

Supplementary Information

The Rgg1518 transcriptional regulator is a necessary facet of sugar metabolism and virulence in *Streptococcus pneumoniae*

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Running title: Rgg 1518 is required for pneumococcal virulence and survival

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STable 1. Microarray analysis of *Δrgg1518* in comparison with wild type D39 grown in CDM supplemented with galactose. The analysis used three independent cultures grown to mid-exponential phase (OD595 of 0.380).

Locus tag	Name	Fold ^a	Gene Name	Motif 1*
Downregulated in <i>Δrgg1518</i>				
SPD_0036		-2.19	hypothetical protein	
SPD_0058	purD	-2.48	phosphoribosylamine--glycine ligase	
SPD_0059	purE	-2.22	phosphoribosylaminoimidazole carboxylase, catalytic subunit	+
SPD_0105		-2.21	hypothetical protein	
SPD_0141		-2.19	hypothetical protein	
SPD_0143		-2.17	UDP-glucose 6-dehydrogenase, putative	
SPD_0163		-2	hypothetical protein	+
SPD_0167	ribB	-2.15	3,4-dihydroxy-2-butanone 4-phosphate synthase/GTP cyclohydrolase II	
SPD_0223		-2.12	iron(III) ABC transporter, permease protein	
SPD_0224		-2	iron(III) ABC transporter, permease protein	
SPD_0228		-2.25	transcriptional regulator, AraC family protein	+
SPD_0276		-2.6	hypothetical protein	
SPD_0293		-2.09	PTS system, IIA component	+
SPD_0364		-2.09	ABC-type polar amino acid transport system, ATPase component	
SPD_0372		-2.09	sodium:alanine symporter family protein	
SPD_0424		-2.04	PTS system, cellobiose-specific IIC component	+
SPD_0425		-2.41	hypothetical protein	
SPD_0426	lacF-1	-2.81	PTS system, lactose-specific IIA component	
SPD_0449		-2.03	hypothetical protein	
SPD_0470	blpC	-2.01	peptide pheromone BlpC	+
SPD_0496		-2.33	cell filamentation protein Fic-related protein	
SPD_0509		-2.07	hypothetical protein	
SPD_0520		-2.21	transposase, putative, truncation	
SPD_0527		-2.36	oxidoreductase, putative	
SPD_0531		-2		+
SPD_0573	msrAB2	-2	peptide methionine sulfoxide reductase msrA/msrB 2	
SPD_0592	rsuA-2	-2.33	ribosomal small subunit pseudouridine synthase A	
SPD_0603		-2.14	peptidase, M50 family protein	
SPD_0613		-2.28	hypothetical protein	
SPD_0616		-2.36	amino acid ABC transporter, ATP-binding protein	
SPD_0626		-2.17	ABC transporter, ATP-binding protein	+
SPD_0640		-2.33		
SPD_0647		-2.05	transcriptional regulator, TetR family protein	
SPD_0744		-2.26		

SPD_0772		-2.32	1-phosphofructokinase, putative	
SPD_0843	celA	-2.09	competence protein CelA	
SPD_0891		-2.15		+
SPD_0893		-2.14	hypothetical protein	
SPD_0903	xylH	-2.03	4-oxalocrotonate tautomerase	
SPD_0929		-2	hypothetical protein	
SPD_0938		-2.34	hypothetical protein	
SPD_0942		-2.27	hypothetical protein	
SPD_0945		-2.13	AMP-binding enzyme, putative	
SPD_0946		-2.06	hypothetical protein	
SPD_0951		-2.07	hypothetical protein	
SPD_1035		-2.1	hypothetical protein	
SPD_1182		-2.07	hypothetical protein	
SPD_1254		-2.38	hypothetical protein	
SPD_1257		-2.32	hypothetical protein	
SPD_1261		-2.18	hypothetical protein	
SPD_1266		-2.13	hypothetical protein	
SPD_1271		-2.13	hypothetical protein	
SPD_1322		-2.04	hypothetical protein	
SPD_1325		-2.57		
SPD_1354		-2.13	hypothetical protein	+
SPD_1424	truA	-2.29	tRNA pseudouridine synthase A	
SPD_1470		-2.01	hypothetical protein	
SPD_1471		-2.1	hypothetical protein	
SPD_1503		-2.25	hypothetical protein	
SPD_1518		-2.07	transcriptional activator, Rgg/GadR/MutR family protein	
SPD_1587		-2.13	transcriptional activator, putative	
SPD_1599	trpC	-2.05	indole-3-glycerol phosphate synthase	
SPD_1611		-2.17	hypothetical protein	
SPD_1614		-2.24	phosphate transport system regulatory protein PhoU, putative	
SPD_1677	rafE	-2.08	sugar ABC transporter, sugar-binding protein	
SPD_1711		-2.23	single-strand binding protein family protein	
SPD_1731		-2.01	hypothetical protein	
SPD_1733		-2.22	hypothetical protein	
SPD_1750	wrbA	-2.13	multimeric flavodoxin WrbA (general function prediction only)	
SPD_1789		-2.01	cell wall surface anchor family protein	
SPD_1796		-2.11	L-asparaginase, putative	
SPD_1798		-2	DNA-binding response regulator	
SPD_1855		-2.17	hypothetical protein	
SPD_1901		-2	transposase, putative	
SPD_1917		-2.12	hypothetical protein	
SPD_1974		-2.06	hypothetical protein	
SPD_1980		-2.34		

SPD_1985		-2.14	alcohol dehydrogenase, iron-containing	
SPD_1987		-2.24	fucolectin-related protein	
SPD_1989		-2.09	PTS system, IID component	
Upregulated in Δrgg1518				
SPD_0013	ftsH	2.82	cell division protein FtsH	
SPD_0017		4.48		+
SPD_0020		2.89		
SPD_0021		2.28		
SPD_0047		3.46	hypothetical protein	
SPD_0064		2.62	transcriptional regulator, GntR family protein	
SPD_0091		2.74	hypothetical protein	+
SPD_0100		2.38		
SPD_0113		5.88		+
SPD_0114		7.19	hypothetical protein	
SPD_0115		8.53	hypothetical protein	
SPD_0116		2.09	hypothetical protein	
SPD_0117		2.33	hypothetical protein	
SPD_0118		2.46	hypothetical protein	
SPD_0120		2.47	hypothetical protein	
SPD_0121		2.82	hypothetical protein	+
SPD_0123		3.6	hypothetical protein	
SPD_0124		2.41	hypothetical protein	
SPD_0126	pspA	2	pneumococcal surface protein A	+
SPD_0145		3.39	hypothetical protein	
SPD_0146		3.66	CAAX amino terminal protease family protein	
SPD_0147		2.74	CAAX amino terminal protease family protein	
SPD_0148		2.27	transporter, major facilitator family protein	
SPD_0150		2.57	ABC transporter, substrate-binding protein	
SPD_0179		2.35	lipoprotein, putative	+
SPD_0180		4.3	hypothetical protein	
SPD_0181		6.06	conserved hypothetical protein TIGR00250	
SPD_0182		2.18	hypothetical protein	
SPD_0192	rpsJ	4.51	ribosomal protein S10	+
SPD_0193	rplC	5.62	ribosomal protein L3	
SPD_0194	rplD	7.46	ribosomal protein L4	
SPD_0195	rplW	4.73	ribosomal protein L23	
SPD_0196	rplB	3.67	ribosomal protein L2	
SPD_0197	rpsS	6.72	ribosomal protein S19	
SPD_0198	rplV	8.56	ribosomal protein L22	
SPD_0199	rpsC	7.81	ribosomal protein S3	
SPD_0200	rplP	3.69	ribosomal protein L16	
SPD_0201	rpmC	7.17	ribosomal protein L29	
SPD_0202	rpsQ	8.11	ribosomal protein S17	
SPD_0203	rplN	6.94	ribosomal protein L14	+

SPD_0204	rplX	5.41	ribosomal protein L24	
SPD_0205	rplE	6.66	ribosomal protein L5	+
SPD_0206	rpsN	4.41	ribosomal protein S14	
SPD_0207	rpsH	5.83	ribosomal protein S8	
SPD_0208	rplF	4.77	ribosomal protein L6	
SPD_0209	rplR	3.12	ribosomal protein L18	
SPD_0210	rpsE	3.66	ribosomal protein S5	
SPD_0211	rpmD	4.34	ribosomal protein L30	+
SPD_0212	rplO	4.82	ribosomal protein L15	
SPD_0213	secY	2.66	preprotein translocase, SecY subunit	
SPD_0215	infA	4.94	translation initiation factor IF-1	
SPD_0216	rpsM	3.89	ribosomal protein S13	+
SPD_0217	rpsK	4.22	ribosomal protein S11	
SPD_0218	rpoA	3.43	DNA-directed RNA polymerase, alpha subunit	
SPD_0219	rplQ	8.71	ribosomal protein L17	+
SPD_0244	cdsA	2.08	phosphatidate cytidylyltransferase	
SPD_0251	rpsL	5.28	ribosomal protein S12	
SPD_0252	rpsG	4.74	ribosomal protein S7	+
SPD_0253	fusA	3	translation elongation factor G	
SPD_0256		2.18	conserved hypothetical protein TIGR00053	
SPD_0257		2.9	hypothetical protein	
SPD_0263	manM	2.59	PTS system, mannose-specific IIC component	
SPD_0264	manL	2.52	PTS system, mannose-specific IIAB components	+
SPD_0274	rplM	3.17	ribosomal protein L13	
SPD_0275	rpsI	3.25	ribosomal protein S9	
SPD_0314		2.01		
SPD_0315	cps2A	5.86	integral membrane regulatory protein Cps2A	+
SPD_0316	cps2B	4.55	tyrosine-protein phosphatase CpsB	
SPD_0317	cps2C	6.36	chain length determinant protein/polysaccharide export protein, MPA1 family protein	
SPD_0318	cps2D	4.11	tyrosine-protein kinase Cps2D cytosolic ATPase domain	
SPD_0319	cps2E	2.5	undecaprenylphosphate glucosephosphotransferase Cps2E	
SPD_0320	cps2T	2.56	glycosyl transferase, group 1 family protein, putative	
SPD_0326	cps2K	4.05	UDP-glucose 6-dehydrogenase, putative	
SPD_0327	cps2P	5.9	UDP-galactopyranose mutase	
SPD_0328	cps2L	5.44	glucose-1-phosphate thymidyltransferase	
SPD_0329	rfbC	3.9	dTDP-4-dehydrorhamnose 3,5-epimerase, putative	
SPD_0330	rfbB	2.96	dTDP-glucose 4,6-dehydratase	
SPD_0331	rfbD	3.45	dTDP-4-dehydrorhamnose reductase	
SPD_0332		2.28		
SPD_0333		2.4	hypothetical protein	
SPD_0334	aliA	4.26	oligopeptide ABC transporter, oligopeptide-binding protein AliA	+

SPD_0336	pbp1A	3.08	penicillin-binding protein 1A	
SPD_0337	recU	4.58	recombination protein U	
SPD_0342		2.02	hypothetical protein	
SPD_0343	gnd	2.51	6-phosphogluconate dehydrogenase, decarboxylating	
SPD_0344		2.85	DNA-binding response regulator	
SPD_0365	tig	4.18	trigger factor	
SPD_0378		3.32	enoyl-CoA hydratase/isomerase family protein	+
SPD_0379		4.45	transcriptional regulator, MarR family protein	
SPD_0380	fabH	7.19	3-oxoacyl-(acyl-carrier-protein) synthase III	
SPD_0381	acpP	2.66	acyl carrier protein	+
SPD_0382	fabK	4.54	trans-2-enoyl-ACP reductase II	
SPD_0383	fabD	4.67	malonyl CoA-acyl carrier protein transacylase	
SPD_0385	fabF	2.96	3-oxoacyl-[acyl-carrier-protein] synthase II	
SPD_0387	fabZ	3.61	beta-hydroxyacyl-(acyl-carrier-protein) dehydratase FabZ	
SPD_0388	accC	2.16	acetyl-CoA carboxylase, biotin carboxylase	
SPD_0389	accD	2.88	acetyl-CoA carboxylase, carboxyl transferase, beta subunit	+
SPD_0390	accA	2.72	acetyl-CoA carboxylase, carboxyl transferase, alpha subunit	
SPD_0394		2.56	hypothetical protein	
SPD_0404	ilvB	2.89	acetolactate synthase, large subunit, biosynthetic type	+
SPD_0406	ilvC	2.83	ketol-acid reductoisomerase	
SPD_0407		3.09	hypothetical protein	
SPD_0408		2.08	hypothetical protein	
SPD_0418		3.19	hypothetical protein	
SPD_0420	pflB	2.73	formate acetyltransferase	
SPD_0448	glnA	2.97	glutamine synthetase, type I	
SPD_0460	dnaK	2.09	chaperone protein DnaK	
SPD_0467	blpS	2.43	BlpS protein	
SPD_0550	rplK	5.61	ribosomal protein L11	+
SPD_0551	rplA	4.58	ribosomal protein L1	
SPD_0554		2.09	ABC transporter, ATP-binding protein	
SPD_0556		2.1	hypothetical protein	
SPD_0557		2.22		
SPD_0560		2.11	PTS system, IIB component, putative	
SPD_0561		2.68	PTS system, IIC component, putative	
SPD_0562	bgaA	2.13	beta-galactosidase precursor, putative	
SPD_0576		2	conserved hypothetical protein TIGR01440	
SPD_0577	zmpB	2.42	zinc metalloprotease ZmpB	+
SPD_0593		2.34	elongation factor Tu family protein	
SPD_0594		2.1	hypothetical protein	
SPD_0620	lysS	2.28	lysyl-tRNA synthetase	
SPD_0636	spxB	2.02	pyruvate oxidase	

SPD_0650	clpP	2.4	ATP-dependent Clp protease, proteolytic subunit ClpP	+
SPD_0652	livJ	2.06	branched-chain amino acid ABC transporter, amino acid-binding protein	
SPD_0667	sodA	3.05	superoxide dismutase, manganese-dependent	
SPD_0674	rpsP	2.89	ribosomal protein S16	
SPD_0690		2.61	hypothetical protein	
SPD_0705		2.16	DnaQ family exonuclease/DinG family helicase, putative	
SPD_0710	ezrA	2.48	septation ring formation regulator EzrA	+
SPD_0716		2.01	IS630-Spn1, transposase Orf1	
SPD_0721	folD	2.19	methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase	
SPD_0724	deoB	2.32	phosphopentomutase	
SPD_0726		2.72	purine nucleoside phosphorylase, family protein 2	+
SPD_0728		2.56	hypothetical protein	
SPD_0750		2.61	hypothetical protein	+
SPD_0751		2.64	hypothetical protein	+
SPD_0752		4.33	hypothetical protein	
SPD_0753	pcp	2.59	pyrrolidone-carboxylate peptidase	
SPD_0756		2.34		
SPD_0757	rpsA	3.4	ribosomal protein S1	
SPD_0760	dnaX	2.14	DNA polymerase III, gamma and tau subunits	
SPD_0762	sufC	2.4	FeS assembly ATPase SufC	
SPD_0763	sufD	3.61	FeS assembly protein SufD	
SPD_0764	sufS	5.72	cysteine desulfurases, SufS subfamily protein	
SPD_0765		4.49	SUF system FeS assembly protein, NifU family protein	
SPD_0766	sufB	3.41	FeS assembly protein SufB	
SPD_0784		2.48	type I restriction-modification system, R subunit, putative	
SPD_0785		2.27	hypothetical protein	
SPD_0790	pyk	2.45	pyruvate kinase	+
SPD_0835	frf	3.8	ribosome recycling factor	
SPD_0838		2.52	PhoH family protein	
SPD_0847	infC	2.24	translation initiation factor IF-3	
SPD_0848	rpmI	4.05	ribosomal protein L35	+
SPD_0900	asd	2.3	aspartate-semialdehyde dehydrogenase	
SPD_0905		2.4	acetyltransferase, GNAT family protein	
SPD_0913		2.66	hypothetical protein	+
SPD_0959		2.81	hypothetical protein	
SPD_0968		2.66	acetyltransferase, GNAT family protein	
SPD_0969		2.65	hypothetical protein	
SPD_0970	map	2.21	methionine aminopeptidase, type I	
SPD_0989	rplU	3.66	ribosomal protein L21	
SPD_0990		3.62	hypothetical protein	

SPD_0991	rpmA	3.83	ribosomal protein L27	
SPD_0996		2.04	DegV family protein	+
SPD_1014		2.27	IS630-Spn1, transposase Orf1	
SPD_1037		2.62	histidine triad protein	
SPD_1039	ptsI	2	phosphoenolpyruvate-protein phosphotransferase	
SPD_1042	nrdE	3.71	ribonucleoside-diphosphate reductase, alpha subunit	
SPD_1049	lacT	2.13	transcription antiterminator LacT	+
SPD_1050	lacD	4.14	tagatose 1,6-diphosphate aldolase	
SPD_1051	lacC	3.57	tagatose-6-phosphate kinase	
SPD_1052	lacB	3.46	galactose-6-phosphate isomerase, LacB subunit	
SPD_1053	lacA	4.73	galactose-6-phosphate isomerase, LacA subunit	+
SPD_1060	lepA	2.32	GTP-binding protein LepA	
SPD_1061		2.7	serine/threonine protein phosphatase	
SPD_1064		2.31	hemolysin A, putative	
SPD_1078	ldh	2.2	L-lactate dehydrogenase	
SPD_1083	vicX	3.29	vicX protein	
SPD_1084		4.52	sensory box sensor histidine kinase	
SPD_1085		2.11	DNA-binding response regulator	
SPD_1099		2.46	amino acid ABC transporter, ATP-binding protein	
SPD_1100	zwf	2.33	glucose-6-phosphate 1-dehydrogenase	
SPD_1106		2.5		
SPD_1125	pck	2.78	choline kinase	
SPD_1127	ispD	3.22	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	+
SPD_1158	gdhA	2.03	NADP-specific glutamate dehydrogenase	+
SPD_1175		2.07	hypothetical protein	
SPD_1188	rplJ	3.23	ribosomal protein L10	
SPD_1193	msrAB1	2.37	peptide methionine sulfoxide reductase msrA/msrB 1	+
SPD_1194	thrB	2.15	homoserine kinase	
SPD_1195	hom	3.21	homoserine dehydrogenase	
SPD_1206		2.31	hypothetical protein	+
SPD_1207	tyrA	3.1	prephenate dehydrogenase	
SPD_1228		2.33	phosphate ABC transporter, ATP-binding protein, putative	
SPD_1245	rpsU	2.31	ribosomal protein S21	
SPD_1246	nagB	2.89	glucosamine-6-phosphate isomerase	
SPD_1268		3.5	hypothetical protein	
SPD_1269		2	hypothetical protein	+
SPD_1290		2.56	amino acid ABC transporter, permease protein	
SPD_1298	nox	2.24	NADH oxidase	
SPD_1309	pgdA	2.26	peptidoglycan GlcNAc deacetylase	
SPD_1310		2.25	hypothetical protein	+
SPD_1318	tuf	2.01	translation elongation factor Tu	
SPD_1319		2.37	hypothetical protein	

SPD_1326	pgm	2.27	phosphoglucomutase/phosphomannomutase family protein	+
SPD_1338	atpH	4.53	ATP synthase F1, delta subunit	
SPD_1339	atpF	4.65	ATP synthase F0, B subunit	
SPD_1340	atpB	2.01	ATP synthase F0, A subunit	
SPD_1344		2.88	hypothetical protein	+
SPD_1346		2.63	Uncharacterized BCR, putative	
SPD_1349	murC	2.04	UDP-N-acetylmuramate--alanine ligase	
SPD_1365		2.08	hypothetical protein	
SPD_1368	rpsR	3.31	ribosomal protein S18	+
SPD_1369	ssb	2.9	single-strand binding protein	
SPD_1370	rpsF	5.23	ribosomal protein S6	
SPD_1400		2.26	hypothetical protein	
SPD_1403	lytC	2.07	1,4-beta-N-acetylmuramidase, putative	
SPD_1404	tpiA	3.33	triosephosphate isomerase	
SPD_1410		3.18		
SPD_1439	rpsO	2.35	ribosomal protein S15	+
SPD_1461	psaB	3.72	manganese ABC transporter, ATP-binding protein	
SPD_1462	psaC	2.56	manganese ABC transporter, permease protein, putative	
SPD_1463		5.74	ABC transporter, substrate binding lipoprotein	
SPD_1473		2.85	hypothetical protein	
SPD_1474	divIVA	5.48	cell division protein DivIVA	
SPD_1475	ylmH	2.85	YlmH protein	
SPD_1477	ylmF	2.63	YlmF protein	
SPD_1479	ftsZ	3.25	cell division protein FtsZ	
SPD_1480	ftsA	2.63	cell division protein FtsA	
SPD_1512	secA	2.64	preprotein translocase, SecA subunit	
SPD_1513		4.09		
SPD_1514		9.48	ABC transporter, ATP-binding protein	
SPD_1515		8.42	hypothetical protein	
SPD_1516		9.86	hypothetical protein	
SPD_1517		4.67	hypothetical protein	+
SPD_1520		2.54	nitroreductase family protein	+
SPD_1522		2.11	hypothetical protein	
SPD_1524		5.06	transcriptional regulator, GntR family protein	+
SPD_1525		6.46	ABC transporter, ATP-binding protein	
SPD_1526		2.87	hypothetical protein	+
SPD_1542		2.23	serine/threonine protein kinase	
SPD_1554		2.3	iojap-related protein	
SPD_1566		3.13	hypothetical protein	
SPD_1581		2.88		
SPD_1588		2.68	hypothetical protein	+
SPD_1626	xth	2.36	exodeoxyribonuclease III	
SPD_1627		2.81		

SPD_1633	galT-2	2.03	galactose-1-phosphate uridylyltransferase	+
SPD_1642	proWX	2.08	choline transporter (glycine betaine transport system permease protein)	
SPD_1644		2.13	hypothetical protein	
SPD_1655	scpB	2.47	segregation and condensation protein B	
SPD_1667	amiF	2.43	oligopeptide ABC transporter, ATP-binding protein AmiF	
SPD_1668	amiE	3.23	oligopeptide ABC transporter, ATP-binding protein AmiE	
SPD_1669	amiD	5.09	oligopeptide ABC transporter, permease protein AmiD	
SPD_1670	amiC	2.38	oligopeptide ABC transporter, permease protein AmiC	
SPD_1671	amiA	7.63	oligopeptide ABC transporter, oligopeptide-binding protein AmiA	
SPD_1683		2.07		
SPD_1686		5.53		
SPD_1687		2.02		
SPD_1688		3.75		
SPD_1689		4.07		
SPD_1691		3.14		
SPD_1692		2.24		
SPD_1694		3.28		
SPD_1696		2.07		
SPD_1698		3.23		
SPD_1707		3.63	hypothetical protein	
SPD_1726	ply	2.57	pneumolysin	
SPD_1727		4.81	hypothetical protein	+
SPD_1728		2.13	hypothetical protein	
SPD_1730		2.1		
SPD_1737	lytA	2.01	autolysin/N-acetylmuramoyl-L-alanine amidase	
SPD_1739	recA	2.44	recA protein	
SPD_1795		2.4	hypothetical protein	
SPD_1870		2.06		
SPD_1879		2.46		
SPD_1898		2.69	hypothetical protein	
SPD_1939		2.07	hypothetical protein	
SPD_1942		2.36	hypothetical protein	
SPD_1949		2.6	hypothetical protein	
SPD_1955		2.08	hypothetical protein	
SPD_1963	rpmF	4.87	ribosomal protein L32	+
SPD_1983		3.41	hypothetical protein	
SPD_2011	glpF	2.2	glycerol uptake facilitator protein	
SPD_2012	glpO	4.02	alpha-glycerophosphate oxidase	
SPD_2013	glpK	4.35	glycerol kinase	
SPD_2016		2.1	TIM-barrel protein, nifR3 family protein, putative	
SPD_2020		2.27	DNA-binding response regulator	

SPD_2032		2.73	DHH subfamily 1 protein	
SPD_2041	tsf	2.99	translation elongation factor Ts	
SPD_2042	rpsB	4.98	ribosomal protein S2	+
SPD_2066		2.6		
SPD_2068		5.84	serine protease	
SPD_2069		2.55	SpoJ protein	
	engB	2.58		
	glmM	2.4		
	ppnK	2.03		
	tpx	2.17		

^aFOLD ≥ 2 or ≤ -2.0 (Δ *rgg1518* compared with D39 wild type). All P-values are <0.001 . *indicates

the list of genes containing motif 1 identified in the regulatory region of SPD_0315.

STable 2 .Microarray analysis of *Δ**rgg1518* in comparison with wild type D39 grown in CDM supplemented with mannose. The analysis used three independent cultures grown to mid-exponential phase (OD595 of 0.380).

Locus	Name	FOLD ^a	Function	Motif 1*
Downregulated in <i>Δ</i><i>rgg1518</i>				
SPD_0316	cps2B	-2.09	tyrosine-protein phosphatase CpsB	
SPD_1149	crcB1	-1.98	CrcB protein	
SPD_0395	efp	-2.07	translation elongation factor P	
	engB	-2.18		
SPD_0343	gnd	-2	6-phosphogluconate dehydrogenase, decarboxylating	
	groEL	-2.2		
SPD_0458	hrcA	-2.01	heat-inducible transcription repressor HrcA	
SPD_1053	lacA	-2.72	galactose-6-phosphate isomerase, LacA subunit	+
SPD_1052	lacB	-4.21	galactose-6-phosphate isomerase, LacB subunit	
SPD_1050	lacD	-3.45	tagatose 1,6-diphosphate aldolase	
SPD_1246	nagB	-2.19	glucosamine-6-phosphate isomerase	
SPD_0542	pepV	-2.06	dipeptidase PepV	
SPD_0420	pflB	-3.23	formate acetyltransferase	
SPD_1461	psaB	-2.27	manganese ABC transporter, ATP-binding protein	
SPD_0550	rplK	-2.2	ribosomal protein L11	+
SPD_0989	rplU	-3.16	ribosomal protein L21	
SPD_1245	rpsU	-1.98	ribosomal protein S21	
SPD_0021		-1.92		
SPD_0104		-2.62	LysM domain protein	

SPD_0114		-2.11	hypothetical protein	
SPD_0116		-2.64	hypothetical protein	
SPD_0146		-2.02	CAAX amino terminal protease family protein	
SPD_0182		-1.9	hypothetical protein	+
SPD_0418		-2.02	hypothetical protein	
SPD_0557		-1.95		
SPD_0765		-2.3	SUF system FeS assembly protein, NifU family protein	
SPD_0913		-2.34	hypothetical protein	+
SPD_0990		-2.24	hypothetical protein	
SPD_1290		-2.31	amino acid ABC transporter, permease protein	
SPD_1291		-2.69	ArsC family protein	
SPD_1293		-2.01	acetyltransferase, GNAT family protein	
SPD_1402		-2.22	non-heme iron-containing ferritin	
SPD_1473		-1.91	hypothetical protein	
SPD_1513		-3.58		
SPD_1514		-6.35	ABC transporter, ATP-binding protein	
SPD_1515		-2.93	hypothetical protein	
SPD_1516		-5.31	hypothetical protein	
SPD_1517		-11.04	hypothetical protein	+
SPD_1566		-1.97	hypothetical protein	
SPD_1834		-2.3	alcohol dehydrogenase, iron-containing	
SPD_1962		-2.3	hypothetical protein	
SPD_0764	sufS	-2.14	cysteine desulfurases, SufS subfamily protein	
SPD_1567	trx	-2.86	thioredoxin	
SPD_2041	tsf	-1.97	translation elongation factor Ts	
Upregulated in Δrgg1518				
SPD_0373		1.98	hypothetical protein	
SPD_0489		1.92	hypothetical protein	
SPD_0519		2.06	hypothetical protein	
SPD_0606		1.95	hypothetical protein	
SPD_1170		-1.5	oligopeptide ABC transporter, oligopeptide-binding protein	
SPD_1783		1.92	hypothetical protein	
SPD_2054	recF	1.9	recF protein	

^aFOLD ≥ 2 or ≤ -2.0 (Δ rgg1518 compared with D39 wild type). All P-values are <0.001 . *indicates

the list of genes containing motif 1 identified in the regulatory region of SPD_0315.

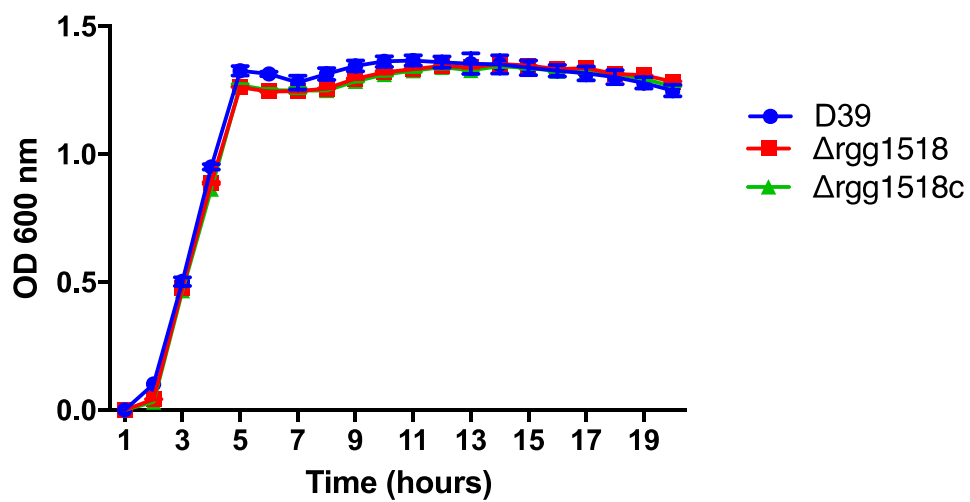
STable 3. The microbial strains and plasmids used in this study.

Strains	Purpose of using	Source
<i>S. pneumoniae</i>		
D39	Serotype 2 strain	Laboratory stock
Δ rgg1518	D39, SPD1518, Spec resistant	This study
Empty pPP2-lacZ-wt	D39, pPP2-lacZ, Tetra resistant	This study
Prgg1518-lacZ-wt	D39, Prgg1518-lacZ, Tetra resistant	This study
Prgg1518-lacZ- Δ rgg0999	Δ rgg0999, Prgg0999-lacZ, Tetra resistant	This study
Prgg1518-lacZ- Δ rgg1518	Δ rgg1518, Prgg1518-lacZ, Tetra resistant	This study
Prgg1518-lacZ- Δ rgg0144	Δ rgg0144, Prgg1518-lacZ, Tetra resistant	This study
Prgg1518-lacZ- Δ rgg0939	Δ rgg0939, Prgg1518-lacZ, Tetra resistant	This study
Pcps-lacZ-wt	D39, Pcps-lacZ, Tetra resistant	This study
Pcps-lacZ- Δ rgg1518	Δ rgg1518, Pcps-lacZ, Tetra resistant	This study
<i>Escherichia coli</i>		
One Shot® TOP10	Plasmid propagation	Invitrogen, UK
One Shot® TOP10-rgg1518	rgg1518-pcep Plasmid, Kan resistant	This study
One Shot® TOP10-Prgg1518	Prgg1518-pPP2 plasmid, Amp resistant	This study
<i>E. coli</i> DH5 α - rgg1518	Cloning rgg1518	PROTEX, University of Leicester
BL21 (DE3) pLysS	Protein expression	Agilent Tech, USA
BL21 (DE3) pLysS-rgg1518	pLEICS-01-rgg1518, Amp resistant	This study
Plasmids		
pCEP	Genetic complementation, Kan resistant	Guiral <i>et al.</i> , 2006
pPP2	lacZ transcriptional report, Amp & Tetra resistant	Halfman <i>et al.</i> , 2007
pLEICS-01	protein expression, Amp resistant	PROTEX, UK

STable 4: The list of primers and peptides used in this study

Primers ID	Mutagenesis primers
Spectinomycin/F	ATCGATTTTCGTTTCGTGAAT
Spectinomycin/R	GTTATGCAAGGGTTTATTGT
SPD01518UP/F	CACAGGTTGTTAACGATTCTCC
SPD1518UP/R	ATTCACGAACGAAAATCGATCATAATCCCATCTCATATTGTT
SPD1518DOWN/F	ACAATAAACCCCTTGCATAACTTTTTCAAACCTATAATTTGTA
SPD1518DOWN/R	GAAAACAATTTAAAAGAGGAGG
Sequencing primers	
SPD1518-seqM/F1	CAAAGTAAGCAAGACATAAA
SPD1518-seqM/F2	ATCCCCAAAACCATGTTTCA
SPD1518-seqM/R1	GGAGCTATAGATACAGTCA
SPD1518-seqM/R2	TTGGTATGAATTGGTAACT
Genetic Complementation primers	
SPD1518F <i>Nco</i> IC	CATGCCATGGTTTTCTGTTTCCTTTTCATT
SPD1518R <i>Bam</i> HI	ACGGATCCTTATAGGTTTGAAAATCAT
MalF	GCTTGAAAAGGAGTATACTT
PCEPR	AGGAGACATTCCTTCCGTATC
EMSA primers	
<i>Pcps</i> EMSA FAMF	ACACATCTGCTTCTAAAATA
<i>Pcps</i> EMSAR	GATTAACACCTATACATTGA
<i>gyrB</i> EMSAFAMF	ATGACAGAAGAAATCAAAAATCTGC
<i>gyrB</i> EMSAR	CCTGGACGCATACGAACAG
<i>Pcps</i> motif1UPR	TTTCACTATCTGCATCTTTA
<i>Pcps</i> motif 1downF	TAGACATTACCGTAAAAAAG
<i>Pcps</i> motif 1downR	TTCGCTTCACTTTCTGTGAA
<i>Pcps</i> motif 2UPR	TAATGTCTACACCTTTTTTC
<i>Pcps</i> motif 2downF	AGTGATATAATCGTAAGATG
<i>Pcps</i> motif 2downR	ACTATATTAACACTTCGCTT
LacZ reporter primers	
Fusion/UF	CTACTTGGAGCCACTATCGA
Fusion/DR	AGGCGATTAAGTTGGGTAAC
Fusion0999F <i>Sph</i> I	GACGCATGCAGCTTTCTAGCTTGGTCAG
Fusion0999R <i>Bam</i> HI	ACGGGATCCC GTTCAATTTTATTAGCTTG
Fusion1518F <i>Sph</i> I	GACGCATGCAAGAAGATTCCTTATAACT
Fusion1518R <i>Bam</i> HI	ACGGGATCCTAAAATTTGTCCATAATCCC
Fusion <i>cps</i> F <i>Sph</i> I	GACGCATGCGATTATACCACATTGTGTAC
Fusion <i>cps</i> R <i>Bam</i> HI	ACGGGATCCAACGTGATTTTTTAAAACGTC
Cloning primers	
T7 Promoter-F	TAATACGACTCACTATAGGG
pLIECS-01-Seq-R	ATTAACATTAGTGGTGGTGGT
ProteinSPD1518F	TACTTCCAATCCATGAGATGGGATTATGGACAAAT
ProteinSPD1518R	TATCCACCTTTACTGTCATAGGTTTGAAAATCATTAA
Peptides	
SHP1518-C12	IQLIWFETWFWG
SHP1518-C16	IWSWIQLIWFETWFWG

SFigure 1



SFigure 1: The growth of pneumococcal strains in BHI under the microaerobic condition at 37°C. There was no difference in growth profiles of wild type D39, Rgg1518 mutant (Δ rgg1518) and the genetically complemented mutant (Δ rgg1518c). The experiment was repeated using 3 replicates of 6 independent biological samples. Error bar indicates standard error of mean.

SFigure 2

P(SPD_0315-SPD_0328)

F →
ACACATCTGCTTCTAAAAATTTGTTAGAAAACGATTGACTGTCCTGATCAATTTGTCATGTTCTTATTTCACTATATTTTTG
GTTCCGCGGAAGTCTACTAAGATACCTTAAAGATGCAGATAGTGA AAAAAGGTG TAGACAATTACCGTAAAAAAGTGATATAATCGT
AAGATGTTCAATGTATAGGTGTTAATC ←
-35 -10
R ←

P(SPD_0378) -35 -10
TTTTTCTTGCTTTCTGATCCTAAAAAGATATACTTTGACAGTGAAATAATTTAACACTCAATAAAAAATTAAGAGCAAACTAAG
AAGCTAGACGAAGTCAGCTCAAAATACGTITTTGAGGTTGCAGATGGAAGCTGACGCGGTTTGAAGAGATTTTCGAAGAGTATAA
ACTGCTTATAAAAAATAAAAGGAGCCCTG

P(SPD_0126) -35 -10
TCGAAAACTTGGAGGGATAAGAAATGAAAGTAAGACCACGGTCAAAACCAATTTGCGAATACTGTAAAGTTATTCGTCGTAATG
GTCGTGTTATGGTAATTTGCCAGCAATCCAAAACACAAACAACGTC AAGGATAAGATAG AAAGGAGAAAAAC

P(SPD_0113)
AAAAATATTGTATCATAGCAATGTAAAAAGTAAAAAGAAAGTCGAAATAAATTTGTGAAAAAGTTAACGTAATTTTAAAAATG
AGCTTAATAAGAGGTACTATGTAGGTAGAAAAATAAGATAGGAGAAAAAGTATGAAATAAAAAATAATTTGTATTCTAGTATGTA
TTATAATTTGATTTAGTCTGGCTATAATCTTTCCATGGGGCTGGCCGATATAATGTAAGGGAGTTTGTAGTTGTTAAAGGAGGCTCAG
TCCTT -35 -10

P(SPD_1127)
CTCTAAAAATTCGACGCCATTTATTAGTAATGCTACAGAATTCCTAGTCAITACTAGAAAATGGACTAGTTCTTTTGAATAATAGA
ACTGCATAAATTCCTCTATTCTAGAAAGGGAGGACCAGTATTTCTTTATGATAGGACTAGATTGTTGGTATAAATAGAGAGAATAAGT
TTTTTTAGTAAGACAAGGAGAAAAATG

P(SPD_1634) -35 -35 -10 -10
AATCCTCCTTGATTAGGTAGTATATCATATTTTTCGCTTTTTACTGATAGTTTAGTAAAAATTTTGTAAAAAGGATTGACCTTGG
GAAATCCCTTGGATACAATAGAAAGAAAACGATTACACGTTAAGGTGGCTTAACGGACAGTC AAAGGAGAAAAATCAT

P(SPD_1053) -35 -10
AAAAAGTAAAAATAAATAACAAAAACAAACACAACTGTTGACTTCTTCAAACAATAGTAGTATAATTATGTTTGTGAGGACTAA
TTGACTGTTTTTAAAGACAGCCAATACACTGCTGGAATCCAGCATAGAAAAATAAAAGGAGTATACAAT

P(SPD_0208)
AATCAAGATACAAAGCTCGTAAAGAACAAAGCAAAATTAGGAAGTTGGAGAAGTTGTTTACAAACAAGCCAACCTTATCTATTTT
GCACAGTCTTAGATCGTGTTCAGTTCAGCTCTTGAACAAAATAGTATCTGAAACCCGTGAAAACCTGGCCGTTCTGGCCTGACAA
TTTAAACAGGAGAAAAATAAAC -35 -10

P(SPD_1461)
TTTGTATTGTCATTATCTTAAACAAAAATCACTAGGGAATGCAATTAATAACCATTTAACTATTATTTTTAGCTATTATAAAAAAT
CTTTCCATGATAAAACGCATAAATCCCAAGTTTTTACACTTGATACTATGCGTTTTATAATTTAGAAAATTTATCCGGAAGTTATCT
TTAGAACTCTATTTTTCAGTTATTTTAAATTTTTTCAA AAAATTAACCTGACTTAATTTTTTTTTTAAATGTAATTAAGAGACAGGAG
GAATACAAGT -35 -10

P(SPD_0404) -10 -35
GATTAATGAAGGACTGAGAAATCAGTTCITTTTTCTTTTTAGTAAATGAAATCGGTATCTTTTTAATAAAAAACAAAAATAACATT
CATAAATAAAAGTTAAATAGAAAAATTCAGAAAATTTCTCTTTTATCTTGAAAAATTTTGAAAAAATGGTATGATAGTAACAAGT
TATTTTTAAGAGGAAAAGAAAGGGGAATA

P(SPD_1512) -10 -35
AGTATCAATAAAGTCAGAGAGGTTAGCTTGGAACTAACCTCTTTTTCTTTTTCAAATGGGGATTCTTCTTGAAAATAATCAGT
AATTGTCATAAAATTAAGGAACATTTCAAATAATCCGGAATTTAAAGTAAGGAAAAAC

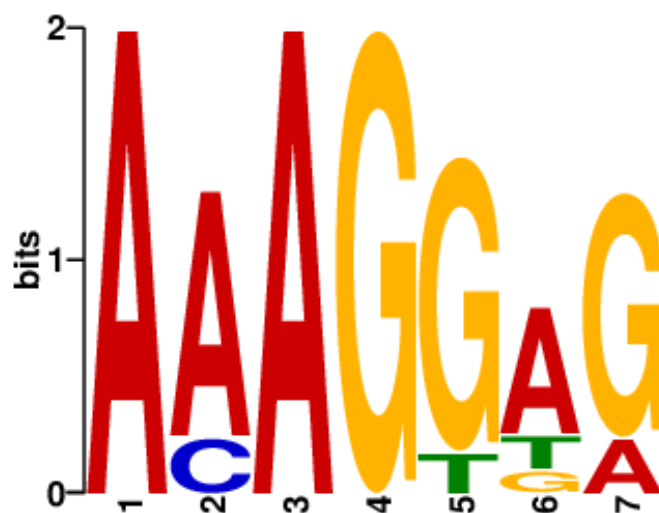
P(SPD_0193) -35 -10
TCTAAAAATAAAAGAGCAGAGGCTGGTGTTCAACTCTAATTGAACACGGGCTAAACTCGGTGTGAAAAAGATAAACTTCTAGT
GTCTGTAGACACTGCGTCAGTTTCTATTTTCACGTTGAGTTTGACGCCCTTTGTATCTTAGACTTGAGCATAAAAACGCTCGTT
AAAAACTTTTTGAATAAAAAATATAGAAAAGGAACATTTTTCTC

P(SPD_0122) -35 -10
CTAGATATGGGTATTA AAAATGGAAAAAATTTTTGTTATTTTTTTTGTATGTCTATTTATATCATCCATAACTTTTTTAGCCCTATG
ATTTTTGTAGCGAAGAAATAAAAAGTTTGATTATTGGATGAATGCGTTTTTTTTGATTTTAAATCATAGCAAATGATAATTTATCCAA
AATTAAGAAAAATGATAAGGACACTGTAAAAAT

P(SPD_1342) -35 -10
GATAAATACCAGTTATAACCTAGAAAATTGTGCTTTCTAGACGATATTGAGGACAATATAATCGTAGCCCAAAATTAGGAATCA
AGACTTATCAGGTTAAGAAAAGAGTGATGTTGTTGATAATTTAAACAATTTATTTAAACTAAAAACTCTCTATCTATTAATCCGA
GATTCCTCCTCGGATTTTGTGTGCTTTCATTAATTTTACAGGTAAGACTTGC

Motif 2

SPD_0315	GATAGTGAAA	AAAGGTG	TAGACATTAC
SPD_0378	TATAAAATAA	AAAGGAG	CCCTG
SPD_0126	GATAAGATAG	AAAGGAG	AAAAC
SPD_0113	TTGAGTTGTT	AAAGGAG	GCTCAGTCCT
SPD_1127	TTAGTAAGAC	AAAGGAG	AAAATAG
SPD_1634	ACGGACAGTC	AAAGGAG	AAATCAT
SPD_1053	GAAAAAATAA	AAAGGAG	TATACAAT
SPD_0208	TGACAATTTA	ACAGGAG	AAAATAAAC
SPD_1461	ATATTAAGAG	ACAGGAG	GAATACAAGT
SPD_0404	AAGAGGAAAG	AAAGGGG	AATA
SPD_1512	TGCTAAAATT	AAAGGAA	CATTCTAAAA
SPD_0193	AAAATATAGA	AAAGGAA	CTATTTTCTC
SPD_0122	AAGAAATAAA	AAAGTTG	ATTATTTGGA
SPD_1342	CATTAAATTT	ACAGGTA	AGACTTGCTA



Motif 1

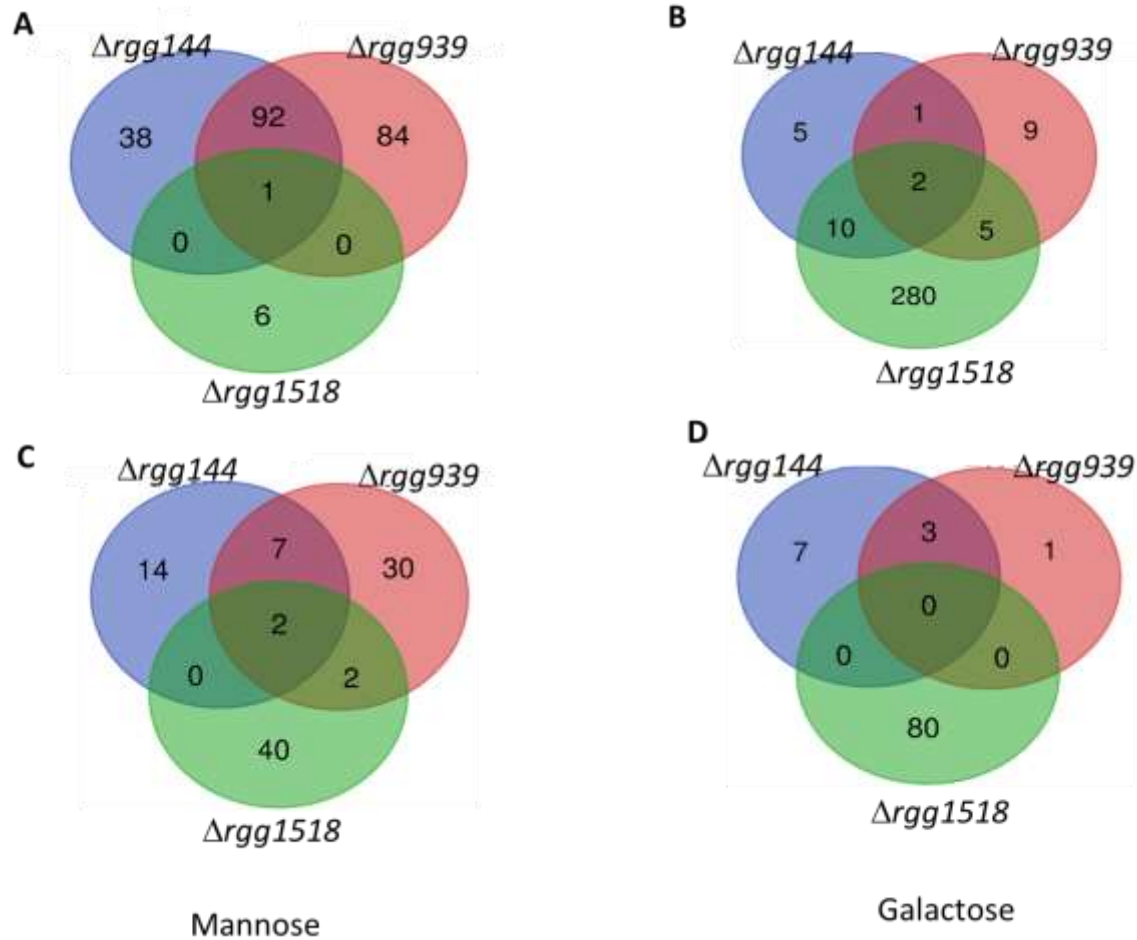
B

SPD_0315	GTAGACATTA	CCGTAAAA	AAGTGATATA
SPD_0378	GCTTTCTGAT	CCTAAAAA	AGATATACTT
SPD_0126	TGGTAATTTG	CCCAGCAA	ATCCAAAACA
SPD_0113	AATATTGTAT	CATAGCAA	TGTAAAAAGT
SPD_1127	CCTAGTCATT	ACTAGAAA	TGGACTAGTT
SPD_1634	AAAGGATTGA	CCTTGGGA	AATCCCTTGG
SPD_1053	TGGAATCCAG	CATAGAAA	AAATAAAAAG
SPD_0208	TATCTGAACC	CCGTGAAA	ACTGGCCGTT
SPD_1461	TTGCATTTAT	CTTAACAA	AAATCACTAG
SPD_0404	TCTTCTTTTA	TCTTGAAA	AATTTTGAAA
SPD_1512	GGGGATTCTT	CCTTGAAA	ATAATCAGTA
SPD_0193	TAGACTTGAG	CATAAAAA	ACGCTCGTTA
SPD_0122	TGATTTTAAT	CATAGCAA	TGATAATTTA
SPD_1342	CCAGTTATAA	CCTAGAAA	TTGTGTCTTT



Figure 2. Binding motif analysis for Rgg1518 (A) The sequences of putative promoter regions of selected genes identified to be differentially expressed by microarray analysis used to identify a putative Rgg1518 binding motif using the MEME suite **(B)**. Two potential binding motives, Motif 1 and Motif 2, were identified. The genetic location of these binding motives in the promoter regions of genes are shown for motif 1 (blue) and motif 2 (red). The core promoter elements -10 and -35 are indicated.

SFigure 3



SFigure 3: A Venn diagram showing regulon overlap among different pneumococcal *Rggs* on mannose (A and C) and galactose (B and D). A and B illustrate the overlap of genes upregulated in *rgg* mutants, whereas C and D show downregulated genes relative to the wild type D39 strain. The diagram was generated by VENNY (<http://bioinfogp.cnb.csic.es/tools/venny/index.html>).

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