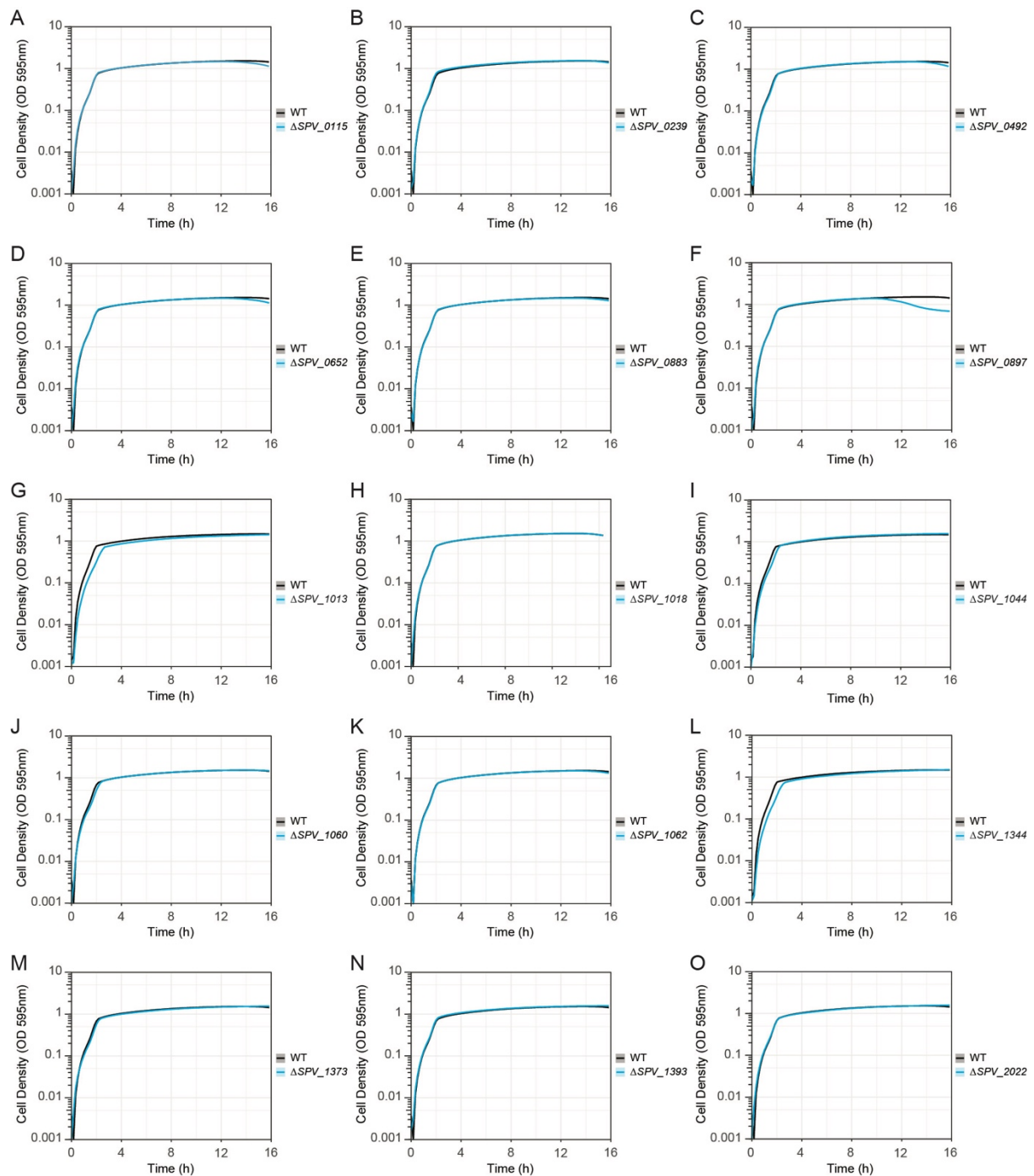
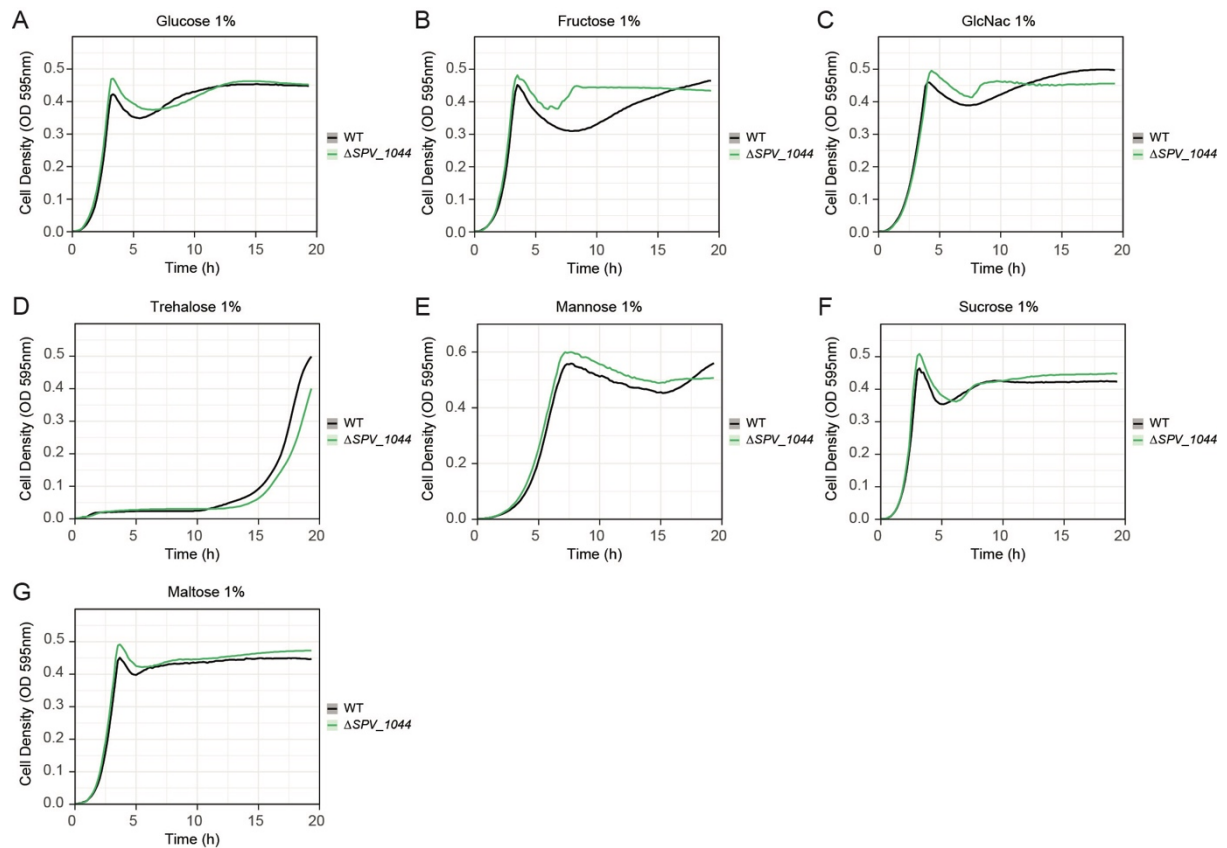


Supplementary information



Supplementary Figure S1. Growth curves of knockout mutant strains compared to the wild-type strain. Growth curves of *Streptococcus pneumoniae* D39V wild-type (WT) or knockout strains in C+Y medium. Strains were grown under approximate microaerophilic conditions in a microplate reader and the OD₅₉₅ was measured every 10 minutes for 16 hours. Compared to the wild-type strain, the knockout mutant strains ΔSPV_1013 and ΔSPV_1044 show delayed growth.



Supplementary Figure S2. Growth curves in various sugars. *Streptococcus pneumoniae* D39V wild-type (WT, black line) and *SPV_1044* knockout (ΔSPV_{1044} , green line) strains were grown in C+Y medium, in which sugars are omitted, supplemented with various sugars (1%). In general, the growth of the knockout strain is comparable to the wild-type strain, although slight differences are seen in the growth curves on fructose and trehalose.

Table S1. Pneumococcal genetic hits from genome-wide association study associated with mortality in patients with pneumococcal meningitis

SNP/k-mer	Signal	Name	Description	Genetic hits mapped to gene(s)	-log10(p value)	p value	Start	End	Remarks
KMER	multiple	SPV_1666	IS1167 transposase Orf1, degenerate	SPV_1666, SPV_2404	4.390192231	4.072E-05	1679099	1679881	No knockout mutant (transposase)
KMER	multiple	SPV_0048	IS1167 transposase Orf1, degenerate	SPV_0048, SPV_2085	4.352812702	0.00004438	40982	41500	No knockout mutant (transposase)
KMER	sparse	SPV_2271	IS1167 transposase Orf2, degenerate	SPV_2271	4.352812702	0.00004438	965072	965590	No knockout mutant (transposase)
KMER	sparse	SPV_1708	IS1167 transposase	SPV_1708	4.264081883	5.444E-05	1709063	1710292	No knockout mutant (transposase)
KMER	multiple	SPV_0115 (rtgB)	HlyD family secretion protein, (Putative) RtgA transporter accessory protein	SPV_0114, SPV_0115	3.808828544	0.0001553	116686	118083	Knockout mutant made (Δ SPV_0114, Δ SPV_0115)
KMER	multiple	SPV_1013	Phage integrase, site-specific tyrosine recombinase	SPV_1012, SPV_1013, SPV_2268	3.643591673	0.0002272	936481	937462	Knockout mutant made (Δ SPV_1013, Δ SPV_2268)
KMER	multiple	intergenic			3.592439151	0.0002556	1032459	1033027	No knockout mutant (intergenic region)
KMER	multiple	intergenic			3.52042469	0.0003017	313112	313621	No knockout mutant (intergenic region)
KMER	sparse	SPV_0507 (rpoD)	Putative integral membrane protein	SPV_0507	3.466863712	0.0003413	517676	518677	Out of top 30 hits
KMER	multiple	SPV_2022 (clpC)	ATP-dependent Clp protease ATP-binding subunit ClpC	SPV_2022, SPV_2023 (operon)	3.309626693	0.0004902	2001918	2003350	See Supplemental Table S2
KMER	multiple	SPV_0615 (glnH3)	Glutamine ABC transporter substrate-binding protein GlnH3, degenerate	SPV_0615, SPV_0616, SPV_0617, SPV_0618 (operon)	3.093341782	0.0008066	637369	637944	Out of top 30 hits
KMER	multiple	SPV_0242	Hypothetical protein	SPV_0241, SPV_0242	3.079511435	0.0008327	233564	234136	Out of top 30 hits
KMER	multiple	SPV_0454 (hsdM)	Type I restriction-modification system DNA-methyltransferase subunit M	SPV_0454, SPV_2170	3.0214543	0.0009518	462890	464353	Out of top 30 hits

Mapped with *Streptococcus pneumoniae* D39V as reference

Supplementary Table 1. Pneumococcal genetic hits from genome-wide association study associated with mortality in patients with pneumococcal meningitis

Table S2. Pneumococcal genetic hits from genome-wide association study associated with unfavourable outcome in patients with pneumococcal meningitis

SNP/k-mer	Signal	Gene	Description	Genetic hits mapped to gene(s)	-log10(p value)	p value	Start	End	Remarks
KMER	multiple	<i>SPV_1180</i>	Tn5253 putative CAAX amino terminal protease	<i>SPV_1180</i>	4.432855955	3.691E-05	1213141	1213728	No knockout mutant (transformation unsuccessful)
KMER	multiple	<i>SPV_1018 (zmpA)</i>	IgA1 protease ZmpA	<i>SPV_1018</i>	4.355955507	4.406E-05	921236	927127	Knockout mutant made (ΔSPV_1018)
KMER	multiple	<i>SPV_0600 (divIB)</i>	Cell division protein DivIB	<i>SPV_0600</i>	4.22366209	5.975E-05	625783	626973	No knockout mutant (essential gene)
KMER	sparse	<i>SPV_0761</i>	Hypothetical protein	<i>SPV_0761</i>	4.174703736	6.688E-05	772763	772957	No knockout mutant (essential gene)
KMER	multiple	<i>SPV_1250 (nadE)</i>	NAD synthetase	<i>SPV_1250</i>	4.170760572	6.749E-05	1279399	1280223	No knockout mutant (essential gene)
KMER	multiple	<i>SPV_2129-2132</i>	csRNA1, csRNA2, Type I addiction module toxin, Fst family	<i>SPV_0240, SPV_2129, SPV_2130</i>	4.010194349	9.768E-05	231544	232612	No knockout mutant (transformation unsuccessful)
KMER	multiple	<i>SPV_1373 (aspC)</i>	Aspartate aminotransferase	<i>SPV_1372, SPV_1373</i>	3.882397308	0.0001311	1385474	1386661	Knockout mutant made (ΔSPV_1372, ΔSPV_1373)
KMER	single	<i>SPV_1060 (lepA)</i>	Translation elongation factor LepA	<i>SPV_1060</i>	3.873543887	0.0001338	1085358	1087181	Knockout mutant made (ΔSPV_1060)
KMER	multiple	<i>SPV_0897 (mesH)</i>	GtrA family protein	<i>SPV_0896, SPV_0897, SPV_0898</i>	3.85823677	0.0001386	1052815	1053807	Knockout mutant made (ΔSPV_0896, ΔSPV_0897, ΔSPV_0898)
KMER	multiple	<i>SPV_2143</i>	IS630-Spn1 transposase, degenerate	<i>SPV_2143</i>	3.842543232	0.0001437	313112	313621	No knockout mutant (transposase)
KMER	multiple	<i>SPV_0883</i>	Hypothetical protein	<i>SPV_0883, SPV_0884, SPV_2263</i>	3.842543232	0.0001437	894813	895615	Knockout mutant made (ΔSPV_0883, ΔSPV_0884, ΔSPV_2263)
KMER	multiple	<i>SPV_0238 (leuS)</i>	Leucyl-tRNA-synthetase	<i>SPV_0238</i>	3.813608784	0.0001536	222726	230227	No knockout mutant (essential gene)
KMER	multiple	<i>SPV_2327</i>	Tn5253 hypothetical protein, degenerate	<i>SPV_2327</i>	3.812197361	0.0001541	1213821	1213964	No knockout mutant (transformation unsuccessful)
KMER	multiple	<i>SPV_0652 (livJ)</i>	High-affinity leucine-specific transport system, Periplasmic binding protein LivJ	<i>SPV_0652</i>	3.783570169	0.0001646	670443	671603	Knockout mutant made (ΔSPV_0652)
KMER	multiple	<i>SPV_0239</i>	Acetyltransferase, GNAT family	<i>SPV_0239</i>	3.739928612	0.000182	230421	231116	Knockout mutant made (ΔSPV_0239)
KMER	sparse	<i>SPV_1393 (trxB)</i>	Thioredoxin reductase	<i>SPV_1393</i>	3.732828272	0.000185	1414602	1415570	Knockout mutant made (ΔSPV_1393)
KMER	sparse	<i>SPV_1344</i>	Hypothetical protein	<i>SPV_1344</i>	3.710188161	0.0001949	1358327	1358734	Knockout mutant made (Δ SPV_1344)
KMER	multiple	<i>SPV_2022 (clpC)</i>	ATP-dependent Clp protease ATP-binding subunit ClpC	<i>SPV_2022</i>	3.7070797	0.0001963	2000918	2003350	Knockout mutant made (ΔSPV_2022)
KMER	multiple	<i>SPV_0492</i>	Acetyltransferase	<i>SPV_0491, SPV_0492</i>	3.648396928	0.0002247	498155	498712	Knockout mutant made (ΔSPV_0491, ΔSPV_0492)
KMER	multiple	<i>SPV_1664 (treP)</i>	Trehalose-specific PTS IIBCA components	<i>SPV_1664</i>	3.638461029	0.0002299	1676081	1678048	No knockout mutant (transformation unsuccessful)
KMER	multiple	<i>SPV_0063 (strH)</i>	Beta-N-acetyl-hexosaminidase	<i>SPV_0063</i>	3.62378815	0.0002378	60528	64466	No knockout mutant (transformation unsuccessful)
KMER	multiple	<i>SPV_1062 (recN)</i>	DNA repair protein RecN	<i>SPV_1062</i>	3.601365675	0.0002504	1087990	1089657	Knockout mutant made (ΔSPV_1062)
KMER	multiple	<i>SPV_1044 (lacR)</i>	Lactose phosphotransferase system repressor	<i>SPV_1043 (1 basepair), SPV_1044</i>	3.595337299	0.0002539	1073586	1074529	Knockout mutant made (ΔSPV_1044)

Supplementary Table 2. Pneumococcal genetic hits from genome-wide association study associated with unfavourable outcome in patients with pneumococcal meningitis

Table S3. Strains

Strain	Description	Reference
D39V	pathogenic, serotype 2	Slager et al. (13)
KR01	D39V Δ SPV_0115::ery ^r	This study
KR02	D39V Δ SPV_0239::ery ^r	This study
KR03	D39V Δ SPV_0492::ery ^r	This study
KR04	D39V Δ SPV_0652::ery ^r	This study
KR05	D39V Δ SPV_0883::ery ^r	This study
KR06	D39V Δ SPV_0897::ery ^r	This study
KR07	D39V Δ SPV_1013::ery ^r	This study
KR08	D39V Δ SPV_1018::ery ^r	This study
KR09	D39V Δ SPV_1044::ery ^r	This study
KR10	D39V Δ SPV_1060::ery ^r	This study
KR11	D39V Δ SPV_1062::ery ^r	This study
KR12	D39V Δ SPV_1344::ery ^r	This study
KR13	D39V Δ SPV_1373::ery ^r	This study
KR14	D39V Δ SPV_1393::ery ^r	This study
KR15	D39V Δ SPV_2022::ery ^r	This study
KR16	D39V Δ SPV_1344::ery ^r , ZIP::SPV_1344(spec) complemented	This study
KR17	D39V Δ SPV_1373::ery ^r , ZIP::SPV_1373(spec) complemented	This study
KR18	D39V, CEP::Plac-luc	This study
KR19	D39V Δ SPV_1044::ery ^r , CEP::Plac-luc	This study

Supplementary Table 3. Strains used in this study