Supplementary information

Supplementary method

Flow cytometry

IL-4 treated or non-treated BV2 cells were cultured in a 15 cm culture dish to a 90% confluent. After trypsinization, we resuspended the cells in PBS, and incubated with ARG-APC antibody (eBioscience, Waltham, MA) at 4 degrees in the dark for 30 minutes. Cells were analyzed on a FACSCalibur (BD Biosciences, San Jose, CA), and flowjo was used for further analysis.

PKH26 staining of exosomes and exosome tracking

For exosome uptake experiment, M2-EXO was labeled with PKH26 Red Fluorescent Cell Linker Kit according to the manufacturer's protocol with minor modifications (Sigma, San Louis, MO). Exosomes diluted in PBS were added to 1 ml Diluent C. In parallel, 4 μl PKH26 dye was added to 1 ml Diluent C and incubated with the exosome solution for 4 minutes. The labeled exosomes were washed and centrifuged at 100000 g for 1 h, and the exosome pellet was diluted in 100 μl PBS for further use. For the exosome tracking, M2-EXO was incubated with neurons for 5 h and imaged by the fluorescent microscope.

HE staining

The mice were perfused with 4% paraformaldehyde, and the heart, liver, spleen and lung were fixed in 4% paraformaldehyde, cryosectioned at a thickness of 30 μm, and stained with hematoxylin-eosin (Beyotime, Jiang Su, China). The instruction protocol was used for the dyeing experiment. After staining, the

sections were treated with 70% ethanol, 80% ethanol, 90% ethanol, 100% ethanol, and xylene.

Exosomal miRNA array

M2-EXO and M0-EXO were isolated as described above. Microarray analysis of the exosomal miRNA profiles including labelling, hybridization, scanning, normalization and data analysis was done by KangChen Bio-tech with the Agilent.

Figures and figure legends

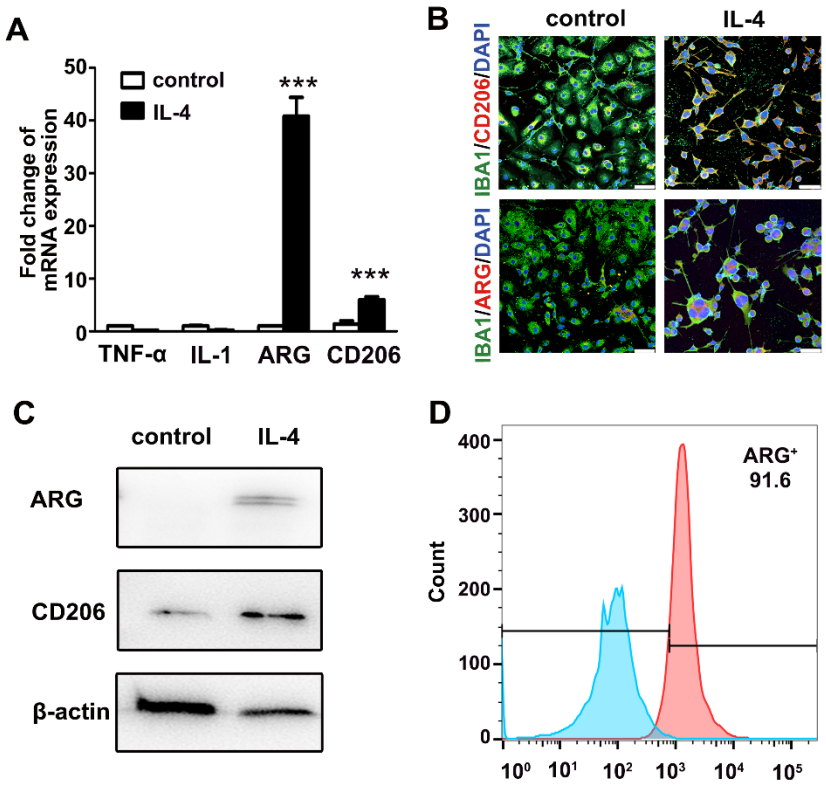

Figure S1. BV2 cells were induced to the M2 type by IL-4 treatment.

A. mRNA levels of ARG and CD206 were detected in BV2 cells with or without IL-4 treatment. **B-C.** Immunohistochemistry and western blot analysis of ARG and CD206 protein levels. **D.** Flow cytometry showing that 91.6% of cells were ARG-positive cells. Scale bar=50 μm. Data are presented as the mean±SD. ***, $p<0.001$.

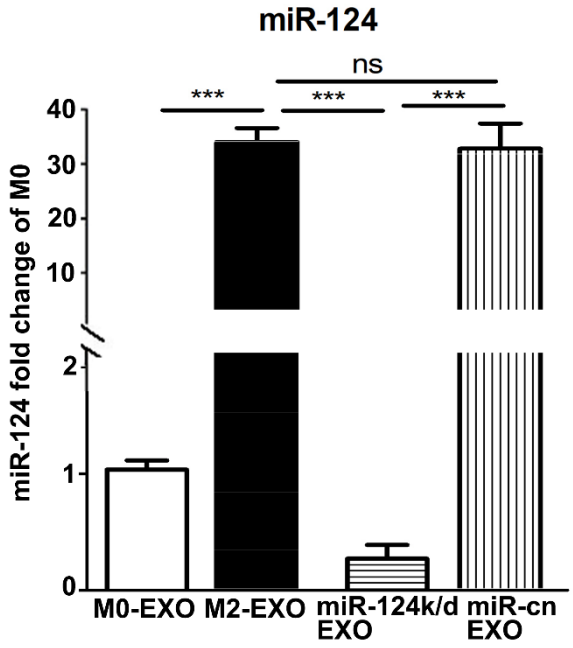

Figure S2. Upregulated expression of miR-124 in M2 BV2-derived exosomes.

Bar graph showing the expression of miR-124 in exosomes derived from M0 BV2, M2 BV2, miR-124 k/d M2 BV2 and miR-cn M2 BV2 cells. miR-124k/d EXO: exosomes isolated from M2-BV2 cells with downregulated miR-124. miR-cn EXO: exosomes isolated from M2-BV2 cells treated with control shRNA. Data are presented as the mean±SD. ***, $p<0.001$.

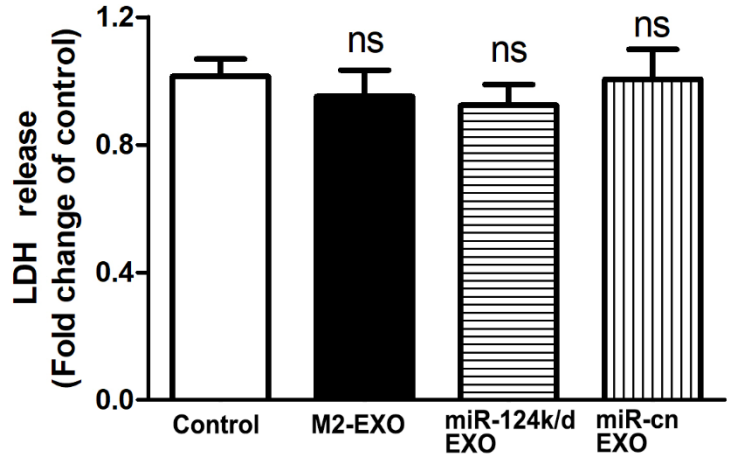

Figure S3. LDH assay under normal conditions.

LDH assay in control neurons, neurons treated with M2-EXO, neurons treated with miR-124k/d EXO and neurons treated with miR-cn EXO. Data were normalized to the control group.

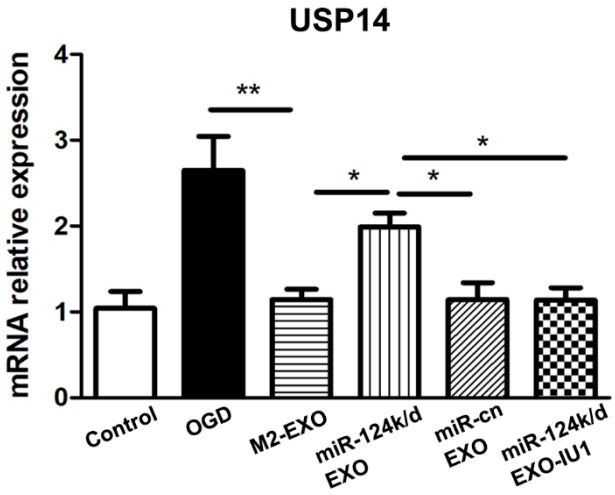

Figure S4. IU1 treatment significantly downregulated the expression of USP14 *in vitro*.

mRNA level of USP14 was detected in control neurons, untreated OGD-exposed neurons, and OGD-exposed neurons treated with M2-EXO, miR-124k/d EXO or miR-124k/d EXO+IU1. Data are presented as the mean±SD. *, $p<0.05$, **, $p<0.01$.

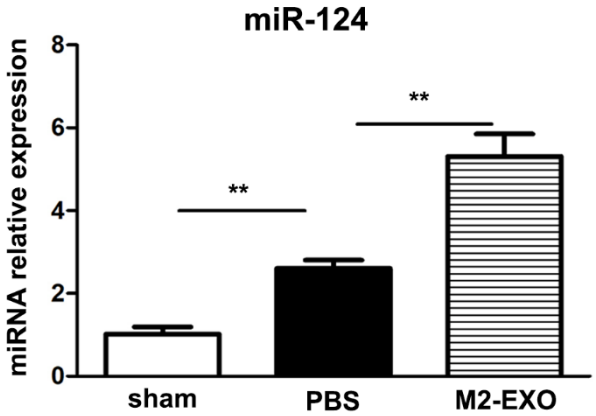

Figure S5. Treatment with M2-EXO significantly upregulated the expression of miR-124 *in vivo*.

miRNA level of miR-124 was detected in sham mice, MCAO mice, and MCAO mice treated with M2-EXO. Data are presented as the mean±SD. **, $p<0.01$.

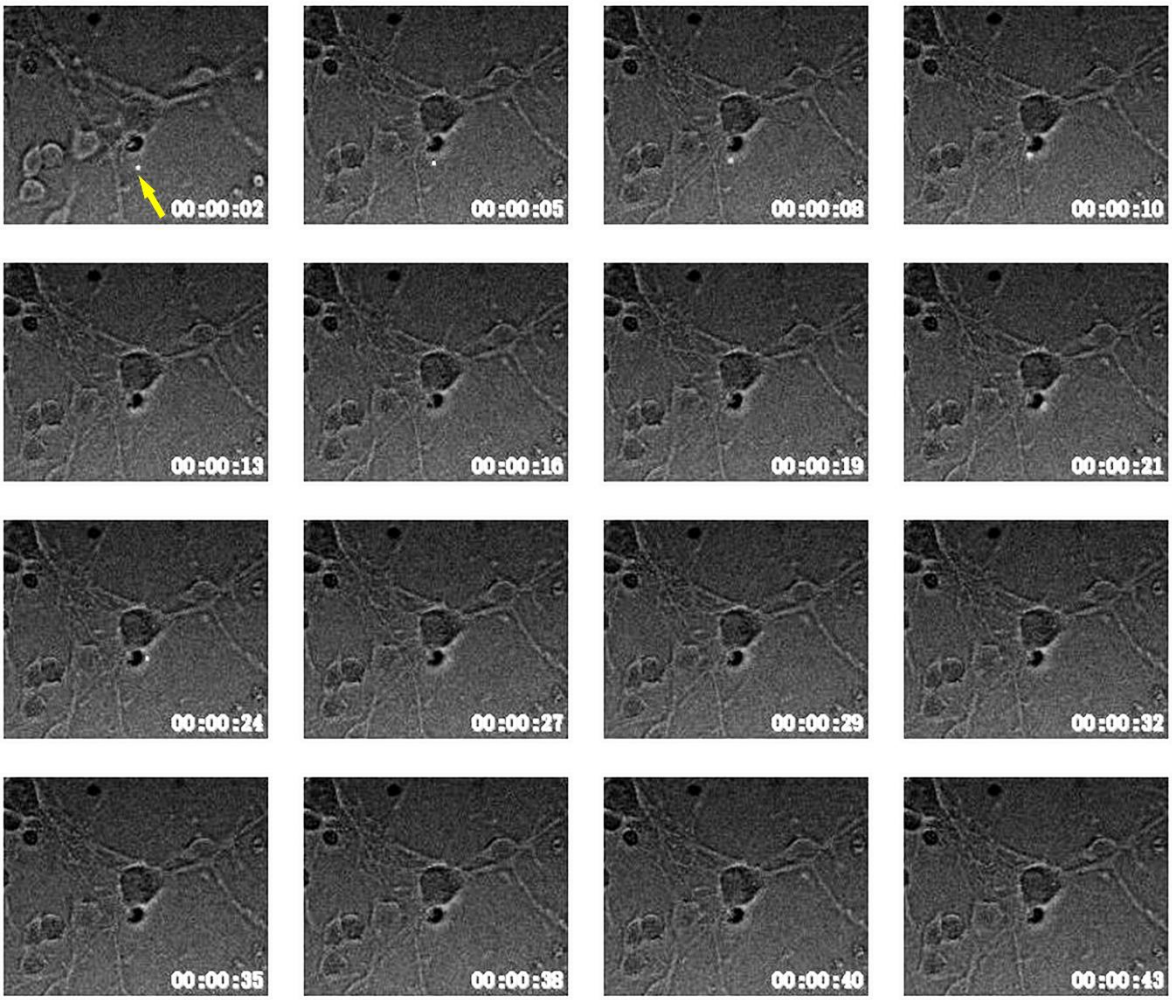

Figure S6. Exosomes were taken up by neurons.

Pictures captured by the video show that exosomes were taken up by neurons as early as 45 s after application. The fluorescent point indicated by the yellow arrow in the picture is an exosome labeled with PKH26.

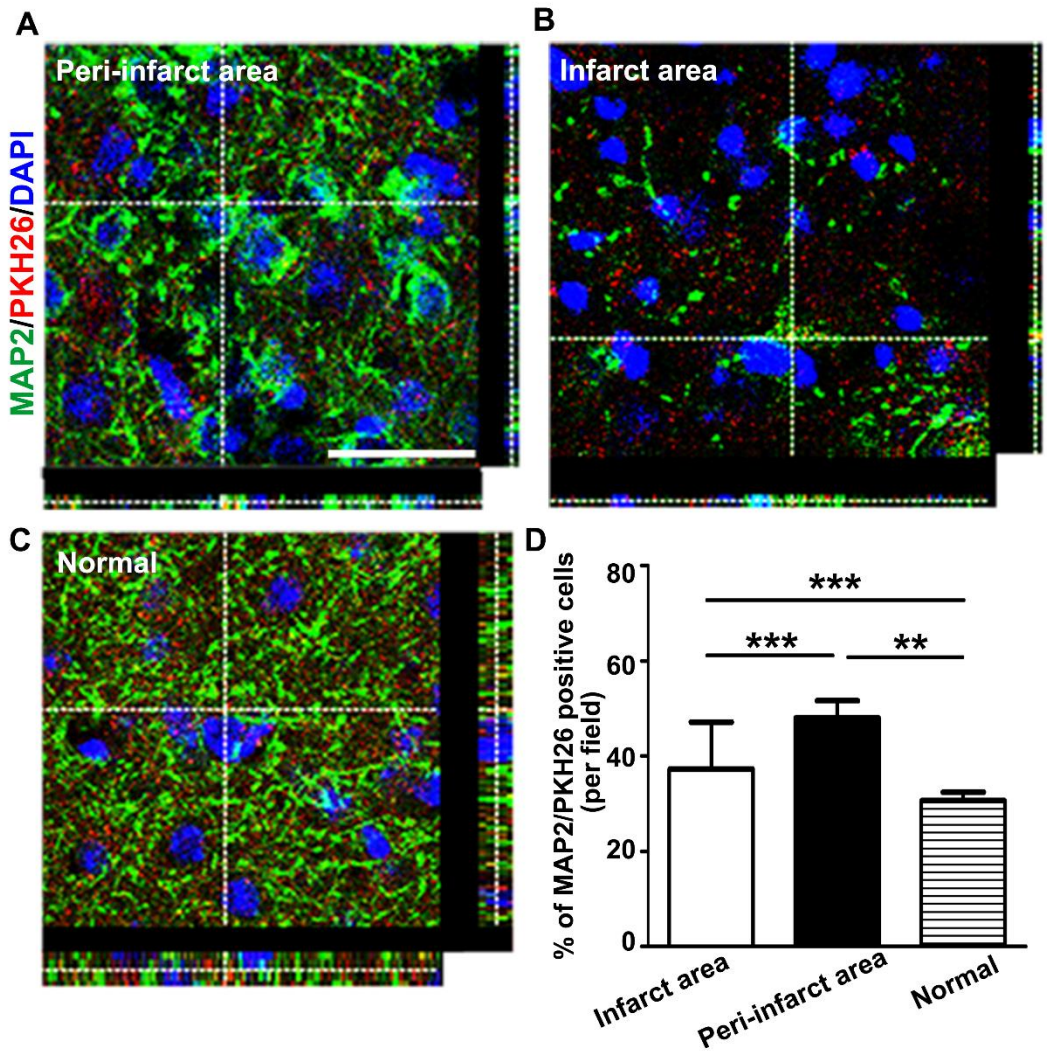

Figure S7. Neurons in different brains areas take up different levels of M2-EXO.

A. Uptake of M2-EXO by neurons in the ischemic core. B. Uptake of M2-EXO by neurons in the ischemic penumbra region. C. Uptake of M2-EXO by neurons in the brains of mice in the sham group. D. Statistical analysis of the percent of MAP2/PKH26-positive cells in different brain areas and the sham mouse brain. Green: MAP2, red: PKH26-labeled exosomes; blue: DAPI. Scale bar=50 μm. **, $p<0.01$, ***, $p<0.001$.

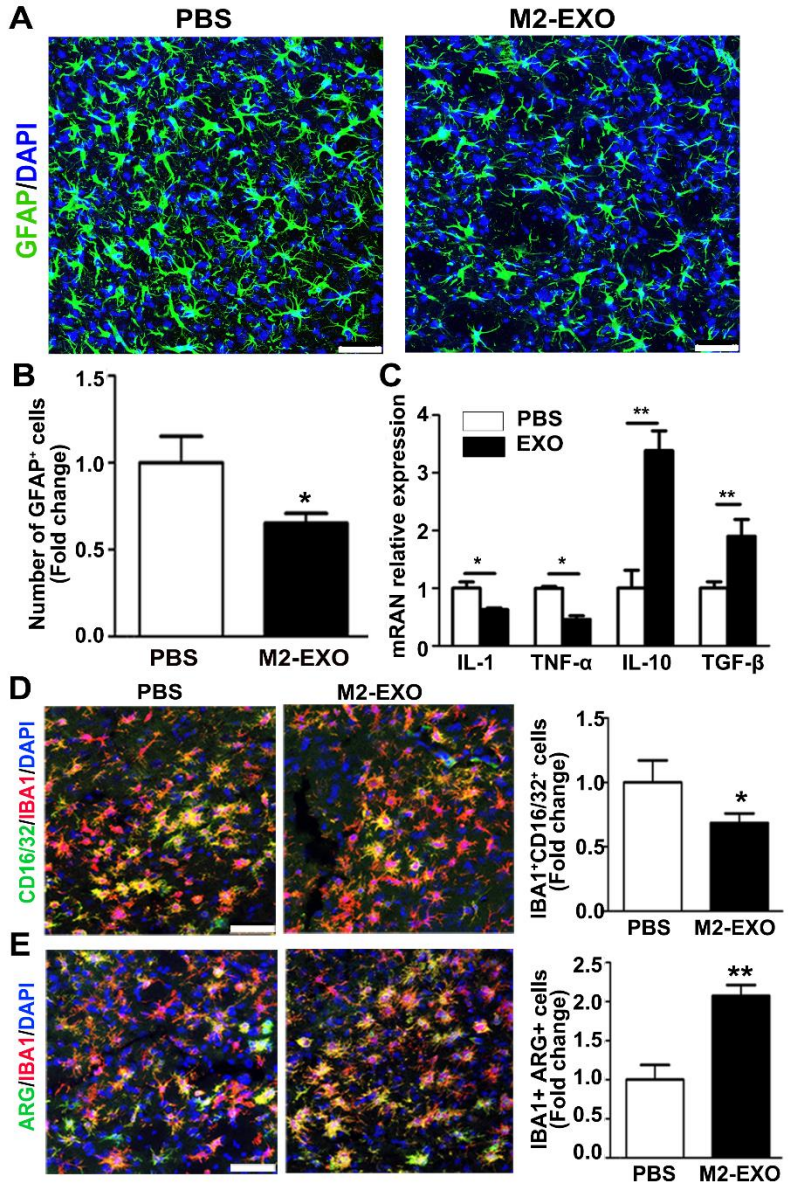

Figure S8. Microglia and astrocyte activation was decreased in the group treated with M2-EXO.

A. GFAP-positive cells in the PBS and EXO group. B. Statistical analysis of GFAP-positive cells. C. Level of inflammatory factors in the PBS and EXO group. D. IBA1 and CD16/32 and IBA1 and ARG double-positive cells in the PBS and EXO group. E. Statistical analysis of the double-positive cells. Scale bar=50 μm, *, $p<0.05$, **, $p<0.01$.

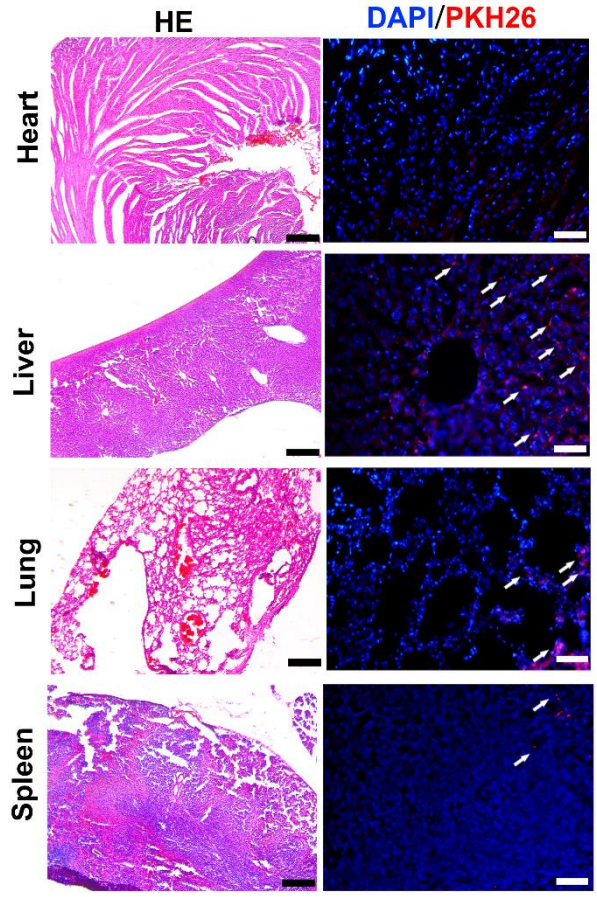

Figure S9. Exosome diffusion in different organs.

The diffusion of exosomes labeled with PKH26 (red) is shown in the heart, liver, lung and spleen. HE staining shows organ morphology (Scale bar=200 um). DAPI and PKH26 labeling are shown in the zoomed-in image (Scale bar=50 μm).

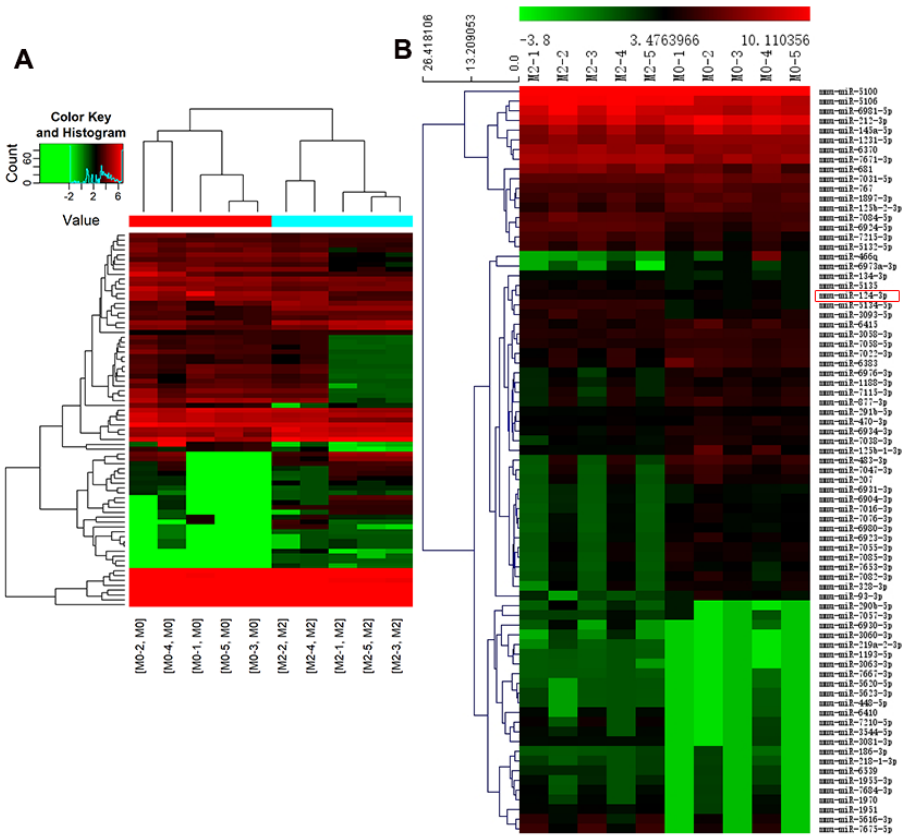

Figure S10. Exosomal miRNA array and significant differentially expressed miRNA.

(A) Cluster map of all M0-EXO miRNAs and M2-EXO miRNAs. (B) In the exosomal miRNA array, we found 38 significantly upregulated and 39 significantly downregulated miRNAs. miR-124 was identified as an upregulated miRNA.

Videos. Neurons uptake exosomes. The video shows neurons taking up PKH26-labeled exosomes in real time under 50 X and 100 X magnification. Spot lights indicate exosomes.