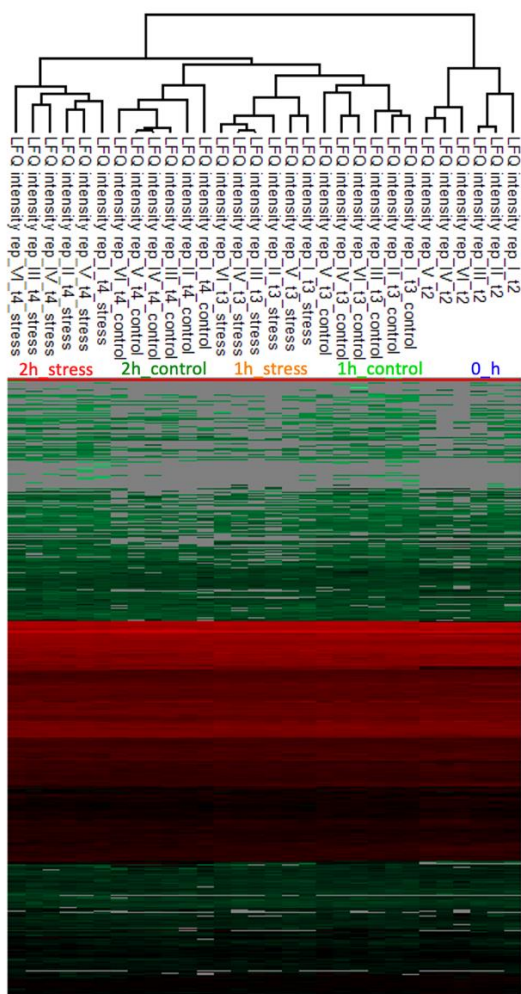


Figure S1: Clustering of independent replicates

(A) Hierarchical clustering before filtering and imputation of missing values using standard settings of the Perseus software [1]



(B) Principal component analysis after filtering (5/6 in at least one exp. group) and imputation of missing values using standard settings of the Perseus software package [1]

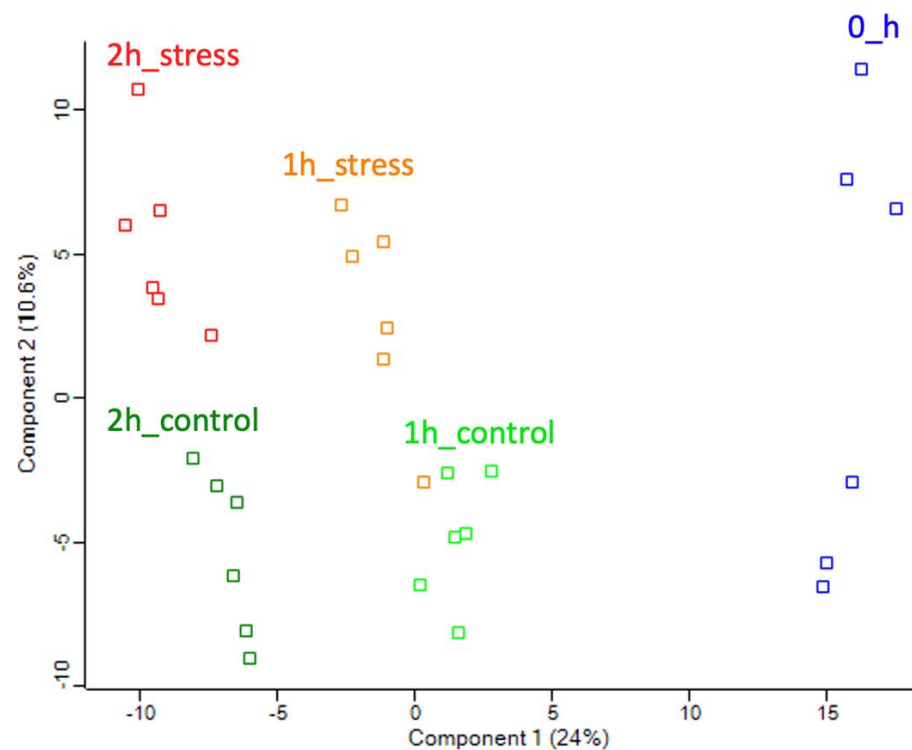


Figure S2: Multi scatter plot of 0 h samples

Pearson correlation

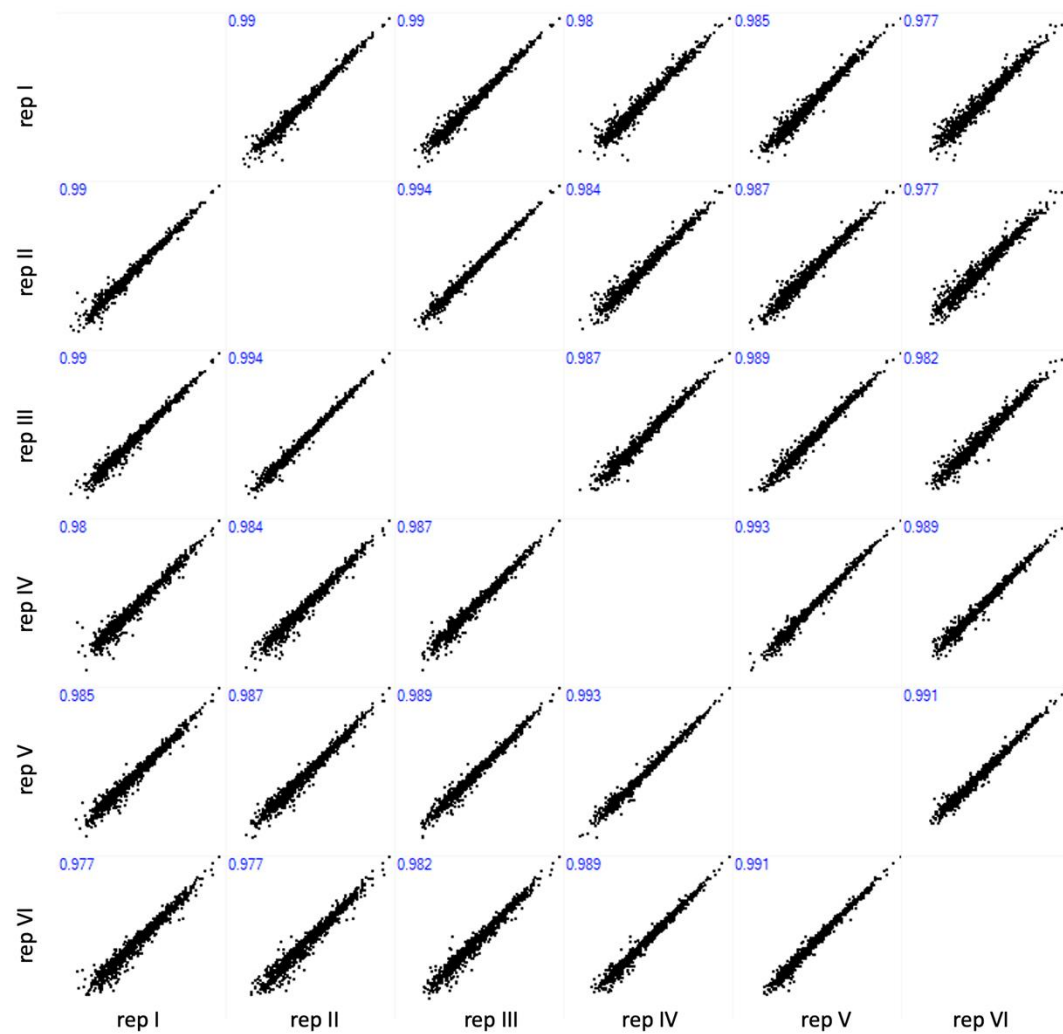


Figure S3: Multi scatter plot of 1 h control samples

Pearson correlation

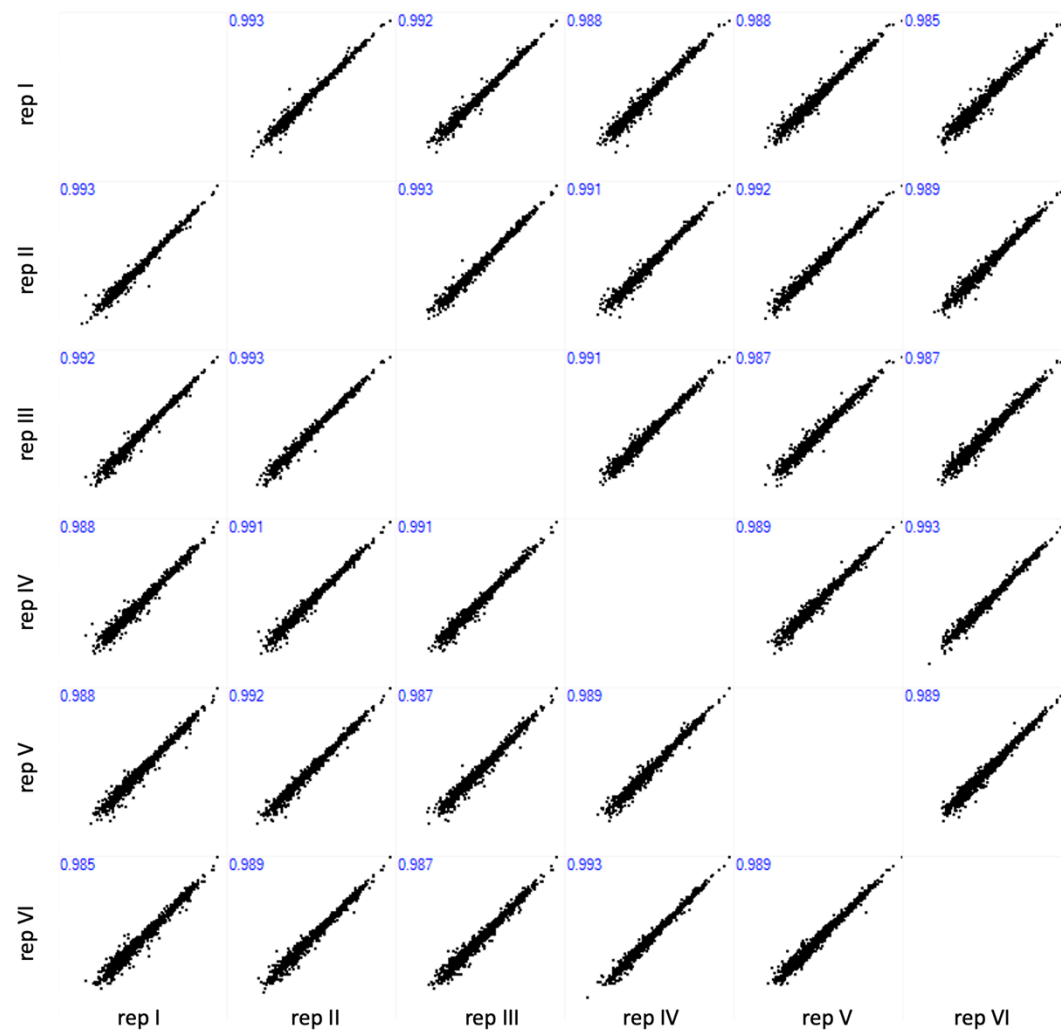


Figure S4: Multi scatter plot of 1 h stress samples

Pearson correlation

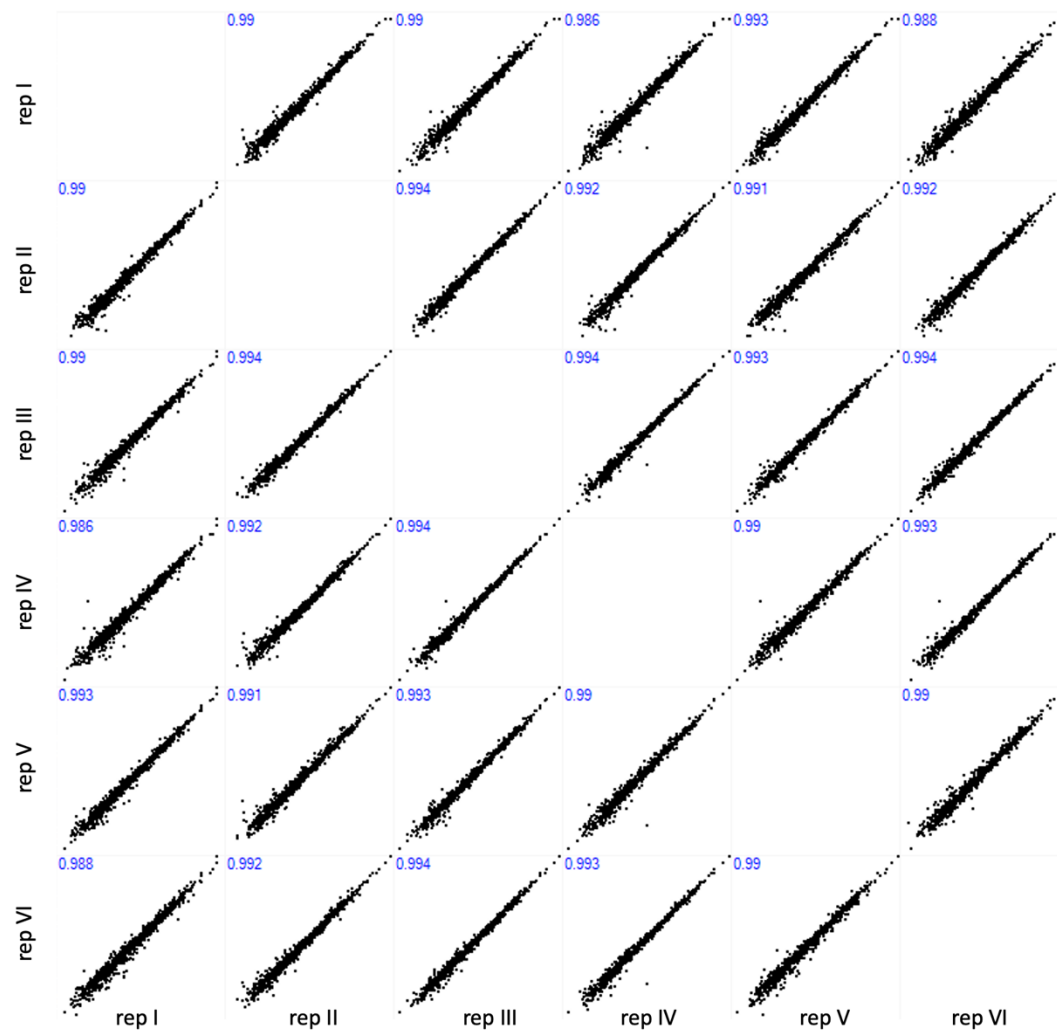


Figure S5: Multi scatter plot of 2 h control samples
Pearson correlation

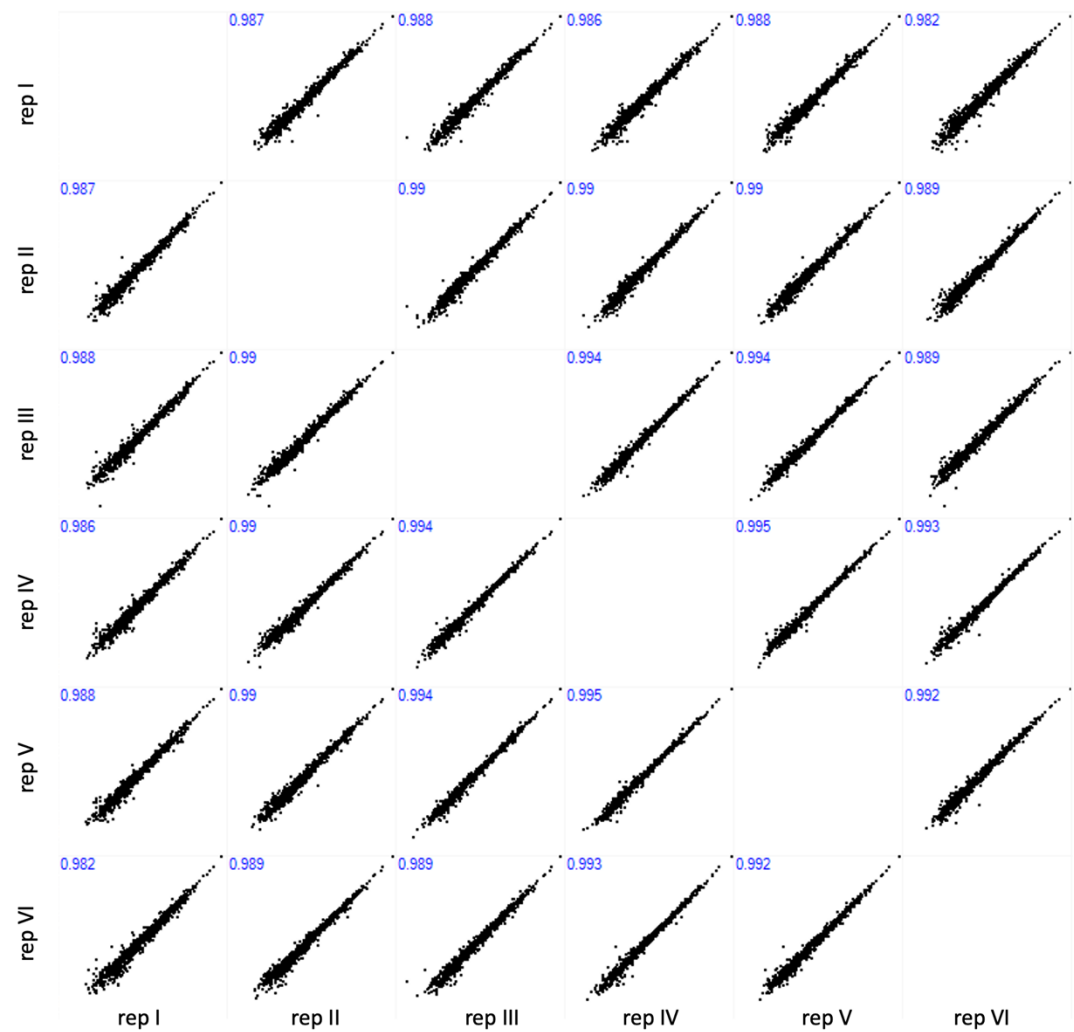


Figure S6: Multi scatter plot of 2 h stress samples
Pearson correlation

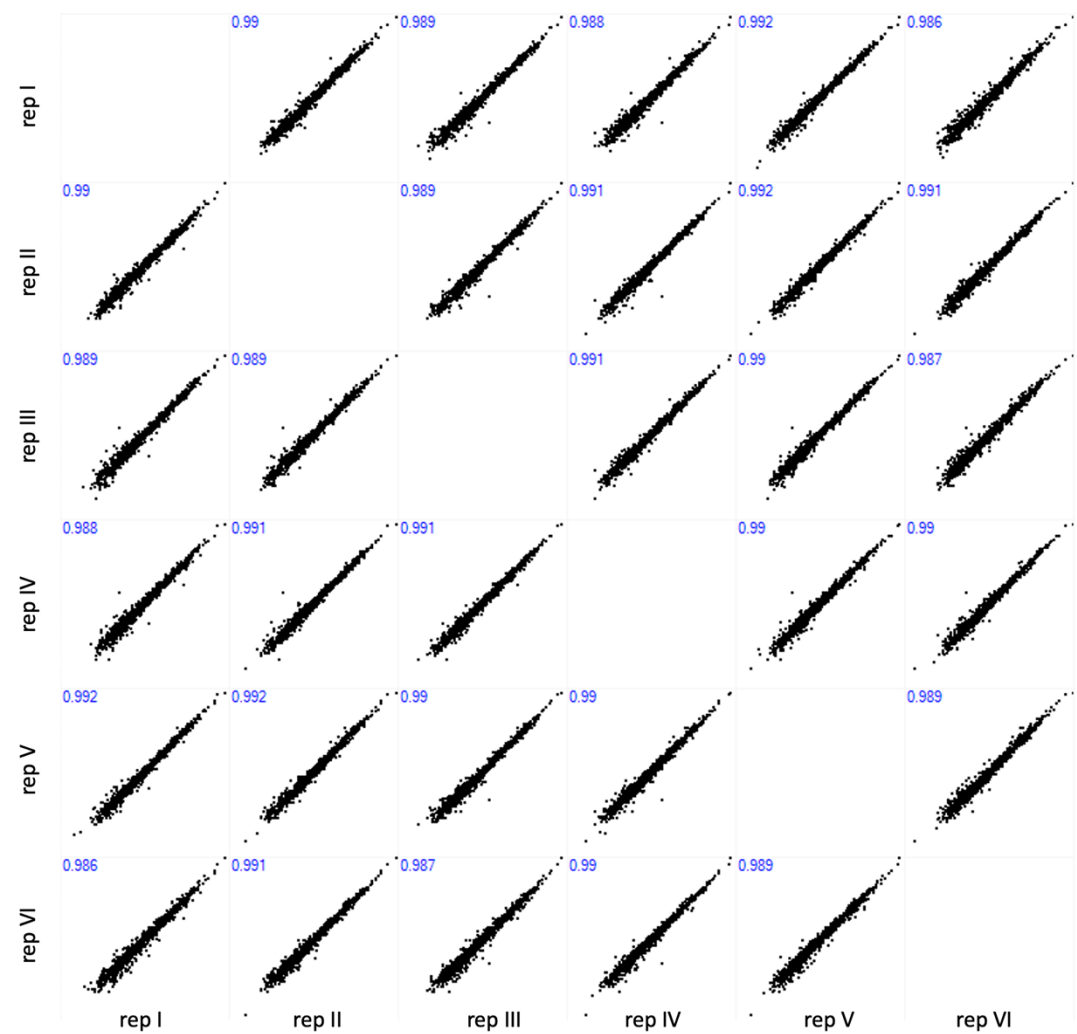


Table S1: Significant changes in protein abundance of *S. pneumoniae* D39 upon LL-37 exposure

locusID	protein	localization	protein fold change	
		PSORTdb_3.0 [2]	<u>1 h stress</u>	<u>2 h stress</u>
14 transporters ↑				
SPD_0076	Potassium uptake protein	Cytoplasmic Membrane	0.9	2.9
SPD_0107	Putative bacteriocin immunity protein	Cytoplasmic Membrane	0.9	1.8
SPD_0464	ABC transporter EcsA	Cytoplasmic Membrane	1.1	1.6
SPD_0684	Biotin transporter	Cytoplasmic Membrane	0.9	4.3
SPD_0686	Efflux transporter	Unknown	1.7	1.5
SPD_0687	ABC transporter	Cytoplasmic Membrane	1.4	1.6
SPD_0688	Efflux ABC transporter	Cytoplasmic Membrane	1.8	2.1
SPD_0861	Protein-export membrane protein SecG	Cytoplasmic Membrane	1.0	2.2
SPD_0887	Cationic amino acid transporter	Cytoplasmic Membrane	1.0	5.4
SPD_1169	Oligopeptide ABC transporter	Cytoplasmic Membrane	0.7	2.8
SPD_1214	ABC transporter	Cytoplasmic Membrane	1.9	4.7
SPD_1525	Multidrug ABC transporter	Cytoplasmic Membrane	8.3	7.5
SPD_1526	Putative ABC transporter	Cytoplasmic Membrane	on	on
SPD_2024	Thiamin ABC transporter	Cytoplasmic Membrane	0.5	2.9
15 transporters ↓				
SPD_0115	Putative efflux transporter	Cytoplasmic Membrane	0.7	0.5
SPD_0161	Putative divalent metal cation transporter	Cytoplasmic Membrane	0.5	0.1
SPD_0374	Putative ABC transporter	Cytoplasmic Membrane	-	off
SPD_0635	Cation-transporting ATPase	Cytoplasmic Membrane	0.6	0.6
SPD_0768	Putative AI-2E transporter	Cytoplasmic Membrane	0.3	2.6
SPD_1176	ABC transporter	Cytoplasmic Membrane	1.1	0.6

SPD_1263	ABC transporter	Cytoplasmic Membrane	0.2	0.6
SPD_1264	ABC transporter	Cytoplasmic Membrane	0.3	0.7
SPD_1465	Efflux ABC transporter	Cytoplasmic Membrane	0.1	0.7
SPD_1466	ABC transporter	Cytoplasmic Membrane	0.9	0.7
SPD_1514	ABC transporter	Cytoplasmic Membrane	0.6	0.2
SPD_1527	Putative sodium ABC exporter	Cytoplasmic Membrane	0.4	0.3
SPD_1528	Putative sodium ABC transporter	Cytoplasmic Membrane	0.6	0.7
SPD_1838	Preprotein translocase YajC subunit	Unknown	1.1	0.6
SPD_1997	Zinc ABC transporter AdcA	Cytoplasmic Membrane	0.7	0.6

6 cell surface modification ↑

SPD_0099	Capsular polysaccharide biosynthesis protein CapD	Cytoplasmic Membrane	1.2	1.5
SPD_0873	Putative lysozyme	Cytoplasmic	1.1	1.6
SPD_1076	Sortase SrtA	Unknown	1.8	1.6
SPD_1128	Putative teichoic acid flippase TacF	Cytoplasmic Membrane	0.8	3.5
SPD_1129	Phosphotransferase LicD1	Cytoplasmic	1.2	1.8
SPD_2002	DltD	Cytoplasmic Membrane	1.6	1.7

1 cell surface modification ↓

SPD_0535	Serine/alanine-adding enzyme MurM	Cytoplasmic	0.3	1.1
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1 protease ↑

SPD_2068	serine protease HtrA	Unknown	2.6	2.0
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3 metabolism ↑

SPD_1932	Alpha-1,4 glucan phosphorylase MalP	Cytoplasmic Membrane	1.8	2.2
SPD_1933	4-alpha-glucanotransferase MalQ	Cytoplasmic	1.1	2.3

SPD_1664	Sugar PTS system	Cytoplasmic Membrane	1.0	1.8
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9 metabolism ↓

SPD_0404	Acetolactate synthase IlvB	Cytoplasmic	0.3	0.9
SPD_0736	Pyrimidine-nucleoside phosphorylase Pdp	Cytoplasmic	1.0	0.6
SPD_0895	Ferrochelataase HemH	Cytoplasmic	0.5	0.3
SPD_1078	L-lactate dehydrogenase L-LDH	Cytoplasmic	0.9	0.7
SPD_1089	Phosphopantothoenoylcysteine decarboxylase CoaC	Unknown	0.6	1.1
SPD_1246	Glucosamine-6-phosphate deaminase NagB	Cytoplasmic	0.8	0.6
SPD_1532	Sugar PTS system	Cytoplasmic Membrane	0.5	1.5
SPD_1834	Aldehyde-alcohol dehydrogenase	Cytoplasmic	0.9	0.7
SPD_1934	Maltose ABC transporter MalX	Cytoplasmic Membrane	0.8	0.6

7 virulence ↓

SPD_0579	Choline binding protein Cbpl	Unknown	0.5	0.8
SPD_0854	Adherence and virulence protein A PavA	Cytoplasmic	0.4	1.3
SPD_0890	Pneumococcal histidine triad protein E PhtE	Unknown	0.6	0.6
SPD_1037	Histidine triad protein B	Unknown	0.6	0.8
SPD_1038	Pneumococcal histidine triad protein A PhpA	Unknown	0.7	0.6
SPD_1064	Putative hemolysin A	Cytoplasmic	0.5	0.7
SPD_1965	Choline binding protein PcpA	Extracellular	0.5	0.8

12 other ↑

SPD_0170	Holliday junction ATP-dependent DNA helicase RuvA	Cytoplasmic	1.0	1.5
SPD_0476	Phosphotransferase enzyme family protein	Cytoplasmic	1.0	1.6
SPD_0534	Tributyryn esterase EstA	Unknown	1.1	1.5
SPD_0863	SsrA-binding protein (Small protein B) SmpB	Cytoplasmic	1.1	1.5

SPD_0868	Foldase PrsA	Cytoplasmic Membrane	1.6	1.6
SPD_0957	DNA primase DnaG	Cytoplasmic	1.0	1.7
SPD_1023	Tyrosine recombinase XerS	Cytoplasmic	1.1	1.7
SPD_1424	tRNA pseudouridine synthase A TruA	Cytoplasmic	1.0	2.8
SPD_1439	30S ribosomal protein S15 RpsO	Cytoplasmic	1.1	1.5
SPD_1546	Primosomal protein PriA	Cytoplasmic	1.5	1.8
SPD_1657	Tyrosine recombinase XerD-like	Cytoplasmic	1.0	2.1
SPD_1822	Ribosomal large subunit pseudouridine synthase	Cytoplasmic	1.3	1.9

15 other ↓

SPD_0128	MutT/nudix family protein	Cytoplasmic	0.7	0.5
SPD_0886	Thioredoxin family protein Etrx2	Cellwall	0.7	0.5
SPD_0968	Acetyltransferase	Cytoplasmic Membrane	0.5	0.9
SPD_1041	Glutaredoxin-like protein NrdH	Unknown	0.5	0.4
SPD_1260	Methyltransferase	Cytoplasmic	1.1	0.7
SPD_1280	Cof family protein	Cytoplasmic	0.7	0.7
SPD_1301	NADPH-dependent FMN reductase	Cytoplasmic	0.3	0.6
SPD_1302	Putative oxidoreductase	Unknown	0.6	0.4
SPD_1375	Putative NADPH-dependent FMN reductase	Unknown	0.7	0.6
SPD_1464	Thioredoxin peroxidase Tpx PsdD	Unknown	1.0	0.6
SPD_1474	Cell division protein DivIVA	Cytoplasmic	0.7	0.8
SPD_1477	Cell division protein SepF	Cytoplasmic	1.1	0.6
SPD_1572	Ribosomal RNA small subunit methyltransferase E	Cytoplasmic Membrane	0.6	0.9
SPD_1590	Putative general stress protein 24	Cytoplasmic	0.7	0.6
SPD_2043	Secreted 45 kDa protein PcsB	Extracellular	0.7	0.7

6 gene regulation ↑

SPD_0467	BlpS	Cytoplasmic	1.5	1.8
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SPD_0701	Response regulator CiaR	Cytoplasmic	1.6	1.6
SPD_0702	Sensor histidine kinase CiaH	Cytoplasmic Membrane	2.1	2.1
SPD_1366	Putative general stress protein 13	Cytoplasmic	1.3	2.2
SPD_1524	Transcriptional regulator GntR	Unknown	10.5	8.3
SPD_2063	Response regulator ComE	Cytoplasmic	1.1	1.5

5 gene regulation ↓

SPD_0309	Autoinducer-2 production protein LuxS	Cytoplasmic	0.7	0.5
SPD_0633	Putative transcriptional regulator CopY	Unknown	0.7	0.5
SPD_1448	Rrf2 family protein	Cytoplasmic	0.2	0.6
SPD_1535	Sucrose operon repressor ScrR	Cytoplasmic	0.4	1.2
SPD_1904	Arginine repressor ArgR	Cytoplasmic	0.6	0.7

1 unknown ↑

SPD_0913	Unknown	Unknown	2.6	2.8
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10 unknown ↓

SPD_0039	Unknown	Cytoplasmic	0.9	0.6
SPD_0188	Unknown	Extracellular	0.4	1.0
SPD_0220	Unknown	Cytoplasmic Membrane	0.3	0.6
SPD_0714	Unknown	Cytoplasmic	0.8	0.6
SPD_0792	Putative lipoprotein	Unknown	0.7	0.6
SPD_1515	Putative membrane protein	Cytoplasmic Membrane	2.0	0.4
SPD_1516	Putative membrane protein	Cytoplasmic Membrane	0.7	0.2
SPD_1517	Unknown	Unknown	0.5	0.3
SPD_1588	Unknown	Cytoplasmic Membrane	0.6	0.7
SPD_1943	Unknown	Cytoplasmic Membrane	0.1	1.6

Table S2: Selected changes in protein abundance of *S. pneumoniae* D39 upon LL-37 exposure after comparison with transcriptomic and genomic data

Proteomic data (this study)					Gene regulation data [3,4]		Transcriptomic data [5]	
locusID	protein	localization PSORTdb_3.0 [2]	protein fold change		genomic regulator	locusID of regulator	RNA fold change	
			<u>1 h</u> <u>stress</u>	<u>2h</u> <u>stress</u>			<u>15 min</u> <u>stress</u>	<u>30 min</u> <u>stress</u>
8 transporters ↑								
SPD_0684	Biotin transporter	Cytoplasmic Membrane	0.9	4.3	RpoD	SPD_0958	2.6	2.3
SPD_0686	Efflux transporter	Unknown	1.7	1.5	GntR, RpoD	SPD_1524, SPD_0958	4.1	5.5
SPD_0687	ABC transporter	Cytoplasmic Membrane	1.4	1.6	GntR, RpoD	SPD_1524, SPD_0958	5.1	5
SPD_0688	Efflux ABC transporter	Cytoplasmic Membrane	1.8	2.1	GntR, RpoD	SPD_1524, SPD_0958	4.6	6.1
SPD_0887	Cationic amino acid transporter	Cytoplasmic Membrane	1.0	5.4	ArgR, RpoD	SPD_1904, SPD_0958		
SPD_1214	ABC transporter	Cytoplasmic Membrane	1.9	4.7	RpoD	SPD_0958	1.5	1.6
SPD_1525	Multidrug ABC transporter	Cytoplasmic Membrane	8.3	7.5	GntR, RpoD	SPD_1524, SPD_0958		
SPD_1526	Putative ABC transporter	Cytoplasmic Membrane	on	on	GntR, RpoD	SPD_1524, SPD_0958	11.4	13
8 transporters ↓								
SPD_0115	Putative efflux transporter	Cytoplasmic Membrane	0.7	0.5				
SPD_0161	Put. divalent metal cation transporter	Cytoplasmic Membrane	0.5	0.1	CodY	SPD_1412		
SPD_0374	Putative ABC transporter	Cytoplasmic Membrane	-	off	CcpA, ComE	SPD_1797, SPD_2063		
SPD_0635	Cation-transporting ATPase	Cytoplasmic Membrane	0.6	0.6	CopY, RpoD	SPD_0633, SPD_0958		
SPD_1514	ABC transporter	Cytoplasmic Membrane	0.6	0.2				
SPD_1527	Putative sodium ABC exporter	Cytoplasmic Membrane	0.4	0.3	CcpA, ComE, RpoD	SPD_1797, SPD_2063, SPD_0958	0.7	n. d.

SPD_1528	Putative sodium ABC transporter	Cytoplasmic Membrane	0.6	0.7	CcpA, ComE , RpoD	SPD_1797, SPD_2063, SPD_0958	0.5	0.6
SPD_1997	Zinc ABC transporter AdcA	Cytoplasmic Membrane	0.7	0.6	AdcR, RpoD	SPD_2000, SPD_0958		

4 cell surface modification ↑

SPD_1076	Sortase SrtA	Unknown	1.8	1.6				
SPD_1128	Putative teichoic acid flippase TacF	Cytoplasmic Membrane	0.8	3.5				
SPD_1129	Phosphotransferase LicD1	Cytoplasmic	1.2	1.8				
SPD_2002	DltD	Cytoplasmic Membrane	1.6	1.7			1.6	n. d.

1 protease ↑

SPD_2068	serine protease HtrA	Unknown	2.6	2.0	CiaR	SPD_0701	3	2.3
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2 metabolism ↑

SPD_1932	Alpha-1,4 glucan phosphorylase MalP	Cytoplasmic Membrane	1.8	2.2	CcpA, MalR , RpoD, CiaR	SPD_1797, SPD_1938, SPD_0958, SPD_0701	3.2	2.6
SPD_1933	4-alpha-glucanotransferase MalQ	Cytoplasmic	1.1	2.3	CcpA, MalR , RpoD, CiaR	SPD_1797, SPD_1938, SPD_0958, SPD_0701	3.4	2.9

4 metabolism ↓

SPD_0404	Acetolactate synthase IlvB	Cytoplasmic	0.3	0.9	CodY, RpoD	SPD_1412, SPD_0958	0.7	0.6
SPD_1532	Sugar PTS system	Cytoplasmic Membrane	0.5	1.5	CcpA, RpoD, ScrR	SPD_1797, SPD_0958, SPD_1535		
SPD_1834	Aldehyde-alcohol dehydrogenase	Cytoplasmic	0.9	0.7	CcpA, Rex , RpoD	SPD_1797, SPD_0976, SPD_0958	0.6	0.4
SPD_1934	ABC transporter MalX	Cytoplasmic Membrane	0.8	0.6	CcpA, MalR , RpoD	SPD_1797, SPD_1938, SPD_0958	0.6	n. d.

3 virulence ↓

SPD_0890	Pneumococcal histidine triad protein E PhtE	Unknown	0.6	0.6	AdcR	SPD_2000	n. d.	0.6
SPD_1037	Histidine triad protein B	Unknown	0.6	0.8	AdcR, RpoD	SPD_2000, SPD_0958		
SPD_1038	Pneumococcal histidine triad protein A PhpA	Unknown	0.7	0.6	AdcR, RpoD	SPD_2000, SPD_0958		

2 others ↑

SPD_0863	SsrA-binding protein (Small protein B) SmpB	Cytoplasmic	1.1	1.5			1.5	1.7
SPD_0868	Foldase PrsA	Cytoplasmic Membrane	1.6	1.6	CiaR	SPD_0701	1.3	n. d.

6 others ↓

SPD_1301	NADPH-dependent FMN reductase	Cytoplasmic	0.3	0.6	RpoD	SPD_0958		
SPD_1302	Putative oxidoreductase	Unknown	0.6	0.4	RpoD	SPD_0958		
SPD_1464	Thioredoxin peroxidase Tpx PsaD	Unknown	1.0	0.6			n. d.	0.5
SPD_1474	Cell division protein DivIVA	Cytoplasmic	0.7	0.8				
SPD_1477	Cell division protein SepF	Cytoplasmic	1.1	0.6				
SPD_1590	Putative general stress protein 24	Cytoplasmic	0.7	0.6	RpoD	SPD_0958		

5 gene regulation ↑

SPD_0467	BlpS	Cytoplasmic	1.5	1.8			1.6	1.8
SPD_0701	Response regulator CiaR	Cytoplasmic	1.6	1.6	CiaR	SPD_0701	2.5	2.1
SPD_0702	Sensor histidine kinase CiaH	Cytoplasmic Membrane	2.1	2.1	CiaR	SPD_0701	2.4	n. d.
SPD_1524	Transcriptional regulator GntR	Unknown	10.5	8.3	GntR, RpoD	SPD_1524, SPD_0958	7.2	9.1
SPD_2063	Response regulator ComE	Cytoplasmic	1.1	1.5	ComE, RpoD	SPD_2063, SPD_0958	n. d.	0.7

3 gene regulation ↓

SPD_0633	Putative transcriptional regulator CopY	Unknown	0.7	0.5	CopY, RpoD	SPD_0633, SPD_0958
SPD_1535	Sucrose operon repressor ScrR	Cytoplasmic	0.4	1.2		
SPD_1904	Arginine repressor ArgR	Cytoplasmic	0.6	0.7	ArgR, RpoD	SPD_1904, SPD_0958

1 unknown ↑

SPD_0913	Unknown	Unknown	2.6	2.8	CiaR	SPD_0701	3.3	2.7
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5 unknown ↓

SPD_0220	Unknown	Cytoplasmic Membrane	0.3	0.6	CcpA	SPD_1797	0.6	n. d.
SPD_1515	Putative membrane protein	Cytoplasmic Membrane	2.0	0.4				
SPD_1516	Putative membrane protein	Cytoplasmic Membrane	0.7	0.2				
SPD_1517	Unknown	Unknown	0.5	0.3				
SPD_1588	Unknown	Cytoplasmic Membrane	0.6	0.7	RpoD	SPD_0958		

References

1. Tyanova, S.; Temu, T.; Sinitcyn, P.; Carlson, A.; Hein, M.Y.; Geiger, T.; Mann, M.; Cox, J. The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nature Methods* **2016**, *13*, 731-740, doi:10.1038/nMeth.3901.
2. Peabody, M.A.; Laird, M.R.; Vlasschaert, C.; Lo, R.; Brinkman, F.S. PSORTdb: expanding the bacteria and archaea protein subcellular localization database to better reflect diversity in cell envelope structures. *Nucleic Acids Res.* **2016**, *44*, D663-D668, doi:10.1093/nar/gkv1271.
3. Slager, J.; Aprianto, R.; Veening, J.W. Deep genome annotation of the opportunistic human pathogen *Streptococcus pneumoniae* D39. *Nucleic Acids Res.* **2018**, *46*, 9971-9989, doi:10.1093/nar/gky725.
4. Halfmann, A.; Kovacs, M.; Hakenbeck, R.; Bruckner, R. Identification of the genes directly controlled by the response regulator CiaR in *Streptococcus pneumoniae*: five out of 15 promoters drive expression of small non-coding RNAs. *Mol. Microbiol.* **2007**, *66*, 110-126, doi:10.1111/j.1365-2958.2007.05900.x.
5. Majchrzykiewicz, J.A.; Kuipers, O.P.; Bijlsma, J.J. Generic and specific adaptive responses of *Streptococcus pneumoniae* to challenge with three distinct antimicrobial peptides, bacitracin, LL-37, and nisin. *Antimicrob. Agents Chemother.* **2010**, *54*, 440-451, doi:10.1128/AAC.00769-09.