The two-component system WalKR provides an essential link between cell wall homeostasis and DNA replication in *Staphylococcus aureus*. - Supplementary Data File -

- Supplementary Data File -

Liam K. R. Sharkey¹, Romain Guerillot¹, Calum J. Walsh¹, Adrianna M. Turner¹, Jean Y. H. Lee¹, Stephanie L. Neville¹, Stephan Klatt², Sarah L. Baines¹, Sacha J. Pidot¹, Fernando J. Rossello^{3,4}, Torsten Seemann^{1,5}, Hamish E. G. McWilliam¹, Ellie Cho⁶, Glen P. Carter¹, Benjamin P. Howden^{1,5}, Christopher A. McDevitt¹, Abderrahman Hachani¹, Timothy P. Stinear^{1,5,*} and Ian R. Monk^{1,*}

Affiliations:

1. Department of Microbiology and Immunology, Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, 3000, Australia

2. The Florey Institute of Neuroscience and Mental Health, Melbourne Dementia Research Centre, The University of Melbourne, Parkville, Victoria, 3010, Australia.

3. University of Melbourne Centre for Cancer Research, The University of Melbourne, Melbourne, Victoria, 3000, Australia.

 Australian Regenerative Medicine Institute, Monash University, Melbourne, Victoria, 3800, Australia.
 Centre for Pathogen Genomics, Department of Microbiology and Immunology, Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, 3000, Australia

6. Biological Optical Microscopy Platform, University of Melbourne, Melbourne, Victoria, 3000, Australia

* Joint senior authors

Timothy P. Stinear and Ian R. Monk. Email: <u>tstinear@unimelb.edu.au</u>; <u>imonk@unimelb.edu.au</u>.

Contents:

		Page
Figure S1	Growth in LB of C-terminally tagged WalR-SmBIT or WalK-LgBIT.	2
Table S1	The indirect WalR regulon defined by RNA-seq	3
Figure S2	S. aureus ChIP-seq reveals binding sites of response regulators.	7
Table S2	Putative response-regulator binding sites identified by ChIPseq	8
Figure S3	Generation of an S. aureus WalR binding motif.	12
Table S3	The predicted WalR direct regulon.	13
Figure S4	Diversity of WalR motifs.	14
Table S4	Relative positions of predicted WalR binding and transcriptional start sites.	15
Figure S5	Purification of recombinant WalR.	16
Figure S6	Competition EMSAs	17
Figure S7	WalR and VraR binding at the <i>ItaS</i> locus.	18
Figure S8	Impact of WalR motif mutation on tarF, tagG and dnaD.	19
Figure S9	The effect of walR, hup, and hla on Ori:Ter ratio.	20
Table S5	Strains and plasmids used in this study	21
Table S6	Oligonucleotides used in this study	24



Figure S1. Growth in LB of the chromosomally C-terminally tagged WalR-SmBIT or WalK-LgBIT in LB compared to the Wt. No difference in growth profile was observed for the mutants.

Table S1. The indirect WalR regulon defined by RNA-seq.Genes undergoing a $\geq 1.5 \log 2$ FC (FDR ≤ 0.05) change in gene expression upon WalK activation(WalKT389A mutant vs wild type) are grouped by function. FDR = P value adjusted for false discovery rate. FC = fold-change.

Cellular function	Locus tag	Gene	Product	RNAseq significance	Log-FC
	Locus tug	dene	House	(FDR)	208210
Amino acid biosynthesis and transport	SAUSA300_2006	ilvD	Dihydroxy-Acid Dehydratase	4.00E-07	2.84
	SAUSA300_2007	ilvB	Acetolactate Synthase Large Subunit	1.00E-07	2.46
	SAUSA300_2013	leuD	Isopropylmalate Isomerase Small Subunit	2.30E-06	2.06
	SAUSA300_0185	argJ	Bifunctional Ornithine Acetyltransferase/N-	3.30E-05	2.02
	SAUSA300_2010	leuA	2-Isopropylmalate Synthase	1.10E-06	2.01
	SAUSA300_0186	argC	N-Acetyl-Gamma-Glutamyl-Phosphate Reductase	1.10E-06	1.95
	SAUSA300_2012	leuC	Isopropylmalate Isomerase Large Subunit	4.00E-07	1.88
	SAUSA300_0359	metC	Trans-Sulfuration Enzyme Family Protein	1.50E-06	1.87
	SAUSA300_2009	ilvC	Ketol-Acid Reductoisomerase	9.00E-07	1.87
	SAUSA300_2011	leuB	3-Isopropylmalate Dehydrogenase	9.00E-07	1.68
	SAUSA300_0184	argB	Acetylglutamate Kinase	2.10E-05	1.58
	SAUSA300_0066	argR	Arginine Repressor	6.30E-05	-1.75
Carbobydrate uptake and metabolism	541154200 0196	araC	N Acotyl Commo Clutomyl Phocobato Poductoco	1 105 06	1.05
carbonydrate uptake and metabolism	541154300 0448	traD	Rts System Trabalosa Specific libs Component	2 205 05	1.55
	SAUSA300_0448	nanA	N Acetylpouraminato Lyaso	1 105 04	1.72
	SAUSA300_0313	alcC	Ptc System libe Components	1.705.06	1.7
	SAUSA300_0230	gice	Pris System, fibe Components	2 205 04	1.04
	SAUSA300_0333	- dmnl	4 Ovalocratorate Tautomorace	2.200-04	1.50
	SAUSA300_1258	ampi aculi	4-Oxalocrotonale Tautomerase	2.60E-04	-1.50
	SAUSA300_0791	gcvn InlA1	Giycine Cleavage System Protein H	1.202-08	-1.70
	SAUSA300_0930	ipiA i	Lipoate-Protein Ligase A Family Protein	5.00E-07	-1.79
	SAUSA300_0030	ugpQ	Giycerophosphoryi Diester Phosphodiesterase	3.00E-07	-2
Cell wall organisation	SAUSA300_2585	asp3	Accessory Secretory Protein Asp3	5.70E-05	1.92
-	SAUSA300_0153	capB	Capsular Polysaccharide Biosynthesis Protein Cap5B	5.10E-05	1.6
	SAUSA300_0156	capE	Capsular Polysaccharide Biosynthesis Protein Cap5E	5.20E-05	1.57
	SAUSA300_0603	-	Hypothetical Protein, Linked To Response To	1.80E-05	-1.59
	SAUSA300_0604	-	Alpha/Beta Fold Family Hydrolase	7.80E-06	-1.61
Cofactor and carrier biosynthesis	SAUSA300_0697	queC	ExsB Protein	8.40E-06	1.85
DNA replication recombination and repair	SAUSA300 0367	ssh	Single-Strand Binding Protein	1 90F-06	1 94
	SAUSA300 1142	dnrA	DNA Protecting Protein DnrA	2 105-06	1.83
	SAUSA300_1142	tnn2	Putative Transposase	3 10E-03	-4.62
Fatty acid biosynthesis	SAUSA300_0320	gehB	Triacylglycerol Lipase	4.00E-07	2.56
	SAUSA300_1563	-	Acetyl-Coa Carboxylase, Biotin Carboxylase	3.20E-05	2.01
	SAUSA300_0177	-	Acyl-Coa/Acyl-Acp Dehydrogenase	5.00E-07	1.96
	SAUSA300_1125	асрР	Acyl Carrier Protein	9.90E-06	-1.92
Metal ion homeostasis	SAUSA300_0720	sstC	Putative Iron Compound ABC Transporter ATP-Binding	9.00E-05	1.6
	SAUSA300_0117	sirA	Iron Compound ABC Transporter Iron Compound-	5.60E-05	-1.51
	SAUSA300_1874	ftnA	Ferritins Family Protein	2.20E-06	-1.69
	SAUSA300_0843	sufA	Fe-S Cluster Carrier	2.30E-04	-1.7
	SAUSA300_1373	fer	Ferredoxin	3.50E-05	-2.14
Nitrogen utilisation	SAUSA300 1566	-	Allonhanate Hydrolase	2 10F-05	1 71
	5/(05/(500_1500		/ inophilitate Hydroidse	2.102 05	1.71
Oxidative stress response	SAUSA300_2502	crt0	Staphyloxanthin Biosynthesis	6.80E-05	-1.65
	SAUSA300_1909	-	Putative Thioredoxin	7.00E-07	-1.86
	SAUSA300_1197	bsaA	Glutathione Peroxidase	3.50E-06	-1.87
	SAUSA300_2463	ddh	D-Lactate Dehydrogenase	1.70E-06	-1.97
	SAUSA300_1044	trxA	Thioredoxin	2.00E-06	-2.2
	SAUSA300_0789	-	Putative Thioredoxin	1.00E-07	-2.3
	SAUSA300_1690	-	Putative Thioredoxin	2.00E-07	-2.35
Dhama and sisted	CALICA200 1047			4 005 02	1 71
Phage associated	SAUSA300_1947	-	Phi// Orf031-Like Protein	4.90E-03	-1./1
	SAUSA500_1944	-	Phi// On026-Like Protein Phage Transcriptional	8.20E-05	-1.71
Protein fate	SAUSA300_1656	uspA1	Universal Stress Protein	2.20E-06	-1.64
	SAUSA300_1984	mroQ	Membrane-Embedded CaaX Protease	2.10E-05	-1.69
	SAUSA300_1790	prsA	Foldase Protein PrsA	4.00E-07	-1.8
	SAUSA300_0752	clpP	ATP-Dependent Clp Protease Proteolytic Subunit	1.40E-06	-1.89
	SAUSA300_1295	cspA	Csd Family Cold Shock Protein	2.00E-07	-3.19
	SAUSA300_0067	-	Universal Stress Protein	2.00E-07	-3.35
	CALIC: 2000				
Purine ribonucleotide biosynthesis	SAUSA300_0973	purM	Phosphoribosylaminoimidazole Synthetase	1.00E-07	2.65
	SAUSA300_0971	purL	Phosphoribosylformylglycinamidine Synthase li	1.20E-06	2.48
	SAUSA300_0970	purQ	Phosphoribosylformylglycinamidine Synthase I	3.10E-05	2.19
	SAUSA300_0972	purF	Amidophosphoribosyltransferase	4.00E-07	2.15
	SAUSA300_0974	purN	Phosphoribosylglycinamide Formyltransferase	5.00E-07	2.01
	SAUSA300_0975	purH	Bitunctional	8.00E-07	1.61

Table S1. The indirect WalR regulon defined by RNA-seq.

	SAUSA300_2183	adk	Adenylate Kinase	4.30E-05	1.57
Respiration	SAUSA300_0960	qoxD	Quinol Oxidase, Subunit Iv	9.90E-05	1.7
Ribosome and Protein synthesis	SAUSA300_2186	rpmD	50S Ribosomal Protein L30	1.70E-06	2.05
	SAUSA300_0531	rpsG	30S Ribosomal Protein S7	2.70E-06	1.79
		, rpsK	30S Ribosomal Protein S11	3.40E-05	1.75
		rpsE	30S Ribosomal Protein S5	7.40E-06	1.7
	SAUSA300 2180	rpsM	30S Ribosomal Protein S13	2.10E-05	1.52
	SAUSA300 2188	rpIR	50S Ribosomal Protein L18	2.10E-05	1.51
	SAUSA300_2648	rnmH	505 Ribosomal Protein I 34	1 10E-03	-1.56
	SAUSA300_0991	def	Peptide Deformylase	1 50E-06	-1.81
	SALISA300 1233	rnmG2	50S Ribosomal Protein L33	1.00E-04	-1.07
	SAUSA300_1233	rncT	205 Ribosomal Protein 255	2 105 05	-1.52
	SAUSA300_1343	rpsr	Sos Ribosofiai Protein 320	5.10L-05	-2.13
	SAUSASU0_0055	sped	Libernation Dramating Factor	6.50E-06	-2.19
	SAUSA300_0736	SUHPF	Fibernation-Promoting Factor	4.00E-07	-2.20
	SAUSA300_1511	rpmG	SUS RIDOSOMAI Protein L33	6.30E-05	-2.33
	SAUSA300_1027	rpmF	50S Ribosomal Protein L32	7.40E-06	-2.38
	SAUSA300_1234	rpsN2	30S Ribosomal Protein S14	4.70E-06	-2.44
	SAUSA300_1535	rpsU	30S Ribosomal Protein S21	7.90E-06	-3.11
Transcriptional regulation	SAUSA300_0691	saeR	Dna-Binding Response Regulator SaeR	5.00E-07	2.28
	SAUSA300_0690	saeS	Sensor Histidine Kinase SaeS	1.00E-07	2.2
	SAUSA300_0928	comK1	Competence Transcription Factor	9.90E-06	1.86
	SAUSA300_0187	argD	Ornithine Aminotransferase	2.10E-06	1.85
	SAUSA300_1992	agrA	Accessory Gene Regulator Protein A	2.40E-06	1.77
	SAUSA300 1991	agrC	Accessory Gene Regulator Protein C	1.40E-06	1.57
	SAUSA300 2337	nreC	Dna-Binding Response Regulator Nrec	3.00E-03	-1.53
	SAUSA300_1708	rot	Accessory Regulator Rot	3.10E-06	-1.63
	SALISA300 0114	sars	Accessory Regulator	1.80E-06	-1.76
	SALISA300_2218	sarV	Stanbylococcal Accessory Regulator V	2 40E-05	-1.89
	SALISA300_0954	-	Marr Family Transcriptional Regulator	1 10E-06	-23
	SALISA300 2599	icaP	Intercellular Adhesion Operon Transcription Regulator	5.00E-07	-2.86
	SAUSA300_2437	sarT	Accessory Regulator T	9.40E-06	-3.14
Transport	CALICA200 0176	ccuC	APC Transporter Dermoses	4 705 06	1.05
Transport	SAUSA300_0176	ssuc	ABC Transporter Permease	4.70E-08	1.95
	SAUSA300_0436	утрв	ABC Transporter Permease	8.00E-07	1.94
	SAUSA300_0202	-	Peptide ABC Transporter Permease	4.40E-05	1.8
	SAUSA300_0203	-	Nickel-Peptide/Transporter Substrate-Binding Protein	1.10E-06	1./4
	SAUSA300_0201	-	Peptide ABC Transporter Permease	1.20E-06	1.68
	SAUSA300_0143	phnE2	Phosphonate ABC Transporter Permease	2.00E-05	1.6
	SAUSA300_0200	-	Peptide ABC Transporter ATP-Binding Protein	1.10E-06	1.59
	SAUSA300_0208	malK	Putative Maltose ABC Transporter ATP-Binding Protein	5.00E-05	1.52
	SAUSA300_2307	hrtB	ABC Transporter Permease	2.00E-05	-1.61
Virulence	SAUSA300_1755	spID	Serine Protease SplD	2.00E-07	5.79
	SAUSA300 1757	splB	Serine Protease SplB	0.00E+00	4.45
		, splA	Serine Protease SplA	0.00E+00	4.15
	SAUSA300 1754	splE	Serine Protease SplE	8.00E-07	3.83
	SAUSA300_1756	splC	Serine Protease SpIC	2.00E-07	3.44
	SAUSA300_1753	snlF	Serine Protease Sple	3.00E-07	3.05
	5/105/300_1/55	icaA	N Glycosyltrapsforaso	3.002.07	2.65
	SALISA200_2000	huke_DV		1 00E 07	2.00
	SALISA200_1202	rung=r V	V/9 Protoco	1.000-07	2.59
	24024200_0251	sspA Iului	Aarahysia // aukocidia Earaik, Daataia	1.00E-07	2.55
	SAUSA300_19/5	іикН ch:		8.50E-06	2.37
	SAUSA300_2364	SDI	Igg-Binding Protein Sbi	1.00E-07	2.15
	SAUSA300_1920	chp	Chemotaxis-Inhibiting Protein Chips	2.90E-06	2
	SAUSA300_1918	hlb-1	Truncated Beta-Hemolysin	3.80E-05	1.93
	SAUSA300_2586	asp2	Accessory Sec System Protein	1.10E-06	1.91
	SAUSA300_0950	sspB	Cysteine Protease	1.20E-06	1.88
	SAUSA300_0285	esxB	Type Vii Secretion System Extracellular Protein B	2.80E-06	1.87
	SAUSA300_2367	hlgB	Gamma-Hemolysin Component B	1.10E-06	1.83
	SAUSA300_1381	-	Panton-Valentine Leukocidin, Lukf-PV	3.00E-07	1.82
	SAUSA300_1058	hla	Alpha-Hemolysin	1.20E-06	1.78
	SAUSA300_2184	secY	Preprotein Translocase Subunit Secy	2.50E-06	1.76
	SAUSA300_1974	lukG	Leukocidin/Hemolysin Toxin Family Protein	5.50E-05	1.71
		sspC	Cysteine Protease	1.80E-05	1.71
	SAUSA300 2587	asp1	Accessory Secretory Protein Asp1	5.30E-05	1.69
	SAUSA300 2601	icaR	Intercellular Adhesion Protein R	1 40F-05	1.05
	SALISA300 2572	aur	Zinc Metalloproteinase Aureolysin	1 10E-06	1.54
	SALICA200_2372	trfA	Adaptor Protein	1.100-00	1.5
	24024300_0899	u jA	Auaptor Protein	1.10E-06	-1.64
	SAUSA300_1/84	u'aP	Signal Transouction Protein Trap	4.90E-06	-1.65
	SAUSA300_0401	SSI/	Superantigen-Like Protein /	9.90E-06	-1.65
	SAUSA300_2262	spdB	Abi Domain-Containing Protein	1.30E-05	-1.69
	SAUSA300_0762	secG	Preprotein Translocase Subunit SecG	4.40E-04	-1.74

Table S1. The indirect WalR regulon defined by RNA-seq.

	SAUSA300_1594	yajC	Preprotein Translocase Subunit YajC	4.60E-05	-1.94
	SAUSA300_0289	esaG	Component Of The Type Vii Secretion System	1.00E-05	-2.02
	SAUSA300_0395	ssl1	Superantigen-Like Protein	2.30E-05	-2.07
	SAUSA300_1988	hld	Delta-Hemolysin	6.50E-05	-2.09
		isaB	Immunodominant Antigen B	8.00E-07	-2.28
	SAUSA300_0399	ss/5	Superantigen-Like Protein 5	7.80E-06	-2.54
	SAUSA300 0654	sarX	Hypothetical Protein	1.30E-06	-3.03
Unknown	SAUSA300 0692	saeO	Hypothetical Protein Of Unknown Function	2 00F-07	3.12
	SAUSA300_0175	ssuA	Putative Linoprotein	1 20E-06	23
	SAUSA300 1877	-	Hypothetical Protein, Transmembrane	5.00E-07	2.06
	SAUSA300_1077	ssuB	Hypothetical Protein, Putative ABC Transporter	3.60E-06	2.00
	SAUSA300_0174	SSUD	Hypothetical Protein, Putative Aber Transporter	0.00E-00	1 70
	SAUSASU0_0520	-	Hypothetical Protein, Protein-Aup-Ribose Hydroidse	9.90E-06	1.79
	SAUSA300_1565	-	Hypothetical Protein, Arisz Domain-Containing	5.10E-05	1.72
	SAUSA300_1734	rppн	Hypothetical Protein, Nudix Hydrolase Domain	2.00E-05	1.52
	SAUSA300_2448	-	Hypothetical Protein, Putative Transmembrane	3.40E-06	1.5
	SAUSA300_0427	mpsC	Hypothetical Protein, Mpsc Domain Containing	2.20E-06	-1.52
	SAUSA300_2352	-	Addiction Module Antitoxin	1.00E-06	-1.53
	SAUSA300_2632	-	Hypothetical Protein, Putative Transmembrane	2.00E-05	-1.53
	SAUSA300_2401	-	Addiction Module Antitoxin	8.10E-05	-1.54
	SAUSA300_0385	-	Hypothetical Protein, Putative Transmembrane	1.80E-05	-1.54
	SAUSA300_2132	-	Hypothetical Protein Of Unknown Function	1.60E-04	-1.54
	SAUSA300_2528	-	Hypothetical Protein, Pepsy Domain-Containing	3.70E-05	-1.54
	SAUSA300_2093	-	Hypothetical Protein, Protein-Disulfide Reductase	8.80E-06	-1.55
	SAUSA300_1054	-	Hypothetical Protein, Putative Transmembrane	7.80E-05	-1.57
	SAUSA300_2624	-	Hypothetical Protein, Putative Transmembrane	2.50E-06	-1.57
	SAUSA300_1977	-	Hypothetical Protein, Putative Methyltransferase	1.50E-04	-1.58
	SAUSA300_0609	-	Phage Integrase Family Protein	1.40E-05	-1.61
	SAUSA300 1685	-	Hypothetical Protein, Putative Transmembrane	1.10E-04	-1.61
	SAUSA300_0940	-	Hypothetical Protein, Putative Transmembrane	1.40E-05	-1.68
	SAUSA300_0304	-	Hypothetical Protein Of Unknown Function	6 70E-06	-1.69
	SAUSA300 1803	-	Hypothetical Protein, Putative Transmembrane	5.00E-07	-17
	SAUSA300_1003	-	Hypothetical Protein, Putitive Antitoxin	1.40E.05	1.7
	SAUSA300_2402	-	Hypothetical Protein, Fullive Antitoxin	1.402-03	-1.71
	SAUSA300_0094	-	Lugethetical Protein Of Onknown Function	1.30L-04	-1.72
	SAUSA300_1495	-	Hypothetical Protein, Rhodanese-Like Domain	5.50E-06	-1.74
	SAUSA300_1213	-	Hypothetical Protein Of Unknown Function	4.20E-04	-1.74
	SAUSA300_2334	-	Hypothetical Protein, Peptidase Activity	1.80E-05	-1.75
	SAUSA300_1606	-	Hypothetical Protein Of Unknown Function	7.50E-06	-1.75
	SAUSA300_2252	-	Hypothetical Protein, Putative Transmembrane	4.00E-06	-1.79
	SAUSA300_1856	-	Hypothetical Protein, Putative Pfpi Endopeptidase	1.70E-06	-1.79
	SAUSA300_1099	-	Hypothetical Protein Of Unknown Function	3.90E-04	-1.8
	SAUSA300_0831	-	Hypothetical Protein Of Unknown Function	2.70E-05	-1.82
	SAUSA300_2080	-	Hypothetical Protein Of Unknown Function	2.50E-06	-1.84
	SAUSA300_2418	-	Hypothetical Protein, Putative Peroxiredoxin	3.60E-06	-1.87
	SAUSA300_1802	-	Hypothetical Protein Of Unknown Function	1.30E-05	-1.89
	SAUSA300_0668	-	Hypothetical Protein Of Unknown Function	1.10E-06	-1.9
	SAUSA300_1057	-	Hypothetical Protein Of Unknown Function	8.10E-06	-1.9
	SAUSA300_2368	bioX	Hypothetical Protein, Putative Transmembrane	1.20E-04	-1.93
	SAUSA300 2637	-	Hypothetical Protein, Putative Phage Tail Protein	6.30E-06	-1.93
	SAUSA300 1272	-	Hypothetical Protein, Swim-Type Domain-Containing	1.80E-05	-1.97
	SAUSA300 1212		Hypothetical Protein, Ntox50 Domain-Containing	5 40F-05	-2
	SALISAR00 2254	dshA	Putative Lipoprotein	1 10E-06	-2 02
	SALISAR00 2354	dshA	Putative Lipoprotein	1 10E-06	-2.02
	SALISA300 0342	-	Hypothetical Protein Of Linknown Function	1 305-05	-2.02
	SALISA200_0242		Hypothetical Protein Or Officiowin Function	1.5UE-U5	-2.04
	SVIIEV300 3300		Hypothetical Protein, Putative Transmembrane	2.4UE-05	-2.05
	SAUSA300_2206		Hypothetical Protein, Putative Transmembrane	4.00E-07	-2.05
	SAUSA300_0942	-	Hypothetical Protein, Pepsy Domain-Containing	3.00E-05	-2.05
	SAUSA300_2527	-	Hypothetical Protein Of Unknown Function	8.60E-06	-2.06
	SAUSA300_1041	-	Hypothetical Protein, Putative Transmembrane	8.80E-06	-2.07
	SAUSA300_0172	-	Hypothetical Protein Of Unknown Function	4.40E-06	-2.07
	SAUSA300_2543	-	Hypothetical Protein, Signal Transduction Protein Trap	1.80E-05	-2.14
	SAUSA300_1692	-	Hypothetical Protein, Putative Transmembrane	2.80E-05	-2.15
	SAUSA300_1230	-	Hypothetical Protein Of Unknown Function	1.10E-05	-2.17
	SAUSA300_2053	ywpF	T6Ss Effector-Like Protein Of Unknown Function	4.00E-06	-2.18
	SAUSA300_1221	-	Hypothetical Protein Of Unknown Function	3.10E-04	-2.18
	SAUSA300_0884	-	Hypothetical Protein Of Unknown Function	9.40E-06	-2.23
	SAUSA300_1004	-	Hypothetical Protein Of Unknown Function	8.40E-06	-2.27
	SAUSA300_0781	-	Hypothetical Protein Of Unknown Function	2.30E-05	-2.3
	SAUSA300_2562	-	Hypothetical Protein Of Unknown Function	6.60E-05	-2,32
	SAUSA300_0565	-	Hypothetical Protein, Putative Transmembrane	1.70E-06	-2.32
	SAUSA300 1326	rnhA	Putative Cell Wall Enzyme Ebsb	8.10E-06	-2.34
	SAUSA300 0929	-	Hypothetical Protein Of Unknown Function	1.30E-05	-2.34
	SAUSA300 0372	-	Hypothetical Protein, Pensy Domain	1.00F-07	-2.34
	SALISA300 1240		Hypothetical Protein Putative Transmembrane	1 10F-05	-2.30
	SAUSA300 0050		Hypothetical Protein, Putative Transmembrane	1 40F-05	-2.37

Table S1. The indirect WalR regulon defined by RNA-seq.

SAUSA300_1107 - Hypothetical Protein, Putative Transmembrane 2.50E-05 2.241 SAUSA300_1277 - Hypothetical Protein Of Unknown Function 1.10E-05 2.460 SAUSA300_1478 - Putative Lipoprotein 8.00E-07 2.260 SAUSA300_1478 - Putative Lipoprotein 8.00E-07 2.260 SAUSA300_2481 - Hypothetical Protein, Yoze Sam Like Domain 2.00E-05 2.267 SAUSA300_2033 - Putative Lipoprotein 5.00E-07 2.267 SAUSA300_20403 - Putative Lipoprotein 5.00E-07 2.267 SAUSA300_2043 - Putative Lipoprotein 5.00E-07 2.267 SAUSA300_2043 - Putative Lipoprotein 5.00E-07 2.267 SAUSA300_2044 - Hypothetical Protein, Noze Sam Like Domain 9.00E-06 2.271 SAUSA300_2544 - Hypothetical Protein Of Unknown Function 1.00E-07 2.267 SAUSA300_1208 - Hypothetical Protein Of Unknown Function 9.00E-06 2.281 SAUSA300_12128					
SAUSA300_1277 · Hypothetical Protein Of Unknown Function 1.10E-05 2.2.46 SAUSA300_1478 · Hypothetical Protein, Putative Transmembrane 3.50E-05 2.2.49 SAUSA300_1478 · Putative Lipoprotein 8.00E-07 2.2.6 SAUSA300_1478 · Putative Lipoprotein 8.00E-07 2.2.6 SAUSA300_2481 · Hypothetical Protein, Yoze Sam Like Domain 2.00E-05 2.2.60 SAUSA300_2403 · Putative Lipoprotein 5.00E-07 2.2.67 SAUSA300_2403 · Putative Lipoprotein 5.00E-07 2.2.67 SAUSA300_2403 · Putative Lipoprotein 5.00E-07 2.2.67 SAUSA300_2403 · Putative Lipoprotein 1.00E-07 2.2.67 SAUSA300_2404 · Hypothetical Protein Mice Domain 9.00E-07 2.7.71 SAUSA300_1432 · Hypothetical Protein Of Unknown Function 1.90E-05 2.7.41 SAUSA300_12026 · Hypothetical Protein Of Unknown Function 9.00E-07 2.80 SAUSA300_1218 · Hypothetical Protein Of Unknown Function 4.00E-07 2.80	SAUSA300_1107	-	Hypothetical Protein, Putative Transmembrane	2.50E-05	-2.41
SAUSA300_1484Hypothetical Protein, Putative Transmembrane3.50E-052.2.6SAUSA300_1478Putative Lipoprotein8.00E-072.2.6SAUSA300_1478Putative Lipoprotein8.00E-072.0.6SAUSA300_2481-Hypothetical Protein, Yoze Sam Like Domain2.00E-052.0.6SAUSA300_2403-Putative Lipoprotein5.00E-072.0.6SAUSA300_2403-Putative Lipoprotein5.00E-072.0.6SAUSA300_2403-Putative Lipoprotein5.00E-072.0.6SAUSA300_2404-Hypothetical Protein, Hh Cro/C1-Type Domain-5.00E-072.0.7SAUSA300_2405-Hypothetical Protein, Yoze Sam Like Domain9.00E-062.7.3SAUSA300_2404-Hypothetical Protein, Yoze Sam Like Domain9.00E-062.7.3SAUSA300_2544-Hypothetical Protein Of Unknown Function1.00E-072.0.7SAUSA300_20241-Hypothetical Protein Of Unknown Function1.00E-072.2.6SAUSA300_2026-Hypothetical Protein Of Unknown Function9.00E-062.8.6SAUSA300_1208-Hypothetical Protein Of Unknown Function5.00E-073.0.6SAUSA300_1333-Hypothetical Protein Of Unknown Function5.00E-073.0.6SAUSA300_2463-Hypothetical Protein Of Unknown Function5.00E-073.0.6SAUSA300_1333-Hypothetical Protein Of Unknown Function5.00E-073.0.6SAUSA300_1333-Hypothetical Protein Of Unknown Function5.00E-0	SAUSA300_1277	-	Hypothetical Protein Of Unknown Function	1.10E-05	-2.46
SAUSA300_1478·Putative Lipoprotein8.00E-072.26SAUSA300_1478·Putative Lipoprotein8.00E-072.26SAUSA300_2478·Hypothetical Protein, Yoze San Like Domain2.00E-052.26SAUSA300_2403·Putative Lipoprotein5.00E-072.267SAUSA300_2403·Putative Lipoprotein5.00E-072.67SAUSA300_2403·Putative Lipoprotein5.00E-072.67SAUSA300_2404·Hypothetical Protein, Bph 3 Domain9.00E-072.67SAUSA300_2414·Hypothetical Protein, Yoze San Like Domain9.09E-062.734SAUSA300_2414·Hypothetical Protein Network Function9.09E-062.734SAUSA300_2414·Hypothetical Protein Of Unknown Function9.09E-062.744SAUSA300_2041·Hypothetical Protein Of Unknown Function9.09E-062.806SAUSA300_2041·Hypothetical Protein Of Unknown Function9.09E-062.806SAUSA300_2043·Hypothetical Protein Of Unknown Function6.70E-062.806SAUSA300_2043·Hypothetical Protein Of Unknown Function6.70E-062.806SAUSA300_2043·Hypothetical Protein Of Unknown Function6.70E-062.925SAUSA300_1373·Hypothetical Protein Of Unknown Function6.70E-062.925SAUSA300_2043·Hypothetical Protein Of Unknown Function6.70E-073.107SAUSA300_1374·Hypothetical Protein Of Unknown Function7.0	SAUSA300_1484	-	Hypothetical Protein, Putative Transmembrane	3.50E-05	-2.49
SAUSA300_1478-Putative Lipoprotein8.00E-072.2.6SAUSA300_2481-Hypothetical Protein, Nto San Like Domain2.00E-052.6SAUSA300_2473-Hypothetical Protein, Hth Cro/C1-Type Domain-9.20E-06-2.66SAUSA300_2403-Putative Lipoprotein5.00E-07-2.67SAUSA300_2246-Hypothetical Protein, Bph_3 Domain1.00E-07-2.67SAUSA300_2246-Hypothetical Protein, Sph_3 Domain1.00E-07-2.67SAUSA300_2247-Hypothetical Protein, Yoze Sam Like Domain9.90E-06-2.73SAUSA300_2248-Hypothetical Protein Of Unknown Function1.90E-05-2.74SAUSA300_2244-Hypothetical Protein Of Unknown Function1.90E-05-2.74SAUSA300_2247-Hypothetical Protein Of Unknown Function9.90E-06-2.89SAUSA300_1208-Hypothetical Protein Of Unknown Function9.90E-06-2.89SAUSA300_2026-Hypothetical Protein Of Unknown Function5.00E-07-2.89SAUSA300_1391-Hypothetical Protein Of Unknown Function5.00E-07-2.89SAUSA300_1393-Hypothetical Protein Of Unknown Function-2.00E-07-2.89SAUSA300_1393-Hypothetical Protein Of Unknown Function-2.00E-07-3.10SAUSA300_2400-Acetyltransferase Family Protein-9.00E-07-3.11SAUSA300_2400-Acetyltransferase Family Protein-9.00E-07-3.11SAUSA300_2400- <td< td=""><td>SAUSA300_1478</td><td>-</td><td>Putative Lipoprotein</td><td>8.00E-07</td><td>-2.6</td></td<>	SAUSA300_1478	-	Putative Lipoprotein	8.00E-07	-2.6
SAUSA300_2481Hypothetical Protein, Yoze Sam Like Domain2.00E-052.2.6SAUSA300_0373Hypothetical Protein, Hth Cro/C1-Type Domain-9.20E-062.6.6SAUSA300_2403Putative Lipoprotein5.00E-072.6.7SAUSA300_2243Hypothetical Protein, Bph_3 Domain1.00E-072.6.7SAUSA300_2244Hypothetical Protein, Yoze Sam Like Domain9.90E-062.7.3SAUSA300_2544Hypothetical Protein, Yoze Sam Like Domain9.90E-062.7.3SAUSA300_1432Hypothetical Protein Of Unknown Function9.30E-062.7.4SAUSA300_1245Hypothetical Protein Of Unknown Function9.30E-062.8.1SAUSA300_2041Hypothetical Protein Of Unknown Function9.30E-062.8.1SAUSA300_2026Hypothetical Protein Of Unknown Function9.30E-062.8.1SAUSA300_1281Hypothetical Protein Of Unknown Function4.00E-072.8.9SAUSA300_1581Hypothetical Protein Of Unknown Function5.80E-062.9.2SAUSA300_1493Hypothetical Protein Of Unknown Function5.80E-062.9.2SAUSA300_1493Hypothetical Protein Of Unknown Function6.40E-062.9.2SAUSA300_1493Hypothetical Protein Of Unknown Function1.00E-073.0.1SAUSA300_1493Hypothetical Protein Of Unknown Function1.00E-073.0.1SAUSA300_1493Hypothetical Protein Of Unknown Function1.00E-073.0.1SAUSA300_1493Hypothetical Protein Of Unknown Function1.00E-073.0.1SAUSA300_1795Hypothetical Protein Of U	SAUSA300_1478	-	Putative Lipoprotein	8.00E-07	-2.6
SAUSA300_0373-Hypothetical Protein, Hth Cro/C1-Type Domain-9.20E-062.20E-06SAUSA300_2403-Putative Lipoprotein5.00E-07-2.67SAUSA300_2403-Putative Lipoprotein5.00E-07-2.67SAUSA300_2246-Hypothetical Protein, Bph_3 Domain1.00E-07-2.67SAUSA300_2544-Hypothetical Protein, Yoze Sam Like Domain9.90E-06-2.73SAUSA300_2544-Hypothetical Protein, Yoze Sam Like Domain9.90E-06-2.74SAUSA300_2041-Hypothetical Protein Of Unknown Function1.90E-05-2.74SAUSA300_2041-Hypothetical Protein Of Unknown Function9.30E-06-2.86SAUSA300_2056-Hypothetical Protein Of Unknown Function9.30E-06-2.86SAUSA300_2057-Hypothetical Protein Of Unknown Function5.00E-07-2.86SAUSA300_1581-Hypothetical Protein Of Unknown Function5.00E-06-2.86SAUSA300_1581-Hypothetical Protein Of Unknown Function5.00E-06-2.92SAUSA300_1793-Hypothetical Protein Of Unknown Function5.00E-06-2.93SAUSA300_1793-Hypothetical Protein Of Unknown Function-2.06-3.03SAUSA300_1793-Hypothetical Protein Of Unknown Function-0.06E-07-3.01SAUSA300_1795-Hypothetical Protein Of Unknown Function-0.06E-07-3.01SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.11SAUSA300_2460 <td>SAUSA300_2481</td> <td>-</td> <td>Hypothetical Protein, Yoze Sam Like Domain</td> <td>2.00E-05</td> <td>-2.6</td>	SAUSA300_2481	-	Hypothetical Protein, Yoze Sam Like Domain	2.00E-05	-2.6
SAUSA300_2403·Putative Lipoprotein5.00E-072.6.67SAUSA300_2403·Putative Lipoprotein5.00E-072.6.67SAUSA300_2264·Hypothetical Protein, Bph.3 Domain1.00E-072.6.67SAUSA300_2142·Hypothetical Protein, Bph.3 Domain9.90E-062.7.73SAUSA300_2143·Phisit Or178-Like Protein1.70E-052.7.44SAUSA300_1242·Hypothetical Protein Of Unknown Function1.90E-052.7.44SAUSA300_1243·Hypothetical Protein Of Unknown Function9.30E-062.8.61SAUSA300_1266·Hypothetical Protein Of Unknown Function6.70E-062.8.61SAUSA300_1281·Hypothetical Protein Of Unknown Function5.80E-062.9.92SAUSA300_2493wwrHypothetical Protein Of Unknown Function5.80E-062.9.92SAUSA300_1293·Hypothetical Protein Of Unknown Function6.40E-062.9.93SAUSA300_1093·Hypothetical Protein Of Unknown Function1.00E-073.01SAUSA300_1093·Hypothetical Protein Of Unknown Function1.00E-073.01SAUSA300_1093·Hypothetical Protein Of Unknown Function2.00E-073.01SAUSA300_1094·Hypothetical Protein Of Unknown Function9.00E-073.11SAUSA300_2460·Acetyltransferase Family Protein9.00E-073.11SAUSA300_1180·Hypothetical Protein Of Unknown Function1.00E-063.01SAUSA300_1180·Hypotheti	SAUSA300_0373	-	Hypothetical Protein, Hth Cro/C1-Type Domain-	9.20E-06	-2.66
SAUSA300_2403·Putative Lipoprotein5.00E-072.067SAUSA300_2246·Hypothetical Protein, Bph_3 Domain1.00E-07-2.67SAUSA300_2544·Hypothetical Protein, Yoze Sam Like Domain9.90E-06-2.73SAUSA300_1432·Phislt Orf78-Like Protein1.70E-05-2.74SAUSA300_2041·Hypothetical Protein Of Unknown Function9.30E-06-2.81SAUSA300_1208·Hypothetical Protein Of Unknown Function9.30E-06-2.81SAUSA300_20266·Hypothetical Protein Of Unknown Function6.70E-06-2.86SAUSA300_2493cwrAHypothetical Protein Of Unknown Function5.80E-06-2.92SAUSA300_2493cwrAHypothetical Protein, Cell Wall Stress Stimulon5.80E-06-2.93SAUSA300_1795·Hypothetical Protein Of Unknown Function6.40E-06-2.95SAUSA300_1795·Hypothetical Protein Of Unknown Function1.00E-07-3.11SAUSA300_1795·Hypothetical Protein Of Unknown Function2.00E-07-3.11SAUSA300_1795·Hypothetical Protein Of Unknown Function2.00E-07-3.11SAUSA300_2460·Acetyltransferase Family Protein9.00E-07-3.11SAUSA300_2460·Acetyltransferase Family Protein9.00E-07-3.11SAUSA300_1786·Hypothetical Protein Of Unknown Function1.00E-06-3.88SAUSA300_2460·Acetyltransferase Family Protein9.00E-07-3.11SAUSA300_1786	SAUSA300_2403	-	Putative Lipoprotein	5.00E-07	-2.67
SAUSA300_2264-Hypothetical Protein, Bph_3 Domain1.00E-07-2.67SAUSA300_2544-Hypothetical Protein, Yoze Sam Like Domain9.90E-06-2.73SAUSA300_1432-Phislt Orf78-Like Protein1.70E-05-2.74SAUSA300_2041-Hypothetical Protein Of Unknown Function1.90E-05-2.74SAUSA300_2041-Hypothetical Protein Of Unknown Function9.30E-06-2.86SAUSA300_20266-Hypothetical Protein Of Unknown Function6.70E-06-2.86SAUSA300_1581-Hypothetical Protein Of Unknown Function5.80E-06-2.92SAUSA300_0937-Hypothetical Protein Of Unknown Function5.80E-06-2.92SAUSA300_1937-Hypothetical Protein Of Unknown Function5.80E-06-2.93SAUSA300_1937-Hypothetical Protein Of Unknown Function6.40E-06-2.95SAUSA300_1937-Hypothetical Protein Of Unknown Function7.80E-06-3.03SAUSA300_1938-Hypothetical Protein Of Unknown Function1.00E-07-3.01SAUSA300_1908-Hypothetical Protein Of Unknown Function1.00E-07-3.01SAUSA300_22460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_1180	SAUSA300_2403	-	Putative Lipoprotein	5.00E-07	-2.67
SAUSA300_2544-Hypothetical Protein, Yoze Sam Like Domain9.90E-062.73SAUSA300_1432-Phist Orf78-Like Protein1.70E-052.74SAUSA300_2041-Hypothetical Protein Of Unknown Function1.90E-052.74SAUSA300_1208-Hypothetical Protein Of Unknown Function9.30E-062.81SAUSA300_1208-Hypothetical Protein Of Unknown Function6.70E-062.86SAUSA300_1281-Hypothetical Protein Of Unknown Function4.00E-072.89SAUSA300_2493cwrAHypothetical Protein, Cell Wall Stress Stimulon5.80E-062.923SAUSA300_1493-Hypothetical Protein Of Unknown Function6.40E-062.93SAUSA300_1493-Hypothetical Protein Of Unknown Function6.40E-062.93SAUSA300_1493-Hypothetical Protein Of Unknown Function7.80E-063.03SAUSA300_1493-Hypothetical Protein Of Unknown Function1.00E-07-3.04SAUSA300_1493-Hypothetical Protein Of Unknown Function1.00E-07-3.01SAUSA300_1285-Hypothetical Protein Of Unknown Function2.00E-07-3.11SAUSA300_2260-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2160-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2160-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2160-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2160	SAUSA300_2246	-	Hypothetical Protein, Bph_3 Domain	1.00E-07	-2.67
SAUSA300_1432·Phislt Orf78-Like Protein1.70E-052.7.4SAUSA300_2041·Hypothetical Protein Of Unknown Function1.90E-052.7.4SAUSA300_1208·Hypothetical Protein Of Unknown Function9.30E-062.8.1SAUSA300_0266·Hypothetical Protein Of Unknown Function6.70E-062.8.6SAUSA300_1581·Hypothetical Protein Of Unknown Function4.00E-072.8.9SAUSA300_2493cwrAHypothetical Protein, Cell Wall Stress Stimulon5.80E-062.9.9SAUSA300_0377·Hypothetical Protein, Putative Transmembrane9.90E-062.9.9SAUSA300_1193·Hypothetical Protein Of Unknown Function6.40E-062.9.9SAUSA300_1193·Hypothetical Protein Of Unknown Function7.80E-063.0.9SAUSA300_1193·Hypothetical Protein Of Unknown Function7.80E-063.0.9SAUSA300_1193·Hypothetical Protein Of Unknown Function9.00E-073.1.1SAUSA300_2460·Acetyltransferase Family Protein9.00E-073.1.7SAUSA300_2460·Acetyltransferase Family Protein9.00E-073.1.7SAUSA300_1180·Hypothetical Protein Of Unknown Function1.00E-073.1.7SAUSA300_1180·Hypothetical Protein Of Unknown Function1.00E-073.1.7SAUSA300_1180·Hypothetical Protein Of Unknown Function1.00E-073.1.7SAUSA300_1180·Hypothetical Protein Of Unknown Function1.00E-073.1.7 <td>SAUSA300_2544</td> <td>-</td> <td>Hypothetical Protein, Yoze Sam Like Domain</td> <td>9.90E-06</td> <td>-2.73</td>	SAUSA300_2544	-	Hypothetical Protein, Yoze Sam Like Domain	9.90E-06	-2.73
SAUSA300_2041-Hypothetical Protein Of Unknown Function1.90E-05-2.74SAUSA300_1208-Hypothetical Protein Of Unknown Function9.30E-06-2.81SAUSA300_0266-Hypothetical Protein Of Unknown Function6.70E-06-2.86SAUSA300_1581-Hypothetical Protein Of Unknown Function4.00E-07-2.89SAUSA300_2493cwrAHypothetical Protein, Cell Wall Stress Stimulon5.80E-06-2.92SAUSA300_0377-Hypothetical Protein, Putative Transmembrane9.90E-06-2.93SAUSA300_11493-Hypothetical Protein Of Unknown Function6.40E-06-2.95SAUSA300_1008-Hypothetical Protein Of Unknown Function7.80E-06-3.03SAUSA300_1008-Hypothetical Protein Of Unknown Function1.00E-07-3.17SAUSA300_2400-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-07-3.17SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-07<	SAUSA300_1432	-	Phislt Orf78-Like Protein	1.70E-05	-2.74
SAUSA300_1208-Hypothetical Protein Of Unknown Function9.30E-062.81SAUSA300_0266-Hypothetical Protein Of Unknown Function6.70E-062.86SAUSA300_1581-Hypothetical Protein Of Unknown Function4.00E-072.89SAUSA300_2493cwrAHypothetical Protein, Cell Wall Stress Stimulon5.80E-062.93SAUSA300_0377-Hypothetical Protein, Putative Transmembrane9.90E-062.93SAUSA300_1193-Hypothetical Protein Of Unknown Function6.40E-052.93SAUSA300_1008-Hypothetical Protein Of Unknown Function7.80E-063.03SAUSA300_1008-Hypothetical Protein Of Unknown Function7.80E-063.03SAUSA300_1008-Hypothetical Protein Of Unknown Function1.00E-073.01SAUSA300_2660-Acetyltransferase Family Protein9.00E-073.17SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-063.78SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-073.17SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-073.17SAUSA300_1180-Hypothetical Protein Of Unknown Function1.20E-063.83SAUSA300_1180-Hypothetical Protein Of Unknown Function1.20E-063.83SAUSA300_1180-Hypothetical Protein Of Unknown Function1.20E-063.83SAUSA300_1180-Hypothetical Protein Of Unknown Function1.20E-063.83 </td <td>SAUSA300_2041</td> <td>-</td> <td>Hypothetical Protein Of Unknown Function</td> <td>1.90E-05</td> <td>-2.74</td>	SAUSA300_2041	-	Hypothetical Protein Of Unknown Function	1.90E-05	-2.74
SAUSA300_0266-Hypothetical Protein Of Unknown Function6.70E-06-2.86SAUSA300_1581-Hypothetical Protein Of Unknown Function4.00E-07-2.89SAUSA300_2493cwrAHypothetical Protein, Cell Wall Stress Stimulon5.80E-06-2.92SAUSA300_0397-Hypothetical Protein, Putative Transmembrane9.90E-06-2.93SAUSA300_1493-Hypothetical Protein Of Unknown Function6.40E-06-2.95SAUSA300_1088-Hypothetical Protein Of Unknown Function7.80E-06-3.03SAUSA300_10795-Hypothetical Protein Of Unknown Function1.00E-07-3.04SAUSA300_2288-Hypothetical Protein Of Unknown Function2.00E-07-3.11SAUSA300_2400-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-06-3.78SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-06-3.78SAUSA300_1180-Hypothetical Protein Of Unknown Function1.20E-06-3.83SAUSA300_2461-Csbd-Like Superfamily Protein1.20E-06-3.83SAUSA300_1180-Hypothetical Protein Of Unknown Function1.20E-06-3.83SAUSA300_20816-Csbd-Like Superfamily Protein4.00E-07-4SAUSA300_1994-Hypothetical Protein, Putative Transmembrane5.00E-07-4.36	SAUSA300_1208	-	Hypothetical Protein Of Unknown Function	9.30E-06	-2.81
SAUSA300_1581-Hypothetical Protein Of Unknown Function4.00E-07-2.89SAUSA300_2493cwrAHypothetical Protein, Cell Wall Stress Stimulon5.80E-06-2.92SAUSA300_0937-Hypothetical Protein, Putative Transmembrane9.90E-06-2.93SAUSA300_1493-Hypothetical Protein Of Unknown Function6.40E-06-2.93SAUSA300_1008-Hypothetical Protein Of Unknown Function7.80E-06-3.03SAUSA300_10795-Hypothetical Protein Of Unknown Function1.00E-07-3.04SAUSA300_22328-Hypothetical Protein Of Unknown Function2.00E-07-3.11SAUSA300_2406-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-06-3.78SAUSA300_1215-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-06-3.78SAUSA300_215-Kcyltransferase Family Protein1.00E-06-3.78SAUSA300_1215-Hypothetical Protein Of Unknown Function1.20E-06-3.83SAUSA300_0816-Csbd-Like Superfamily Protein4.00E-07-4SAUSA300_1904-Hypothetical Protein, Putative Transmembrane5.00E-07-4.36	SAUSA300_0266	-	Hypothetical Protein Of Unknown Function	6.70E-06	-2.86
SAUSA300_2493cwrAHypothetical Protein, Cell Wall Stress Stimulon5.80E-062.92SAUSA300_0937-Hypothetical Protein, Putative Transmembrane9.90E-062.93SAUSA300_1493-Hypothetical Protein Of Unknown Function6.40E-062.95SAUSA300_1008-Hypothetical Protein Of Unknown Function7.80E-06-3.03SAUSA300_10795-Hypothetical Protein Of Unknown Function1.00E-07-3.04SAUSA300_2232-Hypothetical Protein Of Unknown Function2.00E-07-3.11SAUSA300_2400-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2400-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-06-3.78SAUSA300_1215-Kypothetical Protein Of Unknown Function1.20E-06-3.83SAUSA300_2036-Csbd-Like Superfamily Protein4.00E-07-4SAUSA300_1904-Hypothetical Protein, Putative Transmembrane5.00E-07-4.36	SAUSA300_1581	-	Hypothetical Protein Of Unknown Function	4.00E-07	-2.89
SAUSA300_0937Hypothetical Protein, Putative Transmembrane9.90E-062.93SAUSA300_1493Hypothetical Protein Of Unknown Function6.40E-062.95SAUSA300_1008Hypothetical Protein Of Unknown Function7.80E-06-3.03SAUSA300_1795Hypothetical Protein Of Unknown Function1.00E-07-3.04SAUSA300_2232Hypothetical Protein Of Unknown Function2.00E-07-3.1SAUSA300_2460Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460Hypothetical Protein Of Unknown Function1.00E-06-3.78SAUSA300_1180Hypothetical Protein Of Unknown Function1.20E-06-3.83SAUSA300_0816Csbd-Like Superfamily Protein4.00E-07-4SAUSA300_1904Hypothetical Protein, Putative Transmembrane5.00E-07-4.36	SAUSA300_2493	<i>cwrA</i>	Hypothetical Protein, Cell Wall Stress Stimulon	5.80E-06	-2.92
SAUSA300_1493-Hypothetical Protein Of Unknown Function6.40E-06-2.95SAUSA300_1008-Hypothetical Protein Of Unknown Function7.80E-06-3.03SAUSA300_1795-Hypothetical Protein Of Unknown Function1.00E-07-3.04SAUSA300_2328-Hypothetical Protein Of Unknown Function2.00E-07-3.11SAUSA300_22460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-06-3.78SAUSA300_2151-Hypothetical Protein Of Unknown Function1.20E-06-3.83SAUSA300_0816-Csbd-Like Superfamily Protein4.00E-07-4SAUSA300_1904-Hypothetical Protein, Putative Transmembrane5.00E-07-4.36	SAUSA300_0937	-	Hypothetical Protein, Putative Transmembrane	9.90E-06	-2.93
SAUSA300_1008-Hypothetical Protein Of Unknown Function7.80E-06-3.03SAUSA300_1795-Hypothetical Protein Of Unknown Function1.00E-07-3.04SAUSA300_2328-Hypothetical Protein Of Unknown Function2.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Hypothetical Protein Of Unknown Function1.00E-06-3.78SAUSA300_1180-Hypothetical Protein Of Unknown Function1.20E-06-3.88SAUSA300_816-Csbd-Like Superfamily Protein4.00E-07-4SAUSA300_1904-Hypothetical Protein, Putative Transmembrane5.00E-07-4.36	SAUSA300_1493	-	Hypothetical Protein Of Unknown Function	6.40E-06	-2.95
SAUSA300_1795-Hypothetical Protein Of Unknown Function1.00E-07-3.04SAUSA300_2328-Hypothetical Protein Of Unknown Function2.00E-07-3.1SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-06-3.78SAUSA300_1215-Hypothetical Protein Of Unknown Function1.20E-06-3.83SAUSA300_0816-Csbd-Like Superfamily Protein4.00E-07-4SAUSA300_1904-Hypothetical Protein, Putative Transmembrane5.00E-07-4.36	SAUSA300_1008	-	Hypothetical Protein Of Unknown Function	7.80E-06	-3.03
SAUSA300_2328Hypothetical Protein Of Unknown Function2.00E-07-3.1SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460-Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-06-3.78SAUSA300_1215-Hypothetical Protein Of Unknown Function1.20E-06-3.83SAUSA300_0816-Csbd-Like Superfamily Protein4.00E-07-4SAUSA300_1904-Hypothetical Protein, Putative Transmembrane5.00E-07-4.36	SAUSA300_1795	-	Hypothetical Protein Of Unknown Function	1.00E-07	-3.04
SAUSA300_2460Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_2460Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_1180Hypothetical Protein Of Unknown Function1.00E-06-3.78SAUSA300_1215Hypothetical Protein Of Unknown Function1.20E-06-3.83SAUSA300_0816Csbd-Like Superfamily Protein4.00E-07-4SAUSA300_1904Hypothetical Protein, Putative Transmembrane5.00E-07-4.36	SAUSA300_2328	-	Hypothetical Protein Of Unknown Function	2.00E-07	-3.1
SAUSA300_2460Acetyltransferase Family Protein9.00E-07-3.17SAUSA300_1180Hypothetical Protein Of Unknown Function1.00E-06-3.78SAUSA300_1215Hypothetical Protein Of Unknown Function1.20E-06-3.83SAUSA300_0816Csbd-Like Superfamily Protein4.00E-07-4SAUSA300_1904Hypothetical Protein, Putative Transmembrane5.00E-07-4.36	SAUSA300_2460	-	Acetyltransferase Family Protein	9.00E-07	-3.17
SAUSA300_1180-Hypothetical Protein Of Unknown Function1.00E-06-3.78SAUSA300_1215-Hypothetical Protein Of Unknown Function1.20E-06-3.83SAUSA300_0816-Csbd-Like Superfamily Protein4.00E-07-4SAUSA300_1904-Hypothetical Protein, Putative Transmembrane5.00E-07-4.36	SAUSA300_2460	-	Acetyltransferase Family Protein	9.00E-07	-3.17
SAUSA300_1215-Hypothetical Protein Of Unknown Function1.20E-06-3.83SAUSA300_0816-Csbd-Like Superfamily Protein4.00E-07-4SAUSA300_1904-Hypothetical Protein, Putative Transmembrane5.00E-07-4.36	SAUSA300_1180	-	Hypothetical Protein Of Unknown Function	1.00E-06	-3.78
SAUSA300_0816Csbd-Like Superfamily Protein4.00E-07-4SAUSA300_1904Hypothetical Protein, Putative Transmembrane5.00E-07-4.36	SAUSA300_1215	-	Hypothetical Protein Of Unknown Function	1.20E-06	-3.83
SAUSA300_1904 - Hypothetical Protein, Putative Transmembrane 5.00E-07 -4.36	SAUSA300_0816	-	Csbd-Like Superfamily Protein	4.00E-07	-4
	SAUSA300_1904	-	Hypothetical Protein, Putative Transmembrane	5.00E-07	-4.36



Figure S2. S. aureus ChIP-seq reveals binding sites of response regulators. Artemis userplots showing examples of the impact of non-target in silico read-subtraction for the identification of target-specific response regulator DNA-binding regions

Table S2: Putative response-regulator binding sites identified by ChIPseq

Locus ID	Gene name	Product	Function	Impact on gene regulation	Nebraska Tpn insert	ChIP-seq bind coords	Peak prediction score	Motif detected	Motif coords
WalR									
SAUSA300_0136	sasD	Cell-wall associated protein	Contains LPXAG motif – likely covalently anchored to peptidoglycan	Negative (5.8-log2 decrease, 56-fold)	Yes	154986155175	4.24929	Yes	155136155152
SAUSA300_0438	sle1	N-acetylmuramoyl-L-alanine amidase (autolysin)	cleaves amide bond between N-acetylmuramoyl and L-amino acids in peptidoglycan	Positive (3.5-log2 increase, 12-fold)	Yes	491543491550	0.97290	Yes	491559491575
SAUSA300_0524	rplJ	50S ribosomal protein L10	binds the two ribosomal protein L7/L12 dimers and anchors them to the large ribosomal subunit	No regulation	No	583428583441	0.62689	No	
SAUSA300_0602		Hypothetical protein	unknown	Negative (3.3-log2 decrease, 10-fold)	Yes	675677675892	5.77914	Yes	675820675847
SAUSA300_0653		AraC family transcriptional regulator	Transcriptional regulation	Negative (1.2-log2 decrease, 3-fold)	Yes	728668728678	0.64084	No	
SAUSA300_0681		hypothetical protein		Positive (2.3-log2 decrease in down mutant	No	755739755812	2.21698	No	
SAUSA300_0703	ItaS	Glycerol phosphate lipoteichoic acid synthase	Synthesis of polyglycerol- phosphate lipoteichoic acid (LTA) from phosphatidylglycerol	Negative (0.7-log decrease, 1.6- fold)	No	776863776913	2.43150	Yes	776866776882
SAUSA300_0860	rocD	ornithineoxo-acid transaminase		Positive (1.2-log2 increase, 2-fold)	Yes	937633937638	0.462098	Yes	937577937593
SAUSA300_1081		Hypothetical protein		No regulation	Yes	11829121182918	0.610952	No	
SAUSA300_1089	<i>lspA</i>	lipoprotein signal peptidase	lipoprotein signal peptidase; integral membrane protein that removes signal peptides from prolipoproteins during lipoprotein biosynthesis	No regulation	Yes	11916261191643	0.917025	Yes	11916061191622
SAUSA300_1188	mutS	DNA mismatch repair protein MutS	This protein performs the mismatch recognition step during the DNA repair process	No regulation	Yes	13065531306561	0.539114	No	
SAUSA300_1203		Hypothetical protein			Yes	13258321325849	1.02376	No	
SAUSA300_1304		Hypothetical protein		No regulation	Yes	14354031435418	1.04382	No	
SAUSA300_1344	dnaD	putative DNA replication protein DnaD		No regulation	No	15094721509481	0.554517	Yes	15094831509499
SAUSA300_1461		Hypothetical protein		No regulation	Yes	16158531615880	1.549569	No	

Table S2 continued. Putative response-regulator binding sites identified by ChIPseq.

Locus ID	Gene name	Product	Function	Impact on gene regulation	Nebraska Tpn insert	ChIP-seq bind coords	Peak prediction score	Motif detected	Motif coords
SAUSA300_1841 SAUSA300_1890	rrsC	16S rRNA staphopain A		Positive (0.85- log2 increase, 1.8- fold)	Yes	20029082002944 20550152055025	1.675390 0.740715	Yes No	20031592003174
SAUSA300_2124 SAUSA300_2209	rrsE	16S rRNA Hypothetical protein		Negative (0.84- log2 decrease, 1.8-fold)	Yes	22977622297799 23759872375993	2.261965 0.553028	No No	
SAUSA300_2249	ssaA_1	N-acetylmuramoyl-L-alanine amidase (autolysin)	cleaves amide bond between N-acetylmuramoyl and L-amino acids in peptidoglycan	Positive (1.3-log2 increase, 2.5-fold)	Yes	24148652415012	4.061749	Yes (2x)	24149182414933 24150462415061
SAUSA300_2506	isaA	Lytic transglycosylase	cleaves the β-1,4 glycosidic bond between N- acetylmuramic acid and N- acetylglucosamine residues of peptidoglycan	Positive (1.1-log2 increase, 2.2-fold)	No	27128082712836 (peak-1)	1.933075	Yes	27128972712912
SAUSA300_2506	isaA	Lytic transglycosylase		Positive (1.1-log2 increase, 2.2-fold)	No	27128702713227 (peak-2)	4.523005	Yes	27130892713104
VraR SAUSA300_0703	ltaS	Glycerol phosphate lipoteichoic acid synthase	Synthesis of polyglycerol- phosphate lipoteichoic acid (LTA) from			776616776813	5.536103	Yes	
SAUSA300_1357	aroC	chorismate synthase	catalyzes the formation of chorismate from 5-O-(1- carboxyvinyl)-3- phosphoshikimate in aromatic amino acid biosynthesis			15251991525211	0.8984054		
SAUSA300_2016	rrlD	23S ribosomal RNA							
HptR SAUSA300_0151	adhE	bifunctional acetaldehyde-				21790432179049	4.352250		
SAUSA300_0216	uhpT	Sugar phosphate antiporter	cytoplasmic membrane protein that functions as a monomer; catalyzes the active transport of sugar-phosphates such as glucose-6-phosphate with the obligatory exchange of inorganic phosphate or organophosphate"			254804254999	4.867318	Yes	
SAUSA300_0303		Hypothetical protein	5. <u>5</u> 991103911076			352882352918	1.4709476		

Table S2 continued. Putative response-regulator binding sites identified by ChIPseq.

Locus ID	Gene name	Product	Function	Impact on gene regulation	Nebraska Tpn insert	ChIP-seq bind coords	Peak prediction score	Motif detected	Motif coords
SAUSA300_0445	gltB	glutamate synthase, large subunit	an essential enzyme in the nonmevalonate pathway of isopentenyl diphosphate and dimethylallyl diphosphate biosynthesis			497269497311	2.7078340		
SAUSA300_0769		Hypothetical protein				857300857341	0.927730		
SAUSA300_0883		Putative surface protein				969867969932	1.503311		
SAUSA300_1052		fibrinogen-binding protein				11503721150493	2.338896		
SAUSA300_1058		alpha-hemolysin				11578821157919	1.312439		
SAUSA300_1463		Hypothetical protein				16179051617937	0.817594		
SAUSA300_1654		proline dipeptidase				18188971818945	1.959034		
SAUSA300_2012	leuC	isopropylmalate isomerase large subunit	dehydratase component, catalyzes the isomerization between 2-isopropylmalate and 3-isopropylmalate			21722372172454	5.527080		
SAUSA300_2504		acyltransferase				27109792711032 (peak-1)	2.532463		
SAUSA300_2538		amino acid permease family protein				27388472738937 (peak-2)	4.517702		
SAUSA300_2538		amino acid permease family protein				27393122739437	5.807741		
SaeR									
SAUSA300_0220	pflB	formate acetyltransferase				260249260326	2.245046		
SAUSA300_0472	ipk	4-diphosphocytidyl-2-C- methyl-D-erythritol kinase	An essential enzyme in the nonmevalonate pathway of isopentenyl diphosphate and dimethylallyl diphosphate biosynthesis			530663530696	1.313321		
SAUSA300_0892	оррА	oligopeptide ABC transporter oligopeptide-binding protein				979938980000	2.499846		
SAUSA300_0919	murE	UDP-N- acetylmuramoylalanyl-D- glutamateL-lysine ligase	involved in cell wall formation; peptidoglycan synthesis; cytoplasmic enzyme; catalyzes the addition of lysine to UDP- N-acetylmuramoyl-L-alanyl-D- glutamate forming UDP-N- acetylmuramoyl-L-alanyl-D- glutamyl-L-lysine			10083571008398	1.681862		
SAUSA300_1058	hla	Alpha-hemolysin				11577461157818	2.372307	Yes	
SAUSA300_1648		putative NADP-dependent malic enzyme				18111571811173	1.025598		

 Table S2 continued.
 Putative response-regulator binding sites identified by ChIPseq.

Locus ID	Gene name	Product	Function	Impact on gene regulation	Nebraska Tpn insert	ChIP-seq bind coords	Peak prediction score	Motif detected	Motif coords
SAUSA300 2144		Hypothetical protein				23207582320829	2.399629		
SAUSA300 2158		Hypothetical protein				23362112336232	0.992981		
SAUSA300_2423		Hypothetical protein				26090402609080	2.380980		
SAUSA300_2423		Hypothetical protein				(peak-1) 26091822609231 (peak-2)	2.487435		



Figure S3. Generation of an S. aureus WalR binding motif. A) A combined frequency plot of experimentally validated WalR binding motifs and those detected through ChIP-seq analysis. B) Upset plot comparison of overlap between intergenic WalR binding sites identified using a previously published motif (TGTWAHN₅TGTWAH) and that defined in A) (TGTHHNNNNWGTNDD).



Figure S4. A) Diversity of WalR motifs. Analysis of intergenic WalR motif diversity to investigate grouping by alterations to gene expression (left) or the functional class of regulated genes (right). **B) WalR binding site position in relation to expression change upon WalK activation.** The Log₂FC change in expression of genes with a predicted WalR binding site in relation to their distance from the -35 promoter element.

Table S4. Relative positions of predicted WaIR binding and transcriptional start sites (TSS).

Red; WalR binding, green; TSS , underlined; predicted -35 and -10 promoter elements, yellow; sRNA. Direction refers to the orientation of the WalR binding site in relation to the downstream gene.

Gene	Locus tag	Direction	Log2FC		Sequence
-	SAUSA300_2253	Rev	3.90	CTAATTTTTATTACAAAATTAGTCAAAGTTTTTTTTTTT	(79nt)ATG
sle1	SAUSA300_0438	Fwd	3.50	aattataaaatttgatgatacagtatatgatttttt <mark>tgtaatcataatgtcat</mark> caaaca <mark>tcaacc</mark> tattatacataataaaatcg <mark>tataat</mark> gatgtagta	(56nt)ATG
uhpT	SAUSA300_0216	Fwd	3.26	Aatatgtttctaagtatgtgtttatgttcagtattttggataatttaataatttaaggatattaagcgcttacaccgacgtggatgtggcttacggctgacgtgatgtggcttaacggatgtggatgatggatg	(21nt)ATG
-	SAUSA300_2503	Rev	3.09	ATTGGTATATTACATTCAATGAAGCTTTATTAGGAACAGATTACCATTATGATAACAAAGCCCCAAAGCCTAATCTCTGTGTTTTGTCGC	(121nt)ATG
atl	SAUSA300_0955	Fwd	2.85	TTTTTCATTTTTTACAGTGAAAAT <mark>GTAAATTAAGAGTAT</mark> ATTACAAATTGG <u>TTAAAA</u> CGCACAGGTATATAAAACAGGTAC <u>TAAAAT</u> GTTAGTAATAA	(28nt)ATG
amnA	SAUSA300 0435	Fwd	2.58	$\tt A {\tt TGTTAATATTTAGTAATTGCGTTGTGGAGGATTGGTGGACATTTTTATGGATTTAATTTGATAAATGTCATAGTAGTCTCI$	ACAATTCGTC
5p.	CAUSA200_2200	r	2.24	ATTGTCACATGAATTACTTATTTTTTAATTTTTTTAGAAAATTCGGACAATTGAACAATTGAACAATTAGAAATTGCAATTTACAATTATAACATATAATAG	(36nt)ATG
nigc	SAUSA300_2366	FW0	2.34		(31nt)ATG
IULA	3A03A500_2155	rwu/kev	2.20		CTTTAATATT
sasA	SAUSA300_2589	Rev	1.98	aaagatgtattttgcatataatatttatcgtttagt <mark>tgtaan</mark> ttaactatttataaataaaaggtggatggatggatat <u>agatatatt</u> aatacaataa	(255nt)ATG
nptA	SAUSA300_0107	Rev	1.85	CGATATTTTACAAGTCATATACAAATAACATATATTGTTAAATAATTTACCTAATCTTAACATTAAA <mark>ATTACA</mark> ATATAAAGC <mark>GATAAT</mark> CTAAATATAAA	(23nt)ATG
-	SAUSA300_0651	Rev	1.68	TTATAAAAATATGAACCGATATCCTAAAATGTTAATAAT <mark>ATTACAAGATAATAACAA</mark> AGC ACCACA AAGCTACTTATTTTGA TAATAT GGAAATCGTAAT	(83nt)ATG
metX	SAUSA300_0012	Rev	1.60	CGCATGACAATGATAACAACATTTTAAATGATAAAAGTAATTCATCACTGAATCTCAACTAACAC <mark>ATAACA</mark> ATTTCATATT <u>TCTTAATTGT</u> GAGAAGTTGA	(138nt)ATG
plc	SAUSA300_0099	Rev	1.52	төтсаатаалттестат <mark>аатасаттаттааааса</mark> тсааттааатаадетттаааат тттаса сататтттатттаалаадато <mark>татаат</mark> таатегаатта	(23nt)ATG
-	SAUSA300_0277	Fwd	1.48	GTATTTTAGCTTTTTTAAATAAATATATTGAATATACCCATATATTTTAA <mark>TTAACC</mark> ATTCATTTT <mark>GTAATATAAATGTGTAATACTAAAATAGA</mark>	(27nt)ATG
glpF	SAUSA300_1191	Rev	1.45	TTARACTACTTTTATARAGACTTGARARATTARATTTGACARARTTTTTACARARACCA TTGACA	(92nt)ATG
gInP	SAUSA300_1808	Rev	1.38	ACAGTCTTTTAAAAAGAAGCTTTTCTTTATATTGATAAAAATACATTTATAAAACACT <mark>ATTGCA</mark> TCAAAATGTATTAATATG TATTAAT TATGAATATTAA	(18nt) ATG
соа	SAUSA300_0224	Fwd	1.37	TAATTAACTATAACAAAAAGATAGTTAATGCTTTGTTTGT	(26nt) ATG
-	SAUSA300_2482	Rev	1.34	tacaattcaacactttatatgacaacttcattacagttacttttattgttgattg <u>cttaca</u> ttgttttctaaaaaaaatttgttat <u>cataat</u> taacgttg	(32nt) ATG
сорА	SAUSA300_0078	Fwd	1.32	TAACCTTGGTTAGAGTGTATTGCCATGGTGATGGTTAGAGTTAGAGTCTTTTTATTAAAACATGGTTGAGAGGGCGAGAGGCCGAGGGCGAGGGCGAGGGCGAGGGCGAGG	(125nt)ATG
ssaA	SAUSA300_2249	Rev	1.31	ALIACAMATIN'I HEIAVARACHARIN'I ICOAMACATICATIN'I CANAGARICUCUTAN'I MARCHI ILOGAMATOCAMATI TAAAATTGTATGTCAAGATATTAGGARATTAGGAAAAATTACAAAACGTTAACAAGGACGCTAATTTCTGTT <mark>TATGGC</mark>	(94nt)ATG
rocD	541154300 0860	Fwd	1 21	TGTATGCAATTTGTTAGTGGTTTTCAATATTTAT	ГАААТАААА
1000	34034300_0800	r wu	1.21	tattettagtaaaatataettttgattaagtgtaaaattateaattettttge ttgtaa reattaagtaattatgte <u>ttta</u> gaattatge	(142nt) ATG
glpQ	SAUSA300_0862	Fwd	1.18	TAATTCTTTTAAATTTATATTACAAAAATGTTATAAATTGTAAAAGAAATGTGTAAAGCGTT <u>TCACA</u> AGCAGGTTTTGTAGA <u>TATTT</u> AAAATTGTTAG	(32nt) ATG
isaA	SALISA300 2506	Rev/Fwd	1.09	$\label{eq:construction} Transformation and the second se$	facgaaagct fatttcaaca
15UA	54054500_2500	nev/rwa	1.05	TTACAAATAACACTTGATATTGTAATGTTTTGTAAAGAAAG	(100nt)ATG
dnaA	SAUSA300_0001	Rev	1.05	gtggataattagaaattacacaaagttatactatttttagcaacatattcacaggtat <mark>ttgaca</mark> tatagagaactgaaaag <mark>tataat</mark> ggtgata	(200nt)ATG
fadX	SAUSA300_0229	Fwd	1.05	TTATCGACCGTCAAAATG <mark>TGTCTATACTGTGTCTA</mark> TTATCAGAATAAAAAGATATTTCTAA <u>TTGATT</u> TTAAACGTCGTTATGT <u>TATATT</u> TCTTGTAAGGG	(40nt)ATG
ureA	SAUSA300_2238	Fwd	0.98	TCAATATCGGATATGCTAAAAATATAATTTTCTAATTACACATTGAACTTTGTAT <mark>ATTACT</mark> TGTTTATCACAACAATTAGGATTAA	(32nt)ATG
umuC	SAUSA300_1259	Fwd	0.90	TATATCATGTAATACTTAAAAATTCATTTGCAAAATAACAGAACATTTGTTCTAAAATAG TTGAAA TAGAACACGTGTTCGTA TATAAT AAGATTCAAGT	(15nt)ATG
mntH	SAUSA300_1005	Fwd	0.82	GAACGTTCAACATAATAATTCATACTTTTAAAAAAATTAAAATTAAGG <u>TTGACC</u> TAAACATTTTAGGTTATTAGGTTAT <mark>TATAT</mark> GTCCATAAGAAGTAGA	(11nt) ATG
-	SAUSA300_0350	Fwd	0.79	CATAGGTGGATAATCATATTTTTTGGTGTAGATTTGACAAATATAG <mark>TTGTCA</mark> AAAAGACGCATATCATT <mark>TACAGT</mark> GT <mark>AATTAAGGAGTTGA</mark> TGCGATGTG	(12nt) ATG
rplGB	SAUSA300_0529	Fwd	0.77	ATATAACAGAGGCTAATGCTTTAGCCTC <mark>TTGTTATTTTATGTAAA</mark> TTATTTGATTTAATG <mark>TTGACG</mark> AATTCTCTTGTTCAA <mark>TGTTAAT</mark> ATATAAAGGT	(31nt)ATG
				TGTAATGACAJ	ATGTAATGAG
lytM	SAUSA300_0270	Fwd	0.73	TTTAGTAAAACATTTCGGGAATATTAAATAGTTGGAAAATGAGAATTAAATCC <u>TTTACT</u> CAGTTGTCTAATTCTTT <u>TAGTAT</u> GTGCAGTACAG	(45nt)ATG
-	SAUSA300_0178	Fwd	0.72	TAATTTGTTATATCCTTTTAACTAGGAAAATATACATTTCGTAATAATAATAATCGTTATCA <mark>TTGAAA</mark> AAGTGTTAATAAGGTG <mark>TAAAAA</mark> GAAAATGTGA	(30nt)ATG
-	SAUSA300_0464	Fwd	0.71	TGTTATTATTAAGTGTGCACGCAGTATCATTAGTTATAAAATGTAGCTGTTAAAAG <mark>TCAAAA</mark> ATACATCGAATGTAGTAGG CCATATAAT ATAAAAAGAG	(46nt)ATG
prs	SAUSA300_0478	Fwd	0.68	TAGGATAAAAGGATAATCCTAT GTAATATTAATGTAA TCTTTATGATTTAATGA TTCGCA TAGTAATGGAGTTACATTTTATA TATAAT AGTAATTGCGT	(24nt)ATG
-	SAUSA300_0958	Rev	0.63	AAAAATGCTTTTTGATTTTAAC <mark>AAAACATTCAAATTACA</mark> AGGAACTTTGACATAACA <mark>TTTGTA</mark> TTTTTACTATAAAGTAC <u>TACAAAT</u> TTAAGGCTATAAT	(95nt)ATG
-	SAUSA300_0739	Rev	0.61	ATGATAAAAGGTTGTATTCTTTTTATATTGTTAACCATTTG ATTACATCGTTATAACAA TAGC <u>TTTTGA</u> CAAAATGTATTGTGCGT <u>ATAGT</u> ATTTGCATAC	(33nt) ATG
manA	SAUSA300_2096	Fwd	0.60	TATAAATTTTTGAAAA <mark>TGTTAAAGTACTGTAAA</mark> TTTAAGTGAAGCGCTTTAA <mark>TTGGCA</mark> GTACTGCAATGTTAGTAAATATGC <u>TACAAA</u> CACTAATAGTTA	(111nt)ATG
-	SAUSA300_1574	Fwd	0.58	$\texttt{GTACACCACCTGAAAATATCTCAAAAATCATTAAGCTTTATAAGATTACATTAAAAA\underbrace{\texttt{TCTATA}}_{\texttt{A}}\texttt{ATTTAAAGCCATAATAATCTAAGAT}_\texttt{GATAGTGAGTT}$	(27nt)ATG
nos	SAUSA300_1895	Rev	0.55	TTAATTTCCATACTTTTAATTAAAATCAACCAACAATTTAATGACATATACATAATTTT <u>TAAGA</u> GTATTTTAATAATGTAGAC <u>TATAAT</u> ATAAAGCGAGG	(8nt)ATG
rplK	SAUSA300_0522	Fwd	0.54	$\texttt{Atagaaaagctttaattaacaattaaagttattaaactaaccaaaagataaaaaagagta\texttt{tt}ttttaattagaaaagtgt\texttt{tt}aattagaaaagtgt\texttt{tt}$	(95nt)ATG
-	SAUSA300_0606	Fwd	0.46	ATTGTCTAGGGTGATTTTGTTGTAATAATGTCCTGTTTTTTCAACATTAT <mark>TGTAAATGAGT<mark>TGTATA</mark>TTTCTCTATAATGATAATGTAAATATATGAT</mark>	(36nt)ATG
русА	SAUSA300_1014	Fwd	0.46	CTGAAGCGGAGGTTTTAAT <mark>TGTCTCAAGAATGTTAA</mark> ATTTTGAAAATTTGAAACATAACTA <mark>TAGCAA</mark> AACAGAGCGCTTTAAGA <u>TAAAAT</u> TTTATTATCT	(42nt)ATG
fnbB	SAUSA300_2440	Rev	0.43	AAAAACTCAT <mark>TATACATAAAGTTAACA</mark> ACAATCTTAACTTTATTAACTCGCTTT TTTAT	(47nt)ATG
dnaD	SAUSA300_1344	Fwd	0.41	TTAAAAAATACTGTTATTTAAAATTTG <mark>TTTGCTACTAGTTAA</mark> ATATATTAAGCATTTTAG TCGAAA ATTAAAGAAAATGATT TATACT ATTAAGTAATGG	(24nt)ATG
-	SAUSA300_0451	Fwd	0.39	GTTTGAATGATTTTT <mark>GTGTCAATGAAAAGTAAG</mark> AAGTTATAATTTGATGATAAAGAAATGATG <mark>GTGAAA</mark> TGAGGGGGGAGTATCT <u>TACAAT</u> AGAATTATTA	(47nt)ATG
-	SAUSA300_0371	Rev	0.37	TAACTTAGTTTAAAAACGATTCGTATCTTTCAGATTCAAATACCATCATTTTCTCCTAATA <mark>CTTACA</mark> CTTTAATTACAATT <mark>ATGTAAGTT</mark> GTTTTCAGAT	(34mt) ATG
-	SAUSA300_0655	Fwd	0.35	TTTTAAAACTAAGTAACAGTTTGAAGAAATCGTAGTTCAATAATGTTAATTGTGAAAA <u>TGTATA</u> TAAACATAAAAAAATCATG <mark>TATAAT</mark> ATATGTTGTTA	(29nt)ATG
-	SAUSA300_1534	Fwd	0.32	ATTAAAAAAAGTTATATAGTTTATAAATAATCAAATTGATATTCTATAGGTTCTTATAAC TATAAA GTATATTCAATTTCAATTTCAATATAATGTGAGGG	(36nt) ATG
-	SAUSA300_1339	Rev	0.29	ATTTACKGATAACKCATTTGAAAAAATAGTTAAATCAAATTATATAGKGTGTTAAAAC <mark>ATGACG</mark> AATACKGTATATTGGA TAAAT AAAAAATGGTAGG	(10nt) ATG
glpK	SAUSA300_1192	Rev	0.21	AATAAAAGAAACGTAAATAGCATAATTTAACATGTTGATTCATGCATTATCCTATTTTT <u>TCGCA</u> AAATTTAACAGATTTTG <u>TACAAT</u> GGGTTACGAT	(28nt) ATG
SUJB	SAUSA300_0822	FWO	0.20		(56nt)ATG (14nt)ATC
opp-54	SAUSASU0_0035	Rev	0.10		(1411C)AIG (99nt)ATG
	SAUSA300 2456	Rev	-0.01	TTAATGAAATGACCGACACCCTCGCTTCTACTCCAATTTAACAAAA7ACTATA TTAGTA CTAATTTTCATTAACAATGACATGATATCT TACTATCATTA	(204n+) ATG
-	SAUSA300_1905	Rev	-0.02	ACCTCARACCANTTTATATTTCATTTAGTAAAATATAACTTTATTTAACATGACATTCAATATACATAC	(21nt) ATG
putA	SAUSA300 1711	Rev	-0.10	tcarcartartartartartarcataattatttttgattgattgttttgatgataacgctttttttt	(21nt) ATG
-	SAUSA300_0390	Rev	-0.11	tataagccacatgtcactattatcgcagctagctgcgtcactcaaaacgaatacggaacgcg <mark>ttggtt</mark> attcaatcataa <mark>tattactct</mark> gcaaatacac	(74nt)ATG
sdrE	SAUSA300_0548	Fwd	-0.11	TTAATTTATTCAAACCAAAG <mark>TGTAAAATAACAGTCTT</mark> AGATAAATTAAATTATATTAAAGTA <u>TTGTGC</u> TTTATCTAAAAAATGTAT <mark>TACGAT</mark> GGGAATACAA	(59nt)ATG
-	SAUSA300_0383	Rev	-0.13	tttaaacaaacttgaaatcactaacaataacgtttaaactatgtatg	(22nt)ATG
IspA	SAUSA300 1089	Fwd	-0.15	TC	JTCAAAACAT
	CAUCA200, 2054	r	0.10	ASTAGTTIATCAAGTATTGAGTAGTAGCATTGAGTTAATGATTTAATGATTATG	(31nt)ATG
ahns	SAUSASU0_2051	Fwd	-0.18		(19nt) ATG
eops	SAUSA300_1370	Fwd	-0.21		(10nt)ATG
rnz	SAUSA300_1312	Rev	-0.25	GENERATING THE CONTROL OF A C	(13nt)ATG
rqcH	SAUSA300 1101	Rev	-0.36	ATTGAATTGCAAACTTTTAGATAATGTAAAATGTATGGCATAATGTATGGTTCAATAACT <mark>TATACT</mark> GAAAAGTTACAATCATGT TAAAAT GAAACGAATGA	(24nt) ATG
-	SAUSA300_0876	Rev	-0.39	TTCCAAGAGTGTACAATTCATTTTCTAGTTGAATATTTTCTTGAAAATTTTTATAATAAATTTAATTTATA <mark>ATTACAGTTATAT<mark>TACAAT</mark>ACATAATACTT</mark>	(216nt) ATG
ktrB	SAUSA300_1979	Rev	-0.43	TCATTTTCAATTATAAAATATTAAAAGAATTAGTCAACGCCTGTTAGT <mark>AATACACATCA<mark>GTAACA</mark>TTCTATTTTCATTTATGA<mark>TATTAT</mark>CTAATTATTA</mark>	(30nt)ATG
pstS		Rev	-0.45	AAGCATTTTAATTTTACTAATGAAGCAATATTTTTTAGATTAACAAAAATTAATA TTTACA TTTTCTTAACAATTTTTTATG TAACAT TTACAGTTTCTA	(84nt)ATG
adhF	- SAUSA300_0151	Rev	-0.47	AAAACACCGAAATAACAATGATTTCATGAAAACATT	FATTTTAAAA
GUIL	SAUGA200_0151	nev i	0.47	TTTGATATTTGTTCAAATAATATTCGAAATAATAACTTTTTTGTATAGAATTTTCTT <u>TTTTATA</u> TCCTGAGAGACATGTACAATGTTGTGAAAA	(30nt) ATG
- fhc	SAUSA300_1846	Fwd	-0.48	AUTAMAGTITTAUATUGUTATUGUTATUAGTATTATGTATGATTTATTT	(40nt)ATG
JIIS roc^	SAUSA300_1678	Rev	-0.50	alaalachaa diilaalaadigaaliilailgeaaaatgaaagugutaaccoga caaraactigaa guudaa aabaa aabaa aabaa aabaa aabaa aabaa a	(0/NT)ATG
rpsA -	SAUSA300_1365	FWO	-0.60	iaaao igacatati ta igiata igacia ti ilogcaaa to taa tuggaga tagaa tutu tugtaa tugta a tuga a sa sa sa sa sa sa Concepterte a ca ca sa	(18n+)ATG
ltaS	24034200_1902	Rev	-0.64	COLCETETETERCECCECCETERETERTERTERTERTERTERTERTERTERTERTERTE	(262n+) ATG
nhoP	SAUSA300_0705	Fwd	-0.64	TGTCCCAGAGTAAGTCATGCAAATGATAGAAGGAAATATAAGAAGTATTTA TTTATA TGTAAAGTAAGTAAAGTA	(61nt) ATC
prior	20034200_1023	1 100	-0.04		,/MIG

isdB	SAUSA300_1028	Fwd	-0.65	aacctatgtcatagatatttcataatctataacataggttattttttataaaataacg <u>ttgcaa</u> ttaactaacatttcaatg <mark>tacaat</mark> acaagtaatca (52nt) atg
-	SAUSA300_1026	Fwd	-0.66	ATTCGTCCTTTCGGTATTCATTATTAATATTGTAACAGAACATGATATGTTAAGAAAAAATC TTGACA ACTTGTTCTTAGAAAGT TAAAAT AAATTTTGT (21nt)ATG
ackA	SAUSA300_1657	Fwd	-0.66	tgacagagttaaatcagtggatggacacaaatcgtcctaaaaataatcgtgtaatagta <mark>tagtca</mark> taaaactgtatgaatga tat gaaagtgaaaatgaaat (34nt) atg
-	SAUSA300_2592	Rev	-0.66	gagtgaacagaattatatttccatagcaaacattcctaa <mark>actacacatacgttaca</mark> taa <mark>ttgatt</mark> ca tttata tagatat
scdA	SAUSA300_0253	Rev	-0.72	ttgtgagccaacatgattgagggctttatttgcggtt <mark>tatgacatgattatgacat<u>ttccct</u>gattttcatttcatataa<mark>cattaaatt</mark>gtatacact(22nt)atg</mark>
-	SAUSA300_0365	Fwd	-0.72	TTCATATCATTTTTGATATTAATTCATTTGAAACTTTCTATGATATTTTTAAAAATACAC <u>TTCACA</u> AAAGCGAACATAT <mark>GTTC<u>TATAAT</u>AGTTGTGAGGT (8nt)ATG</mark>
-	SAUSA300_0657	Rev	-0.73	tagtaaaataataataataatatttcatatgattaacaaaaactat a aaactgtatc a tgacatcattatgtagtatttat a tgacataataatgatgatgatgatgatgatgatgatgatgatga
-	SAUSA300_1463	Fwd	-0.74	Ataatgtttggtatat <mark>tgttaaaattgtgtctaa</mark> atataggtgtgattcagattagttta ttgac aatatgttattattaattag <u>tagaat</u> gaggatagttt (28nt) atg
tarF	SAUSA300_0248	Fwd	-0.76	TGTTATGTTAA AGTGGTACATTAATCATGTATTTCGTATGATAATTAACGACAAGTGTAA <u>TGGTTA</u> AATGTATTTTATGATGAAATGC <u>TATAAT</u> AGGCATGGTT(57nt)ATG
-	SAUSA300_1278	Fwd	-0.79	$\texttt{GCARTATTCTATCTATCTATAGAAAAATCATTGTTCCTTGCGACATGGAAATC\underline{\texttt{GTAACA}}TTATCGTTTAGGAGACAAAATTATC\underline{\texttt{TATAAT}}GAATGTATTA(18nt)ATG$
-	SAUSA300_1510	Rev	-0.79	aggatttcaattgaatataatatttagaattgtcgcataattcatcatgacaacataatgaca tgatattgtattagaca tgatattgtattaat agaattgaattg 14nt atg tgatattgaattgaattgaatta 14nt atg tgatattgaattgaata tgatattgaattaattaattaattaattaattaattaat
hchA	SAUSA300_0536	Fwd	-0.80	atttagttgtatttttttcaaagaaattcattttgattatttttgataatgagcatt ttaata gtaatacatgtttatagtg <mark>tgtagt</mark> atatgtctatac (35nt) atg
paiA	SAUSA300_2316	Rev/Fwd	-0.91	tgaaaatacggccattttactggaataaatgtatcatttattgatgttacacaacaatc <mark>aattaca</mark> atttgtaattgatttgt <mark>gttaat</mark> gtatttttaaata(63nt)atg
-	SAUSA300_2254	Rev	-1.07	gttatatcacctccatcaaaagtacgcg <mark>cttacatcattgataca</mark> caagacatc <mark>atgaca</mark> tagttgtatactgtctaatcttctac <mark>tatact</mark> aagataaa (14nt) atg
rpsP	SAUSA300_1131	Rev	-1.09	TCACATAGTTATTATTGTAAGAAAAAGGTTCAATAATTTTCCTGGTAAAGAAAAACTC <mark>TTTACA</mark> AACATTCATACACCTGTTAA <u>TATTAT</u> TTCTTGTAG(40nt)ATG
sodA	SAUSA300_1513	Rev	-1.14	CATTICATACICCGTICGTATGATITTATGGCAAATCIG <mark>TTAACITTITATATACIAAAIGTITAAITATITTGATITGAGI<mark>TATATI</mark>AAAIGAGTAGA(96nt)AIG</mark>
walJ	SAUSA300_0024	Fwd	-1.20	cgcatttattcatattttaagtagaaccgcattgtaaaattagtgtaactgttattttaaaaac <u>tttagt</u> atttgtcattgt <mark>taytag</mark> tattggt
bstA	SAUSA300_2626	Fwd	-1.22	ttgagaacttttcgtcaactatcttttat <mark>tgtaaggtagttgttgt</mark> acacattccttaa atg aacaactttgttaatag <mark>ggtaat</mark> acttacggaagt (29nt)atg
sasF	SAUSA300_2581	Fwd	-1.35	ttaaatataagctaagtaataagtagataattactaaccaataactagataga
cspC	SAUSA300_0777	Fwd	-1.45	tattaaaatggaatgttactatatagttcaatgtgtattatcacagaaaataaaatgatagc <mark>t<u>ttact</u>tcatatttaaaagtgtataatgaaagttaagt (109nt) atg</mark>
-	SAUSA300_0374	Fwd	-1.49	aataacgatagctacattgaataaaattgatattcaattactactttaaaaatatt <mark>tggata</mark> aaaataatttgaattt <mark>tagaat</mark> tg <mark>taaa</mark> taagg (50nt) atg
-	SAUSA300_1296	Fwd	-1.53	tttacttaaaaaaactaataattactatagttacttactt
spa	SAUSA300_0113	Rev	-1.60	tttataagttgtaaaacttacctttaaatttaattataaatatagattttagtat $\mathbf{n}_{\mathbf{TGCAA}}$ tacataattcgttatat $\mathbf{t}_{\mathbf{TATGAT}}$ gactttacaaatacata(13nt) atg
tagG	SAUSA300_0625	Fwd	-1.73	TGATTGTATACTATAATGTATTTGTAATAAACTAATATTTTAAAGAACTAGACAATAATT <mark>TTGATA</mark> GCATCCA <mark>TGTATAGTGA<mark>TAGTAT</mark>TTACAACAATT(62nt)ATG</mark>
-	SAUSA300_1070	Fwd	-1.78	tgaaaatcaatttaatcatggaaaatttataatattcattgtttacattttcaaatca \mathbf{atgaa} aacacaagtggtttaat $\mathbf{ctataat}$ aatagtagtagta (2 1nt) atg
csbD	SAUSA300_1582	Rev	-1.83	CATGTTTTATATTTAATCGTATA <mark>AATACTATACCATAAACA</mark> TAAAAACTTCATATTATAATGT <mark>TTAGCG</mark> AACCTCCTTAGTGGTA <u>TAAAA</u> TATATACATC(15nt)ATG
-	SAUSA300_0682	Fwd	-1.86	ttaatttaaaaaggtgaattcaacttataaaatgatgtaaatgttatgtcaaaa \mathbf{r} caaccaatccgtaatgtatttaaaatgt \mathbf{r} agttctgaag(21nt)atg
-	SAUSA300_0985	Fwd	-2.14	gtatttttaaaacattatgcgctatgaaattg <mark>tgtataatttatgtcag</mark> ttcacaatgtg <mark>ttcaca</mark> aatttgaatttcaatg tataat tgtgtatattac (30nt) atg
-	SAUSA300_1965	Fwd	-2.39	gtgataagtacatgtgtttcaggtattggaactgttacgtttaaaatgt $\underline{\mathbf{otggca}}$ tttctatctttcctttcgtg $\underline{\mathbf{tataat}}$ gtt $\underline{\mathbf{ctcttacttact}}$ (14nt) atg
-	SAUSA300_0992	Fwd	-2.48	Atgacgtaactgtcaacagatatacttagtaatgaag <mark>aggtgtaatgtaa</mark>
таоС	SAUSA300_0031	Rev	-2.93	agataaaatgetgeattteteteaatagaaagttagagetgetettecgtttegetgeget t agaettacettaacatgettacettaacatgetaaaaaattaa (24nt) atg
-	SAUSA300_0602	Fwd	-3.24	CAACAAACATCACGATGAAAAGCATGTTTAATTTTAGTGATAAGTGAAAT <mark>ATAAAA</mark> GTAGTAATTATTATTTGTAA <mark>TATAAT</mark> TG <mark>TAATATGACTGTTG</mark> T (66nt)ATG
sasD	SAUSA300_0136	Fwd	-5.82	aarctaaagtgaaarttittataaaaaaatgtaatgattcaaaarttitg \mathbf{trgcat} tictittigtaatcgtat \mathbf{gataat} gtaaa $\mathbf{tgtaaatgataatgataatgataat}$ (16nt) atg
teg41	srn_1080	Rev	#N/A	TACAGGTGCA <mark>TTTACAAAATCTTTACAA</mark> TCATTGGTTTAATAGAGGTTAAGCTCATGAAT <mark>TTGACA</mark> TGAAGCAGAAATTTAAT <mark>TATATT</mark> TTATGTTATAA



Figure S5. Purification of recombinant WalR. WalR was purified by single step immobilized metal-ion affinity chromatography using Cobalt TALON resin. SDS PAGE image shows cellular fractionation and purification. Black arrow denotes 6-His-WalR (28kDa).





Figure S6. DNA competition EMSAs to test binding specificity **of WalR.** Competition electrophoretic mobility shift assay (EMSA) showing the effect on WalR binding of the presence or absence (–) of an excess of non-specific (ns) or specific (s) non-labelled duplex competitor).



Figure S7. WalR and VraR binding at the ItaS locus. A) Artemis userplot showing VraR and WalR ChIP-seq peaks. Box shows sequence 529 bp upstream of ItaS start codon. Bases coloured green correspond to VraR binding peak, red to WalR. Transcriptional start site in orange, -35 and -10 promoter elements are in blue, and VraR and WalR binding motifs are bold and underlined. Bases underlined in green and red correspond to positions of the pltaS_{vraR} and pltaS_{walR} duplex probes respectably. These probes were used for EMSAs (see below). B) Dose dependent band shifts caused by WalR (left) and VraR (right) binding of ItaS promoter regions. C) Competition EMSA showing band shifts for phosphorylated VraR in the presence or absence (–) of an excess of non-specific (ns) or specific (s) non-labelled duplex competitor.



Figure S8. Impact of WalR motif mutation on the expression from tarF, tagG and dnaD. The above genes coupled to bacterial luciferase reporters showing the changes in promoter activity of the Wt (open grey circle: RLU; filled black circles: OD600nm) or the ccc (open red diamond:RLU; filled black diamond: OD600nm) mutated WalR binding site in LB media over time. Data represent the mean of three independent experiments (± standard deviation).



Figure S9. The effect of walR, hup, and hla on Ori:Ter ratio. Graphs show log2-transformed coverage values (y axis) at relative positions across the genome (x axis). Cumulative GC-skew is plotted on the second Y axis. The calculated position of the origin of replication (consensus ori) is marked in red. Four replicates of each condition are shown. Ptr is the peak to trough ratio, which is equal to the Ori:Ter ratio. Graphs and calculations were made using the iRep R package (<u>https://github.com/christophertbrown/iRep</u>).

Table S5. Strains and plasmids used in this study

Escherichia coli strains	Description	Reference / Supplier
IM08B	DH10BAdcm. Expresses CC8 adenine methylation profile for direct transformation of NRS384.	(1)
Rosetta 2(DE3)	B-strain. F ⁻ ompT hsdSB (r _B ⁻ m _B ⁻) gal dcm (DE3); IPTG-inducible T7 RNA polymerase. For protein production.	Novagen
Staphylococcus aureus strains	Description	Reference / Supplier
RN4220	ST8; CC8; chemically mutagenized derivative of 8325-4, transformable with E. coli DNA; premature stop codon in	(2)
NRS384	USA300-14 clone. Tetracycline resistant.	BEI resources
NRS384 ∆spa	Clean deletion of protein A from TTG to TAA.	This study
NRS384 ΔyycHl	Deletion of WalKR positive regulators yycHI. Clean deletion from codon 5 of yycH to the TAA of yycl.	(3)
NRS384 <i>walR</i> ^{T101A}	The mutation introduces a T->A amino acid change at residue 101. Serine threonine kinase phosphorylation site. Exhibits reduced WalKR activity.	This study
NRS384 walk ^{Y32C}	The mutation introduces a Y->C amino acid change at residue 32 (first transmembrane domain) of WalK. Exhibits increased WalKR activity.	This study
NRS384 <i>walK</i> ^{T389A}	The mutation introduces a T->A amino acid change at residue 389 (within the HE/DXXT/N of HisKA histidine kinases) predicted to prevent the desphosphorylation of WalR. Exhibits increased WalKR activity.	This study
NRS384 <i>walR</i> -SmBIT	Small-Bit split luciferase tag incorporated onto the C-terminus of WalR (native location)	This study
NRS384 walK-LgBIT	Large-Bit split luciferase tag incorporated onto the C-terminus	This study
NRS384 walRFLAG	FLAG-tag introduced on the C-terminus of WalR in the Wt strain.	(3)
Plasmids	Description	Reference / Supplier
pIMAY-Z	Allelic exchange plasmid. Cm(R)	(1)
pET28(a)	IPTG inducible protein production plasmid. Kan(R)	Novagen
pET21d	IPTG inducible protein production plasmid. Amp(R)	Novagen
pRAB11-FT	Anhydrotetracycline inducible E. coli / S. aureus shuttle vector. pC194 rep. C-terminal 1x FLAG. Amp(R)	This study and (4)
pIMC8-YFP	Enhanced YFP reporter plasmid. Cm(R)	(3)
pSD1	CRISPRi knockdown plasmid. Amp(R) Cm(R)	(5)
pIMK1-LUX	Bacterial luciferase reporter plasmid. pSK41 low copy number replicon. Kan(R)	This study
pSmBIT	pRAB11 backbone (pC194 replicon) with SmBIT split luciferase. Amp(R) Cm(R).	This study
pLgBIT	pCN34 backbone (pT181 replicon). TetR/tetO ex pRAB11 introduced. LgBIT split luciferase. Amp(R) Kan(R).	This study
pLOW	pSK41 low copy number replicon for IPTG inducible expression in S. aureus. Amp(R) Cm(R).	(6)
pCN34	S. aureus-E. coli shuttle vector, pT181-cop-wt repC CoIE1 Kan(R).	(7)
Allelic exchange const	ructs for S. aureus.	
pIMAY-Z ∆spa	For construction of protein A deficient strain by allelic exchange.	This study
pIMAY-Z walR T101A	For construction of WalR T101A mutation by allelic exchange.	This study
pIMAY-Z walK ^{Y32C}	For construction of WalK Y32C mutation by allelic exchange.	This study
рІМАҮ-Z <i>walK</i> ^{тз89А}	For construction of WalK T389A mutation by allelic exchange.	This study
pIMAY-Z	For construction of a SmBIT C-terminal WalR by allelic exchange.	This study
pIMAY-Z <i>walR</i> (LgBIT)	For construction of a LgBIT C-terminal WalK by allelic exchange.	This study
ChIP-seq response reg	ulator overexpression constructs for <i>S. aureus</i> .	
pRAB11: walR ^{FLAG}	ATc inducible plasmid containing of C-terminally FLAG-tagged WalR, for ChIP-seq.	This study
pRAB11: vraR ^{FLAG}	ATc inducible plasmid containing of C-terminally FLAG-tagged VraR, for ChIP-seq.	This study
pRAB11: <i>hptR</i> ^{FLAG}	ATc inducible plasmid containing of C-terminally FLAG-tagged hptR, for ChIP-seq.	This study

pRAB11: *saeR*^{FLAG}

ATc inducible plasmid containing of C-terminally FLAG-tagged saeR, for ChIP-seq.

This study

YFP reporter plasmids for S. aureus.		
pIMC8: PsasD-YFP	Native sasD driven expression of YFP.	This study
pIMC8: Psle1-YFP	Native <i>sle1</i> driven expression of YFP.	This study
pIMC8: P <i>602</i> -YFP	Native SAUSA300_0602 driven expression of YFP.	This study
pIMC8: P <i>ltaS</i> -YFP	Native ItaS driven expression of YFP.	This study
pIMC8: PssaA1-YFP	Native ssaA1 driven expression of YFP.	This study
pIMC8: P <i>isaA</i> -YFP	Native isaA driven expression of YFP.	(3)
pIMC8: PsasDccc-YFP	Native sasD driven expression of YFP. Mutation in the putative WalR binding site.	This study
pIMC8: Psle1ccc-YFP	Native <i>sle1</i> driven expression of YFP. Mutation in the putative WalR binding site.	This study
pIMC8: P602 _{ccc} -YFP	Native SAUSA300_0602 driven expression of YFP. Mutation in the putative WalR binding site.	This study
pIMC8: P <i>ltaSccc</i> -YFP	Native <i>ItaS</i> driven expression of YFP. Mutation in the putative WalR binding site.	This study
pIMC8: PssaA1 _{cccl} -YFP	Native <i>ssaA1</i> driven expression of YFP. Mutation in the putative WalR binding site (cccl).	This study
pIMC8: P <i>ssaA1_{cccll}-</i> YFP	Native <i>ssaA1</i> driven expression of YFP. Mutation in the putative WalR binding site (cccII).	This study
pIMC8: PisaAcccl-YFP	Native isaA driven expression of YFP. Mutation in the putative WalR binding site (cccl).	(3)
pIMC8: PisaAcccil-YFP	Native <i>isaA</i> driven expression of YFP. Mutation in the putative WalR binding site (cccII).	(3)
Bacterial LUX reporter plasmids for S. aureus.		
pIMK1-LUX: PsasD	Native sasD driven expression of LUX.	This study

pIMK1-LUX: Psle1	Native <i>sle1</i> driven expression of LUX.	This study
pIMK1-LUX: P602	Native SAUSA300_0602 driven expression of LUX.	This study
pIMK1-LUX: PltaS	Native <i>ItaS</i> driven expression of LUX.	This study
pIMK1-LUX: PssaA1	Native ssaA1 driven expression of LUX.	This study
pIMK1-LUX: PisaA	Native <i>isaA</i> driven expression of LUX.	This study
pIMK1-LUX: PsasDccc	Native sasD driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: Psle1ccc	Native <i>sle1</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: P602ccc	Native SAUSA300_0602 driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: PltaS _{ccc}	Native <i>ItaS</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX:	Native <i>ssaA1</i> driven expression of LUX. Mutation in the putative WalR binding site (cccl).	This study
pIMK1-LUX: PisaAcccl	Native isaA driven expression of LUX. Mutation in the putative WalR binding site (cccl).	This study
pIMK1-LUX: Phup	Native <i>hup</i> driven expression of LUX.	This study
pIMK1-LUX: P <i>spa</i>	Native spa driven expression of LUX.	This study
pIMK1-LUX: P <i>prs</i>	Native prs driven expression of LUX.	This study
pIMK1-LUX: P <i>rplK</i>	Native <i>rplK</i> driven expression of LUX.	This study
pIMK1-LUX: PdnaA	Native <i>dnaA</i> driven expression of LUX.	This study
pIMK1-LUX: PdnaD	Native <i>dnaD</i> driven expression of LUX.	This study
pIMK1-LUX: PtarF	Native <i>tarF</i> driven expression of LUX.	This study
pIMK1-LUX: PtagG	Native <i>tagG</i> driven expression of LUX.	This study
pIMK1-LUX: Phup _{ccc}	Native hup driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: Pspaccc	Native spa driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: Pprsccc	Native prs driven expression of LUX. Mutation in the putative WalR binding site.	This study

pIMK1-LUX: P <i>rplKccc</i>	Native <i>rplK</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: PdnaAccc	Native dnaA driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: PdnaD _{cccl}	Native dnaD driven expression of LUX. Mutation in the putative WalR binding site (cccl).	This study
pIMK1-LUX:	Native <i>dnaD</i> driven expression of LUX. Mutation in the putative WalR binding site (cccII).	This study
PanaD _{cccll} pIMK1-LUX: PtarF _{ccc}	Native <i>tarF</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: PtagGccc	Native <i>tagG</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study

Protein overexpression in E. coli.

pET28(a): walR	For production of recombinant WalR in <i>E. coli</i> .	This study
pET28(a): <i>vraR</i>	For production of recombinant VraR in E. coli.	This study
CRISPRi plasmids for g	ene knockdown in <i>S. aureus.</i>	This study
pSD1:walR	CRISPRi knockdown of <i>walR</i> in <i>S. aureus</i> .	This study
pSD1:hup	CRISPRi knockdown of hup in S. aureus.	This study
pSD1: <i>hla</i>	CRISPRi knockdown of <i>hla</i> in <i>S. aureus.</i>	This study
Split luciferase plasmi	ds for protein:protein interaction in <i>S. aureus</i> .	This study
pSmBIT <i>walR</i>	C-terminally tagged WaIR with SmBIT luciferase fragment	This study
pSmBIT <i>walR</i> _{D53A}	C-terminally tagged WalR $_{\mbox{\scriptsize D53A}}$ with SmBIT luciferase fragment	This study
pSmBIT <i>walR</i> _{D53E}	C-terminally tagged WalR_{D53E} with SmBIT luciferase fragment	This study
pSmBIT <i>walR</i> T101A	C-terminally tagged WalR $_{T101A}$ with SmBIT luciferase fragment	This study
pSmBIT <i>walR</i> _{D53A/T101A}	C-terminally tagged WalR ${\tt D53A/101A}$ with SmBIT luciferase fragment	This study
pLgBIT <i>walK</i>	C-terminally tagged WalK with LgBIT luciferase fragment	This study
pLgBIT <i>walK_{Y32C}</i>	C-terminally tagged WalKy32C with LgBIT luciferase fragment	This study
pLgBIT <i>walK</i> _{7389A}	C-terminally tagged WalK_{T389A} with LgBIT luciferase fragment	This study
pLgBIT <i>walK</i> G223D	C-terminally tagged WalK _{G223D} with LgBIT luciferase fragment	This study
pLgBIT <i>walK</i> H385A	C-terminally tagged WalK $_{\mbox{\scriptsize H385A}}$ with LgBIT luciferase fragment	This study

Table CC	Olivervalestides		-
i abie so.	Oligonucleotides	usea in this stuay	

	EMSA	
Primer	Oligonucleotide 5'-3'	Name
LS237	$/5 c_{y}5/cttttgtaatcgtatgataatgtaatgtaatcaaattgtaatataaggggacaagacaatgaaaaaatt$	sasD EMSA Cy5 fwd
LS238	CTTTTGTAATCGTATGATAATGTAAATGTAATCAAATTGTAATATAAGGGGACAAGACAATGAAAAAATT	sasD EMSA fwd
LS239	AATTTTTTCATTGTCTTGTCCCCTTATATTACAATTTGATTACATTTACATTATCATACGATTACAAAAG	sasD EMSA rev
LS234	$/5c_{y}5/tttgatgatacagtatatgatttttttgtaatcataatgtcatcaaacatcaacctattatacataataa$	<i>sle1</i> EMSA Cy5 fwd
LS235	TTTGATGATACAGTATATGATTTTTTTGTAATCATAATGTCATCAAACATCAACCTATTATACATAATAA	<i>sle1</i> EMSA fwd
LS236	TTATTATGTATAATAGGTTGATGTTTGATGACATTATGATTACAAAAAAATCATATACTGTATCATCAAA	sle1 EMSA rev
LS243	/5Cy $5/$ GAAATATAAAAGTAGTAATTATTATTTGTAATATAATTGTAATATGACTGTTGTTTTAGAAATGATTGTT	P602 EMSA fwd Cy5
LS244	GAAATATAAAAGTAGTAATTATTATTTGTAATATAATTGTAATATGACTGTTGTTTTAGAAATGATTGTT	P602 EMSA fwd
LS245	AACAATCATTTCTAAAAACAACAGTCATATTACAATTATTATATAATAATAATTACTACTTTTATATTTC	P602 EMSA rev
LS240	/5Cy $5/$ ATATTTATTAATTGAGCTATGCTTATTATTACAATTTGATTACAAATTTTAAATTTGTTAATTGAATGAT	<i>ltaS</i> EMSA fwd Cy5
LS241	ATATTTATTAATTGAGCTATGCTTATTATTACAATTTGATTACAAATTTTAAATTTGTTAATTGAATGAT	<i>ltaS</i> EMSA fwd
LS242	АТСАТТСААТТААСАААТТТААААТТТGTAATCAAATTGTAATAATAAGCATAGCTCAATTAATAAATA	<i>ltaS</i> EMSA rev
LS246	/5cy5/aaaactgaatgtttcgaaaataagtctgttacaaatttgtaatattactgaaaattctaaatgtatattt	ssaA EMSA fwd Cy5
LS247	AAAACTGAATGTTTCGAAAATAAGTCTGTTACAAATTTGTAATATTACTGAAAAATTCTAAATGTATATTT	<i>ssaA</i> EMSA fwd
LS248	AAATATACATTTAGAATTTTCAGTAATATTACAAATTTGTAACAGACTTATTTTCGAAACATTCAGTTTT	ssaA EMSA rev
LS249	/5Cy5/AAATAACACTTGATATTGTAATGTTTTGTAAAGAAAGTGTAATTTACTGGCTGG	isaA EMSA fwd Cy5
LS250	AAATAACACTTGATATTGTAATGTTTTGTAAAGAAAGTGTAATTTACTGGCTGG	<i>isaA</i> EMSA fwd
LS251	ACTATATCACAAAAAACCAGCCAGTAAATTACACTTTCTTT	isaA EMSA rev
LS477	/5CY5/ATGTTTATATCATTATGGTAAAACTTTAACACAAGCATTACATAAATCGCAATCATTTATGTGGCAAAAA	hup EMSA fwd Cy5
LS479	TTTTTGCCACATAAATGATTGCGATTTATGTAATGCTTGTGTTAAAGTTTTACCATAATGATATAAACAT	hup EMSA rev
LS480	/5CY5/AAGAGTATTGATTTTTTAATTAGAAAAGTGTTAAAATTATGTGGTCGCGCTTTTAGAGCGCCCATTTCGT	rplK EMSA fwd Cy5
LS482	ACGAAATGGGCGCTCTAAAAGCGCGACCACATAATTTTAACACTTTTCTAATTAAAAAATCAATACTCTT	<i>rplK</i> EMSA rev
LS483	/5CY5/AAATGGCTAGGATAAAAGGATAATCCTATGTAATATTAATGTAATCTTTATGATTTAATGATTCGCATAG	prs EMSA fwd Cy5
LS485	CTATGCGAATCATTAAATCATAAAGATTACATTAATATTACATAGGATTATCCTTTTATCCTAGCCATTT	prs EMSA rev
LS486	/5CY5/CATGGTGTGTTTAGAAGTTATCCACGGCTGTTATTTTGTGTATAACTTAAAAATTTAAGAAAGA	dnaA region I EMSA
LS488	ACTCCATCTTTCTTAAATTTTTAAGTTATACACAAAAATAACAGCCGTGGATAACTTCTAAACACACCATG	fwd Cy5 dnaA region I EMSA
LS489	/5CY5/AATAAGCAAGATAAAGTTATCCACCGATTGTTATTAACTTGTGGATAATTATTAACATGGTGTGTTTAGA	dnaA region II EMSA fwd Cy5
LS491	TCTAAACACCACCATGTTAATAATTATCCACAAGTTAATAACAATCGGTGGATAACTTTATCTTGCTTATT	dnaA region II EMSA rev
LS492	/5CY5/AAAGTTATACTATTTTTAGCAACATATTCACAGGTATTTGACATATAGAGAACTGAAAAAGTATAATTGT	dnaA region III EMSA fwd Cy5
LS494	ACAATTATACTTTTTCAGTTCTCTATATGTCAAATACCTGTGAATATGTTGCTAAAAATAGTATAACTTT	dnaA regioin III EMSA
LS267	/5Cy5/CATTAGGAGGAAATTATTTGCATCGGACTCGAGTATGAGCTACGTACCAAATATTATTTCATCATTTCTA	VraR EMSA <i>ltaS</i> fwd Cy5
LS268	CATTAGGAGGAAATTATTTGCATCGGACTCGAGTATGAGCTACGTACCAAATATTATTTCATCATTTCTA	VraR EMSA <i>ltaS</i> fwd
LS269	TAGAAATGATGAAATAATATTTGGTACGTAGCTCATACTCGAGTCCGATGCAAATAATTTCCTCCTAATG	VraR EMSA <i>ltaS</i> rev
LS91	ACTGTGACG <i>GGATCC</i> ATGGCTAGAAAAGTTGTTGTAGTTGATG	walR pET28 F
LS92	CATCGACTTAG <i>AAGCTT</i> TACTCATGTTGGTGGAGGAAATATCCAAC	walR pET28 R
IM423	TTTGTTTAACTTTAAGAAGGAGATATACCATGACGATTAAAGTATTGTTTGT	vraR pET21 F
IM424	<u>GGCTTTGTTAGCAGCCGGATC</u> CTAATGGTGATGATGGTGGTGATGGTGATGTTGAATTAAATTATGTTGGAATGCAT	<i>vraR</i> pET21 R
IM395	GGTATATCTCCTTCTTAAAGTTAAACAAAATTATTTC	pET INV F

IM396 GATCCGGCTGCTAACAAAGCC

pET INV R

	S. aureus mutants	
Primer	Oligonucleotide 5'-3'	Name
IM31	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACC</u> CGTCCATTTTCTTTAAAATGTATGAACC	walR ^{T101A} AF
IM231	aGcaACATAGTCATCTGCACCTAGTTC	<i>walr</i> ^{T101A} BR
IM232	TTAGAACTAGGTGCAGATGACTATGTtgCtAAACCGTTTAGTACGCGTGAATTAATCG	walR ^{T101A} CF
IM233	AGAACTAGGTGCAGATGACTATGTtg	<i>walR</i> ^{T101A} con F
IM181	TTTTTCAAGGTTATTTGTAAAATATAACCC	<i>walR</i> ^{T101A} con R
IM107	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACC</u> ATGGCTAGAAAAGTTGTTGTAG	walK ^{Y32C} AF
IM7	CCTCACTAAAGGGAACAAAAGCTGGGTACCAGGTCGAAACGAATGAAGTGGCTAAAAC	<i>walK</i> ^{T389A} AF
IM121	agcACGTAACTCATGTGATACATTGG	<i>walK</i> ^{7389A} BR
IM122	CAATGTATCACATGAGTTACGTgctCCTTTAACTTCTATGAATAGTTACATTGAAGC	<i>walK</i> ^{T389A} CF
IM10	<u>CGACTCACTATAGGGCGAATTGGAGCTC</u> CTCCTTATTATTCATCCCAATCACCGTC	walR ^{T101A} /walK ^{Y32C} / ^{T389A}
IMT275	CCTCACTAAAGGGAACAAAAGCTGGGTACCAATTCATATGGATGACGCGCAGC	DR Delta <i>spa</i> AF
IMT353	CAAATTAATACCCCCTGTATGTATTTG	Delta <i>spa</i> BR
IMT354	CAAATACATACAGGGGGTATTAATTTGTAAAAACAAACAA	Delta <i>spa</i> CF
IMT278	<u>CGACTCACTATAGGGCGAATTGGAGCTC</u> ATTACTTGTGGCAGCTAACACTGC	Delta <i>spa</i> DR
IM1516	GGTTATAGACTTTTTGAAGAAATTCTATAAAGGTCGAAACGAATGAAGTGGCTAAAAC	walR-SmBIT-walK CF
IM1517	TTATAGAATTTCTTCAAAAAGTCTATAACCTG	walR-SmBIT-walK BR
IM1518	GTTGTTTAGAGTAACTATAAACAGTTAAATGAATAATAAGGAGCATATTAAATCTGTC	walK-LgBIT-yycH CF
IM1519	TTAACTGTTTATAGTTACTCTAAACAACATAGATCC	walK-LgBIT-yycH BR
IM44	<u>CGACTCACTATAGGGCGAATTGGAGCTC</u> TTGTTAATTTTACGTAATCGTGGCGATC	walK-LgBIT-yycHI DR
IM1368	AGCCCGATAATTTGCATACCAATG	walK-LgBIT con R
	ChIP-seq	
Primer	Oligonucleotide 5'-3'	Name
IM512	ATAT <i>GGTACC</i> GATTACAAAGATGATGATGACAAATAAAATAGTCAAAAGCCTCCGGTCGGAGGCTTTTGACTGAATTCAC TGGCCGTCGTTTTAC	pRAB11 FLAG tonB
IM513	ATAT GGTACCTTTCCAATTCCTCCTCATCATACTCTATCAATGATAGAGAGGC	pRAB11 RBS (Kpnl) R
IM514	GATTACAAAGATGATGACAAATAAAATAGTC	INV pRAB11 FLAG F
IM515	TTTCCAATTCCTCCTCATCATACTCTATC	INV pRAB11 FLAG R
IM516	CATTGATAGAGTATGATGAGGAGGAATTGGAAATGGCTAGAAAAGTTGTTGTAGTTG	WalR-FLAG F
IM517	CTATTTTATTTGTCATCATCTTTGTAATCCTCATGTTGTTGGAGGAAATATCC	WalR-FLAG R
IM518	CATTGATAGAGTATGATGAGGAGGAATTGGAAATGACCCACTTACTGATCGTGG	SaeR-FLAG F
IM519	CTATTTTATTTGTCATCATCATCTTTGTAATCTCGGCTCCTTTCAAATTTATATCC	SaeR-FLAG R
IM520	CATTGATAGAGTATGATGAGGAGGAATTGGAAATGACGATTAAAGTATTGTTTGT	VraR FLAG F
IM521	CTATTTTATTTGTCATCATCATCTTTGTAATCTTGAATTAAATTATGTTGGAATGCATAG	VraR-FLAG R
IM522	<u>CATTGATAGAGTATGATGAGGAGGAATTGGAA</u> ATGTTTAAGGTAGTTATTTGTGATGATG	HptR-FLAG F
IM523	<u>CTATTTTATTTGTCATCATCATCTTTGTAATC</u> TTTTGCTTGCTTACAATAATCACTTGG	HptR-FLAG R
	YFP-LUX	
Primer	Oligonucleotide 5'-3'	Name
IM1	GGTACCCAGCTTTTGTTCCCTTTAGTGAGG	pIMC8-YFP F

IM1	GGTACCCAGCTTTTGTTCCCTTTAGTGAGG	pIMC8-YFP F
IM385	TGATTAACTTTATAAGGAGGAAAAACATATG	pIMC8-YFP-R

IM1127	CCTCACTAAAGGGAACAAAAGCTGGGTACCTATAACTTAATATAATTGAGGTGGAGCATC	sasD YFP F
IM1107	ATGTTTTTCCTCCTTATAAAGTTAATCATGTCTTGTCCCCTTATATTACAATTTG	sasD YFP R
IM1108	CCTCACTAAAGGGAACAAAAGCTGGGTACCTTAGAAAAATCAAATTCAGATGCAGTAAAG	sle1 YFP F
IM1109	ATGTTTTTCCTCCTTATAAAGTTAATCATTTAAAATCCTCCTCTTGCTTAACTTTCC	<i>sle1</i> YFP R
IM1129	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACC</u> CAAGAATCAAGTGCATTCCCATTTGTTG	<i>P602</i> YFP F
IM1110	ATGTTTTTCCTCCTTATAAAGTTAATCAAAGCACTCTCTCCTTTTATTTA	<i>P602</i> YFP R
IM1111	CCTCACTAAAGGGAACAAAAGCTGGGTACCTAATATTTGGTACGTAGCTCATACTCG	<i>ltaS</i> YFP F
IM1112	$\underline{\texttt{ATGTTTTTCCTCCTTATAAAGTTAATCA}} \texttt{GATTCTTTCCCCCGTTATTTAGATAATAAATC}$	<i>ltaS</i> YFP R
LS371	CCTCACTAAAGGGAACAAAAGCTGGGTACCCCAAATACCAAAGCTTTCATAATC	ssaA YFP F
LS372	ATGTTTTTCCTCCTTATAAAGTTAATCATTTAAAAATATCCTCCTAAAAATTTTAAATC	<i>ssaA</i> YFP R
IM1128	CCTCACTAAAGGGAACAAAAGCTGGGTACCTAACAGTATGTTTTTTGAAAATATGAGACC	isaA YFP F
IM364	ATGTTTTTCCTCCTTATAAAGTTAATCAAGTAAAAAATCCTCCAGTAATAATTG	<i>isaA</i> YFP R
IMT300	CCTCACTAAAGGGAACAAAAGCTGGGTACCATCCAGATTTAATAATAGGATGGTTAGG	hpt YFP F
IMT301	ATGTTTTTCCTCCTTATAAAGTTAATCACTCTGTCACCTCAATCATTTTCG	<i>hpt</i> YFP R
IM1113	${\tt ATGTTTTTCCTCCTTATAAAGTTAATCATGTCTTGTCCCCTTATATT{\tt GGG}{\tt ATTTGATTAC}$	sasD ccc R
IM1114	GATGATACAGTATATGATTTTTT CCC AATCATAATGTCATCAAACATC	<i>sle1</i> ccc F
IM1115	GGG AAAAAATCATATACTGTATCAACAAAT	<i>sle1</i> ccc R
IM1118	$GAAATATAAAAGTAGTAATTATTATTCCCAATATTGTATTGTGTTTTTGGTTTTTGTGTTTTTGGTGTTTTTGGTGTTTTTGGGTGTTTTGGGGTGTTGTGTGTGTGTGTGTGTGTGGGGGGGGG\mathsf{G$	<i>P602</i> ccc F
IM1119	GGG AATAATTACTACTTTTATATTTCACTTATC	<i>P602</i> ccc R
IM1116	GAGCTATGCTTATTATTACAATTTGATT GGG AATTTTAAATTTGTTAATTGAATG	<i>ItaS</i> ccc F
IM1117	CCC AATCAAATTGTAATAATAAGCATAGCTC	<i>ItaS</i> ccc R
LS375	GGGAAGCACAAAGGACGCTAAT	<i>ssaA</i> cccl F Closest to the <i>ssaA</i> 1 start
LS376	ATTAGCGTCCTTGTGTGCTTCCCTAACGTTTTGTAATTTTTGCTAATATC	ssaA cccl R
IM1120	CAGTAATATTACAAATTTGT GGG GACTTATTTTCGAAACATTCAG	<i>ssaA</i> cccll F Furthest from the <i>ssa</i> A1 start
IM1121	CCC TACAAATTTGTAATATTACTGAAAATTCTAAATG	ssaA cccll R
IM1122	ACACTTGATATTGTAATGTTT CCC AAAGAAAGTGTAATTTACTGGCTGG	<i>isaA</i> cccl F Closest to the <i>isaA</i> start
IM1123	GGG AAACATTACAATATCAAGTGTTATTTG	<i>isaA</i> cccl R
IM1124	CAGATATATTACAGCTATGTA GGG AAAATACAATCTGTAATATTACGAAAGC	<i>isa</i> A cccll F Furthest from the <i>isa</i> A
IM1125	CCCTACATAGCTGTAATATATCTGACATGTAAC	isaA cccll R
Primer	Oligonucleotide 5'-3'	Name
IM1216	ATATGTCGACTATAACTTAATATAATTGAGGTGGAGCATC	sasD LUX F
IM248	CATTGTCTTGTCCCCTTATATTACAATTTG	sasD LUX R
IM1295	ATAT <i>GTCGAC</i> TTAGAAAAATCAAATTCAGATGCAGTAAAG	sle1 LUX F
IM1296	CACTTTAAAAATCCTCCTCTTGCTTAACTTTCC	<i>sle1</i> LUX R
IM1294	A'I'A'I' <i>G'I'CGAC</i> CAAGAATCAAGTGCATTCCCATTTGTTG	0602 LUX F
IM1062	CATAAGCACTCTCTCCTTTTATTATATCG	0602 LUX R
IM1218	ATAT <i>GTCGAC</i> TAATATTTGGTACGTAGCTCATACTCG	<i>ltaS</i> LUX F

CATGATTCTTTCCCCCGTTATTTAGATAATAAATC ATAT*GTCGAC*CTTACATCCTCACATATACAAATATATTG

IM1219

IM1297

ltaS LUX R

ssaA LUX F

IM1298	CATTTTAAAAATATCCTCCTAAAAATTTTAAATC	ssaA LUX R
IM1220	ATAT <i>GTCGAC</i> TAACAGTATGTTTTTTGAAAATATGAGACC	<i>isaA</i> LUX F
IM1221	CATAGTAAAAAATCCTCCAGTAATAATTG	<i>isaA</i> LUX R
IM1222	ATAT <i>GTCGAC</i> TTCTGAGCAATGACGTGCAACTAG	<i>walR</i> LUX F
IM32	CATTTGCATAAACCTCTTTTCTTAAATC	<i>walR</i> LUX R
IM1217	CATTGTCTTGTCCCCTTATATT GGG ATTTGATTAC	<i>sasD</i> ccc LUX R
LS443	TACGCGTCGACACGATGCGAGCAATCAAAT	dnaA LUX F
IM1746	CATAAATTTACTCCATCTTTCTTAAATTTTTAAG	dnaA LUX R
IM1285	ATAT <i>GTCGAC</i> CAATTAGGTGCAGATGTTAAGGTTG	prs LUX F
IM1288	CATTTATAGTCCTCCAATTATTTACTTACG	prs LUX R
IM1289	ATAT <i>GTCGAC</i> TGCCGATCCTATGACATTTCTAGG	hup LUX F
IM1285	CATTAGACATTCACCTCCTGAGG	hup LUX R
LS451	TACGC <i>GTCGAC</i> GATGAGAAGCAGGACTATACAATGAG	tarF LUX F
LS452	CATGTCGTACCTCCGACGTG	tarF LUX R
LS457	TACGC <i>GTCGAC</i> GAAGATTTTGTTATTATCAGAGTGGG	tagG LUX F
LS458	CATTCCATTAAACCACACTTTCAAATG	tagG LUX R
LS471	TACGC <i>GTCGAC</i> GCTGGGCGATTTATTCTTGG	dnaD LUX F
LS472	CATGTTCGTGCCCCCTTTTAA	dnaD LUX R
IM1745	ATAT <i>GTCGAC</i> CTCACTAACAGATACTCTATAGAAGG	dnaA LUX F
IM1746	CATAAATTTACTCCATCTTTCTTAAATTTTTAAG	dnaA LUX R
IM1734	ATAT <i>GTCGAC</i> ATTAACAATTAAAGTTATTAAACTAACCAAAAG	rplK LUX F
IM1735	CACGATGTGCACCTCCTTGATATCG	rplK LUX R
IM1291	CCACATAAATGATTGCGATTTA CCC AATGCTTGTGTTAAAGTTTTACC	hup ccc LUX CF
IM1290	GGGTAAATCGCAATCATTTATGTGGCAAAAACG	hup ccc LUX BR
LS449	GGATAACGATTTTT CCC TATGTTAAAGTGGTAC	tarF ccc LUX CF
LS448	GGG AAAAATCGTTATCCATTCATAACGTATG	tarF ccc LUX BR
LS455	GATAGCATCCA CCC ATAGTGATAGTATTTACAAC	tagG ccc LUX CF
LS454	GGG TGGATGCTATCAAAATTATTGTCTAGTTC	tagG ccc LUX BR
LS467	CCATAAAGTTCAT CCC TAAAATCTAGTGTTAAAAAATAC	dnaD cccl LUX CF
LS466	GGG ATGAACTTTATGGATAATCAGATGAACTA	dnaD cccl LUX BR
LS469	GTTATTTAAAATT CCC TTGCTACTAGTTAAATATATTAAG	<i>dnaD</i> cccII LUX CF Furthest from the start
LS468	GGG AATTTTAAATAACAGTATTTTTAACACTAGATTTTAAC	dnaD cccII LUX BR
LS144	GAGTATTGATTTTTAATTAGAAAAG CCC TAAAATTATGTGGTCGCGC	rplK ccc LUX CF
LS143	GGGCTTTTCTAATTAAAAAATCAATACTCTTTTTA	rplK ccc LUX BR
LS140	TGGCTAGGATAAAAGGATAATCCTA CCC AATATTAATGTAATCTTTATG	prs ccc LUX CF
LS139	GGGTAGGATTATCCTTTTATCCTAGCCATTTTAAATAC	prs ccc LUX BR
LS441	CCC TATTTTTGTGTATAACTTAAAAATTTAAGAAAGATG GTTATACACAAAAATA GGG GCCGTGGATAACTTC	dnaA ccc I LUX CF Closest to the start dnaA ccc I LUX BB
LS439	CCCTATTAACTTGTGGATAATTATTAACATGGTG	dnaA ccc II I I IX CF
LS438	CCACAAGTTAATA GGG ATCGGTGGATAAC	dnaA ccc II I UX BR
IM1744	GTTATACTATTTTAGCAACATATTC GGG GGTATTTGACATATAGAGAACTGAAAAAG	dnaA ccc III LUX C Furthest from the start F

IM1743	CCCGAATATGTTGCTAAAAATAGTATAACTTTGTG	dnaA ccc III LUX BR
IM1241	ATATAGATCTATATAGTTTTTGTATACGGTATTCATTCAT	pLOW rep F
IM1242	ATAT <i>GCATGC</i> TCAACTTTGCAACAGAACC	pLOW rep R
	RT-qPCR	
Primer	Oligonucleotide 5'-3'	Name
IM1026	GGCTCTATGAAAGCAGCAGATA	hla RT-qPCR F
IM1027	CTGTAGCGAAGTCTGGTGAAA	<i>hla</i> RT-qPCR R
IM1020	CGCAGGCGATTTTACCATTA	gyrB RT-qPCR F
IM1021	ATGCTGGAACTTTACTTGCTGG	gyrB RT-qPCR R
IM1153	GAACTAGGTGCAGATGACTATGT	walR RT-qPCR F
IM1154	CTTGTGCTGGTTGTGAGTAATG	walR RT-qPCR R
IM1586	GGTTTCGGTAACTTTGAGGTACG	Hu RT-qPCR F
IM1587	ATGCTGGAACTTTACTTGCTGG	Hu RT-qPCR R
	SmBIT/LgBIT	
Primer	Oligonucleotide 5'-3'	Name
IM1290	ATCATTAATTCCTCCTTTTTGTTGACAcTcTATCATTGATAGAGTTATTTGTCAAACTAG	Fix tetO1 F
IM1291	gAgTGTCAACAAAAAGGAGGAATTAATGATG	Fix tetO1 R
IM1355	ATAT <i>GGTACC</i> GTTAACAGATCTGAGCTCG	Introduce RBS F
IM513	ATAT <i>GGTACC</i> TTTCCAATTCCTCCTCATCATACTCTATCAATGATAGAGAGC	Introduce RBS R
IM515	TTTCCAATTCCTCCTCATCATACTCTATC	INV PCR to clone
IM1356	GTTAACAGATCTGAGCTCGAATTCACTGG	INV PCR to clone
IM1360	GGTTCTAGTGGTGGTGGTGGTTCTGG	SmBIT/LgBIT F INV PCR to clone genes F (IM515)
IM1363	GATAGAGTATGATGAGGAGGAATTGGAAAATGGCTAGAAAAGTTGTTGTAGTTG	WalR pSmBIT F
IM1364	GAACCACCACCACTAGAACCCTCATGTTGTTGGAGGAAATATCC	WalR pSmBIT R
IM1365	GATAGAGTATGATGAGGAGGAATTGGAAAATGAAGTGGCTAAAACAACTACAATCC	WalK pLgBIT F
IM1366	GAACCACCACCACCACTAGAACCTTCATCCCCAATCACCGTCTTCAATGAC	WalK pLgBIT R
	CRISPRI	
Primer	Oligonucleotide 5'-3'	Name
IM1559	CTAGTTCATTAGACATTCACCTCCTG	hup CRISPRi F
IM1560	AACCAGGAGGTGAATGTCTAATGAAC	hup CRISPRi R
IM1180	<i>CTA</i> TTAAATTCTAAAATATCAGCAAT	walR CRISPRi F
IM1181	<i>AAC</i> ATTGCTGATATTTTAGAATTTAA	walR CRISPRi R
IM1182	<i>CTA</i> CATTAGCGACAGGATTCATTAATA	<i>hla</i> CRISPRi F
IM1183	AACTATTAATGAATCCTGTCGCTAATG	hla CRISPRi R
	Itallics: restriction enzyme sites	

Underlined: Tails complementary to plasmid for SLiCE cloning

Lowercase: Mutations introduced

Bold: WalR binding site mutations

REFERENCES

- 1. Monk IR, Tree JJ, Howden BP, Stinear TP, Foster TJ. 2015. Complete Bypass of Restriction Systems for Major *Staphylococcus aureus* Lineages. mBio 6:e00308-15.
- 2. Kreiswirth BN, Lofdahl S, Betley MJ, O'Reilly M, Schlievert PM, Bergdoll MS, Novick RP. 1983. The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. Nature 305:709-12.
- 3. Monk IR, Shaikh N, Begg SL, Gajdiss M, Sharkey LKR, Lee JYH, Pidot SJ, Seemann T, Kuiper M, Winnen B, Hvorup R, Collins BM, Bierbaum G, Udagedara SR, Morey JR, Pulyani N, Howden BP, Maher MJ, McDevitt CA, King GF, Stinear TP. 2019. Zinc-binding to the cytoplasmic PAS domain regulates the essential WalK histidine kinase of *Staphylococcus aureus*. Nat Commun 10:3067.
- 4. Helle L, Kull M, Mayer S, Marincola G, Zelder ME, Goerke C, Wolz C, Bertram R. 2011. Vectors for improved Tet repressor-dependent gradual gene induction or silencing in *Staphylococcus aureus*. Microbiology (Reading) 157:3314-3323.
- 5. Zhao C, Shu X, Sun B. 2017. Construction of a Gene Knockdown System Based on Catalytically Inactive ("Dead") Cas9 (dCas9) in *Staphylococcus aureus*. Appl Environ Microbiol 83.
- 6. Liew ATF, Theis T, Jensen SO, Garcia-Lara J, Foster SJ, Firth N, Lewis PJ, Harry EJ. 2011. A simple plasmidbased system that allows rapid generation of tightly controlled gene expression in *Staphylococcus aureus*. Microbiology (Reading) 157:666-676.
- 7. Charpentier E, Anton AI, Barry P, Alfonso B, Fang Y, Novick RP. 2004. Novel cassette-based shuttle vector system for gram-positive bacteria. Appl Environ Microbiol 70:6076-85.