

Supporting Information

Macrophages signal cross-talks under hyperglycemic conditions and their link to cyclophilins

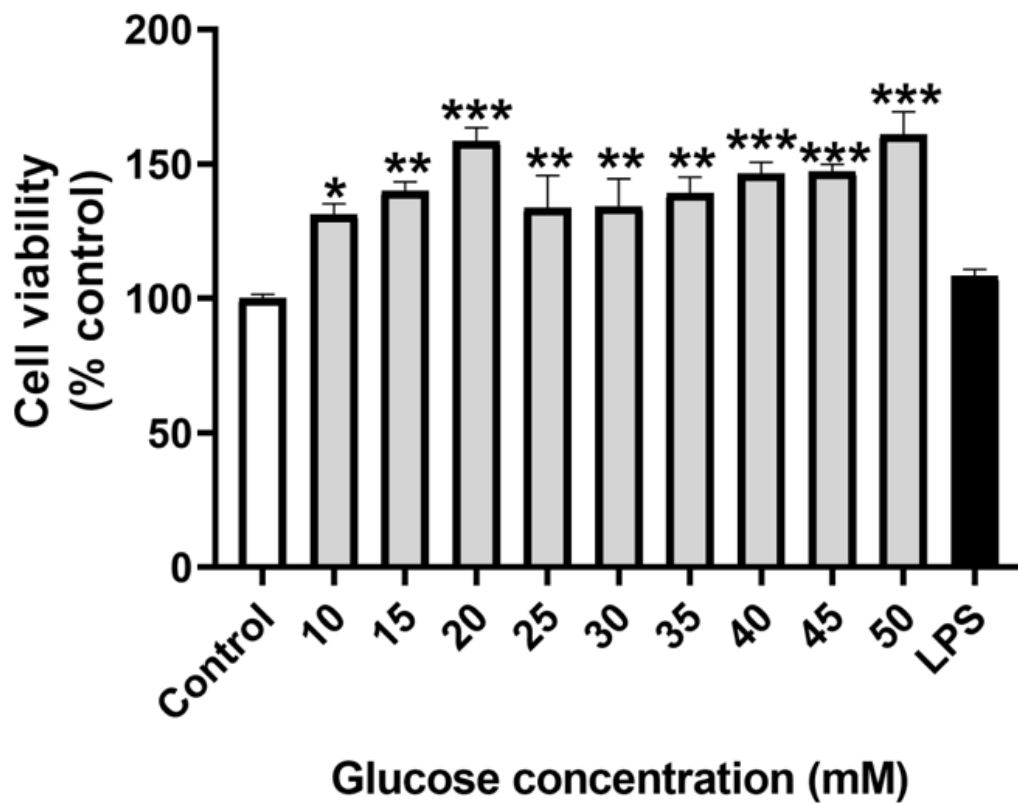
Noelia Castedo¹, Rebeca Alvariño², Amparo Alfonso^{1*}, Mercedes R. Vieytes², Luis M. Botana^{1*}

¹Departamento de Farmacología, Facultad de Veterinaria, IDIS, Universidad de Santiago de Compostela, Lugo 27002, España

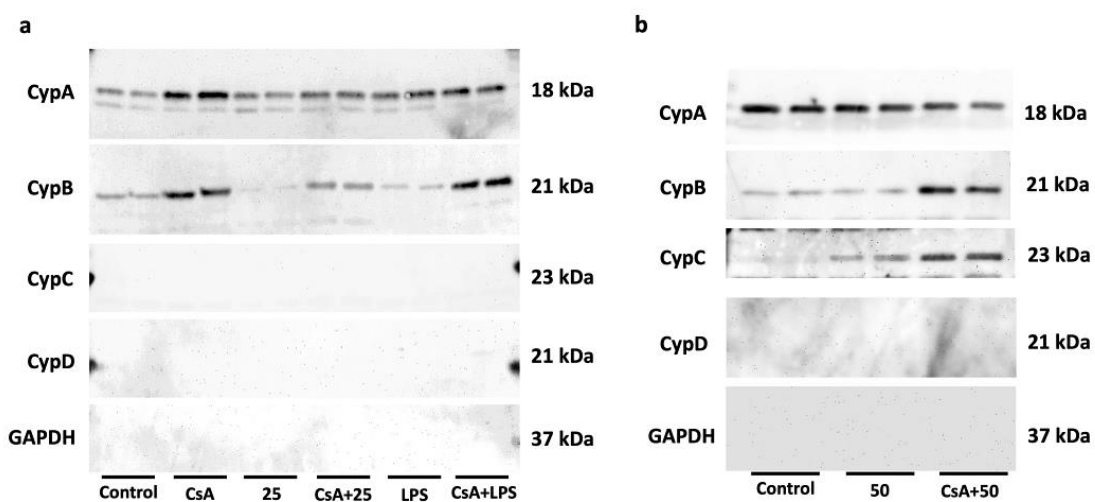
²Departamento de Fisiología, Facultad de Veterinaria, IDIS, Universidad de Santiago de Compostela, Lugo 27002, España

*Correspondence: amparo.alfonso@usc.es ; luis.botana@usc.es

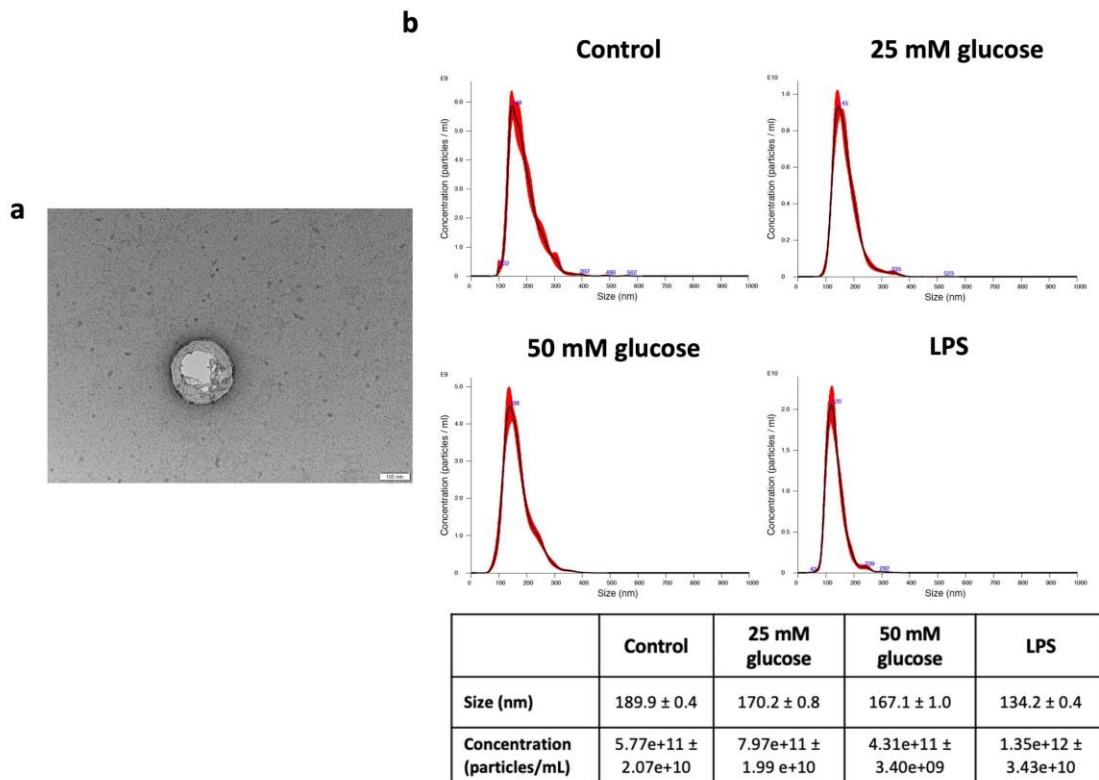
Keywords: Cyclophilins, Extracellular vesicles, Hyperglycemia, Inflammation, Macrophages.



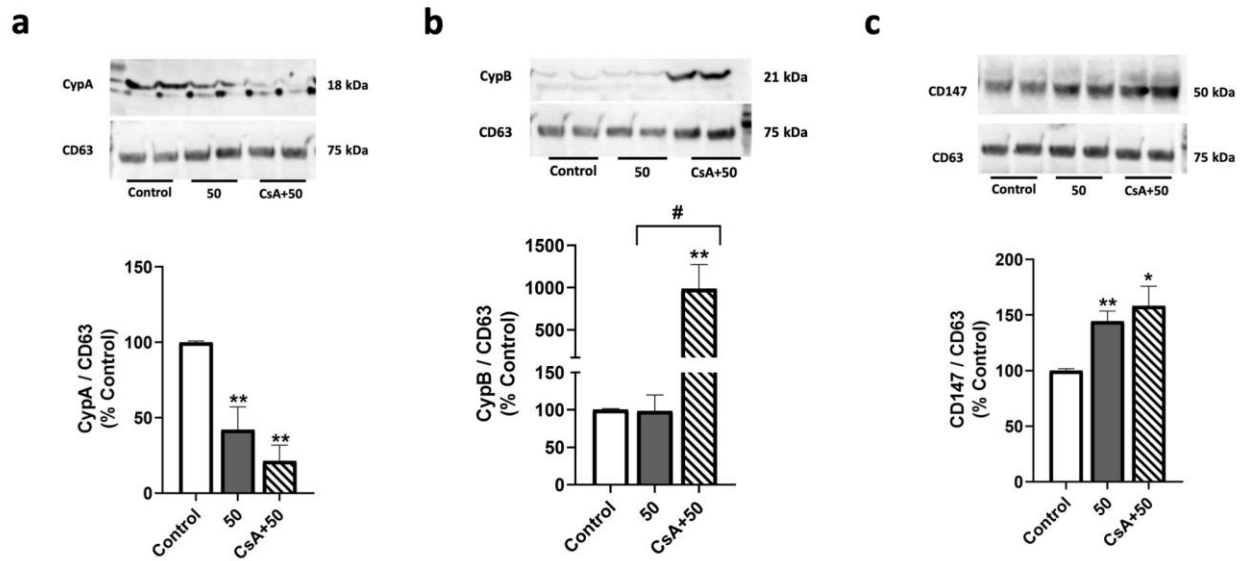
Supplementary Figure 1. Effect of HG treatment on RAW264.7 cells viability. Cells were stimulated with 25 mM and 50 mM glucose for 24 hours and pretreated with 1 μ M CsA for 1 hour, if applicable. LPS at 1 μ g/mL was used as positive control of inflammation. Data are mean $\pm$ SEM of three independent replicates performed by duplicate. Data are expressed as percentage of control cells (5.5 mM glucose). Statistical differences determined by One way ANOVA test and Tuckey's post hoc test. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to control cells.



Supplementary Figure 2. Cyts secretion to extracellular medium under inflammatory conditions. Cells were treated with 25 mM (a) and 50 mM (b) glucose for 24 hours and pretreated 1 hour with 1 μ M CsA if applicable. LPS at 1 μ g/mL was used as positive control. GAPDH was used as a negative control of cell supernatant samples.

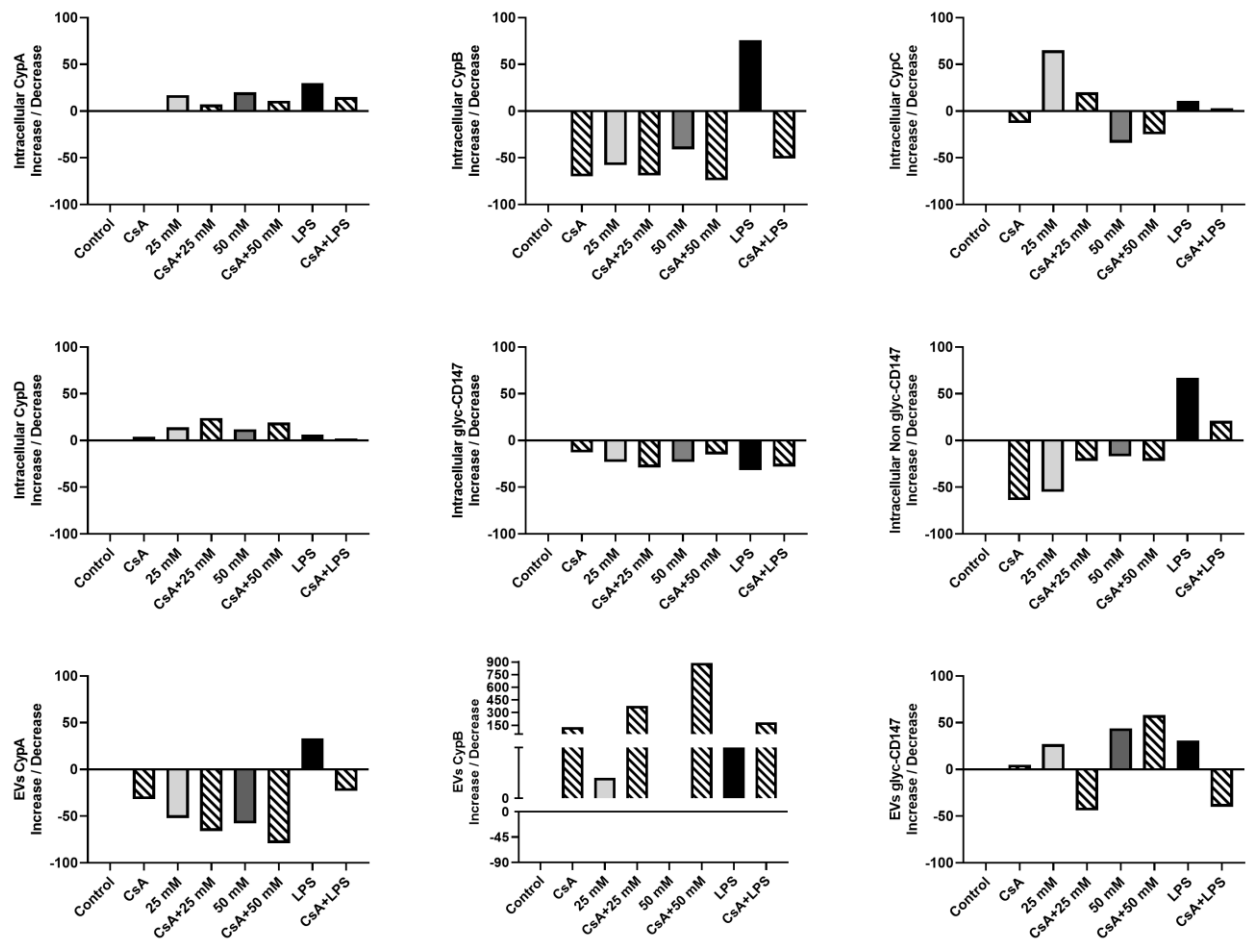


Supplementary Figure 3. Characterization of EVs derived from RAW264.7 macrophages. a) TEM imaging and b) Nanosight analysis of particle size distribution from control EVs and 25 mM glucose, 50 mM glucose and LPS treated EVs.



Supplementary Figure 4. Cyps content inside EVs derived from macrophages under HG conditions.

a) CypA, b) CypB and c) CD147 levels in EVs. Cells were stimulated with 50 mM for 24 hours and pretreated with 1 μ M CsA for 1 hour if applicable. CsA was used as internal control of Cyps route. Band intensity was normalized by CD63, a surface EVs protein. Data are mean $\pm$ SEM of three independent replicates. Data are expressed as percentage of control cells. Statistical differences determined by One way ANOVA test and Tuckey's post hoc test. * $p < 0.05$ and ** $p < 0.01$ compared to control cells. # $p < 0.05$ between groups.



Supplementary Figure 5. Summary of Cyps and CD147 expression in RAW264.7 cells and inside EVs derived from macrophages under all the treatment conditions. The bars represent the increase or decrease of protein expression compared to control cells, which represent a value of 0.