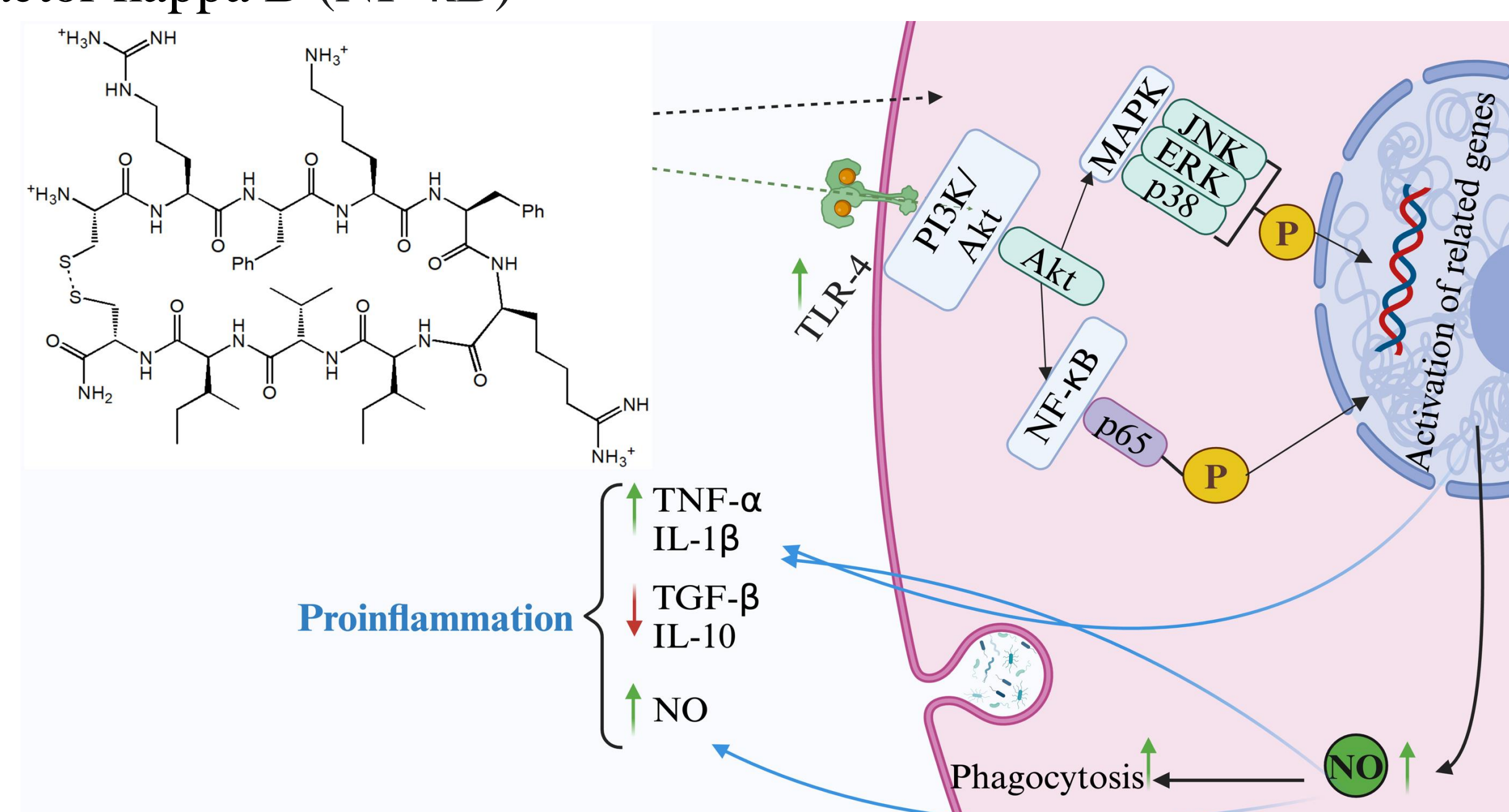


Introduction

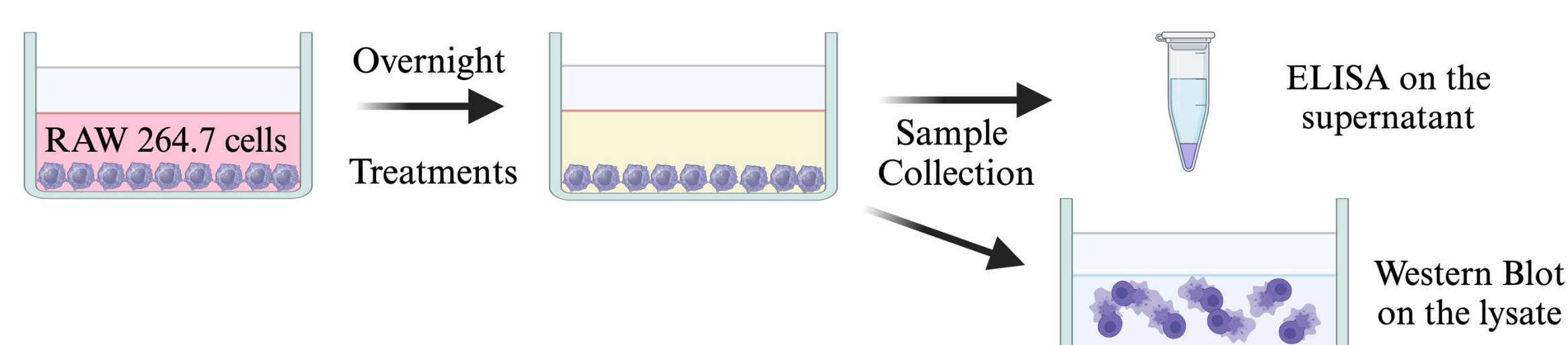
- Host defense peptides (HDPs), small positively charged amphipathic cationic peptides, a group of essential compounds of innate immunity
- Ability to elicit strong anti-infective properties, immune regulation, ubiquitously present in all organisms
- Regulation and modulation of chemotaxis, cell differentiation, pro- and anti-inflammatory cytokine production, activation of various intracellular cell signaling pathways, namely phosphatidylinositol 3-kinase/ protein kinase B (PI3K/Akt), mitogen-activated protein kinase (MAPK), and nuclear factor kappa B (NF-κB)



Objectives

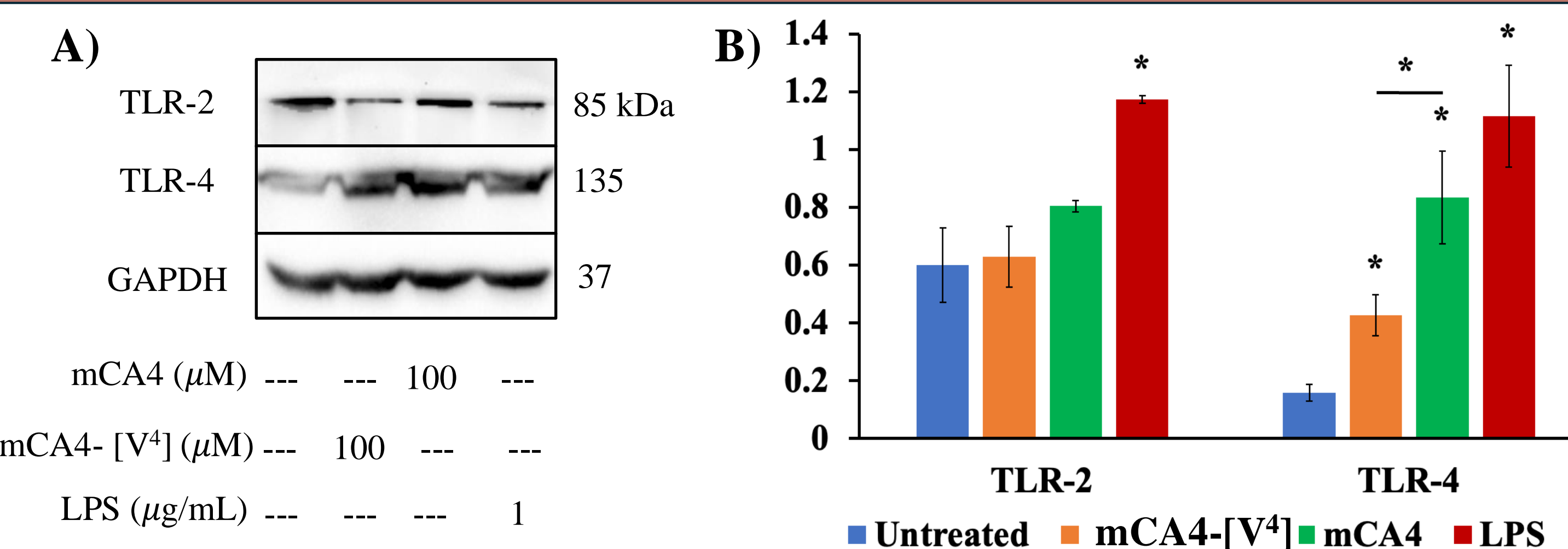
- Treatment of mouse macrophages (RAW 264.7) with mini chicken angiogenin 4 (mCA4) and its inactivated analogue as a control (mCA4-[V⁴]) to evaluate the immunomodulatory potentials of HDPs, derived from chicken angiogenin 4 (CA4)

Methods



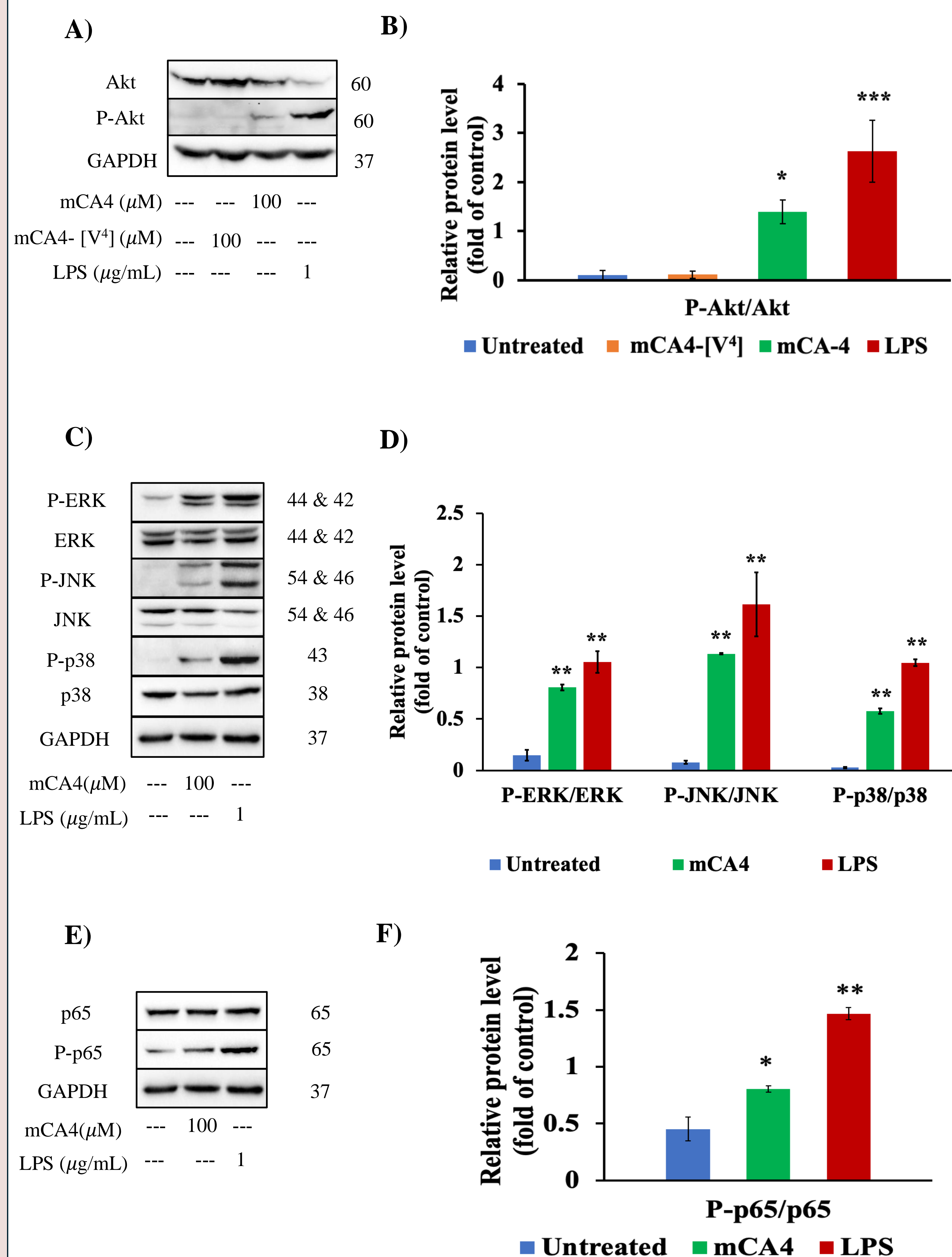
Results

A) Toll-like receptors (TLRs) Activation



- TLR-4 is upregulated in mCA4 treated cells, triggering immunomodulatory intracellular cell-signaling pathways.

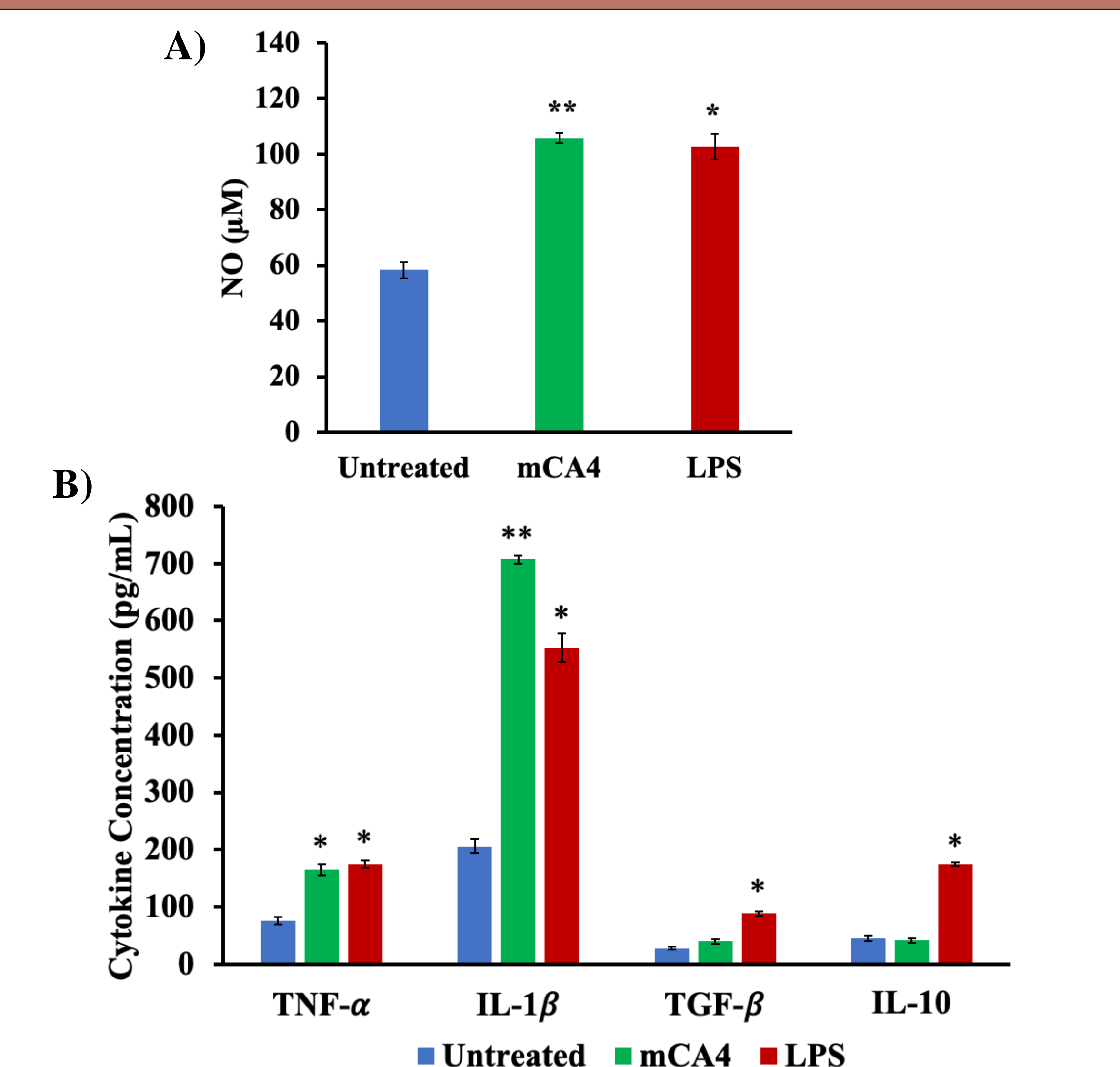
B) PI3K/Akt, NF-κB and MAPK signaling pathways



- Significantly high levels of protein phosphorylation, showing activation of PI3K/Akt, MAPKs, and NFκB intracellular cell-signaling pathways, which result in proinflammatory cytokine production.

Western blot analysis of RAW264.7 cells treated with mCA4, mCA4-[V⁴] (inactive analogue of mCA4) for PI3K/Akt (A), MAPK (C) and NF-κB (E) signaling pathways using primary monoclonal antibodies. LPS was used as a positive control. Gray-scale value ratios of target proteins to loading protein (GAPDH) (B, D, F). Data are presented as the mean ± SD (n = 3). *, p < 0.05; **, p < 0.01; ***, p < 0.001 versus the untreated (control) group.

C) Production of Inflammatory mediators



- Upregulation of pro-inflammatory cytokines and NO production in mCA4 treated macrophages

Effect of mCA4 on secretion of nitric oxide (NO) (A), and other cytokines (B) in RAW 264.7 cells. Macrophages were incubated with mCA4 (100 μM) and LPS (1 μg/mL) as positive control. Data are presented as the mean ± SD (n = 3). *, p < 0.05; **, p < 0.01; versus the untreated (control) group.

Conclusions & Future work

- mCA4 is a pro-inflammatory peptide that activates TLR-4 surface receptors and triggers PI3K/Akt, MAPK and NF-κB signalling cascade, resulting in secretion of pro-inflammatory mediators including IL-1β, NO and TNF-α, in treated macrophages.
- mCA4s can be a promising immunomodulator with potential applications as a therapeutic agent.

Reference

- Kim, J., Cho, B. H., & Jang, Y. S. (2023). Understanding the Roles of Host Defense Peptides in Immune Modulation: From Antimicrobial Action to Potential as Adjuvants. *Journal of microbiology and biotechnology*, 33(3), 288–298. <https://doi.org/10.4014/jmb.2301.01005>
- Moretta, A., Scieuzo, C., Petrone, A. M., Salvia, R., Manniello, M. D., Franco, A., Lucchetti, D., Vassallo, A., Vogel, H., Sgambato, A., & Falabella, P. (2021). Antimicrobial Peptides: A New Hope in Biomedical and Pharmaceutical Fields. *Frontiers in cellular and infection microbiology*, 11, 668632. <https://doi.org/10.3389/fcimb.2021.668632>

Acknowledgements

- This project was supported by University of Prince Edward Island and Natural Sciences and Engineering Research Council of Canada (NSERC)
- Special thanks to all the members of Ahmed Lab