

Figure S1. Microglia-specific miR-146a overexpression improved cognitive deficits in learning and memory in APP/PS1 Tg mice. A-C: Quantification of miR146a, pre-miR146a and miR146a-3p by qRT-PCR of brain (n=6 for each group). Statistical analysis was performed using one-way ANOVA followed by post hoc Tukey's tests for multiple comparisons. D-E: Quantification of swimming speed and escape latency of MWM on the first day (For each group, n=8). F: The escape latencies of each group were tested in the Morris water maze for 5 consecutive days. Statistical analysis was performed using two-way ANOVA followed by post hoc Tukey's tests for multiple comparisons (Interation: Time*Treatment, P = 0.5526, no significant effcet of two factors). G-I: Probe trials were performed at day 6, the number of times crossing in the platform site (G), the time spent in the target quadrant (H), and the more swimming distances in the target quadrant (I) are shown. Statistical analysis was performed using

one-way ANOVA followed by post hoc Tukey's tests for multiple comparisons (H-J). J-K: Quantification of swimming speed and escape latency of MWM on the first day. L-M: Quantification of recognition coefficient (L) and division coefficient (M) in the novel object recognition test of APP/PS1-AAV-M146a mice and APP/PS1-AAV-Mcon mice. one-way ANOVA with Tukey's multiple comparisons test. For each group, n = 6. N: The expression level of NLRP inflammasome (ASC, NLRP and Caspase1) was analyzed by qRT-PCR with the cerebral sample of each group (n=6). Statistical analysis was performed using Two-way ANOVA followed by post hoc Tukey's tests for multiple comparisons. Shown are the means \pm S.E.M values from three independent experiments. *Represents P<0.05, **P<0.01, *** P<0.005, and ****P<0.001.



Figure S2. Increased miR-146a trigger the switch of microglia phenotype and enhance its phagocytosis in LPS induced neuroinflammation. HMC3 cells were transfected with 10 nM miR-146a Mimics /100 nM inhibitor and given 100 ng/ml LPS for 72 h. A: The level of miR-146a increased by overexpressing exogenous miR-146a.

Statistical analysis was performed using one-way ANOVA followed by post hoc Tukey's tests for multiple comparisons, B-C: mRNA levels of proinflammatory factors (IL-6, IL-1 β , TNF- α) secreted by M1 phenotype microglia and M2 phenotype microglia markers (Arg1, TGF-β) were measured by qRT-PCR. Two-way ANOVA followed by post hoc Tukey's tests for multiple comparisons. D: The level of miR-146a declined by exogenous miR-146a inhibitor overexpression. Statistical analysis was performed using one-way ANOVA followed by post hoc Tukey's tests for multiple comparisons. E and F: mRNA levels of proinflammatory factors (IL-6, IL-1 β , TNF- α) secreted by M1 phenotype microglia and M2 phenotype microglia markers (Arg1, TGF-B) under LPS-induced neuroinflammation with miR-146a inhibitor or not. Two-way ANOVA with post hoc Tukey's tests for multiple comparisons. ns: no significance. G and N: HMC3 were treated as above and stained with α-tubulin (green) to visualize cytoskeleton. After a 3 h incubation, phagocytosed microspheres appeared red, and DAPI-stained nuclei appeared blue. Objective magnification: 20X, Zoom: X3, Scale bar: 30 um, H-K and O-R: The cell area, Feret's diameter, circumference and phagocytosis of fluorescent beads were measured by ImageJ. Statistical analysis was performed using one-way ANOVA followed by post hoc Tukey's tests for multiple comparisons. L-M and S-T: The level of phagocytic protein MFG-E8 was measured by Western blot and using ImageJ to quantification. Statistical analysis was performed using one-way ANOVA followed by post hoc Tukey's tests for multiple comparisons. Shown are the means \pm S.E.M values from three independent experiments. *Represents P<0.05, **P<0.01, ***P<0.005, and ****P<0.001.



Figure S3. Increased miR-146a in Microglia prevent neurons apoptosis in LPS induced neuroinflammation. HMC3 cells were transfected with 10 nM miR-146a Mimics / 100 nM inhibitor and given 100 ng/ml LPS for 72 h, then SH-SY5Y cells were added to HMC3 cells at 1:1 ratio, and apoptosis was detected by flow cytometry after 24 h. A-B: SH-SY5Y cells and HMC3 cells were separated by flow cytometry, then go to apoptosis dectection by AnnexinV-PE/7-AAD in miR-146a Mimics (A) or inhibitor (B) group. C-D: LPS treatment of HMC3 increased the apoptosis of SH-SY5Y, an effect reduced by miR-146a mimics (C), but not in the presence of miR-146a inhibitor (D). Statistical analysis was performed using one-way ANOVA followed by post hoc Tukey's tests for multiple comparisons. Data are the means \pm S.E.M values from three independent experiments. *Represents P<0.05, **P< 0.01, ***P<0.005, and ****P<0.001.



Figure S4. Network of protein interactions analysis of the microglial miR-146a overexpressed AD mouse model. A-B: Networks visualizing the functional protein association for gene signatures in APP/PS1-AAV-Mcon mice and APP/PS1-AAV-M146a mice. Nodes in the network represent proteins and edges represent protein–protein interactions, which indicate known or predicted interactions.



Figure S5. Identification and validation of miR-146a target genes. A: Venn diagram of all differential genes downregulating genes (Down), and target genes (Targets) were drew on the website (All), http://bioinformatics.psb.ugent.be/webtools/Venn/. B-C: Luciferase reporter assays were used to detect the interaction between the putative FoxO6, Nkd2, Stpg1 and Cd4 binding sites with miR-146a. The wild-type (wt) FoxO6, Nkd2, Stpg1 and Cd4 3'UTRs constructs were co-transfected with miR-146a mimic in 293T cells and the relative activity of luciferase is presented as Renilla/firefly luminescence units (RLUs). Two-tailed unpaired t test with Welch's correction used for data analysis. D-E: The mRNA levels of Nkd2 and Stpg1 in APP/PS1-AAV-Mcon group and APP/PS1-AAV-M146a group were detected by qRT-PCR. F: The protein level of Nkd2 and Stpg1 were measured by Western blot. G-H: Quantification of Nkd2 and Stpg1 protein levels were measured by ImageJ in APP/PS1-AAV-Mcon group and APP/PS1-AAV-M146a group. Statistical analysis was performed using two-tailed unpaired t test with Welch's correction. I-J: 293T cells were transfected with miR-146a mimics, miRNA NC, inhibitor NC or inhibitor for 24 h, and the mRNA levels of Nkd2 and Stpg1 were detected by qRT-PCR. K-L: HMC3 cells were transfected with miR-146a mimics, miRNA NC, inhibitor NC or inhibitor for 24 h, and the mRNA levels of Nkd2 and Stpg1 were detected by qRT-PCR. Statistical analysis was performed using two-tailed unpaired t test with Welch's correction. M: HMC3 cells were transfected with si-control, Nkd2 siRNA1, siRNA2 or siRNA3 for 24 h, and the mRNA levels of Nkd2 was detected by gRT-PCR. N-Q: HMC3 cells were transfected with si-control or Nkd2 siRNA2 and given A
ß stimulate for 12 h. The mRNA level of IL-1
ß, IL-6, Arg1 and IL-10 were detected by qRT-PCR. R-U: HMC3 cells were transfected with miR-146a inhibitor NC, inhibitor or Nkd2 siRNA2 and given A β stimulate for 12 h. The mRNA level of IL-1 β , IL-6, Arg1 and IL-10 were detected by qRT-PCR. Statistical analysis was performed using one-way ANOVA followed by post hoc Tukey's tests for multiple comparisons. All data are presented as the means \pm S.E.M. At least three independent experiments were performed. *Representsp < 0.05, **p < 0.01, ***p < 0.001, and ****P<0.001.

Table S1 primers sequences

Primer name	Upstream primer $(5' \rightarrow 3')$	Downstream primer $(5' \rightarrow 3')$
U6	ATTGGAACGATACAGAGAAGATT	GGAACGCTTCACGAATTTG
MiR-146a	CCTGAGAAGTGAATTCCATGGG	TGGTGTCGTGGAGTCG
Homo-GAPDH	GAAGGGCTCATGACCACAGTCCAT	TCATTGTCGTACCAGGAAATGAGCTT
Homo-IL-6	CCTTCCAAAGATGGCTGAAA	TGGCTTGTTCCTCACTACT
Homo-IL-1β	CATGGGATAACGAGGCTTATG	CCACTTGTTGCTCCATATCC
Homo-TNF-α	GAGCCAGCTCCCTCTATTTA	GGGAACAGCCTATTGTTCAG
Homo-Arg1	GGGCTACTCTCAGGATTAGAT	CGAAACAAGCCAAGGTTATTG
Homo-TGF-β	CAGTCACCATAGCAACACTC	CCTGGCCTGAACTACTATCT
Mus-NLRP	ACCAGCCAGAGTGGAATGAC	ATGGAGATGCGGGAGAGATA
Mus-Caspase1	GCTTTCTGCTCTTCAACACC	AAAATGTCCTCCAAGTCACAAG
Mus-ASC	GCCAGAACAGGACACTTTGTG	AGTCAGCACACTGCCATGC
Mus-IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Mus-IL-1 β	GGGCCTCAAAGGAAAGAATCT	GAGGTGCTGATGTACCAGTTGG
Mus-TNF-a	GACGTGGAACTGGCAGAAGAG	TTGGTGGTTTGTGAGTGTGAG
Mus-Arg1	CTTGGCTTGCTTCGGAACTC	GGAGAAGGCGTTTGCTTAGTTC
Mus-TGF-β	CAGAGCTGCGCTTGCAGAG	GTCAGCAGCCGGTTACCAAG
Homo-Stpg1	ATCTCCCAGCAGGCTTTGAC	CTCTGGAGAGGCCACAGTTG
Homo-Nkd2	CATGAAGTGAGAGGGGGACAGG	TCTTTCTAGCACTGCTGCCTT
Mus-GAPDH	AAGAGGGATGCTGCCCTTAC	TACGGCCAAATCCGTTCACA
Mus-Nkd2	AGAGGAGAGAGAGTCCCGAAG	CTTGTCCCGTGAGCGATGTT
Mus-CD16	CCAGC TACACGTTTAAGGCC	TAGCGTGATGGTTTCCCCTT
Mus-CD206	GTGGGGACCTGGCAAGTATC	CACTGGGGTTCCATCACTCC
Mus-IL10	TGGTAGAAGTGATGCCCCAG	ACTCTTCACCTGCTCCACTG
Homo-IL-10	GTTGCCAAGCCTTGTCTGAG	CATTCTTCACCTGCTCCACG
Mus-MFG-E8	CGGGCCAAGACAATGACATC	TCTCTCAGTCTCATTGCACACAAG

Table S3 Homo-Nkd2 siRNA target sequences

Name	target sequences
Homo-Nkd2 siRNA1	CGTTCACGCTCTATGACTT
Homo-Nkd2 siRNA2	AGAACACGGAGCGCAGAAA
Homo-Nkd2 siRNA3	GCCTCATGCACACCATCTA