Comment: “The main goal of the authors was to characterize CIMV-MSCs isolated from human adipose-derived MSCs in particular regarding their cytokine content and proteome. One of the major limitations of the study lies on the number of biological replicas. Given the well-know donor variation for MSCs it is paramount to validate the results in at least two more donors.”

Reply: MSCs were isolated from five donors. Based on the morphological and immunophenotype analysis three cultures of human MSCs (passage 4) brightly expressing surface markers (CD90+, CD44+, CD29+, CD73+, STRO-1+) were chosen for the subsequent analysis. In the proteome data (SI Table 1., 2) proteins which were found and confirmed in all 3 repeats was presented. Cytokine content was analysed using 3 biological repeats (MSCs culture from 3 donors). As suggested by the reviewer, we have added the statistical analysis in Fig. 4 and Fig. 5. The error bars represent the mean ± SD.

Comment: “Line 19: mesenchymal stem cells should be mesenchymal stem/stromal cells. Moreover, in the case of the results described herein, the authors should refer to the cells as human adipose-derived mesenchymal stem/stromal cells.”

Reply: We agree that full name of the MSCs is mesenchymal stem/stromal cells. As suggested by the reviewer, we have edited the “mesenchymal stem cells” on “mesenchymal stem/stromal cells” and “adipose-derived mesenchymal stem/stromal cells”.

Comment: “Line 43: I suggest to focus on the “stem” cells used in the study which are MSCs and not other stem cell types such as ES or even iPS.”

“Line 45: reference 2 refers to foetal stem cells and not really MSCs and this could be misleading. I suggest to remove this reference.”

Reply: As suggested by the reviewer, we have specified the type of SCs mostly used in the regenerative medicine - MSCs and removed the reference relevant to fetal stem cells.

Comment: “Line 45: maldifferentiation if probably too strong based on the references (they only show that the cells differentiate into one but not another lineage and they did not test their differentiation potential extensively. Replace with limited differentiation potential sounds more appropriate.”

Reply: As suggested by the reviewer, we have replaced the term “maldifferentiation” with the “differentiation in unwanted direction”.

Breitbach et al. showed unwanted or uncontrolled differentiation of mesenchymal stem cells and whole bone marrow (BM) cells in osteogenic direction after the injection in the infarcted hearts in mice (Breitbach et al. // Blood. 2007. V.110.).

Kunter et al. showed unwanted differentiation of mesenchymal stem cells in adipogenic direction after intrarenal injection in rats. Kunter et al. used term "maldifferentiation" (Kunter et al. // Journal of the American Society of Nephrology. 2007. V.18.).
Comment: “Line 45: application is misspelled.”
Reply: We have edited the typos.

Comment: “Line 46: provide examples that support the use of EVs in detriment of the producing cells. It is also necessary to provide more info regarding the different vesicles. For example, in line 47 the authors refer to EV but Line 50 already refer to micro vesicles.”
Reply: As suggested by the reviewer, we have expanded the Introduction section and added the examples that support the use of EVs instead of cells (References #8,9,13,14). We have also added the information about the three different populations of vesicles (Lines 62-66). We used the term “microvesicles” instead of “EVs” due to namely microvesicles “retain the surface proteins of parental cells” (Line 68).

Comments: “Line 75: provide more details: size of the pieces, weight of the pieces used for digestion and final volume used for digestion. After 1h, the suspension was filtered or simply centrifuged?”
“Line 78: mention seeding density”
Reply: We have added the required details and clarified this point (Lines 94-104). The suspension was first centrifuged and then filtrated through a cell strainer (40 µm). The seeding ratio for solid adipose tissue was 175 cm2 surface area/10–15 ml of adipose tissue.

Comment: “Line 80: replace directions by lineages. Provide the composition of the different differentiation media and the seeding density and culture time for the different differentiation protocols.”
Reply: As suggested by the reviewer we have replaced the “directions” by “lineages” and clarified the differentiation protocol (Lines 105-115).

Comment: “Line 91: provide details regarding the culture conditions (seeding density for MCS, for how long the cells were grown before adding cytochalasin-B, were they trypsinised after the cytoD and if so for how long….)”
Reply: We have provided required details (Lines 123-126).

Comment: “Line 103: independent batches from independent donors or from the same donor?”
Reply: As suggested by the reviewer we have clarified this point (Line 138). For the size analysis, three independent batches of CIMVs-MSCs (MSCs were obtained from three donors) were produced.
Comment: “Line 106: how many CIMVs-MSCs and how many MSCs?”
Reply: We have added this information (Lines 142, 144). CIMVs derived from $3 \times 10^6$ MSCs and $1 \times 10^6$ MSCs were lysed in RIPA buffer, then 20 μg of total protein was loaded per well of gel electrophoresis.

Comment: “Line 111: concentration of trypsin used.”
Reply: We have added required information (Line 148). Gel fragments were rehydrated (200 mM ammonium bicarbonate, 100% acetonitrile, dH2O), placed in sequencing grade-modified trypsin (working concentration was 20 ng/μl) (Promega, USA) and incubated overnight at 37 °C.

Comments: “Line 113: “by adding” should be replaced “by the addition” Line 250: correct the units for DiD labeled CIMVs-MSCs.”
Reply: We have edited the typos.

Comment: “Line 128: given the fact that FBS was used for the expansion of the cells the authors should mention if they used FBS-depleted from EVs or they should also consider the bovine database for their analysis.”
Reply: The protocol of CIMVs production does not require the culture medium centrifugation. Donor MSCs were first washed twice with PBS from the culture medium, maintained in DMEM without serum supplemented with 10 μg/ml of Cytochalasin B (Sigma-Aldrich, USA) for 30 min (37°C, 5% CO₂). After the sequential centrifugation the pellet from last step, containing CIMVs-MSCs, was washed once in PBS.

Comment: “Line 140: How much protein was used?”
Reply: We have added required information (Line 177). Equal protein load (25 μg) was used for the analysis.

Comment: “Line 190: mention in the legend the staining used for each figure.”
Reply: The required information have been added (Lines 232-233).

Comment: Line 258: provide more details in the legend. For example, 5B and 5F represent what? 5E?? For the FACS plots show the controls (unstained cells, single labeled cells). Based on the figure it does not seem that the cells have homogeneous distribution of CD90.
Reply: As suggested by the reviewer we have added missing details in the legend of the Fig.5. The distribution of CD90 on the surface of HEK293FT cells treated with CIMVs-MSCs is not homogeneous. This is due to the presence of CD90 exclusively in the membrane of CIMVs-MSCs. Recipient cells (HEK293FT) treated with CIMVs acquired CD90 positive phenotype due to the
internalization of CIMVs-MSCs membrane into the cytoplasmic membrane of the recipient cells. Therefore HEK293FT showed partial staining with CD90 and blue fluorescence.

**Comment:** Line 282: quantification of blood vessels should within the plug should be done with CD31 antibody or another endothelial specific marker. Explain why immune-competent rats were used given the fact that you transplanted human MSCs…

**Reply:** The classical histological method was used for the quantification of blood vessels which is in use up to now. Hematoxylin and Eosin staining allow to visualize whole tissue structure. Blood vessels were counted based on the presence of erythrocytes in the vessel lumen (Yi et al. // BMC Bioinformatics. 2018. V.19.).

Immunocompetent animals were taken in the experiment due to the 1) low immunogenicity of MSCs (human and rat MSCs do not express class II MHC excluding them as antigen presenting cells to T CD4+ lymphocytes), 2) MSCs are able to reduce the immune response (Rossignol et al. // J Cell Mol Med. 2009. V.13.); 2) duration of the experiment was short and specific immunity on the introduced cells was not developed (Isakova et al. // PLoS One. 2014. V.9.).

**Comment:** Line 315: Re-write the sentence (there’s a “.” in the middle and revise the structure of the sentence. There are other studies, not specifically focus in cytokines, for EV proteome analysis…

**Reply:** As suggested by the reviewer we have edited this part and typos. We agree with the reviewer that we did not mention previous findings in the field of natural EV proteome analysis. This is due to the fact that CIMVs are different from natural EVs. It is known that the mechanism of EVs release provide sorting of bioactive molecules inside of EVs (Yuana et al. // Blood Reviews. 2013. V.27.) (Zhang et al. // Genomics Proteomics Bioinformatics. 2015. V.13.). The CIMVs production protocol does not provide the sorting of molecules inside of CIMVs. CIMVs enclose part of parental MSCs cell cytoplasm. Due to this reason in the present work we compared the cytokine content of CIMVs with parental MSCs.