

Supplementary Materials: *Saccharomyces paradoxus* K66 killer system evidences expanded assortment of helper and satellite viruses

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Sp AML-15-66 16 AATGGAtttttttGTTTTGGCAGGAGCATGAGAGCTTTTACTGGGCAAGAAGACAAGAGA 75
Sp CBS:5829 60 AATGGATTTTTTGTGTTTGGCAGGAGCATGAGAGCTTTTACTGGGCAAGAAGACAAGAGA 119

Sp AML-15-66 76 TGGAGAGTCCAGTCGGGCTGCGCTTAAGTGC GCGGTCTTGCTTGGCTTGTAAAGTTTCTT 135
Sp CBS:5829 120 TGGAGAGTCCAGTCGGGCTGCGCTTAAGTGC GCGGTCTTGCTTGGCTTGTAAAGTTTCTT 179

Sp AML-15-66 136 TCTTGCTATTCCAAACGGTGAGAGATTTCTGTGCTATTGTTATAGGACAATTAACCGT 195
Sp CBS:5829 180 TCTTGCTATTCCAAACGGTGAGAGATTTCTGTGCTATTGTTATAGGACAATTAACCGT 239

Sp AML-15-66 196 TTCAATACAACACACTGTGGAGTTTTCATATCTTTGCAACTTTTTCTTTGGGCATTTCGAG 255
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Sp CBS:5829 300 CAATCGAGGCCAGAGGTAACAAACACAAACAATTTATTTATTCAATTAATTTTGTCA 359

Sp AML-15-66 316 AAAACAAGAATTTTCGTAAC TGGAATTTTAAAAATATTA AAAACTTTCAACACGGATC 375
Sp CBS:5829 360 AAAACAAGAATTTTCGTAAC TGGAATTTTAAAAATATTA AAAACTTTCAACACGGATC 419

Sp AML-15-66 376 TCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAGA 435
Sp CBS:5829 420 TCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTAATGTGAATTGCAGA 479

Sp AML-15-66 436 ATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTGGTATTCCAGGGGGCAT 495
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Sp AML-15-66 496 GCCTGTTTGAGCGTCATTTCTTCTCAAACATTCTGTTTGGTAGTGAGTGATACTCTTTG 555
Sp CBS:5829 540 GCCTGTTTGAGCGTCATTTCTTCTCAAACATTCTGTTTGGTAGTGAGTGATACTCTTTG 599

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Sp CBS:5829 600 GAGTTAACTTGAAATGCTGGCCTTTTCATTGGATGTTTTTTTCCAAAGAGAGGTTTCT 659

Sp AML-15-66 616 CTGCGTGCTTGAGGTATAATGCAAGTACGGTCGTTTTAGGTTTTACCAACTGCGGCTAAT 675
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Sp AML-15-66 676 CTTTTTTGTA CTGAGCGTATTGGAACGTTATCGATAAGAAGAGAGCGCTAGGCGAACAA 735
Sp CBS:5829 720 CTTTTTTGTA CTGAGCGTATTGGAACGTTATCGATAAGAAGAGAGCGCTAGGCGAACAA 779

Sp AML-15-66 736 TGTTCCTTAAAGTTTGACCTCAAATCAGGTAGGAGTACCCGCTGAACCTTAAGCATATCA 793
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B

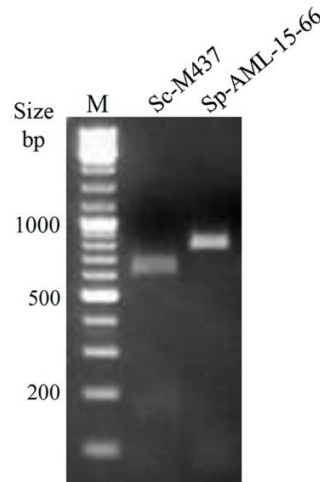


Figure S1. Identification of *S. paradoxus* AML-15-66 strain. A. Similarity of AML-15-66 to *Saccharomyces paradoxus* culture CBS:5829 based on sequences of internal transcribed spacer 1 and 2 including 5.8S ribosomal RNA gene. B. RFLP-PCR profiles of ITS regions of *S. paradoxus* AML-15-66 and *S. cerevisiae* M437 yeasts. FspBI-digested PCR products loaded on 2% agarose gel. M: molecular weight (kb) marker GeneRuler DNA Ladder Mix (Thermo Fisher).

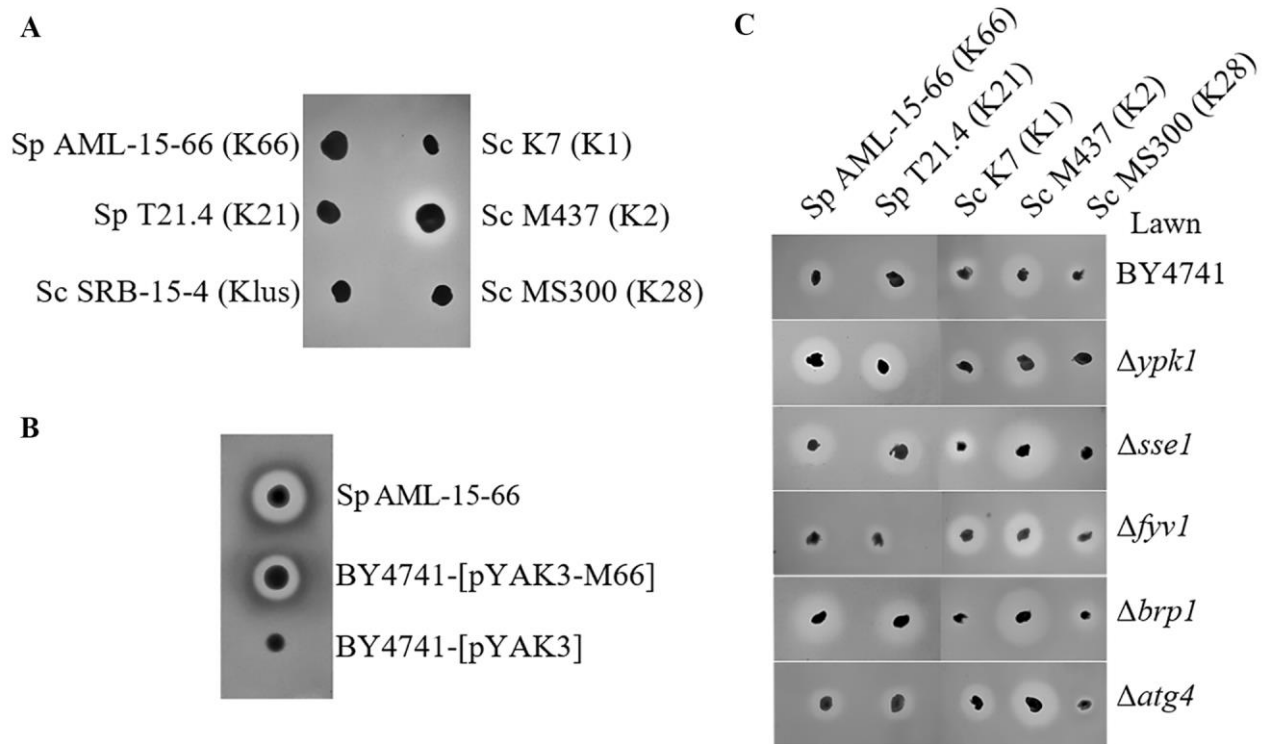


Figure S2. Characterization of killing and immunity features of K66 toxin-producing *S. paradoxus* strain. A. Susceptibility of *S. paradoxus* AML-15-66 strain to the *S. cerevisiae* killer toxins. *S. cerevisiae* strain M437 producing K2 type viral toxin inhibits growth of *S. paradoxus* AML-15-66 cells seeded into MBA agar. Other toxin producing strains did not demonstrate lysis zones on overlay of AML-15-66 cells. B. Killing phenotype of *S. paradoxus* K66 toxin producing *wt* strain and *S. cerevisiae* transformant BY4741-[pYAK3-M66] featuring expressed K66 coding sequence. Plate is seeded with a lawn of the *S. cerevisiae* strain BY4741. C. Representative examples of yeast mutant susceptibility to different killer toxins. The assay was performed by depositing toxin producing cells on the surface of an MBA plate (pH 4.8) inoculated with a mutant strain to test.

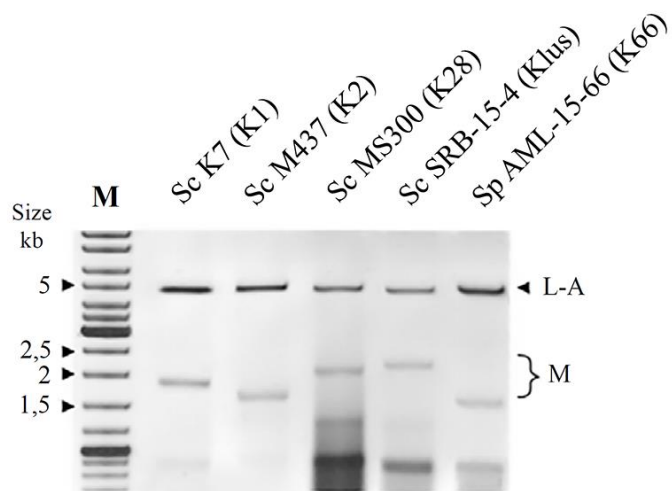


Figure S3. Electrophoretic analysis of dsRNAs extracted from different killer yeast. L-A and M on the right marks type of dsRNA. Source strain and killer type indicated above the picture, M denotes molecular weight marker GeneRuler DNA Ladder Mix (Thermo Fisher Scientific).

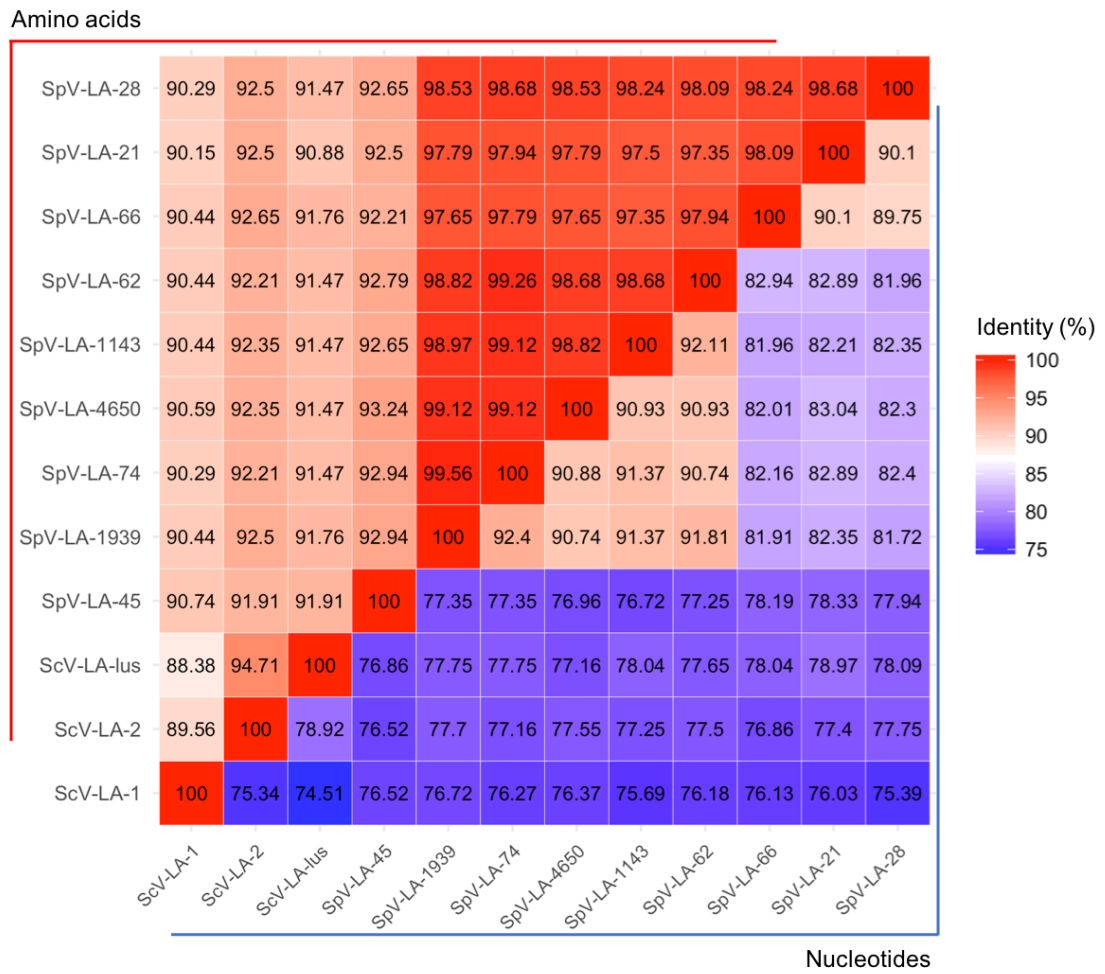


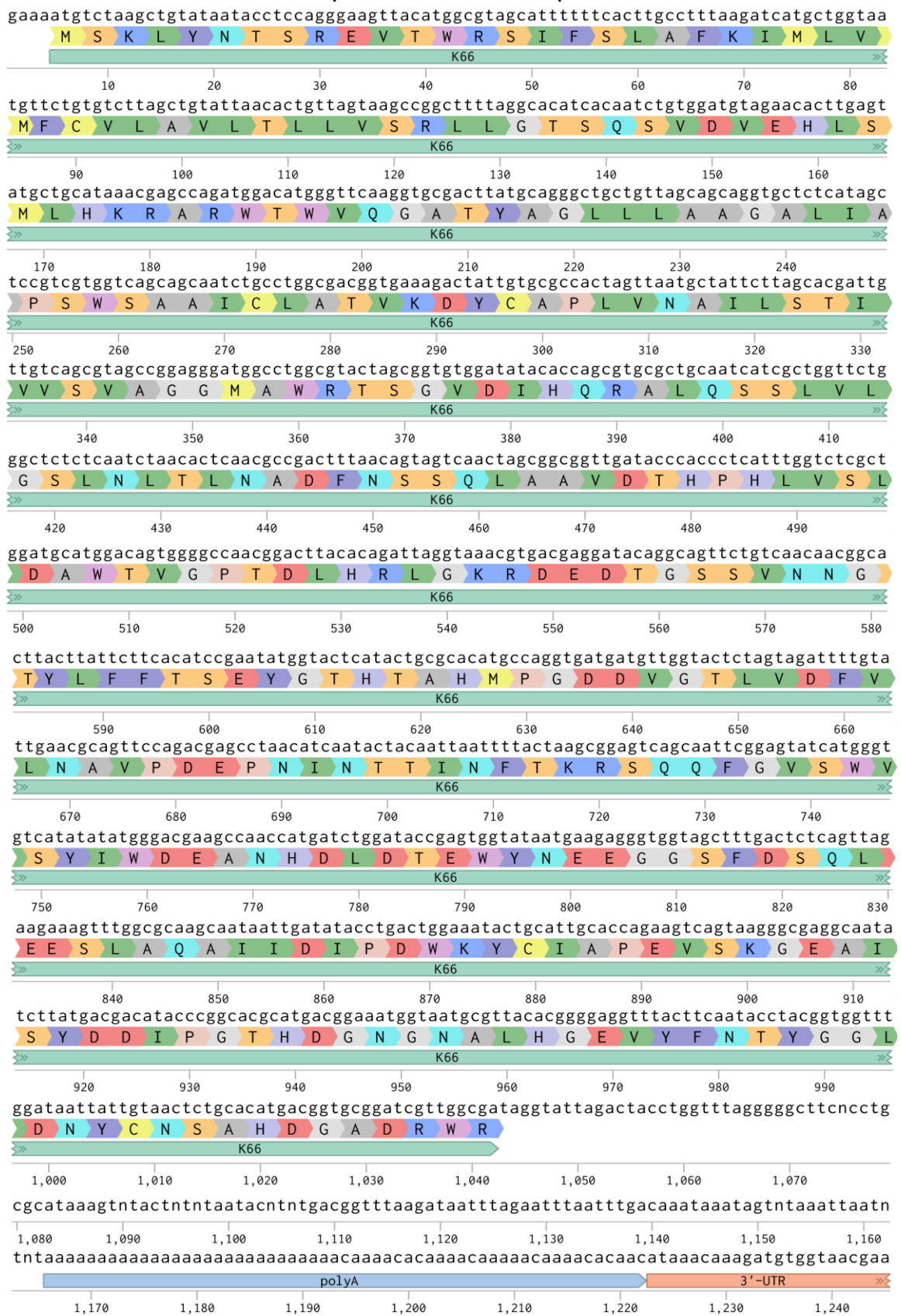
Figure S4. The similarities of *S. cerevisiae* and *S. paradoxus* dsRNA L-A virus-encoded Gag proteins. Identity at nucleotide level is represented in the lower right triangle, amino acid level – in the upper left triangle, framed by corresponding blue and red lines.

	1	10	20	30	40	50
SpV-M66	. . G AAAATGTCTAAGCTGTATAA T ACCTC CAGGGAAGTTACATGGCGTAG C ATTTTTTCA					
SpV-M21	GAA AAAATGTCTAAGCTGTATAA C ACCTC TAGGGAAGTTACATGGCGTAG T ATTTTTTCA					
	60	70	80	90	100	110
SpV-M66	CTTGCC T TTAAGATCATGCTGGTAATGTTCTGTGT C TTAGCTGTATTAAC A CTGTTAGT A					
SpV-M21	CTTGCC C TTAAGATCATGCTGGTAATGTTCTGTGT T TTAGCTGTATTAAC G CTGTTAGT G					
	120	130	140	150	160	170
SpV-M66	AGCCG G CTTT TAGGCACATCACAA T T G TGGATGT A GAACACTTGAGTA T GCTGCATAAA					
SpV-M21	AGCCG A CTTT TAGGCACATCACAA C C A TGGATGT G GAACACTTGAGTA G GCTGCATAAA					
	180	190	200	210	220	230
SpV-M66	CGAGC C AGATGGACATGGGTTCAAGG T GC GACTTATGCAGGGCTG C T TTAGCAGCAGGT					
SpV-M21	CGAGC T AGATGGACATGGGTTCAAGG C GC AACTTATGCAGGGCTG T T A TTAGCAGCAGGT					
	240	250	260	270	280	290
SpV-M66	GCTCTCATAGCTCCGTC G TGGTCAGCAGCAAT C TGC CTGGCGACGG T GAA A GACTA T TGT T					
SpV-M21	GCTCTCATAGCTCCGTC T TGGTCAGCAGCAAT A TGC T TGGCGACGG C GAA G GACTA C TGC C					
	300	310	320	330	340	350
SpV-M66	GC G CCACTAG T TAATGCTATTCTTAGCAGATTGTTGTCAGC G TAGCCGGAGGGAT G GCC					
SpV-M21	GC A CCACTAG C TAATGCTATTCTTAGCAGATTGTTGTCAGC A TAGCCGGAGGGAT A GCC					
	360	370	380	390	400	410
SpV-M66	TGGCGTACTAGCGGTGTGG A T A TACAC C A GCGTGC G CTGCAATCATCGCTG G TTCT G GGC					
SpV-M21	TGGCGTACTAGCGGTGTGG G T G TACAC G A A CGTGC A CTGCAATCATCGCTG A TTCT C GGC					
	420	430	440	450	460	470
SpV-M66	TC T CT CAATCTAACACTCAACGC C GACTT TAACAG T AGTCA A CTAGCGGC G GTTGATACC					
SpV-M21	TC C CT TAATCTAACACTCAACGC T GACTT CAACAG C AGTCA G CTAGCGGC T GTTGATACC					
	480	490	500	510	520	530
SpV-M66	CACCC T CATT TGGTCTCG C TGGATGC A TGGACAGTGGGGCCA A C G GAC T TAC A CAGATTA					
SpV-M21	CACCC G CATT TGGTCTCG T TGGATGC G TGGACAGTGGGGCCA G C A GAC C TAC G CAGATTA					
	540	550	560	570	580	590
SpV-M66	GGTAAACGTGA C G A GGATACAGG C A G TTCTGT C AA C AACGGCACTTA C TTA TTCTTCAC A					
SpV-M21	GGTAAACGTGA T G G GGATACAGG TA A TTCTGT T AA T AACGGCACTTA T TT TTCTTCAC G					
	600	610	620	630	640	650
SpV-M66	TCCGAATATGG T ACTCATACTGC G CACATG C CAGGTGATGATGT T GGTACTCTAGTAGA T					
SpV-M21	TCCGAATATGG C ACTCATACTGC T CACATG T CAGGTGATGATGT C GGTACTCTAGTAGA C					
	660	670	680	690	700	710
SpV-M66	TTTGT A TT G AACGC A G TTCCAGACGAGCCTAACAT C AATA C T ACAATTAATT T T ACTAAG					
SpV-M21	TTTGT G TT A AACGC G A TTCCAGACGAGCCTAACAT T AATA G C ACAATTAATT C T GCTAAG					
	720	730	740	750	760	770
SpV-M66	CGGAGTCA G CAATTCCGAGTATCATGGGTGTCATATATATGGGACGAAGCCAACCATGAT					
SpV-M21	CGGAGTCA A CAATTCCGAGTATCATGGGTGTCATATATATGGGACGAAGCCAACCATGAT					
	780	790	800	810	820	830
SpV-M66	CTGGATACCGAGTGGTATAATGAAGA G GGTGGTAGCTT T GACTCTCAGTTAGAAGAA A GT					
SpV-M21	CTGGATACCGAGTGGTATAATGAAGA A GGTGGTAGCTT C GACTCTCAGTTAGAAGAA G GT					
	840	850	860	870	880	890
SpV-M66	TTG G CGCAAGCAATAA TTGATATACCTGACTGGAA A TACTG C ATTGCACC A GAAGTCAGT					
SpV-M21	TTG A CGCAAGCAATA G TTGATATACCTGACTGGAA G TACTG T ATTGCACC T GAAGTCAGT					
	900	910	920	930	940	950
SpV-M66	AAGGGCGAGGCAA TATCTTATGACGACATACCCGGCAGCA T G ACGGAAATGG T AATGCG					
SpV-M21	AAGGGCGAGGCAA TATCTTATGACGACATACCCGGCAGCA A A ACGGAAATGG C AATGCG					

	960	970	980	990	1000	1010
SpV-M66	TTACACGGGAGGTTTACTTCAATACCTACGGTGGTTTGGATAATTATTGTAACTCTGCA					
SpV-M21	TTACACGGAGAGTCTACTTCAATACCTACGGTGGTTTGGATAGTTATTGCAATCTCTGCA					
	1020	1030	1040	1050	1060	1070
SpV-M66	CATGACGGTGCAGATCGTTGGCGATAGGTATTAGACTACCTGGTTAGGGGCTTCNCCCT					
SpV-M21	CATGACGGTGCAGACCGTTGGCGTAGGTATCACACACCTGGTCTAGAGGAATTCATTT					
	1080	1090	1100	1110	1120	1130
SpV-M66	GCGCATAAAGTNTACTNTNTAATACNTNTGACGGTTTAAGATAATTTAGAATTAAATTTG					
SpV-M21	GCGCATCAAGTCTGTGATTGAATACCTCTGCCGCTTAAGATAAGTCAGAATTAGATTTA					
	1140	1150	1160	1170	1180	1190
SpV-M66	ACAAATAAATAGTNTAAATTA.....ATNTNTAAAAAAAAAAAAAAAAAAAAAAAAA					
SpV-M21	ACAAATAAATAGTATAGGTTAACGTAGTATTTATAAAAAAAAAAAAAAAAAAAAAAAAAA					
	1200	1210				
SpV-M66	AACAAAAACAACAAAAACAAAAACAA.....					
SpV-M21	AAAATAAAAAAATAAAAAAATAAAAAAAGAGAGAGAAAGAAAAAGA					
		1220	1230	1240	1250	
SpV-M66AACACAAACATAAACAAAGATGTGTAACTGAATATTATTACTTGT					
SpV-M21	AAAGAAAGAAAGAGAAACAACTAAATATAAACAAAGATGTAGTAATGATATTATTACTTGT					
	1260	1270	1280	1290	1300	1310
SpV-M66	GTTGTGCTGTACACATGCCTCTAAACAGGTAGTATGGTCTGTACTGTTATCGTCAGTATG					
SpV-M21	GTTGTGCTGTACATATGCCTCTAAACAGGTAGTACGGTCTGTACTATTACCGTCAGTACG					
	1320	1330	1340	1350	1360	1370
SpV-M66	CTAGATGTTTTCATCAGCGTACACTGACTGTTAGGGACGACCGCACTGCCTGTAATTCAG					
SpV-M21	CTAGATGTTTTCATCAGCGTATTCAACTGTCTGGGACAGACCGCACTGCTTGTAAATCAAA					
	1380	1390	1400	1410	1420	1430
SpV-M66	TACGAATCACTGTTTGGCCTGACCGCCGTAGCATTGAGTACTGGGTAGCTGCTGTATTAC					
SpV-M21	TACGAATCACTGTTTGGCCCAACCGTCAATAACATTGAGTACTGGATAGCTGCTGTGTAC					
	1440	1450	1460	1470	1480	1490
SpV-M66	TAGAAAAGACAGGTGTTTCACCGTCTTGATATTGGTAAATTACACTGCTAGCAGGCATGGT					
SpV-M21	TAGAAAAGACAGGTGTTTCACCGTCTCATATCTGGTGGTTACACTGCTAGCAGGCATAGT					
	1500	1510	1520	1530	1540	1550
SpV-M66	GAGCGTAAGCCGTGGCGAACCGACGGCAAACCATGATGGATTCAATTACCTATGCA					
SpV-M21	AAGCGTAAGCCGTGGAGAACCGACGGCAAACCATGATGGATTCAATTACCTATGC.					

Figure S5. Sequence alignment of SpV-M66 and SpV-M21 genomes.

SpV-M66 (1553 bp)



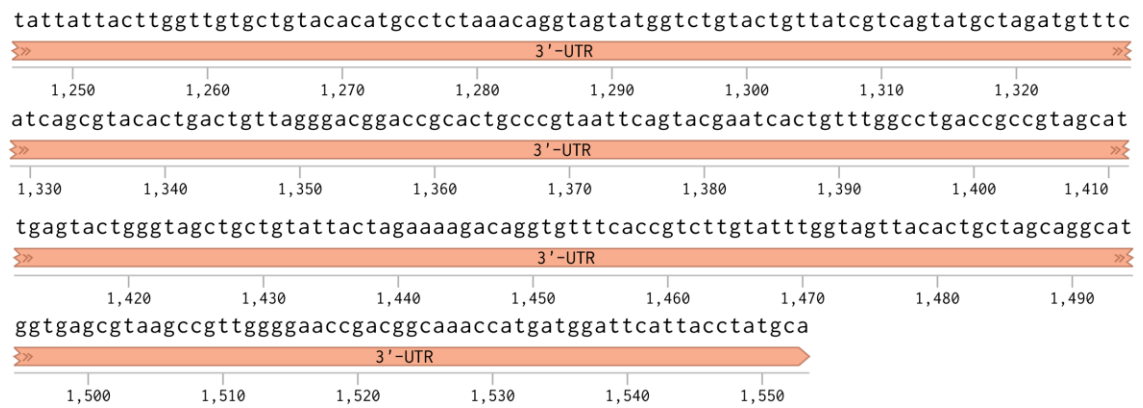


Figure S6. Nucleotide sequence of the SpV-M66 genome and amino acid sequence of the putative ORF of K66 toxin.