

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

- Supplementary Data File -

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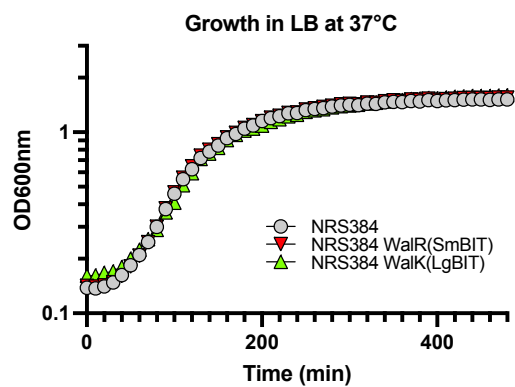


Figure S1. Growth in LB of the chromosomally C-terminally tagged WalR-SmBIT or Walk-LgBIT in LB compared to the Wt. No difference in growth profile was observed for the mutants.

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

Genes undergoing a ≥ 1.5 log₂FC (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 (FDR)	Log ₂ FC
Amino acid biosynthesis and transport	SAUSA300_2006	<i>ilvD</i>	Dihydroxy-Acid Dehydratase	4.00E-07	2.84
	SAUSA300_2007	<i>ilvB</i>	Acetolactate Synthase Large Subunit	1.00E-07	2.46
	SAUSA300_2013	<i>leuD</i>	Isopropylmalate Isomerase Small Subunit	2.30E-06	2.06
	SAUSA300_0185	<i>argJ</i>	Bifunctional Ornithine Acetyltransferase/N-	3.30E-05	2.02
	SAUSA300_2010	<i>leuA</i>	2-Isopropylmalate Synthase	1.10E-06	2.01
	SAUSA300_0186	<i>argC</i>	N-Acetyl-Gamma-Glutamyl-Phosphate Reductase	1.10E-06	1.95
	SAUSA300_2012	<i>leuC</i>	Isopropylmalate Isomerase Large Subunit	4.00E-07	1.88
	SAUSA300_0359	<i>metC</i>	Trans-Sulfuration Enzyme Family Protein	1.50E-06	1.87
	SAUSA300_2009	<i>ilvC</i>	Ketol-Acid Reductoisomerase	9.00E-07	1.87
	SAUSA300_2011	<i>leuB</i>	3-Isopropylmalate Dehydrogenase	9.00E-07	1.68
	SAUSA300_0184	<i>argB</i>	Acetylglutamate Kinase	2.10E-05	1.58
	SAUSA300_0066	<i>argR</i>	Arginine Repressor	6.30E-05	-1.75
	Carbohydrate uptake and metabolism	SAUSA300_0186	<i>argC</i>	N-Acetyl-Gamma-Glutamyl-Phosphate Reductase	1.10E-06
SAUSA300_0448		<i>treP</i>	Pts System, Trehalose-Specific Iibc Component	2.20E-05	1.72
SAUSA300_0315		<i>nanA</i>	N-Acetylneuraminate Lyase	1.10E-04	1.7
SAUSA300_0236		<i>glcC</i>	Pts System, Iibc Components	1.70E-06	1.64
SAUSA300_0333		-	Bglg Family Transcriptional Antiterminator	2.20E-04	1.56
SAUSA300_1258		<i>dmpI</i>	4-Oxalocrotonate Tautomerase	2.60E-04	-1.56
SAUSA300_0791		<i>gcvH</i>	Glycine Cleavage System Protein H	1.20E-06	-1.76
SAUSA300_0930		<i>lplA1</i>	Lipoate-Protein Ligase A Family Protein	5.00E-07	-1.79
SAUSA300_0030		<i>ugpQ</i>	Glycerophosphoryl Diester Phosphodiesterase	3.00E-07	-2
Cell wall organisation		SAUSA300_2585	<i>asp3</i>	Accessory Secretory Protein Asp3	5.70E-05
	SAUSA300_0153	<i>capB</i>	Capsular Polysaccharide Biosynthesis Protein Cap5B	5.10E-05	1.6
	SAUSA300_0156	<i>capE</i>	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	<i>queC</i>	ExsB Protein	8.40E-06	1.85
DNA replication, recombination, and repair	SAUSA300_0367	<i>ssb</i>	Single-Strand Binding Protein	1.90E-06	1.94
	SAUSA300_1142	<i>dprA</i>	DNA Protecting Protein DprA	2.10E-06	1.83
	SAUSA300_1732	<i>tnp2</i>	Putative Transposase	3.10E-03	-4.62
Fatty acid biosynthesis	SAUSA300_0320	<i>gehB</i>	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	<i>acpP</i>	Acyl Carrier Protein	9.90E-06	-1.92
Metal ion homeostasis	SAUSA300_0720	<i>sstC</i>	Putative Iron Compound ABC Transporter ATP-Binding	9.00E-05	1.6
	SAUSA300_0117	<i>sirA</i>	Iron Compound ABC Transporter Iron Compound-	5.60E-05	-1.51
	SAUSA300_1874	<i>ftnA</i>	Ferritins Family Protein	2.20E-06	-1.69
	SAUSA300_0843	<i>sufA</i>	Fe-S Cluster Carrier	2.30E-04	-1.7
	SAUSA300_1373	<i>fer</i>	Ferredoxin	3.50E-05	-2.14
Nitrogen utilisation	SAUSA300_1566	-	Allophanate Hydrolase	2.10E-05	1.71
Oxidative stress response	SAUSA300_2502	<i>crtO</i>	Staphyloxanthin Biosynthesis	6.80E-05	-1.65
	SAUSA300_1909	-	Putative Thioredoxin	7.00E-07	-1.86
	SAUSA300_1197	<i>bsaA</i>	Glutathione Peroxidase	3.50E-06	-1.87
	SAUSA300_2463	<i>ddh</i>	D-Lactate Dehydrogenase	1.70E-06	-1.97
	SAUSA300_1044	<i>trxA</i>	Thioredoxin	2.00E-06	-2.2
	SAUSA300_0789	-	Putative Thioredoxin	1.00E-07	-2.3
	SAUSA300_1690	-	Putative Thioredoxin	2.00E-07	-2.35
Phage associated	SAUSA300_1947	-	Phi77 Orf031-Like Protein	4.90E-03	-1.71
	SAUSA300_1944	-	Phi77 Orf026-Like Protein Phage Transcriptional	8.20E-03	-1.71
Protein fate	SAUSA300_1656	<i>uspA1</i>	Universal Stress Protein	2.20E-06	-1.64
	SAUSA300_1984	<i>mroQ</i>	Membrane-Embedded CaaX Protease	2.10E-05	-1.69
	SAUSA300_1790	<i>prsA</i>	Foldase Protein PrsA	4.00E-07	-1.8
	SAUSA300_0752	<i>clpP</i>	ATP-Dependent Clp Protease Proteolytic Subunit	1.40E-06	-1.89
	SAUSA300_1295	<i>cspA</i>	Csd Family Cold Shock Protein	2.00E-07	-3.19
	SAUSA300_0067	-	Universal Stress Protein	2.00E-07	-3.35
Purine ribonucleotide biosynthesis	SAUSA300_0973	<i>purM</i>	Phosphoribosylaminoimidazole Synthetase	1.00E-07	2.65
	SAUSA300_0971	<i>purL</i>	Phosphoribosylformylglycinamide Synthase Ii	1.20E-06	2.48
	SAUSA300_0970	<i>purQ</i>	Phosphoribosylformylglycinamide Synthase I	3.10E-05	2.19
	SAUSA300_0972	<i>purF</i>	Amidophosphoribosyltransferase	4.00E-07	2.15
	SAUSA300_0974	<i>purN</i>	Phosphoribosylglycinamide Formyltransferase	5.00E-07	2.01
	SAUSA300_0975	<i>purH</i>	Bifunctional	8.00E-07	1.61

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

	SAUSA300_2183	<i>adk</i>	Adenylate Kinase	4.30E-05	1.57
Respiration	SAUSA300_0960	<i>qoxD</i>	Quinol Oxidase, Subunit Iv	9.90E-05	1.7
Ribosome and Protein synthesis	SAUSA300_2186	<i>rpmD</i>	50S Ribosomal Protein L30	1.70E-06	2.05
	SAUSA300_0531	<i>rpsG</i>	30S Ribosomal Protein S7	2.70E-06	1.79
	SAUSA300_2179	<i>rpsK</i>	30S Ribosomal Protein S11	3.40E-05	1.75
	SAUSA300_2187	<i>rpsE</i>	30S Ribosomal Protein S5	7.40E-06	1.7
	SAUSA300_2180	<i>rpsM</i>	30S Ribosomal Protein S13	2.10E-05	1.52
	SAUSA300_2188	<i>rplR</i>	50S Ribosomal Protein L18	2.10E-05	1.51
	SAUSA300_2648	<i>rpmH</i>	50S Ribosomal Protein L34	1.10E-03	-1.56
	SAUSA300_0991	<i>def</i>	Peptide Deformylase	1.50E-06	-1.81
	SAUSA300_1233	<i>rpmG2</i>	50S Ribosomal Protein L33	1.00E-04	-1.92
	SAUSA300_1545	<i>rpsT</i>	30S Ribosomal Protein S20	3.10E-05	-2.13
	SAUSA300_0053	<i>speG</i>	Spermidine N(1)-Acetyltransferase	6.50E-06	-2.19
	SAUSA300_0736	<i>saHPF</i>	Hibernation-Promoting Factor	4.00E-07	-2.26
	SAUSA300_1511	<i>rpmG</i>	50S Ribosomal Protein L33	6.30E-05	-2.33
	SAUSA300_1027	<i>rpmF</i>	50S Ribosomal Protein L32	7.40E-06	-2.38
	SAUSA300_1234	<i>rpsN2</i>	30S Ribosomal Protein S14	4.70E-06	-2.44
	SAUSA300_1535	<i>rpsU</i>	30S Ribosomal Protein S21	7.90E-06	-3.11
Transcriptional regulation	SAUSA300_0691	<i>saeR</i>	Dna-Binding Response Regulator SaeR	5.00E-07	2.28
	SAUSA300_0690	<i>saeS</i>	Sensor Histidine Kinase SaeS	1.00E-07	2.2
	SAUSA300_0928	<i>comK1</i>	Competence Transcription Factor	9.90E-06	1.86
	SAUSA300_0187	<i>argD</i>	Ornithine Aminotransferase	2.10E-06	1.85
	SAUSA300_1992	<i>agrA</i>	Accessory Gene Regulator Protein A	2.40E-06	1.77
	SAUSA300_1991	<i>agrC</i>	Accessory Gene Regulator Protein C	1.40E-06	1.57
	SAUSA300_2337	<i>nreC</i>	Dna-Binding Response Regulator Nrec	3.00E-03	-1.53
	SAUSA300_1708	<i>rot</i>	Accessory Regulator Rot	3.10E-06	-1.63
	SAUSA300_0114	<i>sarS</i>	Accessory Regulator	1.80E-06	-1.76
	SAUSA300_2218	<i>sarV</i>	Staphylococcal Accessory Regulator V	2.40E-05	-1.89
	SAUSA300_0954	-	Marr Family Transcriptional Regulator	1.10E-06	-2.3
	SAUSA300_2599	<i>icaR</i>	Intercellular Adhesion Operon Transcription Regulator	5.00E-07	-2.86
	SAUSA300_2437	<i>sarT</i>	Accessory Regulator T	9.40E-06	-3.14
Transport	SAUSA300_0176	<i>ssuC</i>	ABC Transporter Permease	4.70E-06	1.95
	SAUSA300_0436	<i>gmpB</i>	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	1.74
	SAUSA300_0201	-	Peptide ABC Transporter Permease	1.20E-06	1.68
	SAUSA300_0143	<i>phnE2</i>	Phosphonate ABC Transporter Permease	2.00E-05	1.6
	SAUSA300_0200	-	Peptide ABC Transporter ATP-Binding Protein	1.10E-06	1.59
	SAUSA300_0208	<i>malk</i>	Putative Maltose ABC Transporter ATP-Binding Protein	5.00E-05	1.52
	SAUSA300_2307	<i>hrtB</i>	ABC Transporter Permease	2.00E-05	-1.61
Virulence	SAUSA300_1755	<i>splD</i>	Serine Protease SplD	2.00E-07	5.79
	SAUSA300_1757	<i>splB</i>	Serine Protease SplB	0.00E+00	4.45
	SAUSA300_1758	<i>splA</i>	Serine Protease SplA	0.00E+00	4.15
	SAUSA300_1754	<i>splE</i>	Serine Protease SplE	8.00E-07	3.83
	SAUSA300_1756	<i>splC</i>	Serine Protease SplC	2.00E-07	3.44
	SAUSA300_1753	<i>splF</i>	Serine Protease SplF	3.00E-07	3.05
	SAUSA300_2600	<i>icaA</i>	N-Glycosyltransferase	3.40E-06	2.66
	SAUSA300_1382	<i>lukS-PV</i>	Panton-Valentine Leukocidin, Luks-Pv	1.00E-07	2.59
	SAUSA300_0951	<i>sspA</i>	V8 Protease	1.00E-07	2.55
	SAUSA300_1975	<i>lukH</i>	Aerolysin/Leukocidin Family Protein	8.50E-06	2.37
	SAUSA300_2364	<i>sbi</i>	IgG-Binding Protein Sbi	1.00E-07	2.15
	SAUSA300_1920	<i>chp</i>	Chemotaxis-Inhibiting Protein Chips	2.90E-06	2
	SAUSA300_1918	<i>hly-1</i>	Truncated Beta-Hemolysin	3.80E-05	1.93
	SAUSA300_2586	<i>asp2</i>	Accessory Sec System Protein	1.10E-06	1.91
	SAUSA300_0950	<i>sspB</i>	Cysteine Protease	1.20E-06	1.88
	SAUSA300_0285	<i>esxB</i>	Type VII Secretion System Extracellular Protein B	2.80E-06	1.87
	SAUSA300_2367	<i>hlyB</i>	Gamma-Hemolysin Component B	1.10E-06	1.83
	SAUSA300_1381	-	Panton-Valentine Leukocidin, Lukf-PV	3.00E-07	1.82
	SAUSA300_1058	<i>hlyA</i>	Alpha-Hemolysin	1.20E-06	1.78
	SAUSA300_2184	<i>secY</i>	Preprotein Translocase Subunit Secy	2.50E-06	1.76
	SAUSA300_1974	<i>lukG</i>	Leukocidin/Hemolysin Toxin Family Protein	5.50E-05	1.71
	SAUSA300_0949	<i>sspC</i>	Cysteine Protease	1.80E-05	1.71
	SAUSA300_2587	<i>asp1</i>	Accessory Secretory Protein Asp1	5.30E-05	1.69
	SAUSA300_2601	<i>icaB</i>	Intercellular Adhesion Protein B	1.40E-05	1.54
	SAUSA300_2572	<i>aur</i>	Zinc Metalloproteinase Aureolysin	1.10E-06	1.5
	SAUSA300_0899	<i>trfA</i>	Adaptor Protein	1.10E-06	-1.64
	SAUSA300_1784	<i>traP</i>	Signal Transduction Protein TraP	4.90E-06	-1.65
	SAUSA300_0401	<i>ssl7</i>	Superantigen-Like Protein 7	9.90E-06	-1.65
	SAUSA300_2262	<i>spdB</i>	Abi Domain-Containing Protein	1.30E-05	-1.69
	SAUSA300_0762	<i>secG</i>	Preprotein Translocase Subunit SecG	4.40E-04	-1.74

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

	SAUSA300_1594	<i>yajC</i>	Preprotein Translocase Subunit YajC	4.60E-05	-1.94
	SAUSA300_0289	<i>esaG</i>	Component Of The Type VII Secretion System	1.00E-05	-2.02
	SAUSA300_0395	<i>ssl1</i>	Superantigen-Like Protein	2.30E-05	-2.07
	SAUSA300_1988	<i>hld</i>	Delta-Hemolysin	6.50E-05	-2.09
	SAUSA300_2573	<i>isaB</i>	Immunodominant Antigen B	8.00E-07	-2.28
	SAUSA300_0399	<i>ssl5</i>	Superantigen-Like Protein 5	7.80E-06	-2.54
	SAUSA300_0654	<i>sarX</i>	Hypothetical Protein	1.30E-06	-3.03
Unknown	SAUSA300_0692	<i>saeQ</i>	Hypothetical Protein Of Unknown Function	2.00E-07	3.12
	SAUSA300_0175	<i>ssuA</i>	Putative Lipoprotein	1.20E-06	2.3
	SAUSA300_1877	-	Hypothetical Protein, Transmembrane	5.00E-07	2.06
	SAUSA300_0174	<i>ssuB</i>	Hypothetical Protein, Putative ABC Transporter	3.60E-06	2
	SAUSA300_0326	-	Hypothetical Protein, Protein-Adp-Ribose Hydrolase	9.90E-06	1.79
	SAUSA300_1565	-	Hypothetical Protein, Ahs2 Domain-Containing	5.10E-05	1.72
	SAUSA300_1734	<i>rppH</i>	Hypothetical Protein, Nudix Hydrolase Domain	2.00E-05	1.52
	SAUSA300_2448	-	Hypothetical Protein, Putative Transmembrane	3.40E-06	1.5
	SAUSA300_0427	<i>mpsC</i>	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	-1.7
	SAUSA300_2402	-	Hypothetical Protein, Putative Antitoxin	1.40E-05	-1.71
	SAUSA300_0094	-	Hypothetical Protein Of Unknown Function	1.30E-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	<i>bioX</i>	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	5.40E-05	-2
	SAUSA300_2354	<i>dsbA</i>	Putative Lipoprotein	1.10E-06	-2.02
	SAUSA300_2354	<i>dsbA</i>	Putative Lipoprotein	1.10E-06	-2.02
	SAUSA300_0342	-	Hypothetical Protein Of Unknown Function	1.30E-05	-2.04
	SAUSA300_0292	-	Hypothetical Protein, Putative Transmembrane	2.40E-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	<i>ywpF</i>	T6S 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	<i>rnhA</i>	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, Pepsy Domain	1.00E-07	-2.36
	SAUSA300_1240	-	Hypothetical Protein, Putative Transmembrane	1.10E-05	-2.37
	SAUSA300_0050	-	Hypothetical Protein, Putative Transmembrane	1.40E-05	-2.41

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

SAUSA300_1107	-	Hypothetical Protein, Putative Transmembrane	2.50E-05	-2.41
SAUSA300_1277	-	Hypothetical Protein Of Unknown Function	1.10E-05	-2.46
SAUSA300_1484	-	Hypothetical Protein, Putative Transmembrane	3.50E-05	-2.49
SAUSA300_1478	-	Putative Lipoprotein	8.00E-07	-2.6
SAUSA300_1478	-	Putative Lipoprotein	8.00E-07	-2.6
SAUSA300_2481	-	Hypothetical Protein, Yoze Sam Like Domain	2.00E-05	-2.6
SAUSA300_0373	-	Hypothetical Protein, Hth Cro/C1-Type Domain-	9.20E-06	-2.66
SAUSA300_2403	-	Putative Lipoprotein	5.00E-07	-2.67
SAUSA300_2403	-	Putative Lipoprotein	5.00E-07	-2.67
SAUSA300_2246	-	Hypothetical Protein, Bph_3 Domain	1.00E-07	-2.67
SAUSA300_2544	-	Hypothetical Protein, Yoze Sam Like Domain	9.90E-06	-2.73
SAUSA300_1432	-	Phislt Orf78-Like Protein	1.70E-05	-2.74
SAUSA300_2041	-	Hypothetical Protein Of Unknown Function	1.90E-05	-2.74
SAUSA300_1208	-	Hypothetical Protein Of Unknown Function	9.30E-06	-2.81
SAUSA300_0266	-	Hypothetical Protein Of Unknown Function	6.70E-06	-2.86
SAUSA300_1581	-	Hypothetical Protein Of Unknown Function	4.00E-07	-2.89
SAUSA300_2493	<i>cwrA</i>	Hypothetical Protein, Cell Wall Stress Stimulon	5.80E-06	-2.92
SAUSA300_0937	-	Hypothetical Protein, Putative Transmembrane	9.90E-06	-2.93
SAUSA300_1493	-	Hypothetical Protein Of Unknown Function	6.40E-06	-2.95
SAUSA300_1008	-	Hypothetical Protein Of Unknown Function	7.80E-06	-3.03
SAUSA300_1795	-	Hypothetical Protein Of Unknown Function	1.00E-07	-3.04
SAUSA300_2328	-	Hypothetical Protein Of Unknown Function	2.00E-07	-3.1
SAUSA300_2460	-	Acetyltransferase Family Protein	9.00E-07	-3.17
SAUSA300_2460	-	Acetyltransferase Family Protein	9.00E-07	-3.17
SAUSA300_1180	-	Hypothetical Protein Of Unknown Function	1.00E-06	-3.78
SAUSA300_1215	-	Hypothetical Protein Of Unknown Function	1.20E-06	-3.83
SAUSA300_0816	-	Csbd-Like Superfamily Protein	4.00E-07	-4
SAUSA300_1904	-	Hypothetical Protein, Putative Transmembrane	5.00E-07	-4.36

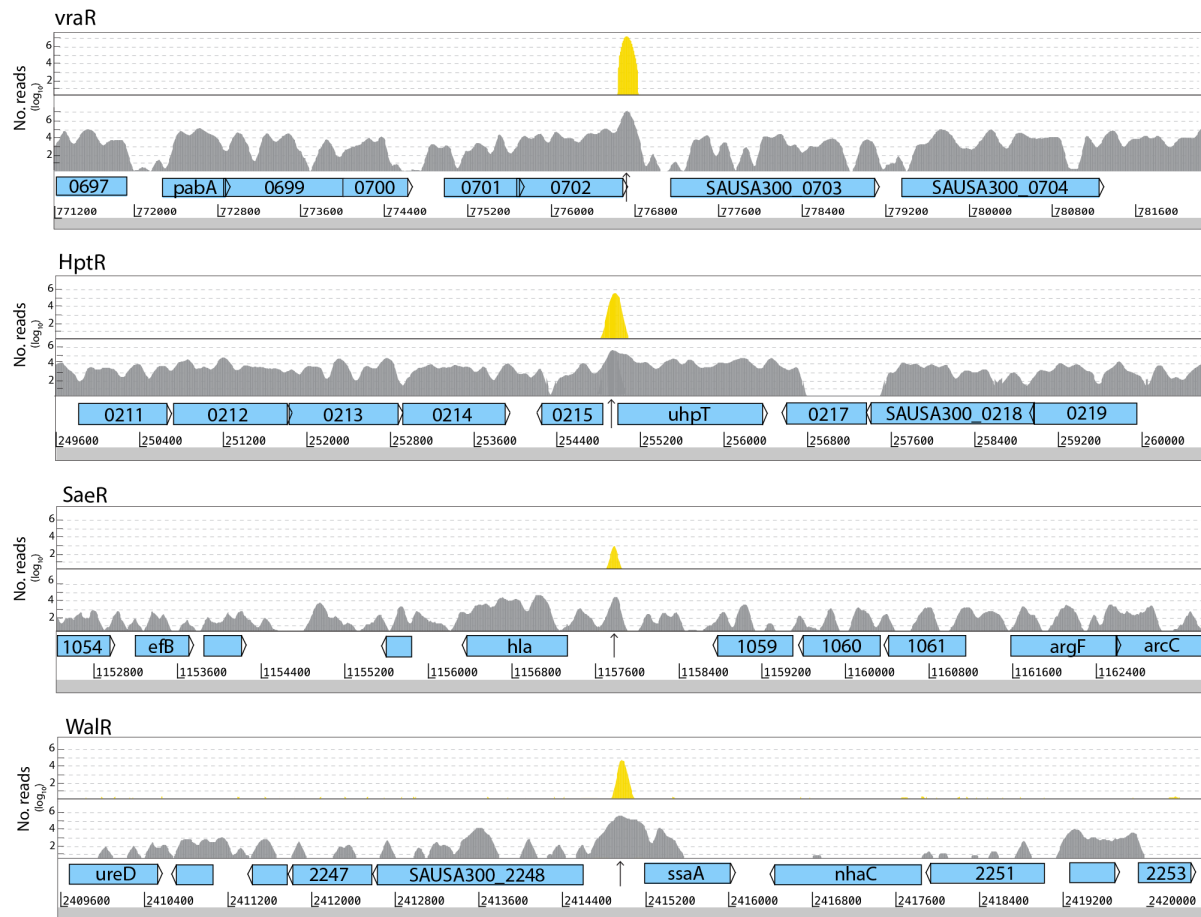


Figure S2. *S. aureus* ChIP-seq reveals binding sites of response regulators. Artemis userplots showing examples of the impact of non-target in silico read-subtraction for the identification of target-specific response regulator DNA-binding regions

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	<i>sasD</i>	Cell-wall associated protein	Contains LPXAG motif – likely covalently anchored to peptidoglycan	Negative (5.8-log2 decrease, 56-fold)	Yes	154986..155175	4.24929	Yes	155136..155152
SAUSA300_0438	<i>sle1</i>	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	491543..491550	0.97290	Yes	491559..491575
SAUSA300_0524	<i>rplJ</i>	50S ribosomal protein L10	binds the two ribosomal protein L7/L12 dimers and anchors them to the large ribosomal subunit	No regulation	No	583428..583441	0.62689	No	
SAUSA300_0602		Hypothetical protein	unknown	Negative (3.3-log2 decrease, 10-fold)	Yes	675677..675892	5.77914	Yes	675820..675847
SAUSA300_0653		AraC family transcriptional regulator	Transcriptional regulation	Negative (1.2-log2 decrease, 3-fold)	Yes	728668..728678	0.64084	No	
SAUSA300_0681		hypothetical protein		Positive (2.3-log2 decrease in down mutant)	No	755739..755812	2.21698	No	
SAUSA300_0703	<i>ltaS</i>	Glycerol phosphate lipoteichoic acid synthase	Synthesis of polyglycerol-phosphate lipoteichoic acid (LTA) from phosphatidylglycerol	Negative (0.7-log decrease, 1.6-fold)	No	776863..776913	2.43150	Yes	776866..776882
SAUSA300_0860	<i>rocD</i>	ornithine--oxo-acid transaminase		Positive (1.2-log2 increase, 2-fold)	Yes	937633..937638	0.462098	Yes	937577..937593
SAUSA300_1081		Hypothetical protein		No regulation	Yes	1182912..1182918	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	1191626..1191643	0.917025	Yes	1191606..1191622
SAUSA300_1188	<i>mutS</i>	DNA mismatch repair protein MutS	This protein performs the mismatch recognition step during the DNA repair process	No regulation	Yes	1306553..1306561	0.539114	No	
SAUSA300_1203		Hypothetical protein		No regulation	Yes	1325832..1325849	1.02376	No	
SAUSA300_1304		Hypothetical protein		No regulation	Yes	1435403..1435418	1.04382	No	
SAUSA300_1344	<i>dnaD</i>	putative DNA replication protein DnaD		No regulation	No	1509472..1509481	0.554517	Yes	1509483..1509499
SAUSA300_1461		Hypothetical protein		No regulation	Yes	1615853..1615880	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	<i>rrsC</i>	16S rRNA				2002908..2002944	1.675390	Yes	2003159..2003174
SAUSA300_1890		staphopain A		Positive (0.85-log ₂ increase, 1.8-fold)	Yes	2055015..2055025	0.740715	No	
SAUSA300_2124	<i>rrsE</i>	16S rRNA				2297762..2297799	2.261965	No	
SAUSA300_2209		Hypothetical protein		Negative (0.84-log ₂ decrease, 1.8-fold)	Yes	2375987..2375993	0.553028	No	
SAUSA300_2249	<i>ssaA_1</i>	N-acetylmuramoyl-L-alanine amidase (autolysin)	cleaves amide bond between N-acetylmuramoyl and L-amino acids in peptidoglycan	Positive (1.3-log ₂ increase, 2.5-fold)	Yes	2414865..2415012	4.061749	Yes (2x)	2414918..2414933 2415046..2415061
SAUSA300_2506	<i>isaA</i>	Lytic transglycosylase	cleaves the β-1,4 glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine residues of peptidoglycan	Positive (1.1-log ₂ increase, 2.2-fold)	No	2712808..2712836 (peak-1)	1.933075	Yes	2712897..2712912
SAUSA300_2506	<i>isaA</i>	Lytic transglycosylase		Positive (1.1-log ₂ increase, 2.2-fold)	No	2712870..2713227 (peak-2)	4.523005	Yes	2713089..2713104
VraR									
SAUSA300_0703	<i>ltaS</i>	Glycerol phosphate lipoteichoic acid synthase	Synthesis of polyglycerol-phosphate lipoteichoic acid (LTA) from phosphatidylglycerol			776616..776813	5.536103	Yes	
SAUSA300_1357	<i>aroC</i>	chorismate synthase	catalyzes the formation of chorismate from 5-O-(1-carboxyvinyl)-3-phosphoshikimate in aromatic amino acid biosynthesis			1525199..1525211	0.8984054		
SAUSA300_2016	<i>rrlD</i>	23S ribosomal RNA							
HptR									
SAUSA300_0151	<i>adhE</i>	bifunctional acetaldehyde-CoA/alcohol dehydrogenase				2179043..2179049	4.352250		
SAUSA300_0216	<i>uhpT</i>	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"			254804..254999	4.867318	Yes	
SAUSA300_0303		Hypothetical protein				352882..352918	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	<i>gltB</i>	glutamate synthase, large subunit	an essential enzyme in the nonmevalonate pathway of isopentenyl diphosphate and dimethylallyl diphosphate biosynthesis			497269..497311	2.7078340		
SAUSA300_0769		Hypothetical protein				857300..857341	0.927730		
SAUSA300_0883		Putative surface protein				969867..969932	1.503311		
SAUSA300_1052		fibrinogen-binding protein				1150372..1150493	2.338896		
SAUSA300_1058		alpha-hemolysin				1157882..1157919	1.312439		
SAUSA300_1463		Hypothetical protein				1617905..1617937	0.817594		
SAUSA300_1654		proline dipeptidase				1818897..1818945	1.959034		
SAUSA300_2012	<i>leuC</i>	isopropylmalate isomerase large subunit	dehydratase component, catalyzes the isomerization between 2-isopropylmalate and 3-isopropylmalate			2172237..2172454	5.527080		
SAUSA300_2504		acyltransferase				2710979..2711032 (peak-1)	2.532463		
SAUSA300_2538		amino acid permease family protein				2738847..2738937 (peak-2)	4.517702		
SAUSA300_2538		amino acid permease family protein				2739312..2739437	5.807741		
SaeR									
SAUSA300_0220	<i>pflB</i>	formate acetyltransferase				260249..260326	2.245046		
SAUSA300_0472	<i>ipk</i>	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	An essential enzyme in the nonmevalonate pathway of isopentenyl diphosphate and dimethylallyl diphosphate biosynthesis			530663..530696	1.313321		
SAUSA300_0892	<i>oppA</i>	oligopeptide ABC transporter oligopeptide-binding protein				979938..980000	2.499846		
SAUSA300_0919	<i>murE</i>	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--L-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			1008357..1008398	1.681862		
SAUSA300_1058	<i>hla</i>	Alpha-hemolysin				1157746..1157818	2.372307	Yes	
SAUSA300_1648		putative NADP-dependent malic enzyme				1811157..1811173	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				2320758..2320829	2.399629		
SAUSA300_2158		Hypothetical protein				2336211..2336232	0.992981		
SAUSA300_2423		Hypothetical protein				2609040..2609080	2.380980		
SAUSA300_2423		Hypothetical protein				2609182..2609231 (peak-1)	2.487435		
						(peak-2)			

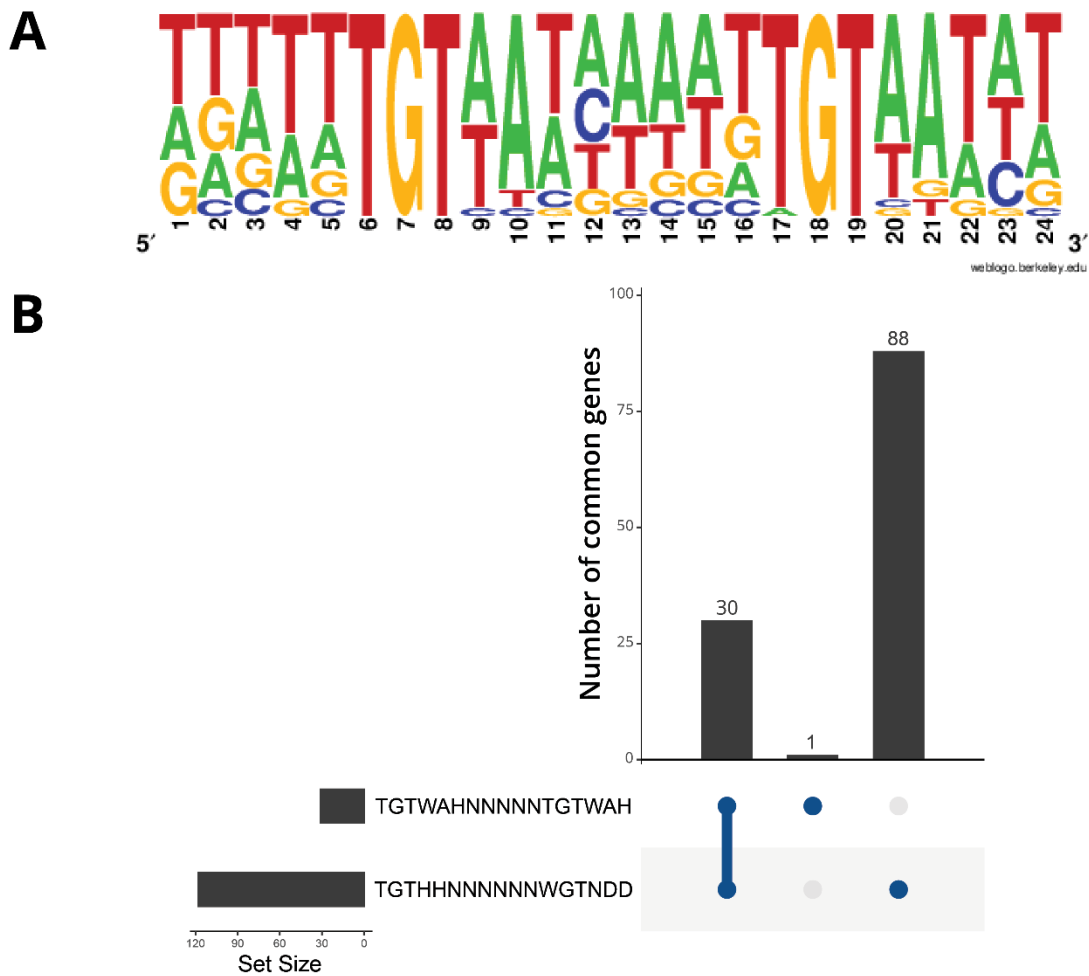


Figure S3. Generation of an *S. aureus* WalR binding motif. A) A combined frequency plot of experimentally validated WalR binding motifs and those detected through ChIP-seq analysis. B) Upset plot comparison of overlap between intergenic WalR binding sites identified using a previously published motif (TGTWAH₅TGTWAH) and that defined in A) (TGTHHNNNNNNWGTNDD).

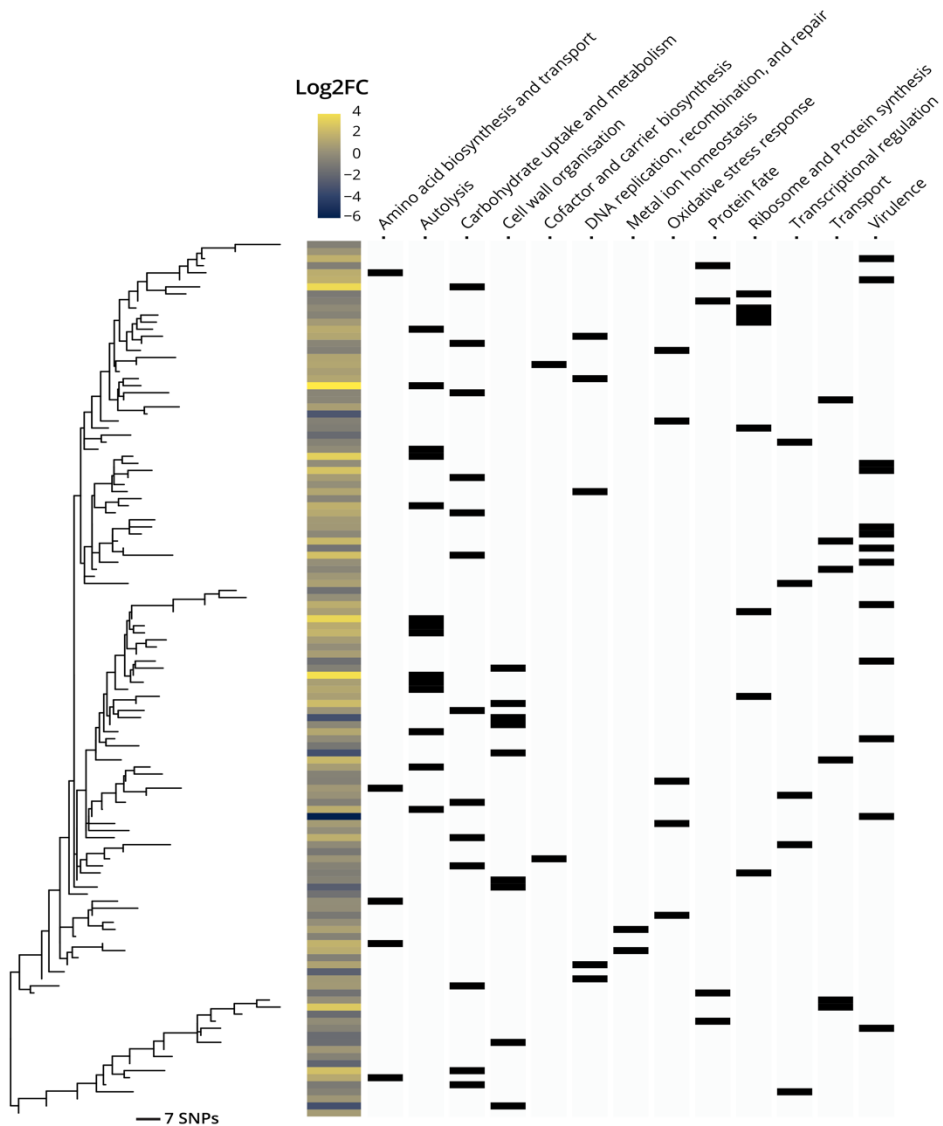
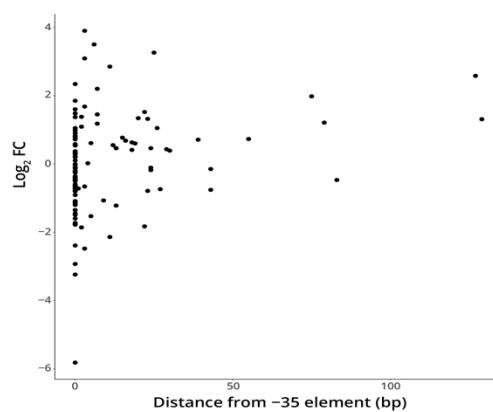
A**B**

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 Log_2FC 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 WalR 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.

Table with columns: Gene, Locus tag, Direction, Log2FC, and Sequence. The table lists 137 genes from SAUSA300_2253 to SAUSA300_1639, providing their relative positions, directions, and Log2FC values. The sequence column contains DNA sequences with predicted WalR binding sites (red), TSS (green), and promoter elements (yellow) highlighted.

<i>isdB</i>	SAUSA300_1028	Fwd	-0.65	AACCTATGTCATAGATATTTCATAATCTATAACATAGGTTATTTTTTATAAATAACG TTGCAAT TAACTAACATTTCAAT CTACAATACAAGTAATCA (52nt) ATG
-	SAUSA300_1026	Fwd	-0.66	ATTCGTCTTCCGTTATTCATTATTAAATTTGTAACAGAACATGATATGTTAAGAAAAAT CTTGACA ACT TTGTTCTTAGAAAGT TAAAT AAATTTTG (21nt) ATG
<i>ackA</i>	SAUSA300_1657	Fwd	-0.66	TGACAGAGTTAAATCAGTGGATGGACACAAATCGTCTAAAAATAAT CTGTAATAGTA TAGTCA TAAAACGTATGATGAT TAATAAT GAAAAATGAAAT (34nt) ATG
-	SAUSA300_2592	Rev	-0.66	GAGTGAACAGAATTATATTTCCATAGCAAAATTCCTAA ACTACACATACGTTACATAA TGGATTCA TTTTTA TAGAAACGGGTAAAAATGATAAAGTAA (40nt) ATG
<i>scdA</i>	SAUSA300_0253	Rev	-0.72	TTGTGAGCCAAATGATTTGAGGGCTTATTTTGTGT TTATGACATGATTAGACA TTCCCT GATTTTCATTTTCATATA CATTAAAT GTATACACT (22nt) ATG
-	SAUSA300_0365	Fwd	-0.72	TTCATATCATTTTTGATATTAATTCATTTGAACTTTCATGATATTTTTAAAAATACAC TTGACA AAAGCGAATAT GTCTATAAT AGTTGT GAGGT (8nt) ATG
-	SAUSA300_0657	Rev	-0.73	TAGTAAAAATAATAAATTTTCATATGATTAACAAAACTATA AAACTGTATCA TGACA TGACATCATTTATGATTT TAATAAT AAATGACAAG (11nt) ATG
-	SAUSA300_1463	Fwd	-0.74	ATAATGTTGGTATAT TGTTAAAAAT TGCTCA AAATATAGGTGTGATTCAGATAGTT TTA TTGAC CAATATGTTATTAATTA CTAGAAT GAGGATAGTT (28nt) ATG
<i>tarF</i>	SAUSA300_0248	Fwd	-0.76	AGTGGT ACATTAATCATGTATTTCCGTATGATAATTAACGACAAGTGA TTGGTTA AAATGATTTTATGATGAAAT GC TATAAT AGGCATGGT (57nt) ATG
-	SAUSA300_1278	Fwd	-0.79	GCAATATTCCTACTACTAATAGAAAAATCATTGTTCCCTGGACATGGAAAT CTAACA TATATCGTTTAGGACAAAAT ATG TATAAT GAATGATTA (18nt) ATG
-	SAUSA300_1510	Rev	-0.79	AAGATTTTCAATTTGAAATAATAAATTTTGAATTTGCGCATAATTCAT CTAGACACATATAGACA TGATTTGTTATTAACG ATATAAT AGAATTGAATA (14nt) ATG
<i>hchA</i>	SAUSA300_0536	Fwd	-0.80	ATTTAGTTGATTTTTTCAAGAAATTCATTTTGATATTTTTGATAATGACAT TTAAT AGTAATACAT TTATAGT GTAGT ATATGCTATAC (35nt) ATG
<i>paiA</i>	SAUSA300_2316	Rev/Fwd	-0.91	TGAAAAACGCCATTTTACTGGAATAAATGTATCATTTTATGAT TTACACACAATCAATACA ATT TTGAAT TGATTTGTT TTAAT GTATTTTAAATA (63nt) ATG
<i>bstA</i>	SAUSA300_2254	Rev	-1.07	GTATATCACCTCCATCAAAGTACGG CTTACATCATTGATACACA AGACAT ATGACA TAGTTGATAGCTAACTCTAC TATACT TAAGATAA (14nt) ATG
<i>rpsP</i>	SAUSA300_1131	Rev	-1.09	TCACATAGTTATTTGTAAGAAAAAGTTCAATAATTTCCGTGTAAGAAAA CTCTTACA AACTTCATACACCTGTAA TATAT TTCTGTAG (40nt) ATG
<i>sodA</i>	SAUSA300_1513	Rev	-1.14	CATTTCACTCCGTCGTATGATTTTATGGCAA CTGTTAACT TTTTAT TACAAA ATGTTTAAATATTTTGGATTTGAGT TATAT AAATGAGTAGA (96nt) ATG
<i>walI</i>	SAUSA300_0024	Fwd	-1.20	CGCATTTATCATATTTTAAAGTAGAACCGCATTGAAAATAGTGTAACTGTTATTTTAAAA CTTTAGT ATT TTGCTAATCAT TTGTTAAT AAATTAAG (182nt) ATG
<i>bstA</i>	SAUSA300_2626	Fwd	-1.22	TTGAGA CTTTT CGTCAACTATCTTTT TGTAAGGTAGTTGTTG TACACATTCCT TAATGACT TAACAAC TTT GTAATAG GGTAAT CTTACGGAA GT (29nt) ATG
<i>sasF</i>	SAUSA300_2581	Fwd	-1.35	TTAAATATAAGCTAAGTAATAAGTAGATAA TACTAACACA TA ACT AGATAGATAAG TTGTAAT TTCTCTGTAAC AGGTA TATAAT AGTATGTAATC (33nt) ATG
<i>cspC</i>	SAUSA300_0777	Fwd	-1.45	TATTAATGGAATGTACTATATAGTTC AAATG ATTTATCACAGAAAA TAAATAAT GC TTTACT CTATATTTAA AGCTATAAT GAAAGTTAAG (109nt) ATG
-	SAUSA300_0374	Fwd	-1.49	AATAACGATAGCTACATTGAATAA ATTTGATAT CA ATTACTACT TTTTAAAA ATTTGGATA AAAA TAATTTGAAT TGTTT TAGAAT TGTAAT TAAG (50nt) ATG
-	SAUSA300_1296	Fwd	-1.53	TTTACTTAAAA AACTAATA ACTATAGTACTACT TTGTTGTT TTCAAGTCG CAAA CTTGATT TTACAGGATAAAG CTATAAA AAATAGTATAGA (30nt) ATG
<i>spa</i>	SAUSA300_0113	Rev	-1.60	TTTATAAGTTGTA AACTACCT TTAAATTTA ATTA AAATATAGATTTTAGT TTGCAA TACATAATTCGTTATAT TATGAT ACTTT TACA ATACATA (13nt) ATG
<i>tagG</i>	SAUSA300_0625	Fwd	-1.73	TGATTTGATACTATA ATGATTTGTA AA AACTAATA TTTTTAAAGAACTAGACA ATAATTTGATA GCATCC ATGATAGTAT TAGTAT TACAACAAT (62nt) ATG
-	SAUSA300_1070	Fwd	-1.78	TGAAAA CAATTTAAT CATGGAA ATTTATA AT TCA TTGTTTACATTTTCA AACTATGABA AA ACACA AGTGGTT TAATCTATAAT AAATAGTAGTAA (21nt) ATG
<i>csbD</i>	SAUSA300_1582	Rev	-1.83	CATGTTTTATTTA ATCGTATA AA TACTATA CTATA CACTAAAA ACTCATATTTAAT TTTACGG AA CTCCT TAGTGGTA TATAAA TATATACATC (15nt) ATG
-	SAUSA300_0682	Fwd	-1.86	TTAATTTAA AAAGTGAAT CA ACTTATA AAAT GATGTA AA TTGTTATGCA AA ATCAACC AA ATCCGTAAT GTATTTAA ATGTAAAT AGTTCTGAAG (21nt) ATG
-	SAUSA300_0985	Fwd	-2.14	GTATTTTAA AAACAT TATCGCGTATGA ATTTGTTATA TT TATGTCAG TTCA CAATGTCTCACA AA TTGAATTTCAAT TATAAT GTGTATATTAC (30nt) ATG
-	SAUSA300_1965	Fwd	-2.39	GTGATAAGTACATGTT TCAGGAT TTGGA ACTGTACG TTAA ATGTGGCA TTTCTATCTTT CTCTT CGT TATAAT GT TTATCTCTCC TAGTGA (14nt) ATG
-	SAUSA300_0992	Fwd	-2.48	ATGACGTA CTGTCA ACGATAT ACTTAG TAATGAAGAT GTAAATGTAAT TGTTTAAAT TTGATT CCAA GCAGAT TTTAT TATCAT TTAATTTAAAT (20nt) ATG
<i>maoC</i>	SAUSA300_0031	Rev	-2.93	AGATAAA ATGGTGCAT TTGCTCA ATGAA AGT TAGAGGT GAGTCTT AGTT CA GTGACG CTAGACT TACCTTAA CA CTTTACAT ACTTAAAA NTTA (24nt) ATG
-	SAUSA300_0602	Fwd	-3.24	CAACAACAT CA CGATGAA AGCAT TT TAATTTT AGTGA TAAGTGAAT TATAAA GTAGTAA TATATTTGTAAT TATAAT TGTAAT TGTAAT ATG ACTGTTG (66nt) ATG
<i>sasD</i>	SAUSA300_0136	Fwd	-5.82	AATCTAA AGTGAAT TTTTTAA AAAAAT GTAA ATGAT CA AAAAATTT TTGCAT TTCTTT GTAA TCG TATATAAT GTAA ATGTAAT CAAAAT TGTAAT (16nt) ATG
<i>teg41</i>	srm_1080	Rev	#N/A	TACAGTGCATTT TACAAA CTTT TACA ATCAT TGGTT TAATAGAG TTAAGCT CATGA TTTGACA TGAAGCAGAA TTTAA T TATAT TTATGTTATA AA

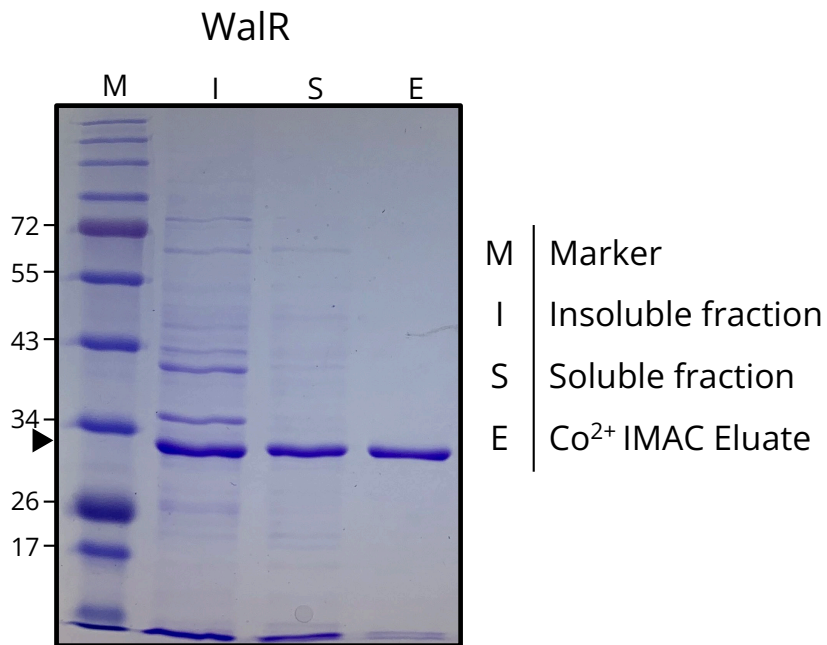


Figure S5. Purification of recombinant WalR. WalR was purified by single step immobilized metal-ion affinity chromatography using Cobalt TALON resin. SDS PAGE image shows cellular fractionation and purification. Black arrow denotes 6-His-WalR (28kDa).

A

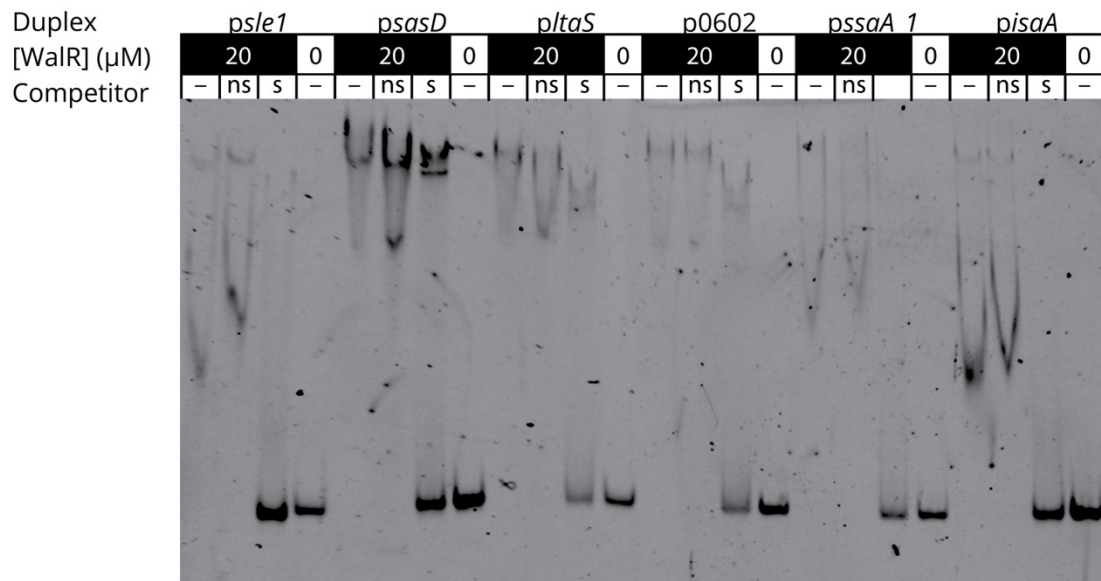


Figure S6. DNA competition EMSAs to test binding specificity of WalR. Competition electrophoretic mobility shift assay (EMSA) showing the effect on WalR binding of the presence or absence (–) of an excess of non-specific (ns) or specific (s) non-labelled duplex competitor).

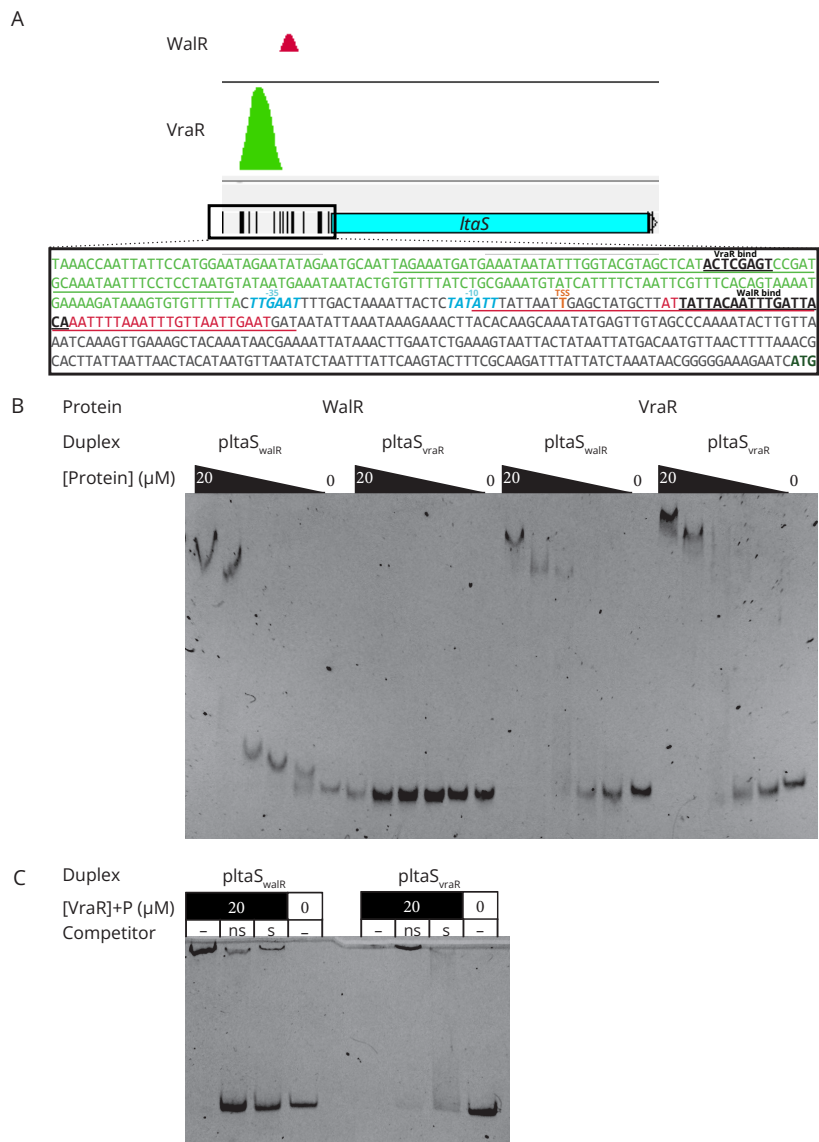


Figure S7. WalR and VraR binding at the *ItaS* locus. A) Artemis userplot showing VraR and WalR ChIP-seq peaks. Box shows sequence 529 bp upstream of *ItaS* start codon. Bases coloured green correspond to VraR binding peak, red to WalR. Transcriptional start site in orange, -35 and -10 promoter elements 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 respectively. 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.

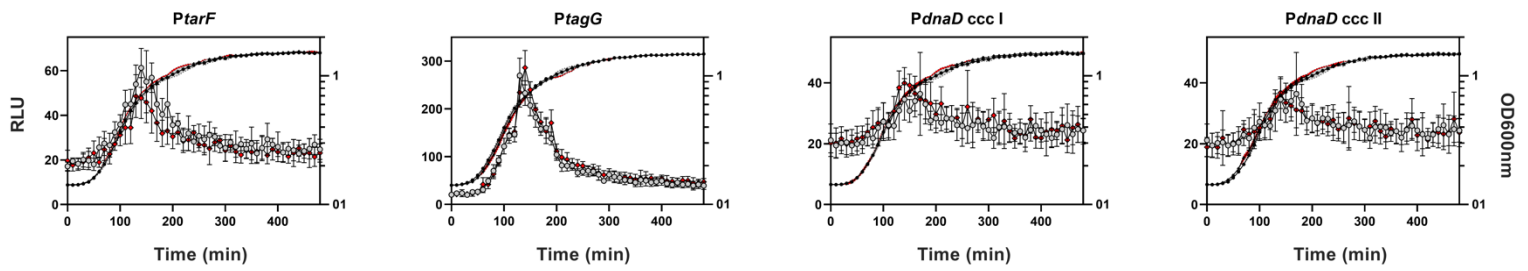


Figure S8. Impact of WalR motif mutation on the expression from *tarF*, *tagG* and *dnaD*. The above genes coupled to bacterial luciferase reporters showing the changes in promoter activity of the Wt (open grey circle: RLU; filled black circles: OD600nm) or the ccc (open red diamond: RLU; filled black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

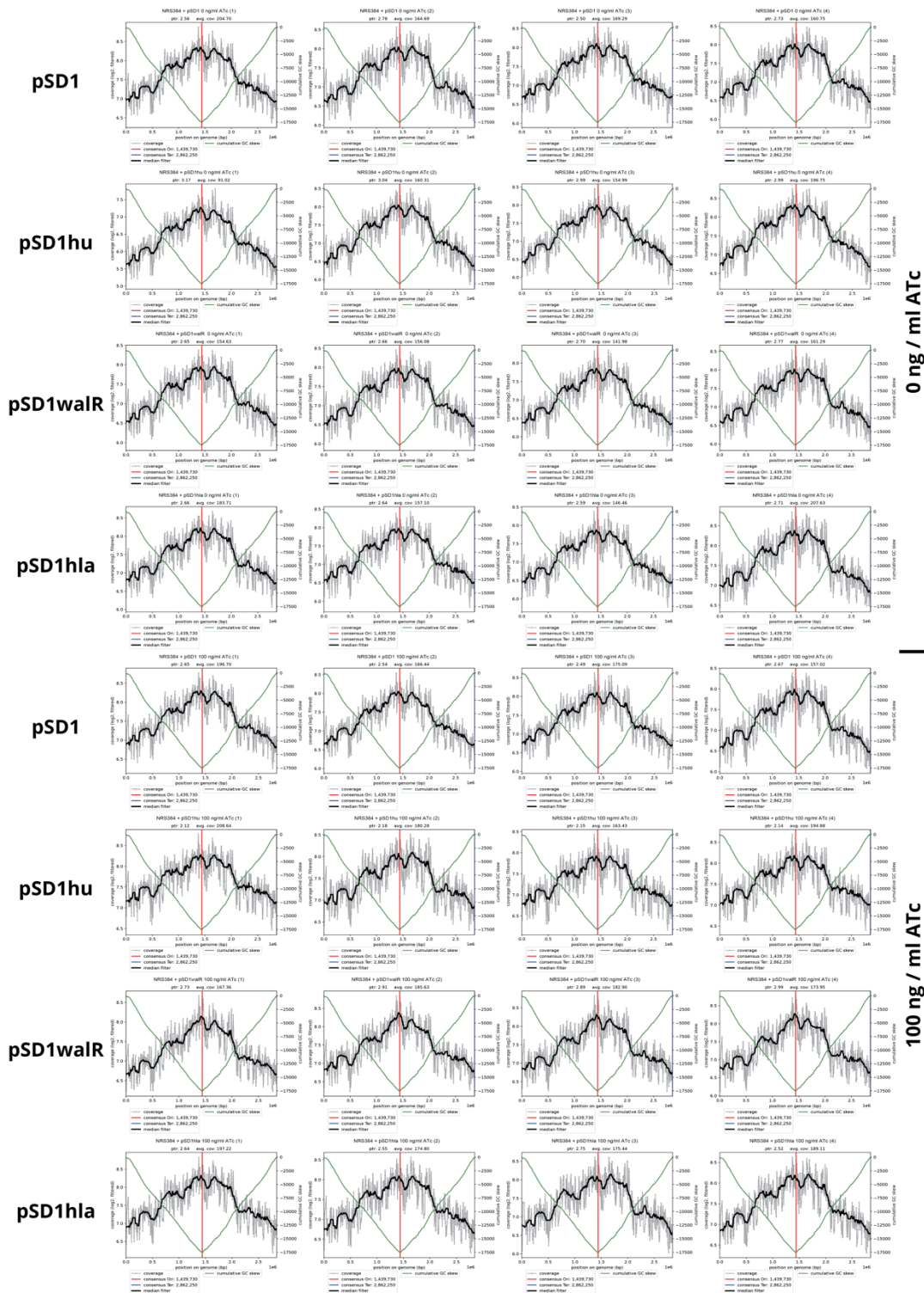


Figure S9. The effect of waIR, hup, and hla on Ori:Ter ratio. Graphs show log₂-transformed coverage values (y axis) at relative positions across the genome (x axis). Cumulative GC-skew is plotted on the second Y axis. The calculated position of the origin of replication (consensus ori) is marked in red. 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 (<https://github.com/christophertbrown/iRep>).

Table S5. Strains and plasmids used in this study

<i>Escherichia coli</i> strains	Description	Reference / Supplier
IM08B	DH10BΔ <i>dcm</i> . Expresses CC8 adenine methylation profile for direct transformation of NRS384.	(1)
Rosetta 2(DE3)	B-strain. F ⁻ <i>ompT hsdSB</i> (r _B ⁻ m _B ⁻) <i>gal dcm</i> (DE3); IPTG-inducible T7 RNA polymerase. For protein production.	Novagen
<i>Staphylococcus aureus</i> strains	Description	Reference / Supplier
RN4220	ST8; CC8; chemically mutagenized derivative of 8325-4, transformable with <i>E. coli</i> DNA; premature stop codon in both <i>hsdR</i> and <i>sauUSI</i> .	(2)
NRS384	USA300-14 clone. Tetracycline resistant.	BEI resources
NRS384 Δ <i>spa</i>	Clean deletion of protein A from TTG to TAA.	This study
NRS384 Δ <i>yycHI</i>	Deletion of WalKR positive regulators <i>yycHI</i> . Clean deletion from codon 5 of <i>yycH</i> to the TAA of <i>yycI</i> .	(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 <i>walK</i> ^{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 <i>walK</i> -LgBIT	Large-Bit split luciferase tag incorporated onto the C-terminus	This study
NRS384 <i>walR</i> ^{FLAG}	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 <i>E. coli</i> / <i>S. aureus</i> shuttle vector. pC194 rep. C-terminal 1x FLAG. Amp(R) Cm(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 <i>S. aureus</i> . Amp(R) Cm(R).	(6)
pCN34	<i>S. aureus-E. coli</i> shuttle vector, pT181-cop-wt <i>repC</i> Cole1 Kan(R).	(7)
Allelic exchange constructs for <i>S. aureus</i>.		
pIMAY-Z Δ <i>spa</i>	For construction of protein A deficient strain by allelic exchange.	This study
pIMAY-Z <i>walR</i> ^{T101A}	For construction of WalR T101A mutation by allelic exchange.	This study
pIMAY-Z <i>walK</i> ^{Y32C}	For construction of WalK Y32C mutation by allelic exchange.	This study
pIMAY-Z <i>walK</i> ^{T389A}	For construction of WalK T389A mutation by allelic exchange.	This study
pIMAY-Z <i>walR</i> (SmBIT)	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 regulator overexpression constructs for <i>S. aureus</i>.		
pRAB11: <i>walR</i> ^{FLAG}	ATc inducible plasmid containing of C-terminally FLAG-tagged WalR, for ChIP-seq.	This study
pRAB11: <i>vraR</i> ^{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 <i>hptR</i> , 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: <i>PsasD</i> -YFP	Native <i>sasD</i> driven expression of YFP.	This study
pIMC8: <i>Psle1</i> -YFP	Native <i>sle1</i> driven expression of YFP.	This study
pIMC8: <i>P602</i> -YFP	Native SAUSA300_0602 driven expression of YFP.	This study
pIMC8: <i>PItaS</i> -YFP	Native <i>ItaS</i> driven expression of YFP.	This study
pIMC8: <i>PssaA1</i> -YFP	Native <i>ssaA1</i> driven expression of YFP.	This study
pIMC8: <i>PisaA</i> -YFP	Native <i>isaA</i> driven expression of YFP.	(3)
pIMC8: <i>PsasD_{ccc}</i> -YFP	Native <i>sasD</i> driven expression of YFP. Mutation in the putative WalR binding site.	This study
pIMC8: <i>Psle1_{ccc}</i> -YFP	Native <i>sle1</i> driven expression of YFP. Mutation in the putative WalR binding site.	This study
pIMC8: <i>P602_{ccc}</i> -YFP	Native SAUSA300_0602 driven expression of YFP. Mutation in the putative WalR binding site.	This study
pIMC8: <i>PItaS_{ccc}</i> -YFP	Native <i>ItaS</i> driven expression of YFP. Mutation in the putative WalR binding site.	This study
pIMC8: <i>PssaA1_{cccI}</i> -YFP	Native <i>ssaA1</i> driven expression of YFP. Mutation in the putative WalR binding site (cccl).	This study
pIMC8: <i>PssaA1_{cccII}</i> -YFP	Native <i>ssaA1</i> driven expression of YFP. Mutation in the putative WalR binding site (ccclI).	This study
pIMC8: <i>PisaA_{cccI}</i> -YFP	Native <i>isaA</i> driven expression of YFP. Mutation in the putative WalR binding site (cccl).	(3)
pIMC8: <i>PisaA_{cccII}</i> -YFP	Native <i>isaA</i> driven expression of YFP. Mutation in the putative WalR binding site (ccclI).	(3)

Bacterial LUX reporter plasmids for *S. aureus*.

pIMK1-LUX: <i>PsasD</i>	Native <i>sasD</i> driven expression of LUX.	This study
pIMK1-LUX: <i>Psle1</i>	Native <i>sle1</i> driven expression of LUX.	This study
pIMK1-LUX: <i>P602</i>	Native SAUSA300_0602 driven expression of LUX.	This study
pIMK1-LUX: <i>PItaS</i>	Native <i>ItaS</i> driven expression of LUX.	This study
pIMK1-LUX: <i>PssaA1</i>	Native <i>ssaA1</i> driven expression of LUX.	This study
pIMK1-LUX: <i>PisaA</i>	Native <i>isaA</i> driven expression of LUX.	This study
pIMK1-LUX: <i>PsasD_{ccc}</i>	Native <i>sasD</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: <i>Psle1_{ccc}</i>	Native <i>sle1</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: <i>P602_{ccc}</i>	Native SAUSA300_0602 driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: <i>PItaS_{ccc}</i>	Native <i>ItaS</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: <i>PssaA1_{cccI}</i>	Native <i>ssaA1</i> driven expression of LUX. Mutation in the putative WalR binding site (cccl).	This study
pIMK1-LUX: <i>PisaA_{cccI}</i>	Native <i>isaA</i> driven expression of LUX. Mutation in the putative WalR binding site (cccl).	This study
pIMK1-LUX: <i>Phup</i>	Native <i>hup</i> driven expression of LUX.	This study
pIMK1-LUX: <i>Pspa</i>	Native <i>spa</i> driven expression of LUX.	This study
pIMK1-LUX: <i>Pprs</i>	Native <i>prs</i> driven expression of LUX.	This study
pIMK1-LUX: <i>PrpIK</i>	Native <i>rplK</i> driven expression of LUX.	This study
pIMK1-LUX: <i>PdnaA</i>	Native <i>dnaA</i> driven expression of LUX.	This study
pIMK1-LUX: <i>PdnaD</i>	Native <i>dnaD</i> driven expression of LUX.	This study
pIMK1-LUX: <i>PtarF</i>	Native <i>tarF</i> driven expression of LUX.	This study
pIMK1-LUX: <i>PtagG</i>	Native <i>tagG</i> driven expression of LUX.	This study
pIMK1-LUX: <i>Phup_{ccc}</i>	Native <i>hup</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: <i>Pspa_{ccc}</i>	Native <i>spa</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: <i>Pprs_{ccc}</i>	Native <i>prs</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study

pIMK1-LUX: <i>PrpK_{ccc}</i>	Native <i>rplK</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: <i>PdnaA_{ccc}</i>	Native <i>dnaA</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: <i>PdnaD_{cccI}</i>	Native <i>dnaD</i> driven expression of LUX. Mutation in the putative WalR binding site (cccl).	This study
pIMK1-LUX: <i>PdnaD_{cccII}</i>	Native <i>dnaD</i> driven expression of LUX. Mutation in the putative WalR binding site (cccll).	This study
pIMK1-LUX: <i>PtarF_{ccc}</i>	Native <i>tarF</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study
pIMK1-LUX: <i>PtagG_{ccc}</i>	Native <i>tagG</i> driven expression of LUX. Mutation in the putative WalR binding site.	This study

Protein overexpression in *E. coli*.

pET28(a): <i>walR</i>	For production of recombinant WalR in <i>E. coli</i> .	This study
pET28(a): <i>vraR</i>	For production of recombinant VraR in <i>E. coli</i> .	This study

CRISPRi plasmids for gene knockdown in *S. aureus*.

pSD1: <i>walR</i>	CRISPRi knockdown of <i>walR</i> in <i>S. aureus</i> .	This study
pSD1: <i>hup</i>	CRISPRi knockdown of <i>hup</i> in <i>S. aureus</i> .	This study
pSD1: <i>hla</i>	CRISPRi knockdown of <i>hla</i> in <i>S. aureus</i> .	This study

Split luciferase plasmids for protein:protein interaction in *S. aureus*.

pSmBIT <i>walR</i>	C-terminally tagged WalR with SmBIT luciferase fragment	This study
pSmBIT <i>walR_{D53A}</i>	C-terminally tagged WalR _{D53A} with SmBIT luciferase fragment	This study
pSmBIT <i>walR_{D53E}</i>	C-terminally tagged WalR _{D53E} with SmBIT luciferase fragment	This study
pSmBIT <i>walR_{T101A}</i>	C-terminally tagged WalR _{T101A} with SmBIT luciferase fragment	This study
pSmBIT <i>walR_{D53A/T101A}</i>	C-terminally tagged WalR _{D53A/T101A} 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 Walk _{Y32C} with LgBIT luciferase fragment	This study
pLgBIT <i>walk_{T389A}</i>	C-terminally tagged Walk _{T389A} with LgBIT luciferase fragment	This study
pLgBIT <i>walk_{G223D}</i>	C-terminally tagged Walk _{G223D} with LgBIT luciferase fragment	This study
pLgBIT <i>walk_{H385A}</i>	C-terminally tagged Walk _{H385A} with LgBIT luciferase fragment	This study

Table S6. Oligonucleotides used in this study

Primer	EMSA	
	Oligonucleotide 5'-3'	Name
LS237	/5Cy5/CTTTTGTAAATCGTATGATAATGTAATGTAATCAAATTGTAATATAAGGGGACAAGACAATGAAAAAAT	<i>sasD</i> EMSA Cy5 fwd
LS238	CTTTTGTAAATCGTATGATAATGTAATGTAATCAAATTGTAATATAAGGGGACAAGACAATGAAAAAAT	<i>sasD</i> EMSA fwd
LS239	AAATTTTTCATTGTCTTGCCCTTATATTACAATTTGATTACATTTACATTATCATAACGATTACAAAAG	<i>sasD</i> EMSA rev
LS234	/5Cy5/TTTGATGATACAGTATATGATTTTTTTTGTAAATCATAATGTCATCAAACATCAACCTATTATACATAATAA	<i>sle1</i> EMSA Cy5 fwd
LS235	TTTGATGATACAGTATATGATTTTTTTTGTAAATCATAATGTCATCAAACATCAACCTATTATACATAATAA	<i>sle1</i> EMSA fwd
LS236	TTATTATGTATAAATAGGTTGATGTTTGTGACATTATGATTACAAAAAATCATATACTGTATCATCAA	<i>sle1</i> EMSA rev
LS243	/5Cy5/GAAATATAAAAGTAGTAATTATTATTTTGTAAATATAATGTAATATGACTGTTGTTTTAGAAATGATTGTT	<i>P602</i> EMSA fwd Cy5
LS244	GAAATATAAAAGTAGTAATTATTATTTTGTAAATATAATGTAATATGACTGTTGTTTTAGAAATGATTGTT	<i>P602</i> EMSA fwd
LS245	AACAATCATTTCTAAAACAACAGTCATATTACAATTATATTACAAATAAATAATTACTACTTTTTATATTTT	<i>P602</i> EMSA rev
LS240	/5Cy5/ATATTTTATTAATTGAGCTATGCTTATTATTACAATTTGATTACAAATTTTAAATTTGTTAATTGAATGAT	<i>ItaS</i> EMSA fwd Cy5
LS241	ATATTTTATTAATTGAGCTATGCTTATTATTACAATTTGATTACAATTTTAAATTTGTTAATTGAATGAT	<i>ItaS</i> EMSA fwd
LS242	ATCATTTCAATTAACAAATTTAAAATTTGTAATCAAATTGTAATAATAAGCATAGCTCAATTAATAAATAT	<i>ItaS</i> EMSA rev
LS246	/5Cy5/AAAAGCTGAATGTTTCGAAAATAAGTCTGTTACAAATTTGTAATATTACTGAAAATTTCTAAATGTATATTT	<i>ssaA</i> EMSA fwd Cy5
LS247	AAAAGCTGAATGTTTCGAAAATAAGTCTGTTACAAATTTGTAATATTACTGAAAATTTCTAAATGTATATTT	<i>ssaA</i> EMSA fwd
LS248	AAATATAACATTTAGAATTTTCAGTAATATTACAATTTGTAACAGACTTATTTTCGAAACATTCAGTTTT	<i>ssaA</i> EMSA rev
LS249	/5Cy5/AAATAACACTTGATATTGTAATGTTTGTAAAGAAAGTGAATTTACTGGCTGGTTTTTTGTGATATAGT	<i>isaA</i> EMSA fwd Cy5
LS250	AAATAACACTTGATATTGTAATGTTTGTAAAGAAAGTGAATTTACTGGCTGGTTTTTTGTGATATAGT	<i>isaA</i> EMSA fwd
LS251	ACTATATCACAAAAACCAGCCAGTAAATTACACTTTCTTTACAAAACATTACAATATCAAGTGTATTTT	<i>isaA</i> EMSA rev
LS477	/5CY5/ATGTTTATATCATTTATGGTAAAATTTAACACAAGCATTACATAAATCGCAATCATTTATGTGGCAAAAA	<i>hup</i> EMSA fwd Cy5
LS479	TTTTTGGCCACATAAATGATTGGCATTTATGTAATGCTTGTGTTAAAGTTTACCATAATGATATAAACAT	<i>hup</i> EMSA rev
LS480	/5CY5/AAGAGTATTGATTTTTTAATTAGAAAAGTGTAAAATTTATGTGGTCGCGCTTTTAGAGCGCCCATTTTCGT	<i>rplK</i> EMSA fwd Cy5
LS482	ACGAAATGGGCGCTCTAAAAGCGCGACCACATAATTTAACACTTTCTAATTAATAAATCAATACTCTT	<i>rplK</i> EMSA rev
LS483	/5CY5/AAATGGCTAGGATAAAAGGATAATCCTATGTAATATTAATGTAATCTTTATGATTTAATGATTGCGATAG	<i>prs</i> EMSA fwd Cy5
LS485	CTATGCGAATCATTAATCATAAAGATTACATTAATATTACATAGGATTATCCTTTTATCCTAGCCATTT	<i>prs</i> EMSA rev
LS486	/5CY5/CATGGTGTGTTTAGAAGTTATCCACGGCTGTATTTTTGTGTATAACTTAAAAATTTAAGAAAGATGGAG	<i>dnaA</i> region I EMSA fwd Cy5
LS488	ACTCCATCTTTCTAAATTTTTAAGTTATACACAAAAATAACAGCCGTGGATAACTTCTAAACACACCATG	<i>dnaA</i> region I EMSA rev
LS489	/5CY5/AATAAGCAAGATAAAGTTATCCACCGATTGTTATTAACCTTGTGGATAATTATTAACATGGTGTGTTTAGA	<i>dnaA</i> region II EMSA fwd Cy5
LS491	TCTAAACACACCATGTTAATAATTATCCACAAGTTAATAACAATCGGTGGATAACTTTATCTTGCTTATT	<i>dnaA</i> region II EMSA rev
LS492	/5CY5/AAAGTTACTACTTTTTAGCAACATATTCACAGGTATTGACATATAGAGAAGTAAAAAGTATAATTGT	<i>dnaA</i> region III EMSA fwd Cy5
LS494	ACAATTACTTTTTTCAGTTCTCTATATGTCAAATACCTGTGAATATGTTGCTAAAAATAGTATAAATTTT	<i>dnaA</i> region III EMSA rev
LS267	/5Cy5/CATTAGGAGGAAATTTTGCATCGGACTCGAGTATGAGCTACGTACCAAATATTATTTTCATCATTTCTA	VraR EMSA <i>ItaS</i> fwd Cy5
LS268	CATTAGGAGGAAATTTTGCATCGGACTCGAGTATGAGCTACGTACCAAATATTATTTTCATCATTTCTA	VraR EMSA <i>ItaS</i> fwd
LS269	TAGAAATGATGAAATAATATTTGGTACGTAGCTCATACTCGAGTCCGATGCAAATAATTTCCCTCCTAATG	VraR EMSA <i>ItaS</i> rev
LS91	ACTGTGACGGGATCCATGGCTAGAAAAGTTGTTGTAGTTGATG	<i>walR</i> pET28 F
LS92	CATCGACTTAGAAGCTTTACTCATGTTGTTGGAGGAAATATCCAAC	<i>walR</i> pET28 R
IM423	<u>TTTGTTTAACTTTAAGAAGGAGATATACCATGACGATTAAGTATTGTTTGTGGATG</u>	<i>vraR</i> pET21 F
IM424	<u>GGCTTTGTTAGCAGCCGGATCCTAATGGTGTATGATGGTGGTGTGGTGTATGTTGAATTAATTTATGTTGGAATGCATAG</u>	<i>vraR</i> pET21 R
IM395	GGTATATCTCCTTCTTAAAGTTAAACAAAATTTATTTT	pET INV F

IM396	GATCCGGCTGCTAACAAAGCC	pET INV R
S. aureus mutants		
Primer	Oligonucleotide 5'-3'	Name
IM31	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACCCGTCATTTTCTTTAAAATGTATGAACC</u>	<i>walR</i> ^{T101A} AF
IM231	aGcaACATAGTCATCTGCACCTAGTTC	<i>walR</i> ^{T101A} BR
IM232	TTAGAACTAGGTGCAGATGACTATGTTgCtAAACCGTTTAGTACGCGTGAATTAATCG	<i>walR</i> ^{T101A} CF
IM233	AGAACTAGGTGCAGATGACTATGTTg	<i>walR</i> ^{T101A} con F
IM181	TTTTTCAAGGTTATTTGTAAAATATAACCC	<i>walR</i> ^{T101A} con R
IM107	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACCATGGCTAGAAAAGTTGTTGTAG</u>	<i>walk</i> ^{Y32C} AF
IM7	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACCAGGTCGAAACGAATGAAGTGGCTAAAAC</u>	<i>walk</i> ^{T389A} AF
IM121	agcACGTAACCTCATGTGATACATTGG	<i>walk</i> ^{T389A} BR
IM122	CAATGTATCACATGAGTTACGTgctCCTTTAACTTCTATGAATAGTTACATTGAAGC	<i>walk</i> ^{T389A} CF
IM10	<u>CGACTCACTATAGGGCGAATTGGAGCTCCTCCTTATTATTCATCCCAATCACCGTC</u>	<i>walR</i> ^{T101A} / <i>walk</i> ^{Y32C/T389A} DR
IMT275	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACCAATTCATATGGATGACGCGCAGC</u>	Delta <i>spa</i> AF
IMT353	CAAATTAATACCCCTGTATGTATTTG	Delta <i>spa</i> BR
IMT354	CAAATACATACAGGGGGTATTAATTTGTAAAAACAACAATACACAACGATAGATATC	Delta <i>spa</i> CF
IMT278	<u>CGACTCACTATAGGGCGAATTGGAGCTCATTACTTGTGGCAGCTAACACTGC</u>	Delta <i>spa</i> DR
IM1516	GGTTATAGACTTTTTGAAGAAATCTATAAAGGTCGAAACGAATGAAGTGGCTAAAAC	<i>walR</i> -SmBIT- <i>walk</i> CF
IM1517	TTATAGAATTTCTTCAAAAAGTCTATAACCTG	<i>walR</i> -SmBIT- <i>walk</i> BR
IM1518	GTTGTTTAGAGTAACTATAAACAGTTAAATGAATAATAAGGAGCATATTAATCTGTG	<i>walk</i> -LgBIT- <i>yycH</i> CF
IM1519	TTAACTGTTTATAGTTACTCTAAACAACATAGATCC	<i>walk</i> -LgBIT- <i>yycH</i> BR
IM44	<u>CGACTCACTATAGGGCGAATTGGAGCTCTGTGTTAATTTTACGTAATCGTGGCGATC</u>	<i>walk</i> -LgBIT- <i>yycHI</i> DR
IM1368	AGCCCGATAATTTGCATACCAATG	<i>walk</i> -LgBIT con R
ChIP-seq		
Primer	Oligonucleotide 5'-3'	Name
IM512	ATATGGTACCGATTACAAAAGATGATGATGACAAATAAAATAGTCAAAGCCCTCCGGTCGGAGGCTTTTGACTGAATTCAC TGGCCGTCGTTTTAC	pRAB11 FLAG tonB (KpnI) F
IM513	ATATGGTACCTTTCCAATTCCTCCTCATCATACTCTATCAATGATAGAGAGC	pRAB11 RBS (KpnI) R
IM514	GATTACAAAAGATGATGATGACAAATAAAATAGTC	INV pRAB11 FLAG F
IM515	TTTCCAATTCCTCCTCATCATACTCTATC	INV pRAB11 FLAG R
IM516	<u>CATTGATAGAGTATGATGAGGAGGAATTGGAAATGGCTAGAAAAGTTGTTGTAGTTG</u>	WalR-FLAG F
IM517	<u>CTATTTTATTTGTCATCATCATCTTTGTAATCCTCATGTTGTTGGAGGAAATATCC</u>	WalR-FLAG R
IM518	<u>CATTGATAGAGTATGATGAGGAGGAATTGGAAATGACCCACTTACTGATCGTGG</u>	SaeR-FLAG F
IM519	<u>CTATTTTATTTGTCATCATCATCTTTGTAATCTCGGCTCCTTTCAAATTTATATCC</u>	SaeR-FLAG R
IM520	<u>CATTGATAGAGTATGATGAGGAGGAATTGGAAATGACGATTAAGTATTTGTTGTGGATG</u>	VraR FLAG F
IM521	<u>CTATTTTATTTGTCATCATCATCTTTGTAATCTTGAATTAATTTATGTTGGAATGCATAG</u>	VraR-FLAG R
IM522	<u>CATTGATAGAGTATGATGAGGAGGAATTGGAAATGTTTAAAGGTAGTTATTTGTGATGATG</u>	HptR-FLAG F
IM523	<u>CTATTTTATTTGTCATCATCATCTTTGTAATCTTTTGCTTGCTTACAATAATCACTTGG</u>	HptR-FLAG R
YFP-LUX		
Primer	Oligonucleotide 5'-3'	Name
IM1	GGTACCCAGCTTTTGTTCCTTTTAGTGAGG	pIMC8-YFP F
IM385	TGATTAACCTTATAAGGAGGAAAAACATATG	pIMC8-YFP-R

IM1127	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACCTATAACTTAATATAATTGAGGTGGAGCATC</u>	<i>sasD</i> YFP F
IM1107	<u>ATGTTTTTCCTCCTTATAAAGTTAATCATGTCTTGCCCTTATATTACAATTTG</u>	<i>sasD</i> YFP R
IM1108	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACCTTAGAAAAATCAAATTCAGATGCAGTAAAG</u>	<i>sle1</i> YFP F
IM1109	<u>ATGTTTTTCCTCCTTATAAAGTTAATCATTTAAAATCCTCCTCTGCTTAACTTTCC</u>	<i>sle1</i> YFP R
IM1129	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACCCAAGAATCAAGTGCATTCCCATTGTTG</u>	<i>P602</i> YFP F
IM1110	<u>ATGTTTTTCCTCCTTATAAAGTTAATCAAAGCACTCTCTCCTTTATTTATATCG</u>	<i>P602</i> YFP R
IM1111	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACCTAATATTTGGTACGTAGCTCATACTCG</u>	<i>ItaS</i> YFP F
IM1112	<u>ATGTTTTTCCTCCTTATAAAGTTAATCAGATTCTTTCCCCCGTTATTTAGATAATAAATC</u>	<i>ItaS</i> YFP R
LS371	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACCCAATACCAAAGCTTTCATAATC</u>	<i>ssaA</i> YFP F
LS372	<u>ATGTTTTTCCTCCTTATAAAGTTAATCATTTAAAATATCCTCCTAAAAATTTTAAATC</u>	<i>ssaA</i> YFP R
IM1128	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACCTAACAGTATGTTTTTTGAAAAATAGAGACC</u>	<i>isaA</i> YFP F
IM364	<u>ATGTTTTTCCTCCTTATAAAGTTAATCAAGTAAAAATCCTCCAGTAATAATTG</u>	<i>isaA</i> YFP R
IMT300	<u>CCTCACTAAAGGGAACAAAAGCTGGGTACCATCCAGATTTAATAATAGGATGGTTAGG</u>	<i>hpt</i> YFP F
IMT301	<u>ATGTTTTTCCTCCTTATAAAGTTAATCACTCTGTACCTCAATCATTTTCG</u>	<i>hpt</i> YFP R
IM1113	ATGTTTTTCCTCCTTATAAAGTTAATCATGTCTTGCCCTTATATTGGGATTTGATTAC	<i>sasD</i> ccc R
IM1114	GATGATACAGTATATGATTTTTTCCCAATCATAATGTCATCAAACATC	<i>sle1</i> ccc F
IM1115	GGGAAAAATCATATACTGTATCATCAAAT	<i>sle1</i> ccc R
IM1118	GAAATATAAAGTAGTAATTATTATTCCCAATATAATTGTAATATGACTGTTGTTTTAG	<i>P602</i> ccc F
IM1119	GGGAATAATAATTACTACTTTTATATTTCACTTATC	<i>P602</i> ccc R
IM1116	GAGCTATGCTTATTATTACAATTTGATTGGGAATTTTAAATTTGTTAATTGAATG	<i>ItaS</i> ccc F
IM1117	CCCAATCAAATTGTAATAATAAGCATAGCTC	<i>ItaS</i> ccc R
LS375	GGGAAGCACACAAGGACGCTAAT	<i>ssaA</i> ccll F Closest to the <i>ssaA1</i> start
LS376	ATTAGCGTCCTTGTTGCTTCCCTAACGTTTTGTAATTTTTTGCTAATATC	<i>ssaA</i> ccll R
IM1120	CAGTAATATTACAAATTTGTGGGACTTATTTTCGAAACATTGAG	<i>ssaA</i> ccll F Furthest from the <i>ssaA1</i> start
IM1121	CCCTACAAATTTGTAATATTACTGAAAATTTCTAAATG	<i>ssaA</i> ccll R
IM1122	ACACTTGATATTGTAATGTTTCCCAAAGAAAGTGAATTTACTGGCTGG	<i>isaA</i> ccll F Closest to the <i>isaA</i> start
IM1123	GGGAAACATTACAATATCAAGTGTATTG	<i>isaA</i> ccll R
IM1124	CAGATATATTACAGCTATGTAGGGAAAATACAATCTGTAATATTACGAAAGC	<i>isaA</i> ccll F Furthest from the <i>isaA</i> start
IM1125	CCCTACATAGCTGTAATATATCTGACATGTAAC	<i>isaA</i> ccll R

LUX

Primer	Oligonucleotide 5'-3'	Name
IM1216	ATATGTCGACTATAACTTAATATAATTGAGGTGGAGCATC	<i>sasD</i> LUX F
IM248	CATTGCTTGTTCCCTTATATTACAATTTG	<i>sasD</i> LUX R
IM1295	ATATGTCGACTTAGAAAAATCAAATTCAGATGCAGTAAAG	<i>sle1</i> LUX F
IM1296	CACTTTAAAATCCTCCTCTGCTTAACTTTCC	<i>sle1</i> LUX R
IM1294	ATATGTCGACCAAGAATCAAGTGCATTCCCATTGTTG	0602 LUX F
IM1062	CATAAGCACTCTCTCCTTTTATTTATATCG	0602 LUX R
IM1218	ATATGTCGACTAATATTTGGTACGTAGCTCATACTCG	<i>ItaS</i> LUX F
IM1219	CATGATTCTTTCCCCCGTTATTTAGATAATAAATC	<i>ItaS</i> LUX R
IM1297	ATATGTCGACCTTACATCCTCACATATACAAATATATTG	<i>ssaA</i> LUX F

IM1298	CATTTTAAAAATATCCTCCTAAAAATTTTAAATC	<i>ssaA</i> LUX R
IM1220	ATATGTCGACTAACAGTATGTTTTTTGAAAATATGAGACC	<i>isaA</i> LUX F
IM1221	CATAGTAAAAAATCCTCCAGTAATAATTG	<i>isaA</i> LUX R
IM1222	ATATGTCGACTTCTGAGCAATGACGTGCAACTAG	<i>walR</i> LUX F
IM32	CATTTGCATAAACCTCTTTTCTTAAATC	<i>walR</i> LUX R
IM1217	CATTGTCTTGTCCCCTTATATTGGGATTTGATTAC	<i>sasD</i> ccc LUX R
LS443	TACGCGTCGACACACGATGCGAGCAATCAAAT	<i>dnaA</i> LUX F
IM1746	CATAAATTTACTCCATCTTTCTTAAATTTTAAAG	<i>dnaA</i> LUX R
IM1285	ATATGTCGACCAATTAGGTGCAGATGTTAAGGTTG	<i>prs</i> LUX F
IM1288	CATTTATAGTCCTCCAATTATTTACTTACG	<i>prs</i> LUX R
IM1289	ATATGTCGACTGCCGATCCTATGACATTTCTAGG	<i>hup</i> LUX F
IM1285	CATTAGACATTCACCTCCTGAGG	<i>hup</i> LUX R
LS451	TACGCGTCGACGATGAGAAGCAGGACTATACAATGAG	<i>tarF</i> LUX F
LS452	CATGTCGTACCTCCGACGTG	<i>tarF</i> LUX R
LS457	TACGCGTCGACGAAGATTTTGTATTATCAGAGTGGG	<i>tagG</i> LUX F
LS458	CATTCCATTAAACCACACTTTCAAATG	<i>tagG</i> LUX R
LS471	TACGCGTCGACGCTGGGCGATTTATTCTTGG	<i>dnaD</i> LUX F
LS472	CATGTTTCGTGCCCCCTTTTAA	<i>dnaD</i> LUX R
IM1745	ATATGTCGACCTCACTAACAGATACTCTATAGAAGG	<i>dnaA</i> LUX F
IM1746	CATAAATTTACTCCATCTTTCTTAAATTTTAAAG	<i>dnaA</i> LUX R
IM1734	ATATGTCGACATTAACAATTAAGTTATTAACCTAACCAAAAG	<i>rplK</i> LUX F
IM1735	CACGATGTGCACCTCCTTGATATCG	<i>rplK</i> LUX R
IM1291	CCACATAAATGATTGCGATTTACCCAATGCTTGTGTTAAAGTTTACC	<i>hup</i> ccc LUX CF
IM1290	GGGTAAATCGCAATCATTTATGTGGCAAAAACG	<i>hup</i> ccc LUX BR
LS449	GGATAACGATTTTTCCCTATGTTAAAGTGGTAC	<i>tarF</i> ccc LUX CF
LS448	GGGAAAAATCGTTATCCATTCATAACGTATG	<i>tarF</i> ccc LUX BR
LS455	GATAGCATCCACCCATAGTGATAGTATTTACAAC	<i>tagG</i> ccc LUX CF
LS454	GGGTGGATGCTATCAAAATTTATGTCTAGTTC	<i>tagG</i> ccc LUX BR
LS467	CCATAAAGTTCATCCCTAAAATCTAGTGTTAAAAAATAC	<i>dnaD</i> cccl LUX CF
LS466	GGGATGAACCTTTATGGATAATCAGATGAACCTA	Closest to the start <i>dnaD</i> cccl LUX BR
LS469	GTTATTTTAAATTTCCCTTGCTACTAGTTAAATATATTAAG	<i>dnaD</i> cccll LUX CF
LS468	GGGAATTTTAAATAACAGTATTTTTTAACTAGATTTTAAAC	Furthest from the start <i>dnaD</i> cccll LUX BR
LS144	GAGTATTGATTTTTTAATTAGAAAAGCCCTAAAATTATGTGGTCGCGC	<i>rplK</i> ccc LUX CF
LS143	GGGCTTTTCTAATTAATAAATACTACTCTTTTTTA	<i>rplK</i> ccc LUX BR
LS140	TGGCTAGGATAAAAAGGATAATCCTA CCCA ATATTAATGTAATCTTTATG	<i>prs</i> ccc LUX CF
LS139	GGGTAGGATTATCCTTTTATCCTAGCCATTTTAAATAC	<i>prs</i> ccc LUX BR
LS441	CCCTATTTTTGTGTATAACTTAAAAATTAAGAAAGATG	<i>dnaA</i> ccc I LUX CF
LS440	GTTATACACAAAATA GGG GCCGTGGATAACTTC	Closest to the start <i>dnaA</i> ccc I LUX BR
LS439	CCCTATTAACCTGTGGATAATTATTAACATGGTG	<i>dnaA</i> ccc II LUX CF
LS438	CCACAAGTTAATA GGG ATCGGTGGATAAC	<i>dnaA</i> ccc II LUX BR
IM1744	GTTATACTATTTTTAGCAACATATTC GGG GGTATTTGACATATAGAGA ACTG AAAAAG	<i>dnaA</i> ccc III LUX C
		Furthest from the start F

IM1743	CCCGAATATGTTGCTAAAAATAGTATAACTTTGTG	<i>dnaA</i> ccc III LUX BR
IM1241	ATATAGATCTATATAGTTTTTGTATACGGTATTCATTCATG	pLOW rep F
IM1242	ATATGCATGCTCAACTTTGCAACAGAACC	pLOW rep R

RT-qPCR

Primer	Oligonucleotide 5'-3'	Name
IM1026	GGCTCTATGAAAGCAGCAGATA	<i>hla</i> RT-qPCR F
IM1027	CTGTAGCGAAGTCTGGTAAA	<i>hla</i> RT-qPCR R
IM1020	CGCAGGCGATTTTACCATTA	<i>gyrB</i> RT-qPCR F
IM1021	ATGCTGGAACCTTACTTGCTGG	<i>gyrB</i> RT-qPCR R
IM1153	GAAC TAGGTGCAGATGACTATGT	<i>walR</i> RT-qPCR F
IM1154	CTGTGCTGGTTGTGAGTAATG	<i>walR</i> RT-qPCR R
IM1586	GGTTTCGGTAACTTTGAGGTACG	Hu RT-qPCR F
IM1587	ATGCTGGAACCTTACTTGCTGG	Hu RT-qPCR R

SmBIT/LgBIT

Primer	Oligonucleotide 5'-3'	Name
IM1290	ATCATTAATTCCTCCTTTTTGTTGACAcTcTATCATTGATAGAGTTATTTGTCAAACCTAG	Fix tetO1 F
IM1291	gAgTGTC AACAAAAAGGAGGAATTAATGATG	Fix tetO1 R
IM1355	ATATGGTACCGTTAACAGATCTGAGCTCG	Introduce RBS F
IM513	ATATGGTACCTTTCCAATTCCTCCTCATCATACTCTATCAATGATAGAGAGC	Introduce RBS R
IM515	TTTCCAATTCCTCCTCATCATACTCTATC	INV PCR to clone SmBIT/LgBIT R
IM1356	GTTAACAGATCTGAGCTCGAATTCACTGG	INV PCR to clone SmBIT/LgBIT F
IM1360	GGTTCTAGTGGTGGTGGTCTCTGG	INV PCR to clone genes F (IM515)
IM1363	<u>GATAGAGTATGATGAGGAGGAATTGGAAAATGGCTAGAAAAGTTGTTGTAGTTG</u>	WalR pSmBIT F
IM1364	<u>GAACCACCACCACCCTAGAACCTCATGTTGTTGGAGGAAATATCC</u>	WalR pSmBIT R
IM1365	<u>GATAGAGTATGATGAGGAGGAATTGGAAAATGAAGTGGCTAAAACAAC TACAATCC</u>	WalK pLgBIT F
IM1366	<u>GAACCACCACCACCCTAGAACCTTCATCCCAATCACCGTCTTCAATGAC</u>	WalK pLgBIT R

CRISPRi

Primer	Oligonucleotide 5'-3'	Name
IM1559	CTAGTTCATTAGACATTCACCTCCTG	<i>hup</i> CRISPRi F
IM1560	AACCAGGAGGTGAATGTCTAATGAAC	<i>hup</i> CRISPRi R
IM1180	CTATTAAATCTAAAATATCAGCAAT	<i>walR</i> CRISPRi F
IM1181	AACATTGCTGATATTTTAGAATTTAA	<i>walR</i> CRISPRi R
IM1182	CTACATTAGCGACAGGATTCATTAATA	<i>hla</i> CRISPRi F
IM1183	AACTATTAATGAATCCTGTCGCTAATG	<i>hla</i> CRISPRi R

Italics: restriction enzyme sites

Underlined: Tails complementary to plasmid for SLICE cloning

Lowercase: Mutations introduced

Bold: WalR binding site mutations

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