

Supplementary data

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Methylation warfare: interaction of pneumococcal bacteriophages with their host

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Table S1. Bacterial strains and primers

Name	Background	Knock out	Relevant properties ^a	Reference
D39	-	-	Type 2 Avery's strain	(3, 27)
DP1004	Rx1	-	D39 unencapsulated derivative; Sm ^r	(27, 59)
FP486	D39	SPD_0449-455	<i>spnD39III</i> deleted mutant; Sp ^r	This work
FP470	DP1004	SPD_0449-455	<i>spnDP1004III</i> deleted mutant; Sp ^r ; Sm ^r	This work
FP422	DP1004	SPD_0450-452	<i>spnDP1004IIIB</i> locked mutant; Sp ^r ; Sm ^r	This work
FP423	DP1004	SPD_0450-452	<i>spnDP1004IIIC</i> locked mutant; Sp ^r ; Sm ^r	This work
FP424	DP1004	SPD_0450-452	<i>spnDP1004IIIA</i> locked mutant; Sp ^r ; Sm ^r	This work
FP425	DP1004	SPD_0450-452	<i>spnDP1004IIID</i> locked mutant; Sp ^r ; Sm ^r	This work
MRO557	DP1004	SPD_0450-452	<i>spnDP1004IIIE</i> locked mutant; Sp ^r ; Sm ^r	This work
MRO558	DP1004	SPD_0450-452	<i>spnDP1004IIIF</i> locked mutant; Sp ^r ; Sm ^r	This work
MRO587	FP470	-	SpSL1 prophage carrying strain	This work
MRO575	FP424	SPD_0450-452; SPD_455	<i>spnDP1004IIIA</i> locked:: <i>hsdR</i> frameshifted; Sp ^r ; Sm ^r	This work
MRO594	FP424	SPD_0450-452; SPD_0930-1	<i>spnDP1004IIIA</i> locked:: <i>pezAT</i> deleted mutant; Sp ^r ; Km ^r	This work
MRO598	FP424	SPD_0450-452; SPD_1081-2	<i>spnDP1004IIIA</i> locked:: <i>relBE2</i> deleted mutant; Sp ^r ; Cm ^r	This work
MRO602	FP424	SPD_0450-452; SPD_1550-1	<i>spnDP1004IIIA</i> locked:: <i>yefM-yoeB</i> deleted mutant; Sp ^r ; Km ^r	This work
MRO589	FP422	SPD_0450-452; SPD_1108-9	<i>spnDP1004IIIB</i> locked:: <i>spnDP1004mcrBC</i> deleted mutant; Sp ^r ; Sm ^s ; Km ^r	This work
MRO590	FP424	SPD_0450-452; SPD_1108-9	<i>spnDP1004IIIA</i> locked:: <i>spnDP1004mcrBC</i> deleted mutant; Sp ^r ; Sm ^s ; Km ^r	This work
MRO628	FP424	SPD_0450-452; SPD_1737	<i>spnDP1004IIIA</i> locked:: <i>lytA</i> deleted mutant; Sp ^r ; Sm ^s ; Km ^r	This work
MRO629	FP470	SPD_0449-445; SPD1259-60	<i>spnDP1004III</i> deleted mutant:: <i>spnDP1004I</i> deleted mutant; Sp ^r ; Sm ^s ; Km ^r	This work
R6x <i>Δivr tvr::Janus</i>	R6x	-	R6x <i>Δivr tvr::Janus</i> ; Km ^r	(38)
R6x <i>Δivr hsdS::tvr_R-MV5ΔtvrR</i>	R6x	-	R6x <i>Δivr hsdS::tvr_R-MV5ΔtvrR</i>	(38)
	Target	Template GenBank ID	Sequence (5' - 3')	
IF100	<i>Sp cassette</i>	KJ955483	GCTCTAGAACTAGTGGATC	(46)
IF101	<i>Sp cassette</i>	KJ955483	TTCCCTTCAAGAGCGATAC	(46)
LG70	<i>spnD39III</i>	NC_008533	GCAGTCTAAGCCATCAAATAC	(46)
LG71	<i>spnD39III</i>	NC_008533	GATCCACTAGTTCTAGAGCTTTCTGCCTGTAATTGTT CATC	(46)
ASM	<i>spnD39III</i>	NC_008533	GTATCGCTCTTGAAGGGAAGCCATCGTTTGGTCTACT AAGATGT	This work
ASM	<i>spnD39III</i>	NC_008533	AGCATATCGCTTACGAAGAATACTT	This work
LF83	SpSL1	KM882824	GTCACAACACAAAACTTTCCAGAGT	This work
LF84	SpSL1	KM882824	ACCATATCGAGCCAACTTCTCCT	This work
LF88	SpSL1	KM882824	ACGGGCTATACTGCATCTTAGAGA	This work
LF89	SpSL1	KM882824	CTACTTTGACCAGTCAGGCGAAAT	This work
LF90	<i>csRNA3</i>	NC_008533	AGTAGGTCCTGGTCGTGAACAA	This work
LF91	<i>csRNA5</i>	NC_008533	AGGAAGTCACCTGTTATGAAGGT	This work
LF92	<i>csRNA2</i>	NC_008533	GTATAGGGATGACTTGGATGAATCGT	This work
LF93	<i>csRNA4</i>	NC_008533	CAGTTGGACTGCGCTTGAATGA	This work
LF94	<i>csRNA3</i>	NC_008533	GAGCAATCTCAGCCTCTCTCAA	This work
LF94	<i>csRNA5</i>	NC_008533	GCGCGACCAATCGTGTAAAC	This work
LF96	<i>csRNA2</i>	NC_008533	GATTAAGAGAACGTTAGGGATTCTATCA	This work
LF97	<i>csRNA4</i>	NC_008533	CTCTACTTCCACAGCCTTATCTTTC	This work
LF106	<i>spnMcrBC</i>	NC_008533	CTTGATATCCGCTTCTGCACG	This work

LF107	<i>spnMcrBC</i>	NC_008533	ATCAAACGGATCCCCAGCTTGTGAGATAGGAAACCTA GCAGGT	This work
LF108	<i>spnMcrBC</i>	NC_008533	CGCCTACGAGGAATTTGTATCCCCAGTGTAGGAGCT TTAATATC	This work
LF109	<i>spnMcrBC</i>	NC_008533	ATCCTTCCTTGGAACCCAGAAG	This work
LF149	<i>lytA</i>	NC_008533	CTTAAATCCATTTCGTGATATTCC	This work
LF150	<i>lytA</i>	NC_008533	ATATCACATTATCCATTAAAAATCAAACCTTCCTTCA ATGTTTGCTACTATATT	This work
LF151	<i>lytA</i>	NC_008533	GCATAAGGAAAGGGGCCATCCAGAACCAGAACTCCT	This work
LF152	<i>lytA</i>	NC_008533	TCTTATCTGGGTTGCCATGA	This work
hsdRU2	<i>hsdR</i>	NC_008533	CAAAATTAGAAAACATGCCTAGCG	This work
janus- hsdRR	<i>hsdR</i>	NC_008533	ATCAAACGGTCATATTGAACCCCTCCGCATTC	This work
hsdrD	<i>hsdR</i>	NC_008533	CAAACCGCTTTTCGAAGGCG	This work
janus- hsdRF	<i>hsdR</i>	NC_008533	AGGGATCCCAGAGAAAGAAATGAGTGAGGAGG	This work
hsdRfram eF	<i>hsdR</i>	NC_008533	TTGAGTTTATAAGTCTAAGAAAGAAATGAGTG	This work
hsdRfram eR	<i>hsdR</i>	NC_008533	CTTTCTTAGACTTATAAACTCAACCATATTGAACC	This work
hsdR- janusF	<i>kan-rpsL</i>	(71)	GTTCAATATGACCGTTTGATTTTAAATGGATAATGTG	This work
hsdR- janusR	<i>kan-rpsL</i>	(71)	ATTTCTTTCTCGGGATCCCTTTCCTTATGC	This work

^a Sm, streptomycin; Sp, spectinomycin; Km, kanamycin, Cm, chloramphenicol.

Table S2. Phage and host proteins identified with LC-MS/MS

Pahge CDS	Function	GenBank ID	Molecular weight (kDa)	Total unique peptide count*
<i>Cds32</i>	portal protein	723007184	44	14
<i>Cds33</i>	prohead maturation protease	723007185	20	3
<i>Cds34</i>	capsid protein	723007186	47	17
<i>Cds35</i>	hypothetical protein	723007187	13	2
<i>Cds36</i>	head-tail adapter protein	723007188	12	3
<i>Cds37</i>	hypothetical protein	723007189	14	4
<i>Cds39</i>	major tail protein	723007191	24	20
<i>Cds42</i>	tail length tape-measure protein	723007194	98	16
<i>Cds43</i>	tail protein	723007231	27	10
<i>Cds44</i>	tail fiber PblB-like protein	723007195	179	43
<i>Cds47</i>	holin	723007198	11	3
<i>Cds49</i>	N-acetylmuramoyl-L-alanine amidase	723007200	36	3
<i>Cds21</i>	hypothetical protein	723007222	28	12
<i>Cds24</i>	hypothetical protein	723007225	10	2

* Total number of different amino acid sequences that are associated only with this protein. Scaffold's calculated probability of correct protein identification threshold was set to >95%. With these settings the probability that a protein is correctly identified with a single peptide is of ~50%, while it increases with more uniquely associated peptides (e.g. to ~99.75% with two unique peptides).

Table S3. Time course of host differentially regulated genes during SpSL1 lytic infection

Gene D39 ^a	Name	Fold change ^b			Function	Lowes q-value
		10	50	90		
SPD_0001	<i>dnaA</i>	1.33	2.28	2.72	chromosomal replication initiator protein DnaA	2.20E-22
SPD_0039		0.66	0.90	0.48	conserved hypothetical protein	4.86E-27
SPD_0063	<i>strH</i>	1.15	0.13	0.06	beta-N-acetylhexosaminidase	0.00E+00
SPD_0066		1.46	0.51	0.46	PTS system, IIB component	1.03E-43
SPD_0068		1.86	0.52	0.47	PTS system, IID component	1.37E-83
SPD_0069		1.95	0.41	0.60	PTS system, IIA component	6.02E-140
SPD_0070	<i>agaS</i>	2.09	0.55	0.52	sugar isomerase domain protein AgaS	6.00E-107
SPD_0071	<i>galM</i>	2.48	0.52	0.57	aldose 1-epimerase	3.22E-124
SPD_0081		0.70	0.58	0.47	DNA-binding response regulator	4.01E-22
SPD_0088		1.84	0.34	0.22	ABC transporter, permease protein	0.00E+00
SPD_0088		1.84	0.34	0.22	ABC transporter, permease protein	0.00E+00
SPD_0089		2.32	0.34	0.19	ABC transporter, permease protein	0.00E+00
SPD_0089		2.32	0.34	0.19	ABC transporter, permease protein	0.00E+00
SPD_0090		1.90	0.24	0.12	ABC transporter, substrate-binding protein	0.00E+00
SPD_0092		1.38	0.45	0.33	conserved hypothetical protein	0.00E+00
SPD_0092		1.38	0.45	0.33	conserved hypothetical protein	0.00E+00
SPD_0104		0.73	0.43	0.31	LysM domain protein	6.83E-76
SPD_0118		1.82	2.00	1.00	conserved hypothetical protein	5.50E-06
SPD_0138		1.49	0.20	0.15	glycosyl transferase, group 1 family protein	0.00E+00
SPD_0139		1.60	0.16	0.14	glycosyl transferase, group 2 family protein	0.00E+00
SPD_0140		2.29	0.19	0.25	ABC transporter, ATP-binding protein	0.00E+00
SPD_0140		2.29	0.19	0.25	ABC transporter, ATP-binding protein	0.00E+00
SPD_0187	<i>nrdD</i>	1.77	18.23	42.50	anaerobic ribonucleoside-triphosphate reductase	0.00E+00
SPD_0188		1.81	20.28	44.72	conserved hypothetical protein	0.00E+00
SPD_0189		1.77	17.15	35.92	acetyltransferase, GNAT family protein	0.00E+00
					anaerobic ribonucleoside-triphosphate reductase activating protein	
SPD_0190	<i>nrdG</i>	1.92	18.31	47.31		0.00E+00
SPD_0191		1.71	16.10	43.05	conserved hypothetical protein	0.00E+00
SPD_0247		1.27	0.25	0.17	glycosyl hydrolase, family protein 1	0.00E+00
SPD_0249		1.00	0.61	0.48	conserved hypothetical protein	3.32E-10
SPD_0261	<i>pepC</i>	0.62	0.69	0.49	aminopeptidase C	6.13E-33
					S-ribosylhomocysteine lyase (autoinducer-2 production	
SPD_0309	<i>luxS</i>	0.44	0.60	0.42	protein luxS) (AI-2 synthesis protein	7.14E-29
SPD_0310		0.66	0.63	0.46	conserved hypothetical protein	1.92E-51
SPD_0375	<i>serS</i>	0.74	0.54	0.48	seryl-tRNA synthetase	4.35E-18
SPD_0381	<i>acpP</i>	0.58	0.46	0.33	acyl carrier protein	2.94E-69
SPD_0386	<i>accB</i>	1.15	1.36	0.95	acetyl-CoA carboxylase, biotin carboxyl carrier protein	1.63E-04
SPD_0388	<i>accC</i>	1.20	1.36	1.03	acetyl-CoA carboxylase, biotin carboxylase	3.09E-04
SPD_0389	<i>accD</i>	1.12	1.37	0.82	acetyl-CoA carboxylase, carboxyl transferase, beta subunit	1.18E-14
SPD_0390	<i>accA</i>	1.12	1.37	1.23	acetyl-CoA carboxylase, carboxyl transferase, alpha subunit	1.05E-11
SPD_0391		1.02	1.34	1.53	conserved hypothetical protein	4.18E-17
SPD_0442	<i>pyrG</i>	0.61	0.32	0.29	CTP synthase	2.33E-106
SPD_0444		1.77	0.17	0.07	endo-beta-N-acetylglucosaminidase, putative	0.00E+00
SPD_0473	<i>blpY</i>	2.83	1.22	1.33	immunity protein BlpY	1.27E-21
SPD_0474		2.53	1.13	1.19	conserved hypothetical protein	7.85E-19
SPD_0546	<i>brnQ</i>	1.08	0.45	0.29	branched-chain amino acid transport system II carrier protein	3.51E-72
SPD_0575		0.68	0.63	0.39	sensor histidine kinase, putative	7.03E-27
SPD_0594		1.02	0.66	0.49	conserved hypothetical protein	8.22E-14
SPD_0608	<i>pyrF</i>	0.16	0.62	0.38	orotidine 5'-phosphate decarboxylase	9.31E-100
SPD_0609	<i>pyrE</i>	0.17	0.62	0.42	orotate phosphoribosyltransferase	2.48E-91
SPD_0612		2.50	3.36	2.71	lipoprotein, putative	1.32E-39
SPD_0614		2.07	2.43	2.36	ABC transporter, ATP-binding protein	4.12E-17
SPD_0632	<i>thiD</i>	0.71	0.55	0.49	phosphomethylpyrimidine kinase	8.96E-16
SPD_0636	<i>spxB</i>	0.70	0.44	0.23	pyruvate oxidase	2.57E-94
SPD_0637		0.55	0.43	0.26	glyoxalase family protein	3.02E-97
SPD_0641	<i>manA</i>	1.08	0.37	0.36	mannose-6-phosphate isomerase, class I	7.19E-222
SPD_0667	<i>sodA</i>	0.52	0.48	0.31	superoxide dismutase, manganese-dependent	1.24E-78
SPD_0731		0.19	0.56	0.49	DNA topology modulation protein FlaR, putative	1.10E-267

SPD_0732	<i>rpsT</i>	0.37	0.73	0.47	ribosomal protein S20	1.65E-42
SPD_0739		0.49	0.47	0.49	probable membrane lipoprotein TmpC precursor	1.86E-12
SPD_0762	<i>sufC</i>	0.72	0.52	0.45	FeS assembly ATPase SufC	5.73E-33
SPD_0763	<i>sufD</i>	0.79	0.56	0.46	FeS assembly protein SufD	7.07E-32
SPD_0764	<i>sufS</i>	0.91	0.56	0.43	cysteine desulfurases, SufS subfamily protein	1.47E-37
SPD_0765		0.97	0.49	0.41	SUF system FeS assembly protein, NifU family protein	4.48E-30
SPD_0799		0.63	0.67	0.43	conserved hypothetical protein	5.41E-10
SPD_0851	<i>pyrK</i>	0.20	0.60	0.30	dihydroorotate dehydrogenase electron transfer subunit	2.31E-97
SPD_0852	<i>pyrDb</i>	0.20	0.56	0.32	dihydroorotate dehydrogenase, catalytic subunit	1.01E-75
SPD_0853	<i>lytB</i>	0.48	0.68	0.49	endo-beta-N-acetylglucosaminidase precursor, putative	1.17E-10
SPD_0895	<i>hemH</i>	2.33	4.11	4.44	ferrochelatase	9.02E-86
SPD_0896	<i>mscL</i>	0.38	0.38	0.40	large conductance mechanosensitive channel protein MscL	4.15E-55
SPD_0898		0.63	0.35	0.32	membrane protein, putative	2.94E-44
SPD_0902	<i>trmE</i>	0.54	0.52	0.48	tRNA modification GTPase TrmE	2.09E-23
SPD_0903	<i>xylH</i>	0.45	1.13	0.56	4-oxalocrotonate tautomerase	3.59E-12
SPD_0913		0.52	0.35	0.34	conserved hypothetical protein	2.47E-66
SPD_0987		0.66	0.64	0.48	conserved hypothetical protein TIGR00103	8.84E-14
SPD_1027		0.54	0.28	0.31	acetoin dehydrogenase, E1 component, beta subunit, putative	1.31E-60
SPD_1028	<i>acoA</i>	0.71	0.48	0.41	TPP-dependent acetoin dehydrogenase alpha-subunit	2.09E-23
SPD_1041	<i>ncdH</i>	0.89	4.17	6.35	glutaredoxin-like protein NrdH	7.95E-297
SPD_1042	<i>ncdE</i>	1.10	3.96	6.41	ribonucleoside-diphosphate reductase, alpha subunit	5.98E-159
SPD_1043	<i>ncdF</i>	1.10	3.56	6.91	ribonucleoside-diphosphate reductase, beta subunit	8.98E-252
SPD_1051	<i>lacC</i>	0.87	0.73	0.50	tagatose-6-phosphate kinase	1.19E-17
SPD_1052	<i>lacB</i>	0.79	0.63	0.38	galactose-6-phosphate isomerase, LacB subunit	6.28E-47
SPD_1053	<i>lacA</i>	0.79	0.61	0.48	galactose-6-phosphate isomerase, LacA subunit	1.83E-20
SPD_1075		2.26	2.04	2.61	transporter, FNT family protein, putative	5.08E-23
SPD_1078	<i>ldh</i>	0.53	0.50	0.26	L-lactate dehydrogenase	1.98E-135
SPD_1100	<i>zwf</i>	0.74	0.76	0.50	glucose-6-phosphate 1-dehydrogenase	7.32E-26
SPD_1107	<i>guaC</i>	0.45	0.60	0.86	guanosine monophosphate reductase	5.85E-28
SPD_1131	<i>carB</i>	0.24	0.83	0.59	carbamoyl-phosphate synthase, large subunit	2.82E-26
SPD_1132	<i>carA</i>	0.25	0.76	0.54	carbamoyl-phosphate synthase, small subunit	1.66E-29
SPD_1133	<i>pyrB</i>	0.21	0.65	0.45	aspartate carbamoyltransferase	2.56E-37
SPD_1134	<i>pyrR</i>	0.20	0.52	0.41	pyrimidine operon regulatory protein/uracil phosphoribosyltransferase	1.48E-68
SPD_1141	<i>uraA</i>	0.19	0.35	0.24	uracil-xanthine permease	3.86E-152
SPD_1158	<i>gdhA</i>	0.81	0.74	0.40	NADP-specific glutamate dehydrogenase	3.87E-35
SPD_1217		0.44	0.54	0.67	conserved hypothetical protein	7.15E-23
SPD_1242		0.83	0.78	0.50	conserved hypothetical protein	2.67E-20
SPD_1286		0.47	0.63	0.63	TrmH family RNA methyltransferase group 3	3.30E-25
SPD_1318	<i>tuf</i>	0.66	0.57	0.45	translation elongation factor Tu	1.19E-14
SPD_1327	<i>bta</i>	0.53	0.55	0.31	bacterocin transport accessory protein	1.37E-52
SPD_1334	<i>atpC</i>	1.15	0.72	0.38	ATP synthase F1, epsilon subunit	8.93E-45
SPD_1337	<i>atpA</i>	1.01	0.66	0.48	ATP synthase F1, alpha subunit	4.88E-29
SPD_1338	<i>atpH</i>	0.84	0.51	0.42	ATP synthase F1, delta subunit	1.05E-23
SPD_1340	<i>atpB</i>	0.80	0.71	0.49	ATP synthase F0, A subunit	2.67E-26
SPD_1360		0.60	0.55	0.34	conserved hypothetical protein	7.91E-43
SPD_1378		0.50	0.71	0.65	conserved hypothetical protein	1.01E-13
SPD_1402		0.78	0.43	0.38	non-heme iron-containing ferritin	1.63E-53
SPD_1413		0.95	0.44	0.82	ATP-dependent RNA helicase, putative	1.33E-30
SPD_1415		0.46	0.39	0.17	oxidoreductase, pyridine nucleotide-disulfide, class I	2.06E-164
SPD_1418	<i>pepQ</i>	0.59	0.54	0.32	proline dipeptidase PepQ	7.81E-75
SPD_1419		0.44	0.43	0.32	conserved hypothetical protein	5.92E-19
SPD_1439	<i>rpsO</i>	0.63	0.65	0.49	ribosomal protein S15	3.03E-17
SPD_1461	<i>psaB</i>	0.51	0.40	0.24	manganese ABC transporter, ATP-binding protein	6.36E-87
SPD_1462	<i>psaC</i>	0.67	0.45	0.29	manganese ABC transporter, permease protein, putative	1.51E-53
SPD_1463		0.70	0.51	0.40	ABC transporter, substrate binding lipoprotein	1.10E-46
SPD_1464	<i>psaD</i>	0.60	0.31	0.18	thiol peroxidase	8.68E-200
SPD_1467		0.31	0.79	0.54	conserved hypothetical protein	1.59E-19
SPD_1524		0.88	0.36	0.29	transcriptional regulator, GntR family protein	1.23E-53
SPD_1525		0.91	0.32	0.31	ABC transporter, ATP-binding protein	1.87E-71
SPD_1526		1.03	0.32	0.29	membrane protein, putative	5.69E-74
SPD_1527		1.06	0.73	0.50	membrane protein, putative	1.53E-33
SPD_1531	<i>scrK</i>	1.18	0.27	0.11	fructokinase	0.00E+00

SPD_1588		1.02	0.46	0.31	conserved hypothetical protein	2.03E-53
SPD_1589		0.89	0.38	0.27	lipoprotein, putative	1.83E-53
SPD_1590		1.07	0.57	0.33	general stress protein 24, putative	1.26E-59
SPD_1591		1.02	0.73	0.37	conserved hypothetical protein	1.41E-40
SPD_1594		0.80	2.16	4.16	transcriptional regulator	0.00E+00
SPD_1595		0.66	2.64	4.11	conserved hypothetical protein	0.00E+00
					choline transporter (glycine betaine transport system	
SPD_1642	<i>proW</i>	1.10	0.68	0.43	permease protein)	2.06E-32
SPD_1643	<i>proV</i>	0.88	0.59	0.39	choline transporter	2.61E-32
SPD_1644		0.84	0.53	0.40	conserved hypothetical protein	1.52E-27
SPD_1645		0.78	0.54	0.41	transcriptional regulator, MarR family protein	4.75E-17
SPD_1789		0.27	0.84	0.82	cell wall surface anchor family protein	1.92E-83
SPD_1795		0.15	0.44	0.43	conserved hypothetical protein	3.82E-111
SPD_1799		1.58	1.83	2.50	sensor histidine kinase, putative	3.31E-18
SPD_1823	<i>gap</i>	0.79	0.63	0.46	glyceraldehyde-3-phosphate dehydrogenase, type I	6.08E-16
SPD_1830		2.81	0.48	0.30	glycosyl hydrolase, family protein 1	0.00E+00
SPD_1830		2.81	0.48	0.30	glycosyl hydrolase, family protein 1	0.00E+00
SPD_1831		4.13	0.58	0.29	PTS system, IIC component	0.00E+00
SPD_1831		4.13	0.58	0.29	PTS system, IIC component	0.00E+00
SPD_1832		4.93	0.77	0.63	PTS system, IIB component	6.30E-215
SPD_1833		4.08	0.92	0.56	PTS system, IIA component	2.52E-136
SPD_1838	<i>yajC2</i>	0.48	0.90	0.66	preprotein translocase, YajC subunit	2.67E-13
SPD_1839	<i>tkl</i>	0.61	0.64	0.47	transketolase	2.20E-28
SPD_1865		2.09	1.98	1.31	alcohol dehydrogenase, zinc-containing	5.67E-27
SPD_1868	<i>tgt</i>	0.61	0.39	0.29	queuine tRNA-ribosyltransferase	4.24E-71
SPD_1874		0.76	0.21	0.17	LysM domain protein	0.00E+00
SPD_1895		0.48	0.78	0.61	cytidine/deoxycytidylate deaminase family protein	1.49E-25
SPD_1896	<i>glxX</i>	0.48	0.67	0.54	glutamyl-tRNA synthetase	6.02E-20
SPD_1968		2.26	0.12	0.08	conserved hypothetical protein	0.00E+00
SPD_1968		2.26	0.12	0.08	conserved hypothetical protein	0.00E+00
SPD_1969		1.39	0.17	0.08	glycosyl hydrolase-related protein	0.00E+00
SPD_1970		1.48	0.17	0.08	ROK family protein	0.00E+00
SPD_1971		1.61	0.17	0.10	glycosyl hydrolase-related protein	0.00E+00
SPD_1972		1.70	0.21	0.11	conserved hypothetical protein	0.00E+00
SPD_1972		1.70	0.21	0.11	conserved hypothetical protein	0.00E+00
SPD_1973		1.07	0.17	0.14	alpha-1,2-mannosidase, putative	0.00E+00
SPD_1974		1.75	0.25	0.11	conserved hypothetical protein	0.00E+00
SPD_2009		1.66	2.84	1.72	conserved hypothetical protein	2.53E-27
SPD_2010		1.13	0.40	0.39	conserved hypothetical protein	5.77E-17
SPD_2011	<i>glpF</i>	1.07	0.29	0.32	glycerol uptake facilitator protein	5.63E-97
SPD_2012	<i>glpO</i>	0.77	0.22	0.25	alpha-glycerophosphate oxidase	5.97E-200
SPD_2013	<i>glpK</i>	0.59	0.33	0.31	glycerol kinase	4.86E-73
SPD_2014		0.87	0.54	0.42	conserved hypothetical protein	7.33E-36
SPD_2015	<i>hslO</i>	0.62	0.50	0.46	chaperonin, 33 kDa	5.16E-30
SPD_2016		0.71	0.47	0.43	TIM-barrel protein, nifR3 family protein, putative	8.68E-31
SPD_2046		2.54	1.69	1.92	cobalt ABC transporter, permease protein	9.75E-19
SPD_2053		0.44	0.85	0.78	conserved hypothetical protein	4.36E-16

^a Gene names refer to *S. pneumoniae* D39 genome (NC_008533). ^b Fold change following gene expression variations after 10, 50, and 90 minutes post phage infection with respect to non-infected cells.

Table S4. Operon structure of the SpSL1 bacteriophage highlighting the CDSs and their predicted product expression time based upon RNA-Seq data.

CDS	Protein	Expression
<i>cds30</i>	hypothetical protein	Late
<i>cds31</i>	terminase, large subunit	Late
<i>cds32</i>	portal protein	Late
<i>cds33</i>	prohead maturation protease	Late
<i>cds34</i>	capsid protein	Late
<i>cds35</i>	hypothetical protein	Late
<i>cds36</i>	head-tail adapter protein	Late
<i>cds37</i>	hypothetical protein	Late
<i>cds38</i>	putative tail-component	Late
<i>cds39</i>	major tail protein	Late
<i>cds40</i>	hypothetical protein	Late
<i>cds41</i>	hypothetical protein	Late
<i>cds42</i>	tail length tape-measure protein	Late
<i>cds43</i>	tail protein	Late
<i>cds44</i>	tail fiber P1B-like protein	Late
<i>cds45</i>	hypothetical protein	Late
<i>cds46</i>	hypothetical protein	Late
<i>cds47</i>	holin	Late
<i>cds48</i>	holin 1	Late
<i>cds49</i>	N-acetylmuramoyl-L-alanine amidase	Late
<i>cds50</i>	hypothetical protein	hypothetical
<i>cds01</i>	integrase	Lysogeny
<i>cds02</i>	hypothetical protein	Lysogeny
<i>cds03</i>	hypothetical protein	Lysogeny
<i>cds04</i>	CI repressor	Lysogeny
<i>cds05</i>	hypothetical protein	Early
<i>cds06</i>	hypothetical protein	Early
<i>cds08</i>	prophage antirepressor protein	Early
<i>cds09</i>	hypothetical protein	Early
<i>cds10</i>	hypothetical protein	Early
<i>cds11</i>	DnaD	Early
<i>cds12</i>	hypothetical protein	Early
<i>cds13</i>	DnaC	Early
<i>cds14</i>	hypothetical protein	Early
<i>cds15</i>	C-5 cytosine-specific DNA methylase	Early
<i>cds16</i>	N-6 adenine-specific DNA methylase	Early
<i>cds17</i>	DNA-binding protein	Early
<i>cds18</i>	hypothetical protein	Early
<i>cds19</i>	hypothetical protein	Early
<i>cds20</i>	hypothetical protein	Early
<i>cds21</i>	hypothetical protein	Early
<i>cds22</i>	hypothetical protein	Early
<i>cds23</i>	hypothetical protein	Early
<i>cds24</i>	hypothetical protein	Early
<i>cds25</i>	Cro repressor	Early
<i>cds26</i>	hypothetical protein	Early
<i>cds27</i>	site-specific recombinase	Early
<i>cds28</i>	hypothetical protein	Very Late
<i>cds29</i>	endonuclease	Very Late

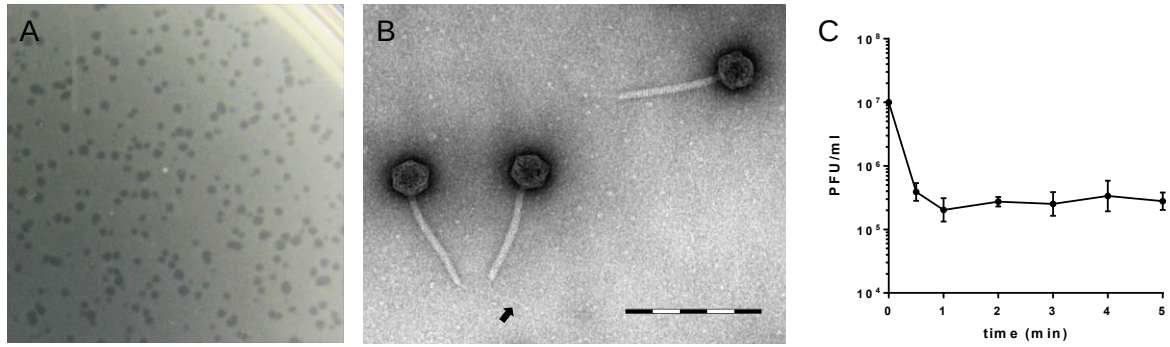


Figure S1. Plaques produced by SpSL1 on CAT-Galactose agar plates using a *spnDP1004III* deletion mutant as bacterial cell lawn (panel A). Electron micrographs of purified SpSL1 showing *Siphoviridae* structure; arrow indicates the tail fiber. Bar 200 nm (panel B). Adsorption of SpSL1 to whole *spnDP1004III* deleted mutant cells (panel C).

A

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SpSL1 attP      CTTTTTCATAATAATCTCCCT
csRNA3          24014 TTCTTTTTCATAATAATCTCCCTAT 24038
csRNA5          212373 AACTTTTTCATAATAATCTCCCTCA 212397
csRNA2          231376 TTCTTTTTCATAATAATCTCCCTTA 231400
csRNA4          233748 AACTTTTTCATAATAATCTCCCTAT 233772
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B

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D39      CTTTTTCATAATAATCTCCCTTaactccaccCAATCAGgtggagttTTTtagctctATTT 60
TIGR4    CTTTTTCATAATAATCTCCCTTaactccaccGAATCAGgtggagttTTTtagctctATTT 60
φSpn_H_1 CTTTTTCATAATAATCTCCCTTAACtCCACCCAATCAGGTGGAGTTTTTtagctctATTT 60
SpSL1    CTTTTTCATAATAATCTCCCTTAACtCCACCCAATCAGGTGGAGTTTTTGGCTCTATTT 60
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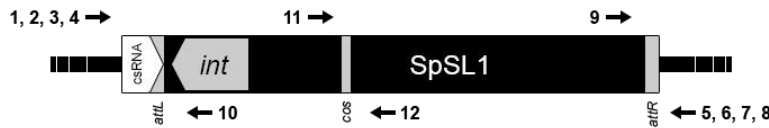
D39      CAGGCTTTTGGGGACTATTCTAAAAATAATTTTTCGATATTTTTCGGTATTTTTCGGATT 120
TIGR4    CAGGATTTTGGGGACTATTCTAAAAATAATTTTTCGATATTTTTCGGTATTTTTCGGATT 120
φSpn_H_1 CAGGCTTTTGGGGACTATTCTAAAAATAATTTTTCGATATTTTTCGGTATTTTTCGGATT 120
SpSL1    CAGGCTTTTGGGGACTATTCTAAAAATCATTTTTCGATATTTTTCGGTATTTTTCGGATT 120
*****

D39      TTGGTCGGGGAATTGGCGGGGACTTTT-----TTAGCGAATAT 158
TIGR4    TTGGTCGGGGAATTGGCGGGGACTTTTGTAGATTTTGGCGGGGATTTTtagcgaatat 180
φSpn_H_1 TTGGTCGGGGAATTGGTCGGGACTTTT-----TTAGCGAATAT 158
SpSL1    TTAGTCGGGGAATTGGCGGGGACTTTTGTAGATTTTGGCGGGGATTTTtagcgaatat 180
*****

D39      AACTAAGAAATAGGTCTGTGGTCGCTTCAGCTAGTTCAAACTCAATTGATTGTAA---- 214
TIGR4    GACTAAGAAATAGGTCTGTGGTCGCTTCAGCTAGTTCAAACTCAATTGATTGTAA---- 236
φSpn_H_1 GACTAAGAAATAGGTCTGTGGTCGCTTCAGCAAGTTCGTCCTCAACTTGGTTATAACGAT 218
SpSL1    GACTAAGAAATAGGTCTGTGGTCGCTTCAGCGAGTTCGTCCTCTACTTGATTATAACGAT 240
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C



D

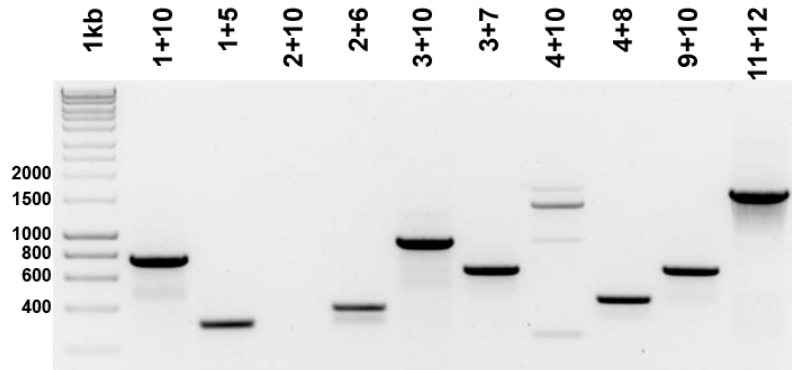


Figure S2. Attachment site analysis. Alignment of phage *att* sequence and complementary regions in the *S. pneumoniae* genome (panel A). The positions of the sequences belonging to the four csRNAs are reported based on D39 genome sequence (GenBank accession number NC_008533). Comparison between the *S. pneumoniae* genome sequence following csRNA2 coding region and the sequence downstream of the *attP* in bacteriophages (panel B). The *attL* sequence is highlighted in bold. The final portion of the csRNA2 coding region is underlined and nucleotides forming the stem of the transcriptional terminator hairpin are shown in lowercase. The 5' phage integrase coding region is italicized. D39 and TIGR4 are representative of phage-free *S. pneumoniae* genome sequences, while φSpn_H_1 is one of the two prophages of Hungary19A-6 strain. Panel C shows the schematic map of lysogenic SpSL1. Internal *cos* site (red), integrase gene (grey), duplicated attachment sites (cyan) and a generic csRNA (orange) are showed. Primers marked 1-8 in the picture correspond to LF90-97 (Table S1), which are designed to specifically amplify the *S. pneumoniae* genome region around csRNA2, 3, 4, and 5. Primers labelled 9-12 coincide with LF89, 88, 83, and 84 (Table S1) respectively. Panel D shows PCR amplification of the four predicted attachment sites of a SpSL1 lysogenic (MRO587 mutant), with and without the integrated phage (well 1-8). Control PCRs for the identification of excised phage

DNA and phage in the lysogenic form are in wells 9 and 10 respectively. Predicted band sizes from left to right are the following: 775, 319, 880, 408, 953, 692, 895, 469, 680, and 1638 base pairs. Note that the third (well 3) and fourth (well 7) samples are negative with the latter showing amplified fragments that are not of the expected size. DNA ladder sizes are reported in base pairs.

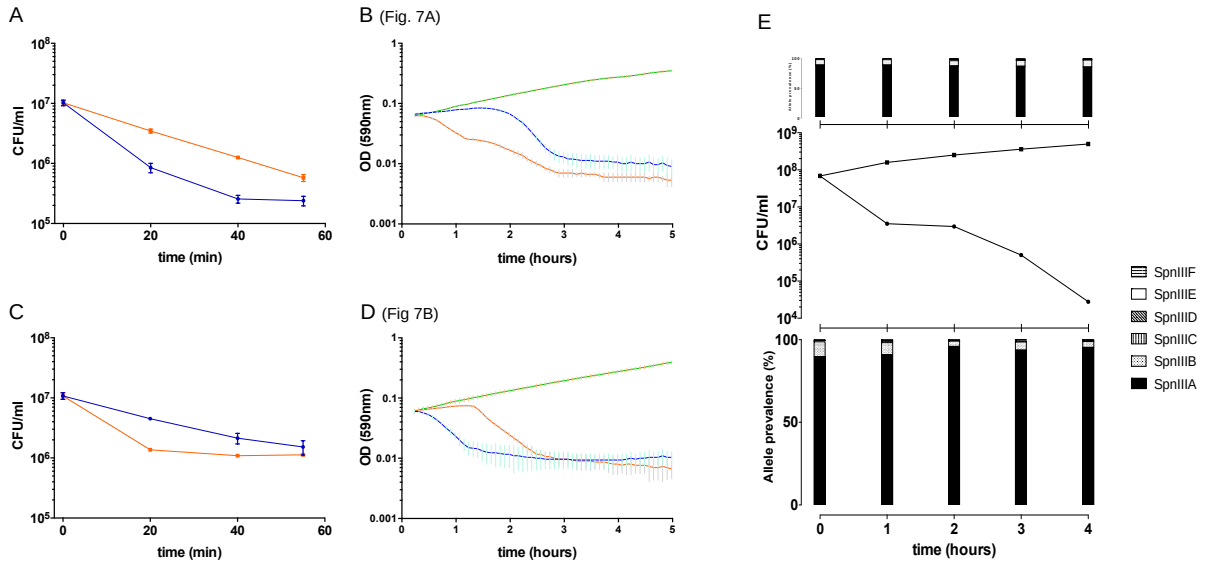


Figure S3: Abortive infection associated phenotypes. Data show the killing (left panels) in comparison to the culture turbidity (right panels; for comparative analysis panels of Fig.7 are reproduced here [Fig.S5B is Fig.7A and Fig.5D is Fig.7B]) of *spnDP1004IIIA* (panel A and B) and *spnDP1004IIIB* (panel C and D) locked mutants following infection with SpnIIIA-methylated (blue) and SpnIIIB-methylated (orange) SpSL1 phage (MOI=2). CFUs of survived bacteria were measured at 20, 40, and 55 minutes after the infection (panel A and C). OD₅₉₀ was read for 5 hours, with green line representing the non-infected controls (panel B and D). Panel E shows lack of any *spnIII* allele selection upon SpnIIIA-methylated phage infection (MOI=0.25). In the central panel CFU counts of the *wt* strain are shown (circles, phage infected strain; squares, negative control). Alleles in live bacteria were quantified for *s.spnDP1004III* variants after overnight incubation of an agar plate for each time point. SpnIII variants composition in the non-infected controls are shown above and of the infected cells below the growth curve. The patterns represent each allele conformation (*s.spnDP1004IIIA* black, *s.spnDP1004IIIB* dots, *s.spnDP1004IIIC* vertical stripes, *s.spnDP1004IIID* diagonal stripes, *s.spnDP1004IIIE* white, *s.spnDP1004IIIF* horizontal stripes).

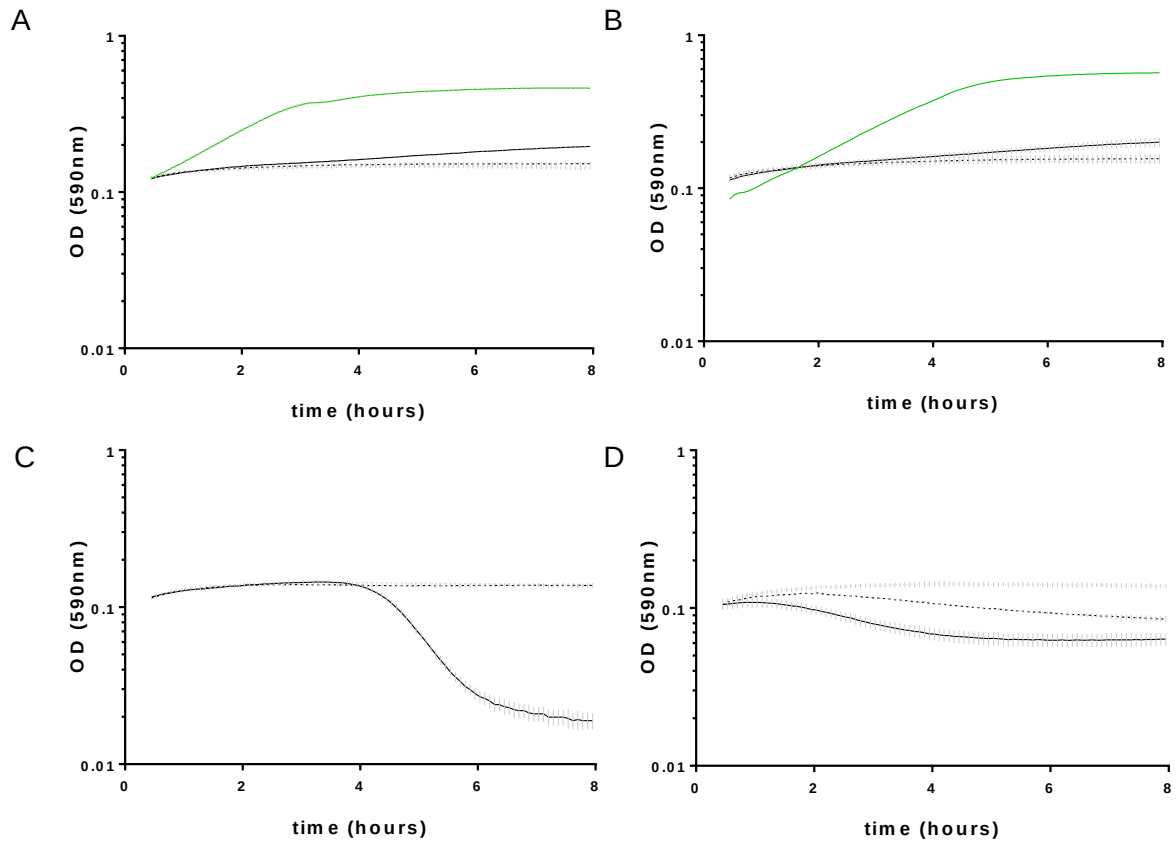


Figure S4: The Abi mechanism requires the *de novo* synthesis of cellular constituents to produce cell death. Control experiments, using *spnDP1004IIIA* (panel A) and *spnDP1004IIIB* (panel B) locked mutants, demonstrate the inhibition of cell replication in the presence of 4 mg/L (straight black line), 16 mg/L (dashed black line), and 64 mg/L (dotted black line) of chloramphenicol (Cm). The *spnDP1004IIIA* locked mutant was infected with SpnIIIA-methylated SpSL1 phage (MOI=2.5) in presence of similar Cm concentrations (panel C). Bacteriophage lytic cycle was delayed with 4 mg/L of Cm and was blocked at 16 mg/L (dashed line). When the *spnDP1004IIIB* locked mutant was infected with SpnIIIA-methylated SpSL1 phage (MOI=2.5) the Abi phenotype was slower with 4 and 16 mg/L Cm and arrested in the presence of 64 mg/L Cm.

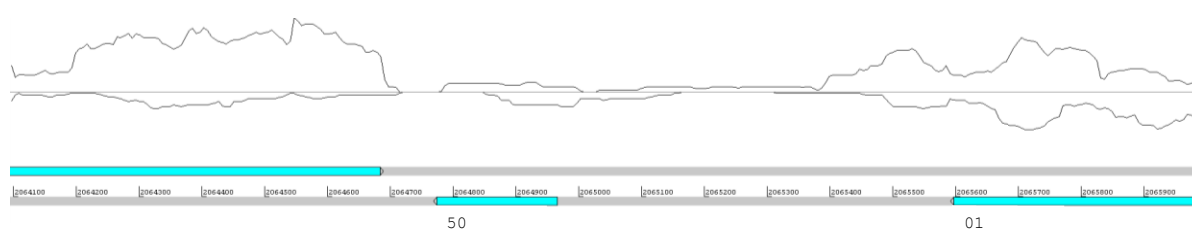


Figure S5. Zoomed in alignment of RNA-Seq of the lysogen at the hypothetical protein (*cds50*) in the centre, *cds49* (left) and *cds01* (right) highlighting the high degree of antisense RNA at *cds50* and throughout this region.