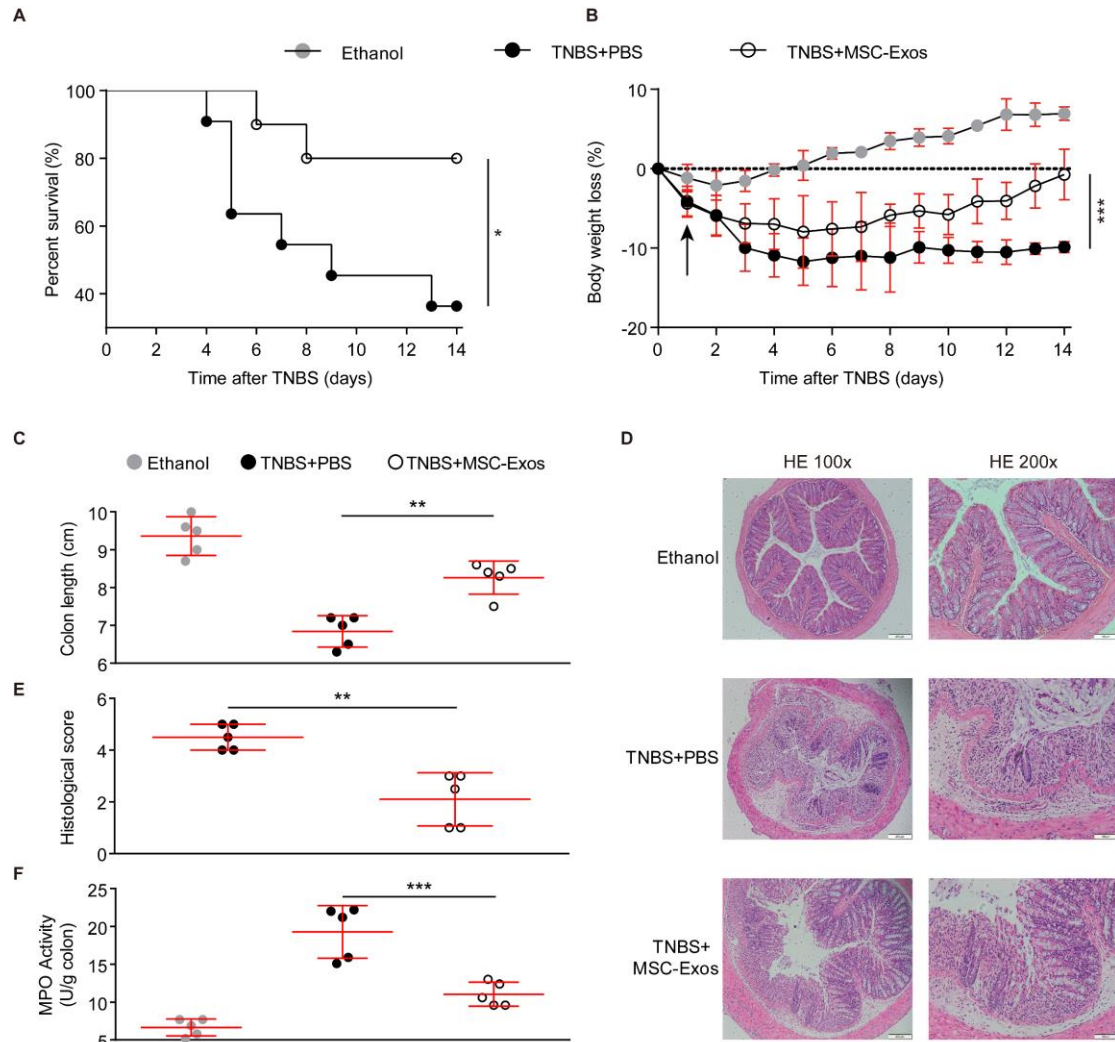# Supplemental Material

Supplemental Figures and Figure Legends
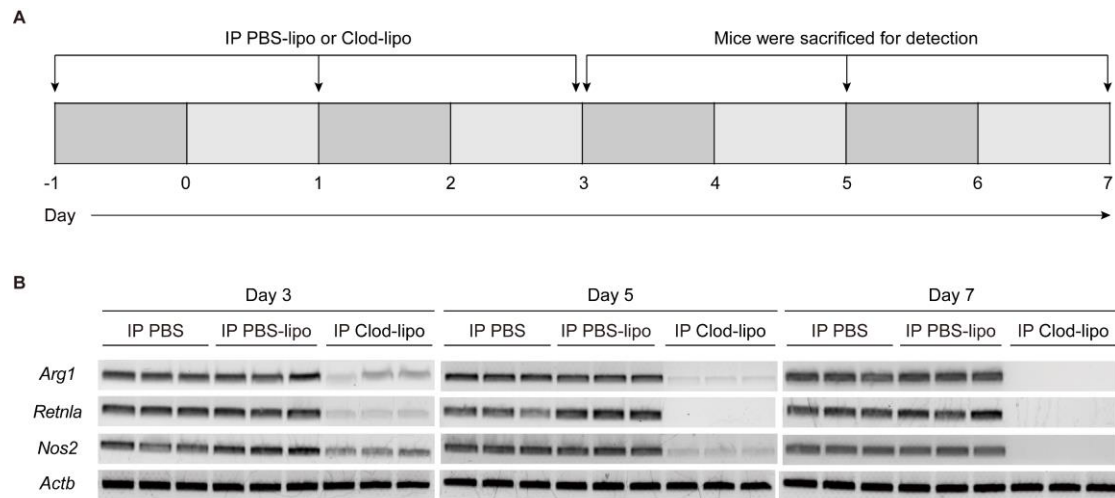
Supplemental Figure 1

Supplemental Figure 1: MSC-Exos inhibit TNBS-induced colitis. Male Balb/c mice at 7-9 weeks of age (n = 12-16 mice per group) were subjected to intrarectal infusion of TNBS (2.0 mg per mouse) in 50% ethanol. Mice injected only with 50% ethanol were used as basal controls. MSC-Exos (200 μg per mouse) were infused intravenously 24 h after the TNBS infusion (arrow in panel **B**). Colitis progression monitored by survival (**A**) and weight loss daily (**B**). At the peak of disease (day 5), colons were subjected to evaluation of colon lengths (**C**), histopathological signs (**D-E**) and colonic MPO activity (**F**). In panel D, left, magnification: 100x; right, magnification: 200x. The number of animals studied is shown in each figure. *$P \leq$ 0.05, **$P \leq$ 0.01 and ***$P \leq$ 0.001, by Log-rank test (A) or Kruskal-Wallis test (B and F) or 1-way ANOVA (C) or Mann-Whitney test (E).
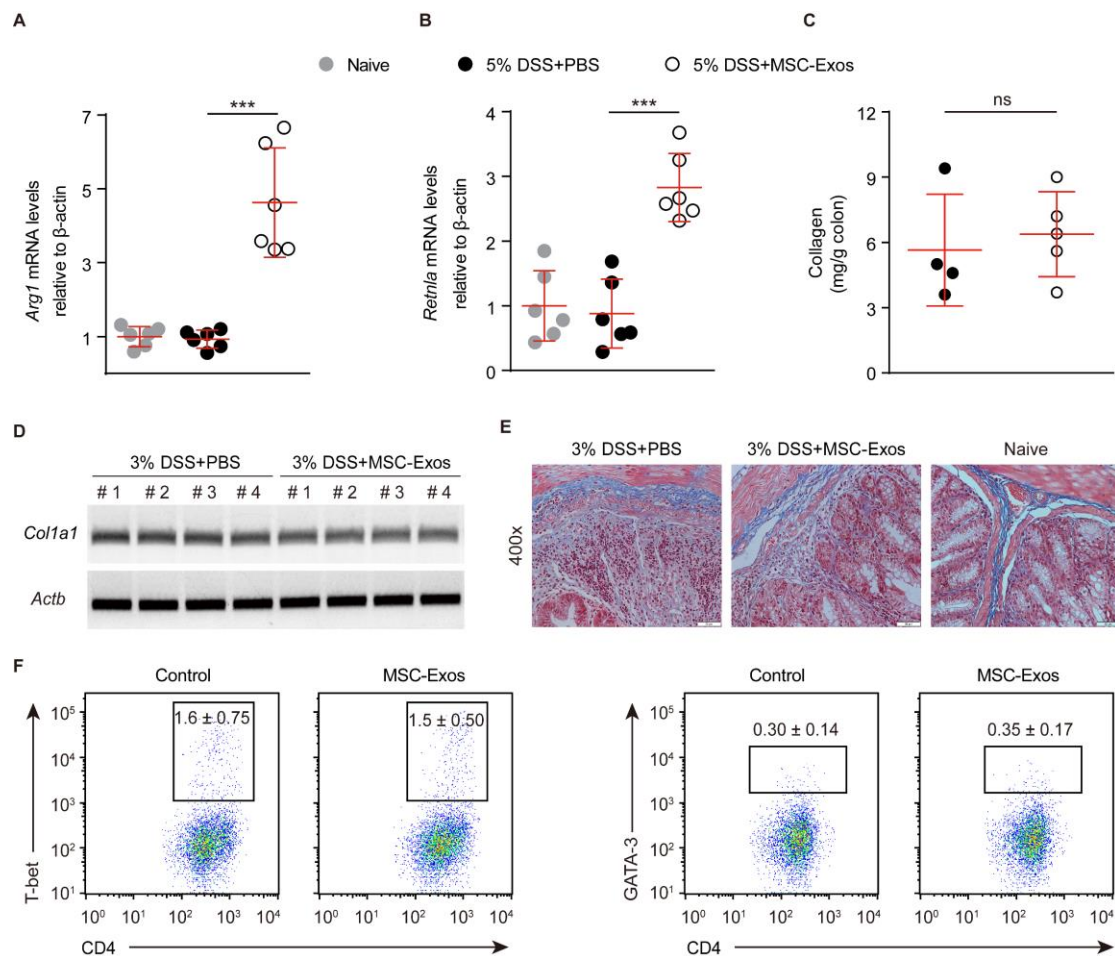
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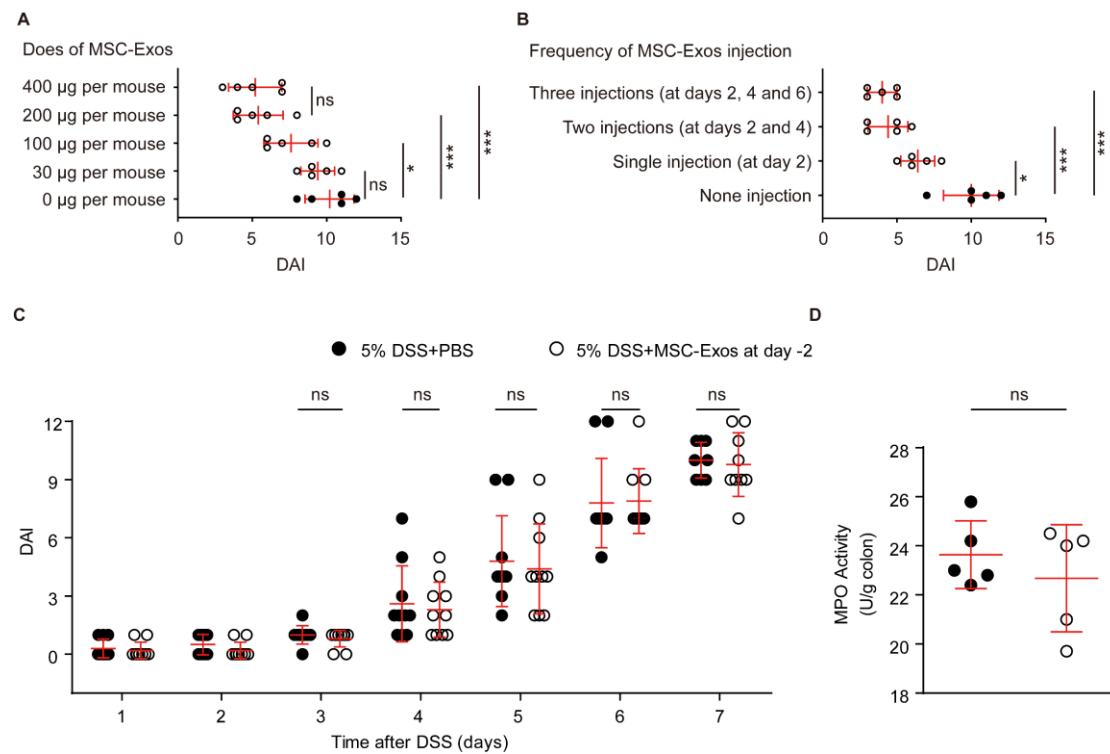
Supplemental Figure 2: (A-B) Male C57BL/6 mice at 6-8 weeks of age were subjected to 5% DSS in drinking water for 7 days. n = 5 mice per group. (**A**) MSC-Exos were injected intravenously on day 2 and the dose-response effects of MSC-Exos on colitis was determined at day 7. (**B**) MSC-Exos (200 μg/mouse/injection) were injected intravenously at the indicated times and frequency response of MSC-Exos was evaluated at day 7. (C-D) Male C57BL/6 mice at 6-8 weeks of age (n = 10 mice per group) were subjected to 5% DSS in drinking water for 7 days, and MSC-Exos (200 μg per mouse) were infused intravenously on day 2 before DSS administration. (**C**) Colitis was assessed by DAI daily (n = 8-10 mice per group). On day 7, colonic MPO activity (**D**) was determined (n = 5 mice per group). $*P \leq 0.05$, $***P \leq 0.001$ and ns indicates $P > 0.05$, by Kruskal-Wallis test (A and B) or Mann-Whitney test (C) or 2-tailed Student's t test (D).

Supplemental Figure 3

Supplemental Figure 3: Administration of Clod-lipo is effective in macrophage depletion of colons. (**A**) Schematic overview of Clod-lipo mediated depletion of colonic macrophages. Male C57BL/6 mice at 6-8 weeks of age received Clod-lipo or PBS-lipo (0.2 ml per mouse) via IP at days -1, 1 and 3. (**B**) RT-PCR gels showing the levels of macrophage-related markers (*Arg1*, *Retnla* and *Nos2*) in the colons at the indicated times, with *Actb* as a housekeeping gene. n = 3 mice per group. IP, intraperitoneal injection.

Supplemental Figure 4

Supplemental Figure 4: (**A-B**) mRNA levels of *Arg1* and *Retnla* in colonic tissues obtained on day 7, with *Actb* as a housekeeping gene (n = 6 mice per group). (**C**) ELISA assays demonstrating the protein levels of collagen in colons of mice with chronic colitis on day 28. n = 4-5 mice per group. (**D**) RT-PCR gels showing mRNA levels of collagen in colons of mice with chronic colitis on day 28, with *Actb* serving as a housekeeping gene. n = 4-5 mice per group. (**E**) Representative Mason's trichrome staining of collagen in colons of mice with chronic colitis on day 28, magnification: 400x. n = 4-5 mice per group. (**F**) Representative flowcytometric profile of Th1 and Th2 in CD4$^+$ T cells, which were isolated from human peripheral blood mononuclear cells and then were treated with or without MSC-Exos (30 μg/ml) for 2 days. Numerical values represent mean ± SD (n = 4 independent experiments). ***$P \leq 0.001$, ns indicates $P > 0.05$, by 1-way ANOVA (A and B) or 2-tailed Student's t test (C).

Supplemental Figure 5
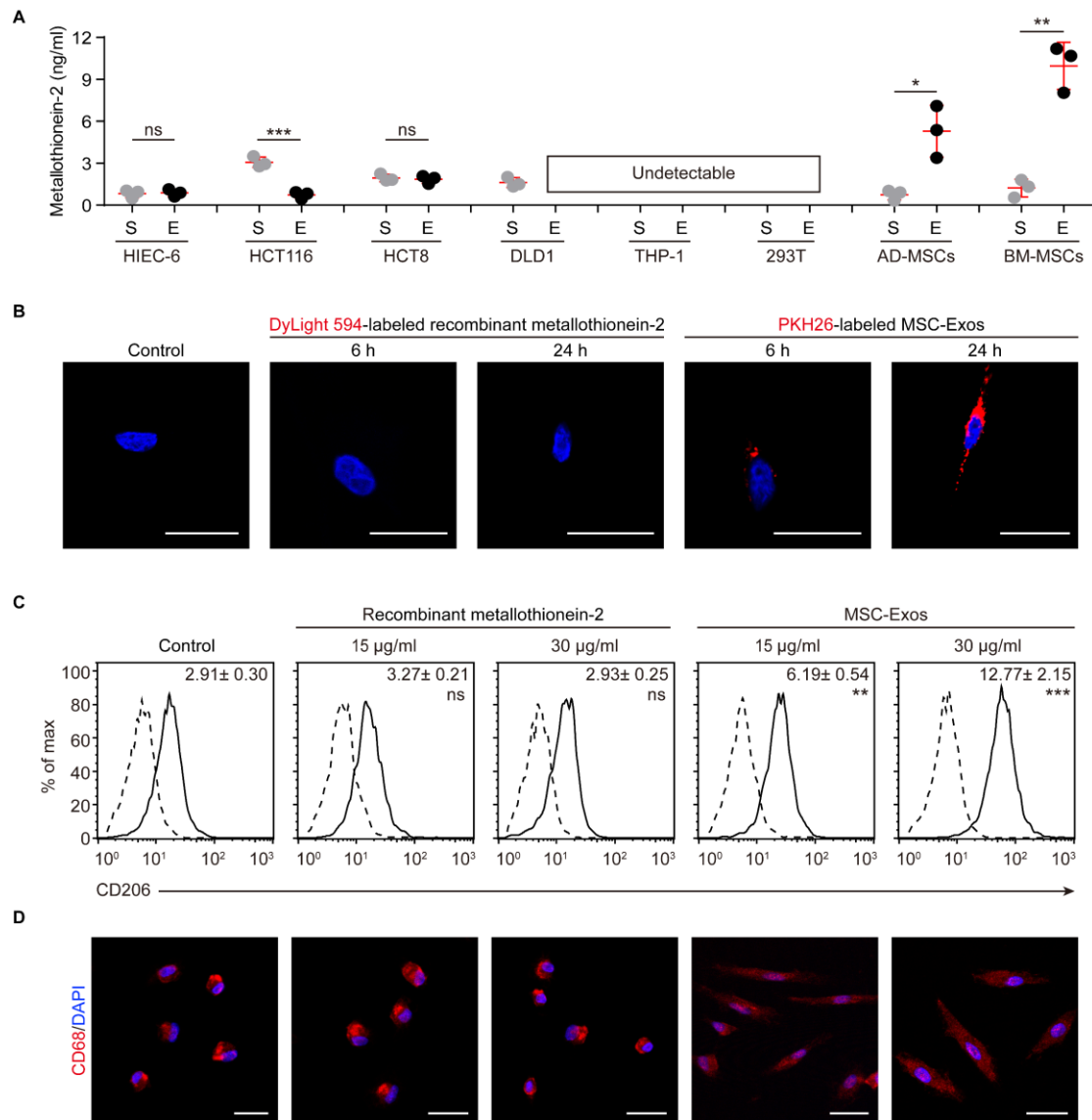
**Supplemental Figure 5**: (**A**) The levels of metallothionein-2 in exosomes versus supernatants from the indicated cell lines. All supernatants were depleted of exosomes by ultracentrifugation and all samples were adjusted to have an equal total protein concentration, and then ELISA assays were performed. BM-MSCs, bone marrow-derived MSCs; AD-MSCs, adipose-derived MSCs from surgical-resection mesenteric tissues of patients with Crohn's disease; S, supernatants; E, exosomes. "Undetectable" indicates that the level of metallothionein-2 was under the detection limit of the ELISA kit used. n = 3 independent experiments. (**B**) MDMs were treated with 15 µg/ml DyLight 594-labeled recombinant metallothionein-2 or PKH26-labeled MSC-Exos for the indicated time and then confocal microscopy image of the internalization of recombinant metallothionein-2 or MSC-Exos. "Control" indicates that MDMs were treated with an equal volume of PBS for 24 h. Scale bars: 20 µm. (C-D) MDMs were treated with the indicated dose of recombinant metallothionein-2

or MSC-Exos for 2 days. "Control" indicates MDMs were treated with an equal volume of PBS. (**C**) Representative flow cytometry staining for CD206 in MDMs. Shown are the representative images for 3 independent experiments of MDMs isolated from four different donors. Numerical values (mean ± SD) denote the relative mean fluorescence intensity normalized to isotype control. *P* values were obtained compared with "Control". (**D**) Immunofluorescence images of CD68/DAPI in MDMs. Scale bars: 20 μm. In panels B and D, 3 independent experiments were performed and yielded similar results. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$, ns indicates $P > 0.05$, by 2-tailed Student's t test (A) or Kruskal-Wallis test (C).

Supplemental Figure 6
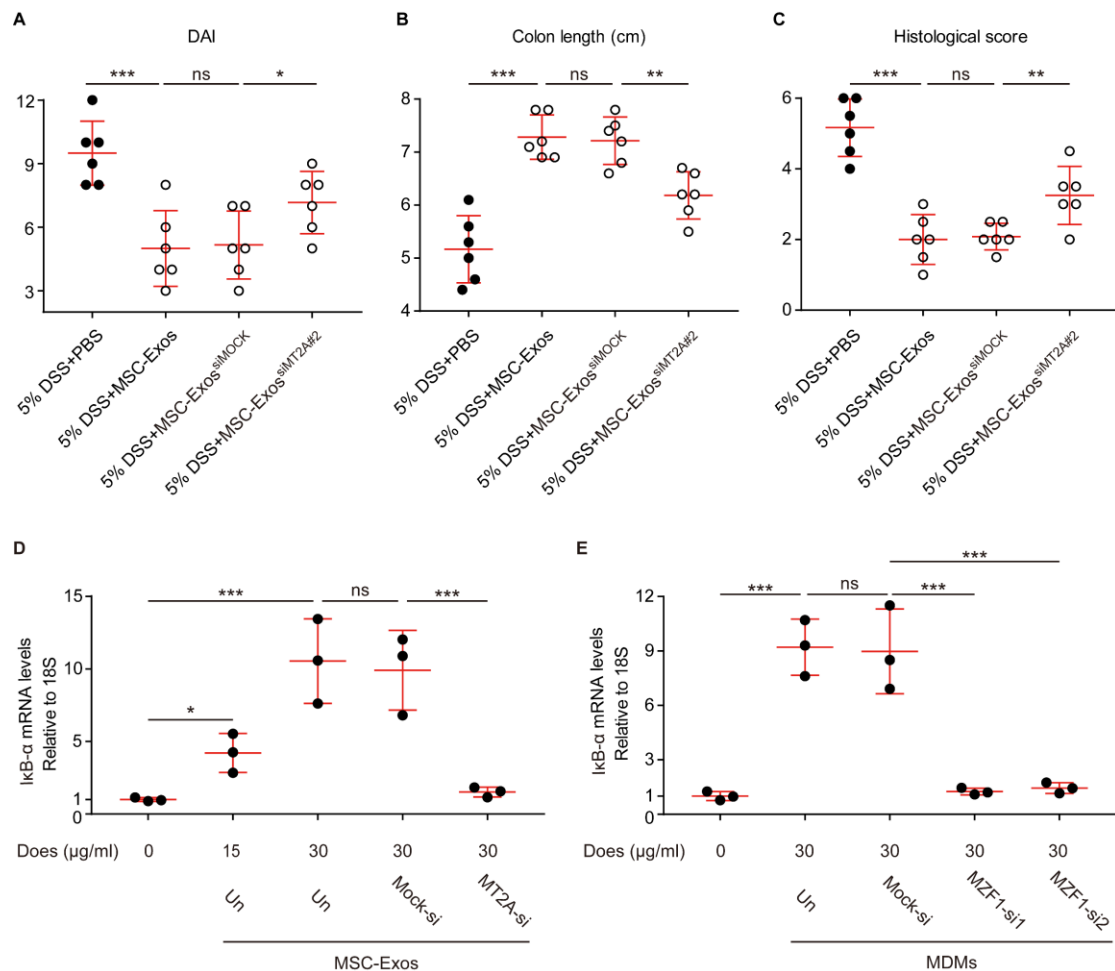
Supplemental Figure 6: (A-C) Male C57BL/6 mice at 6-8 weeks of age (n = 6 mice per group) were subjected to 5% DSS in the drinking water for 7 days and MSC-Exos or MSC-Exos^{siMOCK} or MSC-Exos$^{siMT2A\#2}$ (200 μg per mouse) or an equal volume of PBS were infused intravenously on day 2. (**A**) DAI scored from body weight loss, stool consistency and bleeding, (**B**) colon length and (**C**) histopathological scores in colon tissues analyzed in HE staining were determined on day 7. (**D**) mRNA levels of IκB-α in macrophages derived from human peripheral blood monocytes, which were treated for 2 days with MSC-Exos from MSCs that were that were untransfected (Un), mock transfected (Mock-si), or transfected with *MT2A*-siRNAs (MT2A-si). n=3 independent experiments. (**E**) Similar to (D), macrophages were untransfected (Un), mock transfected (Mock-si), or transfected with either of the two *MZF1*-siRNAs (MZF1-si1 and MZF1-si2) before MSC-Exos treatment. 18S rRNA served as a housekeeping gene. n=3 independent experiments. *$P ≤ 0.05$, **$P ≤ 0.01$, ***$P ≤ 0.001$ and ns indicates $P > 0.05$, by Kruskal-Wallis test (A and C) or 1-way ANOVA (B, D and E).

Supplemental Figure 7
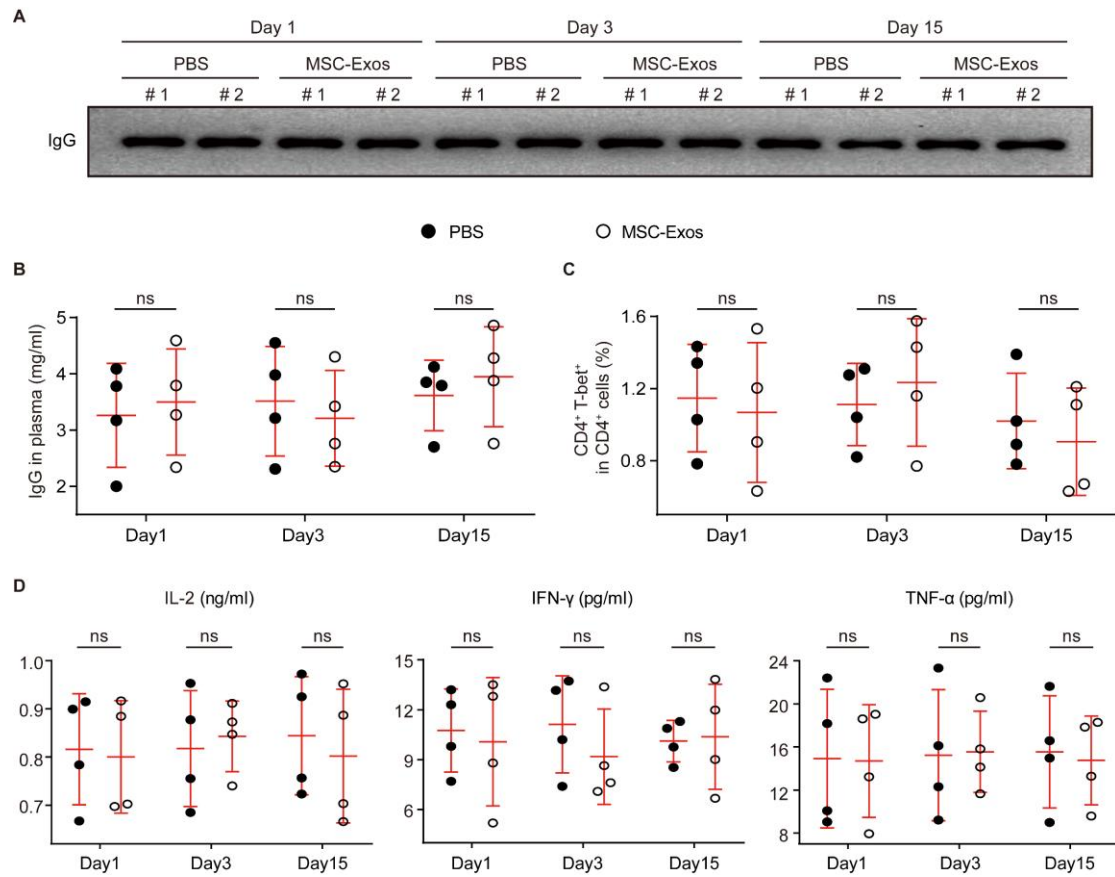
Supplemental Figure 7: MSC-Exos from human samples are immunotolerated by the host mice. MSC-Exos (200 µg per mouse) or an equal volume of PBS were infused intravenously in naïve male C57BL/6 mice at 6-8 weeks of age (n=4 mice per group). (**A**) Western blots for IgG antibody in plasma were determined in the indicated time. As a loading control, 10 µg per lane protein from mouse plasma was loaded. (**B**) ELISA assays for IgG antibody in plasma. (**C**) The profile of Th1 cells in the peripheral blood mononuclear cells of mice. (**D**) ELISA assays for IL-2, IFN-γ and TNF-α in in plasma. The number of animals studied is shown in each figure. ns indicates $P > 0.05$, by 2-tailed Student's t test (B, C and D).

Supplemental Experimental Procedures

Mesenchymal stromal cells (MSCs) preparation and cultures. Human bone marrow-derived MSCs (hereinafter referred to as MSCs) were used in the current study. Briefly, fresh bone marrow was obtained from the iliac crest of three adult healthy volunteers (China, male, with an age range of 24-30 years), followed by culture of obtained cells in MEM Alpha (GIBCO) supplemented with 10% heat inactivated fetal bovine sera (FBS), 1% glutamine, and 1% penicillin/streptomycin. After 3 h, nonadherent cells were removed carefully and fresh medium was replaced. The culture medium was changed every other day. For subsequent passage, the culture was treated with TrypLE™ Express Enzyme (GIBCO) for 2 min at 37 ℃ when primary cultures became almost confluent. The expression of MSCs surface antigens (passage 5) and cell viability were identified by flow cytometry analysis (described as follows).

Exosome extraction and identification. Exosomes were isolated from the supernatants of MSCs (hereinafter referred to as MSC-Exos) by ultracentrifugation methods as previously described (1, 2). Briefly, the cells (passage 5-6) were cultured in the normal medium until about 70-80% confluent; thereafter medium was replaced with defined medium supplemented with 10% exosome-depleted FBS (System Bioscience, USA), and then cells were cultured under 5% CO_2 at 37 ℃. After 3 days of culture, the cell culture medium was harvested. After centrifugation at 300×g for 15 min, 2,000×g for 20 min, 10,000×g for 30 minutes at 4 ℃, the supernatants were filtrated through 0.22 µm filters (Millipore). The obtained medium was at 100,000×g

for 70 min at 4 °C (Beckman Coulter) to pellet exosomes. The resulting supernatant was discarded without disturbing the pellet, which was then washed with a large volume of PBS, and then again ultracentrifuged at the same conditions, before final resuspension in a specific volume of PBS. The identification of purified exosomes was performed using Particle Metrix (PMX), transmission electron microscopy (TEM) and western blotting for detection of two known exosomes markers (TSG101 and CD9). Exosomes were quantified using Micro BCA™ Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The harvested exosomes were stored at -80 °C until further use.

In parallel, prior to exosome extraction, MSCs were subjected to detect the apoptosis analysis via flow cytometry analysis (described as follows) to rule out apoptotic bodies or random cell debris. The apoptosis of MSCs was induced by 10 μM etoposide (a well-known apoptotic drug, Abcam, Cambridge, UK) as a positive control. Also, MSCs were collected to analyze the MSC surface markers (CD73, CD90, CD34, CD45, CD11b, CD19, CD14, CD105, CD79a and HLA-DR) via flow cytometry analysis. The source and catalog number of all antibodies used were listed in Supplemental Table 1.

Exosomes in the supernatants of MSCs were depleted using centrifugation-based depletion protocol as previously described (1), with slight modifications. Briefly, culture medium of MSCs was centrifuged at 300×g for 15 min, 2,000×g for 20 min, 10,000×g for 30 minutes at 4 °C, and filtered through a 0.22 μm filter to remove cellular debris, and the obtained medium was then was depleted of exosomes by

ultracentrifugation for 120 min at 100,000×g at 4 °C (Beckman Coulter).

Isolation and culture of monocytes/macrophages. Human peripheral blood monocytes (PBMs) from healthy volunteer donors were isolated by density-gradient centrifugation using Ficoll-Hypaque (Pharmacia, Peapack, NJ) as previously described (3, 4). Harvested PBMs were seeded at a density of 2×10^6 cells/well per 24-well plate in DMEM medium (GIBCO) supplemented with 10% heat-inactivated human AB serum (Gemini Bio-Products, West Sacramento, CA), 50 U of penicillin per ml, 50 μg of streptomycin per ml, 2 mM L-glutamine and 20 ng/ml human M-CSF (R&D) to stimulate macrophage differentiation. After 5 days of culture, nonadherent cells were removed by repeated gentle washing with a warm medium, and more than 95% of the adherent cells generated from current procedures were CD14$^+$ monocytes/macrophages (data not shown), indicating a good purity of monocytes/macrophages.

Co-cultures of macrophages and CD4$^+$ T cells. Human peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll density gradient (GE Healthcare, Buckinghamshire, UK) by centrifugation at 450 g for 30 min. Cells were washed with PBS containing 10% of heat-inactivated FBS twice. CD4$^+$ T lymphocytes were purified using human CD4$^+$ T Cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, DE). To assess the purity, cells were stained with anti-CD3 and anti-CD4 antibodies (BD Bioscience, San Diego, CA, USA) and analyzed by flow cytometry which revealed that more than 95% of the purified cells were CD4$^+$ T cells (data not shown). Before co-culture, PBMs-derived macrophages were pre-treated

with 30 μg/ml MSC-Exos or IL-4 (20 ng/ml, R&D) for 2 days. Then Transwell chambers with a 0.4 µm pore size membrane (Corning Costar, Cambridge, MA) were used to separate the macrophages (in the bottom chamber) and human CD4$^+$ T cells (in the upper chamber). CD4$^+$ T cells at 2×10^6 cells/well were stimulated with 5 µg/ml phytohaemagglutinin (PHA, Sigma), whereas macrophages were added at the ratio of 1:10 (macrophages: CD4$^+$ T cells). To analyze the involvement of IL-10 in the suppressive activity of macrophages educated by MSC-Exos on CD4$^+$ T cells, the 10 μg/ml anti-IL-10 antibodies (R&D) and an isotype-matched IgG control were in the absence or presence in the Transwell system. After co-culture for 3 days, cytokines and proliferation assays of CD4$^+$ T cells were performed (described as follows).

Proliferation Assays for CD4$^+$ T cells. For the proliferation assays of CD4$^+$ T cells, 5, 6-carboxyfluorescein diacetatesuccinimidyl ester (CFSE; Invitrogen, Carlsbad, CA) staining (10 μmol/l) was performed according to the manufacturer's protocol. The CFSE-labeled human CD4$^+$ T cells were co-cultured with macrophages as described above. After 3 days of co-culture, the CD4$^+$ T cells were collected and analyzed via flow cytometry.

Cytokine assays. For analysis of cytokine production of CD4$^+$ T cells, after 3 days of co-culture with macrophages, human CD4$^+$ T cells were retrieved from the co-culture system and washed twice, and subsequently transferred to fresh culture medium in the presence of PHA (5 µg/ml). After 24 h, the culture medium of CD4$^+$ T cells was collected and subjected to quantitative analysis of secreted proteins using Enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's

protocol.

siRNA preparation and transfection. The target cells were transfected with Lipofectmine 3000 (Invitrogen) in serum-free OPTI-MEMI (Invitrogen, Germany) according to the manufacturer's protocol. All siRNAs were obtained from (Sangon Biotech (Shanghai) Co., Ltd). The sense and anti-sense strands of siRNAs were listed in Supplemental Table 2.

Animal experiments. Male C57BL/6 and Balb/c mice were purchased from the Animal Experiment Center of Sun-Yat-Sen University. Acute colitis was induced in C57BL/6 mice (6-8 weeks) by oral administration of 5% dextran sodium sulfate (DSS, molecular weight, 36-50 kDa, MP Biomedicals, USA) in the drinking water for 7 days. Chronic and recurrent colitis was established by 3% DSS in drinking water in a cyclic manner. Each cycle consisted of 7 days of DSS followed by a 7-day phase without DSS supplementation. Mice receiving tap water instead of DSS were used as controls (naïve).

As for 2, 4, 6-trinitrobenzenesulfonic acid solution (TNBS, Sigma-Aldrich) induced-colitis, presensitization step was performed in Balb/c mice (7-9 weeks) through the abdomen skin with TNBS at 40 mg/kg body weight in 50% ethanol on day 0. On day 7 after treating mice with pre-sensitization procedure, mice were anaesthetized by intraperitoneal injection of 4% pentobarbital sodium (0.1 ml per 10 g mouse body weight) and then the mice were treated with TNBS (2.0 mg per mouse) in 50% ethanol via intrarectal infusion (4 cm from the anus) with a catheter as previously described (5). Mice treated with 50% ethanol were used as basal controls.

Transfer of MSC-Exos to colitic mice. The finally harvested exosome pellet was resuspended in PBS and quantified by measuring protein concentration via BCA protein assays as previously described (6). For exosome treatments, 200 µg of total exosomal protein in a total volume of 200 µl phosphate-buffered saline (PBS) were intravenously administrated to per mouse at the indicated time, and naïve mice received intravenous injections of an equal volume of PBS. Naïve mice were treated with an equal volume of PBS.

In vivo depletion of colonic macrophages. Liposome-mediated macrophage depletion is a well-recognized strategy to delete macrophages in the gut (7-9). C57BL/6 mice intraperitoneally received 200 µl of clodronate-liposomes or PBS-liposomes (From Vrije Universiteit Amsterdam) on 1 day before exposure to DSS, and on day 1 and 3 during 7-day 3% DSS administration (Supplemental Figure 3A). 3% DSS was used to induced a colitis model, because 5% DSS for mice with macrophage depletion caused a mortality of ~85% by day 7. The efficacy for depletion of macrophage in the colon was confirmed via detection of *Arg1*, *Retnla* and *Nos2* by reverse-transcription polymerase chain reaction (RT-PCR) gels (Supplemental Figure 3B).

In vivo neutralization of IL-10. In some experiments, C57BL/6 mice were intravenously treated with MSC-Exos (200 µg per mouse) on day 2, and received intraperitoneal injections of a neutralizing anti-IL-10 antibody, or the appropriate isotype control IgG (R&D; 200 µg antibody per mouse per injection) on alternate days from day 3 to day 6 during 7-day 5% DSS administration. In mice treated with

anti-IL-10 antibody, the levels of IL-10 in the colonic mucosa on day 7 were under the detection limit of the ELISA kit used (< 45 ng/ml).

Assessment of the disease activity index in experimental colitis. The clinical disease activity was determined by the disease activity index scored from weight loss, stool consistency and bleeding as previously described (10). Briefly, weight loss was scored as 0 = weight loss of < 1%; 1 = weight loss of 1-5%; 2 = weight loss of 5-10%; 3 = weight loss of 10-20%; 4 = weight loss of >20%. Stool scores were determined as 0 = well-formed pellets; 2 = soft stools that did not adhere to the anus; 4 = liquid stools that adhered to the anus. Bleeding were determined via Hemoccult (Beckman Coulter) test and scored as 0 = negative blood; 2 = positive hemoccult; 4 = gross bleeding or visible. All scores were determined daily by the same person at similar time points in a blinded manner (unaware of the groups).

Determination of histological score. Mice were sacrificed at the indicated time and the entire colon from cecum to anus was quickly excised, followed by measurement of the colon lengths. For histological analysis, sections (5 µm thick) of paraformaldehyde-fixed paraffin-embedded colon tissues were stained with hematoxylin and eosin (HE). Histopathology scores were determined in colonic sections by two blinded trained pathologists (unaware of the groups), with a combined score for inflammatory cell infiltration (score, 0-3) and tissue injury (score, 0-3). Briefly, for infiltration of inflammatory cells: 0 = the inflammatory cells in the lamina propria present occasionally; 1 = the numbers of inflammatory cells increased in the lamina propria; 2 = accumulation of inflammatory cells, extending into the

submucosa; 3 = transmural extension of the infiltrate. For tissue injury: 0 = normal colonic mucosa; 1 = discrete lymphoepithelial lesions; 2 = surface mucosal erosion or focal ulceration; 3 = extensive mucosal damage and extension into deeper structures of the bowel wall. The combined inflammatory score ranged from 0 (normal) to 6 (including cell infiltration and tissue injury).

Analysis of the activity of myeloperoxidase (MPO). The infiltration of neutrophils into the colon tissue (each sample was analyzed in triplicate) was measured by MPO colorimetric activity assay kit (Sigma-Aldrich) according to the manufacturer's instructions. Briefly, colon tissue was homogenized with MPO assay buffer. After centrifuging at 13,000 g for 10 minutes at 4 °C to remove insoluble material, 10 μl supernatant of the tissue lysates containing 1mg colonic tissue extracts was added to 96-well plate to start the reaction. The absorbance was then measured at 412 nm after 120min incubation at 25 °C. One unit of MPO activity is defined as the amount of enzyme that hydrolyzes the substrate and generates taurine chloramine to consume 1.0 micromole of TNB per minute at 25 °C. The amount of TNB consumed by the enzyme assay was generated from the TNB standard for colorimetric detection.

Gut permeability assay using FITC-dextran. For assessing gut permeability, DSS-colitic and naïve mice were orally gavaged with FITC-labeled dextran (mol wt 4,000, Sigma-Aldrich, 50 mg per 100 mg mouse body weight) after a 24 h fast. Four hours later after the oral gavage of FITC-Dextran, mice were anesthetized and then intracardiac punch was used to collect their blood. The collected blood samples were allowed to clot, and then spun at 2,000 g for 10 minutes, the serum was read on a

varioskan flash (Thermo Scientific) at 485 nm excitation and 535 nm emission. The intestinal permeability was expressed as the FITC-dextran concentration in the serum, which was generated from a standard curve using serial dilution of FITC-Dextran in PBS.

Isolation of macrophages from the lamina propria of colons in mice. Colonic macrophages were isolated as previously described (11). Briefly, C57BL/6 mice were sacrificed at the indicated time and then the large intestines were taken with a sterile operation. The colons were opened longitudinally and all excess feces were removed by flushing in petri dishes with cold PBS using a 5 ml syringe and a 23G needle. After removing the maximum of mucus via abrading the colon on several petri dishes (three to four petri dishes are often needed), the freshly obtained colons were cut into very small pieces and then placed in Hanks' balanced salt solution (HBSS, without Ca^{2+} and Mg^{2+}, containing 5% fetal bovine serum, 2 mM EDTA and 0.15 mg/ml Dithiothreitol) with shaking at 220 rpm for 15 min at 37 °C to remove the epithelium. Then the finely chopped tissue was digested with type IV collagenase (1 mg/ml) and DNase I (0.1 mg/ml) for 20 min at 37 °C with shaking at 220 rpm. The obtained resulting cell suspensions were filtered via a 70 μm cell strainer and then were centrifuged at room temperature at 300 g for 5 minutes. Colonic macrophages were further isolated using immunomagnetic separation via anti-F4/80 mAb (Miltenyi Biotec, the best and most commonly used marker to identify macrophages in mice). The harvested cells were confirmed by flow cytometry for $F4/80^{+}CD11b^{+}$ detection. Isolated $F4/80^{+}$ macrophages from colons of mice were cultured in complete DMEM

medium at a concentration of 10^5 cells/well per 48-well plate. Then harvested cells were ex vivo cultured with or without 100 ng/ml LPS (Sigma-Aldrich). After 24 h the cytokine contents in culture supernatants were determined in duplicate via ELISA assays according to the manufacturer's protocols.

Isolation of CD4$^+$ T lymphocytes from mesenteric lymph nodes. Mesenteric lymph nodes (MLNs) were collected from 5% DSS-colitic mice at the disease peak of DSS-induced colitis (day 7). Then CD4$^+$ T lymphocytes were further isolated from single-cell suspensions of MLNs cells using immunomagnetic separation via anti-CD4 mAb (Miltenyi Biotec). More than 95% of the purified cells were CD4$^+$ T cells, confirmed by CD3/CD4 double staining (BD Bioscience, San Diego, CA, USA, and data not shown). Then CD4$^+$ T lymphocytes were incubated in complete medium (RPMI-1640 with 10% FBS). The harvested CD4$^+$ cells were analyzed for the production of IL-10 with or without 5 µg/ml PHA stimulation for 24 h via ELISA assays, as described below.

Enzyme-linked immunosorbent assay (ELISA). Colonic tissues from DSS-colitic mice treated with or without MSC-Exos and supernatants of mice-derived cells were collected for detection of TNF-α, IL-6, IFN-γ, IL-1β, IL-10 and Collagen. TNF-α, IL-1β, IL-6, IFN-γ and IL-10 ELISA kits were from Raybiotech, and Collagen ELISA kits were from Cusabio Biotech Co. Cytokine contents in culture supernatants of CD4$^+$ T cells were collected at the indicated time for TNF-α and IFN-γ, and these ELISA kits were from Abcam. MT2A ELISA Kits (Aviva Systems Biology) were used to determine the levels of the levels of metallothionein-2. The standard curves

were generated using ELISACalc software. All procedures were performed according to the manufacturer's recommendations.

Masson's trichrome stain. The colonic collagen content was determined by Masson trichrome staining. In brief, paraformaldehyde-fixed paraffin-embedded colon tissues were cut into sections of 5µm thickness, followed by staining in Weigert's iron hematoxylin working solution for 10 min and in Biebrich scarlet-acid fuchsin solution for 10 min. Then after differentiation in a phosphomolybdic-phosphotungstic acid solution for 10 min, the sections were directly transferred to aniline blue solution and stain for 10 min, followed by differentiation in 1% acetic acid solution for 5 min and dehydration through 95% ethyl alcohol and 100% ethyl alcohol. Then the sections were cleared in xylene and mounted with a resinous mounting medium for visualization and analysis.

Exosome labeling and tracking. PKH26 Red Fluorescent membrane linker dye (Sigma-Aldrich) was used for MSC-Exos labeling. Briefly, MSC-Exos resuspended in 0.5 ml PBS were mixed with an equal volume of Diluent C. In parallel, 4 µl PKH26 dye was mixed with 0.5 ml Diluent C and incubated with the mixture of MSC-Exos and Diluent C for 3 min at room temperature, followed by the addition of 2 ml 0.5% bovine serum albumin/PBS to bind excess PKH26 dye. The labelled MSC-Exos were washed at $100,000 \times g$ for 70 min, and the labeled exosome pellets were resuspended with sterile PBS and used for further experiments. For in vivo tracking, 200 µg PKH26-labeled MSC-Exos were intravenously administrated to per mouse on day 2 during 7-day 5% DSS administration. At the indicated days, single-cell suspensions

obtained from colons of DSS-colitic mice were prepared to detect fluorescence signals via flow cytometry analysis (BD Biosciences FACSCalibur).

Determination of gene expression by qRT-PCR and RT-PCR gels. Total RNA was extracted from cells or tissue samples using the TRIzol Reagent (Thermo Fisher Scientific, St Peters, MO, USA) according to the manufacturer's recommendations. The concentrations and purities of obtained RNA were determined using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, St Peters, MO, USA). Reverse transcription was performed using ReverTra Ace qPCR RT Kit (Toyobo Biochemicals, Kita-ku, Osaka, Japan) according to the manufacturer's instructions with 2000 ng of RNA in a 20 µL reaction volume. Quantitative real-time reverse transcription PCR (qRT-PCR) then was performed the SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) on the Applied Biosystems 7500 Sequence Detection system using the SYBR Green detection protocol as outlined by the manufacturer. All reactions were performed in a 10 µl reaction volume in triplicate. Standard curves were generated and the relative amount of target gene mRNA was calculated using the $2^{-\triangle\triangle CT}$ method with normalization to *Actb*. The specificity was verified by melt curve analysis and agarose gel electrophoresis (data not shown). For RT-PCR gels, products from qRT-PCR reactions in triplicate were pooled together and then electrophoresed via 1% agarose gels containing 0.5 µg/ml ethidium bromide, and bands were visualized under ultraviolet light. All of our primers were obtained from Invitrogen and the primer sequences were listed in Supplemental Table 3.

Flow cytometry analysis. Target cell suspensions were incubated with

fluorochrome-conjugated antibodies against interested markers and isotype-matched control IgG for 30 min at room temperature. When intracellular staining was performed, intracellular fixation and permeabilization buffer set (eBioscience) were used. Fluorescence signals were detected and recorded via flow cytometry (BD Biosciences, New Jersey, USA), and obtained raw data were further analyzed and visualized using FlowJo v10.0.7.

To determine the viability of MSCs, propidium iodide (PI)-annexin-V double staining assay kits (Invitrogen) were used to detect cell viability. Briefly, target cells were resuspended in 100 μl binding buffer and then added 5 μl Annexin V-FITC and 10 μl PI in the solution. Then, the above solution was incubated in the dark for 10min at room temperature. Following the incubation, FACScan flow cytometry was performed after another 400 μl of binding buffer was added.

Western blotting. Protein samples were lysed in radio-immunoprecipitation assay buffer containing 150 mM NaCl, 1% Triton X-100, 0.5% Sodium Deoxycholate, 0.1% SDS, 50 mM Tris (pH = 8.0) and protease inhibitors cocktail (Promega, Fitchburg, WI, USA). Protein was separated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to polyvinylidene fluoride (PVDF) by the Trans-Blot System (Bio-Rad, CA, USA). Then the membranes were blocked with 5% w/v skim milk (BD Biosciences, San Jose, CA, USA) in Tris-buffered saline with 0.1% v/v Tween 20 (TBST) for 1h at room temperature, followed by incubation overnight at 4 °C with anti-CD9 (1:1000), anti-TSG101 (1:1000), anti-IκB-α (total, 1:1000), anti-IκB-α(phosphorylated, 1:1000), anti-NF-κB p65 (1:1000) anti-Lamin A

(1:1000) and β-actin (1:1000). The secondary antibodies (1:5000, Abcam) were incubated for 1 hour at room temperature. Specific bands were visualized with ECL Blotting Detection Reagents (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). The source and catalog number of all antibodies used were listed in Supplemental Table 1.

Proteomic analysis. MSCs (passage 5, isolated from three healthy male volunteers) were seeded in 75 cm^2 culture flasks and washed with PBS when they reached 70-80% confluence. After incubation in exosome-depleted complete medium (10 ml per flask) for 3 days, MSCs and their supernatants were collected. Three MSC-Exos samples (called E1, E2 and E3, isolated from the three MSCs) were isolated from the supernatants (called S1, S2 and S3), respectively. Before proteomic analysis, exosomes in the supernatants of MSCs were depleted by ultracentrifugation for 120 min at 100,000×g unless they are specifically mentioned as non-depleted. An equal amount of total protein from each sample (MSC-Exos versus supernatants) was processed for TMT-based quantitative proteomic analysis by Jingjie PTM BioLab (Hangzhou, China). The resulting MS/MS data were processed using Maxquant search engine (v.1.5.2.8). The data have been deposited in PRIDE Archive as PXD015386 (https://www.ebi.ac.uk/pride/archive/). To identify the predominant biological process of the identified proteins of MSC-Exos, gene ontology (GO) analysis was performed and the GO analysis with a corrected *p* values ≤ 0.05 were considered statistically significant.

References

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macrophages to mediate protection against nematode parasites. *Nature medicine.* 2006;12(8):955-60.

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Supplemental Tables

Supplemental Table 1. The source and catalog number of antibodies

Product name	Source	catalog number
FITC-CD73	Thermo Fisher Scientific	11-0739-41
FITC-CD90	Thermo Fisher Scientific	11-0909-42
FITC-CD34	Thermo Fisher Scientific	11-0349-41
FITC-CD45	Thermo Fisher Scientific	11-0459-41
FITC-CD11b	Thermo Fisher Scientific	11-0118-41
FITC-CD19	Thermo Fisher Scientific	11-0199-41
FITC-CD14	Thermo Fisher Scientific	11-0149-42
FITC-IgG1 Isotype	Thermo Fisher Scientific	11-4714-81
PE-CD105	Thermo Fisher Scientific	12-1057-42
PE-CD79a	Thermo Fisher Scientific	12-0792-42
PE-IgG1 Isotype	Thermo Fisher Scientific	12-4714-81
APC-HLA-DR	Thermo Fisher Scientific	17-9956-41
APC-IgG2b Isotype	Thermo Fisher Scientific	17-4732-81
APC-F4/80	Thermo Fisher Scientific	MF48005
FITC-CD11b	Thermo Fisher Scientific	11-0112-82
PE-Cyanine7-CD206	Thermo Fisher Scientific	25-2061-82
PE-Cyanine7-arginase-1	Thermo Fisher Scientific	25-3697-82
APC-CD4	Thermo Fisher Scientific	17-0049-42
PE-T-bet	Thermo Fisher Scientific	12-5825-80

PE-Gata-3	Thermo Fisher Scientific	12-9966-42
Mouse IL-10 Antibody	R&D Systems	MAB417
Rat IgG1 Isotype Control	R&D Systems	MAB005
Human IL-10 Antibody	R&D Systems	MAB217
Mouse IgG2B Isotype Control	R&D Systems	MAB004
MT2A ELISA Kit	Aviva Systems Biology	OKEH01331
IκB-α (total)	Cell signaling technology	4814
phosphorylated IκB-α	Cell signaling technology	9246
NF-κB p65	Cell signaling technology	8242
β-actin	Cell signaling technology	4970
Lamin A	Abcam	ab226198
TSG101	Abcam	ab125011
CD9	Abcam	ab195422
Human TNF alpha ELISA Kit	Abcam	ab181421
Human IFN gamma ELISA Kit	Abcam	ab46025
MPO Activity Assay Kit	Sigma-Aldrich	MAK068
Mouse IFN-γ ELISA Kit	Raybiotech	ELM-IFNg
Mouse IL-1β ELISA Kit	Raybiotech	ELM-IL1b
Mouse IL-6 ELISA Kit	Raybiotech	ELM-IL6
Mouse TNF-α ELISA Kit	Raybiotech	ELM-TNFa
Mouse IL-10 ELISA Kit	Raybiotech	ELM-IL10
Mouse Collagen ELISA Kit	Cusabio Biotech Co	CSB-EL005727MO

Recombinant metallothionein-2	Abnova	ABIN2725864

Supplemental Table 2. The sense and anti-sense strands of siRNAs

siRNA	Strands	Sequences
MT2A siRNA1	sense	5′-GAUCCCAACUGCUCCUGCGCCGCCGTT-3′
	anti-sense	5′-CGGCGGCGCAGGAGCAGUUGGGAUCTT-3′
MT2A siRNA2	sense	5′-AACCCGCGUGCAACCUGUCCCGACUTT-3′
	anti-sense	5′-AGUCGGGACAGGUUGCACGCGGGUUTT-3′
MT2A siRNA3	sense	5′-CCUGCUGCCCUGUGGGCUGUGCCAATT-3′
	anti-sense	5′-UUGGCACAGCCCACAGGGCAGCAGGTT-3′
MZF1 siRNA1	sense	5′-AGAGCCUGGGCCCAAGACACCUCCUTT-3′
	anti-sense	5′-AGGAGGUGUCUUGGGCCCAGGCUCUTT-3′
MZF1 siRNA2	sense	5′-GGUUACAGAGGACUCAGAUUUCCUGTT-3′
	anti-sense	5′-CAGGAAAUCUGAGUCCUCUGUAACCTT-3′

Supplemental Table 3. Primers used for RT-PCR analysis

Genes	Primers	Sequences
Actb	Forward primer	5′-GGCTGTATTCCCCTCCATCG-3′
	Reverse primer	5′-CCAGTTGGTAACAATGCCATGT-3′
Arg1	Forward primer	5′-ACATTGGCTTGCGAGACGTA-3′
	Reverse primer	5′-ATCACCTTGCCAATCCCCAG-3′
Retnla	Forward primer	5′-GGGATGACTGCTACTGGGTG-3′
	Reverse primer	5′-TCAACGAGTAAGCACAGGCA-3′
Nos2	Forward primer	5′-GTTCTCAGCCCAACAATACAAGA-3′
	Reverse primer	5′-GTGGACGGGTCGATGTCAC-3′
Lyz1	Forward primer	5′-GGGAACCTGTGACCTGTCTT-3′
	Reverse primer	5′-ATGCCTCATGACACTGGGAA-3′
Ang4	Forward primer	5′-GCCAAATGGCCGGGACGACA-3′
	Reverse primer	5′- GGCCTGGGAGACGCTCCTGA-3′
Defa20	Forward primer	5′-TGTAGAAAAGGAGGCTGCAATAG-3′
	Reverse primer	5′-AGAACAAAAGTCGTCCTGAGC-3′
Defa29	Forward primer	5′- TGCCCTCGTTCTGCTGGCCT-3′
	Reverse primer	5′- AGCAGAGCCTTCTGTGCCTCCA-3′
Col1a1	Forward primer	5′- GCTCCTCTTAGGGGCCACT-3′
	Reverse primer	5′- CCACGTCTCACCATTGGGG-3′
TNF	Forward primer	5′-CCCATCTATCTGGGAGGGGT-3′
	Reverse primer	5′-GCGTTTGGGAAGGTTGGATG-3′

IL6	Forward primer	5′-TCAATATTAGAGTCTCAACCCCCA -3′
	Reverse primer	5′-CAGGGAGAAGGCAACTGGAC-3′
IL1B	Forward primer	5′-CAGAAGTACCTGAGCTCGC -3′
	Reverse primer	5′-AGATTCGTAGCTGGATGCCG -3′
NFKBIA	Forward primer	5′-GAGCTCCGAGACTTTCGAGG-3′
	Reverse primer	5′-ACACGTGTGGCCATTGTAGT-3′
18S rRNA	Forward primer	5′-CGGCTACCACATCCAAGGAA-3′
	Reverse primer	5′-GCTGGAATTACCGCGGCT-3′