Supplementary data

MiR-431 attenuates synaptic plasticity and memory deficits in APPswe/PS1dE9 mice

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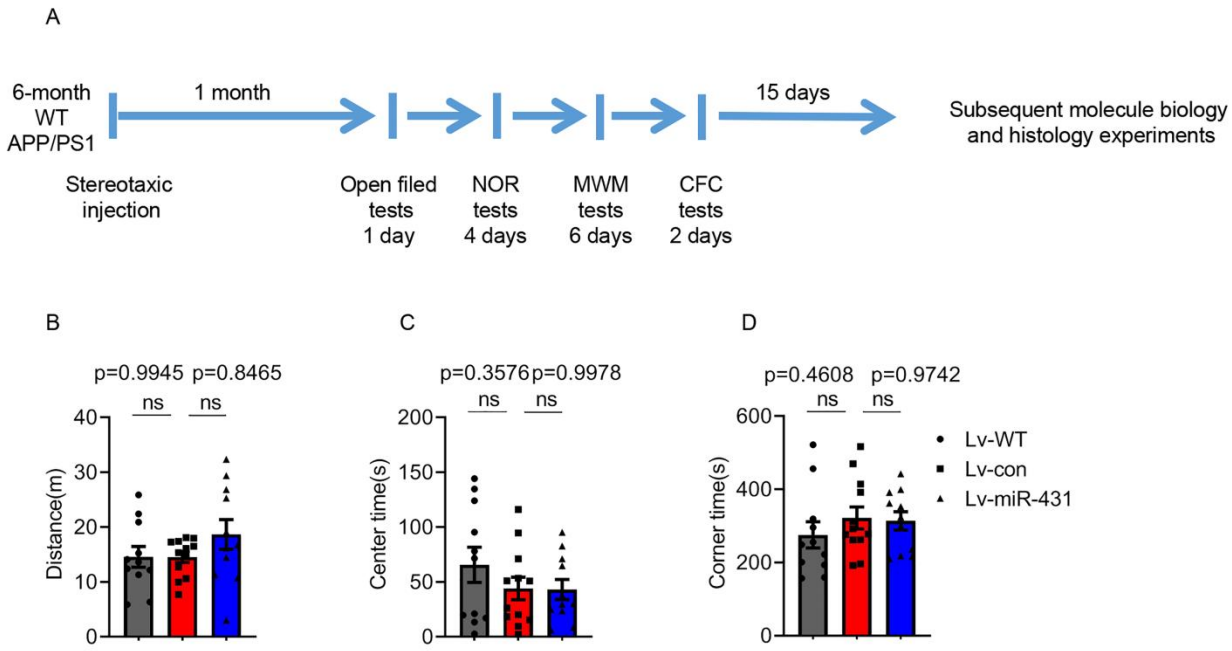

Figure S1 MiR-431 overexpression doesn't modulate the motor function and emotional state of 6-month-old APP/PS1 mice. (A) The flow chart of the experiments. The total distance (B), time spent in the center (C) and corner area (D) were recorded of Lv-miR-431 treated APP/PS1 mice by Any-maze software in the OF test. n = 11-12 for each group, ns: not significant. All data were presented as means ± SEM. One-way ANOVA (B-D) was used.

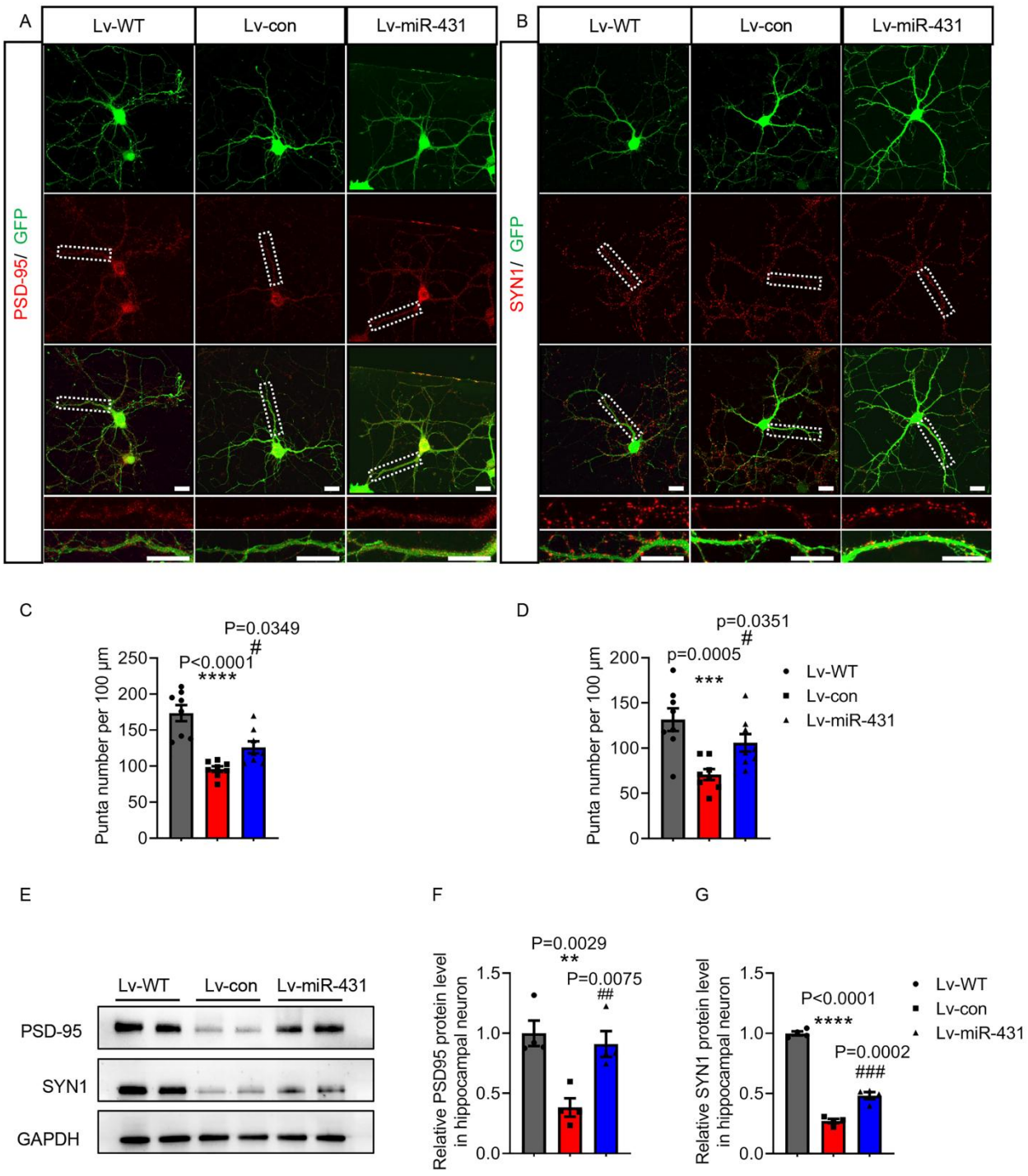

Figure S2 MiR-431 overexpression improves the synaptic density, the PSD-95 and SYN1 protein levels in APP/PS1 hippocampal neurons. (A) Representative confocal images of PSD-95 (red) and GFP (green). Upper bar = 20μm, lower bar = 20μm. (B) Representative confocal images of SYN1 (red) and GFP (green). Upper bar = 20μm, lower bar = 20μm. (C) Quantification of PSD-95-positive puncta per 100μm of GFP-positive neurites. n = 8 neurons per group. (D) Quantification of SYN1-positive puncta per 100μm of GFP-positive neurites. n = 8 neurons per group. (E) Representative western blot of PSD-95 and SYN1 in WT or APP/PS1 hippocampal neurons. (F) Quantification of PSD-95 blots. n = 4. (G) Quantification of SYN1 blots. n = 4. **p<0.01, ***p＜0.001, ****p ＜0.0001 vs. Lv-WT group; # p< 0.05, ## p< 0.01, ### p＜0.001 vs. Lv-con group. All data were presented as means ± SEM. One-way ANOVA (C, D, F and G) was used.

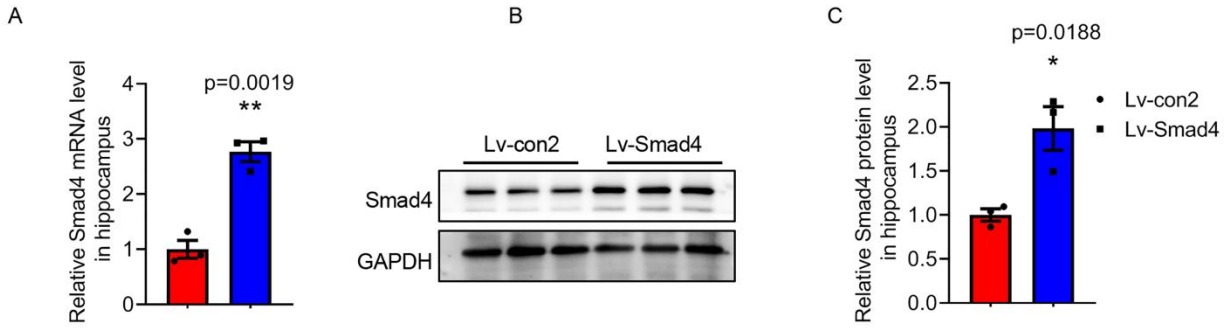

Figure S3 Smad4 overexpression efficiency in Lv-Smad4 treated hippocampus of APP/PS1 mice. (A) The mRNA level of Smad4 in Lv-Smad4 treated hippocampus. n = 3 (B, C). The protein level of Smad4 in Lv-Smad4 treated hippocampus. n = 3, * $p<0.05$, ** $p<0.01$ vs. Lv-con2. All data were presented as means ± SE

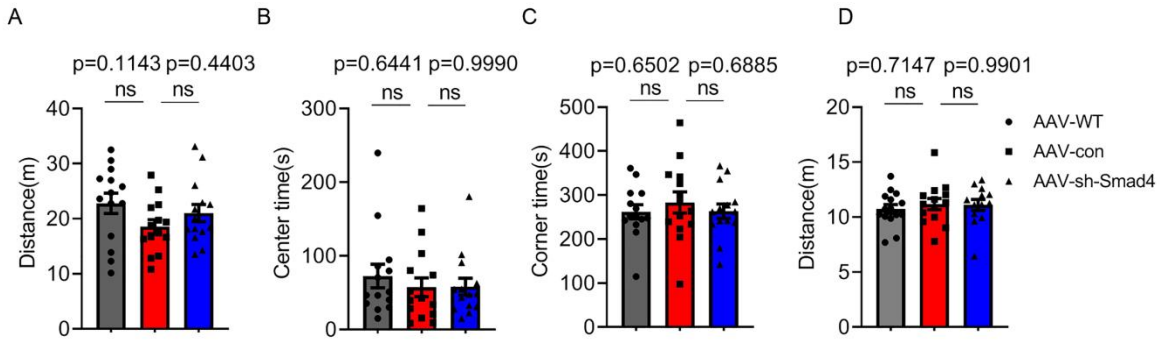

Figure S4 Smad4 inhibition doesn't affect the motor function and emotional state of 6-month-old APP/PS1 mice. The total distance (A), time spent in the center (B) and corner area (C) were recorded of AAV-sh-Smad4 treated APP/PS1 mice by Any-maze software in the OF test. (D) Distance in MWM tests was detected in the acquisition trial, n = 14, ns: not significant. All data were presented as means ± SEM. One-way ANOVA (A-D) was used.

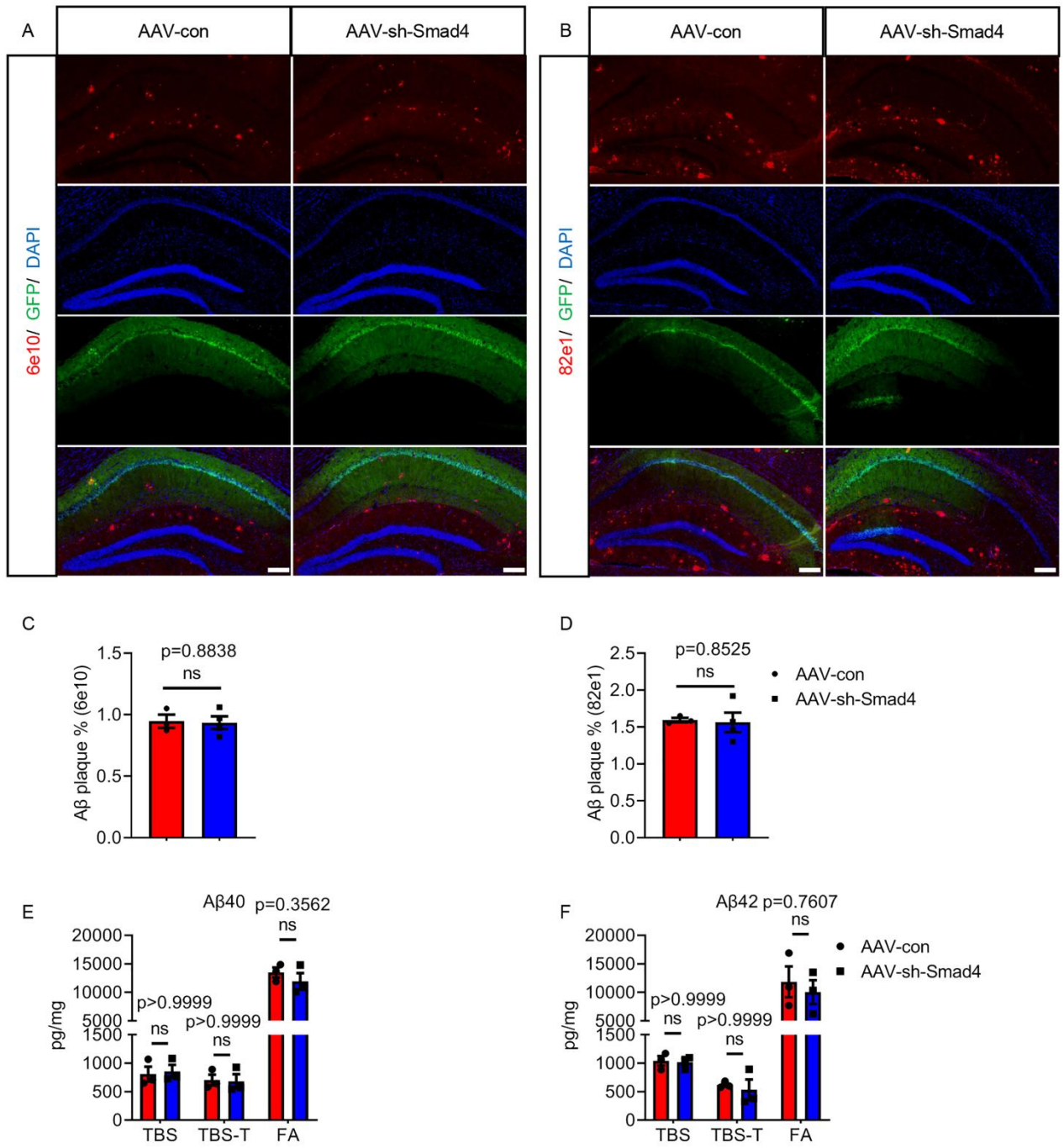

Figure S5 Smad4 inhibition doesn't affect the Aβ levels in the hippocampus of 6-month-old APP/PS1 mice. (A) The level of Aβ 6e10 in the hippocampus of AAV-sh-Smad4 treated APP/PS1 mice were detected by immunofluorescence staining. (B) The level of Aβ 82e1 in the hippocampus of AAV-sh-Smad4 treated APP/PS1 mice were detected by immunofluorescence staining. (C) Quantitative analysis of the percentage of Aβ 6e10 positive area. n = 3-4 mice per group. Bar = 200μm. (D) Quantitative analysis of the percentage of Aβ 82e1 positive area. n=3-4 mice per group. Bar = 20 μm. The protein levels of TBS-soluble, TBS-T-soluble and FA-soluble $A\beta_{40}$ (E) and $A\beta_{42}$ (F) were measured by ELISA in the hippocampus of AAV-sh-Smad4 treated APP/PS1 mice. n=3, ns: not significant. All data were presented as means ± SEM. Two-tailed unpaired Student's t test (C, D) and two-way ANOVA (E, F) were used.

Supplement Table 1. Demographic and neuropsychological data.

Items	HC (n=23)	aMCI (n=20)	AD (n=25)	*p*	*p*(Mann-Whiney U Test)		
					NC versus aMCI	NC versus AD	aMCI versus AD
Demographics							
Age(y)	**63.33±1.5**	**68.65±2.4**	**66.28±1.5**	**0.1383**	**0.1200**	**0.4434**	**0.6259**
Education(y)	**11.78±0.9**	**10.88±1.0**	**6.520±0.85**	**0.0011***	**0.4304**	**0.0093***	**0.0003***
Gender (male/female)	**12/11**	**10/10**	**10/15**	**0.7747**	**>0.9999**	**0.5572**	**0.7715**
General cognition							
MMSE	**29.17±0.2**	**26.93±0.7**	**14.00±1.6**	**<0.0001***	**0.0078***	**<0.0001***	**<0.0001***
MoCA	**24.39±0.7**	**20.00±1.1**	**8.83±1.5**	**<0.0001***	**0.0019***	**<0.0001***	**<0.0001***

Abbreviations: HC, Healthy control; aMCI, amnestic Mild cognitive impairment; AD, Alzheimer's disease; MMSE, Mini-Mental State Exam; MoCA, Montreal Cognitive Assessment.

Values were presented as the average ± standard error (SE).

p-value, the data do not satisfy the normal distribution in the one-way ANOVA, so the p-value is obtained by Kruskal-Wallis test. *p* (Mann-Whiney U Test) was used here due to the fact that the data were not normally distributed in the Post-hoc tests. ***** indicates a statistical difference between groups, $p < 0.05$.