Supplementary Material

## Supplementary material: MISEV2018 checklist

Done

**1-Nomenclature**

Mandatory

* Generic term extracellular vesicle (EV): With demonstration of extracellular (no intact cells) and vesicular nature per these characterization (Section 4) and function (Section 5) guidelines OR
* Generic term, e.g., extracellular particle (EP): no intact cells but MISEV guidelines not satisfied

Encouraged (choose one)

* Generic term extracellular vesicle (EV) + specification (size, density, other)
* Specific term for subcellular origin: e.g., ectosome, microparticle, microvesicle (from plasma membrane), exosome (from endosomes), with demonstration of the subcellular origin
* Other specific term: with definition of specific criteria

**2-Collection and pre-processing**

***Tissue Culture Conditioned medium (CCM, Section 2-a)***

General cell characterization (identity, passage, mycoplasma check…). Medium used before and during collection (additives, serum, other)

* exact protocol for depletion of EVs/EPs from additives in collection medium
* Nature and size of culture vessels, and volume of medium during conditioning
* A T150 flask with 15 ml of medium without FBS was used during conditioning
* specific culture conditions (treatment, % O2, coating, polarization…) before and during collection
* Number of cells/ml or /surface area and % of live/dead cells at time of collection (or at time of seeding with estimation at time of collection)
* 3x105 cells/15ml were seeded in a T150 per condition with estimation at time of collection of ± 6,5x106 cells and ±97% of live cells.
* Frequency and interval of CM harvest
* 6 h.

***Storage and recovery (Section 2-d)***

* Storage and recovery (e.g., thawing) of CCM, biofluid, or tissue before EV isolation (storage temperature, vessel, time; method of thawing or other sample preparation)
* The CCM was stored at 4˚C before starting the experiments.After 16 h, the recovered CCM was used at 4°C during centrifugations.
* Storage and recovery of EVs after isolation (temperature, vessel, time, additive(s)…)
* After EVs isolation, samples were resuspended in medium RPMI1640 supplemented with 10% dimethyl sulfoxid, and frozen at -80ºC for the following applications.
* **3-EV separation and concentration**
* ***Experimental details of the method***
* Centrifugation: reference number of tube(s), rotor(s), adjusted k factor(s) of each centrifugation step (= time+ speed+ rotor, volume/density of centrifugation conditions), temperature, brake settings
* Reference number of tubes: Polypropylene Centrifuge Tubes, Beckman Coulter 337986.
* Each tube contained 30ml of CCM.
* Rotor: SW32Ti
* Centrifugation steps:
* 3000 g for 20 min at 4˚C
* Supernatants filtered through 0.22 μm pore filter
* Samples ultracentrifuged (Optima L100XP, Beckman) at 100,000 g for 1 h at 4˚C

**4-EV characterization**

***Quantification (Table 2a, Section 4-a)***

* Volume of fluid, and/or cell number, and/or tissue mass used to isolate EVs NTA
* 30 ml of CCM were used to isolate EVs for NTA
* Global quantification by at least 2 methods: protein amount, particle number, lipid amount, expressed per volume of initial fluid or number of producing cells/mass of tissue
* Ratio of the 2 quantification figures

***Global characterization (Section 4-b, Table 3)Citometria y los marcadores***

* Transmembrane or GPI-anchored protein localized in cells at plasma membrane or endosomes
* The CD63 marker was observed by Flow Cytometry
* Cytosolic protein with membrane-binding or -association capacity
* The CD9 and CD81 markers were observed by Flow Cytometry
* Assessment of presence/absence of expected contaminants
* A total absence of contaminants was observed by Electron Microscopy

 (At least one each of the three categories above)

* Presence of proteins associated with compartments other than plasma membrane or endosomes
* No presence of proteins was observed.
* Presence of soluble secreted proteins and their likely transmembrane ligands
* Topology of the relevant functional components (Section 4-d)

***Single EV characterization (Section 4-c)***

* Images of single EVs **by wide-field and close-up**: e.g. electron microscopy, scanning probe microscopy, super-resolution fluorescence microscopy
* Non-image-based method analyzing large numbers of single EVs: NTA, TRPS, FCS, high-resolution flow cytometry, multi-angle light-scattering, Raman spectroscopy, etc.

**Reporting**

* Submission of methodologic details to EV-TRACK (evtrack.org) with EV-TRACK number provided (strongly encouraged)
* Submission of data (proteomic, sequencing, other) to relevant public, curated databases or open-access repositories
* Data submission to EV-specific databases (e.g., EVpedia, Vesiclepedia, exRNA atlas)
* Temper EV-specific claims when MISEV requirements cannot be entirely satisfied (Section 6-b)

## Supplementary Tables

**Supplementary Table S1:** Flow cytometry antibodies for characterization of BM-MSCs and their EVs.

|  |  |  |  |
| --- | --- | --- | --- |
| **Marker** | **Dye** | **Clone** | **Company** |
| **CD44** | APC | IM7 | BD Biosciences |
| **CD29 (Integrin 1)** | APC | HMb1-1 | eBioscience |
| **Sca1 (Ly-6A/E)** | PE | E13-161.7 | BD Biosciences |
| **CD9** | unstained | H-110 | Santa Cruz |
| **CD63** | unstained | H-193 | Santa Cruz |
| **Secondary antibody** | FITC | Anti-rabbit IgG | Invitrogen |
| **Isotype** | PE | IgG2a | Thermo Scientific |
| **Isotype** | APC | IgG | Thermo Scientific |
| **Isotype** | APC | Arm Ham IgG eBio299Arm | eBioscience |
| **Isotype** | FITC | IgG1 | Thermo Scientific |

**Supplementary Table S2:** Primers used in quantitative Real-Time PCR.

|  |  |
| --- | --- |
| **Transcript** | **Sequence (5’-3’)** |
| **TIMP-1** | F: ACCTGGTCATAAGGGCTAAATTCA  R: GTCATCTTGATCTTATAACGCTGGTAT |
| **PAI-1** | F: AGGTCAGGATCGAGGTAAACGAG  R: GGATCGGTCTATAACCATCTCCGT |
| **IFN-γ** | F: GAGGTCAACAACCCACAGGT  R: ATCTCTTCCCCACCCCGAAT |
| **HPRT** | F: CCTAAGATGAGCGCAAGTTGAA  R: CCACAGGACTAGAACACCTGCTAA |

## Supplementary Figure

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**Supplementary Figure 1.** Survival curves in CsA-treated mice. **(A)** Survival curve of CsA-treated mice with preventive cell therapies (BM-MSC, EVs or dCM) and CsA monotherapy. **(B)** Survival curve of CsA-treated mice with curative cell therapies (BM-MSC, EVs or dCM) and CsA monotherapy. Survival curve was generated using the Kaplan-Meier method and compared using the long-rank (Mantel-Cox) test.