# THE USE OF ANTIMICROBIAL PEPTIDES, LL-37 AND DERIVATIVES, TO TARGET RIFT VALLEY FEVER VIRUS INFECTION

by

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Biology

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# The Use of Antimicrobial Peptides, LL-37 and Derivatives, to Target Rift Valley Fever Virus Infection

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at George Mason University.

By

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## Table of Contents

## List of Tables

## List of Figures

## Abstract

THE USE OF ANTIMICROBIAL PEPTIDE, LL-37 AND DERIVATIVES, TO TARGET RIFT VALLEY FEVER VIRUS INFECTION

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Rift Valley Fever Virus (RVFV) is an enveloped negative and ambisense, single-stranded RNA phlebovirus of the family Phenuiviridae that primarily infects domestic livestock, but can be transmitted to, and infect, humans. Disease following RVFV infection causes near 100% abortion in pregnant livestock, dramatically affecting economic livelihoods in RVFV endemic areas. In humans, RVFV infection results in a mild febrile illness; however, in a small number of cases, a severe form of the disease may develop which manifests as meningoencephalitis and hemorrhagic fever that is occasionally fatal. Additionally, RVFV is highly infectious in aerosol form solidifying its potential as a biological weapon. In 2015, RVFV was added to the World Health Organization's list of "World's Most Dangerous Emerging Pathogens." There are currently no approved vaccines or therapeutics available for the treatment of RVFV infection. We propose the use of antimicrobial peptides (AMP), both naturally occurring and synthetic, as a

therapeutic strategy against RVFV infection. AMPs are broad-spectrum oligopeptides that are produced as a first line of defense by the innate immune response. The AMP LL-37, the sole member of the human cathelicidin family of AMPs, has demonstrated antiviral activity against a number of viruses. LL-37 inhibits viral infections through several mechanisms including: directly binding to and disrupting viral envelopes, an activity facilitated by its amphipathic structure; inhibiting the upregulation and production of inflammatory mediators; and modulating host signaling pathways. In this study, we assessed the efficacy of LL-37 against RVFV in Human Small Airway Epithelial Cells (HSAECs), using plaque assays and polymerase chain reaction (PCR) techniques, and found that LL-37 significantly reduced RVFV titers. In addition, a library of synthetic peptides was screened against RVFV, which resulted in the identification of several LL-37 derivative peptides with potent anti-RVFV activity. As such, LL-37 and derivative peptides offer an intriguing therapeutic strategy for the treatment of RVFV infections.

## Specific Aims

1. Utilize a proof-of-concept study to exhibit that LL-37, and its synthetic derivative peptides, are efficacious against Rift Valley Fever Virus.
2. Elucidate the mechanism of action of LL-37 and determine if LL-37 exerts its effects on the host cell, the virus, or a combination of the two.

## Introduction

*Rift Valley Fever Virus*

Rift Valley Fever Virus (RVFV) is an enveloped negative and ambisense, single-stranded RNA phlebovirus of the family Phenuiviridae that primarily infects domestic livestock, but can be transmitted to, and infect, humans. Disease following RVFV infection causes near 100% abortion in pregnant livestock, dramatically affecting meat supply chains in endemic areas and their economies as a result. In humans, RVFV infection results in a mild febrile illness; however, in a small number of cases, a severe form of the disease may develop which manifests as meningoencephalitis and hemorrhagic fever that is occasionally fatal. Additionally, RVFV is highly infectious in aerosol form solidifying its potential as a biological weapon. In 2015, RVFV was added to the World Health Organization's list of "World's Most Dangerous Emerging Pathogens." There are currently no approved vaccines or therapeutics available for the treatment of RVFV infection. It has numerous hosts including goats, camels, cattle, sheep, domesticated buffalos, and humans. The disease is endemic in Kenya, Tanzania, Mauritania, South

Africa, Egypt, Saudi Arabia, Yemen, Senegal, Gambia, Sudan, South Sudan, Zambia, Zimbabwe, Mozambique, Madagascar, and Namibia.

The typical incubation period for RVFV is 2-6 days followed by self-limiting symptoms of mild febrile illness, weakness, joint pain, muscle pain, headaches, or no obvious symptoms at all. Severe symptoms of the disease include ocular disease, meningoencephalitis, and hemorrhagic fever. RVFV can be transmitted by direct or indirect contact with contaminated or infected livestock, meat, or milk. It is capable of replicating in both vertebrate and invertebrate hosts and can also be transmitted horizontally by Aedes (primary vector) and Culex mosquitoes (secondary vector), and vertically by Aedes mosquitoes. During dry seasons (endemic cycle), infected Aedes mosquitoes deposit transovarially-infected eggs into their environment introducing RVFV. When rainy season occurs (epidemic cycle), heavy rainfall causes the hatching of infected mosquito eggs (Aedes & Culex) resulting in Rift outbreaks and making transmission most likely at this time.

Similar to other phenuiviruses, RVFV has a diameter of 90-110 nm and a helical nucleocapsid. The genome of RVFV consists of an S, M, and L segment. The ambisense S segment, 1-3 kilobases in length, encodes for a nucleocapsid protein (N) and a nonstructural protein (NSs). The M segment, 3-5 kilobases in length, encodes for two viral envelope glycoprotein precursors (Gn and Gc) and a nonstructural protein (NSm). The L segment, 6-12 kilobases in length, encodes for RNA polymerase. Research has shown that RVFV entry into cells is facilitated by caveolar/lipid raft-mediated

endocytosis. Gn and Gc have been implicated in viral cell attachment, and there is evidence that a conformational change in Gn on the viral surface facilitates the fusion of the virus with cellular membranes at acidic pH levels. Once taken into cells by caveolae, virions are enveloped into endosomes. The acidity of the endosome allows viral uncoating to occur. Afterwards, there is fusion of the viral and endsomomal membranes, leading to the release of the viral nucleocapsid into the host cytoplasm. The virus has a cytoplasmic site of replication. This is where the synthesis of viral mRNA synthesis is primed by stealing the capped 5' ends of host cell mRNAs. Virion assembly has been shown to occur at the golgi apparatus, however assembled virions bud directly at the host cell surface.

Nonstructural protein NSs has been shown to be the main virulent factor contributing to the pathogenicity of RVFV, although viral replication is not dependent on this protein. NSs facilitates the establishment of RVFV, prevents the expression of Interferon-β, and induces filament formation in the host nucleus causing cell cycle arrest and apoptosis. To establish RVFV, NSs utilizes the ubiquitin-proteasome pathway to prevent the assembly of the transcription factor TFIIH complex, thereby inhibiting its association with DNA, which is required for RNA Polymerase II to exit the promoter. The TFIIH complex is composed of two subcomplexes, the Core and CAK, bridged by an XPD subunit. NSs destroys the p62 subunit of the Core, thus shutting down host transcription that would enable antiviral responses. NSs also induces the degradation of RNA-dependent eIF2 kinase PKR. Under normal conditions, PKR senses double-stranded RNA longer than 30 base pairs. It then works by phosphorylating inhibitory eLF2α serine residues, thus

reducing the dissociation rate of eIF2B. This inhibits the GDP-GTP exchange reaction and decreases the rate of viral protein synthesis. Elimination of PKR allows for efficient viral protein synthesis.

To combat RVFV, I propose the use of antimicrobial peptides, both natural and synthetic in origin, as a therapeutic strategy.

*Antimicrobial Peptides*

Antimicrobial peptides (AMP) are protein members of the innate immune response that provide a non-specific, swift, and efficient defense against invading pathogens. They have a diverse array of targets (bacteria, viruses, fungi) and can be natural or synthetic in origin. Typically concentrated in tissues and organs exposed to aerosolized pathogens, AMPs have a variety of functions which are often dependent on their size. Large AMPs typically act as lytic enzymes or binding molecules of either nutrients or microbial macromolecules. Whereas, small AMPs target microbial cell membranes destroying their structure thereby inhibiting their function. Antimicrobial peptides have been shown to act as immunomodulators that enhance or suppress the immune response. Not surprisingly, research has also shown they manage inflammatory responses during infection. There are four main types of AMPs delineated by their secondary structures: α-helix, β-sheet, extended, and loop.

AMPs are well known for their antibacterial effects achieved through membrane disruption and the destruction of bacterial biofilms that release harmful toxins. However, recent studies are suggesting that AMPs can also exhibit antiviral function and work synergistically with the adaptive immune system to combat viruses. Research has shown that AMPs increase the susceptibility of viruses to neutralization by antibodies by bringing normally sequestered viral antigens to the cell surface.

Antimicrobial peptide LL-37 is a 37-residue α-helical human cathelicidin peptide found in the lysosomes of macrophages and polymorphonuclear leukocytes. LL-37 is derived from the precursor hCAP-18, from which it is cleaved by proteinase-3 to release its active form. It has a highly conserved N-terminal cathelin domain. LL-37 upregulation occurs in response to infection and inflammation and evidence suggests that in epithelial cells and macrophages, its expression is upregulated by Vitamin-D. LL-37 expression has been shown to modulate cytokines such as IL-17A, tumor necrosis factor α, toll-like receptor agonists, and vitamin D receptor agonists. It has also been shown to downregulate interleukin-6, interferon –γ, and bacterial endotoxins.

LL-37, along with other AMPs, has been shown to disrupt viral outer membranes. This is essential in regards to enveloped viruses like RVFV, as they are more difficult to target than naked viruses because surface antigens are obscured. As such, this study hopes to evaluate if LL-37 is efficacious against RVFV and elucidate the mechanisms used by this peptide.

## Results

### Evaluation of LL-37 as a Therapeutic Candidate

In order to establish LL-37 as an antiviral candidate, a peptide cytotoxicity screen was first performed on human small airway epithelial cells (HSAECs) to determine cell viability at different concentrations of LL-37. This was done to ensure that the peptide drug, LL-37, is nontoxic to cells and to provide a clear understanding of our data results. To do this, HSAECs were seeded for 24 hours then treated with increasing concentrations of LL-37. After 24 hours of incubation, cell viability was determined with a Cell Titer-Glo Assay. Results (Fig.1) show cell viability was 90% or greater at LL-37 concentrations of 10 ug/mL and 50 ug/mL. A concentration of 50 ug/mL of LL-37 was chosen for infection. Scrambled and reverse sequences of LL-37 (see figure caption) were also tested for toxicity. These sequences were used to ensure that if efficacy was observed, it was due to sequence-specific LL-37 treatment.

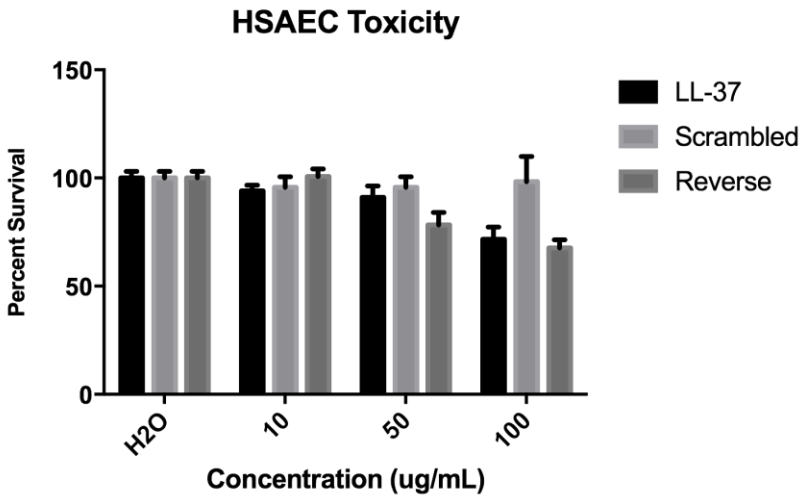


**Figure 1: Toxicity for LL-37 treated HSAECs**
Cell viability was 90% or greater at LL-37 concentrations of 10 ug/mL and 50 ug/mL.
LL-37 Sequence: LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES
Scrambled LL-37 Sequence: GLKLRFEFSKIKGEFLKTPEVRFRDIKLKDNRISVQR
Reverse LL-37 Sequence: SETRPVLNRLFDKIRQVIRKFEKGIKEKSKRFFDGLL

Next, a proof of concept study was performed. HSAECs were seeded at a cell density of 5.00 x 10^4 and incubated for 24 hours. They then underwent an infection that sequentially involved a 2-hour LL-37 pre-treatment, RVFV infection with the MP-12 strain of RVFV for 1 hour, then LL-37 post-treatment for 9 or 16 hours. Plaque assay results (Fig 2) show that LL-37 is efficacious against RVFV replication, as seen through

a decrease in plaque forming units per milliliter (pfu/mL) with LL-37 treatment. Compared to the water control, the data shows that LL-37 treated cells displayed a 3 log decrease in pfu/mL at 9 hpi and a 5.5 log decrease in pfu/mL at 16 hpi. There is a minimal reduction in pfu/mL seen at the 9-hour timepoint for scrambled and reverse LL-37 treatment. However, this phenotype is reversed at the 16-hour timepoint, indicating that sequence-specific LL-37 treatment is responsible for the longitudinal reduction in the number of infectious RVFV virions exiting the host cell.

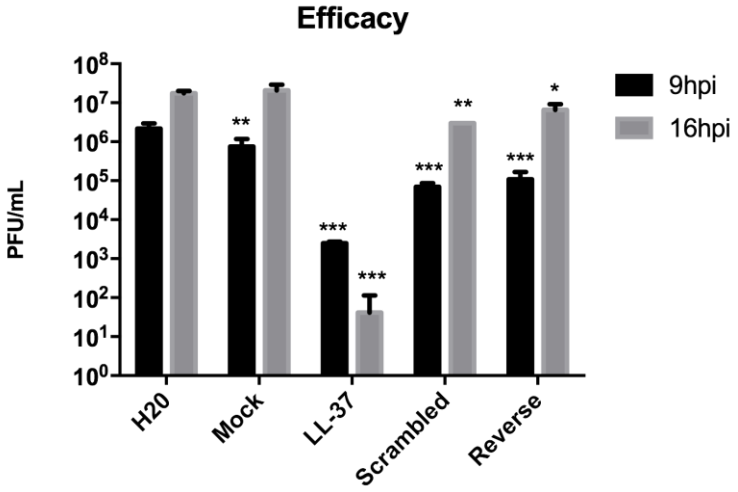


**Figure 2: Efficacy for LL-37 treated HSAECs**
Compared to the water control, the data shows that LL-37 treated cells displayed a 3 log decrease in pfu/mL at 9 hpi and a 5.5 log decrease in pfu/mL at 16 hpi.

**LL-37 Reduces Intracellular Viral Genomic Copies More Robustly at 9 HPI than Extracellular Viral Genomic Copies**

Since it was established that LL-37 treatment reduces the number of infectious virions exiting the host cell, it was important to elucidate if LL-37 treatment is capable of decreasing the number of genomic copies inside the host cell. In order to do this, an intracellular PCR was performed. An infection was carried out, similar to the initial efficacy infection, then cells were lysed and viral RNA was extracted to perform a PCR. For this infection, there was a pre-treatment only condition (LL-37) and a pre-treatment and post-treatment condition (pLL-37). Compared to the water control, the pre-treatment only condition (LL-37) showed a 3 log decrease in intracellular viral genomic copies due to LL-37 treatment at the 9 hpi and a 2.5 log decrease at 16 hpi. (Fig. 3). For the pre-treatment and post-treatment condition (pLL-37), there was a 3.5 log decrease at 9 hpi and a 4.5 log decrease at 16 hpi. This could indicate that increased availability of LL-37, which we see with pLL-37, allows LL-37 to exert its effects longitudinally leading to a greater decrease in intracellular viral genomic copies at the 16 hour timepoint for the

pLL-37 condition . As such, we see that LL-37 treatment of RVFV infection, whether with pretreatment alone or with an additional post-treatment, results in a decrease in the copy number of RVFV inside the host cell.

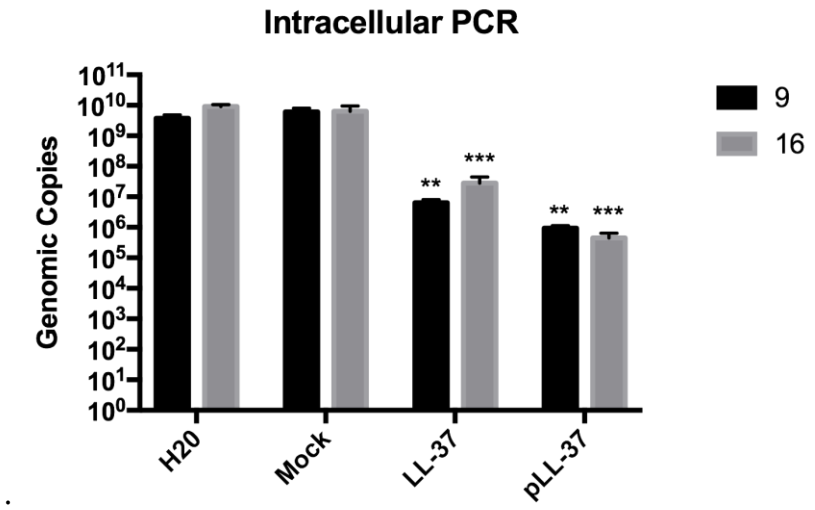


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**Figure 3: 9 hpi and 16 hpi Intracellular PCR**
Compared to the water control, the pre-treatment only condition (LL-37) showed a 3 log decrease in intracellular viral genomic copies due to LL-37 treatment at the 9 hpi and a 2.5 log decrease at 16 hpi. (Fig. 3). For the pre-treatment and post-treatment condition (pLL-37), there was a 3.5 log decrease at 9 hpi and a 4.5 log decrease at 16 hpi.

In order to examine the effects of LL-37 on the number of viral genomic copies exiting the host cell, an extracellular PCR was performed. The purpose was to determine the copy number of RVFV outside the cell in order to evaluate if there is a correlation with the number of infectious virions exiting the host cell seen through the plaque assay.

The hope was that this information would elucidate if extracellular LL-37 exerts its effects on virions exiting the host cell thereby preventing them from being infectious or if peptide treatment leads to defective new virions.

The extracellular PCR infection was performed similarly to the intracellular extraction, however in this case, supernatants were collected at 9 and 16 hpi, and viral RNA was extracted from the supernatant before a PCR was performed. Compared to the water control, the pre-treatment only condition (LL-37) displayed a 2.5 log decrease in extracellular viral genomic copies at 9 and 16 hpi. The pre-treatment and post-treatment condition (pLL-37), compared to the water control, displayed a ~2.75 log decrease in extracellular viral genomic copies at 9 hpi and a 4 log decrease at 16 hpi. Again, the stronger decrease with the pLL-37 condition could be attributable to increased availability of LL-37, particularly in the extracellular environment in this case, which may allow LL-37 to continuously exert its effects on new exiting virions. As such, the data shows that LL-37 treatment of RVFV infection, whether with pretreatment alone or with an additional post-treatment, results in a decrease in the copy number of RVFV outside the host cell.

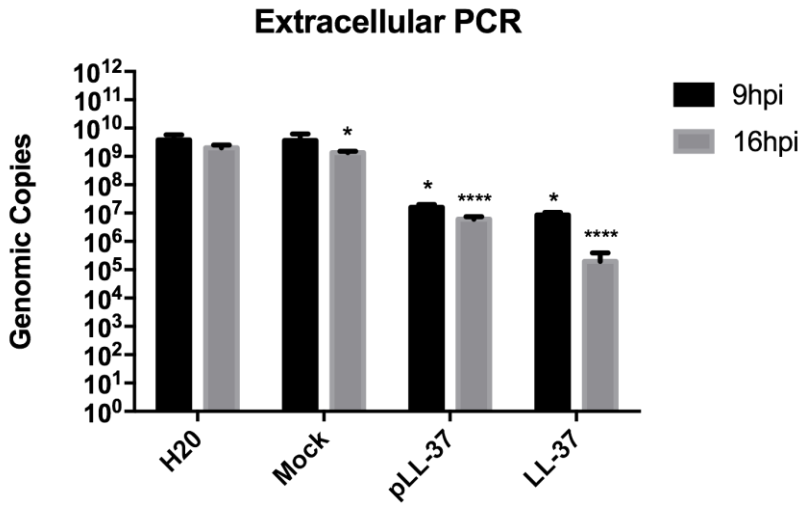


**Figure 4: 9 hpi and 16 hpi Extracellular PCR**
Compared to the water control, the pre-treatment only condition (LL-37) displayed a 2.5 log decrease in extracellular viral genomic copies at 9 and 16 hpi. The pre-treatment and post-treatment condition (pLL-37), compared to the water control, displayed a ~2.75 log decrease in extracellular viral genomic copies at 9 hpi and a 4 log decrease at 16 hpi.

Based on our efficacy studies, sequence-specific LL-37 treatment has proven to be efficacious against RVFV, as seen through the reduction in infectious virions exiting the host cell and the reduction in intracellular and extracellular viral genomic copies.

**There is Decreased Efficacy of LL-37 with Increased Time After Infection**

To determine if the efficacy of LL-37 changes as RVFV infection progresses, a time of addition assay was performed (Fig. 5). LL-37 was given at the following times: 2

hours before infection (labeled LL-37) and 0, 2, 4, and 6 hours post-infection. Supernatants were collected at 16 hpi. As shown through an extracellular PCR, there is a 4.5 log decrease in extracellular viral genomic copies when cells are pretreated with LL-37 2 hours before infection. There is 2 log decrease with LL-37 treatment immediately after infection (0 hpi) and a 1.5 log decrease 2 hours after infection (2 hpi). The decrease observed in viral genomic copies, compared to the water control, decreases with increased time of infection, indicating that LL-37 treatment efficacy has a negative correlation with time after infection. Additionally, our data shows that LL-37 pre-treatment results in a lower number of extracellular viral genomic copies. Taken together, this indicates that LL-37 exerts its maximal effect at early time points, possibly interfering with viral entry.

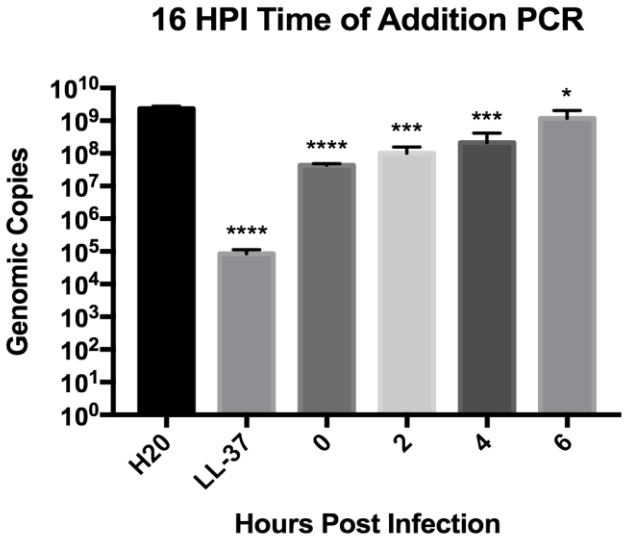


**Figure 5: Time of Addition Assay**
Compared to the water control, there is a 4.5 log decrease in extracellular viral genomic copies when cells are pretreated with LL-37 2 hours before infection. There is 2 log

decrease with LL-37 treatment immediately after infection (0 hpi) and a 1.5 log decrease 2 hours after infection (2 hpi).

**Synthetic Peptides are Efficacious Against RVFV**

LL-37 is a naturally occurring peptide. As such, it has broad innate immunity applications, which in the case of infection treatment results in a lack of specificity. However, optimized synthetic peptides could provide a tailored strategy to targeting Rift Valley Fever Virus infection. To evaluate the efficacy of synthetic peptides against RVFV, a proof-of-concept plaque assay was performed using 1$^{st}$ generation synthetic antimicrobial peptides, based on LL-37. Prior to the plaque assay, 19 synthetic peptides underwent cytotoxicity studies (not shown) to determine if they were toxic to HSAECs. Of these peptides, 5 were found to be nontoxic and showed consistent HSAEC viability at these concentrations: C9 (10 ug/mL), DJK5 (50 ug/mL), F3 (10 ug/mL), F8 (100 ug/mL), and F10 (50 ug/mL). These peptides were used to treat HSAECs infected with RVFV at an MOI of 1. Results (Fig 6) show that pre-treatment results in a lower pfu/mL for most of the synthetic peptides, except C9 and F8. Compared to the water control, RVFV treatment with F8 and F10 resulted in the lowest pfu/mL with F8 treatment resulting in a 2 log decrease with pretreatment and when added at the time of infection and F10 treatment resulting in a 2.25 log decrease with pretreatment and a 2 log decrease when added at the time of infection. This data shows that synthetic peptide treatment of RVFV decreases the number of viral infectious particles exiting the host cell. Since

efficacy has been established, the goal is to modify these synthetic peptides to maximize their anti-Rift functions.

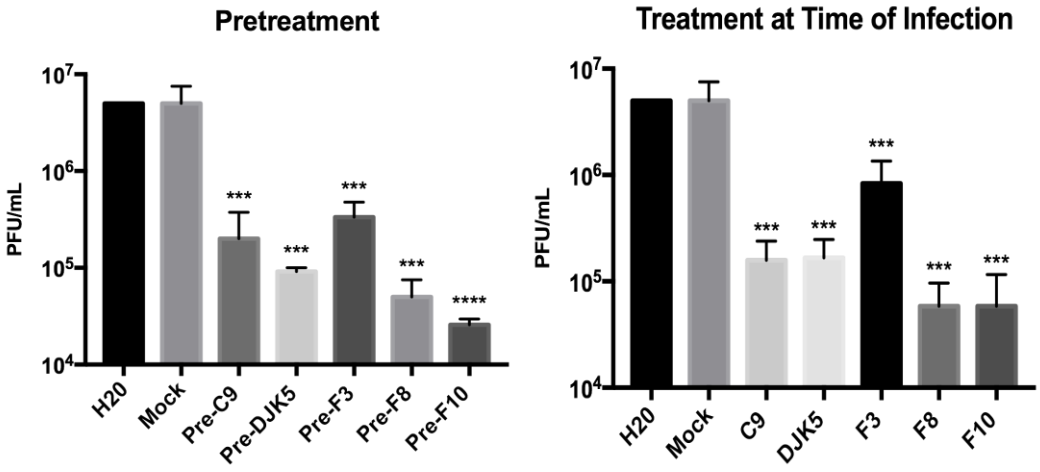


**Figure 6: Plaque Assay for Synthetic Peptides**
Compared to the water control, RVFV treatment with F8 and F10 resulted in the lowest pfu/mL with F8 treatment resulting in a 2 log decrease with pretreatment and when added at the time of infection and F10 treatment resulting in a 2.25 log decrease with pretreatment and a 2 log decrease when added at the time of infection.

The goal of specific aim 1 was efficacy determination. Through the experiments presented, we have shown that sequence-specific LL-37 treatment of RVFV longitudinally decreases the number of viral infectious particles exiting the host cell, decreases the copy number of RVFV inside the host cell, and decreases the copy number of RVFV outside the host cell. Through these studies, it has also been shown that there is

a negative correlation between LL-37 efficacy against RVFV and time after infection, displaying that LL-37 pre-treatment results in a lower number of viral copies. Lastly, the data showed that synthetic peptide treatment of RVFV is also capable of decreasing the number of viral infectious particles exiting the host cell. Next, the focus shifts to specific aim 2, which is the elucidation of the mechanisms LL-37 uses to exert the aforementioned events.

First, LL-37 will be evaluated to determine if it has a viral-based mechanism of action.

**LL-37 has a Viral-Based Mechanism of Action**

The time of addition assay performed showed that pre-treatment with LL-37 greatly impacted RVFV replication, indicating that LL-37 may be interfering with viral entry at earlier time points. Additionally, previous literature by Dean et al. showed that LL-37 is known to interact with the viral envelope, either by poking holes in the viral membrane or through disintegration. To further assess whether LL-37 has a viral-based mechanism of action, an entry assay was performed. Two new conditions were added to the entry assay. The first is a "Pre-incubated LL-37" condition in which LL-37 and RVFV were pre-incubated together for 30 minutes before being used to infect HSAECs. The second new condition was called "LL-37 & Pre-incubation" and it combined the "LL-37" condition, which involved pre- and post-treatment with LL-37, and the "Pre-incubated LL-37" condition. The entry assay was done at an MOI of 1 and 5. Results (Fig. 7) show that,

compared to the water control, all LL-37 treated conditions had a 1- 1.5 log decrease in intracellular genomic copies. These results show that LL-37 directly impacts RVFV. Therefore, introducing RVFV in the presence of LL-37, or incubating it with LL-37, both yield the same effect – a reduction of the RVFV copy number inside the host cell.

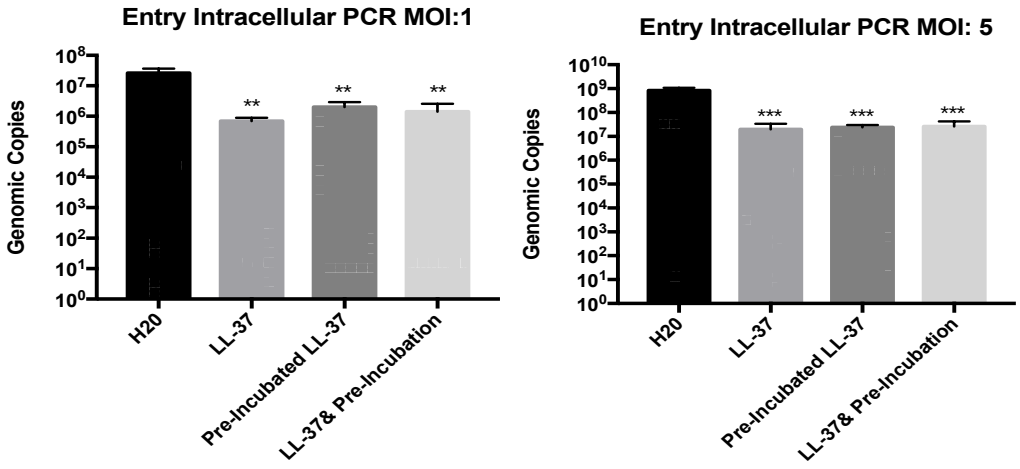


**Figure 7: Intracellular Entry Assay (MOI of 1 & 5)**
Compared to the water control, all LL-37 treated conditions had a 1- 1.5 log decrease in intracellular genomic copies.

**LL-37 has a Host-Based Mechanism of Action**

**Interferon-β Expression**

Previous studies have shown that LL-37 can regulate the immune response, and is upregulated during infection and injury. In Hepatitis C infection and Zika infection, LL-37 bolstered the IFN response leading to an attenuation of the viruses. From the literature, two concepts have been solidified: 1) RVFV represses interferon-β expression 2) LL-37 enhances interferon-β expression. Therefore, we hypothesize that LL-37 treatment will alleviate IFN-β inhibition induced by RVFV and possibly provide a positive feedback loop on LL-37 expression. To assess this, an expression assay was performed. The infection was executed at an MOI of 0.1 with the following conditions: H20, Uninfected Mock (LL-37 treatment without RVFV infection), pLL-37 (pre- & post-treatment), and LL-37 (only pre-treatment). The results (Fig. 8) show that pre- and post-treatment with LL-37 resulted higher expression of interferon-β at 9 HPI (+0.25 fold change) compared to the water control. Therefore, IFN-β expression increased with increased availability of LL-37 after infection, indicating that IFN-β inhibition during RVFV infection is lifted by LL-37 treatment at later time points.

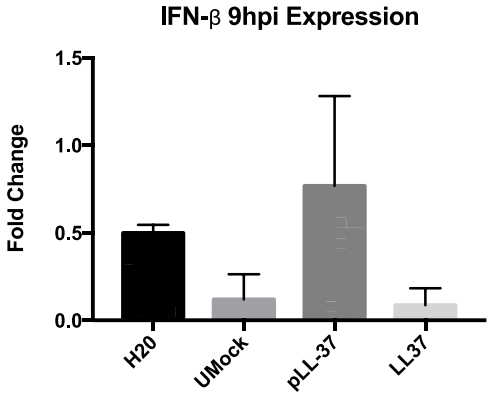


**Figure 8: IFN-β Expression at 9 HPI**
Compared to the water control, pre- and post-treatment with LL-37 resulted higher expression of interferon-β at 9 HPI (+0.25 fold change).

**hCAMP Expression**

Next, it was important to determine if there is a positive feedback loop between exogenous LL-37 and endogenous LL-37. The question being examined was: Does treatment with LL-37 cause the host cell to produce more intrinsic LL-37? This was evaluated through an infection at 3 hpi. Afterwards, cells were lysed and a hCAMP gene expression assay was performed (Fig. 7). The conditions were as follows: H20, Mock (RVFV infection without treatment), Uninfected Mock (LL-37 treatment without RVFV infection), and LL-37 (only pre-treatment). The results showed that, compared to the water control, LL-37 treatment of RVFV does not result in an increase in endogenous LL-37.

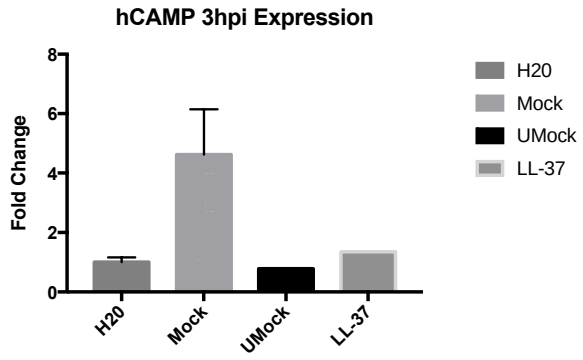


**Figure 9: LL-37 Expression at 3 HPI**
Compared to the water control, LL-37 treatment of RVFV does not result in an increase in endogenous LL-37.

**LL-37 Treatment of RVFV Alters Host Protein Signaling Pathways**

In order to further elucidate LL-37's host-based mechanism of action, Reverse Phase Protein Microarray (RPMA) was used to analyze 195 select proteins. RPMA is a proteomic tool used to detect protein phosphorylation. HSAECs were infected and treated similarly to all other experiments performed. They were then lysed, spread onto a microarray and bound to antibodies that emitted a signal allowing for the detection of phosphorylation levels. Ingenuity Pathway Analysis was used to analyze protein expression data acquired from the RPMA. Of the 195 proteins analyzed, 22 displayed a 50% or greater up- or down-phosphorylation due to LL-37 treatment of RVFV (shown in

Table 1 in Appendix). The top two pathways containing these dramatically altered proteins are the UVB-Induced MAPK Pathway (altered at the 3, 9, and 16 hpi time points) and the ERK/MAPK Signaling Pathways (altered at the 3 and 9 hpi time points). For the graphs below, the proteins in white were not present in the RPMA dataset. The proteins in gray were present in the dataset but did not display a 50% or greater up- or down-phosphorylation with LL-37 treatment of RVFV. The highlighted green proteins are down-phosphorylated and the highlighted pink proteins are up-phosphorylated. Proteins shown encircled by double rings are protein complexes with multiple subunits. It is important to note that for these proteins one subunit may be altered while another is not. In the UVB-Induced MAPK Pathway at 9 HPI (Fig. 10), MSK1 and eIF4E are up-phosphorylated, while EGFR, P13K, 4EBP1, Histone H3 are down-phosphorylated. In the ERK/MAPK Signaling Pathway at 9 HPI (Fig. 11), SRC, FAK, eIF4E, and MSK ½ are up-phosphorylated, while P13K, 4EBP1, and Histone H3 are down-phosphorylated. Taken together, this data suggests that at the 9 hour time point, there may be continued repression of transcription (evidenced by an upregulation of 4E-BP1) and an abundance of chaperone proteins (evidenced by an increase in HSP27 and HSP90a (not pictured)). Additionally, the host cell may be conserving energy (evidenced by the upregulation of AMPPKa1- an energy conserving kinase) and implementing a pro-survival mode (as seen through an increase in MSK1, which activates the pro-survival CREB protein). Lastly, we see a decrease in Cleaved Caspase 9 and ASK 1 (not pictured), also pointing to a pro-survival mode. Collectively, this information suggests that the host cell may be

conserving energy to fight viral infection and produce antiviral proteins, suggesting a pro-survival mode.

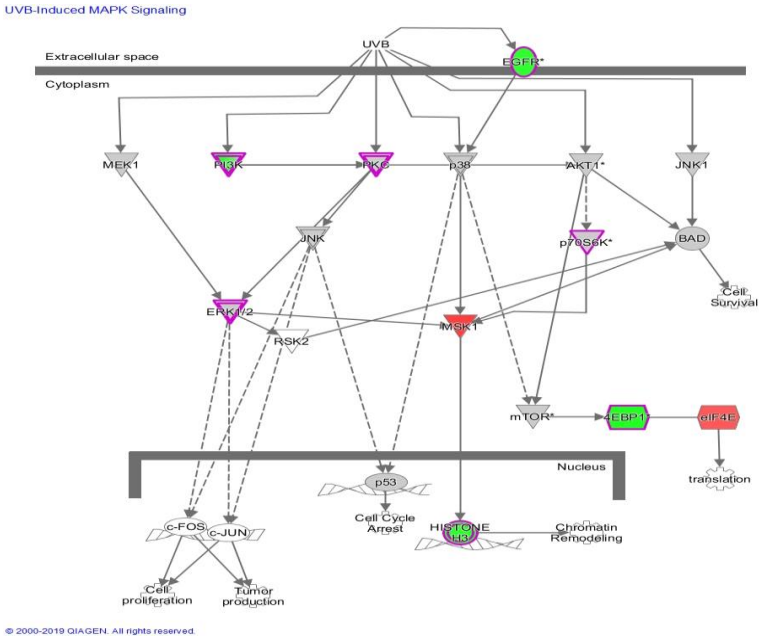


**Figure 10: UVB-Induced MAPK Pathway**
Compared to the mock, MSK1 and eIF4E are up-phosphorylated, while EGFR, P13K, 4EBP1, Histone H3 are down-phosphorylated.

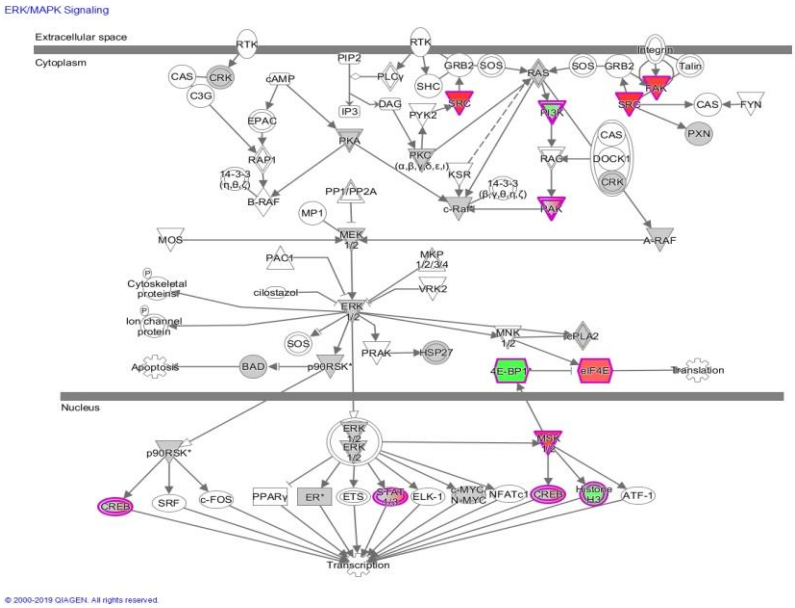


**Figure 11: ERK/MAPK Signaling Pathway**
Compared to the mock, SRC, FAK, eIF4E, and MSK ½ are up-phosphorylated, while P13K, 4EBP1, and Histone H3 are down-phosphorylated.

## Discussion

RVFV is arthropod-borne virus that has many hosts and modes of transmission. It causes outbreaks in many countries, particularly African countries. Transmission is exacerbated in areas that experience intermittent periods of heavy rainfall. RVFV disease in humans can lead to occasionally fatal symptoms of hemorrhagic fever and ocular disease. At this time, there is no FDA-approved treatment for RVFV. To aid in treatment efforts, this study examined the sole human cathelicidin peptide, LL-37, to determine if this peptide can be used as an antiviral therapy to combat RVFV.

Through this study, we exhibited the efficacy of LL-37 against RVFV and established LL-37 as an important regulator of the human immune response. In the data presented here, LL-37 was shown to be nontoxic to HSAECs, but potent against RVFV. While it was expected that LL-37 would be efficacious against RVFV, due to previous studies demonstrating LL-37's antiviral capabilities against other viruses (ie. Hepatitis C virus, Zika Virus), this study revealed that LL-37 efficacy has a negative correlation with time after infection, suggesting that LL-37 may interfere with early infection stages such as viral entry. To assess this further, an entry assay was performed, which confirmed that LL-37 does directly interact with the RVFV virion, which is consistent with other literature asserting a carpet-based model of AMP activity.

This study also demonstrated that LL-37 treatment, in the context of RVFV infection, alters host signaling pathways, thus changing the host cell environment. While the data presented here, only displayed alterations at the 9 hour time point in the two most dramatically changed pathways, there were changes at the 3 and 16 hour time points and alterations in other pathways. There are ongoing studies to determine how these changes in the host cell environment optimize or inhibit the host immune response to RVFV infection.

## Conclusion

In conclusion, LL-37 is efficacious against RVFV, as seen through a decrease in viral titers and genomic copies due to LL-37 treatment. This data shows that the earlier LL-37 is introduced, the less viral replication occurs. Additionally, this study demonstrates that a potent mechanism of LL-37 is viral entry inhibition. This research also provides preliminary evidence that LL-37 treatment of RVFV infection alters host protein signaling pathways. Lastly, these results provide alternatives to LL-37 by exhibiting that synthetic peptides are also efficacious against RVFV, thereby offering a tailored, specific strategy to target RVFV, which in the future, could be more beneficial than the broad innate immunity approach LL-37 provides.

Future studies hope to use electron microscopy to validate viral entry inhibition of RVFV. Additionally, efficacy studies will be formed on other cell types, and with virulent strains of RVFV. Lastly, in vivo studies will be explored.

## Appendix

**Table 1: Reverse Phase Protein Microarray Table**
Displays analyzed proteins that exhibited significant upregulation or downregulation due to LL-37 treatment. Highlighted proteins displayed a 50% or greater upregulation or downregulation with LL-37 treatment.

| | Sample | % Change 3 HPI | % Change 9 HPI | % Change 16 HPI |
|---|---|---|---|---|
| P < 0.05 | eIF4E S209 | **70%** | 44% | -3% |
| | Ack1 Y857/858 | 49% | **86%** | -36% |
| | PAK 1/2 S199/204 S192/197 | 10% | **77%** | -15% |
| | FAK Y576/577 | 42% | **74%** | -22% |
| | MSK1 S360 | 24% | **66%** | -17% |
| | Cleaved Caspase-9 D330 | -34% | -32% | **68%** |
| | MSH6 | -41% | -37% | **65%** |
| | EGFR Y1148 | -22% | -1% | **65%** |
| | Jak1 Y1022/1023 | -42% | **-52%** | **63%** |
| | ATM S1981 | -22% | -33% | **61%** |
| | ASK1 S83 | -18% | -40% | **58%** |
| | Pan-Methyl-Histone H3 K9 | 3% | 1% | **51%** |
| | 4E-BP1 T70 | -32% | **81%** | 44% |
| | HSP90a T5/7 | -22% | **69%** | 39% |
| | Androgen Rec | -19% | **59%** | 28% |
| | *HER4* | **-55%** | **128%** | -1% |
| | AMPKa1 S485 | -8% | **59%** | 7% |
| P < 0.10 | FOX01/03 T24/32 | -30% | **53%** | 28% |
| | Histone H3 S10 | -15% | -32% | **55%** |
| | Src Y527 | 23% | **86%** | 5% |
| | Stat3 S727 | 12% | **58%** | -13% |
| | Ret Y905 | -14% | **55%** | 5% |

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## Biography

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