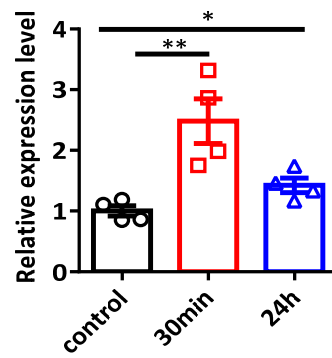


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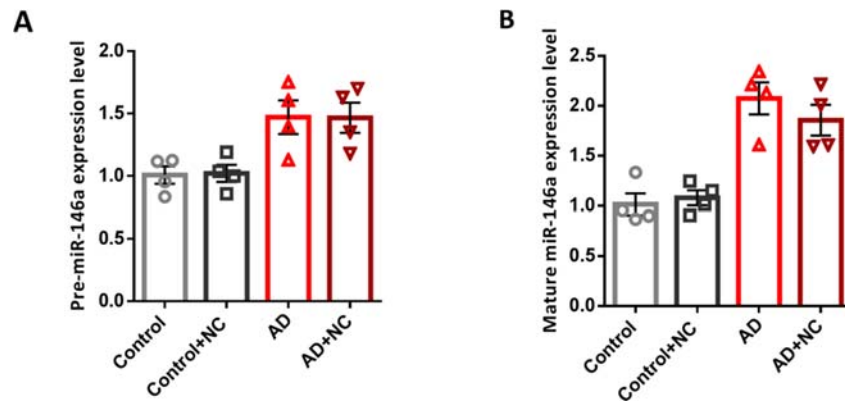
## **Supplemental Information**

### **Intranasal Administration of miR-146a Agomir Rescued the Pathological Process and Cognitive Impairment in an AD Mouse Model**

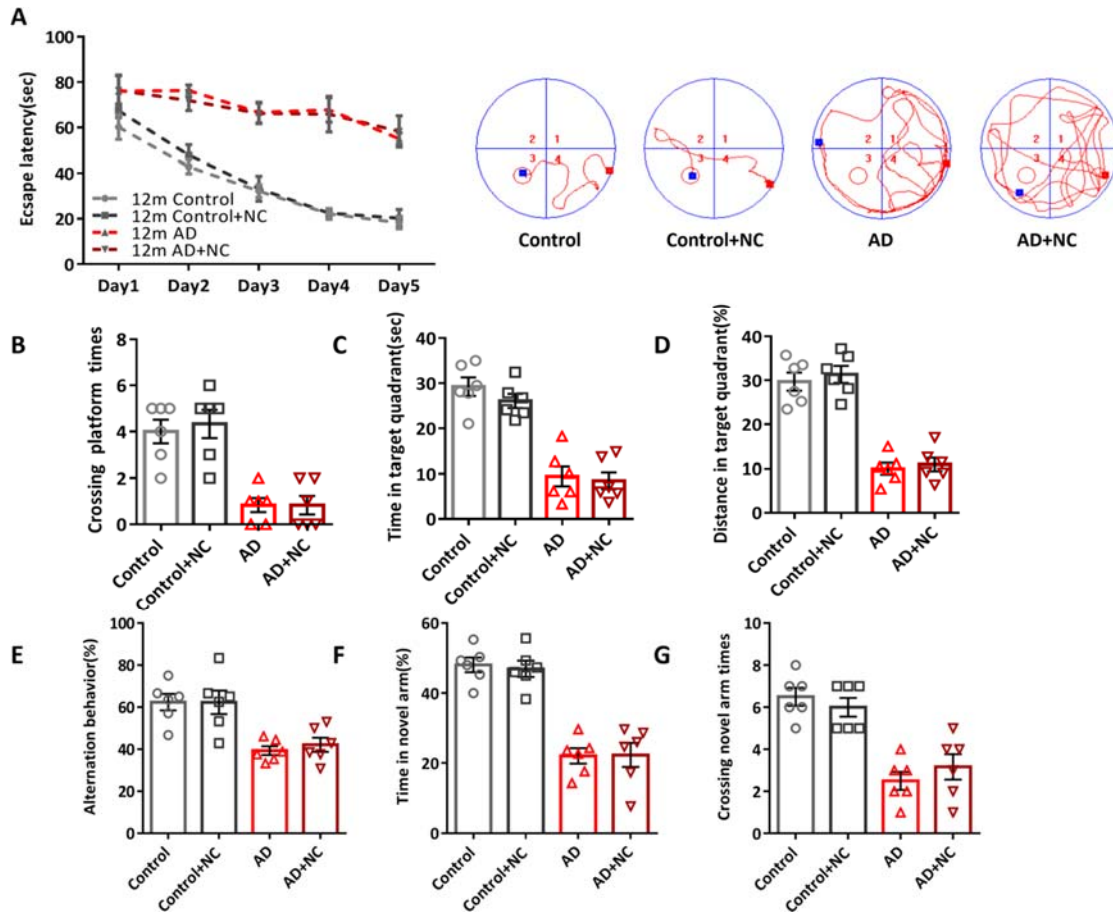
**Hui Mai, Weihao Fan, Yan Wang, Yujie Cai, Xiaohui Li, Feng Chen, Xiongjin Chen, Jingqi Yang, Pei Tang, Huiyi Chen, Ting Zou, Tingting Hong, Conghua Wan, Bin Zhao, and Lili Cui**



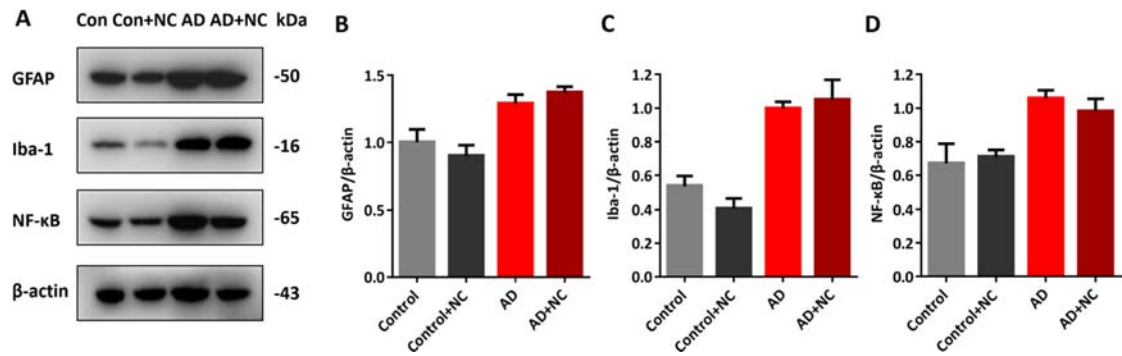
**Figure S1. Nasal administration of miR-1306 agomir effectively delivery to the hippocampus of mouse.** The expression levels of miR-1306 in the hippocampus of 12-month-old C57BL/6 mice with 1nmol miR-1306 agomir and at different time points detected by qRT-PCR. Data are presented as the mean  $\pm$  SEM. At least three independent experiments were performed. N=4 mice per group; \* $p < 0.05$ , \*\* $p < 0.01$ .



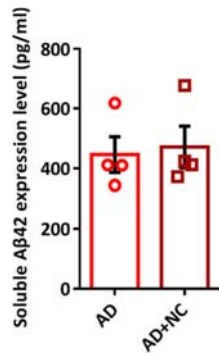
**Figure S2. M146AG NC didn't alter the pre-miR-146a and mature miR-146a expression in the hippocampus of control and APP/PS1 transgenic mice.** (A-B) The expression levels of pre-miR-146a (A) and mature miR-146a (B) in the hippocampus of control, control+NC, AD, AD+NC groups mice was detected by qRT-PCR, GAPDH was used as the control of pre-miR-146a, U6 was used as the control of mature miR-146a. ALL data are presented as the mean  $\pm$  SEM. At least three independent experiments were performed. N=4 mice per group.



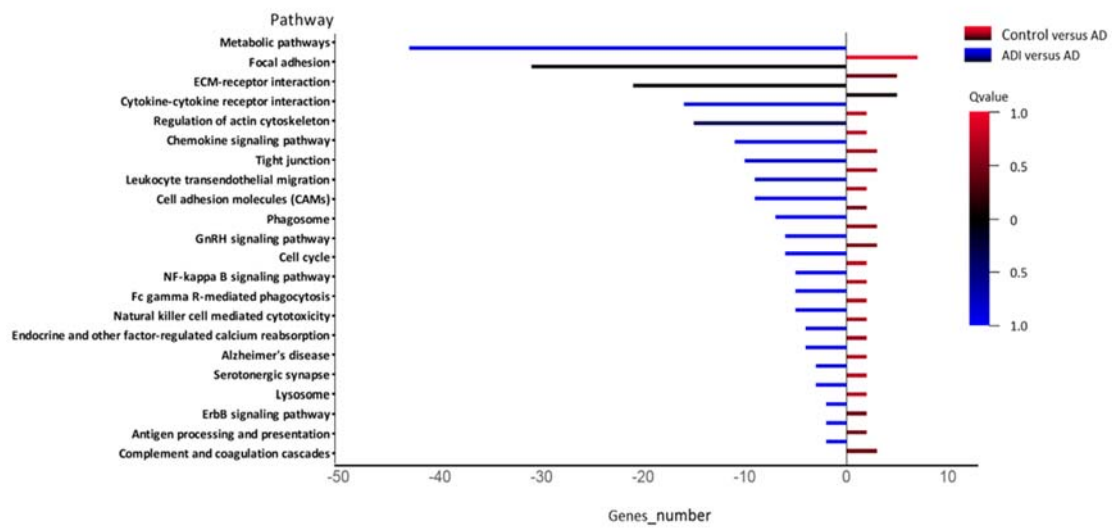
**Figure S3. The memory deficits existed in APP/PS1 transgenic mice with M146AG NC treatment.** (A) The escape latencies and path diagram of control mice (C57 mice), control+NC mice (M146AG –NC C57 mice), AD mice (APP/PS1 Transgenic mice) and AD+NC mice (M146AG-NC APP/PS1 mice) at 12-month-old were tested in the MWM for 5 consecutive days. (B-D) Probe trials performed at day 6, the crossing times in the platform site (B), the time spent in the target quadrant (C), and the more swimming distances in target quadrant (D). (E-G) Y-maze tests were performed at day 6 after MWM test and the spontaneous alternation (E), the time in novel arm (F) and the number of crossing novel arm (G) were measured during a 5-minute session. All data are presented as the mean ± SEM. At least three independent experiments were performed. M146AG-NC: Scrambled miR-146a agomir, N=6 mice per group.



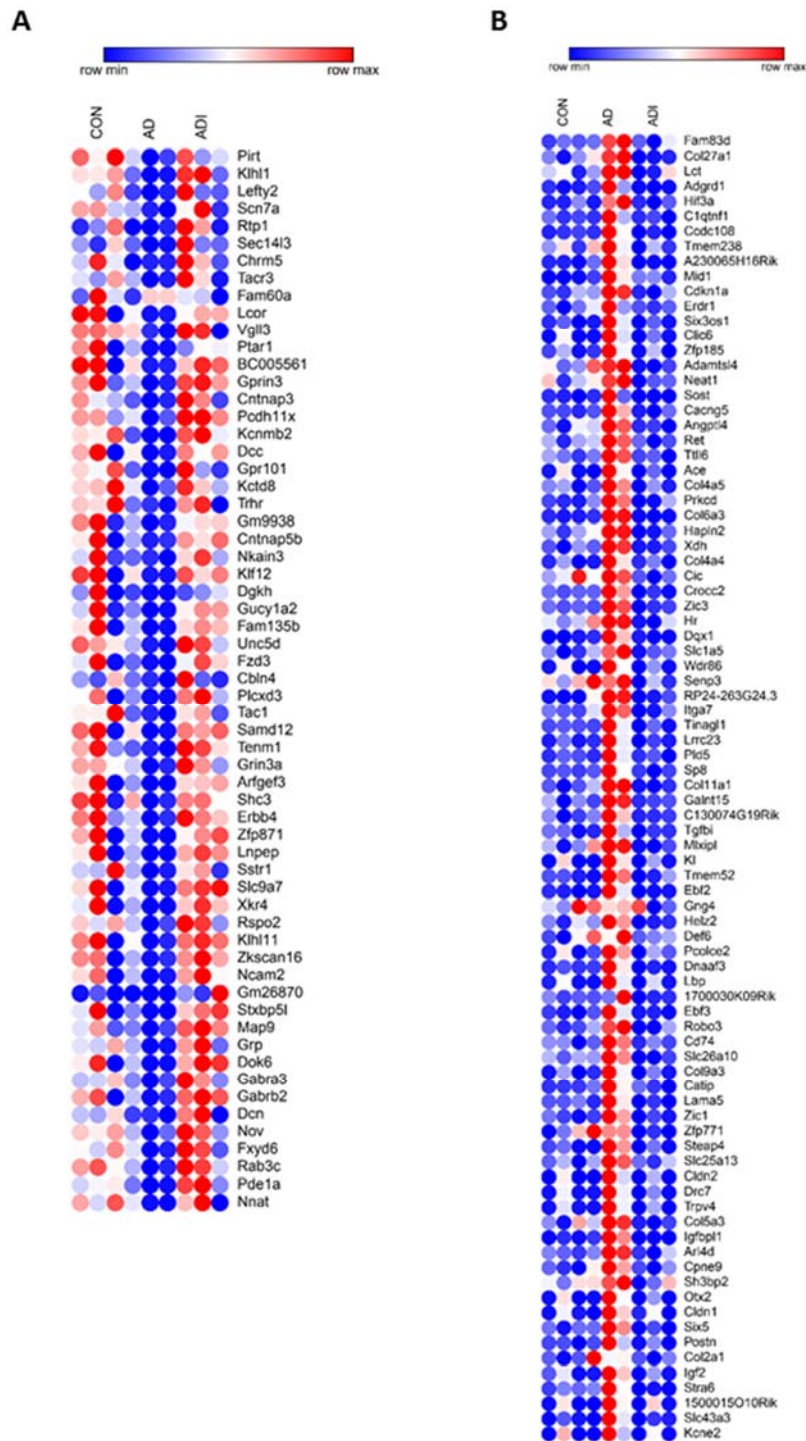
**Figure S4. The activation of glia and inflammation were existed in hippocampi of 12-month-old APP/PS1 mice with M146AG NC treatment.** (A-D) The expression levels of GFAP, Iba-1 and NF-κB were detected by western blotting, β-actin was used as the control. All data are presented as the mean ± SEM. At least three independent experiments were performed. Control represent 12-month-old C57 mice, Control+NC represent 12-month-old C57 mice with M146AG-NC administration, AD represent 12-month-old APP/PS1 mice and AD+NC represent 12-month-old APP/PS1 mice with M146AG NC administration; N=3-4 mice per group.



**Figure S5. Aβ level was unchanged in 12-month-old APP/PS1 mice with M146AG-NC administration.** The expression level of soluble Aβ42 was detected in hippocampi of AD, AD+NC groups by ELISAs. All data are presented as the mean ± SEM. N=4 mice per group.

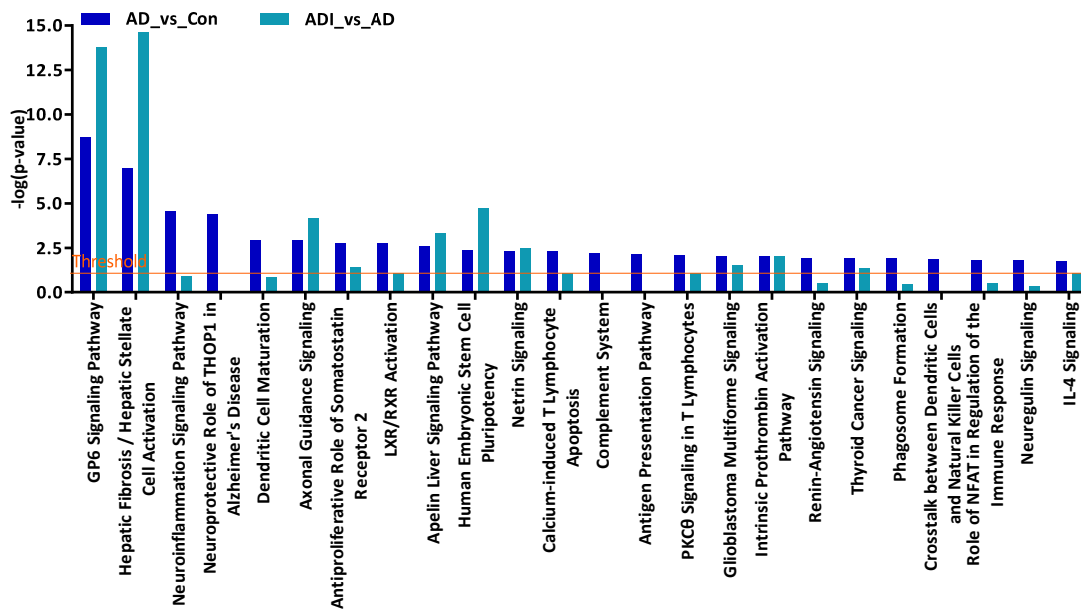


**Figure S6. KEGG Pathway analysis of the biological processes that were rescued in hippocampi of 12-month-old mice after the M146AG administration.** The closer the value is to zero, the more significant it is. AD represented 12-month-old APP/PS1 mice; ADI represented 12-month-old M146AG-APP/PS1 mice; Control represented 12-month-old C57 mice. N=3 mice per group.



**Figure S7. Genome-Wide transcriptional microarray analysis of differential genes that were rescued in hippocampi of 12-month-old mice after the M146AG administration.** (A) The up-regulated gene in 12-month-old M146AG-APP/PS1 mice compared to the APP/PS1 mouse. (B) The down-regulated gene in 12-month-old M146AG-APP/PS1 mice compared to the APP/PS1 mouse. Red indicating higher expression (FPKM) and navy indicating lower expression (FPKM). AD represented 12-month-old APP/PS1 mice; ADI represented 12-month-old M146AG-APP/PS1 mice; Control represented 12-month-old C57 mice. N=3 mice per group. \* $p < 0.05$ .





**Figure S8. The functional pathway analyses of the M146AG “rescued” genes of AD by IPA software.** The significant enrichment of the differentially expressed genes in the IPA-based classical pathway was compared among the three groups in pairs. Abscissa is the path name, and ordinate is the significance level of enrichment (negative logarithmic transformation with base 10). AD represented 12-month-old APP/PS1 mice; ADI represented 12-month-old M146AG-APP/PS1 mice; Control represented 12-month-old C57 mice. N=3 mice per group. \*p <0.05.

**Table S1. Primers Sequence for qRT-PCR**

Category	Sequences
miR-146a	Forward:5'- CCTGAGAAGTGAATTCCATGGG -3' Reverse:5'- TGGTGTCGTGGAGTCG -3'
Pre-miR-146a	Forward:5'- TGAATTCCATGGGTTATATCAA -3' Reverse:5'- ATTCACAGGTCTGACATTGA -3'
miR-1306	Forward:5'- CTCACTACGTTGGCTCTGGTG -3' Reverse:5'- TATGCTTGTCTCTGTCTCTGTGTC -3'
TLR4	Forward:5'- ATGGCATGGCTTACACCACC -3' Reverse:5'- GAGCCAATTTTGTCTCCACA -3'
NF-κB	Forward:5'- CTGACCTGAGCCTTCTGGAC -3' Reverse:5'- GCAGGCTATTGCTCATCACA -3'
IRAK1	Forward:5'- GCTGTGGCACCGATACCT -3' Reverse:5'- GCTACACCCACCCACAGAGT -3'
TRAF6	Forward:5'- GATGGTTGTGTGTGTCTG -3' Reverse:5'- AGACACCCAGCAGCTAAGA -3'
U6	Forward:5'- ATTGGAACGATACAGAGAAGATT -3' Reverse:5'- GGAACGCTTCACGAATTTG -3'
GAPDH	Forward:5'- GAAGGGCTCATGACCACAGTCCAT -3' Reverse:5'- TCATTGTCGTACCAGGAAATGAGCTT -3'