

# Supplementary Information

## Peptide Inhibitor of Complement C1, RLS-0071, Reduces Zosteriform Spread of Herpes Simplex Virus Type 1 Skin Infection and Promotes Survival in Infected Mice

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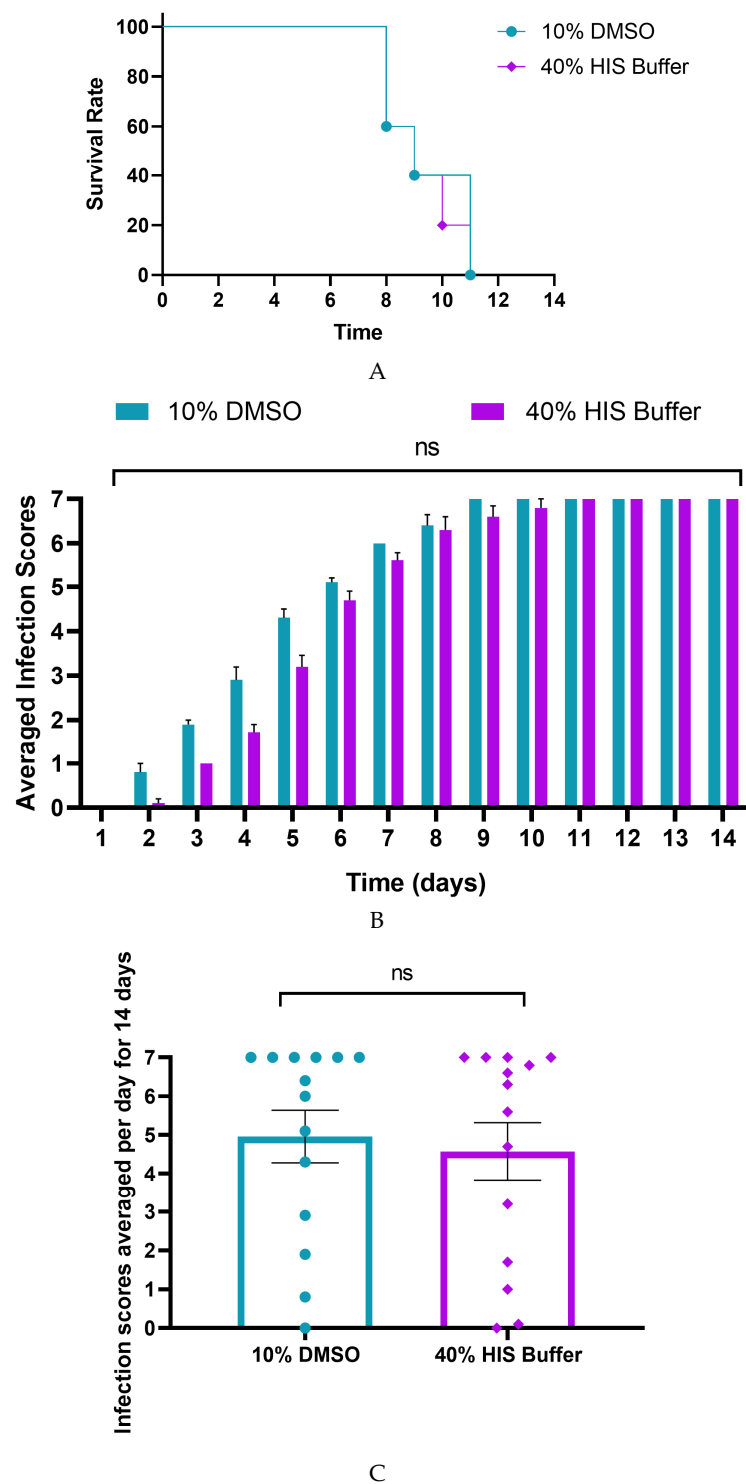
**Table S1.** Testing the antiviral effect of RLS-0071 using plaque assays. 80-85% Vero cells were pretreated with varying concentrations of RLS-0071 or HIS Buffer (control treatment) for 1 hour at 37°C. After incubation, cells were infected with 0.1MOI of GFP-HSV-1 for 1 hour at 37°C in the presence of the respective treatments. Following infection, Vero cells were washed with DPBS 1x and incubated with fresh 199V media (without treatments) for 16 hours. The viruses from the infected cells were extracted and titered using plaque assay.

Treatment	0.1MOI GFP-HSV-1	Viral Titer (PFU/mL)
-	+	$2.1 \times 10^6$
1 mM RLS-0071	+	$5.2 \times 10^6$
2 mM RLS-0071	+	$7.05 \times 10^6$
3 mM RLS-0071	+	$7.85 \times 10^6$
4 mM RLS-0071	+	$5.3 \times 10^6$
5 mM RLS-0071	+	$4.8 \times 10^6$
1 mM HIS Buffer	+	$3.9 \times 10^6$
2 mM HIS Buffer	+	$2.9 \times 10^6$
3 mM HIS Buffer	+	$7.5 \times 10^6$
4 mM HIS Buffer	+	$4.95 \times 10^6$
5 mM HIS Buffer	+	$3.35 \times 10^6$

**Table S2.** Testing the virucidal effect of RLS-0071 using plaque assay. 0.1MOI GFP-HSV-1 cell-free virus was incubated with varying concentrations of RLS-0071 or HIS Buffer (control treatment) for 1 hour at 37°C. Following incubation, viruses with the respective treatments were used to infect 80-85% confluent Vero cells for 1 hour at 37°C, then washed with DPBS 1x and incubated with fresh 199V media (without treatments) for 16 hours. The viruses from infected cells were extracted and titered using plaque assay.

Treatment	0.1MOI GFP-HSV-1	Viral Titer (PFU/mL)
-	+	$2.5 \times 10^6$
1 mM RLS-0071	+	$5.9 \times 10^6$
2 mM RLS-0071	+	$7.05 \times 10^6$
3 mM RLS-0071	+	$9.45 \times 10^6$
4 mM RLS-0071	+	$2.85 \times 10^6$
5 mM RLS-0071	+	$7.15 \times 10^6$
1 mM HIS Buffer	+	$5.3 \times 10^6$
2 mM HIS Buffer	+	$4.8 \times 10^6$
3 mM HIS Buffer	+	$13.3 \times 10^6$
4 mM HIS Buffer	+	$3.9 \times 10^6$
5 mM HIS Buffer	+	$2.8 \times 10^6$

No difference observed in skin infection scores of BALB/cJ mice treated with DMSO or Histidine buffer



**Figure S1.** GFP-HSV-1 zosteriform infection scores of BALB/cJ mice (N = 5/treatment) following the application of 10% DMSO or 40% HIS buffer in 2.5% hydroxyethyl cellulose (HEC) gel. **(A)** Following inoculation with  $6.0 \times 10^4$  PFU of GFP-HSV-1, BALB/cJ mice received respective treatments and were monitored for survival for 14 days. **(B)** Infection scores averaged in each treatment group across 14 days. **(C)** Distribution of averaged infection score of all animals per day for 14 days. **(B/C)** Student independent t-tests (2-tailed); *ns*- non-significant. All error bars represent SEM.

ORF of sequenced ACV<sup>R</sup>-HSV-1 Thymidine kinase gene

ATGGCTTCGTACCCCTGCCATCAACACGCGTCTGCGTTTCGACCAGGCTGCGCGTTCTCGCGGCCATAACAACCGACGTAC  
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ATAGACGGTCCCCACGGGATGGGGAAAACCACCACACGCAACTGCTGGTGGCCCTGGGTTTCGCGCGACGATATCGTCT  
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CCTCGACCAGGGTGAGATATCGGCCGGGGACGCGGCGGTGGTAATGACAAGCGCCCAGATAACAATGGGCATGCCTTA  
TGCCGTGACCGACGCCGTTCTGGCTCCTCATATCGGGGGGAGGCTGGGAGCTCACATGCCCCGCCCCGGCCCTCACCC  
TCATCTTCGACCGCCATCCCATCGCCGCCCTCCTGTGCTACCCGGCCGCGGATACCTTATGGGCAGCATGACCCCCAG  
GCCGTGCTGGCGTTCTGGCCCTCATCCCGCCGACCTTGCCCGGCACAAACATCGTGTTGGGGGGCCCTTCCGGAGGACAG  
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GCCTCCGTCCCATGCACGTCTTTATCCTGGATTACGACCAATCGCCCCCGGGTCCCGGGACGCCCTGCTGCAACTTACCT  
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GATGGGGGAGGCTAACTGA

A

Alignment statistics				
Score		Expect	Identities	Gaps
2084 bits (1128)		0.0	1130 / 1131 (99%)	0 / 1131 (0%)
				Strand
				Plus / Minus
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Sbjct	47803	ATGGCTTCGTACCCCTGCCATCAACACGCGTCTGCGTTTCGACCAGGCTGCGCGTTCTCGC	47744	
Query	61	GGCCATAACAACCGACGTACGGCGTTGCGCCCTCGCCGGCAGCAAAAAGCCACGGAAGTC	120	
Sbjct	47743	GGCCATAACAACCGACGTACGGCGTTGCGCCCTCGCCGGCAGCAAAAAGCCACGGAAGTC	47684	
Query	121	CGCCTGGAGCAGAAAATGCCACGCTACTGCGGGTTTATATAGACGGTCCCCACGGGATG	180	
Sbjct	47683	CGCCTGGAGCAGAAAATGCCACGCTACTGCGGGTTTATATAGACGGTCCCCACGGGATG	47624	
Query	181	GGGAAAACCACCACCACGCAACTGCTGGTGGCCCTGGGTTTCGCGCGACGATATCGTCTAC	240	
Sbjct	47623	GGGAAAACCACCACCACGCAACTGCTGGTGGCCCTGGGTTTCGCGCGACGATATCGTCTAC	47564	
Query	241	GTACCCGAGCCGATGACTTACTGGCGGGTGTGGGGGCTTCCGAGACAATCGCGAACATC	300	
Sbjct	47563	GTACCCGAGCCGATGACTTACTGGCGGGTGTGGGGGCTTCCGAGACAATCGCGAACATC	47504	
Query	301	TACACCACACAACACCGCCTCGACCAGGGTGAGATATCGGCCGGGGACGCGGCGGTGGTA	360	
Sbjct	47503	TACACCACACAACACCGCCTCGACCAGGGTGAGATATCGGCCGGGGACGCGGCGGTGGTA	47444	
Query	361	ATGACAAGCGCCCAGATAACAATGGGCATGCCTTATGCCGTGACCGACGCCGTTCTGGCT	420	
Sbjct	47443	ATGACAAGCGCCCAGATAACAATGGGCATGCCTTATGCCGTGACCGACGCCGTTCTGGCT	47384	
Query	421	CCTCATATCgggggggAGGCTGGGAGCTCACATGCCCCGCCCCGGCCCTCACCCCTCATC	480	
Sbjct	47383	CCTCATATCGGGGGGAGGCTGGGAGCTCACATGCCCCGCCCCGGCCCTCACCCCTCATC	47324	
Query	481	TTCGACCGCCATCCCATCGCCGCCCTCCTGTGCTACCCGGCCGCGGATACCTTATGGGC	540	

Sbjct 47323 TTCGACCGCCATCCCATCGCCGCCCTCCTGTGCTACCCGGCCGCGGATACCTTATGGGC 47264

Query 541 AGCATGACCCCCCAGGCCGTGCTGGCGTTTCGTGGCCCTCATCCCCGCGACCTTGCCCGGC 600  
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Sbjct 47263 AGCATGACCCCCCAGGCCGTGCTGGCGTTTCGTGGCCCTCATCCCCGCGACCTTGCCCGGC 47204

Query 601 ACAAACATCGTGTTGGGGGCCCTTCCGGAGGACAGACACATCGACCGCCTGGCCAAACGC 660  
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Sbjct 47203 ACAAACATCGTGTTGGGGGCCCTTCCGGAGGACAGACACATCGACCGCCTGGCCAAACGC 47144

Query 661 CAGCGCCCCGGCGAGCGGCTTGACCTGGCTATGCTGGCCGCGATTTCGCCGCGTTTATGGG 720  
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Sbjct 47143 CAGCGCCCCGGCGAGCGGCTTGACCTGGCTATGCTGGCCGCGATTTCGCCGCGTTTATGGG 47084

Query 721 CTGCTTGCCAATACGGTGCGGTATCTGCAGGGCGGCGGGTCGTGGCGGGAGGATTGGGGA 780  
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Sbjct 47083 CTGCTTGCCAATACGGTGCGGTATCTGCAGGGCGGCGGGTCGTGGCGGGAGGATTGGGGA 47024

Query 781 CAGCTTTCGGGGGCGGCCGTGCCGCCCCAGGGTGCCGAGCCCCAGAGCAACGCGGGCCCA 840  
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Sbjct 47023 CAGCTTTCGGGGGCGGCCGTGCCGCCCCAGGGTGCCGAGCCCCAGAGCAACGCGGGCCCA 46964

Query 841 CGACCCCATATCGGGGACA TGTATTTACCCTGTTTCGGGCCCCCGAGTTGCTGGCCCCC 900  
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Sbjct 46963 CGACCCCATATCGGGGACA C GTTATTTACCCTGTTTCGGGCCCCCGAGTTGCTGGCCCCC 46904

Query 901 AACGGCGACCTGTATAACGTGTTTGCCTGGGCTTTGGACGTCTTGGCCAAACGCCTCCGT 960  
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Sbjct 46903 AACGGCGACCTGTATAACGTGTTTGCCTGGGCTTTGGACGTCTTGGCCAAACGCCTCCGT 46844

Query 961 CCCATGCACGTCTTTATCCTGGATTACGACCAATCGCCCGCGGCTGCCGGGACGCCCTG 1020  
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Sbjct 46843 CCCATGCACGTCTTTATCCTGGATTACGACCAATCGCCCGCGGCTGCCGGGACGCCCTG 46784

Query 1021 CTGCAACTTACCTCCGGGATGGTCCAGACCCACGTACCCACCCCAGGCTCCATACCGACG 1080  
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Sbjct 46783 CTGCAACTTACCTCCGGGATGGTCCAGACCCACGTACCCACCCCAGGCTCCATACCGACG 46724

Query 1081 ATCTGCGACCTGGCGCGCACGTTTGCCCGGGAGATGGGGGAGGCTAACTGA 1131  
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Sbjct 46723 ATCTGCGACCTGGCGCGCACGTTTGCCCGGGAGATGGGGGAGGCTAACTGA 46673

B

	Score	Expect	Method	Identities	Positives	Gaps	Frame
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Sbjct	1	MASYPCHQHASAFDQAARSRGHNNRR	TALRPRRQQKATEVRLEQKMPTLLRVYIDGPHGM			60	
Query	181	GKTTTTQLLVALGSRDDIVYVPEPMTYWRVLGASETIANIYTTQHRLDQGEISAGDAAVV				360	
Sbjct	61	GKTTTTQLLVALGSRDDIVYVPEPMTYWRVLGASETIANIYTTQHRLDQGEISAGDAAVV				120	
Query	361	MTSAQITMGMPYAVTDAVLAPHIGGEAGSSHAPPPALTLIFDRHPIAALLCYPAARYLMG				540	
Sbjct	121	MTSAQITMGMPYAVTDAVLAPHIGGEAGSSHAPPPALTLIFDRHPIAALLCYPAARYLMG				180	

Query	541	SMTPQAVLAFVALIPPTLPGTNIVLGALPEDRHIDRLAKRQRPGERLDLAMLAAIRRVYG	720
		SMTPQAVLAFVALIPPTLPGTNIVLGALPEDRHIDRLAKRQRPGERLDLAMLAAIRRVYG	
Sbjct	181	SMTPQAVLAFVALIPPTLPGTNIVLGALPEDRHIDRLAKRQRPGERLDLAMLAAIRRVYG	240
Query	721	LLANTVRYLQGGGSWREDWGQLSGAAVPPQGAEPQSNAGPRPHIGD <b>M</b> LFTLFRAPPELLAP	900
		LLANTVRYLQGGGSWREDWGQLSGAAVPPQGAEPQSNAGPRPHIGD LFTLFRAPPELLAP	
Sbjct	241	LLANTVRYLQGGGSWREDWGQLSGAAVPPQGAEPQSNAGPRPHIGD <b>T</b> LFTLFRAPPELLAP	300
Query	901	NGDLYNVFAWALDVLAKRLRPMHVFILDYDQSPAGCRDALLQLTSGMVQTHVTTTPGSIPT	1080
		NGDLYNVFAWALDVLAKRLRPMHVFILDYDQSPAGCRDALLQLTSGMVQTHVTTTPGSIPT	
Sbjct	301	NGDLYNVFAWALDVLAKRLRPMHVFILDYDQSPAGCRDALLQLTSGMVQTHVTTTPGSIPT	360
Query	1081	ICDLARTFAREMGEAN 1128	
		ICDLARTFAREMGEAN	
Sbjct	361	ICDLARTFAREMGEAN 376	

C

**Figure S2.** Analysis of ACV<sup>R</sup>-HSV-1 thymidine kinase gene sequence using PubMed BLASTn and BLASTx. **(A)** Open reading frame of sequenced acyclovir-resistant HSV-1 thymidine kinase gene. **(B)** Nucleotide sequence alignment generated using ORF of viral TK gene and NCBI Human alphaherpesvirus 1 strain 17, complete genome (Sequence ID: BK012101.1); match with the range of 46673 to 47803. The mutation in ACV<sup>R</sup>-HSV-1 TK gene (query 1) occurring at nt 860 (C→ T) is highlighted in yellow. **(C)** Amino acid sequence analysis using BLASTx of mutated TK of ACV<sup>R</sup>-HSV-1 with wild-type thymidine kinase [Human alphaherpesvirus 1] (NCBI Sequence ID: YP\_009137097.1), with length of 376aa and 1 possible match. The mutation sequence (in query 1) occurring at aa 287 (T→ M) is highlighted in yellow.