was analyzed with 369W (immunoreactive with both mouse and human APP; provided by Dr. S. Gandy, The Farber Institute for Neuroscience, Philadelphia).

Immunocytochemical and histochemical analyses were performed using standard methods. After 6 min of perfusion with saline, brains were divided sagittally in half; one half was frozen at -80°C, and the other was immersed in paraformaldehyde (4%) for 26 hr. Serial vibratome sections (20 µm) were made and immunocytochemical analyses were performed using rabbit anti-RAGE IgG (50 µg/ml). To detect plaque-associated microglia and astrocytes, sections were processed by free-floating dual-color peroxidase immunohistochemistry following our published procedures (Kobayashi et al., 1994; Arch Pathol. Lab Med. 118:1127-1129). Microglia were identified by staining with a mouse monoclonal antibody to CD11b (1:1000 dilution; Serotec, Durham, NC) or mouse monoclonal anti-phosphotyrosine antibody (dilution 1:800; Sigma-Aldrich, St. Louis MO). Reactive astrocytes were visualized with rabbit anti-GFAP antibody (dilution 1:3000; Dako, Carpinteria, CA). Sections were subsequently reacted with anti-Aß

antibody 3D6 (dilution 1:3000; provided by Eli Lilly) to identify Aß deposits. The numbers of plaque-associated microglial or astrocytic clusters were counted in the entire hippocampus and cortex from multiple sections from the same level in each Tg animal group. The area of each region counted for each animal at each age group was determined by image analysis using Image Pro Plus software. AChE activity was determined histochemically as described (Kobayashi et al., 1994). For on-slide immunohistochemistry, sites of primary antibody binding were visualized using peroxidase-conjugated goat anti-rabbit IgG and 3-amino-9-ethyl carbozole (Sigma) as the detection system. Analysis of synaptophysin immunoreactivity was studied as described (Hsia A et al., 1999; PNAS[USA] 96:3228-3233; Buttini et al., 1999; J. Neurosci 19:4867-4880). Microscopy was performed using a Biorad confocal microscope (Radian 2000) and image analysis employed the Lap Top System (Bio-Rad Labs, Richmond, CA). For quantitation, Confocal Assistant and NIH Image (to determine the percent area occupied) was employed.

Immunoblotting was performed on protein extracts of hippocampus or cerebral cortex using rabbit anti-phosphoCREB IgG (1:100; Cell Signaling), rabbit anti-CREB IgG (1:100; Cell Signalling), anti-phosphoCAMKII IgG (1:2000; Promega), anti-CAMKII IgG (1:2000; Promega), anti-phosphoErk1/2 IgG (1:1000; Cell Signaling), anti-Erk1/2 IgG (1:1000; Cell Signaling), anti-phosphoJnk/sapk IgG (1:200; Cell Signaling), anti-Jnk/sapk IgG (1:200; Cell Signaling), anti-phospho-p38 (1:200; Cell Signaling), anti-p38 (1;500; Cell Signaling), anti-RAGE IgG (6.5 µg/ml), and 6E10 (1:1000; Signet). SDS-PAGE (10%; reduced) following by electrophoretic transfer of proteins to nitrocellulose was performed with the indicated antibody. Sites of binding of primary antibody were visualized with affinity-purified peroxidase-conjugated goat anti-rabbit IgG or goat anti-mouse IgG (1:5000; Sigma) followed by addition of ECL substrate (Amersham-Pharmacia, Piscataway, NJ) and measurement of chemiluminescence. Relative intensity of gel bands was determined by scanning autoradiograms and quantitation by NIH image.

Behavioral studies in transgenic mice in the radial arm water maze. Experimenters were not aware of the genotype of the animals at the time of testing. The apparatus for testing spatial memory was a round steel white tank (120 cm x 120 cm) filled with water maintained at 24-25°C and made opaque by the addition of powdered milk. Within the tank were stainless steel walls (30 cm long and 20 cm wide) that extended from the floor to a height of 50 cm. The walls were

positioned so as to produce six swim paths, or “arms", radiating from a central area. Spatial cues were present on the walls and ceiling of the testing room. At the end of one of the arms was a clear 10 cm plexiglas platform (submerged 1.5 cm beneath the surface of the water). The platform remained in the same location for every trial in one day, but was moved about randomly from day to day. For each trial, the mouse started the task from a different randomly chosen arm. The mouse could not use its long-term memory of the location of the platform on previous days, but had to rely on the short-term memory of its location on the day in question based on spatial cues that were present in the room. Each trial lasted one minute and errors were counted each time the mouse entered the wrong arm with all four paws or needed more than 20 sec to reach the platform. After each error, the mouse was gently pulled back to the start arm for that trial. At the end of the trial, the mouse remained on the platform for 30 sec. After four consecutive trials, the mouse was placed in its home cage for 30 min, then returned to the maze and administered a retention trial. Testing was considered complete when wild-type mice reached an asymptotic performance (below 2 errors on trials 4 and 5). This generally took about 10 training days. The scores for each mouse on the last three days of testing were averaged and used for statistical analysis.

Visual and motor deficits were tested through visible-platform training. This was performed in the same pool, but without arms and with the platform marked by a black flag. Testing was performed as the studies with the radial arm water maze were completed. Platform location was varied randomly from trial-to-trial to eliminate the potentially confounding contribution of extra-maze spatial cues. Four trials per day were administered during the two days of testing. Each animal was allowed to swim for 1 min from a randomly chosen location in the pool. Once the mouse reached the platform (or was helped to be there if he did not reach it on his own), the animal was allowed to rest there for 30 sec. Failures to reach the platform were scored as 60 sec. In both visible-platform and hidden-platform versions, mice were placed in the pool facing toward the wall at the beginning of testing. Mice were monitored by a camera mounted in the ceiling directly above the pool, and all trials were stored on videotape. Data obtained with visible platform training were recorded and analyzed with a video-tracking system (HVS-2020, HVS Image, UK).

Electrophysiologic studies on hippocampal slices from Tg mice. Briefly, transverse hippocampal slices of a thickness of 400 micron were maintained in an interface chamber at 29ºC. They were perfused with saline solution (124.0 mM NaCl, 4.4 mM KCl, 1.0 mM Na_2HPO_4, 25.0 mM $NaHCO_3$, 2.0 $CaCl_2$, 2.0 mM $MgSO_4$, 10 mM glucose) continuously bubbled with 95% O_2 and 5% CO_2. Slices were permitted to recover for at least 90 minutes before recordings. CA1 extracellularly recorded field-excitatory postsynaptic potentials (fEPSP) were monitored by placing both the stimulating and the recording electrodes in CA1 stratum radiatum (Trinchese et al, 2004). Basal synaptic transmission (BST) was assayed by determining input-output relations from fEPSP; the input was the peak amplitude of the fiber volley and the output as the slope of the fEPSP. For long-term potentiation (LTP) experiments, baseline stimulation was delivered every minute at an intensity that evoked a response of $\approx$ 35% of the maximum evoked response. Baseline responses were recorded for 30 minutes prior to beginning experiments in order to assure stability of the response. LTP was induced using theta-burst stimulation (4 pulses at 100 Hz, with the bursts repeated at 5 Hz and each tetanus including 3 ten-burst trains separated by 15 seconds). In some experiments, ten-burst trains were substituted by twenty-burst trains.

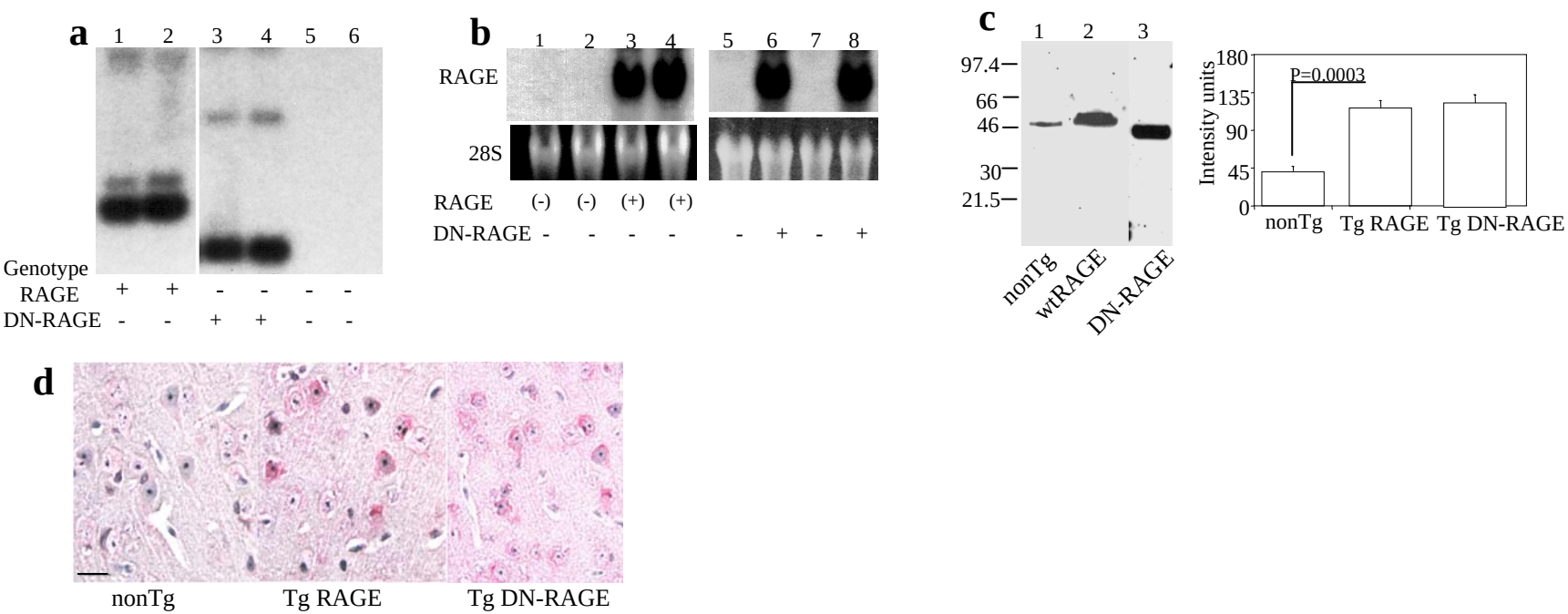


Figure S1a-d. Arancio et al.