# POLYSACCHARIDE PEPTIDE INDUCES AN INTERFERON-DRIVEN RESPONSE WITH NO CYTOTOXICTY IN JURKAT T-CELLS.

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## INTRODUCTION

**INTRODUCTION:** HIV-1 continues to be a significant threat on the adaptive immunity proven to be detrimental in CD4+ T-cells. Our recent findings demonstrated that Polysaccharide peptide (PSP) extracted from the mushroom *Coriolus versicolor* induces potent anti-HIV-1 effects. Specifically, PSP significantly impacts viral entry through the pre-production of Protein Kinase-R (PKR) under Toll-like Receptor 4 (TLR4). The current study sought to determine the anti-HIV roles of PSP in the adaptive immunity. The latest pre-liminary data has revealed the up-regulation of: TLR4, Nuclear Factor Kappa B (NF-kB), and the interferon (IFN) PKR with no PSP-induced cytotoxicity. These new datasets led to the hypothesis that PSP activates the transcription of IFNs under TLR4 signaling in T-cells. The outcome of this research will give insight towards the PSP antiviral effects in the adaptive immunity. **METHODS**: PSP anti-HIV role was evaluated using Jurkat T-cells treated with 50µg-1,000µg for a total of 6 days. Viral load gathered from collaborators at Laboratorio Borinquen were performed to assess total HIV-1 with/without PSP using PKR inhibitors. Immunoblots were performed for: PKR, TLR4 and NF-κB in Jurkat T-cells. MTT-viability were implemented to understand PSP cytotoxicity in Jurkats. **RESULTS**: Viral load revealed an average of 73% and 11% (PKR blocker) PSP-induced restriction in innate immunity. Immunoblotting resulted in the overexpression of PKR, TLR4 and NFκB in PSP-treated Jurkat T-cells. MTT reported no PSP-cytotoxicity during a 6-days treatment. **CONCLUSION**: The data gathered in this research demonstrates the first findings of PSP's immune boosting capabilities in the adaptive immunity.

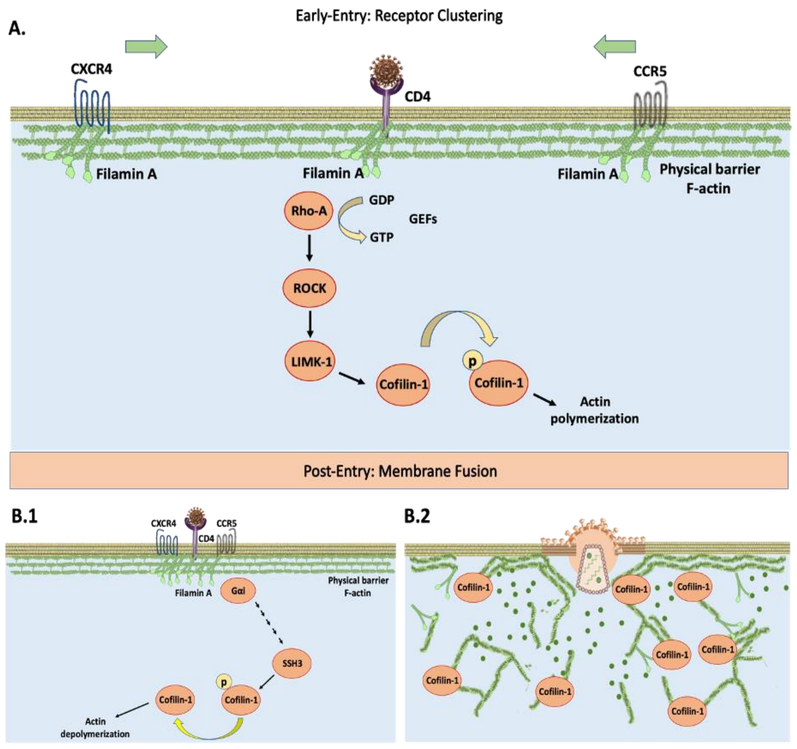


**Figure 1: Diagram depicting HIV-1 entry during infection in CD4+ cells by manipulation of Cofilin1.**

## METHODOLOGY

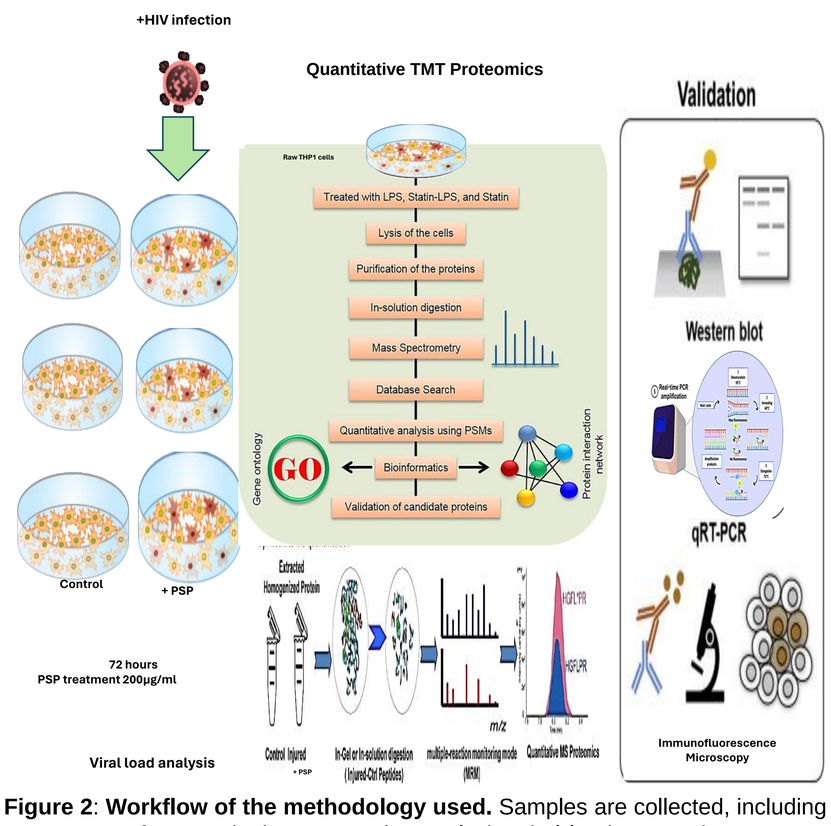


**Figure 2**: **Workflow of the methodology used.** Samples are collected, including supernatant, for quantitative proteomics analysis, viral load expression, western blot and RT-qPCR for validation of key IFN, UPR, and cytoskeleton markers.

## RESULTS

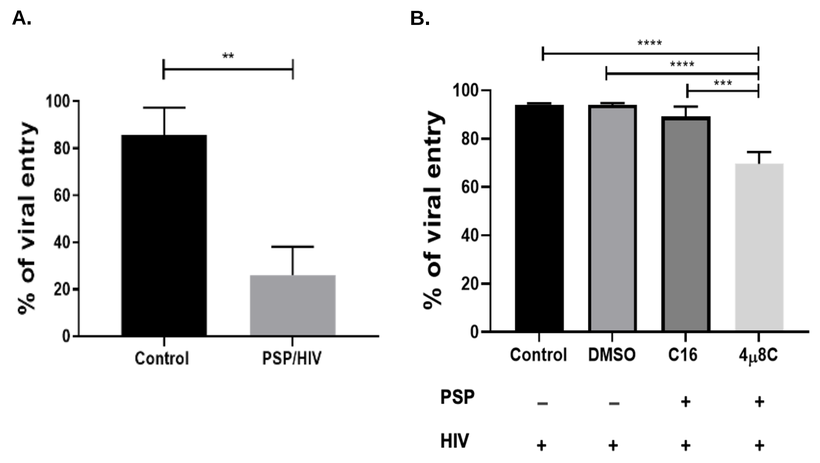


**Figure 3: PSP restricts HIV-1 entry though PKR and IRE1α signaling.** Viral load was performed for PSP-treated THP1 monocytic cells. A) THP-1 cells were treated with 200µg/ml before HIV-1 infection took place. B) THP-1 cells were treated with 56.06nM C16 or 221.8nM of 4µ8C pharmacological inhibitors.

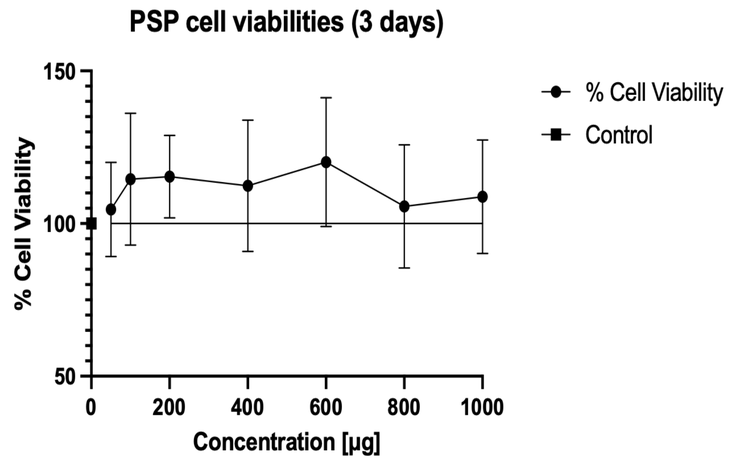


**Figure 4: MTT Cell viability assays demonstrate that PSP is non-toxic with survival/proliferation tendencies.** MTT assays were performed to assess PSP cytotoxicity in Jurkat T-cells.

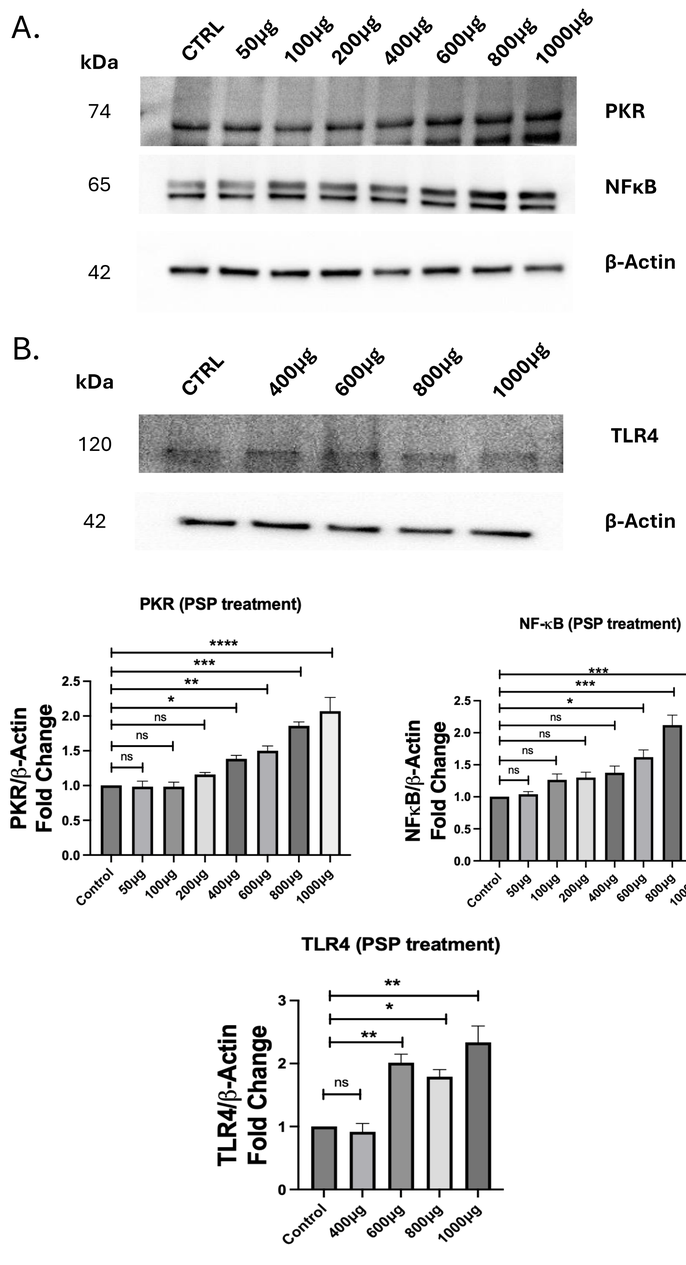


**Figure 5: Western blots figures for key anti-HIV1 signaling markers in Jurkat T-cells.** Western blot was performed in PSP-treated Jurkat T-cells at concentrations ranging from 50µg - 1,000µg: **A-B**) Representative western blots images. **C**) PKR, **D**) NFκB and **E**) TLR4. β-Actin was used as the loading control.

## PSP SIGNALING MODEL

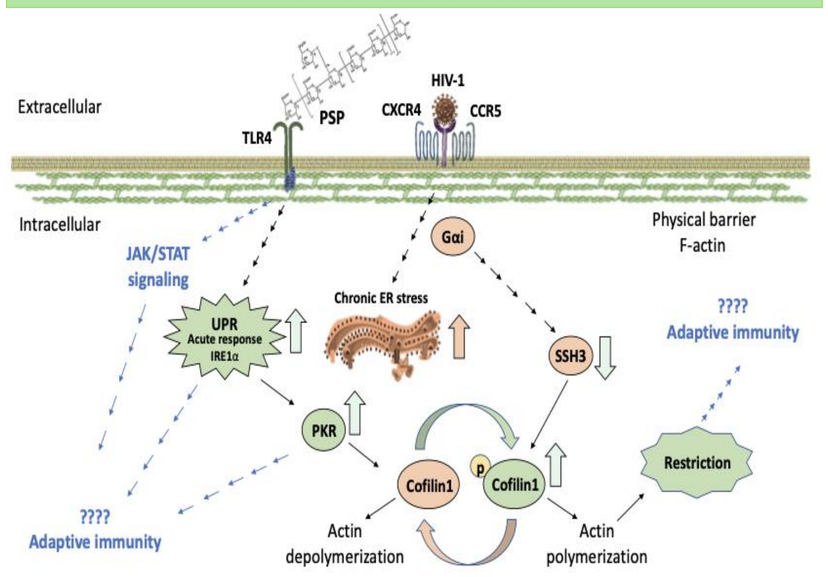


**Figure 6: Proposed model depicting PSP signaling through an IFN-induced and UPR overlapping pathway.** HIV-1 infection results in a chronic ER stress response, while simultaneously dephosphorylating cofilin-1 through SSH3 phosphatase. This results in actin depolymerization for viral entry. Prior infection, PSP treatment induces actin polymerization via an acute UPR. PKR mediates downstream phosphorylation of IRE1α signals and reverses HIV-induced actin remodeling while infection persists. Legend colors: Green – PSP mediated signaling and events. Cream- HIV-downstream pathways. Blue – Hypothesized signaling acting in T cell-mediated immunity with PSP treatment.. Adapted from Alvarez, E; *et al*, 2023.

## DISCUSSION AND CONCLUSION

This study is designed to target the most crucial HIV-1 life cycles such as entry and replication by direct activation of T-cells. PSP has demonstrated potent anti-HIV-1 effects in the innate immunity and the same pattern is being shown towards the adaptive immune response. Specifically, we highlight our novel finding in this section:

**Figure 1**: HIV-1 gains access to CD4+ cells (monocytes, macrophages, and T-lymphocytes) through cytoskeletal re-arrangement using Cofilin-1 as the mediator of this entry.

**Figure 2**: Our workflow has composed of quantitative proteomics (not shown here), quantitative reverse transcriptions polymerase chain reaction (RT-qPCR; not shown here), viral load analysis, MTT cell viability assays and immunoblotting procedures.

**Figure 3**: Viral load has revealed that PSP restricts HIV-1 entry in-vitro THP1 monocytic cells by an average of 73% (Figure 3A). PKR and IRE1α reverses these restrictive effect and facilitates viral entry (Figure 3B). This data has highlighted for the first time the significant immune boosting capabilities of PSP in the innate immune system.

**Figure 4**: To understand the toxicity of PSP in Jurkat T-lymphocytes, MTT cell viability assays were employed. Jurkat T-cells were exposed with PSP for a total period of 3 days. Results have shown that PSP has no cytotoxicity and shows a slight tendency towards survivability and proliferation. This implies a T-lymphocyte associated signaling activation.

**Figure 5**: PSP has been shown in our previous studies to induce its potent antiviral effects through downstream signaling of TLR4 in THP-1 monocytic cells. To determine the PSP-induced signaling being affected in Jurkat T-lymphocytes, western blot approaches were implemented for the following markers: PKR, NF-κB and TLR4. PSP has shown the overexpression of all signaling markers through increased increments of concentrations (50µg – 1,000µg) suggesting a similar pattern taking place in the adaptive immunity. These results combined with figure 4 are implying potent effects in the immune system with no toxicity.

The concept of using PSP as a natural barrier against HIV-1 replication as well as an enhancer of the adaptive immunity is both novel and has not been tested before. The outcome of this research will serve to implement new and effective methods for HIV-1 targeted therapies.

**Our long-term goal is to implement PSP as a future vaccine/preventive method to healthy individuals while also serving as a treatment to infected patients with the ability to slow down viral progression and replication.**

## ACKNOWLEDGEMENTS

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