T.C.

BIRUNI UNIVERSITY

THE GRADUATE EDUCATION INSTITUTE

MOLECULAR BIOLOGY AND GENETICS DEPARTMENT

MOLECULAR AND MEDICAL GENETICS MASTER PROGRAM

INVESTIGATION OF IMMUNOMODULATORY EFFECTS OF MAXIMIN S1 ON MAMMALIAN MACROPHAGES

Ece AYDIN

Prof. Dr. Furkan AYAZ

April, 2025

T.C.

BIRUNI UNIVERSITY

THE GRADUATE EDUCATION INSTITUTE

MOLECULAR BIOLOGY AND GENETICS DEPARTMENT

MOLECULAR AND MEDICAL GENETICS MASTER PROGRAM

INVESTIGATION OF IMMUNOMODULATORY EFFECTS OF MAXIMIN S1 ON MAMMALIAN MACROPHAGES

Ece AYDIN

Prof. Dr. Furkan AYAZ

April, 2025

DECLARATION

I declare that this thesis belongs to me, that I have not committed any unethical behavior at any stage, that I have obtained all the information contained in it within academic and ethical rules, that I have cited all the information I have used and included these sources in the list of sources, and that I have not committed any behavior that would violate patents and copyrights during the execution and writing of this thesis.

Ece AYDIN

DEDICATION

I dedicate this thesis to to my younger self who believed science would make sense of the world — and to all those who still believe. And to my beloved niece and nephew, Hande Mira Gülay and Doğu Kerem Gültürk, for I truly believe that one day, you too will change the world.

ACKNOWLEDGEMENT

I would like to extend my deepest gratitude to Prof. Dr. Furkan Ayaz, my thesis advisor, for his unwavering support, valuable guidance, and constant encouragement throughout my academic journey. His mentorship has been instrumental in shaping this work. I am also sincerely thankful to Esra Aydemir Ayaz for her kind presence and inspiring support during this process.

To my dearest friend Zeyneb Nur Akçay, my constant companion in this academic life since our undergraduate days — thank you for always standing by me with your unwavering support, genuine friendship, and heartfelt encouragement. Your presence has made even the hardest moments easier to bear, and the good ones even more meaningful.

And above all, to my beloved family, whose endless love, strength, and belief in me have been my foundation. Your constant support, even in silence, has meant more than words can ever express. You gave me the space to grow, and the courage to continue. For every sacrifice you made for me — thank you.

This thesis is not only the outcome of academic effort, but also a testament to the strength, kindness, and support I have received from each of you along the way.

LIST OF CONTENTS

LIST OF SYMBOLS AND ABBREVIATIONS

M1	Classically Activated Macrophage
M2	Alternatively Activated Macrophage
LPS	Lipopolysaccharide
NF-kB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
STAT	Signal Transducer and Activator of Transcription
PI3K/Akt	Phosphoinositide 3-Kinase / Protein Kinase B
RNA	Ribonucleic Acid
DNA	Deoxyribonucleic Acid
AMP	Antimicrobial Peptide
CD4*	Cluster of Differentiation 4 Positive
CD8*	Cluster of Differentiation 8 Positive
IFN-y	Interferon-gamma
IL	Interleukin
IL-1ß	Interleukin-1 beta
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-13	Interleukin-13
IL-12p40	Interleukin-12 subunit p40
OD	Optical Density
MHC	Major Histocompatibility Complex
PD-1	Programmed Cell Death Protein 1
PRR	Pattern Recognition Receptor

TAM	Tumor-Associated Macrophage
TGF-ß	Transforming Growth Factor-beta
TGF-ß1	Transforming Growth Factor-beta1
Th1	T helper 1
Th2	T helper 2
Th17	T helper 17
TLR	Toll-Like Receptor
LL-37	Human Cathelicidin Antimicrobial Peptide
PD-1	Programmed Death-1
MIC	Minimum Inhibitory Concentration
MAPK	Mitogen-Activated Protein Kinase
ERK	Extracellular Signal-Regulated Kinase
JNK	c-Jun N-terminal Kinase
DAMP	Damage-Associated Molecular Pattern
PAMP	Pathogen-Associated Molecular Pattern
MyD88	Myeloid Differentiation Primary Response 88
TLR2	Toll-Like Receptor 2
TLR4	Toll-Like Receptor 4
TNF-a	Tumor Necrosis Factor-alpha
p38	p38 Mitogen-Activated Protein Kinase
IkB	Inhibitor of kappa B
IKK	Ikß Kinase
EBV	Epstein-Barr Virus
EMT	Epithelial-Mesenchymal Transition
HCC	Hepatocellular Carcinoma
JAK	Janus Kinase

NSCLC	Non-Small Cell Lung Cancer
Akt1	Protein Kinase B isoform 1
mTOR	Mechanistic Target of Rapamycin
DMEM	Dulbeccos Modified Eagle Medium
FBS	Fetal Bovine Serum
RT-qPCR	Reverse Transcription Quantitative Polymerase Chain Reaction
ECL	Electrochemiluminescence
PCR	Polymerase Chain Reaction
BSA	Bovine Serum Albumin
OD	Optical Density
ANOVA	Analysis of Variance
MSC	Mesenchymal Stem Cell
J774.2	Macrophage cell line
ELISA	Enzyme-Linked Immunosorbent Assay
NK	Natural Killer (cell)
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
PE-A	Phycoerythrin-Area

LIST OF TABLES

LIST of FIGURES

TÜRKÇE ÖZET ve ANAHTAR KELİMELER

Aydın, E (2025). Maximin S1'in Memeli Makrofajları Üzerindeki İmmünomodülatör Etkilerinin İncelenmesi. Yüksek Lisans Tezi, Biruni Üniversitesi Lisansüstü Eğitim Enstitüsü.

Bu çalışma, antimikrobiyal bir peptit olan Maximin S1'in memeli makrofajlarında gösterdiği immünomodülatör etkileri araştırmak amacıyla gerçekleştirilmiştir. Özellikle lipopolisakkarit (LPS) ile birlikte uygulandığında, Maximin S1'in pro-inflamatuar sitokin üretimini anlamlı ölçüde artırdığı gösterilmiştir. Yapılan ELISA analizlerinde, Maximin S1'in GM-CSF ve IL-12p40 sitokin düzeylerini istatistiksel olarak anlamlı şekilde yükselttiği, TNF-α ve IL-6 üretimini ise artan bir eğilimle desteklediği gözlemlenmiştir. Hücre canlılığı analizleri, bu etkilerin sitotoksisiteye bağlı olmadığını, doğrudan bağışıklık sistemi üzerindeki düzenleyici aktiviteden kaynaklandığını ortaya koymuştur. Moleküler düzeyde yapılan akış sitometrisi analizleri, Maximin S1'in PI3K sinyal yolunu belirgin şekilde aktive ettiğini, buna karşın NF-κB sinyal yoluna anlamlı bir etkide bulunmadığını göstermiştir. Bu durum, Maximin S1'in bağışıklık sistemini PI3K-bağımlı ve NF-κB-bağımsız bir mekanizma aracılığıyla aktive ettiğini göstermektedir. Ayrıca, Maximin S1'in LPS olmadan da PI3K aktivitesini artırabilmesi, onun immün hücreleri "önceden hazırlayan" (priming) bir etkisi olabileceğini düşündürmektedir. Sonuç olarak, Maximin S1 seçici ve güçlü bir immünostimülatör profil sergilemektedir. Bu özellikleriyle, kontrollü bağışıklık aktivasyonu gerektiren aşı adjuvanları ya da immünoterapötik stratejilerde kullanılabilecek potansiyel bir aday olarak değerlendirilmektedir.

Anahtar kelimeler: Aşı Adjuvanı; Immünomodülasyon; Makrofajlar; Maximin S1; NF-κB; PI3K; Sitokinler.

ENGLISH ABSTRACT and KEYWORDS

Aydin, E (2025). Investigation of Immunomodulatory Effects of Maximin S1 on Mammalian Macrophages. Master Thesis, Biruni University Graduate Education Institute, Istanbul.

It provides compelling evidence that Maximin S1 possesses strong pro-inflammatory immunomodulatory activity in mammalian macrophages, particularly when co-administered with lipopolysaccharide (LPS). Maximin S1 significantly enhanced the production of key pro-inflammatory cytokines—namely GM-CSF, IL-12p40, TNF-α, and IL-6—following LPS stimulation. Maximin S1's potent capacity to amplify immune activation was seen. These effects occurred without cytotoxicity, confirming that the immunomodulatory actions were not due to cellular stress or damage. Mechanistic analyses revealed that Maximin S1 selectively activated the PI3K signaling pathway, while having negligible impact on NF-κB activation. Flow cytometry results showed a notable increase in PI3K-positive cell populations in Maximin + LPS-treated macrophages, whereas NF-κB-positive cell percentages remained unchanged across treatment conditions. It suggests that Maximin's immunostimulatory effects are mediated specifically through PI3K-dependent intracellular signaling, independent of the canonical NF-κB pathway. Importantly, Maximin S1 alone also moderately increased PI3K activity, suggesting a potential priming effect on immune cells even in the absence of LPS. The specificity of action through PI3K, without engaging NF-κB, indicates a novel immunological mechanism that may be exploited in adjuvant development or in immunotherapies that require heightened but controlled immune activation. So, Maximin S1 demonstrates a promising immunomodulatory profile, making it a candidate for further exploration as an adjuvant in vaccine formulations or as an immune potentiator in therapies targeting infectious diseases, cancer, or immune dysfunction.

Key words: Cytokines; Immune Activation; Immunomodulation; Macrophages; Maximin S1; NF-κB; PI3K; Vaccine Adjuvant

1. INTRODUCTION and PURPOSE

Macrophages play crucial functions in the immune system, functioning as essential components of both innate and adaptive immunity. A distinctive property of macrophages is their capacity to polarize into diverse phenotypes in response to various environmental stimuli. The primary categories of macrophage polarization are M1 and M2. M1 macrophages are typically active and linked to pro-inflammatory responses, whereas M2 macrophages are alternatively activated, facilitating anti-inflammatory responses and tissue healing (Zhao et al., 2021; Miao et al., 2017; Lv et al., 2022). They also affect other physiological processes beyond inflammation, including tissue remodeling, metabolism, and hematopoiesis. Their plasticity enables adaptation to various signaling contexts, which may impact disorders like cancer, where tumor-associated macrophages frequently assume an M2-like phenotype, promoting tumor growth (Miao et al., 2017; Ford et al., 2012). In contrast, M1 macrophages play a crucial role in anti-tumor immunity, indicating a potential therapeutic avenue for altering macrophage morphologies in cancer therapy (Robbe et al., 2015). Consequently, altering macrophage functions using pharmacological agents may provide novel therapeutic strategies for the treatment of immune-mediated disorders.

Maximin S1, the subject of the experiment, is an antimicrobial peptide derived from the skin secretions of the Chinese red-bellied frog (Bombina maxima). (Lai et al., 2002) This 14-amino acid peptide is effective against germs and is a crucial component of the immune system (Wang et al., 2005). Nonetheless, the immunomodulatory effects of Maximin S1 on macrophages have not been thoroughly examined. Antimicrobial peptides are widely recognized for their ability to alter macrophage activation and activities. Strive to create innovative therapeutic strategies by investigating the potential impacts of Maximin S1 on macrophage differentiation, activation, and functionality. This research posits that immunomodulatory medications can substantially modify macrophage activities, hence influencing their ability to respond effectively to infections, tumor cells, and inflammatory stimuli. Research by Mantovani et al. (2002) and Gordon and Taylor (2005) has shown that macrophages possess significant phenotypic plasticity, enabling them to specialize in

reaction to environmental cues. This distinction is essential, especially in the resolution of inflammatory processes and the mechanisms of tissue healing. Nonetheless, it has been underscored that disruptions in the regulatory mechanisms overseeing these phenotypic changes may have a role in the development of pathological disorders, including chronic inflammation and autoimmune illnesses (Mantovani et al., 2002; Gordon & Taylor, 2005). The scientific foundation of the study is to comprehend the effects of Maximin S1 on the immune system and its regulation of macrophage function. This study will specifically investigate signaling pathways, including NF-κB, STAT, and PI3K/Akt, that govern the activation and polarization of macrophages. The nodes in these pathways that are targetable may present prospects for the creation of disease-specific therapeutics.

The main aim of this project is to comprehensively examine the genetic and phenotypic impacts of the antimicrobial peptide Maximin S1 on macrophage cell lines, with the intent of clarifying how this peptide influences macrophage behavior and its immunomodulatory capacity within the immune system. Macrophages are essential immune cells, significantly involved in pathogen response, inflammation regulation, and tissue repair mechanisms. Excessive or dysregulated macrophage activity can significantly contribute to the etiology of numerous diseases, including chronic inflammation, autoimmune disorders, and cancer. This study will specifically investigate the effects of Maximin S1 on macrophage activation, differentiation, and polarization. Special emphasis will be placed on comprehending the regulation of transitions between the pro-inflammatory (M1) and anti-inflammatory (M2) macrophage phenotypes. The study also aims to assess gene expression profiles by molecular techniques, including DNA and RNA isolation, to examine alterations in critical signaling pathways, such as NF-κB, STAT, and PI3K/Akt, which govern macrophage activation and polarization. These analyses will elucidate the molecular processes by which Maximin S1 influences macrophages, thereby establishing a basis for assessing the therapeutic potential of this peptide in immune-related disorders.

This research constitutes a preliminary step in elucidating the essential function of the immune system in illness management and the influence of Maximin S1 on macrophages. The acquired data may enable the formulation of innovative treatment approaches for addressing immune-mediated illnesses, chronic inflammation, autoimmune diseases, and cancer. This study seeks to improve our comprehension of

immune system dynamics and the immunomodulatory properties of antimicrobial peptides.

2. GENERAL INFORMATION

2.1. Macrophages and Immune System

Macrophages are substantial phagocytic cells originating from monocytes, a category of leukocytes. They are strategically located in many tissues throughout the body and are essential for both innate and adaptive immunity. Macrophages act as the initial defense against infections by identifying and phagocytizing pathogens, necrotic cells, and foreign substances, while also playing a role in the modulation of immune responses (Chen & Zhang, 2017; Yin et al., 2017). Macrophages demonstrate significant flexibility, enabling them to assume several functional phenotypes in reaction to environmental cues. They can be categorized principally into two categories according to their activation states:

2.1.1. M1 Macrophages (Classically Activated Macrophages)

Macrophages are activated by pro-inflammatory cytokines, including interferon-gamma (IFN-γ), and microbial products such as lipopolysaccharides (LPS). M1 macrophages are distinguished by their secretion of pro-inflammatory cytokines, including IL-1β and TNF-α, and are mostly engaged in fighting infections and triggering inflammatory responses (Stout & Suttles, 2005; Moghaddam et al., 2018). Their function in phagocytosis aids in the elimination of pathogens and necrotic tissue while concurrently enhancing immunological activation (Moghaddam et al., 2018).

2.1.2. M2 Macrophages (Alternatively Activated Macrophages)

M2 macrophages develop in response to anti-inflammatory signals, including IL-4 and IL-13. They are essential in tissue repair, remodeling, and inflammation resolution by secreting anti-inflammatory cytokines such as IL-10 and TGF-β (Shen et al., 2022; Peña-Martínez et al., 2021). M2 macrophages are crucial for proper wound healing and can enhance angiogenesis, hence aiding tissue regeneration (Safari et al., 2021).

M2 macrophages are predominant in tissue repair, remodeling, and chronic inflammation, releasing anti-inflammatory and growth factors crucial for resolving inflammation and facilitating tissue regeneration. Disturbance of this fragile equilibrium may result in prolonged inflammation, compromised tissue repair, or advancement of chronic illnesses, underscoring the significance of meticulously regulated macrophage polarization as a therapeutic objective (Moghaddam et al., 2018). Macrophages execute numerous biological roles essential for the immune response and the preservation of tissue homeostasis. Macrophages primarily function by engulfing and digesting microorganisms, cellular debris, and apoptotic cells via phagocytosis (Li et al., 2023; Yang et al., 2023). The process commences when macrophages identify pathogens through surface receptors, including pattern recognition receptors (PRRs) like Toll-like receptors (TLRs) (Sinha, 2022). Upon activation, macrophages release several cytokines that coordinate immunological responses. These cytokines not only facilitate the inflammatory response but also affect the activity of other immune cells, including T cells and neutrophils (Stout & Suttles, 2005; Moghaddam et al., 2018). The release of cytokines such as IL-1, IL-6, and IL-12 by M1 macrophages is crucial for enhancing inflammation and attracting more immune cells to areas of infection (Stout & Suttles, 2005). Macrophages are essential in connecting the innate and adaptive immune systems by processing and presenting antigens to T cells through major histocompatibility complex (MHC) molecules (Li et al., 2023; DeRosa & Leftin, 2021). This procedure augments the adaptive immune response and facilitates the formation of immunological memory. In addition to their functions in inflammation and immunological defense, macrophages play a crucial part in tissue repair and regeneration. They facilitate the removal of necrotic cells and detritus, release growth factors, and promote tissue regeneration. Raggatt et al. emphasized their importance to fracture healing, noting that macrophage activity is crucial for the beginning and advancement of ossification during bone regeneration (Raggatt et al., 2014). Moreover, macrophages can govern immunological responses via their secretions, engaging with diverse cell populations and modifying their activity. The equilibrium between M1 and M2 macrophages is essential for facilitating proper immune responses and averting chronic inflammation and autoimmunity (Stout & Suttles, 2005; Moghaddam et al.,

2018).

Macrophages are crucial in adaptive immunological responses, especially through their interactions with T cells and B cells. Macrophages play a crucial role in activating CD4+ and CD8+ T cells by secreting cytokines that facilitate their development into specific subsets, including Th1, Th2, and Th17 cells. M1 macrophages secrete IL-12, an essential cytokine that facilitates the development of naïve T cells into Th1 cells, hence augmenting cell-mediated immunity. This process is crucial in addressing viral infections and intracellular pathogens (Zhang et al., 2019; Ortiz et al., 2015). Moreover, macrophages exhibit regulatory roles in adaptive immune responses. They engage directly with T cells via costimulatory and inhibitory molecules, including CD40-CD40L, thereby influencing T cell activation and proliferation. This relationship is crucial for sustaining immunological homeostasis and averting autoimmunity by facilitating self-tolerance (Yu et al., 2018). They also play a crucial role in immunological memory. Subsequent to an adaptive immune response, they maintain a reservoir of memory T cells, facilitating swift and effective immune responses upon re-exposure to previously encountered pathogens. The active interplay between macrophages and memory T cells enables a rapid response and efficient defense (Xie et al., 2019). Additionally, macrophages affect B cell activation and differentiation, facilitating class switching and affinity development in humoral immune responses. Macrophages facilitate efficient and controlled B cell responses through cytokine release and antigen presentation, hence enhancing the efficacy and specificity of antibody-mediated immunity (Yu et al., 2018; Lin et al., 2022). The various and essential roles of macrophages highlight their multifunctional capacity within adaptive immunity, emphasizing their significance in immune regulation, memory, and effector responses. Comprehending these pathways offers critical insights for formulating treatment methods aimed at modulating macrophage activities in diverse immune-related illnesses.

Macrophages play a crucial part in chronic inflammation, which is marked by extended inflammatory reactions that do not resolve, frequently resulting in tissue damage and the advancement of numerous chronic illnesses. In chronic inflammatory situations, macrophages frequently assume a pro-

inflammatory M1 phenotype, leading to the release of cytokines such as TNF-α and IL-1β (Jha & Moore, 2021). This continuous activation results in the enlistment of supplementary immune cells, sustaining the inflammatory cycle. Jha and Moore established that certain collagen-derived peptides can influence macrophage polarization, directing the immune response towards regeneration instead of chronic inflammation (Jha & Moore, 2021). Extended macrophage activation leads to the persistent secretion of inflammatory mediators that exacerbate tissue injury. TGF-β1 is significant in directing macrophages to a phenotype that worsens chronic inflammation, as demonstrated by Lei et al., who found that TGF-β1 increases PD-1 expression, facilitating immunological exhaustion in chronic inflammatory microenvironments (Lei et al., 2024). The failure of macrophages to effectively shift from an inflammatory to a reparative phenotype may result in clinical diseases, including fibrosis and organ dysfunction. Macrophages facilitate the development of fibrotic tissue by continuously synthesizing extracellular matrix components and pro-fibrotic cytokines, potentially impairing organ function over time (Chung et al., 2020). In autoimmune disorders, when the immune system erroneously targets healthy tissues, macrophages play essential roles in both facilitating and modulating inflammation. The distinction between M1 and M2 macrophages is especially pertinent in autoimmune disorders. Chronic inflammation in conditions like rheumatoid arthritis can be exacerbated by an overabundance of M1 macrophages, which intensify joint inflammation and damage. M2 macrophages, conversely, facilitate healing and tissue regeneration (Kuo et al., 2019). Shibaguchi et al. elucidated that macrophages polarized to the M1 phenotype may aggravate autoimmune disorders by cytokine secretion and direct interactions with other immune cells (Jha & Moore, 2021). Macrophages can initiate autoimmunity by functioning as antigen-presenting cells that stimulate autoreactive T cells. Furthermore, they secrete inflammatory cytokines that enhance the survival and proliferation of these T cells, establishing a feedback loop that sustains the autoimmune response (London et al., 2013). Comprehending the pathways via which macrophages facilitate autoimmune responses has prompted the investigation of targeting these cells in therapeutic techniques. Modulating macrophage activities using biological agents or small chemicals is a viable strategy for the more effective

management of autoimmune illnesses (Brady & Thamm, 2023). In the realm of cancer, macrophages demonstrate a multifaceted role that can either inhibit or facilitate tumor advancement. In numerous malignancies, macrophages develop into tumor-associated macrophages (TAMs), frequently assuming an M2-like phenotype that facilitates tumor proliferation and immune evasion. Tumor-associated macrophages (TAMs) release many growth factors and immunosuppressive cytokines that promote tumor proliferation, angiogenesis, and metastasis (Sós et al., 2022; Zhou et al., 2018). Research has demonstrated that tumor-associated macrophages (TAMs) might foster a pro-tumor milieu via their interactions with tumor cells, hence facilitating progressive malignancies (Chung et al., 2020). Chronic inflammation, driven by macrophages, is becoming acknowledged as a pivotal role in cancer progression. Chronic exposure to inflammatory stimuli can induce genetic changes in adjacent cells, creating an environment favorable for neoplastic transformation (Stewart et al., 2024). Due to their multiple functions, targeting tumor-associated macrophages (TAMs) is a viable therapeutic approach in cancer treatment. Strategies seek to reprogram macrophages from a pro-tumor phenotype to an anti-tumor phenotype or to eliminate them from the tumor microenvironment altogether (Sós et al., 2022; Wang et al., 2021). Recent research suggests that therapeutic strategies aimed at altering TAM polarization may augment the effectiveness of current cancer therapies, hence enhancing patient outcomes (Lei et al., 2024).

2.2 Antimicrobial Peptides and Their Immunomodulatory Potential

Antimicrobial peptides (AMPs) generally consist of 12 to 50 amino acids and have a net positive charge owing to a high concentration of basic residues like lysine and arginine, enhancing their interaction with negatively charged microbial membranes. Antimicrobial peptides (AMPs) can be categorized into several types, including cationic peptides, which are further subdivided into subclasses such as linear peptides that adopt helical conformations, cysteine-rich peptides featuring disulfide bonds, and peptides abundant in specific amino acids like proline or tryptophan, as well as non-cationic peptides that operate through different mechanisms (Dziuba & Dziuba, 2014). The structural

variety and classification of antimicrobial peptides are comprehensively depicted in (Fig.1) (Zhang & Gallo, 2016).

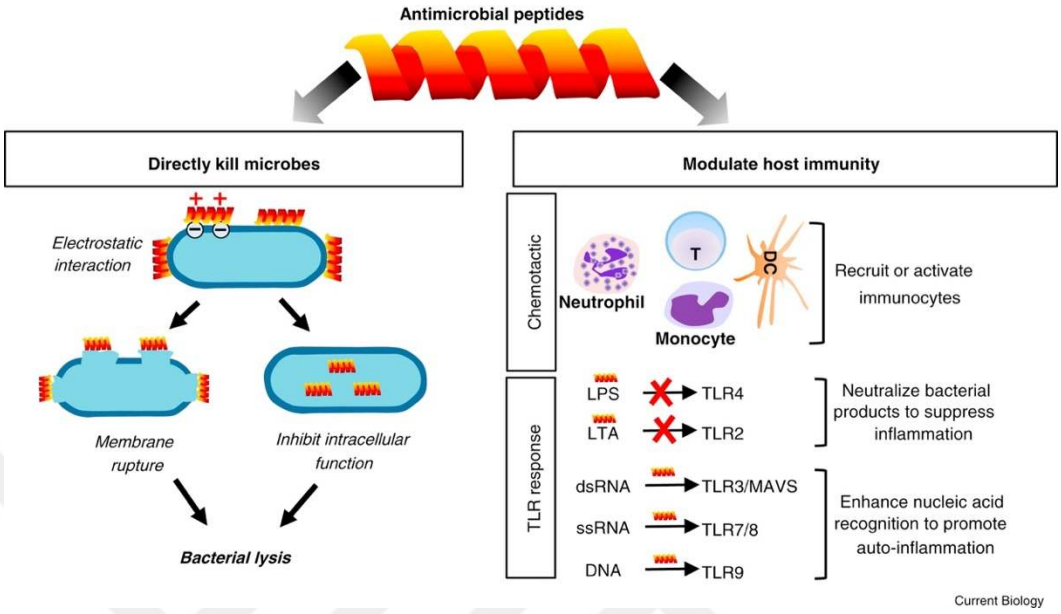


Figure 2.2.1 Biological function of antimicrobial peptides. AMPs exhibit diverse biological functions, including direct antimicrobial activity through disruption of microbial membranes, modulation of immune cell activities (e.g., macrophage activation and polarization), enhancement of wound healing, and influence on inflammatory processes. These multifaceted roles position AMPs as potential therapeutic candidates for infections, chronic inflammatory conditions, autoimmune diseases, and cancer.

The efficacy of AMPs principally resides in their capacity to break microbial membranes, resulting in cell lysis and mortality. Antimicrobial peptides (AMPs) engage with and disrupt the membranes of bacteria, fungi, and viruses, resulting in compromised membrane integrity and eventual cell death. Research demonstrates that the cationic antimicrobial peptide LL-37 compromises bacterial membranes, hence improving bacterial elimination during infections (Kim et al., 2016). Moreover, AMPs can regulate cellular pathways associated with immune responses by interacting with immune cells like macrophages, therefore affecting cytokine release and polarization states (Mohanty et al., 2021; Capparelli et al., 2012). The immunomodulatory

capability of AMPs enables them to either amplify or inhibit inflammatory responses based on the setting. In addition to their antibacterial function, AMPs exhibit significant immunomodulatory effects. Peptides such as LL-37 are essential in regulating inflammation by promoting anti-inflammatory cytokines and inhibiting pro-inflammatory responses (Mohanty et al., 2021). This dual functionality places AMPs as prospective therapeutic agents for the treatment of inflammatory disorders, such as psoriasis and rheumatoid arthritis. Moreover, AMPs play a crucial role in wound healing and tissue repair by facilitating cell migration, proliferation, and the production of new tissue. LL-37 specifically promotes keratinocyte proliferation and migration, which are essential for skin regeneration after injury (Kim et al., 2016).

Antimicrobial peptides significantly influence immune cell functions by augmenting the ability of neutrophils, macrophages, and dendritic cells to combat infections and regulate their activities. Antimicrobial peptides promote the resolution of inflammation by facilitating the change of macrophages from pro-inflammatory (M1) to anti-inflammatory (M2) phenotypes, hence assisting tissue healing (Mohanty et al., 2021; Capparelli et al., 2012). Their importance is further emphasized in disease scenarios like as diabetes, where the expression of antimicrobial peptides like LL-37 is frequently modified. Studies demonstrate that hypoxia-inducible factors can enhance LL-37 expression in diabetic individuals, highlighting its essential function in antimicrobial defense (Mohanty et al., 2021). Due of their medicinal potential, much research is being conducted to create synthetic antimicrobial peptides or improve the effectiveness of existing peptides by structural alterations. The objective is to create peptides that function as antimicrobial medicines while minimizing toxicity to human cells (Ptaszyńska et al., 2019). Research on synthetic modifications encompasses the creation of innovative hybrid compounds based on natural antimicrobial peptides, designed to improve antimicrobial effectiveness while reducing hemolytic effects (Wei et al., 2016; Berkowitz et al., 2019). Antimicrobial peptides (AMPs) offer a promising approach for novel antimicrobial therapies, especially in light of increasing antibiotic resistance in pathogens. Their broad-spectrum efficacy and immunomodulatory properties may effectively target illnesses that are difficult to treat with traditional

antibiotics (Lira et al., 2013). Numerous AMPs are presently under clinical trials, underscoring their potential for addressing infections, especially in immunocompromised individuals or chronic illnesses unresponsive to conventional therapy (Wang et al., 2015; Rodina et al., 2013).

2.2.1 Antimicrobial Peptides: Magainins, Dermaseptins, Temporins, and Bombinins

Antimicrobial peptides (AMPs) are integral to the innate immune response across several species, including amphibians, insects, and mammals. Among the numerous antimicrobial peptides, magainins, dermaseptins, temporins, and bombinins have been identified by their unique structures and biological functions. This page offers a summary of these peptides, emphasizing their modes of action, antibacterial characteristics, and prospective medicinal uses.

2.2.1.1. Magainins: Magainins are cationic peptides initially extracted from the integument of the South African clawed frog (Xenopus laevis). They consist of 23 to 25 amino acids and are distinguished by their amphipathic alpha-helical structure, which is essential for their biological function (Brand et al., 2013). The principal method via which magainins demonstrate their antibacterial activity is the breakdown of microbial membranes. Their cationic properties promote adhesion to the negatively charged elements of microbial membranes, resulting in pore development and eventual cell lysis (Diaconescu et al., 2018). Moreover, magainins exhibit immunomodulatory effects, augmenting the functionality of immune cells, including macrophages. In addition to their direct antibacterial effects, magainins facilitate wound healing by stimulating fibroblast migration and proliferation (Abed et al., 2013). Their efficacy against a wide range of pathogens, encompassing Gram-positive and Gram-negative bacteria, fungi, and viruses, highlights their potential as therapeutic agents in infectious disorders.

2.2.1.2 Dermaseptins: Dermaseptins are a group of peptides extracted from the skin secretions of multiple frog species, including those of the Phyllomedusa genus. These peptides, often comprising 28 to 34 amino acids, possess a cationic and amphipathic structure that enhances their interaction with microbial membranes (Proaño-Bolaños et al., 2019). Like magainins, dermaseptins compromise microbial membranes, resulting in cell death. They create transmembrane holes, resulting in the depolarization of the target cell (Diaconescu et al., 2018). Dermaseptins can interact with immune cells, regulating their functions, including cytokine production, to refine the immune response (Shi et al., 2016). Recent research has underscored the anticancer efficacy of dermaseptins. Dermaseptin B2 and B3 exhibit specific cytotoxicity against many cancer cell lines while preserving healthy cells (Shi et al., 2016; Couty et al., 2021). The process has been ascribed to membrane rupture and the induction of death in tumor cells, positioning dermaseptins as potential chemotherapeutic drugs (Tan et al., 2018).

2.2.1.3 Temporins: Temporins are diminutive, cationic antimicrobial peptides (about 10–13 amino acids) predominantly extracted from the skin of several frog species, especially Ranid frogs. Their small size and unique amino acid structure confer notable antiviral and antibacterial activities (Brand et al., 2013). Temporins typically exert their effects by disrupting bacterial membranes. They can traverse lipid bilayers and facilitate pore development, resulting in cell lysis. Temporins' capacity to swiftly and efficiently target pathogens renders them an essential component of the innate immune defense (Zhu et al., 2018). Besides its antibacterial properties, temporins are believed to modulate inflammation, potentially augmenting the overall immune response to infections (Couty et al., 2021). Investigations into their immunomodulatory functions are ongoing, underscoring their prospective therapeutic uses in managing inflammatory disorders.

2.2.1.4 Bombinins: Bombinins are a distinct category of antimicrobial peptides extracted from the integument of Bombina species. These peptides vary in length from 16 to 28 amino acids and exhibit a distinctive structure featuring an amphipathic alpha-helical section (Zoggel et al., 2010). Bombinins function akin to other antimicrobial peptides by targeting microbial membranes, instigating hole development, and resulting in cellular demise. Their cationic properties allow for preferential interaction with negatively charged bacterial membranes (Huang et al., 2017). Recent research reveal that bombinins exhibit a diverse array of biological actions, encompassing anti-inflammatory and wound-healing properties. Their engagement with immune cells may facilitate pathogen clearance and augment tissue healing (Jiang et al., 2014). This complex action highlights their potential use as therapeutic agents in infectious illnesses and inflammatory disorders.

Magainins, dermaseptins, temporins, and bombinins illustrate the many and powerful functions of antimicrobial peptides in host defense. Their direct antibacterial effects, along with immunomodulatory and anticancer characteristics, underscore their promise as innovative therapeutic agents. The continuous investigation into their mechanisms and activities underscores the significance of AMPs in formulating novel ways for addressing infections, regulating inflammation, and perhaps treating cancer.

2.3 Maximin S1 Peptide

Maximin S1 is defined by its short amino acid sequence, generally comprising around 13 to 21 residues. This peptide possesses a unique amphipathic structure, consisting of hydrophobic and positively charged amino acids. The amphipathic characteristic is essential for its interaction with microbial membranes, enabling the peptide to penetrate lipid bilayers and compromise membrane integrity (Lee et al., 2005). The sequence of Maximin S1 may be modified to enhance its stability and efficacy, as demonstrated in research investigating alterations to improve antibacterial characteristics. Maximin S1 is an antimicrobial peptide derived from the skin secretions of the Chinese red-bellied toad, Bombina maxima, and is attracting interest for its notable antibacterial and immunomodulatory properties. Maximin S1 largely

exerts its antibacterial effects by disrupting microbial cell membranes. Research demonstrates that its cationic properties facilitate robust interactions with negatively charged bacterial membranes, resulting in pore formation and subsequent cell lysis (Dennison et al., 2013; Wang et al., 2021). The peptide exhibits extensive activity against various pathogens, encompassing both Gram-positive and Gram-negative bacteria, as well as certain fungal species, underscoring its potential as an alternative therapeutic agent in the fight against antibiotic-resistant infections (Savoia et al., 2008; Wang et al., 2021).In comparison to other antimicrobial peptides (AMPs) such as magainins or dermaseptins, Maximin S1 has comparable membrane-disrupting properties, albeit with noted differences in efficacy and contextual applications (Chu et al., 2015). In addition to its direct antimicrobial effect, Maximin S1 exhibits immunomodulatory characteristics, affecting macrophage activity via fostering anti-inflammatory responses and augmenting phagocytic function. These multiple functions facilitate the preservation of immunological homeostasis, especially in inflammatory situations (Dennison et al., 2015; Dennison et al., 2017). The peptide facilitates wound healing by directing macrophage polarization towards an anti-inflammatory (M2) phenotype, therefore enhancing tissue repair and alleviating inflammation (Dennison et al., 2017). Initial research suggests its potential use in oncology, as Maximin S1 preferentially triggers apoptosis in cancer cells while minimally impacting healthy tissues (Dennison et al., 2017). Current investigations into the structure-activity relationship of Maximin S1 encompass multiple alterations, such as amino acid substitutions, C-terminal modifications, and peptide cyclization, with the objective of improving its pharmacological stability, selectivity, and bioavailability (Ortiz-Gómez et al., 2020; Lee et al., 2005). These synthetic derivatives may result in more potent, efficacious, and therapeutically viable medicinal medicines (Kurpe et al., 2020; Kravchenko et al., 2023). Amidst the rising challenge of antibiotic resistance, Maximin S1 and analogous peptides present encouraging potential as multifunctional therapeutic agents that can bypass conventional resistance mechanisms via their many modes of action (Kravchenko et al., 2023; Liu et al., 2011).

2.3.1 Biological Activities of Maximin S1

Maximin S1 exerts its antimicrobial effects primarily through membrane disruption. Possessing a positive charge and amphipathic structure, Maximin 1 readily interacts with the negatively charged microbial membranes (Dennison et al., 2015). Upon binding, the peptide inserts into the lipid bilayer, disrupting membrane integrity through pore formation and subsequently leading to microbial cell lysis (Laverty et al., 2010). This peptide exhibits broad-spectrum antimicrobial activity, effectively targeting various pathogens, including Gram-positive and Gram-negative bacteria. Specifically, studies have documented that Maximin 1 inhibits the growth of clinically significant pathogens such as Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa, highlighting its utility as a potent antimicrobial agent (Tikhonov et al., 2024). Moreover, its antimicrobial effects are concentration-dependent, with the Minimum Inhibitory Concentration (MIC) varying across different bacterial species, indicating differential susceptibilities. These findings support the therapeutic potential of Maximin 1 as a candidate for future antimicrobial drug development (Ravallec-Plé et al., 2001). Beyond its direct antimicrobial functions, Maximin 1 has demonstrated significant immunomodulatory properties. It enhances the activation, migration, and phagocytic capabilities of macrophages, contributing to a stronger and more effective immune response against infections (García et al., 2024). Additionally, it may play a crucial role in resolving inflammation by modulating cytokine production, thereby maintaining a balance between pro-inflammatory and anti-inflammatory signals during infection and wound healing processes (Lee et al., 2005).

Considering clinical applications, Maximin 1's broad-spectrum antimicrobial activity positions it as a promising alternative or adjunctive treatment, especially in scenarios where antibiotic resistance presents a significant challenge. Ongoing research aims to enhance its stability and effectiveness through structural modifications such as fatty acylation or conjugation to drug delivery systems (Dennison et al., 2016). Furthermore, preliminary studies exploring its anticancer properties suggest that Maximin 1 may exhibit selective cytotoxicity towards specific cancer cell lines, opening new avenues for potential anticancer therapies (Dennison et al., 2017).

2.4 Immunomodulation and Signaling Pathways in Macrophages

Several critical signaling pathways are involved in the immunomodulatory functions of macrophages, notably the Toll-like receptor (TLR) pathways, Notch signaling pathway, as well as MAPK and PI3K/Akt pathways. TLRs are essential components of the innate immune response, recognizing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). When TLRs, particularly TLR2 and TLR4, are activated, they initiate intracellular signaling cascades that lead to the production of pro-inflammatory cytokines and chemokines (Son et al., 2020). The activation of TLRs leads to the recruitment of adaptor proteins such as MyD88, subsequently activating MAPK pathways (ERK, JNK, and p38) and NF-κB transcription factors (Shen et al., 2017). These pathways promote the expression of pro-inflammatory cytokines (e.g., TNF-α, IL-6) and enhance the functional responses of macrophages (Wang et al., 2018). This results in macrophage activation towards the M1 phenotype, characterized by inflammatory responses and pathogen clearance. Conversely, inappropriate or excessive TLR activation may contribute to chronic inflammatory conditions and tissue damage. The Notch signaling pathway significantly influences macrophage polarization. Activation of Notch can skew macrophage differentiation toward the M2 phenotype, promoting anti-inflammatory responses and tissue repair while inhibiting excessive M1 activation (Wang et al., 2023; Prayitno & MS, 2016). Additionally, cross-talk between Notch signaling and other pathways enhances macrophage responsiveness to cytokines like IL-4 and IL-13, which are crucial for alternative macrophage activation. This makes Notch signaling an attractive target for therapeutic interventions in diseases characterized by dysregulated inflammatory responses (Wang et al., 2023). The PI3K/Akt signaling pathway also plays a vital role in macrophage survival, proliferation, and function. Activation of this pathway often promotes the M2 macrophage phenotype, enhancing anti-inflammatory signaling and tissue repair processes. Conversely, inhibitors of this pathway can skew macrophages toward a more inflammatory phenotype, highlighting its regulatory potential. Macrophage polarization is influenced by an interplay

between the PI3K/Akt pathway and MAPK pathways, with the PI3K/Akt pathway supporting M2 polarization and MAPK pathways driving M1 polarization, underscoring the complexity of macrophage responses to various stimuli (Du et al., 2022). Macrophages exhibit several immunomodulatory functions influenced by these signaling pathways. Depending on their polarization state, macrophages can secrete various cytokines that modulate immune responses. M1 macrophages produce pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α, promoting inflammation, whereas M2 macrophages secrete anti-inflammatory cytokines like IL-10 and TGF-β, contributing to the resolution of inflammation and tissue repair (Son et al., 2020; Wang et al., 2018). Macrophages also respond to pathogens through phagocytosis, a process enhanced by TLR signaling and other pathways, essential for infection control and tissue homeostasis (Kawanaka & Taylor, 2011). After the initial inflammatory response, macrophages transition to an M2 polarized state to facilitate wound healing by contributing to tissue remodeling, producing growth factors, and promoting recruitment and differentiation of cells necessary for repair (Shen et al., 2017). Moreover, macrophages shape adaptive immunity through antigen presentation, influencing T cell activation, differentiation, and memory formation, thus bridging innate and adaptive immune responses (Kawanaka & Taylor, 2011). (Fig2)

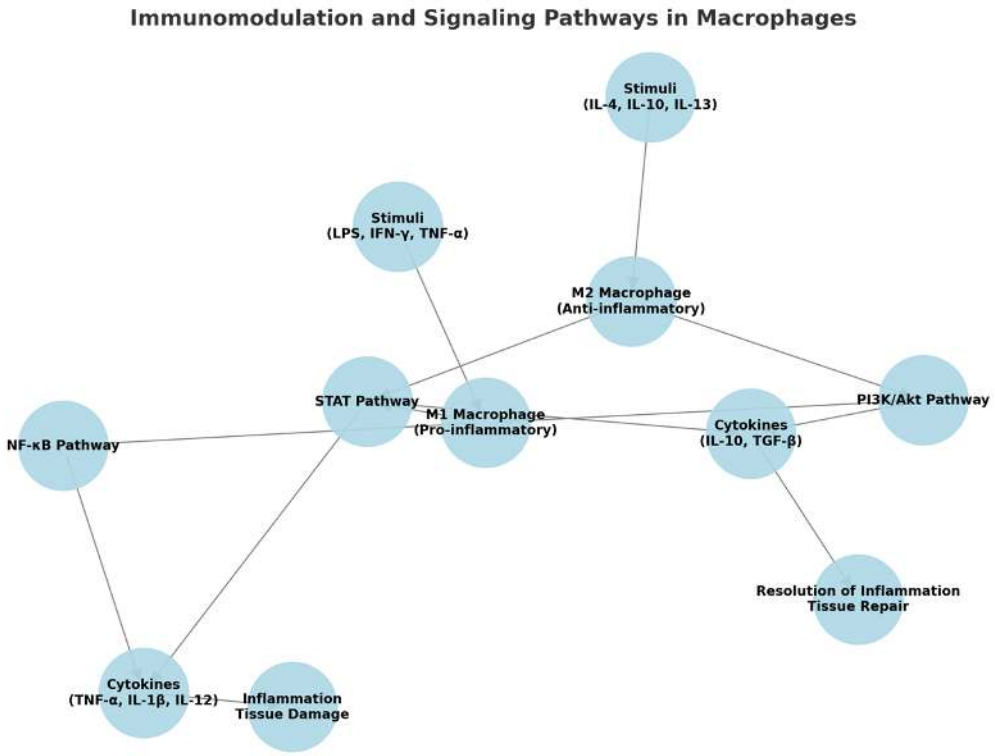


Figure 2.4.1 Signaling Pathways in Macrophages This figure shows the M1 and M2 phenotypes of macrophages polarized in response to different stimuli (e.g. pro-inflammatory, such as LPS, IFN-γ and TNF-α; anti-inflammatory, such as IL-4, IL-10 and IL-13) and the NF-κB, STAT and PI3K/Akt signaling pathways involved in this polarization process. Pro-inflammatory M1 macrophages secrete cytokines that trigger inflammation and tissue damage, while anti-inflammatory M2 macrophages produce cytokines that promote resolution of inflammation and tissue repair.

Understanding these regulatory pathways in macrophage function opens avenues for therapeutic interventions. Drugs targeting TLR, Notch, or PI3K/Akt pathways could enhance macrophage function in diseases such as cancer, chronic inflammatory disorders, and infections. These strategies aim to promote M2 polarization to facilitate tissue repair while limiting M1-driven pathological inflammation (Wang et al., 2018; Wang et al., 2023). Enhancing macrophage function through bioactive compounds, such as polysaccharides, which activate macrophages via TLR-dependent pathways and upregulate essential pro-inflammatory cytokines, has also gained interest as an immunotherapy approach (Shen et al., 2017). Additionally, utilizing mesenchymal stem cells (MSCs) or biomaterials to modulate macrophage

activity represents a promising strategy for tissue regeneration in diseases characterized by chronic inflammation (Lin et al., 2017).

2.4.1. Key Signaling Pathways: NF-κB, STAT, and PI3K/Akt

The intricate web of signaling pathways that govern cellular functions is crucial for understanding both normal physiological processes and the pathological events contributing to diseases such as cancer, inflammation, and metabolic disorders. Among these pathways, NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), STAT (signal transducer and activator of transcription), and PI3K/Akt (phosphoinositide 3-kinase/protein kinase B) signaling pathways are particularly significant due to their central roles in modulating diverse cellular responses, including cell proliferation, apoptosis, differentiation, inflammation, and immune responses (Yu et al., 2020; Liu et al., 2017; Porta et al., 2014).

2.4.1.1 Overview of NF-κB signaling and its role: NF-κB is typically found in the cytoplasm in an inactive state bound to its inhibitor, IκB. Upon activation by stimuli such as pro-inflammatory cytokines (e.g., TNF-α, IL-1), pathogen-associated molecular patterns, or stress signals, IκB is phosphorylated and subsequently degraded through the ubiquitin-proteasome pathway. This degradation allows NF-κB dimers, predominantly p65/p50, to translocate to the nucleus where they initiate transcription of target genes linked to inflammation, cell survival, and immune responses (Wan & Lenardo, 2009), Chen et al., 2020). Emerging studies emphasize how NF-κB regulates various genes, including those responsible for the synthesis of pro-inflammatory cytokines, indicating its strategic position as a central regulatory hub for inflammatory responses (Chen et al., 2017). The nuclear translocation of NF-κB not only activates genes involved in inflammation but also regulates pathways crucial for cell proliferation, demonstrating its role in promoting survival and resistance to apoptosis in cancers (Guo et al., 2022). In cancer, aberrant activation of NF-κB signaling has been implicated in promoting tumor cell proliferation and metastasis. For instance, NF-κB activation has been associated with enhanced cancer cell survival and invasiveness, suggesting that

it contributes to a more aggressive cancer phenotype (Guo et al., 2022; , Fu et al., 2018). This underscores the significance of NF-κB signaling in the pathology of cancers, particularly those exhibiting extensive metastasis and therapeutic resistance.

The crucial role that NF-κB signaling plays in tumorigenesis is further illustrated in various types of cancers, including breast cancer and nasopharyngeal carcinoma. NF-κB activation has been documented in nasopharyngeal carcinoma as a consequence of Epstein-Barr virus (EBV) infection, wherein EBV latent membrane protein 1 induces a positive feedback loop enhancing constitutive NF-κB activation, leading to heightened malignancy (Verhoeven et al., 2018; , Verhoeven et al., 2016). This relationship between viral pathology and NF-κB signaling exemplifies how viral oncogenes can manipulate host signaling pathways to facilitate cancer progression. Additionally, specific inhibitors targeting the NF-κB pathway can disrupt its oncogenic functions. Inhibitors of IκB kinase (IKK) have demonstrated potential as therapeutic agents by blocking NF-κB activation, thereby diminishing tumor growth and enhancing sensitivity to chemotherapy in models of lung and breast cancers (Cherry et al., 2015; , Derudder et al., 2016). Furthermore, modulation of NF-κB by agents such as silibinin has shown promise in alleviating myocardial ischemia/reperfusion injuries, implicating this pathway in not just oncogenic but also cytoprotective phenomena (Chen et al., 2020).

NF-κB does not operate in isolation; it interacts with various other signaling pathways, including the STAT and PI3K/Akt pathways. The integration of these pathways is crucial for determining the ultimate cellular response to external stimuli. For instance, IL-6 can activate both STAT3 and NF-κB pathways, leading to synergistic effects that promote survival and proliferation in cancer cells (Sun et al., 2022). This coordination highlights the complexity of signaling networks, where NF-κB may cooperatively enhance both inflammatory responses and tumorigenesis by working in concert with other oncogenic pathways. Moreover, the NF-κB pathway has been linked to chemoresistance mechanisms observed in gastric and colorectal cancers, where the epithelial-mesenchymal transition (EMT) is driven by NF-κB activation.

This suggests that NF-κB contributes to a more invasive phenotype, resistant to conventional therapeutic modalities, thereby complicating treatment strategies (Fu et al., 2018; , Xing et al., 2016). Given the multifaceted roles of NF-κB in health and disease, therapeutic strategies that inhibit its pathway represent an attractive avenue for intervention. The specificity of targeting NF-κB could minimize side effects while preserving critical physiological functions. However, careful consideration of the tissue-specific roles of NF-κB is paramount, as complete inhibition could have detrimental effects on immune functions and wound healing processes (Wan & Lenardo, 2009). Various agents, including both synthetic and natural compounds, have emerged as promising inhibitors of NF-κB signaling. These include curcumin, resveratrol, and more specific small molecules targeting IKK or downstream transcriptional activities of NF-κB (Chen et al., 2020). These advances open up possibilities for novel pharmacological interventions aimed at manipulating NF-κB activity in a context-dependent manner.

2.4.1.2 STAT signaling pathways and their involvement in macrophages:

The JAK/STAT signaling pathway is initiated when cytokines or growth factors bind to their corresponding receptors on the cell surface. This binding facilitates the aggregation of JAK proteins with these receptors, leading to the activation of the JAKs through mutual phosphorylation. Subsequently, the activated JAKs phosphorylate specific tyrosine residues on the receptors, creating docking sites for the STAT proteins (Tang et al., 2018). Upon their recruitment, the STAT proteins become phosphorylated by JAKs, promoting the dimerization of STAT molecules, which then translocate to the nucleus to modulate the expression of target genes associated with a variety of cellular functions (Li et al., 2020). STAT proteins, including STAT1, STAT3, and STAT5, exhibit distinct roles in regulating cellular processes. For instance, STAT3 activation has been extensively documented to promote the proliferation and survival of cancer cells, while also inhibiting apoptosis (Pan et al., 2020; , Zhu et al., 2019). The aberrant activation of STAT3 is especially prevalent in solid tumors such as non-small cell lung cancer (NSCLC) and breast cancer, where it underlies aspects of tumor progression, including

angiogenesis and immune evasion (Pan et al., 2020; , An & Guo, 2022). Dysfunction in the JAK/STAT pathway is frequently observed in various cancers. For example, in colon cancer, activation of the JAK/STAT signaling pathway plays a pivotal role in regulating cancer cell cycle and promotes oncogenic processes (Tang et al., 2018). Similarly, studies have indicated that constitutively activated STAT3 in breast cancer enhances malignancy by supporting tumor cell proliferation and survival (Reipschläger et al., 2012). The findings regarding STAT3 have prompted therapeutic approaches to target this protein or the upstream JAK kinases as potential cancer therapies (Li, 2008). In melanoma, the prognostic significance of the JAK/STAT pathway has been highlighted, where specific STAT family genes may serve as biomarkers for patient outcomes (Pan et al., 2020). Likewise, in hepatocellular carcinoma (HCC), the JAK/STAT pathway is implicated in driving tumor progression, and disruption of this pathway has shown potential for enhancing sensitivity to therapeutics (Dong et al., 2019). These collective insights underscore the significance of the JAK/STAT pathway in various cancers and illuminate the mechanisms through which it influences prognosis. The JAK/STAT pathway does not function in isolation; it exhibits considerable cross-talk with other signaling cascades, such as the PI3K/Akt and MAPK pathways. Such interactions can lead to complex regulatory networks that influence tumor progression. For instance, aberrant activation of JAK/STAT signaling can cooperatively enhance signaling through the PI3K pathway, promoting cellular survival and evasion from apoptosis (Jaganathan et al., 2014; , Birnie et al., 2008). This interplay further complicates the treatment landscape, as therapies aimed at disrupting one pathway may inadvertently impact others, necessitating a comprehensive understanding of these networks for effective therapeutic strategies. Moreover, specific inhibitors of JAK kinase activity have been developed and are being employed in clinical settings. These inhibitors have shown promise in treating various malignancies by blocking the aberrant JAK/STAT signaling, leading to reduced tumor growth and enhanced apoptosis in cancer cells (Furqan et al., 2013). The efficacy of such inhibitors highlights the therapeutic potential of targeting these pathways in treating cancers characterized by dysregulated JAK/STAT signaling. The expression levels of various components of the JAK/STAT pathway can serve as

prognostic markers in various malignancies. For instance, elevated levels of phosphorylated STAT3 have been correlated with poor outcomes in glioblastoma and renal cancer (Chang & Lai, 2019). Understanding the status of JAK/STAT pathway components not only provides insights into disease prognosis but also aids in identifying patients who might benefit from targeted therapies. Additionally, ongoing research aims to explore the therapeutic potential of natural compounds that modulate JAK/STAT signaling. For example, studies have demonstrated that compounds like curcumin can inhibit the JAK/STAT pathway, reducing cell proliferation and tumor growth in various cancer models (Saydmohammed et al., 2010). Furthermore, the development of small-molecule inhibitors specifically designed to target the JAK/STAT pathway is advancing rapidly, and their combination with existing therapies may enhance overall treatment efficacy (Kim et al., 2008).

2.4.1.3 PI3K/Akt pathway's importance in macrophage survival, polarization, and inflammatory responses: The PI3K/Akt pathway is integral in promoting macrophage survival, particularly in the context of oxidative stress and inflammation. Activation of this pathway enhances cell proliferation and protects against apoptotic signals, enabling macrophages to thrive under adverse conditions (Cui et al., 2021; Li et al., 2022). Activation of Akt, a key downstream effector of PI3K, is critical for inhibiting pro-apoptotic signaling pathways, thus ensuring macrophage longevity in tissue environments where they are needed for immune responses (Zhang & Dong, 2022). Studies have shown that macrophage-specific deletion of PI3K results in elevated apoptotic rates, confirming its essential role in macrophage survival (Li et al., 2022). Moreover, macrophages that are primed with pro-inflammatory signals, such as lipopolysaccharides (LPS), experience enhanced PI3K/Akt activation, further supporting their functional viability during inflammatory episodes. This survival signal allows macrophages to effectively respond to pathogens and participate in the inflammatory process (Li et al., 2017). In addition to facilitating survival, the PI3K/Akt pathway is crucial for macrophage polarization into distinct phenotypes, specifically M1 and M2 macrophages. M1 macrophages are typically associated with pro-inflammatory

responses, while M2 macrophages are linked to anti-inflammatory processes and tissue repair (Fan et al., 2019; Wang et al., 2022). The balance between these polarization states is critical in determining the overall immune response and inflammatory landscape. The activation of PI3K/Akt signaling has been shown to promote M2 macrophage polarization, which is vital for resolving inflammation and encouraging tissue repair (Zhou et al., 2020; Wang et al., 2018). For instance, Zhou et al. demonstrated that the secreted protein HSPA12B from tumor-associated endothelial cells activates the PI3K/Akt/mTOR pathway, leading to M2 polarization of macrophages. This highlights how external signals can modulate macrophage phenotype through signaling pathways to adapt to the tissue microenvironment (Zhou et al., 2020). Conversely, evidence suggests that inhibition of the PI3K/Akt pathway can drive macrophages towards an M1 phenotype, which secretes pro-inflammatory cytokines, further elucidating the pathway's regulatory role in macrophage energetics and immune responses during inflammation (Wang et al., 2022; Yu et al., 2021). The differential roles of Akt isoforms (Akt1 and Akt2) in this polarization process have also been highlighted, suggesting that modulation of specific Akt isoforms could be a therapeutic target in diseases characterized by macrophage polarization dysregulation (Lin et al., 2024).

The role of the PI3K/Akt pathway in mediating inflammatory responses is significant. Activation of this pathway is associated with the production of pro-inflammatory cytokines such as TNF-α and IL-6 from M1 macrophages, which are essential for orchestrating an effective immune response (Dong et al., 2014; Zhao et al., 2014). In macrophages, PI3K/Akt signaling enhances the transcription of genes involved in innate immunity and inflammation, thereby amplifying the body's response to pathogens. For instance, studies examining macrophage responses to LPS show that PI3K inhibition leads to diminished production of pro-inflammatory cytokines, indicating that the PI3K/Akt pathway is a critical mediator of inflammatory signaling (Luyendyk et al., 2008). Similarly, research indicates that the PI3K/Akt pathway modulates responses to various pathogens, including viral infections, by influencing macrophage polarization and their ensuing inflammatory responses (Zhao et al., 2014; Rocher & Singla, 2013). Furthermore, the cross-talk between the

PI3K/Akt pathway and other signaling cascades, such as NF-κB and MAPK, illustrates the complexity of immune response regulation. This cross-regulation can determine whether macrophages adopt a pro-inflammatory or anti-inflammatory role, depending on the stimuli encountered (Arranz et al., 2012; Luyendyk et al., 2008). Given the central role of the PI3K/Akt pathway in macrophage function, it presents a promising target for therapeutic interventions in various disease contexts. For instance, in chronic inflammatory diseases and cancer, modulating PI3K/Akt signaling could help skew macrophage polarization towards beneficial phenotypes that promote tissue repair and resolution of inflammation (Zhou et al., 2020; Tan et al., 2018). Additionally, the use of PI3K inhibitors has been considered in treating diseases characterized by excessive M1 polarization and insufficient M2 responses, offering a means to restore balance in macrophage functions (Wang et al., 2022). Natural compounds such as resveratrol have also been shown to regulate macrophage polarization through the PI3K/Akt pathway, providing a potential avenue for dietary or supplemental interventions to modulate macrophage activity in disease states (Xu et al., 2023). These insights underscore the therapeutic potential of targeting PI3K/Akt signaling not just for macrophage-related diseases but for a broad range of immunopathologies. The PI3K/Akt pathway is critically involved in the survival, polarization, and inflammatory responses of macrophages. By influencing these processes, the pathway plays a vital role in shaping the immune landscape and ensuring effective responses to various stimuli. Understanding the intricacies of this signaling pathway offers promising avenues for therapeutic interventions aimed at modulating macrophage function in both inflammatory diseases and cancers.

2.4.2 Potential therapeutic implications of NF-κB, STAT, and PI3K/Akt

The NF-κB signaling pathway is frequently activated in various cancers, promoting tumorigenesis and resistance to therapies. Targeting NF-κB has emerged as a potential therapeutic strategy in cancers where this pathway plays a pivotal role. For instance, inhibiting NF-κB activity can sensitize cancer cells to chemotherapeutic agents, as shown in studies where NF-κB inhibitors enhanced the efficacy of treatments in breast and lung cancer models (Zhou et

al., 2018). Furthermore, the modulation of NF-κB signaling can help in managing chronic inflammatory conditions, where its overactivation contributes to inflammation and tissue damage (Yang et al., 2018). The JAK/STAT pathway, particularly STAT3, is known to be constitutively activated in many cancers, leading to enhanced survival, proliferation, and immune evasion (Fasouli & Katsantoni, 2021; , Chen et al., 2020). Targeting the JAK/STAT pathway serves as a promising therapeutic strategy, particularly for hematological malignancies such as leukemia. By inhibiting JAKs or STAT proteins, it is possible to diminish the oncogenic effects mediated by this pathway, thereby reducing tumor growth and enhancing sensitivity to conventional therapies (Chen et al., 2020). The PI3K/Akt signaling pathway is involved in regulating numerous cellular processes, including metabolism, growth, survival, and migration, which are particularly relevant in cancer biology. Given that aberrant activation of this pathway is implicated in a significant proportion of human cancers, including ovarian and prostate cancers, targeting the PI3K/Akt pathway has been identified as a promising therapeutic strategy (Yuan et al., 2019; , Miao et al., 2020). Notable strategies include the development of selective PI3K inhibitors and dual inhibitors targeting both the PI3K and mTOR pathways, which have shown varying degrees of success in clinical trials. In summary, the therapeutic implications of the NF-κB, STAT, and PI3K/Akt pathways are substantial, encompassing a range of possible strategies to improve treatment efficacy across various disease states. The ongoing research into specific inhibitors, natural compounds, and combination therapies highlights the potential for novel interventions in modulating these critical signaling cascades and enhancing patient outcomes in cancer and inflammatory diseases.

2.5 Significance of Research

The significance of research on the NF-κB, STAT, and PI3K/Akt pathways lies in their fundamental roles in regulating key cellular processes linked to inflammation, immune responses, and cancer progression. As our understanding of these pathways deepens, the potential for developing targeted therapies grows, offering new hope for treating a variety of diseases. Integrative approaches that consider the crosstalk between these pathways may enhance treatment efficacy and provide novel therapeutic strategies that could transform patient care.

3. METHODS

The J774.2 macrophage cell line provides a robust and versatile platform for exploring macrophage functionality due to its characteristic features, such as efficient phagocytosis, responsive cell signaling, and cytokine production—traits essential for understanding immune responses (O'Rourke et al., 2001). The ease of manipulation and consistent behavior of these cells allow researchers to dissect molecular mechanisms underpinning macrophage activation and modulation across various disease contexts, including infections and inflammatory conditions (Degrossoli & Giorgio, 2007). Research employing J774.2 macrophages has notably expanded our comprehension of inflammatory processes, especially in scenarios involving bacterial infections, chronic inflammatory diseases, and cancer. For example, these cells respond robustly to lipopolysaccharides (LPS) and pro-inflammatory cytokines by activating critical signaling cascades such as NF-κB, which orchestrate the production of cytokines pivotal to inflammation (Ohtsu et al., 2017). Insights derived from these studies facilitate the development of targeted anti-inflammatory therapies and innovative treatments for diseases characterized by dysregulated inflammation. Additionally, J774.2 cells are instrumental in cancer research, specifically regarding the role of macrophages within the tumor microenvironment. The interactions between tumor-associated macrophages and cancer cells significantly influence tumor progression, metastasis, and therapeutic resistance (Park et al., 2013). Research utilizing J774.2 macrophages continues to shed light on how macrophages either promote or inhibit tumor growth, thereby informing therapeutic strategies aimed at reprogramming macrophage polarization within tumors. In drug development and pharmacological screening assays, the J774.2 cell line is frequently employed due to its predictable responses to various therapeutic agents. Studies have demonstrated how multiple compounds, including phytochemicals and novel synthetic drugs, can modulate macrophage activity by attenuating inflammation or influencing polarization toward either the pro-inflammatory (M1) or anti-inflammatory (M2) phenotype (Szabó et al., 2008). This ability to reliably assess drug effects facilitates the identification of promising therapeutic candidates for subsequent preclinical and clinical

evaluation. Furthermore, J774.2 macrophages have proven critical for elucidating signaling pathways involved in macrophage biology, such as PI3K/Akt, and NF-κB pathways. Investigations into these pathways have deepened our understanding of macrophage functions and illuminated potential therapeutic targets (Degrossoli & Giorgio, 2007; Ohtsu et al., 2017). Studying these signaling cascades in J774.2 cells has helped reveal intricate pathway interactions and their collective impact on macrophage behavior under various pathological conditions. The J774.2 macrophage cell line also serves as an invaluable model for assessing vaccine efficacy and immunotherapy potential, allowing researchers to investigate immune responses elicited against specific antigens. Studies have explored how various vaccine formulations modulate macrophage activation and have used functional assays to evaluate cellular immune responses within controlled environments (Guns et al., 2010; Moreno-Altamirano et al., 2007).

Moreover, research utilizing J774.2 cells has significantly advanced our understanding of pathogen-macrophage interactions. Investigations into pathogens such as Brucella spp. have utilized these macrophages to elucidate how microorganisms manipulate macrophage signaling and functions to evade the host immune system and facilitate intracellular survival (Park et al., 2013; Moreno-Altamirano et al., 2007). Findings from these studies are pivotal in developing targeted therapeutic approaches and effective vaccines against infectious diseases. Also, J774.2 macrophages are extensively used for assessing the biocompatibility and immunogenicity of new biomaterials, including nanoparticles and advanced drug delivery systems. Researchers evaluate how these materials interact with macrophages to determine their potential cytotoxicity and inflammatory responses (Sobierajska et al., 2021). Such evaluations are crucial for guaranteeing the safety and effectiveness of biomaterials designed for medical implants and drug administration systems. Lastly, J774.2 macrophages significantly contribute to stem cell and regenerative medicine research. By examining how macrophage polarization affects processes like wound healing, fibrosis, and tissue repair, researchers can devise strategies aimed at promoting effective tissue regeneration following injury (Wang et al., 2022). Collectively, these diverse applications underscore the extensive utility of the J774.2 macrophage cell line as a foundational model

in immunological and biomedical research.

J774.2 immortalized mouse macrophage cell lines were used throughout the study. These cells were cultured in standard cell culture conditions (37°C, 5% CO2) in complete DMEM medium supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin-streptomycin). The macrophage cells were treated with varying concentrations of the immunomodulatory peptide Maximin S1, obtained from Novopro Bioscience Inc. Control groups received no treatment or vehicle controls. The treatment duration was optimized based on preliminary experiments to ensure effective modulation of macrophage phenotypes.

3.1 ELISA

Enzyme-Linked Immunosorbent Assay (ELISA) is a pivotal immunological technique utilized for detecting and quantifying specific antigens or antibodies within a sample. It operates on the principle of antigen-antibody interaction, utilizing an enzyme as a label for detecting the binding event. The versatility of ELISA in various fields, particularly in medical diagnostics, food safety, and environmental monitoring, highlights its relevance in contemporary scientific research. The ELISA methodology typically involves several sequential steps: coating a microplate with the target antigen or antibody, blocking non-specific binding sites, adding the sample, followed by introducing a secondary antibody coupled to an enzyme. After an incubation period, a substrate solution is added, which reacts with the enzyme to yield a quantifiable signal, often measured as optical density using spectrophotometry (Peng et al., 2019; Levy et al., 2023). This colorimetric change is proportional to the amount of the target analyte present in the sample, thus allowing for quantitative analysis (Levy et al., 2023). Various types of ELISA exist, including direct, indirect, sandwich, and competitive formats, each tailored to meet specific needs and types of samples. For instance, the sandwich ELISA utilizes two different antibodies that recognize distinct epitopes of the target analyte, providing high specificity and sensitivity, making it suitable for complex samples such as serum or food matrices (Mast et al., 2016; El-Toukhy et al., 2022). Indirect ELISA techniques have emerged, where the antigen is attached to the plate, and the detection relies on an indirect antibody response—this has proven advantageous in

certain diagnostic applications, such as the detection of bacterial infections (Yin et al., 2021; Zhong et al., 2022). The applications of ELISA are immensely broad. It has been employed for detecting various pathogens, including Vibrio parahaemolyticus and Coxiella burnetii, where its sensitivity and specificity surpass traditional methods (Pinto et al., 2012; Meletis et al., 2021). The adaptability of ELISA has led to the development of hybrid techniques, such as PCR-ELISA, which combines the specificity of polymerase chain reaction (PCR) with the ease of ELISA, facilitating the detection of nucleic acids alongside proteins (Peng et al., 2019; Sue et al., 2014). This incorporation of molecular techniques enriches the ELISA framework, particularly for applications in microbial pathogen detection.

Recent advances in ELISA technology include novel formats such as electrochemiluminescence (ECL) and microfluidic systems, which enhance the sensitivity and throughput of assays. The use of gold nanocluster probes in an electrochemical platform allows for highly sensitive measurements even in complex biological fluids (Peng et al., 2019; Wu et al., 2022). These developments significantly improve the feasibility of ELISA in clinical settings where rapid results are paramount. The importance of specificity in ELISA cannot be overstated, especially in clinical diagnostics where cross-reactivity can lead to false positives. Research emphasizes the need for rigorous validation of antibodies and assay conditions to ensure accurate results (Meletis et al., 2021; Mast et al., 2016; Selman et al., 2012). As the landscape of diseases evolves, so too does the necessity for ELISA to adapt, leveraging monoclonal antibodies and optimizing conditions for various analytes, as seen in ongoing studies focusing on viral infections and foodborne pathogens.

In line with these principles, cell viability and various biological parameters were quantitatively analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) techniques. Commercially available ELISA kits (Thermo Fisher, Sigma-Aldrich, Invitrogen) were used to measure cytokines and other biomarkers. Measurements were carried out using a calibrated ELISA plate reader, ensuring accurate and reproducible results.

3.2 Flow Cytometry

Flow cytometry is a sophisticated analytical technique that enables the simultaneous measurement of multiple physical and chemical characteristics of individual cells or particles as they flow in a fluid stream past a laser or another light source. This technology has revolutionized cellular analysis by allowing researchers to assess various properties, including cell size, granularity, and surface marker expression, with remarkable speed and precision (Adan et al., 2016; Tan et al., 2023). The fundamental principle underlying flow cytometry is based on light scattering and fluorescence emission, which occur when cells are illuminated by laser light as they pass through the illuminated region. The use of flow cytometry spans a vast array of applications in both research and clinical settings. In clinical diagnostics, flow cytometry is employed for immunophenotyping, which is crucial for the diagnosis and classification of hematological malignancies such as leukemias and lymphomas. For instance, studies have demonstrated the utility of flow cytometry in assessing the presence and proportion of specific cell populations, such as identifying malignant B cells in chronic lymphocytic leukemia (Abdel-Ghafar et al., 2012). This technique allows for the rapid analysis of thousands of cells, leading to accurate quantification of various tumor markers and facilitating timely interventions. Additionally, flow cytometry has shown significant promise in immunological research. By analyzing different immune cell populations, researchers can uncover insights into immune responses during infections, autoimmune disorders, and vaccine efficacy. For example, flow cytometric analysis has been instrumental in measuring the frequency and functional status of T cells, providing deep insights into the mechanisms of immune regulation (Jyoti et al., 2023). The use of standardized protocols and carefully selected fluorochromes enhances reproducibility and data reliability, which is critical for robust scientific inference (Lucas et al., 2019). In this study, Flow cytometry was utilized to evaluate macrophage activation states, polarization (M1/M2 phenotype), and cell surface markers. Cells were stained with fluorescently labeled antibodies specific to macrophage markers and analyzed using calibrated flow cytometers. The resulting data were analyzed with FlowJo software, and regular calibration checks ensured the accuracy and reliability of results.

3.2.1 Data Collection and Analysis

Data obtained from RT-qPCR, Western blot, ELISA, and flow cytometry analyses were systematically collected, organized, and analyzed to ensure comprehensive evaluation of macrophage responses following treatment. Each dataset was subjected to rigorous statistical assessment to determine the significance and consistency of observed biological effects. All statistical analyses were performed using GraphPad Prism, a widely recognized and validated software platform for biostatistical computations in biomedical research. To compare differences between two experimental groups, the Student's t-test was employed, providing insight into pairwise variations in measured parameters. For analyses involving more than two groups, One-Way Analysis of Variance (ANOVA) was utilized to detect overarching differences among multiple experimental conditions. When significant differences were detected via ANOVA, appropriate post-hoc tests such as Tukey's or Bonferroni correction were applied to identify specific group-to-group disparities with controlled error rates.

In addition to hypothesis testing, regression and correlation analyses were conducted to explore the relationships between gene expression levels and phenotypic responses, including cytokine production, surface marker expression, and macrophage polarization profiles. These analyses further enabled the evaluation of dose-response effects, offering valuable insight into the pharmacodynamics of the applied treatment. To maintain high standards of data quality and integrity, all analytical instruments—including real-time PCR machines and flow cytometers—were regularly subjected to calibration and quality control checks in accordance with manufacturer guidelines and institutional laboratory protocols. Protein quantification from Western blot assays was conducted using ImageJ software, which allowed for accurate densitometric analysis and normalization against housekeeping proteins, thus ensuring quantitative robustness.

3.3 Materials

To ensure the accuracy, reproducibility, and reliability of the experimental procedures, all materials and reagents used in the study were selected based on their established performance in molecular biology research. The macrophage cell line J774.2, a widely used immortalized mouse macrophage model, was chosen due to its well-characterized immune functions and responsiveness to immunomodulatory agents. These cells were procured and maintained under standard cell culture conditions in accordance with established protocols. The immunomodulatory agent used in the study, Maximin S1 peptide, was obtained from Novopro Bioscience Inc., a trusted commercial supplier known for providing high-purity peptides suitable for in vitro experimentation. In addition, all chemicals and reagents employed throughout the research—including those used for reverse transcription quantitative PCR (RT-qPCR), Western blotting, and enzyme-linked immunosorbent assay (ELISA)—were acquired from reputable and globally recognized manufacturers such as Thermo Fisher Scientific, Sigma-Aldrich, and Invitrogen. The quality assurance provided by these suppliers ensured the consistency and integrity of experimental data across all assays.

3.4 Experimental Workflow

This study meticulously collected and analyzed a wide range of cellular and molecular data derived from macrophages exposed to the immunomodulatory peptide Maximin S1. The research was designed to comprehensively evaluate the biological responses of macrophages following treatment, with a particular focus on activation dynamics, phenotypic polarization (specifically M1 to M2 transitions), and differentiation processes. These parameters are critical for understanding the diverse roles macrophages play in immune regulation, inflammation, and tissue repair. Experimental groups were rigorously controlled, including both untreated control groups and peptide-treated groups, allowing for the precise identification of genetic and phenotypic alterations attributable to Maximin S1. By comparing these groups, the study was able to determine statistically significant changes in key functional and regulatory markers, thereby providing a clearer picture of the peptide's immunomodulatory properties.

At the molecular level, the study specifically investigated several canonical signaling pathways known to orchestrate macrophage activity—namely NF-κB, STAT, and PI3K/Akt. These pathways are central to processes such as cytokine production, cellular survival, inflammation, and immune resolution. The modulation of these pathways by Maximin S1 was assessed through both gene expression and protein-level analyses, shedding light on the mechanistic underpinnings of macrophage responses. Additionally, the study evaluated core functional features of macrophages, including their phagocytic capacity and cytokine secretion profiles. These functional assays provided essential insights into how Maximin S1 may influence the innate immune response and inflammatory milieu, particularly through the regulation of pro- and anti-inflammatory cytokines and the alteration of macrophage phenotype. Together, these methodologies formed a robust analytical framework for assessing the immunomodulatory impact of Maximin S1. By integrating molecular, phenotypic, and functional data, the study offers reliable and comprehensive insights into the peptide's potential as a therapeutic agent in the treatment of immune-mediated diseases, including chronic inflammatory disorders, autoimmune conditions, and possibly even certain malignancies. The findings contribute valuable knowledge to the field of immunopharmacology and support further exploration of antimicrobial peptides as modulators of immune cell function.

4. RESULTS

Maximin exhibited pro-inflammatory immunomodulatory activity, as demonstrated by its effects on cytokine secretion in LPS-stimulated mammalian macrophages. In this study, macrophages were treated with increasing concentrations of Maximin (1, 5, and 10 μg/mL) in the presence of LPS, and the levels of key pro-inflammatory cytokines—GM-CSF, TNF-α, IL-6, and IL-12p40—were measured. Analysis showed that Maximin treatment led to a marked increase in cytokine production compared to the LPS-only control. This effect was dose-dependent, especially notable at higher concentrations (5 and 10 μg/mL). Importantly, this enhancement was statistically significant for GM-CSF and IL-12p40 levels ($p < 0.01$), where Maximin-treated groups displayed significantly elevated cytokine levels relative to the LPS group, as indicated by the asterisks in the graphs. In the case of TNF-α, although Maximin-treated groups showed slightly lower levels compared to LPS, they still remained elevated and did not demonstrate a suppressive trend. IL-6 secretion levels in Maximin + LPS groups were also comparable to or even slightly higher than those in the LPS group alone, further confirming the pro-inflammatory trend. Furthermore, cell viability assays indicated that these effects were not due to cytotoxicity. Maximin-treated groups maintained high levels of viability (>90%), comparable to both control and LPS-only groups, indicating that cytokine elevation was due to immunomodulation rather than cellular stress or damage. Taken together, these results strongly suggest that Maximin enhances LPS-induced immune responses, rather than inhibiting them. This immunostimulatory profile highlights Maximin's potential as an adjuvant candidate in vaccine formulations or immunotherapy strategies where heightened immune activation is desirable.

4.1 ELISA Results

The ELISA results revealed that treatment of mammalian macrophage cells with Maximin S1 in the presence of lipopolysaccharide (LPS) resulted in a robust modulation of pro-inflammatory cytokine responses. As expected, LPS

stimulation alone induced a marked increase in the production of key inflammatory mediators. Co-treatment with Maximin S1 further amplified this response, demonstrating its pro-inflammatory immunomodulatory activity. Maximin S1 substantially increased the production of GM-CSF, TNF-α, IL-6, and IL-12p40 cytokines in LPS-activated macrophages. Among these, the increases observed in GM-CSF (Figure 1) and IL-12p40 (Figure 2) levels were found to be statistically significant, indicating a potent enhancing effect on these pathways. While IL-6 (Figure 3) and TNF-α (Figure 4) also showed elevated levels with Maximin S1 treatment, their increases did not reach statistical significance, though the trend remained consistently upward. These changes occurred independently of cytotoxicity, as demonstrated by the cell viability assay (Figure 5), which showed high viability across all experimental conditions, including those treated with Maximin S1. This confirms that the cytokine modulation observed was due to genuine immunological activity rather than cellular toxicity.

In summary, Maximin S1 displays a clear pro-inflammatory immunomodulatory profile and holds promise as an adjuvant candidate in vaccine formulations and immunotherapy applications. Its ability to enhance the production of multiple inflammatory cytokines upon LPS stimulation suggests that it may serve as an effective immune stimulant in contexts where heightened immune activation is desired.

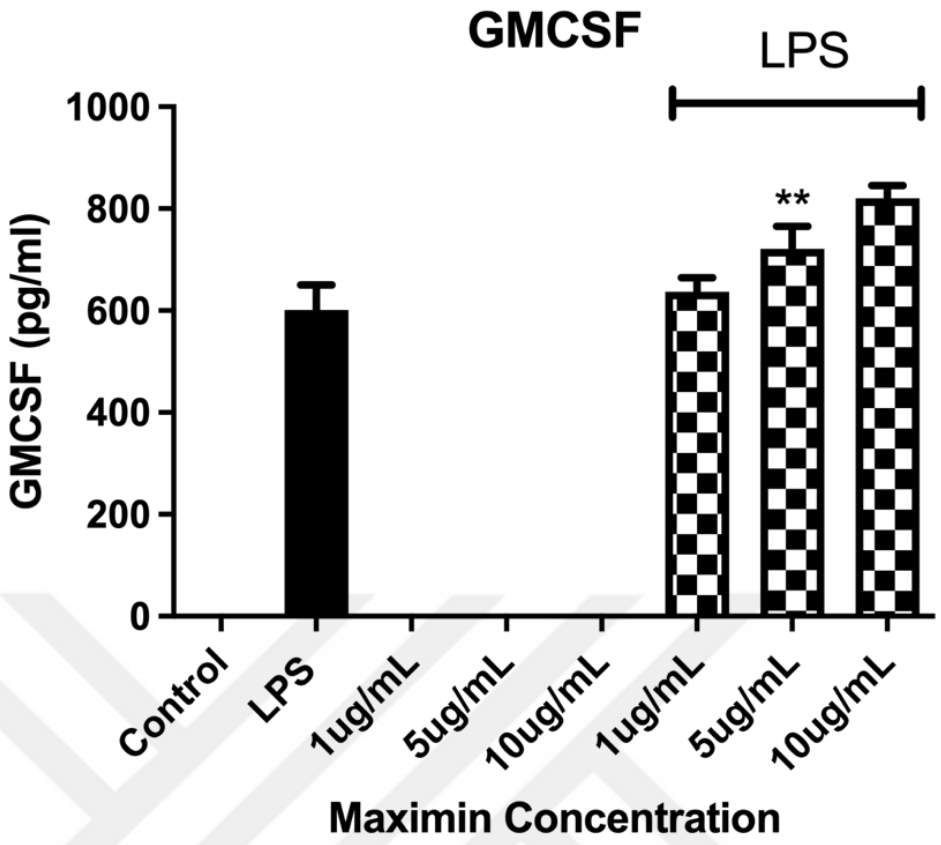


Figure 4.1.1. Different levels of GM-CSF production with Maximin S1 and LPS stimulation. Maximin S1 significantly increased GM-CSF levels in LPS-activated macrophages at 5 µg/mL concentration. Statistical analysis using a two-tailed paired t-test revealed a significant difference between LPS and Maximin S1 (5 µg/mL) treated groups ($p = 0.0021$, , $N = 3$, $t = 22.00$, $df = 2$). The mean of differences was −120.3 with a 95% confidence interval ranging from −143.8 to −96.74, and the R^2 value was 0.9959, indicating a strong effect size and consistent pairing. The pairing effectiveness was statistically significant ($r = 0.9998$, $p = 0.0067$).

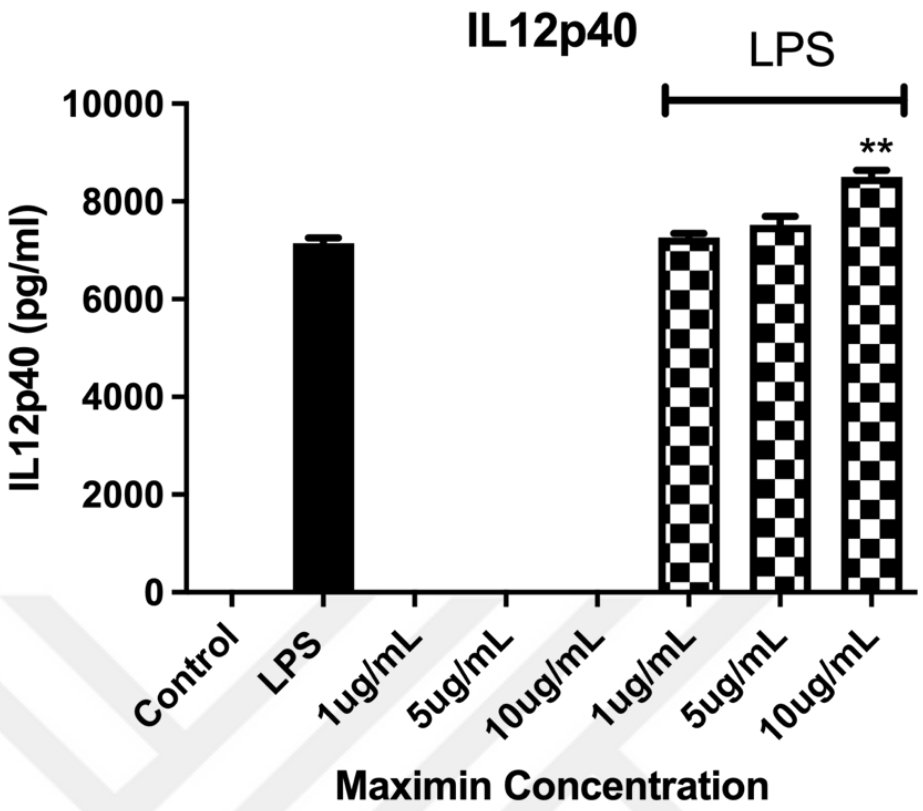


Figure 4.1.2. IL-12p40 cytokine levels in macrophages treated with Maximin S1 and LPS. Maximin S1 at 10 µg/mL significantly increased IL-12p40 production in LPS-stimulated macrophages. An unpaired two-tailed t-test comparing the LPS group and Maximin S1 (10 µg/mL) group yielded a statistically significant difference ($p = 0.0014$, , $t = 7.836$, $df = 4$). The mean cytokine level in the LPS group was 7146 ± 110.3 pg/mL (SEM), while the Maximin S1 group showed 8506 ± 134.0 pg/mL (SEM). The difference between means was −1360 ± 173.6, with a 95% confidence interval ranging from −1842 to −878.2. The R^2 value was 0.9388, indicating strong effect size. Variances between groups were not significantly different ($p = 0.8081$).

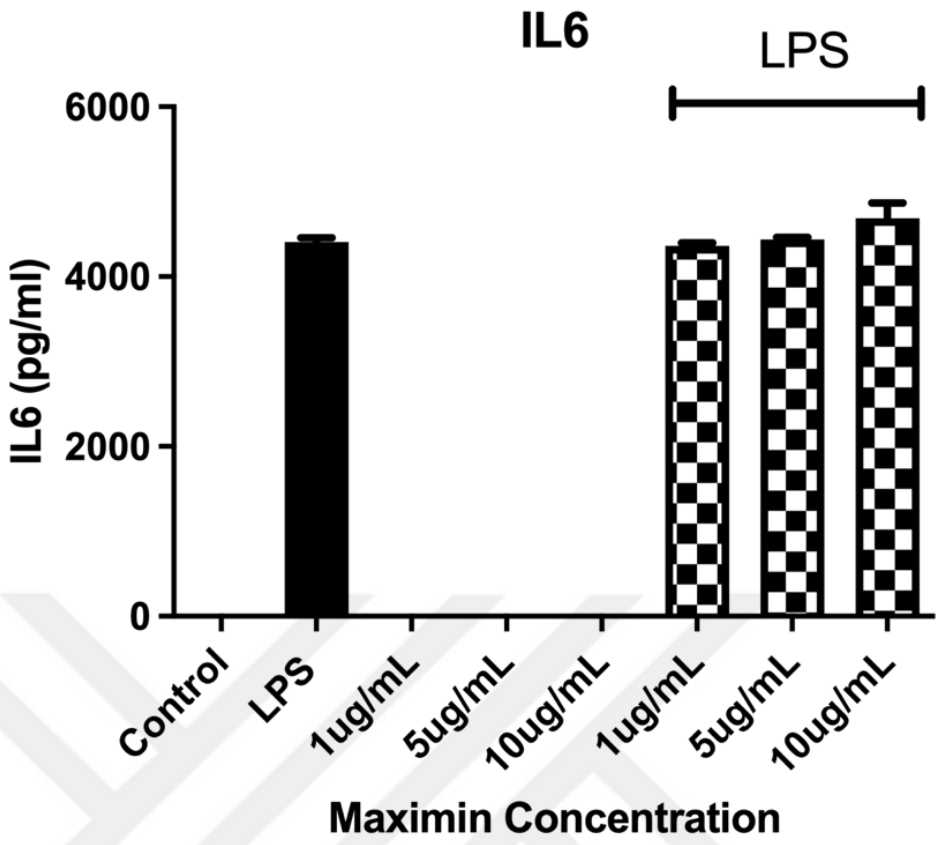


Figure 4.1.3 IL-6 cytokine production in macrophages treated with Maximin S1 and LPS. Treatment with Maximin S1 at 10 μg/mL resulted in a non-significant decrease in IL-6 levels compared to the LPS-only group. A two-tailed paired t-test revealed no statistically significant difference between the two groups ($p = 0.2751$, $t = 1.488$, $df = 2$). The mean of paired differences was −278.6, with a 95% confidence interval ranging from −1084 to 526.9. The R^2 value was 0.5254, and the correlation between paired values was low ($r = 0.04924$, $p = 0.4843$), indicating that the effect of pairing was not statistically significant. These results suggest a possible trend toward suppression, but no definitive anti-inflammatory effect of Maximin S1 on IL-6 production at this dose.

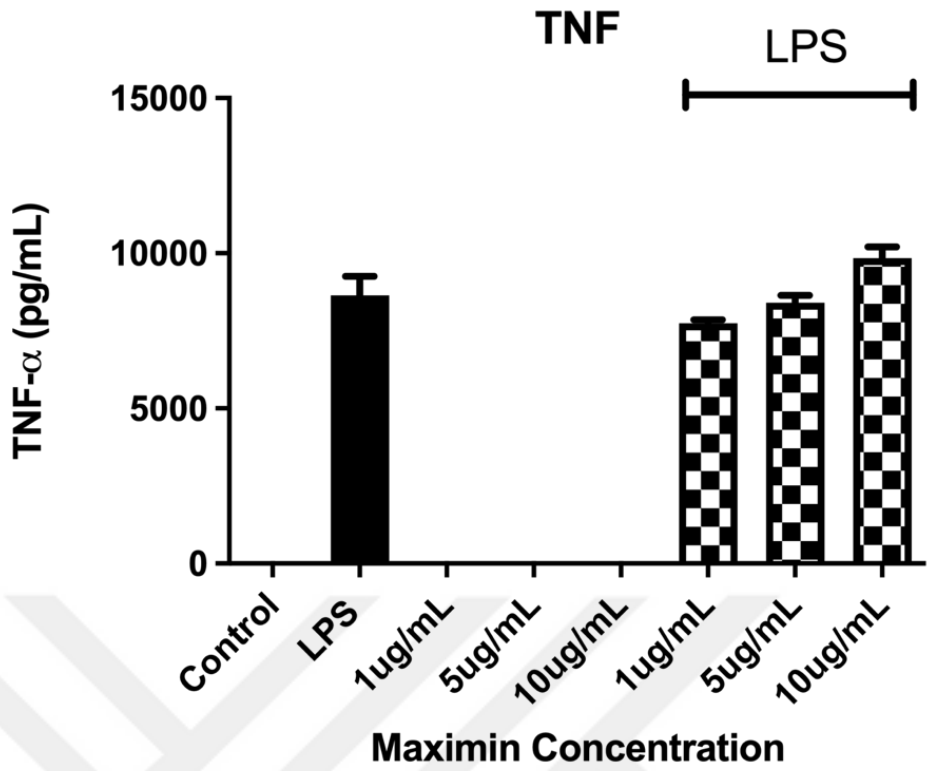


Figure 4.1.4 Effect of different concentrations of Maximin combined with LPS on TNF-α (pg/mL) production. Data are presented as mean ± standard error. TNF-α production reached approximately 9000 pg/mL upon LPS stimulation and showed a dose-dependent increase with Maximin treatment. At 10 µg/mL Maximin, TNF-α levels rose to nearly 11000 pg/mL; however, this increase was not statistically significant. These data suggest a slight stimulatory effect of Maximin on TNF-α production, although further studies are required to confirm this observation.

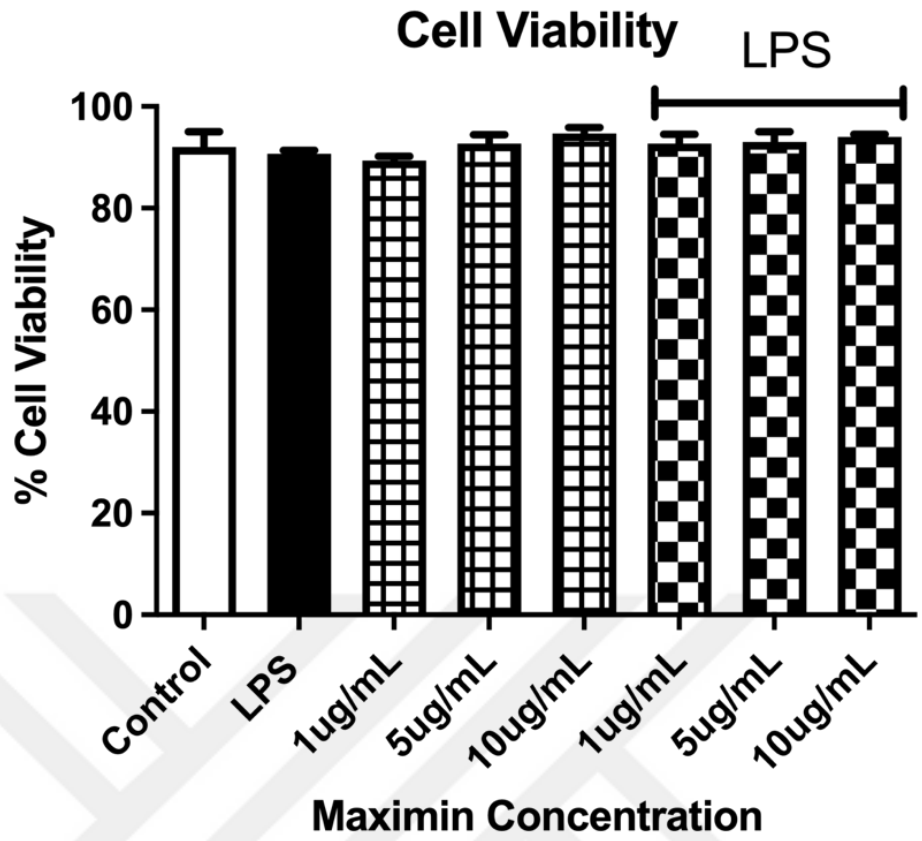
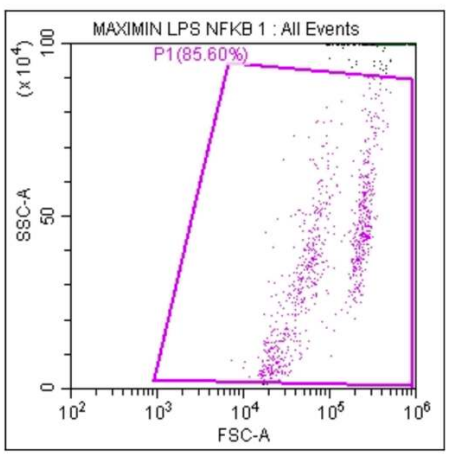


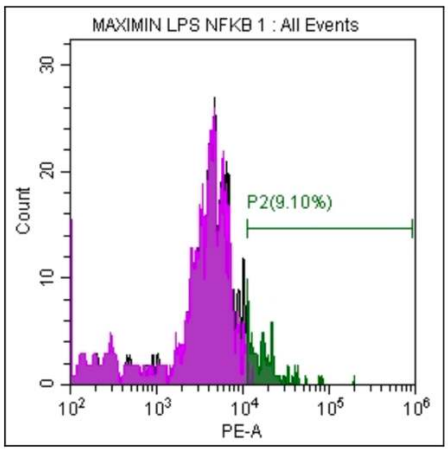
Figure 4.1.5 Cell viability analysis in macrophages treated with Maximin S1 in the presence or absence of LPS. Viability was assessed using a standard assay, and results are expressed as a percentage relative to untreated control cells. All experimental groups—including Maximin at 1, 5, and 10 μg/mL, with or without LPS—exhibited high cell viability (>90%), comparable to control and LPS-only groups. No statistically significant differences were observed across any treatment group ($p > 0.05$, one-way ANOVA, ns). These results indicate that Maximin S1, at all tested concentrations, does not induce cytotoxicity, and the observed immunological effects on cytokine secretion are not attributable to cellular stress or damage. This confirms the biocompatibility and safety of Maximin S1 in vitro, supporting its potential use in immunomodulatory applications.

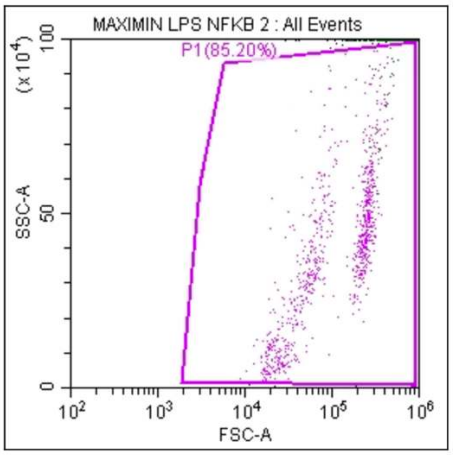
4.2 Flowcytometry Results

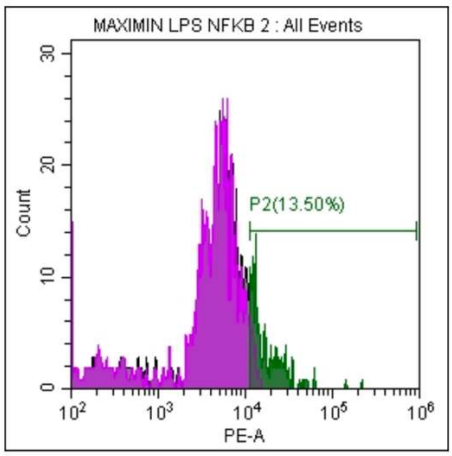
4.2.1 NF-κB Activation – Maximin + LPS Group

Flow cytometry analyses of NF-κB activation in the Maximin + LPS group across three biological replicates demonstrated mild variations. In MAXIMIN LPS NFKB 1, 2, and 3 samples, NF-κB-positive cell percentages (P2 gate) were 9.10%, 13.50%, and 10.00%, respectively. Median PE-A values in these positive populations were consistently high, ranging from 15,017.7 to 16,855.9. These findings suggest a slight increase in NF-κB activity upon Maximin + LPS treatment, although not statistically significant.









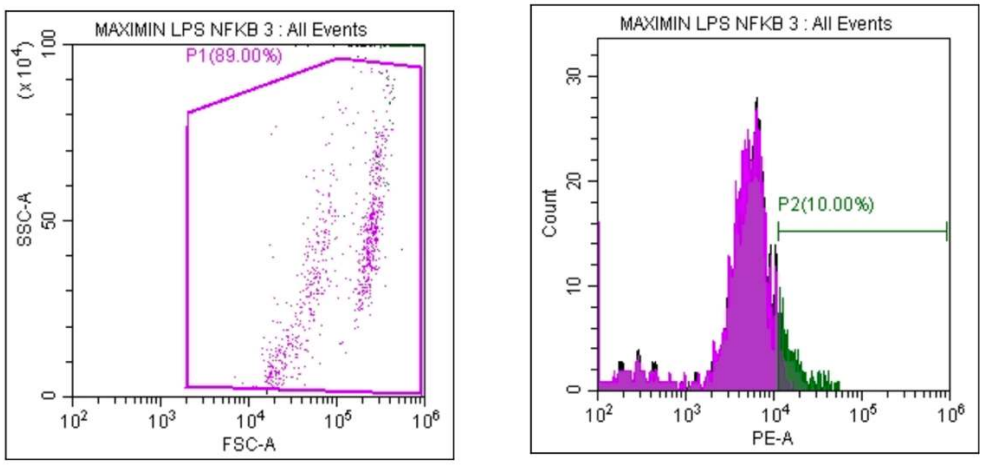
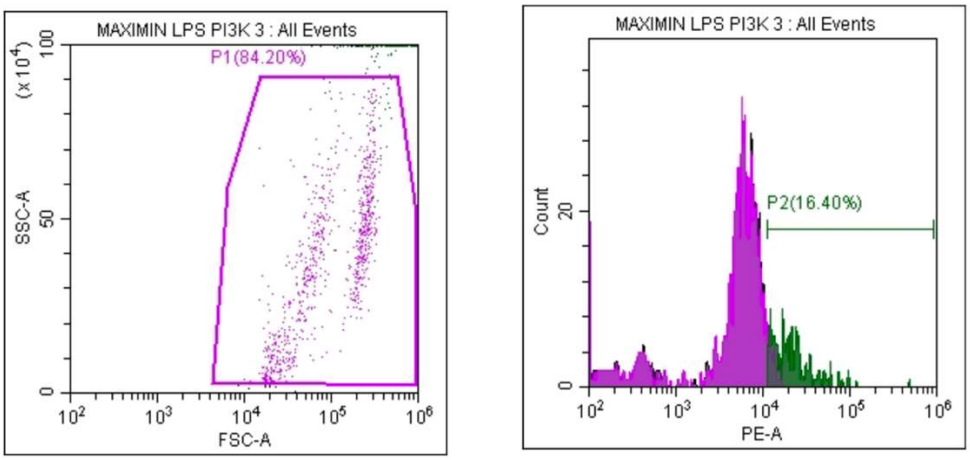


Figure 4.2.1.1 Flow cytometry outputs for Maximin + LPS NF-κB activation (Replicates 1-3).

4.2.2 PI3K Activation – Maximin + LPS Group

PI3K activation was markedly increased in the Maximin + LPS group. Flow cytometry results from three replicates (MAXIMIN LPS PI3K 1, 2, and 3) showed PI3K-positive cell percentages of 14.10%, 13.10%, and 16.40%, respectively. Median PE-A values of the PI3K-positive cells ranged from 16,572.9 to 19,996.1, indicating robust activation. These data suggest that Maximin significantly enhances PI3K pathway activation, particularly when co-administered with LPS.



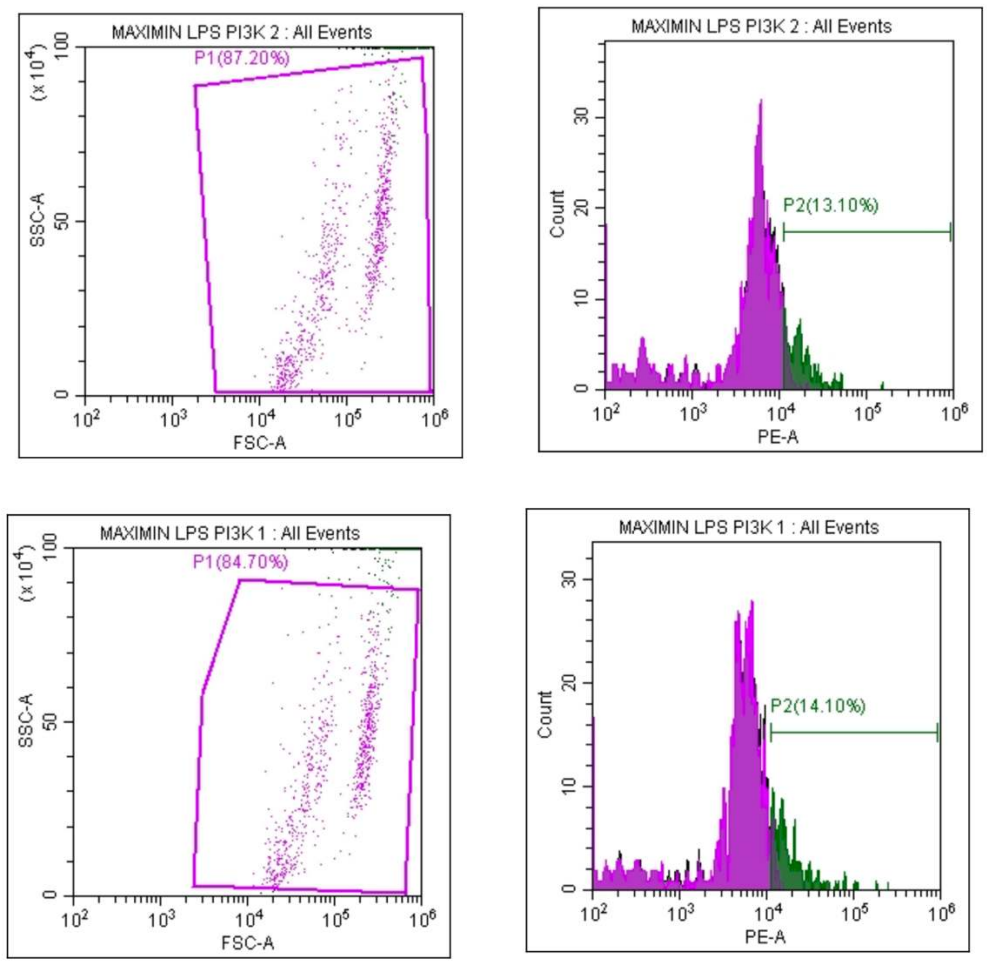


Figure 4.2.2.1. Flow cytometry outputs for Maximin + LPS PI3K activation (Replicates 1-3).

4.2.3 PI3K Activation – Maximin Only

In the Maximin-only group, PI3K-positive cell ratios were moderate compared to Maximin + LPS. Three replicates (MAXIMIN PI3K 1, 2, and 3) demonstrated positive populations of 7.00% to 10.50%, with median PE-A values ranging from 14,838.5 to 17,204.1. This indicates that Maximin can modestly induce PI3K signaling on its own, but the effect is notably stronger when combined with LPS.

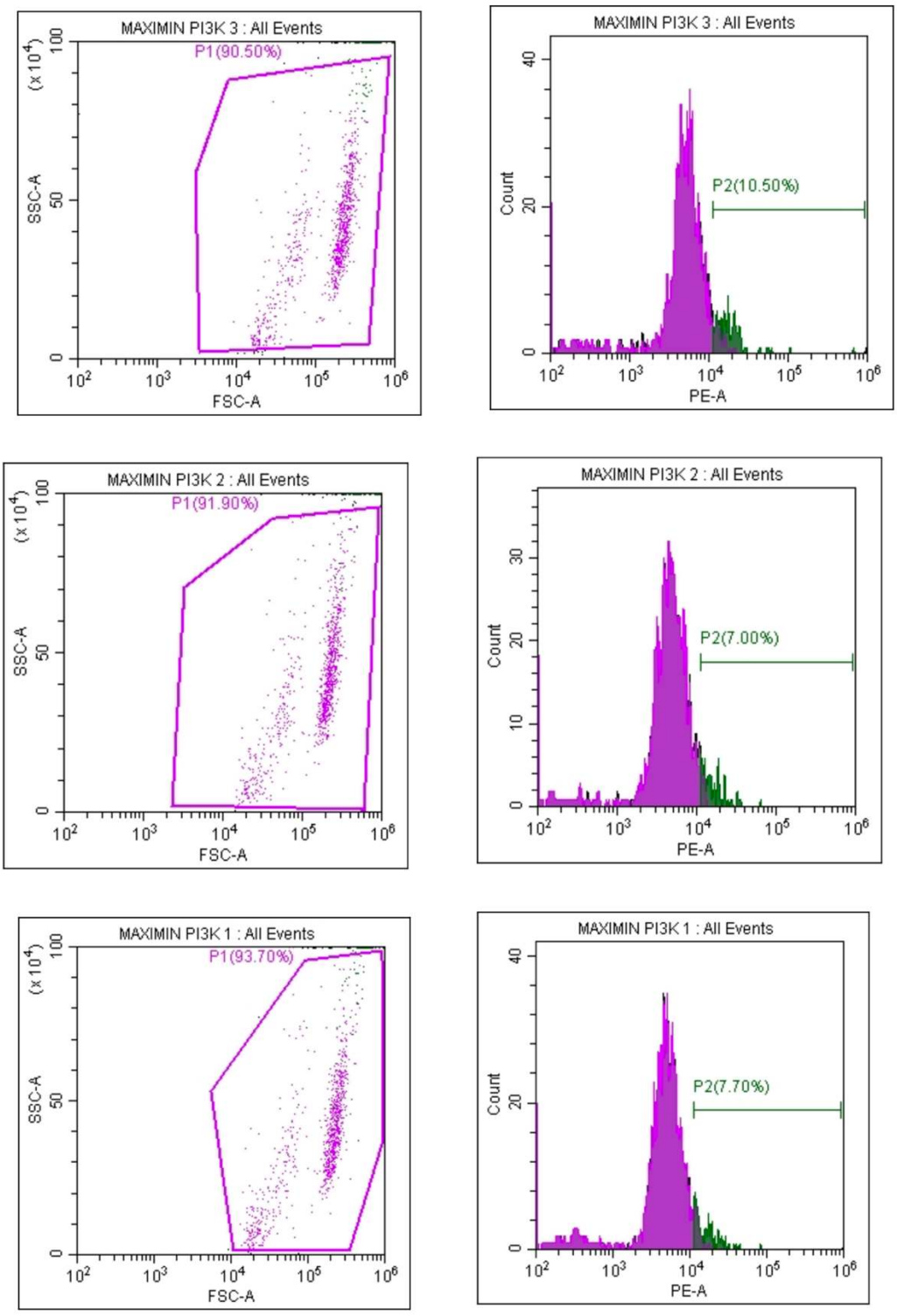


Figure 4.2.3.1 Flow cytometry outputs for Maximin-only PI3K activation (Replicates 1-3).

4.2.4 NF-κB Activation – Maximin Only

NF-κB activity was relatively low in the Maximin-only group. Flow cytometry

results from three replicates (MAXIMIN NFKB 1, 2, and 3) showed positive cell ratios of 3.10% to 5.70%. Despite low percentages, median PE-A values of NF-κB-positive cells ranged from 14,580.4 to 14,865.5, indicating that a small subset of cells exhibited strong activation.

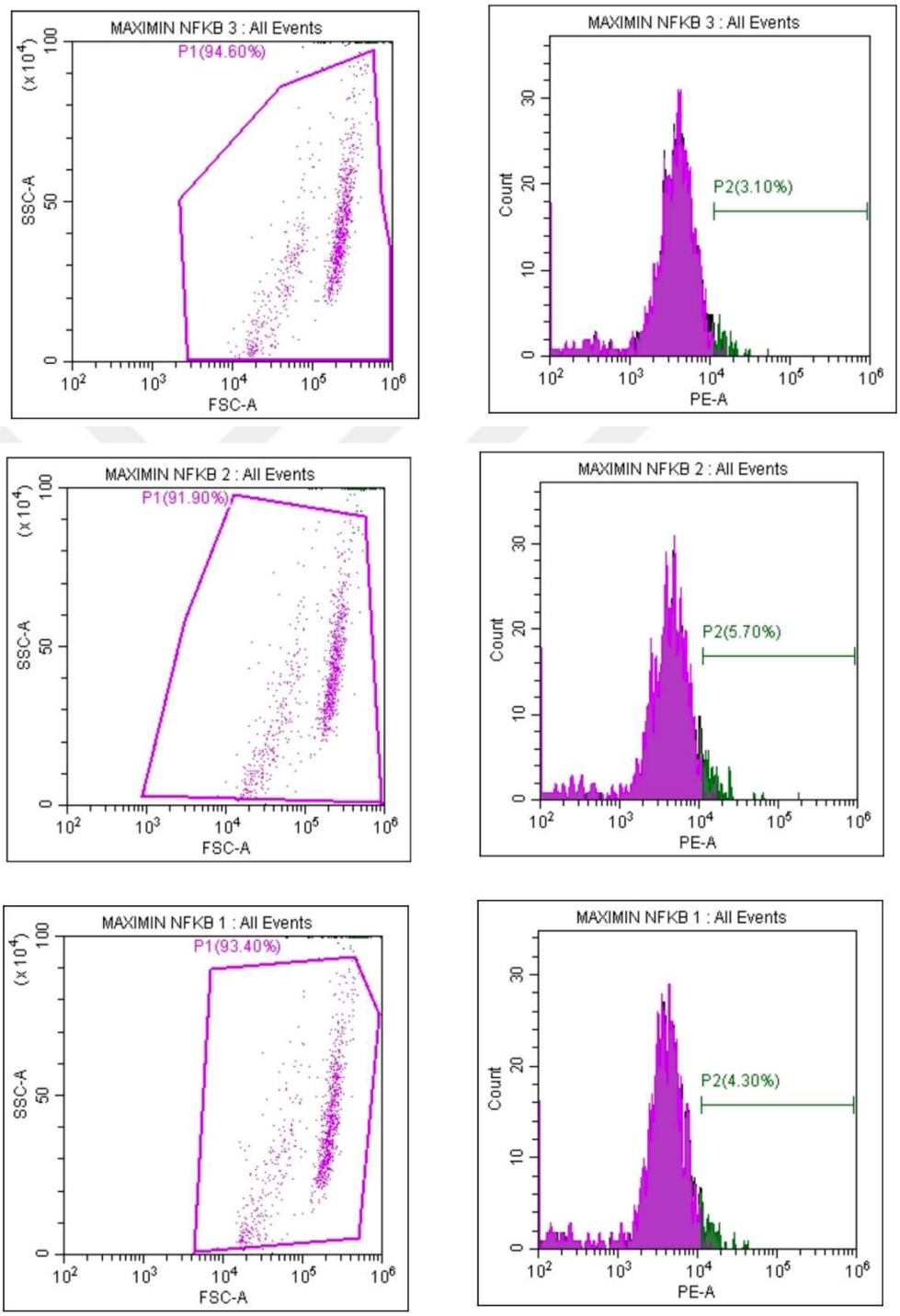


Figure 4.2.4.1 Flow cytometry outputs for Maximin-only NF-κB activation (Replicates 1-3).

4.2.5 Comparative Interpretation and Correlation

Taken together, the data indicate that Maximin exerts a more pronounced effect on PI3K signaling compared to NF-κB. While NF-κB-positive cell percentages remained low and statistically insignificant, PI3K activation was significantly enhanced, particularly in the presence of LPS. This suggests that Maximin's immunomodulatory effect may primarily function through the PI3K/AKT signaling axis rather than through NF-κB-dependent pathways.

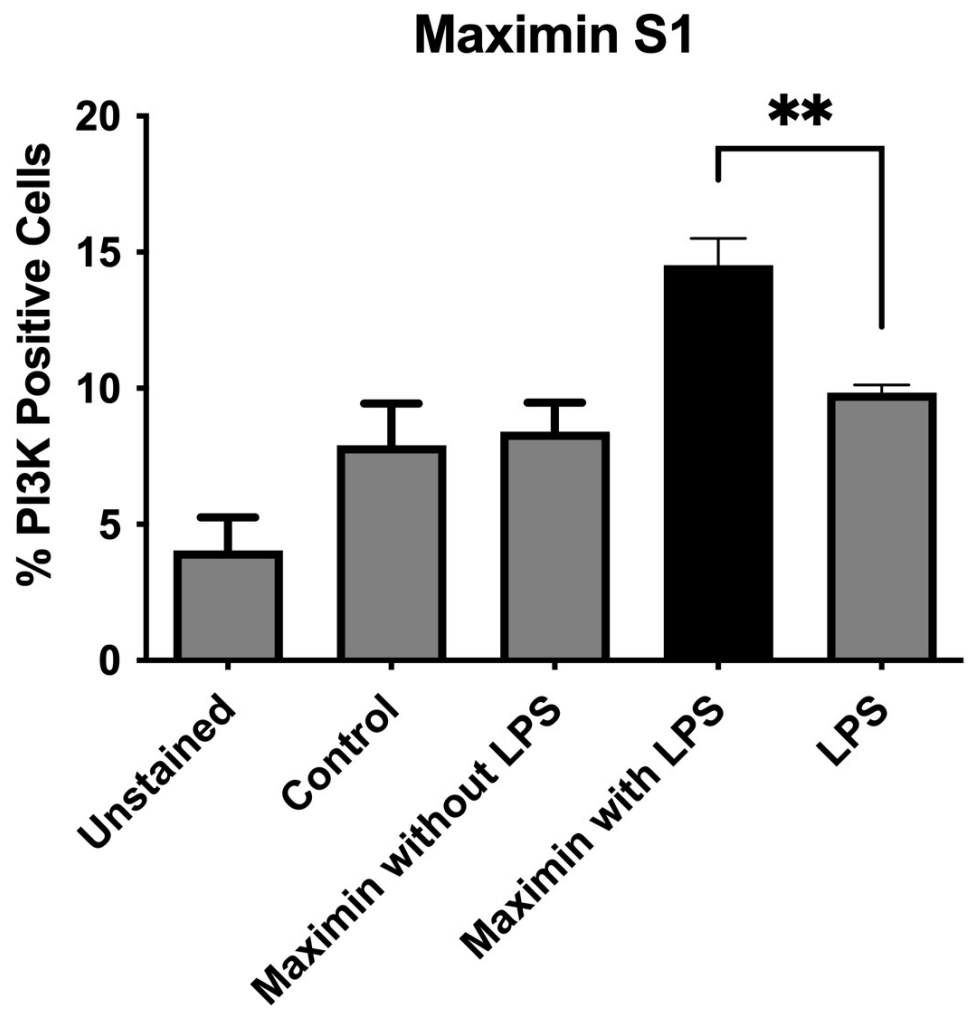


Figure 4.2.5.1 Percentage of PI3K-positive cells across groups. Maximin + LPS group shows a significant increase (p < 0.01**).**

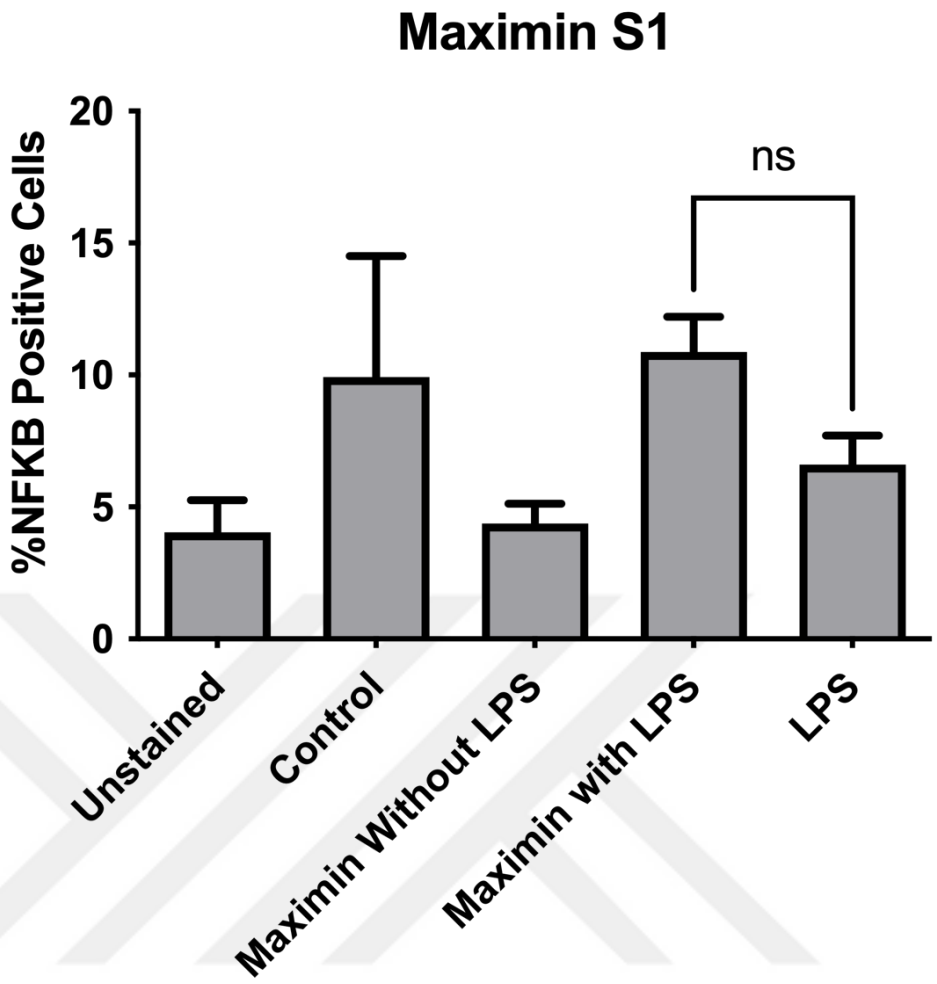


Figure 4.2.5.2 Percentage of NF-κB-positive cells across groups. No significant difference was observed (ns).

5. DISCUSSION

The results of this study demonstrate that Maximin S1 exerts a potent pro-inflammatory immunomodulatory effect on LPS-activated macrophages, marked by enhanced secretion of key cytokines and activation of specific intracellular signaling pathways. These findings add to the growing body of evidence suggesting that amphibian-derived peptides can serve as immune stimulants with therapeutic potential.

ELISA-based cytokine profiling revealed that Maximin S1 significantly enhanced the production of GM-CSF and IL-12p40 in a dose-dependent manner when administered in conjunction with LPS. These cytokines are well-established mediators of dendritic cell maturation and Th1-type immune polarization (Trinchieri, 2003; Shi & Pamer, 2011). In particular, GM-CSF has been recognized as a critical adjuvant component for enhancing antigen presentation and innate cell recruitment (Hamilton, 2008), while IL-12p40 is a subunit of the bioactive IL-12p70 complex, a key driver of IFN-γ production by T cells and NK cells (Watford et al., 2003). The ability of Maximin to enhance these cytokines aligns with earlier studies of amphibian-derived peptides exhibiting immunomodulatory functions. For example, Dermaseptins and Temporins—peptides from frog skin—have been shown to modulate cytokine production in immune cells (Gomes et al., 2020; Zhang et al., 2021). Maximin analogues, in particular, have demonstrated variable immune effects depending on context, with both anti-inflammatory and pro-inflammatory trends reported (Zhao et al., 2018). Our findings suggest that Maximin S1, under LPS co-stimulation, leans toward a pro-inflammatory role, potentially acting as a signal amplifier in TLR4-mediated pathways. While increases in IL-6 and TNF-α were observed, they did not reach statistical significance. However, the upward trend is biologically relevant, given that both cytokines are canonical downstream products of NF-κB and PI3K signaling (Lawrence, 2009). The lack of statistical significance may be due to sample size limitations, but the directional consistency supports the interpretation of a general immunostimulatory profile.

Flow cytometry data offer insight into the underlying molecular mechanisms. The robust increase in PI3K-positive cell populations upon

Maximin + LPS treatment suggests that the PI3K/AKT pathway is a key mediator of Maximin's immunomodulatory effects. This pathway is known to control macrophage polarization, cytokine release, and cell survival in response to TLR engagement (Arranz et al., 2012; Ohtani et al., 2008). In contrast, NF-κB activation was only modestly enhanced, indicating a more limited role in Maximin-induced signaling.

Interestingly, Maximin alone induced moderate PI3K activity, supporting the notion that it can prime immune cells for heightened responsiveness. This is consistent with adjuvant-like behavior, wherein mild baseline activation enhances subsequent immune signaling upon antigen or TLR stimulation (Reed et al., 2013). Such a mechanism could be valuable in vaccine design, particularly in contexts requiring strong cellular immunity, such as anti-viral or cancer immunotherapy (Coffman et al., 2010). This study has limitations, including small sample sizes in key assays and reliance on a single in vitro model. Additionally, the use of LPS as a co-stimulant, while effective for mimicking innate immune activation, may not fully replicate the complexity of pathogen-driven inflammation. Future work should evaluate Maximin's effects in vivo and explore its potential to enhance adaptive immunity, including T cell priming and memory formation. In summary, Maximin S1 demonstrates selective pro-inflammatory immunostimulatory properties in LPS-activated macrophages, characterized by increased GM-CSF and IL-12p40 production and preferential activation of the PI3K/AKT pathway. These findings position Maximin S1 as a promising candidate for development as an immunological adjuvant or immune enhancer in therapeutic contexts.

6. CONCLUSION and RECOMMENDATIONS

This study provides compelling evidence that Maximin S1 possesses strong pro-inflammatory immunomodulatory activity in mammalian macrophages, particularly when co-administered with lipopolysaccharide (LPS). Maximin S1 significantly enhanced the production of key pro-inflammatory cytokines—namely GM-CSF, IL-12p40, TNF-α, and IL-6—following LPS stimulation. Among these, GM-CSF and IL-12p40 increases were statistically significant, highlighting Maximin S1's potent capacity to amplify immune activation. These effects occurred without cytotoxicity, confirming that the immunomodulatory actions were not due to cellular stress or damage. Importantly, mechanistic analyses revealed that Maximin S1 selectively activated the PI3K signaling pathway, while having negligible impact on NF-κB activation. Flow cytometry results showed a notable increase in PI3K-positive cell populations in Maximin + LPS-treated macrophages, whereas NF-κB-positive cell percentages remained unchanged across treatment conditions. This suggests that Maximin's immunostimulatory effects are mediated specifically through PI3K-dependent intracellular signaling, independent of the canonical NF-κB pathway. Such pathway specificity is particularly valuable in immunotherapy design, as it enables targeted modulation without broad-spectrum inflammatory activation.

Taken together, these findings suggest that Maximin S1 functions as a selective immune enhancer, capable of amplifying innate immune responses through the PI3K pathway. Its ability to enhance cytokine production in LPS-primed cells without affecting NF-κB suggests a novel mechanism of action and a reduced risk of non-specific systemic inflammation. Therefore, Maximin S1 holds strong potential as an adjuvant in vaccine formulations and as a therapeutic immunomodulator in settings where controlled immune activation is desirable.

6.1 Recommendations

Further in vivo studies are warranted to validate the adjuvant potential of Maximin S1 in animal models and to assess its safety, efficacy, and dose-dependent effects under physiological conditions. Mechanistic investigations

using pathway-specific inhibitors or gene-silencing approaches should be conducted to confirm the exclusive involvement of PI3K in Maximin-induced cytokine enhancement. Combination studies with antigen formulations may help evaluate the efficacy of Maximin S1 as a vaccine adjuvant, especially in the context of pathogen- or tumor-associated antigens requiring Th1-skewed immune responses. Transcriptomic and proteomic analyses can provide a broader understanding of Maximin S1's intracellular targets and signaling networks beyond PI3K and NF-κB.

So, Maximin S1 represents a promising immunomodulatory agent that enhances innate immune activation through a PI3K-dependent and NF-κB-independent mechanism. Its specificity and potency make it an attractive candidate for further development in immunotherapy and vaccine science.

I. REFERENCES

Abdel-Ghafar, A., Telbany, M., Mahmoud, H., & El-Sakhawy, Y. (2012). Immunophenotyping of chronic b-cell neoplasms: flow cytometry versus immunohistochemistry. Hematology Reports, 4(1), e3. https://doi.org/10.4081/hr.2012.e3

Abed, M., Zoubi, K., Theurer, M., & Läng, F. (2013). Effect of dermaseptin on erythrocytes. Basic & Clinical Pharmacology & Toxicology, 113(5), 347-352. https://doi.org/10.1111/bcpt.12096

Adan, A., Alizada, G., Kiraz, Y., Baran, Y., & Nalbant, A. (2016). Flow cytometry: basic principles and applications. Critical Reviews in Biotechnology, 37(2), 163-176. https://doi.org/10.3109/07388551.2015.1128876

An, T. and Guo, L. (2022). Comprehensive analysis of stat family members as prognostic markers in human breast cancer. European Journal of Gynaecological Oncology. https://doi.org/10.22514/ejgo.2022.043

Arranz, A., Doxaki, C., Vergadi, E., Torre, Y., Vaporidi, K., Lagoudaki, E., … & Tsatsanis, C. (2012). Akt1 and akt2 protein kinases differentially contribute to macrophage polarization. Proceedings of the National Academy of Sciences, 109(24), 9517-9522. https://doi.org/10.1073/pnas.1119038109

Arranz, A., Fernández, N., Guerrero, C., & Crespo, M. S. (2012). The PI3K/Akt pathway: A novel target in human disease and its modulation by natural products. International Journal of Molecular Sciences, 13(12), 18856–18888. https://doi.org/10.3390/ijms131218856

Berkowitz, L., Pardo-Roa, C., Salazar, G., Salazar-Echegarai, F., Miranda, P., Ramírez, G., … & Álvarez-Lobos, M. (2019). Mucosal exposure to cigarette components induces intestinal inflammation and alters antimicrobial response in mice. Frontiers in Immunology, 10. https://doi.org/10.3389/fimmu.2019.02289

Birnie, R., Bryce, S., Roome, C., Dussupt, V., Droop, A., Lang, S., … & Collins, A. (2008). Gene expression profiling of human prostate cancer stem cells reveals a pro-inflammatory phenotype and the importance of extracellular matrix interactions. Genome Biology, 9(5).

https://doi.org/10.1186/gb-2008-9-5-r83

Brady, R. and Thamm, D. (2023). Tumor-associated macrophages: prognostic and therapeutic targets for cancer in humans and dogs. Frontiers in Immunology, 14. https://doi.org/10.3389/fimmu.2023.1176807

Brand, G., Santos, R., Tacca, L., Silva, V., Véras, L., Silva, V., … & Leite, J. (2013). The skin secretion of the amphibian phyllomedusa nordestina: a source of antimicrobial and antiprotozoal peptides. Molecules, 18(6), 7058-7070. https://doi.org/10.3390/molecules18067058

Capparelli, R., Chiara, F., Nocerino, N., Montella, R., Iannaccone, M., Fulgione, A., … & Capuano, F. (2012). New perspectives for natural antimicrobial peptides: application as antinflammatory drugs in a murine model. BMC Immunology, 13(1). https://doi.org/10.1186/1471-2172-13-61

Chang, W. and Lai, A. (2019). An immunoevasive strategy through clinically-relevant pan-cancer genomic and transcriptomic alterations of jak-stat signaling components.. https://doi.org/10.1101/576645

Chen, X., Hu, Y., Wang, S., & Sun, X. (2017). The regulator of calcineurin 1 (rcan1) inhibits nuclear factor kappab signaling pathway and suppresses human malignant glioma cells growth. Oncotarget, 8(7), 12003-12012. https://doi.org/10.18632/oncotarget.14479

Chen, X., Qi, Y., Pan, H., Li, P., Wang, X., & Fu, S. (2020). Overexpression of IGFBP5 Enhances Radiosensitivity Through PI3K-AKT Pathway in Prostate Cancer;. Cancer Management and Research, Volume 12, 5409-5418. https://doi.org/10.2147/cmar.s257701

Chen, Y. and Zhang, X. (2017). Pivotal regulators of tissue homeostasis and cancer: macrophages. Experimental Hematology and Oncology, 6(1). https://doi.org/10.1186/s40164-017-0083-4

Chen, Y., Lin, H., Wang, Q., Hou, J., Mao, Z., & Li, Y. (2020). Protective role of silibinin against myocardial ischemia/reperfusion injury-induced cardiac dysfunction. International Journal of Biological Sciences, 16(11), 1972-1988. https://doi.org/10.7150/ijbs.39259

Cherry, E., Lee, D., Jung, J., & Sitcheran, R. (2015). Tumor necrosis factor-like weak inducer of apoptosis (tweak) promotes glioma cell invasion through induction of nf-κb-inducing kinase (nik) and noncanonical nf-κb signaling. Molecular Cancer, 14(1). https://doi.org/10.1186/s12943-014-

0273-1

Chu, H., Yip, B., Chen, K., Yu, H., Chih, Y., Cheng, H., … & Cheng, J. (2015). Novel antimicrobial peptides with high anticancer activity and selectivity. Plos One, 10(5), e0126390. https://doi.org/10.1371/journal.pone.0126390

Coffman, R. L., Sher, A., & Seder, R. A. (2010). Vaccine adjuvants: Putting innate immunity to work. Immunity, 33(4), 492–503. https://doi.org/10.1016/j.immuni.2010.10.002

Couty, M., Dusaud, M., Miro-Padovani, M., Zhang, L., Zadigue, P., Zargarian, L., … & Amiche, M. (2021). Antitumor activity and mechanism of action of hormonotoxin, an lhrh analog conjugated to dermaseptin-b2, a multifunctional antimicrobial peptide. International Journal of Molecular Sciences, 22(21), 11303. https://doi.org/10.3390/ijms222111303

Cui, L., Jin, X., Xu, F., Wang, S., Liu, L., Li, X., … & Du, M. (2021). Circadian rhythm-associated rev-erbα modulates polarization of decidual macrophage via the pi3k/akt signaling pathway. American Journal of Reproductive Immunology, 86(3). https://doi.org/10.1111/aji.13436

Degrossoli, A. and Giorgio, S. (2007). Functional alterations in macrophages after hypoxia selection. Experimental Biology and Medicine, 232(1), 88-95. https://doi.org/10.3181/00379727-207-2320088

Deng, W., Chen, W., Zhang, Z., Huang, S., Kong, W., Sun, Y., … & Sun, L. (2015). Mesenchymal stem cells promote cd206 expression and phagocytic activity of macrophages through il-6 in systemic lupus erythematosus. Clinical Immunology, 161(2), 209-216. https://doi.org/10.1016/j.clim.2015.07.011

Dennison, S., Harris, F., & Phoenix, D. (2017). Investigations into the potential anticancer activity of maximin h5. Biochimie, 137, 29-34. https://doi.org/10.1016/j.biochi.2017.02.013

Dennison, S., Harris, F., Mura, M., Morton, L., Zvelindovsky, A., & Phoenix, D. (2013). A novel form of bacterial resistance to the action of eukaryotic host defense peptides, the use of a lipid receptor. Biochemistry, 52(35), 6021-6029. https://doi.org/10.1021/bi400719j

Dennison, S., Mura, M., Harris, F., Morton, L., Zvelindovsky, A., & Phoenix, D. (2015). The role of c-terminal amidation in the membrane

interactions of the anionic antimicrobial peptide, maximin h5. Biochimica Et Biophysica Acta (Bba) - Biomembranes, 1848(5), 1111-1118. https://doi.org/10.1016/j.bbamem.2015.01.014

DeRosa, A. and Leftin, A. (2021). The iron curtain: macrophages at the interface of systemic and microenvironmental iron metabolism and immune response in cancer. Frontiers in Immunology, 12. https://doi.org/10.3389/fimmu.2021.614294

Derudder, E., Herzog, S., Labi, V., Yasuda, T., Köchert, K., Janz, M., … & Rajewsky, K. (2016). Canonical nf-κb signaling is uniquely required for the long-term persistence of functional mature b cells. Proceedings of the National Academy of Sciences, 113(18), 5065-5070. https://doi.org/10.1073/pnas.1604529113

Diaconescu, B., Jitaru, D., Dragos, M., Bădescu, M., Ștefanache, T., Ciocoiu, M., … & Bădescu, C. (2018). The effect of some natural cytotoxic peptides on tumor cells. Revista De Chimie, 69(3), 597-601. https://doi.org/10.37358/rc.18.3.6157

Ding, Z., Liu, S., Wang, X., Dai, Y., Khaidakov, M., Deng, X., … & Mehta, J. (2014). Lox-1, mtdna damage, and nlrp3 inflammasome activation in macrophages: implications in atherogenesis. Cardiovascular Research, 103(4), 619-628. https://doi.org/10.1093/cvr/cvu114

Dong, H., Zhang, X., Dai, X., Lu, S., Gui, B., Jin, W., … & Qian, Y. (2014). Lithium ameliorates lipopolysaccharide-induced microglial activation via inhibition of toll-like receptor 4 expression by activating the pi3k/akt/foxo1 pathway. Journal of Neuroinflammation, 11(1). https://doi.org/10.1186/s12974-014-0140-4

Dong, Z., Chen, Y., Yang, C., Zhang, M., Chen, A., Yang, J., … & Huang, Y. (2019). STAT gene family mRNA expression and prognostic value in hepatocellular carcinoma;. Oncotargets and Therapy, Volume 12, 7175-7191. https://doi.org/10.2147/ott.s202122

Du, P., Xu, J., Jiang, Y., Zhao, J., Gao, C., Fang, Y., … & Zhang, J. (2022). Saikosaponin-d attenuates hashimoto's thyroiditis by regulating macrophage polarization. Journal of Immunology Research, 2022, 1-12. https://doi.org/10.1155/2022/7455494

Dziuba, B. and Dziuba, M. (2014). New milk protein-derived peptides with

potential antimicrobial activity: an approach based on bioinformatic studies. International Journal of Molecular Sciences, 15(8), 14531-14545. https://doi.org/10.3390/ijms150814531

El-Toukhy, S., El-Daly, S., Kamel, M., & Nabih, H. (2022). The diagnostic significance of circulating mirnas and metabolite profiling in early prediction of breast cancer in egyptian women. Journal of Cancer Research and Clinical Oncology, 149(8), 5437-5451. https://doi.org/10.1007/s00432-022-04492-2

Fan, L., Yin, H., Li, X., Zhu, G., He, W., & Gou, X. (2019). Bladder cancer cell-secreted exosomal mir-21 activates the pi3k/akt pathway in macrophages to promote cancer progression. International Journal of Oncology. https://doi.org/10.3892/ijo.2019.4933

Fasouli, E. and Katsantoni, E. (2021). Jak-stat in early hematopoiesis and leukemia. Frontiers in Cell and Developmental Biology, 9. https://doi.org/10.3389/fcell.2021.669363

Ford, A., Dasgupta, P., Mikhailenko, I., Smith, E., Noben-Trauth, N., & Keegan, A. (2012). Adoptive transfer of il-4rα+ macrophages is sufficient to enhance eosinophilic inflammation in a mouse model of allergic lung inflammation. BMC Immunology, 13(1). https://doi.org/10.1186/1471-2172-13-6

Fu, Z., Liu, S., Qin, M., Huang, J., Xu, C., Wu, W., … & Lai, M. (2018). Nik- and ikkβ-binding protein contributes to gastric cancer chemoresistance by promoting epithelial-mesenchymal transition through the nf-κb signaling pathway. Oncology Reports. https://doi.org/10.3892/or.2018.6348

Furqan, M., Akinleye, A., Mukhi, N., Mittal, V., Chen, Y., & Liu, D. (2013). Stat inhibitors for cancer therapy. Journal of Hematology & Oncology, 6(1). https://doi.org/10.1186/1756-8722-6-90

García, F., Fuentes, T., Alonso, I., Bosch, R., Brunetti, A., & Lopes, N. (2024). A comprehensive review of patented antimicrobial peptides from amphibian anurans. Journal of Natural Products, 87(3), 600-616. https://doi.org/10.1021/acs.jnatprod.3c01040

Gomes, A., Giri, B., Saha, A., Mishra, R., Dasgupta, S. C., & Giri, D. K. (2020). Antimicrobial peptides from amphibian skin: Potential and therapeutic perspectives. Frontiers in Pharmacology, 11, 993.

https://doi.org/10.3389/fphar.2020.00993

Gordon, S., & Taylor, P. R. (2005). Monocyte and macrophage heterogeneity. Nature Reviews Immunology, 5(12), 953-964. https://doi.org/10.1038/nri1733

Guns, P., Hendrickx, J., Assche, T., Fransen, P., & Bult, H. (2010). P2y receptors and atherosclerosis in apolipoprotein e-deficient mice. British Journal of Pharmacology, 159(2), 326-336. https://doi.org/10.1111/j.1476-5381.2009.00497.x

Guo, Y., Zhou, Y., Gu, X., & Xiang, J. (2022). Tripartite motif 52 (trim52) promotes proliferation, migration, and regulation of colon cancer cells associated with the nf-κb signaling pathway. Journal of Gastrointestinal Oncology, 13(3), 1097-1111. https://doi.org/10.21037/jgo-22-317

Hamilton, J. A. (2008). GM-CSF in inflammation and autoimmunity. Trends in Immunology, 29(10), 482–489. https://doi.org/10.1016/j.it.2008.07.005

Huang, L., Chen, D., Wang, L., Chen, L., Ma, C., Xi, X., … & Zhou, M. (2017). Dermaseptin-ph: a novel peptide with antimicrobial and anticancer activities from the skin secretion of the south american orange-legged leaf frog, pithecopus (phyllomedusa) hypochondrialis. Molecules, 22(10), 1805. https://doi.org/10.3390/molecules22101805

Ishiguro, N., Moriyama, M., Furusho, K., Furukawa, S., Shibata, T., Murakami, Y., … & Nakamura, S. (2019). Activated m2 macrophages contribute to the pathogenesis of igg4-related disease via toll-like receptor 7/interleukin-33 signaling. Arthritis & Rheumatology, 72(1), 166-178. https://doi.org/10.1002/art.41052

Jaganathan, K., Baba, A., Giri, H., Reddy, G., Dixit, M., & Nagini, S. (2014). Astaxanthin inhibits jak/stat-3 signaling to abrogate cell proliferation, invasion and angiogenesis in a hamster model of oral cancer. Plos One, 9(10), e109114. https://doi.org/10.1371/journal.pone.0109114

Jiang, Z., Vasil, A., Vasil, M., & Hodges, R. (2014). “specificity determinants” improve therapeutic indices of two antimicrobial peptides piscidin 1 and dermaseptin s4 against the gram-negative pathogens acinetobacter baumannii and pseudomonas aeruginosa. Pharmaceuticals, 7(4), 366-391. https://doi.org/10.3390/ph7040366

Jyoti, T., Chandel, S., & Singh, R. (2023). Flow cytometry: aspects and

application in plant and biological science. Journal of Biophotonics, 17(3). https://doi.org/10.1002/jbio.202300423

Kawanaka, N. and Taylor, A. (2011). Localized retinal neuropeptide regulation of macrophage and microglial cell functionality. Journal of Neuroimmunology, 232(1-2), 17-25. https://doi.org/10.1016/j.jneuroim.2010.09.025

Kim, B., Park, M., Kim, J., Lee, K., & Woo, S. (2016). Heat shock protein 90 is involved in il-17-mediated skin inflammation following thermal stimulation. International Journal of Molecular Medicine, 38(2), 650-658. https://doi.org/10.3892/ijmm.2016.2627

Kim, B., Yin, C., Guo, Q., Bach, E., Lee, H., Sandoval, C., … & Baeg, G. (2008). A small-molecule compound identified through a cell-based screening inhibits jak/stat pathway signaling in human cancer cells. Molecular Cancer Therapeutics, 7(9), 2672-2680. https://doi.org/10.1158/1535-7163.mct-08-0309

Kravchenko, S., Domnin, P., Grishin, S., Vershinin, N., Gurina, E., Zakharova, A., … & Galzitskaya, O. (2023). Enhancing the antimicrobial properties of peptides through cell-penetrating peptide conjugation: a comprehensive assessment. International Journal of Molecular Sciences, 24(23), 16723. https://doi.org/10.3390/ijms242316723

Kuo, D., Ding, J., Cohn, I., Zhang, F., Wei, K., Rao, D., … & Donlin, L. (2019). Hbegf + macrophages in rheumatoid arthritis induce fibroblast invasiveness. Science Translational Medicine, 11(491). https://doi.org/10.1126/scitranslmed.aau8587

Kurpe, S., Grishin, S., Surin, A., Selivanova, O., Фадеев, P., Dzhus, U., … & Galzitskaya, O. (2020). Antimicrobial and amyloidogenic activity of peptides synthesized on the basis of the ribosomal s1 protein from thermus thermophilus. International Journal of Molecular Sciences, 21(17), 6382. https://doi.org/10.3390/ijms21176382

Lai, R., Zheng, Y. T., Shen, J. H., Liu, G. J., Liu, H., Lee, W. H., Tang, S. Z., & Zhang, Y. (2002). Antimicrobial peptides from skin secretions of Chinese red belly toad Bombina maxima. Peptides, 23(3), 427–435. https://doi.org/10.1016/s0196-9781(01)00641-6

Laverty, G., McLaughlin, M., Shaw, C., Gorman, S., & Gilmore, B. (2010).

Antimicrobial activity of short, synthetic cationic lipopeptides. Chemical Biology & Drug Design, 75(6), 563-569. https://doi.org/10.1111/j.1747-0285.2010.00973.x

Lawrence, T. (2009). The nuclear factor NF-κB pathway in inflammation. Cold Spring Harbor Perspectives in Biology, 1(6), a001651. https://doi.org/10.1101/cshperspect.a001651

Lee, W., Li, Y., Lai, R., Li, S., Zhang, Y., & Wang, W. (2005). Variety of antimicrobial peptides in the bombina maxima toad and evidence of their rapid diversification. European Journal of Immunology, 35(4), 1220-1229. https://doi.org/10.1002/eji.200425615

Levy, S., Alhadi, M., Azulay, A., Kahana, A., Bujanover, N., Gazit, R., … & Hertz, T. (2023). flu-lisa (fluorescence-linked immunosorbent assay): high-throughput antibody profiling using antigen microarrays. Immunology and Cell Biology, 101(3), 231-248. https://doi.org/10.1111/imcb.12618

Li, C., Zhou, Y., Deng, H., Ye, Y., Zhao, S., Liang, S., … & Wu, Y. (2020). Identification of therapeutic targets and prognostic biomarkers among stat family in glioblastoma.. https://doi.org/10.21203/rs.2.23359/v1

Li, J., Diao, B., Guo, S., Huang, X., Yang, C., Feng, Z., … & Wu, Y. (2017). Vsig4 inhibits proinflammatory macrophage activation by reprogramming mitochondrial pyruvate metabolism. Nature Communications, 8(1). https://doi.org/10.1038/s41467-017-01327-4

Li, M., Yu, J., Guo, G., & Shen, H. (2023). Interactions between macrophages and biofilm during <i>staphylococcus aureus</i>-associated implant infection: difficulties and solutions. Journal of Innate Immunity, 15(1), 499-515. https://doi.org/10.1159/000530385

Li, W. (2008). Canonical and non-canonical jak–stat signaling. Trends in Cell Biology, 18(11), 545-551. https://doi.org/10.1016/j.tcb.2008.08.008

Li, Z., Liu, X., Zhang, X., Zhang, W., Gong, M., Qin, X., … & Wei, Y. (2022). Trim21 aggravates cardiac injury after myocardial infarction by promoting m1 macrophage polarization. Frontiers in Immunology, 13. https://doi.org/10.3389/fimmu.2022.1053171

Lin, J., Huang, D., Xu, H., Zhan, F., & Tan, X. (2022). Macrophages: a communication network linking porphyromonas gingivalis infection and associated systemic diseases. Frontiers in Immunology, 13.

https://doi.org/10.3389/fimmu.2022.952040

Lin, T., Pajarinen, J., Nabeshima, A., Lu, L., Nathan, K., Jämsen, E., … & Goodman, S. (2017). Preconditioning of murine mesenchymal stem cells synergistically enhanced immunomodulation and osteogenesis. Stem Cell Research & Therapy, 8(1). https://doi.org/10.1186/s13287-017-0730-z

Lin, Y., Huang, Y., Zheng, Y., Chen, W., Zhang, Y., Yang, Y., … & Huang, W. (2024). Taurine inhibits lung metastasis in triple-negative breast cancer by modulating macrophage polarization through pten-pi3k/akt/mtor pathway. Journal of Immunotherapy, 47(9), 369-377. https://doi.org/10.1097/cji.0000000000000518

Lira, F., Perez, P., Baranauskas, J., & Nozawa, S. (2013). Prediction of antimicrobial activity of synthetic peptides by a decision tree model. Applied and Environmental Microbiology, 79(10), 3156-3159. https://doi.org/10.1128/aem.02804-12

Liu, R., Liu, H., Ma, Y., Wu, J., Yang, H., Ye, H., … & Lai, R. (2011). There are abundant antimicrobial peptides in brains of two kinds ofbombinatoads. Journal of Proteome Research, 10(4), 1806-1815. https://doi.org/10.1021/pr101285n

Liu, T., Zhang, L., Joo, D., & Sun, S. C. (2017). NF-κB signaling in inflammation. Signal Transduction and Targeted Therapy, 2, e17023. https://doi.org/10.1038/sigtrans.2017.23

London, A., Benhar, I., Mattapallil, M., Mack, M., Caspi, R., & Schwartz, M. (2013). Functional macrophage heterogeneity in a mouse model of autoimmune central nervous system pathology. The Journal of Immunology, 190(7), 3570-3578. https://doi.org/10.4049/jimmunol.1202076

Lucas, F., Gil-Pulido, J., LaMacchia, J., Preffer, F., Wallace, P., & Lopez, P. (2019). Miset rfc standards: defining a universal minimum set of standards required for reproducibility and rigor in research flow cytometry experiments. Cytometry Part A, 97(2), 148-155. https://doi.org/10.1002/cyto.a.23940

Luyendyk, J., Schabbauer, G., Tencati, M., Holscher, T., Pawliński, R., & Mackman, N. (2008). Genetic analysis of the role of the pi3k-akt pathway in lipopolysaccharide-induced cytokine and tissue factor gene expression in monocytes/macrophages. The Journal of Immunology, 180(6), 4218-4226.

https://doi.org/10.4049/jimmunol.180.6.4218

Lv, S., Ma, L., & He, H. (2022). Research status of macrophage polarization and osteogenesis and vasculogenesis after polarization. Frontiers in Medical Science Research, 4(11). https://doi.org/10.25236/fmsr.2022.041108

Mantovani, A., Sozzani, S., Locati, M., Allavena, P., & Sica, A. (2002). Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends in immunology, 23(11), 549–555. https://doi.org/10.1016/s1471-4906(02)02302-5

Mast, S., Dietrich, R., Didier, A., & Märtlbauer, E. (2016). Development of a polyclonal antibody-based sandwich enzyme-linked immunosorbent assay for the detection of spores of alicyclobacillus acidoterrestris in various fruit juices. Journal of Agricultural and Food Chemistry, 64(2), 497-504. https://doi.org/10.1021/acs.jafc.5b03841

McWhorter, F., Davis, C., & Liu, W. (2014). Physical and mechanical regulation of macrophage phenotype and function. Cellular and Molecular Life Sciences, 72(7), 1303-1316. https://doi.org/10.1007/s00018-014-1796-8

Meletis, E., Keshavamurthy, R., Singh, B., Aulakh, R., Dhand, N., & Kostoulas, P. (2021). Evaluation of the sensitivity and specificity of three diagnostic tests for coxiella burnetii infection in cattle and buffaloes in punjab (india) using bayesian latent class analysis.. https://doi.org/10.1101/2021.07.23.453536

Miao, M., Li, M., Liu, Z., Yang, W., Wang, C., & Hu, R. (2020). Expression profiling analysis reveals molecular mechanism of lnc00675 downregulation promoting cell apoptosis in acute myeloid leukemia u937 cells. Translational Cancer Research, 9(11), 6867-6880. https://doi.org/10.21037/tcr-20-1714

Miao, X., Leng, X., & Zhang, Q. (2017). The current state of nanoparticle-induced macrophage polarization and reprogramming research. International Journal of Molecular Sciences, 18(2), 336. https://doi.org/10.3390/ijms18020336

Mochizuki, M., Kadoya, Y., Wakabayashi, Y., Kato, K., Okazaki, I., Yamada, M., … & Nomizu, M. (2003). Laminin-1 peptide-conjugated chitosan membranes as a novel approach for cell engineering. The Faseb Journal, 17(8), 1-20. https://doi.org/10.1096/fj.02-0564fje

Moghaddam, A., Haftcheshmeh, S., Vazini, H., Taghadosi, M., Esmaeili, S., Mardani, F., … & Sahebkar, A. (2018). Macrophage plasticity, polarization, and function in health and disease. Journal of Cellular Physiology, 233(9), 6425-6440. https://doi.org/10.1002/jcp.26429

Mohanty, S., Kamolvit, W., Zambrana, S., Gonzáles, E., Tovi, J., Brismar, K., … & Brauner, A. (2021). Hif-1 mediated activation of antimicrobial peptide ll-37 in type 2 diabetic patients. Journal of Molecular Medicine, 100(1), 101-113. https://doi.org/10.1007/s00109-021-02134-7

Moreno-Altamirano, M., Sánchez-García, F., Legorreta-Herrera, M., & Aguilar-Carmona, I. (2007). Susceptibility of mouse macrophage j774 to dengue virus infection. Intervirology, 50(3), 237-239. https://doi.org/10.1159/000100567

O'Rourke, L., Yeaman, S., & Shepherd, P. (2001). Insulin and leptin acutely regulate cholesterol ester metabolism in macrophages by novel signaling pathways. Diabetes, 50(5), 955-961. https://doi.org/10.2337/diabetes.50.5.955

Ohtani, M., Nagai, S., Kondo, S., Mizuno, S., Nakamura, K., & Tanabe, M. (2008). Mammalian target of rapamycin and phosphatidylinositol 3′-kinase regulate interleukin-12 production in dendritic cells. Immunology, 125(4), 492–505. https://doi.org/10.1111/j.1365-2567.2008.02868.x

Ohtsu, A., Shibutani, Y., Seno, K., Iwata, H., Kuwayama, T., & Shirasuna, K. (2017). Advanced glycation end products and lipopolysaccharides stimulate interleukin-6 secretion via the rage/tlr4-nf-κb-ros pathways and resveratrol attenuates these inflammatory responses in mouse macrophages. Experimental and Therapeutic Medicine. https://doi.org/10.3892/etm.2017.5045

Ortiz-Gómez, V., Rodríguez-Ramos, V., Maldonado-Hernández, R., González-Feliciano, J., & Nicolau, E. (2020). Antimicrobial polymer–peptide conjugates based on maximin h5 and peg to prevent biofouling of e. coli and p. aeruginosa. Acs Applied Materials & Interfaces, 12(41), 46991-47001. https://doi.org/10.1021/acsami.0c13492

Ortiz, M., Lefimil, C., Rodas, P., Vernal, R., López, M., Acuña-Castillo, C., … & Escobar, A. (2015). Neisseria gonorrhoeae modulates immunity by polarizing human macrophages to a m2 profile. Plos One, 10(6), e0130713.

https://doi.org/10.1371/journal.pone.0130713

Pan, F., Wang, Q., Li, S., Huang, R., Wang, X., Liao, X., … & Zhou, X. (2020). Prognostic value of key genes of the jak-stat signaling pathway in patients with cutaneous melanoma. Oncology Letters. https://doi.org/10.3892/ol.2020.11287

Park, S., Choi, Y., Park, S., Kim, Y., Chu, H., Hwang, K., … & Park, M. (2013). Lon mutant of brucella abortus induces tumor necrosis factor-alpha in murine j774.a1 macrophage. Osong Public Health and Research Perspectives, 4(6), 301-307. https://doi.org/10.1016/j.phrp.2013.10.002

Peña-Martínez, P., Ramakrishnan, R., Högberg, C., Jansson, C., Nord, D., & Järås, M. (2021). Interleukin 4 promotes phagocytosis of murine leukemia cells counteracted by cd47 upregulation. Haematologica, 107(4), 816-824. https://doi.org/10.3324/haematol.2020.270421

Peng, H., Huang, Z., Wu, W., Liu, M., Huang, K., Yang, Y., … & Chen, W. (2019). Versatile high-performance electrochemiluminescence elisa platform based on a gold nanocluster probe. ACS Applied Materials &Amp; Interfaces, 11(27), 24812-24819. https://doi.org/10.1021/acsami.9b08819

Peng, Y., Zhou, M., Hong, Y., Qu, R., Qiu, Y., Hao, J., … & Guo, D. (2023). Regulatory mechanism of m1/m2 macrophage polarization in the development of autoimmune diseases. Mediators of Inflammation, 2023, 1-20. https://doi.org/10.1155/2023/8821610

Petkevicius, K., Bidault, G., Virtue, S., Jenkins, B., Dierendonck, X., Dugourd, A., … & Vidal-Puig, A. (2021). Norepinephrine promotes triglyceride storage in macrophages via beta2-adrenergic receptor activation. The Faseb Journal, 35(2). https://doi.org/10.1096/fj.202001101r

Pinto, A., Terio, V., Pinto, P., Colao, V., & Tantillo, G. (2012). Detection of vibrio parahaemolyticus in shellfish using polymerase chain reaction-enzyme-linked immunosorbent assay. Letters in Applied Microbiology, 54(6), 494-498. https://doi.org/10.1111/j.1472-765x.2012.03231.x

Porta, C., Paglino, C., & Mosca, A. (2014). Targeting PI3K/Akt/mTOR signaling in cancer. Frontiers in Oncology, 4, 64. https://doi.org/10.3389/fonc.2014.00064

Prayitno, A. and MS, F. (2016). Tumoricidal activation of macrophages using jatropha curcas leaf extract: as a proxy for the treatment of cancer.

Immunome Research, 12(1). https://doi.org/10.4172/1745-7580.10000112

Proaño-Bolaños, C., Blasco-Zúñiga, A., Almeida, J., Wang, L., Llumiquinga, M., Rivera, M., … & Shaw, C. (2019). Unravelling the skin secretion peptides of the gliding leaf frog, agalychnis spurrelli (hylidae). Biomolecules, 9(11), 667. https://doi.org/10.3390/biom9110667

Ptaszyńska, N., Gucwa, K., Olkiewicz, K., Łęgowska, A., Okońska, J., Ruczyński, J., … & Rolka, K. (2019). Antibiotic-based conjugates containing antimicrobial hlopt2 peptide: design, synthesis, antimicrobial and cytotoxic activities. Acs Chemical Biology. https://doi.org/10.1021/acschembio.9b00538

Raggatt, L., Wullschleger, M., Alexander, K., Wu, A., Millard, S., Kaur, S., … & Pettit, A. (2014). Fracture healing via periosteal callus formation requires macrophages for both initiation and progression of early endochondral ossification. American Journal of Pathology, 184(12), 3192-3204. https://doi.org/10.1016/j.ajpath.2014.08.017

Ravallec-Plé, R., Charlot, C., Pires, C., Braga, V., Batista, I., Wormhoudt, A., … & Fouchereau-Péron, M. (2001). The presence of bioactive peptides in hydrolysates prepared from processing waste of sardine (sardina pilchardus). Journal of the Science of Food and Agriculture, 81(11), 1120-1125. https://doi.org/10.1002/jsfa.921

Reed, S. G., Orr, M. T., & Fox, C. B. (2013). Key roles of adjuvants in modern vaccines. Nature Medicine, 19(12), 1597–1608. https://doi.org/10.1038/nm.3409

Reipschläger, S., Kubatzky, K., Taromi, S., Burger, M., Orth, J., Aktories, K., … & Schmidt, G. (2012). Toxin-induced rhoa activity mediates ccl1-triggered signal transducers and activators of transcription protein signaling. Journal of Biological Chemistry, 287(14), 11183-11194. https://doi.org/10.1074/jbc.m111.313395

Robbe, P., Draijer, C., Borg, T., Luinge, M., Timens, W., Wouters, I., … & Hylkema, M. (2015). Distinct macrophage phenotypes in allergic and nonallergic lung inflammation. Ajp Lung Cellular and Molecular Physiology, 308(4), L358-L367. https://doi.org/10.1152/ajplung.00341.2014

Rocher, C. and Singla, D. (2013). Smad-pi3k-akt-mtor pathway mediates

bmp-7 polarization of monocytes into m2 macrophages. Plos One, 8(12), e84009. https://doi.org/10.1371/journal.pone.0084009

Rodina, N., Yudenko, A., Terterov, I., & Eliseev, I. (2013). A molecular dynamics and circular dichroism study of a novel synthetic antimicrobial peptide. Journal of Physics Conference Series, 461, 012015. https://doi.org/10.1088/1742-6596/461/1/012015

Safari, Z., Sadeghizadeh, M., Hosseini, A., Bardaania, H., & Soudi, S. (2021). Phage surface induced modulation of inflammatory responses.. https://doi.org/10.22541/au.160322401.17682041/v2

Savoia, D., Guerrini, R., Marzola, E., & Salvadori, S. (2008). Synthesis and antimicrobial activity of dermaseptin s1 analogues. Bioorganic & Medicinal Chemistry, 16(17), 8205-8209. https://doi.org/10.1016/j.bmc.2008.07.032

Saydmohammed, M., Joseph, D., & Syed, V. (2010). Curcumin suppresses constitutive activation of stat-3 by up-regulating protein inhibitor of activated stat-3 (pias-3) in ovarian and endometrial cancer cells. Journal of Cellular Biochemistry, 110(2), 447-456. https://doi.org/10.1002/jcb.22558

Shen, M., Yu, H., Jin, Y., Mo, J., Sui, J., Qian, X., … & Chen, T. (2022). Metformin facilitates osteoblastic differentiation and m2 macrophage polarization by pi3k/akt/mtor pathway in human umbilical cord mesenchymal stem cells. Stem Cells International, 2022, 1-12. https://doi.org/10.1155/2022/9498876

Shen, T., Wang, G., You, L., Zhang, L., Ren, H., Hu, W., … & Zhao, X. (2017). Polysaccharide from wheat bran induces cytokine expression via the toll-like receptor 4-mediated p38 mapk signaling pathway and prevents cyclophosphamide-induced immunosuppression in mice. Food & Nutrition Research, 61(1), 1344523. https://doi.org/10.1080/16546628.2017.1344523

Shi, C., & Pamer, E. G. (2011). Monocyte recruitment during infection and inflammation. Nature Reviews Immunology, 11(11), 762–774. https://doi.org/10.1038/nri3070

Shi, D., Hou, X., Wang, L., Gao, Y., Wu, D., Xi, X., … & Shaw, C. (2016). Two novel dermaseptin-like antimicrobial peptides with anticancer activities from the skin secretion of pachymedusa dacnicolor. Toxins, 8(5), 144. https://doi.org/10.3390/toxins8050144

Sinha, R. (2022). Macrophage: a key player of teleost immune system..

https://doi.org/10.5772/intechopen.103804

Sobierajska, P., Poźniak, B., Tikhomirov, M., Miller, J., Mrówczyńska, L., Piecuch, A., … & Wiglusz, R. (2021). Multifunctionality of nanosized calcium apatite dual-doped with li+/eu3+ ions related to cell culture studies and cytotoxicity evaluation in vitro. Biomolecules, 11(9), 1388. https://doi.org/10.3390/biom11091388

Son, H., Eo, H., Park, G., & Jeong, J. (2020). heracleum moellendorffii root extracts exert immunostimulatory activity through tlr2/4-dependent mapk activation in mouse macrophages, raw264.7 cells. Food Science & Nutrition, 9(1), 514-521. https://doi.org/10.1002/fsn3.2020

Stout, R. and Suttles, J. (2005). Immunosenescence and macrophage functional plasticity: dysregulation of macrophage function by age-associated microenvironmental changes. Immunological Reviews, 205(1), 60-71. https://doi.org/10.1111/j.0105-2896.2005.00260.x

Stout, R. and Suttles, J. (2005). Immunosenescence and macrophage functional plasticity: dysregulation of macrophage function by age-associated microenvironmental changes. Immunological Reviews, 205(1), 60-71. https://doi.org/10.1111/j.0105-2896.2005.00260.x

Sue, M., Yeap, S., Omar, A., & Tan, S. (2014). Application of pcr-elisa in molecular diagnosis. Biomed Research International, 2014, 1-6. https://doi.org/10.1155/2014/653014

Sun, X., Zhou, J., & Zhang, Z. (2022). Cullin 4a/protein arginine methyltransferase 5 (cul4a/prmt5) promotes cell malignant phenotypes and tumor growth in nasopharyngeal carcinoma. Bioengineered, 13(4), 8712-8723. https://doi.org/10.1080/21655979.2022.2054756

Szabó, R., Mezö, G., Pállinger, É., Kovács, P., Kõhidai, L., Bősze, S., … & Hudecz, F. (2008). in vitro cytotoxicity, chemotactic effect, and cellular uptake of branched polypeptides with poly[l-lys] backbone by j774 murine macrophage cell line. Bioconjugate Chemistry, 19(5), 1078-1086. https://doi.org/10.1021/bc7004544

Tan, G., Xiao, P., Zhu, H., Meng, Y., & Gong, P. (2023). Optical system design of single laser flow cytometer.. https://doi.org/10.1117/12.2667510

Tan, Y., Chen, X., Ma, C., Xi, X., Wang, L., Zhou, M., … & Chen, T. (2018). Biological activities of cationicity-enhanced and hydrophobicity-optimized

analogues of an antimicrobial peptide, dermaseptin-ps3, from the skin secretion of phyllomedusa sauvagii. Toxins, 10(8), 320. https://doi.org/10.3390/toxins10080320

Tang, S., Yuan, X., Song, J., Chen, Y., Tan, X., & Li, Q. (2018). Association analyses of the jak/stat signaling pathway with the progression and prognosis of colon cancer. Oncology Letters. https://doi.org/10.3892/ol.2018.9569

Tikhonov, S., Чернуха, И., & Dunchenko, N. (2024). Comparative evaluation antimicrobial and antitumor activities of natural colostrum peptide and its synthesized analogue. Food Science and Applied Biotechnology, 7(2), 333. https://doi.org/10.30721/fsab2024.v7.i2.283

Trinchieri, G. (2003). Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nature Reviews Immunology, 3(2), 133–146. https://doi.org/10.1038/nri1001

Verhoeven, R., Tong, S., Zhang, G., Zong, J., Chen, Y., Jin, D., … & Chen, H. (2016). Nf-κb signaling regulates expression of epstein-barr virus bart micrornas and long noncoding rnas in nasopharyngeal carcinoma. Journal of Virology, 90(14), 6475-6488. https://doi.org/10.1128/jvi.00613-16

Verhoeven, R., Tong, S., Zong, J., Chen, Y., Tsao, S., Pan, J., … & Chen, H. (2018). Nf-κb signaling regulates epstein–barr virus bamhi-q-driven ebna1 expression. Cancers, 10(4), 119. https://doi.org/10.3390/cancers10040119

Wan, F. and Lenardo, M. (2009). The nuclear signaling of nf-κb: current knowledge, new insights, and future perspectives. Cell Research, 20(1), 24-33. https://doi.org/10.1038/cr.2009.137

Wang, B., Zhong, Y., Li, Q., Cui, L., & Huang, G. (2018). Autophagy of macrophages is regulated by pi3k/akt/mtor signalling in the development of diabetic encephalopathy. Aging, 10(10), 2772-2782. https://doi.org/10.18632/aging.101586

Wang, C., Wang, S., Li, D., Chen, P., Han, S., Zhao, G., … & Wang, J. (2021). Human cathelicidin inhibits sars-cov-2 infection: killing two birds with one stone. Acs Infectious Diseases, 7(6), 1545-1554. https://doi.org/10.1021/acsinfecdis.1c00096

Wang, H., Morales, R., Cui, X., Huang, J., Qian, W., Tong, J., … & Chen, W. (2018). A photoresponsive hyaluronan hydrogel nanocomposite for

dynamic macrophage immunomodulation. Advanced Healthcare Materials, 8(4). https://doi.org/10.1002/adhm.201801234

Wang, J., Wang, N., Zheng, Z., Che, Y., Suzuki, M., Kano, S., … & Homma, A. (2022). Exosomal lncrna hotair induce macrophages to m2 polarization via pi3k/ p-akt /akt pathway and promote emt and metastasis in laryngeal squamous cell carcinoma. BMC Cancer, 22(1). https://doi.org/10.1186/s12885-022-10210-5

Wang, J., Wang, Y., Huang, R., Li, W., Fan, W., Hu, X., … & Liu, G. (2022). Uncovering the pharmacological mechanisms of zizhu ointment against diabetic ulcer by integrating network analysis and experimental evaluation in vivo and in vitro. Frontiers in Pharmacology, 13. https://doi.org/10.3389/fphar.2022.1027677

Wang, J., Zhong, W., Lin, D., Xia, F., Wu, W., Zhang, H., … & He, J. (2015). Antimicrobial peptides derived from fusion peptides of influenza a viruses, a promising approach to designing potent antimicrobial agents. Chemical Biology & Drug Design, 86(4), 487-495. https://doi.org/10.1111/cbdd.12511

Wang, S., Han, L., Li, J., Liu, Y., & Wang, S. (2022). Inflammatory molecules facilitate the development of docetaxel-resistant prostate cancer cells in vitro and in vivo. Fundamental &Amp; Clinical Pharmacology, 36(5), 837-849. https://doi.org/10.1111/fcp.12773

Wang, T., Zhang, J., Shen, J. H., Jin, Y., Lee, W. H., & Zhang, Y. (2005). Maximins S, a novel group of antimicrobial peptides from toad Bombina maxima. Biochemical and biophysical research communications, 327(3), 945–951. https://doi.org/10.1016/j.bbrc.2004.12.094

Wang, W., Shen, Y., Leng, Q., Ding, W., Peng, Z., Zhou, Y., … & Wu, C. (2023). Role of the notch signaling pathway in regulating macrophage polarization in a rat model of fibrotic pigeon breeder's lung. European Journal of Inflammation, 21. https://doi.org/10.1177/1721727x231202431

Watford, W. T., Moriguchi, M., Morinobu, A., & O'Shea, J. J. (2003). The biology of IL-12: Coordinating innate and adaptive immune responses. Cytokine & Growth Factor Reviews, 14(5), 361–368. https://doi.org/10.1016/S1359-6101(03)00034-8

Wei, X., Wu, R., Si, D., Liao, X., Zhang, L., & Zhang, R. (2016). Novel

hybrid peptide cecropin a (1–8)-ll37 (17–30) with potential antibacterial activity. International Journal of Molecular Sciences, 17(7), 983. https://doi.org/10.3390/ijms17070983

Wu, C., Dougan, T., & Walt, D. (2022). High-throughput, high-multiplex digital protein detection with attomolar sensitivity. Acs Nano, 16(1), 1025-1035. https://doi.org/10.1021/acsnano.1c08675

Xie, Y., Wang, M., Tian, J., Li, X., Yang, M., Zhang, K., … & Tang, A. (2019). Long non-coding rna expressed in macrophage co-varies with the inflammatory phenotype during macrophage development and polarization. Journal of Cellular and Molecular Medicine, 23(10), 6530-6542. https://doi.org/10.1111/jcmm.14557

Xing, Y., Wang, X., Jameson, S., & Hogquist, K. (2016). Late stages of t cell maturation in the thymus involve nf-κb and tonic type i interferon signaling. Nature Immunology, 17(5), 565-573. https://doi.org/10.1038/ni.3419

Xu, C., Guo, R., Hou, C., Ma, M., Dong, X., Ouyang, C., … & Huang, T. (2023). Resveratrol regulates macrophage recruitment and m1 macrophage polarization and prevents corneal allograft rejection in rats. Frontiers in Medicine, 10. https://doi.org/10.3389/fmed.2023.1250914

Yang, C., You, L., Yin, X., Liu, Y., Leng, X., Wang, W., … & Ni, J. (2018). Heterophyllin b ameliorates lipopolysaccharide-induced inflammation and oxidative stress in raw 264.7 macrophages by suppressing the pi3k/akt pathways. Molecules, 23(4), 717. https://doi.org/10.3390/molecules23040717

Yang, S., Zhao, M., & Jia, S. (2023). Macrophage: key player in the pathogenesis of autoimmune diseases. Frontiers in Immunology, 14. https://doi.org/10.3389/fimmu.2023.1080310

Yin, D., Bai, Q., Wu, X., Li, H., Shao, J., Sun, M., … & Zhang, J. (2021). A multiepitope fusion protein-based p-elisa method for diagnosing bovine and goat brucellosis.. https://doi.org/10.1101/2021.03.04.434020

Yin, J., Valin, K., Dixon, M., & Leavenworth, J. (2017). The role of microglia and macrophages in cns homeostasis, autoimmunity, and cancer. Journal of Immunology Research, 2017, 1-12. https://doi.org/10.1155/2017/5150678

Yu, H., Bai, Y., Qiu, J., He, X., Xiong, J., Dai, Q., … & Zhang, K. (2021). Pseudomonas aeruginosa pcrv enhances the nitric oxide-mediated tumoricidal activity of tumor-associated macrophages via a tlr4/pi3k/akt/mtor-glycolysis-nitric oxide circuit. Frontiers in Oncology, 11. https://doi.org/10.3389/fonc.2021.736882

Yu, H., Pardoll, D., & Jove, R. (2020). STATs in cancer inflammation and immunity: a leading role for STAT3. Nature Reviews Cancer, 9(11), 798–809. https://doi.org/10.1038/nrc2734

Yu, X., Zhang, H., Yu, L., Liu, M., Zuo, Z., Han, Q., … & Zhang, C. (2018). Intestinal lamina propria cd4 + t cells promote bactericidal activity of macrophages via galectin-9 and tim-3 interaction during salmonella enterica serovar typhimurium infection. Infection and Immunity, 86(8). https://doi.org/10.1128/iai.00769-17

Yuan, S., Dong, Y., Peng, L., Yang, M., Niu, L., Liu, Z., … & Xie, J. (2019). Tumor-associated macrophages affect the biological behavior of lung adenocarcinoma a549 cells through the pi3k/akt signaling pathway. Oncology Letters. https://doi.org/10.3892/ol.2019.10483

Zhang, L.-J., & Gallo, R. L. (2016). Antimicrobial peptides. Current Biology, 26(1), R14–R19. https://doi.org/10.1016/j.cub.2015.11.017

Zhang, X. and Dong, S. (2022). Protective effect of growth differentiation factor 15 in sepsis by regulating macrophage polarization and its mechanism. Bioengineered, 13(4), 9687-9707. https://doi.org/10.1080/21655979.2022.2059903

Zhang, X., He, D., Gao, S., Wei, Y., & Wang, L. (2019). Aspergillus□fumigatus enhances human nk cell activity by regulating m1 macrophage polarization. Molecular Medicine Reports. https://doi.org/10.3892/mmr.2019.10365

Zhang, Z., Chen, L., Li, Z., Yan, L., & Wang, H. (2021). Structure and immunological function of amphibian antimicrobial peptides: Potential for novel adjuvant development. Peptides, 143, 170609. https://doi.org/10.1016/j.peptides.2021.170609

Zhao, X., Dai, J., Xiao, X., Wu, L., Zeng, J., Sheng, J., … & Li, K. (2014). Pi3k/akt signaling pathway modulates influenza virus induced mouse alveolar macrophage polarization to m1/m2b. Plos One, 9(8), e104506.

https://doi.org/10.1371/journal.pone.0104506

Zhao, Y., Li, M., Yang, Y., Wu, T., Huang, Q., Wu, Q., … & Ren, C. (2021). Identification of macrophage polarization-related genes as biomarkers of chronic obstructive pulmonary disease based on bioinformatics analyses. Biomed Research International, 2021(1). https://doi.org/10.1155/2021/9921012

Zhao, Y., Ma, W., Zhang, Y., Zhang, L., & Wang, H. (2018). Maximin-H5 from Bombina maxima exhibits anti-inflammatory activity via inhibition of the NF-κB pathway. Journal of Ethnopharmacology, 224, 178–185. https://doi.org/10.1016/j.jep.2018.06.027

Zhong, K., Zhu, M., Yuan, Q., Deng, Z., Feng, S., Liu, D., … & Yuan, X. (2022). Development of an indirect elisa to detect african swine fever virus pp62 protein-specific antibodies. Frontiers in Veterinary Science, 8. https://doi.org/10.3389/fvets.2021.798559

Zhou, J., Zhang, A., & Liang, F. (2020). HSPA12B Secreted by Tumor-Associated Endothelial Cells Might Induce M2 Polarization of Macrophages via Activating PI3K/Akt/mTOR Signaling;. Oncotargets and Therapy, Volume 13, 9103-9111. https://doi.org/10.2147/ott.s254985

Zhou, P., Xie, W., Luo, Y., Lu, S., Dai, Z., Wang, R., … & Sun, X. (2018). Protective effects of total saponins of aralia elata (miq.) on endothelial cell injury induced by tnf-α via modulation of the pi3k/akt and nf-κb signalling pathways. International Journal of Molecular Sciences, 20(1), 36. https://doi.org/10.3390/ijms20010036

Zhu, D., Yu, Y., Wang, W., Wu, K., Liu, D., Yang, Y., … & Zhao, S. (2019). Long noncoding rna part1 promotes progression of non-small cell lung cancer cells via jak-stat signaling pathway. Cancer Medicine, 8(13), 6064-6081. https://doi.org/10.1002/cam4.2494

Zhu, H., Ding, X., Li, W., Lu, T., Ma, C., Xi, X., … & Chen, T. (2018). Discovery of two skin-derived dermaseptins and design of a tat-fusion analogue with broad-spectrum antimicrobial activity and low cytotoxicity on healthy cells. Peerj, 6, e5635. https://doi.org/10.7717/peerj.5635

Zoggel, H., Hamma-Kourbali, Y., Galanth, C., Ladram, A., Nicolas, P., Courty, J., … & Delbé, J. (2010). Antitumor and angiostatic peptides from frog skin secretions. Amino Acihttps://doi.org/10.1007/s00726-010-0815-9

II. APPENDICES

1- Ethics Committee Decision

BİRUNİ ÜNİVERSİTESİ
"Bilimin Geleceği"

Date: 20 June 2025

Study Title: Investigation of Immunomodulatory Effects of Maximin S1 on Mammalian Macrophages

Study Decision No: 2024-BİAEK/06-21

To

Prof. Furkan Ayaz,

Biruni University Ethics Committee of Scientific Research received, reviewed and discussed the above mentioned study related documents. The study have been approved in the meeting held on **20 Jan 2025** with study decision number **2024-BİAEK/06-21** by the Ethics Committee of Scientific Research.

This document has been translated in English and issued upon the request of researcher, and I kindly submitted for your information and necessary actions.

Merkezefendi Mah. G/75 Sok. No.1-13 Zeytinburnu / İSTANBUL
T: 444 8 276 (BRN) F: (212) 416 46 46 E: info@biruni.edu.tr
www.biruni.edu.tr

BİRUNİ ÜNİVERSİTESİ BİLİMSEL ARAŞTIRMALAR ETİK KURULU KARAR FORMU

ARAŞTIRMANIN AÇIK ADI	Maximin S1'in İmmünomodülatör Etkilerinin Makrafajlar Üzerinde İncelenmesi

KARAR BİLGİLERİ	Karar No: 2024-BİAEK/06-21	Tarih: 20.01.2025
	Yukarıda bilgileri verilen başvuru dosyası ile ilgili belgeler araştırmanın/çalışmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş ve uygun bulunmuş olup araştırmanın/çalışmanın başvuru dosyasında belirtilen merkezlerde gerçekleştirilmesinde etik ve bilimsel açıdan bir sakınca bulunmadığına toplantıya katılan etik kurul üye tam sayısının salt çoğunluğu ile karar verilmiştir. Biruni Üniversitesi Bilimsel Araştırmalar Etik Kurul Yönergesi kapsamında yer alan anket ve ölçek araştırmaları/çalışmaları ile kurum ya da kurumlardan alınması gerekli olan tüm izin ve sorumluluk etik kurul başvuru formunda belirtilen araştırmacı/lara aittir.	

BİRUNİ ÜNİVERSİTESİ BİLİMSEL ARAŞTIRMALAR ETİK KURULU	
ETİK KURULUN ÇALIŞMA ESASI	BİRUNİ ÜNİVERSİTESİ BİLİMSEL ARAŞTIRMALAR ETİK KURUL YÖNERGESİ
BAŞKANIN UNVANI / ADI / SOYADI:	Prof. Dr. AHMET BELCE

Unvanı/Adı/Soyadı	Uzmanlık Alanı	Kurumu	İmza
Prof. Dr. Ahmet Belce Etik Kurul Başkanı	Tıp Fakültesi / Tıbbi Biyokimya	Biruni Üniversitesi	TOPLANTIYA KATILMADI
Prof. Dr. Nezihe Kızılkaya Beji	Sağlık Bilimleri Fakültesi / Hemşirelik	Biruni Üniversitesi	
Prof. Dr. İlker Tolga Özgen	Tıp Fakültesi / Çocuk Endokrinolojisi	Biruni Üniversitesi	
Prof. Dr. Zeynep Hoşbay Etik Kurul Başkan Yardımcısı	Sağlık Bilimleri Fakültesi / Fizyoterapi ve Rehabilitasyon	Biruni Üniversitesi	
Dr. Öğr. Üyesi Manolya Sağlam	Eğitim Fakültesi / İngilizce Öğretmenliği	Biruni Üniversitesi	
Dr. Öğr. Üyesi Nur Ekimci Gürcan	Mühendislik ve DB Fakültesi / Moleküler Biyoloji ve Genetik	Biruni Üniversitesi	
Dr. Öğr. Üyesi Tambay Taşkın	Eczacılık Fakültesi / Farmakoloji	Biruni Üniversitesi	
Dr. Öğr. Üyesi Fatma Büşra Doğan	Diş Hekimliği Fakültesi / Ağız, Diş ve Çene Radyolojisi	Biruni Üniversitesi	
Dr. Öğr. Üyesi Safa Heybet	Meslek Yüksekokulu / Fizyoterapi	Biruni Üniversitesi	

Etik Kurul Başkan Yardımcısı
Unvanı/Adı/Soyadı: PROF. DR. ZEYNEP HOŞBAY
İmza:

2- Institutional Permission Approval

T.C
BİRUNİ ÜNİVERSİTESİ
LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ
ANABİLİM DALI KURUL TOPLANTISI

TOPLANTI SAYISI	KARAR TARİHİ	KARAR SAYISI
37	**11.12.2024**	**37**

Lisansüstü Eğitim Enstitüsü Moleküler ve Tıbbi Genetik ana bilim dalı **11.12.2024** tarihinde saat **09:00'de** aşağıdaki gündem maddesini görüşmek üzere Doç. Dr. Furkan Ayaz başkanlığında toplanmıştır.

Toplantıya Katılanlar
Doç. Dr. Furkan Ayaz (Başkan)
Prof. Dr. İsmail Tuncer Değim *(Üye)*
Dr. Öğr. Üyesi Elif Sibel ASLAN
Dr. Öğr. Üyesi Nur Ekimci Gürcan
Dr. Öğr. Üyesi İlayda Duru

Toplantıya Katılmayanlar
Prof. Dr. Hakan CANGÜL *(Üye)*

GÜNDEM :

Moleküler ve Tıbbi Genetik Anabilim Dalı bünyesinde yapılacak olan

" Maximin S1'in İmmünomodülatör etkilerinin makrofajlar üzerinde incelenmesi; başlıklı yüksek lisans tez çalışmanın yapılması

KARARLAR :

Moleküler ve Tıbbi Genetik Anabilim Dalı bünyesinde yapılacak olan;

" Maximin S1'in İmmünomodülatör etkilerinin makrofajlar üzerinde incelenmesi"; başlıklı araştırma için hazırlanan etik kurul dosyasının kabulüne;

Mevcudun oybirliği ile karar verilmiştir.

1

III. PLAGIARISM REPORT

INVESTIGATION OF IMMUNOMODULATORY EFFECTS OF MAXIMIN S1 ON MAMMALIAN MACROPHAGES

ORİJİNALLİK RAPORU

%12	%7	%10	%3
BENZERLİK ENDEKSİ	İNTERNET KAYNAKLARI	YAYINLAR	ÖĞRENCİ ÖDEVLERİ

BİRİNCİL KAYNAKLAR

1	www.frontiersin.org İnternet Kaynağı	%2
2	Adeeb Shehzad. "Cell Signaling - Interplay, Mechanisms, and Therapeutic Implications", CRC Press, 2025 Yayın	%1
3	Khawaja Husnain Haider. "Handbook of Regenerative Medicine - Cell-Free Therapy Approach", CRC Press, 2025 Yayın	%1
4	www.mdpi.com İnternet Kaynağı	%1
5	Sajal Chakraborti. "Handbook of Proteases in Cancer - Therapeutic Aspects", CRC Press, 2024 Yayın	<%1
6	www.preprints.org İnternet Kaynağı	<%1
7	Umesh Kumar, Deepak Parashar, Subodh Kumar. "Viral Oncology - New Approaches to Molecular Cancer Therapeutics", CRC Press, 2025 Yayın	<%1
8	MD Faiyazuddin, Sumel Ashique. "The Role of Probiotics in Cancer Management", Apple Academic Press, 2025 Yayın	<%1