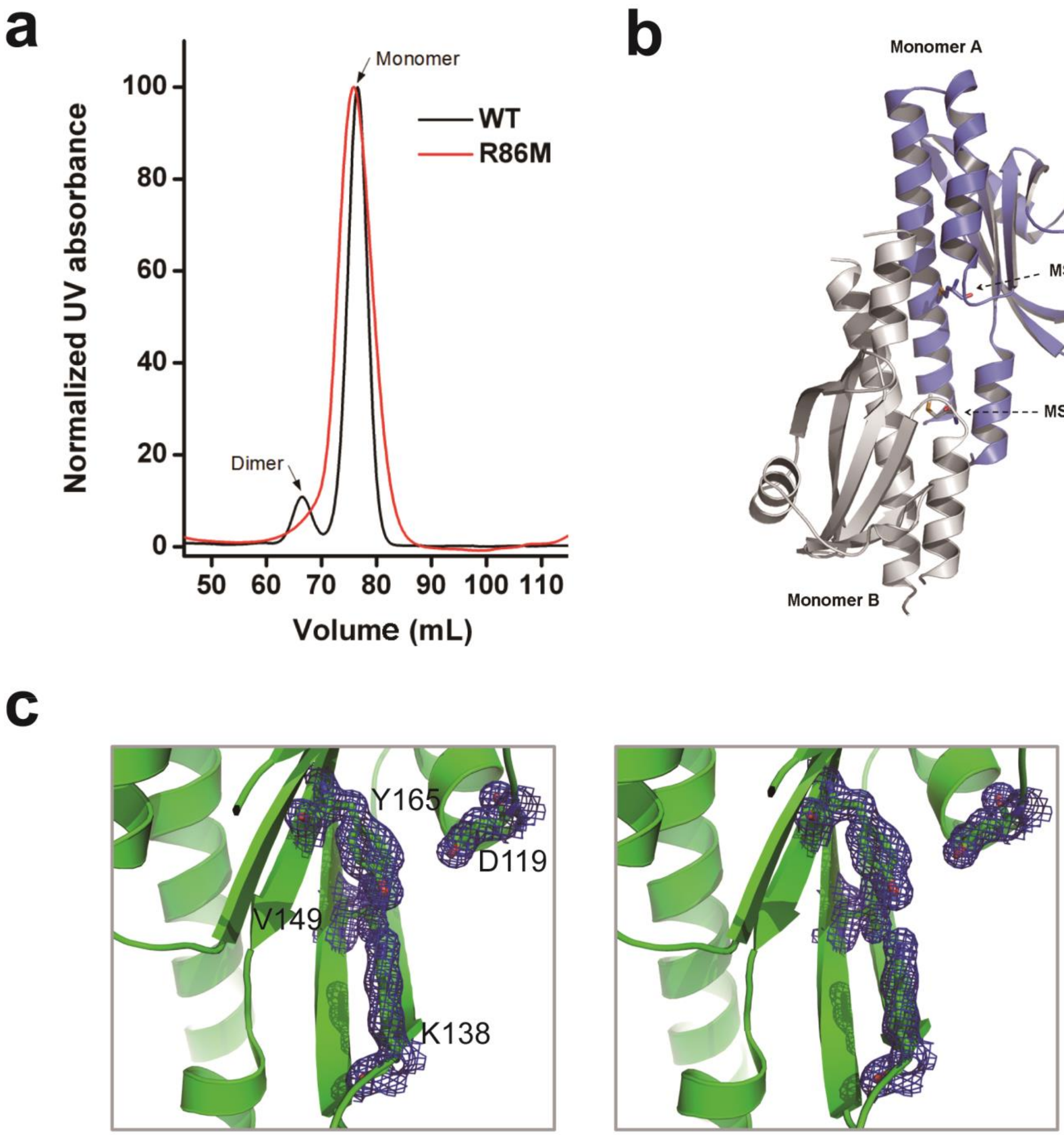
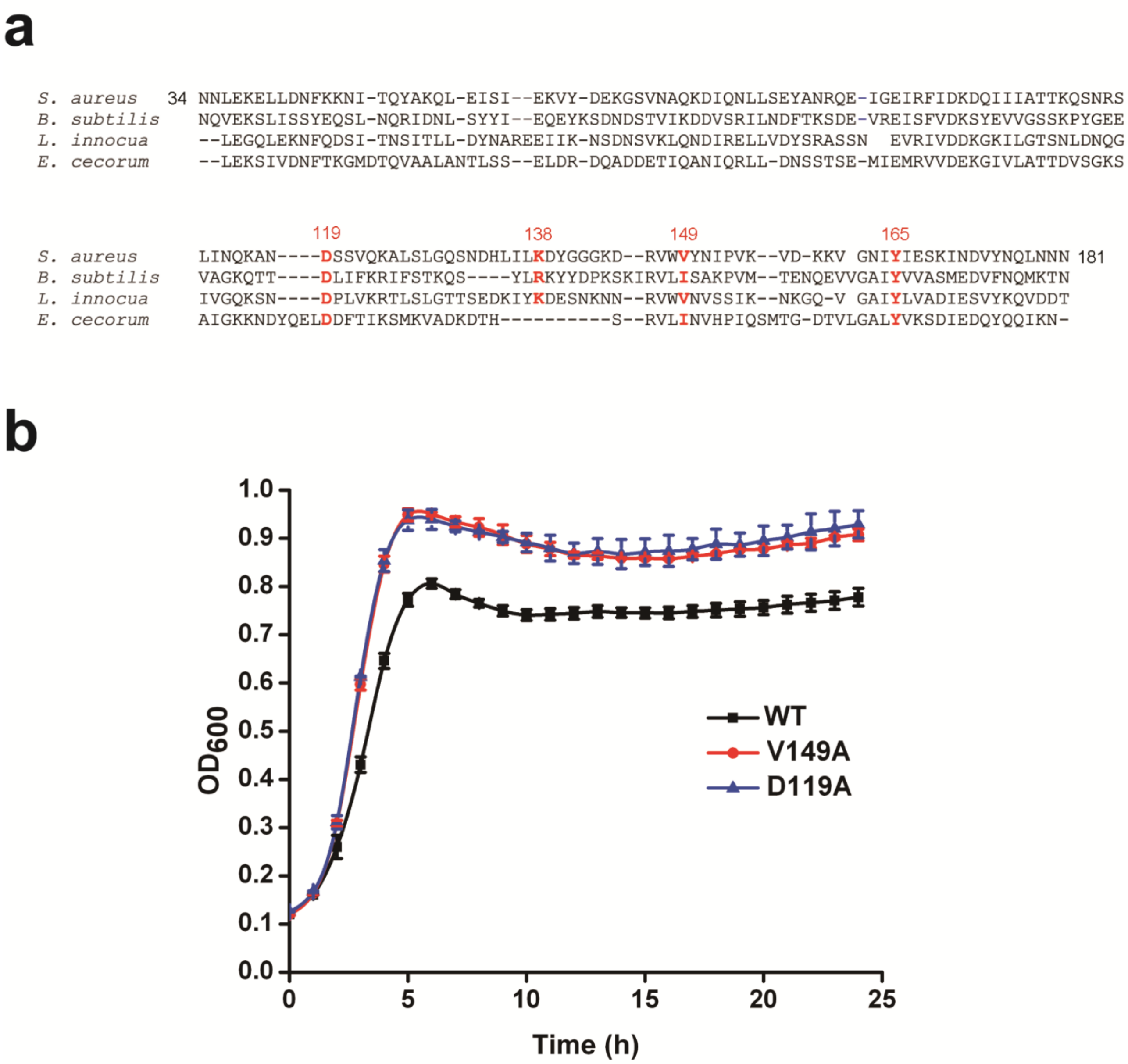


Supplementary Figure 1. The mutant protein erWalKR86M adopts wild-type folding. (**a**) Size exclusion chromatography analysis of wild-type erWalK (WT) and R86M mutant (R86M). (**b**) Localization of the mutation site in erWalK. (**c**) Stereoview of the electron density of the potential signal-transduction residues; 2Fo-Fc map (1.0 σ) is shown as blue mesh.



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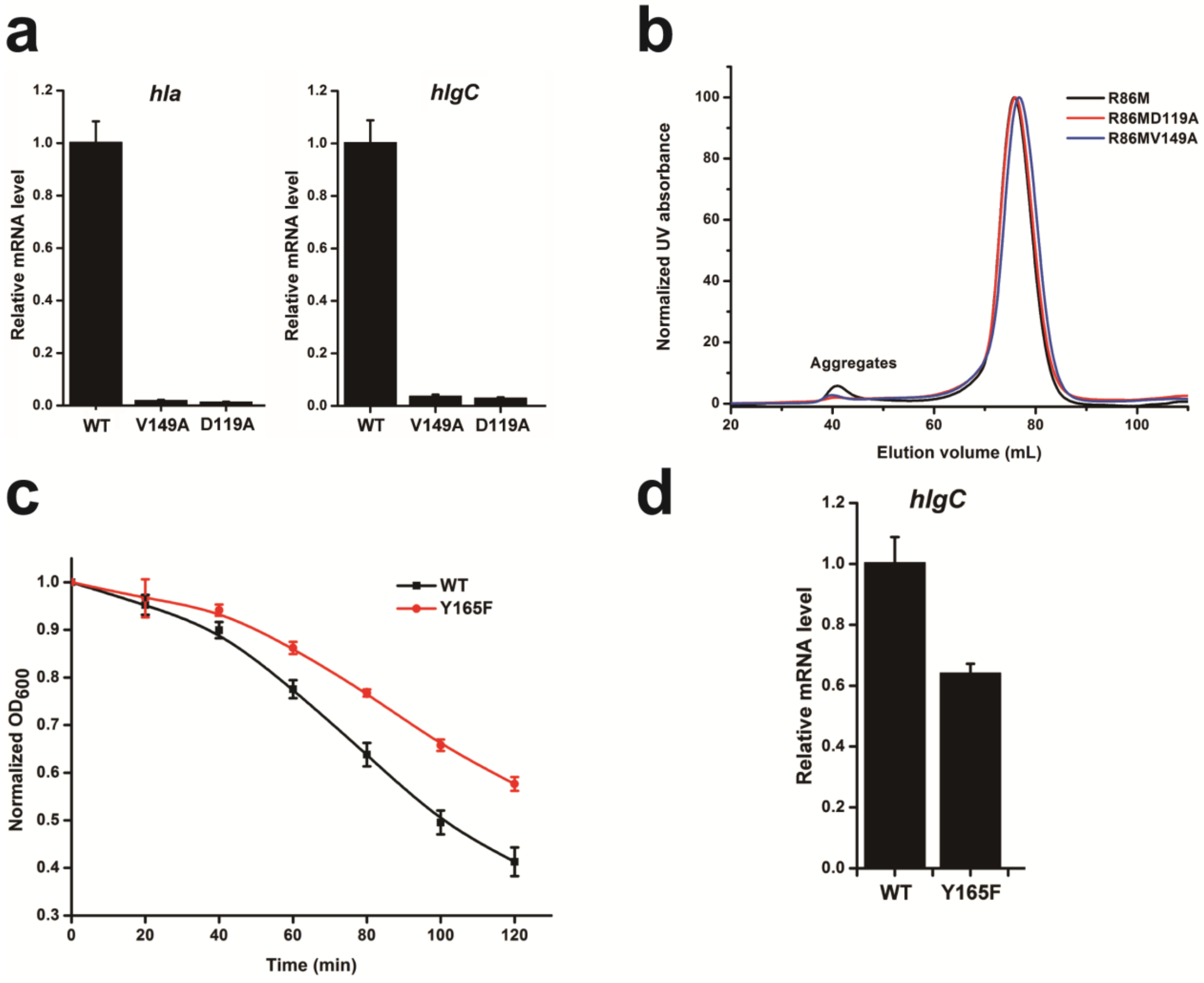
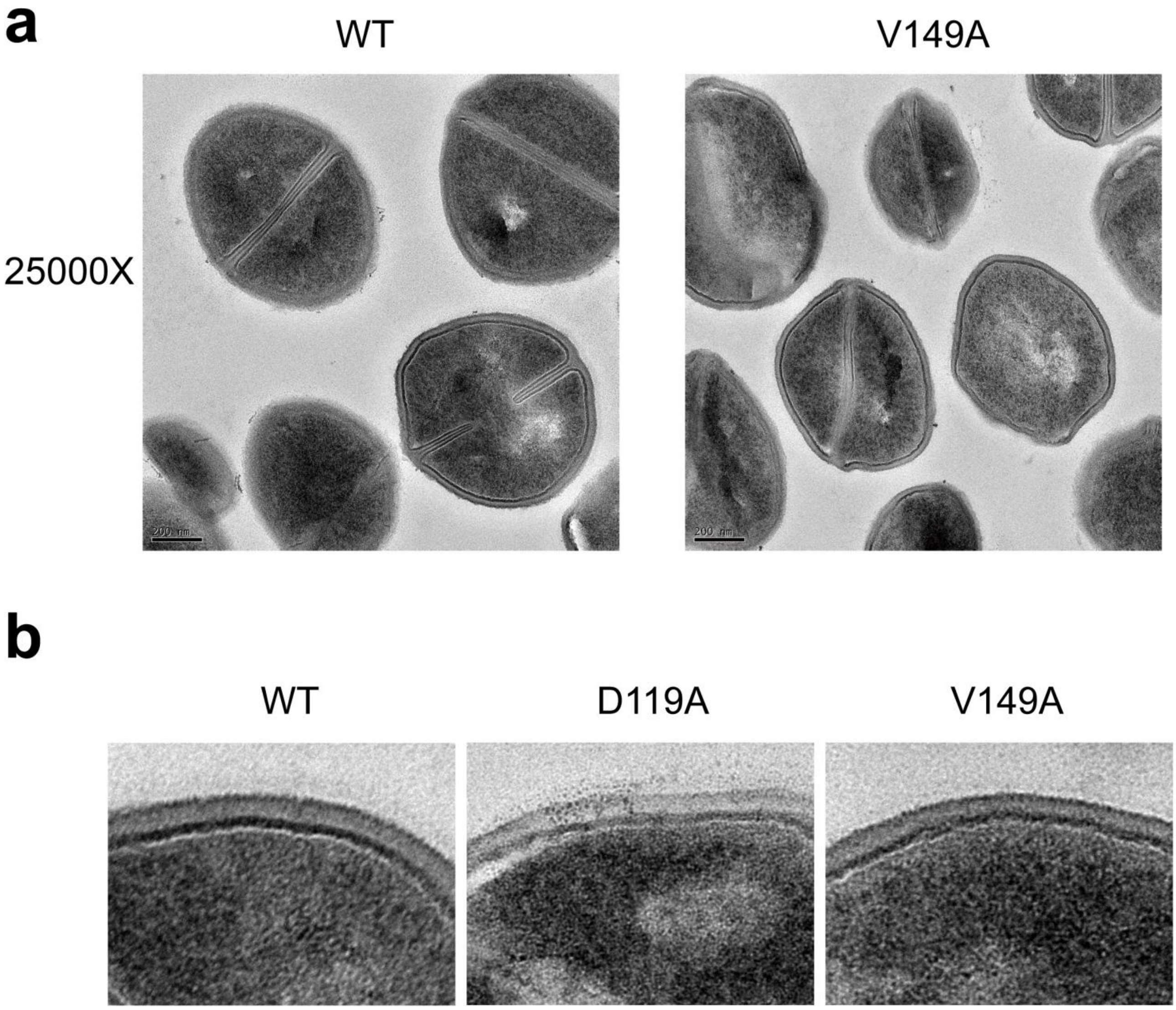
S. aureus   34 NNLEKELLDNFKKNI-TQYAKQL-EISI--EKVY-DEKGSVNAQKDIQNLLSEYANRQE-IGEIRFIDKDQIIIATTKQSNRS
B. subtilis    NQVEKSLISSYEQSL-NQRIDNL-SYYI--EQEYKSDNDSTVIKDDVSRILNDFTKSDE-VREISFVDKSYEVVGSSKPYGEE
L. innocua     --LEGQLEKNFQDSI-TNSITLL-DYNAREEIIK-NSDNSVKLQNDIRELLVDYSRASSN  EVRIVDDKGKILGTSNLDNQG
E. cecorum     --LEKSIVDNFTKGMDTQVAALANTLSS--ELDR-DQADDETIQANIQRLL-DNSSTSE-MIEMRVVDEKGIVLATTDVSGKS

                          119                  138          149                 165
S. aureus      LINQKAN----DSSVQKALSLGQSNDHLILKDYGGGKD--RVWVYNIPVK--VD-KKV GNIYIESKINDVYNQLNNN 181
B. subtilis    VAGKQTT----DLIFKRIFSTKQS----YLRKYYDPKSKIRVLISAKPVM--TENQEVVGAIYVVASMEDVFNQMKTN
L. innocua     IVGQKSN----DPLVKRTLSLGTTSEDKIYKDESNKNN--RVWVNVSSIK--NKGQ-V GAIYLVADIESVYKQVDDT
E. cecorum     AIGKKNDYQELDDFTIKSMKVADKDTH----------S--RVLINVHPIQSMTG-DTVLGALYVKSDIEDQYQQIKN-
```

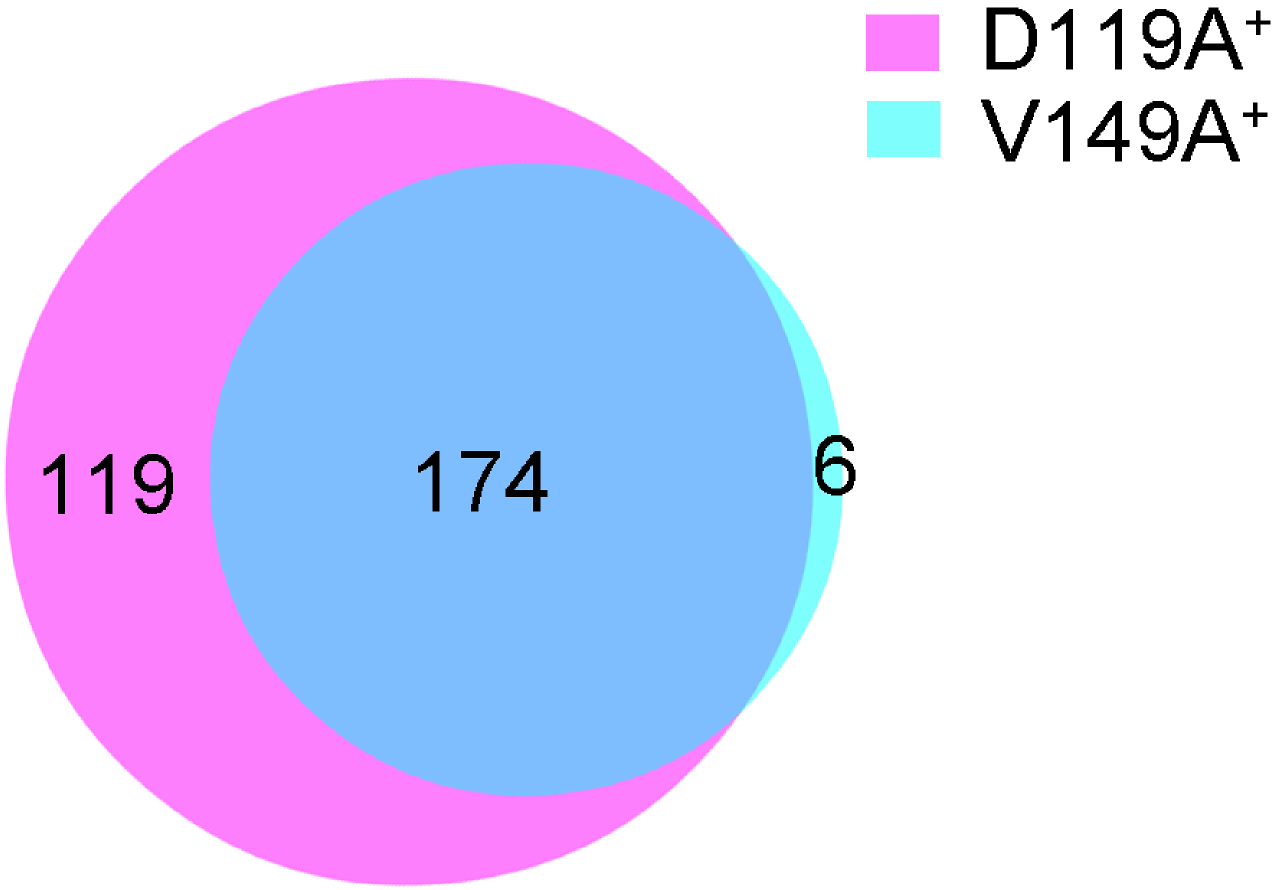
Supplementary Figure 2. The conservation of potential signal recognition residues and the role of two potential signal binding residues in cell growth. (**a**) Sequence alignment of four erWalK proteins from four distinct low GC Gram-positive bacteria reveals the high conservation of potential signal binding residues. (**b**) Mutation of D119 or V149 to Ala slightly accelerates *S. aureus* growth. The growth was tested in TSB. The experiments were performed in triplicate. The error bars represent the standard deviations of analyzed data.



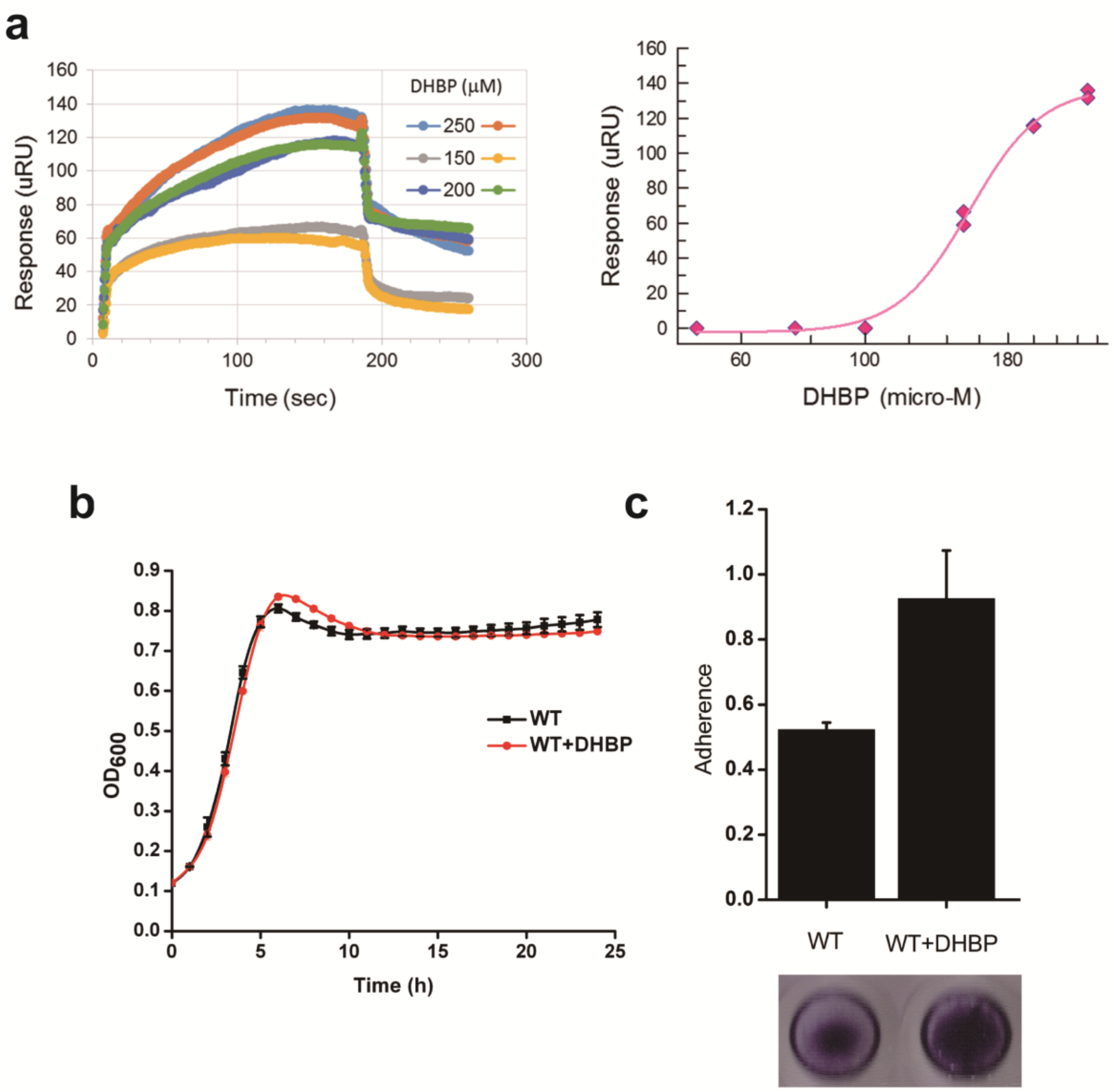
Supplementary Figure 3. Analysis of hemolysin gene expressions in the mutants and wild-type strain and size exclusion chromatography analysis of erWalKR86M, R86MD119A and R86MV149A. (**a**) qRT-PCR analysis of the expressions of hemolysin genes (*hla* and *hlgC*) in the wild-type strain and two mutants, V149A and D119A. Cells were grown to mid-log phase before harvest for analysis. (**b**) Mutation of D119 or V149 to A does not trigger protein aggregation. The gel-filtration assay was performed in a buffer containing 100 mM NaCl, 10 mM Tris.HCl (pH 7.5) and 1 mM DTT. (**c**) Mutation of Y165 to F slightly reduces the lysostaphin-induced lysis activity. (**d**) Mutation of Y165 to F slightly affects the expression of the hemolysin gene *hlgC*. The experiments were performed in triplicate. The error bars represent the standard deviations of analyzed data.



Supplementary Figure 4. Transmission electron microscopy test of the Newman wild-type strain and the V149A and D119A mutants. (**a**) Mutation of V149 to Ala slightly alters cell shape. Cells were grown to OD_{600} of ~0.6 before harvest for analysis. Scale bars: 200 nm. (**b**) Mutation of D119 or V149 to Ala does not affect cell wall thickness. Cells were grown to OD_{600} of ~0.6 before harvest for analysis.

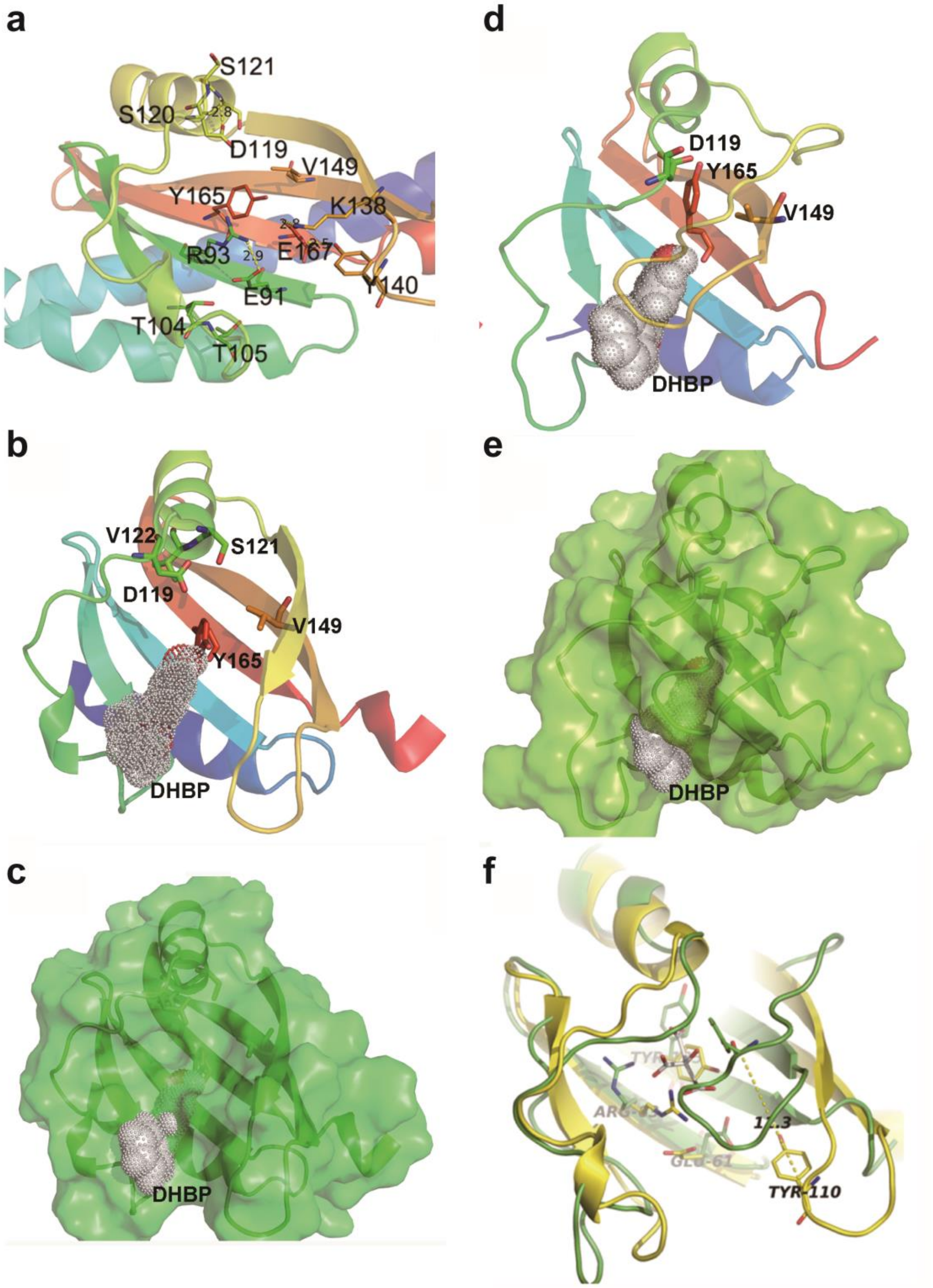


Supplementary Figure 5. Venn diagram of the number of genes whose expressions are up-regulated in the D119A mutant overlapping genes whose expression are up-regulated in the V149A mutant as compared with the wild-type strain. +: genes whose expressions are up-regulated.

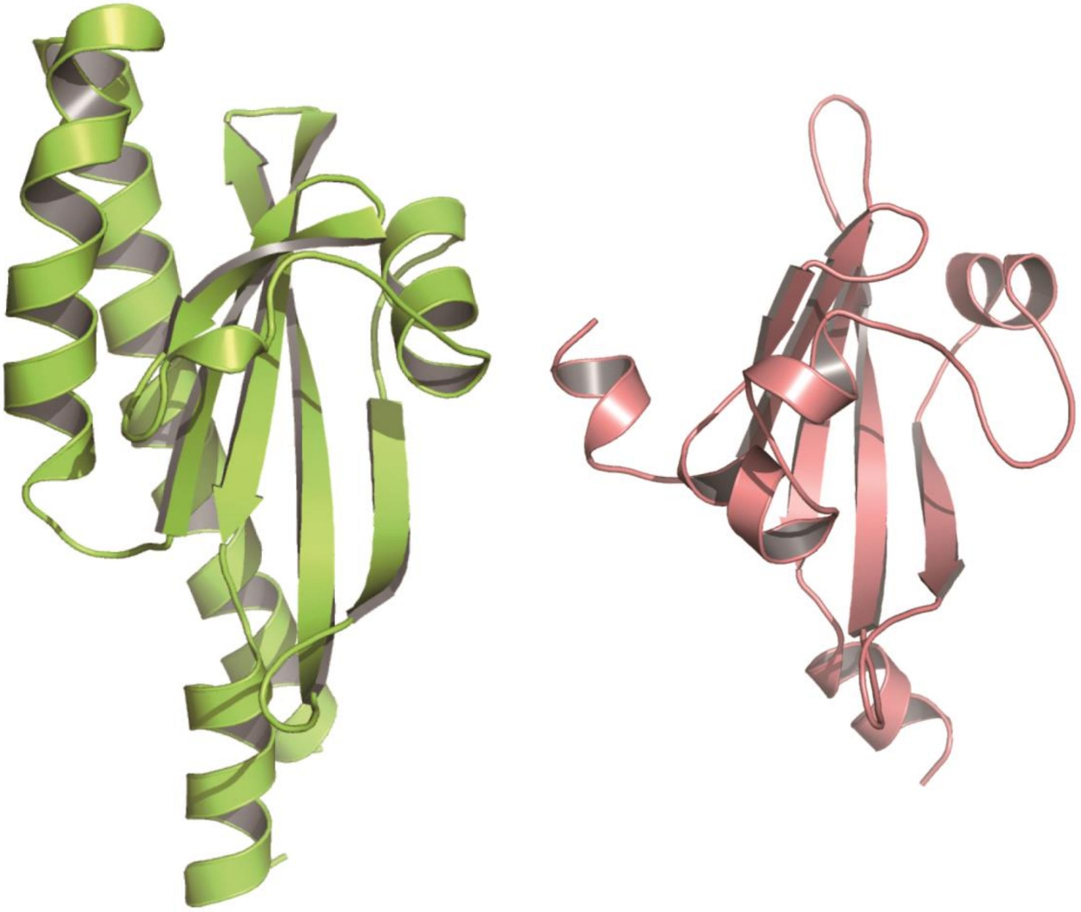


Supplementary Figure 6. DHBP activates WalKR TCS. (**a**) SPR analysis of DHBP binding to erWalK. (Left) Reference-corrected SPR binding curves for the indicated concentrations of DHBP and biotinylated erWalK immobilized on the surface of a Neutravidin sensor chip. Responses (uRU) from the reference surface were subtracted from those from the erWalK coated surface to monitor DHBP-specific binding. (Right) Plot of the binding response values vs the concentrations of DHBP. The experiments were performed in duplicate. (**b**) DHBP treatment does not affect cell growth of the Newman wild-type strain. The Newman wild-type strain was supplemented with 75 µM DHBP for the test. The experiments were performed in triplicate. (**c**) DHBP treatment enhances the biofilm production in the Newman wild-type strain. The biofilm assay was performed in a polystyrene 96-well in TSB with the supplementation of 0.5% glucose and 3% NaCl. When indicated, 75 µM

DHBP was supplemented. The experiments were performed in duplicate. The error bars represent the standard deviations of analyzed data.



Supplementary Figure 7. Modeling of DHBP binding mode in erWalK. (**a**) Ligand-free structure of erWalK. (**b,c**) Free erWalK structure with putative DHBP binding site. (**b**: cartoon representation; **c**: surface representation). (**d**,**e**): DHBP-bound erWalK structure. (**d**: cartoon representation; **e**: surface representation). (**f**) Structural comparison of ligand-free erWalK with DHBP-bound erWalK.



S. aureus WalK
extracellular PAS domain

Streptococcus mutans WalK
intracellular PAS domain

Supplementary Figure 8. The crystal structures of WalK PAS domains. Structure comparison of *S. aureus* WalK extracellular PAS domain with *Streptococcus mutans* WalK intracellular PAS domain (PDB code: 4I5S) reveals distinct signal binding cores.

Supplementary Table 1. Constructs screened for crystallization of erWalK.

Name	Sequence	Expressed	Crystallized	Crystallization condition
erWalK1	T34-E167	Yes	No	/
erWalK2	T34-I170	Yes	No	/
erWalK3	T34-D172	Yes	Yes, 7 Å	2.9 M sodium malonate, pH 6.0
erWalK4	T34-Y174	Yes	No	/
erWalK5	T34-Q176	Yes	No	/
erWalK6	T34-Q182	Yes	Yes, 2.1 Å	10% v/v 2-propanol, 0.1 M bicine pH 8.9, 30% w/v polyethylene glycol 1500
erWalK7	T34-A189	Yes	No	/
erWalK6I58M	T34-Q182, I58M	Yes	No	/
erWalK6R86M	T34-Q182, R86M	Yes	Yes, 1.7 Å	0.2 M ammonium citrate dibasic, 20% w/v polyethylene glycol 3350, pH 5.1

Supplementary Table 2. 190 genes with transcript levels decreased more than two fold in the D119A mutant as compared with the wild-type strain.

Gene	Description	Biological process	Fold change
lytM	Peptidoglycan hydrolase	Cell wall metabolism	0.4954
NWMN_0150	Metalloendopeptidase	Cell wall metabolism	0.4936
NWMN_2203	Secretory amidohydrolase/peptidase precursor SsaA	Cell wall metabolism	0.3336
NWMN_2469	Soluble lytic transglycosylases	Cell wall metabolism	0.2846
chp	Chemotaxis-inhibiting protein CHIPS	Pathogenesis	0.0049
coa	Staphylocoagulase	Pathogenesis	0.1552
fnbA	Fibronectin binding protein A precursor	Pathogenesis	0.0615
fnbB	Fibronectin binding protein B	Pathogenesis	0.0893
hla	Alpha-hemolysin precursor	Pathogenesis	0.0622
hlgA	Gamma-hemolysin subunit I	Pathogenesis	0.0198
hlgB	Gamma-hemolysin component B	Pathogenesis	0.0823
hlgC	Gamma-hemolysin component C	Pathogenesis	0.0239
lukD	Leukotoxin LukD	Pathogenesis	0.0464
lukE	Leukotoxin LukE	Pathogenesis	0.0360
lukF	Leukocidin/hemolysin toxin subunit F	Pathogenesis	0.0091
lukS	Leukocidin/hemolysin toxin subunit S	Pathogenesis	0.0102
NWMN_0165	Staphylococcal complement inhibitor SCIN	Pathogenesis	0.0497
NWMN_0362	Staphylococcal/Streptococcal toxin	Pathogenesis	0.0270
NWMN_0757	Secreted coagulase	Pathogenesis	0.3779
NWMN_1075	Superantigen-like protein	Pathogenesis	0.2707
NWMN_1076	superantigen-like protein	Pathogenesis	0.2551
NWMN_1077	superantigen-like protein	Pathogenesis	0.1861
NWMN_1873	Truncated beta-hemolysin	Pathogenesis	0.0054

NWMN_2396	C-terminal part of fibronectin binding protein B	Pathogenesis	0.1285
NWMN_2398	C-terminal part of fibronectin binding protein A	Pathogenesis	0.1453
sbi	IgG-binding protein SBI	Pathogenesis	0.0084
scn	Complement inhibitor SCIN	Pathogenesis	0.0274
set10nm	superantigen-like protein	Pathogenesis	0.2906
set11nm	Superantigen toxin	Pathogenesis	0.0633
set1nm	superantigen-like protein	Pathogenesis	0.3890
set2nm	Superantigen-like protein	Pathogenesis	0.0921
set4nm	Superantigen-like protein	Pathogenesis	0.0813
set7nm	Superantigen toxin	Pathogenesis	0.2308
set8nm	Superantigen toxin	Pathogenesis	0.1892
set9nm	superantigen-like protein	Pathogenesis	0.1768
splA	Serine protease SplA	Pathogenesis	0.0638
splB	Serine protease SplB	Pathogenesis	0.1010
splC	Serine protease SplC	Pathogenesis	0.3186
splE	Serine protease SplE	Pathogenesis	0.3078
ald	Alanine dehydrogenase	Metabolism	0.0486
aldA	Aldehyde dehydrogenase homologue	Metabolism	0.4099
arcB	Ornithine carbamoyltransferase	Metabolism	0.3855
aroE	Shikimate 5-dehydrogenase	Metabolism	0.4337
cysE	Serine acetyltransferase	Metabolism	0.3688
cysG	Precorrin-2 dehydrogenase	Metabolism	0.1347
geh	Glycerol ester hydrolase	Metabolism	0.3204
gntK	Gluconokinase	Metabolism	0.3043
ilvA	Threonine dehydratase	Metabolism	0.0546
ipdC	Indole-3-pyruvate decarboxylase	Metabolism	0.2824
mvaS	3-hydroxy-3-methylglutaryl-CoA	Metabolism	0.3708

	synthase		
NWMN_0071	Acetoin reductase	Metabolism	0.3941
NWMN_0619	Dihydroxyacetone kinase subunit DhaK	Metabolism	0.4005
NWMN_0853	3-oxoacyl-(acyl carrier protein) synthase III	Metabolism	0.1682
NWMN_0854	3-oxoacyl-(acyl-carrier-protein) synthase II	Metabolism	0.3415
NWMN_0920	Fucose 4-O-acetylase and related acetyltransferases	Metabolism	0.4111
NWMN_2201	Dehydrogenase family protein	Metabolism	0.3356
NWMN_2341	NAD dependent epimerase/dehydratase family protein	Metabolism	0.4707
pabA	para-aminobenzoate synthase component II	Metabolism	0.3755
pabB	Anthranilate/para-aminobenzoate synthase component I	Metabolism	0.4217
pabC	Aminodeoxychorismate lyase	Metabolism	0.4410
panB	3-methyl-2-oxobutanoate hydroxymethyltransferase	Metabolism	0.3826
purA	Adenylosuccinate synthetase	Metabolism	0.4619
putA	Proline dehydrogenase	Metabolism	0.3531
pyrG	CTP synthase	Metabolism	0.3964
sbnA	O-Acetyl serine sulfhydrylase	Metabolism	0.2675
sbnB	Ornithine cyclodeaminase	Metabolism	0.1901
sbnC	Siderophore biosynthesis IucC family protein	Metabolism	0.4079
sbnF	Siderophore biosynthesis IucC family protein	Metabolism	0.3063
sbnG	2-dehydro-3-deoxyglucarate aldolase	Metabolism	0.4004
sbnH	Diaminopimelate decarboxylase	Metabolism	0.3985
sucC	Succinyl-CoA synthetase subunit beta	Metabolism	0.4182
cydB	cytochrome d terminal oxidase, subunit II	Oxidation reduction	0.1817

cydA	Cytochrome d terminal oxidase, subunit I	Oxidation reduction	0.1899
gntR	Gluconate operon transcriptional repressor	Regulatory pathways	0.1707
lrgA	Murein hydrolase regulator LrgA	Regulatory pathways	0.1335
nirR	Transcriptional regulator NirR	Regulatory pathways	0.0588
saeR	DNA-binding response regulator SaeR	Regulatory pathways	0.0269
saeS	Sensor histidine kinase SaeS	Regulatory pathways	0.0162
tcaR	TcaR transcription regulator	Regulatory pathways	0.1100
isdD	Heme ABC transporter, membrane component IsdD	Transporter	0.3632
isdE	Iron compound ABC transporter	Transporter	0.3047
isdF	Iron/heme permease	Transporter	0.4820
nhaC	Na+/H+ antiporter NhaC	Transporter	0.4864
NWMN_0151	Similar to ABC transporter ATP-binding protein	Transporter	0.4034
NWMN_0423	Sodium-dependent symporter protein	Transporter	0.3947
NWMN_0691	ABC transporter permease	Transporter	0.4439
NWMN_0702	Ferrichrome ABC transporter permease	Transporter	0.3000
NWMN_0703	Iron compound ABC transporter permease	Transporter	0.2733
NWMN_0704	ABC transporter ATP-binding protein	Transporter	0.3212
NWMN_0705	Ferrichrome ABC transporter lipoprotein	Transporter	0.2678
NWMN_1261	Glycine betaine transporter 1	Transporter	0.2905
NWMN_1347	Amino acid permease	Transporter	0.0780
NWMN_2077	Iron compound ABC transporter permease	Transporter	0.3641
NWMN_2078	Ferrichrome ABC transporter lipoprotein	Transporter	0.3069
NWMN_2253	Drug resistance transporter EmrB/QacA subfamily protein	Transporter	0.4754
NWMN_2288	Nitrite transport protein	Transporter	0.1296
sbnD	Membrane transporter protein	Transporter	0.3479

sirA	Siderophore compound ABC transporter binding protein	Transporter	0.3762
rplA	50S ribosomal subunit protein L1	Translation	0.4102
rplB	50S ribosomal subunit protein L2	Translation	0.4967
rplE	50S ribosomal subunit protein L5	Translation	0.4066
rplF	50S ribosomal subunit protein L6	Translation	0.4045
rplJ	50S ribosomal subunit protein L10	Translation	0.4699
rplN	50S ribosomal subunit protein L14	Translation	0.3426
rplO	50S ribosomal subunit protein L15	Translation	0.4527
rplP	50S ribosomal subunit protein L11	Translation	0.3542
rplR	50S ribosomal subunit protein L18	Translation	0.4030
rplW	50S ribosomal subunit protein L23	Translation	0.3311
rplX	50S ribosomal subunit protein L24	Translation	0.4506
rpmC	50S ribosomal subunit protein L29	Translation	0.3611
rpmD	50S ribosomal subunit protein L30	Translation	0.3965
rpsC	30S ribosomal subunit protein S3	Translation	0.4237
rpsE	30S ribosomal subunit protein S5	Translation	0.4614
rpsJ	30S ribosomal subunit protein S10	Translation	0.4884
rpsQ	30S ribosomal subunit protein S17	Translation	0.3623
rpsS	30S ribosomal subunit protein S19	Translation	0.4033
bsaA1	Lantibiotic precursor	Others	0.2234
clpX	ATP-dependent protease ATP-binding subunit ClpX	Others	0.4021
def	Peptide deformylase	Others	0.4001
entB	Isochorismatase	Others	0.4613
gatA	Aspartyl/glutamyl-tRNA amidotransferase subunit A	Others	0.4691
hisS	Histidyl-tRNA synthetase	Others	0.4593
isdC	Iron-regulated cell surface protein	Others	0.3372

isdG	Heme-degrading monooxygenase IsdG	Others	0.3544
leuS	Leucyl-tRNA synthetase	Others	0.4099
lip	Triacylglycerol lipase	Others	0.4831
lrgB	Antiholin-like protein LrgB	Others	0.1304
map	Methionine aminopeptidase	Others	0.0026
nagA	N-acetylglucosamine-6-phosphate deacetylase	Others	0.4857
narG	Respiratory nitrate reductase subunit alpha	Others	0.0440
narH	Nitrate reductase subunit beta NarH	Others	0.0532
narI	Respiratory nitrate reductase, gamma subunit	Others	0.3128
narJ	Respiratory nitrate reductase delta chain	Others	0.0517
nirB	Assimilatory nitrite reductase	Others	0.0744
nirD	Assimilatory nitrite reductase [NAD(P)H], small subunit	Others	0.1407
NWMN_0760	Thermonuclease precursor	Others	0.1397
NWMN_0870	Oligo endopeptidase F	Others	0.3184
NWMN_0897	Lipoate-protein ligase A	Others	0.4753
NWMN_1066	Extracellular fibrinogen binding protein	Others	0.0462
NWMN_1067	Formyl peptide receptor-like 1 inhibitory protein	Others	0.0671
NWMN_1069	Extracellular fibrinogen binding protein	Others	0.0028
NWMN_1343	RNase_HI_like protein	Others	0.4052
NWMN_1368	tRNA CCA-pyrophosphorylase	Others	0.4905
NWMN_1496	Nicotinate (nicotinamide) nucleotide adenylyltransferase	Others	0.4073
NWMN_1499	GTP-binding protein YqeH	Others	0.4766
NWMN_1621	Trypsin-like serine proteases	Others	0.4497
NWMN_2274	Pyridine nucleotide-disulfide oxidoreductase family protein	Others	0.4465

NWMN_2474	Glyoxalase family protein	Others	0.4753
NWMN_2587	Putative rhodanese-related sulfurtransferase	Others	0.4332
NWMN_2609	Chromosome partioning ParB family protein	Others	0.4820
panC	Pantoate--beta-alanine ligase	Others	0.4631
pcrA	ATP-dependent DNA helicase PcrA	Others	0.4900
pfs	5'-methylthioadenosine nucleosidase	Others	0.4626
pyrH	Uridylate kinase	Others	0.4975
rpoA	DNA-directed RNA polymerase subunit alpha	Others	0.4929
sbnI	Siderophore staphylobactin biosynthesis protein SbnI	Others	0.4828
secA	Preprotein translocase subunit SecA	Others	0.4135
spsB	Type-1 signal peptidase 1B	Others	0.4569
srtB	NPQTN-specific sortase B	Others	0.4169
ssp	Extracellular matrix and plasma binding protein	Others	0.0345
tgt	Queuine tRNA-ribosyltransferase	Others	0.4976
tig	Trigger factor	Others	0.4424
tyrS	Tyrosyl-tRNA synthetase	Others	0.4908
NWMN_0006	Hypothetical protein	Hypothetical protein	0.4603
NWMN_0157	Hypothetical protein	Hypothetical protein	0.0967
NWMN_0208	Hypothetical protein	Hypothetical protein	0.4801
NWMN_0363	Hypothetical protein	Hypothetical protein	0.1923
NWMN_0401	Hypothetical protein	Hypothetical protein	0.2254
NWMN_0402	Hypothetical protein	Hypothetical protein	0.0033
NWMN_0434	Hypothetical protein	Hypothetical protein	0.4815
NWMN_0537	Hypothetical protein	Hypothetical protein	0.1461
NWMN_0677	Hypothetical protein	Hypothetical protein	0.0026

NWMN_0681	Hypothetical protein	Hypothetical protein	0.3381
NWMN_0759	Hypothetical protein	Hypothetical protein	0.0104
NWMN_0775	Hypothetical protein	Hypothetical protein	0.4731
NWMN_0990	Hypothetical protein	Hypothetical protein	0.4772
NWMN_1346	Hypothetical protein	Hypothetical protein	0.0832
NWMN_1548	Hypothetical protein	Hypothetical protein	0.4922
NWMN_1552	Hypothetical protein	Hypothetical protein	0.3538
NWMN_1570	Hypothetical protein	Hypothetical protein	0.4573
NWMN_1708	Hypothetical protein	Hypothetical protein	0.2845
NWMN_1874	Hypothetical protein	Hypothetical protein	0.1987
NWMN_1875	Hypothetical protein	Hypothetical protein	0.2618
NWMN_2074	Hypothetical protein	Hypothetical protein	0.4548
NWMN_2075	Hypothetical protein	Hypothetical protein	0.4219
NWMN_2254	Hypothetical protein	Hypothetical protein	0.4109
NWMN_2259	Hypothetical protein	Hypothetical protein	0.4266
NWMN_2436	Hypothetical protein	Hypothetical protein	0.4920
NWMN_0676	Hypothetical protein	Hypothetical protein	0.0077
NWMN_1070	Hypothetical protein	Hypothetical protein	0.0030

Supplementary Table 3. 123 genes with transcript levels decreased more than two fold in the V149A mutant as compared with the wild-type strain.

Gene	Description	Biological process	Fold change
lytM	Peptidoglycan hydrolase	Cell wall metabolism	0.4894
NWMN_2203	Secretory amidohydrolase/peptidase precursor SsaA	Cell wall metabolism	0.2774
NWMN_2469	Soluble lytic transglycosylases	Cell wall metabolism	0.3771
chp	Chemotaxis-inhibiting protein CHIPS	Pathogenesis	0.0045
coa	Staphylocoagulase	Pathogenesis	0.1597
fnbB	Fibronectin binding protein B	Pathogenesis	0.0637
hla	Alpha-hemolysin precursor	Pathogenesis	0.0184
hlgA	Gamma-hemolysin subunit I	Pathogenesis	0.0202
hlgB	Gamma-hemolysin component B	Pathogenesis	0.0534
hlgC	Gamma-hemolysin component C	Pathogenesis	0.0176
lukD	Leukotoxin LukD	Pathogenesis	0.0402
lukE	Leukotoxin LukE	Pathogenesis	0.0203
lukF	Leukocidin/hemolysin toxin subunit F	Pathogenesis	0.0081
lukS	Leukocidin/hemolysin toxin subunit S	Pathogenesis	0.0078
NWMN_0165	Staphylococcal complement inhibitor SCIN	Pathogenesis	0.1339
NWMN_0362	Staphylococcal/Streptococcal toxin	Pathogenesis	0.0252
NWMN_0757	Secreted coagulase	Pathogenesis	0.3132
NWMN_1075	Superantigen-like protein	Pathogenesis	0.3847
NWMN_1076	superantigen-like protein	Pathogenesis	0.2808
NWMN_1077	superantigen-like protein	Pathogenesis	0.1806
NWMN_1873	Truncated beta-hemolysin	Pathogenesis	0.0115
NWMN_2396	C-terminal part of fibronectin binding protein B	Pathogenesis	0.1288
NWMN_2398	C-terminal part of fibronectin binding protein A	Pathogenesis	0.1197
sbi	IgG-binding protein SBI	Pathogenesis	0.0084

scn	Complement inhibitor SCIN	Pathogenesis	0.0216
set10nm	superantigen-like protein	Pathogenesis	0.3221
set11nm	Superantigen toxin	Pathogenesis	0.1045
set1nm	superantigen-like protein	Pathogenesis	0.4584
set2nm	Superantigen-like protein	Pathogenesis	0.1708
set4nm	Superantigen-like protein	Pathogenesis	0.1094
set7nm	Superantigen toxin	Pathogenesis	0.2549
set8nm	Superantigen toxin	Pathogenesis	0.1028
set9nm	superantigen-like protein	Pathogenesis	0.1816
splA	Serine protease SplA	Pathogenesis	0.0572
splB	Serine protease SplB	Pathogenesis	0.1957
splC	Serine protease SplC	Pathogenesis	0.2272
ald	Alanine dehydrogenase	Metabolism	0.0355
aldA	Aldehyde dehydrogenase homologue	Metabolism	0.4817
geh	Glycerol ester hydrolase	Metabolism	0.2584
gntK	Gluconokinase	Metabolism	0.3440
ilvA	Threonine dehydratase	Metabolism	0.0500
ipdC	Indole-3-pyruvate decarboxylase	Metabolism	0.3372
mvaS	3-hydroxy-3-methylglutaryl-CoA synthase	Metabolism	0.4790
NWMN_0071	Acetoin reductase	Metabolism	0.2873
NWMN_0619	Dihydroxyacetone kinase subunit DhaK	Metabolism	0.3624
NWMN_0853	3-oxoacyl-(acyl carrier protein) synthase III	Metabolism	0.2386
NWMN_0854	3-oxoacyl-(acyl-carrier-protein) synthase II	Metabolism	0.3775
NWMN_0920	Fucose 4-O-acetylase and related acetyltransferases	Metabolism	0.4626
NWMN_2201	Dehydrogenase family protein	Metabolism	0.3908
pabB	Anthranilate/para-aminobenzoate synthase component I	Metabolism	0.0459
panB	3-methyl-2-oxobutanoate	Metabolism	0.3684

	hydroxymethyltransferase		
putA	Proline dehydrogenase	Metabolism	0.4003
sbnA	O-Acetyl serine sulfhydrylase	Metabolism	0.1819
sbnB	Ornithine cyclodeaminase	Metabolism	0.1839
sucC	Succinyl-CoA synthetase subunit beta	Metabolism	0.4844
pflB	Formate acetyltransferase	Metabolism	0.4791
NWMN_1315	Acylphosphatase	Metabolism	0.4991
gntR	Gluconate operon transcriptional repressor	Regulatory pathways	0.1755
lrgA	Murein hydrolase regulator LrgA	Regulatory pathways	0.1159
nirR	Transcriptional regulator NirR	Regulatory pathways	0.2704
saeR	DNA-binding response regulator SaeR	Regulatory pathways	0.0245
saeS	Sensor histidine kinase SaeS	Regulatory pathways	0.0225
tcaR	TcaR transcription regulator	Regulatory pathways	0.1099
lytS	Autolysin sensor histidine kinase	Regulatory pathways	0.4514
isdD	Heme ABC transporter, membrane component IsdD	Transporter	0.2840
isdE	Iron compound ABC transporter	Transporter	0.2704
isdF	Iron/heme permease	Transporter	0.4147
nhaC	Na+/H+ antiporter NhaC	Transporter	0.4643
NWMN_0151	Similar to ABC transporter ATP-binding protein	Transporter	0.3781
NWMN_0423	Sodium-dependent symporter protein	Transporter	0.4179
NWMN_0691	ABC transporter permease	Transporter	0.4199
NWMN_0705	Ferrichrome ABC transporter lipoprotein	Transporter	0.4294
NWMN_1261	Glycine betaine transporter 1	Transporter	0.4776
NWMN_1347	Amino acid permease	Transporter	0.0519
NWMN_2077	Iron compound ABC transporter permease	Transporter	0.4015
NWMN_2078	Ferrichrome ABC transporter lipoprotein	Transporter	0.3001
sirA	Siderophore compound ABC transporter	Transporter	0.2999

	binding protein		
opp1A	Oligopeptide permease, peptide-binding protein	Transporter	0.4700
NWMN_0690	Osmoprotectant ABC transporter ATP-binding protein	Transporter	0.4722
NWMN_2076	FecCD iron compound ABC transporter permease family protein	Transporter	0.4819
NWMN_0813	Na+/H+ antiporter family protein	Transporter	0.4940
cydA	Cytochrome d terminal oxidase, subunit I	Oxidation reduction	0.2478
cydB	cytochrome d terminal oxidase, subunit II	Oxidation reduction	0.2703
bsaA1	Lantibiotic precursor	Others	0.2136
def	Peptide deformylase	Others	0.4993
entB	Isochorismatase	Others	0.4894
lip	Triacylglycerol lipase	Others	0.4647
lrgB	Antiholin-like protein LrgB	Others	0.1338
map	Methionine aminopeptidase	Others	0.0020
nirB	Assimilatory nitrite reductase	Others	0.4474
NWMN_0760	Thermonuclease precursor	Others	0.0510
NWMN_0870	Oligo endopeptidase F	Others	0.3188
NWMN_1066	?Extracellular fibrinogen binding protein	Others	0.0339
NWMN_1067	Formyl peptide receptor-like 1 inhibitory protein	Others	0.0595
NWMN_1069	Extracellular fibrinogen binding protein	Others	0.0014
NWMN_2274	Pyridine nucleotide-disulfide oxidoreductase family protein	Others	0.4855
NWMN_2609	Chromosome partioning ParB family protein	Others	0.4902
panC	Pantoate--beta-alanine ligase	Others	0.4797
sbnI	Siderophore staphylobactin biosynthesis protein SbnI	Others	0.4543
secA	Preprotein translocase subunit SecA	Others	0.4764
ssp	Extracellular matrix and plasma binding	Others	0.0349

	protein		
nadC	Nicotinate phosphoribosyltransferase	Others	0.4647
NWMN_1755	Bacterioferritin comigratory protein	Others	0.4955
NWMN_0006	Hypothetical protein	Hypothetical protein	0.4095
NWMN_0157	Hypothetical protein	Hypothetical protein	0.1133
NWMN_0363	Hypothetical protein	Hypothetical protein	0.1636
NWMN_0401	Hypothetical protein	Hypothetical protein	0.2544
NWMN_0402	Hypothetical protein	Hypothetical protein	0.0016
NWMN_0681	Hypothetical protein	Hypothetical protein	0.4715
NWMN_0759	Hypothetical protein	Hypothetical protein	0.0157
NWMN_0775	Hypothetical protein	Hypothetical protein	0.4535
NWMN_1346	Hypothetical protein	Hypothetical protein	0.0668
NWMN_1552	Hypothetical protein	Hypothetical protein	0.4801
NWMN_1708	Hypothetical protein	Hypothetical protein	0.2516
NWMN_1874	Hypothetical protein	Hypothetical protein	0.2355
NWMN_1875	Hypothetical protein	Hypothetical protein	0.3517
NWMN_2074	Hypothetical protein	Hypothetical protein	0.4916
NWMN_2259	Hypothetical protein	Hypothetical protein	0.4811
NWMN_2435	Hypothetical protein	Hypothetical protein	0.4569
NWMN_0134	Hypothetical protein	Hypothetical protein	0.4982
NWMN_0874	Hypothetical protein	Hypothetical protein	0.4992
NWMN_0676	Hypothetical protein	Hypothetical protein	0.0074
NWMN_1070	Hypothetical protein	Hypothetical protein	0.0026

Supplementary Table 4. 293 genes with transcript levels increased more than two fold in the D119A mutant as compared with the wild-type strain.

Gene	Description	Biological process	Fold change
NWMN_2463	Glycosyl transferase, group 2 family protein	Cell wall biosynthesis	4.18
murA	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	Cell wall biosynthesis	2.67
capD	Capsular polysaccharide synthesis protein CapD	Pathogenesis	13.83
capB	Capsule biosynthesis protein CapB	Pathogenesis	11.94
capC	Capsular polysaccharide synthesis protein CapC	Pathogenesis	11.66
capA	Capsular polysaccharide synthesis protein CapA	Pathogenesis	11.45
capE	Capsular polysaccharide synthesis protein CapE	Pathogenesis	9.35
capF	Capsular polysaccharide synthesis protein CapF	Pathogenesis	8.32
capG	Capsular polysaccharide synthesis protein CapG	Pathogenesis	6.16
aur	Zinc metalloproteinase aureolysin	Pathogenesis	5.77
sdrD	Ser-Asp rich fibrinogen/bone sialoprotein-binding protein sdrD	Pathogenesis	5.33
capH	Capsular polysaccharide synthesis enzyme O-acetyl transferase CapH	Pathogenesis	4.89
capI	Capsular polysaccharide biosynthesis proteinCapI	Pathogenesis	3.99
capM	Capsular polysaccharide biosynthesis proteinCapM	Pathogenesis	3.85
capL	Capsular polysaccharide biosynthesis protein glycosyltransferase CapL	Pathogenesis	3.72
clfA	Clumping factor A	Pathogenesis	3.67
capN	Capsular polysaccharide biosynthesis protein CapN	Pathogenesis	3.63
capJ	Capsular polysaccharide biosynthesis proteinCapJ	Pathogenesis	3.5
capK	Capsular polysaccharide biosynthesis protein CapK	Pathogenesis	3.24
capO	Capsular polysaccharide synthesis enzyme CapO	Pathogenesis	2.46
isaB	Immunodominant antigen B	Pathogenesis	2.32

capP	Capsular polysaccharide synthesis enzyme CapP	Pathogenesis	2.13
eta	Exfoliative toxin A	Pathogenesis	2.03
NWMN_2286	MarR family regulatory protein	Regulatory pathways	6.1
sarA	Accessory regulator A	Regulatory pathways	4.98
NWMN_2105	Transcriptional regulator MerR family protein	Regulatory pathways	3.88
argR	ArgR family transcriptional regulator	Regulatory pathways	3.72
spoVG	Regulatory protein SpoVG	Regulatory pathways	3.55
NWMN_0921	ATL autolysin transcriptional regulator	Regulatory pathways	3.25
agrD	Accessory gene regulator protein D	Regulatory pathways	2.83
kdpE	KDP operon transcriptional regulatory protein	Regulatory pathways	2.74
agrA	Accessory gene regulator protein A	Regulatory pathways	2.64
NWMN_2225	Phosphosugar-binding transcriptional regulator	Regulatory pathways	2.54
agrC	Accessory gene regulator protein C	Regulatory pathways	2.43
rbsR	Ribose transcriptional repressor RbsR	Regulatory pathways	2.38
lexA	LexA repressor	Regulatory pathways	2.32
sarR	Accessory regulator R	Regulatory pathways	2.3
agrB	Accessory gene regulator B	Regulatory pathways	2.3
NWMN_1655	Accessory regulator Rot	Regulatory pathways	2.24
kdpD	Sensor histidine kinase KdpD	Regulatory pathways	2.23
NWMN_0326	MarR family regulatory protein	Regulatory pathways	2.19
NWMN_2371	Carboxymuconolactone decarboxylase family protein	Metabolism	4.3
thrA	Aspartate kinase	Metabolism	4.07
NWMN_2229	Oxidoreductase, short chain dehydrogenase/reductase family protein	Metabolism	4.07
NWMN_0171	Acetyl-CoA/acetoacetyl-CoA transferase	Metabolism	3.86
NWMN_2419	Acetyltransferase, GNAT family protein	Metabolism	3.82
argC	N-acetyl-gamma-glutamyl-phosphate reductase	Metabolism	2.27

metE	5-methyltetrahydropteroyltriglutamate/homocysteine S-methyltransferase	Metabolism	3.62
metL	Homoserine dehydrogenase	Metabolism	3.49
metH	Bifunctional homocysteine S-methyltransferase/5,10-methylenetetrahydrofolate reductase	Metabolism	3.47
thrC	Threonine synthase	Metabolism	3.36
thrB	Homoserine kinase	Metabolism	3.33
crtI	Phytoene dehydrogenase	Metabolism	3.29
crtM	Squalene desaturase	Metabolism	3.28
NWMN_1929	Succinyl-diaminopimelate desuccinylase	Metabolism	2.91
hutG	Formimidoylglutamase	Metabolism	2.89
NWMN_2210	Formate dehydrogenase-like protein	Metabolism	2.89
NWMN_2501	4-aminobutyrate aminotransferase	Metabolism	2.88
gpmA	Phosphoglycero mutase	Metabolism	2.85
mtlD	Mannitol-1-phosphate 5-dehydrogenase	Metabolism	2.85
crtN	Squalene synthase	Metabolism	2.85
NWMN_2350	para-nitrobenzyl esterase chain A	Metabolism	2.82
NWMN_2369	Short chain dehydrogenase	Metabolism	2.73
fabZ	(3R)-hydroxymyristoyl-ACP dehydratase	Metabolism	2.68
NWMN_0672	Aldo/keto reductase family protein	Metabolism	2.68
NWMN_1746	Similar to glucosamine-6-phosphate isomerase	Metabolism	2.67
leuA	2-isopropylmalate synthase	Metabolism	2.52
hipO	Hippurate hydrolase	Metabolism	2.51
ilvC	Ketol-acid reductoisomerase	Metabolism	2.47
dapA	Dihydrodipicolinate synthase	Metabolism	2.37
dapD	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase	Metabolism	2.37
NWMN_0119	Acyl-CoA dehydrogenases	Metabolism	2.36

ilvB	Acetolactate synthase large subunit	Metabolism	2.35
NWMN_1275	4-oxalocrotonate tautomerase	Metabolism	2.35
dapB	Dihydrodipicolinate reductase	Metabolism	2.22
leuD	Isopropylmalate isomerase small subunit	Metabolism	2.19
leuB	3-isopropylmalate dehydrogenase	Metabolism	2.14
glnA	Glutamine synthetase	Metabolism	2.12
adhE	Bifunctional acetaldehyde-CoA/alcohol dehydrogenase	Metabolism	2.1
argD	Ornithine aminotransferase	Metabolism	2.08
ilvA	Threonine dehydratase	Metabolism	2.08
NWMN_2416	Phospholipase/carboxylesterase family protein	Metabolism	2.07
alr2	Alanine racemase 2	Metabolism	2.04
NWMN_0601	ABC-type metal ion transport system protein	Transporter	20.86
NWMN_0602	ABC-type Mn2+/Zn2+ transport system protein	Transporter	19.26
NWMN_0603	ABC transporter ATP-binding protein	Transporter	16.37
NWMN_0251	ABC transporter ATP-binding protein	Transporter	4.3
ulaA	PTS system ascorbate-specific transporter subunit IIC	Transporter	4.26
NWMN_0114	Cation efflux family protein	Transporter	4.02
NWMN_2424	ABC transporter ATP-binding protein	Transporter	3.92
NWMN_2500	Amino acid permease family protein	Transporter	3.57
bsaE	Lantibiotic ABC transporter protein	Transporter	3.57
NWMN_0428	ABC transporter substrate-binding protein	Transporter	3.3
bsaG	Lantibiotic ABC transporter protein	Transporter	3.22
NWMN_0250	ABC transporter permease	Transporter	3.19
NWMN_2268	L-lactate permease 2	Transporter	3.14
NWMN_2089	Osmoprotectant transporter	Transporter	3.13
oppD	Oligopeptide transport ATP-binding protein	Transporter	3.03
NWMN_0856	Oligopeptide transport system permease	Transporter	3.02

NWMN_0860	ABC-type oligopeptide transport system	Transporter	2.9
oppC	Oligopeptide transport system permease	Transporter	2.87
NWMN_2595	High-affinity nickel transporter	Transporter	2.86
NWMN_2352	ABC-type uncharacterized transport system	Transporter	2.86
azi	ABC transporter ATP-binding protein	Transporter	2.82
mtlA	Mannitol-specific IIA component	Transporter	2.75
NWMN_0863	Oligopeptide ABC transporter ATP-binding protein	Transporter	2.65
NWMN_0971	Manganese transport protein MntH	Transporter	2.58
NWMN_0084	Phosphonates ABC transporter permease	Transporter	2.56
NWMN_0696	Di-/tripeptide ABC transporter	Transporter	2.39
NWMN_0117	ABC-type nitrate/sulfonate/bicarbonate transport system protein	Transporter	2.26
oppF	Oligopeptide ABC transporter ATP-binding protein	Transporter	2.25
NWMN_0782	ABC transporter substrate-binding protein	Transporter	2.21
NWMN_0338	High-affinity Fe2+/Pb2+ permease	Transporter	2.19
NWMN_0049	Na+/phosphate symporter	Transporter	2.19
NWMN_2581	Cobalt transport family protein	Transporter	2.19
lctP	L-lactate permease	Transporter	2.18
NWMN_2242	ABC transporter like protein	Transporter	2.18
NWMN_2224	PTS system, alpha-glucoside-specific IIBC component	Transporter	2.16
NWMN_2353	ABC transporter ATP-binding protein	Transporter	2.13
rlp	RGD-containing lipoprotein	Transporter	2.08
NWMN_2241	ABC-type Na+ efflux pump, permease component	Transporter	2.07
comK	Competence transcription factor ComK	Transcription	8.72
rpmB	50S ribosomal protein L28	Translation	2
sspA	V8 protease, glutamyl endopeptidase precursor	Others	18.92
sspB	Cysteine protease precursor	Others	13.98

sspC	Cysteine protease	Others	11.93
NWMN_2109	Truncated MHC class II analog protein	Others	6.52
NWMN_1831	Ferritin	Others	5.83
sak	Staphylokinase precursor	Others	5.27
NWMN_2550	Accessory Sec system protein Asp2	Others	4.46
NWMN_0783	CsbD-like superfamily protein	Others	4.43
qoxB	Quinol oxidase polypeptide I QoxB	Others	4.23
secY	Preprotein translocase subunitSecY	Others	4.15
qoxA	Quinol oxidase polypeptide II QoxA	Others	4.04
NWMN_2549	Accessory Sec system protein Asp3	Others	3.97
fofB	Fosfomycin resistance protein FosB	Others	3.8
cspB	Cold shock protein CspB	Others	3.76
cspC	Cold-shock protein CSD family protein	Others	3.56
qoxC	Quinol oxidase polypeptide III QoxC	Others	3.51
NWMN_2594	Endonuclease III	Others	3.34
NWMN_2512	Metallo-beta-lactamase superfamily protein	Others	3.29
tnp	Transposase	Others	3.28
NWMN_2551	Accessory secretory protein Asp1	Others	3.28
mscL	Large-conductance mechanosensitive channel	Others	2.97
NWMN_1382	DNA-binding protein HU	Others	2.95
NWMN_0332	NADH-dependent FMN reductase	Others	2.93
sdrE	Ser-Asp rich fibrinogen/bone sialoprotein-binding protein SdrE	Others	2.93
bsaF	Lantibiotic immunity protein F	Others	2.93
sdrC	Ser-Asp rich fibrinogen/bone sialoprotein-binding protein SdrC	Others	2.9
NWMN_2086	Alkaline shock protein 23	Others	2.88
recA	Recombinase A	Others	2.74
NWMN_1819	Low molecular weight phosphotyrosine protein	Others	2.7

	phosphatase		
NWMN_1821	Ribonuclease BN	Others	2.66
NWMN_1888	Phage tail tape measure protein	Others	2.62
NWMN_2547	Glycosyl transferase, group 1 family protein	Others	2.6
radC	DNA repair protein RadC	Others	2.53
NWMN_0374	Predicted Na+/dicarboxylate symporter	Others	2.51
qoxD	Quinol oxidase polypeptide IV	Others	2.51
acuC	Acetoin utilization protein AcuC	Others	2.48
NWMN_0331	Luciferase-like monooxygenase	Others	2.43
bsaA2	Lantibiotic precursor	Others	2.34
NWMN_2480	Hydrolase	Others	2.3
veg	Veg protein	Others	2.3
NWMN_1600	Universal stress protein family protein	Others	2.29
NWMN_1088	Cell division protein MraZ	Others	2.25
NWMN_0330	Glyoxalase family protein	Others	2.2
NWMN_1525	Luciferase family protein	Others	2.17
NWMN_0995	Phage anti-repressor protein	Others	2.16
NWMN_0771	OsmC-like protein	Others	2.07
orfX	rRNA large subunit methyltransferase	Others	2.06
NWMN_0527	Glycosyl transferase, group 1 family protein	Others	2.03
nrdI	Ribonucleotide reductase stimulatory protein	Others	2
NWMN_2502	Hypothetical protein	Hypothetical protein	13.68
NWMN_0753	Hypothetical protein	Hypothetical protein	13.41
NWMN_0752	Hypothetical protein	Hypothetical protein	9.73
NWMN_1848	Hypothetical protein	Hypothetical protein	8.01
NWMN_0219	Hypothetical protein	Hypothetical protein	7.96
NWMN_0232	Hypothetical protein	Hypothetical protein	7.2
NWMN_1526	Hypothetical protein	Hypothetical protein	6.62

NWMN_0216	Hypothetical protein	Hypothetical protein	6.46
NWMN_2406	Hypothetical protein	Hypothetical protein	6.42
NWMN_1731	Hypothetical protein	Hypothetical protein	6.37
NWMN_0220	Hypothetical protein	Hypothetical protein	5.93
NWMN_0221	Hypothetical protein	Hypothetical protein	5.84
NWMN_0739	Hypothetical protein	Hypothetical protein	5.49
NWMN_0225	Hypothetical protein	Hypothetical protein	4.99
NWMN_0223	Hypothetical protein	Hypothetical protein	4.87
NWMN_0041	Hypothetical protein	Hypothetical protein	4.82
NWMN_0738	Hypothetical protein	Hypothetical protein	4.79
NWMN_2553	Hypothetical protein	Hypothetical protein	4.64
NWMN_2597	Hypothetical protein	Hypothetical protein	4.6
NWMN_0234	Hypothetical protein	Hypothetical protein	4.54
NWMN_0695	Hypothetical protein	Hypothetical protein	4.5
NWMN_0222	Hypothetical protein	Hypothetical protein	4.39
NWMN_0224	Hypothetical protein	Hypothetical protein	4.32
NWMN_0323	Hypothetical protein	Hypothetical protein	4.24
NWMN_0382	Hypothetical protein	Hypothetical protein	4.11
NWMN_0352	Hypothetical protein	Hypothetical protein	4.06
NWMN_0765	Hypothetical protein	Hypothetical protein	3.94
NWMN_2270	Hypothetical protein	Hypothetical protein	3.93
NWMN_0366	Hypothetical protein	Hypothetical protein	3.88
NWMN_1989	Hypothetical protein	Hypothetical protein	3.86
NWMN_2368	Hypothetical protein	Hypothetical protein	3.85
NWMN_2555	Hypothetical protein	Hypothetical protein	3.65
NWMN_1527	Hypothetical protein	Hypothetical protein	3.61
NWMN_1688	Hypothetical protein	Hypothetical protein	3.59
NWMN_1689	Hypothetical protein	Hypothetical protein	3.57

NWMN_1632	Hypothetical protein	Hypothetical protein	3.56
NWMN_2005	Hypothetical protein	Hypothetical protein	3.54
NWMN_1074	Hypothetical protein	Hypothetical protein	3.51
NWMN_2209	Hypothetical protein	Hypothetical protein	3.51
NWMN_0227	Hypothetical protein	Hypothetical protein	3.48
NWMN_0673	Hypothetical protein	Hypothetical protein	3.45
NWMN_2557	Hypothetical protein	Hypothetical protein	3.45
NWMN_1861	Hypothetical protein	Hypothetical protein	3.45
NWMN_2591	Hypothetical protein	Hypothetical protein	3.34
NWMN_0460	Hypothetical protein	Hypothetical protein	3.3
NWMN_2087	Hypothetical protein	Hypothetical protein	3.28
NWMN_0364	Hypothetical protein	Hypothetical protein	3.25
NWMN_1225	Hypothetical protein	Hypothetical protein	3.24
NWMN_2538	Hypothetical protein	Hypothetical protein	3.22
NWMN_0226	Hypothetical protein	Hypothetical protein	3.2
NWMN_0246	Hypothetical protein	Hypothetical protein	3.12
NWMN_2556	Hypothetical protein	Hypothetical protein	3.1
NWMN_2465	Hypothetical protein	Hypothetical protein	3.08
NWMN_2417	Hypothetical protein	Hypothetical protein	3.07
NWMN_0948	Hypothetical protein	Hypothetical protein	3.07
NWMN_2389	Hypothetical protein	Hypothetical protein	3.03
NWMN_0648	Hypothetical protein	Hypothetical protein	3.01
NWMN_0556	Hypothetical protein	Hypothetical protein	3
NWMN_0048	Hypothetical protein	Hypothetical protein	3
NWMN_0383	Hypothetical protein	Hypothetical protein	2.93
NWMN_1510	Hypothetical protein	Hypothetical protein	2.91
NWMN_2088	Hypothetical protein	Hypothetical protein	2.89
NWMN_1235	Hypothetical protein	Hypothetical protein	2.87

NWMN_0045	Hypothetical protein	Hypothetical protein	2.8
NWMN_2282	Hypothetical protein	Hypothetical protein	2.79
NWMN_1123	Hypothetical protein	Hypothetical protein	2.75
NWMN_2579	Hypothetical protein	Hypothetical protein	2.75
NWMN_2392	Hypothetical protein	Hypothetical protein	2.71
NWMN_0078	Hypothetical protein	Hypothetical protein	2.7
NWMN_2243	Hypothetical protein	Hypothetical protein	2.62
NWMN_0767	Hypothetical protein	Hypothetical protein	2.61
NWMN_1820	Hypothetical protein	Hypothetical protein	2.6
NWMN_0904	Hypothetical protein	Hypothetical protein	2.58
NWMN_1720	Hypothetical protein	Hypothetical protein	2.58
NWMN_0637	Hypothetical protein	Hypothetical protein	2.56
NWMN_0908	Hypothetical protein	Hypothetical protein	2.55
NWMN_0970	Hypothetical protein	Hypothetical protein	2.5
NWMN_2585	Hypothetical protein	Hypothetical protein	2.49
NWMN_2330	Hypothetical protein	Hypothetical protein	2.49
NWMN_0376	Hypothetical protein	Hypothetical protein	2.47
NWMN_0784	Hypothetical protein	Hypothetical protein	2.43
NWMN_0118	Hypothetical protein	Hypothetical protein	2.41
NWMN_0429	Hypothetical protein	Hypothetical protein	2.41
NWMN_0766	Hypothetical protein	Hypothetical protein	2.4
NWMN_2558	Hypothetical protein	Hypothetical protein	2.4
NWMN_2370	Hypothetical protein	Hypothetical protein	2.4
NWMN_0754	Hypothetical protein	Hypothetical protein	2.39
NWMN_0586	Hypothetical protein	Hypothetical protein	2.34
NWMN_0770	Hypothetical protein	Hypothetical protein	2.34
NWMN_1730	Hypothetical protein	Hypothetical protein	2.33
NWMN_2487	Hypothetical protein	Hypothetical protein	2.33

NWMN_1663	Hypothetical protein	Hypothetical protein	2.31
NWMN_2305	Hypothetical protein	Hypothetical protein	2.31
NWMN_1745	Hypothetical protein	Hypothetical protein	2.3
NWMN_1860	Hypothetical protein	Hypothetical protein	2.3
NWMN_2001	Hypothetical protein	Hypothetical protein	2.3
NWMN_2228	Hypothetical protein	Hypothetical protein	2.26
NWMN_2546	Hypothetical protein	Hypothetical protein	2.25
NWMN_0356	Hypothetical protein	Hypothetical protein	2.2
NWMN_0115	Hypothetical protein	Hypothetical protein	2.19
NWMN_2554	Hypothetical protein	Hypothetical protein	2.19
NWMN_0779	Hypothetical protein	Hypothetical protein	2.19
NWMN_0050	Hypothetical protein	Hypothetical protein	2.18
NWMN_2002	Hypothetical protein	Hypothetical protein	2.17
NWMN_0053	Hypothetical protein	Hypothetical protein	2.15
NWMN_1124	Hypothetical protein	Hypothetical protein	2.14
NWMN_1631	Hypothetical protein	Hypothetical protein	2.13
NWMN_0245	Hypothetical protein	Hypothetical protein	2.12
NWMN_0561	Hypothetical protein	Hypothetical protein	2.11
NWMN_0274	Hypothetical protein	Hypothetical protein	2.11
NWMN_0377	Hypothetical protein	Hypothetical protein	2.09
NWMN_0737	Hypothetical protein	Hypothetical protein	2.08
NWMN_2283	Hypothetical protein	Hypothetical protein	2.08
NWMN_0734	Hypothetical protein	Hypothetical protein	2.07
NWMN_0562	Hypothetical protein	Hypothetical protein	2.06
NWMN_0548	Hypothetical protein	Hypothetical protein	2.06
NWMN_1002	Hypothetical protein	Hypothetical protein	2.04
NWMN_0125	Hypothetical protein	Hypothetical protein	2.04
NWMN_2115	Hypothetical protein	Hypothetical protein	2.03

NWMN_1072	Hypothetical protein	Hypothetical protein	2.02
NWMN_0147	Hypothetical protein	Hypothetical protein	2.01

Supplementary Table 5. 180 genes with transcript levels increased more than two fold in the V149A mutant as compared with the wild-type strain.

Gene	Description	Biological process	Fold change
murA	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	Cell wall biosynthesis	2.17
NWMN_2463	Glycosyl transferase, group 2 family protein	Cell wall biosynthesis	3.6
aur	Zinc metalloproteinase aureolysin	Pathogenesis	3.69
capA	Capsular polysaccharide synthesis protein CapA	Pathogenesis	7.31
capB	Capsule biosynthesis protein CapB	Pathogenesis	7.34
capC	Capsular polysaccharide synthesis protein CapC	Pathogenesis	7.36
capD	Capsular polysaccharide synthesis protein CapD	Pathogenesis	8.61
capE	Capsular polysaccharide synthesis protein CapE	Pathogenesis	6.39
capF	Capsular polysaccharide synthesis protein CapF	Pathogenesis	5.65
capG	Capsular polysaccharide synthesis protein CapG	Pathogenesis	4.43
capH	Capsular polysaccharide synthesis enzyme O-acetyl transferase CapH	Pathogenesis	3.14
capI	Capsular polysaccharide biosynthesis protein CapI	Pathogenesis	3.07
capJ	Capsular polysaccharide biosynthesis protein CapJ	Pathogenesis	2.58
capK	Capsular polysaccharide biosynthesis protein CapK	Pathogenesis	2.62
capL	Capsular polysaccharide biosynthesis protein glycosyltransferase CapL	Pathogenesis	2.53
capM	Capsular polysaccharide biosynthesis proteinCapM	Pathogenesis	3.31
capN	Capsular polysaccharide biosynthesis protein CapN	Pathogenesis	3.46
capO	Capsular polysaccharide synthesis enzyme CapO	Pathogenesis	2.36
clfA	Clumping factor A	Pathogenesis	3
eta	Exfoliative toxin A	Pathogenesis	2.01
isaB	Immunodominant antigen B	Pathogenesis	2.07
sdrD	Ser-Asp rich fibrinogen/bone sialoprotein-binding protein sdrD	Pathogenesis	4.84

crtI	Phytoene dehydrogenase	Metabolism	2.83
crtM	Squalene desaturase	Metabolism	2.98
crtN	Squalene synthase	Metabolism	2.34
fabZ	(3R)-hydroxymyristoyl-ACP dehydratase	Metabolism	2.29
glnA	Glutamine synthetase	Metabolism	2.11
gpmA	Phosphoglycero mutase	Metabolism	2.38
hipO	Hippurate hydrolase	Metabolism	2.01
hutG	Formimidoylglutamase	Metabolism	2.26
metE	5-methyltetrahydropteroyltriglutamate/homocysteine S-methyltransferase	Metabolism	2.26
metL	Homoserine dehydrogenase	Metabolism	2.34
mtlD	Mannitol-1-phosphate 5-dehydrogenase	Metabolism	2.61
NWMN_0119	Acyl-CoA dehydrogenases	Metabolism	2.28
NWMN_1746	Similar to glucosamine-6-phosphate isomerase	Metabolism	2.42
NWMN_1929	Succinyl-diaminopimelate desuccinylase	Metabolism	2.12
NWMN_2210	Formate dehydrogenase-like protein	Metabolism	2.54
NWMN_2229	Oxidoreductase, short chain dehydrogenase/reductase family protein	Metabolism	3.02
NWMN_2350	para-nitrobenzyl esterase chain A	Metabolism	2.13
NWMN_2369	Short chain dehydrogenase	Metabolism	2.28
NWMN_2371	Carboxymuconolactone decarboxylase family protein	Metabolism	3.26
NWMN_2419	Acetyltransferase, GNAT family protein	Metabolism	3.21
thrA	Aspartate kinase	Metabolism	3.28
thrB	Homoserine kinase	Metabolism	2.65
thrC	Threonine synthase	Metabolism	2.41
agrA	Accessory gene regulator protein A	Regulatory pathways	2.27
agrC	Accessory gene regulator protein C	Regulatory pathways	2.06
lexA	LexA repressor	Regulatory pathways	2.04

NWMN_0326	MarR family regulatory protein	Regulatory pathways	2.07
NWMN_0921	ATL autolysin transcriptional regulator	Regulatory pathways	2.81
NWMN_2225	Phosphosugar-binding transcriptional regulator	Regulatory pathways	2.26
NWMN_2286	MarR family regulatory protein	Regulatory pathways	5.3
sarA	Accessory regulator A	Regulatory pathways	4.07
spoVG	Regulatory protein SpoVG	Regulatory pathways	3.03
NWMN_2198	Transcriptional regulator AraC family protein	Regulatory pathways	2.47
sarY	Staphylococcal accessory regulator Y	Regulatory pathways	2.16
azi	ABC transporter ATP-binding protein	Transporter	2.29
lctP	L-lactate permease	Transporter	2.1
NWMN_0114	Cation efflux family protein	Transporter	3.19
NWMN_0250	ABC transporter permease	Transporter	2.69
NWMN_0251	ABC transporter ATP-binding protein	Transporter	3.61
NWMN_0428	ABC transporter substrate-binding protein	Transporter	2.11
NWMN_0601	ABC-type metal ion transport system protein	Transporter	13.49
NWMN_0602	ABC-type Mn2+/Zn2+ transport system protein	Transporter	11.7
NWMN_0603	ABC transporter ATP-binding protein	Transporter	9.2
NWMN_0696	Di-/tripeptide ABC transporter	Transporter	2.54
NWMN_0856	Oligopeptide transport system permease	Transporter	2.51
NWMN_0860	ABC-type oligopeptide transport system	Transporter	2.67
NWMN_0971	Manganese transport protein MntH	Transporter	2.3
NWMN_2089	Osmoprotectant transporter	Transporter	2.74
NWMN_2241	ABC-type Na+ efflux pump, permease component	Transporter	2.02
NWMN_2268	L-lactate permease 2	Transporter	2.96
NWMN_2352	ABC-type uncharacterized transport system	Transporter	2.29
NWMN_2500	Amino acid permease family protein	Transporter	3.05
NWMN_2595	High-affinity nickel transporter	Transporter	2.44
oppC	Oligopeptide transport system permease	Transporter	2.34

oppD	Oligopeptide transport ATP-binding protein	Transporter	2.82
ulaA	PTS system ascorbate-specific transporter subunit IIC	Transporter	3.72
comK	Competence transcription factor ComK	Transcription	4.34
bsaF	Lantibiotic immunity protein F	Others	2.15
cspB	Cold shock protein CspB	Others	3.21
cspC	Cold-shock protein CSD family protein	Others	2.95
NWMN_0332	NADH-dependent FMN reductase	Others	2.33
NWMN_0783	CsbD-like superfamily protein	Others	3.14
NWMN_1382	DNA-binding protein HU	Others	2.4
NWMN_1819	Low molecular weight phosphotyrosine protein phosphatase	Others	2.08
NWMN_1821	Ribonuclease BN	Others	2.56
NWMN_1831	Ferritin	Others	4.61
NWMN_1888	Phage tail tape measure protein	Others	2.2
NWMN_2086	Alkaline shock protein 23	Others	2.73
NWMN_2109	Truncated MHC class II analog protein	Others	4.07
NWMN_2512	Metallo-beta-lactamase superfamily protein	Others	2.85
NWMN_2547	Glycosyl transferase, group 1 family protein	Others	2.33
NWMN_2594	Endonuclease III	Others	2.49
qoxA	Quinol oxidase polypeptide II QoxA	Others	3.5
qoxB	Quinol oxidase polypeptide I QoxB	Others	3.57
qoxC	Quinol oxidase polypeptide III?QoxC	Others	3.08
recA	Recombinase A	Others	2.34
sak	Staphylokinase precursor	Others	4.24
sdrC	Ser-Asp rich fibrinogen/bone sialoprotein-binding protein SdrC	Others	2.24
sdrE	Ser-Asp rich fibrinogen/bone sialoprotein-binding protein SdrE	Others	2.79
secY	Preprotein translocase subunitSecY	Others	3.82

sspA	V8 protease, glutamyl endopeptidase precursor	Others	15.09
sspB	Cysteine protease precursor	Others	10.13
sspC	Cysteine protease	Others	7.88
veg	Veg?protein	Others	2.14
NWMN_1212	RNA chaperone, host factor-1 protein	Others	2.16
comEA	Competence protein?ComEA	Others	2.07
NWMN_0041	Hypothetical protein	Hypothetical protein	3.08
NWMN_0045	Hypothetical protein	Hypothetical protein	3.02
NWMN_0048	Hypothetical protein	Hypothetical protein	2.43
NWMN_0078	Hypothetical protein	Hypothetical protein	2.97
NWMN_0118	Hypothetical protein	Hypothetical protein	2.27
NWMN_0219	Hypothetical protein	Hypothetical protein	5.26
NWMN_0220	Hypothetical protein	Hypothetical protein	4.92
NWMN_0221	Hypothetical protein	Hypothetical protein	4.05
NWMN_0222	Hypothetical protein	Hypothetical protein	3.71
NWMN_0223	Hypothetical protein	Hypothetical protein	4.44
NWMN_0224	Hypothetical protein	Hypothetical protein	3.63
NWMN_0225	Hypothetical protein	Hypothetical protein	4.86
NWMN_0226	Hypothetical protein	Hypothetical protein	3.16
NWMN_0232	Hypothetical protein	Hypothetical protein	4.99
NWMN_0246	Hypothetical protein	Hypothetical protein	2.91
NWMN_0323	Hypothetical protein	Hypothetical protein	4.29
NWMN_0352	Hypothetical protein	Hypothetical protein	3.23
NWMN_0364	Hypothetical protein	Hypothetical protein	2.97
NWMN_0366	Hypothetical protein	Hypothetical protein	2.77
NWMN_0376	Hypothetical protein	Hypothetical protein	2.28
NWMN_0377	Hypothetical protein	Hypothetical protein	2
NWMN_0382	Hypothetical protein	Hypothetical protein	3.41

NWMN_0460	Hypothetical protein	Hypothetical protein	2.54
NWMN_0556	Hypothetical protein	Hypothetical protein	3.03
NWMN_0562	Hypothetical protein	Hypothetical protein	2.11
NWMN_0648	Hypothetical protein	Hypothetical protein	2.87
NWMN_0673	Hypothetical protein	Hypothetical protein	2.83
NWMN_0695	Hypothetical protein	Hypothetical protein	3.61
NWMN_0738	Hypothetical protein	Hypothetical protein	3.92
NWMN_0739	Hypothetical protein	Hypothetical protein	4.27
NWMN_0752	Hypothetical protein	Hypothetical protein	7.5
NWMN_0753	Hypothetical protein	Hypothetical protein	9.89
NWMN_0765	Hypothetical protein	Hypothetical protein	2.72
NWMN_0784	Hypothetical protein	Hypothetical protein	2.07
NWMN_0948	Hypothetical protein	Hypothetical protein	2.81
NWMN_1074	Hypothetical protein	Hypothetical protein	3.74
NWMN_1123	Hypothetical protein	Hypothetical protein	2.16
NWMN_1526	Hypothetical protein	Hypothetical protein	5.45
NWMN_1527	Hypothetical protein	Hypothetical protein	3.32
NWMN_1632	Hypothetical protein	Hypothetical protein	3.27
NWMN_1663	Hypothetical protein	Hypothetical protein	2.17
NWMN_1688	Hypothetical protein	Hypothetical protein	3.22
NWMN_1689	Hypothetical protein	Hypothetical protein	3.08
NWMN_1731	Hypothetical protein	Hypothetical protein	5.22
NWMN_1820	Hypothetical protein	Hypothetical protein	2.02
NWMN_1848	Hypothetical protein	Hypothetical protein	4.99
NWMN_1860	Hypothetical protein	Hypothetical protein	2.11
NWMN_1861	Hypothetical protein	Hypothetical protein	2.68
NWMN_1989	Hypothetical protein	Hypothetical protein	3.18
NWMN_2005	Hypothetical protein	Hypothetical protein	2.82

NWMN_2087	Hypothetical protein	Hypothetical protein	2.89
NWMN_2088	Hypothetical protein	Hypothetical protein	2.46
NWMN_2243	Hypothetical protein	Hypothetical protein	2.43
NWMN_2270	Hypothetical protein	Hypothetical protein	3.4
NWMN_2282	Hypothetical protein	Hypothetical protein	2.07
NWMN_2368	Hypothetical protein	Hypothetical protein	2.66
NWMN_2389	Hypothetical protein	Hypothetical protein	2.58
NWMN_2392	Hypothetical protein	Hypothetical protein	2.91
NWMN_2406	Hypothetical protein	Hypothetical protein	3.76
NWMN_2502	Hypothetical protein	Hypothetical protein	9.21
NWMN_2538	Hypothetical protein	Hypothetical protein	4.08
NWMN_2553	Hypothetical protein	Hypothetical protein	4.07
NWMN_2555	Hypothetical protein	Hypothetical protein	2.82
NWMN_2556	Hypothetical protein	Hypothetical protein	2.5
NWMN_2557	Hypothetical protein	Hypothetical protein	3.15
NWMN_2558	Hypothetical protein	Hypothetical protein	2.1
NWMN_2579	Hypothetical protein	Hypothetical protein	2.92
NWMN_2585	Hypothetical protein	Hypothetical protein	2.02
NWMN_2591	Hypothetical protein	Hypothetical protein	3.33
NWMN_2597	Hypothetical protein	Hypothetical protein	3.75
NWMN_2202	Hypothetical protein	Hypothetical protein	2.05
NWMN_1645	Hypothetical protein	Hypothetical protein	2

Supplementary Table 6. 95 genes with transcript levels increased more than two fold triggered by DHBP treatment in the Newman wild-type strain.

Gene	Description	Biological process	Fold change
NWMN_2469	Soluble lytic transglycosylases	Cell wall metabolism	2.60
NWMN_2199	Secretory antigen precursor SsaA	Cell wall metabolism	2.47
NWMN_0429	N-acetylmuramoyl-L-alanine amidase	Cell wall metabolism	2.05
set11nm	Superantigen toxin	Pathogenesis	6.25
NWMN_0757	Secreted coagulase	Pathogenesis	5.43
coa	Staphylocoagulase	Pathogenesis	5.39
set7nm	Superantigen toxin	Pathogenesis	5.02
set8nm	Superantigen toxin	Pathogenesis	2.45
fnbB	Fibronectin binding protein B	Pathogenesis	2.13
hlgA	Gamma-hemolysin subunit I	Pathogenesis	2.12
NWMN_1075	Superantigen-like protein	Pathogenesis	2.09
spa	Immunoglobulin G binding protein A	Pathogenesis	5.07
arcB	Ornithine carbamoyltransferase	Metabolism	5.02
pyrG	CTP synthase	Metabolism	2.59
argG	Argininosuccinate synthase	Metabolism	28.41
argH	Argininosuccinate lyase	Metabolism	18.50
arcC	Carbamate kinase	Metabolism	2.27
adk	Adenylate kinase	Metabolism	2.05
fruB	Fructose 1-phosphate kinase	Metabolism	2.00
cydA	Cytochrome d terminal oxidase, subunit I	Oxidation reduction	2.48
cydB	cytochrome d terminal oxidase, subunit II	Oxidation reduction	2.47
NWMN_2478	Oxidoreductase	Oxidation reduction	2.25
arcA	DNA-binding response regulator	Regulatory pathways	5.99

NWMN_2530	Transcriptional regulator Crp/Fnr family protein	Regulatory pathways	2.19
NWMN_0667	Fructose operon transcriptional regulator	Regulatory pathways	2.05
NWMN_0423	Sodium-dependent symporter protein	Transporter	2.70
NWMN_1261	Glycine betaine transporter 1	Transporter	2.19
NWMN_2253	Drug resistance transporter EmrB/QacA subfamily protein	Transporter	3.41
NWMN_2276	YhgE/Pip N-terminal domain containing protein	Transporter	61.94
NWMN_2261	ABC transporter ATP-binding protein	Transporter	26.70
NWMN_1749	Glutamine transport ATP-binding protein	Transporter	12.85
NWMN_1231	ABC transporter ATP-binding protein	Transporter	10.17
arcD	Arginine/oirnithine antiporter	Transporter	3.78
NWMN_1232	ABC transporter permease	Transporter	3.18
NWMN_2412	ABC transporter ATP-binding protein	Transporter	2.55
NWMN_2246	Sodium/glutamate symporter	Transporter	2.25
NWMN_2413	ABC-2 family transporter protein	Transporter	2.20
rplW	50S ribosomal subunit protein L23	Translation	2.97
rpsC	30S ribosomal subunit protein S3	Translation	2.78
rplP	50S ribosomal subunit protein L11	Translation	2.71
rplX	50S ribosomal subunit protein L24	Translation	2.64
rplF	50S ribosomal subunit protein L6	Translation	2.61
rplR	50S ribosomal subunit protein L18	Translation	2.59
rplA	50S ribosomal subunit protein L1	Translation	2.53
rpmC	50S ribosomal subunit protein L29	Translation	2.52
rpsS	30S ribosomal subunit protein S19	Translation	2.51

rplN	50S ribosomal subunit protein L14	Translation	2.47
rpsQ	30S ribosomal subunit protein S17	Translation	2.45
rpsE	30S ribosomal subunit protein S5	Translation	2.44
rpmD	50S ribosomal subunit protein L30	Translation	2.43
rplE	50S ribosomal subunit protein L5	Translation	2.43
rplB	50S ribosomal subunit protein L2	Translation	2.38
rplJ	50S ribosomal subunit protein L10	Translation	2.36
rpsJ	30S ribosomal subunit protein S10	Translation	2.13
rplO	50S ribosomal subunit protein L15	Translation	2.11
rpsB	30S ribosomal subunit protein S2	Translation	2.95
rpsH	30S ribosomal subunit protein S8	Translation	2.74
rplV	50S ribosomal subunit protein L22	Translation	2.68
rplY	50S ribosomal subunit protein L25	Translation	2.56
rpsN	30S ribosomal subunit protein S14	Translation	2.56
rplL	50S ribosomal subunit protein L7	Translation	2.46
rplC	50S ribosomal subunit protein L3	Translation	2.46
rpsL	30S ribosomal subunit protein S12	Translation	2.43
rpsG	30S ribosomal subunit protein S7	Translation	2.42
rplK	50S ribosomal subunit protein L11	Translation	2.37
rplD	50S ribosomal subunit protein L4	Translation	2.37
rpsD	30S ribosomal subunit protein S4	Translation	2.26
rpsI	30S ribosomal subunit protein S9	Translation	2.25
rplM	50S ribosomal subunit protein L13	Translation	2.24
rpmI	50S ribosomal subunit protein L35	Translation	2.22
rplS	50S ribosomal subunit protein L19	Translation	2.19
rplU	50S ribosomal subunit protein L21	Translation	2.09
rpsM	30S ribosomal subunit protein S13	Translation	2.09
rplT	50S ribosomal subunit protein L20	Translation	2.09

rpsF	30S ribosomal subunit protein S6	Translation	2.06
rpmH	50S ribosomal subunit protein L34	Translation	2.03
rplQ	50S ribosomal subunit protein L17	Translation	2.00
NWMN_1750	Extracellular glutamine-binding protein	Others	15.21
NWMN_2468	Acetyltransferase, GNAT family protein	Others	2.82
fus	Elongation factor G	Others	2.46
NWMN_0506	Putative ribosomal protein L7Ae-like	Others	2.46
NWMN_0358	Single-strand DNA-binding family protein	Others	2.32
NWMN_1985	ATP-dependent RNA helicase DEAD box family protein	Others	2.17
NWMN_0401	Hypothetical protein	Hypothetical protein	5.70
NWMN_0157	Hypothetical protein	Hypothetical protein	3.78
NWMN_2075	Hypothetical protein	Hypothetical protein	9.44
NWMN_2254	Hypothetical protein	Hypothetical protein	3.08
NWMN_0990	Hypothetical protein	Hypothetical protein	2.38
NWMN_1548	Hypothetical protein	Hypothetical protein	2.11
NWMN_2262	Hypothetical protein	Hypothetical protein	18.22
NWMN_1510	Hypothetical protein	Hypothetical protein	2.74
NWMN_2475	Hypothetical protein	Hypothetical protein	2.32
NWMN_0503	Hypothetical protein	Hypothetical protein	2.24
NWMN_0759	Hypothetical protein	Hypothetical protein	2.07
NWMN_1352	Hypothetical protein	Hypothetical protein	2.03

Supplementary Table 7. 50 genes with transcript levels decreased more than two fold triggered by DHBP treatment in the Newman wild-type strain.

Gene	Description	Biological process	Fold change
capD	Capsular polysaccharide synthesis protein CapD	Pathogenesis	0.4964
splC	Serine protease SplC	Pathogenesis	0.4183
splA	Serine protease SplA	Pathogenesis	0.4125
capB	Capsular polysaccharide synthesis protein CapB	Pathogenesis	0.4120
capA	Capsular polysaccharide synthesis protein CapA	Pathogenesis	0.4095
capC	Capsular polysaccharide synthesis protein CapC	Pathogenesis	0.4041
splB	Serine protease SplB	Pathogenesis	0.3989
NWMN_1084	Staphylococcus haemolytic protein	Pathogenesis	0.2625
lukE	Leukotoxin LukE	Pathogenesis	0.2439
lukD	Leukotoxin LukD	Pathogenesis	0.2287
ribB	Riboflavin synthase subunit alpha	Metabolism	0.4912
NWMN_0029	Uncharacterized NAD(FAD)-dependent dehydrogenases	Metabolism	0.4761
ribD	Riboflavin specific deaminase	Metabolism	0.4649
trpB	Tryptophan synthase subunit beta	Metabolism	0.4412
adhE	Bifunctional acetaldehyde-CoA/alcohol dehydrogenase	Metabolism	0.4411
sbnH	Diaminopimelate decarboxylase	Metabolism	0.4211
sbnB	Ornithine cyclodeaminase	Metabolism	0.2710
NWMN_2515	Anaerobic ribonucleoside triphosphate reductase	Metabolism	0.2545
NWMN_2514	Anaerobic ribonucleotide reductase, small subunit	Metabolism	0.2467
NWMN_2049	Zinc and cobalt transport repressor protein	Regulatory pathways	0.4522
NWMN_0117	ABC-type nitrate/sulfonate/bicarbonate transport systems	Transporter	0.4973

NWMN_0971	Manganese transport protein MntH	Transporter	0.4933
isdE	Iron compound ABC transporter	Transporter	0.4799
opuCD	Glycine betaine/carnitine/choline ABC transporter opuCD	Transporter	0.4528
NWMN_0116	ABC_NrtD_SsuB_transporters	Transporter	0.4297
NWMN_0601	ABC-type metal ion transport system	Transporter	0.3531
opuCC	Glycine betaine/carnitine/choline-binding protein OpuCC	Transporter	0.3431
NWMN_0603	ABC transporter ATP-binding protein	Transporter	0.3410
opuCB	Glycine betaine/carnitine/choline-binding protein OpuCB	Transporter	0.3360
sbnD	Membrane transporter protein	Transporter	0.3313
opuCA	Glycine betaine/carnitine/choline-binding protein OpuCA	Transporter	0.3222
ahpC	Alkyl hydroperoxide reductase	Others	0.4961
ahpF	Alkyl hydroperoxide reductase	Others	0.4819
sbnF	Siderophore biosynthesis IucC family protein	Others	0.4208
NWMN_2050	Cation efflux family protein	Others	0.4113
nrdI	Ribonucleotide reductase stimulatory protein	Others	0.3505
sbnC	Siderophore biosynthesis IucC family protein	Others	0.3435
NWMN_2109	Truncated MHC class II analog protein	Others	0.3365
bsaA1	Lantibiotic precursor	Others	0.2958
sbnA	O-Acetyl serine sulfhydrylase	Others	0.1856
NWMN_1196	Hypothetical protein	Hypothetical protein	0.4873
NWMN_0605	Hypothetical protein	Hypothetical protein	0.4846
NWMN_0115	Hypothetical protein	Hypothetical protein	0.4745
NWMN_0651	Hypothetical protein	Hypothetical protein	0.4740
NWMN_0118	Hypothetical protein	Hypothetical protein	0.4724
NWMN_0542	Hypothetical protein	Hypothetical protein	0.4260

NWMN_0602	Hypothetical protein	Hypothetical protein	0.4133
NWMN_0122	Hypothetical protein	Hypothetical protein	0.4080
NWMN_0041	Hypothetical protein	Hypothetical protein	0.3557
NWMN_1228	Hypothetical protein	Hypothetical protein	0.2269

Supplementary Table 8. Bacterial strains and plasmids used in this study.

Plasmids or strains	Genetype, relevant characteristics	source
Plasmids		
pMCSG7	T7 *lac* promoter, N-terminal His tag, Ap^r	[1]
pCL55	*E.coli-S. aureus* shuttle cloning vector, single-copy integration vector in *S. aureus*, Ap^r, Cm^r	[2]
pKOR1	Allelic replacement vector, Ap^r, Cm^r	[3]
pMCSG7-His-erWalK1	pMCSG7 derivative carrying extracellular receptor domain ofWalKof amino acid from T34-E167	This study
pMCSG7-His-erWalK2	pMCSG7 derivative carrying extracellular receptor domain ofWalKof amino acid from T34-I170	This study
pMCSG7-His-erWalK3	pMCSG7 derivative carrying extracellular receptor domain ofWalKof amino acid from T34-D172	This study
pMCSG7-His-erWalK4	pMCSG7 derivative carrying extracellular receptor domain ofWalKof amino acid from T34-Y174	This study
pMCSG7-His-erWalK5	pMCSG7 derivative carrying extracellular receptor domain ofWalKof amino acid from T34-Q176	This study
pMCSG7-His-erWalK6	pMCSG7 derivative carrying extracellular receptor domain ofWalKof amino acid from T34-Q182	This study
pMCSG7-His-erWalK7	pMCSG7 derivative carrying extracellular receptor domain ofWalKof amino acid from T34-A189	This study
pMCSG7-His erWalK6R86M	pMCSG7 derivative carrying extracellular receptor domain ofWalKof amino acid from T34-Q182 with amino acid R86 mutated to M	This study
pMCSG7-His erWalK6I58M	pMCSG7 derivative carrying extracellular receptor domain ofWalKof amino acid from T34-Q182 with amino acid I58 mutated to M	This study

p-*walKR*	pCL55 derivative carrying *walKR* with its own promoter	This study
pKOR1::*walK*	pKOR1 derivative containing wild-type WalK	This study
pKOR1::*walKV149A*MU	pKOR1 derivative for mutating WalKV149 to A	This study
pKOR1::*walKD119A*MU	pKOR1 derivative for mutating WalKD119 to A	This study
pKOR1::*walKY165A*MU	pKOR1 derivative for mutating WalKY165 to A	This study
pKOR1::*walKY165F*MU	pKOR1 derivative for mutating WalKY165 to F	This study
Strains		
S. aureus		
RN4220	Restriction-deficient transformation recipient	Lab stock
Newman	Wild type	Lab stock
WT	Newman wild-type carrying pCL55	This study
V149A	*walKV149A* carrying pCL55	This study
D119A	*walKD119A* carrying pCL55	This study
V149A/p-*walKR*	V149A carrying p-*walKR*	This study
D119A/p-*walKR*	D119A carrying p-*walKR*	This study
Y165F	*walKY165F* mutant	This study

E. coli		
DH5α	*endA hsdR17 supE44 thi-1 recA1 gyrA relA1* D (*lacZYA-argF*) *U169 deoR* (f*80dlac*D (*lacZ*) *M15*)	Lab stock
BL-21	BL-21 star (DE3) for recombinant protein expression	Lab stock

Supplementary Table 9. Primers used in this study.

Description	Sequence (5’-3’)
erWalK1F	tacttccaatccaatgccacaaataaccttgaaaaagagctgct
erWalK1R	ttatccacttccaatgtta ttcgatataaatattaccaattacctttttat
erWalK2R	ttatccacttccaatgttaaatttttgattcgatataaatattaccaattacc
erWalK3R	ttatccacttccaatgtta gtcattaatttttgattcgatataaatattacc
erWalK4R	ttatccacttccaatgtta ataaacgtcattaatttttgattcgatataaa
erWalK5R	ttatccacttccaatgtta ttggttataaacgtcattaatttttgattc
erWalK6R	ttatccacttccaatgttattgatttatattatttaattggttataaacgtc
erWalK7R	ttatccacttccaatgtta agctgtaccaacaatgaatatttgattt
erWalK6R86MF	agatattcaaaatttattaagtgagtatgccaacatgcaagaaattggagaaattcgttttatagataaag
erWalK6R86MR	ctttatctataaaacgaatttctccaatttcttgcatgttggcatactcacttaataaattttgaatatct
erWalK6I58MF	gtcatatactttttcaatactcatttctaattgtttcgcgtactgcgt
erWalK6I58MR	acgcagtacgcgaaacaattagaaatgagtattgaaaaagtatatgac
erWalK6D119AF	tctaatcaatcaaaaagcgaatgctagttctgtccaaaaagcactat

erWalK6D119AR	atagtgctttttggacagaactagcattcgctttttgattgattaga
erWalK6V149AF	ctttaactgggatattatatgcccagacacggtccttacc
erWalK6V149AR	ggtaaggaccgtgtctgggcatataatatcccagttaaag
erWalK6Y165AF	cgtcattaatttttgattcgatagcaatattaccaattacctttttatcgactttaactgggat
erWalK6Y165AR	atcccagttaaagtcgataaaaaggtaattggtaatattgctatcgaatcaaaaattaatgacg
erWalK6Y165FF	cgtcattaatttttgattcgataaaaatattaccaattacctttttatcgactttaactgggat
erWalK6Y165FR	atcccagttaaagtcgataaaaaggtaattggtaatatttttatcgaatcaaaaattaatgacg
WalKattB1	gggg acaagtttgtacaaaaaagcaggctcatcattgtgttaaatatcattgtcac
WalKattB2	gggg accactttgtacaagaaagctgggt accatatatgtcaatacgacactcat
WalKR_pCL55F	tcg ggatcc gtccattttctttaaaatgtatgaac
WalKR_pCL55R	tcg ggtacc ttattcatcccaatcaccgtc
hlgCF	tgagtcagacattaggatac
hlgCR	ttgttgttctacttcacttac
hlaF	gcctggccttcagcctttaaggtacagttg
hlaR	ggttgaacatatttcagtgtatgaccaatc
rRNAF	acgtggataacctacctataagactgggat
rRNAR	taccttaccaactagctaatgcagcg

SUPPLEMENTARY REFERENCES

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