Supplementary Information

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Methods

Isolation of hUCB-MNCs. All hUCB samples were obtained upon signed informed consent, in compliance with Portuguese legislation. The samples were stored and transported to the laboratory in sterile bags with anticoagulant solution (citrate-phosphate-dextrose) and processed within 48 h after collection. MNCs were isolated by density gradient separation (Lymphoprep TM – StemCell Technologies SARL, Grenoble, France) as previously described by us[1].

Isolation of WJ-MSCs. Human umbilical cord WJ was provided by Crioestaminal (Cantanhede, Portugal). WJ was sliced into pieces of 1-2 mm^2 with a sterile scalpel, placed into 60 mm petri dishes and grown in MEM alpha modification with L-glutamine (GE Healthcare) supplemented with 10% FBS (Invitrogen) and 100 U/mL of penicillin and streptomycin (Invitrogen). Medium was changed every 2-3 days. Adherent cells were passaged upon reaching 80% confluence and reseeded at $5.5\times10^3/cm^2$ in 75 cm^2 or 175 cm^2 tissue culture flasks. Cells in passage 4 or 5 were used for the experiments.

Isolation of rat cortical neurons. Primary cortical neuronal cultures were prepared from the cortex of Wistar embryos at embryonic day 18-19 (E18-E19), as previously described[2]. Briefly, the brain was exposed and placed on calcium and magnesium-free hank's balanced salt solution (HBSS). Meninges and hippocampus were removed, and the cortices were treated with 0.06% trypsin (Gibco Invitrogen, USA) for 15 min at 37ºC. Tissue was then thoroughly washed with HBSS to stop trypsin activity and mechanically dissociated with a pipette. Cells were plated in neuronal plating medium (MEM supplemented with 10% horse serum, 0.6% glucose and 1 mM pyruvic acid) onto poly-D-lysine coated 24 well plates at a density of 180 000 cells per well. After 2-4 h, culture medium was changed to neurobasal medium (Gibco, Life Technologies, USA) supplemented with 1:50 of SM1 (Stem Cell Technologies, Canada), 0.5 mM glutamine (Invitrogen, USA) and 0.12 mg/mL gentamycin (Sigma-Aldrich, USA). Cultures were kept in a humidified incubator with 5% CO_2, at 37ºC for 14 days.

sEV isolation from hUCB-MNCs. For sEV isolation, MNCs were thawed and diluted into XVIVO15^{TM} (Lonza, Switzerland) supplemented with 50 μg/mL of DNAse I (Sigma Aldrich, USA). Cells (2×10^6/mL) were seeded into T75 flasks (Costar, USA) in X-VIVO15^{TM} medium

supplemented with 50 ng/mL of stem cell factor (SCF, Peprotech, USA) and 50 ng/mL of fms-like tyrosine kinase 3 (FLT-3, Peprotech, USA) and incubated in a hypoxia chamber (BioSpherix culture chamber, BCA Scientific, USA) under humidified atmosphere with 0.5% O_2 and 5% CO_2, at 37ºC. After 18 h, cell culture medium was collected and sEVs isolated by differential ultracentrifugation[3]. First, conditioned medium was centrifuged at 300 g for 10 min at 4 ºC to pellet cells and supernatant was again centrifuged at 2000 g for 20 min, also at 4ºC to pellet cell debris. Using an Optima™ XPN 100K ultracentrifuge with a swinging bucket rotor SW 32 Ti (Beckham Coulter, USA), supernatant was two times centrifuged at 10000 g for 30 min at 4 ºC in order to pellet larger vesicles. sEVs were then pelleted with a 100000 g centrifugation for 120 min at 4ºC. Pelleted sEVs were washed with cold PBS, centrifuged at the same speed, resuspended in 150-200 µL of cold sterile PBS and stored at -80ºC.

sEV isolation from MSCs. MSCs (passages 4 and 5) were cultured in alpha MEM (GE-Healthcare) supplemented with 10% FBS (GIBCO, Invitrogen, USA) and antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin; GIBCO, Invitrogen, USA). After reaching 80% confluence, cells were cultured in medium without FBS. Medium was collected for sEV isolation after 24h. First, conditioned medium was centrifuged at 300 g for 10 min at 4 ºC and supernatant was centrifuged again at 2000 g for 20 min to pellet cell debris. The supernatant was filtered using a polyethersulfone membrane filter (0.2 µm pore size) and then concentrated to 500 µL by centrifugation with Centricon Plus-70 Centrifugal filter units (Merck, Germany) according to the manufacturer's instructions. Contaminant protein was removed by size exclusion chromatography with qEV original columns (Izon Science, Cambridge, MA, USA). Purified sEVs were concentrated by ultracentrifugation for 120 min at 100000 g at 4ºC. sEVs were resuspended in 100-150 µL of cold sterile PBS and stored at -80ºC.

Characterization of sEVs by Nanoparticle Tracking Analysis (NTA). The concentration and particle size distribution of sEVs were measured by NTA (NanoSight NS300, Malvern instrument Ltd, UK). sEVs were diluted in PBS (1 mL), in order to achieve a final concentration between 5×10^8 and 1×10^9 particles/mL, representing between 30 and 60 particles per frame. Temperature was monitored across all samples. All samples were recorded 5 times (30 sec each). Camera level was set at 14 and detection threshold at 5. Videos were processed using NTA 3.2 analytical software.

Characterization of sEVs by flow cytometry. For the analysis of sEV surface markers by flow cytometry, between 1×10^9 and 3×10^9 sEVs were incubated with aldehyde-functionalized latex beads (0.2 µL; Life Technologies, USA). After 15 min, PBS was added to achieve a final volume of 100 µL and the mixture was incubated in a rotate shaker overnight at 4ºC. In the next day, a solution of glycine (11 µL; 1 M) was added for 30 min to saturate all the free binding sites on the beads. Next, sEVs and beads were centrifuged at 1,300 g for 6 min and then resuspended in 100 µL of 0.5% bovine serum albumin (BSA) in PBS. This centrifugation step was repeated 3 times. After the final centrifugation, beads were resuspended in 50 µL of 0.5% BSA/PBS. Then, sEV-coated beads (10 µL) were mixed with an antibody of interest (1 µL) for 30 min at 4ºC. Labelled sEVs were washed three times with 0.5% BSA/PBS (150 µL) and suspended in 0.5% BSA/PBS (100 µL). sEV analyses were performed in a BD Accuri 6 (BD Bioscience, USA) cytometer and plotted using FlowJoTM (v10, FlowJo, LLC). Commercial antibodies used for flow cytometry were: CD9-FITC (FAB1880F, RD Systems. USA), CD63-PE (12-0639-42, Invitrogen, USA) and HLA-DR-FITC (11-9952-42, Invitrogen, USA).

Characterization of sEVs by Transmission Electron Microscopy (TEM). For TEM, sEVs samples were processed as previously described[4]. sEVs were fixed in 2% paraformaldehyde (PFA) (w/v) and then deposited over EM-Tec formvar-carbon support film on copper 300 square mesh grills (Innovative Microscopy Supplies). The grids were then turned upside down, to make contact with uranyl-acetate 2%, 1 min at rt, TEM images were taken using a Tecnai G2 Spirit BioTwin electron microscope (FEI) at 80 KV.

Western blot analyses. Protein cell lysates were obtained by incubation on ice for 30 min with RIPA buffer (R 0278, Sigma-Aldrich) and a cocktail of phosphatase/protease inhibitors (5872, Cell Signaling Technologies). Samples were centrifuged at 14.000 g for 15 min at 4º C and the supernatant was stored at -80ºC. Total protein from cells extracts and sEVs was quantified by microBCA (Thermo fisher). Protein cell lysates and sEVs were diluted in loading buffer (Laemmli sample buffer, 0.25 M Tris base, 8% SDS, 40% glycerol, 200 mg bromophenol blue, 10% 2-mercaptoethanol) and 4 µg were applied to a 12% polyacrylamide gel. Protein was transferred to a PVDF membrane (Amersham™ Hybond™ 0.45 PVDF) at 100V. At the end of the run, the membranes were blocked with 5% (w/v) milk in TBST, and incubated with monoclonal antibodies: anti-human CD63 (556019, BD Biosciences); anti-human CD9

(555370, BD Biosciences); anti-GAPDH (sc-365062, Santa Cruz Biotechnology) or anti-Calnexin (ab31290, Abcam) overnight at 4ºC. Then, membranes were incubated with polyclonal goat anti-mouse immunoglobulins/HRP antibody (Cell Signaling) at a final concentration of 1:10,000 for 1 h at room temperature. The protein detection was accomplished by a chemiluminescence reaction using Western Bright Quantum HRP substrate (Advansta).

Cell culture. Human umbilical vein endothelial cells (HUVECs, Lonza, Switzerland; between passage 4 and 7) were used to investigate the bioactive effects of sEVs. Endothelial cells were cultured in EGM-2 (Lonza, Switzerland) with all the factors and 2% FBS. BV-2 cells (murine microglia; ICLC Cat# ATL03001, RRID: CVCL_0182) were maintained in Roswell Park Memorial Institute (RPMI) medium (GIBCO, Invitrogen, USA) supplemented with 10% FBS (GIBCO, Invitrogen, USA) and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin; GIBCO, Invitrogen, USA). For cells treated with sEVs, the cell culture media was centrifuged overnight at 100,000 g and filtered (0.2 μm pore) in order to deplete the sEVs from FBS.

Cell characterization by immunofluorescence. Endothelial cells seeded in a 96 well plate were fixed with 4% PFA for 15 min, washed with PBS and blocked with 1% BSA for 1h. Then, cells were incubated with mouse anti-human CD31 antibody (1:50; Dako, Agilent, USA) for 1h at room temperature, followed by incubation with Alexa-fluor488® conjugated rabbit anti-mouse (1:1000; Invitrogen). Cells were imaged using IN Cell Analyzer.

For characterization of microglia cells, BV-2 cells were fixed with 4% PFA, followed by permeabilization with 0.1% Triton X-100 for 10 min and blocking with 1% BSA for 1h. Afterwards, cells were incubated for 2h with rabbit anti-mouse IBA-1 (1:200; 019-19741, Wako, USA). Alexa-fluor 488® conjugated goat anti-rabbit (1:1000; Invitrogen) was used as secondary antibody. Cells were imaged using IN Cell Analyzer.

Cortical cultures after 15 days *in vitro* were fixed with 4% PFA/ 4% sucrose for 15min, at room temperature, followed by 6 sequential washes with PBS. After permeabilization with 0.25% Triton X-100 for 5 min, 4ºC, neurons were washed with PBS and blocked with 10% BSA for 30 min, 37ºC. Cortical cells were incubated with rabbit anti-rat GFAP primary antibody (1:1000; Z0334, Dako) diluted in 3% BSA for 2h at 37ºC. Cells were then washed 6x with PBS and incubated for 45min with Alexa fluor®647 conjugated anti-rabbit (1:500, Invitrogen). Upon 6 washes with PBS, cells were re-incubated with primary antibodies staining for anti-GABA (1:50; Rabbit, A2052, Sigma Aldrich) and anti-CAMKII alpha (1:500, mouse IgG1;

ab134041; Thermofisher Scientific) antibodies. Cells were then washed 6x with PBS and re-incubated for 45min with secondary antibodies in 3% BSA at 37°C. The following secondary antibodies from Invitrogen Molecular Probes were used: Alexa fluor® 568 conjugated anti-mouse IgG1 (1:500), Alexa Fluor® 488 conjugated anti-rabbit (1:500). Following six washes, coverslips were mounted with fluorescence mounting media. Nuclei were labelled with Bisbenzimide Hoechst 33342 (1 μg/mL) diluted in the mounting media. Images were manually quantified using image analysis software FIJI. Hoescht labelling enabled total cell count. Glial cells count was calculated as the GFAP+ staining. Excitatory and inhibitory neurons count was determined as the CAMKII alpha+ and the GABA+ staining, respectively.

Cell survival after oxygen and glucose deprivation (OGD). The bioactivity of sEVs isolated from MNC or WJ-MSC was evaluated in HUVECs, rat cortical neurons and microglia subjected to OGD[5]. HUVECs at passage 4 were seeded in a 96 well plate, at a density of 10000 cells per well, in EGM-2 and allowed to grow for 24 h before the insult. Cortical neurons were used after 15 days *in vitro*. BV-2 cells were seeded in RPMI medium supplemented with 2% FBS in a 96 well plate at a density of 4000 cells per well. In the day of the insult, inside an anaerobic chamber (OGD chamber, Thermo Forma 1029, Thermo Fisher Scientific, Waltham, MA), cells were placed for 2h in a glucose-free deoxygenated buffer medium (10 mM HEPES, 116 mM NaCl, 5.4 mM KCl, 0.8 mM NaH2PO4, 25 mM sodium bicarbonate, 25 mM sucrose, 1.8 mM CaCl2, 0.04% phenol red, pH 7.1). For microglia, RPMI medium without glucose was used to keep cells under OGD conditions for 6h. Normoxia-control cells, called sham along this work, were incubated in oxygenated conditions with a similar medium, containing 25 mM glucose instead of sucrose. Two hours later, cells were removed from oxygen deprivation conditions. For neurons, medium was immediately changed for their conditioned medium and sEVs added at 1.5, 3 or 4.5×10^9 particles per mL (corresponding to 8.3×10^3, 1.7×10^4 and 2.5×10^4 sEVs/cell, respectively). In the case of HUVECs, insult medium was replaced by endothelial basal medium (EBM-2) (Lonza, Switzerland) supplemented with sEVs added at 1.5, 3 or 4.5×10^9 particles per mL (corresponding to 1.5×10^4, 3×10^4 and 4.5×10^4 sEVs/cell). Cells were treated with sEVs from three different donors.

Microglia activation after OGD. BV-2 cells were seeded in 24 well plates at (2.5×10^4 cells/well^2) and left to adhere before incubation under OGD for 6 h. After OGD, cells were incubated with different concentrations of sEVs (1.5, 3 or 4.5×10^9 particles per mL,

corresponding to 3×10^4, 6×10^4 and 9×10^4 sEVs/cell) for 16 h and finally fixed in 4% PFA. Translocator protein (TSPO) was used as a readout of microglia activation[6]. Briefly, cells were permeabilized with 0.1% Triton X-100 for 10 min, blocked with 1% BSA for 30 min followed by incubation with primary antibody goat anti-TSPO (1:500; Abcam 92291, UK) or anti-CD206 (1:200; AF2535, R&D Systems). Alexa Fluor 488 donkey anti-goat (1:1000; Invitrogen A-11055, USA) or donkey anti-goat (1:1000, A21432, Invitrogen) were used as secondary antibodies. Cells were imaged using a fluorescence microscope (IN Cell Analyzer 2200, GE Healthcare, USA).

sEV uptake by cells *in vitro.* For uptake studies, cortical neurons were used after 15 days *in vitro* (24 well plate, 1.8×10^5 cells/well). Endothelial cells (3×10^4 per well) and microglia (1.25×10^4 per well) were cultured in 48 well-plates for 18-24 h, after which they were incubated with DiO-labelled sEVs for 24 h in sEV-depleted medium, at final concentration of 5×10^8 EVs/mL. Fluorescence images were acquired at 2 h, 4 h, 6 h, 18 h and 24 h of incubation using a high content microscope (IN Cell 2200 Analyzer, GE Healthcare). Cell fluorescence was quantified using the IN Cell developer toolbox. To block EV uptake by microglia, MSC-EVs and MNC-EVs (2.1×10^{10} particles/mL) were pre-incubated with anti-HLA-DR antibody (10 µg/mL) for 30 min at room temperature before incubation with cells.

For colocalization studies, endothelial cells and microglia were seeded in a 15-well IBIDI plate at a desired density of 9.6×10^3 and 4.0×10^3 cells per cm^2, respectively, and allowed to adhere for 24 h. Cells were then incubated with a concentration of 5.0×10^8 particles per mL of DiO-labelled sEVs for 24 h in sEV-depleted medium. After the incubation period, the cells were washed with culture medium to remove non-internalized sEVs. Following that, the cells were stained with Lysotracker Red DND-99 (L7528, Invitrogen) for 30 min at 37° C. After Lysotracker staining, cells were washed again to remove excess dye, and immediately. Images were acquired using a Zeiss LSM 710 confocal microscope (Carl Zeiss, Jena, Germany) with a 40× objective/ 1.4 numerical aperture oil Plan Apochromat immersion lens. Z-stacks were acquired to confirm the intracellular localization of sEVs. The thickness of the slices and the interval between slices were set to 0.5 µm.

MCAO surgery and stereotaxic administration of sEVs. Adult CD57BL/6J mice (10 to 12 weeks old; 28 to 32 g) were subjected to transient unilateral focal ischemia for 45 min followed by reperfusion and stereotaxic administration of sEVs in a PBS solution or PBS only. Focal

ischemia was induced by MCAO[7] by transient intraluminal filament. Animals were randomly assigned to receive either MNC-sEVs or PBS only. Mice were anesthetized with 4% Isoflurane with O_2 by inhalation in an anaesthesia induction chamber. After reaching the anaesthetic state, mice were transferred to the surgery table and anaesthesia was maintained with 1,5% isoflurane administered by an inhalation mask. Prior to the surgery, analgesic treatment (buprenorphine 0,1 mg/kg) was administered through intraperitoneal injection and the hair was removed in the neck region with a hair trimer. Under a dissecting microscope, the right common carotid artery (CCA) bifurcation was carefully exposed through a midline incision in the neck region. The CCA was ligated distal to the bifurcation with a temporary ligature and the external carotid artery (ECA) was blocked distal to the bifurcation with a permanent ligature. A microvascular clip was temporarily placed across the internal carotid artery (ICA) to block the blood flow in the bifurcation area and prevent bleeding during the filament insertion. A 11 mm 6-0 silicon coated (coating diameter: 0.23 +/- 0.02 mm) monofilament (Doccol) was inserted into the ECA lumen through a puncture close to the permanent ligature and the microvascular clip removed. The filament was then gently advanced (9.0-10.0 mm) into the ICA to occlude the origin of the MCA. After 45 min of occlusion, the filament was withdrawn, the ECA was permanently ligated at its origin and the temporary ligature of the CCA was removed to allow reperfusion. The surgical incision was closed and animals were prepared to the stereotaxic administration. Immediately following the MCAO, mice were placed in a stereotaxic frame under anesthesia with 1,5% Isoflurane administered by face mask. A middle incision was made in the top of the skull following a craniotomy on the top of the ischemic region (coordinates: AP +0.38, ML +2.8). A total of 3×10^9 particles in 3 µL PBS, or PBS alone (control group), were administered in 14 different sites across the striatum's cortical border (coordinates: DV -3.5 to 2.1) over 15 min, with 1 min rest between injections and 10 min rest before withdrawal. The goal was to administer the sEVs within the border between the core (corpus striatum) and the penumbra (cortex) of the ischemic lesion. After recovering form anesthesia, mice were allowed to recover in a recovery cage for 24 h. The mortality rate in the induction of MCAO was 44%. Animals showing no signs of dysfunction at day 1 post-ischemia were excluded from the study.

Biodistribution studies of sEVs. To evaluate the accumulation of sEVs in the brain by PET-MRI, MNC-sEVs were surface modified with maleimide-DOTA and then complexed with a radioactive copper (Cu^{2+}), using a methodology previously reported by us[8]. Labelled sEVs were injected in the tail vein of mice with and without induction of brain ischemia. Radioactivity in the brain was measured by scintillation, 1 h after injection.

Behavioral dysfunction analysis. The sensory-motor deficits after brain ischemia were evaluated using the Clark score[9]. The Clark score is battery of tests specifically designed to monitor mice post-ischemic sensory-motor recovery by gathering data that takes into account a wide range of brain functions affected by the induced focal ischemia. The score consists of a 56 scale where a higher score corresponds to a more severe lesion.

Brain sections. Sections were obtained from mice brains 6 h or 3 days after MCAO, from five different regions as indicated in **Fig 2A**. Briefly, the animals were deeply anesthetized using 5% isoflurane and then perfused with PBS and 4% PFA. Brains were dissected and dehydrated through sequential sucrose gradient, 15% sucrose in PBS for 24 h followed by 30% sucrose for 48 h and frozen at -80°C until further processing. Brains were mounted using OCT cryomatrix (Thermo fisher scientific, USA) and cryosections of 50 µm in the coronal plane were obtained using a Leica CM1950 cryostat and collected to glass slides (Superfrost plus, VWR, USA), one representing each region per slide. Slides were kept at -80°C until labelling.

Immunofluorescence analyses. Frozen sections were defrosted at room temperature and re-hydrated by soaking in PBS for 10 min. Brain sections were contoured using a hydrophobic pen (Immedge™ Hydrophobic Barrier Pen, Vector Laboratories, ImmEdge™), permeabilized using 0.2% Triton X-100 in PBS for 30 min and blocked with 5% BSA and 0.1% Triton X-100 in PBS for 1 h. In slides containing labelled sEVs (6 h time point) the permeabilization step was omitted. Slides were incubated overnight at 4°C with anti-mouse primary antibodies in 5% BSA and 0.2% Triton X-100 in PBS. Endothelial cells were stained with goat anti-mouse CD31 (1:200; AF3628-SP, R&D Systems, USA), microglia with rabbit anti-mouse IBA-1 (1:1000; 019-19741, Wako, USA), astrocytes with mouse anti- GFAP (1:200; 3670S, Cell Signaling Technology, USA), neurons were stained with mouse anti-NeuN (1:200; MAB377, Sigma, USA), proliferative cells were stained with rat anti-Ki67 (1:500, 14-5698-82, Thermo Fisher Scientific, uSA), TSPO was stained with goat anti-TSPO (1:200; ab92291, Abcam, UK). Sections were washed with PBS, three times for 10 minutes and incubated with secondary antibodies for 90 min at room temperature. The following secondary antibodies were used: Alexa-Fluor 488 donkey anti-goat (1:1000, Invitrogen, USA), Alexa-Fluor 488 goat anti-rabbit (1:1000, Invitrogen, USA), Alexa-Fluor 488 donkey anti-mouse (1:1000, Invitrogen, USA), Alexa-Fluor 555 goat anti-rat (1:1000, Invitrogen, USA). Following incubation slides were washed with PBS and nuclei were labelled with DAPI (Sigma Aldrich, USA) for 5 min. A final

washing step was performed, and the sections were mounted using fluorescent mounting media (Dako, Agilent, USA). Tissue sections were imaged using IN Cell Analyzer (GE Healthcare, USA). For colocalization studies, images were acquired using a Zeiss LSM 710 confocal microscope (Carl Zeiss, Jena, Germany) with a 40× objective/ 1.4 numerical aperture oil Plan Apochromat immersion lens. DiO fluorescence was detected using the 488 nm laser line of an Ar laser (25 mW nominal output) and an LP 505 filter. Lysotracker Red fluorescence was detected using a 561 nm HeNe laser (1 mW) and an LP 560 filter. Z-stacks were acquired to confirm the intracellular localization of DiO labelled sEVs. The thickness of the slices and the interval between slices were set to 0.7 μm.

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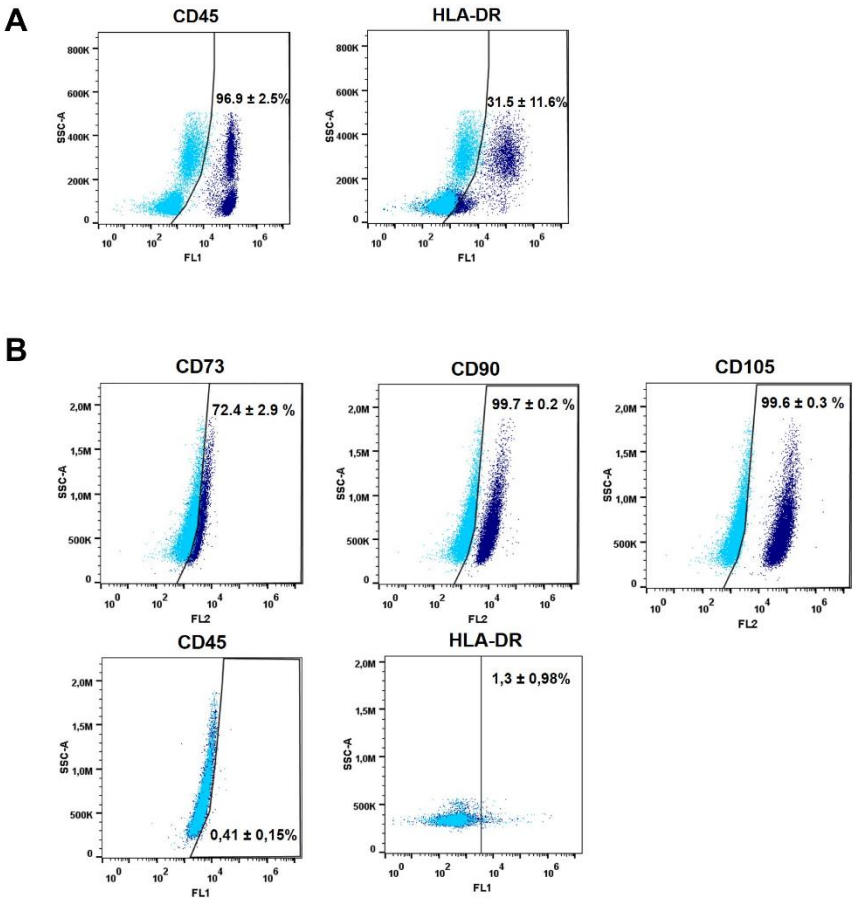
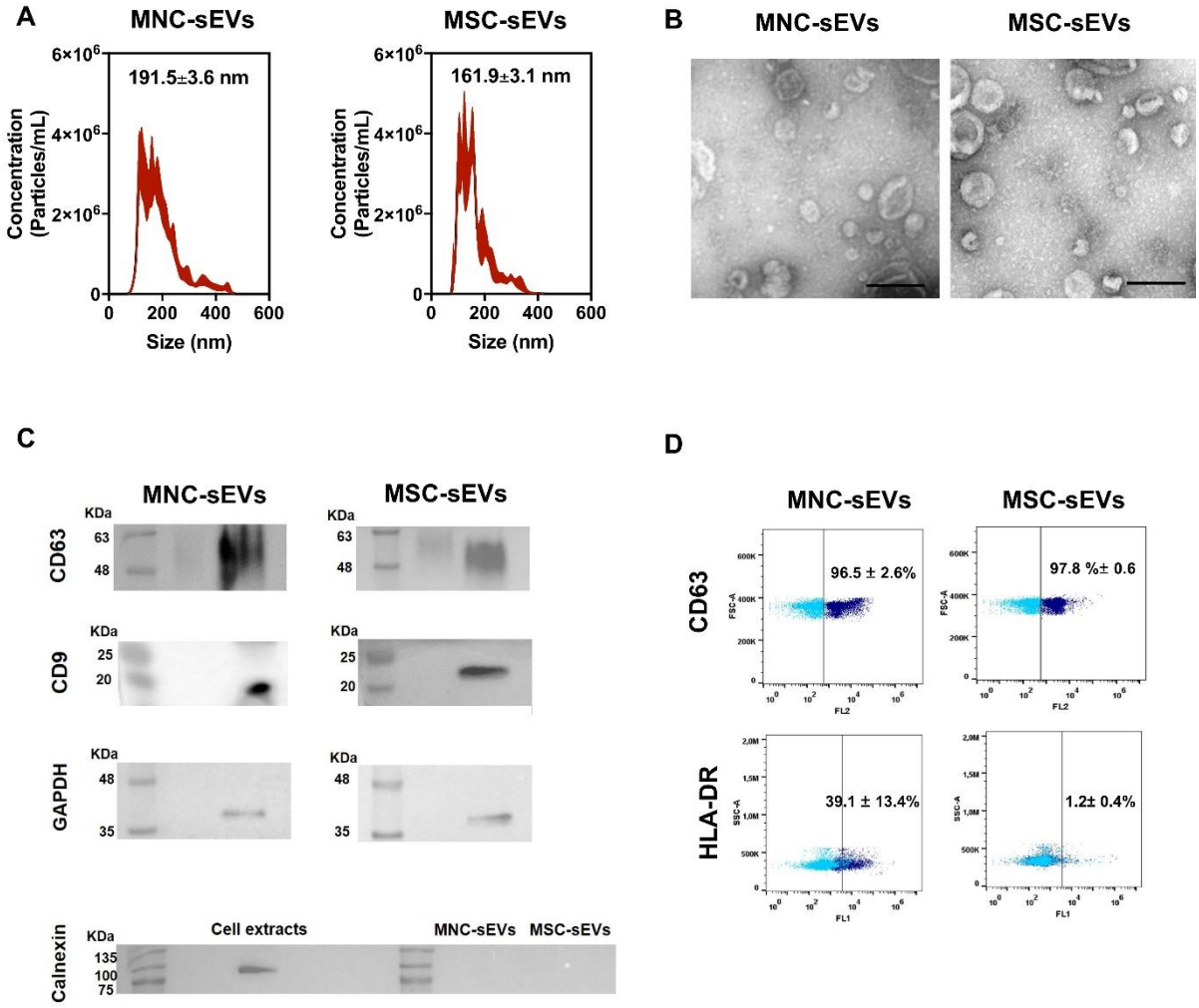
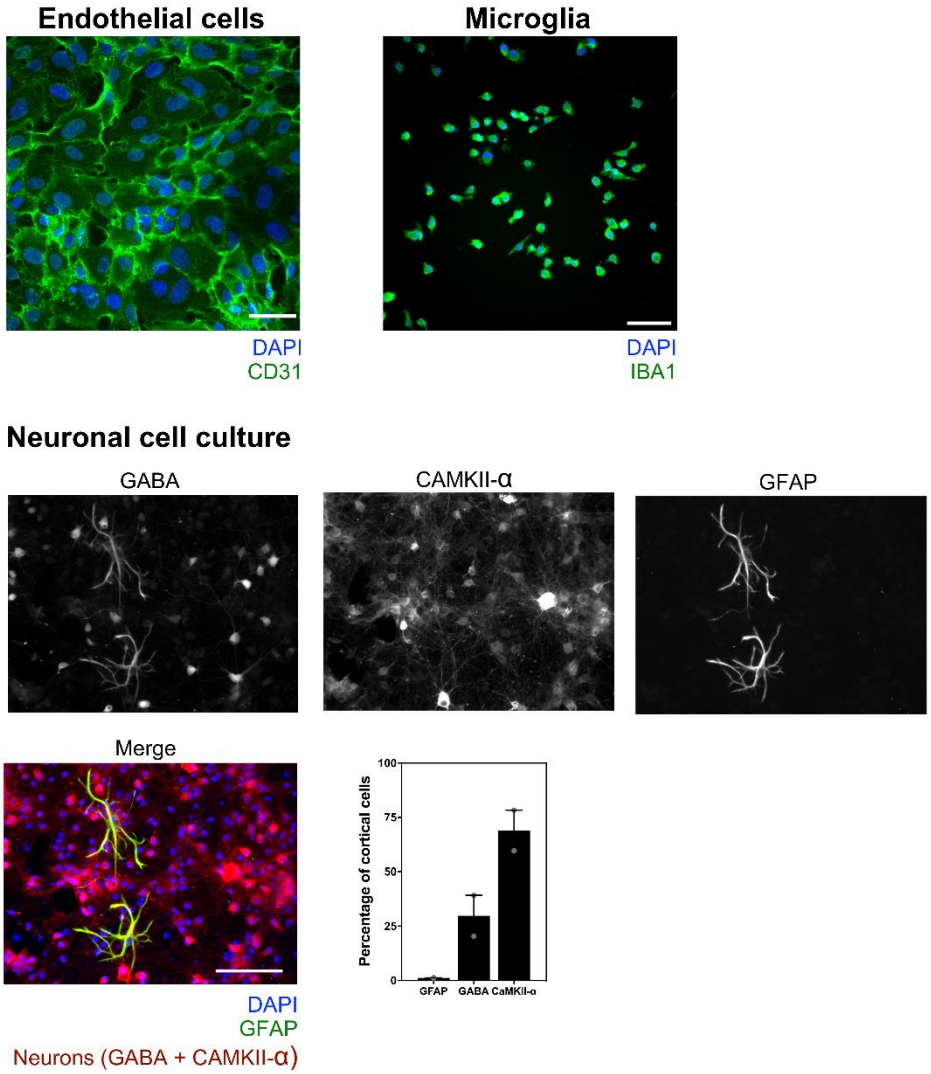


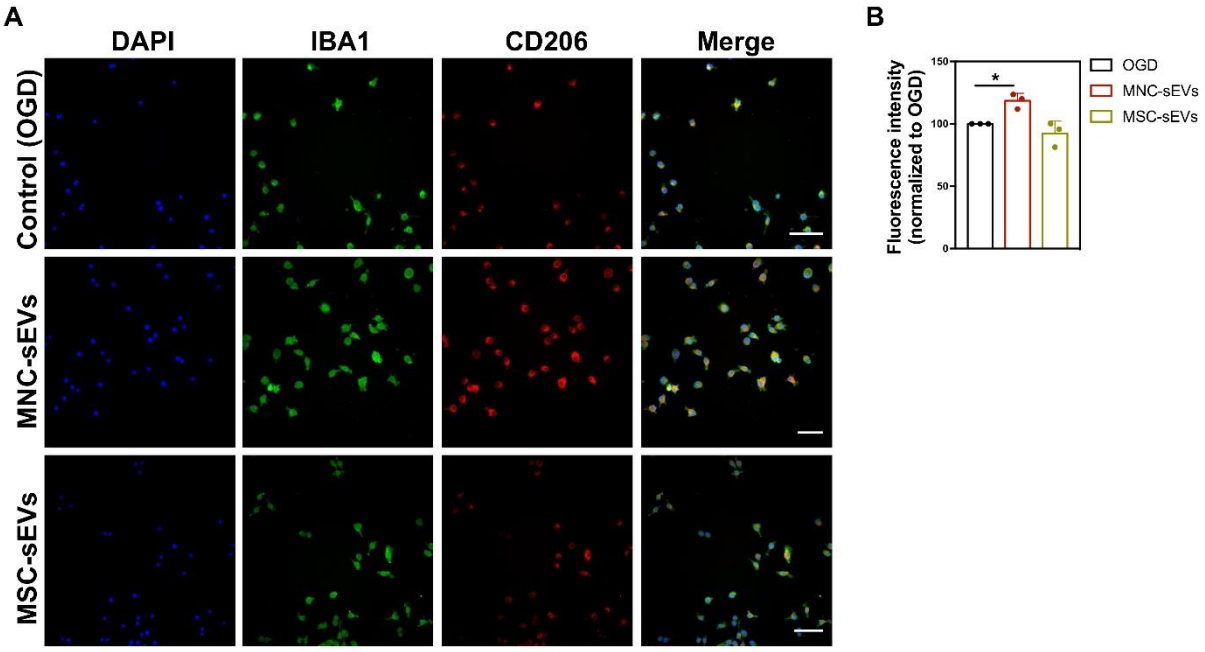
Supplementary Figure 1. Characterization of MNCs and WJ-MSCs by flow cytometry. A) MNCs express hematopoietic marker CD45 and the glycoprotein HLA-DR. **B)** WJ-MSCs express MSC markers CD73, CD90 and CD105 but not the hematopoietic marker CD45 and HLA-DR. Light blue represents the control and dark blue represents cells stained with fluorescence-conjugated antibodies. Results are average ± SEM (n = 6 donors for MNCs and n=3 donors for MSCs).



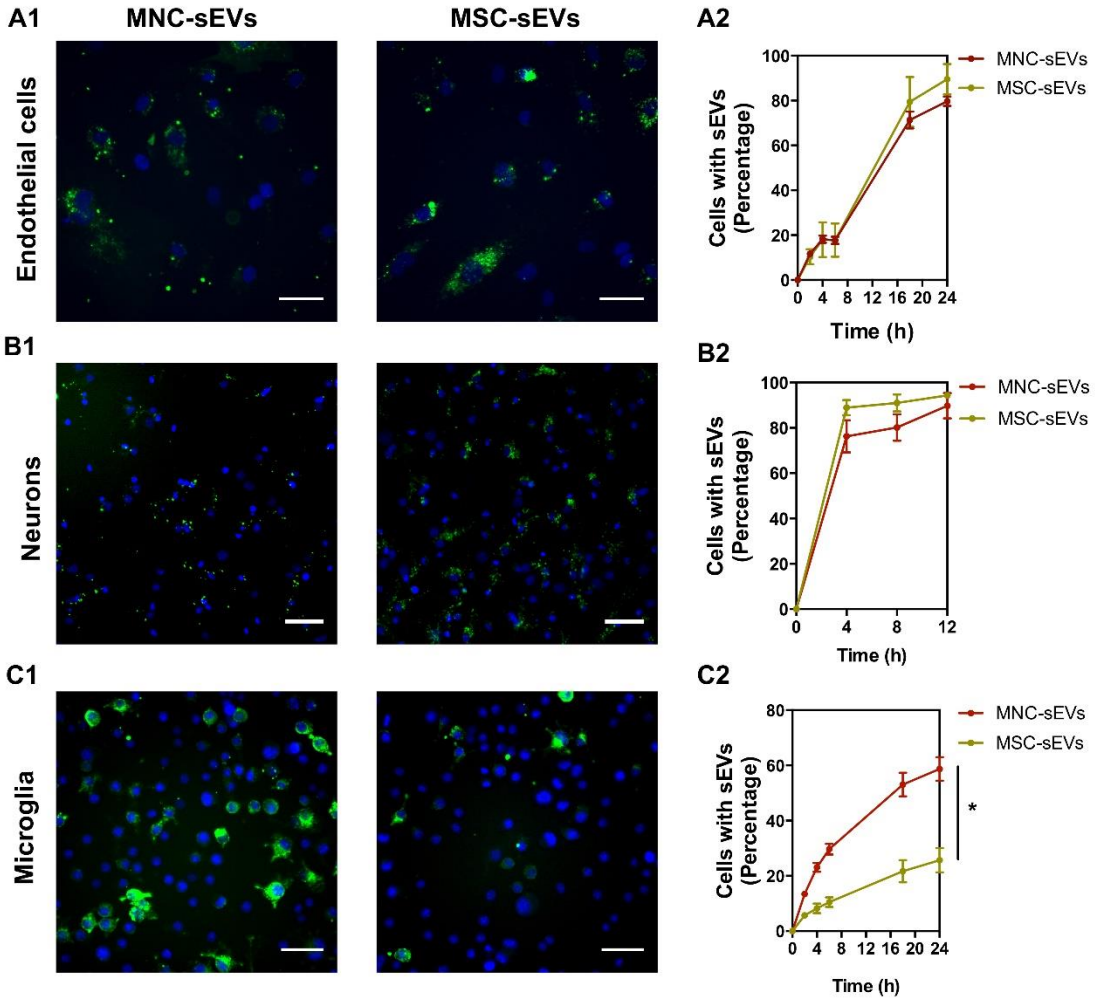
Supplementary Figure 2. Characterization of sEVs. A) sEV size distribution evaluated by NTA. MNC-sEVs have a mean diameter of 191.5±3.6 nm while MSC-sEVs have a mean diameter of 161.9±3.1 nm. **B**) TEM images of MNC-sEVs and MSC-sEVs. Scale bar represents 200 nm. EV markers and contaminants were assessed by **(B)** Western Blot and by **(C)** flow cytometry. Dark blue represents sEVs incubated with fluorescence-conjugated anti-CD63 or anti-HLA-DR antibodies and light blue represents the control (sEVs that were not incubated with antibodies)



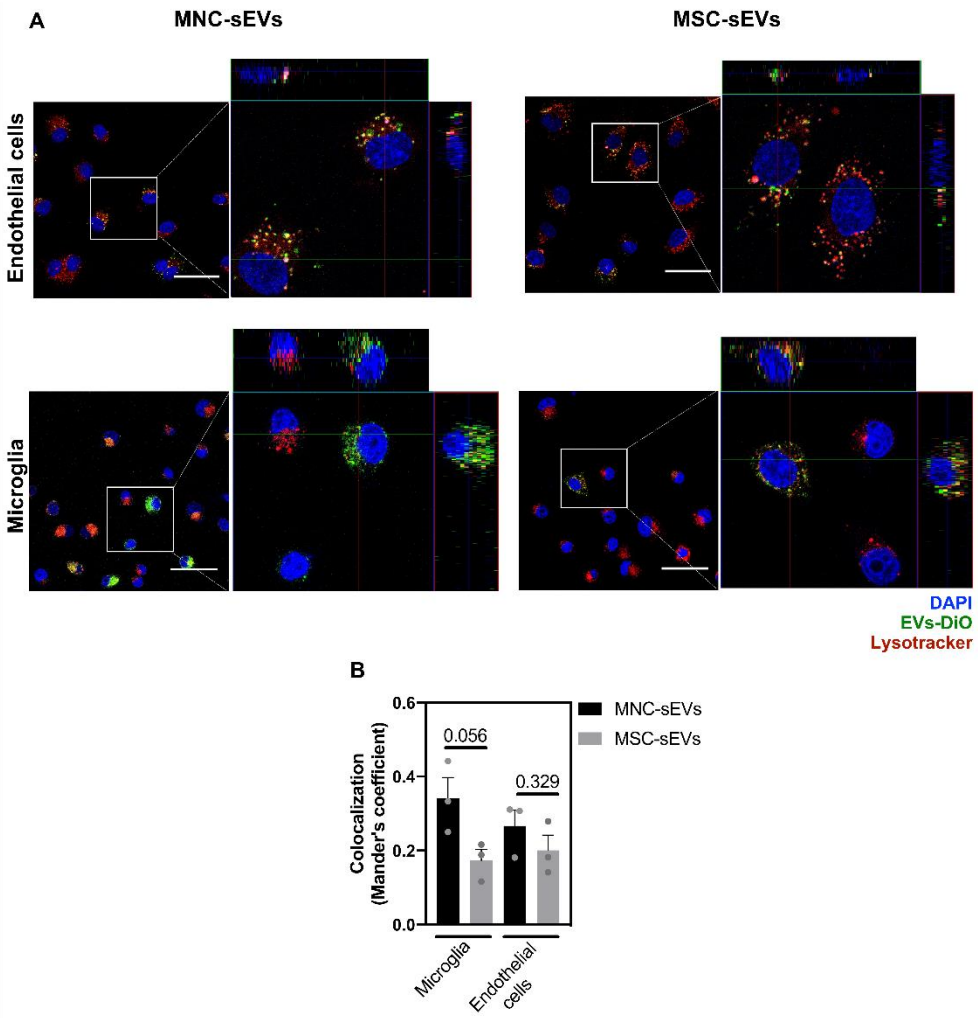
Supplementary Figure 3. Characterization of cell markers. Cells used for *in vitro* assays were first characterized by immunofluorescence. Endothelial cells were stained for CD31 (scale bar corresponds to 50 µm), microglia were stained for IBA1 (scale bar corresponds to 100 µm) and rat cortical cultures were stained for GFAP (glial marker), CaMKII-α and GABA (neuronal markers). Scale bar corresponds to 100 µm. Glial cells ($GFAP^+$ cells) and neurons ($GABA^+$ and $CAMKII\text{-}\alpha^+$) in cortical cultures were quantified from 2 independent cultures (n=2, n>300 cells counted). Results are presented as mean ± SEM.



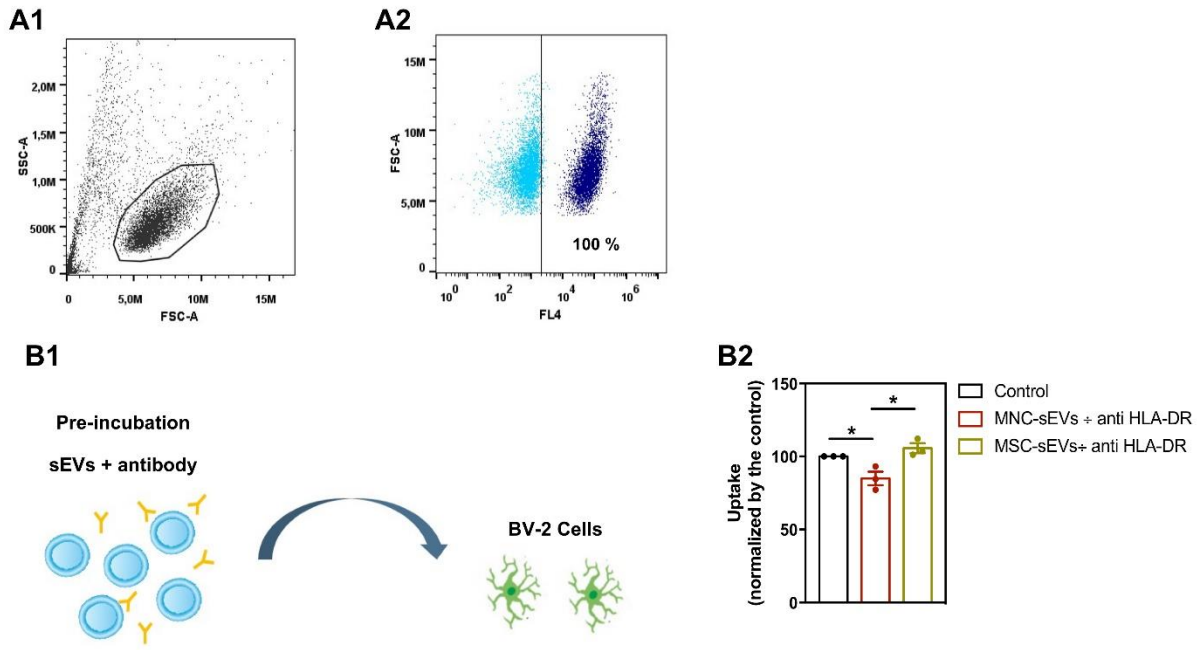
Supplementary Figure 4. Expression of CD206 in microglia. Microglia cells were cultured under OGD conditions for 6 h and then treated with MNC-sEVs and MSC-sEVs (4.5×10^9 part/mL) for 16 h under normoxia. Cells were fixed, stained for CD206 and **(A)** imaged in a high-content microscope. **B)** CD206 fluorescence intensity was calculated using IN Cell developer toolbox and normalized to the control (OGD). Scale bar corresponds to 50 µm. Results are expressed as mean ± SEM (n=3). * denotes statistical significance ($p<0.05$) assessed by one-way ANOVA followed by Tukey's post Test.



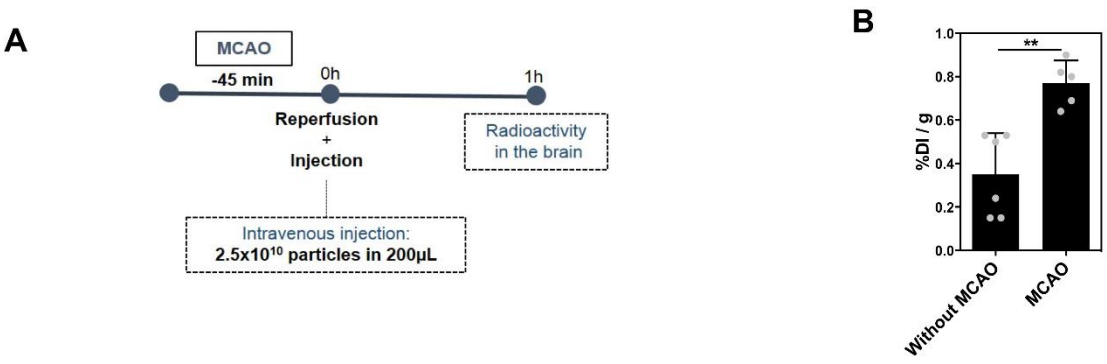
Supplementary figure 5- Uptake kinetics of sEVs labelled with DiO in endothelial cells and microglia. A1, B1 and **C1**) Fluorescence microscopy images of endothelial cells, neurons and microglia cells incubated with 5×10^8 EVs/mL sEVs from MSCs and sEVs from MNCs for up to 24 h. **A2, B2** and **C2)** Internalization kinetics. Quantification of the percentage of cells with internalized sEVs. Results are expressed as mean ± SEM (n=3). * denotes statistical significance ($p<0.05$) between both curves for all the time points assessed by paired t-test. Scale bar corresponds to 50 µm.



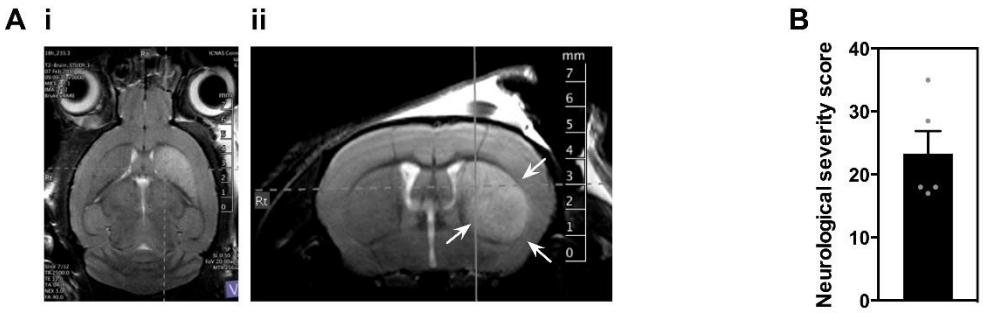
Supplementary Figure 6 - Uptake and colocalization of sEVs with endolysosomal in endothelial cells and microglia. A) sEV internalization was monitored by confocal microscopy after 4 h incubation. Endothelial cells and microglia cells were incubated with sEVs labelled with DiO and then incubated for 30 min with Lysotracker red. Z-stack images were acquired to confirm sEV colocalization with Lysotracker. Scale bar corresponds to 50 µm. **B)** Colocalization (Mander's coefficient) between Lysotracker and sEVs was determined using ImageJ: a coefficient equal to 0 means no colocalization and a coefficient equal to 1 means total colocalization. Results are expressed as mean ± SEM (n=1; 3 technical replicates) Statistical analyses were performed by a t-test.



Supplementary Figure 7 - Uptake of MNC-sEVs in microglia cells is mediated, at least in part, by the axis HLA-DR (sEVs): CD4 (microglia). A1) Scatter plot and gating of BV-2 cells as analysed by flow cytometry. **B2)** Expression of CD4 in microglia cells assessed by flow cytometry. Dark blue are cells labeled with antibody and light blue are unlabeled cells. **B1)** The internalization of MNC-sEVs in microglia is partially mediated by the axis HLA-DR: CD4. MNC-sEVs or MSC-sEVs (5×10^8 sEVs/mL) were pre-incubated with anti HLA-DR (10 µg/mL) for 30 min and then incubated with BV-2 cells. Cell fluorescence was monitored after 4 h incubation in a fluorescence microscope (IN Cell Analyser, GE Healthcare) and quantified using the IN Cell Analyzer developer toolbox. **B2**) Cell fluorescence was normalized to the control (cells incubated with sEVs without pre-incubation with the antibody). Results are expressed as mean ± SEM (n=3). * denotes statistical significance ($p<0.05$) assessed by one-way ANOVA followed by Tukey's post Test.



Supplementary Figure 8 - Brain accumulation of sEVs *in vivo*. A) Biodistribution of DOTA-Cu-SEV after intravenous injection. sEVs (2.5×10^{10}) were injected in the tail vein. One hour after injection mice were sacrificed and radioactivity was measured in several organs by scintillation. **B)** Accumulation of sEVs in the brain after intravenous administration. DOTA-Cu-SEVs (2.5×10^{10}) were injected in the tail vein in animals with and without ischemic stroke. Stroke was induced by MCAO for 45 min and 1 h after sEVs were injected. Radioactivity in the brain was measured by scintillation. Results are expressed as mean ± SEM (n=5-6 mice). ** denotes statistical significance assessed by unpaired t-test.



Supplementary Figure 9 - Brain infarct lesion and neurological score after MCAO. A) brain ischemic lesion caused by 45 min of MCAO was assessed using magnetic resonance imaging 24 h after inducing the lesion. (i) transversal section and (ii) coronal section. White arrows indicate the site of the ischemic brain lesion. **B)** Neurological severity score was evaluated 24 h after MCAO using a functional outcome scale (Clark's score) that measures general deficits and focal deficits (ranging from 0 to 56).