

Table S1. Strains used in this study

Strains	Description	Reference
RN4220	Laboratory strain, restriction-defective derivative of RN450	(1)
RN450	NTCT8325 cured of ϕ 11, ϕ 12 and ϕ 13	(2)
RN451	RN450 lysogenic for ϕ 11	(2)
RN10359	RN450 lysogenic for ϕ 80 α	(3)
JP1794	RN451 (SaPI _{bov1} <i>tst::tetM</i>)	(4)
JP4028	RN451 Δ <i>rinA</i>	This study
JP4128	JP4028 (SaPI _{bov1} <i>tst::tetM</i>)	This study
JP4221	JP4028 (pJP740)	This study
JP5961	JP4128 (pJP740)	This study
JP4027	RN451 Δ <i>rinB</i>	This study
JP4127	JP4027 (SaPI _{bov1} <i>tst::tetM</i>)	This study
JP3602	RN10359 (SaPI1 <i>tst::tetM</i>)	(5)
JP3603	RN10359 (SaPI _{bov1} <i>tst::tetM</i>)	(5)
JP4717	RN10359 Δ <i>rinA</i>	This study
JP5293	JP4717 (SaPI _{bov1} <i>tst::tetM</i>)	This study
JP5294	JP4717 (SaPI1 <i>tst::tetM</i>)	This study
JP5418	JP4717 (pJP740)	This study
JP5419	JP5293 (pJP740)	This study
JP5420	JP5294 (pJP740)	This study
JP5011	RN4220 lysogenic for ϕ SLT <i>pvl::tetM</i>	This study
JP6895	JP5011 Δ <i>rinA</i>	This study
JP6391	JP6895 (pJP838)	This study
JP4223	RN451 (pJP742)	This study
JP4222	JP4028 (pJP742)	This study
JP4216	RN4220 (pJP743)	This study
JP5963	RN4220 (pJP744)	This study
JP5619	RN4220 (pJP746)	This study
JP5620	RN4220 (pJP747)	This study
JP5925	RN4220 (pJP748)	This study
JP5926	RN4220 (pJP749)	This study
JP7188	RN451 chimera ϕ 11- ϕ 69 (ϕ 11 carrying <i>Pter</i> from ϕ 69)	This study
JP7242	JP7188 (SaPI _{bov1} <i>tst::tetM</i>)	This study
JP7218	JP7188 (pJP741)	This study
JP7243	JP7242 (pJP741)	This study
JP3960	<i>Enterococcus faecalis</i> V583	Lab strain
JP5093	<i>Streptococcus pyogenes</i> NZ131	Lab strain
JP3345	<i>Lactococcus lactis</i> IL1403	Lab strain
JP3762	RN4220 (pJP535)	This study
JP3763	RN4220 (pJP537)	This study
JP3766	RN4220 (pJP543)	This study
JP3767	RN4220 (pJP544)	This study
JP3779	RN4220 (pJP564)	This study
JP3780	RN4220 (pJP565)	This study

Table S2. Plasmids used in this study

Plasmids	Description	Reference
pMAD	Vector for efficient allelic replacement	(6)
pJP508	pMAD derivative. Deletion of <i>rinA</i> from ϕ 11 and 80 α	This study
pJP835	pMAD derivative. Deletion of <i>rinA</i> from ϕ SLT	This study
pJP956	pMAD derivative. Generation of the chimera ϕ 11- ϕ 69 (ϕ 11 carrying <i>Pter</i> from ϕ 69)	This study
pCN51	Expression vector	(7)
pCN41	Used in transcriptional fusions to the staphylococcal β -lactamase <i>blaZ</i>	(7)
pCN42	Used in transcriptional fusions to the staphylococcal β -lactamase <i>blaZ</i> . Contains the <i>Pcad</i> promoter	(7)
pJP740	Expression of ϕ 11 RinA, pCN51 derivative	This study
pJP741	Expression of ϕ 69 RinA, pCN51 derivative	This study
pJP838	Expression of ϕ SLT RinA, pCN51 derivative	This study
pJP742	Transcriptional analysis of ϕ 11 <i>terS</i> , pCN41 derivative	This study
pJP743	Transcriptional analysis of ϕ 11 <i>terS</i> in presence of ϕ 11 RinA, pCN42 derivative	This study
pJP744	Transcriptional analysis of ϕ 11 <i>terS</i> in absence of RinA, pCN42 derivative	This study
pJP746	Transcriptional analysis of ϕ 69 <i>terS</i> in presence of ϕ 69 RinA, pCN42 derivative	This study
pJP747	Transcriptional analysis of ϕ 69 <i>terS</i> in absence of RinA, pCN42 derivative	This study
pJP748	Transcriptional analysis of ϕ 69 <i>terS</i> in presence of ϕ 11 RinA, pCN42 derivative	This study
pJP749	Transcriptional analysis of ϕ 11 <i>terS</i> in presence of ϕ 69 RinA, pCN42 derivative	This study
pJP750	Expression in <i>E. coli</i> of ϕ 11 RinA, pGEX-4T-1 derivative	This study
pJP535	Transcriptional analysis of <i>terS</i> from <i>E. faecalis</i> phage, in presence of the phage-encoded homologous RinA, pCN42 derivative	This study
pJP537	<i>rinA</i> mutant of pJP535	This study
pJP543	Transcriptional analysis of <i>terS</i> from <i>S. pyogenes</i> phage, in presence of the phage-encoded homologous RinA, pCN42 derivative	This study
pJP544	<i>rinA</i> mutant of pJP543	This study
pJP564	Transcriptional analysis of <i>terS</i> from <i>L. lactis</i> phage, in presence of the phage-encoded homologous RinA, pCN42 derivative	This study
pJP565	<i>rinA</i> mutant of pJP564	This study

Table S3. Oligonucleotides used in this study

Plasmid	Oligonucleotides	Sequence (5'-3')
pJP508	orf28phi11-1mB orf28phi11-2c orf28phi11-3m orf28phi11-4cE	CGCGGATCCGTGATAAGAAGTGACGC TTTAGTCATGAATACCCTCCG CGGAGGGTATTCATGACTAAAAGATTGGCGGAAGAGTTAGGG CCGGAATTCGTACTTCAGTGAAGTTATCG
pJP835	phiSLTp36-1mB phiSLTp36-2c phiSLTp36-3m phiSLTp36-4cE	CGCGGATCCATCAGAGCTGAAGTTTCATGG GATATCATATATTGTGTTCCC GGAACACAATATATGATATCAACTTTGTTAAAGCGGTAGCG CCGGAATTCAAACATTTTAAGCCGATGGGC
pJP956	orf29phi11-11mB orf28phi11-15c orf30phi69-18m orf25phi69-17cS	CGCGGATCCATCAGTCTGATTTGATGAGGGC GTCTTCCCCTAACTCTCCG CGGAAGAGTTAGGGGAAGACTAAGTTTGGAAAAAGTCTGG ACGCGTCTGACTAATGTACTTCAGTGAAGTTATCG
pJP740	orf28phi11-6mB orf28phi11-5cK	CGCGGATCCGGTGATGTAAAAGTTAAAGAGCG CGGGGTACCACTGCCAATTTTCAGTCTTCCC
pJP741	orf30phi69-1mB orf30phi69-6cK	CGCGGATCCGTTTGGTGTGATGTTGCAAGC CGGGGTACCATGTATTATACCTAATTCATCTGC
pJP838	phiSLTp36-5mB phiSLTp36-6cE	CGCGGATCCTTGCAGAATAAAGAAGTAACG CCGGAATTCGCCTTTTGCACAATCTTTGC
pJP742	orf28phi11-5mB orf29phi11-3cK	CGCGGATCCATCAAGCAGGAAAGAACGC CGGGGTACCTAACCTGCTGTAATTGCTGC
pJP743	orf28phi11-6mB orf29phi11-3cK	CGCGGATCCGGTGATGTAAAAGTTAAAGAGCG CGGGGTACCTAACCTGCTGTAATTGCTGC
pJP744	orf28phi11-6mB orf29phi11-3cK	CGCGGATCCGGTGATGTAAAAGTTAAAGAGCG CGGGGTACCTAACCTGCTGTAATTGCTGC
pJP746	orf30phi69-1mB orf25phi69-2cK	CGCGGATCCGTTTGGTGTGATGTTGCAAGC CGGGGTACCTCATTACCACCAACTCTCGC
pJP747	orf30phi69-1mB orf30phi69-3c orf30phi69-4m orf25phi69-2cK	CGCGGATCCGTTTGGTGTGATGTTGCAAGC CCTCATATCATAAGCAGTAGACATTGCTTTCACCCTATCTCC GTCTACTGCTTATGATATGAGG CGGGGTACCTCATTACCACCAACTCTCGC
pJP748	orf28phi11-6mB orf28phi11-7cK orf30phi69-5mK orf25phi69-2cE	CGCGGATCCGGTGATGTAAAAGTTAAAGAGCG CGGGGTACCGTCTTCCCCTAACTCTTCCG CGGGGTACCATTAAGTTTGGAAAAAGTCTGG CCGGAATTCCAAATTAATTAATATGCCATCAG
pJP749	orf30phi69-1mB orf30phi69-6cK orf28phi11-8mK orf29phi11-3cE	CGCGGATCCGTTTGGTGTGATGTTGCAAGC CGGGGTACCATGTATTATACCTAATTCATCTGC CGGGGTACCCTGAAATTGGCAGTAAAGTGGC CCGGAATTCCTAACCTGCTGTAATTGCTGC
pJP750	orf28phi11-5cX orf28phi11-7mB/b	CCGCTCGAGACTGCCAATTTTCAGTCTTCCC CGCGGATCCATGACTAAAAGAAATACGG
pJP535	EF_V583-Pla-1mS EF_V583-Pla-4cB	ACGCGTCTGACAATCCATCAAGGCCGCATCG CGCGGATCCTGTCTGGCAGTTCTCTAGC
pJP537	EF_V583-Pla-1mS EF_V583-Pla-2c EF_V583-Pla-3m EF_V583-Pla-4cB	ACGCGTCTGACAATCCATCAAGGCCGCATCG TAATTCTTCTTCCCCTTGGACG TCAACGGGAAGAAGAATTACATTAATTGGACTTGCCCAGC CGCGGATCCTGTCTGGCAGTTCTCTAGC
pJP543	Spy_NZ131-Pla-1mB Spy_NZ131-Pla-4cE	CGCGGATCCCGTGCTAGTGATGATAGAGC CCGGAATTCCTTAATAGCCGCTGCTGTCTGC

Plasmid	Oligonucleotides	Sequence (5'-3')
pJP544	Spy_NZ131-Pla-1mB Spy_NZ131-Pla-2c Spy_NZ131-Pla-3m Spy_NZ131-Pla-4cE	<u>CGCGGATCC</u> CGTGCTAGTGATGATAGAGC AAATTTGCTCTTCTCCACCG GTGGAGGAAGAGCAAATTTGCCTATACAAGACATCTGTCCG <u>CCGGAATTC</u> TTTAATAGCCGCTGCTGTCCG
pJP564	bIL309-Alt-1mB bIL309-Alt-5cE	<u>CGCGGATCC</u> CACACTTGGTCAGTAGTATGG <u>CCGGAATTC</u> TATAAATTTCTTGAACCTCACC
pJP565	bIL309-Alt-1mB bIL309-Alt-2c bIL309-Alt-3m bIL309-Alt-5cE	<u>CGCGGATCC</u> CACACTTGGTCAGTAGTATGG AGAATATGCTGTACGAATGCC CATTTCGTACAGCATATTCTGGTTACGGTGGTTTTACATGG <u>CCGGAATTC</u> TATAAATTTCTTGAACCTCACC

* Sequences recognized by the restriction enzymes used in cloning are underlined.

Oligonucleotide	Sequence (5'-3')	Description
gyr-L gyr-U	CACCATGTAAACCACCAGATA TTATGGTGCTGGGCAAATACA	qPCR. Expression of <i>gyrB</i> gene
ORF29-phi11-4m ORF30-phi11-2c	CAGCAGAGTCTTTAGCAAGTCG CTTACCGCTCGAACCTCCACC	qPCR. Expression of ϕ 11 <i>terS</i> gene
ORF32-phi11-7m ORF32-phi11-8c	GATGAGTTCGATGTAAAAGCG TGAACACGTGCCGATTCCG	qPCR. Expression of ϕ 11 ORF32
ORF34-phi11-4m ORF34-phi11-5c	ATTAGGTAAGTACGAACCAATGG GTCTAACGAATCACTGTTACGG	qPCR. Expression of ϕ 11 ORF34
ORF37-phi11-1mB ORF37-phi11-7c	<u>CGCGGATCC</u> AGTTGAACAAATACCGGAA AGG GTACCAAATTCTAAGAAACCAC	qPCR. Expression of ϕ 11 ORF36
ORF39-phi11-1m ORF39-phi11-2c	TACAGAGGATAACAATGGACGG GTGGAATCTGTATCCAGTCGC	qPCR. Expression of ϕ 11 ORF39
ORF45-phi11-2m ORF45-phi11-3c	AACGGTCGATTTAGCAACATGG TCTCCATCAATGTATCTATACGC	qPCR. Expression of ϕ 11 ORF45
ORF49-phi11-6m ORF48-phi11-4cE	TTTTGTACCACAACCAGGCG <u>CCGGAATTC</u> GTTTTTCGAGTCGTCGATTA CC	qPCR. Expression of ϕ 11 ORF49
ORF53-phi11-7m ORF53-phi11-8c	AGTGGTTGAAAACCTTCTGAGGG TACCTTTAGGGTTACTACCACG	qPCR. Expression of ϕ 11 ORF53
ORF1-phi11-1m ORF1-phi11-2c	AGCGTACGAGTGTCTTAAATGG CTGTGTGGGCAGGTTTTAAAGC	qPCR. Expression of ϕ 11 <i>int</i>
ORF29-phi11-sp1c ORF29-phi11-sp2c ORF29-phi11-sp3c	TGGTCAATAGATCTCTGACGC AACCTCTTTTTCCACTTCATCG TAGAAGCAGATAACGCTAAAGC	5' RACE ϕ 11 <i>terS</i>
MH22 MH23	CCTCTTTTTCCACTTCATCG GTCGATATACGCACCATGAAC	5' RACE 80 α <i>terS</i>
UpTerSau-1m DwTerSau-2c	AAAAATCATATCTAGATTGGCGG TCATTATATATTCATCTGCGAATCTC	EMSA

Figure S1. Comparison of the RinA protein sequences of ϕ 11, 80 α , ϕ SLT and ϕ 69.

Colours indicate relative sequence conservation at each position, with red being most conserved and violet being least (adapted from alignment generated by PRALINE (8)). The scoring scheme works from 0 for the least conserved alignment position, up to 10 (*) for the most conserved alignment position.

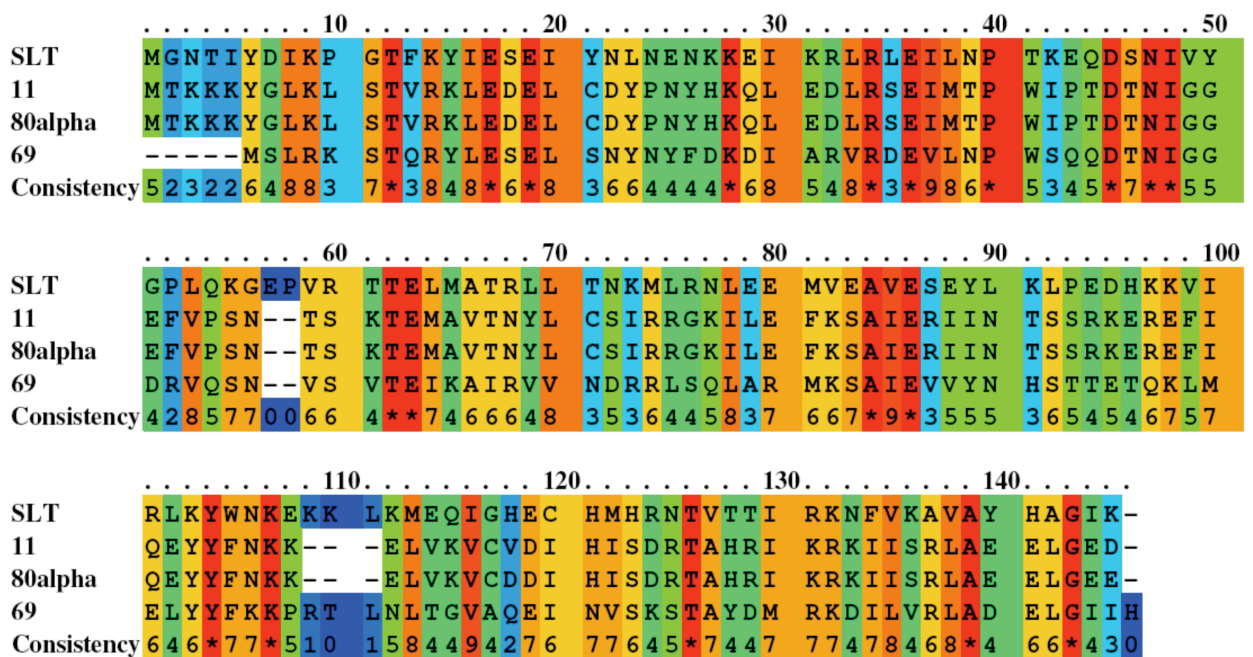
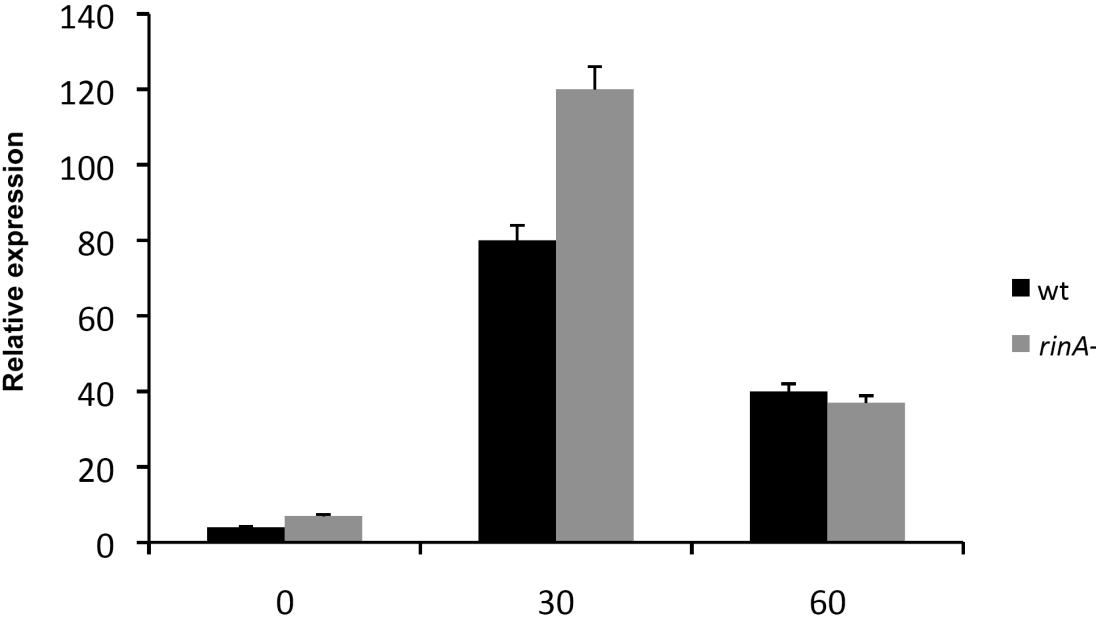


Figure S3. RinA does not control expression of ϕ 11 *int*. Real time quantification of the expression of *int* in wild-type ϕ 11 and a *rinA* mutant at different times (0, 30 or 60 min) after MC induction of the phage lytic cycle. Expression was normalized to *gyrB*, as described in Materials and Methods.



References

1. Kreiswirth, B.N., Lofdahl, S., Betley, M.J., O'Reilly, M., Schlievert, P.M., Bergdoll, M.S. and Novick, R.P. (1983) The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. *Nature*, **305**, 709-712.
2. Novick, R. (1967) Properties of a cryptic high-frequency transducing phage in *Staphylococcus aureus*. *Virology*, **33**, 155-166.
3. Ubeda, C., Barry, P., Penades, J.R. and Novick, R.P. (2007) A pathogenicity island replicon in *Staphylococcus aureus* replicates as an unstable plasmid. *Proc Natl Acad Sci U S A*, **104**, 14182-14188.
4. Ubeda, C., Maiques, E., Knecht, E., Lasa, I., Novick, R.P. and Penades, J.R. (2005) Antibiotic-induced SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence factors in staphylococci. *Mol Microbiol*, **56**, 836-844.
5. Ubeda, C., Maiques, E., Barry, P., Matthews, A., Tormo, M.A., Lasa, I., Novick, R.P. and Penades, J.R. (2008) SaPI mutations affecting replication and transfer and enabling autonomous replication in the absence of helper phage. *Mol Microbiol*, **67**, 493-503.
6. Arnaud, M., Chastanet, A. and Debarbouille, M. (2004) New vector for efficient allelic replacement in naturally nontransformable, low-GC-content, gram-positive bacteria. *Appl Environ Microbiol*, **70**, 6887-6891.
7. Charpentier, E., Anton, A.I., Barry, P., Alfonso, B., Fang, Y. and Novick, R.P. (2004) Novel cassette-based shuttle vector system for gram-positive bacteria. *Appl Environ Microbiol*, **70**, 6076-6085.
8. Simossis, V.A., Kleinjung, J. and Heringa, J. (2005) Homology-extended sequence alignment. *Nucleic Acids Res*, **33**, 816-824.