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.
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.
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      |
| Accession  | Description                                | Category  | Score  |
|------------|--------------------------------------------|-----------|--------|
| 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      | Precorрин-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 |
| Gene ID       | Protein Name                                                                 | Category      | E-value |
|--------------|------------------------------------------------------------------------------|---------------|---------|
| 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 |
| Gene  | Description                                      | Category               | Score |
|-------|--------------------------------------------------|------------------------|-------|
| 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|
| Gene   | Description                                      | Category     | Score  |
|--------|--------------------------------------------------|--------------|--------|
| 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 |
| Gene   | Description                                      | Domain     | Score   |
|--------|--------------------------------------------------|------------|---------|
| 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  |
| GenBank ID   | Description                                           | Group    | Score  |
|-------------|-------------------------------------------------------|----------|--------|
| NWMN_2474   | Glyoxalase family protein                            | Others   | 0.4753 |
| NWMN_2587   | Putative rhodanese-related sulfurtransferase         | Others   | 0.4332 |
| NWMN_2609   | Chromosome partitioning 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 |
| Gene         | Protein Type | Value     |
|--------------|--------------|-----------|
| NWMN_0681    | Hypothetical protein | 0.3381    |
| NWMN_0759    | Hypothetical protein | 0.0104    |
| NWMN_0775    | Hypothetical protein | 0.4731    |
| NWMN_0990    | Hypothetical protein | 0.4772    |
| NWMN_1346    | Hypothetical protein | 0.0832    |
| NWMN_1548    | Hypothetical protein | 0.4922    |
| NWMN_1552    | Hypothetical protein | 0.3538    |
| NWMN_1570    | Hypothetical protein | 0.4573    |
| NWMN_1708    | Hypothetical protein | 0.2845    |
| NWMN_1874    | Hypothetical protein | 0.1987    |
| NWMN_1875    | Hypothetical protein | 0.2618    |
| NWMN_2074    | Hypothetical protein | 0.4548    |
| NWMN_2075    | Hypothetical protein | 0.4219    |
| NWMN_2254    | Hypothetical protein | 0.4109    |
| NWMN_2259    | Hypothetical protein | 0.4266    |
| NWMN_2436    | Hypothetical protein | 0.4920    |
| NWMN_0676    | Hypothetical protein | 0.0077    |
| NWMN_1070    | 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      |
| Gene ID | Description                                      | Pathway   | Score  |
|--------|--------------------------------------------------|-----------|--------|
| 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 |
| Gene Name | Description | Pathway | Score |
|-----------|-------------|---------|-------|
| 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 | 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 |
| Gene ID     | Description                                           | GO Term             | Score  |
|------------|-------------------------------------------------------|---------------------|--------|
| 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 partitioning 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 |
| Gene          | Description                               | Others          | Score  |
|--------------|-------------------------------------------|-----------------|--------|
| *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 protein CapI | Pathogenesis       | 3.99        |
| *capM*    | Capsular polysaccharide biosynthesis protein CapM | 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 protein CapJ | 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        |
| Gene   | Description                                      | Pathway     | Score |
|--------|--------------------------------------------------|-------------|-------|
| capP   | Capsular polysaccharide synthesis enzyme CapP    | Pathogenesis| 2.13  |
| eta    | Exfoliative toxin A                              | Pathogenesis| 2.03  |
| NWMN_2286 | MarR family regulatory protein                  | Regulatory  | 6.1   |
| sarA   | Accessory regulator A                            | Regulatory  | 4.98  |
| NWMN_2105 | Transcriptional regulator MerR family protein    | Regulatory  | 3.88  |
| argR   | ArgR family transcriptional regulator            | Regulatory  | 3.72  |
| spoVG  | Regulatory protein SpoVG                         | Regulatory  | 3.55  |
| NWMN_0921 | ATL autolysin transcriptional regulator         | Regulatory  | 3.25  |
| agrD   | Accessory gene regulator protein D               | Regulatory  | 2.83  |
| kdpE   | KDP operon transcriptional regulatory protein    | Regulatory  | 2.74  |
| agrA   | Accessory gene regulator protein A               | Regulatory  | 2.64  |
| NWMN_2225 | Phosphosugar-binding transcriptional regulator | Regulatory  | 2.54  |
| agrC   | Accessory gene regulator protein C               | Regulatory  | 2.43  |
| rbsR   | Ribose transcriptional repressor RbsR            | Regulatory  | 2.38  |
| lexA   | LexA repressor                                   | Regulatory  | 2.32  |
| sarR   | Accessory regulator R                            | Regulatory  | 2.3   |
| agrB   | Accessory gene regulator B                       | Regulatory  | 2.3   |
| NWMN_1655 | Accessory regulator Rot                          | Regulatory  | 2.24  |
| kdpD   | Sensor histidine kinase KdpD                     | Regulatory  | 2.23  |
| NWMN_0326 | MarR family regulatory protein                  | Regulatory  | 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  |
| Gene   | Enzyme Description                                                                 | Category       | Score |
|--------|------------------------------------------------------------------------------------|----------------|-------|
| metE   | 5-methyltetrahydropteroylglutamate/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  |
| Gene   | Protein Name                                      | Function                           | Description               | Log2 fold change |
|--------|--------------------------------------------------|------------------------------------|---------------------------|------------------|
| ilvB   | Acetolactate synthase large subunit               | Metabolism                         |                           | 2.35             |
| NWMN_1275 | 4-oxalocrotonate tautomerase                       | Metabolism                         |                           | 2.35             |
| dapB   | Dihydridipicolinate 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             |
| Gene/Protein Description                              | Function Description                                      | Type   | Value |
|------------------------------------------------------|-----------------------------------------------------------|--------|-------|
| ABC-type oligopeptide transport system                | Transporter                                               |        | 2.9   |
| oppC                                                  | Oligopeptide transport system permease                    | Transporter | 2.87  |
| High-affinity nickel transporter                      | Transporter                                               |        | 2.86  |
| ABC-type uncharacterized transport system              | Transporter                                               |        | 2.86  |
| ABC transporter ATP-binding protein                   | Transporter                                               |        | 2.82  |
| Mannitol-specific IIA component                       | Transporter                                               |        | 2.75  |
| Oligopeptide ABC transporter ATP-binding protein      | Transporter                                               |        | 2.65  |
| Manganese transport protein MntH                       | Transporter                                               |        | 2.58  |
| Phosphonates ABC transporter permease                 | Transporter                                               |        | 2.56  |
| Di-/tripeptide ABC transporter                        | Transporter                                               |        | 2.39  |
| ABC-type nitrate/sulfonate/bicarbonate transport system protein | Transporter                                               |        | 2.26  |
| Oligopeptide ABC transporter ATP-binding protein      | Transporter                                               |        | 2.25  |
| ABC transporter substrate-binding protein             | Transporter                                               |        | 2.21  |
| High-affinity Fe2+/Pb2+ permease                     | Transporter                                               |        | 2.19  |
| Na+/phosphate symporter                              | Transporter                                               |        | 2.19  |
| Cobalt transport family protein                       | Transporter                                               |        | 2.19  |
| L-lactate permease                                   | Transporter                                               |        | 2.18  |
| ABC transporter like protein                          | Transporter                                               |        | 2.18  |
| PTS system, alpha-glucoside-specific IIBC component  | Transporter                                               |        | 2.16  |
| ABC transporter ATP-binding protein                   | Transporter                                               |        | 2.13  |
| RGD-containing lipoprotein                            | Transporter                                               |        | 2.08  |
| ABC-type Na+ efflux pump, permease component          | Transporter                                               |        | 2.07  |
| Competence transcription factor ComK                  | Transcription                                             |        | 8.72  |
| 50S ribosomal protein L28                             | Translation                                               |        | 2    |
| V8 protease, glutamyl endopeptidase precursor         | Others                                                    |        | 18.92 |
| Cysteine protease precursor                           | Others                                                    |        | 13.98 |


| Protein ID   | Description                                      | Function     | Score |
|-------------|--------------------------------------------------|--------------|-------|
| 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  |
| tmp         | 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   |
| Gene Symbol | Description                                      | Similarity score |
|-------------|--------------------------------------------------|------------------|
| NWMN_1821   | Ribonuclease BN                                  | 2.66             |
| NWMN_1888   | Phage tail tape measure protein                  | 2.62             |
| NWMN_2547   | Glycosyl transferase, group 1 family protein     | 2.6              |
| radC        | DNA repair protein RadC                          | 2.53             |
| NWMN_0374   | Predicted Na+/dicarboxylate symporter            | 2.51             |
| qoxD        | Quinol oxidase polypeptide IV                    | 2.51             |
| acuC        | Acetoin utilization protein AcuC                 | 2.48             |
| NWMN_0331   | Luciferase-like monooxygenase                    | 2.43             |
| bsaA2       | Lantibiotic precursor                            | 2.34             |
| NWMN_2480   | Hydrolase                                        | 2.3              |
| veg         | Veg protein                                      | 2.3              |
| NWMN_1600   | Universal stress protein family protein          | 2.29             |
| NWMN_1088   | Cell division protein MraZ                       | 2.25             |
| NWMN_0330   | Glyoxalase family protein                        | 2.2              |
| NWMN_1525   | Luciferase family protein                        | 2.17             |
| NWMN_0995   | Phage anti-repressor protein                     | 2.16             |
| NWMN_0771   | OsmC-like protein                                | 2.07             |
| orfX        | rRNA large subunit methyltransferase            | 2.06             |
| NWMN_0527   | Glycosyl transferase, group 1 family protein     | 2.03             |
| nrdI        | Ribonucleotide reductase stimulatory protein     | 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   |
| Accession | Description | Score |
|-----------|-------------|-------|
| NWMN_0216 | Hypothetical protein | 6.46  |
| NWMN_2406 | Hypothetical protein | 6.42  |
| NWMN_1731 | Hypothetical protein | 6.37  |
| NWMN_0220 | Hypothetical protein | 5.93  |
| NWMN_0221 | Hypothetical protein | 5.84  |
| NWMN_0739 | Hypothetical protein | 5.49  |
| NWMN_0225 | Hypothetical protein | 4.99  |
| NWMN_0223 | Hypothetical protein | 4.87  |
| NWMN_0041 | Hypothetical protein | 4.82  |
| NWMN_0738 | Hypothetical protein | 4.79  |
| NWMN_2553 | Hypothetical protein | 4.64  |
| NWMN_2597 | Hypothetical protein | 4.6   |
| NWMN_0234 | Hypothetical protein | 4.54  |
| NWMN_0695 | Hypothetical protein | 4.5   |
| NWMN_0222 | Hypothetical protein | 4.39  |
| NWMN_0224 | Hypothetical protein | 4.32  |
| NWMN_0323 | Hypothetical protein | 4.24  |
| NWMN_0382 | Hypothetical protein | 4.11  |
| NWMN_0352 | Hypothetical protein | 4.06  |
| NWMN_0765 | Hypothetical protein | 3.94  |
| NWMN_2270 | Hypothetical protein | 3.93  |
| NWMN_0366 | Hypothetical protein | 3.88  |
| NWMN_1989 | Hypothetical protein | 3.86  |
| NWMN_2368 | Hypothetical protein | 3.85  |
| NWMN_2555 | Hypothetical protein | 3.65  |
| NWMN_1527 | Hypothetical protein | 3.61  |
| NWMN_1688 | Hypothetical protein | 3.59  |
| NWMN_1689 | Hypothetical protein | 3.57  |
| Protein ID     | Description     | Value  |
|---------------|-----------------|--------|
| NWMN_1632     | Hypothetical protein | 3.56   |
| NWMN_2005     | Hypothetical protein | 3.54   |
| NWMN_1074     | Hypothetical protein | 3.51   |
| NWMN_2209     | Hypothetical protein | 3.51   |
| NWMN_0227     | Hypothetical protein | 3.48   |
| NWMN_0673     | Hypothetical protein | 3.45   |
| NWMN_2557     | Hypothetical protein | 3.45   |
| NWMN_1861     | Hypothetical protein | 3.45   |
| NWMN_2591     | Hypothetical protein | 3.34   |
| NWMN_0460     | Hypothetical protein | 3.3    |
| NWMN_2087     | Hypothetical protein | 3.28   |
| NWMN_0364     | Hypothetical protein | 3.25   |
| NWMN_1225     | Hypothetical protein | 3.24   |
| NWMN_2538     | Hypothetical protein | 3.22   |
| NWMN_0226     | Hypothetical protein | 3.2    |
| NWMN_0246     | Hypothetical protein | 3.12   |
| NWMN_2556     | Hypothetical protein | 3.1    |
| NWMN_2465     | Hypothetical protein | 3.08   |
| NWMN_2417     | Hypothetical protein | 3.07   |
| NWMN_0948     | Hypothetical protein | 3.07   |
| NWMN_2389     | Hypothetical protein | 3.03   |
| NWMN_0648     | Hypothetical protein | 3.01   |
| NWMN_0556     | Hypothetical protein | 3     |
| NWMN_0048     | Hypothetical protein | 3     |
| NWMN_0383     | Hypothetical protein | 2.93   |
| NWMN_1510     | Hypothetical protein | 2.91   |
| NWMN_2088     | Hypothetical protein | 2.89   |
| NWMN_1235     | Hypothetical protein | 2.87   |
| Gene   | Protein Description | Value |
|--------|---------------------|-------|
| NWLMN_0045 | Hypothetical protein | 2.8   |
| NWLMN_2282 | Hypothetical protein | 2.79  |
| NWLMN_1123 | Hypothetical protein | 2.75  |
| NWLMN_2579 | Hypothetical protein | 2.75  |
| NWLMN_2392 | Hypothetical protein | 2.71  |
| NWLMN_0078 | Hypothetical protein | 2.7   |
| NWLMN_2243 | Hypothetical protein | 2.62  |
| NWLMN_0767 | Hypothetical protein | 2.61  |
| NWLMN_1820 | Hypothetical protein | 2.6   |
| NWLMN_0904 | Hypothetical protein | 2.58  |
| NWLMN_1720 | Hypothetical protein | 2.58  |
| NWLMN_0637 | Hypothetical protein | 2.56  |
| NWLMN_0908 | Hypothetical protein | 2.55  |
| NWLMN_0970 | Hypothetical protein | 2.5   |
| NWLMN_2585 | Hypothetical protein | 2.49  |
| NWLMN_2330 | Hypothetical protein | 2.49  |
| NWLMN_0376 | Hypothetical protein | 2.47  |
| NWLMN_0784 | Hypothetical protein | 2.43  |
| NWLMN_0118 | Hypothetical protein | 2.41  |
| NWLMN_0429 | Hypothetical protein | 2.41  |
| NWLMN_0766 | Hypothetical protein | 2.4   |
| NWLMN_2558 | Hypothetical protein | 2.4   |
| NWLMN_2370 | Hypothetical protein | 2.4   |
| NWLMN_0754 | Hypothetical protein | 2.39  |
| NWLMN_0586 | Hypothetical protein | 2.34  |
| NWLMN_0770 | Hypothetical protein | 2.34  |
| NWLMN_1730 | Hypothetical protein | 2.33  |
| NWLMN_2487 | Hypothetical protein | 2.33  |
| ID         | Hypothetical protein | Hypothetical protein | Value  |
|------------|----------------------|----------------------|--------|
| 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   |
|            | Hypothetical protein | Hypothetical protein | 2.02 |
|------------|----------------------|----------------------|------|
| NWMN_1072  | 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 protein CapM                           | 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        |
| Gene   | Enzyme/Protein                      | Gene/Protein Function                     | Regulation Type |
|--------|------------------------------------|------------------------------------------|-----------------|
| *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-methyltetrahydropteroylglutamate/homocysteine S-methyltransferase | Metabolism                                | 2.26            |
| *metL* | Homoserine dehydrogenase           | Metabolism                                | 2.34            |
| *mtdD* | 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            |
| Accession | Description                                      | Category         | Score |
|-----------|--------------------------------------------------|------------------|-------|
| 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  |
| Gene   | Description                                                                 | Module        | Score |
|--------|------------------------------------------------------------------------------|---------------|-------|
| 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  |
| Gene       | Protein Description                                      | Function                      | Others | 1209 |
|------------|----------------------------------------------------------|-------------------------------|--------|------|
| **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 |
| Gene ID     | Description       | Value  |
|------------|-------------------|--------|
| NWMN_0460  | Hypothetical protein | 2.54   |
| NWMN_0556  | Hypothetical protein | 3.03   |
| NWMN_0562  | Hypothetical protein | 2.11   |
| NWMN_0648  | Hypothetical protein | 2.87   |
| NWMN_0673  | Hypothetical protein | 2.83   |
| NWMN_0695  | Hypothetical protein | 3.61   |
| NWMN_0738  | Hypothetical protein | 3.92   |
| NWMN_0739  | Hypothetical protein | 4.27   |
| NWMN_0752  | Hypothetical protein | 7.5    |
| NWMN_0753  | Hypothetical protein | 9.89   |
| NWMN_0765  | Hypothetical protein | 2.72   |
| NWMN_0784  | Hypothetical protein | 2.07   |
| NWMN_0948  | Hypothetical protein | 2.81   |
| NWMN_1074  | Hypothetical protein | 3.74   |
| NWMN_1123  | Hypothetical protein | 2.16   |
| NWMN_1526  | Hypothetical protein | 5.45   |
| NWMN_1527  | Hypothetical protein | 3.32   |
| NWMN_1632  | Hypothetical protein | 3.27   |
| NWMN_1663  | Hypothetical protein | 2.17   |
| NWMN_1688  | Hypothetical protein | 3.22   |
| NWMN_1689  | Hypothetical protein | 3.08   |
| NWMN_1731  | Hypothetical protein | 5.22   |
| NWMN_1820  | Hypothetical protein | 2.02   |
| NWMN_1848  | Hypothetical protein | 4.99   |
| NWMN_1860  | Hypothetical protein | 2.11   |
| NWMN_1861  | Hypothetical protein | 2.68   |
| NWMN_1989  | Hypothetical protein | 3.18   |
| NWMN_2005  | Hypothetical protein | 2.82   |
| Protein ID | Description       | Value |
|------------|-------------------|-------|
| NWMN_2087  | Hypothetical protein | 2.89  |
| NWMN_2088  | Hypothetical protein | 2.46  |
| NWMN_2243  | Hypothetical protein | 2.43  |
| NWMN_2270  | Hypothetical protein | 3.4   |
| NWMN_2282  | Hypothetical protein | 2.07  |
| NWMN_2368  | Hypothetical protein | 2.66  |
| NWMN_2389  | Hypothetical protein | 2.58  |
| NWMN_2392  | Hypothetical protein | 2.91  |
| NWMN_2406  | Hypothetical protein | 3.76  |
| NWMN_2502  | Hypothetical protein | 9.21  |
| NWMN_2538  | Hypothetical protein | 4.08  |
| NWMN_2553  | Hypothetical protein | 4.07  |
| NWMN_2555  | Hypothetical protein | 2.82  |
| NWMN_2556  | Hypothetical protein | 2.5   |
| NWMN_2557  | Hypothetical protein | 3.15  |
| NWMN_2558  | Hypothetical protein | 2.1   |
| NWMN_2579  | Hypothetical protein | 2.92  |
| NWMN_2585  | Hypothetical protein | 2.02  |
| NWMN_2591  | Hypothetical protein | 3.33  |
| NWMN_2597  | Hypothetical protein | 3.75  |
| NWMN_2202  | Hypothetical protein | 2.05  |
| NWMN_1645  | 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       | Regulartory pathways        | 5.99        |
| Gene ID     | Description                                      | Category             | Fold Change |
|------------|--------------------------------------------------|----------------------|-------------|
| 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/ornithine 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        |
| Gene   | Protein Type            | Translation Rate |
|--------|-------------------------|-----------------|
| rplN   | 50S ribosomal subunit protein L14 | 2.47            |
| rpsQ   | 30S ribosomal subunit protein S17 | 2.45            |
| rpsE   | 30S ribosomal subunit protein S5  | 2.44            |
| rpmD   | 50S ribosomal subunit protein L30 | 2.43            |
| rplE   | 50S ribosomal subunit protein L5  | 2.43            |
| rplB   | 50S ribosomal subunit protein L2  | 2.38            |
| rplJ   | 50S ribosomal subunit protein L10 | 2.36            |
| rpsI   | 30S ribosomal subunit protein S10 | 2.13            |
| rplO   | 50S ribosomal subunit protein L15 | 2.11            |
| rpsB   | 30S ribosomal subunit protein S2  | 2.95            |
| rpsH   | 30S ribosomal subunit protein S8  | 2.74            |
| rplV   | 50S ribosomal subunit protein L22 | 2.68            |
| rplY   | 50S ribosomal subunit protein L25 | 2.56            |
| rpsN   | 30S ribosomal subunit protein S14 | 2.56            |
| rplL   | 50S ribosomal subunit protein L7  | 2.46            |
| rplC   | 50S ribosomal subunit protein L3  | 2.46            |
| rpsL   | 30S ribosomal subunit protein S12 | 2.43            |
| rpsG   | 30S ribosomal subunit protein S7  | 2.42            |
| rplK   | 50S ribosomal subunit protein L11 | 2.37            |
| rplD   | 50S ribosomal subunit protein L4  | 2.37            |
| rpsD   | 30S ribosomal subunit protein S4  | 2.26            |
| rpsI   | 30S ribosomal subunit protein S9  | 2.25            |
| rplM   | 50S ribosomal subunit protein L13 | 2.24            |
| rpmI   | 50S ribosomal subunit protein L35 | 2.22            |
| rplS   | 50S ribosomal subunit protein L19 | 2.19            |
| rplU   | 50S ribosomal subunit protein L21 | 2.09            |
| rpsM   | 30S ribosomal subunit protein S13 | 2.09            |
| rplT   | 50S ribosomal subunit protein L20 | 2.09            |
| Gene       | Description                          | Category     | Value  |
|------------|--------------------------------------|--------------|--------|
| 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      | Others       | 15.21  |
|            | protein                              |              |        |
| NWMN_2468  | Acetyltransferase, GNAT family       | Others       | 2.82   |
|            | protein                              |              |        |
| fus        | Elongation factor G                  | Others       | 2.46   |
| NWMN_0506  | Putative ribosomal protein L7Ae-like | Others       | 2.46   |
| NWMN_0358  | Single-strand DNA-binding family     | Others       | 2.32   |
|            | protein                              |              |        |
| NWMN_1985  | ATP-dependent RNA helicase DEAD box  | Others       | 2.17   |
|            | family protein                       |              |        |
| NWMN_0401  | Hypothetical protein                 | Hypothetical | 5.70   |
| NWMN_0157  | Hypothetical protein                 | Hypothetical | 3.78   |
| NWMN_2075  | Hypothetical protein                 | Hypothetical | 9.44   |
| NWMN_2254  | Hypothetical protein                 | Hypothetical | 3.08   |
| NWMN_0990  | Hypothetical protein                 | Hypothetical | 2.38   |
| NWMN_1548  | Hypothetical protein                 | Hypothetical | 2.11   |
| NWMN_2262  | Hypothetical protein                 | Hypothetical | 18.22  |
| NWMN_1510  | Hypothetical protein                 | Hypothetical | 2.74   |
| NWMN_2475  | Hypothetical protein                 | Hypothetical | 2.32   |
| NWMN_0503  | Hypothetical protein                 | Hypothetical | 2.24   |
| NWMN_0759  | Hypothetical protein                 | Hypothetical | 2.07   |
| NWMN_1352  | Hypothetical protein                 | Hypothetical | 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/sulfonatebicarbonate transport systems | Transporter | 0.4973      |
| Gene ID       | Description                                           | Category        | Score  |
|--------------|-------------------------------------------------------|-----------------|--------|
| 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 |
| bsuA1        | Lantibiotic precursor                                 | Others          | 0.2958 |
| sbnA         | O-Acetyl serine sulphydrylase                         | 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 |
| Accession  | Description    | Description    | Value    |
|-----------|----------------|----------------|----------|
| NWMN_0602 | Hypothetical   | Hypothetical   | 0.4133   |
|           | protein        | protein        |          |
| NWMN_0122 | Hypothetical   | Hypothetical   | 0.4080   |
|           | protein        | protein        |          |
| NWMN_0041 | Hypothetical   | Hypothetical   | 0.3557   |
|           | protein        | protein        |          |
| NWMN_1228 | Hypothetical   | Hypothetical   | 0.2269   |
|           | protein        | protein        |          |
### Supplementary Table 8. Bacterial strains and plasmids used in this study.

| Plasmids or strains | Genotype, relevant characteristics | source |
|---------------------|-----------------------------------|--------|
| **Plasmids**        |                                   |        |
| pMCSG7              | T7 lac promoter, N-terminal His tag, Ap<sup>f</sup> |        |
| pCL55               | *E. coli*-*S. aureus* shuttle cloning vector, single-copy integration vector in *S. aureus*, Ap<sup>f</sup>, Cm<sup>f</sup> | 2      |
| pKOR1               | Allelic replacement vector, Ap<sup>f</sup>, Cm<sup>f</sup> | 3      |
| pMCSG7-His-erWalK1  | pMCSG7 derivative carrying extracellular receptor domain of WalK<sub>f</sub> amino acid from T34-E167 | This study |
| pMCSG7-His-erWalK2  | pMCSG7 derivative carrying extracellular receptor domain of WalK<sub>f</sub> amino acid from T34-I170 | This study |
| pMCSG7-His-erWalK3  | pMCSG7 derivative carrying extracellular receptor domain of WalK<sub>f</sub> amino acid from T34-D172 | This study |
| pMCSG7-His-erWalK4  | pMCSG7 derivative carrying extracellular receptor domain of WalK<sub>f</sub> amino acid from T34-Y174 | This study |
| pMCSG7-His-erWalK5  | pMCSG7 derivative carrying extracellular receptor domain of WalK<sub>f</sub> amino acid from T34-Q176 | This study |
| pMCSG7-His-erWalK6  | pMCSG7 derivative carrying extracellular receptor domain of WalK<sub>f</sub> amino acid from T34-Q182 | This study |
| pMCSG7-His-erWalK7  | pMCSG7 derivative carrying extracellular receptor domain of WalK<sub>f</sub> amino acid from T34-A189 | This study |
| pMCSG7-His-erWalK6R86M | pMCSG7 derivative carrying extracellular receptor domain of WalK<sub>f</sub> amino acid from T34-Q182 with amino acid R86 mutated to M | This study |
| pMCSG7-His-erWalK6I58M | pMCSG7 derivative carrying extracellular receptor domain of WalK<sub>f</sub> 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:: walKV149AMU | pKOR1 derivative for mutating WalKV149 to A | This study |
| pKOR1:: walKD119AMU | pKOR1 derivative for mutating WalKD119 to A | This study |
| pKOR1:: walKY165AMU | pKOR1 derivative for mutating WalKY165 to A | This study |
| pKOR1:: walKY165FMU | pKOR1 derivative for mutating WalKY165 to F | This study |

**Strains**

*S. aureus*

| Strain        | Description                        | Source    |
|---------------|------------------------------------|-----------|
| 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**

| Strain        | Description                                                                 | Source          |
|---------------|-----------------------------------------------------------------------------|-----------------|
| DH5α          | **endA  hsdR17  supE44  thi-1  recA1  gyrA  relA1** D (lacZYA-argF) U169 deoR (f80dlacD (lacZ) M15) | Lab stock       |
| BL-21         | BL-21 star (DE3) for recombinant protein expression                         | Lab stock       |

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**Supplementary Table 9. Primers used in this study.**

| Description | Sequence (5’-3’) |
|-------------|------------------|
| erWalK1F    | tacctccaatccaatgccacaaaataaccttgaaaaaagagctgtct | |
| erWalK1R    | ttatccacttccaatgta ttgcataaatccattacaccttttat  |
| erWalK2R    | ttatccacttccaatgtaaatttttgattcgatatataatccattaccc |
| erWalK3R    | ttatccacttccaatgta gtcattaatttttgattcgatatataatcc |
| erWalK4R    | ttatccacttccaatgta ataacaagctcataattttggatcgataaa |
| erWalK5R    | ttatccacttccaatgta ttgtataaagctcataattttggatctc  |
| erWalK6R    | ttatccacttccaatgtattgtatttatatttatggattaatggac  |
| erWalK7R    | ttatccacttccaatgta agctgtaccaacagtatcttgccttctgctct | |
| erWalK6R86MF| agatatccaaatttaattaatgtattagatgcaacacatgggaattcgttgtttgatagaaag  |
| erWalK6R86MR| ctttatctaaaaagcattttcttctatttcgttggcatctgatattatctatttatct | |
| erWalK6I58MF| gctatatctttttcaacttctactttcattttgattctctgtctgctt | |
| erWalK6I58MR| acgcagtcagcgaacacattaggagattgattggaagattatagacct | |
| erWalK6D119AF| ttaatcataaaagcgaattctgtcttgctcaaaaagcacta | |
erWalK6D119AR  atagtgcttttggacagaactagcattcgcttttgattgattaga

erWalK6V149AF  ctttaactgggatattatatgccagacacggtccttacc

erWalK6V149AR  ggttaaggaggctgtgctggcataataatatccagtaag

erWalK6Y165AF  cgtaaatatttttgatagtaataattacaattacactttttatcgactttaactgggat

erWalK6Y165AR  atccagtttaaagtctaaaaaggttaattgtaaatattgctatcgaataatccagtaag

erWalK6Y165FF  cgtaaatatttttgatagtaataattacaattacactttttatcgactttaactgggat

erWalK6Y165FR  atccagtttaaagtctaaaaaggttaattgtaaatatttattcgaatcataatccagtaag

WalKattB1  gggg acaaatgtgacaaaaaacagtccatcatatgtatcagac

WalKattB2  gggg accatctttgacaagaagctgggttacatatgtacataagacagac

WalKR_pCL55F  tcg ggatcc gttccattttctttttaaggtatgac

WalKR_pCL55R  tcg ggtacc tttattcatcccaatcaccgtc

hlgCF  tgcattcagacattaggata

hlgCR  tgcttcactttacactac

hlaF  gcctggccttcagcttaaggtacagtgg

hlaR  ggttaaatatttcatgtgatagccaaate

rRNAF  acgtggataacttcataagactgggat

rRNAR  taccttaaactagcataatgcagcg

SUPPLEMENTARY REFERENCES

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