

**Extracellular vesicles derived from astrocyte-treated with haFGF₁₄₋₁₅₄ attenuate
Alzheimer phenotype in AD mice**

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Supplemental Figure legends

Figure S1 Isolation and characterize of AEVs. (A) A representative immunofluorescence staining with the antibody of glial fibrillary acidic protein (GFAP, Green, Scale: 50 μm). (B) A schematic diagram displayed the treatment for astrocytes with $\text{A}\beta_{1-42}$ and haFGF₁₄₋₁₅₄. (C-E) Representative immunoblots (C) and quantification (D-E) of exosome biomarker proteins (CD9, CD63, and clathrin) in astrocytes and AEVs lysates, $n = 4$. Representative Endoplasmic reticulum biomarker calnexin used as negative control. (F) Representative particle size and distribution of AEVs observed by NanoSight Tracking Analysis (NTA). (G) Representative Atomic force microscopy (AFM) image of AEVs. $*p < 0.05$, $**p < 0.01$. P values are calculated by one-way ANOVA with Tukey's post-hoc test.

Figure S2 Tracking AEVs labeled by PKH26. (A) Representative images of AEVs labeled by PKH26 (red) in primary cortical neurons stained by green as MAP2⁺ cells (Scale bars: 20 μm). Neurons and AEVs were co-cultured for 0.5 h. (B) The schematic diagram of intranasal administration of AEVs (60 μg) labeled by PKH26 (red). (C) The distribution of AEVs was detected in olfactory bulb (OB) and entorhinal cortex (EC) of APP/PS1 mice in 6 h after single intranasal administration. The negative control mice were given volume-matched PBS.

Figure S3 Supplementary data for APP/PS1 mice in Y-maze and MWM test. In Y-maze test, total arm entries (A), total traveled distance (B), the correlation between total arm entries and spontaneous alternation (C), the correlation between total traveled distance and spontaneous alternation (D) in APP/PS1 mice, $n = 6-8$. (E) The movement distance of escape latency for APP/PS1 mice in MWM test, $n = 8$. $*p < 0.05$, $**p < 0.01$. P values are calculated by two-way ANOVA with Tukey's post-hoc test.

Figure S4 AEV_{S-Aβ+H} ameliorate learning and memory deficits in AD mice with brain injection of Aβ₁₋₄₂. (A) Experimental protocols of intranasal administration and behavior test of AD mice with hippocampal injection of Aβ₁₋₄₂. (B) Spontaneous alternation in Y-maze, n = 8-10. (C-F) In Y-maze test, total arm entries (C), total traveled distance (D), the correlation between total arm entries and spontaneous alternation (E), the correlation between total traveled distance and spontaneous alternation (F) in AD mice with Aβ brain injection, n = 8-10. (G-I) Navigation test in MWM, n = 7/8. Representative movement tracks of escape latency on day 5 in navigation test (G) and the escape latency (H, I). (J) The movement distance of escape latency for AD mice with Aβ brain injection in MWM test, n = 7/8. (K-M) Spatial probe test was performed 24 h after the navigation test. Representative movement tracks in spatial probe test (K), numbers of crossing the removed platform area (L) and time spend in target quadrant (M) in spatial probe test of MWM, n = 7/8. **p* < 0.05, ***p* < 0.01, ****p* < 0.001. *P* values are calculated by two-way ANOVA with Tukey's post-hoc test.

Figure S5 AEV_{S-Aβ+H} enhance expression of synaptic proteins in AD mice with brain injection of Aβ₁₋₄₂. (A, E) Representative immunoblotting images of PSD95, GAP43 and SYN in the PFC (A) and hippocampus (E) of AD mice. The quantified expression of the above proteins in the PFC (B-D) and hippocampus (F-H), n = 5-6. Data are mean ± SEM. ***p* < 0.01, ****p* < 0.001. *P* values are calculated by one-way ANOVA with Tukey's post-hoc test.

Figure S6 AEV_{S-Aβ+H} reduce Aβ deposition in AD mice with brain injection of Aβ₁₋₄₂. (A) Aβ plaque in the hippocampal DG, CA1 and CA3 regions of AD mice detected by immunohistochemistry. (B-D) The area of Aβ plaque analyzed by image-pro plus software, n = 4-6. (E-G) The level of Aβ in the PFC and hippocampus detected

by Western blot, $n = 4-6$. Data are mean \pm SEM. $*p < 0.05$, $**p < 0.01$. P values are calculated by one-way ANOVA with Tukey's post-hoc test.

Figure S7 Analysis of miRNAs in AEVs by small RNA sequence. (A) Representative density distribution map of miRNAs in different AEVs. Light orange indicates AEVs_{-Ctrl}, light green indicates AEVs_{-A β} and light gray indicates AEVs_{-A β +H}. (B-D) Volcanic maps exhibit statistically differentially expressed miRNAs between the AEVs_{-Ctrl} and AEVs_{-A β} (B), AEVs_{-A β} and AEVs_{-A β +H} (C), AEVs_{-Ctrl} and AEVs_{-A β +H} (D). The red and green dots indicate miRNAs that are up-regulated and down-regulated respectively, and the gray dots indicate miRNAs that are no statistical differences.

Figure S8 Supplementary data for A β brain injection mice treated with miR-206-3p antagomir in Y-maze and MWM test. (A) Representative the movement distance of escape latency for A β brain injection mice treated with miR-206-3p antagomir in MWM test, $n = 9-12$. In Y-maze test, total arm entries (B), total traveled distance (C), the correlation between total arm entries and spontaneous alternation (D), the correlation between total traveled distance and spontaneous alternation (E) in A β brain injection mice treated with miR-206-3p antagomir, $n = 9-12$. Data are mean \pm SEM. $*p < 0.05$. P values are calculated by one-way ANOVA with Tukey's post-hoc test and two-way ANOVA with Tukey's post-hoc test.

Figure S9 AEVs_{-A β +H} increases BDNF levels and inhibits the AEP activity in the brain of AD mice. (A-C) BDNF expression in PFC and hippocampus of AD mice with A β ₁₋₄₂ brain injection, $n = 9-11$. (D-E) BDNF level in the PFC and hippocampal tissues detected by Elisa, $n = 4$. (F) Representative immunoblotting images of AEP (full-length and cleaved AEP) in the PFC and hippocampus. (G-H) The quantified expression of AEP, $n = 7-8$. Data are mean \pm SEM. $*p < 0.05$, $**p < 0.01$, $***p <$

0.001. *P* values are calculated by one-way ANOVA with Tukey's post-hoc test.

Figure S10 No direct target relationship between miR-206-3p and the synaptic proteins (PSD95, GAP43 and SYN). Dual-luciferase reporter assay has showed that miR-206-3p does not directly bind to the 3'UTR of PSD95 (A), GAP43 (B) and SYN (C). Data are mean \pm SEM. *n* = 3. *P* values are calculated by one-way ANOVA with Tukey's post-hoc test (n.s.: not significant).

Figure S11 AEVs- $A\beta$ +H and miR-206-3p antagomir have no effects on the expression of APP cleaving enzymes (ADAM10, BACE1) and $A\beta$ clearance proteins (IDE). Representative the immunoblots (A) and quantified expression of ADAM10, BACE1 and IDE in PFC (B-D) and hippocampus (E-H) of APP/PS1 mice with intranasal administration of AEVs, *n* = 5-6. (I-O) The expression of ADAM10, BACE1 and IDE in the PFC (I, J-L) and hippocampus (I, M-O) of $A\beta$ brain injection mice with intranasal administration of AEVs, *n* = 5-6. The representative immunoblots (P) and quantified expression of ADAM10, BACE1, and IDE in the PFC (Q-S) and hippocampus (T-V) of AD mice injected with miR-206-3p antagomir, *n* = 9. Data are mean \pm SEM. **p* < 0.05 and ***p* < 0.01. *P* values are calculated by one-way ANOVA with Tukey's post-hoc test.

Figure S12 The correlation between plasma miR-206-3p or BDNF and gender in the whole cohort. The correlation between plasma miR-206-3p and gender in the healthy individuals (A, Ctrl), AD patients (B), PD patients (C) and Schizophrenia patients (D), *n* = 25, 18, 15 and 12 for normal control and patients with AD, PD and Schizophrenia respectively. The correlation between plasma BDNF and gender in the healthy individuals (E, Ctrl), AD patients (F), PD patients (G) and Schizophrenia patients (H), *n* = 16, 18, 13 and 8 for normal control and patients with AD, PD and Schizophrenia respectively. Data are calculated by Pearson correlations and best

fitted with linear regression curve.

Figure S1

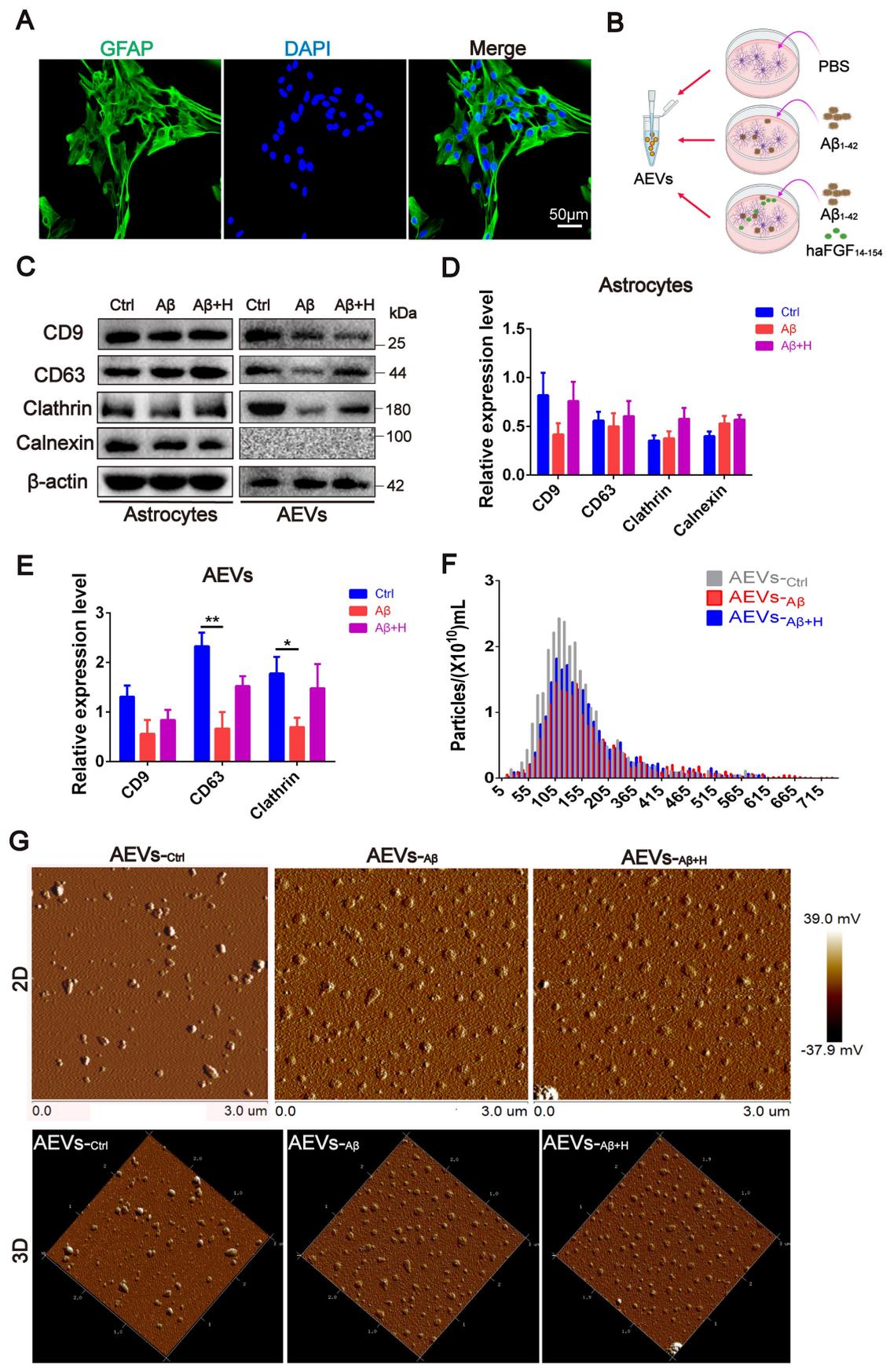


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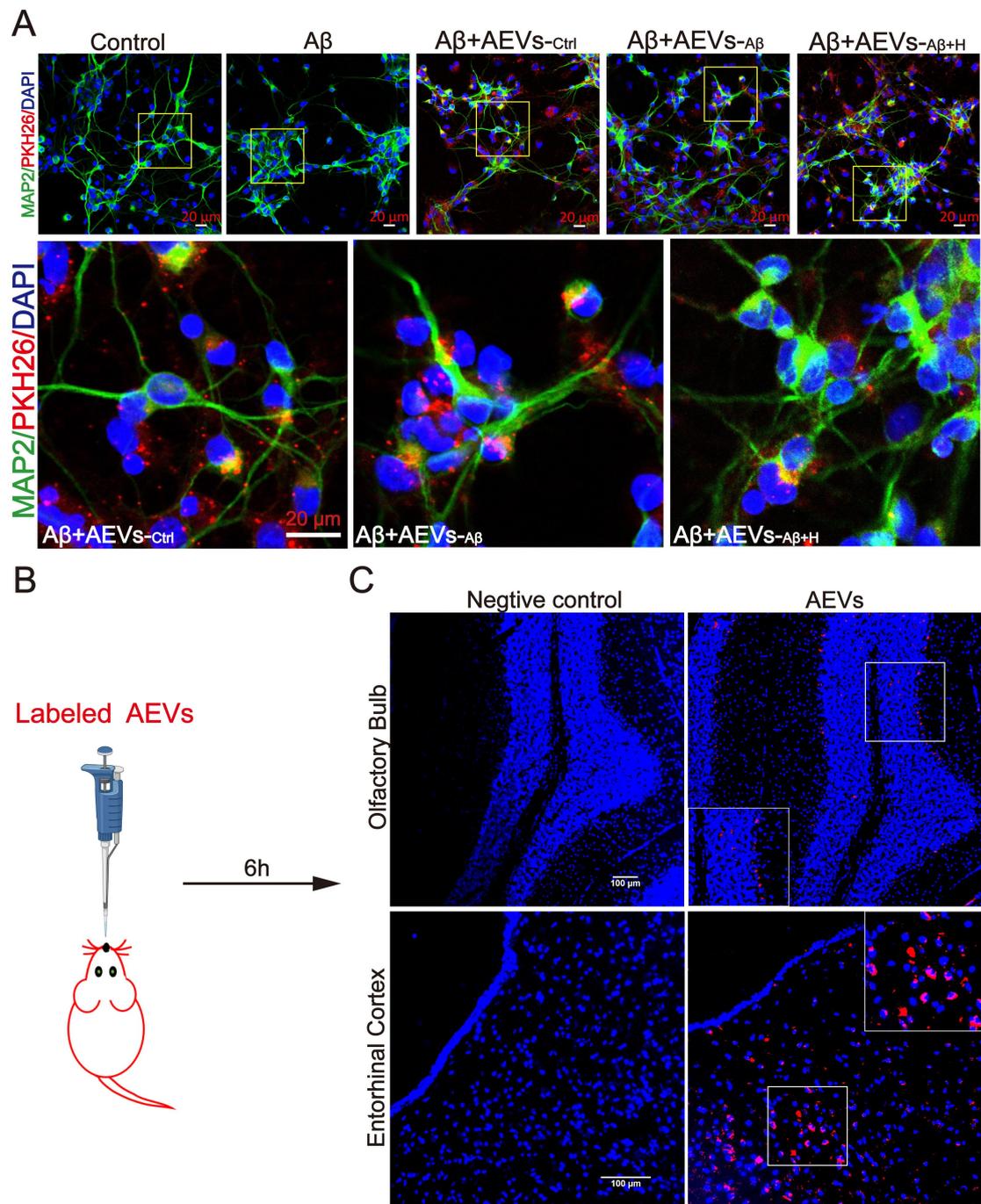


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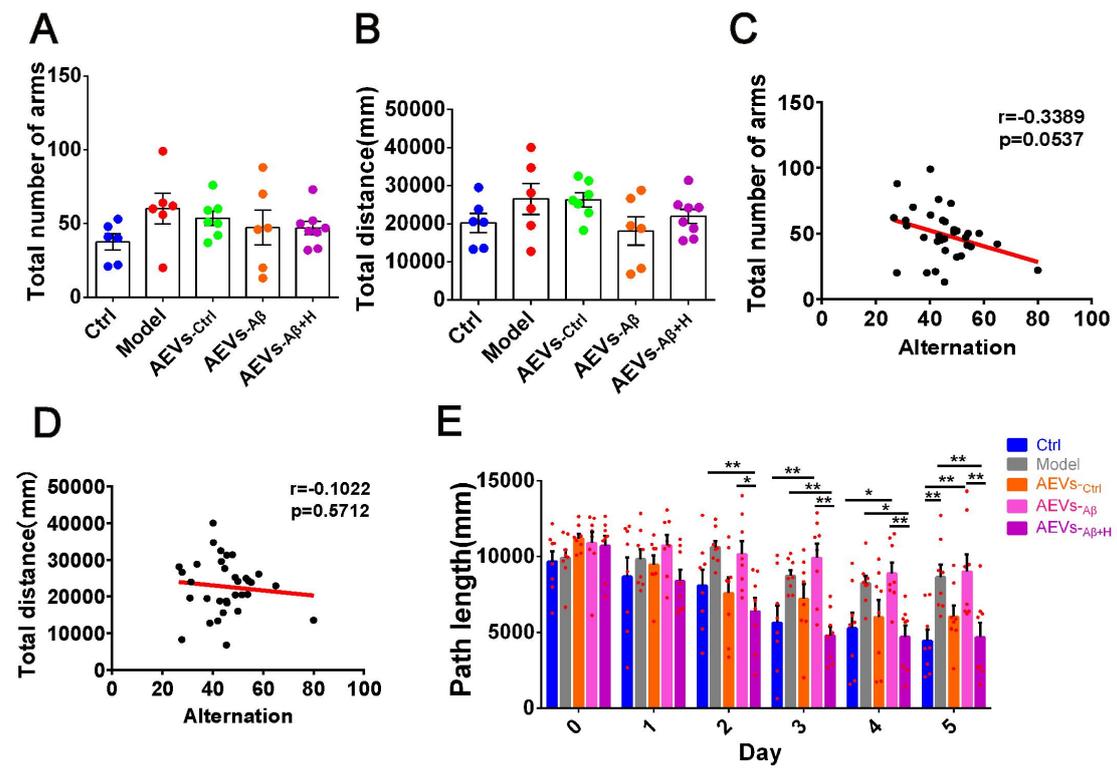


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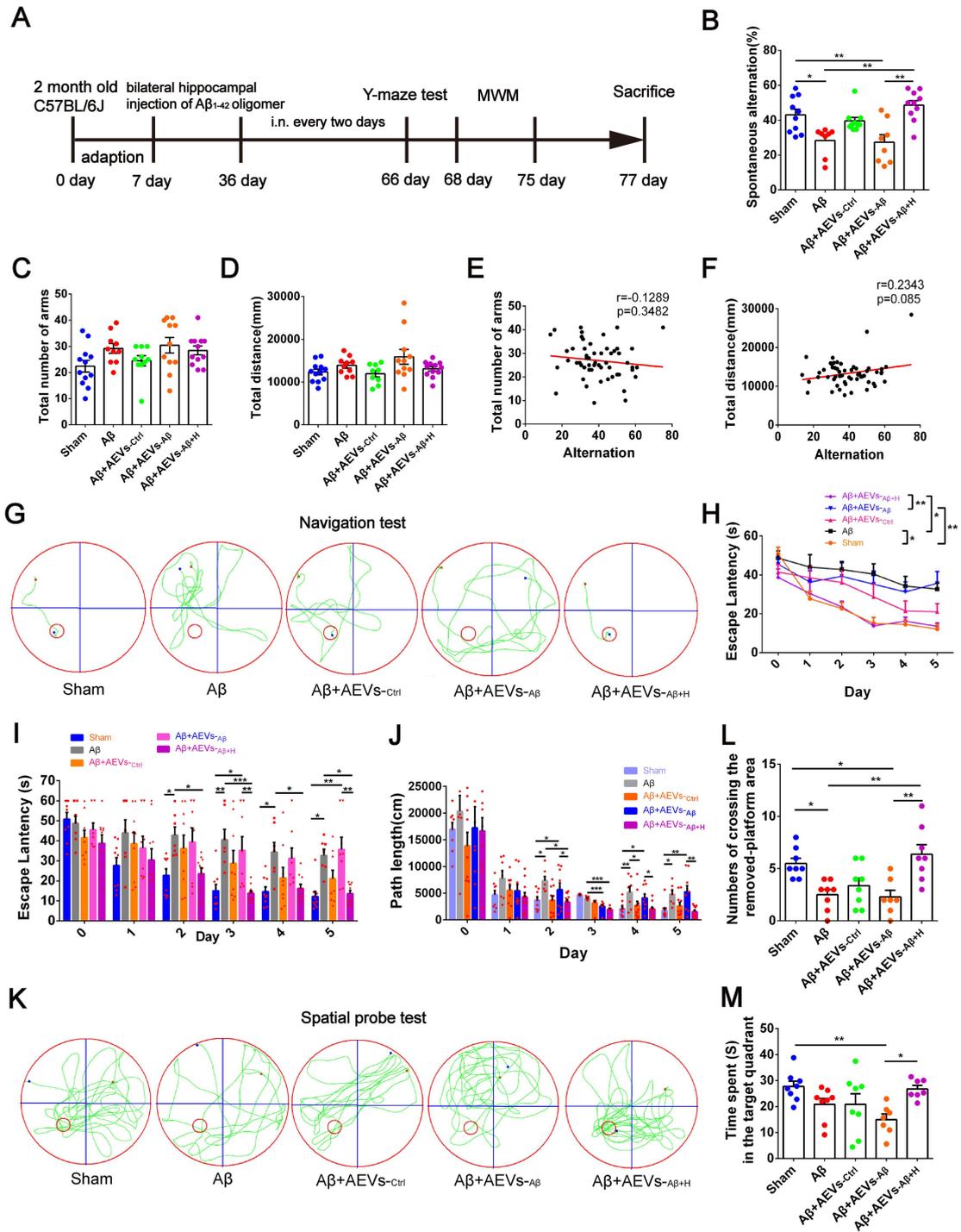


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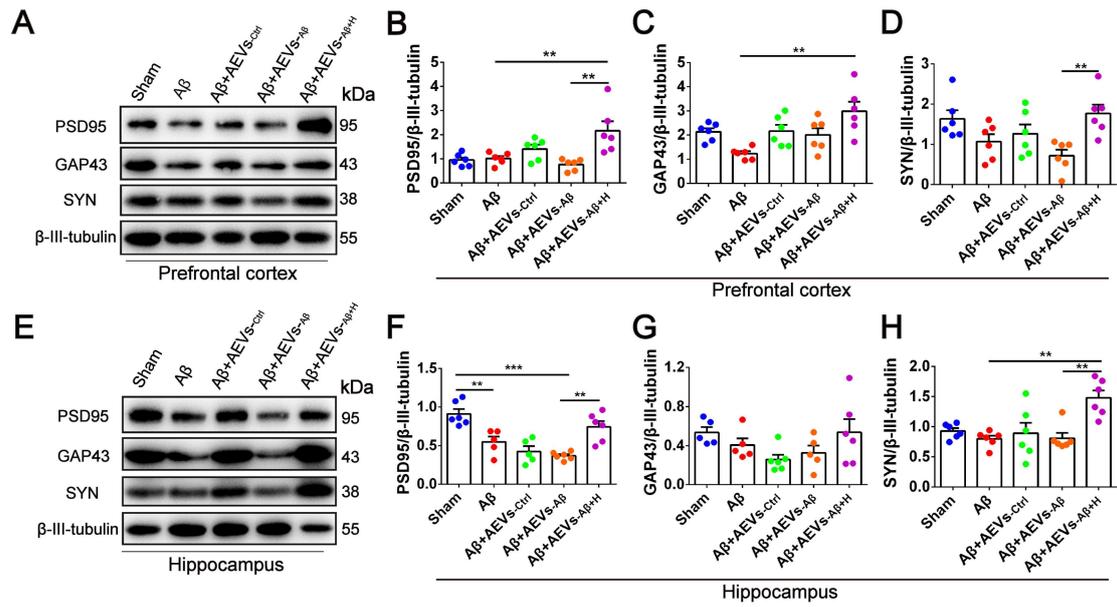


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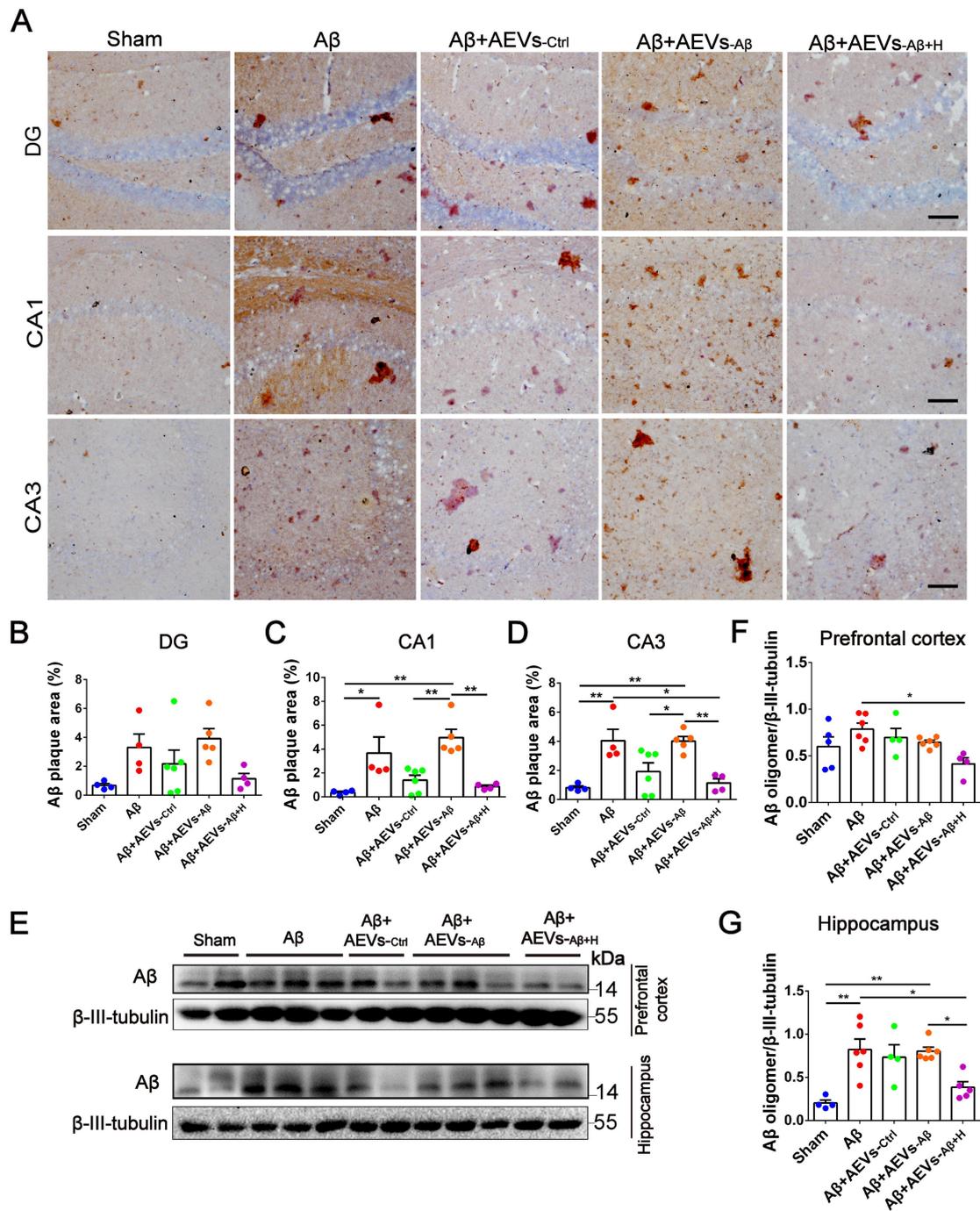
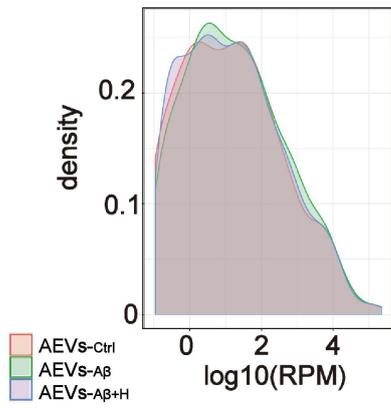
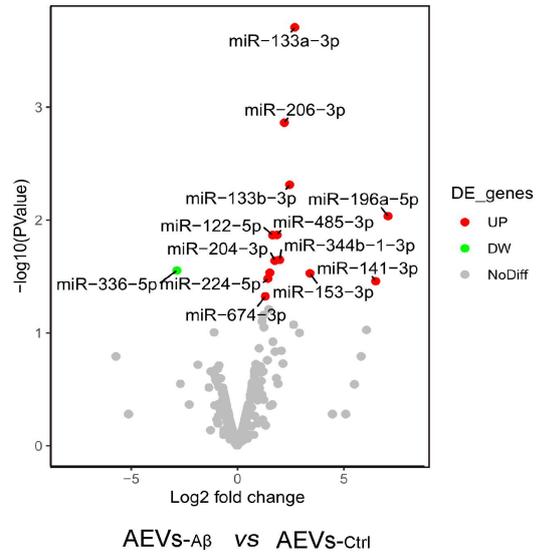


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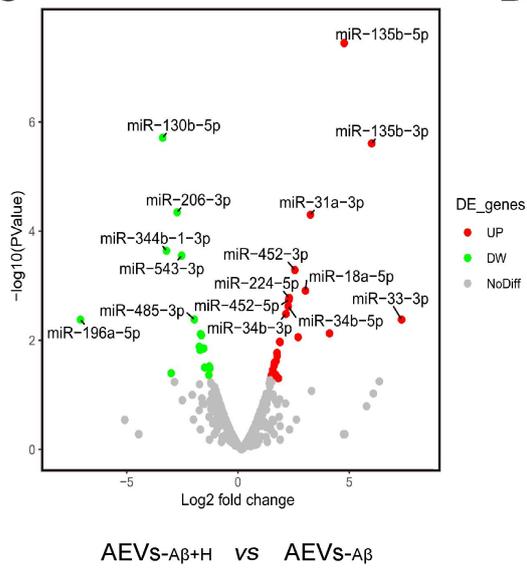
A



B



C



D

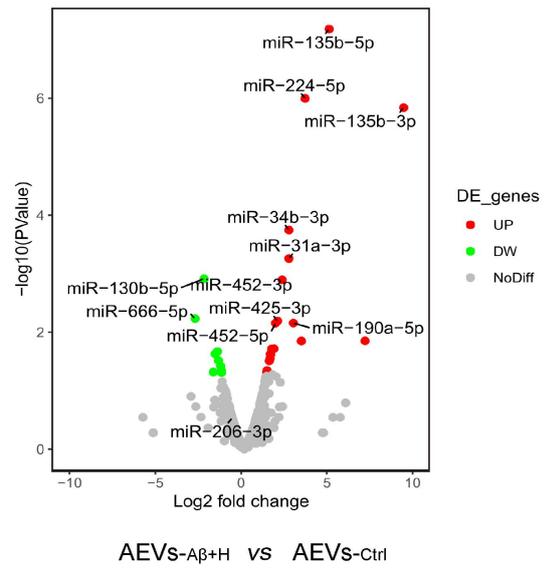


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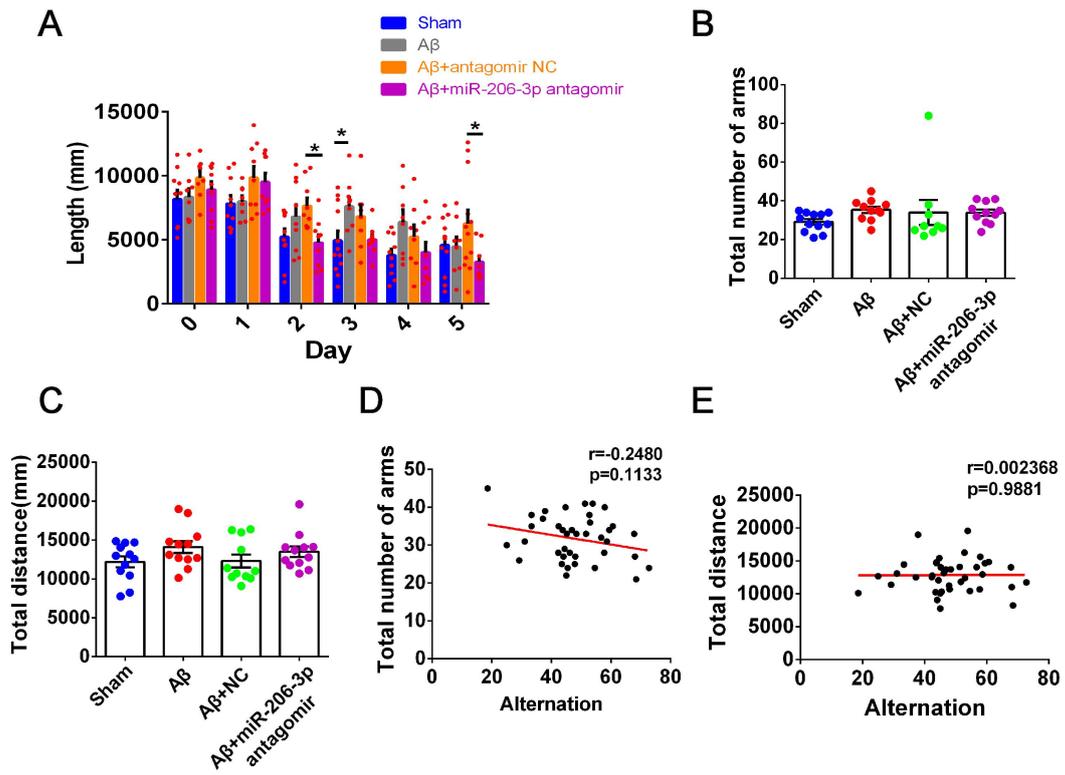


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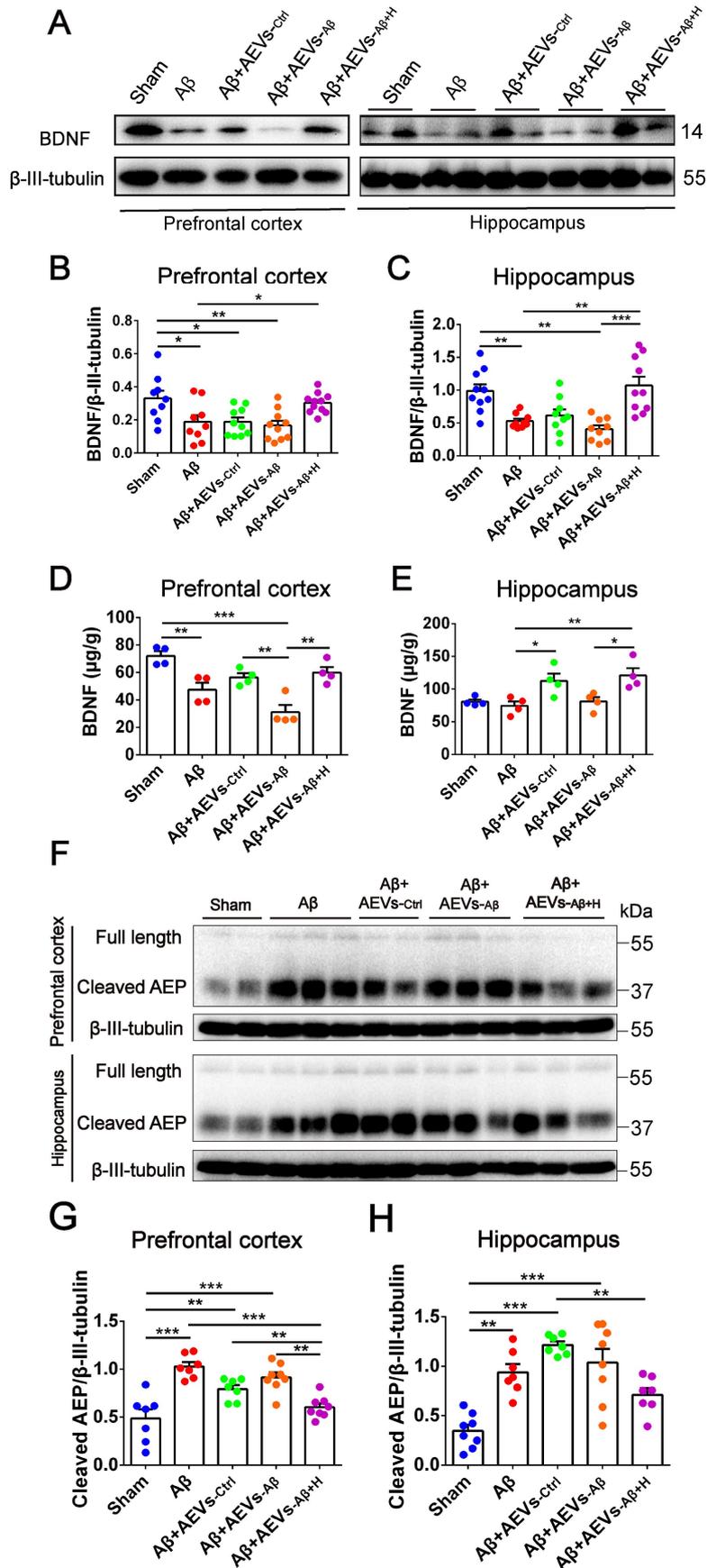


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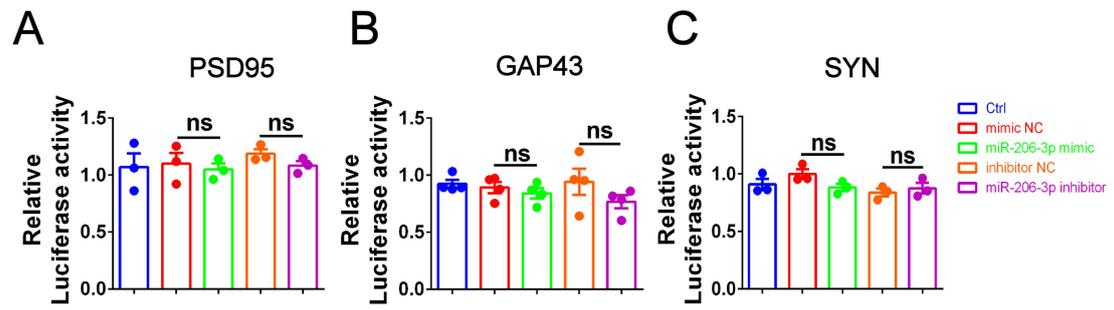


Figure S11

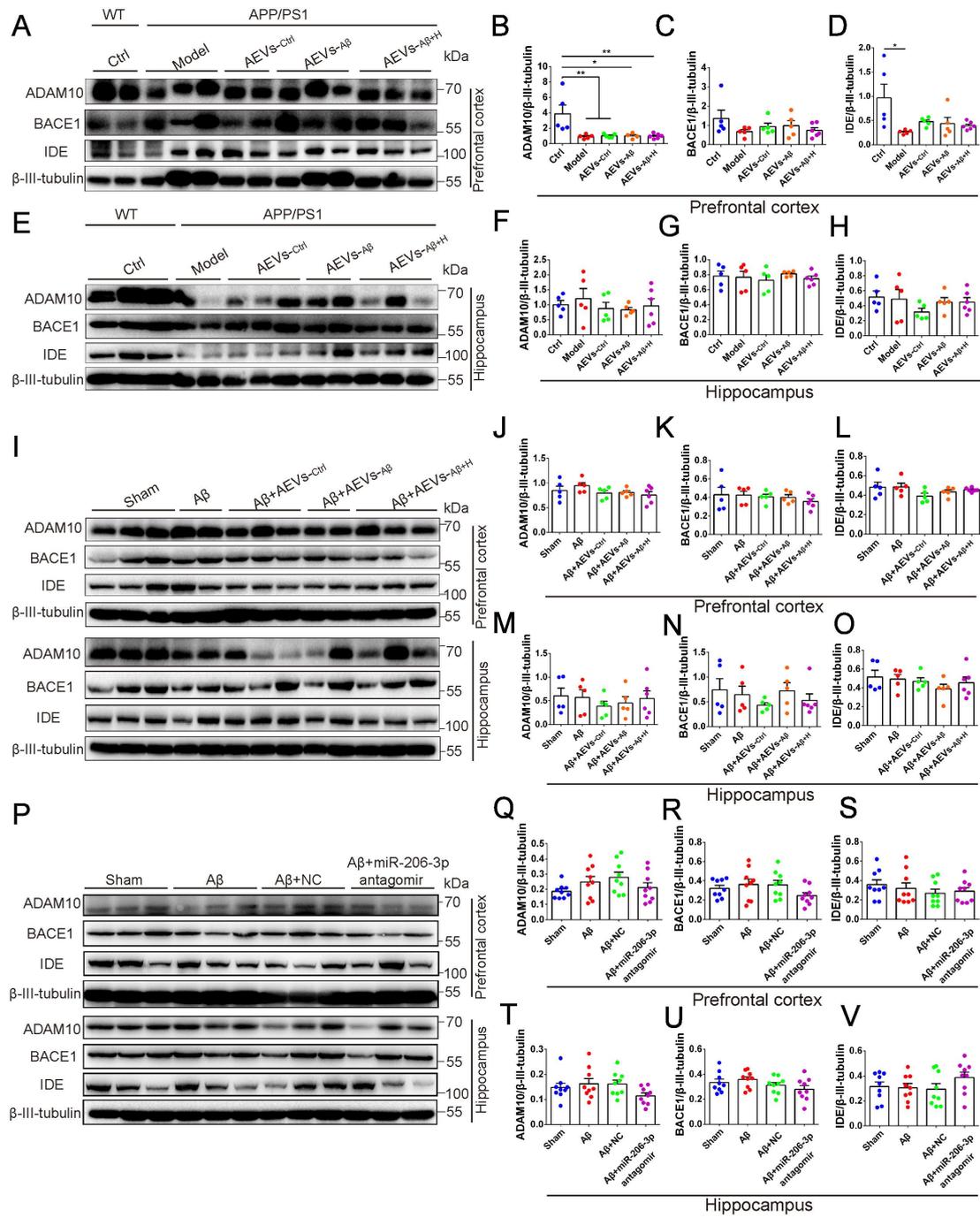


Figure S12

