

## **Supplementary Materials**

### **Amniotic extracellular vesicles' effect on equine endometrial cells challenged with LPS: a proteomic analysis**

**Giulia Gaspari<sup>1,#</sup>, Alessio Soggiu<sup>2,#</sup>, Paola Gagni<sup>3</sup>, Salvatore Desantis<sup>4</sup>, Fausto Cremonesi<sup>1</sup>, Anna Lange-Consiglio<sup>1</sup>**

<sup>1</sup>Department of Veterinary Medicine and Animal Science (DIVAS), Laboratory of Reproduction and Regenerative Medicine, Università degli Studi di Milano, Lodi 26900, Italy.

<sup>2</sup>Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Università degli Studi di Milano, Milan 20133, Italy.

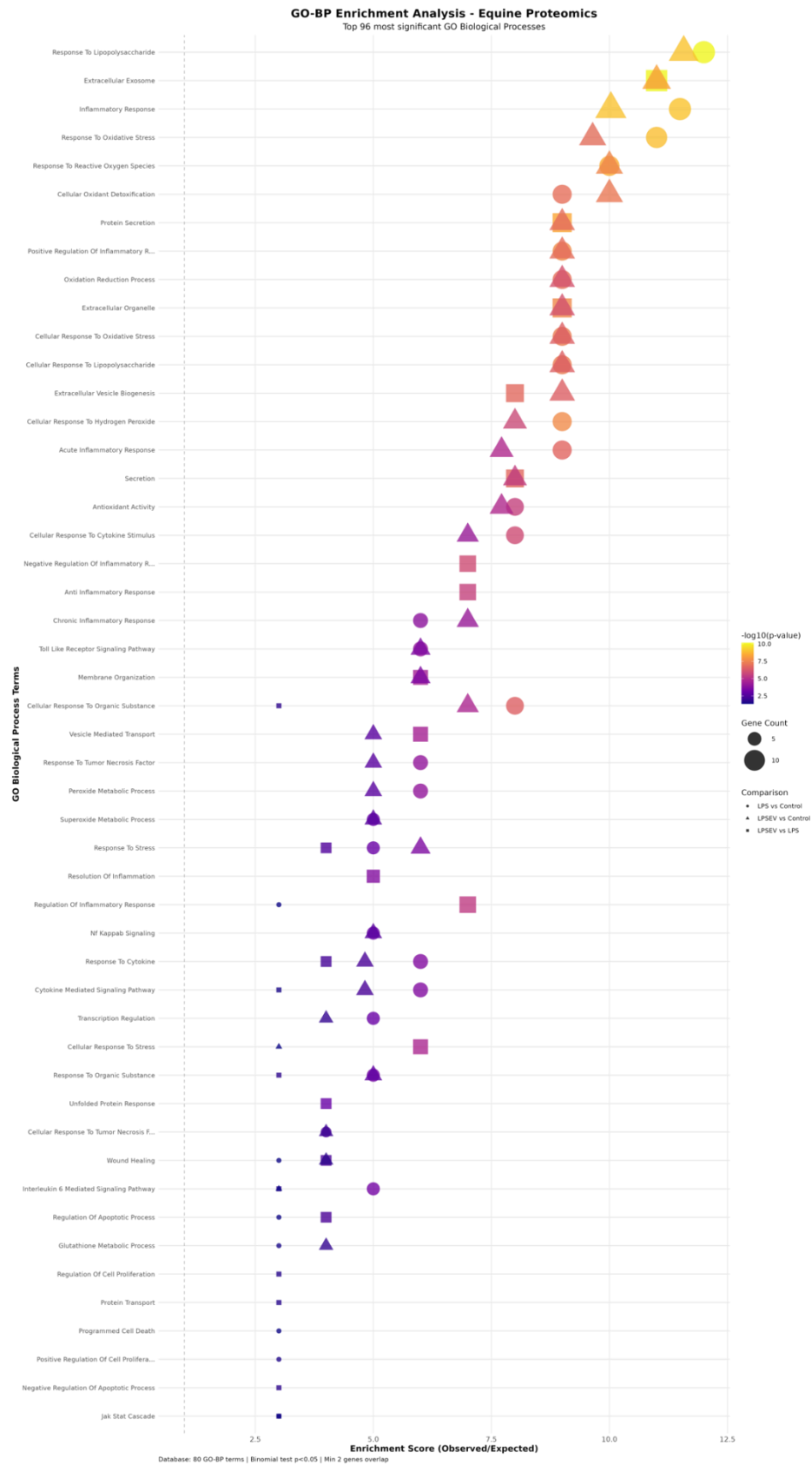
<sup>3</sup>Istituto di Scienze e Tecnologie Chimiche “Giulio Natta” (SCITEC), Consiglio Nazionale delle Ricerche (CNR), Milan 20133, Italy.

<sup>4</sup>Department of Regenerative and Precision Medicine-Jonian Area (DiMePRE-J), University of Bari Aldo Moro, Bari 70124, Italy.

<sup>#</sup>These authors contributed equally to this work.

**ORCID:** Anna Lange-Consiglio (0000-0002-7748-3413)

**Correspondence to:** Prof. Anna Lange-Consiglio, Department of Veterinary Medicine and Animal Science (DIVAS), Laboratory of Reproduction and Regenerative Medicine, Università degli Studi di Milano, Lodi 26900, Italy. E-mail: [anna.langeconsiglio@unimi.it](mailto:anna.langeconsiglio@unimi.it)



**Figure 1.** Most significant Gene Ontology Biological processes enriched among comparisons between LPS and control EC groups (circular dots), LPS/EV and control EC groups (triangular dots), LPS/EV and LPS groups (square dots).

**Heatmap Data Summary:**

- Y-axis (Genes):** 100 genes listed, including FBN1, GAT, ANKRD1, GSNAP2, VSN, FSN, FSN2, FSN3, FSN4, FSN5, FSN6, FSN7, FSN8, FSN9, FSN10, FSN11, FSN12, FSN13, FSN14, FSN15, FSN16, FSN17, FSN18, FSN19, FSN20, FSN21, FSN22, FSN23, FSN24, FSN25, FSN26, FSN27, FSN28, FSN29, FSN30, FSN31, FSN32, FSN33, FSN34, FSN35, FSN36, FSN37, FSN38, FSN39, FSN40, FSN41, FSN42, FSN43, FSN44, FSN45, FSN46, FSN47, FSN48, FSN49, FSN50, FSN51, FSN52, FSN53, FSN54, FSN55, FSN56, FSN57, FSN58, FSN59, FSN60, FSN61, FSN62, FSN63, FSN64, FSN65, FSN66, FSN67, FSN68, FSN69, FSN70, FSN71, FSN72, FSN73, FSN74, FSN75, FSN76, FSN77, FSN78, FSN79, FSN80, FSN81, FSN82, FSN83, FSN84, FSN85, FSN86, FSN87, FSN88, FSN89, FSN90, FSN91, FSN92, FSN93, FSN94, FSN95, FSN96, FSN97, FSN98, FSN99, FSN100.
- X-axis (Treatments):** EC\_1, EC\_2, EC\_3, LPS\_1, LPS\_2, LPS\_3, LPS\_4, LPS\_5, LPS\_6, LPS\_7, LPS\_8, LPS\_9, LPS\_10, LPS\_11, LPS\_12, LPS\_13, LPS\_14, LPS\_15, LPS\_16, LPS\_17, LPS\_18, LPS\_19, LPS\_20, LPS\_21, LPS\_22, LPS\_23, LPS\_24, LPS\_25, LPS\_26, LPS\_27, LPS\_28, LPS\_29, LPS\_30, LPS\_31, LPS\_32, LPS\_33, LPS\_34, LPS\_35, LPS\_36, LPS\_37, LPS\_38, LPS\_39, LPS\_40, LPS\_41, LPS\_42, LPS\_43, LPS\_44, LPS\_45, LPS\_46, LPS\_47, LPS\_48, LPS\_49, LPS\_50, LPS\_51, LPS\_52, LPS\_53, LPS\_54, LPS\_55, LPS\_56, LPS\_57, LPS\_58, LPS\_59, LPS\_60, LPS\_61, LPS\_62, LPS\_63, LPS\_64, LPS\_65, LPS\_66, LPS\_67, LPS\_68, LPS\_69, LPS\_70, LPS\_71, LPS\_72, LPS\_73, LPS\_74, LPS\_75, LPS\_76, LPS\_77, LPS\_78, LPS\_79, LPS\_80, LPS\_81, LPS\_82, LPS\_83, LPS\_84, LPS\_85, LPS\_86, LPS\_87, LPS\_88, LPS\_89, LPS\_90, LPS\_91, LPS\_92, LPS\_93, LPS\_94, LPS\_95, LPS\_96, LPS\_97, LPS\_98, LPS\_99, LPS\_100.
- Legend:**
  - EV\_Effect\_Direction:** Red (Up), Blue (Down), Grey (NS).
  - LPS\_Background:** Red (LPS\_UP), Blue (LPS\_DOWN), Grey (LPS\_NS).
  - Significance:** p < 0.001 (Black), p < 0.01 (Dark Grey), p < 0.05 (Light Grey), NS (White).

**Treatment**

2 EC  
1 LPS  
0 LPSEV

**EV\_Effect\_Direction**

0 Up  
-1 Down  
-2 NS

**Significance**

■  $p < 0.001$   
■  $p < 0.01$   
■  $p < 0.05$   
■ NS

**LPS\_Background**

■ LPS\_UP  
■ LPS\_DOWN  
■ LPS\_NS

**Figure 2.** Heatmap representation of all 121 proteins with significant LPS/EV *vs.* LPS changes, representing the “pure EV effect”. Z-score normalized expression values are displayed, with red indicating higher and blue lower relative expression. Left annotation columns: EV\_Effect (green = enhanced by EVs, red = suppressed by EVs, white = not significant in LPS/EV *vs.* LPS comparison); Significance (in gray scale ranging from black =  $P < 0.001$  to white = not significant); LPS\_Response (red = upregulated by LPS, blue = downregulated by LPS, white = not significant in LPS *vs.* ECs comparison). Upper colored bars indicate experimental groups: light blue (ECs), red (LPS), orange (LPS/EV).