Supplementary Information

Microglial Clearance of Alzheimer's Amyloid-Beta Obstructed by Nanoplastics

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Adsorption of Aβ onto nanoplastics

Aβ (25 μM) was prepared in phosphate buffered saline (PBS) solution and incubated separately at 37 °C for 1, 3, and 6 h with or without the nanoplastics (0.2 mg/mL). Samples were ultracentrifuged at 12,000 *g* for 15 min, and the supernatant was collected. A BCA kit (Thermo Fisher, USA) was used to measure Aβ concentration at 562 nm using a microplate reader (BioTek Synergy H1, USA). For confocal fluorescence imaging, HMC3 cells (8×10^4 cells/well) were seeded in 35 mm dishes. After 12 h, the cells were treated with FITC-Aβ (25 μM) and red fluorescently-labeled PS nanoplastics (0.2 mg/mL) for 24 h. The cells were then washed three times with 1×PBS, fixed in 4% paraformaldehyde solution (Solarbio, China) for 1 h, and rinsed once with 1×PBS for 5 min. Afterwards, the cells were stained with DAPI (4',6-diamidino-2-phenylindole, Solarbio, China) at room temperature for 5 min. Images were captured with a confocal fluorescence microscope (Nikon Eclipse Ti2-E, Japan) at a 60× magnification. DAPI: Ex 405 nm/Em 460 nm, PS: Ex 560 nm/Em 620 nm, FITC-Aβ: Ex 488 nm/Em 525 nm.

Cellular uptake mechanism

HMC3 cells (3×10^6 cells/well) were seeded in 6-well plates and cultured until attachment. The cells were pre-treated with methyl-β-cyclodextrin (MβCD, 5 mM), 5-(N-Ethyl-N-isopropyl)-Amiloride (EIPA, 30 μM), or monodansylcadaverine (MDC, 10 μM) for 2 h. FITC-labeled Aβ or PS nanoplastics were used to visualize their intracellular uptake. The cells were incubated with FITC-PS (0.2 mg/mL) or FITC-Aβ (25 μM) for 3 h. Flow cytometry was used to determine the proportion of positive cells.

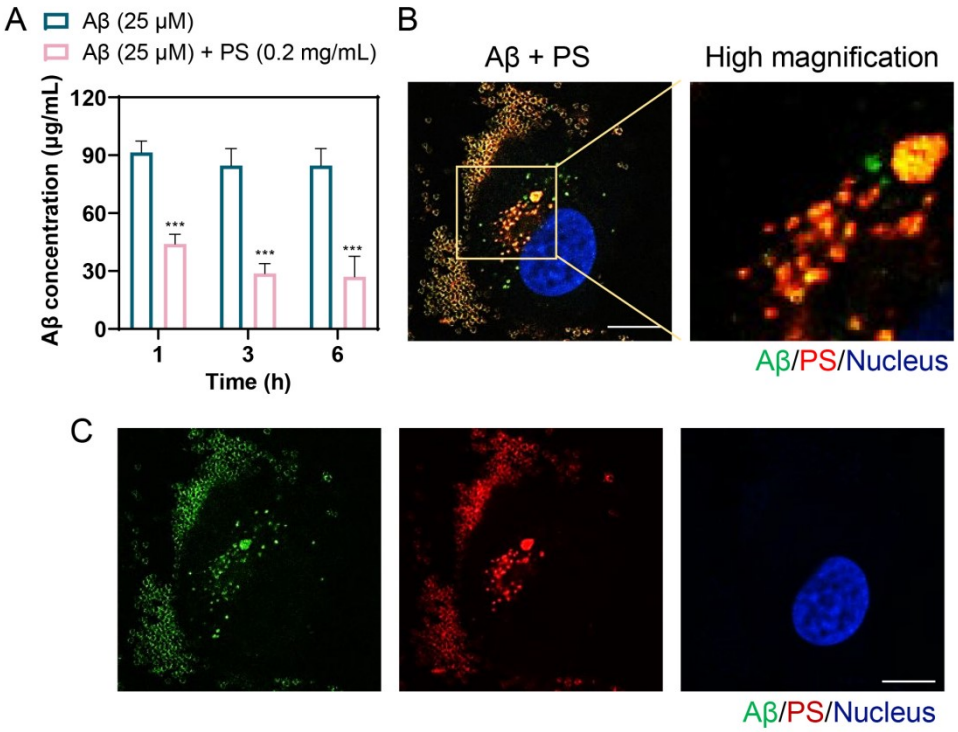

Fig. S1 Adsorption capacity of Aβ to PS nanoplastics. (A) A BCA assay in phosphate-buffered saline (PBS) buffer (n = 3). (B) Representative confocal fluorescence images of HMC3 cells treated with Aβ (25 μM) and PS nanoplastics (0.2 mg/mL) for 24 h. (C) Single-channel images of **Fig. S1B**. Green: FITC-labeled Aβ. Red: Fluorescently-labeled PS nanoplastics. Blue: nucleus. Scale bar: 10 μm. Data are presented as mean ± SD and analyzed using two-tailed Student's t-tests. * $P < 0.05$, *** $P < 0.001$ represent significant differences compared with the Aβ group.

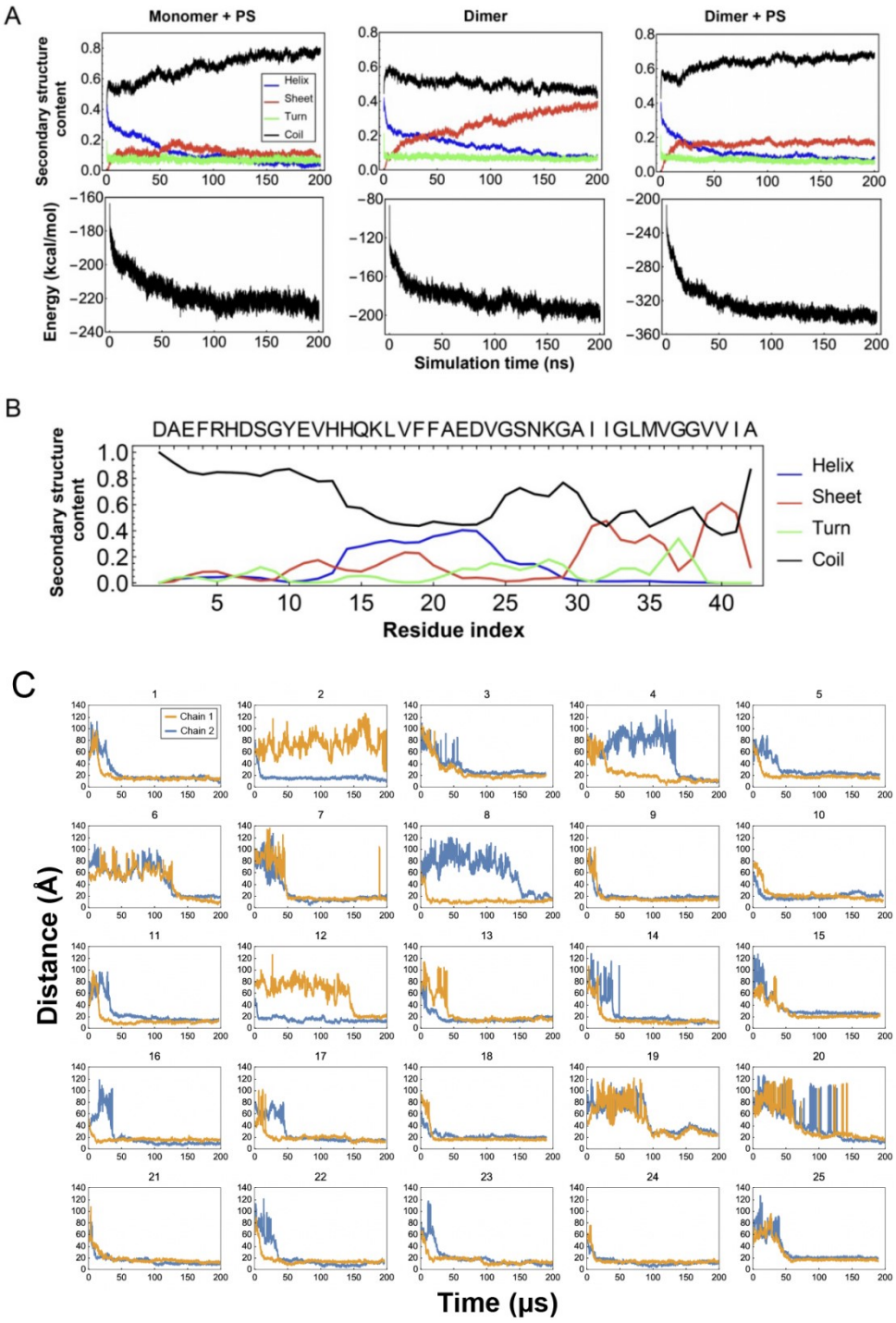

Fig. S2 Secondary structure, energy and dynamics of the simulation systems. (A) Time evolutions of the secondary structure and energy of an Aβ monomer with a PS nanoplastic and an Aβ dimer with and without a PS nanoplastic. (B) Residue-wise secondary structure content of Aβ in the presence of a nanoplastic, averaged across the two chains of the Aβ dimer. (C) Time evolution of the distance between two Aβ peptide chains and the PS nanoplastic for all 25 trajectories involving the interactants. The orange and blue colors correspond to the two separate Aβ peptide chains, respectively.

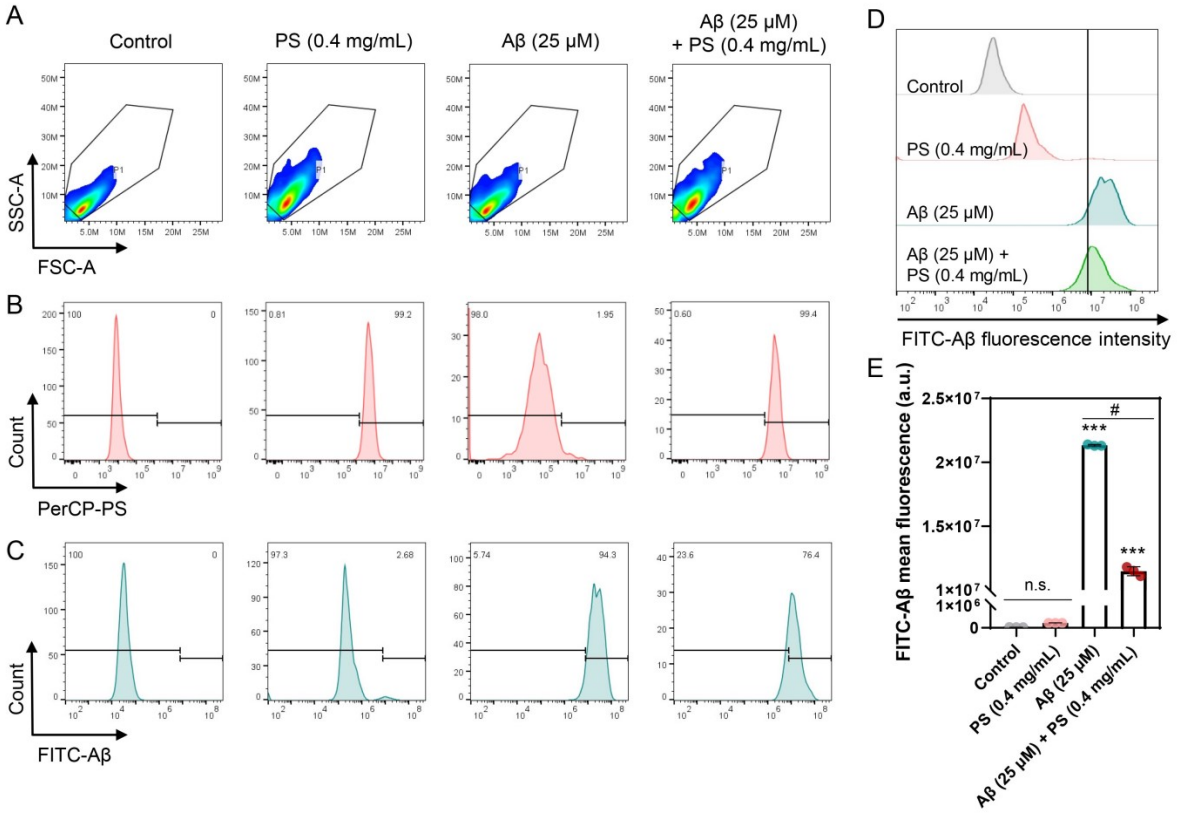

Fig. S3 Aβ promoted microglial uptake of PS nanoplastics. (A) Intracellular fluorescence of Aβ and PS nanoplastics in HMC3 cells determined by flow cytometry. (B, C) Histograms from panel A showing cellular uptake of the nanoplastics (B) or Aβ (C). (D) Overlay of the histograms in panel C. (E) Statistical analysis of intracellular fluorescence intensity of Aβ (n = 3). Data are presented as mean ± SD and were analyzed using one-way ANOVA, followed by Tukey's post-hoc tests. *** $P < 0.001$ represents significant differences compared with control, # $P < 0.05$ represents significant differences between treatment groups, and n.s. represents no significant difference.

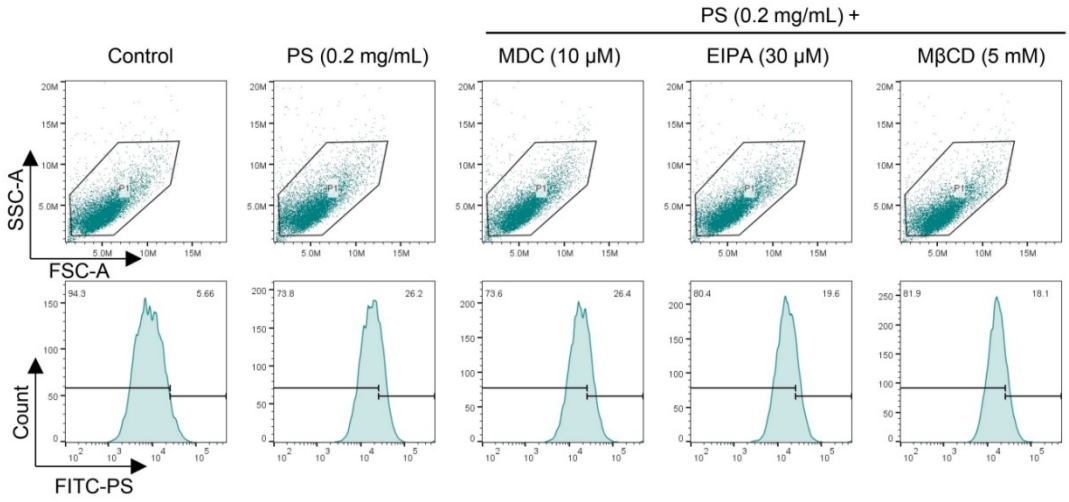

Fig. S4 MβCD (5 mM) and EIPA (30 μM) inhibited microglial uptake of PS nanoplastics. Cells were pre-treated with different endocytosis inhibitors (5 mM MβCD, 30 μM EIPA, 10 μM MDC) for 2 h. The cells were then treated with FITC-labeled PS nanoplastics (0.2 mg/mL) for 3 h. A flow cytometer was used to determine the proportion of FITC-PS positive cells (n = 3).

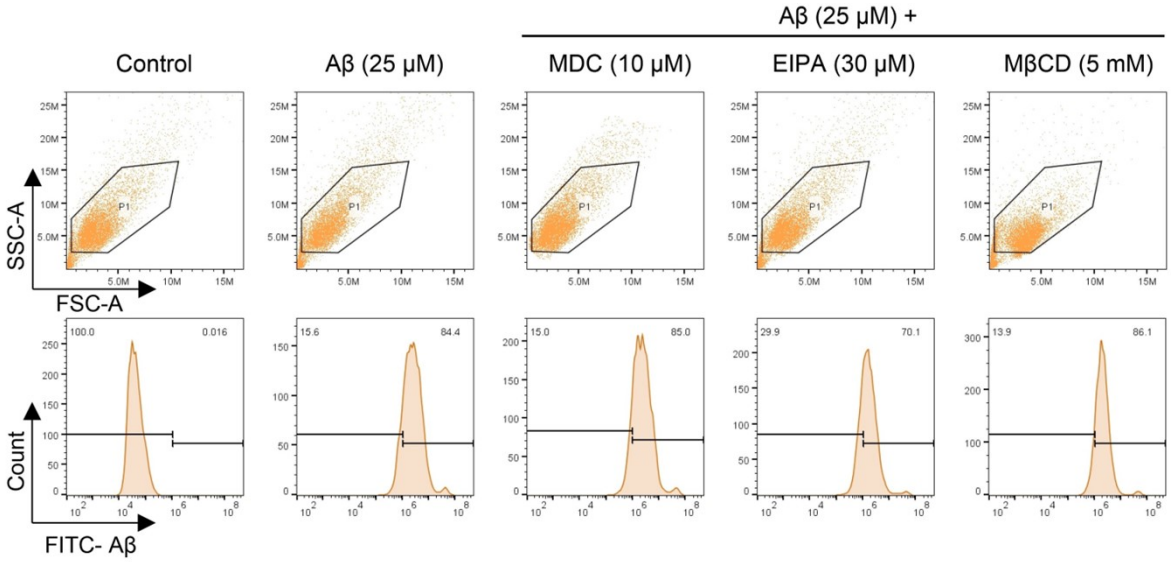

Fig. S5 EIPA (30 μM) inhibited microglial uptake of Aβ. HMC3 cells were pre-treated with different endocytosis inhibitors (5 mM MβCD, 30 μM EIPA, 10 μM MDC) for 2 h. Then the cells were exposed to FITC-labeled Aβ (25 μM) for 3 h. A flow cytometer was used to determine the proportion of FITC-PS-positive cells (n = 3).

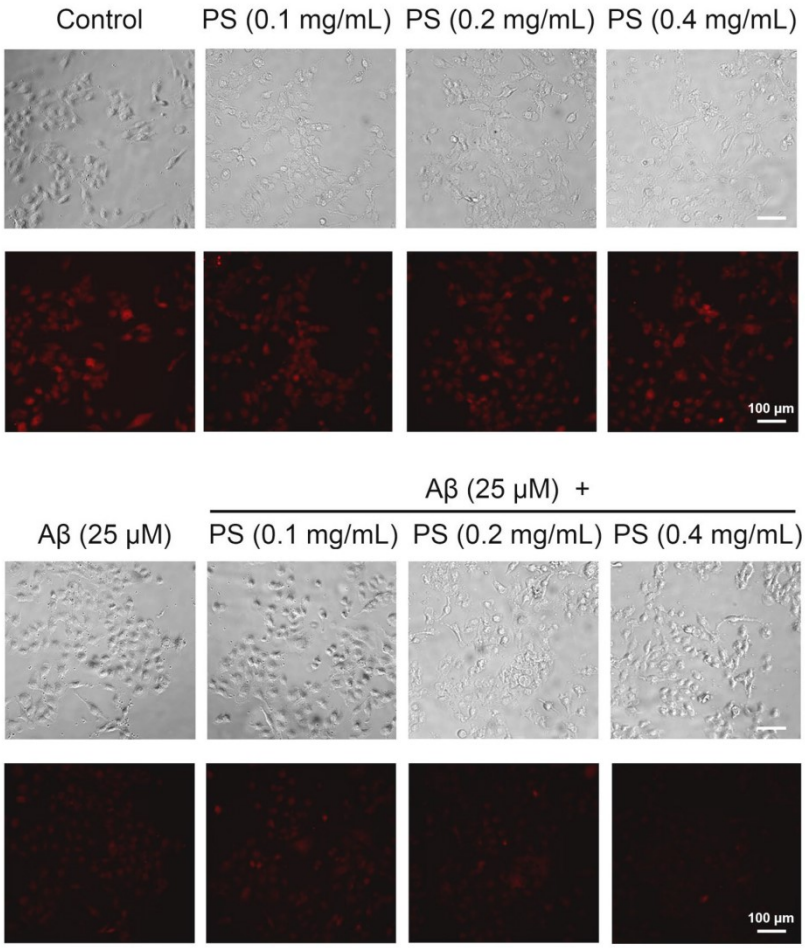

Fig. S6 Split bright field or fluorescence images of Fig. 3C. Scale bar: 100 μm.

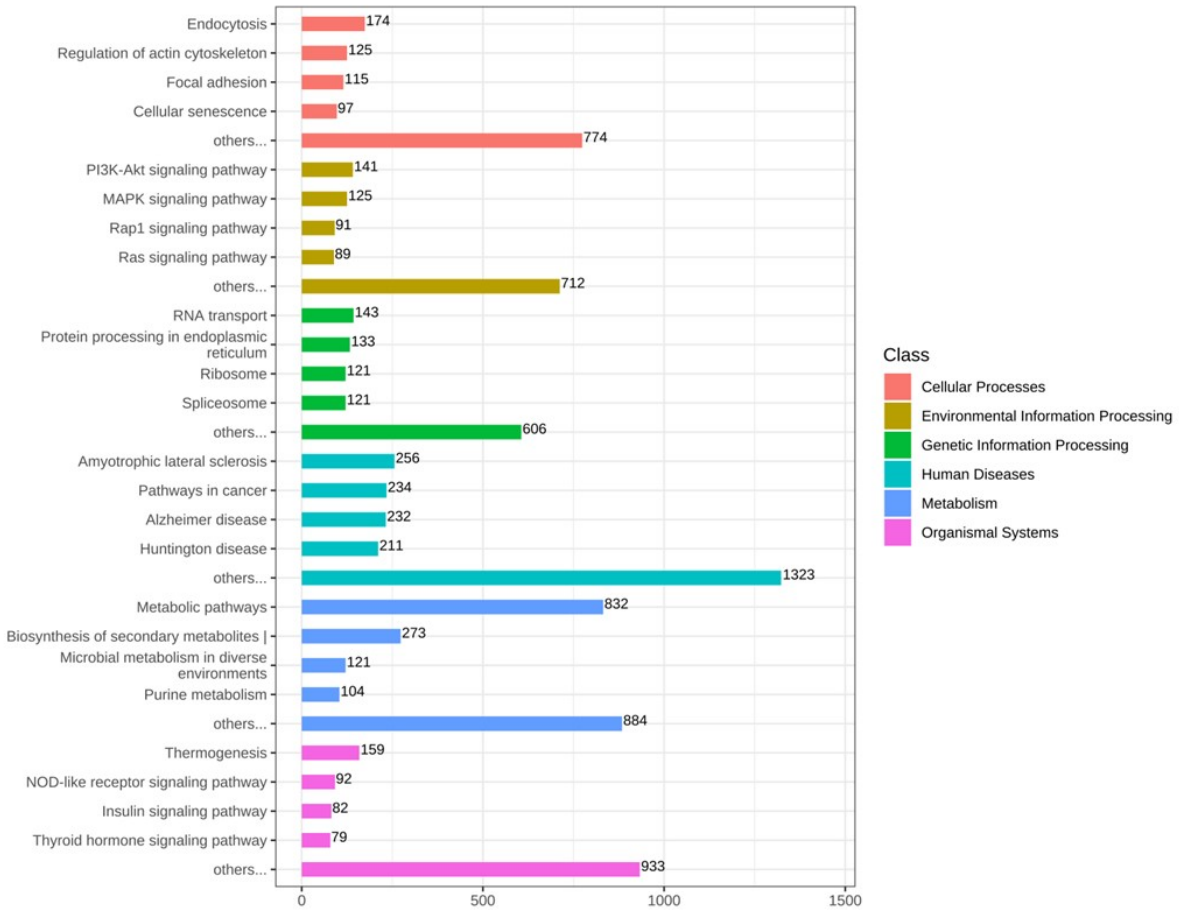

Fig. S7 KEGG pathway enrichment analysis from all detected proteins. The horizontal axis represents the number of proteins in the KEGG annotation entries and the vertical axis displays the enriched terms.

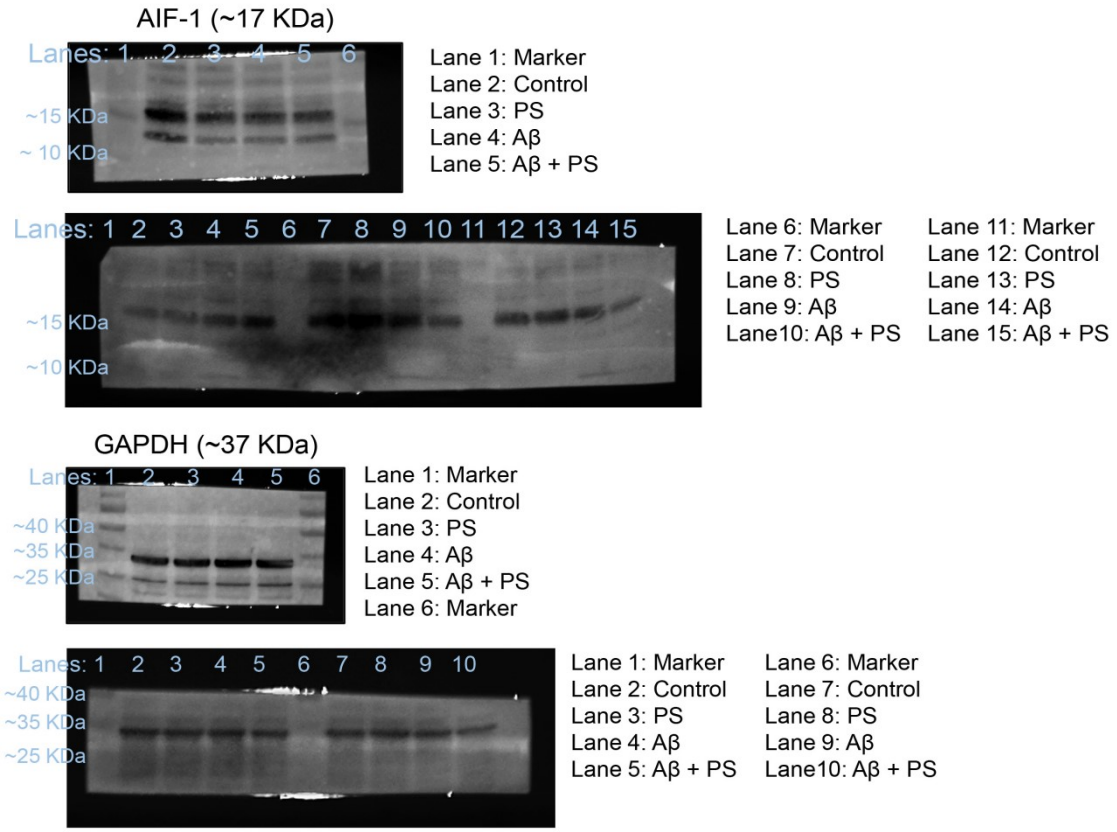

Fig. S8 Raw images of western blot bands in Fig. 3D.

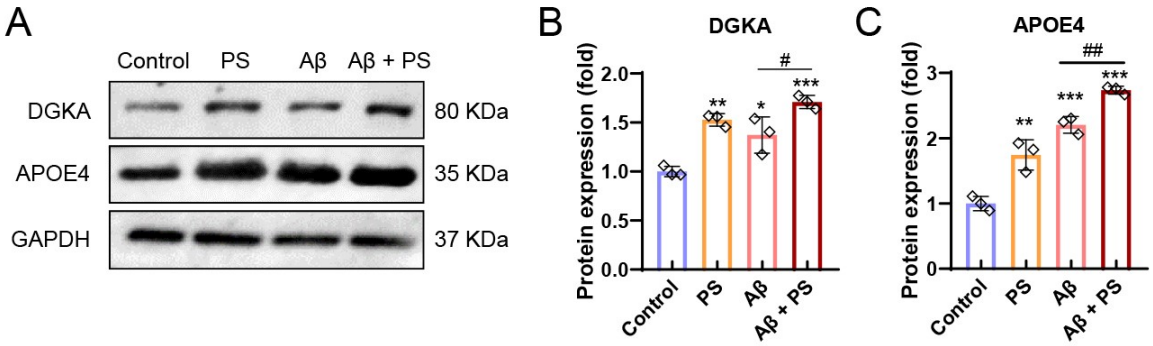

Fig. S9 Western blot assays (A) and quantifications of DGKA (B) and APOE4 (H) expressions. Data are shown as mean ± SD and were analyzed using one-way ANOVA, followed by Tukey's post-hoc tests (n = 3). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ represent significant differences compared with the control, and #$P < 0.05$, ##$P < 0.01$ represent significant differences between treatment groups.

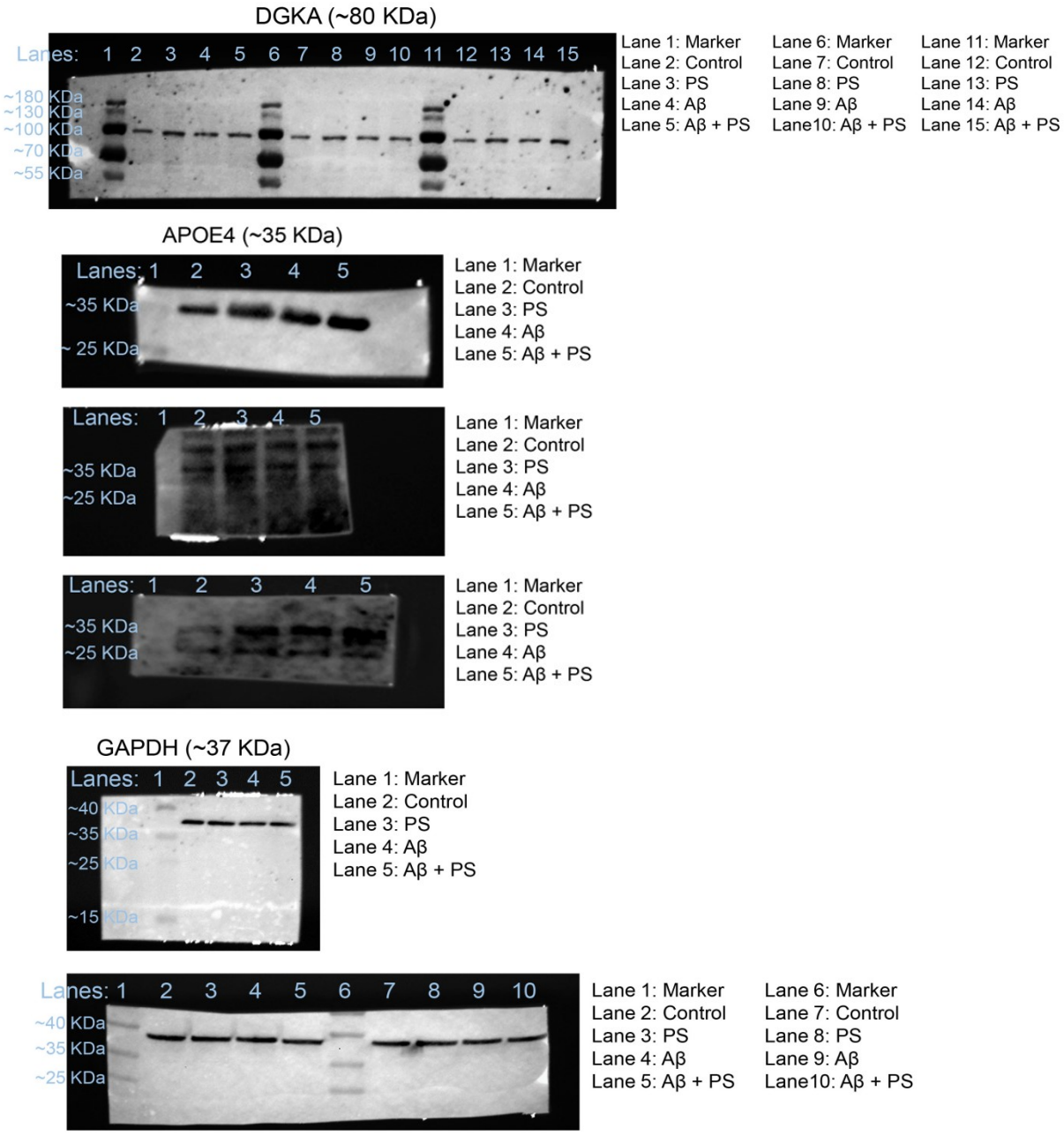

Fig. S10 Raw images of western blot bands in Fig. S9.

Table S1. Primer sequences applied in RT-qPCR.

Gene	Primer sequences
INOS	F: AGAAACCTGCTCTACGAACTGT
	R: GGGAAGCGAGTCTTTCAGAAG
TNF	F: CCTCTCTCTAATCAGCCCTCTG
	R: GAGGACCTGGGAGTAGATGAG
IL-10	F: TCAAGGCGCATGTGAACTCC
	R: GATGTCAAACTCACTCATGGCT
Arg-1	F: TGGACAGACTAGGAATTGGCA
	R: CCAGTCCGTCAACATCAAAACT
GAPDH	F: ACAACTTTGGTATCGTGGAAGG
	R: GCCATCACGCCACAGTTTC

Table S2. Antibodies used in the study.

Antibodies	Product origin
Anti-Iba1/AIF-1	Cell Signaling Technology
Anti-DGKA	Abcam
Anti-Apolipoprotein E4	Abcam
Anti-GAPDH	Abcam
IgG-HRP	Absin