Aggregation Inhibiting Test and Disaggregation Test. To test the inhibitory effect of Edaravone on αβ aggregation, 1 μM Aβ42 monomer was first incubated with a serial gradient of Edaravone (Boda Pharmaceutical) in DMEM at 37 °C for 10 d. For the Thioflavin T (ThT) fluorescence assay, the results were then incubated with 5 μM ThT solution. Fluorescence intensity was monitored at an excitation wavelength of 450 nm and an emission wavelength of 482 nm by a spectrophotometer (Synergy H4; BioTek). Each experiment was performed in triplicate and repeated three times. For Western blot assays, the resultant solutions were mixed with the same volume of 2× loading buffer without reducing agent and subjected to electrophoresis. Nonincubation Aβ samples (Aβ oligomers) were used as controls. All samples were loaded on SDS/PAGE [4–10–15% (wt/vol) acrylamide] gradient gels, and separated Aβ species of whole gels were transferred to nitrocellulose membranes. The blots were probed with the Aβ-specific antibodies 6E10 (Covance) followed by incubation with IRDye 800CW secondary antibodies (Li-COR). The membranes were scanned using the Odyssey fluorescent scanner. For transmission electron microscopy (TEM) negative staining, copper grids were preplaced on the bottom of wells in a 24-well plate where 1 μM Aβ42 monomer and Edaravone were incubated. Thereafter, the mixtures were stained with 2% (wt/vol) aqueous phosphotungstic acid for 30 s. The Aβ fibril images were collected using a Joel 1200 EX TEM equipped with Megaview 3 Digital Camera. To investigate the disaggregation effect of Edaravone on preformed Aβ fibrils, 1 μM Aβ42 monomer was preincubated at 37 °C for 10 d to form Aβ fibril, followed by incubation with the same gradients of Edaravone mentioned in aggregation inhibiting tests for an additional 3 d at 37 °C. For ThT and TEM assays, the following processing steps were performed as methods mentioned above. For all experiments, Aβ42 monomer without preincubation was set as a control.

Aβ Fragment Competition Assay. Full-length Aβ42 was obtained from American Peptide, and Aβ42 fragments (aa1-6, aa 7–12, aa 13–18, aa 19–24, aa 25–30, aa 31–36, and aa 37–42) were synthesized by GL Biochem. Aβ42 (1 μM) was incubated with Edaravone (3 μM) in the presence or absence of Aβ peptide fragments (3 μM) for 10 d, followed by 5 μM ThT solution incubation. Fluorescence was measured by a spectrophotometer (Synergy H4; BioTek). For controls, different Aβ peptide fragments (3 μM) were incubated in DMEM with or without Aβ42 (1 μM) for 10 d. ThT solution was added, and the fluorescence was measured.

Cell Culture and Relevant Assays. To investigate the antagonistic effects of Edaravone against Aβ toxicity in vitro, human neuroblastoma cell line SH-SY5Y was applied. After 72-h seeding, the cells were incubated with 5 μM Aβ oligomers with or without Edaravone at different concentrations for an additional 72 h. A cell viability assay using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] methods was performed as described previously (1). For neurite outgrowth, SH-SY5Y cells were cultured for 7 d in a medium with 1% FBS and 10 μM all-trans-retinoic acid (RA) (Sigma), followed by incubation with 1 μM Aβ oligomers with or without Edaravone at different concentrations for 72 h. The cell images were taken under microscopy, the length of the five longest neurites per view field were measured, and data from six view fields per group were analyzed. To further test the protective effects of Edaravone against Aβ toxicity in vitro, primary cortical neurons were isolated from newborn 129sv mice brain and cultured on poly-D-lysine precoated coverslips for 7-d seeding, followed by the incubation with 1 μM Aβ oligomers with or without Edaravone at different concentrations for 72 h. After the length of axons was measured by ImageJ software, the cortical neurons were subjected to ROS assay according to the protocol provided by the manufacturer (Cat STA-347, Cell Biolabs). The samples were read and quantified with Fluostar OPTIMA from BMG Labtech. Primary cortical neurons after the 72-h treatments were also stained in live with propidium iodide (PI). Briefly, live neurons were treated with PI diluted (2 μg/mL) in the Hepes buffer for 15 min and then thoroughly washed with Hepes buffer. Cells were fixed with 2% (wt/vol) paraformaldehyde in PBS for 20 min and counterstained with DAPI (1:1,000) for 5 min. Cell images were collected with B50 Fluorescence microscope. To investigate the effects of Edaravone on Aβ metabolism, SH-SY5Y-APP695 cells overexpressing APP were cultured with 10% (vol/vol) FBS in DMEM and treated with or without Edaravone (0.3, 1, or 3 μM) for 24 h, and the cell lysates per group were subjected to Western blot for BACE1, CTU, CTFβ, sAPPα, and sAPPβ. To further investigate the effects of Edaravone on Tau phosphorylation, the lysates of SH-SY5Y cells treated with 1 μM Aβ oligomers with or without Edaravone at different concentrations for 72 h were subjected to Western blot for pS396-Tau, total Tau, pS9-GSK3β, and GSK3β. The detailed steps of Western blot are described below.

Animals. APP/PS1 transgenic mice were obtained from Jackson Laboratory and bred in the Animal House of the Third Military Medical University. All mice husbandry procedures performed were approved by the Third Military Medical University Animal Welfare Committee. Immediately after completion of medication and behavioral tests, all mice were humanely killed with an overdose of anesthetics and perfused transcardially with saline, and the brains were harvested as previously described (2). The left hemisphere per animal was frozen for biochemical analysis, and the right hemisphere was fixed in 4% paraformaldehyde for histological analysis.

Behavioral Tests. Mice in the prevention experiment underwent multiple behavioral tests including the Morris water maze, Y-maze, and open field, and mice in the treatment experiment (time schedule was shown in Fig. S3A) and the oral medication experiment underwent the Morris water maze. In a spontaneous alternation test, mice were allowed to move freely through a Y-maze during a 5-min session. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The percentage of alternation was calculated as the total number of alternation × 100/(total number of arm entries − 2). In open field test, each mouse was placed in the center of the open field apparatus for 3 min. Rearing, grooming, defecation, and urination per mouse were recorded. Additionally, paths were tracked using a computer tracking system (Limelight; ActiMetrics) and the distance traveled was collected. Before the Morris water maze test, the swimming abilities of all mice were tested, and one mouse in the Tg control group in treatment experiments was excluded due to a disability in swimming. The Morris water maze test consisted of three platform trials per day for 5 consecutive days, followed by a probe trial. Performance was video-recorded and analyzed by image analyzing software (ANY-maze; Stoelting). In platform trials, the distance of path and escape latency were measured. In probe trials, the time spent in each quadrat and annulus crossings was measured. The novel arm exploration test was also performed in a Y-maze.

Supporting Information

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SI Materials and Methods

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SI Materials and Methods
One arm was blocked (defined as novel arm), and mice were allowed to explore the other two arms (home arm and familiar arm) for 5 min. After a 2-h interval, mice were allowed to explore freely all three arms for 3 min. The number of novel arm entries and time spent in the novel arm were recorded.

**Pathological Staining for Parenchymal and Vascular Aβ Deposition.** For Aβ plaques in parenchyma, a series of five equally spaced tissue sections (~1.3 mm apart) spanning the entire brain was stained with Congo red and 6E10 (Aβ antibody) using a free-floating method as described previously (3). The area fraction and number of Congo red- or 6E10-positive Aβ plaques in neocortex and hippocampus was quantified by ImageJ software. To assess the degree of vascular Aβ deposition, a novel method was applied in the present study. Using 1a4 (smooth muscle actin antibody) and 6E10 double immunofluorescence, image per vessel were collected with a confocal fluorescence microscope (Radiance 2000MP; Bio-Rad). Each vessel was scored on a four-grade scale for the severity of CAA: grade 0, no deposition; grade 1, mild deposition, the area of Aβ deposition is less than one third of the vessel perimeter; grade 2, moderate deposition, the area of Aβ deposition is greater than one third but less than 75% of the vessel perimeter; grade 3, severe deposition, the area of Aβ deposition is greater than 75% of the vessel perimeter. The grade of each vessel in five equally spaced sections per animal was scored, and the percentage of each grade was calculated. Prussian blue staining was also conducted according to the manufacturer’s protocols (Polysciences), microhemorrhage profiles were counted on five equally spaced sections of each animal under microscopy, and the average number of hemosiderin deposits per section was calculated.

**ELISA and Secretase Activity Assay.** Frozen brain was homogenized in liquid nitrogen, and part of the resultant powder was successively extracted with TBS, 2% (wt/vol) SDS, and 70% (vol/vol) formic acid (FA) solutions. Concentrations of Aβ40, Aβ42 (Covance), IL-6, IL-1β, INF-γ, TNF-α (eBioscience, BMS603, BMS6002, BMS606, and BMS607, respectively) in brain extracts were quantitatively measured by ELISA according to the manufacturer’s instructions. α- and β-secretase activities in fresh brain tissues were measured by relevant kits according to the manufacturer’s instructions (R&D Systems). In addition, the remaining part of the brain powder was dissolved in specific buffers provided by KeyGEN Biotech. The resultant supernatant was subjected to assays for CuZn-superoxide dismutase (SOD) activity, glutathione peroxidase (GSH-Px) activity, hydroxyl radicals scavenging ability, and malondialdehyde (MDA) concentration, according to the relevant protocols provided by the manufacturer (KeyGEN Biotech).

**Histological Staining.** For immunostaining, the free-floating immunohistochemistry method was used. The sections were stained for NeuN, ChAT, MAP-2, CD45, GFAP, pSer396-Tau, 3-NT, and Caspase-3 antibodies in 1:100–1:200 dilution, visualized by DAB for ChAT, CD45, GFAP, 3-NT, and pSer396-Tau or by Alexa Fluor fluorescent dyes for NeuN and MAP-2 and Caspase-3. Images per staining were collected and quantified by ImageJ, yielding the area fraction of positive staining against the area of tissue analyzed. TUNEL staining was used to detect apoptotic cells. The sections were labeled by in the situ Death Detection Kit, POD (Roche) according to the manufacturer’s instructions. Animals in the treatment experiment underwent Golgi staining using the manufacturer’s protocols (FD rapid GolgiStain kit).

**Western Blot.** The levels of molecules or enzymes involving Aβ production and degradation, phosphorylated Tau, oxygen stress, and synapse-related proteins were analyzed using Western blot. Proteins in the animal brain homogenate were extracted with RIPA buffer. Samples were loaded on SDS/PAGE [4–10% or 4–10–15–18% (wt/vol) acrylamide] gels, and separated proteins were transferred to nitrocellulose membranes. The blots were probed with the following antibodies: anti-APP C-Terminal (171610; Millipore), which recognizes APPI and sAPPβ, CTFα and CTFβ; anti-BACE1 (Millipore); anti-Aβ (6E10, which also recognizes sAPPα; Abcam); anti-NEP (Millipore); anti-RAGE (Millipore); anti-LRP (5A6; Calbiochem); anti-IDE (Epitomics); antiphosphorylated Tau including pS396 (Signalway), pT231 (Signalway), pS199 (Epitomics), and pS262 (Abcam); anti-total tau (tau-5; Abcam); anti-GSK3β (Abcam); anti-pS9-GSK3β (Abcam); anti–4-HNE (Abcam); anti–3-NT (Abcam); anti–3-DNPH (Millipore); anti-SNAP25 (Millipore); anti-Synaptophysin (Millipore); anti-Synapsin I (Millipore); anti-VAMP1 (Epitomics); anti–PSD95 (Millipore); anti–β-actin (Sigma-Aldrich); and anti-GAPDH (Millipore). The membranes were incubated with IRDye 800CW secondary antibodies (Li-COR) and incubated with IRDye 800CW secondary antibodies (Li-COR) and scanned using the Odyssey fluorescent scanner. The band density was all normalized to β-actin or GAPDH when analyzing. It is worth noting that for detection of DNPH, 5-μL samples was pretreated with 5 μL 12% (wt/vol) SDS and 10 μL DNPH solution for 20 min at room temperature. Then, the resultant was neutralized with 7.5 μL neutralization solution and subjected to electrophoresis.

Fig. S1. Competition assays mapping the putative Edaravone binding site on Aβ42. The Aβ fibrillation was assessed by Thioflavin T (ThT) assay. (A) Effect of different Aβ42 peptide fragments (six amino acids, 3 μM) on the suppression of Edaravone (3 μM) on Aβ1–42 fibrillation (n = 3, mean ± SEM, one-way ANOVA, Tukey’s test, *P < 0.05, **P < 0.01, vs. Aβ1–42+EDA+ Aβ13–18; *P < 0.05, **P < 0.01, vs. Aβ1–42). Only the peptide of Aβ42 fragment aa 13–18 interfered with the impact of Edaravone on the fluorescence intensity of Aβ42, indicating the peptide aa 13–18 competitively abolished the suppressive effect of Edaravone on Aβ fibrillation. (B) Effect of different Aβ42 peptide fragments on Aβ42 fibrillation (n = 3, mean ± SEM, one-way ANOVA, Tukey’s test). All Aβ42 peptide fragments have no effect on the formation of Aβ fibrils. (C) Fibrillation ability of different Aβ peptide fragments (n = 3, mean ± SEM, one-way ANOVA, Tukey’s test, **P < 0.01, vs. Aβ1–42). All Aβ42 peptide fragments showed comparable fluorescence intensity to blank, suggesting that none of them forms fibrils by itself. (D) Schematic of Edaravone binding site in the sequences of Aβ42. The putative Edaravone binding site falls within the β-strand conformation region, which explains why Edaravone suppresses Aβ42 fibril formation.
Fig. S2. Earavone antagonizes Aβ neurotoxicity in vitro. (A) Representative images of SH-SY5Y cells treated with 5 μM Aβ with or without 15 μM Edaravone. (Scale bar, 25 μm.) (B) Quantitative analyses of cell viability in different treatment groups. SH-SY5Y cells were treated with 5 μM Aβ plus different concentrations of Edaravone, and the control group was treated with the same volume of culture medium. Each assay was performed in triplicates, and the experiment was repeated three times (mean ± SEM, one-way ANOVA, Tukey’s test, **P < 0.01). (C) Comparison of neurite length among different treatment groups. SH-SY5Y cells were pretreated with 10 μM all-trans-retinoic acid (RA) for 7 d to stimulate neurite outgrowth, followed by treatment with 1 μM Aβ plus different concentrations of Edaravone. The length of five longest neurites per view field were measured, and data from six view fields per group were analyzed (mean ± SEM, one-way ANOVA, Tukey’s test, **P < 0.01). (D–H) Primary cortical neurons isolated from newborn 129sv mice brain were cultured on poly-λ-lysine precoated coverslips for 7 d when living neurons have matured, followed by neurite length measurement, ROS assay, and PI staining. (D) Representative images of primary mouse cortical neurons cultured in 1 μM Aβ with or without 3 μM Edaravone. (Scale bar, 50 μm.) (E) Quantification of neurite length of cortical neurons (mean ± SEM, one-way ANOVA, Tukey’s test, **P < 0.01). (F) Representative images of PI staining in cultured primary cortical neurons treated with 5 μM Aβ with or without 15 μM Edaravone. The neurons were counterstained with DAPI. (Scale bar, 100 μm.) (G) Quantification of percentages of PI-labeled neurons (mean ± SEM, one-way ANOVA, trend test). (H) Quantification of the ROS products in SH-SY5Y cells by ROS assays (mean ± SEM, one-way ANOVA, trend test).
Fig. S3. Edaravone improves behavioral performances of AD mice in the prevention experiment. (A) Time schedule of behavioral tests. (B) Comparison of distance to platform among groups \((n = 7\) for 9-mo Wt Ctrl, \(n = 11\) for 9-mo Tg EDA, \(n = 8\) for 9-mo Tg Ctrl, mean ± SEM, two-way ANOVA, Tukey’s test, \(*P < 0.05\)). (C) Swim speed (cm/s) calculated by the equation velocity = distance/time during platform trials in the Morris water maze were analyzed (mean ± SEM, two-way ANOVA, Tukey’s test). There was no difference in swim speed among groups. (D) Comparison of time spent in quadrants among groups (mean ± SEM, one-way ANOVA, Tukey’s test, \(*P < 0.05\), **\(P < 0.01\)). Q3, quadrant where the platform is located; o.a., average values of the time spent in all other quadrants. Nine-month Wt Ctrl and 9-mo Tg EDA but not 9-mo Tg Ctrl mice showed a clear preference for Q3. (E) Comparison of total number of entries into arms in spontaneous alternation test among groups \((n = 7\) for 9-mo Wt Ctrl, \(n = 11\) for 9-mo Tg EDA, \(n = 8\) for 9-mo Tg Ctrl, mean ± SEM, one-way ANOVA, Tukey’s test, \(*P < 0.05\), **\(P < 0.01\)). (F and G) Quantification of total entries (F) and percentage of time spent in novel arm (G) (mean ± SEM, one-way ANOVA, Tukey’s test, \(*P < 0.05\), **\(P < 0.01\)). (H) Comparison of grooming number in open field test \((n = 7\) for 9-mo Wt Ctrl, \(n = 11\) for 9-mo Tg EDA, \(n = 8\) for 9-mo Tg Ctrl, mean ± SEM, one-way ANOVA, Tukey’s test, \(*P < 0.05\), **\(P < 0.01\)).
Fig. S4. Edaravone improves AD-like phenotypes of male APP/PS1 mice. Male APP/PS1 mice were administered with Edaravone (12-mo Tg EDA-Male) or saline (12-mo Tg Ctrl-Male) by i.p. injection from 9 mo of age to 12 mo of age. Age-matched male WT mice with an i.p. injection of saline were used as a control (12-mo Wt Ctrl-Male), n = 6 per groups. (A) Escape latency (seconds) during platform trials in the Morris water maze (mean ± SEM, two-way ANOVA, Tukey’s test, *P < 0.05, **P < 0.01). (B and C) Comparison of the number of annulus crossing in the probe test (B) and rearing in the open field test (C) among groups (mean ± SEM, one-way ANOVA, Tukey’s test, **P < 0.01). (D and E) Representative images and quantification of Congo red- (D) and 6E10-positive Aβ plaques (E) (mean ± SEM, Student t test, **P < 0.01 vs. 12-mo Tg Ctrl-Male). (Scale bar, 1 mm.) (F–H) ELISA analyses of Aβ40 and Aβ42 in TBS fraction (F), SDS fraction (G), and FA fraction (H) of brain homogenates in 12-mo Tg Ctrl-Male and 12-mo Tg EDA-Male groups (mean ± SEM, Student t test, *P < 0.05, vs. 12-mo Tg Ctrl-Male).
Fig. S5. Effects of Edaravone on Aβ plaque density, microhemorrhage, and expression of NEP, IDE, LRP, and RAGE. (A and B) Comparisons of the density of Congo red- and 6E10-positive Aβ plaques in the neocortex (NC) and hippocampus (HC) between Edaravone-treated and control groups in prevention (A) and treatment (B) experiments (n = 9 for 9-mo Tg EDA, n = 8 for 9-mo Tg Ctrl, n = 9 for 12-mo Tg EDA, n = 9 for 12-mo Tg Ctrl, mean ± SEM, Student t test, *P < 0.05, **P < 0.01, vs. control). (C) Representative images of microhemorrhage in 12-mo Tg EDA and 12-mo Tg Ctrl groups. (Scale bar, 50 μm.) (D) Comparisons of microhemorrhage profiles between the Edaravone-treated group and the control group in prevention (Left) and treatment (Right) experiments (mean ± SEM, Student t test, *P < 0.05, vs. age-matched Control). (E) Representative Western blot images and quantitative analyses of Aβ-degrading enzymes NEP and IDE in brain homogenates of treatment experiment (n = 8 per group, mean ± SEM, Student t test). There was no difference among NEP, IDE, LRP, and RAGE between 12-mon Tg Ctrl and 12-mo Tg EDA groups.
Fig. S6. Edaravone alleviates neuronal and synaptic degeneration in the prevention experiment. (A) Representative images of NeuN immunofluorescence (Left) and ChAT immunohistochemistry (Right) in hippocampus and its subregions of CA1, CA2, and CA3 in the prevention experiment. (Scale bar, 400 μm.) (B) Quantification of area fraction of NeuN- or ChAT-positive staining in 9-mo Tg Ctrl and 9-mo Tg EDA groups (n = 8 for 9-mo Tg Ctrl, n = 9 for 9-mo Tg EDA, mean ± SEM, Student t test, *P < 0.05, **P < 0.01, vs. 9-mo Tg Ctrl). (C–E) Representative Western blot images and quantification of postsynaptic density protein 95 (PSD-95), synaptosomal-associated protein 25 (SNAP-25), major synaptic vesicle protein p38 synaptophysin (SYP), the vesicle-associated membrane protein-1 (VAMP-1), and synapsin I (Syn I) in brain homogenates (n = 8 for each group, mean ± SEM, Student t test, **P < 0.01, vs. 9-mo Tg Ctrl).

Fig. S7. Edaravone ameliorates Tau pathologies of APP/PS1 mice in the prevention experiment. (A) Representative pS396-Tau immunostaining images of 9-mo Tg Ctrl and 9-mo Tg EDA groups. [Scale bar, 400 (hippocampus) and 100 μm (subregion.)] (B) Quantification of area fraction of pS396-Tau-positive staining in whole hippocampus and its subregions (n = 8 for 9-mo Tg Ctrl, n = 9 for 9-mo Tg EDA, mean ± SEM, Student t test, *P < 0.05, **P < 0.01, vs. 9-mo Tg Ctrl). (C and D) Representative Western blot images (C) and quantification (D) of phosphorylated Tau assessed by Western blot at multiple sites including pS199-Tau, pS262-Tau, pS396-Tau, pT231-Tau, and total Tau (T-Tau) in brain homogenates (n = 8 for each group, mean ± SEM, Student t test, *P < 0.05, **P < 0.01, vs. 9-mo Tg Ctrl).
Edaravone attenuates oxidative stress in the brains of APP/PS1 mice. (A and B) Comparison of the activities of SOD (A) and GSH-Px (B) between 12-mo Tg Ctrl and 12-mo Tg EDA groups (n = 9 per group, mean ± SEM, Student t test, *P < 0.05, **P < 0.01, vs. 12-mo Tg Ctrl). (C) Hydroxyl radicals scavenging ability of brain tissues from 12-mo Tg Ctrl and 12-mo Tg EDA groups (n = 9 per group, mean ± SEM, Student t test, **P < 0.01, vs. 12-mo Tg Ctrl). (D) Levels of lipid peroxidation product MDA in brain homogenates of 12-mo Tg Ctrl and 12-mo Tg EDA groups (n = 9 per group, mean ± SEM, Student t test, **P < 0.01, vs. 12-mo Tg Ctrl). (E and F) Representative Western blot image (E) and quantification (F) of lipid peroxidation product 4-HNE in brain homogenates of 12-mo Tg Ctrl and 12-mo Tg EDA groups (n = 8 per group, mean ± SEM, Student t test, **P < 0.01, vs. 12-mo Tg Ctrl). (G–J) Representative Western blot image and quantification of protein peroxidation products DNPH (G and H) and 3-NT (I and J) in brain homogenates of 12-mo Tg Ctrl and 12-mo Tg EDA groups (n = 8 per group, mean ± SEM, Student t test, **P < 0.01, vs. 12-mo Tg Ctrl). (K–N) To further detect the changes in different brain regions, separate groups of APP/PS1 mice aged 9 mo were treated with or without Edaravone for 30 d (10-mo Tg EDA, n = 6; 10-mo Tg Ctrl, n = 6). (K and L) Levels of 3-NT in hemibrain homogenate mixture vs. in cortex and hippocampus homogenates of 10-mo Tg Ctrl and 10-mo Tg EDA groups show the representative Western blot images and quantification results, respectively (mean ± SEM, Student t test, *P < 0.05, **P < 0.01, vs. 10-mo Tg Ctrl). (M and N) Representative images and quantification of 3-NT immunostaining in neocortex, entorhinal cortex, and CA3 region of hippocampus from 10-mo Tg Ctrl and 10-mo Tg EDA groups (n = 6 per group, mean ± SEM, Student t test, **P < 0.01, vs. 10-mo Tg Ctrl). (Scale bar, 100 μm.)
Fig. S9. Oral Edaravone administration alleviates cognitive deficits and Aβ burden of APP/PS1 mice. APP/PS1 mice were orally administered with Edaravone in drinking water from 3 to 12 mo of age; 12-mo Tg EDA-Oral, 12-mo-old APP/PS1 transgenic mice treated with Edaravone (n = 8); 12-mo Tg Ctrl, 12-mo-old APP/PS1 transgenic mice without Edaravone treatment (n = 8); 12-mo Wt Ctrl, 12-mo-old WT mice without Edaravone treatment (n = 7). (A) Pharmacokinetic curves of Edaravone through different administration routes. The bioavailability of oral Edaravone was 38% of the i.v. delivery. (B) Escape latency (seconds) during platform trials in the Morris water maze (mean ± SEM, two-way ANOVA, Tukey’s test, *P < 0.05). (C) Comparison of the number of annulus crossing in the probe trial among groups (mean ± SEM, one-way ANOVA, Tukey’s test, **P < 0.01). (D and F) Representative images of Congo red- (D) and 6E10-positive Aβ plaques (F) of 12-mo Tg Ctrl and 12-mo Tg EDA-Oral groups. (Scale bar, 1 mm.) (E and G) Comparison of the area fractions of Congo red- or 6E10-positive Aβ plaques in the neocortex (NC) and hippocampus (HC) between 12-mo Tg Ctrl and 12-mo Tg EDA-Oral groups (mean ± SEM, Student t test, *P < 0.05, **P < 0.01, vs. 12-mo Tg Ctrl). (H–J), ELISA analyses of Aβ40, Aβ42, and total Aβ in the TBS fraction (H), SDS fraction (I), and FA fraction (J) of brain homogenates of 12-mo Tg Ctrl and 12-mo Tg EDA-Oral groups (mean ± SEM, Student t test, *P < 0.05, **P < 0.01, vs. 12-mo Tg Ctrl).