

## Supplemental Material

### Cell culture

Cryopreserved iPSC-CPC were thawed and plated at a density of 78,000 cells/cm<sup>2</sup> on fibronectin-coated flasks (ref.: 11051407001, Roche Applied Sciences, Indianapolis, IN, USA) and cultured 4 days at 7% CO<sub>2</sub> and 37°C in serum-free media according to the proliferation protocol recommended by the manufacturer: William's E medium (ref.: A12176-01, Life Technologies, Saint Aubin, France) with Cocktail B from hepatocyte maintenance supplement pack (ref.: CM4000, Life Technologies), 25µg/mL Gentamicin (ref.: 15750, Life Technologies) and bFGF, 1µg/mL (Miltenyi, ref.: 130-093-842). The particle concentration in the media prior to exposure to cells (virgin media) was 4x10<sup>8</sup> particles/mL. During CPC culture, the media was refreshed on day 2, and at day 4, the conditioned media (containing the EV secreted over the previous 48 hours) was collected for EV isolation. The particle concentration in the conditioned media was 1000-fold higher than in the virgin media alone.

### Quantification and size distribution of EV

Nanoparticle tracking analysis (NTA) (NanoSight LM-14, Malvern) of EV-CPC preparation detected a poly-disperse population of particles ranging primarily from approximately 50 to 550 nm, with a small population at around 1000 nm. The majority of particles (~70%) in our preparations were in the size range of small particles (approximately 50–200 nm) as shown in Supplemental Figure 1b.

### Dose of EV-CPC

The doses used are expressed in different metrics according to MISEV guidelines. The EV-CPC secreted by an equivalent amount of 1.4x10<sup>6</sup> of CPC correspond to 1x10<sup>10</sup> (+/-3000) particles, as quantified by NTA and 100 µg of proteins, as measured by BCA (Thermo Scientific).

### Bioactivity assays for EV-CPC

Each batch of EV-CPC was tested *in vitro* using angiogenesis and viability assays described in our previous work <sup>6</sup> and which basically entail the measurements of scratch-induced endothelial cell wound healing and viability of H9c2 cardiomyoblasts stressed by serum deprivation, respectively.

### EV-CPC characterization

To test if the effect on the secretome of EV-CPC is not due to components of the cell culture media alone (composed by William's E medium; Cocktail B and human bFGF), we used the H9c2 cell viability assay described in our previous work <sup>6</sup>. As expected, the viability of stressed cells following their exposure to EV-CPC robustly outweighed those incubated in the presence of the media alone (362 % *vs.* 234%).

To separate the protein load co-purified with EV from EV, we used size exclusion chromatography (qEV isolation system, Izon Science, Oxford, UK) and assessed the bioactivity of the isolated fractions with the above-mentioned cell viability assay <sup>6</sup>. The EV –CPC + protein bulk yielded a viability of 398% compared to the negative control. This effect was decreased by 2.6-fold with the only EV-enriched fraction and 1.3-fold with the protein-enriched fraction, thereby supporting the use of the whole secretome to leverage the synergistic protection afforded by each fraction separately.

Concerning EV-CPC stability, we incubated EVs during 9 days at 37°C and tested their bioactivity at different time points: Day 0, Day 2, Day 6 and Day 9, using a cell viability assay previously described <sup>6</sup> and in which H9c2 cells are stressed by serum deprivation. Compared with the control, viability increased by 3.6-fold at Day 0; 2.8-fold at Day 2; 2.5-fold at Day 6 and 2.0-fold at Day 9.

EV-CPC did not express Calnexin, as expected for EV (data not shown).

## Supplemental Figures

**Supplemental Figure 1:** a) Fluorescence intensity of EV-CPC surface marker. EVs from 4 independent productions were captured by a set of 39 bead-linked antibodies, followed by staining with a cocktail of anti-CD9, anti-CD63 and anti CD81-APC antibodies. REA and mIgG1 indicate isotype control-beads, i.e. beads linked to an antibody which does not bind EV. CPC: iPS cell-derived cardiovascular progenitor cells; EV: Extracellular vesicles from iPS cell-derived cardiovascular progenitor cells. Data are presented as box-and-whisker plots, with the midline representing the median and the whiskers representing maximum and minimum values. b) Nanoparticle tracking analysis of EV-CPC preparation detected a poly-disperse population of particles ranging primarily from approximately 50 to 550 nm, with predominantly small particles.

**Supplemental Figure 2:** Circulating blood levels of inflammatory cells and cytokines in the chronic myocardial infarction model. Three weeks after MI, mice were untreated (n=5) or injected with PBS (n=6-8), EV (n=7), CPC (n=6) or LPS (n=4). Three days after injections, the plasma was processed by flow cytometry for the assessment of immune cells (a) and inflammatory mediators (b), using a luminex bead-based multiplex assay; untreated (n=5), PBS (n=4), EV (n=5), CPC (n=4) or LPS (n=3). Results are given as mean  $\pm$  SD. PBS: Phosphate-Buffered Saline; LPS: Lipopolysaccharide; other abbreviations as in Supplemental Figure 1. The histogram at the far left corresponds to the group of mice which underwent myocardial infarction but did not receive any treatment.

Wilcoxon signed rank test was used, a p-value  $<0.05$  was considered statistically significant. \*  $p \leq 0.01$ .

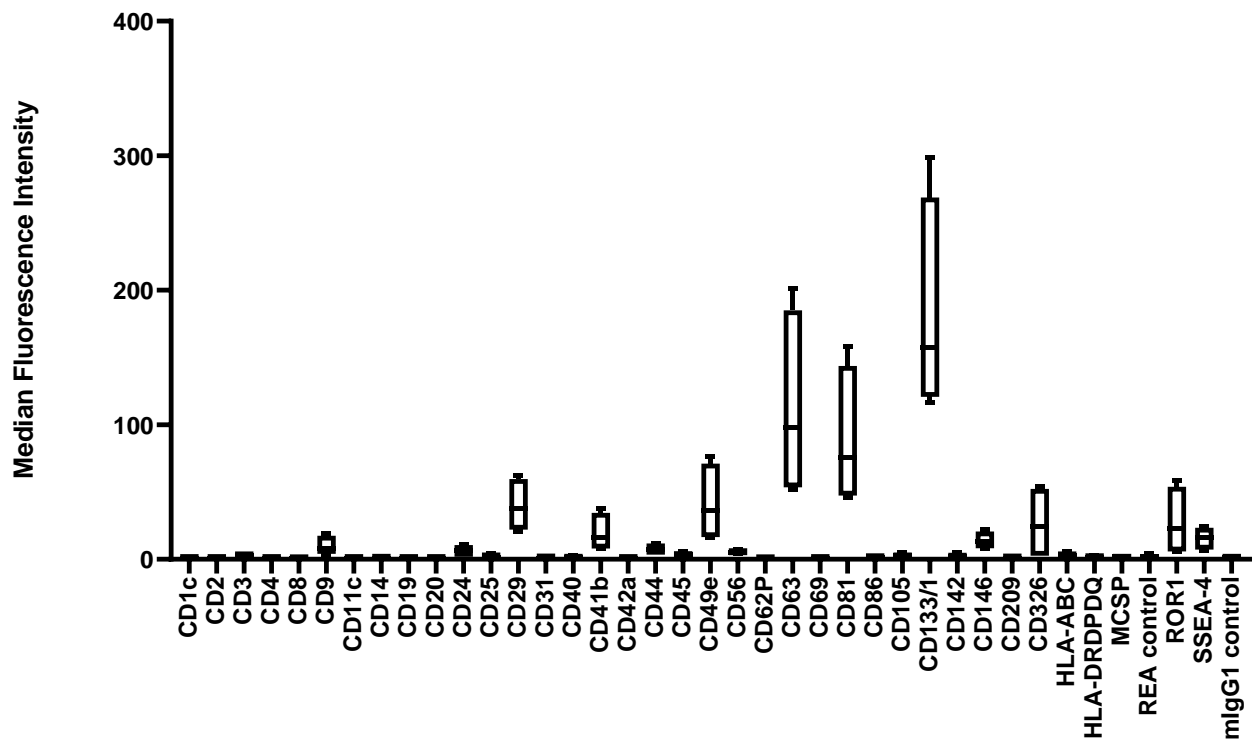
**Supplemental Figure 3:** Spleen and Bone marrow inflammatory cells in the chronic myocardial infarction model. a) Three weeks after MI, mice were untreated (n=5) or injected with PBS (n=6-7), EV (n=7), CPC (n=6) or LPS (n=4). Three days after injections, the spleen was processed by flow cytometry for the assessment of immune cells. The results represent the mean  $\pm$  SD of the number of cells/g of tissue. b) Three days after treatment, the bone marrow was collected flushing one femur of each mice and immune cells were analyzed by flow cytometry. Untreated (n=5), PBS (n=6-8), EV (n=7), CPC (n=6) or LPS (n=4) \*  $p \leq 0.05$ ; Same abbreviations as in Supplemental Figure 2. Wilcoxon signed rank test was used, a p-value  $<0.05$  was considered statistically significant. \* $p < 0.05$ ; \*\*  $p \leq 0.01$ .

**Supplemental Figure 4:** Spleen and Bone marrow inflammatory cell number in the acute myocardial infarction model. a) Two days after MI, mice were injected with PBS (n=6) or EV (n=6). Three days after injections, the bone marrow was collected by flushing one femur and processed by flow cytometry for the assessment of immune cells. b) Three days after injections, the spleen was processed by flow cytometry for the assessment of immune cells.

**Supplemental Figure 5:** Schematic representation of polarization of human macrophages into M0, M1 or M2 macrophages with or without exposure to EV-CPC. EV= Extracellular vesicles from iPS cell-derived cardiovascular progenitor cells.

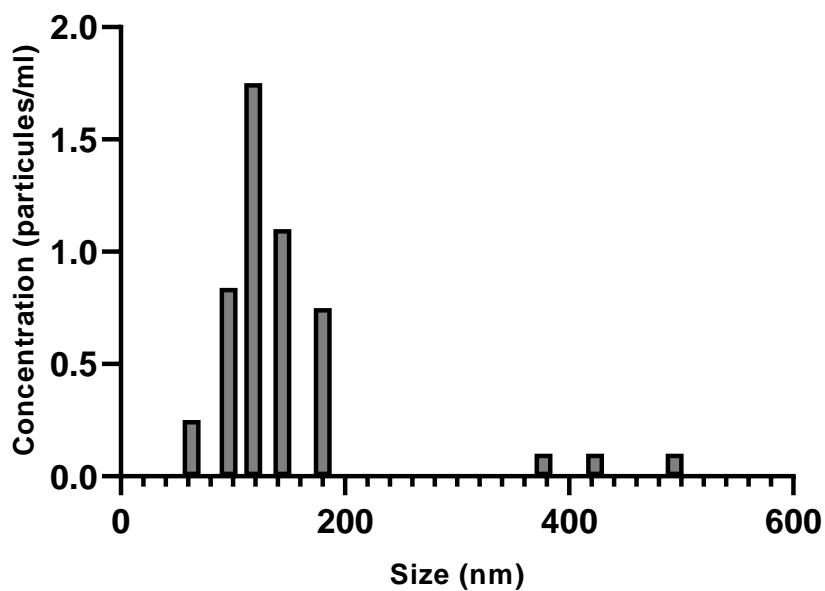
Supplemental Figure 1

a)



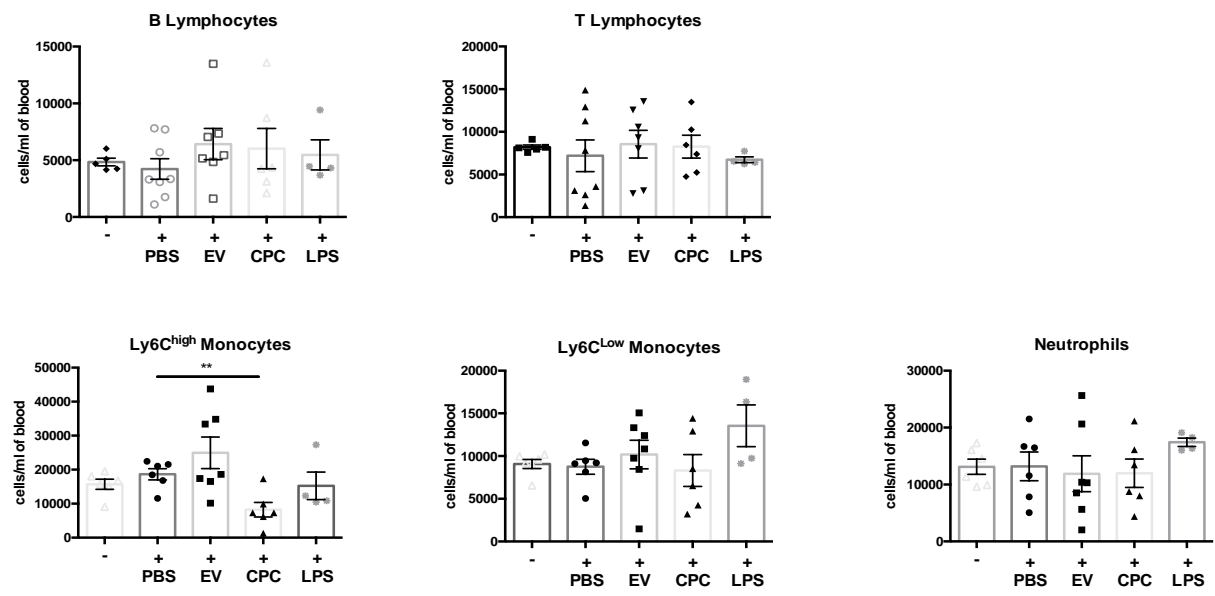
b)

### Nanoparticle tracking analysis (NTA)

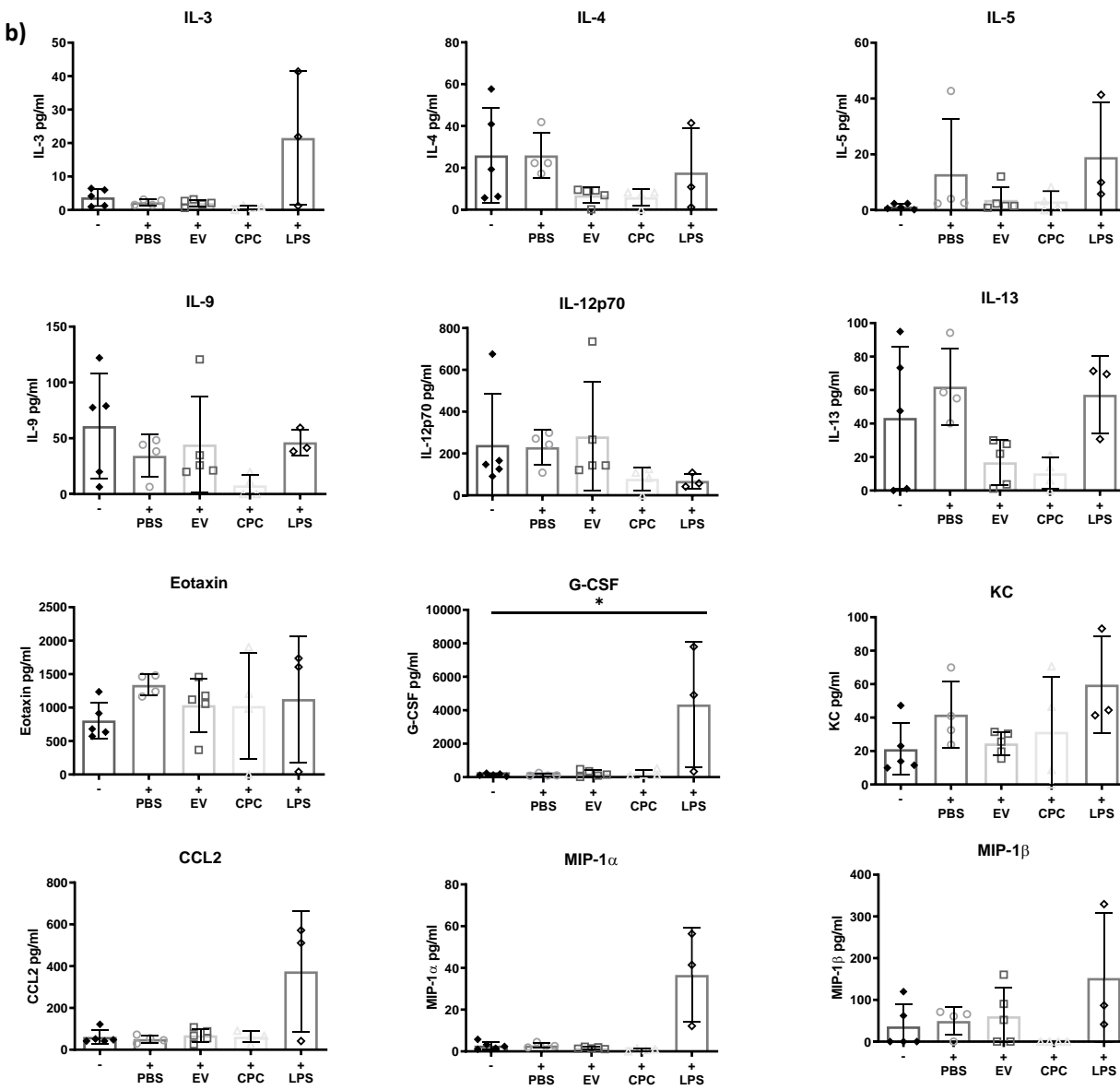


# Supplemental Figure 2

a)

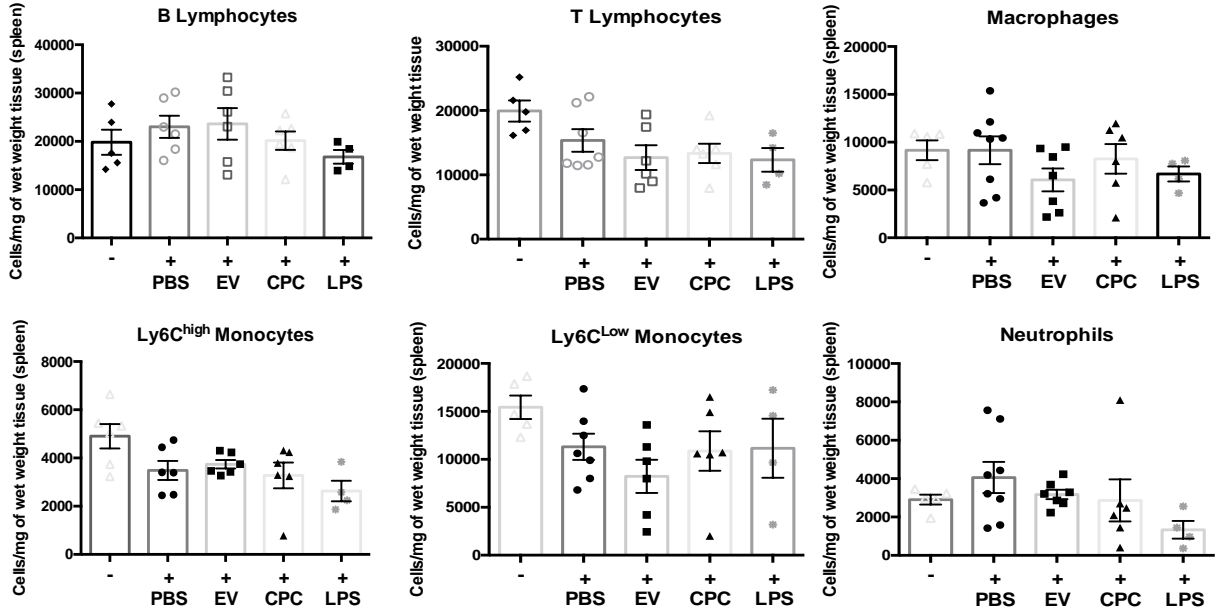


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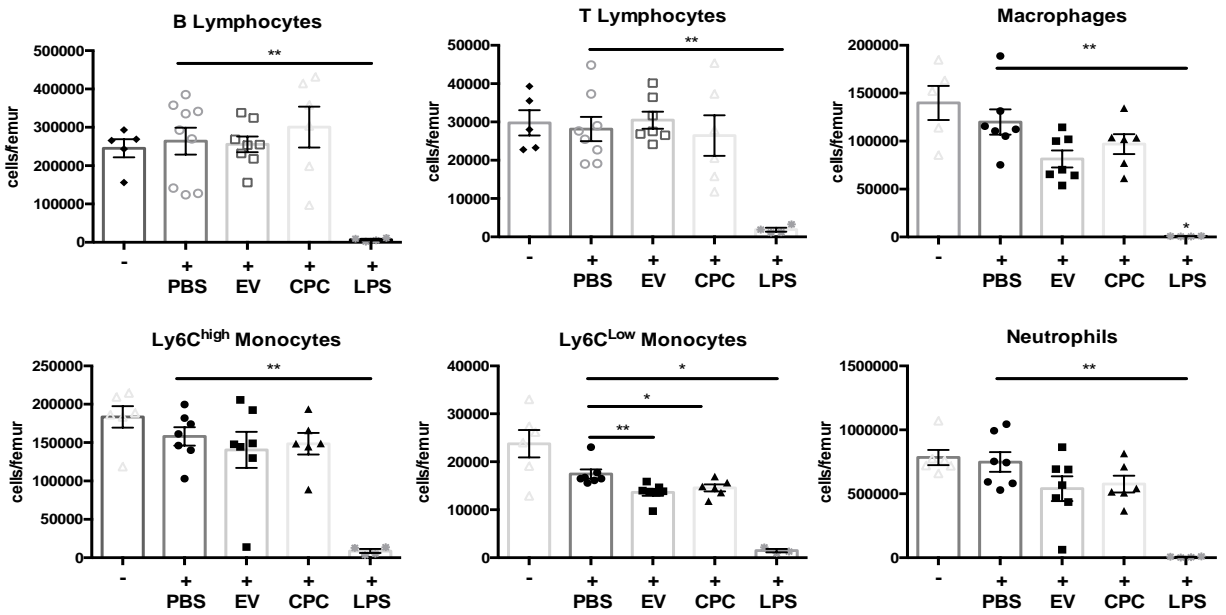


# Supplemental Figure 3

a)

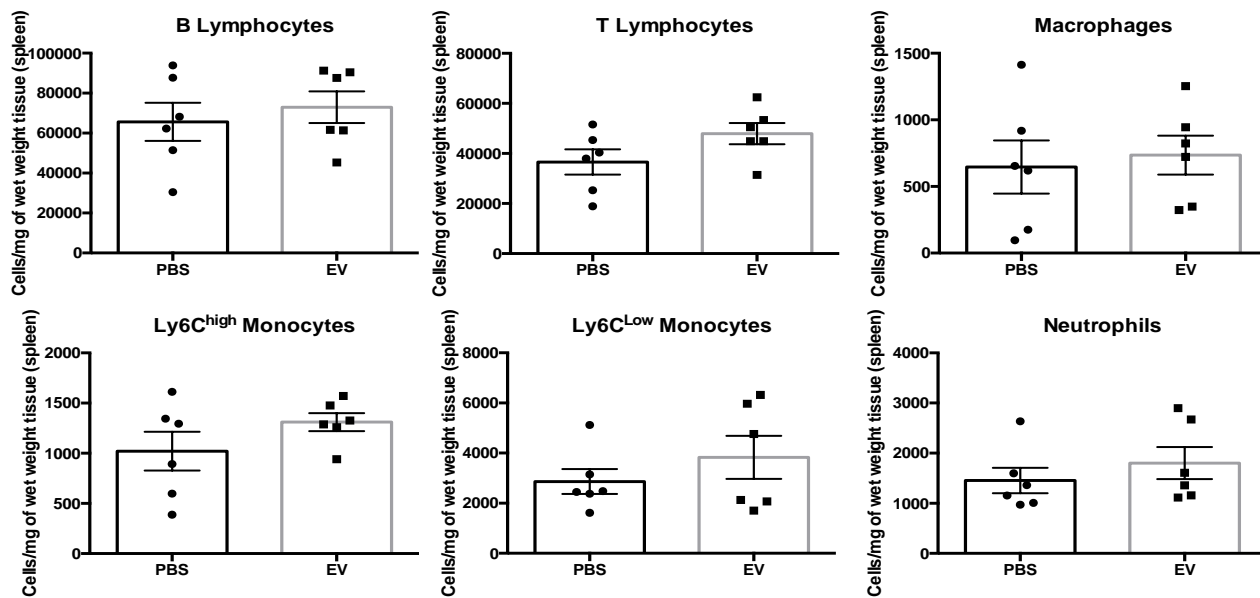


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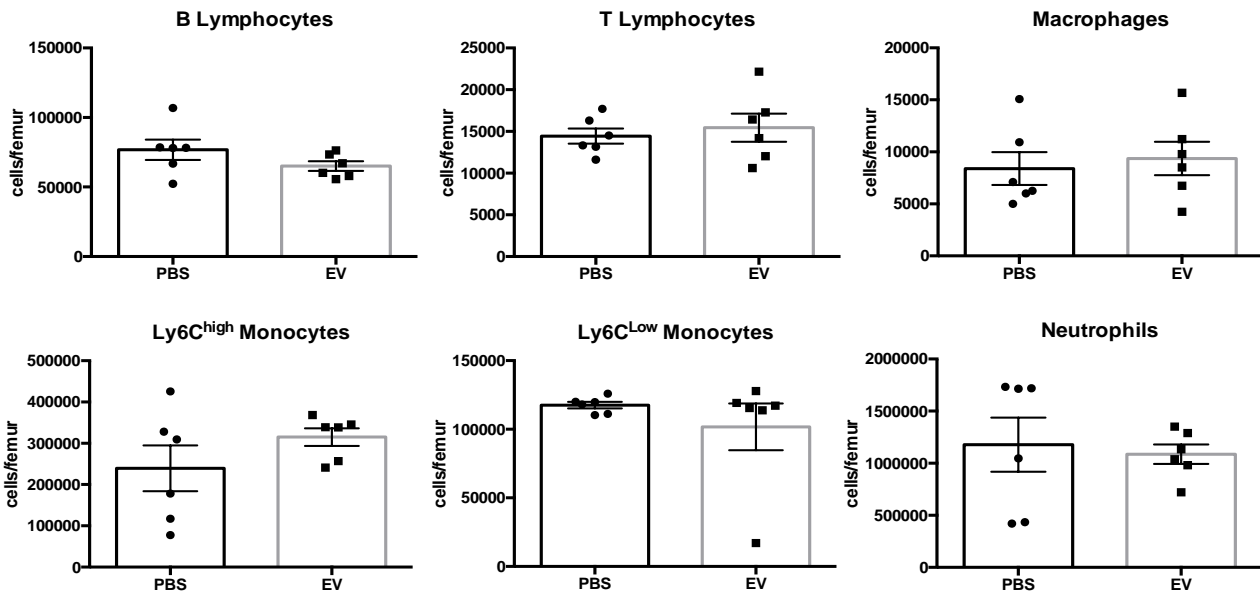


# Supplemental Figure 4

a)



b)



# Supplemental Figure 5

