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Supplemental Information

miR-351-5p/Miro2 axis contributes to hippocampal neural progenitor cell death via unbalanced mitochondrial fission

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Figure S1

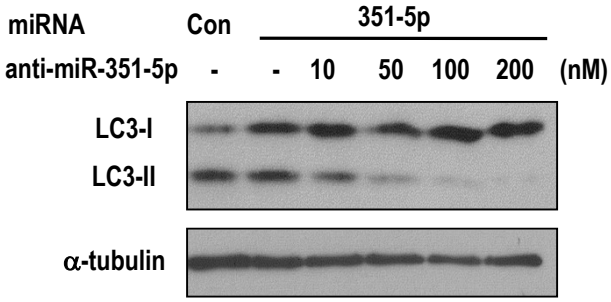


Figure S1. Co-treatment of miR-351-5p with anti-miR-351-5p restored LC3 conversion

HCN cells were treated with miR-Con or miR-351-5p together with anti-miR-351-5p at the concentration of 15, 50, 100, 200 nM, respectively. 24 h after transfection, cells were analyzed by western blotting using anti-LC3 or anti-α-tubulin antibody.

Figure S2

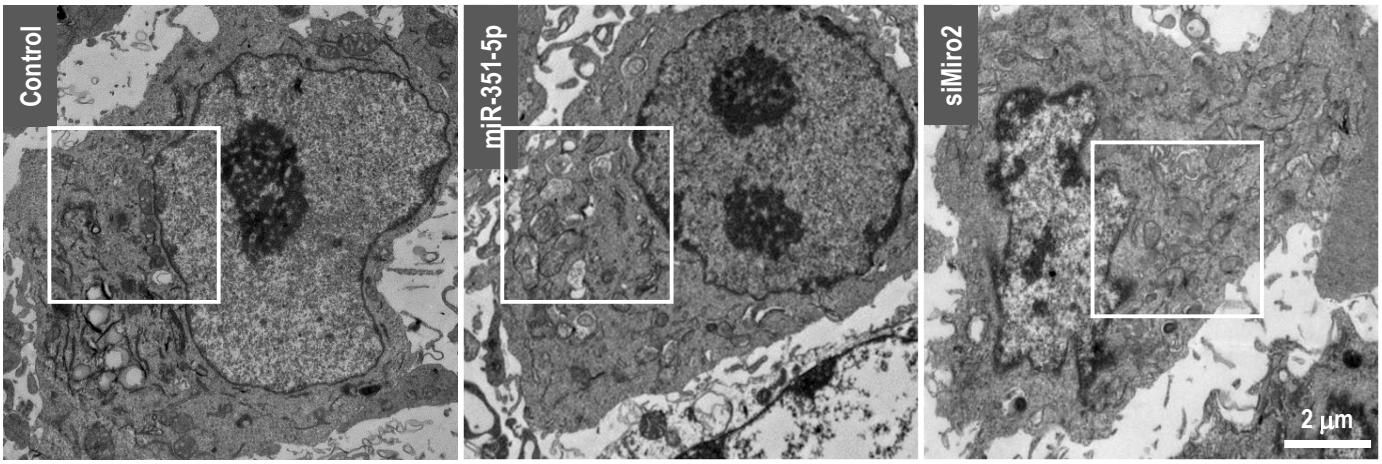


Figure S2. miR-351-5p and siMiro2 induced mitophagy in HCN cells

(A) Cells were transfected with miR-Con, miR-351-5p, or siMiro2. At 24 h after transfection, cells were prepared for transmission electron microscopy. Multiple autophagosome-containing mitochondria are observed in miR-351-5p and siMiro2-treated cells. White boxed area were magnified and showed in Figure 4A.

Figure S3

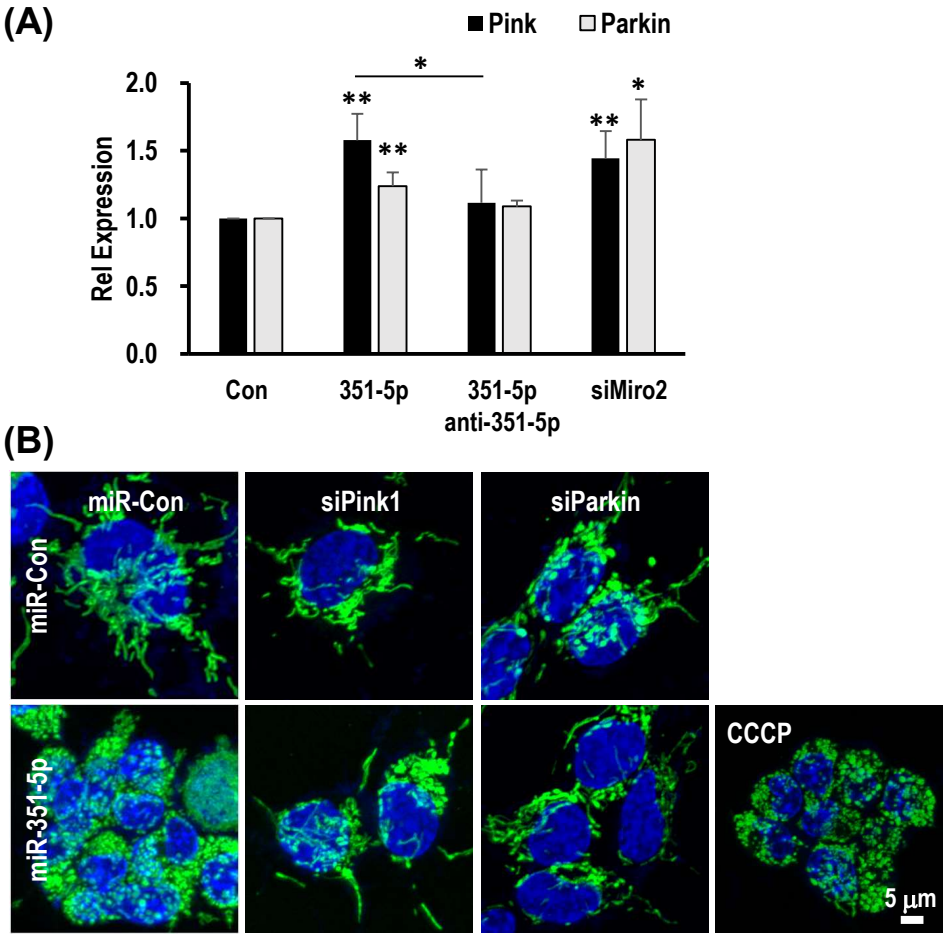


Figure S3. Pink1 and parkin are involved in mitochondrial dysfunction and mitophagy induced by miR-351-5p and siMiro2

HCN cells were treated with miR-Con, miR-351-5p, anti-miR-351-5p, or siMiro2. (A) 24 h after transfection, cell lysates were prepared and assayed by western blotting using anti-Pink1 or anti-parkin antibody. Bands were analyzed by ImageJ software. (B) Cells were treated with Mitotracker green and Hoechst 33258. The cells were then observed under confocal fluorescence microscope. * $p < 0.05$; **, $p < 0.01$

Figure S4

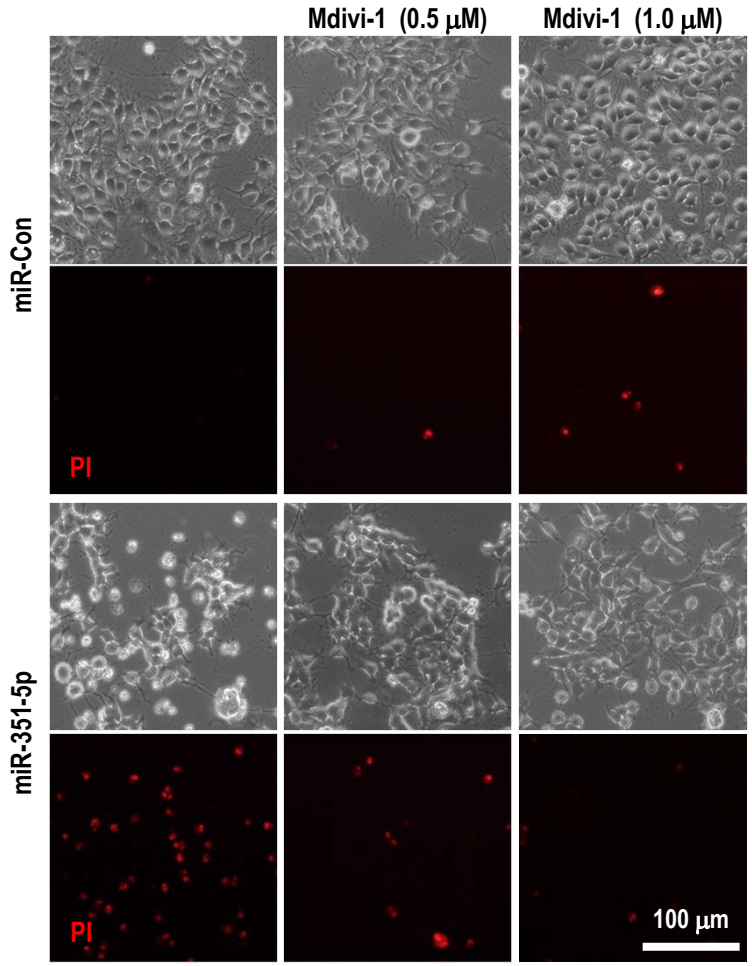


Figure S4. Inhibition of mitochondrial fission abolished cell death induced by miR-351-5p

Cells were pretreated with the mitochondrial fission inhibitor Mdivi-1 for 1 h before transfection. Next, cells were transfected with miR-Con or miR-351-5p. At 24 h after transfection, cells were stained with PI. Cell death was analyzed as described above.

Figure S5

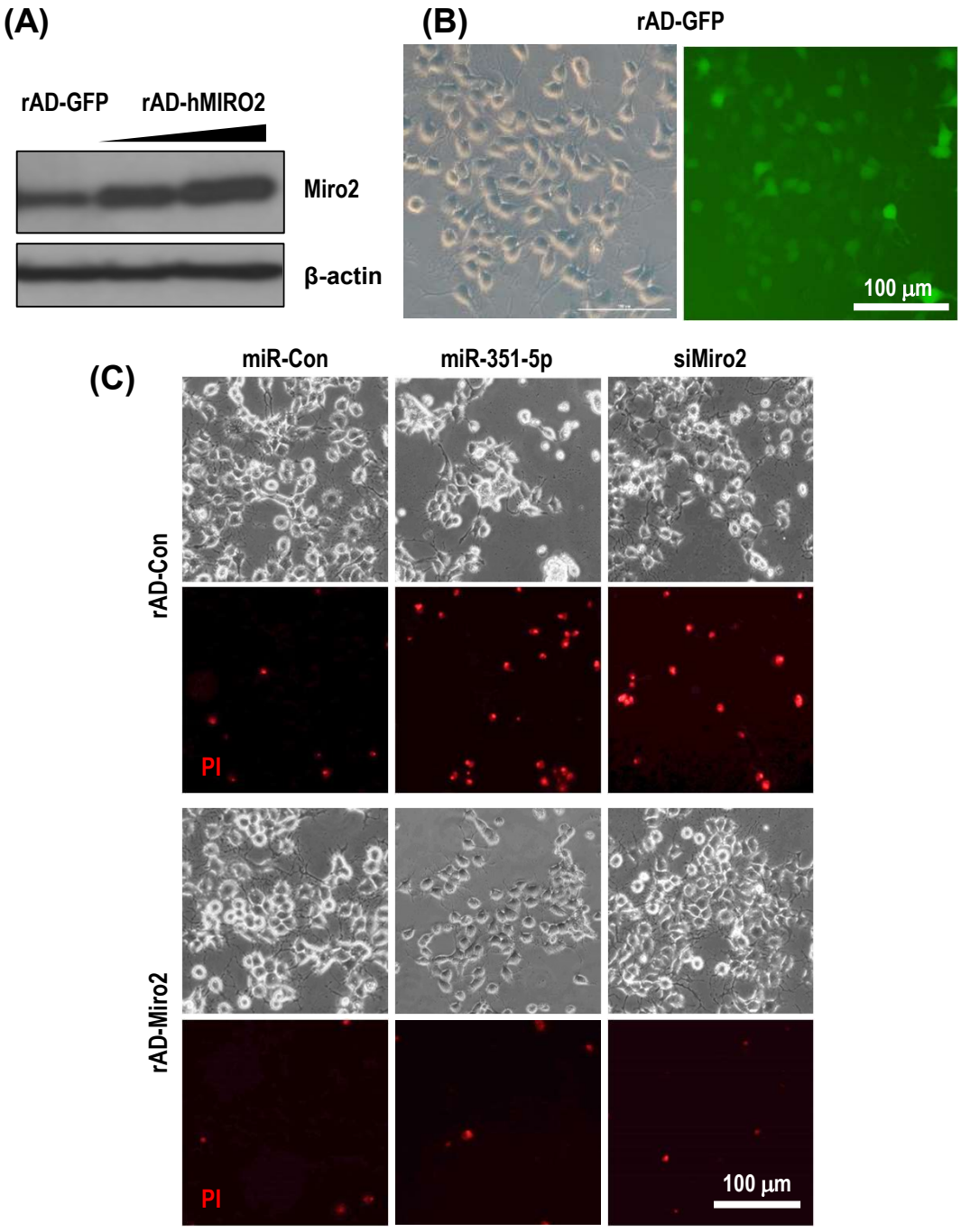


Figure S5. rAD-Miro2 restored mitochondrial fission induced by miR-351-5p

(A-B) Adenovirus expressing Miro2 was prepared. Infection efficiency of rAD in HCN cells was confirmed by using rAD-GFP. The expression levels of Miro2 was investigated by western blotting. β-actin was used as loading control. (C) Cells were transfected with miR-Con, miR-351-p, or siMiro2, together with either rAD-Con or rAD-Miro2. After 24 h, cells were stained with PI and observed. Cell death was calculated by counting PI-positive cells.

Figure S6

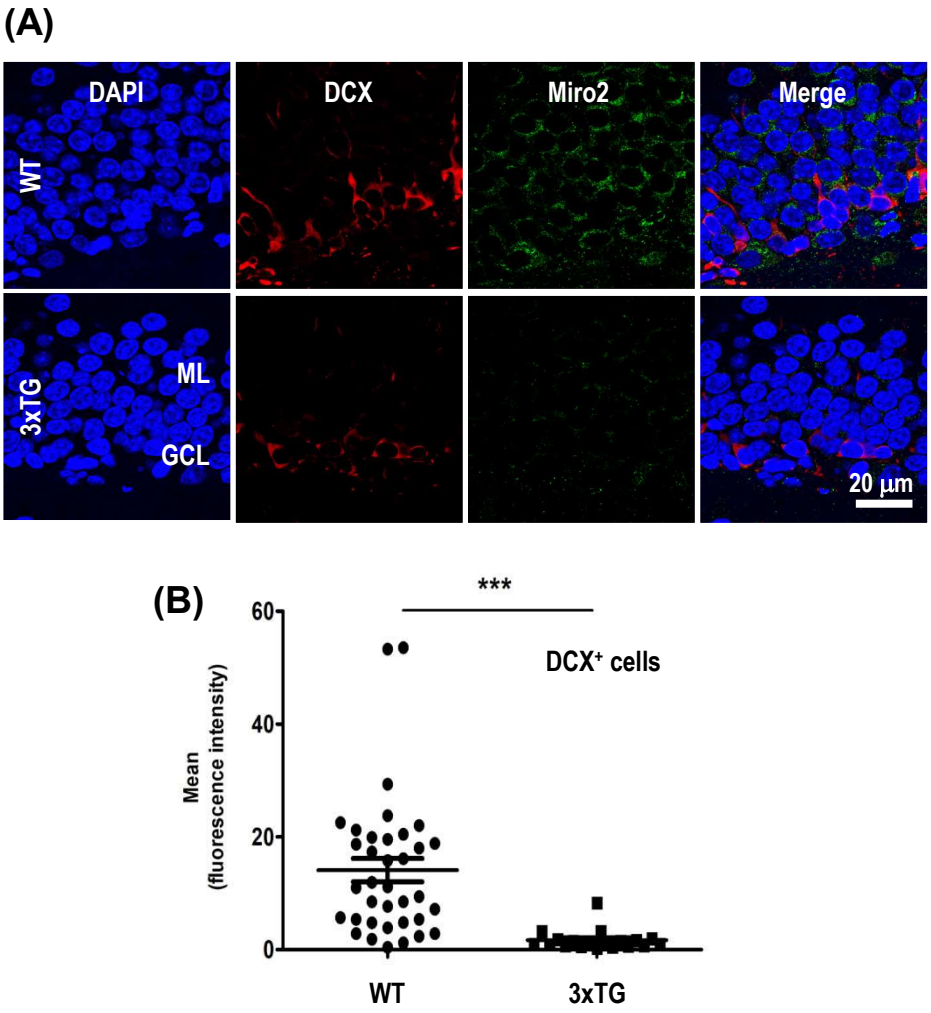


Figure S6. Miro2 expression was deregulated in AD model mice

(A) To analyze the level of Miro2 expression in proliferating cells in the hippocampus of mouse brain, 15-weeks age of WT and 3xTG AD model mice were examined by immunohistochemistry using anti-Miro2 and anti-DCX antibody. ML is molecular layer and GCL is granule cell layer in dentate gyrus. (B) The levels of Miro2 were quantified specifically in DCX+ cells using ImageJ software. The dot plot graph data represent the mean±SD values (n=36 cells for WT, 24 cells for 3xTG from 5 independent experiments). *** $p < 0.001$.

Figure S7

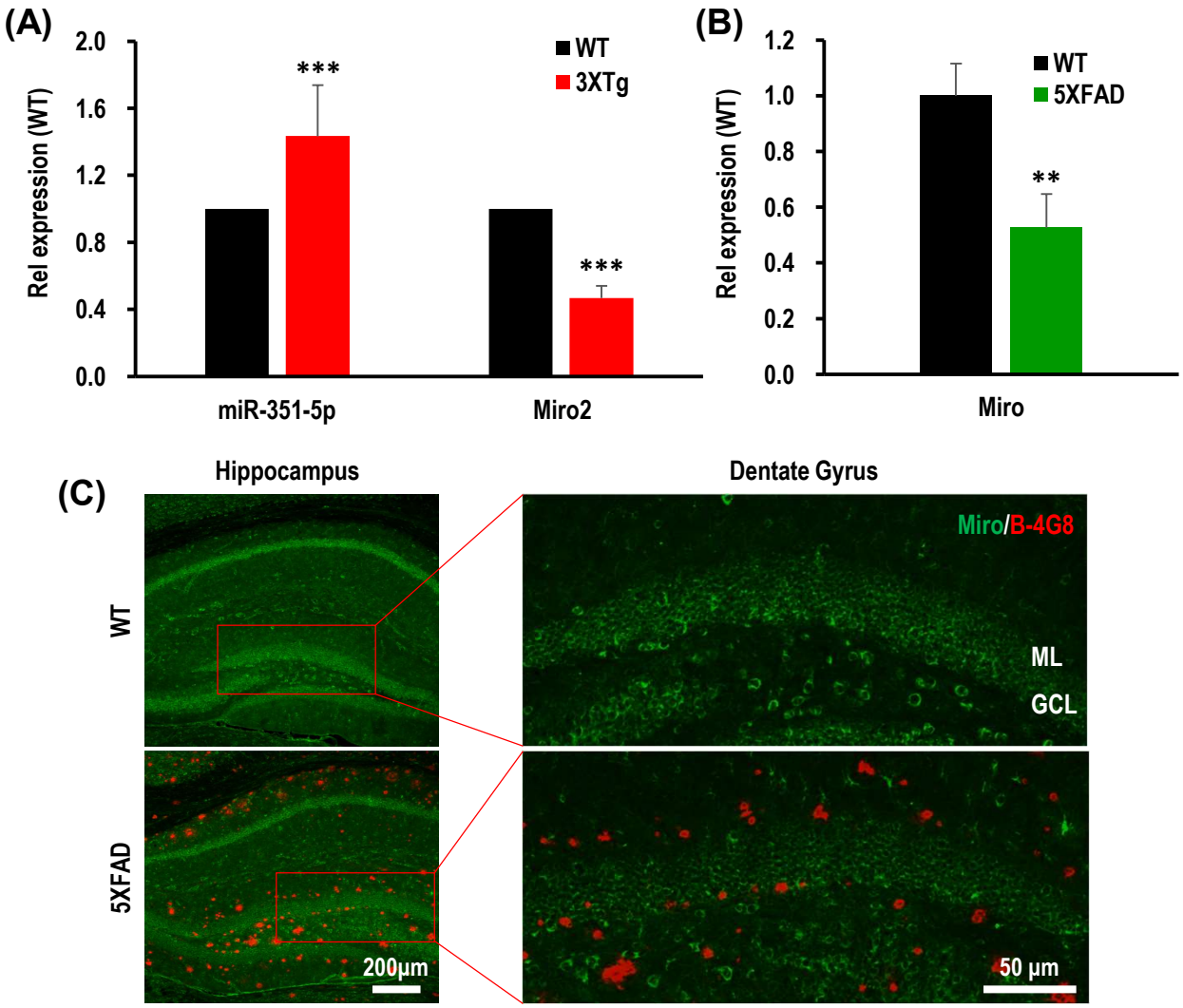
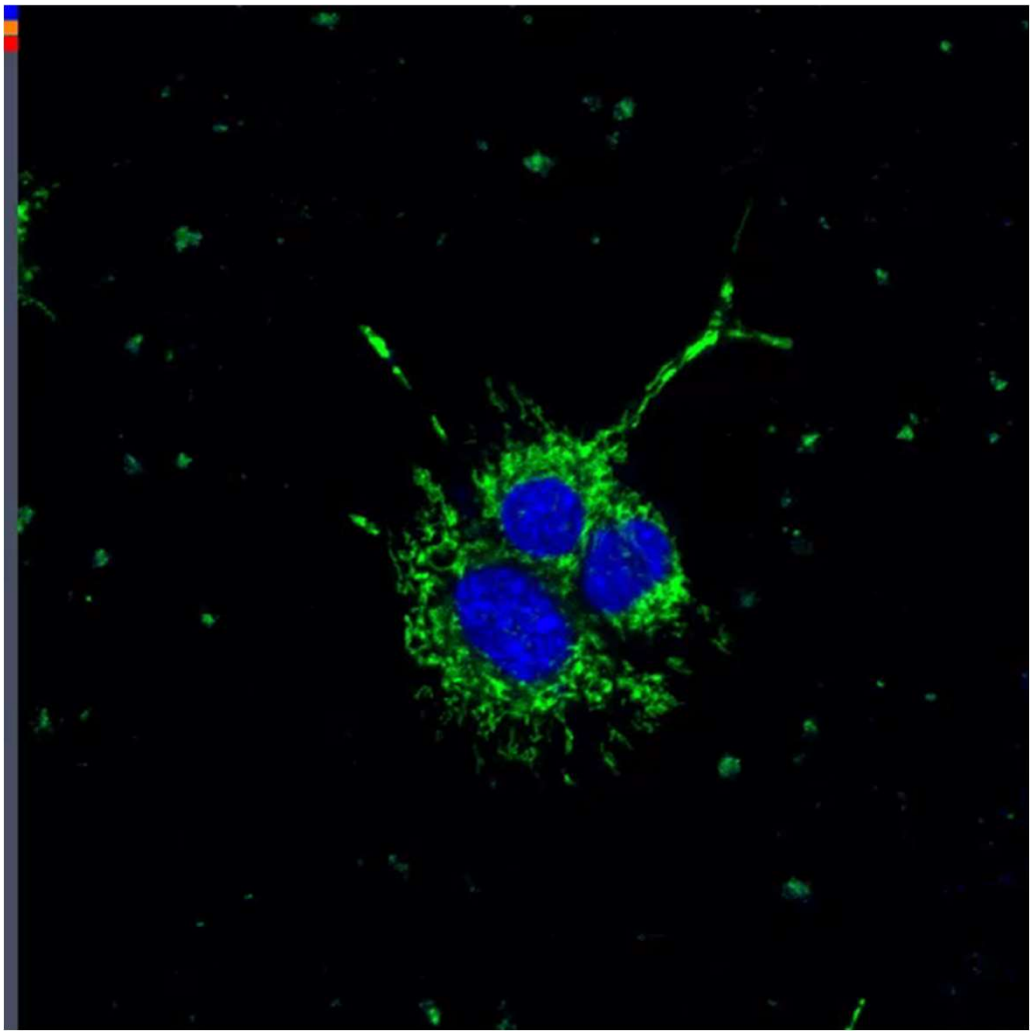


Figure S7. miR-351-5p and Miro2 expression showed opposite correlation in AD model mice

(A) To compare the expression level of miR-351-5p and Miro2, hippocampus obtained from 10 months age of WT and 3xTG AD model mice was examined by quantitative PCR. (B-C) In addition, cryo-sections of hippocampus were stained with anti-Miro (green) and anti-amyloid β antibody (red). ML is molecular layer and GCL is granule cell layer in dentate gyrus. The expression level of Miro was measured by ImageJ software. The graph data represent the mean±SD values (n>3). ** $p < 0.01$; *** $p < 0.001$.



Movie 1. miR-351-5p induced excessive mitochondrial fission in HCN cells

HCN cells were transfected with miR-351-5p and stained with mitotracker green. Cells were observed for 16 h under a fluorescence microscope supported by a mini-scale CO_2 incubation chamber. Photos were taken every 20 seconds.