

## Supplementary information

### **Versatile lipoproteins-inspired nanocomposites rescue Alzheimer's cognitive dysfunction by promoting A $\beta$ degradation and lessening oxidative stress**

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## Experimental Section

### Materials

Phospholipid was purchased from Avanti (Shanghai, China). RLA (RLASHLRKLRKRLLEEQAAQQIRLQAEAFQARLKSWFEPLVEDM) and A $\beta$ <sub>1-42</sub> were from Top-Peptide Co., Ltd (Shanghai, China). DSPE-NH<sub>2</sub>, HOOC-TK-COOH, NH<sub>2</sub>-PEG<sub>2000</sub>, DSPE-NHS, NH<sub>2</sub>-TK-NH<sub>2</sub>, NHS-PEG<sub>3400</sub>-MAL and ANG (Angiopep-2, TFFYGGSRGKRNNFKTEEY) were purchased from Ruixi biotechnology Co., Ltd (Xi'an, China). Mouse anti-human A $\beta$ <sub>1-42</sub> antibody and FITC-A $\beta$ <sub>1-42</sub> were purchased from China Peptides Co., Ltd (Shanghai, China). Colloidal gold labeled goat anti-mouse IgG and peroxidase-conjugated goat anti-mouse IgG were from Beijing Biodragon Immunotechnologies Co., Ltd (Beijing, China). Thioflavin-T was purchased from Aladdin (Shanghai, China). The rest were commercially qualified reagents.

### Synthesis and identification of DSPE-TK-mPEG<sub>2000</sub>

In a typical DSPE-TK-mPEG<sub>2000</sub> synthesis, the carboxyl group of HOOC-TK-COOH was reactivated with EDC and DMAP at 1.1:1.1:0.2 molar ratios in 10 mL of DMF, followed by adding dropwise 10 mL of DMF containing 2.0 g of mPEG<sub>2000</sub>-NH<sub>2</sub>. The reaction continuously proceeded for 48 h at 37 °C with gentle agitation. The reacted mixture was dialyzed against distilled water for 48 h to remove free EDC and DMAP (MWCO:1000). The expected HOOC-TK-mPEG<sub>2000</sub> was obtained after lyophilization with the freeze dryer (Beijing Boyikang Laboratory Instrument Co., Ltd., Beijing, China). Then, DSPE-NH<sub>2</sub> was conjugated with HOOC-TK-mPEG<sub>2000</sub> to obtain DSPE-TK-mPEG<sub>2000</sub> with the same reaction conditions except for an extra reverse dialysis (MWCO:3500) for 24 h to remove mPEG<sub>2000</sub>-TK-mPEG<sub>2000</sub>. The structure of the product was verified by <sup>1</sup>H-NMR (AVANCEIII HD 400 spectrometer, Bruker, Germany) and biomass spectrometry (Bio-MS) (Autoflex speed MALDI-TOF, Bruker, Germany)

#### **Synthesis and identification of DSPE-TK-PEG<sub>3400</sub>-ANG**

To synthesize DSPE-TK-PEG<sub>3400</sub>-ANG, Mal-PEG<sub>3400</sub>-NHS and NH<sub>2</sub>-TK-NH<sub>2</sub> at a molar ratio of 1:2 were dissolved in trichloromethane, and an adequate amount of triethylamine were added and reacted for 24 h at room temperature. The reaction mixture was concentrated by reducing pressure and subsequently large amounts of ice diethyl ether were introduced to precipitate the product, and the expected Mal-PEG<sub>3400</sub>-TK-NH<sub>2</sub> was obtained by vacuum drying process. DSPE-TK-PEG<sub>3400</sub>-MAL was then synthesized by linking Mal-PEG<sub>3400</sub>-TK-NH<sub>2</sub> with DSPE-NHS referring to the same procedure. Finally, DSPE-TK-PEG<sub>3400</sub>-MAL and ANG were dispersed in PBS buffer (0.1 M, pH 7.4) and reacted for 24 h at room temperature. The reaction mixture was

dialyzed against distilled water for 48 h to remove extra impurities (MWCO 3500 Da).

The expected DSPE-TK-PEG<sub>3400</sub>-ANG was obtained after lyophilization, followed by <sup>1</sup>H-NMR and Bio-MS measurements by to confirm the target structure.

### **ROS responsibility of DSPE-TK-mPEG<sub>2000</sub> and DSPE-TK-PEG<sub>3400</sub>-ANG**

5 mg of DSPE-TK-mPEG<sub>2000</sub> was dispersed in 2 mL PBS buffer (10 mM, pH 7.4), followed by adding H<sub>2</sub>O<sub>2</sub> at the final concentration of 100 μM and incubating for 3 h at 37°C. After dialysis and lyophilization, Bio-MS was involved to identify the molecular weight (MW) of the H<sub>2</sub>O<sub>2</sub>-treated product. The MW change of DSPE-TK-PEG<sub>3400</sub>-ANG before and after H<sub>2</sub>O<sub>2</sub> incubation was also detected according to the same procedure.

### **Preparation and characterization of RLA-rHDL@ANG**

Phospholipid, RLA and DSPE-TK-mPEG<sub>2000</sub> in the mass ratio of 24:1:4 were dissolved in methanol and dichloromethane (1:3, v/v) mixture. The suspension was evaporated at 50°C under a high vacuum using a rotary evaporator (RE2000B, Yarong Biochemical Instrument Factory, Shanghai, China). The lipid film was then rehydrated in 15 mL of PBS buffer (10 mM, pH 7.4). The turbid emulsion was subsequently probe sonicated using a SCIENTZ-IID sonifier (SCIENTZ, China) at 250 W output for 3 min. Here, rHDL fabricated with RLA (designated as RLA-rHDL) was obtained after filtration with 0.22 μm millipore filter and ultracentrifugation (100 kD, Millipore). The carrier RLA-rHDL without DSPE-TK-mPEG<sub>2000</sub> addition (termed as RLA-Lip) was prepared following the same method, and applied to *in vitro* investigation of neuroprotection experiment and *in vivo* imaging.

Next, 0.5 mg of DSPE-TK-PEG<sub>3400</sub>-ANG was added in RLA-rHDL and further incubated at 4°C for 6 h. RLA-rHDL assembling with ANG (designated as RLA-rHDL@ANG) was obtained after 0.22 µm filtration and ultracentrifugation (100 kD, Millipore). RLA-rHDL@ANG without RLA addition was termed as Lip@ANG and applied to *in vivo* and *in vitro* therapeutic efficacy evaluation.

Size distribution and Zeta potential of RLA-rHDL and RLA-rHDL@ANG were performed using a NICOMP 380/ZLS Zeta potential/particle size analyzer (PSS. NICOMP Particle Systems, Santa Barbara, CA, USA). Plasma colloidal stability of RLA-rHDL, Lip@ANG and RLA-rHDL@rHDL was determined in terms of the variations in particle size under the simulative physiological condition of DMEM containing 10% FBS. In brief, three vehicles were suspended at a concentration of 10% FBS solution in DMEM at 37°C and 100 rpm. At predetermined time points (0, 12, 24, 36, 48, 60 and 72 h), the particle sizes were measured and recorded.

### **ROS scavenging of RLA-rHDL@ANG**

ROS scavenging ability of RLA-rHDL@ANG was evaluated through monitoring the change of H<sub>2</sub>O<sub>2</sub> concentration. In briefly, 2 mL of RLA-rHDL@ANG was mixed with 2 mL of H<sub>2</sub>O<sub>2</sub> (200 µM) with gentle agitation at 37°C. Then, 100 µL of the mixture was withdrawn at predetermined time points and detected with the hydrogen peroxide assay kit to calculate the percentage of H<sub>2</sub>O<sub>2</sub> degradation. H<sub>2</sub>O<sub>2</sub> mixed with the same volume PBS 7.4 buffer was served as control.

To further speculate the potential effects of ROS scavenging ability on microstructure transformation, the morphology of RLA-rHDL@ANG with and without H<sub>2</sub>O<sub>2</sub>

treatment was observed under transmission electron microscopy (TEM) (FEI Company, Hillsboro, OR, USA). Briefly, 100  $\mu\text{L}$  of RLA-rHDL@ANG was incubated with  $\text{H}_2\text{O}_2$  at the final concentration of 100  $\mu\text{M}$  for 3 h at  $37^\circ\text{C}$ , and to obtain the product of RLA-rHDL@ANG after  $\text{H}_2\text{O}_2$  incubation (termed as RLA-rHDL@ANG $_{\text{H}_2\text{O}_2}$ ). Then, RLA-rHDL@ANG and RLA-rHDL@ANG $_{\text{H}_2\text{O}_2}$  were added on the copper grid and then negatively stained with 1% uranyl acetate. After drying at room temperature, TEM was employed to observe and compare the morphological differences. RLA-rHDL@ANG $_{\text{H}_2\text{O}_2}$  was further applied to *in vitro*  $\text{A}\beta_{1-42}$  binding assay and intracellular  $\text{A}\beta_{1-42}$  degradation.

### **Preparation of $\text{A}\beta_{1-42}$ monomer and oligomer**

$\text{A}\beta_{1-42}$  was dissolved in precooling hexafluoroisopropanol (HFIP) at 1 mM and reposed for 1 h at room temperature. HFIP was evaporated overnight in the fuming cupboard and the resultant peptide film was stored at  $-20^\circ\text{C}$ . Before use, the peptide film was resuspended in dimethyl sulfoxide (DMSO) at the concentration of 5 mM and bath sonicated for 10 min to obtain  $\text{A}\beta_{1-42}$  monomer solution. For the preparation of  $\text{A}\beta_{1-42}$  oligomers,  $\text{A}\beta_{1-42}$  monomer DMSO solution (5 mM) was diluted with PBS 7.4 buffer to 100  $\mu\text{M}$  and incubated at  $4^\circ\text{C}$  for 24 h.

### **Immuno-electron microscopy observation**

Immuno-electron microscope (IEM) colloid gold labelling technique was applied to confirm the combining capacity between RLA-rHDL@ANG $_{\text{H}_2\text{O}_2}$  and  $\text{A}\beta_{1-42}$  monomers. Specifically, 500  $\mu\text{L}$  of RLA-rHDL@ANG $_{\text{H}_2\text{O}_2}$  was mixed with 20  $\mu\text{L}$  of  $\text{A}\beta_{1-42}$  monomer solution at  $37^\circ\text{C}$  for 2 h. After removing uncombined  $\text{A}\beta_{1-42}$  with mini-

column centrifugation method, mouse anti-human A $\beta$ <sub>1-42</sub> antibody was added into prior suspension, and then incubated at 37°C for 1 h. The primary antibody was removed with the same centrifugal condition. Eluent containing nanoparticles was collected, then mixed with colloidal gold-labelled goat anti-mouse IgG and incubated at 37°C for 2 h. After centrifugation at 10000 rpm and 4°C for 20 min to remove dissociative secondary antibody, the precipitate was resuspended in 500  $\mu$ L PBS 7.4 and observed under TEM after negatively staining with 1% uranyl acetate. Control group was conducted as above procedure except for the absence of primary antibody incubation.

#### **Fluorescence co-localization assay**

DiI was selected as hydrophobic fluorescent probe for labelling RLA-rHDL@ANG<sub>H<sub>2</sub>O<sub>2</sub></sub>. 1 mg/mL of FITC-A $\beta$ <sub>1-42</sub> was mixed with DiI-labeled RLA-rHDL@ANG<sub>H<sub>2</sub>O<sub>2</sub></sub> at volume ratio of 1/5 and incubated for 2 h at 37°C. Mini-column centrifugation method was utilized to remove free FITC-A $\beta$ <sub>1-42</sub>. Eluent was gathered, diluted and dropped onto the microslide. Fluorescence images were observed under inverted fluorescence microscope (Olympus Corporation, Tokyo, Japan).

#### **Enzyme linked immunosorbent assay**

A $\beta$ <sub>1-42</sub> monomer solution was diluted with carbonate buffer (0.05 M, pH 9.6) to the final concentration of 25  $\mu$ g/mL, followed by dropwise adding into enzyme linked immunosorbent assay (ELISA) plate. After preincubation at 37°C for 3 h and further incubation at 4°C overnight, A $\beta$ <sub>1-42</sub> solution was withdrawn and washed three times. 5% BSA solution was added, incubated at 37°C for 3 h and rinsed thrice. 200  $\mu$ L RLA-rHDL@ANG<sub>H<sub>2</sub>O<sub>2</sub></sub> assembling with different RLA concentrations from 5  $\mu$ g/mL to 250

$\mu\text{g/mL}$  was added and incubated for 0.5, 1 and 2 h, respectively. 100  $\mu\text{L}$  anti-apoE antibody and peroxidase-conjugated goat anti-mouse IgG were successively added and incubated at  $37^\circ\text{C}$  for 1 h. Then the suspension was discarded, rinsed five times and patted dry. Ultimately, the chromogenic solution A and B were added, mixed uniformly and developed at  $37^\circ\text{C}$  for 15 min in dark. The optical density (OD) at 450 nm was measured with a multi-mode microplate reader (ELx808, BioTek Instruments Inc., Agilent Technologies, CA, USA). Relative binding amount (RBA) was calculated according to the following formula (1). Control group was set as above without adding RLA-rHDL@ANG<sub>H<sub>2</sub>O<sub>2</sub></sub>.

$$\text{RBA} = \frac{\text{OD}_{\text{experiment group}} - \text{OD}_{\text{control group}}}{\text{OD}_{\text{control group}}} \quad (1)$$

where RBA is the relative binding amount;  $\text{OD}_{\text{experiment group}}$  and  $\text{OD}_{\text{control group}}$  are the OD of experiment group and control group, respectively.

### **Thioflavin-T fluorescence assay**

Thioflavin-T (ThT) fluorescence assay was applied to investigate the effects of RLA-rHDL@ANG<sub>H<sub>2</sub>O<sub>2</sub></sub> on A $\beta_{1-42}$  monomer and oligomer. In brief, RLA-rHDL@ANG<sub>H<sub>2</sub>O<sub>2</sub></sub> at different RLA concentrations from 0 to 250  $\mu\text{g/mL}$  was blended with 1  $\text{mg/mL}$  A $\beta_{1-42}$  monomer solution in volume ratio of 20/1, and incubated at 100 rpm and  $37^\circ\text{C}$ . Simultaneously, A $\beta_{1-42}$  monomer was mixed with Lip@ANG after incubation of H<sub>2</sub>O<sub>2</sub> (termed as Lip@ANG<sub>H<sub>2</sub>O<sub>2</sub></sub>) and PBS 7.4 as control. 80  $\mu\text{L}$  of samples were withdrawn at set interval points and injected into 400  $\mu\text{L}$  ThT solution (10  $\mu\text{M}$ , pH 7.4) for determination of the fluorescence intensity (Ex: 400 nm, Em: 480 nm). A $\beta_{1-42}$  monomer was substituted for A $\beta_{1-42}$  oligomer to investigate the dissociation effects as the same

procedure.

### **Evaluation of brain-targeting ability *in vitro***

The mouse brain microvascular endothelial cells (bEnd.3) were seeded into a 1.2 cm<sup>2</sup> cell culture insert at a density of 1×10<sup>5</sup> cells/well and incubated for several days till transepithelial electrical resistance (TEER) reached to 250 Ω/cm<sup>2</sup> monitored with Millicell electrical resistance system (Millipore, USA). Coumarin-6 labeled RLA-rHDL and RLA-rHDL@ANG were prepared with the same method as described previously. 0.5 mL of Coumarin-6 labeled carriers diluted with Hanks buffer were added to the donor chamber and 1.5 mL Hanks buffer was added to the reception pool. 200 μL of samples were withdrawn from reception pool at predetermined time points for detecting Coumarin-6 fluorescence intensity and fresh Hanks buffer was supplemented. TEER was determined before and after experiment to judge the integrity of BBB. The apparent permeability (P<sub>app</sub>) was calculated as the following formula (2):

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{C_0} \times \frac{1}{A} \quad (2)$$

where  $\frac{dQ}{dt}$  was the permeability rate (nmol/s),  $C_0$  was the initial concentration (nmol/mL) in the donor chamber, and  $A$  was the surface area (cm<sup>2</sup>) of the membrane filter.

### **Microglia and neuron degradation of Aβ<sub>1-42</sub>**

Mouse microglial cells (BV2) and mouse hippocampal neurons (HT22) were cultured in confocal culture dishes (Corning, New York) at a density of 1×10<sup>4</sup> cells/well and incubated overnight. All dishes were treated with 10 μg/mL FITC-Aβ<sub>1-42</sub> for another 15 min with or without RLA-rHDL@ANG<sub>H<sub>2</sub>O<sub>2</sub></sub> addition. DAPI and LysoTracker Red were



applied as the indicators of cell nucleus and lysosome under a confocal microscope (Olympus, Tokyo, Japan). Colocalization coefficient was analyzed by Image J software.

### **Quantification of intracellular A $\beta$ <sub>1-42</sub> elimination**

BV2 and HT22 cells were seeded in a 24-well plate at a density of  $2.5 \times 10^4$  cells/well and incubated for 24 h. The cells were further treated with 10  $\mu\text{g}/\text{mL}$  A $\beta$ <sub>1-42</sub> in the presence or absence of RLA-rHDL@ANG<sub>H<sub>2</sub>O<sub>2</sub></sub>. After incubation for another 3 h, the cells were washed with normal saline thrice and then lysed using RIPA lysis buffer. The total protein content of cell lysates was determined with BCA protein assay kit, and the remaining intracellular A $\beta$ <sub>1-42</sub> was quantified via an ELISA kit and normalized to total protein in the lysates.

### **Investigation of neuroprotective effect**

Two different neurotoxic models were applied in this study. In the first model, BV2 and HT22 cells were seeded in 96-well plates at a density of  $1 \times 10^4$  cells/well and incubated for 24 h. Cells were treated with RLA-Lip and RLA-rHDL@ANG for 1 h before 24 h incubation of A $\beta$ <sub>1-42</sub> oligomers (25  $\mu\text{M}$ ). ROS level was monitored with DCFH-DA probe and imaged under inverted fluorescence microscope. In the second model, BV2 and HT22 cells were incubated with RLA-rHDL, Lip@ANG and RLA-rHDL@ANG for 1 h, cells were extensively rinsed and processed with H<sub>2</sub>O<sub>2</sub> at a final concentration of 100  $\mu\text{M}$  at 37°C for 12 h. H<sub>2</sub>O<sub>2</sub> was replenished every 4 h in this process. 100  $\mu\text{L}$  CCK8 working solution was added for another 1 h incubation, and OD was determined at 450 nm. Cells without above formulations treatment and H<sub>2</sub>O<sub>2</sub> incubation served as control group. Blank group was considered and set up simultaneously. Cell viability

was calculated referring to the equation (3).

$$\text{Cell viability} = \frac{\text{OD}_{\text{experiment group}} - \text{OD}_{\text{blank group}}}{\text{OD}_{\text{control group}} - \text{OD}_{\text{blank group}}} \times 100\% \quad (3)$$

where  $\text{OD}_{\text{experiment group}}$ ,  $\text{OD}_{\text{control group}}$  and  $\text{OD}_{\text{blank group}}$  are the OD of experiment group, control group and blank group, respectively.

## **Animals**

Kunming female mice ( $20 \pm 2$  g) were purchased from the Laboratory Animal Center of Xuzhou Medical University (Xuzhou, China), and maintained in a sterile environment, and allowed free access to sterilized food and distilled water. All experiments were performed in accordance with the Regulations of the Experimental Animal Administration, issued by the State Committee of Science and Technology of the People's Republic of China (November 14, 1988), and with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Xuzhou Medical University and had received approval from the Animal Investigation Ethics Committee of Xuzhou Medical University (202208S109).

## ***In vivo* imaging of brain-targeted properties**

Thirty-six Female Kunming mice were randomly divided into three groups and received a tail intravenous injection of Cy5.5-labeled RLA-Lip, RLA-rHDL and RLA-rHDL@ANG at the Cy5.5 dosage of 0.1 mg/kg. Images were taken after 0.5, 2, 4 and 12 h post injection with the NightOWL LB 983 *in vivo* Image System (Berthold Technologies, Germany). The brains as well as major organs were dissected from the mice after euthanasia at 4 h and subjected for *ex vivo* imaging.

To determine *in vivo* biodistribution, individual tissues, including the brain, heart,

liver, spleen, lung and kidney were collected, washed and weighed. Equivalent amount of methanol was mixed with the organs and homogenized with a homogenizer (Changzhou Yuexin Instrument Manufacturing Co. LTD, China) to extract Cy5.5 from the tissues. The turbid fluid was centrifuged at 8000 rpm for 10 min. The supernatant was prepared for measuring Cy5.5 fluorescence intensity.

### **Treatment of AD Model Mice**

Accumulative evidences showed that continuous stimulation of toxic  $A\beta_{1-42}$  to hippocampal cholinergic neurons would trigger amyloid metabolism disorders, drastic neurotoxicity and cognitive function impairment, which was recognized as a classical AD model to investigate AD pathological mechanisms.<sup>1,2</sup> Here, *in vivo* AD mice models were well-established via micro-injection of  $A\beta_{1-42}$  into the left hippocampus of mice.<sup>3</sup> Morris water maze test and pathological assay including hematoxylin-eosin staining (HE) and immunofluorescence analysis were subjected to testify the success of animal modeling. AD mice were divided into five groups ( $n = 10$  per group): sham + saline, AD + saline, AD + RLA-rHDL, AD + Lip@ANG, AD + RLA-rHDL@ANG. The mice were treated with different formulations at RLA dosage of 10 mg/kg via tail vein daily for 4 weeks.

### **Morris water maze behavioral test**

The Morris water maze composed of a round pool which was evenly divided into four quadrants (diameter: 120 cm, height: 50 cm). One of quadrants was equipped with a 9-cm platform under the surface of water 1 cm to make it invisible. Each quadrant contained one geometric figure on the walls. An automatic heater was utilized to

maintain the water temperature at 24°C~26°C to avoid hypothermia. Performance of each mouse was recorded via a digital camera and the data was obtained by the equipped software. On the first 4 days, the mice were trained four times daily at four different quadrants before the experimental test. The cutoff time for the escape latency to reach the platform was set as 60 s. If the platform was not found within 60 s, the escape latency was recorded as 60 s and the mouse was gently guided to the platform and allowed to remain on the platform for 15 s. On the fifth day, each mouse was placed into water from the opposite position of the escape platform and permitted to swim freely for 60 s. Swimming path, escape latency, swimming time spent in the targeted quadrant as well as crosses over the platform site were counted with a computerized tracking system.

### **Immunofluorescence analysis**

Immunofluorescence analysis was performed on paraformaldehyde-fixed paraffin-embedded sections. The mice were sacrificed, followed by heart perfusion with 100 mL normal saline and 50 mL 4% paraformaldehyde. The brains were gently harvested, immobilized in 4% paraformaldehyde for 24 h, dehydrated and embedded in paraffin. The brains were serially sectioned at 5  $\mu$ m in thickness. For immunofluorescence, the brain sections were incubated with pH 6.0 citrate buffer for 15 min, and then treated with 0.3% peroxide for 10 min. Subsequently, the sections were blocked with 10% goat serum for 1 h, and then incubated with the anti-A $\beta$  and 8-OHG antibody overnight at 4°C. Secondary antibodies including goat anti-mouse IgG Dylight649 and goat anti-rabbit IgG Alexa Flour 488 were applied for fluorescence microscope imaging. Then,

the slices were stained with DAPI for 10 min. The fluorescence images were observed under fluorescence microscope and the fluorescence intensity was analyzed by Image J software.

### **Biosafety evaluation**

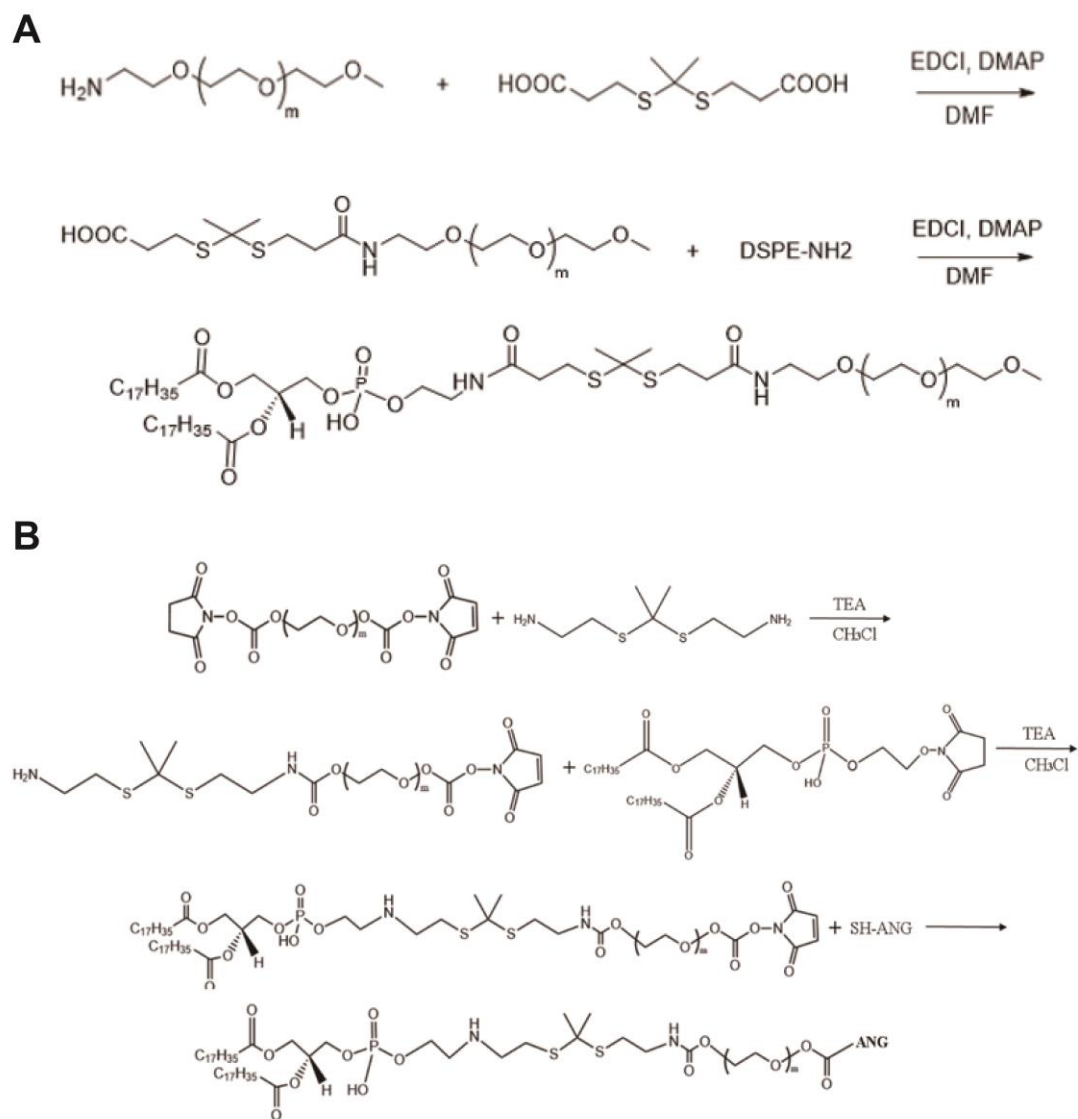
To evaluate the potential toxicity of different formulations after treatment for 4 weeks, the mice were sacrificed and the major organs were collected and fixed in 4% paraformaldehyde for 24 h. Then the tissues were dehydrated, embedded in paraffin and sliced into 5 µm in thickness. After dewaxing, the sections were further stained with hematoxylin and eosin before subjected to microscope observation.

### **Statistical analysis**

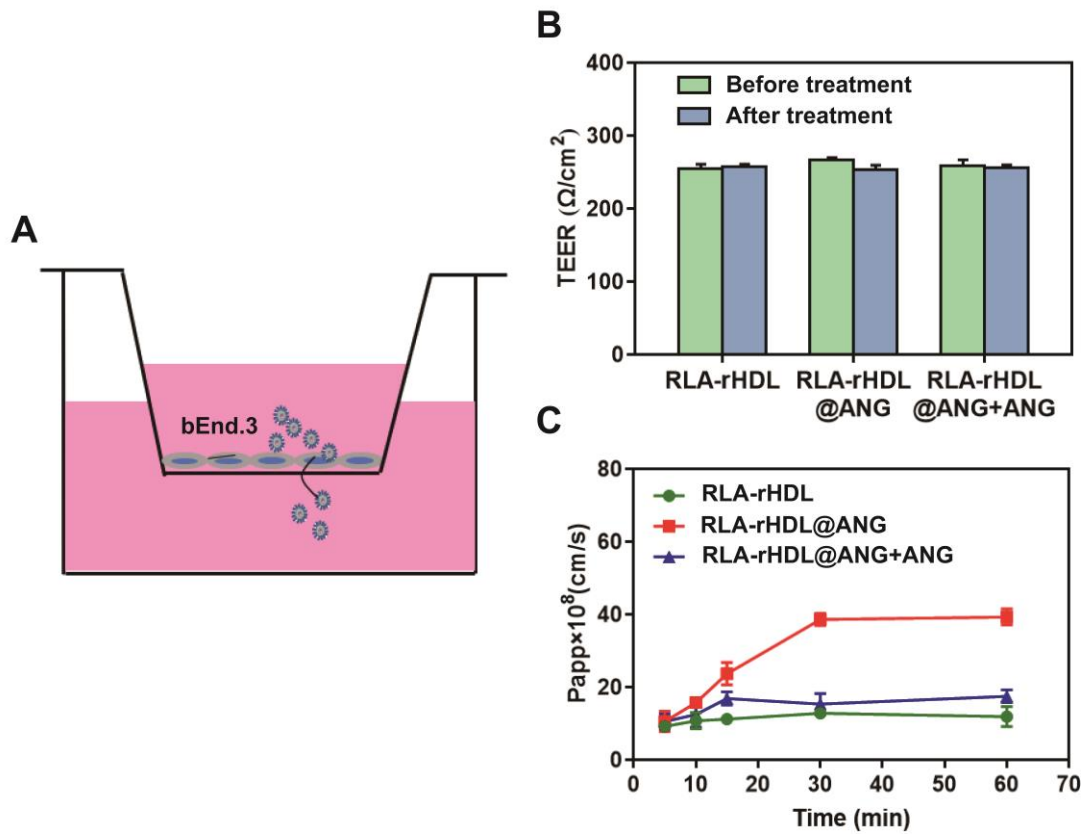
Results were analyzed by SPSS 19.0 and presented as mean ± SD. The statistical significance of group differences was evaluated using two-tailed Student's t-test or one-way ANOVA. Statistical significance was noted as follows: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

### **Reference**

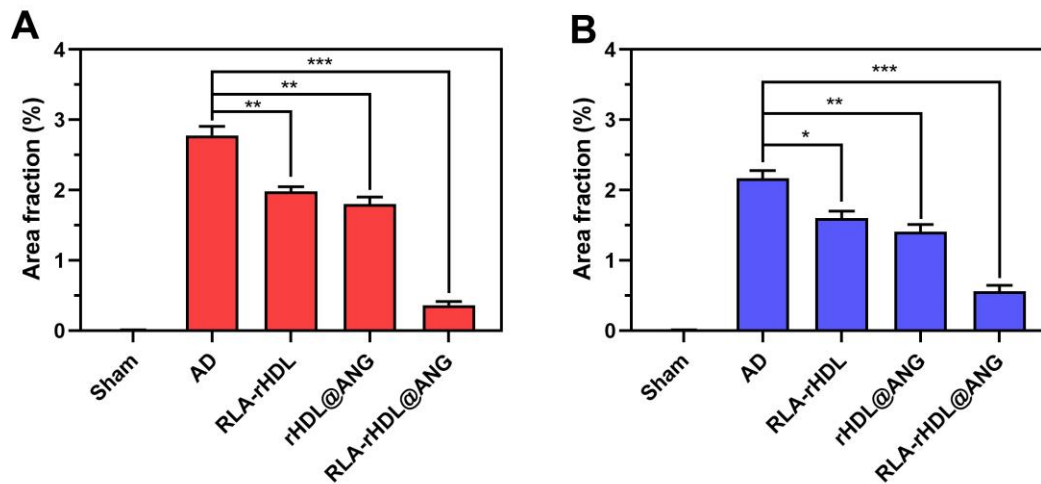
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- 2 M. Yizibula, Z. Wusiman, N. Abudouhalike, B. Maimaitiming, *Folia Neuropathol*, 2020, **58**, 365-376.
- 3 J. Guo, L. Chang, C. Li, M. Li, P. Yan, Z. Guo, C. Wang, Q. Zha, Q. Wang, *Metab Brain Dis*, 2017, **32**, 57-68.



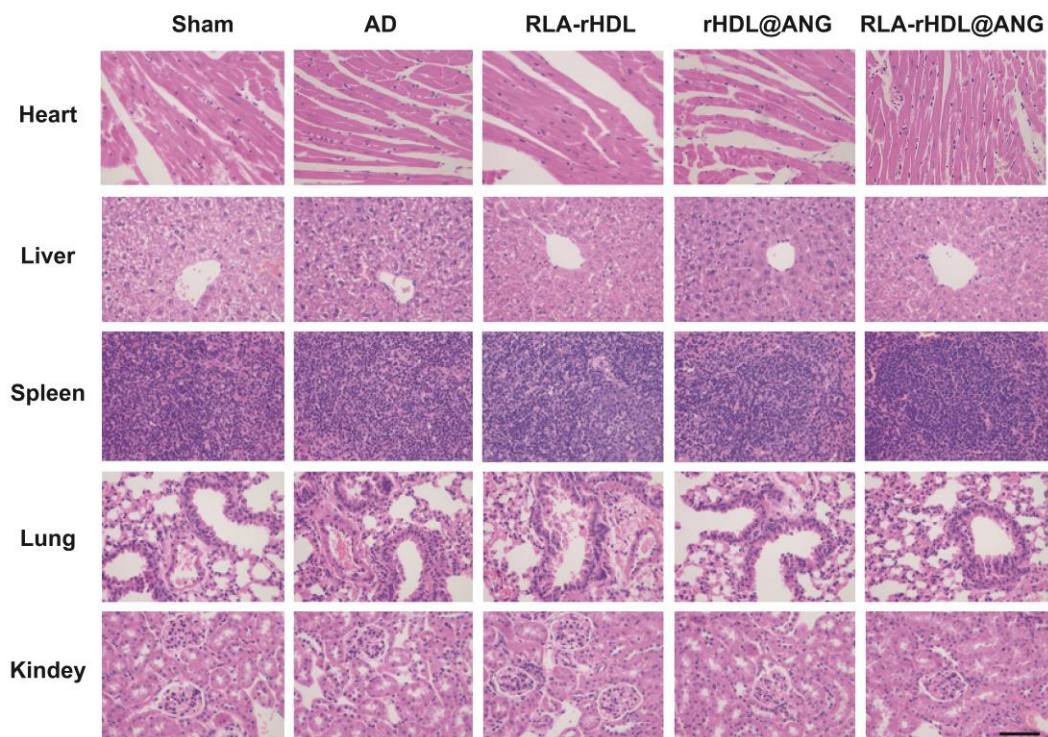
**Fig. S1** The synthetic route of DSPE-TK-mPEG<sub>2000</sub> (A) and DSPE-TK-PEG<sub>3400</sub>-ANG (B).



**Fig. S2** Transcellular internalization of RLA-rHDL@ANG across BBB model. (A) Illustration of the bEnd.3 monolayer transwell system. (B) TEER measurement before and after the experiment. (C) The permeability of RLA-rHDL and RLA-rHDL@ANG with or without free ANG preincubation.



**Fig. S3** Quantitative analysis of (A)  $A\beta_{1-42}$  and (B) 8-OHG from different groups of mice. (\*\* $p < 0.01$ , \*\*\* $p < 0.001$ )



**Fig. S4** HE staining of major organs from the AD mice after treatment of different formulations. The bar was 100  $\mu\text{m}$ .