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Introduction

- Extracellular vesicles (EVs) produced by probiotics are emerging as key mediators of the health benefits provided by these beneficial bacteria.
- Lactocaseibacillus casei* BL23 produces EVs ranging in size from 50 to 200 nm, which contain proteins associated with their probiotic effect¹.
- Their nanometric size would allow EVs to reach the host's tissues after penetrating the intestinal barrier² and interact with different types of cells (transkingdom communication) (Figure 1).
- The aim of this study was to purify EVs from *L. casei* BL23, characterize them, assess their biocompatibility and immunomodulatory effects on human cells, and evaluate their impact on the survival of *Caenorhabditis elegans* exposed to toxins, in order to determine the protective potential of probiotic EVs in a whole-organism model

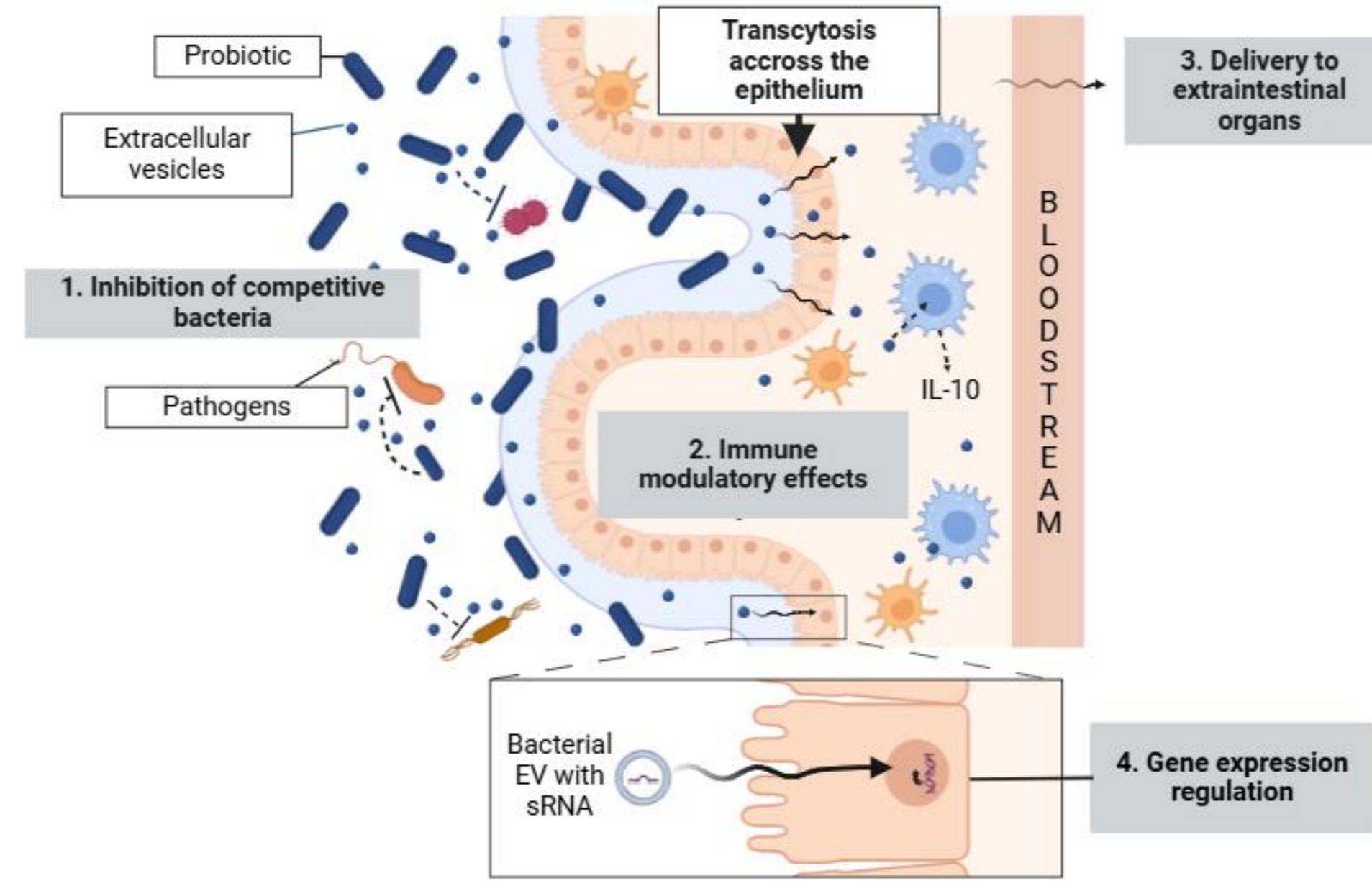
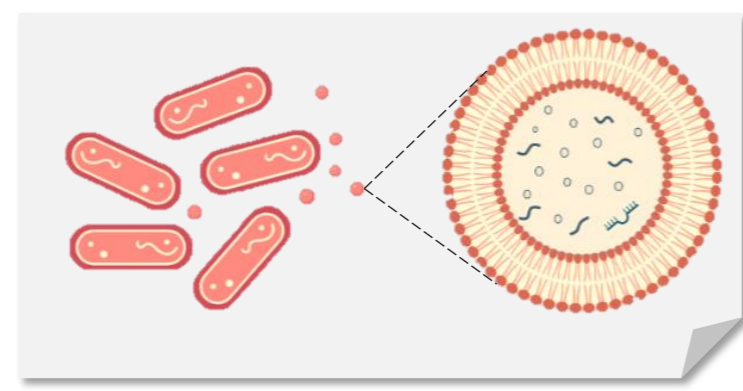
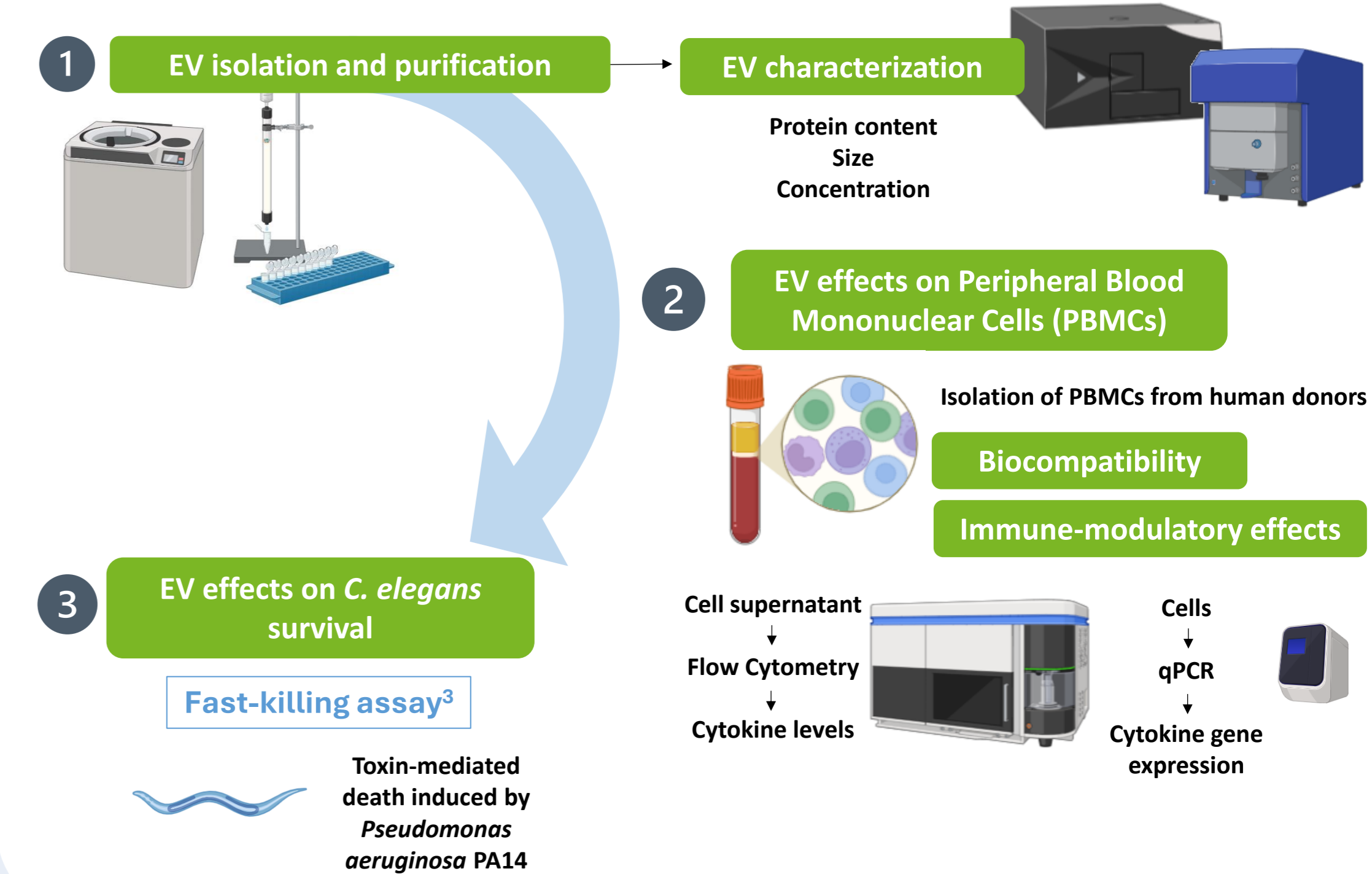
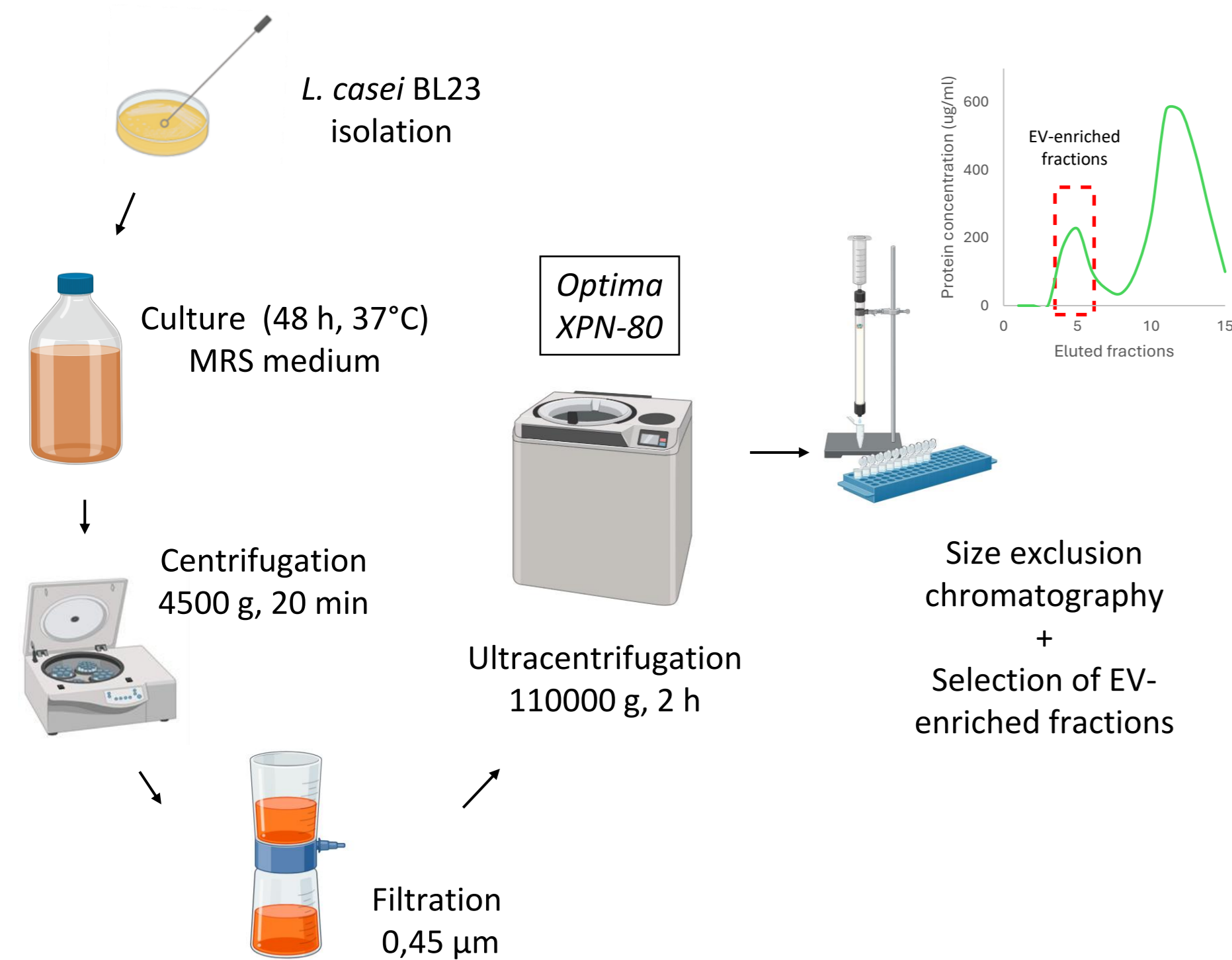


Figure 1. Graphical representation of the potential targets of EVs produced by probiotics

Materials and Methods



EV isolation and purification



EV characterization

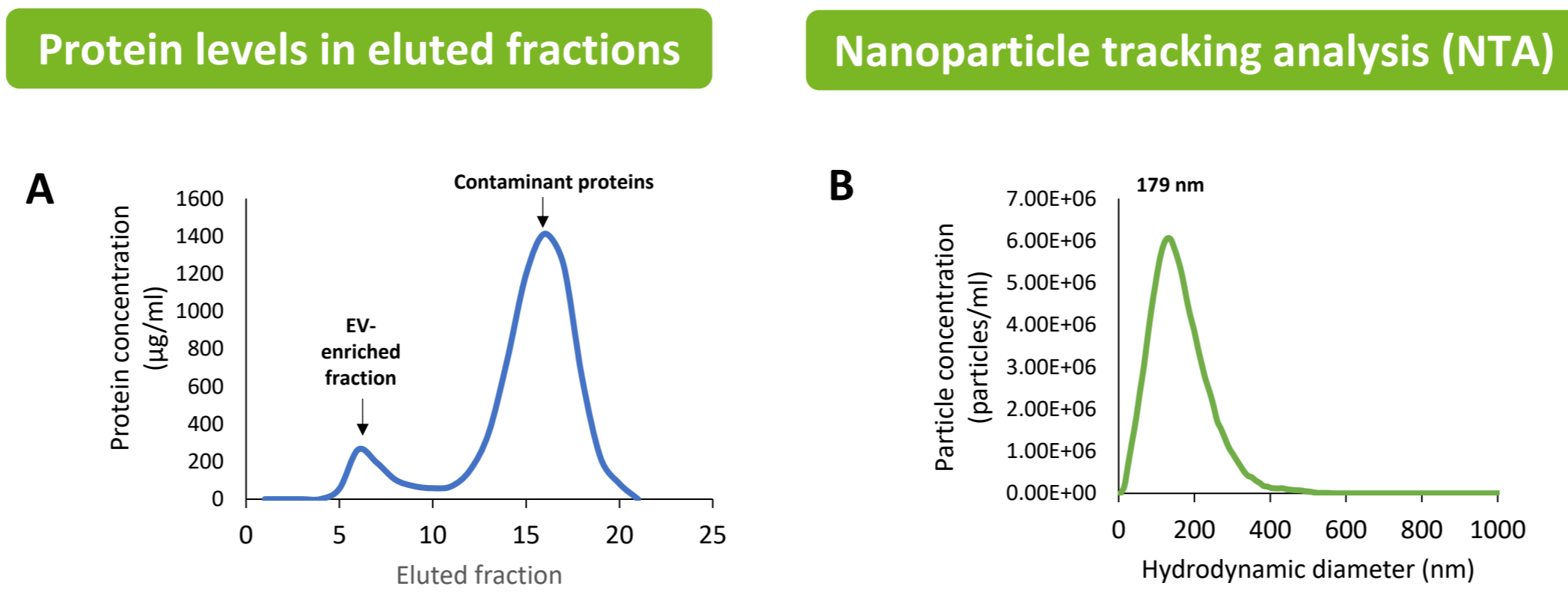


Figure 2. A. Protein concentration in the EV-enriched fraction (fraction 6) was 271 ± 89 µg/ml, as determined by the bicinchoninic acid assay. B. The mean size of EVs was 179 ± 13 nm, and the particle concentration was 2.53 × 10¹¹ particles/ml.

Biocompatibility

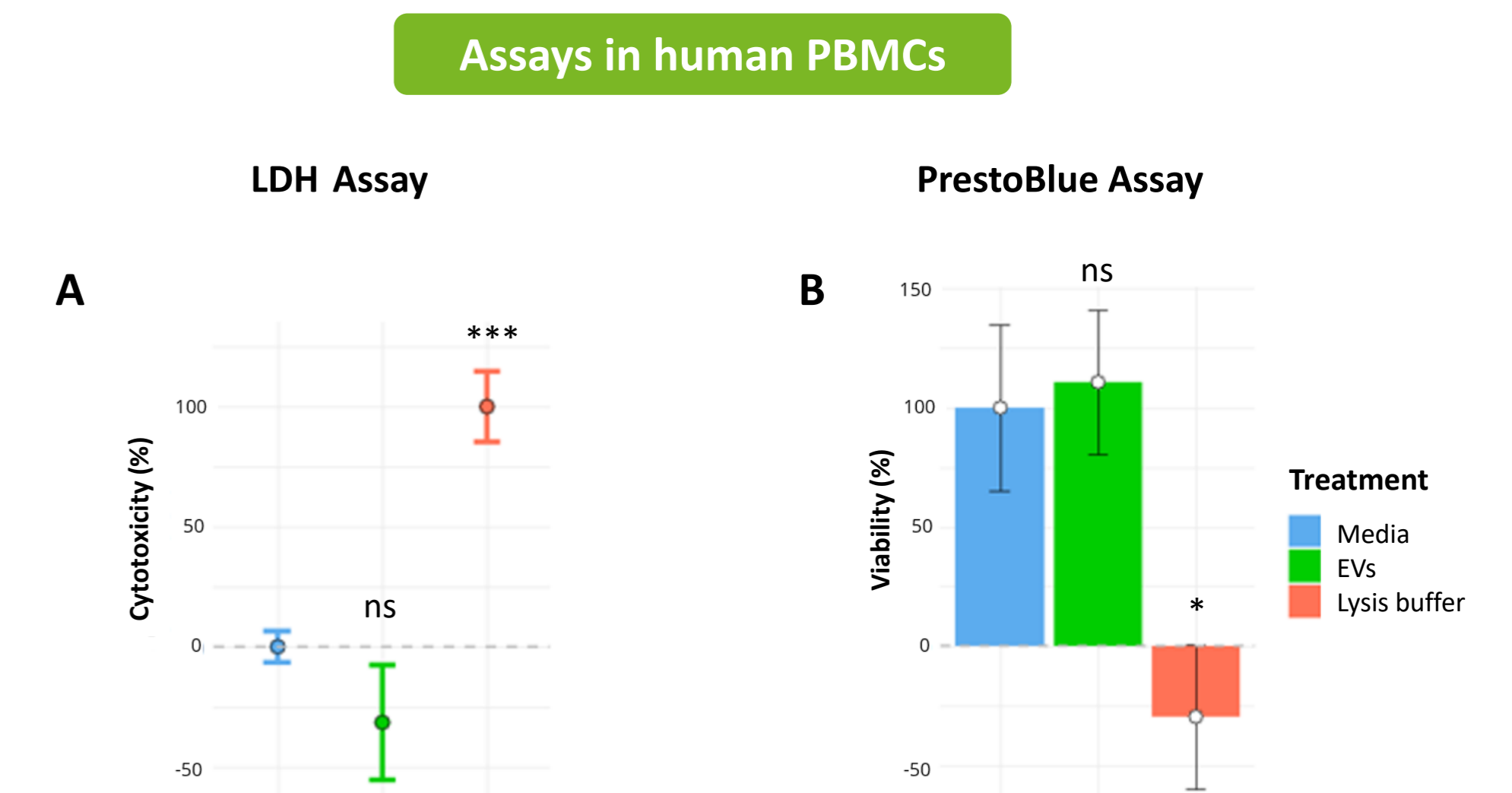


Figure 3. A. The EVs did not induce LDH release, indicating the absence of cytotoxicity. B. The cells maintained their viability after 24 hours of exposure to EVs.

Immunomodulatory effects

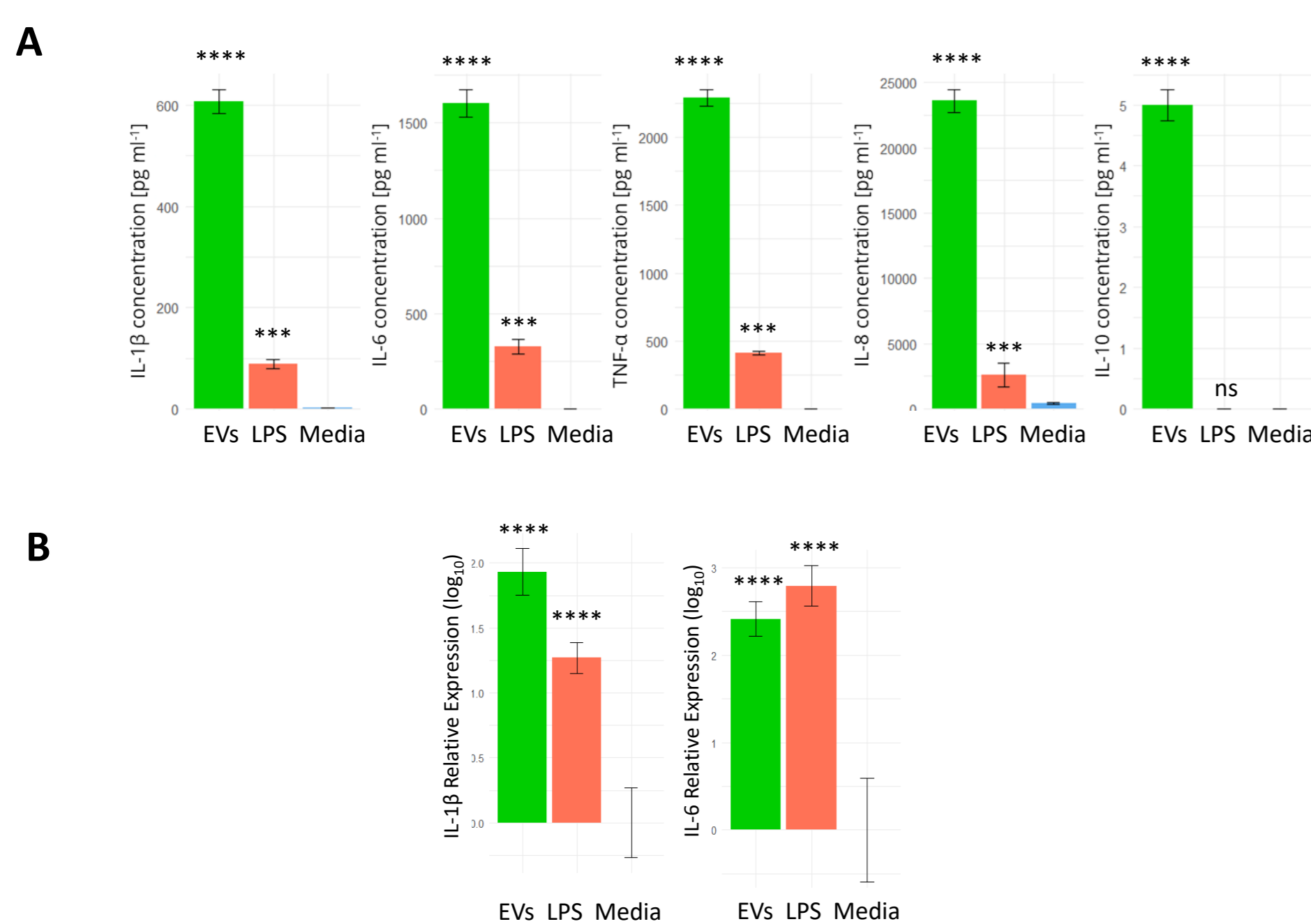


Figure 4. A. EVs induce a proinflammatory response (IL-1β, IL-6, TNF-α, IL-8) and an anti-inflammatory response (IL-10) in PBMC, as determined by flow cytometry using the BD™ Cytometric Bead Array (CBA) Human Inflammatory Cytokine Cytometric Bead Array (CBA) kit. B. qPCR confirmed the upregulation of proinflammatory genes IL-1β and IL-6.

Lifespan extension on *C. elegans*

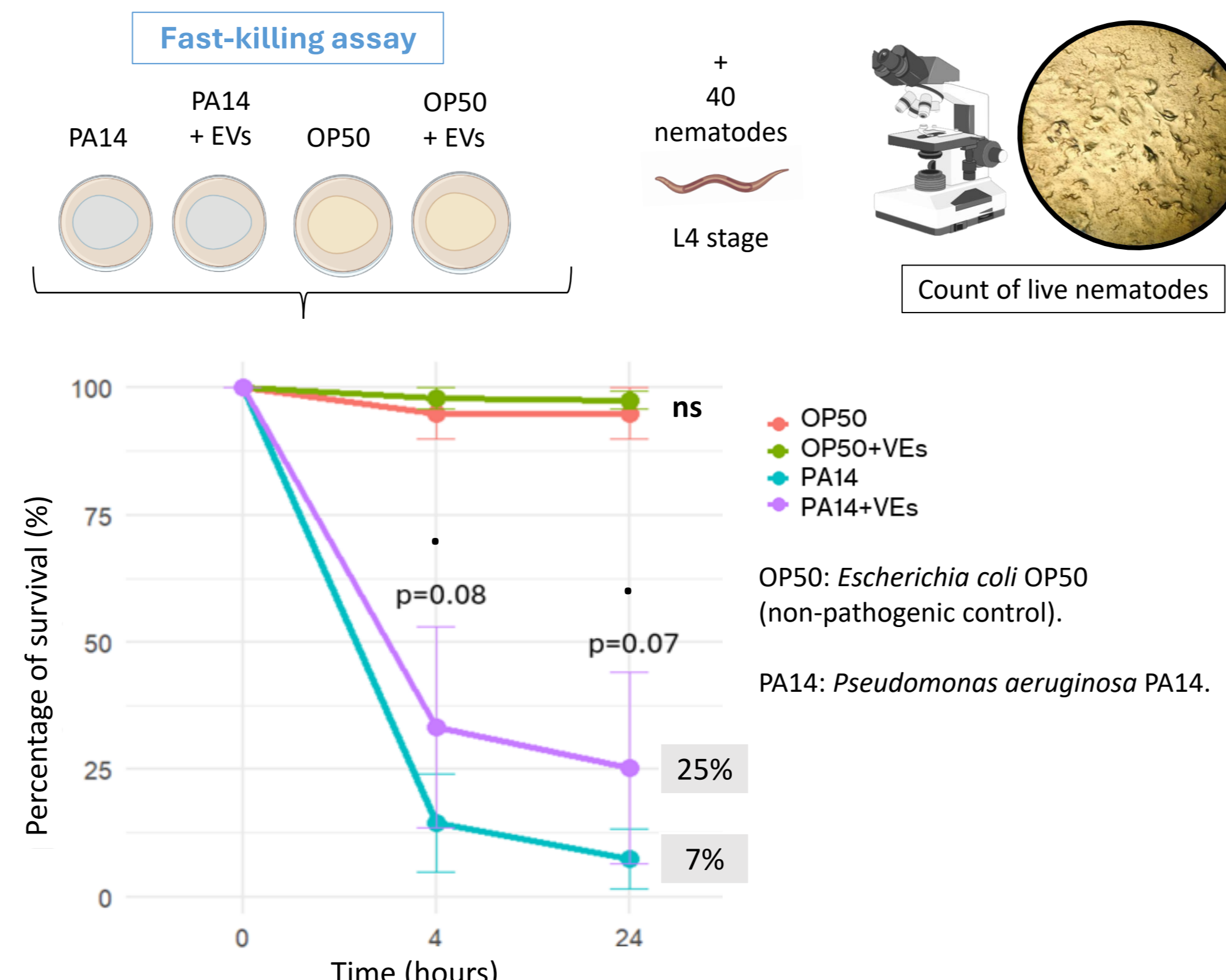


Figure 7. *L. casei* EVs slightly increased nematode survival against death induced by *P. aeruginosa* PA14 toxins, confirming their biocompatibility and potential protective effect.

Conclusions

- L. casei* BL23 EVs resulted biocompatible and safe as demonstrated by PBMCs assay and by *C. elegans* model, probably due to their probiotic origin.
- L. casei* BL23 EVs exhibited a marked immunomodulatory effect in PBMCs, with the ability to induce both pro- and anti-inflammatory responses, suggesting their potential as immune modulators.
- These results position *L. casei* BL23 EVs as promising candidates for developing innovative and safe therapies in the field of nutraceutical and nanomedicine, such as immunotherapy.

However, further studies are needed, including more complex studies in *in vivo* models and detailed investigations to better understand how these EVs modulate the immune response.

References

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