Supplementary information, Data S1 Methods

Isolation and culture of CD4+ T lymphocytes from the spleen and peripheral blood
Freshly isolated spleens and peripheral blood were placed in cold PBS and homogenized immediately. The lymphocyte subsets were purified with a CD4+ T cell isolation kit (MiltenyiBiotec) following the manufacturer’s instructions. The CD4+ T cells were cultured in RPMI-1640 medium supplemented with 1 mM HEPES, 1 µg/mL anti-mouse CD3e (BD Bioscience), 1 µg/mL anti-mouse CD28 (BD Bioscience), and 12% fetal FBS in a 5% CO2 water-saturated atmosphere.

Flow cytometry analysis of Tregs
A mouse Treg staining kit (eBioscience) was used to analyze the CD4+CD25^{high}Foxp3^{+}Tregs. The cells were analyzed with a BD FACSCaliburflow cytometer using CellQuest™Pro software (BD Biosciences).

MV isolation and administration
MV isolation was performed as previously described (Zhang et al., 2010). Briefly, after removing the cells and other debris by centrifugation at 300 \times g, 1,200 \times g, and 10,000 \times g, the supernatant was centrifuged at 110,000 \times g for 70 min (all of these steps were performed at 4°C). The MVs were collected from the pellet and resuspended in PBS or FBS-free medium. The MVs were resuspended in PBS and administered to C57BL/6J mice via tail vein injection at a concentration of 20 µg of MV protein in 100 µL of PBS per mouse. The mice were injected with the MVs once every 2 days for 2 weeks.

Cell transfection
LLC or 293T cells were seeded in 60-mm dishes and transfected the following day using
Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. To knock down miR-214, 300 pmol of anti-miR-214 or a scrambled negative control anti-miRNA (anti-ncRNA) was used. To overexpress miR-214, 300 pmol of pre-miR-214 or a scrambled negative control pre-miRNA (pre-ncRNA) was used. To transfect miR-214\textsuperscript{mut}, 300 pmol of miR-214\textsuperscript{mut} or a scrambled negative control miRNA (ncRNA) was used. After 6 h, the media was changed to DMEM supplemented with 1% fetal bovine serum with or without MVs from the 16 h centrifugation. The cells were harvested 48 h after transfection.

RNA isolation and qRT-PCR of the mature miRNAs

Total RNA was extracted from MVs derived from $10^8$ cells using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). qRT-PCR was performed using Taqman microRNA probes (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions. Briefly, 1 µg of total RNA was reverse-transcribed into cDNA using AMV reverse transcriptase (TaKaRa, Dalian, China) and a stem–loop RT primer (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed using a TaqMan PCR kit with an Applied Biosystems 7900 Sequence Detection System (Applied Biosystems). All reactions, including the no-template controls, were analyzed in triplicate. After the reaction, the C\textsubscript{T} values were determined using fixed threshold settings. To calculate the absolute expression levels of the target miRNAs, a series of synthetic miRNA oligonucleotides of known concentrations were also reverse-transcribed and amplified. The absolute amounts of each miRNA were then calculated in reference to the standard curve. In these experiments, the miRNA expression in the cells was normalized to U6 snRNA, which has been frequently used in previous studies. However, because there is no current consensus regarding the use of housekeeping genes for qRT-PCR analysis in MVs and the expression level of U6 snRNA was low in the MVs, the expression levels of the target miRNAs in the MVs were directly normalized to the total protein content of the MVs.
**qRT-PCR of the pre-miRNAs and PTEN**

Total RNA (1 µg) was reverse-transcribed into cDNA using AMV reverse transcriptase (TaKaRa, Dalian, China) and a reverse primer (Invitrogen). Real-time PCR was performed using a forward and reverse primer on an Applied Biosystems 7900 Sequence Detection System (Applied Biosystems). The probe sequences were as follows: mmu-pre-miR-214 forward, 5’-CCTGGCTGGACAGAGTTG-3’, mmu-pre-miR-214 reverse, 5’-TACAGGTGAGCGGATGTT-3’; mmu-PTEN forward, 5’-TTTGAAGACCATAACCCACCACA-3’, and mmu-PTEN reverse, 5’-AATCATTACACCAGTCCGTCCCT-3’.

**IL-10, IL-12, and TGF-β1 ELISAs**

ELISA development reagents (duo-set kit) for mouse IL-10, mouse IL-12, and mouse TGF-β were purchased from R&D Systems, and the assay was performed according to the manufacturer’s instructions. Absorbance was measured with a wavelength correction (A450 nm) using a microplate reader (Bio-Rad).

**Fluorescent labeling of MVs and confocal microscopy**

LLC cells were labeled with DiIC18(3) (1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate, Sigma) for 1 h and then washed three times with PBS. The cells were then cultured overnight in high-glucose (4.5 g/L) DMEM supplemented with 10% FBS. The supernatants were collected and centrifuged to harvest the MVs. The LLC MVs were resuspended in PBS and administered to C57BL/6J mice via tail vein injection at a concentration of 100 µg MV protein in 200 µl PBS per mouse. One hour after the injection, the mouse peripheral lymphocytes were isolated and washed three times with PBS. The lymphocytes were then stained with a FITC-labeled CD4+ antibody (eBioscience) for 30 min at 4°C. After staining, the lymphocytes were washed, fixed, and observed via confocal microscopy (FV1000; Olympus, Tokyo). The images were obtained under the following conditions: objective lens: PLAPON 60X Oil, NA: 1.42; scan mode: XY; excitation
wavelengths: 405 nm for DAPI, 488 nm for FITC, and 543 nm for DiIC18(3); and image size: 1,024 × 1,024 pixels.

Western blotting

PTEN protein levels were quantified via Western blot analysis of the whole-cell extracts using an anti-PTEN antibody (Cell Signaling, catalogue no. 9552). Normalization was performed by blotting the same samples with an anti-GAPDH antibody (Santa Cruz, catalogue no. sc-32233). Anti-CD63 (catalogue no. sc-31214) and anti-TGF-β1 (catalogue no.ab53169) were purchased from Santa Cruz Biotechnology and Abcam, respectively.

Quantitative proteomic analysis of protein expression levels in MVs by iTRAQ

Protein digestion and iTRAQ labeling: The MV proteins harvested from the 293T and LLC cells were solubilized in lysis buffer (8 M urea, 30 mM HEPES, 10 mM DTT, 2 mM EDTA, 1 mM PMSF, pH 8.0), and the protein concentration was measured using the Bradford method. For each sample, 100 μg of protein was labeled using an iTRAQ commercial kit (Applied Biosystems, Foster City, CA). iTRAQ labeling was performed according to the product manual with slight modifications. Briefly, 100 μg of protein obtained from the five samples was precipitated in 6 volumes of cold acetone for 2 h at -20°C. After centrifugation at 2,000 × g for 5 min at 4°C, the precipitates were dried, resuspended in 10 μL 8 M urea, and dissolved in buffer-tetraethylammonium bromide (TEAB) in a total volume of 20 μL. Following resuspension, the protein samples were reduced in 5 mMtris(2-carboxyethyl)phosphine (TCEP) at 60°C for 1 h and blocked in methyl methanethiosulfonate (MMTS) at room temperature for 10 min. After reducing and blocking the protein samples, TEAB was added to reduce the urea concentration to 1 M. Next, trypsin was added at an enzyme:protein ratio of
1:30 for digestion at 37°C overnight, and the reaction was terminated by incubation at -20°C for 30 min. Following digestion, the samples were concentrated to a few microliters and re-solubilized in 30 μL TEAB dissolution buffer. Next, 70 μL of ethanol-treated iTRAQ reagent was added to the previously treated samples and incubated for an additional 2 h at room temperature. Three samples from the 293T MVs were each labeled with one of three types of reporter reagents. Two samples from the LLC MVs were labeled with one of two types of reporter reagents. After labeling, the iTRAQ reagent-labeled samples from the 293T and LLC MVs were combined, respectively.

Peptide desalting and fractionation: after labeling, 300 μL of pure water was added to 100 μg of the peptide mixture from each of the combined samples and concentrated to 30 μL under vacuum. Next, 500 μL 0.1% formic acid was added to the samples, and the samples were pressure-loaded onto a two-phase silica capillary column packed with 3 cm of C18 resin (Sunchrom 5 μm, Germany) and 3 cm of strong cation exchange resin (Luna 5 mm, SCX 100A, Phenomenex). Next, the column was initially washed with buffer A (5% ACN/0.1% formic acid) for 15 min and desalted with a 60-min ACN gradient to remove the excess iTRAQ reagent and other labeling chemicals that could interfere with the LC/MS/MS analysis. Next, the peptides were fractionated using a 10-step salt gradient ranging from 0 to 700 mM ammonium acetate/5% ACN/0.1% formic acid (buffer C) for 8 min at a flow rate of 1.5 μL/min. The percentages of buffer C during the 10 steps of the 8-min elution were 10, 20, 25, 30, 35, 40, 50, 60, 80, and 100, respectively. A total of 10 fractions from each of the LLC cell samples and 293T cell samples were collected for further analysis.
LC/MS/MS Analysis: the fractionated peptides were analyzed on an LTQ Orbitrapvelos (Thermo Fisher Scientific, Waltham, MA). A total of 10 fractions from each sample were eluted with a 120-min gradient profile on the Agilent 1200 HPLC, consisting of a 105-min gradient from 5% to 30% buffer B (100% ACN/0.1%FA), a 1-min gradient from 30% to 90% buffer B, a 4-min gradient from 90% to 5% buffer B, and 10 min at 5% buffer B. The LTQ-Orbitrapvelos mass spectrometer was operated in positive ionization mode. A spray voltage of 2.1 kV and a heated capillary temperature of 250 °C were applied. The MS survey for all of the experiments was performed in the FT cell recording a window between 300 and 2,000 m/z. The resolution was set to 60,000, and the automatic gain control (AGC) was set to 1,000,000 ions. HCD fragmentation was used for the MS/MS, and the 10 most intense signals in the survey scan were fragmented. Fragmentation was performed with normalized collision energies of 40%. The minimum MS signal for triggering the MS/MS was set to 5,000 counts.

Data analysis with Mascot: the data were processed using the MSconvert into mgf files and searched using Mascot Daemon (Matrix Sciences, London, U.K). All searches were performed against the International Protein Index (IPI) human database v3.81 (84,808 sequences) for the samples from the 293T MVs and IPI mouse database v3.87 (39,602 sequences) for the samples from the LLC MVs using the following settings: trypsin as the cleavage enzyme; two missed cleavages; methylthiocysteine, iTRAQ (N terminal), and iTRAQ (K) as fixed modifications; and the oxidation of methionine as a variable modification. The mass error tolerance for the precursor ions was set to 10 ppm and 0.02 Da for the
fragment ions. For both of the analyses, an automatic decoy database search was also performed to assess the false discovery rate (FDR) of protein identification. An automatic isotope correction was performed using the values supplied with the ABSciex reagents. The following settings were used to process the quantification results: the protein ratio type was the “weighted” geometric mean, normalization was “summed intensity,” outlier removal was “automatic,” and the peptide threshold was “above homology.”

**Analysis of mRNA and miRNA expression levels in the MVs using microarrays**

Total RNA from $10^8$ cells was extracted from the MVs using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions.

A microarray analysis of mRNA expression in the LLC MVs was performed using GeneChip Mouse Genome 430 2.0 arrays (Affymetrix), which permitted the expression analysis of more than 39,000 transcripts (corresponding to 34,000 mouse genes) derived from sequences in the entire GenBank, dbEST, and RefSeq databases.

A microarray analysis of the mRNA expression in the 293T MVs was performed using GeneChip Human Genome U133 Plus 2.0 arrays (Affymetrix), which permitted the expression analysis of more than 47,000 transcripts (corresponding to 38,500 human genes) derived from sequences in the entire GenBank, dbEST, and RefSeq databases.

A microarray analysis of the miRNA expression in the LLC and 293T MVs was performed
using GeneChip miRNA 2.0 arrays (Affymetrix), which contain 15,644 probe sets and provide complete coverage of the mature miRNAs of all 131 organisms in miRBase, version 15.0, including 1,205 human miRNAs and 722 mouse miRNAs.

Labeling, hybridization, probe array washing and staining, and signal detection were performed according to standard protocols. The array scanning and generation of raw signal data files were performed using the GeneChip Operating Software (Affymetrix).