SUPPLEMENTARY INFORMATION

ORF4 of the temperate archaeal virus SNJ1 governs the lysis-lysogeny switch and superinfection immunity

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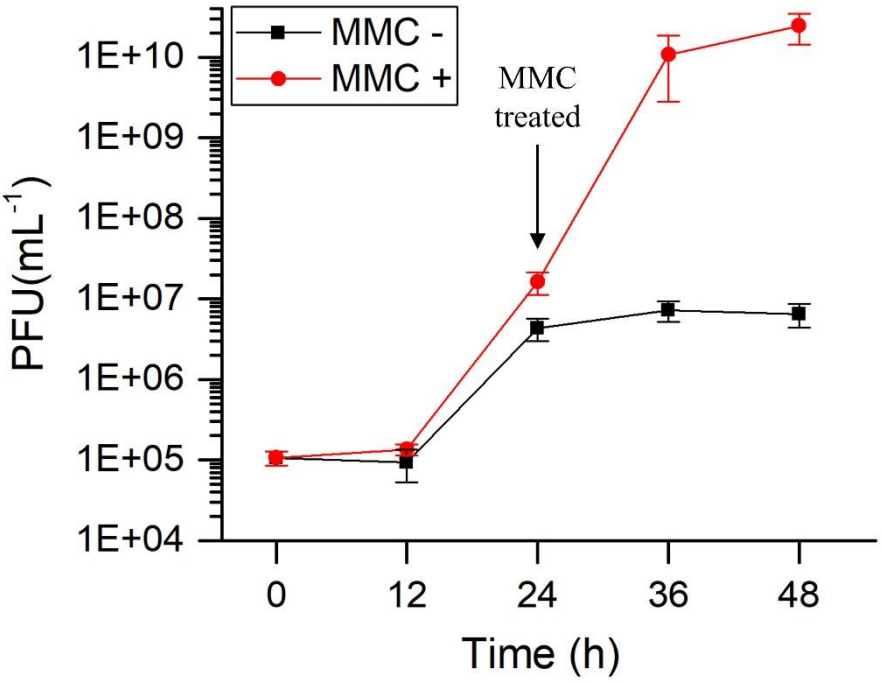
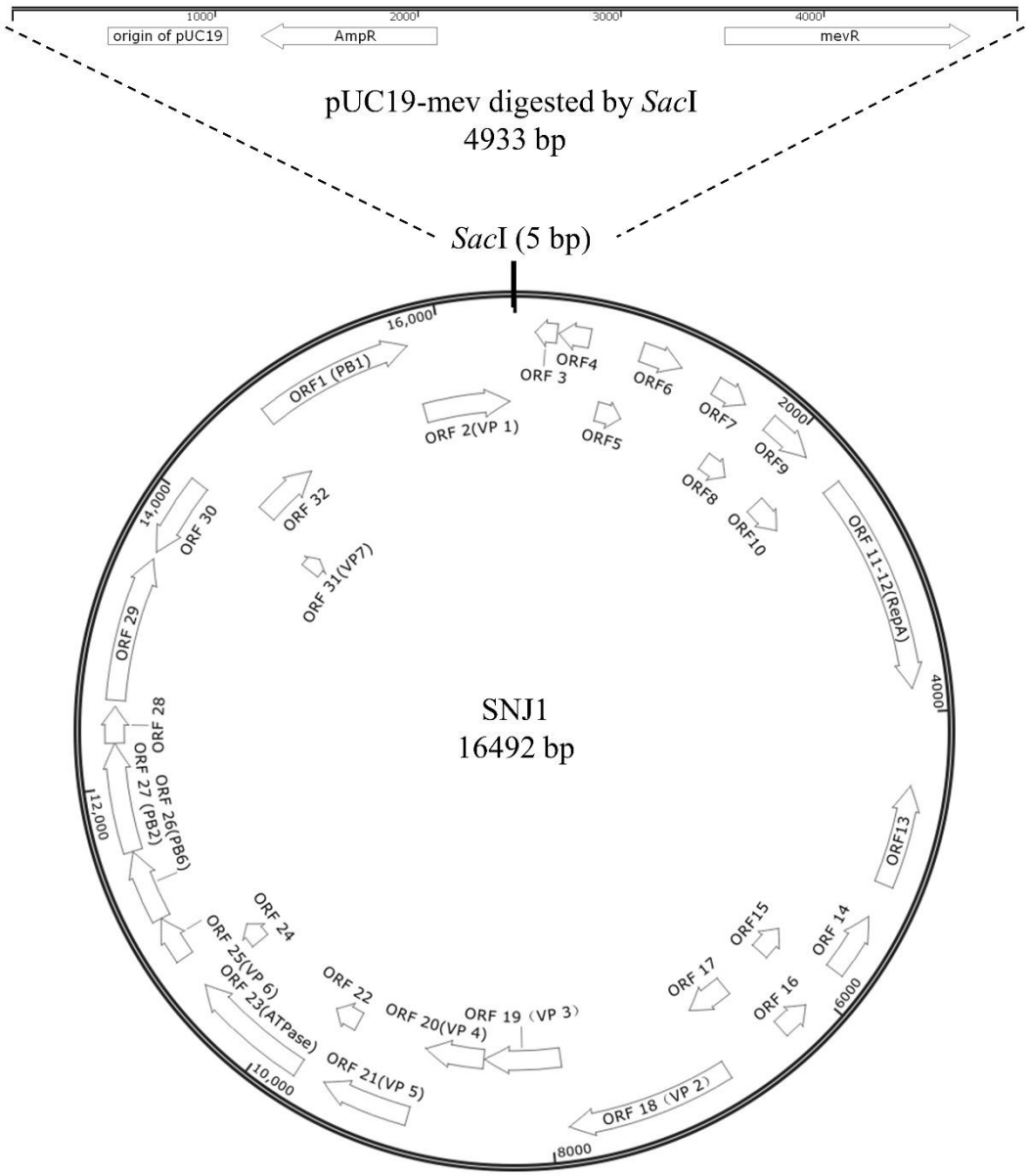


Supplementary Figure 1. The titer of SNJ1 produced by the J7-1 strain with/without MMC treatment.

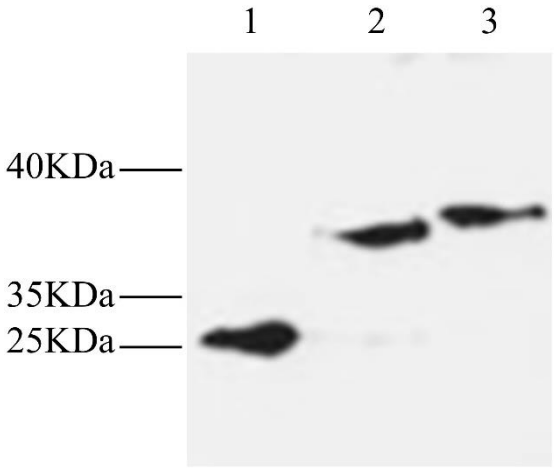
J7-1 was cultured in Halo-2 medium for 24 h and then treated with or without MMC for 30 min. Cells were washed with fresh Halo-2 medium to remove MMC and then cultivated for another 24 h. Supernatants were saved as virus stocks and titers were determined by double-layer plaque assay. Three independent experiments were performed, and error bars indicated standard deviations.



Supplementary Figure 2. Schematic map of the *E. coli-Natrinema* shuttle vector pYC-S.

pYC-S was obtained by ligation of *Sac*I digested pUC19-mev into *Sac*I digested pHH205 (proviral genome of SNJ1). Scheme of SNJ1 genome and pUC19-mev plasmid were shown. The putative ORFs of SNJ1 and pUC19-mev were indicated by arrows.

a



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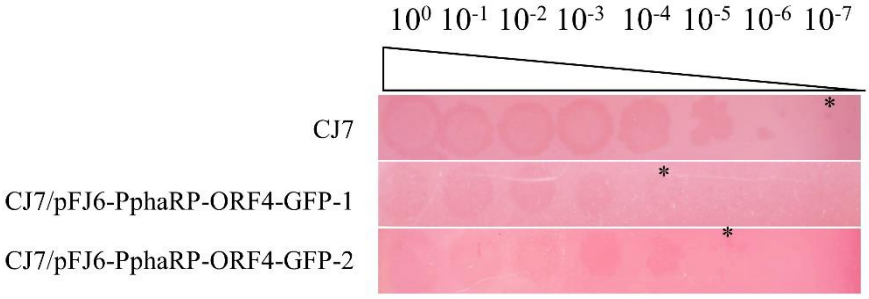

Supplementary Figure 3. Western blot of ORF4-GFP and functional test in superinfection immunity.

a. Samples from CJ7/pFJ6-PphaRP-GFP, CJ7/pFJ6-PphaRP-ORF4-GFP-1 and CJ7/pFJ6-PphaRP-ORF4-GFP-2 were collected at late-exponential phase and crude extracts were loaded for SDS-PAGE, lane 1 through lane 3 respectively. Anti-GFP antibodies was used for Western blot assay of GFP and ORF4-GFP fusion protein. **b.** Expression of ORF4-GFP fusion protein in CJ7 did not show resistance to SNJ1 infection. Ten-fold serial dilutions of SNJ1 viruses were spotted onto the lawns of CJ7, CJ7/pFJ6-PphaRP-ORF4-GFP-1 and CJ7/pFJ6-PphaRP-ORF4-GFP-2. Plates were incubated at 37°C for 48 h and photographed. The maximum dilution for observed plaques was highlighted by asterisk.

Supplementary Table 1: Strains used in this study

Strains	Description	Source and reference
Natrinema sp. J7-1[a]	Lysogenic strain of SNJ1	[1-3]
Natrinema sp. CJ7	SNJ1 cured strain of J7-1	[2,3]
Natrinema sp. CJ7-F	CJ7Δ*pyrF* (orotidine 5'-phosphate decarboxylase, NJ7G_2091), uracil auxotrophic strain	[4]
Escherichia coli DH5α	For plasmids construction *supE*44 *ΔlacU*169(*φ*80*lacZ*ΔM15) *hsd*R17 *rec*A1 *end*A1 *gyr*A96 *thi*-1 *rel*A1	CCTCC[b]
Escherichia coli JM110	For plasmids demethylation *dam dcm supE*44 *hsdR*17 *thi leu rps*L *lac*Y *gal*K *gal*T *ara ton*A *thr tsx* Δ(l*ac-pro*AB) F'(*tra*D36 *pro*AB^{+}*lac*I^{q} *lac*Z ΔM15)	CCTCC

a: GenBank Acc. No. AJVG00000000.1. b: CCTCC, China Center for Type Culture Collection.

Supplementary Table 2: Plasmids used in this study

Plasmids	Description	Source and reference
pUC19-mev	pUC19 with mevinolin resistance marker (mevR).	[2]
pYC-S	An *E. coli-Natrinema* shuttle vector based on SNJ1 genome, pUC19-mev was digested with *Sac*I and inserted into SNJ1 proviral genome.	[2]
pYC-S-4M	pYC-S with start codon mutated of *orf4*.	This study
pYC-S Δ1-575	pYC-S with 1-575 deleted within SNJ1.	This study
pYC-1	pUC19-Mev with the insertion of 5.9 kb *Sac*I-*Bam*HI 575-6,482 fragment of SNJ1, used for pYC-S Δ1-575 construction	[2]
pYC-SHS	An *E. coli-Natrinema* shuttle vector based on SNJ1 replication and regulation elements. pUC19-mev was digested with *Sac*I-*Hin*dIII and linked with *Sac*I-*Hin*dIII digested SHS fragment (1-6,482) of SNJ1.	[2]
pYC-SHS-4M	pYC-SHS with start codon mutated of *orf4*.	[2]
pFJ6-MCS	An *E. coli-Natrinema* shuttle vector based on replication element of CJ7 chromosome, containing pUC19, *pyrF* marker and multiple cloning site.	[4]
pFJ6-1-656	pFJ6-MCS with *Mun*I-*Sph*I fragment containing 1-656 of SNJ1	This study
pFJ6-1-656-3FSM	pFJ6-1-656 with frame-shift mutation of *orf3*	This study
pFJ6-1-656-4FSM	pFJ6-1-656 with frame-shift mutation of *orf4*	This study
pFJ6-1-656-3FSM 4FSM	pFJ6-1-656 with frame-shift mutation of *orf3* and *orf4*	This study
pYCJ-H	pUC19-Mev with the insertion of 4.5 kb *Sac*I-*Sma*I 1-4,481 fragment of SNJ1 and heat-shock protein 70 promoter (Hpro) from *Haloferax volcanii* DS52, used for pFJ6-Hpro construction	[2]
pFJ6-Hpro	pFJ6-MCS with *Mun*I-*Not*I fragment containing Hpro	This study
pFJ6-Hpro-*orf4*	pFJ6-Hpro with *Not*I-*Sph*I fragment containing *orf4* (499-293)	This study
pFJ6-Hpro-*orf4*-4FSM	pFJ6-Hpro-*orf4* containing frame-shift mutation of *orf4*	This study
pFJ6-Hpro-*orf4*^{x}	pFJ6-Hpro with *Not*I-*Sph*I fragment containing a series of truncation of *orf4*. (x = 1-50, 1-33, 1-24, 1-16, 1-8, 34-68)	This study
pFJ6-PphaRP-GFP	pFJ6-MCS with *Afl*II-*Sph*I fragment containing promoter of the *phaRP* operon from *Haloferax mediterranei* and GFP	This study
pFJ6-PphaRP-ORF4-GFP-1	pFJ6-MCS with *Afl*II-*Sph*I fragment containing promoter of the *phaRP* operon from *Haloferax mediterranei* and ORF4-GFP fusion protein linked by A(EAAAK)*3A polypeptide.	This study
pFJ6-PphaRP-ORF4-GFP-2	pFJ6-MCS with *Afl*II-*Sph*I fragment containing promoter of the *phaRP* operon from *Haloferax mediterranei* and ORF4-GFP fusion protein linked by (GGGGS)*3 polypeptide.	This study

Supplementary Table 3: Primers used in this study

Primers	sequences (5'-3')	Description
HJ-F	TGAATCAAGATGCCGCCGTG	primers used to check the genome integrity of the mutant viruses generated from pYC-S
HJ-R	CGGCTTCTGATCCCACTCG	
pYC-S-AflII-F	GTACCCTCTAGTCAAGGCCTTAAGTGAGTCGTATTACGGACT	primers used for pYC-S-4M construction
pYC-S-NcoI-R	CGCTAGCTAGGAGGTTTCCCATGGTTCCCGGCCGTCGGCCCG	
4M-F	GTGCCATCTGAATCCTCTTACC	
4M-R	GGTAAGAGGATTCAGATGGCAC	
SacI-1-F	AAAGAGCTCGCGATCGTTGACGTCGTC	primers used for pYC-SHS-4M construction
NcoI-1554-R	TTCCCATGGTTCCCGGCCGTCGGCCCG	
SphI-1-F	AAAGCATGCGAGCTCGCGATCGTTGAC	primers used for pFJ6-1-656, pFJ6-1-656-3FSM, pFJ6-1-656-4FSM and pFJ6-1-656-3FSM 4FSM construction
MunI-656-ORF4-R	AAACAATTGACACGTAGTATCGTATTATTGTTAG	
ORF3-FSM-F	GTCTAAGAGGCCATCGGTCA	primers used for frame-shift mutation of *orf3*
ORF3-FSM-R	TGACCGATGGCCTCTTAGAC	
ORF4 FSM-F	TCAATATCAAGGCCATCTGCATCCT	primers used for frame-shift mutation of *orf4*
ORF4 FSM-R	GATGCAGATGGCCTTGATATTGATC	
MunI-Hpro-F	AAACAATTGTCTTCGCTGCCGCCGTTC	primers used for pFJ6-Hpro construction
NotI-Hpro-R	AAAGCGGCCGCCCTTGCCCGCAAATACCGT	

NotI-ORF4-499-F	AATGCGGCCGCAATGCAGATGGCACTTGAT	primers used for pFJ6-Hpro-*orf4*, pFJ6-Hpro-*orf4*-4FSM and pFJ6-Hpro-*orf4*^{x} construction
SphI-ORF4-293-R	AAAGCATGCCTACTCTTCGTCGGTACC	
NotI-ORF4-FSM-F	AATGCGGCCGCAATGCAGATGGCCTTGATATTG	primer used for pFJ6-Hpro-*orf4*-4FSM construction
SphI-ORF4-150-R	TAAGCATGCCTAAACCATCTCAAGTTCGTCGTCTT	primers used for pFJ6-Hpro-*orf4*^{x} construction
SphI-ORF4-99-R	TAAGCATGCCTAGGGCGGGATCGAGAGGATATTGCTA	
SphI-ORF4-72-R	AAAGCATGCTCACCCAGAGGACCGGATTGCGCGCT	
SphI-ORF4-48-R	AAAGCATGCTCATGCTTTCACCGTCGATTCATTCG	
SphI-ORF4-24-R	AAAGCATGCTCAATCAATATCAAGTGCCATCT GCAT	
NotI-ORF4-400-F	AAAGCGGCCGCATGCAGATGATGCAGTTGA	
radA-F	ATCCACGTCGCGAAGGCGTTCA	primers used in qPCR assays
radA-R	CTGCTTGTTGAGTTTCTGCTGTCGGTC	
ORF14-F	GACAGATGTGAATAGCGGCGAAAG	
ORF14-R	AGAAAGCGACGGAGACGATGGA	
vector-F	GGGGATAACGCAGGAAAGAACA	
vector-R	GGCAGGGTCGGAACAGGAG	
ORF7-F	CGTTGAGGCGACGAAGCTCGGTGA	primers used in southern blot assay
ORF11-R	CCTCTTTCGTCAGCGTGTCCAAGCTCT	
ORF4-GFP-1-F	GACGAACCCTTGCAATTGCTTAAGTAATCTCGTGGTATCT CT	primers used for pFJ6-PphaRP-GFP
ORF4-	ATCAAGTGCCATCTGCATTCAGGATCCCATCTCCTA	

GFP-1-R		and pFJ6-PphaRP-ORF4-GFP-1/2 construction
ORF4-GFP-2-F	TAGGAGATGGGATCCTGAATGCAGATGGCACTTGAT	
ORF4-GFP-2-R	GGCCTTGGCGGCGGCTTCCTTGGCGGCGGCTTCCTTGGCGGCGGCTTCGGCCTCTTCGTCGGTACCGGC	
ORF4-GFP-3-F	GCCGAAGCCGCCGCCAAGGAAGCCGCCGCCAAGGAAGCCGCCGCCAAGGCCATGAGTAAAGGAGAAGAACTTTTCACTGGA	
ORF4-GFP-3-R	ACCATGATTACGCCAAGCTTGCATGCTTATTTGTATAGTTCATCCATGCCATGTGT	
ORF4-GFP-4-F	GGCGGCGGTGGGTCGGGCGGCGGTGGGTCGGGCGGCGGTGGGTCGATGAGTAAAGGAGAAGAACTTT	
ORF4-GFP-4-R	AAAGTTCTTCTCCTTTACTCATCGACCCACCGCCGCCCGACCCACCGCCGCCCGACCCACCGCCGCCCTCTTCGTCGGTACCGGCC	

1 Zhang, Z. et al. Temperate membrane-containing halophilic archaeal virus SNJ1 has a circular dsDNA genome identical to that of plasmid pHH205. *Virology* **434**, 233-241, (2012).

2 Wang, Y. et al. Identification, characterization, and application of the replicon region of the halophilic temperate sphaerolipovirus SNJ1. *J. Bacteriol.* **198**, 1952-1964, (2016).

3 Liu, Y. et al. Identification and characterization of SNJ2, the first temperate pleolipovirus integrating into the genome of the SNJ1-lysogenic archaeal strain. *Mol. Microbiol.* **98**, 1002-1020, (2015).

4 Wang, Y., Chen, B., Sima, L., Cao, M. & Chen, X. Construction of Expression Shuttle Vectors for the Haloarchaeon Natrinema sp. J7 Based on Its Chromosomal Origins of Replication. *Archaea* **2017**, 4237079, (2017).