## Supplementary Data for

# Cellular and Molecular Life Sciences

## Circulating cardiomyocyte-derived extracellular vesicles reflect cardiac injury during systemic inflammatory response syndrome in mice

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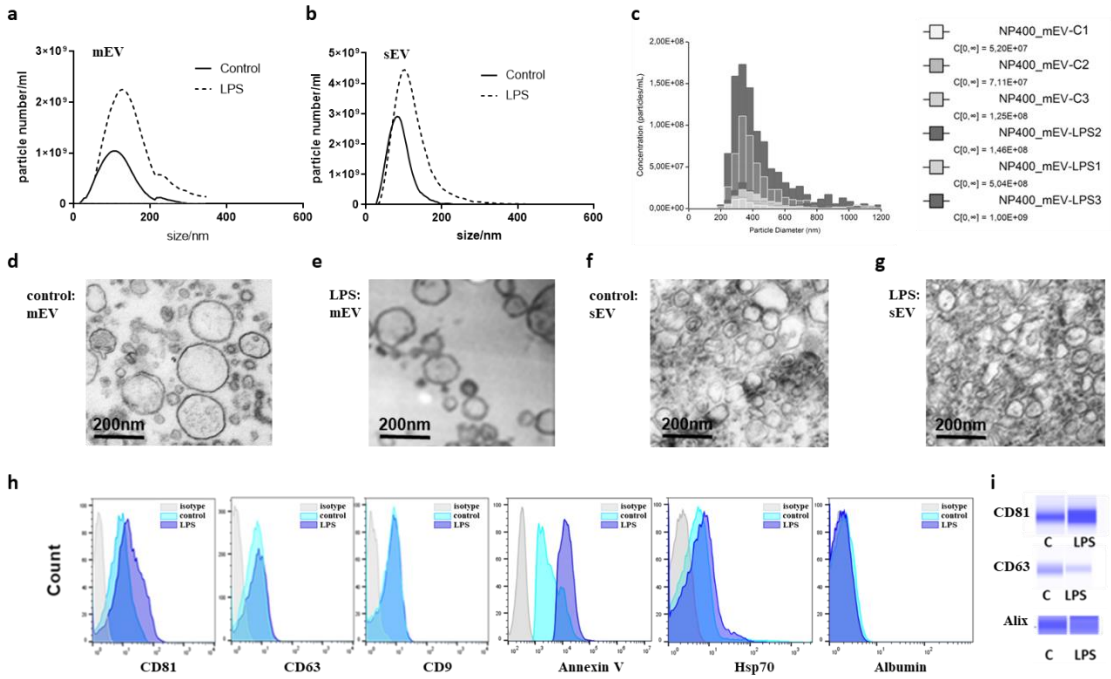
**Supplementary Table I. List of antibodies used for immunofluorescence studies**

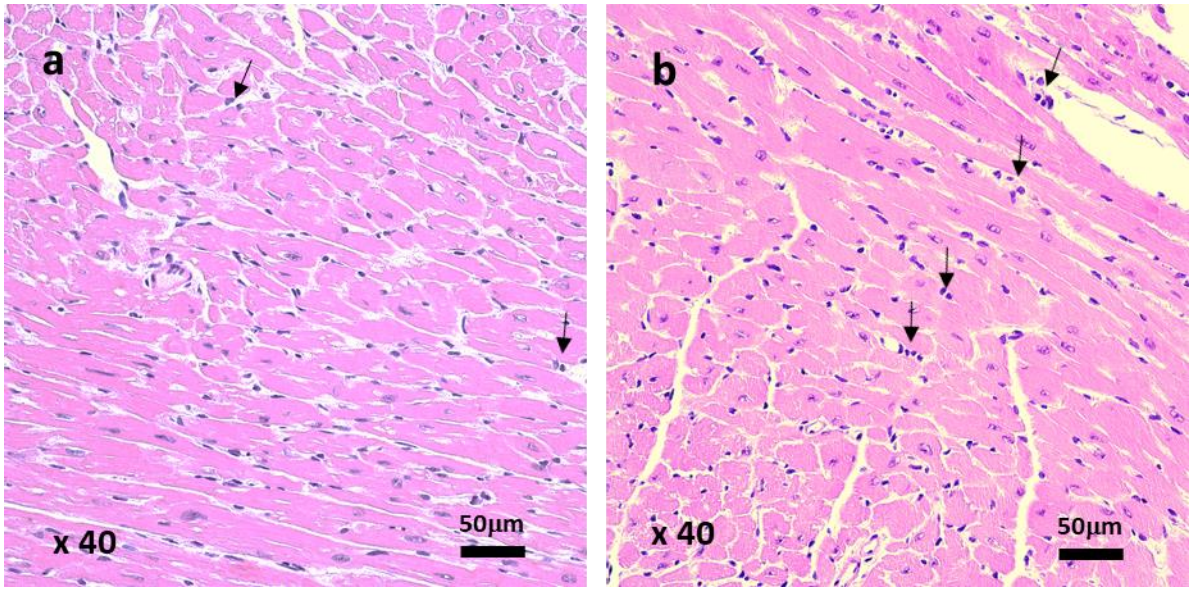
| Antibodies | Conjugate | Supplier | Clone | Species | Dilution |
|---|---|---|---|---|---|
| Albumin | No | abcam | ab207327 | rabbit | 1:100 |
| HSP-70 | No | invitrogen | Ma3-006 | mouse | 1:100 |
| PYGM | FITC | Biorbyt | orb9674 | rabbit | 1:100 |
| PYGM | No | FineTest | FNab06977 | rabbit | 1:100 |
| CPN1 | No | AntibodyGenie | CAB78887 | rabbit | 1:100 |
| CLU | No | AntibodyGenie | CAB12913 | rabbit | 1:100 |
| CD63 | APC | Sony | 1319525 | rat | 1:100 |
| CD63 | PE | Sony | 143903 | rat | 1:100 |
| CD9 | APC | Sony | 1224060 | rat | 1:100 |
| CD81 | PE | Sony | 1124530 | hamster | 1:100 |
| CD63 | No | Santa Cruze | sc-15363 | rabbit | 1:50 |
| CD81 | No | Sigma | SAB3500454 | rabbit | 1:50 |
| alpha-actinin | FITC | Frank | 106936 | human | 1:1000 |
| cTnI | biotin | Abbexa | abx272698 | rabbit | 1:100 |
| Alix | No | Sigma | SAB4200477 | rabbit | 1:50 |
| Caveolin-3 | No | ThermoFisher | PA1-066 | rabbit | 1:100 |
| Rabbit IgG | APC | abcam | ab232814 | rabbit | 1:100 |
| Mouse IgG1 | FITC | Sony | 2630005 | mouse | 1:500 |
| Armenian hamster | APC | Sony | 2604635 | hamster | 1:100 |
| Rat IgG2a | APC | Sony | 2603115 | rat | 1:100 |
| Rat IgG1 | AlexaFluor-647 | Sony | 2602090 | rat | 1:100 |
| REA control | FITC | Frank | 104611 | human | 1:100 |
| Rabbit IgG H&L | Cy2 | abcam | ab6940 | goat | 1:1000 |
| Rabbit IgG H&L | APC | ThermoFisher | 31984 | goat | 1:500 |
| Rabbit IgG H&L | AlexaFluor-647 | abcam | ab150079 | goat | 1:500 |
| Mouse IgG | FITC | invitrogen | AB_2536524 | goat | 1:1000 |
| Mouse IgG | HRPO | invitrogen | AB_2536527 | goat | 1:10000 |
| Rat IgG | HRPO | invitrogen | AB_228356 | goat | 1:10000 |
| streptavidin | APC | Sony | 2626215 | | 1:1000 |
| AnnexinV | FITC | Sony | 3804530 | | 1:500 |

**Supplementary Figure 1 Validation of the EVs based on MISEV proposal**

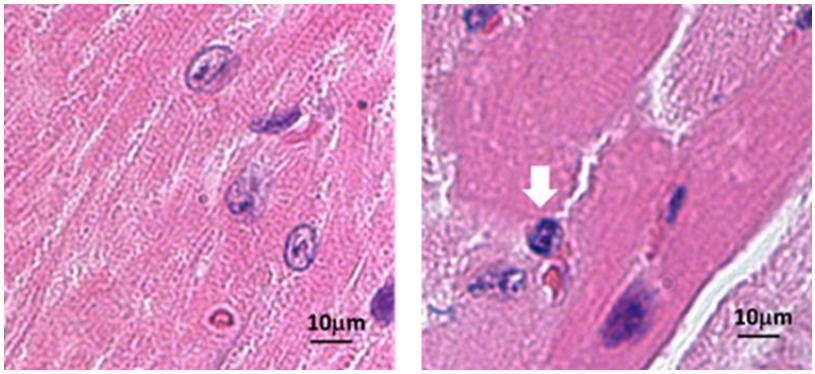



**Supplementary Fig. 1** Characterization of the mEVs by the MISEV2018 guidelines [3]
**a, b** shows concentration and particle sizes of platelet-free plasma EV fractions measured by NTA. **c** Size distibution of a 3-3 samples before and after LPS injection determined by qNano. **d-g** TEM images of control and LPS-challenged samples for both mEV and sEV fractions; **h** Flow cytometry histograms for five positive and one negative EV markers: light grey indicates the isotype control, light blue is the control, and violet shows the LPS group. **i** WES detection of three EV markers CD81= 22kDa, CD63= 30-65kDa, Alix= 95-105kDa.

**Supplementary Figure 2. Effect of LPS on inflammatory cell infiltration**




Heart histopathology H&E staining. Panels **a** on the left panel is example of control heart and on the right **b** is the images of the LPS group. Inflammatory cells pointed with black arrows. Magnification 40x (Eclips, Nikon).




Representative images from formalin-fixed and paraffin-embedded sections (4-µm thickness) of mouse hearts stained with H&E. Twenty four h following LPS injection, diffuse leukocyte infiltration was observed in the hearts. The white arrow points to a granulocyte (objective magnification was 40 X and optical magnification was further 5X).

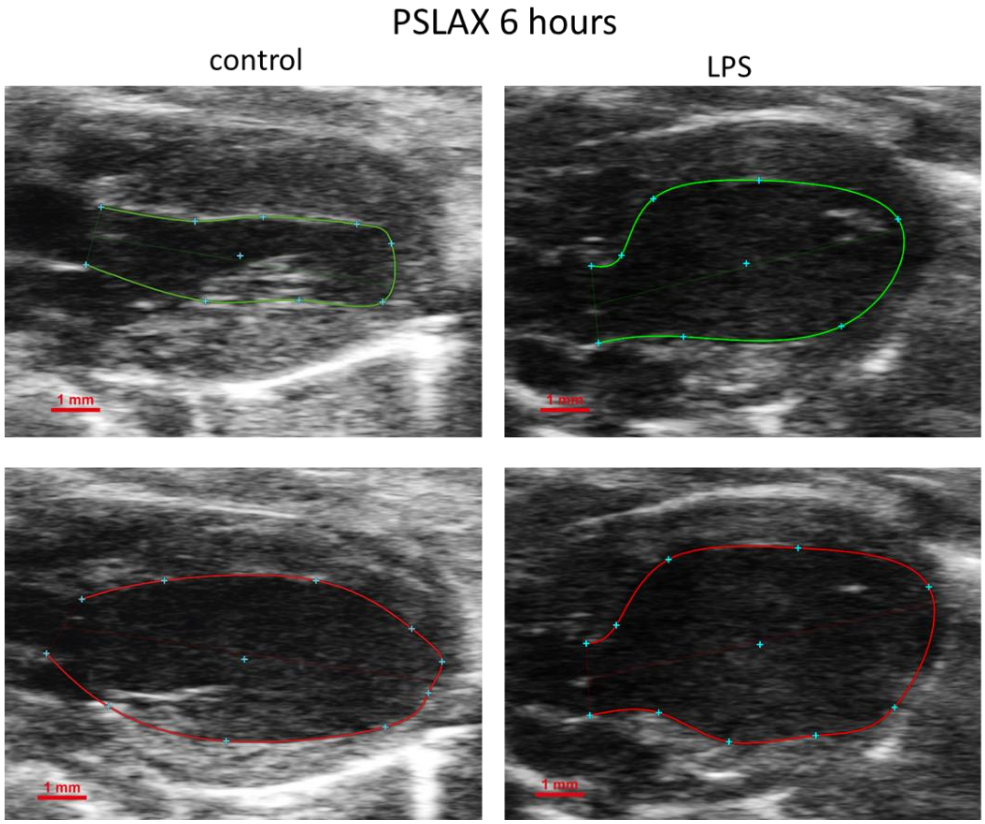
**Supplementary Table 2.**

**Echocardiographic evaluations were performed one day before (baseline), 6 and 24 hours after intraperitoneal injection of LPS or NaCl (n= 6 mouse/group).**

| | Baseline | | | | | | 6 hours | | | | | | 24 hours | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Vehicle | | | LPS | | | Vehicle | | | LPS | | | Vehicle | | | LPS | | |
| **BW (g)** | 25,34 | ± | 1,89 | 23,45 | ± | 4,01 | 25,21 | ± | 1,65 | 22,64 | ± | 3,72 | 25,19 | ± | 1,76 | 20,97 | ± | 3,62 |
| **HR (b.p.m.)** | 529,31 | ± | 23,43 | 508,37 | ± | 188,43 | 535,08 | ± | 27,76 | 516,48 | ± | 186,74 *# | 505,89 | ± | 47,05 | 491,05 | ± | 174,29 |
| **LV mass (mg)** | 97,35 | ± | 26,25 | 95,14 | ± | 31,10 | 100,70 | ± | 28,67 | 107,39 | ± | 44,99 | 115,18 | ± | 22,50 | 90,91 | ± | 28,14 |
| **LVPWd (mm)** | 0,84 | ± | 0,07 | 0,77 | ± | 0,28 | 0,81 | ± | 0,10 | 1,12 | ± | 0,49 *# | 0,91 | ± | 0,07 | 0,83 | ± | 0,30 |
| **LVPWs (mm)** | 1,38 | ± | 0,18 | 1,21 | ± | 0,42 | 1,31 | ± | 0,14 | 1,32 | ± | 0,57 | 1,35 | ± | 0,09 | 1,22 | ± | 0,43 |
| **LVAVd (mm)** | 1,03 | ± | 0,15 | 0,96 | ± | 0,33 | 1,03 | ± | 0,25 | 0,92 | ± | 0,27 | 1,09 | ± | 0,12 | 0,95 | ± | 0,33 |
| **LVAVs (mm)** | 1,72 | ± | 0,17 | 1,47 | ± | 0,50 | 1,64 | ± | 0,23 | 1,20 | ± | 0,40 *# | 1,77 | ± | 0,20 | 1,53 | ± | 0,55 |
| **LVIDd (mm)** | 3,56 | ± | 0,49 | 3,74 | ± | 1,26 | 3,70 | ± | 0,47 | 3,49 | ± | 1,24 | 3,74 | ± | 0,35 | 3,46 | ± | 1,21 |
| **LVIDs (mm)** | 1,96 | ± | 0,57 | 2,25 | ± | 0,69 | 2,19 | ± | 0,56 | 2,81 | ± | 1,03 | 2,20 | ± | 0,35 | 2,03 | ± | 0,68 |
| **LVESV (µL)** | 16,46 | ± | 7,71 | 19,96 | ± | 7,21 | 23,40 | ± | 11,62 | 32,16 | ± | 13,90 | 21,13 | ± | 11,41 | 19,34 | ± | 8,00 |
| **LVEDV (µL)** | 47,69 | ± | 17,18 | 51,76 | ± | 15,48 | 58,17 | ± | 16,25 | 48,01 | ± | 17,21 | 55,26 | ± | 12,95 | 46,81 | ± | 15,86 |
| **FS (%)** | 45,81 | ± | 7,38 | 39,86 | ± | 13,13 | 41,47 | ± | 7,55 | 20,41 | ± | 8,09 *# | 41,27 | ± | 4,53 | 41,44 | ± | 14,33 |
| **SV (µL)** | 31,23 | ± | 9,74 | 31,80 | ± | 9,72 | 34,77 | ± | 7,21 | 15,86 | ± | 5,62 *# | 34,13 | ± | 4,00 | 27,47 | ± | 9,35 |
| **CO (mL/min)** | 16,63 | ± | 5,66 | 16,08 | ± | 4,57 | 18,64 | ± | 4,21 | 8,26 | ± | 3,07 *# | 17,22 | ± | 2,13 | 13,43 | ± | 4,49 |
| **E (mm/s)** | 787,77 | ± | 75,51 | 818,23 | ± | 294,19 | 811,75 | ± | 65,57 | 565,91 | ± | 206,82 *# | 751,21 | ± | 100,20 | 657,59 | ± | 217,11 # |
| **IVCT (ms)** | 15,22 | ± | 2,74 | 12,41 | ± | 4,14 | 12,98 | ± | 3,25 | 16,69 | ± | 5,32 | 11,97 | ± | 3,99 | 12,38 | ± | 4,68 |
| **e' (mm/s)** | -26,87 | ± | 6,12 | -27,65 | ± | 12,98 | -26,36 | ± | 4,60 | -18,40 | ± | 9,68 # | -24,50 | ± | 5,42 | -18,25 | ± | 9,81 # |
| **E/e'** | -30,20 | ± | 5,01 | -29,65 | ± | 13,36 | -31,68 | ± | 6,74 | -31,54 | ± | 15,30 | -31,50 | ± | 5,69 | -38,11 | ± | 19,57 |

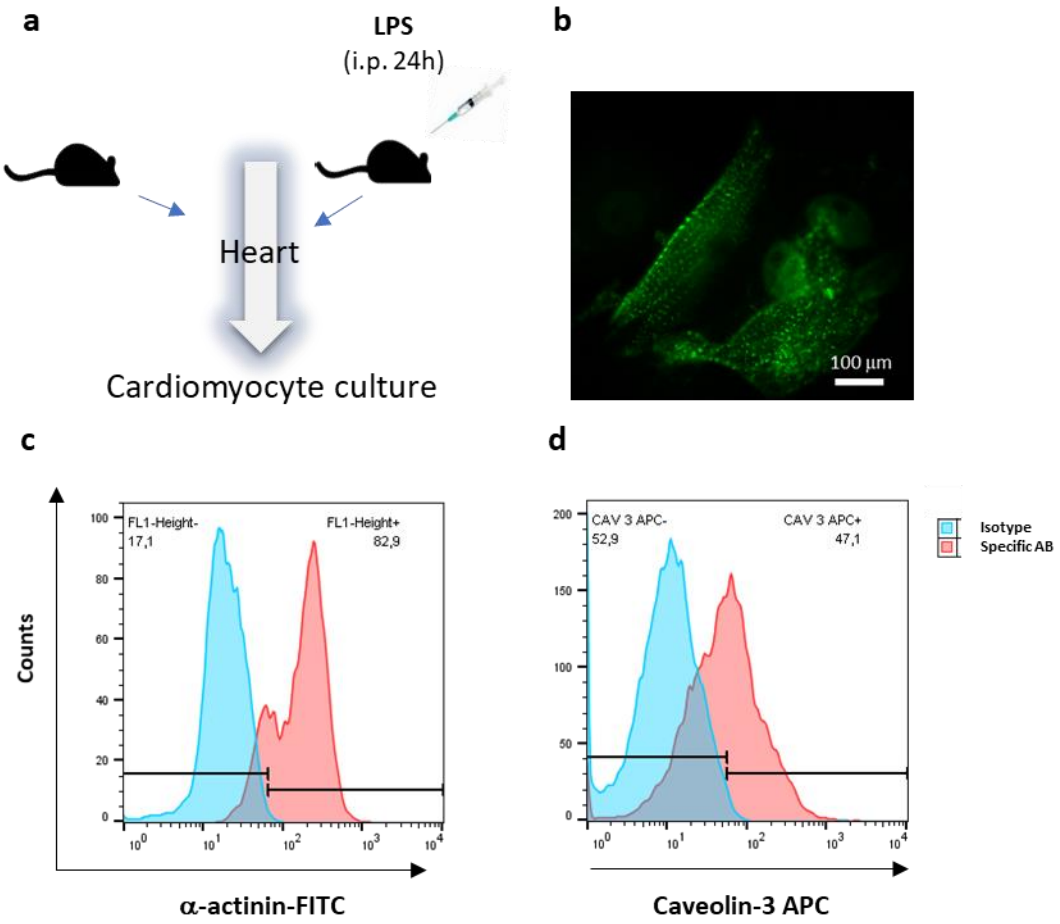
Two way anova with Tukey post-hoc test. * and # represents $p < 0.05$ significance, * VS Vehicle 6 hours, # VS LPS baseline

**Supplementary Figure 3. Representative frames from B-mode imaging (labeled PSLAX)**




**Supplementary Fig. 3** Representative frames from B-mode imaging (labeled PSLAX) are shown for the two (control, LPS ) groups.

**Supplementary Figure 4. Confirmation of the cardiomyocyte nature of the isolated cells**




Experimental setup is outlined on Panel (**a**). The microscopy (Eclipse E600, Nikon) image of immunfluorescent stained cultured cardiomyocytes are shown on (**b**); the green fluorescent staining portrays α-actinin-FITC. (**c,d**) representative flow cytometry histograms